Methods for the Asymmetric Synthesis
of α-Fluoro and α,α-Difluoro-β³-Amino Acids

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A thesis submitted in fulfilment of the requirements for the degree
Doctor of Philosophy

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2010

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SUMMARY

Fluorinated β^3-amino acids are becoming increasingly important due to their potential therapeutic applications, particularly with regard to enzyme inhibition, however research in this area is somewhat limited by a lack of adequate methods for their preparation. Strategies to prepare β-amino acids that feature complex side chains e.g., lysine and arginine, receive even less attention, and are thus the focus of this work, which details the development of two complementary methodologies for the asymmetric synthesis of α-fluoro- and α,α-difluoro-β^3-amino acids.

Chapter 1 discusses the tandem conjugate addition of a chiral lithium amide to an α,β-unsaturated ester, whereby the intermediate enolate is quenched with an electrophilic source of fluorine. A series of α-fluoro-β^3-amino esters were subsequently prepared using this method, with silyl-protected substrates proving the most suitable (*Figure 1*). A stepwise method involving separate deprotonation and fluorination reactions was also investigated, however this failed to match the diastereoselectivity of the tandem reaction.
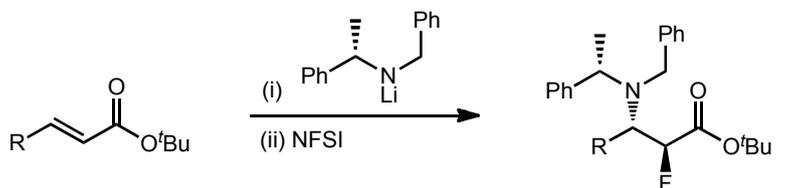
R = Me, TBSO(CH$_2$)$_2$, TBSO(CH$_2$)$_3$, Boc$_2$N(CH$_2$)$_3$, 4-Si(Ph$_2$)-piperidine(CH$_2$)$_3$

Figure 1 Tandem conjugate addition-fluorination method for the preparation of α-fluoro-β^3-amino esters.

Demonstrating the applicability of this tandem conjugate addition-fluorination reaction to the preparation of α-fluoro-β^3-amino acids, two silyl-protected conjugate adducts were then transformed into orthogonally protected α-fluoro-β^3-lysine and α-fluoro-β^3-arginine derivatives (*Figure 2*), as described in Chapter 2.
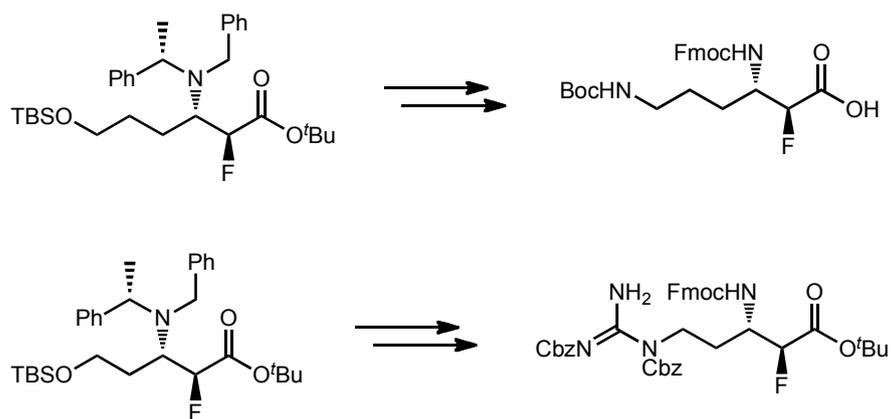
Figure 2 Fluorinated derivatives of β-lysine and β-arginine prepared from silyl-protected conjugate adducts

Access to the corresponding α,α-difluoro-β³-amino esters was achieved via the development of a Reformatsky reaction employing chiral aliphatic imines derived from phenylglycine (*Figure 3*), which is discussed in Chapter 3. This method improves upon the Reformatsky methodologies currently available for the preparation of α,α-difluoro-β³-amino esters bearing aliphatic β-substituents, which have historically given unsatisfactory yields and diastereoselectivities.

R = ^{i}Pr, ^{i}Bu, ^{n}C$_7$H$_{15}$, ^{c}C$_6$H$_{11}$, Ph(CH$_2$)$_2$, CH$_2$=CH(CH$_2$)$_2$, TBSO(CH$_2$)$_2$, TBSO(CH$_2$)$_3$, Phth(CH$_2$)$_3$
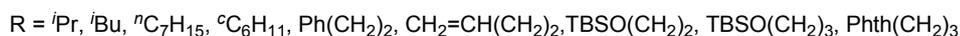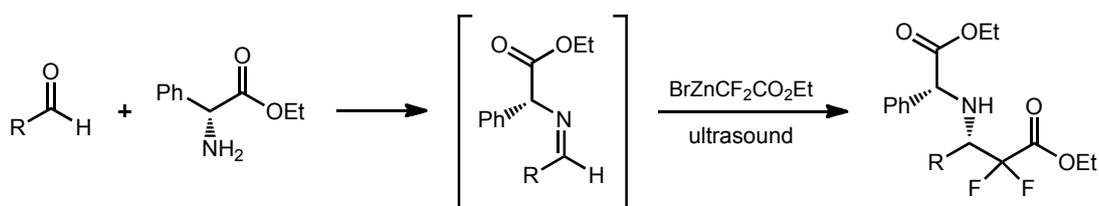
Figure 3 Preparation of α,α-difluoro-β³-amino esters via the Reformatsky reaction.

The versatility of these conjugate addition and Reformatsky methodologies was further demonstrated in Chapter 4, in which the preparation of three β³-arginine analogues is detailed. An unfluorinated and α-fluoro analogue were both prepared from substrates synthesised via the conjugate addition methodology, while the corresponding α,α-difluoro analogue was prepared using the Reformatsky reaction.

The complementarity of the two techniques is demonstrated by the fact that each of the analogues was derived from the same achiral aldehyde (*Figure 4*). Given the successful synthesis of these compounds, each of the two methodologies discussed in this thesis should find greater use amongst the synthetic community for the preparation of α-fluoro and α,α-difluoro-β^3-amino acids.
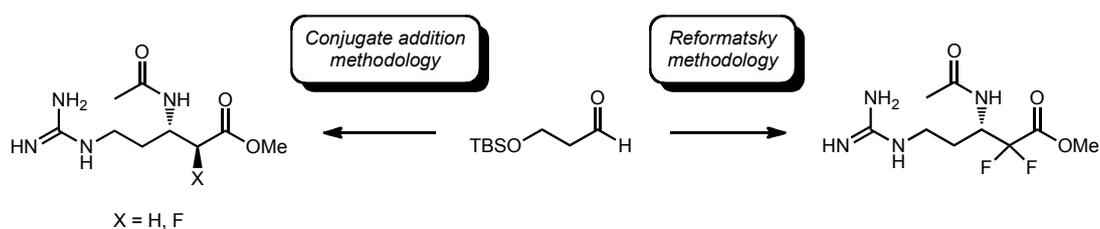
Figure 4 Preparation of unfluorinated, monofluoro and difluoro analogues of β^3-arginine.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Taryn March

Dated: …………………………………………

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

2,4-DNP	2,4-dinitrophenylhydrazine
9-BBN	9-borabicyclo[3.3.1]nonane
Ac_2O	acetic anhydride
AcCl	acetyl chloride
AcOH	acetic acid
AIBN	2,2-azo-bis-isobutyronitrile
Alloc	allyloxycarbonyl
atm	atmospheres
Bn	benzyl
BnBr	benzyl bromide
Boc	*tert*-butyloxycarbonyl
Boc_2O	*tert*-butyl dicarbonate
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate
bs	broad singlet
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
Cl-Cbz	2-chlorobenzyloxycarbonyl
COSY	correlation spectroscopy
d	doublet
DAST	diethylaminosulfur trifluoride
DCC	*N,N*-dicyclohexylcarbodiimide
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublets of doublets
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine

DME	dimethyl ether
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DPSide	diphenylsilyldiethylene
dq	doublet of quartets
dr	diastereomeric ratio
dt	doublet of triplets
ee	enantiomeric excess
ESI	electrospray ionisation
Et	ethyl
Et$_2$Zn	diethylzinc
Et$_3$N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	9-fluorenylmethoxycarbonyl
Fmoc-OSu	9-fluorenylmethoxycarbonyl succinimide
HIU	high intensity ultrasound
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
J	coupling constant (Hz)
KHMDS	potassium hexamethyldisilazide
LDA	lithium diisopropylamine
LiHMDS	lithium hexamethyldisilazide
m	multiplet
Me	methyl
MeCN	acetonitrile
MeI	methyl iodide
MeNH$_2$	methylamine
MeO	methoxy
MeOH	methanol

MIS	1,2-dimethoxyindole-3-sulfonyl
^{n}BuLi	*n*-butyllithium
NFOBS	*N*-fluoro-*o*-benzenedisulfonimide
NFSI	*N*-fluorobenzenesulfonimide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
Pbf	2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl
PG	protecting group
Ph	phenyl
PMB	*p*-methoxybenzyl
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
PMP	*p*-methoxyphenyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
q	quartet
R_f	retention factor
RT	room temperature
s	singlet
SAR	structure activity relationship
SPPS	solid phase peptide synthesis
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
TBSO	*tert*-butyldimethylsilyloxy
^{t}BuOH	*tert*-butanol
TFA	trifluoroacetic acid
TFMK	trifluoromethyl ketone
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TolSH	thiotoluene
TsCl	*p*-toluenesulfonyl chloride

CHAPTER 1:

INTRODUCTION

1.1   Fluorinated serine protease inhibitors

The serine proteases are a family of enzymes that all feature in their active site three key amino acids—histidine, serine and aspartic acid, which together make up what is known as the catalytic triad. Responsible for catalysing the hydrolysis of amide bonds, they are grouped according to the type of substrate amino acid residue adjacent to the scissile. For example, the protease chymotrypsin features a hydrophobic pocket in its active site that makes it selective for peptide bonds adjacent to bulky, hydrophobic amino acid residues, while the enzyme trypsin prefers positively charged amino acid residues like arginine and lysine as a result of an additional aspartic acid residue in one of its substrate binding pockets.

Serine proteases feature a common catalytic cycle involving initial attack on the amide bond by the hydroxy group of the highly reactive serine residue, which results in the formation of a tetrahedral intermediate (*Figure 1*, A). This reaction is facilitated by the histidine residue, whose imidazole ring acts as a base by accepting a proton from serine. Subsequent proton transfer to the peptide splits it in two, giving a terminal amine fragment and a covalently bound acyl-enzyme complex (*Figure 1*, B). A water molecule then enters the active site and attacks the complex, forming a second tetrahedral intermediate. Proton transfer from histidine to serine releases the carboxylic acid fragment and the active site is regenerated. An analogous method of catalysis exists for the cysteine proteases which, as their name suggests, possess the thiol-functionalised cysteine residue instead of serine.

An important feature of the active site is a region called the oxyanion hole. This site is formed from the backbone amide protons of the serine 195 and glycine 193 residues, which are perfectly situated to participate in hydrogen bonding with the negatively charged oxygen atom of the unstable tetrahedral intermediate. This interaction provides additional stability and enhances the rate of hydrolysis. As this mode of stabilisation is confined to the active sites of serine and cysteine proteases, compounds capable of specifically interacting with the oxyanion hole of the target enzyme potentially make good inhibitors.
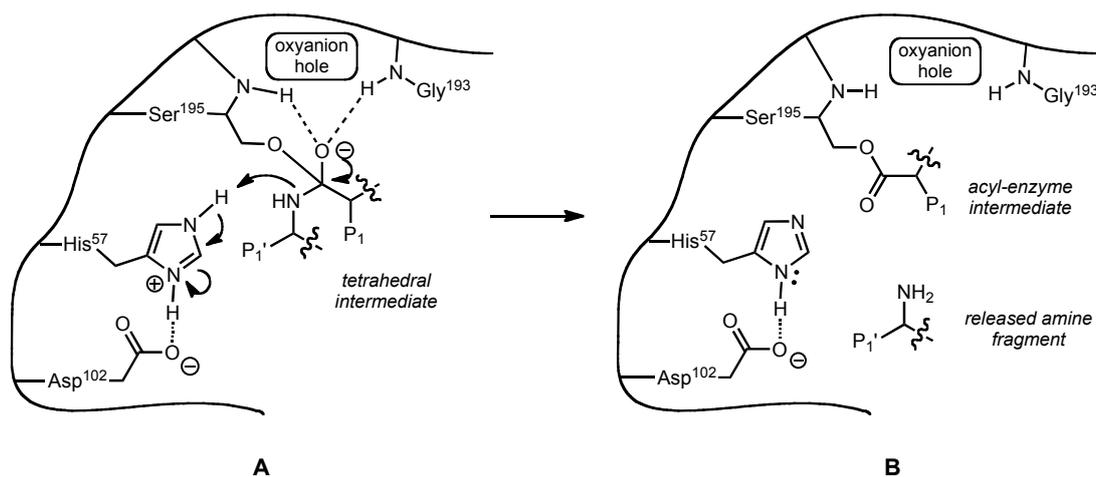
Figure 1 Part of the catalytic cycle that takes place in the active site of a serine protease.[1]

A substrate capable of interacting with the protease in such a way that it remains either irreversibly bound to serine or dissociates only very slowly from the active site can act as an enzyme inhibitor. The enzyme is effectively deactivated and unable to catalyse further reactions, which can significantly affect major physiological processes, particularly if the enzyme is part of a cascade pathway within the body. As the active site of enzymes are often very specific with regard to the type of substrate they accept, an enzyme can be selectively targeted for inhibition if enough is known about the structural characteristics of its active site (e.g., through X-ray analysis); the best inhibitors are generally very similar in structure to the endogenous substrate, as the higher the number of binding interactions within the active site (i.e., the specificity), the more effective the inhibition is likely to be.

Given the nature of the mechanism involved in proteolysis by the serine proteases, the presence of a carbonyl moiety in an inhibitor is highly desirable. While esters and amides are susceptible to hydrolytic cleavage, aldehydes and ketones form tetrahedral adducts instead. Hence, once they are bound to the enzyme, there is no feasible way to progress towards the two products. This is advantageous when seeking to design an irreversible inhibitor that remains covalently bound to the enzyme. Ketones are preferable to aldehydes because of the ability to extend the chain length on either side of the carbonyl group, however their lower reactivity

makes them much less prone to nucleophilic attack by the serine residue. It was postulated that placing highly electron withdrawing substituents adjacent to the carbonyl group would increase the carbonyl carbon's electrophilicity, thus promoting nucleophilic attack by serine. As it was already known that difluoro- and trifluoromethyl ketones form tetrahedral hydrates in solution, it seemed reasonable to assume that the introduction of fluoroalkyl moieties to these ketones could enhance their inhibitory effects.

