Sustainable Approaches to the Synthesis of Aromatic Compounds and α-Hydroxyketones

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Declaration

I, Sally Higson, 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.

Sally Higson

06/11/2017

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Abstract

The unifying theme of this thesis is a sustainable approach to synthetic chemistry, focussing on two areas; the synthesis of aromatic compounds from biomass-derived furfural derivatives using environmentally friendly methodology, and the synthesis of α-hydroxyketones by expanding the substrate scope of transketolase enzymes by medium throughput mutant screening.

Chapter I gives an overview of the principles and importance of sustainability in synthetic chemistry and presents a broad discussion of biomass sources and uses, the use of water as a solvent, and enzymatic catalysis in organic synthesis.

Chapter II discusses the production of furfural derivatives from biomass, including the potential role of sugar beet pulp as a feedstock, and an overview of furfurals in synthesis. A new route to substituted benzene rings from furfurals via a three-step, one-pot cascade in water is then described, its scope explored, and an intramolecular variant developed. A 'Design of Experiment' optimisation of a furfural Michael addition is also presented.

Chapter III concerns the reactivity and potential uses of the phthalimide hydrazones produced by the methodology introduced in Chapter II, presenting an overview of potential downstream products, a broad investigation of functional group manipulation, and a new synthetic route to a poly(ADP-ribose) ribose polymerase inhibitor and substituted phthalocyanines.

Chapter IV explores the use of transketolase enzymes in the synthesis of α-hydroxyketones, investigating the role of enzyme variants in enhancing the acceptance of previously unreported non-natural keto-acid donor substrates. The development of a medium throughput methodology and its application in enzyme mutant screening is described.

Chapter V gives a summary of the research carried out, presents some general conclusions, and describes future work, and Chapter VI contains the details of experimental procedures and compound characterisation for the results discussed in Chapters II-IV.

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Summary of Abbreviations

°C	degrees centigrade
ACS	American Chemical Society
ADA	*N*-(2-acetamido)iminodiacetic acid
aq.	aqueous
Ar	generic aryl group
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
b.p.	boiling point
br.	broad
Bu	butyl
CPME	cyclopentyl methyl ether
conc.	concentrated
DIEA	*N,N*-diisopropylethylamine
DMSO	dimethyl sulfoxide
DoE	design of experiment
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	ethyl
EtOAc	ethyl acetate
FPP	farnesyl pyrophosphate
g	gram(s)
Gly-gly	glycylglycine
GST	*Geobacillus stearothermophilus*
h	hours
HMDS	hexamethyldisiloxane
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMF	hydroxymethylfurfural
HOBt	1-hydroxybenzotriazole
HPA	hydroxypyruvic acid
hv	ultraviolet irradiation
Hz	hertz
i	*iso*
M	molar
Me	methyl

MES	2-(*N*-morpholino)ethanesulfonic acid
MF	methylfurfural
mg	milligram(s)
MMPP	magnesium monoperoxyphthalate
mol	mole(s)
MOPS	3-morpholinopropane-1-sulfonic acid
mw	molecular weight
MW	microwave
ND	not determined
NMR	nuclear magnetic resonance
NR	no reaction
OTf	trifluoromethanesulfonate
p	*para*
PET	poly(ethylene terephthalate)
pet. ether	petroleum ether
Ph	phenyl
PIPES	piperazine-*N*,*N'*-bis(2-ethanesulfonic acid)
PLA	poly(lactic acid)
Pn	pentyl
ppm	parts per million
Pr	propyl
R	generic alkyl group
R_f	retention factor
rt	retention time
RT	room temperature
sat.	saturated
SBP	sugar beet pulp
t	*tert*
T	temperature
THF	tetrahydrofuran
TK	transketolase
TLC	thin layer chromatography
TRIS	tris(hydroxymethyl)aminomethane
UV	ultraviolet light

Acknowledgements

Chapter I. General Introduction

1.1. Sustainable Synthesis

The field of green chemistry has emerged over the last thirty years in response to concerns over the effect of the chemical industry on the environment and its inhabitants. Chemists are becoming increasingly concerned with developing more sustainable synthetic processes that minimise the use and generation of hazardous substances and reduce the consumption of non-renewable resources.

Twelve key principles of this approach have been proposed by Anastas and Warner, and endorsed by the American Chemical Society (ACS), which read as follows;[1]

" 1. **Prevention** - It is better to prevent waste than to treat or clean up waste after it has been created.

2. **Atom Economy** - Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. **Less Hazardous Chemical Syntheses -** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. **Designing Safer Chemicals -** Chemical products should be designed to affect their desired function while minimizing their toxicity.

5. **Safer Solvents and Auxiliaries -** The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

6. **Design for Energy Efficiency -** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. **Use of Renewable Feedstocks -** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. **Reduce Derivatives -** Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. **Catalysis -** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. **Design for Degradation -** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. **Real-time analysis for Pollution Prevention -** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

12. **Inherently Safer Chemistry for Accident Prevention -** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires. ”

Similar principles have been set out by other bodies, such as the UN. The overall aim is to be more efficient and responsible in the use of resources and to design products, processes, materials and molecules that are safer and less harmful for both the people exposed to them and the environment. A common metric for evaluating the environmental impact of a synthetic process is the "E-factor", defined by the ratio of the mass of waste per mass of product.

This thesis is concerned with sustainable synthesis, and particularly focuses on atom efficiency, the use of renewable feedstocks, and the use of safer solvents, particularly water.

1.2. The Use of Renewable Feedstocks

In Europe, approximately ninety percent of compounds used in commercial synthetic processes currently derive from petrochemicals. This is a finite, depleting resource whose usage is implicated in the release of harmful greenhouse gases.[2] In addition to environmental concerns and the subsequent ethical obligations, it is thought that alternative feedstocks will become increasingly economically desirable as crude oil becomes a more limited resource.[3] As biomass is the most abundant source of sustainable

organic carbon, and its generation consumes atmospheric carbon, it presents a versatile and desirable alternative feedstock.[3,4]

Biomass can be purpose-grown, like energy crops, but then must compete for land use. Alternatively, it can be harvested from natural resources such as aquatic biomass,[5] or derived from waste products of other industries, including agriculture and foresting.[4] The food industry currently produces approximately 1.3 petagrams of biowaste per annum, with the majority used as low-value livestock feed or crop fertilizer.[6] This biomass is predominantly plant-based and comprised mostly of carbohydrates, with small amounts of other plant products such as oils, lipids and terpenes.

Processing of these carbohydrates largely focuses on creating biofuels or polymers. The three major routes are: pyrolysis into liquid fuels; gasification into SynGas, a fuel gas that can be converted into alkanes and methanol; and hydrolysis to monosaccharides. The most common fate of these monosaccharides is fermentation to various small molecule platform chemicals. These include ethanol, a common solvent and biofuel, and organic acids such as lactic acid **1**, succinic acid **2**, and glutamic acid **3** (Figure 1).[4]
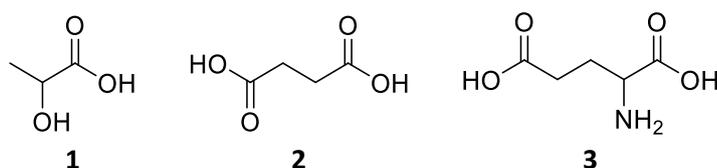
Figure 1. Structures of products of monosaccharide fermentation

Monosaccharides and their derivatives can be processed into biopolymers. Sucrose is used in the synthesis of rigid polyurethanes,[3] and in the US, lactic acid **1** is used to produce polylactic acid (PLA) on the million kilogram scale.[7] 5-Hydroxymethylfurfural is produced by the dehydration of C_6 sugars and has emerging potential as a progenitor for a wide range of polymer materials.[8]

Direct synthetic routes to highly functionalised compounds, such as pharmaceutical intermediates, have been less well studied,[3] and an example of this approach is explored in this work.

1.3. Water as a Solvent

Solvents and Green Chemistry

Solvents are consumed in huge quantities in the chemical industry, both in performing synthetic reactions and in purification techniques. Many traditional solvents are toxic and hazardous to the environment, requiring expensive disposal or recycling procedures.[9] Several non-classical solvents are gaining recognition and greater use for their more sustainable profiles. These include 2-methyltetrahydrofuran **4** and cyclopentyl methyl ether (CPME) **5**, which present alternatives to more harmful ethereal solvents such as THF and 1,4-dioxane,[10,11] and dimethylcarbonate **6**, which has been described as a suitable substitute for dichloromethane.[12] The GSK Solvent Selection Guide provides an excellent assessment of the 'green' credentials of a wide range of solvents.[13]

4   **5**   **6**
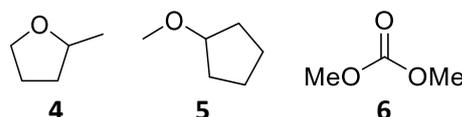
Figure 2. Structures of 2-methyltetrahydrofuran **4**, *cyclopentyl methyl ether,* **5**, *and dimethylcarbonate* **6**

The use of supercritical fluids, ionic liquids, and biomass-derived solvents such as limonene and glycol are also gaining interest as green alternatives.[14] In many ways, however, water can be considered the ultimate 'green' solvent, as it is totally benign and nonflammable, easily disposed of or recycled, and is the cheapest and most abundant solvent available.[15] It also has the largest known specific heat capacity, allowing for excellent temperature control and safety.[16] In many instances, the use of water as a solvent can actually have an accelerating effect on organic reactions, improving rates and yields.[15,17]

While Diels and Alder performed cycloadditions in water in 1931,[18] it wasn't until 1980 that the rate enhancing properties of water on cycloadditions were studied. Breslow *et al.* found that the cycloaddition of cyclopentadiene **7** with methyl vinyl ketone (MVK) and acrylonitrile occurred 1-2 orders of magnitude faster than with isooctane or methanol (Scheme 1, Table 1).[19]
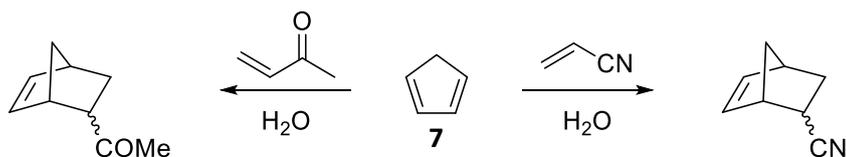
Scheme 1. Literature cycloaddition reactions of cyclopentadiene with methyl vinyl ketone (MVK) or acrylonitrile[19]

SOLVENT	RELATIVE RATE		SOLVENT	RELATIVE RATE
isooctane	1		isooctane	1
methanol	12.7		methanol	2.11
water	740		water	31.2

Table 1. Rates of the literature cycloaddition reactions of cyclopentadiene with methyl vinyl ketone (MVK) (left) or acrylonitrile (right)[19]

These reactions were carried out at low concentrations in order to fully dissolve the reagants in water. In 2005, Sharpless *et al.* carried out a series of Claisen rearrangements and cycloaddition reactions in which non-polar reagents were vigourously stirred with water to create an aqueous/organic suspension.[20] The reaction of quadricyclane **8** with dimethyl azodicarboxylate **9** in various solvents and solvent mixtures suggested that the heterogenous suspension was responsible for the accelerating effect of water in these reactions (Scheme 2, Table 2).
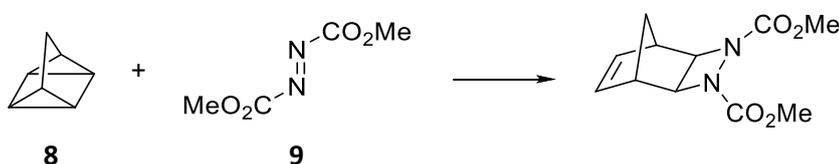
*Scheme 2. Literature cycloaddition reaction of quadricyclane **8** with dimethyl azodicarboxylate **9**[20]*

SOLVENT	TIME TO COMPLETION		SOLVENT	TIME TO COMPLETION
toluene	>120 h		on water	10 min
acetonitrile	84 h		methanol/water (3:1, homogenous)	4 h
CH_2Cl_2	72 h			
DMSO	36 h		methanol/water (1:1, heterogenous)	10 min
methanol	18 h			
neat	48 h		methanol/water (1:3, heterogenous)	10 min
on C_6F_{14}	36 h			

*Table 2. Time to completion for literature cycloaddition reaction of quadricyclane **8** with dimethyl azodicarboxylate **9** in a range of solvents and solvent mixtures[20]*

Small rate accelerations were observed in homogeneous polar solutions, but large accelerations required heterogeneity. In water/methanol solvent mixtures, the methanol did not affect the speed of the reaction unless there was enough to solubilise the reagents, at which point the rate drops dramatically. The term "on water" was coined to describe the reactions with a heterogeneous organic/aqueous suspension, and previous reactions in which the reagents were dissolved in water were described as occurring "in water". However, in most accelerated reactions in water the reagent solubility, or lack thereof, is not absolute, and it is thought that a mixture of "on water" and "in water" reaction effects are common.[21]

Mechanism of Acceleration

Several studies have suggested contributing factors to this accelerating effect. Breslow attributed it to hydrophobic effects, but this didn't explain why the cycloaddition with MVK occurred at a much higher rate than acrylonitrile (Scheme 1, Table 1). Jorgensen *et al.* studied Breslow's reactions computationally and proposed that hydrogen bonding to the dienophiles and the transition states had a greater stabilising effect on the latter (Figure 3).[22] This would lower the activation energy and increase rate of reaction, and the effect would be greater on MVK than acrylonitrile due to superior hydrogen bonding.
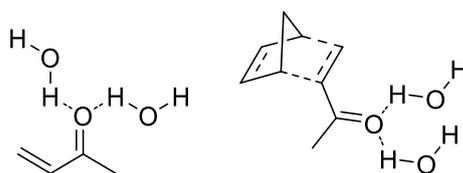
Figure 3. Stabilisation of MVK and its transition state by hydrogen bonding to water

Marcus *et al.* performed similar computational analyses of Sharpless' 'on water' reactions and suggested that the acceleration was caused by the transition state hydrogen bonding to 'protruding hydroxyl groups' at the organic/aqueous interface.[23]

The accelerating effect of water on pericyclic reactions has been well-studied, but several other types of reaction have also shown improved rates and yields with water as the solvent.[15] These include Michael additions,[24] Bayliss-Hillman reactions,[25] Wittig reactions with stabilised ylids,[21,26] and nucleophilic ring openings, which are a key step in many biosyntheses.[27] The use of aqueous solvents has been criticised as less environmentally friendly than suggested due to isolation of the reaction product often requiring large amounts of organic solvent, or significant organic cosolvent being required in the

reaction.[28]   In some cases, these drawbacks can be avoided by the insolubility or immiscibility of the reaction product in water facilitating isolation by filtration or phase separation.[15]

Reactions in water have particular relevance in biology-related fields, such as protein modification, biosynthesis, and the origins of life.[29-31]   A highly active area of green chemistry is biocatalysis, the use of enzymes in synthetic chemistry, which predominantly uses aqueous reaction conditions.

1.4. Enzyme Catalysis in Organic Synthesis

Biocatalysis is an attractive approach in sustainable synthesis, as enzymatic reactions often provide exquisite regio- and stereochemical control and can be performed at ambient temperatures and pressures in aqueous media. While biocatalysis has technically been in use for millennia, with the oldest records of using yeast in brewing dating back 6000 years, scientific reports date back to Liebig and Wöhler's application of hydroxynitrile lyases 180 years ago.[32]   Since then, biocatalysts have been used in a wide range of syntheses and industrial processes, including for the food, cosmetic, pharmaceutical and agrochemical industries.[33-36]

Biocatalysis has been described as "one of the greenest technologies",[37] due to its minimal use of organic solvents and energy input, high efficiency, and the complete biodegradability and renewable sourcing of the enzyme catalysts themselves. Biocatalytic processes are also considered 'natural' by marketing laws and this often leads to the product having a higher market value than a chemically identical, non-biologically synthesised compound.[38]

Biocatalysis can be carried out *in vitro* using isolated enzymes or cell extracts such as lysate, or *in vivo* using the metabolic pathways of an organism. These 'whole cell' *in vivo* processes can be naturally present in the organism, or designed and introduced to the cell in an act of synthetic biology pathway engineering. Some advantages of this approach are that enzyme purification costs are avoided, cofactors and complimentary redox reactions are built into the system, and whole reaction pathways can be engineered. However, uptake and release of the reagants and products must also be engineered into the cell, and the system is much more complicated with less control over the reaction environment, lengthening development time for the process.

Many isolated enzymes are commercially available, such as Lipolase 100L®, a lipase from *Thermomyces lanuginosus*. This is used in the production of pregabalin **12**, a neuropathic pain treatment worth $3 billion per year.[39,40] In 2007 the manufacturer, Pfizer, redesigned its synthetic route with a stereospecific biocatalytic step using Lipolase®. This provided early stage chiral resolution while allowing recycling of the unreacted enantiomer, (*R*)-**10**. The enzymatic product, **11**, could be converted into pregabalin in only two steps, both also in aqueous media. This reduced the volume of organic solvent required by 90% and the amount of starting material needed by 50%.
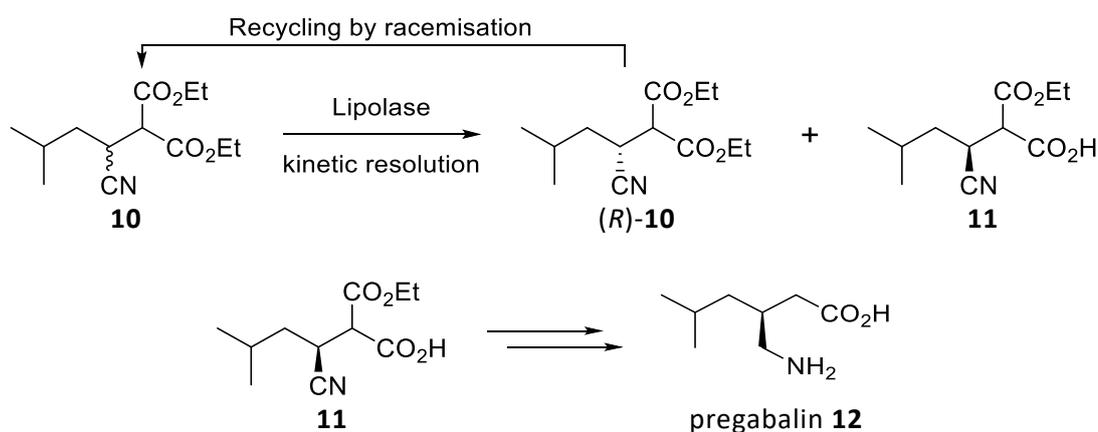
*Scheme 3. Enzymatic synthesis of **11**, a precursor to pregabalin **12***

An example of the use of whole cell synthetic biology pathways is the synthesis of artemisinic acid **15** by engineered Baker's yeast. Artemisinic acid **15** is a direct precursor to the antimalarial compound artemisinin **16**, a natural product of the wormwood *Artemisia annua*. The yeast was designed to overproduce farnesyl pyrophosphate **13** (FPP)**,** a natural intermediate of the mevalonate pathway, and convert it into **15** via the intermediate amorpha-4,11-diene **14** using enzymes taken from *A. annua*; amorphadiene synthase and a cytochrome P450 monooxygenase (Scheme 4).[41] The artemisinin this produces is referred to as "semi-synthetic" and a factory in Italy is able to produce up to 50 tonnes per year; approximately a third of global annual demand.[42]
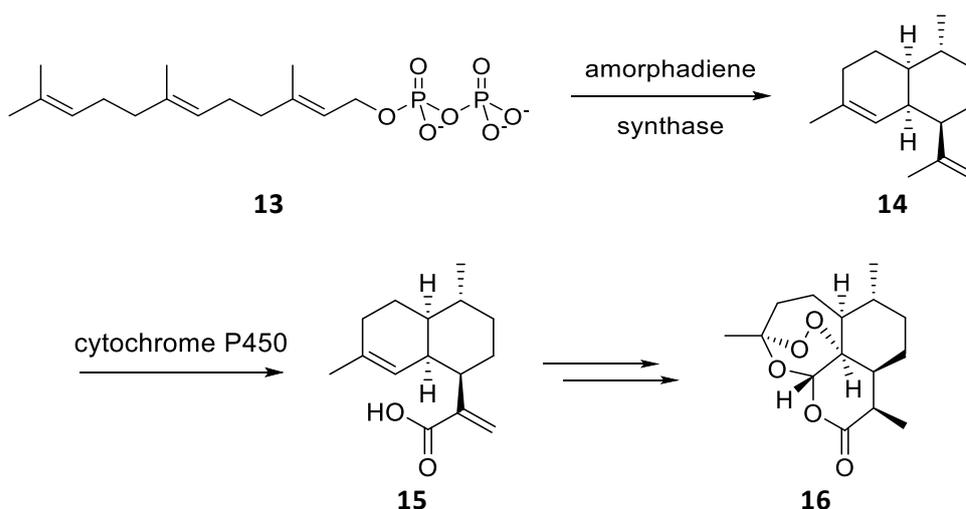
Scheme 4. *Enzymatic synthesis of artemisinic acid **15**, a precursor to artemisinin **16**, from FPP **13***

While this is a powerful example of the use of natural enzymes to create natural products in an engineered environment, over the last two decades drug discovery has been increasingly focused on non-natural products. This shift has been largely driven by the improvement of combinatorial chemistry techniques allowing more efficient searching of non-natural chemical space for bioactive compounds.[43,44] For biocatalysis to be widely applicable in synthesis, a diverse and flexible enzymatic 'toolkit', able to perform non-natural transformations on non-natural substrates, is therefore required.[45] To this end, a significant amount of research has been carried out on discovering new enzymes and on the directed evolution of existing enzymes to carry out new biotransformations.[45-47]

1.5. Overall Summary and Project Aims

Sustainable chemistry is an increasingly prevalent concern in the chemical industry, with environmental and economical pressures driving the development of new synthetic routes and methodologies. The use of biomass is an increasingly important aspect of sustainability in chemical synthesis, as traditional petrochemical sources become depleted and their detrimental environmental effects accumulate. Biocatalysis is also of significant interest as a green approach to synthesis, with widespread applicability in industry.

This work consists of two major project aims. The first is to develop new methodology for the utilisation of biomass, particularly the use of biomass-derived furfural and analogues in the synthesis of polysubstituted aromatic compounds (Scheme 7).

28   **31**
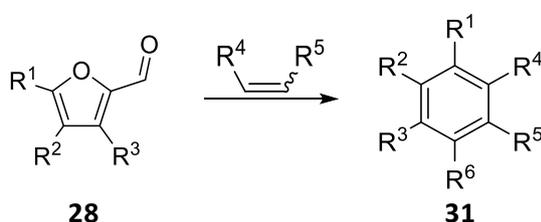
Scheme 5. General scheme for the use of furfural and its analogues in the synthesis of polysubstituted aromatic compounds

The second project aim is to expand the enzymatic toolkit by investigating the enzyme Transketolase (TK) and its acceptance of novel donor subtrates for the stereoselective synthesis of α-hydroxyketones (Scheme 6).

donor   acceptor
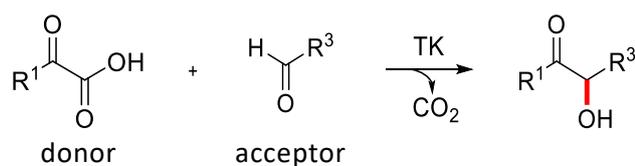
Scheme 6. General scheme for the use of novel donor substrates in transketolase biotransformations for the synthesis of α-hydroxyketones

These topics will be further introduced in their respective chapters. In both approaches, the use of water as a solvent, for its green profile and potential reaction accelerating properties, was a key aim and consideration.

Chapter II. Synthesis of Substituted Benzene Rings from Furfurals

2.1. Introduction

2.1.1. Sources of Furfural and Furfural Analogues

Furfural **17** is obtained exclusively from the dehydration of aldopentoses such as xylose, with no existing synthetic route from petrochemicals (Scheme 7). In nature, the majority of pentoses exist in hemicellulose, a heterogeneous polysaccharide that constitutes approximately 20-35% of biomass; the second most abundant bio-based polymer in nature, after cellulose.[4]
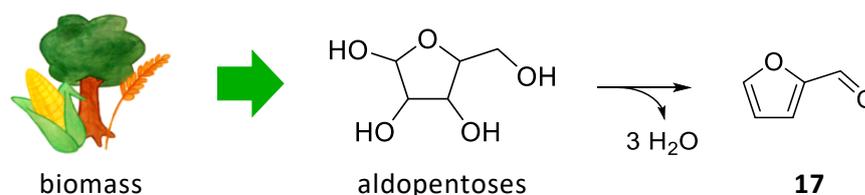
Scheme 7. Representation of the accessibility of furfural from biomass-derived aldopentoses

5-Methylfurfural **18** and 5-hydroxymethylfurfural **19** (HMF) can be similarly produced by the dehydration of aldohexoses such as glucose and mannose, found in cellulose and hemicellulose, respectively.[8,48] This widens the source material for furfural analogues and lends some diversity to furfural species directly obtainable from biomass (Figure 4). The synthesis of 5-chloromethylfurfural **20** from saccharides was first reported in 2009, and it has since gained significant attention as an alternative biomass-derived furfural.[49] It has been described as "functionally equivalent [to HMF] but more practical in terms of its production from biomass" due to its lipophilicity facilitating extraction from aqueous media, and its stability under the acidic conditions commonly used to dehydrate monosaccharides.[50]
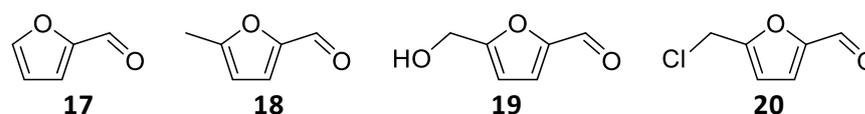
*Figure 4. Structures of furfural **17**, 5-methylfurfural **18**, HMF **19** and 5-chloromethylfurfural **20**, the furfural analogues directly obtainable by the dehydration of sugars*

The monosaccharide subunit composition of different hemicelluloses varies significantly by source; for example, hardwoods are rich in xylans and softwoods contain glucomannans.[51] Different sources of biomass therefore have varying suitability as sources of aldopentoses and thus furfural **17**. For example, the potential yield of furfural, measured per metric tonne of dried biomass, is reported as 220 kg for corncobs, 170 kg for bagasse, 160 kg for cornstalks and sunflower hulls, which are available as by-products of the food industry, and between 150 kg and 170 kg for hardwoods.[51] The ubiquitous nature of carbohydrates in plants, however, makes any plant or crop a potential source of furfural and its analogues. Indeed, many food commodities contain "several mg per kg" of furfurals, including cereal derivatives, fruit juices, dried fruits, honey, milk and coffee,[52] and it has been estimated that on a daily basis the average American consumes 300 µg of furfural per kg of bodyweight.[53]

Typical industrial processing methods give approximately 40-50% of a feed crop's total potential furfural yield despite stoichiometric yields being possible.[4,54] Over 300,000 tonnes of furfural are produced per annum, with approximately 200,000 tonnes originating from corncobs in China and 20,000 tonnes from bagasse in South Africa.[51,55] Furfural is the only unsaturated chemical produced from carbohydrates on this order of magnitude.[2]

Some substituted furfurals can be obtained directly from biomass (Figure 4), and others are relatively easy to synthesise. Substituents can be added to furfural **17** by Friedel-crafts alkylation or acylation,[56] bromination followed by a palladium-catalysed cross-coupling reaction such as a Suzuki[57,58] or Negishi[59] coupling, or palladium acetate-catalysed oxidative coupling alkenylation.[56,60]
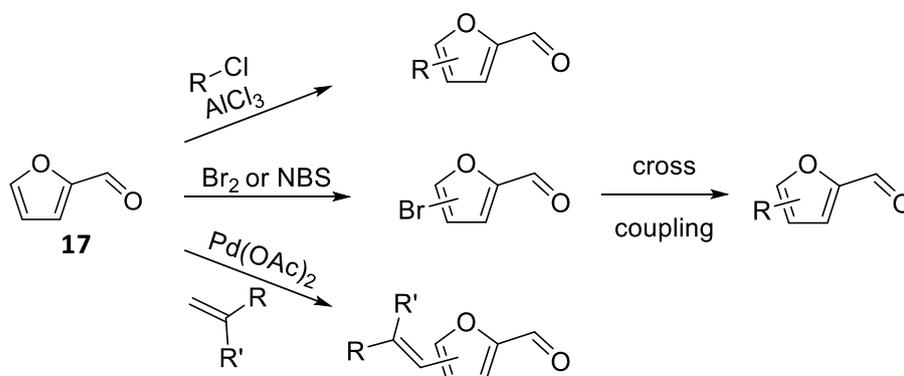
*Scheme 8. Functionalisation and diversification of furfural **17** on the furan ring by i) Freidel-Crafts reaction, ii) bromination followed by Suzuki coupling, and iii) palladium acetate-catalysed oxidative coupling alkenylation*

Sugar beet pulp as a source of furfural derivatives

There is growing interest in maximising the economical use of waste or byproducts and minimising the land usage of 'purpose-grown' feedstock crops by obtaining feedstock chemicals from the 'leftover' biomass of other industries, such as sawdust,[61] bagasse,[55] grain hulls and nut shells and husks,[56] and even used coffee grounds.[62]

Sugar beet pulp (SBP) is a byproduct of processing sugar beets into refined sugar; the leftover vegetable matter after the glucose content has been extracted. It is comprised mainly of cell-wall polysaccharides, including cellulose and hemicelluloses such as arabinan and aribinoxylan,[63] and pectins.[64,65] It currently, it finds low-value use as livestock feed, with around 500,000 tonnes produced and sold for this purpose by British Sugar in 2016.[66] Drawbacks of this usage include the low-protein content of SBP limiting its value as feed, the surplus of SBP produced, and the expense of first drying the pulp into pellets for sale.[65] Alternative uses have been investigated, including as a cultivation substrate[67] and as paper pulp.[68] Extraction of isolated components has also been explored, particularly cellulose microfibrils,[69] ferulic acid,[65,70] and polyols, for polyurethane production.[71]

This PhD work is part of a large multi-disciplinary project across several universities, with the aim of isolating sugars from SBP and creating added value products from them in an economically viable and environmentally sustainable way. This work focuses on novel ways to utilise isolated furfurals.

2.1.2. Current Applications of Furfural and its Analogues

Resin production accounts for around 70% of the market demand for furfural.[4] Other large-scale uses include decarbonylation to furan, a feedstock chemical that can then be optionally hydrogenated to give tetrahydrofuran (THF). THF is a common solvent and monomer precursor to poly(tetramethylene ether) glycol, a polymer used in making elastane fibres.[72] Other furfural derivatives have been upgraded to 'drop-in fuels' such as 2,5-dimethylfuran, 2-methylfuran, 5-ethoxymethylfurfural, γ-valerolactone, ethyl levulinate and long chain hydrocarbon alkanes.[73]

Furfural is considered toxic, with an oral LD_{50} in rats of 65 mg/kg and an inhalation LD_{50} of 175 ppm.[74,75] Despite this, furfurals are in everyday foodstuffs in "several mg per kg" quantities,[52] as previously mentioned, and are in fact used as additives in a wide range of

foods, from meat and gravy to confectionary.[76] Furfural is described as providing "brown, sweet, woody, bready, nutty, caramellic with a burnt astringent nuance" flavours.[77]

An increasingly common application of furfural derivatives is as building blocks in plastic polymer synthesis, with significant ongoing research in this area.[78] Poly(ethylene terephthalate) (PET) and poly(ethylene furanoate) **21** can both be prepared from 5-hydroxymethylfurfural (HMF) **19** (Figure 5).[79,80]
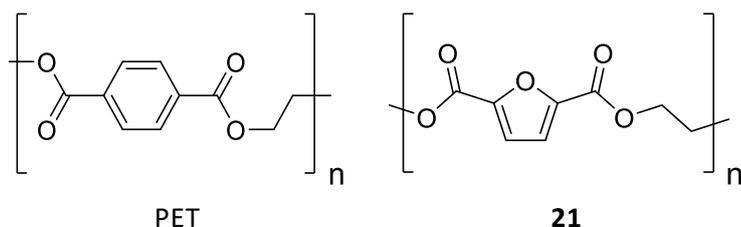
Figure 5. Structures of poly(ethylene terephthalate) (PET) and poly(ethylene furanoate) 21

PET is typically industrially synthesised from petrochemical-derived *p*-xylene, which is oxidised to produce either terephthalic acid **22** or its dimethyl ester **23**, depending on the process used. This oxidised product is then reacted with ethylene glycol in an esterification or transesterification reaction (Scheme 9).[81]
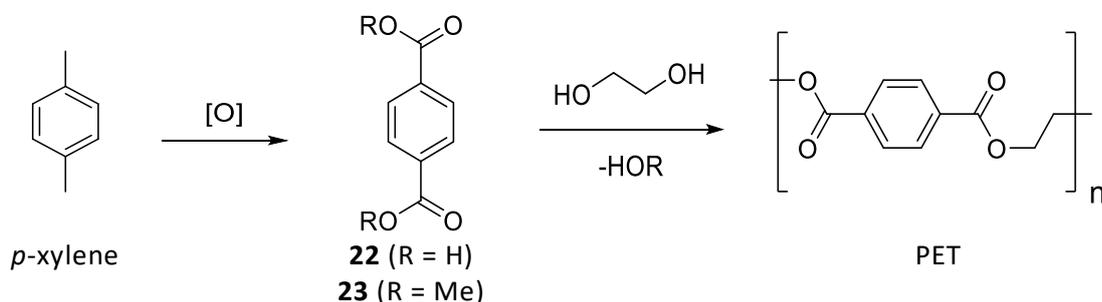
Scheme 9. Synthesis of PET from p*-xylene via a terephthalic acid/ester intermediate*

To make bio-derived PET, a process was developed to convert HMF **19** into *p*-xylene. First, **19** was reduced to give dimethylfuran **24**, which underwent a Diels-Alder reaction with bio-derived acrolein to give a formyl-substituted oxanorbornene **25**. This was aromatised and the aldehyde group was oxidised, giving 2,5-dimethylbenzoic acid **26**, and removal of the carboxylic acid gave *p*-xylene (Scheme 10).[79,82]
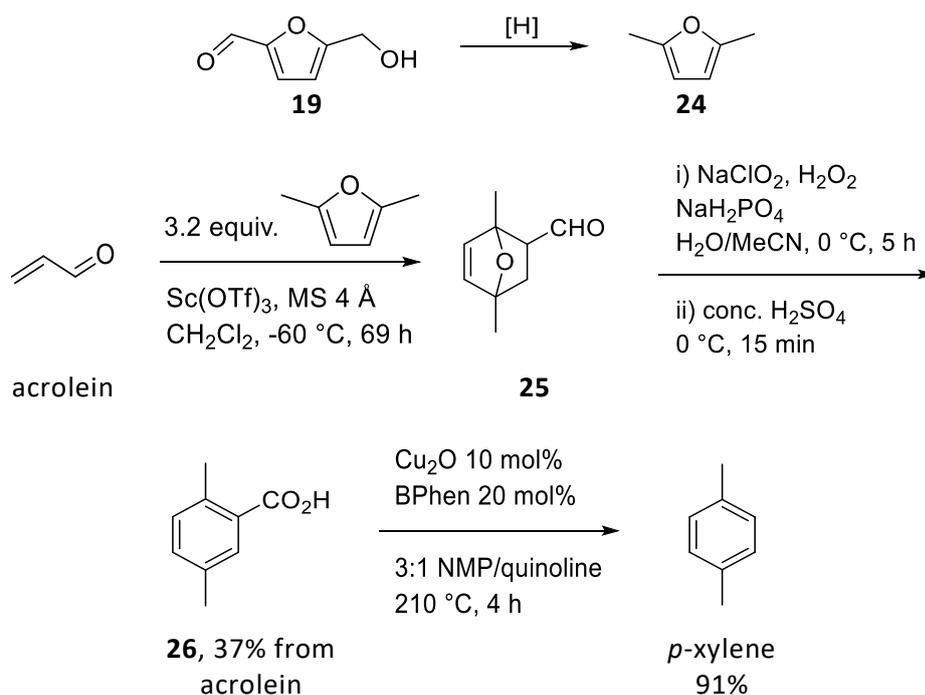
Scheme 10. Synthesis of p-*xylene from 5-hydroxymethylfurfural*

In contrast, the synthesis of **21** from HMF **19** is much more concise; oxidation of **19** to **27** and subsequent esterification with ethylene glycol gave **21** in two steps (Scheme 11).[80,83]
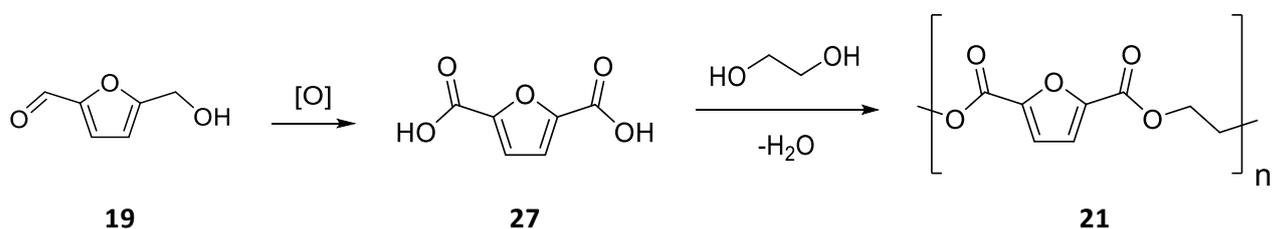
Scheme 11. Synthesis of **21** from HMF *via* **27**

These two polymer syntheses illustrate alternative approaches to the development of biomass-derived end products. The first is the conversion of sustainable, biomass-derived feedstocks into traditional petrochemical feedstock compounds, allowing them to be fed into pre-existing production streams which make well-established commercial products. The second approach is to harness the feedstock's inherent reactivity to create novel end-products less laboriously and via greener chemistry than employed in the pre-existing production streams.[78] For the latter to be commercially viable against well-established streams, however, the novel product must have advantages over the pre-existing, traditional mass-produced petrochemical-based product.

Poly(ethylene furanoate) **21** was recently described as being "a fully biosourced alternative to PET with greatly improved barrier properties and attractive thermal and

mechanical properties",[84] providing the qualitative advantages to make its production competitive with PET.

2.1.3. Reactivity of Furan Rings

While a significant amount of work has been reported on the use of furfural derivatives as precursors to biomass-derived polymers, work on converting these furfurals to added-value synthons for the pharmaceutical or agrochemical industries has been limited and largely focuses on the reactivity of the aldehyde group or, in the case of HMF, the hydroxyl group, rather than utilising the reactivity of the furan ring. [8,85]

Notable reactions of furan rings include reduction to the tetrahydrofuran moeity, hydrolysis to 1,4-dicarbonyls, electrophilic aromatic substitution, cycloaddition reactions, nucleophilic substituion of furans with suitable leaving group substituents and, in the case of hydroxymethyl furans, the Achmatowicz reaction to dihydropyrans (Scheme 12). [86-89]
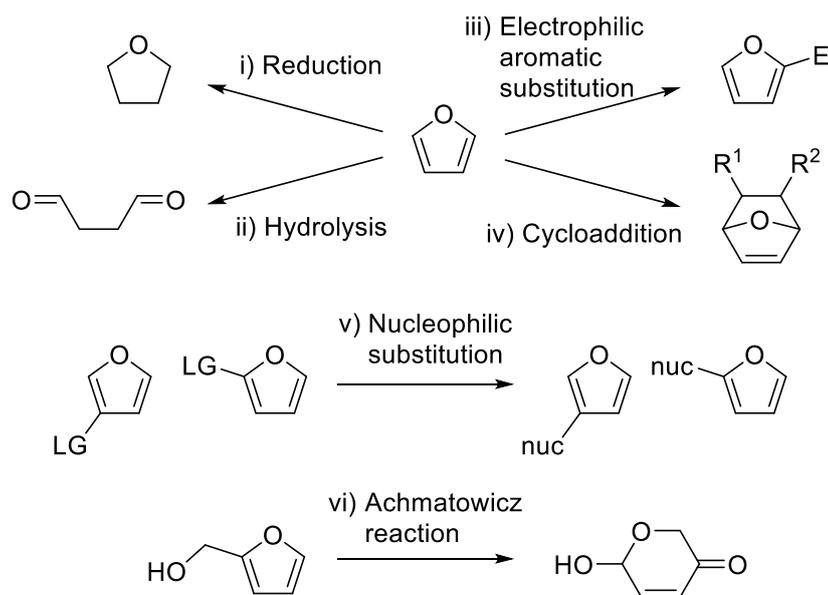
Scheme 12. Representation of the potential reaction of a furan by i) reduction to tetrahydrofurans, ii) hydrolysis to 1,4-dicarbonyls, iii) electrophilic aromatic substitution, iv) cycloaddition with dienophiles to 7-oxanorbornenes, v) nucleophilic substitution and vi) Achmatowicz reaction to dihydropyrans

Of these, Diels-Alder cycloadditions and electrophilic aromatic substitutions were selected to study for their potential in creating complex, high-value chemicals from sugar-derived furfurals.

Cycloadditions of furan rings

Diels-Alder cycloadditions using furan as a diene were among the first reactions carried out by Diels and Alder over eighty years ago.[90] These reactions initially give rise to substituted 7-oxanorbornenes (7-oxabicyclo[2.2.1]heptenes), which have the potential to undergo further reaction to a variety of interesting molecules (Scheme 13). Basic conditions can cause opening of the oxygen bridge to give a hydroxyl-substituted cyclohexa-1,3-diene (Scheme 13, i), while nucleophilic attack into the oxygen bridge and addition reactions across the olefin can provide additional substituents on the cyclohexyl ring (Scheme 13, iii). A variety of different conditions can instigate complete loss of the bridging oxygen centre as water, aromatising the ring and creating aromatic compounds (Scheme 13, ii) as previously shown in the synthesis of *p*-xylene (Scheme 10, step 4).
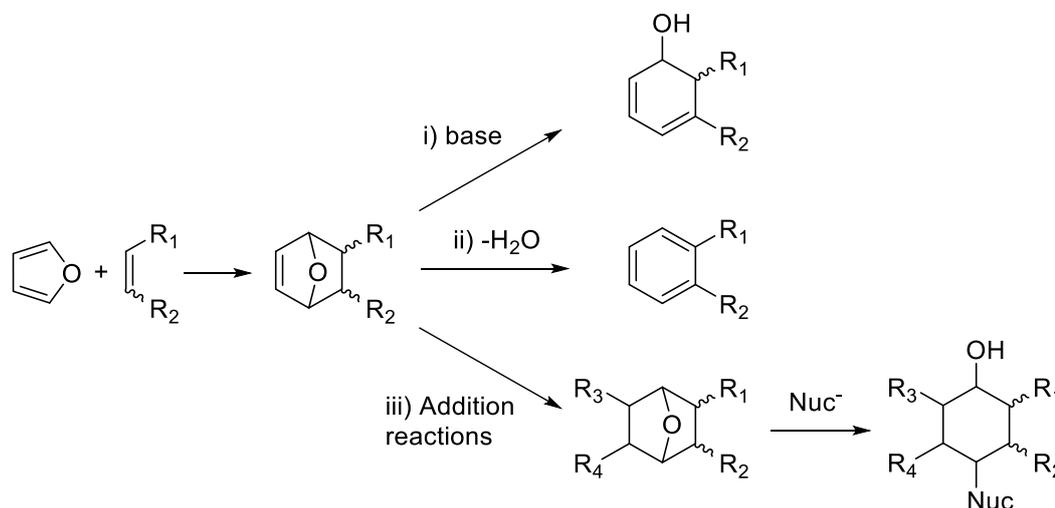
Scheme 13. Reaction of furan with dienophiles to give a substituted 7-oxanorbornenes, and some examples of potential subsequent reactions: i) base catalysed β-elimination of the heteroatom bridge, ii) dehydration to a substituted benzene ring, and iii) addition reaction followed by nucleophilic attack on the heteroatom bridge to give a stereoselectively highly

Over the years, the use of furan rings in cycloadditions has been well-documented and several applications in synthesis have been developed, with recent interest in the use of intramolecular Diels-Alder reactions of furan (IMDAFs) in total synthesis.[86,91-93]

Furan is a poor diene that only gives high yields of cycloaddition products with reactive dienophiles, and the retro-Diels-Alder is a known problem.[91,94-96] Lewis acids are often used to catalyse these reactions by activating the dienophile, and high-pressure reaction conditions have also been reported.[97-100] A recent report describes the activation of 2-furfuryl ketones towards Diels-Alder reactions and Friedel-Crafts alkylations by reaction with an amine, forming a trienamine motif that donates electron density into the furan

ring (Scheme 14a).[101] Diels-Alder reactions with furfurals have been promoted by forming a linker between the aldehyde motif and an unsaturated carboxylic acid via a four-component Ugi reaction, setting up an intramolecular cycloaddition (Scheme 14b).[102] In a small number of cases from both approaches, the resulting 7-oxanorbornenes were then successfully aromatised by treating with acids in toluene. However, the aromatisation of 7-oxanorbornenes (Scheme 13, ii) often requires harsh reaction conditions; it has been effected using strong, concentrated acids,[103,104] selenium activation,[105] and, in cases with electron-withdrawing groups present, basic treatment in presence of lithium iodide.[106]
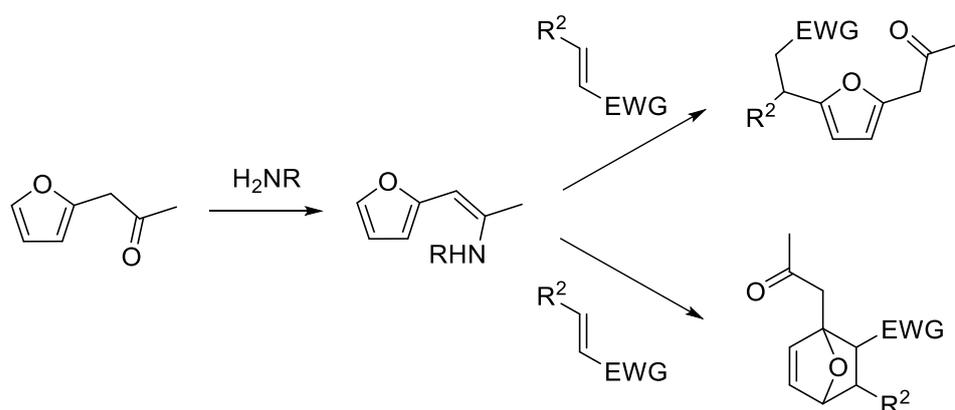
a)   General reaction scheme for amine-activated 2-furfuryl ketones
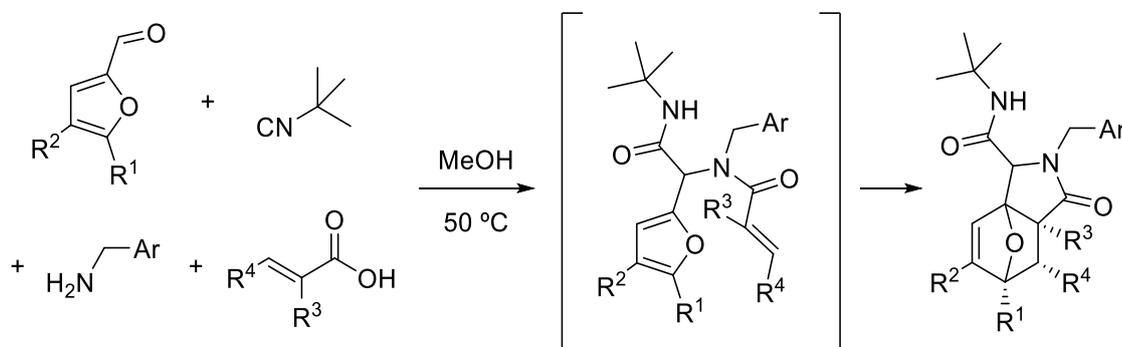
b)   Ugi/Diels-Alder reaction cascade of furfurals
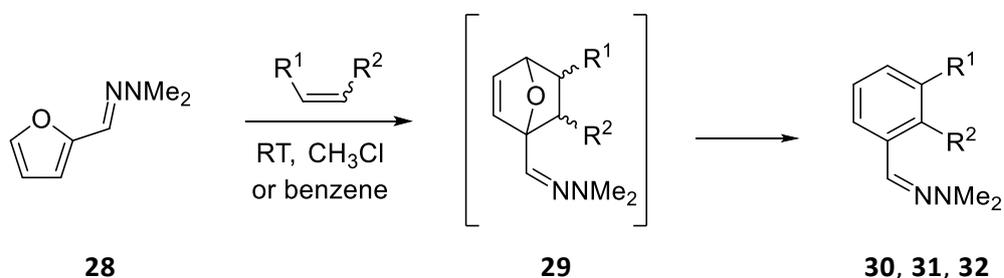
c)   Diels-Alder/aromatisation reaction cascade of furfural hydrazones

Scheme 14. Methods of activating furan rings towards Diels-Alder reaction

In 1984, Potts et al. found that furfural **17** can also be activated as a Diels-Alder diene by conversion of the aldehyde into a hydrazone group. Furfural dimethylhydrazone **28** was found to undergo Diels-Alder reactions with a selection of dienophiles, with the subsequent hydrazone-substituted 7-oxanorbornenes **29** undergoing immediate spontaneous aromatisation at room temperature to give substituted benzene rings **30**, **31** and **32** (Scheme 14c, Figure 6).[107]
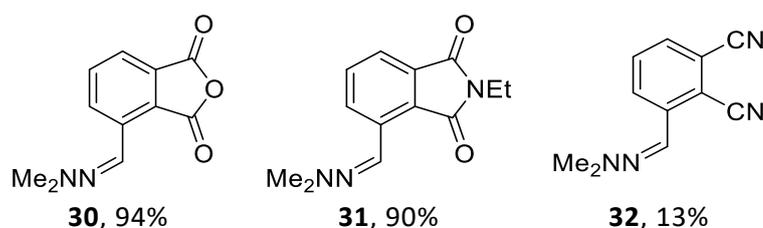
*Figure 6. Structures of **30**, **31** and **32**, products of Diels-Alder/aromatisation reactions by Potts et al.[107]*

This Diels-Alder/aromatisation approach was subsequently reported in a patent for the synthesis of **35**, a compound used in the treatment of cutaneous lupus (Scheme 15).[108]
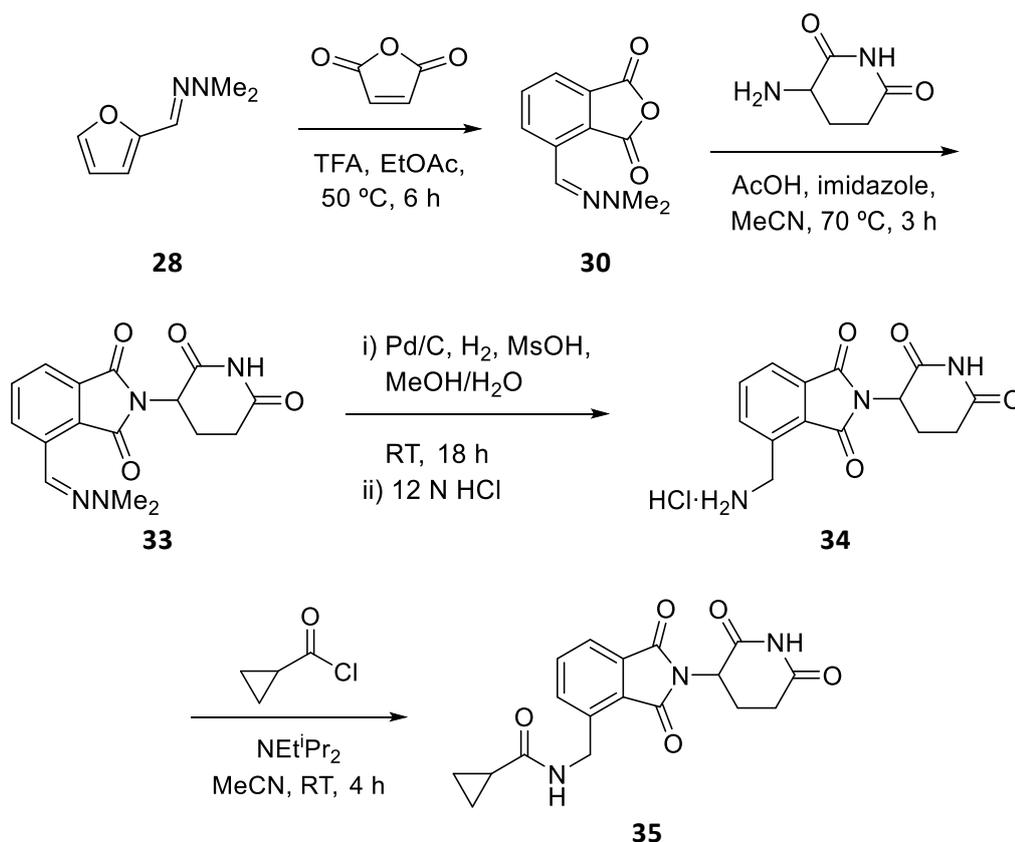
*Scheme 15. Synthesis of **35**, a treatment for cutaneous lupus*

Potts reported molecular orbital calculations to explain this reactivity, showing an increase in the HOMO energy level and C-5 HOMO substituent of **28** compared to both furan and 2-vinylfuran (Figure 7).[109]

28
α + 0.37β

2-vinyl furan
α + 0.44β
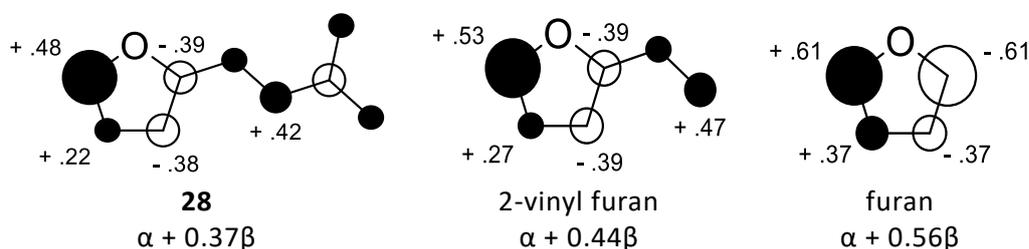
furan
α + 0.56β

*Figure 7. Coefficients of the HOMO of furfural dimethylhydrazone **28**, 2-vinylfuran, and furan. Redrawn from a diagram by Potts et al., 1988[109]*

This is consistent with π-donation of election density into the furan ring from the hydrazone substituent, which can also be illustrated by electron resonance structures (Scheme 16).
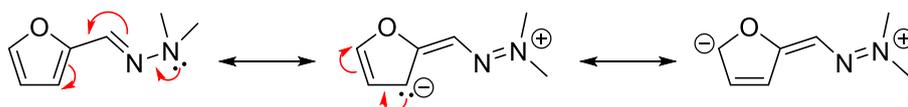
*Scheme 16. Representation of electronic resonance structures of **28***

This electron density donation effect explains the increased reactivity observed, as the furan ring will be activated towards a Diels-Alder reaction regardless of whether the mechanism is step-wise or concerted (Scheme 17).
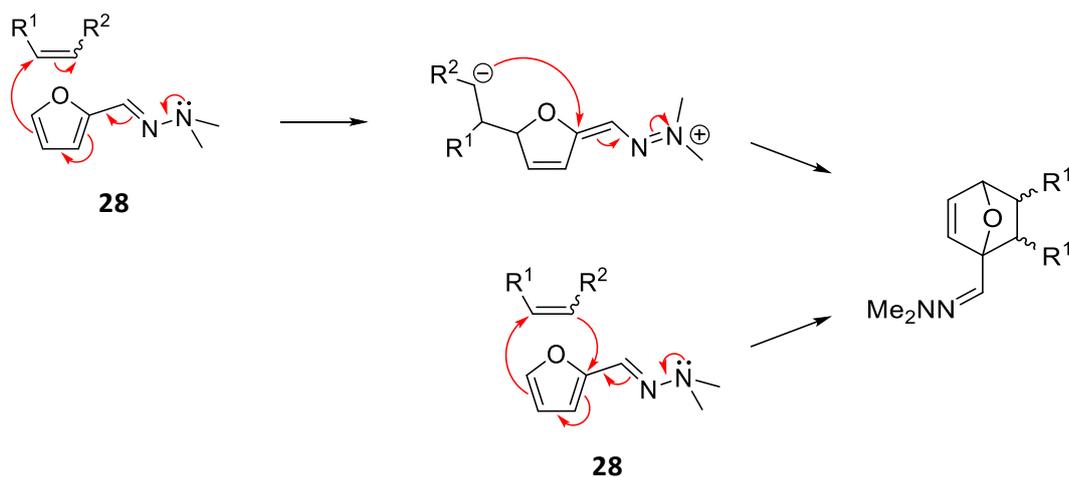
*Scheme 17. Mechanism for the activation of the furan ring of **28** towards Diels-Alder reaction via a step-wise and a concerted mechanism by the hydrazone group's electron donation*

The same electron donation effect then activates the resulting oxanorbornene towards aromatisation by dehydration (Scheme 18).
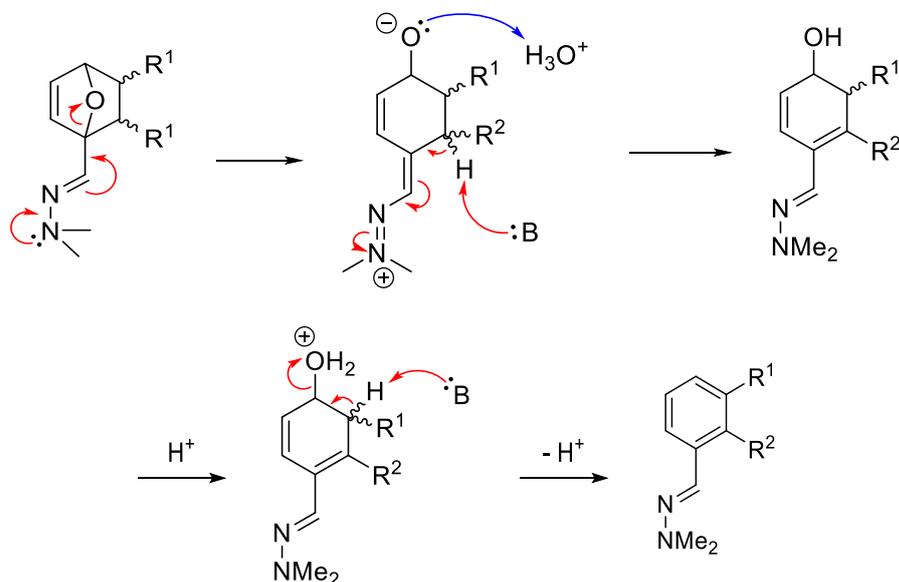
Scheme 18. Mechanism for the activation of a hydrazone oxanorbornene intermediate towards aromatisation by dehydration

In 1999, Amarasekara *et al.* used furfural phenylhydrazone **36** in a series of Diels-Alder/aromatisation reactions and found that the mono-substituted hydrazone was able to act as a 1,3-dipole in [3+2] cycloadditions. Whether the [4+2] or [3+2] cycloaddition occurred – or both – depended on the dienophile/dipolarophile used (Scheme 19).[110]

36

37, R = Me, 25%
38, R = Et, 26%
39, R = Ph, 22%

40, R = Me, 21%
41, R = Et, 22%
42, R = Ph, 20%
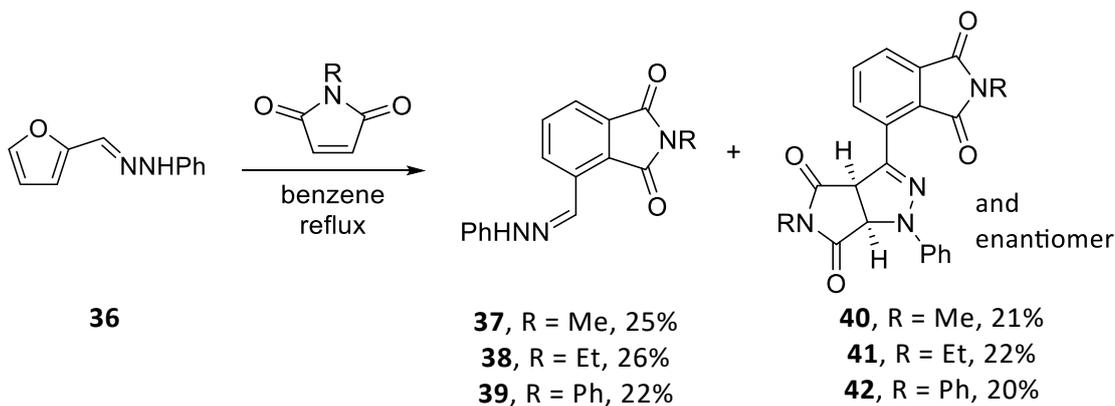
*a)   Cycloaddition with N-substituted maleimides to give a mixture of the DA/aromatisation product **9** and the 'double cycloaddition' product **10***
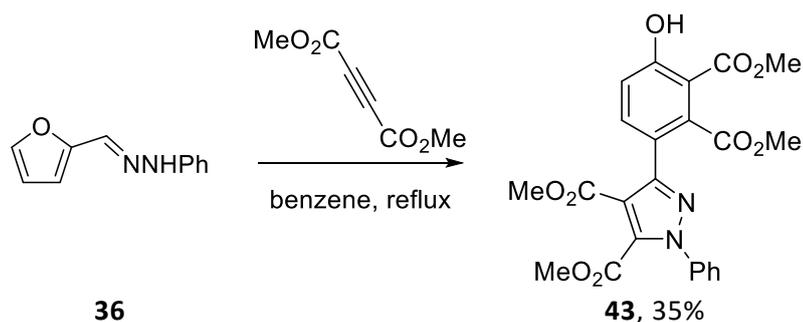
b)   Cycloaddition with dimethyl acetylenedicarboxylate to give the 'double cycloaddition' product 11

44, R = CO₂Me, 18%
45, R = CN, 16%
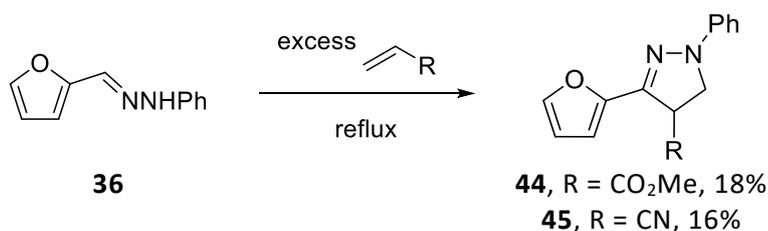
c)   Cycloaddition with acrylates to give the [3+2] cycloaddition product 12

Scheme 19. Cycloadditions of furfural phenylhydrazone with a) N-substituted maleimides, b) dimethyl acetylenedicarboxylate, and c) acrylates

Using two equivalents of an *N*-substituted maleimide and heating to reflux in dry benzene gave a mixture of the DA/aromatisation product, **37** - **39**, in 22-26% yield, and the double cycloaddition product **40** - **42** in 20-22% yield (Scheme 19, a). In contrast, using two equivalents of dimethyl acetylenedicarboxylate under the same conditions gave only the double cycloaddition product **43** in 35% yield (Scheme 19, b). Using acrylates in a large excess as the solvent and heating to reflux gave only the [3+2] hydrazone cycloaddition products **44** and **45** in 16-18% yield (Scheme 19, c).

The general hydrazone-promoted Diels-Alder/aromatisation cascade reaction sequence was selected for further study due to the availability of furfurals as products of biomass, and to interest in forming polysubstituted aromatic compounds from non-petrochemical sources.

During this project, related work was published on cycloadditions between furan derivatives and *N*-substituted maleimides 'on water', including furfural dimethylhydrazone **28**, which reacted with selected maleimides to give substituted phthalimides **31** and **47** in quantitative yields (Scheme 20). However, the main focus of this publication was the synthesis of 7-oxanorbornenes such as **46**.[111]
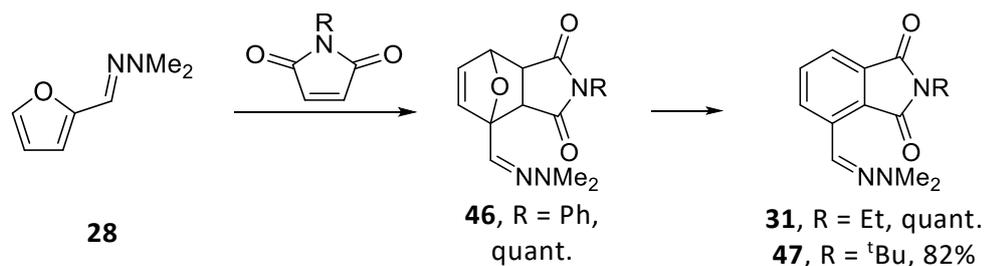
Scheme 20. 'On water' reaction between furfural dimethylhydrazone and N-substituted maleimides

2.1.4. Electrophilic Aromatic Substitution Reactions of Furans

Furan rings undergo electrophilic aromatic substitution reactions much more readily than benzene, due to the heteroatom donating electron density into the ring, and reactions of furan and its derivatives with a variety of electrophiles are well-established.[88] Furan readily undergoes lithiation at the C-2 position, increasing the nucleophilicity of the furan ring and allowing it to successfully react with most electrophiles.[88]

Potts *et al.* found that when trying to carry out Diels-Alder/aromatisation reactions between **28** and napthoquinone, the enone preferentially reacted as a Michael addition acceptor. Addition onto the furan ring at the C-5 position was observed, followed by immediate oxidation to give **48** (Scheme 21).[109]
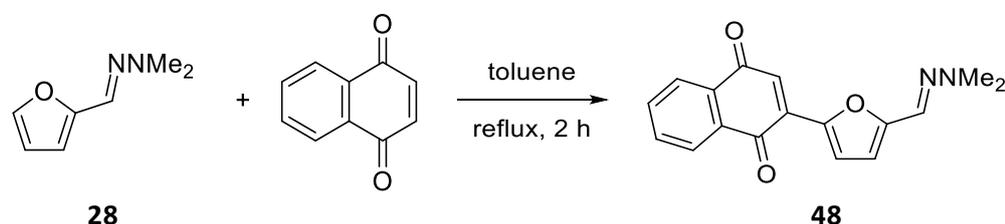
Scheme 21. Michael addition of napthoquinone to **28** to give **48**

The same result was observed with benzoquinone, quinoline-5,8-dione, isoquinoline-5,8-dione, and 5-acetoxy-1,4-naphthoquinone, to give the oxidised Michael adducts **49**, **50**, **51** and **52**, respectively (Figure 8).

50 (R = H, Y = CH, X = N)
51 (R = H, Y = N, X = CH)
52 (R = OAc, Y = X = CH)
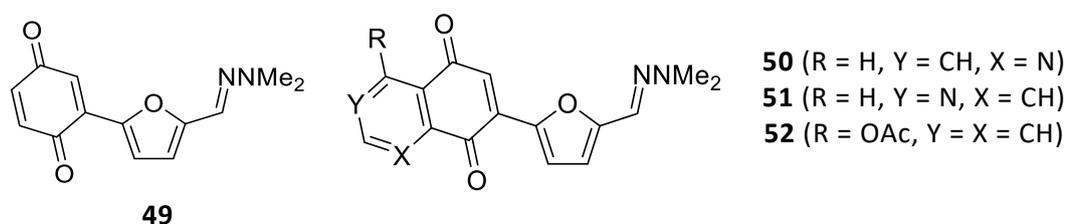
Figure 8. Structures of the Michael addition products of reacting furfural dimethylhydrazone with benzoquinone and napthoquinone analogues

These results are consistent with the increase in electron density at C-5, and show that the hydrazone group of **28** activates its furan ring as both a Diels-Alder diene and a nucleophile in electrophilic aromatic substitutions.

This dual reactivity allows the simultaneous study of potential dienophiles for Diels-Alder/aromatisation reactions, and potential Michael acceptors for electrophilic aromatic subtitution onto the furan ring. These two strategies could be combined to give further functionalised aromatic compounds (Scheme 22).
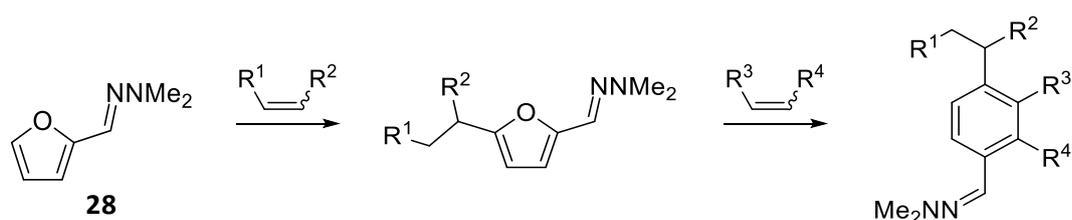
*Scheme 22. Furfural dimethylhydrazone **28** in a Michael addition reaction followed by a Diels-Alder/aromatisation reaction sequence, to give a highly funtionalised aromatic compound*

2.1.5. Chapter Aims

More sustainable, 'greener chemistry' methodology for the utilisation of this Diels-Alder/aromatisation sequence was sought, with an emphasis on the use of water as a solvent wherever possible. The scope of this methodology was investigated, with the aim of using green chemistry and bio-based feedstocks to synthesise as wide a range of polysubstituted benzene rings as possible by variation of the furfural species, the dienophile, and the hydrazine species. The use of Michael acceptors to produce novel functionalised furfural derivatives was also explored.

2.2. Results and Discussion

2.2.1. Initial Reactions Based on Literature

Furfural dimethylhydrazone **28** was initially synthesised from furfural **17** and *N,N*-dimethylhydrazine in ethanol, as according to the literature (Scheme 23).[112]
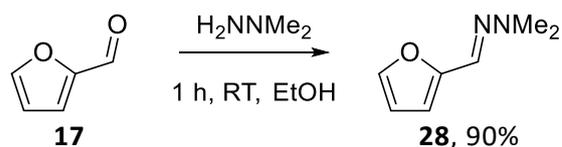
Scheme 23. Synthesis of furfural dimethylhydrazone 28 from furfural 17

A selection of key reactions reported by Potts *et al.* in 1984 were carried out and **28** was reacted with either maleic anhydride in chloroform, forming **30**, or *N*-ethylmaleimide in ethanol, forming **31** (Scheme 24).[107,109]
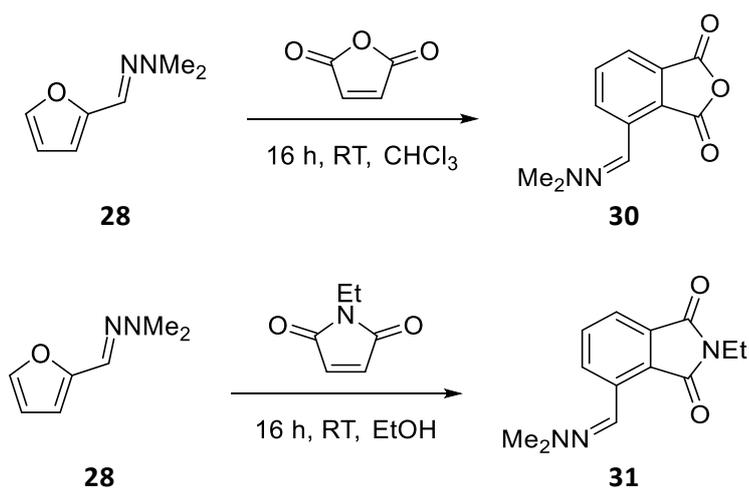
*Scheme 24. Diels-Alder/aromatisation reactions of furfural dimethylhydrazone **28** with maleic anhydride and N-ethylmaleimide to give **30** and **31***

Isolation of these products was by trituration, and the yields were comparable to those in the literature (85% of **30** c.f. 94%[109]; 82% of **31** c.f. 90%[109]).

