Evaluation of Novel Arginine Based Inhibitors of DDAH and Investigations into Radical Hydroacylation of Vinyl Sulfonates

A thesis submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

At the Chemistry Department of University College London

by Nurjahan Khanom

DECLARATION

I, Nurjahan Khanom, 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.

Nurjahan Khanom

May 2010

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In the name of Allah (swt), the most beneficent, the most merciful.

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ABSTRACT

The thesis is in two main sections.

In the first section, studies on methylarginine processing enzymes are presented. Dimethyalrginine dimethylaminohydrolase (DDAH) is a class of enzymes involved in the metabolism of methylarginines ADMA and L-NMMA, which indirectly regulate physiological nitric oxide levels. It is desirable to inhibit excess NO in pathological situations, and the arginine mimetic L-257 is a DDAH inhibitor which reduces levels of NO. Synthesis of ester analogues of L-257 proved to be troublesome with a low yielding key guanidine forming reaction. However, amide analogues were readily synthesised, and testing for DDAH inhibition showed the dimethylamide analogue possessed similar activity to L-257. Further design and synthesis of a 7-membered cyclic analogue, based on the crystal structure of *hu*DDAH1 with L-257, provided a novel analogue with no significant inhibition for rat kidney DDAH. Purified and isolated *hu*DDAH2 protein showed activity after incubation with substrate L-NMMA.

In the second part studies on aldehyde auto-oxidation are presented.

Aldehydes autoxidise to their acids, *via* an acyl radical, which can undergo addition reactions with electron-deficient acceptors in a radical hydroacylation reaction. An α-iodo and α-chloro hexanal failed to autoxidise, however 7-hydroxycitronellal readily autoxidised and added to pentafluorophenyl(PFP)-vinyl sulfonate. Further studies on hydroacylation of butanal with PFP-vinyl sulfonate led to functionalised β-keto-sulfonates which undergo elimination to form an enone and can then undergo further conjugate addition *in situ* by nucleophiles. Conjugate addition was carried out using carbon, nitrogen, oxygen and phosphorus nucleophiles, providing a method of obtaining products which are challenging to make *via* hydroacylation of electron-rich alkenes. Decarbonylation of pivaldehyde to the *t*-butyl radical, *via* auto-oxidation, was optimised and the alkyl radical captured by a number of electron-deficient acceptors, providing a complementary method to current methods of *t*-butyl addition using metal reagents.

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Investigations into Radical Hydroacylation of Vinyl Sulfonates

ABBREVIATIONS

ADI	Arginine deiminase
ADMA	Asymmetric dimethylated L-arginine
ADP	Adenosine diphosphate
AIBN	2,2'-azobisisobutyronitrile
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
BHT	2,6-Di-*tert*-butyl-4-methylphenol
Boc	*tert*-Butoxycarbonyl
BPO	Benzoyl peroxide
BrCN	Cyanogen bromide
Bn	Benzyl
Bu	Butyl
CAL	Calmodulin
Cbz	Benzyloxycarbonyl
cGMP	Cyclic guanosine monophosphate
CI	Chemical ionisation
cNOS	Constitutive nitric oxide synthase
Dab	2,4-Diaminobutyric acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDAH	Dimethylarginine dimethylamino hydrolase
DEAD	Diethyl azodicarboxylate
DEA-NONOate	(2-(*N*,*N*-diethylamino)-diazenolate-2-oxide, sodium salt
DIBAL	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	*N*,*N*-Dimethylaminopyridine
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium

DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DTT	Dithiothreitol
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EI	Electron ionisation
eNOS	Endothelial nitric oxide synthase
ES	Electrospray
Et	Ethyl
FAB	Fast atom bombardment
FAD	Flavin adenine dinucleotide
FBS	Foetal bovine serum
FMN	Flavin mononucleotide
Fmoc	Fluorenylmethyloxycarbonyl
GTP	Guanosine-5'-triphosphate
HcyNO	*S*-Nitroso-L-homocysteine
HOBt	1-Hydroxybenzotriazole
4-HNE	4-Hydroxynonenal
HRMS	High resolution mass spectrum
Hz	Hertz
ICS	Intersystem crossing
IC_{50}	Half maximal inhibitory concentration
iNOS	Inducible nitric oxide synthase
IP	Immunoprecipitation
L-IPO	N^5-(1-iminopropyl)-L-ornithine
IR	Infra-red
k_{cyc}	Rate of cyclisation
K_i	Inhibitory equilibrium constant
k_{red}	Rate of reduction
L-NMMA	Monomethylated L-arginine

LRMS	Low resolution mass spectrometry
Me	Methyl
mM	Millimolar
μM	Micromolar
mp	Melting point
MW	Microwave
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NHPI	*N*-Hydroxyphthalimide
NMP	*N*-Methyl-2-pyrrolidinone
NMR	Nuclear magnetic resonance
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
L-Orn	L- Ornithine
Pa	*Pseudomonas aeruginosa*
PAD4	Peptidyl argininedeiminase
PBS	Phosphate buffer solution
PFP	Pentafluorophenyl
PFPOH	Pentafluorophenol
Ph	Phenyl
PINO	Phthalimide *N*-oxyl
PRMT	Protein arginine methyltransferase
RT	Room temperature
SAR	Structure-activity relationships
SDMA	Symmetric dimethylated L-arginine
TBADT	Tetrabutylammonium decatungstate
TBAF	Tetrabutylammonium flouride
TBDPS	*tert*-Butyldiphenylsilyl

TBHN	Di-*tert*-butyl hyponitrite
TBS-T	Tris-buffered saline with Tween
TCP	2,4,6-Trichlorophenyl
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
UV	Ultra violet

Evaluation of Novel Arginine Based Inhibitors of DDAH

Chapter 1: Introduction

1.1 Nitric oxide and its effects

Nitric oxide (NO) is a key biological mediator involved in the physiology of the human body.[1] Its *in vivo* effects have led the scientific community to spend considerable effort on its modulation.[2] NO is a highly reactive gaseous molecule formed in the body from L-arginine by the nitric oxide synthase (NOS) family of enzymes. There are three isoforms of NOS: endothelial NOS (eNOS) constitutively expressed in endothelial cells, neuronal NOS (nNOS) constitutively expressed in the nervous system, and inducible form of NOS (iNOS). In the endothelial system NO has a vital role regulating vascular homeostasis by relaxing smooth muscle cells in blood vessels thereby controlling blood pressure, and preventing platelet aggregation.[3] In the central nervous system NO produced acts as a neurotransmitter.[4] NO produced from iNOS is involved in inflammatory responses by attacking invading organisms.

The NOS enzymes function as dimers around a central zinc atom, and contain a reductase domain that binds the cofactors flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and NADPH, and an oxygenase domain which binds tetrahydrobiopterin (BH_4) and haem.[5] Molecular oxygen is required for the oxidation of L-arginine **1** to L-citrulline **2** and NO. eNOS and nNOS activity is dependent on calmodulin binding (CAL) which in turn is dependent on a intracellular calcium concentration controlled by receptor-mediated calcium channels. Contrastingly, iNOS already has tightly bound Ca^{+}/calmodulin and is regulated on the transcriptional level.

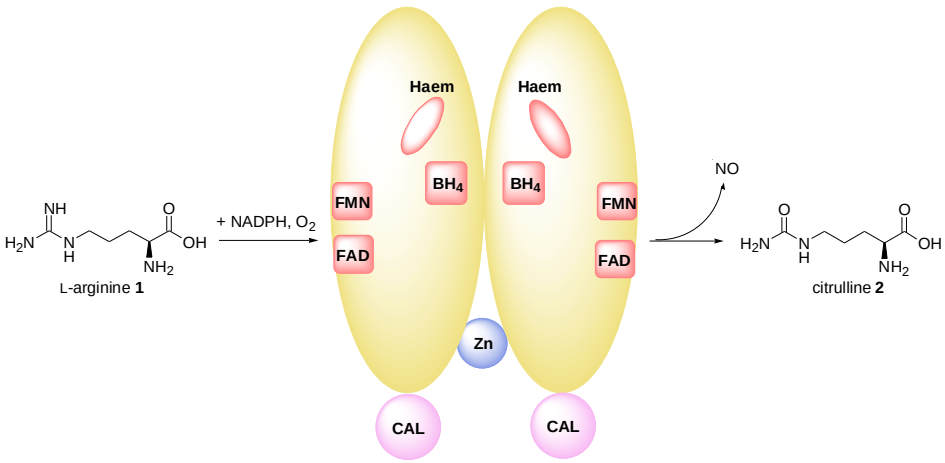


Figure 1: Conversion of L-arginine to citrulline by the NOS enzymes.[2]

In endothelial cells, increase in NO leads to vasodilatation by the following mechanism (Figure 2):[6] NO synthesis is triggered by a rise in Ca^{2+} concentration due to receptor activation on the cell membrane by various chemical signals. When it is formed it diffuses across the plasma membranes of neighbouring smooth muscle cells, where it activates the enzyme guanylate cyclase, converting guanosine monophosphate (GTP) into cyclic guanosine monophospate (cGMP). Increase in cGMP then leads to relaxation of the smooth muscle and eventual vasodilatation, which plays an important role in regulating blood pressure.

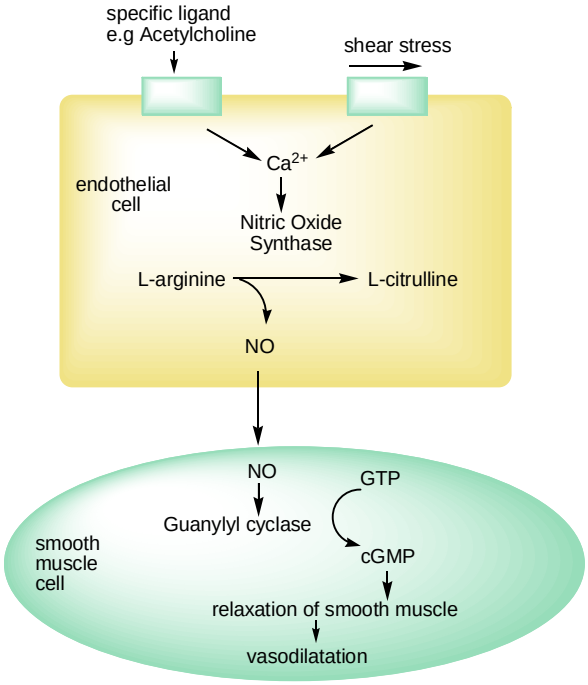


Figure 2: Mechanism of smooth muscle relaxation.

Although NO is an important molecule required for various functions, excess levels of NO have been implicated in many disease states. The harmful effects of excess NO induced by nNOS is implicated in migraine, stroke and neurodegenerative diseases such as Alzheimers disease. NO induced by iNOS is implicated in septic shock, ischeamia and cerebral inflammation, and NO induced by eNOS has an effect on diabetes and inflammation.[7] More recently it has been found that low levels of NO are found in malignant human breast, neuronal, gastric, cervical and ovarian cancers, but not in the surrounding benign tissues.[8] It is therefore clinically desirable to modulate levels of NO in certain disease states. The most obvious target is to inhibit the NOS enzymes, but to date no clinically useful inhibitors have been discovered, as there are issues of selectivity of a single NOS isoform. Examples of NOS inhibitors include the endogenous inhibitor monomethylated L-arginine (L-NMMA **3**, Figure 3). L-NMMA has been evaluated in phase III clinical trials to treat septic shock patients but a poor survival rate impeded its further development. A substrate based inhibitor 1400W showed considerable selectivity for iNOS over the other isoforms, ~10,000 fold selectivity for iNOS over eNOS and ~30 fold selectivity for iNOS over nNOS (Figure 3). However, toxicity problems in humans hindered its development. Other selective iNOS inhibitors with toxicity problems preventing their development include GW273629 **5**, L-NIL **6**, and the isothiourea 1,4-PBITU **7**. Another class of NOS

inihibitors are dimerization inhibitors such as Miconazole **8**. However, dimerization inhibitors show poor selectivity of one NOS isoform over another, and are not viable candidates for development for clinical use.[2]

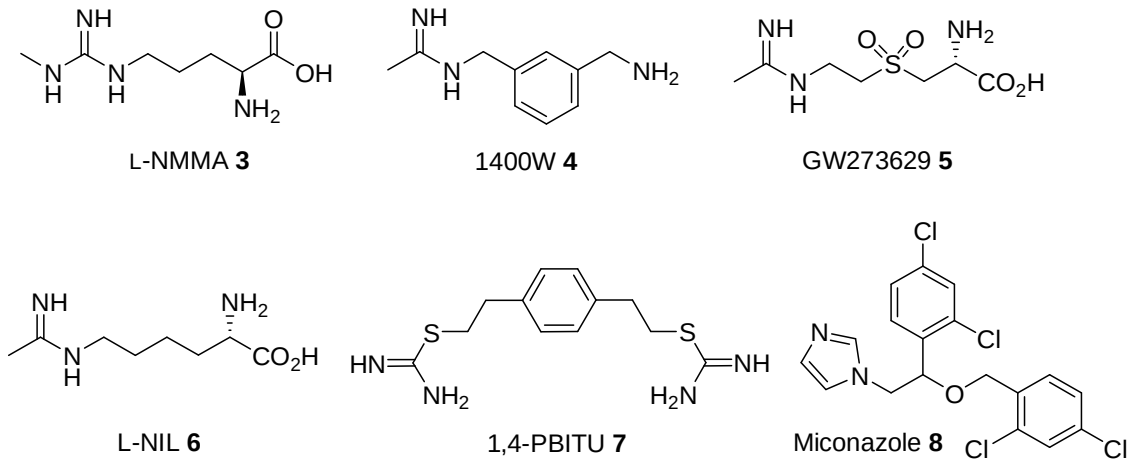


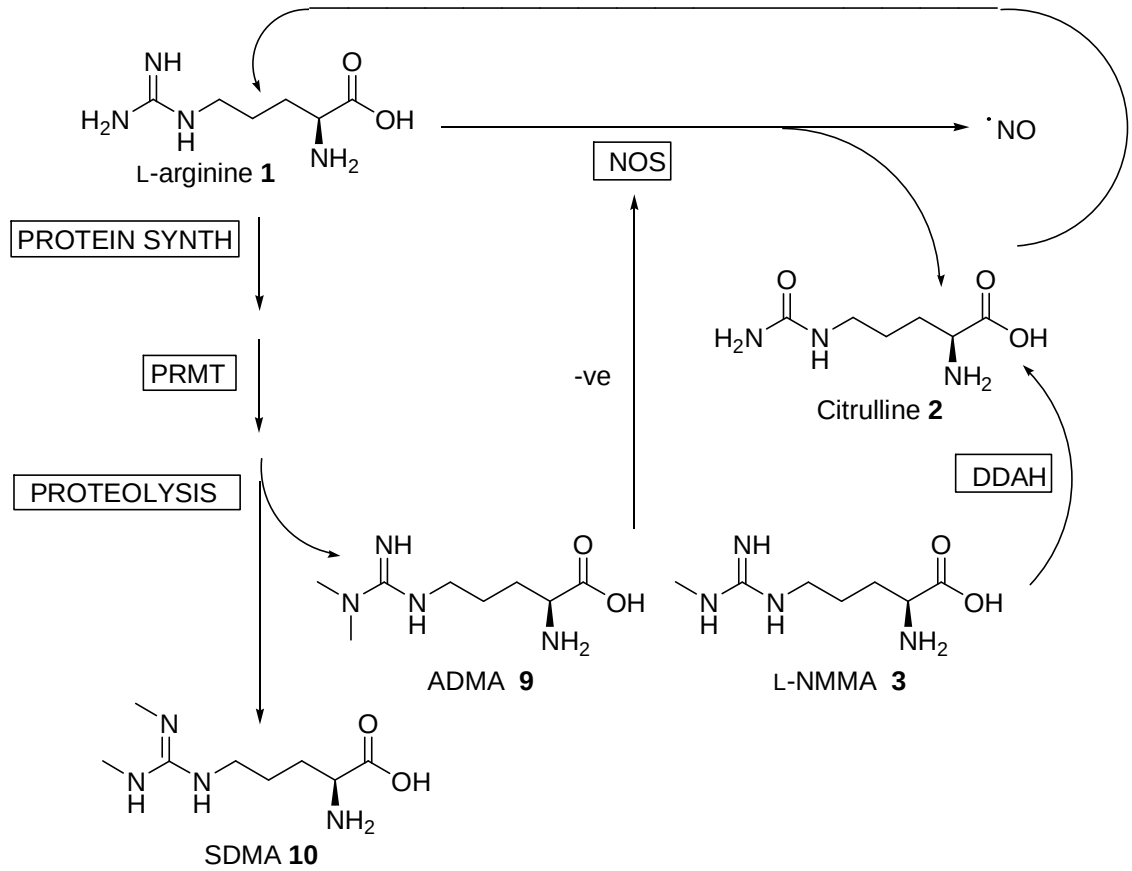
Figure 3: NOS inhibitors.

To date there have been no NOS inhibitors developed for widespread clinical use for the control of excess NO production. Since most NOS inhibitors show poor isoform selectivity or are associated with toxicity problems, developing NOS inhibitors for the control of excess NO does not show promise. However, control of the metabolism of endogenous NOS inhibitors offers an alternative approach.

1.2 The ADMA/DDAH pathway

L-Arginine **1** is converted to nitric oxide by NOS, which can be inhibited by the methylarginines asymmetric dimethylarginine (ADMA) **9** and L-NMMA **3** (Scheme 1). These methylarginines are metabolised to citrulline and the corresponding alkylamine by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes. Inhibiting DDAH would increase the concentrations of ADMA and L-NMMA which would lead to the inhibition of NOS and therefore reduce levels of NO in pathological situations where NO is overproduced (Scheme 1).[9] Both L-NMMA and ADMA are formed *via* protein methylation on the terminal nitrogen of arginine residues. The protein methylation here is accomplished by a post translational modification by protein-arginine methyltransferases (PRMTs). Using *S*-adenosyl methionine (SAM) as methyl group donors type I PRMTs produce ADMA and L-NMMA, whilst type II produce L-NMMA

and symmetric dimethylarginine (SDMA) **10** which does not inhibit NOS. After methylation of arginine residues in proteins, free methylarginines are generated by proteolysis and are present in the cytosol.



Scheme 1: The ADMA/DDAH pathway.

DDAH dysfunction leads to accumulation of ADMA which acts as a marker of various disease states of the vascular system,[10] as well as for diabetes and renal failure.[11,12] In a recent study by Vallance *et al.* it was found in *ddah1* knock out mice that ADMA accumulates in endothelial cells and there is a reduction in NO signalling.[13] This in turns leads to increased blood pressure, endothelial dysfunction and systemic vascular resistance. It is proposed that the vascular dysfunction associated with sepsis can be improved by inhibition of DDAH. In a study by Konishi *et al.* it was found that DDAH can promote endothelial repair after vascular injury; hence vascular dysfunction can perhaps be improved by targeting DDAH.[14]

Although NO levels can be regulated by targeting NOS or DDAH, NO itself can self-regulate to some extent in a potential homeostatic mechanism, when present at high

levels. Vallance *et al.* showed that DDAH enzyme activity is regulated by *S*-nitrosylation.[15] Recombinant *Pa*DDAH is reversibly inhibited after incubation with NO donors *in vitro*, and mammalian DDAH in cytosolic extracts is reversibly inhibited the NO donor DEA-NONOate. This effect was reversed 92% by incubating with the reducing agent DTT. In cultured endothelial cells, cytokine induced iNOS expression of NO, *S*-nitrosylates human DDAH II. However, the significance of this regulatory mechanism is yet to be determined.

1.3 Function and structure of DDAH

DDAH exists as two isoforms: DDAH1 and DDAH2.[16] The isoforms share 62% homology in their amino acid sequence and have a conserved active site sequence, but have low sequence homology with the bacterial *Pseudomonas aeruginosa* DDAH (*Pa*DDAH). DDAH1 and DDAH2 also differ in their locality. DDAH1 is widely expressed especially in the brain, liver, skeletal muscle, macrophages and pancreas and is roughly co-localised with nNOS. DDAH2 is generally co-localised with eNOS; it has a wider distribution but is most densely expressed in the heart, placenta and the endothelium. DDAH2 is also expressed in the immune tissues that express iNOS and both of the enzymes are densely expressed in the kidneys.[17]

The first crystal structure of DDAH was solved by Vallance *et al.*[18] This was the DDAH crystal structure from the *Pa*DDAH complexed with L-Citrulline. *Pa*DDAH is 245 amino acids long and its structure is a barrel comprised of five repeats of a ββαβ structural motif (Figure 4, **A** and **B**). Structure **A** shows a bird's eye view and structure **B** shows a side view of the crystal structure of *Pa*DDAH. The N and C terminus project out of one side of the barrel ('bottom side') and the 'top' side consists of a protruding loop region (magenta) acting as a lid to the active site. The active site is formed from a central channel closed in the middle by a salt bridge, which acts as the bottom of the active site; and the other side of a channel is a water filled pore. The crystal structure showed a catalytic triad present at the active site involving a cysteine residue: Cys249, His162 and Glu114. The crystal structure also showed homology with Arginine Deiminase (ADI) and Arginine:Glycine Amidinotransferase which also share a common catalytic triad and catalyse similar reactions. Hence, DDAH is part of a superfamily of arginine modifying enzymes.

A DDAH1 structure of the mammalian bovine brain was later solved by Grütter *et al.* who showed, that like the *Pa*DDAH1, Bovine DDAH1 is also comprised of five repeats of a ββαβ structural motif (Figure 4, **C** and **D**).[19] L-Citrulline is bound in the active site of bovineDDAH1 as a space-filling model with yellow carbon atoms, blue nitrogen atoms and red oxygen atoms. The magenta loop is the 'lid' to the active site and the bottom of the active site is closed by a salt bridge between Glu77 and Lys174 shown in structure **D**. The water channel pore is thought to exist to reload molecules of water needed for the hydrolysis mechanism.

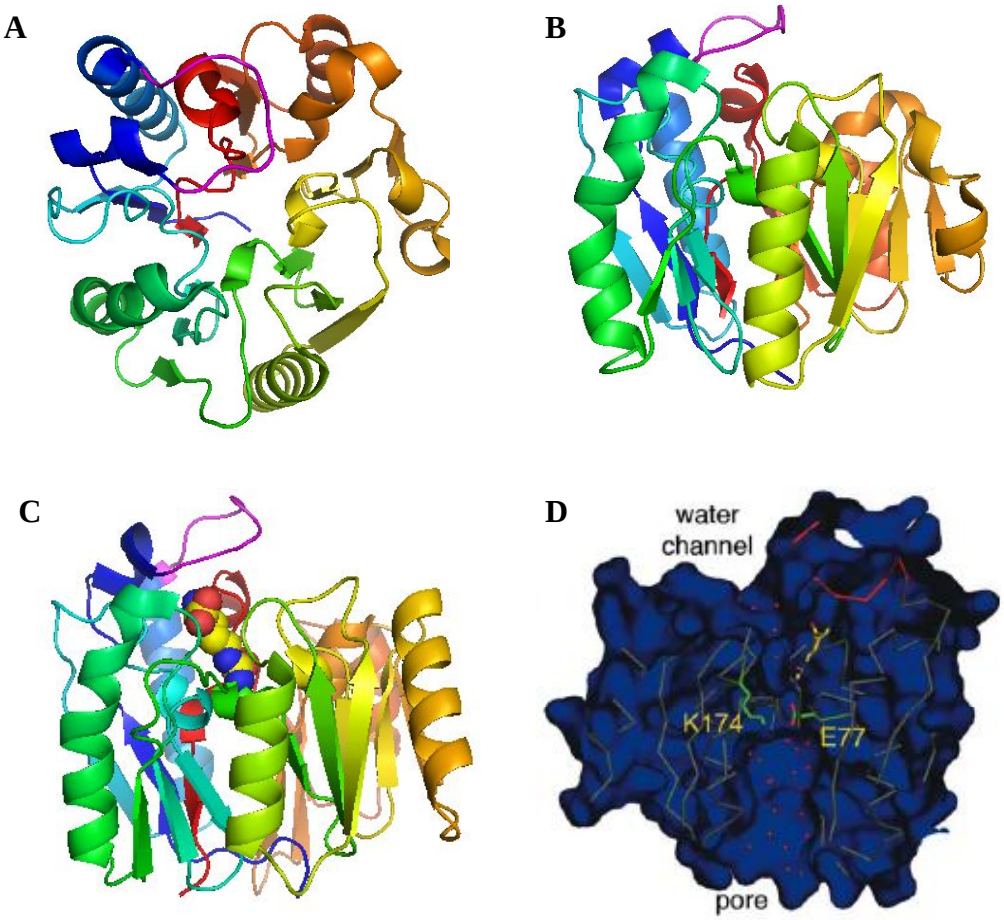


Figure 4 A: bird's eye view of *Pa*DDAH; **B**: side on view of *Pa*DDAH; **C**: bovine DDAH1 complexed with citrulline; **D**: bovine DDAH1 showing a salt bridge between a glutamic acid and a lysine residue.[18]

The crystal structure of L-Citrulline bound bovine DDAH in the closed lid conformation shows that the Cα-carboxyl group forms three salts bridges: two with the guanidine

group of Arg144 and one with the guanidine group of Arg97 (Figure 5). The Cα-amino group forms two H-bonds with the main chain carbonyls of Val267 and Leu29 and a salt bridge to Asp72. The terminal hydrogens of the ureido moiety are held in place in the active site by hydrogen bonding to Asp78 and Glu77. Phe75 and Leu171 shield the middle alkyl chain on both sides in a small hydrophobic pocket. The positions in the catalytic triad of bovineDDAH differ from those of *Pa*DDAH, and they are Cys273, His172 and Asp126.

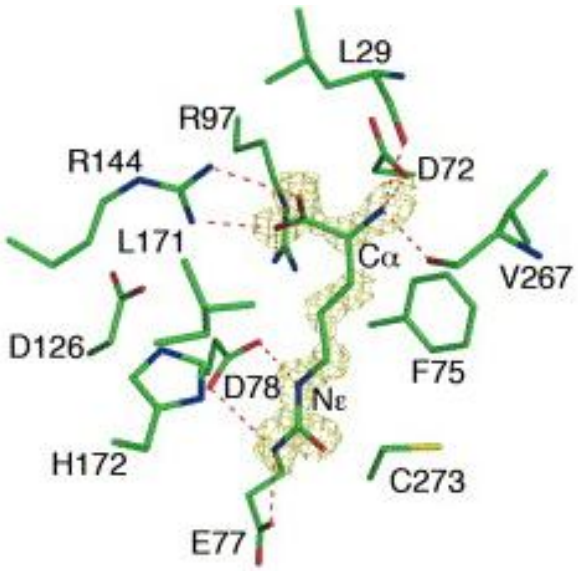
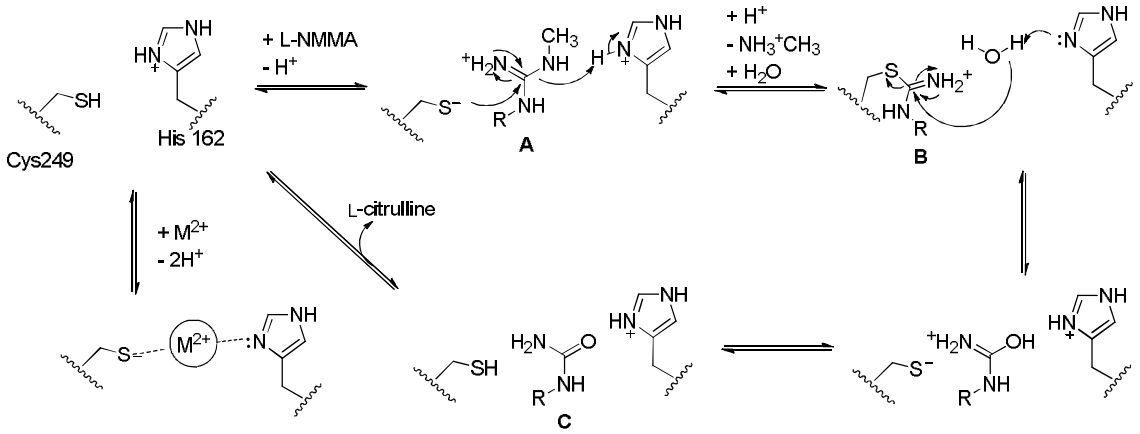


Figure 5: Binding of citrulline in DDAH active site.

1.4 Mechanistic studies

Vallance *et al.* proposed the hydrolysis mechanism of substrates such as ADMA in the active site of *Pa*DDAH goes *via* an imine hydrolysis, whereby the dimethylated nitrogen is protonated by the active site His162.[18] This imine is then attacked by the thiolate of Cys249, which they propose is deprotonated by His162, suggesting that Cys-249 and His162 form an ion pair. However, Fast *et al.* argue against this proposing that the deprotonation of the cysteine thiolate is substrate assisted as crystal structures show that Cys246 and His162 are too far away from each other to form an ion pair.[8] The authors propose the Cys249 in the resting enzyme is only deprotonated when bound to cationic substrates, lysine and metals (Scheme 2). Binding of the substrate L-NMMA with a positively charged guanidine residue is thought to induce the deprotonation of the thiol. It is not known whether this proton is lost to the solvent or to an unknown base. The thiolate attacks at the guanidinium carbon of the substrate (state **A**) and expels

methylamine leading to the formation of a covalent thiouronium intermediate (state **B**). The methylamine leaving group picks up a proton from the protonated histidine. Subsequent deprotonation of water by the His162, for the hydrolysis of the thiouronium species, leads to the formation of citrulline, and regeneration of the protonated thiol of Cys249 and protonated His162 (state **C**).

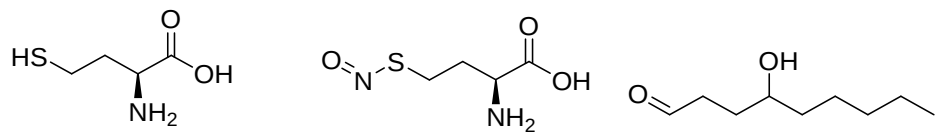


Scheme 2: Hydrolysis mechanism at the active site of *Pa*DDAH.

1.5 DDAH inhibitors

1.5.1 Endogenous Inhibitors

Endogenous inhibitors of DDAH include Zn^{2+}, L-homocysteine **11**, *S*-nitroso-L-homocysteine (HcyNO) **12** and 4-hydroxynonenal (4-HNE) **13** (Figure 6).

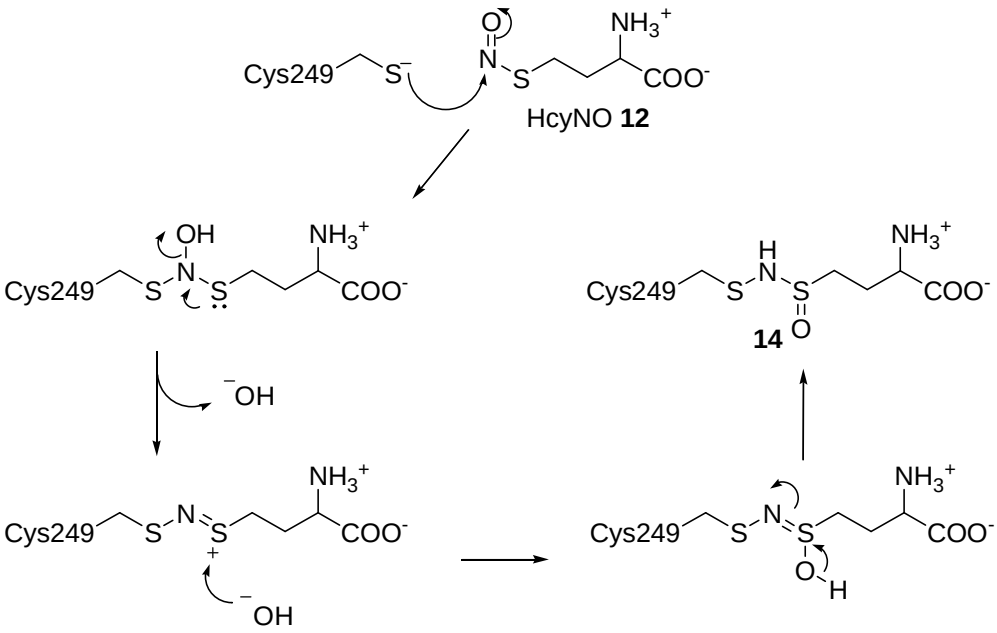


L-Homocysteine **11** *S*-Nitroso-L-Homocysteine **12** 4-HNE **13**

Figure 6: Endogenous inhibitors of DDAH.

Knipp *et al.* describe the inhibition of bovine DDAH1 by *S*-nitroso-L-homocysteine by its covalent attachment to the active site of the enzyme.[20] They propose the mechanism goes through the attack of the active site Cys249 thiolate on HcyNO **12** (Scheme 3). The covalently attached molecule is a dead-end complex **14** (Scheme 3) and the authors propose that this compound may help in the design of covalently attached inhibitors for

DDAH and the structurally and mechanistically related enzyme, arginine deiminase, ADI.



Scheme 3: Reaction of HcyNO at DDAH active site.

DDAH does not have any cofactor requirements for its catalytic activity, but exists as an inactive enzyme bound to a single Zn^{2+} ion. Removal of the Zn^{2+} ion from the enzyme by phosphate or imidazole increases its activity. Hence, it appears that Zn^{2+} has a stabilising and regulatory role in the activation of the DDAH enzymes.[21] The crystal structure of DDAH1 from bovine brain shows it is a monomeric enzyme with a molecular mass of 31.2 kDa. Although the structure of bovine brain DDAH1 showed it to have bound zinc, a more recent paper disclosing the crystal structure of human DDAH1 did not show any bound zinc.[13] Hence, the exact role of zinc in the regulation of DDAH activity seems uncertain.

4-HNE **13** is a reactive aldehyde and is the product of oxidative degradation of polyunsaturated fatty acids. It is produced in pathological situations of oxidative stress especially in cardiovascular diseases. It was shown by Cardounel *et al.* that exposure in pathological situations of 4-HNE to huDDAH1 leads to a dose dependent inhibition of the enzyme by forming an adduct at the histidine residues (His15 and His173) of *hu*DDAH1.[22] This leads to inhibition of DDAH and hence leads to lowering of NO

levels. It is thought that this mechanism plays a critical role in mediating endothelial dysfunction seen in disease states involving oxidative stress.

1.5.2 Structurally distinct inhibitors

Inhibitors which are structurally different to the natural substrate include the pentafluorophenylsulfonates **15** and **16**, chloroacetamidine **17**, and the indolylthiobarbiturates **18** and **19** (Figure 7). Prompted by the work of Roush *et al.* on sulfonates and sulfonamides as cysteine protease inhibitors, Vallance *et al.* have reported pentafluorophenyl (PFP) sulfonates and their activity against DDAH and ADI.[23, 24] The sulfonates **15** and **16** showed significant activity against DDAH and ADI, with IC_{50} values of 16 μM of **15** against *Pa*DDAH, and 74 μM of **16** against ADI (Figure 7). These compounds represent the first structurally different inhibitors of DDAH, and the first exogenous small molecules to inhibit ADI.

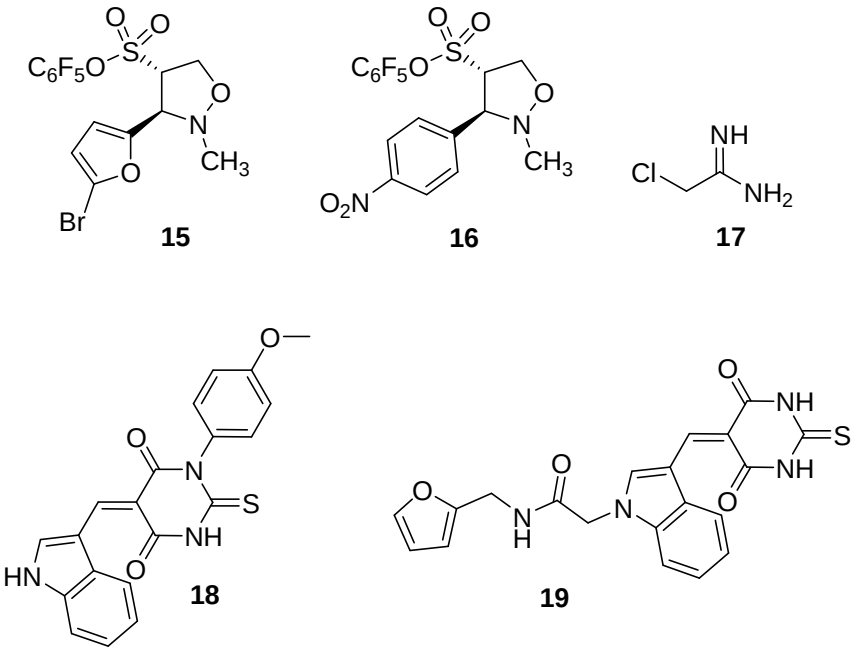


Figure 7: DDAH inhibitors.

Chloroacetamidine **17** (Figure 7) is an irreversible time-dependent inhibitor which targets the active site Cys249.[25] It also inhibits peptidyl argininedeiminase (PAD4) and is a widely applicable inhibitor for the superfamily of arginine modifying enzymes. The authors suggest incorporation of additional functional groups may lead to the design of future therapeutics.

Selwood *et al.* more recently took a different approach for the discovery of DDAH inhibitors.[26] They used *in silico* virtual screening and hit analysis, which does not require high throughput screening and has additional advantages of lower cost, higher speed and lower resource requirements. Filtering of physicochemical parameters from a 308,000 compound library and biological screening identified the barbituric acids **18** and **19** (Figure 7) as active *Pa*DDAH inhibitors. Compound **18** was the most active compound identified $IC_{50(PaDDAH)}$ = 2 μM and represents the most active *Pa*DDAH inhibitor to date. However, in a more recent study Clement *et al.* reported no activity for **18** against *hu*DDAH1.[27] Although the active site is highly conserved in both *Pa*DDAH and *hu*DDAH1, sequence similarity is only ~25% and the work of Clement highlights the limitations of using *Pa*DDAH as a model for mammalian DDAH.

1.5.3 Substrate based inhibitors

One of the first exogenous molecules found to inhibit DDAH was a chain shortened analogue of L-NMMA, 4124W **21** (Figure 8), a weak inhibitor with activity at millimolar concentrations and poor selectivity.[28] Based on the structure of 4124W Rossiter *et al.* have developed structural analogues of 4124W to improve DDAH inhibition and enhance selectivity for DDAH over NOS.[29] Modifications of 4124W made include changing the number of carbon atoms in the middle alkyl chain and changing the R groups on the guanidine moiety of **20** (Figure 8).

From a library of 4124W analogues it was found that guanidine **23** (Figure 8) bearing a 2-methoxyethyl group on the guanidine moiety, and a benzyl ester on the acid, was the most potent (IC_{50} = 27 μM) out of a series of analogues with a two carbon length alkyl chain. Analogues with amide groups in place of the ester moiety were not as effective at inhibiting DDAH. One carbon chain extension of **23** to give free acid L-257 **22** (IC_{50} = 22 μM) and methyl ester L-291 **24** (IC_{50} = 20 μM) exhibited higher activity for DDAH, without any significant effect on NOSs. Treatment of rats with L-257 **22** and L-291 **24** led to an increase in ADMA concentrations as compared with control animals, [ADMA] ~ 1.2 μM, vs ~ 0.8 μM in controls. Hence, compounds L-257 **22** and L-291 **24** show potential as DDAH inhibitors and for exploring the significance of the DDAH enzymes.

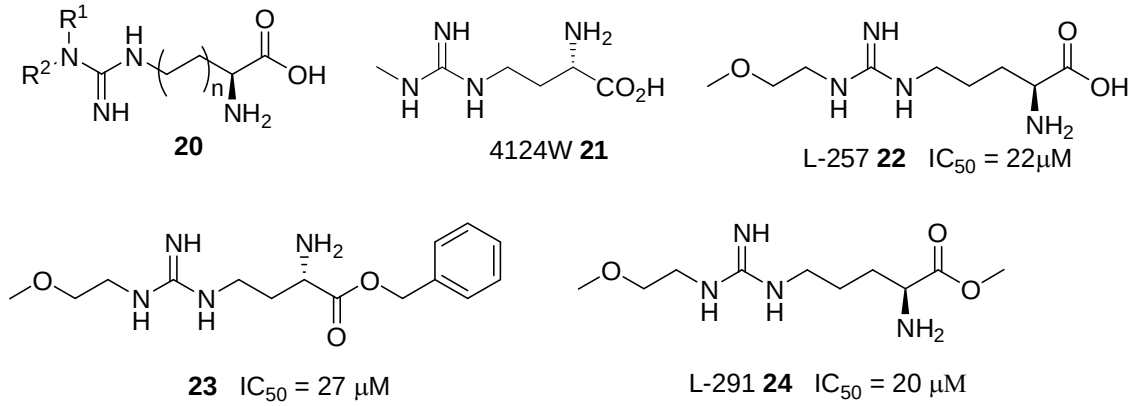


Figure 8: Substrate based DDAH inhibitors.