The stable and highly electron withdrawing trifluoromethyl group was the first to be investigated. Early reports of trifluoromethyl ketones (TFMKs) for use as serine protease inhibitors appeared in the mid 1980s as two independent research groups[2,3] sought to prepare metabolically stable analogues of unfluorinated inhibitors containing highly electrophilic carbonyl groups. It was shown that these compounds were excellent slow-binding inhibitors of the proteolytic enzymes acetylcholinesterase and elastase, and much more potent than the hydrocarbon analogues. In one example it was found that **1**—the trifluoromethyl analogue of known angiotensin converting enzyme (ACE) inhibitor **2**, was 300 times more potent than this unfluorinated substrate (*Figure 2*). The effect of varying the number of fluorine atoms in the TFMK was also investigated by synthesising ketones with terminal CFH_2 and CF_2H groups.[4] It was found that trifluoro > difluoro > monofluoro in terms of inhibitory activity, with the TFMK possessing a K_i 30 times lower than that of the difluoro analogue. One theory to explain this was that the additional inductive effects of the CF_3 lowered the pK_a of the hemiketal hydroxy group, enabling a more effective interaction with the oxyanion hole.

Figure 2 Proline-derived inhibitors of angiotensin converting enzyme.

Trifluoromethyl ketones generally inhibit proteases in one of three forms: (i) the keto structure, which results in binding to the active site without the formation of a covalent bond between the substrate and enzyme (**3**); (ii) as a covalently bound hemiketal intermediate that acts as an analogue of the transition state formed during the hydrolysis of natural peptide substrates (**4**) and (iii) as the hydrated *gem*-diol species (**5**) which, like the keto form, results in non-covalent interactions with amino acid residues in the active site (*Figure 3*). The CF_3 moiety destabilises the ketone, ensuring that the hydrate is the predominant form in aqueous solutions. This allows the substrate to effectively mimic the structure of the hemiketal intermediates, which generally increases their effectiveness as inhibitors, although the preferred form of the substrate is highly enzyme-dependent. The inductive effects of the CF_3 group also lower the pK_a of the hemiketal OH, ensuring it is ionised, as well as help to stabilise tetrahedral adduct **4**, slowing dissociation of the inhibitor out of the active site.

Figure 3 Trifluoromethylketones as part of a covalently bound hemiketal intermediate (**4**) and in their hydrated form (**5**).

Unfortunately CF_3 groups can only be placed on the carboxyl-terminus, which prevents chain elongation and a lack of specificity on one side of the cleavage site. Building on the fact that α-keto acids were known inhibitors of protease enzymes and can be extended at either end, Abeles and Parisi prepared a series of fluorinated α-keto derivatives based on phenyl and benzylpyruvic acid (*Figure 4*).[5] Here the reactive carbonyl is flanked by two electron withdrawing groups—either fluoromethine or difluoromethylene, and a second carbonyl group, which ensures that the α-keto moiety is hydrated. While fluorinated α-keto esters were found to be better inhibitors of chymotrypsin than the corresponding α-keto acids, the most potent and selective inhibitors were those containing a peptide chain, which had

been included to encourage binding interactions. Again, difluoro substrates were better inhibitors than the corresponding monofluoro analogues. These results indicate that fluorination itself is often not enough to accomplish good inhibition, but should be combined with peptidyl substituents that meet the specificity requirements of the active site.

6

R = H: K_i = 270 μM
R = Et: K_i = 15 μM

7

K_i = 0.19 μM
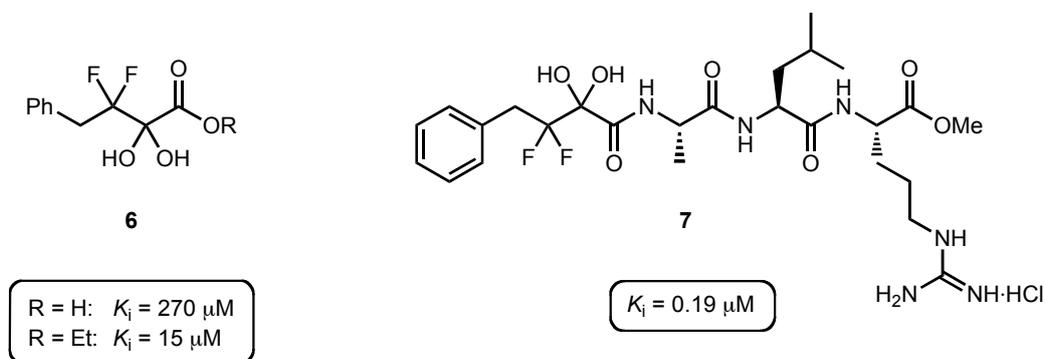
Figure 4 Examples of hydrated fluorobenzylpyruvic acid derivatives and their inhibition constants for the protease chymotrypsin.

High specificity is essential when trying to minimise undesired interactions between an inhibitor and other enzymes and nucleophiles within the body, which can result in unwanted consequences for other physiological processes. Obviously those inhibitors bearing a close structural resemblance to the natural peptide substrate are likely to be amongst the most selective, and various inhibitors feature the peptide sequences required for binding with only the scissile amide bond replaced with a more stable (e.g., non-hydrolysable) component. One example of a highly selective fluorinated peptidomimetic is the difluoromethylene ketone **8** (*Figure 5*), an inhibitor of the protease human heart chymase reported by Eda *et al.*,[6] where favourable interactions involving the Val-Pro-Phe peptide sequence were found to be a key factor in the observed potency. Likewise, Skiles *et al*. devised potent inhibitors of human neurophil elastase (HNE), such as the difluorostatone **9**, which bore several amino acid residues capable of interacting with the enzymes subsites.[7] However, peptidic substrates tend to make poor bioactives *in vivo*—absorption, solubility and degradation issues often occur due to the inherent limitations of the peptide structure, which in the case of oral administration, are exacerbated by the need to pass through the gastrointestinal

tract before reaching the site of action. Therefore, heterocyclic components such as pyridone or pyrimidine rings are commonly introduced to peptide-based leads to improve their stability and solubility. Such compounds are termed peptidomimetics, and are usually designed through extensive structure activity relationship (SAR) studies that identify the key functional groups required for optimal binding interactions.
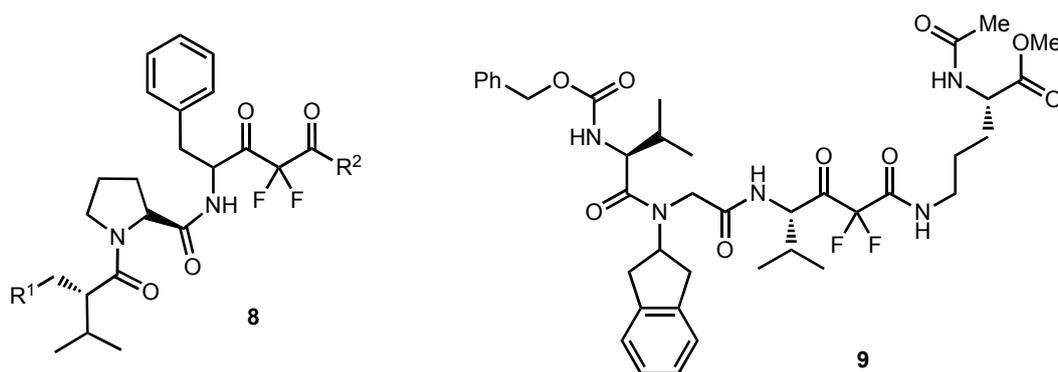
Figure 5 Fluorinated peptidomimetics successful in the inhibition of serine proteases.

While difluorinated and trifluorinated compounds account for the majority of fluorinated protease inhibitors because of their high stability and relative ease of synthesis, a few examples of inhibitors possessing a single fluorine atom do exist. Ohba *et al.* designed a series of mono- and difluoro homologated phenylalanine derivatives as potential α-chymotrypsin inhibitors hoping to exploit fluorine's ability to participate in hydrogen bonding.[8] It was postulated that replacing the oxygen atom of the scissile amide bond with fluorine would result in either a slow-binding mechanism, whereby the enzyme-inhibitor complex was stabilised by hydrogen bonding between fluorine and the oxyanion hole, or irreversible inhibition in which said hydrogen bonding could facilitate the usually difficult displacement of F$^-$ upon nucleophilic attack by the active site serine (*Figure 6*).

In replacing this oxygen atom and introducing a methyl ester moiety adjacent to fluorine, Ohba's group effectively created several α-fluoro-β^3-amino esters, a class of compound that had received very little attention due to a lack of effective
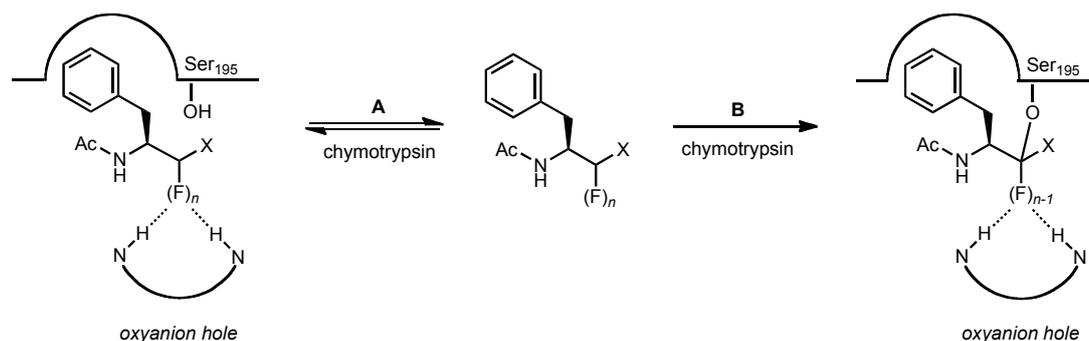
Figure 6 Ohba's postulated mechanisms of reversible (**A**) and irreversible (**B**) inhibition of the serine protease chymotrypsin by fluorinated phenylalanine derivatives.[8]

synthetic methods. In addition to the esters, two allylic analogues were also prepared to compare the inhibitory activities of the two functional groups (*Figure 7*). Inhibition assays revealed that while allylic monofluoro derivative **10** possessed no inhibitory activity towards α-chymotrypsin, difluoro analogue **11** was a slow-binding inhibitor with a K_i of 0.46 mM. On the other hand, the three β^3-amino esters displayed reversible competitive inhibition; *syn*-isomer **12** possessed a K_i of 19.1 mM, while the *anti*-isomer was clearly involved in more favourable binding interactions, recording a K_i of 6.44 mM and demonstrating the importance of configuration. However it was the α,α-difluoro-β-amino ester **14** that proved the most potent inhibitor, with a K_i of 0.034 mM. These findings are in general agreement with those of Abeles discussed earlier, which showed that a greater number of fluorine atoms generally results in higher levels of inhibitory activity.

10 $n = 1$	No inhibition
11 $n = 2$	$K_i = 0.046$ mM

12 $n = 1$ (*syn*)	$K_i = 19.1$ mM
13 $n = 1$ (*anti*)	$K_i = 6.44$ mM
14 $n = 2$	$K_i = 0.034$ mM
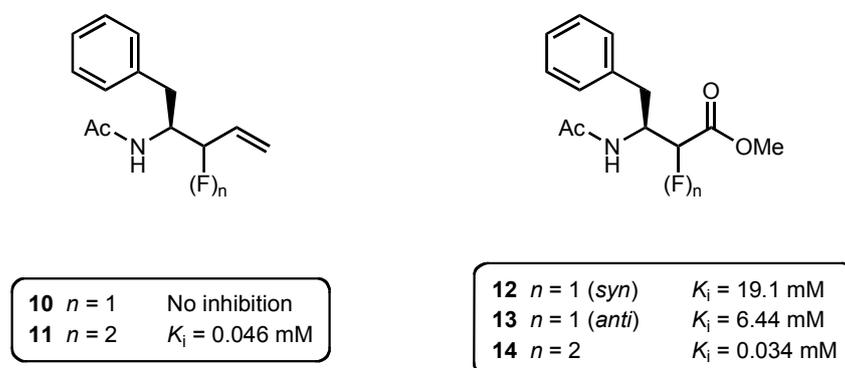
Figure 7 Fluorinated phenylalanine derivatives prepared by Ohba *et al.* as potential α-chymotrypsin inhibitors.

Given Ohba's promising results, Bromfield synthesised and assayed two related α-fluoro-β-phenylalanine derivatives (*Figure 8*).[1,9] Featuring a phenyl ring as the β-substituent rather than the benzyl group of Ohba's inhibitors, these compounds more closely resemble phenylalanine, a preferred substrate of α-chymotrypsin. It was hoped that such fluorinated β-amino acids could be stable substitutes for the scissile α-amino acid of a peptide, preventing or at least slowing the rate of enzymatic degradation. The unfluorinated β-phenylalanine ester **15** displayed reversible *non-competitive* inhibition of α-chymotrypsin, with the observed K_i of 3.60 ± 0.83 indicating that it was twice as potent as Ohba's *anti*-fluoro **13**. However, as the type of inhibition was different (non-competitive versus **13**'s competitive), it is difficult to compare the two results and ascertain whether the shorter side chain was responsible for the improved inhibitory activity. The results obtained with fluorinated analogue **16** allow a better comparison, however: possessing a K_i of 0.88 ± 0.40 mM and displaying reversible *competitive* inhibition, **16** showed a seven-fold increase in potency over **13**, a substrate whose structure differed by only one methylene unit. Together, the work of Ohba and Bromfield demonstrates two things: fluorination notwithstanding, β-amino esters themselves are effective inhibitors of serine proteases (conditional upon optimal side chain structure), and that further modification of these β-amino esters through α-fluorination can enhance their inhibitory activity substantially. Individually, both fluorine and β-amino acids have had significant roles in the field of medicinal chemistry. The following sections are dedicated to explaining the reasons behind this, and then how a combination of the two has led to a promising new class of bioactives—fluorinated β-amino acids.

