Novel methods in [2+2] photocycloadditions and cysteine modification

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Declaration

I, Lauren Mirella Tedaldi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Acknowledgements

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Abstract

The [2+2] photocycloaddition is a powerful but underused synthetic route to cyclobutanes. The underuse of this reaction can stem from unreliable yields, which can, in part, be attributed to secondary photoreactions of the desired product. The first part of this thesis describes investigations into new methods of trapping out primary photoproducts before secondary photoreactions can take place. Lithium borohydride was demonstrated as a successful trapping agent in intramolecular [2+2] enone-olefin photocycloadditions. Eight ketone products from the [2+2] photocycloaddition of vinylogous esters were trapped as their analogous alcohols, in moderate to good yields (27-83%). Without the conditions, the ketones were all afforded in lower yields (0-50%). This is the first example of the addition of a reducing agent to trap out products during the [2+2] photocycloaddition. Initial investigations into other trapping agents and trapping the products from other [2+2] photocycloadditions are discussed.

Selective modification of proteins is a sought after goal in chemical biology. The second part of this thesis describes the use of bromomaleimide to selectively and reversibly label cysteine in small molecule substrates as thiomaleimides. Addition of a second thiol to the thiomaleimides can afford dithiosuccinimides, or addition of three equivalents of potassium carbonate in methanol can afford dehydroalanine motifs. Cysteine can be added to dibromomaleimide to afford bromothiomaleimides and dithiomaleimides. The thiomaleimides are photoactive and can be quantitatively dimerised to head-to-head *trans* adducts, or conjugated with a variety of olefins and alkynes. The regio- and diastereochemical outcomes of these reactions are discussed. The cyclobutanes thus formed render the modification irreversible to reductive conditions. The absorptions of thiomaleimides and their suitability for protein modification are considered very useful (>360 nm). A [2+2] photocycloaddition of styrene to RNAP sub-unit H was successful with good conversions, demonstrating the first use of this reaction to modify proteins.

Contents

Abbreviations

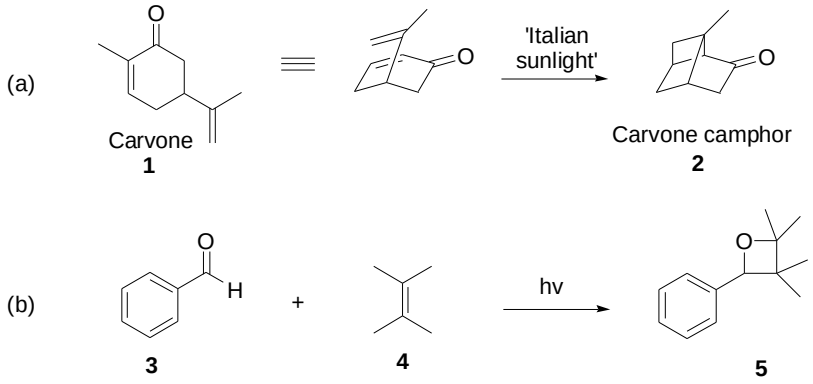
2M-SST	Doubly-labelled thiomaleimide somatostatin
aaRss	Aminoacyl *t*RNA synthetases
Ac	Acetyl
Aha	Azidohomoalanine
Ac	Acetyl
AU	Arbitrary units
Boc	Di-*tert*-butyl carbonate
Bu	Butyl
Bn	Benzyl
COSY	Correlation spectroscopy
CuAAC	Copper catalysed azide-alkyne [3+2] cycloaddition
Cy	Cyclohexane
Cys	*L*-Cysteine
D	Drug
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
Dha	Dehydroalanine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DTE	1,2-dithioethane
DTT	Dithiothreitol
E	Energy
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
eq	Equivalents
FMO	Frontier molecular orbital
GC	Gas chromatography
Grb2	Growth factor receptor-bound protein 2
GSH or G-SH	Glutathione
HMBC	Heteronuclear multiple bond coherence
HMQC	Heteronuclear multiple quantum coherence
HOBt	Hydroxybenzotriazole
HOMO	Highest occupied molecular orbital
Hpg	Homopropargylglycine
Hag	Homoallylglycine
IC	Internal conversion
ISC	Intersystem crossing
ISMB	Institute of structural molecular biology
LC	Liquid chromatography-mass spectrometry
LG	Leaving group
LUMO	Lowest unoccupied molecular orbital
Mal-SUH	Monobromomaleimide labelled P10C subunit-H
MB-SST	Monobromomaleimide-bridged somatostatin
Me	Methyl
*m*RNA	Messenger RNA
Ms	Mesyl
MSH	*O*-Mesitylenesulfonylhydroxylamine
n	Integer number

n	normal
NMM	*N*-Methylmorpholine
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
NOeSY	Nuclear Overhauser effect spectroscopy
p.	Page
p	*para*
PEG	Polyethylene glycol
Ph	Phenyl
*p*TsOH	*para*-Toluenesulfonic acid
RNAP	RNA-polymerase
RT	Room temperature (19-22 ºC)
SST	Somatostatin
SUH	P10C subunit-H
t or *tert*	Tertiary
TA	Trapping agent
TCEP	Tris(2-carboxyethyl)phosphine
Tf	Triflyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
*t*RNA	Transfer RNA
UCL	University college London
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
w/v	Weight/volume

1. Introduction

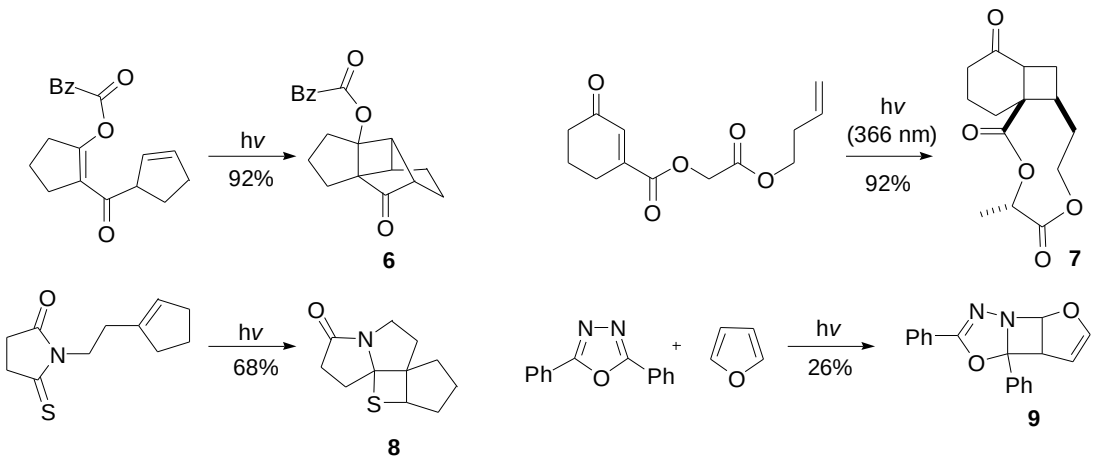
1.i. Introduction to the [2+2] photocycloaddition

The first documented photocycloaddition was observed by Ciamician and Silber[1-3] in 1908, who left carvone on the roof in Rome in direct sunlight for a period of months. Possessing both an enone and an alkene functionality, carvone **1** converted to carvone camphor **2** (structure confirmed in 1957[4]), **Scheme 1 (a)**. Shortly after Ciamician's description of the [2+2] intramolecular photocycloaddition of carvone, Paterno and co-workers disclosed the first example of a [2+2] photocycloaddition of an aldehyde **3** to an alkene **4** to give an oxetane[5] **5**, **Scheme 1 (b)**.



Scheme 1

From these first stepping stones, a vast number of [2+2] photocycloadditions have been developed, involving heteroatoms as well as carbon centres. The [2+2] photocycloaddition can involve the reaction of a range of double bond motifs. For example, a variety of enones can react with alkenes to produce cyclobutanes, *e.g.* **6** and **7**,[6, 7] thiocarbonyls and alkenes will undergo the [2+2] photocycloaddition to afford thietanes, *e.g.* **8**,[8] and the reaction of oxadiazoles and furans can provide azetidines, *e.g.* **9**.[9] An illustrative set of examples of these reactions is shown in **Scheme 2**.



Scheme 2

Before reviewing the literature further on [2+2] photocycloadditions, the fundamentals behind photochemistry will be discussed.

1.i.i. Fundamentals of photochemistry

Absorption of a photon by an organic molecule affords an electronically excited state. The excited molecule is a species whose electrons are arranged in way that is not the lowest energy conformation, **Figure 1**.

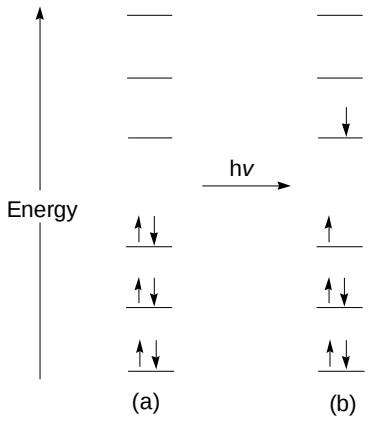


Figure 1: Stylized molecular orbital diagram for a molecule, showing some of the orbitals and their occupancy in (a) the ground state and in (b) the excited state

Once a molecule is excited, the fate of the excited electron can result in a number of different processes that can be divided into two categories, namely non-radiative and radiative processes.

Non-radiative decay processes involve conversion of one electronic state into another without emission of light (depicted by wavy lines on the Jablonski diagram, **Figure 2**). They can be further divided into two types according to whether or not there is an overall change in spin multiplicity during the process. If no spin change occurs, the transition is 'spin allowed', and the non-radiative process is called internal conversion (IC). This is the means by which higher excited singlet states decay to the lowest excited singlet states prior to further photochemical reaction, **Figure 2**. Similarly, higher excited triplet states decay to their lowest excited triplet state by IC, **Figure 2**. IC can also occur from the lowest excited singlet state to the ground singlet state. When non-radiative decay involves a change in spin multiplicity it is called intersystem crossing (ISC) and is 'spin forbidden'. ISC can convert the lowest excited triplet state to the ground state or it can convert the lowest excited singlet state to the lowest excited triplet state, **Figure 2**. The latter example is important in that it provides the means by which many triplet states are produced prior to phosphorescence or photochemical reaction. An additional non-radiative transition is vibrational relaxation, where the electron falls from higher vibrational states to lower vibrational states within one electronically excited state, **Figure 2**.

During radiative processes (depicted by straight lines on the Jablonski diagram, **Figure 2**) a photon is emitted. When an excited singlet state (S_x, where $x \neq 0$) emits a photon, the state is normally converted to the singlet ground state (S_0). Such a radiative process, when there is no overall change of spin, is called fluorescence, **Figure 2**. Most commonly it is the lowest excited singlet state (in its lowest vibrational level) whose emission is observed, regardless of which singlet state is formed initially by absorption, this is known as Kasha's Rule.[10] In phosphorescence, the spin multiplicity of the species changes. Almost all phosphorescence in organic compounds originates from the lowest excited triplet state collapsing to the singlet ground state, **Figure 2**. This process is 'spin forbidden', thus, phosphorescence generally has an associated lifetime that is considerably longer than for fluorescence.

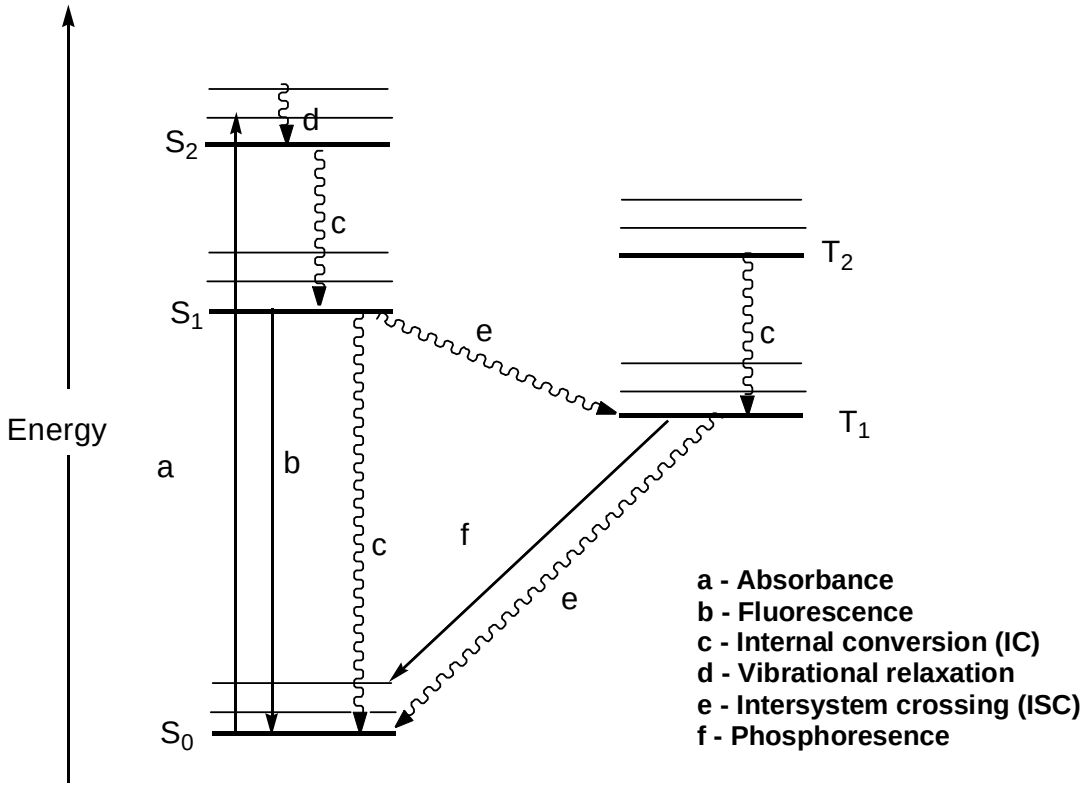


Figure 2: Jablonski diagram

As there are a number of competing processes that occur after the electron has been excited, not all absorptions lead to the eventual formation of a new product. The efficiency with which absorbed radiation causes a compound to undergo a specified photochemical process can be expressed in terms of the quantum yield (Φ) which, for a given process, is defined as;

$$\Phi = \frac{\text{Number of molecules of product (of a given process) produced}}{\text{Number of photons of light absorbed}}$$

High quantum yields are desirable for photochemical transformations to avoid extended reaction times.

The altered orbital occupancies in an excited molecule can lead to different reactions than when a molecule is in its ground state. For example, the ground state configuration of a two electron π-system is shown in **Figure 3**.

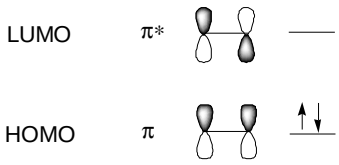


Figure 3: Ground state alkene configuration

The interactions of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of two 2π systems lead to anti-bonding interactions and no reaction takes place, **Figure 4**.

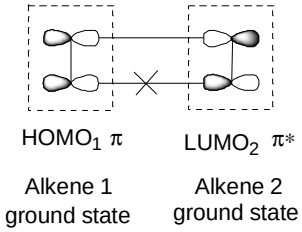


Figure 4: In the ground state, the orbital symmetries do not allow suprafacial overlap

This outcome is summarized by the simplified Woodward-Hoffman rules[11] whereby 4n electron processes are forbidden to proceed *via* suprafacial overlap in the ground state. As [2+2] cycloadditions are 4n electron processes (n = 1), in the ground state they are forbidden to proceed *via* suprafacial overlap and, as the system is too small to twist into an antarafacial configuration, there is no reaction.

However, [2+2] photocycloadditions are allowed due to the reconfiguration of the HOMO and LUMO of the ground state systems upon excitation by a photon. When a photon of the correct wavelength, and therefore correct quanta of energy, excites the system, one electron from the HOMO is excited into the LUMO, and thus the excited molecule has a new set of 'HOMO' and 'LUMO' molecular orbitals, **Figure 5**.

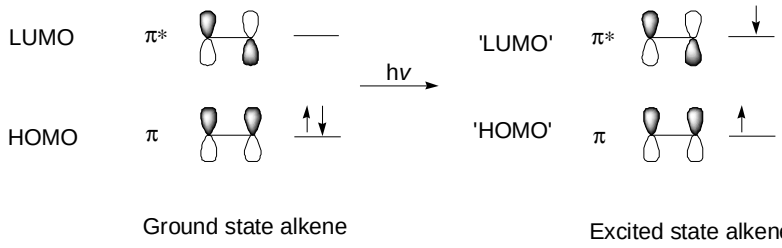


Figure 5: Excitation of an alkene changes the electronic distribution

The new ‘HOMO’ and ‘LUMO’ have favourable interactions with the HOMO and LUMO of another alkene molecule, leading to interactions that have a lower total energy than the individual molecules combined. The interacting molecules can then undergo suprafacial addition to form a cyclobutane ring, **Figure 6**. The inverted commas are used here to remind the reader that the orbitals are not the actual HOMO and LUMO at the time of the reaction, but that they were the HOMO and LUMO before excitation took place

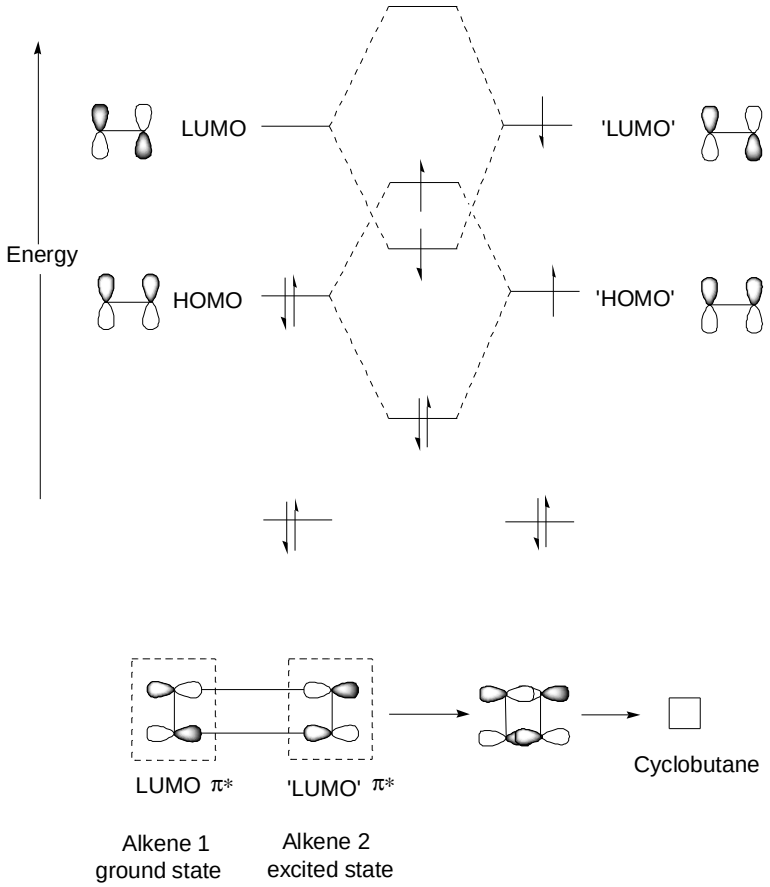


Figure 6: Photochemically-mediated cyclobutane formation

1.i.ii. Photochemical apparatus

To carry out irradiations, photochemical apparatus has been largely standardised, although there are some variations between laboratories. The equipment usually consists of a reaction solution, a light source, and if necessary, a source of cooling. Light sources in photochemistry have moved on considerably from the use of 'italian sunlight' on a roof in Rome. The more reproducible, if less exotic, approach now encompasses a variety of mercury discharge lamps. Different internal pressure lamps emit different spectral output. The most commonly used lamp is the medium pressure mercury lamp with a spectral output between around 240 nm to 600 nm. The output is not uniform across this range and there are key 'spikes' of spectral power, particularly at 365 nm, 405 nm and 436 nm, **Figure 7**.[12]

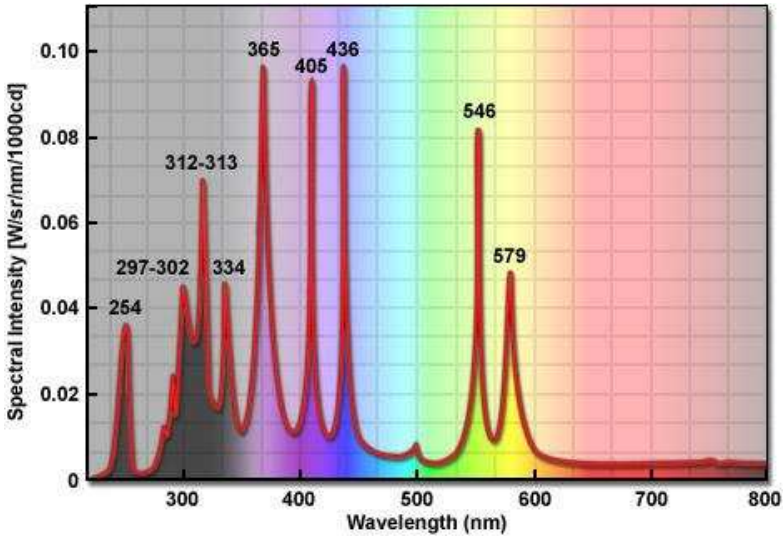


Figure 7: Spectral output from a medium pressure mercury discharge lamp[12]

Lasers are also used in photochemical research,[13] especially for time-resolved studies as they provide a very fine wavelength and distinct time control, but reactions can only be carried out on a very small scale and they do not provide a realistic choice for synthetic photochemistry.

An immersion well reactor is amongst the most common type of reactor employed in photochemistry and was used in the work reported in this thesis. In the immersion well reactor, the lamp is partitioned from the reaction solution via a water-well, **Figure 8**. This 'well' serves two functions - it keeps the reaction solution cool, as the lamp can reach temperatures in excess of 900 ^{o}C, and it also acts as a filter. The choice of glass

for the well will usually determine the lower cut-off of the range of light that the reaction is subjected to. For mercury lamps, typically the glass is Pyrex® with a cut off of around 280 nm, whilst a quartz well is mostly transparent allowing practically all of the emitted light to reach the reaction solution. Rarer uranium and Vycor® glass filters cut out wavelengths below 350 nm and 230 nm respectively. Although there are a vast number of types of glass available, there is limited choice of water-wells made out of these glasses.

Secondary to the glass-based filter is the solution filter, whereby the solution in the well acts as a filter. Solution filters can be used for a broad range of cut-offs, they can range from 1.5% w/v aqueous copper sulfate with a cut-off around 290 nm all the way up to 0.2% w/v aqueous rhodamine B which blocks out wavelengths below 620 nm.[14] However, for photochemical synthesis, the use of solution filters is not straightforward, due to the fact that the solution serves as a coolant as well as a filter and must therefore be pumped from a chilled reservoir.

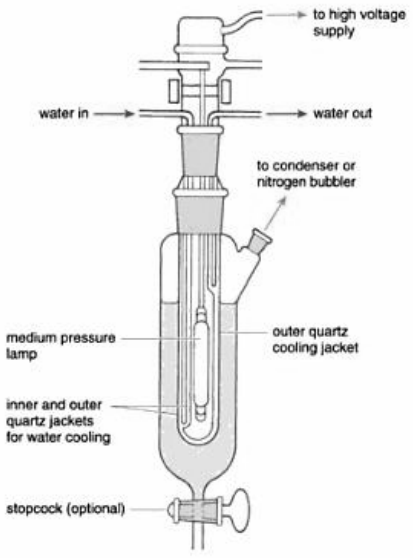


Figure 8 : Standard photochemical apparatus[15]

The reaction vessel is sealed to allow an inert gas to be bubbled through the reaction solution, removing a large proportion of dissolved oxygen from the solution prior to irradiation. This attenuates a major degradation pathway, which is the reaction of the substrate with highly reactive singlet oxygen that forms upon irradiation. This process is

referred to as ‘degassing’; it is usually carried out before the irradiation event but can be continuously employed if stringent removal of oxygen is required.

Recently, there have been some developments in flow chemistries for photochemistry.[16, 17] These allow the photochemist to irradiate samples by flowing the reaction solution past or around a light source. Residence time is therefore defined by flow rate, and the wavelength used to irradiate the substrate is characterized by the light source and the filtering properties of the tubing used.

Solvents for UV irradiation should be chosen with care. Water, alkanes and acetonitrile are transparent and usually unreactive to electronically excited substrates. Alcohols and ethers are mostly transparent but can form reactive alkoxy radicals and halogenated solvents run the risk of forming halide radicals in the course of the reaction.[10]

Sensitizers can be used when the substrate cannot achieve the correct energy state required for a particular reaction *via* irradiation on its own. For example, although many reactions take place from the triplet state, not all molecules can undergo efficient singlet-triplet intersystem crossing as the energy gap is too large, **Figure 9**. When this is the case, a triplet sensitizer is used. This is a molecule that undergoes facile intersystem crossing, as the energy gap between the two states is small, and it can then confer this triplet energy to another molecule, **Figure 9**.

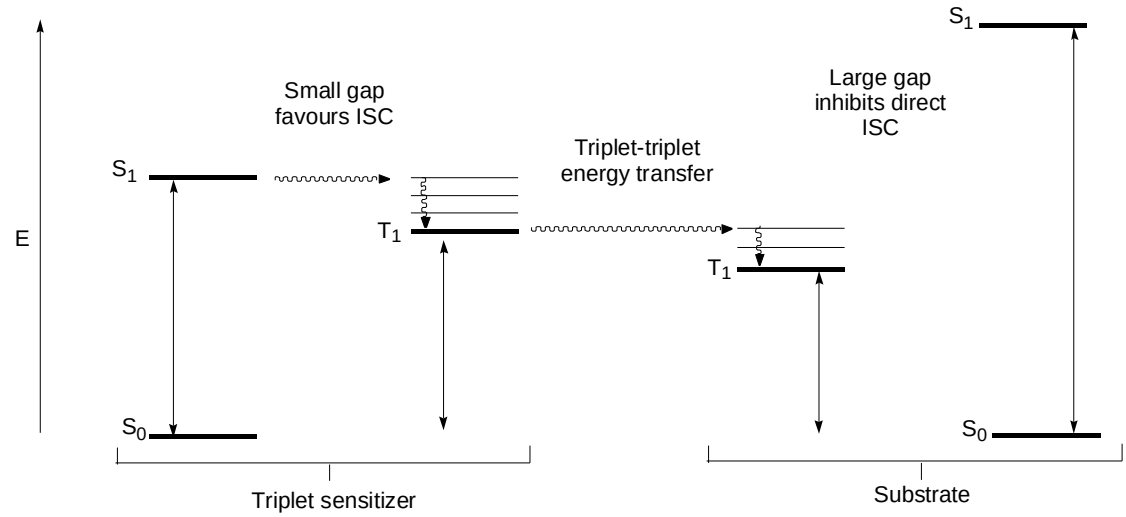


Figure 9: Energy level diagram for typical energy transfer between a triplet sensitizer and a substrate

Conversely, an excited molecule can also be quenched. Interaction between a molecule in its excited state, A*, **Eq. 1**, and a molecule in its ground state, Q, can lead to deactivation of the electronically excited state and generation of the excited state of the other molecule, **Eq. 1**. This phenomenon is known as quenching.

Eq. 1 $A^* + Q \longrightarrow Q^* + A$

Selective quenching can remove an unwanted reaction pathway from a photochemical transformation when there is more than one excited state possible, leading to more than one outcome. For example, in **Figure 10**, the singlet or triplet excited state of a substrate transfers its energy to the quencher to form the quencher's own excited states. Thus, the substrate is prevented from reacting through its own triplet state. This is particularly useful in inhibiting unwanted triplet state reactions in the presence of a desired singlet state reaction

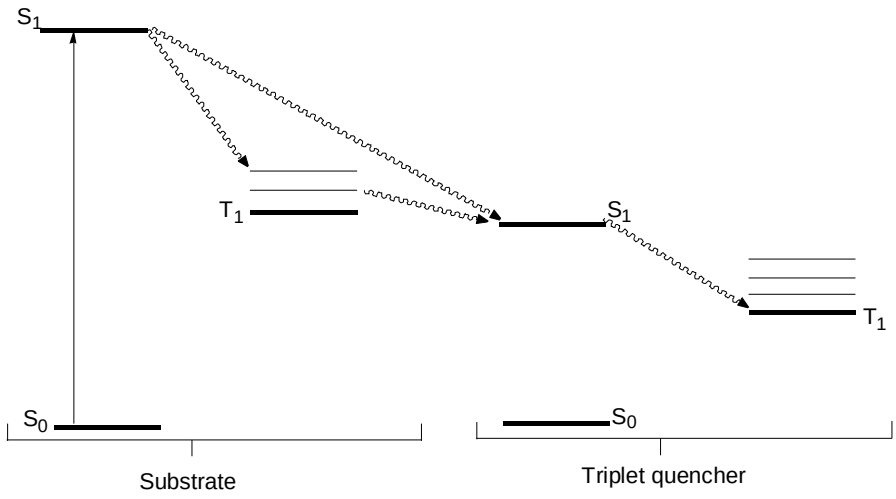


Figure 10: Energy level diagram for a typical energy transfer between a substrate and a triplet quencher

1.i.iii. [2+2] Photocycloaddition of enones

One of the most common [2+2] photocycloadditions observed involves an enone as the chromophoric alkene. The range of reactions that occur with the enone system are thought to be largely due to the formation of the n → π* singlet state (S_1) followed by efficient intersystem crossing (ISC) to the n → π* and π → π* triplet states (T_1, T_2, etc),

illustrated by the simplified Jablonski diagram and structures in **Figure 11**. Other transitions are possible, but only those involved in the singlet to triplet transitions are shown for clarity. These excitations are possible due to the n-bonding electrons available from the oxygen lone pair of the carbonyl group.[18]

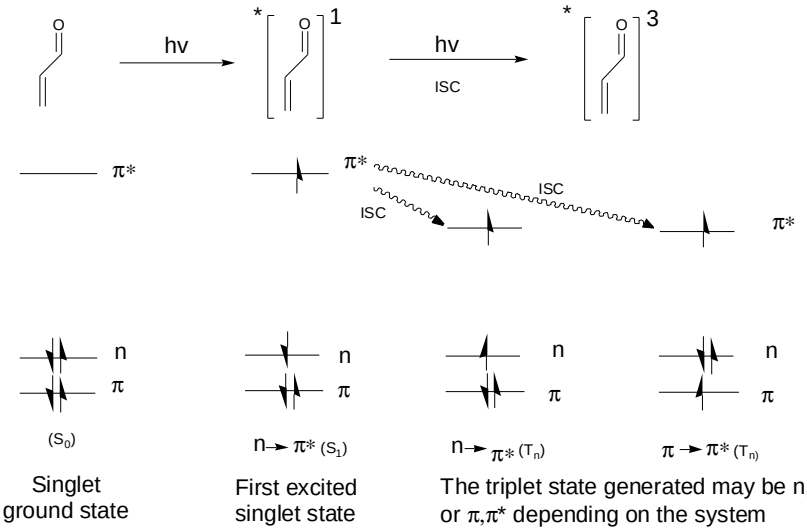
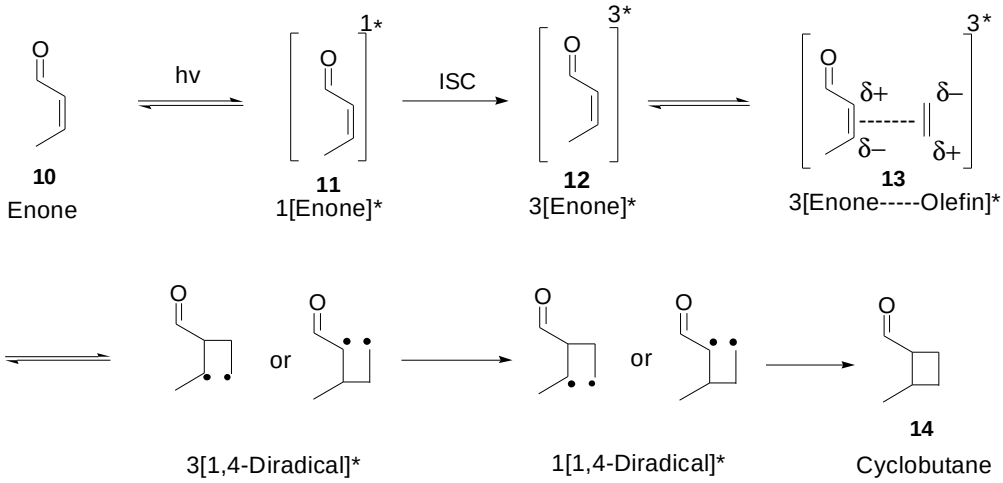


Figure 11: Simplified Jablonski diagram of the excited states of an enone system

Once excited, the enone can lose energy and collapse back to the ground state *via* IC, which is very efficient if *cis*/*trans* isomerisation is possible. However, if the enone is held in a small cyclic system, such as a cyclopentenone or cyclohexenone there is little opportunity to expend the absorbed energy *via cis/trans* isomerism of the double bond[19] and intersystem crossing becomes rapid and gives reasonable quantum efficiencies for photoaddition.

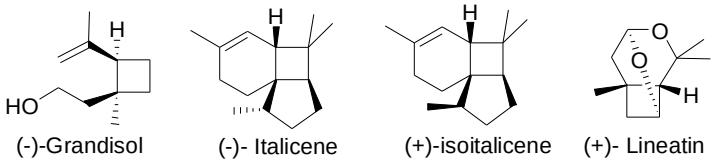
As discussed, when ground state enone **10** is photochemically excited, the singlet state enone **11** is formed, whereby intersystem crossing can take place to the triplet enone **12**, **Scheme 3**. This can then lead to the formation of a triplet 'exciplex' **13** between the enone and an olefin.[20-23] An exciplex merely describes a complex of an excited state molecule with a reaction partner, usually aligned according to new dipoles formed from excitation.[20] The exciplex has not been directly observed, but can be implied from the regioselectivity of certain intermolecular photocycloadditions and explains why the photocycloaddition can be much faster than 'standard' radical additions to olefins.[23, 24] The exciplex formed can collapse to a 1,4-diradical through bond formation at the C_α or C_β of the enone[2] and ultimately lead to the cyclobutane **14**. Relaxation back to the

ground state enone **10** can occur (or the *cis/trans* isomer in the case of straight chain enones[25]) at several stages of this transformation. However, once the cyclobutane **14** is formed it cannot undergo photochemical reversion. Therefore, whilst there are many reversible intermediate steps to this process, the overall reaction is irreversible, **Scheme 3**.



Scheme 3[20-23]

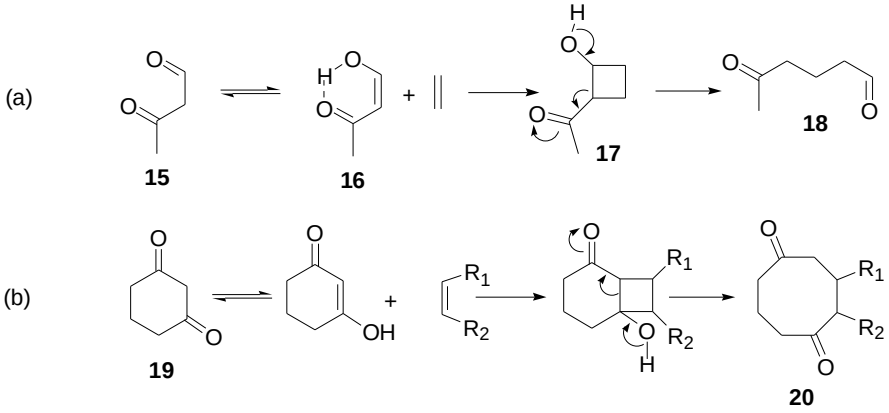
The [2+2] addition of an enone with an alkene, exemplified by Ciamician, remains one of the most exploited of the photocycloadditions in natural product synthesis due to the wide range of species that contain the cyclobutane system,[26] including grandisol,[27] (+)- and (-)-italicene,[6] and lineatin,[28] **Scheme 4**.



Scheme 4

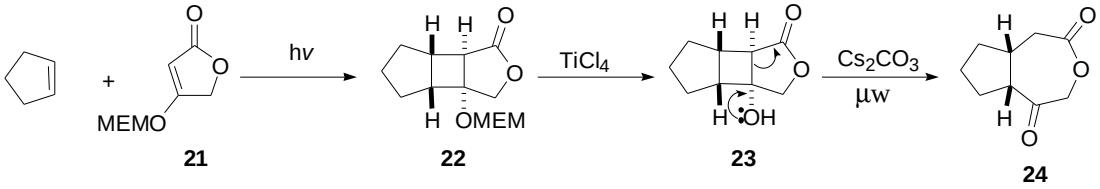
What has often made this reaction attractive to the synthetic chemist is the formation of strained and, or, sterically hindered products, which are often difficult targets to access through 'standard' thermochemical means. Furthermore, the subsequent release of ring strain can drive ensuing reactions to provide further useful scaffolds. For example, the cyclobutane can be exploited due to its propensity to be used as a reactive intermediate, as observed in the De Mayo reaction,[29, 30] **Scheme 5**. The initial reaction involves the [2+2] photocycloaddition of an enolisable 1,3-diketone **15** with an alkene **16**, **Scheme 5**

(a). The newly formed cyclobutane **17** contains an alcohol and ketone in the correct orientation to rearrange and afford 1,5-diketone **18**, **Scheme 5 (a)**. The initial 1,3-diketone can be cyclic. For example, the diketone can be part of a six-membered ring **19**, **Scheme 5 (b)**. When this is the case the 1,5-dione product **20**, after the [2+2] cycloaddition and fragmentation, is also cyclic, **Scheme 5 (b**).



Scheme 5

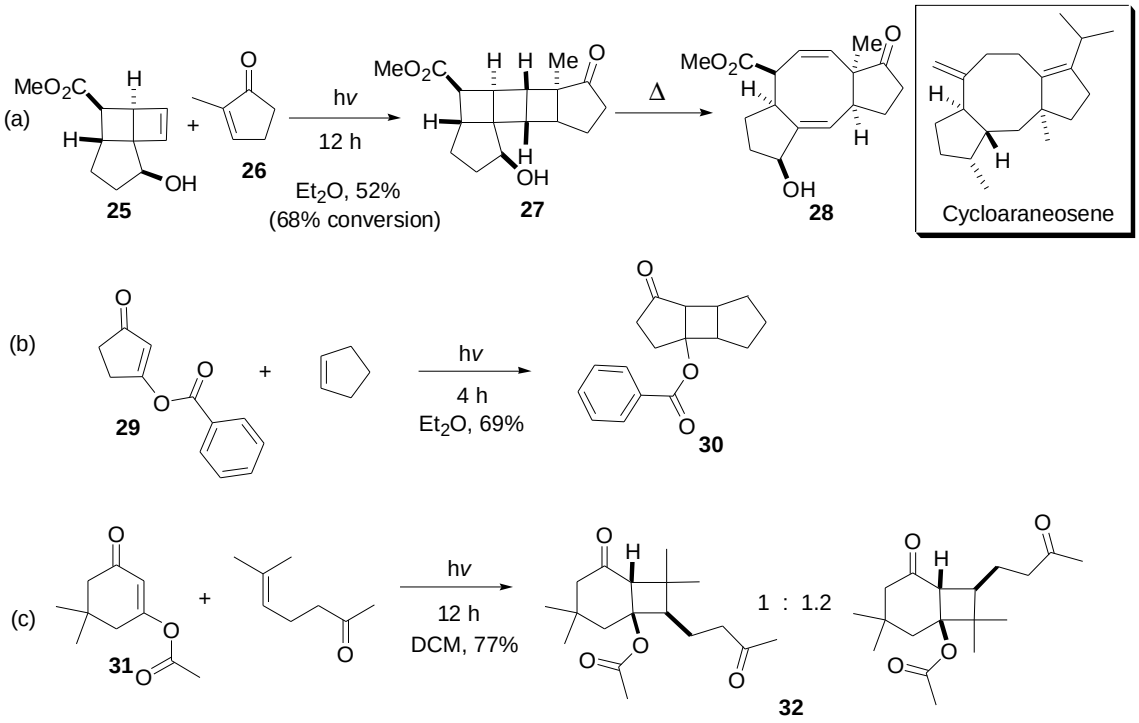
The De Mayo fragmentation has been employed towards a variety of 7,5 structures[31, 32] including lactone **24**[33] from the intermolecular reaction of cyclopentene and tetronate **21**, **Scheme 6**. In this example the photocycloaddition product **22** is a strained 5,4,5-ring. The deprotected hydroxyl of cyclobutane **23** allows fragmentation of the strained structure to 7,5-lactone **24**.



Scheme 6

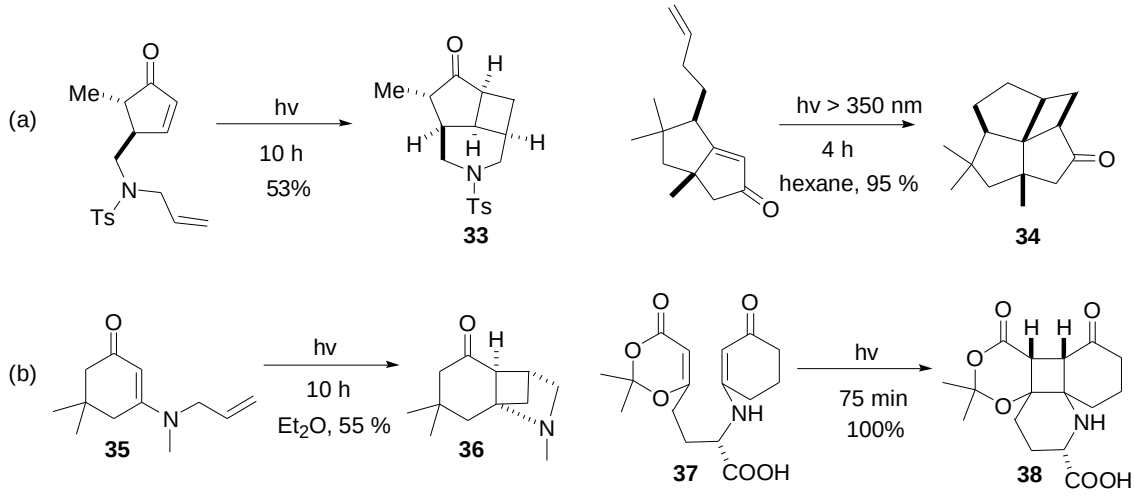
The diverse range of [2+2] enone-olefin photocycloadditions in the literature can be divided into two categories, namely inter- and intramolecular variations. Intermolecular examples of the enone-olefin [2+2] photocycloaddition are quite varied,[26, 34-38] and include the formation of complex ring systems and subsequent fragmentation products toward natural products. An example of this is the intermolecular [2+2] photocycloaddition of cyclobutene **25** with enone **26** to afford 5,4,4,4,5-ring system **27**,

which undergoes fragmentation upon heating to 5,8,5-ring system **28**. The fragmented ring **28** was used in studies towards cycloaraneosene, **Scheme 7 (a)**.[39] The intermolecular [2+2] photocycloaddition tends to involve five- and six-membered enones that are often part of a vinylogous ester system, affording oxy-substituted cyclobutanes. Examples of such scaffolds can be seen in **Scheme 7 (b**) and **(c)**, where vinylogous esters **29** and **31** undergo photocycloadditions upon irradiation to cyclobutanes **30** and **32**, respectively, **Scheme 7 (b)** and **(c)**.[40, 41]



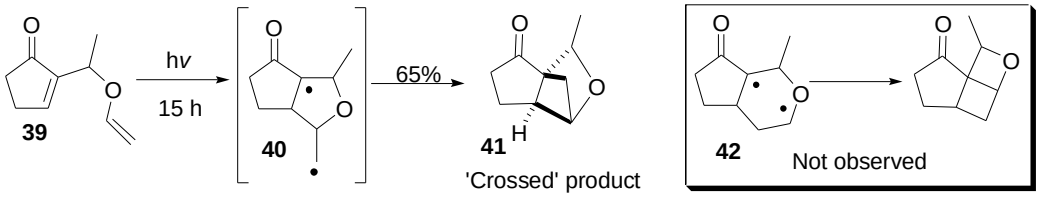
Scheme 7

The intramolecular variant of the enone-olefin [2+2] photocycloaddition has also been used to form polycycles, similarly starting from, and often forming, five- and six-membered rings.[2, 6, 26, 35, 42-56] Enones have been utilised to form complex structures such as 5,4,6-rings **33**[57] and strained fenestranes **34**,[58] **Scheme 8 (a**). Vinylogous amides are more common in the intramolecular reaction than in the intermolecular variant, an example of their use can be seen in **Scheme 8 (b)**, where vinylogous amides **35** and **37** are used to afford amino-substituted cyclobutanes **36** and **38**, respectively. In addition, the occurrence of enones as vinylogous esters is still widespread and is also demonstrated in structure **37**, **Scheme 8 (b)**.[59, 60]



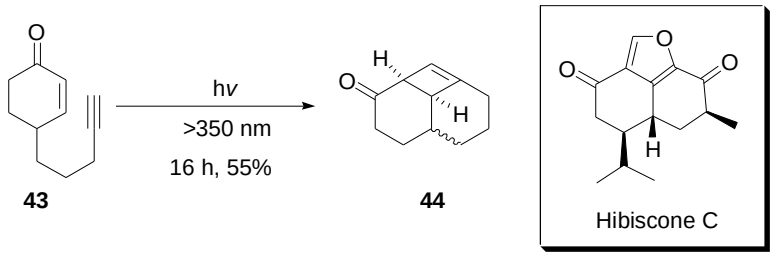
Scheme 8

For intramolecular [2+2] photocycloadditions such as these, regioselectivity is quite high in systems where the two double bonds are connected by two, three, or four atoms. Termed the 'Rules of Five' by Hammond and Srinivasan,[61, 62] the observed trend is that five-membered rings are formed, if possible, from the initial radical addition to the olefin, if not, then the six-membered ring is formed. This trend is exemplified by the work of Matlin *et al*..[48, 49, 63] When enone **39** was irradiated**,** the strained 'crossed' product **41** was formed *via* initial formation of the five membered ring diradical **40**, and no product from the formation of the six-membered ring diradical **42** was seen, **Scheme 9**.



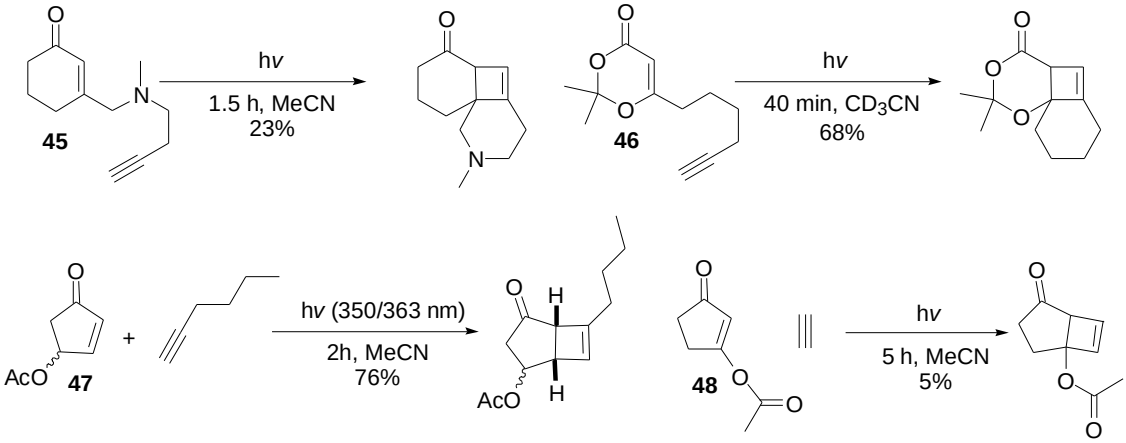
Scheme 9

Many of the photoreactions that occur with alkenes can also be carried out with alkynes, including the addition of an alkyne to an enone to yield a cyclobutene.[2, 10, 64] However, in comparison to the enone-olefin variant, surprisingly few syntheses have been reported utilizing enone-alkyne photocycloaddition as a route to cyclobutenes. The first example of the intramolecular enone-alkyne [2+2] photocycloaddition was published as recently as 1982 by Koft *et al*. towards the synthesis of hibiscone C,[46, 65, 66] where enone-alkyne **43** was converted to tri-cycle **44** in a key step, **Scheme 10**.



Scheme 10

As discussed, successful examples of the enone-alkyne [2+2] photocycloaddition are less common than the enone-olefin variation, and, as such, the inter- and intramolecular variations of this reaction will be discussed together. Those examples that are in the literature primarily include the cyclohexenone species,[50, 54, 55, 65-67] such as **45** and **46**, **Scheme 11**.[55, 67] Interestingly, whilst not completely unknown, **47** and **48**, **Scheme 11**,[68, 69] there are far fewer examples with five-membered rings either in the starting material or forming in the product, than with six-membered rings. With analogy to the enone-olefin reaction, the enone motifs in enone-alkyne [2+2] photocycloadditions are often incorporated as vinylogous esters, such as **46** and **48**, **Scheme 11**.



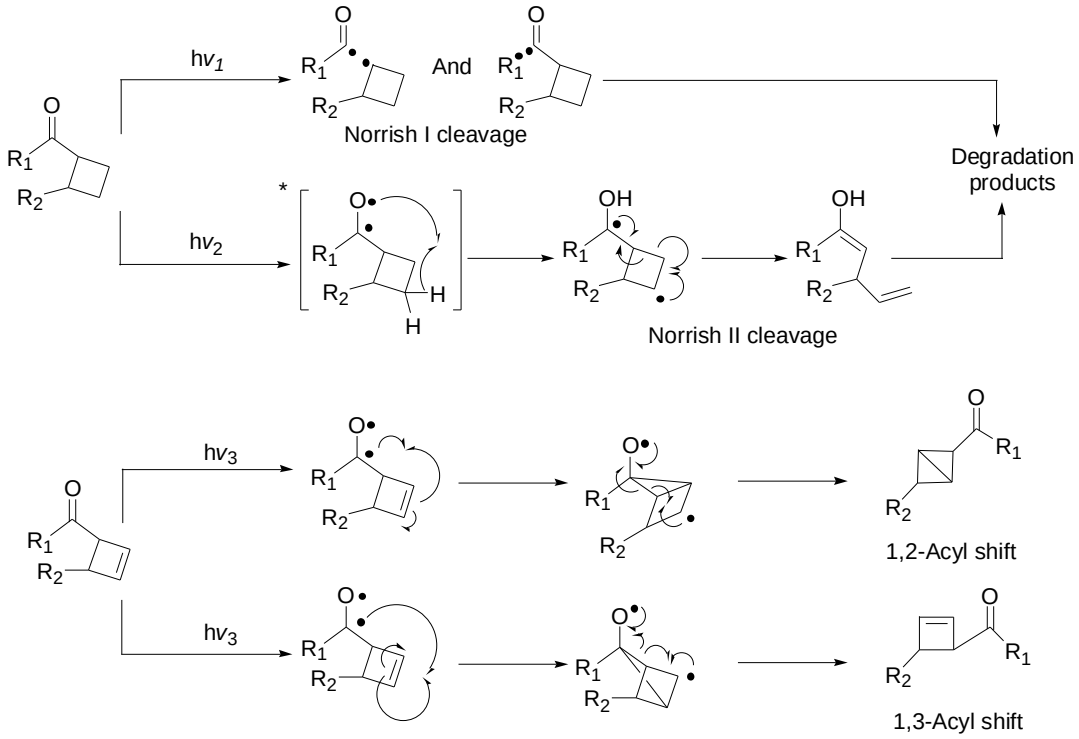
Scheme 11

1.i.iii.i. Secondary photoreactions in [2+2] photocycloaddition of enones

Whilst [2+2] photocycloadditions are very powerful routes to cyclobutane systems, they are underused in synthesis. One of the main reasons for this is that many of these reactions suffer from unpredictable yields. These low yields can often be attributed to low quantum yields and/or photochemical side-reactions that consume the product as it is being formed (secondary photoreactions).

When a low quantum yield of a phototransformation is a problem, the irradiation time can be extended. However, this is only useful if competing processes do not form other products. When other photoprocesses are occurring alongside the desired outcome, extending the reaction time merely increases the yield of by-products and secondary photoreactions. Such secondary photoreactions are the focus of the following work.

During the [2+2] enone-olefin photocycloaddition, complications can arise from continued photoactivity of the desired product, usually due to the chromophoric activity of the carbonyl in the products. Common photoreactions of carbonyls can include Norrish cleavages and 1,2- or 1,3- acyl shifts - the 1,2 acyl shift is also known as the di-π-methane rearrangement,[10, 35, 64] **Scheme 12**. When a carbonyl is retained in the product of a photochemical reaction, photochemically-mediated reactions such as these can lead to product degradation. They are often undesired and they are usually termed 'secondary photoreactions'.[46, 47, 70]

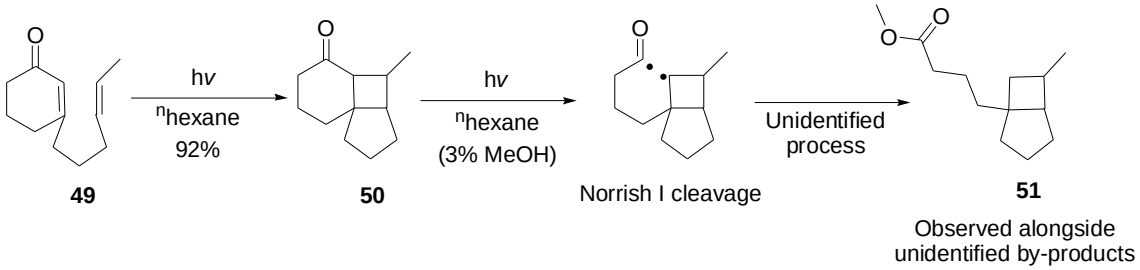


Scheme 12

Secondary photoreactions are not always reported in the literature as they can lead to complete degradation of identifiable material. However, there are some research groups

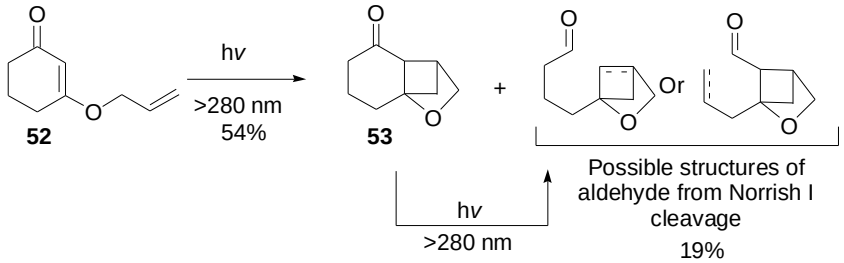
that have investigated and identified distinct secondary photoreactions of carbonyl-containing photoproducts.

Two different research groups have discussed the photochemical formation of ester **51** from a saturated ketone system,[13, 71] the scaffold of which is a recurring motif in [2+2] enone-olefin photocycloadditions, **Scheme 13**. The enone **49** is irradiated to initially form ketone **50**. However, the ester **51** is isolated upon prolonged irradiation of enone **49**, or irradiation of the ketone product **50**, in a 3% methanol solution. The ester **51** is thought to be the result of a Norrish I cleavage of ketone **50**, followed by a radical abstraction process involving the methanolic solvent, **Scheme 13**.



Scheme 13

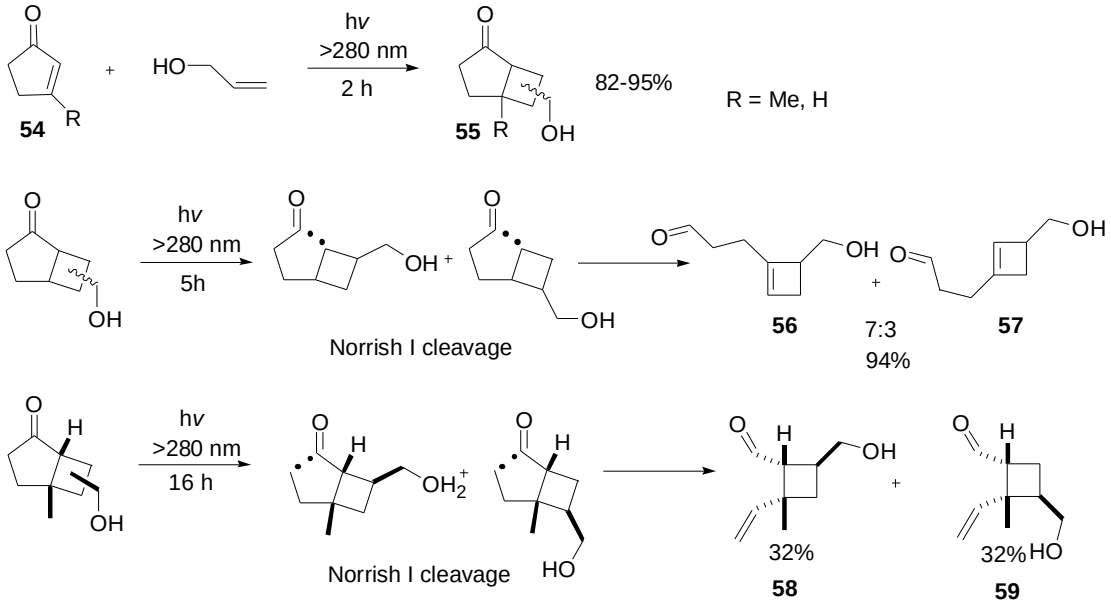
In extensive work on the photochemical behaviour of vinylogous esters, Matlin and co-workers[49] discovered the formation of an aldehyde upon irradiation of hexenone **52** or its isolated photoproduct, ketone **53**, **Scheme 14**. Whilst the aldehyde was not fully characterised, this was deemed as evidence of the occurrence of Norrish I side reactions of ketone **53**.



Scheme 14

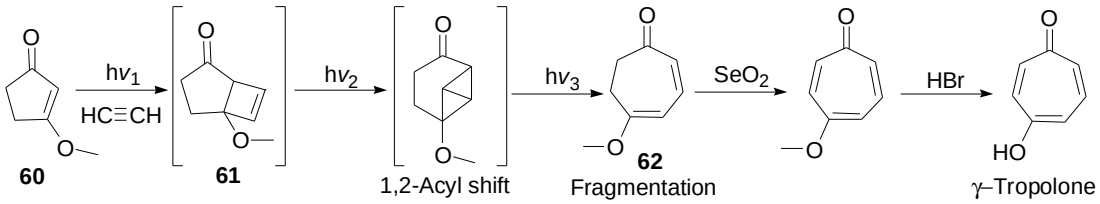
Work carried out by Sydnes *et al.*[72, 73] perfectly exemplifies the intermolecular reaction of cyclopentenones with allyl alcohol, and the different outcomes that can be achieved

when secondary photoreactions are attenuated or exploited, **Scheme 15**. By terminating reactions before the enone **54** was completely consumed, they isolated the 5,4-products **55** from the [2+2] photocycloaddition (reactions are described as containing 'some' unchanged enone). By exposing the various photoadducts to prolonged irradiation, they could isolate a mixture of Norrish I cleavage products, **56** and **57**, or **58** and **59**, depending on the nature of R, **Scheme 15**. These products were also identified if the initial enones **54** were irradiated with allyl alcohol for prolonged periods of time to complete consumption of enone **54** (10-25 hours).



Scheme 15

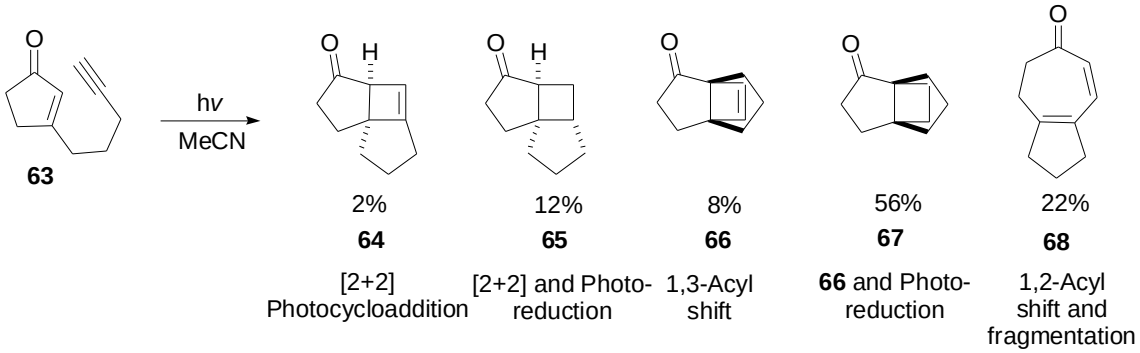
In addition to the photoactivity of saturated ketones, unsaturated ketones are also photoactive. This has been turned towards a positive result by Cavazza *et al.*.[69, 74, 75] They have exploited the 1,2-acyl shift of a 1,4-unsaturated system towards a 3-alkyl tropone. Photochemical reaction of methoxycyclopentenone **60** with acetylene affords cyclobutene **61**, **Scheme 16**. Further irradiation forced the cyclobutene **61** to undergo a 1,2-acyl shift and fragmentation to dienone **62**. Subsequent oxidation and demethylation of the secondary photoproduct, dienone **62**, afforded γ-tropolone, **Scheme 16**.[75] However, the photochemical transformation of vinylogous ester **60** to cyclobutene **61**, and thus dienone **62**, was only carried out to 19% completion. The reason behind the deliberately low conversion was that '*an excess of enone* (**60**) *is needed throughout the process in order to protect dienone* (**62**) *from phototransformation*'.[75]



[Reaction deliberately stopped at 19% conversion]

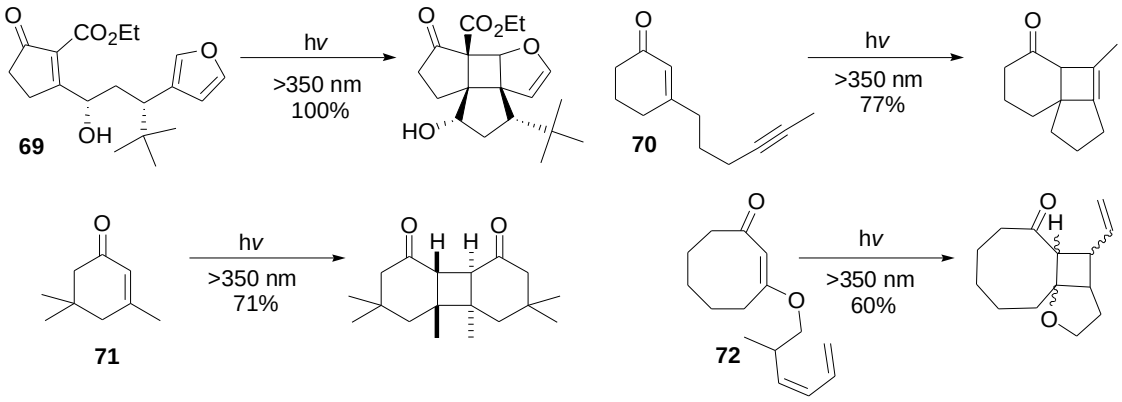
Scheme 16

Further study on an intramolecular variant of the enone-alkyne [2+2] photocycloaddition afforded a variety of products from several secondary photoreaction pathways, **Scheme 17**. Whilst the initial photoproduct **64** is afforded from the intramolecular addition of the enone in **63** to the alkyne tether, the 1,4-unsaturated system **64** formed can then undergo photoreduction to **65**, a 1,3-acyl shift and photoreduction to **66** and **67**, respectively, or a 1,2-acyl shift and subsequent fragmentation to afford **68**, [47] **Scheme 17**.



Scheme 17

A variety of [2+2] photocycloadditions in the literature are carried out using uranium glass filters, keeping incident irradiation above 350 nm. The glass is used in the photocycloadditions of enones, *e.g.* **69**, **70** and **71** as well as vinylogous esters, *e.g.* **72**, in intramolecular, intermolecular and dimerisation reactions, with a variety of partners,[2, 26, 45, 47, 48, 51, 54, 65, 66, 69, 75] illustrated in **Scheme 18**.

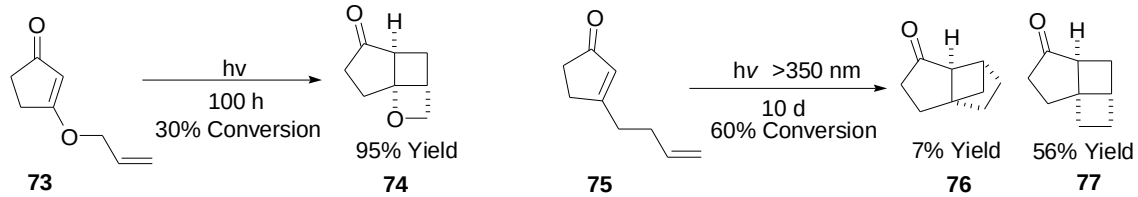


Scheme 18

Koft *et al.* explain the use of uranium filters in the irradiation of hexenone **70** by saying that '*central to the success of this photolysis was the use of a uranium glass filter to avoid secondary photochemical decomposition of the resultant β,γ-unsaturated ketone'*.[46, 65] It can therefore be postulated that when uranium filters are used, it is to minimise secondary photochemical reactions of carbonyl-containing scaffolds. Unfortunately, uranium glass is in increasingly short supply as there is limited production due to safety considerations related to the handling of uranium compounds.[76]

It can be seen that filters are sometimes used to minimise secondary photoreactions. However, the close overlap in absorptions of carbonyl containing products with that of enone starting materials often makes finding a filter suitable for these reactions very difficult or even impossible. For example, the absorption maxima for cyclopentenone is at 300 nm[77] whilst cyclopentanone is 304 nm,[78] therefore finding a filter that would be suitable for distinguishing between such compounds is unattainable.

Another, commonly employed technique to minimize product degradation is to isolate any formed product(s) prior to the reaction reaching completion,[48, 49, 54, 56, 79] *e.g.* when enones **73** and **75** are converted to products **74** and **76**/**77**, **Scheme 19**.[48] In the latter example, the reaction is also carried out with a uranium glass filter, **Scheme 19**.



Scheme 19

In this manner, the starting material always remains the major absorptive component of the solution, and, as it usually has a higher extinction coefficient than the product, the product is effectively shielded from absorption and thus decomposition. However, whilst stopping a reaction at incomplete conversion may be sufficient to study mechanisms and determine if something new is forming, synthetically, this is not an ideal method of arriving at a usable quantity of product. Halting the reaction prior to completion also adds the often non-trivial purification of the remaining starting material from the desired product. Currently, there are a limited number of resolutions to the problem of sustained photoreactivity.

Further to the reactions that have been described in this text it is postulated that there are numerous other unsuccessful reactions that have not been reported due to secondary photoreactions completely degrading any identifiable material.

Analysis of the literature therefore shows that whilst the enone-alkene and enone-alkyne [2+2] photocycloadditions are powerful routes to four-membered rings, the reactions are not without their significant difficulties. Fundamentally, such enone-based reactions retain a chromophore in the product (the carbonyl) and further absorption of this chromophore can lead to photochemically-mediated degradation. Current methods of preventing this degradation, such as filters and incomplete conversions, are not generally applicable for synthesis. If the synthetic viability of the [2+2] enone-olefin and the [2+2] enone-alkyne photocycloadditions could be increased then the reactions could, and would, be employed more widely to a vast array of synthetic targets.

1.ii. Introduction to protein modification

Whilst the synthetic chemist has a vast number of reactions with which to synthesise small molecules under a variety of conditions, the scientific community's ability to directly modify biomolecules remains relatively limited. To use the varied 'tool-box' of the synthetic chemist and make reactions applicable to proteins, peptides, nucleotides and other biological macromolecules has become an increasingly prevalent area of research.[80-87] Modifications can be carried out on a variety of naturally occurring biomolecules including nucleotides,[88, 89] polymeric sugars or 'glycans',[90, 91] proteins[92] and even whole cell membranes[93, 94] and antibodies.[89] The modification of amino acid-containing targets, *e.g.* proteins and peptides, will be discussed in this text as it was the focus of the following work.

The chemical modification of proteins is desirable to mimic endogenous post-translational modifications like acetylation[95, 96] and glycosylation,[97] as well as to impart exogenous synthetic modifications such as fluorophores,[93] therapeutics,[90, 98] affinity[89, 99] or photoaffinity tags,[100] in addition to tethering molecules of interest to surfaces,[101-103] **Figure 12**.

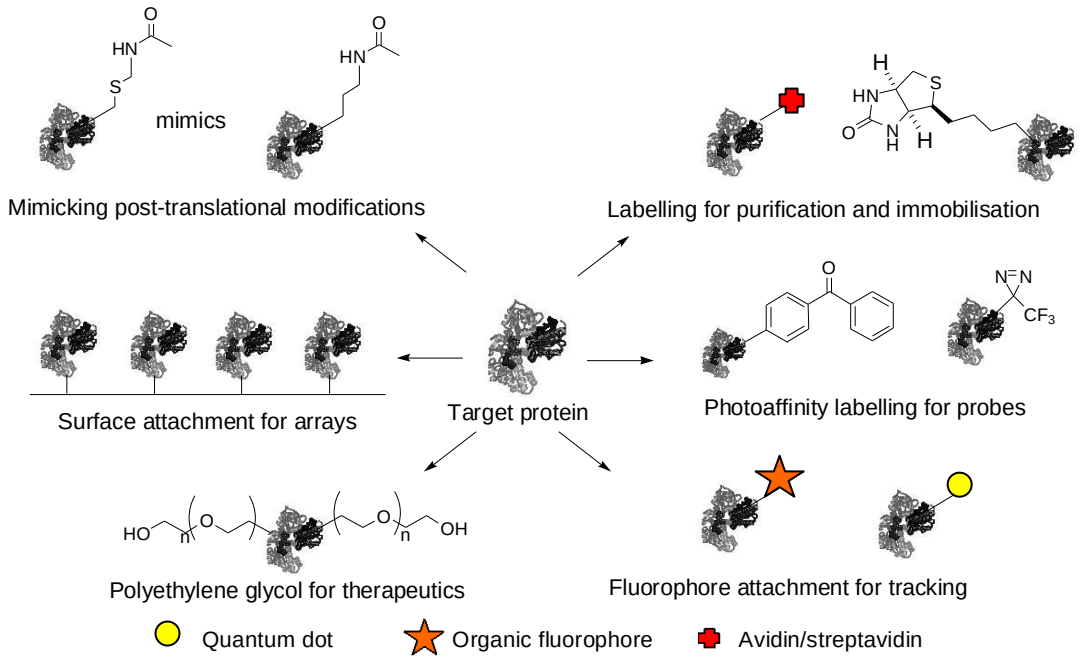


Figure 12: Modification strategies for bioconjugates

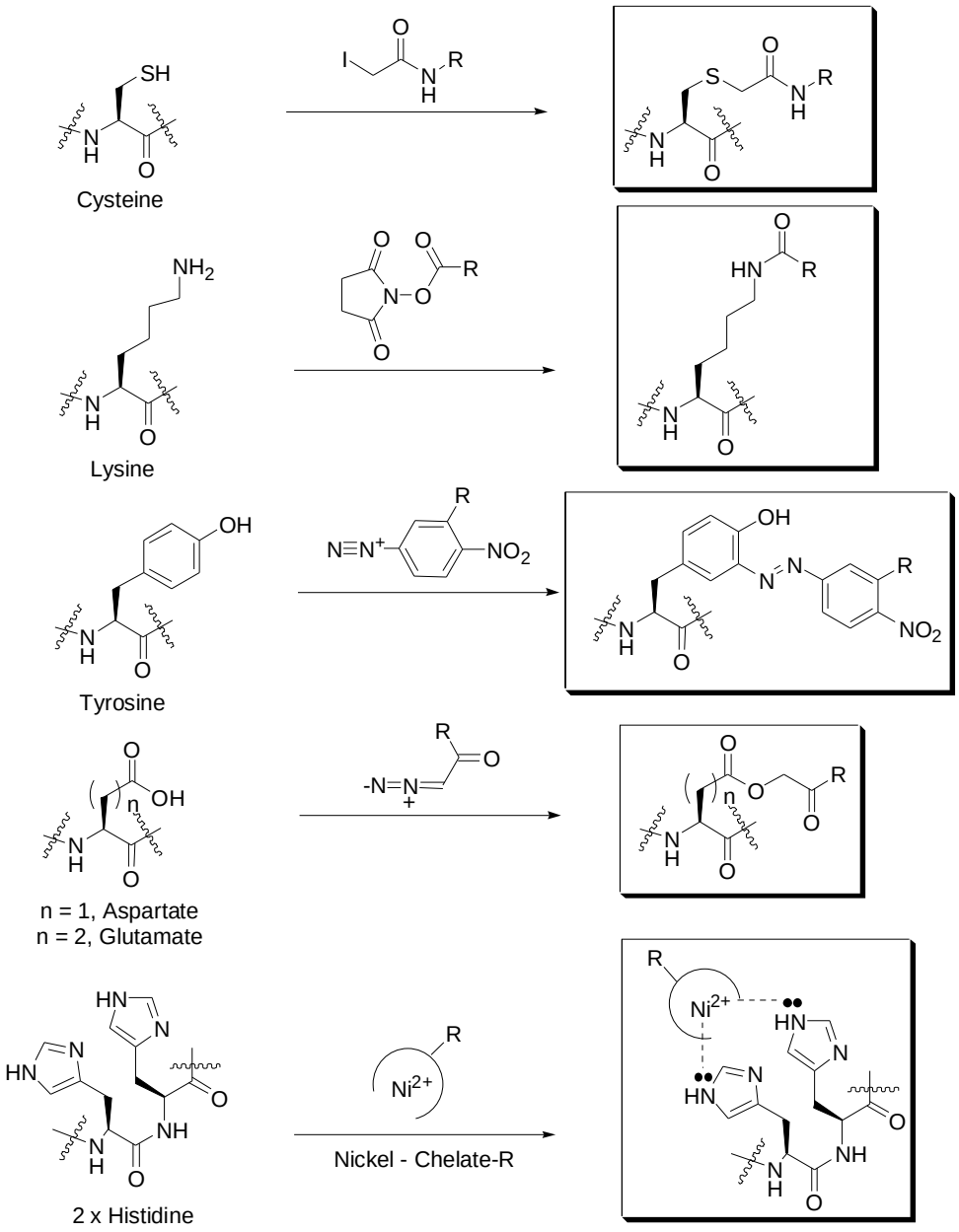
Proteins are synthesized from twenty natural amino acids, each with different functionalities. For a reaction to be of general use in protein modification it must selectively modify the residue of interest in the presence of other amino acids contained in the target protein. This selectivity must also be borne out in conditions that are conducive to protein stability, such as aqueous media, temperatures not usually exceeding 37 ^{o}C and at a pH that is near neutral. In addition, the reactions should tolerate any salts or surfactants that might be needed for protein stability.[104] Lastly, proteins are often available at much lower concentrations than conventional chemistries. This means that the model reactions must be rapid when they are tested at higher concentrations with small molecules, so that when the conditions are transferred to a protein, full conversions can be achieved in reasonable times. These requirements result in a considerable challenge to the biochemist or chemical biologist.

Since selectivity is an integral part of protein modification, one alternative solution of note is to incorporate an unnatural amino acid into the target protein, (discussed in **1.ii.ii.**) This unnatural amino-acid is designed to contain 'bioorthogonal' functionality and thus imparts selectivity to the reaction.[80] This residue must not perturb the biological system in any way, other than that intended.

1.ii.i. Modifications of native amino acids

Extensive research into selective reactions for protein modifications focusing on native amino acids is underway throughout the biochemical society[80-82, 84, 85, 105] as incorporation of non-native amino acid into a protein brings inherent complications to the biological system.

For modification of native amino-acids, some residues are more easily targeted than others. For example, owing to their nucleophilicities,[81] cysteine and lysine are amongst the most widely targeted residues as they can be reacted with carefully chosen electrophiles. Other amino-acids targeted include tyrosine *via* aromatic substitution;[80] glutamate and aspartate which can form esters;[86] and histidine's tendency to co-ordinate transition metals has also been utilised,[81, 106] **Scheme 20**.

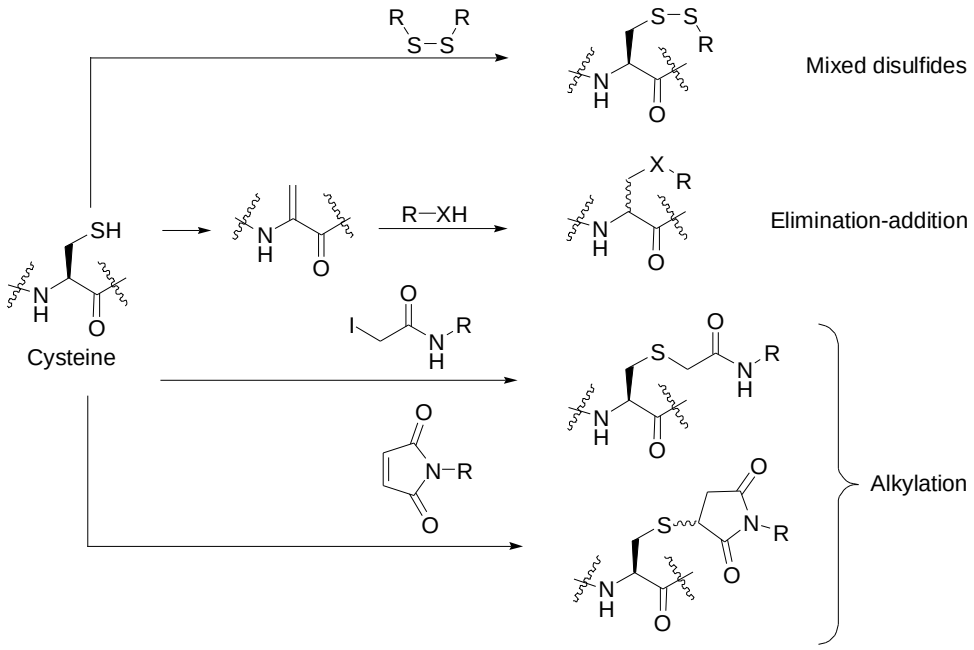


Scheme 20

The fact that lysine, aspartate and glutamate residues are present in relatively high abundance in proteins limits their utility when single specific modifications are required. Conversely, cysteine's relatively low natural abundance[107, 108] makes the introduction of a single cysteine *via* site directed mutagenesis and subsequent chemoselective reaction a very effective method to access selectively modified proteins. Cysteine is therefore increasingly becoming the target of choice for protein modification,[82, 87, 109] and is the focus of the work described in this text.

Cysteine modification

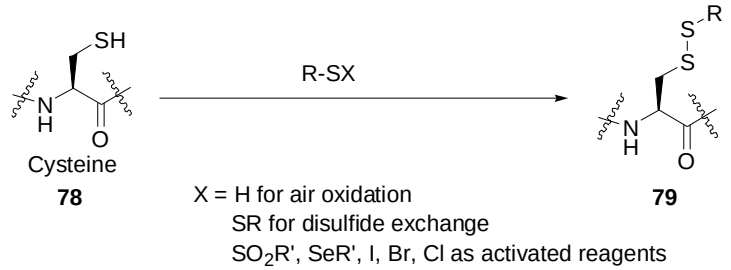
Methods of cysteine modification are varied, but there are a few that have gained more widespread use. These include oxidation of cysteine to form disulfides,[110, 111] base-mediated elimination to dehydroalanine and subsequent conjugate addition,[82, 112-115] and alkylation *via* iodoacetamide[86, 87, 116] or Michael acceptors such as maleimide,[117-119] **Scheme 21**.



Scheme 21

Disulfide formation

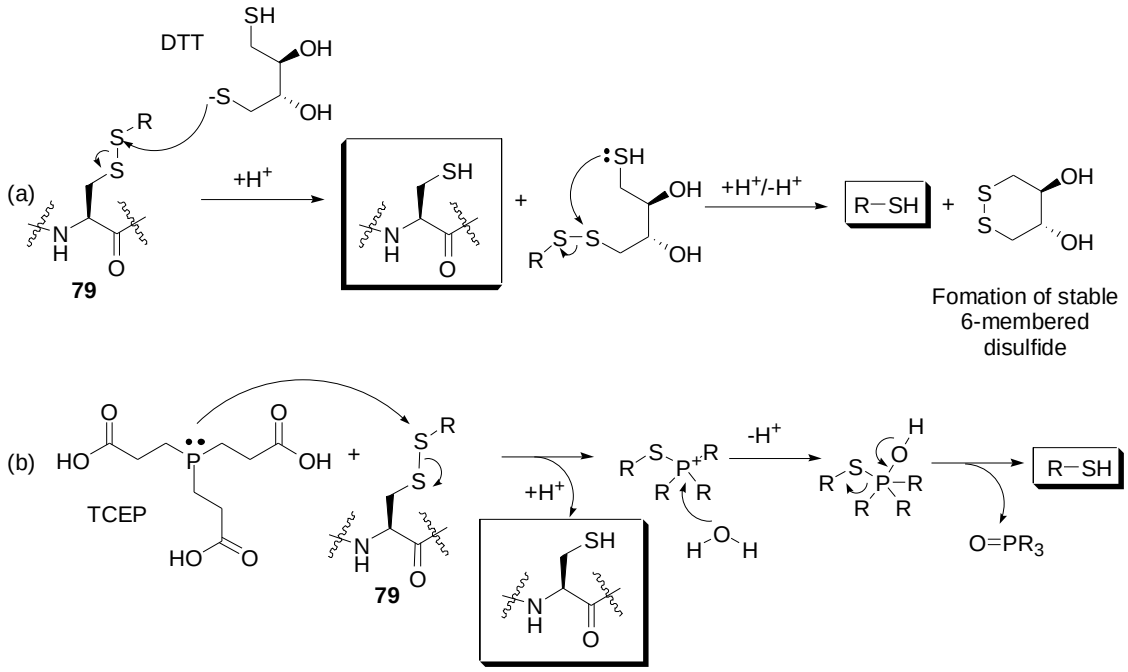
Disulfides are naturally occurring motifs that have been adopted by the scientific community as a simple method of cysteine modification. The disulfide can be formed from air oxidation - simply mixing the modifying thiol, R-SX, and the cysteine containing molecule **78** in basic buffers, open to air, will often generate disulfides **79**, **Scheme 22**. This method is used to regenerate folded proteins as well as to conjugate small molecules.[82] Disadvantages of this method include long reaction times and a limited control of the product distribution (*e.g.* mixed disulfides *versus* dimer). To speed up the conjugation event, the added thiol can be activated *via* formation of the sulfenyl halide[120, 121] or methanethiosulfonate reagents can be used, **Scheme 22**.[122]



Scheme 22

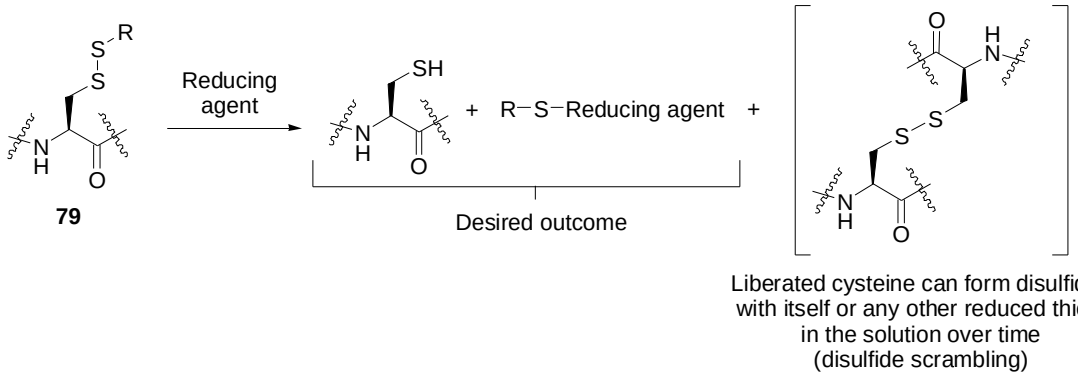
Disulfide formation has gained popularity because of its selectivity and relative simplicity and recent uses of disulfide conjugation include labelling of glycoproteins[123] and nanoparticle functionalization.[111]

A feature of disulfides is their reversibility upon reaction with reducing agents. The newly formed disulfides **79** can be cleaved with reducing reagents such as DTT (dithiothreitol), **Scheme 23 (a)**, or TCEP (tris(2-carboxyethyl)phosphine), **Scheme 23 (b)**.



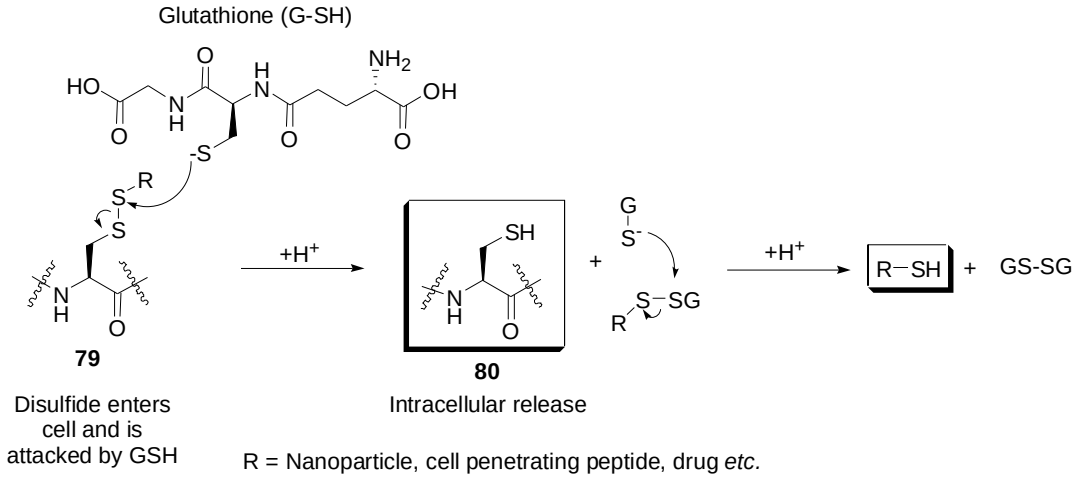
Scheme 23

However, as with the initial labelling technique, the use of such disulfide reducing agents can lead to undesired mixed disulfides from associated opening of other endogenous disulfides, **Scheme 24**.[124] This is known as disulfide 'scrambling'.



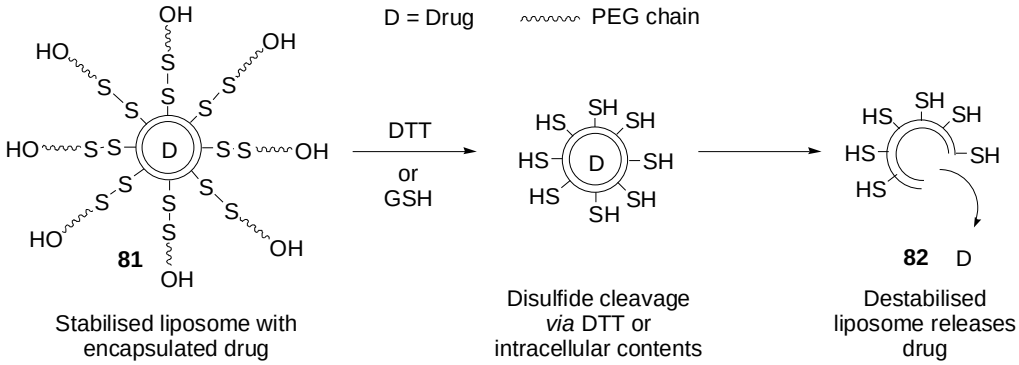
Scheme 24

The reversibility of disulfide labelling has been exploited as the cysteine-containing 'cargo' **80** is released from the disulfide **79** upon exposure to the reducing conditions of the cell, which contains up to 5 mM glutathione, G-SH,[125] **Scheme 25**.



Scheme 25

Strategies that exploit the reversibility of the disulfide linkage are gaining prevalence for the study of formation and cleavage of disulfides in proteins[124, 126] and in drug delivery.[111, 127] For example, disulfides can be used to conjugate polyethylene glycol (PEG) to liposomes **81** to stabilise them to systemic conditions and thus inhibit the release of an encapsulated drug **D**, **Scheme 26**. When the liposome reaches its target, the disulfide is cleaved at a higher rate by lysosomal enzymes within the target cells, and the drug is released by the destabilised liposome **82** in the targeted region, **Scheme 26**.[127]

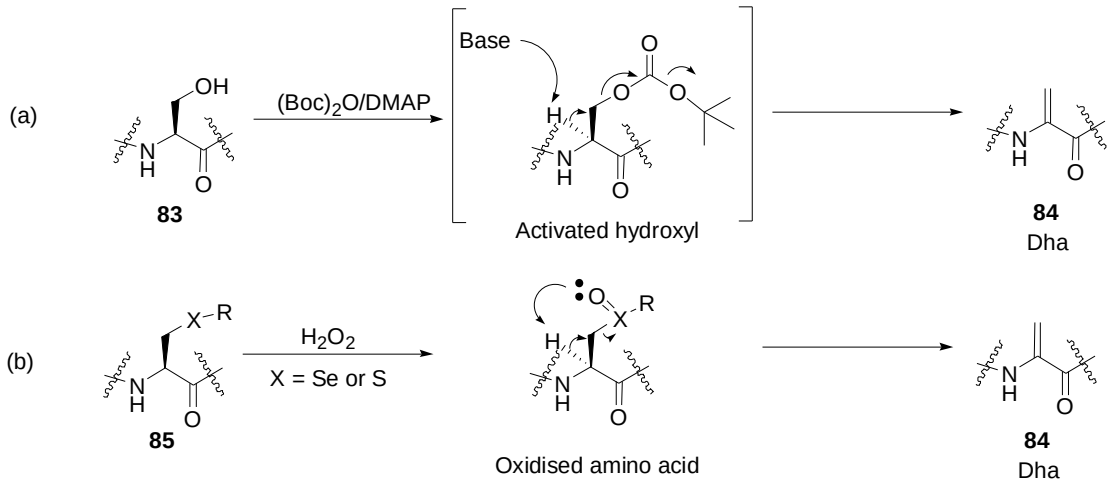


Scheme 26

If the reversibility is under tight control and disulfide scrambling limited, then there are potential applications for such temporary modifications with protein targets, ranging from impermanent blocking of active cysteine residues,[128] protein purification,[129] quantitative proteomic analyses,[124] probing binding sites,[130] enabling structural studies[131] and in drug delivery.[110]

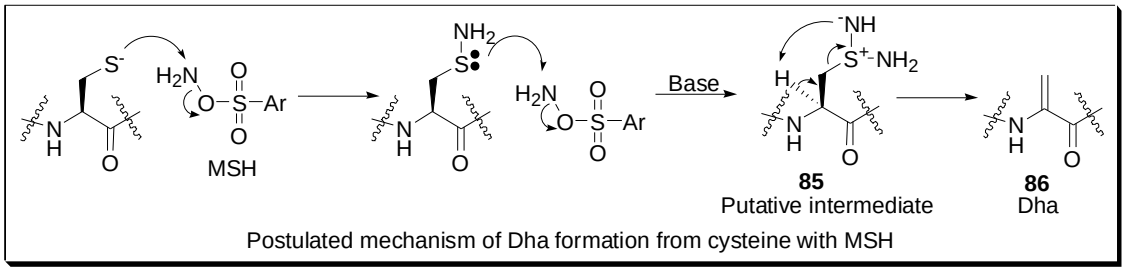
Dehydroalanine formation

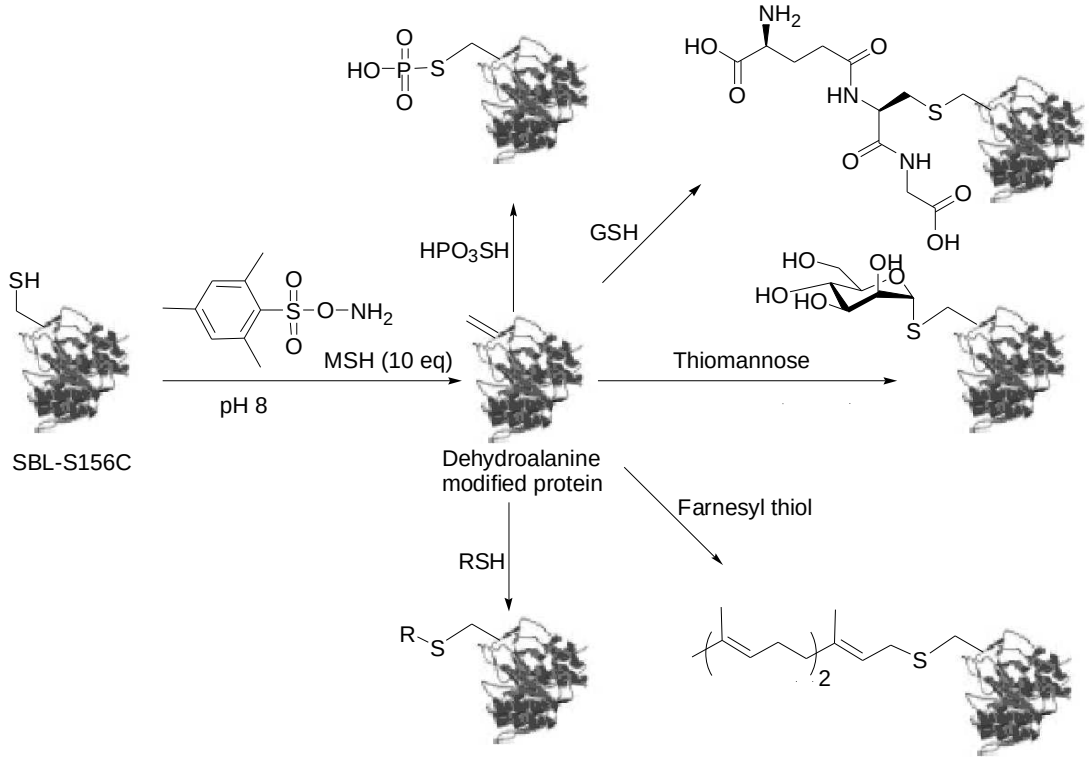
Dehydroalanine (Dha) does not belong to the twenty natural amino acids but the residue is found in nature in several peptide antibiotics, where it is formed by post-translational modification.[113, 115, 132-135] The Dha motif is thought to provide rigidity and a reactive site for nucleophilic attack that can be crucial for biological activity.[135] Synthetically, Dha residues **84** are usually formed from activation and subsequent elimination reaction of serines **83**,[132] **Scheme 27 (a)**, cysteines[114, 132] and selenocysteines **85**, [113, 134] **Scheme 27 (b)**.



Scheme 27

Biochemists have exploited the reactivity of Dha by using the Michael acceptor properties of the double bond to add in a nucleophile of their choice.[82, 112, 114, 115, 132] The Davis research group, in particular, has carried out extensive research on the use of *O*-mesitylenesulfonylhydroxylamine (MSH) to form the Dha motif within a protein structure from a cysteine residue. MSH acts as an electrophilic source of NH_2 and it is thought that it doubly labels cysteine residues to form sulfonium species **85**, **Scheme 28**. They postulate that the NH_2 is deprotonated by a base to form the NH^-, that, in turn, deprotonates the α-carbon. The activated sulfur is then eliminated to form Dha **86**. They have subsequently functionalised the Dha modified protein with a thiophosphate, a thiomannose, glutathione (GSH), farnesyl thiol and a variety of alkyl thiols,[114] **Scheme 28**.





Scheme 28[114]

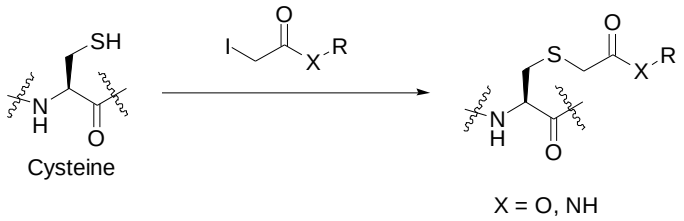
Whilst the reaction of MSH is rapid, selective for cysteine over methionine residues (unlike peroxide based methods[134]) and can be used in open air, there are several incidents of violent explosions and exothermic degradations reported,[136] limiting the reagent's utility. All modifications on Dha set a new chiral centre, thus diastereomeric products will be present. Local stereochemical environments can have an effect on the stereochemistry of this addition.[137]

Alkylation

Alkylation is a very common method of cysteine modification. Whilst this overview is by no means exhaustive, two methodologies in particular merit further discussion as they have gained widespread use throughout chemical biology.

α-Halocarbonyls

α-Halocarbonyl electrophiles such as iodoacetate and iodoacetamide have been used to modify sulfhydryl molecules and cysteine residues for decades,[116, 138] **Scheme 29**.



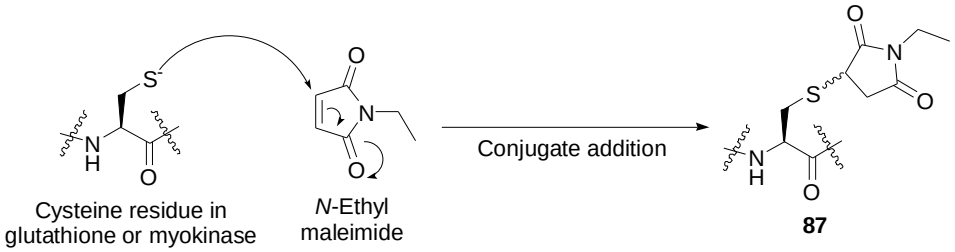
Scheme 29

They are usually used at pH 8 to 9.[87] There are documented problems with non-specific additional labelling of lysine residues at higher pH[86, 87] and incomplete cysteine conversion with concomitant reaction of methionine at lower pH.[86, 87] Thus, to abrogate the non-cysteine modifications, strict pH control is necessary. The α-halo acids used to synthesise the α-halo carbonyls decompose in water, increasingly in alkaline solutions, thus for storage reagents should be kept dry over P_2O_5. Most of the haloalkyl compounds are photolabile, so preparations should be carried out in the dark to avoid complications arising from the decomposition of the reagent and possible reactions of the decomposition products with the protein.[86] Iodoacetamides have been widely used for peptide 'mapping' of cysteine containing proteins.[139] Alkylation of cysteines is a key

step in this multi-step process that aims to define a protein's primary structure and its level of post translational modification by mass spectroscopy.

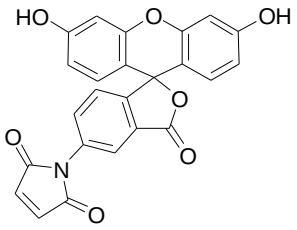
Maleimide

The conjugate addition of cysteine to Michael acceptors such as maleimide is a useful way to modify cysteine – exploiting the nucleophilicity of the sulfhydryl. Historically, this was first demonstrated in the 1950s with *N*-ethyl maleimide and its irreversible reaction with glutathione and myokinase to form thiosuccinimides **87**, **Scheme 30**.[140]

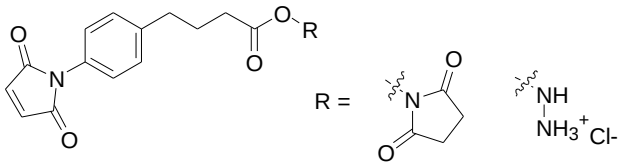


Scheme 30

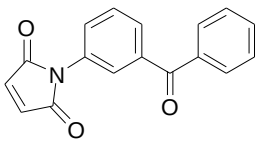
Conjugations using maleimides are extremely rapid and usually highly cysteine selective leading to a substantial adoption of these reagents in many laboratories.[117, 141-144] Cysteine modification with maleimide is regarded as irreversible[141, 145] and can suffer from lysine and histidine cross-reactivities above pH 7.[142] Importantly, in contrast to the disulfide strategy, the irreversible nature of the maleimide addition prevents the possibility of using the formed succinimide constructs **87** to release the cysteine-containing biomolecule at a later date. The use of maleimides is now a widely employed methodology in cysteine alkylation and there are numerous *N*-functionalised maleimide commercial reagents available,[82, 86] illustrative examples are shown in **Figure 13**.



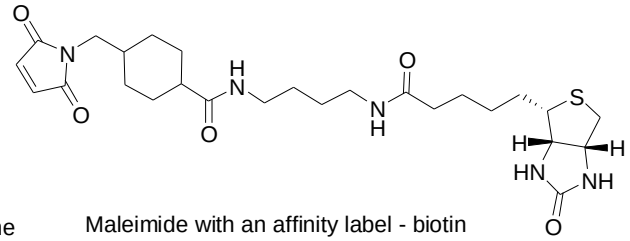
Maleimide with a fluorophore - fluorescein



Maleimides as bifunctional linkers - amine or aldehyde reactive



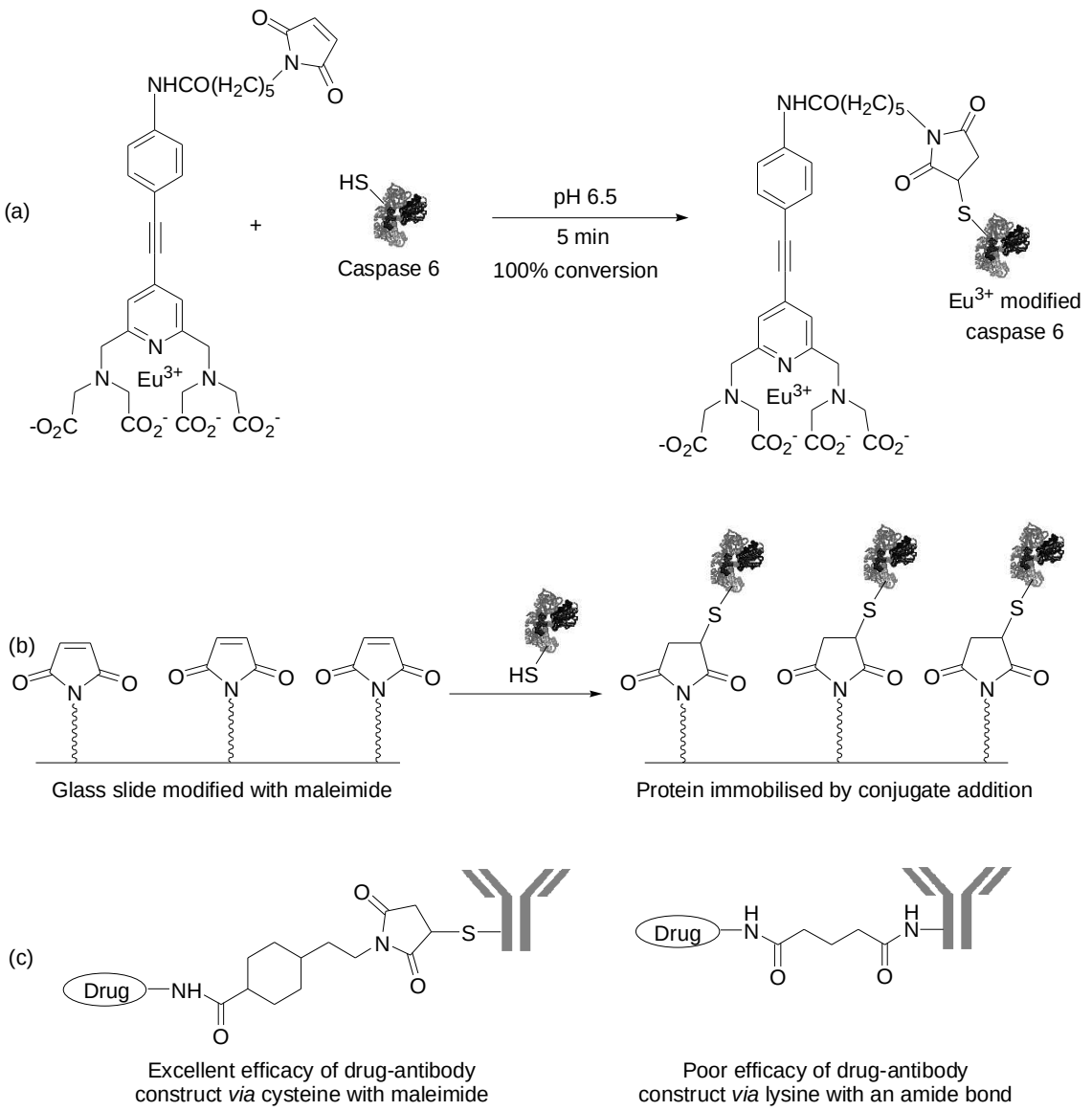
Maleimide with a photoaffinity label - benzophenone



Maleimide with an affinity label - biotin

Figure 13: Commercially available *N*-functionalised maleimides[82, 86]

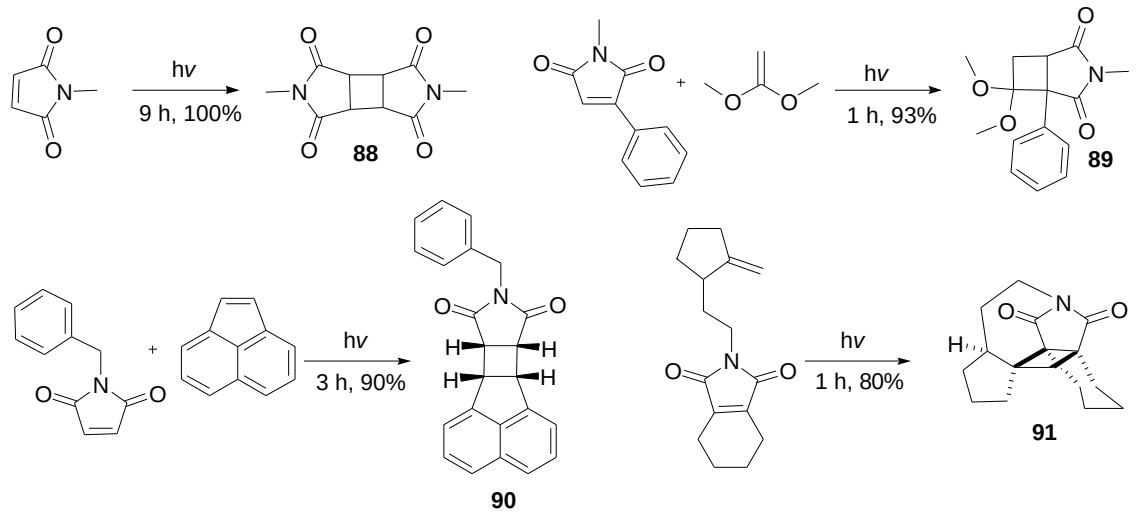
Recently, maleimides have been used to create complex fluorescent lanthanide (III) chelates,[119] **Scheme 31 (a)**, and to modify surfaces in order to immobilise thiol containing biomolecules,[144] **Scheme 31 (b**). A testament to the utility of maleimide is its use in synthetic cancer vaccine constructs[143] - the use of an analogous construct *via* a lysine on the antibody, **Scheme 31 (c)**, was unsuccessful, creating a conjugate with very poor efficacy.



Scheme 31

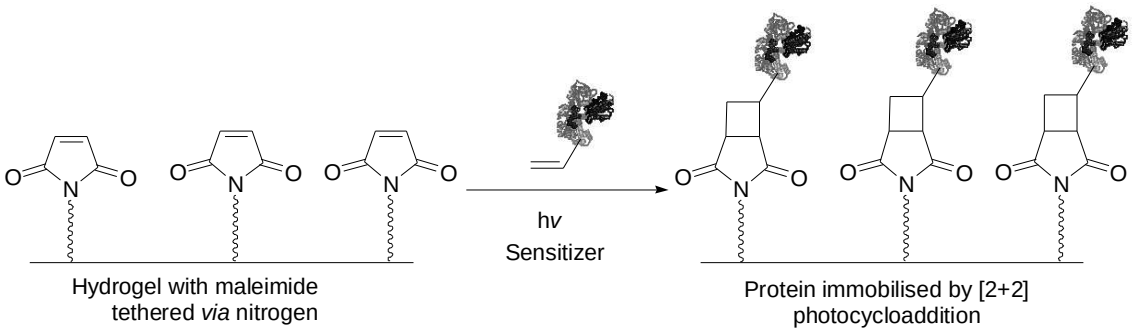
Photochemistry of maleimide

Maleimides are excellent photochemical partners in a variety of photocycloadditions. They can undergo efficient [2+2] photocycloadditions with themselves to form dimers, *e.g.* **88**,[146, 147] or with substituted olefins to afford an array of complex products, as exemplified by **89**, **90** and **91**, **Scheme 32**.[148-150]



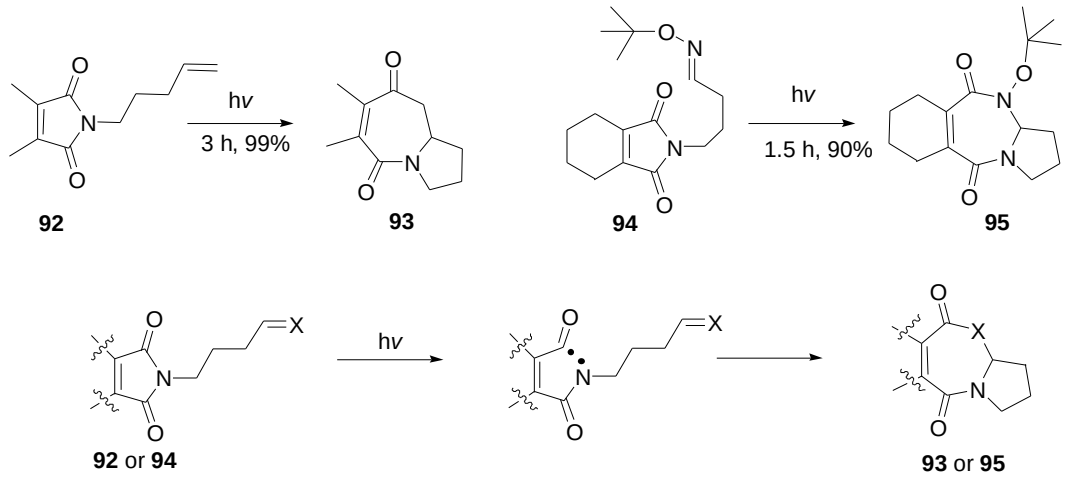
Scheme 32

The [2+2] photocycloaddition of maleimide has been exploited towards biomolecule immobilisation strategies.[151-153] Photosensitizers are employed to afford a rapid conjugation of alkene modified proteins to a maleimide-labelled surface upon irradiation, **Scheme 33**.



Scheme 33

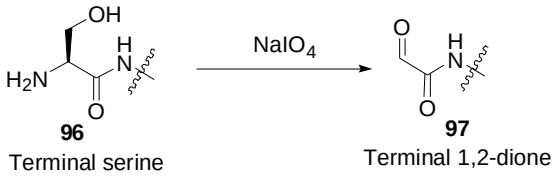
In addition to the [2+2] photocycloaddition, maleimides can also undergo a [5+2] photocycloaddition, referring to the atoms involved in the reaction, and not the number of electrons, **Scheme 34**.[149, 154] Examples of this transformation are where the *N*-substituted maleimides **92** and **94** undergo a photochemical N-CO cleavage to a diradical, followed by insertion of the nearby double bond, to afford seven-membered rings **93** and **95**. To date, the [5+2] reactivity of maleimides has only been observed as an intramolecular reaction when bulky groups hinder the [2+2] transformation.



Scheme 34

1.ii.ii. Incorporation of non-native amino acids

The modifications discussed thus far have dealt with alterations of native amino acids. An alternative approach that will be briefly summarised here involves the incorporation of non-native functionality into a protein, which can then undergo highly selective, bioorthogonal reactions. For example, an oxidative cleavage can be carried out on protein *N*-termini **96** to afford terminal 1,2-diones **97**, **Scheme 35**, which can undergo subsequent dione specific conjugations.



Scheme 35

With the advent of sophisticated biochemical techniques, single locations or single residues can be targeted for conversion to a range of non-native motifs including ketones, alkynes and azides.[80] Three methods of incorporating unnatural amino acids are described below, although there are many more.[105]

Site specific incorporation of non-native amino acids

Translation is the process of converting the DNA transcript, the *m*RNA sequence, into an amino acid polypeptide chain. In cells, an amino acid is attached to the *t*RNA that codes for it *via* an ester bond. The *m*RNA is directed to a ribosome where it is attached

to the corresponding *t*RNA molecule. Thereby delivering the correct amino acids governed by the codon-anticodon interaction between the *m*RNA and *t*RNA molecules. Site specific unnatural amino acids can be incorporated by modifying a *t*RNA of a regular codon with the desired unnatural amino acid. This codon is then used in the *m*RNA transcript, dictating the placement of the target analogue within the target protein. This technique is limited to cases where only small amounts of protein are required because the production of the modified *t*RNA is not trivial.[80]

Residue specific incorporation of non-native amino acids

Residue specific incorporation relies on competition with natural amino acids throughout the step at which they are connected to *t*RNA. The ribosome pairs *m*RNA codons to anticodons from *t*RNA with high precision and accuracy; however it is largely insensitive to the nature of the amino acid attached to the 3'-end of the *t*RNA, leaving the responsibility of bonding the correct amino-acid with the corresponding *t*RNA to the aminoacyl *t*RNA synthetases (aaRSs). Whilst this family of enzymes has excellent and essential fidelity against each of the twenty natural amino acids, they demonstrate a high level of promiscuity towards unnatural analogues.[80] As a result of this, unnatural amino acids that are structurally and, crucially, electronically similar to natural analogues can be introduced into the media, where they will be attached to *t*RNA and will be 'fed' into growing polypeptides.[155] Larger, structurally deviant amino acids have been incorporated using aaRSs with altered substrate specificity generated from rational design and directed evolution.[156] A variety of residues have been incorporated *via* this method, but particularly useful for conjugation strategies are residues containing ketones, azides and terminal alkynes.[105, 156, 157]

Auxotrophic bacterial strains to incorporate unnatural amino acids.

An auxotrophic bacterial strain is an artificially engineered species whose metabolic pathway for a particular organic compound, often essential for growth, has been interrupted. Such an organism is dependant on external sources to incorporate this compound. If an auxotrophic strain is supplied with a non-native version of the essential compound during protein expression, the bacteria can instead incorporate this molecule. Typical auxotrophic targets are amino-acids and substitutions include the replacement of hydrogen with fluorine, methylene with oxygen or sulfur and *vice versa*, and ring substitutions.[105] For example, leucine has been replaced with trifluoroleucine and

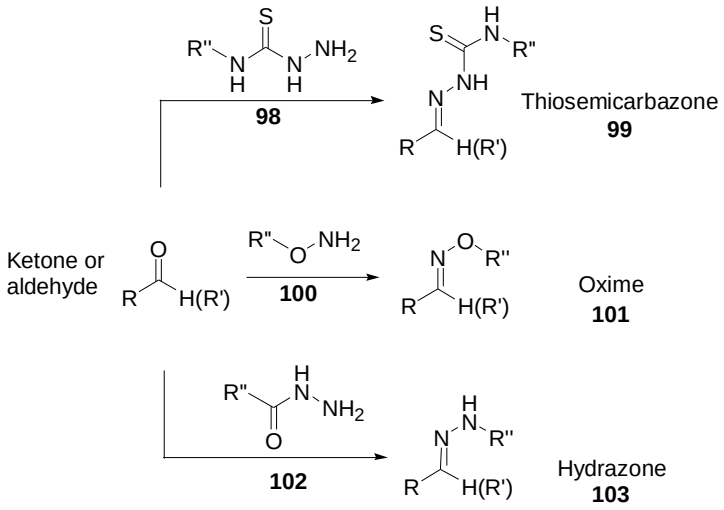
methionine has been substituted for an alkynyl amino acid (homopropargylglycine-Hpg), an alkenyl amino acid (homoallylglycine-Hag) and an azido amino acid (azidohomoalanine-Aha).[81, 105]

1.ii.iii. Bioorthogonal modification of non-native amino acids

The newly incorporated unnatural amino acid(s) should now render the subsequent reactions specific to that particular residue, leading to no perturbation of the rest of the biological system - this is known as 'bioorthogonality'.

Ketones and aldehydes

Ketones and aldehydes in proteins are reversibly reactive towards amine, thiol and alcohol nucleophiles, but the equilibrium in water generally favours the free carbonyl.[80] Nucleophiles that are enhanced by the α-effect, *i.e.*, they are flanked by a heteroatom containing a lone-pair[158] form stable conjugates with aldehydes and ketones. For example, thiosemicarbazide **98**, aminoxy **100** and hydrazide compounds **102** react rapidly to form stable thiosemicarbazones **99**,[159] oximes **101**,[160] and hydrazones **103**,[161] respectively, **Scheme 36**.



Scheme 36

Due to the reversible nature of potential interferences, such reactions are tolerant of a wide range of competing functionality. The reactions proceed in high yield to stable adducts, but modifications above pH 6 are slow, representing the major hurdle for these reactions.[80] These carbonyl-exploiting methods have not been widely used inside cells

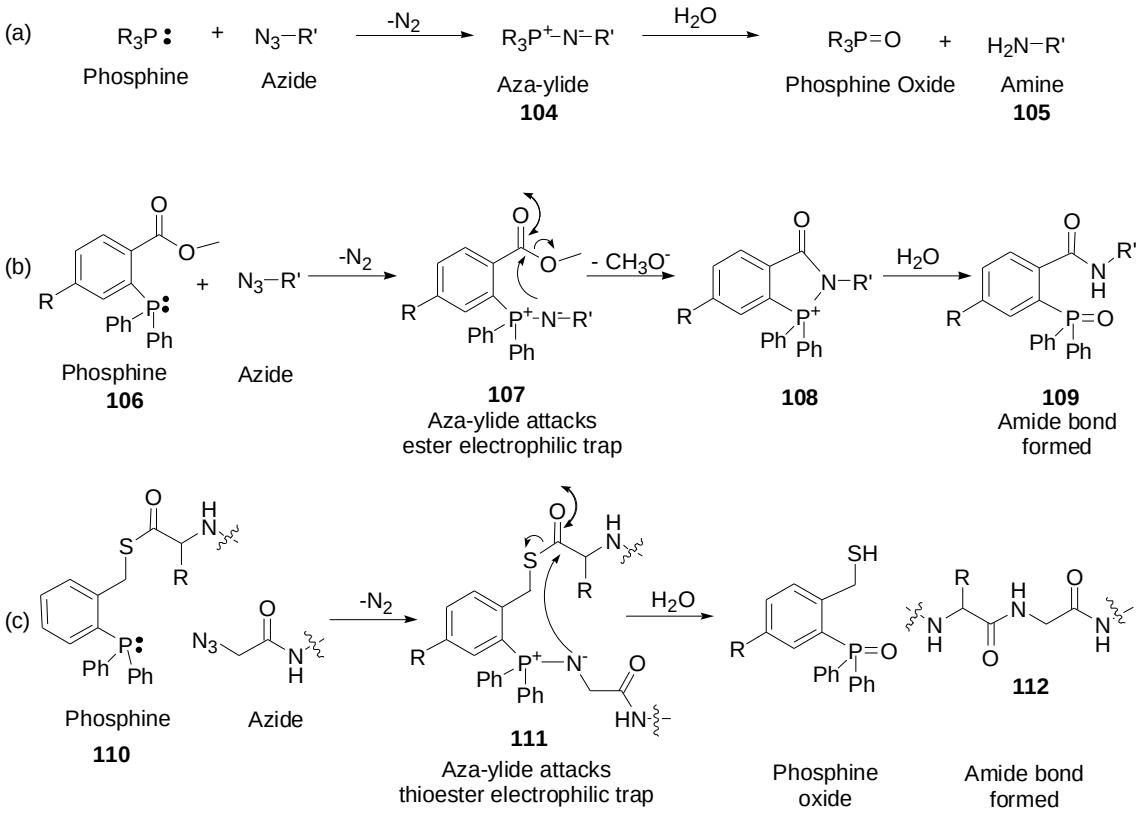
due to potential competition from endogenous aldehydes, such as glucose and pyruvate.[81] However, ketones are virtually absent from cell surfaces[162] and thus, for this application, ketone modifications have some utility. For example, Bertozzi and co-workers have shown that certain keto-sugars are metabolized by cells and integrated into cell surface glycans, where they can be treated with aminoxy and hydrazide probes, and they report no interferences from endogenous aldehydes.[163]

Azides

Azide modification has become increasingly prevalent in the literature as azides are small, kinetically stable under physiological conditions, easily synthesized, absent from the biological milieu and fundamentally bioorthogonal. They are usually introduced *via* metabolic incorporation, described in **1.ii.ii.**, and subsequently modified *via* the Staudinger ligation or [3+2] cycloadditions.

Staudinger ligation

The Staudinger ligation is a great example of synthetic chemistry furthering biochemistry. The Staudinger reaction[164] involves reduction of azides with triphenyl phosphine, proceeding through an aza-ylide intermediate **104**, **Scheme 37 (a)**, to afford amines **105**. The Staudinger *ligation*[165] exploits a triphenylphosphine reagent with a proximal ester **106**. An intramolecular reaction of the formed aza-ylide intermediate **107** and hydrolysis of the cyclic amino phosphonium **108** affords a stable amide **109** in good yields, **Scheme 37 (b)**.[80] The triarylphosphine does not reduce disulfide bonds[166] as other phosphines (TCEP) have been known to, and reduction of the azide by cellular thiols has been shown to be slow.[167] A variation on this reaction is the traceless Staudinger ligation, whereby a phosphinothioester **110** is used. The formed aza-ylide **111** is intramolecularly trapped, and this results in elimination of the triarylphosphine oxide and formation of a native amide backbone **112**, **Scheme 37 (c)** – hence the term 'traceless.'

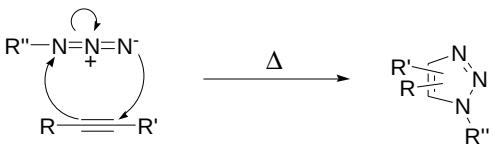


Scheme 37

Like any reaction, the Staudinger ligation is not without its limitations. It suffers from relatively slow reaction rates, necessitating concentrations of the phosphine of 250 μM and above. Efforts to speed up the ligation have not been particularly successful but the desirable properties of the azide have consequently led to considerable interest in azide chemistry for biological applications.

Azides and alkynes

The reaction of alkynes and azides to form triazoles was first reported at the end of the 19th century,[168] and has been considered a [2+4] cycloaddition since the 1950s when Rolf Huisgen introduced the concept of 1,3-dipolar cycloadditions,[169] **Scheme 38**. These reactions are often referred to as [3+2] cycloadditions, describing the number of atoms involved in the transformation rather than the electrons.

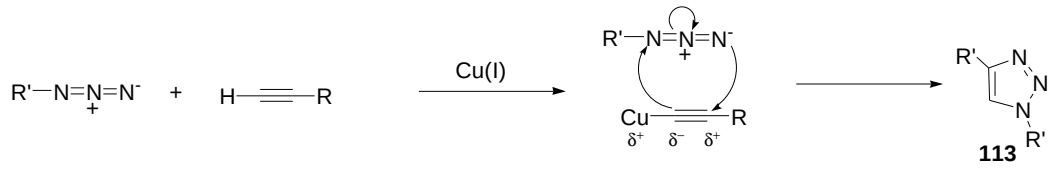


Scheme 38

Whilst the reaction tolerates a variety of functionality and aqueous conditions, it requires elevated temperatures to proceed at an appreciable rate. Such elevated temperatures are not suitable for application to protein chemistries and thus investigations have been undertaken to circumvent the need for such temperatures. To date, two routes have gained prevalence i. copper catalyzed azide-alkyne [3+2] cycloadditions (CuAAC) and ii. strain promoted azide-alkyne [3+2] electrocyclisation.

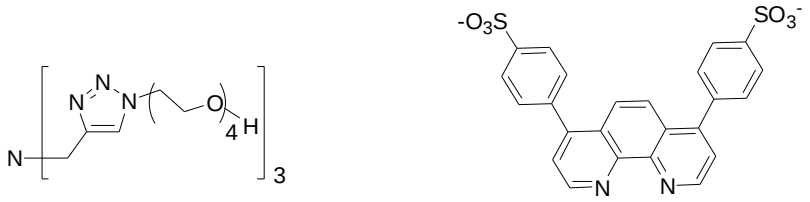
i. Copper catalyzed azide-alkyne [3+2] cycloadditions (CuAAC)

In 2002, Meldal *et al.*[170] and Sharpless *et al.*[171] simultaneously reported dramatic acceleration of azide-alkyne electrocyclisations by addition of a Cu(I) catalyst, **Scheme 39**. The catalysed cycloaddition is thought to proceed through a series of copper acetylide intermediates but the precise mechanism is still under investigation. The reaction produces the 1,4 regioisomer **113** exclusively and tolerates a range of functional groups, aqueous conditions and is fairly insensitive to pH, although better reaction rates are afforded between pH 7 and 9, **Scheme 39**.



Scheme 39

The reaction has gained such high standing in the chemical community that it is often referred to simply as a 'Click' reaction - implying the ease with which the reaction proceeds, especially with the addition of accelerating ligands, **Figure 14**.



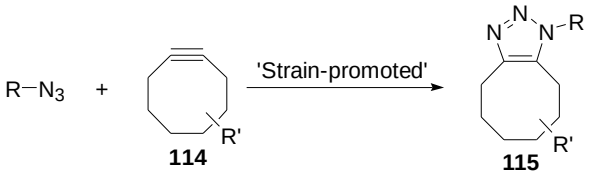
Tris(benzyltriazolylmethyl)amine (TBTA) Sulfonated bathophenanthroline

Figure 14: Ligands used to accelerate the 'CuAAC' reaction

Click chemistry has been widely adopted in organic synthesis and combinatorial chemistry,[172] polymer and materials science,[173] and chemical biology,[80, 105, 174] but some complications with this Cu(I) mediated reaction have been reported. Residual protein bound Cu(I) can interfere with mass spectroscopic analysis, and the best stabilizing and accelerating ligands[80, 175] are often poorly soluble[176] or need oxygen-free conditions.[177] As mentioned, both alkyne and azide motifs can be inserted into a protein motif *via* a range of techniques, allowing flexibility of reaction partners, *i.e*. the label can contain the azide and the protein the alkyne or *vice versa*. To date, the use of this method in cells has been limited by the inherent toxicity of Cu(I)[81, 178] and this has sparked investigations into Cu(I)-free azide-alkyne reactions.

ii. Strain promoted azide-alkyne [3+2] electrocyclisation.

To promote the cyclisation without the need for copper, the alkyne must be activated. This could be done with the use of electron deficient alkynes, but they could potentially act as Michael acceptors, lowering the specificity and functional group tolerance of the reaction. However, incorporating the alkyne into the smallest stable cycloalkyne, cyclooctyne **114**, has been shown to activate the electrocyclisation to triazole **115**, **Scheme 40**, with comparable efficiencies to the Staudinger ligation, thus requiring similar high concentrations of probe.[179]



Scheme 40

Poor solubility of the cyclo-octyne reagent has lead to the addition of stabilization agents like cyclodextrin[180] and variations on the cyclooctyne scaffold including geminal-difluorinated (DIFO),[181] dibenzo (DIBO),[93] amine (DIMAC),[181] amide **116** and **117**,[182] disulfide **116**[183] containing species and combinations thereof (DIFBO), **Figure 15**.[81, 179-183]

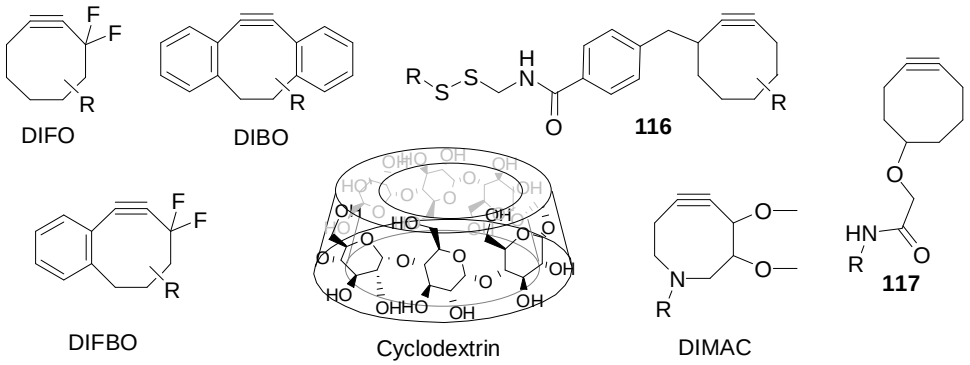


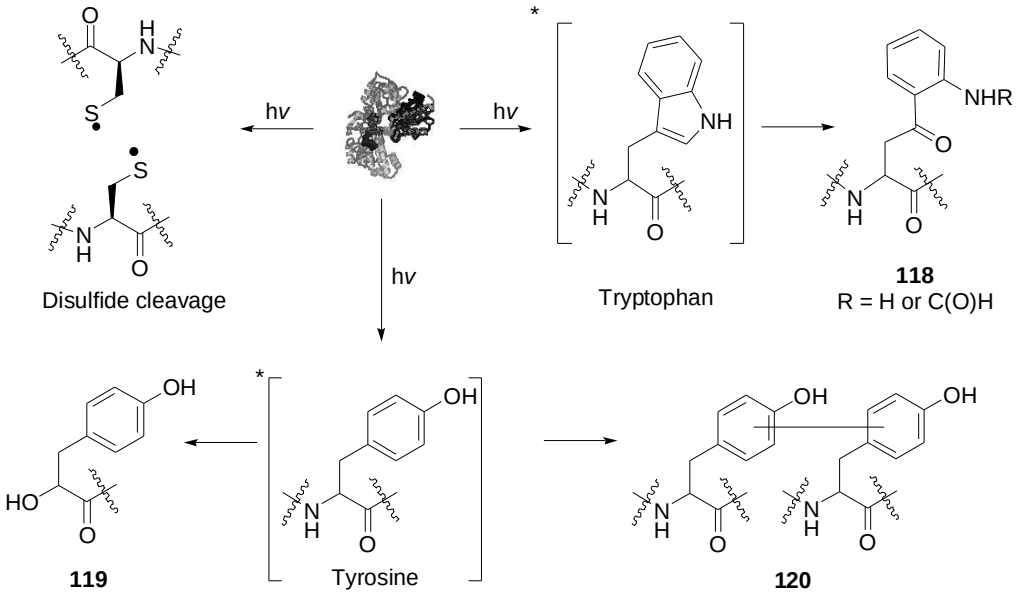
Figure 15: Variations on the cyclooctyne and a stabilising additive used in the electrocyclisation

Reaction rates are comparable to the copper-catalyzed variant when *gem*-difluorination of the alkyne is employed.[180] A limiting factor of this strategy is currently the complexity of the synthesis of cyclooctyne reagents. An additional restriction, borne out of the complexity of the cyclooctyne, is that the azide motif must be the partner that is incorporated into the target protein, whilst the cyclooctyne assumes the identity of the tag moiety. Encompassing the cyclo-octyne as a photochemically-masked moiety will be discussed in **1.ii.iv.**.

1.ii.iv. Modifications of proteins using photochemistry

In order to achieve high levels of specificity for biochemical applications, attention has increasingly turned towards the use of light as an external stimulus to bring about reactions. Since photoreactive reagents are 'silent' under normal conditions and become chemically reactive only upon UV irradiation, they can render a modification precise in space and time. This 'spatiotemporal' control could potentially provide the ability to regulate a reaction in a particular compartment of a system, *e.g.* either extra- or intracellularly. Therefore, using light as a stimulus is also gaining increasing attention in investigations of cellular processes.[184-187] The reagent can be allowed to concentrate in a homogenous matter at the site to be investigated, allowing for background to be taken from the real system prior to the irradiation event. Another powerful application of photochemistry is the patterning of biomolecules - whereby the shape, size and distribution of features on a surface could be controlled *via* a laser or a photomask.[188] In fact, photochemical reactions are increasingly termed 'photoclick' when they are high yielding and robust, so well-recognised is their potential.[189-191]

Proteins possess some inherent UV activity. The amino acids tryptophan (λ_{max} ~280 nm),[192] tyrosine (λ_{max} ~275 nm),[193] phenylalanine (λ_{max} ~257 nm),[193] and cysteine (λ_{max} ~280 nm – disulfide bond)[193] all absorb to some extent in the UV range of 250 – 400, as the absorption profile of a given species is usually somewhat Gaussian about the $\lambda_{max.}$ There are also some weak absorptions from the peptide bonds themselves.[193] Upon absorption, these residues can undergo photochemical reactions and degradation. Commonly observed degradation pathways include, but are not exclusive to, S-S bond fission, conversion of tryptophan to kynurenine species **118** and the conversion of tyrosine to 3-(4-hydroxyphenyl) lactic acid **119** and various dityrosines **120**, **Scheme 41**.



Scheme 41

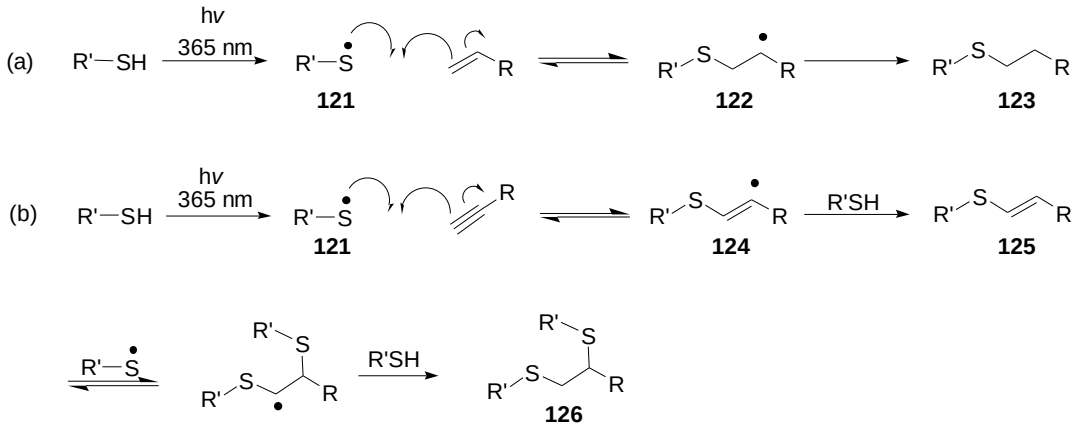
However, control of the range of wavelengths of UV irradiation that the protein is exposed to can negate such degradative pathways and make photochemistry a viable modification strategy. Photochemical transformations are usually regarded as suitable for utilisation with a protein substrate if there are moderate to good conversions to the desired conjugate at 365 nm, a commonly used wavelength for irradiation.[193-197] However, increasingly higher conversion rates at longer wavelength are sought after to minimise degradations. Wells *et al.* have carried out irradiation studies on human cells (IMR-91), showing that there is a marked decrease in cell death as irradiation increases from 334 nm to 365 nm, and again when going from 365 nm to 402 nm.[197] The cell death can be attributed to both DNA and protein degradation. As the native absorptions

of the amino acids in a protein can lead to side-reactions, photochemical modifications of proteins are usually carried out on modified amino acids or non-native amino acids, whereby the applied wavelength can be tuned to outside, or at the tail-end of, the natural absorbance range.