Vallance *et al.* recently disclosed the first crystal structure of *hu*DDAH1 complexed with citrulline and the DDAH inhibitor L-257 (Figure 9).[13] The residues in the catalytic triad in *hu*DDAH1 consist of Cys273, His172 and Asp126 which differ from the catalytic triad residues in *Pa*DDAH (Cys249, His162 and Glu114). Crystal structure **A** shows the electron density of L-257 in the active site of DDAH (DDAH carbon atoms in green) with a closely bound water molecule near the active site cysteine Cys273. This is needed for the hydrolysis of the thiouronium species (Scheme 2, **B**). Crystal structure **B** shows a comparison of L-257 (carbon atoms in cyan, oxygen atoms in red) and L-citrulline (in yellow) bound in the active site. Both molecules are anchored to the active site by H-bonding to Asp78. Binding of L-257 induces changes in the active site. Movement of His172 creates a pocket for the 2-methoxyethyl moiety of the L-257 to fit in, and increase its affinity for the active site, and hence impede the hydrolysis mechanism of DDAH. Whereas the carboxyl end of citrulline is bonded to Arg144 (Figure 5), L-257 induces a large change in the position of Arg144 away from the carboxyl end of the inhibitor, and creates a large pocket. This pocket can potentially be filled by a functional group at the acid end of L-257.

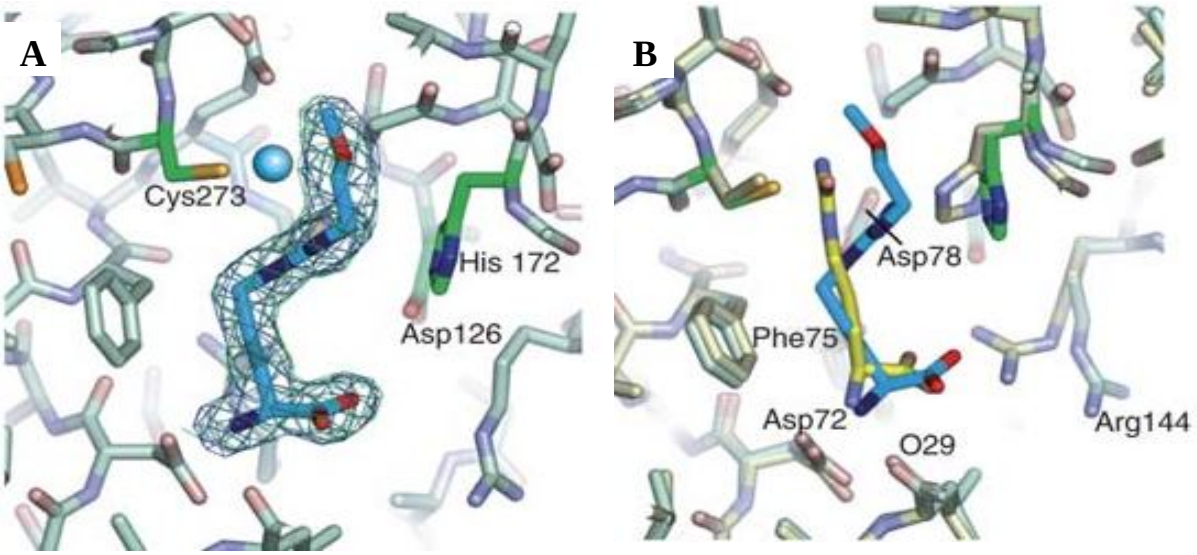


Figure 9: **A** Crystal structures of *hu*DDAH1 bound with inhibitor L-257; **B** Comparison of bound L-257 and citrulline.

A more recent investigation into the binding pocket of the guanidine moiety by screening substituted guanidines, amidines and isothioureas was carried out by Clement *et al.*[27] Replacing the guanidine moiety of ornithine with amidine as a bioisostere showed improvement in activity of inhibitors **25**. The alkenyl amidine **26** (Table 1, entry 1) showed 97% inhibition at 1 mM with an IC_{50} value of 13 µM and a K_i of 2 µM when assayed against *hu*DDAH1 and **26** is the most potent *hu*DDAH1 inhibitor to date. Compounds **27** and **28** (entry 2 and 3 respectively) also showed modest activity with 78% and 73% inhibition at 1 mM respectively. The authors suggest that having a double bond in the side chain increases potency as is evident when compound **26** (entry 1) is compared to **27** (entry 2). N^{ω}-Substituted L-arginines **29** and **30** (entries 4 and 5) were also investigated where groups with π-systems, alkenyl and alkynyl, were added but none of the synthesised compounds showed anymore potency than the amidines.

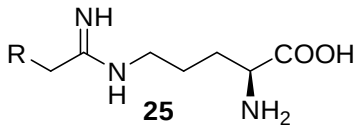
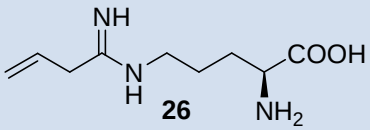
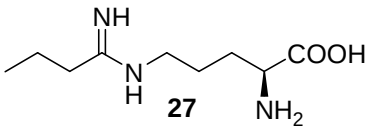
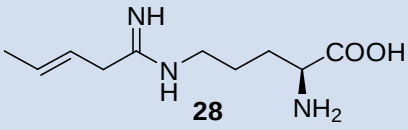
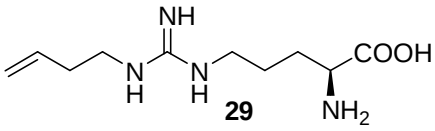
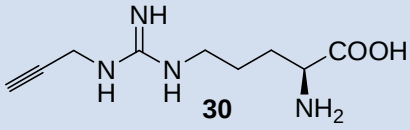
Entry	Compound	Inhibition at 1 mM (%)	IC_{50} (µM)	K_i (µM)
	 **25**			
1	 **26**	97 ± 3	13 ± 3	2 ± 1
2	 **27**	78 ± 7	70 ± 21	32 ± 6
3	 **28**	73 ± 8	79 ± 25	36 ± 3
4	 **29**	71 ± 3	189 ± 10	57 ± 9
5	 **30**	83 ± 7	55 ± 8	17 ± 5

Table 1: Alkenyl amidine inhibitors and guanidine inhibitors

Fast *et al.* recently surveyed a range of DDAH and NOS inhibitors as dual targeted NO inhibitors, as inhibiting both DDAH and NOS would lead to a decrease in levels of NO.[30] They found that the amidino group was a promising scaffold to build a dual-targeted inhibitor. Extending the alkyl chain on the amidines **31**, **32** and **33** (Figure 10) from the iminoethyl through to the iminopentyl side chains, increased the inhibition of *hu*DDAH1 (990, 52, 7.5 µM K_i values respectively) and decreases the inhibition for nNOS (1.7, 3.0, 20 µM K_i). Further extension to the iminohexyl side chain on compound **34** led to a decrease in inhibition (100 µM K_i), but an increase in selectivity for DDAH. Similar length compound L275 also showed selectivity for DDAH over NOS, and this suggests a smaller binding pocket at the active site of NOS compared to that of DDAH.

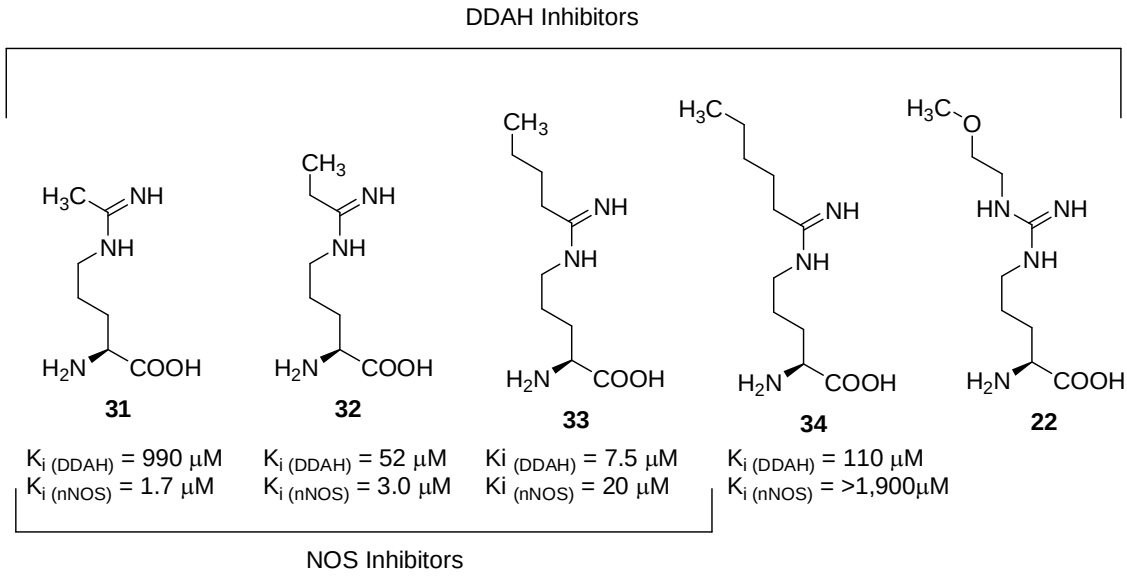


Figure 10: Amidine inhibitors.

Further studies on N^5-(1-iminopropyl)-L-ornithine (L-IPO) **32** revealed that this compound is a competitive nonhydrolyzable substrate analogue. A crystal structure of this compound in the active site *hu*DDAH1 shows it to adopt a similar conformation to that of L-257 in the active site, the only difference being continous electron density between the active site cysteine thiol and the C^{ζ} of L-IPO, suggesting a tetrahedral intermediate is formed (Figure 11). This covalent adduct is thought to be in rapid equilibrium with the parent compound and enzyme and hence, has not been detected *via* mass spectrometry.

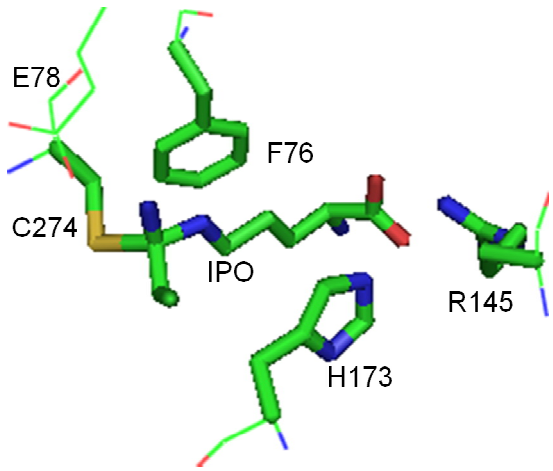


Figure 11: Binding of L-IPO at DDAH active site.

1.6 Synthesis of guanidines

Key to the synthesis of L-arginine derivatives is the synthesis of guanidines. The ability of the guanidinium moiety to form H-bond, charge-pair, and cation π-interactions makes it a vital amino acid involved in many proteins and their active sites. Many isolated natural products contain the guanidine motif and their synthesis and biological activities have been well investigated.[31] One such natural product is Batzelladine A **38** (Figure 12) which contains three guanidine moieties. Guanidine groups are also important in the pharmaceutical industry forming parts of various drug molecules, including those used for diabetes, cardiovascular drugs, antihistamines, anti-inflammatory agents, antibacterial and antifungal agents.[32] Metformin **35** is an anti-diabetic for type II diabetes, Amiloride **36** is a potassium-conserving drug used to manage hypertension, and Cimetidine **37** is a H_2-receptor antagonist which was previously used for the treatment of heartburn and peptic ulcers (Figure 12). Guanidine acts as a strong base, with a pKa_H of 12.5, hence many organic transformations are carried out with guanidines and substituted guanidines.[33] One example is 1,1,3,3-tetramethylguanidine (TMG) **39** which catalyses alkylation of carboxylic acids.[34]

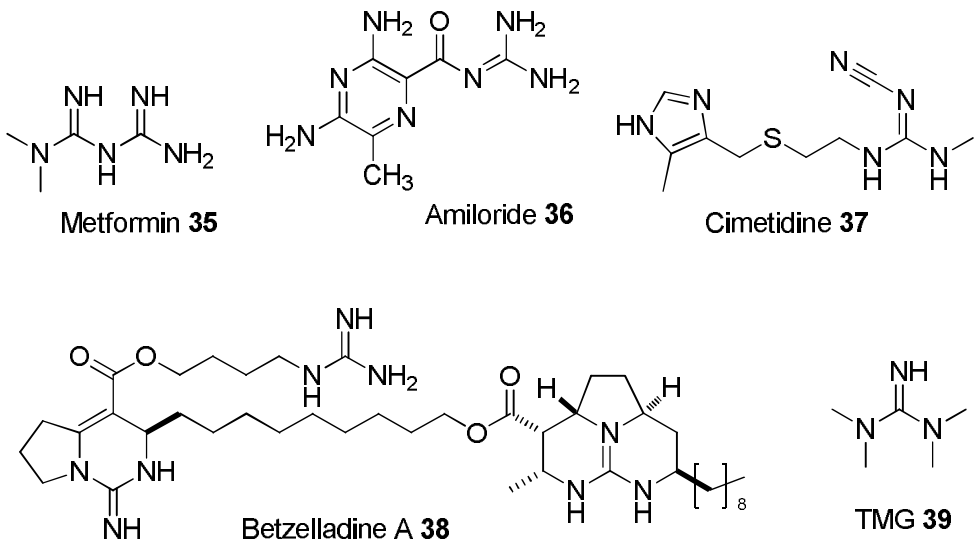


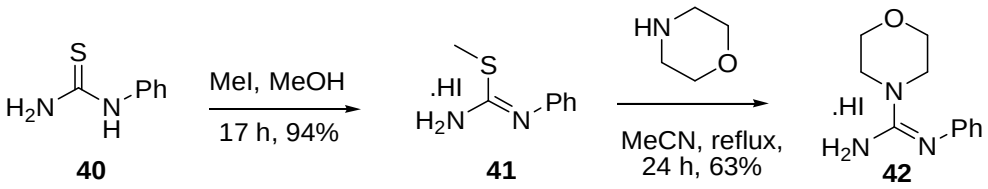
Figure 12: Drugs and natural products containing the guanidine moiety.

There are numerous ways to synthesise guanidines and not all the methods can be covered in the scope of this introduction, but these have been extensively reviewed in the literature.[31-33] Many of these methods have also been adapted to solid-phase synthesis.[32] The most common ways to synthesise guanidines include synthesis from thioureas, isothioureas, pyrazoles and isothiocyanates. Less common syntheses include the use of cyanamides and triflyl guanidines. All of these will be examined briefly in this introduction.

1.6.1 Guanidines from thioureas and isothioureas

Many of the examples in the literature for the synthesis of guanidines employ isothioureas, especially methylisothiourea, which are converted to guanidines using guanylating reagents. Some of the reagents used for this purpose include Mukaiyama's reagent, thiophiles such as $HgCl_2$, HgO, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and 1-fluoro-2,4-dinitrobenzene (Sanger's reagent).[32]

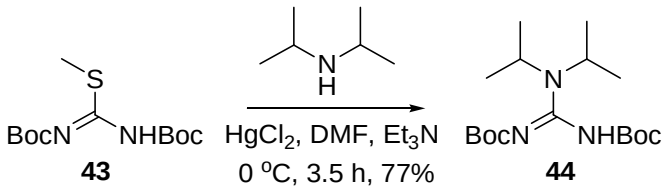
The substitution pattern of the resulting guanidine can be modified to afford tri- and tetra- substituted guanidines by starting with di-substituted guanidines and primary or secondary amines. Isothioureas are easy to prepare from thioureas, and subsequent reaction with an amine with concomitant loss of a thiol affords guanidines. For example, treatment of thiourea **40** with methyl iodide in methanol yields the methylisothiouronium salt **41,** which when treated with morpholine, forms the di-substituted guanidine **42** (Scheme 4).[35]



Scheme 4: Synthesis of a 1,2-disubstituted guanidine using an isothiourea.

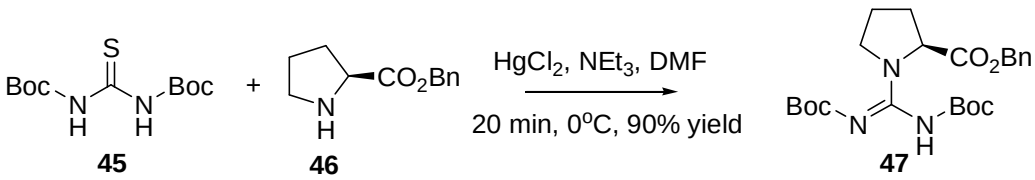
Long reaction times were observed for the formation of the isothiouronium salt **41** as well as for the formation of the guanidine **42**. The guanidine forming reaction can be accelerated by using $HgCl_2$, as shown by Guo *et al.* Treatment of commercially available *N,N'*-bis-Boc-*S*-methylisothiourea **43** with $HgCl_2$ and amines lead to the formation of bis-Boc-guanidines (up to 3.5 h, Scheme 5).[36] Sterically hindered amines

such as disopropyl amine work well affording **44** in 77% yield in 3.5 h, as well as aromatic amines such as aniline. One of the disadvantages to using methylisothioureas in guanidine synthesis is the formation of the malodorous by-product methanethiol.



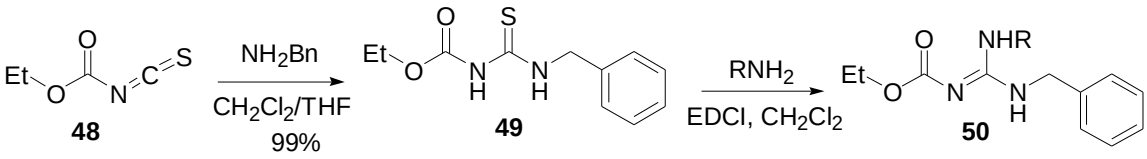
Scheme 5: Synthesis of a guanidine from an isothiourea.

The addition of $HgCl_2$ to thioureas aids desulfurization leading to the formation of an intermediate carbodiimide. This is then attacked by amine nucleophiles to form a guanidine. For example, treatment of bis-Boc protected thiourea **45**, $HgCl_2$, Et_3N and proline **46** as the amine nucleophile in DMF leads to the quick formation of the bis-Boc-protected guanidine **47** (Scheme 6).[37] This is a fast reaction and other amine nucleophiles diisopropylamine and *o*-chloroaniline also reacted in 0.5 h and 2 h respectively, to give good yields of their corresponding guanidines. Hence, it can be seen that the rate of guanidine formation is enhanced by using $HgCl_2$. However, purification of the guanidine from the mercury waste presents a drawback in using this reagent.



Scheme 6: Synthesis of a guanidine from a thiourea.

Isothiocyanates are often used to form thioureas which are subsequently converted to guanidines. Hamilton *et al.* describe the reaction of carbamoyl isothiocyanate **48** with benzylamine to form thiourea **49**.[38] This is subsequently converted to a guanidine *via* treatment of an amine and EDCI as the coupling agent. For example, reaction of thiourea **49** with the hindered *tert*-butyl amine furnished the guanidine **50** in near quantitative yield (Scheme 7). This protocol was used to synthesise 1,3-disubstituted guanidines using a range of amines with varying reactivities in high yields by condensation with the thiourea **49** (Table 2).



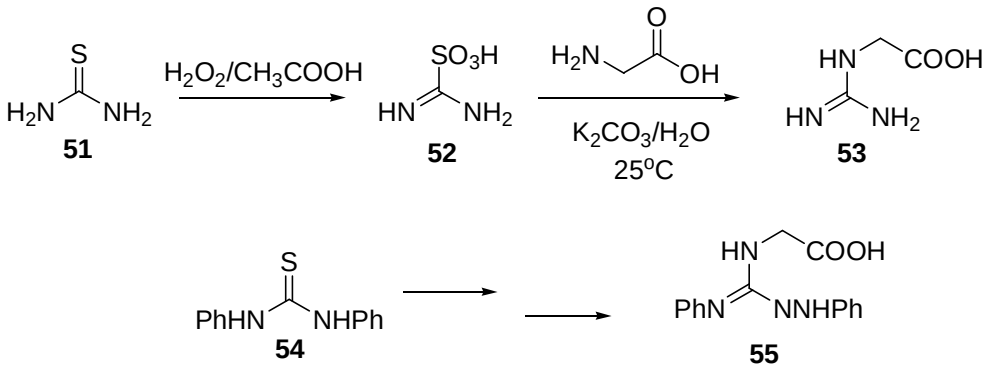
Scheme 7: Synthesis of a guanidine from an isothiocyanate**.**

Entry	Amine (RNH_2)	Guanidine yield (%)
1	*t*-butyl amine	99
2	Morpholine	95
3	Diisoproplyamine	76
4	4-methoxyaniline	82

Table 2: Amines used in synthesis of guanidines from an isothiocyanate.

The carbamoyl moiety acts as a protecting group for the isothiocyanate and offers the advantages of increasing its reactivity, and makes the purification of the polar guanidine compounds easier.

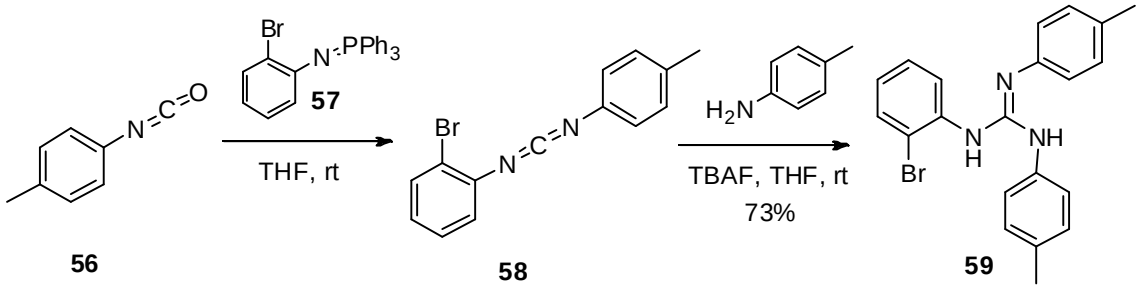
Thioureas can also be oxidised to sulfonic acids to convert the sulfur into an enhanced leaving group, and hence promote nucleophilic displacement by an amine to form a guanidine. The treatment of thiourea **51** with hydrogen peroxide and acetic acid by Miller *et al*. lead to the oxidation of thiourea to sulfonic acid **52**. This can be easily displaced by glycine to form guanidino acid **53** (Scheme 8).[39] Reaction of **52** with amino acids DL-methionine, L-phenylalanine, DL-serine and DL-valine gave lower yields (60, 45, 50 and 55% respectively). An advantage of this method is the easy isolation and purification, whereby the products precipitated out and could be purified by recrystallization. However, reaction of sulfonic acid **52** with DL-alanine, L-isoleucine and L-leucine led to products which were soluble in the reaction mixture and therefore difficult to isolate. 1,2-Disubstituted and 1,2,3-trisubstituted guanidino acids can also be formed *via* functionalisation of the starting thiourea. Miller *et al.* oxidised diphenylthiourea **54** to the sulfonic acid and condensed it with glycine to form a 1,2,3-trisubstituted guanidino acid **55** but with a lower yield of 35% (Scheme 8). It can be concluded that substituted guanidines are formed in low yields using this protocol.



Scheme 8: Synthesis of a guanidines from sulfonic acids.

1.6.2 Guanidines from isocyanates

Isocyanates are also used in guanidine synthesis and can be readily converted to their corresponding carbodiimides. For example, Molina *et al.* have reacted the aromatic isocyanate **56** with *N*-aryliminophosphorane **57** to afford carbodiimide **58**.[40] This was treated with an aromatic amine in the presence of tetrabutylammonium fluoride (TBAF), to form the desired guanidine **59** (Scheme 9). This method is highly efficient in synthesising 1,2,3-trisubstituted guanidines in good yields.

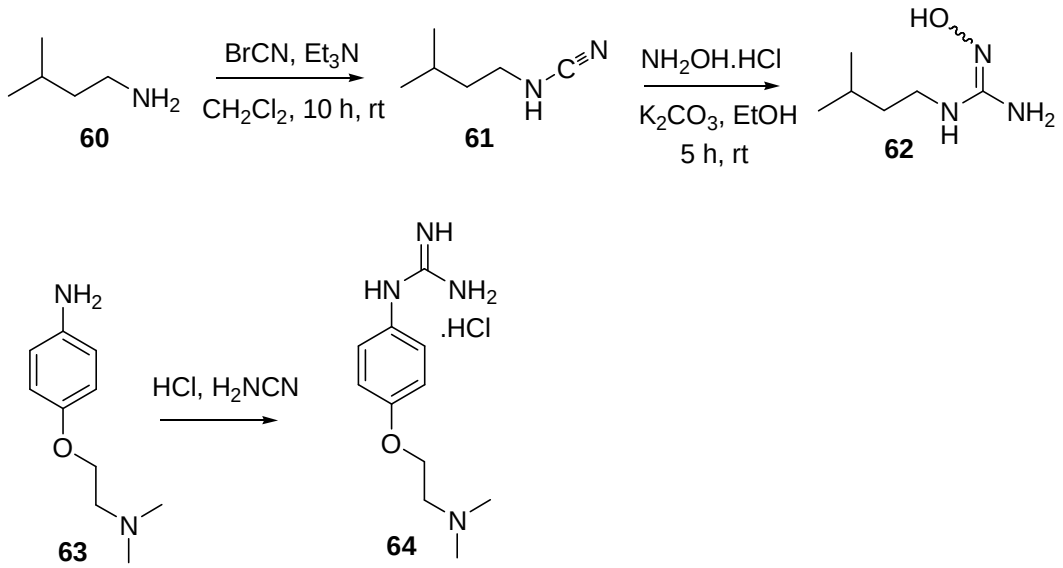


Scheme 9: Synthesis of a guanidine from an isocyanate.

1.6.3 Guanidines from cyanamides

Cyanamides are widely used in the synthesis of guanidines. They can be formed from amine condensation on cyanogen bromide, where further treatment with a nitrogen nucleophile forms a guanidine. For example, Wang *et al.* treated the primary amine **60** with cyanogen bromide to form the cyanamide **61**, which on further treatment with hydroxylamine hydrochloride furnished the hydroxyguanidine **62** (Scheme 10).[41] The authors also report producing cyanamide from a range of alkyl and aryl amines. Mono- and 1,2-disubstituted guanidines are reported to have been made using this method, but

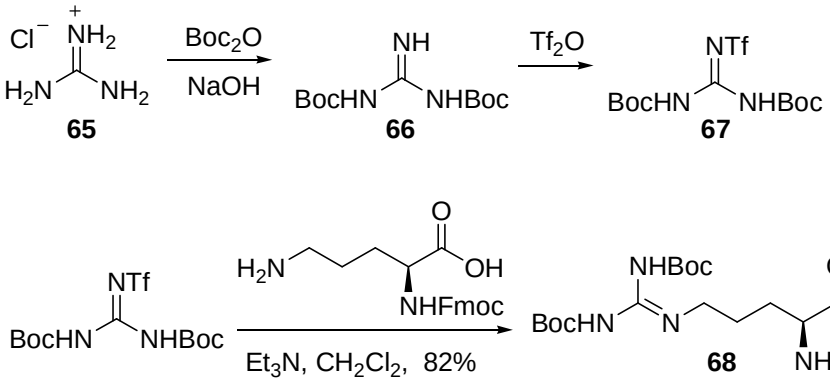
this is the limit of the substitution that can be achieved. Another useful cyanamide employed in the synthesis of monosubstituted guanidines is hydrogen cyanamide. Reaction of an amine with hydrogen cyanamide leads to a monosubstituted terminal guanidine. For example, Paul *et al.* converted amine **63** into guanidine **64** using hydrochloric acid and hydrogen cyanamide (Scheme 10).[42]



Scheme 10: Synthesis of guanidines from cyanamides.

1.6.4 Guanidines from triflyl guanidines

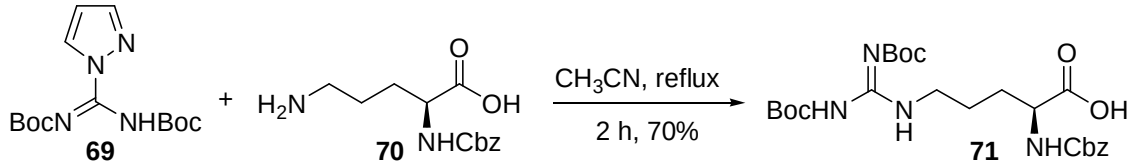
Recently the use of triflyl guanidines for the synthesis of monosubstituted guanidines has been reported by Goodman *et al.*[43] These can be prepared from guanidine hydrochloride **65** *via* reaction with Boc-anhydride to yield the Boc-protected guanidine **66**, or CBzCl to form the CBz protected analogue (Scheme 11). Further treatment with triflic anhydride converts the diprotected guanidine into the triflyl guanidine **67**. The triflyl amine is a very good leaving group and can be displaced with amine nucleophiles. For example, Fmoc-protected L-ornithine can be condensed with triflyl guanidine **67** to furnish Boc-protected arginine **68** in a high yield.



Scheme 11: Synthesis of triflyl guanidine and its use in guanidine formation.

1.6.5 Guanidines from Pyrazoles

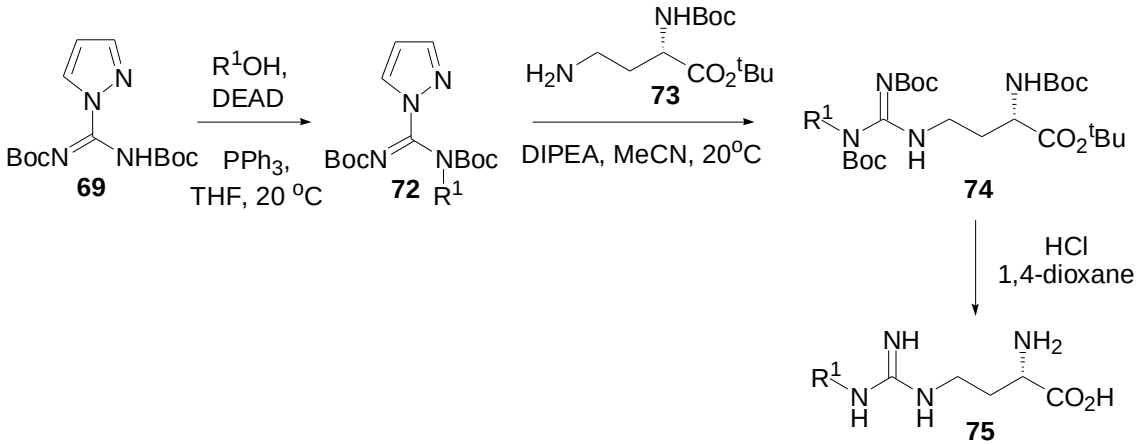
N,*N*'-bis-*tert*-butoxycarbonylpyrazole-1-carboxamidine **69** (Scheme 12) can be reacted with a variety of amines and amino acids to form protected guanidines.[44] For example, Drake *et al*. treated CBz- protected ornithine **70** with pyrazole **69** to form the Boc-protected guanidine **71** in 70% yield. Employment of simple amines butylamine, *tert*-butylamine and benzylamine in this reaction yielded the corresponding guanidines in yields in excess of 90% yields in 1-3 h. Reaction with secondary amine proline took 16 h at room temperature and yielded its corresponding guanidino acid in 73%. Hence, both primary and secondary amines yield mono-substituted guanidines in good yields. Disubstituted guanidines can also be produced by modification of the pyrazole **69** *via* the Mitsunobu reaction, whereby alkylating at the disubstituted nitrogen of **69** and reaction with an amine leads to the formation of 1,2-disubstituted guanidines (see section 1.7).



Scheme 12: Synthesis of a guanidino acid from a pyrazole.

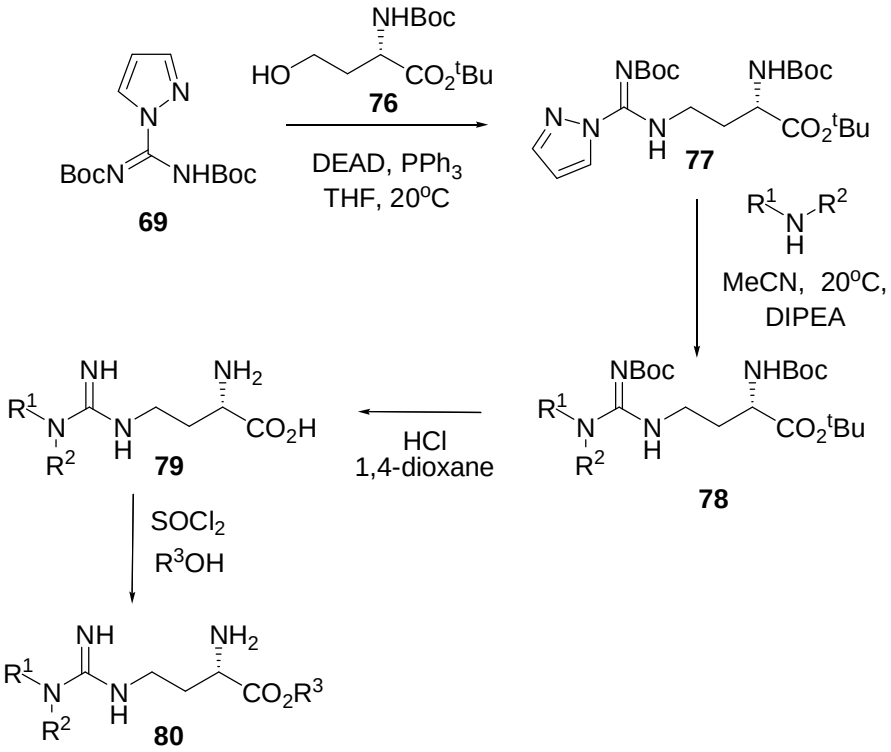
1.7 Previous syntheses of arginine based DDAH inhibitors

Rossiter *et al.* used pyrazole **69** to synthesise disubstituted guanidines in the synthesis of analogues of 4124W in the search for more potent inhibitors of DDAH.[29] The alkylation of pyrazole **69** was achieved *via* Mitsunobu reaction with an alcohol. The carboxamidine **72** was then reacted with the amine **73** to displace the pyrazole and yield the protected guanidine amino acid **74**. This could be deprotected in 4 M HCl in dioxane to give the monosubstituted guanidine **75** where R^1 = H, and 1,2-disubstituted guanidine where R^1 = alkyl group (Scheme 13).



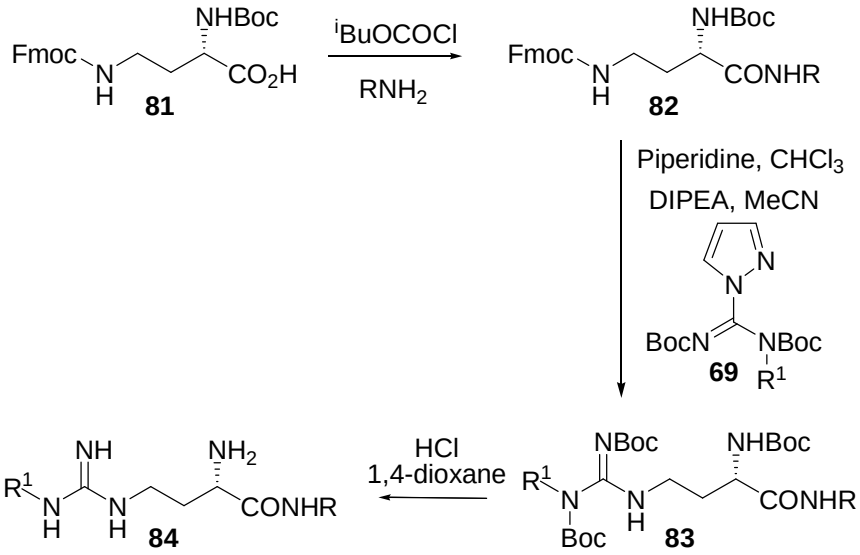
Scheme 13: Synthetic route to analogues of 4124W.

1,2,2-Trisubstituted guanidines were also synthesised *via* the following synthetic route (Scheme 14): A Mitsonobu reaction was performed, to attach the amino acid **76** onto pyrazole **69**. Displacement of the pyrazole group on **77** using a secondary amine, led to the formation of guanidine **78**. The Boc groups were removed with HCl to yield the 1,2,2-trisubstituted guanidino acid **79**. Rossiter *et al.* also made some ester analogues of these compounds by reacting them with thionyl chloride and an alcohol to form esters **80**.[29]



Scheme 14: Synthetic route to ester analogues.

Amide analogues were also synthesised (Scheme 15); the amide groups were formed on the carboxylic acid of amino acid **81** to form the amides **82**. The Fmoc groups were removed using piperidine, and the amino group thus revealed was used to displace the pyrazole from compound **69** to yield the protected amides **83**. These were then deprotected to furnish 1,2-disubstituted guanidine amide analogues **84** of arginine.

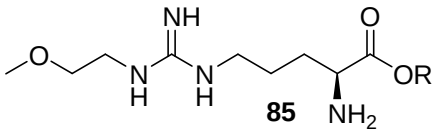


Scheme 15: Synthetic route to amide analogues.

Chapter 2: Results and discussion

2.1 Aims

Analogues of **85** have been synthesised by Rossiter *et al.* where R = H, and OMe (Figure 13).[29] The aim of this project is to synthesise further ester analogues of **22** where R = allyl and substituted aromatic groups.



R = H L-257 **22**
R = OMe L-291 **24**

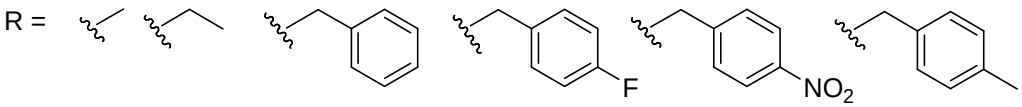
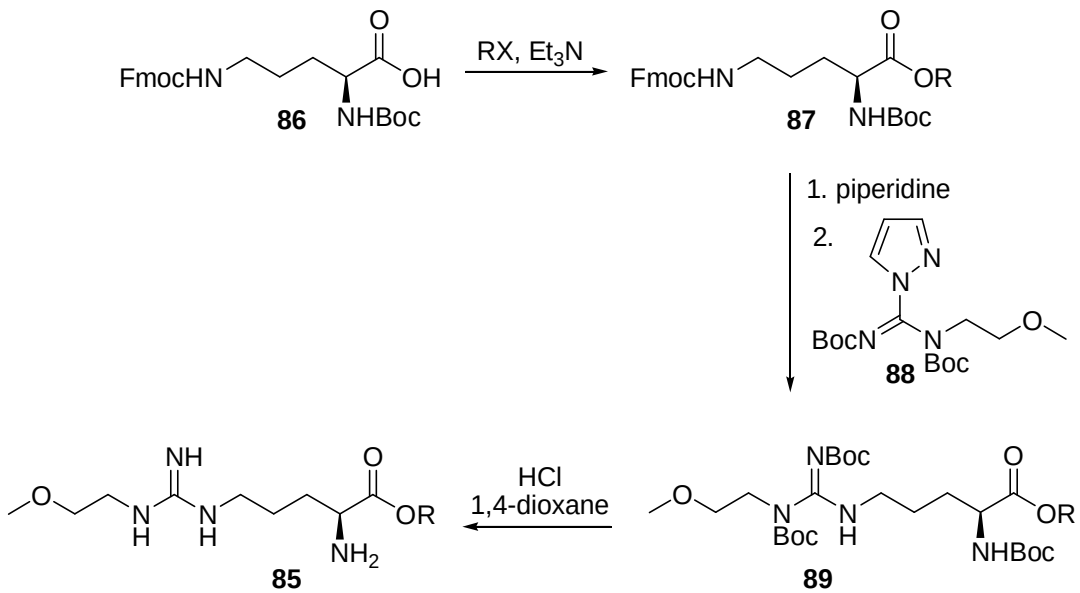


Figure 13: Ester analogues.

A route similar to that taken by Rossiter *et al*. will be adopted (Scheme 16). Ester analogues will be synthesised on amino acid **86**. Fmoc-deprotection of **87** and reaction with pyrazole **88** to give Boc-protected guanidines **89** can be deprotected using HCl to give guanidines **85**.



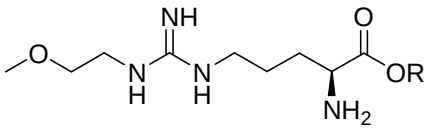
Scheme 16: Proposed synthetic strategy.

This synthetic strategy will also be tested on the chain shortened analogue **75**. Substrate based design will further be investigated by attaching amide groups on the carboxyl end, using the strategy empolyed by Rossiter *et al.* for amide analogues (Scheme 15).[29]

Drug design will also be carried out using the crystal structure of DDAH to design possible inhibitors, and it is foreseen that drug design based on the crystal structure of *hu*DDAH1 with the inhibitor L-257 in the active site (Figure 9) will give rise to conformational structures that could be synthetically mimicked. This approach would potentially give DDAH1 specific inhibitors which could be useful as DDAH1 is roughly colocalised with nNOS (Section 1.3). Crystal structures of DDAH2 have not been elucidated to date and biological work on the extraction and purification of the DDAH2 enzyme will be attempted. If successful then the DDAH2 enzyme will be subjected to crystal structure studies and drug design specific to DDAH2.