15

K_i = 3.60 ± 0.83 mM

16

K_i = 0.88 ± 0.40 mM
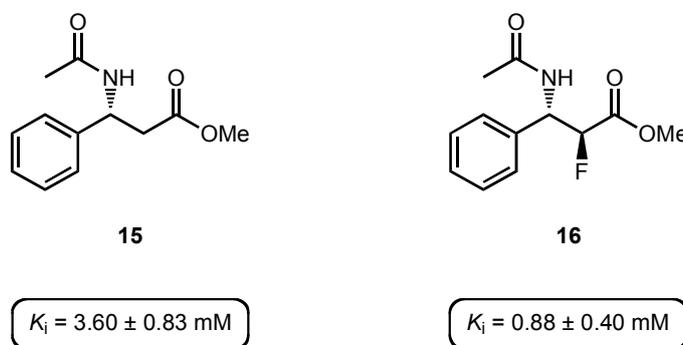
Figure 8 β³-amino esters prepared by Bromfield as potential α-chymotrypsin inhibitors.

1.2   Fluorine in medicinal chemistry

The use of fluorine in medicinal chemistry is far from limited to enzyme inhibitors—it is estimated that between 20-25% of all drugs currently in use feature one or more fluorine atoms.[10] The impact of organofluorine chemistry on the pharmaceutical industry has been so great that almost every class of drug features at least one fluorinated compound. This is despite the fact that naturally occurring organofluorine compounds are virtually non-existent; simple fluorinated substrates have been discovered in the biosynthetic processes of certain plants and microorganisms[11-13], however the total number barely exceeds one dozen. The over-representation of fluoro-organics amongst therapeutically useful bioactive compounds is for good reason, however—the unique properties possessed by fluorine can significantly alter the attributes of neighbouring functional groups, which can then profoundly affect the overall pharmacological properties of a compound. Such are the number of potential changes imparted on a drug candidate through the addition of fluorine that, given a random selection of fluorinated therapeutic agents, it is likely that the role played by the fluorine moiety is different in each case. These roles can range from increasing a drug's bioavailability (via changes in solubility and lipophilicity) to improving binding efficacy and metabolic stability, the effects of which are all well recognised. As a result, medicinal chemists have become particularly adept at optimising the physicodynamics and pharmacokinetics of a drug through the selective introduction of one or more fluorine atoms. A brief discussion of some of fluorine's properties can aid in understanding how fluorinated compounds have managed to gain such a comparatively large share of the pharmaceutical market.

1.2.1   The properties of fluorine

Perhaps one of the most valuable characteristics of fluorine—and the one that makes it such a successful replacement for oxygen or hydrogen—is its relative size. With a van der Waals radius of 1.47 Å,[14] fluorine has historically been considered isosteric with hydrogen (van der Waals radius = 1.2 Å), although this misconception has generally been overcome in recent decades as the field of fluorine chemistry has continued to grow. In actual fact, the size of fluorine more closely resembles that of oxygen (van der Waals radius = 1.52 Å), although fluorine

is still considered an invaluable monovalent replacement for hydrogen when seeking to alter the properties of a compound. While the replacement of an individual hydrogen or oxygen atom has very little steric impact, fluoroalkyl groups are much more sterically demanding; the trifluoromethyl group is almost twice the size of a methyl group, laying somewhere in size between the isopropyl[15] and isobutyl[16] groups. The introduction of such fluoroalkyl groups can be used to bring about conformational changes in the molecule, which is particularly useful when seeking to improve ligand-receptor interactions in the active site of a protein. The effect tends to be most notable for aromatic compounds, with fluoroalkyl substituents commonly adopting out of plane conformations in contrast to the corresponding alkyl analogues, which are usually planar.[17]

Often the enhanced binding affinity that results from a change in the preferred molecular conformation is not due solely to steric factors, but also to electronic effects. Upon replacement of the poorly electronegative hydrogen with a highly electronegative fluorine atom, a number of alternative bonding interactions become possible. The most contentious is the hydrogen bond, with debate continuing to surround the existence of such bonds. However, there are now many reports in the literature providing evidence in support of both inter- and intramolecular hydrogen bonding to the C-F moiety.[18-21] The reason fluorine can participate in hydrogen bonding is the electron density surrounding the atom, although as the C–F bond is relatively non-polarisable, it can act as a weak acceptor only. In fact, a Csp^3–F$\cdots$H–O bond (10 kJ/mol) is only half the strength of a Csp^3–O$\cdots$H–O bond (21 kJ/mol), while Csp^2–F$\cdots$H–O (6 kJ/mol) is even weaker.[22] In addition to hydrogen bonding, fluorine is also capable of electrostatic interactions, particularly within the active site of enzymes. These are frequently revealed by X-ray crystal structures of cocrystallised enzyme-ligand complexes, with dipolar C–F$\cdots$H–C$_\alpha$ and C–F$\cdots$C=O interactions between the substrate and amino acid residues amongst the most common.[23-25] These interactions provide extra stability, and often contribute to an increase in potency as a result of enhanced binding affinities. The interactions described here are particularly relevant to fluorinated β-amino acids as they may be partly responsible for enhanced binding within the active site of an enzyme.[8]

The pK_a of a compound can influence many pharmacokinetic parameters, especially solubility and lipophilicity, as well as binding affinities. Given that fluorine is the most electronegative element, fluorination of a compound can have a profound impact on the acidity and basicity of neighbouring functional groups—amines tend to be much less basic, while acids increase in strength.[23] Often the difference in pK_a between the fluorinated and non-fluorinated compound can be several orders of magnitude, with the effects additive i.e., each additional fluorine atom lowers the pK_a by a similar amount. The exact extent to which the pK_a is affected has been found to depend largely on the geometry of the conjugate carbanion and site of fluorination.[24] Surprisingly, however, fluorine substitution does not always acidify adjacent protons to the extent that other halogens do—while fluorine is always inductively electron withdrawing, its lone-pair electrons contribute by resonance. As such, α-fluorination is less acidifying of an α-proton than other α-halogenations because of a combination of inductive and resonance effects.[24]

One of the most important properties of a drug relates to its lipophilicity—orally administered drugs must be lipophilic enough to be passively transported through the lipid bi-layer of cells, however too high a lipophilicity and the drug suffers from poor solubility and thus incomplete absorption. The addition of a fluorine atom or fluoroalkyl group to a phenyl ring always increases lipophilicity, as does fluorination adjacent to a π-system or heteroatom-containing functional group.[23] Conversely, the strong dipoles in C–F and C–CF$_3$ bonds of alkanes serve to decrease lipophilicity, particularly in the case of terminal mono-, di- or trifluorination of an alkane.[24] One exception to the general increase in lipophilicity observed upon fluorination of π-systems is fluorinated α-carbonyl compounds, such as those prepared during this work, whose relative lipophilicities depend on the solvent used to measure them. This makes it quite difficult to predict the effect fluorination has on the absorption of these types of compounds in biological systems.

Finally, the strength of the C–F bond is well known, and the replacement of a labile hydrogen atom with fluorine can inhibit a specific metabolic pathway (as F$^+$ is not readily lost from a molecule).[25] The primary outcome is an increase in the drug's half-life (and hence efficacy), but it can also help to prevent the formation of toxic metabolites that would otherwise render the drug unsuitable for clinical use.

1.2.2   Methods of fluorine introduction

As the popularity of organofluorine chemistry grew, so too did the demand for reagents that could be used for the effective introduction of fluorine. The significant safety hazards posed by the early fluorinating reagents, especially elemental fluorine itself, necessitated the development of safer, easier to handle reagents; by their very nature, these milder reagents were also more regioselective. Aside from the hazards associated with these reagents (especially on a large-scale), selectivity is perhaps the greatest issue—the extreme oxidising potential of fluorine and, by association, many other fluorinating reagents, means that careful optimisation of a procedure is required to prevent complete destruction of the starting material. This is particularly true of elemental fluorine, which is still used in industry today— typically as a 10% mixture in N_2, which makes it easier to handle. However, with the advent of more stable reagents specialised equipment is no longer necessary and the synthesis of organofluorine compounds is now commonplace.

The method of fluorination employed in any given synthesis depends on a range of factors including the type of material to be fluorinated and its availability, as well as cost, the synthetic step at which fluorine is to be introduced and safety hazards. It thus follows that there is a marked difference between the techniques used in industrial and lab-scale fluorinations; the strategy employed by industry is typically dictated by cost and often involves either halogen exchange with HF or KF or direct fluorination with F_2, whereas research laboratories are much more likely to make use of safer but more expensive reagents. For the purposes of large-scale industrial synthesis, the use of commercially available fluorinated building blocks, which either act as a scaffold or are introduced later as part of a convergent synthesis is common. This is particularly true of pharmaceutical products, which often involve laborious multi-step syntheses and expensive starting materials, and helps to avoid the unpredictability of fluorination when using the harsh reagents commonly employed during large-scale operations.

Newly developed reagents and techniques mean that asymmetric fluorination is also now possible, generally via one of four methods: the first involves the more traditional approach towards diastereoselective synthesis—the use of a natural product derived from the chiral pool. This stereocontrolling group is a core

component of the compound and comprises part of the final structure. A common alternative is to use a chiral auxiliary (typically also natural product based), which is introduced solely for its stereodirecting properties and then removed upon completion of the reaction. Amongst the most well known and versatile auxiliaries are Evans' oxazolidinones, which have already proven effective in directing the fluorination of enolates in the asymmetric synthesis of α-carbonyl compounds.[26,27,9,28] The remaining two methods are less traditional in nature, and reflect the recent developments in fluorine chemistry, as well as organic chemistry in general. The first involves the use of chiral stoichiometric reagents, which direct the face of fluorination themselves, while the newest—and hence least well developed—makes use of chiral catalysts as co-reagents to non-chiral fluorinating agents. The latter is particularly useful when the enantioselective introduction of fluorine is desired, and generally uses either transition metal complexes or organocatalysts. Such has been the interest in developing these asymmetric reactions that several extensive reviews on the subject have appeared over recent years.[29-31]

Fluorination can be accomplished via nucleophilic, electrophilic, radical or electrochemical means, although the former two comprise most of the standard methods in use. Nucleophilic fluorinating reagents include HF, Olah's reagent (70% HF/pyridine),[32,33] KF, tetrabutylammonium fluoride (TBAF)[34] and diethylamino-sulfur trifluoride (DAST),[35] plus the newer, less hazardous reagents such as those shown in *Figure 9*.[36-40] While these are all important in their own right, the following section will focus exclusively on methods of electrophilic fluorination—the technique employed during the work presented in this thesis.

| **17** | **18** | **19** | **20** | **21** |
| DAST | Deoxofluor | Yarovenko reagent | Ishikawa reagent | DFI |
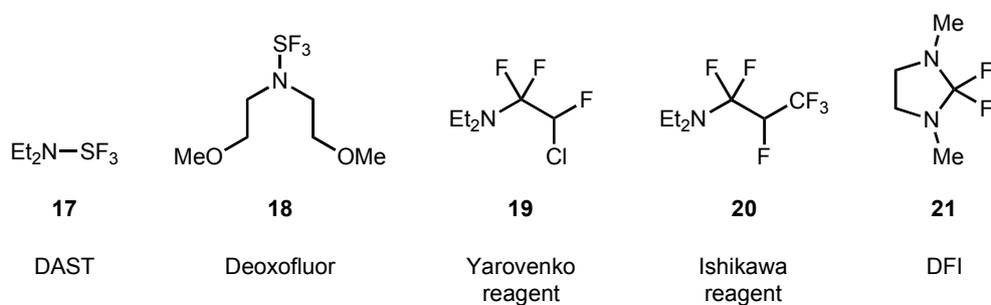
Figure 9 The nucleophilic fluorinating reagent DAST and a series of more recently developed alternative reagents.

1.2.2.1 Electrophilic fluorinating reagents

Aside from fluoroaromatics, one of the most important classes of organofluorine substrates is the α-fluorocarbonyls. As substitution adjacent to a carbonyl group often involves the formation of a metal enolate that is then quenched by an electrophile, reagents that effectively transfer "F$^+$", not only to enolates but to any carbanion, are hugely popular. Of course, it must be borne in mind that there is no actual evidence supporting the existence of the F$^+$ cation in solution—to date it has only been observed spectroscopically in the gas phase.[41] As α-fluorocarbonyl compounds are the focus of the work presented in this thesis, a discussion of the types of reagents used to prepare such compounds is necessary.

The simplest source of electrophilic fluorine is F$_2$, a highly toxic and corrosive gas that reacts explosively with trace amounts of organic compounds. Its extreme reactivity rules it out for the selective fluorination of highly functionalised compounds, while handling difficulties associated with the gas also reduce its utility in industrial fluorinations. Another of the earliest reagents was the more selective XeF$_2$, a stable and easy to handle solid capable of many transformations including the fluorination of aromatic rings and alkenes, and the fluorodecarboxylation of carboxylic acids.[42,43] Perchloryl fluoride (FClO$_3$) and hypofluorites (e.g., CH$_3$COOF and CF$_3$COOF) tended to be used in early industrial processes for the preparation of fluorinated pharmaceuticals, however their toxicity and explosive properties make them largely unsuitable for large-scale syntheses.

The emergence of ternary N–F and quaternary [N–F]$^+$ reagents in the late 1980s revolutionised electrophilic fluorination, with the reagents' high stability facilitating their commercialisation, which in turn led to a surge of interest in the development of new reactions. These compounds feature fluorine bound to an electronegative nitrogen atom, with further activation often provided by the attachment of strongly electron withdrawing groups to nitrogen, e.g., sulfonyl. Most of these N–F reagents are prepared by fluorination of the corresponding N–H compound with elemental fluorine, but their commercial availability means fluorine gas no longer has to be handled in research laboratories.

Umemoto is considered one of the pioneers of the field through his introduction of a series of *N*-fluoropyridinium salts (*Figure 10*).[44] It was found that the fluorinating power can be tuned by altering the ring substituents and thus the electron density surrounding the nitrogen atom; electron withdrawing substituents increase the fluorination power, while bulky, electron donating alkyl groups reduce it.[45-47] The same substituent effects can generally be observed throughout the series of N–F reagents, and contribute to the chemoselectivity of a reagent.
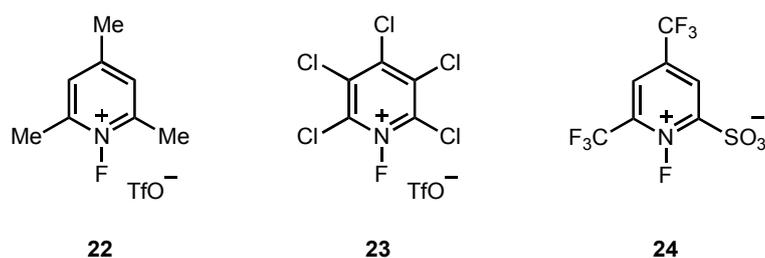
Figure 10 Second generation substituted *N*-fluoropyridinium salts developed by Umemoto.