2.2.2. Screening Conditions with Fumaronitrile

Reaction of **28** with fumaronitrile has previously been reported to produce **32** in 13% yield, using tin tetrachloride (SnCl$_4$) in benzene (Scheme 25).[109]

28   SnCl$_4$, benzene, 5 h   **32**, 13%
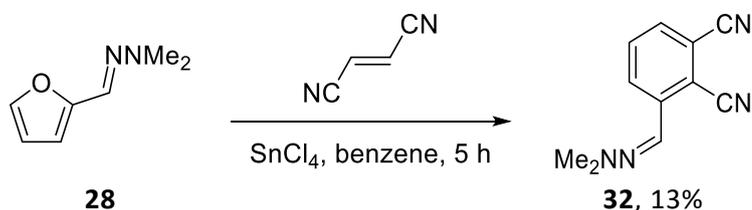
Scheme 25. Literature conditions for the Diels-Alder/aromatisation reaction of furfural dimethylhydrazone 28 to 32

This reaction was selected for optimisation due to its low yield and toxic solvent and catalyst. Reaction conditions with optimised yields and improved sustainability profiles were sought, with the aim of then applying these conditions to a range of other dienophiles. Solvents selected were toluene, due to its similarity to, and lower toxicity than, the previously used benzene; ethanol, due to its previous use with N-ethylmaleimide (Scheme 24), and water, for its desirable environmental profile and the precedence for acceleration of pericyclic reactions in aqueous media.

Three Lewis acid catalysts were used in screening: hafnium tetrachloride (HfCl$_4$), as a selective activator of furan species towards Diels-Alder reactions,[98] ytterbium triflate (Yb(OTf)$_3$), a water-tolerant Lewis acid previously used in Diels-Alder reactions;[113] and the previously used catalyst, SnCl$_4$, which was included for comparison. Reactions were performed for 24-72 hours at either RT or 50 °C (Scheme 26, Table 3).

28   **32**
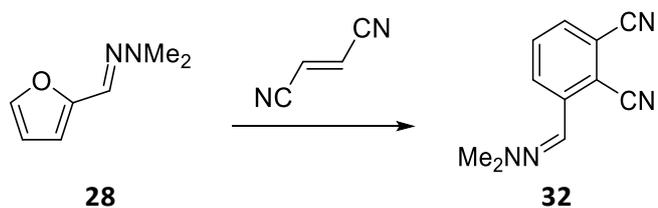
Scheme 26. Diels-Alder/aromatisation reaction of 28 with fumaronitrile to give 32 under various conditions

Entry	Solvent	Temp.	Catalyst	Time /H	Yield
1	dry benzene	RT	SnCl$_4$	5	13% (lit.)[109]
2	toluene	RT	SnCl$_4$	24	7%
3	toluene	RT	HfCl$_4$	24	13%
4	toluene	RT	Yb(OTf)$_3$	24	5%
5	ethanol	RT	SnCl$_4$	72	no conversion
6	ethanol	RT	HfCl$_4$	72	no conversion
7	ethanol	RT	Yb(OTf)$_3$	72	no conversion
8	water	RT	Yb(OTf)$_3$	48	44%
9	water	50 °C	Yb(OTf)$_3$	48	trace
10	water	RT	/	40	39%*
11	water†	RT	/	40	27%*
12	water	50 °C	/	48	68%
13	toluene:water, 9:1	50 °C	/	48	no conversion
14	CPME:water, 9:1	50 °C	/	48	no conversion

*Conversion yield only, by quantitative NMR. 1,3,4-methoxybenzene used as an internal standard, confirmed as an accurate approximation of conversion yield by subsequent product isolation in three instances. †For entry 11, water was adjusted to pH 5 – other aqueous reactions used Millipore water at pH 6.

*Table 3. Results of the Diels-Alder/aromatisation reaction of **28** with fumaronitrile to give **32** under various conditions*

Using Lewis acid catalysts in organic solvents gave similar or lower yields than the literature reaction (Table 3, entries 2-7 c.f. entry 1), while the use of Yb(OTf)$_3$ in water gave a significant increase in yield, to 44% (Table 3, entry 8). Increasing the temperature from ambient to 50 °C, however, gave minimal conversion and instead the formation of a black, insoluble material was observed (Table 3, entry 8), suggesting that the furan species **28** had polymerised.

The reaction in water without a Lewis acid gave a 39% yield; almost as high as with the Yb(OTf)$_3$ catalyst present (Table 3, entry 10). To investigate potential Brønsted acid catalysis, the pH was lowered from 6 to 5, but this gave a decreased yield of 27% (Table 3, entry 11). Heating the reaction to 50 °C in water without catalyst, however, gave a considerably improved yield of 68% (Table 3, entry 12).[114]

As the starting material **28** is an oil with partial miscibility in water, these reactions could occur 'on water' or 'in water' (Section 1.3.). To investigate these potential effects, 2-phase reactions in a 9:1 ratio of organic solvent (toluene or CPME) to water, with rapid stirring, were carried out (Table 3, entries 13 & 14). In both cases, no product was formed, presumably due to **28** being sequestered into the organic phase with the water playing no role.

The polymerisation of **28** in water with Yb(OTf)$_3$ highlights a potential problem with using lewis acid catalysts in these Diels-Alder/aromatisation reactions. A similar compound, furfural hydrazone **54** (Figure 9), is reported to chelate metal ions by electron lone pair donation from its furan oxygen heteroatom and hydrazone nitrogen groups.[115] Compound **28** could form similar complexes with lewis acids and thus lose electron density from the furan ring, deactivating it as a Diels-Alder diene. This could also result in side-reactions such as the polymerisation observed with Yb(OTf)$_3$. To investigate this effect, **28** was mixed with each of the screened lewis acids in ethanol and in toluene, and in each case a significant colour change was observed, suggesting the formation of a complex.

54   **28**•Yb(OTf)$_3$
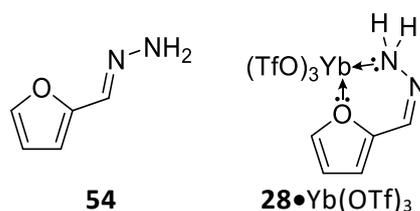
*Figure 9. Structure of furfural hydrazone **54** and of a proposed complex between furfural dimethylhydrazone **28** and ytterbium triflate Yb(OTf)$_3$*

In the reactions with Yb(OTf)$_3$, temperature was a key factor in determining whether the desired Diels-Alder/aromatisation reaction occurred, or side reactions and polymerisation. However, as the highest yield was attained without a catalyst, the issue was avoided in this instance.

The reaction in water at 50 °C was later scaled up twenty-fold and isolation carried out by filtration of the precipitate, giving **32** in an improved yield of 77% on a 10 gram scale.

2.2.3. A Three-step, One-pot Cascade Route to Phthalimides

The optimised reaction conditions in water were applied to the previously carried out literature reactions (Section 2.2.1.). While maleic anhydride did not react with furfural dimethylhydrazone **28** under these conditions, both the synthesis of **28** from furfural **17** and the Diels-Alder/aromatisation reaction of **28** with *N*-ethylmaleimide took place in water at 50 °C (Scheme 27). Unless otherwise stated, all aqueous reactions were carried out in millipore water, at approximately pH 6.
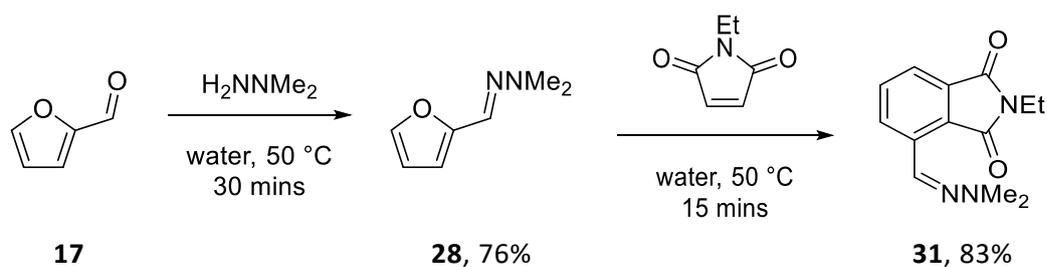
Scheme 27. Synthesis of **28** in water and subsequent Diels-Alder/aromatisation to give **31**

The products **28** and **31** were isolated by organic extraction in yields of 76% and 83% respectively, giving a two-step yield of 63%. Next, these two reactions were combined into a one-pot reaction (Scheme 28).
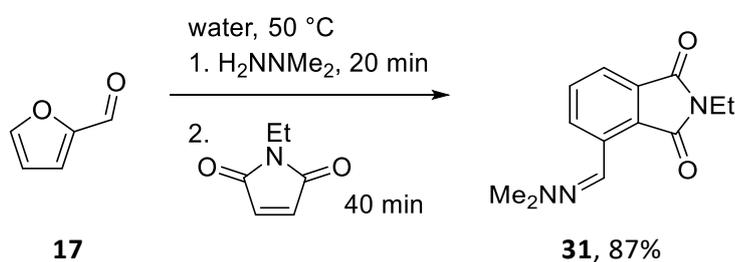
Scheme 28. One-pot, three-step synthesis of **31** from furfural

Carrying out the steps sequentially in one pot enhanced the two-step yield from 63% to 87%, and the *in situ* depletion of the intermediate **28** seemed to improve the conversion of furfural **17** into **28**. The product **31** precipitated out of the reaction solvent and was isolated by filtration in high purity. No organic solvents were therefore needed in either the reaction or the work-up.

To optimise the reaction conditions further, the number of equivalents of *N,N*-dimethylhydrazine and *N*-ethylmaleimide were varied, and the reaction carried out with either the simultaneous addition of reagents (one-step) or sequential addition (two-step) as previously described (Scheme 28). The possibility of using catalytic quantities (0.1 equivalents) of *N,N*-dimethylhydrazine and obtaining the product as aldehyde **55** (Figure 10) rather than a hydrazone was also investigated.
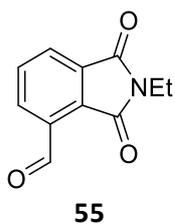
Figure 10. The potential aldehyde product **55**

H$_2$NNMe$_2$ Equiv.	Reagent Addition	N-ETHYLMALEIMIDE EQUIVALENTS				
		1	1.25	1.5	1.75	2
1	One step	80%	/	/	/	/
	Two steps	87%	85%	87%	84%	89%
1.2	One step	85%	/	/	/	/
	Two steps	95%	/	96%	/	96%
0.1	One step	9% **31** 0% **55**	/	/	/	/

Table 4. Varying the ratio of reactants and one-/two-step reagent addition in the one-pot synthesis of **31**

Increasing the number of equivalents of N-ethylmaleimide did not increase the yield beyond normal variation, while increasing the equivalents of N,N-dimethylhydrazine from 1 to 1.2 gave a noticeably increased yield of 95% (Table 4). Attempting to use catalytic quantities of N,N-dimethylhydrazine gave only a 9% yield of **31** and no formation of **55**, suggesting that the hydrazone group of **31** is not labile under these conditions. Carrying out the reaction with the simultaneous addition of reagents reduced the yield by 7-10%. The optimum conditions gave a final isolated yield of 95% and, when the reaction was repeated on a 20 g scale to investigate scalability, a near-quantitative yield of 97% was obtained.[114]

This reaction optimisation process was repeated with maleimide (Scheme 29) and the equivalents of reactants were varied similarly (Table 5).
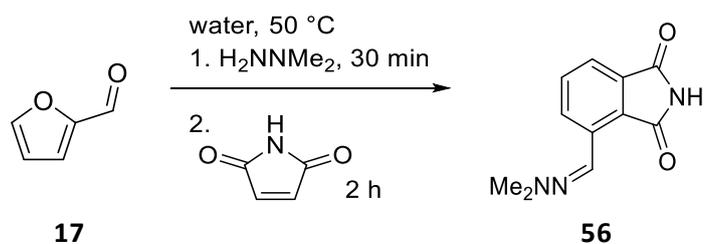
Scheme 29. One-pot synthesis of **56** from furfural

H$_2$NNMe$_2$ Equiv.	MALEIMIDE EQUIVALENTS		
	1	1.2	2
1	66%	72%	76%
1.2	60%	70%	86%
1.6	52%	65%	85%

Table 5. Varying the ratio of reactants in a one-pot synthesis of **56**

The highest yield was 86% and obtained by using 1.2 equivalents of N,N-dimethylhydrazine and 2 equivalents of maleimide. Interestingly, when using 1 - 1.2 equivalents of

maleimide, increasing the equivalents of *N,N*-dimethylhydrazine reduced the yield. This suggests that excess *N,N*-dimethylhydrazine can undergo undesirable side-reactions with the maleimide; the nucleophilic attack of hydrazines with both imides and enones is well documented.[116]   In future reactions, 1.2 equivalents of *N,N*-dimethylhydrazine and 1 equivalent of dienophile were initially used, and excess dienophile added where low yields were observed.

2.2.4. The Scope of Furfural Analogues and Derivatives

To access a wider variety of substituted benzene ring products via this Diels-Alder/aromatisation reaction sequence, the furfural species was varied. As potential by-products of the sugar industry, 5-methylfurfural **18** and 5-hydroxymethylfurfural (HMF) **19** are of particular interest.   To explore the scope of the reaction, several other commercially available furfurals were also screened and some additional furfural derivatives were synthesised by Dr. Fabiana Subrizi.

Initially, the ability to form a hydrazone in water was tested with **18**, **19**, and 4,5-dimethylfurfural **57** and in each case the expected product was formed in similar yields to furfural dimethylhydrazone **28** (Scheme 30, Table 6).
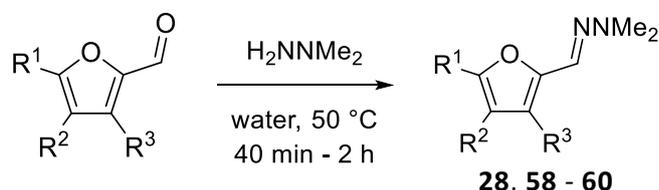
Scheme 30. Varying the furfural species for hydrazone formation in water

FURFURAL SPECIES	R^1	R^2	R^3	PRODUCT	YIELD
Furfural **17**	H	H	H	**28**	76%
18	Me	H	H	**58**	56%
HMF **19**	CH$_2$OH	H	H	**59**	77%
57	Me	Me	H	**60**	62%

Table 6. Results of varying the furfural species for hydrazone formation in water

Next, a wider range of furfurals was investigated for their ability to participate in the three-step, one-pot hydrazone formation and Diels-Alder/aromatisation reaction sequence (Scheme 31, Table 7, Figure 11). Some of these reactions, as indicated, were carried out by Dr. Fabiana Subrizi.
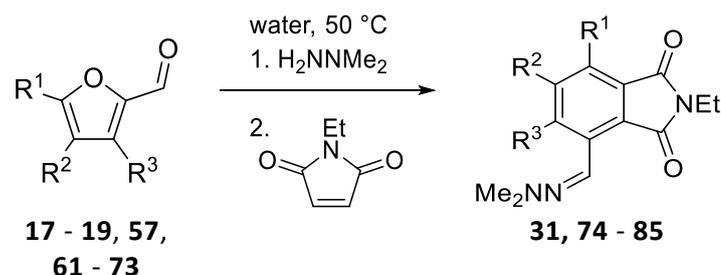
Scheme 31. Varying the furfural species in Diels-Alder/aromatisation phthalimide synthesis

FURFURAL SPECIES	R^1	R^2	R^3	PRODUCT	YIELD
Furfural **17**	H	H	H	**31**	97%
18	Me	H	H	**74**	93%
HMF **19**	CH$_2$OH	H	H	**75**	95%
57	Me	Me	H	**76**	69%
61	Et	H	H	**77**	75%
62	CH$_2$OEt	H	H	**78**	70%
63	Ph	H	H	NR	N/A
64	—◯—Br	H	H	NR	N/A
65	—◯—OEt	H	H	NR	N/A
66	—◯—CF$_3$	H	H	NR	N/A
67	H	Ph	H	**79**	81%[†]
68	—◯O (tetrahydropyran)	H	H	**80**	72%[†]
69	—N◯O (morpholine)	H	H	**81**	41%[†]
70	CF$_3$	H	H	**82**	72%[†*]
71	H	H	Br	**83**	79%[†]
72	H	Br	H	**84**	84%[†]
73	Br	H	H	**85****	54%[†]

[†]This reaction carried out by Dr. Fabiana Subrizi and included here with her permission. [*]This reaction carried out as a two-pot reaction with the Diels-Alder/aromatisation step being conducted in CPME. [**]This reaction gave an alternative product to the expected bromo-substituted benzene ring, with a hydroxyl group instead of a bromine centre.

Table 7. Results of varying the furfural species in Diels-Alder/aromatisation phthalimide synthesis[114]

Reaction yields were generally high for furfural species without aromatic substituents. In the case of 5-trifluoromethylfurfural **70**, the hydrazone intermediate successfully formed in water but did not proceed to react with the *N*-ethylmaleimide. The intermediate was

isolated and reacted further in CMPE in a two-pot reaction, giving the expected aromatic product **82**.
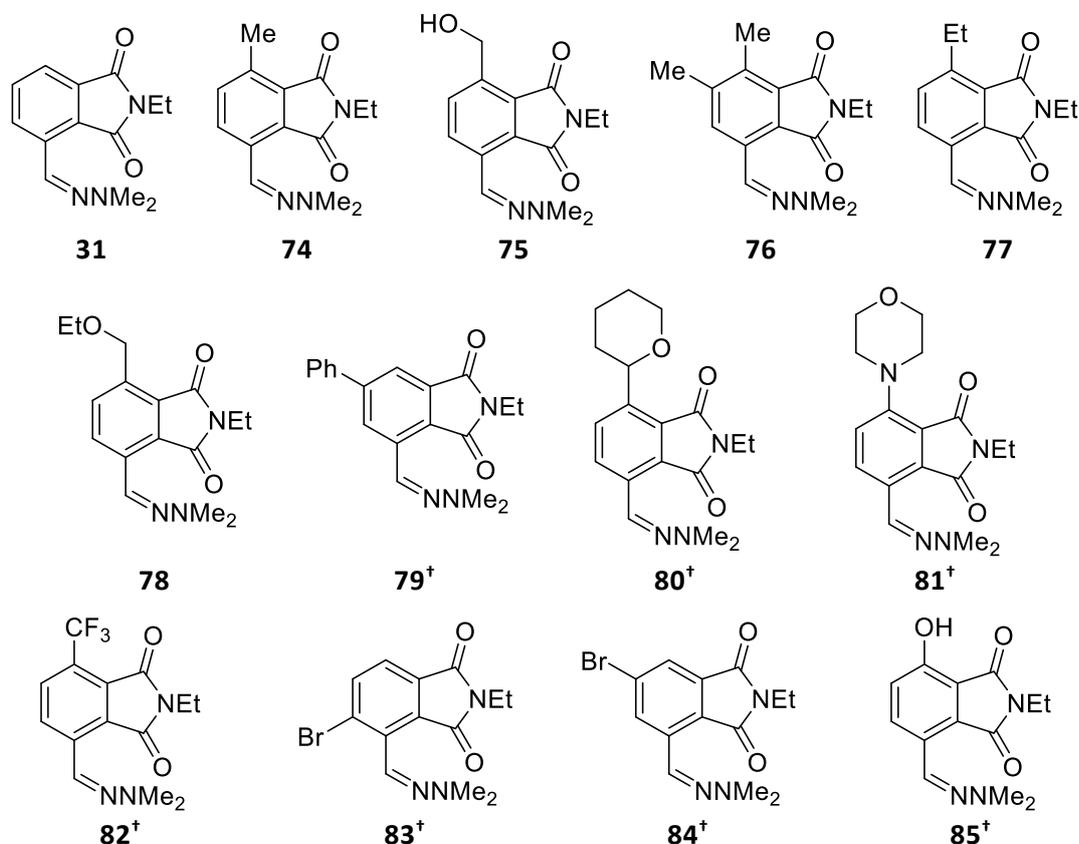
Figure 11. Structures of the products of varying the furfural species in Diels-Alder/aromatisation phthalimide synthesis[114]

5-Phenylfurfural **63** had very limited solubility in water and no depletion of the starting material was observed. Furfural derivatives **64 - 66** have substituted aromatic groups at the same position, with electron-donating or withdrawing groups, but these also showed no reaction. However, **68** and **69** have similarly sized substituents at the C-5 position and reacted to give the expected products **80** and **81**, and 4-Phenylfurfural **67** has similar solubility in water to 5-phenylfurfural but successfully reacted to give **79**. It is therefore unclear if the lack of reactivity of **63 - 66** is due to electronic, steric, or solubility factors, or a combination of all three.

3- and 4-bromofurfural, **71** and **72**, reacted to give the expected bromo-substituted phthalimides **83** and **84**, but 5-bromofurfural **73** was converted into the hydroxyl substituted aromatic product **85**. This can be explained by the usual mechanism of aromatisation by dehydration, wherein the bridging oxygen of the oxanorbornene generates a hydroxyl group that is then eliminated as water. When a bromo group is present at the bridging site, an unstable intermediate is formed in which a bromide and

hydroxide group are bonded to the same carbon (Scheme 32). While multiple mechanisms for the elimination of the bromide group are feasible, it seems likely that the bromide was spontaneously eliminated intramolecularly in the formation of a ketone. This would then tautomerise to the observed aromatic product.

Scheme 32. Mechanism for producing the hydroxyl-substituted product from 5-bromofurfural

3-(2-Furyl)acrolein **86** was successfully reacted both with *N,N*-dimethylhydrazine, giving hydrazone **87** in 67% yield, and in the two-step, one-pot Diels-Alder/aromatisation reaction, giving **88** in 64% yield (Scheme 33).[114] The geometry of the alkene bond of **88** was not clear from spectroscopic data of the compound. However, downstream products were found to be (*E*)-alkenes (Section 3.2.1.), with no reason to suspect a change in geometry during reaction. It is therefore likely that **88** also exists as the (*E*) isomer.

Scheme 33. Reacting 3-(2-furyl)acrolein with N,N-dimethylhydrazine to give **87**; and in Diels-Alder/aromatisation phthalimide synthesis to give **88**

Reacting each of acetylfuran **89**, 3-furaldehyde **90**, and 2,5-furandicarboxaldehyde **91** with *N,N*-dimethylhydrazine in either water or ethanol led to formation of the hydrazones **92** - **95** in yields of 41 - 88 %, (Scheme 34), which are comparable to those achieved with 5-substituted furfurals (Table 6). In the case of 2,5-furandicarboxaldehyde, it is possible to form either the single hydrazone product **94** or the double hydrazone product **95**, and both were isolated by using different quantities of *N,N*-dimethylhydrazine.
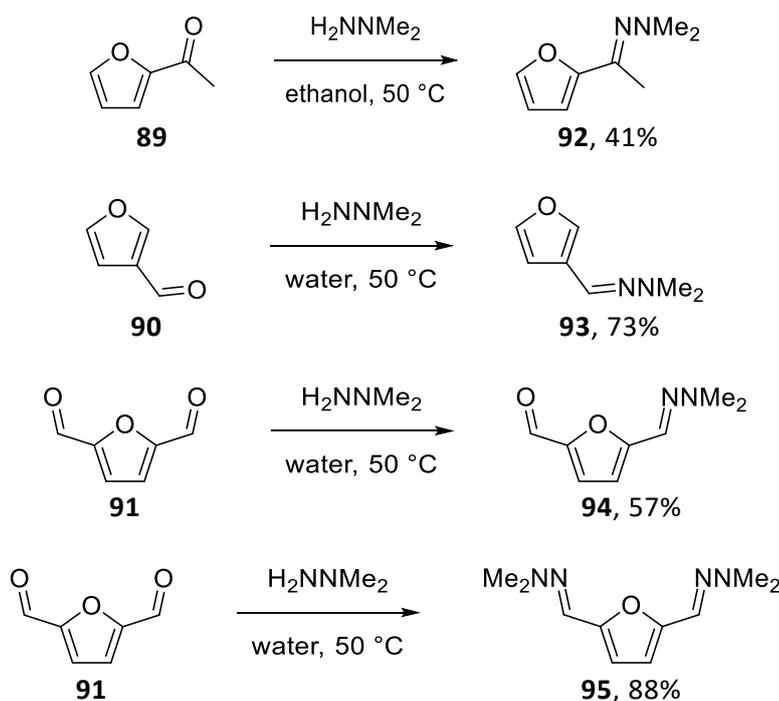
*Scheme 34. The reactions of furfural derivatives **62** - **65** with* N,N-*dimethylhydrazine in water to give the hydrazones **62** - **65***

However, hydrazones **92** - **95** were not observed to undergo a Diels-Alder reaction when heated with *N*-ethylmaleimide in water at various temperatures, or when heated to reflux in CPME, dichloromethane, or ethanol. Steric effects or reduced electron donation could deactivate **92** to Diels-Alder reaction compared to the aldehyde-based hydrazone, while the lack of reactivity of **93** and **95** suggests that the position of the electron density on the furan ring plays an important role. Previous calculations show a particular increase at the C-5 position of furfural dimethylhydrazone **28** (Figure 7), which would be diminished in **93** and **95**. Compound **94** is likely deactivated due to the electron-withdrawing aldehyde group.

2.2.5. Alternatives to *N,N*-Dimethylhydrazine

Alternatives to *N,N*-dimethylhydrazine were explored, and hydrazine **96**, hydroxylamine **97**, and methoxylamine **98** were selected based on their price, availability, and similar reactivities. Phenylhydrazine **99** was used as it has previously been used in similar literature reactions and mono-substituted hydrazones are of interest due to their to previously reported reactivity as 1,3-dipoles in [2+3] cycloadditions (Section 3.1.1.). For further diversity, methylhydrazine **100**, acetic hydrazide **101** and diphenylhydrazine **102** were also screened (Scheme 35, Table 8).
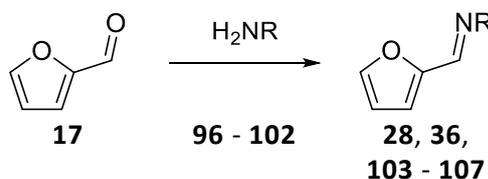
Scheme 35. Reaction of furfural with hydrazine alternatives

NITROGEN SPECIES	R	PRODUCT	CONDITIONS	YIELD
H$_2$NNMe$_2$		**28**	ethanol, RT, 1 h	90%
H$_2$NNMe$_2$		**28**	water, 50 °C, 40 min	76%
96	-NH$_2$	**103**	water, 50 °C, 30 min	72%
97	-OMe	/	water, 50 °C, 30 min	Not isolated
98	-OH	**104**	water, 50 °C, 30 min	81%
99	-NHPh	**36**	water, 50 °C, 1 h	93%
100	-NPh$_2$	**105**	water, 50 °C, 1 h	88%
101	-NHMe	**106**	water, 50 °C, 2 h	44%
102	-NHAc	**107**	water, 50 °C, 2 h	67%

Table 8. Results of the reaction of furfural with hydrazine alternatives

Formation of hydrazone **103** and oxime **104** was achieved in yields of 76% and 72% from hydrazine **96** and hydroxylamine **98**, respectively. Methoxylamine **97** appeared to react with furfural **17** but the product could not be isolated, possibly due to volatility. Phenylhydrazine **99** and diphenylhydrazine **100** both reacted to form hydrazones **36** and **105** in yields of 93% and 88% despite the limited solubility of the solid hydrazines in water, and methylhydrazine **101** and acetic hydrazide **102** also gave the expected products **106** and **107** in yields of 44% and 67%. Next, these furfural hydrazone/oxime species were screened in cycloadditions with *N*-ethylmaleimide (Scheme 36, Table 9).

36, 28,
103 - 107

38, 31, 108
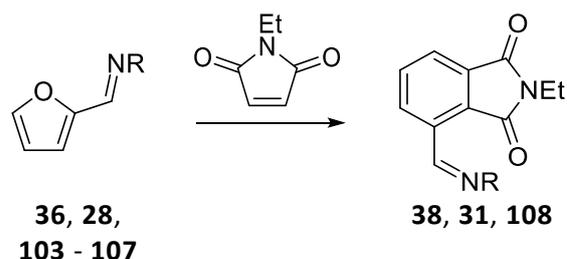
Scheme 36. Reaction of furfural hydrazones/oxime with N-*ethylmaleimide*

STARTING MATERIAL	R	PRODUCT	YIELD*
28	-NMe$_2$	31	97%
103	-NH$_2$	NR	/
104	-OH	NR	/
36	-NHPh	38	16%
		108	58%
105	-NPh$_2$	NR	
106	-NHMe	Unresolved mixture	/
107	-NHAc	Unresolved mixture	/

Calculated yields determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

Table 9. Results of reacting furfural hydrazones/oxime with N-*ethylmaleimide*

For **103** and **104**, no reaction was observed over 72 hours, suggesting that the alkyl groups on furfural dimethylhydrazone **28** are important to the electron donating effect that activates the furan ring. The reaction with **36** resulted in the formation of the expected aromatic product **38** in 16% calculated yield and also the oxanorbornene **108** in 58% calculated yield, along with 22% of the initial starting material **36** (Scheme 37).
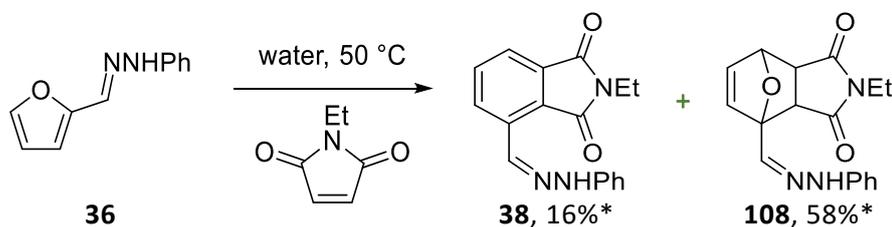
36

38, 16%*   **108**, 58%*

Yields determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

*Scheme 37. Reaction of furfural phenylhydrazone **36** with* N-*ethylmaleimide to give **38** and **108***

Furfural diphenylhydrazone **105** did not react under these conditions, probably due to its lack of solubility in water. Finally, **106** and **107** reacted to give complex mixtures of products which were not isolated, quantified, or characterised, though a combination of positive electrospray LC-MS analysis and ^{1}H NMR spectroscopic analysis of the mixtures

was used to propose probable identities of the major components. For methylhydrazone **106** these were, in order of abundance, the double cycloaddition product **109** (m/z = 355.26), the unaromatised Diels-Alder product **110** (m/z = 248.17), and the double cycloaddition product without aromatisation, **111** (m/z = 373.28) (Scheme 38).

*Scheme 38. Reaction of **106** with N-ethylmaleimide to give **109**, **110** and **111***

In the case of acetylhydrazone **107** the proposed probable products were, in order of abundance, the expected Diels-Alder/aromatisation product **112** (m/z = 260.20) and the unaromatised Diels-Alder product **113** (m/z = 278.22) (Scheme 39).

*Scheme 39. Reaction of **107** with N-ethylmaleimide to give **112** and **113***

Compared with the phenylhydrazone **36**, the methylhydrazone group of **106** seemed to have a higher propensity to undergo [3+2] cycloaddition, possibly due to a lack of steric bulk. This converts the hydrazone into a dihydropyrazole ring, reducing its electron donating effect and disrupting the Diels-Alder reaction. The acetylhydrazone **107** was less reactive to cycloaddition at both the furfural and hydrazone, and heating at reflux for 5 h did not increase conversion to the aromatic product **113**. Only phenylhydrazine **99** was studied further as an alternative to *N,N*-dimethylhydrazine.

2.2.6. Optimisation of the Use of Phenylhydrazine

To optimise the synthesis of **38**, a series of one-pot, two-step Diels-Alder/aromatisation reactions between furfural **17** and phenylhydrazine **99** were carried out at different temperatures, with one or two equivalents of *N*-ethylmaleimide (Scheme 40). The resulting reaction mixtures were analysed by ¹H NMR spectroscopic analysis to determine

the ratios of products (Table 10). In two cases, the aromatic product **38** was then isolated by column chromatography.
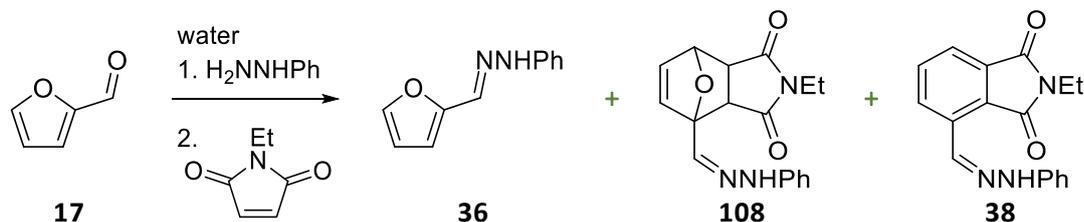
Scheme 40. One-pot reaction of furfural with phenylhydrazine and N-ethylmaleimide to give **36**, **108** and **38**

Temp. /°C	Time /h	Maleimide equiv.	Additive	Ratio of Products			Isolated yield of 38
				36	108	38	
50	24	1		1	3	0.3	
50	48	1		0.2	1	0.4	30%
50	24	2		0	1	0.6	35%
80	24	2		0	1	1	
80	24	2	0.1 M HCl	0	1	1	
80	24	2	1 M HCl	0	1	1	
Reflux	24	2		Polymerisation occured			

Table 10. Results of the one-pot reaction of furfural with phenylhydrazine and N-ethylmaleimide

Allowing the reaction to proceed for 48 h rather than 24 h led to an improved conversion to the second intermediate **108** and the desired product **38**, for which an isolated yield of 30% was obtained. This was slightly more than the calculated yield from analysis of the crude reaction mixture, due to aromatisation of **108** into **38** during flash column chromatography.

However, intermediate **36** was still not fully consumed and so the number of equivalents of N-ethylmaleimide were increased, to enhance the Diels-Alder step of the reaction. This led to the full conversion of **36** to **108** and an improved conversion of **108** to **38**, with a slightly improved isolated yield of 35%.

Performing the reaction at 80 °C gave a 1:1 mixture of **108** and **38**. In the hopes of acid-catalysing the oxobridge opening, reactions were carried out at 80 °C with 0.1 M and 1 M hydrochloric acid, but a 1:1 mixture was still obtained. Heating the reaction at reflux resulted in polymerisation of the materials and little recoverable product.

As the reaction was not successfully driven to completion *in situ*, efforts were made to isolate a mixture of **108** and **38** and then achieve the aromatisation under alternative conditions.

Using reaction conditions that led to the full conversion of intermediate **36**, furfural was successfully converted to a mixture of **108** and **38** which were then separated by column chromatography to give yields of 47% and 39%, respectively (Scheme 41).
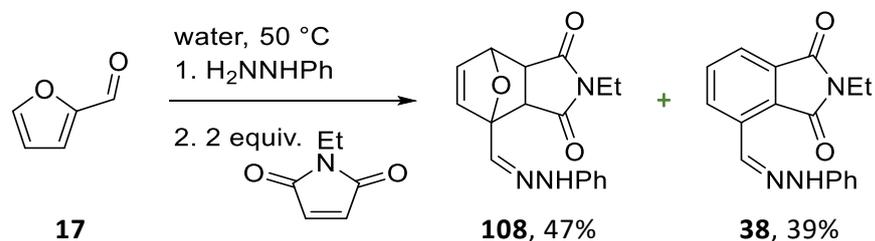
Scheme 41. Synthesis of a mixture of 108 and 38 from a one-pot reaction of furfural with phenylhydrazine and N-ethylmaleimide

Isolated **108** was then used to screen for reaction conditions that promoted aromatisation of the oxanorbornene motif (Scheme 42). To begin with, water was used as the solvent and the oxanorbornene heated to different temperatures with or without 0.1 M hydrochloric acid (Table 15). To determine if aromatisation had taken place, samples were assessed by quantitative ^{1}H NMR spectroscopic analysis using an internal standard.
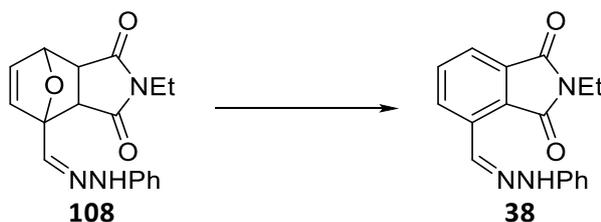
Scheme 42. Aromatisation by dehydration of 108 to give 38

Time /h	Temp. /°C	0.1 M HCl	Remaining 108*	Yield of 38*	Mass Conversion
18 h	100	N	N/A – insoluble polymerisation		
18 h	60	N	47%	50%	97%
18 h	60	Y	41%	44%	85%
18 h	80	N	44%	48%	92%
18 h	80	Y	42%	42%	84%
2 h	100	N	N/A – insoluble polymerisation		
2 h	100	N	N/A – insoluble polymerisation†		

Calculated yield, determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard. †This reaction carried out solvent-free.

Table 11. Results of the aromatisation by dehydration of 108 to give 38 in water at varying temperatures and with or without acid

The starting material was partially soluble in the water and the undissolved precipitate appeared to melt at around 100 °C, leading to an immiscible liquid organic layer above the

water. Rapid stirring created droplets and it was hoped that this might facilitate 'on water' interactions, but the organic material was observed to quickly polymerise. Carrying out the reaction solvent-free did not mitigate this.

Varying the temperature and using an acid catalyst did not significantly affect the outcome of the reaction, and the use of acid led to a slight decrease in mass conversion. In each case, approximately half of the oxanorbornene **108** was successfully aromatised. In the hopes that improved solubility would enable the reaction, a series of water-miscible organic cosolvents were investigated. The reactions were carried out with 10% v/v cosolvent in water at 50 °C for 24 hours (Table 12).

Cosolvent	Remaining 108*	Yield of 38*	Mass Conversion
1,4-dioxane	40%	55%	95%
THF	5%	87%	92%
acetonitrile	21%	74%	95%
ethanol	39%	54%	93%
DMSO	29%	65%	94%
none	49%	47%	96%
solvent-free	N/A – insoluble polymerisation		

Calculated yield, determined by quantitative ^1H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard

*Table 12. Results of the aromatisation of **108** to give **38** using aqueous cosolvent systems or a solvent-free system*

Each organic cosolvent system improved the conversion of **108** into **38**, though THF gave the most significant improvement and was selected for further optimisation. Different ratio solvent mixtures of THF and water were screened, and reactions in organic solvents THF, ethanol and methanol were also carried out, each at 50 °C for 24 hours (Table 13).

Organic Solvent %	Organic Solvent	Remaining 108*	Yield of 38*	Mass Conversion
5%	THF	5%	92%	97%
10%	THF	5%	94%	99%
50%	THF	8%	90%	98%
100%	THF	12%	83%	95%
100%	ethanol	11%	85%	96%
100%	methanol	8%	91%	99%

Calculated yield, determined by quantitative ^1H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard

*Table 13. Results of the aromatisation of **108** to give **38** using a series of aqueous cosolvent or organic solvent systems*

Each reaction gave significant conversion of **108** into **38**, though 10% THF in water gave the highest yield. In the hope that the reaction could be carried out in one pot without changing solvent, the DA/aromatisation of furfural with phenylhydrazone and *N*-ethylmaleimide was attempted in 10% THF in water, but only furfural phenylhydrazone **36** was isolated; the Diels-Alder reaction did not proceed in the presence of the cosolvent.

The reaction was then carried out in water with the addition of 10% THF after the depletion of furfural and furfural phenylhydrazone was observed by TLC analysis. However, product **38** was isolated in only 64% yield, along with a 33% yield of unaromatised **108**, potentially due to excess hydrazine and maleimide.

Finally, the synthesis of a mixture of **108** and **38** was carried out as previously described (Scheme 41), and the precipitated product mixture was collected by filtration, washed with water, and then resuspended in 10% THF for further reaction with no intermediate drying step. This gave **38** in 83% total yield from furfural, with minimal workup and predominantly using water as the solvent (Scheme 44).
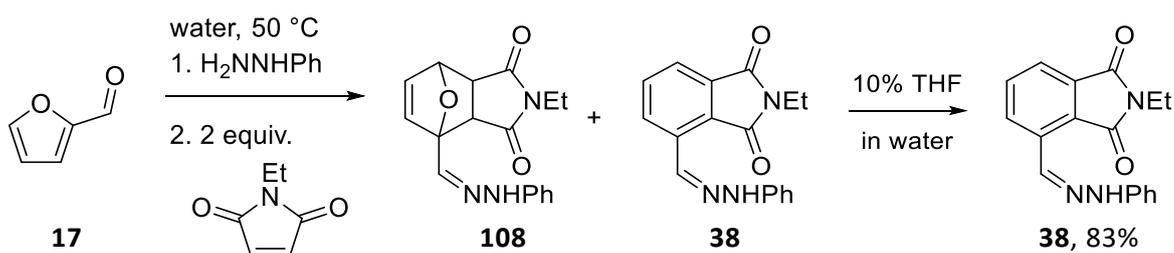
*Scheme 43. Synthesis of **38** from furfural **17** in a two-pot, three-step DA/partial-aromatisation followed by total aromatisation reaction sequence*

This is a significant improvement upon the literature preparation of **38**, which involved refluxing pre-prepared furfural phenylhydrazone in dry benzene with *N*-ethylmaleimide for 48 hours and produced **38** in 26% yield alongside the double cycloaddition product **41** in 22% yield (Scheme 19, a).[110]

As previously discussed, mono-substituted hydrazones are potential 1,3-dipoles in cycloadditions, and the reactivity of **38** is described in Chapter III.

2.2.7. Dienophile Scope

Maleimides

To investigate the use of alternative *N*-substituted maleimides, **114** and **115** were synthesised from maleic anhydride in 37% and 25% yield, respectively (Scheme 44).

114 (R = cyclopropyl), 38%
115 (R = 4-methylbenzyl), 26%
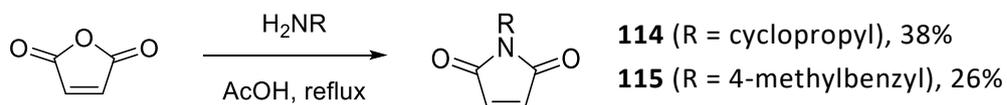
Scheme 44. Synthesis of N-*substituted maleimides from maleic anhydride*

These *N*-substituted maleimides, along with the commercially available *N*-phenylmaleimide **116**, were reacted with the *in situ* generated furfural dimethylhydrazone **28** as previously described (Scheme 45, Table 14).
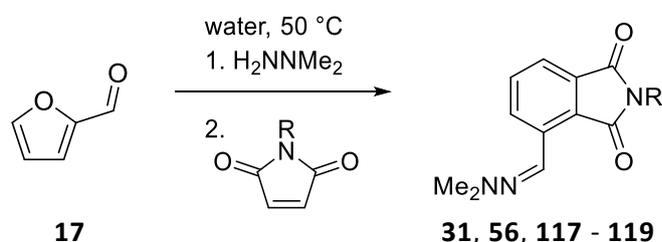
Scheme 45. Varying the maleimide in the Diels-Alder/aromatisation phthalimide synthesis

STARTING MATERIAL	R	MALEIMIDE EQUIV.	PRODUCT	YIELD
N-ethylmaleimide	-Et	1	**31**	97%
Maleimide	-H	2	**56**	86%
114	cyclopropyl	1	**117**	72%
114	cyclopropyl	2	**117**	80%
115		1	**118**	68%
116	-Ph	1	**119**	73%
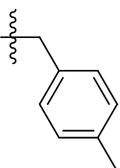
Table 14. Results of varying the maleimide in the Diels-Alder/aromatisation phthalimide synthesis[114]

With *N*-cyclopropylmaleimide **114**, an initial yield of 72% was increased to 80% by the use of two equivalents of the maleimide rather than one. Aromatically substituted maleimdies **115** and **116**, however, were less soluble in water and so could not be separated from the precipitated product by filtration when added in excess. For these reactions, a 1 : 1 ratio

of furfural to maleimide was therefore used, and the products **117** - **119** were obtained in good yields of 68-80%. No unreacted maleimide was observed under these conditions, suggesting side reaction with the *N,N*-dimethylhydrazine as previously observed.

Other dienophiles

An ideal approach to introducing more structural diversity would be to use other dienophiles, modifying the substituents on the resulting aromatic ring. A range of dienophiles were selected for screening based on reactivity and availability, each of which could also potentially react as a Michael acceptor to funtionalise the furan ring of **28** instead (Scheme 46).
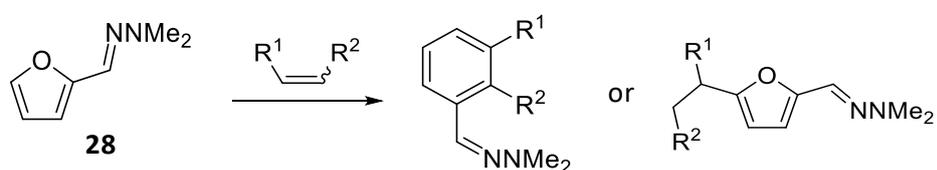
Scheme 46. Varying the dienophile in the Diels-Alder/aromatisation reaction, with potential Michael addition alternate product

Initially, acrolein, methyl vinyl ketone (MVK), dimethyl fumarate and ethyl acrylate were each combined with **28** in ratios of between 1:1 and 5:1, in ethanol, chloroform, or under solvent-free conditions, with 40 mol% copper triflate (Cu(OTf)$_2$), Yb(OTf)$_3$, or no catalyst, and reactions were carried out at room temperature or at reflux, for 48 hours in each case. No potential product formation was observed under any of these conditions. After the success of using aqueous media in the reactions with fumaronitrile, a further screening of acrolein, MVK, diethyl maleate, and acrylonitrile in water was carried out (Table 15).
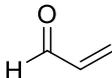
DIENOPHILE	TEMP. /°C	TIME	CATALYST	PRODUCT	YIELD
acrolein	RT	18 h	/	No conversion	N/A
	50	72 h	/	Polymerisation occurred	N/A
diethyl maleate	RT	18 h	/	No conversion	N/A
	50	72 h	/	**120**	7%
	80	30 min	/	Trace alternate product	N/A
	120 (MW)	30 min	/	Trace alternate product and polymerisation	N/A
	200 (MW)	30 min	/	Polymerisation	N/A
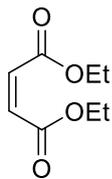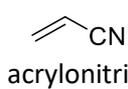
Dienophile	Temp. /°C	Time	Catalyst	Product	Yield
acrylonitrile (CH₂=CH-CN)	RT	18 h	/	No conversion	N/A
	50	72 h	/	**121**	trace
	100	24 h	/	**121**	24%
methyl vinyl ketone	RT	18 h	/	No conversion	N/A
	50	72 h	/	Unidentified	Trace
	50	72 h	Yb(OTf)$_3$	Unidentified	Trace
	50	72 h	Y(OTf)$_3$	Unidentified	Trace
	50	72 h	Sc(OTf)$_3$	Unidentified	Trace
	80	72 h	Yb(OTf)$_3$	Unidentified	Trace
	80	72 h	Y(OTf)$_3$	Unidentified	Trace
	80	72 h	Sc(OTf)$_3$	**122**	28%
	80 (MW)	30 min	/	Polymerisation	N/A
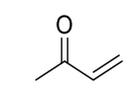
Table 15. Results of varying the dienophile in the Diels-Alder/aromatisation reaction in water

At room temperature, no product formation was observed with of the dienophiles. At 50 °C, diethyl maleate reacted to give **123** in 7% isolated yield (Figure 12), while TLC and ^{1}H NMR spectroscopy analysis of the reactions with acrylonitrile and MVK showed trace amounts of then-unidentified products that were not successfully isolated due to low yields. Under the same conditions, acrolein polymerised and its use as a dienophile was not investigated further. When acrylonitrile used at 100 °C, the previously unidentified product **124** was formed in 24% isolated yield (Figure 12). No *meta*-substituted regioisomer was observed in the crude reaction mixture, indicating selective orientation of the dienophile during Diels-Alder reaction driven by electronic factors rather than steric.

120   **121**
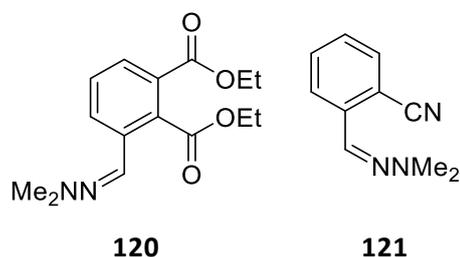
*Figure 12. Structures of **120** and **121***

There are many reports of reactions in water being accelerated by heating with microwaves,[117] and so this strategy was applied to these Diels-Alder/aromatisation reactions. Diethyl maleate and MVK were each reacted with **28** in water at 80 °C, 120 °C or 200 °C using microwaves. In each case, amorphous polymerisation of the reagents was

observed. The reaction with diethyl maleate at 120 °C also gave an unidentified aromatic product, by TLC and ^{1}H NMR spectroscopic analysis of the crude reaction mixture, but the reaction scale and yield were insufficient for isolation.

The reaction of MVK with **28** was performed with three water-tolerant Lewis acid catalysts; ytterbium triflate, Yb(OTf)$_3$, yttrium triflate (Y(OTf)$_3$), and scandium triflate (Sc(OTf)$_3$). Sc(OTf)$_3$ is reported as a superior activator of dienophiles for cycloaddition reactions, with specific reference to MVK.[113] Due to previous concerns over the formation of a chelation complex between **28** and Lewis acids, the dienophile, MVK, was pre-mixed with the catalyst before the addition of **28**. The reactions were carried out at 50 °C for 20 hours, using 40 mol% of catalyst. As this did not result in any product formation, the temperature was increased to 80 °C for 72 hours. Only the reaction with Sc(OTf)$_3$ showed significant consumption of the starting material, and the Michael addition/hydrazone hydrolysis product **122** was isolated in 28% yield (Figure 13). The further optimisation of this Michael addition reaction by Design of Experiment approach is discussed in Section 2.2.9.

122
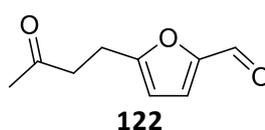
*Figure 13. Structure of **122***

The previously developed three-step, one-pot reaction sequence was then applied to diethyl maleate and dimethyl maleate, giving the phthalates **120** and **123** in yields of 15% and 19%, respectively (Scheme 47).

120 (R = Et), 15%
123 (R = Me), 19%
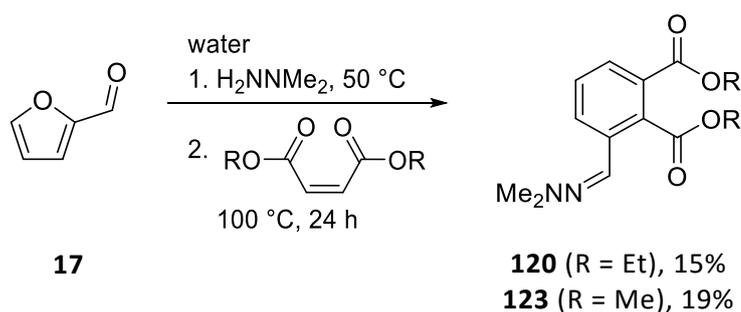
*Scheme 47. Three step, one pot Diels-Alder/aromatisation reaction with maleates to give phthalates **120** and **123***

Another dienophile screen was carried out, this time looking at the use of: dimethyl acetylenedicarboxylate **124** and methyl propiolate **125**, to see if alkynes could be accepted as dienophiles; 1,2-dichloroethene **126**, to synthesise a potential precursor to the pharmaceutical drug lamictal **154** (Figure 22); 2-chloroacrylonitrile **127**, for its similarity

to the successfully applied dienophile acrylonitrile; and 2,5-dimethoxy-2,5-dihydrofuran **128**, as a masked dialdehyde dienophile. Two equivalents of each of these were combined with **28** in water at various temperatures for 24 h each (Table 16).

DIENOPHILE	TEMP. /°C	RESULTS
MeO_2C———CO_2Me **124**	50	starting material, some side products
	80	insoluble solids
———CO_2Me **125**	50	starting material, some side products
	80	Side products
Cl⧵═⧸Cl *cis*-**126**	50	no change
	80	no change
	100	no change
Cl⧵═⧸Cl 1:1 *cis*-**126**/ *trans*-**126**	50	no change
	80	no change
	100	no change
Cl / CN **127**	50	no change
	100	hydrazone hydrolysis
MeO⧵O⧸OMe **128**	50	no change
	100	hydrazone hydrolysis
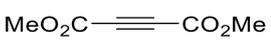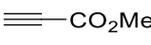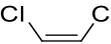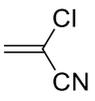
Table 16. Results of second screen varying the dienophile in the Diels-Alder/aromatisation reaction in water

The alkynes **124** and **125** showed minimal reactivity at 50 °C, with some minor unidentified side-products and no product formation observed. At 80 °C, full depletion of **28** was observed with formation of either insoluble solids or a complex mixture of unidentified side products. For 1,2-dichloroethene **126**, both the *cis* isomer and a 1:1 *cis*/*trans* mixture were used, but no reactivity was observed at 50 °C, 80 °C or 100 °C.

For both 2-chloroacrylonitrile **127** and dimethoxydihydrofuran **128**, no reaction was observed at 50 °C and at 100 °C hydrazone hydrolysis of **28** to furfural occurred. It was hypothesised that this was due to the addition of the dienophile affecting the pH of the solution. To investigate this, the reaction between **28** and dimethoxydihydrofuran was carried out at a range of pHs at 100 °C (Scheme 48).
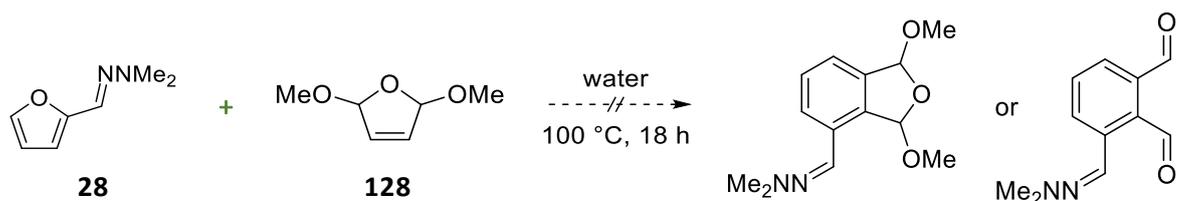
Scheme 48. Varying the pH in an attempt to catalyse a Diels-Alder/aromatisation reaction between **28** *and dimethoxydihydrofuran*

At pH 1 and 3, full hydrazone hydrolysis was observed, while at both pH 5 and 7, 21% of the starting material was hydrolysed. However, no product formation was observed at any pH, and these dienophiles were not investigated further. Of the dienophiles successfully screened for DA/aromatisation reactivity, acrylonitrile was selected for further optimisation, as the product **121** is of synthetic interest.

2.2.8. Optimisation of Acrylonitrile as a Dienophile

To improve the yield of the DA/aromatisation reaction between furfural dimethylhydrazone **28** and acrylonitrile, the use of an organic cosolvent was investigated (Scheme 49). Methanol, ethanol, tetrahydrofuran, 1,4-dioxane, acetonitrile and dimethyl sulfoxide were selected as readily available water-miscible organic solvents, and these cosolvents were screened at both 50 °C and 100 °C for 48 h (Table 17).

Yields were determined by quantitative ^{1}H NMR spectroscopic analysis using an internal standard. At 50 °C, the yields of **121** were low (1-14%), but significant amounts (19-32%) of an alternate product that contained the hydrazone moiety were present. Mass conversions of the furan species were high, but significant depletion of the acrylonitrile, which was present in excess, occurred.

At 100 °C, yields of the alternate product were significantly lower (0-5%) while the expected product **121** was produced in much higher yields (5-84%) despite mass conversion of the furan species and acrylonitrile tending to be lower due to side reactions.
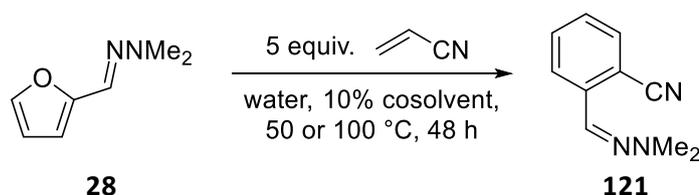
Scheme 49. Reacting **28** *with acrylonitrile in various water/cosolvent mixtures to synthesise* **121**

T /°C	Cosolvent	'Yields'*				
		28	121	Alternate Product	Mass Conversion	Acrylonitrile
50	methanol	59%	14%	26%	99%	35%
	ethanol	65%	4%	26%	95%	10%
	THF	57%	1%	22%	80%	39%
	1,4-dioxane	63%	2%	32%	97%	39%
	acetonitrile	65%	2%	28%	95%	59%
	DMSO	44%	3%	19%	65%	12%
100	methanol	6%	55%	0%	61%	12%
	ethanol	9%	69%	3%	81%	2%
	THF	3%	23%	1%	27%	7%
	1,4-dioxane	2%	5%	2%	9%	0%
	acetonitrile	11%[†]	84%[†]	5%[†]	100%[†]	28%
	DMSO	9%	6%	4%	19%	21%

*Calculated yield, determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard. †Due to inherent error margins in using internal standards in quantitative spectroscopy, the mass conversion was initially calculated to 105%, and all yield calculation values were scaled down appropriately to be within possibility.

Table 17. Results of reacting **28** with acrylonitrile in various water/cosolvent mixtures to synthesise **121**

In particular, use of 10% acetonitrile at 100 °C appeared to be very promising, with an 84% yield of **121**. This reaction was selected for five-fold scale-up, in a sealed pressure tube to avoid the evaporation of acrylonitrile at 100 °C (b.p. 77 °C). However, the resulting reaction mixture was analysed and found to contain the starting material and the alternate product previously noted at 50 °C, which was isolated and identified as the non-aromatised product (Z)-**129** in 29% yield (Scheme 50). The different results upon scale-up of the reaction could potentially be explained by a combination of the partial immiscibility of the starting material in the solvent mixture, and the fact that mixing was less effective at larger volumes, giving worse droplet dispersion. The reactions at different scales could therefore have experienced different combinations of 'in water' and 'on water' effects.
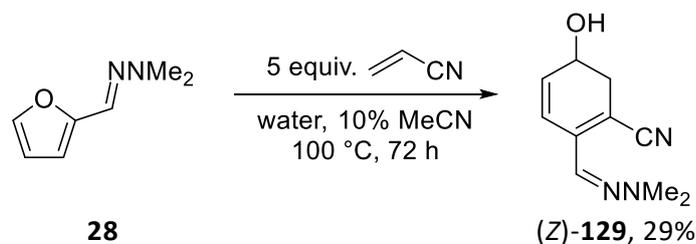
Scheme 50. Scaling up the reaction of **28** with acrylonitrile in 10% acetonitrile in water, producing **129**

In this reaction, 69% of the starting material **28** was recovered, giving a mass conversion of 98%. However, no acrylonitrile was observed in the crude reaction mixture by ^{1}H NMR analysis, suggesting that the reaction stopped due to depletion of the dienophile in side reactions. A significantly improved yield could therefore potentially be obtained by adding the acrylonitrile dropwise, but first the product (Z)-**129** and its utility were investigated.

The structure was characterised by positive electrospray LC-MS ([MH]$^+$ m/z 192.23), IR spectroscopy (br 3381 cm^{-1} (OH), 2188 cm^{-1} (CN)), and ^{1}H, ^{13}C, DEPT, COSY, HMBC and HMQC NMR spectroscopy analysis, with the annotated ^{1}H and ^{13}C NMR spectra below (Figure 14).
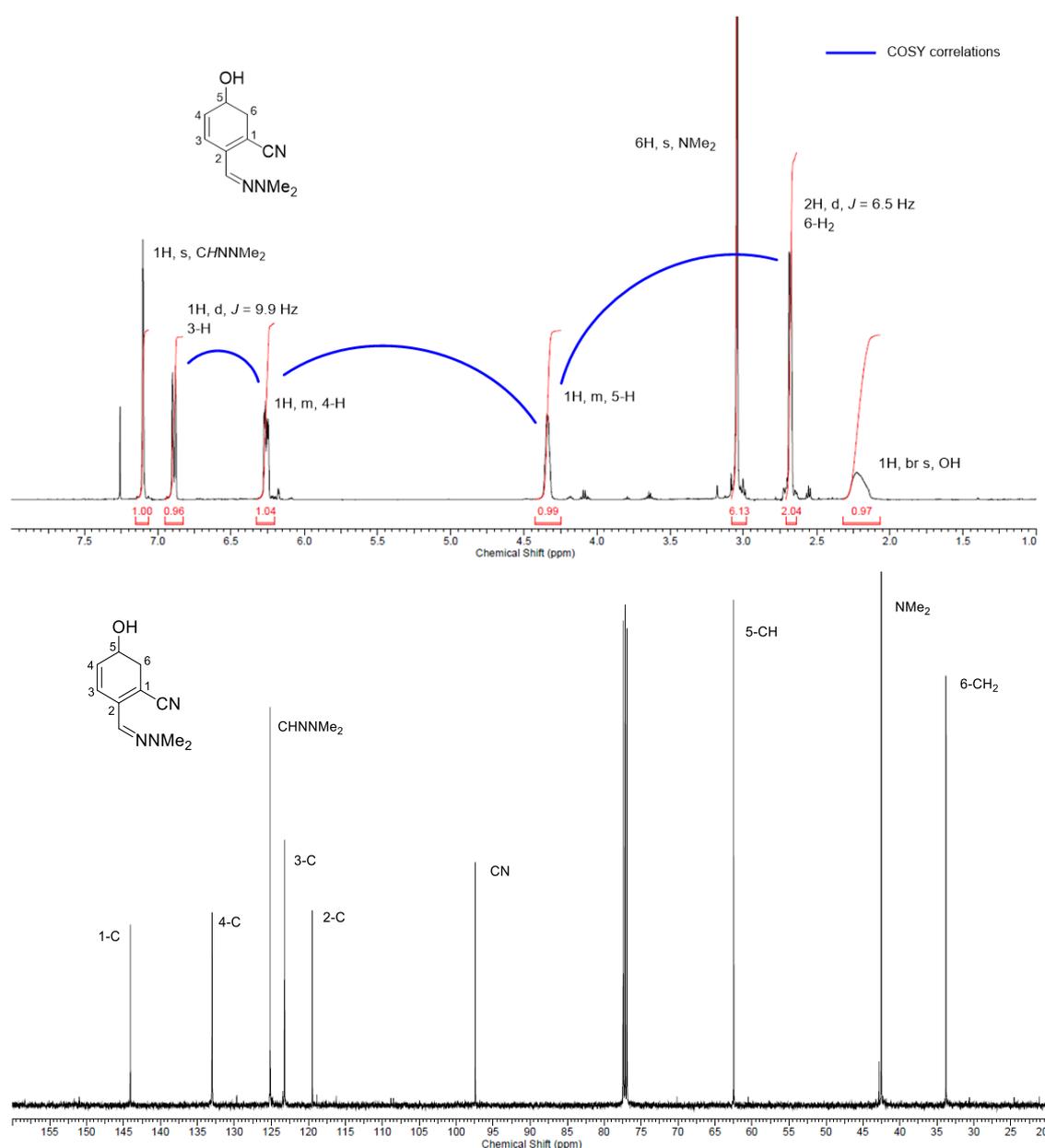
*Figure 14. Annotated ^{1}H and ^{13}C NMR spectroscopy analysis spectra for compound **129***

Compound **129** is an expected intermediate in the Diels-Alder/aromatisation reaction sequence between between **28** and acrylonitrile, formed by the oxo-bridge opening of **130** (Scheme 51).
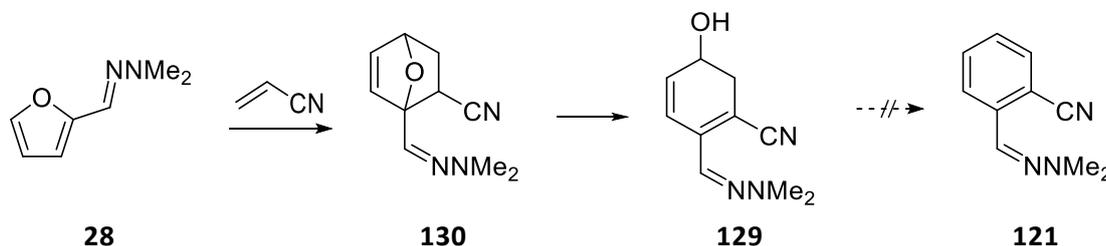
Scheme 51. *Proposed reaction scheme of the synthesis of* **129** *from* **28** *via* **130**

However, if (*Z*)-**129** were an intermediate in the synthesis of **121**, it should convert into **121** under the reaction conditions used to synthesise **121**. When **129** was heated at 100 °C in water for 24 h, no reaction was observed. It was therefore proposed that the synthesis of **121** from **28** and acrylonitrile proceeds via (*E*)-**129**, the hydrazone geometric isomer of (*Z*)-**129** (Figure 15).
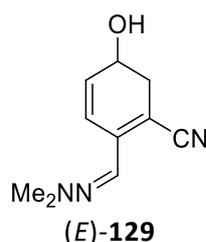
(*E*)-**129**

Figure 15. *Structure of (*E*)-***129**, *a proposed intermediate in the synthesis of* **121** *from* **28**

Isomer (*Z*)-**129** could be stabilised by interactions between the hydrazone and nitrile (Scheme 52), thus reducing its propensity to aromatise. The selective synthesis of (*Z*)- or (*E*)-**129** could be determined by disruption of this hydrazone/nitrile interaction by solvent effects such as hydrogen bonding between the hydrazone and water.
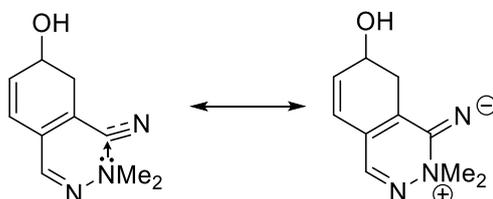
Scheme 52. *Resonance structures illustrating the proposed interaction between the hydrazone and nitrile of* **129**

This proposed interaction is supported by the ^{13}C NMR and IR data for **129**; the ^{13}C NMR nitrile signal (97 ppm) is upfield of the typical 113-117 ppm nitrile shift, while the IR nitrile stretch (2188 cm^{-1}) is lower than the typical 2230-2250 cm^{-1}, both consistent with electron donation into the nitrile.

The analytical spectra of the original reactions between **28** and acrylonitrile in just water (Table 15) were re-examined for the presence of (Z)-**129** and none was observed. This suggests that this geometric isomer forms only in the presence of a cosolvent. Regardless of the rationale behind its synthesis, (Z)-**129** could be a useful precursor to **121** and this conversion by aromatisation was investigated (Scheme 53, Table 18).
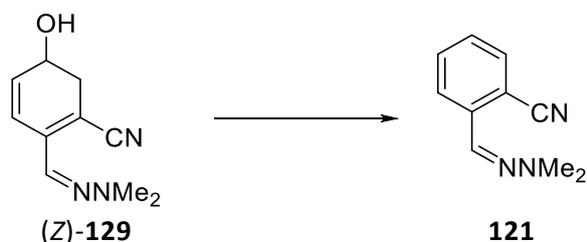
*Scheme 53. Varying reaction conditions to synthesise **121** by aromatisation of (Z)-**129***

SOLVENT	TEMP.	ADDITIVE	RESULT
water	100 °C	/	no reaction
CPME	reflux	/	no reaction
CPME	reflux	200 mM HCl	Insoluble solids, trace product
CPME	RT - 60 °C	20 - 200 mM HCl	no reaction
CPME	reflux	78 mM HCl	46% **121** with some insoluble solid
CPME	RT – 60 °C	acetic acid	no reaction
CPME	reflux	acetic acid	small amount of side reactions
CPME	RT	acetic anhydride	starting material and side products
CPME	60 °C	acetic anhydride	side products

*Table 18. Results of varying reaction conditions to synthesise **121** by aromatisation of (Z)-**129***

As mentioned, the reaction of **129** to **121** did not proceed at 100 °C in water. CPME was selected as an alternative 'green' organic solvent. Heating at reflux in CPME also resulted in no reaction, while adding 400 mM HCl caused the reaction to form insoluble solids with a trace of **121** by TLC analysis. Reacting at RT or 60 °C with 20 or 200 mM HCl gave no reaction, but refluxing with 200 mM HCl gave a 46% yield of **121**, in addition to some insoluble solid. Acetic acid was also used, but no formation of product occured at RT, 60 °C or at reflux, and small quantities of unidentified side products were observed. The use of acetic anhydride to convert the hydroxyl into an acetyl group and thus enhance its ability to act as a leaving group was investigated, but no product or acetyl intermediate formation was observed. Due to time constraints, further optimisation was not

attempted, but other methods of promoting the hydroxyl as a leaving group, such as tosylation, could be explored. The use of base could also potentially catalyse elimination.

Later, while investigating the use of **121** as a precursor to phthalocyanines (Section 3.2.1.), more material was required and a petrochemical-derived route to **121** was carried out. 2-Cyanobenzaldehyde **131** was stirred in ethanol with *N*,*N*-dimethylhydrazine at 50 °C for 3 h (Scheme 54). However, an alternative geometric isomer was instead isolated in 73% yield. ^{1}H NMR NOESY analysis showed this to be the (*E*)-isomer while the previously isolated **121** was the (*Z*)-isomer.
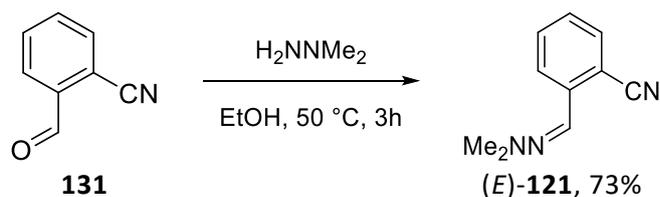
*Scheme 54. Synthesis of the (*E*)-isomer of **121** from 2-cyanobenzaldehyde*

Interestingly, this (*E*)-isomer exists as fine colourless crystals, while the (*Z*)-isomer is a pale green oil. To investigate whether (*E*)-**121** could be converted into (*Z*)-**121**, and thus demonstrate the thermodynamically favoured product, the former was heated in water at 100 °C. However, (*E*)-**121** decomposed under these conditions.