2.2 Synthesis of ester analogues of L-257

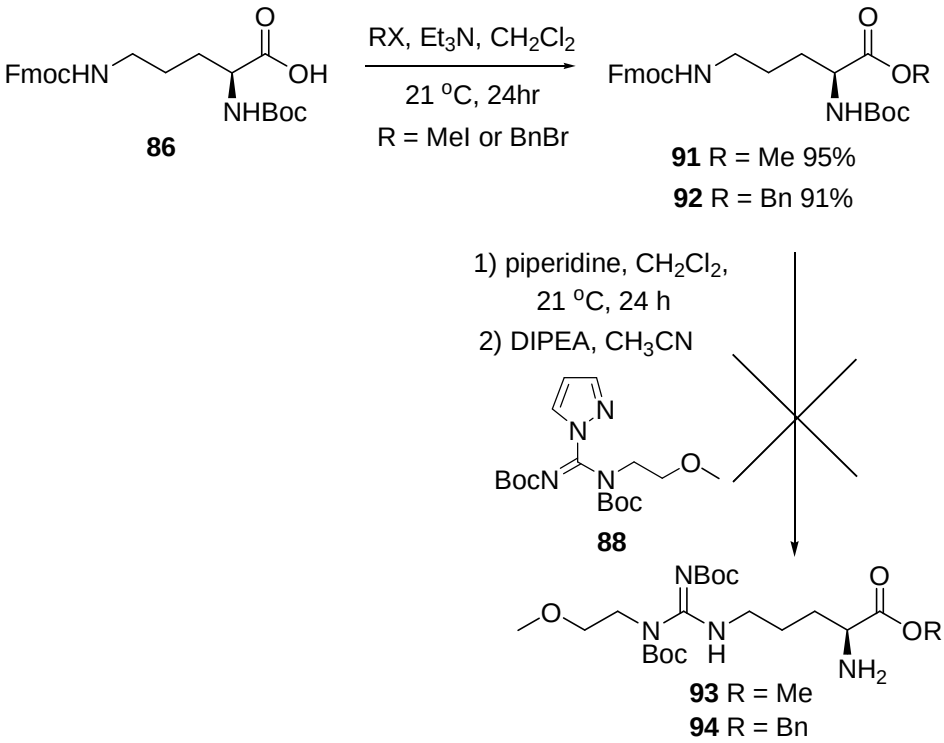
The initial aim of the project was to synthesise esters of L-257 **22**, namely the methyl and benzyl ester, and hence validate the synthetic route for the synthesis of further ester analogues (Figure 14).



22 R = H L-257
24 R = Me L-291
90 R = OBn

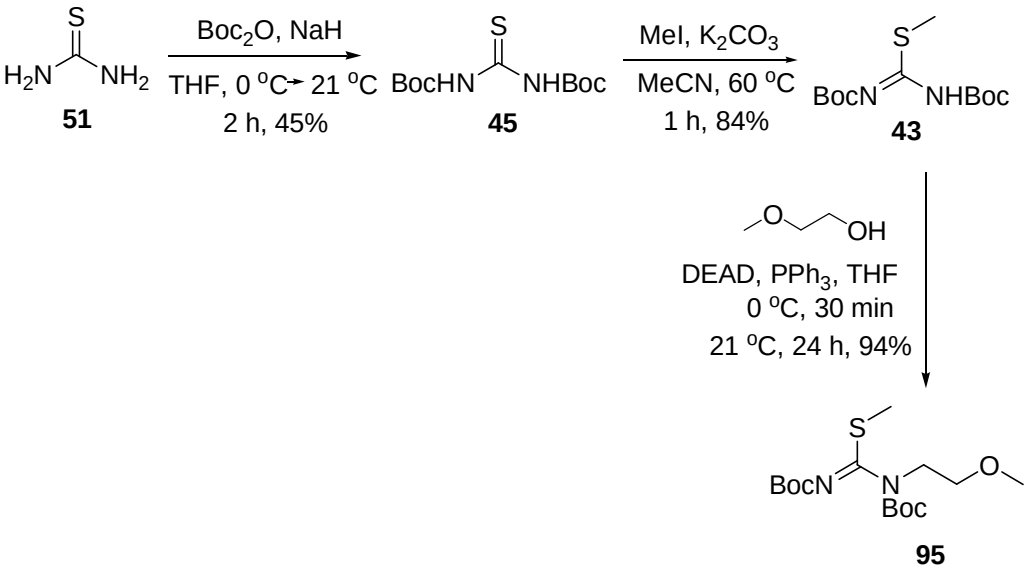
Figure 14: Ester analogues of L-257.

Initial efforts were concentrated on synthesising the methyl ester **24** (L-291) and the benzyl ester **90** starting from Boc-Orn(Fmoc)-OH **86** (Scheme 17). The methyl ester **91** and benzyl ester **92** were synthesised in 95% and 91% yields respectively. The Fmoc groups were then cleaved using piperidine, and subsequent treatment with the *N,N'*-bis-Bocpyrazole-1-carboxamidine **88** failed to form the products **93** and **94**.[45,29]



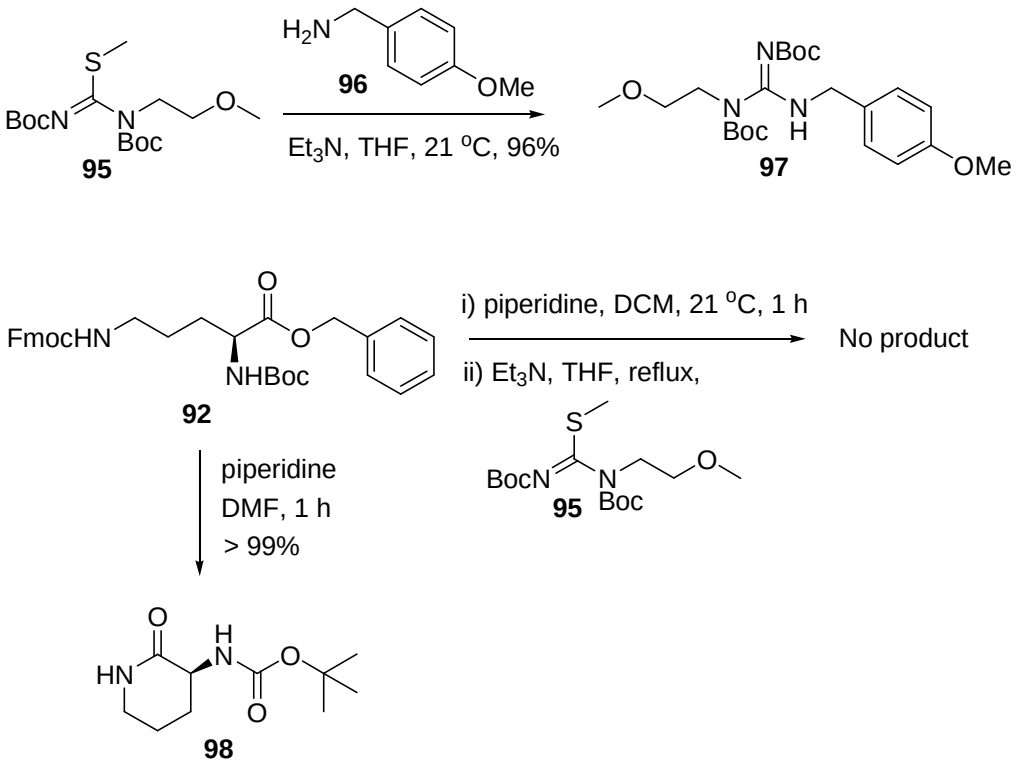
Scheme 17: Attempted synthesis of esters **93** and **94**.

The failure of the guanidine forming reaction, coupled with the expense of the pyrazole **88**, led us to consider the use of a cheaper alternative guanylating agent. The Bocthiourea **45** was readily synthesised starting from thiourea **51** (Scheme 18). Conversion of the Bocthiourea **45** to the Bocmethylisothiourea **43** was readily achieved with methyl iodide in 84% yield, and subsequent treatment of the urea with 2-methoxyethanol, under Mitsunobu reaction conditions, furnished the methylisothiourea **95** in 94% yield.[46,47,48,49] This guanylating agent served as a cheaper alternative to the pyrazole **88**. It was necessary to attach the 2-methoxyethyl group on the methylisothiourea **43**, for selective *N*-alkylation as treatment of thiourea with base and 1-bromo-2-methoxyethane leads to *S*-alkylation despite literature precedence.



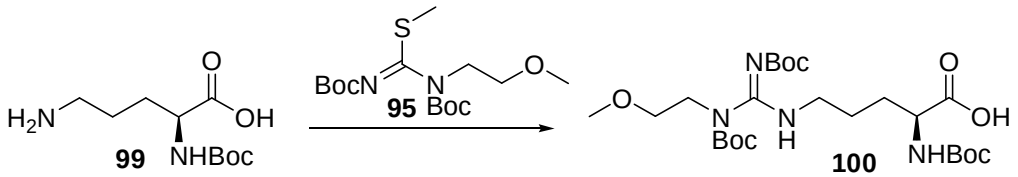
Scheme 18: Synthesis of guanylating agent **95**.

It was previously established that formation of guanidine **97** using the methylisothiourea **95**, with *para*-methoxybenzylamine **96** could be easily achieved in 96% yield (Scheme 19).[45] Deprotection of the Fmoc group of ester **92**, and treatment of the subsequent crude reaction mixture, under the same reaction conditions under reflux, led to a complex reaction mixture with no isolable products. At this point it became clear that Fmoc-deprotection from ester **92** would liberate an amine, which could easily undergo cyclisation, to form a six membered cyclic lactam. This was indeed observed when reaction of the ester with piperidine in DMF for 1 h led to the formation of the lactam **98**, isolated in a quantitative yield.



Scheme 19: Guanidine formation using methylisothiourea **95**.

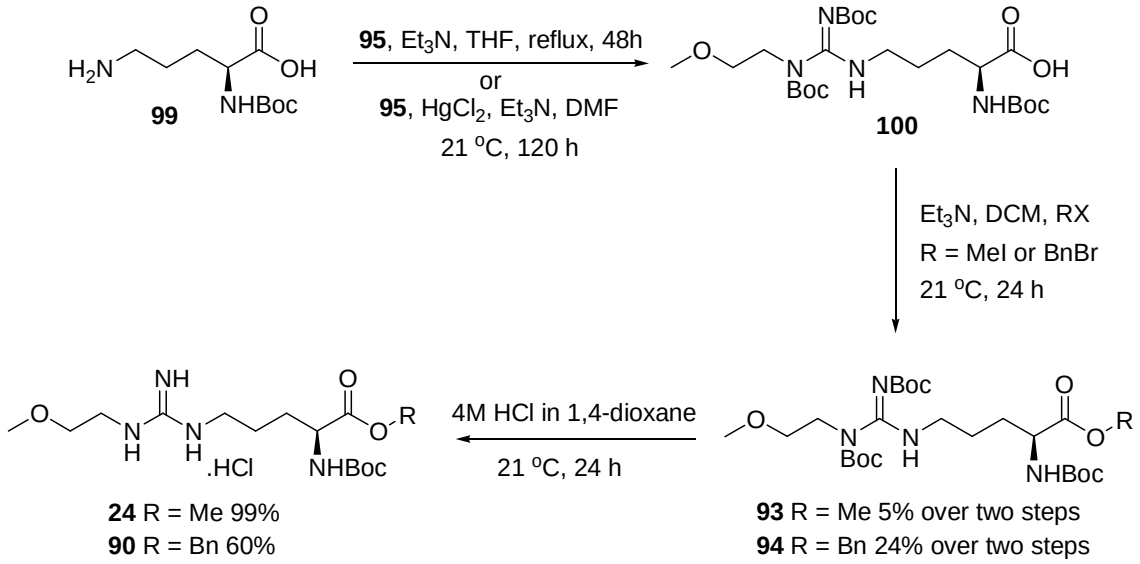
The design of the synthetic approach to ester analogues of L-257 was flawed. To circumvent the cyclisation problem, the ester moiety would have to be installed on the acid **99** (Scheme 20) after guanidine formation, by coupling Boc-Orn-OH with methylisothiourea **95**. The subsequent acid **100** could also be used to make a range of esters and amides.



Scheme 20: Synthesis of acid **100**.

Two different conditions were initially employed for the coupling of Boc-Orn-OH to methylisothiourea **95**; 1. THF, Et_3N, reflux and, 2. $HgCl_2$, Et_3N, DMF (Scheme 21). Neither reaction went to completion which made it extremely difficult to purify the product acid **100** from Boc-Orn-OH, as they were both very polar products, and had similar retention factors on silica gel. Nevertheless, semi purification of the reaction mixture generated in THF, *via* flash chromatography, gave a mixture of the two polar compounds which were treated with MeI and Et_3N to furnish the methyl ester **93** in 5%

yield over the two steps. Similarly, the semi purified acid **100** from reaction conditions (2), and further treatment with benzyl bromide and Et_3N, gave the benzyl ester **94** in 24% yield over two steps. The extremely low yields achieved over two steps for esters **93** and **94** were thought to have occurred due to the incomplete reaction of Boc-Orn-OH with methylisothiourea **95** to form acid **100**, as opposed to the ester formation step. Esters **93** and **94** were then deprotected in 4 M HCl to yield the hydrochloride salts of the arginine esters, **24** and **90**, in 99 and 60% yields respectively.

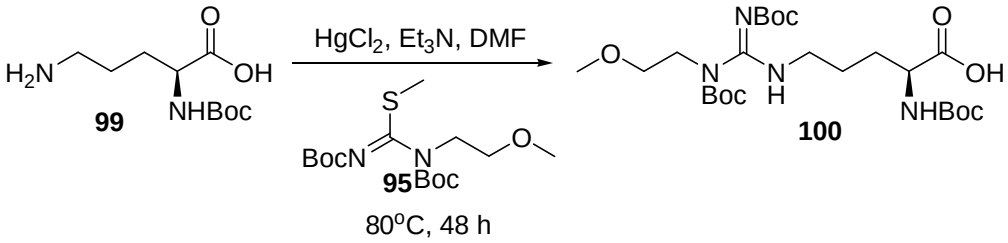


Scheme 21: Synthesis of methyl and benzyl ester analogues of L-257.

2.3 Optimisation of guanidine formation

Boc-protected guanidine **100** formation with **95** using $HgCl_2$ was carried out at 80 °C for 48 h, and monitoring by tlc showed the reaction did not reach completion. However, due to biological systems being sensitive to any mercuric residue left after purification, optimisation of this reaction without the use of mercury was pursued. Several reaction conditions were employed to try and optimise guanidine formation (Table 3). Coupling agent EDCI was employed using conditions used by Hamilton *et al.*, but the reaction failed to proceed (Table 3, entry 1).[38] Guanidine formation was also attempted using water as a polar solvent in the first instance, for two days at 21 °C, and thence at 80 °C for two days. Unfortunately, in both cases no reaction was observed with only methylisothiourea **95** recovered in 90% (Table 3, entry 2). Reactions in DMF with Et_3N led to an incomplete reaction, but with DBU led to decomposition of **95** with no product

formation. Further reactions in the microwave, also led to incomplete reactions with 90% starting material recovered each time (Table 3, entries 5-7).



Entry	1 (eq.)	Boc-Orn-OH (eq.)	Reaction conditions	Observation
1	1	1.5	DIPEA (2eq), EDCI (3eq), DMF, RT, 5days [50]	No reaction
2	1	1.5	Et_3N, H_2O, RT, 2 days, 80°C, 2 days	~ 90% **95** recovered
3	1	2	Et_3N, DMF, 80 °C, 6 days	77% starting material recovered
4	1	2	DBU (2 eq), DMF, 80°C, 48 h,	Complete decomposition of **95**
5	1	2	NMP, MW, 90°C, 100°C, 110°C, (30min at each temp.)	~ 90% starting material **95** recovered
6	1	2	NMP, MW, Et_3N (2 eq), 100°C, 110°C, (30min at each temp.)	~ 90% starting material **95** recovered
7	1	2	DMF, MW, Et_3N (2 eq), 90°C, 100°C, 110°C, (30 min at each temp.)	~ 90% starting material **95** recovered

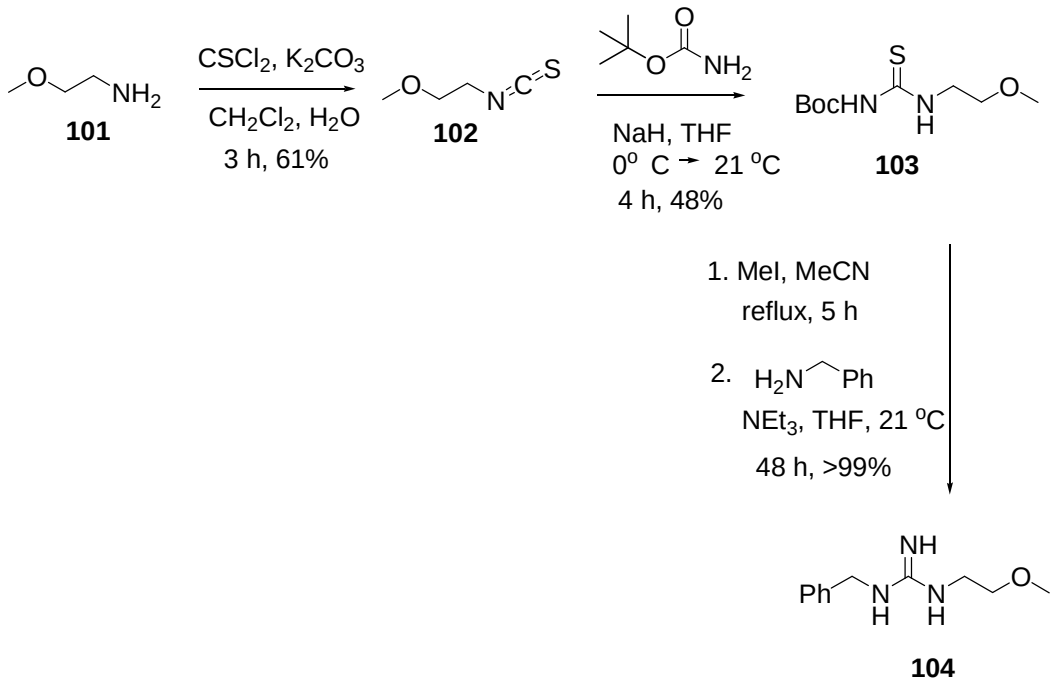
Table 3: Optimisation conditions for guanidine formation.

A number of reasons may be attributed to the prevention of guanidine formation. The Boc groups on methylisothiourea **95** may have been sterically hindering the carbon at which the amine was supposed to attack; the free acid was perhaps hindering the reaction; or the methanethiol was not a good enough leaving group. The latter seemed unlikely as the reaction worked well with amine **96** (Scheme 19).

2.4 Synthesis of mono-Boc guanylating agent 103

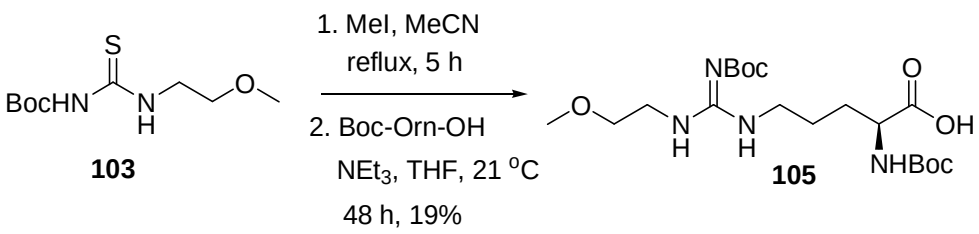
In order to establish whether the two large Boc groups were sterically hindering the reaction, mono-Boc analogue **103** was synthesised to see if this improved the guanidine formation. A different synthetic route had to be used for the synthesis of the mono-Boc protected substrate **103** (Scheme 22). The 2-methoxy ethyl group was attached *via* treatment of 2-methoxyethyl amine **101** with thiophosgene, which furnished the

isothiocyanate **102** in 61% yield. This was then treated with sodium hydride and *t*-butyl carbamate, to yield the mono-Boc protected thiourea **103** in 48% yield.[51] Reaction with methyliodide to give the methylisothiouronium salt, and subsequent treatment with benzylamine, led to the formation of the guanidine **104** in quantitative yield. Hence, the mono-Boc protected thiourea **103** seemed to be a promising substrate for the formation of a guanidine, and it was thought that this reduction in steric bulk, around the carbon atom to be attacked, might aid nucleophilic attack by the amine of Boc-Orn-OH.



Scheme 22: Synthesis of guanidine using mono-Boc thiourea **103**.

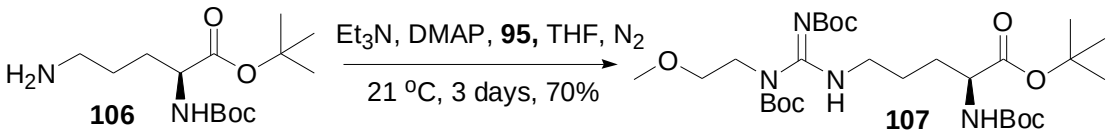
However, treatment of the thiourea **103** with methyliodide, and then Boc-Orn-OH, led to the formation of the desired guanidine but with a low yield, 19% (Scheme 23). Purification of the product guanidine **105** was also difficult, as the product was extremely polar. The yield of this synthetic route was deemed insufficient for the synthesis of a larger library of arginine mimetics.



Scheme 23: Synthesis of guanidine **105**.

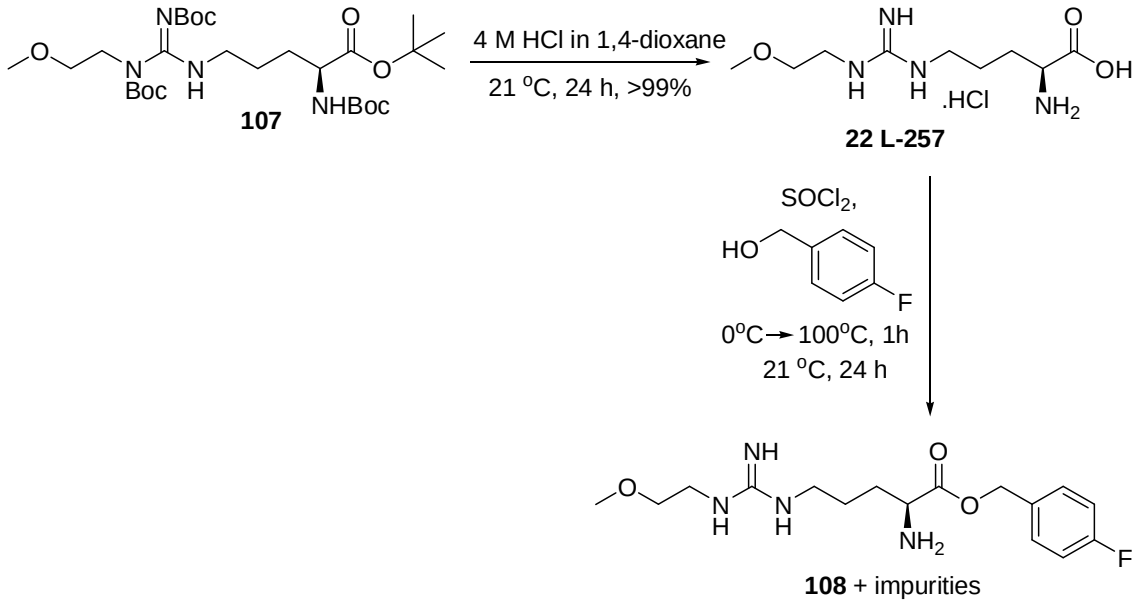
2.5 Guanidine formation using Boc-Orn-O-^{t}Bu

An ester stable to cyclization is the *t*-butyl ester formed on the carboxyl end of Boc-Orn-OH. The *t*-butyl ester **106** is commercially available and was employed in the guanylating reaction with the methylisothiourea **95**, which pleasingly underwent smooth conversion to the guanidine **107** in 70% yield after three days (Scheme 24). Moreover, the isolation of this compound was readily achieved by flash chromatography. This suggested that protecting the acid improved the guanidine forming reaction and hence, that the free acid in Boc-Orn-OH was hindering product formation.



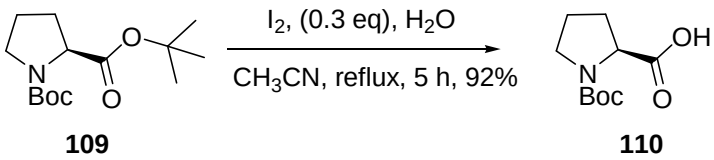
Scheme 24: Protecting groups for the acid of Boc-Orn-OH.

Following the ester formation example by Rossiter *et al.* (Section 1.7, Scheme 14) the *t*-butyl ester **107** was deprotected along with all the Boc groups, using 4 M HCl in dioxane to reveal arginine analogue **22** (Scheme 25).[29] On treatment of this compound with thionyl chloride and with 4-fluorobenzyl alcohol as the solvent, the reaction seemed to yield the desired product **108** but impurities in the compound, as well as the high boiling point of 4-fluorobenzyl alcohol, meant that the 4-fluorobenzyl alcohol could not be evaporated *in vacuo* as reported by Rossiter *et al.* Numerous attempts and failure at purification of this compound led us to conclude this esterification procedure was unsuitable to be carried out on arginine analogue **22**. Hence, synthesis of ester analogues required a different approach with easier purification of end products.



Scheme 25: Synthesis of impure fluorobenzyl analogue **108.**

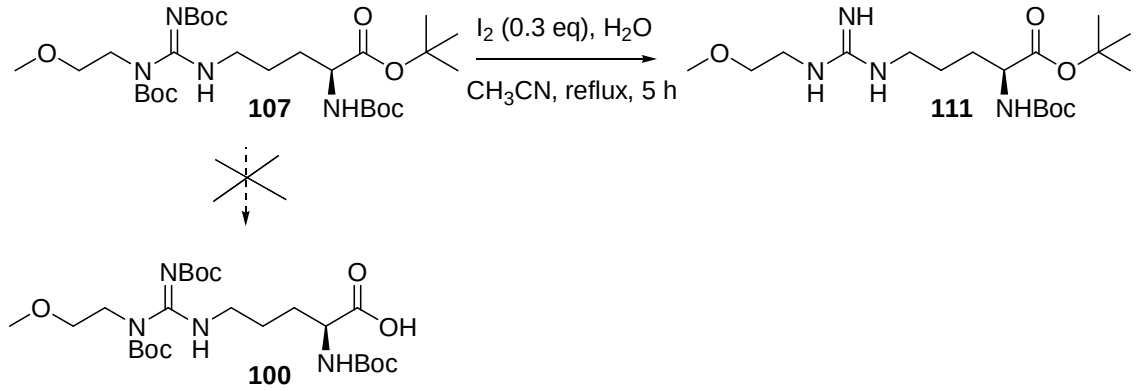
Since, the synthesis of the *t*-butyl ester **107** proceeded with a relatively high yield, it was perceived that selective *t*-butyl ester deprotection in the presence of the carbamate groups, could lead to the desired guanidino acid **100** (Table 3). Yadav *et al.* describe a mild and efficient cleavage of *t*-butyl esters in the presence of Boc groups using molecular iodine in water.[52] For example, the *t*-butyl ester **109** was cleaved to the acid **110** using 30 mol% molecular iodine and water, 92% yield (Scheme 26).



Scheme 26: Selective cleavage of *t*-butyl ester in presence of carbamate.

The *t*-butyl ester **107** was subjected to the same conditions with 30 mol% iodine in water and acetonitrile under reflux for 5 h (Scheme 27). This yielded a small amount of a compound containing signals consistent with two *t*-butyl groups in the ^{1}H NMR spectrum. However, infra red analysis failed to show an acid peak, hence, these conditions failed to cleave the *t*-butyl ester and gain access to the acid **100**, suggesting instead that compound **111** was formed. Analysis by mass spectrometry also supported this as it showed a molecular ion at 389, which suggests that two Boc groups were cleaved leaving one Boc group and the *t*-butyl ester intact. This suggests that the Boc groups on the guanidine moiety are extremely labile in these mildly acidic conditions,

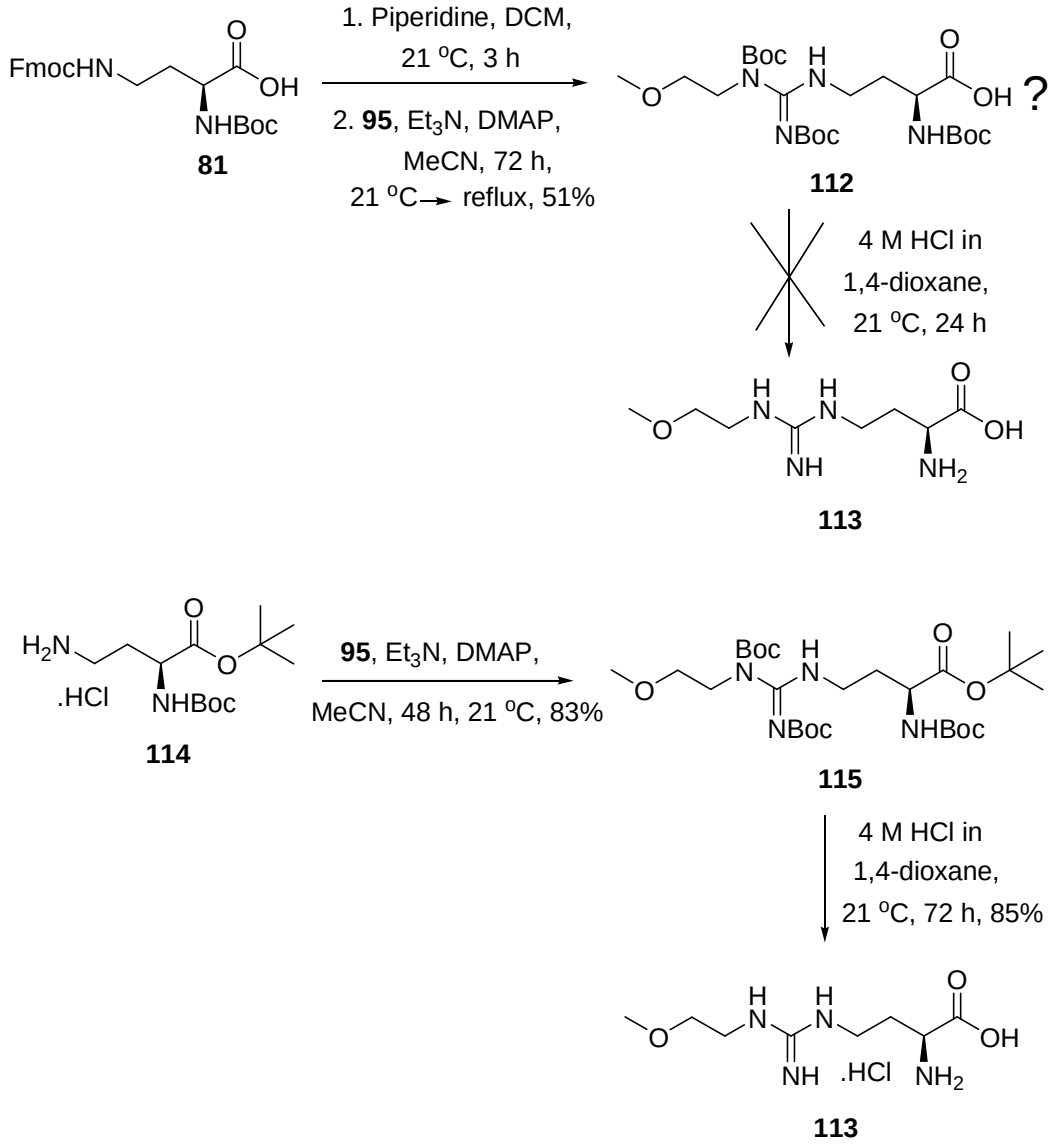
where a small amount of hydroiodic acid is thought to be produced. Deprotection of these Boc groups also lead to compounds with such high polarity, it makes purification extremely difficult.



Scheme 247: Selective cleavage of *t*-butyl ester.

2.5.1 Synthesis of the chain shortened analogue of L-257

Synthesis of the chain shortened analogue of L-257, **22**, was carried out by treating the methylisothiourea **95** with the deprotected Boc-Dab(Fmoc)-OH **81,** which was thought to yield the guanidine **112** in 51% yield (Scheme 28). However, the NMR spectrum of this compound was not easy to interpret because of the presence of Boc groups which broaden all the other proton peaks in the ^{1}H NMR. In order to improve this situation, the compound was deprotected using 4 M HCl in dioxane, which yielded a compound that had similar peaks to that of **113**, but contained an unknown impurity which could not be separated from the desired product. Hence, the synthesis of the chain shortened analogue **113** was carried out by the method of Rossiter *et al.* using the *t*-butyl ester **114**.[45] This was treated with the methylisothiourea **95** and after 48 h at 21 °C furnished the guanidine **115** in 83% yield. The Boc groups and *t*-butyl ester were then cleaved by 4 M HCl, to reveal the chain shortened analogue **113** in 85% yield.



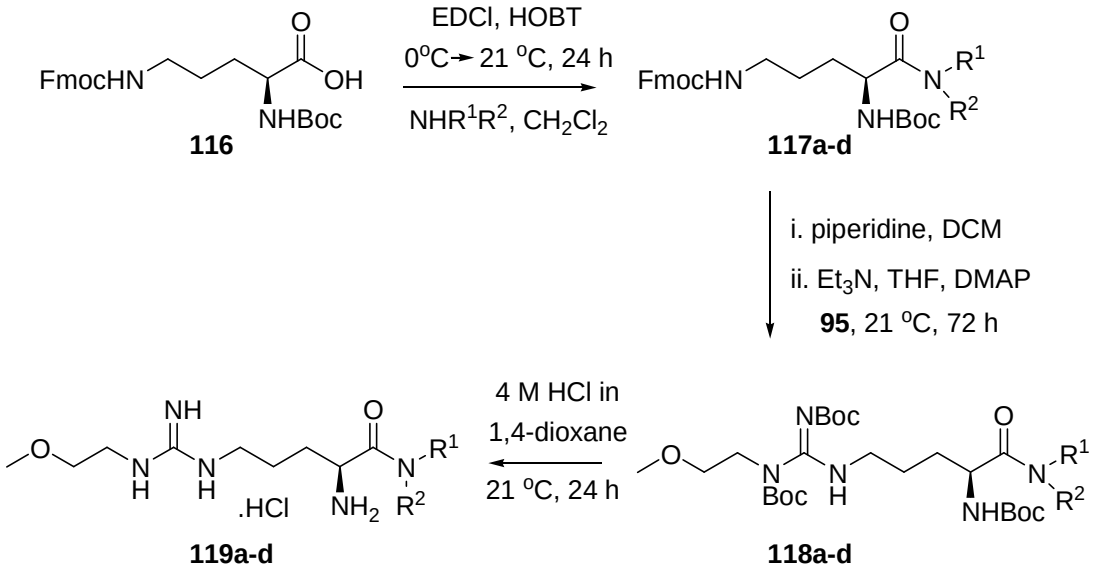
Scheme 28: Synthesis of the chain shortened analogue **113**.

2.6 Synthesis of amide analogues

Crystallization conditions led to the hydrolysis of the ester moieties of the methyl ester **24** and benzyl ester **90** (Scheme 21).[53] It was therefore envisaged that the more stable amide analogues of arginine should be synthesised for crystal structure studies, as well as testing for biological activity.

Amides were synthesised *via* the route outlined in Scheme 15 (section 1.6.6), using the methylisothiourea **95** instead of pyrazole **88**.[29] Amide formation on Boc-Orn(Fmoc)-OH *via* an EDCI and HOBT coupling reaction gave moderate yields of the amides **117a-d**,

(Scheme 29, Table 4).[54,55] These amides were then treated with piperidine to cleave the Fmoc groups, and subsequent reaction with methylisothiourea **95**, furnished the guanidines **118b-d**. The guanidine forming reactions were low yielding, the benzylamide **118c** was furnished in 70% yield, however the methylbenzyl amide **118d** was only achieved in 24% yield. The dimethyl amide **118b** could not be isolated in pure form, even after attempted purification by flash chromatography, and was therefore used impure in the next step. The amide **117a** failed to form the guanidine **118a**; the primary amide is not expected to be as nucleophilic as the primary amine formed from Fmoc cleavage, for nucleophilic attack at the thiourea. However, the presence of a primary amide seemed to have hindered the reaction. The Boc protected guanidines **118b-d** were then deprotected to yield the amide analogues **119b-d**.



Scheme 29: Synthesis of amide analogues.

R^1	R^2	Amide	Yield (%)	Guanidine	Yield (%)	Deprotected guanidine	Yield (%)
H	H	**117a**	89	**118a**	-	**119a**	-
Me	Me	**117b**	46	**118b**	8	**119b**	3
H	Bn	**117c**	70	**118c**	70	**119c**	62
Me	Bn	**117d**	92	**118d**	24	**119d**	71

Table 4: Yields in the synthesis of amide analogues.

2.7 Biological results

2.7.1 Biological results for DDAH synthesised DDAH inhibitors

In order to determine the inhibitory activity of the esters **24** and **90**, and of the amides **119b**, **119c** and **119d**, they were tested on rat kidney lysates which contained both DDAH1 and DDAH2. The assay measured the conversion of [^{14}C]-L-NMMA to [^{14}C]-citrulline. Inhibitors were added to the lysate and incubated at 37 °C, and ^{14}C Citrulline formation was measured by scintillation counting, and the IC_{50} values were calculated using Graphpad Prism software.

The results in table 5 show that the benzyl ester **90** was not as active as the methyl ester in competitively inhibiting the activity of DDAH. The methyl ester was not as active as the literature compound L-291(IC_{50} = 20 μM).[29,56] This may be attributed to errors in measuring out small concentrations of the inhibitor, as well as the DDAH enzymes in the kidney lysate loosing activity. The benzyl amide **119c** and the methyl benzyl amide **119d** did not show significant inhibition at 500 μM, with the benzyl amide being a better inhibitor than the methyl benzyl amide. However, the dimethyl amide **119b** seemed to have similar inhibition to L-257 and the methyl ester L-291, with an IC_{50} value of 23 μM. These results indicate that smaller R groups give better inhibition of DDAH, with the amide **119b** showing increased inhibition than the larger amides **119c** and **119d**, and the larger benzyl ester being a weaker inhibitor than the methyl ester.

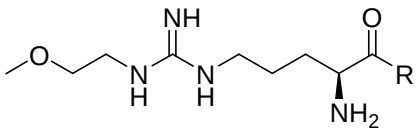
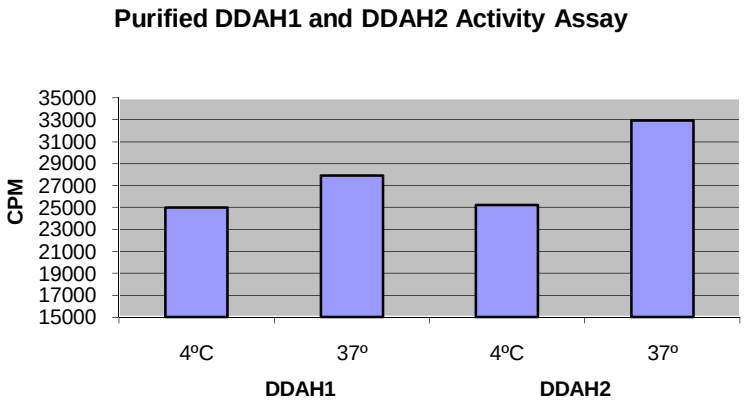
Analogue	 R =	Inhibition at 500 µM	IC_{50} (µM)
22	–OH	–	20 ± 2 [29]
24	–OMe	–	37 ± 4
90	–OBn	–	78 ± 3
119c	–NHBn	65%	300 ± 7
119d	–NMeBn	29%	–
119b	$–NMe_2$	–	23 ± 3

Table 5: Biological results of synthesised analogues.

2.7.2 Studies on DDAH2

The crystal structure of DDAH2 has not been solved to date and it was thought we could express and purify DDAH2, for this purpose. Myc-DDAH1 and Myc-DDAH2 were overexpressed in sEND cells and then purified using an immunoprecipitation technique using a Myc-antibody. Ten T75 flasks of the cells had to be grown in order to obtain 1mL of the cell lysate containing the Myc-DDAH protein. The aim was to purify enough DDAH2, ~1 mg, for crystallisation studies. In previous purifications in the group, western blots showed that DDAH1 and DDAH2 were present in the purified protein solution from three T75 flasks. However, once the DDAH2 was purified, a standard protein assay could not detect any protein in the lysate, as the concentration of the protein was too small. To purify larger amounts of DDAH2, it would have required many more T75 flasks of cells than is possible. Hence, purification of DDAH2 *via* the immunoprecipitation method was not a viable method to get enough protein for crystal structure studies.