Photochemical modifications will be dealt with in three categories - (i) highly efficient photochemically-mediated additions; (ii) compounds that form non-specific, highly-reactive species upon irradiation, known as 'photoaffinity labels' and (iii) inert compounds that are rendered active upon an irradiation event and undergo defined chemistries, also called 'caged' compounds.

i. Photoaddition reactions

Photoaddition reactions can be described as highly efficient photochemically-mediated additions, such as the thiol-ene and thiol-yne reactions, **Scheme 42 (a)** and **(b)**, respectively. Whilst the reactions were described over 100 years ago,[198] they have recently taken on 'Click' status as they are highly efficient and tolerate a wide range of groups in water and withstand the presence of oxygen.[199] The thiol-ene/yne reactions proceed with either an initiator or with irradiation wavelengths near to visible light[102] and in buffered solutions. The thiyl radical **121** is formed from irradiation, which can then reversibly add to an alkene or alkyne to afford the addition products **122** and **124**, **Scheme 42**. The reversion of this step can be affected by the structure of both the thiol and the alkene/alkyne and can be sensitive to reaction conditions.[199] The alkyl radical **122** abstracts a H· from a remaining thiol and affords the thioether **123**. However, once the radical product **124** from the alkyne addition is quenched it is still an alkene **125**, and can therefore undergo a second radical addition to afford dithioether **126**, **Scheme 42 (b)**. The reactions have been used to synthesise cyclic peptides both in solution phase and on-resin,[190, 200] to pattern surfaces,[102, 201] to prepare macrocycles[202] and for dendrimer construction.[199, 203] A similar reaction is the addition of thiols to electron deficient alkynes without the need for irradiation.[204] However, the reaction is much slower and does not benefit from the spatiotemporal control of irradiation-mediated reactions. The main side products and added complication of the thiol-ene/yne reactions are the disulfide compounds arising from thiol-thiol coupling.

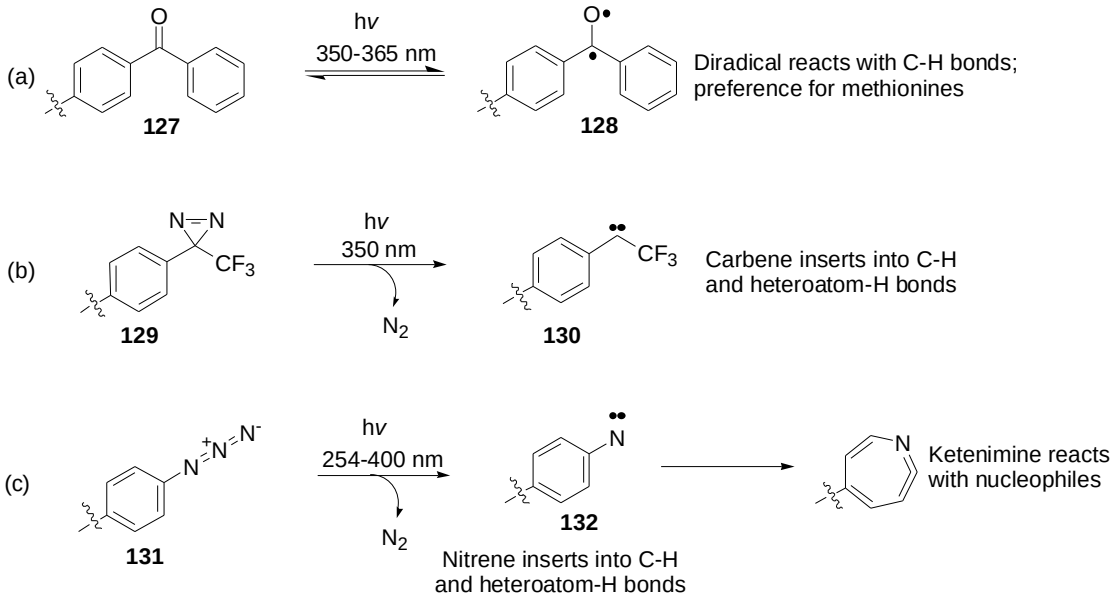


Scheme 42

Optimum conditions must be sought to further the utility of this promising reaction that proceeds at exposure to high wavelength, and thus low-energy, light.

ii. Photoaffinity labels

A powerful tool to define interactions between biomolecules is photoaffinity labelling.[194, 195, 205] Photoaffinity labels are characterized by the absorption of irradiation and subsequent formation of a highly reactive species. The reactive intermediate can then undergo largely non-specific reactions (*e.g.* C-H or X-H insertion) with surrounding biomolecules to form cross-linked products. By capturing interactions in this way, a 'snapshot' of a molecule's environment is obtained. The most common photoactivatable groups include aryl ketones **127**, **Scheme 43 (a)**, diazirines **129**, **Scheme 43 (b)**, and aryl azides **131**,[86, 194, 195] **Scheme 43 (c)**, forming reactive diradicals **128**, carbenes **130** and nitrenes **132**, respectively.



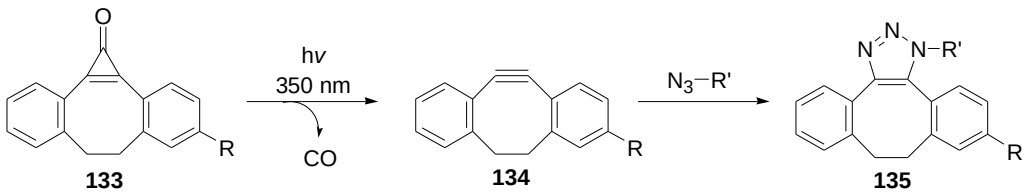
Scheme 43

The labels can be introduced *via* standard chemical means, or genetically or metabolically incorporated into target molecules.[195] The cross-linking reaction is usually brought about by a pulse of light. The λ_{max} of these species is usually too low for biological uses[196] and can damage the biomolecules to which conjugation is intended. Therefore, so-called 'off-maximum' values are used to approach irradiation around 400 nm and hence conversion rates of conjugation are compromised.[195] As the lifetime of an excited species increases so does its crosslinking efficiency. However, longer-lived reactive species often lead to less specific interactions and a compromise between these characteristics must be sought when choosing a photoaffinity label. Benzophenone groups generally have a higher degree of bond formation to the intended target molecule compared to yields obtained using phenyl azides, due to their ability to be repeatedly irradiated without breakdown of the precursor to an inactive form.[86] A recent use of photoaffinity labels incorporated modified aryl azides into a protein for site-specific mapping of protein-protein interactions.[100]

iii. Photoactivatable residues and 'caged' functionality

The term 'photoactivatable' is used in this text to describe molecules that are inactive prior to irradiation but, upon irradiation undergo selective reactions with specific reaction partners. The term 'caged' is usually used when the active molecule is an endogenous substrate or receptor that has a natural function that is inhibited by modification with a 'cage'.

As a direct result of the popularity of the 'Click' reaction in protein modifications efforts have been focused on ways to mediate this reaction without the use of copper. The utility of the azide-alkyne 'Click' reaction has been further extended by the development of a photochemically triggered adaptation[191] as it allows combination of the fast and bioorthogonal 'Click' reaction with the spatiotemporal control of photochemistry. The cyclopropenone **133**, upon irradiation at 350 nm, forms the strained alkyne **134** which allows copper-free triazole **135** formation from the resultant cycloaddition, **Scheme 44**.



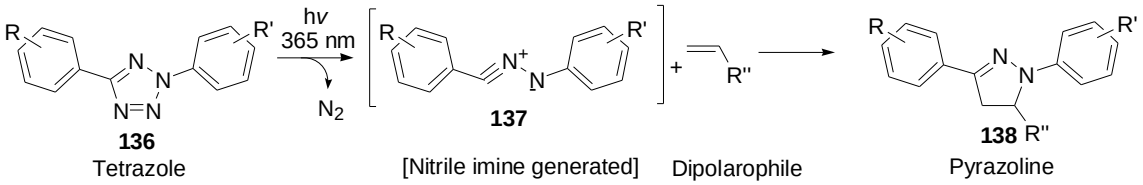
133 **134** **135**

Scheme 44

The masked alkyne can be tethered to an affinity moiety (biotin), a fluorophore or any manner of molecule of interest. A recent development in this area, is the use of the photoactivated reaction to modify surfaces with complex patterns *via* photomasks.[206] Similarly to all cyclo-octyne based cycloaddition strategies, a major drawback of this route is the complexity of synthesis of eight-membered ring substrates. Additionally, as the conjugation event is not itself light-mediated, the kinetics of the cycloaddition are the same as the standard 'strain'-mediated reaction. This results in a time delay between generation of the alkyne and occurrence of the cyclisation reaction – allowing for some diffusion to areas that have not been specifically targeted. The use of this technique with spatiotemporal control has therefore not yet been perfected and ways to combat this aspect of the methodology are sought after.[191]

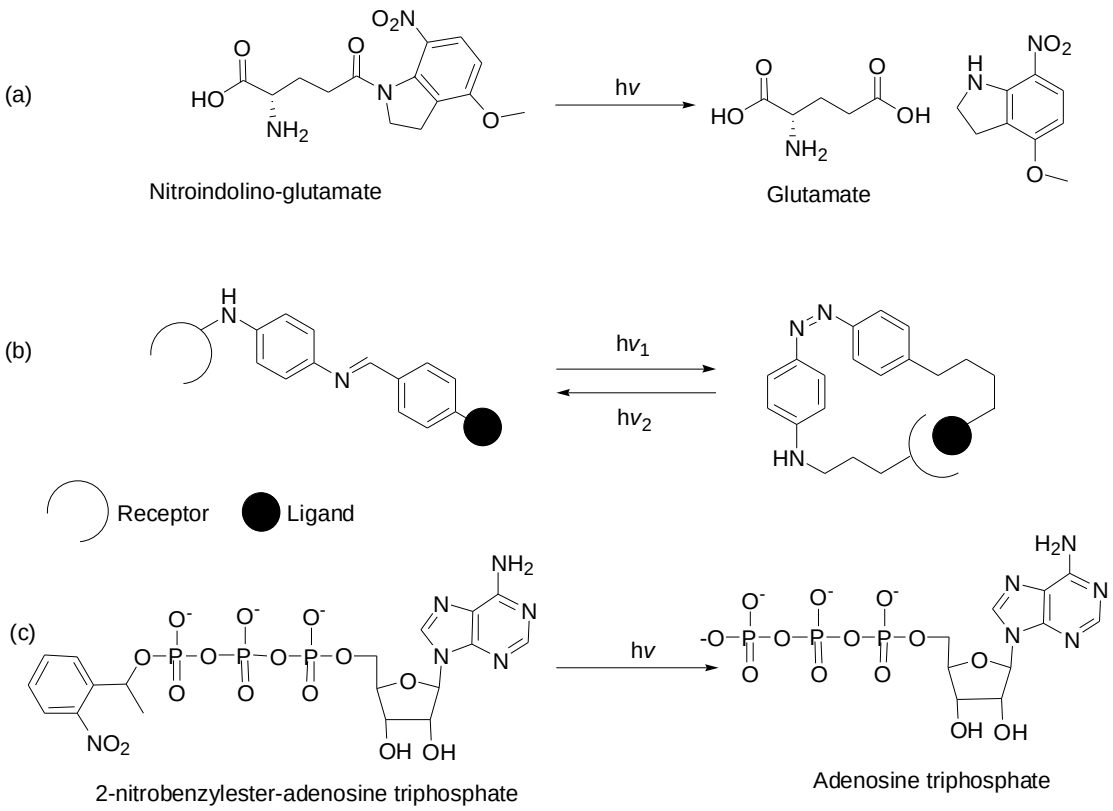
Lin and co-workers[189, 196, 207-211] have developed a photochemical conjugation strategy that proceeds at usable wavelengths for application to proteins. Their work utilises the 1,3-dipolar cycloaddition of nitrile imines that are generated *in situ*, from the photolysis of tetrazoles.[196, 207] Tetrazole **136** liberates nitrogen to form the dipole **137**, which then undergoes a highly regiospecific cycloaddition with a range of alkenes to form fluorescent pyrazoline products **138**, **Scheme 45**. The reaction is specific to alkenes and

therefore bioorthogonal. Tetrazole-labelled amino acids[208] and proteins[209] are easily accessed synthetically[196, 208] and can be reacted with a range of dipolarophiles, or, conversely, the dipolarophile alkene can be incorporated into the target protein and reacted with a range of tetrazoles.[189, 210, 211] Whilst the maximum absorbance varies from substrate to substrate, labels have been identified that have good conjugation yields at 365 nm, *i.e.* low enough energy irradiation for biological application. As the conjugation affords fluorescent pyrazoline cycloadducts, with a different absorption profile to the starting materials, the bioconjugations can be monitored *via* fluorimetry.[196]



Scheme 45

The rationale behind the caging technique is that a molecule of interest can be rendered biologically inert (caged) by chemical modification with a photoremovable or photoisomerable[186] group. The functionality of the caged molecule is then revealed upon irradiation. A variety of caged motifs have been made and used, including amino acids,[212] **Scheme 46 (a)**, whole receptors,[213] **Scheme 46 (b**), and nucleotides,[214] **Scheme 46 (c)**.



Scheme 46

A molecule that is to be 'caged' is initially modified *via* standard conjugation techniques and the use of photochemistry infers strict control on its subsequent activity. This strategy has recently been used to fabricate a photoresponsive drug carrier that releases an encapsulated drug upon irradiation.[215]

The work described in this thesis is presented in two parts. Firstly, there is a discussion of investigations into novel methods in [2+2] photocycloadditions, followed by research into new reagents for selective cysteine modification and their photoactivity.

2.i. Novel methods in [2+2] photocycloadditions

2.i.i. Aims

It was hypothesised that the scope of synthetically viable enone-olefin and enone-alkyne [2+2] photocycloadditions could be broadened by removing the chromophoric products *in situ*, after the initial photoreaction, thus maximizing the yields and potential of these types of reactions, **Figure 16**.

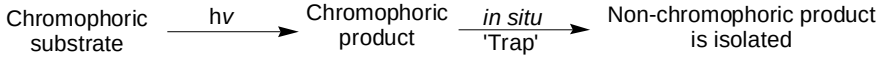
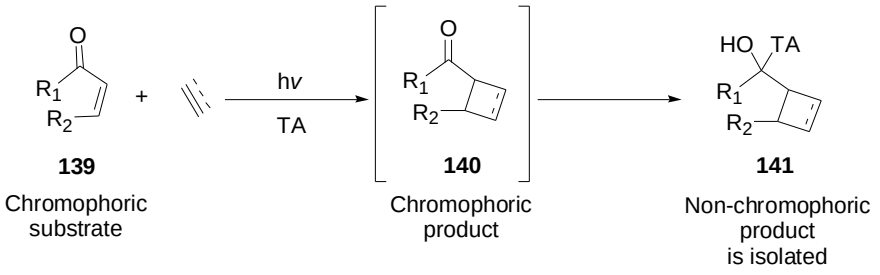


Figure 16: Hypothesis

For example, after the initial [2+2] photocycloaddition of enone **139**, an *in situ* trap *via* a trapping agent (TA) of the newly formed ketone **140**, in preference to the enone **139**, would afford a non-chromophoric species **141** which would not undergo photochemical degradation, **Scheme 47**.



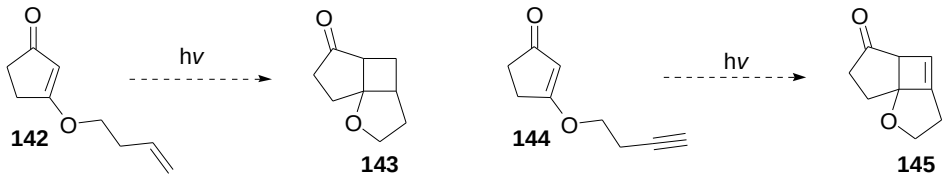
TA = Trapping agent that is selective for ketone **140** over enone **139**

Scheme 47

To attain the goal of finding a nucleophile that would be a suitable 'trap' in these photochemical reactions, the chosen nucleophile had to be photochemically unreactive and be selective for the ketone products **140** of these reactions rather than the enone substrates **139**.

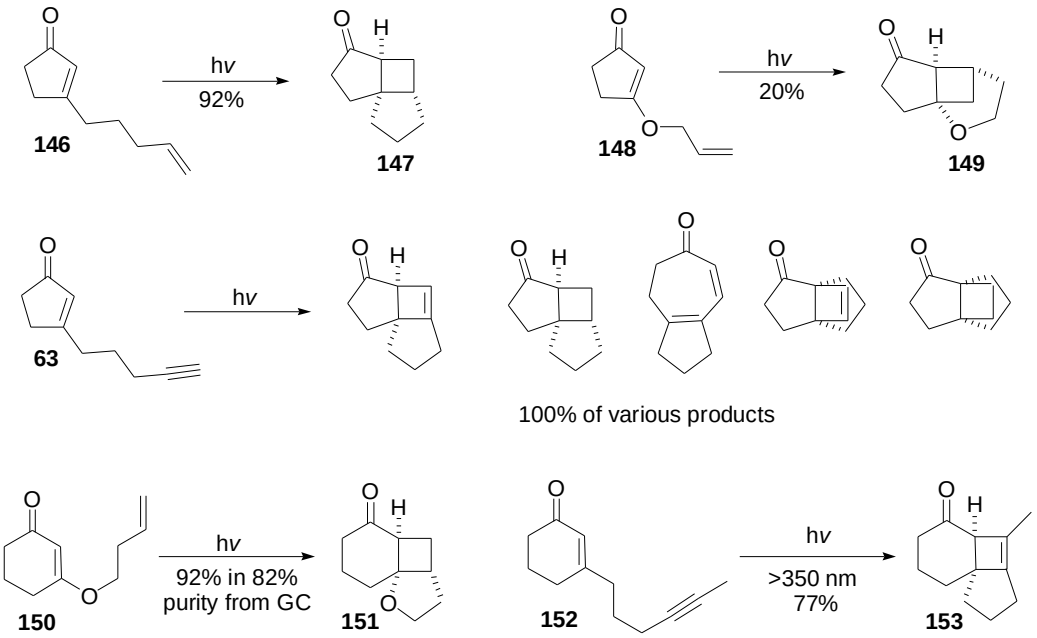
Model system for [2+2] photocycloadditions

The vinylogous esters **142** and **144** were chosen to test the hypothesis as they would provide significant reactivity differences towards nucleophiles between the carbonyls in the vinylogous ester substrates **142** and **144** and the carbonyls in the ketone products **143** and **145, Scheme 48**.



Scheme 48

The photochemical transformations to the ketones **143** and **145** are also conspicuously missing from the literature, as other, very similar, closely-related motifs have been reported, **Scheme 49**.[47-49, 63] For example, the enone **146** differs from the vinylogous ester model substrate **142** by just one atom, and affords the ketone product **147** in excellent yields.[63] When the vinylogous ester **148**, with one methylene less than the model substrate **142**, was irradiated, the crossed adduct **149** was formed in moderate yield.[49] Enone **63** affords a variety of four-membered ring photoproducts after irradiation.[47] It differs from the alkyne model **144** by just one atom (for further discussion on this reaction see **Scheme 17**, p. 28). When the vinylogous ester contains an extra methylene in the ring system **150**, 92% of the ketone **151** is afforded in 82% purity (from GC analysis).[50] The analogous six-membered ring enone-alkyne **152** affords the 1,4-unsaturated ketone **153** in 77% when uranium glass is used,[66] **Scheme 49**.

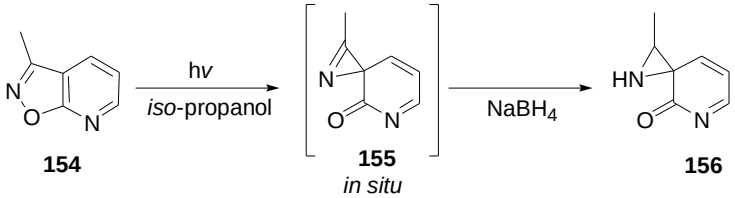


Scheme 49

It was thus postulated that the secondary photoreactions of ketones **143** and **145** had prevented the photochemical reaction of vinylogous esters **142** and **144** from being successfully reported in the literature. The intramolecular reactions also promised to provide much quicker reaction times than if the intermolecular analogous reactions were used.

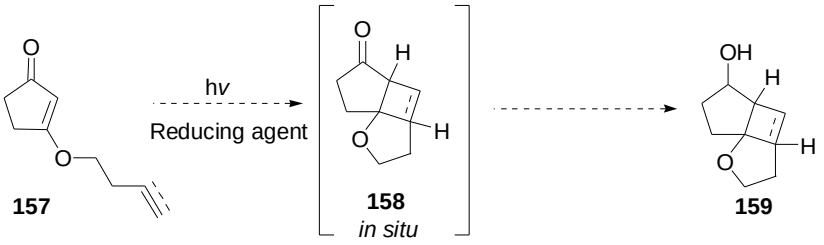
Reagents proposed for *in situ* trapping

The first trapping agents proposed for investigation were borohydride reducing agents. Donati *et al.*[216] had shown that borohydride reduction could be performed *in situ* in photochemical reactions,[216] **Scheme 50**, suggesting that borohydrides were compatible with irradiation conditions. In their work they had trapped the photoreactive azirine intermediate **155** during irradiation of isoxazolopyridine **154** as the photochemically stable aziridine **156**, **Scheme 50**.



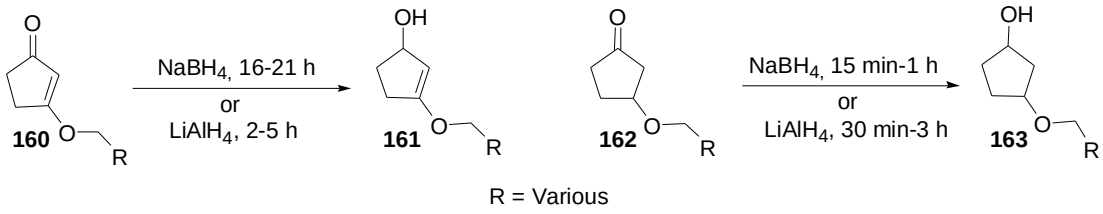
Scheme 50

Therefore, it was proposed that the use of borohydride reducing agents under photochemical conditions was theoretically possible, trapping ketones **158** as the analogous alcohols **159**, **Scheme 51**.



Scheme 51

For the *in situ* trapping reaction to be successful, the vinylogous esters **157** had to be less reactive towards hydride reducing agents than the ketone photoproducts **158**, and literature precedent suggested that this would be the case. Across various vinylogous ester systems **160**, reduction with sodium borohydride to the alcohols **161** took between sixteen and twenty-one hours, and lithium aluminium hydride reduction of similar systems took between two and five hours. Conversely, similar ketones **162** were much quicker to react with both sodium borohydride and lithium aluminium hydride, taking between fifteen minutes and one hour and between thirty minutes and three hours, respectively, to afford the alcohols **163**,[217-226] **Scheme 52**.

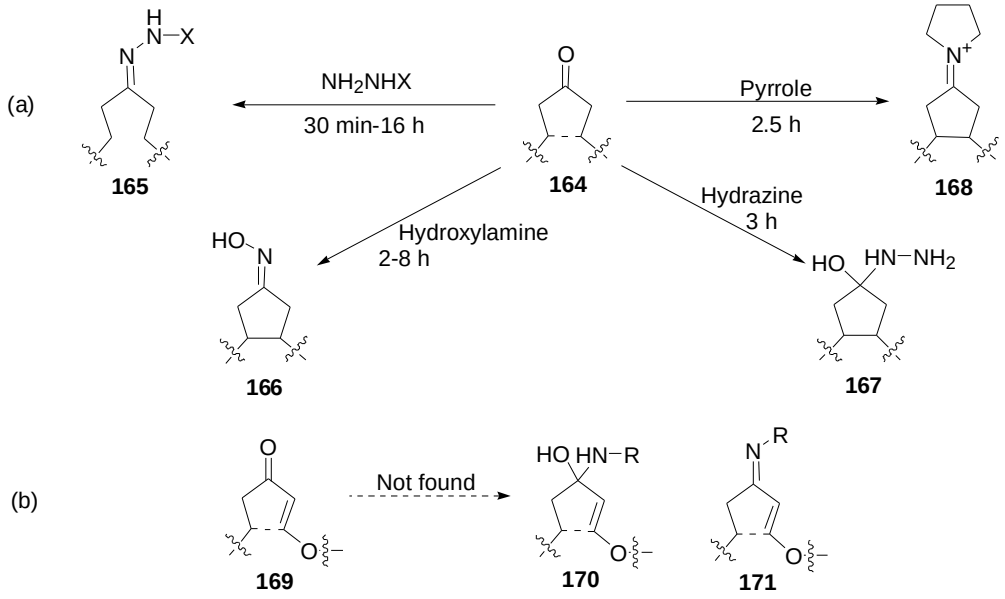


Scheme 52

It was thus suggested that the selective reduction of ketone products **158**, rather than vinylogous esters **157**, could be carried out during irradiation, and that this would remove the possibility of secondary photoreactions and degradation in [2+2] photocycloadditions of such substrates.

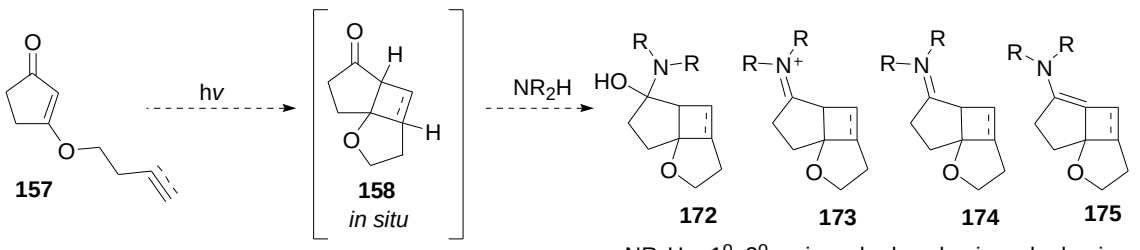
In addition to hydride-based reducing agents, nitrogen nucleophiles were postulated as useful reagents for trapping studies. The literature described rapid addition of a variety

of nitrogen nucleophiles to cyclic and acyclic ketones **164** to afford hydrazones **165**, oximes **166**, hemiaminals **167**, and iminiums **168**, **Scheme 53 (a)**.[227-231] In contrast, there was no evidence found that implied that nitrogen nucleophiles would react rapidly, if at all, with vinylogous esters **169** from cyclic or acyclic scaffolds, to afford the analogous hemiaminal **170** or imine-based products **171**, **Scheme 53 (b)**.



Scheme 53

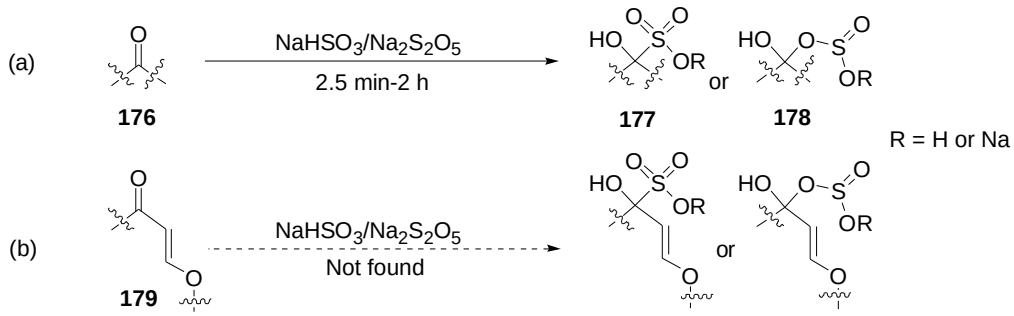
With these examples in mind, it was postulated that nitrogen nucleophiles such as amines, hydroxylamines and hydrazines could be utilised during irradiation to trap out photoproduct ketones **158** in preference to the vinylogous esters **157**, affording hemiaminals **172**, iminiums **173**, oximes or hydrazones **174** and enamines **175**, **Scheme 54**.



Scheme 54

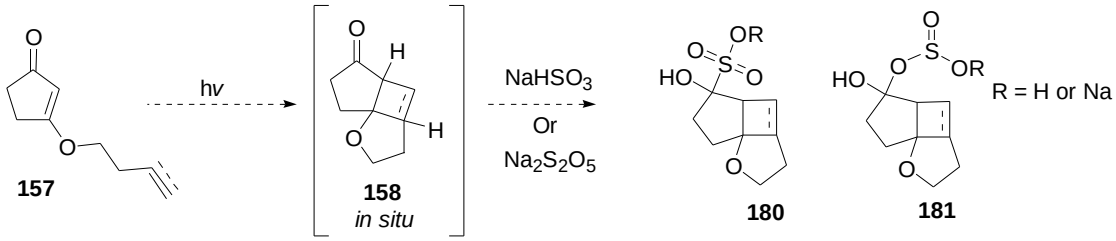
Sodium bisulfite and metabisulfite were also suggested as possible trapping agents. The reaction of a variety of ketones **176** with bisulfites and metabisulfites was quoted as

rapid, forming α-hydroxy sulfonic acids **177** and bisulfite adducts **178** in as little as two and a half minutes in some cases, **Scheme 55 (a)**.[232-235] The analogous reaction of sodium bisulfite and metabisulfite with vinylogous esters **179** was not found, **Scheme 55 (b)**.



Scheme 55

These examples were taken to suggest that the bisulfite-based reagents would be selective for the ketone products **158** of the photochemical transformation over the vinylogous ester substrates **157**, **Scheme 56**. The products, **180** and **181**, of the reaction of the bisulfites with ketones **158** were predicted to be non-chromophoric, and therefore would not degrade in the photochemical reaction.



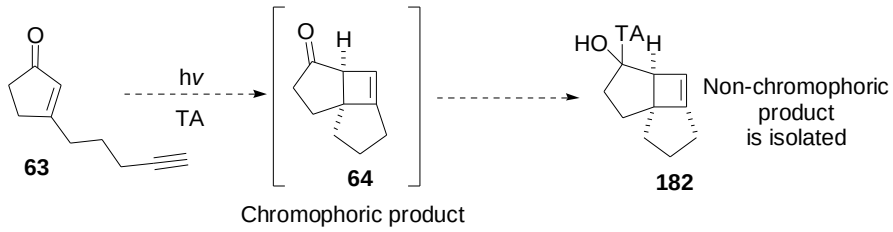
Scheme 56

It was the premise of this work that removing the carbonyl functionality *via* trapping agents would preclude all secondary photoreactions, including Norrish cleavage reactions and the 1,2- and 1,3- acyl shifts that are seen in 1,4 unsaturated systems.[47, 48, 236]

<u>Alternative enone systems for use in trapping studies</u>

If trapping conditions could be established with the intramolecular vinylogous ester models **142** and **144**, then enone **63** would be synthesised in efforts towards isolating

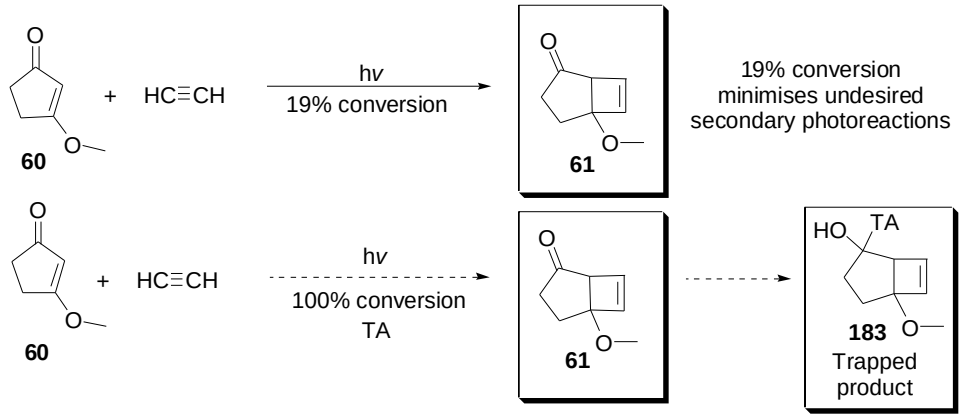
the primary photoproduct **64** as a 'trapped' analogue **182**, , **Scheme 57**, for further details on the by-products of this reaction see **Scheme 17**, p. 28.



TA = Trapping agent that is selective for ketone **64** over enone **63**

Scheme 57

It was also planned that any successful trapping agents (TA) discovered in the course of the investigations into the intramolecular reactions would be transferred to intermolecular [2+2] photocycloadditions that were either reported as low yielding or not reported in the literature at all. For example, in literature examples the photocycloaddition of vinylogous ester **60** with acetylene was only carried out to 19% conversion in order to minimise secondary photoreactions,[75] **Scheme 58**, for further details on this reaction see **Scheme 16**, p. 28. It was postulated that once *in situ* trapping conditions were in hand from model studies, the photochemical step from this synthesis could be carried out to completion, thus the yield of the transformation to trapped cyclobutene **183** would be greatly increased, **Scheme 58**.

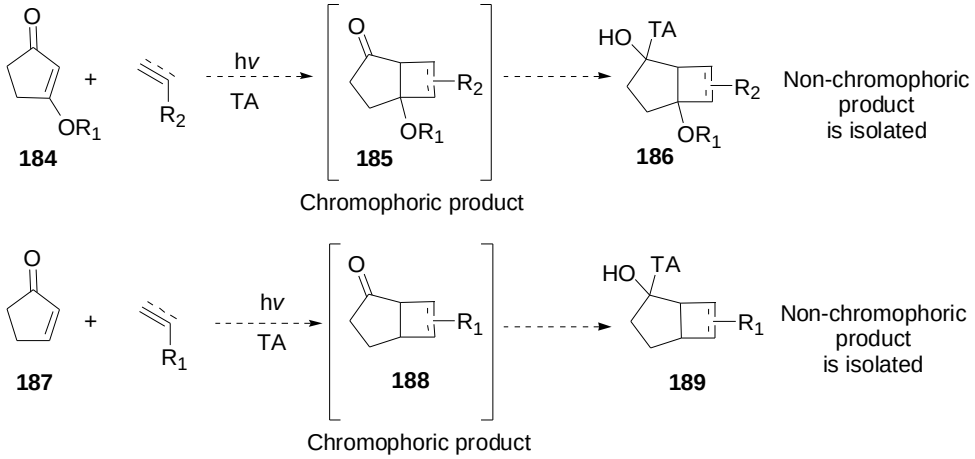


TA = Trapping agent that is selective for ketone **61** over vinylogous ester **60**

Scheme 58

To trap out chromophoric photoproducts of the intermolecular [2+2] photocycloaddition, enones such as vinylogous esters **184** and cyclopentenone **187**,

Scheme 59, would also be irradiated under any new conditions with alkenes and alkynes in attempts to afford both cyclobutane and cyclobutene products. It was envisaged that a variety of chromophoric ketones, **185** and **188**, could be trapped as analogous non-chromophoric products, **186** and **189**, **Scheme 59.**

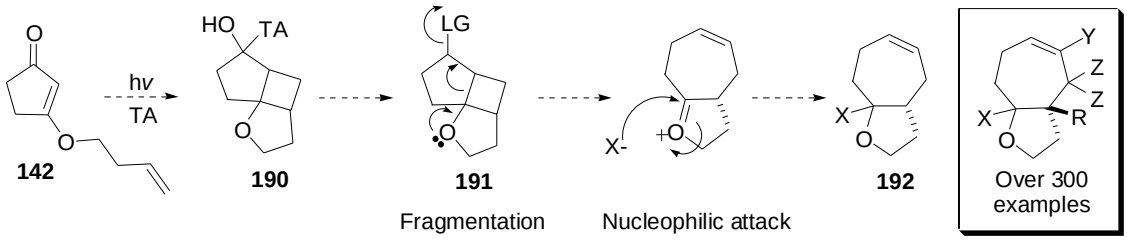


TA = Trapping agent that is selective for ketones **185** and **188** over enones **184** and **187**

Scheme 59

Ring fragmentation of photocycloaddition products

If the trapping strategy could be successfully demonstrated, structures such as alcohol **190** could potentially be accessed for the first time, **Scheme 60**. It was envisaged that these motifs could be exploited towards useful scaffolds such as 7,5-ring **192** *via* functional group interconversions and directed fragmentation. There were over three hundred literature examples of highly substituted 7,5-rings in natural products[237-239] such as those that could potentially be afforded from Grob-like[240] or step-wise fragmentation of the 5,4,5-ring system **191**, **Scheme 60**.

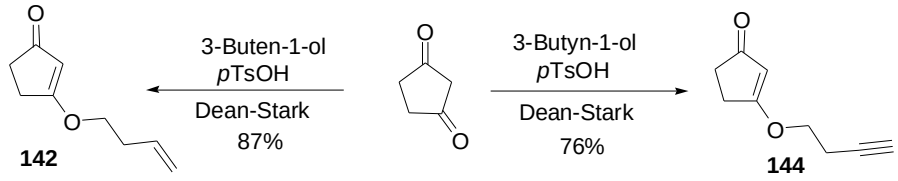


TA = Trapping agent, e.g. borohydride reducing agent

Scheme 60

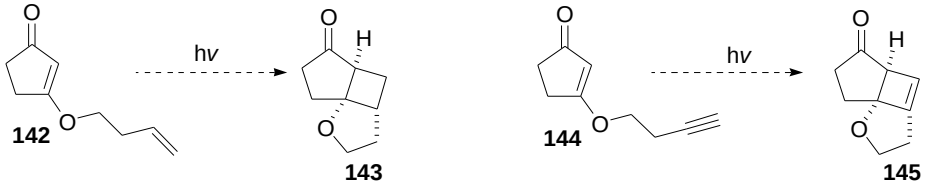
2.i.ii. Results and discussion for novel methods in [2+2] photocycloadditions

The vinylogous esters **142** and **144** used in this study were synthesized by acid catalysed condensation of either 3-buten-1-ol or 3-butyn-1-ol with 1,3-cyclopentanedione, **Scheme 61**.



Scheme 61

Scheme 62 shows the initial photochemical transformations that were proposed to be exploited in the trapping studies, converting vinylogous esters **142** and **144** into ketones **143** and **145**, both previously unreported reactions.



Scheme 62

Depending on the success of these transformations other substrates would be chosen for investigation.

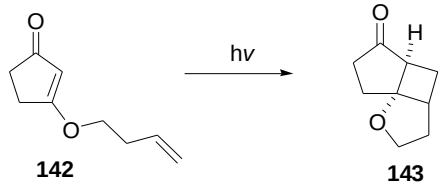
2.i.ii.i. Model validation

To be certain that the photochemical transformations of vinylogous esters **142** and **144** to ketones **143** and **145** suffered from secondary photoreactions as predicted, and thus were relevant model systems for trialling the trapping agent methodology, investigations into the photochemical conversions were initiated.

The initial irradiation of vinylogous ester **142** was carried out in 0.04 M acetonitrile, affording a mere 13% yield of ketone **143** after column chromatography, entry 1, **Table 1**. Solvent plays a large part in photochemistry due to filtering effects, sensitizing[241] and solubilities, thus carrying out the reaction in other solvents was attempted. However, mixtures of unidentifiable material were all that could be seen by ^{1}H NMR spectra of

the crude reaction mixtures from reactions in benzene, water, methanol, and *iso*-propanol. All reactions were carried out to complete consumption of vinylogous ester **142** and then subjected to column chromatography, but no ketone **143** was isolated from any of the reactions in solvents other than acetonitrile, entries 2-5, **Table 1**. The concentrations used are low compared to 'standard' chemistries as intramolecular photochemistry is usually undertaken at lower concentrations to minimize competing intermolecular reactions. Lowering the concentration of the reaction in acetonitrile further had no positive affect on the yield, entry 6, **Table 1**, whilst increasing the concentration led to no isolation of any ketone **143**, entry 7, **Table 1**.

Table 1: Irradiation of vinylogous ester 142



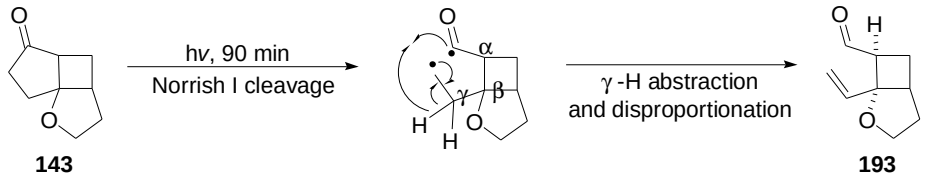
142 **143**

Entry	Time (h)	Solvent	Concentration	Yield of 143
1	2	Acetonitrile	0.04 M	13%
2	4	Benzene	0.04 M	--
3	4	Water	0.04 M	--
4	4	Methanol	0.04 M	--
5	1	*iso*-Propanol	0.04 M	--
6	4.5	Acetonitrile	0.02 M	3%
7	4	Acetonitrile	0.08 M	--

All reaction solutions were irradiated to complete consumption of the starting material and analysed by TLC and ^{1}H NMR, prior to column chromatography of the reaction residue.

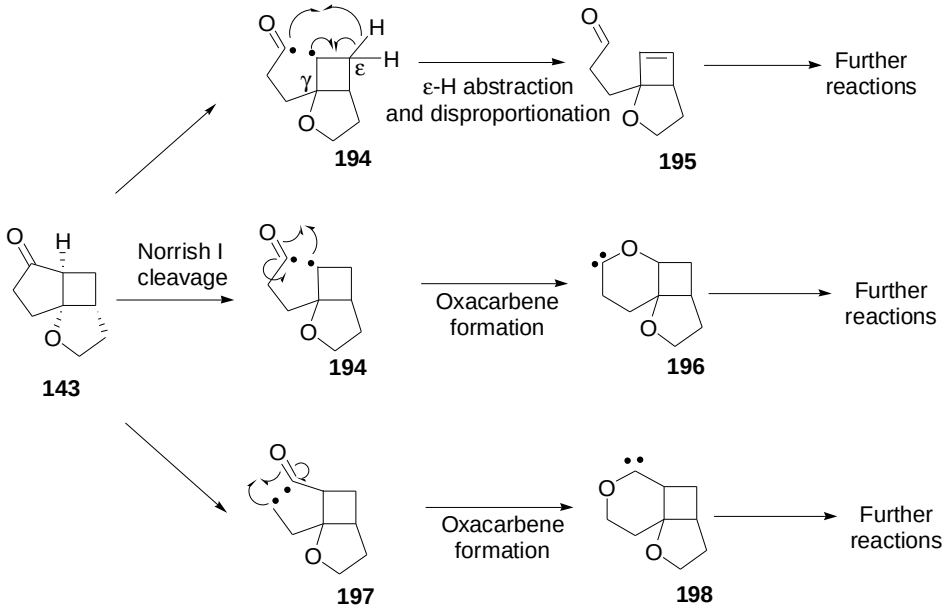
Once isolated and subjected to further irradiation, the ketone **143** degraded to mostly unidentifiable material in ninety minutes, confirming that this compound was sensitive to photochemical degradation over the time course for the [2+2] photocycloaddition. One side product, tentatively proposed to be aldehyde **193**, could be seen in the crude reaction mixture by ^{1}H NMR, **Scheme 63**. Peaks were visible that suggested a terminal alkene was present in the complex mixture (m, 1H, 6.2-6.0; m, 2H, 5.3-5.1) as well as a number of peaks above 9 ppm that could be attributed to a variety of aldehydes. The aldehyde **193** could neither be completely characterised nor isolated from the reaction

mixture, but could have been derived from ketone **143** undergoing Norrish I cleavage, and γ-hydrogen abstraction and disproportionation, **Scheme 63**. This sequence is well described in the photochemical literature.[10, 18, 64, 242, 243]



Scheme 63

Whilst no other products could be isolated for characterisation, it was postulated that ketone **143** would have also undergone Norrish I cleavage towards the alternative α-carbon, **Scheme 64**. This could then afford the secondary radical **194** and subsequent disproportionation to further reactive products **195** that were not isolated, but would have lowered the yield of the desired ketone **143**. Both Norrish I cleavage diradicals **194** and **197** could also have formed reactive oxacarbenes, which can form in strained systems.[243] The oxacarbenes **196** and **198** would be expected to have limited stability as they are highly reactive and so were not directly observed, but could further account for the low yield observed in this reaction.

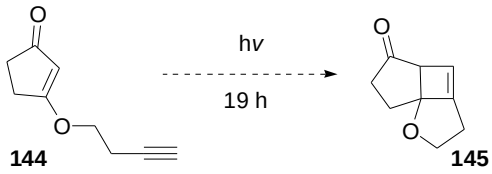


Scheme 64

It had been shown that the intramolecular [2+2] photocycloaddition of vinylogous ester **142** was low yielding, suffered from secondary photoreactivity of the ketone product **143** and could potentially benefit from attempts to increase the yield. The [2+2] photocycloaddition of vinylogous ester **142** was thus validated as a model system.

Initial photochemistry of the alternate alkynyl vinylogous ester **144** in acetonitrile, **Scheme 65**, suggested a very slow reaction with formation of only very few baseline products after eight hours in both Pyrex® and quartz glassware. It was necessary to irradiate vinylogous ester **144** for nineteen hours in acetonitrile to afford complete reaction. No identifiable products could be seen in the crude reaction mixture by ^{1}H NMR or after column chromatography of the reaction mixture, entry 1, **Table 2**. Benzene, hexane, water, methanol, acetone and a mixed solvent system of acetone and acetonitrile were also employed as solvents in this reaction. However, the reaction was not deemed to go to completion (judged by disappearance of vinylogous ester **144** by TLC and ^{1}H NMR) until nineteen hours of irradiation had passed in all solvents tested and no identifiable product was ever observed, entries 2-7, **Table 2**. A range of concentrations from 0.01 to 0.40 M were implemented to determine if changing the concentration of the reaction could afford cyclobutene **145**, but no identifiable products were observed or isolated, entry 8-10, **Table 2**.

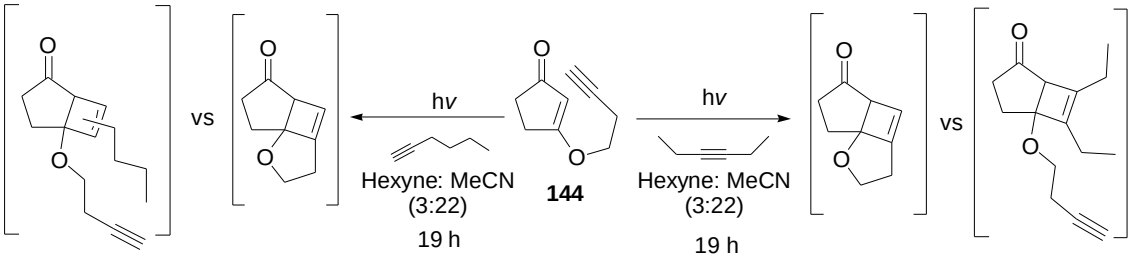
Table 2: Irradiation of vinylogous ester 144



Entry	Solvent	Concentration	Yield of 145
1	Acetonitrile	0.04 M	--
2	Benzene	0.04 M	--
3	Hexane	0.04 M	--
4	Water	0.04 M	--
5	Methanol	0.04 M	--
6	Acetone	0.04 M	--
7	Acetone: acetonitrile (1:1)	0.04 M	--
8	Acetonitrile	0.02 M	--
9	Acetonitrile	0.01 M	--
10	Acetonitrile	0.40 M	--

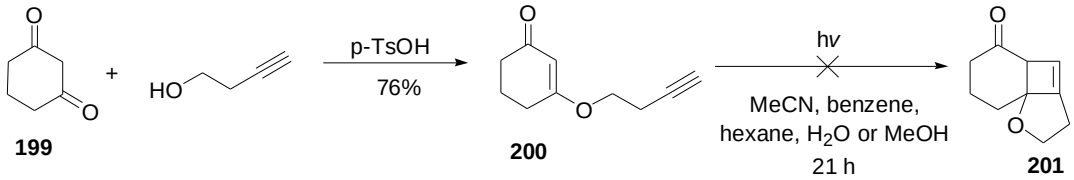
All reaction solutions were irradiated to complete consumption of the starting material and analysed by TLC and ^{1}H NMR, prior to column chromatography of the reaction residue.

It was contemplated that the slower reaction time could be explained by the alkyne tether being held in an undesirable conformation to undergo intramolecular reaction with the excited enone. Therefore, intermolecular reactions were investigated with both hex-3-yne and hex-1-yne to determine if the cyclisation was hindered by the length and, or, conformation of the pendant chain, *i.e.* if the enone would react more favourably intermolecularly. The two hexynes were used to resolve whether the cycloaddition would preferentially occur with the less hindered terminal alkyne, or its symmetrical internal counterpart, **Scheme 65**. The complete consumption of vinylogous ester **144** was observed after nineteen hours in both reactions, but no cyclobutene products were observed with either hex-3-yne or hex-1-yne, (merely degraded product was observed). This implied that it was neither a conformational problem, nor a steric problem with the substrate **144**. This suggested that the [2+2] photocycloaddition of the cyclopentenone and an alkyne was slow, *i.e.* the quantum yield was low, as the extinction coefficient should not present a problem as it was in the expected region for such compounds (see **3.iii.i.144**).[18]



Scheme 65

Literature suggested that the intramolecular [2+2] photocycloaddition of six-membered ring enone-alkynes were somewhat faster than their five-membered counterparts,[46, 54, 65, 66, 74, 75] therefore the six-membered analogue **200** was synthesised from 1,3-cyclohexanedione **199** and irradiations to form cyclobutene **201** were attempted, **Scheme 66**. Unfortunately, the analogue **200** behaved in exactly the same way as the five-membered ring variant **144 -** a slow reaction occurred upon irradiation, which, upon prolonged reaction times, formed unidentifiable degradation products with no ketone **201** witnessed, **Scheme 66**.



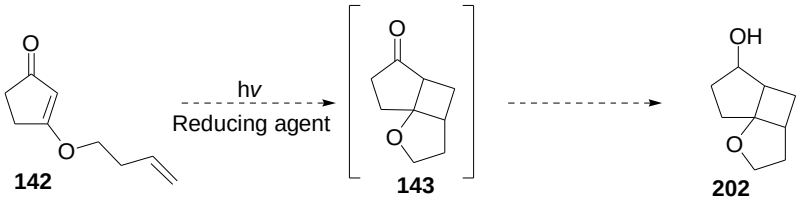
Scheme 66

It was thus hypothesized that the photochemical reaction of vinylogous esters with alkynes was very slow, and as soon as any reaction was taking place, the problem of secondary photoactivity was again interfering and the desired 1,4-unsaturated ketones **145** and **201** were being degraded. The vinylogous ester **144** had therefore been proven as problematic in photochemistry; hence it was deemed a useful model of enone-alkyne [2+2] photocycloadditions.

2.i.ii.ii. Product 'trapping' during [2+2] photocycloadditions

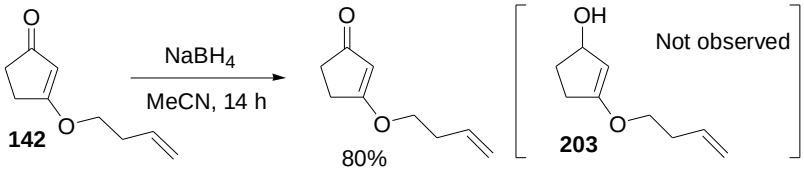
Initial investigations were made into trapping out the product from the [2+2] photocycloaddition of vinylogous ester **142** as alcohol **202** with reducing agents, **Scheme 67**, due to the reasons discussed in **2.i.i.**, namely, that borohydride reagents had

been used in photochemistry.[216] A literature search had also suggested that the reagents were more reactive towards ketones than towards vinylogous esters, **Scheme 52**, p. 62.[217-226]



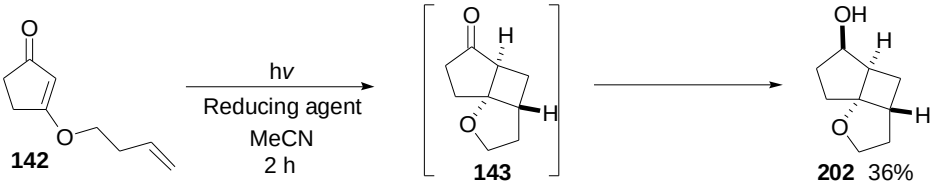
Scheme 67

For this methodology to be successful, vinylogous ester **142** needed to be tolerant to the reducing agent used in the trapping reaction. Therefore, vinylogous ester **142** was subjected to a solution of sodium borohydride for fourteen hours, **Scheme 68**. Preliminary tests showed that vinylogous ester **142** was recovered in 80% after this prolonged stirring with the reducing agent. Assuming the loss of vinylogous ester **142** was purely from reduction to alcohol **203** (not observed), this afforded a maximum of 20% reduction over fourteen hours. As the photochemical reaction of vinylogous ester **142** was to take place over a maximum of two hours, this was deemed chemoselective enough for initial trial reactions.



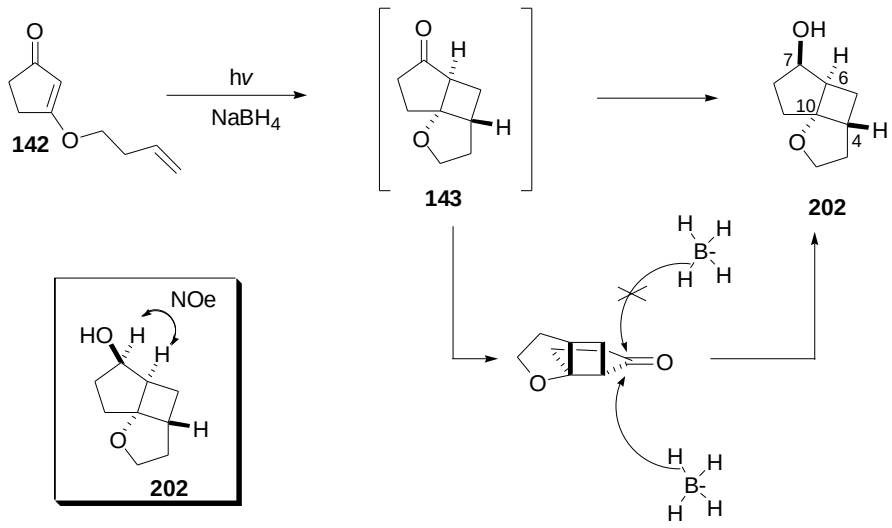
Scheme 68

Vinylogous ester **142** was thus irradiated to complete consumption (two hours) in the presence of one equivalent of sodium borohydride, affording alcohol **202** in 36% yield, **Scheme 69**, entry 2, **Table 3**.



Scheme 69

A fascinating outcome of the trapping study was apparent at this stage. The alcohol **202** isolated from the reaction was exclusively one diastereomer. Whilst the relative stereochemistry at carbons 4, 6 and 10 were all set in the photochemical reaction and governed by the achievable bond angles of a fused five-four ring, the resulting chirality at C7 was defined in the reduction. The hydroxyl group was in *cis* configuration with respect to the newly formed cyclobutane. When a three-dimensional model of the structure was constructed, the reason for this diastereoselectivity was apparent. The newly formed 5,4,5-ring ketone **143** was extremely rigid. The cyclobutane effectively shielded one face of the carbonyl from attack by the reducing agent, and the 'hydride' was delivered to the opposite face, **Scheme 70**. The structure was confirmed by NOeSY analysis of alcohol **202**, **Scheme 70**.



Scheme 70

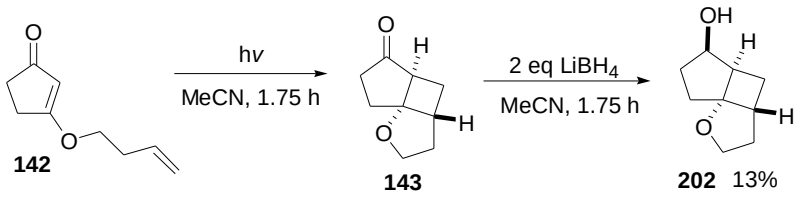
The near tripling of the yield of isolated product from this transformation with sodium borohydride as a trapping agent suggested scope for further improvement of this reaction, thus increased equivalents and different reducing agents were investigated. Two equivalents of sodium borohydride increased the yield of alcohol **202**, but only to 42%, entry 3, **Table 3**, thus a stronger reducing agent, namely lithium borohydride, was used. One equivalent of lithium borohydride increased the yield to 57%, entry 4, **Table 3**. Furthermore, two equivalents of lithium borohydride afforded the desired product in 64% yield, entry 5, **Table 3**. It was then established that the reducing agent should be added after the degassing step. This shortened the time the starting material was in the

presence of the reducing agent before the irradiation began, and thus minimized any reduction of the starting material, taking the achieved yield to 71%, entry 6 *cf.* 5, **Table 3**. Increasing the equivalents of lithium borohydride to three merely reduced the yield of alcohol **202**, entry 7, **Table 3**, most likely due to increased reduction of the starting material, and slight clouding of the solution resulting in retarded photochemistry and slightly longer reaction times. The use of an even stronger reducing agent, lithium aluminium hydride, was wholly unsuccessful as no alcohol **202** could be isolated from the reaction mixture, entry 8, **Table 3**. This was probably due to excessive reduction and degradation of the vinylogous ester **142**.

Zinc borohydride has been reported in the literature as a selective reducing agent for ketones and conjugated aldehydes in the presence of an enone,[219, 220] hence it was chosen to be utilized in the optimization studies. Ultimately, however, the synthesis of the reagent necessitated the addition of another solvent, namely ether, into the system. Ether is somewhat transparent to UV light but is likely to fragment and produce reactive radicals[10] and, as such, is not ideal for use in photochemistry. The reagent was prepared in solution and was immediately added *via* syringe in dry ether to a solution of the substrate in dry acetonitrile. Whilst zinc borohydride was quoted as selective[219, 220] and no reduction of the vinylogous ester **142** was identified after the irradiation, the yield of alcohol **202** was only 19%, entry 9, **Table 3**, most likely due to the fact that the reduction is simply not fast enough to intervene in secondary photoreactions.

Alternative reducing agents such as aminoborohydrides[244] are quoted as powerful and selective reducing agents, but their synthetic viability was questioned due to their sensitivity to moisture and air, and the fact that they are recommended for use in solvents unsuitable for photochemistry (*e.g.* THF).

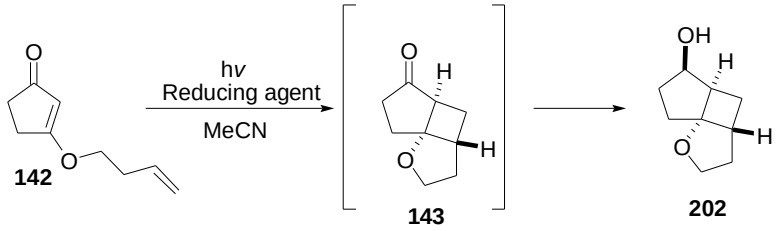
It was deemed necessary to determine that the low yield of ketone **143** was from photochemically-mediated degradation and not a matter of improved isolation of the alcohol **202** over the ketone **143**. Thus, $LiBH_4$ was added at the end of the photochemical reaction to afford alcohol **202**, **Scheme 71**. This yield would solely represent the outcome of the photochemical transformation.



Scheme 71

The yield obtained of alcohol **202**, 13%, was the same as that observed for ketone **143** when no reducing agent was used, entry 10 *cf.* 1, **Table 3**, suggesting that the degradation pathway of the ketone **143** was indeed photochemical.

Table 3: [2+2] Photocycloaddition of vinylogous ester 142 with reducing agents

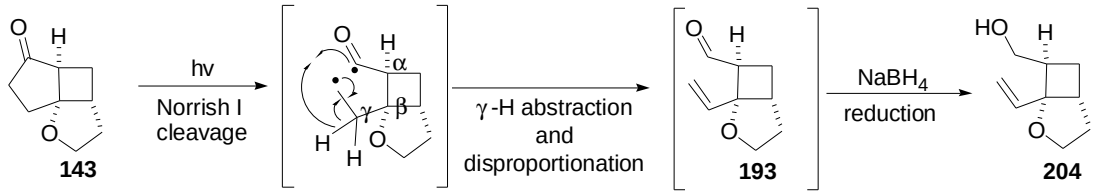


Entry	Reducing agent	Equivalent(s)	Time	Yield of 143	Yield of 202
1	--	--	2 h	13%	--
2	$NaBH_4$[a]	1	1.5 h	--	36%
3	$NaBH_4$[a]	2	1.5 h	--	42%
4	$LiBH_4$[a]	1	1.75 h	--	57%
5	$LiBH_4$[a]	2	1.75 h	--	64%
6	$LiBH_4$[b]	2	1.75 h	--	71%
7	$LiBH_4$[b]	3	2 h	--	60%
8	$LiAlH_4$[b]	1	1.5 h	--	0%
9	$Zn(BH_4)_2$[b,c]	2	1.5 h	--	19%
10	$LiBH_4$[d]	2	2 h	--	13%

All reactions carried out at 0.04 M in Acetonitrile (MeCN). [a]Reducing agent added prior to degassing. [b]Reducing agent added after degassing. [c]Reaction carried out in 9:1 MeCN:Ether. [d]Reducing agent added after photochemistry.

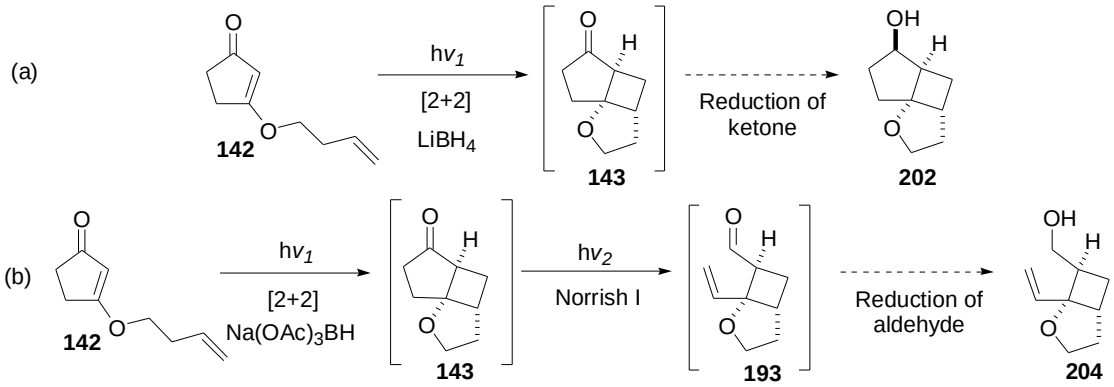
At this stage of the investigations into trapping agents, a recurrent by-product was observed in less than 1% yield in the reaction. A large scale reaction was undertaken to isolate this material in quantities that could be fully characterized. The by-product was identified as olefinic alcohol **204**. Whilst this by-product represented a loss of yield of

the desired compound, it was present in very small amounts and was separable from the alcohol **202**. Indeed, the identification of this compound was a useful result for the work. It was deemed that this product was formed from the predicted Norrish I cleavage of the product ketone **143**, followed by disproportionation *via* a well-precedented γ-H abstraction.[10, 64, 242, 243] This olefinic aldehyde **193** could then be reduced by the *in situ* reducing agent to the isolated alcohol **204**, **Scheme 72**. This was in accordance with the tentative observation of aldehyde **193** in the initial photochemical trials, **Scheme 63**, p. 69.



Scheme 72

As sodium borohydride in acetic acid media had been quoted as selective for aldehydes over ketones,[218] it was postulated that using sodium triacetoxyborohydride could trap out the Norrish degradation product **193** in preference to ketone **143**, **Scheme 73 (b)** *cf.* **(a)**. This would allow the product of this reaction to be altered merely by changing the nature of the reducing agent.

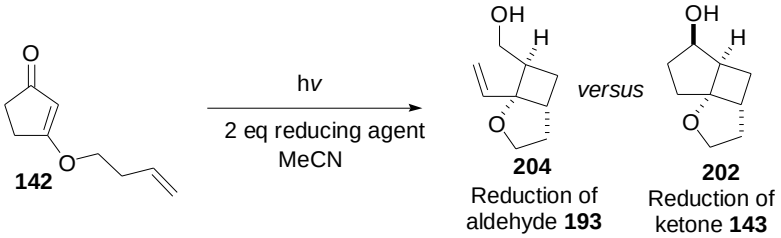


Scheme 73

However, when the irradiation of vinylogous ester **142** was carried out with two equivalents of sodium borohydride in the presence of six equivalents of acetic acid, entry 1, **Table 4**, an 18% yield of the usual reduced photoaddition product **202** was seen and only a trace amount of the reduced Norrish I degradation product **204** was isolated

(<1%). The reaction was attempted with commercial sodium triacetoxyborohydride, entry 2, **Table 4**. However, whilst the yield of the reduced photocyclisation product, alcohol **202**, was better than when the sodium triacetoxyborohydride was formed *in situ*, only minor traces of Norrish cleavage alcohol **204** were observed (<1%). Sodium monoacetoxy borohydride was utilized in this reaction as it was quoted as more reactive than the sodium triacetoxyborohydride in some systems,[218, 245] but neither alcohol **202** nor alcohol **204** were observed when it was used, entry 3, **Table 4**.

Table 4: Attempts to trap out Norrish I cleavage product 193 from the [2+2] photocycloaddition of vinylogous ester 142 with reducing agents



Entry	Reducing agent	Time	Yield of 204	Yield of 202
1	$NaBH(OAc)_3$[a,b]	1.5 h	<1%	18%
2	$NaBH(OAc)_3$[a,c]	1.5 h	<1%	40%
3	$NaBH_3(OAc)$[a]	2 h	--	--

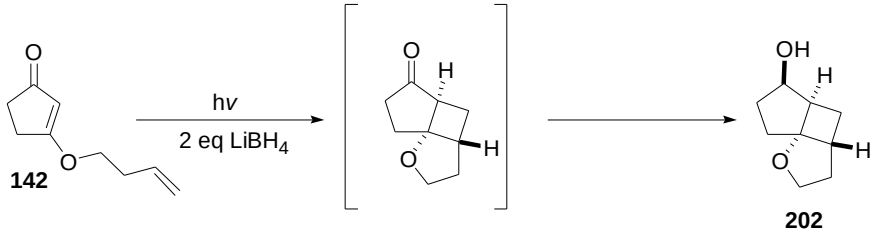
All reactions carried out at 0.04 M in Acetonitrile (MeCN). [a]Reducing agent added after degassing. [b]$NaBH(OAc)_3$ formed *in situ* from two equivalents of $NaBH_4$ and six equivalents of acetic acid. [c]$NaBH(OAc)_3$ purchased from Aldrich.

Ultimately, as a readily available, easily handled reducing agent, lithium borohydride was deemed a perfect reagent for the *in situ* trapping methodology, and conveniently, whilst a number of unusual reagents had been investigated, it was this commercially available reagent that afforded the best yield, 71%, entry 6, **Table 3**.

For investigations into the optimal solvent for the new tandem photocycloaddition-reduction, it was decided to use 'standard' photochemical solvents such as acetonitrile, hexane, benzene and dichloromethane, alongside *iso*-propanol. *Iso*-propanol was proposed as it had been used in the one previous example of sodium borohydride employment in photochemistry.[216] Benzene and hexane afforded successively lower yields of alcohol **202** when they were used as the solvents, 52% and 28%, respectively,

entries 2 and 3, **Table 5**. These results are both likely to be due to the poor solubility of the reducing agent in these solvents. Solvents such as *iso*-propanol and dichloromethane, afforded much lower yields than acetonitrile, entries 4 and 5, **Table 5**. Precluding water from the reaction did not increase the yield of the reduction, entry 6, **Table 5**. From the solvent screen, the best results achieved were in acetonitrile (71%), entry 6, **Table 5**, and acetonitrile:water (3:1), (72%), entry 7, **Table 5**, fairly mediocre yields were found with all other solvents used (20 - 52%).

Table 5: Solvent screen in [2+2] photocycloaddition of vinylogous ester 142 with $LiBH_4$



Entry	Solvent	Yield of 202
1	Acetonitrile	71%
2	Benzene	52%
3	Hexane	28%
4	*iso*-Propanol	39%
5	Dichloromethane	20%
6	Dry acetonitrile	63%
7	Acetonitrile:water (3:1)	72%

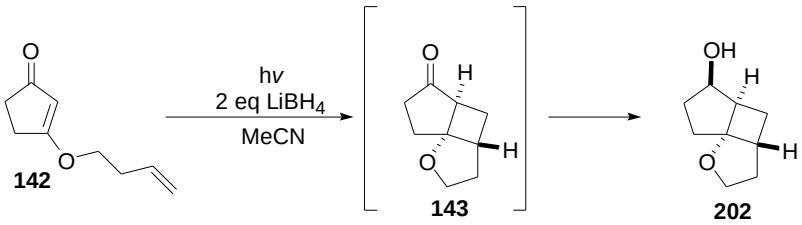
All reactions were carried out with two equivalents $LiBH_4$ (added after degassing) at 0.04 M.

The interpretation of the exciplex as a dipolar arrangement of the two reaction components indicates that in a polar solvent, the dipolarity of the exciplex is increased to the point where ions can form from direct electron transfer.[18] The decrease in exciplex binding energy is offset by the new Coulombic attractions,[18] so the cycloaddition can take place from a lower energy exciplex, and should be favoured by polar solvents. The exciplex mechanism can still take place in non-polar solvents, through a much 'tighter' complex, but with no stabilization from the solvent the energy barrier is much higher. The variation in exciplex formation with solvent polarity, led to the prediction that the photocycloaddition would be more likely in polar solvents where

pre-arrangement of the dipolar components was stabilized, but this was not borne out in the trends and yields observed, **Table 5**. This was probably due to the fact that the solvent had major effects in several other areas of the reaction. For example, in instances where the reducing agent was poorly soluble in the solvent, apart from leading to poor reduction of the product, a cloudy dispersion formed. This borane salt dispersion could have acted as a partial light filter, slowing down the photochemical reaction and increasing the time that the vinylogous ester **142** was exposed to the reducing agent – further decreasing the yield of ketone **143**. In addition, different solvents convey their own photochemical profile to the irradiated system. For example, using *iso*-propanol as a solvent did not merely increase the polarity of the solvent, but it also provided a route of product degradation through H· abstraction from the solvent.

For the desired intramolecular transformation, the concentration had to be kept low to minimize competition from intermolecular reactions. Unfortunately, at low concentrations, the reduction rate of chromophoric ketone **143** to photostable alcohol **202** is slowed. The concentration also needed to be such that the reducing agent was dissolved. It was therefore necessary to balance the reaction concentrations to favour the reduction of ketone **143** but not to force the reduction of the vinylogous ester **142**, and to favour quick photoreaction. Additionally, if the concentration was too low, then the reaction became less synthetically viable. A trial of concentrations was undertaken to see if the 71% yield could be improved upon without extending the reaction times or dilutions too adversely. Reducing the concentration of the irradiation below the standard 0.04 M, increased the yield of alcohol **202**, entries 1 and 2 *cf.* 3, **Table 6**. However, no significant difference in the yield was seen between 0.01 M to 0.02 M. Increasing the concentration to 0.08 M significantly decreased the yield of alcohol **202**, entry 4, **Table 6**. This was probably due to increased reduction of vinylogous ester **142** and increased 'clouding' of the solution, slowing the time to completion, lengthening the time the ketone **143** was further exposed to irradiation, and further reducing vinylogous ester **142**. The best yields were thus achieved with a 0.02 M solution of vinylogous ester **142**, entry 2, **Table 6**, affording 79% of alcohol **202**.

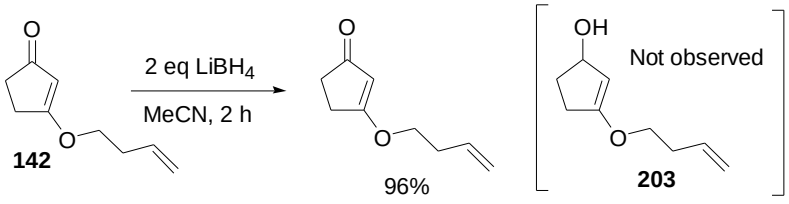
Table 6: [2+2] Photocycloaddition of vinylogous ester 142 with $LiBH_4$



Entry	Concentration (M)	Time to completion	Yield of 202
1	0.01	1.75 h	78%
2	0.02	1.75 h	79%
3	0.04	1.5 h	71%
4	0.08	2 h	12%

All reactions were carried out with two equivalents of $LiBH_4$ (added after degassing) in acetonitrile.

It was deemed necessary to establish where the loss of yield in the model reaction was occurring. If the vinylogous ester substrate **142** was sensitive to reduction by lithium borohydride it would form the alcohol **203** and would not undergo the enone-mediated photocycloaddition. Therefore, in order to determine the extent to which vinylogous ester **142** was reduced throughout the course of the reaction, it was stirred in acetonitrile without irradiation in the presence of two equivalents of lithium borohydride for two hours, **Scheme 74**. There was 96% starting material **142** recovery from this reaction, therefore indicating that the loss of yield in the current best conditions (21%) was not wholly from reduction of the vinylogous ester **142**.

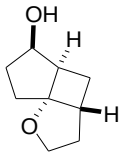


Scheme 74

The isolated product of the *in situ* hydride trapping was alcohol **202**. To determine if alcohol **202** was sensitive to column chromatography, 100 mg was resubjected to flash chromatography. Around 16% 'loss' to the silica gel column was observed, entry 1, **Table 7**, implying that some of the losses observed in the previous trials had indeed been due to isolation rather than just the reaction profile. The losses to the silica-gel were possibly from an unidentified fragmentation of the strained cyclobutane, promoted

by the inherent acidity of the silica. Alternatively, the strained alcohol **202** may have had a strong interaction with the silica gel and merely remained adsorbed rather than eluted. This prompted an investigation into purification techniques. Firstly, the crude reaction mixture was examined. Simple work-ups were attempted but it seemed that a large proportion of the alcohol **202** was lost in washes, whether neutral, basic or acidified solvents were used, and the recovered material was not clean. The crude reaction mixture needed to be subjected to flash chromatography to remove the borane salts as well as the recurrent Norrish I by-product alcohol **202** that prior reducing agent trials couldn't eliminate. A sample of the isolated alcohol **202** was subjected to flash chromatography in various other stationary phases to ascertain if the loss could be minimized. When alumina and fluorosil were implemented as stationary phases, the recoveries of alcohol **202** were dramatically lowered, entries 2 and 3, **Table 7**. However, when base-neutralised silica-gel was implemented, along with a base-neutralised eluent, the recovery of alcohol **202** was improved to 89%, entry 4, **Table 7**.

Table 7: Flash chromatography of alcohol 202



202

Entry	Gel	Recovery of 202
1	Silica	84%
2	Alumina[a]	29%
3	Fluorosil	34%
4	Silica[b]	89%

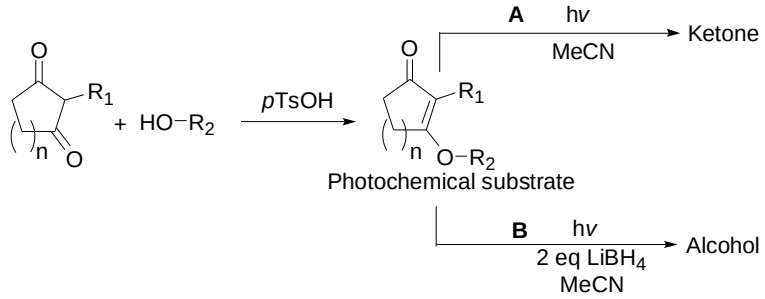
Various stationary phases were investigated in flash chromatography and were carried out with an eluent of 20% ethyl acetate in petroleum ether. [a]Eluent of 10% ethanol in ethyl acetate was required to isolate alcohol **202** from this stationary phase. [b]Neutralized with a solution of 1% triethylamine prior to, and during, chromatography.

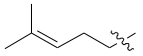
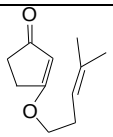
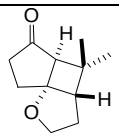
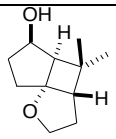
Across the flash chromatography systems trialled, only silica-gel had proven useful, particularly when used with a basic eluent, thus a reaction was carried out, followed by the modified method of purification. This increased the yield of alcohol **202** to 83%. Thus the optimum conditions to isolate the product from the enone-olefin [2+2] photocycloaddition were therefore defined at this stage as a 0.02 M solution of

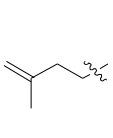
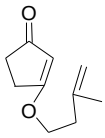
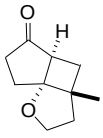
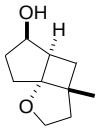
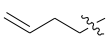
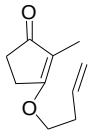
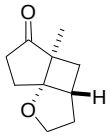
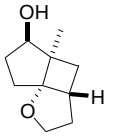
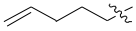
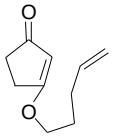
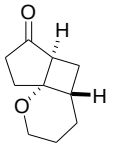
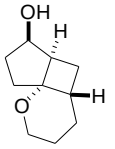
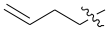
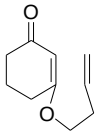
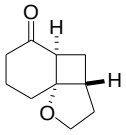
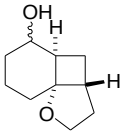
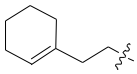
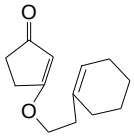
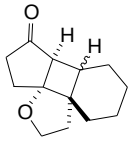
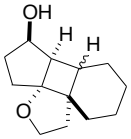
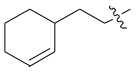
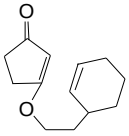
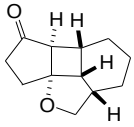
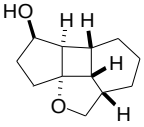
vinylogous ester **142** in acetonitrile, degassed and then two equivalents of lithium borohydride should be added. Immediate irradiation for 105 minutes at this stage afforded the alcohol **202** in 83% after flash chromatography, employing a 1% triethylamine-neutralised silica-gel column and eluent. Further optimization of the yield was not deemed likely due to the potential instability of the product and inherent losses during chromatography, Hence, investigations into alternative reducing agents and purification was halted here.

With these conditions in hand, a broader range of vinylogous esters were synthesized to probe the scope of this reaction. This was a very important step to carry out as photochemical reactions can be frustratingly substrate specific. A range of vinylogous esters were synthesized analogously to the model compounds **142** and **144**, *i.e.* by condensing either 1,3-cyclopentanedione or 1,3-cyclohexanedione with the appropriate alcohols, in yields ranging from 73-95%, **Table 8**. Substrates were chosen to substitute the olefinic tether terminally, entry 1, **Table 8**, and internally, entry 2, **Table 8**, to substitute the alkene in the enone system, entry 3, **Table 8**, to increase the tether to the terminal olefin, entry 4, **Table 8**, to increase the ring size of the enone, entry 5, **Table 8**, and to create more complex tethers that could produce tetracyclic products, entries 6 and 7, **Table 8**.

Table 8: Synthesis and photochemical reactions of vinylogous esters



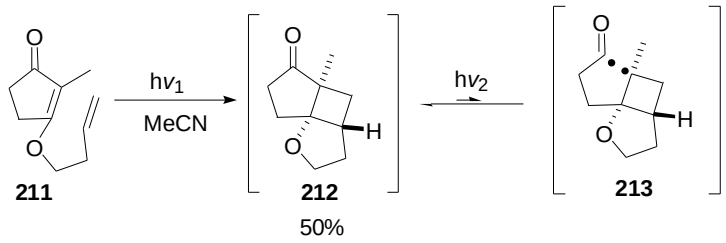
Entry	n	R_1	R_2	Photochemical substrate	Ketone from route A, yield	Alcohol from route B, yield
1	1	H		 **205**, 95%	 **206**, 0%	 **207**, 78%

2	1	H		 **208**, 58%	 **209**, 0%	 **210**, 71%
3	1	Me		 **211**, 95%	 **212**, 50%	 **214**, 72%
4	1	H		 **215**, 88%	 **216**, 27%	 **217**, 46%
5	2	H		 **150**, 75%	 **151**, 28%	 **218**, 59%[a]
6	1	H	 **219**[b]	 **220**, 88%	 **221**, 13%[c,d]	 **224**, 72%[e,f]
7	1	H	 **226**[g]	 **227**, 91%	 **228**, 0%	 **229**, 24%[f]

All irradiations were carried out to complete consumption of the vinylogous ester. [a]2:1 mixture of inseparable diastereomers isolated (see text). [b]Alcohol **219** was synthesised from the commercial carboxylic acid in 87% yield. [c]One diastereomer of undefined stereochemistry at C9. [d]13% De Mayo fragmentation product **223** also isolated (see text). [e]1:1 mixture of inseparable diastereomers isolated (see text). [f]A further equivalent of $LiBH_4$ was added at three hours of irradiation. [g]Alcohol **226** was synthesised from the benzyl alcohol in 90% yield

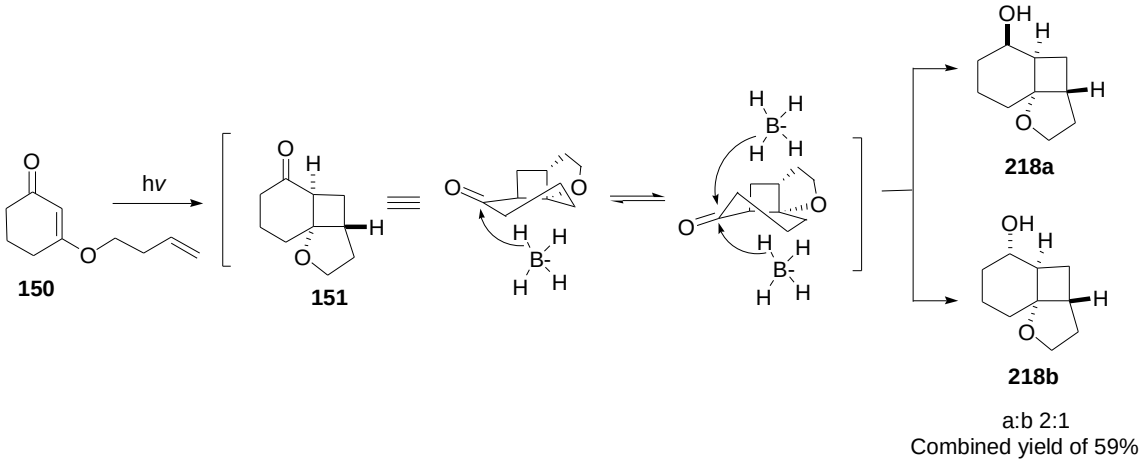
Photochemical reaction of vinylogous esters **205** and **208**, entries 1 and 2, **Table 8**, showed the impressive scope of utilizing the modified conditions. Without the conditions, ketones **206** and **209** could not be identified or isolated from the crude reaction mixtures. However, once the modifications were implemented, the analogous alcohols **207** and **210** could be afforded in good yields. Vinylogous esters **211** and **215**

showed more moderate increases in yield, but nevertheless, increases were observed, entries 3 and 4, **Table 8**. Interestingly, a methyl substituent on the enone, afforded the ketone **212** in much higher yields from irradiation without the modified conditions, entry 3, **Table 8**. It was postulated that this may be due to the fact that the hindered tertiary radical **213** formed from α-cleavage toward the methyl substituent would be more stable, longer lived, and could perhaps recombine, rather than lead to degradation products, **Scheme 75**.



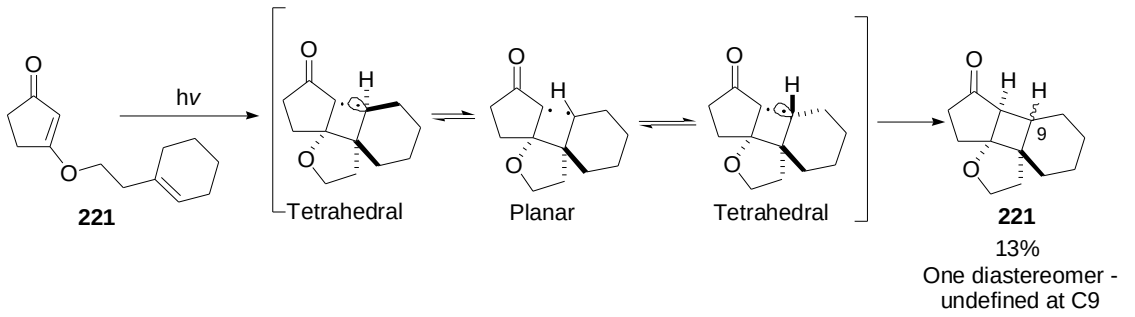
Scheme 75

Transformation of vinylogous ester **150** to ketone **151**, entry 5, **Table 8**, was in the literature as a successful reaction taking place in 97% yield at 82% purity.[51] However, this yield and purity was estimated from gas chromatographic analysis and not actually isolated material so the reaction was chosen for investigation. It was found that the maximum yield obtainable of this ketone **151** was only 28%. For this example there is minimal diastereoselectivity of the reduction and alcohol **218** was an inseparable 2:1 mixture of diastereomers, obtained in a combined yield of 59%. Again, three-dimensional modelling of the ketone **151** was useful to deduce the cause of this. Ketone **151** was far less rigid than the five-membered ring analogues, with the six-membered ring able to flip into two different conformations, **Scheme 76**. The two interconverting conformations rendered the subsequent reduction poorly diastereoselective. The major diastereomer was tentatively assigned as **218a** due to similarities between the chemical shifts and coupling constants of **218a** in ^{1}H NMR to other isolated substrates.



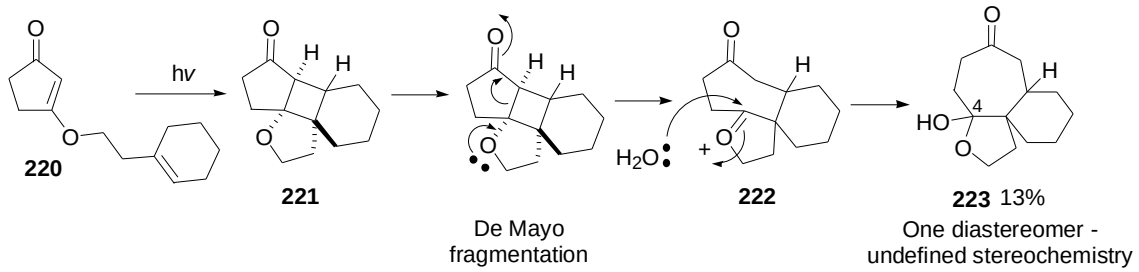
Scheme 76

The irradiation of vinylogous ester **220** was the first of the reactions to afford tetracyclic products, entry 6, **Table 8**. One diastereomer of ketone **221** was isolated in 13%, but the relative stereochemistry at carbon 9 was uncertain. Singlet-mediated reactions are concerted and all bonds are formed in the same instance, inferring strict diastereocontrol. However, the enone-olefin [2+2] photocycloaddition is nearly always triplet-mediated, and is thus usually non-concerted. This means that there is a time-gap between the first and second bond formations that form the cyclobutane ring. This gives the molecule chance to rotate bonds and create mixed stereochemistries at the newly formed radical centres, **Scheme 77**. As both diastereomers were theoretically possible from this transformation, but only one diastereomer was isolated, it was postulated that the other diastereomer was more prone to degradation, either through photochemistry or other means, and was not isolated. Ketone **221** was an oil and, as such, could not be crystallised for X-ray crystallographic analysis.



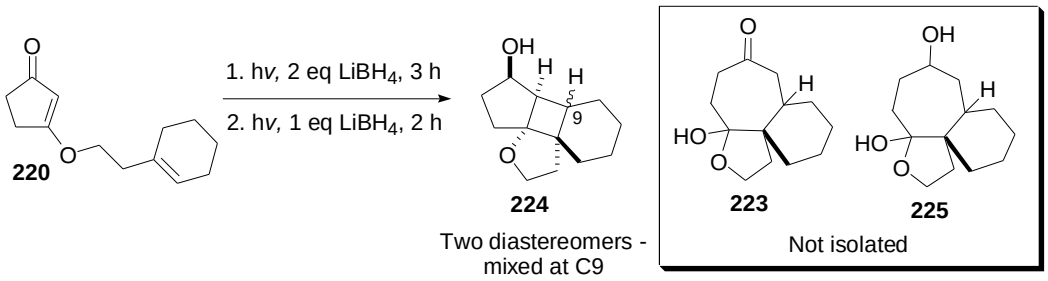
Scheme 77

Tricyclic compound **223** was also isolated in this reaction, also as one unidentified diastereomer, in 13% yield, **Scheme 78**. It was postulated that this fragmentation was from the decomposition of a different diastereomer of ketone **221**, which would account for why only one diastereomer of both tetracycle **221** and tricycle **223** were isolated. Tricyclic ketone **223** could have originated from the De Mayo fragmentation of the tetracyclic ketone **221**, followed by quenching of the resultant cation **222** with water, **Scheme 78**. As only one diastereomer of tricycle **223** was isolated, this suggested that water attacked only from one face of the intermittent cation **222**. Tricycle **223** was a paste, thus crystallisation was not possible and the relative stereochemistry of this molecule was not defined.



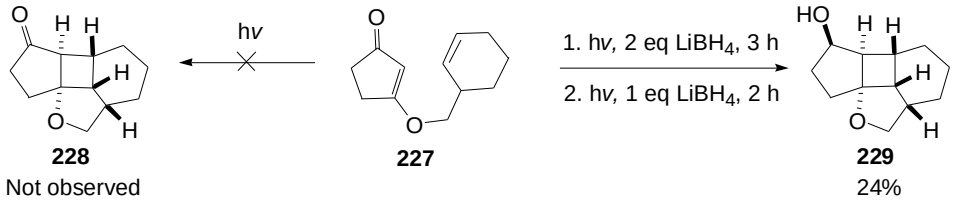
Scheme 78

The alcohol **224** was isolated in 72% from the irradiation of vinylogous ester **220** under reducing conditions, entry 7, **Table 8**. In this instance, at three hours of irradiation, the ketone product **221** could be seen by TLC. Therefore a further equivalent of $LiBH_4$ was added and irradiation continued for a further two hours until all vinylogous ester **220** was consumed. Two diastereomers of alcohol **224** were isolated, representing both possible stereochemical configurations of carbon 9 in a ratio of 1:1. In contrast to when the reaction was carried out without a reducing agent, the De Mayo product **223** was not isolated, and neither was its reduced form **225**, **Scheme 78**. This suggested that the reduction of ketone **221** was more rapid than the De Mayo fragmentation. The utility of the modified conditions were therefore further bolstered in that, not only were photochemically-mediated secondary pathways halted, but, by removal of the carbonyl, other unwanted side-reactions could also be abrogated.



Scheme 79

The second tetracyclic alcohol **229**, entry 7, **Table 8**, was of particular interest. Whilst the yield of the alcohol **229**, at 24%, was fairly mediocre, the analogous ketone **228** could not be isolated without the use of the modified conditions. Furthermore, six contiguous stereocentres had been set from an achiral substrate, simply synthesized in two steps from commercially available material,[246] **Scheme 80**. Similarly to the irradiation to afford the other tetracyclic alcohol **224**, a further equivalent of $LiBH_4$ was added at three hours of irradiation, when another compound, postulated to be ketone **228**, could be seen by TLC.

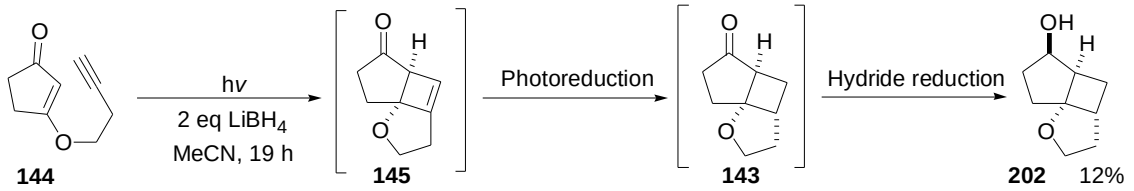


Scheme 80

The stereochemistry of alcohol **229** was assigned by established trends and achievable bond angles of the strained 5,4,5,6-ring system.

The solvent, concentration, identity and initial equivalents of reducing agent for the irradiation of all the vinylogous esters were taken directly from the optimization of the photochemical transformation of vinylogous ester **142** to alcohol **202** (0.02 M in acetonitrile with two initial equivalents of $LiBH_4$). Thus, there is potentially scope to increase the yields somewhat with further optimization on a case-by-case basis. It was not felt that such optimization was necessary at this stage as these reactions had proven the concept and illustrated the potential scope of this tandem photocycloaddition-reduction methodology.

With the success of the olefinic vinylogous ester model **142**, the hydride trapping methodology was turned towards the elusive cyclobutene scaffold from alkynyl vinylogous ester **144**. However when the alkynyl vinylogous ester **144** was irradiated with the reducing conditions, the same alcohol product as that isolated from the olefinic vinylogous ester **142** photochemistry was isolated, **202** in 12% yield, **Scheme 81**. This product was formed, as in previous reactions, from the hydride reduction of the carbonyl in the photocyclisation product **143**, but in this case, prior photoreduction of the double bond of the cyclobutene **145** had been observed. A similar photochemical reduction was reported by Mancini *et al.*[47] as H· abstraction from the solvent, seen in **Scheme 17**, p 28.



Scheme 81

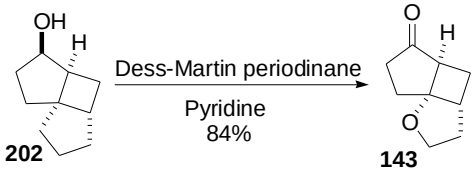
Carrying out the reaction in hexafluorobenzene to eliminate proton abstraction from the solvent was not successful, and no identifiable products could be isolated, it seemed that without the photoreduction, the 1,4-unsaturated system underwent photochemical degradation before the hydride-reduction could take place.

Although the yield of alcohol **202** was low and the cyclobutene product **145** had been photoreduced, the results were deemed somewhat promising as they showed that the photoproduct **145** could be formed, and it was merely necessary to stop the side reactions. It was postulated that if the reduction of the double bond was photochemically mediated and therefore occurred whilst the carbonyl was in place, then this could be avoided by merely removing the carbonyl by a more rapid means. Alternate trapping conditions for the vinylogous ester-alkyne system **144** would be identified from explorations with the vinylogous ester-alkene analogue **142**.

Alternative trapping methodologies for vinylogous ester 142

Having determined that hydride reducing agents successfully prevented secondary photoreactions in the photocycloaddition of a range of vinylogous esters, by reacting selectively with ketones, it was considered what other reagents could be employed in a similar manner. The [2+2] photocycloaddition of the initial vinylogous ester model **142** was chosen to test potential alternative trapping agents.

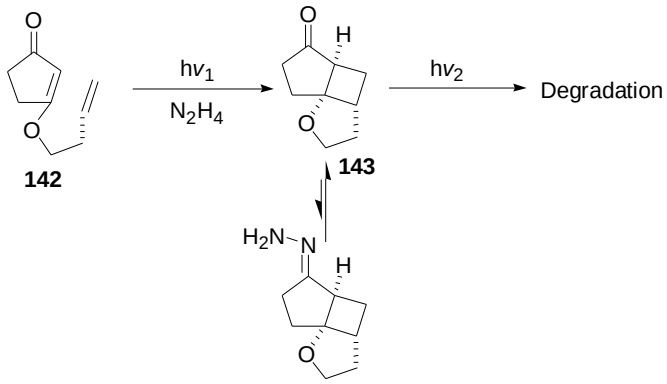
To determine the reagents that would react with ketone **143** in the course of the model photochemical reaction, it was decided to carry out simple trapping reactions directly with the isolated ketone **143**, without irradiation. In order to do this, larger quantities of ketone **143** were required than could be provided by the direct [2+2] photocycloaddition, therefore alcohol **202**, now available in increased amounts due to the newly modified conditions, was oxidised with Dess Martin periodinane to ketone **143** in good yield, **Scheme 82**.



Scheme 82

Small scale experiments were carried out in deuterated acetonitrile and analysed by ^{1}H NMR to identify different species that might 'trap' the carbonyl in ketone **143**. The screens were carried out by adding just 1.2 equivalents of the potential trapping agent to ketone **143** in deuterated acetonitrile and emergence of the new product monitored by ^{1}H NMR, **Table 9**. In the event that the trapping event was slow, ten equivalents of the additives were added to the ketone **143** and, again, the reactions were monitored by ^{1}H NMR. In line with the intentions set out in the aims, ketone **143** was reacted with a range of nitrogen nucleophiles, sodium bisulfite and sodium metabisulfite and **Table 9** summarises the best conversion achieved for each reagent. Attempts to react ketone **143** with hydrazine[231] and pyrrolidine[228] trapped around 20% of the ketone after six hours, entries 1 and 2, **Table 9**. This was not deemed a suitable timeframe for such a small amount of trapping. Additionally, prolonged exposure to ten equivalents of pyrrolidine led to overall degradation. When ten equivalents of hydroxylamine[230] were utilised,

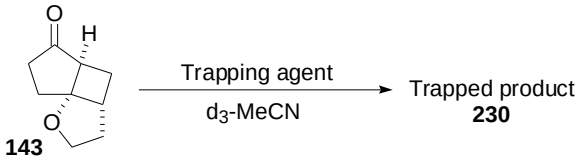
after six hours there was just 30% conversion to a new product, which, again, was not deemed a suitable conversion for trapping methodologies in photochemistry, entry 3, **Table 9**. These results were not deemed useful as such a significant proportion of the untrapped ketone **143** in the equilibria of the trapping event would eventually lead to its photochemical degradation during irradiation, **Scheme 83**. Acid was not added to these trapping reactions to enhance the proportion of trapping because the vinylogous ester **142** was not stable to acidic conditions, and it was postulated that acid would favour De Mayo fragmentation of ketone **143**.



Scheme 83

When morpholine[247] was used in ten equivalents, entry 4, **Table 9**, in just thirty minutes there was almost complete conversion to a new product. Sodium bisulfite and metabisulfite were also utilised to ascertain if they would complex well to the strained ketone.[233] Gratifyingly, just 1.2 equivalents of sodium metabisulfite formed a new product as 87% of the reaction mixture in thirty minutes, and just 1.2 equivalents of sodium bisulfite appeared to form just one product in thirty minutes, entries 5 and 6, **Table 9**.

Table 9: Screen of trapping agents for ketone 143

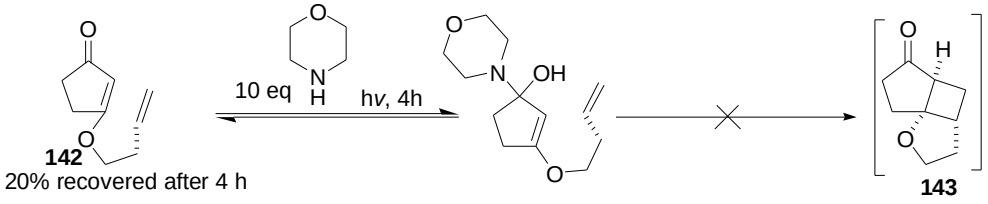


Entry	Trapping agent (eq)	Time	Ratio of ketone 143:230[a]
1	Hydrazine (10)	6 h	80:20
2	Pyrrolidine (10)	6 h	80:20[b]
3	Hydroxylamine (10)	6 h	70:30
4	Morpholine (10)	30 min	1:10
5	Sodium bisulfite (1.2)	30 min	--:1
6	Sodium metabisulfite (1.2)	10 min	13:87

[a]Ratio determined from ^{1}H NMR. [b]Degradation of products witnessed after longer than six hours.

As there was no literature found on the reaction of morpholine or the bisulfite reagents with similar vinylogous esters, it was deemed that morpholine and both bisulfite reagents could be added to the photochemical reaction to determine if the trapping reaction would sufficiently 'protect' the ketone **143** from secondary photoreactions.

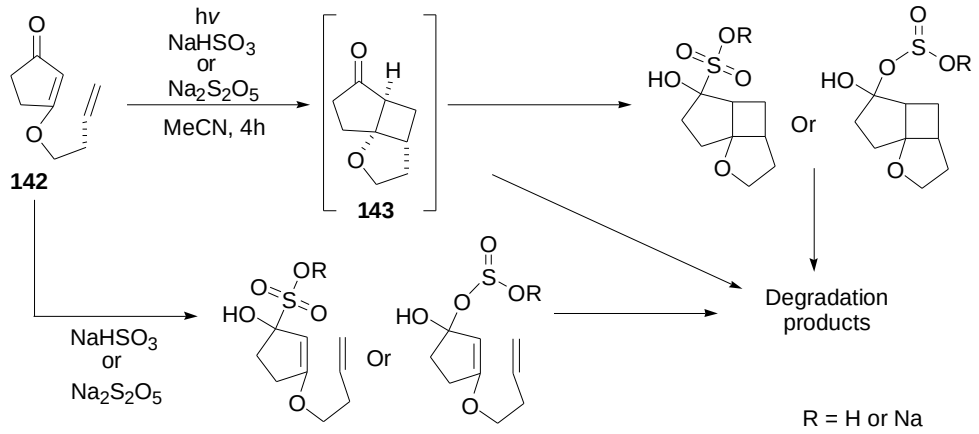
Morpholine was added to vinylogous ester **142** and the solution was irradiated until vinylogous ester **142** could not be seen by TLC. This took four hours, but unfortunately only recovered starting material **142** was isolated (20%). This indicated that the vinylogous ester **142**, rather than the ketone **143**, was being reversibly trapped by the morpholine and that the desired photochemical reaction was being prohibited, **Scheme 84**.



Scheme 84

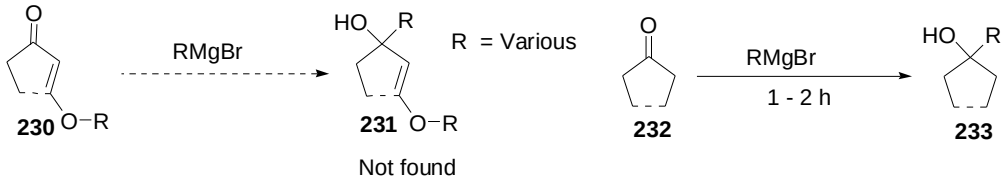
When either sodium bisulfite or metabisulfite were added to the reaction the result was the same for either reagent, in that, complete degradation to unidentifiable products occurred after two hours of irradiation, with 1.2 or ten equivalents. This could be due to

reaction of the compounds with the vinylogous ester **142** or the ketone **143**, followed by further degradation, or because degradation was just too rapid to be trapped by the reagents, **Scheme 85**.



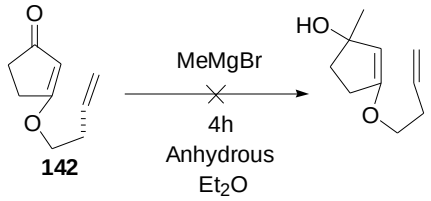
Scheme 85

Morpholine and the bisulfite reagents had all been identified as potentially useful trapping agents from screens undertaken on the isolated ketone **143**. An alternative approach was to use reagents that were known to react with ketones, but would be analysed for poor reactivity with vinylogous ester **142**. For example it is well-known that Grignard reagents react with ketones.[248] Specifically, whilst the reaction of a Grignard reagent with vinylogous esters **230** to alcohol**s 231** was not found, **Scheme 86**, the addition of a Grignard to a range of ketones **232** was reported as rapid (1 – 2 h), affording a variety of alcohols **233**.[249]



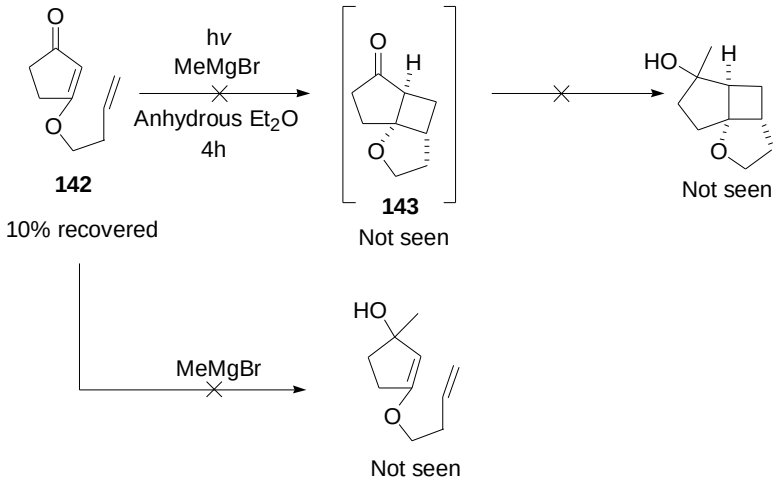
Scheme 86

Therefore, vinylogous ester **142** was exposed to methyl magnesium bromide for four hours, **Scheme 87**. TLC analysis showed no reaction of vinylogous ester **142** after this time.



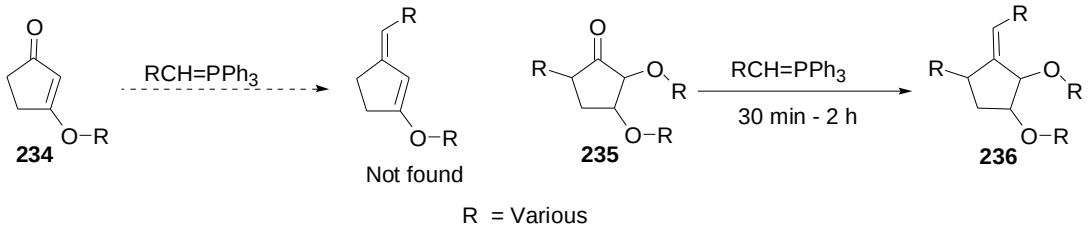
Scheme 87

This indicated that in the maximum observed timescale for the [2+2] photocycloaddition, *i.e.* two hours, the Grignard reagent would not react with vinylogous ester **142**. As Grignard reagents were known as reactive towards ketones, the trapping reaction was carried out without testing the methyl magnesium bromide with ketone **143**. Owing to the use of the moisture sensitive Grignard reagent, the photochemical reaction was carried out in a non-ideal solvent for photochemistry, namely, anhydrous diethyl ether. When the [2+2] photocycloaddition was carried out in the presence of the Grignard reagent, 10% vinylogous ester **142** was recovered, indicating that, although the Grignard was poorly reactive towards the starting material, the photochemical transformation to ketone **143** itself was not optimal in this solvent system, **Scheme 88**.



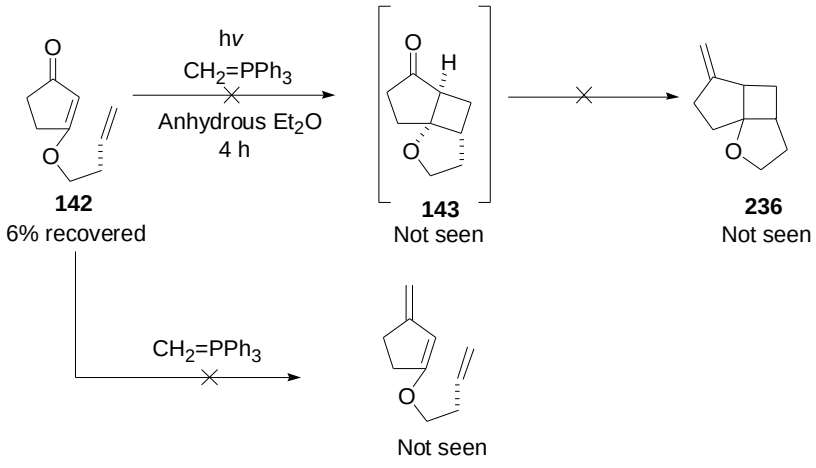
Scheme 88

Attention was turned to a different reaction that showed selectivity between vinylogous esters and ketones. There was sparse literature on the Wittig reaction of vinylogous esters **234**, but there was evidence that the reaction of phosphorus ylides with cyclic ketones **235** should take place in between thirty minutes and two hours to afford alkenes **236**,[250] **Scheme 89**.



Scheme 89

This was taken to suggest that phosphorus ylides would not react with vinylogous ester **234** and could potentially trap out ketones **235** instead, during the [2+2] photocycloaddition. The methyl phosphorus ylide was chosen as the trapping agent to minimise any potential steric interaction that might retard the trapping reaction. Trialling the trapping agents with vinylogous ester **142** and ketone **143** had not proven successful so it was decided to test the phosphorus ylide directly in the photochemical reaction. One equivalent of the phosphorus ylide was added to the vinylogous ester **142** and the solution irradiated for four hours, **Scheme 90**. No trapped product **236** was isolated, **Scheme 90**, and the vinylogous ester **142** was recovered in 6%. This suggested that, as in the reaction with Grignard reagents, whilst the trapping agent had been poorly reactive towards the starting material **142**, the use of anhydrous ether as a solvent had adversely affected the desired photochemical reaction, **Scheme 90**.



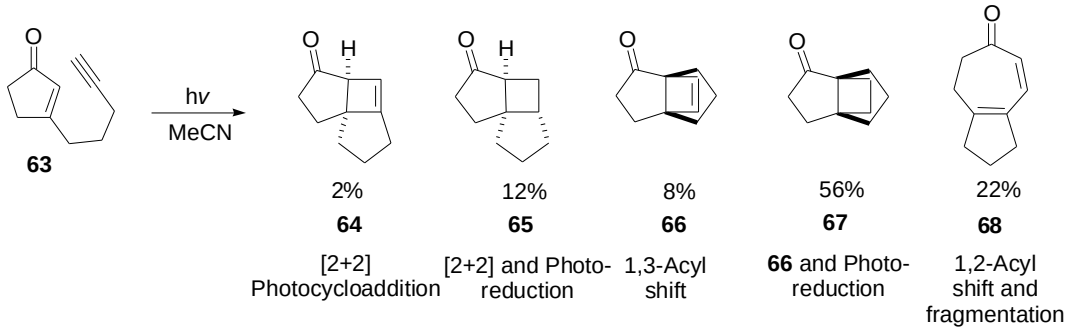
Scheme 90

The investigation into alternative trapping agents in the intramolecular [2+2] photocycloaddition of vinylogous esters was halted here as no reagents had been

successful in trapping out an isolable analogue of ketone **143**. Attention was instead turned to intramolecular systems that demonstrated the [2+2] photocycloaddition of enones with olefins. The intermolecular reaction of vinylogous esters in discussed in **2.i.ii.iv.**.

2.i.ii.iii. Intramolecular [2+2] photocycloadditions with enones

As discussed in **2.i.i.**, it was envisaged that the hydride reducing agents that had successfully trapped the ketone products from irradiation of alkenyl vinylogous esters could be used to trap out the ketone products from simpler enone systems. The [2+2] photocycloaddition of the carbon analogue **146** of the alkenyl vinylogous ester **142** was successful in the literature in 92%, see **Scheme 49**, p. 61, but the carbon analogue **63** of the alkynyl vinylogous ester **144** had quite a different photochemical profile. As previously described, investigations into the intramolecular [2+2] photocycloaddition between the enone of a cyclopentenone system and a terminal alkyne have been carried out by Mancini *et al.*,[47] **Scheme 91**.

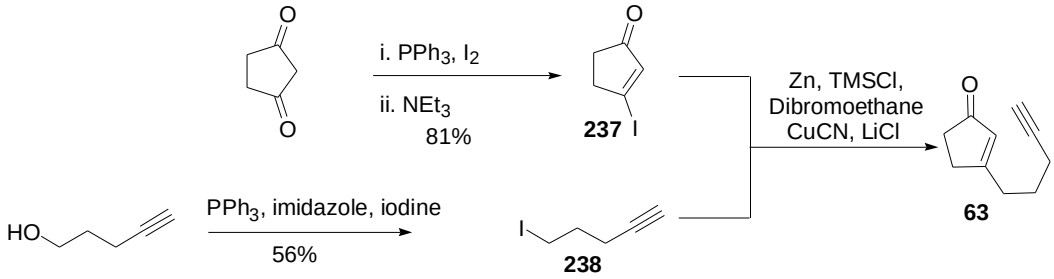


Scheme 91

A variety of products that all derive from the initial photoadduct **64** were identified. Therefore, it was postulated, that if the photoactivity of the ketone in the photoadduct **64** could be removed, then secondary photochemical reactions would also be halted, and the yield of cyclobutene **64** enhanced. The scaffold differed from the alkynyl vinylogous ester model **144**, **Scheme 48** p. 60, in that it had no heteroatom in the pendant group. It was envisaged that this reaction could be carried out with the reducing conditions, demonstrating the power of the reducing conditions by increasing the yield of a known reactions that had previously suffered from problems with secondary photoreactivity. However, this substrate raised a new challenge as there was expected to be a lower level of chemoselectivity between α,β-enone, **63**, and β,γ-enone **64**. Trapping

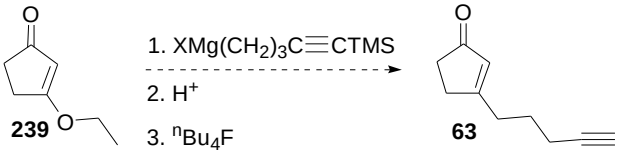
trials of this reaction were to be carried out with the optimized trapping agent, namely lithium borohydride, from studies with the vinylogous ester **142**.

The synthesis of enone **63** was reported by Mancini,[47] **Scheme 92**. The iodo-pentyne **237** provided isolation problems due to its volatility, but was prepared in 60% yield, and the cyclopentenone **238** was afforded in 85%, **Scheme 92**.[251] The procedure for synthesizing the enone **63** from these two primary products was complex[47] and, whilst repeated attempts were made, the synthesis of enone **63** *via* an activated zinc and copper synthesis was not reproducible.



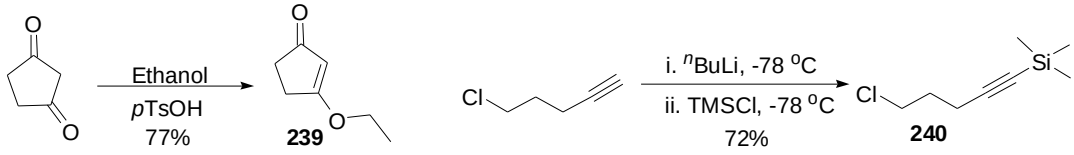
Scheme 92

An alternative route to enone **63** was investigated,[252] whereby the C-C bond was formed *via* a Grignard reaction on vinylogous ester **239** with a TMS protected alkyne, followed by hydrolysis and deprotection, **Scheme 93**.



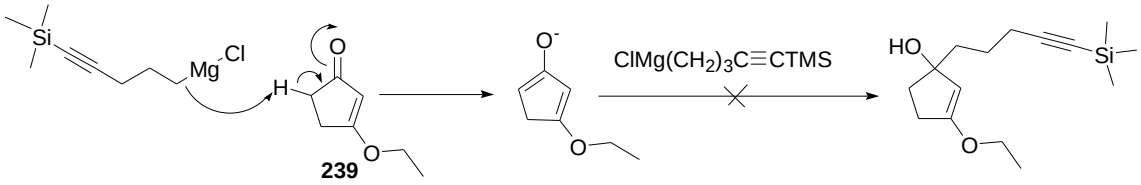
Scheme 93

To this end the enone **239** and silylated alkyne **240** were synthesized in good yields from commercial substrates, **Scheme 94**.



Scheme 94

However, reaction of the Grignard reagent with vinylogous ester **239** was unsuccessful. In similar systems, this had been attributed to the Grignard reagent merely acting as a base and forming the enolate of the carbonyl, inhibiting the desired reaction,[46] **Scheme 95**.

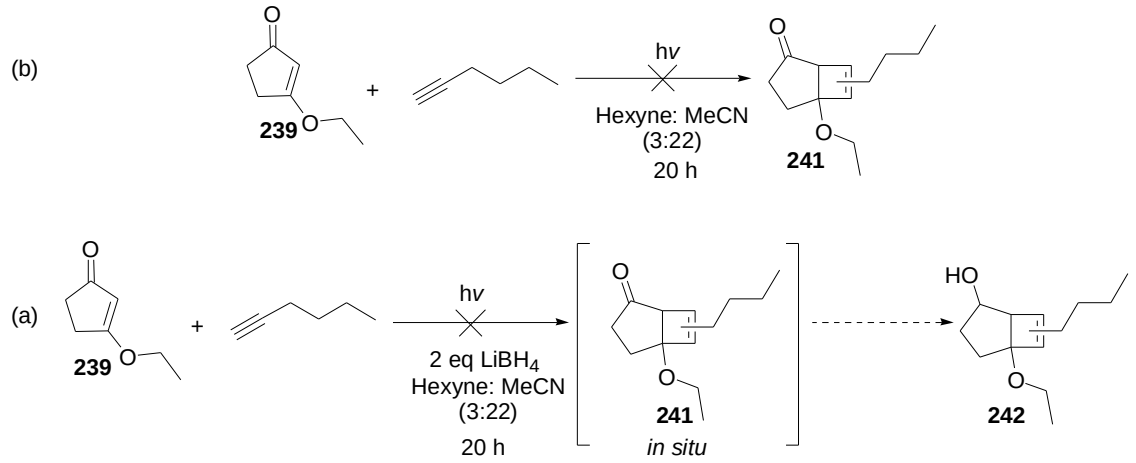


Scheme 95

There were many other desired systems with which to utilise the hydride-based methodology, thus investigations into the synthesis of the troublesome alkynyl enones was terminated here, in order to investigate other systems. Investigations into the intermolecular reactions of enones will be discussed in **2.i.ii.v.**.

2.i.ii.iv. Intermolecular [2+2] photocycloadditions with vinylogous esters

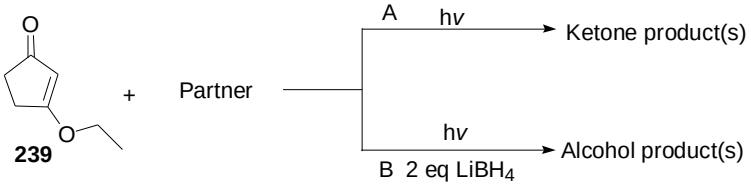
The work thus far had focused on intramolecular [2+2] photocycloadditions, due to their proposed speed compared to the intermolecular counterparts. However, the intermolecular reaction would also provide access to numerous useful products. Attention was thus turned to the intermolecular photochemical reaction of vinylogous ester **239** with alkynes, with the γ-tropolones as an ultimate goal, **Scheme 16** p. 28. Vinylogous ester **239** was irradiated in a solution of hex-1-yne:MeCN (3:22) and no isolable products were afforded after extended irradiation to complete consumption of vinylogous ester **239** (twenty hours), **Scheme 96 (a)**. This was promising for the methodology as it was envisaged that adding a reducing agent could trap out the reactive 1,4-unsaturated product **241** as alcohol **242**, **Scheme 96 (b**). Unfortunately, irradiation of vinylogous ester **239** in a solution of hex-1-yne:MeCN (3:22) with two equivalents of lithium borohydride led to extended reaction times (twenty hours) and no isolable products were afforded, **Scheme 96 (a)**. At this juncture, the use of acetylene was suggested, as an unhindered alkyne, but due to its highly flammable and potentially explosive nature, it was decided to validate the intermolecular methodology with other substrates prior to its use.



Scheme 96

To determine if the slow reaction was particular to alkynes, vinylogous ester **239** was irradiated with hex-1-ene, cyclopentene and cyclohexene, **Table 10**. The reactions were lengthy and no identifiable material was isolated either without or with the addition of a reducing agent to the photochemical reaction, entries 1-3, **Table 10**.

Table 10: Screen of partners for intermolecular [2+2] photocycloaddition of vinylogous ester 239, without and with $LiBH_4$



Entry	Partner	Time	Result of route A	Result of route B
1	Hex-1-ene	19 h	No identifiable product	No identifiable product
2	Cyclopentene	12 h	No identifiable product	No identifiable product
3	Cyclohexene	25 h	No identifiable product	No identifiable product

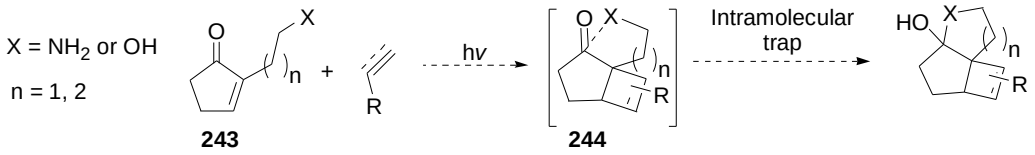
All irradiations were carried out at 0.02 M in 22 mL MeCN with 3 mL of partner as a co-solvent until complete consumption of vinylogous ester **239** was observed.

The lack of identifiable material indicated that the lithium borohydride *in situ* trapping was limited to the intramolecular reaction of vinylogous ester substrates and that, for the intermolecular variation, lithium borohydride could not trap out the ketone products. This was potentially due to the fact that over such extended times the vinylogous ester substrates were being reduced and thus the [2+2] photocycloaddition was not occurring, although no compounds to corroborate this were isolated. Alternatively, the degradative

secondary photoreactions could just be faster than the borohydride reduction. With no identifiable products identified from these reactions, this called a halt to investigations into intermolecular reactions with vinylogous esters.

2.i.ii.v. Intermolecular [2+2] photocycloaddition with enones

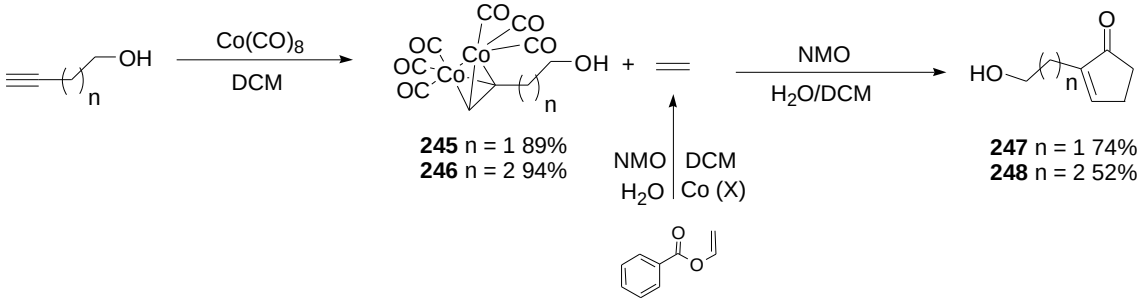
As they are very powerful reactions, intermolecular [2+2] photocycloadditions of enones with suitable partners were also transformations that merited further investigation. Alongside the use of reducing agents, it was envisaged that the trapping event would be favoured if it was intramolecular in nature. Substrates were therefore chosen that contained an hydroxyl in such a position that it would (speculatively) be prevented from 'closing' onto the carbonyl when in an sp^2 configuration **243** in the starting material, but was free to react with the carbonyl in the product as an sp^3 tether to form a five- or six-membered ring **244**, **Scheme 97**.



243 **244**

Scheme 97

Synthesis of suitable enone substrates **247** and **248** was undertaken *via* Pauson-Khand methodology, with vinyl benzoate acting as an ethylene equivalent from an unknown mechanism involving water, *N*-methylmorpholine *N*-oxide (NMO) and a low oxidation state cobalt species,[253] **Scheme 98**.



245 n = 1 89%
246 n = 2 94%

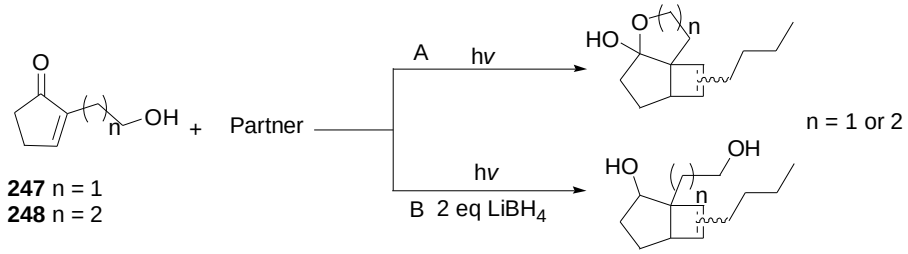
247 n = 1 74%
248 n = 2 52%

Scheme 98

Whilst the synthesis of compounds **247** and **248** was successful, none of the attempts to utilise these species in photochemistry afforded any isolable material, **Table 11**. The

enone was dissolved in a solution of the reaction partner:MeCN (3:22) and irradiated until complete consumption of the enone was witnessed by TLC. Each reaction was carried out without and with two equivalents of lithium borohydride for comparison to the earlier hydride-based reducing agent conditions. Irrespective of which enone was used, whether the reaction was with alkenes or alkynes, or whether a reducing agent was used or not, a complex mixture of unidentifiable products was observed in every case from analysis of the crude ^{1}H NMR and after column chromatography of the reaction residue, entries 1 – 4, **Table 11**.

Table 11: Screen of partners for intramolecular trapping with enones 247 and 248, without and with $LiBH_4$



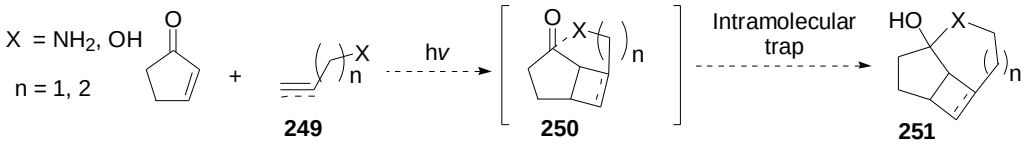
247 n = 1
248 n = 2

Entry	n	Partner	Time	Result of route A	Result of route B
1	1	Hex-1-yne	1.75 h	No identifiable product	No identifiable product
2	2	Hex-1-yne	1 h	No identifiable product	No identifiable product
3	1	Hex-1-ene	2 h	No identifiable product	No identifiable product
4	2	Hex-1-ene	1.5 h	No identifiable product	No identifiable product

All irradiations were carried out at 0.02 M in 22 mL MeCN with 3 mL of partner as a co-solvent until complete consumption of enone **247** or **248** was observed.

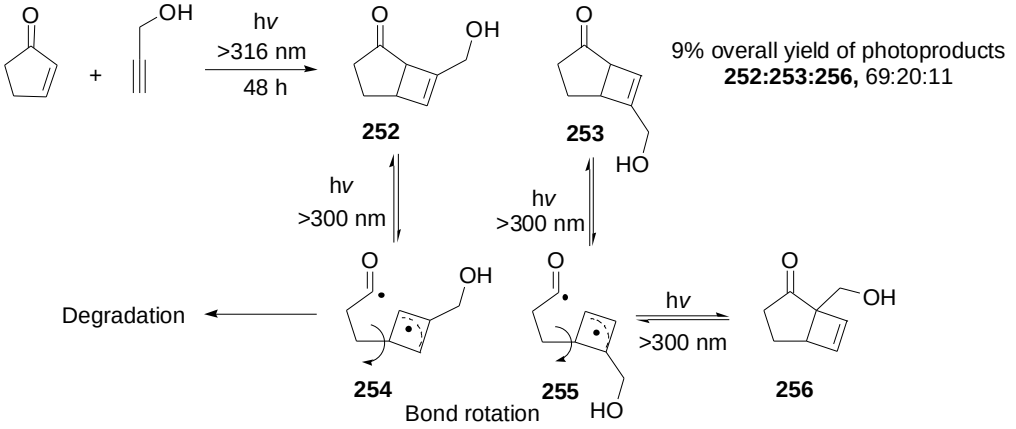
These results indicated that incorporating a nucleophilic 'trap' on the enone was not a successful route to trapping out photoproducts from these types of reactions. In addition, these results further supported the previous deduction that lithium borohydride *in situ* trapping was limited to intramolecular vinylogous ester substrates.

It was also suggested that the trapping nucleophile could be incorporated on the reaction partner **249**, rather than on the enone. After irradiation, the nucleophile would be in close proximity to the carbonyl in product **250**, potentially trapping it as a stable adduct **251**, **Scheme 99**.



Scheme 99

As discussed, it was known that the reaction of cyclopentenone and allyl alcohol afforded products that were prone to secondary photoreactions,[72, 73] **Scheme 15**, p. 27. There was also literature precedent that the photoproducts **252** and **253** from the reaction of cyclopentenone with propargyl alcohol would also undergo secondary photoreactions,[70] **Scheme 100**. In the literature examples, 1,4-unsaturated ketones **252** and **253** did not undergo any sort of intramolecular trap from the free hydroxyl, merely undergoing a variety of Norrish I cleavages to diradicals **254** and **255.** The diradicals **254** and **255** could then degrade or, in the case of **255,** rearrange to form ketone **256**, **Scheme 100**. Allyl and propargyl alcohol had therefore already been shown as unable to stop secondary photoreactions by any intramolecular trapping and, hence, were not utilised in trapping studies.

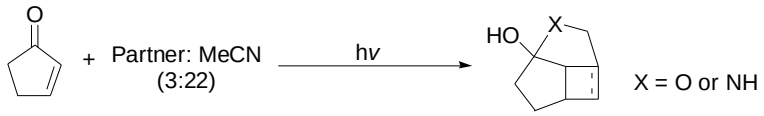


Scheme 100

Therefore, instead of allyl and propargyl alcohol, the longer chain analogues, 3-buten-1-ol and 3-butyn-1-ol were chosen for investigation, alongside propargyl amine, to afford a nitrogen nucleophile. Cyclopentenone was dissolved in a solution of the reaction partner:acetonitrile (3:22) and irradiated until complete consumption of the enone was witnessed by TLC. The crude reaction mixture was analysed by ^{1}H NMR and then subjected to column chromatography. When cyclopentenone was irradiated with 3-buten-1-ol as a co-solvent no identifiable material was isolated from the crude reaction

mixture, entry 1, **Table 12**. The same result was observed when the analogous alkyne, 3-butyn-1-ol, was utilised as a reaction partner, affording no isolable products after one and a half hours of irradiation, entry 2, **Table 12**. When propargyl amine was utilised in these reactions, a complex mixture of unidentifiable products was afforded again, entry 3, **Table 12**.

Table 12: Screen of partners for intramolecular trapping with cyclopentenone

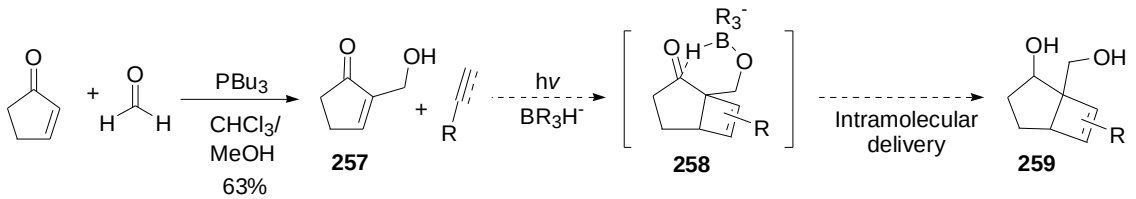


Entry	Partner	Time	Result
1	3-Buten-1-ol	1.75 h	No identifiable product
2	3-Butyn-1-ol	1.5 h	No identifiable product
3	Propargyl amine	12 h	No identifiable product

All irradiations were carried out at 0.02 M in 22 mL MeCN with 3 mL of partner as a co-solvent until complete consumption of cyclopentenone was observed.

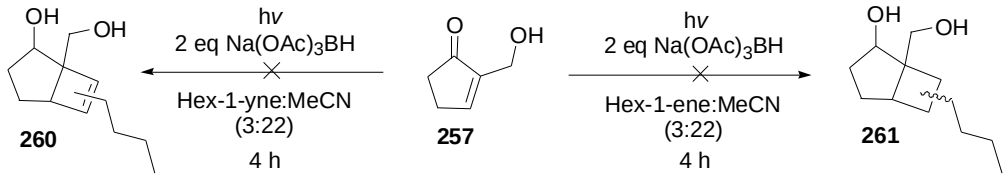
It appeared from these results that hydroxyl and amine nucleophiles could not trap the carbonyl, even if they were incorporated onto the reacting scaffolds.

Attention was now turned to the reaction of molecules that contained their own hydride "delivery system",[218, 254] *i.e.* enones were proposed that, through co-ordination, could deliver an external hydride to the carbonyl after a photocycloaddition had taken place and a new configuration formed. For example, it was hoped that enone **257**, afforded by a Morita-Bayliss Hillman reaction,[255] would be less able to convey a hydride to the carbonyl when in sp^2 configuration, **Scheme 101**. However, once the photocycloaddition had taken place to afford cyclobutane **258**, the new sp^3 tether might convey the reducing agent through a six-membered ring that is inhibited in the starting enone **257**, affording alcohol **259**.



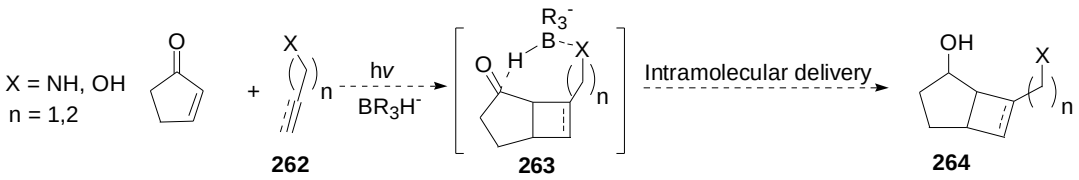
Scheme 101

Triacetoxyborohydride had been quoted as useful for such hydride 'delivery' and was utilised in these reactions.[218] However, disappointingly, when the enone **257** was irradiated in the presence of two equivalents of sodium triacetoxyborohydride and either 1-hexyne or 1-hexene as co-solvents, no isolable products **260** or **261** were produced, **Scheme 102**.



Scheme 102

Previous attempts at nucleophilic trapping of the carbonyl had been attempted from utilising the enone and the reaction partner as the trapping motifs, thus the hydride-delivery hypothesis was also tested from the reaction partner **262** as well as from the enone. It was envisaged that an hydroxyl or amine could deliver the hydride intramolecularly **263** after the cycloaddition had taken place, to afford alcohol **264**, **Scheme 103**.

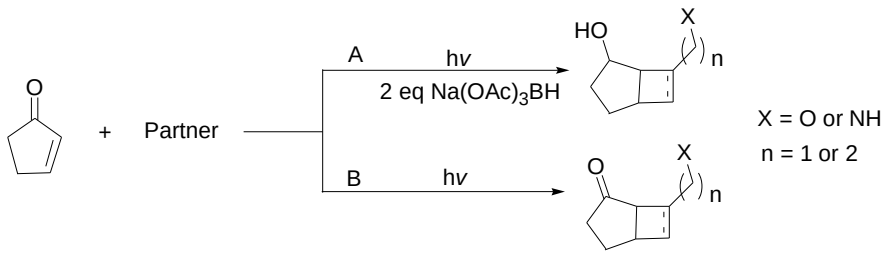


Scheme 103

Similarly to known literature reactions,[70, 72] but with the addition of the reducing agent, allyl and propargyl alcohol, as well as propargyl amine, were chosen to attempt to deliver the hydride from the reaction partner. Cyclopentenone was dissolved in a solution of the reaction partner:acetonitrile (3:22) with two equivalents of sodium triacetoxyborohydride and the solution irradiated until complete consumption of the enone was witnessed by TLC. The crude reaction mixture was analysed by ^{1}H NMR and then subjected to column chromatography. The reactions were also carried out without the reducing agent for comparison. Unfortunately, no isolable material was

afforded from any of the reactions, regardless of whether the reducing agent was used or not, **Table 13**.

Table 13: Screen of partners for hydride delivery with and without $Na(OAc)_3BH$



Entry	Partner	Time	Result of route A	Result of route B
1	Allyl alcohol	12 h	No identifiable product	No identifiable product
2	Propargyl alcohol	12 h	No identifiable product	No identifiable product
3	Propargyl amine	12 h	No identifiable product	No identifiable product
4	3-Buten-1-ol	1.5 h	No identifiable product	No identifiable product
5	3-Butyn-1-ol	1.5 h	No identifiable product	No identifiable product

All irradiations were carried out at 0.02 M in 22 mL MeCN with 3 mL of partner as a co-solvent until complete consumption of cyclopentenone was observed.