Further optimisation of the synthesis of **121** could occur by successful scale up of the 84% yield small scale reaction, or by producing **129** and optimising the aromatisation conditions.

2.2.9. Functionalisation of Furfural by Michael Addition

Section 2.2.7. described the reaction of methyl vinyl ketone (MVK) and furfural dimethylhydrazone **28** at 80 °C with 40 mol% of Sc(OTf)$_3$ to give the Michael addition and hydrazone hydrolysis product **122** (Scheme 55).
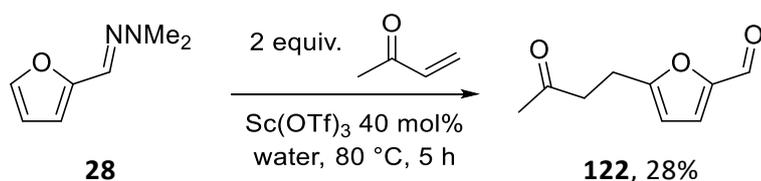
*Scheme 55. Michael Addition and hydrazone hydrolysis reaction of furfural dimethylhydrazone and MVK to give **122***

Control reactions showed that furfural did not react in this way with MVK in the presence of Sc(OTf)$_3$, suggesting that the hydrazone hydrolysis occured after the Michael addition.

However, furfural dimethylhydrazone did undergo incomplete hydrolysis to furfural in the presence of Sc(OTf)₃ and absence of MVK. Reactions investigating the potential of Sc(OTf)₃ as a general hydrazone hydrolysis catalyst were carried out, using aromatic hydrazone **31**, but no change was observed after 72 h at 80 °C (Scheme 56).
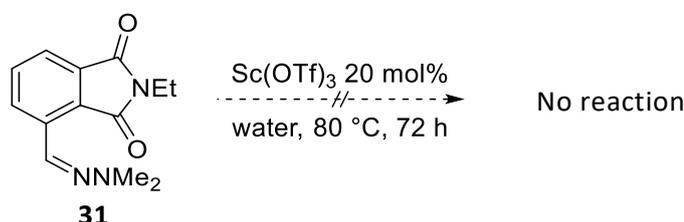
Scheme 56. Attempted hydrolysis of the hydrazone group of 31 using scandium triflate

A reaction using 20 mol% of Sc(OTf)₃ on a larger scale was conducted with the aim of synthesising more of **122**. The hydrazone product **132** was instead formed in 24% yield, showing that lower catalyst loadings can avoid the hydrazone hydrolysis (Scheme 57). Retention of the hydrazone motif was desirable, to allow the furan ring to then undergo Diels-Alder reactions.
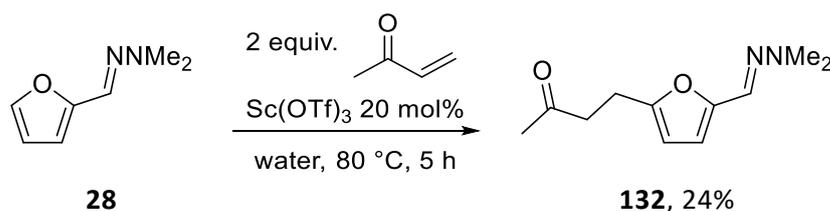
Scheme 57. Michael addition reaction between furfural dimethylhydrazone 28 and methyl vinyl ketone, with lower catalyst loading, resulting in the hydrazone product 132

'Design of Experiment' optimisation

To optimise the synthesis of **132**, a Design of Experiment (DoE) screen was carried out, varying the volume, temperature, catalyst loading, and equivalents of MVK (Table 8).

VARIABLE	LOWER BOUND	MID-POINT	UPPER BOUND
Temperature /°C	80	90	100
Solvent volume /mL	1	5.5	10
Catalyst Loading /mol%	1	10.5	20
MVK Equiv.	1	3	5

Table 19. Range and midpoints of factors varied in the DoE screen

To model the reaction space for four variables, eleven reactions were carried out. Calculated yields and mass conversions were obtained using analytical reverse phase high pressure liquid chromatography (HPLC) of the reaction mixture after organic extraction (Table 20).

ENTRY	TEMP. /°C	SOLVENT VOLUME /mL	CATALYST LOADING /mol%	MVK EQUIV.	YIELD*	MASS CONVERSION
1	80	1	1	1	12%	60%
2	80	1	20	5	26%	28%
3	80	10	20	1	13%	43%
4	80	10	1	5	33%	79%
5	100	10	20	5	22%	33%
6	90	5.5	10.5	3	25%	44%
7	90	5.5	10.5	3	28%	49%
8	90	1	1	5	42%	80%
9	100	1	20	1	13%	26%
10	100	10	1	1	21%	83%
11	90	5.5	10.5	3	26%	45%

*Calculated yield, determined by quantitative ^1H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

Table 20. Conditions, calculated yields, and mass conversions of reactions performed for DoE analysis

Calculated yields of 12-42% were found, compared to the previous isolated reaction yield of 24%. The data collected was processed Dr. Tom D. Sheppard and a model of the reaction space was constructed. The quality of the model was assessed and found to be within acceptable boundaries. For a good model, a Q2 value of greater than 0.6, an R2 - Q2 value of less than 0.3, a model validity of greater than 0.25, and a reproducibility of greater than 0.5 is desired. Each of these criteria were met in the modelling of this reaction space (Figure 16).
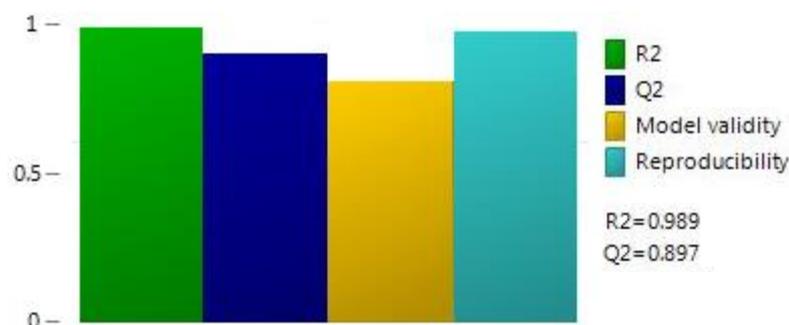
Figure 16. Assessment of the quality and fit of the DoE model produced†

The effects of each variable on the yield of the reaction were calculated and interactions between these variables modelled (Figure 17).
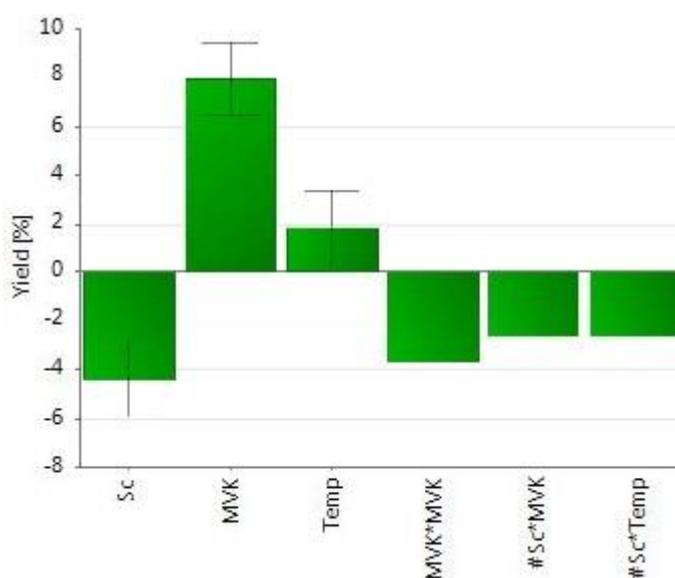
Figure 17. Coefficients of the effect of each condition variable on yield†

The number of equivalents of MVK had the greatest correlation with yield. At low temperatures, a higher catalyst loading improved the yield, while at high temperatures, lower catalyst loading favoured formation of **132**. The effect of catalyst loading on yield became more pronounced when more equivalents of MVK are used. These observations suggested that the scandium catalyst may have promoted the polymerisation of MVK, lowering the yield. The optimal conditions suggested by the model were high temperatures, low catalyst loading, and high numbers of equivalents of MVK.

Further optimisation was carried out, with a highest yield of 47% (Table 21, entry 6). Similar yields were obtained in the absence of catalyst at 100 °C (Table 21, entry 5, 8 & 9), in contrast to previous experiments which found no product formation in the absence of

†Produced by Dr. Tom D. Sheppard

catalyst at 80 °C (Table 15 on page 68). This was attributed to the strong relationship between temperature and catalyst loading, as found in the DoE.

ENTRY	TEMP. /°C	SOLVENT VOLUME /mL	CATALYST LOADING /MOL%	MVK EQUIV.	YIELD*	MASS CONVERSION
1	80	1	1	5	15%	29%
2	80	5	1	5	19%	51%
3	80	1	0.5	5	16%	47%
4	80	1	1	5	30%	61%
5	100	5	0	5	41%	72%
6	100	5	0.1	5	47%	78%
7	100	5	1	5	42%	79%
8	100	5	0	5†	46%	69%
9	100	5	0	5	40%	69%

*Calculated yield, determined by quantitative ^1H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard. †Added portionwise

Table 21. Conditions, calculated yields, and mass conversions of the second round of DoE reactions

Portion-wise addition of the MVK was found to enhance the yield (Table 21, entry 8 c.f. entry 5 & 9). The reaction was repeated with no catalyst and dropwise addition of five equivalents of MVK at 100 °C, giving **132** in 42% isolated yield (Scheme 58).
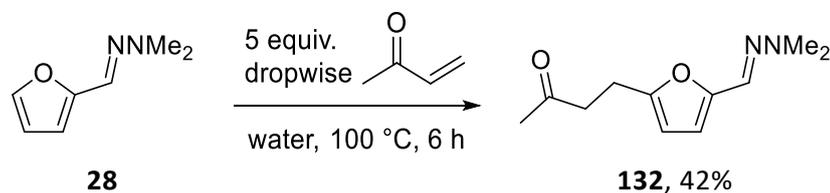
Scheme 58. Optimised synthesis of **132** by Michael addition of furfural dimethylhydrazone **28** with methyl vinyl ketone[114]

A four-step route to functionalised aromatics

The formation of **28** from furfural, and subsequent Michael addition with MVK, were successfully combined into a one-pot, two-step reaction that gave **132** in an isolated yield of 39%. Compound **132** was then successfully reacted with N-ethylmaleimide to give the substituted aromatic product **133**, with an unoptimised yield of 47%. This gave a two-pot, four-step route from furfural to a 1,2,3,4-tetrasubstituted benzene ring (Scheme 59), which could be adapted to alternative Michael acceptors and dienophiles to vary the aromatic substituents.
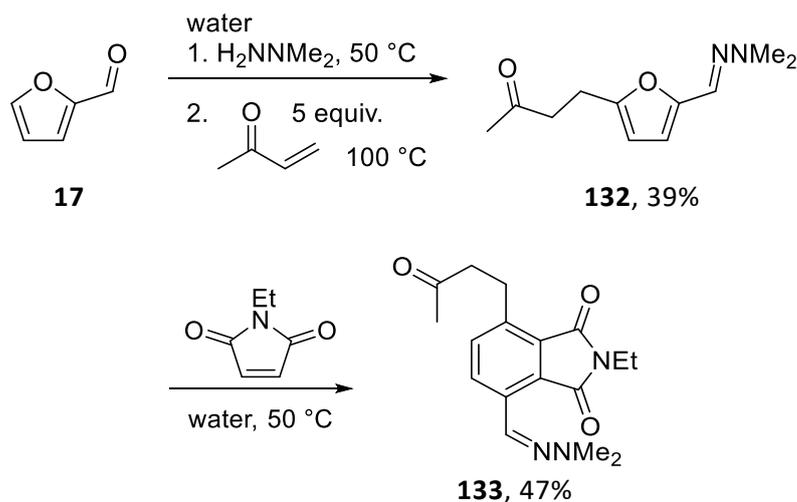
Scheme 59. Two-pot, four-step synthesis of 133[114]

Alternative michael acceptors

To expand the scope of the functionalisation of furfural dimethylhydrazone **28** by Michael addition, a series of alternative Michael acceptors were investigated. Initially, ethyl vinyl ketone **134** and phenyl vinyl ketone **135** were selected due to their similarity to MVK. It was hoped that their additional bulk would minimise the vinyl ketone polymerisation seen with MVK and thus improve product yields. A literature procedure was adapted to synthesise **135** from 3-chloropropiophenone **136** in 85% yield (Scheme 60).[118]
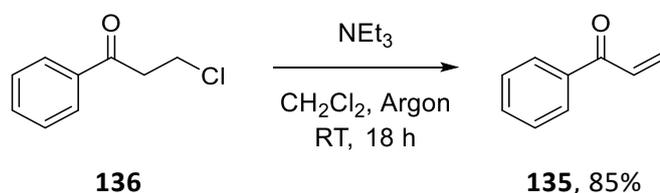
Scheme 60. Synthesis of PVK from 3-chloropropiophenone

Using the optimised conditions from the DoE screen with MVK, **134** and **135** were reacted with **28**. No reaction was observed with **135**, possibly due to the bulky phenyl substituent. Vinyl ketone **134** successfully reacted to give **137**, but in a yield of only 14% compared to the 42% achieved with MVK (Scheme 61).
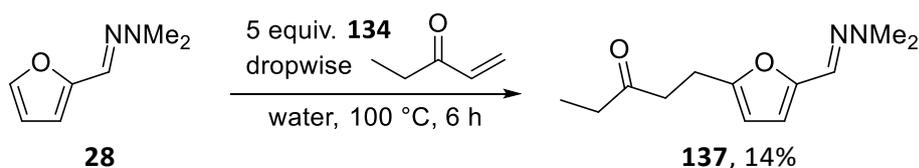
Scheme 61. Michael addition of furfural dimethylhydrazone with ethyl vinyl ketone

As mentioned (Section 2.1.4.), Potts *et al.* previously reported the Michael addition of **28** with 1,4-napthoquinone to produce **48** by refluxing in toluene, giving a yield of 77%.[109] This literature reaction was repeated in 68% yield, compared to the literature yield of 77% (Scheme 62).
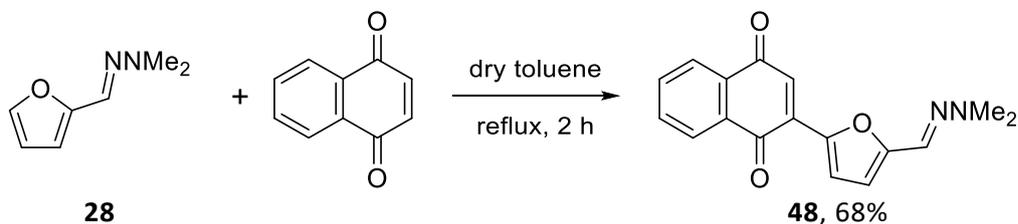
Scheme 62. Michael addition of furfural dimethylhydrazone with 1,4-napthoquinone

Performing the reaction in water at 100 °C showed a small trace of product formation by both TLC and ^{1}H NMR analysis of the crude mixture after 3 hours, but attempts to isolate the product were unsuccessful. Allowing the reaction to proceed over more time led to the disappearance of **48**. The use of water-miscible organic cosolvents in quantities of 10-50% did not improve product formation.

1,4-Benzoquinone **138**, 2-cyclopenten-1-one **139**, and 2-cyclohexen-1-one **140** were also screened for reactivity as Michael acceptors in the reaction (Figure 18).
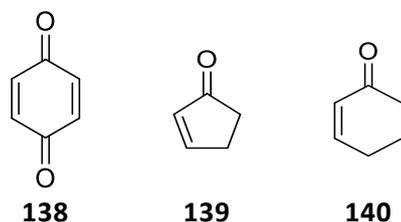
*Figure 18. Structures of Michael acceptors 1,4-benzoquinone **138**, 2-cyclopenten-1-one **139**, and 2-cyclohexen-1-one **140***

No product formation was observed under a range of reaction conditions, using both water and cosolvent mixtures. Water-soluble lewis acid catalysts Sc(OTf)$_3$, Yb(OTf)$_3$ and Y(OTf)$_3$ were applied but still no product formation was observed. The reactions were then carried out at reflux in toluene according to Potts' literature conditions for napthoquinone, and again no reaction occurred.

Finally, **48** was tested in the Diels-Alder/aromatisation reaction sequence in an attempt to synthesise the highly conjugated phthalimide **141** (Scheme 63).
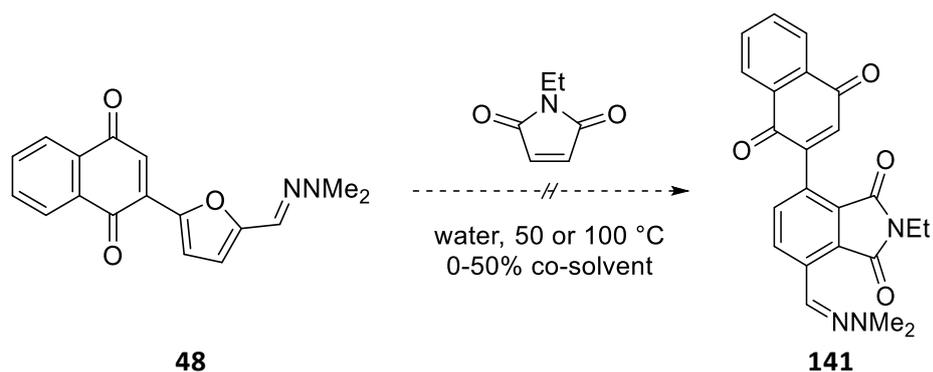
Scheme 63. Attempted Diels-Alder/aromatisation reaction of 48 with N-ethylmaleimide to give 141

Disappointingly, the reaction was found not to occur in water at 50 or 100 °C and with 0-50% of water-miscible cosolvents, and this Michael addition strategy as a potential route to highly functionalised aromatic compounds was not explored further.

2.2.10. Intramolecular Diels-Alder Reactions

Intramolecular Diels-Alder furan reactions are well known,[86,91-93] and the synthesis of a furfural derivative capable of undergoing an intramolecular Diels-Alder reaction was investigated. The hydroxyl group on HMF **19** provides a handle on which to link and tether a dienophile motif, such an allyl or acrolyl group by ester or ether linkage (Scheme 64).
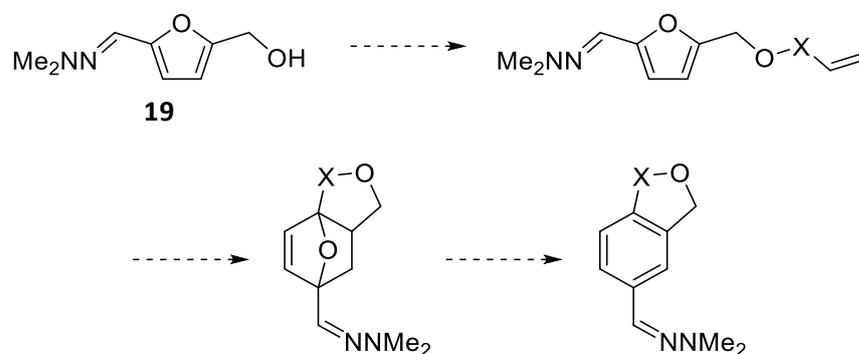
Scheme 64. Hypothetical reaction scheme for introducing an allyl or acrolyl group onto the hydroxyl group of 19 by ester or ether linkage, and subsequent intramolecular Diels-Alder/aromatisation reaction

A Williamson ether synthesis was carried out between **59** and allyl bromide using potassium hydroxide in water. However, this resulted in conversion to HMF **19** by hydrolysis of the hydrazone group (Scheme 65).
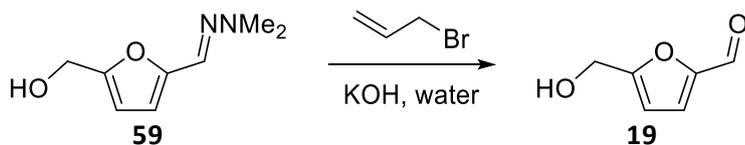
Scheme 65. Attempted Williamson ether synthesis between 59 and allyl bromide, resulting in formation of furfural 19

Alternate literature conditions using sodium hydroxide in acetonitrile were then adapted and **142** was isolated in 22% yield, alongside the alcohol **143** in 18% yield (Scheme 66). The alcohol is likely a product of a Cannizzaro reaction, though no carboxylic acid was isolated, possibly due to a high retention factor (R_f) during column chromatography. Allyl ether **142** was then reacted with *N,N*-dimethylhydrazine to form **144** in 92% yield.
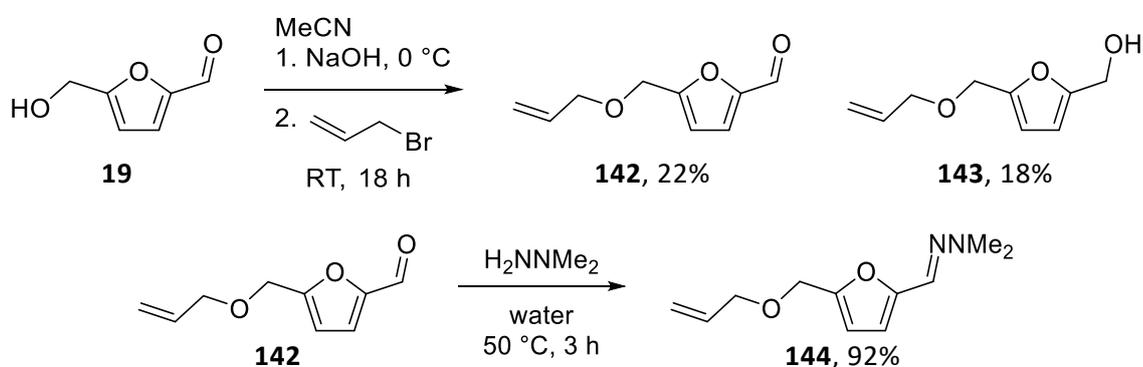
Scheme 66. Synthesis of 142 from HMF and allyl bromide, with side-product 143, and reaction of 142 with N,N-dimethylhydrazine to give hydrazone 144

Ester **145** was synthesised from HMF and acryloyl chloride in 43% yield using Schotten-Baumann-like conditions, and then reacted with *N,N*-dimethylhydrazine (Scheme 67). However, attempting the hyrazone formation in both water and ethanol lead to the breaking of the ester bond, producing **59**.
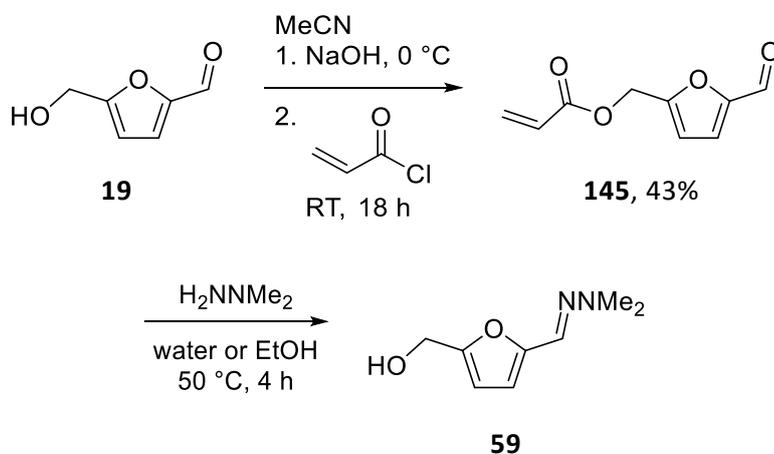
Scheme 67. Schotten-Baumann-like ester formation between HMF and acryloyl chloride to give 145, followed by reaction with N,N-dimethylhydrazine

To avoid this, ester formation with hydrazone **59** was attempted. As strong bases cause hydrolysis of the hydrazone, a Steglich esterification was attempted. DCC and EDCI were both applied, but in both cases a complex mixture of products was produced, with no formation of **146** observed (Scheme 68).
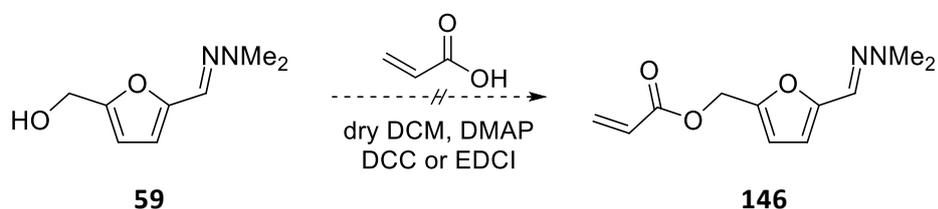
Scheme 68. Attempted Steglich esterification between 59 and acrylic acid

Due to time constraints, this was not pursued further and focus shifted to the intramolecular Diels-Alder reaction of **144** (Scheme 69, Table 22).

Scheme 69. Attempted intramolecular Diels-Alder/aromatisation reaction of 144 to produce aromatic compound 147

SOLVENT	TEMP.	TIME	RESULT
water	50 °C	24 h	no reaction
water	reflux	24 h	no reaction
1:1 water /ethanol	reflux	24 h	no reaction
water	200 °C (MW)	20 min	23% **148**

Table 22. Results of attempted intramolecular Diels-Alder/aromatisation reactions of 144

Previous results of furfural hydrazones successfully undergoing DA/aromatisation in water at 50 °C prompted a similar approach here. However, heating in water or a 1:1 water/ethanol mixture, to improve solubility, gave no reaction. The use of microwave heating to promote Diels-Alder reaction, as has previously been reported,[119,120] gave the hydrazone-hydrolysed product **148** in 23% yield (Scheme 70).
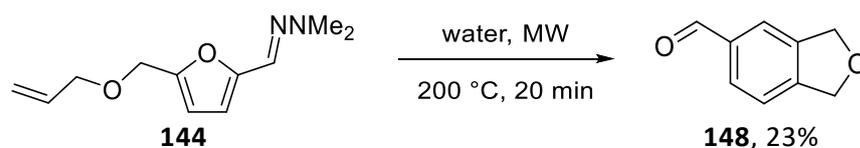
Scheme 70. Intramolecular Diels-Alder/aromatisation reaction and hydrazone hydrolysis of 144 to produce aromatic compound 148

The reaction product mixture contained significant amounts of insoluble solids, and the extreme 200 °C temperature likely caused significant decomposition and/or polymerisation. Reducing the temperature and fine-tuning the microwave's parameters could likely lead to a significant improvement in yield for this DA/aromatisation reaction. Due to time constraints, this strategy was not explored further, but the successful synthesis of **148** illustrates its potential.

2.3. Summary of Chapter II

A three-step, one-pot synthesis of substituted phthalimide **31** from furfural **17**, *N,N*-dimethylhydrazine, and *N*-ethylmaleimide has been developed in water with near-quantitative yields, no loss of yield on a 20 g scale, and facile isolation of the precipitated product in high purity by filtration from the reaction solution (Scheme 71).
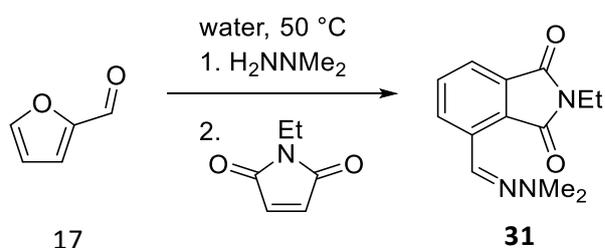
*Scheme 71. Three-step, one-pot synthesis of substituted phthalimide **31** from furfural **17**,* N,N-*dimethylhydrazine, and* N-*ethylmaleimide in water*

The scope of this methodology was then explored extensively, with alternative furfurals and derivatives, *N*-substituted maleimides and other dienophiles, and hydrazine alternatives applied. Select reactions were extensively optimised. Modification of the furfural species by Michael addition to add substituents, or by extension of a hydroxyl to induce intramolecular Diels-Alder reaction, was also investigated, and a wide range of aromatic and non-aromatic compounds synthesised (Figure 19).
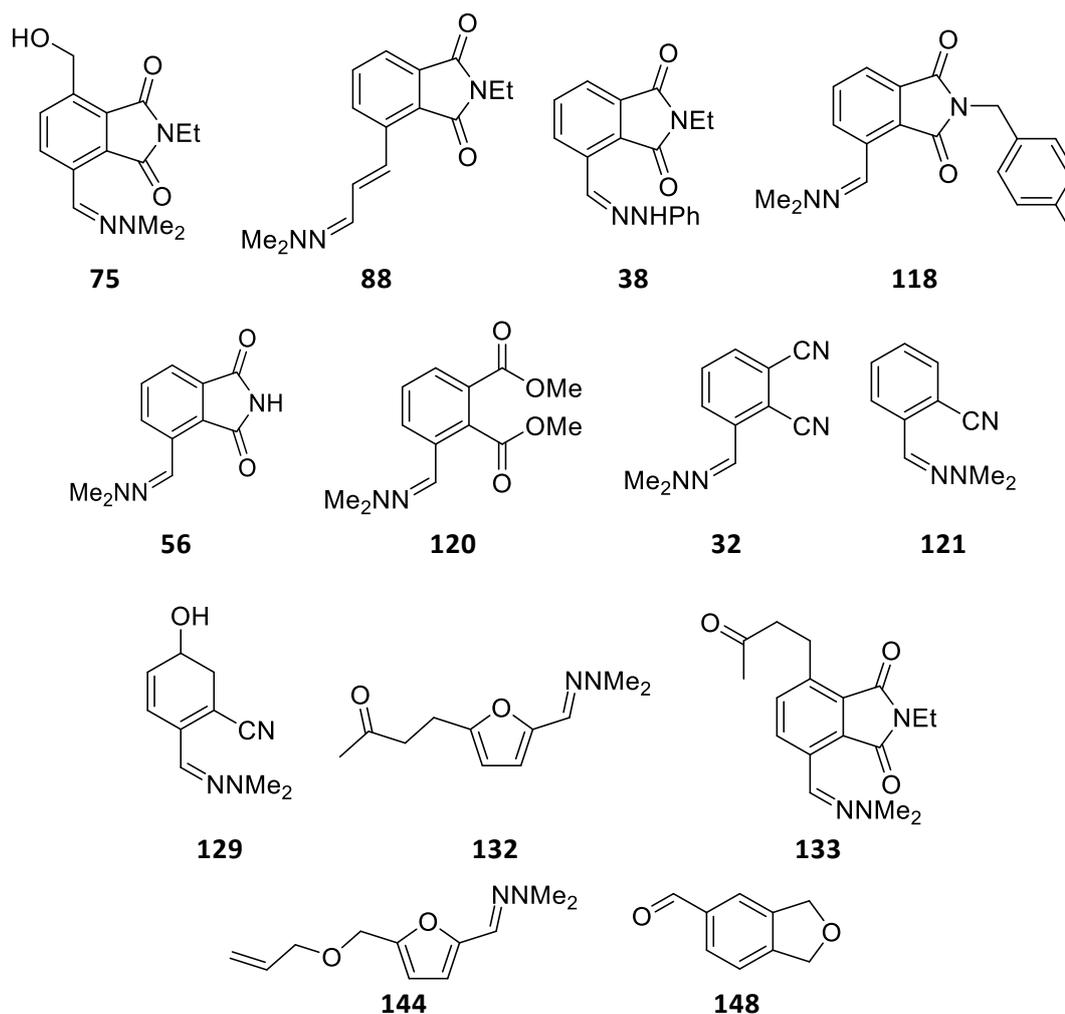
Figure 19. Structures of compounds synthesised in Chapter II

Fourteen furfural derivatives and five maleimides were used to create a range of phthalimides such as **82**, **88**, **118** and **56**. Other dienophiles successfully applied include maleates, giving phthalates such as **120**; fumaronitrile, giving **32**; and acrylonitrile, whose use gave **121** but also, under alternative conditions, the non-aromatised Diels-Alder product **129**.

Mono-substituted hydrazines were successfully used as *N,N*-dimethylhydrazine substitutes and the synthesis of **38** was optimised. This compound has the ability to undergo cycloaddition at the mono-substituted hydrazone 1,3-dipole (Section 3.1.1.).

The Michael addition of dimethylhydrazone with methyl vinyl ketone to give **132** was discovered and optimised using Design of Experiment techniques, and then used to develop a four-step, two-pot synthesis of tetra-substituted phthalimides such as **133**.

Finally, extension of the hydroxyl group of HMF **19** gave compounds such as **146**, which have the potential to undergo intramolecular Diels-Alder reactions to give aromatic

compounds such as **148**, a pharmaceutically relevant chemical scaffold present in widely prescribed drugs such as the anti-depressant citalopram **149** (Figure 20).

149
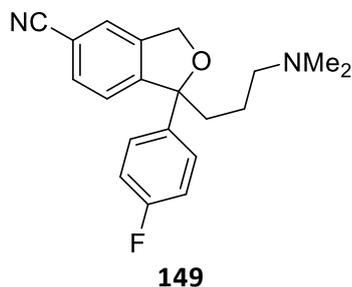
*Figure 20. Structure of citalopram **149***

There has been interest from industrial sources in this Diels-Alder/aromatisation methodology as a way of quickly synthesising a large and varied phthalimide library for drug fragment screening. A large portion of Chapter III describes further diversification of these phthalimides as well as other aromatic hydrazones.

Future Work

A substituted hydrazine bearing a dienophile motif could be synthesised and used to form a furfural hydrazone able to undergo intramolecular Diels-Alder/aromatisation reactions, forming an interesting heterocyclic structure (Scheme 72).
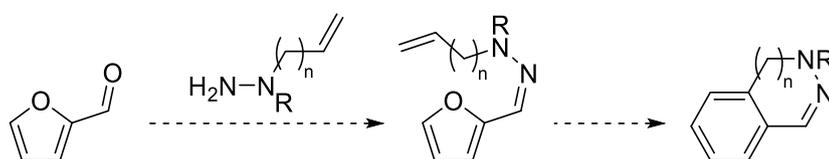
Scheme 72. Proposed synthesis of a dienophile-bearing hydrazone and subsequent intramolecular Diels-Alder/aromatisation

Compound **121** is a potential precursor to phthalonitrile, which has industrial applications in phthalocyanine pigment synthesis (Section 3.1.3.). It would therefore be desirable to optimise the synthesis of **121** and extend the methodology to substituted furfurals in order to produce substituted cyanobenzenes **150** and phthalonitriles **151** (Scheme 73). These have applications in the synthesis of substituted phthalocyanines (Section 3.1.3.).
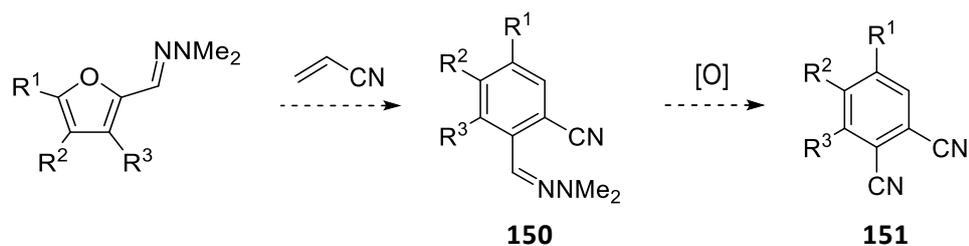
Scheme 73. Proposed synthesis of substituted cyanobenzenes from substituted furfurals, and subsequent oxidation to give substituted phthalonitriles

Finally, further reaction condition screening for intramolecular Diels-Alder reactions in compounds such as **144** and **146**, and diversification of these compounds, could allow for a wide range of aromatic compounds to be produced (Scheme 74).

144 ($R^1 = R^2 = R^3 = H$, $X = CH_2$)
146 ($R^1 = R^2 = R^3 = H$, $X = C(O)$)
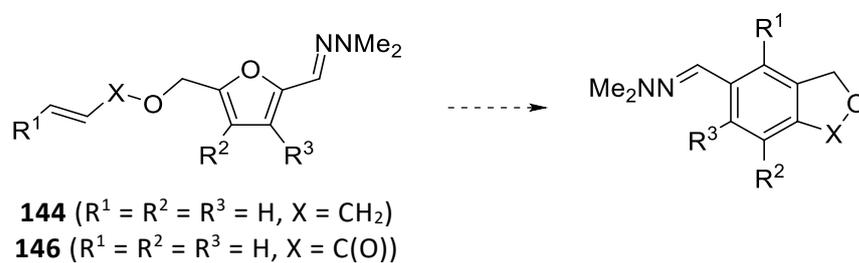
*Scheme 74. Proposed synthesis of aromatic compounds by intramolecular Diels-Alder reactions of compounds such as **144** and **146***

Chapter III. Reactivity of Aromatic Products and Synthesis of Target Molecules

3.1. Introduction

The reactivity of the products synthesised in Chapter II was explored, with the aim of diversifying the compounds accessible via this sustainable methodology and synthesising industrially relevant target molecules. Particular focus was on manipulating the hydrazone group present in most of the products, to give more drug-like structures. The reactivity of the imide ring of the phthalimides was also explored, as these represent the largest family of compounds produced. Target molecules were selected for synthesis to demonstrate the utility of these compounds as intermediates to commercially valuable products such as pharmaceuticals and colourants.

3.1.1. Substituted Hydrazone Reactivity

The hydrolysis of the hydrazone into an aldehyde (Scheme 75a, ii) would be desirable, as this could then be converted into a wide range of functionalities by oxidation, reduction, nucleophilic attack, Wittig reaction, etc. Reduction to an amine (Scheme 75a, iii) would also give potential for further reactivity, e.g. by amide coupling or intramolecular aminolysis to give an isoindolinone, a common molecular core in biologically active compounds (Scheme 75b).

Hydrazones can also be reduced without full N-N bond cleavage, giving hydrazines (Scheme 75a, iv),[121] or oxidised to a variety of functional groups. Alcohols can be generated by ozone oxidation followed by reductive workup with borane dimethylsulfide (BMS) (Scheme 75 a, v).[122] Aldehyde-derived hydrazones can be oxidised by magnesium monoperoxyphthalate (MMPP) to give a nitrile group (Scheme 75 c, i),[123] and they can also act as 1,3-dipoles in [3+2] cycloadditions,[110] generating five-membered rings (Scheme 75 c, ii). While the α-positions of ketone-based hydrazones are mildly electrophilic, aldehyde-based hydrazones are known to show umpolung reactivity and act as d^1 nucleophiles, allowing the formation of a wide range of carbon-carbon and carbon-heteroatom bonds (Scheme 75 c, iii).[123,124]
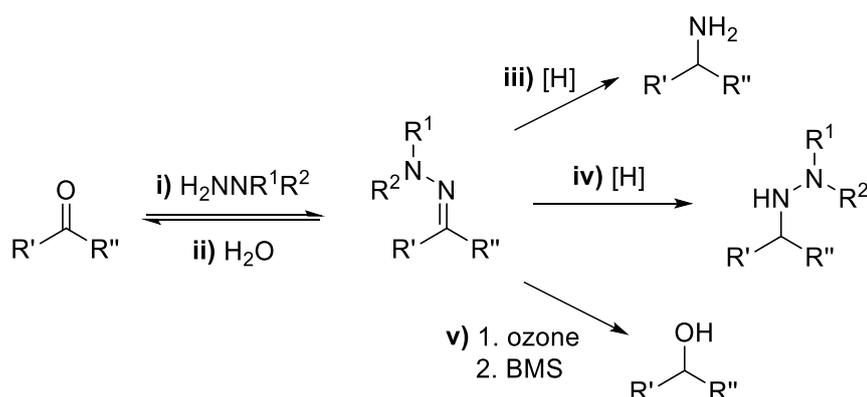
a) Synthesis of i) a hydrazone from a carbonyl, and reactivity of a hydrazone by ii) hydrolysis to a carbonyl; iii) reduction to a primary amine; iv) reduction to a hydrazine; and v) ozone oxidation with reductive workup using borane dimethylsulfide (BMS) to give an alcohol
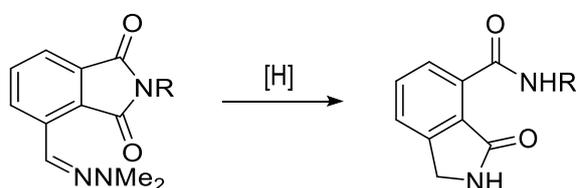
b) Hydrogenation and intramolecular transamidation of a hydrazone-substituted phthalimide to give an isoindolinone
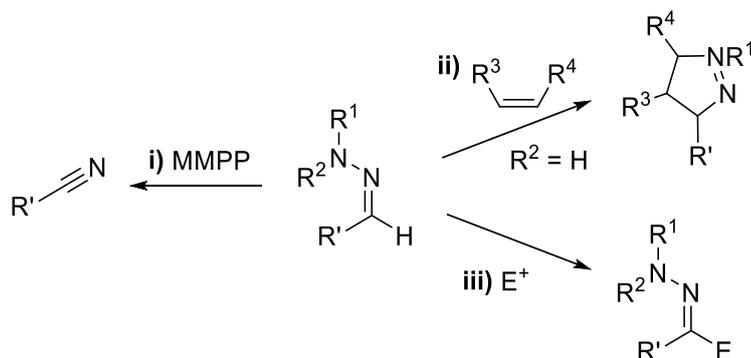
c) Reactivity of an aldehyde-derived hydrazone by i) oxidation to a nitrile by magnesium monoperoxyphthalate (MMPP); ii) a [3+2] cycloaddition reaction; and iii) nucleophilic attack into an electrophile from the d^1 centre

Scheme 75. Reactivity of the hydrazone chemical motif

3.1.2. Phthalimide Reactivity

Phthalimide is a precursor to anthranilic acid, used in the production of saccharin and some azo dyes (Scheme 76a, i).[125] In the Gabriel synthesis of primary amines from alkyl halides, the potassium salt of phthalimide is formed under mild conditions (Scheme 76a, ii) and then attacks the halide in a nucleophilic substitution (Scheme 76a, iii), forming a tailored *N*-substituted phthalimide. The primary amine is then liberated by removal of the phthaloyl group.[116,126] Phthalimide is also used as an amine protecting group, e.g. in

protein synthesis, and is introduced by transamidation (Scheme 76a, iv).[127] Phthaloyl group removal can typically be achieved by hydrazinolysis, acid hydrolysis, further transamidation with methylamine, or reduction and lactonisation of *N*-substituted phthalimides (Scheme 76b, i-iii).[116,127,128] Phthalimides can also undergo aminolysis, wherein the imido ring is opened by nucleophilic attack of an amine (Scheme 76b, iv).[127]
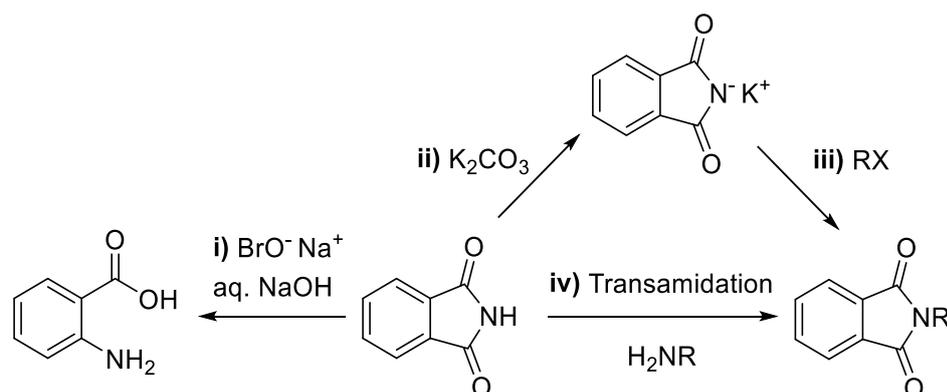
a)   *Reactivity of phthalimide by i) conversion into anthranilic acid, ii) conversion to the potassium salt, iii) subsequent reaction with an alkyl halide as the first step in the Gabriel synthesis, and iv) transamidation*
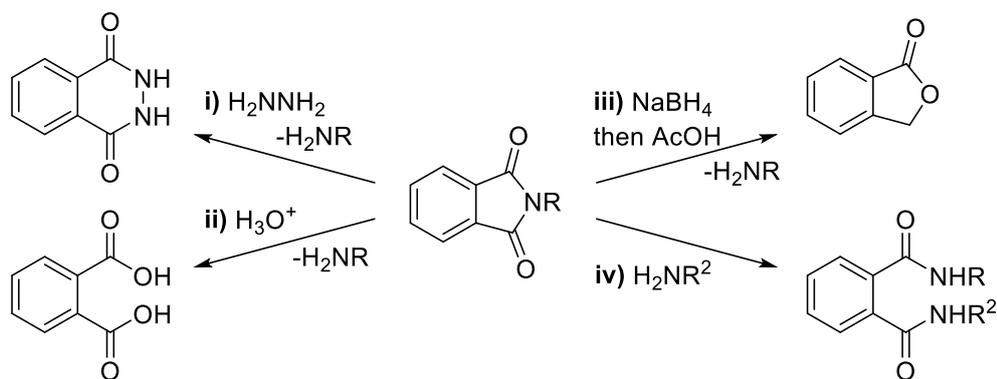
b)   *Reactivity of N-substituted pthalimides by i) hydrazinolysis, ii) acid hydrolysis, iii) reduction and lactonisation, and iv) aminolysis*

Scheme 76. Reactivity of phthalimide compounds

The transamidation of phthalimides and aminolysis of the imide ring present synthetically straightforward routes to the diversification of the products, and were explored with the hydrazone-substituted phthalimides produced in Chapter II.

3.1.3. Commercially Valuable Potential Products

Pharmaceutically relevant structures

Phthalimides and their derivatives, such as isoindolines and indoles, are common pharmaceutical cores. Possibly the most well-known example is thalidomide, a drug still prescribed for the treatment of certain cancers and leprosy complications but which is infamous for the teratogenic effects of its *S*-enantiomer (Figure 21).
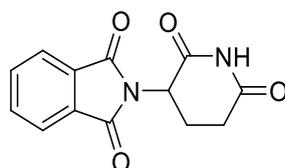
Figure 21. Structure of thalidomide, a phthalimide-based drug

Hydrazone-substituted phthalimides have potential uses as precursors to various pharmaceutically relevant compounds and a few examples are given here, with the relevant structural core highlighted in red (Figure 22). The literature synthesis of **35** from furfural dimethylhydrazone has been described (Scheme 15). Other potential target molecules include: isoindolinone **152**, a poly(ADP-ribose) polymerase inhibitor and potential chemotherapeutic;[129] Citalopram **153**, a widely prescribed antidepressant drug of the selective serotonin reuptake inhibitor (SSRI) class;[130] Lamotrigine **154,** an anticonvulsant used in the treatment of epilepsy and bipolar disorder;[131,132] Conioimide **155**, a marine fungi natural product and anti-inflammatory agent;[133] compound **156**, a tumour necrosis factor-alpha and phosphodiesterase inhibitor with potential use in the treatment of cancer and inflammatory or autoimmune diseases;[134] phthalimide **157**, a protease inhibitor;[135] and isoindolinone **158**, a potential inhibitor of prolyl oligopeptidase and thus treatment of neurodegenerative and psychiatric disorders.[136]
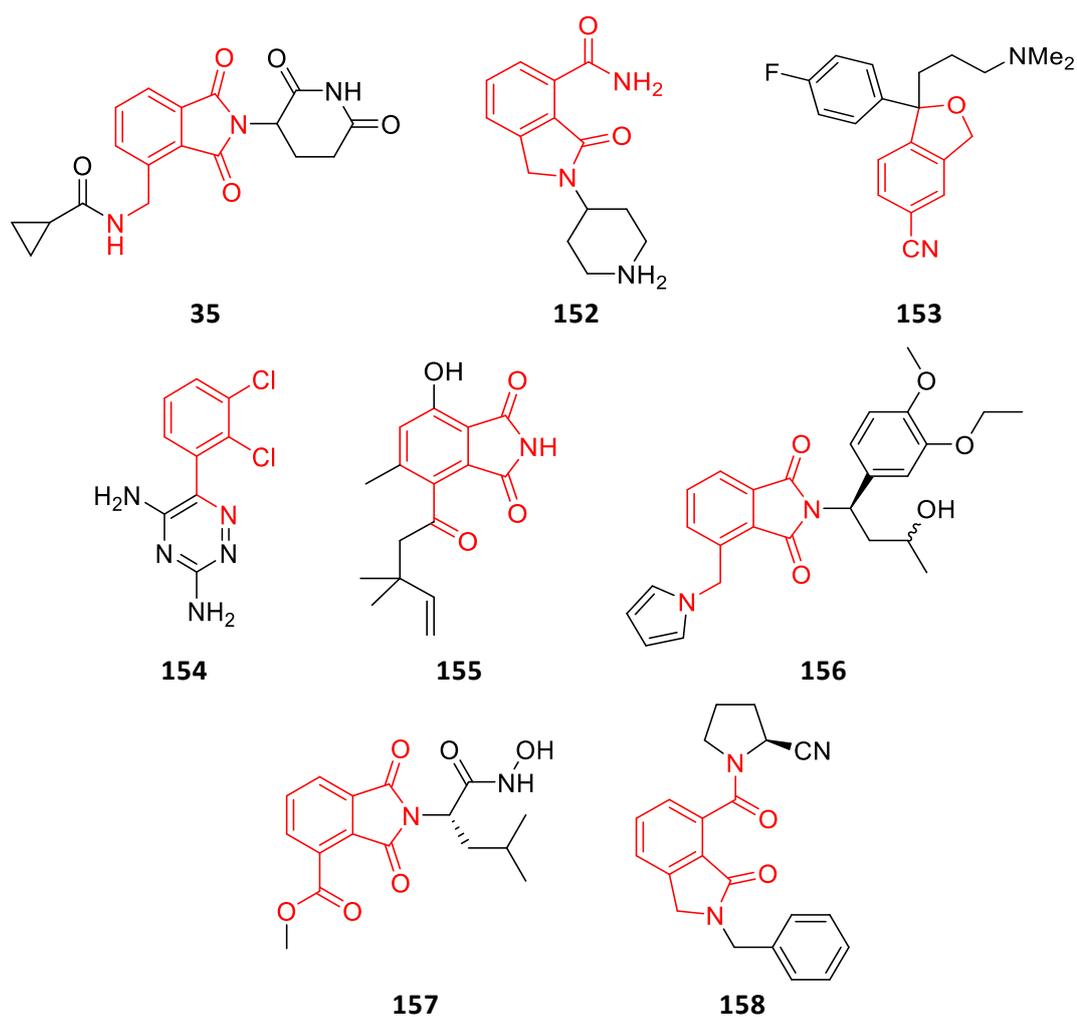
Figure 22. Structures of potential downstream target molecules of furfural derivative Diels-Alder/aromatisation products

Isoindolinone **152**, an inhibitor of poly(ADP-ribose) ribose polymerase PARP1, was selected as a target for synthesis. PARPs are involved in the repair of DNA damaged by radiation therapy or chemotherapeutic agents, and are typically upregulated in tumour cells, diminishing the effectiveness of these therapies.[137] PARP1 inhibition has been shown to increase the efficacy of DNA-damaging antitumor agents in mice[138] and human glioblastoma stem cells.[139]

Phthalocyanines

Phthalimides, phthalamides, phthalic acids and anhydrides, phthalonitriles and cyanobenzamides are all used to produce phthalocyanines, synthetic analogues of porphyrin comprising a ring of four isoindole rings linked through their nitrogen atoms (Scheme 77).
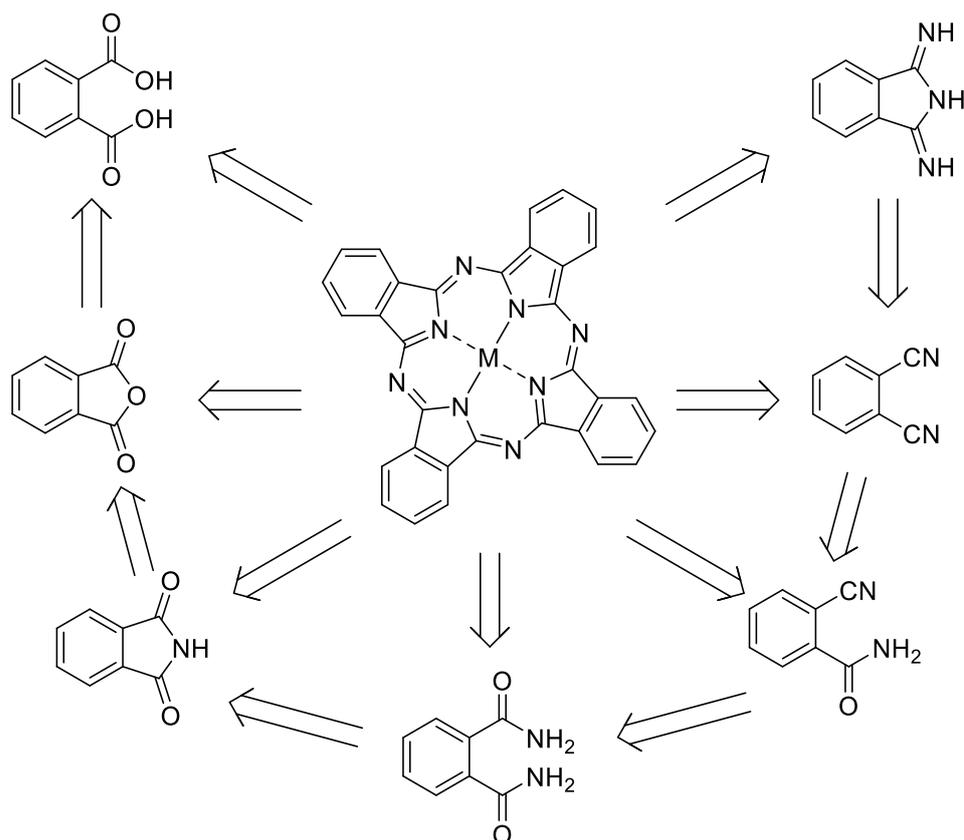
Scheme 77. Synthesis of a phthalocyanine compound from (counter-clockwise) phthalic acid, phthalic anhydride, phthalimide, phthalamide, 2-cyanobenzamide, phthalonitrile, or isoindoline-1,3-diimine

Phthalocyanines make up approximately 25% of global synthetic organic pigment production. Copper phthalocyanine is an extremely deep blue compound that has widespread use in pen inks, with 80,000 tonnes produced per annum.[140,141] Phthalocyanine rings are of increasing interest in the fields of electronics and optoelectronics due to their highly delocalised 18 π-electron systems and the fact that their electronic properties can be easily tailored by the addition of peripheral functional groups or the use of different core metal ions.[142] Over the last ten years, significant research has been carried out on the use of phthalocyanines in optical switching and

limiting devices, sensors, light-emitting devices, low band gap molecular solar cells, optical information recording media, and photodynamic therapy, among others.[141]

Many of the products of Chapter II could act as direct precursors to novel substituted phthalocyanines. In particular, the synthesis of cyanobenzenes from substituted furfurals and acrylonitrile has the potential to give access to a wide range of novel tailored substituted phthalocyanines (Scheme 78). As acrylonitrile can be produced from glycerol,[143,144] these phthalocyanine rings could be significantly biomass-derived.
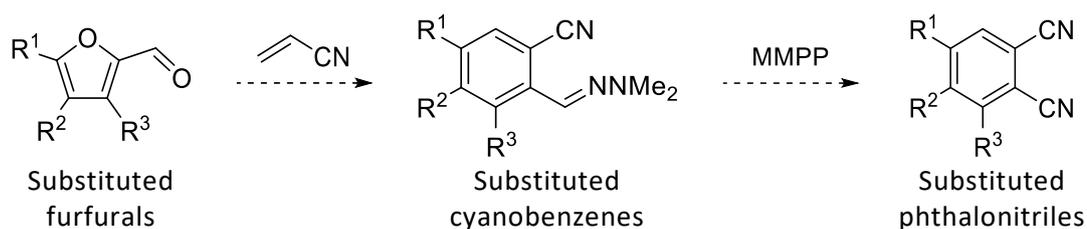
Scheme 78. Synthesis of substituted phthalonitriles from substituted furfurals via a cyanobenzene

3.1.4. Chapter Aims

The aim of the work presented in Chapter III was to further diversify the range of aromatics compounds synthesised primarily from biomass by exploring the reactivity of the compounds produced in Chapter II, introducing new functional groups and adjusting the chemical core to access a wider range of potential downstream products. Some of these products, as described, were pursued and sustainable synthetic routes developed.

3.2. Results and Discussion

3.2.1. Hydrazone Functional Group Conversions

Hydrogenation

A previously described literature reduction of a hydrazone-substituted phthalimide (Scheme 15, step 3) used palladium on carbon (Pd/C) and hydrogen with a methanesulfonic acid (MsOH) catalyst, followed by the addition of hydrochloric acid to give the chloride salt of the amine (Scheme 79).[108]
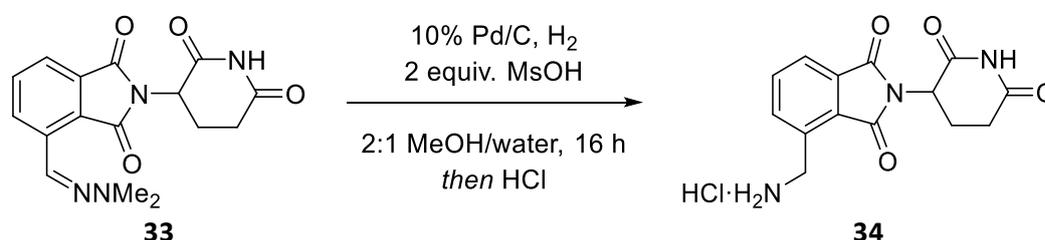
Scheme 79. Literature reduction of a phthalimide hydrazone by hydrogenation to the amine, followed by conversion to the chloride salt using hydrochloric acid

These literature conditions were applied to **31**, but without the addition of hydrochloric acid, in the hope of obtaining the free amine **159**. However, multiple products were observed (Scheme 80).
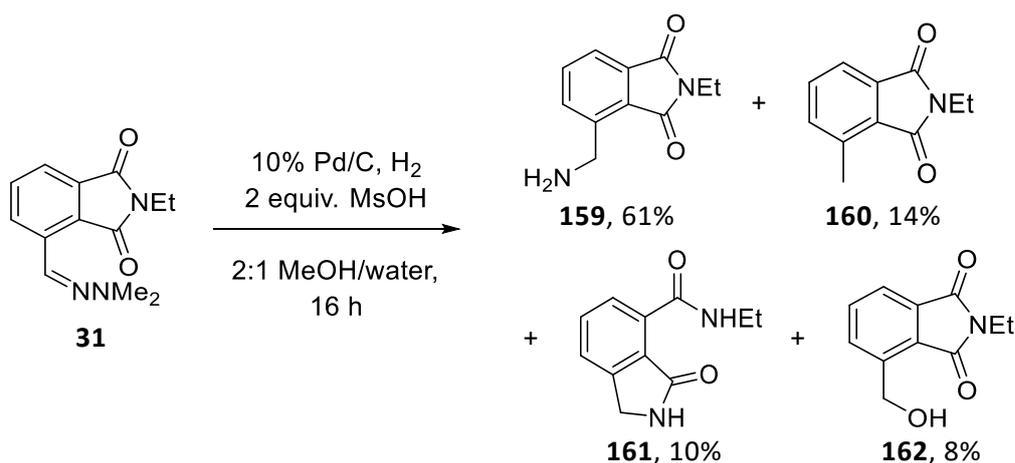
*Scheme 80. Hydrogenation of **31** using literature conditions, giving **159, 160, 161** and **162***

The products were isolated by flash column chromatography and identified as: the fully reduced product **160**, in which the hydrazone was converted into a methyl group, in 14% yield; lactam **161**, in which the free amine had been formed and then opened up the imide ring, in 10% yield; the partially reduced hydrolysis product **162**, with a hydroxyl group in

place of the hydrazone, in 8% yield; and the expected product **159** with an initial yield of 61%. However, it was not possible to fully characterise an isolated sample of **159** as it converted into **161** at room temperature and in the deuterated solvents used.

The formation of **160** and **162** suggested that the hydrolysis of **31** had occurred as a side reaction, with the resulting aldehyde then reduced to the hydroxyl and methyl groups. To avoid this hydrolysis the reaction was repeated without the acid catalyst, but no reaction was observed. The reaction was then carried out in CPME, but after 48 h significant amounts of starting material were still observed, along with the aldehyde and hydroxyl products, suggesting that the trace water in the solvent, reagents, and air were sufficient to cause hydrolysis of the hydrazone. Attempting the reaction without an acid catalyst in CPME resulted in no reaction.

Before carrying out the reaction under anhydrous conditions, an alternative approach using milder acids was investigated. Acetic and formic acid were used and both resulted in the full conversion of hydrazone **31** to the amine **159** and lactam **161** only, successfully avoiding hydrolysis of the hydrazone group. Rather than isolating **159** and **161**, the product mixtures were heated at 80 °C to encourage the conversion of amine **159** to the lactam **161**. However, this seemed to cause undesirable side-reactions, and isolated yields for **161** were 31% with acetic acid and 39% with formic acid.

The use of hydrochloric acid to obtain the hydrochloride salt of **159** was then attempted, using formic acid in place of methanesulfonic acid. However, the sole product obtained was the lactam, **161**, in 64% yield (Scheme 81).

$$10\% \text{ Pd/C, H}_2$$
$$2 \text{ equiv. HCO}_2\text{H}$$

2:1 MeOH/water,
16 h, *then* HCl

31   **161**, 64%
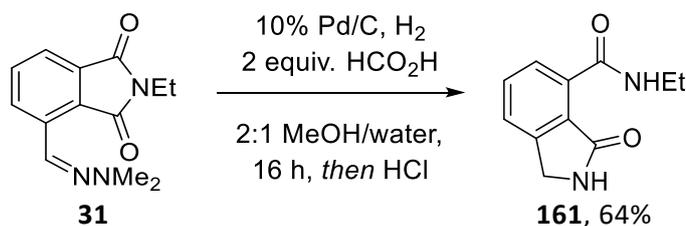
*Scheme 81. Hydrogenation of **31** under literature conditions, including addition of hydrochloric acid, producing the lactam product, **161***

Addition of the hydrochloric acid during the hydrogenation was attempted, but this resulted in hydrolysis of the hydrazone. Next, the reaction was carried out without hydrochloric acid and the resulting product mixture of amine **159** and lactam **161** was separated by flash column chromatograhy. Hydrochloric acid was added to the isolated amine **159** in an attempt to generate the amine salt, but this resulted in full conversion of

159 to lactam **161**. The literature precedent of generating the amine salt *ortho* to an imide was not investigated further, but an *in situ* protection of the amine with a tert-butyloxycarbonyl (Boc) group was successfully carried out, generating the Boc-protected amine, **163**, in 77% isolated yield (Scheme 82).
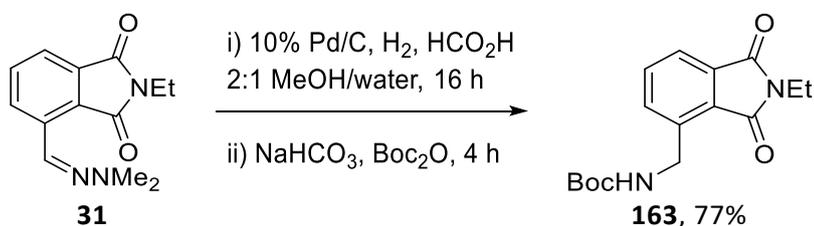
*Scheme 82. Hydrogenation and subsequent Boc-protection of **31**, to give **163***

As isoindolinones are common pharmaceutical cores, synthesis of lactam **161** was optimised by varying the hydrogenation acid catalyst and isolation method. A 92% yield was achieved by the use of formic acid and isolation by removal of palladium on carbon by celite followed by organic extraction, then addition of sulphuric acid to the filtrate to promote lactam formation. The lactam precipitated out and was collected by filtration in high purity. This procedure was then applied to phthalimides **56** and **78** (Scheme 83, Table 23).
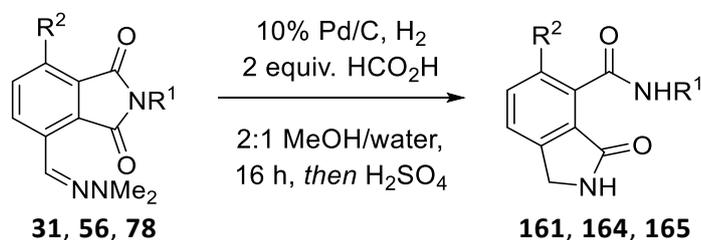
*Scheme 83. Hydrogenation and intramolecular transamidation of phthalimides **31**, **56** and **78**, to give the corresponding lactams*

STARTING MATERIAL	R^1	R^2	PRODUCT	YIELD
31	Et	H	**161**	92%
56	H	H	**164**	98%
78	Et	CH_2OEt	**165**	62%

*Table 23. Results of the hydrogenation and intramolecular transamidation of phthalimides **31**, **56** and **78**, to give the corresponding lactams*

Compound **56** reacted to give **164** in near-quantitative isolated yield, while the bulkier **78** gave **165** in a lower yield of 62%. Phthalimide **166** was synthesised from HMF **19** and maleimide in 87% yield using the previously developed Diels-Alder/aromatisation reaction sequence (Scheme 84).
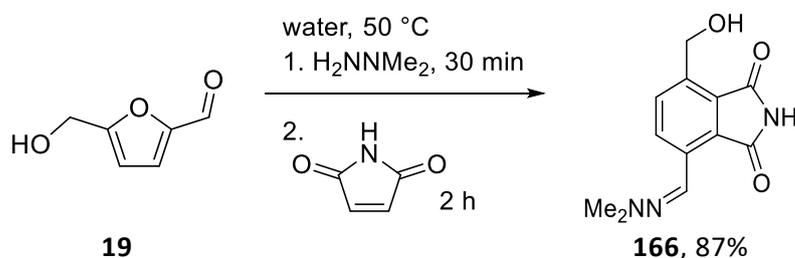
Scheme 84. One-pot DA/aromatisation synthesis of **166** from HMF and maleimide

Phthalimides **75** and **166** were selected for hydrogenation because their expected products, **167** and **169**, have a hydroxymethyl group *ortho* to the newly formed non-cyclic amide, and it was hypothesised that they could undergo further intramolecular cyclisation to give **168**, another potential pharmacore (Scheme 85).
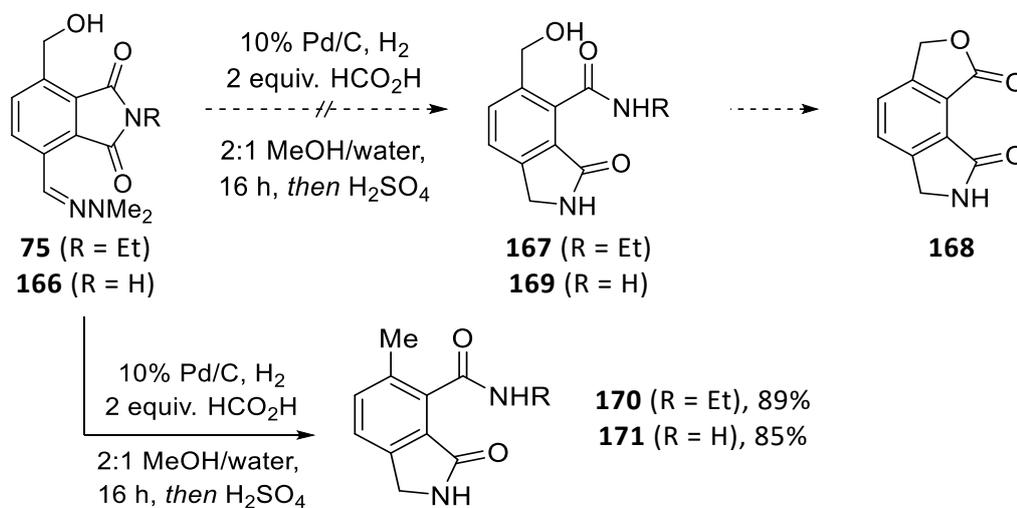
Scheme 85. Attempted synthesis of **168** via **167** and **169** by hydrogenation and intramolecular transamidation of **75** and **166**, and actual lactam products **170** and **171**

However, the expected products **167** and **169** were not formed due to the hydrogenation conditions reducing the hydroxymethyl group to a methyl group, giving lactams **170** and **171** in 89% and 85% isolated yields, respectively. The 'extended' hydrazone **88** was also reacted under these conditions and the 7-membered lactam ring product **172** was successfully formed in 45% yield (Scheme 86).

88   **172**, 45%
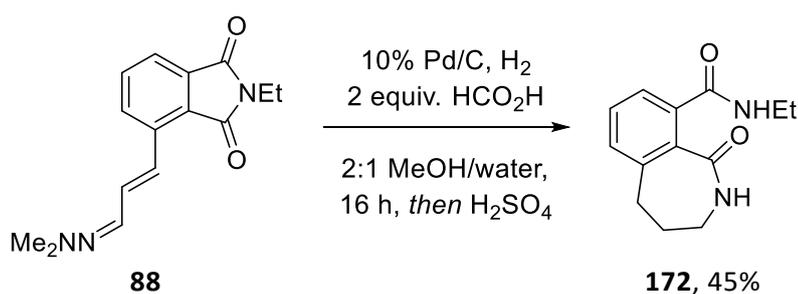
Scheme 86. Hydrogenation and intramolecular transamidation of a series of hydrazone phthalimides to give the corresponding lactam

This reduced yield relative to the reaction of **31** could be due to the kinetically less favourable formation of a seven-membered ring, or reduction of the alkene before the hydrazone, which would disrupt the conjugation and could make the hydrazone less susceptible to reduction.

With the Boc-protected amine and a range of lactams synthesised in high yield, focus was then shifted to other hydrazone funtional group conversions.

Hydrolysis to the aldehyde

In previous experiments, hydrazone hydrolysis was observed as a side-reaction when using scandium triflate (Sc(OTf)$_3$), Brønsted acids, and under aqueous microwave conditions. Compound **31** was therefore stirred with 40 mol% scandium triflate but no reaction was observed (Scheme 87).