The purified protein was nevertheless incubated with [^{14}C]-L-Citrulline overnight at 4 ºC as a control, and at 37 ºC to check for activity of the protein. The graph below shows a difference in the radiocounts between the two reactions, for both purified DDAH1 and DDAH2 (Graph 1). Purified DDAH1 showed a difference of 2935 cpm between the 4 ºC and the 37 ºC reaction. The difference in counts was higher with the purified DDAH2. These results showed that DDAH1 and DDAH2 remain active after purification, and this purified DDAH2 can perhaps be developed into a DDAH2 specific assay for development of DDAH2 specific inhibitors. This result however could not be repeated as subsequent purification of DDAH2 using immunoprecipitation, and activity assay failed to show any activity in the purified enzyme.



Graph 1: Activity of purified DDAH1 and DDAH2.

2.8 Inhibitor design from *hu*DDAH1 crystal structure

Publication of the crystal structure of *hu*DDAH1 complexed with the inhibitor L-257 by Leiper *et al.* showed the 2-methoxy ethyl chain of L-257 sat in a 7-membered ring *via* a hydrogen bond between the hydrogen of the guanidine and the oxygen of the 2-methoxyethyl chain (Figure 15 **22**, Figure 16).[56] This suggested that constraining the guanidine group up into a 7-membered ring i.e. analogue **120**, may lead to a more potent inhibitor (Figure 16).

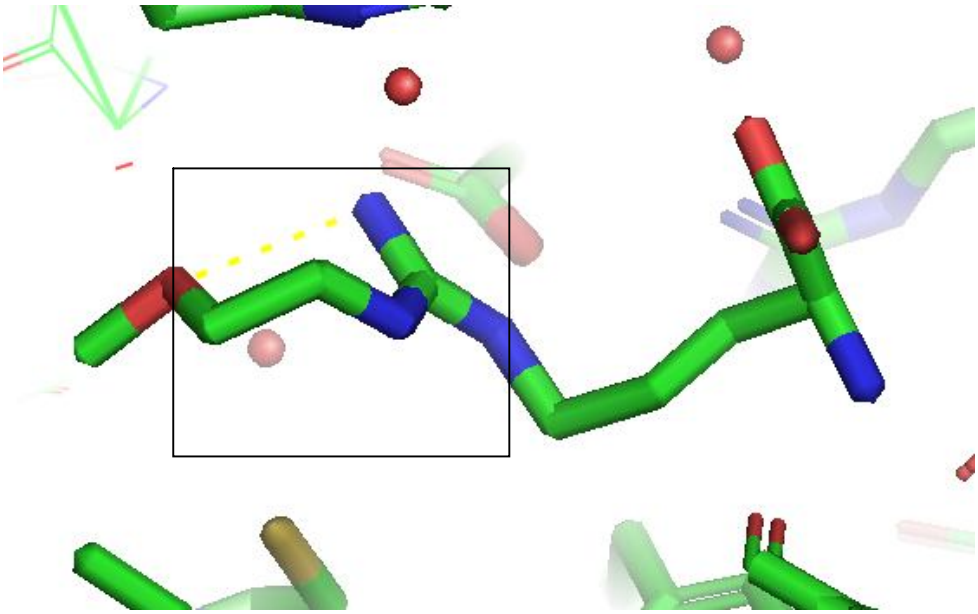


Figure 15: Crystal structure of L-257 in *hu*DDAH1.

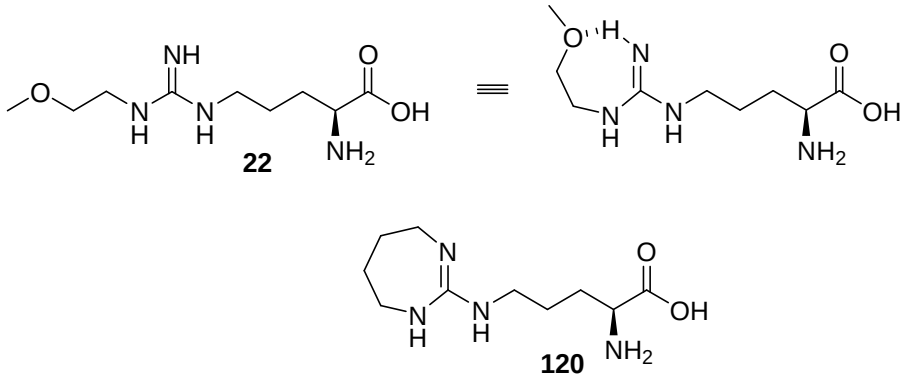
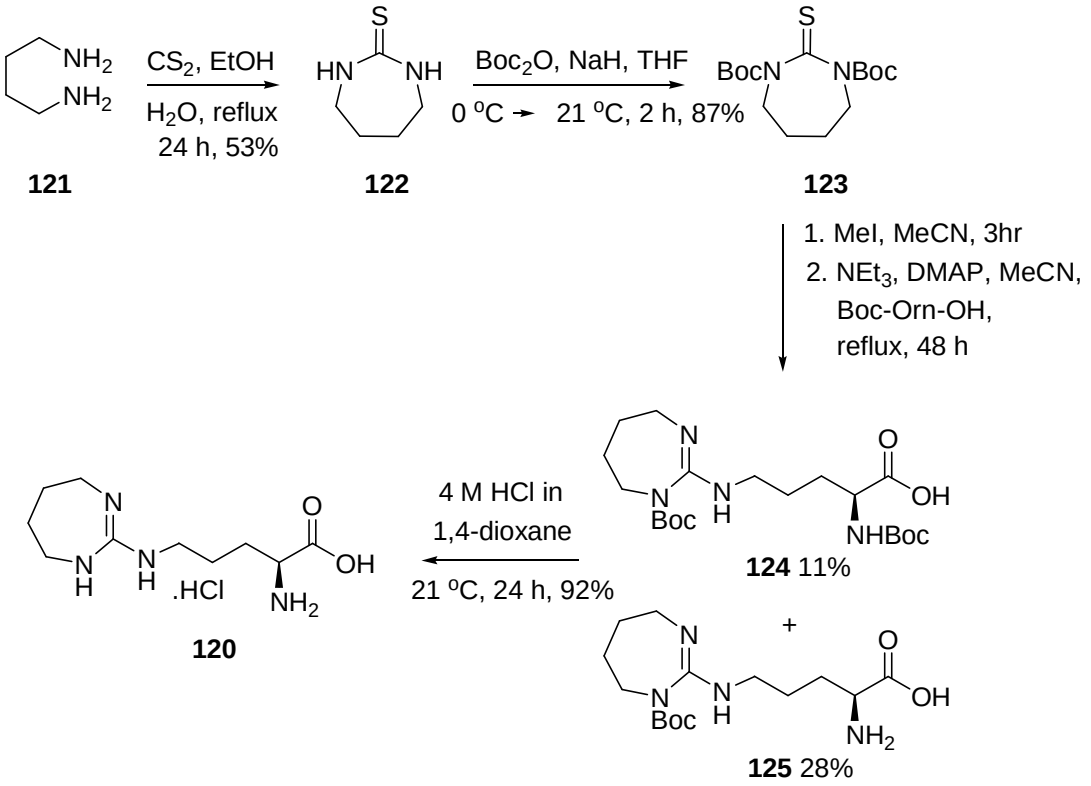


Figure 16: Conformation of L-257 in *hu*DDAH1 and proposed 7-membered analogue.

Although cyclic analogues have been synthesised previously and didn't exhibit significant activity, cyclic analogue **120** had not been evaluated. The 7-membered cyclic thiourea **122** was synthesised starting from carbon disulphide with 1,4-diaminobutane. Condensation of thiourea **122** with Boc_2O furnished bright yellow thiourea **123** in 87% yield (Scheme 30). Subsequent treatment of this thiourea with methyl iodide, and then refluxing with Boc-Orn-OH for 48 h, furnished the Boc protected cyclic guanidine **124** in 11% yield, and the bisBoc protected guanidine **125** in 28% yield. Presumably, the refluxing conditions lead to the loss of the additional Boc group of guanidine **125**. The Boc groups were then deprotected using 4 M HCl in dioxane to yield the cyclic analogue **120**. The inhibitory activity of this compound was assayed against rat kidney DDAH and was found to have 92% activity at 500 μM, but disappointingly displayed no inhibition at 100 μM. It seems plausible that the large 7-membered ring may restrict the ability of **120** to dock into the active site of the DDAH1.



Scheme 30: Synthesis of 7-membered cyclic analogue **120**.

2.9 Summary

Our investigations into the synthesis of ester analogues on the carboxyl end of L-257 proved to be troublesome. Initial synthesis of the methyl and benzyl ester, **24** and **90** respectively, were extremely low yielding. This was due to the key guanidine forming reaction being difficult to effect with high yields. Different conditions using mercury and EDCI as a coupling agent, and differing solvents, temperature and reaction times did not seem to have any significant effect on increasing the small yield of the guanidine formed (Table 3). The high polarity of the intermediates also resulted in purification problems. The key problem in the guanidine forming reaction was proved to be the free acid on Boc-Orn-OH used in the coupling reaction as the t-Butyl ester protected Ornithine **106** underwent smooth conversion to the guanidine in high yields (Scheme 24). These difficulties with the synthesis of esters led us to attempt synthesis of other functional groups on the carboxyl end of L-257. A number of amide analogues were synthesised on Boc-Orn-OH which underwent good conversion to their corresponding guanidines (Scheme 29, Table 4). However, the amide analogues prevented a drawback as testing for inhibition of DDAH showed that apart from the

dimethylamide analogue which showed similar activity to L-257 and L-291, larger amide analogues did not possess any significant activity.

As substrate based inhibitor design did not prove fruitful, a crystal structure approach to designing an inhibitor was adopted. This was prompted by the publication of the crystal structure of *hu*DDAH1 with L-257 at the active site. This allowed for the design of a 7-membered cyclic analogue, by mimicking the conformation taken by the 2-methoxyethyl chain of L-257. The synthesis of this compound was relatively facile. However, subsequent biological evaluation showed disappointing inhibition of rat kidney DDAH with no activity at 100 μM. It is anticipated that further analogues based on the crystal structure of L-257 in *hu*DDAH1 will lead to more promising inhibitors of DDAH.

Alongside the chemical synthesis of inhibitors of DDAH, some biological investigations into DDAH2 was also carried out. The crystal structures of bacterial and mammalian DDAH1 had been solved but the crystal structure of DDAH2 is unresolved thus far. Hence, an attempt was made to purify *hu*DDAH2 for crystal structure studies *via* an immunoprecipitation technique. This failed to produce enough protein for characterisation of its crystal structure. However, purification of DDAH2 and incubation with substrate L-NMMA showed some activity. This result however, could not be further reproduced.

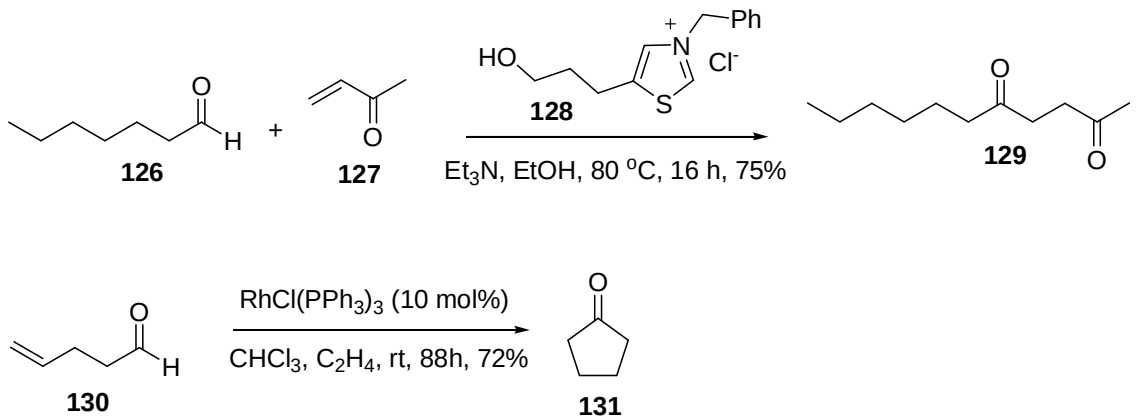
It is envisaged that the search for DDAH inhibitors will be carried out in the future *via* crystal structure based design. Indeed, there are many more conformations of L-257 in the active site of *hu*DDAH1 that could potentially be mimicked, or another useful way is by attaching a group onto L-257 to displace the closely bound water molecule needed for hydrolysis seen in Figure 9, A. Development of inhibitors with high potency could lead to clinically useful drugs for NO reduction in pathological situations such as ischemia, migraine and septic shock.

Investigations into Radical Hydroacylation of Vinyl Sulfonates

Chapter 3: Introduction

3.1 Hydroacylation

Hydroacylation reactions can be carried out using a number of methods. These methods include the Stetter reaction, transition metal catalysed and radical mediated hydroacylation. The Stetter reaction employs an aldehyde, an α,β-unsaturated ketone with a thiazolium catalyst which leads to the formation of a 1,4 dicarbonyl *via* an umpolung mechanism. For example, the aldehyde **126** was treated with methylvinyl ketone **127** and the thiazolium catalyst **128** to yield 1,4 dione **129** as the intermediate in the synthesis of dihydrojasmone by Stetter *et al.* (Scheme 31).[57] Transition metals, in particular rhodium, have been used, to catalyse hydroacylation reactions. For example, treating pentenal **130** with Wilkinson's catalyst leads to the formation of cyclopentenone **131** (Scheme 31).[58] The use of other transition metal catalysed hydroacylation reactions has been reviewed by Willis.[59]



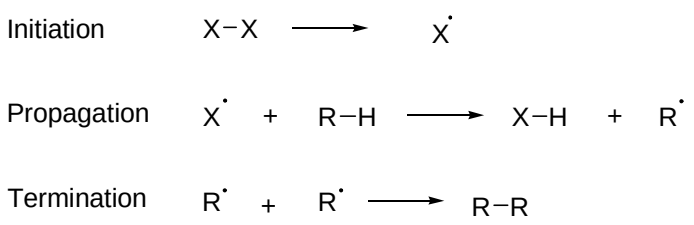
Scheme 31: Stetter reaction and rhodium catalysed hydroacylation.

Hence, there are a number of distinct mechanisms for hydroacylation of alkenes and these include a free radical pathway involving acyl radicals.

3.2 Radical Chemistry

Radicals are atoms, molecules or ions with an unpaired electron and are formed from the homolysis of a two electron bond. They are generally highly reactive as the unpaired electron can readily 'pair' with another electron to form a more stable species, and hence they are reactive intermediates. Radical intermediates are found in many naturally occurring processes, for example in the human body the formation of OH radical leads to indiscriminate destruction of enzymes, fats and DNA. Molecular oxygen, which exists as a stable biradical is involved in autoxidation processes such as drying of paints and deterioration of foods.

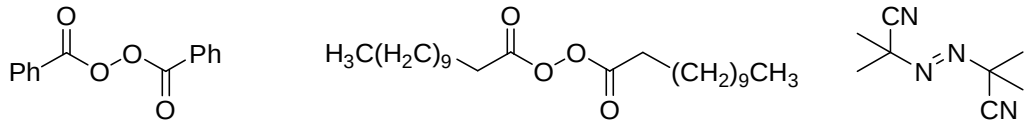
To form a radical, a relatively weak covalent bond must be broken, with the supply of energy to overcome bond dissociation coming from heating (thermolysis) or UV light (photolysis). Once initiated, radicals can react with other non-radical species such as alkenes, to form another radical (propagation). The reaction of a radical with another radical leads to a stable unreactive species which terminates the radical chain reaction (Scheme 32).



Scheme 32: Steps in a radical reaction.

In synthetic chemistry radicals are formed from molecules containing relatively weak bonds i.e. bond energies of 125-165 kJ mol^{-1}. These bonds include heteroatom-heteroatom, heteroatom-carbon and metal-metal bonds, and can be homolysed at relatively low temperatures. One of the most common examples is the single O-O bond in peroxides. Diacyl peroxides, in particular benzoyl peroxide (BPO) **132** and lauroyl peroxide **133** have low bond energies (~125 kJ mol^{-1}). BPO has a half life of 1 h at 92 °C and 1 min at 131 °C, and lauroyl peroxide has a half life of 1 h at 80 °C and 1 min at 120 °C (Figure 17).[60] Other common radical initiators include the azobisnitrile azobisisobutyronitrile (AIBN) **134** and organometallics such as $(SnBu_3)_2$ and $(SePh)_2$.

Irradiation by visible and UV light of certain wavelengths can also promote homolysis of peroxides and nitriles.

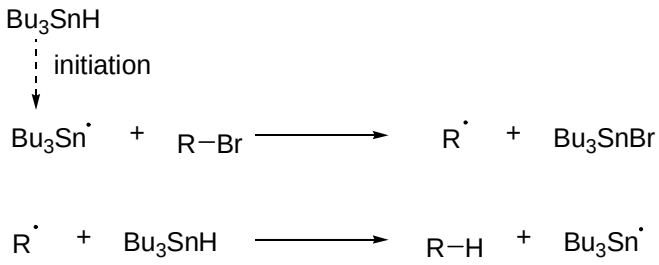


benzoyl peroxide **132** lauroyl peroxide **133** AIBN **134**

Figure 17: Common radical initiators.

3.3 Organotin in radical reactions

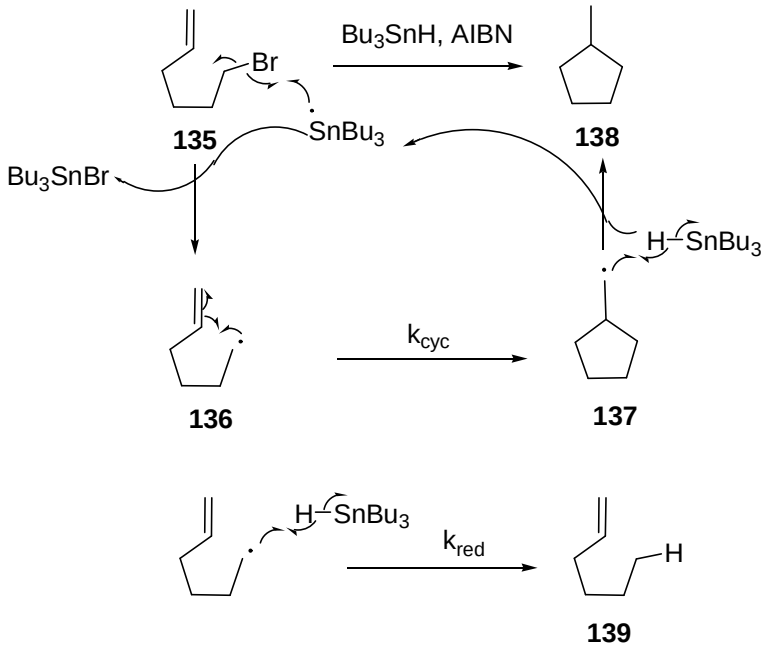
Chain reactions using organotin hydrides are the most common application of radical chemistry in organic synthesis, the most common reagent being tributyltin hydride (Bu_3SnH). These are used in radical reduction, addition, cyclisation and elimination reactions, with initiation of the radical chain by AIBN. The reduction of an alkyl halide such as an alkyl bromide, using Bu_3SnH proceeds *via* abstraction of the bromine atom by a tributyltin radical (Scheme 33).[61] This reaction proceeds to form a strong tin-halide bond which provides a driving force for the reaction. The radical adduct R can then abstract a hydrogen from the Bu_3SnH, forming a stable C-H bond and producing a Bu_3Sn radical which further propagates the chain reaction.



Scheme 33: Radical reduction by Bu_3SnH.

Intramolecular radical addition reactions are synthetically useful for the formation of cyclic structures. In the cyclisation of the alkene **135** (Scheme 34), the radical generated from abstraction of the bromine atom leads to radical **136**, which can either cyclise to form **137** or reduce to **139**. The rate of cyclisation (k_{cyc}) must be higher than the rate of reduction (k_{red}) for cyclisation to take place. This is achieved by maintenance of a low concentration of Bu_3SnH which can be achieved by its slow addition over the course of

the reaction. In this way it can be possible to maximise the cyclisation pathway and minimise the undesired reduction.

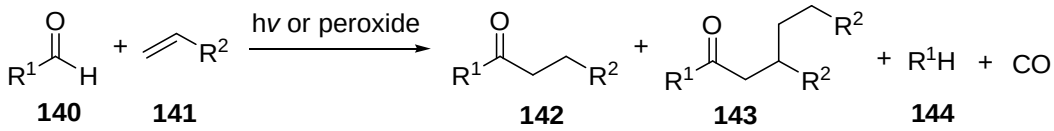


Scheme 34: Intramolecular radical addition.

Radicals are frequently used in synthesis due to the advantages they offer over ionic reactions. They are unsolvated and this can create more sterically hindered systems than ionic species; they are compatible with functional groups (protecting groups are not needed for NH or OH), less prone to rearrangement, and their ambiphilic nature allows them to react with both electrophilic and nucleophilic reagents. An area of organic chemistry where radicals are widely used is in hydroacylation of alkenes for the formation of ketones.

3.4 Radical hydroacylation

One of the first reports of radical hydroacylation was by Kharasch *et al.* in 1949 whereby addition of aldehyde **140**, to alkene **141**, (6:1 respectively) led to the formation of ketone **142** (Scheme 35).[62] Small amounts of a double addition product **143**, the alkane **144** derived from decarbonylation of the acyl radical, and carbon monoxide were also formed.



Scheme 35: Kharasch's hydroacylation of alkenes.

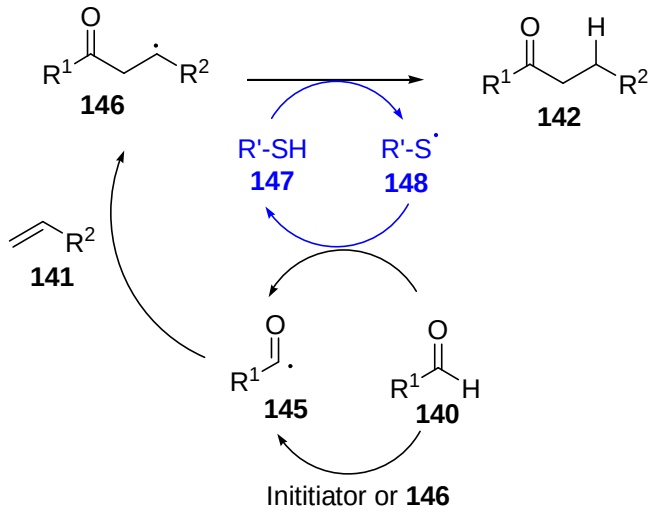
The reaction occurs *via* the following sequence (Scheme 36):

(a) An initiator abstracts the aldehydic hydrogen from the aldehyde **140** to form an acyl radical **145**.

(b) The acyl radical **145** adds to an alkene forming radical adduct **146**.

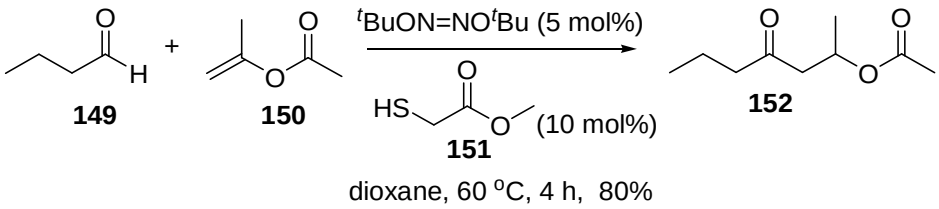
(c) Abstraction of an aldehydic hydrogen from another molecule of aldehyde by adduct **146** generates the ketone **142** and another acyl radical.

It has now widely established that ketones can be synthesized from acyl radicals which are nucleophilic and add to electron poor alkenes.[63] Addition of the acyl radical to neutral or electron rich alkenes i.e. if R^2 = alkyl is sluggish (Scheme 36). This is because the nucleophilic adduct **146** is not efficient in abstracting the aldehydic hydrogen. However, hydroacylation is relatively fast when R^2 is an electron-withdrawing group. When the rate of reaction is slow due to the inefficiency of the abstracting radical adduct **146**, additional chain carriers can be used. For example, Roberts *et al.* used a thiol as a chain carrier and found that thiol catalysis was effective for hydroacylation of electron-rich, -neutral and -deficient alkenes, in particular for addition to electron-rich double bonds.[64] The thiol acts as a polarity-reversal catalyst, whereby the adduct radical **146** abstracts a hydrogen from the thiol **147** to form the ketone **142**, and the thiyl radical **148** thus formed is polarity matched to abstract the aldehydic hydrogen to form an acyl radical and regenerate itself (Scheme 36).



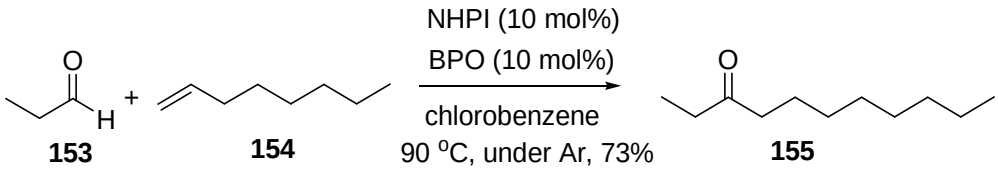
Scheme 36: Hydroacylation reaction mechanism (black), and thiol catalysis (blue).

An example using butanal **149** (2 eq.) and isopropenyl acetate **150**, with di-*tert*-butyl hyponitrite TBHN as the initiator and methylthioglycolate **151** as the thiol chain carrier, gave the ketone **152** in 80% yield (Scheme 37), whereas reaction without a thiol catalyst gave only 8% yield, indicating the effectiveness of the thiol chain carrier.



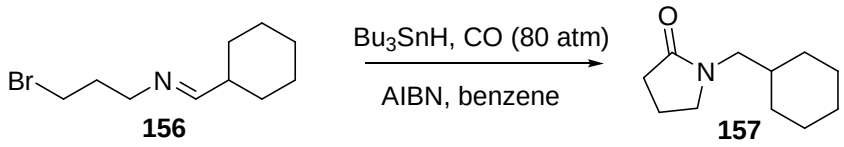
Scheme 37: Hydroacylation of an electron-rich alkene using methylglycolate.

Another such chain carrier used by Ishii *et al.* is *N*-hydroxyphthalimide (NHPI). This molecule also acts as a polarity reversal catalyst.[65] In a similar manner to the thiol catalyst protocol, adduct radical **146** (Scheme 36) abstracts a hydrogen from the NHPI to form the ketone and the phthalimide *N*-oxyl (PINO) radical. This radical can then abstract an aldehydic hydrogen to form an acyl radical and regenerate the NHPI. The example below shows the hydroacylation of propanal with 1-hexene **154**. Catalytic amounts of NHPI and BPO were used to give the ketone **155** in 73% yield (Scheme 38).



Scheme 38: Hydroacylation using polarity-reversal catalyst NHPI.

Although most of the hydroacylations of alkenes involve electron poor acceptors *via* what is thought to be a nucleophilic acyl radical, it was recently shown that acyl radicals add to imines solely *via* reaction on the nitrogen. Ryu *et al.* showed that the acyl radical is *N*-philic and reacts exclusively at the nitrogen of imines (Scheme 39).[66] For example, treatment of the alkyl bromide **156** with Bu_3SnH and carbonylation to form an acyl radical, led to cyclisation at the more electron rich end of the C=N π-bond, yielding the lactam **157**.



Scheme 39: Cyclisation of *N*-philic acyl radical.

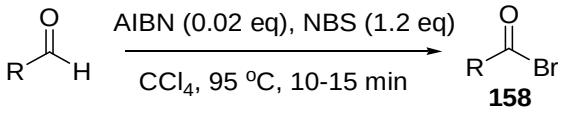
3.5 Generation of acyl radicals

Chatgilialoglu *et al.* broadly classify the generation of acyl radicals into three routes: i) from the homolysis of a C-X bond; ii) carbonylation of alkyl radicals; and iii) homolysis of an R-R' bond.[63]

3.5.1 Acyl radical generation *via* homolysis of R-X where X = H

Acyl radical formation directly from aldehydes can occur *via* homolysis of the aldehydic C-H bond. Homolysis of this bond requires activators, such as peroxide reagents and Bu_3SnH. Kharasch used the peroxide initiator diacetyl peroxide (Scheme 35). Marko *et al.* reported the reaction of aldehydes with AIBN and *N*-bromosuccinimide (NBS) to afford acid bromide **158** (Table 6).[67] Heptanal, cyclohexanal and trimethyl acetaldehyde were converted to their acid bromides in 10-15

min in good yields (Table 6). This is a synthetically useful reaction as the acid bromides can be further converted to amides upon addition of amines.



158

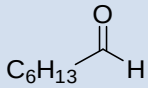
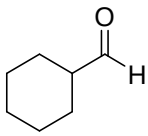
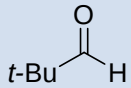
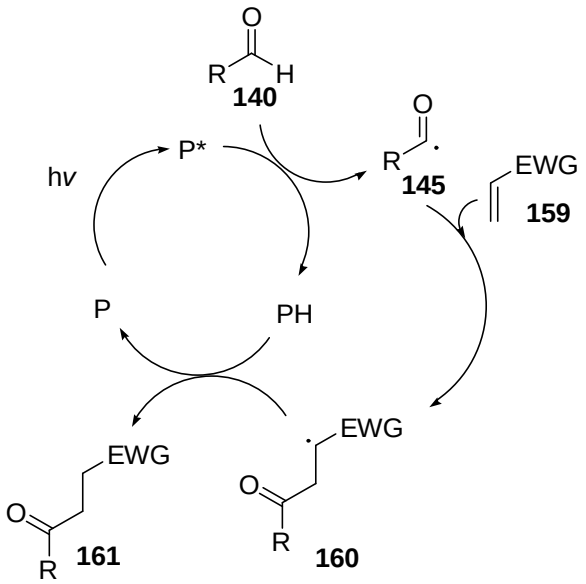
Entry	Substrate	Product yield
1		78%
2		83%
3		85%

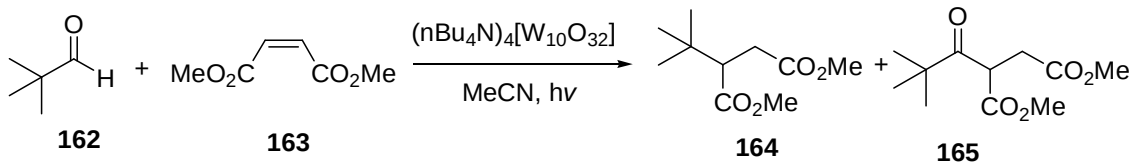
Table 6: Acid bromide formation.

Recently it was shown that tetrabutylammonium decatungstate (TBADT) acts as an efficient activator of the aldehydic hydrogen in photocatalyzed hydroacylation of electron poor alkenes.[68] The process is thought to be initiated by excited TBADT (P*) which abstracts the aldehydic hydrogen of aldehyde **140**, leading to the formation of the acyl radical **145** (Scheme 40). The acyl radical is captured by the electron-poor alkene **159** to form adduct **160**. Hydrogen atom transfer by PH completes the cycle forming ketone **161**. Hydroacylation using primary, secondary and tertiary aldehydes were successful in the reaction as well as using a variety of different activated alkenes.



Scheme 40: Hydroacylation using TBADT.

Decarbonylation of trimethylacetaldehyde **162** led to the alkyl addition product **164** in 70% yield at room temperature (Scheme 41). Predominance in the acyl addition product **165** over the alkyl addition was achieved by lowering the temperature of the reaction to -20 °C, which reduced decarbonylation, to give a ratio of 3:1.

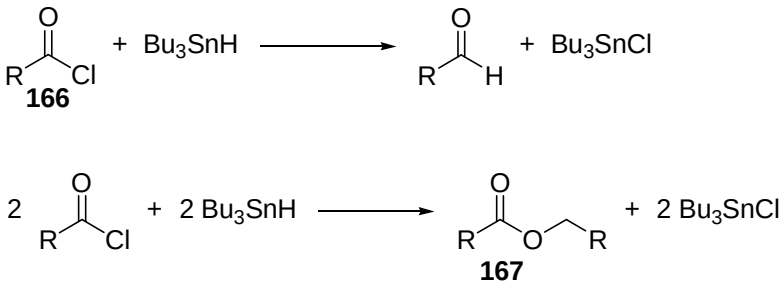


Scheme 41: TBADT mediated hydroacylation of pivaldehyde.

Equimolar amounts of aldehyde and alkene were used, with only 2 mol% of the TBADT present. Foul smelling auxiliaries such as thiols were also not needed and hence the authors suggest these properties qualify the synthetic method as 'green'. This approach mediated by TBADT was recently used to attach acyl groups to [60]fullerene.[69]

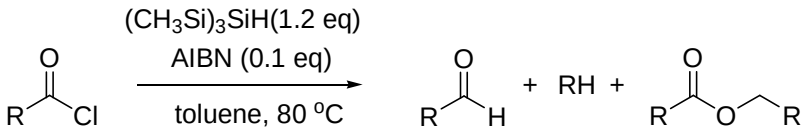
3.5.2 Acyl radical generation *via* homolysis of R-X where X ≠ H

Acyl radicals can be formed from acyl chlorides as demonstrated by a number of groups.[63] Chatgilialoglu showed that reduction of an acid chloride **166** with Bu_3SnH is plagued by the formation of an ester by-product **167** (Scheme 42).[70]



Scheme 42: Reduction of acid chlorides with Bu_3SnH.

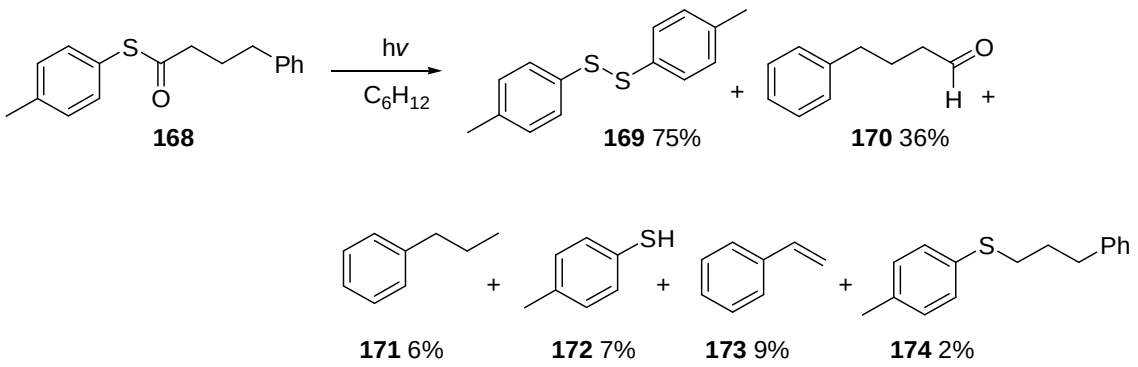
Treating acid chlorides with tris(trimethylsilyl)silane ($(CH_3Si)_3SiH$) and AIBN leads to the reduction of acid chlorides to the aldehyde or decarbonylated alkane, and reduces ester by-product formation. For example, Chatgilialoglu *et al.* showed that nonanoyl chloride could be reduced to the corresponding aldehyde and decarbonylated product, RH, in 37 and 63% yields respectively with only a small amount of ester formation (Table 7, entry 1).[71] However, reaction of cyclohexanecarbonyl chloride (entry 2) and pivaloyl chloride (entry 3) led to almost exclusive formation of their decarbonylated products *via* the acyl radical pathway.



Entry	RCOCl	Time (min)	RCHO yield	RH yield	Ester yield
1	$CH_3(CH_2)_8COCl$	30	37	63	Unquantified small amounts
2	*c*-$C_6H_{11}COCl$	40	<1	99	-
3	$(CH_3)_3COCl$	30	-	98	-

Table 7: Acid chloride reduction using $(CH_3Si)_3SiH$.

Thioesters can also be employed as acyl radical precursors. Grunwell *et al.* reported the photolysis of aromatic thiol esters using a 254 nm light source to generate the corresponding thiyl radical and an acyl radical.[72] For example, photolysis of 4'-tolyl thiol-4-phenylbutyrate **168** gave, primarily disulfide **169** and aldehyde **170**, presumably *via* generation of an acyl radical and a thiyl radical (Scheme 43). Minor amounts of other products were also formed including propylbenzene **171**, and sulfide **174**, presumably from decarbonylation of the acyl radical, and recombination of the thiyl radical and the decarbonylated acyl radical respectively.



168

169 75%

170 36%

171 6%

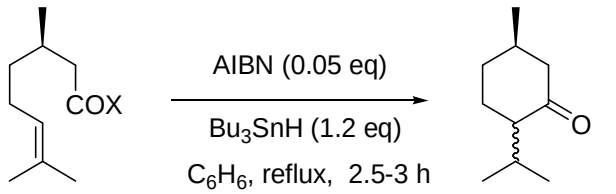
172 7%

173 9%

174 2%

Scheme 43: Acyl radical generation by photolysis of a thioester.

Simple thioesters such as *S*-phenyl thioesters show a lack of reactivity towards stannane mediated chain reactions. Boger *et al.* showed that treatment of the thiol ester **175** with AIBN and Bu_3SnH led to no reaction; however, treatment of the corresponding seleno ester under the same conditions led to the formation of the cyclised product **176** in 84% yield (Scheme 44).[73] Selenoesters are more useful for this purpose than thioesters as the RCO-SeR' bond is weak and reacts readily with stannyl radicals.



175

X = SPh

X = SePh

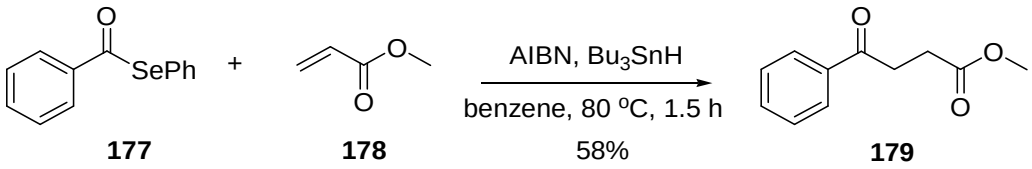
176

no product

84%

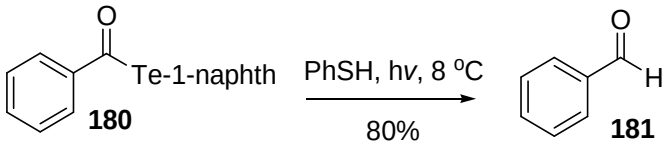
Scheme 44: Cyclisation of acyl radicals generated from thioester and selenoester.

Boger *et al.* also showed that a range of phenyl selenide acyl precursors underwent hydroacylation to a range of alkenes without decarbonylation.[74] *Se*-Phenyl benzenecarboselenoate **177** and methyl acrylate **178** underwent hydroacylation to give ketone **179** in 58% yield under standard radical conditions using AIBN and Bu_3SnH (Scheme 45).



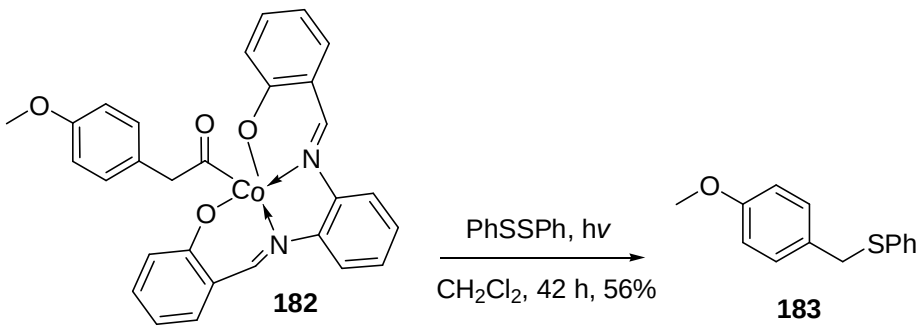
Scheme 45: Phenylselenide as an acyl radical precursor.

Telluroesters are also commonly used and acyl radicals from telluroesters can be generated *via* white light photolysis. For example, telluroester **180** is reduced to aldehyde **181** by treatment with thiophenol under white light (Scheme 46).[75]



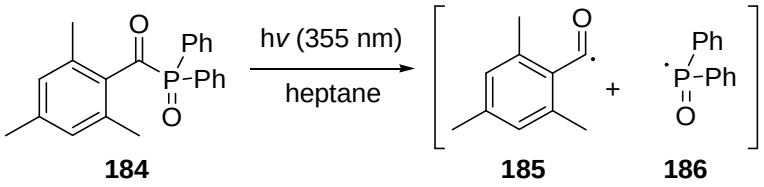
Scheme 46: Telluroester as an acyl radical precursor.