Despite their versatility, the *N*-fluoropyridinium family of electrophilic fluorinating reagents never reached the popularity of another [N–F]+ salt, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor® or F-TEDA-BF$_4$, **25**), most likely due to Selectfluor's greater reactivity (it was found to react up to 100 times faster than the *N*-fluoropyridinium BF$_4$ salt[48]). First reported in 1992,[49] the reagent is now prepared in multi-ton quantities annually.[50] Substitution of the *N*-chloromethyl group is used to modulate the fluorinating power of the reagent by altering the electron density—the highly electron withdrawing 2,2,2-trifluoroethyl derivative even fluorinates benzene.[51] One such commercially available derivative is Accufluor (**26**, *Figure 11*), in which the *N*-chloromethyl group is substituted by a hydroxy group. Over the last two decades Selectfluor has become one of the best general-purpose, selective and stable electrophilic fluorinating reagents available to chemists.[52-54]
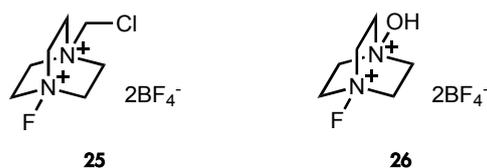
Figure 11 The electrophilic fluorinating reagents
Selectfluor (*left*) and Accufluor (*right*).

One disadvantage to the use of Selectfluor is its limited solubility in most organic solvents—being a dication the reagent is only soluble in acetonitrile, water and nitromethane, and only moderately soluble in DMF. This can pose a problem when seeking to fluorinate carbanions that are more effectively prepared in ethereal solvents such as THF (e.g., metal enolates). Another family of electrophilic fluorinating reagents—those that are sulfonyl-based—provide a good alternative in such cases. Although they are generally weaker F⁺ donors than the quaternary salts as a result of their uncharged N–F moiety, these reagents are soluble in most organic solvents. DesMarteau *et al.* were amongst the first to describe the preparation and use of such compounds, publishing the synthesis of a series of cyclic and acyclic *N*-fluoro-*N*-perfluoroalkylsulfonimides in 1987.[55] These workers recognised that the electron withdrawing sulfonyl groups significantly reduced the electron density surrounding the nitrogen atom and also provided excellent stabilisation of the anion formed post-fluorination. Both of these properties combined to give highly powerful electrophilic fluorinating reagents—the triflimide $(CF_3SO_2)_2NF$ is even more powerful than most [N–F]⁺ reagents, however it is not available commercially.

Shortly thereafter two aryl bis-sulfonyl N–F reagents—Differding's *N*-fluorobenzenesulfonimide (NFSI, **27**)[56] and Davis' *N*-fluoro-*o*-benzene-disulfonimide (NFOBS, **28**)[57,58]—were reported, with the former now equally as popular as Selectfluor due to its versatility. Both compounds are easily prepared, stable and display comparable reactivity, although NFOBS is yet to be commercialised, making NFSI the reagent of choice. In addition to its intermediate reactivity (it lies between the *N*-fluoro-perfluoroalkylsulfonimides and the *N*-fluoro-*N*-alkylsulfonimides), NFSI is favoured because of its one-step preparation

and its solubility in most organic solvents, with the latter making it the preferred reagent for the work presented in this thesis. The higher solubility results from the presence of a second phenyl ring, although the lower solubility of NFOBS in organic solvents can prove advantageous—the sulfonimide by-product is water-soluble, allowing aqueous extraction and thus aiding product purification.[59]

27 **28**
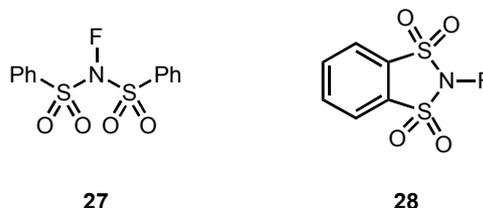
Figure 12 N-Fluorobenzenesulfonimide (*left*)
and *N*-fluoro-*o*-benzene-disulfonimide (*right*).

Each of the previous electrophilic fluorinated reagents are achiral, relying on the stereodirecting properties of the substrate to achieve stereoselective fluorination. Alternatively, enantioselective fluorinating reagents such as the *N*-fluorosultams, which are based on either camphor (**29a, 29b**)[60,61] or saccharin (**30**)[62,63] structures can be used. When acting as the sole source of chirality they succeeded in fluorinating metal enolates with up to 70% ee. The saccharin-based substrates were later modified to include bulkier alkyl groups, such as the cyclohexane ring of **31**, which saw an increase in both yield (up to 79%) and ee (up to 88%).[64,65] However, like the earliest sulfonamide N–F reagents these compounds feature only one electron withdrawing sulfonyl group, making the fluorination of relatively unreactive substrates somewhat difficult.

29a R^1 = Me, R^2 = H **30** **31**
29b R^1 = H, R^2 = Cl
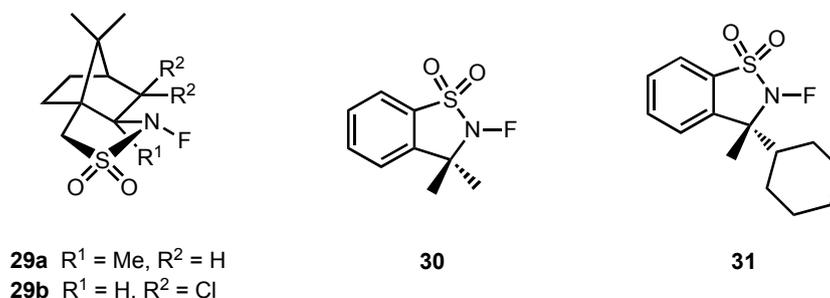
Figure 13 Early enantioselective electrophilic fluorinating reagents.

Despite some of the promising results obtained with these enantioselective N–F reagents, they generally require multi-step syntheses using either molecular fluorine or perchloryl fluoride, while their scope is also somewhat limited. The relatively recent introduction of a practical new class of reagents based on the naturally occurring cinchona alkaloids has encouraged renewed interest in reagent-controlled asymmetric fluorinations. Reported simultaneously yet independently by the groups of Cahard[66] and Shibata,[67] they are [N–F]+ reagents and thus generally more powerful than their predecessors. This enables the fluorination of less reactive substrates like silyl enol ethers, as well as metal enolates and β-ketoesters,[68,29] with high yields and ee's generally obtained for most substrates. Furthermore, as the cinchona alkaloids exist as two pairs of readily available diastereomers (cinchonine/cinchonidine and quinidine/quinine), the stereochemistry of the product can be determined by choosing the appropriate alkaloid isomer. As with any reagent there are some limitations, however: like Selectfluor, these [N–F]+ salts are limited to use in either acetonitrile or acetone, and low temperatures are generally required to achieve a high degree of stereoselectivity.

1.3   α-Fluoro and α,α-difluoro-β-amino acids

As with most other classes of organic compounds, fluorine has found its way into the field of amino acid chemistry, which is unsurprising given the element's practical uses in medicinal chemistry. Fluorinated α-amino acids have long been used in biomedical applications and are particularly valuable as intermediates in the synthesis of larger bioactives.[69,70] However, despite the fact that α,α-difluoro-β3-amino acids appeared in the literature as early as 1987 in relation to peptidic enzyme inhibitors,[71] fluorinated β-amino acids have generally received little attention. This is attributable not only to the fact that the therapeutic potential of β-amino acids themselves has only been realised relatively recently, but also to a general lack of effective synthetic methods for their preparation. This comparatively undeveloped field is dominated by the work of Seebach, whose group has studied the conformational effects that a central α-fluoro or α,α-difluoro-β-amino acid residue has on β-peptides.[72,73] Further studies on such fluorinated β-peptides found that these particular residues were resistant to hydrolysis by a

number of proteolytic enzymes.[74] This is despite the presence of an electron-withdrawing moiety activating the adjacent carbonyl.

Fluorinated β-amino acids have also been incorporated into naturally occurring pharmacophores. The substitution of a hydroxyl group by the difluoromethylene moiety has enabled the structure activity relationship of the taxol side chain to be probed,[75] while 3,3-difluoroazetidin-2-ones (β-lactams) have demonstrated biological activity[76,71] and can act as versatile scaffolds and synthetic intermediates.[77-79] As fluorine is essentially a foreign element in natural systems, the use of [^{18}F]-radiolabelled markers, which possess a relatively long half-life, are valuable tools in nuclear medicine,[80] as is the ^{19}F NMR study of protein structure and enzyme-drug interactions.[10,81] It is anticipated that interest in the field would be enhanced and significant developments made should fluorinated derivatives of both natural and unnatural β-amino acids be more easily accessible. Advancement of this underdeveloped field provided the impetus for the work presented in this thesis.

1.3.1 β-amino acids in medicinal chemistry

The importance and therapeutic potential of fluorinated β-amino acids cannot be established without a discussion of β-amino acids themselves, and their role in medicinal chemistry. While α-amino acids are considered nature's building blocks, comprising the peptides and proteins that make up all forms of life, very few naturally occurring β-amino acids exist. A small number of peptides featuring a β-amino acid residue have been isolated from marine organisms and prokaryotes,[82] while non-peptidic natural products like the potent anti-tumor agent paclitaxel (Taxol, **32**) and the aminopeptidase inhibitor bestatin (**33**) contain an α-hydroxy-β-amino acid fragment. Cyclic derivatives of β-amino acids—β-lactams—are produced by many mould species. Their 4-membered amide ring forms the core structure of the β-lactam antibiotics, and are usually fused to a saturated or unsaturated ring. This family of broad spectrum antibiotics, which includes the penicillins, cephalosporins and monobactams (*Figure 14*), inhibit bacterial cell-wall synthesis in Gram positive, and to a lesser extent Gram negative bacteria. Despite their relative scarcity, β-amino acids possess many fascinating properties, and thanks to intensive research efforts over the past two decades their therapeutic potential is now well recognised. This biochemical interest has been paralleled by

an explosion in the number of techniques developed to synthesise these unnatural β-amino acids, and is a testament to their great promise.
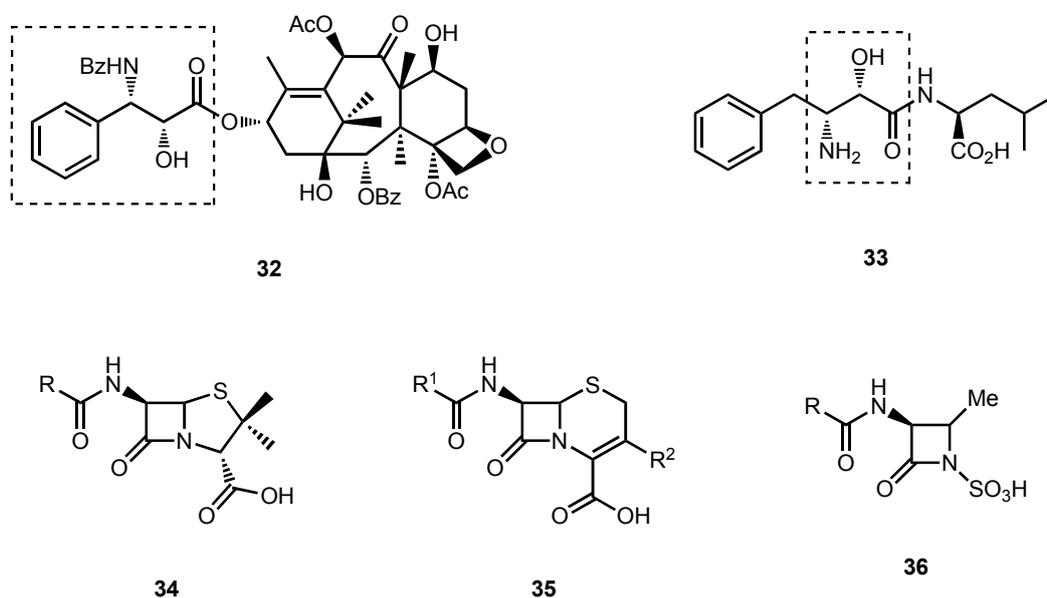
Figure 14 The natural products taxol (**32**) and bestatin (**33**), and the penicillin (**34**), cephalosporin (**35**) and monobactam (**36**) families of β-lactam antibiotics.

β-Amino acids are essentially homologated α-amino acids—they possess an additional methylene unit adjacent to the carbonyl group, which effectively shifts the amino moiety from the α-carbon to the β-carbon (*Figure 15*). With substitution possible at both the α- and β-carbons, plus the associated four-fold increase in the number of available isomers, β-amino acids are much more structurally diverse than α-amino acids. This backbone extension confers an extra degree of conformational freedom on the molecule, with the precise conformation adopted by a β-amino acid dependent upon the number and type of substituents it bears. As a result β-peptides tend to be relatively flexible, as compared to natural α-peptides, whose structures are more rigid. Despite their increased flexibility, β-peptides are generally conformationally stable, and the ability of β-peptides to adopt stable helical structures is well-known.[83] Even the incorporation of a single β-amino acid residue in an α-peptide can influence the overall shape of the peptide by inducing additional turns or folding. This allows the peptide to adopt unique secondary structures, and can be manipulated to improve its binding properties.[84] In a similar

manner, the inclusion of cyclic β-amino acids can be used to restrict the conformation of a peptide, improving its bioactivity.[85]
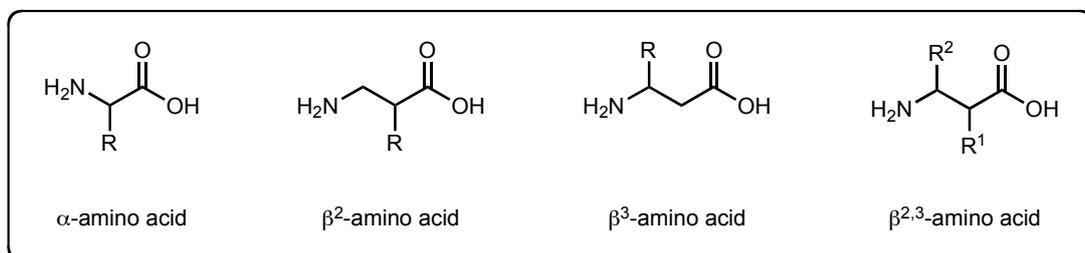
Figure 15 A comparison of α-amino and β-amino acid structures, with the latter classified according to the nomenclature introduced by Seebach.