31   **173**
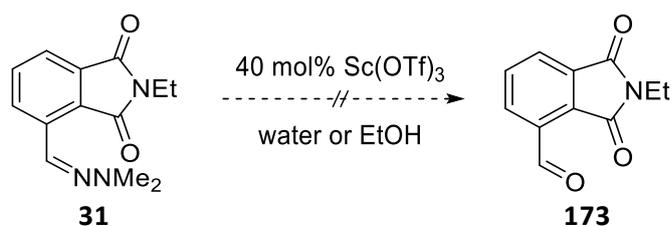
Scheme 87. Attempted conversion of 31 into 55 by scandium triflate catalysed hydrazone hydrolysis

Hydrochloric acid was then used with a solvent mixture of 1:1 water/acetone, as this provided excess H$_2$O to drive the reaction while still solubilising starting materials **31** and **56** (Scheme 88).
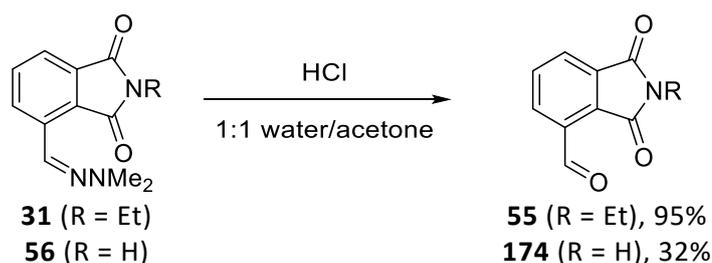
Scheme 88. Brønsted acid-catalysed hydrazone hydrolysis of 31 and 56 into 55 and 174

Aldehyde **55** was obtained from **31** in a favourable 95% yield, while the reaction with **56** gave **174** in only 32% yield after separation from a mixture of unidentified side products. As **174** is a desirable intermediate to a chosen target compound (Section 3.1.3.), optimisation of this reaction was pursued.

Acidic resins are an attractive, sustainable option for acid catalysis, as they are easy to remove for a facile workup, and also reusable. Amberlyst® 15, a sulfonic acid resin, was therefore investigated, using a range of solvents (Scheme 89, Table 24).
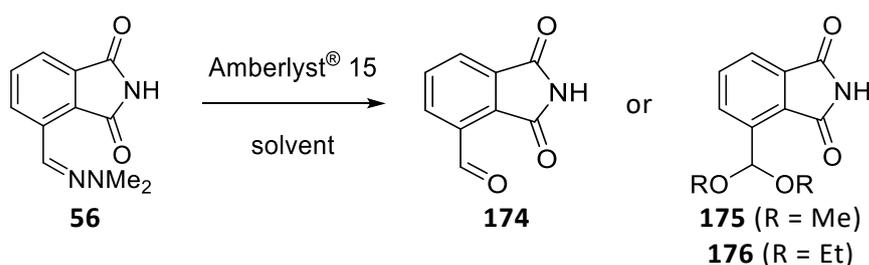
Scheme 89. Amberlyst® 15 catalysed hydrazone hydrolysis of 56 in a range of solvents

SOLVENT	WATER CONTENT	TEMP.	TIME /h	PRODUCT	YIELD
methanol	4%	RT	4	**175**	86%
isopropanol	4%	RT	4	No reaction	
EtOAc	4%	RT	4	**174**	61%*
EtOAc	4%	RT	18	**174**	63%*
EtOAc	4%	50 °C	24	**174**	45%*
				176	39%*
acetone	4%	RT	18	**174**	77%*
acetone	50%	RT	18	**174**	63%*
acetone	0%	RT	3	**174**	97%

*Calculated yield, determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard

Table 24. Results of Amberlyst® 15 catalysed hydrazone hydrolysis of 56 in a range of solvents

Of the reactions in methanol, isopropanol, and ethyl acetate, only ethyl acetate resulted in formation of **174**, in 61% calculated yield. Carrying the reaction out in methanol

resulted in formation of the dimethoxy acetal **175** in 86% isolated yield, while no reaction occurred in isopropanol. In an attempt to improve formation of **174** in ethyl acetate, the reaction was left for 18 hours (c.f. 4 h), but no significant increase in yield was observed. The reaction was then carried out at the higher temperature of 50 °C (c.f. RT), which gave **174** in a reduced calculated yield of 45%, and an alternate product believed to be the diethoxy acetal product **176** in 39% calculated yield. The acetal was identified from ^{1}H NMR spectroscopic analysis of the crude reaction mixture and not isolated or further characterised. The isolated methoxy acetal **175** was successfully converted into aldehyde **174** in 97% yield by stirring in acetone with HCl for two hours (Scheme 90), giving a two-step yield of 83% from the hydrazone **56**.
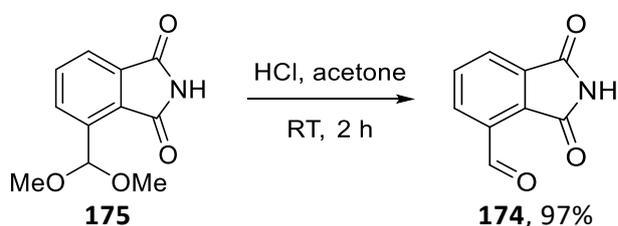
*Scheme 90. Acid catalysed conversion of acetal **175** to aldehyde **174***

Reaction of hydrazone **56** with Amberlyst® 15 in 4% water in acetone gave **174** in 77% calculated yield, with no acetal formation (Table 24). It seems likely that the byproduct *N,N*-dimethylhydrazine can react with the acetone to give the corresponding hydrazone and water. This would create a system in equilibrium in which free *N,N*-dimethylhydrazine is significantly more likely to react with the excess acetone than with aldehyde **174**, thus driving the hydrolysis of **56**.

Increasing the water content from 4% to 50% gave a lower yield of 63%, while carrying out the reaction with no added water led to an isolated yield of 97%. This suggests that sufficient water for the reaction is provided by a combination of background moisture in the solvent and atmosphere and, potentially, the water produced by the reaction between the *N,N*-dimethylhydrazine and acetone. This is consistent with previous results showing partial hydrolysis of the hydrazone in the absence of added water.

The use of Amberlyst® 15 was then applied to the hydrazone hydrolysis of **31**, giving a 94% yield of **55** (Scheme 91). While this is not an improvement in yield compared to carrying this reaction out with hydrochloric acid (Scheme 88), the sustainability advantages of resins make this route preferable.
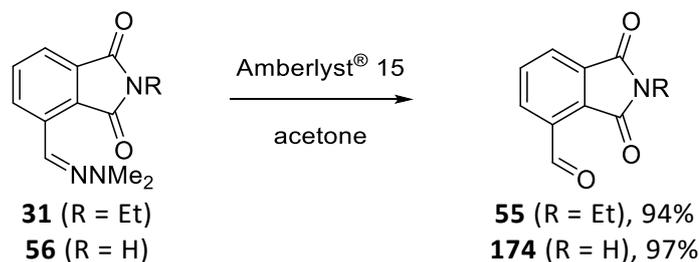
Scheme 91. Amberlyst® 15 catalysed hydrazone hydrolysis of **31** and **56** into **55** and **174** in acetone

This procedure was also applied to **88**, the 'extended' hydrazone, to form enone **177** (Scheme 92) in 91% yield. This enone grants an additional route of potential reactivity, for example via conjugate addition, cycloadditions, or reduction and cyclisation to a 7-membered lactone ring.
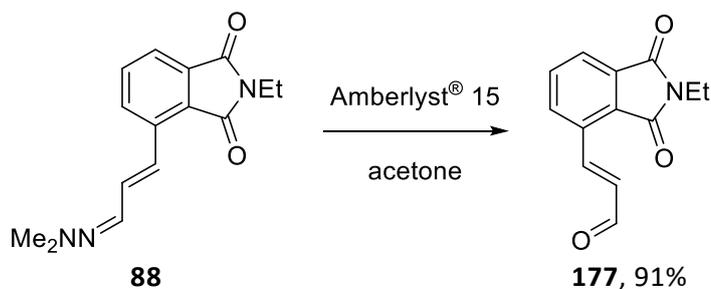
Scheme 92. Amberlyst® 15 catalysed hydrazone hydrolysis of **88** into **177** in acetone

The hydrolysis of biomass-derived aromatic hydrazone products was thus achieved in near-quantitative yields, allowing potential access to a range of downstream products via the flexible reactivity of the resulting aldehyde.

Oxidation to the nitrile

A literature procedure for the oxidation of hydrazones to nitriles using magnesium monoperoxyphthalate (MMPP) was applied to **31**, and **178** was obtained in 97% yield (Scheme 93).[145]
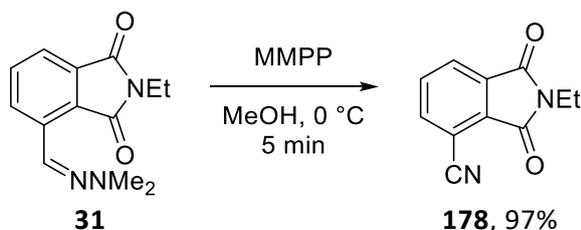
Scheme 93. Conversion of **31** into **178** by oxidation with MMPP

This procedure was then applied to **121** and **32** to investigate the synthesis of biomass-derived phthalonitrile **179** and benzene-1,2,3-tricarbonitrile **180**, which are commonly used industrial compounds. The expected products were obtained in near-quantitative yields (Scheme 94).

121 (R = H)   **179** (R = H), 99%
32 (R = CN)   **180** (R = CN), 98%
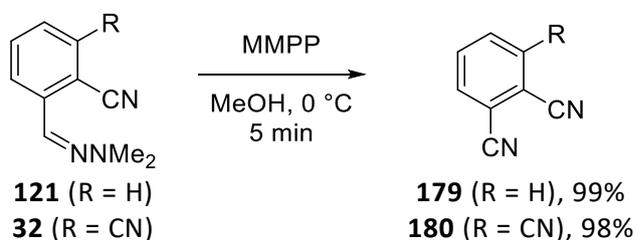
Scheme 94. Conversion of 121 and 32 into phthalonitrile 179 and benzene-1,2,3-tricarbonitrile 180, respectively, by oxidation with MMPP

This demonstrates promise for the previously proposed strategy for the synthesis of substituted phthalonitriles from biomass-derived furfurals and acrylonitrile (Scheme 78).

This oxidation procedure was also applied to **88**, giving **181**, a substituted acrylonitrile, in 88% yield (Scheme 95). This type of novel aromatic-substituted acrylonitrile has potential use in, for example, cycloadditions, conjugate additions, and in forming substituted polyacrylonitriles.

88   **181**, 93%
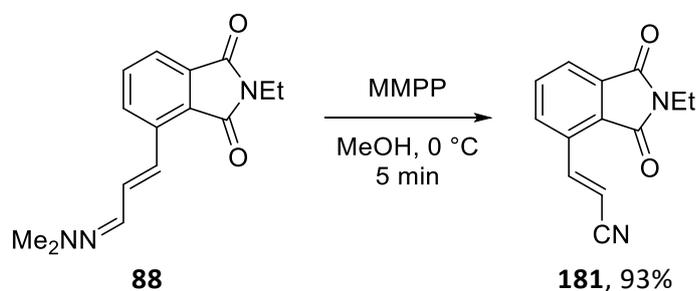
Scheme 95. Conversion of 88 into 181 by oxidation with MMPP

[3+2] cycloadditions of mono-substituted hydrazones

Compound **38** has been reported to act as a 1,3-dipole in [3+2] cycloadditions at the hydrazone group upon refluxing in benzene or an acrylates (Scheme 19). More sustainable conditions for reactions of this type were sought, with the aim of developing optimised conditions that could be applied to a wide range of dipolarophiles, thus increasing the diversity of these aromatic products.

Initially, **38** was heated at reflux with 1.2 equivalents of *N*-ethylmaleimide for 48 hours in a variety of different solvents or solvent mixtures (Scheme 96, Table 25). Several were

selected for their favourable sustainability profile, such as CPME, 2-MeTHF, DMC, propionitrile (EtCN), and ethyl lactate. Yields of 41 were calculated by quantitiative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

The coupling between the ^{1}H NMR peaks of the two stereocentres generated was 11.1 Hz, indicating relative *cis* stereochemistry, and it is likely that 41 exists as a mixture of the (*R*,*R*) and (*S*,*S*) enantiomers.

38   **41**
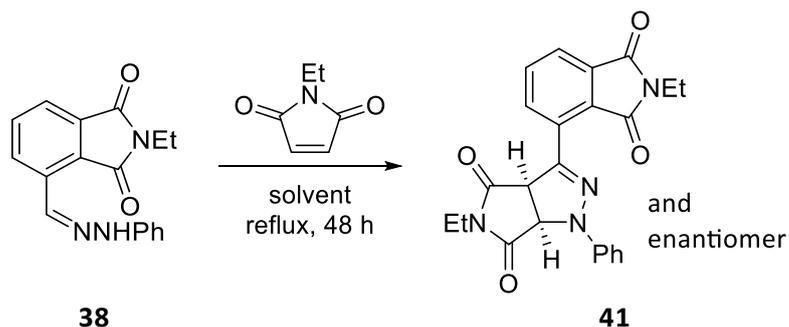
*Scheme 96. The [3+2] cycloaddition between **38** and N-ethylmaleimide to produce 41*

Solvent	T /°C	Yield*	Solvent	T /°C	Yield*
toluene	111	48%	methanol	65	51%
CPME	106	40%	ethanol	78	25%
THF	66	21%	DMC	90	27%
2-MeTHF	80	15%	EtCN	98	23%
n-heptane	98	6%	ethyl lactate	155	31%
water	100	/	1-butanol	118	69%
MeCN	82	65%	1-pentanol	139	55%
1:1 MeCN/water	77	18%	2-methyl-1-butanol	128	61%
2:1 MeCN/water	75	71%			

*Calculated yield, determined by quantitative ^{1}H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

*Table 25. Results of the [3+2] cycloaddition between **38** and N-ethylmaleimide to produce 41*

Calculated yields of up to 71% were obtained, with the more polar solvents appearing to give higher yields. No correlation between reflux temperature and yield was found (R^2 = 0.0085). The highest yielding solvents/solvent mixtures were 2:1 acetonitrile/water, 1-butanol and acetonitrile, and these were selected for reaction scale-up. Ethyl lactate was also selected despite its low yield of 31% as isolation issues during workup may have caused the loss of product. The reactions in these four solvents were scaled up five-fold (Scheme 97, Table 26).
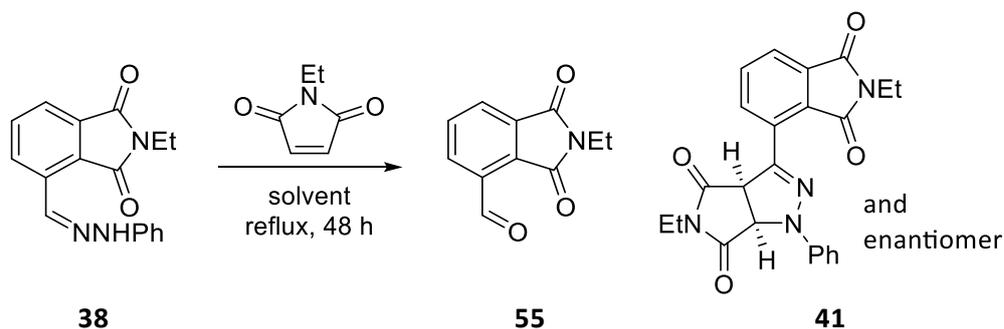
Scheme 97. Scaled up [3+2] cycloaddition reactions of **38** and N-ethylmaleimide to produce 41

SOLVENT	REMAINING 38*	YIELD OF 55*	YIELD OF 41*
2:1 MeCN/water	6%	60%	29%
1-butanol	56%	16%	23%
MeCN†	/	83%	9%
ethyl lactate	/	89%	/

Calculated yield, determined by quantitative ¹H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard. †This reaction carried out for 72 hours rather than 48 hours

Table 26. Results of the scaled up [3+2] cycloaddition reactions of **38** and N-ethylmaleimide to produce 41

The desired product **41** was produced in no higher than 29% yield, and each reaction mixture contained significant quantities of the aldehyde **55**. Indeed, this was the only product of the reaction in ethyl lactate, in 89% calculated yield.

It was proposed that the difference observed between the small and large scale reactions could be due to the former being performed in small, sealed carousel reaction tubes, while the scaled up reactions had been open to the air. To investigate this, a scaled up reaction was carried out in 2:1 acetonitrile/water in a sealed reaction tube. However, results were similar to those of the unsealed reaction, with calculated yields for **55** and **41** of 56% and 33%, respectively (c.f. 60% and 29%). The rationale behind the change in reactivity upon scale-up of the reactions was unclear. Further experiments under these conditions were not investigated, however, as concurrent experiments using microwave conditions were more promising.

Solvent mixtures of acetonitrile and water were used in these microwave reactions due to water having excellent absorption of standard microwave wavelengths and acetonitrile previously performing well in the synthesis of **41**. Compound **38** was treated with

microwaves at 200 °C in different combinations of the two solvents, including a reaction in acetonitrile only, to investigate what effect the presence of water has (Scheme 98, Table 27).
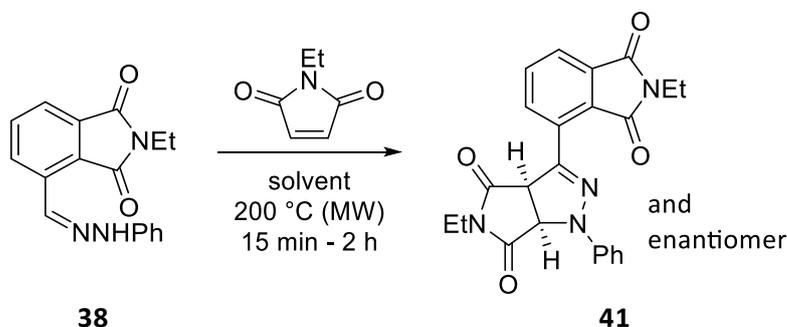
Scheme 98. Screening microwave conditions for the [3+2] cycloaddition between the hydrazone of **38** and N-ethylmaleimide, to produce 41

SOLVENT	TIME	MALEIMIDE EQUIV.	YIELD*
2:1 MeCN/water	15 min	2	42%
1:9 MeCN/water	15 min	2	36%
MeCN	15 min	2	25%
2:1 MeCN/water	2 h	2	41%
1:9 MeCN/water	2 h	2	40%
MeCN	2 h	2	21%
2:1 MeCN/water	15 min	10	73%
MeCN	15 min	10	58%

*Calculated yield, determined by quantitative ^1H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard.

Table 27. Results of screening microwave conditions for the [3+2] cycloaddition between the hydrazone of **38** and N-ethylmaleimide, to produce 41

The reactions were initially carried out for fifteen minutes, giving yields of 25-42%, and a correlation between water content and yield was observed. No decomposition was observed, so the same high temperature was used in future experiments. Carrying out the reactions for 2 hours did not improve yields, but increasing the number of equivalents of N-ethylmaleimide from two to ten increased the calculated yield from 42% to 73%. While this is a significant excess of N-ethylmaleimide, it was possible to recycle most of the unreacted material by collecting the precipitated product **41** using filtration and then performing an organic extraction on the filtrate. In one such reaction, 8.7 equivalents of N-ethylmaleimide was isolated, accounting for 94% of the unreacted reagent.

As the reaction is likely in equilibrium between the cycloaddition and reverse cycloaddition reactions, a lower microwave temperature could perhaps favour the forward reaction. However, due to time constraints, this was not pursued. The use of other dipolarophiles in this cycloaddition could be useful in rapidly producing a range of novel compounds, with use in, for example, generating compound libraries for drug screening. Alkynes have been known to partake in similar [3+2] cycloaddition reactions to give pyrazole rings, and acrylates to give tri-substituted dihydropyrazoles (Scheme 19).

3.2.2. Transamidation Reactions of the Imide Ring

In Section 2.1.3., alternate *N*-substituted maleimides were used to tailor the group on the imide nitrogen of the aromatic product. Such compounds can also be generated by transamidation with primary amines (Scheme 76, ii). Transamidation of the phthalimides produced in Chapter II was explored, initially using *n*-butylamine and benzylamine (Scheme 99). The reaction was carried out either by dissolving **56** in an excess of amine, or by using one of two literature procedures using two different transamidation catalysts; boric acid ($B(OH)_3$) or iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$) (Table 28).[146,147]
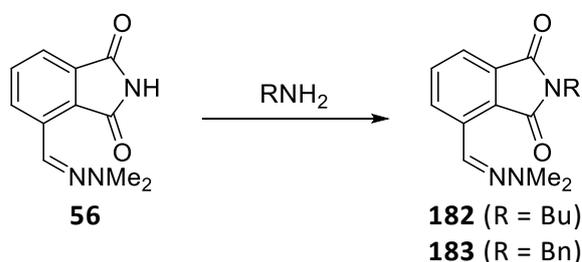
Scheme 99. The transamidation between **56** and n-butylamine or benzylamine

ENTRY	CATALYST	SOLVENT	T /°C	t /h	R	PRODUCT	YIELD
1	$B(OH)_3$	/	50	16	^nBu	No reaction	
2	$B(OH)_3$	/	50	16	Bn	No reaction	
3	$B(OH)_3$	toluene/ 1,4-dioxane	100	16	^nBu	**182**	40%
4	$B(OH)_3$	toluene/ 1,4-dioxane	100	16	Bn	**183**	31%
5	$Fe(NO_3)_3$	toluene	reflux	16	^nBu	**182**	68%†
6	$Fe(NO_3)_3$	toluene	reflux	16	Bn	**183**	35%†
7	/	excess amine	RT	16	^nBu	**184*** **185***	66% 27%

ENTRY	CATALYST	SOLVENT	T /°C	t /h	R	PRODUCT	YIELD
8	$B(OH)_3$	toluene/ 1,4-dioxane	100	72	^nBu	**182**	92%
9	$B(OH)_3$	toluene/ 1,4-dioxane	100	72	Bn	**183**	62%
10	$Fe(NO_3)_3$	toluene	reflux	72	^nBu	**182**	63%†
11	$Fe(NO_3)_3$	toluene	reflux	72	Bn	**183**	37%†
12	$B(OH)_3$	CH_2Cl_2	40	72	^nBu	**182**	58%
13	$B(OH)_3$	CH_2Cl_2	40	72	Bn	**183**	29%

†Calculated yield, determined by quantitative ¹H NMR spectroscopic analysis using 1,3,5-trimethoxybenzene as an internal standard. *Not expected product; alternative imide ring opened diamide product obtained instead, see Figure 23.

Table 28. Results of the transamidation between 56 and n-butylamine or benzylamine

Initially, a general, solvent-free boric acid literature procedure was used,[146] but no reaction was observed (entries 1 & 2). A phthalimide-specific boric acid procedure was then employed, using a 1:1 toluene/1,4-dioxane solvent mixture at 100 °C, and this gave **182** and **183** in isolated yields of 40% and 31%, respectively (entries 3 & 4). The iron(III) nitrate literature procedure, which involves heating to reflux in toluene, resulted in calculated yields of 68% and 35%, respectively (entries 5 & 6). Dissolving **56** in an excess of butylamine as a solvent and stirring at room temperature for 16 h resulted in a mixture of alternative products **184** and **185** in 66% and 27% yields, respectively (entry 7), illustrating opening of the imide ring with either single or double transamidation (Figure 23).
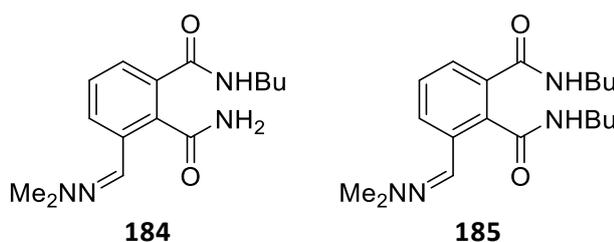
Figure 23. Structures of 184 and 185, products of stirring 56 in n-butylamine

To improve the yields of **182** and **183**, the boric acid and iron(III) nitrate procedures were carried out for 72 h. In the case of boric acid, this led to improved yields of 92% and 62% for **182** and **183**, respectively (entries 8 & 9), while the iron(III) nitrate reactions showed no significant change in yield (entries 10 & 11). Finally, due to the limited solubility of the starting material **56** in 1:1 toluene/1,4-dioxane, the boric acid procedure was repeated in dichloromethane to explore whether improved solubility could enhance reactivity. Reduced yields of 58% and 29% for **182** and **183** were observed (entries 12 & 13), possibly

due to the lower solvent boiling point limiting reaction temperature. The favoured boric acid procedure was then applied to a range of other amines (Scheme 100, Table 29).
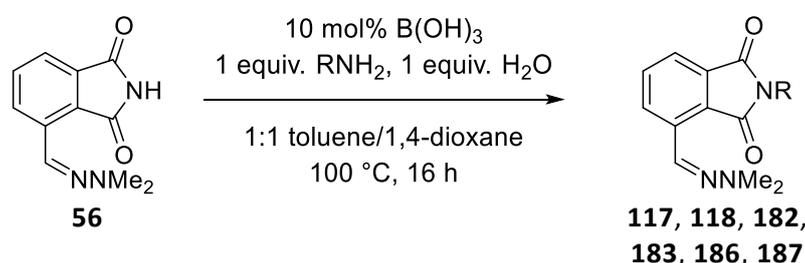
Scheme 100. Screening amines in the boric acid catalysed transamidation of 56

AMINE	R	PRODUCT	YIELD
n-butylamine	^{n}Bu	**182**	92%
benzylamine	Bn	**183**	62%
isopropylamine	^{i}Pr	no conversion	N/A
allylamine		no conversion	N/A
aniline	Ph	side reactions	N/A
cyclopropylamine		**117**	41%
4-methylbenzylamine		**118**	75%
3-morpholinopropan-1-amine		**186**	78%
sec-butylamine	^{s}Bu	**187**	57%

Table 29. Results of screening amines in the boric acid catalysed transamidation of 56

No reaction was observed with isopropylamine or allylamine, perhaps due to their volatility at 100 °C causing them to become sequestered in the gaseous phase. Reaction with aniline produced a complex mixture of side-reaction products. The use of 3-morpholinopropan-1-amine, 4-methylbenzylamine, *sec*-butylamine or cyclopropylamine as the amine, however, resulted in the expected *N*-substituted phthalimide products 117, 118, 186 and 187 in 41-78% isolated yields.

As these boric acid transamidation reactions were water-tolerant and the synthesis of their starting material, **56**, is conducted in water, a reaction sequence without intermediary workup was envisaged. Carrying the two reactions out in a single pot

resulted in a mixture of side reaction products, perhaps due to reactions of the excess maleimide and *N,N*-dimethylhydrazine with the transamidation reagents. The synthesis of **56** was then carried out with collection of the product by filtration, but without washing or drying. This wet, crude product was directly used in the transamidation reaction with n-butylamine, giving the product, **182**, in 78% yield from furfural. This is comparable to the previous overall yield of 83% from furfural over two reaction pots.

The synthesis of diamides such as **184** and **185** (Figure 23) was then considered. Diamides **184** and **185** were formed from *N*-unsubstituted phthalimide **56**, but *N*-ethyl-substituted phthalimide **31** was chosen for further investigation, in the hope that its alkyl substituted imide nitrogen centre would act as a better leaving group.   A series of amines were investigated and used in excess as the solvent. In each reaction, the amine was added to **31** until the starting material was fully dissolved, and then the mixture was stirred at room temperature for 3 hours (Scheme 101, Table 30). Water was then added, causing the products to precipitate, and they were collected by filtration.
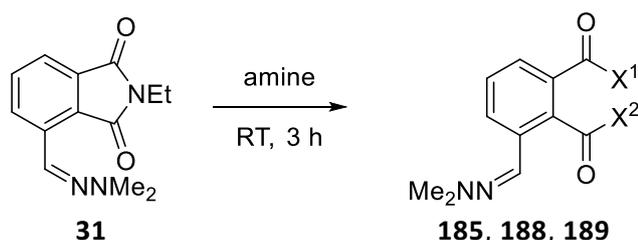
Scheme 101. Reacting **31** with an excess of a series of amines

Amine	Product	X^1	X^2	Yield
n-butylamine	**185**	NH^nBu	NH^nBu	96%
benzylamine	**188**	NHBn	NHBn	75%
isopropylamine	**189**	NH^iPr	NHEt	87%
pyrrolidine	No reaction	/	/	/
morpholine	No reaction	/	/	/

Table 30. Results of reacting **31** with an excess of a series of amines

Dissolving **31** in *n*-butylamine or benzylamine gave the double transamidation diamide product, **185** or **188**, in 96% or 75% yield, respectively. Using isopropylamine resulted in the single transamidation diamide **189** in 87% yield, possibly due to the increased steric bulk of an α-branched primary amine preventing a second transamidation. Only a single regioisomer was obtained, with the isopropyl amide *meta* to the hydrazone, due to this

carbonyl being more sterically accessible. Pyrrolidine and morpholine did not react with **31**, suggesting that secondary amines are too hindered for this transamidation reaction.

While investigating facile isolation methods, an ammonium chloride solution was used in place of water, to quench and cause precipitation. This resulted in the ring closure of the diamide into an imide, for example in the synthesis of phthalimide **182** from **31** via diamide **185**, in 89% yield (Scheme 102).
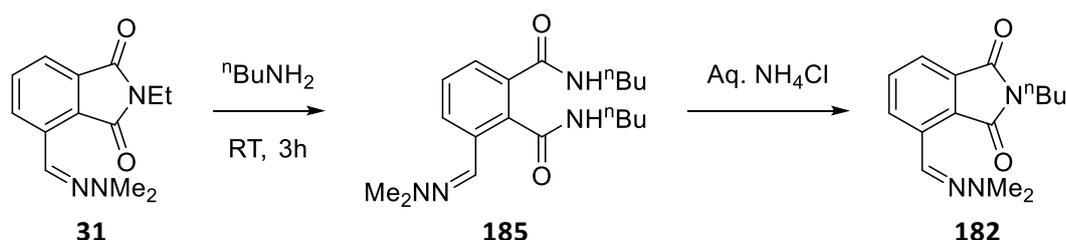
*Scheme 102. Transamidation of **31** with n-butylamine followed by acid-catalysed ring closure to give **182** via **185***

This provided an alternative to the boric acid synthesis that does not require a catalyst or organic solvents, though an excess of the amine as solvent is required. It is possible, however, that the excess amine could be re-isolated and recycled from the aqueous filtrate solution. This approach was then applied to a selection of other amines (Scheme 103, Table 31).
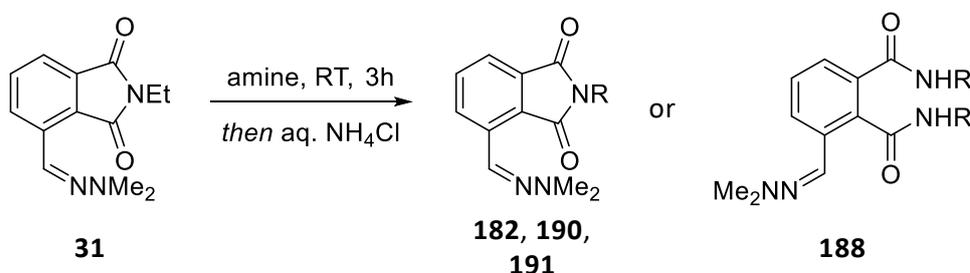
*Scheme 103. Screening amines in the catalysis-free transamidation of **56** in excess amine*

AMINE	R	PRODUCT	YIELD
n-butylamine	^{n}Bu	**182**	89%
allylamine		**190**	93%
isopropylamine	^{i}Pr	**191**	47%
benzylamine	Bn	**188**	62%

*Table 31. Results of screening amines in the catalysis-free transamidation of **56** in excess amine*

The use of allylamine gave phthalimide **190** in 93% yield. This product could not be synthesised by the boric acid procedure due to volatility of the amine. Isopropylamine,

however, gave a mixture of starting material **31** and phthalimide product **191**, which was
isolated by flash column chromatography in 47% yield. It was thought that the *N*-ethyl-*N*-isopropyl diamide was formed, and then underwent ring closure to give both **31** and **191**,
depending on which imide's nitrogen centre acted as the nucleophile and which as the
leaving group. The use of benzylamine gave the diamide **188**, probably due to steric bulk
blocking ring closure.

A range of phthalamides and phthalimides were thus synthesised by transamidation of the
unsubstituted or ethyl-susbstituted phthalimide, using either boric acid catalysis or an
excess of amine.

3.2.3. Synthesis of a PARP Inhibitor

As discussed in Section 3.1.3., isoindolinone **152** is a pharmaceutically relevant poly(ADP-ribose) polymerase inhibitor and was selected as a target molecule to demonstrate the
synthetic utility of furfural-derived aromatic compounds. The synthetic route proposed
involves the reductive amination of the aldehyde-substituted phthalimide **192** (Section
3.2.1.) with commercially available amine **192**, followed by acid-catalysed intramolecular
transamidation to form the lactam ring and then deprotection of the piperidynl nitrogen
group to give **152** (Scheme 101).
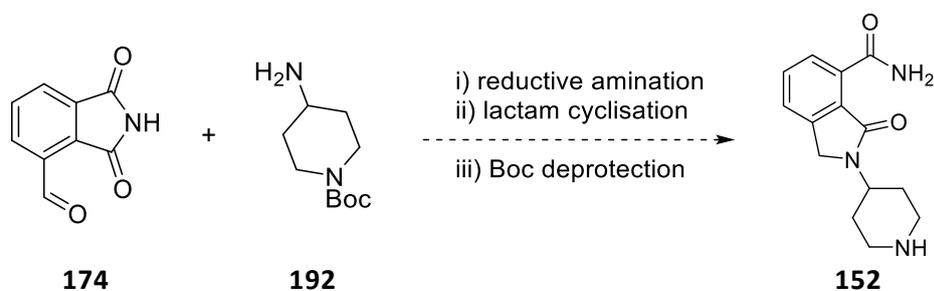
Scheme 104. Proposed strategy for the synthesis of **152** from **56** and **192** by reductive amination
and intramolecular transamidation cyclisation/lactam formation, followed by Boc deprotection

The reductive aminiation between **174** and **192** was initially attempted in methanol with
palladium on carbon (Pd/C) (Scheme 105). Deuterated methanol was used so that the
imine formation could be followed *in situ* by ^{1}H NMR spectroscopic analysis.
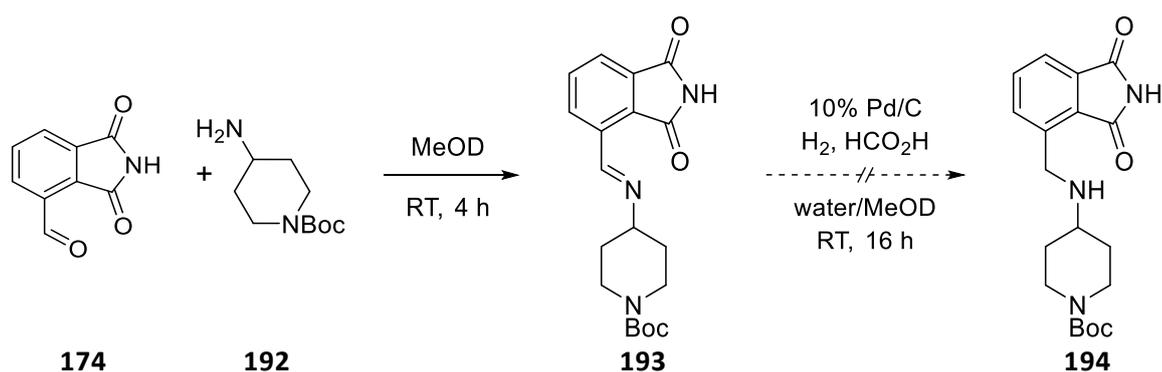
Scheme 105. Attempted synthesis of 194 from 174 and 192 by uncatalysed imine formation followed by hydrogenation with palladium on carbon

The *in situ* analysis of the reaction mixture showed that imine formation proceeded to approximately 50% completion within one hour, with no further reaction in the following three hours. Hydrogenation was attempted *in situ* using the previously optimised hydrazone hydrogenation conditions (Section 3.2.1.) but no product or product derivative formation was observed by LC-MS or ^{1}H NMR analysis after 16 hours.

Tris(2,2,2-trifluoroethyl) borate (B(OCH$_2$F$_3$)$_3$), also known as the Sheppard reagent, has been reported to promote both amide formation between amines and carboxylic acids[148-150] and imine formation between amines or amides and carbonyls.[151] Using a literature procedure for a similar phthalimide compound,[151] this reagent was used to generate 193 (Scheme 106).
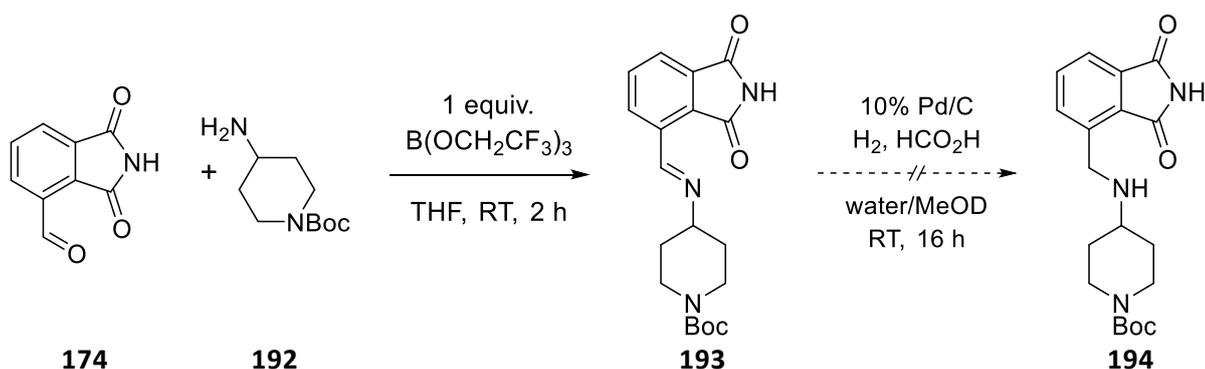
Scheme 106. Attempted synthesis of 194 from 174 and 192 by Sheppard reagent catalysed imine formation followed by hydrogenation with palladium on carbon

In situ ^{1}H NMR spectroscopic analysis showed full conversion to the imine 193 after 2 hours. However, attempts to carry out *in situ* hydrogenation with palladium on carbon (10%, dry) were still unsuccessful and alternative reduction procedures were therefore investigated. Sodium cyanoborohydride is frequently used in reductive aminations, and

resulted in a crude reaction mixture containing imine **193**, amine **194** and lactam **195**, suggesting partial completion of the reduction reaction (Scheme 107).
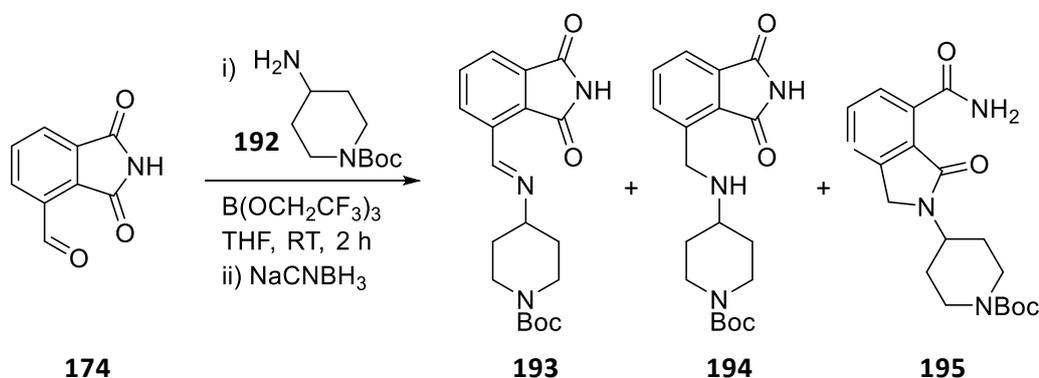
Scheme 107. Imine formation between **174** and **192**, catalysed by the Sheppard reagent, followed by reduction by sodium cyanoborohydride, giving a mixture of **193**, **194** and **195**

When the reaction was repeated with two equivalents of acetic acid added with the sodium cyanoborohydride, this successfully catalysed the reduction step, with only **194** and **195** observed in the crude product mixture. Addition of HCl at this point catalysed lactam formation while simultaneously deprotecting the Boc-protected piperidine amine, and a mixture of **152** and **152•HCl** was obtained. Additional HCl was added to form solely the amine salt **152•HCl**, which precipitated and was collected by filtration.

These optimised conditions were then combined into a single pot reaction sequence that gave **152•HCl** in 74% yield from the aldehyde **174**. The overall yield of **152•HCl** from biomass-derived furfural **17** was therefore 62% over seven reaction steps (Scheme 108).
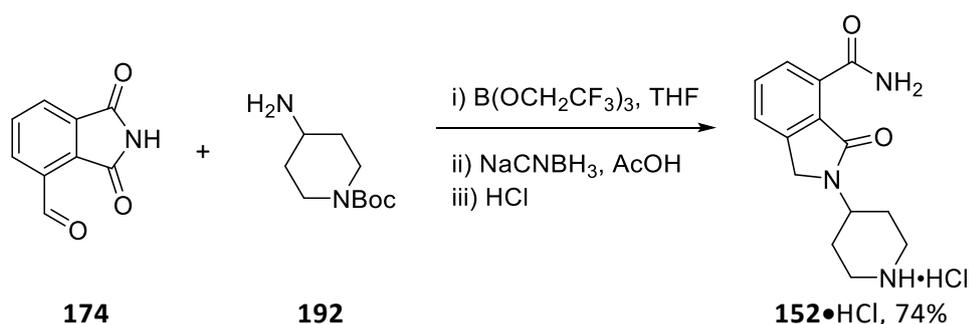
Scheme 108. Three step, one-pot synthesis of **152•HCl** from **174** and **192** by Sheppard reagent catalysed imine formation, acid-catalysed sodium cyanoborohydride reduction, and hydrochloric acid catalysed lactam formation, Boc deprotection, and formation of the hydrochloride salt

After this synthesis was developed and shortly before it was published, another synthesis of isoindolinone **152** from furfural **17** was published (Scheme 109). This synthesis also utilised a cycloaddition and aromatisation reaction to generate the aromatic ring, though using much harsher conditions. The yield was not specified.[152]
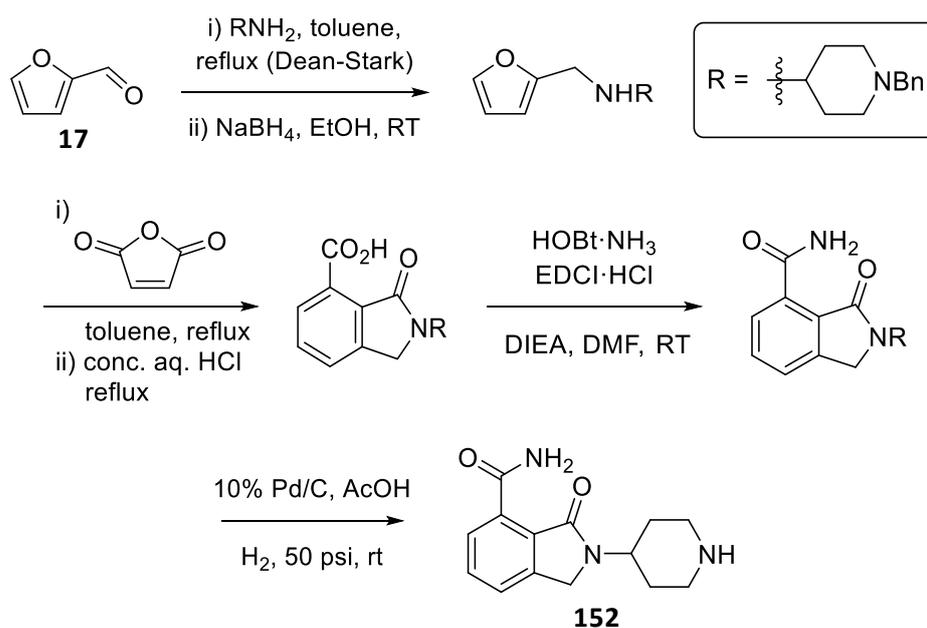
*Scheme 109. Recent literature synthesis of **152** from furfural*

3.2.4. Synthesis of Phthalocyanines

A literature procedure using microwaves for the synthesis of copper phthalocyanine from phthalonitrile and phthalimide was applied to substituted phthalonitrile **32** and phthalimide **56** (Scheme 110).[153] This procedure uses solvent-free conditions and, in the case of phthalimides, urea and ammonium molybdate are added as nitrogen sources.
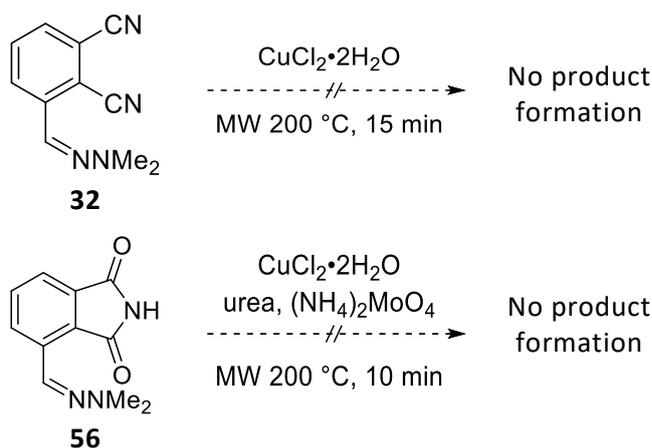
*Scheme 110. Attempted application of a literature phthalocyanine synthesis to **31** and **32***

No formation of phthalocyanine observed under these reaction conditions. An alternative literature procedure for phthalimides was applied to phthalimide, **31**, and **56**, using *para*-toluenesulfonic acid as a catalyst and hexamethyldisilazane (HMDS) with

dimethylformamide as both the solvent and additional nitrogen source (Scheme 111).[154] A similar procedure for phthalonitriles was then applied to **32**.[155]
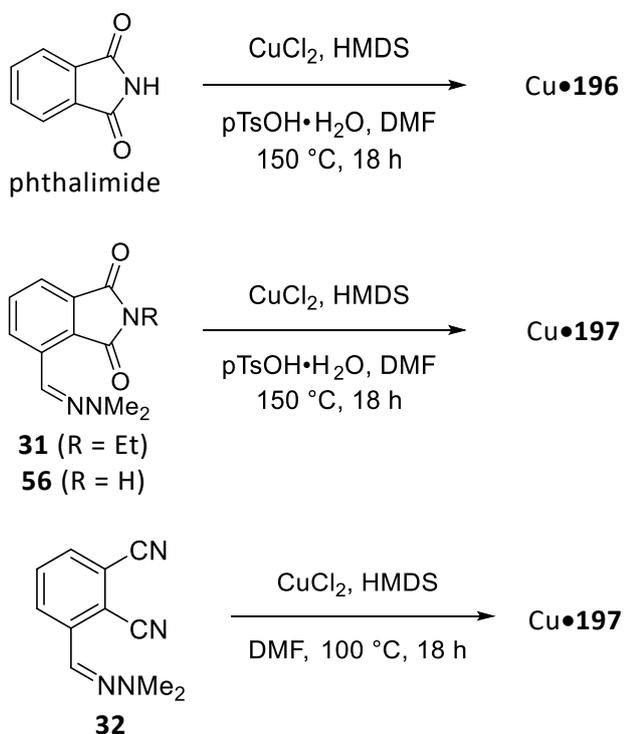
Scheme 111. Synthesis of copper phthalocyanines from phthalimide, 31, 56 and 32 using literature conditions

In each case, the colourless, yellow or green starting material was converted into a deep blue solid that was insoluble in water and most organic solvents, characteristic of phthalocyanines. NMR spectroscopic analysis was impeded by the long relaxation times of the extensive π system and the paramagnetic copper(II) ion. MALDI and crystal structure analyses were attempted but no conclusive data were obtained.

In several relevant publications, the characterisation of phthalocyanines is limited to the ultraviolet, visible and infra-red spectra, with particular note of the 'Q-band', the strongest absorption in the visible region.[154,156,157] This data was therefore used to provide preliminary characterisation of these phthalocyanines without full structural determination. The product Cu•**196** of the reaction with unsubstituted phthalimide matched the literature data for unsubstituted copper phthalocyanine (Cu•Pc), with a Q-band of 675 nm in DMF compared to a literature value of 678 nm in the same solvent and similarly consistent IR peaks.[158] If **196** is indeed phthalocyanine, this would give an isolated yield of 50% for this reaction, compared to a literature yield of 63%.[154]

The reactions with **31**, **56** and **32** seemed to all give the same product, Cu•**197**, which exhibited a blue-shifted Q-band of 629 nm. This is consistent with the presense of electron-donating groups such as dimethylhydrazone, and extra IR peaks were present in the 1500 – 1600 cm^{-1} region (1501, 1543, 1582).

To explore whether the hydrazone is likely to remain in-tact during these procedures, aromatic hydrazone **198** was synthesised from benzaldehyde using *N,N*-dimethylhydrazine and then subjected to the reaction conditions used in the synthesis of phthalocyanines (Scheme 112).
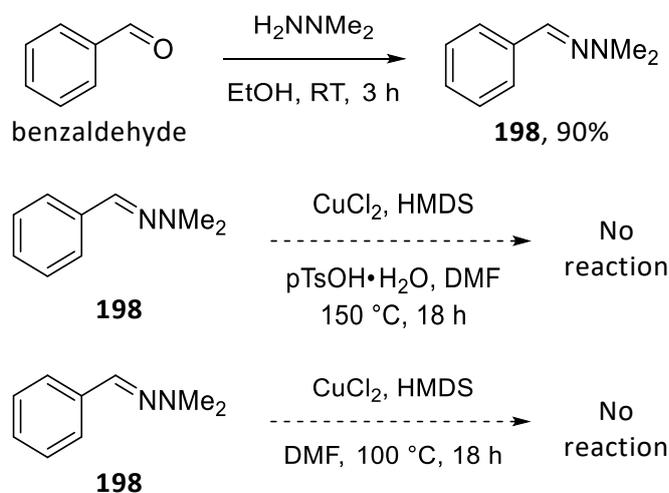
Scheme 112. Synthesis of aromatic hydrazone **198** from benzaldehyde, and subsequent exposure to phthalocyanine synthesis reaction conditions

Under these conditions, no reaction was observed, suggesting that the phthalocyanines likely retain the hydrazone groups of the starting material. The substitution pattern is unknown and likely a mixture of the four possibilities, denoted by their symmetry groups (Figure 24). If this structure is accurate, then isolated yields of 41% from **31**, 37% from **56**, and 61% from **32** were obtained.
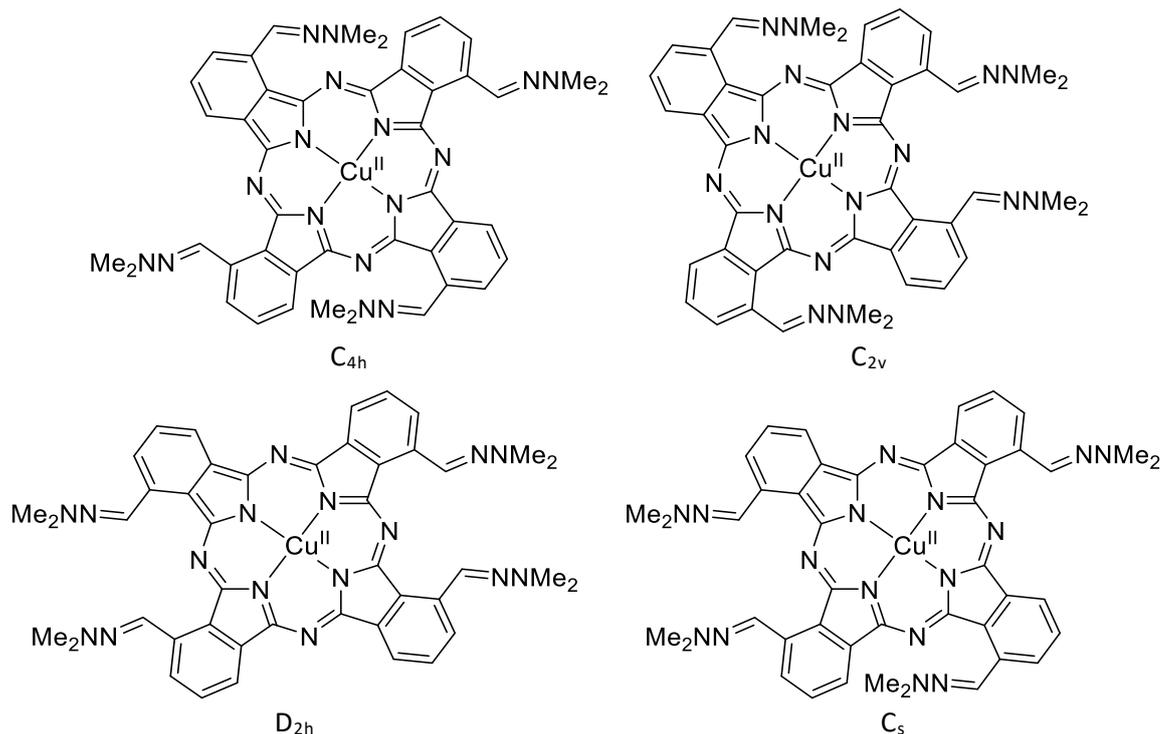
Figure 24. Structures of the four possible hydrazone phthalocyanine substitution patterns

The reaction was repeated using zinc ions, in the hope that the diamagnetic product would be easier to characterise by NMR spectroscopy, but peaks were still too broad to assign. The use of phthalimide **174** resulted in a compound Cu•**199** with a slightly red-shifted Q-band at 684 nm and an IR peak at 1695 cm^{-1}, both consistent with the retention of the electron-withdrawing aldehyde on the phthalocyanine ring
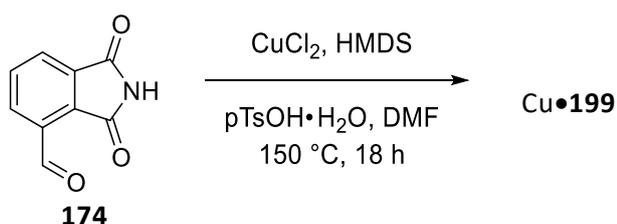
Scheme 113. Synthesis of putative aldehyde-substituted copper phthalocyanine Cu •199

3.3. Summary of Chapter III

By applying a range of functional group transformations, the range of aromatic compounds that can be sustainably synthesised from furfural and its derivatives has been significantly expanded, including the introduction of chemical motifs that allow for further manipulation. This both demonstrates the flexibility of this approach, and improves its

potential as a means of rapidly generating a library of novel, medium-weight aromatic compounds.

To illustrate the utility of these aromatic products, a poly(ADP-ribose) polymerase inhibitor promising potential chemotherapeutic agent was synthesised in an overall yield of 62% from furfural over seven reaction steps. The utility of these products in the synthesis of phthalocyanines was also explored, with promising preliminary results.

3.3.1. Future Work

Aromatic compounds synthesised from 5-hydroxymethylfurfural **19** using the methodology described in Chapter II, such as phthalimide **166**, have a hydrazone group *para* to a hydroxyl group. Direct oxidation of a hydrazone to a carboxylic acid has previously been achieved using hydrogen peroxide with silica-supported selenamide,[159] or hydrolysis to an aldehyde followed by a conventional oxidation could be applied to produce a monomer such as **200**. This phthalimide could undergo homopolymer formation via ester linkage to give tailored aromatic polymers such as **201** (Scheme 114).
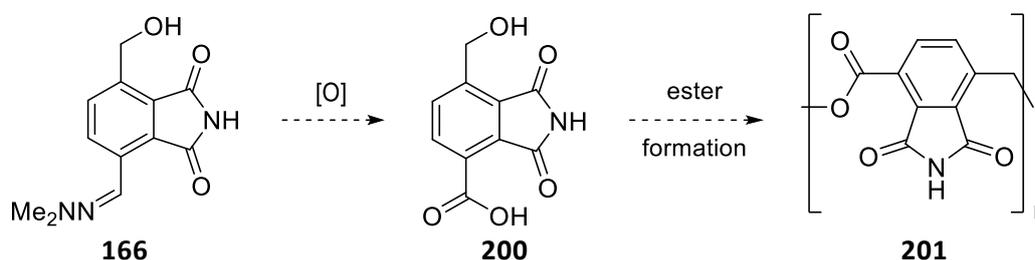
*Scheme 114. Proposed synthesis of a homopolymer by oxidation of a HMF-derived aromatic compound such as **166**, followed by polymerisation via formation of ester bonds*

Additional work is also required to develop a sustainable route to phthalocyanines from biomass-derived furfurals, including further characterisation of the structures and substitution patterns of the resulting phthalocyanine rings. Investigation into the synthesis and use of a range of substituted phthalonitriles would broaden the diversity of accessible biomass-derived phthalocyanines (Section 3.1.3.).

Finally, optimisation of the [3+2] cycloaddition of the aromatic hydrazones would allow this approach to be used in conjunction with a range of dienophiles to produce a wide range of benzene rings with pyrazole and dihydropyrazole substituents.

Chapter IV. Use of Novel Donors in Transketolase Reactions

4.1. Introduction

As mentioned in Chapter I, enzymatic chemistry provides an excellent solution to the need for sustainable, green chemistry, being typically carried out in water at ambient temperatures and pressures, often with the benefit of high regio- and stereoselectivity.

4.1.1. Thiamine Diphosphate Dependent Enzymes

Thiamine diphosphate (ThDP) **202** (Figure 25) is an enzyme cofactor used by a broad and diverse family of enzymes that takes its name from this use; the thiamine diphosphate dependent enzymes.[160,161]

202
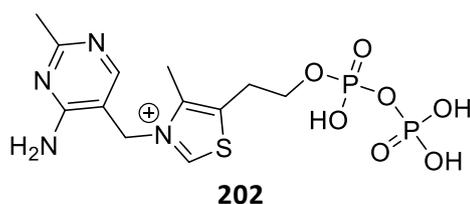
*Figure 25. Structure of thiamine diphosphate **202***

ThDP-dependent enzymes are a useful tool in biocatalytic reactions as they can perform powerful transformations, breaking and forming carbon-carbon, carbon-oxygen, carbon-nitrogen or carbon-sulphur bonds with high specificity and creating new stereocentres with high selectivity.[162] Members of this enzyme family accept different selections of substrates. Many readily accepting a range of non-natural substrates and thus have significant scope for broad utility.[161-163]

As the enzymes derive their reactivity largely from their ThDP cofactor, most demonstrate a similar pattern of reactivity and reaction mechanism. In general, a carbonyl-containing substrate loses a group bonded to the carbonyl carbon and is thus converted from an a_1 synthon which acts as an electron pair acceptor at its carbonyl carbon to a d_1 synthon which acts as an electron pair donor at this position. The d_1 synthon then acts as either a base or a nucleophile (Scheme 115).[161]
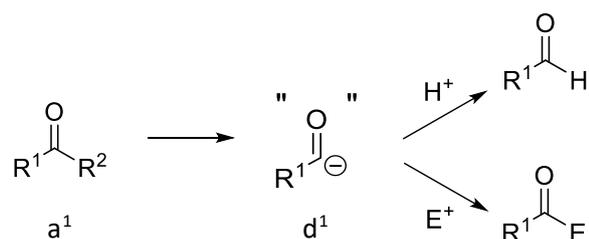
Scheme 115. General depiction of ThDP-dependent enzyme reactivity

In many cases, the electrophile (E⁺) in this scheme is an aldehyde species acting as a classical a^1 synthon, and is referred to as the 'acceptor substrate', while the carbonyl species converted into a d^1 synthon is referred to as the 'donor substrate'. A general scheme for these donor/acceptor reactions is shown below, with the new stereocentre highlighted in red (Scheme 116).[161]
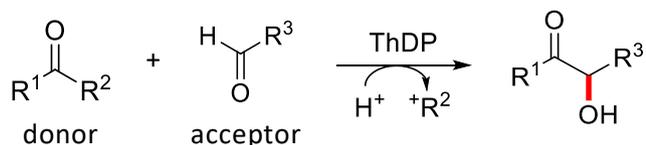
Scheme 116. General scheme for the coupling of a donor and acceptor substrate catalysed by a ThDP-dependent enzyme

Mechanism of ThDP-dependent Enzymes

The general mechanism for ThDP-dependent enzymes is shown below (Scheme 117). It begins with the deprotonation of ThDP at the C-2 position of the thiazolium ring, often by a nearby lysine residue in the active site (step i).[164]
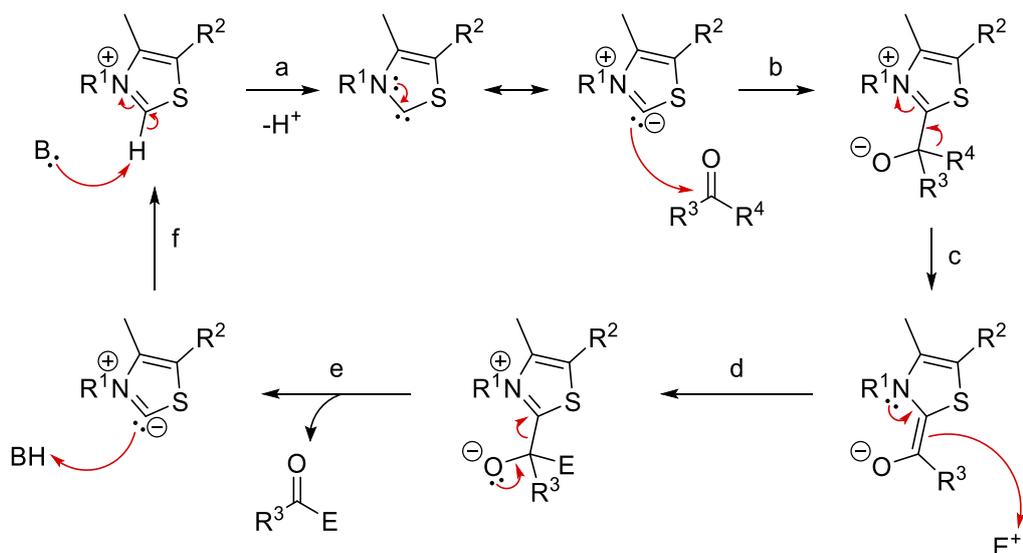
Scheme 117. General reaction scheme for the mechanism of ThDP-dependent enzymes

The resulting intermediate can be depicted and considered as a carbene next to a neutral nitrogen centre, or as an ylid with a carbocation stabilised by the adjacent positively charged nitrogen. The resonance structures are depicted above. This carbene/ylid intermediate then nucleophillically attacks the carbonyl group of the donor substrate, forming a quaternary centre with an oxyanion (Step b). The loss of R^4 is then facilitated by the positively charged thiazolium ring acting as an electron sink, forming an enamine (step c). This enamine is the 'd^1' synthon, also known a Breslow intermediate,[165] and attacks into the acceptor substrate (step d). The thiazole then acts as a leaving group, breaking the bond between the substrate and cofactor and releasing the product from the active site (step e). The enzyme is returned to its starting state by reprotonation of the ThDP (step f). It is the ability of the deprotonated thiazolium ring ylid to act as both a nucleophile and an electron sink that enables it to catalyse these reactions.[164]

Decarboxylases

ThDP-dependent enzymes are commonplace in nature and everyday life. One example is the decarboxylases, in which the R^4 'leaving group' as shown in Scheme 117 is a carboxylate motif (Scheme 118).[164]
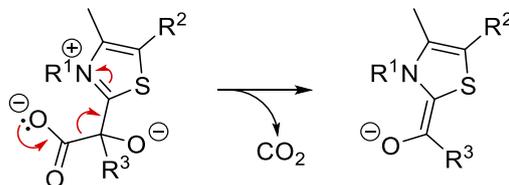
Scheme 118. General loss of a carboxylate in a ThDP-dependent decarboxylase enzyme

One such enzyme, pyruvate decarboxylase, converts pyruvate into acetaldehyde and carbon dioxide. This is part of the fermentation process that takes place in yeast under anaerobic conditions, as the acetaldehyde is then reduced by alcohol dehydrogenase to produce ethanol.[166] Another example is α-ketoglutarate decarboxylase, the first component of the α-ketoglutarate dehydrogenase enzyme complex. This complex catalyses the conversion of α-ketoglutarate into succinyl-CoA as part of the Krebs cycle, also known as the citric acid or tricarboxylic acid cycle.[161]

Transketolase

One of the most ubiquitous and well-known examples of a ThDP-dependent enzyme is transketolase (TK, EC 2.2.1.1), a key enzyme in the non-oxidative pentose-phophate pathway and the Calvin cycle.[167] In each of these biosynthetic pathways, TK catalyses key

reactions in which a two carbon glycoaldehyde fragment is transferred from one phosphorylated sugar to another. In the pentose phosphate pathway, xylulose 5-phosphate **203** and ribose 5-phosphate **204** are converted into glyceraldehyde 3-phosphate **205** and sedoheptulose 7-phosphate **206**. Similarly, **203** and erythrose 4-phosphate **207** are converted into **205** and fructose 6-phosphate **208** (Scheme 119). In the Calvin cycle, the reverse of these two reactions are catalysed.
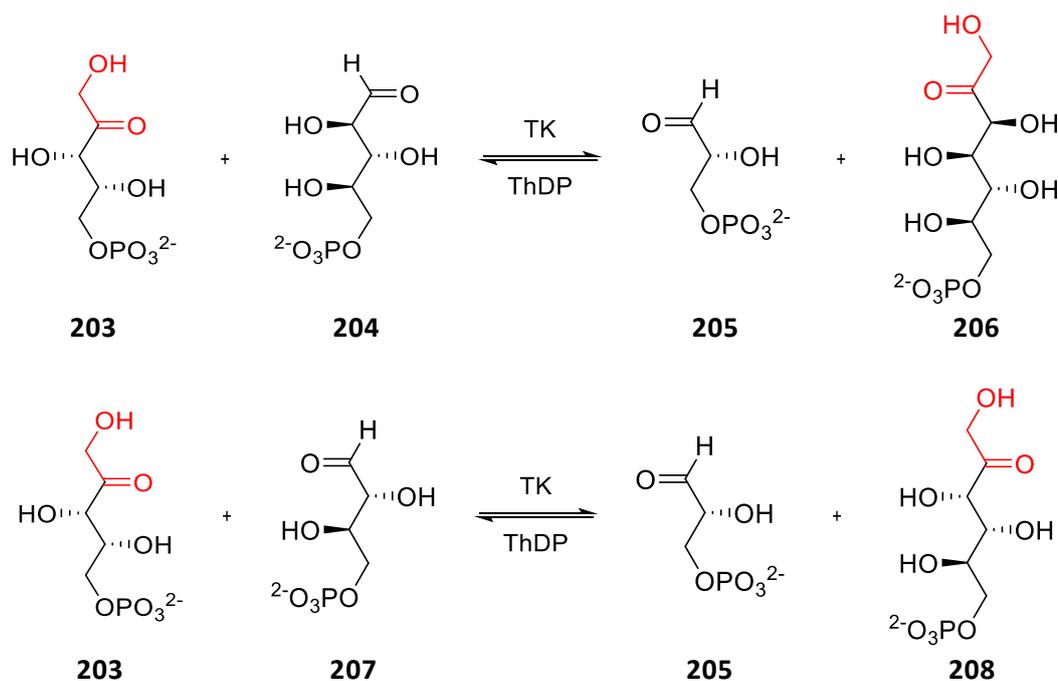
Scheme 119. Steps in the non-oxidative pentose phosphate pathway (forwards) and Calvin cycle (backwards) catalysed by transketolase

4.1.2. Transketolase in Synthesis

Spinach TK has been used in the preparation of 'natural'-labelled furaneol, **212**, an industrially used caramel flavouring compound. The TK catalyses the synthesis of furaneol precursor 6-deoxy-L-sorbose **211** from 4-deoxy-L-threose **210** and hydroxypyruvate (HPA) **209** (Scheme 120).[168]
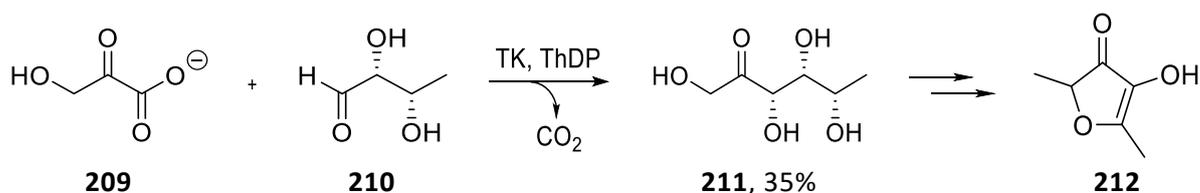
*Scheme 120. Synthesis of 6-deoxy-L-sorbose **211**, a precursor to furaneol **212**, from 4-deoxy-L-threose **210** and hydroxypyruvate **209** using spinach transketolase*

E. coli TK has been widely used in synthesis, including in the multi-gram synthesis of **215**, a novel *N*-hydroxypyrrolidine and potential glycosidase inhibitor. Here, the TK enzyme was used to catalyse the coupling of 3-*O*-benzylglyceraldehyde **213** and lithium hydroxypyruvate Li-**209** to give triol **214**, which was then converted to **215** over 8 steps (Scheme 121).[169,170]
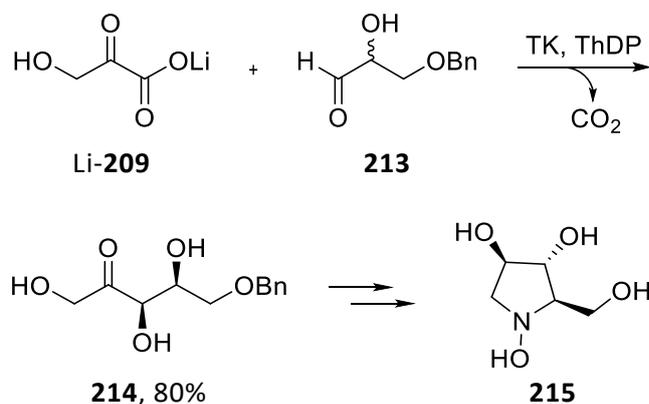
Scheme 121. Synthesis of triol 214, a precursor to N-hydroxypyrrolidine 215, from lithium hydroxypyruvate Li-209 and 3-O-benzylglyceraldehyde 213 using E. coli transketolase

Another example of a synthesis using *E. coli* TK, from our research group, is the one-step, stereoselective conversion of L-arabinose **216** to L-*gluco*heptulose **217**, a potential therapeutic in cancer and hypoglycaemia (Scheme 122).[171,172]
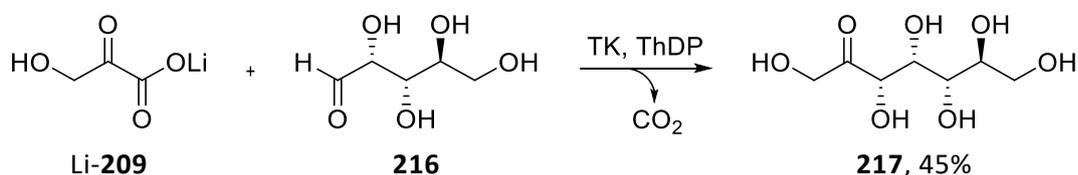
Scheme 122. Synthesis of L-glucoheptulose 259 from L-arabinose 258 and lithium hydroxypyruvate Li-209 using E. coli transketolase

4.1.3. Transketolase Substrate Scope and Directed Evolution

Acceptor scope

Compared to spinach and yeast transketolase, *E. coli* WT TK has a broad acceptance of hydroxylated aliphatic aldehyde acceptor substrates.[167,173] Its tolerance for non-α-hydroxylated substrates is typically significantly lower, with 5-35% less activity reported than for their α-hydroxylated counterparts.[167,169] Directed evolution has been used to generate *E. coli* TK mutants with increased non-natural acceptor substrate tolerance.[174,175]

In one study, a crystal structure of the *E. coli* TK-ThDP complex (1QGD.pdb) was used to select active site residues for modification based on both proximity to the bound

substrates, and on a phylogenetic basis. These included all residues within 4 Å of the bound substrates (first shell mutants) and those residues within 10 Å which are varied across the phylogenetic tree of bacterial and yeast TK evolution (second shell mutants) (Figure 26).[174]
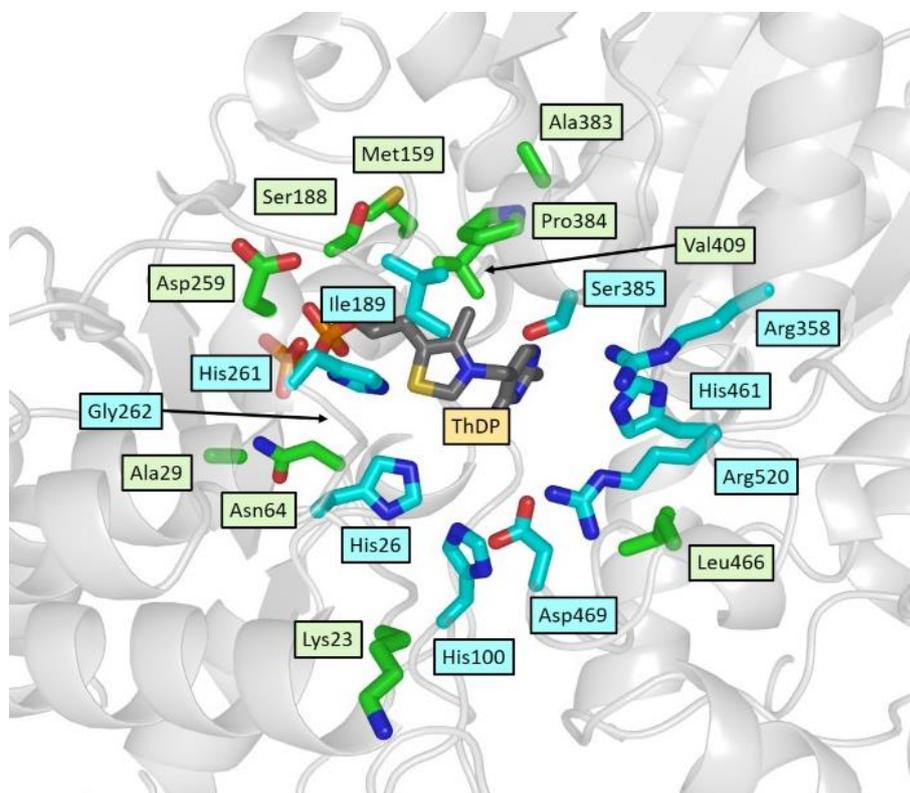
Figure 26. Structure of the active site of the TK-ThDP complex with first and second shell residues highlighted in cyan and green, respectively. Image generated from 1QGD.pdb using data published by Hibbert et al.[174]

Mutant libraries of these residues were created and screened for their ability to convert Li-**209** and glycolaldehyde **218** to erythrulose **219** (Scheme 123). Four mutants were identified to have activities superior to WT; A29D, A29E, H461S and R520V. The increased activity of H461S and R520V could be explained by their location in the phosphate-binding region close to the active site entrance, improving entry of non-phosphorylated substrates.[174]

Li-**209**   **218** (R = OH)   **219** (R = OH)
             220 (R = Me)   **221** (R = Me)
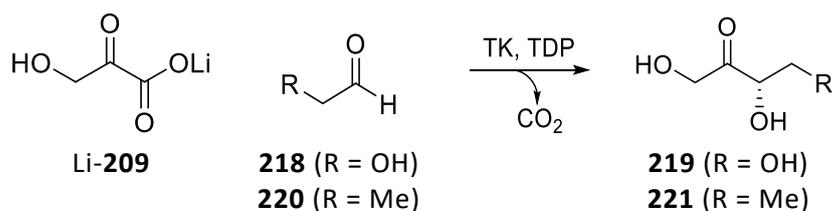
*Scheme 123. Transketolase biotransformation between Li-**209** and glycoaldehyde **218** or propanal **220** to give erythrulose **219** or ketodiol **221**, respectively*

Further work followed the reaction of propanal **220** and Li-HPA Li-**209** to form ketodiol **221** (Scheme 123), and found five mutants with increased activity relative to wild-type; D469E, D469A, D469Y, D469T and R520V.[175] The D477 residue in yeast TK is analogous to the D469 site in *E. coli* TK and was already known to be important in binding hydroxylated aldehyde substrates.[176] Crystal structure studies of *S. cerevisiae* TK complexed to natural acceptor substrate erythrose-4-phosphate (1NGS.pdb) show that binding site residues that are analogous to the H29, H100 and D469 residues in *E. coli* form key interactions with the C2-hydroxyl group of the substrate.[174,177] As propanal has a methyl group in place of the C2 hydroxyl of natural substrates, this could explain why mutations replacing the charged aspartic acid residue with less polar groups enhance interaction with the non-polar methyl group.