Pattenden *et al.* showed that white light photolysis of aryl and acetyl cobalt salophens yield acyl radicals, and that these can undergo decarbonylation to alkyl radicals, and can then be trapped by various electrophiles including phenyldisulphides in moderate to good yields.[76] For example, cobalt-salophen **182** was converted to the phenylsulfide **183** in 63% (Scheme 47).



Scheme 47: Cobalt salophen as an acyl radical precursor.

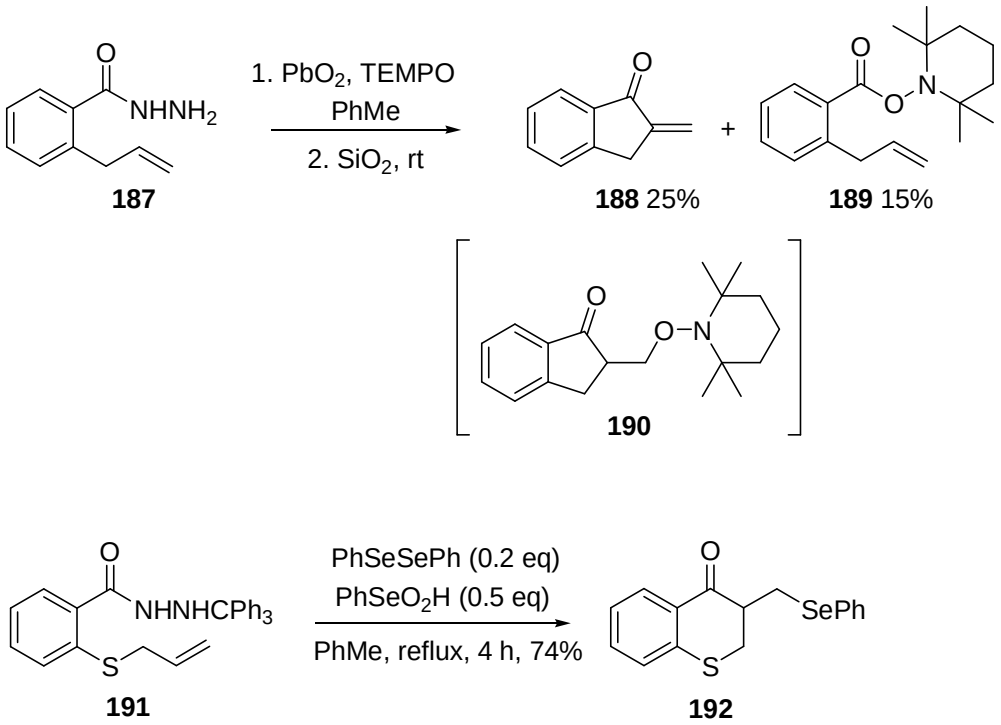
There are many more examples of acyl radical precursors in the literature, including those from metal carbene complexes and acylphosphine oxides. The phosphine oxide (2,4,6-trimethylbenzoyl)diphenylphosphine oxide **184** is a commercial photoinitiator used in polymerization processes (Scheme 48).[77] This molecule forms benzoyl **185** and phosphonyl **186** radicals with initiation by UV light of 355 nm wavelength.[78]



184 **185** **186**

Scheme 48: Acyl radical generation from a phosphine oxide.

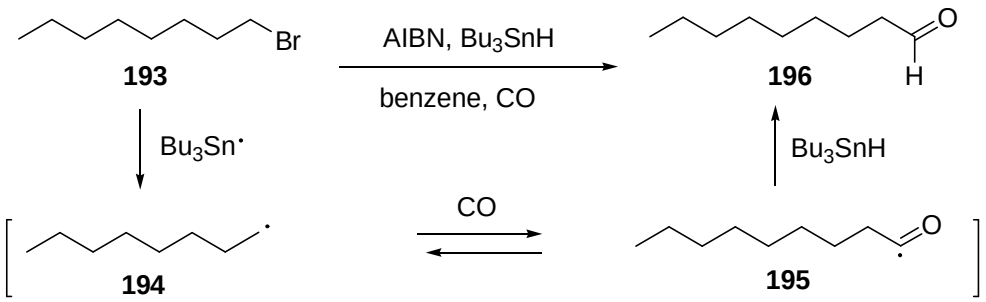
More recently acyl hydrazines and hydrazides have been employed as acyl radical precursors. Braslau *et al.* employed acyl hydrazines in cyclisation reactions whereby the hydrazine **187** was treated with lead oxide (PbO_2) to form the acyl radical which was then trapped by 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO).[79] This yielded the acyloxyamine **189**, and the α,β-unsaturated system **188** formed *via* elimination of the 5-exo-cyclisation product **190** (Scheme 49). Zard *et al.* also used acyl hydrazide analogues to carry out cyclisation reactions. [80] In this case the acyl radicals were trapped by phenyl selenide, and this seemed to give improved yields over hydrazines. For example, the hydrazide **191** was treated with diphenyl diselenide and phenylseleninic anhydride to yield the cyclised and trapped product **192** in 74% yield.



Scheme 49: Acyl hydrazine and acyl hydrazides as acyl radical precursors.

3.5.3 Acyl radical generation *via* carbonylation

Alkyl and aryl radicals can be carbonylated with CO usually under high pressure, to generate acyl radicals. Ryu *et al.* showed that alkyl radicals, generated from alkyl halides such as 1-bromoctane **193**, could be carbonylated under CO pressures of 70-90 atm (Scheme 50).[81] Dehalogenation by $Bu_3Sn^{\bullet}$ led to the formation of the alkyl radical **194** which carbonylated to form an acyl radical **195**. Abstraction of a hydrogen from Bu_3SnH furnished the aldehyde **196** in 61% yield.

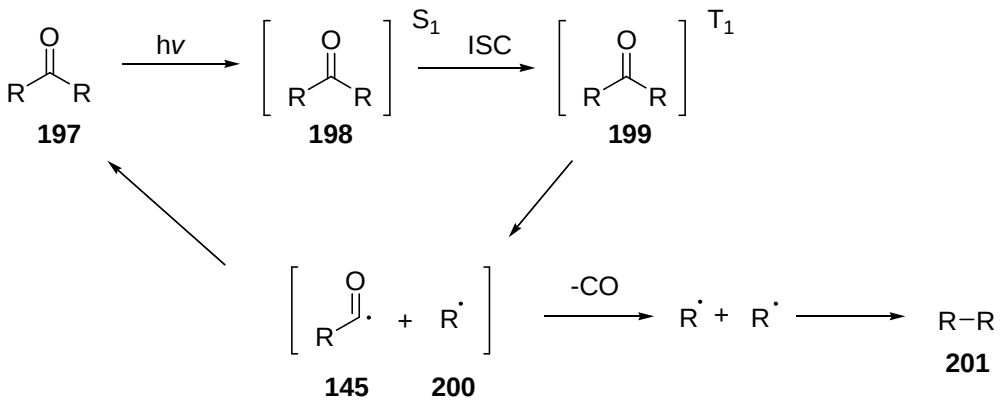


Scheme 50: Acyl radical formed *via* carbonylation.

Further development of this reaction by substituting tris(trimethylsilylsilane) $(TMS)_3SiH$ for Bu_3SnH reduced the need for the high CO pressure, instead requiring 15 atm of CO to produce a similar yield of product (65%).

3.5.4 Acyl radical generation through fragmentation of C-C bonds

Fragmentation of ketones and diketones by a Norrish type I photocleavage mechanism leads to the formation of acyl radicals (Scheme 51). The carbonyl group of the ketone can be excited to the singlet state S_1 **198** by accepting a photon. It can then reach the triplet state **199** through intersystem crossing (ICS) and cleave forming two radical fragments **145** and **200**. The acyl radical can undergo decarbonylation to form an alkyl radical R , and then recombine with another alkyl radical to form **201**, or recombine to form the original ketone **197**.

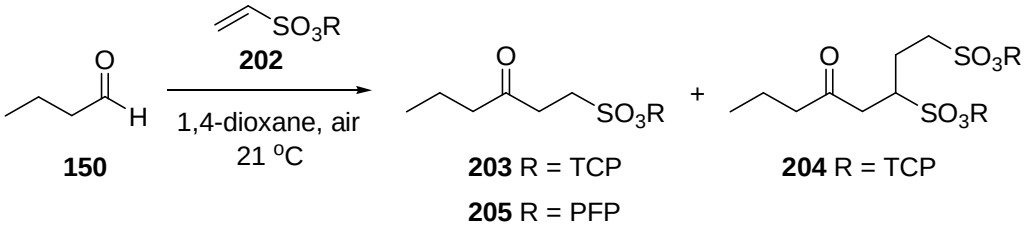


Scheme 51: Acyl radical formation *via* Norrish type 1.

3.6 Acyl radical generation using air

Although it seems from the literature precedence that an added initiator is needed for the activation of the C-H bond of aldehydes to form the acyl radical for addition to olefins, a discovery made by Caddick *et al.* provides evidence for the C-H activation of aldehydes without the need of this additive.[82] This reaction was detected when an aldehyde was reacted with a vinyl sulfonate in the presence of air which led to the formation of an unexpected unsymmetrical ketone. The initial reaction used 2 equivalents of butanal and one equivalent of trichlorophenyl(TCP)-vinyl sulfonate in dioxane at room temperature. This furnished the corresponding unsymmetrical ketone

203 in a 40% yield, with a small amount of the double addition product **204** (Scheme 52).



Scheme 52: Hydroacylation using TCP-vinyl sulfonate as acceptor.

Further investigations into the reaction parameters revealed higher concentrations and a high ratio of aldehyde to alkene (5:1 respectively) increased the yield of the product **203** to 74% after 24 h. A much smaller reaction time was needed for the reaction of butanal with pentafluorophenyl(PFP)-vinyl sulfonate as the acceptor, yielding the ketone **205** in 65% after just 1 h. This can be attributed to the strong electron withdrawing nature of the PFP moiety. The generality of the reaction with respect to aldehydes was also investigated, and it was found that primary and secondary aldehydes underwent conversion to their corresponding ketones in good yields with TCP-vinyl sulfonate, and in lower yields but faster reaction times with PFP-vinyl sulfonate as the acceptor. Further optimisation of the reaction with PFP-vinylsulfonate in water at room temperature led to an increase in yield for a range of aldehydes employed in the reaction. The butanal reaction with PFP-vinyl sulfonate in these conditions increased the yield of **205** to 78%.[83]

Evidence for a radical mechanism in this reaction comes from the complete inhibition of this reaction, when the radical inhibitor 2,6-di-*tert*-butyl-4-methylphenol (BHT 1%) is added. In addition, reaction of the pivaldehyde led to the formation of **206** and the decarbonylated product (**207**, Figure 18) which is indicative of radical decarbonylation and addition.

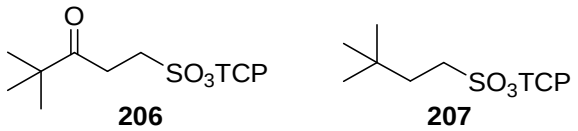
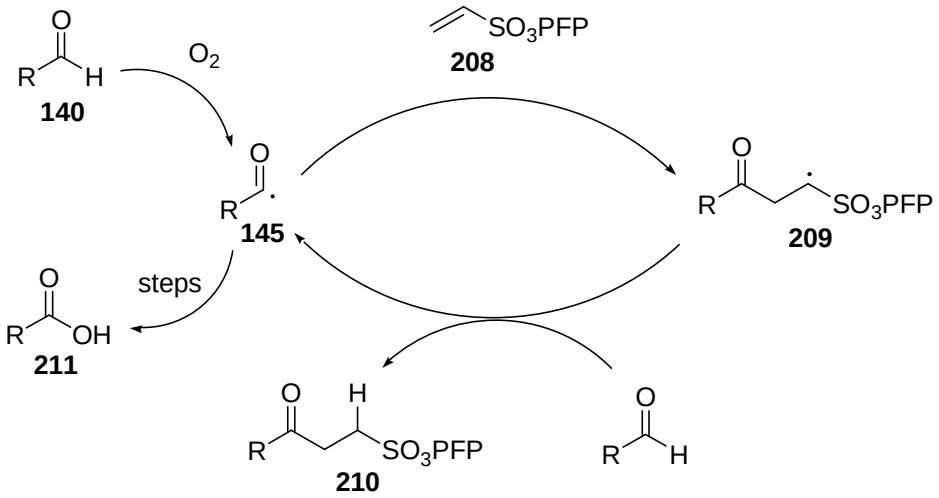


Figure 18: Reaction products of pivaldehyde.

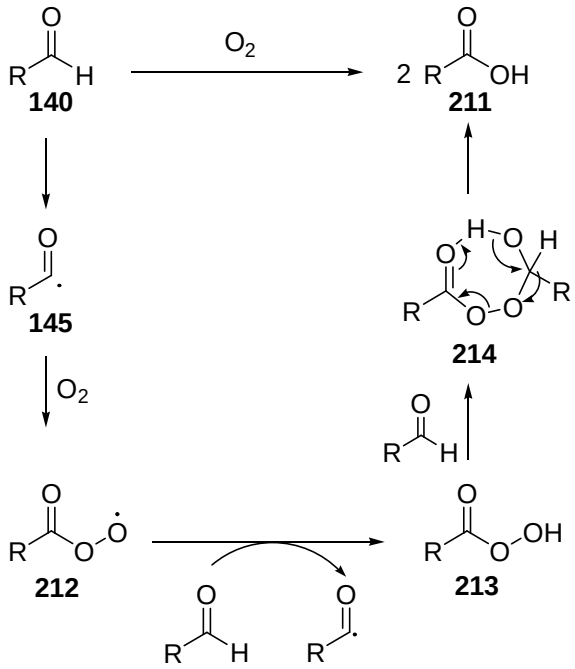
The authors postulate that the reaction proceeds though a radical mechanism (Scheme 53). The acyl radical **145** is formed by autoxidation of the aldehyde **140** by molecular oxygen in air. The radical **145** adds to the vinylsulfonate **208** to form the radical adduct **209**. This then abstracts the aldehydic hydrogen to form ketone **210**. The acyl radical formed *via* autoxidation of the aldehyde, can also react further with molecular oxygen resulting in the formation of the carboxylic acid **211**.



Scheme 53: Postulated mechanism by Caddick *et al*.

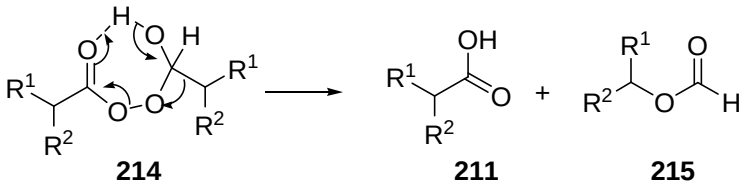
3.7 Autoxidation

It is known that aldehydes autoxidise to their corresponding acids *via* aerobic oxidation involving acyl radicals. Melville and Cooper have shown that *n*-decanal autoxidises to a peroxy radical by reacting with molecular oxygen. [84,85] Formation of peroxyradicals **212** occurs *via* reaction of an acyl radical **145** with molecular oxygen (Scheme 54). The peroxyradical **212** abstracts the aldehydic hydrogen from a molecule of aldehyde leading to the formation of the acyl radical and a peroxy acid **213**. The peroxyacid then reacts with a molecule of aldehyde forming an α-hydroxyperester **214** which decomposes to 2 molecules of carboxylic acid **211**. This overall pathway represents the autoxidation of aldehydes to their respective acids.



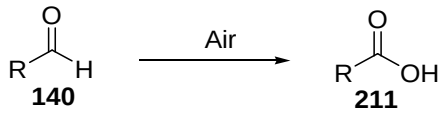
Scheme 54: Autoxidation of aldehydes.

The α-hydroxyperoxyacid **214** can also break down to give a formate **215** and an acid, as shown by Lehtinan *et al.* through a Bayer-Villiger mechanism if the aldehyde is α-branched.[86] The α-branched aldehyde 2-ethyl hexanal reacts to give a mixture of the corresponding acid and formate in a ratio of 1:0.43 (acid: formate) (Scheme 55).



Scheme 55: Alternative degredation pathway of the α-hydroxyperester

Autoxidation studies of simple aldehydes were carried out by Caddick *et al.*, by mixing aldehyde in air for 2 h, examining the ratio of the unreacted aldehyde to the acid formed (Table 8) and comparing them with hydroacylation reaction rates.[83] For the same aldehyde, it can be seen that the hydroacylation reaction rates depend on the autoxidation rate of the aldehyde, which in turn depends on the length of the aldehyde i.e. for the longer aldehydes decanal and hexanal, autoxidation rates were slower and therefore reaction rates were slower. However, too fast a rate of autoxidation leads to a smaller yield of addition product, as can be seen with isobutaraldehyde (Table 8, entry 1).



Entry	R =	Ratio of 140 : 211 after autoxidation (2h)	Reaction time (h) of hydroacylation to PFP-vinyl sulfonate in water	Yield of hydroacylation product 210 (Scheme 53) (%)
1	$-CH(CH_3)_2$	1:5.11	3h	40
2	$-CH(C_2H_5)(CH_2)_3CH_3$	1: 2.16	3h	83
3	$-CH_2CH(CH_3)_2$	1:1.20	3h	74
4	$-(CH_2)_2CH_3$	1:1.08	3h	78
5	$-{}^cC_6H_{11}$	1:0.66	3h	74
6	$-(CH_2)_4CH_3$	1:0.37	6h	75
7	$-(CH_2)_8CH_3$	1.0.04	6h	62

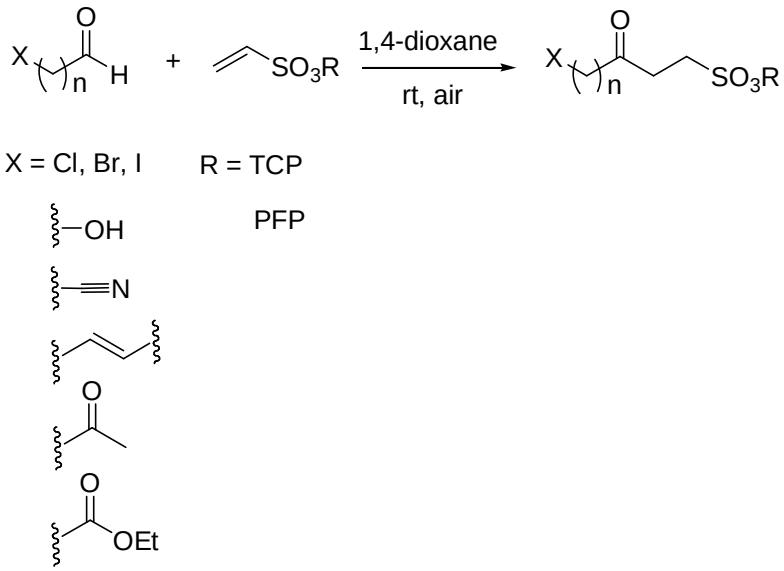
Table 8: Comparison of aldehyde autoxidation rates with hydroacylation reaction times.

Aerobic oxidation of aldehydes to their respective acyl radicals and subsequent reaction to give acids has been used synthetically by many researchers, including Shapiro *et al.*[87,88,89] They used the autoxidation of aldehydes to carry out the Passerini reaction on water. The hydroacylation of aldehydes to vinyl sulfonates discovered by Caddick *et al.* exploits the inherent tendency of aldehydes to be autoxidised by molecular oxygen in air.[82] Compared to radical hydroacylation reactions being carried out in which various reagents for acyl radical formation are needed (section 3.4), this reaction represents a 'green' reaction using only air as the oxidant for the formation of an acyl radical.

Chapter 4: Results and discussion

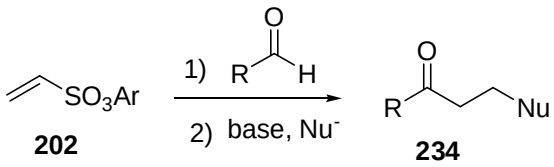
4.1 Aims

Caddick *et al.* have reported the hydroacylation of aldehydes to vinylsulfonates, *via* autoxidation of aldehydes in air.[82] The scope of aldehydes employed thus far is extremely limited, with no reports of the use of aldehydes bearing functional groups such as halides, esters, alcohol etc. The focus of this work is to investigate the tolerance of the aerobic hydroacylation of vinyl sulfonates to a range of functional groups, hence expanding the utility of this method. Functional groups that will be examined include halides, alcohols, alkene and alkynes, nitriles, esters and ketones (Scheme 56).



Scheme 56: Use of functionalised aldehydes in hydroacylation.

Hydroacylation of nucleophilic alkenes is sluggishand difficult to carry out (Section 3.3). [63,90] Another method of gaining entry to ketone **234** is *via* hydroacylation of vinylsulfonates **202**, followed by base catalysed elimination and conjugate addition (Scheme **57**). This is a new method of gaining entry to such compounds and the scope of the nucleophile will be investigated using oxygen, nitrogen, sulphur and phosphorus nucleophiles.



202 **234**

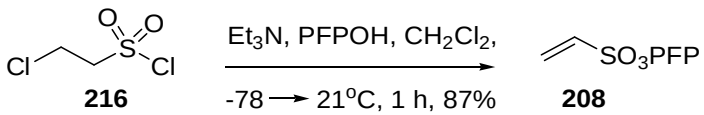
Scheme 57

Caddick et al. also report that the reaction of pivaldehyde led to the formation of **206** and the decarbonylated product (**207**, Figure 18) which is indicative of radical decarbonylation *via* aldehyde autoxidation, and addition to an acceptor. This autoxidation of aldehydes will be further investigated to gain entry to alkyl radicals *via* decarbonylation. Further capture of the alkyl radical by electron-deficient alkenes will be examined.

4.2 Functional group compatibility in hydroacylation

4.2.1 Functional group compatibility tests

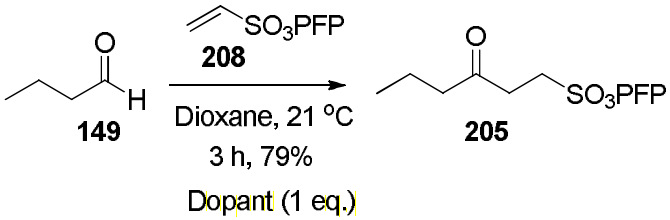
With the limited functional groups used in the hydroacylation reaction by Caddick *et al.* it was envisaged that functional group compatibility of aldehydes could be expanded.[82] Hence, PFP-vinyl sulfonate **208** was synthesised from **216** by the method of Caddick *et al.* in 87% yield (Scheme 56), and evaluated in hydroacylation with butanal, in order to rapidly evaluate functional group tolerance by simply doping the hydroacylation reaction with molecules containing potentially sensitive functional groups. A single equivalent of dopant was added, and conversion of PFP-vinyl sulfonate and yield of ketone **205** measured by ^{1}H NMR using pentachlorobenzene as an internal standard (Scheme 59, Table 9).



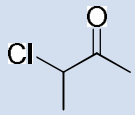
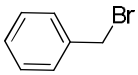
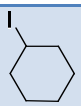
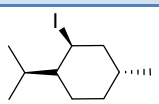
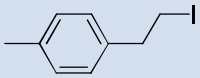
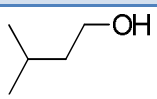
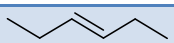
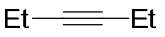
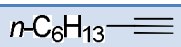
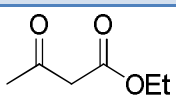
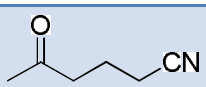
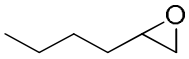
Scheme 58: Synthesis of PFP-vinyl sulfonate.

3-Chlorobutan-2-one and benzyl bromide had no effect on the reaction, whereas cyclohexyliodide inhibited the reaction (Table 9, entries 1-3). If the iodide from cyclohexyl iodide was abstracted under free-radical conditions then; cyclohexane should have formed and it would be of interest to isolate this by-product. However, because of its volatility it can be difficult to detect and hence another experiment using a less volatile species, menthyl iodide, was carried out and this appeared to slow the reaction down (Table 9, entry 4).[91] An alternative iodide, 1-(2-iodoethyl)-4-methylbenzene, also seemed to inhibit the reaction (Table 9, entry 5), and the iodides used seem to present no obvious pattern to inhibition of the reaction. Thus we are unable to make a definitive conclusion on the compatibility of the reaction to iodides.

3-Methylbutan-1-ol, (*E*)-hex-3-ene and hex-3-yne seemed to have no effect on the reaction, whereas a terminal acetylene, oct-1-yne, seemed to hinder the reaction (Table 9, entries 6-9). Ester, nitrile and epoxide functionalities also had no effect on the reaction (Table 9, entries 10-12). Incorporation of these functional groups on aldehydes with subsequent hydroacylation, would validate these doping experiments.



Scheme 59: Hydroacylation of butanal with PFP-vinyl sulfonate.

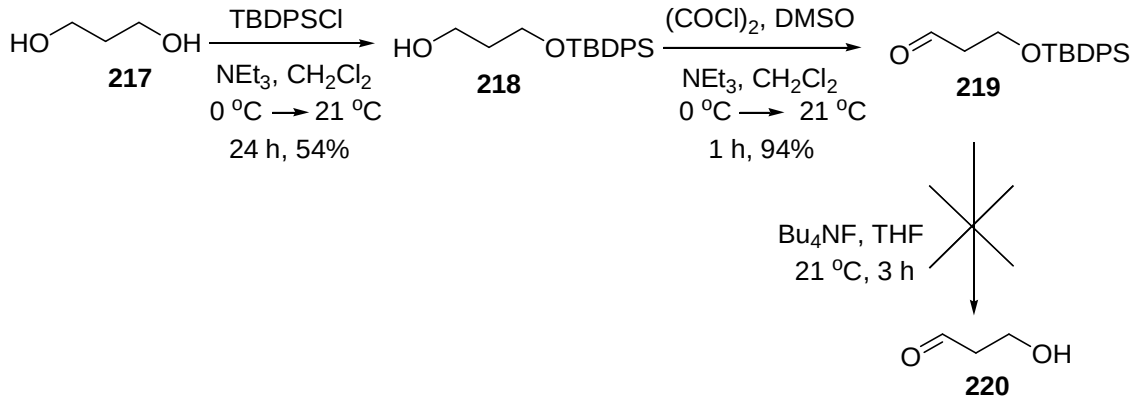
Entry	Dopant	PFP-vinyl sulfonate conversion (%)	Product yield (%)*
1		100	95
2		100	79
3		0	0
4		48	39
5		15	0
6		100	88
7		91	78
8		100	71
9		75	28
10		100	91
11		100	92
12		100	82

* ^{1}H NMR yields using pentachlorobenzene as internal standard

Table 9: Functional group compatibility testing.

4.2.2 Synthesis of functionalised aldehydes

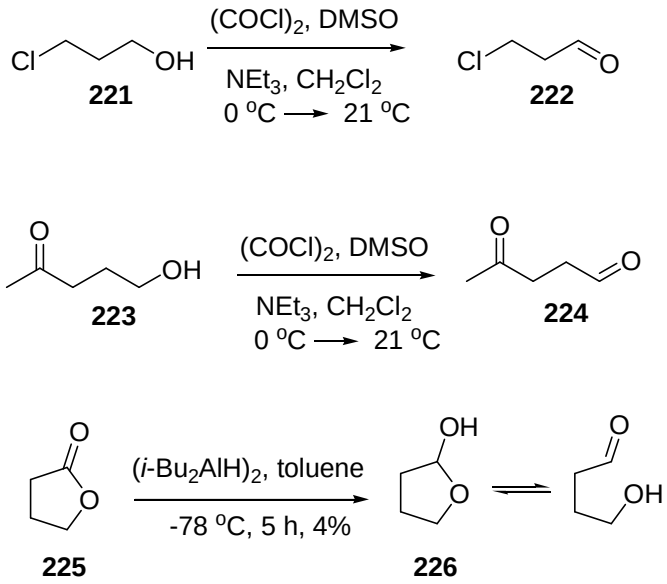
A strategy to functionalised aldehydes was *via* diol monoprotection, oxidation of the alcohol to the aldehyde and deprotection of the alcohol to reveal a hydroxy substituted aldehyde. Diol **217** monoprotection using TBDPS-Cl gave the protected alcohol **218** in 54% yield (Scheme 60). The alcohol **218** was oxidised to the aldehyde **219** using a Swern oxidation, in 94% yield. Subsequent deprotection of the silyl group with tetrabutyl ammonium fluoride failed to furnish the desired alcohol substituted aldehyde **220**.



Scheme 60: Synthesis of an alcohol substituted aldehyde.

With the success of the Swern oxidation it was perceived that alcohols containing functional groups could simply be oxidised to their corresponding aldehydes. Oxidation of 3-chloro-1-propanol **221** furnished the product aldehyde **222** in a disappointing 2% yield (Scheme 61).[92] However, the instability of the aldehyde led to its decomposition, most probably *via* an elimination to acrolein. The keto-alcohol 3-acetyl-1-propanol **223** was also oxidised *via* the Swern oxidation to the corresponding aldehyde **224**. However, this aldehyde was also extremely sensitive and decomposed when the solvent was being evaporated *in vacuo* after purification by flash chromatography. Hence, this synthetic approach was not successful and an alternative was sought.

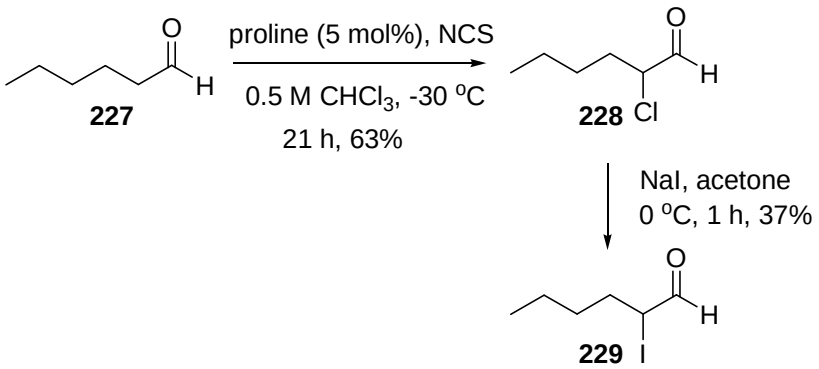
Another method for generation of an α-hydroxy aldehyde was by reduction of a lactone to a lactol.[93] Hence, γ-butyrolactone **225** was reduced to the lactol using DIBAL to give tetrahydrofuran-2-ol **226** in a disappointing 4% yield (Scheme 61).



Scheme 61: Synthesis of functionalised aldehydes by oxidation and reduction.

Enamine alkylation has been adopted by many chemists for the synthesis of chiral functionalised aldehydes. Macmillan *et al.* have carried out extensive research on enamine and iminium catalysis to form products resulting from α-halogenation, α-oxyamination, α-vinylation and others.[94,95,96] For example, organocatalytic α-chlorination of aldehydes was carried out using *N*-chlorosuccinimide (NCS) as the chlorinating agent.[97]

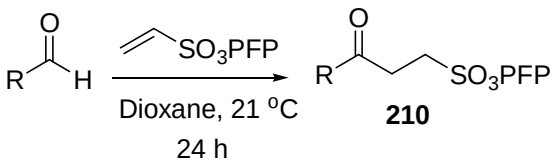
Using proline, NCS and hexanal **227**, an α-chlorinating reaction was carried out (Scheme 62). This reaction had to be carried out under carefully controlled conditions at -30 °C, and due to the sensitivity of the aldehyde the products had to be maintained at low temperature and solvents had to be evaporated ice-cold, to give α-chlorohexanal **228** in 63% yield. α-Chlorohexanal could further be converted to α-iodo-hexanal **229** on treatment with NaI, in 37 % yield.

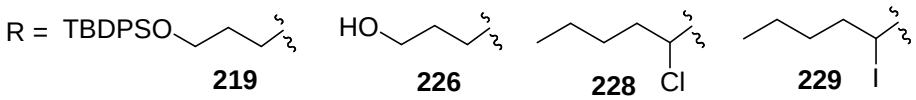


Scheme 62: Synthesis of α-chloro and α-iodo hexanal by method of MacMillan *et al*.

4.2.3 Hydroacylation of functionalised aldehydes

Synthesis of functionalised aldehydes proved quite difficult as aldehydes can be difficult to handle, and prone to self condensation or decomposition. The functionalised aldehydes **219**, **226**, **228**, **229** and the commercially available aldehyde **231** were treated with PFP-vinyl sulfonate in dioxane (Scheme 63 and 65). Treatment of the silyl-protected aldehyde **219** in dioxane with PFP-vinyl sulfonate at 21 °C showed no product formation after 3 days, and subsequent heating at 60 °C led to its decomposition. Reaction of the lactol **226** with PFP-vinyl sulfonate led to no reaction even after mixing for 5 days at 21 °C and reflux for 24 h.



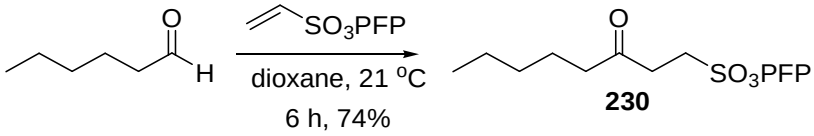


Scheme 63: Functionalised aldehydes tested in the radical reaction.

α-Chlorohexanal **228** was evaluated in the hydroacylation reaction with stirring for two days at 21 °C, then at 40 °C for two days, and then at reflux for 1 h. No product formation was observed. Crude ^{1}H NMR of the reaction mixtures suggested that elimination of HCl from aldehyde **228** had taken place. Doping the reaction with 3-chlorobutan-2-one (Table 9, entry 1) showed that the chlorinated molecule had no effect

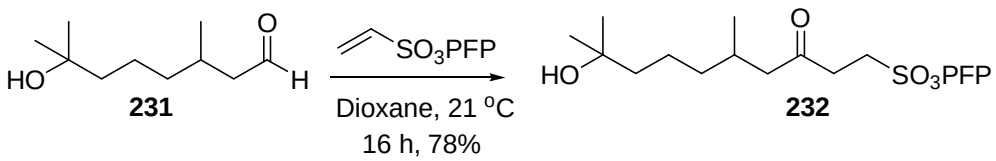
on hydroacylation, and treatment of hexanal in dioxane with PFP-vinyl sulfonate resulted in the formation of the addition product **230** in 74% after 6 h (Scheme 63), suggesting that the reaction with the α-chloroaldehyde should occur, hence having a chlorine at the α-position has an inhibitory effect on the autoxidation of the aldehyde.

Similarly, α-iodoaldehyde **229** failed to autoxidise and react with PFP-vinyl sulfonate in dioxane. The crude NMR did not show any change after 5 days at 21 °C. This suggests the iodide prevents autoxidation of aldehyde **229**, and hence addition to PFP-vinyl sulfonate. This was indeed indicated by the doping experiments with iodide molecules, which predicted that hydroacylation will be inhibited.



Scheme 64: Hydroacylation of hexanal.

The commercially available 7-hydroxycitronellal **231** underwent the addition leading to product **232** in 78% yield after 16 h (Scheme 65). This showed that the presence of an alcohol functionality can be tolerated and appears not to hinder the autoxidation of the aldehyde, or its subsequent addition to PFP-vinyl sulfonate. This is consistent with the doping experiments, which showed that the alcohol functional group is tolerated in radical hydroacylation.

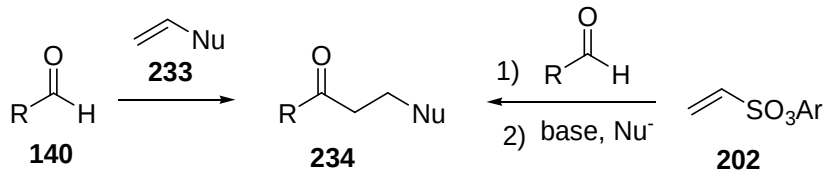


Scheme 65: Hydroacylation of 7-hydroxycitronellal.

4.3 *In situ* elimination and conjugate addition of ketosulfonate 205

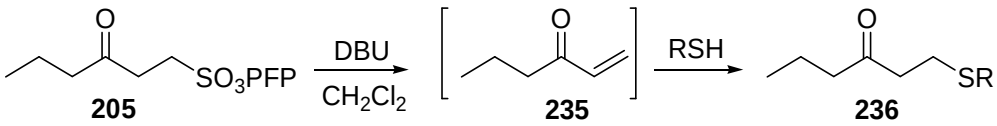
Acyl radicals, which are nucleophilic, add readily to electron poor alkenes but addition to neutral or electron rich alkenes **233** for the formation of ketone **234**, is sluggish and difficult to carry out (Section 3.3). [63,98] Another method of gaining entry to ketone **234** is *via* hydroacylation of vinylsulfonates **202**, followed by base catalysed elimination and conjugate addition (Scheme 66). This potentially circumvents the difficulty associated

with hydroacylation of electron rich alkenes, and can produce molecules which would otherwise be difficult to access.



Scheme 66: Formation of ketone **234**.

Caddick *et al.* showed that an aldehyde could be converted to an enone *via* hydroacylation and elimination.[83] Treatment of hydroacylation product **205** with DBU led to the formation of enone **235** which could be trapped *in situ* by thiols, to form β-keto sulfides **236** (Scheme 67). Enone synthesis from aldehydes usually requires oxidising conditions or metal reagents,[99,100] and hence this is a clean method of enone generation, where the subsequent enone can potentially undergo a wide range of conjugate addition reactions.[101]



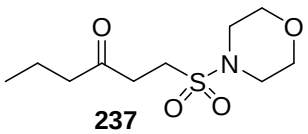
Scheme 67: Elimination and *in situ* conjugate addition.

Caddick *et al.* have carried this out with thiols, and it was envisaged that the set of nucleophiles could be utilised for conjugate addition to enone **235**, formed *in situ via* elimination of ketone **205**.[83] Nucleophiles that could be used for conjugate addition include nitrogen, oxygen, carbon, and phosphorus nucleophiles and an initial attempt with hexane thiol worked smoothly to furnish **243** in quanititative yield (Table 10, entry 1).

Treatment of ketone **205** with DBU and dimethyl malonate failed to produce any new spots by tlc after 24 h, and enone peaks were not visible in the crude ^{1}H NMR. Hence, DBU may not have been a suitable base to deprotonate the acidic proton from the malonate, or the enone may have polymerised before addition of malonate occurred. Other bases were hence tried; K_2CO_3 is used by Gervais *et al.* as a base for the addition

of malonate to enones at room temperature.[102] This base was used in methanol to promote the elimination and malonate deprotonation steps, furnishing conjugate addition product **244** in 23% yield after 3 h (Table 10, entry 2). However, use of NaH as the base resulted in no product formation, and the use of NaOEt in ethanol, led to the displacement of methyl esters to form product **245** in 11% yield (Table 10, entry 4). Campana *et al.* used sodium tetramethoxyborate to catalyse Michael reactions at room temperature.[103] Sodium tetramethoxyborate was readily synthesised by refluxing sodium borohydride in methanol and used in the conjugate addition of malonate to enone **205**, which furnished the product **244** in 63% yield over 24 h (Table 10, entry 3).

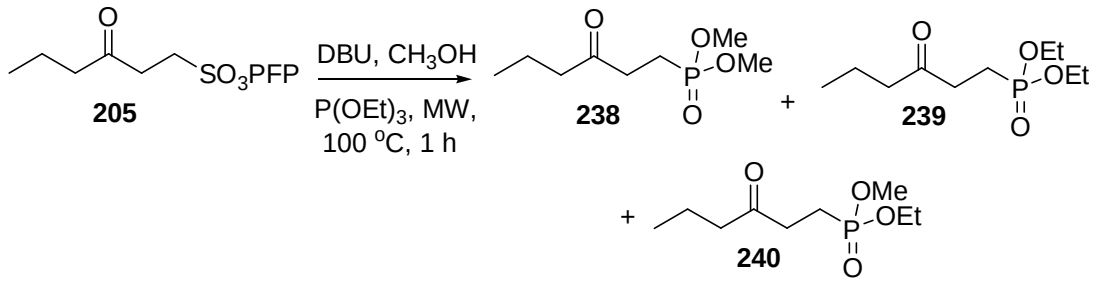
Two nitrogen nucleophiles were evaluated, a good nitrogen nucleophile, morpholine, and a weaker nucleophile, aniline. Reaction with morpholine yielded an impure addition product after 1 h, which could not be purified to homogeneity by flash chromatography, the impurity was presumed to be the sulfonamide **237** arising from the attack of the morpholine of the PFP-sulfonate moiety (Figure19). Hence, to circumvent sulfonamide formation, DBU and ketone **205** were first stirred for 20 minutes before the addition of morpholine, and this led to the clean formation of **246** in 80% yield after 1 h (Table 10, entry 5). Conjugate addition with aniline did not go to completion after 24 h. The absence of enone peaks in the crude NMR, suggested that the enone may have polymerised before the aniline could add, hence the conjugate addition product **247** was isolated in 38% yield (Table 10, entry 6).