Unlike their α-amino counterparts, β-amino acids are highly resistant to attack by protease enzymes. In fact, β-peptides can survive incubation with various proteases for days, whereas α-peptides are typically degraded within minutes.[86,87] However rather than inactivating enzymes, their inherent stability is thought to be due to the inability of the enzymes to recognise these unnatural substrates. If necessary, this can be overcome through the use of β-amino acid-containing α-peptides, which retain their affinity for the target enzymes. Thus the replacement of an α-amino acid with the analogous β-amino acid can stabilise the scissile bond whilst retaining the overall biological activity and physicochemical properties of the original peptide.[74,88,89] One explanation for this phenomenon is that the addition of a single carbon atom into the backbone of the peptide displaces the scissile bond from the active site, significantly interfering with its ability to interact with the active site residues.[90] An example of this features the short peptide sequence **37** (*Figure 16*), a potent inhibitor of endopeptidase EP24.15.[91] Unfortunately this substrate is completely degraded by a related enzyme, so Steer *et al.* sought to modify the peptide in such a way that it retained its inhibitory properties while surviving proteolysis by the other enzyme.[92,93] By sequentially replacing each of the α-amino acid residues around the scissile bond with a β²- or β³-amino acid (see *Figure 15* for an explanation of this nomenclature[94]), analogues that still possessed inhibitory activity (albeit slightly reduced) but were totally resistant to the degradation were successfully identified.
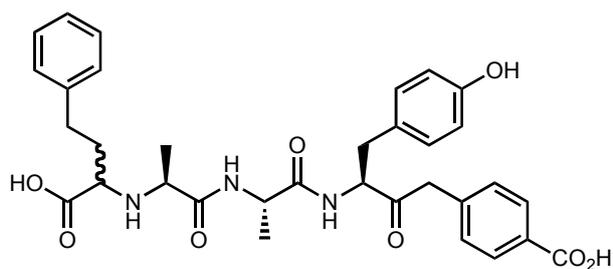
37

Figure 16 The structure of endopeptidase EP24.15 inhibitor cpp-Ala-Ala-Tyr-pAb.

Over the past decade β-amino acids and β-peptides have increasingly been used in a broad range of pharmacological roles. These applications usually take advantage of either the β-amino acid's ability to induce conformational changes or their proteolytic stability, and the number of peptidomimetics based on the β-amino acid structure will only increase as more knowledge about these compounds becomes available. Their role in protease inhibition has been well studied, however, and α-hydroxy-β-amino acids (isoserines)—one of the most important classes of β-amino acid—are commonly used as transition state inhibitors due to their ability to mimic tetrahedral intermediates.[82] They have also been used to stabilise the scissile bond of peptides that suffer from undesirable proteolysis, leading to the development of selective enzyme inhibitors.[95] Inhibition via the covalent phosphorylation of the active site serine residue has been accomplished using phosphoramidate-substituted β-amino acids, which are much more effective than those containing α-amino acids.[96] However the potential shown by β-amino acids extends far beyond the inhibition of protease enzymes. Paclitaxel and its analogue docetaxel, two of the most effective drugs for the treatment of ovarian and breast cancer, each bear an isoserine side chain, with the precise structure of this side chain critical to the compound's bioactivity. Modification of the amino and β^3-substituents in this side chain has resulted in an array of second generation taxoids that show more potent activity against a range of cancer cell lines, even those displaying multi-drug resistance.[97]

Potent agonists and antagonists of various receptors have been identified,[90] with peptides in which the scissile bonds are stabilised through the substitution of β-amino acids proving successful antagonists.[98] Small peptides stabilised in this manner[99] and those which mimic the natural substrates[100] have found use as platelet aggregation inhibitors by acting as selective antagonists.

Gellman, a pioneering figure in the study of β-amino acids, has demonstrated that a β-peptide comprised of cyclic β-amino acids forms a helix that possesses significant antimicrobial activity against a range of microorganisms.[101] While its activity was comparable to natural antibiotics, its haemolytic activity (the destruction of mammalian red blood cells) was substantially lower. At a time when bacterial resistance to current antibiotics is increasing, Gellman's work provides an alternate class of compounds that may prove extremely useful in the future.

1.3.1.1   Current methods for the synthesis of α,α-difluoro-β³-amino acids

The preparation of fluorinated β-amino acids has traditionally involved one of two methods: direct fluorination of the precursor amino acid or through a building block approach, which usually involves a convergent synthesis using fluorinated components. The latter, which often involves the selective formation of a C–C bond instead of a C–F bond, is more common as the stereoselective generation of fluorinated quaternary carbons is quite difficult (and hence rare). However, these reactions can suffer from low diastereoselectivity as a result of ineffective stereocontrol. Methods for the preparation of β-fluoroalkyl-β³-amino compounds—substituted or unsubstituted at the α-position—are now relatively well established,[102] however β-amino acids featuring α-fluoro substituents have received considerably less attention. Of these, most of the focus has been on α,α-difluoro-β³-amino compounds due to their ease of synthesis relative to α-fluoro-β³-amino compounds, which feature an additional stereogenic centre.

The standard preparation of α,α-difluoro derivatives perfectly exemplifies the building block approach as the CF_2 synthon can be conveniently introduced via the cheap and readily available ethyl bromodifluoroacetate. This is generally performed under Reformatsky-type conditions in which an imine is treated with an organozinc species formed from the haloacetate. The structure of the imine is a

major determinant of reaction success, and a number of variations on imine structure have been reported. These range from simple achiral *N*-aryl imines[103] to oxazolidinones, which act as imine equivalents (page 128).[104] Even iminium salts prepared using Katritzky's benzotriazole methodology have been shown to act as stable imine precursors that undergo Reformatsky reaction, although a lack of effective stereocontrol is an issue.[103,105]

A significant advance was made with the *p*-toluenesulfinimines of Soloshonok *et al.* (*Scheme 1*)[106,107] and the *tert*-butylsulfinimines of Staas *et al.* (*Scheme 2*),[108] which were reported virtually simultaneously. Unlike many imines they are stable and thus isolable, and the sulfinyl groups are easily hydrolysed under acidic conditions to give the free amine. While they prove to be highly effective for the preparation of β-aryl-substituted derivatives, sulfinimines with *C*-alkyl substituents give much poorer yields and de's. As the Reformatsky reaction comprises a large part of the work conducted for this thesis, a more extensive discussion of its application to the synthesis of α,α-difluoro-β-amino acids is presented in Chapter 4.

R = Aryl: 77-85% yield; 80-98% de
R = Alkyl: 59-65% yield; 72-76% de
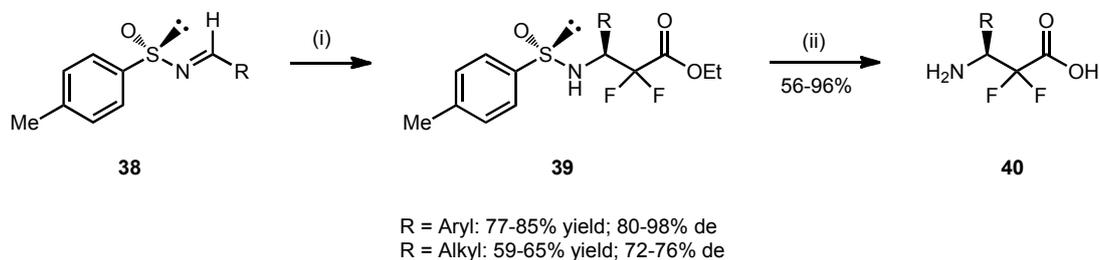
Scheme 1 Reagents and conditions: (i) Zn, BrCF$_2$CO$_2$Et, THF, RT, 18 hr; (ii) 4M HCl.

R = Aryl: 58-82% yield; 80-90% de
R = Alkyl: 51-81% yield; 60-74% de
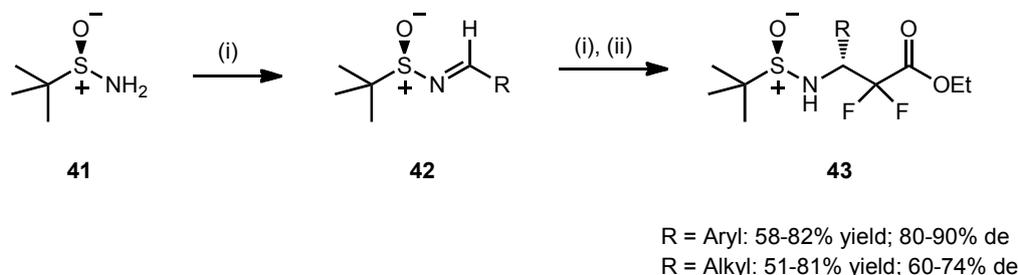
Scheme 2 Reagents and conditions: (i) RCHO, CuSO$_4$, DCM; (ii) Zn, BrCF$_2$CO$_2$Et, THF, reflux.

Alternatively, α,α-difluoro-β³-hydroxy esters—compounds typically prepared via the Reformatsky reaction of aldehydes with ethyl bromodifluoroacetate—can be subjected to Mitsunobu conditions in order to introduce the amino substituent.[75] An example is shown in *Scheme 3*,[109] where β-hydroxy ester **44** is converted to the phthalimide functionalised **45** in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD). The β-amino and carboxyl groups are then deprotected over two steps to give the free β³-amino acid **46**.
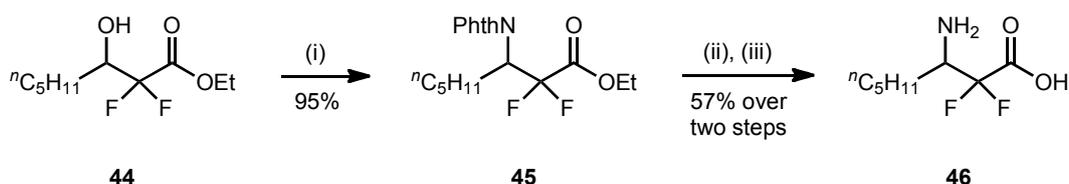
Scheme 3 Reagents and conditions: (i) Ph₃P, DEAD, phthalimide, toluene, 0°C, 1h; (ii) HBr, AcOH; (iii) MeOH, propylene oxide.

A variation of this involves an intramolecular Mitsunobu reaction using amides instead of esters.[71] The β-hydroxy ester is converted to the *p*-methoxyphenyl (PMP) derivative (**47**) before the Mitsunobu reaction, with the activated hydroxyl group this time displaced by the amide nitrogen. This forms a β-lactam (**48**), which is then subjected to ring-opening hydrolysis to give the acyclic PMP-protected β-amino acid (*Scheme 4*).[110] However, in this case a lack of stereocontrol led to an enantiomeric mixture of products.
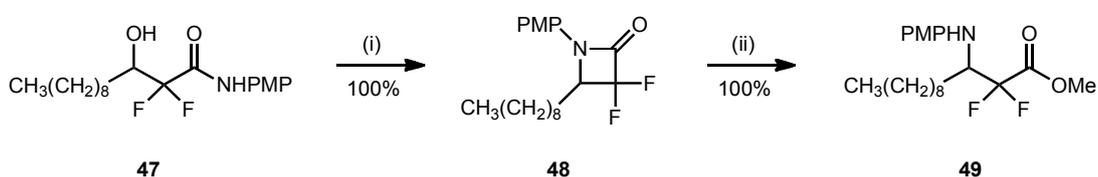
Scheme 4 Reagents and conditions: (i) Ph₃P, DEAD, THF; (ii) MeONa, MeOH.

An alternative to the Reformatsky reaction is the Mannich reaction, which also employs an enolisable carbonyl compound. However, an iminium ion formed *in situ* from the requisite amine and aldehyde components is the electrophilic species. Welch and co-workers[111] developed a non-stereoselective Mannich reaction between sulfonylimines and a difluorinated silyl enol ether (**51**), however yields were only moderate (*Scheme 5*).

49-63% when R = alkyl
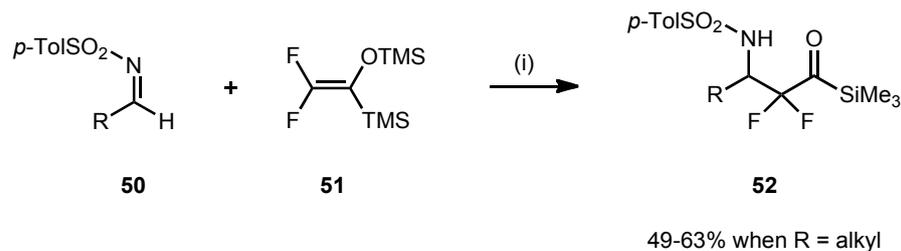
Scheme 5 Reagents and conditions: (i) TMSOTf (10 mol %), DCM, -78°C RT, 24 hr.

While each of the previous examples involve the CF_2-synthon method, direct fluorination provides an alternative route. Typical of this method is the conversion of α-keto compounds to α,α-difluoro substrates using DAST, a technique employed by Ohba *et al.* in the preparation of the α,α-difluoro-β³-amino esters mentioned on page 8 (*Scheme 6*).[8]
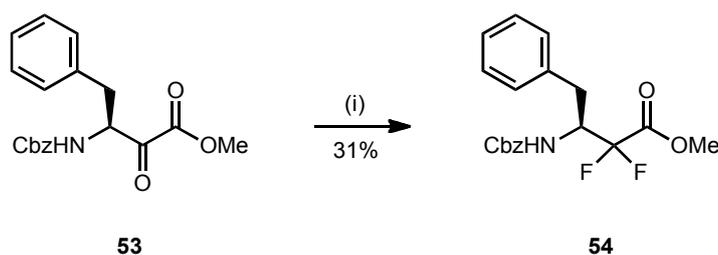
Scheme 6 Reagents and conditions: (i) DAST, DME, RT.