Stereochemical studies showed that WT TK produces ketodiol **221** with only 58% *ee*, favouring the (*S*)-enantiomer. D469E was shown to give 90% *ee* of the 3(*S*)-enantiomer, while H26 mutants gave reversed stereoselectivity, with the H26Y mutant resulting in 88% *ee* favouring the 3*R*- isomer. C_4 - C_9 linear aldehydes and C_3, C_5 and C_6 cyclic aldehydes were also accepted by D469E, with isolated yields comparable to, or better than, those obtained with WT TK and high *ee*s favouring the 3*S*- enantiomer. H26Y showed reversed stereoselectivities for C_3 and C_8 linear aldehydes.[178]

In an attempt to further improve the acceptance of both glycoaldehyde **218** and propanal **220**, Stafford *et al.* created double mutants combining single-site mutations that had previously shown increased activity towards these substrates.[179] However, each mutant showed reduced activity towards both substrates due to loss of protein stability, and statistical coupling analysis revealed nine residues to be part of a co-evolved, synergistic network; these were G467, D469, G470, T472, P482, P493, D495, E498 and R520. Introducing mutations into such networks can often lead to impaired stability and function. To avoid this, new double mutants were created from promising single-site mutations by introducing a second mutation that was limited to variants found in naturally occurring, related ThDP-dependant enzymes, as determined by analysis of 382 aligned sequences. Of these, only the D469/R520 library gave improved activity over WT TK, with the highest activity achieved by the D469T/R520Q mutant.[179]

A thermostable transketolase enzyme from the bacterium *Geobacillus stearothermophilus* (TK$_{GST}$) was recently characterised.[180] As with *E. coli* TK, wild-type TK$_{GST}$ has low acceptance of non-α-hydroxylated aldehyde acceptors, though double-site saturation mutagenesis

studies have produced mutants with up to 7.4 times higher acceptance of propanal and butanal.[181] These are mutated at the L191, F435 and D470 sites, which are analogous to the *E. coli* I189, F434 and D469 residues. The F435L/D470E mutant exhibited improved activity and 3*S* selectivity while the L191V/D470I gave inverted 3*R* selectivity.

TK mutants with improved acceptance of aromatic aldehyde acceptors have also been developed, particularly the F434A mutant,[182] R358 and S385 mutants[183] and combination mutants such as the R358X/D469T/R520Q and S385X/D469T/R520Q triple mutants.[184] However, for this project linear aldehyde acceptors were chosen for combination with novel keto-acid donors, as they produce synthetically interesting α-hydroxyketones and typically give higher yields, particularly with single-site *E. coli* TK mutants.[183,185]

Donor scope

While a significant body of work has been carried out on developing TK mutants that accept alternative acceptor substrates, relatively little work has been reported on improving the tolerance of donor substrates, which have the potential to open up an even more diverse range of TK products. Wild-type *E. coli* TK shows high specificity for its natural substrates, xylulose-5-phosphate **203**, sedoheptulose 7-phosphate **206**, and fructose 6-phosphate **208**, and for a single non-natural substrate, hydroxypyruvate **209** (Figure 27).[162,186]
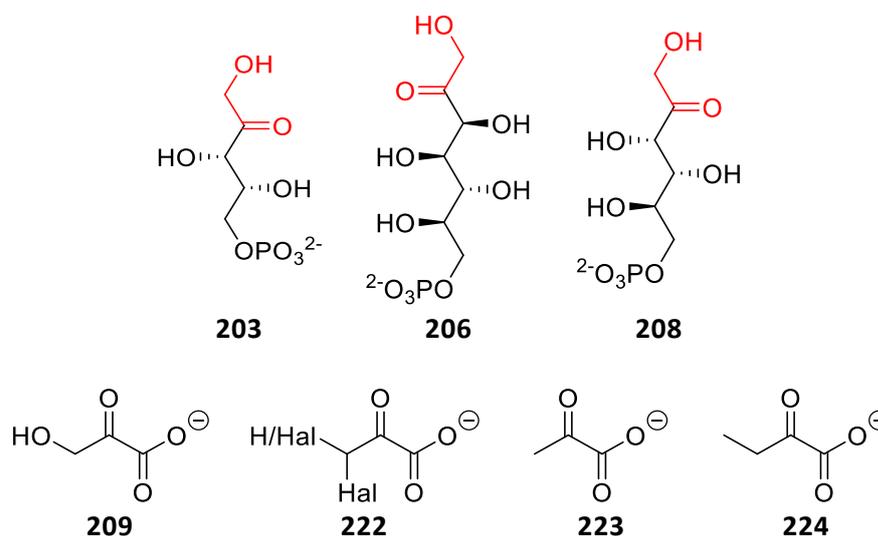
Figure 27. Keto-acid donor substrates for TK biotransformations

In the significant majority of literature examples, hydroxypyruvate **209** has been used as the donor. Reasons for this include the ready acceptance of HPA **209** by TK and the fact that the resulting ketodiol motif is synthetically useful and pharmaceutically relevant. The

use of HPA **209** also renders the reaction irreversible due to the loss of CO_2 as the 'leaving group', thus promoting the reaction.[187,188] *E. coli* TK accepts HPA **209** with 30-fold higher activity than yeast and spinach TKs.[167]

Recent work with yeast TK has shown that chloro-, bromo- and fluoro- pyruvate derivatives **222** will convalently bond to the TK-ThDP complex. However, only formation of the active ThDP-enamine intermediate was monitored, using circular dichroism, and no products were isolated or characterised.[189] This demonstrated some potential for the acceptance of alternate keto-acid donors, though the lack of characterisation and product formation limits the conclusions that can be drawn from this work.

Crystal structures of TK bound to its natural donor substrates (2R8O.pdb and 2R8P.pdb) show key interactions between the ketol group and residues H100 and H473; the 3-hydroxyl group and both H26 and H261; the 4-hydroxyl group and D469; and the phosphate group and both S385 and R358 (Figure 28).[160,190]
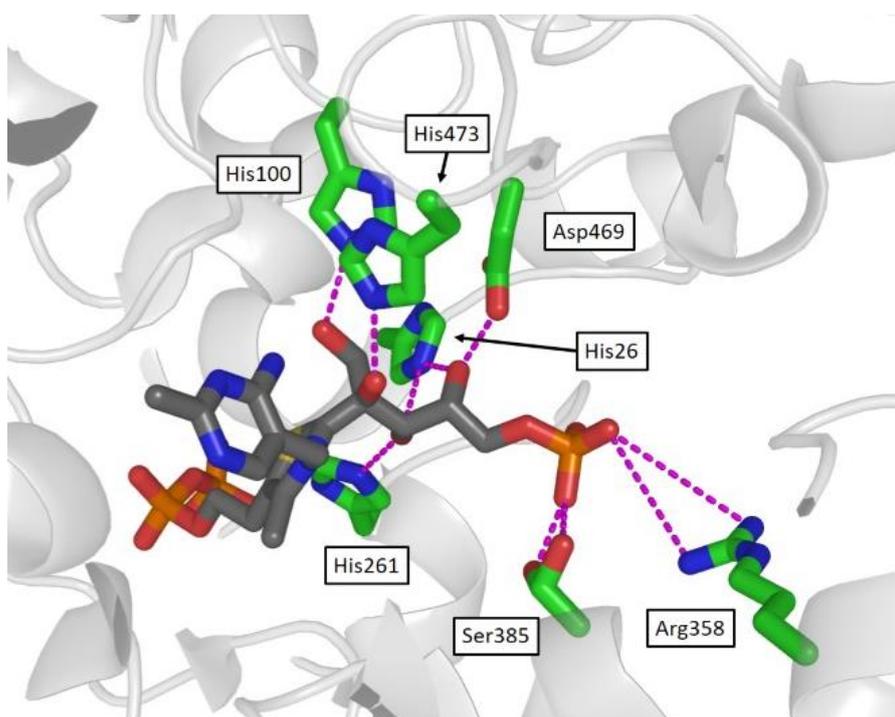
Figure 28. Crystal structure of the binding of D-xylulose-5-phosphate to wild-type E. coli *transketolase, with key interaction residues labelled, generated from 2R8O.pdb*

It was hypothesised that the retention of the ketol interactions combined with the disruption of hydroxyl and phosphate binding could improve acceptance of substrates lacking these groups, such as pyruvate **223** and ketobutyrate **224**. The alteration of peripheral residues close to the active site (Figure 26) could also be considered, as a means to increase the spatial capacity of the active site and thus lower specificity.

Unpublished preliminary work within our group has uncovered promising TK mutants that accept pyruvate **223** and ketobutyrate **224** as donor substrates with pentanal and hexanal as the aldehyde acceptors, giving α-hydroxyketones **225** - **228** (Scheme 124, Table 32).[185] These products were purified by flash column chromatography and their stereochemistry determined by a modified Mosher's ester analysis.[185,191] In each case, the (*S*)-enantiomer was produced with an *e.e.* of greater than 95%.

223, R = H   pentanal, n = 3
224, R = Me   hexanal, n = 4
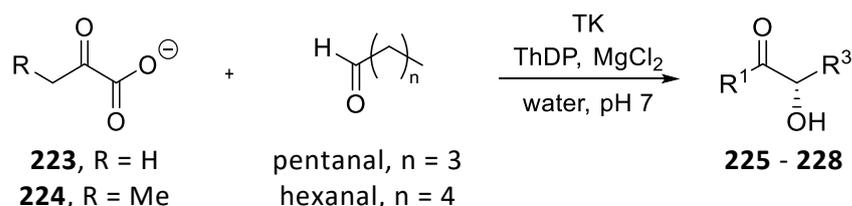
*Scheme 124. Previous transketolase reactions within the Hailes group, between sodium pyruvate Na-**223** or ketobutyric acid **224**-H and pentanal or hexanal*

DONOR	ACCEPTOR	PRODUCT	MUTANT	YIELD
	Pentanal	(3*S*)-**225**	WT	NR
			D469T	5%
			D469Y	Trace
			D469E	5%
			D469E/R520Q	trace
223			S385T/D469T/R520Q	trace
	Hexanal	(3*S*)-**226**	WT	NR
			D469T	4%
			D469Y	trace
			D469E	6%
			D469E/R520Q	trace
			S385T/D469T/R520Q	trace
	Pentanal	(4*S*)-**227**	D469T	4%
			D469Y	trace
			D469E	trace
			D469E/R520Q	6%
224			S385T/D469T/R520Q	4%
	Hexanal	(4*S*)-**228**	D469T	trace
			D469Y	8%
			D469E	7%
			D469E/R520Q	3%
			S385T/D469T/R520Q	trace

*Table 32. Preliminary results of previous transketolase reactions between sodium pyruvate Na-**223** or ketobutyric acid **224**-H and pentanal or hexanal*

During the writing of this thesis, work on introducing donor promiscuity to the thermostable TK$_{GST}$ was published.[192] Saturation mutagenesis was carried out on four residues with direct contact to the donor's 3-hydroxyl group, and library screening for activity towards pyruvate **223** as a donor showed that the H102L/H474S variant had a ten-fold higher catalytic efficiency than WT TK$_{GST}$. Preparative scale reactions were carried with donors pyruvate **223** and ketobutyrate **224** using the H102L/H474S mutant, and 3-methyl-2-oxanoate **229** using a H102T mutant. These were combined with a series of hydroxylated acceptors and isolated yields of 48 – 88% were obtained (Scheme 125).[192]

223 (R^1 = Me)
224 (R^1 = Et)
229 (R^1 = ^{i}Pr)

R^2 = CH$_2$OH,
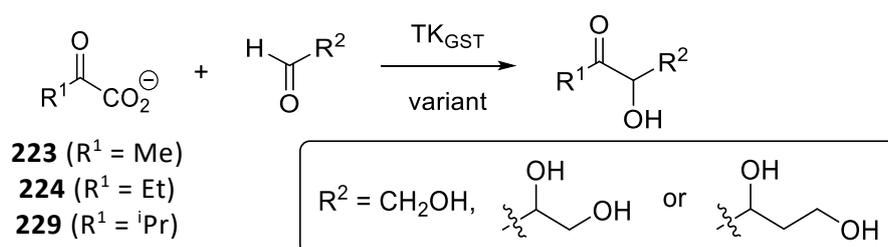
*Scheme 125. Literature reactions of TK$_{GST}$ variants with donor substrates **223**, **224** and **230**[192]*

4.1.4. Accessible Compounds of Interest

The use of pyruvate **223** or ketobutyrate **224** as a donor substrate results in the formation of an α-hydroxyketone with a methyl- or ethyl-ketone. This motif and its derivatives are found in a selection of biologically active compounds (Figure 29). For example, combining ketobutyrate **224** with natural, sugar-based aldehyde acceptors could produce deoxy-sugars such as **231** and **232**, which are potential antimycobacterial agents,[193] and **233**, which is a precursor to the β-mannosidase inhibitor **234**.[194,195] Using unnatural TK acceptor substrates could also give compounds such as **235**, a natural product and potential cholera treatment.[196,197]

Combined with a transaminase to stereospecifically convert the ketone into an amine, drugs such (+)-spisulosine **236**, an anti-cancer agent,[198] and phenylpropanolamine **237**, a nasal decongestant also known as norephedrine or pseudonorephedrine depending on stereochemistry, could also be synthesised. Hydrogenation of the α-hydroxyketone carbonyl could result in diols such as artemidiol **238** and pezizolide E **239**, natural fungal products.[199,200]
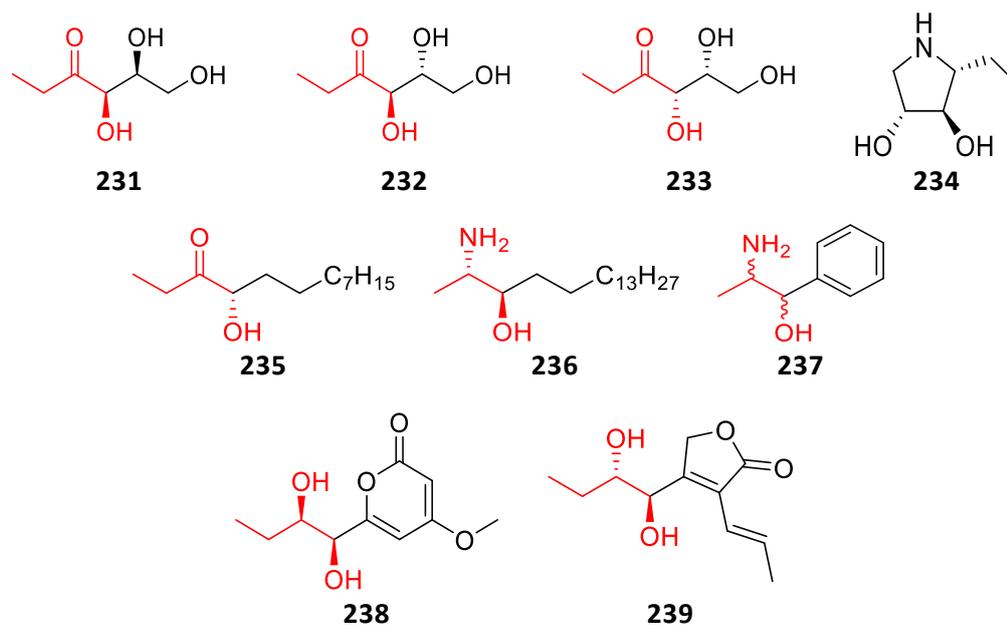
*Figure 29. Compounds accessible from transketolase reactions with **223** or **224**, either directly or from subsequent transaminase biotransformation or hydrogenation of the ketone; the methyl-/ethyl- α-hydroxyketone motif is highlighted in red*

4.1.5. Chapter Aims

Building on previous work within our research group, the use of pyruvate **223** and ketobutyrate **224** as *E. coli* TK donor substrates to synthesise α-hydroxyketones was further explored. TK mutants were selected from pre-existing libraries and screened for improved catalytic activity and to investigate the role of mutations in allowing the binding of less hydroxylated donor substrates.

4.2. Results and Discussion

4.2.1. Mutant Selection

Based on previous directed evolution studies (Section 4.1.3.), a selection of *E. coli* TK mutants were chosen for investigation into their relative abilities to accept novel keto-acid donor substrates. Linear aldehydes were selected for use as acceptor substrates due to their ready acceptance by a range of TK mutants and to compliment and build upon previous work within our research group that used pentanal and hexanal (Table 32). Therefore, enzyme variants known to accept these acceptor substrates were used as a starting point for mutant selection. These included D469 mutants and D469/R520Q double mutants. The F434A/R520Q mutant and the S385/D469T/R520Q and R358/D469T/R520Q triple mutants were also investigated. These have typically been notable for their acceptance of aromatic aldehydes. Amino acids S385 and R358 both bind the phosphate moiety and, as this binding region is close to the active site entrance, such residues have been implicated in allowing substrates into the active site.[174] Of the mutants selected, five have previously shown acceptance of pyruvate **223** and ketobutyrate **224** (Table 32).

Access to these cell lines was provided by Panwajee Payongsri and Pierre Affaticati, and a new generation of stocks were generated, maintained, and used to produce cell lysate samples as needed throughout this project. All cell lysate was kept at -20 °C for a maximum of one month. The TK protein concentration was determined to be approximately 1 mg/mL and, in all TK biotransformations described, 1 mL of lysate was used per 50 mmol of substrate.

4.2.2. Preliminary Reaction Screening

To ensure enzyme and method functionality, literature reactions were replicated. Lithium hydroxypyruvate (Li-HPA) **209** was synthesised from bromopyruvic acid **240** according to previously reported protocols and a TK biotransformation between Li-HPA and propanal **220** was carried out using wild-type (WT) *E. coli* TK, producing **221** (Scheme 126).[185]
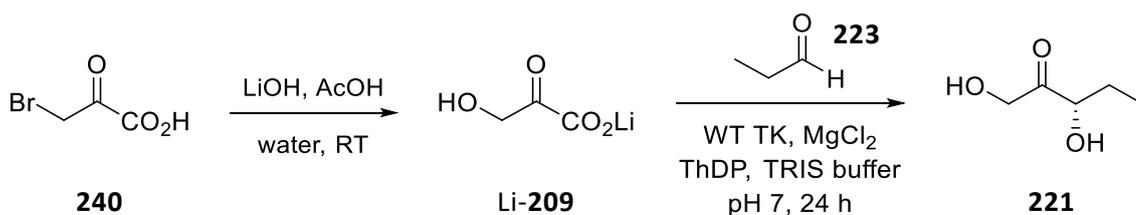
Scheme 126. *Literature synthesis of Li-HPA **209** and subsequent transketolase biotransformation with propanal **220** to give ketodiol **221***

Both reactions proceeded in similar yields to previous reports; 63% cf. 70% for step 1, and 31% cf. 36% for step 2,[185] confirming the functionality of the enzyme stock produced.

A selection of chosen transketolase variants were then screened for their ability to accept novel donor ketobutyric acid **224**-H. In previous preliminary reactions, linear aldehydes pentanal and hexanal were used as acceptor substrates (Table 32), but there were concerns over the volatility of the product causing isolation issues and reducing the yield. For these screening reactions, longer-chain aldehydes heptanal and octanal were therefore used, to give longer, less volatile products. Hexanal was also included in screening to ensure comparability with prior results.

Thirty small-scale biotransformation reactions in tris(hydroxymethyl)aminomethane (TRIS) buffer were carried out, with each of the ten mutants being screened against each of the three aldehydes (Scheme 127).
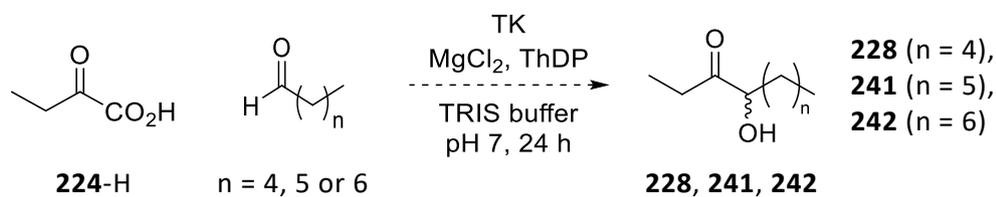
Scheme 127. *Attempted mutant screening for the biotransformation of ketobutyric acid **224**-H with a series of linear aldehydes*

Initially, thin later chromatography analysis was used to follow conversion to potential products and ^{1}H NMR analysis used to confirm product identity. However, in every reaction an alternative product was observed, with no formation of the expected products **228**, **241** and **242**.

Control reactions omitting each of the reaction components in turn revealed that only the TRIS buffer and the aldehyde were involved in producing this product. An investigation of the literature revealed that despite being a frequently used enzymatic buffer, TRIS has previously been reported to react with aliphatic aldehydes to form oxazolidines (Scheme

128).[201] ^{1}H NMR spectroscopic analysis of the unknown product was consistent with this hypothesis and it was not characterised further.
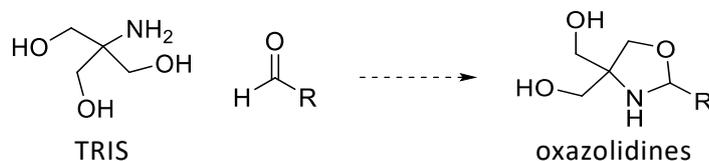
Scheme 128. General scheme for the reaction of TRIS with aliphatic aldehydes to give the corresponding oxazolidine products

These TK reactions require *in situ* control of the pH due to the byproduct CO_2 acting as a base. To find an alternative buffer system that would not interfere with the desired biotransformations, five were selected for screening in a TK reaction with ketobutyric acid and hexanal. Buffers 2-(*N*-morpholino)ethanesulfonic acid (MES), glycylglycine (Gly-Gly), *N*-(2-acetamido)iminodiacetic acid (ADA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) were selected for their availability, cost, pH ranges, and minimal presence of nucleophilic centres that might react with a linear aldehyde (Figure 30). Phosphate buffers were not investigated due to concerns over the ions binding into the phosphate pocket of the TK active site.
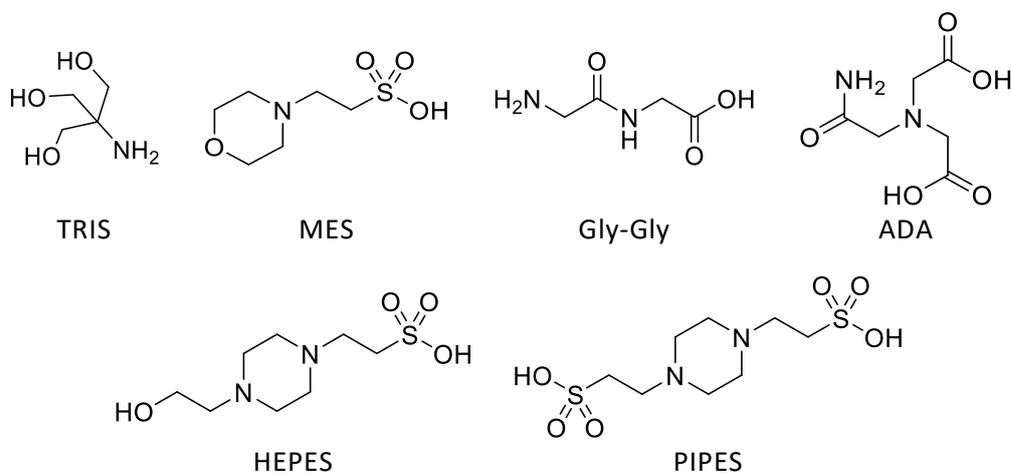
Figure 30. Structures of TRIS, MES, Gly-gly, ADA, HEPES and PIPES, a selection of biological buffers

Ten small scale reactions were carried out; a biotransformation and a lystate-free control in each buffer. In each of the biotransformations, product formation was followed by TLC analysis and was consistent with the expected product, while the control reactions showed no observable change by TLC analysis. These results suggested that any of the buffer systems would be appropriate for use in further reactions, and MES was selected due to availability and cost. The preliminary mutant screening was repeated as previously described, substituting the TRIS buffer for MES (Scheme 129, Table 33).

228 (n = 4),
241 (n = 5),
242 (n = 6)

*Scheme 129. Mutant screening for the biotransformation of ketobutyric acid **224**-H with a series of linear aldehydes using MES buffer*

MUTANT	n		
	4	5	6
D469T	✓	✓	✓
F434A/R520Q	✗	✗	✗
D469Y/R520Q	✗	~	~
R358P/D469T	✗	✗	✗
R358H/D469T/R520Q	~	~	~
R358I/D469T/R520Q	✗	✗	~
R358P/D469T/R520Q	~	✗	~
S385T/D469T/R520Q	~	~	~
S385Y/D469T/R520Q	~	~	~
S385E/D469T/R520Q	~	~	~

✓ = product observed by TLC analysis; ~ = faint indication of product by TLC analysis; ✗ = no product observed by TLC analysis.

*Table 33. Crude results of mutant screening for the biotransformation reactions of ketobutyric acid **224**-H with a series of linear aldehydes using MES buffer*

These results suggested that single mutants at the D469 position would provide a good starting point for optimising these biotransformations. D469T, D469Y and D469E mutants were available, and so these were selected for use in larger scale reactions to determine reaction yields.

D469 TK reactions

Biotransformations with single D469 mutant TK enzymes and ketobutyric acid with heptanal and octanal were scaled up in order to isolate the products (Scheme 130, Table 34).

228 (n = 4),
241 (n = 5),
242 (n = 6)

*Scheme 130. Scaled up biotransformation reactions of ketobutyric acid **224**-H with a series of linear aldehydes*

MUTANT	YIELD		
	n = 4	n = 5	n = 6
WT	NR	NR	NR
D469T	Trace	8%	14%
D469Y	8%	13%	9%
D469E	7%	5%	10%

Table 34. Results of scaled up biotransformation reactions of ketobutyric acid **224**-H with a series of linear aldehydes

Isolation was carried out by flash column chromatography, initially using petroleum ether 40-60 and ethyl acetate to elute. However, negligible yields were obtained despite crude mixture analysis indicating the presence of the expected products, and it was hypothesised that loss of product was occurring during removal of the chromatography solvents. They were therefore substituted for pentane and diethyl ether, solvents with lower volatility and similar polarities, and the expected products **228**, **241**, and **242**, produced in 5 - 14% isolated yield. This is an improvement on the yields previously obtained for transketolase reactions between ketobutyric acid and hexanal and pentanal, which was attributed to the lower volatility of the products.

In each case, an *e.e.* of greater than 95% was achieved, as determined by chiral gas chromatography (GC). The stereochemistry was assigned by comparison to the α-hydroxyketones synthesised in preliminary work by Dr. David Steadman (Table 32). Each of these were reported to demonstrate positive optical rotations, regardless of chain length,[185] and so it was determined that the positive optical rotations of **228**, **241** and **242** indicated the same (*S*)-enantioselectivity.

At this point, it was decided that the development of a medium throughput procedure for quantitative mutant screening was desirable.

4.2.3. Medium Throughput Mutant Screening Method Development

Gas chromatography (GC) analysis with flame ionisation detection was chosen for the quantification of crude reaction mixtures as this method allows the direct anaylsis of the products and aldehyde starting materials directly, with no prior derivatisation. In comparison, UV-based detection methods would require the addition of a UV-visible group, e.g. by benzoylation of the hydroxyl groups. The use of chiral GC columns also

allows for the concurrent determination of reaction stereoselectivies, which is not possible with colorimetric assays, and provides a high degree of sensitivity.

As α-hydroxyketones **241** and **242** were already available, they were used to generate calibration curves for the development of a GC-based assay. Biotransformation reactions between ketobutyric acid and heptanal or octanal were carried out on a 2.5 mmol scale in 500 μL of water and the reaction mixtures analysed by gas chromatography (GC).

Direct analysis of the reaction mixture gave inconsistent results due to non-homogeniety of the largely water-insoluble product and aldehyde starting material, and so small-scale organic extraction work-ups were employed. This involved adding 500 μL of ethyl acetate to each reaction mixture and agitating. As the lysate seemed to have an emulsifying effect, brief centriguation of the samples was required to achieve a separate organic phase. A sample of this organic phase was then collected for analysis. To minimise inconsistency caused by inherent errors in the volumes of the samples analysed, decane was used as an internal standard and added to the ethyl acetate used in the workup procedure.

However, inconsistencies of up to ±15% were still observed in reaction duplicates. This was attributed to the fact that the aldehyde acceptor substrates were pipetted directly into the reaction mixtures in 3 - 4 μL volumes, with significant error. In comparison, aqueous solutions of the other reagents were prepared, to allow addition of greater volumes and thus greater accuracies. This was not possible with the aldehydes due to their poor solubility in water. An organic reaction cosolvent would circumvent this issue as an aldehyde solution could then be prepared using this solvent, and so a series of organic cosolvents were screened.

Solvents 1,4-dioxane, tetrahydrofuran, acetonitrile, ethanol, methanol, and dimethylsulfoxide were selected as water-miscible solvents, along with ethyl acetate as an example of an immiscible solvent, and water as a control. These solvents were screened for their effect on the yield of the small-scale reaction between octanal and ketobutyric acid **224**-H, and the consistency between reaction duplicates (Scheme 131, Table 35).

*Scheme 131. Screening of cosolvents in the small-scale biotransformation of ketobutyric acid **224**-H with octanal to give **242***

Cosolvent	Product peak (Average) /pA	Variation
1,4-dioxane	252	±5%
THF	232	±3%
acetonitrile	291	±3%
ethanol	254	±2%
methanol	284	±3%
DMSO	346	±2%
EtOAc	trace	N/A
water	114	±11%

*Table 35. Results of the screening of cosolvents in the small-scale biotransformation of ketobutyric acid **224**-H with octanal to give **242***

The use of a water-miscible cosolvent significantly reduced the discrepancy between duplicate reactions and increased conversion to the product, with the highest relative 'yield' obtained with dimethylsulfoxide as the cosolvent. Future reactions were therefore carried out with 10% dimethylsulfoxide as cosolvent.

While the product peaks now showed consistency, the aldehyde starting material peaks still had high variance, which was attributed to their higher volatility. It was therefore decided that quantitative yields would be determined using the product peak rather than depletion of the starting material peak. This required isolated samples of each of the products to be synthesised, in order to create calibration curves and allow quantification.

4.2.4. Non-enzymatic Synthesis of α-Hydroxyketone Products

In previous studies, non-enzymatically synthesised samples of transketolase products have been generated using a biomimetic reaction with 3-(*N*-morpholino)propanesulfonic acid (MOPS).[202] In this reaction, a portion of HPA **209** is present as its tautomer, a dihydroxyacrylic acid **243**, which MOPS can undergo Michael addition into, forming enolate **244**. This then attacks into the acceptor substrate, achieving carboligation in a Bayliss-Hillman-like fashion (Scheme 132a). Loss of the carboxylate and MOPS groups from **245** then yields the α,α-dihydroxyketone product.
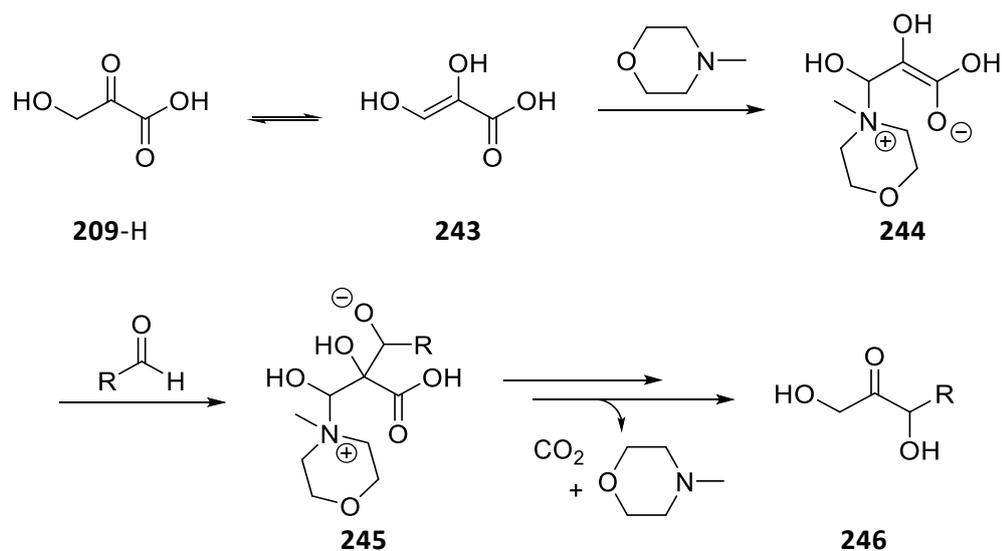
a) *MOPS-catalysed biomimetic synthesis of α,α-dihydroxyketones from HPA **209** and an aldehyde*

b) *Tautomerisation of ketobutyric acid **224**-H*

Scheme 132. The transketolase biomimetic reaction

Non-hydroxylated donor substrates do not form the enone tautomer as readily (Scheme 132b), and attempts to perform the biomimetic reaction with ketobutyric acid and octanal using literature conditions were unsuccessful (Scheme 133).[202]
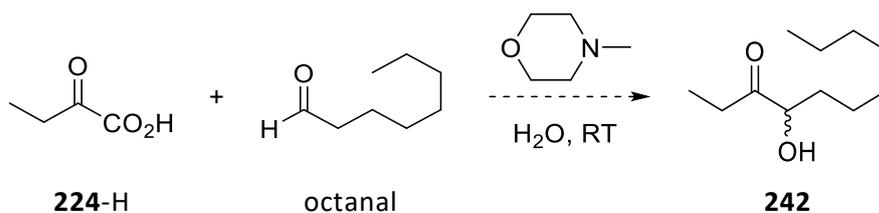
Scheme 133. Attempted biomimetic synthesis of **242**

Next, scaled-up enzyme reactions were used to synthesise α-hydroxyketone products **241** and **242** as previously described (Table 34). However, these reactions were relatively low yielding and could not easily be significantly scaled up, and the volatility of the small amounts of products caused isolation and handling issues. Therefore, a method to synthesise larger volumes of the α-hydroxyketone products was sought.

An acyloin condensation between propanal and heptanal or octanal was attempted, using a literature procedure that involves a catalytic thiamine analogue **247** (Scheme 134).[203] This reaction proceeds in a similar fashion to the TK mechanism.
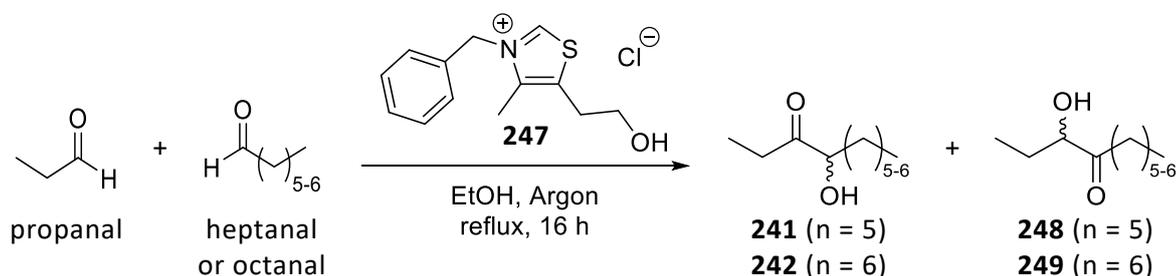
*Scheme 134. Acyloin condensation between propanal and heptanal or octanal, giving a mix of hydroxyketone regioisomers **241** and **248**, or **242** and **249***

Due to a lack of selectivity over which aldehyde is used as the d^1 synthon, this produced mixtures of regioisomers **241** and **248**, for heptanal, or **242** and **249**, for octanal. These regioisomers were not successfully separated by column chromatography and this approach was not investigated further.

The use of thioacetals as equivalent d_1 synthons in the synthesis of the target hydroxyketones was then investigated (Scheme 135).[204]
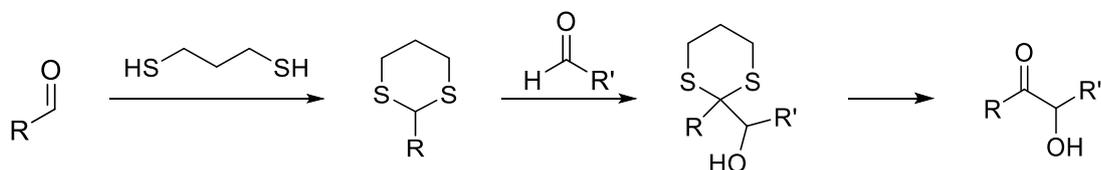
Scheme 135. General scheme for the synthesis of target hydroxyketones using thioacetal umpolung chemistry

Thioacetals **250** and **251** were synthesised relatively straightforwardly using an adapted literature procedure.[205] The aldehydes acetaldehyde and propanal were respectively stirred in dichloromethane at 0 °C with propane-1,3-dithiol and boron trifluoride diethyl etherate added dropwise (Scheme 136). Thioacetals **250** and **251** were isolated in yields of 94% and 97%, respectively.
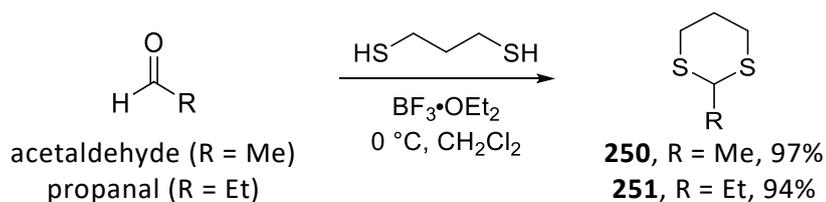
*Scheme 136. Synthesis of thioacetals **250** and **251** from acetaldehyde and propanal, respectively*

These thioacetals were then reacted with a series of long-chain aldehydes using another literature procedure.[206] *n*-Butyllithium was added dropwise to a solution of the thioacetal at -78 °C, followed by the dropwise addition of the aldehyde (Scheme 137). This gave the α-hydroxythioacetals **252** - **262** in moderate isolated yields of 38-55% (Table 36).
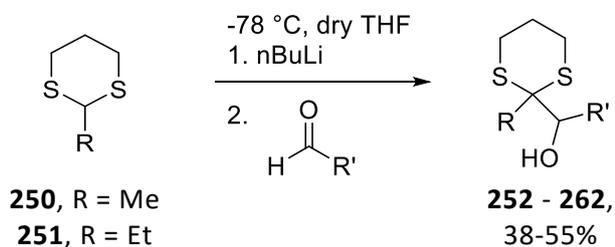
Scheme 137. Synthesis of α-hydroxythioacetals **252** - **262** by reacting **250** and **251**, respectively, with a series of six aldehydes

R	R'	PRODUCT	YIELD
Me	Pentyl	**252**	43%
	Hexyl	**253**	39%
	Heptyl	**254**	45%
	Octyl	**255**	52%
	Pentenyl	**256**	49%
Et	Pentyl	**257**	38%
	Hexyl	**258**	40%
	Heptyl	**259**	45%
	Octyl	**260**	55%
	Pentenyl	**261**	39%
	Decyl	**262**	53%

Table 36. Results of the synthesis of α-hydroxythioacetals **252** - **262**

This series of 10 α-hydroxythioacetals were then subjected to literature conditions for the conversion of the thioacetal back into a carbonyl. Initially, a literature procedure using *N*-chlorosuccinimide and silver nitrate was applied,[197] but found not to fully remove the thioacetal. An alternative procedure using iodomethane and calcium carbonate was then used,[207] giving α-hydroxyketones **225** - **228**, **241**, **242**, **235** and **263** - **266** in 69 - 80% yield (Scheme 138, Table 37).
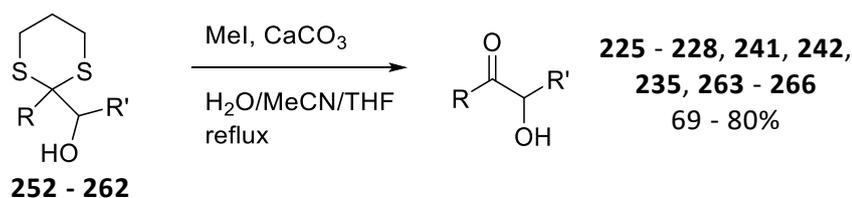
Scheme 138. Synthesis of α-hydroxyketones **225** - **228**, **241**, **242**, **235** and **263** - **266** from α-hydroxythioacetals **252** - **262** by removal of the thioacetal using iodomethane and calcium carbonate

R	R'	STARTING MATERIAL	PRODUCT	YIELD
Me	Pentyl	**252**	**225**	74%
	Hexyl	**253**	**226**	71%
	Heptyl	**254**	**263**	73%
	Octyl	**255**	**264**	77%
	Pentenyl	**256**	**265**	71%
Et	Pentyl	**257**	**227**	69%
	Hexyl	**258**	**228**	80%
	Heptyl	**259**	**241**	71%
	Octyl	**260**	**242**	72%
	Pentenyl	**261**	**266**	72%
	Decyl	**262**	**235**	75%

Table 37. Results of the synthesis of α-hydroxyketones **225** - **228**, **241**, **242**, **235** and **263** - **266** from α-hydroxythioacetals **252** - **262** by removal of the thioacetal using iodomethane and calcium carbonate

The overall yields of these α-hydroxyketones from acetaldehyde or propionaldehyde were relatively low at 25 - 39% and the synthetic route used is not ideal in terms of sustainable, 'green' chemistry. However, this was considered acceptable as only small volumes of the α-hydroxyketones were required as standards.

This synthetic route is non-stereoselective, giving racemic samples of the α-hydroxyketones. It was therefore possible to develop chiral GC methods that allowed separation of the two enantiomers (Figure 31a). This enables the determination of *ee*s for these products.
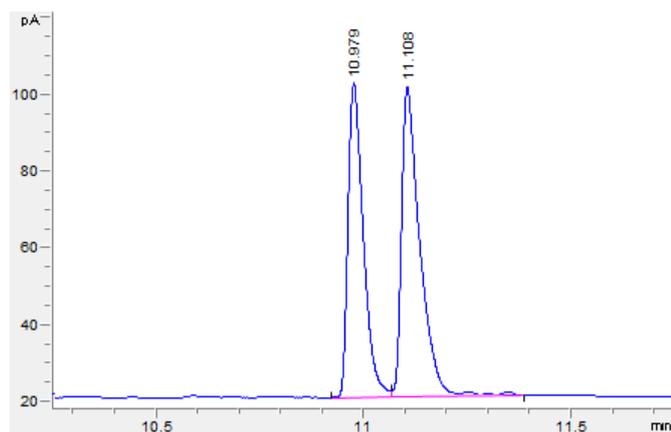
*a)   Separation of the two enantiomers of α-hydroxyketone **264***
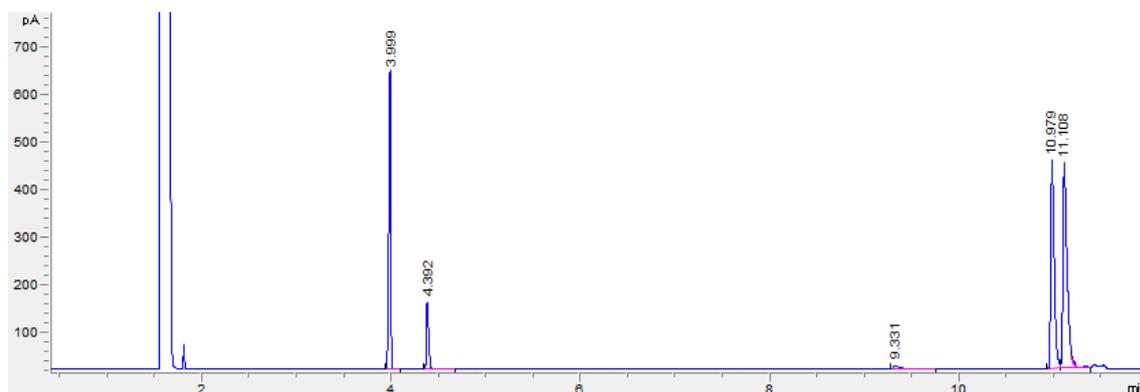
b)   Separation of a mixture of octanal, decane, and the two enantiomers of α-hydroxyketone
264

*Figure 31. Example chiral GC spectra of octanal, decane, and racemic α-hydroxyketone **264***

For each of the eleven products, a tailored GC instrument method was developed by varying the times and the temperatures used across the course of each run. Separation of the two product enantiomers, the starting aldehyde, and the chosen internal standard was thus achieved, and a typical chromatogram is depicted above (Figure 31b).

4.2.5. Mutant Screening for Acceptance of Novel Keto-acid Donors

For the medium throughput screening, eight mutants were chosen based on previous criteria (Section 4.2.1.) and an additional two were included that a were developed by Pierre Affaticati.[208]   These were a quadruple and a quintuple mutant based on the S385Y/D469T/R520Q triple mutant.   The quadruple contained a rationally designed mutation that stabilises the protein, while the quintuple included this stabilising mutation

and an additional mutation found by single-site screening to improve the acceptance of aromatic acceptor compounds.

The substrate donor sodium pyruvate Na-**223** was screened alongside C_5 - C_8 linear aldehydes to give α-hydroxyketone products **225**, **226**, **263** and **264**, and product yields were analysed by gas chromatography (Scheme 139, Table 38).

Scheme 139. Screening of sodium pyruvate Na-223 with C_5 - C_8 linear aldehydes

MUTANT	YIELD			
	n = 3	n = 4	n = 5	n = 6
D469T	72%	65%	6%	10%
D469Y	72%	65%	19%	19%
D469E	69%	61%	5%	9%
D469T/R520Q	73%	65%	9%	10%
D469Y/R520Q	72%	66%	21%	20%
S385T/D469T/R520Q	68%	63%	8%	8%
S385Y/D469T/R520Q	68%	68%	10%	13%
S385E/D469T/R520Q	72%	64%	Trace	Trace
quadruple	51%	48%	Trace	Trace
quintuple	43%	43%	Trace	Trace

Table 38. Results of the screening of sodium pyruvate Na-223 with C_5 - C_8 linear aldehydes

Many of the yields calculated were much higher than anticipated, and confirmation by scale-up and product isolation is required. It is possible than the use of a cosolvent had a significant positive effect on yield, or that the analysis has systematically overestimated yields. Regardless of this, trends can still be observed in the data and used to guide directed evolution for the acceptance of non-hydroxylated donor substrates.

In contrast to the larger scale reactions' isolated yields, which showed higher yields for longer chain aldehydes (Table 34 c.f. Table 32), the small-scale screening indicated a sharp decline in yield when using linear aldehydes longer than hexanal. This was attributed to the original reactions losing significant quantities of product in the workup, due to volatility. This observation could also be due to subsequent changes in experimental procedure, such as the use of 10% dimethylsulfoxide cosolvent.

Surprisingly little variance was found between the mutants used, though a few trends in the data were observable. Of the three single-point mutants, D469Y gave the best yields, while the introduction of a second mutation, R520Q, to the D469T and D469Y mutants increased the product yields, especially for longer chain aldehyde acceptors. The R520Q mutation is known to enhance acceptance of aliphatic aldehyde acceptors, and so this increase in yield could be due to superior aldehyde acceptance rather than donor acceptance, or could be partly due to the loss of positive charge allowing the more hydrophobic, non-α-hydroxylated donors to better bind the active site.

The introduction of a third mutation to the D469T/R520Q double mutant at the S385 site gave slightly worse yields for the S385T mutant, slightly better for S385Y, while the S385E mutant showed only trace product formation for heptanal and octanal. This could be due to the negatively charged aspartic acid residue causing poorer binding of the non-hydroxylated donor substrates. Tyrosine could be favoured over serine and threonine due to interactions involving the aromatic ring, or placement of the hydroxyl group.

The quadruple and quintuple mutants screened showed reduced yields for all aldehydes, potentially due to too significant a change to the enzyme for retention of activity in this instance. Combining the advantageous mutants from this data, an S385Y/D469Y/R520Q triple mutant could potentially give further improved yields. In all cases, high enantiomeric excesses were achieved, with no quantifiable levels of the minor enantiomer present (Figure 32).

*a) Sample of racemic **225** from non-enzymatic synthesis*

*b) Small-scale TK reaction of pentanal and pyruvate to give **225***
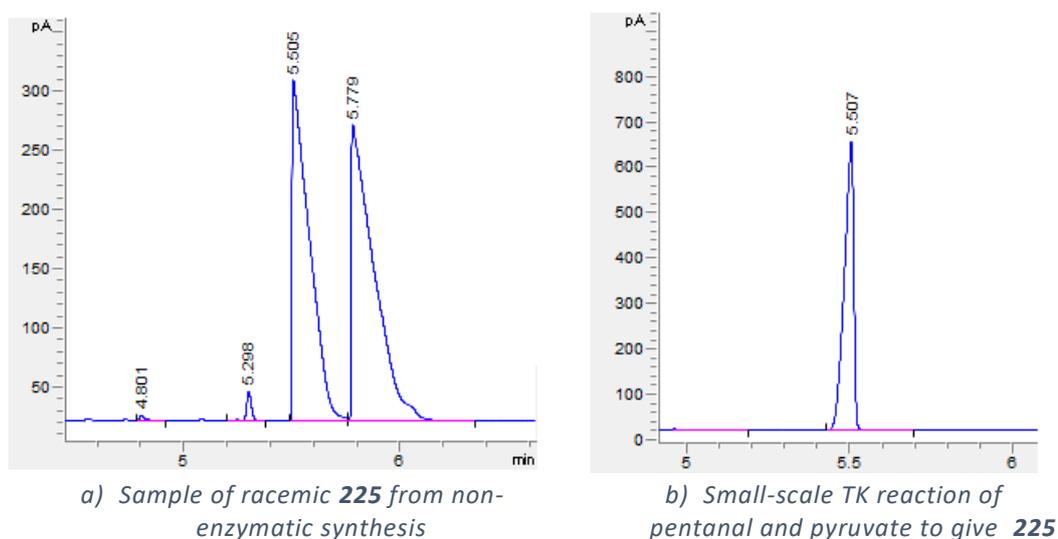
*Figure 32. GC spectra of α-hydroxyketone **225***

Next, the donor substrate ketobutyric acid **224**-H was screened in the same way (Scheme 140, Table 39). In addition to the previously used aldehydes, decanal was also screened due to the product, **235**, being desirable as a natural product and potential cholera treatment (Figure 29).[196,197]

227 (n = 3),
228 (n = 4),
241 (n = 5),
242 (n = 6),
235 (n = 8)

224-H   n = 3 – 6, 8

TK, MgCl$_2$, ThDP

50 mM MES buffer, pH 7, RT, 24 h
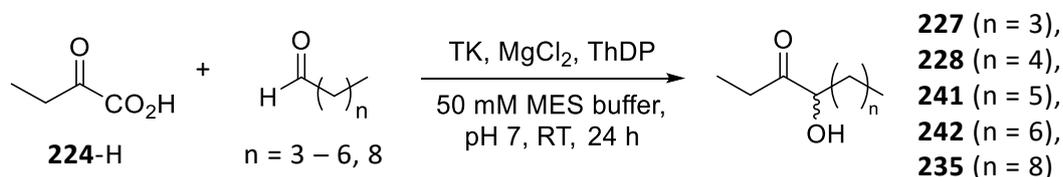
*Scheme 140. Screening of ketobutyric acid **224**-H with C$_5$ - C$_8$ linear aldehydes*

MUTANT	YIELD (*e.e*)				
	n = 3	n = 4	n = 5	n = 6	n = 8
D469T	72%	13%	3%	1%	/
D469Y	72%	26%	5%	2% (66%)	Trace
D469E	69%	10%	2%	Trace	/
D469T/R520Q	72%	21% (52%)	3%	Trace	Trace
D469Y/R520Q	71%	36% (72%)	6%	1%	Trace
S385T/D469T/R520Q	68%	16% (50%)	4% (50%)	Trace	/
S385Y/D469T/R520Q	69%	18% (44%)	5%	Trace	Trace
S385E/D469T/R520Q	70%	5%	Trace	Trace	Trace
quadruple	48%	Trace	Trace	/	/
quintuple	37%	Trace	Trace	Trace	/

*Table 39. Results of the screening of ketobutyric acid **224**-H with C$_5$ - C$_8$ linear aldehydes*

Similar trends were observed, though acceptance of the hepantal aldehyde acceptor was much lower, possibly due to the bulkier donor leaving less space in the active site. For C$_6$ - C$_8$ aldehydes (n = 4 - 6), lower enatiomeric excesses were observed for several mutants. Unfortunately, no quantifiable yields were observed with decanal.

Finally, 4-pentenal was screened with Na-**223** and **224**-H (Scheme 141, Table 40).
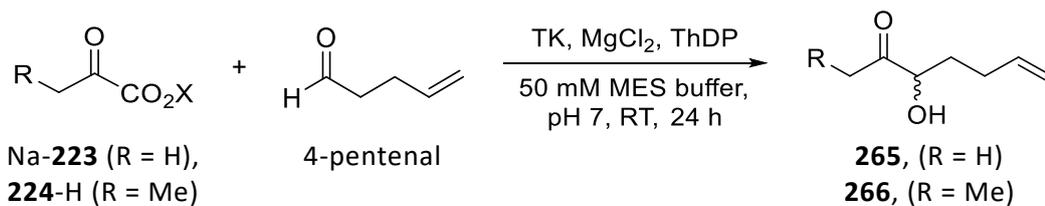
Scheme 141. Screening of sodium pyruvate Na-**223** or ketobutyric acid **224**-H with 4-pentenal

MUTANT	YIELD	
	265	266
D469T	67%	66%
D469Y	69%	67%
D469E	67%	65%
D469T/R520Q	66%	67%
D469Y/R520Q	67%	67%
S385T/D469T/R520Q	61%	59%
S385Y/D469T/R520Q	64%	61%
S385E/D469T/R520Q	67%	66%
quadruple	45%	40%
quintuple	39%	44%

Table 40. Results of the screening of sodium pyruvate Na-**223** or ketobutyric acid **224**-H with 4-pentenal

High yields were observed, similar to those obtained when using pentanal as the aldehyde. This demonstrates, as expected, that the terminal alkene has little effect of acceptance of the acceptor substrate. As with pentanal, similar yields were observed for all mutants except for the quadruple and quintuple mutants, which have consistently shown reduced yields throughout the screening.

To determine the stereoselectivity of these reactions, the GC spectra were compared to those of the racemic products and the isolated enantiopure products from the scaled up TK reactions. Of the two enantiomer peaks observed in the racemic samples' spectra, the isolated TK products' GC peaks matched the peak with the earlier elution time. In every reaction mixture analysed during the medium-throughput screening, the elution times of the major enantiomers also matched the enantiomer peak with the earlier elution time. This indicates that they all share the same stereoselectivity, which was determined, for the larger scale TK products, to favour the (*S*)-enantiomer.

4.3. Summary of Chapter IV

A medium throughput procedure for screening transketolase mutants was developed, using chiral gas chromatography for the quantification of yield and enantiomeric excess. Eleven products were synthesised using thioacetal umpolung chemistry and used to quantify the results of this medium throughput screen. Ten mutants and wild-type transketolase were then screened for their acceptance of two novel keto-acid donor substrates, pyruvate **223** and ketobutyrate **224**, using a series of linear aldehydes as the acceptor substrates. Results showed high acceptance of the donor substrates with shorter chain aldehydes, with yields of up to 73%, possibly aided by the organic cosolvent.

Future Work

Using this medium throughput methodology to carry out a screen of the same mutants but using the widely accepted donor HPA **209** would allow a direct comparison to the results found for pyruvate **223** and ketobutyrate **224**. This could be used to determine to what extent differences in yield were due to differences in donor tolerance rather than aldehyde tolerance.

Further mutant screening based on these findings could further improve the acceptance of these novel donor substrates, and combining with other aldehyde acceptors could give a range of pharmaceutically relevant compounds.

The transketolase reactions described here were highly selective for the (*S)*- enantiomers of the α-hydroxyketone products. Studies into the use of known stereoselectivity-inverting mutations, such as H26Y, could allow the formation of the (*R)*- enantiomers.

Chapter V. Thesis Conclusions

This thesis has described detailed investigations into the synthesis of aromatic compounds by Diels-Alder reactions of furfural hydrazones and their analogues (Chapter II), downstream reactivity of these aromatic compounds and example syntheses of commercially relevant compounds (Chapter III), and the use of novel donor substrates in transketolase enzyme biotransformations in the synthesis of α-hydroxyketones (Chapter IV). Potential future work was described at the end of each chapter, as relevant.
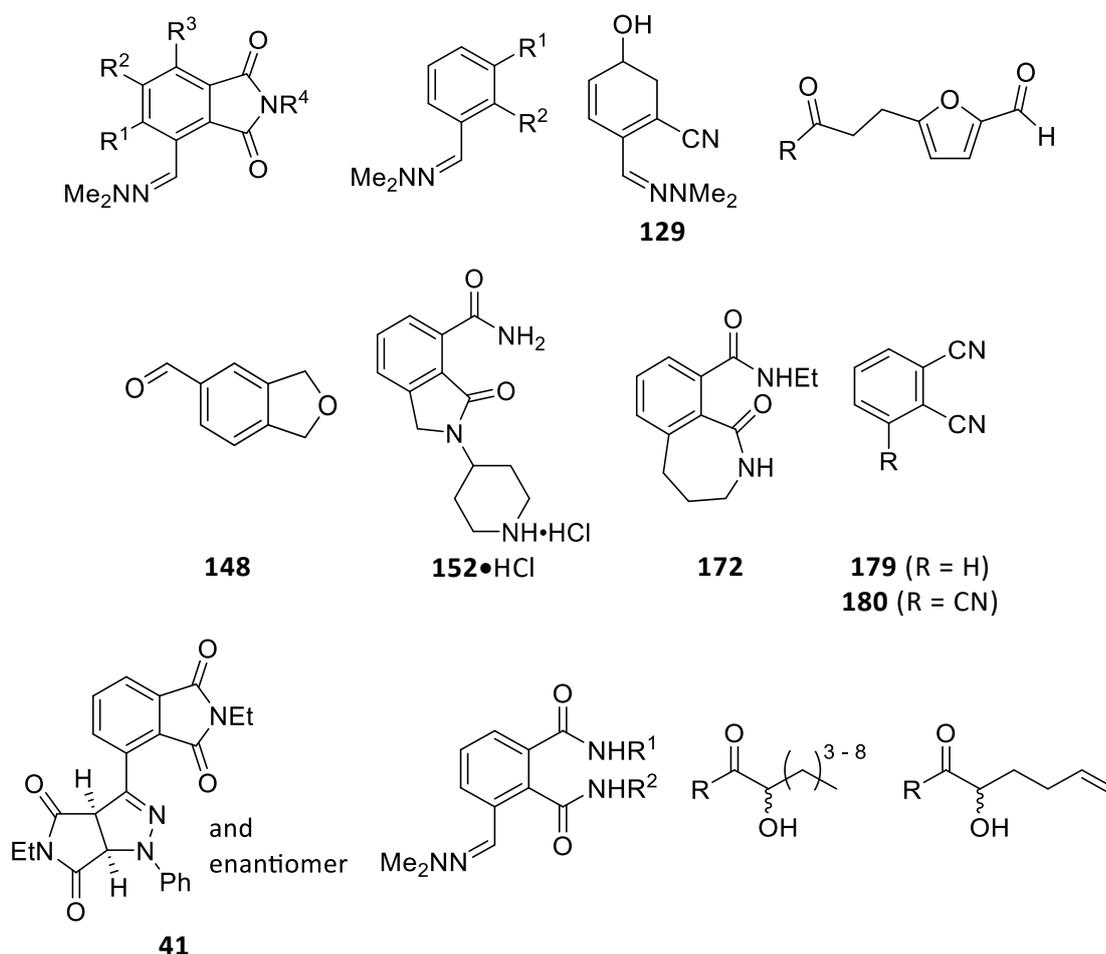
Figure 33. A selection of compounds generated during the PhD

A range of structures have been generated, some of which have current applications, such as the PARP-inhibitor **152**, and the aromatic dinitriles phthalonitrile **179** and benzene-1,2,3-tricarbonitrile **180**. Others serve as examples of accessible motifs that could be further manipulated to produce compounds of interest or generated as part of a compound library (Figure 33).

5.1.1. Adherence to the Principles of Green Chemistry

Within Chapter II, a Diels-Alder/aromatisation reaction sequence was developed with adherence to the principles of green chemistry, using water as the solvent and no catalysts, with facile isolation by filtration, near total atom efficiency, and water as the sole by-product.

As described in Chapter III, more concessions were required to achieve the desired reactivity, particularly regarding the use of organic solvents. However, in most cases a relatively sustainable option was found, such as the use of acetone in hydrazone hydrolysis reactions, a methanol/water mixture for hydrogenations, and methanol in MMPP oxidations. These solvents all have acceptable profiles according to the 2010 GSK Solvent Selection Guide (Table 41).[13] When a less 'green' solvent, THF, was required for the synthesis of target molecule **152**, the three-step synthesis was performed in 74% yield in a single pot, minimising solvent use and need for intermediate purifications. The product **152** was also collected as a salt by filtration and purified by recrystallization in a mixture of water and methanol, minimising peripheral solvent use.

SOLVENT	WASTE	ENVIRONMENTAL IMPACT	HEALTH	FLAMMABILITY & EXPLOSION	REACTIVITY/ STABILITY	LIFE CYCLE SCORE
water	4	10	10	10	10	10
acetone	3	9	8	4	9	7
methanol	4	9	5	5	10	9
ethanol	3	8	8	6	9	9
THF	3	5	6	3	4	4
DMSO	5	5	7	9	2	6

Table 41. A selection of solvents' ratings in the 2010 GSK Solvent Selection Guide[13]

For the biocatalysis in Chapter IV, a concession was made in the use of 10% dimethyl sulfoxide cosolvent during mutant screening. This was deemed acceptable for small-scale screening purposes, as cosolvent screens suggested that an alternative, greener organic solvent would similarly improve aldehyde acceptor solubility (Table 35), and biotransformation reactions are still excellent examples of sustainable, green chemistry regardless of the use of 10% cosolvent.

Chapter VI. Experimental Details

6.1. General Experimental

All reagents were obtained from commercial sources and used as received unless otherwise stated. Analytical thin layer chromatography was performed on aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F_{254}) and compounds visualised by exposure to UV light, potassium permanganate, phosphomolybdic acid or ninhydrin. Flash column chromatography was carried out using silica gel 60, SDS, 0.04–0.06 mm.

Melting points (m.p.) were established using a Gallenkamp apparatus and measured in °C. ^1H and ^{13}C NMR spectra were recorded at 298 K at the field indicated using Bruker AMX 300, AMX 400, Avance 500 and Avance 600 machines. Coupling constants were measured in Hertz (Hz) and referenced to the deuterated solvent used. Infrared spectra were recorded on Perkin Elmer Spectrum 100 FTIR spectrometer. Mass spectra were recorded on Thermo Finnegan MAT 900XP and Micro Mass Quattro LC electrospray mass spectrometers VG ZAB 2SE, or LTQ orbitrap XL (EPSRC National Mass Spectrometry Service Centre, Swansea University). Optical rotations were recorded on a Perkin Elmer 343 model polarimeter at 589 nm at 20 °C using the indicated solvent and quoted in deg cm^2 g^{-1} and concentration (*c*) in g/100 mL.

Chiral gas chromatography spectra were recorded using a 7820A GC system with Beta Dex 225 capillary Supelco column, using flame ionisation detection. Injection volumes of 5 µL were used with temperature gradients of 50 – 200 °C were used, and quantitative analysis was performed with respect to chemical standards. Further method details, retention times, calibration curves, and example traces are provided (Section 6.4.).

6.2. Biocatalysis Procedures

Preparation of cell lysate

Glycerol stocks of TK mutants (25% v/v glycerol) were obtained from Panwajee Payongsri or propagated from stocks provided by Pierre Affaticati and stored at -80 °C until needed. These were then used to inoculate 250 mL shake flasks containing 50 mL LB broth containing ampicillin (150 µg/mL) and incubated overnight at 37 °C and 160 rpm using an SI 50 orbital shaker (Stuart Scientific, Redhill, UK.) This culture was then used to inoculate

a 1 L shake flask containing 200 mL LB-Amp media and incubated at 37 °C and 200 rpm. The culture was then centrifuged at 4500 rpm for 20 minutes at 4 °C and approximately 4 g of cell paste was obtained and subsequently re-suspended in cold buffer (5 mM, pH 7) to a final concentration of 1 g of cell paste per 10 mL buffer. The cells were then lysed by sonication for 4 minutes (cycles of 10 seconds on, 10 seconds off) using a Soniprep 150 sonicator (MSE, Sanyo, Japan), and centrifuged at 4500 rpm for 20 minutes at 4 °C. Supernatant cell-free lysate (approximately 40 mL total) was stored in 2 mL eppendorf tubes and kept at -20 °C for a maximum of 1 month.   TK protein concentration was determined to be approximately 1 mg/mL.