Whilst a number of substrates were utilised that were postulated to provide six-, seven- and eight-membered transition states in these studies, no identifiable products could be isolated either from nucleophilic or hydride-delivery methodologies. It appeared that the degradation pathways were simply faster than the postulated intramolecular nucleophilic and hydride-delivery trapping reactions. Even if this methodology had been successful, it would not have been generically useful to the synthetic chemist as it required the 'self-trapping' moieties to be designed into the scaffold and in most cases this would not be required or desired in the final motif.

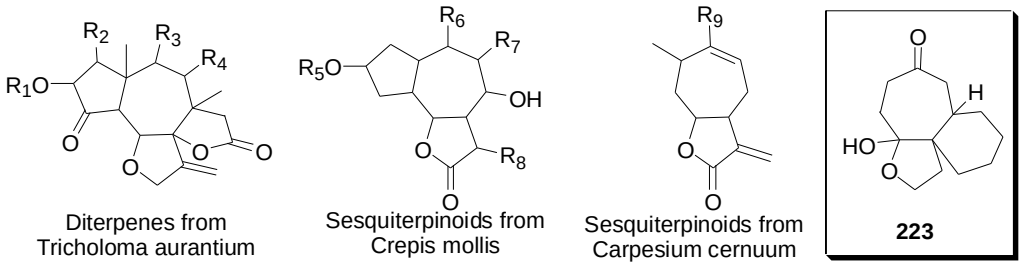
Thus, this avenue of research was terminated at this juncture. For further work, alternative trapping agents would represent a more significant result as new reagents, rather than modifications on the scaffold, are far more likely to be used by other chemists in the bid to make photochemistry more accessible.

It was interesting to note at this stage the large number of unsuccessful photocycloadditions that had been witnessed. This further suggested that secondary

photoreactions are indeed a widespread problem in photochemistry.

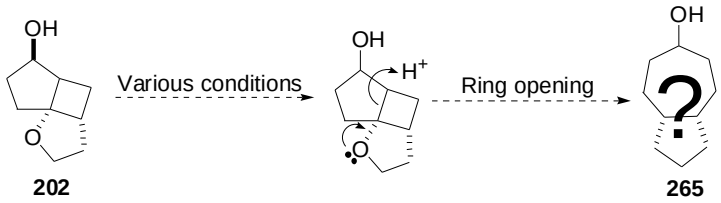
2.i.ii.vi. Ring fragmentation of photocycloaddition products

The successful hydride trapping reactions had provided a variety of polycyclic cyclobutanes, and it was envisaged that fragmentation of these strained structures would provide examples of the use of photocycloadditions as early steps towards complex structures. Therefore, fragmentation of the tricyclic ring systems to afford more ubiquitous structures for natural product chemistry was investigated. Attention was turned to the De Mayo fragmentation product **223** – isolated in the purification of the products of the [2+2] photocycloaddition of vinylogous ester **220**, **Scheme 78** p. 87. Similar 7,5 ring systems are present in a number of natural product cores, **Scheme 104**.[237-239]



Scheme 104

It was envisaged that the oxy-substituted strained cyclobutane ring **202** could be prone to ring-opening to afford similar 7,5-rings **265**, **Scheme 105**.

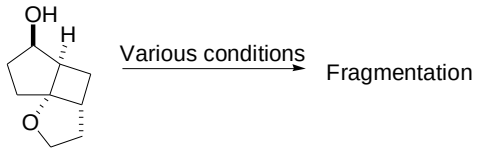


Scheme 105

Therefore alcohol **202** was subjected to a variety of conditions with the goal of forming new products, particularly the 7,5-ring systems **265**. Prolonged stirring of alcohol **202** in methanol led to no reaction, entry 1, **Table 14**. The same lack of reaction was witnessed when alcohol **202** was stirred with acid or base, even when the solution was heated to reflux for one week, entries 2 and 3, **Table 14**. Interestingly, although a proportion of

alcohol **202** was 'lost' upon column chromatography, stirring with silica and even adsorbing the alcohol **202** onto silica gel led to no reaction after one week, entries 4 and 5, **Table 14**.

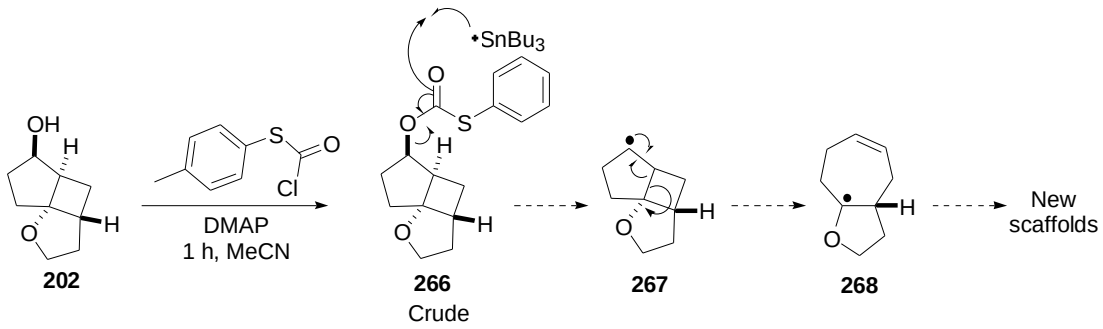
Table 14: Attempted fragmentation of alcohol 202



202

Entry	Conditions	Result
1	Stirred in methanol, RT	No reaction after 1 week
2	2 M HCl in methanol, RT – 70°C	No reaction after 1 week
3	5 eq NEt_3 in methanol, RT – 70°C	No reaction after 1 week
4	Stirred in DCM with silica, RT	No reaction after 1 week
5	Adsorbed onto silica, RT	No reaction after 1 week

At this stage it was decided to synthesise a substrate that could undergo a radical-mediated cleavage. To this end, thioloformate **266** was synthesised from the alcohol **202**,[256] **Scheme 106**. Unfortunately, whilst the thioloformate **266** was the major component of the crude reaction mixture, distinguished by ^{1}H NMR, it could not be purified as it degraded under aqueous work-ups and column chromatography. The intention was to submit this material to Barton McCombie conditions[257] to afford a radical **268** that could go on to fragment the 5,4,5-ring **267** to other scaffolds, *e.g.* **268**, **Scheme 106**.

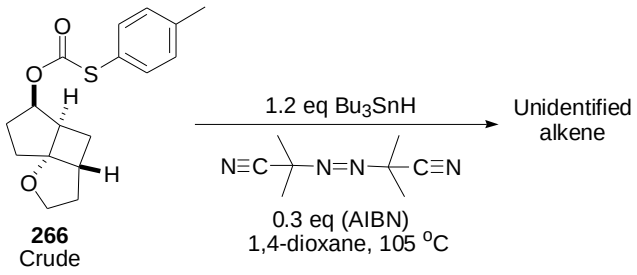


202 **266** Crude **267** **268**

Scheme 106

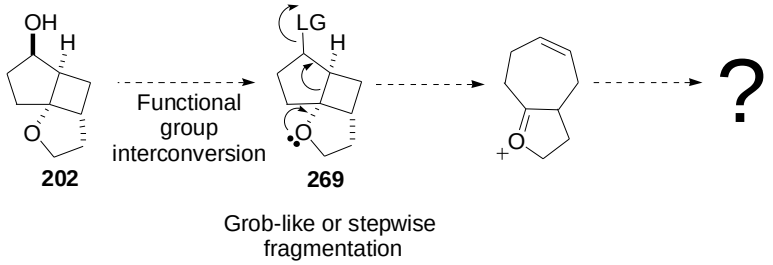
The crude thioloformate **266** was taken on without further purification and when treated

under Barton McCombie[257] conditions, complete consumption of the starting material was witnessed in five hours, **Scheme 107**. A trace amount (<1%) of an unidentifiable alkene was observed upon column chromatography of the reaction residue, **Scheme 107**, but the ring did not undergo fragmentation to identifiable products in good yields with this strategy.



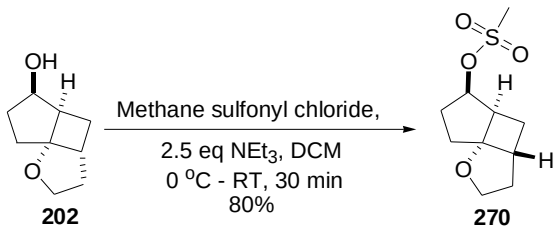
Scheme 107

It was postulated that the fragmentation would be more facile if the alcohol in **202** was converted to a good leaving group **269**, favouring a Grob-like[240] or step-wise fragmentation, **Scheme 108**.



Scheme 108

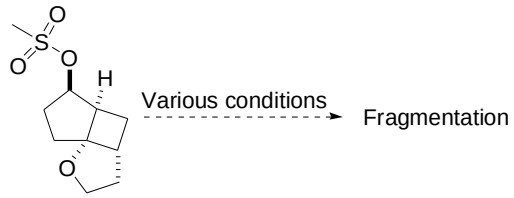
Mesylate **270** was proposed as a useful scaffold to provide a good leaving group for cleavages that could lead to new ring systems and mesylation[258] of alcohol **202** was successful in 80%, **Scheme 109**.



Scheme 109

The intention was to undertake fragmentation studies of the mesylate by subjecting it to a variety of conditions to determine if the ring would fragment. However, heating of the mesylate **270** afforded no new products after conventional heating to 85 ^{o}C for sixteen hours, entry 1, **Table 15**. Subjecting the mesylate **270** to microwave heating resulted in complete product degradation in two hours, entry 2, **Table 15**. Activation of the mesylate with Lewis acids such as silver triflate, boron trifluoride, aluminium chloride were all unsuccessful, entries 3-8, **Table 15**. Upon conventional heating, the mesylate **270** underwent no reaction but once subjected to microwave heating, the onset of degradation to a complex mixture of unidentifiable products was observed in ten minutes.

Table 15: Attempted fragmentation of mesylate 270



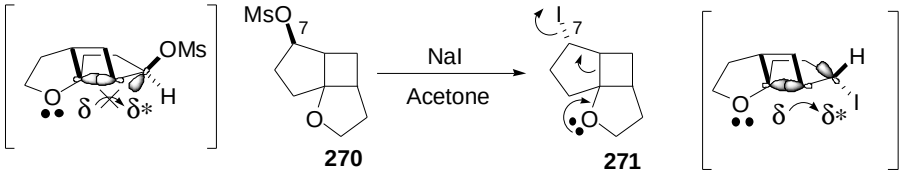
270

Entry	Conditions	Result
1	85 ^{o}C, 16 h[a]	No reaction
2	85 ^{o}C, 2 h[b]	Complete degradation
3	$AlCl_3$, 85 ^{o}C, 16 h[a]	No reaction
4	$AlCl_3$, 85 ^{o}C, 10 min[b]	Partial degradation
5	AgOTf, 85 ^{o}C, 16 h[a]	No reaction
6	AgOTf, 85 ^{o}C, 10 min[b]	Partial degradation
7	$BF_3.Et_2O$, 85 ^{o}C, 16 h[a]	No reaction
8	$BF_3.Et_2O$, 85 ^{o}C, 10 min[b]	Partial degradation

All reactions were carried out in acetonitrile. [a]Heated with a stirrer hotplate. [b]Heated *via* a microwave reactor.

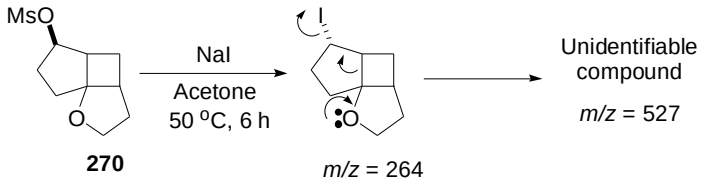
A potential problem with this fragmentation was that the lone pair of the oxygen possibly couldn't get into the correct alignment with the anti-bonding orbital of the C-C bond to initiate the desired fragmentation. This was a direct result of the orientation of the 5,4,5-tricycle itself and was not deemed 'solvable'. However, it was also suggested

that the stereochemistry at carbon centre 7 was problematic – it was postulated that the bonding orbitals of the C-C bond that was targeted to break could not get into the correct alignment with the anti-bonding orbital of the C-O bond, **Scheme 110**. To attempt to solve the latter problem, it was decided to carry out a Finkelstein-type reaction to afford the iodide **271** with concomitant inversion of stereochemistry in the hope that this would make subsequent reactions more facile, **Scheme 110**.



Scheme 110

The Finkelstein reaction was carried out and over the course of the conversion a new product was observed by TLC. Mass spectrometry distinguished a new compound formed in the crude reaction mixture as approximately double the mass of the expected iodide (527 *cf.* 264), **Scheme 111**. However, after column chromatography no identifiable compounds could be isolated.



Scheme 111

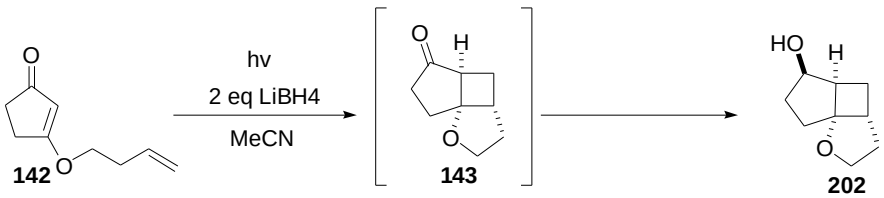
This suggested that under the conditions utilised the mesylate **270** was reactive, and, potentially, in some manner, a dimeric structure was forming. This reaction was repeated several times in an effort to afford isolable and identifiable material, but to no avail. Whilst the results were inconclusive, as no compounds could be fully characterised from this reaction, it did suggest that the ring was reactive and alternative conditions or nucleophiles may afford an isolable useful scaffold from a similar reaction.

It was concluded at this stage that the alcohol **202** and mesylate **270** were very stable compounds, but upon subjection of mesylate **270** to the Finkelstein conditions an

unknown reaction mechanism had afforded a product or products that could not be identified. Due to time constraints the fragmentation of the ring system was not further investigated but, in the future, alternative nucleophiles could be examined to ascertain if they could afford isolable products from the mesylate.

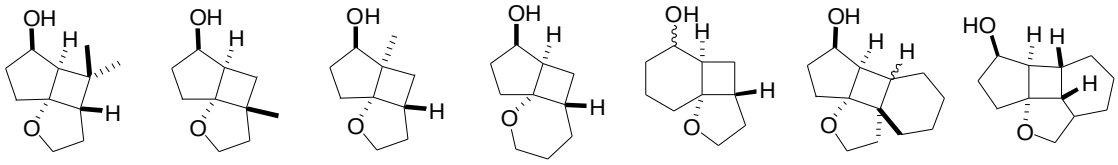
2.i.iii. Summary

Work to date has proven that inclusion of a hydride trapping agent in a photochemical reaction can prevent secondary photoreactions, providing previously inaccessible cyclobutanes, **Scheme 112**. This is the first example of the [2 + 2] photocycloaddition of these vinylogous esters and the first example of the use of reducing agents in photochemistry to minimise secondary photoreactions.[259]



Scheme 112

Overall yields for vinylogous esters tested showed a marked increase when the methodology was used, **Scheme 113**, and the alcoholic products can be oxidized back to the ketone in good yields, if desired, or implemented as the alcohol.



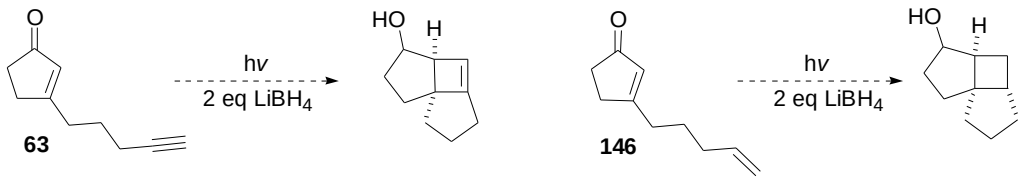
207, 78% (**206**, 0%) | **210**, 71% (**209**, 0%) | **214**, 72% (**212**, 50%) | **217**, 46% (**216**, 27%) | **218**, 59% (**151**, 28%) | **224**, 72% (**221**, 13%) | **229**, 27% (**228**, 0%)

Yield of corresponding ketone isolated in absence of reducing agent given in parentheses

Scheme 113

The fragmentation of these structures should be possible with further investigation towards complex scaffolds for natural products.

Whilst the hydride-based trapping agent solves the secondary photoreactivity problem for reactions where reduction of a ketone is required over a vinylogous ester in intramolecular reactions, it probably would not have the selectivity to trap out a ketone in preference to a simple enone. To fully determine selectivity of reagents, the carbon analogous compounds **63** and **146** should be synthesized and irradiated with two equivalents of $LiBH_4$ for comparison, **Scheme 114**.

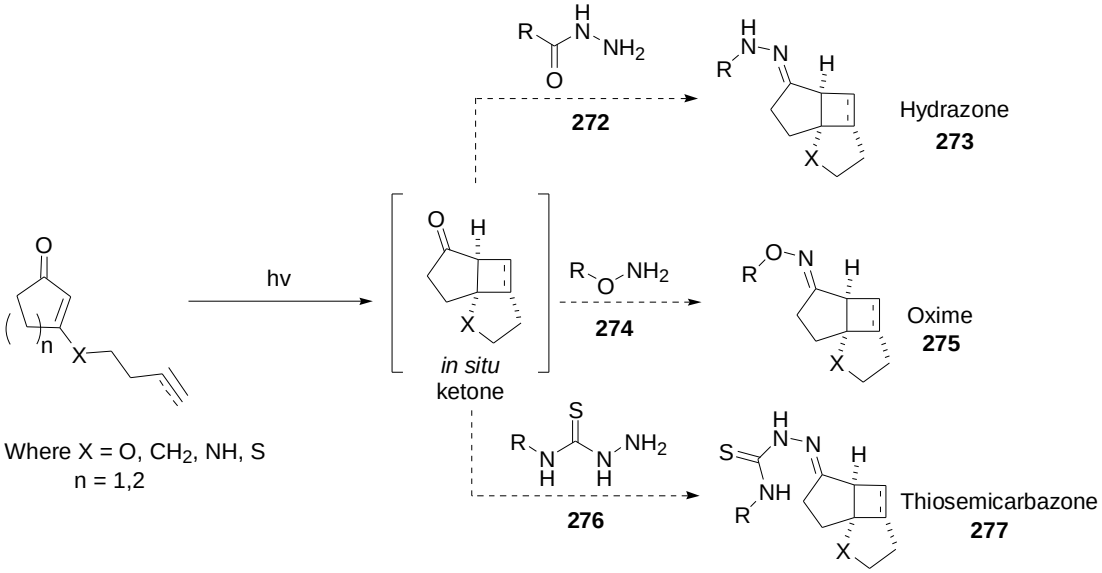


Scheme 114

The hydride reducing conditions did not successfully trap out any products from intermolecular [2+2] photocycloadditions of either vinylogous esters or simple enones.

The most worthwhile future goal of this project would be to develop a selective trapping agent that can be used in all enone-based photoreactions where the latent photoactivity of the carbonyl in the product prevents the reaction from widespread use.

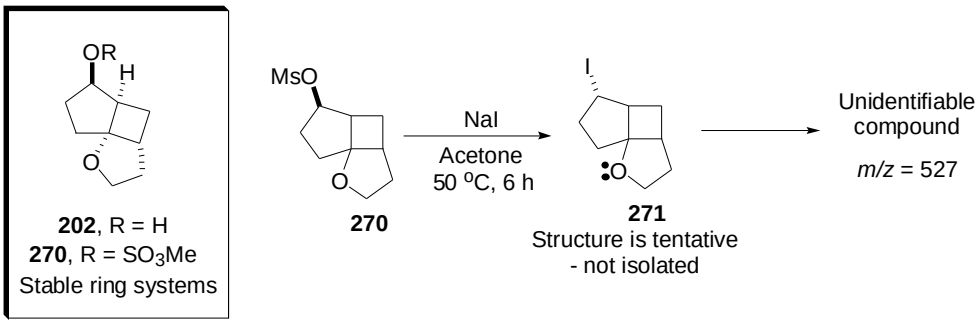
To this end, it is felt that following strategies from irreversible and ketone selective reactions may be useful. Ketones react with hydrazides **272**, aminoxy **274** and thiosemicarbazide compounds **276**[80] to afford stable conjugates as hydrazones **273**, oximes **275** and thiosemicarbazones **277**, respectively. Conversely, there is much less literature on the reaction of enones with similar reagents, unless the enone is incorporated into an aromatic system.[260-262] Thus, reagents such as these may prove to be a fruitful area of future investigation, **Scheme 115**. They were not implemented in this work due to time constraints.



Scheme 115

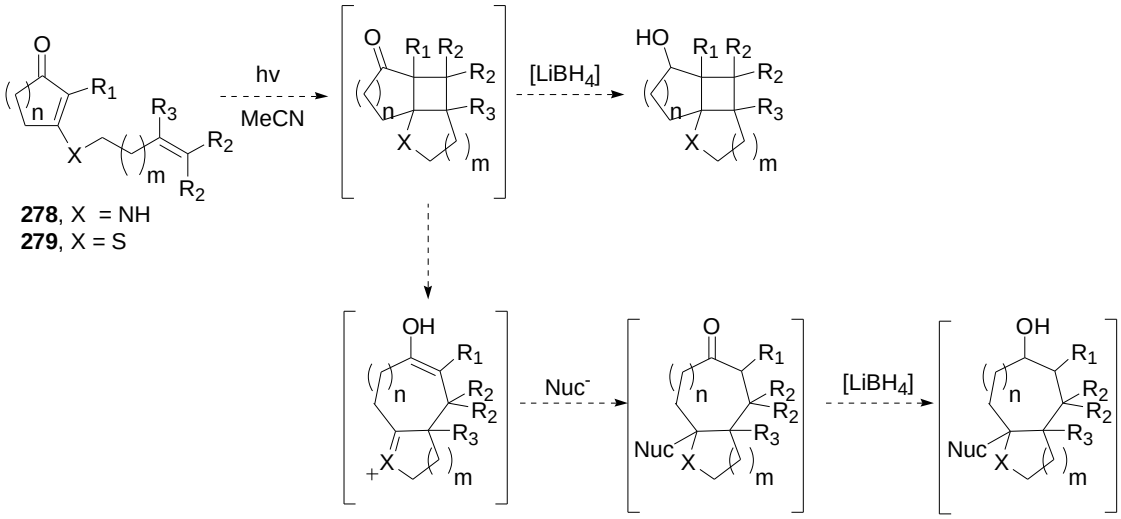
Ideally, future trapping agents would be commercially available or easily synthesized (one or two simple steps) and would be implemented in inter- and intramolecular reactions, for both cyclobutane and cyclobutene syntheses.

Initial studies into the fragmentation of the 5,4,5-ring systems afforded from the tandem photocycloaddition-reduction methodology were interesting. The results show that the strained 5,4,5-ring system is stable in alcohol **202** and as mesylate **270**, **Scheme 116**. However, once subjected to sodium iodide the mesylate **270** is reactive and can form complex products, **Scheme 116**. Unfortunately, the nature of the complex products could not be identified at this stage. Further investigation into the identity of the fragmentation product(s) from the 5,4,5-systems, and trial of other nucleophiles could lead to the formation of useful scaffolds for natural product synthesis.



Scheme 116

In efforts towards fragmentation, it is the opinion of the author that nitrogen **278** or sulfur analogues **279** of the vinylogous esters used in the trapping studies would be interesting explorations. The vinylogous amides **278** and vinylogous thioesters **279** would have a different reactivity towards reducing agents and the lone pairs may behave differently in fragmentation reactions, **Scheme 117**.



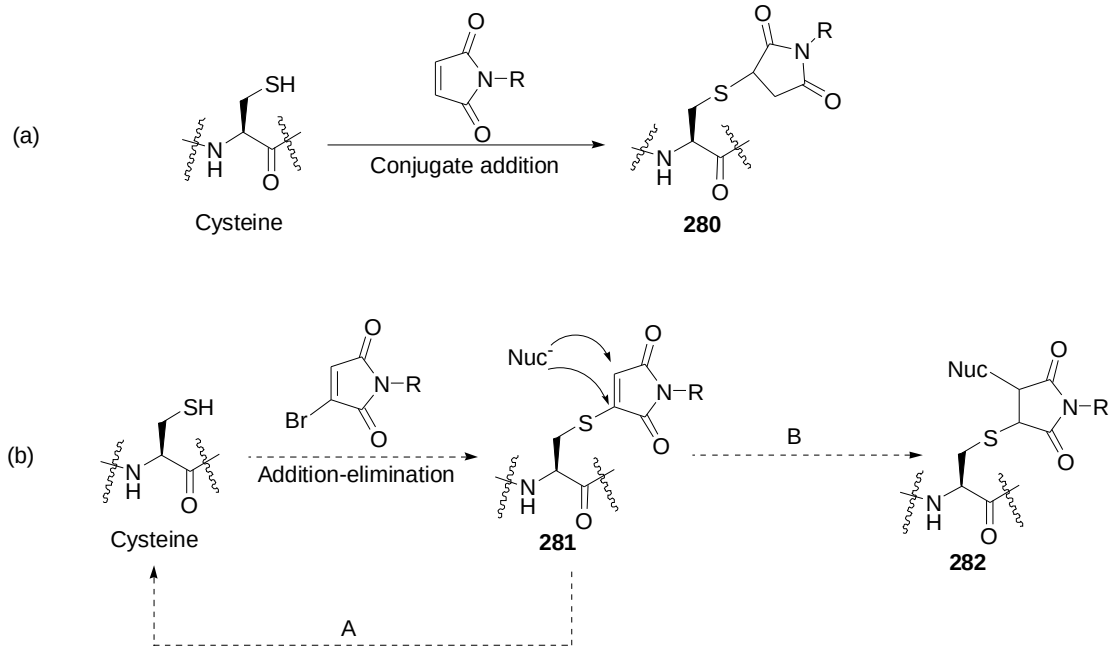
Scheme 117

Future areas for the development of this work would therefore include synthesis and irradiation of an intramolecular enone system to test the scope of the hydride-based reduction methodology; development of trapping strategies that are rapid and selective in all enone-based [2+2] photocycloadditions, with both alkenes and alkynes; directed fragmentation of the strained cyclobutane products of the successful [2+2] photocycloaddition towards natural products; and the synthesis and irradiation of vinylogous amides and thioesters under the new reducing agent conditions. Development of these avenues would further bolster the use of photochemistry as a truly powerful method in synthetic chemistry.

2.ii. Novel methods in cysteine modification

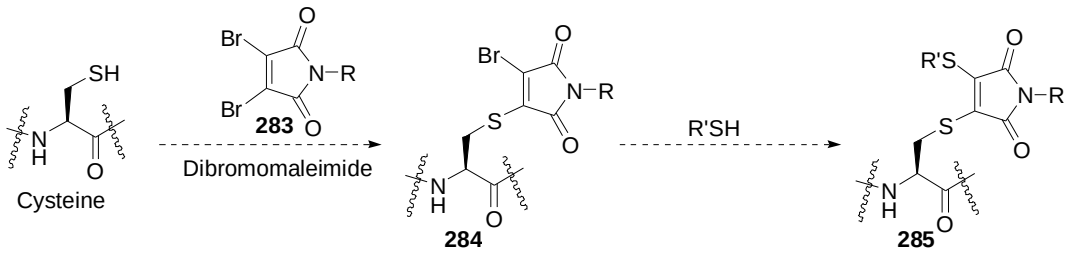
2.ii.i. Aims

As discussed, maleimides are one of the most widely used motifs for cysteine modification. Upon reaction with cysteine residues, maleimides form stable thiosuccinimide conjugates **280** in short time-periods, **Scheme 118 (a**). Molecules labelled *via* maleimide reagents can therefore not be regenerated in their native 'pre-labelled' state. The hypothesis of the work described in this text was based on the incorporation of a leaving group, namely a bromide, on the maleimide. This would enable reformation of the double bond upon an addition-elimination reaction with cysteine, **Scheme 118 (b**), forming thiomaleimides **281**, rather than thiosuccinimides **280**. It was envisaged that thiomaleimides **281** might be reactive towards nucleophiles, either to displace the cysteine (route A) or to conjoin three molecules *via* a second conjugate addition on the reformed double bond, to form succinimides **282** (route B), **Scheme 118 (b)**.



Scheme 118

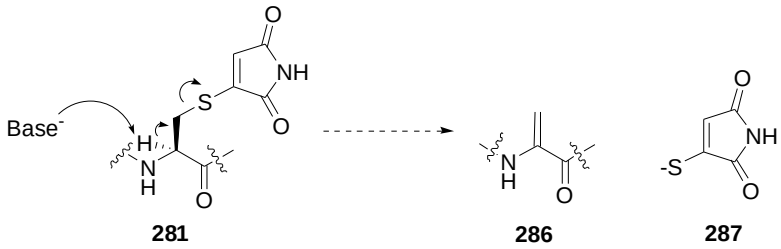
Alternatively, addition of cysteine residues to dibromomaleimides **283** would form bromothiomaleimides **284**, which, upon addition of a further thiol, would form dithiomaleimides **285**, **Scheme 119**.



Scheme 119

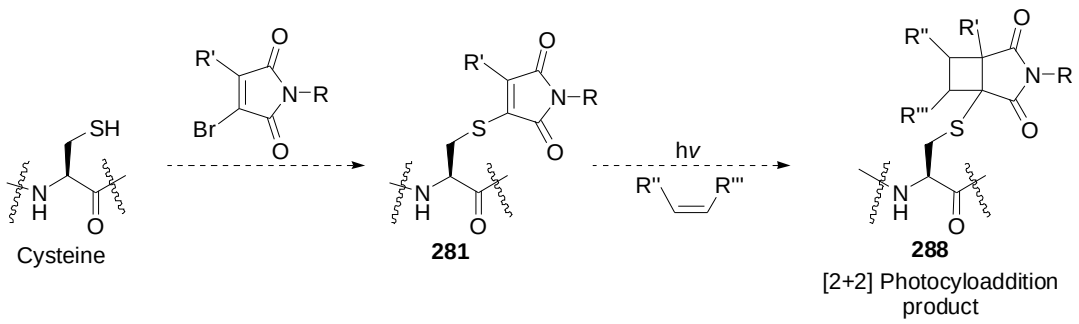
If successful, these reactions would represent a new approach to the modification of cysteine residues in proteins. If shown to be reversible, the modification would then be applicable as a rival to disulfide formation for temporary modification of cysteine residues.

It was also postulated that the thiomaleimides **281** formed might be prone to a base-mediated elimination to afford Dha motifs **286**, **Scheme 120**, such as those investigated by Davis *et al*..[114] It was envisaged that the thiolate **287** would be a good leaving group due to the electron withdrawing properties of the maleimide.



Scheme 120

Maleimides are excellent photochemical partners in a variety of photocycloadditions,[147, 196, 263] and have also been used as a photochemical tether for immobilisation strategies,[151] see p. 42. Thus, it was envisaged that thiomaleimides **281** might retain the photoactivity that is well-documented for maleimide[146-154] and that the motif could be exploited for photochemical conjugation of cysteine derivatives to afford cyclobutanes **288**, **Scheme 121**. This would potentially afford a novel mode of photochemical bioconjugation for peptides and proteins that have been modified to contain a thiomaleimide.

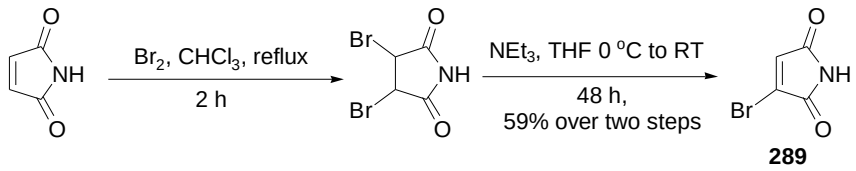


Scheme 121

It was envisaged that these reactions, if successful on small molecule models, could be transferred to polypeptides or proteins.

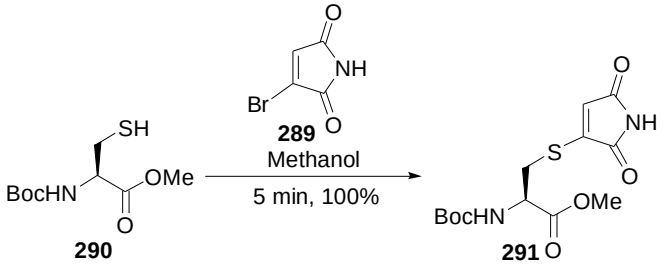
2.ii.ii. Results and discussion for novel methods in cysteine modification

In order to investigate the reaction of bromomaleimide with cysteine residues, bromomaleimide **289** was synthesised according to literature precedent, **Scheme 122**.[264]



Scheme 122

In accordance with publications on cysteine modifications[114, 204] a protected form of the amino acid cysteine **290** was used as a model to determine the work's utility for protein and peptide conjugation strategies. The addition of cysteine **290** to bromomaleimide **289** occurred quantitatively in five minutes in methanol, affording thiomaleimide **291**, **Scheme 123**.

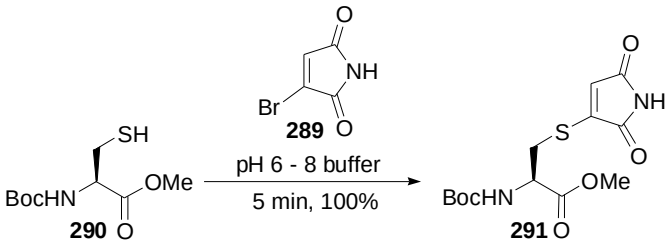


Scheme 123

It was discovered that when the reaction was concentrated from methanol some reversion was occasionally observed to regenerate bromomaleimide in up to 30%, presumably due to the HBr present. Henceforth, when the reaction was carried out in methanol, one equivalent of sodium acetate was added to quench the eliminated HBr and halt any reaction reversion.

To ascertain if the reaction could be applicable to protein chemistry, it was deemed necessary to carry it out in aqueous media. Aqueous buffers are utilised to maintain a constant pH during the course of a reaction and the buffers used in this work were 150 mM NaCl and 100 mM sodium phosphate. The addition of cysteine **290** to bromomaleimide **289** was successful in buffers from pH 6 – 8 to form thiomaleimide

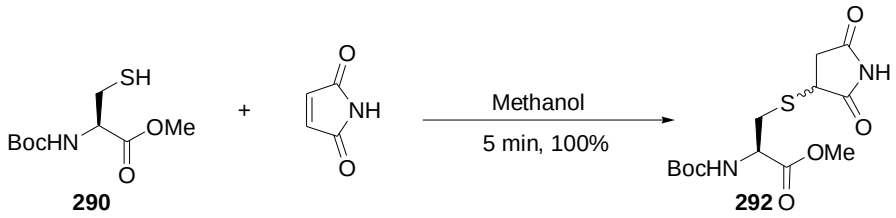
291 in five minutes in 100% yield, **Scheme 124**. This was deemed very promising as such a rapid reaction in aqueous solutions suggested that it could be transferred to protein chemistries with relative ease.



Scheme 124

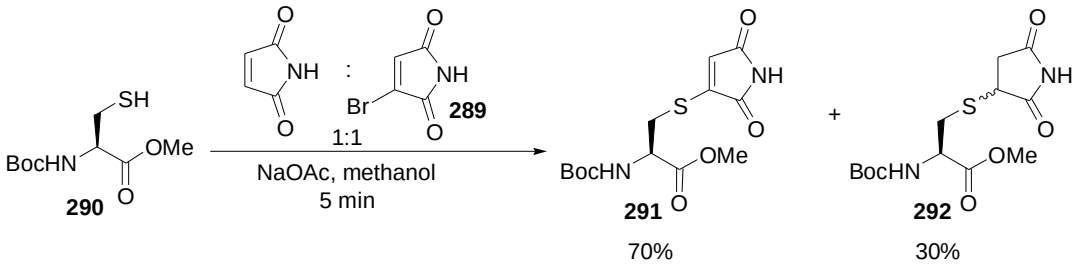
Polarimetry of the thiomaleimide **291** showed that the stereogenic α-carbon had not been racemised during the addition.

As discussed, maleimides are widely used as a method of cysteine modification. To enable comparison, maleimide was reacted with the same cysteine **290** to afford succinimide **292**, **Scheme 125**. This reaction was also complete in five minutes to afford a quantitative yield of diastereomeric thiosuccinimides **292**.



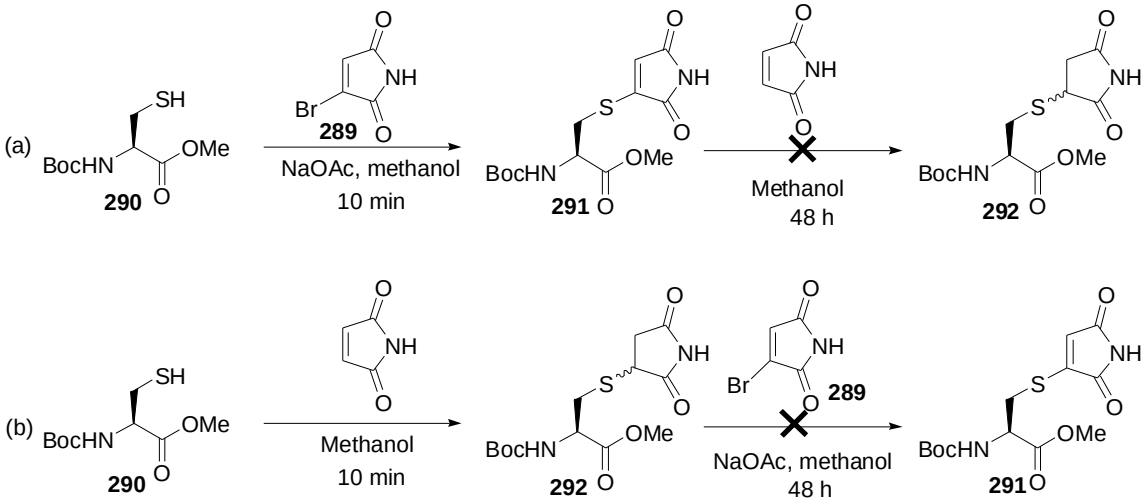
Scheme 125

As the reactions of maleimide and bromomaleimide **289** with the cysteine **290** were both complete in five minutes, a competition reaction was carried out to determine the relative reactivities of bromomaleimide **289** and maleimide with the cysteine **290**. Cysteine **290**, in the presence of sodium acetate in methanol, was treated with a 1:1 mixture of maleimide and bromomaleimide **289**, **Scheme 126**. The result was 7:3 in favour of the thiomaleimide **291** compared to thiosuccinimide **292**, **Scheme 126**. This suggested that bromomaleimide **289** reacted faster than maleimide with cysteine **290**.



Scheme 126

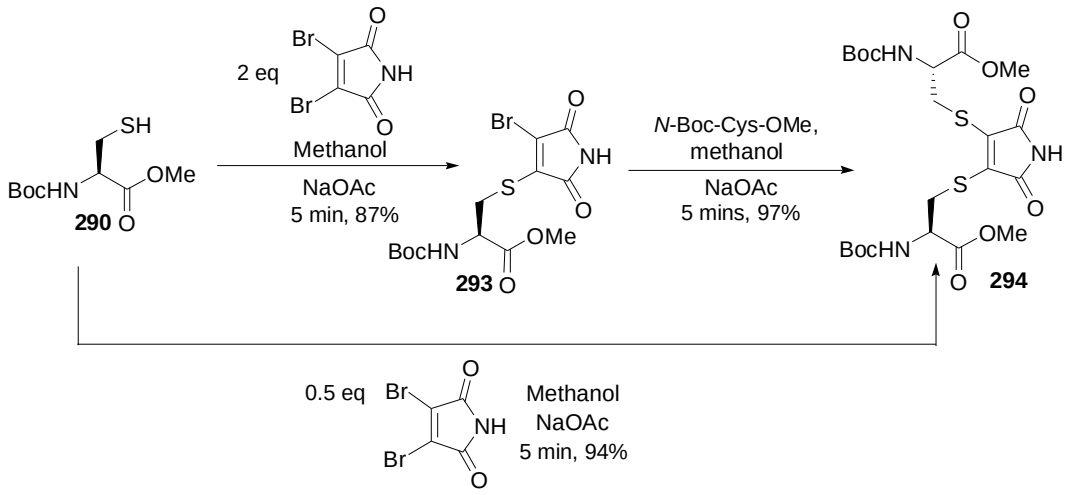
To determine that this was the kinetic and not the thermodynamic outcome of the reaction, cysteine **290** was reacted with bromomaleimide **289** and then treated with maleimide ten minutes later. The only product isolated from this reaction was thiomaleimide **291**, **Scheme 127 (a)**. In addition, the reaction of cysteine **290** with maleimide, followed by addition of bromomaleimide **289**, resulted in isolation of only thiosuccinimide **292**, **Scheme 127 (b**). This indicated that the products did not interconvert under the reaction conditions utilised, and that the previous result did indeed demonstrate that the cysteine **290** was more reactive towards bromomaleimide **289** than maleimide.



Scheme 127

Complementary to the work with bromomaleimide **289**, addition of cysteine **290** to two equivalents of commercially available dibromomaleimide afforded the single mixed bromothioconjugate **293** in 87% yield (with respect to the cysteine **290**), **Scheme 128**. Addition of cysteine **290** to the isolated bromothiomaleimide **293** afforded the dithiomaleimide **294** in 97%. The dithiomaleimide **294** could also be prepared in 94%

from simply adding two equivalents of the cysteine **290** to the dibromomaleimide, **Scheme 128**.



Scheme 128

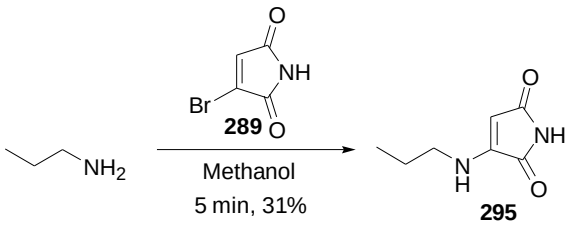
At this early stage it had been demonstrated that the reaction of bromomaleimide **289** with cysteine **290** was rapid, and that cysteine **290** was more reactive towards bromomaleimide **289** than maleimide. Dibromomaleimide could also be used to rapidly label one or two molecules of cysteine **290**.

With thiomaleimide adducts **291** and **294** in hand, there were several avenues that were of further interest for the use of such constructs; (i) determination of the selectivity of the bromomaleimide **289** addition to thiols; (ii) reactivity of the thiomaleimide **291** towards thiols; (iii) probing the reversibility of thiomaleimide **291** formation; (iv) manipulation of thiomaleimide **291** to provide dehydroalanine; (v) use of the strategy for the functionalisation of peptides; and (vi) exploring the potential photoactivity of the thiomaleimide adducts towards bioconjugation strategies.

2.ii.ii.i. Reaction selectivity

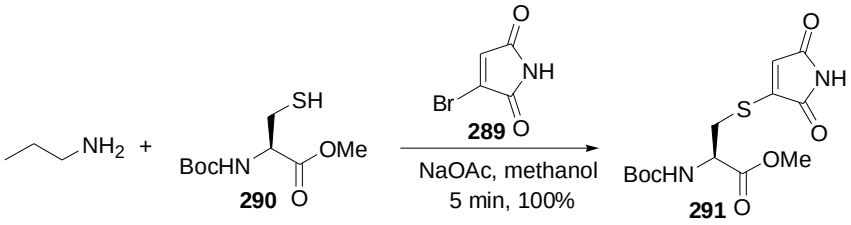
The impetus behind this work was to afford reactions that would be applicable to proteins, and thus would be successful in the presence of competing amino acids, particularly lysine.[86] In order to ascertain whether the bromomaleimide reagent **289** could react with amines, the bromomaleimide **289** was reacted with propylamine in

methanol, **Scheme 129**. The bromomaleimide **289** did react with the propylamine in around ten minutes to afford aminomaleimide **295** in a low yield.



Scheme 129

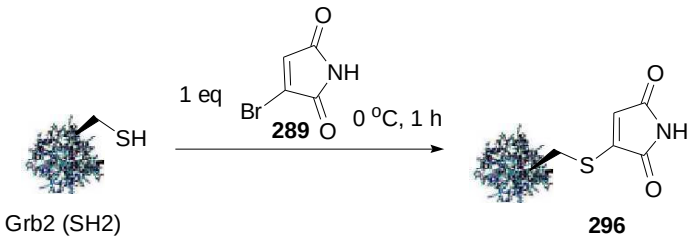
With the intention of determining if bromomaleimide **289** was selective for thiols over amines, and thus potentially cysteine over lysine, bromomaleimide **289** was added to a mixture of propylamine and the cysteine **290**, **Scheme 130**. Gratifyingly, the thiomaleimide adduct **291** was formed in 100% yield, implying that bromomaleimide **289** would be selective for cysteine.



Scheme 130

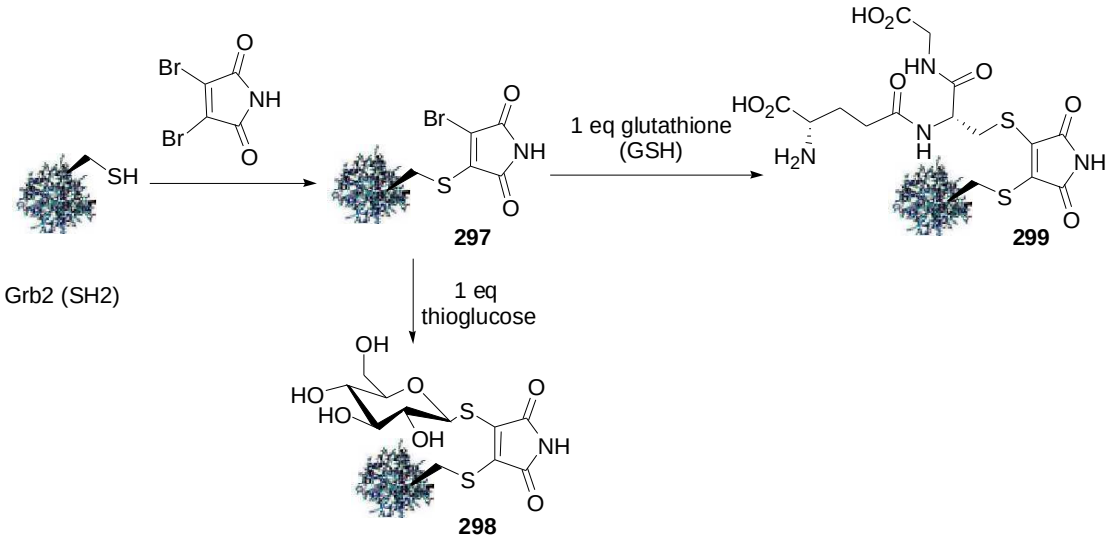
<u>Collaboration with other scientists</u>

At this stage in the work, a collaboration was undertaken between the Baker group (UCL, Chemistry) and the groups of Professors Caddick (UCL, Chemistry) and Waksman (Birkbeck, ISMB). As part of this collaboration Dr Mark Smith (UCL, Chemistry, Caddick Group) transferred the cysteine labelling to a single cysteine residue in a protein domain (Grb2, SH2 domain). Bromomaleimide **289** rapidly labelled the single cysteine residue, affording the protein labelled with a thiomaleimide **296**,[265] **Scheme 131**. The selectivity of the cysteine labelling in small molecule work was supported by this work as the protein domain used contained eight lysine residues, none of which were labelled by bromomaleimide.[265]



Scheme 131

It was also envisaged that dithiomaleimides such as those previously synthesised, **294** **Scheme 128**, p. 122, could be used as a mode of bioconjugation of two thiol-containing biomolecules. Therefore, as part of the work undertaken in the aforementioned collaboration, Dr Mark Smith (UCL, Chemistry, Caddick group) demonstrated that a protein (Grb2, SH2 domain) could be labelled with dibromomaleimide to afford a bromothiomaleimide labelled protein **297**, **Scheme 132**. This bromothiomaleimide protein **297** could be further conjugated to either thioglucose or glutathione *via* a dithiomaleimide **298** or **299** , **Scheme 132**.[265]

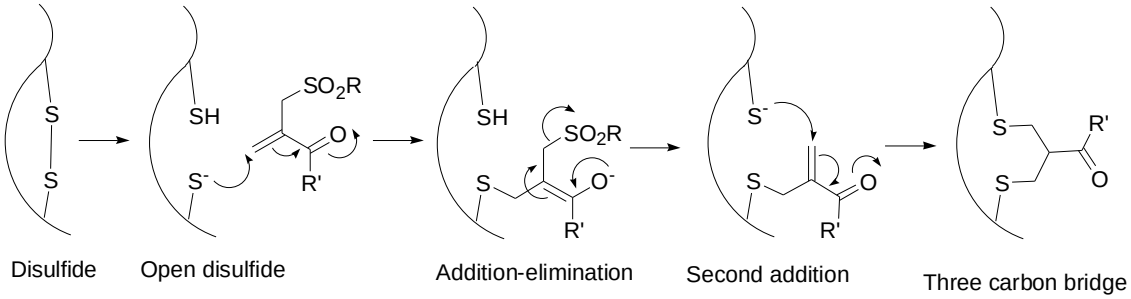


Scheme 132

The collaboration was ongoing throughout the work presented in this thesis, and when relevant, specific outcomes of the collaborators' work will be discussed in more detail.

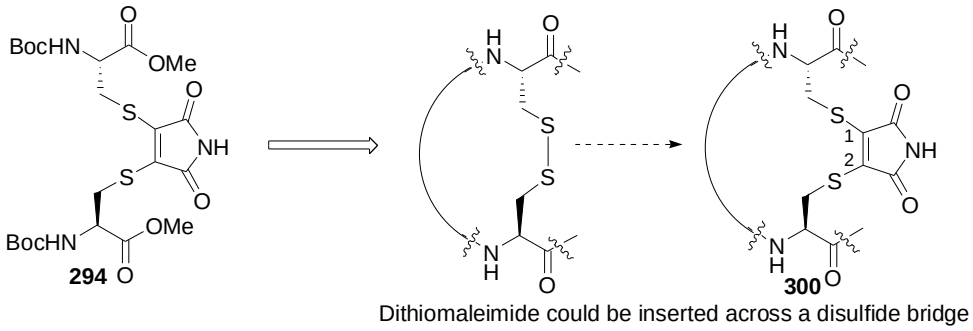
Work carried out by Brocchini *et al.*[266, 267] has site-specifically labelled a disulfide-containing protein with a three-carbon bridge. In their work it was highlighted that modifications of disulfides should be specific and the disulfide bridge maintained

relatively undisturbed in terms of distance. Whilst their methodology did form a relatively short bridge (three carbon atoms) across a disulfide, it also introduced a chiral centre, **Scheme 133**.



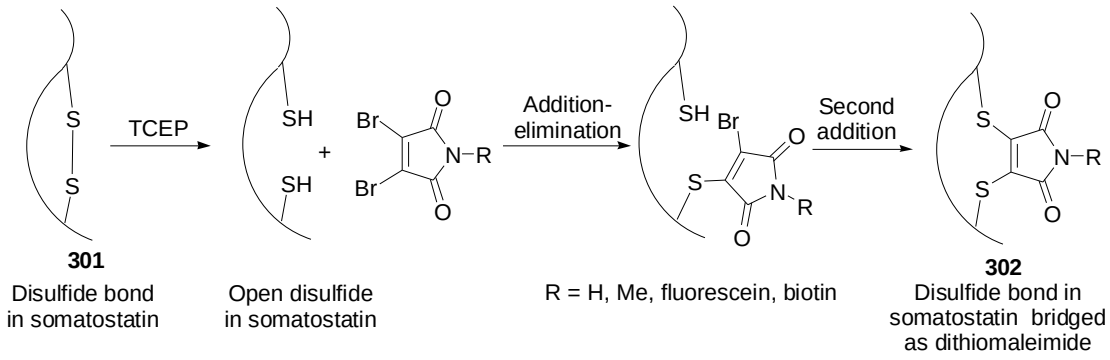
Scheme 133

In contrast, in the small molecule work described in this text, it was postulated that the dithiomaleimide **294** could be used to bridge a disulfide bond *via* just two achiral carbons **300**, **Scheme 134**.



Scheme 134

The extension of this work was carried out by Felix Schumacher (UCL, Chemistry) in the Baker research group. He successfully transferred the small molecule work to a polypeptide **301** (somatostatin), and showed that a variety of *N*-functionalised dibromomaleimides could be incorporated into its single disulfide bridge **302**, **Scheme 135**.[265]

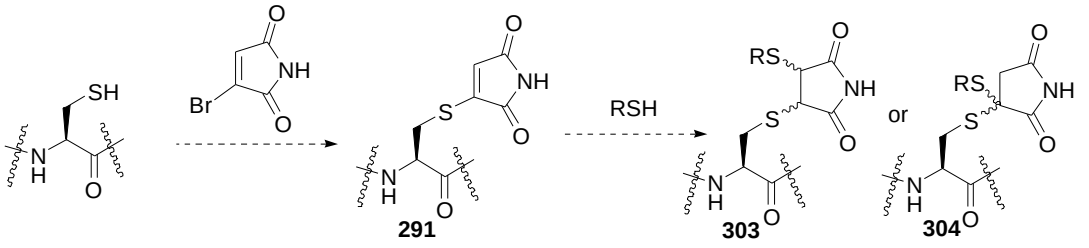


Scheme 135

These highly promising results suggested that the bromomaleimides could be employed in protein and peptide modification. It was the intention, at this stage, that this PhD project would retain focus on single amino acid studies and thus the early protein work was carried out by other scientists, as described over the course of this thesis.

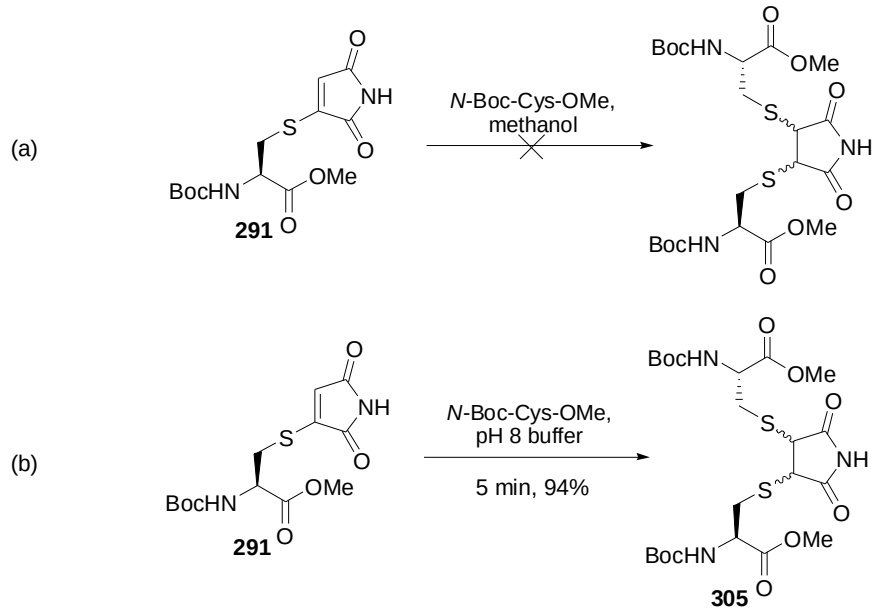
2.ii.ii.ii. Reactivity of the thiomaleimides towards thiols

If thiomaleimides **291** were reactive to thiols, then dithiosuccinimides **303** or **304** could be formed which would represent the conjugation of two thiols, **Scheme 136**.



Scheme 136

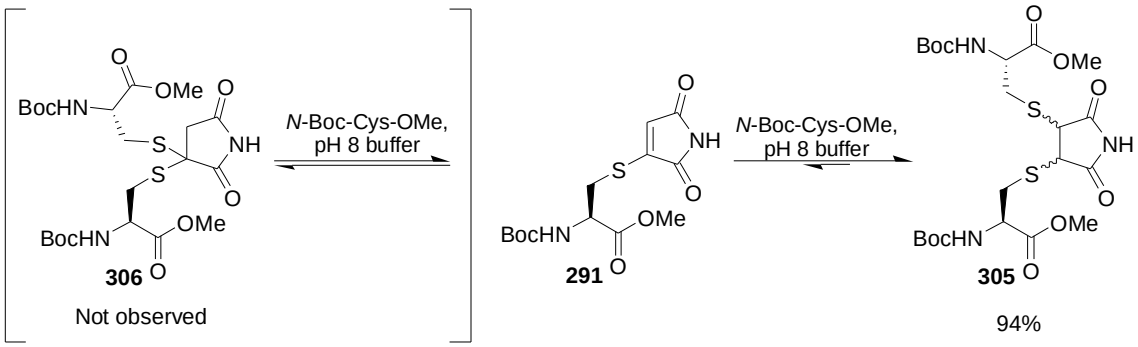
When the thiomaleimide adduct **291** was subjected to cysteine **290** in methanol, no addition was seen, **Scheme 137 (a)**. This suggested that the hypothesis that thiomaleimides could act as Michael acceptors, and undergo a second nucleophilic addition was incorrect. However, when the reaction was carried out in pH 8 aqueous sodium phosphate buffer, the addition of the thiol occurred rapidly, affording dithiosuccinimide **305**, **Scheme 137 (b)**.



Scheme 137

Literature suggests that thiol addition to maleimides occurs through the thiolate and not the sulfhydryl,[140] and that the thiolate would start to become a significant component of the solution at pH 8,[86, 268] explaining the increased rate in **Scheme 137 (b**). Notably, the cysteine **290** addition to bromomaleimide **289** had occurred rapidly in methanol and aqueous solutions from pH 6 - 8, suggesting that bromomaleimide **289** was more reactive than thiomaleimide **291** towards thiols.

The addition of the second thiol could potentially be geminal or vicinal but analysis of the ^{13}C NMR spectrum indicated that the isolated products were vicinal. Mechanistically, the geminal adduct **306** was not ruled out as an intermediate, but if it was formed, it must be a reversible process that leads to the vicinal adduct **305** as the thermodynamic product, **Scheme 138**.



Scheme 138

The addition of the second thiol formed two adjacent chiral centres. The two thiols could be *cis* or *trans* to one another. This afforded three possible product stereochemistries, as the two *cis* succinimides are identical, **Figure 17**.

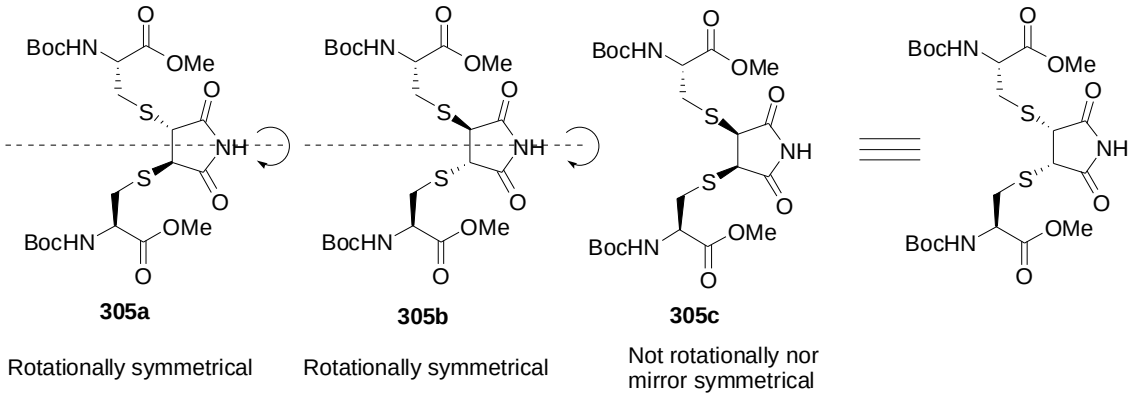


Figure 17: Possible vicinal addition succinimides

The ^{1}H NMR spectrum indicated that the two major products from this reaction were symmetrical, thus only **305a** and **305b** were possible (as rotationally symmetrical), as compound **305c** was neither symmetrical through rotation nor a mirror plane.

Very small peaks could be identified in the ^{1}H NMR spectrum alongside the peaks attributed to *trans* succinimides **305a** and **305b**, **Figure 18**. They were tentatively attributed to small amounts of the *cis* succinimide **305c**. It was postulated that the reaction was reversible and that, over time, the adducts had preferentially adopted the least hindered *trans* configurations, **305a** and **305b**.

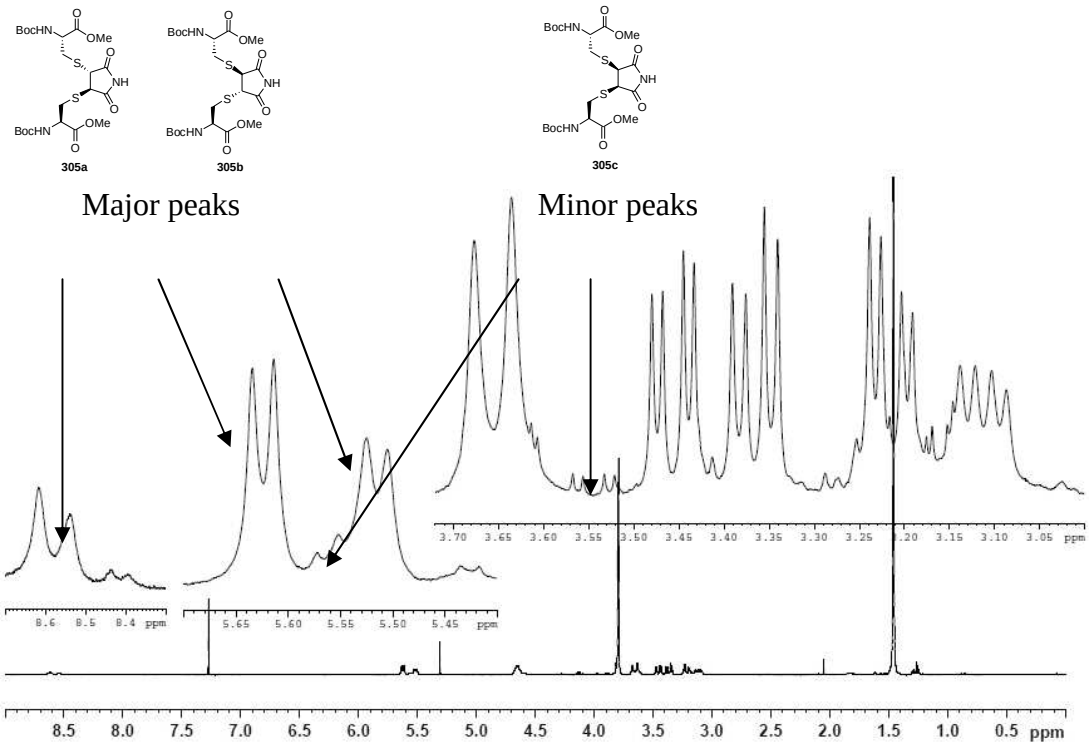
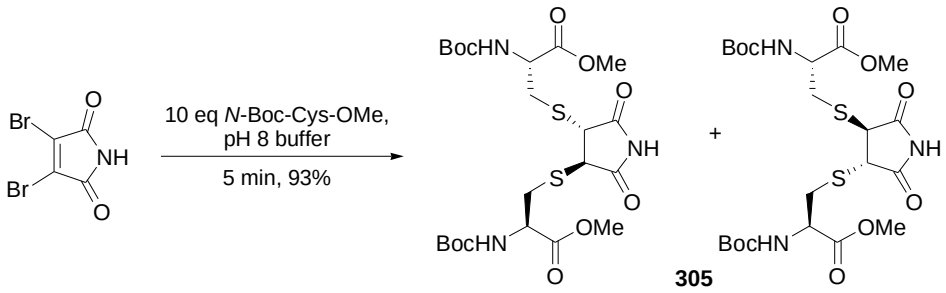


Figure 18: ^{1}H NMR spectrum of 305a and 305b

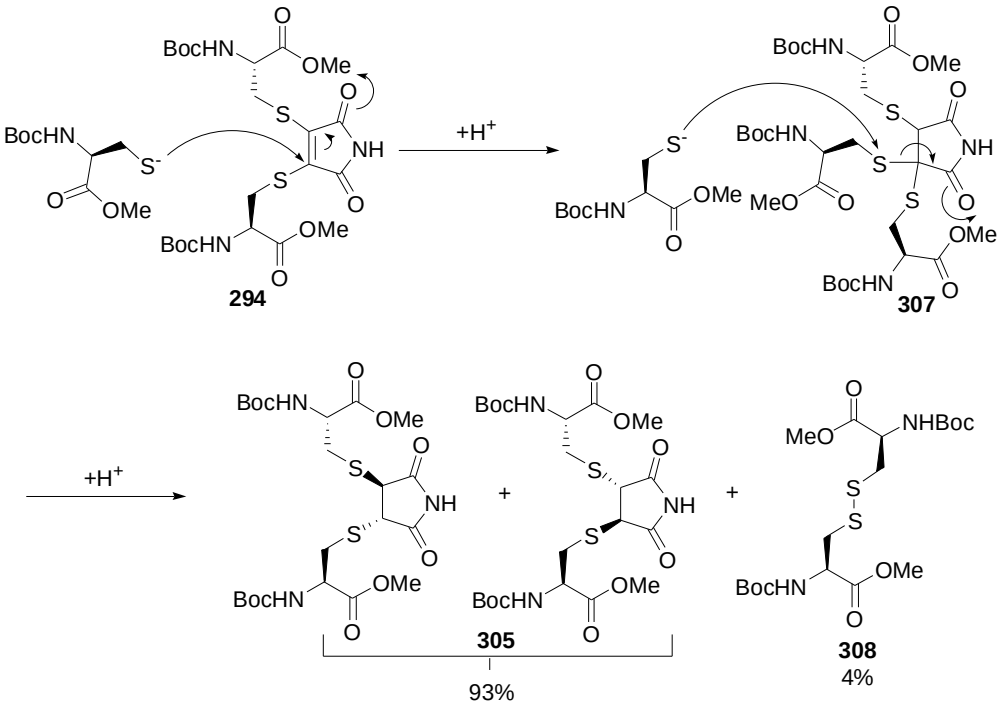
Interestingly, when dibromomaleimide was treated with a large excess of the cysteine **290**, *i.e.* ten equivalents or more, in basic buffer, the succinimide adducts **305** were isolated from the reaction in 93%, **Scheme 139**.



Scheme 139

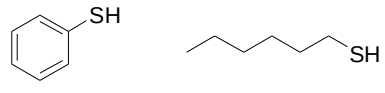
A postulated mechanism for this outcome was that a third addition of the cysteine took place to afford succinimide **307**. This succinimide **307** can then be attacked by latent thiolate to afford the dicysteine succinimide adduct **305** and cysteine disulfide **308**, **Scheme 140**. Thus overall, a reduction of the dithiomaleimide had taken place, and an oxidation of the cysteine. Indeed, the cysteine disulfide **308** was isolated in 4%, although this was considered inconclusive as the disulfide can form from free cysteine

over time. This indicated that in buffered solutions, like the thiomaleimide **291**, dithiomaleimide **294** was reactive towards thiols.



Scheme 140

It had previously been ascertained that a second cysteine thiol would add to the thiomaleimide **291** in pH 8 buffered solutions. Thus, to probe the reactivity of thiomaleimide **291** towards various other thiols, one and ten equivalents of several thiols were separately added to the adduct **291** in the pH 8 buffer. The simple thiols chosen were thiophenol and hexanethiol, **Figure 19**.

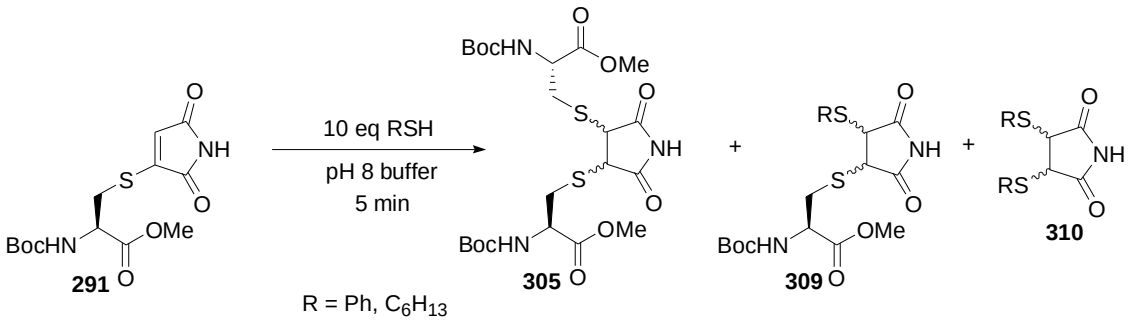


Thiophenol Hexanethiol

Figure 19: Thiols used to probe the reactivity of thiomaleimide 291

An apparent problem with these thiols was that they were slow to react with thiomaleimide **291** when just one equivalent was used. When ten equivalents were used, a complex mixture of products could be seen by thin layer chromatography. Column chromatography could not separate the products but ^{1}H NMR of the mixtures described

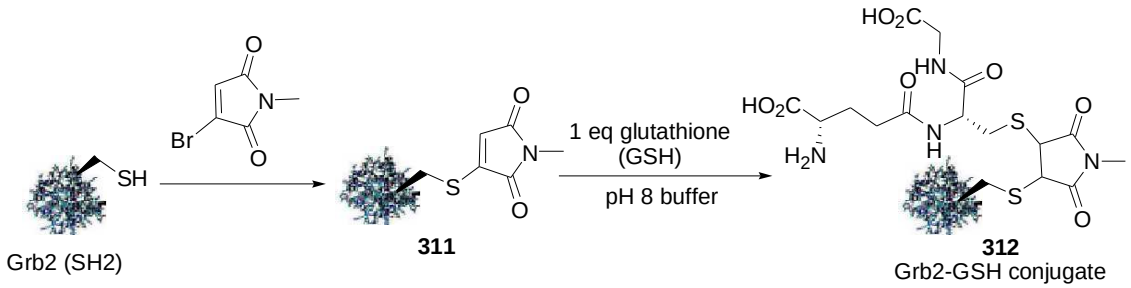
the reactions as containing inseparable, diastereomeric and vicinal addition products **305**, **309** and **310**, **Scheme 141**.



Scheme 141

As the dithiosuccinimides **305** and **310** were isolated, this suggested that, to some extent, there was some elimination of the cysteine thiol upon addition of a second thiol.

As part of the collaboration described, Dr Mark Smith (UCL, Chemistry, Caddick group) demonstrated the reactivity of thiomaleimides in proteins by conjugating a thiomaleimide labelled protein **311** with glutathione (GSH) to afford the dithiosuccinimide conjugate **312**, **Scheme 142**.[265]

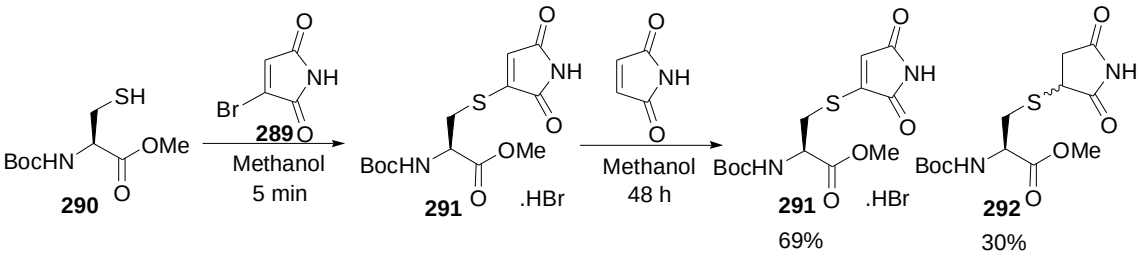


Scheme 142

2.ii.ii.iii. Reversibility of thiomaleimides and thiosuccinimides

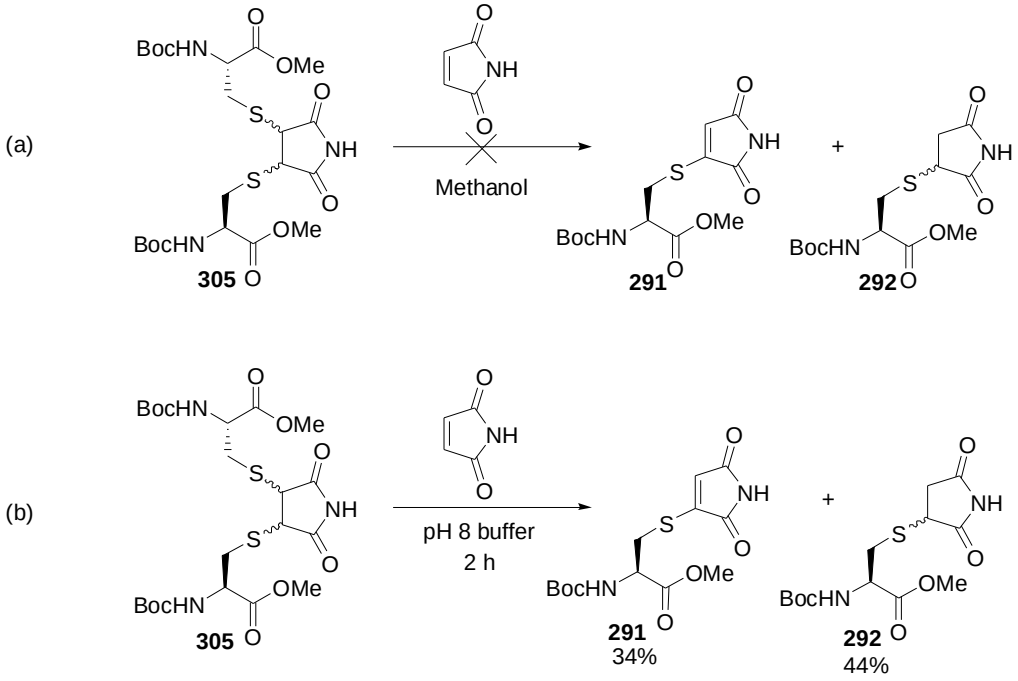
The thiosuccinimides formed from the addition of maleimides to cysteine are not reversible.[86, 265] Conversely, it had previously been observed that the thiomaleimide **291** was susceptible to reversion in the presence of HBr, if a base wasn't present in the reaction, p. 119. To confirm this outcome, the thiomaleimide adduct **291** was formed without addition of base in methanol, and maleimide added to ascertain if any free cysteine **290** was trapped by the maleimide, **Scheme 143**. After forty eight hours, the

reaction was subjected to column chromatography and 69% of the original thiomaleimide **291** was recovered. Thiosuccinimide **292** was also isolated from this reaction in 30%, **Scheme 143**. This further suggested that the construct **291** was reversible in the presence of HBr, and eliminated cysteine thiol could react with the maleimide. From previous results, it was known that in pH 8 buffer or with addition of one equivalent of sodium acetate, the reversion was negated, and, upon addition of maleimide after the base, no thiosuccinimide products **292** were seen, **Scheme 127**, p 121.



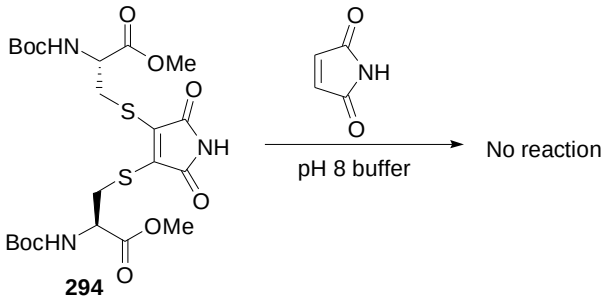
Scheme 143

Subjection of the dithiosuccinimide **305** to a solution of maleimide in methanol resulted in no thiosuccinimide **292**, and thus the dithiosuccinimide **305** was deemed irreversible in methanol, **Scheme 144 (a**). However, in the presence of a one equivalent of sodium acetate, or under aqueous conditions, both neutral and basic, reversion was witnessed. For example, both thiomaleimide **291** and thiosuccinimide **292** were isolated in 34% and 44% yield, respectively, when dithiosuccinimide **305** was dissolved with maleimide in aqueous buffer at pH 8, **Scheme 144 (b**). This suggested that the dithiosuccinimide construct **305** could be reversible or irreversible depending on the conditions.



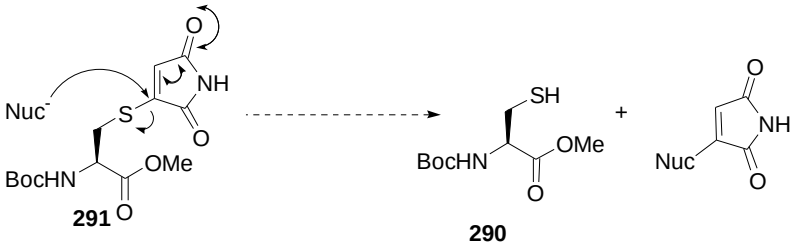
Scheme 144

Subjection of the dithiomaleimide adduct **294** to aqueous basic conditions in the presence of maleimide afforded no reaction, **Scheme 145**. In contrast to dithiosuccinimides **305**, this suggested that this construct was stable in buffer at pH 8.



Scheme 145

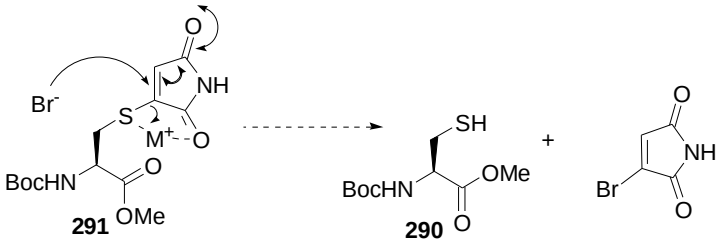
As discussed in p.36 there are many potential uses of reversible cysteine labelling. Therefore, with the idea that reversible cysteine labelling *via* bromomaleimide **289** could be a rival to the current method of using disulfides, potential reversion conditions were sought. Inspired by the reversion of thiomaleimide **291** when the eliminated HBr was not quenched by a base, it was predicted that addition of a nucleophile to thiomaleimide **291** could result in deliberate reversion of the conjugation, **Scheme 146**.



Scheme 146

Halide nucleophiles to displace cysteine

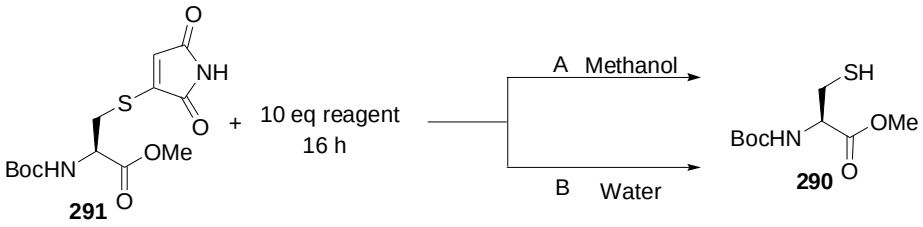
In order to probe potential nucleophiles that could reverse the reaction, bromide, from HBr, was an initial choice due to the aforementioned reversion of the formation of thiomaleimide **291** upon concentration of the reaction in methanol. However, addition of HBr and concentration of the resultant solution were not suitable reaction conditions, as this would not be transferrable to protein chemistries and buffered solutions. Therefore metallic cations were sought that could substitute the proton and counter the bromide ion. It was postulated that the Lewis acid activity of the metallic cation would aid the elimination of cysteine **290** through co-ordination with the sulfur or carbonyl, or even both, **Scheme 147**.



Scheme 147

Zinc bromide and sodium bromide both failed to react with the thiomaleimide and no cysteine **290** was eliminated, whether the reaction was carried out in water or methanol, entries 1 and 2, **Table 16**. Mercury is quoted as forming a strong bond with sulfur,[269] thus mercuric acetate was added to the thiomaleimide **291**, with and without added HBr. However, again, this afforded no reaction and no elimination of cysteine **290** was observed in either methanol or water, entries 3 and 4, **Table 16**.

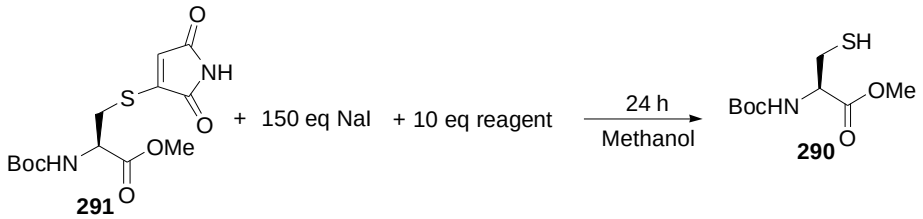
Table 16: Use of various bromides to displace cysteine 290 from thiomaleimide 291



Entry	Reagent	Route A	Route B
1	$ZnBr_2$	No reaction	No reaction
2	NaBr	No reaction	No reaction
3	$Hg(OAc)_2$	No reaction	No reaction
4	HBr and $Hg(OAc)_2$	No reaction	No reaction

When one hundred and fifty equivalents of sodium iodide were used, some reversion was witnessed by TLC. After twenty four hours the reaction had not gone to completion but nonetheless it was extracted to determine the result. A mixture of thiomaleimide **291**, iodomaleimide and cysteine **290** was afforded in ratios that indicated 60% elimination of the cysteine **290** had occurred, entry 1, **Table 17**. Whilst this result was promising, it was irreproducible and no reversion with sodium iodide could ever be afforded again. The addition of mercuric acetate in an attempt to co-ordinate to the sulfur and encourage elimination, alongside the sodium iodide, was not successful, entry 2, **Table 17**. When a second stronger nucleophile was added, either propylamine or hexanethiol, alongside sodium iodide to attempt to displace the thiolate, no elimination was observed, entries 3 and 4, **Table 17**.

Table 17: Use of sodium iodide to displace cysteine 290 from thiomaleimide 291

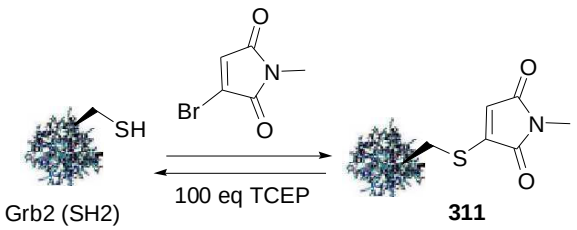


Entry	Reagent	Result
1	--	60% reversion observed once (not reproducible)
2	$Hg(OAc)_2$	No reaction
3	Propylamine	No reaction
4	Hexanethiol	No reaction

A range of bromides and iodides had not proven successful in elimination of cysteine **290** from thiomaleimide **291**, and thus no further halides were investigated.

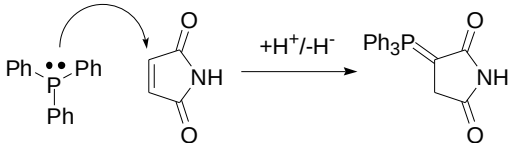
Phosphorus nucleophiles to displace cysteine

As part of the work undertaken in the collaboration, Dr Mark Smith (UCL, Chemistry, Caddick group) had identified a phosphine, tris(2-carboxyethyl)phosphine (TCEP), that could reverse the formation of a thiomaleimide in Grb2 (SH2) **311** in protein studies, releasing the free thiol, **Scheme 148**.[265]



Scheme 148

It was deemed important at this stage to confirm that this also worked on the small molecule model, this would indicate if the thiomaleimide **291** was serving as a valid model for protein studies and potentially optimise the conditions for studies on small molecules. TCEP behaves as a nucleophile in the cleavage of disulfides, **Scheme 23** p. 35, and phosphines are reactive as nucleophiles towards maleimides,[270] **Scheme 149**, this it was deemed likely that the phosphine would react with the thiomaleimide **291** and potentially eliminate cysteine **290**, as it had done in the protein studies.

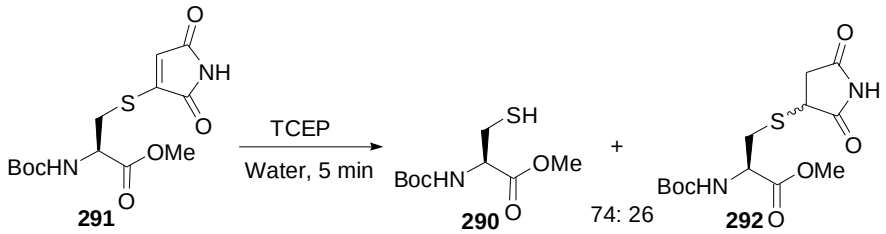


Scheme 149[270]

Investigations with TCEP encompassed increasing the added equivalents of the phosphine, use of different concentrations and the use of different solvents, as it was known that TCEP could oxidise in aqueous environments,[271] **Table 18**. As the reaction was being compared to protein chemistries it was deemed necessary to carry out the trial

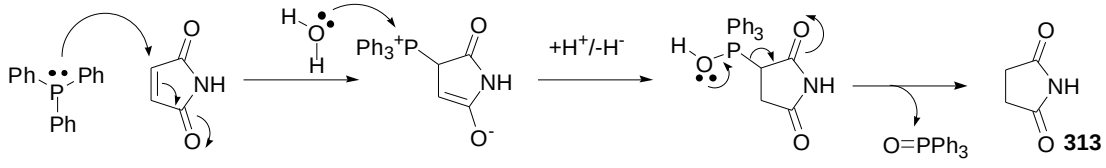
reactions initially in water and buffered solutions at dilute concentrations. At that time, the protein work carried out by Dr Mark Smith (UCL, Chemistry, Caddick group) was undertaken at pH 8, thus this was the pH of the buffer of choice for trial studies. If adequate conditions could be found then the reaction would be concentrated to ascertain if the conditions were applicable to smaller molecule chemistries, where concentrations are usually much larger. The dilute concentrations used (3.5 mM) were not quite as dilute as the protein chemistry (150 μM) undertaken in the collaboration, due to practicality, but they were around an order of magnitude lower than the concentrations in standard synthetic chemistry.

When one equivalent of TCEP was added to thiomaleimide **291** in water, it became apparent that the phosphine could eliminate cysteine **290** but it could also reduce the thiomaleimide **291** to succinimide **292**, **Scheme 150** and entry 1, **Table 18**.



Scheme 150

Examination of the literature found reference to phosphine-mediated reduction of maleimide to succinimide 313, **Scheme 151**,[272] so this result was perhaps not so surprising.



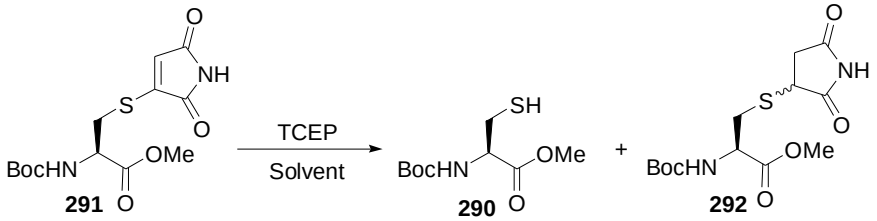
Scheme 151

Interestingly, moving to an aqueous buffer of pH 8 afforded a much higher proportion of the cysteine **290** (92%), entry 2, **Table 18**, and only a small amount of succinimide **292** was observed. It was necessary to increase the equivalents of TCEP to ten in order to completely negate the succinimide **292** formation and afford quantitative recovery of

the cysteine **290**, entries 3 - 5, **Table 18**. The reaction had to be carried out at pH 8, in order to buffer the highly acidic TCEP, entries 5 and 6 *cf.* 7 and 8, **Table 18**.

The elimination reaction also had to be carried out at a dilution of 10 mM. When the reactions were carried out in more concentrated solutions, the use of pH 8 buffer, water and DMF all afforded no cysteine **290**, entries 9 - 11, **Table 18**. This was because when ten equivalents of TCEP were used, TCEP was at a concentration of 0.1 M. The 100 mM phosphate solution could not buffer the reaction at such high concentration of the acidic phosphine and the pH of the solution was in effect approaching pH 3 in the 'buffered' solution, water and in DMF. This indicated a key breakthrough in that the TCEP equivalents and overall pH of the solution, after addition of all the reagents, were identified as the truly crucial factors in the elimination reaction.

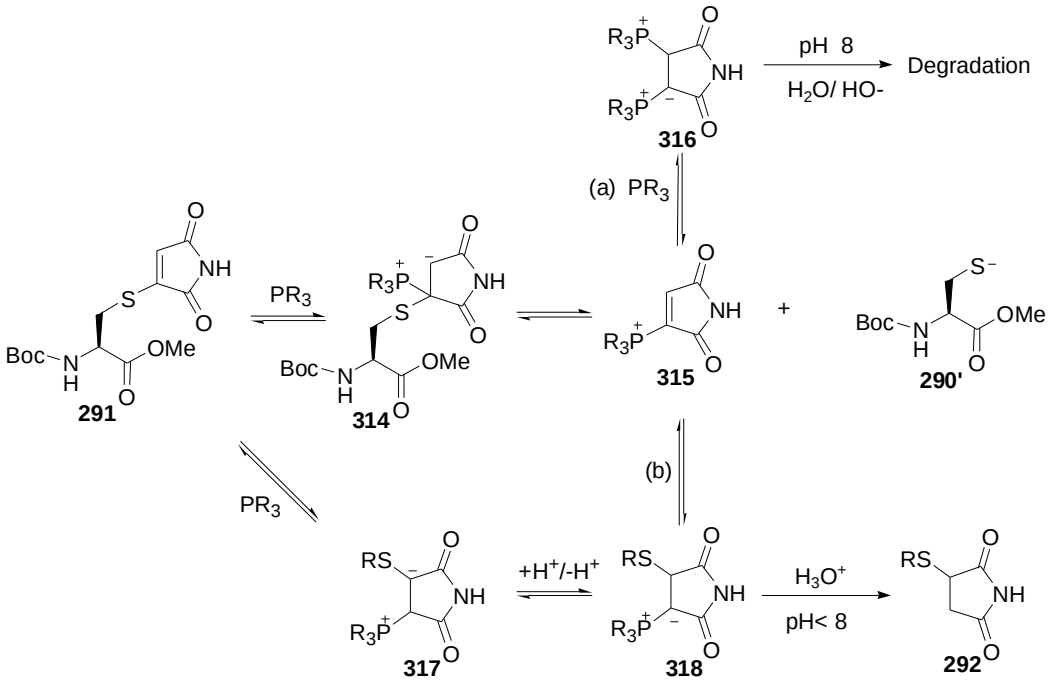
Table 18: Investigation into the use of TCEP to eliminate cysteine 290 from thiomaleimide 291



Entry	TCEP	Solvent	Concentration of 291	Yield of 290	Yield of 292
1	1 eq	Water	3.5 mM	74%[a]	26%[a]
2	1 eq	pH 8 buffer	3.5 mM	92%[a]	2%[a]
3	3 eq	pH 8 buffer	3.5 mM	97%[a]	1%[a]
4	5 eq	pH 8 buffer	3.5 mM	98%[a]	1%[a]
5	10 eq	pH 8 buffer	3.5 mM	100%[b]	--
6	10 eq	Water[c]	3.5 mM	91%[b]	--
7	10 eq	Water	3.5 mM	--	35%[b]
8	10 eq	DMF	3.5 mM	--	--
9	10 eq	pH 8 buffer[d]	10 mM	--	70%[b]
10	10 eq	Water	10 mM	--	68%[b]
11	10 eq	DMF	10 mM	--	8%[b]

The buffer used was aqueous 150 mM NaCl, 100 mM NaH_2PO_4 at pH 8.0. [a]Yield estimated from NMR. [b]Isolated yield. [c]Taken to pH 8 with sodium acetate. [d]Solution was at pH 3 after addition of TCEP.

Whilst the intermediates in the elimination reaction were not isolated or observed, one mechanism was postulated, **Scheme 152**. It was predicted that the addition of the phosphine would afford either of the phosphino thiosuccinimides **314** or **317**. The subsequent elimination of the cysteine as its thiolate **290'** from phosphino succinimide **314** is shown as reversible but the fate of the phosphino maleimide **315** and phosphino succinimide **318** govern the true outcome of the reaction. In the presence of excess phosphine, route (a), **Scheme 152**, *e.g.* ten equivalents in the case of the most successful reactions, a second addition to phosphino maleimide **315** can take place forming succinimides **316** that were postulated to degrade at pH 8. This pushes the reaction towards the formation of the free cysteine **290**. Phosphino maleimide **315** can be afforded from elimination from phosphino succinimides **314** or **318**. When the equivalents of the phosphine reagent are limited, phosphino succinimide **318** has a major role in the outcome of the reaction. It is formed either *via* the vicinal addition product **317** and proton transfer, or *via* route (b), **Scheme 152**, where the thiolate **290'** has attacked the phosphino maleimide **315**. This succinimide **318** can be protonated in acidic media and then reduced by addition of water to irreversibly afford the succinimide **292**, **Scheme 152**



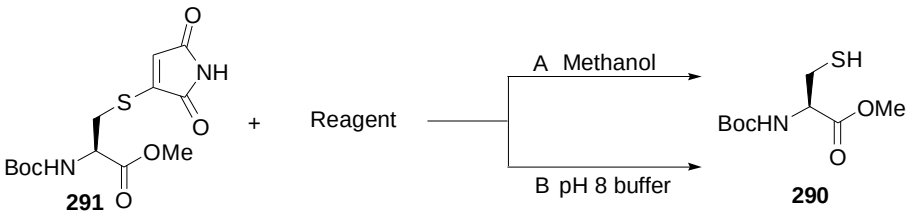
Scheme 152

Both routes (a) and (b) would both be occurring in solution, but excess phosphine would force the equilibrium of the reactions towards route (a) and thus cysteine **290** elimination. In addition, it was postulated that the succinimide **316** might be unstable in basic media, explaining why no by-products were isolated, further contributing to shifting the equilibrium via route (a). In contrast, it was envisaged that in acidic media the phosphino thiosuccinimide product **318** of route (b) was protonated and thus reduced more readily, shifting the equilibrium towards the isolated succinimide **292**. Whilst the mechanism was not proven by isolation of any of the intermediates, it fitted well with the empirical findings.

Importantly, polarimetry of the eliminated cysteine **290** indicated that the chiral centre had not been scrambled or inverted in the reaction, see **3.iii.ii.290.**.

To investigate the effect of other phosphorus nucleophiles, triphenylphosphine (PPh_3) and trimethylphosphite ($POMe_3$) were implemented. Unfortunately, although thoroughly evaluated in methanol and pH 8 buffer, these reagents afforded no eliminated cysteine **290** in six hours and led to degradation products that could not be isolated after prolonged exposure to the thiomaleimide **291**, **Table 19**.

Table 19: Investigations into the use of phosphorus nucleophiles to eliminate cysteine 290 from thiomaleimide 291



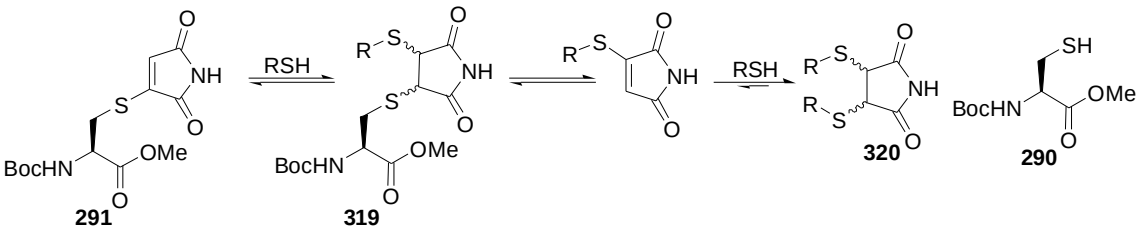
Entry	Reagent	Eq	Concentration of 291 (mM)	Route A	Route B
1	$POMe_3$	1	3.5	No elimination at 6 hours[a]	No elimination at 6 hours[a]
2	$POMe_3$	1	10	No elimination at 6 hours[a]	No elimination at 6 hours[a]
3	$POMe_3$	10	3.5	No elimination at 6 hours[a]	No elimination at 6 hours[a]
4	$POMe_3$	10	10	No elimination at 6 hours[a]	No elimination at 6 hours[a]
5	PPh_3	1	3.5	No elimination at 6 hours[a]	No elimination at 6 hours[a]
6	PPh_3	1	10	No elimination at 6 hours[a]	No reaction at 6 hours[a]
7	PPh_3	10	3.5	No elimination at 6 hours[a]	No elimination at 6 hours[a]
8	PPh_3	10	10	No elimination at 6 hours[a]	No elimination at 6 hours[a]

[a]Complete degradation of all material at 16 hours.

At the close of investigations into phosphorus nucleophiles to eliminate cysteine **290** from thiomaleimide **291**, it could be seen that triphenyl phosphine and trimethyl phosphite were not successful. However, when used in pH 8 buffer at dilute concentrations, ten equivalents of TCEP could quantitatively afford cysteine **290** from thiomaleimide **291**. If the pH was not closely controlled, or the equivalents of TCEP were lowered, then reduction of the thiomaleimide to form thiosuccinimide **292** was witnessed.

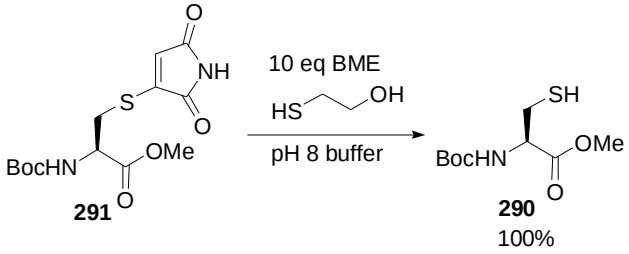
Thiol nucleophiles to displace cysteine.

Thiomaleimide **291** had been demonstrated as reactive to thiols, **Scheme 137**, p. 127. It had also been previously observed that dithiosuccinimide **305** was reversible in base, **Scheme 144**, p. 133 Therefore, it was postulated that if a nucleophilic thiol (RSH) was added in high enough equivalents, the equilibrium of the dithiosuccinimides **319** and **320** formation should eventually lead to cysteine **290** elimination and the dithiosuccinimide **320** of the excess thiol, **Scheme 153**.



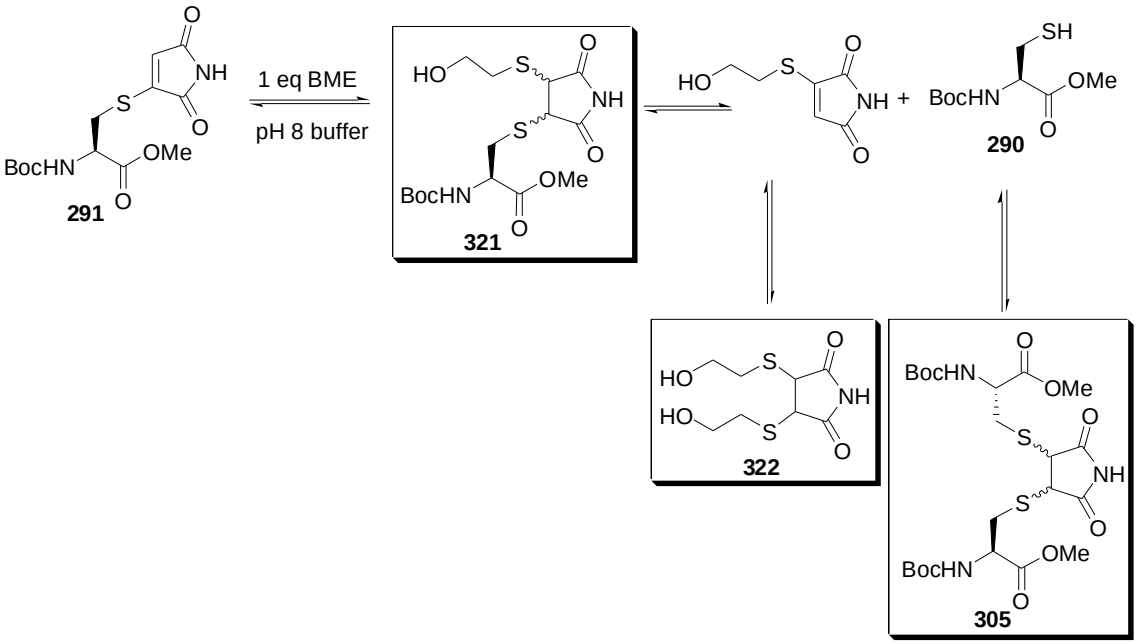
Scheme 153

β-mercaptoethanol (BME) acts as a good nucleophile in disulfide cleavage[86, 87] and is water soluble, thus it was utilised in an attempt to eliminate cysteine **290** from thiomaleimide **291**. Gratifyingly, when used in ten equivalents at pH 8, at a variety of concentrations, BME afforded the cysteine **290** in quantitative yield, **Scheme 154**.



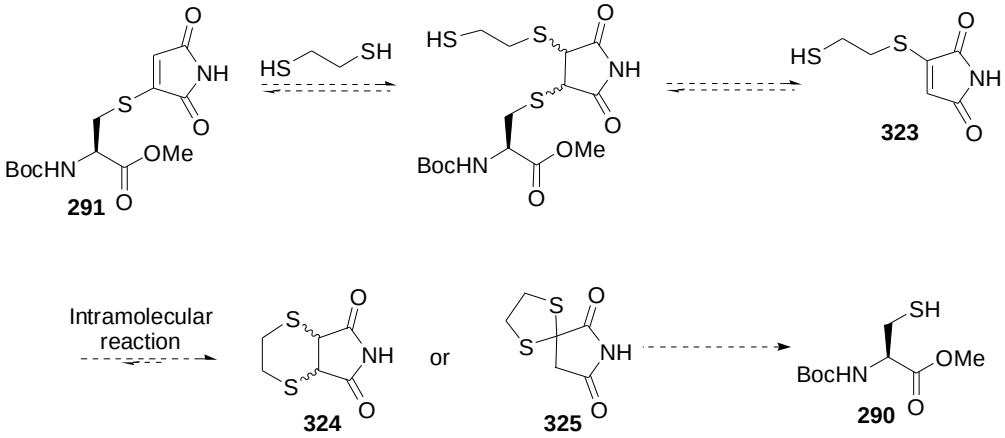
Scheme 154

Notably, using just one equivalent of BME gave a complex mixture of inseparable vicinal succinimide adducts, postulated to be diastereomers **321**, **322** and **305**, seen by NMR after unsuccessful purification by column chromatography, **Scheme 155**. This was seen as further evidence that the addition of the thiol was vicinal as, even though the succinimide products isolated, **322** and **305**, indicated the reaction of the vicinal addition was reversible, no geminal products were observed, **Scheme 155**.



Scheme 155

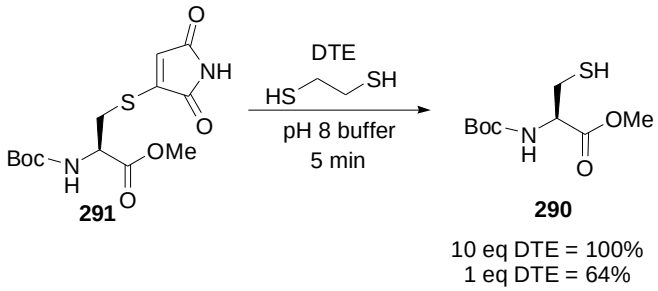
It was envisaged that 1,2-dithioethane would form succinimides **324** or **325** from the intramolecular reaction of thiomaleimide **323**, and eliminate cysteine **290** upon reaction with thiomaleimide **291**, **Scheme 156**. The succinimides formed could either be from vicinal **324** or geminal **325** addition, **Scheme 156**.



Scheme 156

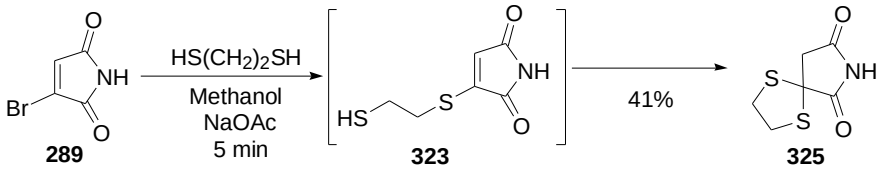
Therefore, 1,2-dithioethane (DTE) was added to the thiomaleimide **291**. As forecast, ten equivalents of DTE afforded quantitative yields of the cysteine **290**, **Scheme 157**. No succinimide adducts were isolable from this reaction. When just one equivalent of DTE

was used, a yield of 64% of the cysteine **290** was isolated, as well as a crude mixture of succinimide products (not isolable), **Scheme 157**.



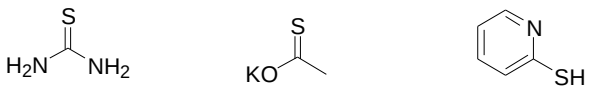
Scheme 157

As no succinimide compounds had been isolated from the reaction of DTE with thiomaleimide **290**, the fate of the DTE had not been identified. Therefore, in order to ascertain if the dithiol had formed a geminal or vicinal succinimide in this reaction, one equivalent of DTE was added to bromomaleimide **289**. A 41% yield of the geminal spiro compound **325** was isolated, from initial formation of the thiomaleimide **323** and intramolecular geminal attack of the thiol, **Scheme 158**.



Scheme 158

The use of other potential thiol nucleophiles such as thiourea, potassium thioacetate and 2-mercaptopyridine, **Figure 20**, where also investigated, to distinguish if they could eliminate cysteine **290** from thiomaleimide **291**

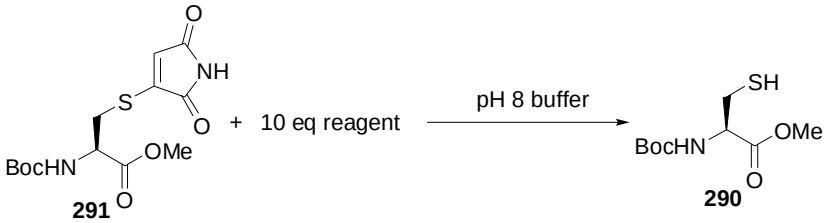


Thiourea Potassium thioacetate 2-Mercaptopyridine

Figure 20: Thiol nucleophiles utilised in elimination attempts

Unfortunately these reagents afforded no elimination in six hours and led to degradation products that could not be isolated after sixteen hours, **Table 20**.

Table 20: Investigations into the use of thiols to eliminate cysteine 290 from thiomaleimide 291



Entry	Reagent	Result
1	Thiourea	No elimination at 6 hours[a]
2	Potassium thioacetate	No elimination at 6 hours[a]
3	2-Mercaptopyridine	No elimination at 6 hours[a]

[a]Complete degradation of all material at 16 hours.

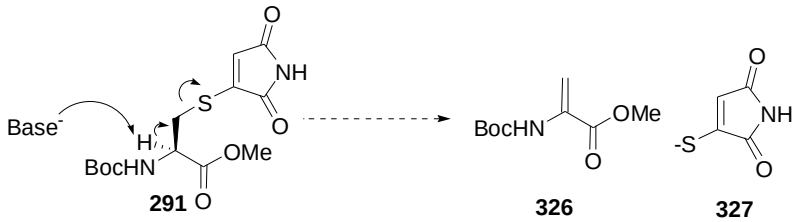
Therefore, in studies on the reversibility of thiomaleimide **291** formation, it had been ascertained that the thiomaleimide could reverse in the presence of HBr, upon concentration. However, if the addition of bromomaleimide **289** to cysteine **290** was carried out in pH 8 buffer, or in methanol with one equivalent of sodium acetate, the reversion was negated. Halide nucleophiles could not displace the cysteine **290** from thiomaleimide **291**. Ten equivalents of TCEP at 3.5 mM in pH buffer quantitatively eliminated cysteine **290** from thiomaleimide **291**, whilst triphenylphosphine and trimethylphosphite could not mediate cysteine **290** elimination. Ten equivalents of BME or DTE in pH 8 buffer could afford eliminated cysteine **290** in quantitative yields from thiomaleimide **291**, but thiourea, thioacetate and 2-mercaptopyridine could not. These results, coupled with the early work from the collaboration indicated that the use of bromomaleimides for cysteine labelling could be reversible, with potential applications for impermanent labelling of a thiols and cysteine residues in a range of biomolecules.

2.ii.ii.iv. Manipulation of thiomaleimides to form dehydroalanine

Base-mediated dehydroalanine formation

As discussed on p.37, the formation of Dha in proteins is useful as it creates a reactive Michael acceptor that can be used to react with selected nucleophiles. Inspired by recently published work by Davis and co-workers,[114] it was postulated that the thiomaleimide **291** might be a route to dehydroalanine motifs **326** *via* a base induced

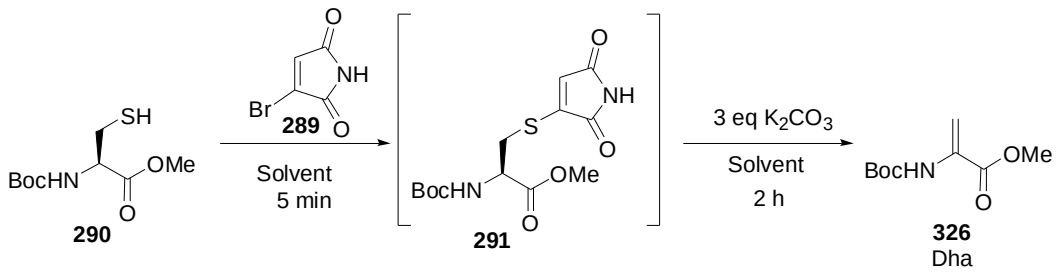
elimination to expel the thiolate **327**, **Scheme 159**. The thiomaleimide was predicted to be a good leaving group due to the electron withdrawing properties of the maleimide.



Scheme 159

To afford Dha **326**, the Davis research group had employed MSH and five equivalents of potassium carbonate as their base of choice (see p.38). Therefore, guided by these conditions, the thiomaleimide **291** was treated with three equivalent of potassium carbonate in methanol. Immediately the solution turned a bright yellow and by TLC a new, high-running spot could be seen that was believed to be the desired dehydroalanine product (hereafter referred to as Dha **326**). The yellow colour was attributed to the thiolate anion **327**, although this compound proved impossible to isolate. The reaction was complete in two hours and when the solvent was removed *in vacuo*, Dha **326** was afforded in 65%, entry 1, **Table 21**. Attempts to improve the yield and make the conditions more applicable to protein chemistries were investigated. Using different solvents such as methanol:water (1:1), and DMF:water (1:1) only served to significantly lower the yield of Dha **326**, entries 2 and 3, **Table 21**. To rule out aqueous instability, the reaction was carried out in anhydrous methanol but it appeared that the base was only sparingly soluble in this solvent and yields were poor, entry 4, **Table 21**.

Table 21: The use of different solvents to afford Dha 326 from basic media

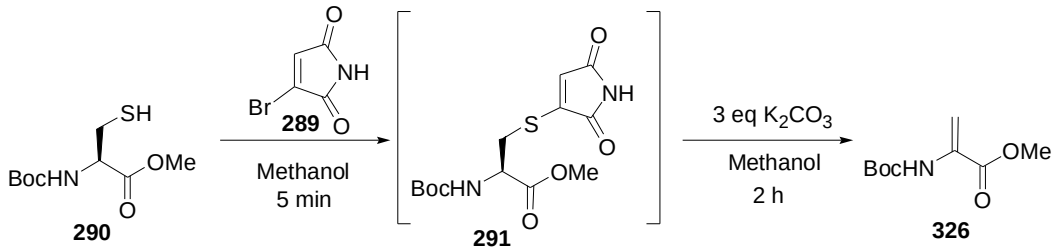


Entry	Solvent	Yield of 326
1	Methanol	65%
2	Methanol:water (1:1)	8%
3	DMF:water (1:1)	12%
4	Anhydrous methanol	13%

In an attempt to minimise the exposure of Dha **326** to the basic environment, the use of less equivalents of base were attempted, but this merely confirmed that three equivalents of base were definitely necessary, entries 1-3, **Table 22**. When the base was added sequentially, lower yields of Dha **326** were observed, entry 4 and 5, **Table 22**. To be certain of at least three equivalents it was deemed appropriate at this stage to use 3.3 equivalents as the standard base addition, entry 6, **Table 22**. If a mild base, namely sodium acetate, was used to quench the eliminated HBr first and then 2.2 equivalents of potassium carbonate added, then the yield was lower, entry 7, **Table 22**.

It was postulated that hydroxide would be a good base to promote elimination from the thiomaleimide **291**. Thus 3.3 equivalents of sodium hydroxide in methanol and methanol:water (1:1) were used in an attempt to afford Dha **326** in higher yields, entries 8 and 9, **Table 22**. The formation of Dha **326** was overshadowed in these reactions by the formation of baseline impurities that appeared to be degradation of the thiomaleimide **291** – this was not unexpected as the pH of the solution was well above 8, in a region that maleimides are prone to hydrolysis.[273]

Table 22: Investigations into the use of different bases to afford Dha 326



Entry	Base	Equivalents	Yield of 326
1	K_2CO_3	3	65%
2	K_2CO_3	2	Incomplete reaction
3	K_2CO_3	1	Incomplete reaction
4	K_2CO_3	3[a]	12%
5	K_2CO_3	3[b]	20%
6	K_2CO_3	3.3	65%
7	NaOAc then K_2CO_3	1.1 then 2.2[c]	47%
8	NaOH	3.3	--
9	NaOH[d]	3.3	--

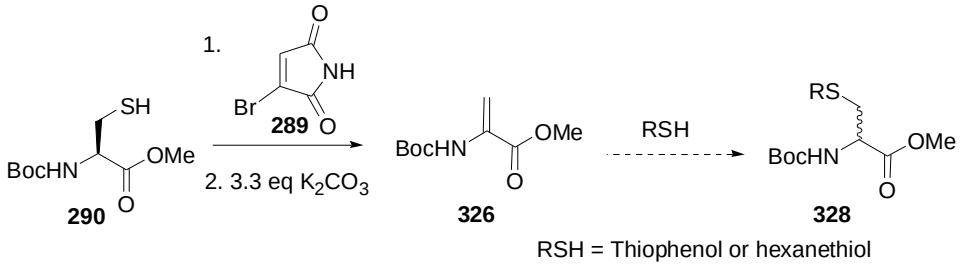
[a]1 equivalent of base was added after bromomaleimide **289**. After 10 minutes, 2 further equivalents of base were added. [b]1 equivalent of base was added after bromomaleimide **289**. After 10 minutes, 1 further equivalent of base was added. Another equivalent of base was added after a further hour. [c]1.1 equivalents of NaOAc were added after bromomaleimide **289**. After 10 minutes, 2.2 equivalents of K_2CO_3 were added. [d]Reaction carried out in methanol:water (1:1).

When the thiomaleimide **291** was subjected to three equivalents of potassium carbonate in methanol, the formation of Dha **326** and a bright yellow baseline spot was all that could be seen by TLC but, frustratingly, the yields of the reaction thus far did not reflect this. All previous reactions had isolated Dha **326** from an extraction with ethyl acetate on the concentrated reaction residue. By diluting the reaction with ethyl acetate instead, and washing the organic solvent to remove aqueous soluble impurities, the yield was increased to 75%. Whilst this was promising, it did not reflect the quantitative yields that the reaction analysis suggested should be possible, and the reaction was still only successful in methanol, thus not transferrable to proteins.

To try to minimise Dha **326** degradation the reaction was carried out at 0 ^{o}C in methanol, methanol:water (1:1) and water: DMF (1:1). No benefit was seen from this as

the yield in methanol was unchanged by the lower temperature and no Dha **326** product was afforded from the reactions in the latter two solvents

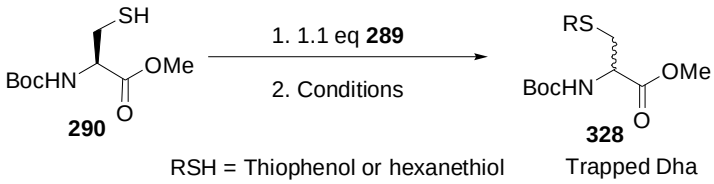
It was postulated that, as a reactive Michael acceptor, Dha **326** may have been too unstable to be easily isolated. Therefore it was suggested that the reactive product **326** may be isolable by reaction with an *in situ* nucleophile such as thiophenol or hexanethiol, to afford thioether **328**, **Scheme 160**.



Scheme 160

Whilst these reactions were attempted in methanol, methanol:water (1:1) and DMF:water (1:1), the trapping reactions all afforded multiple products that could neither be isolated in high yields nor completely characterised due to the mixtures obtained, entries 1-4, **Table 23**. Addition of the nucleophile was attempted both before and after the addition of the base but the trapping reactions were not successful under these conditions.

Table 23: Attempts to trap Dha 326 with thiols

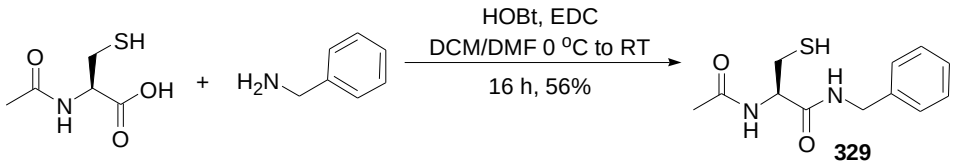


Entry	Conditions	Result
1	3.3 eq K_2CO_3, 10 min then 10 eq thiophenol, 6 h	Complex mixture[a]
2	10 eq thiophenol, 10 min then 3.3 eq K_2CO_3, 6 h	Complex mixture[a]
3	3.3 eq K_2CO_3, 10 min then 10 eq hexanethiol, 6 h	Complex mixture[a]
4	10 eq hexanethiol, 10 min then 3.3 eq K_2CO_3, 6 h	Complex mixture[a]

[a]The same result was observed whether the reaction was carried out in methanol, water: methanol (1:1) or DMF:water (1:1).

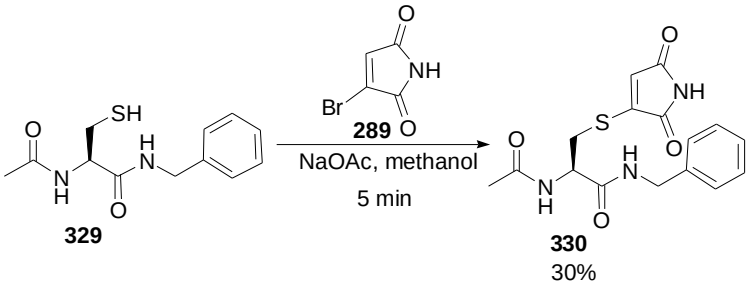
Instead of undergoing the desired conjugate addition to Dha **326**, it was postulated that the thiol nucleophiles could have merely reacted with the intermediate thiomaleimide **291**, formed disulfides from oxidation, or reacted with the thiolate **327** or its degradation products.

It was speculated that the *N*-Boc-Cys-OMe thiomaleimide **291**, as a model for cysteine residues in proteins might be problematic as the ester bond may be sensitive to basic conditions. In addition the acidity of the α-carbon between the carbamate and the ester was not necessarily a true representation of the acidity of the α-carbon in a peptide, *i.e.* between two amides, although this model had been successfully used by Davis *et al.*.[114] Therefore, an alternative model cysteine system was designed with two amide protecting groups. *N*-Ac-Cys-NHBn cysteine **329**, **Scheme 161**, was synthesised from the commercially available *N*-protected cysteine carboxylic acid *via* carbodiimide-mediated coupling with benzylamine, **Scheme 161**.



Scheme 161

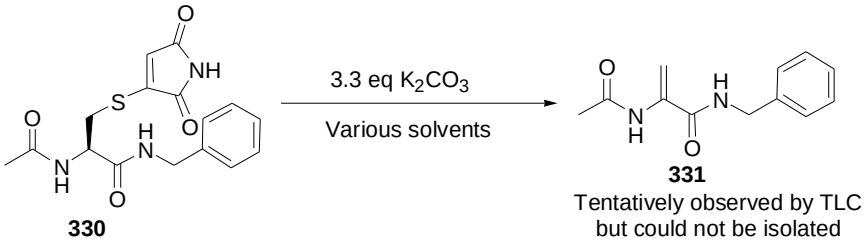
The *N*-Ac-Cys-NHBn model cysteine **329** proved to be quite testing in its own right as it formed its disulfide much more readily than the commercial *N*-Boc-Cys-OMe cysteine analogue **291**. Reactions on the *N*-Boc-Cys-OMe thiomaleimide **291** had been carried out on the isolated thiomaleimide **291**, or by forming it *in situ* and then merely adding the base. However, with the *N*-Ac-Cys-NHBn cysteine **329**, the proportion of material that was effectively 'lost' to disulfide formation during any reaction necessitated that the attempted elimination reactions always be carried out on the isolated thiomaleimide **330**, **Scheme 162**. Unreacted bromomaleimide could be recovered from this reaction in 70%.



Scheme 162

The formation of a Dha species **331** was attempted *via* addition of 3.3 equivalents of potassium carbonate to the thiomaleimide **330**, **Table 24**. The elimination conditions were carried out in methanol, methanol:water (1:1) and water:DMF (1:1) but this did not afford any Dha **331**, entries 1-3, **Table 24**. In fact, whilst a high-running compound was identified by TLC in all solvents, postulated to be Dha **331**, it could never be isolated from these reactions. The formation of the postulated Dha **331** also appeared to be slower, taking at least three hours in all solvents. This model **330** was also much less soluble in the solvents used than the *N*-Boc-Cys-OMe thiomaleimide **291** and the low yields could, in part, be attributed to poor solubility of the reagent. This could have led to overexposure of solvated material to base and subsequent degradation of the *N*-Ac-Cys-NHBn thiomaleimide **330**.

Table 24



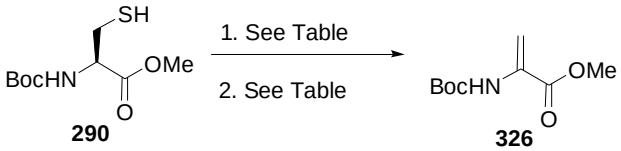
Entry	Solvent	Time	Result
1	Methanol	3 h	No Dha **331** isolated
2	Methanol:water (1:1)	4 h	No Dha **331** isolated
3	DMF: water (1:1)	4.5 h	No Dha **331** isolated

The elimination reaction of the *N*-Ac-Cys-NHBn thiomaleimide **330** was further investigated by attempting the reaction in deuterated methanol so that progress could be monitored by ^{1}H NMR. In doing so, it was noticed that the thiomaleimide **330**

underwent degradation upon exposure to the basic conditions of the reaction. This observation, in addition to the poor solubility profile of *N*-Ac-Cys-NHBn thiomaleimide **330** and the susceptibility of *N*-Ac-Cys-NHBn cysteine **329** to increased disulfide formation meant that use of the *N*-Ac-Cys-NHBn model system **330** was terminated.

Returning to the initial *N*-Boc-Cys-OMe thiomaleimide **291**, it was deemed that the isolation of the Dha **326** was potentially the problem rather than its formation. The highest reproducible yield thus far was 75% from dilute ethyl acetate extraction after carrying out the reaction with 3.3 equivalents of potassium carbonate in methanol, entry 1, **Table 25**. At this stage, attention was more closely turned to the paper by Davis *et al.*,[114] and several key differences were noted. In particular, the reactive intermediate that was used in their work was formed in the presence of base, rather than forming the reactive species and then treating it with base. Therefore, the cysteine **290** was stirred in the basic methanol conditions and then bromomaleimide added to form the thiomaleimide **291** in the presence of the base. No improvement was observed with this method of addition and a yield of 73% isolated Dha **326** was afforded over the same time period (two hours), entry 2, **Table 25**. Other than order of addition, another noted difference was that the Davis group extracted the formed Dha **326** in large quantities of diethyl ether (40 mg in 150 mL), where up to this point only ethyl acetate had been used in this work. Repeat of the conditions with the dilute diethyl ether extraction finally afforded the near quantitative yields that were expected, *i.e.* 93%, entry 3, **Table 25**.

Table 25: Investigations into the formation of Dha 326 using literature guidance



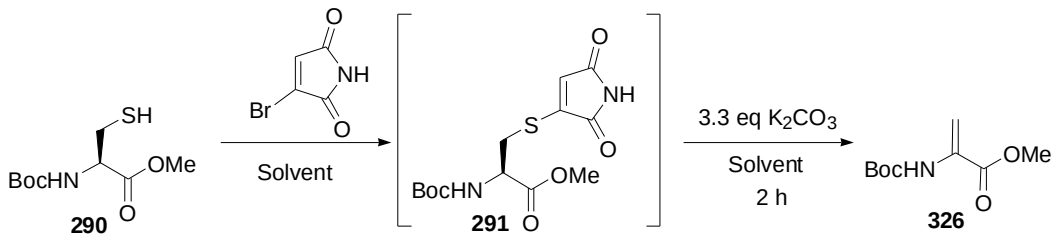
290 **326**

Entry	1	2	Extraction	Yield of 326
1	1.1 eq **289**	3.3 eq K_2CO_3	Ethyl acetate (3 x 15 mL)	75%
2	3.3 eq K_2CO_3	1.1 eq **289**	Ethyl acetate (3 x 15 mL)	73%
3	1.1 eq **289**	3.3 eq K_2CO_3	Diethyl ether (3 x 50 mL)	93%

Now that conditions and isolation techniques had been ascertained that could afford good yields of Dha **326** from the thiomaleimide **291**, the reaction was again attempted in water, methanol: water (1:1), DMF:water (1:1) and also in DMF, **Table 26**. In all

solvent systems that contained an appreciable amount of water there was substantial degradation of the thiomaleimide **291**, and Dha **326** was not isolable in any significantly higher yields than in initial attempts, entry 2-4, **Table 26** (*cf*. **Table 21**. p. 147). Interestingly, in neat DMF the reaction did not reach completion, even at extended times (six hours), and merely afforded an inseparable mixture of thiomaleimide **291** and Dha **326**, entry 5, **Table 26**. NMR investigations indicated that the mixture was 3:2 thiomaleimide **291**:Dha **326**. It became apparent that the thiomaleimide **291** was simply not stable to the aqueous basic conditions of around pH 10.

Table 26: The use of different solvents to afford Dha 326 from basic media



Entry	Solvent	Yield of 326
1	Methanol	93%
2	Water	-
3	Methanol:water (1:1)	10%
4	DMF:water (1:1)	15%
5	DMF	40%[a]

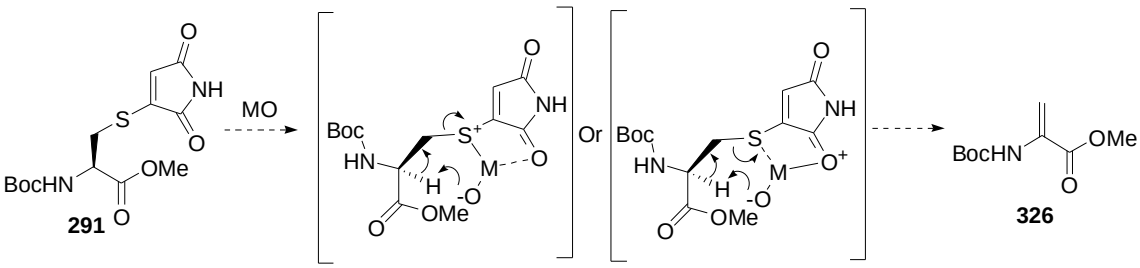
[a]Estimated conversion by ^{1}H NMR, as mixture with unreacted thiomaleimide **291**.

Therefore, the formation of Dha **326** from thiomaleimide **291** could be achieved in 93% by addition of 3.3 equivalents of potassium carbonate in methanol, followed by extraction of the Dha **326** in large quantities of diethyl ether. The reaction was not successful in aqueous systems, most likely due to the instability of thiomaleimide **291** to strong base, and, as such, cannot be transferred to Dha formation in proteins.

Metal oxide promoted formation of dehydroalanine formation

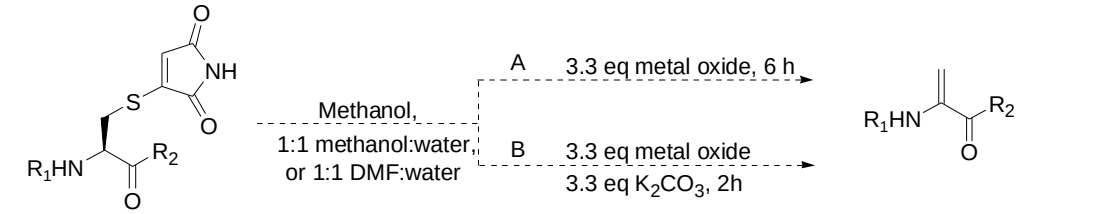
As basic conditions were not suitable in the solvent systems required to carry out widely applicable protein modifications, attention was turned to other methods of promoting elimination. A range of metal oxides were screened in the hope that the Lewis acidity of

the metal would aid co-ordination between the sulfur and, or, the oxygen and the oxide might act as a base, as illustrated in **Scheme 163**.



Scheme 163

The thiomaleimides **291** and **330** were both dissolved in either methanol, methanol:water (1:1) or DMF:water (1:1). A screen of metal oxides was carried out whereby 3.3 equivalents of a metal oxide was added to the resulting solutions and the reaction monitored for six hours, **Scheme 164**, route A. In addition to the sole addition of metal oxide, 3.3 equivalents of potassium carbonate were added with the metal oxide to ascertain if the elimination of Dha **326** occurred in less than two hours, **Scheme 164**, route B. The reactions were initially monitored by TLC as they were small scale (2 mg of thiomaleimides **291** and **330**). Reactions that showed evolution of any new products were scaled up for further investigation and isolation of Dha **326** or **331**.

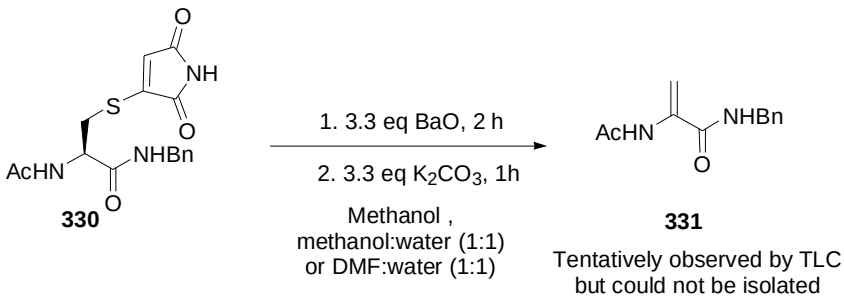


Scheme 164

The metal oxides investigated were silver (I) oxide, hafnium (I) oxide, copper (I) oxide, tin (I) oxide, barium (II) oxide, calcium (II) oxide, nickel (II) oxide, mercury (II) oxide, titanium (II) oxide, cobalt (II/III) oxide, iron (II/III) oxide, chromium (III) oxide, lead (IV) oxide, ruthenium (IV) oxide, platinum (IV) oxide and manganese (IV) oxide, chromium (VI) oxide. A full breakdown of the results, over two hundred small scale experiments, is not presented as, with one exception, the metal oxides alone all failed to

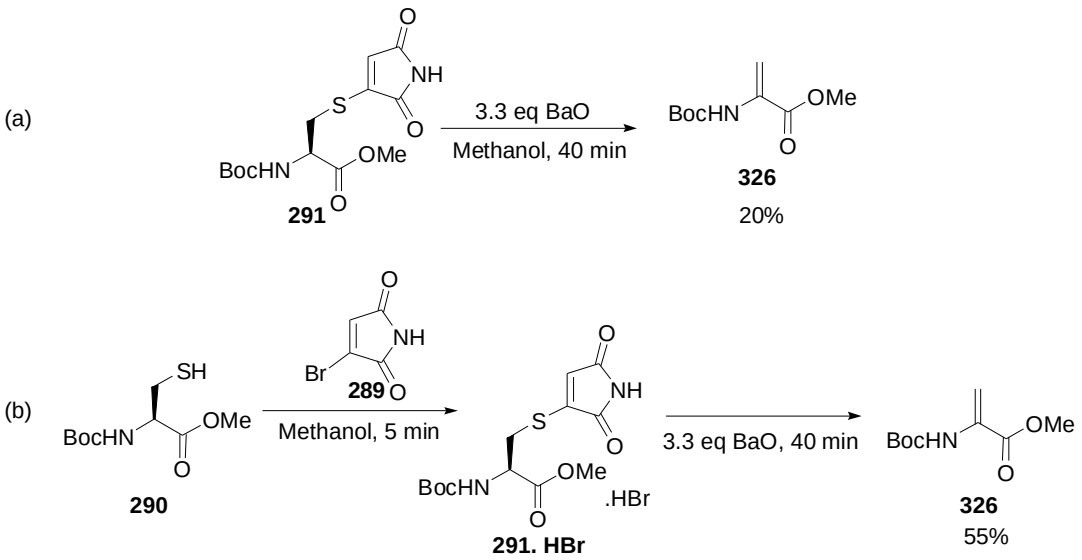
react with both thiomaleimides **291** and **330** and, in the presence of base, led to no acceleration of the reaction to form either Dha **326** or **331**. The exception to this trend was the reaction of barium (II) oxide with both of the thiomaleimide models.

In the case of the *N*-Ac-Cys-NHBn thiomaleimide model **330**, it was thought that Dha **331** started to form upon addition of barium oxide but the reaction was slow and could only be taken to completion with addition of base, **Scheme 165**. Unfortunately, with analogy to the reaction using this model and base alone, the postulated Dha **331**, seen by TLC, could never be isolated from the crude reaction mixtures.



Scheme 165

When BaO was added to the *N*-Boc-Cys-OMe thiomaleimide model **291** in methanol, all of the thiomaleimide **291** had been consumed at forty minutes and only Dha **326** could be seen by TLC. However, isolation of Dha **326** was successful in only 20% yield, **Scheme 166 (a)**. For completeness, these conditions were attempted on the same system, but where the thiomaleimide **291** was formed *in situ*, **Scheme 166 (b)**. This yield was not as high as the potassium carbonate-mediated reaction with this thiomaleimide but it did reach 55%, **Scheme 166 (b**). It was postulated that over the course of the reactions, the BaO was undergoing hydrolysis to basic $Ba(OH)_2$. The higher yield of Dha **326** observed in the latter case could be due to the eliminated HBr, from the addition of bromomaleimide **289** to cysteine **289**, quenching any excess $Ba(OH)_2$ that was degrading the unstable Dha **326**.



Scheme 166

The best yields of Dha **326** afforded in methanol from using 3.3 equivalents barium oxide (55%) were lower than those achieved from using 3.3 equivalents of potassium carbonate (93%). Neither conditions were successful in aqueous conditions and therefore were not yet deemed suitable for protein chemistries.

2.ii.ii.v. Use of the conjugation strategy for functionalisation of peptides

Although it was recognised that the current Dha **326** elimination conditions weren't suitable for aqueous systems, it was deemed important to attempt to transfer the basic conditions to larger peptide systems. This would ascertain if Dha motifs could be afforded from peptides that could be manipulated in methanol. It was also planned to transfer the reversion conditions, with TCEP and BME, to any peptide thiomaleimides formed.

To transfer the previously successful reactions to peptides, a simple cysteine-containing synthetic tripeptide was designed, namely an alanine-cysteine-alanine conjugate **332**, **Figure 21**.