Seebach *et al.* used the same method for the preparation of their fluorinated β-amino esters.[74] As with Ohba's example, the route is based on the use of enantiopure α-amino acids, ruling out the need for asymmetric reactions. The appropriate α-amino acid is reduced to the aldehyde, which is then converted to a cyanohydrin, effectively adding an extra carbon to the chain (*Scheme 7*). Acid hydrolysis in the presence of MeOH gives the β-amino ester **56**. Swern oxidation followed by DAST fluorination of the intermediate α-keto ester produces the α,α-difluoro compound **58**. Acid hydrolysis, removal of the benzyl protecting groups and reprotection as the Boc derivative completes the synthesis. However there are a couple of drawbacks to this method, namely the use of DAST, which is unfavourable because of the hazards associated with its use. Another is the fact that the method generally relies on the use of naturally occurring α-amino acids as starting materials, which can limit the diversity of the β-amino acid products.
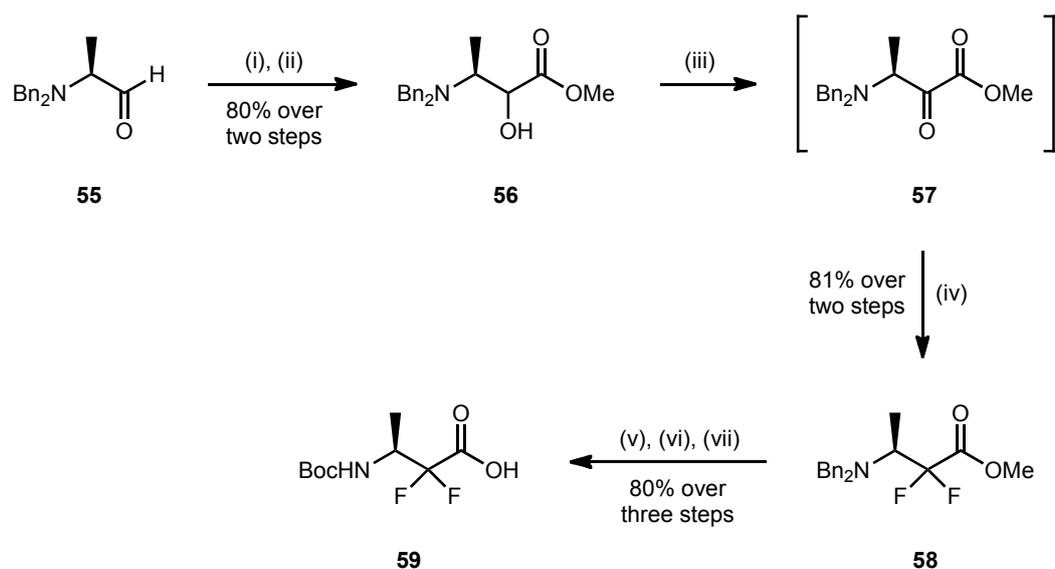
Scheme 7 Reagents and conditions: (i) acetone cyanohydrin, KCN cat., Bu$_4$NI cat., hexane-H$_2$O, RT, 2 hr; (ii) HCl-MeOH, RT, 12 hr; (iii) (COCl)$_2$, DMSO, Et$_3$N, DCM, -78°C; (iv) DAST, DCM, RT, 4 hr; (v) LiOH·H$_2$O, EtOH-H$_2$O, RT, 1 hr; (vi) Pd/C, H$_2$, MeOH, 24 hr; (vii) Boc$_2$O, Et$_3$N, MeOH, RT, 12 hr.

1.3.1.2   *Synthesis of α-fluoro-β-amino acids*

The number of stereoselective syntheses of α-fluoro-β-amino compounds is limited, with the majority of these involving fluorination of hydroxy compounds with DAST. The first example was reported in 1982 by Somekh and Shanzer,[112] who successfully prepared α-fluoro-β-amino ester **63** in 90% yield from *N,N*-dibenzyl-L-serine benzyl ester (**60**) (*Scheme 8*). Upon conducting an analogous experiment with diastereomeric starting materials (not shown), only a single isomer was produced. The stereochemical outcome of this reaction was subsequently rationalised by the formation of an intermediate aziridinium ion (**62**), which is susceptible to regioselective ring-opening by fluoride anion. To confirm this, the experiment was repeated with α-hydroxy ester **61**, with (*R*)-**63** again formed as a single isomer.
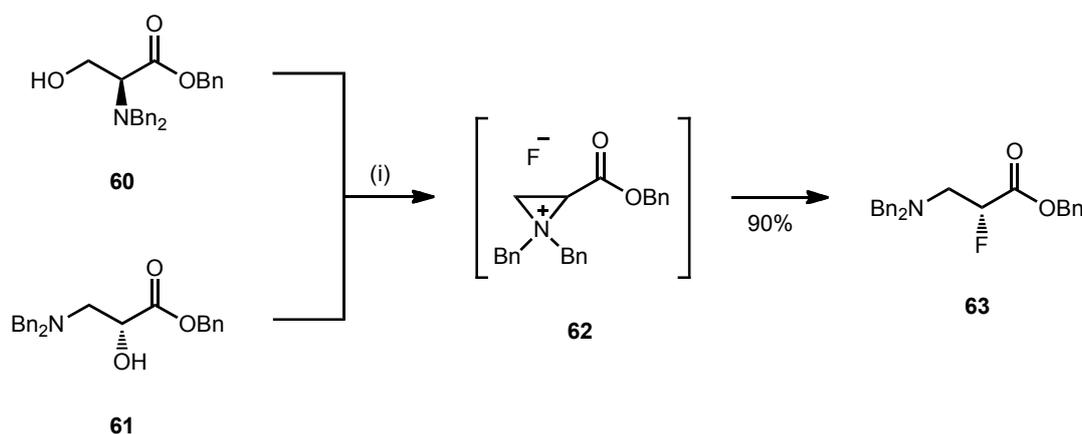
Scheme 8 Reagents and conditions: (i) DAST, THF, RT, 50 min.

This methodology involving the homologation of α-amino acids has been used by a further three research groups[113,84,74] over the years to prepare α-fluoro-β-alanine derivatives, with varying degrees of success. Of those, Seebach's work is the most recently reported (2004), with one example using L-threonine (**64**) as the starting material (*Scheme 9*). Benzyl protection of the amino and carboxylic acid moieties gives **65**, which is then fluorinated with DAST to give the rearranged α-fluoro-β-amino ester **66**. Global debenzylation followed by Boc protection of the amine affords the amino acid **67**. While the fluorination reaction is highly stereoselective, each of the steps occurs in only moderate yield, and it is reasonable to expect that

yields would only decrease in the presence of more complex side chains. Furthermore, the aziridinium ion was found to suffer from non-selective attack in some cases, producing a mixture of regioisomers.
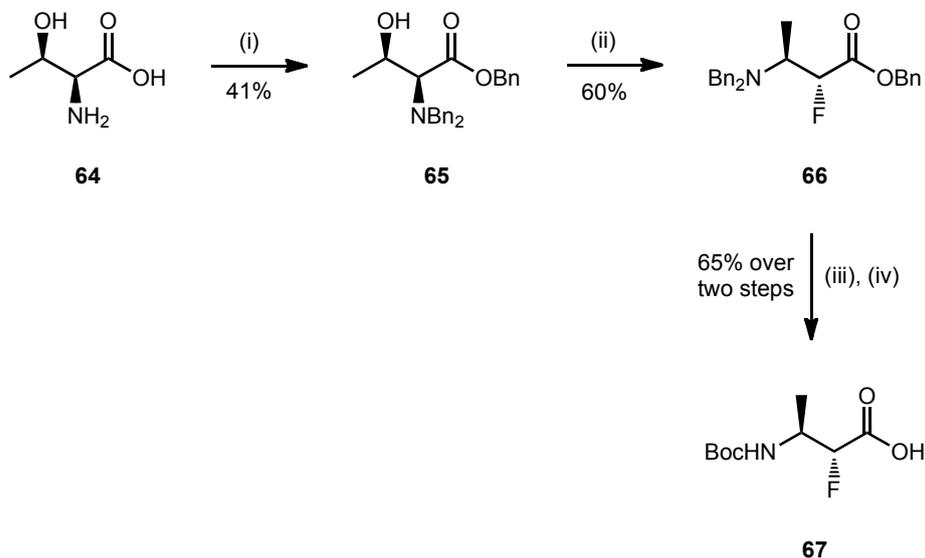
Scheme 9 Reagents and conditions: (i) K_2CO_3, NaOH, BnBr, H_2O, reflux, 1 hr; (ii) DAST, THF, RT, 30 min; (iii) Pd/C, H_2, MeOH, 24 hr; (iv) Boc_2O, Et_3N, MeOH, RT, 12 hr.

Several examples employing electrophilic fluorination have also been reported. The first was published by Davis *et al.*,[114] who sought to devise a method for the preparation of fluorinated analogues of the taxol C-13 side chain, (3*S*)-*N*-benzoyl-3-isoserine. Their method involved deprotonating β-amino ester **68** with LDA then quenching the enolate with an electrophilic fluorinating reagent (*Scheme 10*).

NFOBS: 94%, syn:anti = 44:56
NFSI: 65%, syn:anti = 19:81
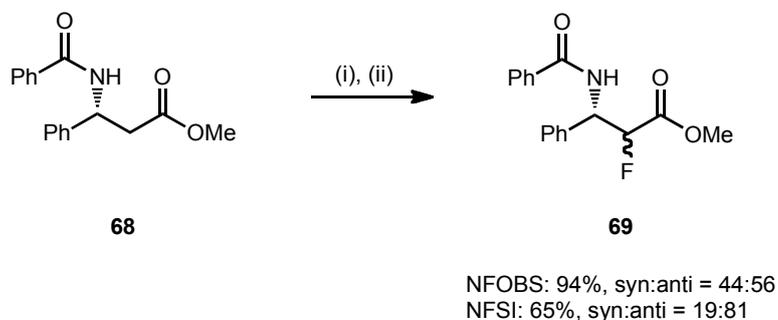
Scheme 10 Reagents and conditions: (i) LDA (2 eq.), LiCl, THF; (ii) NFOBS or NFSI, -78°C, 6 hr.

Fluorination with NFOBS gave the product in excellent yield (94%), however, as the C3 stereocentre was the only source of stereocontrol a 1:1 mixture of isomers resulted. Upon switching to NFSI the yield dropped to 65%, but the selectivity was improved to 19:81 in favour of the *anti* isomer. Abell and co-workers also employed this deprotonation-fluorination strategy using LDA and NFSI for the preparation of a series of *N*-protected α-fluoro-β-alanine esters.[9] Yields ranged from 51-75% with de's ranging from 26-66%. Applying analogous conditions to a β-homoalanine derivative resulted in a yield of 73% and a much higher de of 90%. A similar stepwise protocol involving deprotonation and fluorination of a β^3-amino ester was investigated during the course of this work (Section 2.3.1).

A method developed by Abell *et al.* for the preparation of $\beta^{2,2,3}$-amino acids, in which the α-carbon is both benzylated and fluorinated, demonstrates the use of chiral auxiliaries in controlling the stereofacial addition of fluorine.[28] Their use of an oxazolidinone auxiliary resulted in a high dr of 12:1 in favour of the *anti* product. An extensive series of steps was then required to install the CH_2NH_2 moiety, which proceeded via the azido intermediate **73**. Further synthetic manipulation eventually led to the Fmoc protected β^3-amino acid **74**.
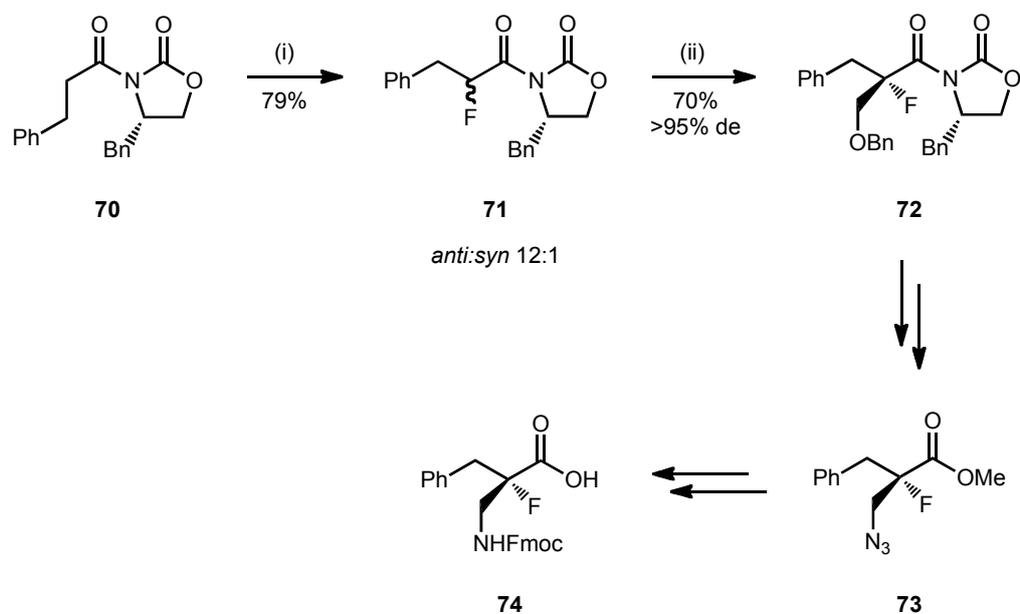
Scheme 11 Reagents and conditions: (i) LDA, then NFSI, THF, -78°C; (ii) TiCl₄, DIEA, BnOCH₂Cl, DCM, 0°C.

Pan *et al.* developed a highly stereoselective Mannich reaction between α-fluoro-β-keto acetyloxazolidinones and aromatic imines that employs a chiral bicyclic guanidine catalyst (**77**). The oxazolidinone group of the α,α-disubstituted products can be cleaved in good yield using K_2CO_3 to give the corresponding esters, while increasing the amount of K_2CO_3 also results in deacylation of the α-carbon (*Scheme 12*). Replacing K_2CO_3 with a biphasic NaOH mixture allows the preparation of the deacylated α-fluoro-$β^3$-amino ketones as a result of decarboxylation.
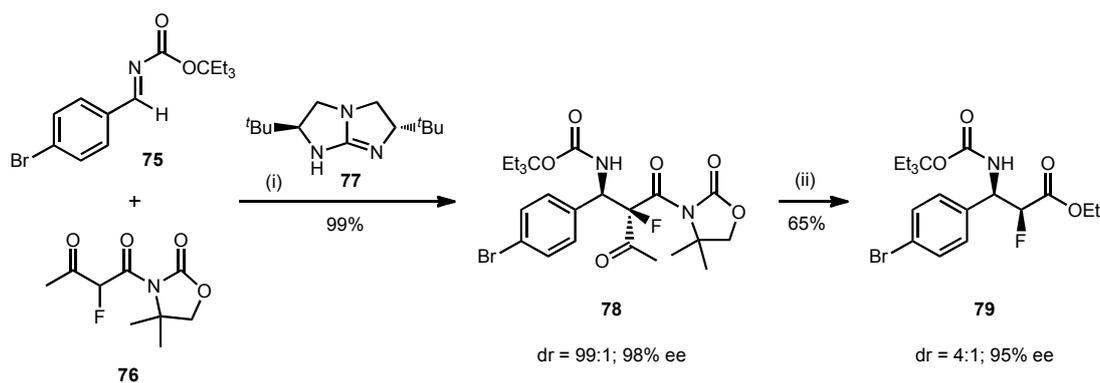
Scheme 12 Reagents and conditions: (i) **77** (10 mol %), DCM, RT, 24hr; (ii) K_2CO_3 (2 eq.), EtOH, -20°C, 24 hr.