Standard TK biotransformation procedure

A stock "10x" cofactor solution of $MgCl_2$ (0.39 g) and ThDP (0.22 g) in water (10 mL) was adjusted to pH 7 and stored at 4 °C. 1 mL of 10x cofactor solution was mixed with TK cell-free lysate (2 mL or 4 mL, as indicated) and water added to bring the volume to 10 mL. The lysate mixture was incubated for 20 minutes. In a separate flask, a mixture of 100 mM desired donor and desired acceptor was made up and adjusted to pH 7, then added to the enzyme mixture, to an end substrate concentration of 50 mM each. Reactions were then stirred at room temperature for 24 hours or 48 hours, as indicated, either in buffer at pH 7, or in an autotitrator programmed to add 1 M HCl when the pH increased above 7.

6.3. Compound Synthesis: Experimental Details and Compound Characterisation

2-Furaldehyde dimethylhydrazone **28**[107]

28
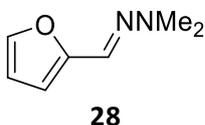
To a solution of furfural (28.8 mg, 25.4 μL, 300 μmol) in water (6 mL), *N,N*-dimethylhydrazine (24.0 mg, 30.4 μL, 400 μmol) was added and the mixture was stirred at RT for 40 min. The organic component was extracted with diethyl ether (3 x 30 mL), dried ($MgSO_4$), filtered, and the solvent removed under vacuum to give **28** as a red-brown liquid (36.5 mg, 76%).

R_f 0.67 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm^{-1}) 2850, 1524, 1445; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.95 (6H, s, N(CH$_3$)$_2$), 6.37 (1H, d, J = 3.3 Hz, 5-H), 6.39 (1H, dd, J = 3.3, 1.6 Hz, 4-H), 7.10 (1H, s, N=CH), 7.39 (1H, d, J = 1.6 Hz, 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 107.1 (C-4), 111.2 (C-5), 123.2 (N=CH), 141.8 (C-3), 152.1 (C-2); m/z HRMS (ESI+) found [MH]$^+$ 139.0875, C$_7$H$_{11}$N$_2$O requires 139.0871.

4-((2,2-Dimethylhydrazono)methyl)isobenzofuran-1,3-dione 30[107]

30
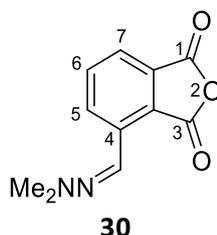
To a solution of 2-furaldehyde dimethylhydrazone **28** (1.93 g, 140 mmol) in chloroform (30 mL), maleic anhydride (1.18 g, 120 mmol) was added and a colour change from yellow to bright red-orange was observed. The mixture was stirred at RT for 16 h, the solvent removed under vacuum, and the residue triturated with anhydrous diethyl ether (30 mL), giving **30** as yellow needles (2.61 g, 85%).

R_f 0.22 (pet. ether 40-60/EtOAc, 17:3); M.p. 172-174 °C (CDCl$_3$), Lit[107] 173-174 °C; IR (film, cm^{-1}) 1830, 1758, 1580; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.18 (6H, s, N(CH$_3$)$_2$), 7.72 (2H, m, 6-H and 7-H), 7.86 (1H, s, N=CH), 8.37 (1H, dd, J = 6.8, 2.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 121.9 (CH), 122.5 (N=CH), 123.4 (C), 130.3 (C), 131.5 (C), 135.3 (CH), 138.3 (C), 163.4 (C=O), 163.5 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 219.0768, C$_{11}$H$_{11}$N$_2$O$_3$ requires 219.0770.

4-((2,2-Dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31**[107]

31
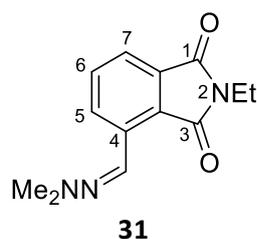
To a solution of furfural (17.2 mL, 208 mmol) in water (500 mL), *N,N*-dimethylhydrazine (19.0 mL, 250 mmol) was added and the mixture was stirred at 50 °C for 30 min. *N*-Ethylmaleimide (26.0 g, 208 mmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 100 mL) and dried to give **31** as yellow needles (49.6 g, 97%).

R_f 0.22 (pet. ether 40-60/EtOAc, 17:3); M.p. 142-143 °C (water), Lit[107] 141-142 °C (CHCl$_3$); IR (film, cm^{-1}) 2920, 2875, 1755, 1695, 1590; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.27 (3H, t, *J* = 7.3 Hz, CH$_2$C*H*$_3$), 3.14 (6H, s, N(CH$_3$)$_2$), 3.72 (2H, q, *J* = 7.3 Hz, C*H*$_2$CH$_3$), 7.55 (1H, t, *J* = 7.7 Hz, 6-H), 7.61 (1H, dd, *J* = 7.7, 1.1 Hz, 7-H), 8.14 (1H, s, N=CH), 8.22 (1H, dd, *J* = 7.7, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (CH$_2$*C*H$_3$), 32.9 (*C*H$_2$CH$_3$), 42.9 (N(CH$_3$)$_2$), 120.9 (C-7), 124.7 (N=CH), 124.9 (C), 129.0 (C-5), 132.6 (C), 133.4 (C-6), 136.4 (C), 168.4 (C=O), 169.4 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 246.1341, C$_{13}$H$_{16}$N$_3$O$_2$ requires 246.1243.

3-((2,2-Dimethylhydrazono)methyl)phthalonitrile **32**[109]

32
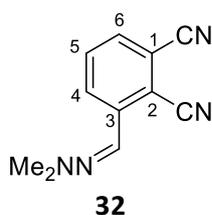
To a solution of 2-furaldehyde dimethylhydrazone, **28** (9.72 g, 928 µL, 7.00 mmol), in water (50 mL), fumaronitrile (10.9 g, 14.0 mmol) was added and the reaction stirred at 50 °C for 48 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (3 x 200 mL) and dried to give **32** as pale green needles (10.8 g, 77%).

R_f 0.22 (pet. ether 40-60/EtOAc, 17:3); M.p. 168-169 °C (EtOAc), Lit[109] 165-166 °C (CHCl$_3$); IR (film, cm^{-1}) 2219 (C≡N), 1570, 1546; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.16 (6H, s, N(CH$_3$)$_2$), 7.29 (1H, s, N=CH), 7.52 (1H, dd, *J* = 7.4, 1.4 Hz, 6-H), 7.55 (1H, m, 5-H), 8.19 (1H, dd, *J* = 8.0,

1.4 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 110.7 (C), 115.0 (C), 116.1 (CN), 116.2 (CN), 122.3 (N=CH), 128.3 (C-4), 130.5 (C-6), 132.4 (C-5), 142.6 (C); *m/z* HRMS (ESI+) found [MH]$^+$ 199.0979, C$_{11}$H$_{11}$N$_4$ requires 199.0984.

1-(Furan-2-ylmethylene)-2-phenylhydrazine **36**[209]

36
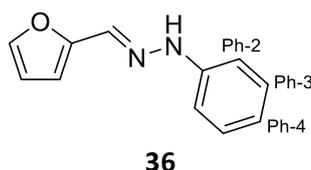
To a solution of furfural (96.0 mg, 84.7 μL, 1.00 mmol) in water (20 mL), phenylhydrazine (108 mg, 1.00 mmol) was added and the mixture was stirred at 50 °C for 1 h. The organic component was extracted with diethyl ether (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **36** as an orange-brown solid (173 mg, 93%).

R$_f$ 0.74 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 3317 (N-H), 1601, 1584, 1511; M.p. 84 - 85 °C (Et$_2$O), Lit[209] 98 °C; ^{1}H NMR (CDCl$_3$; 500 MHz) 6.46 (1H, dd, *J* = 3.3, 1.6 Hz, 4-H), 6.56 (1H, d, *J* = 3.3 Hz, 3-H), 6.88 (1H, t, *J* = 7.3 Hz, Ph-4-H), 7.09 (2H, d, *J* = 7.7 Hz, Ph-2-H), 7.27 (2H, m, Ph-3-H), 7.47 (1H, s, N=CH), 7.57-7.61 (2H, m, NH & 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 109.4 (CH, C-3), 111.7 (CH, C-4), 112.9 (CH, Ph-2), 120.4 (CH, Ph-4), 127.8 (CH, C-5), 129.4 (CH, Ph-3), 143.1 (N=CH), 144.4 (Ph-1), 150.6 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 187.0921, C$_{11}$H$_{11}$N$_2$O requires 187.0871.

2-Ethyl-4-((2-phenylhydrazono)methyl)isoindoline-1,3-dione **38**[110]

38
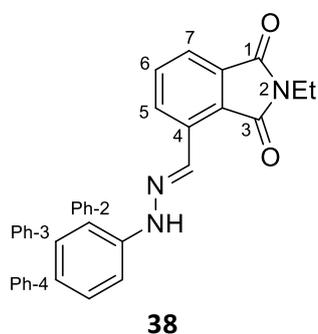
To a solution of furfural (8.28 mL, 100 mmol) in water (250 mL), phenylhydrazine (9.83 mL, 100 mmol) was added and the mixture was stirred at 50 °C for 1 h. *N*-Ethylmaleimide (25.0 g, 200 mmol) was added and the reaction stirred at 50 °C for 16 h. The reaction was cooled

to RT and the precipitate was collected by filtration, washed with cold water (2 x 50 mL) and then resuspended in THF/water (1:9 v/v, 250 mL). The mixture was stirred at 100 °C for 72 h then cooled and water (250 mL) added. The precipitate was collected by filtration, washed with cold water (2 x 50 mL) and dried to give **38** as an orange solid (24.3 g, 83%).

R_f 0.76 (pet. ether 40-60/EtOAc, 3:2); M.p. 164 - 166 °C (water), Lit[110] 168 - 169 °C; IR (film, cm^{-1}) 3274 (N-H), 1761, 1696, 1596, 1547, 1527; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.28 (3H, t, J = 7.3 Hz, CH$_2$CH$_3$), 3.74 (2H, q, J = 7.3 Hz, CH$_2$CH$_3$), 6.93 (1H, tt, J = 7.3, 1.0 Hz, Ph-4-H), 7.15 (2H, dt, J = 8.0, 1.0 Hz, Ph-2-H), 7.31 (2H, m, Ph-3-H), 7.64 (1H, m, 6-H), 7.71 (1H, dd, J = 7.3, 1.0 Hz, 7-H), 8.13 (1H, s br, NH), 8.38 (1H, dd, J = 8.0, 1.0 Hz, 5-H), 8.73 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (CH$_2$CH$_3$), 33.0 (CH$_2$CH$_3$), 113.2 (2 × Ph-2), 121.2 (Ph-4), 122.2 (C-7), 126.2 (C), 129.5 (2 x Ph-3), 129.8 (C-5), 131.1 (N=CH), 132.6 (C), 133.5 (C-6), 134.5 (C-4), 143.9 (Ph-1), 168.1 (C=O), 169.1 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 294.1265, C$_{17}$H$_{16}$N$_3$O$_2$ requires 294.1243.

(R,R)- and (S,S)- 2-Ethyl-4-(5-ethyl-4,6-dioxo-1-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-c] pyrazol-3-yl)isoindoline-1,3-dione **41**[110]

41
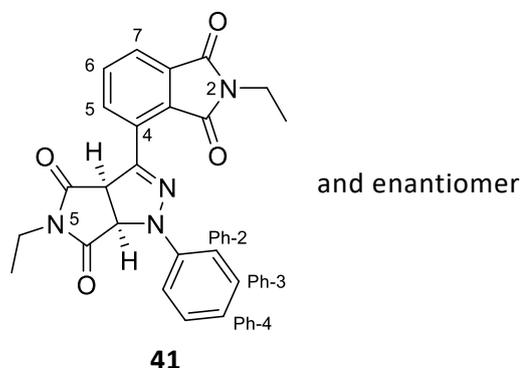
2-Ethyl-4-((2-phenylhydrazono)methyl)isoindoline-1,3-dione, **15** (147 mg, 500 μmol), and *N*-ethylmaleimide (626 mg, 5.00 mmol) were combined in acetonitrile/water (2:1, 5 mL) in a microwave tube. The tube was sealed and heated to 200 °C by microwaves for 15 min. The mixture was added to water (50 mL) and the precipitate collected by filtration, washed with cold water (2 x 20 mL) and dried to give **41** as a dark orange solid (152 mg, 73%).

R_f 0.53 (pet. ether 40-60/EtOAc, 4:1); M.p. 218 °C (water), Lit[110] 214-215 °C; IR (film, cm^{-1}) 2920, 1764, 1759, 1694, 1590, 1532; ^{1}H NMR (CDCl$_3$; 500 MHz) 1.15 (3H, t, J = 7.2 Hz, CH$_2$CH$_3$), 1.30 (3H, t, J = 7.2 Hz, CH$_2$CH$_3$), 3.54 (2H, q, J = 7.2 Hz, CH$_2$CH$_3$), 3.77 (2H, q, J = 7.2 Hz, CH$_2$CH$_3$), 5.26 (1H, d, J = 11.1 Hz, CH), 6.34 (1H, d, J = 11.1 Hz, CH), 7.04 (1H, t, J =

7.3, Ph-4-H), 7.37 (2H, m, Ph-3-H), 7.60 (2H, d, J = 8.5 Hz, Ph-2-H), 7.71 (1H, m, 6-H), 7.89 (1H, d, J = 7.4 Hz, 7-H), 8.14 (1H, d, J = 7.9 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 12.9 (5-CH$_2$CH$_3$), 14.0 (2-CH$_2$CH$_3$), 33.3 (2-CH$_2$CH$_3$), 34.7 (5-CH$_2$CH$_3$), 54.5 (CH), 65.8 (CH), 114.7 (2 x Ph-2), 122.3 (Ph-4), 123.9 (C-5), 128.2 (C), 129.0 (C), 129.4 (2 x Ph-3), 133.5 (C), 133.8 (C-6), 134.8 (C-7), 141.6 (C), 143.9 (Ph-1), 167.7 (C=O), 168.4 (C=O), 172.3 (C=O), 173.0 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 417.1581, C$_{23}$H$_{21}$N$_4$O$_4$ requires 417.1563.

2-(5-((2,2-Dimethylhydrazono)methyl)furan-2-yl)naphthalene-1,4-dione **48**[109]

48
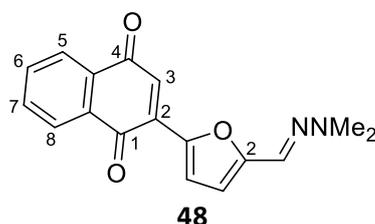
Synthesis adapted from literature procedure.[109] The reaction was carried out under anhydrous conditions. To a solution of 2-furaldehyde dimethylhydrazone **28** (266 µL, 2.00 mmol) in anhydrous toluene (10 mL), 1,4-napthoquinone (333 mg, 2.00 mmol) was added and the mixture was heated at reflux for 48 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 20 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **48** as dark purple crystals (400 mg, 68%).

R$_f$ 0.45 (pet. ether 40-60/EtOAc, 3:2); M.p. 172 - 173 °C (water), Lit[109] 175 - 176 °C (CH$_2$Cl$_2$/hexanes); IR (film, cm^{-1}) 2860, 1671, 1651, 1587; ^{1}H NMR (CDCl$_3$; 500 MHz) 3.08 (6H, s, N(CH$_3$)$_2$), 6.59 (1H, d, J = 3.7 Hz, furan 3-H), 7.03 (1H, s, N=CH), 7.33 (1H, s, napthyl 3-H), 7.66 (1H, d, J = 3.7 Hz, furan 4-H), 7.69-7.75 (2H, m, napthyl 5-H & 8-H), 8.06 − 8.13 (2H, m, napthyl 6-H & 7-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 109.8 (furan C-3), 119.9 (N=CH), 122.1 (furan C-4), 126.0 (napthyl CH), 126.4 (napthyl C-3), 126.8 (napthyl CH), 132.5 (napthyl C), 132.6 (napthyl C), 133.4 (napthyl CH), 134.0 (napthyl CH), 134.8 (napthyl C-4), 145.8 (furan C-5), 156.2 (furan C-2), 183.7 (C=O), 184.8 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 295.1080, C$_{17}$H$_{15}$N$_2$O$_3$ requires 295.1083.

2-Ethyl-1,3-dioxoisoindoline-4-carbaldehyde 55[114]
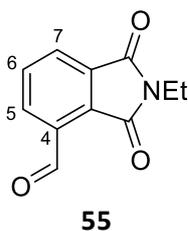
55

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in acetone (30 mL), Amberlyst® 15 (1.00 g) was added, and the mixture was stirred at RT for 3 h. The Amberlyst® 15 was removed by filtration and the solvent removed under vacuum to give **55** as fine colourless crystals (192 mg, 94%).

R_f 0.44 (pet. ether 40-60/EtOAc, 2:1); M.p. 126-127 °C (acetone); IR (film, cm^{-1}) 1773, 1692, 1610; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.31 (3H, t, J = 7.3 Hz, CH$_2$CH_3), 3.80 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 7.84 (1H, t, J = 7.5 Hz, 6-H), 8.02 (1H, d, J = 7.5 Hz, 7-H), 8.24 (1H, d, J = 7.5 Hz, 5-H), 11.05 (1H, s, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.0 (CH$_2$CH$_3$), 33.4 (CH$_2$CH$_3$), 128.0 (C-6), 131.4 (C-7), 132.3 (C), 133.1 (C), 133.7 (C), 134.3 (C-5), 167.3 (C=O), 167.7 (C=O), 188.9 (CHO); m/z HRMS (ESI+) found [MH]$^+$ 204.0652, C$_{11}$H$_{10}$NO$_3$ requires 204.0661.

4-((2,2-Dimethylhydrazono)methyl)isoindoline-1,3-dione 56[114]

56
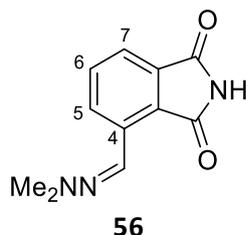
To a solution of furfural (4.81 g, 4.13 mL, 50.0 mmol) in water (100 mL), *N,N*-dimethylhydrazine (3.60 g, 4.56 mL, 60.0 mmol) was added and the mixture was stirred at 50 °C for 30 min. Maleimide (7.32 g, 75.0 mmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 500 mL) and dried to give **56** as yellow needles (9.33 g, 86%).

R_f 0.41 (pet. ether 40-60/EtOAc, 3:2); M.p. 238-240 °C (water); IR (film, cm^{-1}) 3196, 1759, 1715, 1544; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.13 (6H, s, N(CH$_3$)$_2$), 7.55 (1H, s br, NH), 7.60 (1H, t, J = 7.4 Hz, 6-H), 7.62 (1H, dd, J = 7.4, 1.1 Hz, 7-H), 8.05 (1H, s, N=CH), 8.27 (1H, dd, J =

7.4, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.8 (N(CH$_3$)$_2$), 121.2 (CH), 124.1 (N=CH), 125.1 (C), 129.4 (C), 133.0 (C), 133.8 (CH), 137.0 (C), 168.1 (C=O), 169.0 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 218.0940, C$_{11}$H$_{14}$N$_3$O$_2$ requires 218.0930.

1,1-Dimethyl-2-((5-methylfuran-2-yl)methylene)hydrazine 58[109]
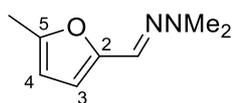
58

5-Methylfurfural, **18**, (29.8 µL, 300 µmol) and *N,N*-dimethylhydrazine (30.4 µL, 400 µmol) were added to water (2 mL) and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **58** as a yellow oil (28.3 mg, 56%).

R$_f$ 0.67 (pet. ether 40-60/EtOAc, 3:17); IR (film, cm^{-1}) 1521, 1468; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.33 (3H, s, CH$_3$), 2.93 (6H, s, N(CH$_3$)$_2$), 5.99 (1H, d, *J* = 3.0 Hz, 3-H), 6.25 (1H, d, *J* = 3.0 Hz, 4-H), 7.10 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.9 (CH$_3$), 42.0 (N(CH$_3$)$_2$), 107.5 (C-4), 109.2 (C-3), 124.6 (N=CH), 150.4 (C-5), 152.4 (C-2). *m/z* HRMS (ESI+) found [MH]$^+$ 153.1022, C$_8$H$_{13}$N$_2$O requires 153.1028.

(5-((2,2-Dimethylhydrazono)methyl)furan-2-yl)methanol 59
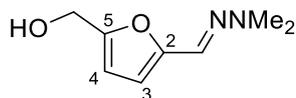
59

Hydroxymethylfurfural (30.4 µL, 300 µmol) and *N,N*-dimethylhydrazine (30.4 µL, 400 µmol) were added to water (2 mL) and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **59** as a dark yellow oil (35.2 mg, 77%).

R$_f$ 0.46 (EtOAc); IR (film, cm^{-1}) 3237 (br), 1525, 1432; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.92 (6H, s, N(CH$_3$)$_2$), 4.58 (2H, s, C*H*$_2$OH), 6.28 (2H, m, 3-H & 4-H), 7.06 (1H, s, N=CH); ^{13}C NMR

(CDCl$_3$; 151 MHz) 42.9 (N(CH$_3$)$_2$), 57.6 (CH$_2$OH), 108.3 (C-4), 109.6 (C-3), 123.5 (N=CH), 152.0 (C-5), 153.7 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 169.0982, C$_8$H$_{13}$N$_2$O$_2$ requires 169.0977.

2-((4,5-Dimethylfuran-2-yl)methylene)-1,1-dimethylhydrazine **60**
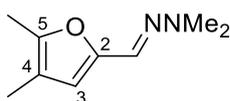
60

4,5-Dimethylfurfural, **57** (36.6 µL, 37.3 mg, 300 µmol), and *N,N*-dimethylhydrazine (30.4 µL, 400 µmol) were added to water (2 mL) and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **60** as a yellow oil (31 mg, 62%).

R$_f$ 0.64 (pet. ether 40-60/EtOAc, 3:17); IR (film, cm^{-1}) 1524, 1465; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.31 (3H, s, CH$_3$), 2.40 (3H, s, CH$_3$), 2.92 (6H, s, N(CH$_3$)$_2$), 6.07 (1H, s, 3-H), 7.09 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.8 (CH$_3$), 14.3 (CH$_3$), 41.9 (N(CH$_3$)$_2$), 107.8 (C-4), 110.7 (C-3), 124.6 (N=CH), 150.2 (C-5), 152.1 (C-2). *m/z* HRMS (ESI+) found [MH]$^+$ 167.1182, C$_9$H$_{15}$N$_2$O requires 167.1184.

4-((2,2-Dimethylhydrazono)methyl)-2-ethyl-7-methylisoindoline-1,3-dione **74**[109]
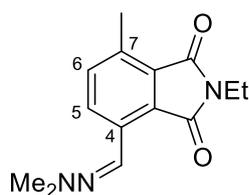
74

To a solution of 5-methylfurfural, **18** (110 mg, 99.5 µL, 1.00 mmol), in water (4 mL), *N,N*-dimethylhydrazine (72.0 mg, 91.2 µL, 1.20 mmol) was added and the mixture was stirred at 50 °C for 2 h. *N*-Ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **74** as yellow needles (260 mg, 93%).

R$_f$ 0.70 (pet. ether 40-60/EtOAc, 17:3); M.p. 144-146 °C (CDCl$_3$), Lit[109] 145-146 °C (CHCl$_3$); IR (film, cm^{-1}) 2976, 2937, 2868, 1750, 1685, 1590; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.26 (3H, t, *J* = 7.3 Hz, CH$_2$C*H*$_3$), 2.65 (3H, s, Ar-CH$_3$), 3.09 (6H, s, N(CH$_3$)$_2$), 3.70 (2H, q, *J* = 7.3 Hz, C*H*$_2$CH$_3$), 7.32 (1H, d, *J* = 8.2 Hz, 6-H), 8.09 (1H, d, *J* = 8.2 Hz, 5-H), 8.17 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (CH$_2$*C*H$_3$), 17.7 (Ar-CH$_3$), 32.7 (*C*H$_2$CH$_3$), 42.9 (N(CH$_3$)$_2$), 125.3 (C), 125.6 (N=CH), 128.7 (C), 128.8 (C-5), 134.1 (C-6), 135.7 (C), 136.2 (C), 169.1 (C=O), 169.3 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 260.1480, C$_{14}$H$_{18}$N$_3$O$_2$ requires 260.1399.

4-((2,2-Dimethylhydrazono)methyl)-2-ethyl-7-(hydroxymethyl)isoindoline-1,3-dione **75**[114]

75
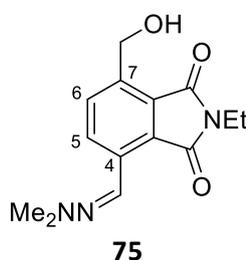
To a solution of 5-hydroxymethylfurfural (126 mg, 97.8 µL, 1.00 mmol) in water (4 mL), *N,N*-dimethylhydrazine (72.0 mg, 91.2 µL, 1.20 mmol) was added and the mixture was stirred at 50 °C for 2 h. *N*-Ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at 50 °C for 30 min. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **75** as yellow needles (251 mg, 95%).

R$_f$ 0.14 (pet. ether 40-60/EtOAc, 17:3); M.p. 152-154 °C (CDCl$_3$); IR (film, cm^{-1}) 3306 (br), 2937, 2871, 1750, 1682, 1592; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.27 (3H, t, *J* = 7.2 Hz, CH$_2$C*H*$_3$), 3.12 (6H, s, N(CH$_3$)$_2$), 3.72 (2H, q, *J* = 7.2 Hz, C*H*$_2$CH$_3$), 4.89 (2H, s, C*H*$_2$OH), 7.45 (1H, d, *J* = 8.2 Hz, 6-H), 8.14 (1H, s, N=CH), 8.18 (1H, d, *J* = 8.2 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (CH$_2$*C*H$_3$), 33.0 (*C*H$_2$CH$_3$), 42.7 (N(CH$_3$)$_2$), 62.7 (CH$_2$OH), 124.3 (N=CH), 125.2 (C), 129.4 (C-5), 133.2 (C-6), 134.3 (C), 136.0 (C), 138.5 (C), 169.2 (C=O), 170.2 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 275.1268, C$_{14}$H$_{18}$N$_3$O$_3$ requires 275.1270.

7-((2,2-Dimethylhydrazono)methyl)-2-ethyl-4,5-methylisoindoline-1,3-dione 76[114]

76
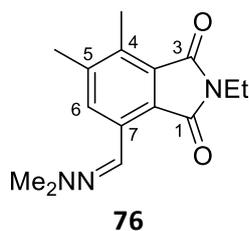
To a solution of 4,5-dimethylfurfural **57** (124 mg, 122 µL, 1.00 mmol) in water (4 mL, pH 6), *N,N*-dimethylhydrazine (72.0 mg, 91.2 mL, 1.20 mmol) was added and the mixture was stirred at 50 °C for 2 h. *N*-Ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **76** as yellow needles (195 mg, 72%).

R_f 0.10 (pet. ether 40-60/EtOAc, 4:1); M.p. 153-154 °C (CDCl$_3$); IR (film, cm^{-1}) 2938, 2864, 1748, 1691, 1546; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.25 (3H, t, *J* = 7.1 Hz, CH$_2$C*H*$_3$), 2.36 (3H, s, Ar-CH$_3$), 2.61 (3H, s, Ar-CH$_3$), 3.09 (6H, s, N(CH$_3$)$_2$), 3.69 (2H, q, *J* = 7.1 Hz, C*H*$_2$CH$_3$), 7.97 (1H, s, 6-H), 8.15 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.9 (CH$_2$*C*H$_3$), 14.1 (Ar-CH$_3$), 20.1 (Ar-CH$_3$), 32.6 (*C*H$_2$CH$_3$), 42.8 (N(CH$_3$)$_2$), 123.5 (C), 126.1 (N=CH), 129.0 (C), 129.3 (C-6), 133.5 (C), 135.6 (C), 144.5 (C), 169.2 (C=O), 169.6 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 274.1552, C$_{15}$H$_{20}$N$_3$O$_2$ requires 274.1556.

4-((2,2-Dimethylhydrazono)methyl)-2,7-diethylisoindoline-1,3-dione 77[114]

77
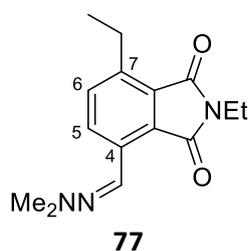
To a solution of 5-ethyl-2-furaldehyde, **61** (124 mg, 1.00 mmol), in water (4 mL) *N,N*-dimethylhydrazine (78 mg, 99 µL, 1.30 mmol) was added and the mixture was stirred at 50 °C. After 2.5 h, *N*-ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at the same temperature with a yellow precipitate forming within 5 min. After 4 h, the reaction was cooled to RT and the precipitate collected by filtration, washed with cold water (100 mL), and dried under vacuum to give **77** as yellow needles (205 mg, 75%).

R_f 0.38 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 2935, 2879, 2864, 2785, 1748, 1690, 1545, 1439; M.p. 109-111 °C (water); ^{1}H NMR (CDCl$_3$; 600 MHz) 1.26 (6H, m, 2 x CH$_2$CH_3), 3.07 - 3.10 (8H, m, N(CH$_3$)$_2$ & Ar-CH_2CH$_3$), 3.70 (2H, q, J = 7.2 Hz, NCH_2CH$_3$), 7.36 (1H, d, J = 8.4 Hz, 6-H), 8.12 (1H, d, J = 8.4 Hz, 5-H), 8.17 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (NCH$_2$$CH_3$), 15.1 (Ar-CH$_2$$CH_3$), 24.4 (Ar-$CH_2CH_3$), 32.6 (N$CH_2CH_3$), 42.8 (N(CH$_3$)$_2$), 125.4 (C), 125.7 (N=CH), 128.1 (C), 129.1 (C-5), 134.1 (C-6), 134.7 (C), 142.4 (C), 168.9 (C=O), 169.4 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 274.1559, C$_{15}$H$_{20}$N$_3$O$_2$ requires 274.1555.

4-((2,2-Dimethylhydrazono)methyl)-7-(ethoxymethyl)-2-ethylisoindoline-1,3-dione 78[114]

78
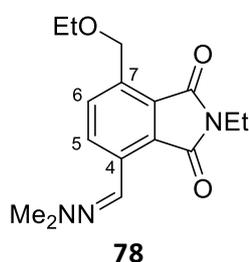
To a solution of 5-(ethoxymethyl)-2-furaldehyde, **62** (154 mg, 1.00 mmol), in water (4 mL, pH 6), N,N-dimethylhydrazine (78 mg, 99 µL, 1.30 mmol) was added and the mixture was stirred at 50 °C. After 4 h, N-ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at the same temperature with a yellow precipitate forming within 5 min. After 1 h, the reaction was cooled to RT and the precipitate collected by filtration, washed with cold water (100 mL), and dried under vacuum to give **78** as yellow solid (217 mg, 70%).

R_f 0.40 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 2980, 2938, 2870, 2795, 1751, 1692, 1543, 1437; M.p. 104-106 °C (water); ^{1}H NMR (CDCl$_3$; 600 MHz) 1.25 (3H, t, J = 7.2 Hz, NCH$_2$CH_3), 1.28 (3H, t, J = 7.0 Hz, OCH$_2$CH_3), 3.11 (6H, s, N(CH$_3$)$_2$), 3.64 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.69 (2H, q, J = 7.2 Hz, NCH_2CH$_3$), 4.97 (2H, s, Ar-CH$_2$), 7.70 (1H, d, J = 8.4 Hz, 6-H), 8.14 (1H, s, N=CH), 8.20 (1H, d, J = 8.4 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (NCH$_2$$CH_3$), 15.4 (OCH$_2$$CH_3$), 32.7 (N$CH_2CH_3$), 42.7 (N(CH$_3$)$_2$), 66.6 (O$CH_2CH_3$), 67.4 (Ar-CH$_2$), 124.9 (C), 125.0 (N=CH), 127.9 (C), 129.1 (C-5), 132.7 (C-6), 135.3 (C), 136.4 (C), 168.7 (C=O), 169.4(C=O); m/z HRMS (ESI+) found [MH]$^+$ 304.1653, C$_{16}$H$_{22}$N$_3$O$_3$ requires 304.1661.

2-(3-(Furan-2-yl)allylidene)-1,1-dimethylhydrazine **87**

87
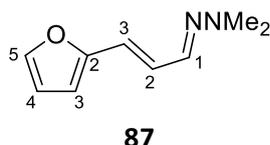
To a solution of 3-(2-furyl)acrolein **86** (61.1 mg, 500 µmol) in water (2 mL), *N,N*-dimethylhydrazine (36.0 mg, 46.0 µL, 600 µmol) was added and the mixture was stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **87** as a dark yellow oil (55 mg, 67%).

R$_f$ 0.69 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 3037, 2953, 2857, 1675, 1626, 1573, 1537; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.91 (6H, s, N(CH$_3$)$_2$), 6.27 (1H, d, *J* = 3.3 Hz, furan 5-H), 6.37 (1H, dd, *J* = 3.3, 1.7 Hz, furan 4-H), 6.40 (1H, d, *J* = 15.9 Hz, alkene 3-H), 6.82 (1H, dd, *J* = 15.9, 9.2 Hz, alkene 2-H), 7.05 (1H, d, *J* = 9.2 Hz, N=CH), 7.35 (1H, d, *J* = 1.7 Hz, furan 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.9 (N(CH$_3$)$_2$), 108.0 (furan C-3), 111.7 (furan C-4), 119.4 (alkene C-3), 126.2 (alkene C-2), 134.8 (N=CH), 142.2 (furan C-5) 148.2 (furan C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 165.1024, C$_9$H$_{13}$N$_2$O requires 165.1028.

4-(3-(2,2-Dimethylhydrazono)prop-1-en-1-yl)-2-ethylisoindoline-1,3-dione **88**[114]

88
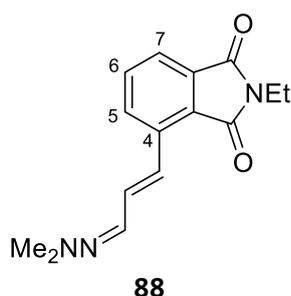
To a solution of 3-(2-furyl)acrolein **86** (104 mg, 850 µmol) in water (4 mL), *N,N*-dimethylhydrazine (61.3 mg, 77.6 µL, 1.02 mmol) was added and the mixture was stirred at 50 °C for 30 min. *N*-Ethylmaleimide (189 mg, 1.70 mmol) was added and the reaction stirred at 50 °C for 18 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **88** as yellow needles (147 mg, 64%).

R_f 0.56 (pet. ether 40-60/EtOAc, 2:3); M.p. 145-146 °C (CDCl₃); IR (film, cm⁻¹) 3057, 2919, 2864, 1764, 1695, 1608; ¹H NMR (CDCl₃; 600 MHz) 1.26 (3H, t, J = 7.2 Hz, CH₂C*H₃*), 3.00 (6H, s, N(CH₃)₂), 3.72 (2H, q, J = 7.2 Hz, C*H₂*CH₃), 7.12-7.19 (2H, m, alkene C*H*C*H*), 7.58 (1H, t, J = 7.3 Hz, 6-H), 7.64 (1H, d, J = 7.3 Hz, 7-H), 7.70 (1H, d, J = 14.3 Hz, N=CH), 7.88 (1H, d, J = 7.3 Hz, 5-H); ¹³C NMR (CDCl₃; 151 MHz) 14.1 (CH₃), 32.9 (CH₂), 42.7 (N(CH₃)₂), 121.4 (C-7), 123.7 (N=CH), 126.0 (C), 129.4 (C-5), 132.7 (alkene CH), 133.0 (C), 133.4 (alkene CH), 133.6 (C-6), 136.8 (C), 168.3 (C=O), 169.3 (C=O); *m/z* HRMS (ESI+) found [MH]⁺ 272.1403, C₁₅H₁₈N₃O₂ requires 272.1399.

2-(1-(Furan-2-yl)ethylidene)-1,1-dimethylhydrazine **92**
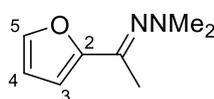
92

To a solution of 2-acetylfuran **89** (55.1 mg, 500 μmol) in ethanol (5 mL), *N,N*-dimethylhydrazine (76.0 μL, 1.00 mmol) was added and the mixture stirred at 50 °C for 3 h. The solvent was removed under vacuum and the crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **92** as a dark yellow oil (31 mg, 41%).

R_f 0.60 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm⁻¹) 2852, 1531, 1438; ¹H NMR (CDCl₃; 600 MHz) 2.32 (3H, s, CH₃), 2.36 (6H, s, N(CH₃)₂), 6.54 (1H, dd, J = 3.4, 1.7 Hz, 4-H), 6.68 (1H, d, J = 3.4, Hz, 5-H), 7.01 (1H, s, N=CH), 7.59 (1H, d, J = 1.7 Hz, 3-H); ¹³C NMR (CDCl₃; 151 MHz) 34.2 (CH₃), 39.5 (N(CH₃)₂), 122.4 (C-4), 123.5 (N=C), 124.5 (C-3), 136.6 (C-5), 151.9 (C-2); *m/z* HRMS (ESI+) found [MH]⁺ 153.1028, C₈H₁₃N₂O requires 153.1028.

2-(Furan-3-ylmethylene)-1,1-dimethylhydrazine **93**
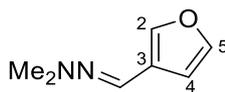
93

To a solution of 3-furancarboxaldehyde **90** (43.3 μL, 500 μmol) in water (5 mL), *N,N*-dimethylhydrazine (45.6 μL, 600 μmol) was added and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL) and the combined

organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **93** as a dark yellow oil (50 mg, 73%).

R$_f$ 0.60 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm^{-1}) 2845, 1525, 1442; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.88 (6H, s, N(CH$_3$)$_2$), 6.71 (1H, d, J = 1.6 Hz, 4-H), 7.22 (1H, s, N=CH), 7.36 (1H, d, J = 1.6 Hz, 5-H), 7.52 (1H, s, 2-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 43.1 (N(CH$_3$)$_2$), 107.6 (C-4), 124.7 (C-3), 126.2 (N=CH), 140.7 (C-2), 143.5 (C-5); m/z HRMS (ESI+) found [MH]$^+$ 139.0868, C$_7$H$_{11}$N$_2$O requires 139.0871.

5-((2,2-Dimethylhydrazono)methyl)furan-2-carbaldehyde **94**
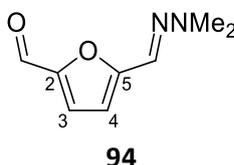
94

To a solution of 2,5-furandicarboxaldehyde **91** (62.0 mg, 500 µmol) in water (5 mL), *N,N*-dimethylhydrazine (30.4 µL, 400 µmol) was added and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **94** as a dark yellow oil (47.4 mg, 57%).

R$_f$ 0.43 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm^{-1}) 2850, 1775, 1528; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.08 (6H, s, N(CH$_3$)$_2$), 6.58 (1H, d, J = 3.7 Hz, 4-H), 7.01 (1H, s, N=CH), 7.24 (1H, d, J = 3.7 Hz, 3-H), 9.53 (1H, s, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 43.5 (N(CH$_3$)$_2$), 110.6 (C-4), 116.7 (C-3), 122.9 (N=CH), 142.3 (C-2), 153.6 (C-5), 179.0 (CHO); m/z HRMS (ESI+) found [MH]$^+$ 167.0825, C$_8$H$_{11}$N$_2$O$_2$ requires 167.0821.

2-((2,2-Dimethylhydrazono)methyl)-5-((2,2-dimethylhydrazono)methyl)furan 95
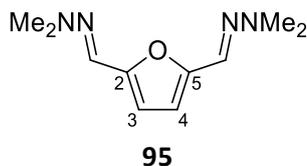
95

To a solution of 2,5-furandicarboxaldehyde, **91** (62.0 mg, 500 μmol), in water (5 mL), *N,N*-dimethylhydrazine (91.2 μL, 1.20 mmol) was added and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL) and the combined organic phase was dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **95** as a dark yellow oil (91.6 mg, 88%).

R$_f$ 0.77 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm^{-1}) 2844, 1526, 1445; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.84 (12H, s, 2 x N(CH$_3$)$_2$), 6.29 (2H, s, 3-H & 4-H), 7.99 (2H, s, 2 x N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.2 (2 x N(CH$_3$)$_2$), 108.7 (C-3 & C-4), 148.1 (C-2 & C-5), 123.3 (2 x N=CH); *m/z* HRMS (ESI+) found [MH]$^+$ 209.1407, C$_{10}$H$_{17}$N$_4$O requires 209.1402.

(Furan-2-ylmethylene)hydrazine 103[210]

103
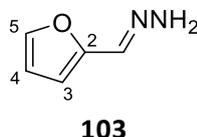
Furfural (25.4 μL, 300 μmol) and hydrazine **93** (12.4 μL, 400 μmol) were combined in water (4 mL) and the mixture stirred at 50 °C for 30 min, with a yellow precipitate forming after 10 min. The organic component was extracted with ethyl acetate (3 x 30 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum, giving **103** as light orange crystals (24 mg, 72%).

R$_f$ 0.11, 0.18 (pet. ether 40-60/EtOAc, 3:17); IR (film, cm^{-1}) 1637, 1547; ^{1}H NMR (CDCl$_3$; 600 MHz) 6.54 (1H, dd, *J* = 3.5, 1.5 Hz, 4-H), 6.90 (1H, d, *J* = 3.5 Hz, 3-H), 7.60 (1H, d, *J* = 1.5 Hz, 5-H), 8.52 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 112.4 (C-4), 117.0 (C-3), 145.9 (C-5), 149.5 (N=CH), 151.0 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 111.0562, C$_5$H$_7$N$_2$O requires 111.0558.

Furan-2-carbaldehyde oxime **104**[211]

104
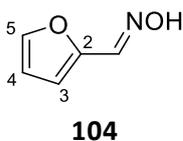
Furfural (25.4 µL, 300 µmol) and hydroxylamine **95** (27.8 mg, 400 µmol) were combined in water (4 mL) and the mixture stirred at 50 °C for 30 min. The organic component was extracted with ethyl acetate (3 x 30 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum, giving **104** as fine colourless needles (27 mg, 81%).

R$_f$ 0.23, 0.34 (pet. ether 40-60/EtOAc, 3:17); IR (film, cm^{-1}) 3164 (br), 1644, 1475; ^{1}H NMR (CDCl$_3$; 600 MHz) 6.46 (1H, dd, J = 3.3, 1.9 Hz, 4-H), 6.64 (1H, d, J = 3.3 Hz, 3-H), 7.35 (1H, d, J = 1.9 Hz, 5-H), 8.02 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 112.4 (C-4), 118.4 (C-3), 143.6 (N=CH), 144.4 (C-5), 147.2 (C-2); m/z HRMS (ESI+) found [MH]$^+$ 112.0412, C$_5$H$_6$NO$_2$ requires 112.0399.

2-(Furan-2-ylmethylene)-1,1-diphenylhydrazine **105**[212]

105
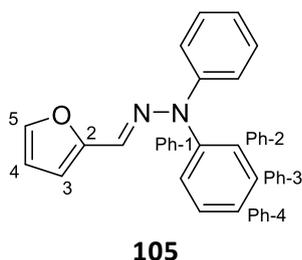
N,N-diphenylhydrazine hydrochloride **100**•HCl (221 mg, 1.00 mmol) was added to water (4 mL) and sodium carbonate added until the solution was pH 7. Furfural (82.8 µL, 1.00 mmol) was added and the mixture stirred at 50 °C for 1 h with a yellow-green precipitate forming after 10 min. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **105** as a dark yellow-green solid (231 mg, 88%).

R$_f$ 0.68 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 1586, 1546, 1492; M.p. 86 - 87 °C (water), Lit[212] 90 °C (aq. ethanol); ^{1}H NMR (CDCl$_3$; 500 MHz) 6.42 (1H, dd, J = 3.3, 1.8 Hz, 4-H), 6.46 (1H, d, J = 3.3 Hz, 5-H), 7.05 (1H, s, N=CH), 7.17 – 7.22 (6H, m, 2 x Ph-4-H & 4 x Ph-2-H), 7.40 - 7.45 (5H, m, 4 x Ph-3-H & 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 109.0 (C-5), 111.7

(C-4), 122.7 (2 × Ph-4), 124.8 (4 × Ph-2), 126.3 (N=CH), 130.0 (4 × Ph-3), 142.8 (C-3), 143.4 (2 × Ph-1), 151.7 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 263.1180, $C_{17}H_{15}N_2O$ requires 263.1184.

1-(Furan-2-ylmethylene)-2-methylhydrazine **106**[213]
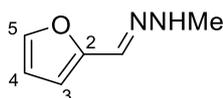
106

To a solution of 2-furfuraldehyde (25.4 µL, 300 µmol) in water (4 mL), monomethylhydrazine, **101** (20.9 µL, 18.4 mg, 400 µmol), was added and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL), dried (MgSO$_4$), filtered, and the solvent was removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **106** as a dark yellow oil (16 mg, 44%).

R$_f$ 0.42 (EtOAc); IR (film, cm^{-1}) 3002 (NH), 2854, 1665, 1620, 1520; ^{1}H NMR (CDCl$_3$; 500 MHz) 2.36 (3H, s, NCH$_3$), 6.49 (1H, dd, *J* = 3.3, 1.6 Hz, 4-H), 6.70 (1H, d, *J* = 3.4 Hz, 3-H), 7.52 (1H, d, *J* = 1.6 Hz, 5-H), 7.65 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 20.5 (NCH$_3$), 112.0 (C-4), 113.2 (C-3), 133.3 (N=CH), 144.7 (C-5), 149.1 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 125.0717, $C_6H_9N_2O$ requires 125.0715.

N'-(Furan-2-ylmethylene)acetohydrazide **107**[214]
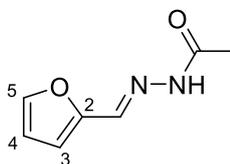
107

To a solution of 2-furfuraldehyde (25.4 µL, 300 µmol) in water (4 mL), acetic hydrazide, **102** (29.6 mg, 400 µmol), was added and the mixture stirred at 50 °C for 2 h. The organic component was extracted with ethyl acetate (3 x 30 mL), dried (MgSO$_4$), filtered, and the solvent was removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **107** as a dark yellow oil (30.6 mg, 67%).

R_f 0.48 (EtOAc); IR (film, cm^{-1}) 3119 (NH), 2958, 1654 (C=O), 1588, 1561, 1523; ^{1}H NMR (CDCl$_3$; 500 MHz) 2.17 (3H, s, CH$_3$), 5.61 (1H, br, NH), 6.39 (1H, d, J = 3.3 Hz, 3-H), 6.40 (1H, dd, J = 3.3, 1.8 Hz, 4-H), 7.38 – 7.41 (2H, m, N=CH & 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 31.1 (CH$_3$), 107.0 (C-3), 111.4 (C-4), 125.6 (N=CH), 142.4 (C-5), 151.6 (C-2), 207.4 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 153.0666, C$_7$H$_9$N$_2$O$_2$ requires 153.0664.

2-Ethyl-4-((2-phenylhydrazono)methyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-epoxyisoindole-1,3(2*H*)-dione **108**
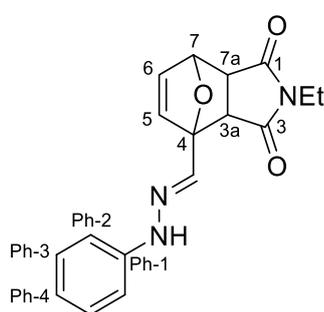
108

To a solution of furfural (828 μL, 961 mg, 10 mmol) in water (25 mL), phenylhydrazine (98.3 mL, 1.08 g, 10 mmol) was added and the mixture was stirred at 50 °C for 1 h. *N*-Ethylmaleimide (2.50 g, 20 mmol) was added and the reaction stirred at 50 °C for 24 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 4:1) to give **108** as an orange solid (2.30 g, 47%).

R_f 0.42 (pet. ether 40-60/EtOAc, 3:2); M.p. 90 - 92 °C (EtOAc); IR (film, cm^{-1}) 2980, 1769 (C=O), 1689, 1598, 1550, 1518; ^{1}H NMR (dmso-d$_6$; 500 MHz) 1.04 (3H, t, J = 7.3 Hz, CH$_2$CH_3), 3.05 (1H, d, J = 6.4 Hz, 3a-H), 3.13 (1H, d, J = 6.4 Hz, 7a-H), 3.40 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 5.16 (1H, d, J = 1.7 Hz, 7-H), 6.62 (1H, m, 6-H), 6.74 (1H, tt, J = 7.3, 1.0 Hz, Ph-4-H), 6.82 (1H, d, J = 5.7 Hz, 5-H), 6.99 (2H, dt, J = 8.2, 1.0 Hz, Ph-2-H), 7.19 (2H, m, Ph-3-H), 7.34 (1H, s, N=CH), 10.47 (1H, s, NH); ^{13}C NMR (dmso-d$_6$; 151 MHz) 12.8 (CH$_2$CH$_3$), 33.0 (CH$_2$CH$_3$), 50.2 (C-3a), 50.3 (C-7a), 80.6 (C-7), 89.5 (C-4), 111.9 (2 x Ph-2), 119.0 (Ph-4), 129.1 (Ph-3), 131.1 (C-6), 136.4 (C-5), 136.9 (Ph-1), 145.0 (N=CH), 174.6 (C=O), 175.9 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 312.1364, C$_{17}$H$_{18}$N$_3$O$_3$ requires 312.1348.

1-Cyclopropyl-1*H*-pyrrole-2,5-dione **114**[215]

114
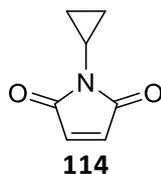
Synthesis adapted from literature procedure.[215] Cyclopropylamine (693 μL, 10.0 mmol) and maleic anhydride (1.00 g, 10.2 mmol) were combined in acetic acid (20 mL) and heated at reflux for 2 h. The reaction was cooled to RT and concentrated under vacuum, and ethyl acetate (50 mL) and aq. sat. NaHCO$_3$ (50 mL) were added. The organic component was extracted with ethyl acetate (3 x 50 mL). The solvent was dried (MgSO$_4$), filtered, and removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **114** as fine colourless crystals (526 mg, 38%).

R$_f$ 0.53 (pet. ether 40-60/EtOAc, 1:1); M.p. 57 - 59 °C (EtOAc), Lit.[215] 57 - 59 °C; IR (film, cm^{-1}) 1777, 1759; ^{1}H NMR (DMSO-d6; 600 MHz) 0.75 (2H, m, 2 x C*H*H), 0.82 (2H, m, 2 x CH*H*), 2.49 (1H, m, NCH), 6.93 (2H, s, HC=CH); ^{13}C NMR (DMSO-d6; 151 MHz) 4.4 (2 x CH$_2$), 19.7 (NCH), 134.2 (C=C), 171.4 (2 × C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 138.0551, C$_7$H$_8$NO$_2$ requires 138.0555.

1-(4-Methylbenzyl)-1*H*-pyrrole-2,5-dione **115**[215]

115
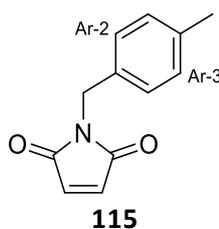
Synthesis adapted from literature procedure.[215] 4-Methylbenzylamine (1.27 ml, 10.0 mmol) and maleic anhydride (1.00 g, 10.2 mmol) were combined in acetic acid (20 ml) and heated at reflux for 3 h. The reaction was cooled to RT and concentrated under vacuum, and ethyl acetate (50 mL) and aq. sat. NaHCO$_3$ (50 mL) were added. The organic component was extracted with ethyl acetate (3 x 50 mL). The solvent was dried (MgSO$_4$), filtered, and removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **115** as fine colourless crystals (533 mg, 26%).

R$_f$ 0.60 (pet. ether 40-60/EtOAc, 1:1); M.p. 101 - 103 °C (EtOAc), Lit.[215] 100 - 102 °C; IR (film, cm^{-1}) 1702, 1515; ^{1}H NMR (MeOH-d4; 600 MHz) 2.27 (2H, s, CH$_3$), 4.55 (2H, s, CH$_2$), 7.06 (2H, s, HC=CH), 7.10-7.15 (4H, m, 2 x Ar-2-H & 2 x Ar-3-H); ^{13}C NMR (MeOH-d4; 151 MHz) 20.6 (CH$_3$), 40.7 (CH$_2$), 127.2 (2 × Ar-2), 129.1 (2 × Ar-3), 133.7 (Ar), 134.6 (C=C), 136.6 (Ar), 170.8 (2 × C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 200.0866, C$_{12}$H$_{12}$NO$_2$ requires 200.0868.

2-Cyclopropyl-4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **117**[114]

117
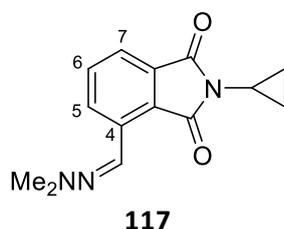
To a solution of furfural (29.0 μL, 350 μmol) in water (2 mL), *N,N*-dimethylhydrazine (32.0 μL, 420 μmol) was added and the mixture was stirred at 50 °C for 30 min. *N*-Cyclopropylmaleimide **114** (96.0 mg, 700 μmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **117** as yellow needles (72 mg, 80%).

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 μmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 μL, 2.0 mmol) and cyclopropylamine (347 μL, 5.00 mmol) were added and the mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **117** as a yellow solid (105 mg, 41%).

R$_f$ 0.30 (pet. ether 40-60/EtOAc, 4:1); M.p. 174-176 °C (water); IR (film, cm^{-1}) 2861, 1764, 1706, 1548; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.01 (4H, m, 2 x cyclopropyl CH$_2$), 2.67 (1H, m, cyclopropyl CH), 3.12 (6H, s, N(CH$_3$)$_2$), 7.55 (1H, t, *J* = 7.6 Hz, 6-H), 7.59 (1H, dd, *J* = 7.6, 1.1 Hz, 7-H), 8.11 (1H, s, N=CH), 8.22 (1H, dd, *J* = 7.6, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz)

5.3 (2 x CH_2), 20.9 (NCH), 42.7 ($N(CH_3)_2$), 120.9 (C-7), 124.4 (C), 124.7 (N=CH), 129.1 (C-5), 132.2 (C), 133.5 (C-6), 136.5 (C), 169.1 (C=O), 170.2 (C=O); *m/z* HRMS (ESI+) found $[MH]^+$ 258.1240, $C_{14}H_{17}N_3O_2$ requires 258.1242.

4-((2,2-Dimethylhydrazono)methyl)-2-(4-methylbenzyl)isoindoline-1,3-dione 118[114]
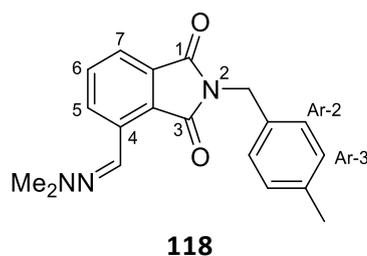
118

To a solution of furfural (48.0 μL, 700 μmol) in water (2 mL), *N,N*-dimethylhydrazine (64.0 μL, 840 μmol) was added and the mixture was stirred at 50 °C for 30 min. *N*-(4-Methylbenzyl) maleimide **115** (141 mg, 700 μmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **118** as yellow needles (153 mg, 68%).

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 μmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 μL, 2.0 mmol) and 4-methylbenzylamine (255 μL, 2.00 mmol) were added and the mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **118** as a yellow solid (241 mg, 75%).

R_f 0.40 (pet. ether 40-60/EtOAc, 4:1); M.p. 135-137 °C (water); IR (film, cm^{-1}) 2928, 1758, 1697; ^1H NMR ($CDCl_3$; 600 MHz) 2.31 (3H, s, CH_3), 3.11 (6H, s, $N(CH_3)_2$), 4.79 (2H, s, CH_2), 7.13 (2H, d, *J* = 7.9 Hz, 2 × Ar-3-H), 7.33 (2H, d, *J* = 7.9 Hz, 2 × Ar-2-H), 7.54 (1H, t, *J* = 7.7 Hz, 6-H), 7.60 (1H, d, *J* = 7.7 Hz, 7-H), 8.12 (1H, s, N=CH), 8.21 (1H, d, *J* = 7.7 Hz, 5-H); ^{13}C NMR ($CDCl_3$; 151 MHz) 41.4 (NCH_2), 42.8 ($N(CH_3)_2$) , 121.1 (C-7), 124.9 (C), 125.2 (N=CH), 128.7 (2 × Ar-3), 129.1 (C-5), 129.5 (2 × Ar-2), 132.5 (C), 133.4 (C-6), 133.8 (C), 136.5 (C), 137.6 (C), 167.6 (C=O), 168.6 (C=O); *m/z* HRMS (ESI+) found $[MH]^+$ 322.1567, $C_{19}H_{20}N_3O_2$ requires 322.1556.

4-((2,2-Dimethylhydrazono)methyl)-2-phenylisoindoline-1,3-dione **119**[216]
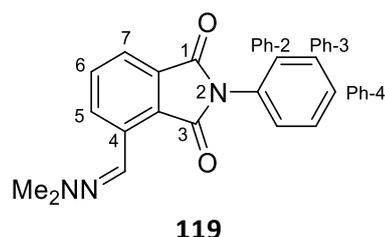
119

To a solution of furfural (48.0 µL, 700 µmol) in water (2 mL), *N,N*-dimethylhydrazine (64.0 µL, 840 µmol) was added and the mixture was stirred at 50 °C for 30 min. *N*-Phenylmaleimide **116** (121 mg, 700 µmol) was added and the reaction stirred at 50 °C for 2 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **119** as yellow needles (150 mg, 73%).

R_f 0.34 (pet. ether 40-60/EtOAc, 4:1); M.p. 210-212 °C (water), Lit[216] 206 °C (EtOAc); IR (film, cm^{-1}) 2941, 1766, 1710, 1687; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.13 (6H, s, N(CH$_3$)$_2$), 7.40 (1H, tt, *J* = 7.3, 1.3 Hz, Ph-4-H), 7.43 (2H, dd, *J* = 7.3, 1.3 Hz, 2 × Ph-2-H), 7.50 (2H, t, *J* = 7.3 Hz, 2 × Ph-3-H), 7.64 (1H, t, *J* = 7.6 Hz, 6-H), 7.72 (1H, d, *J* = 7.6 Hz, 7-H), 8.16 (1H, s, N=CH), 8.31 (1H, d, *J* = 7.6 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 120.8 (C), 121.4 (C-7), 124.2 (C), 124.4 (N=CH), 126.8 (Ph-4), 128.1 (2 × Ph-2), 129.2 (C-5), 129.4 (2 × Ph-3), 131.9 (C), 132.1 (C), 133.9 (C-6), 137.0 (C), 167.5 (C=O), 168.5 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 294.1251, C$_{17}$H$_{16}$N$_3$O$_2$ requires 294.1243.

Diethyl-3-((2,2-dimethylhydrazono)methyl)phthalate **120**
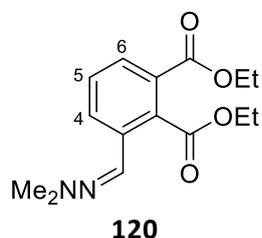
120

To a solution of furfural (82.9 µL, 1.00 mmol) in water (2 mL), *N,N*-dimethylhydrazine (91.4 µL, 1.20 mmol) was added and the mixture was stirred at 50 °C for 30 min. Diethyl maleate (344 mg, 322 µL, 2.00 mmol) was added and the reaction stirred at 100 °C for 24 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 50

mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 4:1) to give **120** as a yellow oil (44 mg, 15%).

R$_f$ 0.23 (pet. ether 40-60/EtOAc, 17:3); IR (film, cm^{-1}) 1447, 1557, 1721; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.37 (6H, m, 2 x CH$_2$CH_3), 2.99 (6H, s, N(CH$_3$)$_2$), 4.34 (2H, q, J = 7.1 Hz, CH_2CH$_3$), 4.42 (2H, q, J = 7.1 Hz, CH_2CH$_3$), 7.17 (1H, s, N=CH), 7.39 (1H, t, J = 7.9 Hz, 5-H), 7.82 (1H, d, J = 7.9 Hz, 6-H), 8.06 (1H, d, J = 7.9 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.2 (CH$_3$), 14.3 (CH$_3$), 42.8 (N(CH$_3$)$_2$), 61.5 (CH$_2$), 61.6 (CH$_2$), 127.2 (N=CH), 128.6 (C), 129.1 (C-6), 129.1 (C-5), 129.9 (C-4), 132.8 (C), 135.1 (C) 166.0 (C=O), 169.0 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 293.1498, C$_{15}$H$_{21}$N$_2$O$_4$ requires 293.1501.

(*Z*)-2-((2,2-Dimethylhydrazono)methyl)benzonitrile (*Z*)-**121**[114]

(*Z*)-**121**
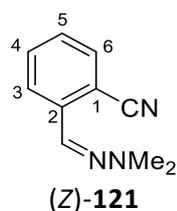
To a solution of 2-furaldehyde dimethylhydrazone **28** (46.4 μL, 350 μmol) in water (2 mL), acrylonitrile (46.4 μL, 700 μmol) was added and the reaction stirred at reflux for 24 h. The mixture was then cooled to RT and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give (*Z*)-**121** as a pale green oil (8.5 mg, 24%).

To a solution of 2-((2,2-dimethylhydrazono)methyl)-5-hydroxycyclohexa-1,3-diene-1-carbonitrile, **129** (25 mg, 131 μmol), in CPME (25 mL), HCl (4 M in hexane, 0.5 mL) was added and the mixture stirred at 100 °C for 16 hours. The mixture was then neutralized by addition of NaHCO$_3$, filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give (*Z*)-**121** as a pale green oil (10 mg, 46 %).

R$_f$ 0.66 and 0.74 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 2827, 2219, 1570; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.09 (6H, s, N(CH$_3$)$_2$), 7.21 (1H, t, J = 7.8 Hz, 4-H), 7.37 (1H, s, N=CH), 7.48 (1H, t, J = 7.8 Hz, 5-H), 7.56 (1H, d, J = 7.8 Hz, 6-H), 7.95 (1H, d, J = 7.8 Hz, 3-H); ^{13}C NMR

(CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 109.0 (C), 118.3 (CN), 124.4 (C), 125.7 (C-3), 126.6 (C-4), 132.7 (C-6), 132.8 (C-5), 140.4 (C); *m/z* HRMS (ESI+) found [MH]$^+$ 174.1023, C$_{10}$H$_{12}$N$_2$ requires 174.1031.

(*E*)-2-((2,2-Dimethylhydrazono)methyl)benzonitrile (*E*)-121

(*E*)-**121**
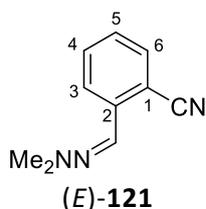
To a solution of 2-cyanobenzaldehyde, **131** (131 mg, 1.00 mmol) in ethanol (10 mL), *N,N*-dimethylhydrazine (91.3 µL, 1.20 mmol) was added and the mixture stirred at 50 °C for 3 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 20 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give (*E*)-**121** as fine colourless crystals (126 mg, 73%).

R$_f$ 0.41 (EtOAc); M.p. 145 - 146 °C (EtOAc) IR (film, cm^{-1}) 2861, 2219, 1647, 1616, 1572, 1542; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.97 (6H, s, N(CH$_3$)$_2$), 7.20 (1H, td, *J* = 7.5, 1.2 Hz, 5-H), 7.37 (1H, m, 4-H), 7.43 (1H, d, *J* = 7.5 Hz, 6-H), 7.64 (1H, s, N=CH), 7.93 (1H, dd, *J* = 7.8 Hz, 1.2 Hz, 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.9 (N(CH$_3$)$_2$), 123.6 (C≡N), 125.7 (C-3), 126.9 (C-5), 127.4 (C-6), 129.8 (N=CH), 130.6 (C-4), 133.0 (C-1), 135.2 (C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 174.1027, C$_{10}$H$_{12}$N$_2$ requires 174.1031.

5-(3-Oxobutyl)furan-2-carbaldehyde 122

122
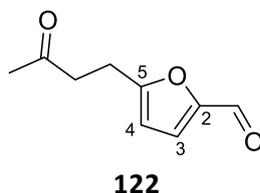
Methyl vinyl ketone (170 µL, 2.10 mmol) and scandium triflate (207 mg, 420 µmol) were added to water (4 mL) and stirred at room temperature for 30 min. Furfural dimethylhydrazone (140 µL, 1.05 mmol) was added and the reaction mixture heated to 80 °C and stirred for 5 h. A colour change to dark yellow was observed. The organic

component was extracted with ethyl acetate (3 x 50 mL). The combined organic phases were dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 4:1) to give **122** as a yellow oil (49 mg, 28%).

R_f 0.05 (pet. ether 40-60/EtOAc, 17:3); IR (film, cm^{-1}) 1518, 1671, 1714; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.14 (3H, s, C(O)CH_3), 2.85 (2H, t, J = 7.2 Hz, CH_2CH$_2$), 2.97 (2H, t, J = 7.2 Hz, CH$_2$CH_2), 6.23 (1H, d, J = 3.5 Hz, 3-H), 7.13 (1H, d, J = 3.5 Hz, 4-H), 9.47 (1H, s, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 22.4 (Ar-CH$_2$), 29.9 (CH$_3$), 40.8 (Ar-CH$_2$CH$_2$), 109.3 (C-3), 123.8 (C-4), 152.0 (C-5), 162.0 (C-2), 177.0 (C=O), 206.3 (C(O)H). *m/z* HRMS (ESI+) found [MH]$^+$ 167.0704, C$_9$H$_{11}$O$_3$ requires 167.0708.

Dimethyl-3-((2,2-dimethylhydrazono)methyl)phthalate **123**[114]
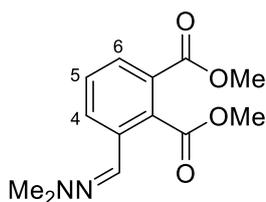
123

To a solution of furfural (82.9 μL, 1.00 mmol) in water (2 mL), *N,N*-dimethylhydrazine (91.4 μL, 1.20 mmol) was added and the mixture was stirred at 50 °C for 30 min. Dimethyl maleate (250 μL, 2.00 mmol) was added and the reaction stirred at 100 °C for 24 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **123** as a yellow oil (44.8 mg, 19%).

R_f 0.39 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 1721 (str), 1555; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.99 (6H, s, N(CH$_3$)$_2$), 3.88 (3H, s, OCH$_3$), 3.93 (3H, s, OCH$_3$), 7.12 (1H, s, N=CH), 7.40 (1H, t, J = 7.9 Hz, 5-H), 7.81 (1H, dd, J = 7.9, 1.1 Hz, 6-H), 8.02 (1H, dd, J = 7.9, 1.1 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.7 (N(CH$_3$)$_2$), 52.6 (OCH$_3$), 52.7 (OCH$_3$), 127.1 (N=CH), 128.4 (C), 128.4 (C-6), 129.3 (C-5), 129.5 (C-4), 132.4 (C), 134.9 (C), 136.4 (C), 166.5 (C=O), 169.7 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 265.1182, C$_{13}$H$_{17}$N$_2$O$_4$ requires 265.1188.

2-((2,2-Dimethylhydrazono)methyl)-5-hydroxycyclohexa-1,3-diene-1-carbonitrile 129
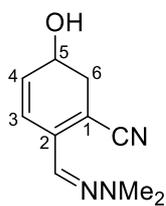
129

To a solution of 2-furaldehyde dimethylhydrazone **28** (691 mg, 663 µL, 5.00 mmol) in acetonitrile/water (1:9, 25 mL) in a high-pressure tube, acrylonitrile (1.33 g, 1.64 mL, 25.0 mmol) was added and the reaction stirred at 100 °C for 72 h. The reaction was cooled to RT and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **129** as a yellow oil (277 mg, 29%).

R_f 0.35 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 3381 br (OH), 2833 (CH), 2188 (C≡N), 1627 (N=CH); ^{1}H NMR (CDCl$_3$; 600 MHz) 2.23 (1H, s br, OH), 2.68 (2H, d, J = 6.8 Hz, 6-H$_2$), 3.04 (6H, s, N(CH$_3$)$_2$), 4.34 (1H, m, 5-H), 6.26 (1H, m, 4-H), 6.89 (1H, d, J = 9.9 Hz, 3-H), 7.10 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 33.8 (C-6), 42.5 (N(CH$_3$)$_2$), 62.5 (C-5), 97.4 (C-1), 119.4 (C≡N), 123.2 (C-3), 125.2 (N=CH), 133.0 (C-4), 144.1 (C-2); m/z HRMS (ESI+) found [MH]$^+$ 192.1157, C$_{10}$H$_{13}$N$_3$O requires 192.1137.

4-(5-((2,2-Dimethylhydrazono)methyl)furan-2-yl)butan-2-one 132[114]
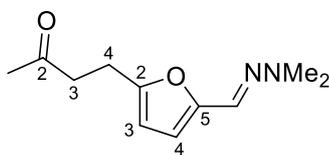
132

To a solution of furfural (166 µL, 2.00 mmol) in water (10 mL), *N,N*-dimethylhydrazine (183 µL, 2.40 mmol) was added and the mixture was stirred at 50 °C for 30 min. The solution was heated at reflux and methyl vinyl ketone (834 µL, 10.0 mmol) added dropwise over 5 h. The solution was stirred at reflux for a further 1 h then cooled to RT, and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the

solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **132** as a yellow oil (162 mg, 39%).

R_f 0.21 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 2925, 1713, 1674; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.11 (3H, s, C(O)CH_3), 2.76 (2H, t, J = 7.5 Hz, butyl 4-H$_2$), 2.86-2.91 (8H, m, N(CH$_3$)$_2$ and butyl 3-H$_2$), 5.97 (1H, d, J = 3.3 Hz, furan 3-H), 6.21 (1H, d, J = 3.3 Hz, furan 4-H), 7.03 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 22.5 (butyl C-4), 30.1 (CH$_3$), 41.9 (butyl C-3), 43.0 (N(CH$_3$)$_2$), 107.4 (furan C-3), 108.8 (furan C-4), 124.3 (N=CH), 150.9 (furan C-5), 154.6 (furan C-2), 207.8 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 209.1289, C$_{11}$H$_{17}$N$_2$O$_2$ requires 209.1290.