237

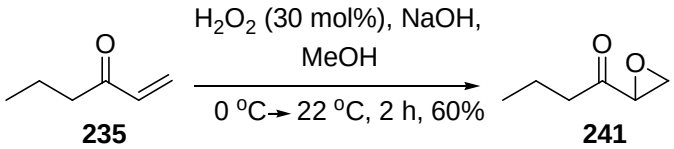
Figure 19: Possible sulfonamide from attack of morpholine on PFP-sulfonate.

Triethylphosphite was used in an Arbuzov-type reaction. Treatment of ketone **205** with DBU in triethylphosphite in methanol heated at 100 °C under microwave heating conditions, gave 77% of a mixture of products **238**, **239** and **240** (Scheme 68). Hence carrying the reaction out in ethanol led to the clean formation of product **239** in 90% yield (Table 10, entry 8).



Scheme 68: Mixture of product from reaction in methanol.

Peroxide can act as an oxygen nucleophile and adds to conjugated systems to form peroxides. For example, Antczak *et al.* reacted 3-butenone with hydrogen peroxide and NaOH to get the epoxy-ketone **241** in 60% yield (Scheme 69).[104] However, treatment of ketone **205** with DBU, with H_2O_2 and NaOH did not succeed in forming the epoxy-ketone product.

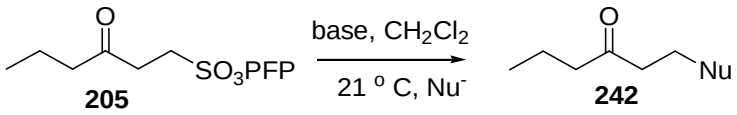


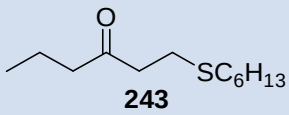
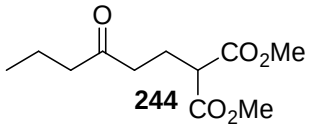
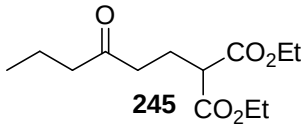
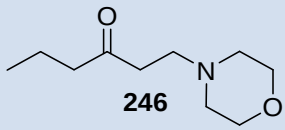
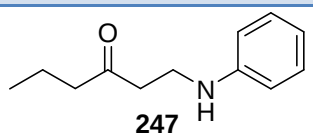
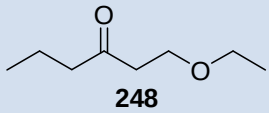
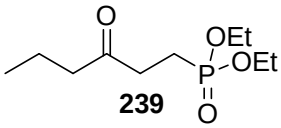
Scheme 69: Epoxide formation *via* conjugate addition.

We also considered sodium ethoxide as an alternative oxygen nucleophile which might add to enone **235**. Treatment of ketone **205** with DBU and NaOEt in ethanol led to the formation of the addition product **248** in 41% by NMR. However, the product could not be isolated as the product seemed to decompose on silica (Table 10, entry 7).

Hence, these reactions show carbon, nitrogen, phosphorus and oxygen nucleophiles are successful in conjugate addition reactions of enone **235**, generated *in situ* from the sulfonate **205**. Only a limited number of hydroacylations of vinyl phosphonates and enamides have been carried out successfully. For example, treatment of butanal with benzoyl peroxide and diethylvinyl phosphonate by Rakhimov *et al.*, led to the formation

of the hydroacylation product in 54% yield after 8 h of heating.[105] Hence, the present approach utilising, the elimination and conjugate addition approach by triethylphosphate, seems a much faster and higher yielding reaction as compared to the hydroacylation of electron rich vinyl phosphonate. Roberts *et al.* used a thiol catalyst methylthioglycolate for hydroacylation of butanal with enamides *N*-vinylpyrrolidin-2-one and *N*-vinylphthalimide in 62% and 50% yields repectively.[106] Hence, conjugate addition to enone **235** with nitrogen nucleophiles is a complimentary method to existing methods based on direct hydroacylation reaction of electron rich enamides. The results with triethylphosphite and morpholine and aniline, show that this method can be used for the formation of products that would otherwise be difficult to generate by direct hydroacylation of electron-rich alkenes.



Entry	Nucleophile	Base	Solvent	Time (h)	Product yield (%)	yield
1	$C_6H_{13}SH$	DBU	DCM	1	 **243**	>99
2	Dimethyl malonate	K_2CO_3	MeOH	3	 **244**	23
3	Dimethyl malonate	DBU, $NaB(OMe)_4$	MeCN	24	**244**	63
4	Dimethyl malonate	NaOEt	EtOH,	48	 **245**	11
5	Morpholine	DBU	DCM	1.2	 **246**	80
6	$PhNH_2$	DBU	DCM	24	 **247**	38
7	NaOEt in EtOH	DBU	EtOH	1	 **248**	38[1]
8	$P(OEt)_3$	DBU	EtOH	1[2]	 **239**	90

[1]Based on internal standard pentachlorobenzene [2]Heated in MW at 100 °C

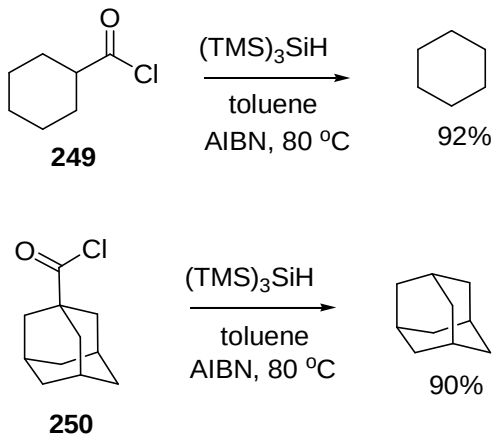
Table 10: Elimination and conjugate addition by different nucleophiles.

4.4 Pivaldehyde decarbonylation

4.4.1 Decarbonylation of acyl radicals

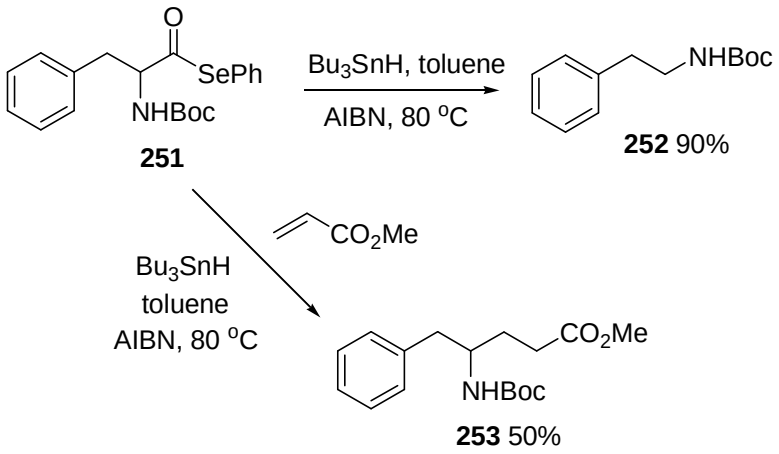
One of the difficulties associated with the synthetic utility of acyl radicals is their propensity to undergo decarbonylation to generate alkyl radicals. For decarbonylation to occur, it must be faster than the rate of acyl radical reduction to the aldehyde, and acyl radical addition to an acceptor, and the ease of decarbonylation depends on the stability of the newly generated radical.

Decarbonylation of acid chlorides and selenoesters occur with direct reduction to give the corresponding alkane, with tributyl tinhydride or trimethylsilylhydride as reducing agents.[63] For example, Chatgilialoglu *et al.* treats acid chlorides **249** and **250** with $(TMS)_3SiH$ to furnish the secondary and tertiary alkanes cyclohexane and adamantane in 92% and 90% yields respectively (Scheme 70).[107]



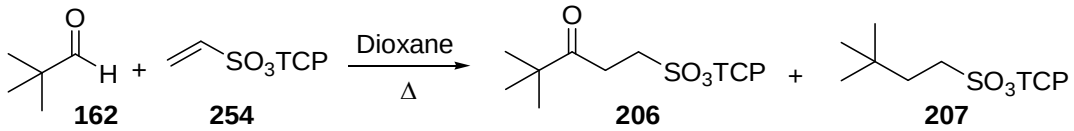
Scheme 70: Decarbonylation of acid chlorides.

An example of decarbonylation of a selenoester is demonstrated by Stojanovic *et al.* who show that the α-amino phenyl selenoester **251** can be reduced to the corresponding alkane **252** under tributyltin hydride conditions in 90% yield, and the alkyl radical can be captured by methyl acrylate to furnish **253** in 50% yield (Scheme 71).[108]



Scheme 71: Decarbonylation of selenoester.

Although authors have used acyl radical precursors for the formation of acyl radicals (Section 3.4) and the subsequent decarbonylation to alkyl radicals, for the formation of alkyl compounds, Caddick *et al.* reported the reaction of pivaldehyde with TCP-vinyl sulfonate **254** in air, which resulted in the a 1:8 mixture of alkylated:acylated product **207** and **206** at 50 °C (Scheme 72).[82] Selectivity as high as 1:11 of **206**:**207** could be achieved at 60 °C, and the authors propose that at higher temperatures, the unimolecular decarbonylation of the acyl radical would be accelerated.

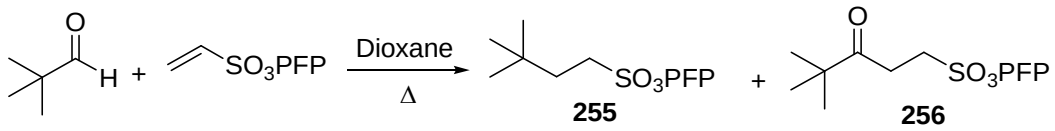


Scheme 72: Pivaldehyde **162** addition to TCP-vinyl sulfonate **254**.

4.4.2 Decarbonylation of pivaldehyde

The aim of this work was to show that it can be possible to use aldehyde autoxidation of aldehydes as a route to alkyl radicals *via* decarbonylation, and initial investigations concentrated on attaining a higher selectivity for this reaction *via* complete decarbonylation to the *t*-butyl radical, and thence capture by PFP-vinyl sufonate. Optimisation of this reaction under thermal conditions was carried out in water with two equivalents of aldehyde to alkene, and 0.05 mol% peroxide but these conditions failed to give complete decarbonylation of pivaldehyde (Table 11, entries 1-3). Selectivity as high as 23:1 for the **255**:**256** product was achieved at 100 °C using 5 equivalents of

pivaldehyde to 1 equivalent of PFP-vinyl sulfonate in dioxane, and an isolated yield of 70% (Table 11, entry 5).



Entry	Temperature (°C)	Solvent	Initiator	Ratio of 255:256
1	75	H_2O	H_2O_2	4:1
2	100	H_2O	H_2O_2	7:1
3	125	H_2O	H_2O_2	9:1
4	75	dioxane	–	16:1
5	100	dioxane	–	23:1

Table 11: Optimisation of decarbonylation of pivaldehyde.

4.4.3 Optimisation of reaction in microwave

When carried out under microwave heating at 100 °C for 3 h, the reaction does not proceed to completion due to pressure build-up in the microwave tube (Table 12). However, repeated reactions in the microwave for 10 mins x 3, with venting after each 10 min, led to the complete consumption of the PFP-vinyl sulfonate. This suggested that high CO pressure in the reaction vessel from the decarbonylation of the pivaldehyde prevented the forward reaction. The reaction was further carried out in the microwave in 1 min intervals with regular venting. After 7 x 1 min the reaction was complete with a 65% yield of only the alkylated product **255**.

Aldehyde scale	Time (h)	Temperature (°C)	PFP-vinyl sulfonate conversion[1]	% Yield[2] (255:256)
1 mmol Scale	3 h	100	35	–
	1 h	150	40	45% (1:0.04)
	3 x 10 min	100	100	–
	30 min	100	30	–
	10 x 1 min	100	100	61% (1:0)
	7 x 1 min	100	100	65% (1:0)
0.25 mmol	10 min	100	100	–
	5 min	100	100	–
	2 min	100	100	80% isolated (1:0)

[1] tlc monitoring of PFP-vinyl sulfonate consumed [2] NMR yields using pentachlorobenzene as an internal standard unless otherwise stated.

Table 12: Optimisation of pivaldehyde deacrbonylation in the microwave.

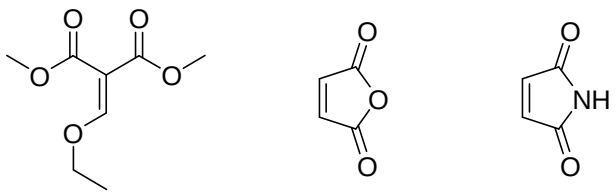
Scaling down the reaction to 0.25 mmol of pivaldehyde led to further optimisation of the reaction at 2 mins, with an isolated yield of 80% alkylated product. Scaling down the amount of pivaldehyde used kept the CO pressure in the microwave tube low enough for the reaction to proceed to completion.

The microwave reactions showed that the reaction needed an open vessel, or a large enough vessel to fill the overhead space with the carbon monoxide produced. However, in order to produce alkyl radicals from acyl radicals under microwave conditions there was a clear requirement for venting the reaction mixture, and despite the reduced reaction time and good yield, it was felt that this protocol was not particularly practical. Hence, the thermal reaction optimised at 100 °C with 5 equivalents of pivaldehyde and one equivalent of PFP-vinyl sulfonate in dioxane, was chosen to investigate *t*-butyl radical addition to different acceptors (Table 11, entry 5).

4.4.4 Investigations on the reaction of *tert*-butyl radical addition reactions using pivaldehyde as a precursor

Addition of the *t*-butyl radical to electron deficient alkenes was evaluated using the aforementioned thermal conditions. The *t*-butyl radical added to PFP and TCP-vinyl sulfonates in relatively good yields, 70% and 64% respectively at 100 °C after 3 h (Table 13, entries 1 and 2). The addition to ethyl vinyl sulfone is relatively fast compared to all the other alkenes used, proceeding to completion in only 40 min to furnish **261** (Table 13, entry 5).

Addition to maleate and fumarates required longer reaction times of, 6 and 7 h, and addition to dimethyl maleate is relatively low yielding furnishing **164** in 46% yield (Table 13, entries 6-8). *t*-Butyl addition to dimethylvinyl phosphonate first led to a 34% yield of **264**, and took 4 h for 100% conversion of the phosphonate, however purification of the compound from its polymer residue led to an increase in yield to 50% in 2.5 h (Table 13, entry 10). Addition to dimethyl 2-ethylidenemalonate only proceeded with a 10% yield of **263** and needed an increase in temperature and reaction time (Table 13, entry 9). This is presumably due to steric hindrance provided by the allyl group on the malonate. Further steric bulk on the malonate on compound **265** (Figure 20), appears to prevent the addition of the *t*-butyl radical altogether. Addition to maleic anhydride **266,** and maleimide **267**, does not occur, and polymeric residue is observed in these reactions presumably due to polymerisation of these compounds.

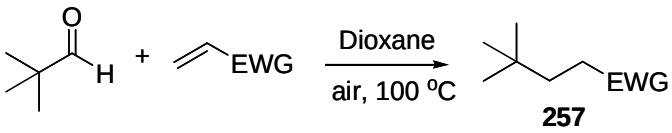


265 **266** **267**

Figure 20: Acceptors for *t*-butyl addition.

These results are comparable to literature methods for the addition of the *t*-butyl group to vinylsulfones and sulfonates, which include tin-mediated *t*-butyl iodide addition to TCP-vinyl sulfonate in 88% yield, and in a 64% yield under EPHP-BEt_3/air mediated conditions.[109] Addition of *t*-butyl to vinyl sulfones, sulfonates, and maleate is slightly lower than those achieved by Zhao *et al.* using Zn/Cu reagents and by Esposti *et al.* who

uses decatungstate agent TBADT.[68,122] However, considering the only reagent used in the reaction is air, these reactions are atom economical and avoid waste production or expensive reagents. Hence, although the addition reaction of the *t*-butyl group derived from pivaldehyde are lower yielding, they are advantageous in that they don't require metal reagents, especially tin which is toxic and difficult to get rid of, and hence this method of *t*-butyl addition is complimentary to current methods in the literature.



257

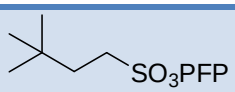
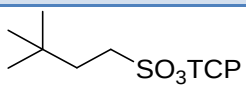
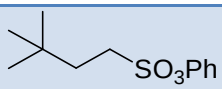
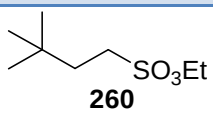
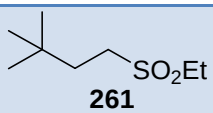
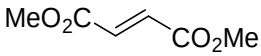
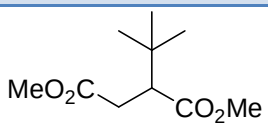
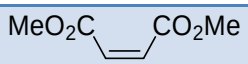
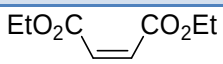
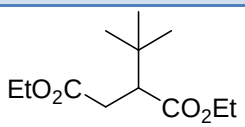
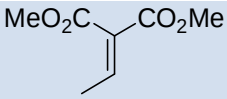
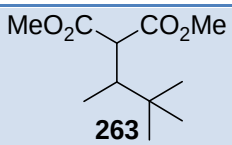
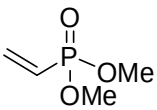
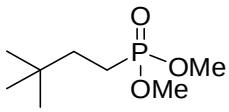
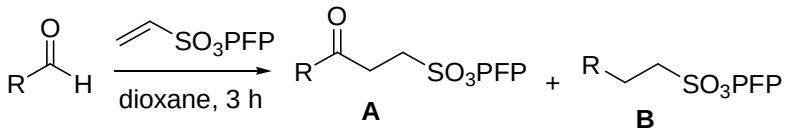
Entry	Acceptor	Time (h)	Decarbonylated product	yield (%)
1		3	 **258**	70
2		3	 **207**	64
3		3	 **259**	76
4		4	 **260**	54
5		0.66	 **261**	53
6		6	 **164**	63
7		6	**164**	46
8		7	 **262**	55
9		120	 **263**	10
11		2.5	 **264**	50

Table 13: Addition of *t*-butyl radical to electron-deficient alkenes.

4.4.5 Secondary aldehydes

With the success of the decarbonylation of pivaldehyde to the *t*-butyl radical and its subsequent addition to alkenes, it was considered that decarbonylation of secondary aldehydes may provide a viable route to secondary alkyl radicals. Three secondary aldehydes, cyclohexanecarbaldehyde **269**, 2-ethylhexanal **270**, and 2-phenyl propanal **271**, were screened for decarbonylation and capture by PFP-vinyl sulfonate at 100 °C (Table 14). At 100 °C aldehyde **271** failed to decarbonylate, aldehydes **269** and **270** showed more promising results with decarbonylated products seen in the crude NMR. Addition product from the cyclohexyl radical seemed to decrease with temperature. Addition products from the 2-ethyl hexane radical seemed to form both the alkyl and acyl addition products without much selectivity. Hence, it was clear that this method was not efficient for decarbonylation for secondary aldehydes.



	**Temperature (°C)**	**A(acyl)***	**B(alkyl)***
269 R = *c*-C_6H_6	100	0	46
	130	0	38
270 R = $C_4H_9CH(C_2H_5)$	100	3	4
	130	13	15
271 R = $PhCH(CH_3)$	100	0	0

*Percentage isolated

Table 14: Decarbonylation of secondary aldehydes.

4.5 Summary

Functional group compatibility on the aldehyde in the hydroacylation reaction was carried out. In the first instance experiments using small molecule dopants, predicted iodo and acetylene functionalities would hinder hydroacylation, and a large number of dopants containing alcohol, keto and ester functionalities did not effect hydroacylation (Table 9). Synthesis of functionalised aldehydes proved difficult, with decomposition observed in short chain aldehydes. However, Swern oxidation of an alcohol provided a silyl protected aldehyde **219** and DIBAL reduction provided a lactol **226**, unfortunately neither of these were successful in autoxidation/ hydroacylation. Enamine alkylation led to the synthesis of α-iodo and α-chloro hexanal, which did not seem to autoxidise or add to PFP-vinyl sulfonate in dioxane. Commercially available 7-hydroxycitronellal did, however add readily to PFP-vinyl sulfonate in a 78% yield demonstrating the feasibility of autoxidation of functionalised aldehydes.

Hydroacylation of pivaldehyde led to two products, the addition of the acyl radical as well the addition of the decarbonylated t-butyl radical. Further investigations into hydroacylation of pivaldehyde led to the optimisation of the thermal decarbonylation of pivaldehyde at 100 °C. Microwave optimisation also led to the optimisation of this decarbonylation after just two minutes with an 80% isolated yield when carried out on a small scale. However, neither the protocol or scale were practical for synthetic use. Hence, thermal conditions were used to add the *t*-butyl radical to a number of different acceptors in relatively good yields. This is a novel and extremely simple method of attaching the t-butyl moiety on to a wide range of alkenes including vinyl sulfonates, malonates and vinyl phosphonates. These results are as yet unpublished and is complementary to literature methods used for the addition of *t*-butyl species using metal reagents.

The hydroacylation product ketone **205** undergoes elimination *in situ* and conjugate addition by nucleophiles. Conjugate additions were carried out using carbon, nitrogen, oxygen and phosphorus nucleophiles, and establishes this approach as an effective method for the synthesis of products which are the products derived from formal hydroacylation of electron-rich alkenes.

Functional group compatibility experiments using small molecule dopants, predicted iodo and acetylene functionalities would hinder hydroacylation. Synthesis of functionalised aldehydes proved difficult, with decomposition observed in short chain aldehydes. However, Swern oxidation of an alcohol provided a silyl protected aldehyde and DIBAL reduction provided a lactol, unfortunately neither of these were successful in autoxidation/ hydroacylation. Enamine alkylation led to the synthesis of α-iodo and α-chloro hexanal, which did not seem to autoxidise or add to PFP-vinyl sulfonate in dioxane. Commercially available 7-hydroxycitronellal did, however add readily to PFP-vinyl sulfonate in a 78% yield demonstrating the feasibility of autoxidation of functionalised aldehydes.

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The hydroacylation product ketone **205** undergoes elimination *in situ* and conjugate addition by nucleophiles. This was carried out using carbon, nitrogen, oxygen and phosphorus nucleophiles, and establishes this approach as an effective method for the synthesis of products which are the products derived from formal hydroacylation of electron-rich alkenes.

Chapter 5: Experimental

5.1 Biology for Chapter 2

Tissue Culture: DDAH1 and D2M2 cells (Skin Endothelial cells over-expressing Myc-tagged DDAH1 and Myc-tagged DDAH2, respectively) were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 1 mM glutamine, 10% FBS and penicillin in a humidified incubator at 37 ^{o}C with 5% CO_2. The cells were divided every 2 or 3 days, when they became confluent.

Immunoprecipitation of DDAH1 and DDAH2: Immunoprecipitation of Myc-DDAH1 and Myc-DDAH2 was achieved with a ProFound mammalian c-Myc Tag IP/co-IP Kit (Pierce Biotechnology, Rockford, IL, USA). The cells from T75 flasks (x 10) were collected and lysed in 1 ml of lysis buffer (supplied with the kit). Lysates were then incubated with anti-Myc antibody (overnight at 4 °C), and then after washes with TBS-T, the protein was eluted with gentle elution buffer (Pierce Biotechnology, Rockford, IL, USA).

Protein Assay: Six serial dilutions of standard protein were made by dilutions with PBS to give a standard curve: 0.5 µM, 0.4 µM, 0.25 µM, 0.20 µM, 0.10 µM, 0.05 µM, 0 µM. The standard protein and the cell lysate were added to a 96 well plate and 200 µL of Bradford Reagent (Biorad, Hemel Hempstead, UK) was added. The absorbance was measured at 630 nm using a plate reader and the protein concentration of each sample were calculated.

Preparation of Homogenates: The rat kidneys were homogenised by crushing under liquid nitrogen using a mortar and pestle that had been already placed in -80 ^{o}C. The homogenised tissues were then resuspended in ice-cold PBS with Protease Inhibitor (Roche, Hertfordshire, UK). The homogenates were then ultracentrifuged for 40 min, at 4 ^{o}C, >50,000 RPM. After spinning, the fat from the supernatant was removed, and the supernatant (protein lysate) removed into a new tube and analysed for DDAH activity. CH_2Cl_2

DDAH Assay: For DDAH assays, [^{14}C]-L-NMMA was used as a substrate and [^{14}C]-citrulline was detected as a product. Concentrations of 1 µM, 10 µM, 30 µM, 100 µM,

300 μM and 500 μM of the DDAH inhibitors, L291, **22** and **24**, were added to 50 μL of rat kidney tissue lysate or to the purified DDAH1 and DDAH2. The standard assay mixture contained 1500 μL PBS, 3 μL L-NMMA (100 mM stock) and 1.5 μL ^{14}C L-NMMA. 50 μL of the assay mixture was added to all the lysate and PBS samples, and then the reaction was initiated by incubation at 37 ^{o}C and 4 °C for 1 hr. All assays were performed in triplicate. The reaction was then terminated by the addition of 500 μL of cation exchange resin (Dowex 50wx8, pH 7.0). The samples were vortexed and centrifuged at 13000 rpm for 3 min. 100 μL of the supernatant was then taken and mixed with 5 mL of liquid scintillation fluid and the [^{14}C] L-Citrulline content was determined by using B-scintillation counting (Hewlett Packard).

5.2 Chemistry

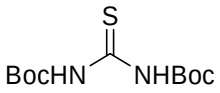
5.2.1 General Experimental

All reagents and solvents were used as received without further purification, unless otherwise stated. Reactions were monitored by TLC on Merck SIL G/UV_{254} plates purchased from VWR and were visualised under UV lamp operating at short and long wavelength ranges with alkaline potassium permanganate solution. Flash column chromatography was carried out with Kieselgel 60M 0.04/0.063nm (230-400 mesh) silica gel. All yields quoted are isolated yields. All reactions were carried out at room temperature (rt) unless otherwise stated.

Instrumentation: Microwave reactions were carried out in the CEM Discover™ system. ^{1}H NMR spectra were recorded at 300 MHz, 400 MHz, 500 MHz and 600 MHz and ^{13}C NMR at 75 MHz, 100 MHz, 125 MHz and 150 MHz on a Bruker AMX300, AMX400, AMX500 and AMX600 respectively at ambient temperature unless otherwise stated. The chemical shifts (δ) for ^{1}H and ^{13}C are quoted relative to residual signals of the solvent on the ppm scale. Coupling constants (*J* values) are reported in Hertz (Hz). Multiplicies for ^{1}H NMR are reported as singlet (s), doublet (d), triplet (t), quartet (q), sextet, broad (br) and multiplet (m); or some combination of these. Multiplicies for ^{13}C NMR were determined using distortionless enhancement by phase transfer (DEPT) spectral editing technique and are reported as singlet (s), doublet (d), triplet (t), quartet (q), based on the anticipated multiplicity in a non-decoupled spectrum. **For compounds bearing a pentafluorophenyl the ^{13}C NMR signals from this group are not reported due to a high degree of C-F coupling. Mass spectra were obtained on a VG70-SE mass spectrometer, a MAT 900 XP spectrometer or at National Mass Spectrometry Service, Swansea. Infrared spectra were obtained on a Shimadzu FTIR-8700 Fourier Transform Infrared Spectrophotometer or Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode. Melting points were measured with a Gallenkamp apparatus and are uncorrected. Elemental analysis was performed at the Department of Chemistry, University College London.

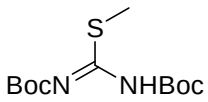
5.2.2 Experimental for Chapter 2

1,3-Bis(*tert*-butoxycarbonyl) thiourea[110] **45**



NaH, 60% in mineral oil (1.94 g, 80.9 mmol, 4.5 eq.) was added to a stirred solution of thiourea (1.37 g, 17.9 mmol, 1 eq.) in dry THF (250 mL) under argon at 0 °C. The ice bath was removed after 5 min and the reaction mixture was stirred at rt for 10 min. The reaction mixture was cooled to 0 °C again, di-*tert*-butyl dicarbonate (4.35 g, 39.6 mmol, 2.2 eq.) was added, and the ice bath was removed after 30 min of stirring at that temperature. The resulting slurry was stirred for another 2 h at 21 °C and quenched with saturated $NaHCO_3$ solution (70 mL). The reaction mixture was poured onto water (350 ml) and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography eluting with petrol/Et_2O (1/1) gave the title compound as a white solid (2.3 g, 45% yield): m.p. 102-106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (br s, 2H), 1.51 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7 (s), 150.3 (s), 84.1 (s), 27.9 (q); IR (neat) 3178, 2983, 1770, 1720 cm^{-1}; LRMS (ES$^+$) 299 (10%, [M+Na]$^+$), 199 (12), 176 (100), 154 (23).

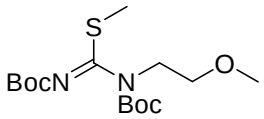
1,3-Bis-(*tert*-butoxycarbonyl)-*S*-methyl-thiopseudourea[111] **43**



Solid K_2CO_3 (7.9 g, 57 mmol) and MeI (2.2 mL, 36 mmol) was added to a solution of 1,3-bis(*tert*-butoxycarbonyl) thiourea **45** (8.0 g, 28 mmol) in dry acetonitrile (250 ml) and refluxed at 60 °C for 1 h. The reaction mixture was filtered under vacuum and the solvent removed *in vacuo*. Purification by flash chromatography eluting with petrol/Et_2O (9/1) gave the title compound as a white solid (6.83 g, 84% yield): m.p. 126-128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.10 (s, 1H), 2.37 (s, 3H), 1.53 (s, 9H), 1.51

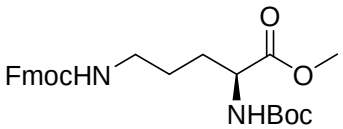
(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (s), 160.7 (s), 150.7 (s), 83.2 (s), 80.9 (s), 28.0 (2 x q), 14.4 (q): IR (neat) 3100, 2983, 1747, 1651 cm^{-1}; LRMS (EI) 290 (19% $[M]^{+\bullet}$), 234 (68), 178 (91), 161 (100), 134 (56).

1,3-Bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea 95



2-Methoxy ethanol (1.7 mL, 21.7 mmol) and PPh_3 (5.4 g, 21.7 mmol) were added to a solution of 1,3-bis-(*tert*-butoxycarbonyl)-*S*-methyl-thiopseudourea **43** (4.2 g, 14.4 mmol) in THF (75 mL). The solution was cooled to 0 °C and treated dropwise with Diethyl azodicarboxylate (3.4 mL, 21.7 mmol). The reaction was further stirred at 0 °C for 30 min and then at 21 °C for 24 h. The solvent was removed *in vacuo* and purification by flash chromatography eluting with petrol/EtOAc (9/1) gave the title compound as a colourless oil (7.74 g, 94% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (t, J = 6.0 Hz, 2H), 3.55 (t, J = 6.0 Hz, 2H), 3.33 (s, 3H), 2.39 (s, 3H), 1.50 (s, 9H), 1.47 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4 (s), 158.0 (s), 151.9 (s), 82.5 (s), 81.9 (s), 70.0 (t), 58.6 (q), 47.8 (t), 28.0 (2 x q), 15.6 (q); IR (neat) 2979, 2931, 1716, 1616 cm^{-1}; LRMS (ES^+) 349 (100%, $[M+H]^+$), 193 (7), 101 (95); HRMS (ES^+) calc. For $C_{15}H_{29}O_5N_2S$ 349.1797 $[M+H]^+$, found 349.1799.

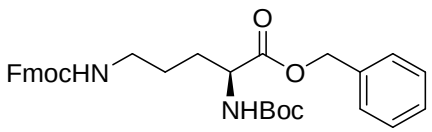
(S)-Methyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanoate 91



Triethylamine (0.37 mL, 2.64 mmol) and MeI (0.16 mL, 2.64 mmol) were added to a stirred solution of Boc-L-Orn(Fmoc)-OH (400 mg, 0.88 mmol) in CH_2Cl_2 (5 mL) and the reaction was stirred for 24 h. The solvent was removed *in vacuo*. Purification by

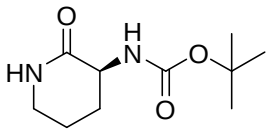
flash chromatography eluting with petrol/Et_2O (1/2) gave the title compound as a white solid (391 mg, 95% yield): m.p. 120-122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 6.0 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H), 7.42-7.37 (m, 2H), 7.33-2.25 (m, 2H), 5.07 (br s, 1H), 4.91 (br s, 1H), 4.40 (d, J = 9.0 Hz, 2H), 4.32-4.30 (m, 1H), 4.20 (t, J = 9.0 Hz, 1H), 3.73 (s, 3H), 3.22-3.12 (m, 2H), 1.82-1.59 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (s), 156.5 (s), 155.5 (s), 143.9 (s), 141.3 (s), 127.7 (d), 127.0 (d), 125.1 (d), 119.9 (d), 80.0 (s), 66.5, (t), 66.0 (t), 52.4 (q), 47.3 (d), 40.5 (t), 30.3 (d), 28.3 (q), 25.9 (t) ; IR (neat) 3361, 2961, 2925, 1744, 1690 cm^{-1}; LRMS (ES^+) 491 (100%, $[M+Na]^+$), 435 (94), 391 (89); HRMS (ES^+) calc. For $C_{26}H_{32}O_6N_2Na$ 491.2158 $[M+Na]^+$, found 491.2169.

(*S*)-Benzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanoate 92



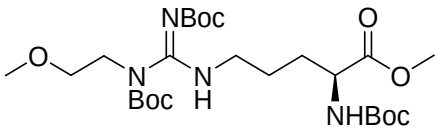
Triethylamine (0.18 mL, 1.32 mmol) and benzylbromide (0.16 mL, 1.32 mmol) were added to a solution of Boc-L-Orn(Fmoc)-OH (0.2 g, 0.44 mmol) in CH_2Cl_2 (5 mL) and the reaction was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with sat. $NaHCO_3$ (2 x 5 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent evaporated *in vacuo.* Purification by flash chromatography eluting with petrol/Et_2O (1/2) gave the title compound as a white solid (0.22 g, 91% yield): m.p. 91-93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.0 Hz, 2H), 7.58 (d, J = 7.0 Hz, 2H), 7.42-7.28 (m, 9H), 5.22-5.14 (m, 3H), 4.83 (br s, 1H), 4.40-4.38 (m, 3H), 4.2 (t, J = 7.0 Hz, 1H), 3.20-3.15 (m, 2H), 1.92-1.82 (m, 1H), 1.65-1.61 (m, 2H), 1.49-1.46 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4 (s), 156.5 (s), 155.5 (s), 144.0 (s), 141.4 (s), 135.4 (s), 128.7 (d), 128.6 (d), 128.52 (d) 127.7 (d), 127.1 (d), 125.1 (d), 120.0 (d), 80.0 (s), 67.1 (t), 66.5 (t), 53.2 (d), 47.3 (d), 40.4 (t), 30.0 (t), 28.3 (q), 25.8 (t); IR (neat) 3500, 3055, 2985, 1716 cm^{-1}; LRMS (FAB) 545 (10 % $[M+H]^+$), 445 (24), 338 (35), 178 (100): HRMS (FAB) calc. for $C_{32}H_{37}O_6N_2$ 545.2651 $[M+H]^+$, found 545.2634.

(S)-*tert*-Butyl 2-oxopiperidin-3-ylcarbamate[112] **98**



Piperidine (1 mL) was added to a stirred solution of (*S*)-benzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanoate **92** (145 mg, 0.27 mmol) in DMF (3 mL) and stirred for 1 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with $CHCl_3$/MeOH (99/1) gave the title compound as a clear oil (57 mg, >99% yield): ^{1}H NMR (300 MHz, D_2O): δ 3.98 (br s, 1H), 3.31-3.28 (m, 2H), 2.13-2.06 (m, 1H), 1.97-1.89 (m, 3H), 1.45 (s, 9H); ^{13}C NMR (300 MHz, D_2O): δ 173.8 (s), 157.8 (s), 82.0 (s), 50.8 (d), 42.0 (t), 28.0 (q), 27.6 (t), 21.0 (t); IR (neat) 3287, 2974, 1705, 1662 cm^{-1}; LRMS (ES$^+$) 237 (100% $[M+Na]^+$), 176 (23%); HRMS (ES$^+$) calcd for $C_{10}H_{18}N_2O_3Na$ $[M+Na]^+$ 237.1215, observed 237.1211.

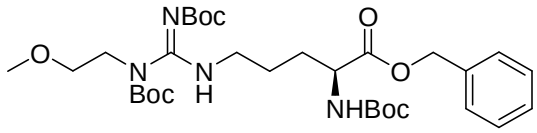
(*S*)-Methyl-5-(*N'*, *N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino)pentanoate[113] **93**



Boc-L-Orn-OH (0.51 g, 2.20 mmol) and triethylamine (150 µL, 1.10 mmol) were added to a solution of 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (0.38 g, 1.10 mmol) in dry THF (8.0 mL) and refluxed for 48 h. The solvent was evaporated *in vacuo* and a mixture of the product and starting material acid was obtained by flash chromatography eluting first with petrol/EtOAc (4/1), then with CH_2Cl_2/MeOH (4/1). The crude product (0.81 g) was dissolved in CH_2Cl_2 (8 mL) to which was added Triethylamine (640 µL, 4.57 mmol) and MeI (280 µL, 4.57 mmol) and stirred for 24 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/EtOAc (1/1) gave an impure product. Further purification was needed by flash chromatography eluting with $CHCl_3$/Et_2O (3:1) which

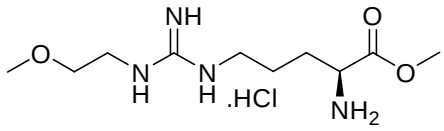
gave the title compound as an off-white waxy solid (29 mg, 5% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (br s, 1H), 4.30-4.20 (m, 1H), 3.91 (br s, 1H), 3.78-3.77 (m, 2H), 3.72 (s, 3H), 3.64-3.49 (m, 2H), 3.32 (br s, 3H), 3.31-3.27 (m, 2H), 1.85-1.83 (m, 2H), 1.67-1.63 (m, 2H), 1.47 (s, 9H), 1.45 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8 (s), 156.4 (s), 155.3 (s), 155.3 (s), 143.9 (s), 80.0 (3 x s), 70.8 (t), 58.5 (d), 53.0 (q), 52.4 (q), 40.8 (t), 40.4 (t), 29.6 (t), 25.8 (t), 28.3 (q), 28.2 (q), 28.1 (q); IR (neat) 3337, 3055, 2931, 2858, 1702, 1697, 1685, 1655 cm^{-1}; LRMS (ES^+) 569 (58%, $[M+Na]^+$), 469 (40), 347 (100); HRMS (ES^+) calcd for $C_{25}H_{46}N_4O_9Na$ $[M+Na]^+$ 569.3162, observed 569.3172.

(*S*)-Benzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino) pentanoate[29] 94



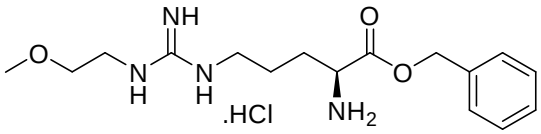
Boc-L-Orn-OH (250 mg, 1.09 mmol) and triethylamine (300 µL, 2.19 mmol) was added to a solution of 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (250 mg, 0.73 mmol) in DMF (8 mL). The solution was stirred as a clear solution for 10 min after which $HgCl_2$ (0.36 g, 1.32 mmol) was added and the reaction mixture turned into a milky off-white suspension. The reaction mixture was further stirred for 120 h. The solvent was evaporated *in vacuo* and a mixture of the product and starting material acid obtained by flash chromatography eluting first with petrol/EtOAc (4/1), then with CH_2Cl_2/MeOH (4/1). The crude product obtained (0.64 g) was dissolved in CH_2Cl_2 (10 mL) with stirring, to which was added triethylamine (430 µL, 3.09 mmol) and benzyl bromide (370 µL, 3.09 mmol). The reaction mixture was stirred for 24 h. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography eluting with petrol/EtOAc (1/1). Further purification by flash chromatography eluting with $CHCl_3/Et_2O$ (3:1) gave the title compound as an off-white waxy solid (110 mg, 24% over two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.33 (m, 5H), 5.17 (d, J = 15.6 Hz, 1H), 5.13 (d, J = 15.6 Hz, 1H), 4.35-4.33 (m, 1H), 3.76 (br s, 2H), 3.48-3.46 (m, 2H), 3.26 (m, 5H), 1.86-1.85 (m, 2H), 1.63-1.53 (m, 2H), 1.47 (s, 9H), 1.43 (s, 9H), 1.41 (s, 9H);); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2 (s), 155.4 (s), 153.5 (s) 152.4 (s), 135.3 (s), 128.6 (d), 128.5 (d), 128.3 (d), 83.5 (3 x s), 82.1 (s), 79.9 (t), 79.2 (t), 70.8 (t), 67.2 (t), 58.4 (d), 53.2 (q), 29.9 (t), 26.0 (t), 28.3 (q), 28.2 (q), 28.1 (q); IR (neat) 3330, 2980, 2927, 1712, 1623, 1605 cm^{-1}; LRMS (ES^+) 645 (90%, $[M+Na]^+$), 623 (100%, $[M]^+$), 523 (98), 432 (92).