A novel multicomponent organocascade reaction reported recently describes the synthesis of α-fluoro-β-amino aldehydes—useful precursors to the corresponding amino acids or esters.[115] This one-pot tandem methodology involves the *in situ* conversion of an α,β-unsaturated aldehyde to an iminium ion using a MacMillan catalyst (**83**) prior to the conjugate addition of an achiral amine (**81**). The intermediate enamine is then fluorinated with NFSI, with the chiral catalyst responsible for the stereoselectivity obtained in both the conjugate addition and fluorination steps. Reduction of the crude aldehyde immediately after work-up provided the more stable α-fluoro-β-amino alcohols, which could be obtained in yields of 24-73%; dr's ranged from between 87:13 to 98:2 in favour of the *syn* isomer while ee's were almost exclusively 99%. *Scheme 13* shows how an α,β-unsaturated aldehyde was converted to the α-fluoro-β-amino methyl ester **82**. Rather than reducing the aldehyde, it is instead oxidised to the carboxylic acid, which is subsequently esterified with TMS diazomethane. Unfortunately the *N*-methoxy amine was the only nucleophile examined—obtaining the unprotected β-amino would necessitate cleavage of the N–O bond, which could be problematic.
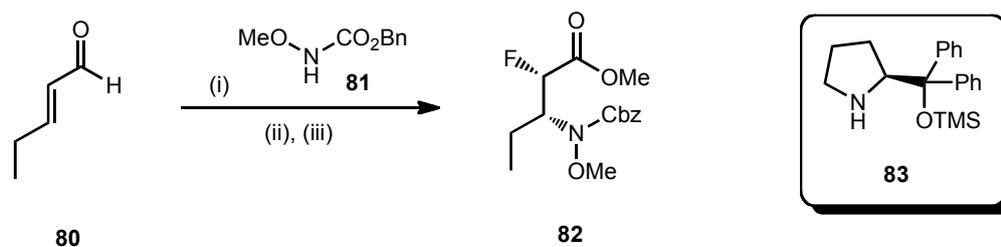
Scheme 13 Reagents and conditions: (i) **83** (20 mol %), **81**, RT, then NFSI, 0°C; (ii) NaClO$_2$, NaH$_2$PO$_4$, cyclohexene, ^{t}BuOH-H$_2$O, RT, 2.5 hr; (iii) TMSCHN$_2$, MeOH, 2 hr.

Another example of the conjugate addition methodology—and one that forms the basis for much of the work in this thesis—is that of Bromfield *et al.* who adapted the tandem conjugate addition-alkylation methodology developed by Davies[116-118] to prepare α-fluoro-β^3-amino esters, including **16** (page 9).[1,119] This is a one-pot procedure in which chiral lithium amide **86** undergoes highly stereoselective 1,4-addition to an α,β-unsaturated ester, with the intermediate enolate quenched with NFSI. The β-amino and α-fluoro moieties are thus installed in one step, with the product featuring an *anti* relationship between the two as a result of the bulky amine substituents directing the fluorination (assuming a chelated cyclic enolate intermediate). Ethyl and methyl esters are susceptible to nucleophilic attack by the amide, but when carried out with *tert*-butyl esters the 1,4-adduct can be obtained in excellent yield (**87**, *Scheme 14*). With both enantiomers of the α-methylbenzylamine precursor to the lithium amide available commercially, both of the *anti* enantiomers can in principle be prepared.

84 R^1 = Ph, R^2 = ^{t}Bu   **86**   **87** 100%, 64% de
85 R^1 = Me, R^2 = Et   **88** 39%, 66% de
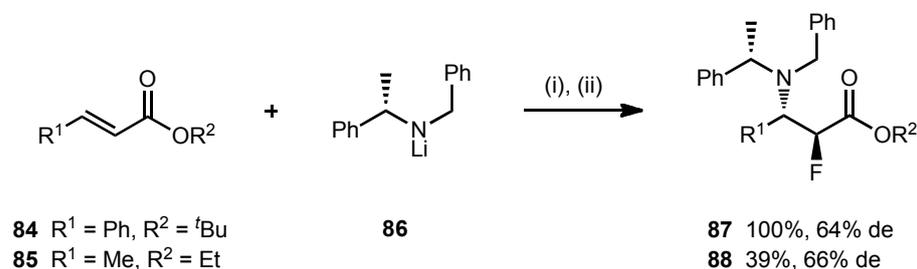
Scheme 14 Reagents and conditions: (i) THF, -78°C, 30 min; (ii) NFSI, -78°C, 5 hr.

With the conjugate adducts in hand, the free β-amino moiety could be obtained upon cleavage of the two benzyl groups under standard hydrogenolysis conditions (*Scheme 15*). After TFA hydrolysis of the *tert*-butyl ester, the amine was protected as the Fmoc derivative to ensure compatibility with peptide synthesis techniques.
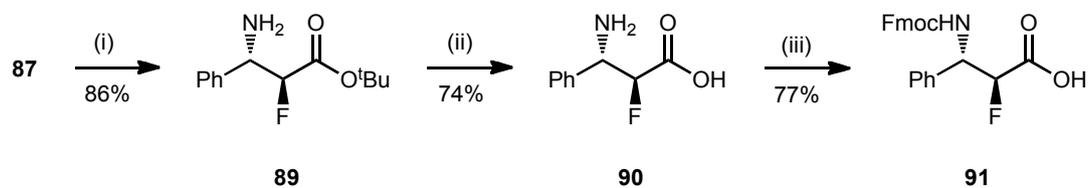
Scheme 15 Reagents and conditions: (i) Pd(OH)$_2$/C, H$_2$, MeOH, 3 hr; (ii) TFA; (iii) Fmoc-OSu, DIEA, MeCN, 0°C to RT, overnight.

1.4   Project aims

Each of the examples covered in the previous section broadly encompass the existing methods for the preparation of α-fluoro and α,α-difluoro-β³-amino acids/esters. It should be noted that in many cases there is great room for improvement with regard to the yields and/or stereoselectivities obtained. Given the efficiency of the tandem conjugate addition-fluorination reaction and the fact that the Fmoc-protected derivatives can be obtained in only a few steps, the work in this project aims to expand the methodology beyond its current scope to include more complex substrates. Specific focus was placed on the preparation of fluorinated β-amino acids featuring basic side chains, such as lysine and arginine (*Figure 17*)—with Bromfield and Ohba demonstrating that α-fluoro-β³-amino esters with hydrophobic side chains showed inhibitory activity against proteases specific for such residues, α-fluoro and α,α-difluoro analogues of β³-lysine and β³-arginine could potentially be used to target proteases that cleave at these positively charged residues, e.g., trypsin, thrombin and factor Xa. The latter two both play important roles in the blood coagulation pathway, and as such their selective inhibition is highly desirable.
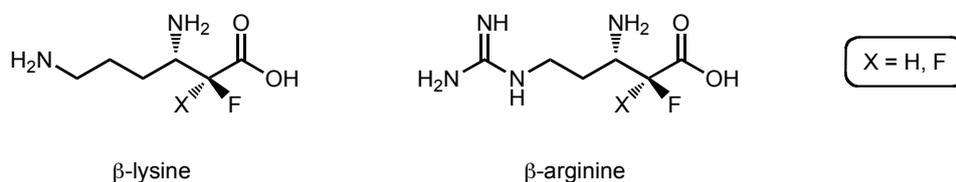
Figure 17 Fluorinated analogues of β-lysine and β-arginine, compounds that potentially possess inhibitory activity against certain serine protease enzymes.

For these fluorinated β³-amino acids to be synthetically useful their highly reactive side chains must be suitably protected to prevent undesirable inter- and intramolecular reactions from occurring. Furthermore, for the amino acid to be incorporated into a peptide, current peptide synthesis techniques require that the β-amino moiety be protected with a group that permits orthogonal cleavage with regard to the side chain protecting group. Thus the challenge is to design an efficient synthetic route to the desired α-fluoro-β³-amino acids that will allow the

installation of the required functionalities and protecting groups in a minimum number of synthetic steps. Using the experimental conditions developed by Bromfield as a basis for the preparation of the α-fluoro-β³-amino framework, the monofluoro β³-lysine and β³-arginine derivatives could be prepared from the appropriately functionalised α,β-unsaturated *tert*-butyl esters (*Figure 18*). Amino-functionalised unsaturated esters would provide the most direct route to the target amino acids, however this is contingent on the protecting groups being resistant to the conditions of the tandem conjugate addition-fluorination reaction. As an alternative, non-amino based substrates capable of surviving these highly basic conditions could be employed initially, with the required functional group interconversions carried out in the latter stages of the synthesis.
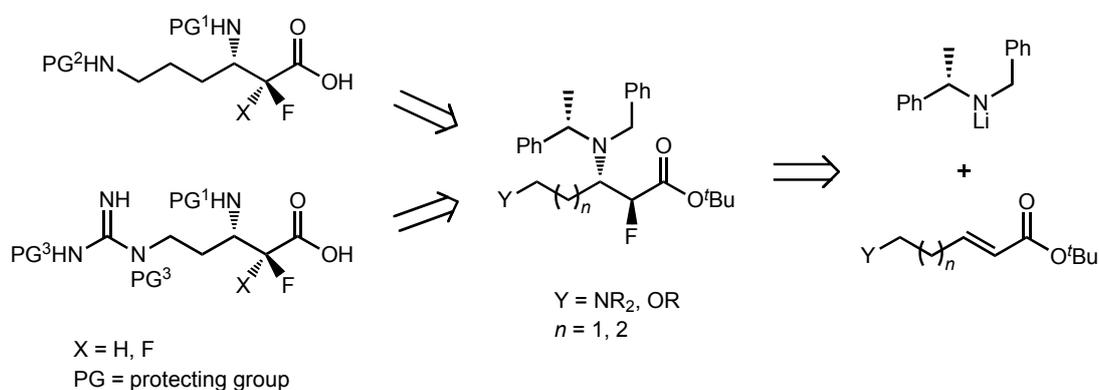
Figure 18 Proposed route to the desired α-fluoro and α,α-difluoro-β³-amino acids via the conjugate addition-fluorination methodology.

Hypothetically the α,α-difluoro derivatives should be available from the α-fluoro conjugate adducts via a subsequent deprotonation-fluorination process. However, should the electronic effects of the α-fluorine atom already present make this reaction difficult to accomplish, an alternative method is necessary. With the success of the CF_2-synthon method already widely demonstrated, the use of Reformatsky-type conditions may represent the best approach. As shown in *Figure 19*, this would involve the condensation of the requisite aldehyde and amine components—the aldehyde bearing the desired side chain functionality and the amine acting as a chiral auxiliary—with an organometallic species generated from ethyl bromodifluoroacetate. The auxiliary would then be cleaved and the side chain

functionality manipulated using similar techniques to those applied in the conjugate addition route to give the requisite side chains for the desired lysine and arginine derivatives.
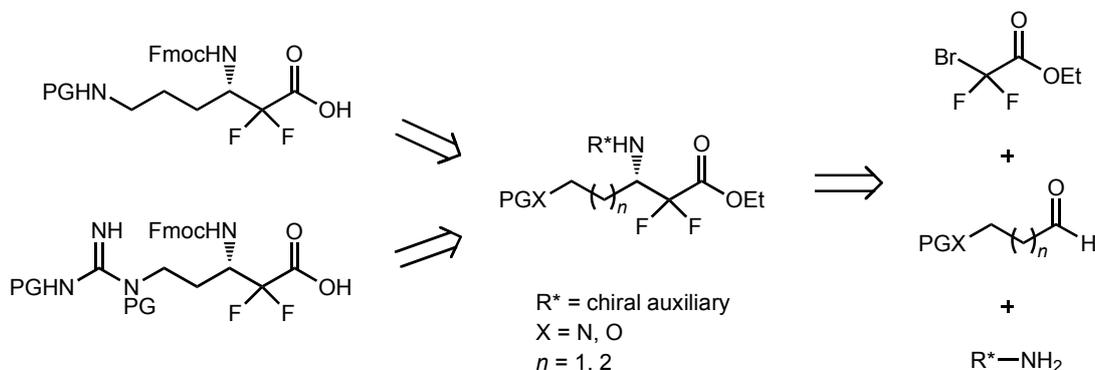
Figure 19 Proposed alternative route to α,α-difluoro-β³-amino acids via Reformatsky reaction, where PG = undefined protecting group.

Both the conjugate addition and Reformatsky methodologies utilise the same achiral aldehyde as a starting material, with this substrate the prime determinant of amino acid identity because it forms the side chain. Thus, the two techniques are complimentary: by taking the same starting material and applying different sets of conditions, either α-fluoro or α,α-difluoro derivatives of the target β³-amino acid could be prepared. The following chapters will discuss the systematic investigation of each of these two methodologies, as well as their application to the synthesis of fluorinated β³-amino acids suitably derivatised for further use.

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CHAPTER 2:

PREPARATION OF α-FLUORO-β³-AMINO ESTERS VIA CONJUGATE ADDITION

2.1   Introduction

The conjugate addition of a nucleophile to an α,β-unsaturated carbonyl compound has historically been a popular method for new carbon-carbon and carbon-nitrogen bond formation, with the first case reported by Komnenos as early as 1883.[1] This popularity stems from the wide variety of both carbon and heteroatom nucleophiles that can be utilised, which includes enolates (Michael reaction[2,3]), enamines (Stork enamine reaction), cyanides (Nagata hydrocyannation[4]) and lithium dialkylcuprates (Gilman reagents).[5] Even simple amines like ammonia are sufficiently nucleophilic to undergo conjugate addition,[6,7] resulting in perhaps the simplest direct synthesis of β-amino type compounds.