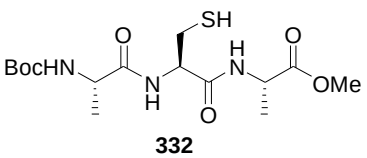
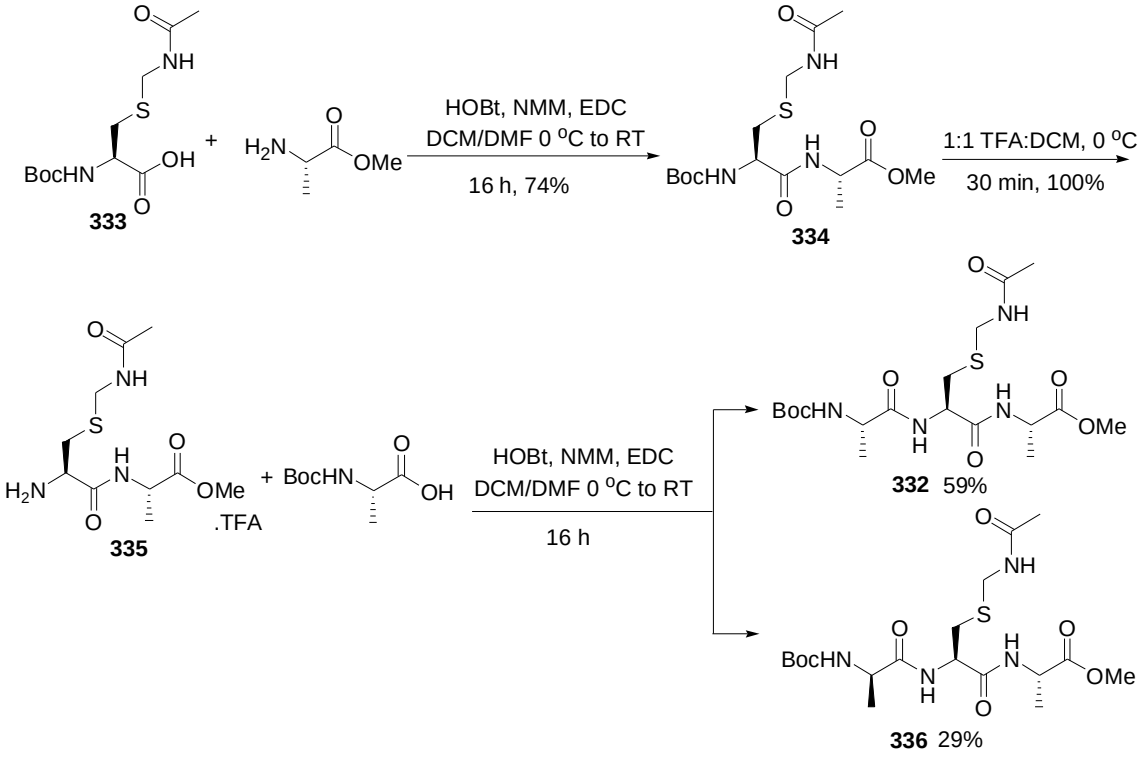


Figure 21: *N*-Boc-Ala-Cys-Ala-OMe proposed model tripeptide

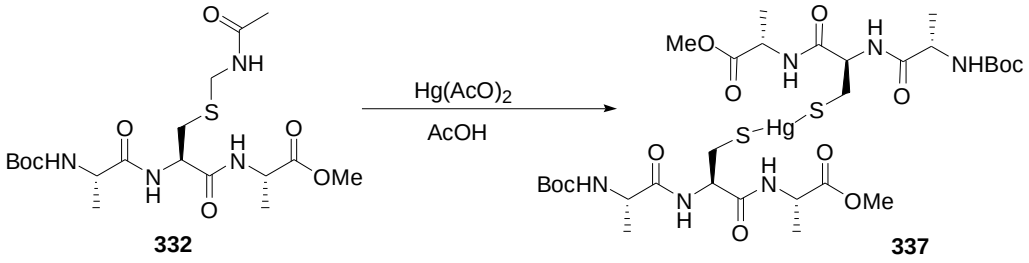
The synthesis was initiated with an *N*- and *S*-protected cysteine carboxylic acid **333** and an acid-protected alanine amine, **Scheme 167**. A cysteine with a protected thiol was chosen to minimise the thiol's interference as a nucleophile in the coupling reactions. Standard coupling conditions were undertaken to form the first amide bond, **Scheme 167**. Subsequent treatment of dipeptide **334** with trifluoroacetic acid afforded TFA salt **335** and a second coupling under similar conditions afforded protected tripeptides **332** and **336**, **Scheme 167**.



Scheme 167

HOBt (hydroxybenzotriazole) is used in carbodiimide reactions to minimise racemisation of the α-carbon in the activated carboxylic acid. However, even though HOBt was used there was some concomitant scrambling of the last chiral centre added, seen by shifts of the corresponding α-proton in the ^{1}H NMR spectrum. This was not deemed too problematic as the by-product **336** could be separated from the naturally configured tripeptide and represented a further model that could be used if extension of the methodology was needed.

At this stage, the cysteine thiol had to be deprotected to afford the free sulfhydryl. Mercuric acetate was quoted for thio-acetamide deprotection[274] so use of this reagent was undertaken to afford the mercury bridged disulfide **337**, **Scheme 168**. ^{1}H NMR analysis of the reaction mixture suggested the desired product had been formed but the broad signals in the NMR prevented confident assignment. The peak broadening was due to rotational constrictions imposed by the amide bonds in the structures **332** and **337** and the presence of salts in the crude reaction mixture.

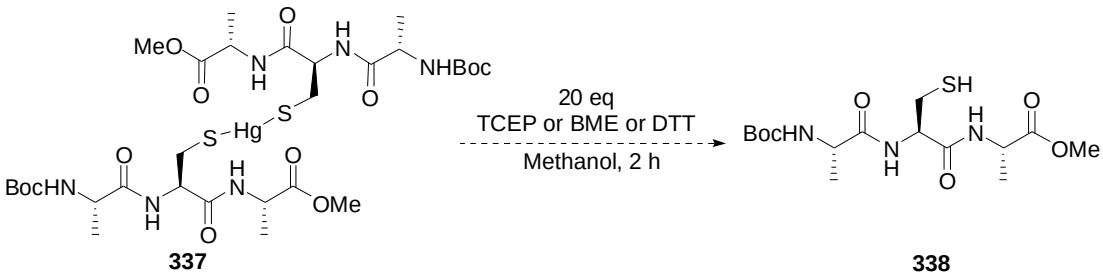


332 **337**

Scheme 168

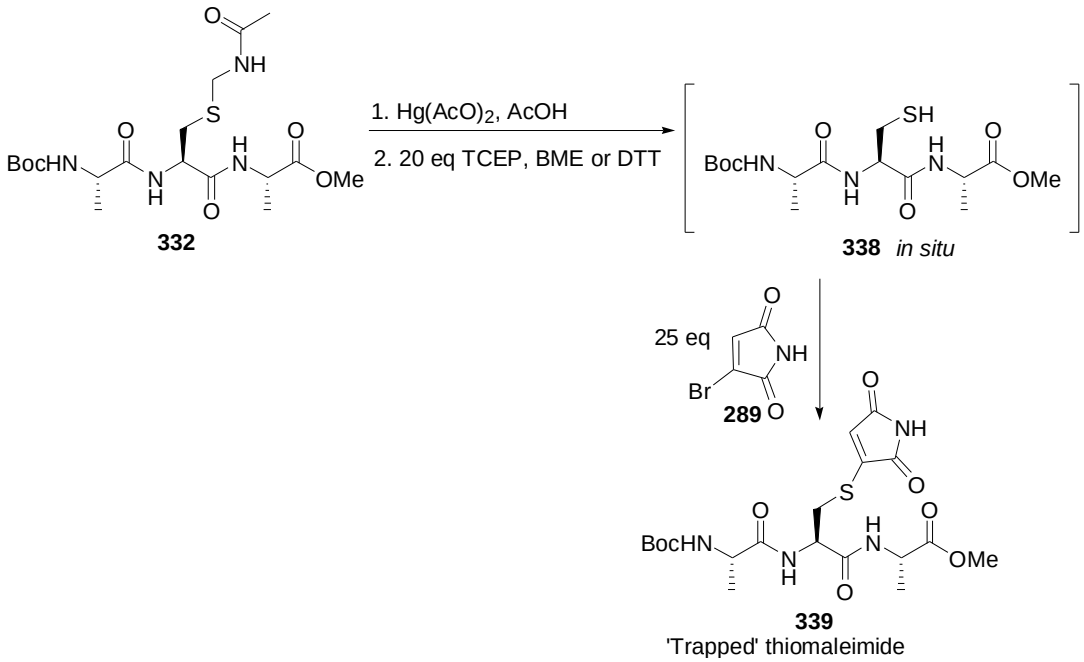
It was difficult to ascertain at this stage if the reaction had reached completion, and the material could not be purified by column chromatography. It was postulated that any unreacted acetamido tripeptide **332** could be removed in later steps. Therefore, at this stage, no further purification was undertaken and the material was taken on in an attempt to afford the free sulfhydryl **338**, **Scheme 169**. The crude mercuric disulfide **337**, **Scheme 169**, was treated with twenty equivalents of TCEP[274] in methanol and the resultant solution partitioned between aqueous and organic solvent. The desired outcome was to extract the more lipophilic cleaved tripeptide and leave mercuric salts in the aqueous layer. Analysis of the ^{1}H NMR spectrum of the organic layer suggested that the free thiol tripeptide had been successfully formed but that there was more than one impurity in the residue. One of these impurities appeared to still have the acetamido protecting group, indicating that the initial formation of the mercuric bridged disulfide **337** had not reached completion. Due to the impurities in the reaction residue, it was subjected to column chromatography. However, in the process of purification the thiol **338** formed its disulfide and still maintained unknown impurities, probably the original protected tripeptide **332** and the mercuric disulfide **337**. It was deemed necessary to find another method of cleaving the mercuric disulfide that would be clean and require minimal purification. The use of twenty equivalents of BME[274] and DTT[275] were attempted, **Scheme 169**, but the reactions were never clean and attempts to purify the

material merely lead to a mixture of acetamido, mercuric bridged and disulfide bridged tripeptides.



Scheme 169

In a last attempt to afford the tripeptide for model studies, it was thought that bromomaleimide **289** could be added to the cleavage reaction mixture to trap out any free thiol tripeptide. Even though this added another potential product to the mixture, it was envisaged that the thiomaleimide **339** would be stable, and thus could be isolated from the mixture, **Scheme 170**.



Scheme 170

Column chromatography of the resultant mixtures from using TCEP, BME or DTT and then adding bromomaleimide **289**, afforded a whole host of degradation products and it became apparent that the tripeptides formed were not stable to silica.

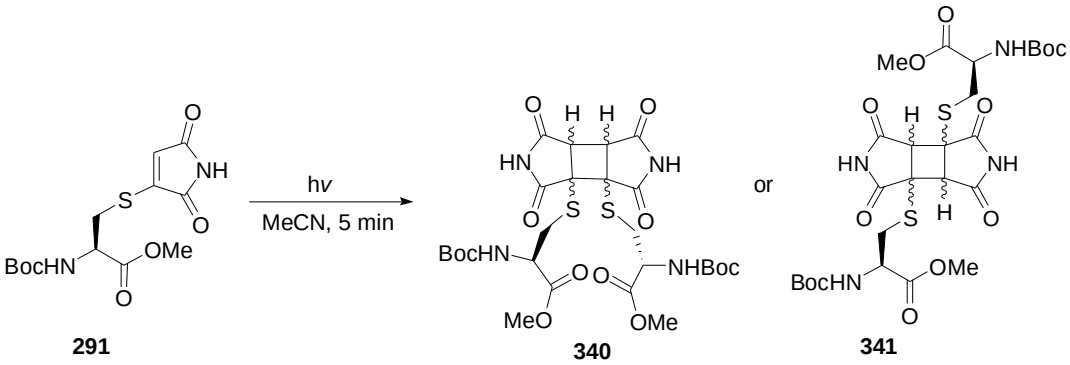
The overall goal of this work was to demonstrate potential cysteine modifications on small molecules for eventual transferral to protein systems *via* the aforementioned collaboration. As the conditions had been shown as successful on a protein, it was decided not to spend any more time on synthesising a tripeptide, and instead focus attention on more novel reactions in the small molecule work.

2.ii.ii.vi. Exploring the potential photoactivity of thiomaleimides towards bioconjugation strategies

Photochemical dimerisation of thiomaleimides

As discussed in **1.ii.iv.**, interest in the use of light to mediate bioconjugations has increased in recent years. Photochemical modifications are mediated by light and, as such, the reactions have 'spatiotemporal' control – *i.e.* the exact time and location of a reaction can be specified. As discussed in the hypothesis, it was envisaged that thiomaleimides would be photoactive, opening up routes to photochemical conjugation of proteins already modified with thiomaleimides.

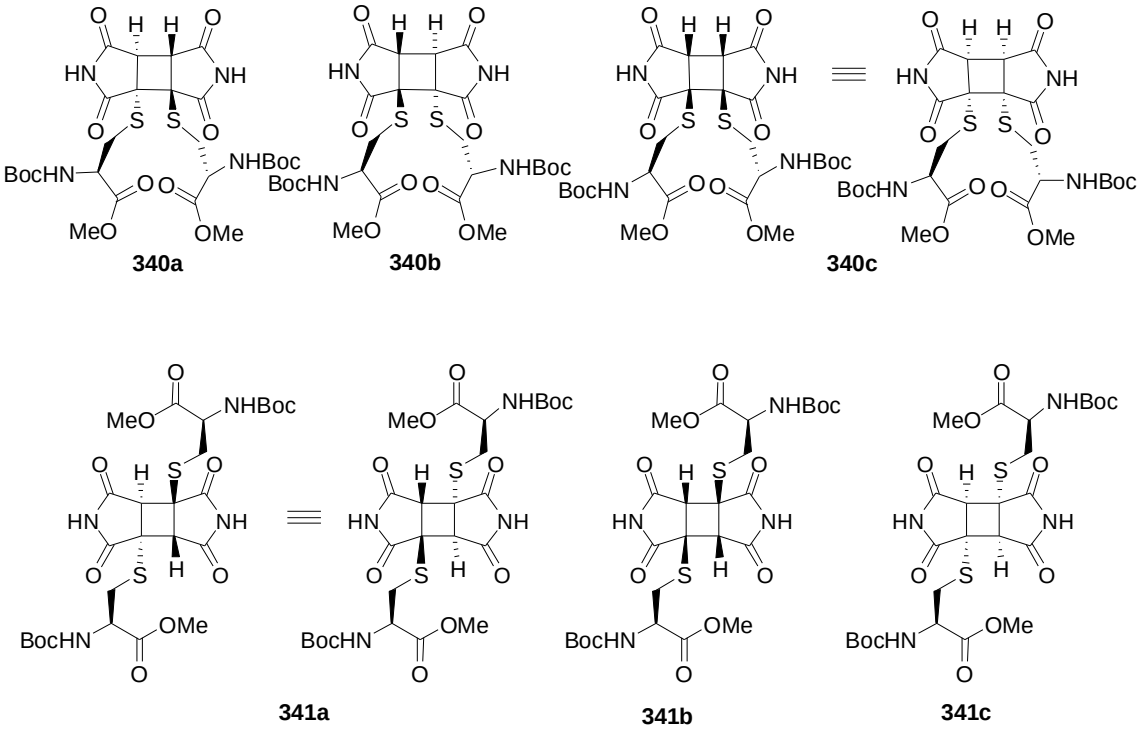
Irradiations were carried out under a standard lamp for photochemical reactions, a 250 W medium pressure (MP) Hg discharge lamp (Photochemical Reactors Ltd.), see **1.i.ii.** for further discussion. In order to determine the photochemical behaviour of thiomaleimides in proteins, thiomaleimide **291** was initially chosen for investigation. After five minute of irradiation, the thiomaleimide **291** had undergone complete conversion to what appeared to be one product. ^{1}H NMR and mass spectroscopy indicated that the thiomaleimide **291** had successfully dimerised, **Scheme 171**, although the ^{1}H NMR spectrum showed quite broad peaks due to the hindered rotation of the bonds afforded by the carbamate. Analysis of the ^{13}C NMR indicated that the product was actually a mixture of two very similar compounds, most likely diastereomers of one of the dimer products **340** or **341**, **Scheme 171**.



291 **340** **341**

Scheme 171

There were a variety of possible products in this reaction. Theoretically, the reaction could proceed in head-to-tail or head-to-head fashion, and the dimerisation stereochemistry could be *cis* or *trans*-affording six diastereomers, **Figure 22**, as several apparently different structures are in fact identical.



340a **340b** **340c**

341a **341b** **341c**

Figure 22: Potential products from dimerisation of thiomaleimide 291

After consulting the literature on photochemical dimerisations, it became apparent that the head-to-head interactions are often favoured.[276-281] This can be explained by examination of the frontier molecular orbitals (FMO) of the species involved in

photochemistry. Photochemical dimerisations are facile because the two important orbitals that interact are of identical energies and coefficients. This is because when a molecule becomes excited, important interactions are between the 'HOMO'/HOMO and the 'LUMO'/LUMO. In a dimerisation, these interactions arise from two of the same molecules, thus the 'LUMO' has the perfect configuration and energy to interact with the other molecule's LUMO, and the 'HOMO' has the perfect configuration and energy to interact with another molecule's HOMO, **Figure 23**. Keeping in mind that large-large and small-small coefficient interactions are energetically favoured[158] it can be seen that the head-to-head bonding orientation is highly favoured, regardless of the substituents on the double bond, **Figure 23**.

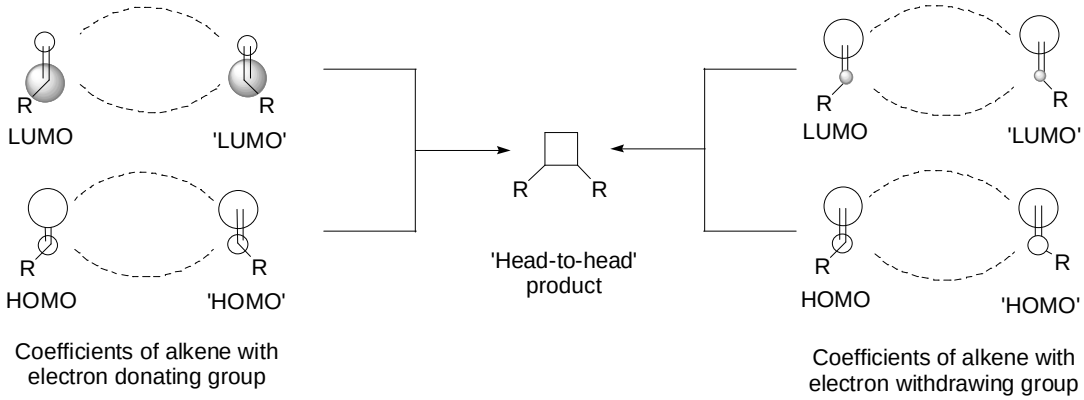
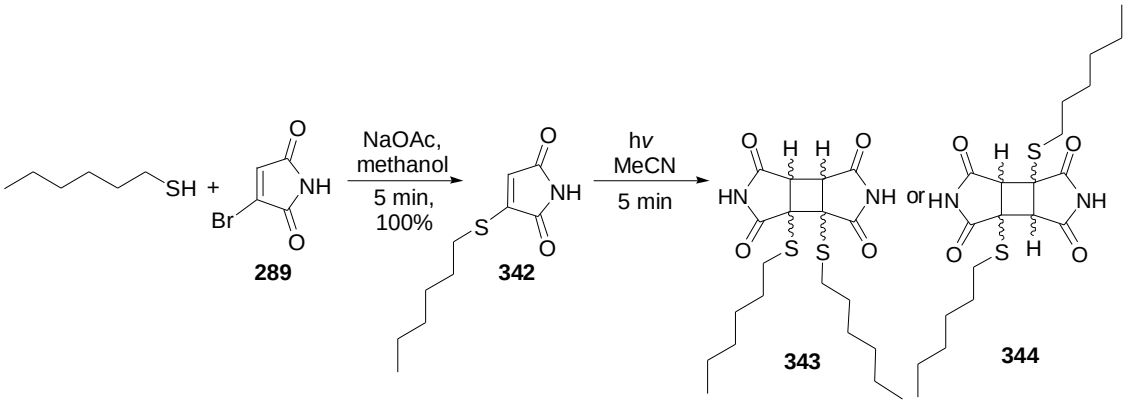


Figure 23: Stylised 'LUMO'/LUMO and 'HOMO'/HOMO interactions both afford the head-to-head product

As a mixture of two diastereomers, the newly-formed species (**340** or **341**) could not be crystallised for X-ray crystallographic analysis. Analysis of the NMR spectra was inconclusive due to the symmetries in the potential dimers and the large number of possible products.

Consequently, in order to ascertain in-depth information on the nature of the dimerisation of thiomaleimides, attention was turned to the achiral hexylthiomaleimide **342**, synthesised by addition of hexanethiol to bromomaleimide **289**, in 100% yield, **Scheme 172**. This hexylthiomaleimide **342** was also consumed in just five minutes of irradiation, **Scheme 172**.



Scheme 172

In this case however, analysis of the crude reaction mixture was far clearer. Merely removing the solvent from the reaction mixture afforded an off-white solid that was one pure compound by ^{1}H and ^{13}C NMR and mass spectroscopy. In this reaction, four possible isomers could be formed, the *cis* and *trans* adducts of the head-to-head photocycloaddition, **343a** and **343b**, and *cis* and *trans* adducts of the head-to-tail photocycloaddition, **344a** and **344b**. In all four cases, the symmetrical nature of the molecules ensured that all cyclobutane hydrogens were identical; precluding observation of coupling constants or NOes, **Figure 24**.

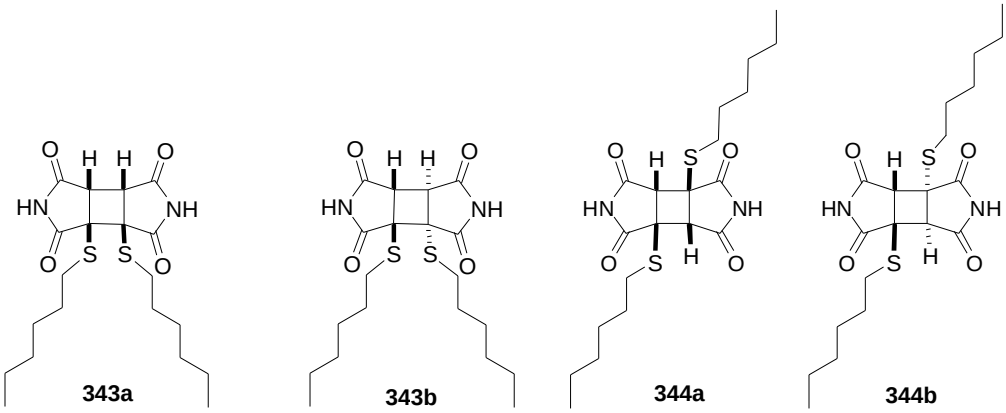
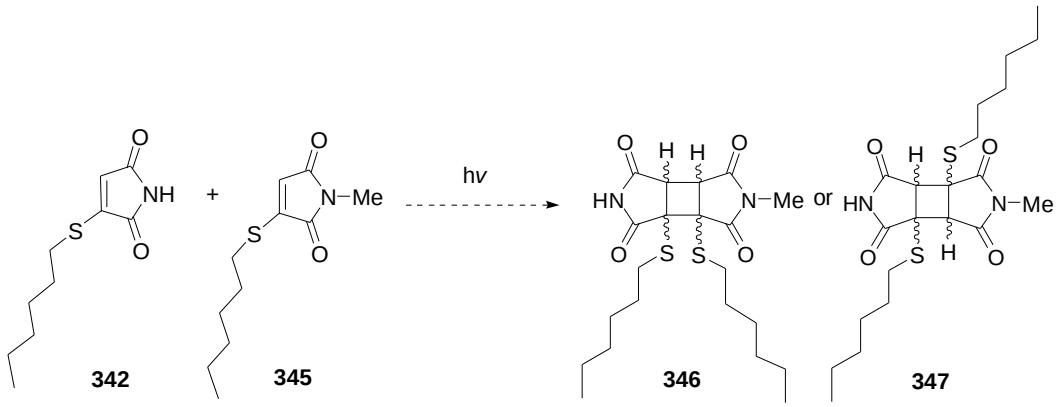


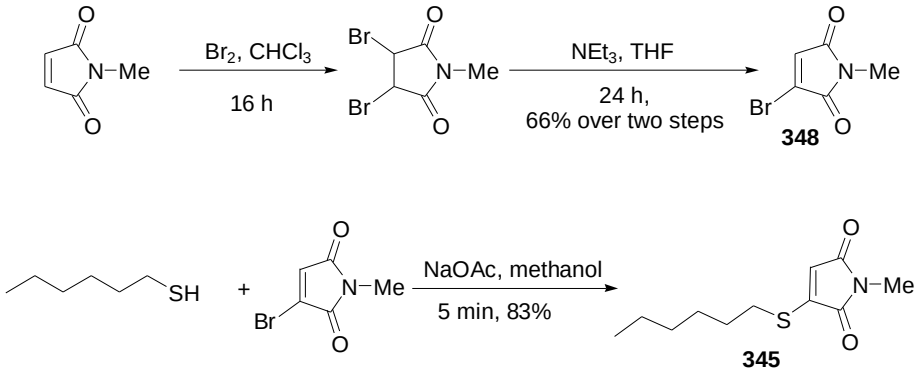
Figure 24: Potential products from dimerisation of thiomaleimide 342 and lack of through-space interactions

It was proposed that the thiomaleimide **342** would react in a similar manner with another subtly different thiomaleimide **345**, methyl-substituted on the nitrogen, **Scheme 173**. This would resolve the protons on the two sides of the cyclobutane (**346** or **347**), allowing the use of NMR correlation experiments to describe the molecule more fully.



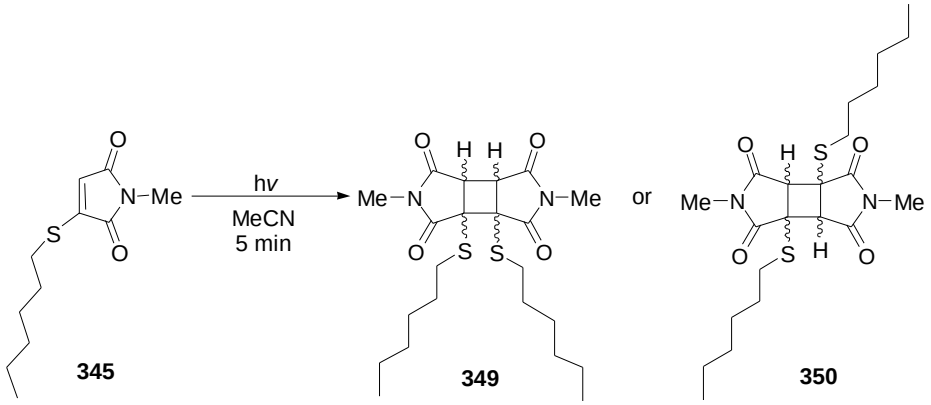
Scheme 173

Prior to carrying out this investigative reaction, it was deemed necessary to establish that the *N*-methyl derivative **345**, would dimerise in a similar time frame. Therefore the *N*-methyl bromomaleimide **348** was synthesised in the same fashion as the standard bromomaleimide **289** and reacted with hexanethiol, **Scheme 174**.



Scheme 174

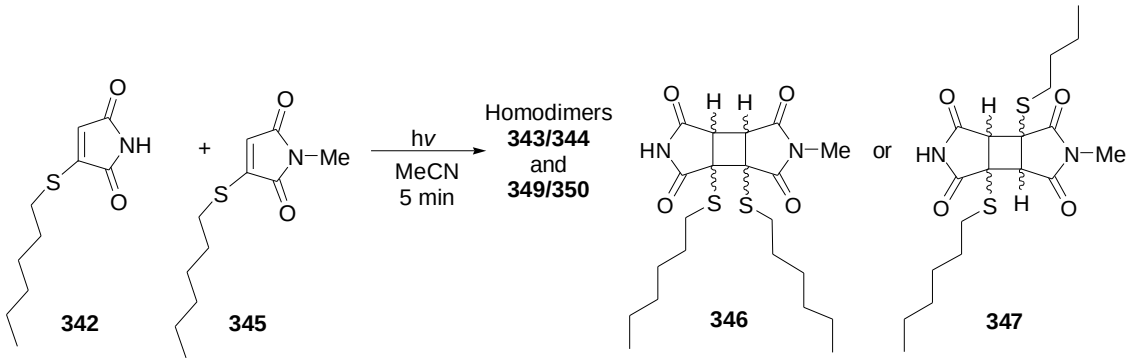
A dimeric compound (**349** or **350**) was the sole product after irradiation of the *N*-methyl thiomaleimide **345**, **Scheme 175**, seen by ^{1}H and ^{13}C NMR, and mass spectroscopy. Therefore, this reaction showed that the dimerisation was not just specific to the *N*-protic thiomaleimide **342**, and that the *N*-methyl derivative **345** could be used.



Single product of an unknown dimeric structure

Scheme 175

A 1:1 mixture of thiomaleimides **342** and **345** was irradiated for five minutes and complete consumption of the starting materials was observed, to afford a mixture of the same dimeric structures obtained previously **343/344** and **349/350**, alongside a hetero-coupled product, either **346** or **347**, **Scheme 176**.



Scheme 176

Separation of the two dimers from the hetero-coupled product (**346** or **347**) was not successful so analysis was turned to the NMR spectra of the crude mixture. Fortunately, as previously described, the dimers afforded no useful 2-D correlations and thus the only standout correlations were those of the hetero-coupled product (**346** or **347**). HH COSY (COrrelation SpectroscopY) analysis described a strong coupling between the two protons on the cyclobutane ring in the hetero-coupled product. Consistent with FMO predictions, this showed that the products formed in this reaction were head-to-head, with the hexylthiyl groups adjacent (**346**), **Figure 25**.

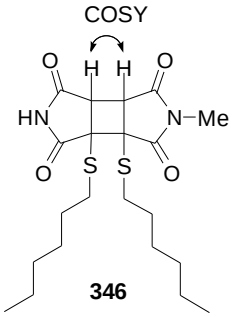


Figure 25: Adjacent protons observed by HH COSY

At this stage, possible orientations of the hetero-coupled product **346** were reduced to two, the *cis* **346a** or *trans* **346b** diastereomers, **Figure 26**.

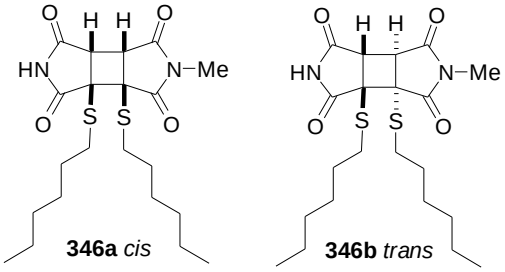
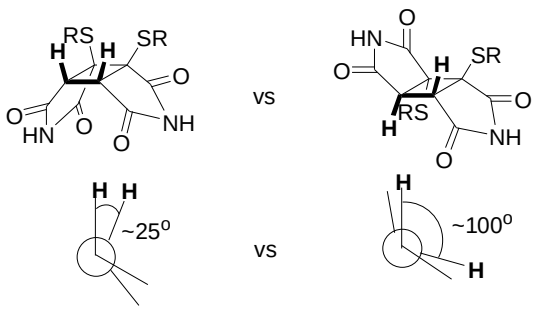


Figure 26: Possible stereochemical configuration of head-to-head dimers of thiomaleimide 346

NOeSY (Nuclear Overhauser effect SpectroscopY**)** experiments showed a medium strength coupling between the two cyclobutane protons indicating that they were 5 Å apart or less.[282] However, in such a strained molecule, this could have still been from either the *cis* **346a** or *trans* **346b** configurations,[283-285] and the literature suggested both could be possible.[281, 286] When a three-dimensional model of the structure was constructed, it could be seen that the flanking five-membered rings maintained a very flat conformation of the cyclobutane ring. On such a ring, a Newman projection helps depict the approximate angles between the protons in the ring, **Figure 27**. Analysis of the coupling constants of the doublets arising from these protons was very useful.



346a *cis* conformation and Newman projection

346b *trans* conformation and Newman projection

Figure 27: Newman projections of the cyclobutane in *cis* 346a and *trans* 346b configurations

The Newman projections depict the *cis* protons as existing at a very acute angle, whilst the *trans* protons are greater than 90°, approaching 100°. The Karplus equation,[287] **Eq. 2** describes the relationship between the dihedral angle (ϕ) and the expected coupling constants ($^3J_{HH}$).[287, 288]

Eq. 2

$$^3J_{HH}(\phi) = A + B\cos\phi + C\cos 2\phi$$

The lower curve of **Figure 28** corresponds approximately to the theoretical Karplus curve. The empirical range within which $^3J_{HH}$ is found to vary in practice is shown in the shaded reagion.[289]

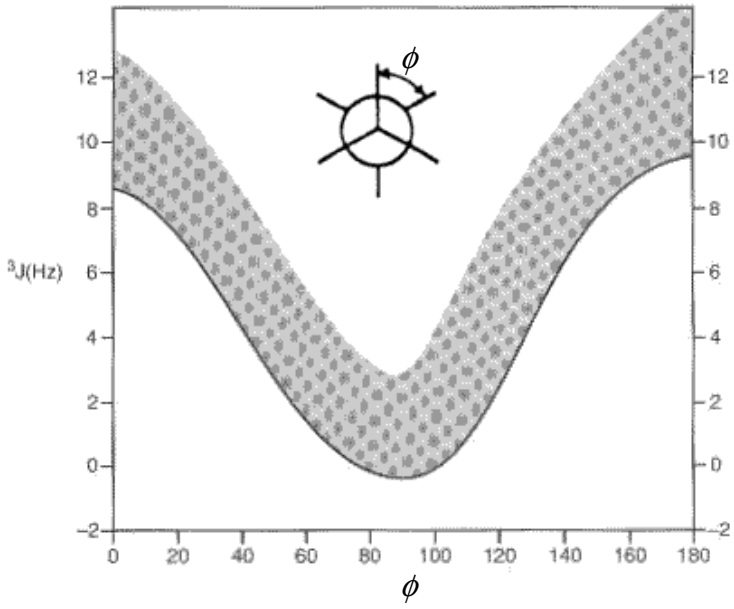


Figure 28: Graphical representation of the Karplus equation[289]

It can be seen from **Figure 28** that the *cis* protons in **346a** should afford a coupling constant of at least 7, whilst the *trans* dimer **346b** should produce a coupling constant of nearer to 3. Fortuitously, the coupling constant of the relevant protons in the hetero-coupled product **346** was 3.1, perfectly in-line with the *trans* prediction (**346b)**.

Whilst there was no literature on similar non-symmetric maleimide dimers, there was literature that described the relationships of similar protons in some flat cyclobutane rings. The *trans* protons generally coupled with a coupling constant of 3 - 4 Hz and the *cis* protons coupled with a much higher value of 8 - 11 Hz,[148, 290-292] some examples are shown in **Figure 29**.

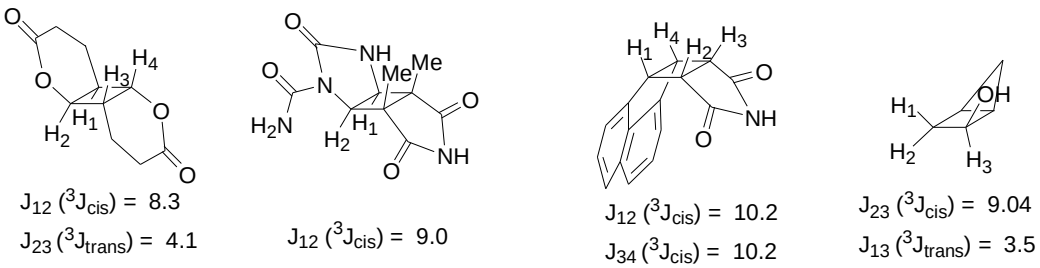


Figure 29: Literature values for *cis* and *trans* protons in flat cyclobutanes[290]

From these values it was deduced that, with a coupling constant of 3.1, the hetero-coupled product **346** had adopted the *trans* configuration **346b**, **Figure 30**.

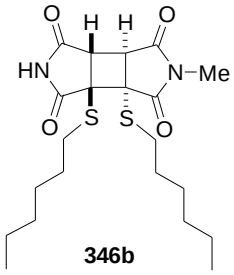


Figure 30: Assigned structure of dimer 346b

After this deduction, the structure of the homodimers **343b** and **349**, in the previous examples, **Scheme 172**, p. 163 and **Scheme 175**, p.165, could also be inferred to be head-to-head and *trans* dimers, formed in 100% yield, **Figure 31**.

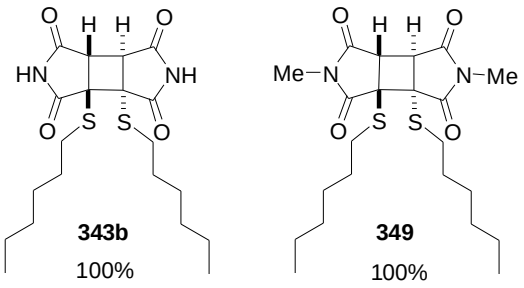
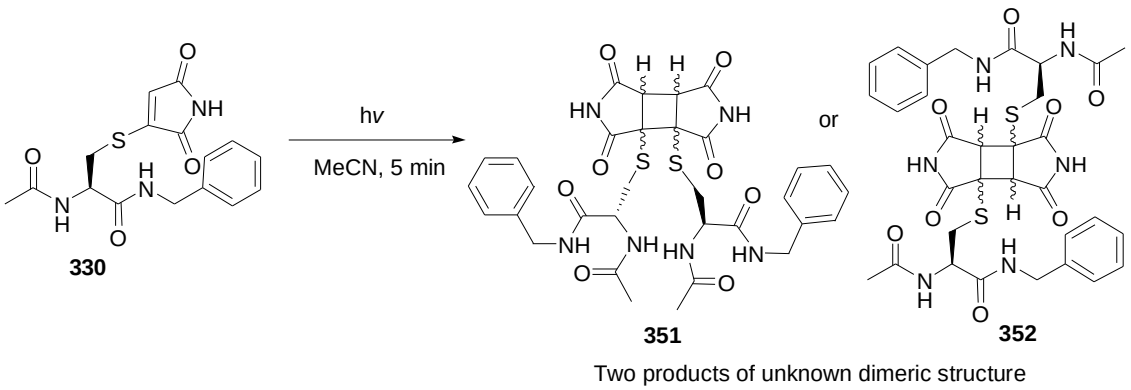


Figure 31: Assigned structure of dimers 343 and 349

With these findings in mind, attention was turned to the analysis of the product from the initial *N*-Boc-Cys-OMe thiomaleimide **291** irradiation. It was deemed somewhat presumptive to immediately assume the results of the achiral model **342** could be transferred to the chiral bulky amino acid-containing thiomaleimide **291**. Thus, further validation of the structures of the dimers **340**/**341** was sought.

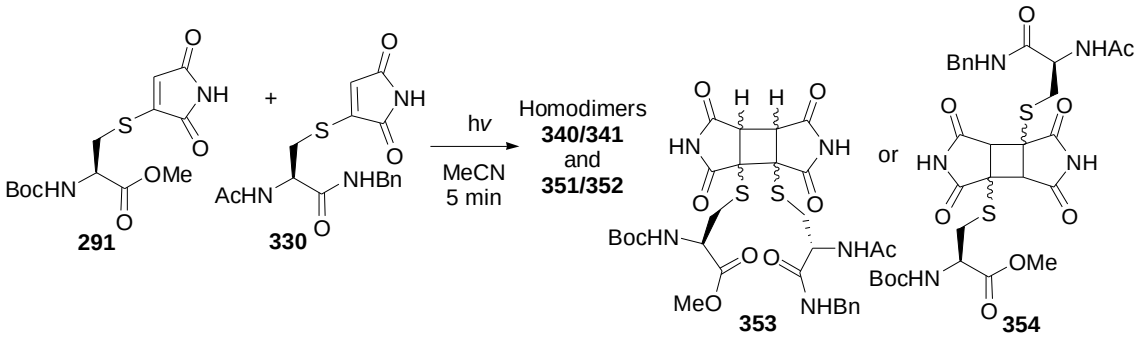
The *N*-Ac-Cys-NHBn thiomaleimide **330** was examined. It, too, was completely consumed in five minutes to what appeared to be one symmetrical product by ^{1}H NMR, **Scheme 177**. Again, however, the ^{1}H NMR spectrum showed quite broad peaks, due to the hindered rotation of the bonds afforded by the two amide bonds. Analysis of the ^{13}C NMR indicated that the product was actually a mixture of two very similar compounds, most likely diastereomers of the dimer products **351** or **352**, **Scheme 177**.



Scheme 177

To test the head-to-head hypothesis with two different bulky amino acid thiomaleimides, a 1:1 mixture of amino acid thiomaleimides **291** and **330** was irradiated for five minutes, **Scheme 178**. Unfortunately, whilst the thiomaleimides **291** and **330** were consumed in five minutes, the large number of potential compounds that could be

formed in this reaction afforded numerous peaks in the ^{1}H NMR spectrum. In addition, the free rotation of the bonds was again limited due to the carbamate and amide bonds in the amino acid protecting groups, resulting in broad peaks. These factors made analysis *via* 2-D NMR experiments, *e.g.* COSY and NOeSY, particularly inconclusive.



Scheme 178

An alternative approach to determine the structure and stereochemistry of these symmetrical products involved analysis of ^{13}C satellite peaks in the ^{1}H NMR spectrum. The analysis was undertaken with the assistance of Dr Abil Aliev (UCL, Chemistry). Effectively, the 1.1% of carbon atoms that existed as ^{13}C in the cyclobutane ring, C1-C4, **Figure 32**, would render a small percentage of the dimeric molecules unsymmetrical, and splitting from non-identical protons could be seen in the satellite peaks of the CH in the ^{1}H NMR.

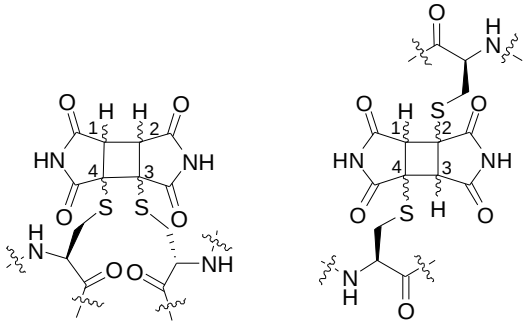


Figure 32: Dimers are rendered unsymmetrical by 1.1% ^{13}C

This analysis is only successful when the peaks in question are distinct from all other signals. Fortunately, for cyclobutane dimer **351/352** in deuterated acetonitrile, two different satellite peaks from the cyclobutane CH were in isolated regions of the spectrum and both showed coupling constants of 3.4. This was in accordance with the

achiral model **346b** and led to the determination of the amino acid thiomaleimide dimers as head-to-head and in *trans* configuration, **351a** and **351b**, **Figure 33**. As no other peaks were present in the NMR, the yield was 100% across the two compounds.

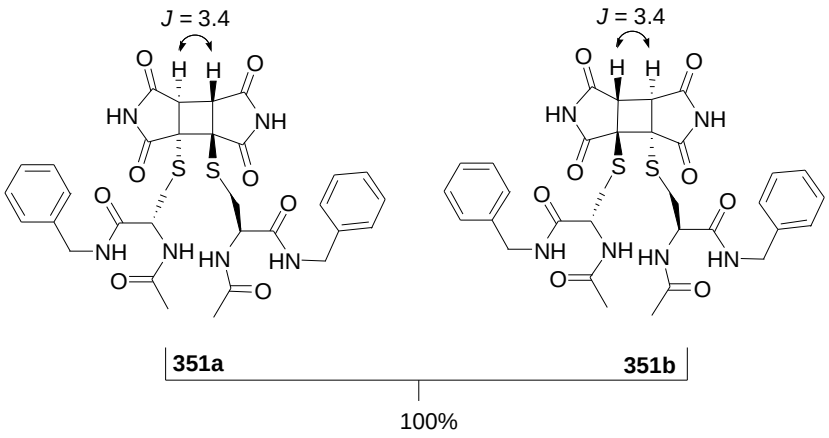


Figure 33: Assigned structure of dimers 3511 and 351b

Whilst the ^{13}C satellite peaks for dimers from irradiation of thiomaleimide **291** could not be resolved from the rest of the spectral peaks, it was deemed a justifiable comparison to assume the dimers had adopted the same head-to-head *trans* configuration as the two model systems **346** and **351**, **Figure 34**. Again, as no other peaks were present in the NMR, the yield was 100% across the two compounds.

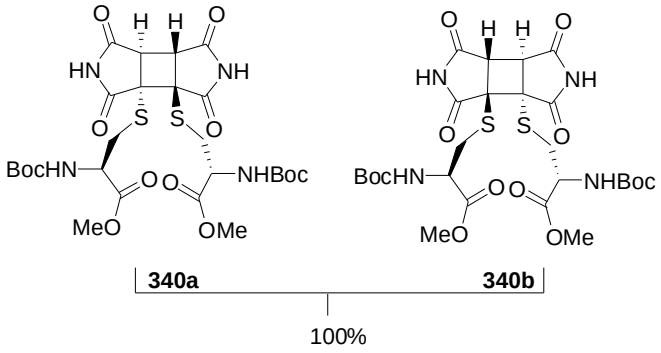
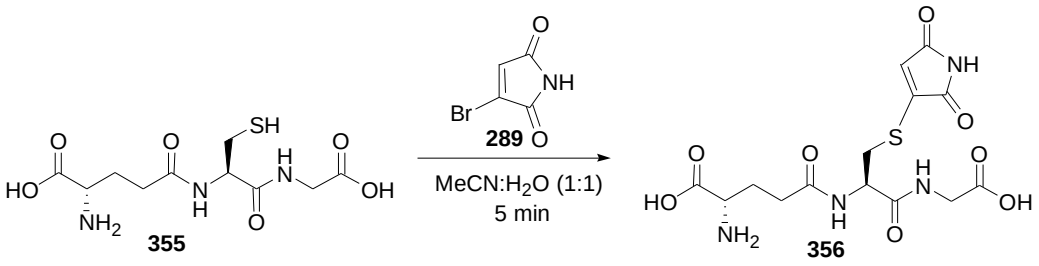


Figure 34: Assigned structure of dimer 340

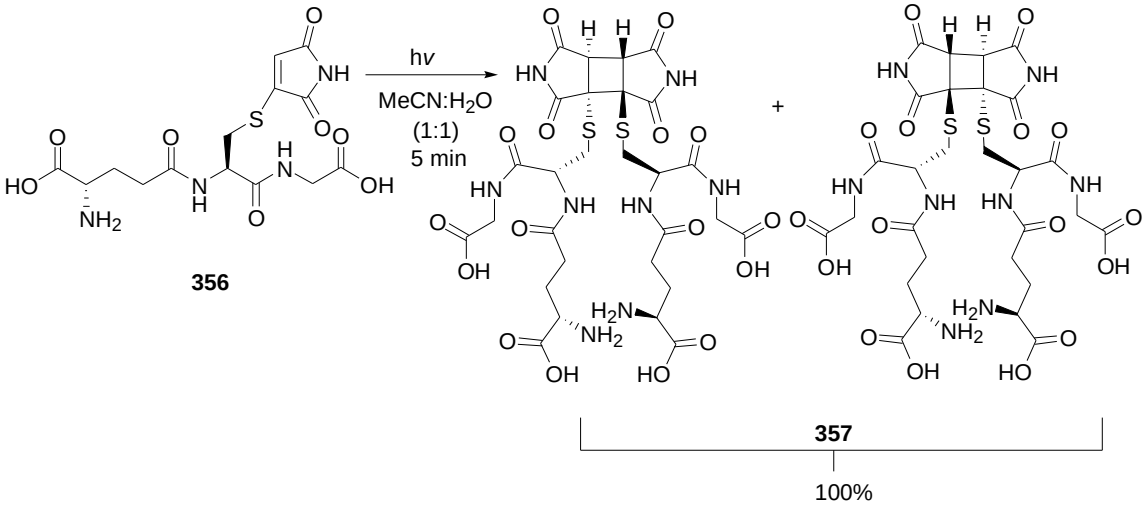
To transfer the dimerisation on the single amino acid to peptides, the use of the tripeptide glutathione was proposed. Bromomaleimide **289** was added to the commercially available glutathione **355**, affording a tripeptide thiomaleimide model **356**, **Scheme 179**. This reaction was carried out in MeCN:H_2O to solubilise the glutathione **355**. Unfortunately, the polarity of the conjugate **356** caused isolation

problems and this necessitated use without further purification. NMR spectroscopy indicated that the thiomaleimide **356** had the correct structure.



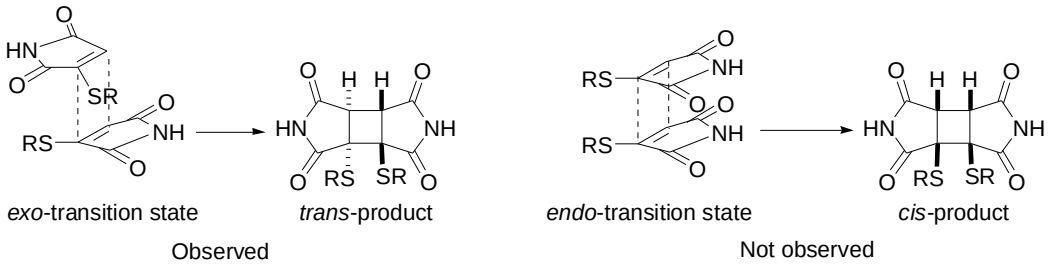
Scheme 179

The reaction solution from **Scheme 179** was irradiated to complete consumption in five minutes, **Scheme 180**. The dimer **357** was not subjected to further purification as the high polarity of glutathione made column chromatography very challenging. The ^{1}H NMR spectrum was complex but indicated the dimerisation products had formed and mass spectroscopy supported the formation of the dimer. The stereochemistry of the addition is shown as *trans* as well as head-to-head by analysis of the NMR spectra, and with analogy to the model systems. The use of the glutathione maleimide conjugate **356** further suggested that the reaction could be applied to bulky substituents and potentially even proteins. As no other peaks were present in the NMR, the yield was tentatively suggested as 100% across the two compounds.



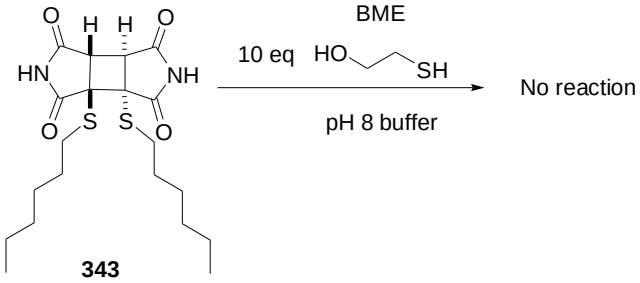
Scheme 180

The observed formation of the *trans* dimers can be explained by looking at the possible transition states. The *trans* dimers would originate from *exo*-transition states, whilst the *cis*-dimers (not observed) would be formed from the *endo*-transition states, **Scheme 181**. If there is a lesser steric clash between the SR groups and the maleimides, than between the SR groups with each other and the maleimides with each other, then the *exo*-transition state is the least sterically hindered approach. In addition, if there are no secondary orbital effects between the molecules that draw the maleimides to eclipse one another, this would be expected. The *trans*-products were exclusively observed, indicating that the reaction proceeded through the *exo*-transition state, and therefore, that the smallest steric clash was indeed between the SR group and the maleimide.



Scheme 181

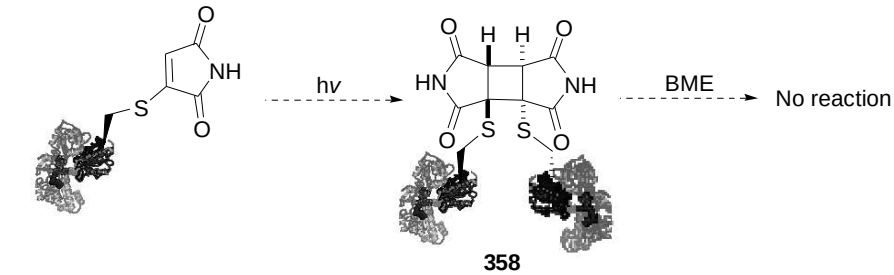
The thiomaleimide **291** eliminated cysteine when exposed to ten equivalents of *β*-mercaptoethanol (BME), **Scheme 154**, p. 142. To determine the dimers' reactivity towards BME, dimer **343** was treated with ten equivalents of BME in pH 8 buffer. No elimination of the thiol was observed, **Scheme 182**. This indicated that the photochemical reaction had conferred irreversibility on the modified thiol.



Scheme 182

The dimerisation of thiomaleimides to cyclobutanes could potentially be applied to dimerisation of proteins, **Scheme 183**. The dimerised protein **358** would contain a two-

carbon bridge between two cysteine residues, mimicking a disulfide bridge that would be stable to reducing conditions. This stable, photochemically-mediated linkage could potentially be useful to investigate the differences in protein behaviour between monomers and dimers. The tight control that the use of light imparts on this reaction could be used to define the exact time and location at which the dimerisation takes place as, prior to irradiation, the thiol is 'trapped' in the thiomaleimide and a disulfide cannot form. Once formed, the new two-carbon disulfide 'mimic' would be stable to reducing conditions.



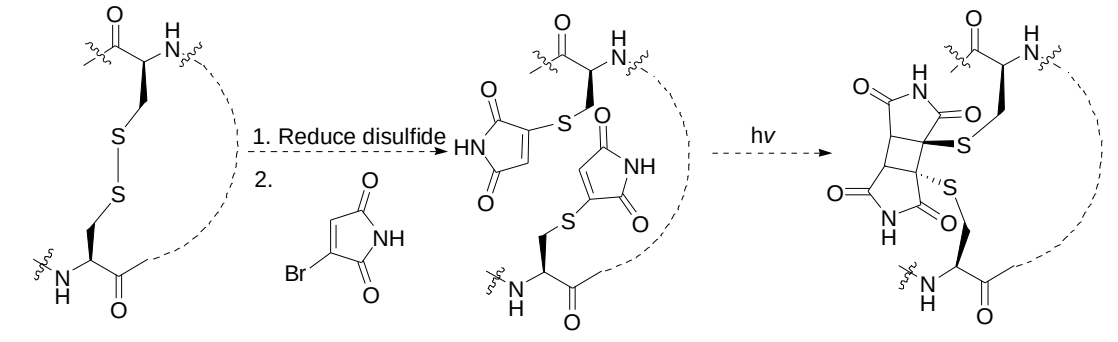
358

Protein-thiomaleimide monomer

Stable protein-cyclobutane dimer

Scheme 183

Alternatively, such dimerisation could be used to dimerise thiomaleimides within the same protein. For example, disulfide bridges in a protein are susceptible to cleavage by reducing conditions *e.g.* excess thiols, TCEP *etc.*. If a disulfide bond in a protein **359** was cleaved and reacted with thiomaleimide, a double labelled protein **360** would arise from labelling of both thiols, **Scheme 184**. This construct would still be cleavable by addition of excess thiols or TCEP. However, upon irradiation, the newly formed cyclobutane **361** would render the 'disulfide' linkage stable to reducing conditions, **Scheme 184**. Again, the use of light to promote the disulfide mimic could define the exact time and place whereby such 'disulfides' would be rendered irreversible. The formation of specifically the head-to-head dimers is particularly useful when this strategy is proposed as a disulfide mimic. In the cyclobutane product **361** the thiyl groups are close to one another and it is envisaged that this would perturb folding less than if the dimer formed was head-to-tail.



359
Protein with a disulfide - unstable to reducing conditions

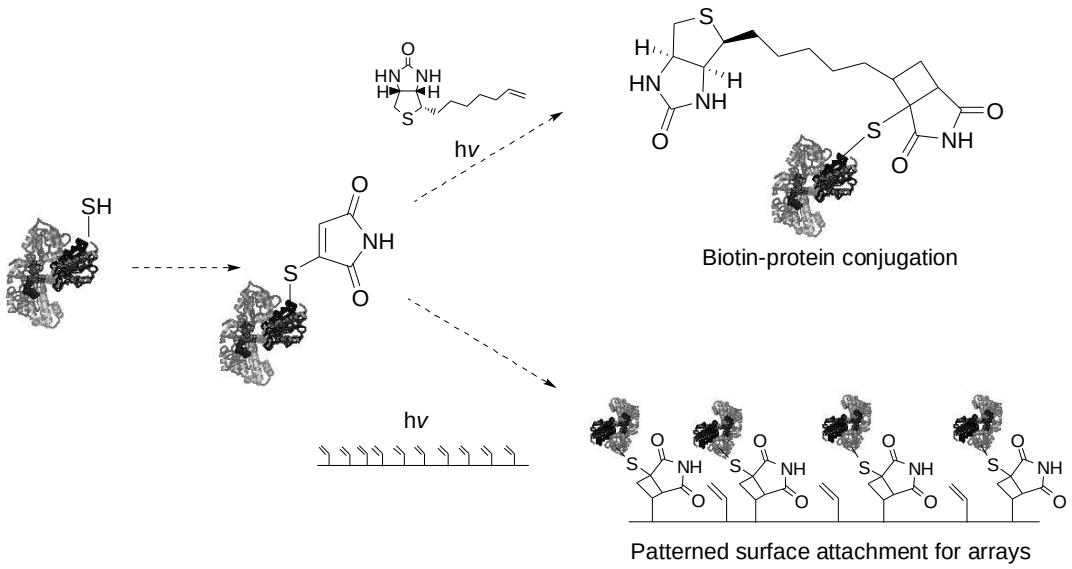
360
Protein with thiomaleimides - unstable to reducing conditions

361
Protein bridged *via* cyclobutane - **stable** to reducing conditions

Scheme 184

Photochemical conjugation of thiomaleimides with olefins

As it was now known that thiomaleimides were indeed photoactive, it was a goal of the work to apply thiomaleimides as partners for conjugations *via* [2+2] photocycloadditions to olefins. The small molecule work would serve as a model towards the development of a novel photochemical bioconjugation strategy. For example, if thiomaleimides were found to undergo successful photocycloadditions with olefins, it was envisaged that thiomaleimide-labelled proteins could be tethered to a molecule of interest such as biotin or to a solid surface *via* an alkene, **Scheme 185**. This would allow photochemical bioconjugation starting from modification of a native amino acid, compared to current methods in **1.ii.iv.** which largely occur from modification of non-native amino acids.

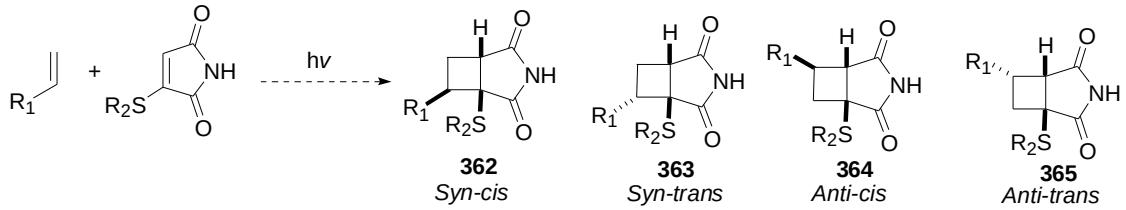


Biotin-protein conjugation

Patterned surface attachment for arrays

Scheme 185

It was recognised at this stage that the conjugation of a thiomaleimide with a substituted alkene could afford two different regiomeric outcomes. In line with literature describing dimerisation and photochemical reactions,[276-279, 284, 286] when the two bulky groups are adjacent, the structure is referred to as *syn* **362** and **363**, **Scheme 186**. When the bulky groups exist across the ring from one another, the structure is referred to as *anti*, **364** and **365**, **Scheme 186**. The diastereomers that are then afforded are referred to as *cis*, **362** and **364** or *trans*, **363** and **365**, depending on the alignment, or lack thereof, of defined substituents. For example in **Scheme 186**, *cis* and *trans* are defined with respect to the R_1 and R_2 groups.



Scheme 186

Regiomeric preferences of the photochemical conjugation

The outcomes of photochemical conjugations can often be explained with the use of frontier molecular orbital (FMO) perturbation theory. Using FMO perturbation theory, the coefficients of the atoms involved in bond formations can be estimated. Alkenes bearing an electron donating group, X, can have their HOMO and LUMO estimated by combination of the HOMOs and LUMOs of ethylene and the allyl anion,[158] **Figure 35**.

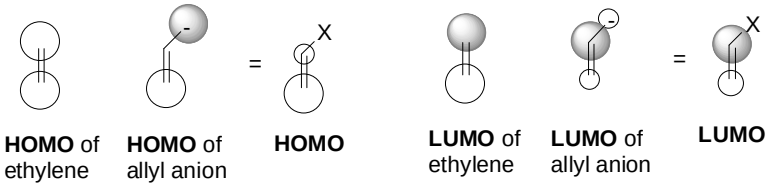


Figure 35: Estimations of the HOMO and LUMO coefficients of an alkene substituted with an electron donating group, X[158]

Alkenes in conjugation can be approximated by examining the HOMO and LUMO of butadiene,[158] **Figure 36**.

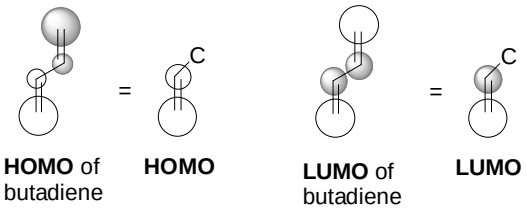


Figure 36: Estimations of the HOMO and LUMO coefficients of an alkene substituted with a conjugating group, C[158]

Alkenes with an electron withdrawing substituent, Z, can be estimated by merging the HOMOs and LUMOs of butadiene and the allyl cation,[158] **Figure 37**.

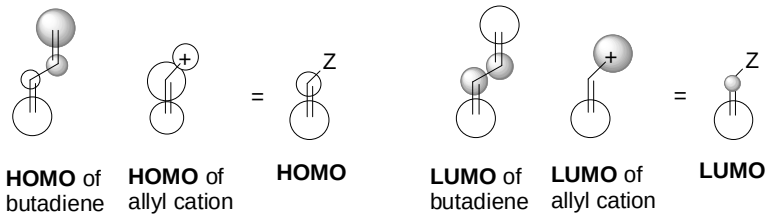
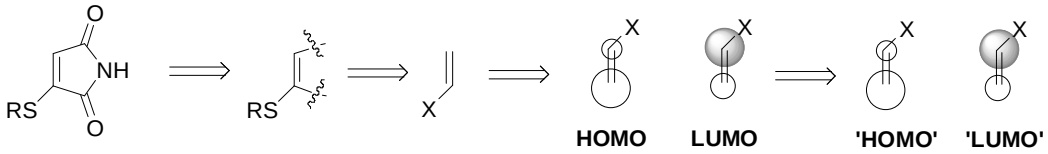


Figure 37: Estimations of the HOMO and LUMO coefficients of an alkene substituted with an electron withdrawing group, Z[158]

When a molecule is photochemically excited, an electron is promoted to the next available molecular orbital. Thus the orbital configuration and coefficients of the HOMO and LUMO become those of the 'HOMO' and 'LUMO', (see **1.i.** for further discussion). As discussed the interactions that are important in photochemistry are those between the 'HOMO'/HOMO and the 'LUMO'/LUMO of the excited molecule/ground state molecules.

To determine the expected preference of the reaction of thiomaleimide **342**, the coefficients of its 'LUMO' and 'HOMO' had to be estimated. In order to do this, the system had to be likened to one of the three types of alkene, namely, either substituted with an electron donating group, a withdrawing group or as an alkene in conjugation. The thiomaleimide alkene had several substituents, all exerting an effect on the coefficients of the orbitals. However, it was predicted that the thiomaleimide alkene coefficients would have the same effect exerted upon either side by the imide system of the maleimide. Therefore for the purpose of likening orbital coefficients, the maleimide was ignored. The double bond's coefficients were therefore likened to an alkene

substituted with an electron donating group, **Scheme 187**. Once excited, the coefficients of the HOMO and LUMO of this double bond become the coefficients of the 'HOMO' and 'LUMO', respectively. It was these orbitals that were used to explain the resultant regiochemical outcome.



Scheme 187

The [2+2] photocycloadditions would require dominant interactions either between the 'LUMO' with the LUMO of the reactive partner and the 'HOMO' with the HOMO of the reactive partner. It was uncertain how the thiomaleimide systems would behave so the outcomes of HOMO/'HOMO' domination and LUMO/'LUMO' domination were predicted, **Figure 38**.

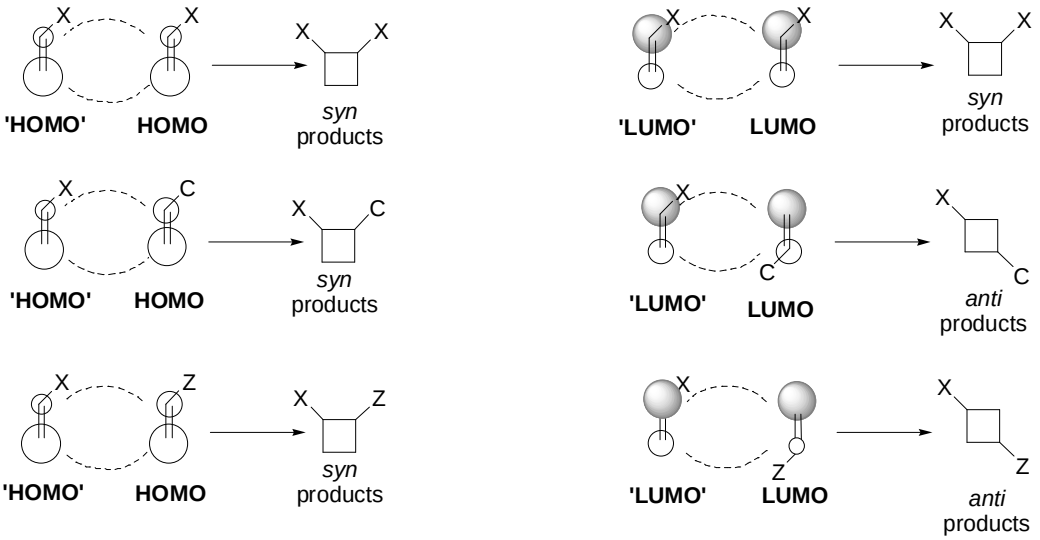
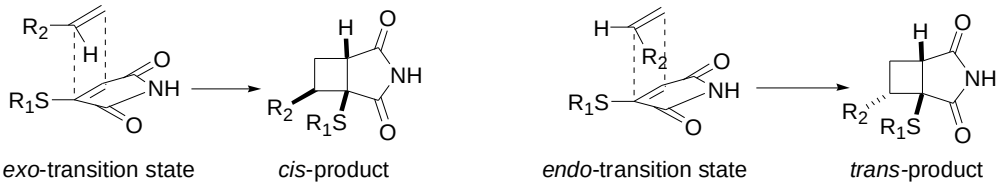


Figure 38: 'HOMO'/HOMO *versus* 'LUMO'/LUMO interactions and products

As can be seen in **Figure 38**, if 'HOMO'/HOMO interactions are dominant, whether the reactive partner is substituted with an electron donating group (X, alkyl), a conjugating system (C, aryl) or an electron withdrawing group (Z, acrylate), overlap of largest coefficients always places the substituent groups adjacent to each other, in *syn* configuration. If 'LUMO'/LUMO interactions dominate the transition state, then the outcome is mixed depending on the nature of the alkene partner.

As for the stereochemistry observed, the two substituents can also be *cis* or *trans* to each other, **Scheme 188**. In the following examples, the *exo*-transition state is defined as where the substituent on the alkene (R_2) is away from the maleimide in the transition state, and affords the *cis* product, **Scheme 188**. The *endo*-transition state is where R_2 sits over the maleimide in the transition state, and leads to the *trans* product, **Scheme 188**. If R_1 and R_2 create a lesser clash, the *exo*-transition state describes the least sterically hindered approach of the two molecules, and this would usually be expected. If R_1 and R_2 create a significant steric clash or secondary orbital effects draw the substituent on the alkene over the maleimide, then the *endo*-transition state and *trans* product is expected. Secondary orbital effects can occur from orientation of the molecules in the ground state or the excited state.[158, 293]



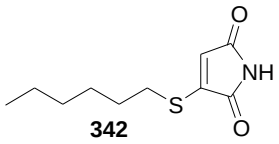
exo-transition state *cis*-product *endo*-transition state *trans*-product

Scheme 188

As discussed, there is a real benefit for constructing bonds using irradiation. The tight spatiotemporal control that light affords can allow the design of complex patterned arrays or specify the exact time and location for a reaction to occur. Therefore, the investigation of good alkene partners commenced. To minimise complications from the different regioisomers from the orientation of the alkene addition to the thiomaleimide, initially some symmetrical alkenes would be investigated. A literature search suggested that electron poor alkenes would also be useful partners for these reactions,[151] as they had been observed to undergo less side-reactions during photochemistry. Therefore, acrylonitrile was chosen as an electron deficient alkene with few protons that could complicate subsequent ^{1}H NMR analysis. In research of photochemistry for surface modification strategies,[151] acrylates had been employed, thus they were also chosen for investigation. Terminal alkenes[210] and alkynes[174] have been incorporated into proteins for subsequent bioorthogonal modifications, including those that are photochemically mediated,[189, 210] thus simple olefins and alkynes were proposed as potential partners. In

addition to alkenes with literature precedent, several other alkenes were also chosen to probe the photochemical reactivity of thiomaleimides.

In the small molecule studies, the conversion times and yields of the dimerisations had all mirrored one another in that they were all complete in five minutes to afford high yields of head-to-head dimers. Therefore, a simpler example to the amino acid containing thiomaleimides, an achiral hexylthiomaleimide **342**, **Figure 39**, was adopted as a model structure to probe the conjugation reactions of thiomaleimides. It was envisaged that this would minimise the complications of further diastereomeric products possible when a chiral centre is already present in the thiomaleimide. The achiral thiomaleimide **342** was used as a simple model to probe ideal conditions for eventual transferral to amino acid containing variants.



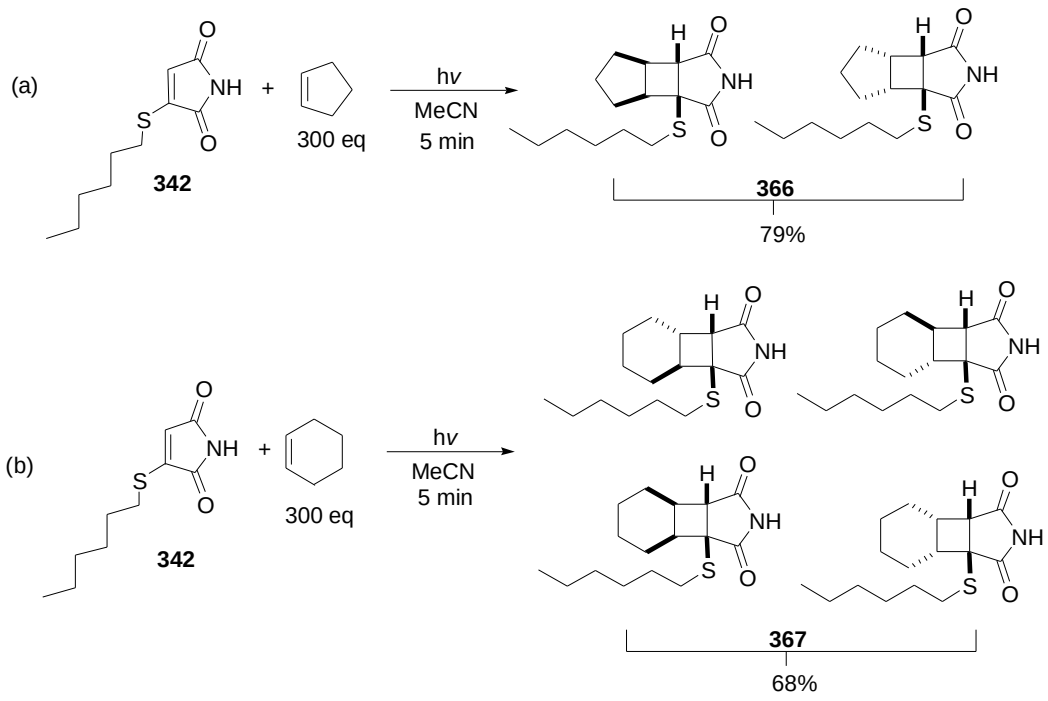
342

Figure 39: The achiral thiomaleimide was proposed for further model studies

Symmetrical olefins

As discussed, in an effort to minimise complications arising from regioisomers, some symmetrical olefinic partners were initially investigated. Varying the equivalents of the photochemical partners was implemented in an attempt to overcome the competing facile dimerisation of thiomaleimide **342**. The irradiations were carried out and the presence of dimer **343** was checked by analysis of the ^{1}H NMR spectrum of the crude reaction mixture. When it was deemed that there was no dimerisation, the corresponding crude mixtures were subjected to column chromatography to afford isolated material.

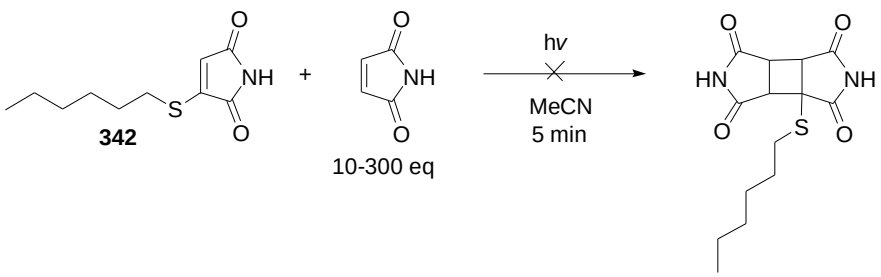
Cyclopentene was a good partner at three hundred equivalents, affording a mixture of two diastereomers **366** in 79%, **Scheme 189 (a**). Cyclohexene afforded a mixture of four diastereomeric compounds **367** in 68% yield in five minutes, **Scheme 189 (b**). In each case the diastereomers were inseparable and specific signals from the NMR spectra could not be attributed to a particular structure. The formation of the different diastereomers in these reactions indicated that both the *endo*- and *exo*-transition states were possible for these partners.



Scheme 189

Lowering the equivalents of either of the cyclic alkenes below three hundred, *e.g.* to one hundred equivalents, led to emergence of the dimerisation product **343** alongside the conjugation products.

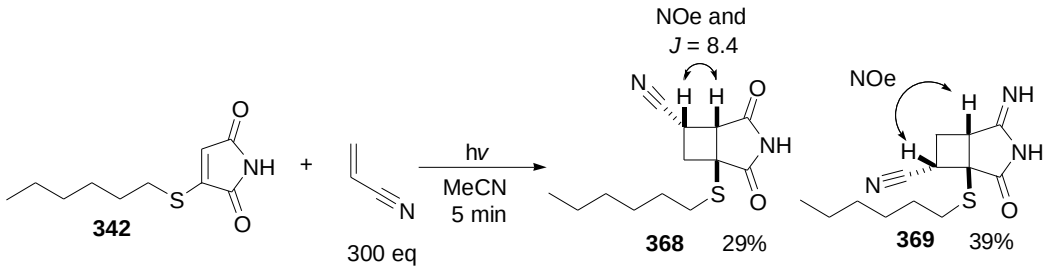
Maleimide had been used as a partner for research into cycloadditions for bioconjugation[196] and surface strategies,[153] thus it was chosen as a possible partner. Unfortunately, regardless of the number of equivalents, it was never a successful reaction partner, **Scheme 190**. It was found that maleimide polymerisation dominated the reaction at high equivalents and dimerisation of thiomaleimide **342** occurred when lower equivalents of maleimide were used.



Scheme 190

Electron deficient olefins

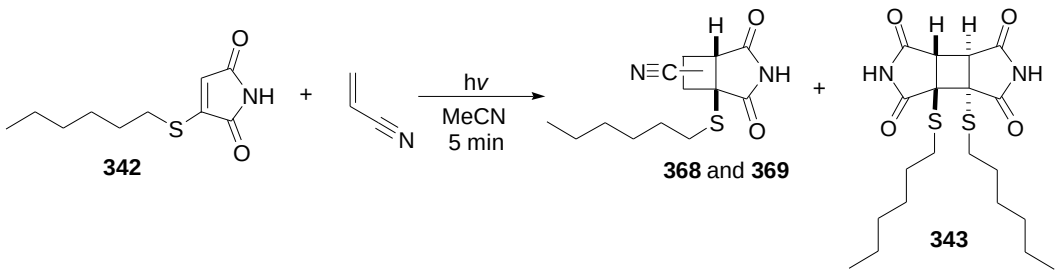
A patent by Liu *et al.*[152] described the use of maleimide and electron deficient alkenyl partners in [2+2] photocycloaddition, thus electron poor olefins were investigated. Acrylonitrile was chosen as an initial partner as it was both electron poor and contained few protons that could complicate the resultant analysis. Three hundred equivalents of acrylonitrile were required to completely negate dimerisation. The products formed in this reaction were the two isolable regioisomers of the addition of the acrylonitrile **368** and **369**, but each regioisomer was one particular diastereomer, in 29% and 39% yield, respectively, **Scheme 191**. From through-space interactions deduced from NOeSY experiments and coupling constants, it was determined that the nitrile was *trans* to the thiyl group in both cases.



Scheme 191

Owing to the lower number of diastereotopic protons in the conjugation products of this reaction compared to when cyclohexene and cyclopentene were used, the crude reaction mixtures were easily analysed, and the results are shown in **Table 27**. Analyses of the crude reaction mixtures by ^{1}H NMR clearly showed that decreasing the equivalents of acrylonitrile allowed the dimerisation reaction to take place in increasing amounts, **Table 27**.

Table 27: Photoconjugation of thiomaleimide 342 with varying equivalents of acrylonitrile



Entry	Equivalents	Ratio of (368 + 369):343 [a]
1	300	100:0[a]
2	100	73:27[a]
3	10	23:77[a]
4	1	10:90[a]

[a]Ratio obtained from analysis of the crude reaction mixture by ^{1}H NMR, these values are not yields.

An interesting result with acrylonitrile was that the two different regioisomers **368** and **369** had been formed in similar yields. There are two postulated explanations for this outcome. Firstly, the formation of both products could be attributed to the reaction occurring from competing interactions of both the 'LUMO'/LUMO of the reactive partners as well as the 'HOMO'/HOMO, leading to both products, **Figure 40**.

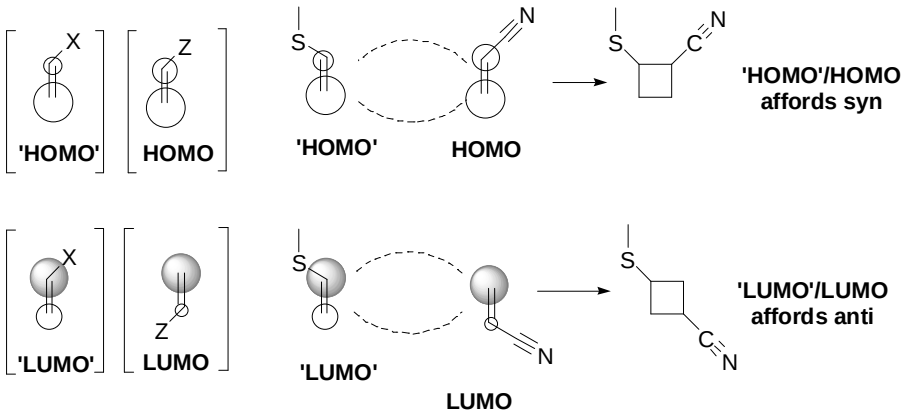


Figure 40

An alternative explanation for this outcome could be due to the coefficients assigned to acrylonitrile. As a Z-substituted alkene, its coefficients are modelled on a combination of the butadiene and ally cation orbitals, **Figure 37**, p. 177. The model is taken from Fleming's interpretation of perturbation theory and is a generalisation for Z-substituted

olefins.[158] However, acrylonitrile is a complex example of a Z-substituted olefin, whereby, the allyl cation can be given more weight in the construction of estimated orbitals, as the C≡N bond is highly polarised.[158, 294] This subtle change can alter the polarisation of the HOMO of acrylonitrile, and thus this alternate view of the orbitals can afford the *anti* product from 'HOMO'/HOMO interactions, **Figure 41**. As there are two contrasting interpretations of acrylonitrile's polarisation, it could be postulated that the double bond is not heavily polarised towards either end of the double bond,[293, 295] and both orientations are possible from the same 'HOMO'/HOMO interaction.

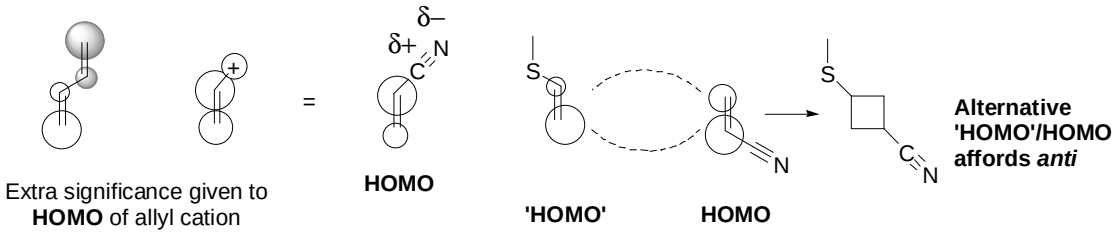


Figure 41

The *trans* diastereomers **368** and **369** of both regioisomers were formed, suggesting that the reactions had proceeded through the *endo*-transition state. In the case of acrylonitrile, the olefin substituent was very small. This could have resulted in steric interactions becoming negligible and even small increased interactions between a π- or π*-orbital in the nitrile system and the excited or ground state maleimide carbonyl affording the *trans* configuration of both regioisomeric compounds, **Figure 42**.

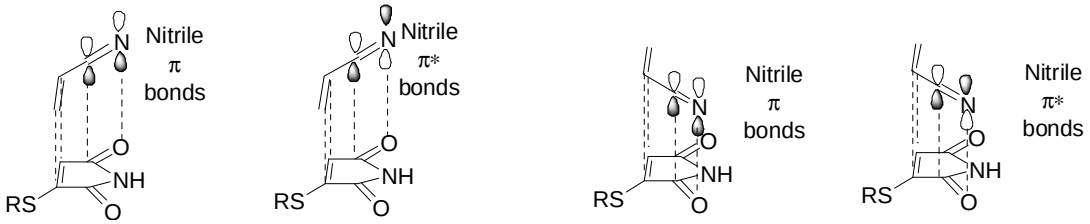
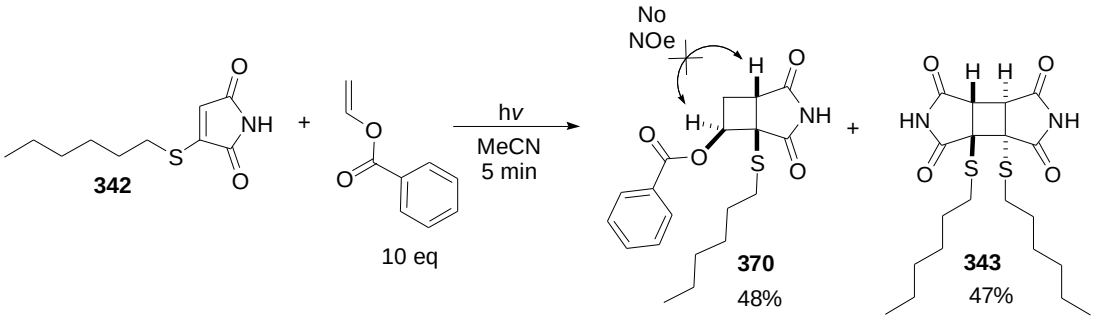


Figure 42: Postulated secondary orbital interactions that could afford the *endo* transition state

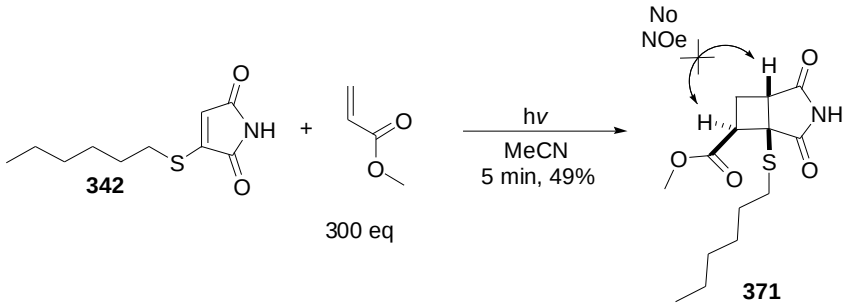
Acrylates had been implemented in [2+2] strategies towards surface modifications,[151-153] therefore phenyl and methyl acrylate were explored as possible partners. Phenyl acrylate itself caused problems in isolation of the conjugation product when used in large excess (100-300). It co-eluted with all products in column chromatographic

purification and dominated any attempts at analysis. The best result obtainable was to use ten equivalents of phenylacrylate. Whilst 47% dimerisation product **343** was also isolated, 48% of a conjugation product **370** was afforded. This product was just one regioisomer and just one diastereomer, **Scheme 192**. The *syn* addition of the acrylate was in accordance with dominant 'HOMO'/HOMO interactions rather than 'LUMO'/LUMO interactions. The diastereomer was assigned as *cis* with respect to the thiyl and the benzoate group due to the lack of through space interactions seen between the protons shown in **Scheme 192**. The *cis* configuration of this diastereomer **370** supported the formation of the cyclobutane through the least sterically hindered *exo*-transition state, as expected. If the orientation of the benzoate and the thiyl were *trans*, the distance between the two protons shown should be less than 3 Å.[283, 284] This would afford a strong through-space interaction that would be seen by NOeSY NMR experiments. Indeed, this coupling was seen when *trans* configurations were formed, **Scheme 191**, p. 182, thus its absence was taken to infer *cis* orientation of diastereomer **370**.



Scheme 192

Methyl acrylate was employed in three hundred equivalents to afford 49% of the single conjugation product **371**, **Scheme 193**, again as one regioisomer and one diastereomer. Lowering the equivalents of methyl acrylate also afforded dimer **343** as a by-product. Again, the *syn* addition of the acrylate was in accordance with dominant 'HOMO'/HOMO interactions rather than 'LUMO'/LUMO interactions. The *cis* configuration of this diastereomer **371** supported that this reaction proceeded through the *exo*-transition state, as expected.

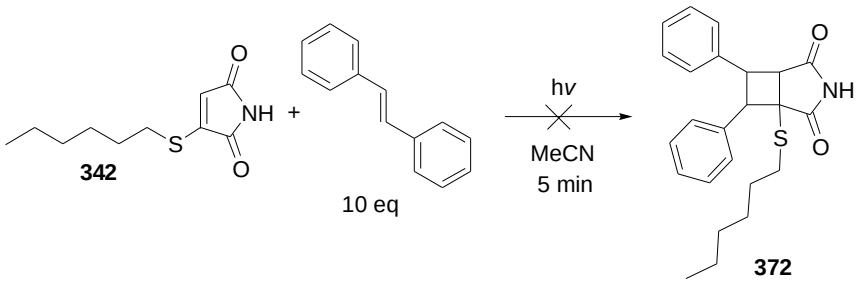


Scheme 193

At this stage, both *syn* and *anti* addition of the alkenes had been observed, as well as *cis* and *trans* orientation of the substituents on the cyclobutane ring. It was envisaged that, in the course of investigations into olefinic partners, further trends in the addition and orientation of the substituents would be established

Conjugated olefins

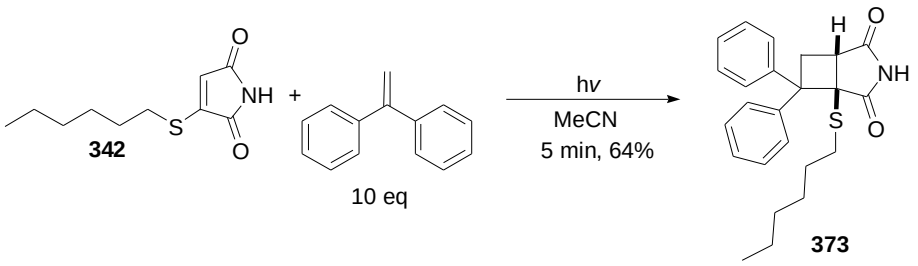
Stilbene, 1,1-diphenylethylene and styrene were chosen as conjugated alkenes that could be useful photocycloaddition partners as their own absorptions were minimal in the region targeted by the lamp.[296-299] When ten equivalents of *trans*-stilbene were used, no starting material **342** or dimer **343** were observed by analysis of the crude reaction mixture by ^{1}H NMR spectrum. However, after column chromatography, neither the cyclobutane **372** nor any identifiable products were isolated, **Scheme 194**. Increased steric hindrance on both positions of the alkene could have precluded efficient photocycloadditions with other reactions occurring instead.



Scheme 194

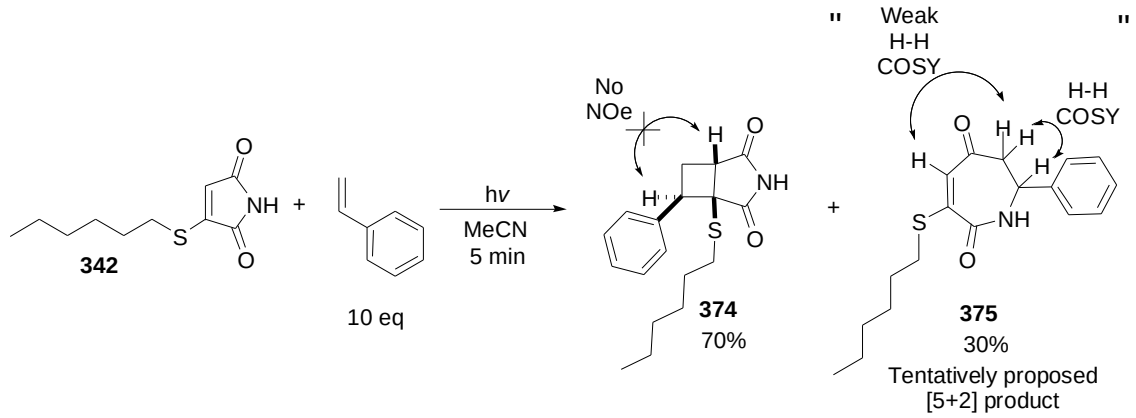
Ten equivalents of 1,1-diphenylethylene afforded 64% of the *syn* adduct **373** after five minutes irradiation, **Scheme 195**. Whilst this orientation had been observed with other partners, it was interesting to see that even with two large substituents on the alkene, the

preference was for the product whereby both the substituent on the alkene and the thiyl are adjacent.



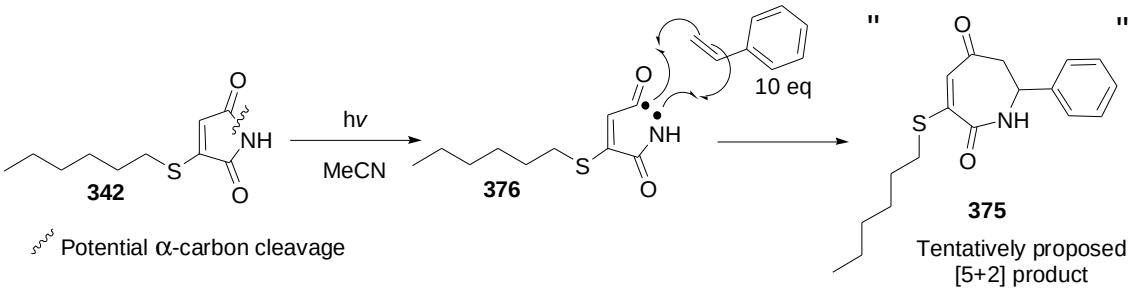
Scheme 195

When styrene was used for the conjugation reaction there was a very interesting outcome. The use of just ten equivalents of styrene afforded 70% of the [2+2] adduct in one regioisomer as one diastereomer, the *syn-cis* product **374**, **Scheme 196**. Additionally, the remaining mass was just one isolable compound. Mass spectroscopy indicated that the second compound **375** was of the same mass as the desired conjugate **374** and thus an alternative conjugation product was afforded in 30% yield, **Scheme 197**. At this stage, minor product **375** was assigned as the product of a [5+2] photocycloaddition,[149, 300] **Scheme 196**, but as the compound was an oil it could not be crystallised. Adduct **375** represented another mode of conjugation for the molecules of interest, indicated by both high and low resolution mass spectroscopy. The [5+2] adduct was suggested as ^{1}H and ^{13}C NMR compound described the structure as still containing an alkene peak, but it had gained a methylene and methine group, alongside the aromatic ring. This suggested that the thiomaleimide **342** had incorporated the styrene *via* loss of the styrene double bond, but not *via* a [2+2] photocycloaddition of the thiomaleimide double bond. The [5+2] photocycloaddition had only been reported from intramolecular systems by Booker-Milburn *et al.*,[149, 154, 300] however, at this stage, it was deemed the most likely side reaction. Structure **375** was proposed as the alkene CH had a weak correlation with the new CH_2 and not with the new CH, and the new CH_2 and CH were adjacent, seen by HH COSY, **Scheme 196**.



Scheme 196

The postulated [5+2] mechanism would involve homolytic cleavage of one of the N-CO bonds (α-cleavage) to create a di-radical **376** across five atoms, **Scheme 197**. Subsequent insertion of the two atoms of the styrene double bond would result in formation of the seven-membered ring **375**.



Scheme 197

The intention of this work was to tether two molecules together and, at this point, styrene represented a 100% conjugation of the pendant thiyl and aromatic ring, albeit with 30% of this conjugation *via* a proposed [5+2] conjugation.

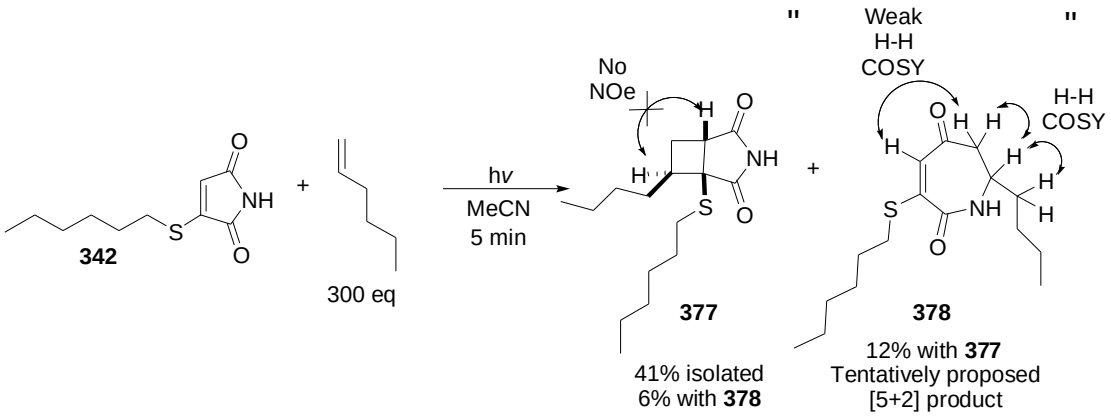
A recurring pattern seemed to be that in the [2+2] photocycloaddition the addition of the photochemical partner occurred with the bulky group adjacent to the thiyl pendant group - a *syn* addition. Also, it appeared that there was an emerging preference for the substituent on the alkene to adopt a *cis* configuration with the thiyl group. Further partners were thus investigated to see if, and when, these trends altered, with the goal of furthering the scope of this reaction. It was envisaged that through the trial of partners, further [5+2] products would be isolated that would support the assignment. Ideally, a

solid [5+2] adduct would be afforded and crystallised to confirm the structure by X-ray crystallography

Terminal and internal olefins and alkynes

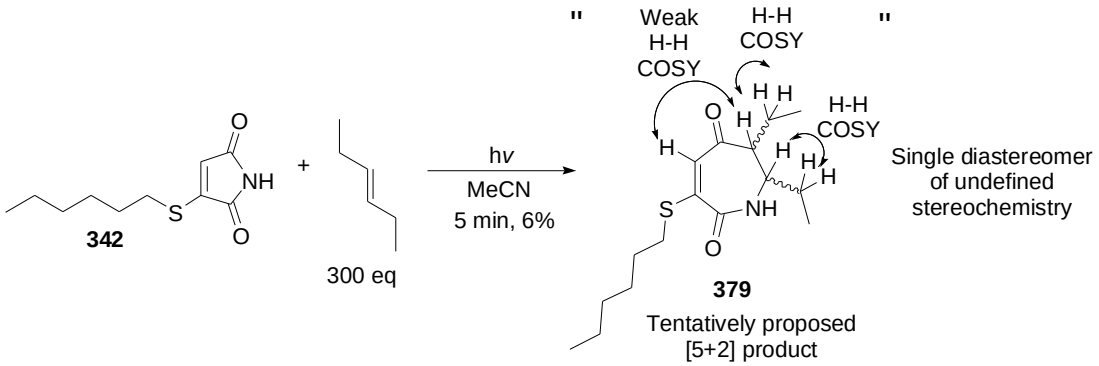
Terminal alkenes and alkynes were investigated as there was literature precedent that they could be incorporated into proteins for subsequent bioorthogonal modifications.[174, 189, 210]

The terminal alkene, hex-1-ene, could overcome the facile dimerisation if used in three hundred equivalents, and 41% of the *syn-cis* conjugation product **377** was afforded, **Scheme 198**. However, a further 6% of the [2+2] product **377** was inseparable from another compound **378**, **Scheme 198**. Mass spectroscopy indicated that, similarly to when styrene was used, this second compound was of the same mass as the desired conjugate **377** and was therefore afforded in 12% yield. This compound was tentatively identified as the [5+2] adduct **378** as, similarly to the styrene [5+2] adduct **375**, NMR investigations indicated that the thiomaleimide **342** had incorporated the butene *via* the butenes double bond, but the thiomaleimide alkene CH was in tact. HH COSY NMR indicated a weak coupling between the alkene CH and the new CH_2, which again coupled to the new CH. In this instance, the new CH could be seen to couple to the $CH_2CH_2CH_2CH_3$ alkyl chain, **Scheme 198**, further evidence that the structure **378** was correctly assigned. A sample of the proposed [5+2] adduct **378** was isolated pure for analysis but as an oil it could not be crystallised.



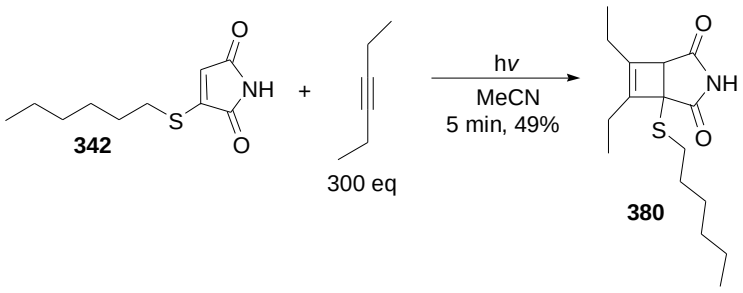
Scheme 198

When the internal alkene counterpart was used, three hundred equivalents of *trans*-hex-3-ene, a similar proposed [5+2] product was afforded in just 6% **379**, **Scheme 199**. However, in this instance, there was no expected [2+2] conjugation product observed. The [5+2] product **379** was proposed as the newly formed compound still maintained the CH from the thiomaleimide **342** alkene with a new methine and methylene, alongside the alkyl chain of the hex-3-ene. However, the compound was again isolated as an oil, precluding recrystallisation and thus the stereochemistry of the single diastereomer was not defined. It was postulated at this stage, that with analogy to the use of *trans*-stilbene, the desired [2+2] cyclobutane may have been too congested to form under the reaction conditions, or perhaps too strained to withstand isolation.



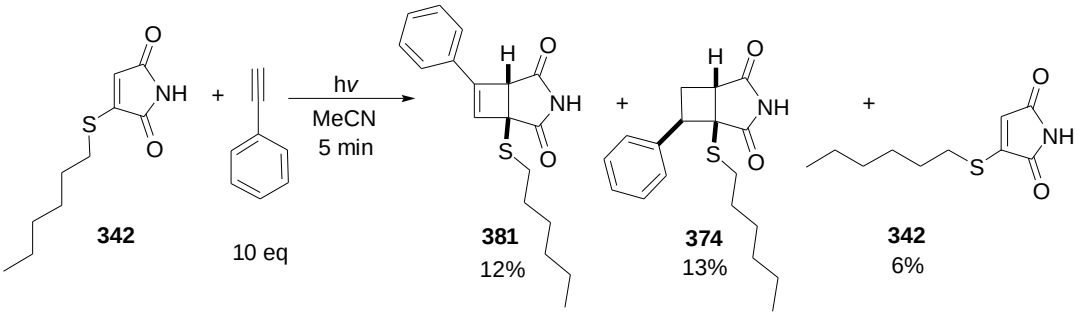
Scheme 199

In contrast to the terminal alkene, the use of hex-1-yne afforded neither conjugation products nor dimer at three hundred equivalents, but a complex mixture of products that could not be characterised. Conversely hex-3-yne afforded 49% of the [2+2] conjugation product **380** when used in three hundred equivalents, the first cyclobutene afforded from these reactions, **Scheme 200**.



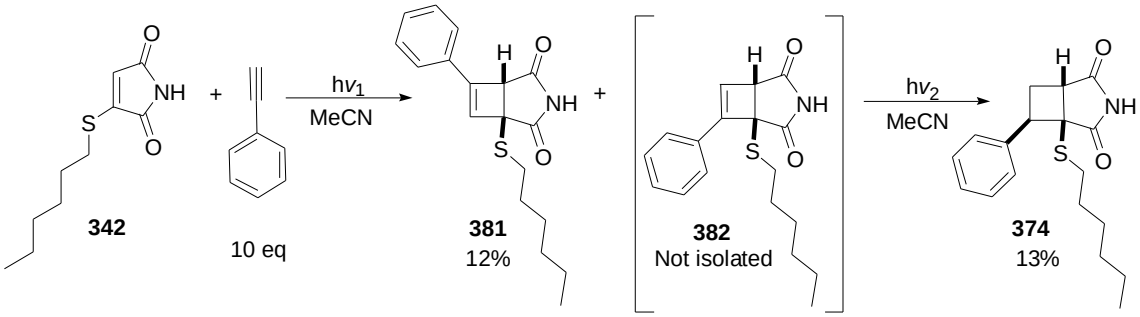
Scheme 200

Encouraged by the success of this alkyne and styrene in this reaction, phenylacetylene was chosen for irradiation with thiomaleimide **342**. When ten equivalents of phenylacetylene were used, a [2+2] adduct **381** was formed in 12%, **Scheme 201**. In this unusual case, the substituent on the alkyne, the aromatic ring, was 'opposite' the thiyl pendant as the result of an *anti* addition. This could be due to the linear bulky nature of phenyl acetylene forcing the least sterically hindered approach of the two groups. Interestingly, 6% of the thiomaleimide **342** starting material was recovered in this reaction and no dimer **343** was isolated. This potentially indicated the aryl alkyne was slightly quenching the photochemical activation of the thiomaleimide **342** and thus the dimerisation. In addition to the cyclobutene **381**, a 13% yield of cyclobutane **374** was isolated, **Scheme 201**.



Scheme 201

This indicated that the *syn* addition did take place, but that the resulting *syn* cyclobutene product **382**, was more prone to photoreduction than the *anti* addition product **381**, **Scheme 202**. Such photoreductions can occur in photochemical reactions that form 1,4-conjugated systems, whereby the excited state can abstract protons from the solvent.[47]



Scheme 202

An interesting feature of cyclobutane formed in this reaction **374** was that it had the same stereochemistry as the product from reaction of styrene with thiomaleimide **342**, **Scheme 196**, p. 188. This was postulated to occur due to the reduction of the cyclobutene **382** from the face of the succinimide rather than the thiyl pendant group.

Allyl and propargyl alcohol were not successful partners in this reaction, regardless of the equivalents employed. It appeared that the alcohols behaved as nucleophiles, either with the thiomaleimide **342**, or with any formed product, as there were a number of alkenyl and alkynyl peaks in the crude spectra.

At this stage a summary of the alkene and alkyne partners employed shows the scope of this conjugation strategy, **Table 28.**

Table 28: Photoconjugation of thiomaleimide 342 with different partners

Entry	Partner	Eq	Yield of [2+2] product	Proposed [5+2] product [d]
1	Cyclopentene	300	**366**, 78%[a]	--
2	Cyclohexene	300	**367**, 68%[a]	--
3	Acrylonitrile	300	**368** and **369**, 68%[b]	--
4	Phenyl acrylate	10	**370**, 48%[c]	--
5	Methyl acrylate	300	**371**, 49%	--
6	1,1-diphenyl ethylene	10	**373**, 64%	--
7	Styrene	10	**374**, 70%	**375**, 30%
8	Hex-1-ene	300	**377**, 47%	**378**, 12%
9	*trans*-Hex-3-ene	300	--	**379**, 6%
10	Hex-3-yne	300	**380**, 49%	--
11	Phenylacetylene	10	**381**,12% (13%[e])	--

[a]Inseparable mix of diastereomers. [b]Combined yield of two isolable regioisomers. [c]47% dimer **343** also isolated. [d]Structures were assigned at this stage as [5+2] adducts. [e]Cyclobutane **374**. All reactions were carried out at 0.005 M in acetonitrile for 5 minutes.

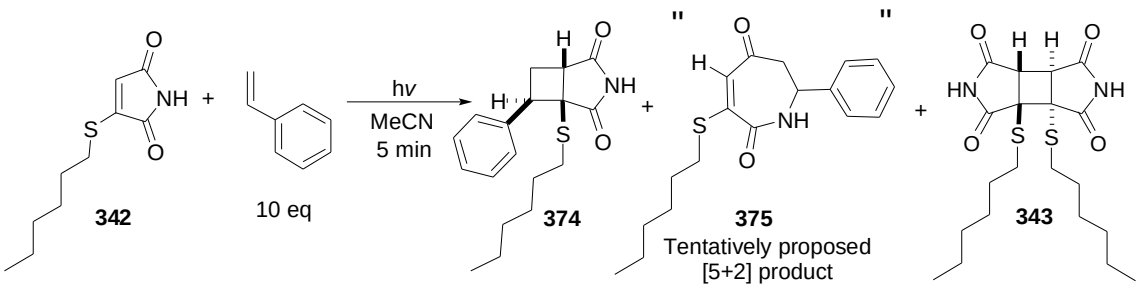
Whilst the formation of the proposed [5+2] conjugation products **375**, **378** and **379** was unexpected, the adducts still represented conjugation of the substituent on the thiomaleimide **342** with the substituent on the alkene. It could be seen that, overall,

styrene afforded the best yield of conjugation. When styrene was used, combining the yields of the two products obtained afforded 100% conjugation of the two substituents. For synthetic chemistry, where tight control of the structure of the formed products is necessary, the forming of two different products might be sub-optimal. However, the goal of this work was to investigate model structures for biochemical conjugation. As discussed in **1.ii.iv.**, photoaffinity labelling, a current method of bioconjugation, has largely non-specific modes of reactivity. The key factor in tethering biomolecules is the yield of modification and not the mode, or modes, of that conjugation. Thus, this modification strategy could prove useful for protein methodologies. The formation of just two products also meant that the reaction was easy to analyse *via* the ^{1}H NMR spectrum of the crude reaction mixture and thus styrene was chosen as the model reaction partner with which to probe the reaction profile.

In-depth investigation of the photochemical conjugation with styrene

Investigations were undertaken into the photochemical reaction of thiomaleimide **342** with varying equivalents of styrene, **Table 29**. When the equivalents of styrene were increased to three hundred, entry 1, **Table 29**, the yield of isolated conjugation product **374** decreased, potentially due to continued reaction with excess styrene leading to degradation. Decreasing the equivalents of styrene led to increased formation of dimer **343**, whilst the ratio of other products remained largely unchanged, entries 3 - 6, **Table 29**. Interestingly, with just one equivalent of styrene there was still a preference for conjugation over dimerisation - indicating that styrene was a very powerful partner for photochemical reaction with thiomaleimide **342**.

Table 29: Photoconjugation of thiomaleimide 342 with varying equivalents of styrene



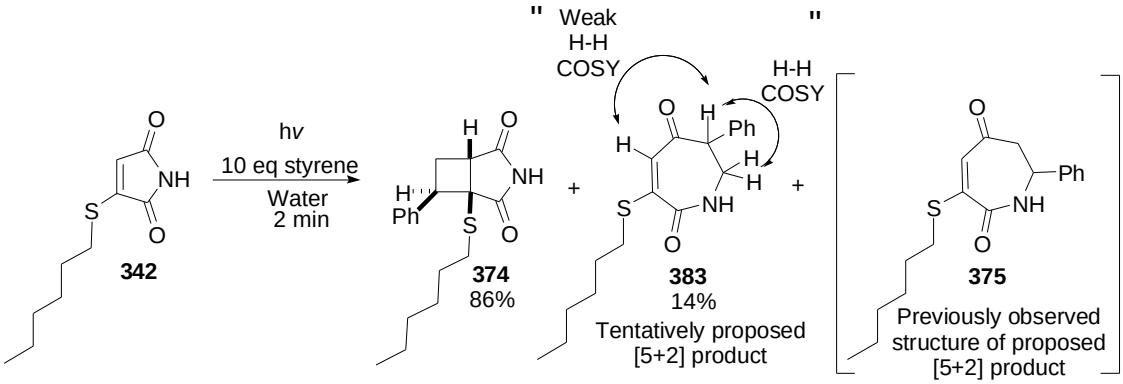
Entry	Equivalents	Yield of 374	Yield of 375	Yield of 343
1	300	49[a]	5[a]	--
2	10	70[a]	30[a]	--
3	4	63[b]	31[b]	5[b]
4	2	66[b]	26[b]	7[b]
5	1.5	55[b]	34[b]	11[b]
6	1	59[b]	14[b]	20[b]

[a]Isolated yield. [b] ^{1}H NMR ratio. All reactions were carried out at 0.005 M in acetonitrile.

Investigations into solvent and concentration were carried out with styrene to ascertain if the conjugation could be applied at the low concentration and in the aqueous solvent systems required for protein chemistries, **Table 30**. Protein modifications, undertaken as part of the aforementioned collaboration, by Dr Mark Smith (UCL, Chemistry, Caddick group), had been carried out at concentrations as low as 72 μM. Therefore, guided by this work, the reaction was tested at this concentration, entry 2, **Table 30**. The reaction still proceeded well in just five minutes with similar ratios of the [2+2] product **374** and the proposed [5+2] product **375**. Whilst the reaction was complete in five minutes, it was deemed necessary to ascertain if the reaction reached completion before this time. At just two minutes the reaction was complete and the ratios of the two compounds remained very similar, entry 3, **Table 30**. However, when thiomaleimide **342** was irradiated in the presence of ten equivalents of styrene for just thirty seconds, only minimal reaction was observed, entry 4, **Table 30**. This indicated that the timescale for this reaction was between thirty seconds and two minutes. It is interesting to note here that the lamp used on these studies takes approximately seven to ten minutes to 'warm-up' and reach its full spectral radiance. Therefore, the reaction was effectively complete before the lamp was at its maximum power. This rapid reaction was perhaps not too

surprising as the λ_{max} of thiomaleimide **342** was 347 nm, with a high extinction coefficient, and the lamp had an intense spectral output near to this at 365 nm. For example, the extinction coefficient of thiomaleimide was around ten times that of maleimide (9500 $cm^{-1}M^{-1}$ at 346 nm *cf.* 720 $cm^{-1}M^{-1}$ at 275 nm).

It was important that the conjugation would proceed in water as most applications for bioconjugations involving proteins must be carried out in aqueous media. After two minutes in water (5% acetonitrile) the reaction was complete, and there was even a slight further preference displayed for the [2+2] product **374**, entry 5, **Table 30**. This could be due to increased interaction of the hydrophobic reagents in water adopting more favourable configurations for the [2+2] photocycloaddition. Interestingly, in water, the proposed [5+2] product **383** had slightly different chemical shifts. Analysis of the NMR spectra from this molecule indicated that the alkene in the ring was weakly coupling to the new methine, rather than the methylene as previously observed, **Scheme 203**. This could arise from a similar seven-membered ring, but where the styrene has been incorporated to afford the alternate regioisomer to that observed previously. It was uncertain why carrying out the reaction in water had changed the configuration of the products obtained.

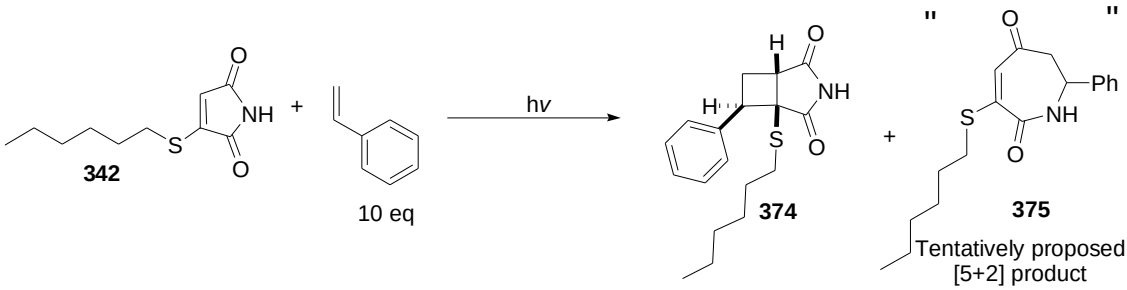


Scheme 203

The photochemical lamp used in these trials was a 250 W MP Hg lamp which had a wide spectral range and intense emissions (see **1.i.ii.** for further details). The intensity of the light source used could potentially limit the use of the strategy for proteins that were particularly sensitive to irradiation. Therefore the experiment was carried out under a much weaker hand-held lamp (UVGL-55, 6 W, UVP Inc.) equipped with a filter, with

only one major emission at 365 nm. Whilst the reaction took considerably longer (1.5 h), it still proceeded in a reasonable time to afford similar ratios of the two products, entry 6, **Table 30**.

Table 30: Investigations into the limits of the photoconjugation of thiomaleimide 342 with styrene

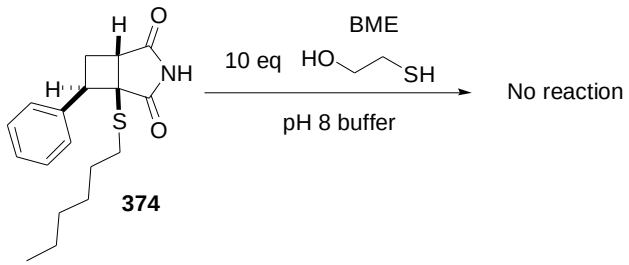


Entry	Concentration (μM)	Time	Solvent	Ratio of 374:375[a]
1	5000	5 min	Acetonitrile	70:30
2	72	5 min	Acetonitrile	80:19
3	72	2 min	Acetonitrile	71:29
4	72	30 s	Acetonitrile	7:0[b]
5	72	2 min	Water[c]	86: 14[d]
6	72	1.5 h[e]	Acetonitrile	69:31

[a]Ratios deduced from ^{1}H NMR. [b]93% thiomaleimide **342** remains. [c]5% acetonitrile. [d]Product had slightly different chemical shifts in ^{1}H NMR spectra indicating [5+2] product **383**. [e]Reaction carried out under 6 W light source.

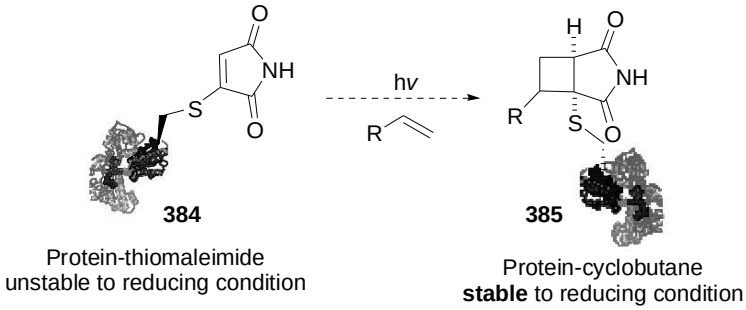
As the product profile was largely unchanged between two and five minutes, the optimum irradiation time was kept at five minutes. This was deemed an adequate compromise between a rapid reaction and minimal stress on the lamp, as repeatedly turning the lamp off before it has fully warmed up (between seven to ten minutes) lowers the lifetime of the filament.

With analogy to the dimeric structure **343**, the cyclobutane **374** was subjected to ten equivalents of BME in pH 8 buffer and no reaction was observed, **Scheme 204**. This indicated that the photochemical transformation had now rendered the cysteine modification irreversible in the presence of excess thiol.



Scheme 204

This had similar implications in the conjugation of olefins to thiomaleimides as it did in the dimerisation. Fundamentally, by carrying out the [2+2] photocycloaddition on any given thiomaleimide, the thiol would no longer be eliminated upon exposure to reducing conditions. This has applications in that the photochemical conjugation of a label *via* photochemistry would be stable to reducing conditions. In addition, any thiomaleimide in a protein **384** could potentially be made stable to reducing environments simply by photochemical formation of a cyclobutane **385**, **Scheme 205**.



Scheme 205

An investigation into the tolerated substituents on the styrene was undertaken at this stage and 4-aminostyrene, 4-methoxystyrene and 2-nitrostyrene were chosen as readily available styrene analogues that would probe the profile of the reaction, **Figure 43**.

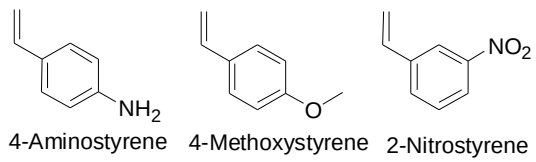
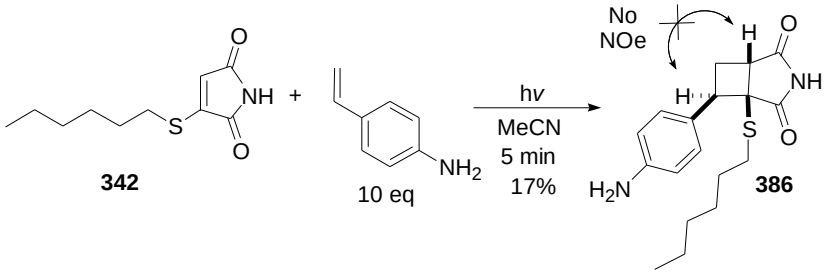


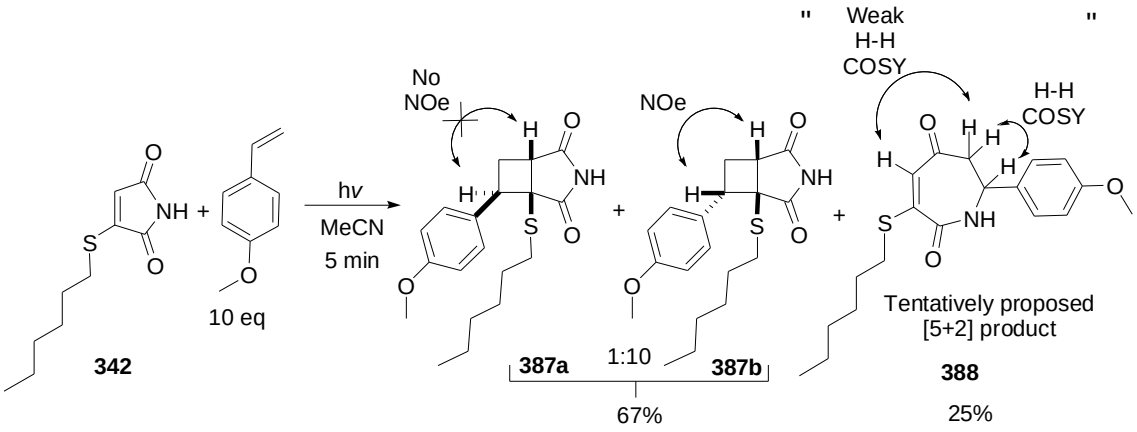
Figure 43: Styrenes proposed to probe the photochemical behaviour of thiomaleimide 342

The only product isolated from reaction with 4-aminostyrene was one regioisomer and one diastereomer **386**, the *syn-cis* product **386** in 17% yield, **Scheme 206**. The low yield of this reaction compared to the standard styrene reaction was attributed to nucleophilic reactions of the aniline group causing many multiple by-products. This was postulated due to the many species observed in the crude ^{1}H NMR spectrum that still contained the styryl alkene peaks, although these side-products were not isolable upon column chromatography. No [5+2] adduct was seen in this reaction, entry 2, **Table 31**.



Scheme 206

In the case of irradiation with 4-methoxystyrene, there was a preference for just the *syn* regioisomer once more. However, in this instance, the product was a 10:1 mix of diastereomers, with the major product **387b** in *trans* configuration, thus existing as the opposite diastereomer to past styrene examples, entry 3, **Table 31**. The through-space interaction of the two protons in *trans* diastereomer **387b** could clearly be seen *via* NOeSY NMR experiments. The [5+2] product **388** was again proposed due to key couplings observed in NMR spectra and was again afforded as an oil in 25%, **Scheme 207.**



Scheme 207

The identification of the major diastereomer **387b** as *trans* indicated that the [2+2] photocycloaddition had proceeded through the *endo*-transition state. It was postulated that this was due to secondary orbital interactions placing the anisole moiety over the maleimide ring. As the aromatic ring of the styrene was polarised by attachment of a methoxy group, increased secondary interactions were hypothesised. Taking the orbital phases of anisole as a model,[158] the now rich HOMO of the aromatic system could perhaps interact further with the maleimide's orbitals, particularly over the aligned carbonyl in the maleimide, **Figure 44**. Coefficients are ignored here for clarity. This could place the aromatic ring over the maleimide ring and afford the *trans* configuration cyclobutane **387b**.

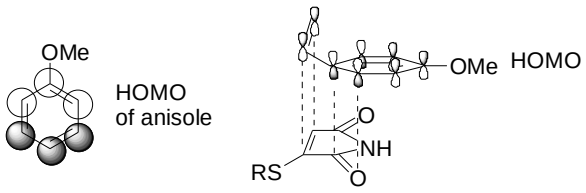
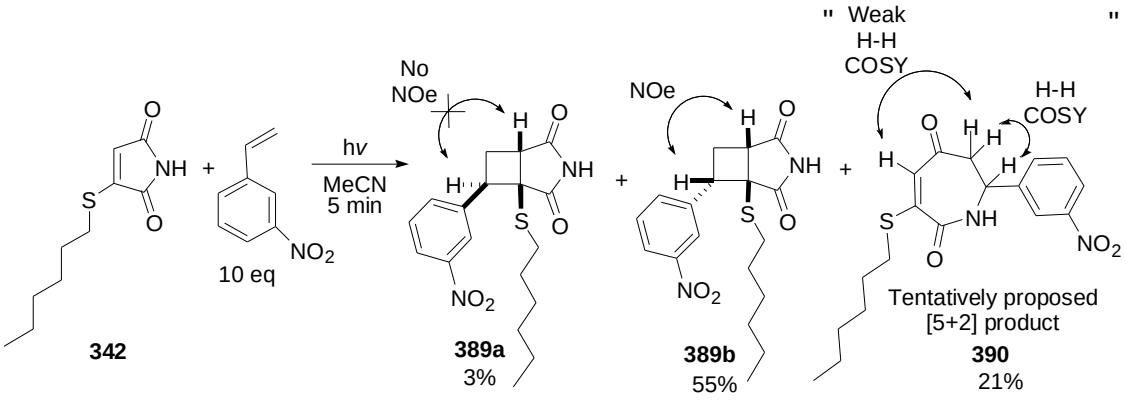


Figure 44: Postulated secondary orbital interactions that could afford the *endo* transition state

A similar result was achieved when the thiomaleimide **342** was irradiated with 2-nitrostyrene. The major [2+2] adduct **389b** was in *syn* configuration but the aryl and thiyl group were *trans*, opposite to the stereochemistry seen with the unsubstituted aryl ring, **Scheme 208**. The [5+2] product **390** was afforded as a thick oil in 21%, entry 4, **Table 31**, proposed due to key couplings observed in NMR spectra, **Scheme 208**.



Scheme 208

The identification of the major diastereomer **389b** as *trans* indicated that this reaction had proceeded through the *endo*-transition state. It was postulated that this was due to secondary orbital interactions placing the nitro-substituted aromatic ring over the maleimide ring. As the aromatic ring of the styrene was polarised by attachment of a nitro group, increased secondary interactions were hypothesised. In the case of 2-nitrostyrene the aromatic ring was now electron deficient. Increased interaction between the LUMO of the aromatic ring, modelled on the benzylic cation[158] and the thiomaleimide, particularly over the carbonyl; could lead to the *endo* configuration in the transition state and the *trans* product witnessed, **Figure 45.**

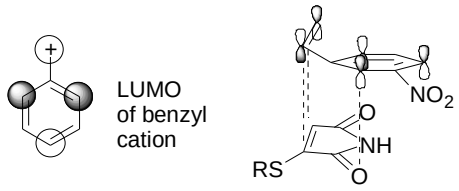
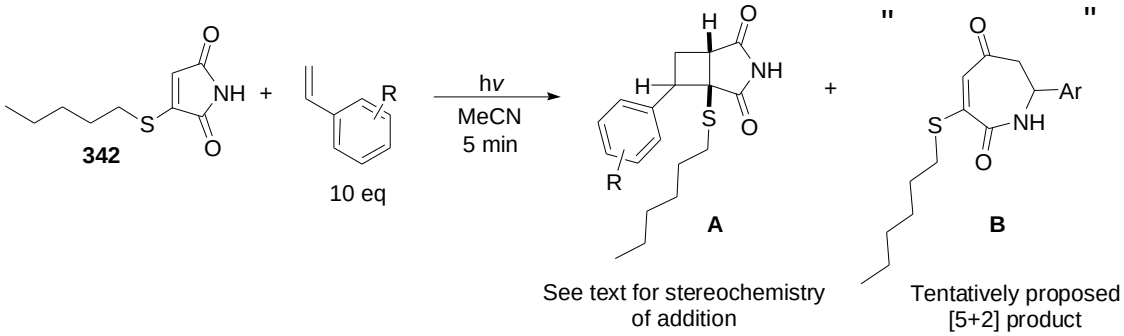


Figure 45: Postulated secondary orbital interactions that could afford the *endo* transition state

Comparison of the results of varying styrene substitution showed that only in the reaction of thiomaleimide **342** with 4-aminostyrene was the [5+2] conjugation product not observed, entry 2, **Table 31**. Interestingly, substituting the aromatic ring with an electron withdrawing (NO_2) and a donating (OMe) group had the same effect on the substitution pattern, specifically, formation of the *trans* diastereomer became favoured, entries 3 and 4, **Table 31**.

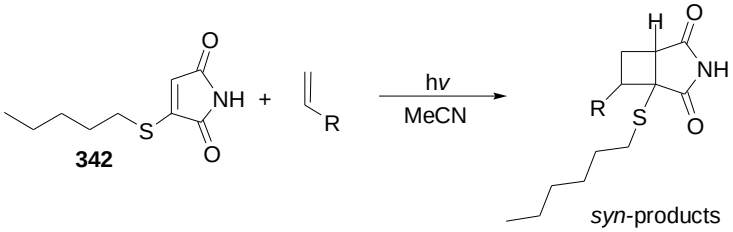
Table 31: Comparison of the photoconjugation products of thiomaleimide 342 with various styrenes



Entry	R	Yield of [2+2] product A (cis:trans)	Yield of proposed [5+2] product B
1	--	70% (*cis*)	30%
2	4-Amino	17% (*cis*)	--
3	4-Methoxy	58%[a] (1:10)	25%
4	2-Nitro[b]	67%[b] (3:55)	21%

[a]Inseparable mix of diastereomers. [b]Combined yield of isolated diastereomers

It could now be seen that throughout the conjugation reactions there was an over-riding preference for the [2+2] conjugation to adopt a *syn* approach, with substituents on the cyclobutane adjacent, **Scheme 209**. In fact only acrylonitrile afforded any of the *anti* product, see **Scheme 191**, p.182.



Scheme 209

This suggested that in most cases the 'HOMO'/HOMO interactions were dominant in the reaction. As 'LUMO'/LUMO interactions tend to be observed when the reaction occurs from an n-π* transition,[158] this could be taken to suggest that the initial n-π* singlet transition collapses to, and reacts from, the π-π* triplet rather than the n-π* triplet. The *exo*-transition state afforded the expected *cis* adducts in the majority of

cases. Where the *trans* adducts were formed, secondary orbital interactions have been postulated as resulting in the *endo*-transition state

The range of olefins and alkenes that could be utilised in this reaction to afford [2+2] conjugation products was very promising as it suggested that the reaction was tolerant to a variety of partners. It was a goal of this work to modify a protein labelled with a thiomaleimide *via* a [2+2] photocycloaddition with an olefin to prove the concept that this was a viable method of bioconjugation.

Photochemical behaviour of *N*-substituted thiomaleimides

Up to this point, the minor products in some of the reactions had been attributed to [5+2] adducts. The [5+2] reaction has been documented purely as an intramolecular phenomenon,[149, 300] but it was the side-reaction that was deemed most likely. As the proposed [5+2] photocycloaddition would have required cleavage at one of the N-CO maleimide bonds, an investigation into varying N-substitutions was undertaken, to ascertain if this increased or decreased the proportion of the proposed [5+2] products.

N-Methyl, phenyl and methylenecyclohexanyl thiomaleimides **345**, **391** and **392** were targeted to probe how the reaction profile altered with changes to the maleimide, from simple *N*-methylation to the addition of an aromatic group, **Figure 46**.

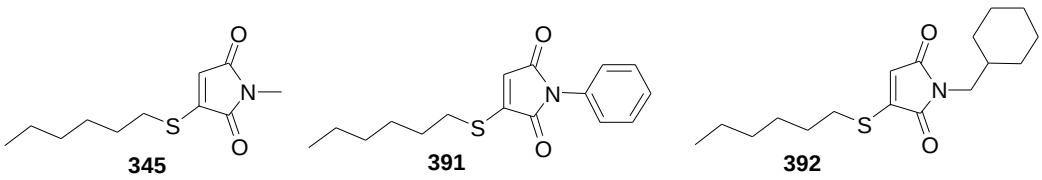
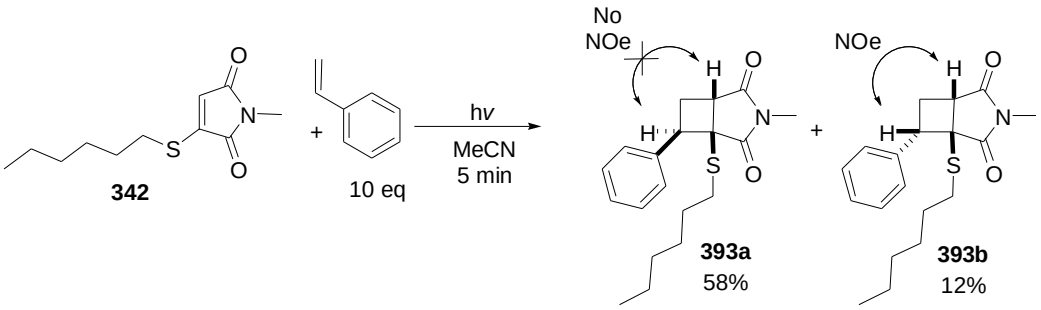


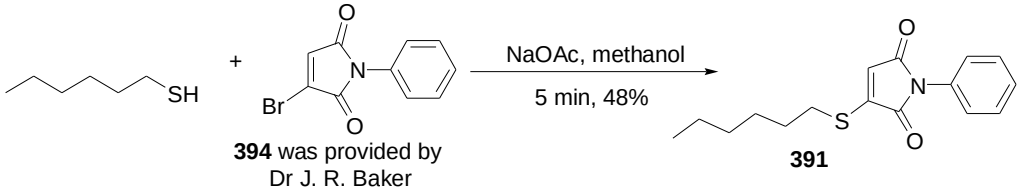
Figure 46: Proposed *N*-substituted maleimides

The *N*-methylthiomaleimide **345**, **Figure 46**, had previously been synthesised in efforts towards investigating the dimerisation of thiomaleimides. When irradiated with ten equivalents of styrene for five minutes, a 70% combined yield of separable diastereomers **393a** and **393b** was isolated. The major diastereomer **393a** was in-line with past trends as it was *syn* and *cis* to the thiyl pendant group, **Scheme 210**. Notably, none of the proposed [5+2] product was observed, entry 1, **Table 32**.



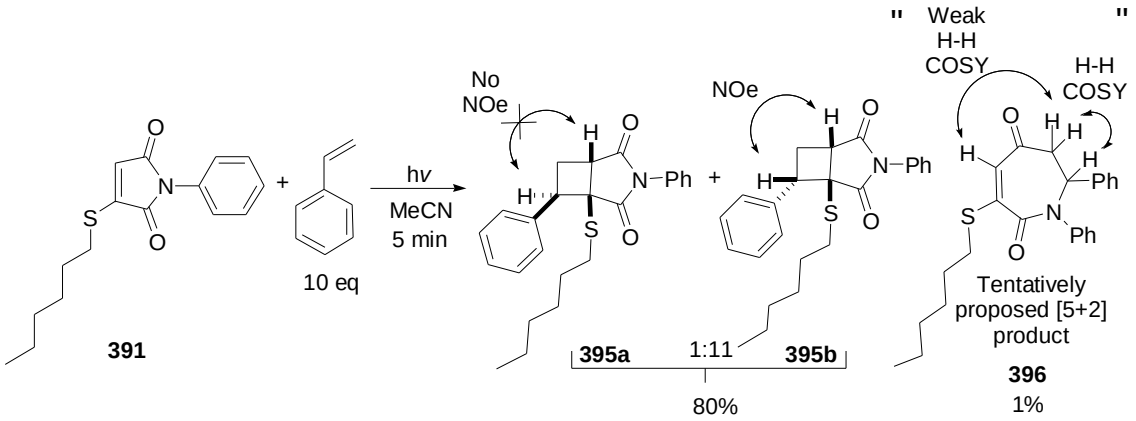
Scheme 210

The *N*-phenylbromomaleimide **394** was obtained from Dr James Baker (UCL, Chemistry) who followed a literature procedure,[301] and the hexylthiomaleimide **391** was synthesised under standard conditions in 48%, **Scheme 211**.



Scheme 211

Irradiation of thiomaleimide **391** with ten equivalents of styrene afforded an inseparable mixture of diastereomers **395a** and **395b** in 80% after five minutes irradiation, **Scheme 212**. A 1% yield of a proposed [5+2] product **396** was also isolated, entry 2, **Table 32**, again key NMR couplings indicated its structure, **Scheme 212**.



Scheme 212

This reaction unusually afforded the *trans* diastereomer **395b** as the major component (80%). In 2008, it was discussed that in structures of less than eleven atoms, what are

often deemed 'π-stacking interactions' from two aryl rings are actually largely due to increased van der Waals interactions due to the ease of orientation of two flat motifs.[302] This increased interaction between the two substituents may have brought the styrene *endo* in the transition state with the maleimide, **Figure 47**.