4-((2,2-Dimethylhydrazono)methyl)-2-ethyl-7-(3-oxobutyl)isoindoline-1,3-dione **133**[114]

133
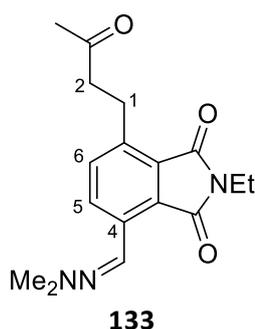
To a solution of 4-(5-((2,2-dimethylhydrazono)methyl)furan-2-yl)butan-2-one **132** (208 mg, 1.00 mmol) in water (4 mL), *N*-ethylmaleimide (250 mg, 2.00 mmol) was added and the reaction stirred at 50 °C for 4 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 25 mL) and dried to give **133** as yellow needles (184 mg, 58%).

R_f 0.32 (pet. ether 40-60/EtOAc, 4:1); M.p. 132-133 °C (H$_2$O); IR (film, cm^{-1}) 2936, 1754, 1694, 1545, 1441; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.26 (3H, t, J = 7.2 Hz, NCH$_2$CH_3), 2.16 (3H, s, C(O)CH$_3$), 2.82 (2H, t, J = 7.6 Hz, butyl 2-H$_2$), 3.10 (6H, s, N(CH$_3$)$_2$), 3.28 (2H, t, J = 7.6 Hz, butyl 1-H$_2$), 3.70 (2H, q, J = 7.2 Hz, NCH_2CH$_3$), 7.37 (1H, d, J = 8.4 Hz, 6-H), 8.11 (1H, d, J = 8.4 Hz, 5-H), 8.13 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (NCH$_2$CH$_3$), 25.8 (butyl C-1), 30.0 (C(O)CH$_3$), 32.7 (NCH$_2$CH$_3$), 42.8 (N(CH$_3$)$_2$), 44.3 (butyl C-2), 125.2 (C), 125.4 (C), 128.6 (C), 129.2 (N=CH), 134.8 (C-5), 135.7 (C-6), 138.9 (C), 168.9 (C=O), 169.3 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 316.1653, C$_{17}$H$_{23}$N$_3$O$_3$ requires 316.1661.

1-Phenylprop-2-en-1-one **135**[118]

135
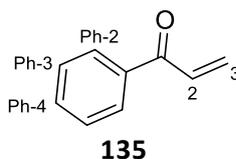
Synthesis adapted from literature procedure.[118] To a solution of 3-chloropropiophenone **136** (1.50 g, 8.90 mmol) in DCM (20 mL) under argon, triethylamine (3.00 mL, 21.4 mmol) was added dropwise over 5 minutes. The mixture was stirred at RT for 18 h, then washed with aqueous hydrochloric acid (0.1 M, 2 x 20 mL), water (2 x 20 mL), saturated aqueous sodium bicarbonate (2 x 20 mL), and brine (20 mL), then dried (Na_2SO_4), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **135** as a colourless oil (9.9 g, 85%).

R_f 0.62 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3061, 2926, 1677, 1597, 1578; ^{1}H NMR ($CDCl_3$; 500 MHz) δ 5.90 (1H, d, J = 10.6 Hz, 3-H_a), 6.40 (1H, d, J = 17.1 Hz, 3-H_b), 7.15 (1H, dd, J = 10.6, 17.1 Hz, 2-H), 7.49 (2H, t, J = 8.0 Hz, Ph-4), 7.58 (1H, m, 2 × Ph-3), 7.95 (2H, d, J = 8.3 Hz, 2 × Ph-2); ^{13}C NMR ($CDCl_3$; 151 MHz) 128.6 (C-3), 128.7 (2 × Ph-2), 130.2 (2 × Ph-3), 132.5 (Ph-4), 133.2 (C-2), 137.4 (Ph-1), 191.0 (C=O); m/z HRMS (ESI+) found $[MH]^+$ 133.0655, C_9H_9O requires 133.0653.

1-(5-((2,2-Dimethylhydrazono)methyl)furan-2-yl)pentan-3-one **137**

137
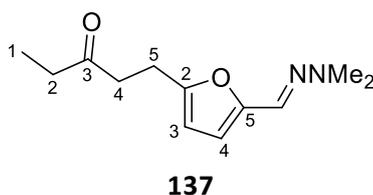
A solution of 2-furaldehyde dimethylhydrazone, **28** (265 µL, 2.00 mmol), in water (10 mL) was heated at reflux and ethyl vinyl ketone, **134** (1.00 mL, 10.0 mmol), added dropwise over 5 h. The solution was stirred at reflux for a further 1 h then cooled to RT, and the organic component extracted with ethyl acetate (3 x 50 mL), dried ($MgSO_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **137** as a yellow oil (67 mg, 14%).

R_f 0.18 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 2922, 1716, 1674; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.01 (3H, t, J = 7.4 Hz, 1-H$_3$), 2.41 (2H, q, J = 7.4 Hz, pentyl 2-H$_2$), 2.75 (2H, t, J = 7.5 Hz, pentyl 4-H$_2$), 2.88-2.94 (8H, m, N(CH$_3$)$_2$ & pentyl 5-H$_2$), 5.98 (1H, d, J = 2.3 Hz, furan 3-H), 6.23 (1H, d, J = 2.3 Hz, furan 4-H), 7.05 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 7.2 (C-1), 22.1 (pentyl C-2), 30.5 (pentyl C-4), 41.9 (pentyl C-5), 43.0 (N(CH$_3$)$_2$), 107.1 (furan C-3), 108.9 (furan C-4), 124.3 (N=CH), 150.8 (furan C-5), 154.4 (furan C-2), 207.6 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 223.1450, C$_{12}$H$_{19}$N$_2$O$_2$ requires 223.1447.

5-((Allyloxy)methyl)furan-2-carbaldehyde 142[217]
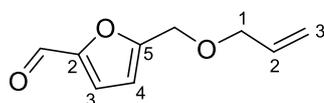
142

A solution of 5-hydroxymethylfurfural (775 μL, 8.00 mmol), in acetonitrile (32 mL) was cooled to 0 °C and finely ground sodium hydroxide (480 mg, 12 mmol) was added. Allyl bromide (1.04 mL, 12.0 mmol) was added dropwise and the reaction mixture stirred for 24 h and allowed to reach RT. Water (100 mL) was added and the organic component extracted with ethyl acetate (3 x 100 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **142** as a yellow oil (292 mg, 22%).

R_f 0.25 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3078, 2881, 1701, 1652, 1520; ^{1}H NMR (CDCl$_3$; 500 MHz) 4.04 (2H, m, allyl 1-H$_2$), 4.51 (2H, s, CH$_2$Oallyl), 5.20 (1H, dq, J = 10.4, 1.6 Hz, allyl 3-H$_a$), 5.28 (1H, dq, J = 17.2, 1.6 Hz, allyl 3-H$_b$), 5.87 (1H, m, allyl 2-H), 6.50 (1H, d, J = 3.5 Hz, furan 4-H), 7.19 (1H, d, J = 3.5 Hz, furan 3-H), 9.58 (1H, s, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 64.0 (allyl C-1), 71.8 (CH$_2$Oallyl), 111.3 (furan C-4), 118.1 (allyl C-3), 122.1 (allyl C-2), 133.9 (furan C-3), 152.6 (furan C-2), 158.5 (furan C-5), 177.8 (CHO); m/z HRMS (ESI+) found [MH]$^+$ 167.0706, C$_9$H$_{11}$O$_3$ requires 167.0708.

(5-((Allyloxy)methyl)furan-2-yl)methanol 143
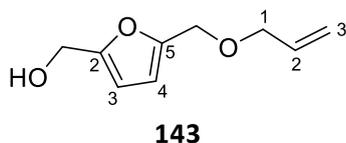
143

A solution of 5-hydroxymethylfurfural (775 µL, 8.00 mmol), in acetonitrile (32 mL) was cooled to 0 °C and finely ground sodium hydroxide (480 mg, 12 mmol) was added. Allyl bromide (1.04 mL, 12.0 mmol) was added dropwise and the reaction mixture stirred for 24 h and allowed to reach RT. Water (100 mL) was added and the organic component extracted with ethyl acetate (3 x 100 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **143** as a yellow oil (242 mg, 18%).

R_f 0.37 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 3242 (br), 2904, 1648; ^{1}H NMR (CDCl$_3$; 500 MHz) 2.64 (1H, br s, O*H*), 3.99 (2H, dt, *J* = 5.7, 1.3 Hz, allyl 1-H$_2$), 4.40 (2H, s, C*H$_2$*OH), 4.53 (2H, s, CH$_2$Oallyl), 5.19 (1H, dq, *J* = 10.4, 1.4 Hz, allyl 3-H$_a$), 5.27 (1H, dq, *J* = 17.2, 1.6 Hz, allyl 3-H$_b$), 5.88 (1H, m, allyl 2-H), 6.20 (1H, d, *J* = 3.2 Hz, furan 4-H), 6.24 (1H, d, *J* = 3.2 Hz, furan 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 57.4 (allyl C-1), 63.9 (CH$_2$OH) 71.1 (CH$_2$Oallyl), 108.4 (furan C-4), 110.2 (furan C-3), 117.8 (allyl C-3), 134.3 (allyl C-2), 151.5 (C), 154.6 (C); *m/z* HRMS (ESI+) found [MH]$^+$ 169.0864, C$_9$H$_{13}$O$_3$ requires 169.0865.

2-((5-((Allyloxy)methyl)furan-2-yl)methylene)-1,1-dimethylhydrazine 144

144
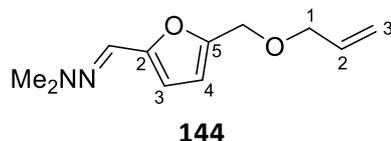
To a solution of 5-((allyloxy)methyl)furan-2-carbaldehyde **142** (162 mg, 970 µmol) in water (5 mL), *N*,*N*-dimethylhydrazine (88.7 µL, 1.17 mmol) was added and the reaction stirred at RT for 3 h. The organic component extracted with ethyl acetate (3 x 100 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **144** as a deep red oil (186 mg, 92%).

R_f 0.31 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3081, 2915, 1646, 1526; ^{1}H NMR (CDCl$_3$; 500 MHz) 2.94 (6H, s, N(C*H$_3$*)$_2$), 4.02 (2H, dt, *J* = 5.72, 1.34 Hz, allyl 1-H$_2$), 4.47 (2H, s, CH$_2$Oallyl), 5.20 (1H, dq, *J* = 10.4, 1.6 Hz, allyl 3-H$_a$), 5.29 (1H, dq, *J* = 17.2, 1.6 Hz, allyl 3-

H$_b$), 5.91 (1H, ddt, *J* = 17.2, 10.4, 5.72 Hz, allyl 2-H), 6.32 (1H, d, *J* = 3.3 Hz, furan 4-H), 6.35 (1H, d, *J* = 3.3 Hz, furan 3-H), 7.09 (1H, s, N=CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 42.8 (N(CH$_3$)$_2$), 64.1 (allyl C-1), 71.1 (CH$_2$Oallyl), 107.4 (furan C-4), 111.1 (furan C-3), 117.6 (allyl C-3), 123.4 (N=CH), 134.5 (allyl C-2), 151.1 (furan C-5), 152.6 (furan C-2); *m/z* HRMS (ESI+) found [MH]$^+$ 209.1294, C$_{11}$H$_{17}$N$_2$O$_2$ requires 209.1290.

(5-Formylfuran-2-yl)methyl acrylate **145**
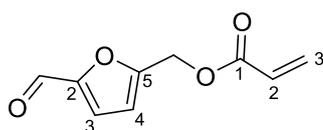
145

A solution of 5-hydroxymethylfurfural (77.5 µL, 800 µmol), in acetonitrile (3 mL) was cooled to 0 °C and finely ground sodium hydroxide (48 mg, 1.20 mmol) was added. Acryloyl chloride (80.8 µL, 1.20 mmol) was added dropwise and the reaction mixture stirred for 24 h and allowed to reach RT. The organic component was extracted with ethyl acetate (3 x 20 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **145** as a yellow oil (62 mg, 43%).

R$_f$ 0.66 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 3031, 2925, 1721, 1697, 1631; ^{1}H NMR (CDCl$_3$; 500 MHz) 5.19 (2H, s, CH$_2$-acrylate), 5.88 (1H, d, *J* = 10.6 Hz, acrylate 3-H$_a$), 6.12 (1H, dd, *J* = 17.3, 10.6 Hz, acrylate 2-H), 6.44 (1H, d, *J* = 17.3 Hz, acrylate 3-H$_b$), 6.60 (1H, d, *J* = 3.5 Hz, furan 4-H), 7.20 (1H, d, *J* = 3.5 Hz, furan 3-H), 9.62 (1H, s, C*H*O); ^{13}C NMR (CDCl$_3$; 151 MHz) 57.9 (CH$_2$-acrylate), 112.8 (furan C-4), 121.9 (furan C-3), 127.6 (acrylate C-2), 132.2 (acrylate C-3), 152.9 (furan C-2), 155.4 (furan C-5), 165.5 (acrylate C=O), 177.9 (C(O)H); *m/z* HRMS (ESI+) found [MH]$^+$ 181.0512, C$_9$H$_9$O$_4$ requires 181.0501.

1,3-Dihydroisobenzofuran-5-carbaldehyde **148**[218]
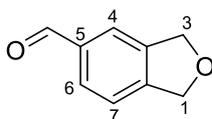
148

A solution of 2-((5-((allyloxy)methyl)furan-2-yl)methylene)-1,1-dimethylhydrazine, **144** (40.0 mg, 192 μmol), in water (5 mL) was sealed in a microwave tube and heated to 200 °C by microwaves for 20 min. The organic component was extracted with ethyl acetate (3 x 20 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **148** as a yellow oil (6.5 mg, 23%).

R_f 0.43 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 2854, 1706, 1584; ^{1}H NMR (CDCl$_3$; 500 MHz) 5.16 (4H, s, 1-H$_2$ & 3-H$_2$), 7.40 (1H, d, J = 7.6 Hz, 7-H), 7.77 (1H, s, 4-H), 7.80 (1H, d, J = 7.6 Hz, 6-H), 10.0 (1H, s, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 73.2 (C-1), 73.5 (C-3), 121.8 (C-7), 122.1 (C-4), 130.2 (C-6), 133.5 (C-7a), 135.7 (C-3a), 146.4 (C-5), 192.5 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 149.0599, C$_9$H$_9$O$_2$ requires 149.0603.

4-(7-Carbamoyl-1-oxoisoindolin-2-yl)piperidin-1-ium chloride **152**·HCl[114]
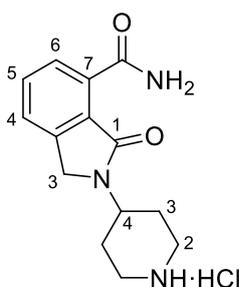
152·HCl

Under anhydrous conditions, 1,3-dioxoisoindoline-4-carbaldehyde **174** (1.05 g, 6.00 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate **192** (1.20 g, 6.00 mmol), and dry THF (15 mL) were combined in a sealed flask. The flask was evacuated, filled with nitrogen, and fitted with a nitrogen balloon. The mixture was stirred at RT and tris-(2,2,2-trifluoroethyl) borate (1.85 g, 1.30 mL, 6.00 mmol) was added dropwise, followed by stirring for a further 2 h. Sodium cyanoborohydride (754 mg, 12.0 mmol) was dissolved in THF (5 mL) and added to the reaction mixture, followed by acetic acid (686 μL, 12.0 mmol), and the mixture stirred for a further 2 h. Hydrogen chloride solution (4 M in 1,4-dioxane,

15 mL) was added slowly and the mixture stirred for 1 h. The precipitate formed was collected by filtration and washed with THF (2 x 50 mL), then recrystallized (ethanol/water, 9:1), giving **152**·HCl as a colourless salt (1.3 g, 74%).

M.p. decomposed at 240-250 °C (ethanol/water); IR (film, cm^{-1}) 2960 (br, NH), 2706, 2592, 2517, 1758, 1720, 1701, 1587, 1549, 1455; ^{1}H NMR (D$_2$O; 600 MHz) 2.06 (2H, d, J = 13.2, 4.0 Hz, 2 x piperidine 3-H$_{ax}$), 2.60 (2H, br d, J = 13.2 Hz, 2 piperidine 2-H$_{eq}$), 3.23 (2H, td, J = 13.2, 2.2 Hz, 2 x piperidine 2-H$_{ax}$), 3.71 (2H, br d, J = 13.2 Hz, 2 x piperidine 3-H$_{eq}$), 3.82 (1H, app tt, J = 11.8, 4.0 Hz, piperidine 4-H), 4.79 (2H, s, lactam 3-H$_2$), 7.92 (1H, dd, J = 7.4, 1.2 Hz, 6-H), 7.95 (1H, t, J = 7.4 Hz, 5-H), 8.02 (1H, dd, J = 7.4, 1.2 Hz, Ar 4-H); ^{13}C NMR (D$_2$O; 151 MHz) 25.7 (2 x piperidine C-3), 42.8 (2 x piperidine C-2), 44.3 (lactam C-3), 53.5 (piperidine C-1), 125.4 (C-6), 129.2 (C), 130.9 (C), 133.6 (C), 136.1 (Ar C-4), 137.2 (C-5), 171.0 (C=O), 171.5 (C=O); m/z HRMS (ESI+) found [**152**H]$^+$ 260.1398, C$_{14}$H$_{18}$N$_3$O$_2$ requires 260.1399.

2-Ethyl-4-methylisoindoline-1,3-dione **160**

160

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (200 mg, 815 µmol) in water (3.3 mL), palladium on carbon (10%, 50% wet, 8 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (6.6 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Methanesulfonic acid (232 µL, 1.63 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 25 mL) and the solvent removed removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **160** as fine colourless crystals (22 mg, 14%).

R$_f$ 0.43 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 2921, 1696, 1650, 1555; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.26 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 2.69 (3H, s, Ar-CH$_3$), 3.72 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 7.43 (1H, d, J = 7.7 Hz, 5-H), 7.54 (1H, app t, J = 7.6 Hz, 6-H), 7.65 (1H, d, J =

7.3 Hz, 7-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (CH$_2$*C*H$_3$), 17.7 (Ar-CH$_3$), 32.8 (*C*H$_2$CH$_3$), 120.8 (C-5), 129.0 (C-4), 132.8 (C), 133.5 (C-6), 136.3 (C-7), 137.9 (C), 168.4 (C=O), 169.2 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 190.0862, C$_{11}$H$_{12}$NO$_2$ requires 190.0868.

N-Ethyl-3-oxoisoindoline-4-carboxamide **161**[114]

161

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in water (7 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (14 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (75.5 μL, 92.1 mg, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 50 mL). Concentrated sulphuric acid (107 μL, 2.00 mmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (100 mL), and dried under vacuum, to give **161** as fine colourless crystals (188 mg, 92%).

R$_f$ 0.30 (CH$_3$OH/EtOAc, 4:1); M.p. decomposed at 205-210 °C; IR (film, cm^{-1}) 2879, 1769, 1697; ^{1}H NMR (CD$_3$OD; 600 MHz) 1.27 (3H, t, *J* = 7.3 Hz, CH$_2$C*H*$_3$), 3.74 (2H, q, *J* = 7.3 Hz, C*H*$_2$CH$_3$), 4.58 (2H, s, CH$_2$), 7.83 (1H, d, *J* = 7.5 Hz, 5-H), 7.86 (1H, t, *J* = 7.5 Hz, 6-H), 7.93 (1H, d, *J* = 7.5 Hz, 7-H); ^{13}C NMR (CD$_3$OD; 151 MHz) 14.0 (CH$_2$*C*H$_3$), 33.9 (*C*H$_2$CH$_3$), 39.8 (C-1), 125.0 (C-5), 131.3 (C), 132.4 (C), 134.2 (C), 135.9 (C-7), 136.5 (C-6), 168.9 (C=O), 170.0 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 205.1094, C$_{11}$H$_{13}$N$_2$O$_2$ requires 205.0977.

2-Ethyl-4-(hydroxymethyl)isoindoline-1,3-dione **162**

162

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (200 mg, 815 μmol) in water (3.3 mL), palladium on carbon (10%, 50% wet, 8 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (6.6 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Methanesulfonic acid (232 μL, 1.63 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 25 mL) and the solvent removed removed under vacuum. The crude reaction mixture was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **162** as a colourless oil (15 mg, 8%).

R_f 0.37 (pet. ether 40-60/EtOAc, 2:1); IR (film, cm^{-1}) 3293 (br), 2912, 1682, 1645, 1508; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.28 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 3.75 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 4.95 (2H, s, CH_2OH), 7.61 (1H, dd, J = 8.2, 1.1 Hz, 5-H), 7.66 (1H, app t, J = 7.5 Hz, 7-H), 7.77 (1H, dd, J = 7.3, 1.1 Hz, 6-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.0 (CH$_2$CH$_3$), 33.2 (CH$_2$CH$_3$), 62.5 (CH$_2$OH), 122.6 (C-7), 129.3 (C), 133.0 (C), 133.2 (C-5), 134.3 (C-6), 141.0 (C), 168.2 (C=O), 170.2 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 206.0814, C$_{11}$H$_{12}$NO$_3$ requires 206.0817.

tert-Butyl-((2-ethyl-1,3-dioxoisoindolin-4-yl)methyl)carbamate **163**[114]

163

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in water (7 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (14 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (92.0 mg, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. Solid $NaHCO_3$ was slowly added until the solution was alkaline, then di-*tert*-butyl dicarbonate (1.15 mL, 5.00 mmol) added and the reaction stirred at RT for 4 h. The organic component was extracted with ethyl acetate (3 x 100 mL), dried ($MgSO_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **163** as a pale yellow oil (234 mg, 77%).

R_f 0.46 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 1807, 1770, 1704, 1508; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.25 (3H, t, J = 7.3 Hz, CH$_2$CH_3), 1.39 (9H, s, C(CH$_3$)$_3$), 3.70 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 4.61 (2H, d, J = 6.5 Hz, ArCH$_2$), 5.63 (1H, br t, J = 6.5 Hz, NH), 7.61 (1H, t, J = 7.40 Hz, 6-H), 7.66 (1H, d, J = 7.4 Hz, 7-H), 7.71 (1H, d, J = 7.4 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.0 (CH$_2$CH$_3$), 28.5 (C(CH$_3$)$_3$), 33.0 (CH$_2$CH$_3$), 40.9 (ArCH$_2$), 79.7 (C(CH$_3$)$_3$), 122.3 (C-7), 128.9 (C), 132.9 (C), 134.1 (C-6), 134.6 (C-5), 138.5 (C), 156.0 (C(O)O^{t}Bu), 168.2 (C=O), 169.1 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 305.1493, C$_{16}$H$_{21}$N$_2$O$_4$ requires 305.1501.

3-Oxoisoindoline-4-carboxamide 164[219]

164

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (217 mg, 1.00 mmol), in water (7 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (14 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (75.5 µL, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 50 mL). Concentrated sulphuric acid (107 µL, 2.00 mmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (100 mL), and dried under vacuum, to give **164** as fine colourless crystals (173 mg, 98%).

R_f 0.28 (CH$_3$OH/EtOAc, 4:1); M.p. decomposed at 205-210 °C (EtOAc); IR (film, cm^{-1}) 3038 (br), 2925 (br), 1763, 1699, 1600, 1515; ^{1}H NMR (CD$_3$OD; 600 MHz) 4.56 (2H, s, 1-H$_2$), 7.83 (1H, dd, J = 7.3, 1.7 Hz, 5-H), 7.86 (1H, t, J = 7.3 Hz, 6-H), 7.90 (1H, dd, J = 7.3, 1.7 Hz, 7-H); ^{13}C NMR (CD$_3$OD; 151 MHz) 39.9 (C-1), 125.1 (C-5), 132.0 (C), 132.5 (C), 135.1 (C), 136.0 (C-7), 136.6 (C-6), 170.1 (C=O), 171.3 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 177.0657, C$_9$H$_9$N$_2$O$_2$ requires 177.0664.

5-(Ethoxymethyl)-*N*-ethyl-3-oxoisoindoline-4-carboxamide 165[114]
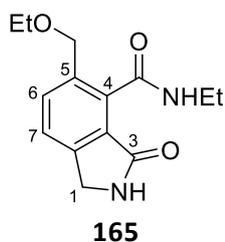
165

To a solution of 4-((2,2-dimethylhydrazono)methyl)-7-(ethoxymethyl)-2-ethylisoindoline-1,3-dione **78** (60.6 mg, 200 µmol) in water (3 mL), palladium on carbon (10%, 50% wet, 10 mg) was added and the reaction vessel evacuated under vacuum and filled with argon.

Methanol (6 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (15.1 μL, 400 μmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 20 mL). Concentrated sulphuric acid (21.4 μL, 400 μmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (40 mL), and dried under vacuum, to give **165** as fine colourless crystals (33 mg, 62%).

R_f 0.35 (CH$_3$OH/EtOAc, 4:1); M.p. decomposed at 115-120 °C (EtOAc); IR (film, cm^{-1}) 1764, 1697, 1598, 1504; ^{1}H NMR (CD$_3$OD; 600 MHz) 1.23-1.29 (6H, m, NCH$_2$C*H*$_3$ and OCH$_2$C*H*$_3$), 3.67 (2H, q, *J* = 7.2 Hz, NC*H*$_2$CH$_3$), 3.71 (2H, q, *J* = 7.3 Hz, OC*H*$_2$CH$_3$), 4.55 (2H, s, 1-H$_2$), 4.99 (2H, s, C*H*$_2$OEt), 7.78 (1H, d, *J* = 8.0 Hz, 7-H), 7.92 (1H, d, *J* = 8.0 Hz, 6-H); ^{13}C NMR (CD$_3$OD; 151 MHz) 14.0 (NCH$_2$*C*H$_3$), 15.4 (OCH$_2$*C*H$_3$), 33.8 (N*C*H$_2$CH$_3$), 39.8 (O*C*H$_2$CH$_3$), 67.7 (*C*H$_2$OEt), 68.1 (C-1), 129.8 (C), 131.1 (C), 131.4 (C), 134.5 (C-7), 136.5 (C-6), 140.9 (C), 169.0 (C=O), 169.9 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 263.1389, C$_{14}$H$_{19}$N$_2$O$_3$ requires 263.1396.

4-((2,2-Dimethylhydrazono)methyl)-7-(hydroxymethyl)isoindoline-1,3-dione **166**

166
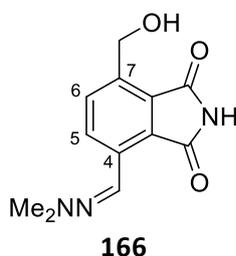
To a solution of 5-hydroxymethylfurfural (775 μL, 7.94 mmol) in water (20 mL), *N,N*-dimethylhydrazine (1.16 mL, 9.52 mmol) was added and the mixture was stirred at 50 °C for 40 min. Maleimide (2.33 g, 15.9 mmol) was added and the reaction stirred at 50 °C for 3 h. The reaction was cooled to RT and the precipitate was collected by filtration, washed with cold water (2 x 100 mL) and dried to give **166** as yellow needles (1.71 g, 87%).

R_f 0.50 (pet. ether 40-60/EtOAc, 1:2); M.p. 202-204 °C (water); IR (film, cm^{-1}) 3187 (br), 3045, 1752, 1688, 1650, 1537; ^{1}H NMR (CD$_3$OD; 600 MHz) 3.06 (6H, s, N(CH$_3$)$_2$), 4.98 (2H, s, CH$_2$O), 7.69 (1H, d, *J* = 8.4 Hz, 6-H), 8.05 (1H, s, N=CH), 8.14 (1H, d, *J* = 8.4 Hz, 5-H); ^{13}C

NMR (CD$_3$OD; 151 MHz) 42.7 (N(CH$_3$)$_2$), 60.5 (CH$_2$OH), 125.7 (N=CH), 126.9 (C), 129.7 (C), 129.9 (C-5), 133.2 (C-6), 136.5 (C), 140.2 (C), 171.2 (C=O), 171.6 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 248.1032, C$_{12}$H$_{14}$N$_3$O$_3$ requires 248.1035.

N-Ethyl-5-methyl-3-oxoisoindoline-4-carboxamide **170**
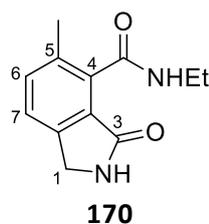
170

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethyl-7-(hydroxymethyl) isoindoline-1,3-dione **75** (274 mg, 1.00 mmol) in water (7 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (14 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (75.5 µL, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 50 mL). Concentrated sulphuric acid (107 µL, 2.00 mmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (100 mL), and dried under vacuum, to give **170** as fine colourless crystals (194 mg, 89%).

R$_f$ 0.27 (CH$_3$OH/EtOAc, 4:1); M.p. decomposed at 205-210 °C; IR (film, cm^{-1}) 2883, 1770, 1698; ^{1}H NMR (CD$_3$OD; 600 MHz) 1.27 (3H, t, *J* = 7.3 Hz, CH$_2$C*H$_3$*), 2.63 (3H, s, Ar-CH$_3$), 3.74 (2H, q, *J* = 7.3 Hz, C*H$_2$*CH$_3$), 4.60 (2H, s, 1-H$_2$), 7.53 (1H, d, *J* = 7.9 Hz, 6-H), 7.57 (1H, d, *J* = 7.9 Hz, 7-H); ^{13}C NMR (CD$_3$OD; 151 MHz) 14.0 (CH$_2$*C*H$_3$), 18.0 (Ar-CH$_3$), 33.8 (*C*H$_2$CH$_3$), 39.7 (C-1), 126.2 (C), 131.9 (C), 132.6 (C), 134.5 (C), 135.9 (C-7), 136.5 (C-6), 169.1 (C=O), 170.2 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 219.1128, C$_{12}$H$_{15}$N$_2$O$_2$ requires 219.1133.

5-Methyl-3-oxoisoindoline-4-carboxamide 171

171
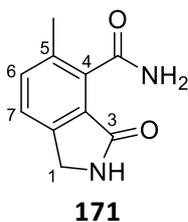
To a solution of 4-((2,2-dimethylhydrazono)methyl)-7-(hydroxymethyl)isoindoline-1,3-dione **166** (247 mg, 1.00 mmol), in water (7 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol (14 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (75.5 μL, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 50 mL). Concentrated sulphuric acid (107 μL, 2.00 mmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (100 mL), and dried under vacuum, to give **171** as fine colourless crystals (162 mg, 85%).

R_f 0.27 (CH$_3$OH/EtOAc, 4:1); M.p. decomposed at 205-210 °C (EtOAc); IR (film, cm^{-1}) 3036 (br), 2925 (br), 1765, 1699; ^{1}H NMR (CD$_3$OD; 600 MHz) 2.72 (3H, s, Ar-CH$_3$), 4.56 (2H, s, 1-H$_2$), 7.83 (1H, d, J = 7.3 Hz, 6-H), 7.90 (1H, d, J = 7.3 Hz, 7-H); ^{13}C NMR (CD$_3$OD; 151 MHz) 18.0 (Ar-CH$_3$), 39.9 (C-1), 126.4 (C), 132.0 (C), 132.5 (C), 135.1 (C), 136.0 (C-7), 136.6 (C-6), 169.9 (C=O), 171.0 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 191.0818, C$_{10}$H$_{11}$N$_2$O$_2$ requires 191.0821.

N-Ethyl-1-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-9-carboxamide 172[114]

172
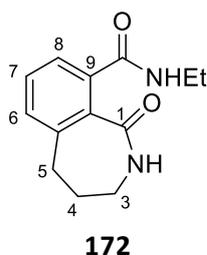
To a solution of 4-(3-(2,2-dimethylhydrazono)prop-1-en-1-yl)-2-ethylisoindoline-1,3-dione **88** (135 mg, 500 μmol), in water (3 mL), palladium on carbon (10%, 50% wet, 20 mg) was added and the reaction vessel evacuated under vacuum and filled with argon. Methanol

(6 mL) was added and the reaction vessel evacuated once more and put under hydrogen using a balloon. Formic acid (37.8 µL, 1.00 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solution collected. The organic component was extracted with ethyl acetate (3 x 25 mL). Concentrated sulphuric acid (53.5 µL, 1.00 mmol) was added and the reaction mixture stirred for 5 min. A colourless precipitate formed and was collected by filtration, washed with cold ethyl acetate (50 mL), and dried under vacuum, to give **172** as fine colourless crystals (52 mg, 45%).

R_f 0.11 (MeOH/EtOAc, 4:1); M.p. 124-126 °C (EtOAc); IR (film, cm^{-1}) 2942 (w, br), 1767, 1697, 1657, 1556; ^{1}H NMR (CD$_3$OD; 600 MHz) 1.23 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 2.02 (2H, qn, J = 7.7 Hz, 4-H$_2$), 2.99 (2H, t, J = 7.7 Hz, 5-H$_2$), 3.17 (2H, t, J = 7.7 Hz, 3-H$_2$), 3.68 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 7.61 (1H, dd, J = 6.8, 1.7 Hz, 8-H), 7.67-7.73 (2H, m, 6-H and 7-H), 8.53 (1H, br s, NH); ^{13}C NMR (CD$_3$OD; 151 MHz) 14.1 (CH$_2$CH$_3$), 29.0 (C-4), 29.9 (C-5), 33.6 (CH$_2$CH$_3$), 40.2 (C-3), 122.5 (C-8), 129.9 (C-5a), 134.2 (C-9), 135.3 (C-7), 136.7 (C-6), 141.6 (C-9a), 169.4 (C=O), 170.2 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 233.1285, C$_{13}$H$_{17}$N$_2$O$_2$ requires 233.1290.

1,3-Dioxoisoindoline-4-carbaldehyde **174**[114]

174
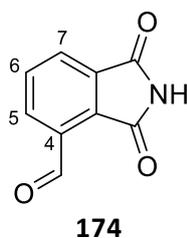
To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (217 mg, 1.00 mmol) in acetone (70 mL), Amberlyst® 15 (1.00 g) was added, and the mixture was stirred at RT for 3 h. The Amberlyst® 15 was removed by filtration and the solvent removed under vacuum to give **174** as a pale yellow solid (169 mg, 97%).

R_f 0.60 (pet. ether 40-60/EtOAc, 2:1); M.p. 226 °C (acetone); IR (film, cm^{-1}) 3212 (br, NH), 1773, 1700, 1610; ^{1}H NMR (acetone-d$_6$; 600 MHz) 8.01 (1H, t, J = 7.3 Hz, 6-H), 8.13 (1H, d, J = 7.3 Hz, 7-H), 8.22 (1H, d, J = 7.3 Hz, 5-H), 10.41 (1H, s br, NH), 10.97 (1H, s, CHO); ^{13}C NMR (acetone-d$_6$; 151 MHz) 128.6 (C-6), 131.5 (C-7), 134.0 (C), 134.7 (C), 135.0 (C), 135.4 (C-5), 168.5 (C=O), 169.3 (C=O), 189.3 (CHO); m/z HRMS (ESI+) found [MH]$^+$ 176.0344, C$_9$H$_6$NO$_3$ requires 176.0348.

4-(Dimethoxymethyl)isoindoline-1,3-dione **175**

175
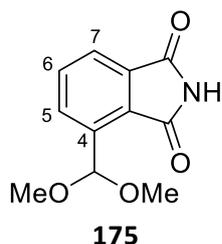
To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (108 mg, 500 µmol) in methanol (24 mL) and water (1 mL), Amberlyst® 15 (500 mg) was added, and the mixture was stirred at RT for 4 h. The Amberlyst® 15 was removed by filtration and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5), giving **175** as fine colourless crystals (95 mg, 86%).

R_f 0.38 (pet. ether 40-60/EtOAc, 3:2); M.p. 138 - 140 °C (EtOAc); IR (film, cm^{-1}) 3185 (br, NH), 2830, 1764 (C=O), 1708 (C=O), 1657, 1598; ^{1}H NMR (CDCl$_3$; 500 MHz) 3.46 (6H, s, 2 x OCH$_3$), 6.20 (1H, s, C*H*(OCH$_3$)$_2$), 7.76 (1H, app t, *J* = 7.6 Hz, 6-H), 7.84 (1H, dd, *J* = 7.4, 0.9 Hz, 7-H), 7.99 (1H, dd, *J* = 7.8, 0.9 Hz, 5-H), 8.42 (1H, s br, NH); ^{13}C NMR (CDCl$_3$; 151 MHz) 54.9 (2 x OCH$_3$), 96.9 (*C*H(OCH$_3$)$_2$), 123.8 (C-7), 129.1 (C-4), 132.3 (C-5), 133.0 (C-3a), 134.4 (C-6), 138.0 (C-7a), 168.1 (C=O), 168.3 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 222.0765, C$_{11}$H$_{12}$NO$_4$ requires 222.0766.

3-(2-Ethyl-1,3-dioxoisoindolin-4-yl)acrylaldehyde **177**

177
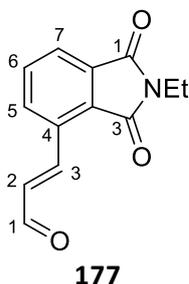
To a solution of 4-(3-(2,2-dimethylhydrazono)prop-1-en-1-yl)-2-ethylisoindoline-1,3-dione **88** (123 mg, 500 µmol) in acetone (40 mL), Amberlyst® 15 (500 mg) was added, and the mixture was stirred at RT for 3 h. The Amberlyst® 15 was removed by filtration and the solvent removed under vacuum to give **177** as fine colourless crystals (104 mg, 91%).

R_f 0.43 (pet. ether 40-60/EtOAc, 3:2); M.p. 143-145 °C (acetone); IR (film, cm^{-1}) 3076, 2945, 1768, 1757, 1698, 1678; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.29 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 3.77 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 6.85 (1H, dd, J = 16.3, 7.7 Hz, alkene 2-H), 7.75 (1H, app t, J = 7.7 Hz, 6-H), 7.90 (1H, d, J = 7.3 Hz, 7-H), 7.96 (1H, d, J = 8.0 Hz, 5-H), 8.64 (1H, d, J = 16.3 Hz, alkene 3-H), 9.84 (1H, d, J = 7.7 Hz, CHO); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.0 (CH$_2$CH$_3$), 33.2 (CH$_2$CH$_3$), 125.1 (C-7), 129.0 (C-4), 131.1 (C-5), 132.4 (alkene C-2), 132.6 (C-3a), 133.2 (C-7a), 134.2 (C-6), 145.0 (alkene C-3), 167.5 (C=O), 168.4 (C=O), 193.9 (CHO); m/z HRMS (ESI+) found [MH]$^+$ 230.0824, C$_{13}$H$_{12}$NO$_3$ requires 230.0817.

2-Ethyl-1,3-dioxoisoindoline-4-carbonitrile 178[114]
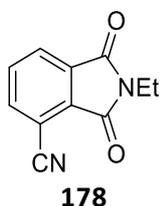
178

To a solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in methanol (10 mL) on ice, magnesium monoperoxyphthalate hexahydrate (1.23 g, 2.50 mmol) was added and the reaction stirred for 5 min. To the reaction mixture, water (20 mL) was added, and the organic component extracted with ethyl acetate (3 x 100 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum, giving **178** as fine colourless crystals (194 mg, 97%).

R_f 0.34 (pet. ether 40-60/EtOAc, 3:2); M.p. 158-159 °C (EtOAc); IR (film, cm^{-1}) 2239 (C≡N), 1777, 1704; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.30 (3H, t, J = 7.3 Hz, CH$_2$CH_3), 3.79 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 7.85 (1H, t, J = 7.6 Hz, 6-H), 7.97 (1H, dd, J = 7.6, 1.1 Hz, 7-H), 8.07 (1H, dd, J = 7.6, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.9 (CH$_2$CH$_3$), 33.7 (CH$_2$CH$_3$), 107.8 (C), 114.4 (C), 127.2 (C-7), 133.4 (C), 133.5 (C), 134.6 (C-5), 137.8 (C-6) 165.2 (C=O), 166.4 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 201.0659, C$_{11}$H$_9$N$_2$O$_2$ requires 201.0664.

Phthalonitrile 179[220]
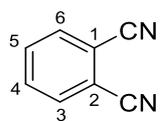
179

To a solution of 2-((2,2-Dimethylhydrazono)methyl)benzonitrile **121** (86.6 mg, 500 μmol) in methanol (5 mL) on ice, magnesium monoperoxyphthalate hexahydrate (618 mg, 1.25 mmol) was added and the reaction stirred for 5 min. To the reaction mixture, water (10 mL) was added, and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO₄), filtered, and the solvent removed under vacuum, giving **179** as fine colourless crystals (63 mg, 99%).

R_f 0.51 (pet. ether 40-60/EtOAc, 3:2); M.p. 140-141 °C (EtOAc), Lit[220] 138-140 °C; IR (film, cm^{-1}) 3082, 2231 (C≡N), 1664, 1590; ^{1}H NMR (CDCl₃; 600 MHz) 7.77 (2H, dd, J = 5.8, 3.3 Hz, 4-H & 5-H), 7.84 (2H, dd, J = 5.8, 3.3 Hz, 3-H & 6-H); ^{13}C NMR (CDCl₃; 151 MHz) 115.4 (C-1 & C-2), 116.1 (2 × CN), 133.3 (C-4 & C-5), 133.7 (C-3 & C-6); m/z HRMS (ESI+) found [MH]$^+$ 129.0452, C₈H₅N₂ requires 129.0453.

Benzene-1,2,3-tricarbonitrile 180[221]
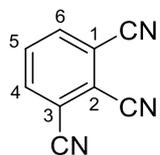
180

To a solution of 3-((2,2-dimethylhydrazono)methyl)phthalonitrile **32** (198 mg, 1.00 mmol) in methanol (10 mL) on ice, magnesium monoperoxyphthalate hexahydrate (1.23 g, 2.50 mmol) was added and the reaction stirred for 5 min. To the reaction mixture, water (20 mL) was added, and the organic component extracted with ethyl acetate (3 x 100 mL), dried (MgSO₄), filtered, and the solvent removed under vacuum, giving **180** as fine colourless crystals (150 mg, 98%).

R_f 0.46 (pet. ether 40-60/EtOAc, 3:2); M.p. 178-179 °C (EtOAc), Lit[221] 182-183 °C; IR (film, cm^{-1}) 3053, 2240 (C≡N), 2235 (C≡N), 1667, 1587; ^{1}H NMR (CDCl₃; 600 MHz) 7.96 (1H, t, J = 5.9 Hz, 5-H), 8.04 (2H, d, J = 5.9 Hz, 4-H & 6-H); ^{13}C NMR (CDCl₃; 151 MHz) 114.4 (C-1 & C-3), 115.2 (C-2), 118.7 (1-CN & 3-CN), 120.6 (2-CN), 133.8 (C-5), 136.8 (C-4 & C-6); m/z HRMS (ESI+) found [MH]$^+$ 154.0406, C₉H₄N₃ requires 154.0405.

3-(2-Ethyl-1,3-dioxoisoindolin-4-yl)acrylonitrile **181**

181
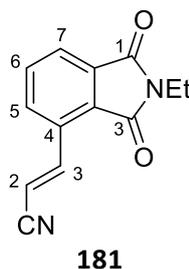
To a solution of 4-(3-(2,2-dimethylhydrazono)prop-1-en-1-yl)-2-ethylisoindoline-1,3-dione **88** (136 mg, 500 µmol) in methanol (5 mL) on ice, magnesium monoperoxyphthalate hexahydrate (618 mg, 1.25 mmol) was added and the reaction stirred for 5 min. To the reaction mixture, water (10 mL) was added, and the organic component extracted with ethyl acetate (3 x 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5), giving **181** as fine colourless crystals (105 mg, 93%).

R$_f$ 0.50 (pet. ether 40-60/EtOAc, 3:2); M.p. 140 - 141 °C (EtOAc); IR (film, cm^{-1}) 3064, 2943, 2231 (C≡N), 1775, 1701, 1670, 1611; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.28 (3H, t, *J* = 7.3 Hz, CH$_2$C*H$_3$*), 3.75 (2H, q, *J* = 7.3 Hz, C*H$_2$*CH$_3$), 7.05 (1H, d, *J* = 15.9, alkene 2-H), 7.73 (1H, d, *J* = 7.9 Hz, 5-H), 7.88 (1H, app t, *J* = 7.8 Hz, 6-H), 8.00 (1H, d, *J* = 7.3 Hz, 7-H), 8.50 (1H, d, *J* = 15.9 Hz, alkene 3-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.0 (CH$_2$*C*H$_3$), 33.0 (*C*H$_2$CH$_3$), 126.5 (C-7), 128.9 (C), 130.7 (C-5), 132.5 (alkene C-2), 133.3 (C), 133.7 (C-6), 134.0 (C), 139.7 (alkene C-3), 167.8 (C=O), 169.0 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 227.0818, C$_{13}$H$_{11}$N$_2$O$_2$ requires 227.0820.

2-Butyl-4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **182**[114]
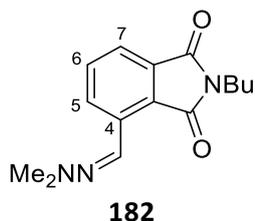
182

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 µmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 µL, 2.0 mmol) and *n*-butylamine (198 µL, 2.00 mmol) were added and the

mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **182** as a yellow solid (252 mg, 92%).

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in butylamine (4 mL) was stirred for 3 h at RT and then added to aqueous ammonium chloride (10% w/v, 40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), and dried to give **182** (244 mg, 89%) as a yellow solid.

R_f 0.41 (pet. ether 40-60/EtOAc, 4:1); M.p. 91-92 °C (EtOAc); IR (film, cm^{-1}) 2896, 1760, 1696, 1547; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.92 (3H, t, J = 7.3 Hz, butyl CH$_3$), 1.37 (2H, sx, J = 7.3 Hz, CH$_2$), 1.65 (2H, qn, J = 7.3 Hz, CH$_2$), 3.12 (6H, s, N(CH$_3$)$_2$), 3.65 (2H, t, J = 7.3 Hz, CH$_2$), 7.55 (1H, t, J = 7.6 Hz, 6-H), 7.60 (1H, d, J = 7.6 Hz, 7-H), 8.11 (1H, s, N=CH), 8.22 (1H, d, J = 7.6 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.8 (butyl CH$_3$), 20.3 (CH$_2$), 30.8 (CH$_2$), 37.8 (CH$_2$), 42.7 (N(CH$_3$)$_2$), 120.9 (C-7), 124.7 (N=CH), 124.8 (C), 128.9 (C-5), 132.5 (C), 133.3 (C-6), 136.3 (C), 168.7 (C=O), 169.7 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 274.1551, C$_{15}$H$_{20}$N$_3$O$_2$ requires 274.3382.

2-Benzyl-4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **183**[114]
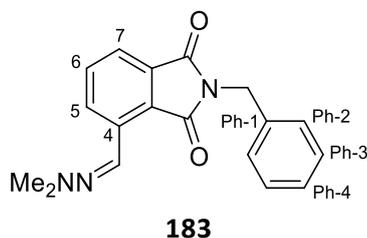
183

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 μmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 μL, 2.0 mmol) and benzylamine (219 μL, 2.00 mmol) were added and the mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the

solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **183** as a yellow solid (191 mg, 62%).

R_f 0.29 (pet. ether 40-60/EtOAc, 4:1); M.p. 136-138 °C (water); IR (film, cm^{-1}) 2951, 1754, 1696, 1546; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.12 (6H, s, N(CH$_3$)$_2$), 4.83 (2H, s, CH$_2$), 7.27 (1H, t, J = 7.5 Hz, Ph-4-H), 7.32 (2H, t, J = 7.5 Hz, 2 × Ph-3-H), 7.43 (2H, d, J = 7.5 Hz, 2 × Ph-2-H), 7.56 (1H, t, J = 7.6 Hz, 6-H), 7.63 (1H, d, J = 7.6 Hz, 7-H), 8.09 (1H, s, N=CH), 8.23 (1H, d, J = 7.6 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 41.5 (CH$_2$), 42.7 (N(CH$_3$)$_2$), 121.0 (C-7), 124.5 (C), 124.7 (N=CH), 127.9 (Ph-4), 128.6 (2 × Ph-3), 128.8 (2 × Ph-2), 129.1 (C-5), 132.4 (C), 133.5 (C-6), 136.6 (C), 136.7 (C), 168.3 (C=O), 169.2 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 308.0831, C$_{18}$H$_{18}$N$_3$O$_2$ requires 308.1399.

N^1-Butyl-3-((2,2-dimethylhydrazono)methyl)phthalamide **184**

184
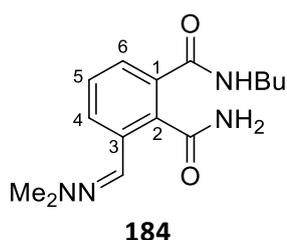
A solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) in butylamine (4 mL) was stirred for 16 h at RT and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **184** as yellow needles (192 mg, 66%).

R_f 0.35 (pet. ether 40-60/EtOAc, 1:1); M.p. 166-167 °C (EtOAc); IR (film, cm^{-1}) 3263 (br, NH), 2899, 1632, 1553; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.94 (3H, t, J = 7.4 Hz, butyl CH$_3$), 1.39 (2H, sx, J = 7.4 Hz, CH$_2$), 1.56 (2H, qn, J = 7.4 Hz, CH$_2$), 2.99 (6H, s, N(CH$_3$)$_2$), 3.420 (2H, m, NCH$_2$), 6.00 (2H, br, NH$_2$), 6.58 (1H, br, NHBu), 7.17 (1H, s, N=CH), 7.38 (1H, m, 5-H), 7.56 (1H, dd, J = 7.7, 1.1 Hz, 6-H), 8.00 (1H, dd, J = 8.0, 1.1 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.1 (butyl CH$_3$), 20.5 (CH$_2$), 31.9 (CH$_2$), 41.6 (CH$_2$), 42.7 (N(CH$_3$)$_2$), 126.1 (N=CH), 127.2 (C-6), 132.3 (C-4), 133.8 (C-5), 134.2 (C), 134.5 (C), 136.7 (C), 169.8 (C=O), 171.5 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 291.1822, C$_{15}$H$_{23}$N$_4$O$_2$ requires 291.1821

N,*N*-Dibutyl-3-((2,2-dimethylhydrazono)methyl)phthalamide **185**[114]
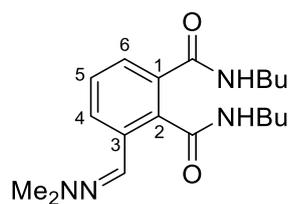
185

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in butylamine (4 mL) was stirred for 3 h at RT and then added to water (40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), and dried to give **185** (313 mg, 96%) as yellow needles.

R_f 0.47 (pet. ether 40-60/EtOAc, 2:1); M.p. 132-133 °C (water); IR (film, cm^{-1}) 3258 (br, NH), 2917, 1630, 1554, 1458; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.932 (3H, t, J = 7.4 Hz, butyl CH$_3$), 0.935 (3H, t, J = 7.4 Hz, butyl CH$_3$), 1.379 (2H, sx, J = 7.4 Hz, CH$_2$), 1.383 (2H, sx, J = 7.4 Hz, CH$_2$), 1.53 (2H, qn, J = 7.4 Hz, CH$_2$), 1.55 (2H, qn, J = 7.4 Hz, CH$_2$), 2.98 (6H, s, N(CH$_3$)$_2$), 3.35 (2H, m, NCH$_2$), 3.40 (2H, m, NCH$_2$), 6.07 (1H, br t, J = 5.0 Hz, NH), 6.51 (1H, br t, J = 5.0 Hz, NH), 7.18 (1H, s, N=CH), 7.34 (1H, t, J = 7.6 Hz, 5-H), 7.46 (1H, d, J = 7.6 Hz, 6-H), 7.95 (1H, d, J = 7.6 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 13.8 (butyl CH$_3$), 13.9 (CH$_3$), 20.2 (CH$_2$), 20.3 (CH$_2$), 31.57 (CH$_2$), 31.61 (CH$_2$), 40.0 (CH$_2$), 40.1 (CH$_2$), 42.7 (N(CH$_3$)$_2$), 126.6 (N=CH), 126.9 (C-6), 127.8 (C-4), 129.3 (C-5), 132.7 (C), 134.2 (C), 134.5 (C), 168.5 (C=O), 169.8 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 347.2442, C$_{19}$H$_{31}$N$_4$O$_2$ requires 347.2447.

4-((2,2-Dimethylhydrazono)methyl)-2-(3-morpholinopropyl)isoindoline-1,3-dione **186**[114]
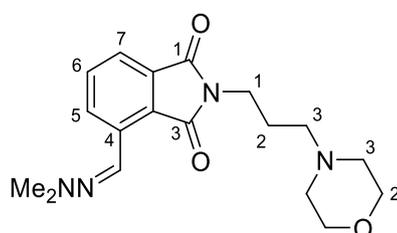
186

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 µmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 µL, 2.0 mmol) and 3-morpholinopropylamine (292 µL, 2.00 mmol) were added

and the mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **186** as a yellow solid (269 mg, 78%).

R_f 0.17 (pet. ether 40-60/EtOAc, 1:2); M.p. 98-99 °C (water); IR (film, cm^{-1}) 2930, 1759, 1697, 1596, 1547; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.87 (2H, t, J = 6.9 Hz, propyl 2-H$_2$), 2.42 (6H, m, propyl 1-H$_2$ & 2 x morpholine 3-H$_2$), 3.12 (6H, s, N(CH$_3$)$_2$), 3.58 (4H, m, 2 x morpholine 2-CH$_2$), 3.74 (2H, t, J = 6.9 Hz, propyl 3-H$_2$), 7.56 (1H, t, J = 7.5 Hz, 6-H), 7.60 (1H, d, J = 7.5 Hz, 7-H), 8.10 (1H, s, N=CH), 8.23 (1H, d, J = 7.5 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 29.6 (propyl C-2), 35.0 (propyl C-3), 37.8 (propyl C-1), 42.8 (N(CH$_3$)$_2$), 51.7 (2 x morpholine C-3), 67.1 (2 x morpholine C-2), 120.9 (C-7), 124.8 (N=CH), 125.1 (C), 127.0 (C), 129.4 (C-5), 131.4 (C-6), 136.4 (C), 167.7 (C=O), 169.2 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 345.1924, C$_{18}$H$_{24}$N$_4$O$_3$ requires 345.1927.

2-(*sec*-Butyl)-4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **187**
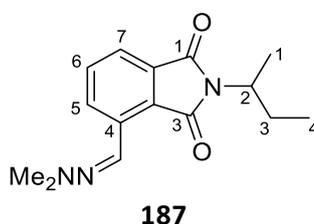
187

To a solution of 4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **56** (229 mg, 1.00 mmol) and boric acid (6.2 mg, 100 µmol) in toluene (0.2 mL) and 1,4-dioxane (0.2 mL), water (36 µL, 2.0 mmol) and *sec*-butylamine (505 µL, 5.00 mmol) were added and the mixture was stirred at 100 °C. After 72 h the reaction was cooled to RT and IRA 743 scavenger (approximately 1 g) and water (0.2 mL) were added, and the mixture stirred for a further 1 h. The scavenger resin was removed by filtration and the filtrate collected and the solvent removed under vacuum. The crude product was dry-loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **187** as a yellow solid (156 mg, 57%).

R_f 0.62 (pet. ether 40-60/EtOAc, 3:2); M.p. 121-122 °C (water); IR (film, cm^{-1}) 2923, 1754, 1694, 1594, 1548; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.88 (3H, t, J = 7.4 Hz, butyl 4-H$_3$), 1.47 (3H, d, J = 6.9 Hz, butyl 1-H$_3$), 1.78 (1H, m, butyl 3-H$_a$), 2.05 (1H, m, butyl 3-H$_b$), 3.12 (6H, s, N(CH$_3$)$_2$), 4.24 (1H, m, butyl 2-H), 7.55 (1H, t, J = 7.5 Hz, 6-H), 7.59 (1H, dd, J = 7.5, 1.1 Hz, 7-H), 8.12 (1H, s, N=CH), 8.22 (1H, dd, J = 7.5, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 11.4 (butyl C-4), 18.5 (butyl C-1) 27.1 (butyl C-3), 42.6 (N(CH$_3$)$_2$), 48.9 (butyl C-2), 120.7 (C-7), 124.6 (C), 124.7 (N=CH), 128.9 (C-5), 132.3 (C), 133.2 (C-6), 136.2 (C), 168.7 (C=O), 169.8 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 274.1547, C$_{15}$H$_{20}$N$_3$O$_2$ requires 274.3382.

N,N-Dibenzyl-3-((2,2-dimethylhydrazono)methyl)phthalamide **188**[114]
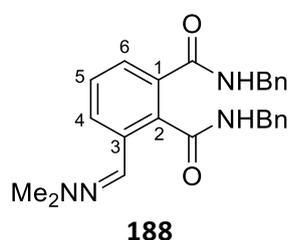
188

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in benzylamine (4 mL) was stirred for 3 h at RT and then added to water (40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), dried, and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **188** (311 mg, 75%) as yellow needles.

R_f 0.53 (pet. ether 40-60/EtOAc, 2:1); M.p. 184-185 °C (EtOAc); IR (film, cm^{-1}) 3254 (br, NH), 2925, 1636, 1554, 1496; ^{1}H NMR ((CD$_3$)$_2$SO; 600 MHz) 2.73 (6H, s, N(CH$_3$)$_2$), 4.33 (2H, d, J = 5.8 Hz, benzyl CH$_2$), 4.41 (2H, d, J = 5.8 Hz, benzyl CH$_2$), 7.01 (1H, s, N=CH); 7.23 - 7.40 (11H, m, 10 x benzyl CH & 5-H), 7.42 (1H, d, J = 7.6 Hz, 6-H), 7.87 (1H, d, J = 7.6 Hz, 4-H), 8.61 (1H, br t, J = 5.8 Hz, NH), 8.81 (1H, br t, J = 5.8 Hz, NH); ^{13}C NMR ((CD$_3$)$_2$SO; 151 MHz) 42.1 (N(CH$_3$)$_2$), 42.5 (benzyl CH$_2$), 42.6 (benzyl CH$_2$), 125.0 (benzyl CH), 125.7 (benzyl CH), 126.7 (N=CH), 126.8 (C-6), 127.2 (benzyl CH), 127.5 (benzyl CH), 127.8 (C-4), 128.16 (benzyl CH), 128.20 (benzyl CH), 128.3 (C), 133.7 (C-5), 134.7 (C), 134.8 (C), 139.4 (C), 167.4 (C=O), 167.6 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 415.1837, C$_{25}$H$_{27}$N$_4$O$_2$ requires 415.2134.

3-((2,2-Dimethylhydrazineylidene)methyl)-N^2-ethyl-N^1-isopropylphthalamide **189**
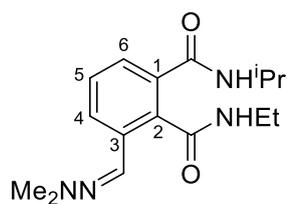
189

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in isopropylamine (4 mL) was stirred for 3 h at RT and then added to water (40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), dried, and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **189** (265 mg, 87%) as yellow needles.

R_f 0.41 (pet. ether 40-60/EtOAc, 2:1); M.p. 144-146 °C (EtOAc); IR (film, cm^{-1}) 3232 (br, NH), 2940, 1630, 1549; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.17 – 1.25 (9H, m, CH$_2$C*H$_3$* & CH(C*H$_3$*)$_2$), 2.98 (6H, s, N(CH$_3$)$_2$), 3.44 (2H, m, C*H$_2$*CH$_3$), 4.18 (1H, m, NC*H*(CH$_2$)$_3$), 6.19 (1H, br t, NH), 6.42 (1H, br t, NH), 7.19 (1H, s, N=CH), 7.35 (1H, m, 5-H), 7.46 (1H, d, *J* = 7.6 Hz, 6-H), 7.95 (1H, d, *J* = 7.9 Hz, 4-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 31.5 (CH$_2$*C*H$_3$), 31.7 (CH(*C*H$_3$)$_2$), 39.8 (*C*H$_2$CH$_3$), 41.0 (*C*H(CH$_3$)$_2$), 42.7 (N(CH$_3$)$_2$), 126.5 (N=CH), 127.1 (C-6), 127.6 (C-4), 129.7 (C-5), 132.3 (C), 133.2 (C), 134.5 (C), 168.9 (C=O), 169.9 (C=O); *m/z* HRMS (ESI+) found [MH]$^+$ 305.1977, C$_{16}$H$_{25}$N$_4$O$_2$ requires 305.1978.

2-Allyl-4-((2,2-dimethylhydrazono)methyl)isoindoline-1,3-dione **190**
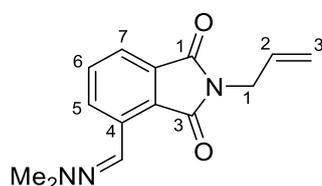
190

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in isopropylamine (4 mL) was stirred for 3 h at RT and then added to aqueous ammonium chloride (10% w/v, 40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), dried, and purified by flash column chromatography (pet. ether 40-60/EtOAc, 95:5) to give **190** (229 mg, 93%) as yellow needles.

R_f 0.73 (pet. ether 40-60/EtOAc, 3:2); M.p. 120-122 °C (water); IR (film, cm^{-1}) 3088, 2925, 1760, 1703, 1613 (w), 1594 (w), 1546; ^{1}H NMR (CDCl$_3$; 600 MHz) 3.12 (6H, s, N(CH$_3$)$_2$), 4.28 (2H, dt, J = 5.6, 1.4 Hz, allyl 1-H$_2$), 5.20 (1H, dd, J = 10.2, 1.2 Hz, allyl 3-H$_a$), 5.25 (1H, dd, J = 17.1, 1.2 Hz, allyl 3-H$_b$), 5.89 (1H, m, allyl 2-H), 7.57 (1H, app t, J = 7.8 Hz, 6-H), 7.63 (1H, dd, J = 7.2, 0.9 Hz, 7-H), 8.11 (1H, s, N=CH), 8.24 (1H, dd, J = 8.1, 0.9 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 40.1 (allyl 1-H$_2$). 42.7 (N(CH$_3$)$_2$), 117.7 (allyl C-3), 121.0 (C-7), 124.5 (N=CH), 124.7 (C), 129.1 (C-5), 131.9 (allyl C-2), 132.4 (C), 133.5 (C-6), 136.6 (C), 168.2 (C=O), 169.2 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 258.1242, C$_{14}$H$_{16}$N$_3$O$_2$ requires 258.1243.

4-((2,2-Dimethylhydrazono)methyl)-2-isopropylisoindoline-1,3-dione **191**
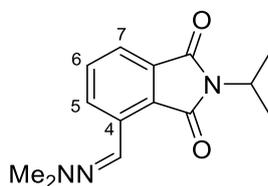
191

A solution of 4-((2,2-dimethylhydrazono)methyl)-2-ethylisoindoline-1,3-dione **31** (245 mg, 1.00 mmol) in allylamine (4 mL) was stirred for 3 h at RT and then added to aqueous ammonium chloride (10% w/v, 40 mL), causing the precipitation of fine yellow crystals. The solution was cooled on ice and the precipitate was collected by filtration, washed with cold water (2 x 50 mL), and dried to give **191** (122 mg, 47%) as a yellow solid.

R_f 0.47 (pet. ether 40-60/EtOAc, 4:1); M.p. 129-131 °C (water); IR (film, cm^{-1}) 2922, 1755, 1694, 1595, 1548; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.49 (6H, d, J = 6.9 Hz, CH(CH$_3$)$_2$), 3.12 (6H, s, N(CH$_3$)$_2$), 4.52 (1H, m, CH(CH$_3$)$_2$), 7.54 (1H, m, 6-H), 7.59 (1H, dd, J = 7.2, 1.1 Hz, 7-H), 8.15 (1H, s, N=CH), 8.22 (1H, dd, J = 7.8, 1.1 Hz, 5-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 26.1 (CH(CH$_3$)$_2$), 42.7 (N(CH$_3$)$_2$), 48.7 (CH(CH$_3$)$_2$), 120.6 (C-7), 124.7 (C), 124.7 (N=CH), 129.2 (C-5), 132.0 (C), 133.2 (C-6), 136.2 (C), 168.5 (C=O), 169.6 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 260.1401, C$_{14}$H$_{18}$N$_3$O$_2$ requires 260.1399.

Copper(II) phthalocyanine Cu•196
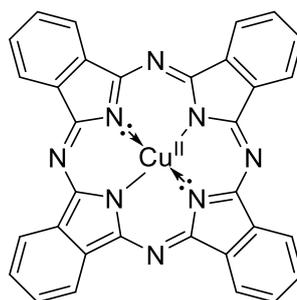
Cu•196

Phthalimide (100 mg, 680 µmol), anhydrous copper chloride (23 mg, 0.25 mmol), p-toluenesulfonic acid monohydrate (13 mg, 70 µmol) and hexadimethyldisilazane (560 µL, 438 mg, 270 mmol) were combined in a pressure tube under argon. The mixture was stirred at 100 °C for 1 h, cooled, and dimethylformamide (50 µL, 0.68 mmol) added. The tube was sealed and the mixture stirred at 150 °C for 18 h. A dark purple solid formed. The reaction was cooled to RT and the precipitate collected by filtration, washed with methanol (2 x 20 mL) and then dissolved in concentrated sulphuric acid (5 mL). This was added to water (100 mL) and the resulting blue precipitate was collected by filtration and washed with dilute sulphuric acid (20 mL), water (20 mL) and methanol (20 mL). The solid was further purified by extraction with methanol by Soxhlet's extractor to give Cu•196 as a blue solid (44 mg, 50%).

Q-band = 675 nm (DMF), Lit[158] 678 nm (DMF); IR (film, cm^{-1}) 3128, 3082, 3044, 1610, 1590; Not further characterised, see Section 3.2.4.

2-Benzylidene-1,1-dimethylhydrazine 198[222]
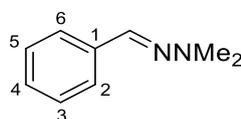
198

To a solution of benzaldehyde (102 µL, 1.00 mmol) in ethanol (10 mL), N,N-dimethylhydrazine (114 µL, 1.50 mmol) was added and the mixture stirred at 50 °C for 3 h. The solvent was removed under vacuum and the crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give 198 as a dark yellow oil (233 mg, 90%).

R_f 0.56 (pet. ether 40-60/EtOAc, 3:2); IR (film, cm^{-1}) 2878, 1600, 1570, 1485; ^{1}H NMR (CDCl$_3$; 600 MHz) 2.98 (6H, s, N(CH$_3$)$_2$), 7.23 – 7.29 (2H, m, N=CH & 4-H), 7.36 (2H, app t, J = 7.7 Hz, 3-H & 5-H), 7.61 (2H, dd, J = 8.4, 1.1 Hz, 2-H & 6-H); ^{13}C NMR (CDCl$_3$; 151 MHz) 43.2 (N(CH$_3$)$_2$), 126.5 (N=CH), 128.0 (C-4), 129.2 (C-3 & C-5), 133.3 (C-2 & C-6), 137.3 (C-1); m/z HRMS (ESI+) found [MH]$^+$ 149.1077, C$_9$H$_{13}$N$_2$ requires 149.1079.

Lithium hydroxypyruvate 209[168]

209
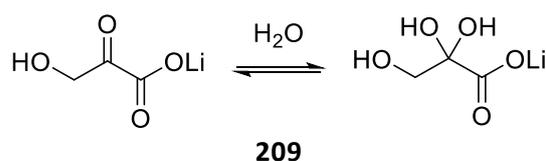
Aqueous lithium hydroxide (1M) was added to bromopyruvic acid (5.00 g, 30.0 mmol) in water (50 mL) until pH 9.5 was achieved. A colour change to pale yellow was observed. Glacial acetic acid was added dropwise until pH 5 was achieved, and the reaction mixture concentrated under vacuum to approximately 15 mL. The solution was left overnight at 4 °C and the resulting precipitate collected by suction filtration and washed with ethanol (25 mL). The crude product was resuspended in ethanol and stirred at 40 °C for 30 min then collected by suction filtration, washed with ethanol (25 mL) and dried to give **209** as fine colourless crystals (2.0 g, 63%).

M.p. decomposed at 115 °C (ethanol); IR (film, cm^{-1}) 3340, 1595, 1533, 1377; ^{1}H NMR (D$_2$O; 600 MHz) 3.60 (2H, s, CH$_2$ (ketone)), 4.61 (2H, s, CH$_2$ (hydrated ketone)); ^{13}C NMR (D$_2$O; 151 MHz) 65.8 (CH$_2$), 66.5 (CH$_2$), 94.7 (C(OH)H_2), 167.9 (C=O), 177.0 (C=O), 203.0 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 111.0268, C$_3$H$_3$O$_4$Li requires 111.0270.

(3S)-1,3-Dihydroxypentane-2-one (3S)-221
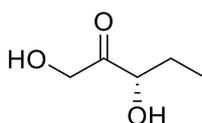
(3S)-**221**

The TK biotransformation procedure was performed on a 20 mL scale using wild-type TK cell-free lysate (2 mL), lithium hydroxypyruvate, **209** (0.110 g, 1.00 mmol, 50 mM), and propanal (58.1 mg, 1.00 mmol, 50 mM) for 48 hours. The crude reaction mixture was dry

loaded onto silica and purified by flash column chromatography (pet. ether 40-60/EtOAc, 7:3) to give (3S)-**221** as a colourless oil (37.1 mg, 31%).

R_f 0.20 (pet. ether 40-60/EtOAc, 1:1); IR (film, cm^{-1}) 3412, 2957, 2934, 2874, 1720; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.91 (3H, t, J = 7.2 Hz, CH$_3$), 1.52-1.61 (1H, m, CHHCH$_3$), 1.72-1.80 (1H, m, CHHCH$_3$), 3.60 (2H, br s, OH), 4.20 (1H, m, CHOH), 4.38 (1H, d, J = 19.7 Hz, CHHOH) 4.40 (1H, d, J = 19.7 Hz, CHHOH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 27.4 (CH$_2$CH$_3$), 65.8 (CH$_2$OH), 76.0 (CHOH), 213.0 (C=O); m/z HRMS (ESI+) found [MH]$^+$ 118.0629, C$_5$H$_{10}$O$_3$ requires 118.0630.

3-Hydroxyheptan-2-one **225**[223]
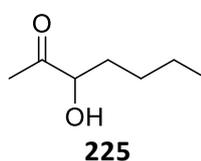
225

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-methyl-1,3-dithian-2-yl)pentan-1-ol **252** (331 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **225** as a pale yellow oil (145 mg, 74%).

R_f 0.22 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3420 (br, OH), 2928, 1709 (C=O), 1457; ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 0.92 (3H, t, J = 7.2 Hz, CH$_2$CH$_3$), 1.28-1.43 (4H, m, (CH$_2$)$_2$CH$_3$), 1.54 (1H, m, CHOHCHH), 1.76 (1H, m, CHOHCHH), 2.18 (3H, s, COCH$_3$), 4.06-4.18 (2H, m, OH & CHOH); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 14.3 (CH$_3$), 23.2 (CH$_2$), 25.4 (CH$_3$), 28.0 (CH$_2$), 34.1 (CH$_2$), 77.7 (CHOH), 211.5 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 148.1332, C$_7$H$_{18}$NO$_2$ requires 148.1332.