(*S*)-Methyl 2-amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanoate hydrochloride[29] **24**



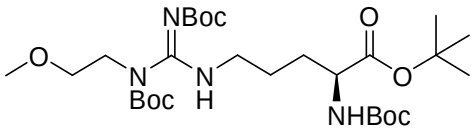
A solution of the (*S*)-methyl-5-(*N'*,*N''*-bis(tert-butoxycarbonyl)-3-(*N'*-methoxyethyl) guanidino)-2-(*tert*-butoxycarbonylamino)pentanoate **93** (29 mg, 0.05 mmol) in 4 M HCl in dioxane (3 mL) was stirred for 36 h. The solvent was removed *in vacuo*, dissolved in distilled water (5 mL) and freeze dried for 48 h giving the title compound as a waxy off-white solid (16 mg, 99% yield): ^{1}H NMR (300 MHz, D_2O) δ 4.19 (t, J = 6.5 Hz, 1H), 3.85 (s, 3H), 3.61 (t, J = 5.0 Hz, 2H), 3.39 (s, 3H), 3.40 (t, J = 5.0 Hz, 2H), 3.27 (t, J = 6.5 Hz, 2H), 2.03-1.95 (m, 2H), 1.76-1.33 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 172.7 (s), 158.6 (s), 72.7 (t), 60.6 (t), 56.0 (d), 54.9 (q), 43.5 (t), 42.7 (t), 29.2 (q), 26.2 (t); LRMS (ES^+) 246 (17%, $[M]^+$), 201 (24), 178 (100), 130 (22).

(*S*)-Benzyl 2-amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanoate hydrochloride[29] **90**



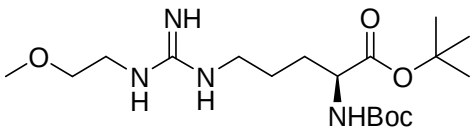
A solution of (*S*)-benzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl) guanidino)-2-(*tert*-butoxycarbonylamino) pentanoate **94** (90 mg, 0.15 mmol) in 4 M HCl in dioxane (5 mL) was stirred for 48 h. The solvent was evaporated *in vacuo*, the compound dissolved in distilled water (5 mL) and freeze dried for 48 h to give the title compound as a waxy off-white solid (36 mg, 60% yield): ^{1}H NMR (400 MHz, D_2O) δ 7.45-7.48 (m, 5H), 5.39 (d, J = 12 Hz, 1H), 5.26 (d, J = 12 Hz, 1H), 4.23 (t, J = 6.3 Hz, 1H), 3.55 (t, J = 5.0 Hz, 2H), 3.36 (s, 3H), 3.34 (t, J = 5.0 Hz, 2H), 3.18 (t, J = 6.9 Hz, 2H), 2.01-1.95 (m, 2H), 1.68-1.64 (m, 1H), 1.55-1.50 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 170.3 (s), 157.0 (s), 135.3 (s), 129.7 (d), 129.5 (d), 129.4 (d), 70.9 (t), 69.2 (t), 58.8 (d), 53.0 (q), 41.7 (t), 40.8 (t), 27.5 (t), 24.4 (t); IR (neat) 2930, 1746, 1660, 1606 cm^{-1}; LRMS (ES^+) 323 (100%, $[M+H]^+$), 306 (15), 259 (40).

(*S*)-*tert*-Butyl 5-(*N'*, *N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino)pentanoate[29] 107



Triethylamine (340µ mL, 2.46 mmol), 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (430 mg, 1.23 mmol), and 4-dimethylaminopyridine (18 mg, 0.12 mmol) were added to a stirred solution of Boc-L-Orn-O^{t}Bu (400 mg, 1.23 mmol) in THF (20 mL) under nitrogen for 72 h. The solvent was evaporated *in vacuo* and purification by flash chromatography starting with petrol/EtOAc (19/1) gave the title compound as a yellow waxy solid. Further purification by freeze drying for 48 h furnished the title compound as a white waxy solid (0.52 g, 70 % yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19-5.12 (m, 1H), 4.20-4.15 (m, 1H), 3.78 (br s, 2H), 3.62-3.55 (m, 2H), 3.31 (s, 3H), 3.31-3.27 (m, 2H), 1.83-1.79 (m, 2H), 1.66-1.54 (m, 2H), 1.47 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0 (s), 158.0 (s), 155.5 (s), 153.0 (s), 144.0 (s), 82.0 (s), 81.9 (s), 81.7 (s), 79.6 (s), 70.7 (t), 58.5 (d), 53.01 (q) 42.0 (t), 40.2 (t), 30.3 (t), 29.7 (q), 28.3 (q), 28.2 (q), 27.9 (q), 26.0 (t); IR (neat) 3335, 2976, 2931, 1711, 1615 cm^{-1}; LRMS (FAB) 588 (100%, $[M]^+$), 489 (20), 415 (48), 359 (23), 303 (68); HRMS (FAB) calcd for $C_{28}H_{52}N_4O_9$ $[M]^+$ 588.3679, observed 588.3660.

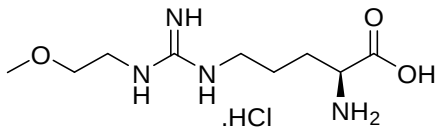
(*S*)-*tert*-Butyl 2-(*tert*-butoxycarbonylamino)-5-(*N'*-(2-methoxyethyl)guanidino) pentanoate 111



Iodine (7 mg, 0.023 mmol) was added to a stirred solution of (*S*)-*tert*-butyl 5-(*N'*, *N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino) pentanoate **107** (55 mg, 0.094 mmol) in water (8 µL) and acetonitrile (1 mL) under reflux for 5 h. The reaction mixture was diluted with aqueous $Na_2S_2O_3$ (1 mL) and

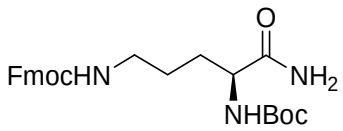
extracted with EtOAc (2 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent evaporated *in vacuo*. Purification by flash chromatography eluting with MeOH/$CHCl_3$ (1/9) gave the title compound as a waxy solid (19 mg, 55%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (br s, 2H), 5.36 (br s, 1H), 4.10-4.09 (m, 1H), 3.54-3.24 (m, 7H), 3.38 (s, 3H), 1.80-1.78 (m, 2H), 1.70-1.67 (m, 2H), 1.46 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (s), 158.2 (s), 156.7 (s), 82.9 (s), 80.6 (s), 73.5 (t), 59.3 (q), 52.5 (d), 43.0 (t), 41.2 (t), 29.4 (t), 28.4 (q), 28.1 (q), 25.1 (t); IR (neat) 3310, 3188, 3054, 2929, 2855, 1712, 1693, 1660, 1633 cm^{-1}; LRMS (CI) 389 (100%, $[M+H]^+$); HRMS (CI) calcd. for $C_{18}H_{37}N_4O_9$ $[M+H]^+$ 389.2764, observed 389.2752.

(*S*)-2-Amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanoic acid hydrochloride[29] **22**



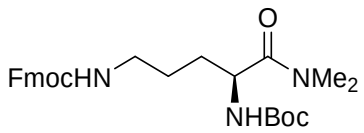
The (*S*)-*tert*-butyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino)pentanoate **107** (0.52 g, 0.88 mmol) was dissolved in 4 M HCl in dioxane (5 mL) and stirred under nitrogen for 24 h. The solvent was evaporated *in vacuo* to give the title compound as a white waxy-solid (0.27 g, 0.88 mmol, >99% yield): ^{1}H NMR (300 MHz, D_2O) δ 4.01 (t, J = 6.0 Hz, 1H), 3.56 (t, J = 5.0 Hz, 2H), 3.36 (t, J = 5.0 Hz, 2H), 3.34 (s, 3H), 3.23 (t, J = 6.0 Hz, 2H), 2.01-1.92 (m, 2H), 1.85-1.70 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 172.7 (s), 156.6 (s), 70.7 (t), 58.6 (d), 53.2 (q), 41.5 (t), 40.8 (t), 27.4 (t), 24.2 (t); IR (neat) 3334, 3195, 3053, 2987, 2929, 1645 cm^{-1}; LRMS (FAB) 233 (100%, $[M+H]^+$), 188 (6), 158 (8); HRMS (FAB) calcd for $C_9H_{21}N_4O_3$ $[M+H]^+$ 233.1613, observed 233.1610.

(*S*)-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide 117a



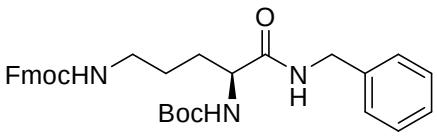
EDC.HCl (370 mg, 1.93 mmol) was added to a stirred solution of Boc-L-Orn(Fmoc)-OH (800 mg, 1.76 mmol) and HOBt (260 mg, 1.93 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The reaction was stirred at 21 ^oC for 30 min. $NH_{3(g)}$ was bubbled through the mixture for 3 min after which the solution turned cloudy and precipitates formed. This was further stirred at 21 ^oC for 1 h. The mixture was concentrated *in vacuo*, dissolved in EtOAc (30 mL), washed with water (50 mL) and $NaHCO_3$ (50 mL) and back extracted with EtOAc (2 x 50 mL). The organic layers were collected and washed with 0.5M HCl (30 mL), brine (50 mL) and then dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to leave the crude product as a yellow solid. Purification by flash chromatography eluting with petrol/EtOAc (6/4) gave the title compound as a white solid (713 mg, 89% yield): m.p. 171-173 °C; ^1H NMR (600 MHz, $CDCl_3$): δ 7.78 (d, J = 7.5 Hz, 2H), 7.58 (dd, J = 1.3, 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 6.48 (br s, 1H), 5.61 (br s, 1H), 5.25 (br s, 1H), 5.12 (br s, 1H), 4.44-4.39 (m, 2H), 4.29 (br s, 1H), 4.21 (t, J = 7.0 Hz, 1H), 3.50 (br s, 1H), 3.18-3.16 (m, 1H), 1.83 (br s, 1H), 1.64-1.63 (m, 1H), 1.60-1.55 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.6 (s), 156.9 (s), 155.8 (s), 143.9 (s), 141.3 (s), 127.7 (d), 127.0 (d), 125.0 (d), 120.0 (d), 80.1 (s), 66.6 (t), 52.7 (d), 47.2 (d), 39.7 (t), 29.9 (t), 28.3 (q), 26.2 (t); IR (neat) 3500, 3054, 2986, 1694, 1682, 1660 cm^{-1}; LRMS (ES^+) 476 (63% $[M+ Na]^+$), 376 (100); HRMS (ES^+) calcd for $C_{25}H_{31}N_3O_5Na$ $[M+Na]^+$ 476.2161, observed 476.2172.

(*S*)-Dimethyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide 117b



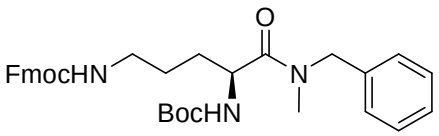
EDC.HCl (230 mg, 1.20 mmol) was added to a stirred solution of dimethylamine (110 µL, 2.11 mmol), HOBt (260 mg, 1.94 mmol) and Boc-L-Orn(Fmoc)-OH (0.80 g, 1.76 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was stirred at this temperature for 1 h after which the reaction mixture was warmed to 21 ^oC and stirred for 3 h. The mixture was concentrated *in vacuo*, dissolved in EtOAc (30 mL), washed with water (50 mL) and $NaHCO_3$ (50 mL) and back extracted with EtOAc (2 x 50 mL). The organic layers were collected and washed with 0.5 M HCl (30 mL), brine (50 mL) and then dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to leave the crude product as a yellow solid. Purification by flash chromatography eluting with petrol/EtOAc (7/3) gave the title compound as a white solid (0.40 g, 46% yield): m p: 76-82 °C; IR (neat) 3500, 3054, 2985, 1713, 1645 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.0 Hz, 2H), 7.64-7.56 (m, 2H), 7.34 (t, J = 7.0 Hz, 2H), 7.31-7.25 (m, 2H), 5.52 (d, J = 8.0 Hz, 1H), 5.27-5.24 (m, 1H), 4.64-4.61 (m, 1H), 4.40-4.29 (m, 2H), 4.19 (t, J = 7.0 Hz, 1H), 3.23-3.21 (m, 2H), 3.00 (s, 3H), 2.92 (s, 3H), 1.68-1.56 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9 (s), 156.5 (s), 155.7 (s), 144.0 (s), 141.3 (s), 127.6 (d), 127.0 (d), 125.1 (d), 119.9 (d), 79.7 (s), 66.6 (t), 49.8 (d), 47.3 (d), 40.8 (t), 37.0 (q), 35.7 (q), 30.9 (t), 28.4 (q), 25.4 (t); LRMS (ES^+) 504 (100%, $[M+Na]^+$), 448 (25), 404 (95); HRMS (ES^+) calcd for $C_{27}H_{35}O_5N_3Na$ $[M+Na]^+$ 504.2474, observed 504.2453.

(*S*)-Benzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide 117c



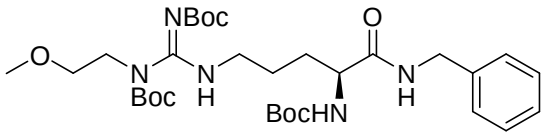
Benzylamine (290 µL, 2.64 mmol) and HOBt (330 mg, 2.42 mmol) were added to a solution of Boc-L-Orn(Fmoc)-OH (1.0 g, 2.20 mmol) in CH_2Cl_2 (15 mL) with stirring. The solution was cooled to 0 °C, EDC.HCl (460 mg, 2.42 mmol) was added and further stirred at 0 °C for 1 h after which the reaction mixture was warmed to 21 ^oC and stirred for 3 h. The mixture was concentrated *in vacuo*, diluted with EtOAc (30 mL), washed with water (2 x 30 mL) and back extracted with EtOAc (2 x 50 mL). The organic layers were collected and washed with 0.5 M HCl (40 mL), brine (50 mL) and then dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to leave the crude product as a yellow solid. Purification by flash chromatography eluting with petrol/EtOAc (7/3) gave the title compound as an off-white solid (854 mg, 70% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.54-7.50 (m, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31-7.12 (m, 7H), 6.90 (br s, 1H), 5.30-5.27 (m, 1H), 5.08-5.04 (m, 1H), 4.44-4.36 (m, 2H), 4.32-4.26 (m, 2H), 4.18-4.13 (m, 2H), 3.40-3.34 (m, 1H), 3.16-3.10 (m, 1H), 1.64-1.51 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5 (s), 155.5 (s), 151.7 (s), 143.9 (s), 143.8 (s), 141.3 (s), 137.9 (s), 128.6 (d), 127.7 (d), 127.6 (d), 127.4 (d), 127.0 (d), 124.9 (d), 119.9 (d), 66.9 (d), 66.6 (t), 47.2 (d), 43.5 (t), 39.7 (t), 31.0 (t), 28.3 (q), 26.3 (t); IR (neat) 3311, 2937, 1683, 1655, 1519 cm^{-1}; LRMS (FAB) 566 (8%, $[M+Na]^+$), 475 (100), 329 (25), 154 (35); HRMS (FAB) calcd for $C_{37}H_{37}N_3O_5Na$ $[M+Na]^+$ 566.2631, observed 566.2645.

(*S*)-Methylbenzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide 117d



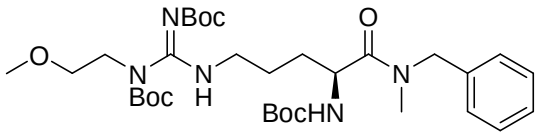
N-Methyl-benzylamine (160 mg, 1.32 mmol) and HOBt (160 mg, 1.20 mmol) were added to a stirred solution of Boc-L-Orn(Fmoc)-OH (0.5 g, 1.10 mmol) in CH_2Cl_2 (15 mL). The solution was cooled to 0 °C, EDC.HCl (230 mg, 1.20 mmol) was added and further stirred at 0 °C for 1 h after which the reaction mixture was warmed to 21 ^oC and stirred for 3 h. The mixture was concentrated *in vacuo*, diluted with EtOAc (10 mL), washed with water (2 x 20 mL) and back extracted with EtOAc (2 x 50 mL). The organic layers were combined and washed with 0.5 M HCl (30 mL), brine (50 mL) and dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Purification by flash chromatography starting with petrol/EtOAc (7/3) gave the title compound as a white solid (0.56 g, 92% yield): ^1H NMR (400 MHz, $CDCl_3$, 55 °C) δ 7.75 (d, J = 7.0 Hz, 2H), 7.58 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.31-7.25 (m, 5H), 7.17-7.09 (m, 2H), 5.35 (m, 1H), 4.89 (br s, 1H), 4.56-4.50 (m, 3H), 4.39-4.35 (m, 2H), 4.19 (t, J = 6.5 Hz, 1H), 3.28-3.30 (m, 2H), 2.96 (s, 3H), 1.68-1.54 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 55 °C) δ 172.1 (s), 156.2 (s), 155.5 (s), 143.9 (s), 141.2 (s), 137.0 (s) 136.6 (s), 128.5 (d), 127.8 (d), 127.4 (d), 127.1 (d), 126.8 (d), 124.8 (d), 119.7 (d), 79.6 (q), 66.5 (t), 51.2 (d), 49.9 (d), 47.3 (t), 40.7 (t), 30.5 (t), 28.2 (q), 25.4 (t); IR (neat) 3316, 2931, 1701, 1638 cm^{-1}; LRMS (FAB) 580 (32%, $[M+Na]^+$), 360 (22), 329 (28), 176 (100), 154 (30); HRMS (FAB) calcd for $C_{33}H_{39}N_3O_5Na$ $[M+Na]^+$ 580.2787, observed 580.2787.

(*S*)-Benzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino) pentanamide 118c



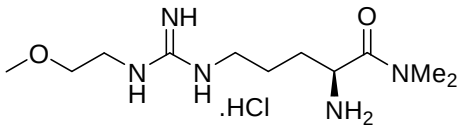
Piperidine (0.74 mL, 7.53 mmol) was added to a solution of (*S*)-benzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide **117c** (0.84 g, 1.54 mmol) in CH_2Cl_2 (15 mL) and stirred for 24 h. The solvent was evaporated *in vacuo* and redissolved in dry THF (20mL) with stirring. Triethylamine (0.43 mL, 3.08 mmol), 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (610 mg, 1.54 mmol) and 4-dimethylaminopyridine (20 mg, 0.15 mmol) were added to this solution and stirred under nitrogen for 72 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/EtOAc (1/4), and further purification by flash chromatography eluting with $CHCl_3/Et_2O$ (1/4) gave the title compound as a white waxy-solid (670 mg, 70 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.18 (m, 5H), 4.43 (t, J = 6.5 Hz, 1H), 4.45-4.41 (m, 2H), 3.78-3.75 (m, 2H), 3.49-3.47 (m, 2H), 3.33-3.30 (m, 2H), 3.30 (br s, 3H), 1.92-1.89 (m, 2H), 1.65-1.55 (m, 2H), 1.45 (s, 9H), 1.43 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9 (s), 156.0 (s), 152.4 (s), 146.5 (s), 138.0 (s), 128.6 (d), 127.7 (d), 127.4 (d), 119.9 (s), 82.2 (s), 79.9 (s), 79.4 (s), 70.6 (t), 67.0 (d), 53.6 (q), 43.5 (t), 31.1 (t), 29.5 (t), 28.3 (q), 28.2 (q), 29.1 (q), 25.3 (t), 20.2 (t); IR (neat) 3430, 3321, 3055, 2981, 2933, 1712, 1674, 1620 cm^{-1}; LRMS (FAB) 644 (100%, $[M+Na]^+$), 422 (18), 344 (34), 176 (98), 154 (43); HRMS (FAB) calcd for $C_{31}H_{51}N_5O_8Na$ $[M+Na]^+$ 644.3635, observed 644.3626.

(*S*)-Methylbenzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-ethoxyethyl)guanidino) -2-(*tert*-butoxycarbonylamino) pentanamide 118d



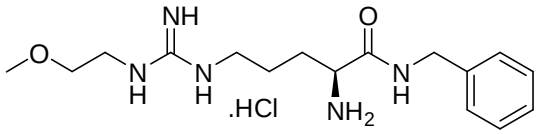
Piperidine (310 µL, 3.43 mmol) was added to a stirred solution (*S*)-methylbenzyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide **117d** (380 mg, 0.68 mmol) in CH_2Cl_2 (10 mL) and stirred for 24 h. The solvent was evaporated *in vacuo* and redissolved in dry THF (5 mL) with stirring. Triethylamine (190 µL, 1.37 mmol), 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (240 mg, 0.68 mmol) and 4-dimethylaminopyridine (10 mg, 0.07 mmol) were added to the reaction mixture under nitrogen for 72 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/EtOAc (1/4) and further purification by flash chromatography eluting with $CHCl_3/Et_2O$ (1/4) gave the title compound as a white waxy-solid (110 mg, 24% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.23 (m, 3H), 7.20-7.14 (m, 2H), 5.50 (d, J = 8.5 Hz, 1H, major), 5.42 (d, J = 8.5 Hz, 1H, minor), 4.67-4.56 (m, 2H), 3.78-3.76 (m, 2H), 3.47-3.45 (m, 2H), 3.28 (br s, 3H), 3.27-3.25 (m, 2H), 2.97 (s, 3H, major), 2.90 (s, 3H, minor), 1.78-1.63 (m, 4H), 1.46 (s, 9H, minor), 1.44 (s, 9H, major), 1.43 (s, 9H), 1.42 (s, 9H, major), 1.39 (s, 9H, minor); ^{13}C NMR (75 MHz, $CDCl_3$, major rotamer) δ 173.0 (s), 155.6 (s), 152.4 (s), 137.0 (s), 136.6 (s), 128.9 (d, minor), 128.7 (d, major), 127.8 (d, major), 127.5 (d, minor), 126.7 (d), 82.1 (s), 79.7 (2 x s), 79.0 (s), 70.8 (t), 58.0 (d), 56.0 (t), 51.2 (t), 50.0 (q), 34.6 (q, major), 33.0 (q, minor), 31.0 (t), 30.0 (t), 28.3 (q), 28.2 (q), 28.1 (q), 24.9 (t); IR (neat) 2970, 2880, 1720, 1650 cm^{-1}; LRMS (FAB) 658 (25 % $[M+Na]^+$), 176 (100); HRMS (FAB) calcd for $C_{32}H_{53}O_8N_5Na$ $[M+Na]^+$ 658.3791, observed 658.3802.

***(S)*-Dimethyl-2-amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanamide hydrochloride 119b**



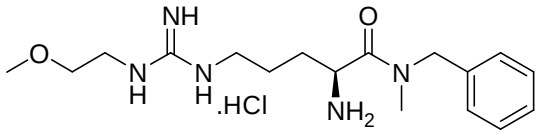
Piperidine (0.26 mL, 2.64 mmol) was added to the (*S*)-dimethyl-5-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(*tert*-butoxycarbonylamino) pentanamide **117b** (255 mg, 0.53 mmol) in CH_2Cl_2 (10 mL) and stirred for 1 h. The solvent was evaporated *in vacuo* and redissolved in dry THF (10 mL) under nitrogen. To this solution was added 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (180 mg, 0.53 mmol), Triethylamine (150 µL, 1.06 mmol) and 4-dimethylaminopyridine (10 g, 0.05 mmol). The solution was stirred for 72 h. The solvent was evaporated *in vacuo* and the reaction mixture was redissolved in DMF (10 mL) and heated at 130 °C for 2 h and at 21 ^{o}C for 24 h. The solvent was evaporated *in vacuo* and purification three times by flash chromatography eluting with $CHCl_3$/MeOH (30/1) gave an impure product as a waxy solid (23 mg, 8 % yield). The product was dissolved 4M HCl in dioxane (2 mL) and stirred for 2 h. The solvent was evaporated *in vacuo*, extracted into water and freeze dried twice to give the title compound as a yellow waxy solid (0.43 mg, 3.3 % yield): ^{1}H NMR (300 MHz, D_2O): δ 4.55 (t, J = 6.0 Hz, 1H), 3.61 (t, J = 5.0 Hz, 2H), 3.40 (t, J = 5.0 Hz, 2H), 3.39 (s, 3H), 3.29-3.24 (m, 2H), 3.11 (s, 3H), 2.99 (s, 3H), 1.99-1.89 (m, 2H), 1.73-1.63 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ 170.0 (s), 156.8 (s), 70.9 (t), 58.8 (q), 50.7 (d), 41.7 (t), 41.0 (t), 37.6 (q), 36.4 (q), 27.8 (t), 23.9 (t); IR (neat) 3390, 2926, 1639 cm^{-1}; LRMS (ES^{+}) 282 (20%, $[M+Na]^{+}$), 260 (100, $[M+H]^{+}$), 243 (45); HRMS (ES^{+}) calcd for $C_{11}H_{26}O_2N_5$ $[M+H]^{+}$ 260.2087, observed 260.2093.

(*S*)-Benzyl-2-amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanamide hydrochloride 119c



(*S*)-Benzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino) pentanamide **118c** (100 mg, 0.32 mmol) was dissolved in 4 M HCl in dioxane (2 mL) with stirring for 24 h. The solvent was evaporated *in vacuo*, dissolved in distilled water (3 mL) and freeze dried for 48 h which gave the title compound as an off-white hydroscopic solid (41 mg, 62 % yield): ^{1}H NMR (300 MHz, D_2O) δ 7.42-7.22 (m, 5H), 4.54 (6, J = 16.0 Hz, 1H), 4.33 (d, J = 16.0 Hz, 1H), 4.04 (t, J = 6.0 Hz, 1H), 3.56 (t, J = 7.0 Hz, 2H), 3.36-3.34 (m, 2H), 3.41 (s, 3H), 3.18 (t, J = 7.0 Hz, 2H), 1.91-1.86 (m, 2H), 1.59-1.43 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 169.7 (s), 156.6 (s), 138.1 (s), 129.5 (d), 128.4 (d), 128.3 (d), 70.9 (t), 58.9 (d), 53.5 (q), 43.9 (t), 41.7 (t), 40.9 (t), 28.6 (t), 24.6 (t); IR (neat) 3363, 3217, 1650 cm^{-1}; LRMS (CI) 322 (13%, $[M+H]^+$), 215 (18), 203 (15), 115 (40), 91 (100); HRMS (CI) calcd for $C_{16}H_{28}O_2N_5$ $[M+H]^+$ 322.2242, observed 322.2234.

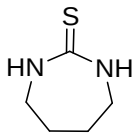
(*S*)-Methylbenzyl-2-amino-5-(*N'*-(2-methoxyethyl)guanidino)pentanamide hydrochloride 119d



(*S*)-Methylbenzyl-5-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino) pentanamide **118d** (100 mg, 0.16 mmol) was dissolved in 4 M HCl in dioxane (2 mL) and stirred for 24 h. The solvent was evaporated *in vacuo*, dissolved in distilled water (3 mL), and freeze drying for 48 h which gave the title compound as an off-white hydroscopic solid (0.045g, 71 % yield, mixture of rotamers): ^{1}H NMR (400 MHz, D_2O, 90 °C) δ 7.59-7.38 (m, 3H), 7.35-7.25 (m, 2H), 4.96 (d, J = 14.0 Hz, 1H, major), 4.85 (br s, 1H, minor), 4.77-4.73 (m, 1H, major), 4.77-4.70 (m, 2H, minor), 4.59 (d, J = 14.0 Hz, 1H, major), 3.76-3.73 (m, 2H), 3.56-3.54 (m, 2H),

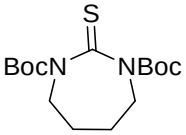
3.54 (br s, 3H), 3.41-3.39 (m, 2H), 3.25 (br s, 3H, major), 3.18 (br s, 3H, minor), 2.12-1.92 (m, 2H), 1.79-1.62 (m, 2H); ^{13}C NMR (100 MHz, D_2O, 90 °C) δ 170.4 (s, minor), 169.8 (s, major), 156.8 (s), 136.7 (s, major), 136.2 (s, minor), 129.8 (d, minor), 129.6 (d, major), 128.7 (d, minor), 128.6 (d, major), 127.3 (d), 70.9 (t), 58.9 (d), 53.6 (t, minor), 52.4 (t, major), 51.2 (q, minor), 51.0 (q, major), 41.8 (t), 41.0 (t), 35.6 (q, major), 35.5 (q, minor), 28.2 (t, minor), 27.7 (t, major), 24.1 (t); IR (neat) 3363, 3207, 1650 cm^{-1}; LRMS (ES^+) 336 (100% $[M+H]^+$), 319 (10); HRMS (ES^+) calcd for $C_{17}H_{30}N_5O_2$ $[M+H]^+$ 336.2400, observed 336.2415.

1,3-Diazepane-2-thione[114,115] **122**



Carbon disulphide (0.68 mL, 11.0 mmol) was added to a stirred solution of 1,4-diaminobutane (1.0 g, 11.0 mmol) in ethanol (15 mL) and water (10 mL). White precipitate immediately formed and the reaction mixture was refluxed overnight at 90 °C. The next day the reaction was warmed to 21 °C before being put in the fridge overnight for crystallization. Filtration and washing with diethyl ether (50 mL) gave the title compound as colourless crystals (0.78 g, 53% yield): m.p. 182-184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (br s, 2H), 3.05 (br s, 4H), 1.58-1.55 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 193.0 (s), 49.9 (t), 32.3 (t); IR (neat) 3217, 2942, 2920; LRMS (EI) 130 (100%, $[M]^{+\bullet}$), 97 (8).

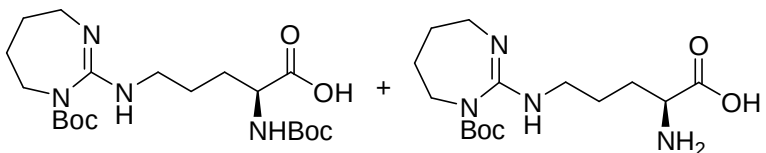
Di-*tert*-butyl 2-thioxo-1,3-diazepane-1,3-dicarboxylate[116] **123**



NaH 60% in mineral oil (0.77 g, 19.28 mmol) was added to a stirred solution of 1,3-diazepane-2-thione **122** (0.56 g, 4.28 mmol, 1 eq.) in dry THF (20 mL) under argon at

0 °C. The ice bath was removed after 5 min and the reaction mixture was stirred at 21 °C for 10 min. The reaction mixture was cooled to 0 °C, and di-*tert*-butyl dicarbonate (2.06 g, 9.43 mmol) was added slowly over 5 min at which the reaction turned bright yellow. The ice bath was removed after 30 min of stirring at that temperature and the resulting slurry was stirred for 2 h at 21 °C and then quenched with saturated $NaHCO_3$ solution (50 mL). The reaction mixture was poured onto water (50 ml) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography eluting with petrol/Et_2O (9/1) gave the title compound as a bright yellow solid (1.23 g, 87 % yield): m.p. 103-106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78-3.75 (m, 4H), 1.58-1.53 (m, 4H), 1.43 (s, 18H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 186.9 (s), 152.7 (s), 82.6 (s), 49.3 (t), 27.3 (q), 24.9 (t); IR (neat) 2978, 2942, 1718, 1120 cm^{-1}; LRMS (ES^+) 353 (100%, $[M+Na]^+$), 253 (85),153 (95); HRMS (ES^+) calcd for $C_{15}H_{26}N_2O_4SNa$ $[M+Na]^+$ 353.1512, observed 353.1502.

(*S*)-5-(1-(*tert*-Butoxycarbonyl)-4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)-2-(*tert*-butoxycarbonylamino)pentanoic acid 124) and **(*S*)-5-(1-(*tert*-Butoxycarbonyl)-4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)-2-aminopentanoic acid 125**



124 **125**

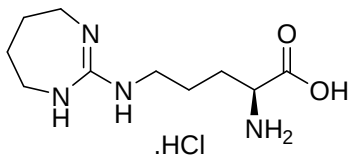
MeI (100 µL, 1.73 mmol) was added to a stirred solution of the (*E*)-*tert*-butyl 2-(methylthio)-4,5,6,7-tetrahydro-1,3-diazepine-1-carboxylate **123** (459 mg, 1.39 mmol) in MeCN (5 mL) and refluxed for 4 h. The solvent was evaporated *in vacuo* and the compound redissolved in dry THF (5 mL). To this solution was added Boc-L-Orn-OH (323 mg, 1.39 mmol), Triethylamine (0.48 mL, 3.47 mmol), and 4-dimethylaminopyridine (10 mg, 0.08 mmol). The solution was stirred at 21 °C for 72 h. The solvent was evaporated *in vacuo* and redissolved in MeCN (5 mL) and stirred at 21 °C for 48 h, and then refluxed for 48 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with $CHCl_3$/MeOH (99/1) and freeze

drying gave (*S*)-5-(1-(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)-2-(*tert*-butoxycarbonyl)pentanoic acid **124** as a white waxy-solid (25 mg, 11% yield) and (*S*)-5-(1-(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)-2-aminopentanoic acid **125** as a white waxy-solid (129 mg, 28% yield).

Data for 124: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98-3.97 (m, 1H), 3.39-3.37 (m, 2H), 3.32-3.39 (m, 4H), 1.84-1.70 (m, 8H), 1.51 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0 (s), 159.3 (s), 157.6 (s), 152.0 (s), 80.2 (s), 79.5 (s), 57.0 (d), 45.7 (t), 44.0 (t), 31.7 (t), 28.8 (q), 28.3 (q), 27.9 (t), 27.0 (2 x t), 25.0 (t); IR (neat) 3258, 2934, 1642, 1581 cm^{-1}; LRMS (ES^+) 429 (15%, $[M+H]^+$), 329 (100), 60 (64); HRMS (ES^+) calcd for $C_{20}H_{36}N_4O_6$ $[M+H]^+$ 429.2708; observed 429.2707.

Data for 125 (Boc Group tentatively assigned): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (br s, 1H), 7.37 (br s, 1H), 5.81 (m, 2H), 3.98 (br s, 1H), 3.40-3.37 (m, 2H), 3.28 (br s, 1H), 3.20-3.10 (m, 2H), 3.01 (br s, 1H), 1.70-1.63 (m, 8H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2 (s), 162.4 (s), 156.8 (s), 79.0 (s), 53.9 (d), 44.6 (t), 41.6 (t), 31.9 (t), 29.7 (t), 28.5 (q), 28.2 (t), (28.0 (t), 26.6 (t); IR (neat) 3315, 2933, 1702, 1657 cm^{-1}; LRMS (ES^+) 329 (100%, $[M+H]^+$); HRMS (ES^+) calcd for $C_{15}H_{29}N_4O_4$ $[M+H]^+$ 329.2183, observed 329.2183.

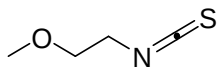
(*S*)-2-Amino-5-(4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)pentanoic acid hydrochloride 120



A solution of (*S*)-5-(1-(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1H-1,3-diazepin-2-ylamino)-2-aminopentanoic acid **125** (120 mg, 0.36 mmol) in 4 M HCl in dioxane (2 mL) was stirred under argon for 24 h. The solvent was evaporated *in vacuo*, extracted into D_2O and freeze dried to give the title compound as a yellow waxy-solid (38 mg, 46% yield); ^{1}H NMR (400 MHz, D_2O) δ 3.97 (t, J = 6.3 Hz, 1H), 3.17-3.14 (m, 6H), 2.15-1.89 (m, 2H), 1.77-1.71 (m,1H), 1.67-1.63 (m, 1H), 1.42-1.40 (m, 4H); ^{13}C NMR

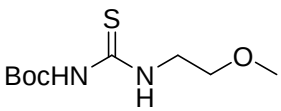
(100 MHz, D_2O) δ 171.7 (s), 160.6 (s), 52.4 (d), 44.2 (2 x t), 41.1 (t), 26.8 (t), 26.7 (2 x t), 24.0 (t); LRMS (ES^+) 229 (100%, $[M+H]^+$), 184 (85), 158 (35), 114 (90); HRMS (ES^+) calcd for $C_{10}H_{21}N_4O_2$ $[M+H]^+$ 229.1732, observed 229.1732.

1-Isothiocyanato-2-methoxyethane[117] **102**



2-Methoxyethylamine (1.0 g, 13.3 mmol) was added to a solution of K_2CO_3 (5.52 g, 39.9 mmol) dissolved in water (25 mL) and $CHCl_3$ (25 mL) and stirred for 5 min. Thiophosgene (1.52 mL, 19.97 mmol) was added and stirred vigorously for 3 h after which the organic layer was separated from the aqueous layer. The aqueous layer was washed with $CHCl_3$ (2 x 50 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification by flash chromatography eluting with petrol/Et_2O (18/1) gave the title compound as a yellow oil (0.94 g, 61 % yield); ^1H NMR (300 MHz, $CDCl_3$) δ 3.39 (s, 3H), 3.65 (t, J = 5.0 Hz, 2H,), 3.56 (t, J = 5.0 Hz, 2H,); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.9 (s), 70.3 (t), 58.8 (q), 45.0 (t); IR (neat) 2931, 2833, 2117, 1124 cm^{-1}; LRMS (CI) 118 (38%, $[M+H]^+$), 91 (100).

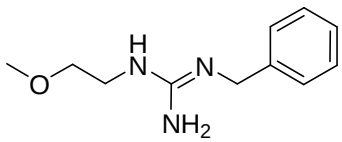
***tert*-Butyl (2-methoxyethyl)carbamothioylcarbamate 103**



NaH (30 mg, 0.94 mmol) was added to a solution of *tert*-butyl carbamate (100 mg, 0.85 mmol) in dry THF (3 mL) at 0° C and stirred under argon for 5 min, and then at 21 °C for 10 min. The reaction mixture was cooled again to 0° C and 1-isothiocyanato-2-methoxyethane **102** (100 mg, 0.85 mmol) was added and stirred for 30 min at this temperature, and then 4 h at 21 °C. The reaction was quenched with water (20 mL) and the organic material was extracted with ethyl acetate (3 x 20 mL). The organic layers

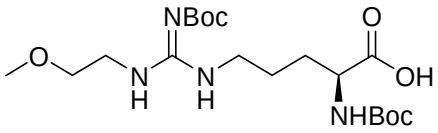
were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification by flash chromatography eluting with petrol/EtOAc (18/1) gave the product as a white solid (95 mg, 48% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 8.32 (br s, 1H), 3.80 (m, 2H), 3.56 (t, J = 5.3 Hz, 2H), 3.34 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.8 (s), 151.7 (s), 83.4 (s), 69.8 (t), 58.8 (q), 45.2 (t), 27.9 (q); IR (neat) 3239, 3191, 2935, 2866, 1718, 1097 cm^{-1}; LRMS (ES^+) 235 (100%, $[M+H]^+$); HRMS (ES^+) calcd for $C_9H_{19}N_2O_3S$ $[M+H]^+$ 235.1111, observed 235.1113.

_N_'-Benzyl-_N_''-(2-methoxyethyl)guanidine 104



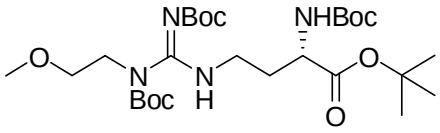
MeI (49 mg, 0.35 mmol) was added to a stirred solution of *tert*-butyl (2-methoxyethyl)carbamothioylcarbamate **103** (54 mg, 0.23 mmol) in MeCN (2 mL) and refluxed for 4 h. To this mixture was added benzylamine (25 mg, 0.23 mmol), triethylamine (64 µL, 0.46 mmol) and stirred at rt for 48 h under argon. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting MeOH/$CHCl_3$ (1/99), and further freeze drying, gave the title compound as a viscous yellow oil (47 mg, >99% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (br s, 1H), 7.34-7.23 (m, 5H), 6.68 (br s, 2H), 4.49 (d, J = 5.6 Hz, 2H), 3.47 (t, J = 4.0 Hz, 2H), 3.37-3.34 (m, 2H), 3.26 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.7 (s), 135.1 (s), 129.0 (d), 128.3 (d), 127.7 (d), 72.8 (t), 59.1 (q), 46.2 (t), 42.9 (t); IR (neat) 3173, 2929, 1626 cm^{-1}; LRMS (ES^+) 208 (100%, $[M+H]^+$); HRMS (ES^+) calcd for $C_{11}H_{18}N_3O$ $[M+H]^+$ 208.1444, observed 208.1443.