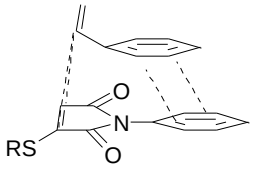
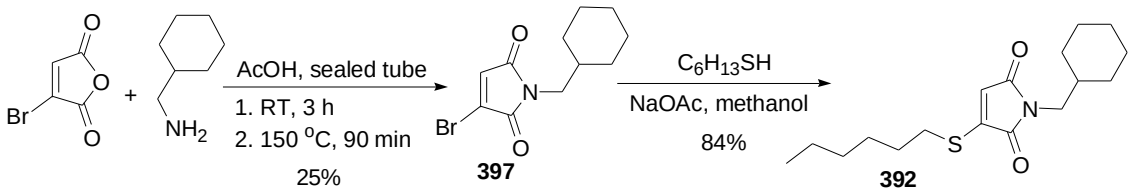


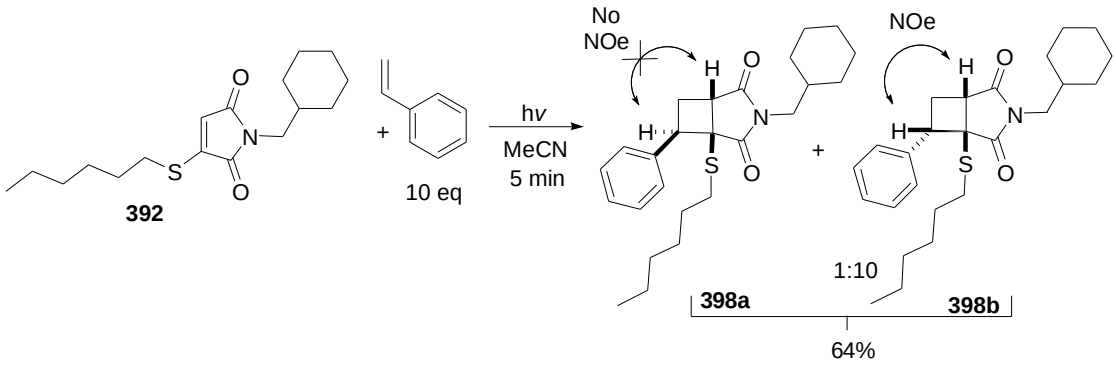
Figure 47: Postulated increased van der Waals interactions between styrene and thiomaleimide 391 that could favour the *endo* transition state

The *N*-methylenecyclohexane bromomaleimide **397** was synthesised *via* condensation of *N*-methylenecyclohexamine with bromomaleic anhydride in a sealed tube at 150 ^{o}C in 25%, **Scheme 213**. The bromomaleimide **397** was then reacted with hexanethiol in methanol in the presence of sodium acetate to afford the thiomaleimide **392** in 84%, **Scheme 213**.



Scheme 213

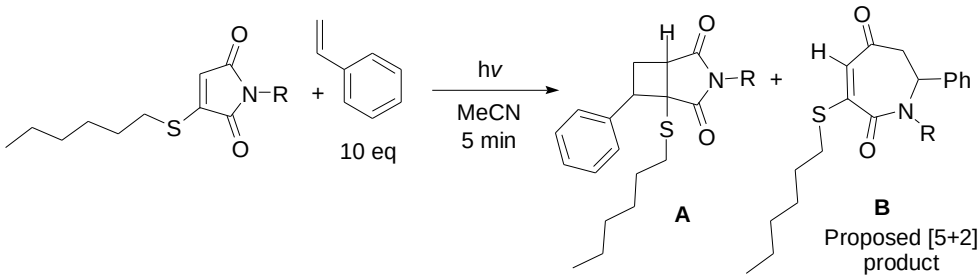
Irradiation with ten equivalents of styrene for five minutes afforded an inseparable mixture of diastereomeric products **398a** and **398b** in 55%, **Scheme 214**. The addition of the alkene was in agreement with established preferences, in that the pendant groups on the cyclobutane were *syn*, entry 3, **Table 32**. However, the major diastereomer **398b** was the *trans* configuration, elucidated from NOeSY NMR experiments, **Scheme 214**. The reason behind this was uncertain as the additional bulk on the nitrogen was not expected to aid the *endo* transition state.



Scheme 214

By adding steric bulk at the nitrogen, the proposed [5+2] product was almost completely negated, **Table 32**.

Table 32: Comparison of the photoconjugation products of styrene with various *N*-substituted thiomaleimides



Entry	R	Yield of [2+2] product A (*cis*:*trans*)	Yield of proposed [5+2] product B
1	Me	70% [a] (29:6)	--
2	Phenyl	80% [b] (1:11)	1%
3	CH_2Cy	64% [b] (1:10)	--

[a]Combined yield of separable diastereomers. [b]Combined yield of inseparable diastereomers.

The proposed [5+2] reaction required interaction of styrene with the diradical, formed from irradiation, in proximity to the nitrogen substituent on the maleimide, **Scheme 198**, p. 189. Therefore, it was postulated that increasing the bulk of the nitrogen substituent had attenuated the formation of the proposed [5+2] products, presumably from steric clash at the reacting bonds.

Photochemical behaviour of various S-substituted thiomaleimides

Attention was turned at this stage to the nature of the thiyl pendant group. It was postulated, that if there was competition between reaction at the double bond, for the [2+2] addition, and reaction at the imide bond, for the [5+2] addition, then adding bulk to the thiyl should favour the [5+2] addition. Therefore, *tert*-butyl, cyclohexyl and phenyl substituents were investigated, **Figure 48**.

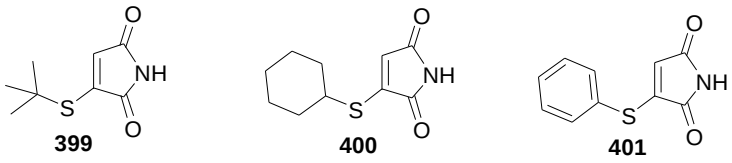
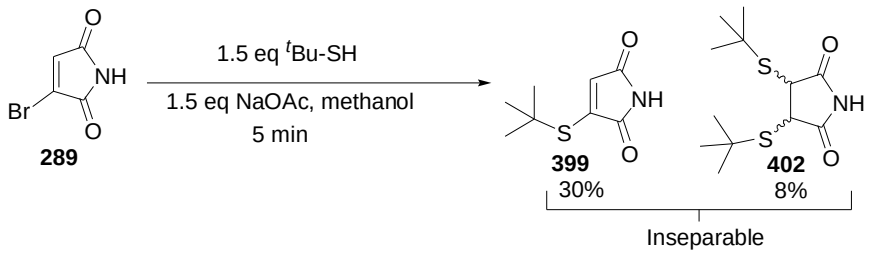


Figure 48: Proposed *S*-substituted maleimides

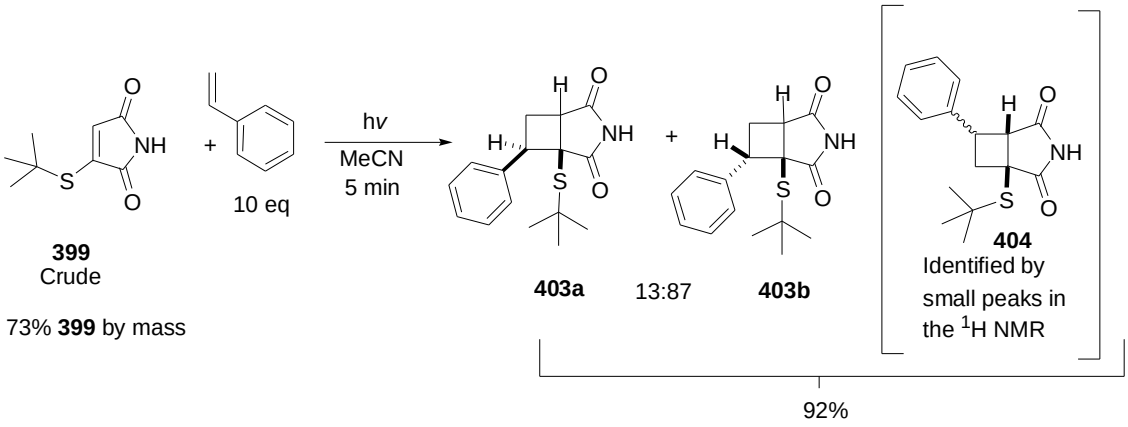
tert-Butylthiomaleimide **399** was synthesised *via* reaction of bromomaleimide **289** with *tert*-butylthiol. It was necessary to add greater than one equivalent as it seemed that the bulk of the thiol was retarding the reaction. As a result, a yield of just 30% of thiomaleimide **399** was isolated with an inseparable impurity of double addition succinimide **402** in 8%, **Scheme 215**. The double addition product **402** could not be removed by column chromatography but this was not deemed too problematic as it wasn't predicted to react with the styrene in the conjugation reaction, thus the material was taken on without further purification.



Scheme 215

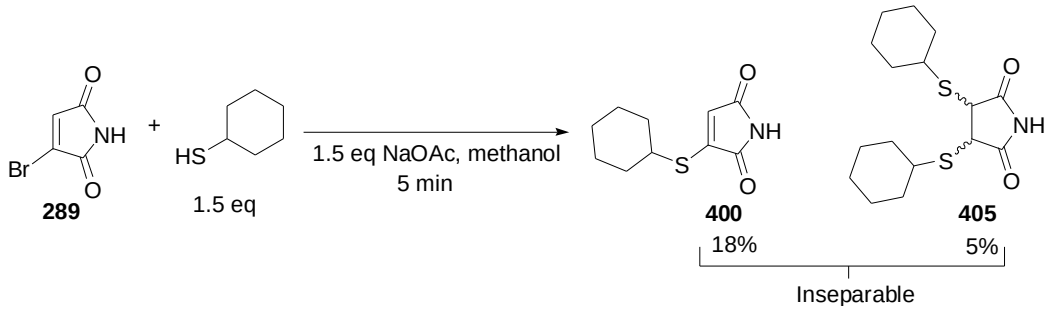
When the crude thiomaleimide **399(402)** was irradiated for five minutes with ten equivalents of styrene, a 92% yield of diastereomers **403a**, **403b** and **404** was obtained from the [2+2] conjugation, based on thiomaleimide **399** in the crude starting material, with no proposed [5+2] product, entry 1, **Table 33**. This yield represented the highest to date as a mix of diastereomers. The two major diastereomers were identified as **403a** and **403b**, in a ratio of 1:4, the *trans* and *cis* diastereomers of the *syn* addition, **Scheme**

216. However, alongside this, there were small amounts of the alternate *anti* regioisomeric diastereomers **404**. The favoured formation of the *trans* product **403** was not in accordance with most of the reactions but was explained as due to the bulk of the pendant *tert*-butylthiyl group. Similarly, the alternate regioisomers' appearance could be explained by the added bulk of the *tert*-butylthiyl group adding more steric constraints on the system. As predicted the double addition succinimide impurity **402** from the crude starting material was recovered in 100% yield at the end of the photochemical reaction, as it had not undergone any photochemical reaction.



Scheme 216

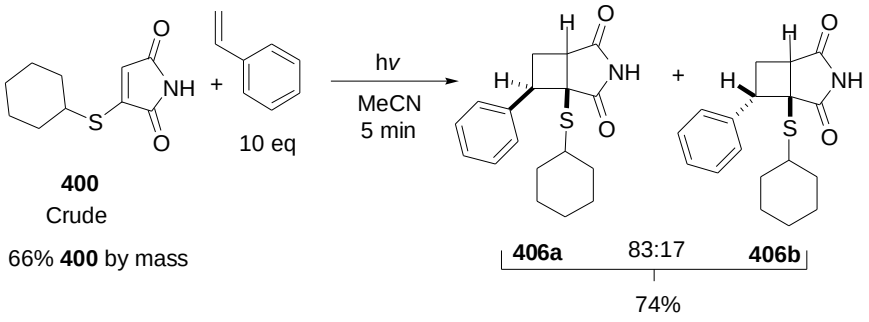
Synthesis of the cyclohexyl analogue afforded the thiomaleimide **400** in 18%. Yet again, the addition required excess thiol and thiomaleimide **400** was inseparable from the double addition product, succinimide **405** in 5%, **Scheme 217**.



Scheme 217

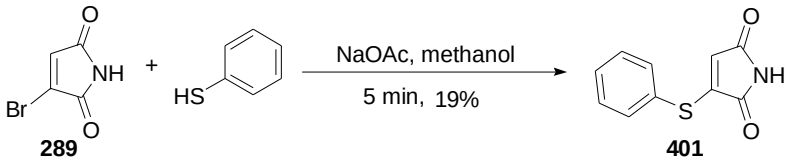
Irradiation of crude thiomaleimide **400(405)** with ten equivalents of styrene resulted in an inseparable mixture of diastereomers **406a** and **406b** in 74%, based on thiomaleimide **400** in the crude starting material, **Scheme 218**. The preference for the expected *cis*

product **406a** re-emerged here as it was by far the dominant species at 83% of the mixture, entry 2, **Table 33**. The succinimide impurity **405** from the crude starting material was recovered in 29% at the end of this reaction, based on the succinimide **405** that was in the crude starting material.



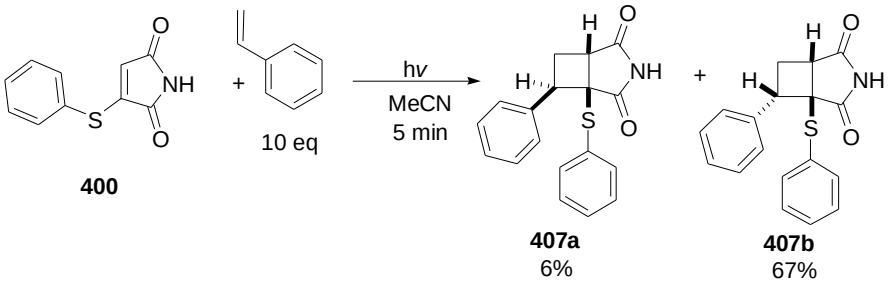
Scheme 218

Use of thiophenol afforded thiomaleimide **401**, from the bromomaleimide **289** in 17% yield, **Scheme 219**.



Scheme 219

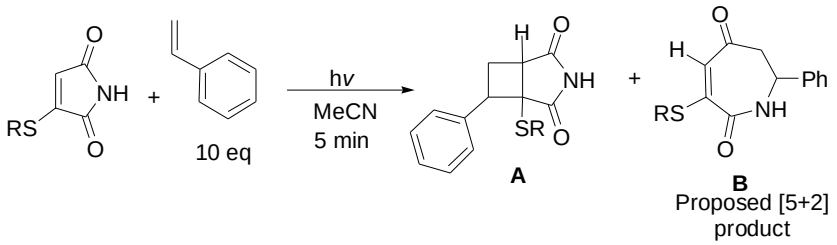
The resultant thiomaleimide **401** was irradiated in the presence of ten equivalents of styrene for five minutes to afford two isolable diastereomers **407a** and **407b**, entry 3, **Table 33**. In accordance with the *tert*-butyl analogue, it now appeared that preference was overwhelmingly towards the *trans* product **407b** in 67%, compared to the *cis* diastereomer **407a** that was only formed in 6%, **Scheme 220**. Somewhat surprisingly, it appeared that any positive interactions of the two aromatic rings did not afford the *cis* isomer as the dominant product. Instead, in accordance with the *tert*-butyl analogue, it was postulated that the bulk of the phenylthiyl pendant clashed with the styryl aromatic ring and afforded the *endo* transition state and the *trans* diastereomer **407b**.



Scheme 220

Interestingly, it was realised at this stage that adding bulk at the thiyl pendant had not minimised the [2+2] conjugation product and it had entirely negated the proposed [5+2] reaction pathway, **Table 33**. This was not expected as adding bulk in proximity to the bonds involved in the [2+2] photocycloaddition should have favoured the [5+2] reaction. This was the first indication that the products proposed as [5+2] photocycloaddition adducts might, in fact, have occurred from a different reaction pathway.

Table 33: Comparison of the photoconjugation products of styrene with various *S*-substituted thiomaleimides



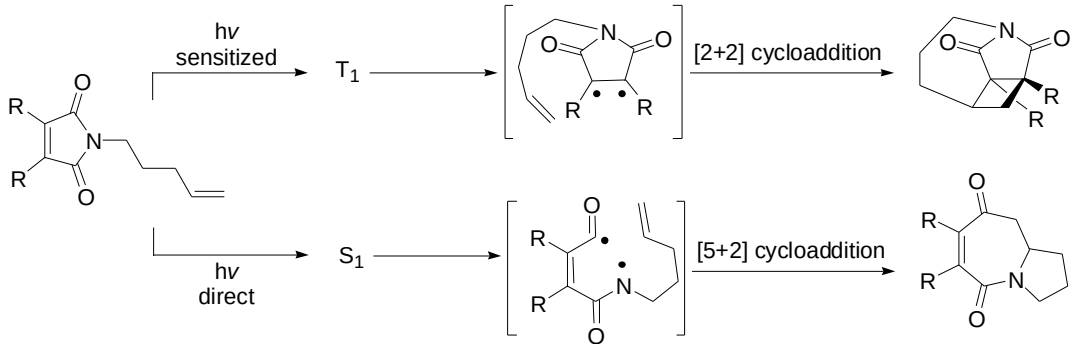
Entry	R	Yield of [2+2] A (cis:trans)	Yield of proposed [5+2] B
1	t-Butyl	92%[a,b] (13:87)	--
2	Cyclohexyl	74% (83:17)[c]	--
3	Phenyl	73% (67:6)[c]	--

[a]Isolated alongside regioisomers (see text). [b]Isolated as an inseparable mixture of diastereomers. [c]Combined yield of isolable diastereoisomers.

<u>Probing the proposed [5+2] photocycloaddition</u>

Substituting the nitrogen had negated the proposed [5+2] pathway, but so had substituting the sulfur, which was unexpected. Up to this point, it had been deemed that the minor conjugation products were from intermolecular [5+2] photocycloadditions.

Whilst intramolecular examples were known,[149] this would have represented the first intermolecular variant of its kind. Work by the Booker-Milburn research group had favoured the formation of [2+2] adducts over [5+2] adducts with the use of triplet sensitizers.[149] It was postulated that the [2+2] reaction proceeded through the triplet (T_1) pathway and the [5+2] proceeded through the singlet pathway (S_1), **Scheme 221**. The outcome of the reactions could be altered by addition of additives that behaved as sensitizers,[10, 18, 64, 242] favouring the [2+2] reaction and shutting down the [5+2] pathway.

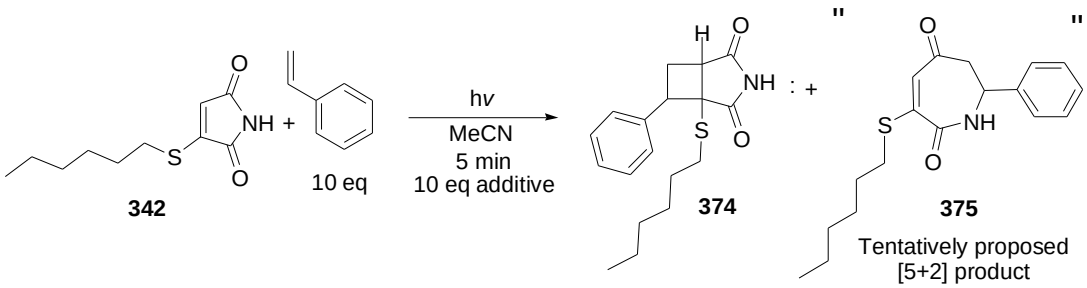


Scheme 221

Similarly, it was envisaged that if the two photochemical reaction pathways of thiomaleimide **342** were [2+2] and [5+2] photocycloadditions, then these additives could be used to favour one of the pathways in the reaction of thiomaleimide **342** with styrene. Reagents were chosen by direct analogy to the work of Booker-Milburn *et al.*,[149] where acetophenone and benzophenone had successfully altered the path of [2+2] *versus* [5+2] mediated reactions,[149] behaving as triplet sensitizers and favouring the [2+2] reaction. Quinoline had not successfully sensitized the triplet reaction in the work carried out by the Booker-Milburn group,[149] but was chosen for comparison to acetophenone and benzophenone. The additives could behave as triplet sensitizers or quenchers,[149] thus the outcome of their addition to the irradiation reaction of thiomaleimide **342** and styrene was not predicted. It was merely thought that they would have a notable effect on the proportion of the [2+2] product **374** to the proposed [5+2] adduct **375**. If the product ratios significantly altered, this could indicate that the side-product **375** was indeed the [5+2] adduct.

The model reaction of thiomaleimide **342** plus ten equivalents of styrene in acetonitrile was used to investigate the effects of these additives. The standard irradiation for five minutes was carried out, and then the solvent removed *in vacuo*. ^{1}H NMR spectroscopy was used to investigate the ratio of [2+2] product to the proposed [5+2] product. No notable effects were observed when acetophenone, benzophenone and quinoline were used, entries 2-4, **Table 34**. This suggested that the addition of these reagents was not a viable method of forcing the reaction to one overwhelming product. In addition to guidance from work carried out by the Booker-Milburn research group,[149] the reaction was also carried out in acetone, as it was a known triplet sensitizer,[64] but no change in the ratio was observed, entry 5, **Table 34**.

Table 34: Investigations into the use of triplet quenchers and sensitizers in the reaction of thiomaleimide 342 with styrene



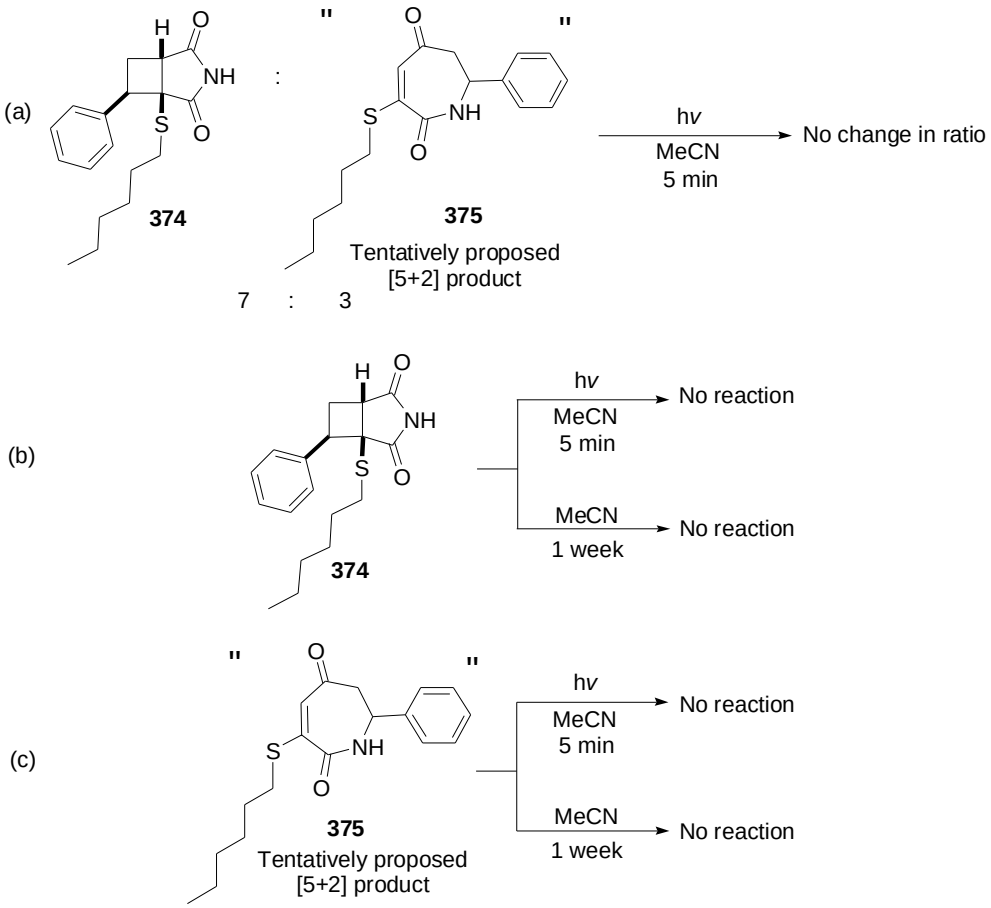
Entry	Additive	Ratio of 374:375[a]
1	No additive	70:30
2	Quinoline	73:27
3	Benzophenone	69:30
4	Acetophenone	63:37
5	Acetone[b]	70:30

[a]Ratios deduced from ^{1}H NMR. [b]Used as reaction solvent.

As the potential sensitizers and quenchers had shown minimal effects on the outcome of the reaction, the identification of the compound as a [5+2] adduct was again brought into question.

It was deemed necessary to establish that the proposed [5+2] product **375** was not formed from a secondary photochemical reaction of the [2+2] adduct **374**. Therefore a mixture of the [2+2] adduct **374** and the proposed [5+2] adduct **375** were subjected to

further irradiation and no change in the ratio was witnessed, **Scheme 222 (a)**. Additionally, both the isolated [2+2] adduct **374** and the proposed [5+2] adduct **375** were irradiated separately and no reaction was seen, **Scheme 222 (b)** and **(c)**. After one week in solution, no change in the isolated products **374** and **375** was witnessed, **Scheme 222 (b)** and **(c)**. These results all indicated that the products did not interconvert.



Scheme 222

As substituting the thiol with bulky groups had shut down the formation of the proposed [5+2] adduct, which was unexpected, and the addition of sensitizers and quenchers had no effect on the outcome of the photochemical addition, the proposed [5+2] products were brought into question. The proposed [5+2] products could not be crystallised as they were all oils, thus X-ray crystallography could not be used to confirm their structure. The proposed [5+2] products were novel and, as such, there were no similar compounds for direct comparison in the literature. Without crystallographic data, NMR spectroscopy was the technique of choice to help confirm the structures of the proposed

[5+2] adducts. The ^{1}H, ^{13}C, HH COSY and HC HMQC (Heteronuclear Multiple Quantum Coherence) spectra all fitted the proposed structure **375**. The HC HMBC spectrum from proposed [5+2] product **375** also seemed to fit well with the proposed structure, **Figure 49 (b)**. However, the HMBC spectrum was difficult to interpret as there was literature that suggested that quaternary carbons, and carbon centres across double bonds could sometimes demonstrate no coupling, even when close-by, and that flat connectivity, amide bonds and π-systems could all increase the number of bonds through which correlations could be seen.[303] Lack of correlation between the SCH_2 and the alkene CH, 1, **Figure 49 (b)**, was a cause for concern but it was explained by the dividing thio-alkene. However, the most speculative of assignments was from the SCH_2 to the CH in the azepine ring, 4, **Figure 49 (b)**. The distant correlation was previously attributed to the dividing heteroatoms and amide bond extending the scope of the observed multiple bond coupling.

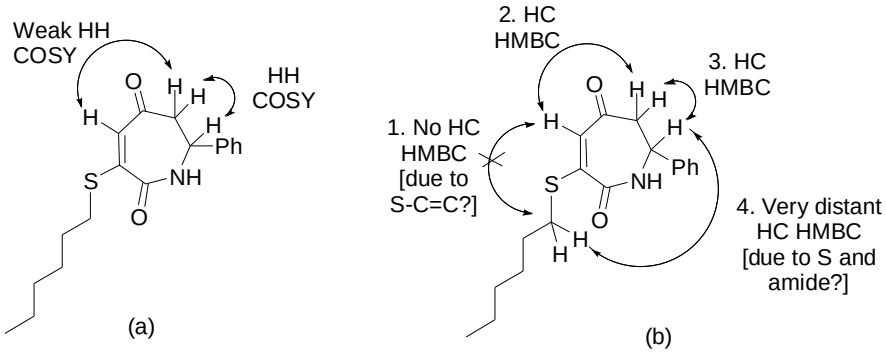


Figure 49: Correlations seen and missing by (a) HH COSY and (b) HC HMBC

At this juncture, it was felt that the proposed [5+2] adduct **375** had perhaps been misassigned. Therefore, all known mechanisms from photochemical activation of maleimides were ignored and the spectra reassessed with all the structural information that had now been obtained.

In order to make an unbiased assignment of the structure, 'missing' H-C correlations were not taken into account and all present H-C correlations were assumed as from 2-4 bond couplings, as is the standard range.[303] Using these parameters, the data indicated that there was a CH bonded both to the thiyl group and the aromatic group originating from the styrene, affording fragment **(a)**, **Figure 50**, this was very different to the original supposition. This CH was bonded to a CH_2 that, through multiple bond

correlation, could couple to the alkene in fragment **(b)**, **Figure 50**. In addition to these two factors, both carbonyl signals appeared around 170 ppm, indicating that they were both maleimidic in nature, fragment **(c), Figure 50**. With these factors in mind, and ignoring what was deemed 'likely' from past precedent, structure **408** was proposed, **Figure 50**.

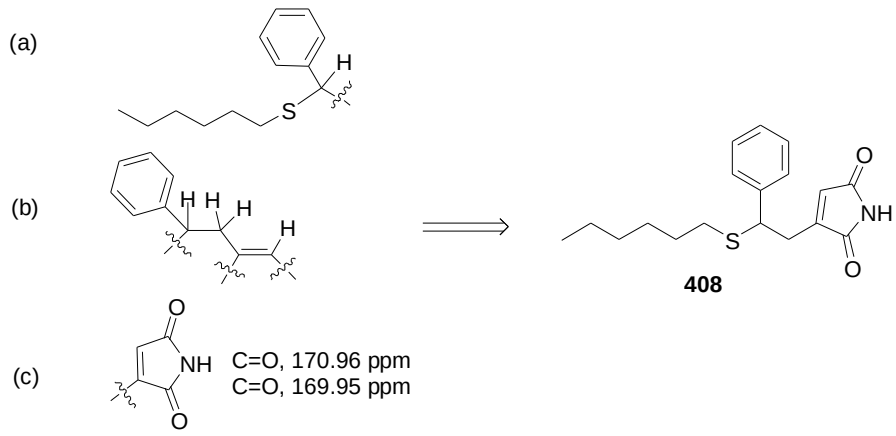


Figure 50: Fragments described by NMR and elucidated structure 408

The previous HH COSY **(a)** and HC HMBC **(b)** correlations could now be attributed to those shown in **Figure 51**. Furthermore, under the guidance of Dr Abil Aliev (UCL, Chemistry), the ^{1}H NMR spectrum was further processed to resolve small coupling constants by minimising line broadening in the spectrum. By minimising line broadening, coupling constants of 1.5 Hz could be seen between the alkene in the maleimide with the CH_2 and the NH **(c)**, **Figure 51**.

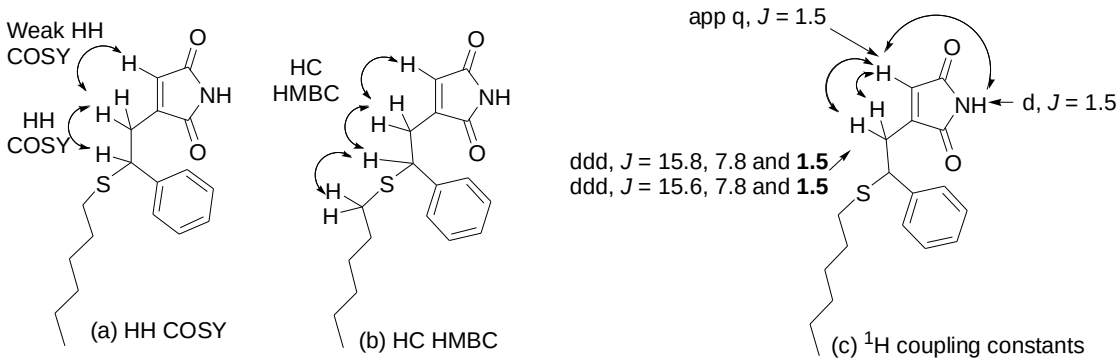


Figure 51: (a) HH COSY and (b) HC HMBC coupling and (c) small coupling constants in structure 408 seen by ^{1}H NMR

Comparison of the proposed structure **408** with known compounds was not completely conclusive as the products were novel and the scaffold quite unusual, but there were some similarities between the literature and the positions of the peaks observed. In ^{1}H NMR, the aryl-thiyl CH in structure **408** was at 4.11 ppm, falling between 3.69-4.78 ppm, the range found for this proton in similar compounds, **Figure 52**.[304-306] Additionally the maleimide-CH_2 in the structure **408** was between 2.96 and 3.01 ppm, a little higher than literature examples (2.40-2.72 ppm),[307-309] but taking the adjacent phenyl ring into account, this was deemed acceptable. In addition, the SCH_2 in the structure **408** was between 2.26-2.36 ppm. In literature examples,[304-306, 310, 311] **Figure 52**, similar protons fell between 2.18-2.45 ppm, and it was thus felt that the structure **408** was now correct. The structure was further confirmed by corroboration of the ^{13}C NMR shifts of compound **408** with the available literature compounds,[308, 312] **Figure 52**. For example, the maleimidic alkenyl carbons were at 129.41 ppm (CH) and 147.26 ppm (C) in **408** *cf.* literature values of 128.3 ppm (CH) and 142.28-146.70 ppm (C).[308, 312] Whilst similar structures with ^{13}C spectra could not be found for most of the comparable carbons, the CH_2 adjacent to the ring in **408** was also close to literature values for this carbon, *e.g.* 28-31 ppm *cf.* 29.24 ppm,[308] **Figure 52**.

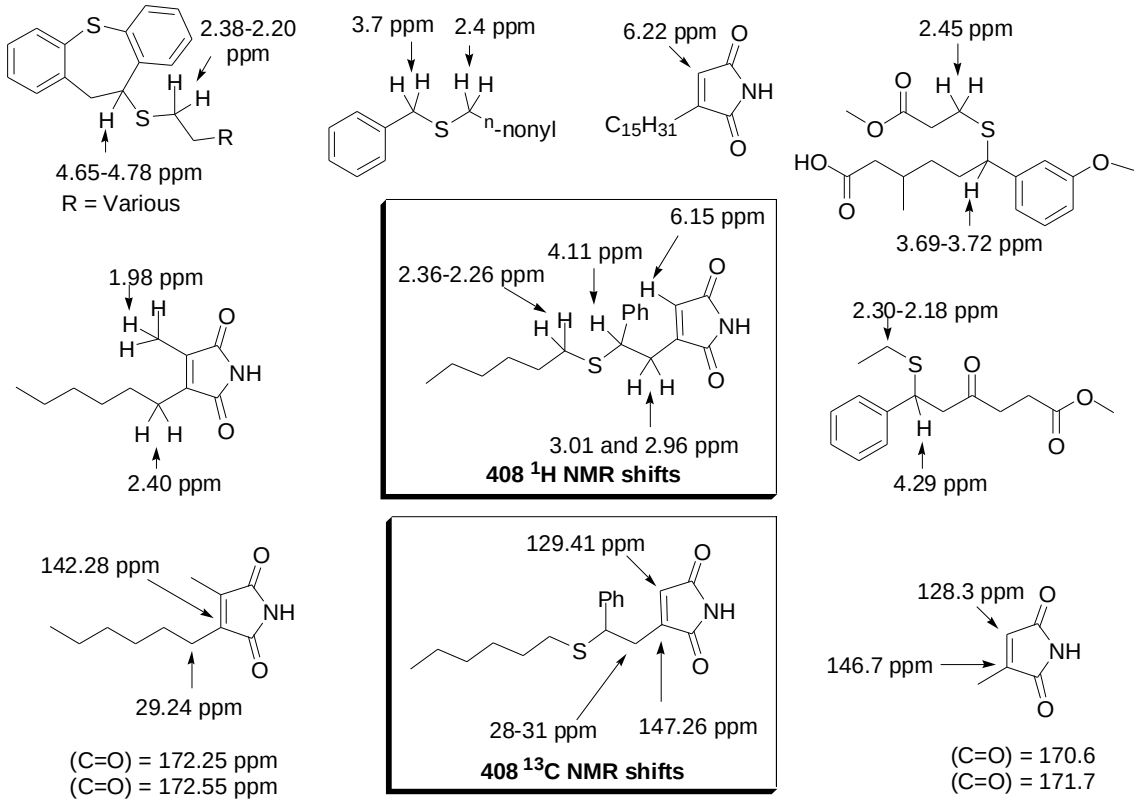
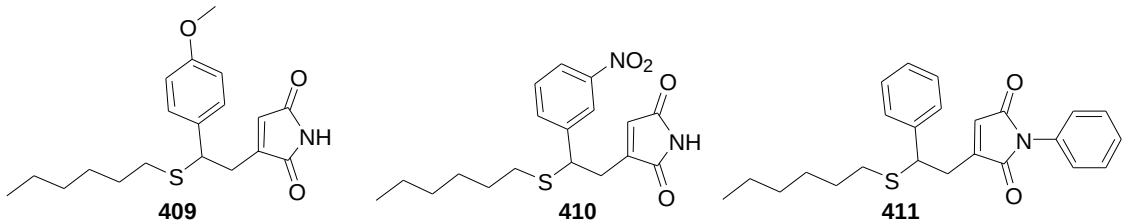


Figure 52: A range of some literature examples for comparison to structure 408

At this stage it was realised that all of the proposed [5+2] adducts with styrenes were in fact of this 'insertion' nature, with the correct corresponding HC HMBC and HH COSY correlations. The structures also all showed the small coupling constants (1.1-1.6 Hz) after further processing of their ^{1}H NMR spectra. Therefore the yields and true structures of the products observed from thiomaleimide **342** and **391** irradiation with different styrenes with were identified as shown in **Figure 53**.



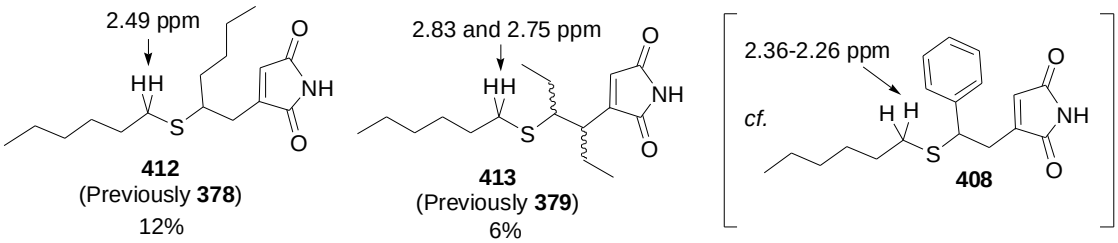
409
(Previously **388**)
25%

410
(Previously **390**)
25%

411
(Previously **396**)
1%

Figure 53: Proposed structures and yields of the products of the minor conjugation

The 'insertion' products from the reaction of thiomaleimide **342** with but-1-ene and *trans*-hex-3-ene also demonstrated the correct HH COSY and HC HMBC NMR spectra for assignment as shown in **Figure 54**, yields are given underneath the structures. Maleimide **412** demonstrated the small coupling constants of 1.4 Hz in the ^{1}H NMR after further processing of the spectrum, but maleimide **413** did not. This could be due to the extra substitution in maleimide **413** affording a slightly different conformation of the structure that lessened the coupling beneath the threshold of the software used to process the spectra (Topspin). In the 'insertion' products **412** and **413**, it was noticed that the SCH_2 appeared further downfield in the aliphatic thioethers than in those substituted with an aromatic ring, **Figure 54**. This was a potential cause for concern as the reverse was expected, with the SCH_2 near an aromatic ring expected to be further downfield.



412
(Previously **378**)
12%

413
(Previously **379**)
6%

408

Figure 54: Comparison of SCH_2 in 412 and 413 with SCH_2 in 408

However, it seemed that this outcome was in accordance with literature examples. For example, it could be seen that the CH_2 in thioethers with an aromatic substituent, **414**, **415** and **416**, were usually upfield compared to CH_2s in simple thioethers, **417** and **418**, **Figure 55**.[311, 313, 314] This was not explained in the literature examples found but could be due to an orientation of the aromatic ring whereby it electronically shields the SCH_2 leading to an upfield shift in the NMR signal.

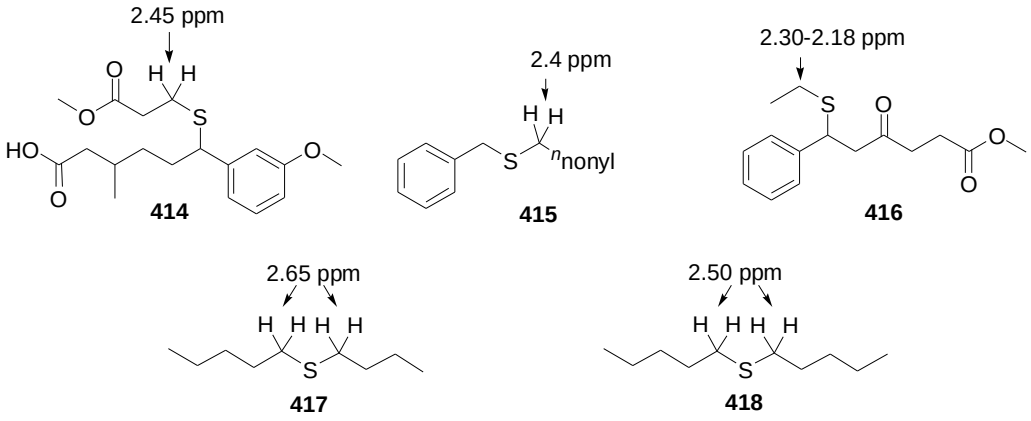
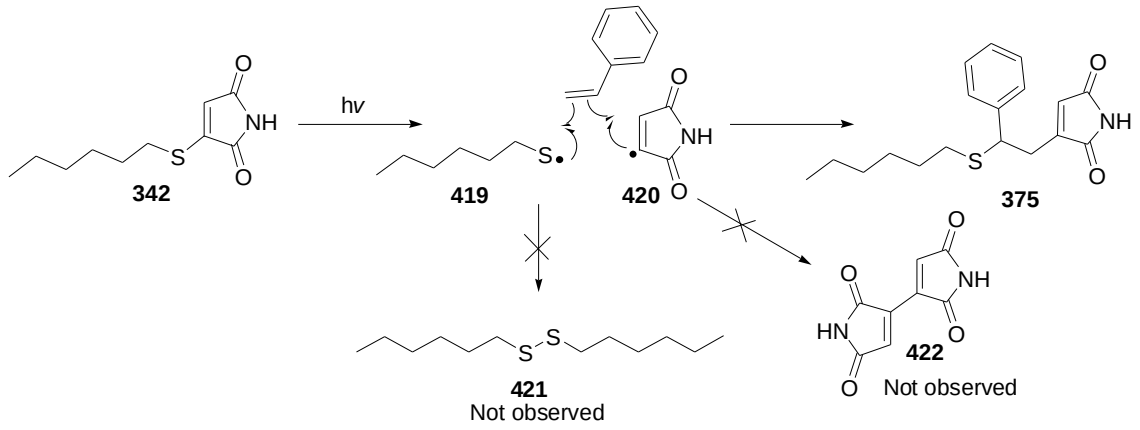


Figure 55: CHs in thioether with and without aromatic substitution

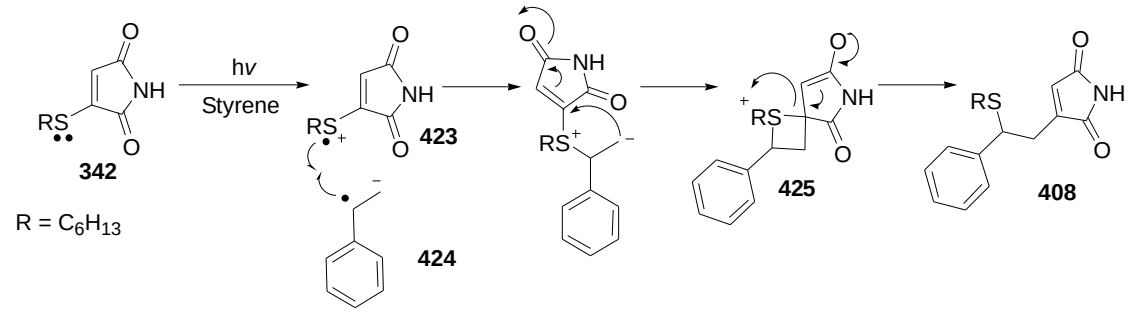
It was uncertain why this subsection of the partners trialled afforded the alternate 'insertion' conjugation. It could be proposed that the 'insertion' products are more stable for these examples, and this merely supported their isolation in these cases. There were no accounts of exactly this transformation in the literature but several mechanisms could be postulated.

For example, aliphatic thioethers can undergo homolytic cleavage of the C-S bond.[315] Cleavage of the C-S bond in thiomaleimide **342** and addition across the styrene double bond, **Scheme 223**, could theoretically afford compounds of the sort identified in these reactions. However, no homocoupled disulfide **421** or dimaleimide **422** were isolated in these reactions, thus radicals **419** and **420** would have had to have undergone a very selective and regiospecific reaction with styrene, in order to afford just one by-product. This was deemed highly unlikely as homocoupling of radicals is usually the major result of such reactions. [315]



Scheme 223

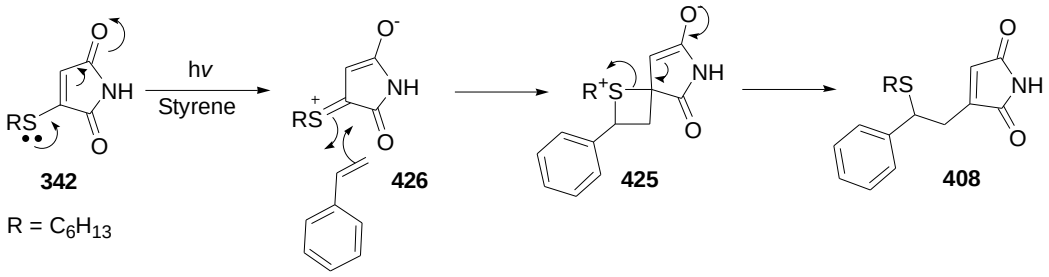
An alternative mechanism was derived from the fact that when irradiated with appropriate electron acceptors, thioethers can behave as electron donors.[316] If the styrene is taken as an electron acceptor, a mechanism can be envisaged whereby the products of the electron transfer event can afford the observed conjugate **408**, **Scheme 224**. Combination of the two radical species **423** and **424** and Michael addition from the terminal position of the styryl species could afford the cyclobutane enolate **425**. Collapse of the enolate **425** would fragment the C-S bond and afford the product isolated **408**, **Scheme 224**. For this mechanism to be viable, radical recombination must take place exclusively at the benzylic position and the Michael addition of the anion from the terminal position of the styrene.



Scheme 224

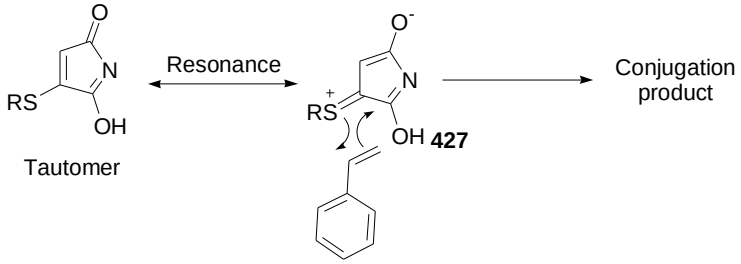
Additionally, thiocarbonyls are capable of [2+2] photocycloaddition[10, 317] and a resonance form of the thiomaleimide can be drawn whereby the C-S bond has double bond character **426**, **Scheme 225**. If this double bond is treated as a thiocarbonyl then [2+2] photocycloaddition can be postulated to take place with the styrene double bond. It has already been discussed that such cycloadditions usually have a preferred

orientation, and this could explain why only one configuration was observed. Collapse of the enolate **425** and concomitant cleavage of the C-S bond would afford the observed product, **Scheme 225**.



Scheme 225

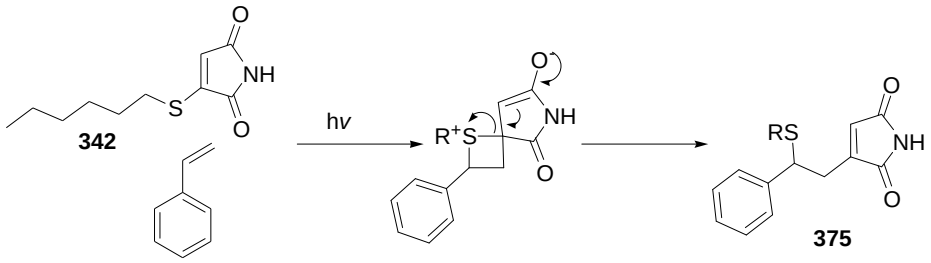
When the nitrogen of the thiomaleimide was substituted, no 'insertion' products were isolated. This could suggest the tautomer shown in **Scheme 226** played a key role in the transformation. It was postulated that tautomer **426** and its resonance form **427** perhaps had a stabilising effect on the S^+=C bond, allowing the thiocarbonyl [2+2] to take place, **Scheme 226**. When the nitrogen was substituted, the formation of these tautomers would have been disrupted. This could potentially account for why, upon substitution of the nitrogen, products of this kind were not isolated.



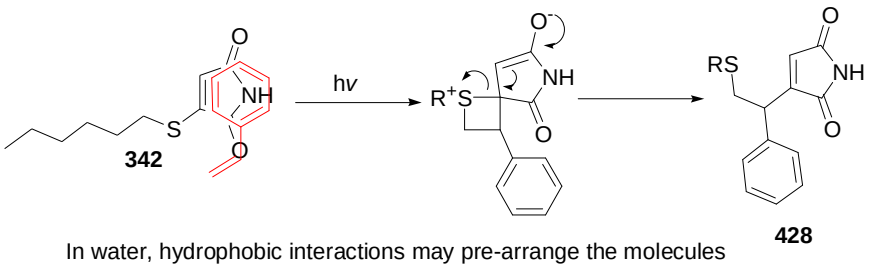
Scheme 226: Tautomers of the thiomaleimide 408 could stabilise S^+=C bond

It was postulated that the electron transfer and [2+2] photocycloaddition mechanisms would both be attenuated if the thiyl group's bulk stopped approach of the styrene. Indeed, when the thiyl pendant was increased in bulk, no products of this kind were isolated, bolstering the likelihood of these mechanisms over the homolytic fission mechanism which should have been less affected by bulky groups on the thiyl pendant group.

Increased solvent polarity could favour the electron transfer mechanism[18] as the charged radicals would have been stabilised by the solvent. However, the opposite result occurred, and, in water, there was, in fact, a preference for the expected [2+2] adduct. This potentially made the electron transfer mechanism less likely. When the reaction was carried out in water, the different chemical shifts of the product could be attributed to maleimide **428**, **Scheme 227**. This product has the opposite orientation with respect to the styrene 'insertion', although this compound was not isolated. One possible explanation why the latter two mechanisms were affected by the aqueous solution was due to the hydrophobicity of the styrene. Both mechanisms were bimolecular processes, whereby the styrene and the thiomaleimide had to be in close proximity for the reactions to occur. When exposed to aqueous media, it was possible that the hydrophobic styrene formed a close-packed system with the organic thiomaleimide **342**. Potentially, this pre-orientation afforded the altered configuration **428**. **Scheme 227** shows how the altered orientation could have provided the different product **428** in the latter mechanism.



In acetonitrile, the molecules are free to adopt the preferred conformation for reaction



In water, hydrophobic interactions may pre-arrange the molecules

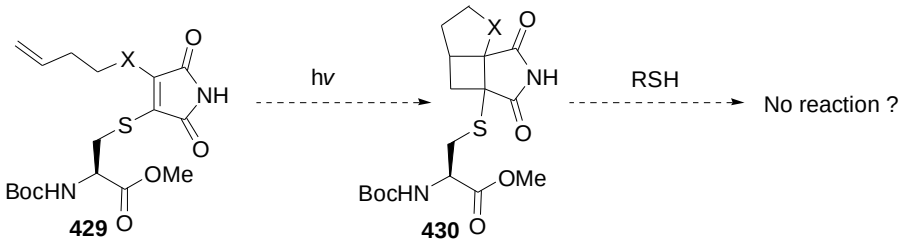
Scheme 227

After the investigations into the spectral data of the previously proposed [5+2] products, it was thus concluded that the products were in fact the result of an unknown mechanism that afforded 'insertion' products. These products were unprecedented and represented a new photochemical reaction. Ultimately, further in-depth mechanistic

studies of this 'insertion' were not the focus of this work and no further investigations were carried out on this.

Photochemistry of intramolecular analogues

The potential of using irradiaton to render a thiomaleimide stable to reducing conditions has already been discussed, see p.174 and p.197. However, in dimerisation and conjugation strategies, the modifications imparted on the thiol are rendered irreversible by photochemical reaction with another molecule. It was postulated that incorporation of an alkenyl tether onto the thiomaleimide would allow the molecule to react with itself upon irradiation, to impart the irreversibility afforded from cyclobutane formation. For example, it was postulated that irradiation of thiomaleimide **429** would form the cyclobutane **430**. It was envisaged that the cyclobutane **430** thus formed would be stable to reducing conditions, **Scheme 228**.



Scheme 228

In order to determine if the [2+2] photocycloaddition would proceed on simple substrates, it was deemed necessary to synthesise scaffolds **431** and **432**, **Figure 56**. The aminomaleimide **431** was chosen with a view to tethering the alkene to the maleimide *via* an amine with eventual transferral to aminothiomaleimides, **Figure 56**. The thiomaleimide **432** was proposed to investigate tethering the alkene to the maleimide *via* sulfur with the eventual goal of synthesising dithiomaleimides that could undergo the proposed intramolecular [2+2] photocycloaddition, **Figure 56**.

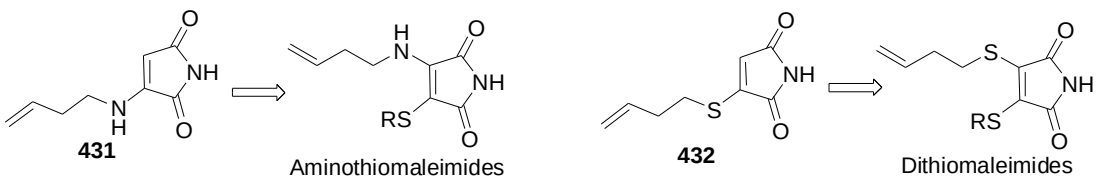
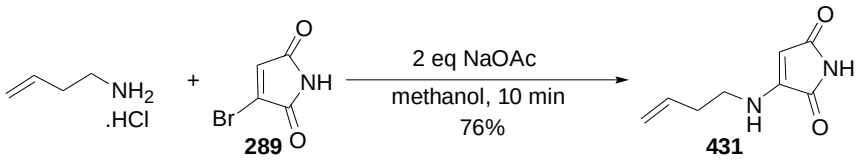


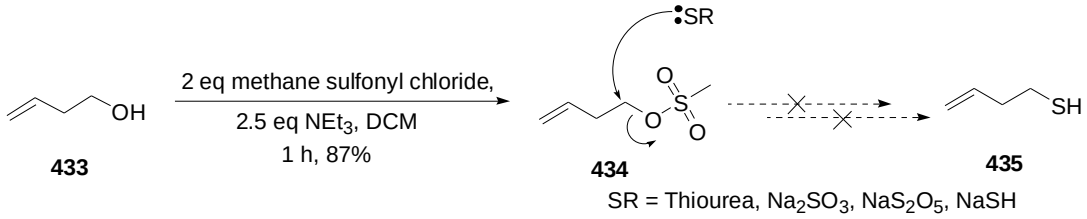
Figure 56: Planned maleimide cores for intramolecular studies

The aminomaleimide **431** was synthesised *via* addition of the commercially available 3-butenylamine hydrochloride salt to bromomaleimide **289** in the presence of two equivalents of base in 76%, **Scheme 229**.



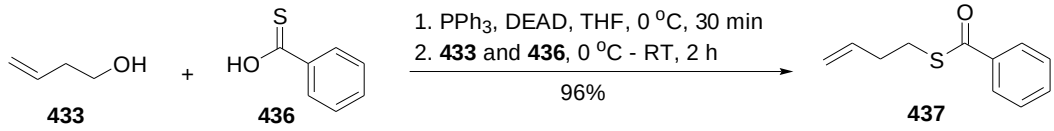
Scheme 229

The thiomaleimide **432** proved more problematic as 3-butenylthiol was not commercially available and had to be synthesised. The synthesis of the thiol **435** was not trivial as it was volatile and prone to forming its disulfide. With the intention of displacing the mesylate with a thio-nucleophile, the appropriate alcohol **433** was converted to the methylsulfonate **434** with methanesulfonyl chloride and triethylamine in 87%, **Scheme 230**. Unfortunately, treatment of this mesylate **435** with any one of thiourea and then base; sodium bisulfite; sodium metabisulfite or sodium hydrosulfide afforded no usable product. The reactions were never clean, and attempts to purify the material only resulted in loss of the volatile thiol **435**.



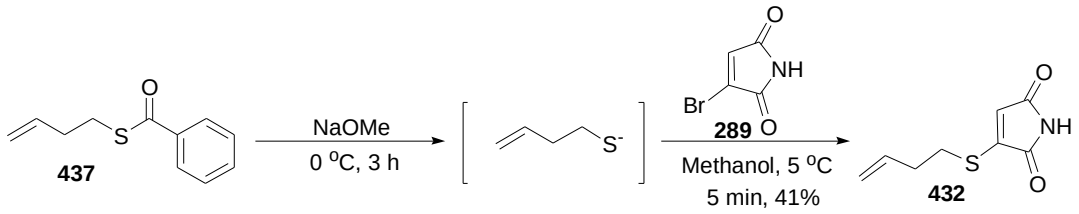
Scheme 230

The eventual route to thiol **434** involved the thio-mitsunobu reaction of 3-buten-1-ol **433** with thiobenzoic acid **436** to afford thioester **437** in 96%, **Scheme 231**. The thioester **437** was less volatile than the thiol **435**, but care was still taken as it was soon noticed that the product could be lost under the strong vacuum used to remove residual solvent.



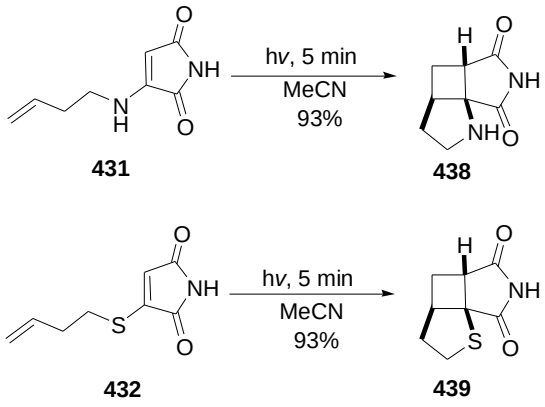
Scheme 231

The thioester **437** resisted hydrolysis in pH 8 phosphate buffer, but underwent hydrolysis after three hours when dissolved in sodium methoxide. The *in situ* thiolate was immediately added dropwise to a cooled solution of bromomaleimide **289** in an effort to minimise double addition of the reactive thiolate, affording thiomaleimide **432**, **Scheme 232**.



Scheme 232

Irradiation of the maleimides **431** and **432** was successful, with both variants affording almost pure crude reaction mixtures. Column chromatography removed a small amount of impurities and the tricyclic structures were both afforded in 93%, **Scheme 233**.



Scheme 233

This indicated that the desired intramolecular [2+2] reaction would occur with similar molecules. The aminothiomaleimide **440** was used as the model for this work rather

than dithiomaleimide **440**, **Figure 57**, as, owing to time constraints, the incorporation of the butenylthiyl to afford dithiomaleimide **441** was not achieved.

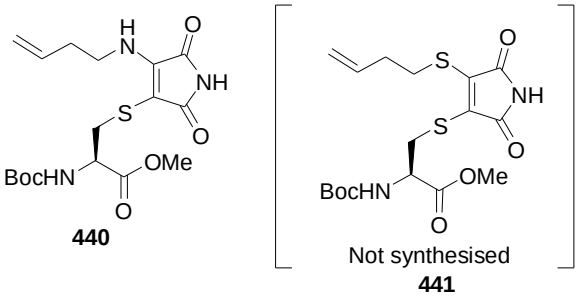
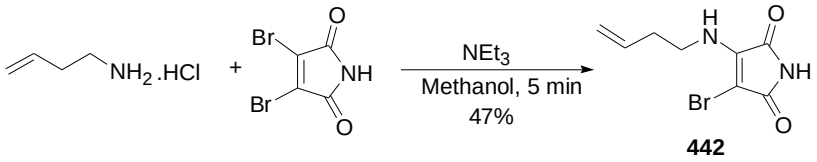


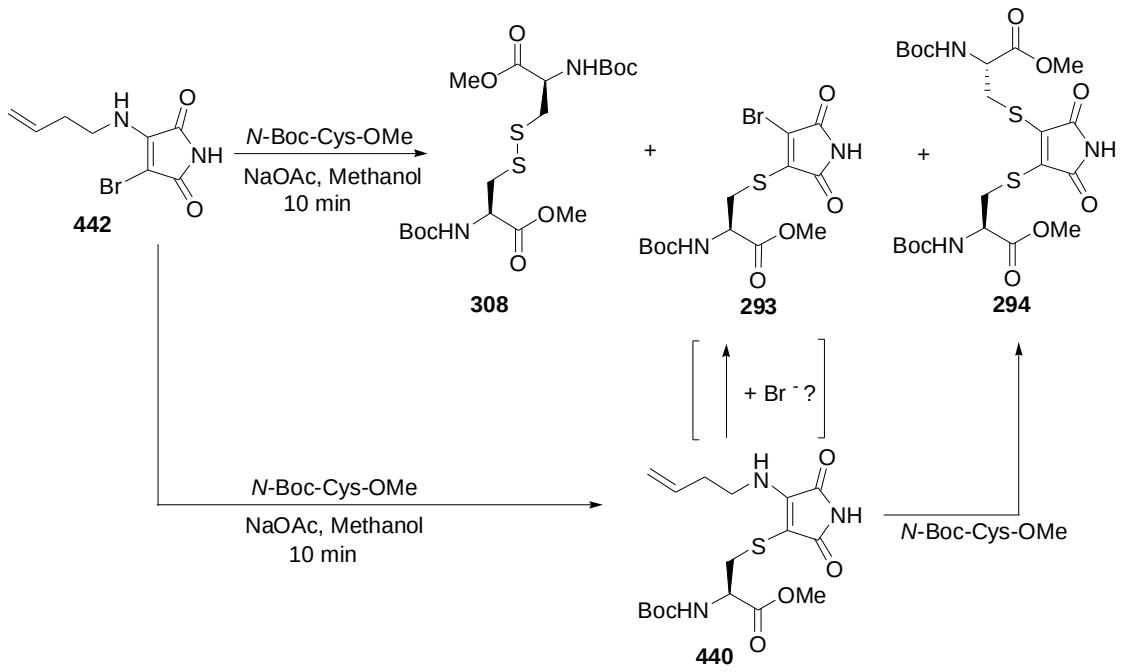
Figure 57: Proposed model for intramolecular studies

Starting from the commercial dibromomaleimide, one equivalent of the 3-butenylamine hydrochloride salt was added to afford a 47% yield of the single addition product **442**, **Scheme 234**.



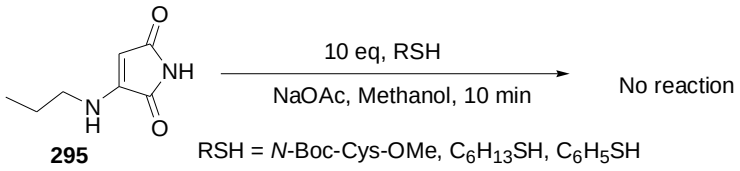
Scheme 234

However, when one equivalent of cysteine was added to this construct, an array of compounds could be seen in the crude reaction mixture after ten minutes, including free cysteine, cysteine disulfide **308**, bromothiomaleimide **293**, and dithiomaleimide **294**, **Scheme 235**. The terminal alkene peak of the amine could not be seen in the crude reaction mixture. This indicated that the cysteine thiol was capable of reacting with the aminobromomaleimide **442** to displace the amine. Additionally, the cysteine could react more than once with the compounds in solution to afford dithiomaleimide **294**. The presence of the mixture of compounds could be through formation of the desired aminothiomaleimide **440** and further reaction to the products seen in the crude, or *via* direct displacement of the amine by the thiol.



Scheme 235

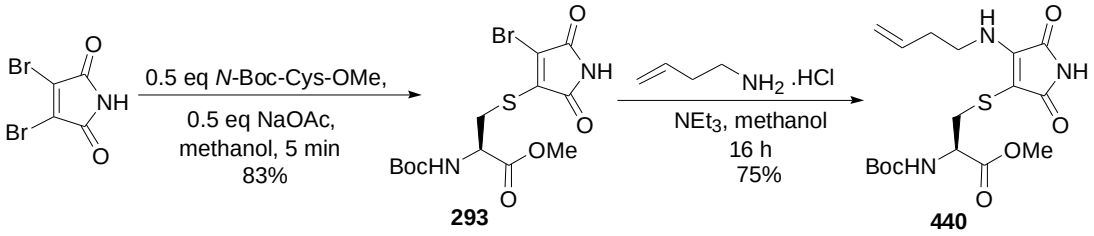
As, at some point in the reaction, aminobromomaleimide **442** had converted to bromothiomaleimide **293**, the use of maleimide as a novel protecting group for amines, with subsequent deprotection by thiols was recognised here. However, addition of a variety of thiols, including cysteine, to the simple aminomaleimide **295**, resulted in no reaction, **Scheme 236**.



Scheme 236

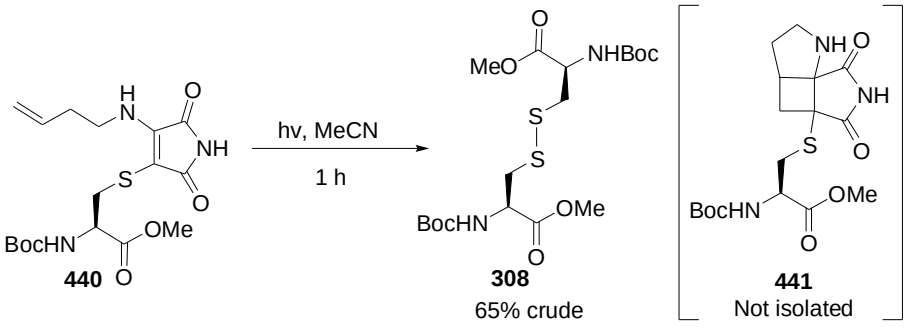
This reaction suggested that the previous displacement of an amine by a thiol was specific to the aminobromomaleimide **442** under those particular reaction conditions. Aminobromomaleimides of similar structure to the aminobromomaleimide **442** were deemed too reactive to be utilised in a realistic protecting strategy, as they could potentially react with other functionalities, and this methodology wasn't taken any further.

It was decided to approach the mixed aminothiomaleimide **440** from the 'opposite' direction, *i.e.* attaching the cysteine motif first and then adding the amine to the bromothiomaleimide **293** thus formed. The bromocysteine adduct **293** was synthesised in 88% yield. Although notably, with sacrificial dibromomaleimide used in excess to limit double addition of the cysteine, **Scheme 237**. With this product in hand, it was reacted with one equivalent of the appropriate amine salt to afford a 75% yield of the aminothiomaleimide **440**, **Scheme 237**.



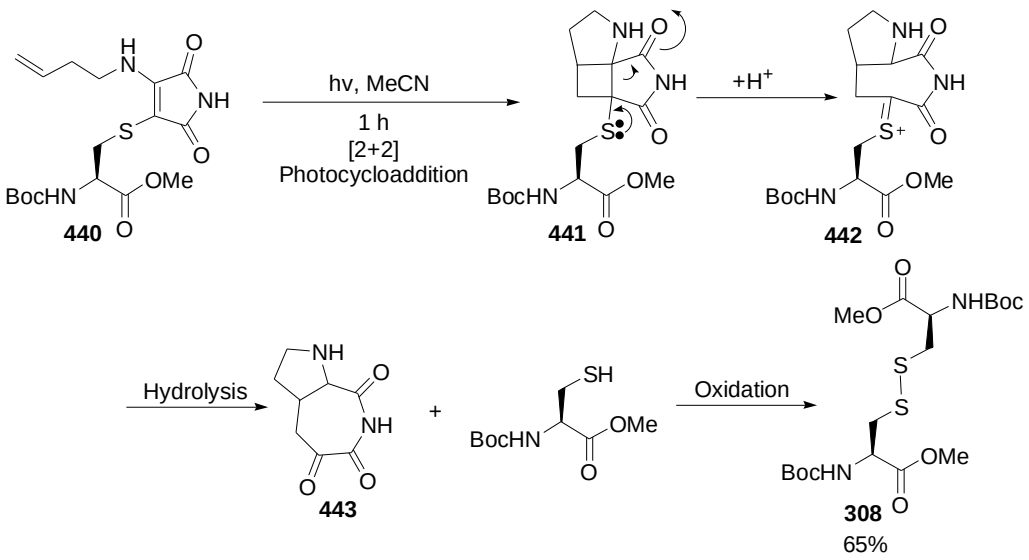
Scheme 237

It took an hour of irradiation for complete consumption of the maleimide **440**, and the result was not the expected cyclobutane **442**, **Scheme 238**. Crude cysteine disulfide **308** was the only isolable material from this reaction, in approximately 65%, but further purification of dicysteine **308** was not successful.



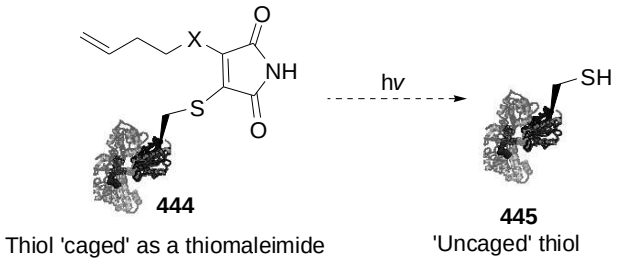
Scheme 238

The cysteine was possibly liberated *via* initial formation of the cyclobutane **441**, followed by the thiol lone-pair allowing the ring-opening of the highly-substituted strained ring to form the sulfonium **442**, **Scheme 239**. This cation could then be hydrolysed to afford the free cysteine, which could, in time, form the disulfide **308** isolated, **Scheme 239**. The fate of the 5,7 ring **443** was unidentified as no other products were isolated from this reaction.



Scheme 239

The cysteine appeared to be liberated from the thiomaleimide *via* irradiation, *i.e.* the cysteine was 'uncaged' *via* photolysis. This could potentially have applications in uncaging of thiomaleimide modified proteins **444** to afford the free thiol **445**, **Scheme 240**.



Scheme 240

This reaction could potentially start investigations into photochemically-mediated elimination of the cysteine from the thiomaleimide constructs, but due to time constraints this avenue was not explored any further in this work.

<u>Photochemical conjugation of amino acid thiomaleimides with styrene</u>

As the [2+2] photocycloaddition of thiomaleimides and a variety of alkenyl partners had proven successful, attention was turned to the use of this reaction for conjugation of biomolecules. The focus of this work was to investigate potential modes of conjugation

of peptides and proteins thus the photochemical conjugation of thiomaleimides **291**, **330** and **356**, containing amino acids was investigated, **Figure 58**. In addition, the diastereochemical purity of the conjugation products in these reactions would not be crucial as this would be less relevant for protein bioconjugation applications.

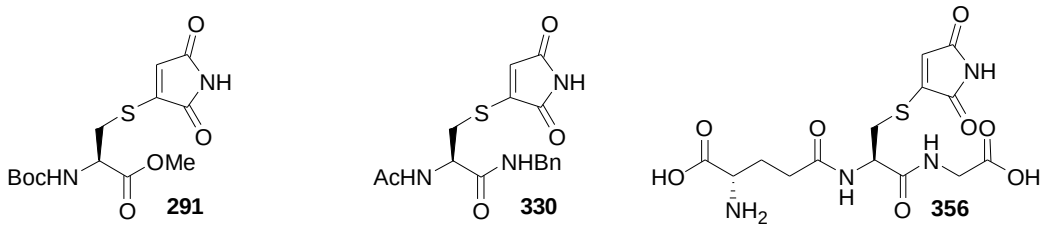
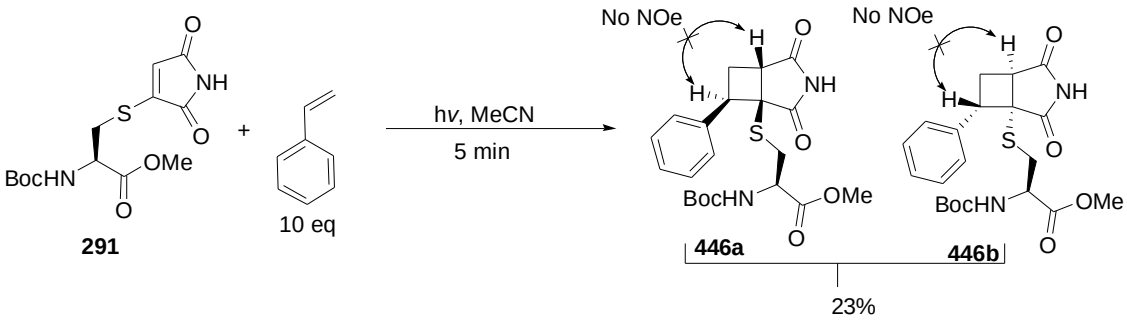


Figure 58: Amino acid based thiomaleimides

When thiomaleimide **291** was irradiated with ten equivalents of styrene in acetonitrile all peaks in the crude NMR could be assigned to expected products from the [2+2] photocycloaddition conjugation reaction. With a chiral centre already in the starting material, the range of diastereoisomers likely to be formed was further increased, to add to the potential regioisomers, thus a complex crude NMR was expected. Mass spectroscopic analysis of the crude reaction indicated that conjugation products were the major products formed. Column chromatography of the residue was difficult as the compounds were similar and prone to degradation on silica. However, after the first column, a mixture of four diastereomers could be seen in the NMR spectra in a combined yield of 63%. Further column chromatography afforded the two major diastereomers **446a** and **446b** as an inseparable mixture in 23% in a ratio of 1:1, **Scheme 241**. The diastereomers were two *syn* addition products, postulated to both have *cis* orientation, as no through-space interaction could be seen by NOeSY NMR experiments between the protons indicated.



Scheme 241

The two other diastereomers seen after the first column chromatographic purification were not fully characterised but were tentatively assigned as **447a** and **447b**, **Figure 59**, due to the similarity of all of the chemical shifts in the crude ^{1}H NMR to the characterised compounds and established trends in this conjugation.

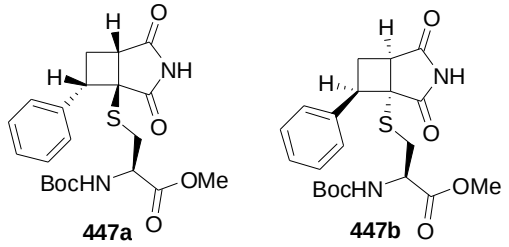
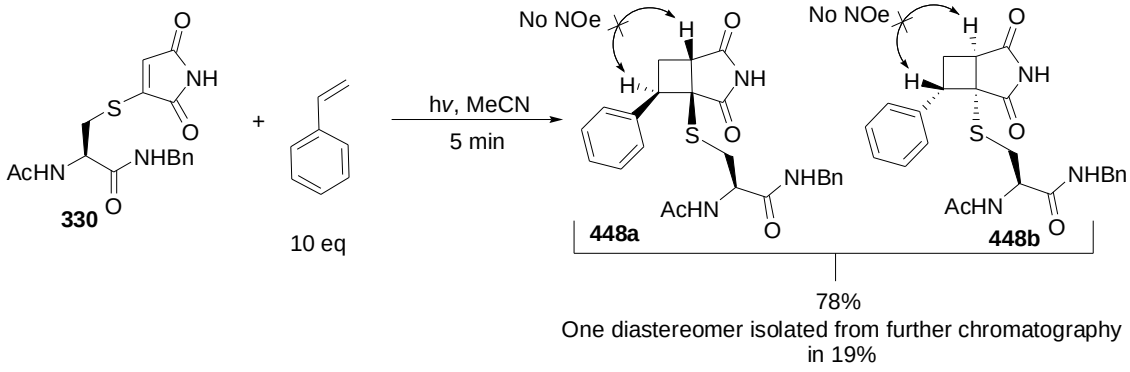


Figure 59: Proposed structure of un-isolable diastereomers

Lowering the styrene equivalents to just one afforded no dimer **340** and very similar crude NMR spectra, with a similar mix of the four diastereomers isolated in 52% after one column chromatographic purification.

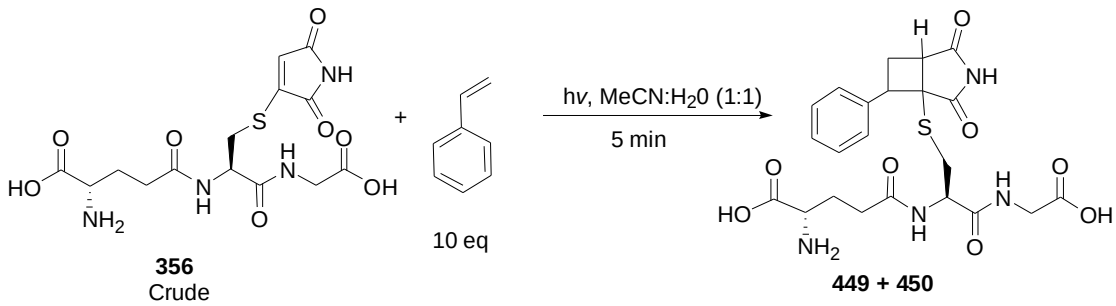
The *N*-Ac-Cys-NHBn cysteine thiomaleimide **330** was completely consumed after irradiation with ten equivalents of styrene in five minutes. Mass spectroscopy of the crude reaction again indicated that the conjugation products were the major products formed. After column chromatography, 78% of two diastereomers were isolated, **448a** and **448b**, as a 1:1 mixture, and further column chromatography afforded 19% of just one diastereomer, **Scheme 242**. It could not be determined which of the diastereomers **448a** or **448b** was isolated, as crystallisation was not successful. Reanalysis of the crude allowed almost all of the peaks to be attributed to the conjugation products isolated.



Scheme 242

Similarly to the *N*-Boc-Cys-OMe thiomaleimide **291**, when the styrene equivalents were lowered to one, the reaction was still successful, with no dimer **351** and the crude NMR spectra indicating high conjugation yields. A similar mixture of the two diastereomers **448a** and **448b** could be isolated from this reaction by column chromatography in 87%, in a ratio of 1:1.

When the crude glutathione thiomaleimide adduct **356** was irradiated with ten equivalents of styrene, NMR analysis of the crude reaction mixture also indicated a high yield of conjugation in five minutes, **Scheme 243**. This reaction was carried out in acetonitrile:water (1:1) to solubilise the glutathione adduct **356**.



Scheme 243

Unfortunately, due to the highly polar nature of glutathione, column chromatographic purification was not suitable for this reaction residue. Whilst NMR spectra indicated that there were several conjugate diastereomers formed in the reaction, LCMS indicated the presence of four compounds, all of mass 507.7 Da. These compounds were present as two pairs, tentatively attributed to two sets of similar diastereomers by analogy with past models, **Figure 60**.

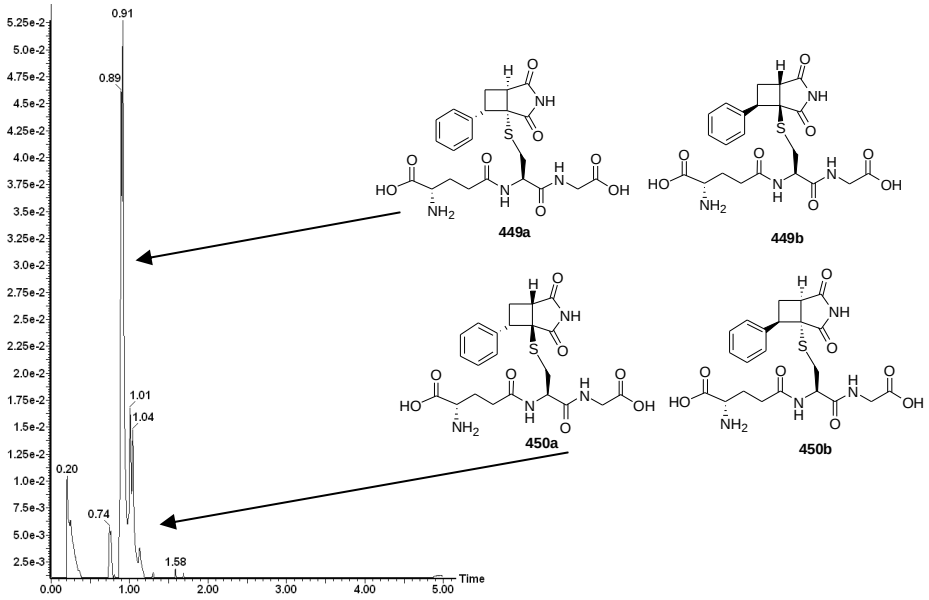
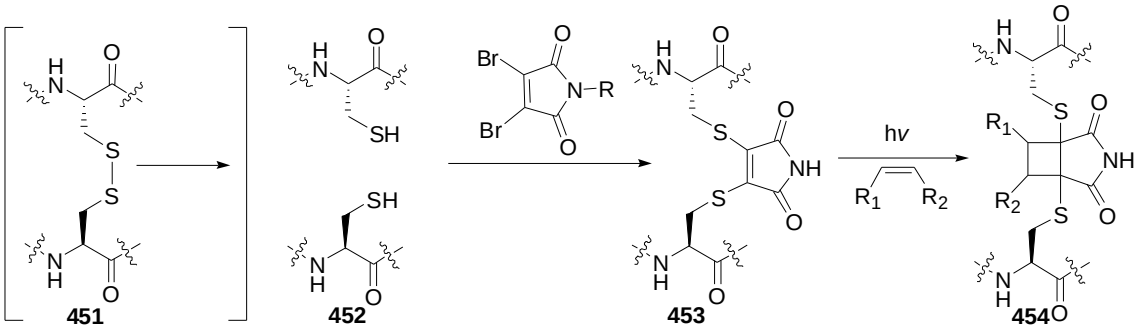


Figure 60: Mass spectrum of reaction mixture after irradiation of thiomaleimide 356 with 10 eq of styrene for 5 minutes

The photochemical conjugation strategy had therefore successfully been shown with thiomaleimides containing amino acids. This was deemed very promising as it suggested that the [2+2] photocycloaddition could potentially be transferred to proteins or peptides containing a thiomaleimide from modification of a single cysteine residue with bromomaleimide **289**.

Previous work by Dr Mark Smith (UCL, Chemistry, Caddick group), **Scheme 132**, p. 124, and Felix Schumacher (UCL, Chemistry, Baker group), **Scheme 135**, p. 126, had shown that dithiomaleimides can be formed from the bioconjugation of two thiol-containing molecules, *e.g.* a protein and a thiosugar, or from the modification of a disulfide in protein. It was decided to investigate if, like the thiomaleimides, dithiomaleimides would be photoactive. If successful, dithiomaleimides **453** formed from the addition of dibromomaleimide to thiols **452**, potentially from a disulfide bond **451**, could be further conjugated as cyclobutanes **454** *via* photochemistry, **Scheme 244**.



Scheme 244

With dithiomaleimide **294** in hand from previous studies, **Figure 61**, conjugation strategies on this molecule were planned. However, it was decided that with two chiral centres already in the molecule, initial investigations into the photoactivity would be clearer with a simpler achiral thiomaleimide.

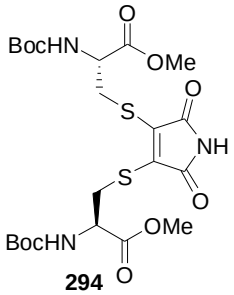
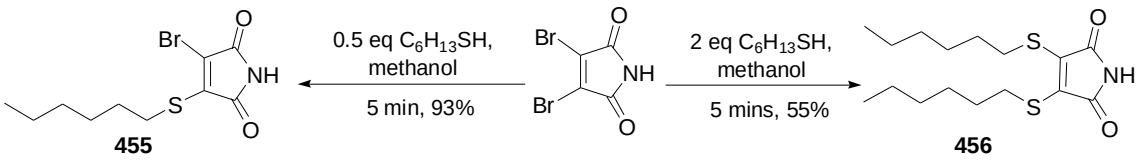


Figure 61: Proposed dithiomaleimide 294 for photochemical investigations

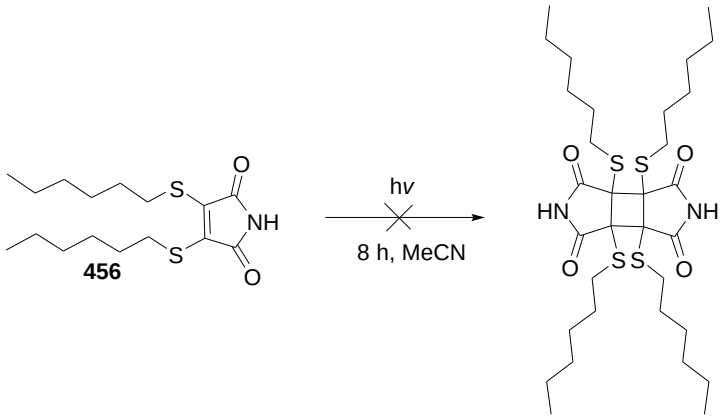
Therefore, with analogy to the monothiomaleimides, the ^{n}hexyl achiral dithiomaleimide derivative **456** was synthesised in 55%, the mixed bromothiomaleimide **455** could also be isolated in 93%, with respect to the thiol, if the thiol was used in half an equivalent, **Scheme 245**.



Scheme 245

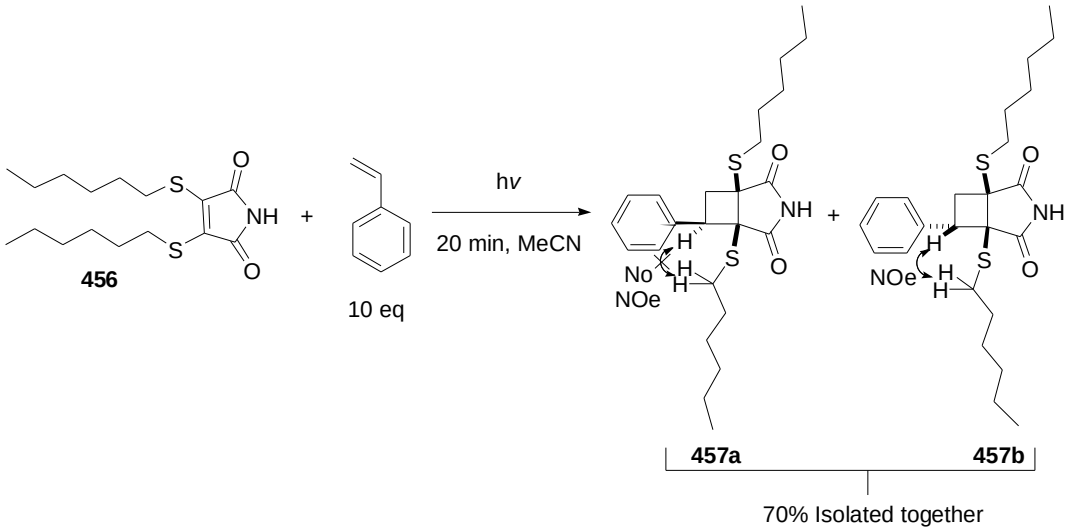
It was predicted that dithiomaleimide **456** would not dimerise upon irradiation due to steric constraints. When subjected to prolonged irradiation of eight hours, this

prediction was proven to be correct as no dimerisation occurred, **Scheme 246**, and only degradation of the dithiomaleimide **456** was witnessed.



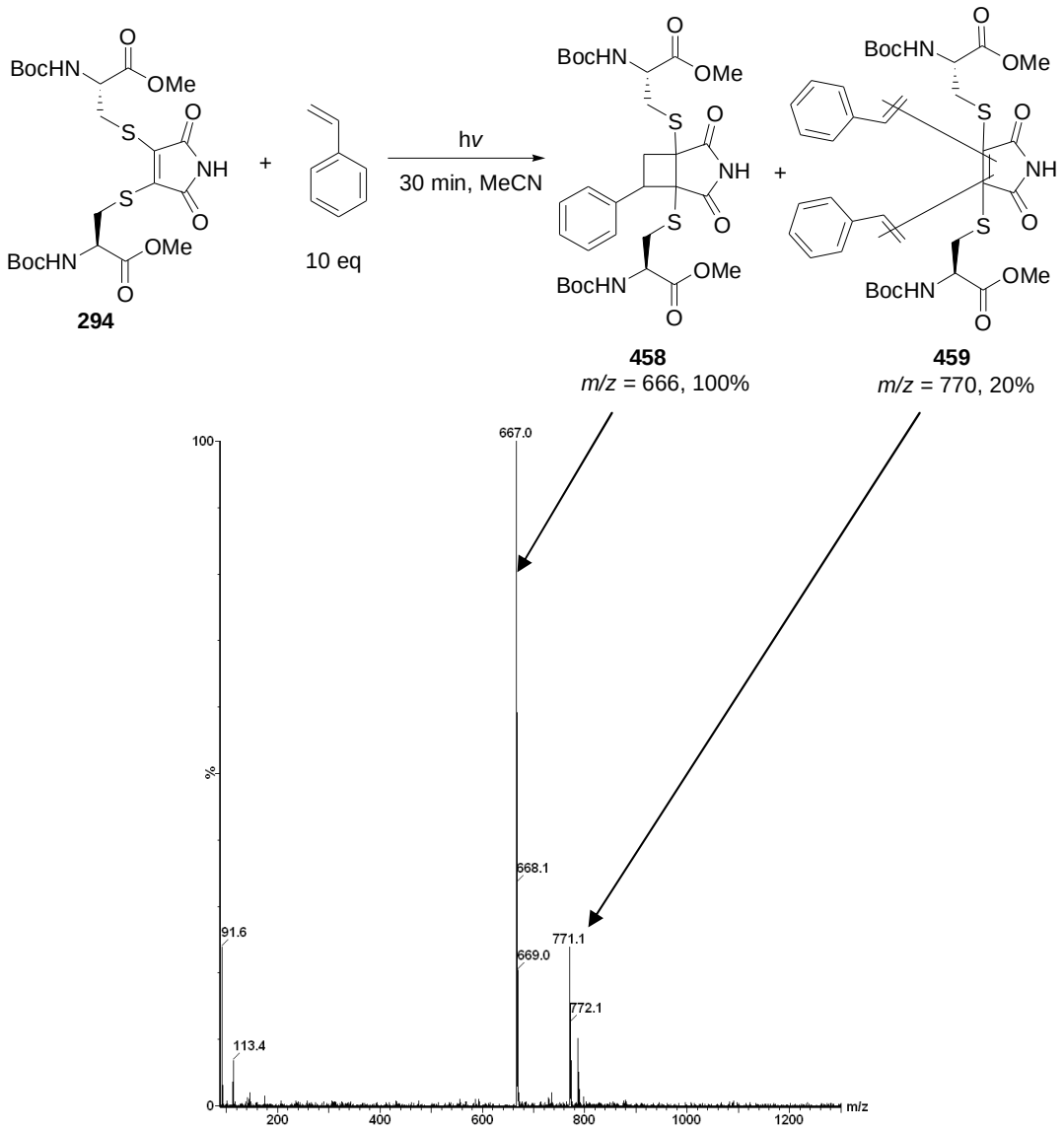
Scheme 246

Dithiomaleimide **456** was thus irradiated with ten equivalents of styrene, as this represented the best conditions for conjugation, **Scheme 247**. Complete consumption of the hindered dithiomaleimide **456** took twenty minutes and single column chromatography afforded a 1:1 mix of two diastereomers **457a** and 4**57b** in 70% yield, **Scheme 247**. Further column chromatography led to isolation of the separated diastereomers **457a** and **457b** in 6% each. The diastereomers were tentatively assigned due to through space interactions seen *via* NOeSY NMR and lack thereof.



Scheme 247

As the reaction had been established on the achiral thiomaleimide **456**, the *N*-Boc-Cys-OMe cysteine dithiomaleimide **294** was irradiated under the same conditions to afford a more complex result. NMR and mass spectroscopic analysis indicated that most of the constituents of the crude reaction mixture were of the desired structure and had the correct mass **458** ([M-H], 666). However, mass spectroscopy also suggested that, in this crude mixture, a significant proportion of the material constituted a species that had incorporated two molecules of styrene **459** ([M-H]+104, 770), **Scheme 248**.

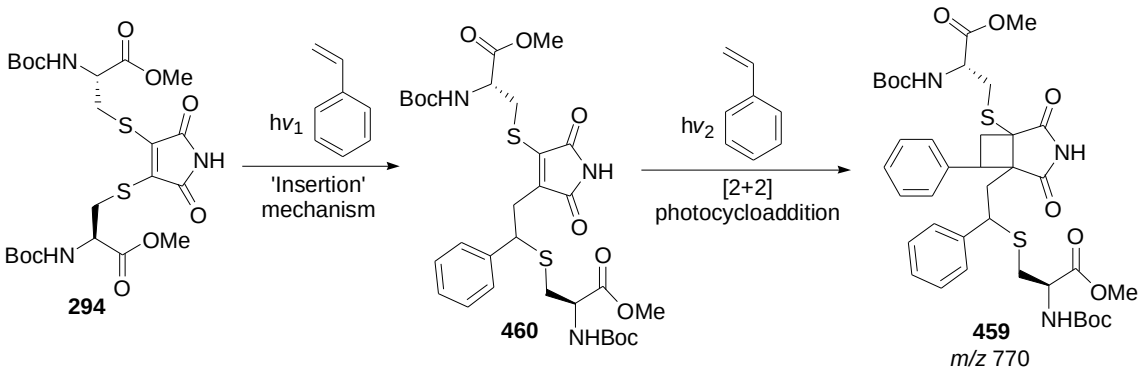


Scheme 248: Mass spectrum of the resultant reaction (above) after irradiation of dithiomaleimide 294 with 10 eq of styrene for 5 min

A 60% yield of a complex mixture of four diastereomers **458** was isolated from this crude reaction mixture by column chromatography. Mass spectroscopy and NMR

analysis indicated that they were the desired conjugation products **458**. The 'double-incorporation' product **459** was not isolated after column chromatography.

It was postulated at this stage, that the two different reaction pathways observed with some of the monothiomaleimides had both occurred to the dithiomaleimide **294** in this case. This could be explained from what had been learnt from identification of the 'insertion' products in previous reactions. For example, once the 'insertion' reaction had taken place, the product **460** was still a thiomaleimide, thus still capable of undergoing the expected [2+2] photocycloaddition, **Scheme 249**.



Scheme 249

When the equivalents of styrene were lowered to one, 35% of the conjugation diastereomers **458** were isolated post column chromatography. Analysis of the crude reaction mixture by NMR and mass spectroscopy had indicated that the double addition product **459** was not formed in this reaction, and that all of the starting material had been consumed. Therefore, the low yield in this reaction could be due to problematic isolation of the strained cyclobutane, rather than low conversion to the desired cyclobutane products **458**.

It had thus been indicated that thiomaleimides and dithiomaleimides could potentially be used to photochemically conjugate labels into modified cysteines and disulfides. Hence, focus shifted to functionalising the *N*-position of the maleimide with a biologically relevant molecule, namely a fluorophore. It was predicted that, with analogy to prior studies, substitution of the nitrogen would shut down the alternative

conjugation mode and thus the pathway through which the double incorporation of styrene was postulated to occur.

Photochemical conjugation of N-functionalised amino acid thiomaleimides

The molecules synthesised thus far had contained *N*-functionalities that merely probed sterics and the nature of the substituent, *e.g*. aliphatic *versus* aromatic. It was decided to synthesise molecules with a significant substituent on the nitrogen to exemplify the utility of the strategy for conjoining complex biologically relevant molecules. Two particular targets were identified as the monothiomaleimide **461** and the dithiomaleimide **462**, **Figure 62**.

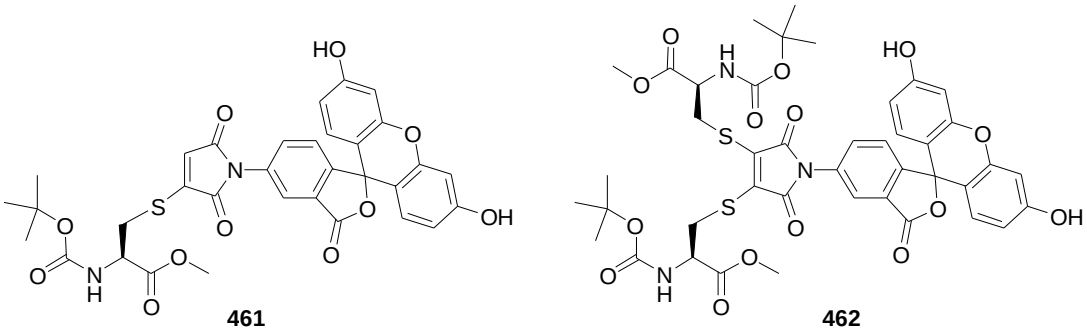
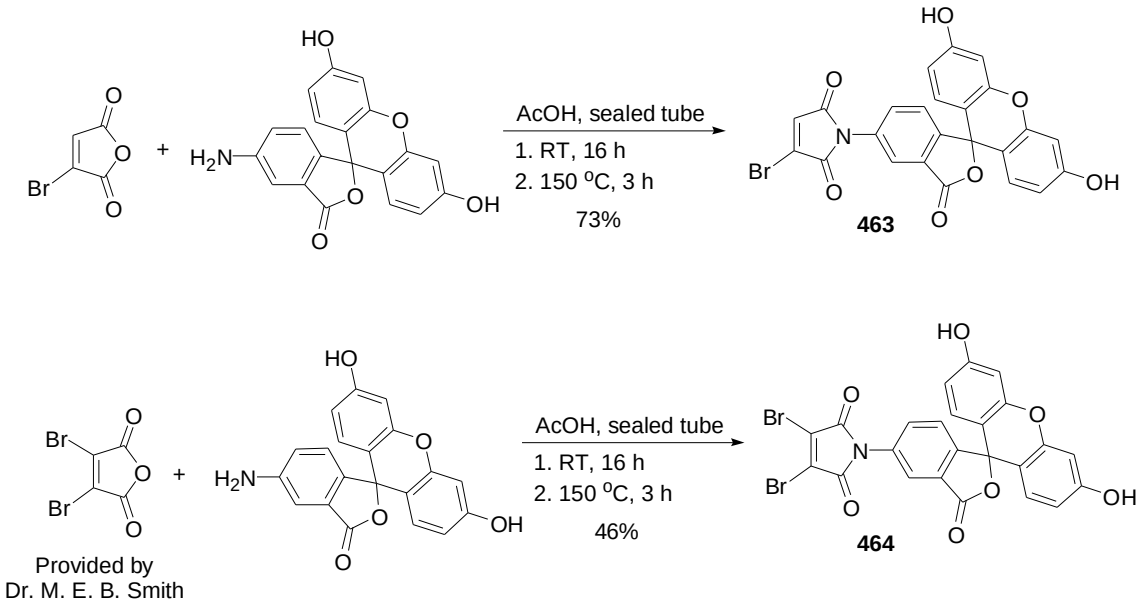


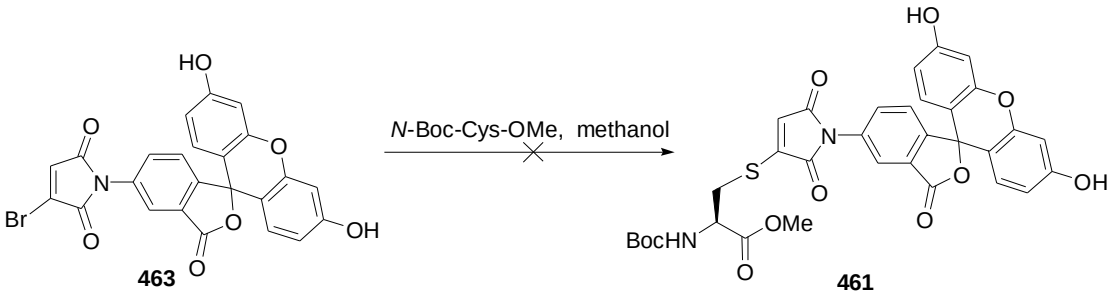
Figure 62: Targeted *N*-functionalised amino acid thiomaleimides

N-Fluorescein monobromomaleimide **463** and dibromomaleimide **464** were prepared according to literature,[265, 318] *via* condensation of the fluorescein amine with commercial bromomaleic anhydride and dibromomaleic anhydride. Dibromomaleic anhydride was provided by Dr Mark Smith (UCL, Chemistry, Caddick group), who prepared it according a to literature procedure.[319] The yields of the monobromomaleimide **463** and dibromomaleimide **464** were 73% and 46% respectively, **Scheme 250**.



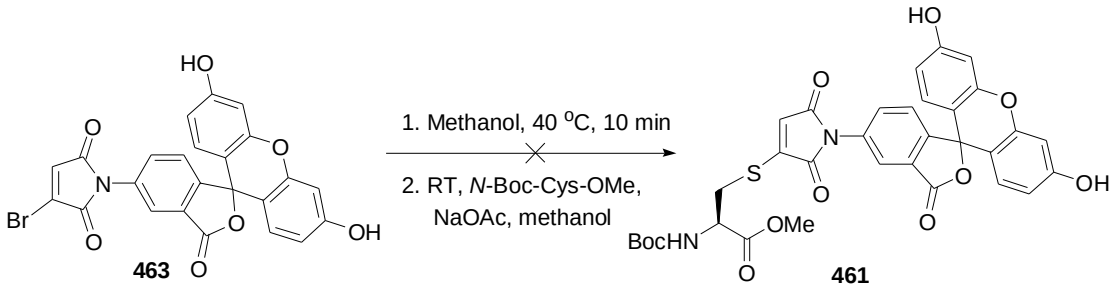
Scheme 250

Formation of the *N*-fluorescein thiomaleimide **461** was quite complex. In an effort to minimise purification of the complex fluorescein-containing product **461**, cysteine was initially added to the bromomaleimide **463** in methanol with no base, **Scheme 251**. However, the *N*-fluorescein compounds underwent degradation under these conditions, most likely due to exposure to the eliminated HBr.



Scheme 251

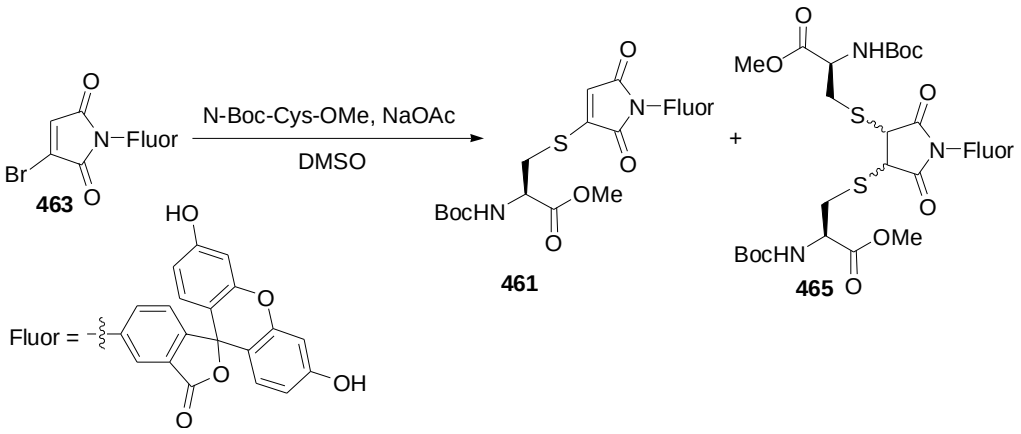
The bromomaleimide **463** was only sparingly soluble and reactions had to be carried out at around 1 mg/mL of solvent. The only useful solvent that could dissolve the compound at even these low dilutions was methanol. The dissolution was very slow, thus bromomaleimide **463** in methanol was heated to 40 °C for ten minutes to aid dissolution, prior to cooling and addition of a basic solution of the cysteine, **Scheme 252**.



Scheme 252

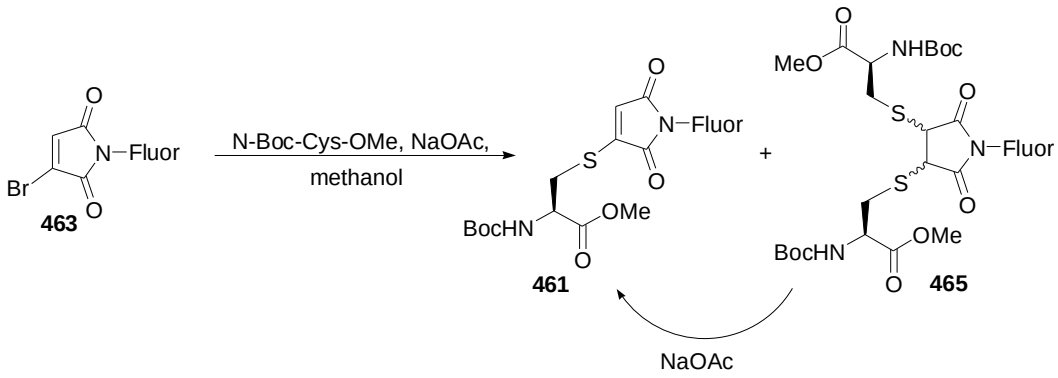
Examination of the ^{1}H NMR spectrum of the crude mixture from this reaction showed many products, some indicated that bromomaleimide **463** had hydrolysed during heating before it had reacted with the thiol. In order to minimise degradation, the bromomaleimide **463** was therefore stirred in methanol with no heating. Unfortunately, suspending the solution in methanol for the eight hours that was required to get complete dissolution also degraded a large proportion of the starting material. In all conditions attempted, subsequent column chromatography to remove impurities only resulted in further degradation of the thiomaleimide product **461**.

Attention was turned to the strong solvent dimethylsulfoxide (DMSO), but again the bromomaleimide **463** was only soluble at 1 mg/mL. Upon dissolution of the bromomaleimide **463** in DMSO, the reaction immediately turned dark brown and no product could be recovered from this reaction. Carrying out the reaction in deuterated DMSO indicated that there were both single and double addition products, **461** and **465** respectively, in the reaction mixture alongside degradation products, **Scheme 253**.



Scheme 253

Up to this point, focus had been on complete dissolution of the bromomaleimide **463** before addition of the cysteine. This was in an attempt to minimise a second addition of the cysteine to the formed thiomaleimide **461**. However, it was realised at this stage that the double addition product **465** would be reversible under the basic conditions of the reaction and should eventually all form the single addition product **461** as the bromomaleimide **463** slowly went into solution, **Scheme 254**.



Scheme 254

To check this hypothesis, the bromomaleimide **463**, sodium acetate and cysteine were added together in an NMR tube in deuterated methanol. As the NMR tube was narrow, addition of a stirrer bar was not feasible, thus, it was attached to a 'shaker' more commonly employed in chemical biology laboratories. Throughout the time taken for the reaction to reach completion, *i.e.* eight hours, sequential ^{1}H NMR spectra were taken to ascertain the progress of the reaction, **Figure 63 (a)-(c**). It could clearly be seen that at the start of the reaction the major components of the solution were cysteine and double addition product **465** alongside the desired thiomaleimide **461**. As the reaction proceeded the cysteine and double addition peaks could be seen to decrease, alongside an increase in the product peaks, **Figure 63 (a)-(c)**.

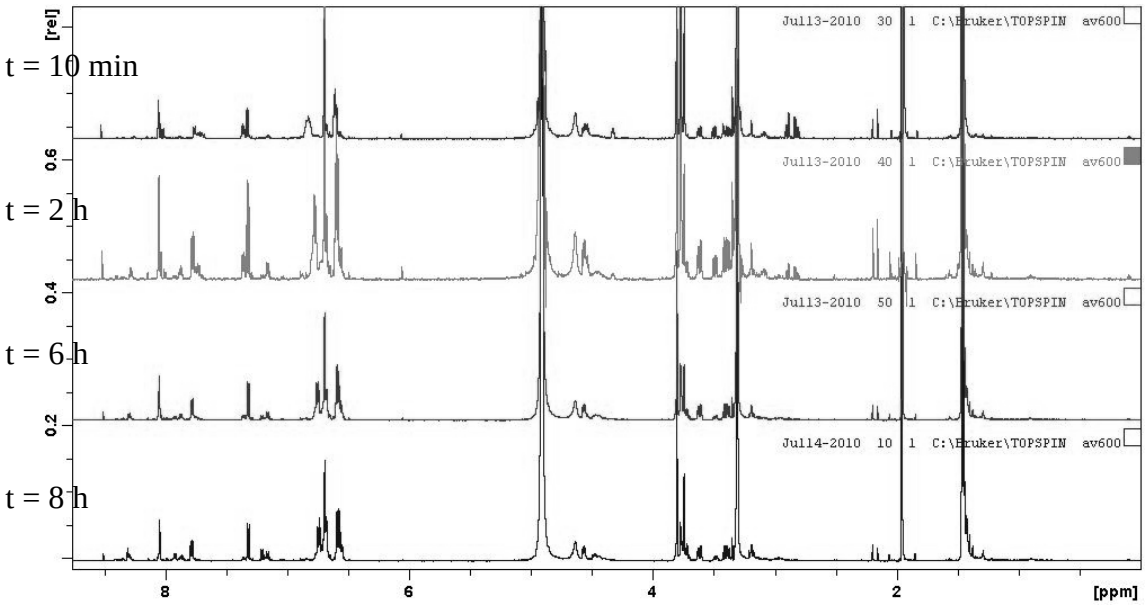


Figure 63 (a): ^{1}H NMR spectra of the reaction shown in Scheme 254 at t = 10 min, 2, 6 and 8 h

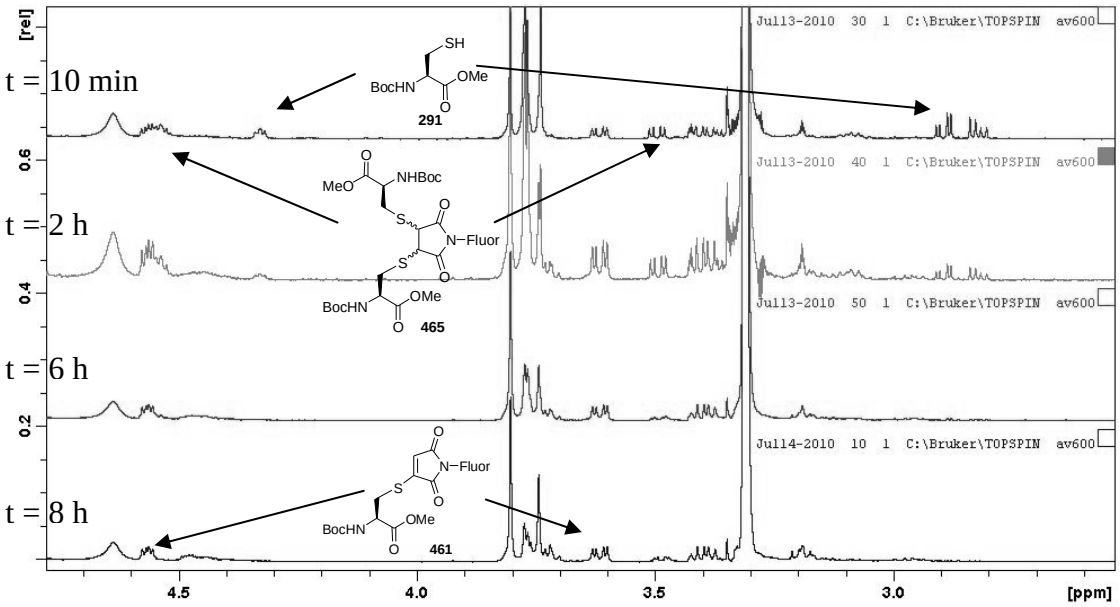


Figure 63 (b): Expanded ^{1}H NMR spectra (4.58-2.5 ppm) of the reaction shown in Scheme 254 at t = 10 min, 2, 6 and 8 h

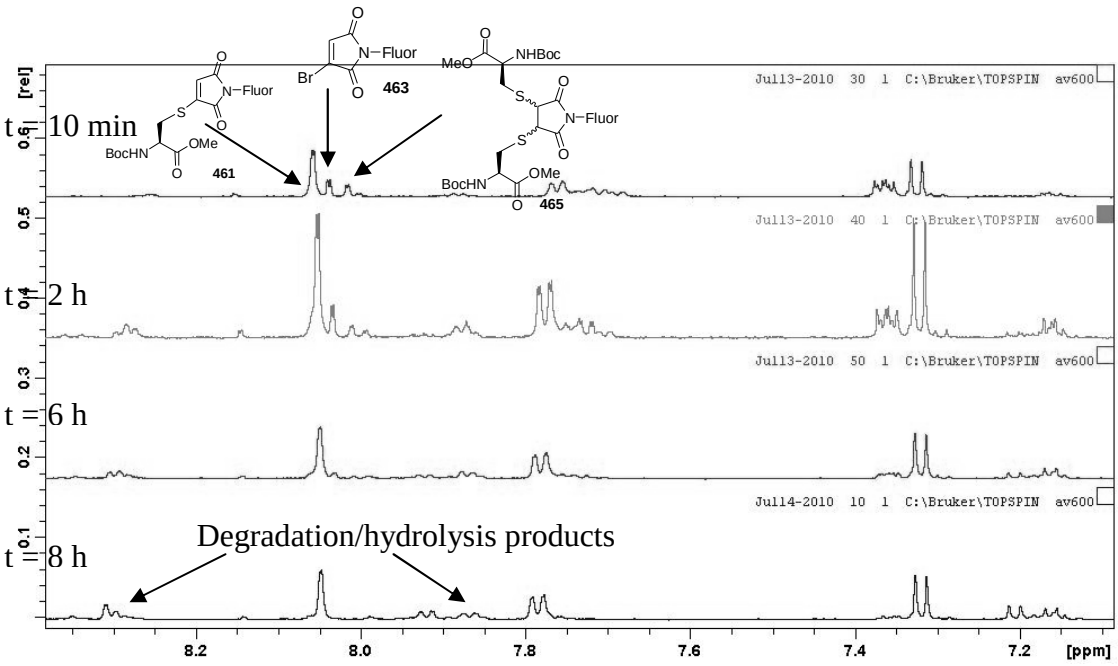


Figure 63 (c): Expanded ^{1}H NMR spectra (8.30-7.15 ppm) of the reaction shown in Scheme 254 at t = 10 min, 2, 6 and 8 h

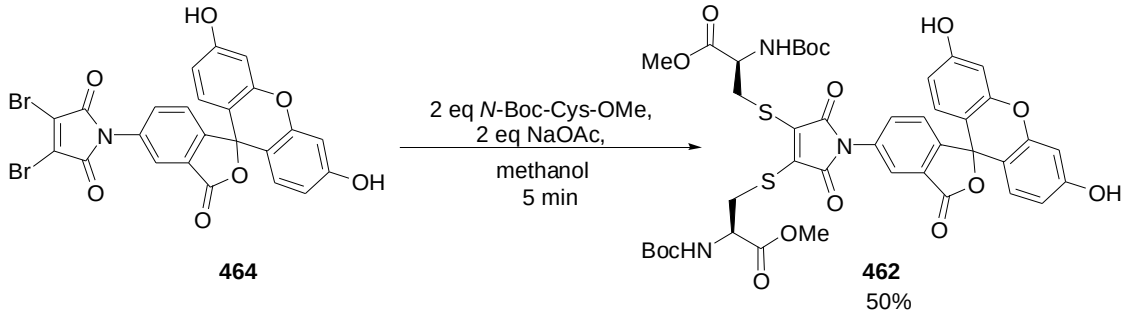
However, before the reaction was complete, NMR analysis also showed emergence of a new product that contained key fluorescein peaks, **Figure 63 (c)**, indicating that either the starting bromomaleimide **463** or the desired thiomaleimide **461** were degrading.

The most likely route for degradation was *via* hydrolysis of the maleimide thus the large-scale reaction was attempted in anhydrous methanol. Regrettably, this did not minimise degradation of the reaction components.

Whilst the conditions in methanol were still sub-optimal, they represented the best outcome so far and thus *N*-fluoroscein monobromomaleimide **463**, cysteine and sodium acetate were combined in methanol and stirred. Unfortunately, this was not successful as the reagents did not go into solution, even though the reaction was at the same concentration as the NMR experiment, *i.e.* 1 mg/mL. No clean product could be isolated from the very complex residue that was obtained from this reaction. It was appreciated at this point that the methods of agitation were different, and thus, the reaction was repeated at large scale but 'shaken' and not stirred. Under these conditions, the reagents went into solution over eight hours and TLC analysis of the reaction mixture showed one major product that could potentially be subjected to column chromatographic

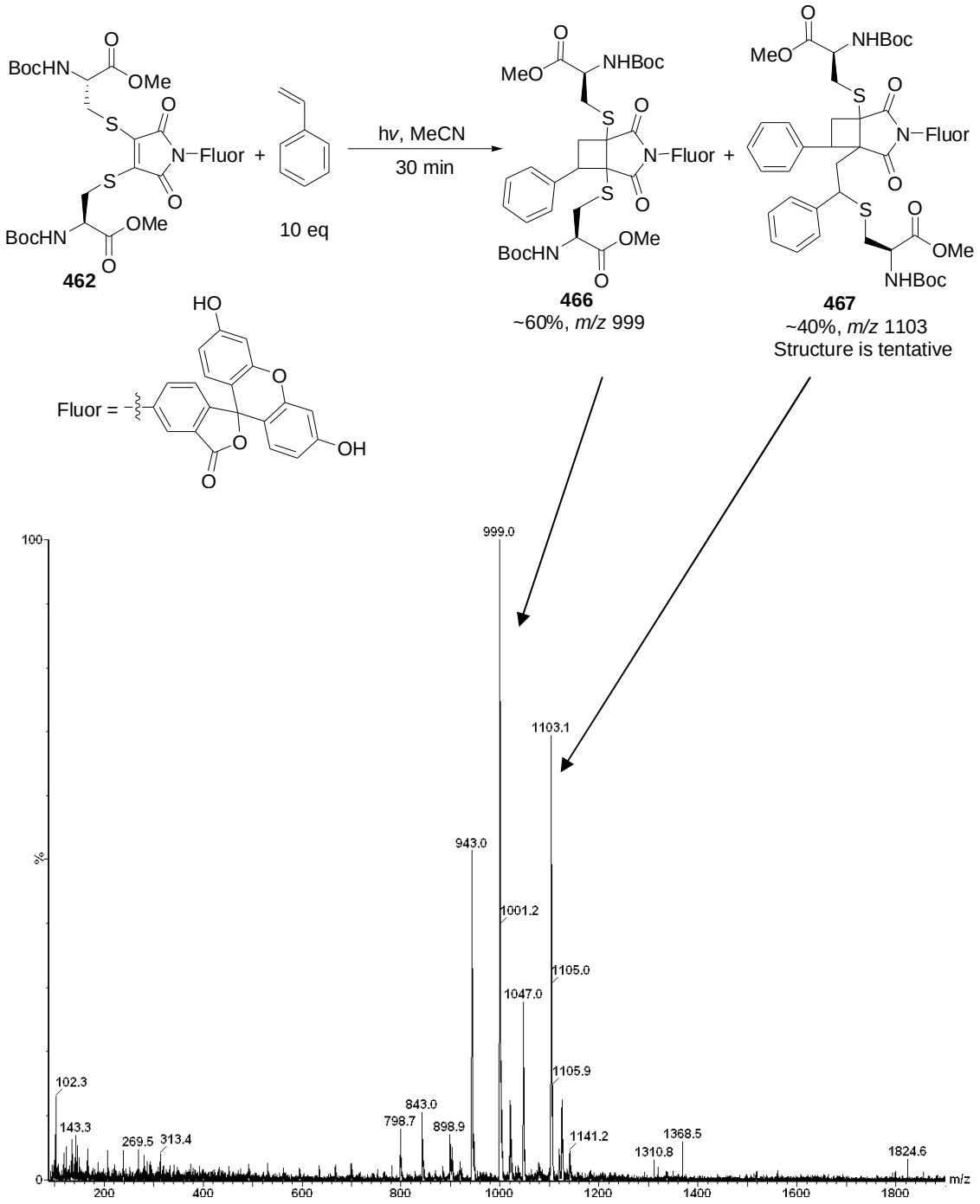
techniques. Initially, aqueous work-ups were attempted on the material but they merely led to further hydrolysis. Column chromatography with alumina and silica-gel systems afforded impure material in low yields after this reaction, and attempts at further purification were unsuccessful. Owing to its poor stability to purification techniques, *N*-fluorescein cysteine thiomaleimide **461** was never isolated.

Similarly to the *N*-fluorescein monobromomaleimide **463**, the *N*-fluorescein dibromomaleimide **464** and the resultant products underwent degradation when the addition reaction was carried out without addition of the base. Therefore, two equivalents of the cysteine were dissolved in methanol with two equivalents of sodium acetate, and then added to the *N*-fluorescein dibromomaleimide **464** to form dithiomaleimide **462**. Although a proportion of the desired material was lost upon column chromatography, as the fluorescein compounds appeared unstable to silica-gel, the dithiomaleimide **462** was isolated in 50%, **Scheme 255**.



Scheme 255

When dithiomaleimide **462** was irradiated with ten equivalents of styrene, a complex mixture was formed that made NMR examination very difficult. The crude reaction residue was subjected to column chromatography but the compounds remained inseparable. Mass spectroscopic analysis of the crude indicated a large proportion of the residue had the correct mass for the desired conjugate **466** ([M+H], 999, 100%), **Scheme 256**. However, there was a substantial peak that could be attributed to double incorporation of styrene **467** ([M+H]+104, 1103, 70%), **Scheme 256**. This was in accordance with what had been witnessed with dithiomaleimide **294**, and was postulated as from similar products, **Scheme 256**.



Scheme 256

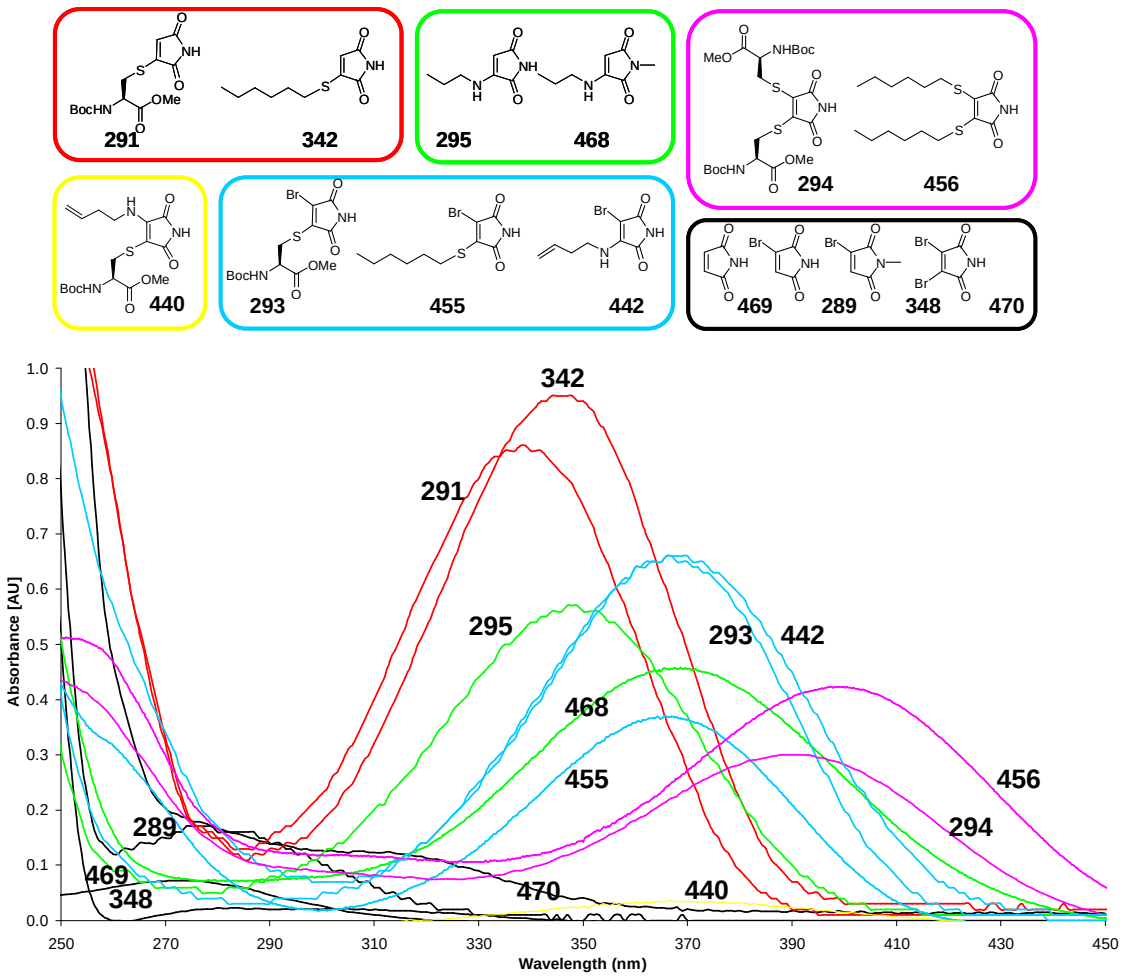
It had been predicted, that with analogy to the reactions on *N*-substituted maleimides, the 'insertion' reaction, and thus the double incorporation of styrene, would not occur, but unfortunately the double incorporation of styrene had still occurred. This perhaps suggested that the second incorporation of styrene was not *via* the 'insertion' mechanism postulated. Using just one equivalent of styrene led to a similar result, with a mixture of single and double incorporation of styrene. Neither reaction could be

purified *via* column chromatography. Whilst the mode of the incorporation was believed to be *via* both conjugation reactions seen in prior work, this was not certain and it was felt that inclusion of a complex fluorophore was confusing interpretation of the reaction outcome. The use of *N*-fluorescein thiomaleimides was thus terminated here.

Applying the photochemical conjugation to proteins and peptides

i. Absorption profiles of substituted maleimides

As the thiomaleimides were to be used in protein chemistries, the absorption profile of the constructs were crucial to their application in chemical biology. The thiomaleimides needed to have sufficient absorption above 360 nm where most proteins have minimal absorption.[192, 193, 196] UV-Vis spectroscopy of the maleimides, thiomaleimides and aminomaleimides made an interesting trend apparent. Successive substitutions of the maleimide caused a sequential bathochromic shift in the λ_{max} of the structures, **Figure 64**. Maleimides and bromomaleimides absorb with λ_{max} values between 275 and 310 nm, monothiomaleimides **291** and **342** have λ_{max} values of 339 and 347 nm, respectively, aminomaleimides **295** and **468** have λ_{max} values of 348 and 368 nm, bromothiomaleimides **293** and **455**, and aminobromomaleimide **442**, all have very similar λ_{max} values of 367 or 368 nm and finally, dithiomaleimides **294** and **456** have λ_{max} values of 393 and 401 nm. Thus it could be seen that sequential substitutions of the maleimide core afforded absorption profiles with better λ_{max} values for irradiation of proteins. In addition, the extinction coefficients (ε) of the substituted thiomaleimides and dithiomaleimides were much larger than those of simple maleimide, although with increasing substitutions the ε did begin to decrease. For example, the measured extinction coefficient of maleimide was just 720 $cm^{-1}M^{-1}$, whilst the thiomaleimides **291** and **342** had ε values of 8600 and 9500 $cm^{-1}M^{-1}$, respectively. However, whilst still much higher than maleimide, dithiomaleimides **294** and **456** had lower ε values than the corresponding thiomaleimides, at 3000 and 4200 $cm^{-1}M^{-1}$, respectively.



All solutions were 0.1 mM in acetonitrile

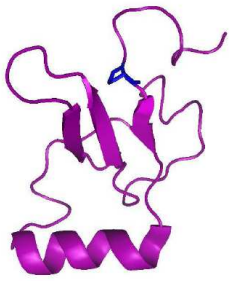
Figure 64: Representative spectra of a variety of maleimides

The strong absorptions and high λ_{max} of dithiomaleimide and aminothiomaleimide structures has been observed prior to this work,[285, 320] but the resultant compounds were not turned towards any biological application.

ii. Photochemistry of maleimide-labelled RNAP subunit-H

Beyond the well known alkyne-azide 'click' reaction, there are several reports[321] of cycloadditions to modify biomolecules[321] - mostly *via* Diels-Alder reactions[322] of furans [323] and maleimides.[321] However, the use of the thiomaleimide [2+2] photocycloaddition would be the first example, to the best of the author's knowledge, of the use of the [2+2] photocycloaddition for biomolecule modification.

It was decided to initially demonstrate the work on a protein that would have an easily accessible cysteine residue. From the lab of Dr Finn Werner (UCL, ISMB), a protein with an accessible cysteine was supplied, which was a subunit of an RNA polymerase (RNAP), known as subunit H. The wild-type protein has a proline at position 10,[324] **Figure 65**.



MKVTDHILVP KHEIVPKEEV EEILKRYNIK IQQLPKIYED DPVIQEIGAK EGDVVRVIRK SPTAGVSIAY RLVIKRII

Figure 65: Crystal structure and amino acid sequence of RNAP subunit H, proline shown in blue

The proline residue was substituted for a cysteine by Dr Dina Grohmann (UCL, ISMB, Werner group), by a technique known as gene splicing by overlap extension, to afford P10C subunit-H. P10C sub-unit H was provided in a complex buffer (20 mM tris(hydroxymethyl)aminomethane, pH 7.5, 300 mM potassium acetate, 0.1 mM zinc sulfate, 10 mM magnesium acetate and 10% glycerol). This milieu of metals and potential proton donors and abstractors was deemed unsuitable for photochemistry, thus the protein needed to be 'desalted'. Desalting is the process of exchanging a buffer and its contents for deionised water *via* osmosis through a size dependant membrane. The process works by diluting the ions to such an extent that, whilst not completely negated, they are deemed negligible in the solution.

To be certain that the protein would not precipitate or degrade in a simple aqueous solution, the P10C sub-unit H (hereafter referred to as SUH) was desalted and analysed prior to any modification. Mass spectroscopy showed no perturbation of the signal observed (9016 Da) and no emergence of new peaks after the protein had been kept in a desalted solution for twelve hours, **Figure 66**. Thus the protein was deemed stable enough for use in non-buffered solutions.

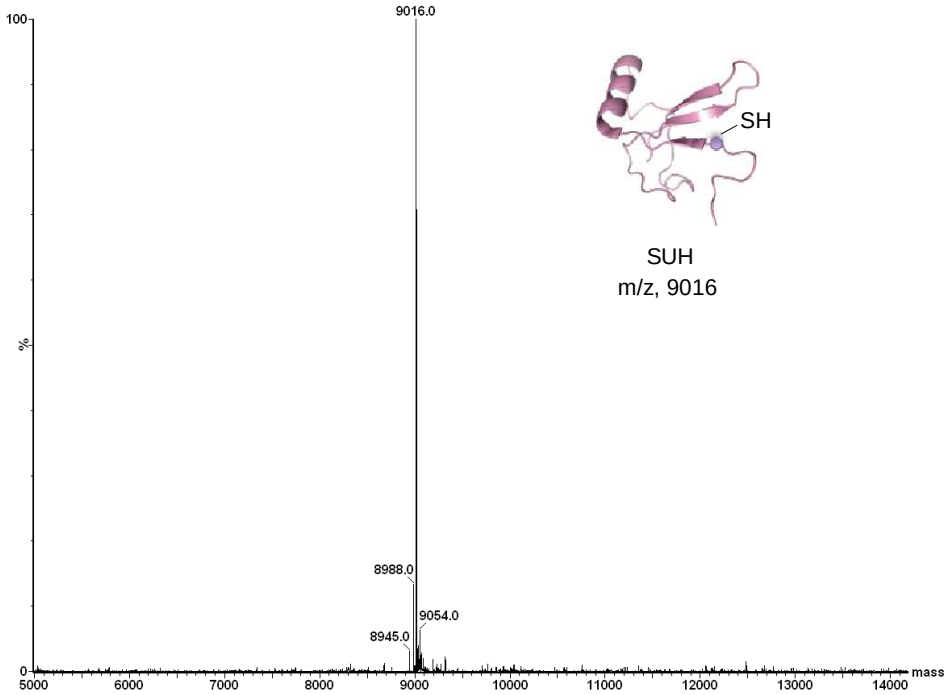
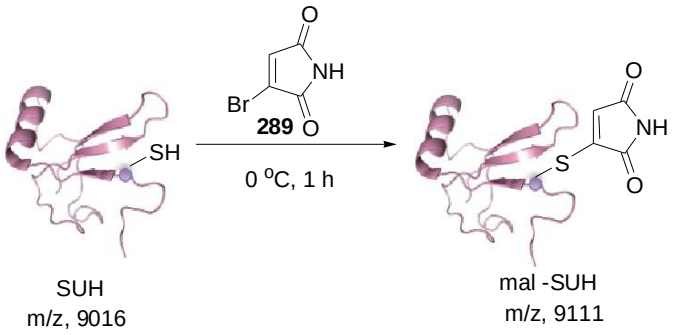


Figure 66: Deconvoluted mass spectrum of desalted SUH

SUH was reacted with monobromomaleimide **289** to modify the cysteine with 100% conversion, according to a protocol developed by Dr Mark Smith (UCL, Chemistry, Caddick group),[325] affording maleimide labelled SUH (hereafter referred to as mal-SUH), **Scheme 257**.



Scheme 257

The mal-SUH was desalted and left for twelve hours and, again, analysis showed that this modified protein was not detrimentally affected by the buffer-free conditions over twelve hours, **Figure 67**.

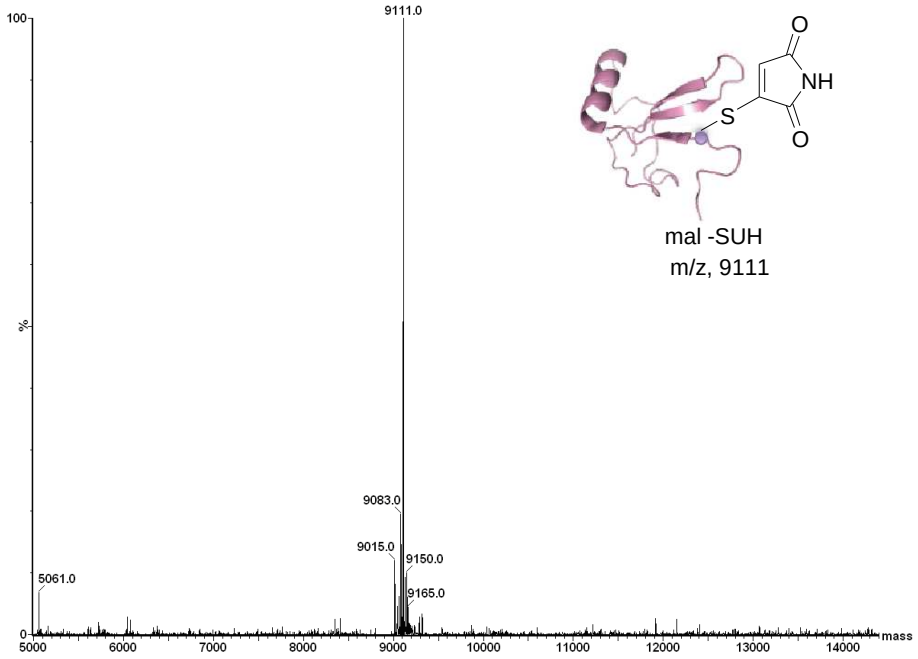


Figure 67: Deconvoluted mass spectrum of desalted mal-SUH

The best photochemical conjugation yields had been achieved with ten equivalents of styrene in small molecule work, thus styrene was chosen as the conjugation partner for the protein. It was recognised at this stage that styrene conjugation had minimal use in biological modifications, and that the reagent was extremely hydrophobic, but this alkene merely represented a 'start-point' for this research. It was envisaged that, if successful, focus could be turned to olefinic molecules with higher biological utility.