3-Hydroxyoctan-2-one **226**[224]
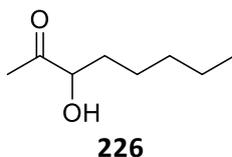
226

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-methyl-1,3-dithian-2-yl)hexan-1-ol **253** (352 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **226** as a pale yellow oil (154 mg, 71%).

R_f 0.22 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3450 (br, OH), 2925, 1710 (C=O), 1457; ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 0.91 (3H, t, J = 6.6 Hz, CH$_2$CH_3), 1.25-1.47 (6H, m, (CH_2)$_3$CH$_3$), 1.53 (1H, m, CHOHCHH), 1.75 (1H, m, CHOHCHH), 2.18 (3H, s, COCH_3), 4.04-4.14 (2H, m, OH & CHOH); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 14.4 (CH$_3$), 23.3 (CH$_2$), 25.4 (CH$_3$), 25.5 (CH$_2$), 32.5 (CH$_2$), 34.3 (CH$_2$), 77.7 (CHOH), 211.4 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 162.14935, C$_8$H$_{20}$NO$_2$ requires 162.14940.

4-Hydroxyoctan-3-one **227**[225]
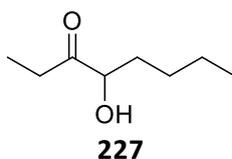
227

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)pentan-1-ol **257** (352 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **227** as a pale yellow oil (149 mg, 69%).

R_f 0.24 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3470 (br, OH), 2930, 1709 (C=O), 1459; ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 0.89 (3H, t, J = 7.1 Hz, CH$_2$CH_3), 1.00 (3H, t, J = 7.3 Hz, C(O)CH$_2$CH_3), 1.25-1.46 (4H, m, (CH_2)$_2$CH$_3$), 1.52 (1H, m, CHOHCHH), 1.74 (1H, m, CHOHCHH), 2.57 (2H, m, COCH$_2$), 4.04 (1H, d, J = 5.2 Hz, OH), 4.08 (1H, m, CHOH); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 7.2 (CH$_3$) 13.7 (CH$_3$), 22.7 (CH$_2$), 27.5 (CH$_3$), 30.7 (CH$_2$), 33.8 (CH$_2$), 76.7 (CHOH), 213.4 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 162.14932, C$_8$H$_{20}$NO$_2$ requires 162.14940.

4-Hydroxynonan-3-one **228**[226]
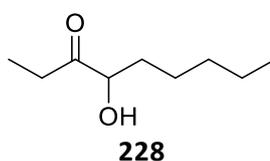
228

Synthesis adapted from literature procedure. [206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)hexan-1-ol **258** (373 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **228** as a pale yellow oil (190 mg, 80%).

R_f 0.25 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3447 (br, OH), 2933, 1709 (C=O), 1459; ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 0.88 (3H, t, J = 7.1 Hz, CH$_2$CH_3), 1.00 (3H, t, J = 7.3 Hz, C(O)CH$_2$CH_3), 1.22-1.47 (6H, m, (CH_2)$_3$CH$_3$), 1.51 (1H, m, CHOHCHH), 1.73 (1H, m, CHOHCHH), 2.58 (2H, m, COCH$_2$), 4.03 (1H, d, J = 5.2 Hz, OH), 4.08 (1H, m, CHOH); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 7.2 (CH$_3$) 13.8 (CH$_3$), 22.7 (CH$_2$), 25.0 (CH$_2$), 30.8 (CH$_2$), 31.9 (CH$_2$), 34.1 (CH$_2$), 76.7 (CHOH), 213.4 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 176.16450, C$_9$H$_{22}$NO$_2$ requires 176.16451.

(3S)-4-Hydroxynonan-3-one (3S)-**228**
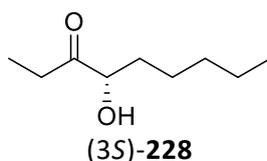
(3S)-**228**

The TK biotransformation procedure was performed on a 20 mL scale using D469Y TK cell-free lysate (2 mL), ketobutyric acid, **209** (102 mg, 1.00 mmol, 50 mM), and hexanal (123 µL, 1.00 mmol, 50 mM) for 24 hours. The crude reaction mixture was dry loaded onto silica and purified by flash column chromatography (pentane/diethyl ether, 9:1) to give (3S)-**228** as a pale green oil (13 mg, 8%).

$[\alpha]_D$ = +79.3 (c 0.32, CDCl$_3$); remaining characterisation data was as found for the racemic product, **228**.

4-Hydroxytridecan-3-one **235**[196]
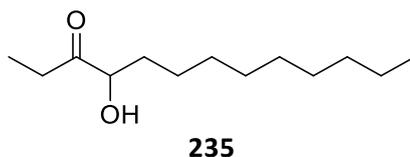
235

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)decan-1-ol **261** (457 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **235** as a pale yellow oil (241 mg, 75%).

R$_f$ 0.30 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3456 (br, OH), 2949, 2930, 2856, 1708 (C=O); ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 0.88 (3H, t, J = 7.2 Hz, (CH$_2$)$_8$CH$_3$), 1.00 (3H, t, J = 7.3 Hz, COCH$_2$CH$_3$), 1.22-1.44 (14H, m, (CH$_2$)$_7$CH$_3$), 1.51 (1H, m, CHOHCHH), 1.73 (1H, m, CHOHCHH), 2.58 (2H, m, COCH$_2$), 4.06-4.10 (2H, m, OH & CHOH); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 7.5 (CH$_3$), 14.4 (CH$_3$), 21.7 (CH$_2$), 22.9 (CH$_2$), 23.6 (CH$_2$), 24.7 (CH$_2$), 25.6 (CH$_2$), 29.1 (CH$_2$), 31.3 (CH$_2$), 32.7 (CH$_2$), 34.4 (CH$_2$), 77.1 (CHOH), 213.8 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 232.22771, C$_{13}$H$_{30}$NO$_2$ requires 232.22765.

4-Hydroxydecan-3-one **241**[227]
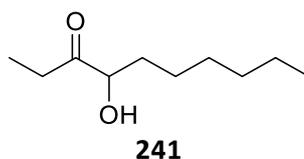
241

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)heptan-1-ol **259** (394 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried ($MgSO_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **241** as a pale yellow oil (183 mg, 71%).

R_f 0.25 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3473 (br, OH), 2925, 1709 (C=O), 1458; ^{1}H NMR ((CD_3)$_2$CO; 600 MHz) 0.88 (3H, t, J = 7.0 Hz, CH$_2$CH_3), 1.00 (3H, t, J = 7.3 Hz, C(O)CH$_2$CH_3), 1.21-1.44 (8H, m, (CH_2)$_4$CH$_3$), 1.52 (1H, m, CHOHCHH), 1.73 (1H, m, CHOHCHH), 2.58 (2H, m, COCH_2), 4.06 – 4.10 (2H, m, OH & CHOH); ^{13}C NMR ((CD_3)$_2$CO; 151 MHz) 7.80 (CH$_3$), 14.4 (CH$_3$), 23.3 (CH$_2$), 25.8 (CH$_2$), 28.9 (CH$_2$), 31.3 (CH$_2$), 32.6 (CH$_2$), 34.7 (CH$_2$), 77.3 (CHOH), 214.0 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 190.1802, C$_{10}$H$_{24}$NO$_2$ requires 190.1802.

(3*S*)-4-Hydroxydecan-3-one (3*S*)-**241**
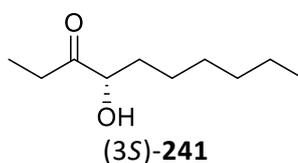
(3*S*)-**241**

The TK biotransformation procedure was performed on a 20 mL scale using D469Y TK cell-free lysate (2 mL), ketobutyric acid, **209** (102 mg, 1.00 mmol, 50 mM), and heptanal (141 µL, 1.00 mmol, 50 mM) for 24 hours. The crude reaction mixture was dry loaded onto silica and purified by flash column chromatography (pentane/diethyl ether, 9:1) to give (3*S*)-**241** as a pale green oil (22 mg, 13%).

[α]$_D$ = +80.6 (*c* 0.27, CDCl$_3$); remaining characterisation data was as found for the racemic product **241**.

4-Hydroxydecan-3-one **241** & 3-hydroxydecan-4-one **248**
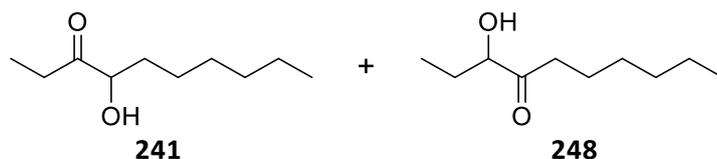
Synthesis adapted from literature procedure.[202] To a solution of propanal (1.08 mL, 15.0 mmol) and heptanal (706 µL, 5.00 mmol) in ethanol (20 mL) under argon, 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (135 mg, 500 µmol) and triethylamine (420 µL, 3.00 mmol) were added. The reaction was heated at reflux for 16 h, and then water (40 mL) added to quench the reaction. The organic component was extracted with diethyl ether (3 × 60 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give a 1:1 mixture of **241** and **248** as a pale yellow oil (379 mg, 44%).

R_f 0.25, 0.26 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3478 (br, OH), 2926, 1711 (C=O), 1461; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.86 (6H, t, J = 6.9 Hz, 2 x (CH$_2$)$_5$CH_3), 0.93 (3H, t, J = 7.4 Hz, CHOHCH$_2$CH_3), 1.08 (3H, t, J = 7.3 Hz, C(O)CH$_2$CH_3), 1.20 – 1.46 (16H, m, 8 x CH$_2$), 1.51 (1H, m, CHOHCHH(CH$_2$)$_4$CH$_3$), 1.59 (1H, m, CHOHCHHCH$_3$), 1.79 (1H, m, CHOHCHH(CH$_2$)$_4$CH$_3$), 1.87 (1H, m, CHOHCHHCH$_3$), 2.40-2.55 (4H, m, 2 x C(O)CH$_2$), 3.08 (1H, s br, OH), 3.35 (1H, s br, OH), 4.12 – 4.17 (2H, m, 2 x CHOH); ^{13}C NMR (CDCl$_3$; 151 MHz) 7.8 (CH$_3$), 8.6 (CH$_3$), 14.2 (CH$_3$), 22.5 (CH$_3$), 24.7 (CH$_2$), 26.7 (CH$_2$), 29.2 (CH$_2$), 29.30 (CH$_2$), 29.36 (CH$_2$), 29.43 (CH$_2$), 29.48 (CH$_2$), 30.6 (CH$_2$), 31.83 (CH$_2$), 31.86 (CH$_2$), 33.9 (CH$_2$), 37.9 (CH$_2$), 76.3 (CHOH), 77.7 (CHOH), 212.5 (CO), 213.0 (CO); m/z HRMS (ESI+) found [M]$^+$ 173.1548, C$_{10}$H$_{21}$O$_2$ requires 173.1542.

4-Hydroxyundecan-3-one **242**[227]
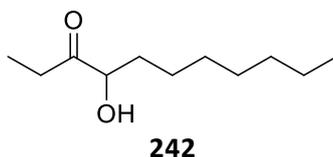
Synthesis adapted from literature procedure.[206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)octan-1-ol **260** (415 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$),

filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **242** as a pale yellow oil (201 mg, 72%).

R_f 0.27 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3468 (br, OH), 2921, 1709 (C=O), 1457; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.84 (3H, t, J = 6.9 Hz, CH$_2$C*H*$_3$), 1.08 (3H, t, J = 7.3 Hz, C(O)CH$_2$C*H*$_3$), 1.17-1.45 (10H, m, (C*H*$_2$)$_5$CH$_3$), 1.50 (1H, m, CHOHC*HH*), 1.77 (1H, m, CHOHCH*H*), 2.45 (2H, m, COC*H*$_2$), 3.05 (1H, s br, OH), 4.14 (1H, dd, J = 7.4, 3.8 Hz, C*H*OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 7.7 (CH$_3$), 14.1 (CH$_3$), 22.7 (CH$_2$), 24.9 (CH$_2$), 29.2 (CH$_2$), 29.5 (CH$_2$), 31.1 (CH$_2$), 31.8 (CH$_2$), 34.0 (CH$_2$), 76.3 (CHOH), 213.0 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 204.19575, C$_{11}$H$_{26}$NO$_2$ requires 204.19580.

(3*S*)-4-Hydroxyundecan-3-one (3*S*)-**242**

(3*S*)-**242**
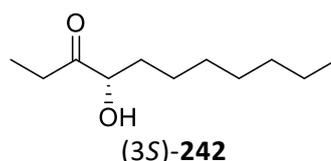
The TK biotransformation procedure was performed on a 20 mL scale using D469T TK cell-free lysate (2 mL), ketobutyric acid, **209** (102 mg, 1.00 mmol, 50 mM), and octanal (156 µL, 1.00 mmol, 50 mM) for 24 hours. The crude reaction mixture was dry loaded onto silica and purified by flash column chromatography (pentane/diethyl ether, 9:1) to give (3*S*)-**242** as a pale green oil (26 mg, 14%).

[α]$_D$ = +82.0 (*c* 0.18, CDCl$_3$); remaining characterisation data was as found for the racemic product **242**.

4-Hydroxyundecan-3-one **242** & 3-hydroxyundecan-4-one **249**
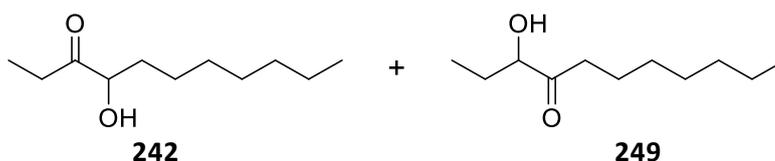
242   +   **249**

Synthesis adapted from literature procedure.[202] To a solution of propanal (1.08 mL, 15.0 mmol) and octanal (738 µL, 5.00 mmol) in ethanol (20 mL) under argon, 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (135 mg, 500 µmol) and triethylamine (420 µL,

3.00 mmol) were added. The reaction was heated at reflux for 16 h, and then water (40 mL) added to quench the reaction. The organic component was extracted with diethyl ether (3 × 60 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give a 1:1 mixture of **242** and **249** as a pale yellow oil (379 mg, 44%).

R$_f$ 0.27, 0.28 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3457 (br, OH), 2923, 1710 (C=O), 1460; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.86 (6H, t, J = 6.9 Hz, 2 x (CH$_2$)$_5$CH_3), 0.92 (3H, t, J = 7.4 Hz, CHOHCH$_2$CH_3), 1.08 (3H, t, J = 7.3 Hz, C(O)CH$_2$CH_3), 1.17 − 1.47 (20H, m, 10 x CH$_2$), 1.50 (1H, m, CHOHCHH(CH$_2$)$_4$CH$_3$), 1.59 (1H, m, CHOHCHHCH$_3$), 1.78 (1H, m, CHOHCHH(CH$_2$)$_4$CH$_3$), 1.86 (1H, m, CHOHCHHCH$_3$), 2.41-2.57 (4H, m, 2 x C(O)CH$_2$), 3.05 (1H, s br, OH), 3.36 (1H, s br, OH), 4.12 − 4.16 (2H, m, 2 x CHOH); ^{13}C NMR (CDCl$_3$; 151 MHz) 7.7 (CH$_3$), 8.6 (CH$_3$), 14.1 (CH$_3$), 22.5 (CH$_3$), 22.7 (CH$_3$), 24.8 (CH$_2$), 26.7 (CH$_2$), 29.2 (CH$_2$), 29.32 (CH$_2$), 29.35 (CH$_2$), 29.44 (CH$_2$), 29.48 (CH$_2$), 30.6 (CH$_2$), 31.0 (CH$_2$), 31.2 (CH$_2$), 31.86 (CH$_2$), 33.9 (CH$_2$), 37.8 (CH$_2$), 76.3 (CHOH), 77.7 (CHOH), 212.4 (CO), 213.0 (CO); *m/z* HRMS (ESI+) found [M]$^+$ 187.1698, C$_{11}$H$_{23}$O$_2$ requires 187.1698.

2-Methyl-1,3-dithiane **250**[204]

250
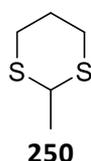
Synthesis adapted from literature procedure.[204] Under anhydrous conditions, acetaldehyde (2.24 mL, 40 mmol) and propane-1,3-dithiol (4.41 mL, 44 mmol) were added to anhydrous dichloromethane (250 mL) and the mixture cooled to 0 °C and stirred. Boron trifluoride diethyl etherate (2.46 mL, 20 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for a further hour, then allowed to reach RT. The reaction was quenched with saturated aqueous sodium carbonate solution (100 mL) and the organic phase was extracted with dichloromethane (3 x 500 mL), washed with aqueous sodium hydroxidesolution (0.1 M, 200 mL), then brine (200 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **250** as a pale yellow oil (5.21 g, 97%).

R$_f$ 0.30 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 2965, 1454; ^{1}H NMR (CDCl$_3$; 500 MHz) 1.40 (3H, d, J = 6.9 Hz, CH$_3$), 1.73 (1H, m, 5-HH), 2.04 (1H, m, 5-HH), 2.74 (2H, m, 2 × SCHH),

2.83 (2H, m, 2 × SCH*H*), 4.06 (1H, q, *J* = 6.9 Hz, CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 21.2 (CH$_3$), 25.2 (C-5), 30.6 (2 × SCH$_2$), 42.0 (CH); *m/z* HRMS (ESI+) found [MH]$^+$ 135.0310, C$_5$H$_{11}$S$_2$ requires 135.0302.

2-Ethyl-1,3-dithiane **251**[204]
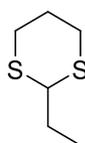
251

Synthesis adapted from literature procedure.[204] Under anhydrous conditions, propionaldehyde (2.86 mL, 40 mmol) and propane-1,3-dithiol (4.41 mL, 44 mmol) were added to anhydrous dichloromethane (250 mL) and the mixture cooled to 0 °C and stirred. Boron trifluoride diethyl etherate (2.46 mL, 20 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for a further hour, then allowed to reach RT. The reaction was quenched with saturated aqueous sodium carbonate solution (100 mL) and the organic phase was extracted with dichloromethane (3 x 500 mL), washed with aqueous sodium hydroxide solution (0.1 M, 200 mL), then brine (200 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum to give **251** as a pale yellow oil (5.58 g, 94%).

R$_f$ 0.37 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 2962, 1444; ^{1}H NMR (CDCl$_3$; 500 MHz) 1.02 (3H, t, *J* = 7.4 Hz, CH$_3$), 1.74 (2H, m, C*H*$_2$CH$_3$), 1.80 (1H, m, 5-*H*H), 2.06 (1H, m, 5-H*H*), 2.80 (4H, m, 2 × SCH$_2$), 3.93 (1H, t, *J* = 6.8 Hz, CH); ^{13}C NMR (CDCl$_3$; 151 MHz) 11.5 (CH$_3$), 20.0 (*C*H$_2$CH$_3$), 28.8 (C-5), 30.4 (2 × SCH$_2$), 49.3 (CH); *m/z* HRMS (ESI+) found [MH]$^+$ 149.0431, C$_6$H$_{13}$S$_2$ requires 149.0459.

1-(2-Methyl-1,3-dithian-2-yl)pentan-1-ol **252**

252

Synthesis adapted from literature procedure.[205] A solution of 2-methyl-1,3-dithiane **250** (479 µL, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed

to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, valeraldehyde (638 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **252** as a pale yellow oil (379 mg, 43%).

R$_f$ 0.30 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3480 (br, OH), 2952; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.91 (3H, t, J = 7.1 Hz, CH$_2$C*H*$_3$), 1.18 – 1.49 (7H, m, CH$_3$ & (CH$_2$)$_2$), 1.59 (1H, m, CHOHC*H*H), 1.83 (1H, m, SCH$_2$C*H*H), 1.93 (1H, m, CHOHCH*H*), 2.06 (1H, m, SCH$_2$CH*H*), 2.59 (2H, m, SC*H*H x 2), 2.97 (2H, m, SCH*H* x 2), 3.55 (1H, m, C*H*OH), 3.91 (1H, d, J = 9.1 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.2 (CH$_3$), 21.9 (CH$_3$), 22.7 (CH$_2$), 24.4 (CH$_2$), 27.8 (CH$_2$), 29.5 (CH$_2$), 30.0 (CH$_2$), 32.2 (CH$_2$), 54.1 (C), 71.3 (CHOH); *m/z* HRMS (ESI+) found [MH]$^+$ 221.1032, C$_{10}$H$_{21}$OS$_2$ requires 221.1034.

1-(2-Methyl-1,3-dithian-2-yl)hexan-1-ol **253**

253

Synthesis adapted from literature procedure.[205] A solution of 2-methyl-1,3-dithiane **250** (479 µL, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, hexanal (737 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide

solution (7%, 20 mL) and brine (20 mL), dried (MgSO₄), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **253** as a pale yellow oil (365 mg, 39%).

R_f 0.31 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3463 (br, OH), 2958; ^{1}H NMR (CDCl₃; 600 MHz) 0.89 (3H, t, J = 7.1 Hz, CH₂CH₃), 1.18 – 1.48 (9H, m, CH₃ & (CH₂)₃), 1.61 (1H, m, CHOHCHH), 1.83 (1H, m, SCH₂CHH), 1.92 (1H, m, CHOHCHH), 2.06 (1H, m, SCH₂CHH), 2.59 (2H, m, SCHH x 2), 2.97 (2H, m, SCHH x 2), 3.56 (1H, m, CHOH), 3.91 (1H, d, J = 9.6 Hz, OH); ^{13}C NMR (CDCl₃; 151 MHz) 14.1 (CH₃), 21.9 (CH₃), 22.8 (CH₂), 24.4 (CH₂), 26.3 (CH₂), 27.8 (CH₂), 29.5 (CH₂), 30.1 (CH₂), 32.0 (CH₂), 54.0 (C), 71.3 (CHOH); m/z HRMS (ESI+) found [MH]$^+$ 235.1190, C₁₁H₂₃OS₂ requires 235.1190.

1-(2-Methyl-1,3-dithian-2-yl)heptan-1-ol **254**

254

Synthesis adapted from literature procedure.[205] A solution of 2-methyl-1,3-dithiane **250** (479 µL, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, heptanal (846 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO₄), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **254** as a pale yellow oil (447 mg, 45%).

R_f 0.31 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3455 (br, OH), 2949; ^{1}H NMR (CDCl₃; 600 MHz) 0.83 (3H, t, J = 7.1 Hz, CH₂CH₃), 1.13 – 1.39 (11H, m, CH₃ & (CH₂)₄), 1.57 (1H, m, CHOHCHH), 1.79 (1H, m, SCH₂CHH), 1.89 (1H, m, CHOHCHH), 2.02 (1H, m, SCH₂CHH), 2.55 (2H, m, SCHH x 2), 2.94 (2H, m, SCHH x 2), 3.52 (1H, m, CHOH), 3.87 (1H, d, J = 9.3 Hz, OH);

^{13}C NMR (CDCl$_3$; 151 MHz) 14.2 (CH$_3$) 21.8 (CH$_3$), 22.8 (CH$_2$), 24.5 (CH$_2$), 25.7 (CH$_2$), 26.3 (CH$_2$), 27.8 (CH$_2$), 29.5 (CH$_2$), 30.1 (CH$_2$), 32.0 (CH$_2$), 54.1 (C), 71.3 (CHOH); *m/z* HRMS (ESI+) found [MH]$^+$ 249.1344, C$_{12}$H$_{25}$OS$_2$ requires 249.1347.

1-(2-Methyl-1,3-dithian-2-yl)octan-1-ol 255

255

Synthesis adapted from literature procedure.[205] A solution of 2-methyl-1,3-dithiane **250** (479 µL, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, octanal (937 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **255** as a pale yellow oil (545 mg, 52%).

R$_f$ 0.34 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3472 (br, OH), 2960; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.86 (3H, t, *J* = 7.1 Hz, CH$_2$C*H*$_3$), 1.16 – 1.42 (13H, m, CH$_3$ & (CH$_2$)$_5$), 1.60 (1H, m, CHOHC*H*H), 1.83 (1H, m, SCH$_2$C*H*H), 1.92 (1H, m, CHOHCH*H*), 2.07 (1H, m, SCH$_2$CH*H*), 2.59 (2H, m, SC*H*H x 2), 2.97 (2H, m, SCH*H* x 2), 3.55 (1H, m, C*H*OH), 3.91 (1H, d, *J* = 9.5 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.3 (CH$_3$), 21.8 (CH$_3$), 22.8 (CH$_2$), 24.5 (CH$_2$), 25.7 (CH$_2$), 26.3 (CH$_2$), 27.9 (CH$_2$), 29.4 (CH$_2$), 29.8 (CH$_2$), 30.1 (CH$_2$), 32.0 (CH$_2$), 54.1 (C), 71.3 (CHOH); *m/z* HRMS (ESI+) found [MH]$^+$ 263.1502, C$_{13}$H$_{27}$OS$_2$ requires 263.1503.

1-(2-Methyl-1,3-dithian-2-yl)pent-4-en-1-ol 256

256

Synthesis adapted from literature procedure.[205] A solution of 2-methyl-1,3-dithiane **250** (479 μL, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, 4-pentenal (592 μL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **256** as a pale yellow oil (427 mg, 49%).

R_f 0.31 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3483 (br, OH), 3078, 2950; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.47 (3H, s, CH$_3$), 1.66 (1H, m, CHOHCHH), 1.73 – 2.05 (5H, m, CH_2CH=CH$_2$ & SCH$_2$CH_2 & CHOHCHH), 2.58 (2H, m, SCHH x 2), 2.97 (2H, m, SCHH x 2), 3.59 (1H, m, CHOH), 3.94 (1H, d, J = 9.9 Hz, OH), 4.94 (1H, dd, J = 10.3, 1.2 Hz, CH=CHH), 5.02 (1H, dd, J = 17.0, 1.2 Hz, CH=CHH), 5.82 (1H, m, CH=CH$_2$); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.2 (CH$_3$), 22.8 (CH$_2$), 27.9 (CH$_2$), 30.1 (CH$_2$), 36.5 (CH$_2$), 37.3 (CH$_2$), 53.9 (C), 71.5 (CHOH), 114.8 (CH=CH$_2$), 138.8 (CH=CH$_2$); m/z HRMS (ESI+) found [MH]$^+$ 219.0875, C$_{10}$H$_{19}$OS$_2$ requires 219.0877.

1-(2-Ethyl-1,3-dithian-2-yl)pentan-1-ol **257**

257

Synthesis adapted from literature procedure.[205]   A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, valeraldehyde (638 μL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **257** as a pale yellow oil (356 mg, 38%).

R$_f$ 0.33 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3477 (br, OH), 2961; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.92 (3H, t, J = 7.1 Hz, (CH$_2$)$_5$CH_3), 1.07 (3H, t, J = 7.5 Hz, CH$_3$) 1.19 − 1.49 (10H, m, CH$_2$ & (CH$_2$)$_4$), 1.68 (1H, m, CHOHCHH), 1.83 (1H, m, SCH$_2$CHH), 1.91 (1H, m, CHOHCHH), 2.05 (1H, m, SCH$_2$CHH), 2.63 (2H, m, SCHH x 2), 2.97 (2H, m, SCHH x 2), 3.71 (1H, m, CHOH), 3.95 (1H, d, J = 9.3 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 24.0 (CH$_3$), 24.6 (CH$_2$), 25.1 (CH$_2$), 25.9 (CH$_2$), 27.8 (CH$_2$), 29.2 (CH$_2$), 29.8 (CH$_2$), 31.1 (CH$_2$), 59.7 (C), 71.0 (CHOH); m/z HRMS (ESI+) found [MH]$^+$ 235.1188, C$_{11}$H$_{23}$OS$_2$ requires 235.1190.

1-(2-Ethyl-1,3-dithian-2-yl)hexan-1-ol **258**

258

Synthesis adapted from literature procedure.[205] A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, hexanal (737 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **258** as a pale yellow oil (397 mg, 40%).

R$_f$ 0.34 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3478 (br, OH), 2953; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.92 (3H, t, J = 7.1 Hz, (CH$_2$)$_4$CH_3), 1.07 (3H, t, J = 7.5 Hz, CH$_3$) 1.21 − 1.48 (8H, m, CH$_2$ & (CH$_2$)$_3$), 1.67 (1H, m, CHOHCHH), 1.83 (1H, m, SCH$_2$CHH), 1.90 (1H, m, CHOHCHH), 2.04 (1H, m, SCH$_2$CHH), 2.63 (2H, m, SCHH x 2), 2.97 (2H, m, SCHH x 2), 3.73 (1H, m, CHOH), 3.97 (1H, d, J = 9.6 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 23.9 (CH$_3$), 24.5 (CH$_2$), 25.1 (CH$_2$), 26.0 (CH$_2$), 26.7 (CH$_2$), 27.8 (CH$_2$), 29.2 (CH$_2$), 29.8 (CH$_2$), 31.5 (CH$_2$), 59.7 (C), 71.1 (CHOH); m/z HRMS (ESI+) found [MH]$^+$ 249.1344, C$_{12}$H$_{25}$OS$_2$ requires 249.1347.

1-(2-Ethyl-1,3-dithian-2-yl)heptan-1-ol **259**

259

Synthesis adapted from literature procedure.[205] A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed

to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, heptanal (846 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **259** as a pale yellow oil (472 mg, 45%).

R$_f$ 0.34 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3449 (br, OH), 2961; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.88 (3H, t, J = 7.1 Hz, (CH$_2$)$_4$CH_3), 1.07 (3H, t, J = 7.5 Hz, CH$_3$) 1.18 – 1.45 (10H, m, CH$_2$ & (CH$_2$)$_4$), 1.67 (1H, m, CHOHCHH), 1.83 (1H, m, SCH$_2$CHH), 1.90 (1H, m, CHOHCHH), 2.03 (1H, m, SCH$_2$CHH), 2.62 (2H, m, SCHH x 2), 2.96 (2H, m, SCHH x 2), 3.73 (1H, m, CHOH), 3.96 (1H, d, J = 9.6 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 23.6 (CH$_3$), 24.2 (CH$_2$), 24.5 (CH$_2$), 25.3 (CH$_2$), 25.9 (CH$_2$), 26.6 (CH$_2$), 27.8 (CH$_2$), 29.1 (CH$_2$), 29.8 (CH$_2$), 31.7 (CH$_2$), 59.7 (C), 71.1 (CHOH); m/z HRMS (ESI+) found [MH]$^+$ 263.1501, C$_{13}$H$_{27}$OS$_2$ requires 263.1503.

1-(2-Ethyl-1,3-dithian-2-yl)octan-1-ol **260**

260

Synthesis adapted from literature procedure.[205] A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, octanal (937 µL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide

solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **260** as a pale yellow oil (607 mg, 55%).

R$_f$ 0.36 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3460 (br, OH), 2963; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.86 (3H, t, J = 7.1 Hz, (CH$_2$)$_4$CH_3), 1.08 (3H, t, J = 7.5 Hz, CH$_3$) 1.17 − 1.46 (12H, m, CH$_2$ & (CH$_2$)$_5$), 1.65 (1H, m, CHOHCHH), 1.82 (1H, m, SCH$_2$CHH), 1.90 (1H, m, CHOHCHH), 2.04 (1H, m, SCH$_2$CHH), 2.62 (2H, m, SCHH x 2), 2.95 (2H, m, SCHH x 2), 3.73 (1H, m, CHOH), 3.97 (1H, d, J = 9.6 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 23.6 (CH$_3$), 24.0 (CH$_2$), 24.5 (CH$_2$), 25.7 (CH$_2$), 26.0 (CH$_2$), 26.6 (CH$_2$), 27.3 (CH$_2$), 27.8 (CH$_2$), 29.4 (CH$_2$), 29.8 (CH$_2$), 31.8 (CH$_2$), 59.7 (C), 71.1 (CHOH); m/z HRMS (ESI+) found [MH]$^+$ 277.1659, C$_{14}$H$_{29}$OS$_2$ requires 277.1660.

1-(2-Ethyl-1,3-dithian-2-yl)decan-1-ol **261**
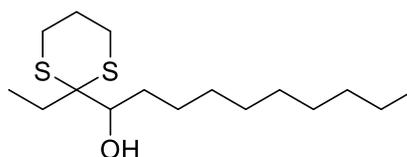
261

Synthesis adapted from literature procedure.[205] A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, decanal (1.13 mL, 6 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **261** as a pale yellow oil (644 mg, 53%).

R$_f$ 0.37 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3475 (br, OH), 2951; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.88 (3H, t, J = 7.1 Hz, (CH$_2$)$_4$CH_3), 1.08 (3H, t, J = 7.5 Hz, CH$_3$) 1.19 − 1.44 (16H, m, CH$_2$ & (CH$_2$)$_7$), 1.68 (1H, m, CHOHCHH), 1.83 (1H, m, SCH$_2$CHH), 1.91 (1H, m, CHOHCHH),

2.05 (1H, m, SCH$_2$CH*H*), 2.63 (2H, m, SC*H*H x 2), 2.97 (2H, m, SCH*H* x 2), 3.73 (1H, m, C*H*OH), 3.97 (1H, d, *J* = 9.6 Hz, OH); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 22.9 (CH$_3$), 23.5 (CH$_2$), 23.8 (CH$_2$), 24.1 (CH$_2$), 24.5 (CH$_2$), 25.3 (CH$_2$), 25.8 (CH$_2$), 26.6 (CH$_2$), 27.3 (CH$_2$), 27.7 (CH$_2$), 29.6 (CH$_2$), 30.1 (CH$_2$), 31.8 (CH$_2$), 59.7 (C), 71.1 (CHOH); *m/z* HRMS (ESI+) found [MH]$^+$ 305.1974, C$_{16}$H$_{33}$OS$_2$ requires 305.1973.

1-(2-Ethyl-1,3-dithian-2-yl)pent-4-en-1-ol 262
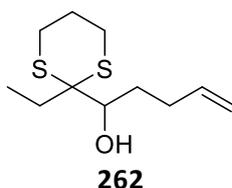
262

Synthesis adapted from literature procedure.[205] A solution of 2-ethyl-1,3-dithiane **251** (593 mg, 4.00 mmol) in dry THF (16 mL) in a dried RBF was cooled to -78 °C and n-butyllithium (2 mL, 2.5 M in hexane, 5 mmol) added dropwise, then stirred at -78 °C for 30 min, warmed to 0 °C and stirred for a further 30 min, and then cooled back down to -78 °C. To this solution, 4-pentenal (592 µL, 6.00 mmol) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 18 h. To quench the reaction, water (16 mL) was carefully added, followed by dichloromethane (16 mL), and the organic phase extracted with dichloromethane (3 × 50 mL). The volume of the combined organic fraction was reduced under vacuum to approximately 50 mL, then washed with aqueous potassium hydroxide solution (7%, 20 mL) and brine (20 mL), dried (MgSO$_4$), filtered, and the solvent fully removed under vacuum. The crude product was purified by flash column chromatography (pet. ether 40-60/EtOAc, 9:1) to give **262** as a pale yellow oil (362 mg, 39%).

R$_f$ 0.33 (pet. ether 40-60/EtOAc, 4:1); IR (film, cm^{-1}) 3456 (br, OH), 3074, 2960; ^{1}H NMR (CDCl$_3$; 600 MHz) 1.07 (3H, t, *J* = 7.5 Hz, CH$_3$), 1.52 (2H, q, *J* = 7.5 Hz, C*H*$_2$CH$_3$), 1.66 (1H, m, CHOHC*H*H), 1.75 − 2.08 (5H, m, C*H*$_2$CH=CH$_2$ & SCH$_2$C*H*$_2$ & CHOHCH*H*), 2.61 (2H, m, SC*H*H x 2), 2.95 (2H, m, SCH*H* x 2), 3.73 (1H, m, C*H*OH), 3.99 (1H, d, *J* = 10.0 Hz, OH), 4.99 (1H, dd, *J* = 10.3, 1.3 Hz, CH=C*H*H), 5.08 (1H, dd, *J* = 17.1, 1.3 Hz, CH=CH*H*), 5.85 (1H, m, C*H*=CH$_2$); ^{13}C NMR (CDCl$_3$; 151 MHz) 9.4 (CH$_3$), 24.5 (CH$_2$), 25.0 (CH$_2$), 26.0 (CH$_2$), 27.3 (CH$_2$), 29.4 (CH$_2$), 31.8 (CH$_2$), 59.8 (C), 70.9 (CHOH), 115.1 (CH=*C*H$_2$), 138.6 (*C*H=CH$_2$); *m/z* HRMS (ESI+) found [MH]$^+$ 233.1032, C$_{11}$H$_{21}$OS$_2$ requires 233.1034.

3-Hydroxynonan-2-one 263[228]
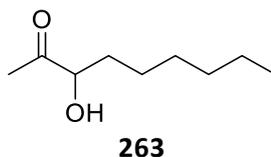
263

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-methyl-1,3-dithian-2-yl)heptan-1-ol **254** (373 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO₄), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **263** as a pale yellow oil (158 mg, 73%).

R_f 0.24 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3425 (br, OH), 2923, 1711 (C=O); ^{1}H NMR (CDCl₃; 600 MHz) 0.85 (3H, t, J = 6.5 Hz, CH₂C*H₃*), 1.19-1.47 (8H, m, (C*H₂*)₄CH₃), 1.52 (1H, m, CHOHC*H*H), 1.80 (1H, m, CHOHCH*H*), 2.17 (3H, s, COC*H₃*), 3.12 (1H, s br, OH), 4.15 (1H, dd, J = 7.5, 3.7 Hz, C*H*OH); ^{13}C NMR (CDCl₃; 151 MHz) 14.1 (CH₃), 22.7 (CH₂), 24.8 (CH₂), 25.3 (CH₃), 29.2 (CH₂), 31.7 (CH₂), 33.6 (CH₂), 77.0 (CHOH), 210.2 (CO); m/z HRMS (ESI+) found [M+NH₄]⁺ 176.1650, C₉H₂₂NO₂ requires 176.1651.

3-Hydroxydecan-2-one 264[229]
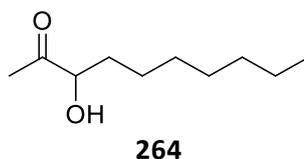
264

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-methyl-1,3-dithian-2-yl)octan-1-ol **255** (394 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO₄), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **264** as a pale yellow oil (183 mg, 77%).

R_f 0.25 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3405 (br, OH), 2922, 1711 (C=O), 1617; ^{1}H NMR (CDCl$_3$; 600 MHz) 0.86 (3H, t, J = 6.9 Hz, CH$_2$CH_3), 1.18-1.46 (10H, m, (CH_2)$_5$CH$_3$), 1.53 (1H, m, CHOHCHH), 1.81 (1H, m, CHOHCHH), 2.18 (3H, s, COCH_3), 2.96 (1H, s br, OH), 4.17 (1H, dd, J = 7.4, 3.8 Hz, CHOH); ^{13}C NMR (CDCl$_3$; 151 MHz) 14.2 (CH$_3$), 22.7 (CH$_2$), 24.9 (CH$_2$), 25.3 (CH$_3$), 29.2 (CH$_2$), 29.5 (CH$_2$), 31.9 (CH$_2$), 33.7 (CH$_2$), 77.0 (CHOH), 210.2 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 190.1806, C$_{10}$H$_{24}$NO$_2$ requires 190.1807.

3-Hydroxyhept-6-en-2-one **265**
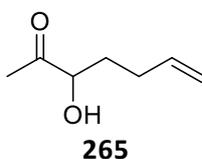
265

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-methyl-1,3-dithian-2-yl)pent-4-en-1-ol **256** (328 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **265** as a pale yellow oil (136 mg, 71%).

R_f 0.21 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3406 (br, OH), 3076, 2924, 1709 (C=O), 1638 (C=C); ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 1.59 (1H, m, CHOHCHH), 1.84 (1H, m, CHOHCHH), 2.12 – 2.19 (5H, m, COCH_3 & CH_2CH=CH$_2$), 4.08 (1H, m, CHOH), 4.14 (1H, d, J = 5.2 Hz, OH), 4.95 (1H, dm, J = 10.3 Hz, CH=CHH), 5.04 (1H, dq, J = 17.2, 1.7 Hz, CH=CHH), 5.85 (1H, m, CH=CH$_2$); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 25.5 (CH$_3$), 31.1 (CH$_2$), 33.7 (CH$_2$), 77.1 (CHOH), 115.5 (CH=CH$_2$), 139.1 (CH), 211.3 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 146.1181, C$_7$H$_{16}$NO$_2$ requires 146.1181.

4-Hydroxyoct-7-en-3-one 266
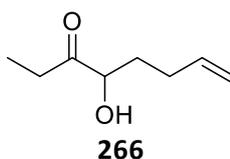
266

Synthesis adapted from literature procedure.[206] To a solution of 1-(2-ethyl-1,3-dithian-2-yl)pent-4-en-1-ol **262** (349 mg, 1.50 mmol) in water (40 mL), acetonitrile (8 mL) and THF (5 mL), iodomethane (2.99 mL, 48.0 mmol) and calcium carbonate (1.80 g, 18.0 mmol) were added and the reaction mixture stirred at reflux for 4 h. The reaction was allowed to cool to RT and the organic component extracted with diethyl ether (3 × 50 mL), dried (MgSO$_4$), filtered, and the solvent removed under vacuum. The crude product was purified by flash column chromatography (pentane/diethyl ether, 9:1) to give **266** as a pale yellow oil (154 mg, 72%).

R_f 0.24 (pet. ether 40-60/EtOAc, 9:1); IR (film, cm^{-1}) 3421 (br, OH), 3076, 2923, 1709 (C=O), 1640 (C=C); ^{1}H NMR ((CD$_3$)$_2$CO; 600 MHz) 1.00 (3H, t, J = 7.3 Hz, CH$_3$), 1.59 (1H, m, CHOHCHH), 1.83 (1H, m, CHOHCHH), 2.16 (2H, m, CH_2CH=CH$_2$), 2.59 (2H, m, CH_2CH$_3$), 4.07 – 4.14 (2H, m, CHOH & OH), 4.94 (1H, dm, J = 10.1 Hz, CH=CHH), 5.03 (1H, dq, J = 17.2, 1.7 Hz, CH=CHH), 5.84 (1H, m, CH=CH$_2$); ^{13}C NMR ((CD$_3$)$_2$CO; 151 MHz) 7.78 (CH$_3$), 30.1 (CH$_2$), 31.4 (CH$_2$), 34.0 (CH$_2$), 76.7 (CHOH), 115.4 (CH=CH$_2$), 139.1 (CH), 213.9 (CO); m/z HRMS (ESI+) found [M+NH$_4$]$^+$ 160.1333, C$_8$H$_{18}$NO$_2$ requires 160.1332.

6.4. Gas Chromatography Screening Details

The following stock solutions were mixed: magnesium chloride hexahydrate (83 mg/mL), thiamine diphosphate (22 mg/mL) and MES buffer (50 mM) in water was adjusted to pH 7 to give the cofactor solution; sodium pyruvate (12.2 mg/mL) or ketobutyric acid (11.3 mg/mL) and MES buffer (50mM) in water was adjusted to pH 7 to give the donor solution; and an aldehyde (250 mM) in DMSO, to give the acceptor solution.

A mixture of MES buffer (50mM, 150 µL), lysate (50 µL) and cofactor solution (50 µL) was shaken at 25 °C for 20 min. Donor solution (225 µL) and acceptor solution (50 µL) were then added and the reaction was shaken at 25 °C for a further 24 h. Ethyl acetate (500 µL) was then added and the mixture agitated, then centrifuged (4000 rpm, 3 min). An aliquot of the organic phase (100 µL) was taken and added to decane in ethyl acetate (50 mM, 900 µL). This solution was then injected into the GC apparatus with an injection volume of 5 µL and one of the following methods used to separate the product, starting material, and decane internal standard.

Method A: A linear temperature gradient was applied, starting at 100 °C and increasing by 5 °C per minute until the retention time of the product was reached. This was used in the quantification of;

3-hydroxyheptan-2-one **225** ((*S*)- rt 5.51 min, (*R*)- rt, 5.78 min)

3-hydroxyoctan-2-one **226** ((*S*)- rt 5.50 min, (*R*)- rt, 5.80 min)

3-hydroxynonan-2-one **263** ((*S*)- rt 9.25 min, (*R*)- rt, 9.38 min)

3-hydroxydecan-2-one **264** ((*S*)- rt 10.98 min, (*R*)- rt 11.11 min min)

3-hydroxyhept-6-en-2-one **265** ((*S*)- rt 5.49 min, (*R*)- rt, 5.69 min)

4-hydroxyoctan-3-one **227** ((*S*)- rt 5.49 min, (*R*)- rt 5.70 min)

4-hydroxynonan-3-one **228** ((*S*)- rt 7.94 min, (*R*)- rt 8.04 min)

4-hydroxydecan-3-one **241** ((*S*)- rt 10.00 min, (*R*)- rt, 10.10 min)

4-hydroxyundecan-3-one **242** ((*S*)- rt 12.00 min, (*R*)- rt, 12.09 min)

4-hydroxyoct-7-en-3-one **266** ((*S*)- rt 5.48 min, (*R*)- rt, 5.67 min)

Method B: A linear temperature gradient of 100 - 170 °C over 14 minutes was applied, followed by a linear gradient of 170 - 195 °C over 2.5 min. This was used in the quantification of 4-hydroxytridecan-3-one **235** ((*S*)- rt 16.03 min, (*R*)- rt, 16.12 min).

6.4.1 Calibration Curves

3-Hydroxyheptan-2-one **225**

R² = 0.9991

3-Hydroxyoctan-2-one **226**

R² = 0.996

3-Hydroxynonan-2-one **263**

R² = 0.9989

3-Hydroxydecan-2-one **264**

R² = 0.9972

3-Hydroxyhept-6-en-2-one **265**

R² = 0.9969

4-Hydroxyoctan-3-one **227**

R² = 0.9976

4-Hydroxynonan-3-one **228**

R² = 0.9869

4-Hydroxydecan-3-one **241**

R² = 0.9981

4-Hydroxyundecan-3-one **242**

4-Hydroxytridecan-3-one **235**

4-Hydroxyoct-7-en-3-one **266**

6.4.2. Spectra for TK Screening of D469T Mutant

3-Hydroxyheptan-2-one **225**

3-Hydroxyoctan-2-one **226**
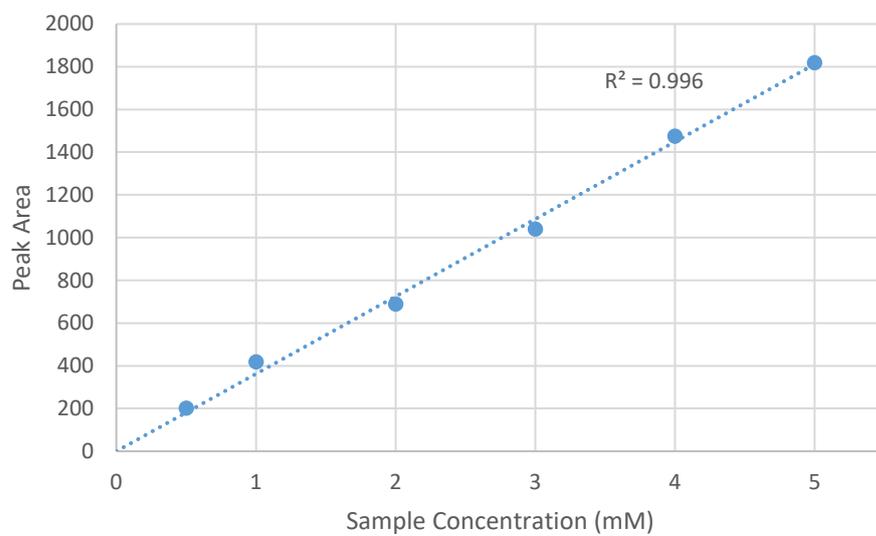
3-Hydroxynonan-2-one **263**
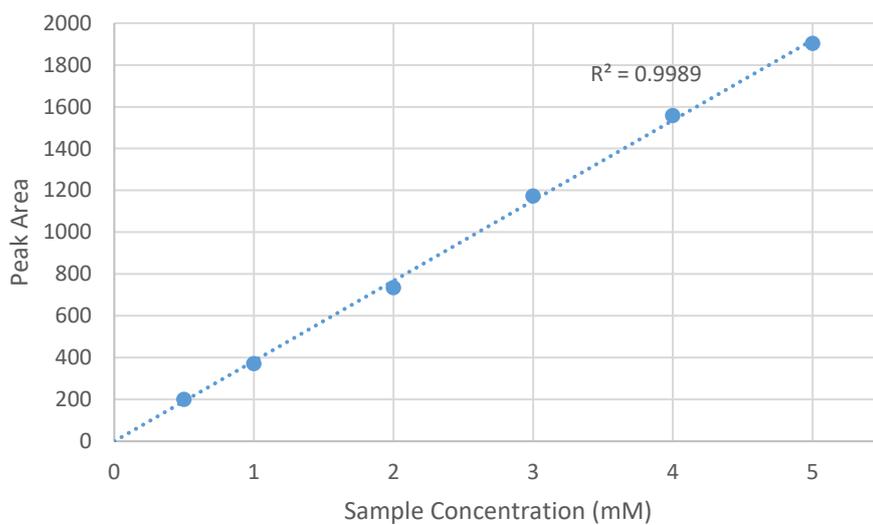
3-Hydroxydecan-2-one **264**
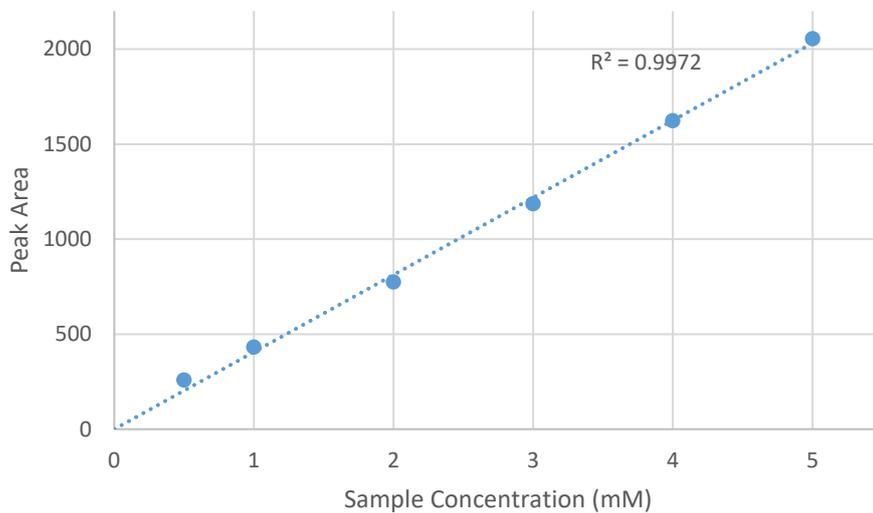
3-Hydroxyhept-6-en-2-one **265**
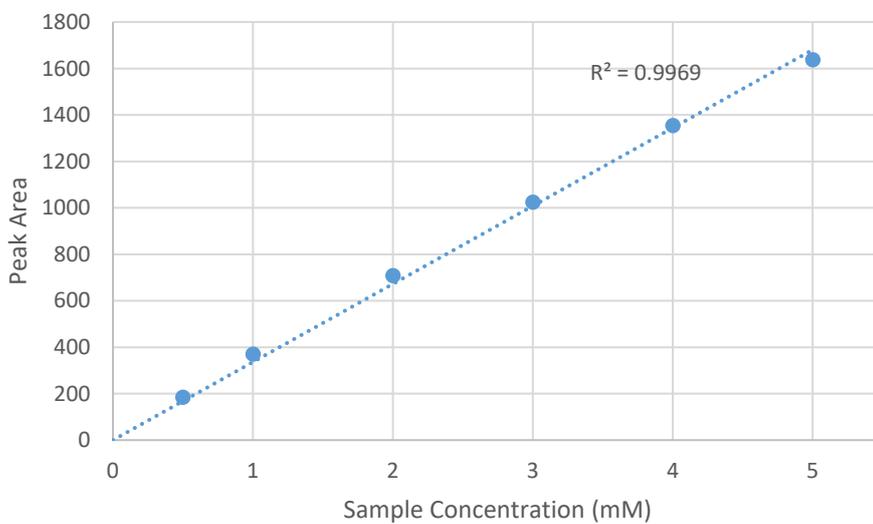
4-Hydroxyoctan-3-one **227**
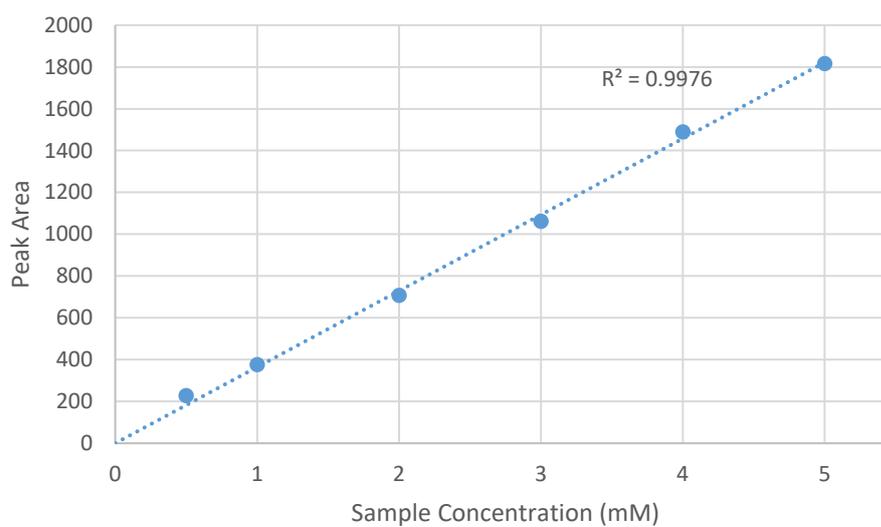
4-Hydroxynonan-3-one **228**
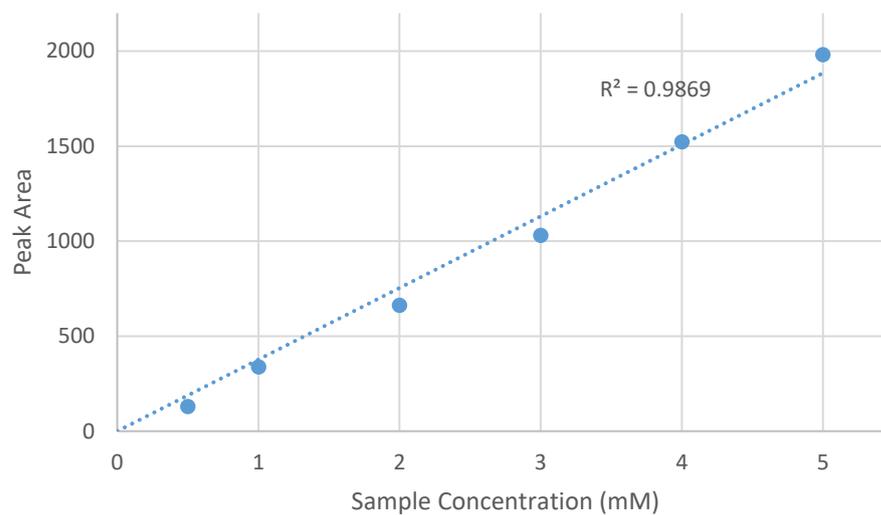
4-Hydroxydecan-3-one **241**
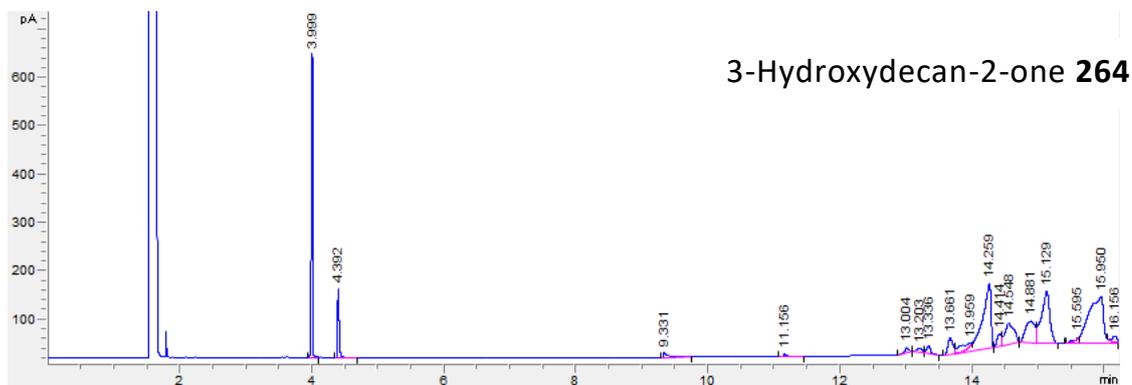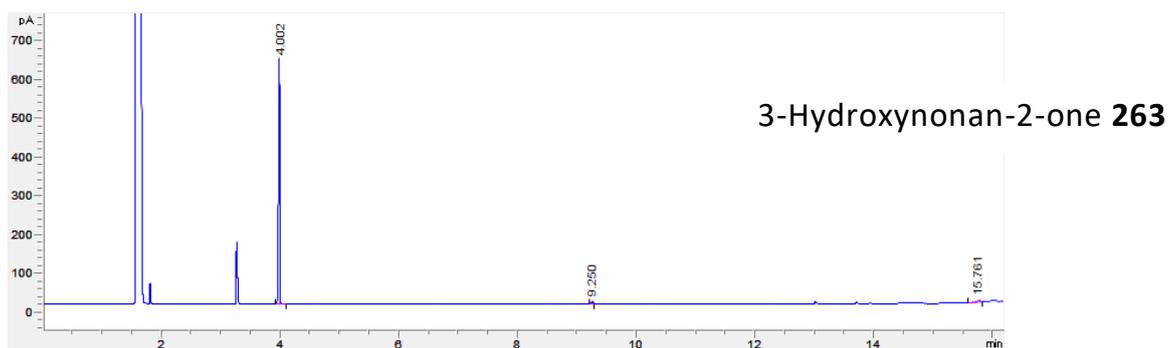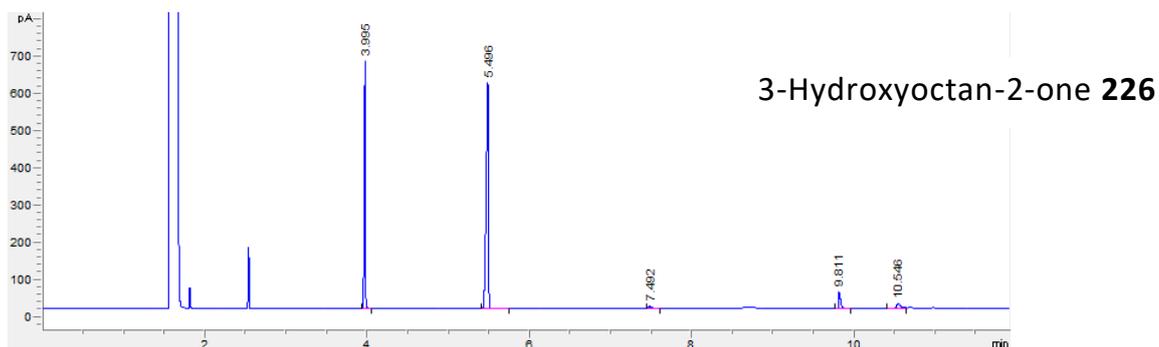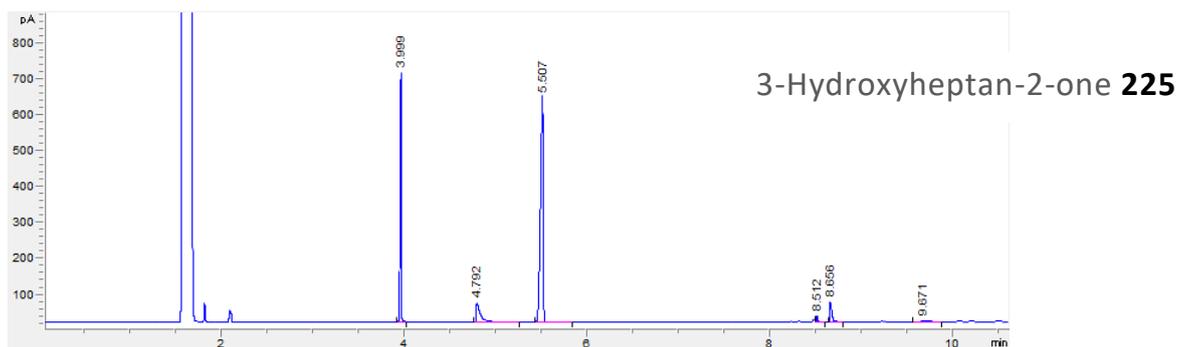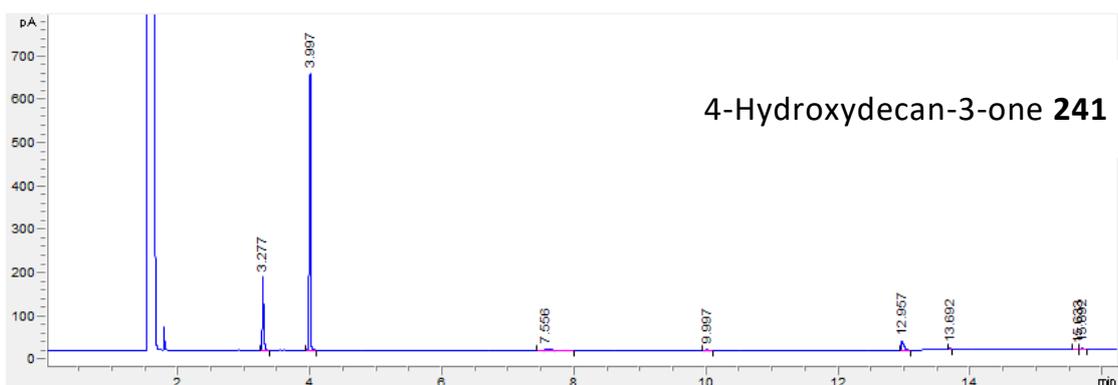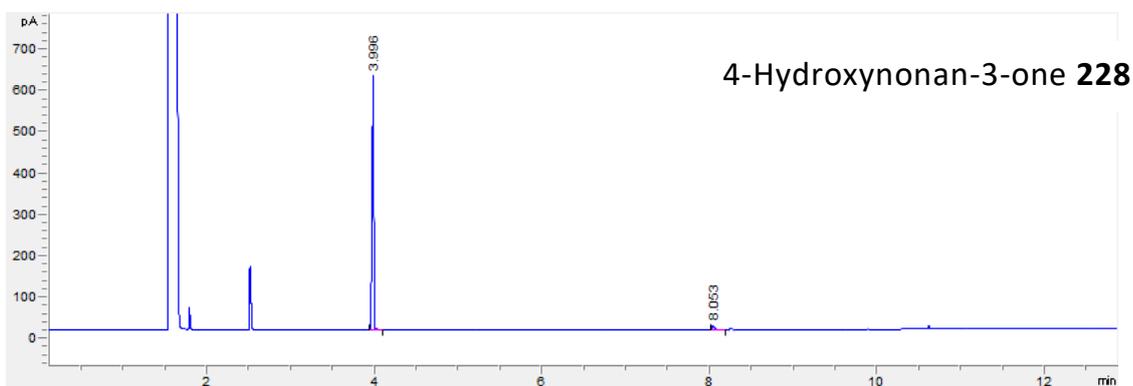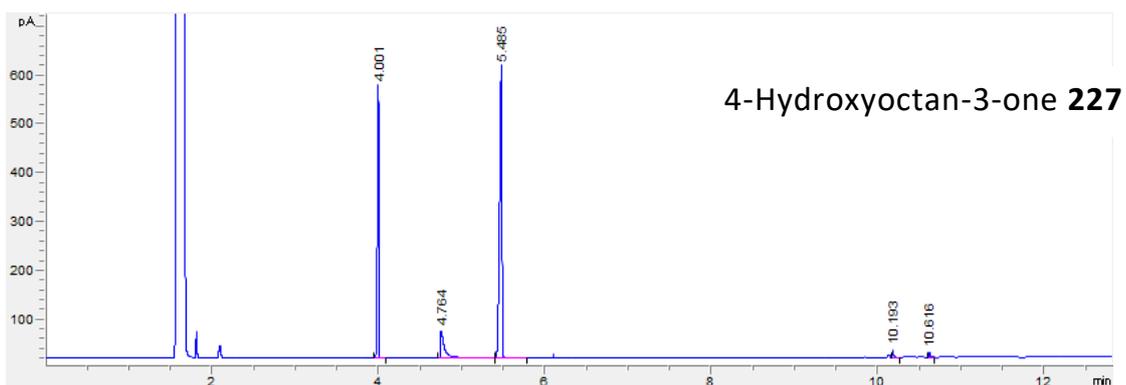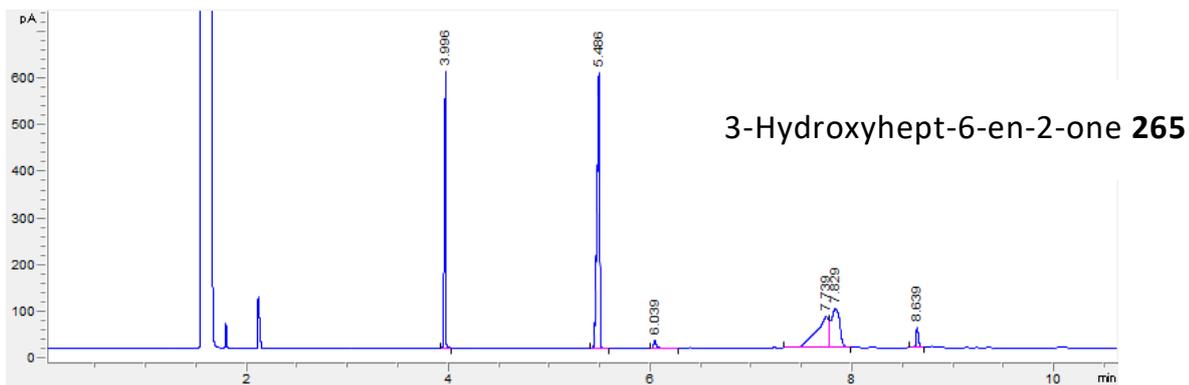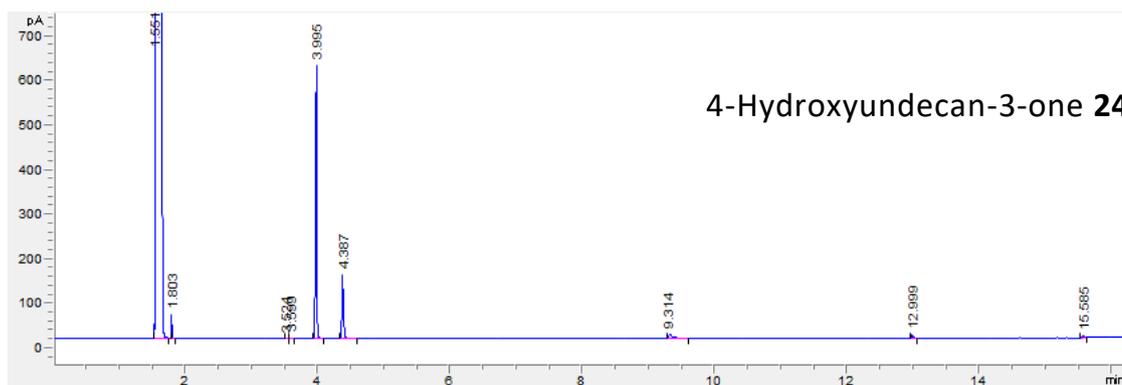
4-Hydroxyundecan-3-one **242**
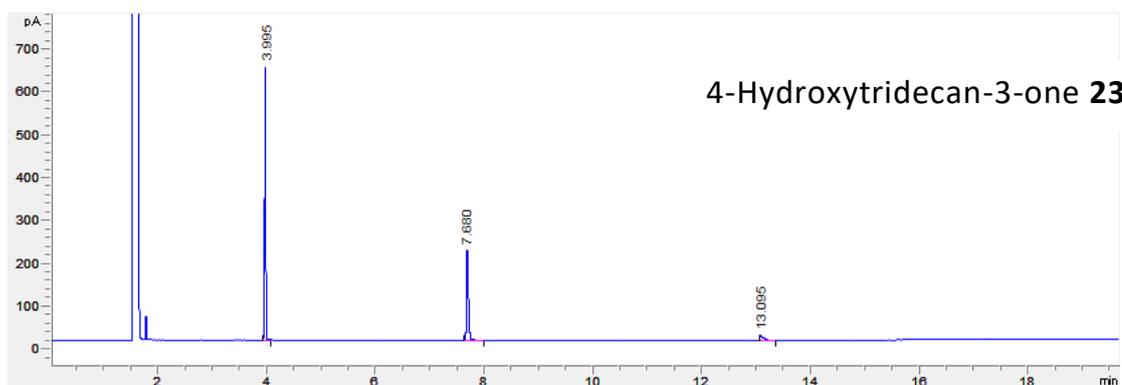
4-Hydroxytridecan-3-one **235**
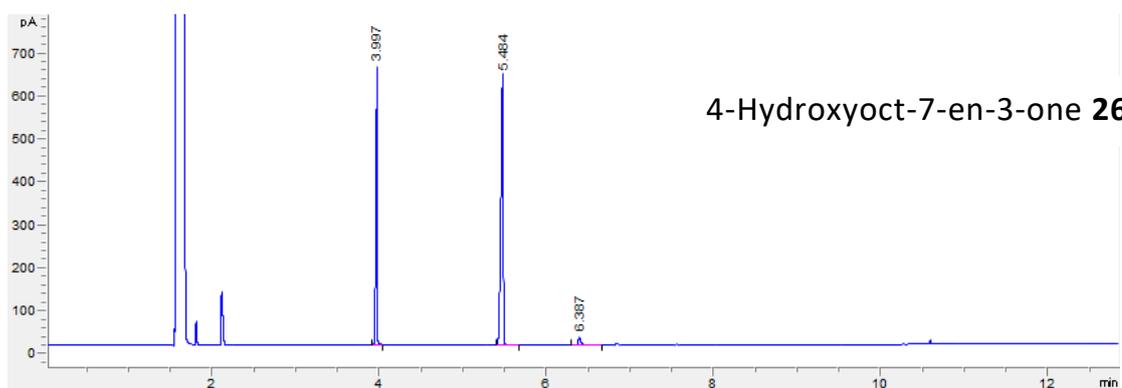
4-Hydroxyoct-7-en-3-one **266**

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