***(S)*-2-(*tert*-Butoxycarbonylamino)-5-(*N'*-(*tert*-butoxycarbonyl)-3-(*N''*-methoxyethyl)guanidino) pentanoic acid 105**



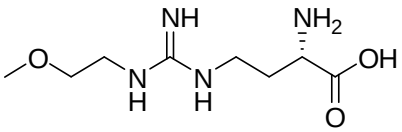
MeI (0.45 mmol, 27 µL) was added to a solution of *tert*-butyl (2-methoxyethyl)carbamothioylcarbamate **103** (84 mg, 0.35 mmol) in MeCN (3 mL) and EtOAc (1 mL). The reaction mixture was stirred under reflux for 7 h. The solvent was evaporated *in vacuo* and the compound was redissolved in dry THF (4 mL) under argon. To this mixture was added Boc-L-Orn-OH (89 mg, 0.38 mmol), triethylamine (0.1 mL, 0.72 mmol) and 4-dimethylaminopyridine (cat.) and stirred for 24 h. The solvent was evaporated *in vacuo*, redissolved in MeCN (5 mL) and refluxed for a further 24 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with MeOH/$CHCl_3$ (1/99) and further freeze drying gave the title compound as an off-white waxy solid (25 mg, 19% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.98 (br s, 1H), 5.67 (br s, 1H), 4.02-4.01 (m, 1H), 3.49-3.48 (m, 2H), 3.34-3.30 (m, 4H) 3.37 (s, 3H), 2.70 (br s, 1H), 1.68 (m, 4H), 1.40 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.6 (s), 159.0 (s), 158.4 (s), 155.5(s), 78.9 (s), 73.8 (s), 59.2 (q), 54.5 (d), 42.5 (t), 40.9 (t), 30.4 (t), 29.8 (t), 28.6 (2 x q), 26.0 (t); IR (neat) 3300, 3200, 2930, 1633, 1578 cm^{-1}; LRMS (ES^+) 433 (10%, $[M+H]^+$), 333 (15), 277 (100); HRMS (ES^+) calcd for $C_{19}H_{37}O_7N_4$ $[M+H]^+$ 433.2664, observed 433.2662.

(*S*)-*tert*-Butyl 4-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl)guanidino)-2-(*tert*-butoxycarbonylamino)butanoate[29] 115



Boc-Dab-O-*tert*-Bu (50 mg, 1.60 mmol), triethylamine (450 µL, 3.22 mmol) and 4-dimethylaminopyridine (18 mg, 0.16 mmol) were added to a stirred solution of 1,3-bis-(*tert*-butoxycarbonyl)-1-(2-methoxyethyl)-*S*-methyl-isothiourea **95** (560 mg, 1.60 mmol) in MeCN (50 mL). The reaction mixture was stirred for 48 h and the solvent was removed *in vacuo*. Purification by column chromatography eluting with petrol/EtOAc (3/1) gave the title compound as a clear viscous liquid (153 mg, 83 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (br s, 1H), 5.14 (br s, 1H), 4.16 (br s, 1H), 3.74 (t, J = 5.2 Hz, 2H), 3.51 (t, J = 5.2 Hz, 2H), 3.29 (s, 3H), 3.42-3.30 (m, 2H), 2.11-2.03 (m, 1H), 1.87-1.78 (m, 1H), 1.46 (s, 9H), 1.45 (s, 18H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0 (2 x s), 155.3 (s), 152.5 (2 x s), 82.1 (s), 81.9 (s), 79.7 (s), 79.0 (s), 70.6 (t), 58.3 (q), 52.4 (d), 47.3 (t), 39.9 (t), 32.4 (t), 28.2 (q), 28.1 (q), 28.1 (q), 27.9 (q); IR (thin film) 3316, 2977, 2933, 1713, 1618 cm^{-1}; LRMS (ES^+) 575 (100% $[M+H]^+$), 475 (6); HRMS (ES^+) calcd for $C_{27}H_{51}N_4O_9$ $[M+H]^+$ 575.3651, observed 575.3645.

(*S*)-2-Amino-4-(*N'*-(2-methoxyethyl)guanidino)butanoic acid hydrochloride[29] 113

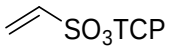


A solution of **(***S*)-*tert*-butyl-4-(*N'*,*N''*-bis(*tert*-butoxycarbonyl)-3-(*N'*-methoxyethyl) guanidino)-2-(*tert*-butoxycarbonylamino)butanoate **115** (150 mg, 0.26 mmol) in 4 M HCl in dioxane (2 mL) was stirred for 2 h. The solvent was evaporated *in vacuo* and the product was extracted into water (10 mL) and freeze dried for 18 h to give the title compound as a white waxy solid (64 mg, 85 % yield).^{1}H NMR (400 MHz, D_2O) δ 3.97 (t, J = 7.0 Hz, 1H), 3.45 (t, J = 5.0 Hz, 2H), 3.31 (t, J = 7.0 Hz, 2H), 3.25 (t, J = 5.0 Hz, 2H), 3.22 (s, 3H), 2.15-2.01 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 171.3 (s), 156.1 (s),

70.1 (t), 58.1 (q), 50.2 (d), 41.1 (t), 37.2 (t), 28.8 (t); IR (thin film) 3316, 2977, 2933, 1713, 1618 cm^{-1}; LRMS (ES^+) 219 (100%, $[M+H]^+$), 218 (12); HRMS (ES^+) calcd for $C_8H_{19}N_4O_3$ $[M+H]^+$ 219.1452, observed 219.1450.

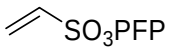
5.2.3 Experimental for Chapter 4

2,4,6-Trichlorophenyl vinylsulfonate[82] 254



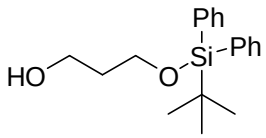
2,4,6-Trichlorophenol (15 g, 0.07 mol) and triethylamine (23 mL, 167 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a solution of 2-chloroethanesulfonyl chloride in CH_2Cl_2 (200 mL) at -78 °C over 1 h. The mixture was then warmed to 21 ^oC and stirred for a further 10 min. The mixture was filtered through a plug of 10% K_2CO_3/silica. The solvent was evaporated *in vacuo* and purification by column chromatography eluting with petrol/Et_2O (9/1) gave the title compound as a white solid (16.3 g, 57 mmol, 74% yield): m.p. 53-55 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 2H), 6.93 (dd, J = 9.8, 16.6 Hz, 1H), 6.54 (dd , J = 0.6, 16.6 Hz, 1H), 6.25 (dd, J = 0.6, 9.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9 (s), 133.8 (d), 133.0 (s), 131.4 (t), 130.7 (s), 129.1 (d); IR (neat) 3080, 1562 cm^{-1}; LRMS (EI) 286 (17% $[M]^{+\bullet}$), 195 (100), 97 (74).

Pentafluorophenyl vinylsulfonate[82] 208



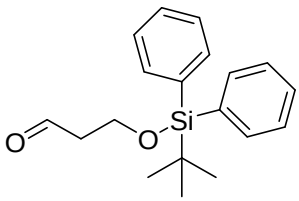
Pentafluorophenol (13.2 g, 0.07 mmol) and triethylamine (22 mL, 158 mmol) in DCM (50 mL) was added dropwise to a solution of 2-chloroethanesulfonyl chloride in CH_2Cl_2 (200 mL) at -78 °C over 1 h. The mixture was then warmed to 21 ^oC and diluted with CH_2Cl_2 (100 mL) and washed with water, 2 M HCl (50 mL x2) and sat. $NaHCO_3$ (50 mL x2), dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification by column chromatography eluting with petrol/Et_2O (6/1) gave the title compound as a clear oil which crystallized upon freezing (17.1 g, 62 mmol, 87% yield): m.p. 24-26 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 6.79 (dd, J = 9.9, 16.5 Hz, 1H), 6.54 (dd, J = 1.0, 16.5 Hz, 1H), 6.33 (dd, J = 1.0, 9.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.8 (s), 133. 1 (t), 131.8 (d); IR (thin film) 3080, 1651, 1514 cm^1; LRMS (EI) 274 (14% $[M]^{+\bullet}$), 184 (82), 91 (100).

3-(*tert*-Butyldiphenylsilyloxy)propan-1-ol[118] **218**



TBDPSCl (4.37 mL, 18.2 mmol) was added dropwise over 5 min to a stirred suspension of 1,3-propanediol (6.75 mL, 90.9 mmol) and NEt_3 (13.9 mL, 100 mmol) in CH_2Cl_2 (100 mL at 0 °C. Stirring was continued for 24 h at 21 °C after which the reaction mixture was washed with 10% HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo* to give a crude oil. Purification by column chromatography eluting with petrol/EtOAc (6/1) gave the product as a yellow oil which solidified upon freezing (3.09 g, 54% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71-7.67 (m, 4H), 7.47-7.38 (m, 6H), 3.87-3.83 (m, 2H), 3.87-3.83 (m, 2H), 2.41 (m, 1H), 1.85- 1.79 (m, 2H), 1.06 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.6 (d), 135.4 (d), 133.3 (2 x s), 129.8 (d), 129.6 (d), 127.8 (d), 127.7 (d), 63.3 (t), 62.0 (t), 34.3 (t), 26.9 (q), 19.1 (s); IR (neat) 3340, 2930, 2857 cm^{-1}; LRMS (CI) 315 (100%, $[M+H]^+$), 237 (10), 117 (12).

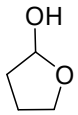
3-(*tert*-Butyldiphenylsilyloxy)propanal[118] **219**



DMSO (2.16 mL, 30.52 mmol) was added dropwise to a stirred solution of oxalyl chloride (0.72 mL, 7.63 mmol) at -78 °C. This was followed by dropwise addition of 3-(*tert*-butyldiphenylsilyloxy)propan-1-ol **218** (2.0 g, 6.4 mmol) in CH_2Cl_2 (10 mL) and 30 min stirring at -78 °C. NEt_3 (4.35 mL, 32.49 mmol) was added dropwise and the reaction was warmed to 21 °C. The reaction mixture was further stirred for 1 h and the solvent was removed *in vacuo*. The remaining residue was triturated with 4:1 petrol: EtOAc (50 mL) before filtering through a small plug of silica. The filtrate was concentrated *in vacuo* and purification by column chromatography eluting with petrol/EtOAc (12/1) gave the title compound as a pale-yellow oil which solidified upon

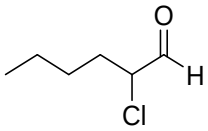
freezing (1.98 g, 94 % yield).^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83 (t, J = 2.2 Hz, 1H), 7.67 (dd, J = 1.5, 7.9 Hz, 4H), 7.47- 7.37 (m, 6H), 4.03 (t, J = 6.0 Hz, 2H), 2.61 (td, J = 2.2, 6.0 Hz, 2H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.9 (d), 135.6 (2 x d), 133.3 (s x 2), 129.8 (d), 129.7 (d), 127.8 (2 x d), 58.3 (t), 46.4 (t), 26.8 (q), 19.2 (s); IR (neat) 2931, 2857, 1727 cm^{-1}; LRMS (CI) 313 (5%, $[M+H]^+$), 94 (100).

Tetrahydrofuran-2-ol[119] **226**



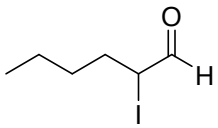
DIBAL (1.0 M solution in toluene) (39 mL, 39 mmol) was added to a stirred solution of γ-butyrolactone (2 mL, 26 mmol) in CH_2Cl_2 (30 mL) at -78 °C. The reaction mixture was stirred at that temperature for 5 h after which it was quenched with methanol (3.3 mL). The mixture was warmed to 0 °C and diluted with water (5 mL). The mixture was stirred with $MgSO_4$ (4.4 g) and Celite (4.4 g) for 20 min. the mixture was filtered through a pad of Celite, the filtered residue was washed with ether and the filtrate was concentrated *in vacuo* to give the crude product as an oil. Purification by column chromatography afforded the product and its aldehyde form in a 1:1 ratio, as a yellow oil (87 mg, 4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H, aldehyde), 5.31-5.28 (m, 1H, lactol), 5.07-5.05 (m, 1H, lactol), 3.68-3.63 (m, 2H, aldehyde), 3.43-3.33 (m, 2H, lactol), 2.50-2.45 (m, 2H, aldehyde), 1.95-1.69 (m, 6H, lactol); ^{13}C (75 MHz, $CDCl_3$) δ 202.0 (d, aldehyde), 103.8 (d, lactol), 66.9 (t, aldehyde), 66.0 (t, lactol), 41.0 (t, lactol), 32.3 (t, aldehyde), 23.4 (t, aldehyde), 22.6 (t, lactol); IR (neat) 3415, 2952, 2876, 1723 cm^{-1}.

2-Chlorohexanal[120] **228**



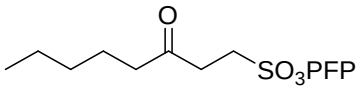
N-Chlorosuccinimide (7.02 g, 52.5 mmol) was added to a solution of proline (0.25 g, 2.19 mmol) in $CHCl_3$ (87 mL) at -30 °C. Hexanal (6.18 mL, 43.7 mmol) was then added and the resulting mixture was stirred at -30 °C for 21 h. The reaction mixture was concentrated *in vacuo* in an ice cold rotary evaporator. The crude product was purified by column chromatography eluting with petrol/Ether (99/1) which afforded the product as a clear oil which solidified upon freezing (3.70 g, 63% yield): m.p. 145-148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.48 (d, J = 2.5 Hz, 1H), 4.15 (ddd, J = 2.5, 5.4. 8.1Hz, 1H), 2.05-1.95 (m, 1H), 1.89-1.82 (m, 1H), 1.59-1.33 (m, 4H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CDCl3) δ 195.4 (s), 64.0 (d), 31.8 (t), 27.7 (t), 22.1 (t), 13.8 (q); IR (neat) 2959, 2932, 2863, 1732 cm^{-1}; $[\alpha]_D^{20}$ +7.9 c = 1.0; LRMS (CI) 135 (26%, $[M+H]^+$), 99 (36), 81 (100); HRMS (CI) calcd for $C_6H_{12}OCl$ $[M+H]^+$ 135.0576, observed 135.0571.

2-Iodohexanal[121] **229**



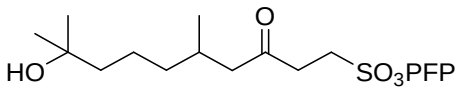
NaI (2.77 g, 18.5 mmol) was added to a solution of 2-chlorohexanal **228** (2.5 g, 18.5 mmol) in acetone (10 mL) at 0 °C and stirred for 1 h. The solvent was evaporated *in vacuo*. The crude product was redissolved in diethyl ether (5 mL) and filtered through a short pad of silica. Further purification by flash chromatography eluting with petrol/Et_2O (4/1) gave the product as a brown oil (1.49 g, 37% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.52 (d, J = 3.2 Hz, 1H), 4.47-4.43 (m, 1H), 2.04-1.85 (m, 2H), 1.39-1.31 (m, 4H), 0.91 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (s), 36.8 (d), 31.9 (t), 31.5 (t), 22.0 (t), 13.8 (q) ; IR (neat) 2957, 2929, 2859, 1720 cm^{-1}; LRMS (EI) 225 (100%, $[M]^{+\bullet}$), 115 (66%); HRMS (EI) calcd for $C_6H_{10}OI$ $[M]^{+\bullet}$ 224.9771, observed 224.9782.

Pentafluorophenyl 3-oxooctane-1-sulfonate 230



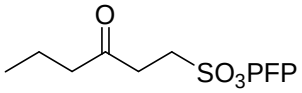
Hexanal (705 µL, 5 mmol) was added to a solution of pentafluorophenyl vinylsulfonate **208**(274 mg, 1 mmol) in dioxane (1 mL) and stirred for 6 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/CH_2Cl_2 (4/1) gave the title compound as a white solid (388 mg, 74% yield): m.p. 53-56 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (t, J = 7.2, 7.7 Hz, 2H), 3.14 (t, J = 7.2, 7.7 Hz, 2H), 2.50 (t, J = 7.4 Hz, 2H), 1.62 (quintet, J = 7.4 Hz, 2H), 1.29-1.25 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.1 (s), 46.9 (t), 42.7 (t), 35.8 (t), 31.2 (t), 23.3 (t), 22.3 (t), 13.8 (q); IR (neat) 2953, 2871, 1709, 1531, 1377 cm^{-1}; LRMS (FAB) 397 (100%, $[M+Na]^+$), 375 (30), 176 (23); HRMS (FAB) calcd for $C_{14}H_{15}F_5NaO_4S$ $[M+Na]^+$ 397.0508, observed 397.0500.

Pentafluorophenyl 9-hydroxy-5,9-dimethyl-3-oxodecane-1-sulfonate 232



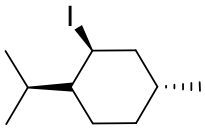
7-Hydrocycitronellal (936 µL, 5 mmol) was added to a solution of pentafluorophenyl vinylsulfonate **208** (274 mg, 1 mmol) in dioxane (1 mL) and stirred for 16 h. The reaction mixture was washed with sodium bisulphite (5 mL x 5), dried with anhydrous $MgSO_4$, filtered and the solvent evaporated *in vacuo.* Purification by flash chromatography eluting with petrol/EtOAc (4/1) gave the title compound as a pale yellow oil (335 mg, 78% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79-3.75 (m, 2H), 3.15-3.11 (m, 2H), 2.53 (dd, J = 16.0, 6.0 Hz, 1H), 2.35 (dd, J = 16.0, 8.0 Hz, 1H), 2.11-2.04 (m, 1H), 1.50-1.19 (m, 13H), 0.93 (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 204.9 (s), 71.0 (s), 50.1 (t), 46.9 (t), 43.8 (t), 37.2 (t), 36.5 (t), 29.4 (q), 29.3 (q), 29.2 (d), 21.6 (t), 19.8 (q); IR (neat) 3417, 2968, 1718, 1518, 1383 cm^{-1}; LRMS (FAB) 469 (85%, $[M+Na]^+$), 176 (100); HRMS (FAB) calcd for $C_{18}H_{23}F_5NaO_5S$ $[M+Na]^+$ 469.1084, observed 469.1090.

Pentafluorophenyl 3-oxohexane-1-sulfonate[82] 205



Butaraldehyde (445 µL, 5 mmol) was added to a solution of pentafluorophenyl vinylsulfonate **208** (288 mg, 1 mmol) in dioxane (1 mL) and stirred for 3 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/CH_2Cl_2 (4/1) gave the title compound as a white solid (273 mg, 79% yield): m.p. 53-55 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.75 (t, J = 7.3 Hz, 2H), 3.13 (t, J = 7.3 Hz, 2H), 2.50 (t, J = 7.3 Hz, 2H), 1.68-1.64 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.0 (s), 47.0 (t), 44.7 (t), 35.9 (t), 17.2 (t), 13.6 (q); IR (thin film) 2960, 2936, 1713 cm^{-1}; LRMS (FAB+) 369 (35% $[M+Na]^+$), 329 (18), 176 (100).

2-Iodo-1-isopropyl-4-methylcyclohexane[91]

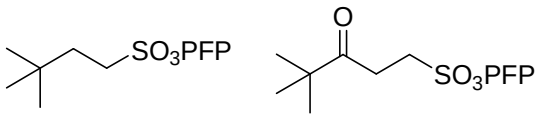


Imidazole (1.96 g, 28.8 mmol) and iodine (7.3 g. 28.8 mmol) were added to a suspension of PPh_3 (7.55 g, 28.8 mmol) in $CHCl_3$ (60 mL) at 0 °C and stirred for 30 min. A solution of L-menthol (3.0 g, 19.0 mmol) in CH_2Cl_2 (40 mL) was added and stirring continued for a further 30 min at 0 °C. The reaction mixture was allowed to warm to 21 ^{o}C and stirred for a further 2 h. The reaction was quenched with water (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with sat. aqueous sodium thiosulphate (2 x 50 mL) dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification twice by flash chromatography eluting with petrol/EtOAc (4/1) gave the title compound as a brown oil (2.91 g, 57% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82 – 4.74 (m, 1H), 2.22 (ddd, J = 3.2, 5.7, 14.4 Hz, 1H), 2.07 – 1.9 (m, 1H), 1.83 – 1.71 (m, 2H), 1.43 – 1.18 (m, 4H), 0.96 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), -0.15 (ddt, J = 2.9, 9.2, 11.8 Hz, 1H); ^{13}C NMR (75 MHz,$CDCl_3$) δ 49.3 (d), 46.7 (d), 45.3 (t), 34.8 (t), 33.8 (d), 28.6 (d), 26.7 (t), 21.6 (q), 20.4 (q), 20.0 (q); IR (thin film) 2948, 2922, 2867 cm^{-1}; LRMS (EI) 266 (60%, $[M]^{+\bullet}$), 139 (100).

General procedure for decarbonylation reactions:

Trimethylacetaldehyde (543 µL, 5 mmol) was added to a solution of alkene acceptor (1 mmol) in 1,4-dioxane (1 mL) at 100 °C and stirred until complete consumption of alkene (TLC). The solvent was evaporated *in vacuo* and purification as described below afforded the desired sulfonate ester.

Pentafluorophenyl 3,3-dimethylbutane-1-sulfonate 255 and **pentafluorophenyl 4,4-dimethyl-3-oxopentane-1-sulfonate**[82] **256**



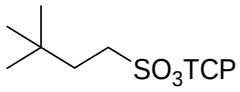
255 **256**

Reaction was complete after 3 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave pentafluorophenyl 3,3-dimethylbutane-1-sulfonate **255** as a clear oil (221 mg, 67 %) and pentafluorophenyl 4,4-dimethyl-3-oxopentane-1-sulfonate **256** as a clear oil (7 mg, 2% yield).

Data for 255: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.34-3.30 (m, 2H), 1.85-1.80 (m, 2H), 0.89 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 49.6 (t), 36.5 (t), 31.3 (s), 28.7 (q) ; IR (thin film) 2972, 2948 cm^{-1}; LRMS (CI) 333 (100%, $[M+H]^+$); HRMS (CI) calc. For $C_{12}H_{13}F_5O_3S$ 333.0583 $[M+H]^+$, observed 333.0587.

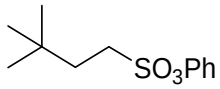
Data for 256: ^{1}H NMR (125 MHz, $CDCl_3$) δ 3.74-3.71 (m, 2H), 3.23-3.20 (m, 2H), 1.19 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.5 (s), 47.5 (t), 44.2 (s), 30.7 (t), 26.3 (q); IR (thin film) 2967, 1707, 1561 cm^{-1}; LRMS (CI) 361 (100% $[M+H]^+$), 177 (41), 113 (42).

2,4,6-Trichlorophenyl 3,3-dimethylbutane-1-sulfonate[82] 207



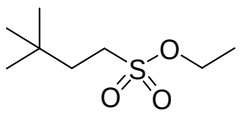
Reaction was complete after 6 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a white solid (220 mg, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (s, 2H), 3.55-3.51 (m, 2H), 2.09-2.01 (m, 2H), 1.99 (s, 9H); ^{13}C NMR (75 MHz,CDCl3) δ 142.0 (s), 133.9 (s), 130.6 (s), 129.1 (d), 51.2 (t), 36.6 (t), 30.3 (s), 28.9 (q); IR (thin film) 3079, 2957, 2866, 1718, 1557 cm-1; LRMS (CI) 347 (100%, $[M+H]^+$), 197 (20).

Phenyl 3,3-dimethylbutane-1-sulfonate[122] 259



Reaction was complete after 2 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a cloudy oil (186 mg, 76%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.41 (m, 2H), 7.33 (t, J = 7.4 Hz, 1H), 7.30-7.27 (m, 2H), 3.25 – 3.22 (m, 2H), 1.91-1.88 (m, 2H), 0.97 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.2 (s), 130.0 (d), 127.2 (d), 122.1 (d), 47.2 (t), 36.6 (t), 30.2 (s), 28.9 (q); IR (thin film) 2957, 2870 cm^{-1}; LRMS (EI) 242 (42% $[M]^{+\bullet}$), 94 (100); HRMS (EI) calcd for $C_{12}H_{18}O_3S$ $[M]^{+\bullet}$ 242.0971, observed 242.0976.

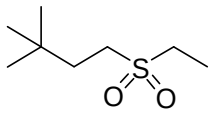
Ethyl 3,3-dimethylbutane-1-sulfonate[122] 260



Reaction was complete after 6 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a pale yellow oil (104 mg, 54%): ^{1}H

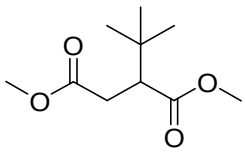
NMR (500 MHz, $CDCl_3$) δ 4.28 (q, J = 7.1 Hz, 2H), 3.08 – 3.02 (m, 2H), 1.76 – 1.70 (m, 2H), 1.40 (t, J = 7.1 Hz, 3H), 0.93 (s, 9H); ^{13}C NMR (125 MHz,$CDCl_3$) δ 65.8 (t), 47.1 (t), 36.5 (t), 30.0 (s), 28.8 (q), 15.1 (q); IR (thin film) 2956, 2871 cm^{-1}; LRMS (CI) 212 (100%, $[M+NH_4]^+$).

1-(Ethylsulfonyl)-3,3-dimethylbutane[122] **261**



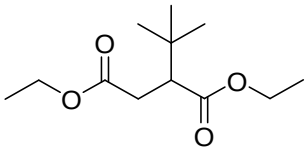
Reaction was complete after 40 min. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a clear oil (94 mg, 53%): ^1H NMR (400 MHz, $CDCl_3$) δ 2.99 (q, J = 7.5 Hz, 2H), 2.95-2.89 (m, 2H), 1.75-1.68 (m, 2H), 1.40 (t, J = 7.5 Hz, 3H), 0.95 (s, 9H); ^{13}C NMR (125 MHz,$CDCl_3$) δ 48.5 (t), 47.0 (t), 35.1 (t), 30.1 (s), 29.0 (q), 6.7 (q); IR (thin film) 2955, 2869 cm^{-1}; LRMS (CI) 179 (100%, $[M+H]^+$), 111 (8).

Dimethyl 2-*tert*-butylsuccinate 164



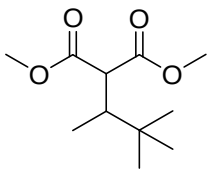
Reaction was complete after 6 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a pale yellow oil (126 mg, 63%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.70 (s, 3H), 3.67 (s, 3H), 2.80 (dd, J = 11.8, 16.5 Hz, 1H), 2.65 (dd, J = 11.8 Hz, 3.0, 1H), 2.49 (dd, J = 3.0, 16.5 Hz, 1H), 0.97 (s, 9H); ^{13}C NMR (125 MHz,$CDCl_3$) δ 174.7 (s), 173.2 (s), 51.8 (q), 51.4 (d), 51.1 (q), 32.6 (t), 32.6 (s), 27.9 (q); IR (thin film) 2954, 1731 cm^{-1}; LRMS (ES^+) 203 (100%, $[M+H]^+$), 171 (64), 143 (4); HRMS (ES^+) calcd for $C_{10}H_{19}O_4$ $[M+H]^+$ 203.1283, observed 203.1283.

Diethyl 2-*tert*-butylsuccinate[123,124] **262**



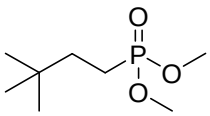
Reaction was complete after 7 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a clear oil (126 mg, 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22-4.07 (m, 4H), 2.77 (dd, J = 11.8, 16.4 Hz, 1H), 2.61 (dd, J = 3.0, 11.8 Hz, 1H), 2.45 (dd, J = 3.0, 16.4 Hz, 1H), 1.29-1.21 (m, 6H), 0.96 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ174.1 (s), 172.7 (s), 60.5 (t), 60.2 (t), 51.3 (d), 32.9 (t), 32.6 (s), 27.8 (q), 14.3 (q), 14.1 (q); IR (thin film) 2965, 1727 cm^{-1}; LRMS (FAB) 253 (25%, $[M+Na]^+$), 185 (100), 176 (30); HRMS (FAB) calcd for $C_{12}H_{22}O_4Na$ $[M+Na]^+$ 253.1415, observed 253.1409.

Dimethyl 2-(3,3-dimethylbutan-2-yl)malonate 263



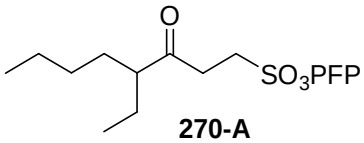
Reaction was complete after 96 h at 100 °C and then 24 h at 120 °C. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a pale yellow oil (21 mg, 10%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H), 3.70 (s, 3H), 3.56 (d, J = 5.4, 1H), 2.31-2.17 (m, 1H), 0.99 (d, J = 7.2, 3H), 0.88 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6 (s), 169.9 (s), 53.0 (q), 52.5 (d), 52.0 (q), 42.8 (q), 33.5 (s), 27.5 (q), 14.0 (d); IR (neat) 2955, 1740, 1750 cm^{-1}; LRMS (ES^+) 234 (62% $[M+NH_4]^+$), 217 (100, $[M+H]^+$), 185 (8); HRMS (ES^+) calc. For $C_{11}H_{21}O_4$ 217.1434 $[M+H]^+$, observed 217.1430.

Dimethyl 3,3-dimethylbutylphosphonate 264

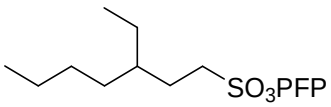


Reaction was complete after 4 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave the title compound as a pale yellow oil (96 mg, 50%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.69 (s, 3H), 1.70-1.63 (m, 2H), 1.46-1.42 (m, 2H), 0.85 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.3 (dq, $J_{C\text{-}P}$ = 25 Hz), 35.7 (dt, $J_{C\text{-}P}$ = 21 Hz), 30.3 (ds, $J_{C\text{-}P}$ = 70 Hz), 28.7 (s), 20.1 (dt, $J_{C\text{-}P}$ = 561 Hz); IR (neat) 2954, 2867 cm^{-1}; LRMS (CI) 195 (100%, $[M+H]^+$), 179 (23), 138 (19); HRMS (CI) calcd for $C_8H_{20}O_3P$ $[M+H]^+$ 195.1150, observed 195.1156.

Pentafluorophenyl 4-ethyl-3-oxooctane-1-sulfonate[82] **(270-A)** and
Pentafluorophenyl 3-ethylheptane-1-sulfonate (270-B)



270-A



270-B

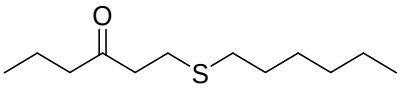
Reaction was stopped after 3 h. Purification by flash chromatography eluting with petrol/CH_2Cl_2 (6/1) gave pentafluorophenyl 4-ethyl-3-oxooctane-1-sulfonate **270-A** as a colourless oil (96 mg, 50% yield) and pentafluorophenyl 3-ethylheptane-1-sulfonate **270-B** as a colourless oil (45 mg, 12% yield).

Data for 270-A: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.77-3.74 (m, 2H), 3.16-3.14 (m, 2H), 2.51-2.47 (m, 1H), 1.71-1.60 (m, 2H), 1.59-1.44 (m, 2H), 1.37-1.27 (m, 2H), 1.26-1.17 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 208.9 (s), 53.9 (d), 46.9 (t), 35.5 (t), 30.9 (t), 29.9 (t), 24.6 (t), 22.7 (t), 13.9 (q), 11.7 (q); IR (neat) 2961, 2876, 1708, 1519, 1387 cm^{-1}; LRMS (CI) 403 (15%, $[M+H]^+$), 145 (10), 127 (100); HRMS (CI) calc. For $C_{16}H_{20}F_5O_4S$ 403.1002 $[M+H]^+$, found 403.1012.

Data for 270-B: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.45-3.38 (m, 2H), 2.04-1.96 (m, 2H), 1.46 (m, 1H), 1.42 – 1.23 (m, 8H), 0.93 – 0.88 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

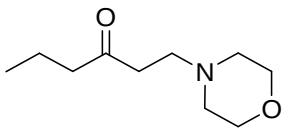
50.9 (t), 37.9 (d), 32.3 (t), 28.6 (t), 26.6 (t), 25.4 (t), 22.9 (t), 14.0 (q), 10.6 (q); IR (thin film) 2962, 2876 cm^{-1}; LRMS (CI) 375 (34% $[M+H]^+$), 297 (100), 142 (86); HRMS (CI) calcd for $C_{15}H_{20}F_5O_3S$ $[M+H]^+$ 375.1053, observed 375.1053.

1-(Hexylthio)hexan-3-one[83] 243



Hexanethiol (52 µL, 0.37 mmol) was added to a solution of pentafluorophenyl 3-oxohexane-1-sulfonate **205** (100 mg, 0.28 mmol) and DBU (86 µL, 0.57 mmol) in CH_2Cl_2 (1 mL) and stirred for 45 min. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/CH_2Cl_2 (3/1) gave the title compound as a yellow oil (62 mg, quantitative yield): ^1H NMR (400 MHz, $CDCl_3$) δ 2.75-2.67 (m, 4H), 2.52 (t, J = 6.0 Hz , 2H), 2.42 (t, J = 6.0 Hz, 2H), 1.67-1.54 (m, 4H), 1.41-1.23 (m, 6H), 0.94-0.86 (6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.8 (s), 45.0 (t), 42.8 (t), 32.5 (t), 31.4 (t), 29.6 (t), 28.6 (t), 25.9 (t), 22.6 (t), 17.2 (t), 14.0 (q), 13.7 (q); IR (neat) 2958, 2927, 2857, 1713 cm^{-1}; LRMS (ES^+) 217 (100%, $[M+H]^+$); HRMS (ES^+) calcd for $C_{12}H_{25}OS$ $[M+H]^+$ 217.1621, observed 217.1622.

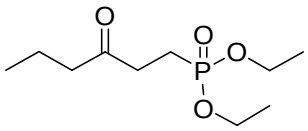
1-Morpholinohexan-3-one[127] 246



Pentafluorophenyl 3-oxohexane-1-sulfonate **205** (200 mg, 0.55 mmol) and DBU (173 µL, 1.09 mmol) in CH_2Cl_2 (1 mL) were stirred at for 20 mins, to which was added morpholine (56 µL, 0.63 mmol) and stirred for 3 h. The solvent was evaporated *in vacuo* and purification three times by flash chromatography eluting with petrol/EtOAc (2/1) gave the title compound as a yellow oil (81 mg, 80% yield): ^1H NMR (600 MHz, $CDCl_3$) δ 3.72 (t, J = 4.3 Hz, 4H), 2.70-2.62 (m, 4H), 2.47 (br s, 4H), 2.42 (t, J = 7.4 Hz, 2H), 1.62 (sextet J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H): ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.8 (s), 66.7 (t), 53.5 (t), 53.0 (t), 45.0 (t), 39.9 (t), 17.1 (t), 13.7 (q); IR (neat) 2959,

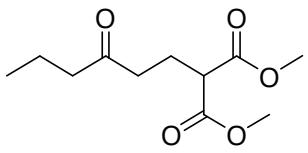
2854, 2808, 1709 cm^{-1}; LRMS (CI) 186 (100% $[M+H]^+$), 88 (43); HRMS (CI) calcd for $C_{10}H_{20}NO_2$ $[M+H]^+$ 186.1489, observed 186.1492.

Diethyl 3-oxohexylphosphonate[125] 239



Triethylphosphite (99 µL, 0.57 mmol) was added to a solution of pentafluorophenyl 3-oxohexane-1-sulfonate **205** (100 mg, 0.28 mmol) and DBU (86 µL, 0.57 mmol) in EtOH (1 mL) in a microwave tube. The tube was sealed and heated at 100 °C (MW) for 1 h. The reaction was washed with $NaHCO_3$ (2 x 5 mL), water (5 mL). The organic layers were collected and dried with $MgSO_4$, filetered, and concentrated *in vacuo*. Purification by flash chromatography eluting with CH_2Cl_2/MeOH (30/1) gave the title compound as a yellow oil (123 mg, 90% yield): ^{1}H NMR (600 MHz, $CDCl_3$) 4.15-4.05 (m, 4H), 2.74 – 2.69 (m, 2H), 2.42 (t, J = 7.4 Hz, 2H), 2.05 – 1.99 (m, 2H), 1.63 (sextet, J = 7.4 Hz, 2H), 1.34 (t, 6H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 208.3 (d, $J_{C\text{-}P}$ = 15 Hz), 61.7 (dt, $J_{C\text{-}P}$ = 6 Hz), 44.5 (t), 35.4 (dt, $J_{C\text{-}P}$ = 4.5 Hz), 19.3 (dt, $J_{C\text{-}P}$ = 144 Hz), 17.3 (t), 16.4 (dq, $J_{C\text{-}P}$ = 6 Hz), 13.7 (q); IR (neat) 2963, 2933, 1716 cm^{-1}; LRMS (ES^+) 237 (100%, $[M+H]^+$); HRMS (ES^+) calcd for $C_{10}H_{22}O_4P$ $[M+H]^+$ 237.1250, observed 237.1250.

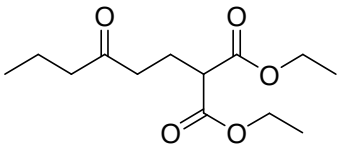
Dimethyl 2-(3-oxohexyl)malonate[126] 244



$NaBH_4$ (0.5 g, 13.21 mmol) was refluxed in MeOH (25 mL) for 40 min. The solvent was evaporated *in vacuo* to give $NaB(OMe)_4$ as a white solid (1.28 g, 8.1 mmol). $NaB(OMe)_4$ (5 mg, 0.03 mmol) and dimethyl malonate (66 µL, 0.57 mmol) was added to a solution of pentafluorophenyl 3-oxohexane-1-sulfonate **205** and DBU (173 µL, 1.14 mmol) in acetonitrile (1.5 mL) under argon and stirred for 48 h. Purification by flash

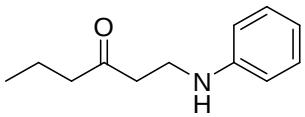
chromatography eluting with petrol/EtOAc (9/1) gave the product as a clear oil (81 mg, 63% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (s, 6H), 3.44 (t, J = 8.0 Hz, 1H), 2.50 (t, J = 8.0 Hz, 2H), 2.37 (t, J = 7.8 Hz, 2H), 2.16 (q, J = 7.8 Hz, 2H), 1.59 (sextet, J = 7.8 Hz, 2H), 0.90 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.0 (s), 169.6 (s), 52.6 (q), 50.4 (d), 44.8 (t), 39.4 (t), 22.5 (t), 17.3 (t), 13.7 (q); IR (neat) 2957, 1720, 1712 cm^{-1}; LRMS (ES^+) 248 (48% $[M+NH_4]^+$), 231 (100, $[M+H]^+$), 199 (35); HRMS (ES^+) calcd for $C_{11}H_{19}O_5$ $[M+H]^+$ 231.1227, observed 231.1225.

Diethyl 2-(3-oxohexyl)malonate 245



Dimethyl malonate (132 µL, 1.15 mmol) was added to a solution of pentafluorophenyl 3-oxohexane-1-sulfonate **205** (200 mg, 0.77 mmol) and NaOEt (869 µL, 1.84 mmol) in ethanol (4 mL) and stirred at for 48 h. The solvent was evaporated *in vacuo* and purification by flash chromatography eluting with petrol/Et_2O (6/1) gave the title compound as a clear oil (22 mg, 11% yield): ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.26 – 4.16 (m, 4H), 3.40 (t, J = 7.3 Hz, 1H), 2.52 (t, J = 3.6 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.17 (q, J = 7.3 Hz, 2H), 1.58 (sextet, J = 7.3 Hz, 2H), 1.28 (t, J = 7.1 Hz, 6H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.7 (s), 169.2 (s), 61.4 (t), 50.7 (d), 44.7 (t), 39.5 (t), 22.4 (t), 17.2 (t), 14.0 (q), 13.7 (q); IR (neat) 2964, 2937, 1750, 1745 cm^{-1}; LRMS (EI) 258 (8% $[M]^{+\bullet}$), 169 (100); HRMS (EI) calcd for $C_{13}H_{22}O_5$ $[M]^{+\bullet}$ 258.1462, observed 258.1472.

1-(Phenylamino)hexan-3-one[127] **247**



DBU (173 µL, 1.14 mmol) was added to a solution of pentafluorophenyl 3-oxohexane-1-sulfonate **205** (200 mg, 0.57 mmol) in CH_2Cl_2 (3 mL) and stirred for 20 min. Aniline

(53 µL, 0.57 mmol) was added to the reaction mixture and stirred for 24 h. The solvent was evaporated *in vacuo* and purification twice by flash chromatography eluting with petrol/EtOAc (19/1) gave the title compound as a white solid (41 mg, 38% yield): m.p. 60-61 °C; ^{1}H NMR (600 MHz, $CDCl_3$) 7.22 – 7.18 (m, 2H), 6.76 (tt, J = 7.3 Hz, 1.0, 1H), 6.67 – 6.65 (m, 2H), 3.44 (t, J = 6.2 Hz, 2H), 2.74 (t, J = 6.2 Hz, 2H), 2.41 (t, J = 7.4 Hz, 2H), 1.61 (sextet, J = 7.3, 2H), 0.92 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.4 (s), 147.2 (s), 129.3 (d), 118.1 (d), 113.4 (d), 45.1 (t), 41.4 (t), 38.8 (t), 17.1 (t), 13.7 (q); IR (neat) 3391, 2957, 2871, 1700, 1605 cm^{-1}; LRMS (EI) 191 (43%, $[M]^{+\bullet}$), 106 (100%); HRMS (EI) calcd for $C_{12}H_{17}ON$ $[M]^{+\bullet}$ 191.1304, observed 191.1301.

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