Ten equivalents of styrene in DMF were added to the mal-SUH solution. Upon addition of the hydrophobic styrene, no perturbation of the protein was witnessed by mass spectroscopic analysis. Thus it was decided that irradiation experiments with styrene could proceed.

The light source for these reactions was the standard 250 W Hg lamp from small molecule studies. The solutions were irradiated in a 1.25 mL cuvette, taped to the immersion well at a height that ensured the reaction solution was in line with the lamp bulb, **Figure 68**. The solution was cooled *via* running water through the immersion well and by submerging the cuvette in an ice-water bath, **Figure 68**.

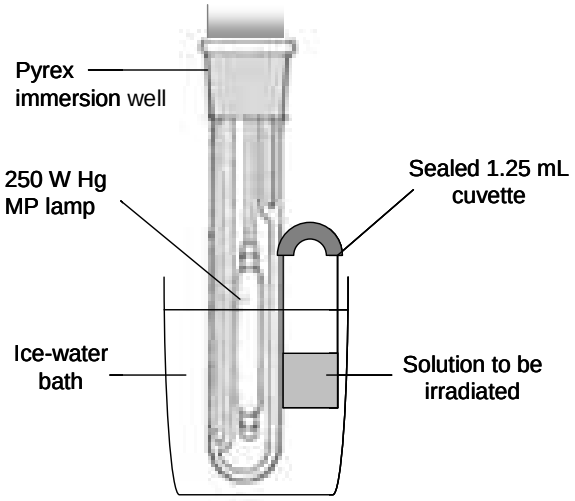


Figure 68: Photochemical set-up for irradiation of protein solutions (not to scale)

Desalted mal-SUH was irradiated in the presence of ten equivalents of styrene for five minutes but LCMS analysis was inconclusive. The peak attributed to mal-SUH (9111 Da) had decreased with emergence of a peak at M+18 (9129 Da), **Figure 69**.

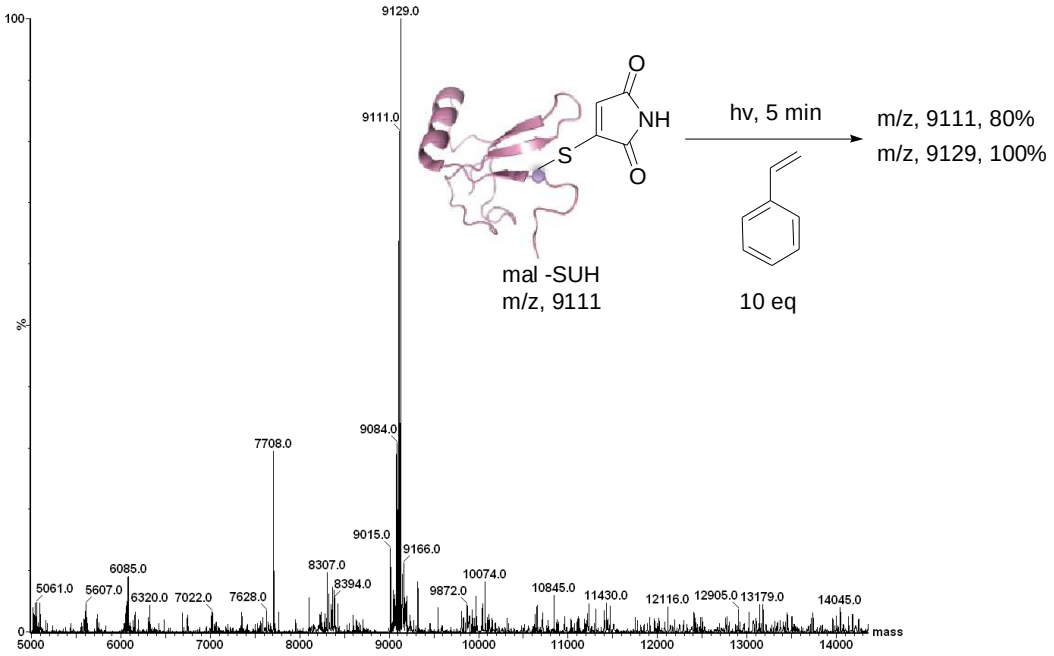
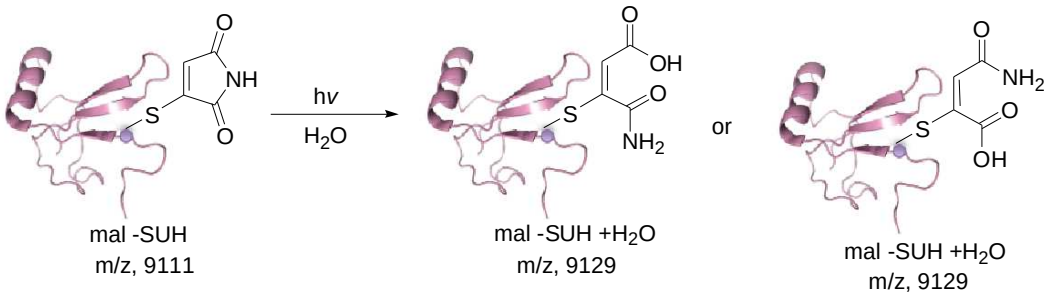


Figure 69: Deconvoluted mass spectrum of mal-SUH after addition with 10 eq of styrene and 5 minutes of irradiation

This peak could perhaps be attributed to hydrolysis of the thiomaleimide, **Scheme 258**, although such a significant extent of hydrolysis in just five minutes of irradiation was

not expected. Apart from the postulated hydrolysis peak, no other modification of mal-SUH was seen, which was very surprising.



Scheme 258

The ionisation technique used in the MS analysis, electrospray ionisation, does not always ionise all species equally in a particular solution when they are subjected to the strong electrostatic field used. It was postulated that perhaps the modification that had been imparted on the mal-SUH rendered the molecule unable or unlikely to ionise under the same conditions as the parent mal-SUH.

At this juncture, attention was turned to alternative methods of analysis, namely matrix assisted laser desorption/ionization-time of flight mass spectroscopy, MALDI-TOF. This technique is often used for fragile biomolecules that can be difficult to analyse by other methods.[326] The ionisation is triggered by a laser that fires at a dried matrix containing the analyte. This matrix facilitates vaporization and ionisation and protects the biomolecule from immediate obliteration by the laser. A downside of MALDI is that the matrix must be chosen carefully to allow for successful analysis and this can be very time consuming. 'Time of flight' (TOF) refers to the method of identifying the mass of the molecule. The TOF analyser converts the time taken for the molecule to reach the detector to its mass, by calibrating to an internal standard of known mass. The two main modes of TOF analysis are linear and reflectron. Linear flight tubes, merely allow detection of molecules of different kinetic energy *via* a linear flight path, this leads to poor resolution as some ions of different mass will have the same kinetic energy and arrive at the detector at the same time. A wide range of masses can be detected in this mode (up to 500,000 Da) with high sensitivity.[326] In contrast, reflectron analyzers also use an electric field (ion mirror) to deflect ions of differing mass even if they have the same kinetic energy. The overall result is that ions of a lower mass are reflected faster

than heavier ions, thus the ions are separated over a longer distance by kinetic energy and by mass. In this manner, reflectron analyzers have much better resolution, but they are not suitable for high molecular masses, *i.e.* those above 2000 Da,[327] and are not as sensitive as ions are lost in the reflection event.[326]

The samples to be analysed are 'spotted' onto a MALDI-TOF plate - an unreactive stainless-steel slide with defined regions for analysis, **Figure 70**.

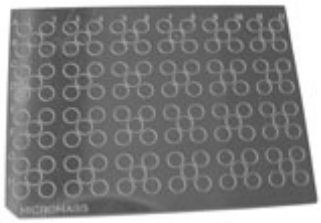


Figure 70[328]

Analysis of the mal-SUH was via linear mode as the protein was greater than 2000 Da, resulting in broad peaks, **Figure 71**.

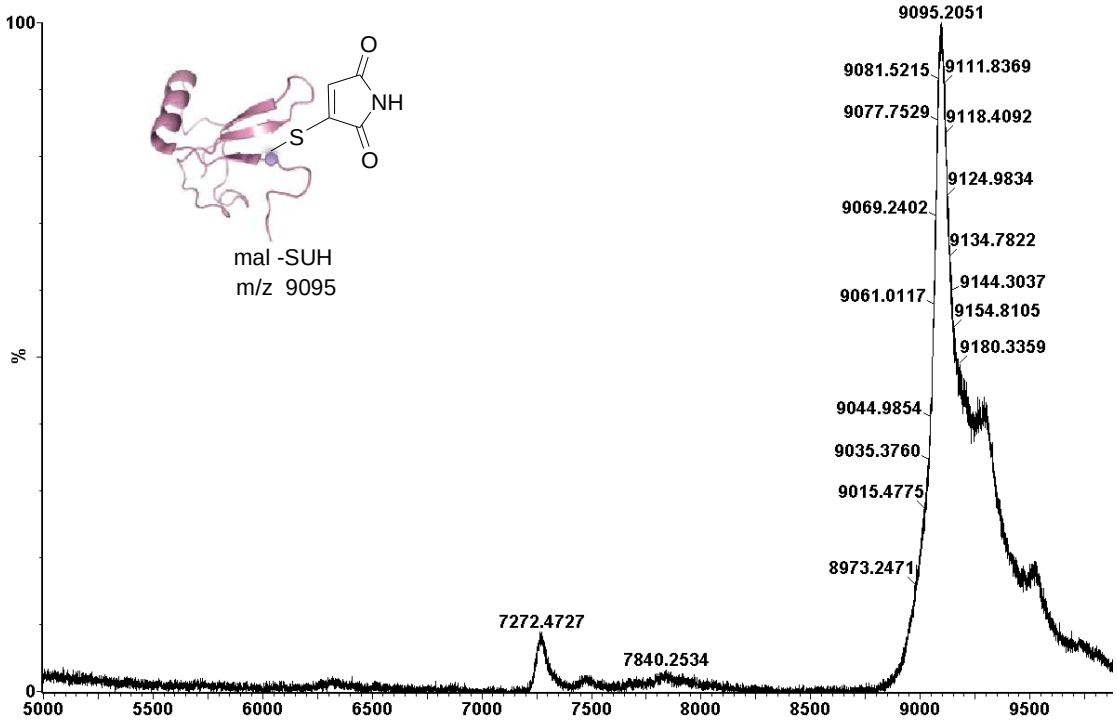


Figure 71: Mass spectrum of desalted mal-SUH obtained by MALDI-TOF operating in linear mode

As linear mode affords such broad peaks, the mass shifts of the peaks of interest are important, rather than the absolute value of the peak maxima. The mal-SUH peaks in the following analyses, at *m/z* 9111 by electrospray ionisation, are between 9087 and 9152 Da.

The use of a matrix in MALDI sample preparation results in a heterogeneous distribution of molecules amongst the sample. Therefore, several scans were taken and those spectra reported represent the average of the observed scans.

It was understood that the MALDI-TOF method used here was not perfectly quantitative but percentage conversions are estimated by comparison of the heights of the major peaks in the spectra. This was deemed acceptable as the conversion percentages estimated merely give an indication of reaction conversion and are not exact figures. The peaks obtained in the following reaction analyses were very close and could affect the height of each other by overlap, thus the resolution of the peaks was also taken into account when determining the peak ratios. The peaks were described in relation to one another, thus an internal standard was not used. This negated any possible complications arising from interactions of the internal standard with the analytes.

When the mal-SUH was irradiated in the presence of ten equivalents of styrene for five minutes, the conjugation could be seen to proceed in approximately 40%, by addition of roughly 104 Da and emergence of a new peak alongside the unmodified mal-SUH, **Figure 72**.

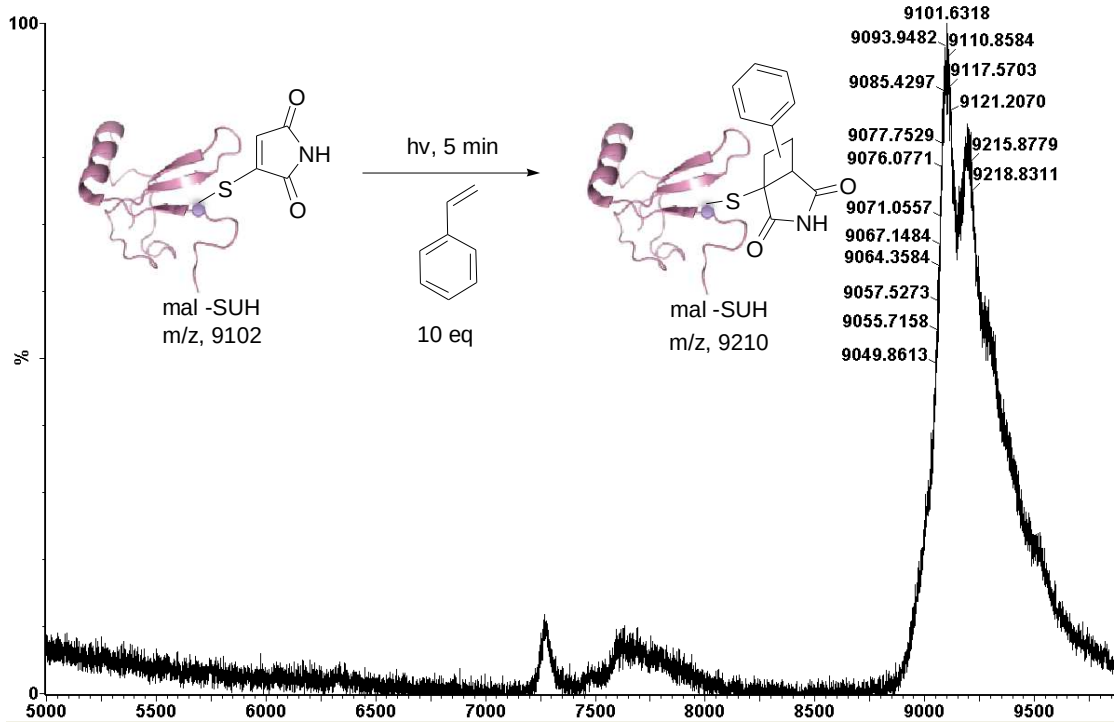


Figure 72: Mass spectrum of mal-SUH after irradiation with styrene

Further irradiation did not afford any higher proportion of the conjugation peak. It was a concern that increasing the equivalents of styrene at the start of the reaction might perturb the protein or lead to lowered yields, as had been witnessed in small molecule work, **Table 29**, p. 194. Therefore the reaction was repeated, but with a second addition of ten equivalents of styrene added at five minutes. At just ten minutes of total irradiation the conjugation yield was almost at 60%, and at twenty minutes the modification percentage was largely unchanged, **Figure 73**. Hereafter, for ease of comparison, figures depict the mass range of approximately 8500 to 10000. Full spectra indicated no other modification, other than that shown, had taken place throughout the experiments.

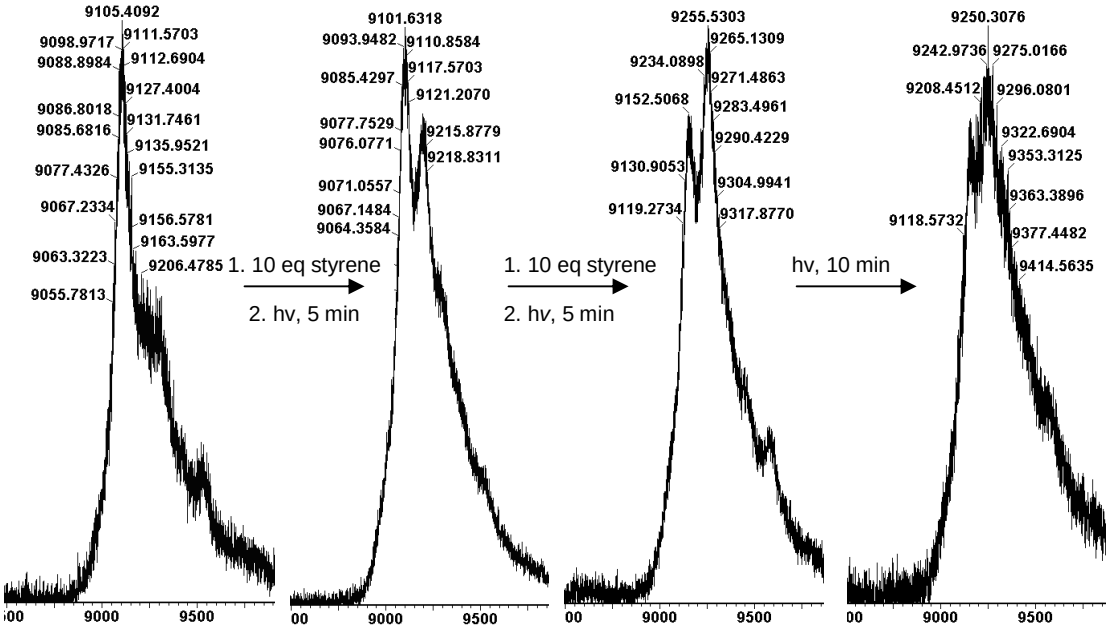


Figure 73: Mass spectra of mal-SUH before and after irradiation with styrene

Interestingly, when the amount of styrene was lowered to five equivalents, there was still an emergence of a peak (9245 Da) that could be attributed to modification, but it was a minor component of the reaction mixture, **Figure 74**.

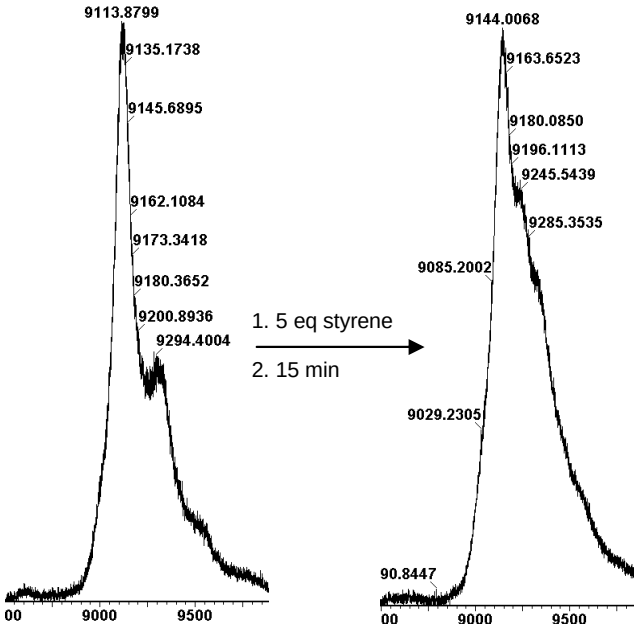


Figure 74: Mass spectra of mal-SUH before and after irradiation with styrene

When five equivalents were followed by five further equivalents after five minutes irradiation this minor modification peak could be seen after fifteen minutes (9274 Da),

Figure 75. It was thus decided that for conjugation yields of over 50%, at least ten equivalents should be used.

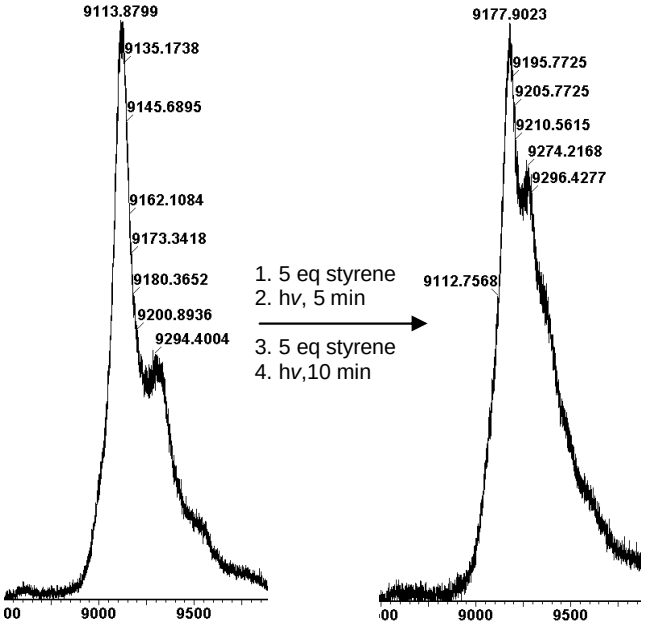


Figure 75: Mass spectra of mal-SUH before and after irradiation with styrene

In an attempt to increase the conjugation yield, one hundred equivalents of styrene were added to the solution and the same time points taken. Quick emergence of the conjugation adduct could be seen at two minutes and at fifteen minutes there was approaching 70% conjugation, with approximately 30% mal-SUH remaining, **Figure 80**. Further irradiation had little positive effect on the observed proportion of modification.

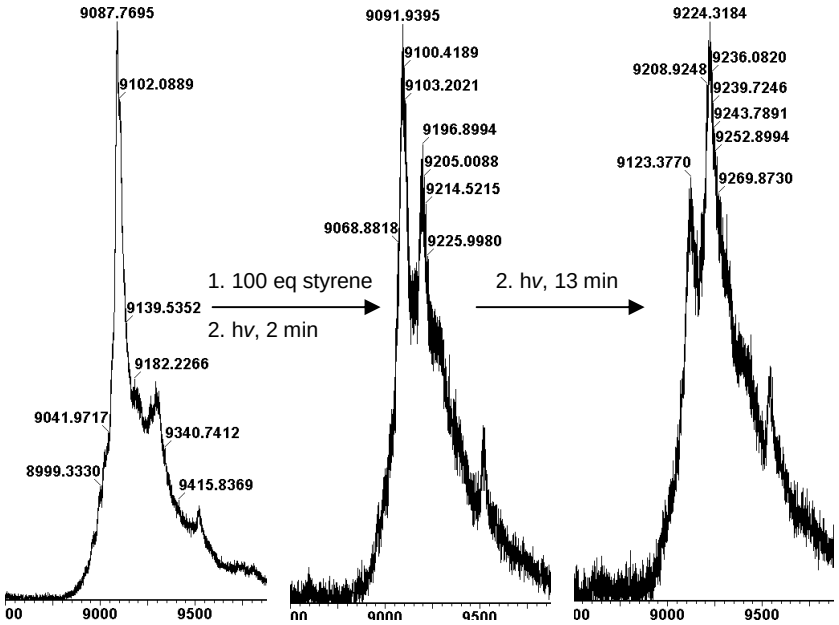


Figure 76: Mass spectra of mal-SUH before and after irradiation with styrene

Adding another one hundred equivalents at five minutes had no effect on the amount of conjugation achieved. Whilst around 70% conjugation was a very useful result, in an attempt to ascertain if full conversion could be achieved, one thousand equivalents of styrene were added to the modified protein and the solution was irradiated, **Figure 77**.

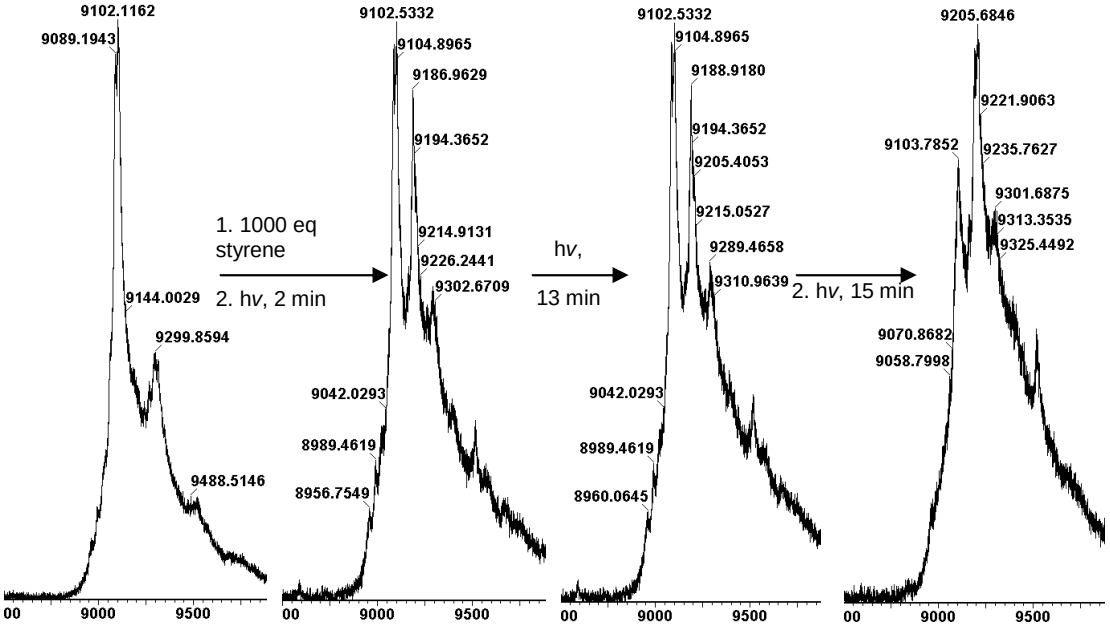
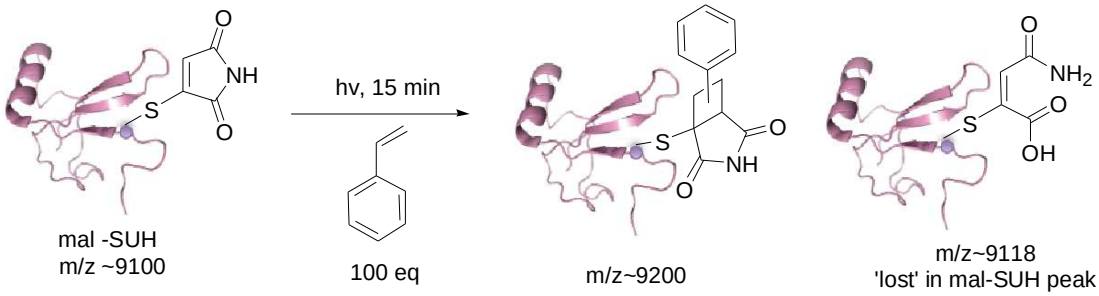


Figure 77: Mass spectra of mal-SUH before and after irradiation with styrene

Unfortunately, this didn't lead to any further conjugation than that observed with one hundred equivalents, potentially due to loss of styrene to side-reactions, or over-reaction of the modified protein with excess styrene. The incomplete conversion could potentially be an artefact of styrene, and a different alkene might afford increased conjugation. Alternatively, it was suggested that the postulated hydrolysis of mal-SUH from **Scheme 258**, p. 250, was correct, and the proportion of the mal-SUH that did not undergo the conjugation was actually hydrolysed mal-SUH. The hydrolysed mal-SUH would not be expected to undergo the [2+2] photocycloaddition and would not be resolved from the broad parent peak of mal-SUH by analysis *via* linear mode, **Scheme 259**.



Scheme 259

It was deemed necessary to ensure that the addition of ~104 Da was not an artefact of SUH or mal-SUH irradiation. Therefore, unmodified SUH was irradiated alone and in the presence of styrene. No modification was seen in either case, **Scheme 260**. After thirty minutes of irradiation, the SUH peak began to broaden further which could be attributed to photochemical degradation of the protein, but no addition peak was seen.

Scheme 260

When mal-SUH was irradiated without styrene for prolonged time periods, no reaction occurred. This not only suggested that the addition of approximately 104 Da was due to

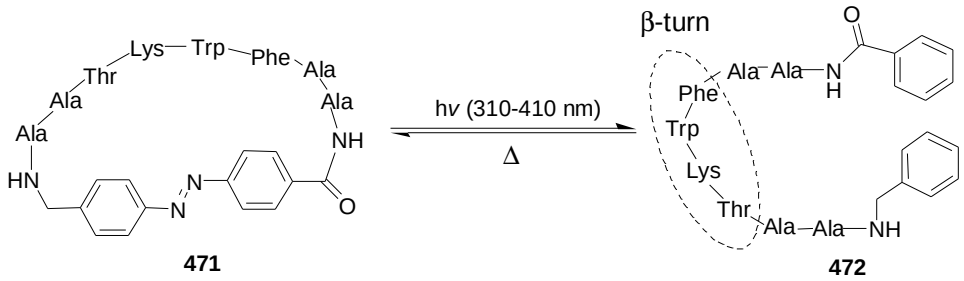
photochemical addition of styrene, but that dimerisation of the thiomaleimide-containing protein was not a competing reaction.

Further research into the photochemical modification of SUH was not carried out due to time constraints, but, from the work carried out, it was very gratifying to conclude that the [2+2] photocycloaddition of thiomaleimides with olefins could be transferred to a protein. With one hundred equivalents of styrene, RNAP P10C sub-unit H could be modified in approximately 70% with just fifteen minutes of irradiation. Future work would include the photochemical conjugation of biologically relevant labels, *e.g.* biotin, **Scheme 185**, p. 175, and investigations into whether the modification imparted on the protein is irreversible, as observed in small molecule studies.

iii. Photochemistry of maleimide labelled somatostatin

Having demonstrated the use of the [2+2] photocycloaddition conjugation strategy on a thiomaleimide incorporated into a protein with a free cysteine, it was intended to demonstrate this work on a peptide that had incorporated a thiomaleimide or thiomaleimides *via* a disulfide bond.

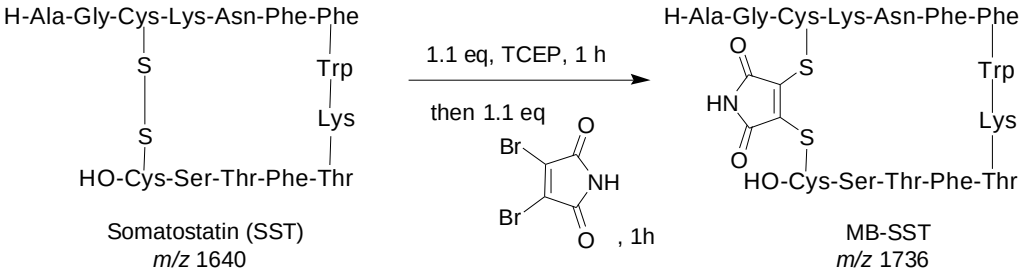
Somatostatin is a fourteen amino acid peptide containing one disulfide bond. It has gained significant interest in the field of disulfide labelling as it has analogues that are used as therapeutic peptides.[266, 267] In work by Ulysse Jr. *et al.*[329] a somatostatin analogue has been modified with photochemically responsive molecules to study its short β-turn.[329] The somatostatin analogue **471** was modified with an azobenzene group **472** and upon irradiation, isomerisation of the azobenzene from *trans* to *cis* allowed formation of the β-turn **472**, **Scheme 261**. Heating the *cis* construct **472** reformed the *trans* isomer **471** and 'unfolded' the turn. In this manner, the different behaviours of the two interconvertable isomers could be studied. The analogue used in this work contained a tryptophan and yet it was not detrimentally affected by the irradiation (310-410 nm).



471 **472**

Scheme 261

Therefore, as somatostatin and its analogues had precedent in both disulfide bridging and photochemical modification, it was decided to use somatostatin to investigate the photoactivity of a polypeptide containing a disulfide. Following a procedure previously set-up by Felix Schumacher (UCL, Chemistry, Baker group),[265] the somatostatin disulfide bond was labelled with dibromomaleimide to form maleimide-bridged somatostatin, (hereafter referred to as MB-SST), **Scheme 262**, and the solution desalted.



Scheme 262

SST could be analysed *via* MALDI-TOF in reflectron mode giving sharp, reproducible peaks and it was indicated that the MB-SST (1736 Da) was not adversely affected by desalting, **Figure 78**.

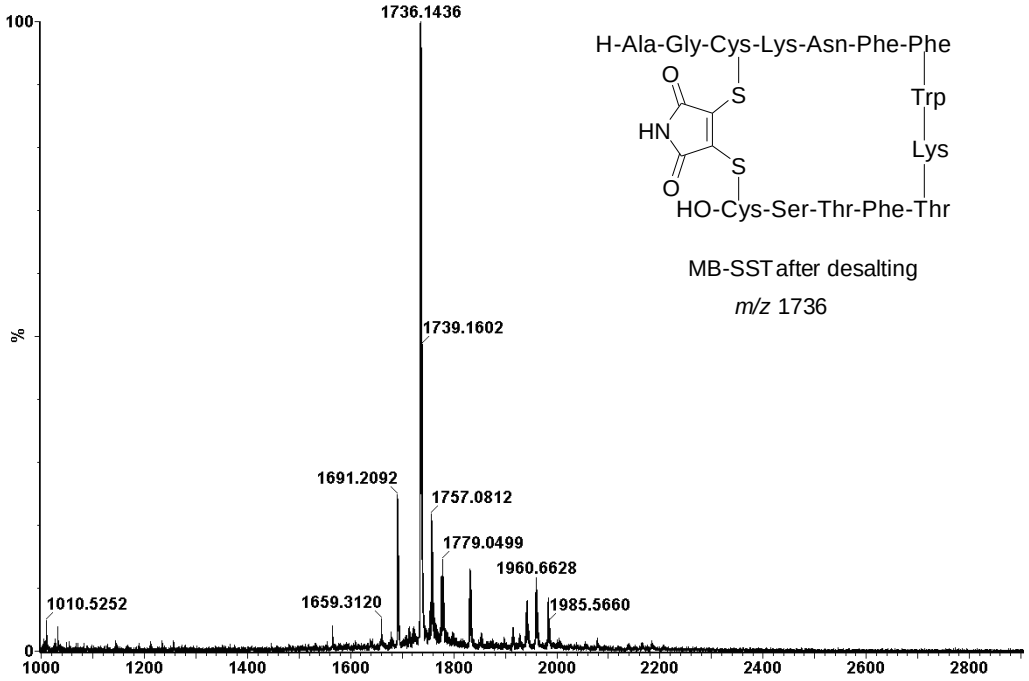
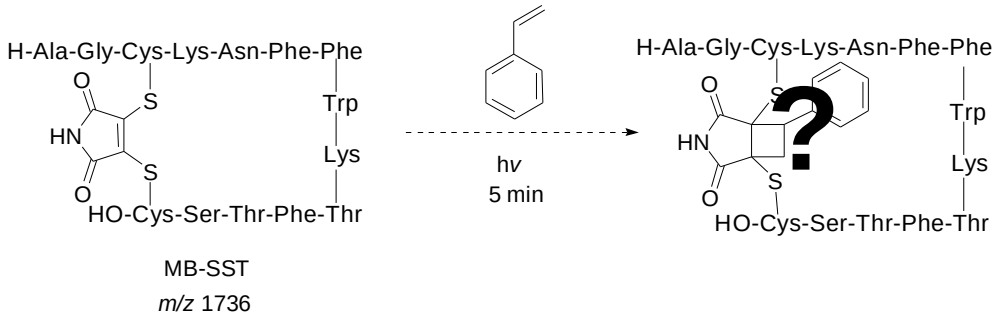


Figure 78: Mass spectrum of desalted MB-SST obtained by MALDI-TOF operating in reflectron mode

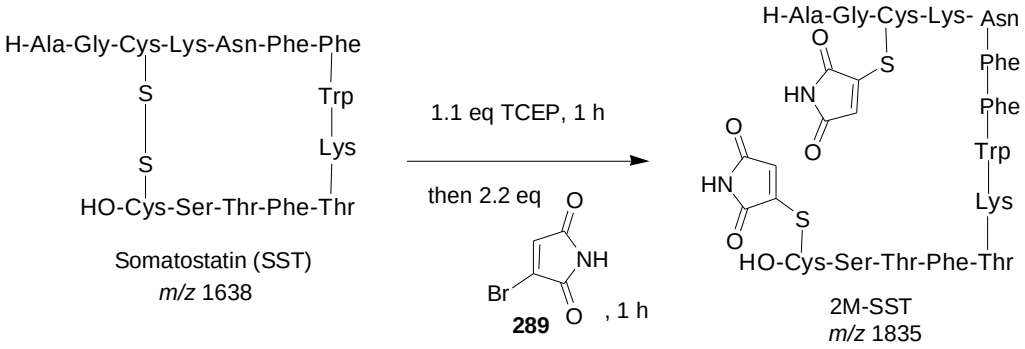
Irradiation of this solution in the presence of ten and one hundred equivalents of styrene for five minutes afforded samples for analysis that were inconclusive, **Scheme 263**. The parent peak (1736 Da) had completely disappeared but there were no other identifiable peaks in the mass spectrum.



Scheme 263

The somatostatin MALDI-TOF protocol had been robustly tested by Felix Schumacher (UCL, Chemistry, Baker group)[330] and, due to time constraints, it was not deemed prudent to undertake a lengthy analysis of new matrices and spotting techniques to attempt to visualise what had happened to the MB-SST. Instead, attention was turned to the doubly-labelled thiomaleimide somatostatin, **Scheme 264** to ascertain if any

modifications imparted by irradiation with styrene could be visualised by the previously established MALDI-TOF protocol. The disulfide bond in somatostatin was cleaved with TCEP[265] and the subsequent free thiols were labelled with bromothiomaleimide **289** to create doubly-labelled thiomaleimide somatostatin (hereafter referred to as 2M-SST), **Scheme 264**. This experiment was also carried out following a procedure developed by Felix Schumacher (UCL, Chemistry, Baker group).[330]



Scheme 264

Again, this solution was desalted and analysis indicated that the molecule was intact, **Figure 79**.

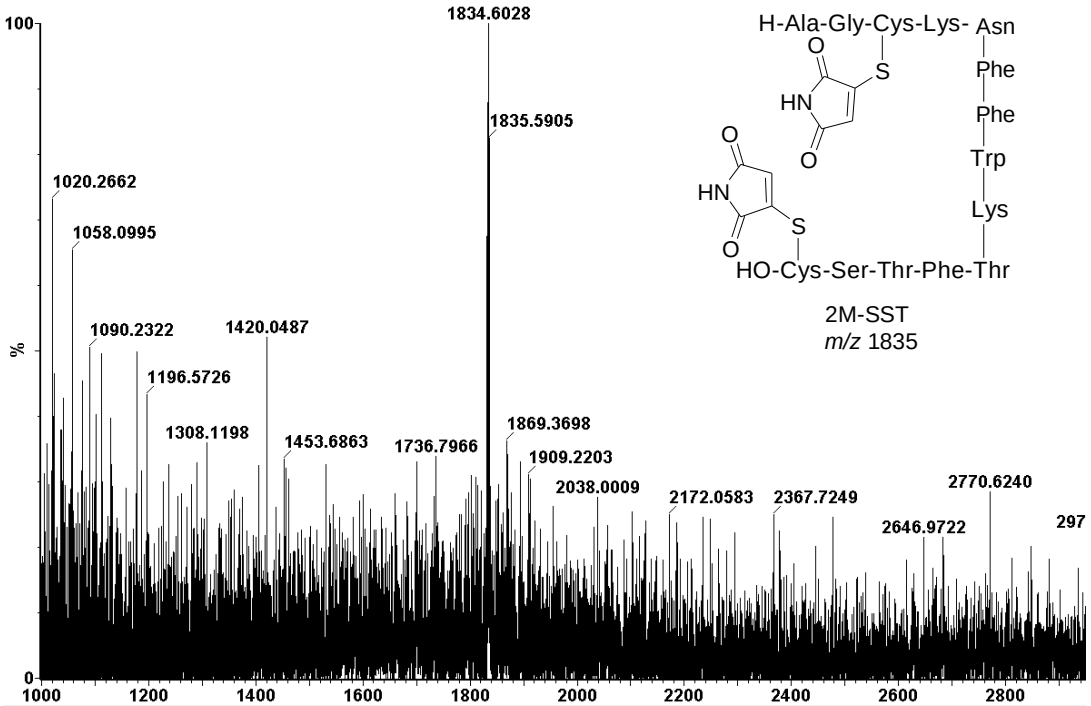
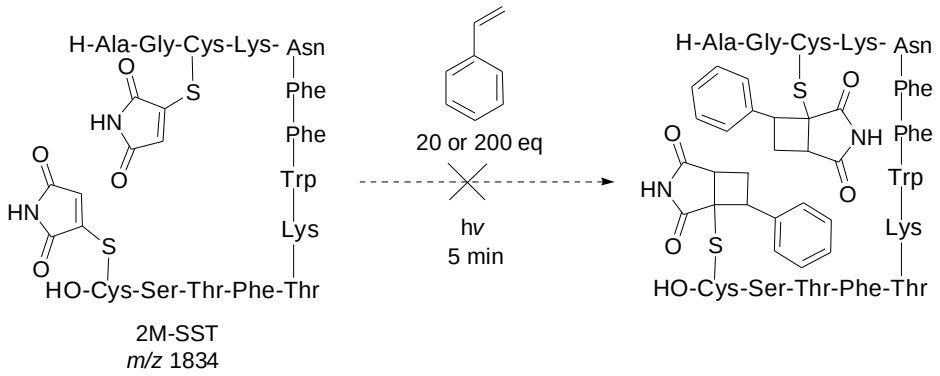


Figure 79: Mass spectrum of desalted 2M-SST obtained by MALDI-TOF operating in reflectron mode

In the first instance, twenty and two hundred equivalents of styrene were added to the mixture, to represent ten and one hundred equivalents with respect to the thiomaleimides, and the sample irradiated for five minutes, **Scheme 265**.



Scheme 265

In both cases, the same surprising result was obtained. The parent peak (1834-1836 Da) started to lose ~173 Da at just two minutes of irradiation, with degradation of both peaks (1834 and 1661 Da) witnessed at thirty minutes, **Figure 80**. Hereafter, for ease of comparison, figures depict the mass range of approximately 1600 to 2300. Full spectra indicated no other modification, other than those shown, had taken place throughout the experiment.

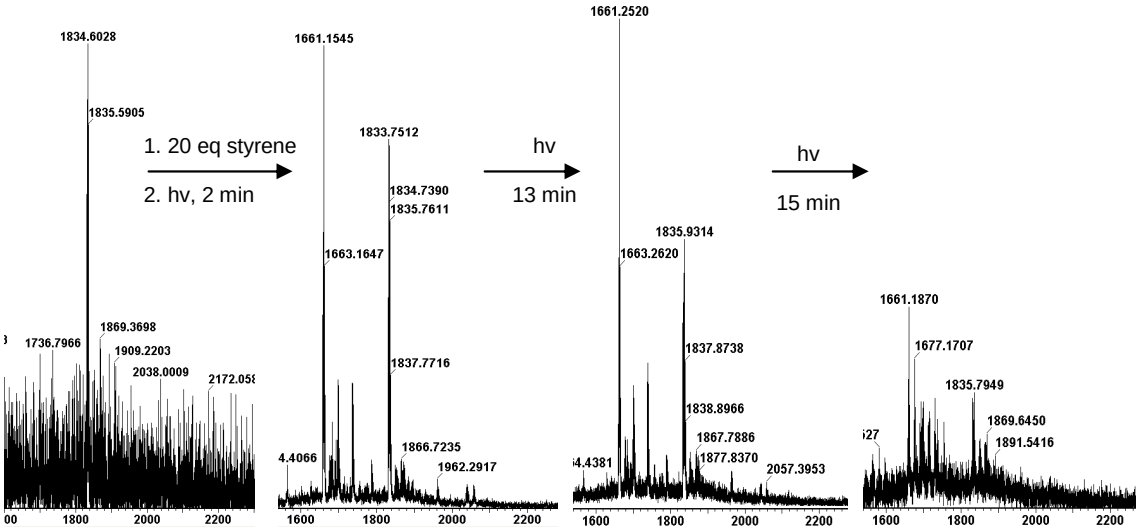


Figure 80: Mass spectra of 2M-SST before and after irradiation with styrene

When the irradiation was carried out without the presence of styrene the loss was still observed in a similar time frame, **Figure 81**, but in this case, at thirty minutes the new peak at 1661 Da still remained intact. This suggested that the cleavage did not occur after incorporation of styrene and that the new product was reactive towards styrene, either *via* irradiation or some other means. To further support the hypothesis of a maleimide-mediated photoreaction, native SST was irradiated and no loss was observed.

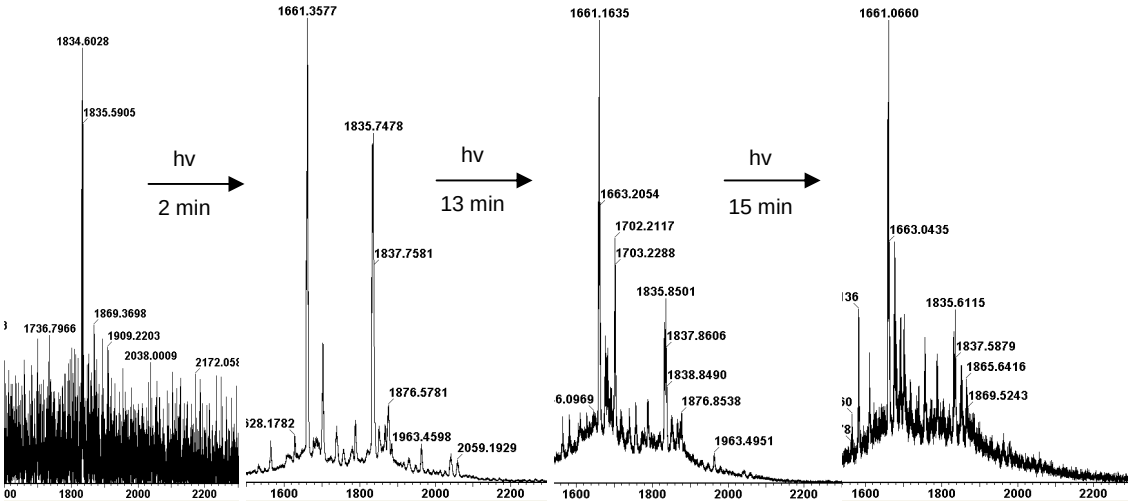
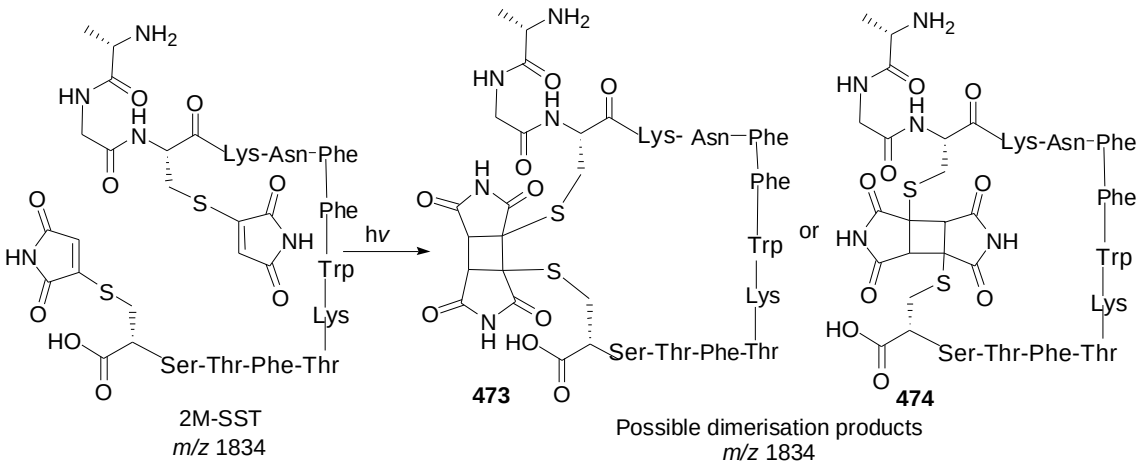


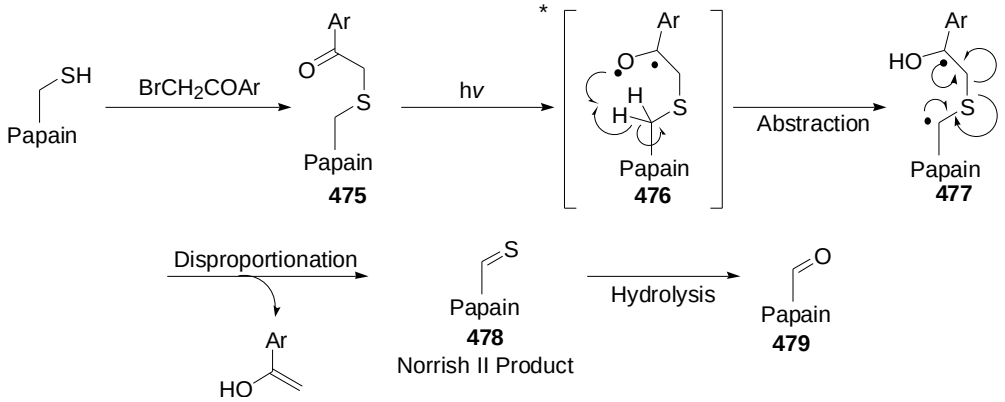
Figure 81: Mass spectra of 2M-SST before and after irradiation

The expected reactions of 2M-SST had been either head-to-head **473** or head-to-tail **474** dimerisation, **Scheme 266**. However, these modifications would not change the mass of the species, thus this was not the identity of the peak at 1661 Da.



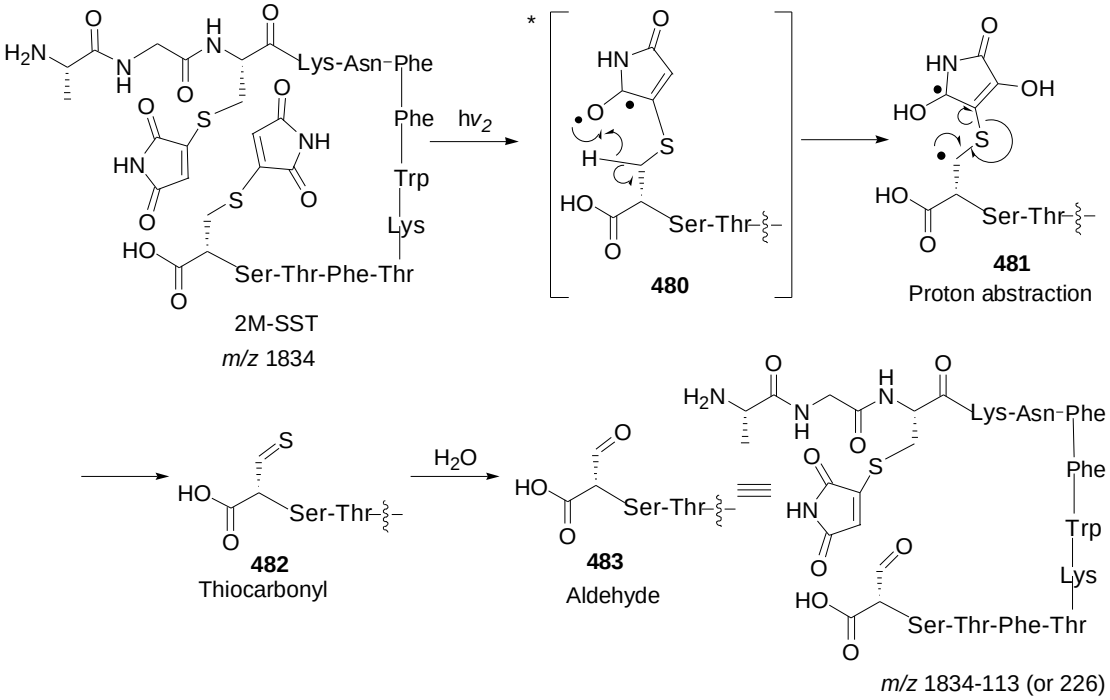
Scheme 266

Alternatively, there was literature precedent that cysteines modified with a carbonyl containing group **475** could undergo an overall Norrish II cleavage upon irradiation, **Scheme 267**. For example, upon irradiation, the excited state **476** abstracts a proton to afford diradical **477**, disproportionation could afford a thiocarbonyl **478**, that could be hydrolysed to afford the aldehyde **479**, **Scheme 267**.[331, 332]



Scheme 267

For 2M-SST the analogous process would involve formation of the excited species **480** upon irradiation and proton abstraction to afford the secondary diradical **481**. The eliminated thiocarbonyl **482** could be hydrolysed to aldehyde **483**, with 2M-SST losing an overall mass of 113 Da, **Scheme 268**. This could occur at one or both of the thiomaleimides but these mass losses were not the mass loss seen.



Scheme 268

At this stage, it was postulated that somatostatin could have undergone an unprecedented photochemical process to cleave the backbone or to remove the thiomaleimide labels. However, none of the indicated cleavages could afford a loss of 173 Da, **Figure 82**.

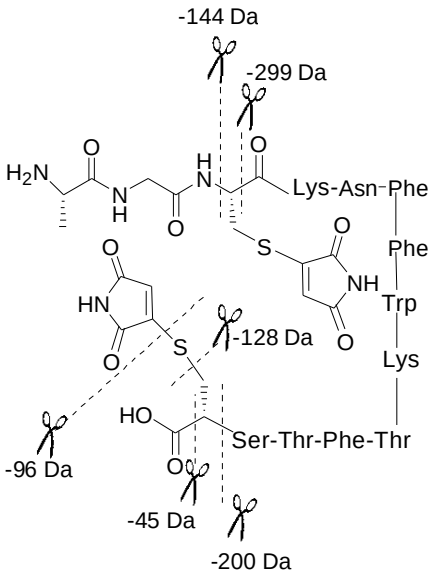
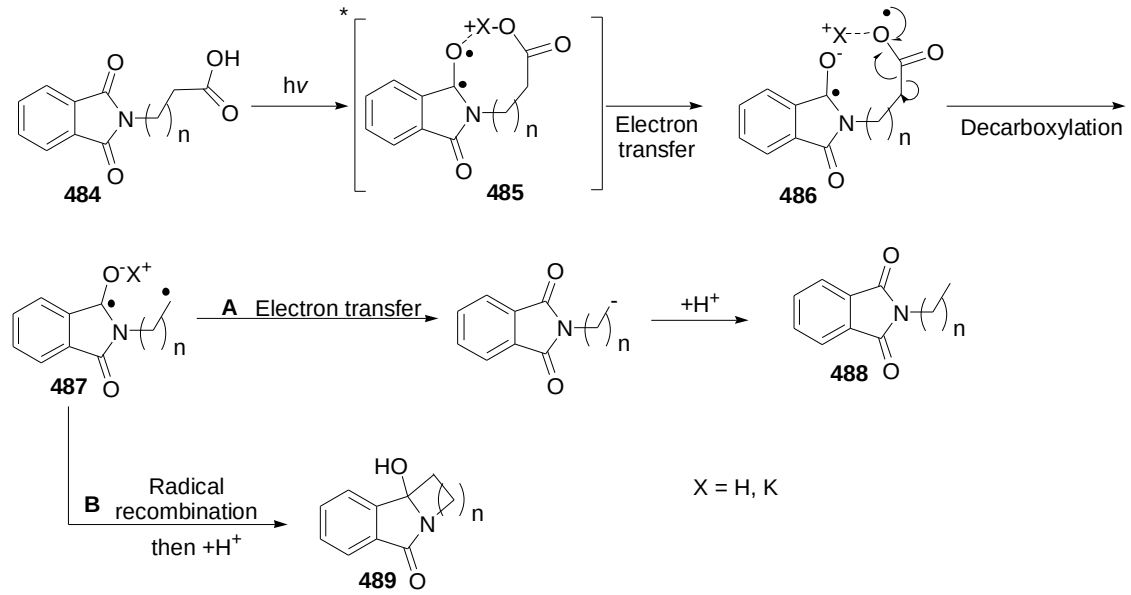


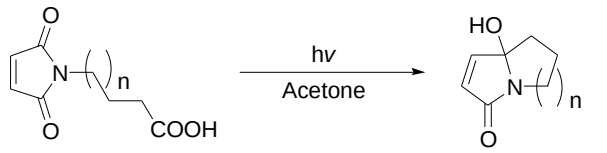
Figure 82: Losses that would be observed in the mass of 2M-SST if the indicated bonds were cleaved

It was realised at this juncture that combining the smaller cleavages could afford a value of 173 (-128 Da and -45 Da), resulting from decarboxylation and loss of one thiomaleimide. Literature precedent suggested that decarboxylation was possible from imide-labelled carboxylic acids,[333, 334] **Scheme 269**. In the reported process, [333, 334] when phthalimides containing a carboxylic acid **484** were excited, the excited species **485** undergoes facile electron transfer to diradical **486**. The diradical **486** decarboxylates to afford species **487** which can go on to react *via* two different pathways. Firstly, a second electron transfer process can occur to afford phthalimide **488**, route A, **Scheme 269**. Alternatively, the diradical **487** can recombine to afford the cyclised species **489**, route B, **Scheme 269**. When X = K^+, the process is much faster as the potassium stabilises the donor-acceptor constrcuct.[333] When the tether between the phthalimide and carboxylic acid contains a sulphur, the thioether can behave as a primary electron donor, 'shuttling' electrons between the systems.[335]



Scheme 269

In the course of research on imide photochemistry Griesbeck *et al*. also make reference to the decarboxylation and cyclisation process occurring with maleimides.[335] The carboxylic acid-substituted maleimides **490** and **492** undergo decarboxylation and cyclisation upon irradiation to form the cyclised species **491** and **493** in good yields, **Scheme 270**.[335] Whilst the mechanism isn't discussed in detail for maleimides, it is assumed as proceeding through a similar electron transfer-decarboxylation process.

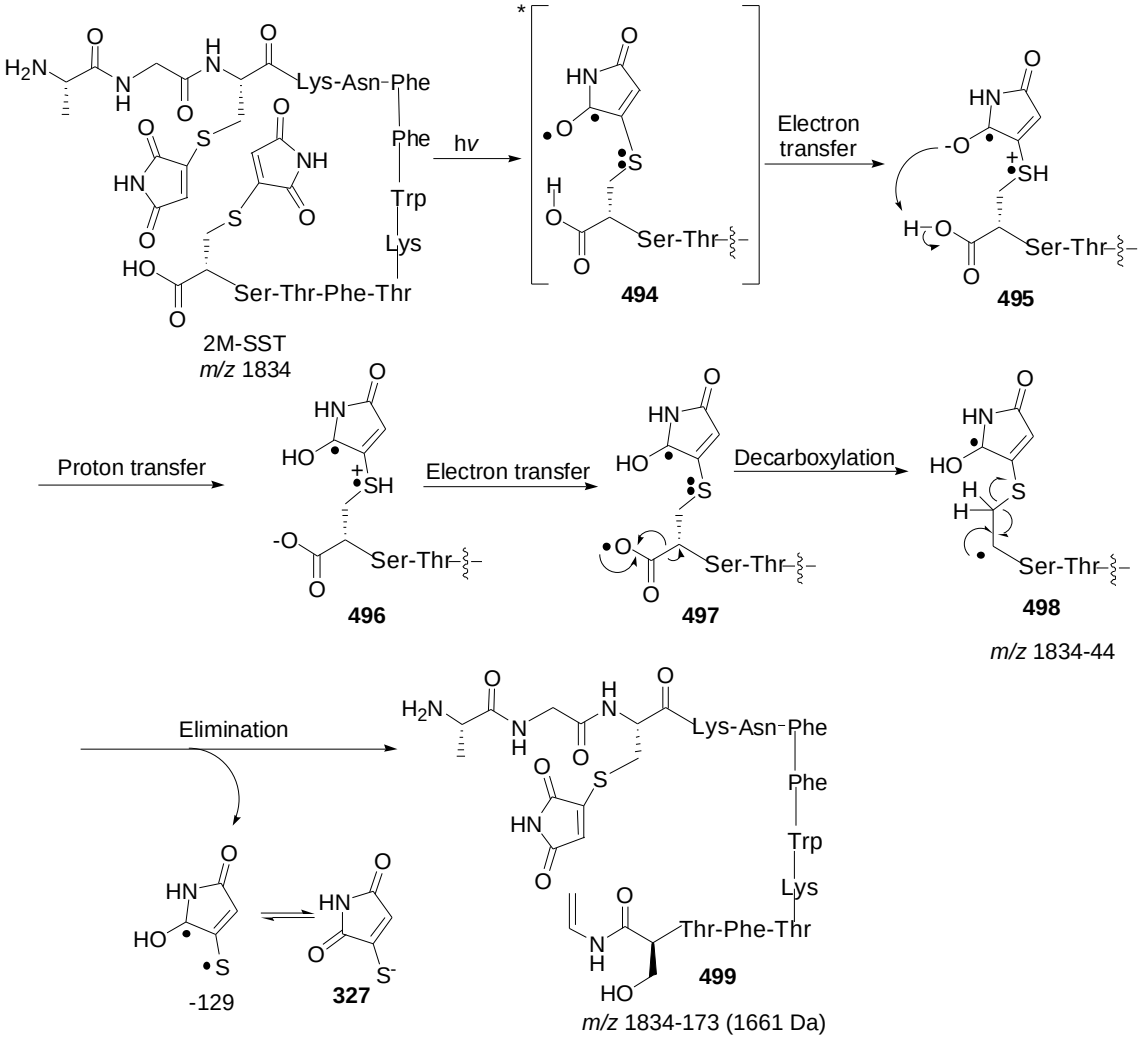


490, n = 1
492, n = 2

491, n = 1, 56%
493, n = 2, 62%

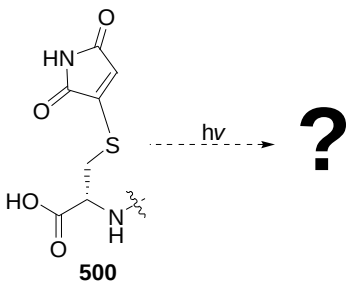
Scheme 270

It was therefore postulated that when 2M-SST was irradiated a similar decarboxylative process had occurred *via* electron transfer involving the sulfur[335] and the carbonyl of the thiomaleimide. The excited species **494** undergoes electron transfer with the thiol lone pair to afford diradical **495**, **Scheme 271**. Proton transfer to **496** and a second electron transfer regenerates the thiol lone pair in diradical **497**, and decarboxylation affords the secondary diradical **498**. Where this mechanism differs from **Scheme 269** is that, in this case, the thiomaleimide radical is a good leaving group, as it is a form of the stabilised thiolate **327**, and elimination occurred to afford alkene **499**, **Scheme 271**. The overall result of the mechanism shown in **Scheme 271** would result in a loss of 173 Da, to afford a species **499** of 1661 Da, thus it was postulated that this was the outcome of irradiation of 2M-SST. The electron transfer mechanism affords the same products regardless of which carbonyl of the thiomaleimide is involved, and the terminal alkene would be part of an enamide, which, unlike enamines, are described as stable in the literature.[336]



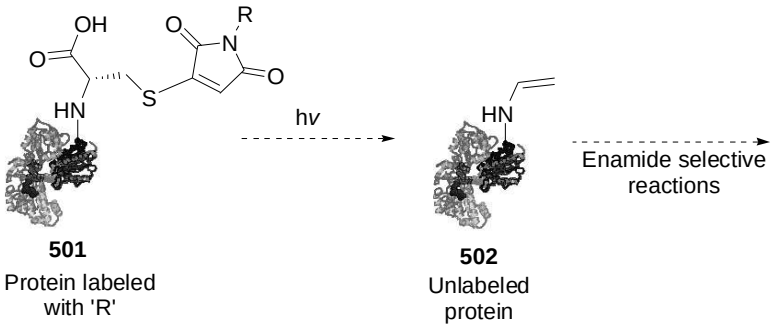
Scheme 271

Unfortunately, due to time constraints, this finding could not be investigated any further but ideally, a small molecule analogue **500** should be synthesised and irradiated to confirm the nature of the loss of 173 Da in the protein experiment.



Scheme 272

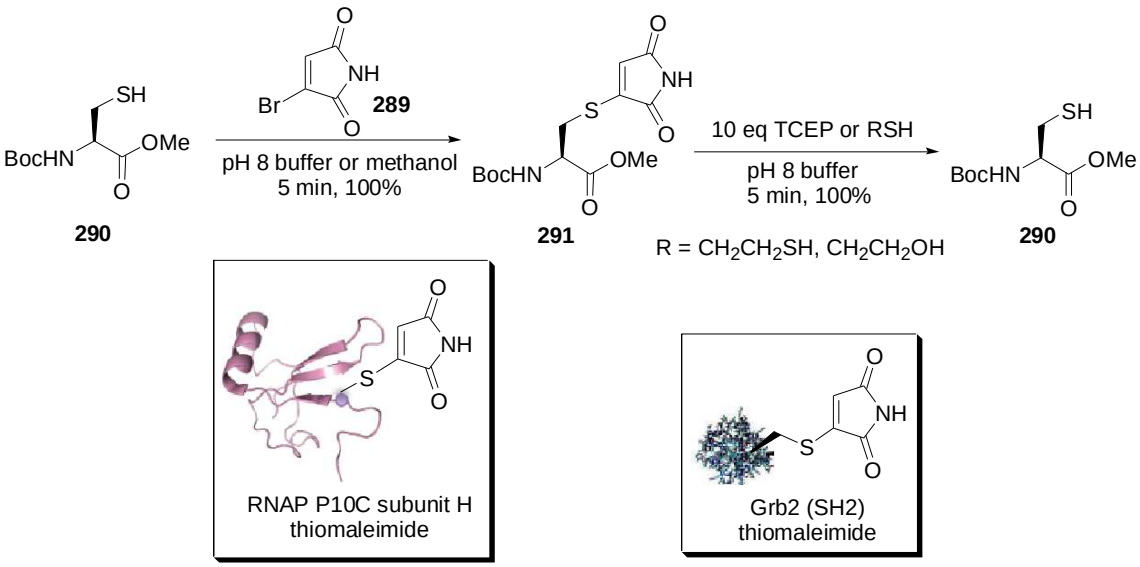
Further investigation of this result could open up avenues of research towards photochemically-mediated thiomaleimide cleavage. Fundamentally, any label that was attached to a terminal thiomaleimide **501**, *i.e. via* the thiomaleimide nitrogen would be cleaved upon irradiation to afford the alkene **502**, although with concomitant decarboxylation of the C-terminus, **Scheme 273**. The 'unmasked' enamide could also then be manipulated in subsequent bioorthogonal reactions, **Scheme 273**.



Scheme 273

2.ii.iii. Summary

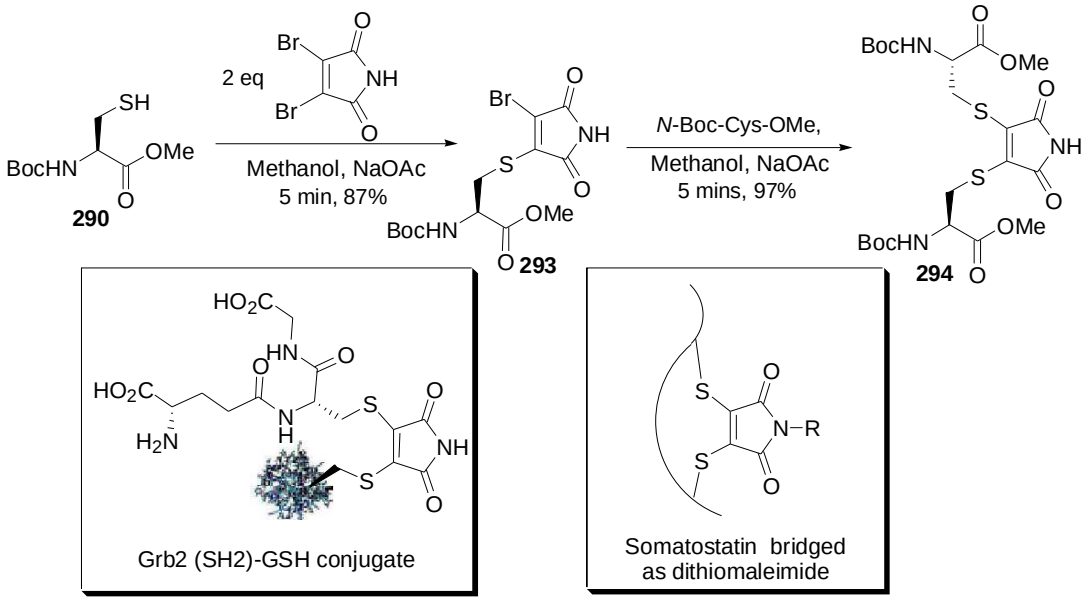
To date, work in small molecule model studies has demonstrated that bromomaleimide **289** is a rapid and selective label for cysteine, **Scheme 274**. Indeed, cysteine **290** was found to be more reactive towards bromomaleimide **289** than maleimide itself. This suggests that bromomaleimide **289** could be successfully transferred to proteins as a biomodification strategy. Initial reactions with Grb2 (SH2) and RNAP P10C sub-unit H carried out by Dr Mark Smith (UCL, Chemistry, Caddick group) and described in this text indicated that the methodology can be successfully applied to proteins containing a single cysteine residue,[265, 325] **Scheme 90**, with no lysine interference. The thiomaleimide **291** can be cleaved by addition of ten equivalents of TCEP, BME or DTE to afford the free thiol **290**, **Scheme 274**. Protein work carried out by Dr Mark Smith showed the reversion of the thiomaleimide label strategy with Grb2 (SH2) when exposed to excess TCEP.[265, 325] For future work in this area, the use of bromomaleimide reagents could and should be investigated as a rival to disulfide formation, the current method of choice for reversible labelling in chemical biology.



Scheme 274

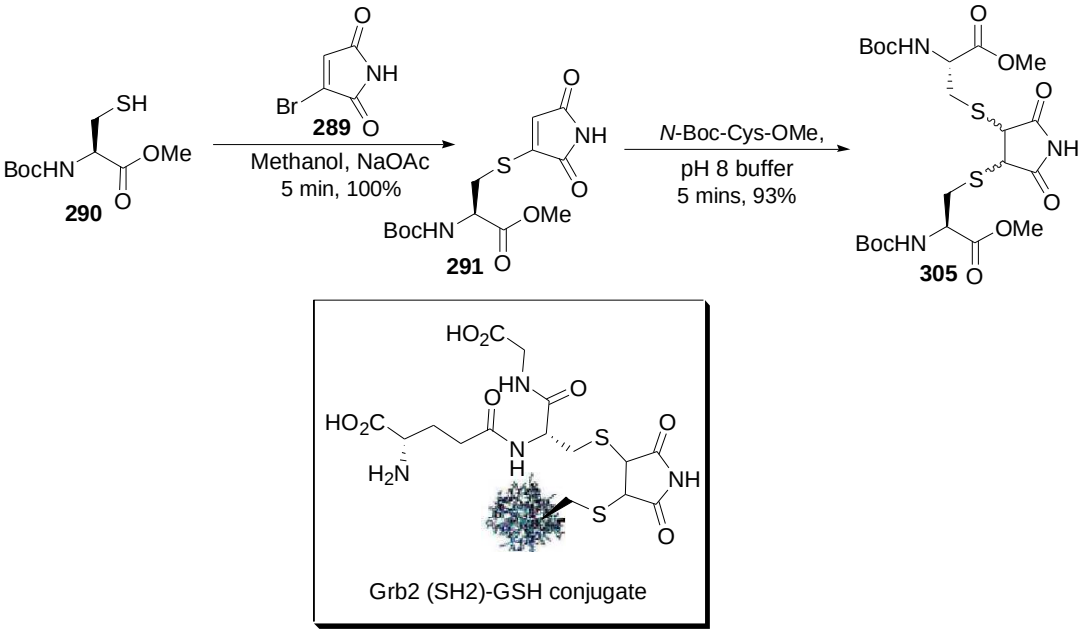
If dibromomaleimides are added to cysteine residues **290**, then bromothiomaleimides **293** can be formed, whereupon, after addition of another thiol, dithiomaleimides **294** are afforded, **Scheme 275**. This has been taken on to protein modification by the conjugation of two different thiol-containing biomolecules by Dr Mark Smith (UCL,

Chemistry, Caddick group) and the bridging of a disulfide in a polypeptide (somatostatin) by Felix Schumacher (UCL, Chemistry, Baker group), **Scheme 275**.[265]



Scheme 275

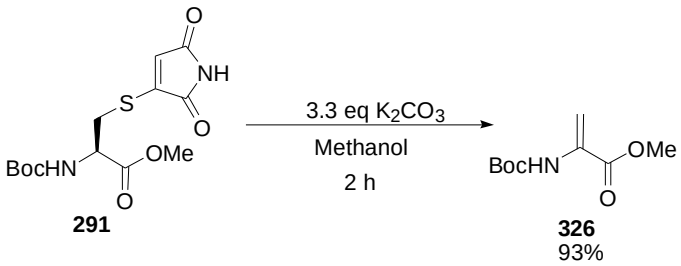
Thiomaleimides **291** are unreactive towards thiols in methanol but in aqueous basic solutions a second thiol can be added to the construct to form vicinal dithiosuccinimides **305**. The dithiosuccinimide **305** can also be formed from addition of ten equivalents of cysteine **290** to dibromomaleimide. The thiosuccinimides thus formed represent the potential for conjugation of two thiol containing. Dr Mark Smith (UCL, Chemistry, Caddick group) has again shown the use of dithiosuccinimides for bioconjugation by conjugating Grb2 (SH2) to glutathione *via* this construct, **Scheme 276**.



Scheme 276

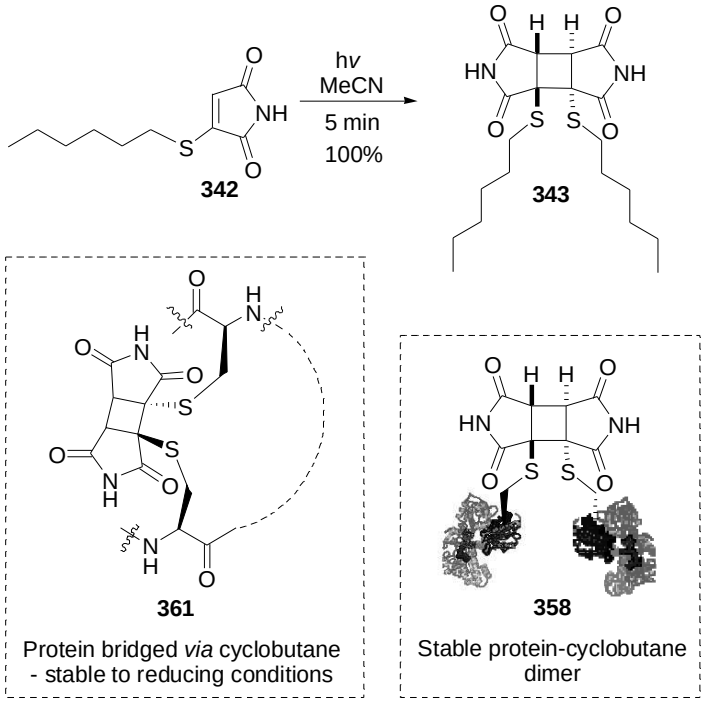
Further work to investigate the labelling strategy with bromomaleimide and dibromomaleimide to form thiomaleimides **291**, dithiomaleimides **294** and dithiosuccinimides **305** should encompass determining how the activity of any given protein is affected by modification to a thiomaleimide. In particular, for disulfide-modified proteins, how the protein folding is affected by the small, but evident extension of the disulfide bridge.

The addition of 3.3 equivalents of potassium carbonate to thiomaleimide **291** in methanol can afford Dha **326**, **Scheme 277**, although currently these conditions are not applicable to proteins. A future goal of this work would be to create thiomaleimide constructs that are better leaving groups and could therefore be eliminated by weaker bases and at pHs that do not degrade the constructs.



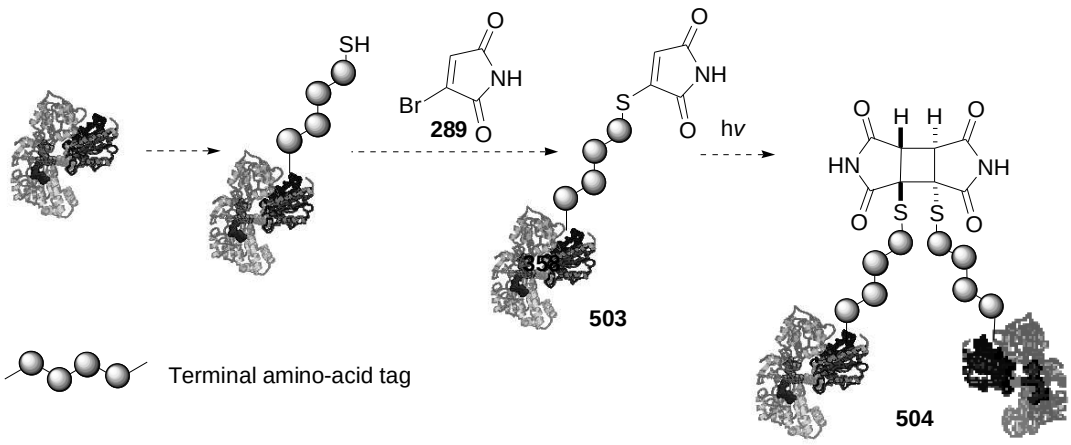
Scheme 277

It has been shown in this work that thiomaleimides are highly photochemically active. The photochemical activity of thiomaleimides can be exploited towards the formation of novel head-to-head *trans* dimers in quantitative yields, **Scheme 278**. The structures were assigned by analysis of coupling constants, NOeSY experiments and ^{13}C satellites in ^{1}H NMR. The dimers formed (**343**) are stable to reducing conditions. As discussed this could open up avenues of research into photochemical formation of a stable disulfide mimic in the place of a disulfide bridge **361** or between two proteins **358**, **Scheme 278**.



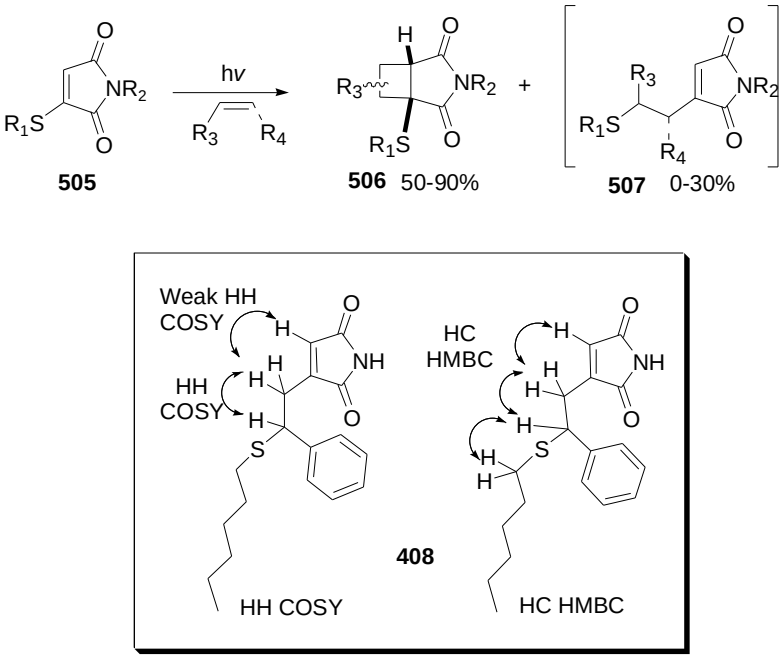
Scheme 278

Initial investigations into dimerisation with amino acid-containing thiomaleimides indicated that the conjugation and dimerisation strategies could be transferred to peptides and proteins. However, whilst RNAP P10C sub-unit H had an accessible cysteine and therefore a thiomaleimide modification that was reactive, no dimerisation was witnessed. This was probably due to the bulk of the protein hindering the approach and correct orientation of two thiomaleimide labels. In efforts towards protein dimerisation, it is the opinion of the author that dimerisation could be achieved if the thiomaleimide was located on a terminal amino acid 'tag' **503**. This tag could bring the dimerisation site away from the main bulk of the protein, potentially allowing two structures to come together and dimerise **504**, **Scheme 279**.



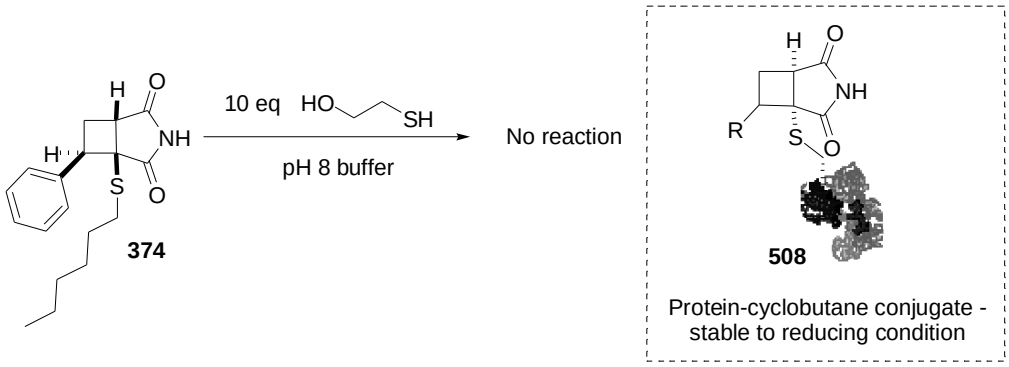
Scheme 279

In addition to dimerisation, conjugation of thiomaleimides **505** with a range of different olefins and alkynes can be achieved in moderate to good yields, **Scheme 280**, to afford novel cyclobutanes and cyclobutenes **506** (R_3 = CO_2Ar, CO_2Me, C≡N, aromatic or aliphatic groups, R_4 = H or Et), **Scheme 280**. The conjugation proceeds well when the thiomaleimide contains a variety of different thiol pendant groups (R_1 = amino acids, aromatic or aliphatic groups) and *N*-substitutions (R_2 = aromatic or aliphatic groups), **Scheme 280**. In general, in the [2+2] photocycloaddition, the reaction proceeded *via* the least sterically hindered transition state to afford the *cis* diastereomers and it appeared that 'HOMO'/HOMO interactions dominated to afford the *syn* adducts in most cases. An unprecedented 'insertion' reaction afforded side products **507** when thiomaleimide **342** was irradiated with styrene, 2-nitrostyrene, 4-methoxystyrene and 1-hexene, and when thiomaleimide **391** was irradiated with styrene. Initially, the 'insertion' products were described as [5+2] adducts but they were later identified as the 'insertion' products, assigned by NMR, **Scheme 280**, as all of the compounds were oils and thus could not be crystallised.



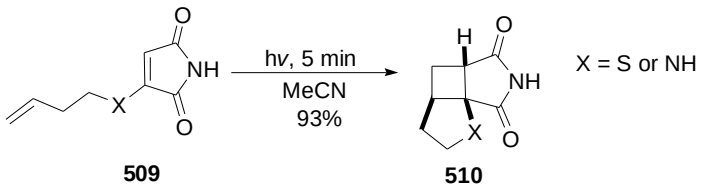
Scheme 280

Similarly to the cyclobutane dimer **343**, the cyclobutane conjugation product **374** was stable to reducing conditions, **Scheme 281**. This could potentially have applications in forming stable bioconjugations **508** *via* photochemistry and for easily rendering any thiomaleimide modification irreversible by irradiation with a simple olefin to form a cyclobutane, **Scheme 281**.



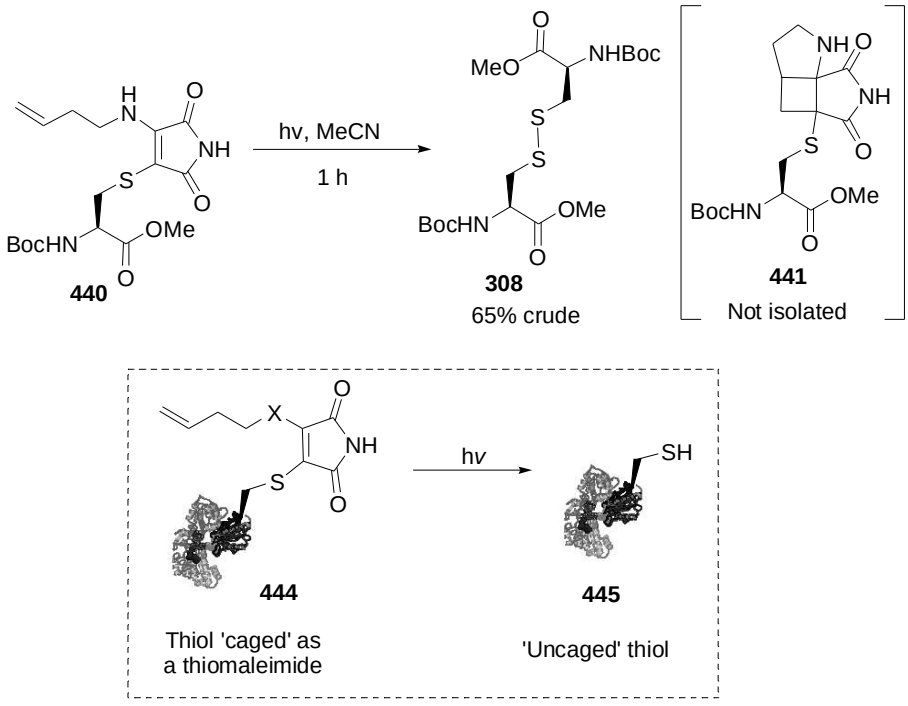
Scheme 281

Initial studies into the synthesis and irradiation of thiomaleimides **509** that could undergo an intramolecular [2+2] photocycloaddition with an alkene tether within the construct were very successful. High yields of cyclobutanes **510** were afforded in 93% yield after five minutes of irradiation, **Scheme 282**.



Scheme 282

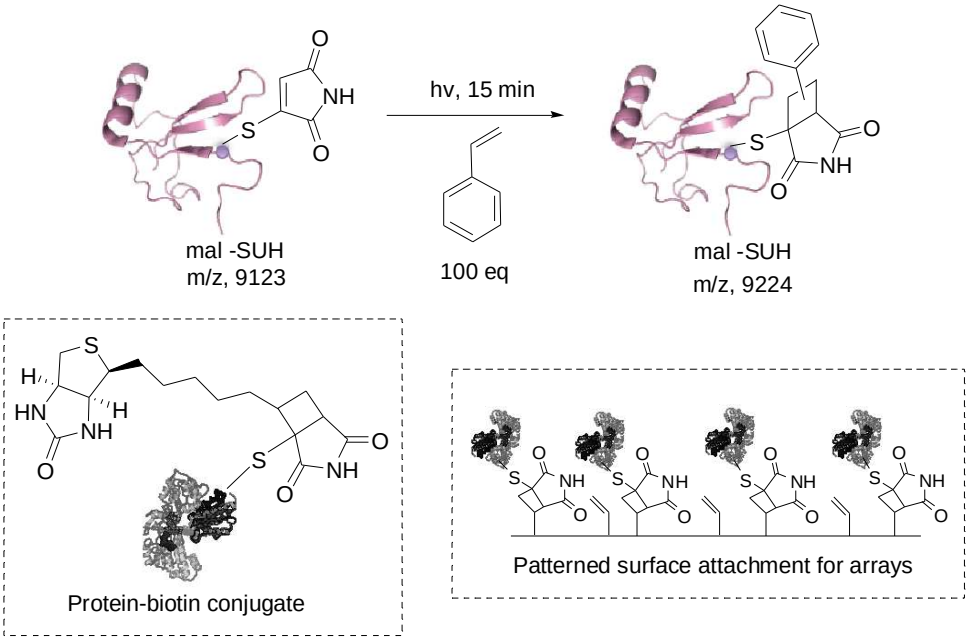
However, when an alkenyl tether was incorporated into a thiomaleimide that also contained a cysteine residue, the expected cyclobutane **441** was not formed, **Scheme 283**. Instead, a moderate yield of the eliminated cysteine was isolated as the analogous disulfide **308** (crude), **Scheme 283**. This could open up potential investigations into the photochemical 'uncaging' of thiols **445** from thiomaleimides **444**, **Scheme 283**.



Scheme 283

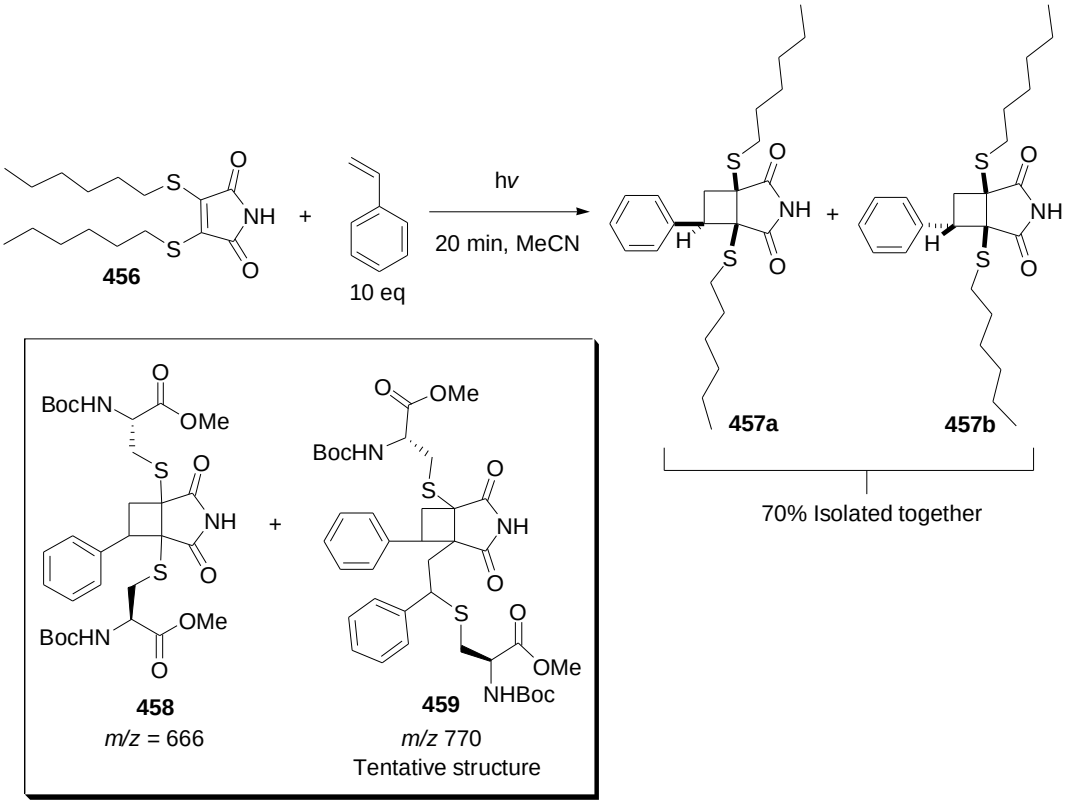
Preliminary results of protein modification *via* the [2+2] photocycloaddition were promising, with styrene modification of RNAP sub-unit H witnessed in just two minutes of irradiation with one hundred equivalents of styrene. The optimal conjugation conditions afforded approximately 70% conjugation after fifteen minutes of irradiation with one hundred equivalents of styrene, **Scheme 284**. Conjugation yields of approximately 70% can also be achieved with twenty equivalents of styrene, added in

two portions, and fifteen minutes of irradiation. Future work would investigate the reversibility of the modification upon exposure to reducing agents. Extension of this work would be towards photochemical conjugation of a thiomaleimide labelled protein with a biologically relevant tag such as a fluorophore, biotin, or a solid surface, **Scheme 284**.



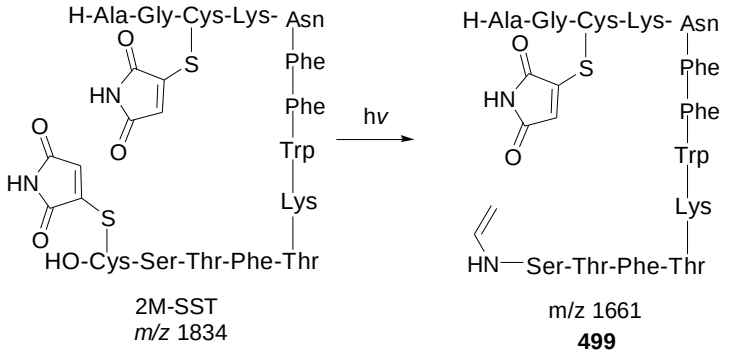
Scheme 284

The photochemistry of dithiomaleimides was also investigated. Dimerisation of dithiomaleimide **456** did not occur upon irradiation, probably due to the steric hindrance in the scaffold, but styrene could be conjugated to dithiomaleimide **456** in good yields, **Scheme 285**. When this strategy was attempted with a dithiomaleimide incorporating cysteines **294**, a product that had doubly incorporated the styrene was also witnessed by mass spectroscopy **459**. The structure of the double incorporation product **459** was only tentatively assigned as it was not isolated post column chromatography, **Scheme 285**.



Scheme 285

When the conjugation strategy was attempted with a peptide labelled as a dithiomaleimide across a disulfide bridge, *i.e.* MB-SST, irradiation afforded a mixture that could not be analysed. When the same disulfide bridge was doubly labelled with two bromomaleimides **289** to afford doubly labelled somatostatin (2M-SST), subsequent irradiation afforded a mass loss of 173 Da, **Scheme 286**. It was postulated that this loss occurred from an unprecedented decarboxylation and thiomaleimide elimination to afford enamide **478**, **Scheme 286**.



Scheme 286

Owing to time constraints, the photochemical ‘cleavage’ could not be further investigated but investigations in this area would involve the synthesis and irradiation of a small molecule thiomaleimide analogue containing a carboxylic acid. This strategy could potentially be used in photochemically-mediated cleavage of a label tethered to a protein *via* a thiomaleimide, and subsequent functionalisation of the resultant enamide.

The wide range of suitable partners in the [2+2] photocycloaddition of thiomaleimides indicates the vast potential for bioconjugation of alkene or alkyne containing labels with biomolecules modified with a thiomaleimide. This was further supported by the high λ_{max} and ε of thiomaleimides and dithiomaleimides, indicating that the constructs could be irradiated at high wavelengths, largely avoiding protein degradation from sub-360 nm irradiation.

Work described in this text opens up many potential avenues for modifications *via* conjugate addition of cysteine to the bromomaleimides to form reversible constructs, or *via* irradiation of the newly formed thiomaleimides to form irreversible cyclobutane adducts. Future goals of this work would encompass numerous potential applications, including use of these strategies towards labelled proteins, immobilisation onto solid support or photochemically-mediated label cleavage.

3. Experimental

All reactions were carried out at atmospheric pressure with stirring unless otherwise stated. Solvents and reagents were purchased from suppliers and used without any further purification.

Normal phase silica gel (BDH) and sand (VWR) were used for flash chromatography. All reactions were monitored by thin layer chromatography (TLC) unless otherwise stated. TLC plates pre-coated with silica gel 60 F_{254} on aluminium (Merck KGaA) were used, detection was by UV (254 or 365 nm) or chemical stain ($KMnO_4$ or vanillin).

High and low resolution mass spectrometry was performed using a VG70 SE operating in modes ES, EI, FAB or CI (+ or -) depending on the sample.

^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 MHz, 400 MHz, 500 MHz and 600 MHz and ^{13}C NMR at 75 MHz, 100 MHz, 125 MHz and 150 MHz on a Bruker AMX300, AMX400, AMX500 and AMX600 at ambient temperature. Chemical shifts were measured in parts per million (ppm) and are quoted as δ. Coupling constants, *J*, are quoted in Hertz (Hz) to 1 decimal place. The multiplicity of the signal is indicated as s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, defined as all multipeak signals where overlap produces complex peaks. All peaks should be taken as sharp unless otherwise described. Where necessary, assignments were made with the aid of DEPT, COSY, HMQC, HMBC or NOESY correlation experiments.

Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode. Melting points were measured with a Gallenkamp apparatus and are uncorrected. UV-Vis spectroscopy was carried out on a UV-2400PC Series. Elemental analyses were carried out by Gillian Maxwell at University College London.

3.i. Abbreviations

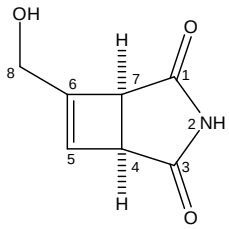
Within this text, room temperature is defined as between 19-22 ºC. The term *in vacuo* is used to describe solvent removal by Büchi rotary evaporation between 17 and 60 ºC at approx 10 mmHg unless otherwise stated. The term ‘degassed’ refers to the process of removing O_2 from a solution by bubbling argon through the solution. Irradiations were carried out using a medium pressure 125W mercury lamp (Photochemical Reactors

Ltd.) and a pyrex immersion well (Photochemical Reactors Ltd.) cooled *via* running water. For NMR experiments, $CDCl_3$ is fully deuterated (d^3) chloroform, DMSO is fully deuterated (d^6) dimethylsulfoxide, and MeOD is fully deuterated (d^4) methanol. Solvents were chosen according to the position of solvent peak in spectra and solubility of substrate.

3.ii. Validation of photochemical equipment

To determine that all new photochemistry apparatus was working efficiently, a well-known photochemical "control reaction"[263] was investigated, the [2 + 2] photocycloaddition of maleimide with propargyl alcohol. The reaction was successful in 65-68% yield (lit. 75%[263]), confirming that each photochemistry set-up was providing sufficient strength and range wavelength, affording 6-hydroxymethyl-2-aza-bicyclo[3.2.0]hept-5-ene-1,3-dione.

(4RS, 7RS)-6-Hydroxymethyl-2-aza-bicyclo[3.2.0]hept-5-ene-1,3-dione[263]



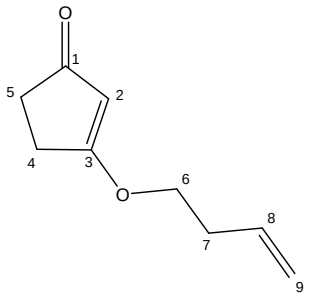
To a solution of maleimide (250 mg, 2.58 mmol) in acetonitrile (50 mL) was added propargyl alcohol (230 μL, 3.99 mmol) and the solution degassed for 15 minutes prior to irradiation in pyrex glassware for 60 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (ethyl acetate) afforded affording 6-hydroxymethyl-2-aza-bicyclo[3.2.0]hept-5-ene-1,3-dione as a white powder (243 mg, 1.59 mmol) in 68% yield.

δ_H (300 MHz, DMSO) 6.21 (br s, 1H, H-5), 5.04 (m, 1H, HO-8), 3.99-3.92 (m, 2H, H_2-8), 3.69-3.66 (m, 1H, CH), 3.59-3.52 (m, 1H, CH) peaks are very broad, thus splittings are not seen; δ_C (75 MHz, DMSO) 182.2 (C=O), 181.0 (C=O), 157.9 (C6), 134.3 (C5), 63.2 (C8), 53.7 (CH), 50.6 (CH); IR (solid, cm^{-1}) 3377 (m), 3074 (m), 2876 (w), 2798 (w), 1764 (m), 1697 (s); MS (EI) *m*/z (relative intensity): 153 (M+, 20), 136 (15), 124 (25), 110 (20), 99 (10), 82 (100); Exact Mass Calcd for $[C_7H_7O_3N]$+ requires *m/z* 153.0420 Found 153.0422 (EI); m.p. 102-106 ºC (Lit[263] m.p. 106-108).

3.iii. Synthesised compounds

3.iii.i. Synthesised compounds for 2.i.

142. 3-(But-3-enyloxy)-cyclopent-2-enone[337]

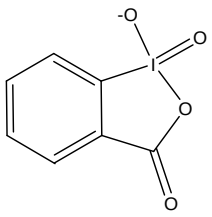


To a stirring dispersion of 1,3-cyclopentanedione (1.00 g, 10.2 mmol) in toluene (10 mL) was added but-3-en-1-ol (2.62 mL, 30.6 mmol). *p*-Toluene sulfonic acid (100 mg, 0.59 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed *in vacuo* and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **142** as a pale yellow oil (1.14 g, 7.50 mmol) in 87% yield.

δ_H (300 MHz, $CDCl_3$) 5.80 (ddt, 1H, *J* = 17.0, 10.2 and 6.7, H-8), 5.27 (s, 1H, H-2), 5.27-5.08 (m, 2H, H_2-9), 4.00 (t, 2H, *J* = 6.6, H_2-6), 2.61-2.57 (m, 2H, CH_2), 2.51 (dt, 2H, *J* = 6.7 and 6.6, H_2-7), 2.43-2.39 (m, 2H, CH_2); δ_C (75 MHz, $CDCl_3$) 205.0 (C1), 190.1 (C3), 133.2 (C2), 117.9 (C9), 104.8 (C8), 70.9 (C6), 34.0 (CH_2), 32.8 (CH_2), 28.5 (CH_2); IR (oil, cm^{-1}) 3080 (w), 2928 (w), 1702 (s), 1586 (s); MS (EI) *m*/z (relative intensity): 152 (M+, 75), 124 (70), 97 (85), 69 (80); Exact Mass Calcd for $[C_9H_{12}O_2]$+ requires *m/z* 152.0831 Found 152.0834 (EI); UV (Acetonitrile) ε_{234} = 36100 and ε_{282} = 7500 $cm^{-1}M^{-1}$.

Synthesis of Dess-Martin periodinane

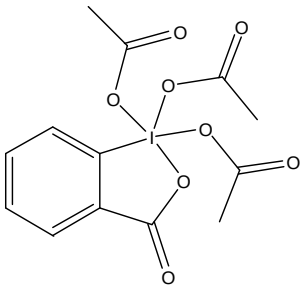
2-Iodoxybenzoic acid[338]



2-Iodoxybenzoic acid was synthesised and isolated according to literature methods[338] and afforded as a white solid that degraded on standing (21.7 g, 0.077 mol) in 97%

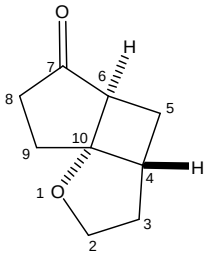
yield. δ_H (500 MHz, DMSO) 8.12 (d, 1H, *J* = 8.0, Ar-H), 8.03-7.97 (m, 2H, 2 x Ar-H), 7.83 (app t, 1H, *J* = 7.4, Ar-H); δ_C (125 MHz, DMSO) 167.52 (C=O), 146.54 (Ar), 133.41 (Ar-H), 132.96 (Ar-H), 131.40 (Ar), 130.06 (Ar-H), 124.99 (Ar-H); Mass ion not found.

Dess-Martin periodinane[338]



Dess-Martin periodinane was synthesised and isolated according to literature methods[338] and afforded as a white solid that degraded on standing (23.6 g, 0.056 mol) in 72% yield. δ_H (500 MHz, DMSO) 8.36 (d, 1H, *J* = 8.6, Ar-H), 8.13-8.05 (m, 2H, 2 x Ar-H), 7.93 (app t, 1H, *J* = 7.4, Ar-H); δ_C (125 MHz, DMSO) 175.87 (C=O), 174.10 (C=O), 166.23 (C=O), 142.36 (Ar), 135.76 (Ar-H), 133.85 (Ar-H), 131.90 (Ar), 126.55 (Ar-H), 126.10 (Ar-H), 20.48 (2 x CH_3), 20.34 (CH_3); Mass ion not found.

143. (4RS, 6SR, 10SR)-1-Oxa-tricyclo[5.3.0.0^{4,10}]decan-7-one



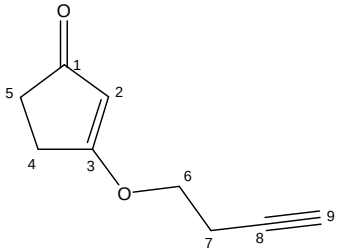
Method 1: 142 (76 mg, 0.50 mmol) was dissolved in acetonitrile (12.5 mL) and the resulting solution was degassed for 30 minutes prior to immediate irradiation in pyrex glassware for 90 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **143** as a colourless oil (10 mg, 0.065 mmol) in 13% yield.

Method 2: To a stirring solution of **202** (50 mg, 0.26 mmol) in dichloromethane at 0 ^oC, was added freshly prepared Dess-Martin periodinane (151 mg, 0.36 mmol). Pyridine

(77 µL, 0.96 mmol) was added and the dispersion stirred at ambient temperature for 15 minutes. The solvent was removed *in vacuo* and the purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **143** as a colourless oil (41 mg, 0.27 mmol) in 84% yield.

δ_H (500 MHz, $CDCl_3$) 4.23 (dd, 1H, *J* = 8.8 and 8.5, H*H*-2), 3.95 (ddd, 1H, *J* = 8.8, 8.5 and 7.0, *H*H-2), 2.83 (ddd, 1H, *J* = 9.1, 8.2 and 6.5, H-4), 2.70-2.61 (m, 1H, H-6), 2.62 (ddd, 1H, *J* = 18.0, 8.8 and 1.7, CH*H*), 2.53-2.47 (m, 1H, CH*H*), 2.20-2.10 (m, 2H, 2 x C*H*H), 2.03-1.83 (m, 3H, H*H*-3 and H_2-5), 1.74 (dd, 1H, *J* = 12.6 and 6.5, *H*H-3); δ_C (125 MHz, $CDCl_3$) 218.47 (C7), 89.28 (C10), 68.52 (C2), 47.91 (C6), 41.30 (C4), 37.88 (CH_2), 32.42 (CH_2), 31.05 (CH_2), 23.94 (CH_2); IR (oil, cm^{-1}) 2932 (m), 2857 (m), 1732 (s); MS (CI+) *m*/z (relative intensity): 153 ([M+H], 100); Exact Mass Calcd for $[C_9H_{12}O_2]$+H requires *m/z* 153.0916 Found 153.0913 (CI+); UV (Acetonitrile) ε_{274} = 1100 $cm^{-1}M^{-1}$.

144. 3-But-3-ynyloxy-cyclopent-2-enone

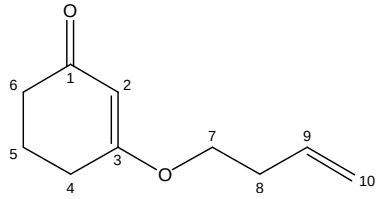


To a stirring dispersion of 1,3-cyclopentanedione (500 mg, 5.10 mmol) in toluene (5 mL) was added 3-butyn-1-ol (1.40 mL, 15.3 mmol). *p*-Toluene sulfonic acid (50.0 mg, 0.263 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **144** as an off-white solid (0.560 g, 3.73 mmol) in 76% yield.

δ_H (300 MHz, $CDCl_3$) 5.31 (s, 1H, H-2), 4.08 (t, 2H, *J* = 6.8, H_2-6), 2.71-2.62 (m, 4H, H_2-4 and H_2-5), 2.45 (td, 2H, *J* = 6.7 and 2.6, H_2-7), 2.05 (t, 1H, *J* = 2.7, H-9); δ_C (75 MHz, $CDCl_3$) 205.7 (C1), 189.7 (C3), 105.1 (C2), 79.3 (C8), 70.5 (C9), 69.3 (C6), 34.1 (CH_2), 28.4 (CH_2), 19.0 (CH_2); IR (solid, cm^{-1}) 3223 (m), 3077 (w), 2926 (w), 1671 (s), 1590 (s); MS (CI+) *m*/z (relative intensity): 151 ([M+H], 25), 122 (42), 108 (100);

Exact Mass Calcd for $[C_9H_{10}O_2]$+H requires *m/z* 151.0759 Found 151.0761 (CI+); m.p. 75-76 ºC; UV (Acetonitrile) ε_{228} = 15600 and ε_{274} = 3900 $cm^{-1}M^{-1}$.

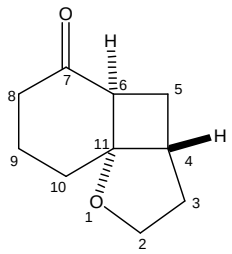
150. 3-(But-3-enyloxy)-cyclohex-2-enone



To a stirring dispersion of 1,3-cyclohexanedione (280 mg, 2.50 mmol) in toluene (3 mL) was added 3-buten-1-ol (642 μL, 7.50 mmol). *p*-Toluene sulfonic acid (25.0 mg, 0.132 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **150** as a pale yellow oil (310 mg, 1.87 mmol) in 75% yield.

δ_H (300 MHz, $CDCl_3$) 5.80 (ddt, 1H, *J* = 17.0, 10.2 and 6.7, H-9), 5.34 (s, 1H, H-2), 5.15-5.08 (m, 2H, H_2-10), 3.87 (t, 2H, *J* = 6.6, H_2-7), 2.49-2.45 (m, 2H, CH_2), 2.39 (t, 2H, *J* = 6.2, CH_2), 2.35-2.32 (m, 2H, CH_2), 1.99-1.94 (m, 2H, CH_2); δ_C (75 MHz, $CDCl_3$) 199.6 (C1), 177.8 (C3), 135.6 (C2), 117.5 (C10), 102.8 (C9), 67.5 (C7), 36.8 (CH_2), 32.8 (CH_2), 29.0 (CH_2), 21.2 (CH_2); IR (oil, cm^{-1}) 2945 (w), 1624 (s), 1563 (s); MS (CI+) *m*/z (relative intensity): 167 ([M+H], 65), 113 (100), 85 (100); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+H requires *m/z* 167.1072 Found 167.1073 (CI+); UV Acetonitrile) ε_{242} = 22600 $cm^{-1}M^{-1}$.

151. (4RS, 6SR, 11SR)-1-Oxa-tricyclo[5.4.0.0^{4,11}]undecan-7-one

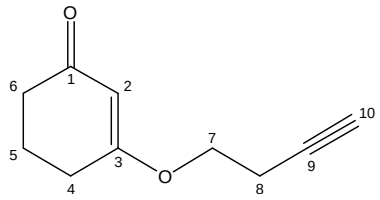


150 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to immediate irradiation in pyrex glassware for 4 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in

petroleum ether (1% NEt_3)) afforded **151** as a colourless oil (46 mg, 0.28 mmol) in 28% yield.

δ_H (500 MHz, $CDCl_3$) 4.14 (ddd, 1H, *J* = 9.1, 8.0 and 1.1, H*H*-2), 3.96 (ddd, 1H, *J* = 11.2, 9.1 and 5.5, *H*H-2), 2.81 (app ddt, 1H, *J* = 11.6, 6.7 and 0.6, H-6), 2.70-2.64 (m, 1H, H-4), 2.48 (app dtd, 1H, *J* = 17.0, 4.5 and 0.6, H*H*-8), 2.24 (app dtd, 1H, *J* = 17.0, 4.5 and 0.6, *H*H-8), 2.09-2.00 (m, 2H, H*H*-5 and H*H*-9), 1.92-1.72 (m, 5H, *H*H-5, *H*H-3, *H*H-9 and H_2-10), 1.67 (dd, 1H, *J* = 5.4 and 1.7, H*H*-3); δ_C (125 MHz, $CDCl_3$) 213.27 (C7), 87.94 (C11), 67.36 (C2), 49.87 (C6), 40.49 (C4), 39.70 (C8), 31.87 (C3), 31.68 (C10), 24.76 (C5), 20.17 (C9); IR (oil, cm^{-1}) 2943 (m), 1701 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 20), 96 (100); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+ requires *m/z* 166.0988 Found 166.0985 (EI); UV (Acetonitrile) ε_{222} = 20100 and ε_{282} = 39 $cm^{-1}M^{-1}$.

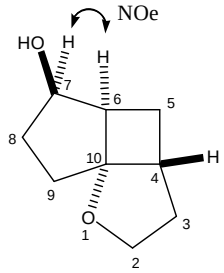
200. 3-But-3-ynyloxy-cyclohex-2-enone



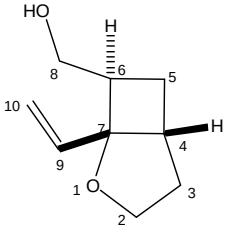
To a stirring dispersion of 1,3-cyclohexanedione (280 mg, 2.50 mmol) in toluene (3 mL) was added 3-butyn-1-ol (0.570 mL, 7.50 mmol). *p*-Toluene sulfonic acid (25 mg, 0.13 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **200** as a pale cream solid (312 mg, 1.90 mmol) in 76% yield.

δ_H (300 MHz, $CDCl_3$) 5.32 (s, 1H, H-2), 3.92 (t, 2H, *J* = 6.8, H_2-7), 2.61 (td, 2H, *J* = 6.8 and 2.7, H_2-8), 2.41 (t, 2H, *J* = 6.2, CH_2), 2.32 (t, 2H, *J* = 6.2, CH_2), 2.02 (t, 1H, *J* = 2.7, H-10), 2.00-1.94 (m, 2H, CH_2); δ_C (75 MHz, $CDCl_3$) 199.5 (C1), 177.2 (C3), 103.1 (C2), 79.6 (C9), 70.2 (C10), 66.1 (C7), 36.7 (CH_2), 28.8 (CH_2), 21.2 (CH_2), 18.9 (CH_2); IR (solid, cm^{-1}) 3237 (m), 2963 (w), 1642(s), 1598 (s); MS (CI+) *m*/z (relative intensity): 165 ([M+H], 35), 113 (100), 85 (90); Exact Mass Calcd for $[C_{10}H_{12}O_2]$+H requires *m/z*: 165.0916 Found 165.0917 (CI+); m.p. 70-75 ºC; UV (Acetonitrile) ε_{242} = 18000 $cm^{-1}M^{-1}$.

202. (4RS, 6RS, 7RS, 10SR)-1-Oxa-tricyclo[5.3.0.0^{4,10}]decan-7-ol



204. (4RS, 6RS, 7SR)-(6-Hydroxymethyl-7-vinyl)-1-oxa-bicyclo[3.2.0]heptane



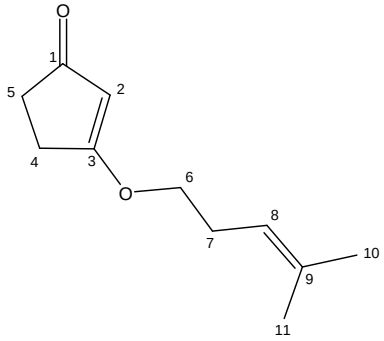
142 (152 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 105 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **202** as a colourless oil (128 mg, 0.83 mmol) in 83% yield and **204** as a colourless oil (2 mg, 0.13 mmol) in 1% yield.

202. δ_H (500 MHz, $CDCl_3$) 4.52 (app dt, 1H, *J* = 8.5 and 8.0, H-7), 4.16 (dd, 1H, *J* = 8.9 and 7.8, *H*H-2), 3.91 (ddd, 1H, *J* = 11.6, 8.9 and 5.4, H*H*-2), 2.55 (app td, 1H, *J* = 8.5 and 5.3, H-6), 2.47 (app td, 1H, *J* = 8.5 and 4.6, H-4), 2.1 (m, 2H, H*H*-9 and H*H*-8), 2.0 (ddd, 1H, *J* = 13.3, 8.7 and 5.2, *H*H-5) 1.9 (dddd, 1H, *J* = 12.4, 11.7, 8.5 and 7.9, H*H*-3), 1.78-1.52 (m, 3H, H*H*-9, H*H*-8 and *H*H-3), 1.25 (ddd, 1H, *J* = 13.4, 9.0 and 4.6, H*H*-5); δ_C (125 MHz, $CDCl_3$) 91.58 (C10), 89.11 (C7), 68.23 (C2), 43.25 (C6), 40.70 (C4), 32.45 (C3), 31.79 (CH_2), 31.21 (CH_2), 17.84 (C5); IR (oil, cm^{-1}) 3373 (br), 2935 (m); MS (EI) *m*/z (relative intensity): 153 ([M-H], 100), 97 (35); Exact Mass Calcd for [$C_9H_{14}O_2$]-H requires *m/z* 153.0910 Found 153.0915 (EI).

204. δ_H (300 MHz, $CDCl_3$) 6.18 (dd, 1H, *J* = 17.3 and 11.0, H-9), 5.31 (dd, 1H, *J* = 17.4 and 1.6, H*H*-10), 5.19 (dd, 1H, *J* = 10.9 and 1.7, *H*H-10), 4.17-4.06 (m, 2H), 3.72-3.50 (m, 1H), 2.88-2.72 (m, 1H), 2.55-2.38 (m, 1H), 1.95-1.21 (m, 5H); IR (oil, cm^{-1}) 3384

(br), 2930 (m), 2856 (m); MS (EI) *m*/z (relative intensity): 153 ([M-H], 100) 98 (50); Exact Mass Calcd for $[C_9H_{14}O_2]$-H requires *m/z* 153.0910 Found 153.0908 (EI).

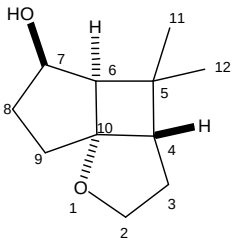
205. 3-(4-Methyl-pent-3-enyloxy)-cyclopent-2-enone



To a stirring dispersion of 1,3-cyclopentanedione (327 mg, 3.34 mmol) in toluene (5 mL) was added 4-methyl-pent-3-en-1-ol (1.17 mL, 10.0 mmol). *p*-Toluene sulfonic acid (40.0 mg, 0.211 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed *in vacuo* and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **205** as a yellow oil (0.568 g, 3.16 mmol) in 95% yield.

δ_H (500 MHz, $CDCl_3$) 5.26 (s, 1H, H-2), 5.10 (t, 1H, *J* = 7.4, H-8), 3.91 (t, 2H, *J* = 6.9, H_2-6), 2.58 (t, 2H, *J* = 5.4, CH_2), 2.45-2.39 (m, 4H, 2 x CH_2), 1.69 (s, 3H, CH_3), 1.62 (s, 3H, CH_3); δ_C (125 MHz, $CDCl_3$) 206.04 (C1), 190.25 (C3), 135.37 (C9), 118.43 (C2), 104.73 (C8), 71.57 (C6), 33.99 (CH_2), 28.55 (CH_2), 27.53 (CH_2), 25.73 (CH_3), 17.85 (CH_3); IR (oil, cm^{-1}) 2925 (w), 1702 (s), 1588 (s); MS (EI) *m*/z (relative intensity): 180 (M+, 50), 109 (17), 83 (100); Exact Mass Calcd for $[C_{11}H_{16}O_2]$+ requires *m/z* 180.1145 Found 180.1143 (EI); UV (Acetonitrile) ε_{234} = 20200 and ε_{274} = 2900 $cm^{-1}M^{-1}$.

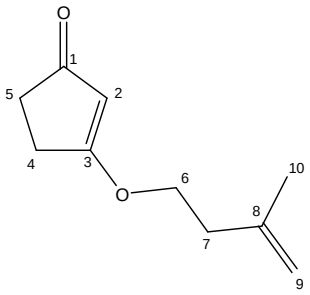
207. (4RS, 5SR, 7SR, 10RS)-5, 5-Dimethyl-1-oxa-tricyclo[5.3.0.0^{4,10}]decan-7-ol



205 (180 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 2 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate (1% NEt_3)) afforded **207** as a colourless oil (142 mg, 0.78 mmol) in 78% yield.

δ_H (500 MHz, $CDCl_3$) 4.33 (app dt, 1H, *J* = 8.6 and 8.0, H-7), 4.04 (ddd, 1H, *J* = 9.1, 8.5 and 1.6, H*H*-2), 3.73 (ddd, 1H, *J* = 11.3, 9.1 and 5.8, *H*H-2), 2.26 (d, 1H, *J* = 8.7, H-4), 2.19-1.98 (m, 2H, H_2-8), 2.09 (d, 1H, *J* = 8.6, H-6), 1.88 (dd, 1H, *J* = 13.5 and 6.1, H*H*-9), 1.79-1.75 (m, 2H, H_2-3), 1.75-1.68 (m, 1H, *H*H-9), 1.55 (br s, 1H, HO-7), 1.32 (s, 3H, CH_3), 1.10 (s, 3H, CH_3); δ_C (125 MHz, $CDCl_3$) 88.13 (C10), 75.77 (C7), 69.56 (C2), 53.13 (C6), 51.97 (C4) 33.29 (C8), 32.34 (C3), 31.02 (C5), 26.97 (CH_3), 26.84 (C9), 26.58 (CH_3); IR (oil, cm^{-1}) 3354 (br), 2932 (m), 2858 (m); MS (EI) *m*/z (relative intensity): 182 (M+, 14), 99 (51), 83 (100); Exact Mass Calcd for $[C_{11}H_{18}O_2]$+ requires *m/z* 182.1301 Found 182.1297 (EI).

208. 3-(3-Methyl-but-3-enyloxy)-cyclopent-2-enone

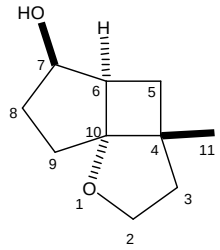


To a stirring dispersion of 1,3-cyclopentanedione (500 mg, 5.10 mmol) in toluene (5 mL) was added 3-methyl-3-buten-1-ol (1.54 mL, 15.3 mmol). *p*-Toluene sulfonic acid (100 mg, 0.59 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed *in vacuo* and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **208** as a pale orange oil (493 mg, 2.97 mmol) in 58% yield.

δ_H (300 MHz, $CDCl_3$) 5.30 (t, 1H, *J* = 1.1, H-2), 4.85 (s, 1H, H*H*-9), 4.76 (s, 1H, *H*H-9), 4.08 (t, 2H, *J* = 6.8, H_2-6), 2.62-2.58 (m, 2H, CH_2), 2.50-2.41 (m, 4H, 2 x CH_2), 1.77 (s, 3H, H_3-10); δ_C (75 MHz, $CDCl_3$) 206.0 (C1), 190.1 (C3), 141.0 (C2), 112.7 (C9), 104.8

(C8), 70.1 (C6), 36.4 (CH_2), 33.9 (CH_2), 28.5 (CH_2), 22.7 (C10); IR (oil, cm^{-1}) 2927 (w), 1702 (s), 1596 (s); MS (CI+) *m*/z (relative intensity): 167 ([M+H], 90), 127 (40), 99 (100); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+H requires *m/z* 167.1072 Found 167.1074 (CI+); UV (Acetonitrile) ε_{232} = 27800 and ε_{274} = 2300 $cm^{-1}M^{-1}$.

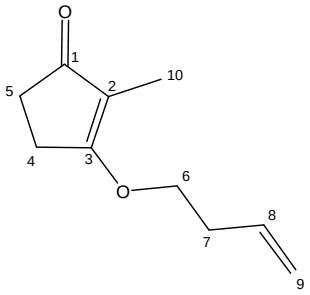
210. (4RS, 6RS, 7RS, 10RS)-4-Methyl-1-oxa-tricyclo[5.3.0.0^{4,10}]decan-7-ol



208 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 90 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **210** as a colourless oil (119 mg, 0.71 mmol) in 71% yield.

δ_H (500 MHz, $CDCl_3$) 4.25 (ddd, 1H, *J* = 14.5, 9.6 and 7.0, H-7), 4.00 (app dt, 1H, *J* = 9.3 and 8.0, H*H*-2), 3.80 (ddd, 1H, *J* = 15.5, 9.3 and 6.2, *H*H-2), 2.51 (app td, 1H, *J* = 8.7 and 7.0, H-6), 2.31 (br s, 1H, HO-7), 2.08-1.97 (m, 1H, H*H*-8), 1.81 (ddd, 1H, *J* = 12.5, 6.0 and 1.3, H*H*-9), 1.77-1.71 (m, 1H, H*H*-3), 1.69-1.57 (m, 5H, *H*H-3, H_2-5, *H*H-8 and *H*H-9), 1.07 (s, 3H, H_3-11); δ_C (125 MHz, $CDCl_3$) 92.51 (C10), 72.09 (C7), 67.10 (C2), 41.66 (C6), 41.66 (C4) 41.57 (C3), 32.27 (C8), 27.25 (C9), 25.85 (C5), 20.51 (C11); IR (oil, cm^{-1}) 3379 (br), 2950 (m), 2862 (m); MS (EI) *m*/z (relative intensity): 168 (M+, 18), 151 (30), 98 (100); Exact Mass Calcd for $[C_{10}H_{16}O_2]$+ requires *m/z* 168.1145 Found 168.1148 (EI).

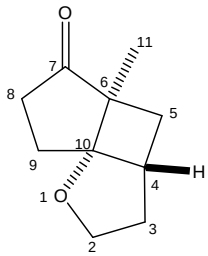
211. 3-(But-3-enyloxy)-2-methyl-cyclopent-2-enone



To a stirring dispersion of 2-methyl-1,3-cyclopentanedione (500 mg, 4.46 mmol) in toluene (5 mL) was added but-3-en-1-ol (1.15 mL, 13.4 mmol). *p*-Toluene sulfonic acid (50.0 mg, 0.263 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **211** as a pale yellow oil (0.700 g, 4.22 mmol) in 95% yield.

δ_H (500 MHz, $CDCl_3$) 5.85 (ddt, 1H, *J* = 16.8, 10.0 and 6.8, H-8), 5.21-5.13 (m, 2H, H_2-9), 4.22 (t, 2H, *J* = 6.2, H_2-6), 2.66-2.64 (m, 2H, CH_2), 2.52 (dt, 2H, *J* = 6.9 and 6.2, H_2-7), 2.46-2.44 (m, 2H, CH_2), 1.65 (s, 3H, H_3-10); δ_C (125 MHz, $CDCl_3$) 205.78 (C1), 183.14 (C3), 133.64 (C8), 118.33 (C9), 116.69 (C2), 68.88 (C6), 34.41 (CH_2), 33.83 (CH_2), 25.56 (CH_2), 6.48 (C10); IR (oil, cm^{-1}) 2922 (w), 1688 (s), 1624 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 37), 138 (22), 112 (28), 97 (100); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+ requires *m/z* 166.0988 Found 166.0984 (EI); UV (Acetonitrile) ε_{248} = 16300 $cm^{-1}M^{-1}$.

212. (4RS, 6SR, 10SR)-6-Methyl-1-oxa-tricyclo[5.3.0.0^{4,10}]decan-7-one

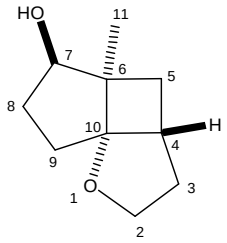


211 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to immediate irradiation in pyrex glassware for 2 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in

petroleum ether (1% NEt_3)) afforded **212** as a colourless oil (83 mg, 0.50 mmol) in 50% yield.

δ_H (500 MHz, $CDCl_3$) 4.17 (app t, 1H, *J* = 8.8, H*H*-2), 3.99 (ddd, 1H, *J* = 8.7, 5.5 and 4.6, *H*H-2), 2.70 (app td, 1H, *J* = 8.2 and 6.3, H-4) 2.56 (ddd, 1H, *J* = 18.4, 12.9 and 9.2, H*H*-8), 2.41 (ddd, 1H, *J* = 18.4, 8.7 and 1.8, *H*H-8), 2.03-1.90 (m, 4H, H_2-9, H*H*-5 and H*H*-3) 1.68 (dd, 1H, *J* = 12.5 and 5.5, *H*H-3), 1.41 (dd, 1H, *J* = 13.2 and 6.2, *H*H-5), 1.00 (s, 3H, H_3-11); δ_C (125 MHz, $CDCl_3$) 220.81 (C7), 91.13 (C10), 69.32 (C2), 49.57 (C6), 38.91 (C4), 37.28 (C9), 32.30 (C5), 31.70 (C3), 29.65 (C8), 14.04 (C11); IR (oil, cm^{-1}) 2955 (m), 2925 (m), 1731 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 30), 111 (100), 96 (46); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+ requires *m/z* 166.0988 Found 166.0986 (EI); UV (Acetonitrile) ε_{274} = 1700 $cm^{-1}M^{-1}$.

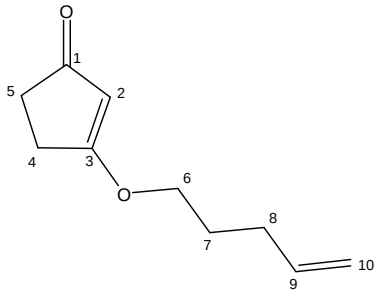
214. (4RS, 6RS, 7RS, 10SR)-6-Methyl-1-oxa-tricyclo[5.3.0.0^{4,10}]decan-7-ol



211 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL). The resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 105 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **214** as a colourless oil (121 mg, 0.72 mmol) in 72% yield.

δ_H (500 MHz, $CDCl_3$) 4.08-3.96 (m, 2H, H_2-2), 3.70 (dd, 1H, *J* = 9.5 and 6.3, H-7), 2.5 (br s, 1H, HO-7), 2.33 (dddd, 1H, *J* = 14.0, 8.8, 5.2 and 1.6, H-4), 2.16 (dd, 1H, *J* = 13.0 and 9.0, H*H*-5), 2.01-1.83 (m, 2H, CH*H* and H*H*-3), 1.67-1.49 (m, 4H, C*H*H, CH_2, *H*H-3), 1.05 (s, 3H, H_3-11), 0.95 (dd, 1H, *J* = 13.0 and 5.2, *H*H-5); δ_C (125 MHz, $CDCl_3$) 92.20 (C10), 79.21 (C7), 69.71 (C2), 46.25 (C6), 39.09 (C4) 32.44 (C8), 31.55 (C9), 30.64 (C3), 27.20 (C5), 20.17 (C11); IR (oil, cm^{-1}) 3371 (br), 2948 (m), 2864 (m); MS (EI) *m*/z (relative intensity): 168 (M+, 47), 150 (100), 135 (60); Exact Mass Calcd for $[C_{10}H_{16}O_2]$+ requires *m/z* 168.1145 Found 168.1155 (EI).

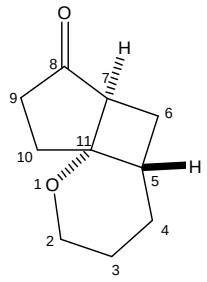
215. 3-(Pent-4-enyloxy)-cyclopent-2-enone



To a stirring dispersion of 1,3-cyclopentanedione (0.750 g, 7.65 mmol) in toluene (10 mL) was added 4-penten-1-ol (2.37 mL, 23.0 mmol). *p*-Toluene sulfonic acid (75.0 mg, 0.395 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **215** as a yellow oil (1.12 g, 6.75 mmol) in 88% yield.

δ_H (300 MHz, $CDCl_3$) 5.78 (ddt, 1H, *J* = 17.0, 10.1 and 6.7, H-9), 5.26 (t, 1H, *J* = 1.1, H-2), 5.06-4.97 (m, 2H, H_2-10), 3.96 (t, 2H, *J* = 6.4, H_2-6), 2.62-2.57 (m, 2H, H_2-4), 2.41 (app td, 2H, *J* = 4.8 and 1.1, H_2-5), 2.17 (td, 2H, *J* = 7.5 and 6.7, H_2-8), 1.84 (tt, 2H, *J* = 7.5 and 6.4, H_2-7); δ_C (75 MHz, $CDCl_3$) 205.9 (C1), 190.2 (C3), 137.0 (C2), 115.7 (C10), 104.7 (C9), 71.1 (C6), 34.0 (CH_2), 29.8 (CH_2), 28.5 (CH_2), 27.7 (CH_2); IR (oil, cm^{-1}) 2925 (w), 1702 (s), 1588 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 100), 119 (65), 111 (92); Exact Mass Calcd for $[C_{10}H_{14}O_2]$+ requires *m/z* 166.0988: Found 166.0990 (EI); UV (Acetonitrile) ε_{234} = 19800 and ε_{274} = 6500 $cm^{-1}M^{-1}$.

216. (**5RS, 7RS, 11RS)-1-Oxa-tricyclo[6.3.0.0^{5,11}]undecan-8-one**

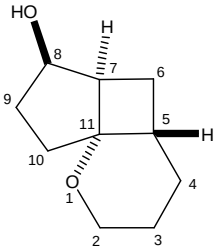


215 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to immediate irradiation in pyrex glassware for 2 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in

petroleum ether (1% NEt_3)) afforded **216** as a colourless oil (43 mg, 0.27 mmol) in 27% yield.

δ_H (500 MHz, $CDCl_3$) 3.77-3.73 (m, 1H, H*H*-2), 3.60-3.56 (m, 1H, *H*H-2), 3.13 (dd, 1H, *J* = 9.8 and 6.9, H-7), 2.56-2.43 (m, 2H, H_2-4), 2.27-2.21 (m, 2H, H-5 and CH*H*), 2.14-2.06 (m, 2H, H_2-3), 1.85 (ddd, 1H, *J* = 11.8, 9.8 and 3.3, H*H*-6) 1.74 (ddd, 1H, *J* = 11.8, 6.9 and 6.0, *H*H-6) 1.58-1.44 (m, 3H, C*H*H and CH_2); δ_C (125 MHz, $CDCl_3$) 219.14 (C8), 79.25 (C11), 63.81 (C2), 48.69 (C7), 38.49 (C6), 37.36 (C5), 34.99 (C3), 27.80 (C4), 25.25 (CH_2), 22.82 (CH_2); IR (oil, cm^{-1}) 2935 (m), 2856 (w), 1730 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 30), 111 (100), 96 (46); Exact Mass Calcd for [$C_{10}H_{14}O_2$]+ requires *m/z* 166.0988 Found 166.0986 (EI); UV (Acetonitrile) ε_{216} = 293 and ε_{278} = 34 $cm^{-1}M^{-1}$.

217. (5RS, 7SR, 8SR, 11RS)-1-Oxa-tricyclo[6.3.0.0^{5,11}]undecan-8-ol

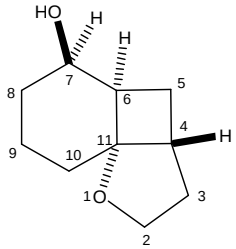


215 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 4 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **217** as a colourless oil (76 mg, 0.46 mmol) in 46% yield.

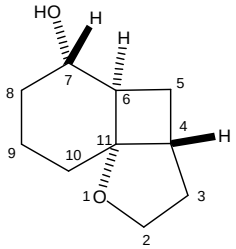
δ_H (500 MHz, $CDCl_3$) 4.34 (app dt, 1H, *J* = 10.1 and 6.6, H-8), 3.76-3.68 (m, 2H, H_2-2), 2.93 (app dt, 1H, *J* = 10.1 and 6.2, H-7), 2.31 (br s, 1H, HO-8), 2.09 (ddd, 1H, *J* = 14.8, 8.0 and 4.3, H-5), 2.05-1.96 (m, 2H, H*H*-3 and H*H*-9), 1.87-1.77 (m, 2H, *H*H-6 and *H*H-10), 1.72 (ddd, 1H, *J* = 13.0, 9.6 and 6.6, *H*H-9), 1.66-1.56 (m, 2H, H*H*-10 and H*H*-4) 1.51-1.41 (m, 2H, *H*H-4 and *H*H-3), 1.37 (ddd, 1H, *J* = 9.6, 8.0 and 6.2, H*H*-6); δ_C (125 MHz, $CDCl_3$) 81.66 (C11), 73.49 (C8), 63.46 (C2), 44.83 (C7), 36.96 (C5) 36.47 (C10), 31.56 (C9), 27.75 (C3), 21.96 (C4), 20.85 (C6); IR (oil, cm^{-1}) 3402 (br), 2958

(m), 2862 (m); MS (EI) *m*/z (relative intensity): 168 (M+, 10), 111 (85), 98 (100); Exact Mass Calcd for $[C_{10}H_{16}O_2]$+ requires *m/z* 168.1145 Found 168.1147 (EI).

218. a(4RS, 6RS, 7RS, 11SR)- and b(4RS, 6RS, 7SR, 11SR)-1-Oxa-tricyclo[5.4.0.0^{4,11}]undecan-7-ol



218a

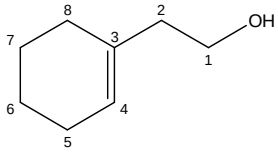


218b

150 (166 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 4 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **218** as a colourless oil (100 mg, 0.59 mmol) in 59% yield, a mixture of inseparable diastereomers **(7RS) 218a** and **(7SR) 218b**. NMR investigations indicated that the mixture was a 2:1 ratio of **218a:218b**. Signals are tentatively assigned as the **(7RS) (a)** and **(7SR) (b)** from comparison with *J* values of similar structures.

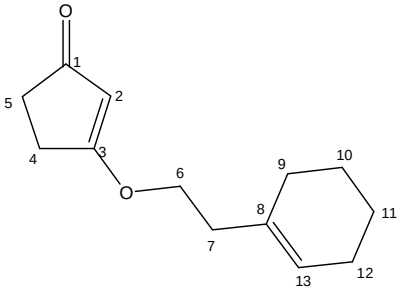
δ_H (500 MHz, $CDCl_3$) 4.15 (app td, 1H, *J* = 7.9 and 1.9, H*H*-2_b), 4.10 (app td, 1H, *J* = 8.0 and 2.11, H*H*-2_a), 4.06-3.98 (m, 3H, *H*H-2_a, H-7_a and *H*H-2_b), 3.72 (ddd, 1H, *J* = 10.2, 6.5 and 4.0, H-7_b), 2.65 (app td, 1H, *J* = 7.2 and 6.5, H-6_b), 2.61 (app td, 1H, *J* = 9.0 and 7.0, H-6_a), 2.50 (app td, 1H, *J* = 8.6 and 3.9, H-4_a), 2.07-1.37 (m, 21H from $10H_a$ and $11H_b$); δ_C (125 MHz, $CDCl_3$) 87.90 ($C11_b$), 86.97 ($C11_a$), 75.58 ($C7_b$), 67.66 ($C2_b$), 67.51 ($C7_a$), 67.45 ($C2_a$), 46.69 ($C6_b$), 42.70 ($C6_a$), 40.25 ($C4_a$), 40.06 ($C4_b$), 32.19 ($C3_a$), 31.64 ($C3_b$), 30.54 ($CH_{2\text{-}b}$), 30.52 ($CH_{2\text{-}b}$), 28.84 ($CH_{2\text{-}a}$), 27.36 ($CH_{2\text{-}a}$), 26.62 ($CH_{2\text{-}b}$), 19.68 ($CH_{2\text{-}b}$), 18.75 ($CH_{2\text{-}a}$), 18.54 ($CH_{2\text{-}a}$); IR (oil, cm^{-1}) 3381 (br), 2936 (m), 2860 (m); MS (CI+) *m*/z (relative intensity): 169 ([M+H], 17), 151 (100); Exact Mass Calcd for $[C_{10}H_{16}O_2]$+H requires *m/z* 169.1229 Found 169.1221 (CI+).

219. 2-Cyclohex-1-enyl-ethanol[339]



2-Cyclohex-1-enyl-ethanol **219** was synthesised and isolated according to literature methods[339] and afforded as an orange oil (1.95 g, 0.015 mol) in 87% yield. δ_H (300 MHz, $CDCl_3$) 5.54-5.53 (br m, 1H, H-4), 3.67-3.64 (br m, 2H, H_2-1), 2.20 (t, 2H, *J* = 6.2, H_2-2), 2.10-1.96, (m, 2H, CH_2), 1.94-1.86 (m, 2H, CH_2), 1.65-1.54 (m, 4H, 2 x CH_2); δ_C (75 MHz, $CDCl_3$) 134.08 (C3), 124.42 (C4), 60.20 (C1), 41.20 (C2), 28.07 (C5), 25.33 (C6), 22.92 (C7), 22.42 (C8); IR (oil, cm^{-1}) 3342 (w), 2924 (m), 1670 (w); MS (EI) *m*/z (relative intensity): 126 (M+, 5), 93 (35), 79 (100); Exact Mass Calcd for $[C_8H_{14}O]$+ requires *m/z* 126.1039 Found 126.1046 (EI).

220. 3-(2-Cyclohex-1-enyl-ethoxy)-cyclopent-2-enone

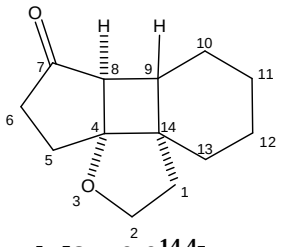


To 1,3-cyclopentanedione (300 mg, 3.06 mmol) in toluene (40 mL) was added known 2-cyclohex-1-enyl-ethanol[339] (1.16 g, 9.18 mmol). *p*-Toluene sulfonic acid (60.0 mg, 0.316 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **220** as a bright orange oil (0.550 g, 2.69 mmol) in 88% yield.

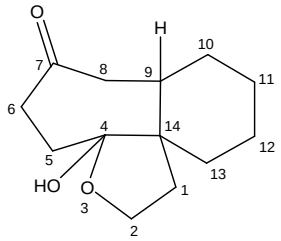
δ_H (500 MHz, $CDCl_3$) 5.46 (br s, 1H, H-13), 5.26 (s, 1H, H-2), 4.00 (t, 2H, *J* = 6.8, H_2-6), 2.57 (t, 2H, *J* = 6.8, H_2-7), 2.43-2.32 (m, 4H, H_2-4 and H_2-5), 2.01-1.87 (m, 4H, H_2-9 and H_2-12), 1.65-1.47 (m, 4H, H_2-10 and H_2-11); δ_C (125 MHz, $CDCl_3$) 206.10 (C1), 190.28 (C3), 133.10 (C8), 124.11 (C13), 104.82 (C2), 70.74 (C6), 36.84 (CH_2), 34.06 (CH_2), 28.64 (CH_2), 28.62 (CH_2), 25.29 (CH_2), 22.86 (CH_2), 22.25 (CH_2); IR (oil, cm^{-1}) 2925 (m), 1705 (m), 1590 (s); MS (CI+) *m*/z (relative intensity): 207 ([M+H], 100);

Exact Mass Calcd for $[C_{13}H_{18}O_2]$+H requires *m/z* 207.1385 Found 207.1388 (CI+); UV (Acetonitrile) ε_{232} = 21800 $cm^{-1}M^{-1}$.

221. (4RS, 8SR, 14SR)-3-Oxa-tetracyclo[8.4.0.0^{4,8}.0^{4,14}]tetradecan-7-one (One diastereomer but stereochemistry at 9 is not defined)



and 223. 4-Hydroxy-3-oxa-tricyclo[8.4.0.0^{14,4}]tetradecan-7-one (one diastereomer of undefined stereochemistry)



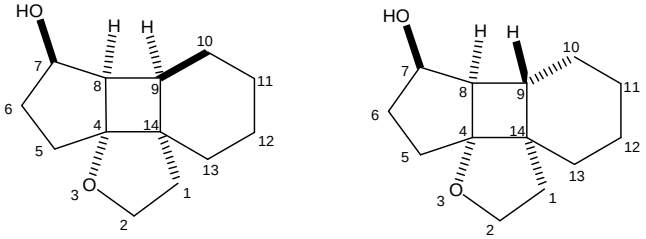
220 (206 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 hours with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetete in petroleum ether (1% NEt_3)) afforded tetracycle **221** as a colourless oil (26 mg, 0.13 mmol) in 13% yield, and tricycle **223** as a pale yellow paste (27 mg, 0.13 mmol) in 13% yield.

221. δ_H (500 MHz, $CDCl_3$) 4.17 (app t, 1H, *J* = 9.1, H*H*-2), 3.89 (ddd, 1H, *J* = 11.6, 9.1 and 5.4, *H*H-2), 2.71 (dd, 1H, *J* = 10.8 and 2.2, H-8), 2.57 (dddd, 1H, *J* = 19.5, 11.3, 2.7 and 2.2, H*H*-6), 2.45 (ddd, 1H, *J* = 19.5, 10.6 and 9.6, *H*H-6) 2.23 (ddd, 1H, *J* = 14.0, 9.6 and 2.8, H*H*-5), 2.15 (app td, 1H, *J* = 10.6 and 7.4, H-9), 2.07 (app dt, 1H, *J* = 14.0 and 10.9, *H*H-5), 1.95 (dd, 1H, *J* = 12.2 and 5.2, *H*H-1), 1.84 (m, 1H, H*H*-12) 1.70-1.62 (m, 2H, *H*H-11 and H*H*-1), 1.60-1.50 (m, 2H, H*H*-10 and H*H*-13), 1.44-1.33 (m, 2H, *H*H-12 and H*H*-11), 1.31-1.17 (m, 2H, *H*H-10 and *H*H-13); δ_C (125 MHz, $CDCl_3$) 219.03 (C7), 90.72 (C4), 67.27 (C2), 52.15 (C8), 48.98 (C14) 40.70 (C6), 30.59 (C1), 36.02 (C9), 27.12 (C5), 26.13 (C12), 21.26 (CH_2), 19.71 (CH_2), 18.64 (CH_2); IR (oil, cm^{-1}) 2934 (m), 1724 (s); MS (CI+) *m*/z (relative intensity): 207 ([M+H], 100); Exact

Mass Calcd for $[C_{13}H_{18}O_2]$+H requires *m/z* 207.1389 Found 207.1389 (CI+); UV (Acetonitrile) ε_{230} = 750 and ε_{294} = 49 $cm^{-1}M^{-1}$.

223. δ_H (500 MHz, $CDCl_3$) 3.97 (ddd, 1H, *J* = 10.1, 8.8 and 6.0, H*H*-2), 3.75 (ddd, 1H, *J* = 9.4, 8.8 and 6.0, *H*H-2), 2.51 (m, 2H, H_2-6), 2.34 (dd, 1H, *J* = 15.4 and 7.7, *H*H-8), 2.17 (dd, 1H, *J* = 18.0 and 12.6, H*H*-5), 1.98 (dd, 1H, *J* = 18.0 and 2.2, *H*H-5), 1.95-1.56 (m, 5H, H-9, H_2-1, H*H*-8 and CH*H*), 1.75-1.70 (m, 2H, 2 x CH*H*), 1.46 (d, 1H, *J* = 13.3, CH*H*), 1.31-1.15 (m, 4H, 4 x C*H*H); δ_C (125 MHz, $CDCl_3$) 209.59 (C7), 107.46 (C4), 64.93 (C2), 51.99 (C14), 46.89 (C5), 38.22 (C9), 37.85 (C6), 32.96 (CH_2), 30.58 (CH_2), 30.34 (CH_2), 29.51 (CH_2), 26.26 (CH_2), 23.66 (CH_2); IR (oil, cm^{-1}) 3392 (br), 2926 (s), 1693 (s); MS (CI+) *m*/z (relative intensity): 225 ([M+H], 56), 207 (100); Exact Mass Calcd for $[C_{13}H_{20}O_3]$+H requires *m/z* 225.1491 Found 225.1495 (CI+); UV (Acetonitrile) ε_{230} = 750 and ε_{294} = 49 $cm^{-1}M^{-1}$.

224. 1:1 mixture of diastereomers (4RS, 7RS, 8RS, 9RS, 14SR)- and (4RS, 7RS, 8RS, 9SR, 14SR)-3-Oxa-tetracyclo[9.3.0.0^{4,8}.0^{4,14}]tetradecan-7-ol



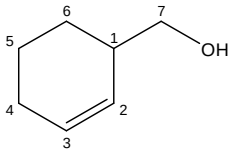
220 (206 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol) and immediate irradiation in pyrex glassware for 3 hours with stirring. Lithium borohydride (22 mg, 1 mmol) was added and irradiation continued for two hours. The dispersion was filtered and the solid washed with 2 M hydrochloric acid (aq.) (5 mL). The filtrate was taken and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **224** as a colourless oil (150 mg, 0.72 mmol) in 72% yield. NMR investigations indicated that the mixture was a 1:1 ratio of diastereomers shown.

N.B. Due to the complex nature of the diastereomers and overlapping NMR signals, peaks cannot be assigned to one diastereomer or the other and are merely labelled as CH

or CH_2. Where notation 'a' and 'b' is used, signals can be determined as arising from different structures but not from which diastereomer. It was proposed that the two diastereomers are epimers at carbon-9 by the notable shift of the 9-H proton and the adoptable configurations of the structure.

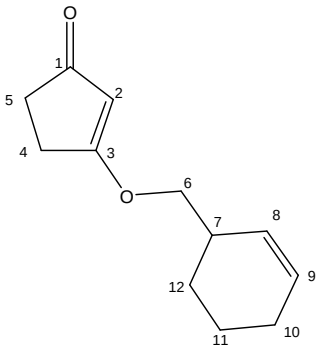
δ_H (500 MHz, $CDCl_3$) 4.41-4.31 (m, 2H, (H-7)$_a$ and (H-7)$_b$), 4.11(app t, 1H, *J* = 7.9, (*H*H-2)$_a$), 3.93 (app td, 1H, *J* = 8.2 and 6.4, (*H*H-2)$_b$), 3.89-3.79 (m, 2H, (H*H*-2)$_a$ and (H*H*-2)$_b$), 2.69-2.60 (m, 2H, H-8$_a$ and H-8$_b$), 2.28-2.13 (m, 3H), 2.13-2.04 (m, 1H), 2.04-1.94 (m, 1H), 1.91-1.85 (m, 5H, contains H-9$_a$ by COSY), 1.84-1.73 (m, 5H), 1.72-1.64 (m, 4H), 1.61-1.50 (m, 6H, contains H-9$_b$ by COSY), 1.44-1.38 (m, 3H), 1.23-1.13 (m, 1H), 1.07 (ddd, 1H, *J* = 13.2, 5.5 and 2.3); δ_C (125 MHz, $CDCl_3$) 96.17 (C4$_a$), 92.58 (C4$_b$), 74.92 (C7$_a$), 72.88 (C7$_b$), 67.31 (C2$_a$), 66.95 (C2$_b$), 53.61 (C8$_a$), 51.38 (C14$_a$), 47.36 (C14$_b$), 46.00 (C8$_b$), 41.10 (CH_2), 36.65 (CH), 35.00 (CH_2), 34.23 (CH_2), 34.01 (CH), 33.94 (CH_2), 28.17 (CH_2), 26.88 (CH_2), 26.55 (CH_2), 26.07 (CH_2), 22.92 (CH_2), 22.88 (CH_2), 21.19 (CH_2), 21.12 (CH_2), 19.55 (CH_2), 17.37 (CH_2); IR (oil, cm^{-1}) 3371 (br), 2929 (s), 2858 (m); MS (EI) *m*/z (relative intensity): 208 (M+, 7), 109 (94), 108 (100); Exact Mass Calcd for $[C_{13}H_{20}O_2]$+ requires *m/z* 208.1458 Found 208.1451 (EI).

226. Cyclohex-2-enyl-methanol[246]



Cyclohex-2-enyl-methanol **226** was synthesised and isolated according to literature methods[246] and afforded as a colourless oil (1.60 g, 7.77 mol) in 90% yield. δ_H (300 MHz, $CDCl_3$) 5.82 (dt, 1H, *J* = 9.6 and 3.3, H-3), 5.59 (dd, 1H, *J* = 9.6 and 2.2, H-2), 3.57-3.53 (m, 2H, H_2-7), 2.36-2.26 (m, 1H, H-1), 2.03-1.97 (m, 2H, H_2-4), 1.83-1.69 (m, 2H, CH_2), 1.61-1.31 (m, 2H, CH_2); δ_C (75 MHz, $CDCl_3$) 129.7 (C3), 127.7 (C2), 67.1 (C7), 38.23 (C1), 25.51 (CH_2), 25.28 (CH_2), 20.96 (CH_2); IR (oil, cm^{-1}) 3312 (m), 2922 (m), 2859 (m), 1702 (w); MS (EI) *m*/z (relative intensity): 112 ([M+], 10), 95 (30), 94 (100); Exact Mass Calcd for $[C_7H_{12}O]$+ requires *m/z* 112.0883 Found 112.0886 (EI);

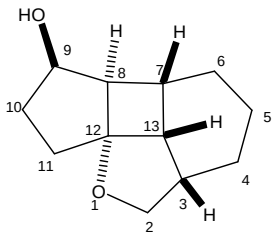
227. 3-(Cyclohex-2-enylmethoxy)-cyclopent-2-enone



To a stirring dispersion of 1,3-cyclopentanedione (400 mg, 4.08 mmol) in toluene (40 mL) was added cyclohex-2-enyl-methanol (1.14 g, 10.2 mmol). *p*-Toluene sulfonic acid (40 mg, 0.211 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **227** as a yellow oil (0.710 g, 3.69 mmol) in 91% yield.

δ_H (500 MHz, $CDCl_3$) 5.84-5.82 (m, 1H, H-9), 5.57 (ddt, 1H, *J* = 10.2, 2.3 and 1.7, H-8), 5.28 (s, 1H, H-2), 3.83 (d, 2H, *J* = 7.0, H_2-6), 2.62-2.60 (m, 3H, H-7 and H_2-10), 2.44-2.42 (m, 2H, H_2-4), 2.03-2.01 (m, 2H, CH_2), 1.86-1.69 (m, 2H, CH_2), 1.62-1.37 (m, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 206.11 (C1), 190.40 (C3), 130.23 (C9), 126.29 (C8), 104.86 (C2), 75.56 (C6), 34.93 (C7), 34.06 (C4), 28.56 (C5), 25.58 (CH_2), 25.18 (CH_2), 20.55 (CH_2); IR (oil, cm^{-1}) 2926 (m), 1702 (m), 1584 (s); MS (CI+) *m*/z (relative intensity): 193 ([M+H], 100), 127 (37), 95 (43); Exact Mass Calcd for $[C_{12}H_{16}O_2]$+H requires *m/z* 193.1229 Found 193.1225 (CI+); UV (Acetonitrile) ε_{232} = 29700 and ε_{280} = 1480 $cm^{-1}M^{-1}$.

229. (3RS, 7SR, 8SR, 9SR, 12RS, 13SR)-1-Oxa-tetracyclo[6.4.1.$0^{8,12}$.$0^{12,13}$]tridecan-9-ol

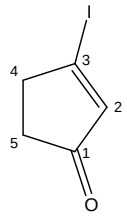


227 (192 mg, 1 mmol) was dissolved in acetonitrile (50 mL) and the resulting solution was degassed for 30 minutes prior to addition of lithium borohydride (44 mg, 2 mmol)

and immediate irradiation in pyrex glassware for 3 hours with stirring. Lithium borohydride (22 mg, 1 mmol) was added at this stage and irradiation continued for two hours. The dispersion was filtered and the solid washed with 2 M hydrochloric acid (aq.) (5 mL). The filtrate was taken and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in petroleum ether (1% NEt_3) to 50% ethyl acetate in petroleum ether (1% NEt_3)) afforded **229** as a colourless oil (46 mg, 0.24 mmol) in 24% yield.

δ_H (500 MHz, $CDCl_3$) 4.34 (app dt, 1H, *J* = 13.4 and 7.1, H-9), 4.00 (dd, 1H, *J* = 9.0 and 8.8, *H*H-2), 3.71 (dd, 1H, *J* = 9.5 and 9.0, H*H*-2), 2.58-2.50 (m, 1H, H-3), 2.48 (t, 1H, *J* = 7.2, H-8), 2.36 (dd, 1H, *J* = 8.7 and 8.0, H-13), 2.14 (app ddt, 1H, *J* = 8.0, 7.6 and 4.0, H-7), 2.05-2.00 (m, 1H, H*H*-10), 1.84-1.75 (m, 3H, H_2-11 and *H*H-10), 1.68-1.58 (m, 2H, H_2-5), 1.51-1.36 (m, 4H, H_2-6 and H_2-4); δ_C (125 MHz, $CDCl_3$) 89.58 (C12), 73.41 (C9), 71.43 (C2), 47.21 (C8), 41.81 (C13), 33.74 (C3), 31.72 (C10), 31.29 (C11), 26.26 (CH_2), 22.91 (CH_2), 19.81 (C7), 15.36 (CH_2); IR (oil, cm^{-1}) 3384 (br), 2929 (s); MS (EI) *m*/z (relative intensity): 194 (M+, 13), 176 (23), 150 (25), 95 (100); Exact Mass Calcd for $[C_{12}H_{18}O_2]$+ requires *m/z* 194.1301 Found 194.1309 (EI).

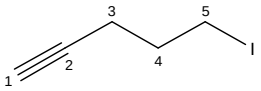
237. 3-Iodo-cyclopent-2-enone[47, 251]



To a solution of triphenylphosphine (0.75 g, 2.87 mmol) in dry acetonitrile (30 mL), iodine (0.73 mg, 2.87 mmol) was added in one portion. The yellow dispersion was stirred for two hours at room temperature and then 1,3-cyclopentanedione (250 mg, 2.55 mmol) added in one portion, immediately followed by triethylamine (400 μL, 2.87 mmol). The yellow dispersion was heated at reflux for 4 hours. Approximately half of the solvent was removed *in vacuo* and diethyl ether (50 mL) added. The brown precipitate was removed *via* filtration and purification of this solid by flash chromatography (50% ethyl acetate in petroleum ether) afforded **237** as a yellow solid that darkens on standing (440 mg, 2.12 mmol) in 81% yield. ^{1}H NMR shows degradation after one week.

δ_H (300Hz, MeOD) 6.68 (s, 1H, H-2), 3.11-3.07 (m, 2H, CH_2), 2.49-2.46 (m, 2H, CH_2); δ_C (75Hz, MeOD) 208.3 (C1), 144.2 (C2), 138.9 (C3), 43.0 (CH_2), 38.4 (CH_2); IR (solid, cm^{-1}) 3066 (m), 1691 (s); MS (EI) *m/z* (relative intensity): 208 (M+, 25), 81 (100); Exact Mass Calcd for $[C_5H_5IO]$+ requires *m/z* 207.9380 Found 207.9375 (EI).

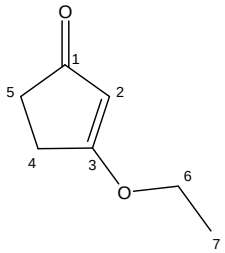
238. 5-Iodo-pent-1-yne[340]



To a stirred solution of triphenylphosphine (1.22 g, 4.65 mmol) in dichloromethane (15 mL) were added in quick succession, imidazole (316 mg, 4.65 mmol) and iodine (1.18 g, 4.65 mmol). The dispersion was stirred at room temperature for 30 minutes and then 4-pentyn-1-ol (370 µL, 4 mmol) added, whereupon the dispersion was stirred for 3 hours. The solvent was removed *in vacuo* (caution: volatile product) to afford **238** as a colourless oil (650 mg, 3.35 mmol) in 72% yield.

δ_H (300 MHz, $CDCl_3$) 3.31 (t, 2H, *J* = 6.7, H_2-5), 2.34 (dt, 2H, *J* = 6.7 and 2.7, H_2-3), 2.00-1.91 (m, 3H, H_2-4 and H-1); δ_C (75 MHz, $CDCl_3$) 82.3 (C2), 69.5 (C1), 31.8 (C3), 19.5 (C4), 5.0 (C5); IR (oil, cm^{-1}) 3293 (m), 2908 (w), 1427 (m); MS (EI) *m/z* (relative intensity): 194 (M+, 100), 134 (80), 127 (65); Exact Mass Calcd for $[C_5H_7I]$+ requires *m/z* 193.9587 Found 193.9590 (EI).

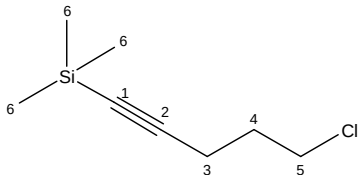
239. 3-Ethoxy-cyclopent-2-enone



To a stirring dispersion of 1,3-cyclopentanedione (500 mg, 5.10 mmol) in toluene (25 mL) was added ethanol (0.890 mL, 15.3 mmol). *p*-Toluene sulfonic acid (100 mg, 0.52 mmol) was added and the resulting yellow solution was refluxed under Dean-Stark conditions for 16 hours. Half of the solvent was removed in vacuo and the solution remaining was purified by flash chromatography (20% ethyl acetate in petroleum ether) affording **239** as a pale orange oil (460 mg, 3.71 mmol) in 77% yield.

δ_H (300 MHz, $CDCl_3$) 5.29 (s, 1H, H-2), 4.05 (q, 2H, *J* = 7.1, H_2-6), 2.61 (t, 2H, *J* = 5.0, CH_2), 2.44 (t, 2H, *J* = 5.0, CH_2), 1.41 (t, 3H, *J* = 7.1, H_3-7); δ_C (75 MHz, $CDCl_3$) 206.1 (C1), 190.2 (C3), 104.7 (C2), 67.7 (C6), 34.0 (CH_2), 28.6 (CH_2), 14.2 (C7); IR (oil, cm^{-1}) 2984 (w), 1674 (m), 1582 (s); MS (CI+) *m*/z (relative intensity): 127 ([M+H], 100), 113 (25), 99 (70); Exact Mass Calcd for $[C_7H_{10}O_2]$+H requires *m/z* 127.0759 Found 127.0760 (CI+); UV (Acetonitrile) ε_{210} = 18200, ε_{232} = 18600, ε_{263} = 1500 and ε_{291} = 300 $cm^{-1}M^{-1}$.

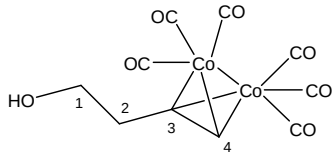
240. (5-Chloro-pent-1-ynyl)-trimethyl-silane[341]



To a stirred solution of 5-chloro-1-pentyne (1.00 g, 1.02 mL, 9.71 mmol) in dry tetrahydrofuran (10 mL) at -78 ºC under argon, was added ^{n}-butyl lithium (6.4 mL, 1.6 M in hexanes, 10.24 mmol) dropwise. After 30 minutes a solution of trimethyl silyl chloride (2.47 mL, 19.4 mmol) in tetrahydrofuran (10 mL) was added dropwise over 30 minutes. The colourless solution was allowed to come to room temperature over 2 hours. The reaction mixture was quenched with saturated ammonium chloride (aq.) (50 mL) and extracted with ether (2 x 50 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification by flash chromatography (petroleum ether) afforded **240** as a colourless volatile oil (0.97 g, 5.42 mmol) in 56% yield.

δ_H (300 MHz, $CDCl_3$) 3.65 (t, 2H, *J* = 6.7, H_2-5), 2.41 (t, 2H, *J* = 6.8, H_2-3), 1.96 (tt, 2H, *J* = 6.7 and 6.7, H_2-4), 0.15 (s, 9H, 3 x H_3-6); δ_C (75 MHz, $CDCl_3$) 105.17 (C2), 85.66 (C1), 43.61 (C5), 31.35 (C3), 17.32 (C4), 0.09 (3 x C6); IR (oil, cm^{-1}) 2922 (w), 1248 (m); Mass ion not found.

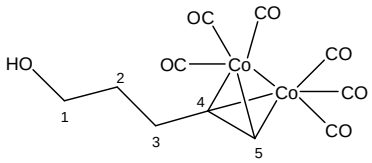
245. Cobalt hexacarbonyl(butyn-4-ol)dicobalt[253]



Cobalt hexacarbonyl(butyn-4-ol)dicobalt **245** was synthesised and isolated according to literature methods,[253] and afforded as a dark brown oil (4.50 g, 13.31 mol) in 89% yield.

δ_H (500 MHz, $CDCl_3$) 6.07 (br s, 1H, HO-1), 3.90 (br s, 2H, H_2-1), 3.11 (br s, 2H, H_2-2), 1.55 (br s, 1H, H-4); ^{13}C NMR could not be performed on this compound due to excessive broadening by cobalt; IR (oil, cm^{-1}) 3338 (m), 2924 (w), 1993 (m); No mass ion found.

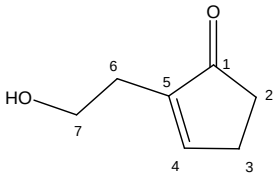
246. Cobalt hexacarbonyl(pentyn-5-ol)dicobalt



To a stirring black solution of cobalt octacarbonyl in dichloromethane (5 mL) at room temperature was added 1-pentyn-5-ol (285 μL, 3 mmol) in dichloromethane (1 mL) over 10 minutes and stirred for five hours. The solvent was removed *in vacuo* and purification by flash chromatography (10% ether in petroleum ether) afforded **246** as a dark red-brown oil (0.99 g, 2.82 mmol) in 94% yield.

δ_H (500 MHz, $CDCl_3$) 6.04 (br s, 1H, HO-1), 3.80 (br s, 2H, H_2-1), 2.98 (br, s, 2H, H_2-2), 1.92 (br s, 2H, H_2-3), 1.41 (br s, 1H, H-5); ^{13}C NMR could not be performed on this compound due to excessive broadening by the cobalt; IR (oil, cm^{-1}) 3315 (w), 2945 (w), 1984 (s); MS (EI) *m/*z (relative intensity): 370 (M+, 15), 343 (15), 314 (100), 287 (95); Exact Mass Calcd for $[C_{11}H_8Co_2O_7]$+ requires *m/z* 369.8929 Found 369.8938 (EI).

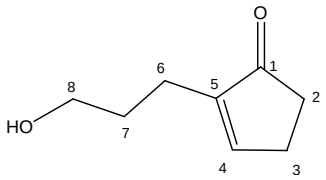
247. 5-(2-Hydroxy-ethyl)-cyclopent-2-enone[253]



5-(2-Hydroxy-ethyl)-cyclopent-2-enone **247** was synthesised and isolated according to literature methods,[253] and afforded as an orange oil (0.95 g, 7.5 mmol) in 74%. δ_H (500 MHz, $CDCl_3$) 7.44 (br s, 1H, H-4), 3.69 (t, 2H, *J* = 5.8, H_2-7), 2.97 (br s, 1H, HO-7), 2.59-2.57 (m, 2H, CH_2), 2.44 (t, 2H, *J* = 5.8, H_2-6), 2.40-2.38 (m, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 211.26 (C1), 160.58 (C4), 143.96 (C5), 61.28 (C7), 34.68 (CH_2), 29.31 (CH_2), 26.92 (CH_2); IR (oil, cm^{-1}) 3410 (m), 2922 (w), 1690 (s); MS (CI+) *m/*z (relative intensity): 127 ([M+H], 100), 109 (65), 97 (47); Exact Mass Calcd for $[C_7H_{10}O_2]$+H

requires *m/z* 127.0759 Found 127.0763 (CI+); UV (Acetonitrile) ε_{221} = 17000 and ε_{264} = 2550 $cm^{-1}M^{-1}$.

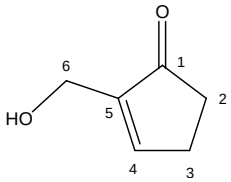
248. 5-(3-Hydroxy-propyl)-cyclopent-2-enone



To **246** (4.80 g, 13.64 mmol) in vinyl benzoate (25 mL) and water (5 mL) was added *N*-methyl morpholine-*N*-oxide (14.20 g, 121 mmol) in dichloromethane (50 mL) dropwise over one hour, whereupon the red/brown solution turned purple. The solution was stirred for 16 hours. The purple dispersion was filtered through a plug of silica and washed through with ether. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in ether to 10% ethanol in ethyl acetate) afforded **248** as a pale orange oil (0.99 g, 7.09 mmol) in 52% yield.

δ_H (500 MHz, $CDCl_3$) 7.37 (ddd, 1H, *J* = 3.9, 2.6 and 1.2, H-4), 3.59 (t, 2H, *J* = 6.2, H_2-8), 2.59-2.57 (m, 2H, CH_2), 2.43-2.41 (m, 2H, CH_2), 2.31-2.27 (m, 2H, CH_2), 2.12 (s, 1H, HO-8), 1.74-1.69 (m, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 210.87 (C1), 158.86 (C4), 145.92 (C5), 61.60 (C8), 34.61 (CH_2), 31.29 (CH_2), 26.66 (CH_2), 20.83 (CH_2); IR (oil, cm^{-1}) 3388 (m), 2929 (w), 1691 (s); MS (CI+) *m/*z (relative intensity): 141 ([M+H], 75), 123 (100); Exact Mass Calcd for $[C_8H_{12}O_2]$+H requires *m/z* 141.0916 Found 141.0916 (CI+); UV (Acetonitrile) ε_{222} = 16300 and ε_{374} = 2110 $cm^{-1}M^{-1}$.

257. 5-(Hydroxy-methyl)-cyclopent-2-enone[255]

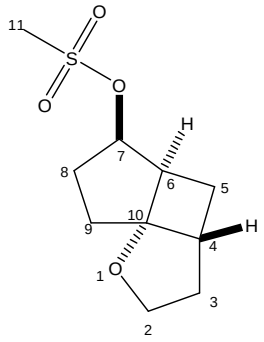


To a solution of 2-cyclopenten-1-one (1.00 g, 12 mmol) in chloroform (15 mL) and methanol (10 mL) was added 37% aqueous formaldehyde (1.2 mL). A solution of tributylphosphine was added to the reaction mixture and stirred at room temperature for 2 hours. Purification by flash chromatography (gradient elution in 20% ethyl acetate in

petroleum ether 33% ethyl acetate in petroleum ether) afforded **257** as a waxy paste (0.83 g, 7.4 mmol) in 63% yield.

δ_H (500 MHz, $CDCl_3$) 7.52 (ddd, 1H, *J* = 4.0, 2.7 and 1.4, H-4), 4.36-4.33 (m, 2H, H_2-6), 2.66 (t, 1H, *J* = 5.9, HO-6), 2.64-2.61 (m, 2H, CH_2), 2.44-2.42 (m, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 209.98 (C1), 159.00 (C4), 145.01 (C5), 57.70 (C6), 35.09 (CH_2), 26.94 (CH_2); IR (solid, cm^{-1}) 3355 (m), 2870 (w), 1664 (s); MS (CI+) *m/*z (relative intensity): 113 ([M+H], 100), 95 (32); Exact Mass Calcd for $[C_6H_8O_2]$+H requires *m/z* 113.0603 Found 113.0609 (CI+); UV (Acetonitrile) ε_{218} = 14700 and ε_{306} = 278 $cm^{-1}M^{-1}$.

270. (4RS, 6RS, 7RS, 10SR)-1-Oxa-7-methylsulfonatyl-tricyclo[5.3.0.0^{4,10}]decane



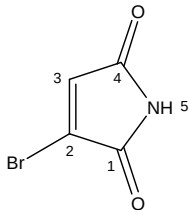
To an ice-cold solution of **202** (50 mg, 0.32 mmol) and triethylamine (107 µL, 0.77 mmol) in dichloromethane (1 mL) was added methane sulfonyl chloride (50 µL, 0.32 mmol) in dichloromethane (1 mL) in four equal portions. The solution was stirred at 0 ^oC for 30 minutes and allowed to come to room temperature. The reaction was diluted with water (5 mL) and then the layers separated. The organic layer was extracted and washed with water (2 x 5 mL), brine (10 mL) and dried over $MgSO_4$. Organic solvent was removed *in vacuo* to afford a pale yellow waxy solid **270** that melts on standing at room temperature (65 mg, 0.28 mmol) in 80% yield.

δ_H (500 MHz, $CDCl_3$) 5.02 (ddd, 1H, *J* = 10.6, 7.8 and 6.8, H-7), 4.14 (app t, 1H, *J* = 7.4, *H*H-2), 3.88 (ddd, 1H, *J* = 11.4, 7.4 and 5.5, H*H*-2), 2.95 (s, 3H, H_3-11), 2.73 (ddd, 1H, *J* = 9.2, 8.0 and 5.2, H-6), 2.51 (app td, 1H, *J* = 8.8 and 4.7, H-4), 2.30 (ddd, 1H, *J* = 12.5, 7.0 and 6.1, H*H*-8), 2.07 (ddd, 1H, *J* = 12.7, 10.8 and 7.3, *H*H-8), 2.00 (ddd, 1H, *J* = 13.8, 8.9 and 5.2, H*H*-5), 1.91 (dddd, 1H, *J* = 12.4, 11.2, 8.8 and 7.4, H*H*-3), 1.80 (app td, 1H, *J* = 12.5 and 7.0, H*H*-9), 1.76 (app td, 1H, *J* = 12.5 and 6.1, *H*H-9), 1.66

(dd, 1H, *J* = 12.5 and 5.4, *H*H-3), 1.42 (ddd, 1H, *J* = 13.8, 9.6 and 4.5, *H*H-5); δ_C (125 MHz, $CDCl_3$) 93.18 (C10), 81.26 (C7), 68.45 (C2), 51.76 (CH), 40.78 (CH), 38.20 (C11), 32.34 (CH_2), 31.30 (CH_2), 29.73 (CH_2), 18.41 (C5); IR (oil, cm^{-1}) 3388 (m), 2929 (w), 1691 (s); MS (CI+) *m/z* (relative intensity): 153 (M-CH_3SO_2, 30), 137 (100); Exact Mass Calcd for $[C_{10}H_{16}O_4S]$-CH_3SO_2 requires *m/z* 153.0910 Found 153.0915 (CI+).

3.iii.ii. Synthesised compounds for 2.ii.

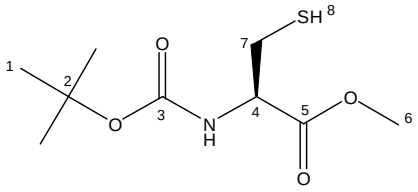
289. 3-Bromo-pyrrole-2,5-dione



To maleimide (2.00 g, 0.02 mol) in chloroform (15 mL) was added bromine (1.16 mL, 0.02 mol) in chloroform (15 mL) dropwise. The reaction mixture was refluxed for 2 hours and left to cool to room temperature over 1 hour. The solid yellow precipitate was filtered off and washed with cold chloroform (2 x 50 mL) to afford off -white crystals of crude 2,3-dibromosuccinimide (4.09 g, 0.016 mol). The crude succinimide was dissolved in tetrahydrofuran (50 mL) and triethylamine (2.4 mL, 0.017 mol) in tetrahydrofuran (10 mL) was added over 5 minutes at 0 ^oC. The reaction mixture was allowed to warm to room temperature and stirred for 48 hours. The solid was filtered off and washed with tetrahydrofuran (50 mL). Purification by flash chromatography (5% ethyl acetate in petroleum ether) afforded **289** as a pale yellow powder (2.14 g, 0.012 mol) in 59% yield.

δ_H (500 MHz, $CDCl_3$) 7.67 (br s, 1H, 5-H), 6.89 (s, 1H, H-3); δ_C (125 MHz, $CDCl_3$) 173.8 (C=O), 170.5 (C=O), 136.9 (C2), 135.4 (C3); IR (solid, cm^{-1}) 3235 (s), 1709 (s); MS (CI+) *m/z*, (relative intensity): 178 ([M+H], 32), 176 ([M+H], 32), 125 (25), 86 (100); Mass calcd for $[C_4H_2O_2N^{79}Br]$+H requires 175.9347 Found 175.9349 (CI+); m.p. 148-151 ^oC; UV (Acetonitrile) ε_{242} = 13800 and ε_{276} = 1700 $cm^{-1}M^{-1}$.

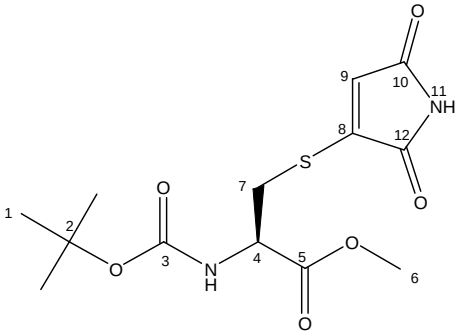
290. 2R-*tert*-Butoxycarbonylamino-3-mercapto-propionic acid methyl ester[2]



To a stirring solution of an aqueous buffer (20 mL, 150 mM NaCl, 100 mM NaH_2PO_4, pH 8.0) was added **291** (50 mg, 0.15 mmol) in *N*,*N*-dimethylformamide (2 mL). Tris(2-carboxyethyl)phosphine (430 mg 1.51 mmol) in an aqueous buffer (20 mL, 150 mM NaCl, 100 mM NaH_2PO_4, pH 8.0) was added to the solution. After 5 minutes the aqueous solution was extracted with ethyl acetate (3 x 25 mL), washed with saturated lithium chloride solution (5 x 25 mL), water (25 mL) and brine (25 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to afford a colourless oil (34.5 mg, 0.15 mmol) in 98% yield. NMR and α_D of this oil showed it to be the commercially available **290**.[2]

δ_H (500 MHz, $CDCl_3$) 5.40 (s, 1H, 3-NH), 4.61 (s, 1H, H-4), 3.78 (s, 3H, H_3-6), 3.01-2.92 (m, 2H, H_2-7), 1.45 (s, 9H, 3 x H_3-1), 1.39 (t, 1H, J = 8.9, H-8); δ_C (125 MHz, $CDCl_3$) 170.49 (C=O), 154.76 (C=O), 79.91 (C2), 54.51 (C4), 52.33 (C6), 27.94 (3 x C1), 26.97 (C7); $^{20}\alpha_D$: -23.6^o (c = 1.0, Methanol). Authentic sample $^{20}\alpha_D$: -23.4^o (c = 1.0, Methanol).

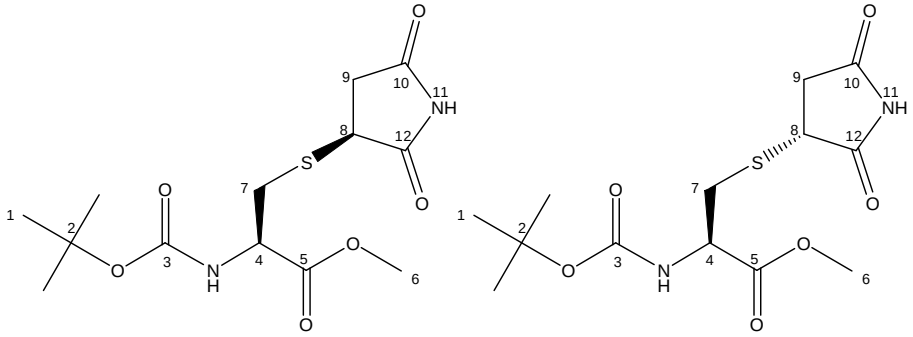
291. 2R-*tert*-Butoxycarbonylamino-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl)-propionic acid methyl ester



To a stirring solution of **290**(36 mg, 0.15 mmol) and sodium acetate (13 mg, 0.15 mmol) in methanol (3 mL) was added **289** (30 mg, 0.17 mmol) in methanol (3 mL). After 1 minute solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 50% ethyl acetate in petroleum ether to ethyl acetate) afforded a pale yellow powder **291** (51 mg, 0.15 mmol) in 100% yield.

δ_H (500 MHz, $CDCl_3$) 7.63 (s, 1H, 11-H), 6.27 (s, 1H, 9-H), 5.40 (d, 1H, *J* = 6.8, 3-NH), 4.67 (ddd, 1H, *J* = 6.8, 5.4 and 5.1, H-4), 3.80 (s, 3H, H_3-6), 3.48 (dd, 1H, *J* = 13.8 and 5.1, *H*H-7), 3.62 (dd, 1H, *J* = 14.1 and 5.4, H*H*-7) 1.45 (s, 9H, 3 x H_3-1); δ_C (125 MHz, $CDCl_3$) 170.2 (C=O), 168.9 (C=O), 167.6 (C=O), 155.2 (C=O), 155.9 (C8), 119.7 (C9), 81.1 (C2), 53.3 (C6), 52.7 (C4), 34.0 (C7), 28.3 (3 x C1); IR (solid, cm^{-1}) 3236 (w), 1715 (s); MS (CI+) *m/z*, (relative intensity): 331 ([M+H], 5), 275 (20), 231 (100); Mass calcd for $[C_{13}H_{18}O_6N_2S]$+H requires 331.0964 Found 331.0968 (CI+); $^{20}\alpha_D$: -41.9^o (c = 1.0, Methanol); Elemental analysis: Calc for $C_{13}H_{18}O_6N_2S$: C: 47.26, H: 5.49, N: 8.48 Found: C: 46.81, H: 5.56, N: 8.15. m.p. 145-147 ^oC; UV (Acetonitrile) ε_{245} = 14200 and ε_{339} = 8600 $cm^{-1}M^{-1}$.

292. 2R-*tert*-Butoxycarbonylamino-3-(2,5-dioxo-pyrrolidin-3S-ylsulfanyl)-propionic acid methyl ester and 2R-*tert*-butoxycarbonylamino-3-(2,5-dioxo-pyrrolidin-3R-ylsulfanyl)-propionic acid methyl ester

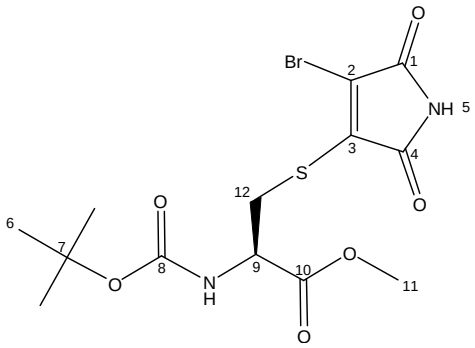


To a stirring solution of 2S-*tert*-butoxycarbonylamino-3-mercapto-propionic acid methyl ester [*N*-Boc-Cys-OMe] (36 mg, 0.15 mmol) in methanol (3 mL) was added maleimide (17 mg, 0.17 mmol) in methanol (3 mL). After 1 minute the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 1% methanol in dichloromethane to 30% methanol in dichloromethane) afforded **292** as a colourless oil (51 mg, 0.15 mmol) in 100% yield, a 1:1 mix of two inseparable diastereomers.

δ_H (500 MHz, $CDCl_3$) 9.00 (s, 1H, 11-H), 8.95 (s, 1H, 11-H), 5.59 (1H, d, *J* = 7.6, 3-NH), 5.41 (d, 1H, *J* = 7.6, 3-NH), 4.65-4.56 (m, 2H, 2 x H-4), 3.93 (dd, 1H, *J* = 4.3 and 3.9, H-8), 3.86 (dd , 1H, *J* = 9.2 and 4.2, H-8), 3.76 (s, 3H, H_3-6), 3.76 (s, 3H, H_3-6), 3.51 (dd, 1H, *J* = 13.8 and 6.6, *H*HC), 3.36 (dd, 1H, *J* = 14.1 and 6.0, *H*HC), 3.19-3.11

(m, 3H, 2 x CH*H* and C*H*H), 2.96 (dd, 1H, *J* = 7.1 and 13.1, C*H*H), 2.54-2.02 (m, 2H, 2 x CH*H*) 1.43 (s, 18H, 6 x H_3-1); δ_C (125 MHz, $CDCl_3$) 177.2 (C=O), 177.1 (C=O), 175.1 (C=O), 175.0 (C=O), 172.0 (C=O), 171.5 (C=O), 155.5 (C=O), 155.3 (C=O), 80.6 (2 x C2), 53.6 (CH), 52.91 (C6), 52.85 (C6), 50.8 (CH), 40.6 (CH), 40.0 (CH), 37.3 (CH_2), 37.0 (CH_2), 34.6 (CH_2), 34.1 (CH_2), 28.3 (6 x CH_3); IR (oil, cm^{-1}) 3233 (w), 2980 (w), 1783 (w), 1709 (s); MS (CI+) *m/z*, (relative intensity): 333 ([M+H], 15), 277 (50), 233 (100); Exact mass calcd for $[C_{13}H_{20}O_6N_2S]$+ requires 332.1042 Found 332.1048 (CI+).

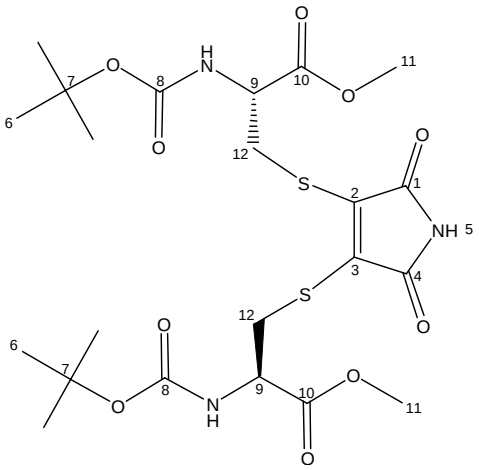
293. 3-(4-Bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl)-2R-*tert*-butoxycarbonylamino-propionic acid methyl ester



To 2,3-dibromomaleimide (200 mg, 0.78 mmol) and sodium acetate (32 mg, 0.39 mmol) in methanol (30 mL) was added *N*-Boc-Cys-OMe (92 mg, 0.39 mmol) in methanol (30 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **293** as a yellow waxy oil (140 mg, 0.34 mmol) in 87% yield.

δ_H (500 MHz, $CDCl_3$) 8.32 (s, 1H, 5-H), 5.36 (d, 1H, *J* = 7.6, 8-NH), 4.70-4.66 (m, 1H, H-9), 3.95 (dd, 1H, *J* = 14.2 and 4.5, H*H*-12), 3.77 (s, 3H, H_3-11), 3.68 (dd, 1H, *J* = 13.8 and 6.6, *H*H-12), 1.42 (s, 9H, 3 x H_3-6); δ_C (125 MHz, $CDCl_3$) 170.23 (C=O), 165.41 (C=O), 163.18 (C=O), 154.72 (C=O), 142.61 (C3), 120.37 (C2), 80.50 (C7), 53.11 (C9), 52.66 (C11), 32.81 (C12), 27.92 (3 x C6); IR (oil, cm^{-1}) 2979 (m), 1732 (s); No mass ion found; UV (Acetonitrile) ε_{242} = 12300 and ε_{367} = 6600 $cm^{-1}M^{-1}$.

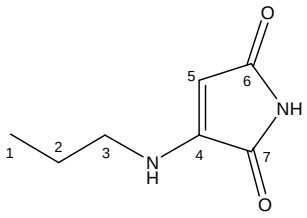
294. 2R-*tert*-Butoxycarbonylamino-3-[4-(2R-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanyl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl]-propionic acid methyl ester



To 2,3-dibromomaleimide (50 mg, 0.19 mmol) in aqueous buffer (100 mM sodium phosphate, 150 mM NaCl, pH 8.0):DMF, 95:5 (54 mL) was added *N*-Boc-Cys-OMe (91 mg, 0.38 mmol). The reaction was stirred until all the cysteine was dispersed by the solvent and after a further 5 minutes the aqueous reaction mixture was extracted with ethyl acetate (3 x 25 mL) and the combined organic layers washed with saturated lithium chloride solution (aq.) (5 x 25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution 20% ethyl acetate in petroleum ether to ethyl acetate) afforded **294** as a bright yellow sticky foam (108 mg, 0.19 mmol) in 100% yield. Carrying out this reaction in methanol afforded a 94% of **294**, with the same data observed.

δ_H (500 MHz, $CDCl_3$) 8.10 (s, 1H, H-5), 5.81 (d, 2H, *J* = 7.4, 2 x 8-NH), 4.69-4.66 (m, 2H, 2 x H-9), 3.81-3.75 (m, 2H, 2 x H*H*-12), 3.76 (s, 6H, 2 x H_3-11), 3.69 (dd, 2H, *J* = 14.3 and 5.2, 2 x *H*H-12), 1.42 (18H, s, 6 x H_3-6); δ_C (125 MHz, $CDCl_3$) 170.88 (2 x C=O), 166.02 (2 x C=O), 155.12 (2 x C=O), 137.05 (C2 and C3), 81.31 (2 x C7), 53.89 (2 x C9), 52.89 (2 x C11), 33.83 (2 x C12), 28.39 (6 x C6); IR (film, cm^{-1}) 3371 (w), 2978 (w), 1724 (s); MS (ES-) *m*/z (relative intensity): 562 ([M-H], 100); Exact Mass Calcd for $[C_{22}H_{33}N_3O_{10}S_2]$-H requires *m/z* 562.1529 Found 562.1532 (ES-); m.p. 75-77 ^oC; UV (Acetonitrile) ε_{210} = 17400, ε_{253} = 4200 and ε_{393} = 3000 $cm^{-1}M^{-1}$.

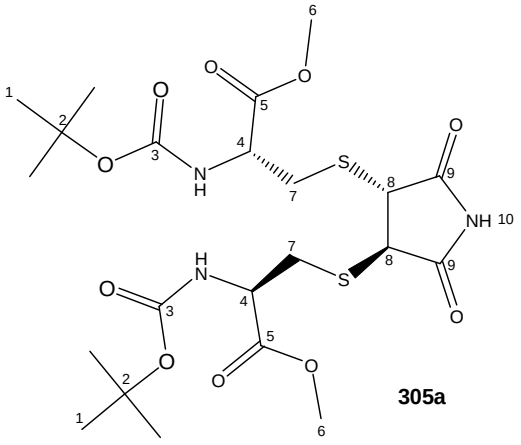
295. 3-Propylamino-pyrrole-2,5-dione



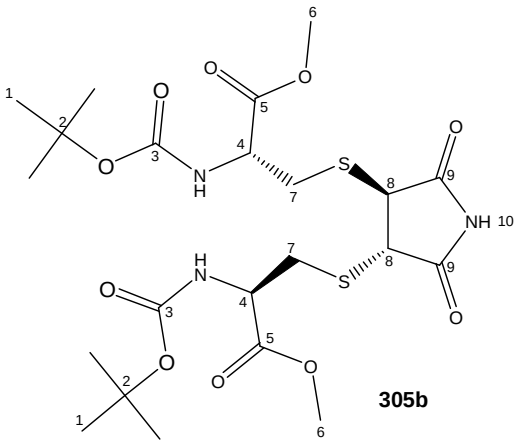
To propylamine (75 μL, 1.09 mmol) and sodium acetate (92 mg, 1.12 mmol) in methanol (15 mL) was added **289** (200 mg, 1.12 mmol) dropwise in methanol (15 mL). After 10 minutes, solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **295** as a bright yellow waxy solid (82 mg, 0.53 mmol) in 49% yield

δ_H (500 MHz, $CDCl_3$) 7.36 (s, 1H, 6-NH), 5.45 (br s, 1H, 3-NH), 4.80 (s, 1H, H-5), 3.14 (td, 2H, *J* = 7.2 and 6.2, H_2-3), 1.71-1.63 (m, 2H, H_2-2), 0.99 (t, 3H, *J* = 7.4, H_3-1); δ_C (125 MHz, $CDCl_3$) 172.31 (C=O), 167.73 (C=O), 149.83 (C4), 85.29 (C5), 46.16 (C3), 21.91 (C2), 11.42 (C1); IR (solid, cm^{-1}) 3190 (m), 2962 (m), 1693 (m), 1627 (s); MS (EI) *m*/z (relative intensity): 154 (M+, 60), 125 (98), 84 (100); Exact Mass Calcd for [$C_7H_{10}N_2O_2$]+ requires *m/z* 154.0737 Found 154.0734 (EI); m.p. 108-118 ^oC; UV (Acetonitrile) ε_{240} = 7400 and ε_{348} = 5700 $cm^{-1}M^{-1}$.

305a. 2R - *tert* - Butoxycarbonylamino – 3 - [4R - (2R – *tert*- butoxycarbonylamino-2-methoxycarbonyl - ethylsulfanyl) - 2,5 - dioxo-pyrrolidin-3R-ylsulfanyl]-propionic acid methyl ester and 305b. 2R-*tert*-butoxycarbonylamino-3-[4S-(2R-*tert*-butoxycarbonylamino - 2 – methoxycarbonyl - ethylsulfanyl) - 2,5 – dioxo – pyrrolidin - 3S - ylsulfanyl] - propionic acid methyl ester

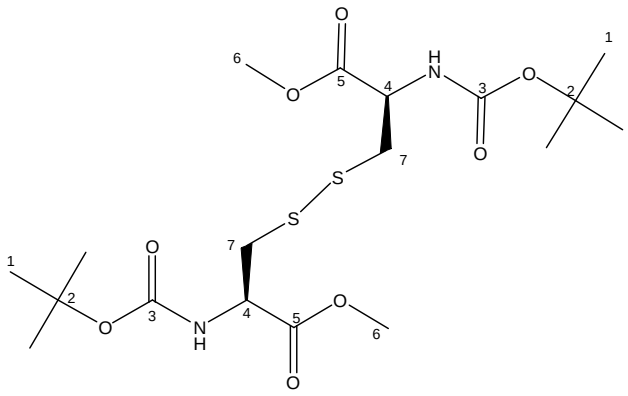


305a



305b

308. 2R – *tert* – Butoxycarbonylamino – 3 - (2R – *tert* – butoxycarbonylamino – 2 - methoxycarbonyl-ethyldisulfanyl)-propionic acid methyl ester



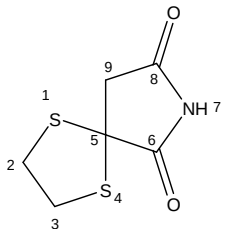
Method 1: To a stirred solution of **289** (50 mg, 0.28 mmol) in aqueous buffer (100 mM sodium phosphate, 150 mM NaCl, pH 8.0):DMF, 95:5 (9.25 mL) was added *N*-Boc-Cys-OMe (660 mg, 2.81 mmol) in DMF (0.25 mL). After 5 minutes the aqueous reaction mixture was extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with saturated lithium chloride solution (aq.) (5 x 25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10 ethyl acetate in petroleum ether to 40% ethyl acetate in petroleum ether) afforded **305** as a yellow oil (150 mg, 0.27 mmol) in 94% yield as an inseparable 1:1 mix of two symmetrical diastereomers. Small peaks seen in the ^{1}H NMR are tentatively assigned as the *cis* diastereomers.

Method 2: To 2,3-dibromomaleimide (100 mg, 0.56 mmol) in aqueous buffer (100 mM sodium phosphate, 150 mM NaCl, pH 8.0):DMF, 95:5 (20 mL) was added *N*-Boc-Cys-OMe (1.32 g, 5.6 mmol) in DMF (20 mL). After 5 minutes the aqueous reaction mixture was extracted with ethyl acetate (3 x 25 mL) and the combined organic layers washed with saturated lithium chloride solution (aq.) (5 x 25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 40% ethyl acetate in petroleum ether) afforded **305** as a yellow waxy oil (238 mg, 0.42 mmol) in 93% yield as an inseparable 1:1 mix of two symmetrical diastereomers (data matched that obtained above for **305**), and **308** as a thick colourless oil (49 mg, 0.10 mmol) in 4% yield.

305. δ_H (400 MHz, $CDCl_3$) 8.66 (s, 1H, 10-H), 8.62 (s, 1H, 10-H), 5.62 (d, 2H, *J* = 8.4, 2 x 3-NH), 5.51 (d, 2H, *J* = 8.0, 2 x 3-NH), 4.72-4.58 (m, 4 x H-4), 3.80 (s, 6H, 2 x H_3-6), 3.79 (s, 6H, 2 x H_3-6), 3.68 (s, 2H, 2 x H-8), 3.64 (s, 2H, 2 x H-8), 3.46 (dd, 2H, *J* = 14.0 and 4.8, 2 x *H*H-7_a), 3.37 (dd, 2H, *J* = 14.0 and 6.0, 2 x *H*H-7_b), 3.21 (dd, 2H, *J* = 14.0 and 4.8, 2 x H*H*-7_b), 3.11 (dd, 2H, *J* = 14.0 and 6.4, 2 x H*H*-7_a), 1.463 (s, 18H, 6 x H_3-1), 1.45 (s, 18H, 6 x H_3-1), $_a$-signals shown as part of the same CH_2 by HMQC data, $_b$-signals shown as part of the same CH_2 by HMQC data, they do not refer to structures **305a** and **305b**; δ_C (125 MHz, $CDCl_3$) 174.32 (4 x C=O), 171.25 (4 x C=O), 155.33 (4 x C=O), 80.61 (2 x C2), 80.58 (2 x C2), 53.51 (2 x C4), 53.18 (2 x C4), 52.91 (2 x C6), 52.90 (2 x C6), 48.45 (2 x C8), 47.89 (2 x C8), 34.66 (2 x C7), 34.59 (2 x C7), 28.37 (6 x C1), 28.36 (6 x C1); IR (oil) 3348, 2978, 1719 cm^{-1}; MS (EI) *m*/z (relative intensity): 566 ([M+H], 20), 564 ([M-H], 100); Exact mass calcd for $[C_{22}H_{35}N_3O_{10}S_2]$-H requires 564.1669 Found 564.1686;

308. δ_H (500 MHz, $CDCl_3$) 5.38 (d, 2H, 2 x NH), 4.61-4.57 (m, 2H, 2 x H-4), 3.75 (s, 6H, 2 x H_3-6), 3.01-2.93 (br m, 4H, 2 x H_2-7), 1.44 (s, 18H, 6 x H_3-1); δ_C (125 MHz, $CDCl_3$) 171.26 (2 x C=O), 155.13 (2 x C=O), 80.37 (2 x C2), 52.86 (2 x C4), 52.71 (2 x C6), 41.35 (2 x C7), 28.36 (6 x C1); IR (solid, cm^{-1}) 3363 (w), 2978 (w), 1696 (s); MS (CI+) *m*/z (relative intensity): 469 ([M+H], 100), 313 (60); Exact Mass Calcd for $[C_{18}H_{32}N_2O_8S_2]$+H requires *m/z* 469.1678 Found 469.1684 (CI+).

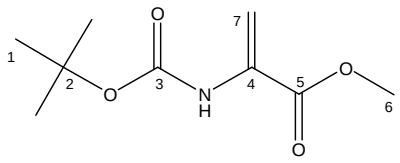
325. 1,4-Dithia-7-aza-spiro[4.4]nonane-6,8-dione



To **289** (30 mg, 0.17 mmol) and sodium acetate (14 mg, 0.17 mmol) in methanol (6 mL) was added 1,2-ethanedithiol (17 μl, 0.17 mmol). After five minutes the solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **325** as a pale yellow powder (13 mg, 0.07 mmol) in 41% yield.

δ_H (500 MHz, $CDCl_3$) 8.39 (s, 1H, 7-H), 3.75-3.69 (m, 2H, H*H*-2 and H*H*-3), 3.60-3.53 (m, 2H, *H*H-2 and *H*H-3), 3.30 (s, 2H, H_2-9); δ_C (125 MHz, $CDCl_3$) 177.93 (C=O), 172.76 (C=O), 61.23 (C5), 43.12 (C9), 41.05 (C2 and C3); IR (solid, cm^{-1}) 3290 (m), 1703 (m), 1629 (s); MS (ES-) *m*/z (relative intensity): 188 ([M-H], 100); Exact Mass Calcd for $[C_6H_7NO_2S_2]$-H requires *m/z* 187.9840 Found 187.9839 (ES-); m.p. 112-115 ^oC.

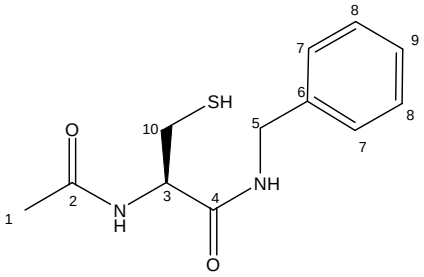
326. 2-*tert*-Butoxycarbonylamino-acrylic acid methyl ester



To a stirring solution of *N*-Boc-Cys-OMe (36 mg, 0.15 mmol) in methanol (3 mL) was added **289** (30 mg, 0.17 mmol) in methanol (3 mL). Potassium carbonate (71 mg, 0.51 mmol) was added after 1 minute and the solution immediately turned bright yellow. Over two hours the solution turned dark orange, at which time the reaction mixture was diluted with diethyl ether (25 mL). The cloudy yellow dispersion was washed with water, and the aqueous layer extracted with diethyl ether (3 x 50 mL). The combined organic extracts were washed with brine (150 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to afford **326** as a colourless oil (29 mg, 0.14 mmol) in 93% yield.

δ_H (500 MHz, $CDCl_3$) 7.00 (s, 1H, 3-NH), 6.15 (br s, 1H, H*H*-7), 5.72 (s, 1H, *H*H-7), 3.82 (s, 3H, H_3-6), 1.48 (s, 9H, 3 x H_3-1); δ_C (125 MHz, $CDCl_3$) 164.6 (C=O), 152.6 (C=O), 131.4 (C4), 105.3 (C7), 80.8 (C2), 52.9 (C6), 28.3 (3 x C1); IR (oil, cm^{-1}) 3423 (w), 2979 (w), 1717 (w); MS (EI) *m/z*, (relative intensity): 201 (M+, 23), 174 (40), 145 (100); Exact mass calcd for $[C_9H_{15}O_4N]$+ requires 201.0996 Found 201.0991 (EI).

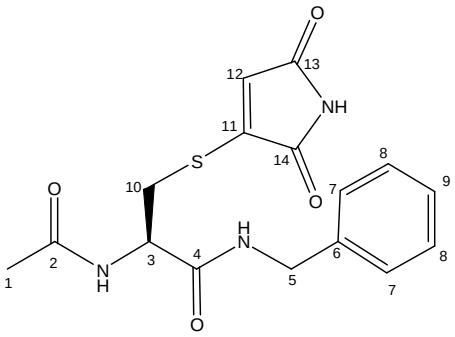
329. 2R-Acetylamino-*N*-benzyl-3-mercapto-propionamide



To 2S-acetylamino-3-mercapto-propionic acid (500 mg, 3.07 mmol) in dichloromethane (25 mL) and DMF (3 mL) at 0 ^{o}C, was added HOBt (456 mg, 3.38 mmol), benzylamine (0.50 mL, 4.60 mmol) and finally EDC (0.65 g, 3.38 mmol). The reaction was brought to room temperature and stirred for 16 hours. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in dichloromethane to 3% methanol in dichloromethane) afforded **329** as a white solid (434 mg, 1.72 mmol) in 56% yield.

δ_H (500 MHz, MeOD) 7.29-7.23 (m, 5H, 5 x Ar-H), 4.48 (dd, 1H, *J* = 7.1 and 5.8, H-3), 4.39 (s, 2H, H_2-5), 2.87 (dd, 1H, *J* = 13.9 and 5.9, *H*H-10), 2.79 (dd, 1H, *J* = 13.9 and 7.1, H*H*-10), 2.01 (s, 3H, H_3-1); δ_C (125 MHz, MeOD) 173.48 (C=O), 172.36 (C=O), 139.70 (C6), 129.49 (2 x Ar-H), 128.47 (2 x Ar-H), 128.19 (C9), 57.32 (C3), 44.11 (C5), 26.83 (C10), 22.46 (C1); IR (oil, cm^{-1}) 3284 (m), 1630 (w); MS (CI+) *m*/z (relative intensity): 253 ([M+H], 12), 211 (15), 146 (20), 108 (100); Exact Mass Calcd for $[C_{12}H_{16}NO_2S]$+H requires *m/z* 253.1011 Found 253.1017 (CI+); m.p. 164 – 166 ^{o}C.

330. 2R-Acetylamino-*N*-benzyl-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl)-propionamide

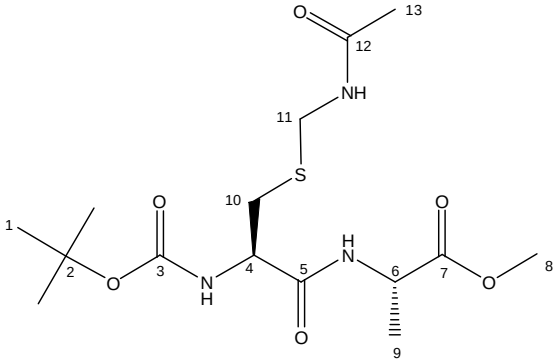


To **329** (1.00 g, 4.00 mmol) in methanol (42 mL), was added **289** (0.78 g, 4.37 mmol) in methanol (42 mL) dropwise over 5 minutes. After 10 minutes, the solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **330** as an off-white solid (429 mg, 1.2 mmol) in 30% yield and unreacted **289** (700 mg, 2.8 mmol) in 70%. Data matched that given above.

δ_H (500 MHz, MeOD) 7.32-7.20 (m, 5H, 5 x Ar-H), 6.45 (s, 1H, H-12), 4.71 (t, 1H, *J* = 7.3, H-3), 4.38 (d, 2H, *J* = 2.7, H_2-5), 3.40 (dd, 1H, *J* = 13.6 and 7.0, H*H*-10), 3.25 (dd, 1H, *J* = 13.6 and 7.0, *H*H-10), 1.99 (s, 3H, H_3-1); δ_C (125 MHz, MeOD) 173.51 (C=O),

172.22 (C=O), 171.44 (C=O), 170.51 (C=O), 151.58 (C11), 139.48 (C6), 129.54 (2 x Ar-H), 128.51 (2 x Ar-H), 128.26 (C9), 121.01 (C12) 53.04 (C3), 44.25 (C5), 33.72 (C10), 22.42 (C1); IR (film, cm^{-1}) 3187 (w), 1717 (s), 1646 (s); MS (ES+) *m*/z (relative intensity): 370 ([M+Na], 20), 337 (50), 325 (90), 309 (100); Exact Mass Calcd for [$C_{16}H_{17}N_3O_4S$]+Na requires *m/z* 370.0873 Found 370.0852 (ES+); White solid decomposes above 180 ^oC; UV (Acetonitrile) ε_{213} = 19400, ε_{247} = 4800 and ε_{337} = 2700 $cm^{-1}M^{-1}$.

334. 2S-[3-(Acetylamino-methylsulfanyl)-2R-*tert*-butoxycarbonylamino-propionylamino]-propionic acid methyl ester

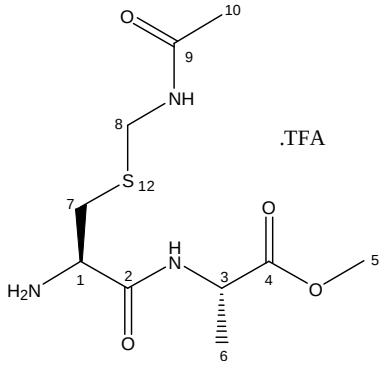


To 2S-*tert*-butoxycarbonylamino-3-mercapto-propionic acid (500 mg, 1.71 mmol) in dichloromethane (25 mL) and *N*,*N*-dimethylformamide (3 mL) at 0 ^oC, was added hydroxybenzotriazole (HOBt) (254 mg, 1.88 mmol), *N*-methylmorpholine (NMM) (206 μL, 1.88 mmol), 2S-amino-propionic acid methyl ester hydrochloride salt (358 mg, 2.56 mmol), and finally 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (361 mg, 1.88 mmol). The reaction was brought to room temperature and stirred for 16 hours. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in dichloromethane to 3% methanol in dichloromethane) afforded **334** as a colourless thick oil (478 mg, 1.27 mmol) in 74% yield.

δ_H (500 MHz, $CDCl_3$) 7.21 (d, 1H, *J* = 6.0, 11-NH), 6.94 (s, 1H, NH), 5.57 (s, 1H, NH), 4.69 (dd, 1H, *J* = 13.0 and 6.0, *H*H-11), 4.54 (t, 1H, *J* = 7.4, H-4), 4.46 (q, 1H, *J* = 6.9, H-6), 4.26 (d, 1H, *J* = 13.0, H*H*-11), 3.73 (s, 3H, H_3-8), 2.93 (dd, 1H, *J* = 14.7 and 7.5, *H*H-10), 2.77 (dd, 1H, *J* = 14.5 and 7.5, H*H*-10), 2.06 (s, 3H, H_3-13), 1.46 (s, 9H, 3 x H_3-1), 1.44 (d, 3H, *J* = 6.9, H_3-9); δ_C (125 MHz, $CDCl_3$) 173.12 (C=O), 171.10 (C=O), 170.73 (C=O), 156.04 (C=O), 80.34 (C2), 53.51 (C4), 52.50 (C8), 48.26 (C6), 40.73 (C11), 34.29 (C10), 28.37 (3 x C1), 23.25 (C13), 17.84 (C9); IR (oil, cm^{-1}) 3287 (m),

2978 (w), 1744 (m), 1657 (s); MS (ES+) *m*/z (relative intensity): 400 ([M+Na], 70), 300 (100); Exact Mass Calcd for $[C_{15}H_{27}N_3O_6S]$+Na requires *m/z* 400.1518 Found 400.1503 (ES+).

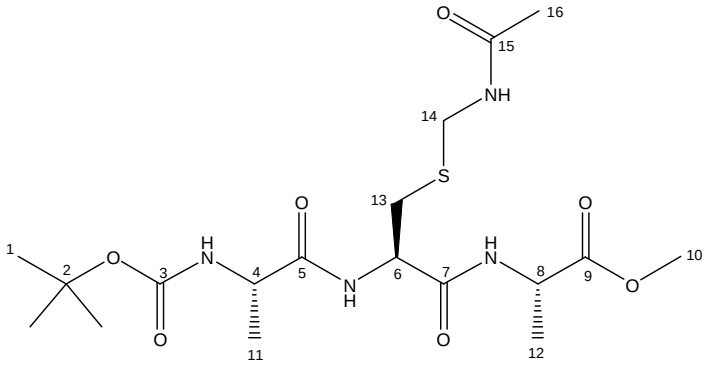
335. 2S-[3-(Acetylamino-methylsulfanyl)-2R-amino-propionylamino]-propionic acid methyl ester trifluoracetic acid salt



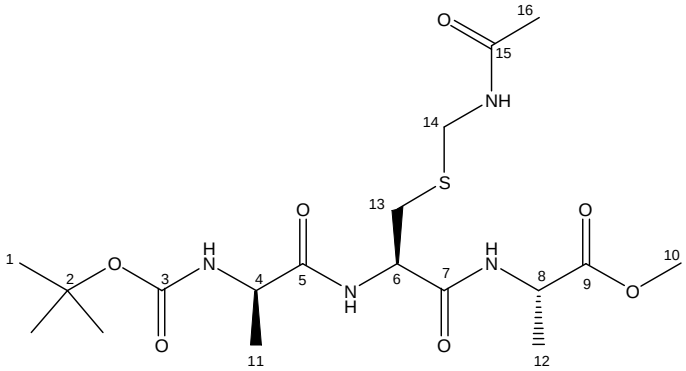
To **334** (150 mg, 0.40 mmol) in dichloromethane (10 mL) at 0 ^oC was added trifluoroacetic acid (10 mL) over 2 minutes at 0 ^oC with stirring. After 30 minutes solvent was removed *in vacuo* to afford **335** as a thick pale yellow oil (156 mg, 0.40 mmol) in 100% yield.

δ_H (500 MHz, MeOD) 4.61 (d, 1H, *J* = 13.9, H*H*-8) 4.47 (q, 1H, *J* = 7.4, H-3), 4.23 (dd, 1H, *J* = 9.5 and 3.9, H-1), 4.19 (d, 1H, *J* = 14.0, *H*H-8), 3.71 (s, 3H, H_3-5), 3.18 (dd, 1H, *J* = 15.1 and 3.9, H*H*-7), 2.86 (dd, 1H, *J* = 15.1 and 9.5, *H*H-7), 2.01 (s, 3H, H_3-10), 1.42 (d, 3H, *J* = 7.4, H_3-6); δ_C (125 MHz, MeOD) 174.29 (C=O), 174.17 (C=O), 168.92 (C=O), 158.95 (q, *J* = 43, TFA, C=O), 118.29 (q, *J* = 284, CF_3), 53.45 (C3), 52.94 (C5), 49.62 (C1), 40.97 (C8), 32.71 (C7), 22.73 (C10), 17.37 (C6); IR (oil, cm^{-1}) 3372 (m), 2491 (w), 1664 (s); Mass ion not found.

332. 2S-[3-(Acetylamino-methylsulfanyl)-2R-(2S-*tert*-butoxycarbonylamino-propionylamino)-propionylamino]-propionic acid methyl ester



336. 2S-[3-(Acetylamino-methylsulfanyl)-2R-(2R-*tert*-butoxycarbonylamino-propionylamino)-propionylamino]-propionic acid methyl ester



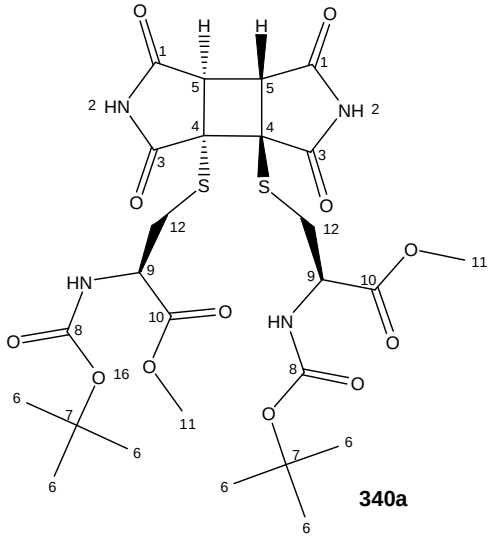
To 2-*tert*-butoxycarbonylamino-propionic acid (0.66 g, 3.50 mmol) in dichloromethane (60 mL) and DMF (3 mL) at 0 ^{o}C, was added HOBt (473 mg, 3.50 mmol), NMM (385 μL, 3.50 mmol), **335** (1.25 g, 3.20 mmol) and finally EDC (0.67 g, 3.50 mmol). The reaction was brought to room temperature and stirred for 16 hours. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in dichloromethane to 3% methanol in dichloromethane) afforded **336** as a thick colourless oil (416 mg, 0.93 mmol) in 29% yield and **332** as a thick colourless oil (0.85 g, 1.89 mmol) in 59%.

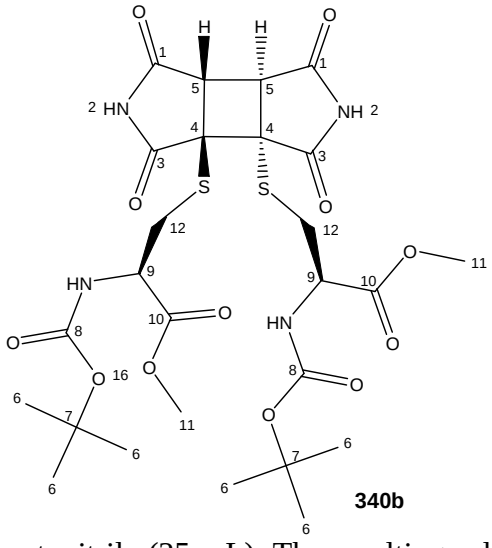
332. δ_H (500 MHz, MeOD) 4.79-4.24 (m, 1H, H-6), 4.45 (d, 1H, *J* = 13.8, H*H*-14), 4.39 (q, 1H, *J* = 7.2, CH), 4.20 (d, 1H, *J* = 13.9, H*H*-14), 4.10-4.06 (m, 1H, CH), 3.70 (s, 3H, H_3-10), 3.04 (dd, 1H, *J* = 14.2 and 4.7, *H*H-13), 2.81 (dd, 1H, *J* = 14.0 and 9.1, H*H*-13), 1.98 (s, 3H, H_3-16), 1.43 (s, 9H, 3 x H_3-1), 1.39 (d, 3H, *J* = 7.3, CH_3), 1.32 (d, 3H, *J* = 7.2, CH_3); δ_C (125 MHz, MeOD) 173.31 (C=O), 173.10 (C=O), 172.92 (C=O), 171.08 (C=O), 170.05 (C=O), 80.45 (C2), 52.54 (C10), 52.30 (CH), 50.64 (CH), 48.43 (CH) 40.99 (C14), 33.41 (C13), 28.37 (3 x C1), 23.37 (CH_3), 18.35 (CH_3), 17.74 (CH_3); IR

(oil, cm^{-1}) 3287 (m), 2980 (w), 1743 (m), 1647 (s); MS (ES+) *m*/z (relative intensity): 471 ([M+23], 55), 415 (45), 371 (100); Exact Mass Calcd for $[C_{18}H_{32}N_4O_7S]$+Na requires *m/z* 471.1902 Found 471.1889 (ES+).

336. δ_H (500 MHz, MeOD) 4.79-4.25 (m, 1H, H-6), 4.57 (d, 1H, *J* = 13.9, H*H*-14), 4.42 (q, 1H, *J* = 7.4, H-8), 4.17 (d, 1H, *J* = 14.1, H*H*-14), 4.10-4.05 (m, 1H, H-4), 3.71 (s, 3H, H_3-10), 3.09 (dd, 1H, *J* = 14.6 and 4.1, *H*H-13), 2.81 (dd, 1H, *J* = 14.6 and 10.4, H*H*-13), 2.00 (s, 3H, H_3-16), 1.43 (s, 9H, 3 x H_3-1), 1.41 (d, 3H, *J* = 7.3, H_3-11), 1.34 (d, 3H, *J* = 7.3, H_3-12); δ_C (125 MHz, MeOD) 176.81 (C=O), 174.42 (C=O), 173.90 (C=O), 171.24 (C=O), 159.32 (C=O), 80.42 (C2), 54.17 (CH), 52.82 (CH), 50.39 (CH), 49.53 (C10) 41.11 (C14), 33.15 (C13), 28.71 (3 x C1), 22.71 (CH_3), 17.92 (CH_3), 17.35 (CH_3); IR (oil, cm^{-1}) 3288 (w), 2983 (w), 1719 (s), 1675 (s); MS (ES+) *m*/z (relative intensity): 471 ([M+23], 10), 456 (95), 396 (100); Exact Mass Calcd for $[C_{18}H_{32}N_4O_7S]$+Na requires *m/z* 471.1902 Found 471.1899 (ES+).

340a. (9R, 10R) – 4S, 5S-Di[2R-*tert*-butoxycarbonylamino-3-sulfanyl-propionic acid methyl ester]-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one and 340b. (9S, 10S) – 4R, 5R-di[2R-*tert*-butoxycarbonylamino-3-sulfanyl-propionic acid methyl ester]-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one. Stereochemistry and regiochemistry defined with analogy to 351.



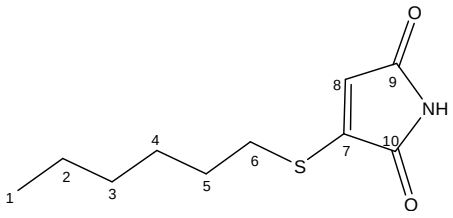


291 (39 mg, 0.117 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* to afford a mixture of **340a** and **340b** as a

yellow solid (39 mg, 0.117 mmol) in 100% yield. NMR investigations showed the mixture was a 1:1 ratio of **340a**:**340b**.

δ_H (600 MHz, $CDCl_3$) 5.49 (s, 2H, 2 x 8-NH), 5.48 (s, 2H, 2 x 8-NH), 4.54-4.52 (m, 2H, 2 x H-9), 4.50-4.47 (m, 2H, 2 x H-9), 3.78 (s, 6H, 2 x H_3-11), 3.76 (s, 6H, 2 x H_3-11), 3.66-3.59 (m, 2H, 2 x H*H*-12), 3.42-3.39 (m, 2H, 2 x *H*H-12), 3.35-3.32 (m, 2H, 2 x *H*H-12), 3.21 (s, 4H, 4 x H-5), 3.16-3.11 (m, 2H, 2 x H*H*-12), 1.43 (s, 36H, 12 x H_3-6); δ_C (150 MHz, $CDCl_3$) 173.17 (2 x C=O), 173.11 (2 x C=O), 172.85 (2 x C=O), 172.76 (2 x C=O), 171.11 (2 x C=O), 171.18 (2 x C=O), 155.53 (2 x C=O), 155.49 (2 x C=O), 80.82 (4 x C7), 55.83 (4 x C4), 53.11 (4 x C11), 52. 89 (4 x C9), 45.57 (4 x C5), 33.34 (2 x C12), 33.22 (2 x C12), 28.40 (12 x C6); IR (film, cm^{-1}) 3171 (w), 2980 (w), 1721 (s); MS (ES+) *m*/z (relative intensity): 683 ([M+Na], 95), 505 (100); Exact Mass Calcd for $[C_{26}H_{36}N_4O_{12}S_2]$+Na requires *m/z* 683.1669 Found 683.1666 (ES+). m.p. 144-146 ^oC;

342. 3-Hexylsulfanyl-pyrrole-2,5-dione

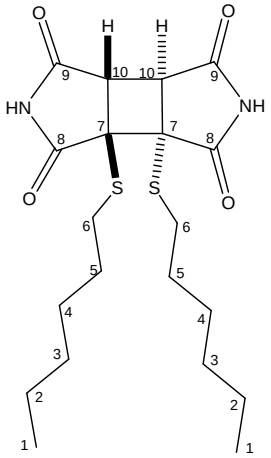


To **289** (300 mg, 1.69 mmol) and sodium acetate (138 mg, 1.69 mmol) in methanol (100 mL) was added hexanethiol (237 μL, 1.69 mmol). After 5 minutes the solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **342** as a cream powder (362 mg, 1.69 mmol) in 100% yield.

δ_H (500 MHz, $CDCl_3$) 7.35 (s, 1H, NH), 6.04 (s, 1H, H-8), 2.91 (t, 2H, J = 7.4, H_2-6), 1.78-1.72 (m, 2H, H_2-5), 1.48-1.42 (m, 2H, CH_2), 1.33-1.30 (m, 4H, 2 x CH_2), 0.90 (t, 3H, J = 6.9, H_3-1); δ_C (125 MHz, $CDCl_3$) 169.06 (C=O), 167.69 (C=O), 152.74 (C7), 118.24 (C8), 32.06 (C6), 31.26 (CH_2), 28.58 (CH_2), 27.70 (CH_2), 22.52 (CH_2), 14.03 (C1); IR (solid, cm^{-1}) 3200 (m), 2918 (m), 1703 (s); MS (ES-) *m*/z (relative intensity): 212 ([M-H], 100); Exact Mass Calcd for $[C_{10}H_{15}NO_2S]$-H requires *m/z* 212.0745 Found 212.0753 (ES-); Elemental analysis: Calc for $C_{10}H_{15}NO_2S$: C: 56.31, H: 7.09, N: 6.57

Found: C: 55.96, H: 7.02, N: 6.56; m.p. 99-101 ^{o}C; UV (Acetonitrile) ε_{247} = 12000 and ε_{347} = 9500 $cm^{-1}M^{-1}$.

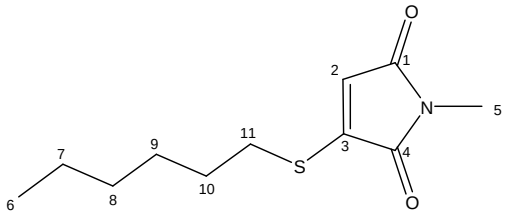
343b. (4RS, 5RS, 9SR, 10SR) - 4, 5-Dihexysulfanyl-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one. Stereochemistry and regiochemistry defined with analogy to 346



342 (25 mg, 0. 116 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* to afford **343** as an off-white solid (53 mg, 0.25 mmol) in 100% yield.

δ_H (500 MHz, $CDCl_3$) 3.14-3.09 (m, 4H, 2 x H-10 and 2 x H*H*-6), 2.84 (dt, 2H, *J* = 11.0 and 7.6, 2 x *H*H-6), 1.56-1.50 (m, 4H, 2 x H_2-5), 1.38-1.33 (m, 4H, 2 x H_2-4), 1.31-1.25 (m, 8H, 2 x H_2-3 and 2 x H_2-2), 0.87 (t, 6H, *J* = 7.0, 2 x H_3-1); δ_C (125 MHz, $CDCl_3$) 173.37 (2 x C=O), 172.82 (2 x C=O), 56.26 (2 x C7), 45.66 (2 x C10), 31.36 (2 x CH_2), 31.16 (2 x H_2-6), 28.64 (2 x CH_2), 28.53 (2 x CH_2), 22.51 (2 x CH_2), 14.07 (2 x C1); IR (solid, cm^{-1}) 3194 (m), 2933 (m), 1719 (s); MS (ES-) *m*/z (relative intensity): 425 ([M-H], 35), 212 (100); Exact Mass Calcd for [$C_{20}H_{30}N_2O_4S_2$]-H requires *m/z* 425.1584 Found 425.1584 (ES-); m.p. 116-118 ^{o}C.

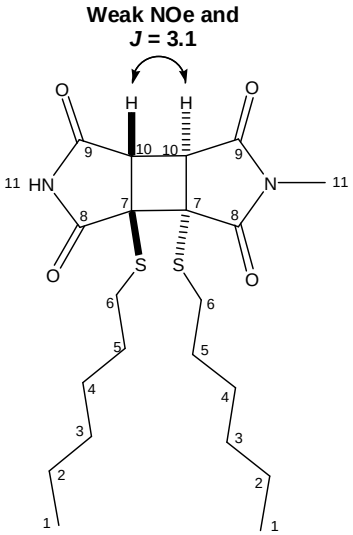
345. 3-Hexylsulfanyl-1-methyl-pyrrole-2,5-dione



To **289** (100 mg, 0.53 mmol) and sodium acetate trihydrate (70 mg, 0.53 mmol) in methanol (15 mL) was added hexanethiol (74 μL, 0.58 mmol) in methanol (100 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **345** as a bright yellow solid (99 mg, 0.44 mmol) in 83% yield.

δ_H (600 MHz, $CDCl_3$) 6.03 (s, 1H, H-2), 3.01 (s, 3H, H_3-5), 2.89 (t, 2H, *J* = 7.6, 2H, H_2-11), 1.76-1.71 (m, 2H, H_2-10), 1.46-1.41 (m, 2H, H_2-9), 1.33-1.27 (m, 4H, H_2-7 and H_2-8), 0.89 (t, 3H, *J* = 6.5, H_3-6); δ_C (125 MHz, $CDCl_3$) 171.47 (C=O), 169.94 (C=O), 151.84 (C3), 117.27 (C2), 31.92 (C11), 31.31 (CH_2), 28.64 (CH_2), 27.75 (CH_2), 24.10 (C5), 24.10 (C7), 14.09 (C6); IR (oil, cm^{-1}) 2727 (w), 1708 (s); MS (FAB+) *m*/z (relative intensity): 250 ([M+Na], 40), 228 (35), 199 (30), 176 (100); Exact Mass Calcd for $[C_{11}H_{17}NO_2S]$+Na requires *m/z* 250.0878 Found 250.0880 (FAB+); m.p. 62-65 ^oC; UV (Acetonitrile) ε_{264} = 8010 and ε_{360} = 3200 $cm^{-1}M^{-1}$.

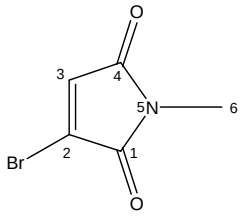
346b. (4RS, 5RS, 9SR, 10SR) - 4, 5-Dihexysulfanyl-2-methyl-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one. Stereochemistry and regiochemistry defined by analysis of coupling constants, see p. 166.



342 (12.5 mg, 0.058 mmol) and **345** (12.5 mg, 0.058 mmol) were dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* to afford an inseparable mixture of dimers **343** and **349**, alongside **346b**. Full spectral

analysis was not possible due to the complex mixture of structures but in an isolated area of the spectra, NOeSY and coupling constant analysis indicated that the structure was of the stereochemistry shown, see p.166 for a full description.

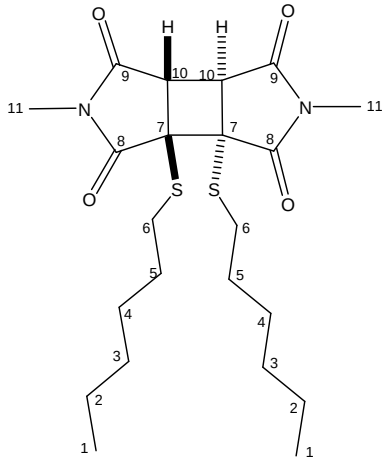
348. 3-Bromo-1-methyl-pyrrole-2,5-dione



To *N*-methyl maleimide (0.50 g, 4.5 mmol) in methanol (10 mL) was added bromine (232 μL, 4.5 mmol) dropwise in methanol (5 mL). The reaction mixture was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and dissolved in tetrahydrofuran (20 mL). Triethylamine (815 μL, 5.9 mmol) in tetrahydrofuran (5 mL) was added over 5 minutes, whereupon a precipitate formed. The reaction mixture was stirred for 24 hours. The solid was filtered off and washed with tetrahydrofuran (50 mL). Purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **348** as a pale yellow powder (0.56 g, 2.96 mmol) in 66% yield.

δ_H (500 MHz, $CDCl_3$) 6.90 (s, 1H, H-3), 3.09 (s, 3H, H_3-6); δ_C (125 MHz, $CDCl_3$) 168.6 (C=O), 165.4 (C=O), 131.9 (C3), 131.4 (C2), 24.7 (C6); IR (solid, cm^{-1}) 3106 (s), 1708 (s); MS (CI+) *m/z*, (relative intensity): 192 ([M+H], 99), 190([M+H], 100); Exact mass calcd for $[C_5H_4O_2N^{79}Br]$+H requires 189.9504 Found 189.9505 (CI+); m.p: 77-79 ^oC; UV (Acetonitrile) ε_{209} = 17100, ε_{238} = 13200 and ε_{299} = 210 $cm^{-1}M^{-1}$.

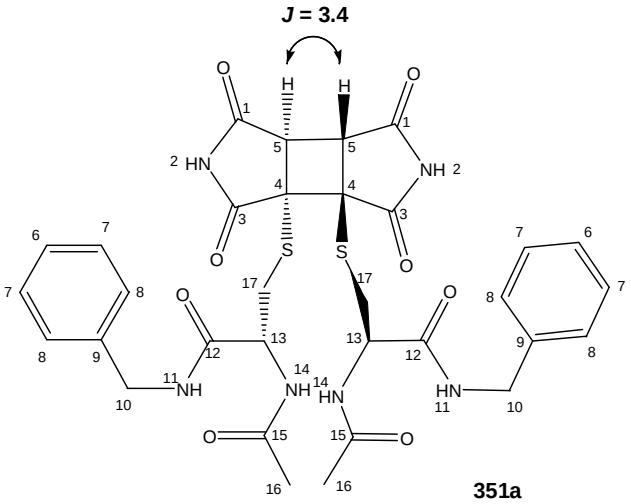
349. (4RS, 5RS, 9SR, 10SR) - 4, 5-Dihexysulfanyl-2,7-dimethyl-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one. Stereochemistry and regiochemistry defined with analogy to 346



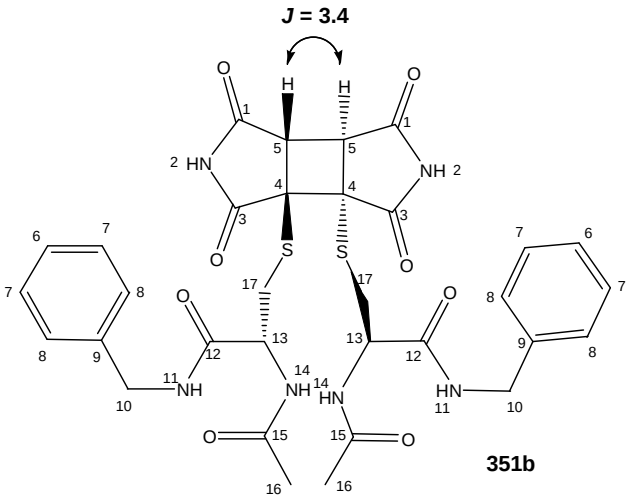
345 (25 mg, 0.11 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* to afford **349** as an off white solid (25 mg, 0.11 mmol) in 100% yield.

δ_H (500 MHz, $CDCl_3$) 3.15 (dt, 2H, *J* = 11.0 and 7.3, 2 x H*H*-6), 3.11 (s, 6H, 2 x H_3-11), 2.97 (s, 2H, 2 x H-10), 2.84 (dt, 2H, *J* = 11.0 and 7.5, 2 x *H*H-6), 1.53-1.47 (m, 4H, 2 x H_2-5), 1.37-1.25 (m, 12H, 6 x CH_2), 0.87 (t, 6H, *J* = 6.7, 2 x H_3-1); δ_C (125 MHz, $CDCl_3$) 173.11 (2 x C=O), 172.87 (2 x C=O), 55.39 (2 x C7), 44.56 (2 x C11), 31.34 (2 x CH_2), 31.16 (2 x CH_2), 28.66 (2 x CH_2), 28.49 (2 x CH_2), 25.76 (2 x C10), 22.53 (2 x CH_2), 14.05 (2 x C1); IR (oil, cm^{-1}) 3463 (w), 2970 (m) 1744 (s), 1720 (s); MS (CI+) *m*/z (relative intensity): 455 ([M+H], 50), 228 (100), 194 (60); Exact Mass Calcd for [$C_{22}H_{34}N_2O_4S_2$]+H requires *m/z* 455.2038 Found 455.2019 (CI+); m.p. 104-106 ^oC.

351a. (9R, 10R) – 4S, 5S-Di[2R-acetylamino-*N*-benzyl-3-sulfanyl-propionamide]-2,7-diaza - bicyclo [3.5.0.0^{5,9}]decantetra - 1,3,6,8 - one and 351b. (9S, 10S) – 4R, 5R - di[2R - acetylamino-*N*-benzyl-3-sulfanyl-propionamide]-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one



351a



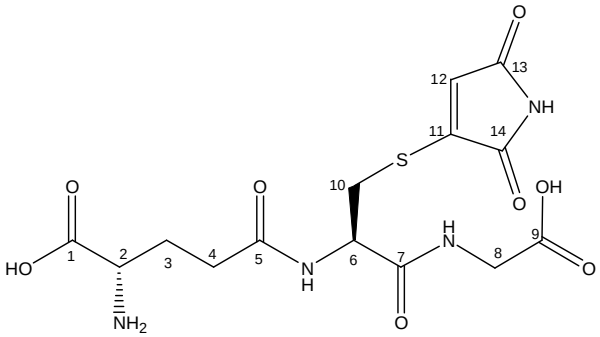
351b

330 (55 mg, 0.16 mmol) was dissolved in acetonitrile (50 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in 30% ethyl acetate in petroleum ether to 10% methanol in ethyl acetate) afforded **351a** and **351b** as a colourless oil (55 mg, 0.16 mmol) in 100% yield. NMR investigations showed the mixture was a 1:1 ratio of **351a**:**351b**.

δ_H (600 MHz, $CDCl_3$) 7.37-7.23 (m, 16H, 16 x Ar-H), 7.21 (d, 4H, *J* = 7.1, 4 x H-6), 4.58 (dt, 4H, *J* = 7.7 and 5.0, 4 x H-13), 4.38-4.36 (m, 8H, 4 x H_2-10), 3.49 (dd, 2H, *J* = 12.8 and 5.3, 2 x H*H*-17), 3.35 (s, 2H, 2 x H-5), 3.31-3.30 (m, 4H, 2 x H_2-17) 3.27 (s,

2H, 2 x H-5), 3.12 (dd, 2H, *J* = 12.8 and 8.7, 2 x *H*H-17), 1.99 (s, 12H, 4 x H_3-16); δ_C (150 MHz, $CDCl_3$) 175.85 (C=O), 175.74 (C=O), 175.72 (C=O), 173.50 (C=O), 173.45 (C=O), 171.98 (C=O), 171.88 (C=O) 139.57 (4 x C9), 129.55 (8 x Ar-H), 128.48 (8 x Ar-H), 128.20 (4 x Ar-H), 57.50 (2 x C4), 57.40 (2 x C4), 54.27 (2 x C13), 54.18 (2 x C13), 47.20 (2 x C5), 47.10 (2 x C5), 44.16 (2 x C10), 44.14 (2 x C10), 33.86 (2 x C17), 33.70 (2 x C17), 22.56 (4 x C16) One carbon signal is missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3437 (w), 1726 (s); MS (FAB+) *m*/z (relative intensity): 695 ([M+H], 10), 439 (10), 286 (100); Exact Mass Calcd for $[C_{32}H_{34}N_6O_8S_2]$+H requires *m/z* 695.1958 Found 695.1964 (FAB+).

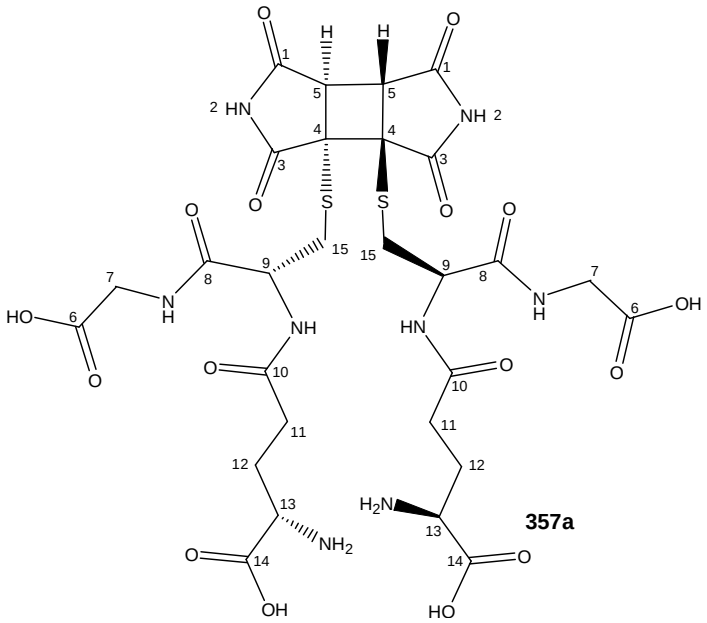
356. 2S-Amino-4-[1R-(carboxymethyl-carbamoyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl)-ethylcarbamoyl]-butyric acid



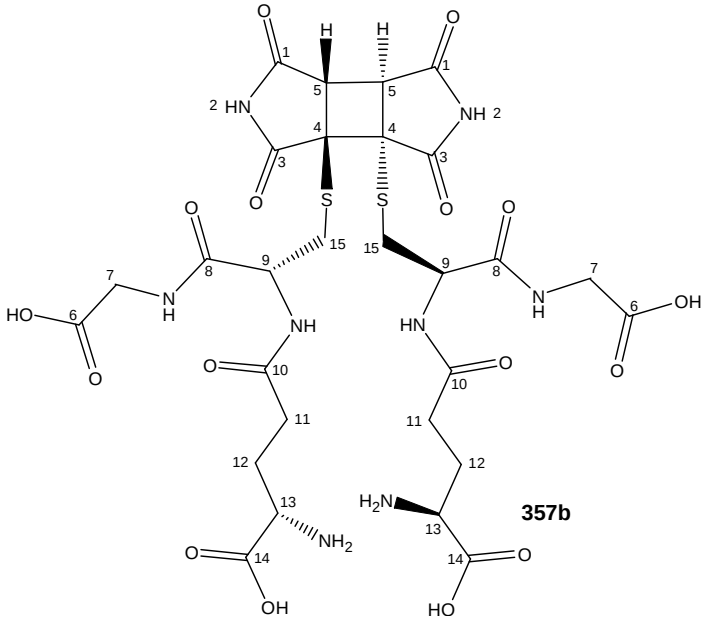
To glutathione (47 mg, 0.15 mmol) in methanol (3 mL) was added **289** (30 mg, 0.15 mmol) in methanol (3 mL). After 5 minutes the solvent was removed *in vacuo* to afford **356** as a thick colourless oil (77 mg) that was used without further purification. The same data was afforded for **356** if this reaction was carried out in acetonitrile:water (1:1).

δ_H (500 MHz, MeOD) 6.47 (s, 1H, H-12), 4.79 (dd, 1H, *J* = 8.2 and 5.7, H-6), 4.06 (t, 1H, *J* = 6.5, H-2), 3.95 (s, 2H, H_2-8), 3.49 (dd, 1H, *J* = 13.9 and 5.8, *H*H-10), 3.29 (dd, 1H, *J* = 13.6 and 8.3, H*H*-10), 2.61 (t, 2H, *J* = 7.1, H_2-4), 2.29-2.15 (m, 2H, H_2-3); δ_C (125 MHz, MeOD) 174.68 (C=O), 172.81 (C=O), 172.39 (C=O), 171.89 (C=O), 171.62 (C=O), 170.59 (C=O), 151.75 (C11), 120.91 (C12), 53.79 (C6), 52.76 (C2), 42.01 (C8), 33.92 (C10), 32.42 (C4), 27.03 (C3); IR (oil, cm^{-1}) 3259 (m), 2928 (m), 1717 (s); MS (ES-) *m*/z (relative intensity): 401 ([M-H], 100), 272 (30); Exact Mass Calcd for $[C_{14}H_{18}N_4O_8S]$-H requires *m/z* 401.0767 Found 401.0773 (ES-); UV (Acetonitrile) ε_{204} = 8100, ε_{253} = 5600 and ε_{342} = 1900 $cm^{-1}M^{-1}$.

357a. (9R, 10R) – 4S, 5S-Di[2S-amino-4-(1R-(carboxymethyl-carbamoyl)-2-sulfanyl-ethylcarbamoyl)-butyric acid]-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one and 357b. (9S, 10S) – 4R, 5R-di[2S-amino-4-(1R-(carboxymethyl-carbamoyl)-2-sulfanyl-ethylcarbamoyl)-butyric acid]-2,7-diaza-bicyclo [3.5.0.0^{5,9}]decantetra-1,3,6,8-one. Stereochemistry and regiochemistry defined with analogy to 351



357a



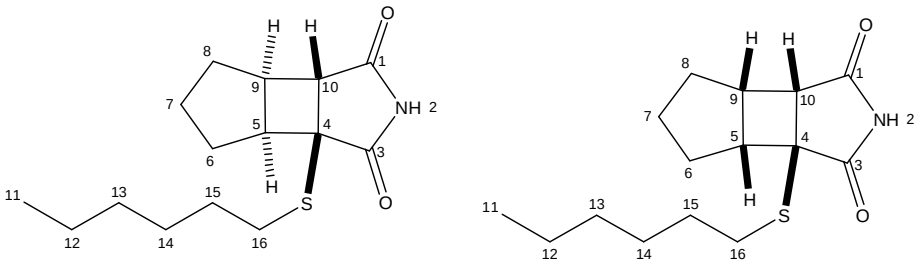
357b

To glutathione (36 mg, 0.12 mmol) in water (12.5 mL) was added **289** (21 mg, 0.12 mmol) in acetonitrile (12.5 mL). The solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in*

vacuo to afford **357** (a mixture of diastereoisomers) as a colourless oil (114 mg). No further purification was carried out on this compound.

δ_H (600 MHz, $CDCl_3$) 4.83-4.63 (m, 4H, 4 x H-9), 4.14-4.10 (m, 4H, 4 x H-13), 4.02-3.95 (m, 8H, 4 x H_2-7), 3.59-3.36 (m, 4H, 4 x *H*H-15), 3.36 (s, 4H, 4 x H-5), 3.29-2.92 (m, 4H, 4 x H*H*-15), 2.69-2.62 (m, 8H, 4 x H_2-11) 2.35-2.15 (m, 8H, 4 x H_2-12); δ_C (150 MHz, $CDCl_3$) 175.85 (C=O), 175.71 (C=O), 174.61 (C=O), 174.56 (C=O), 172.44 (C=O), 172.40 (C=O), 171.76 (C=O) 171.71 (C=O), 171.59 (C=O), 57.55 (4 x C4), 54.17 (4 x CH), 49.90 (4 x CH), 47.13 (2 x C5), 47.01 (2 x C5), 42.06 (2 x C7), 42.01 (2 x C7), 33.84 (2 x C15), 33.84 (2 x C15), 32.53 (2 x C11), 32.45 (2 x C11), 27.02 (2 x C12), 26.92 (2 x C12) Two diastereomers are indicated, thus three carbon signals are missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3460 (br), 2959 (m), 1717 (s), 1646 (m); MS (ES-) *m*/z (relative intensity): 803 ([M-H], 10), 708 (30), 401 (90); Exact Mass Calcd for $[C_{28}H_{36}N_8O_{16}S_2]$-H requires *m/z* 803.1612 Found 803.1634 (ES-).

366. (4RS, 5RS, 9SR, 10SR) - 2-Aza-4-hexylsulfanyl-2-aza-tricyclo[3.5.0.0^{5,9}]di-1,3-one and (4RS, 5SR, 9RS, 10SR) - 2-aza-4-hexylsulfanyl-2-aza-tricyclo[3.5.0.0^{5,9}]di-1,3-one

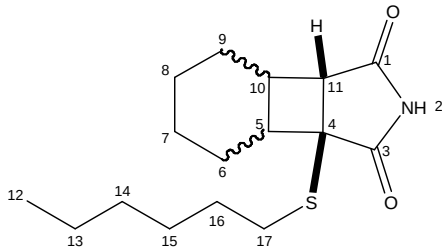


342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (22.5 mL) and cyclopentene (3 mL, 36 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution 10% ethyl acetate in petroleum ether to 50% ethyl acetate in petroleum ether) afforded **366** as a thick colourless oil (12 mg, 0.045 mmol) in 78% yield, a 1:1 mix of two inseparable diastereomers. Overlap of signals prevented NOe analysis.

δ_H (500 MHz, $CDCl_3$) 3.15-3.07 (m, 2H, H-5_a and H-10_a), 3.00 (app t, 1H, *J* = 6.8, H-5_b), 2.94 (app dt, 1H, *J* = 6.6 and 3.9, H-9_b), 2.87-2.82 (m, 2H, H-9_a and H*H*-16), 2.64-2.59 (m, 1H, H*H*-16), 2.52-2.47 (m, 3H, H-10_b, *H*H-16 and *H*H-16), 2.07 (dd, 2H, *J* =

6.9 and 6.3, 2 x H*H*-15), 1.96-1.89 (m, 2H, 2 x *H*H-15) 1.88-1.82 (m, 4H, 2 x H_2-7) 1.64-1.50 (m, 8H, 2 x H_2-6 and 2 x H_2-8), 1.38-1.25 (m, 12H, 2 x H_2-12, 2 x H_2-13 and 2 x H_2-14), 0.89-0.86 (m, 6H, 2 x H_3-11), $_a$-signals shown as part of the same CH_2 by HMQC data, $_b$-signals shown as part of the same CH_2 by HMQC data; δ_C (125 MHz, $CDCl_3$) 179.09 (C=O), 177.12 (C=O), 176.93 (C=O), 171.83 (C=O), 51.31 (C4), 51.33 (C4), 50.68 (C10), 45.32 (C10), 43.26 (C9), 41.70 (C5), 32.38 (CH_2), 30.98 (CH_2), 30.95 (CH_2), 30.78 (CH_2), 28.92 (CH_2), 28.50 (CH_2), 28.30 (CH_2), 28.22 (CH_2), 28.10 (CH_2), 28.13 (CH_2), 25.09 (CH_2), 22.14 (CH_2), 22.11 (CH_2), 13.66 (2 x C11) Two diastereomers are indicated, thus five carbon signals are missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3120 (w), 2927 (m), 1711 (s), 1627 (s); MS (ES-) *m*/z (relative intensity): 280 ([M-H], 50), 212 (100); Exact Mass Calcd for $[C_{15}H_{23}NO_2S]$-H requires *m/z* 280.1371 Found 280.1382 (ES-).

367. (4RS, 11SR)-4-Hexylsulfanyl-2-aza-tricyclo[5.4.0.0^{4,11}]undecandi-1,3-one

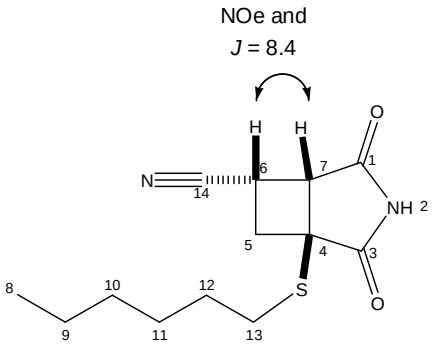


342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (21.5 mL) and cyclohexene (3.5 mL, 36 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in 10% ethyl acetetate in petroleum ether to 50% ethyl acetate in petroleum ether) afforded **367** as a thick colourless oil (24 mg, 0.081 mmol) in 68% yield, a complex unequal mix of 4 inseparable diastereomers. NMR investigations showed the mixture was approximately 4:1:3:1.5 by NH signals.

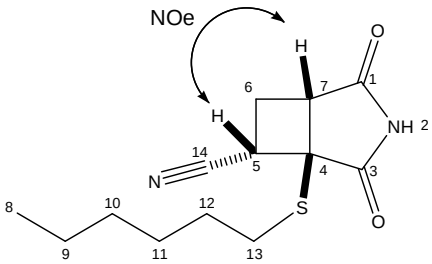
δ_H (500 MHz, $CDCl_3$) 8.34 (s, 1H, NH), 8.05 (s, 1H, NH), 7.99 (s, 1H, NH), 7.92 (s, 1H, NH), 3.24 (app tt, 1H, *J* = 6.4 and 1.1, CH) 3.18 (d, 1H, *J* = 8.7, H-11), 3.12 (app dt, 1H, *J* = 11.0 and 7.4, CH), 3.09 (d, 1H, *J* = 8.5, H-11), 2.89-2.81 (m, 3H, multiplet contains a CH signal by HMQC analysis), 2.74 (dd, 1H, *J* = 8.2 and 8.4), 2.68-2.47 (m, 8H, contains several different H_2-17), 2.21 (app td, 1H, *J* = 12.1 and 3.4, CH), 1.97-1.72 (m, 15H, contains several different H_2-16 and other CH_2s) 1.66-1.44 (m, 15H, contains

several different H_2-15 and other CH_2s) 1.44-1.23 (m, 32H, contains several different H_2-13 and H_2-14 and other CH_2s), 0.89-0.86 (m, 12H, contains several different H_3-12); δ_C (125 MHz, $CDCl_3$) 178.09 (C=O), 177.09 (C=O), 176.71 (C=O), 174.74 (C=O), 171.91 (C=O), 171.68 (C=O), 59.14 (C4), 55.87 (C4), 53.63 (C4), 53.19 (CH), 50.64 (CH), 50.08 (C11), 49.02 (CH), 45.32 (CH), 41.61 (CH), 36.74 (CH), 35.53 (CH), 30.99 (CH), 30.99 (CH_2), 30.95 (CH_2), 30.95 (CH_2), 30.78 (CH_2), 29.77 (CH_2), 29.14 (CH_2), 29.03 (CH_2), 28.91 (CH_2), 28.83 (CH_2), 28.29 (CH_2), 28.22 (CH_2), 28.09 (CH_2), 27.98 (CH_2), 27.78 (CH_2), 25.70 (CH_2), 25.45 (CH_2), 25.32 (CH_2), 22.15 (CH_2), 22.09 (CH_2), 20.48 (CH_2), 20.20 (CH_2), 13.67 (4 x C12) Four diastereomers are indicated, thus twenty one carbon signals are missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3221 (w), 2929 (m), 1711 (s); MS (CI+) *m*/z (relative intensity): 296 ([M+H], 100), 214 (20); Exact Mass Calcd for $[C_{16}H_{25}NO_2S]$+H requires *m/z* 296.1684 Found 296.1678 (CI+).

368. (4RS, 6RS, 7SR)-2-Aza-4-hexylsulfanyl-6-carbonitrile-bicyclo[3.2.0]heptan-1,3-dione



369. (4RS, 5RS, 7SR)-2-Aza-4-hexylsulfanyl-5-carbonitrile-bicyclo[3.2.0]heptan-1,3-dione



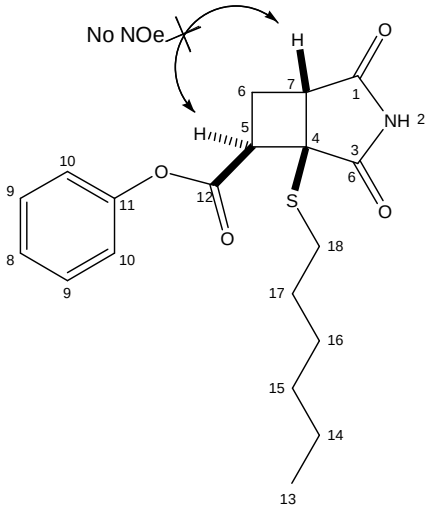
342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (22.5 mL) and acrylonitrile (2.5 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution 10% ethyl acetate in petroleum

ether to 50% ethyl acetate in petroleum ether) afforded **368** as a thick colourless oil (9 mg, 0.034 mmol) in 29% yield and **369** as a thick colourless oil (12 mg, 0.045 mmol) in 39% yield.

368. δ_H (500 MHz, $CDCl_3$) 3.53 (app td, 1H, *J* = 8.4 and 1.4, H-6), 3.16-3.10 (m, 2H, H*H*-5 and H-7), 2.89-2.80 (m, 2H, H_2-13), 2.56-2.50 (m, 1H, *H*H-5), 1.67-1.55 (m, 4H, H_2-12 and H_2-11), 1.42-1.37 (m, 2H, H_2-10), 1.33-1.27 (m, 2H, H_2-9), 0.89 (t, 3H, *J* = 6.9, H_3-8); δ_C (125 MHz, $CDCl_3$) 174.49 (C=O), 172.91 (C=O), 116.82 (C14), 52.38 (C4), 44.16 (C6), 31.33 (C13), 30.87 (C7), 30.29 (CH_2), 29.26 (CH_2), 28.64 (CH_2), 25.92 (CH_2), 22.82 (C5), 14.11 (C8); IR (oil, cm^{-1}) 3223 (w), 2926 (w), 1778 (w), 1714 (s); MS (CI+) *m*/z (relative intensity): 267 ([M+H], 40), 213 (70), 180 (100); Exact Mass Calcd for $[C_{13}H_{18}N_2O_2S]$+H requires *m/z* 267.1167 Found 267.1175 (CI+).

369. δ_H (500 MHz, $CDCl_3$) 3.66 (dd, 1H, *J* = 9.5 and 6.0, H-5), 3.23 (dd, 1H, *J* = 10.9 and 5.2, H-7), 3.01-2.82 (m, 3H, H*H*-6 and H_2-13), 2.67 (ddd, 1H, *J* = 14.7, 9.6 and 5.3, *H*H-6), 1.65-1.60 (m, 2H, H_2-12), 1.42-1.36 (m, 2H, H_2-11), 1.32-1.27 (m, 4H, H_2-9 and H_2-10), 0.88 (t, 3H, *J* = 6.8, H_3-8); δ_C (125 MHz, $CDCl_3$) 175.08 (C=O), 174.82 (C=O), 117.13 (C14), 51.24 (C4), 44.26 (C5), 31.36 (C13), 30.96 (C7), 29.82 (CH_2), 29.19 (CH_2), 28.62 (CH_2), 25.72 (C6), 22.58 (CH_2), 14.26 (C8); IR (oil, cm^{-1}) 3247 (w), 2927 (w), 1717 (s); MS (CI+) *m*/z (relative intensity): 267 ([M+H], 75), 214 (100), 180 (70); Exact Mass Calcd for $[C_{13}H_{18}N_2O_2S]$+H requires *m/z* 267.1167 Found 267.1158 (CI+).

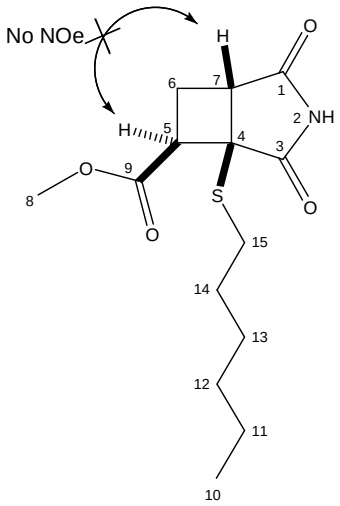
370. (4RS, 5RS, 7RS)-2-Aza-4-hexylsulfanyl-5-carboxylic acid phenyl ester-bicyclo [3.2.0]heptan-1,3-dione



342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, phenyl acrylate (160 μL, 1.20 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **370** as a thick colourless oil (21 mg, 0.058 mmol) in 48% yield and dimer **343** (12 mg, 0.028 mmol) in 48% yield (data matched that obtained above for **343**).

δ_H (600 MHz, $CDCl_3$) 8.41 (s, 1H, NH), 7.41-7.39 (m, 2H, 2 x Ar-H), 7.27-7.25 (m, 1H, H-8), 7.20 (d, 2H, *J* = 7.8, 2 x Ar-H), 3.80 (dd, 1H, *J* = 8.5 and 5.1, H-5), 3.29 (dd, 1H, *J* = 10.7 and 5.1, H-7), 3.20 (ddd, 1H, *J* = 13.0, 10.9 and 5.5, H*H*-6), 2.73 (td, 1H, *J* = 11.5 and 7.5, H*H*-18), 2.62 (td, 1H, *J* = 11.5 and 7.5, *H*H-18), 2.40 (ddd, 1H, *J* = 13.5, 8.8 and 5.6, *H*H-6), 1.54-1.48 (m, 2H, H_2-17), 1.33-1.16 (m, 6H, H_2-14, H_2-15 and H_2-16), 0.84 (t, 3H, *J* = 6.9, H_3-13); δ_C (150 MHz, $CDCl_3$) 176.35 (C=O), 176.19 (C=O), 168.85 (C=O), 150.66 (C11), 129.64 (2 x Ar-H), 126.34 (C8), 121.54 (2 x Ar-H), 52.59 (C4), 44.78 (C7), 44.17 (C5), 31.36 (CH_2), 29.94 (C18), 29.09 (CH_2), 28.68 (CH_2), 23.83 (C6), 22.56 (CH_2), 14.08 (C13); IR (oil, cm^{-1}) 3213 (w), 2927 (w) 1757 (m), 1715 (s); MS (CI+) *m*/z (relative intensity): 362 ([M+H], 35), 268 (100), 149 (25); Exact Mass Calcd for $[C_{19}H_{23}NO_4S]$+H requires *m/z* 362.1426 Found 362.1431 (CI+).

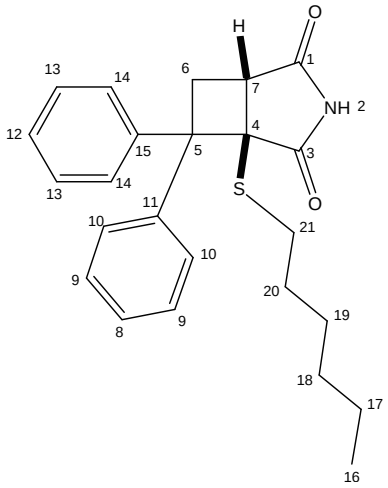
371. (4RS, 7RS, 5RS)-2-Aza-4-hexylsulfanyl-5-carboxylic acid methyl ester-bicyclo [3.2.0]heptan-1,3-dione



342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (21.9 mL) and methyl acrylate (3.1 mL, 36 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 50% ethyl acetate in petroleum ether) afforded **371** as a thick colourless oil (17 mg, 0.056 mmol) in 49% yield.

δ_H (600 MHz, $CDCl_3$) 8.50 (s, 1H, NH), 3.81 (s, 3H, H_3-8), 3.57 (dd, 1H, *J* = 8.5 and 5.8, H-5), 3.18 (dd, 1H, *J* = 10.7 and 5.0, H-7), 3.11 (ddd, 1H, *J* = 12.9, 10.0 and 5.5, *H*H-6), 2.73 (td, 1H, *J* = 11.5 and 7.5, H*H*-15), 2.64 (td, 1H, *J* = 11.5 and 7.5, *H*H-15), 2.29 (ddd, 1H, *J* = 13.2, 8.5 and 5.2, H*H*-6), 1.52-1.47 (m, 2H, H_2-14), 1.35-1.30 (m, 2H, H_2-13), 1.29-1.21 (m, 4H, H_2-11 and H_2-12), 0.87 (t, 3H, *J* = 6.7, H_3-10); δ_C (150 MHz, $CDCl_3$) 176.65 (C=O), 171.13 (C=O), 170.48 (C=O), 52.59 (C8), 52.39 (C4), 44.56 (C7), 44.06 (C5), 31.41 (C15), 29.73 (CH_2), 29.16 (CH_2), 28.68 (CH_2), 23.57 (C12), 22.57 (C11), 14.11 (C10); IR (oil, cm^{-1}) 3244 (w), 2928 (w) 1778 (w), 1714 (s); MS (FAB+) *m*/z (relative intensity): 322 ([M+Na], 100), 300 (30), 214 (25); Exact Mass Calcd for $[C_{14}H_{21}NO_4S]$+Na requires *m/z* 322.1089 Found 322.1082 (FAB+).

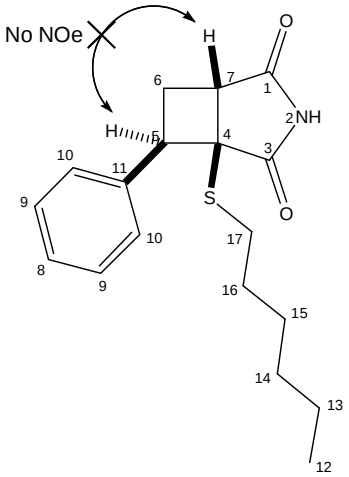
373. (4RS, 7RS)-2-Aza-4-hexylsulfanyl-5,5-diphenyl-bicyclo[3.2.0]heptan-1,3-dione



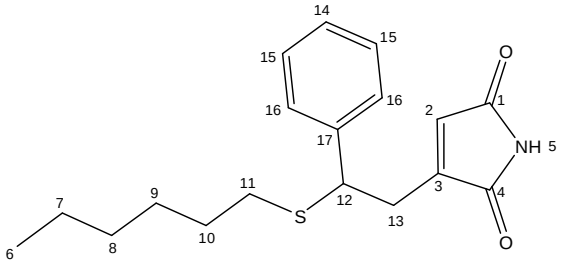
342 (25 mg, 0.116 mmol) was dissolved in acetonitrile (21 mL) and 1,1-diphenylethyene (203 μL, 1.16 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **373** as a colourless oil (30 mg, 0.075 mmol) in 64% yield.

δ_H (600 MHz, $CDCl_3$) 8.15 (s, 1H, NH), 7.42 (m, 2H, H-8 and H-12), 7.36-7.21 (m, 8H, 8 x Ar-H), 3.54 (dd, 1H, *J* = 12.9 and 10.3, H*H*-6), 3.34 (dd, 1H, *J* = 10.3 and 5.7, H-7), 3.18 (dd, 1H, *J* = 12.9 and 5.8, *H*H-6), 2.43 (td, 1H, *J* =11.0 and 7.3, *H*H-21), 2.34 (td, 1H, *J* = 11.0 and 7.4, H*H*-21), 1.40-1.34 (m, 2H, H_2-20), 1.26-1.20 (m, 4H, H_2-18 and H_2-19), 1.18-1.13 (m, 2H, H_2-17), 0.84 (t, 3H, *J* = 7.5, H_3-16); δ_C (150 MHz, $CDCl_3$) 177.16 (C=O), 175.87 (C=O), 142.09 (Ar), 141.80 (Ar), 128.17 (2 x Ar-H), 128.13 (2 x Ar-H), 128.10 (2 x Ar-H), 128.06 (2 x Ar-H), 127.44 (Ar-H), 127.32 (Ar-H), 63.03 (C5), 57.20 (C4), 44.38 (C7), 35.17 (C6), 31.35 (CH_2), 30.07 (C21), 28.77 (CH_2), 28.71 (CH_2) 22.53 (CH_2), 14.11 (C16); IR (oil, cm^{-1}) 2927 (m) 1772 (w), 1709 (s); MS (ES-) *m*/z (relative intensity): 392 ([M-H], 10), 212 (100); Exact Mass Calcd for $[C_{24}H_{27}NO_2S]$-H requires *m/z* 392.1684 Found 392.1674 (ES-).

374. (4RS, 5SR, 7SR)-2-Aza-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



408. 3-(2-Hexylsulfanyl-2-phenyl-ethyl)-pyrrole-2,5-dione



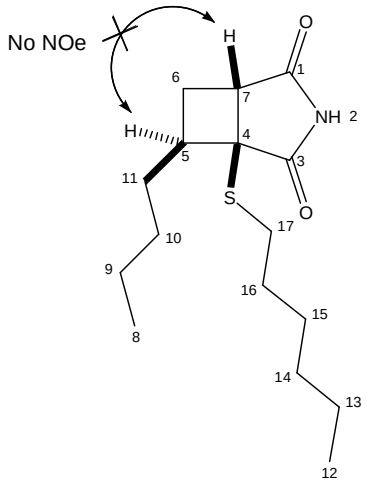
342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, styrene (133 μL, 1.2 mmol) added and the solution irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **408** as a thick yellow oil (11 mg, 0.034 mmol) in 30% yield and **374** as an off-white paste (26 mg, 0.082 mmol) in 70% yield

374. δ_H (600 MHz, $CDCl_3$) 8.77 (s, 1H, NH), 7.39-7.31 (m, 5H, 5 x Ar-H), 4.05 (app t, 1H, *J* = 8.8, H-5), 3.17 (dd, 1H, *J* = 10.9 and 3.4, H-7), 3.04 (ddd, 1H, *J* = 12.7, 11.1 and 8.4, *H*H-6), 2.63 (ddd, 1H, *J* = 12.8, 9.0 and 3.6, H*H*-6), 2.43 (ddd, 1H, *J* = 11.3, 7.9 and 6.7, *H*H-17), 2.13 (ddd, 1H, *J* = 11.3, 8.0 and 6.6, H*H*-17), 1.30-1.08 (m, 8H, H_2-13, H_2-14, H_2-15 and H_2-16), 0.83 (t, 3H, *J* = 7.3, H_3-12); δ_C (150 MHz, $CDCl_3$) 178.76 (C=O), 177.62 (C=O), 136.51 (C11), 128.77 (2 x Ar-H), 128.70 (2 x Ar-H), 128.03 (C8), 57.17 (C4), 45.70 (C5), 43.87 (C7), 31.26 (C17), 28.70 (CH_2), 28.65 (CH_2), 28.52 (CH_2), 26.22 (C6), 22.46 (CH_2), 14.10 (C12); IR (oil, cm^{-1}) 3218 (w), 2926 (w) 1771 (m), 1703 (s); MS (FAB+) *m*/z (relative intensity): 340 ([M+Na], 20), 199 (25), 176 (100); Exact Mass Calcd for $[C_{16}H_{23}NO_2S]$+Na requires *m/z* 340.1347

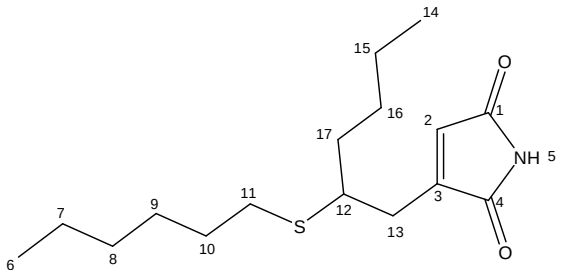
Found 340.1357 (FAB+). Elemental analysis: Calc for $C_{16}H_{23}NO_2S$: C: 68.10, H: 7.31, N: 4.41 Found: C: 68.31, H: 7.41, N: 4.34.

408. δ_H (600 MHz, $CDCl_3$) 7.34-7.24 (m, 5H, 5 x Ar-H), 7.22 (d, 1H, *J* = 1.5, H-5), 6.15 (app q, 1H, *J* = 1.5, H-2), 4.11 (t, 1H, *J* = 7.7, H-12), 3.01 (ddd, 1H, *J* = 15.8, 7.8 and 1.5, *H*H-13), 2.96 (ddd, 1H, *J* = 15.6, 7.8 and 1.5, H*H*-13), 2.36-2.26 (m, 2H, H_2-11), 1.50-1.41 (m, 2H, H_2-10), 1.35-1.33 (m, 6H, H_2-7, H_2-8 and H_2-9), 0.85 (t, 3H, *J* = 7.0, H_3-6); δ_C (150 MHz, $CDCl_3$) 170.96 (C=O), 169.95 (C=O), 147.26 (C3), 141.10 (C17), 129.41 (C2), 128.89 (2 x Ar-H), 127.86 (C14), 127.71 (2 x Ar-H), 47.24 (C12), 32.47 (C11), 31.43 (CH_2), 31.31 (CH_2), 29.19 (CH_2), 28.61 (CH_2), 22.60 (CH_2), 14.10 (C6); IR (oil, cm^{-1}) 3288 (w), 2928 (w), 1775 (w), 1717 (s); MS (FAB+) *m*/z (relative intensity): 340 ([M+Na], 20), 329 (35), 207 (20), 176 (100); Exact Mass Calcd for [$C_{16}H_{23}NO_2S$]+Na requires *m/z* 340.1347 Found 340.1351 (FAB+).

377. (4RS, 5RS, 7SR)-2-Aza-4-hexylsulfanyl-5-butyl-bicyclo[3.2.0]heptan-1,3-dione



412. 3-(2-Hexylsulfanyl-hexyl)-pyrrole-2,5-dione and



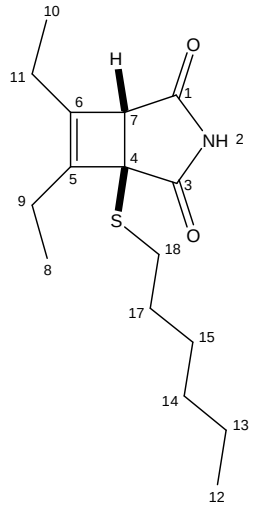
342 (25 mg, 0.116 mmol) was dissolved in acetonitrile (20.7 mL) and hex-1-ene (4.3 mL, 11.6 mmol). The resulting solution was degassed for 30 minutes and irradiated in

pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded a mixture of **377** and **412** (6 mg, 0.021 mmol). NMR investigations showed the mixture was a 1:2 ratio of **377**:**412**, thus representing **412** (4 mg, 0.013 mmol) in 12% yield and **377** (2 mg, 0.0068 mmol) in 6% yield. **377** was also isolated pure as a colourless oil (14 mg, 0.047 mmol) in 41% yield. Further column chromatography afforded a pure sample of **412** as an oil.

377. δ_H (600 MHz, $CDCl_3$) 8.19 (s, 1H, H-2), 3.09 (dd, 1H, *J* = 10.2 and 4.5, H-7), 2.70-2.64 (m, 1H, H-5), 2.53-2.45 (m, 2H, H_2-17), 2.37-2.28 (m, 2H, H_2-6), 1.80-1.73 (m, 1H, H*H*-11), 1.59-1.50 (m, 2H, *H*H-11 and H*H*-10), 1.38-1.20 (m, 11H, *H*H-10 and 5 x CH_2), 0.90 (t, 3H, *J* = 7.1, CH_3), 0.87 (t, 3H, *J* = 7.3, CH_3); δ_C (150 MHz, $CDCl_3$) 178.73 (C=O), 177.81 (C=O), 56.10 (C4), 44.29 (C7), 40.99 (C5), 31.42 (C17), 29.24 (CH_2), 28.99 (CH_2), 28.90 (CH_2), 28.74 (CH_2), 28.73 (CH_2), 27.92 (CH_2), 22.59 (CH_2), 22.58 (CH_2), 14.12 (CH_3), 14.09 (CH_3); IR (oil, cm^{-1}) 3209 (w), 2927 (m) 1774 (w), 1711 (s); MS (CI+) *m*/z (relative intensity): 298 ([M+H], 100); Exact Mass Calcd for $[C_{16}H_{27}NO_2S]$+H requires *m/z* 298.1841 Found 298.1845 (CI+).

412. δ_H (600 MHz, $CDCl_3$) 7.29 (br s, 1H, NH), 6.43 (app q, 1H, *J* = 1.4, H-2), 2.91-2.76 (m, 3H, H-12 and H_2-17), 2.70 (ddd, 1H, *J* = 15.1, 6.5 and 1.4, H*H*-13), 2.64 (ddd, 1H, *J* = 15.1, 6.5 and 1.4, *H*H-13), 2.49 (t, 1H, *J* = 7.4, H_2-11), 1.81-1.25 (m, 12H, 6 x CH_2), 0.93-0.85 (m, 6H, H_3-6 and H_3-14); δ_C (150 MHz, $CDCl_3$) 174.39 (C=O), 171.40 (C=O), 148.19 (C3), 129.28 (C2), 43.90 (C12), 34.97 (CH_2), 31.53 (CH_2), 30.60 (CH_2), 29.70 (CH_2), 29.07 (CH_2), 28.76 (CH_2), 28.51 (CH_2), 22.66 (CH_2), 22.65 (CH_2), 14.16 (CH_3), 14.13 (CH_3); IR (oil, cm^{-1}) 3226 (w), 2927 (m) 1715 (s); MS (CI+) *m*/z (relative intensity): 298 ([M+H], 80), 187 (100); Exact Mass Calcd for $[C_{16}H_{27}NO_2S]$+H requires *m/z* 298.1841 Found 298.1841 (CI+).

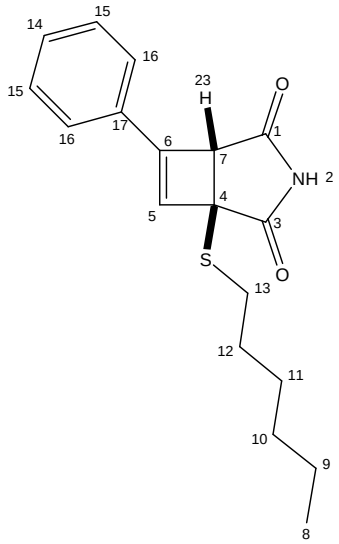
380. (4RS, 7RS)-2-Aza-4-hexylsulfanyl-5-ethyl-6-ethyl-bicyclo[3.2.0]hept-5-ene-1,3-dione



342 (25 mg, 0.116 mmol) was dissolved in acetonitrile (21.1 mL) and hex-3-yne (3.9 mL, 11.6 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **380** as a colourless oil (17 mg, 0.057 mmol) in 49% yield.

δ_H (600 MHz, $CDCl_3$) 8.26 (s, 1H, NH), 3.88 (s, 1H, H-7), 2.99 (ddd, 1H, *J* = 12.8, 9.1 and 7.5, H*H*-18), 2.87 (ddd, 1H, *J* = 12.9, 8.7 and 5.1, *H*H-18), 2.39-2.20 (m, 2H, *H*H-9 and *H*H-11), 1.95-1.75 (m, 2H, H*H*-9 and H*H*-11), 1.56-1.10 (m, 8H, 4 x CH_2), 0.90-0.86 (m, 9H, H_3-8, H_3-10 and H_3-12); δ_C (150 MHz, $CDCl_3$) 172.66 (C=O), 171.34 (C=O), 148.84 (C=C), 142.53 (C=C), 70.45 (C4), 48.86 (C18), 48.09 (C7), 31.45 (CH_2), 28.51 (CH_2), 23.53 (CH_2), 22.50 (CH_2), 21.89 (CH_2), 21.62 (CH_2) 14.09 (C12), 12.03 (CH_3), 11.84 (CH_3); IR (oil, cm^{-1}) 2931 (m) 1717 (s); MS (CI+) *m*/z (relative intensity): 312 ([M+OH], 100), 178 (100); Exact Mass Calcd for $[C_{16}H_{25}NO_2S]$+OH requires *m/z* 312.1633 Found 312.1648 (CI+).

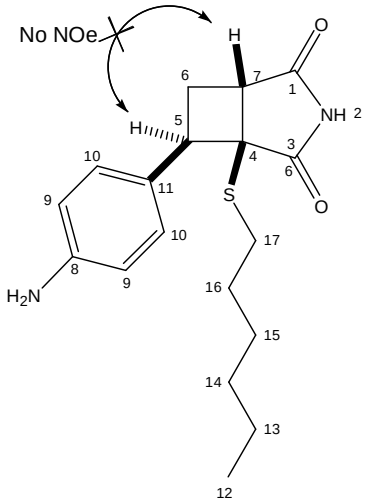
381. (4RS, 7SR)-2-Aza-4-hexylsulfanyl-bicyclo[3.2.0]hept-5-ene-1,3-dione



342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, phenyl acetylene (128 μL, 1.16 mmol) added and irradiated in pyrex glassware for 30 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **381** as a colourless oil (4.5 mg, 0.014 mmol) in 12% yield and **342** (1.5 mg, 0.007 mmol) in 6% yield (data matched that obtained previously).

δ_H (600 MHz, $CDCl_3$) 7.86 (s, 1H, 2-H), 7.68 (d, 2H, *J* = 7.86, 2 x Ar-H), 7.40-7.34 (m, 3H, 3 x Ar-H), 6.58 (s, 1H, H-5), 3.73 (s, 1H, H-7), 2.57 (td, 1H, *J* = 7.4 and 2.3, H_2-13), 1.77-1.19 (m, 8H, 4 x CH_2), 0.85 (t, 3H, *J* = 7.3, H_3-8); δ_C (150 MHz, $CDCl_3$) 173.46 (C=O), 173.28 (C=O), 149.72 (C17), 130.25 (C6), 130.02 (C14), 128.85 (2 x Ar-H), 126.32 (2 x Ar-H), 125.76 (C5), 53.78 (C7), 31.33 (CH_2), 29.70 (CH_2), 29.32 (CH_2), 28.61 (CH_2), 22.55 (CH_2), 14.11 (C8); IR (oil, cm^{-1}) 3228 (w), 2925 (m), 1770 (w) 1709 (s); MS (CI+) *m*/z (relative intensity): 316 ([M+H], 100), 214 (30); Exact Mass Calcd for $[C_{18}H_{21}NO_2S]$+H requires *m/z* 316.0371 Found 316.1365 (CI+).

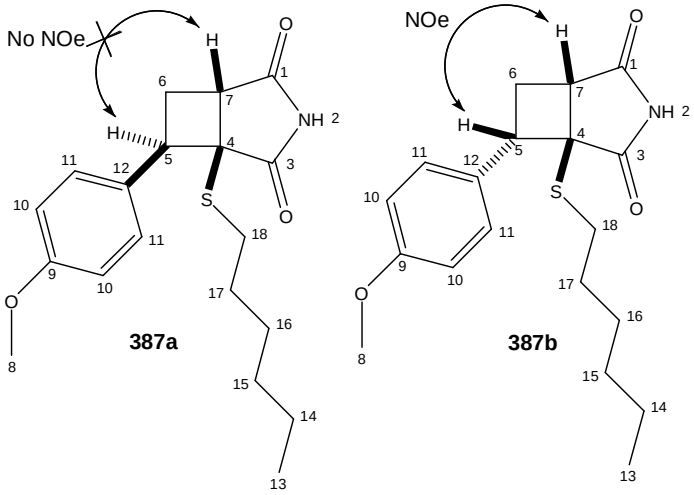
386. (4RS, 5SR, 7SR)-2-Aza-4-hexylsulfanyl-5-(*p*-amino)phenyl-bicyclo[3.2.0]heptan-1,3-dione



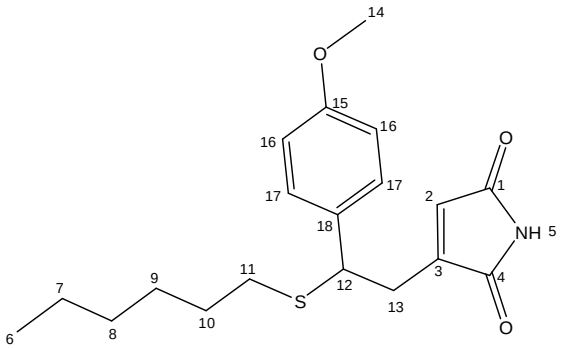
342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, 4-vinyl aniline (136 μL, 1.2 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **386** as a thick colourless oil (7 mg, 0.021 mmol) in 17% yield.

δ_H (600 MHz, $CDCl_3$) 8.17 (s, 1H, NH), 7.10 (d, 2H, *J* = 8.5, 2 x Ar-H), 6.67 (d, 2H, *J* = 8.5, 2 x Ar-H), 3.94 (app t, 1H, *J* = 9.0, H-5), 3.13 (dd, 1H, *J* = 11.1 and 3.7, H-7), 2.98 (ddd, 1H, *J* = 12.9, 11.2 and 8.8, H*H*-6), 2.58 (ddd, 1H, *J* = 12.8, 9.1 and 3.5, *H*H-6), 2.42 (td, 1H, *J* = 11.5 and 7.5, *H*H-17), 2.17 (td, 1H, *J* = 11.5 and 7.5, H*H*-17), 1.34-1.29 (m, 2H, H_2-16), 1.25-1.11 (m, 6H, H_2-13, H_2-14 and H_2-15), 0.80 (t, 3H, *J* = 7.4, H_3-12); δ_C (150 MHz, $CDCl_3$) 178.64 (C=O), 177.48 (C=O), 146.28 (C8), 129.81 (2 x Ar-H), 126.20 (C11), 114.81 (2 x Ar-H), 57.97 (C4), 45.48 (C5), 43.79 (C7), 31.35 (CH_2), 29.07 (CH_2), 28.65 (CH_2), 28.64 (CH_2), 26.41 (C6), 22.54 (CH_2), 14.12 (C12); IR (oil, cm^{-1}) 3214 (w), 2928 (w) 1769 (m), 1715 (s); MS (CI+) *m*/z (relative intensity): 333 ([M+H], 55), 119 (100); Exact Mass Calcd for $[C_{18}H_{24}N_2O_2S]$+H requires *m/z* 333.1637 Found 333.1642 (CI+).

387a. (4RS, 5SR, 7SR) – 2 – Aza – 4 – hexylsulfanyl – 5 - (*p* - methoxy)phenyl-bicyclo[3.2.0]heptan-1,3-dione and 387b. (4RS, 5RS, 7SR) - 2-aza-4-hexylsulfanyl-5-(*p*-methoxy)phenyl-bicyclo[3.2.0]heptan-1,3-dione



409. 3-[2-Hexylsulfanyl-2-(4-methoxy-phenyl)-ethyl]-pyrrole-2,5-dione



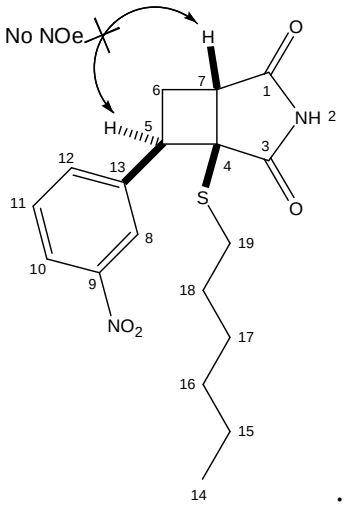
342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, 4-methoxystyrene (154 μL, 1.20 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **409** as a colourless oil (10 mg, 0.037 mmol) in 25% yield and **387a** and **387b** (27 mg, 0.77 mmol) in 67% yield as a mixture of diastereomers 1:10.

387a. and 387b. δ_H (600 MHz, $CDCl_3$) 8.71 (s, 1H, NH_b), 8.45 (s, 1H, NH_a), 7.24 (d, 2H, *J* = 7.9, 2 x Ar-H_b), 7.15 (d, 2H, *J* = 8.7, 2 x Ar-H_a), 6.90 (d, 2H, *J* = 8.6, 2 x Ar-H_b), 6.86 (d, 2H, *J* = 8.6, 2 x Ar-H_a), 3.99 (app t, 1H, *J* = 8.8, H-5_b), 3.94 (dd, 1H, *J* = 10.1 and 8.4, H-5_a), 3.81 (s, 3H, H_3-8_b), 3.77 (s, 3H, H_3-8_a), 3.20 (dd, 1H, *J* = 11.9 and 4.5, H-7_a), 3.13 (dd, 1H, *J* = 11.0 and 3.4, H-7_b), 3.09 (app td, 1H, *J* = 11.9 and 10.6, H*H*-6_a), 2.98 (ddd, 1H, *J* = 12.9, 11.2 and 8.7, H*H*-6_b), 2.67 (td, 1H, *J* = 11.5 and 7.3,

H*H*-18_a), 2.63-2.53 (m, 2H, *H*H-6_b and *H*H-18_a), 2.54 (ddd, 1H, *J* = 11.9, 8.4 and 4.5, *H*H-6_a), 2.43 (ddd, 1H, *J* = 11.3, 8.2 and 6.7, H*H*-18_b), 2.15 (ddd, 1H, *J* = 11.4, 8.4 and 6.7, *H*H-18_b), 1.56-1.52 (m, 2H, H_2-17_a), 1.39-1.33 (m, 2H, $CH_{2\text{-}a}$), 1.31-1.09 (m, 12H, H_2-14_b, H_2-15_b, H_2-16_b, H_2-17_b and 2 x $CH_{2\text{-}a}$), 0.87 (t, 3H, *J* = 7.1, H_3-13_a), 0.83 (t, 3H, *J* = 7.1, H_3-13_b); δ_C (150 MHz, $CDCl_3$) 177.81 (C=O_b), 175.48 (C=O_b), 175.48(C=O_a), 159.39 (C9_b), 159.20 (C9_a), 129.94 (2 x Ar-H_b), 129.01 (2 x Ar-H_a), 128.56 (C12_b), 128.49 (C12_a), 114.11 (2 x Ar-H_a), 113.65 (2 x Ar-H_b), 57.63 (C4_b), 57.38 (C4_a), 55.38 (C8_b), 55.33 (C8_a), 45.25 (C5_b), 47.47 (C5_a), 43.78 (C7_b), 43.93 (C7_a), 31.41 ($CH_{2\text{-}a}$), 31.33 ($CH_{2\text{-}b}$), 30.10 ($CH_{2\text{-}a}$), 29.35 ($CH_{2\text{-}a}$), 29.23 ($CH_{2\text{-}b}$), 29.01 ($CH_{2\text{-}b}$), 28.70 (C18_a), 28.62 (C18_b), 26.53 (C6_b), 26.25 (C6_a), 22.52 ($CH_{2\text{-}b}$), 22.59 ($CH_{2\text{-}a}$), 14.14 (C13_a), 14.10 (C13_b) One carbon signal from **387a** is missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3216 (w), 2928 (m) 1771 (m), 1707 (s); MS (CI+) *m*/z (relative intensity): 348 ([M+H], 20), 135 (20), 134 (100); Exact Mass Calcd for [$C_{19}H_{25}NO_3S$]+H requires *m/z* 348.1633 Found 363.1642 (CI+).

409. δ_H (600 MHz, $CDCl_3$) 7.21 (d, 2H, *J* = 8.5, 2 x Ar-H), 7.18 (br s, 1H, NH), 6.84 (d, 2H, *J* = 9.0, 2 x Ar-H), 6.13 (app q, 1H, *J* = 1.1, H-2), 4.08 (t, 1H, *J* = 7.9, H-12), 3.80 (s, 3H, H_3-14), 2.99 (ddd, 1H, *J* = 15.7, 7.2 and 1.1, *H*H-13), 2.91 (ddd, 1H, *J* = 15.7, 8.7 and 1.1, H*H*-13), 2.35-2.26 (m, 2H, H_2-11), 1.51-1.43 (m, 2H, H_2-10), 1.33-1.16 (m, 6H, H_2-7, H_2-8 and H_2-9), 0.85 (t, 3H, *J* = 7.0, H_3-6); δ_C (150 MHz, $CDCl_3$) 171.03 (C=O), 170.05 (C=O), 159.06 (C15), 147.37 (C3), 132.92 (C18), 129.37 (C2), 128.79 (2 x Ar-H), 114.18 (2 x Ar-H), 55.38 (C14), 46.61 (C12), 32.59 (C13), 31.45 (C11), 31.38 (CH_2), 29.22 (CH_2), 28.64 (CH_2), 22.61 (CH_2), 14.14 (C6); IR (oil, cm^{-1}) 3275 (w), 2927 (m) 1774 (w), 1717 (s); MS (CI+) *m*/z (relative intensity): 347 (M+, 15), 237 (70), 230 (100), 202 (60); Exact Mass Calcd for [$C_{19}H_{25}NO_3S$]+ requires *m/z* 347.1550 Found 363.1553 (CI+).

389a. (4RS, 5SR, 7SR)-2-Aza-4-hexylsulfanyl-5-(*m*-nitro)phenyl-bicyclo[3.2.0]heptan-1,3-dione



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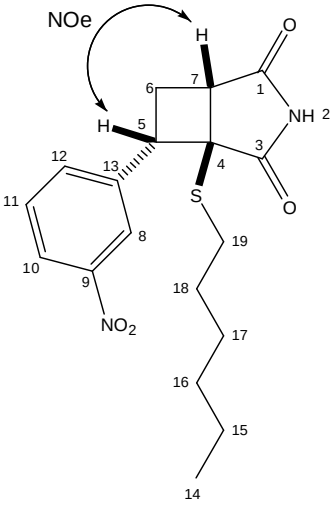
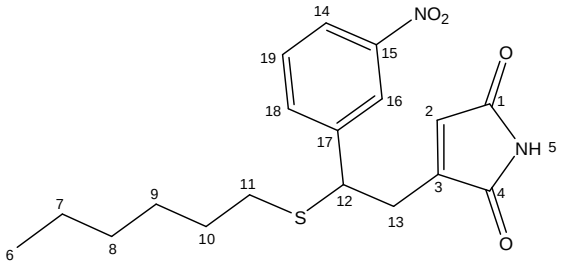
389b. (4RS, 5RS, 7SR) 2-Aza-4-hexylsulfanyl-5-(*m*-nitro)phenyl-bicyclo[3.2.0]heptan-1,3-dione



410. 3-[2-Hexylsulfanyl-2-(3-nitro-phenyl)-ethyl]-pyrrole-2,5-dione



342 (25 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, 3-nitrostyrene (136 μL, 1.2 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl

acetate in petroleum ether) afforded **410** as a thick colourless oil (12 mg, 0.33 mmol) in 21% yield, **389a** as a thick colourless oil (1.5 mg, 0.003 mmol) in 3% yield and **389b** as a thick colourless oil (23 mg, 0.063 mmol) in 55% yield,

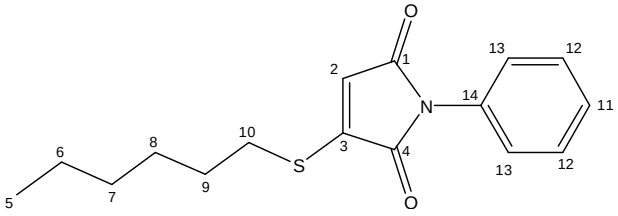
389a. δ_H (600 MHz, $CDCl_3$) 8.15 (d, 1H, *J* = 8.4, Ar-H), 8.07 (s, 1H, H-8), 7.84 (s, 1H, NH), 7.62 (d, 1H, *J* = 7.6, Ar-H), 7.53 (app t, 1H, *J* = 8.0, H-11), 4.10 (app t, 1H, *J* = 10.0, H-5), 3.30 (dd, 1H, *J* = 10.4 and 6.1, H-7), 3.17 (app dt, 1H, *J* = 13.3 and 10.3, H*H*-6), 2.68-2.56 (m, 3H, *H*H-6 and H_2-19), 1.40-1.35 (m, 2H, H_2-18), 1.31-1.23 (m, 6H, H_2-15, H_2-16 and H_2-17), 0.87 (t, 3H, *J* = 6.9, H_3-14); δ_C (150 MHz, $CDCl_3$) 176.25 (C=O), 174.16 (C=O), 148.44 (C9), 139.02 (C13), 133.88 (Ar-H), 129.69 (Ar-H), 123.07 (Ar-H), 121.92 (Ar-H), 57.34 (C4), 46.76 (C5), 44.18 (C7), 31.36 (CH_2), 30.21 (CH_2), 29.33 (C19), 28.68 (CH_2), 26.07 (C6), 22.57 (CH_2) 14.11 (C14); IR (oil, cm^{-1}) 2934 (w), 1719 (s); MS (CI+) *m*/z (relative intensity): 363 ([M+H], 65), 214 (90), 180 (100); Exact Mass Calcd for $[C_{18}H_{22}N_2O_4S]$+H requires *m/z* 363.1379 Found 363.1397 (CI+).

389b. δ_H (600 MHz, $CDCl_3$) 8.59 (s, 1H, NH), 8.22-8.16 (m, 2H, 2 x Ar-H), 7.66 (d, 1H, *J* = 7.5, Ar-H), 7.56 (app t, 1H, *J* = 8.0, H-11), 4.14 (app t, 1H, *J* = 8.6, H-5), 3.23 (dd, 1H, *J* = 11.6 and 3.0, H-7), 3.05 (ddd, 1H, *J* = 13.1, 11.1 and 8.5, H*H*-6), 2.73 (ddd, 1H, *J* = 12.9, 9.0 and 3.6, *H*H-6), 2.45 (ddd, 1H, *J* = 11.4, 8.1 and 6.7, *H*H-19), 2.13 (ddd, 1H, *J* = 11.3, 8.0 and 6.8, H*H*-19), 1.30-1.24 (m, 2H, H_2-18), 1.21-1.08 (m, 6H, H_2-15, H_2-16 and H_2-17), 0.81 (t, 3H, *J* = 7.1, H_3-14); δ_C (150 MHz, $CDCl_3$) 177.94 (C=O), 176.84 (C=O), 148.14 (C9), 138.76 (C13), 135.29 (Ar-H), 129.24 (Ar-H), 123.39 (Ar-H), 123.16 (Ar-H), 56.71 (C4), 45.09 (C5), 43.69 (C7), 31.26 (CH_2), 28.88 (CH_2), 28.84 (C19), 28.51 (C6), 26.33 (CH_2), 22.57 (CH_2), 14.06 (C14); IR (oil, cm^{-1}) 3214 (w), 2928 (w) 1773 (m), 1709 (s); MS (CI+) *m*/z (relative intensity): 363 ([M+H], 10), 214 (15), 84 (100); Exact Mass Calcd for $[C_{18}H_{22}N_2O_4S]$+H requires *m/z* 363.1379 Found 363.1394 (CI+).

410. δ_H (600 MHz, $CDCl_3$) 8.22 (s, 1H, H-16), 8.14 (d, 1H, *J* = 8.5, Ar-H), 7.68 (app t, 1H, *J* = 7.6, Ar-H), 7.53 (app t, 1H, *J* = 7.8, H-19), 7.20 (br s, 1H, NH), 6.29 (app q, 1H, *J* = 1.4, H-2), 4.25 (t, 1H, *J* = 7.9, H-12), 3.03 (ddd, 1H, *J* = 15.4, 8.4 and 1.1, H*H*-13), 2.98 (dd, 1H, *J* = 15.2, 7.4 and 1.1, *H*H-13), 2.37-2.26 (m, 2H, H_2-11), 1.30-1.24 (m, 2H, H_2-10), 1.21-1.08 (m, 6H, 3 x CH_2), 0.81 (t, 3H, *J* = 7.1, H_3-6); δ_C (150 MHz,

$CDCl_3$) 170.70 (C=O), 169.54 (C=O), 148.60 (C15), 146.35 (C3), 143.94 (C17), 133.83 (Ar-H), 129.96 (Ar-H), 129.86 (C2), 122.96 (Ar-H), 122.59 (Ar-H), 46.78 (C12), 32.52 (C13), 31.56 (CH_2), 31.37 (C11), 29.03 (CH_2), 28.52 (CH_2), 22.57 (CH_2), 14.11 (C6); IR (oil, cm^{-1}) 3282 (w), 2928 (m) 1775 (w), 1717 (s); Mass ion not found.

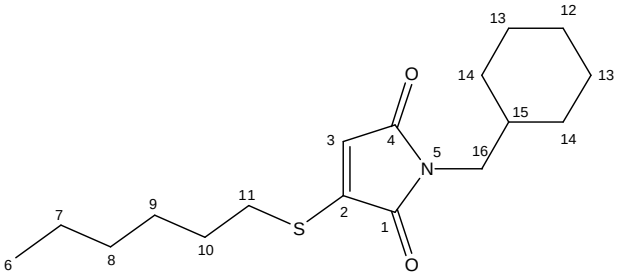
391. 3-Hexylsulfanyl-1-phenyl-pyrrole-2,5-dione



To **394** (200 mg, 0.79 mmol, from Dr J. Baker) in methanol (60 mL) was added hexanethiol (111 μL, 0.79 mmol) and sodium acetate trihydrate (108 mg, 0.79 mmol) in methanol (60 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **391** as a pale yellow solid (109 mg, 0.38 mmol) in 48% yield.

δ_H (600 MHz, $CDCl_3$) 7.45 (dd, 2H, *J* = 8.0 and 7.1, 2 x H-12), 7.36 (t, 1H, *J* = 7.1, H-11), 7.35 (d, 2H, *J* = 8.1, 2 x H-13), 6.19 (s, 1H, H-2), 2.96 (t, 2H, *J* = 7.9, H_2-10), 1.81-1.76 (m, 2H, H_2-9), 1.50-1.45 (m, 2H, H_2-8), 1.34-1.32 (m, 4H, H_2-6 and H_2-7), 0.91 (t, 3H, *J* = 6.9, H_3-5); δ_C (125 MHz, $CDCl_3$) 168.59 (C=O), 166.96 (C=O), 152.20 (C3), 131.53 (C14), 129.21 (2 x Ar-H), 127.93 (C11), 126.09 (2 x Ar-H), 117.24 (C2), 32.03 (C10), 31.33 (CH_2), 28.68 (CH_2), 27.78 (CH_2), 22.59 (CH_2), 14.11 (C5); IR (oil, cm^{-1}) 2931 (w), 1703 (s); MS (CI+) *m*/z (relative intensity): 290 ([M+H], 100); Exact Mass Calcd for $[C_{16}H_{19}NO_2S]$+H requires *m/z* 290.1215 Found 290.1224 (CI+); m.p. 102-104 ^oC; UV (Acetonitrile): ε_{277} = 9150 and ε_{346} = 5880 $cm^{-1}M^{-1}$.

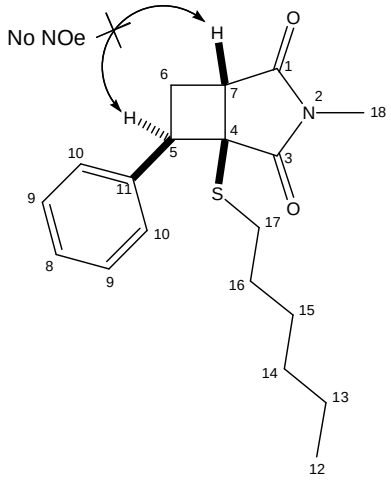
392. 1-Cyclohexylmethyl-3-hexylsulfanyl-pyrrole-2,5-dione



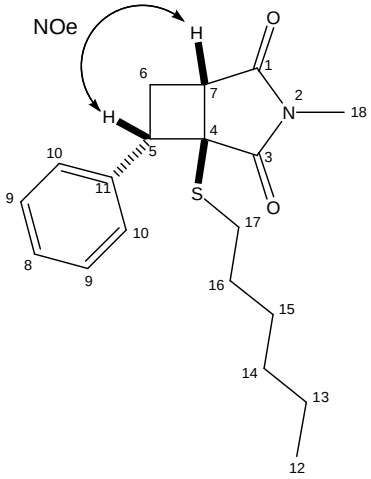
To **397** (50 mg, 0.19 mmol) in methanol (50 mL), was added hexanethiol (52 μL, 0.37 mmol) and sodium acetate (50 mg, 0.37 mmol) in methanol (50 mL) dropwise over 5 minutes. After 10 minutes, the solvent was removed *in vacuo* and purification by flash chromatography (petroleum ether) afforded **392** as an off-white solid (29 mg, 0.09 mmol) in 84% yield.

δ_H (600 MHz, $CDCl_3$) 6.01 (s, 1H, H-3), 3.33 (d, 2H, *J* = 7.2, H_2-16), 2.89 (t, 2H, *J* = 7.3, H_2-11), 1.76-1.60 (m, 8H, H_2-10, H-15 and 5 x CH*H*), 1.45 (q, 2H, *J* = 7.4 H_2-9), 1.33-1.29 (m, 4H, H_2-7 and H_2-8), 1.22-111 (m, 2H), 0.96-0.86 (m, 3H), 0.89 (t, 3H, *J* = 6.9, H_3-6); δ_C (125 MHz, $CDCl_3$) 170.23 (C=O), 168.44 (C=O), 151.49 (C2), 117.08 (C3), 44.36 (C16), 37.00 (C15), 31.91 (2 x CH_2), 31.32 (2 x CH_2), 30.73 (CH_2), 28.66 (CH_2), 27.78 (CH_2), 26.33 (CH_2), 25.73 (CH_2), 22.58 (CH_2), 14.10 (C6); IR (solid, cm^{-1}) 2927 (m), 1701 (s); MS (ES+) *m/z*, (relative intensity): 310 ([M+H], 100), 180 (40); Mass calcd for $[C_{17}H_{27}O_2NS]$+H requires 310.1841 Found 310.1828 (ES+); UV (Acetonitrile): ε_{254} = 6680 and ε_{348} = 5040 $cm^{-1}M^{-1}$.

393a. (4RS, 5SR, 7SR)-2-Aza-2-methyl-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



393b. (4RS, 5RS, 7SR)-2-Aza-2-methyl-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



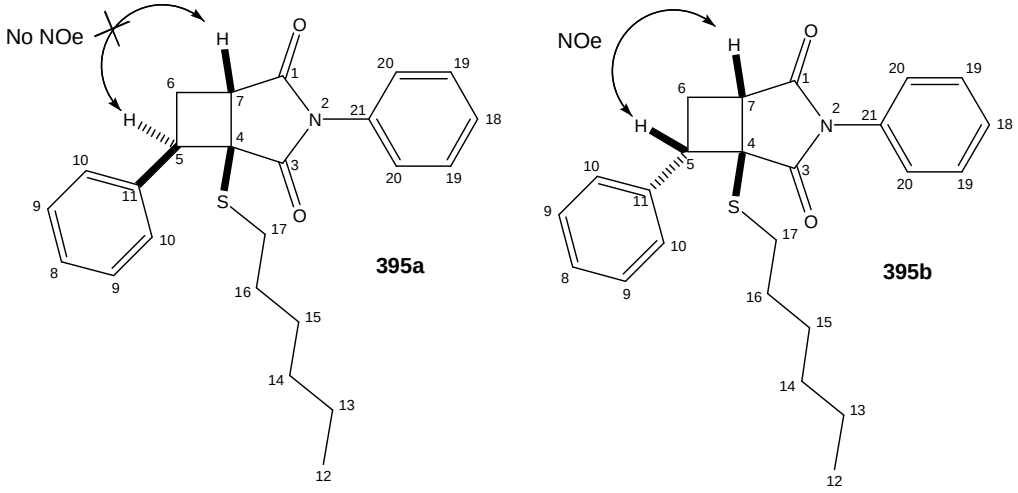
395 (27 mg, 0.119 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, styrene (136 μL, 1.19 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **393a** as a colourless oil (23 mg, 0.069 mmol) in 58% yield and **393b** as colourless oil (5 mg, 0.015 mmol) in 12%

393a. δ_H (600 MHz, $CDCl_3$) 7.39-7.30 (m, 5H, 5 x Ar-H), 3.90 (app t, 1H, *J* = 8.7, H-5), 3.13-3.11 (m, 4H, H_3-18 and H-7), 3.00 (ddd, 1H, *J* = 12.8, 11.0 and 8.5, H*H*-6), 2.53 (ddd, 1H, *J* = 12.8, 9.1 and 3.7, *H*H-6), 2.40 (ddd, 1H, *J* = 11.3, 8.1 and 6.4, *H*H-17), 2.06 (ddd, 1H, *J* = 11.3, 8.3 and 6.5, H*H*-17), 1.25-1.08 (m, 8H, H_2-13, H_2-14, H_2-15 and H_2-16), 0.82 (t, 3H, *J* = 7.4, H_3-12); δ_C (150 MHz, $CDCl_3$) 178.73 (C=O), 177.70 (C=O), 136.77 (C11), 128.89 (2 x Ar-H), 128.27 (2 x Ar-H), 127.99 (C8), 55.69 (C4), 45.62 (C5), 42.61 (C7), 31.29 (CH_2), 28.94 (CH_2), 28.68 (CH_2), 28.55 (C17), 26.26 (C6), 25.70 (C18), 22.58 (CH_2), 14.09 (C12); IR (oil, cm^{-1}) 2927 (w) 1703 (s),; MS (CI+) *m*/z (relative intensity): 332 ([M+H], 100); Exact Mass Calcd for $[C_{19}H_{25}NO_2S]$+H requires *m/z* 332.1684 Found 332.1680 (CI+).

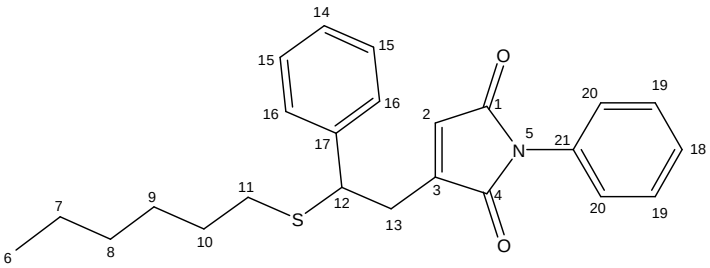
393b. δ_H (600 MHz, $CDCl_3$) 7.32-7.30 (m, 3H, 3 x Ar-H), 7.13 (d, 2H, *J* = 7.9, 2 x H-10), 3.99 (dd, 1H, *J* = 10.2 and 7.8, H-5), 3.20 (dd, 1H, *J* = 10.3 and 4.8, H-7), 3.13-3.07 (m, 1H, H*H*-6), 2.93 (s, 3H, H_3-18), 2.65 (dt, 1H, *J* = 11.4 and 7.5, *H*H-17), 2.58 (dt, 1H, *J* = 11.8 and 7.5, H*H*-17), 2.46 (ddd, 1H, *J* = 12.0, 7.7 and 4.9, *H*H-6), 1.55-1.25 (m, 8H, H_2-13, H_2-14, H_2-15 and H_2-16), 0.87 (t, 3H, *J* = 7.0, H_3-12); δ_C (150

MHz, $CDCl_3$) 177.78 (C=O), 175.17 (C=O), 137.21 (C11), 128.71 (2 x Ar-H), 127.93 (C8), 127.24 (2 x Ar-H), 55.39 (C4), 48.23 (C5), 42.71 (C7), 31.42 (CH_2), 30.07 (C17), 29.33 (CH_2), 28.69 (CH_2), 25.71 (CH_2), 25.25 (C18), 22.58 (CH_2), 14.12 (C12); IR (oil, cm^{-1}) 2927 (w) 1715 (s); MS (CI+) *m*/z (relative intensity): 332 ([M+H], 40), 228 (40), 86 (70), 84 (100); Exact Mass Calcd for $[C_{19}H_{25}NO_2S]$+H requires *m/z* 332.1684 Found 333.1697 (CI+).

395a. (4RS, 5SR, 7SR)-2-Aza-2-phenyl-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and 395b. (4RS, 5RS, 7SR)-2-aza-2-phenyl-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



411. 3-(2-Hexylsulfanyl-2-phenyl-ethyl)-1-phenyl-pyrrole-2,5-dione



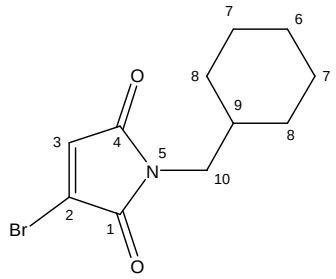
391 (34 mg, 0.12 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, styrene (135 μL, 1.18 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **395a** and **395b** as a colourless oil (37 mg, 0.94

mmol) in 80% (NMR investigations showed the mixture was a 1:11 ratio of a:b) and **411** as a colourless oil (0.5 mg, 0.001 mmol) in 1% yield.

395a. and **395b.** δ_H (600 MHz, $CDCl_3$) 7.54-7.52 (m, 2H, 2 x Ar-H_b), 7.46-7.42 (m, 4H, 4 x Ar-H_a), 7.41-7.37 (m, 10H, 8 x Ar-H_b and 2 x Ar-H_a), 7.27-7.26 (m, 2H, 2 x Ar-H_a), 7.06-7.05 (m, 2H, 2 x Ar-H_a), 4.09 (app t, 1H, *J* = 8.5, H-5_a), 4.08 (app t, 1H, *J* = 8.5, H-5_b), 3.35 (dd, 1H, *J* = 10.5 and 4.7, H-7_a), 3.29 (dd, 1H, *J* = 10.9 and 4.0, H-7_b), 3.20 (ddd, 1H, *J* = 13.3, 10.3 and 8.5, H*H*-6_a), 3.09 (ddd, 1H, *J* = 13.0, 11.0 and 8.2, H*H*-6_b), 2.78 (dt, 1H, *J* = 11.7 and 7.4, H*H*-17_a), 2.72-2.68 (m, 2H, *H*H-6_b and *H*H-6_a), 2.66 (dd, 1H, *J* = 7.4 and 4.8, *H*H-17_a), 2.52 (ddd, 1H, *J* = 11.4, 8.2 and 6.5, *H*H-17_b), 2.16 (ddd, 1H, *J* = 11.4, 8.4 and 6.6, H*H*-17_b), 1.63-1.58 (m, 2H, H_2-16_a), 1.42-1.37 (m, 2H, H_2-15_a), 1.36-1.09 (m, 6H, 4 x $CH_{2\text{-}b}$ and 2 x $CH_{2\text{-}a}$), 0.88 (t, 3H, *J* = 6.7, H_3-12_a), 0.83 (t, 3H, *J* = 7.3, H_3-12_b); δ_C (150 MHz, $CDCl_3$) 177.65 (C=O_b), 176.82 (C=O_a), 176.67 (C=O_b), 174.08 (C=O_a), 136.96 (C11_a), 136.82 (C11_b), 132.06 (C21_b), 131.86 (C21_a), 129.40 (2 x Ar-H_b), 129.22 (Ar-H_a), 128.96 (2 x Ar-H_b), 128.84 (Ar-H_a), 128.79 (Ar-H_a), 128.34 (2 x Ar-H_b), 128.08 (Ar-H_b), 127.51 (Ar-H_a), 126.56 (2 x Ar-H_b), 126.34 (Ar-H_a), 55.57 (C4_a), 55.47 (C4_b), 48.78 (C5_a), 45.87 (C5_b), 44.88 (C7_a), 42.79 (C7_b), 31.45 (C17_a), 31.31 (C17_b), 30.27 ($CH_{2\text{-}a}$), 29.46 ($CH_{2\text{-}a}$), 29.08 ($CH_{2\text{-}b}$), 28.75 ($CH_{2\text{-}a}$), 28.56 ($CH_{2\text{-}b}$), 26.95 ($CH_{2\text{-}b}$), 22.60 ($CH_{2\text{-}a}$), 22.52 ($CH_{2\text{-}b}$), 14.14 (C12_a), 14.10 (C12_b) Four carbon signals are missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 2926 (w) 1709 (s); MS (CI+) *m*/z (relative intensity): 394 ([M+H], 70), 290 (100), 105 (100); Exact Mass Calcd for $[C_{24}H_{27}NO_2S]$+H requires *m/z* 394.1841 Found 394.1834 (CI+).

411. δ_H (600 MHz, $CDCl_3$) 7.54-7.27 (m, 10H, 10 x Ar-H), 6.32 (t, 1H, *J* = 1.0, H-2), 4.19 (t, 1H, *J* = 8.0, H-12), 3.12 (ddd, 1H, *J* = 15.1, 7.5, and 1.0, *H*H-13), 3.07 (ddd, 1H, *J* = 15.6, 8.0 and 1.0, H*H*-13) 2.39-2.29 (m, 2H, H_2-11), 1.61-1.10 (m, 8H, 4 x CH_2), 0.89-0.81 (m, 3H, H_3-6); δ_C (150 MHz, $CDCl_3$) 172.61 (C=O), 169.60 (C=O), 146.31 (C3), 141.19 (Ar-H), 130.19 (Ar), 129.41 (Ar), 129.20 (Ar-H), 128.93 (2 x Ar-H), 128.49 (2 x Ar-H), 127.90 (C2), 127.76 (2 x Ar-H), 126.03 (2 x Ar-H), 47.19 (C12), 32.70 (CH_2), 31.43 (CH_2), 29.83 (CH_2), 29.20 (CH_2), 28.63 (CH_2), 22.61 (CH_2), 14.13 (C6); IR (oil, cm^{-1}) 2926 (m) 1715 (s); MS (CI+) *m*/z (relative intensity): 394 ([M+H], 40), 278 (100); Exact Mass Calcd for $[C_{24}H_{27}NO_2S]$+H requires *m/z* 394.1841 Found 394.1829 (CI+).

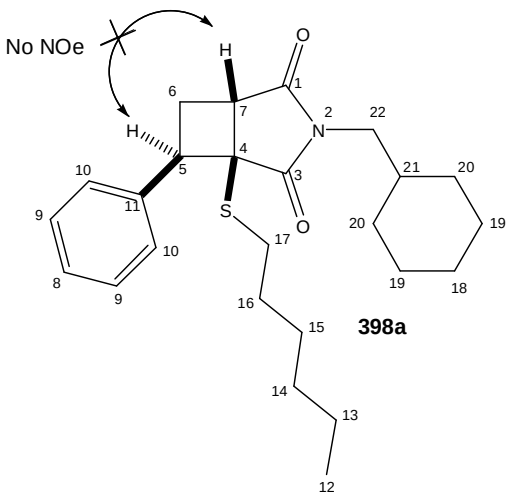
397. 3-Bromo-1-cyclohexylmethyl-pyrrole-2,5-dione

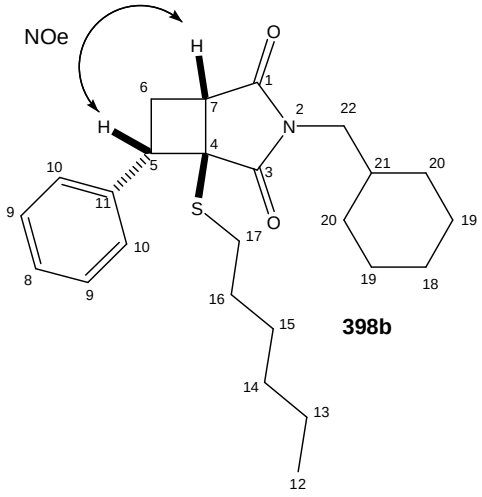


To bromomaleic anhydride (131 μL, 1.41 mmol) in acetic acid (20 mL) was added *N*-methylene cyclohexamine (183 μL, 1.41 mmol). The reaction mixture was stirred in a sealed tube for 3 hours at room temperature and then heated to 150 ^{o}C for 90 minutes. The solvent was removed *in vacuo* and purification by flash chromatography (petroleum ether) afforded **397** as a yellow powder (96 mg, 0.35 mmol) in 25% yield.

δ_H (600 MHz, $CDCl_3$) 6.85 (s, 1H, H-3), 3.39 (d, 2H, *J* = 7.3, H_2-10), 1.72-1.59 (m, 7H, H_2-6, 2 x *H*H-7, 2 x *H*H-8 and H-9), 1.3-1.10 (m, 2H, 2 x H*H*-7), 0.98-0.90 (m, 2H, 2 x H*H*-8); δ_C (150 MHz, $CDCl_3$) 169.03 (C=O), 165.73 (C=O), 131.78 (C3), 131.32 (C2), 45.06 (C10), 36.95 (C9), 30.68 (2 x CH_2), 26.25 (C6), 25.66 (2 x CH_2); IR (solid, cm^{-1}) 2925 (w), 1713 (s); MS (EI) *m/z*, (relative intensity): 273 (M+, 60), 271 (M+, 60), 191 (90), 189 (90), 95 (100); Exact mass calcd for $[C_{11}H_{14}O_2N^{79}Br]$+ requires 271.0202 Found 271.0192 (EI); m.p. 76-78 ^{o}C; UV (Acetonitrile): ε_{237} = 20700 and ε_{274} = 2790 $cm^{-1}M^{-1}$.

398a. (4RS, 5SR, 7SR)-2-Aza-2-methylenecyclohexane-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and 398b. (4RS, 5RS, 7SR)-2-aza-2-methylenecyclohexane-4-hexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



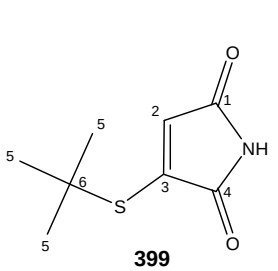


342 (25 mg, 0.116 mmol) was dissolved in acetonitrile (21 mL). The resulting solution was degassed for 30 minutes, styrene (133 μL, 1.16 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **398a** and **398b** as a colourless oil (30 mg, 0.075 mmol) in 64% yield as a mix of diastereoisomers. NMR investigations showed the mixture was a 1:10 ratio of **398a** to **398b**.

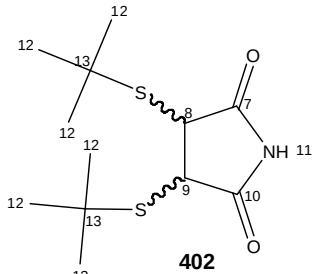
398a. and 398b. δ_H (600 MHz, $CDCl_3$) 7.39-7.29 (m, 7H, 5 x Ar-H_b and 2 x Ar-H_a), 7.24 (d, 1H, *J* = 7.4, H-8_a), 7.16 (d, 2H, *J* = 7.4, 2 x H-10_a), 4.00 (dd, 1H, *J* = 10.5 and 6.9, H-5_a), 3.89 (app t, 1H, *J* = 8.7, H-5_b), 3.46 (d, 4H, *J* = 7.5, H_2-22_b and H_2-22_a), 3.19 (dd, 1H, *J* = 10.5 and 6.9, H-7_a), 3.12 (dd, 1H, *J* = 10.9 and 3.5, H-7_b), 3.07 (app dt, 1H, *J* = 12.0 and 10.5, *H*H-6_a), 3.00 (ddd, 1H, *J* = 12.6, 11.5 and 8.5, *H*H-6_b), 2.64 (ddd, 1H, *J* = 14.0, 11.4 and 6.9, H*H*-17_a), 2.56 (app dt, 1H, *J* = 12.0 and 6.9, H*H*-6_a), 2.53 (ddd, 1H, *J* = 12.9, 9.1 and 3.3, H*H*-6_b), 2.48 (ddd, 1H, *J* = 14.0, 8.0 and 5.4, *H*H-17_a), 2.39 (dt, 1H, *J* = 11.4 and 7.4, H*H*-17_b), 2.08 (dt, 1H, *J* = 11.4 and 7.7, *H*H-17_b), 1.83-0.99 (m, 38H, 19H_b and 19H_a), 0.87 (t, 3H, *J* = 7.0, H_3-12_a), 0.82 (t, 3H, *J* = 7.5, H_3-12_b); δ_C (150 MHz, $CDCl_3$) 179.12 (C=O_b), 178.35 (C=O_a), 178.15 (C=O_b), 175.41 (C=O_a), 137.23 ($C11_a$), 137.12 ($C11_b$), 129.08 (2 x Ar-H_b), 128.76 (2 x Ar-H_a), 128.47 (2 x Ar-H_b), 128.16 ($C8_b$), 127.99 ($C8_a$), 127.51 (2 x Ar-H_a), 55.84 ($C4_a$), 55.69 ($C4_b$), 48.06 ($C5_a$), 45.97 ($C5_b$), 45.63 ($C22_b$), 45.48 ($C22_a$), 42.94 ($C7_a$), 42.83 ($C7_b$), 36.67 ($C21_a$), 36.58 ($C21_b$), 31.64 ($CH_{2\text{-}a}$), 31.49 ($CH_{2\text{-}b}$), 31.08 ($CH_{2\text{-}b}$), 31.01 ($CH_{2\text{-}b}$), 30.96 ($CH_{2\text{-}a}$), 30.34 ($CH_{2\text{-}a}$), 30.04 ($CH_{2\text{-}a}$), 29.67 ($CH_{2\text{-}a}$), 29.28 ($CH_{2\text{-}b}$), 28.98 ($CH_{2\text{-}b}$), 28.82 ($CH_{2\text{-}b}$), 26.76 ($CH_{2\text{-}a}$), 26.63 ($CH_{2\text{-}a}$), 26.52 ($CH_{2\text{-}b}$), 26.45 ($CH_{2\text{-}a}$), 25.96 ($CH_{2\text{-}b}$), 25.94 ($CH_{2\text{-}b}$),

25.82 ($CH_{2\text{-}a}$), 14.34 ($C12_a$), 14.31 ($C12_b$); IR (oil, cm^{-1}) 2925 (m) 1703 (s); MS (CI+) *m*/z (relative intensity): 414 ([M+H], 100), 309 (20); Exact Mass Calcd for $[C_{25}H_{35}NO_2S]$+H requires *m/z* 414.2461 Found 414.2452 (CI+)

399. 3-tert-Butylsulfanyl-pyrrole-2,5-dione and 402. 3,4-bis-*tert*-butylsulfanyl-pyrrolidine-2,5-dione (one diastereomer of undefined stereochemistry)



399

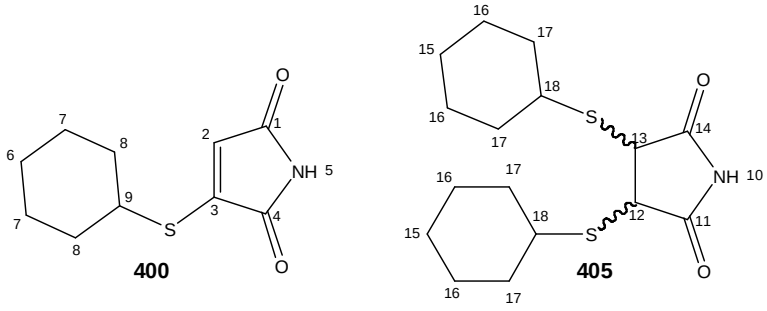


402

To **289** (200 mg, 1.12 mmol) in methanol (50 mL) was added 2-methyl-propane-2-thiol (210 μL, 1.65 mmol) and sodium acetate trihydrate (225 mg, 1.65 mmol) in methanol (50 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded a pale yellow solid (87 mg). ^{1}H NMR and subsequent calculations indicated that this mixture was 73% by mass **399** (63.5 mg, 0.34 mmol, 30% yield) and 25% by mass **402** (23.5 mg, 0.085 mmol, 8% yield).

δ_H (600 MHz, $CDCl_3$) 8.41 (br s, 1H, H-11), 7.90 (br s, 1H, NH), 6.21 (s, 1H, H-2), 3.49 (s, 2H, H-8 and H-9), 1.54 (s, 9H, 3 x H_3-5), 1.43 (s, 18H, 6 x H_3-12); δ_C (125 MHz, $CDCl_3$) 175.98 (C7 and C10), 170.18 (C=O), 169.10 (C=O), 149.73 (C3), 119.49 (C2), 49.32 (C8 and C9), 47.35 (C6), 45.83 (2 x C13), 31.40 (6 x C12), 29.81 (3 x C5); IR (solid, cm^{-1}) 3196 (m), 1770 (m), 1703 (s); MS (CI+) *m*/z (relative intensity): 186 ([M+H], 100), 178 (20); Exact Mass Calcd for $[C_8H_{11}NO_2S]$+H requires *m/z* 186.0589 Found 186.0592 (CI+);

400. 3-Cyclohexylsulfanyl-pyrrole-2,5-dione and 405. 3,4-bis-cyclohexylsulfanyl-pyrrolidine-2,5-dione



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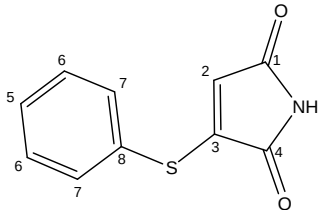
To **289** (200 mg, 1.12 mmol) in methanol (50 mL) was added cyclohexylmercaptan (226 μL, 1.65 mmol) and sodium acetate trihydrate (225 mg, 1.65 mmol) in methanol (50 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded a pale yellow solid (64 mg). ^{1}H NMR and subsequent calculations indicated that this mixture was 66% by mass **400** (42 mg, 0.20 mmol, 18% yield) and 34% **405** (22 mg, 0.06 mmol, 5% yield).

δ_H (600 MHz, $CDCl_3$) 8.16 (br s, 1H, H-10), 7.28 (s, 1H, H-5), 6.01 (s, 1H, H-2), 3.53 (s, 2H, H-12 and H-13), 3.23 (tt, 1H, *J* = 10.0 and 3.6, H-9), 3.14 (tt, 2H, *J* = 10.3 and 3.5, 2 x H-18), 2.11-2.04 (m, 4H), 1.96-1.94 (m, 1H), 1.83-1.74 (m, 5H), 1.66-1.53 (m, 7H), 1.46-1.32 (m, 11H), 1.29-1.24 (m, 2H); δ_C (125 MHz, $CDCl_3$) 175.08 (C11 and C14), 169.54 (C=O), 168.20 (C=O), 151.77 (C3), 118.16 (C2), 47.24 (CH), 45.13 (CH), 44.65 (CH), 33.51 (4 x CH_2), 33.01 (4 x CH_2), 32.18 (2 x CH_2), 25.71 (C6), 25.59 (2 x C15), 25.56 (2 x CH_2); IR (solid, cm^{-1}) 3284 (w), 2931 (m), 1771 (w), 1713 (s); MS (CI+) *m*/z (relative intensity): 212 ([M+H], 100), 130 (10); Exact Mass Calcd for [$C_{10}H_{13}NO_2S$]+H requires *m/z* 186.0745 Found 212.0752 (CI+);

401. 3-Phenylsulfanyl-pyrrole-2,5-dione

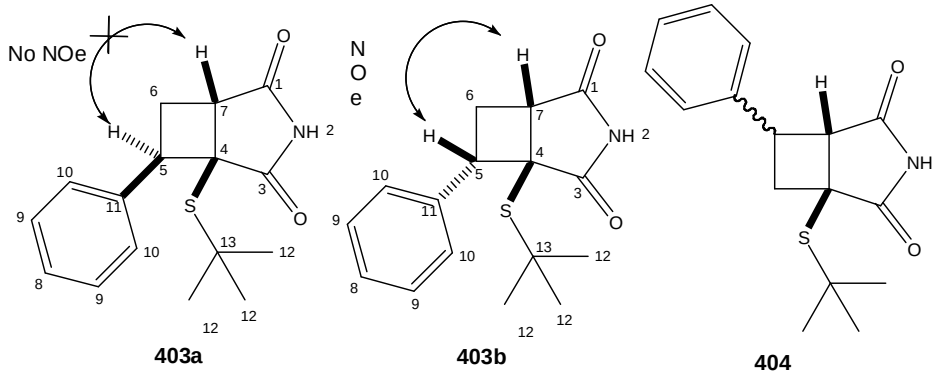


To **289** (100 mg, 0.56 mmol) in methanol (30 mL) was added thiophenol (57 μL, 0.56 mmol) and sodium acetate trihydrate (136 mg, 0.56 mmol) in methanol (30 mL)

dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **401** as a pale yellow solid (22 mg, 0.11 mmol) in 19% yield.

δ_H (600 MHz, $CDCl_3$) 7.56 (dd, 2H, *J* = 7.8 and 1.6, 2 x H-7), 7.50-7.48 (m, 3H, 3 x Ar), 5.63 (s, 1H, H-2); δ_C (125 MHz, $CDCl_3$) 169.42 (C=O), 167.98 (C=O), 153.60 (C3), 134.45 (2 x Ar-H), 130.68 (C5), 130.42 (2 x Ar-H), 127.27 (C8), 119.91 (C2); IR (oil, cm^{-1}) 3265 (m), 1770 (m), 1701 (s); MS (CI+) *m*/z (relative intensity): 206 ([M+H], 100), 111 (40); Exact Mass Calcd for $[C_{10}H_7NO_2S]$+H requires *m/z* 206.0276 Found 206.0273 (CI+); m.p. 120-132 ^oC; UV (Acetonitrile): ε_{335} = 8500 $cm^{-1}M^{-1}$.

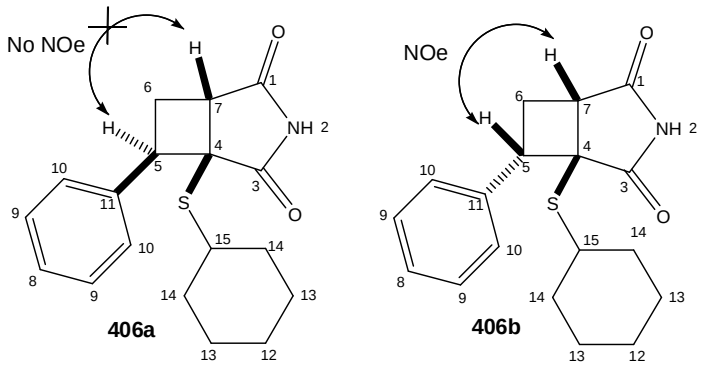
403a. (4RS, 5SR, 7SR)-(2-Aza-4-*tert*butylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione, 403b. (4RS, 5RS, 7SR)-(2-aza-4-*tert*butylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione,and 404. 4RS, 6SR, 7SR)-(2-aza-4-*tert*butylsulfanyl-6-phenyl-bicyclo[3.2.0]heptan-1,3-dione and 404. (4RS, 6RS, 7SR)-(2-aza-4-*tert*butylsulfanyl-6-phenyl-bicyclo[3.2.0]heptan-1,3-dione.



Crude **399** (26 mg, of which 19 mg, 0.102 mmol was **399**) was dissolved in acetonitrile (20 mL). The resulting solution was degassed for 30 minutes, styrene (133 μL, 1.16 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 20% ethyl acetate in petroleum ether) afforded **402** as a yellow solid (7 mg, 0.025 mmol, data matched that previously obtained) in 100% yield and **403a**, **403b** and **404** together as a colourless oil (27 mg, 0.075 mmol) in 92%, based on thiomaleimide **399**, as a mix of diastereoisomers. NMR investigations indicated that the mixture was a 13:87 ratio of **403a**:**403b**.

δ_H (600 MHz, $CDCl_3$) 8.66 (s, 1H, H-2_b), 8.43 (s, 1H, H-2_a), 7.37-7.21 (m, 10H, 5 x Ar-H_b and 5 x Ar-H_a), 4.05 (app t, 1H, *J* = 9.3, H-5_b), 3.96 (app t, 1H, *J* = 9.3, H-5_a), 3.44 (dd, 2H, *J* = 10.8 and 2.8, H-7_a and H-7_b), 3.20-3.11 (m, 2H, H*H*-6_a and H*H*-6_b), 2.65-2.56 (m, 2H, *H*H_a-6 and *H*H_b-6), 1.34 (s, 9H, 3 x H_3-12_a), 1.25 (s, 9H, 3 x H_3-12_b); δ_C (150 MHz, $CDCl_3$) 179.58 (C=O_b), 177.74 (C=O_b), 177.69 (C=O_a), 175.70 (C=O_a), 136.34 ($C11_a$), 135.36 ($C11_b$), 128.88 (2 x Ar-H_b), 128.67 (2 x Ar-H_a), 128.20 (2 x Ar-H_b), 128.07 (Ar-H_b), 128.02 (Ar-H_a), 127.52 (2 x Ar-H_a), 57.81 ($C13_a$), 57.56 ($C13_b$), 48.97 ($C5_b$), 48.90 ($C5_a$), 46.34 ($C4_b$), 45.08 ($C7_b$), 32.56 (3 x $C12_b$), 32.42 (3 x $C12_a$), 26.28 ($C6_a$), 25.82 ($C6_a$) Two carbon signals are missing from the minor diasteromer, proposed as due to overlap of the diastereomers; IR (oil, cm^{-1}) 3210 (w), 2962 (w), 1771 (m), 1708 (s); MS (CI+) *m*/z (relative intensity): 290 ([M+H], 100), 234 (75); Exact Mass Calcd for $[C_{16}H_{19}NO_2S]$+H requires *m/z* 290.1215 Found 290.1206 (CI+)

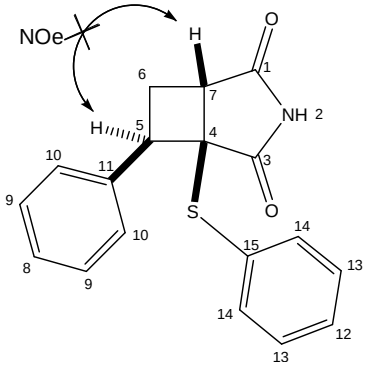
406a. (4RS, 5SR, 7SR)-2-Aza-4-cyclohexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and 406b. (4RS, 5RS, 7SR)-2-aza-4-cyclohexylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



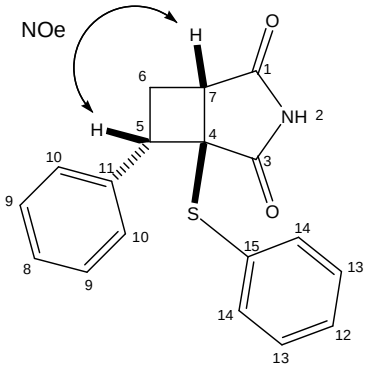
Crude **400** (42 mg, of which 29 mg, 0.14 mmol was **400**) was dissolved in acetonitrile (27 mL). The resulting solution was degassed for 30 minutes, styrene (156 μL, 1.36 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 20% ethyl acetate in petroleum ether) afforded **405** as a yellow powder (4 mg, 0.012 mmol, data matched that previously obtained) in 29% yield and **406a** and **406b** as a colourless oil (32 mg, 0.101 mmol) in 74% as a mix of diastereoisomers. NMR investigation indicated that the mixture was a 83:17 ratio of **406a:406b**.

δ_H (600 MHz, $CDCl_3$) 8.76 (br s, 1H, H-2_a), 8.50 (br s, 1H, H-2_b), 7.39-7.23 (m, 10H, 5 x Ar-H_a and 5 x Ar-H_b), 4.06 (app t, 1H, *J* = 8.9, H-5_a), 3.97 (dd, 1H, *J* = 10.2 and 8.4, H-5_b), 3.26 (dd, 1H, *J* = 10.5 and 5.3, H-7_b), 3.20 (dd, 1H, *J* = 11.0 and 3.4, H-7_a), 3.13-3.04 (m, 2H, *H*H-6_a and *H*H-6_b), 2.90 (tt, 1H, *J* = 10.7 and 3.9, H-15_b), 2.68-2.58 (m, 3H, H*H*-6_a, H*H*-6_a and H-15_a), 1.89-0.82 (m, 20H); δ_C (150 MHz, $CDCl_3$) 178.9 ($C{=}O_a$), 177.93 ($C{=}O_a$), 177.86 ($C{=}O_b$), 175.19 ($C{=}O_b$), 136.75 ($C11_b$), 136.40 ($C11_a$), 128.88 (2 x Ar-H_a), 128.72 (Ar-H_b), 128.31 (2 x Ar-H_a), 128.01 (Ar-H_a), 127.35 (Ar-H_b), 57.56 ($C4_b$), 57.31 ($C4_a$), 48.41 ($C5_b$), 46.74 ($C5_a$), 45.27 ($C7_b$), 44.72 ($C7_a$), 43.80 ($C15_b$), 42.81 ($C15_a$), 35.21 ($CH_{2\text{-}b}$), 34.90 ($CH_{2\text{-}a}$), 34.20 ($CH_{2\text{-}a}$), 33.74 ($CH_{2\text{-}b}$), 26.14 ($C6_a$), 26.02 ($C6_b$), 25.49 ($CH_{2\text{-}b}$), 25.41 ($CH_{2\text{-}a}$) Two diastereomers are indicated, thus one carbon signal is missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3209 (w), 2929 (m), 1771 (m), 1706 (s); MS (CI+) *m*/z (relative intensity): 316 ([M+H], 100), 212 (25); Exact Mass Calcd for $[C_{18}H_{21}NO_2S]$+H requires *m/z* 316.1371 Found 316.1367 (CI+)

407a. (4RS, 5SR, 7SR)-2-Aza-4-phenylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



407b. (4RS, 5RS, 7SR)-2-Aza-4-phenylsulfanyl-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione

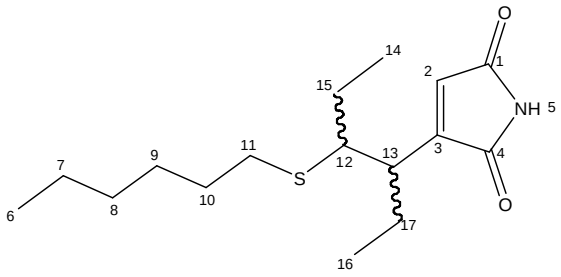


401 (17 mg, 0.082 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, styrene (111 μL, 0.82 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **407a** as a colourless oil (1.5 mg, 0.005 mmol) in 6% yield and **407b** as a colourless oil (17.5 mg, 0.056 mmol) in 67% yield.

407a. δ_H (600 MHz, $CDCl_3$) 8.05 (s, 1H, 2-H), 7.42-7.41 (m, 2H, 2 x Ar-H), 7.35-7.17 (m, 8H, 8 x Ar-H), 4.05 (app t, 1H, *J* = 10.1, H-5), 3.29 (dd, 1H, *J* = 13.0 and 5.5, H-7), 3.01 (app td, 1H, *J* = 13.0 and 10.3, H*H*-6), 2.56 (ddd, 1H, *J* = 13.4, 10.1 and 5.6, *H*H-6); δ_C (150 MHz, $CDCl_3$) 176.69 (C=O), 174.10 (C=O), 136.56 (C11), 136.01 (2 x Ar-H), 130.13 (Ar-H), 129.66 (2 x Ar-H), 129.28 (C15), 128.72 (2 x Ar-H), 128.01 (Ar-H), 127.32 (2 x Ar-H), 60.59 (C4), 46.32 (C5), 43.70 (C7), 25.51 (C6); IR (oil, cm^{-1}) 3226 (w), 2925 (w) 1715 (s); MS (CI+) *m*/z (relative intensity): 310 ([M+H], 10), 206 (30), 111 (100); Exact Mass Calcd for $[C_{18}H_{15}NO_2S]$+H requires *m/z* 310.0902 Found 310.0901 (CI+).

407b. δ_H (600 MHz, $CDCl_3$) 8.24 (s, 1H, NH), 7.43-7.30 (m, 6H, 8 x Ar-H), 7.27-7.24 (m, 2H, 2 x Ar-H), 4.13 (app t, 1H, *J* = 9.0, H-5), 3.20-3.13 (m, 2H, H*H*-6 and H-7), 2.55 (ddd, 1H, *J* = 13.4, 8.3 and 5.6, *H*H-6); δ_C (150 MHz, $CDCl_3$) 177.99 (C=O), 177.17 (C=O), 135.80 (C11), 135.80 (2 x Ar-H), 129.64 (Ar-H), 129.54 (2 x Ar-H), 128.95 (C15), 128.51 (2 x Ar-H), 128.45 (2 x Ar-H), 128.22 (Ar-H), 60.72 (C4), 45.80 (C5), 43.39 (C7), 25.20 (C6); IR (oil, cm^{-1}) 3211 (w), 1772 (w) 1707 (s); MS (CI+) *m*/z (relative intensity): 310 ([M+H], 50), 206 (100), 104 (40); Exact Mass Calcd for $[C_{18}H_{15}NO_2S]$+H requires *m/z* 310.0902 Found 310.0905 (CI+).

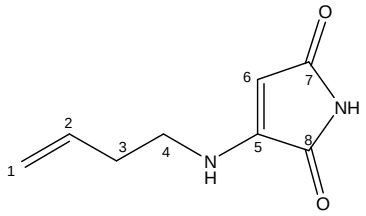
413. 3-(1-Ethyl-2-hexylsulfanyl-butyl)-pyrrole-2,5-dione (one diastereomer of undefined stereochemistry)



342 (25 mg, 0.116 mmol) was dissolved in acetonitrile (21.1 mL) and *trans*-hex-3-ene (3.9 mL, 11.6 mmol). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **413** as a colourless oil (2 mg, 0.007 mmol) in 6% yield, a single diastereomer of undetermined relative stereochemistry.

δ_H (600 MHz, $CDCl_3$) 7.32 (br s, 1H, NH), 6.49 (s, 1H, H-2), 2.92 (ddd, 1H, *J* = 10.8, 6.3 and 4.6, H-12), 2.83 (ddd, 1H, *J* = 12.8, 9.4 and 5.2, *H*H-11), 2.75 (dd, 1H, *J* = 10.9 and 5.9, H-13), 2.62 (ddd, 1H, *J* = 12.7, 9.6 and 6.7, H*H*-11), 2.07-2.00 (m, 1H, H*H*-17), 1.89-1.83 (m, 1H, H*H*-15), 1.79-1.40 (m, 6H, *H*H-15, *H*H-17 and 2 x CH_2), 1.33-1.30 (m, 4H, 2 x CH_2), 1.10 (t, 3H, *J* = 7.5, H_3-16), 0.92 (t, 3H, *J* = 7.4, CH_3), 0.89 (t, 3H, *J* = 7.0, CH_3); δ_C (150 MHz, $CDCl_3$) 171.00 (C=O), 169.53 (C=O), 149.76 (C3), 129.27 (C2), 62.54 (C13), 51.16 (C11), 40.42 (C12), 31.48 (CH_2), 28.65 (CH_2), 23.65 (CH_2), 23.32 (CH_2), 22.52 (CH_2), 17.23 (C17) 14.11 (CH_3), 13.96 (CH_3), 12.33 (CH_3); IR (oil, cm^{-1}) 2962 (m) 1717 (s); MS (CI+) *m*/z (relative intensity): 314 ([M+O], 75), 180 (100); Exact Mass Calcd for $[C_{16}H_{27}NO_2S]$+O requires *m/z* 314.1790 Found 314.1799 (CI+).

431. 3-But-3-enylamino-pyrrole-2,5-dione

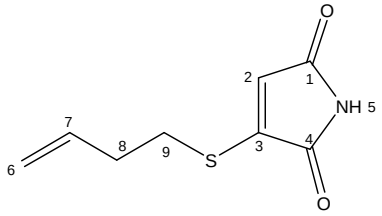


To 3-butenylamine hydrochloride (200 mg, 1.12 mmol) and sodium acetate (184 mg, 2.24 mmol) in methanol (15 mL) was added **289** (200 mg, 1.12 mmol) dropwise in methanol (15 mL). After 10 minutes, solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **431** as a bright yellow waxy solid (142 mg, 0.85 mmol) in 76% yield.

δ_H (500 MHz, $CDCl_3$) 7.10 (s, 1H, NH), 5.77 (ddd, 1H, *J* = 17.4, 10.7 and 6.9, H-2), 5.38 (s, 1H, NH), 5.18-5.15 (m, 2H, H_2-1), 4.83 (s, 1H, H-6), 3.24 (t, 2H, *J* = 6.7, H_2-4), 2.40 (dtd, 2H, *J* = 6.9, 6.9 and 6.8, H_2-3); δ_C (125 MHz, $CDCl_3$) 171.94 (C=O), 167.45

(C=O), 149.53 (C5), 133.89 (C2), 118.51 (C1), 85.80 (C6), 43.30 (C4), 32.68 (C3); IR (solid, cm^{-1}) 3290 (m), 1703 (m), 1629 (s); MS (ES-) *m*/z (relative intensity): 165 ([M-H], 100); Exact Mass Calcd for $[C_8H_{10}N_2O_2]$-H requires *m/z* 165.0659 Found 165.0664 (ES-); m.p. 68-76 ^oC; UV (Acetonitrile) ε_{241} = 8300 and ε_{348} = 6100 $cm^{-1}M^{-1}$.

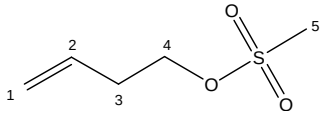
432. 3-But-3-enylsulfanyl-pyrrole-2,5-dione



To a solution of sodium (22 mg, 0.96 mmol) in methanol (20 mL) at 0 ^oC was added **437** (200 mg, 0.96 mmol) in methanol (5 mL) and the solution stirred for three hours. This solution was then added dropwise to a solution of **289** (170 mg, 0.96 mmol) in methanol (20 mL) at 0 ^oC and stirred for ten minutes. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 20% ethyl acetate in petroleum ether) afforded **432** as a pale yellow solid (72 mg, 0.39 mmol) in 41% yield.

δ_H (600 MHz, $CDCl_3$) 7.38 (s, 1H, H-5), 6.06 (s, 1H, H-2), 5.82 (ddt, 1H, *J* = 17.0, 10.2 and 6.6, H-7), 5.18-5.13 (m, 2H, H_2-6), 2.98 (t, 1H, *J* = 7.4, H_2-9), 2.52-2.49 (m, 2H, H_2-8); δ_C (125 MHz, $CDCl_3$) 169.03 (C=O), 167.64 (C=O), 152.37 (C3), 134.77 (C7), 118.46 (C2), 117.91 (C6), 31.88 (C8), 31.32 (C9); IR (solid, cm^{-1}) 3158 (m), 1762 (m), 1703 (s); MS (CI+) *m*/z (relative intensity): 184 ([M+H], 35), 105 (35), 86 (62), 84 (100); Exact Mass Calcd for $[C_8H_9NO_2S]$+H requires *m/z* 184.0432 Found 184.0438 (ES-); m.p. 85-90 ^oC; UV (Acetonitrile) ε_{242} = 6800 and ε_{344} = 4600 $cm^{-1}M^{-1}$.

434. Methanesulfonic acid but-3-enyl ester

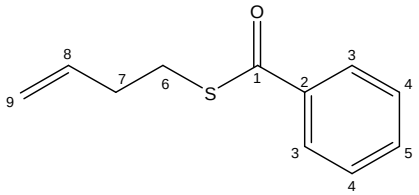


To 3-buten-1-ol (2 mL, 23 mmol) and triethylamine (7.8 mL, 56 mmol) in dichloromethane (50 mL) at 0 ^oC was added methane sulfonyl chloride (3.62 mL, 47 mmol) in dichloromethane (5 mL). The reaction was stirred at 0 ^oC for 1 hour and then allowed to come to room temperature. The solvent was removed *in vacuo* and residue

dissolved in ethyl acetate (25 mL), washed with water (3 x 25 mL), brine (25 mL) and dried over $MgSO_4$. The solvent was removed to afford **434** as a brown oil (2.99 g, 20 mmol) in 87% yield.

δ_H (500 MHz, $CDCl_3$) 5.78 (ddt, 1H, *J* = 17.0, 10.2 and 6.8, H-2), 5.20-5.14 (m, 2H, H_2-1), 4.26 (t, 2H, *J* = 6.7, H_2-4), 3.00 (s, 3H, H_3-5), 2.53-2.48 (m, 2H, H_2-3); δ_C (125 MHz, $CDCl_3$) 132.48 (C2), 118.59 (C1), 68.93 (C4), 37.58 (C5), 31.62 (C3); IR (oil, cm^{-1}) 2942 (m), 1349 (s); No mass ion found.

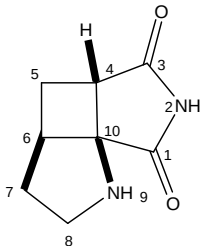
437. Thiobenzoic acid *S*-but-3-enyl ester[342]



To an ice-cold solution of diethyl azodicarboxylate (2.19 mL, 13.8 mmol) in tetrahydrofuran (10 mL) was added a solution of triphenylphosphine (3.64 g, 13.8 mmol) in tetrahydrofuran (40 mL). The yellow solution was stirred at 0 ^oC for 30 minutes. But-3-en-1-ol (500 mg, 6.9 mmol) and thiobenzoic acid (1.92 g, 13.8 mol) in tetrahydrofuran (30 mL) were added at 0 ^oC under argon. The reaction was allowed to come to room temperature and stirred for a further two hours. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 5% ethyl acetate in petroleum ether) afforded **437** as a volatile orange oil (1.3 g, 6.6 mmol) in 96% yield.

δ_H (600 MHz, $CDCl_3$) 7.96 (dd, 2H, *J* = 8.2 and 1.1, 2 x Ar-H), 7.58-7.55 (m, 1H, H-5), 7.45 (t, 2H, *J* = 8.2, 2 x Ar-H), 5.85 (ddt, 1H, *J* = 16.9, 10.2 and 6.6, H-8), 5.13 (dd, 1H, *J* = 17.1 and 1.4, H*H*-9), 5.07 (dd, 1H, *J* = 10.1 and 1.4, *H*H-9), 3.14 (t, 2H, *J* = 7.2, H_2-6), 2.43 (m, 2H, H_2-7); δ_C (150 MHz, $CDCl_3$) 129.02 (C1), 137.22 (C2), 136.27 (C8), 133.43 (C5), 128.70 (2 x Ar-H), 127.32 (2 x Ar-H), 116.69 (C9), 33.77 (C6), 28.39 (C7); IR (film, cm^{-1}) 2979 (w), 1661 (s); MS (CI+) *m*/z (relative intensity): 193 ([M+H], 10), 105 (100); Exact Mass Calcd for $[C_{11}H_{12}OS]$+H requires *m/z* 193.0687 Found 193.0692 (CI+).

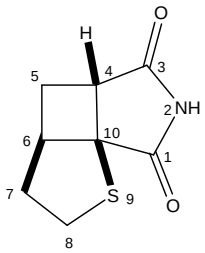
438. (4RS, 6SR, 10RS)-2,9-Diazatricyclo[5.3.0.0^{10,4}]decan-1,3-dione



431 (42 mg, 0.25 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 4 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in ethyl acetate to 5% methanol in ethyl acetate) afforded **438** as an off-white solid (39 mg, 0.23 mmol) in 93% yield.

δ_H (500 MHz, $CDCl_3$) 3.50 (ddd, 1H, *J* = 11.8, 4.8 and 2.6, H*H*-8), 3.18-3.12 (m, 2H, *H*H-8 and H-6), 2.98 (dd, 1H, *J* = 10.7 and 3.9, H-4), 2.21 (ddd, 1H, *J* = 13.2, 8.6 and 4.0, H*H*-5), 2.01 (ddd, 1H, 13.4, 10.5 and 5.8, *H*H-5), 1.79 (m, 2H, H_2-7); δ_C (125 MHz, $CDCl_3$) 179.04 (C=O), 178.95 (C=O), 70.85 (C10), 48.43 (C8), 44.25 (C4), 43.82 (C6), 32.93 (C7) 24.96 (C5); IR (solid, cm^{-1}) 3198 (m), 2944 (m), 1701 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 45), 125 (100); Exact Mass Calcd for $[C_8H_{10}N_2O_2]$+ requires *m/z* 166.07387 Found 166.07386 (EI); m.p. 110-113 ^oC.

439. (4RS, 6SR, 10SR)-2-Aza-9-thiatricyclo[5.3.0.0^{10,4}]decan-1,3-dione 664

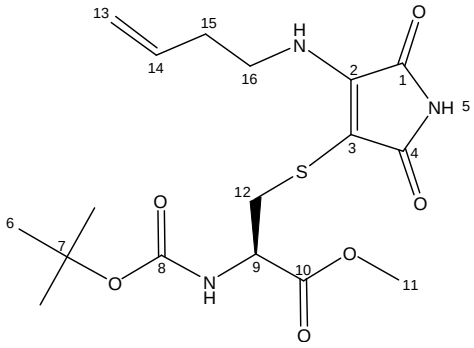


432 (42 mg, 0.25 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes and irradiated in pyrex glassware for 4 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in ethyl acetate to 5% methanol in ethyl acetate) afforded **439** as an off-white solid (39 mg, 0.23 mmol) in 93% yield

δ_H (500 MHz, $CDCl_3$) 3.50 (ddd, 1H, *J* = 11.8, 4.8 and 2.6, H*H*-8), 3.18-3.12 (m, 2H, *H*H-8 and H-6), 2.98 (dd, 1H, *J* = 10.7 and 3.9, H-4), 2.21 (ddd, 1H, *J* = 13.2, 8.6 and

4.0, H*H*-5), 2.01 (ddd, 1H, 13.4, 10.5 and 5.8, *H*H-5), 1.79 (m, 2H, H_2-7); δ_C (150 MHz, MeOD) 181.44 (C=O), 180.54 (C=O), 64.01 (C10), 50.17 (C6), 48.95 (C4), 37.78 (C8), 36.20 (C7), 26.07 (C5); IR (solid, cm^{-1}) 3218 (w), 1768 (m), 1709 (s); MS (EI) *m*/z (relative intensity): 166 (M+, 45), 125 (100); Exact Mass Calcd for $[C_8H_{10}N_2O_2]$+ requires *m/z* 166.07387 Found 166.07386 (EI); m.p. 155-160 ^oC.

440. 3-(4-But-3-enylamino-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl)-2R-tert-butoxycarbonylamino-propionic acid methyl ester

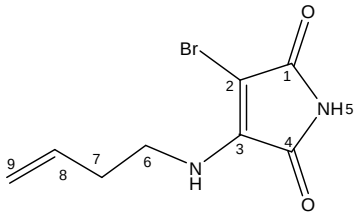


To **293** (50 mg, 0.12 mmol) in methanol (10 mL) was added homoallyl amine hydrochloride (13 mg, 0.12 mmol) and triethylamine (17 μL, 0.12 mmol) in methanol (5 mL), whereupon the solution turned yellow. The reaction was left stirring for 16 hours and the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution 10% ethyl acetate in petroleum ether to 40% ethyl acetate in petroleum ether) afforded **440** as a bright yellow oil (36 mg, 0.09 mmol) in 75% yield and **293** starting material (13 mg, 0.03 mmol) in 14%.

δ_H (500 MHz, $CDCl_3$) 7.68 (s, 1H, H-5), 5.79 (ddt, 1H, *J* = 16.9, 10.1 and 6.8, H-14), 5.63 (d, 1H, *J* = 6.2, 8-NH), 5.20-5.15 (m, 2H, H_2-13), 4.50-4.47 (m, 1H, H-9), 3.88-3.84 (m, 2H, H_2-16), 3.70 (s, 3H, H_3-11), 3.18-3.07 (m, 2H, H_2-12), 2.44-2.40 (m, 2H, H_2-15), 1.85 (br s, 1H, NH), 1.43 (s, 9H, 3 x H_3-6); δ_C (125 MHz, $CDCl_3$) 170.25 (C=O), 169.76 (C=O), 164.55 (C=C), 163.22 (br, C=O), 161.64 (br, C=O), 154.29 (C=C), 132.78 (C14), 117.55 (C13), 79.23 (C7), 52.78 (C9), 51.83 (C11), 41.25 (C16), 30.47 (C15), 28.68 (C12), 27.23 (3 x C6); IR (oil, cm^{-1}) 3305 (w), 2928 (w), 1711 (s),

1627 (s); MS (CI+) *m*/z (relative intensity): 399 (M+, 10), 300 (15), 146 (100); Exact Mass Calcd for $[C_{17}H_{25}N_3O_6S]$+ requires *m/z* 399.1459 Found 399.1464 (CI+); UV (Acetonitrile) ε_{210} = 13900, ε_{375} = 343 $cm^{-1}M^{-1}$.

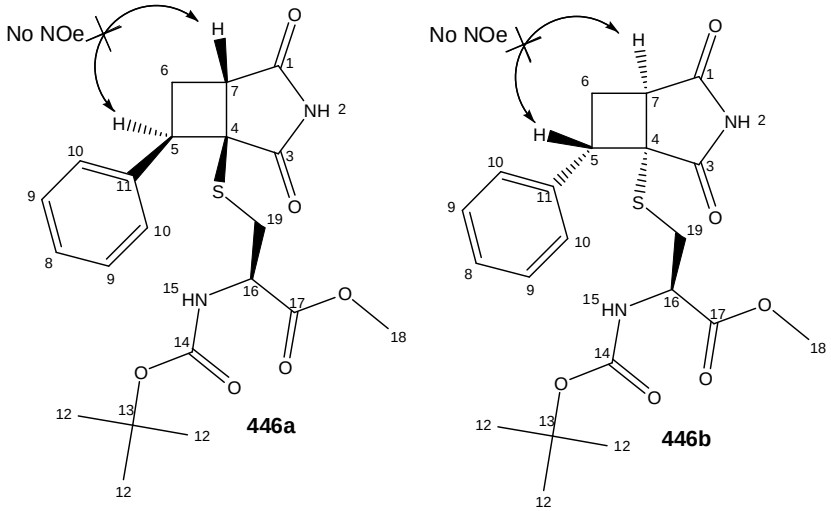
442. 3-Bromo-4-but-3-enylamino-pyrrole-2,5-dione



To 2,3-dibromomaleimide (100 mg, 0.39 mmol) in methanol (10 mL) was added homoallyl amine hydrochloride (42 mg, 0.39 mmol) and triethylamine (55 μL, 0.39 mmol) in methanol (10 mL). After 5 minutes the solvent was removed *in vacuo* and purification by flash chromatography (gradient elution petroleum ether to 20% ethyl acetate in petroleum ether) afforded **442** as a white powder (45 mg, 0.18 mmol) in 47% yield.

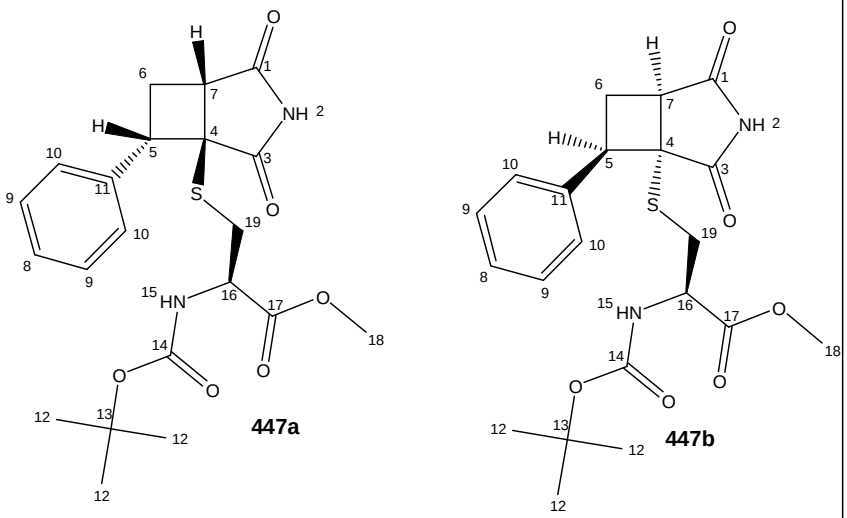
δ_H (500 MHz, $CDCl_3$) 7.58 (s, 1H, H-5), 5.78 (ddt, 1H, *J* = 16.2, 10.4 and 6.9, H-8), 5.47 (s, 1H, 6-NH), 5.19-5.15 (m, 2H, H_2-9), 3.73-3.71 (m, 2H, H_2-6), 2.44-2.39 (m, 2H, H_2-7); δ_C (125 MHz, $CDCl_3$) 172.40 (C=O), 167.42 (C=O), 165.70 (C=C), 143.61 (C=C), 133.63 (C8), 118.69 (C9), 41.89 (C6), 34.64 (C7); IR (solid, cm^{-1}) 3232 (w), 1724 (s); MS (CI+) *m*/z (relative intensity): 245 (M+, 100), 165 (90); Exact Mass Calcd for $[C_8H_9N_2O_2Br]$+ requires *m/z* 244.9926 Found 244.9928 (CI+); m.p. 214-218 ^oC; UV (Acetonitrile) ε_{241} = 8600 and ε_{368} = 6600 $cm^{-1}M^{-1}$.

446a and 446b. (4S, 5R, 7R)-2-Aza-4-([2R-*tert*-butoxycarbonylamino-3-sulfanyl] propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and (4R, 5S, 7S)-2-aza-4-([2R-*tert*-butoxycarbonylamino-3-sulfanyl] propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



Postulated as isolated diastereomers after 2nd column

447a and 447b. (4S, 5S, 7R)-2-Aza-4-([2R-*tert*-butoxycarbonylamino-3-sulfanyl] propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and (4R, 5R, 7S)-2-aza-4-([2R-*tert*-butoxycarbonylamino-3-sulfanyl] propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



Postulated as components of the complex mixture, alongside **446a** and **446b** after 1st column

Method 1: 291 (39 mg, 0.12 mmol) was dissolved in acetonitrile (30 mL). The resulting solution was degassed for 30 minutes, styrene (13.6 μL, 1.2 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and ^{1}H NMR indicated a complex mixture of diastereomers. Purification by flash

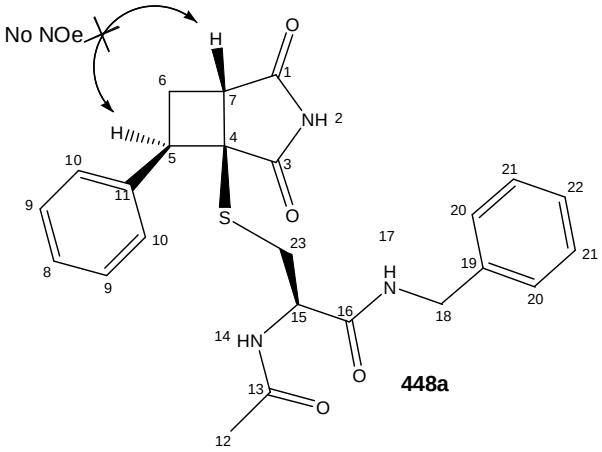
chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded a white solid (34 mg, 0.078 mmol) in 63% yield that was postulated as a mixture of the four diastereomers shown, **446a, 446b, 447a** and **447b**, overlap of signals prevented NOe analysis at this stage. Further column chromatography afforded **446a** and **446b** as a white solid (12 mg, 0.028 mmol) in 23%. NMR investigations showed the mixture was 1:1 of **a:b**. Reanalysis of the crude suggests that almost all peaks are from these four compounds.

Method 2: 291 (39 mg, 0.12 mmol) was dissolved in acetonitrile (30 mL). The resulting solution was degassed for 30 minutes, styrene (13.6 μL, 0.12 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded a white solid (27 mg, 0.062 mmol) in 52% yield as a mixture of the same four major diastereomers as in Method 2, **446a, 446b, 447a** and **447b**. Further purification of this mixture was not undertaken.

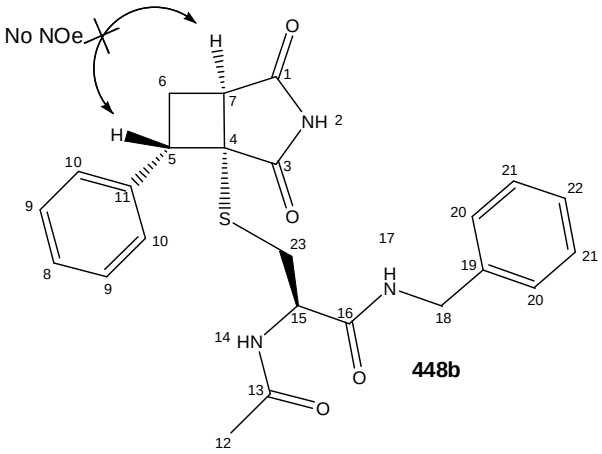
δ_H (600 MHz, $CDCl_3$) 8.08 (s, 2H, 2 x H-2), 7.40-7.31 (m, 10H, 10 x Ar-H), 5.0 (d, 1H, *J* = 8.2, H-15), 4.9 (d, 1H, *J* = 7.5, H-15), 4.26-4.23 (m, 1H, H-16), 4.18-4.12 (m, 1H, H-16), 4.06 (app t, 2H, *J* = 8.5, 2 x H-5), 3.69 (s, 3H, H_3-18), 3.674 (s, 3H, H_3-18), 3.19 (ddd, 1H, *J* = 11.0 and 2.4, H-7), 3.11 (dd, 1H, *J* = 11.0 and 3.2, H-7) 3.04-2.93 (m, 3H, 2 x H*H*-6 and H*H*-19), 2.91 (dd, 1H, *J* = 12.8 and 6.6, H*H*-19), 2.64-2.60 (m, 2H, 2 x *H*H-6), 2.51 (dd, 1H, *J* = 12.8 and 4.6, *H*H-19), 2.43 (dd, 1H, *J* = 13.0 and 7.3, *H*H-19), 1.45 (s, 9H, 3 x H_3-12), 1.43 (s, 9H, 3 x H_3-12); δ_C (150 MHz, $CDCl_3$) 178.41 (C=O), 177.25 (C=O), 177.20 (C=O), 171.40 (C=O), 171.10 (C=O), 170.98 (C=O), 155.28 (C=O), 155.18 (C=O), 136.28 (C11), 136.25 (C11), 128.94 (2 x Ar-H), 128.93 (2 x Ar-H), 128.49 (2 x Ar-H), 128.46 (2 x Ar-H), 128.38 (C8), 128.33 (C8), 80.44 (2 x C13), 56.71 (C4), 56.48 (C4), 53.03 (C16), 52.87 (C16), 52.78 (C18), 52.75 (C18), 45.92 (C5), 45.82 (C5), 43.76 (C7), 43.61 (C7), 31.28 (C6), 31.09 (C6), 28.38 (6 x C12), 26.33 (C19), 26.21 (C19); IR (oil, cm^{-1}) 3215 (w), 2971 (w) 1738 (s), 1715 (s); MS (CI+) *m*/z (relative intensity): 435 ([M+H], 10), 379 (30), 335 (100); Exact Mass Calcd for $[C_{21}H_{26}N_2O_6S]$+H requires *m/z* 435.1590 Found 435.1576 (CI+); m.p. 78-90 ^oC.

448a. (4S, 5R, 7R)-2-Aza-4-(2R-acetylamino-*N*-benzyl-3-sulfanyl-propionamide)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and 448b. (4R, 5S, 7S)-2-aza-4-(2R-

acetylamino-*N*-benzyl-3-sulfanyl-propionamide)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione



448a



448b

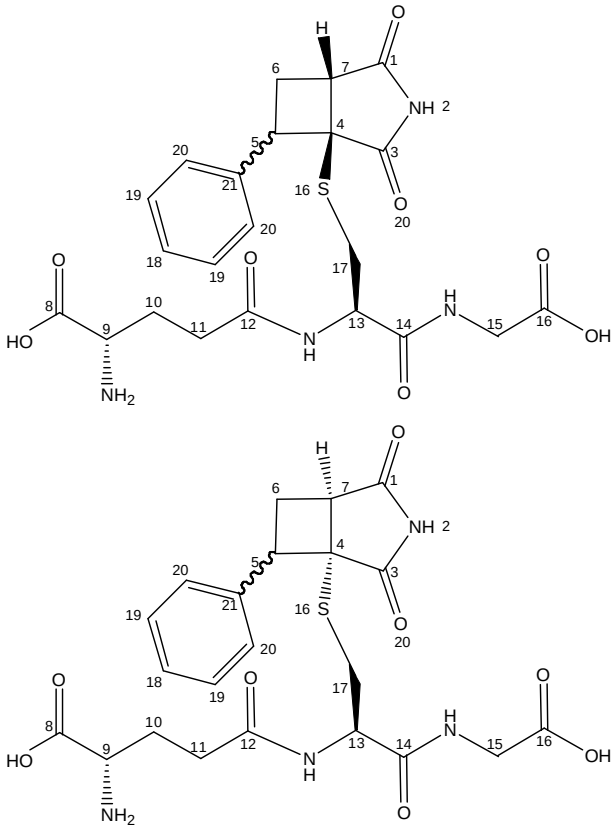
Method 1: 291 (58 mg, 0.17 mmol) was dissolved in acetonitrile (80 mL). The resulting solution was degassed for 30 minutes, styrene (191 μL, 1.70 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in 30% ethyl acetate in petroleum ether to 10% methanol in ethyl acetate) afforded **448a** and **448b** as a 1:1 mixture (57 mg, 0.127 mmol) in 78%. Further column chromatogtraphy afforded an off-white solid (14 mg, 0.031 mmol) in 19% yield, which was one pure diastereomer that showed no through-space interactions between the protons indicated. The data below is for the isolated diastereomer, but it was not certain whether it was a or b. Reanalysis of the crude reaction mixture suggested that almost all peaks are from **448a** and **448b**.

Method 2: 291 (29 mg, 0.084 mmol) was dissolved in acetonitrile (50 mL). The resulting solution was degassed for 30 minutes, styrene (10 μL, 0.084 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* and ^{1}H NMR indicated a complex mixture of diastereomers that appear be the

desired conjugation. Purification by flash chromatography (gradient elution in 30% ethyl acetate in petroleum ether to 10% methanol in ethyl acetate) afforded an off-white solid (33 mg, 0.073 mmol) in 87% as a mixture of the two diastereomers **448a** and **448b**, assigned from lack of through space interactions in 2-D NMR experiments.

δ_H (600 MHz, $CDCl_3$) 8.69 (s, 1H, H-2), 7.36-7.26 (m, 8H, 8 x Ar-H), 7.18 (d, 2H, *J* = 7.0, 2 x Ar-H), 6.65 (t, 1H, *J* = 5.6, H-17), 6.51 (d, 1H, *J* = 1.2, H-14), 4.33 (d, 2H, *J* = 6.0, H_2-18), 4.30 (dt, 1H, *J* = 5.4 and 1.2, H-15), 4.05 (app t, 1H, *J* = 8.9, H-5), 3.16 (dd, 1H, *J* = 11.1 and 3.1, H-7), 3.04 (ddd, 1H, *J* = 12.8, 11.1 and 8.9, H*H*-6), 2.94 (dd, 1H, *J* = 13.3 and 5.6, H*H*-23), 2.59 (ddd, 1H, *J* = 12.5, 8.9 and 3.4, *H*H-6), 2.31 (dd, 1H, *J* = 13.6 and 5.3, *H*H-23), 1.98 (s, 3H, H_3-12); δ_C (150 MHz, $CDCl_3$) 179.07 (C=O), 176.97 (C=O), 171.10 (C=O), 169.67 (C=O), 137.67 (Ar), 136.16 (Ar), 129.02 (2 x Ar-H), 128.80 (2 x Ar-H), 128.55 (2 x Ar-H), 128.35 (Ar-H), 128.78 (2 x Ar-H), 127.66 (Ar-H), 57.44 (C4), 52.61 (C15), 46.09 (C5), 43.68 (C7), 43.66 (C18), 30.58 (C6), 26.15 (C23), 23.17 (C12); IR (oil, cm^{-1}) 3283 (w), 1714 (s); MS (FAB+) *m*/z (relative intensity): 452 ([M+H], 10), 439 (10), 286 (100); Exact Mass Calcd for [C-$_{24}H_{25}N_3O_4S$]+H requires *m/z* 452.1650 Found 452.1644 (FAB+); m.p. 96-104 ^oC.

449. - a mixture of 4 diastereomers. (4S, 5R, 7R)-2-Aza-4-(2S-amino-4-[1R-(carboxymethyl-carbamoyl)-2-sulfanyl)-ethylcarbamoyl]-butyric acid)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione, (4S, 5S, 7R)-2-aza-4-(2S-amino-4-[1R-(carboxymethyl-carbamoyl)-2-sulfanyl)-ethylcarbamoyl]-butyric acid)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione, (4R, 5R, 7S)-2-aza-4-(2S-amino-4-[1R-(carboxymethyl-carbamoyl)-2-sulfanyl)-ethylcarbamoyl]-butyric acid)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and (4R, 5S, 7S) – 2 – aza – 4 - (2S – amino – 4 - [1R - (carboxymethyl - carbamoyl) – 2 - sulfanyl)-ethylcarbamoyl]-butyric acid)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione.

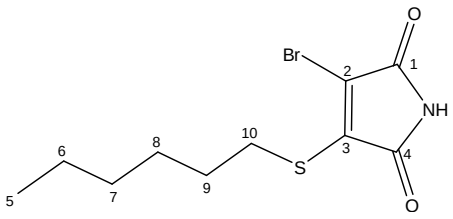


To glutathione (36 mg, 0.12 mmol) in water (12.5 mL) was added **289** (21 mg, 0.12 mmol) in acetonitrile (12.5 mL). The solution was degassed for 30 minutes, styrene (133 μL, 1.20 mmol) added and irradiated in pyrex glassware for 5 minutes with stirring. The solvent was removed *in vacuo* to afford **449** as a mixture of diastereoisomers as a colourless oil (61 mg). No further purification was undertaken. NMR spectra reported below but complexity of spectra prevents clear assignment of the peaks to a paeticular structure

δ_H (600 MHz, $CDCl_3$) 7.42-7.41 (m, 20H, 20 x Ar-H), 4.80-4.28 (m, 4H, 4 x H-13), 4.13-3.91 (m, 12H, 4 x H-9 and 4 x H_2-15), 3.87 (d, 4H, *J* = 6.5, 4 x H-5), 3.57-2.66 (m, 20H, 4 x H-7, 4 x H_2-6 and 4 x H_2-17), 3.13-2.45 (m, 8H, 4 x H_2-11), 2.26-2.11 (m, 8H, 4 x H_2-10); δ_C (150 MHz, $CDCl_3$) 181.77 (2 x C=O), 181.61 (2 x C=O), 180.40 (2 x C=O), 174.58 (2 x C=O), 174.46 (2 x C=O), 174.42 (2 x C=O), 172.78 (2 x C=O), 172.69 (2 x C=O),172.45 (2 x C=O), 172.42 (2 x C=O), 171.90 (2 x C=O), 138.16 (2 x C21), 138.13 (2 x C21), 130.07 (4 x Ar-H), 130.04 (4 x Ar-H), 129.73 (2 x Ar-H), 129.22 (4 x Ar-H), 128.90 (4 x Ar-H), 127.52 (2 x Ar-H), 58.85 (2 x C4), 58.81 (2 x C4), 57.10 (2 x CH), 57.04 (2 x CH), 54.31 (2 x CH), 54.23 (2 x CH), 53.80 (2 x CH), 47.29 (2 x CH), 44.74 (2 x CH), 44.65 (2 x CH), 41.85 (2 x CH_2), 41.81 (2 x CH_2),

32.48 (2 x CH_2), 31.26 (2 x CH_2), 31.11 (2 x CH_2), 27.18 (2 x CH_2), 27.11 (2 x CH_2), 27.05 (2 x CH_2), 26.94 (2 x CH_2), 26.71 (2 x CH_2) Four diastereomers are indicated, thus one carbon signal is missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 3062 (m), 1711 (s), 1654 (m); MS (ES+) *m*/z (relative intensity): 507 ([M+H], 100), 378 (40); Exact Mass Calcd for $[C_{22}H_{26}N_4O_8S_s]$+H requires *m/z* 507.1550 Found 507.1525 (CI+).

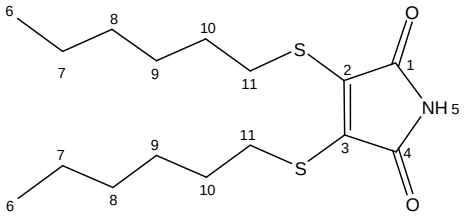
455. 3-Bromo-4-hexylsulfanyl-pyrrole-2,5-dione



To 2,3-dibromomaleimide (300 mg, 1.17 mmol) and sodium acetate (48 mg, 0.59 mmol) in methanol (100 mL) was added hexanethiol (82 μL, 0.58 mmol) in methanol (100 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **455** as a pale orange oil (160 mg, 0.55 mmol) in 93% yield based on hexanethiol.

δ_H (500 MHz, $CDCl_3$) 7.65 (s, 1H, NH), 3.39 (t, 2H, *J* = 7.4, H_2-10), 1.72-1.66 (m, 2H, H_2-9), 1.46-1.40 (m, 2H, H_2-8), 1.32-1.29 (m, 4H, H_2-6 and H_2-7), 0.89 (t, 3H, *J* = 7.0, H_3-5); δ_C (125 MHz, $CDCl_3$) 165.05 (C=O), 163.28 (C=O), 144.39 (C=C), 116.95 (C=C), 30.88 (CH_2), 30.65 (CH_2), 30.09 (CH_2), 27.77 (CH_2), 22.13 (CH_2), 13.64 (C5); IR (oil, cm^{-1}) 3278 (m), 2926 (m), 1777 (m), 1715 (s); MS (EI) *m*/z (relative intensity): 293 ([M+], 15), 291 ([M+], 15), 84 (100); Exact Mass Calcd for $[C_{10}H_{14}NO_2S^{79}Br]$+ requires *m/z* 290.9923 Found 290.9912 (EI); UV (Acetonitrile) ε_{210} = 18000, ε_{232} = 8600, ε_{262} = 3000 and ε_{367} = 3700 $cm^{-1}M^{-1}$.

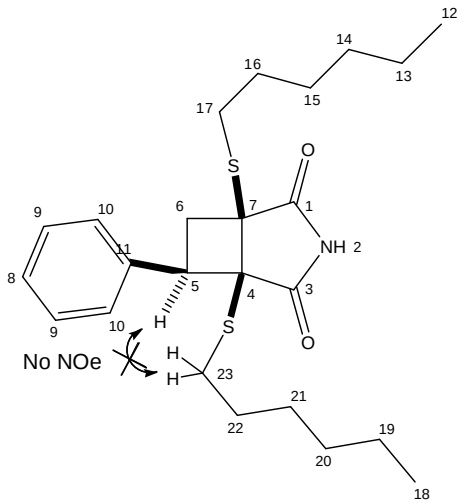
456. 3,4-Bis-hexylsulfanyl-pyrrole-2,5-dione



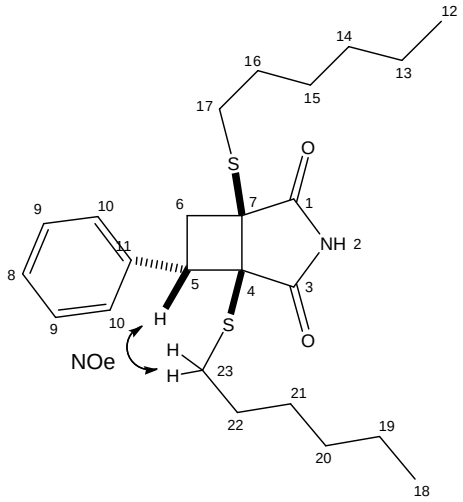
To 2,3-dibromomaleimide (300 mg, 1.17 mmol) and sodium acetate trihydrate (320 mg, 2.35 mmol) in methanol (100 mL) was added hexanethiol (328 μL, 2.34 mmol) in methanol (100 mL) dropwise over 1 hour with vigorous stirring. After 5 minutes the solvent was removed *in vacuo*. Purification by flash chromatography (gradient elution in 10% in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **456** as a bright yellow oil (215 mg, 0.65 mmol) in 55% yield.

δ_H (600 MHz, $CDCl_3$) 7.40 (s, 1H, H-5), 3.27 (t, 4H, *J* = 7.8, 2 x H_2-11), 1.66-1.61 (m, 4H, 2 x H_2-10), 1.44-1.38 (m, 4H, 2 x H_2-9), 1.04 (m, 8H, 2 x H_2-8 and 2 x H_2-7), 0.88 (t, 6H, *J* = 6.5, 2 x H_3-6); δ_C (150 MHz, $CDCl_3$) 166.23 (C1 and C4), 136.80 (C2 and C3), 31.92 (2 x C11), 31.39 (2 x CH_2), 30.56 (2 x CH_2), 28.29 (2 x CH_2), 22.62 (2 x C7), 14.13 (2 x C6); IR (oil, cm^{-1}) 3271 (w), 2927 (m) 1770 (m), 1713 (s); MS (FAB+) *m*/z (relative intensity): 352 ([M+Na], 85), 329 (30), 176 (100); Exact Mass Calcd for $[C_{16}H_{27}N_2O_2S_2]$+Na requires *m/z* 352.1381 Found 352.1385 (FAB+); UV (Acetonitrile) ε_{234} = 5400 and ε_{398} = 4200 $cm^{-1}M^{-1}$.

457a. (4RS, 5RS, 7SR)-2-Aza-4-hexylsulfanyl-5-phenyl-7-hexylsulfanyl-bicyclo[3.2.0]heptan-1,3-dione



457b. (4RS, 5SR, 7SR)-2-Aza-4-hexylsulfanyl-5-phenyl-7-hexylsulfanyl-bicyclo[3.2.0]heptan-1,3-dione

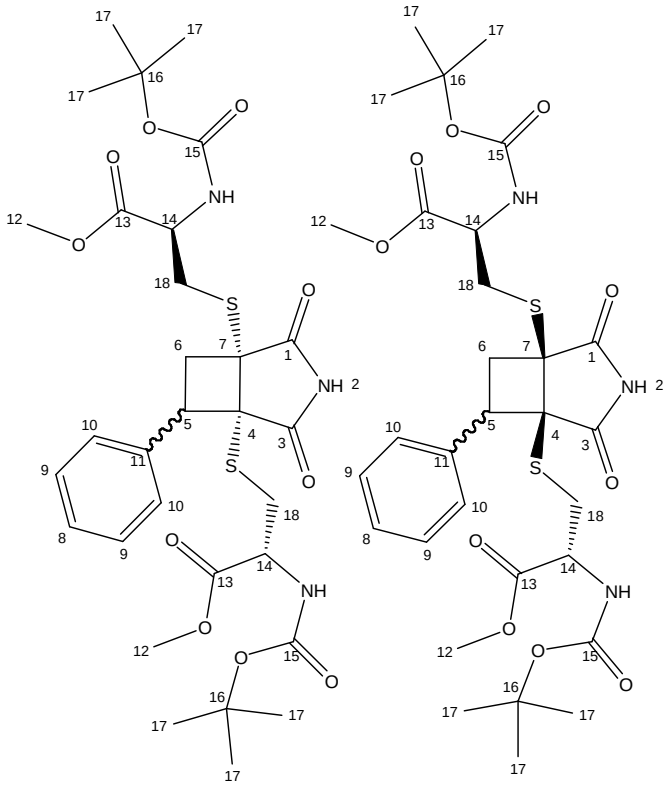


456 (38 mg, 0.115 mmol) was dissolved in acetonitrile (25 mL). The resulting solution was degassed for 30 minutes, styrene (133 μL, 1.2 mmol) added and irradiated in pyrex glassware for 20 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in petroleum ether to 30% ethyl acetate in petroleum ether) afforded **457a** and **457b** together as a colourless oil (35 mg, 0.082 mmol) in 70%. NMR investigations showed the mixture was a 1:1 ratio of **457a** to **457b**. Further purification by flash chromatography afforded **457a** as a colourless oil (3 mg, 0.007 mmol) in 6% yield and **457b** as a colourless oil (3 mg, 0.007 mmol) in 6% yield. Diastereomers were tentatively assigned due to through space interactions seen *via* 2-D NMR and the lack thereof.

457a. δ_H (600 MHz, $CDCl_3$) 8.10 (s, 1H, NH), 7.41 (d, 2H, *J* = 6.9, 2 x Ar-H), 7.37 (t, 1H, *J* = 6.9, H-8), 7.33 (d, 2H, *J* = 6.9, 2 x Ar-H), 3.92 (app t, 1H, *J* = 8.9, H-5), 2.95 (dd, 1H, *J* = 12.9 and 8.9, H*H*-6), 2.86 (td, 1H, *J* = 14.2 and 6.9, -S-CH*H*-), 2.78-2.66 (m, 2H, *H*H-6 and -S-C*H*H-), 2.60 (ddd, 1H, *J* = 10.9, 8.3 and 6.3, -S-CH*H*-) 2.00 (ddd, 1H, *J* = 10.7, 8.6 and 5.6, -S-C*H*H-), 1.65-1.60 (m, 2H, H*H*-16 and H*H*-22), 1.43-1.06 (m, 14H, *H*H-16, *H*H-22 and 6 x CH_2), 0.88 (t, 3H, *J* = 6.7, CH_3), 0.82 (t, 3H, *J* = 7.1, CH_3); δ_C (150 MHz, $CDCl_3$) 176.59 (C=O), 176.44 (C=O), 136.03 (C11), 129.50 (2 x Ar-H), 128.83 (C8), 128.29 (2 x Ar-H), 62.32 (C4), 54.58 (C7), 45.33 (C5), 34.85 (C6), 31.48 (CH_2), 31.33 (CH_2), 30.51 (CH_2), 29.21 (CH_2), 29.06 (CH_2), 28.90 (CH_2), 28.76 (CH_2), 28.53 (CH_2), 22.62 (CH_2), 22.50 (CH_2), 14.16 (CH_3), 14.11 (CH_3); IR (oil, cm^{-1}) 3215 (w), 2926 (m) 1774 (w), 1715 (s); MS (CI+) *m*/z (relative intensity): 432 ([M-H], 5), 329 (60), 207 (100), 161 (60); Exact Mass Calcd for $[C_{24}H_{35}NO_2S_2]$-H requires *m/z* 432.2026 Found 432.2034 (CI+).

457b. δ_H (600 MHz, $CDCl_3$) 7.96 (s, 1H, NH), 7.33 (d, 2H, *J* = 7.0, 2 x Ar-H), 7.28 (t, 1H, *J* = 7.0, H-8), 7.21 (d, 2H, *J* = 7.5, 2 x Ar-H), 4.02 (app t, 1H, *J* = 10.0, H-5), 2.98-2.92 (m, 2H, H*H*-6 and -S-C*H*H-), 2.87 (dd, 1H, *J* = 13.6 and 10.8, *H*H-6), 2.83-2.72 (m, 2H, -S-CH*H*- and -S-C*H*H-), 2.69 (td, 1H, *J* = 10.8 and 7.4, -S-CH*H*-) 1.68-1.57 (m, 4H, H_2-16 and H_2-22), 1.45-1.31 (m, 4H, H_2-15 and H_2-21), 1.31-1.25 (m, 8H, H_2-13, H_2-14, H_2-19 and H_2-20), 0.86 (t, 6H, *J* = 7.0, H_3-12 and H_3-18); δ_C (150 MHz, $CDCl_3$) 176.91 (C=O), 172.96 (C=O), 136.09 (C11), 128.83 (2 x Ar-H), 128.13 (C8), 127.41 (2 x Ar-H), 62.86 (C4), 54.36 (C7), 46.49 (C5), 33.28 (C6), 31.51 (CH_2), 31.47 (CH_2), 30.65 (SCH_2), 30.09 (SCH_2), 29.22 (CH_2), 28.96 (CH_2), 28.80 (CH_2), 28.74 (CH_2), 22.63 (CH_2), 22.60 (CH_2), 14.17 (CH_3), 14.15 (CH_3); IR (oil, cm^{-1}) 3194 (w), 2928 (m) 1774 (w), 1722 (s); MS (CI+) *m*/z (relative intensity): 432 ([M-H], 5), 332 (50), 316 (95), 207 (100); Exact Mass Calcd for $[C_{24}H_{35}NO_2S_2]$-H requires *m/z* 432.2026 Found 432.2029 (CI+).

458 – a mixture of 4 diastereomers. (4R, 5R, 7S)-2-Aza-4,7-di(2R-*tert*-butoxycarbonylamino-3-sulfanyl propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione, (4R, 5S, 7S)-2-aza-4,7-di(2R-*tert*-butoxycarbonylamino-3-sulfanyl propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione, (4S, 5S, 7R)-2-aza-4,7-di(2R-*tert*-butoxycarbonylamino-3-sulfanyl propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione and (4S, 5R, 7R)-2-aza-4,7-di(2R-*tert*-butoxycarbonylamino-3-sulfanyl propionic acid methyl ester)-5-phenyl-bicyclo[3.2.0]heptan-1,3-dione.

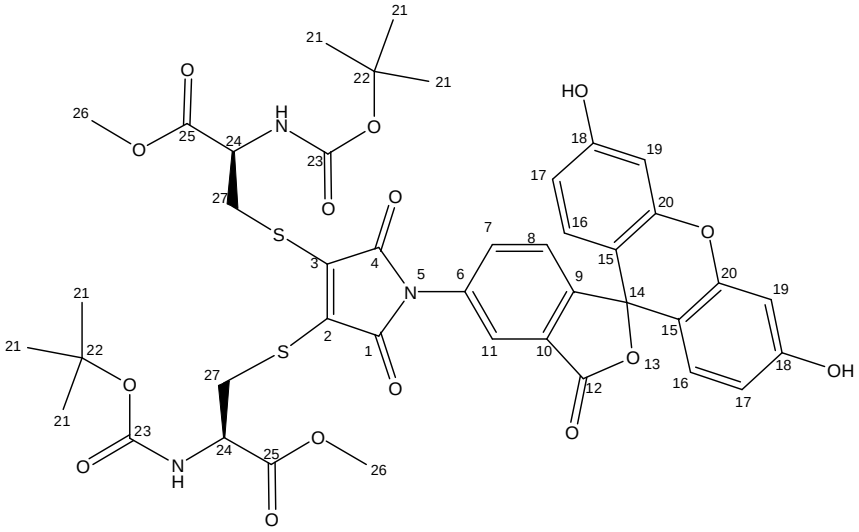


294 (76 mg, 0.135 mmol) was dissolved in acetonitrile (29 mL). The resulting solution was degassed for 30 minutes, styrene (148 μL, 1.35 mmol) added and irradiated in pyrex glassware for 30 minutes with stirring. The solvent was removed *in vacuo* and purification by flash chromatography (gradient elution in 10% ethyl acetate in petroleum ether to 30% ethyl acetate in petroleum ether) afforded a mixture of diastereomers **458** as a colourless oil (47 mg, 0.070 mmol) in 60% yield. NMR spectra reported below but complexity of spectra prevents clear assignment of the peaks. MS confirmed the identity of the compounds as all having a mass that indicated conjugation had taken place.

δ_H (600 MHz, $CDCl_3$) 8.18 (s, 2H, 2 x H-2), 7.95 (s, 2H, 2 x H-2), 7.40-7.26 (m, 16H, multiple Ar-H), 7.20 (d, 4H, *J* = 7.5, Ar-H), 5.56 (d, 2H, *J* = 5.8, 2 x 15-NH), 5.50-5.42 (m, 4H, 4 x 15-NH), 5.40 (d, 2H, *J* = 7.3, 2 x 15-NH), 4.74-4.55 (m, 6H, multiple H-14), 4.08-3.87 (m, 10H), 3.80-3.70 (m, 24H, 8 x H_3-12), 3.70-3.60 (m, 16H), 3.59 (d, 1H, *J* = 4.0), 3.53 (d, 1H, *J* = 3.5), 3.42-2.75 (m, 20H, contains multiple H-6 and H_2-18 by HMQC analysis), 1.46-1.42 (m, 74H, 24 x H_3-17); δ_C (150 MHz, $CDCl_3$) 175.94 (C=O), 175.84 (C=O), 175.82 (C=O), 171.10 (C=O), 171.04 (C=O), 170.95 (C=O), 155.28 (4 x C11), 129.63 (Ar-H), 129.50 (Ar-H), 128.96 (Ar-H), 128.88 (Ar-H), 128.80 (Ar-H), 128.75 (Ar-H), 128.59 (Ar-H), 128.53 (Ar-H), 128.41 (Ar-H), 80.51 (4 x C16),

80.22 (4 x C16), 55.97 (4 x C), 55.83 (4 x C), 52.97 (4 x C12), 52.93 (4 x C12), 52.71 (4 x C14), 52.61 (4 x C14), 45.25 (4 x C5), 32.92 (C18), 32.86 (C18), 31.19 (C18), 31.08 (C18), 29.83 (4 x C6), 28.42 (24 x C17) Four diastereomers are indicated, thus thirteen carbon signals are missing due to overlap of the diastereomers; IR (oil, cm^{-1}) 2924 (m), 1712 (s); MS (CI+) *m*/z (relative intensity): 666 ([M-H], 100); Exact Mass Calcd for $[C_{30}H_{41}N_3O_{10}S_2]$-H requires *m/z* 666.2155 Found 666.2188 (CI+).

462. 2R-*tert*-Butoxycarbonylamino-3-[4-(2R-*tert*-butoxycarbonylamino-2-carbonyl-ethylsulfanyl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-ylsulfanyl-*N*-fluorescein]-propionic acid methyl ester

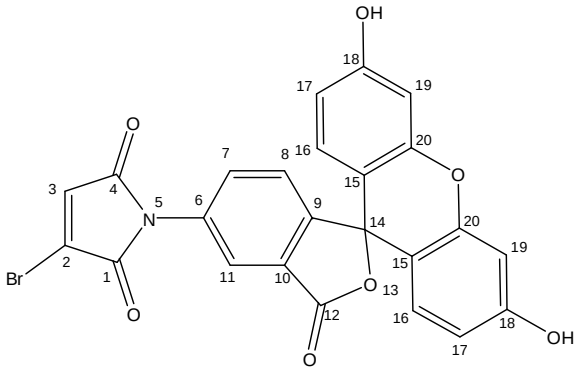


To **464** (200 mg, 0.34 mmol) in methanol (100 mL) was added *N*-Boc-Cys-OMe (159 mg, 0.68 mmol) and sodium acetate trihydrate (93 mg, 0.68 mmol). Purification by flash chromatography (gradient elution dichloromethane to 10% methanol in dichloromethane afforded **462** as a bright orange solid (155 mg, 0.17 mmol) in 50% yield.

δ_H (400 MHz, MeOD) 8.11 (s, 1H, H-11), 7.86 (d, 1H, *J* = 8.5, H-Ar), 8.11 (d, 1H, *J* = 8.1, H-Ar), 6.71 (d, 2H, *J* = 2.4, 2 x H-Ar), 6.70-6.67 (m, 2H, 2 x H-Ar), 6.58 (dd, 2H, *J* = 8.7 and 2.3, 2 x H-Ar), 4.64 (s, 2H, 2 x NH), 4.56 (dd, 2H, *J* = 8.5 and 4.5, 2 x H-24), 3.99 (dd, 2H, *J* = 14.1 and 4.1, 2 x H*H*-27), 3.79 (s, 6H, 2 x H_3-26), 3.56 (dd, 2H, *J* = 14.1 and 8.6, 2H, 2 x *H*H-27), 1.43 (s, 18H, 6 x H_3-21); δ_C (125 MHz, $CDCl_3$) 172.35 (C=O), 170.44 (C=O), 166.16 (C), 161.44 (br, C=O), 157.68 (C), 156.63 (C), 154.09 (C), 152.79 (br, C=O), 137.82 (C2 and C3), 134.76 (C), 134.22 (Ar-H), 130.27 (2 x Ar-H), 129.12 (C), 126.02 (Ar-H), 123.40 (Ar-H), 113.69 (Ar-H), 111.00 (Ar), 103.59 (2 x

Ar-H), 81.05 (2 x C22), 55.68 (2 x 24), 53.17 (2 x C26), 34.10 (2 x C27), 28.74 (6 x 21); IR (solid, cm^{-1}) 3373 (w), 2922 (m), 1742 (m), 1717 (s); MS (ES+) *m*/z (relative intensity): 894 ([M+H], 100); Exact Mass Calcd for $[C_{42}H_{43}N_3O_{15}S_2]$+H requires *m/z* 894.2214 Found 894.2192 (ES+); m.p. 140-145 ^oC; UV (Acetonitrile): ε_{421} = 3100, ε_{452} = 2940 and ε_{490} = 2600 $cm^{-1}M^{-1}$, λ_{em} (intensity, concentration) = 550 nm (81, 1 mM).

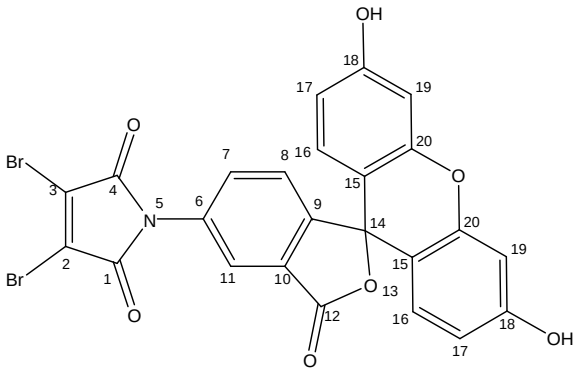
463. 3-Bromo-1-fluorescein-pyrrole-2,5-dione[318]



Dibromomaleic anhydride (346 mg, 1.95 mmol) was added in one portion to a solution of fluoresceinamine isomer 1 (0.68 g, 1.95 mmol) in acetic acid (65 mL) and the reaction mixture was stirred for 16 hours at room temperature in a sealed tube. The reaction mixture was then heated to 150 ^oC for 3 hours. Upon cooling to room temperature the precipitate was filtered and dried to afford **463** as an orange solid (0.72 mg, 1.43 mmol) in 73% yield.

δ_H (600 MHz, DMSO) 7.99 (d, 1H, *J* = 1.7, H-11), 7.77 (dd, 1H, *J* = 8.2 and 1.9, 1H, H-7), 7.73 (s, 1H, H-3), 7.43 (d, *J* = 8.2, 1H, H-8), 6.69 (m, 6H, 2 x H-16, 2 x H-17, 2 x H-19); δ_C (175 MHz, DMSO) 167.93 (C=O), 167.63 (C=O), 164.48 (C=O), 159.62 (2 x C18), 151.79 (2 x C20), 151.52 (C6), 133.68 (C7), 133.02 (C), 132.90 (C3), 131.23 (C), 129.15 (2 x Ar-H), 126.73 (C), 124.82 (C11), 122.29 (C8), 112.77 (2 x Ar-H), 109.08 (2 x 15), 102.30 (2 x Ar-H), 83.36 (C14); IR (solid, cm^{-1}) 3064 (w), 1726 (s); MS (ES+) *m/z*, (relative intensity): 508 ([M+H], 95), 506([M+H], 100); Exact mass calcd for $[C_{24}H_{12}O_7N^{79}Br]$+H requires 505.9875 Found 505.9833 (ES+); Orange solid decomposes above 190 ^oC; UV (Acetonitrile): ε_{455} = 6000 and ε_{480} = 6050 $cm^{-1}M^{-1}$, λ_{em} (intensity, concentration) = 530 nm (36, 10 μM).

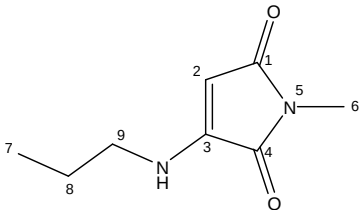
464. 3,4-Dibromo-1-fluorescein-pyrrole-2,5-dione[318]



Dibromomaleic anhydride (500 mg, 1.95 mmol) was added in one portion to a solution of fluoresceinamine isomer 1 (0.68 g, 1.95 mmol) in acetic acid (65 mL) and the reaction mixture was stirred for 16 hours at room temperature. The reaction mixture was then heated to reflux for 3 hours and allowed to cool to room temperature. The dispersion was filtered to remove impurities and the solvent was removed from the solution in *vacuo* to afford **464** as an orange solid (0.53 g, 0.90 mmol) in 46% yield.

δ_H (600 MHz, MeOD) δ 7.97 (d, 1H, *J* = 1.6, H-11), 7.77 (dd, 1H, *J* = 8.2 and 1.7, H-7), 7.43 (d, 1H, *J* = 8.2, H-8), 6.75-6.55 (m, 6H, 6 x Ar-H); δ_C (125 MHz, MeOD) 169.93 (C), 164.40 (C), 164.26 (C1 and C4), 162.81 (C), 141.23 (C), 134.72 (Ar-H), 133.96 (Ar-H), 133.33 (C), 131.04 (2 x C), 130.68 (Ar-H), 129.93 (2 x Ar-H), 129.22 (2 x Ar-H), 126.31 (Ar-H), 121.62 (C), 114.43 (br C), 103.53 (2 x C); IR (solid, cm^{-1}) 3064 (w), 1732 (s); MS (ES-) *m/z*, (relative intensity): 586 ([M-H], 30), 584([M-H], 100), 582([M-H], 100); Exact mass calcd for $[C_{24}H_{11}O_7N^{79}Br_2]$-H requires 581.8824 Found 581.8824 (ES-); Orange solid decomposes above 180 ^oC; UV (Acetonitrile): ε_{452} = 3580 and ε_{479} = 3550 $cm^{-1}M^{-1}$, λ_{em} (intensity, concentration) = 521 nm (396, 0.1M).

477. 1-Methyl-3-propylamino-pyrrole-2,5-dione



To propylamine (52 μL, 0.78 mmol) and sodium acetate (64 mg, 0.78 mmol) in methanol (30 mL) was added **289** (150 mg, 0.78 mmol) dropwise in methanol (30 mL). After 10 minutes, solvent was removed *in vacuo* and purification by flash chromatography (10% ethyl acetate in petroleum ether) afforded **477** as a bright yellow waxy solid (41 mg, 0.24 mmol) in 31% yield.

δ_H (500 MHz, $CDCl_3$) 5.43 (s, 1H, NH), 4.80 (s, 1H, H-2), 3.16-3.13 (m, 2H, H_2-9), 2.98 (s, 3H, H_3-6), 1.71-1.64 (m, 2H, H_2-8), 0.99 (t, *J* = 7.5, H_3-7); δ_C (125 MHz, $CDCl_3$) 172.71 (C=O), 167.66 (C=O), 149.51 (C3), 83.84 (C2), 46.01 (C9), 23.44 (C6), 21.87 (C8), 11.38 (C7); IR (film, cm^{-1}) 3317 (m), 2944 (w), 1698 (s), 1651 (s); MS (EI) *m*/z (relative intensity): 168 (M+, 70), 139 (100), 111 (40); Exact Mass Calcd for [$C_8H_{12}N_2O_2$]+ requires *m/z* 168.0893 Found 168.0887 (EI); UV (Acetonitrile) ε_{368} = 4500 $cm^{-1}M^{-1}$.

3.iv. RNAP sub-unit H and somatostatin modification and analysis

Desalting was via Slide-A-Lyzer mini dialysis units (Thermo Scientific, 2K MWCO) at 5 ^oC. Insulin (5733.4917 Da, 1 mg/1 mL in H_2O) and Adrenocorticotropic Hormone Fragment 18-39 (ACTH, 2465.1989, 1 mg/1 mL in H_2O)) were used as calibrants for RNAP sub-unit H and somatostatin, respectively. The matrix used for this study was 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (sinapinic acid) prepared at a concentration of 10 mg/1 mL of 1:1 v/v H_2O:MeCN. All samples were deposited by air-drying (*i.e.* using the dried drop method) onto a stainless steel MALDI-TOF plate (Waters).

For subunit H analysis, 2 μL of the reaction mixture was removed and diluted with 10 μL of HPLC grade water and mixed well. 2 μL of this diluted sample was mixed with 2 μL of the sinapinic acid matrix and mixed well. From this resultant solution, 2 μL were spotted onto air-dried pre-spotted sinapinic acid (10 mg/1 mL in acetone). Mass spectra were collected on a Micromass MALDI-TOF, operated in linear positive mode using a 337 nm nitrogen laser (Pulse:1250; Detector: 2750, Suppression:1200).

For somatostatin analysis, 2 μL of the reaction mixture was removed and mixed well with 2 μL of the sinapinic acid matrix. From this resultant solution, 2 μL were spotted onto the blank MALDI plate. Mass spectra were collected on a Micromass MALDI-TOF, operated in reflectron positive mode using a 337 nm nitrogen laser (Pulse:1250; Detector: 2750, Suppression:1200).

3.iv.i. Irradiation

In both instances reaction mixtures were irradiated in a 1.25 mL pyrex cuvette. The cuvette was taped to the side of a photochemical immersion well and cooled by

submersion in ice-water. Irradiations were carried out using a medium pressure 125 W mercury lamp (Photochemical Reactors Ltd.) and a pyrex immersion well (Photochemical Reactors Ltd.) cooled *via* running water.

3.iv.i.i. General procedure for preparation and irradiation of sub-unit H

Subunit H (SUH, mass = 9016, in buffer (25 mM tris(hydroxymethyl)aminomethane, 300 mM potassium acetate, 0.1 mM zinc acetate, 10 mM magnesium acetate, 10% glycerol)) was provided by Dr Dina Grohmann (UCL, ISMB) at a concentration of 111 μM. 10 equivalents of **289** (15.3 mM in 5% DMF in H_2O) were added and the reaction maintained at 5 ^oC for 1 hour. Quantitative reaction of the maleimide with cysteine to give mal-SUH was confirmed by MALDI-TOF (mass = 9095 Da). The solution was desalted over 18 hours at 5 °C. Analysis by LC-MS and MALDI-TOF showed that the construct was intact at this stage.

Single addition: 10 equivalents of styrene (153 mM in DMF) were added to the protein solution and the reaction degassed for 30 seconds. The reaction mixture was sealed and then irradiated for 5 minutes. Samples were analysed by MALDI-TOF MS and indicated that the reaction mixture contained mal-SUH (mass = 9102, 100%) and a new peak attributed to incorporation of styrene (mass = 9210, 80%), see p.253 for spectrum.

Sequential addition: 10 equivalents of styrene (153 mM in DMF) were added to the protein solution and the reaction degassed for 30 seconds. The reaction mixture was sealed and then irradiated for 5 minutes. A further 10 equivalents of styrene (153 mM in DMF) were added to the solution and the reaction degassed for 30 seconds. The reaction mixture was sealed and then irradiated for a further 5 minutes. Samples were analysed by MALDI-TOF MS and indicated that the reaction mixture contained mal-SUH (mass = 9153, 80%) and a new peak attributed to incorporation of styrene (mass = 9256, 100%), see p.254 for spectrum.

3.iv.i.ii. General procedure for preparation and irradiation of somatostatin

Lyophilised somatostatin (SST, mass = 1638) was solubilised in buffer (50 mM sodium phosphate, pH 6.2, 40 % MeCN, 2.5 % DMF) to yield a concentration of 152.6 μM and reduced with 1.1 equivalents of TCEP (15.3 mM in 5% DMF in H_2O) for 1 hour at 20 ^oC. 2.2 equivalents of **289** (15.3 mM in 5% DMF in deionised water) were added and

the reaction maintained at 20 ^{o}C for 1 h. Quantitative reaction to 2M-SST was confirmed by MALDI-TOF (mass = 1835). The solution was desalted over 18 hours at 5 °C. Analysis by LC-MS and MALDI-TOF showed that the construct was intact at this stage. The reaction mixture was degassed, sealed and then irradiated for 15 minutes. Samples were analysed by MALDI-TOF MS and indicated that the reaction mixture contained 2M-SST (mass = 1836, 50%) and a new peak (mass = 1661, 100%), see p.262 for spectrum.

4. References

1. Ciamician G., S. P., *Chem. Ber.* **1908,** 41, 1928.
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5. Appendix

The following articles have been published as a result of the work reported in this thesis, contributions are as follows:

1. **In situ Reduction in Photocycloadditions: A Method to Prevent Secondary Photoreactions, Lauren M. Tedaldi and James R. Baker**

L.M.T carried out the chemical synthesis. L.M.T. and J.R.B. designed the experiments. J.R.B. wrote the paper with assistance from L.M.T..

2. Bromomaleimides: New Reagents for the Selective and Reversible Modification of Cysteine, Lauren M. Tedaldi, Mark E. B. Smith, Ramiz I. Nathani and James R. Baker

L.M.T. carried out the chemical synthesis with assistance from R.I.N.. L.M.T., M.E.B.S., R.I.N. and J.R.B. designed the experiments. J.R.B. wrote the paper with assistance from L.M.T, and R.I.N..

3. Protein Modification, Bioconjugation, and Disulfide Bridging Using Bromomaleimides, Mark E. B. Smith, Felix F. Schumacher, Chris P. Ryan, Lauren M. Tedaldi, Danai Papaioannou, Gabriel Waksman, Stephen Caddick, and James R. Baker

M.E.B.S. and F.F.S. carried out protein modification. C.P.R. synthesised the fluorescent and biotinylated reagents for protein modification. L.M.T. carried out the small molecule supportive work. D.P. expressed the protein under supervision from G.W.. M.E.B.S., F.F.S., L.M.T., S.C. and J.R.B. designed the experiments. J.R.B. wrote the paper with assistance from M.E.B.S., F.F.S., L.M.T. and S.C..