For the synthesis of more complex β-amino acids and esters, numerous groups over the years have demonstrated the addition of primary and, to a lesser extent, secondary *N*-alkyl and *N*-arylamines to simple β-alkyl-acrylates with varying results. Weisel *et al.*[8] reported the successful addition of diethylamine and piperidine to ethyl acrylate in 83 and 88% yield, respectively. Johnson *et al.* treated substituted anilines with methyl acrylate and a catalytic amount of acetic acid to prepare the corresponding addition products in good yield (*Scheme 1*)[9]. The conjugate addition of *N*-benzylamines has typically met with more limited success, with high temperatures and/or pressures often required for the reaction to proceed in useful yield.[10,11]

92a R^1 = R^2 = H   **93**   **94a** 69%
92b R^1 = H, R^2 = Cl   **94b** 63%
92c R^1 = OMe, R^2 = H   **94c** 54%
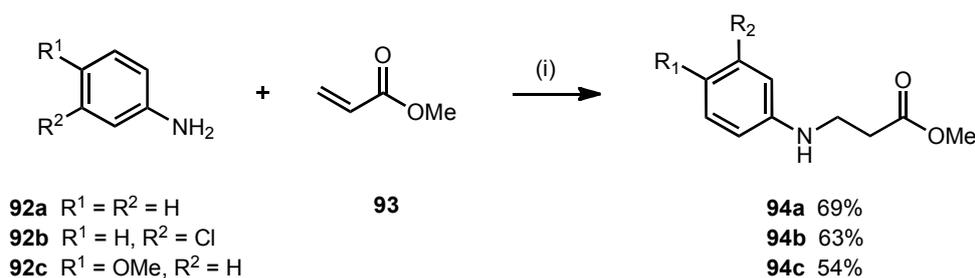
Scheme 1 Reagents and conditions: (i) Methyl acrylate, AcOH, reflux.

While the fact that those reactions mentioned above proceed in relatively good yield is useful, they lack stereocontrol, leading to racemic product mixtures that require some sort of resolution to produce enantiopure materials. Attempts to

introduce a measure of stereocontrol have been relatively successful, with the use of homochiral substrates (either the amino or α,β-unsaturated components) being the main approach. d'Angelo and Maddaluno exploited the steric bulk of the 8-β-naphthyl-menthyl crotonate acceptor **95** to achieve virtually complete stereocontrol (*Scheme 2*).[12] In another example, addition of *N*-benzylamine to glyceraldehyde derivative **97** gave the product as a single diastereomer (*Scheme 3*).[13]

95 → **96**

(i)

50%, >99% de
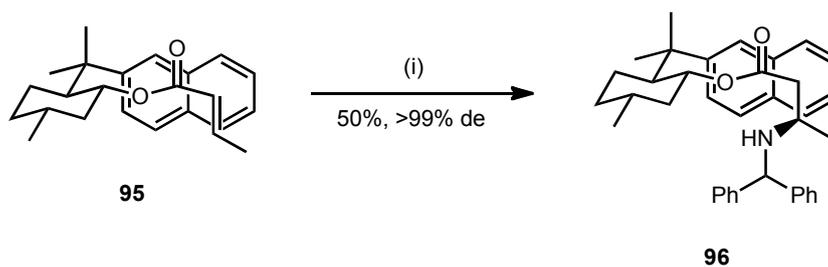
Scheme 2 Reagents and conditions: (i) diphenylmethylamine, MeOH, 15 kbar, RT.

97 → **98**

(ii)

85%, 100% de
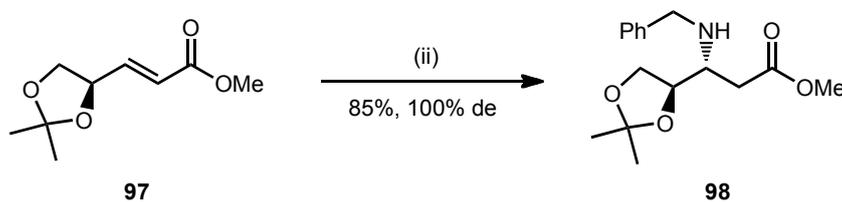
Scheme 3 Reagents and conditions: (i) benzylamine, -50°C, 50 hr.

With regard to chiral amines, Hawkins *et al.* achieved reasonable results with the addition of the rigid and symmetric 3,5-dihydro-4*H*-dinaphth[2,1-c:1',2'-*e*]azepine **100** to methyl crotonate **99** (63%; 55% de),[14] however it was not until the corresponding lithium amide (**103**) was used that significant results were observed—both in terms of yield and de (*Scheme 4*).[15] This is because the use of amines as nucleophiles places the conjugate addition under thermodynamic control, with the reversibility of the addition leading to equilibration and thus poor yields and stereocontrol. With metal amides, however, the reaction becomes kinetically controlled, allowing synthetically useful yields and stereoselectivities to be obtained.

102a $R^1 = R^2 = Me$
102b $R^1 = Hept, R^2 = {}^tBu$
102c $R^1 = {}^iBu, R^2 = {}^tBu$
102d $R^1 = TBSO(CH_2)_2, R^2 = {}^tBu$

104a 83%, >97% de
104b 80%, 96% de
104c 74%, 97% de
104d 86%, 95% de
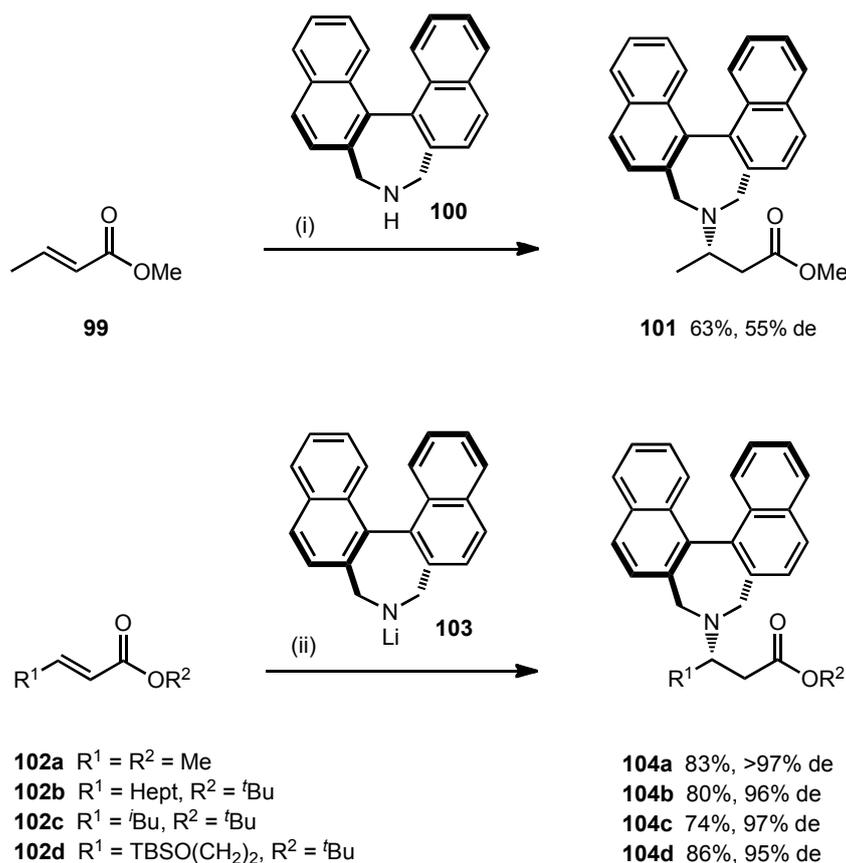
Scheme 4 Reagents and conditions: (i) **100** (0.1 eq), reflux, 21 hr; (ii) **103**, *n*BuLi, THF, -78°C.

Shortly after, Davies and Ichihara published what was to become a seminal paper in the field of lithium amide conjugate addition reactions, describing the addition of (*R*)-*N*-benzyl-*N*-α-methylbenzylamide **106** to benzyl crotonate (*Scheme 5*)[16]. While homochiral (*R*)- or (*S*)-α-methylbenzylamine had previously been used in the thermal conjugate addition to methyl crotonate, the de's of the final products were extremely poor.[17,18] In contrast, Davies' chiral dibenzylamide gave the conjugate adduct in 88% yield and >95% de. Furthermore, the two benzyl groups were easily cleaved using standard hydrogenolytic conditions, unlike the binaphthyl ligand, which was resistant to removal under H$_2$ at atmospheric pressure. In the time since Davies' original paper appeared other reports of lithium amide conjugate addition methodologies have been published,[19,20] but the α-methylbenzylamine-derived lithium amides remain amongst the most effective reagents in use today, and contribute to much of the work presented in this thesis.
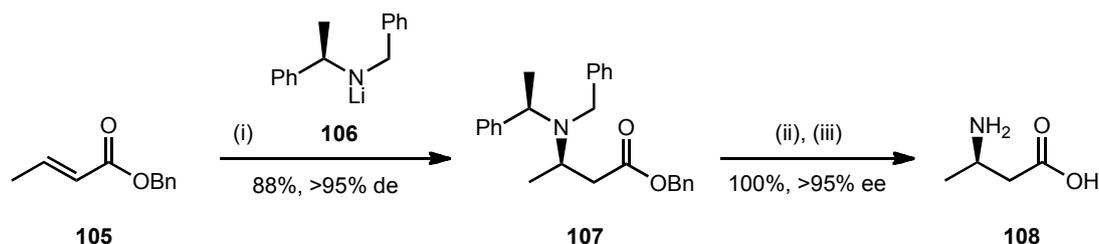
Scheme 5 Reagents and conditions: (i) **106**, THF, -78°C, then NHCl₄; (ii) Pd(OH)₂/C, MeOH-AcOH-H₂O (40:4:1), H₂ (1 atm); (iii) aq HCl, reflux.

2.1.1 Conjugate addition of lithium α-methylbenzylamides to β-substituted acrylates

Davies' protocol appears constantly in the literature since being reported in the early 90's, demonstrating its applicability to a wide range of α,β-unsaturated substrates.[21] It is typically represented by the reaction shown in *Scheme 6*, with the products featuring a *syn* relationship between the β-carbon and α-methyl group. These substrates, or Michael acceptors, range from simple alkyl or aryl substituted compounds, to those featuring more complex side-chains (unsaturated or ethereal, for example). The strong basicity and nucleophilicity of the amide does preclude the use of more sensitive substrates, however even functional groups expected to be adversely affected (e.g., formyl[22] and tosyl[23]) are relatively resistant to the conditions. It is also for this reason that *tert*-butyl esters are typically used in these reactions—the smaller methyl or ethyl esters tend to suffer from competing 1,2-addition at the carbonyl group, reducing yields. Other carbonyl compounds that have proven suitable for these reactions include benzyl esters, and *N,N*-dimethyl,[24] cyclic[25] and Weinreb[26] amides.

109 R = Me **113** 82%, 99% de
110 R = Et **114** 92%, >95% de
111 R = Ph **115** 92%, >95% de
112 R = ᶦBu **116** 96%, 97% de
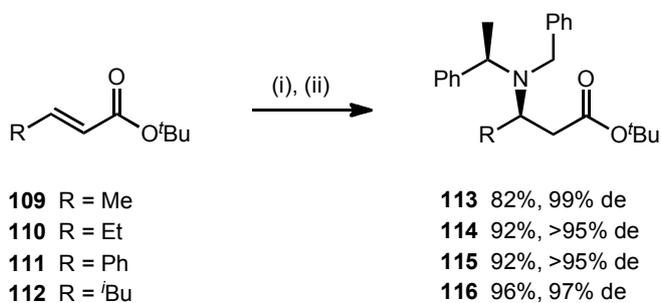
Scheme 6 Reagents and conditions: (i) **106**, THF, -78°C; (ii) NH₄Cl.

The reaction is also tolerant of variations to the lithium amide, with manipulations of the protecting group typically used to take advantage of selective deprotection strategies.[27] Lithium *N*-benzyl-*N*-α-methylbenzylamide is the most commonly used reagent due to the high diastereoselectivities obtained (often >95% de) and the ease of hydrogenolysis with palladium catalysts. However, *N*-allyl-*N*-α-methylbenzylamide gives comparable selectivity in the conjugate addition reaction whilst being susceptible to selective deallylation by Wilkinson's catalyst.[28] As an example, a combination of hydrogenolytic and oxidative cleavage techniques can be used to selectively cleave either of the benzyl groups, as shown for conjugate adduct **117** (*Scheme 7*).[29] Under relatively standard conditions (Pd(OH)$_2$/C and H$_2$), the α-methylbenzylamine protecting group is removed, leaving the dimethoxybenzyl group—which is more resistant to hydrogenolysis—intact. The susceptibility of 4-methoxybenzylamines to oxidative cleavage can also be exploited using either 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) or ceric ammonium nitrate (CAN). This demethoxybenzylation is particularly useful for adducts containing alkenes or other functional groups unstable to hydrogenolysis conditions.
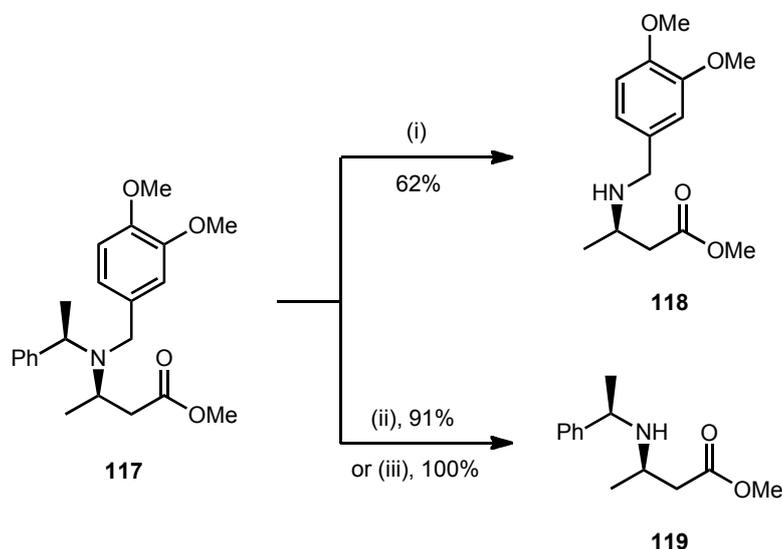
Scheme 7 Reagents and conditions: (i) Pd(OH)$_2$/C, MeOH, H$_2$ (1 atm); (ii) CAN, MeCN-H$_2$O (5:1), RT; (iii) DDQ, DCM-H$_2$O (5:1), RT.

The stereochemical outcome of this type of reaction has a large bearing on the identity of the fluorinated β-amino esters prepared during the course of this project, and thus deserves further explanation. The origins of the high stereoselectivity achieved in these conjugate addition reactions was investigated using molecular modelling of the reaction between lithium (*R*)-*N*-benzyl-*N*-α-methylbenzylamide and *tert*-butyl cinnamate, in which the (*R*)-amide favours addition to the 3-*Si* face of the ester to give the *syn* diastereomer **115** (*Scheme 8*).[30]
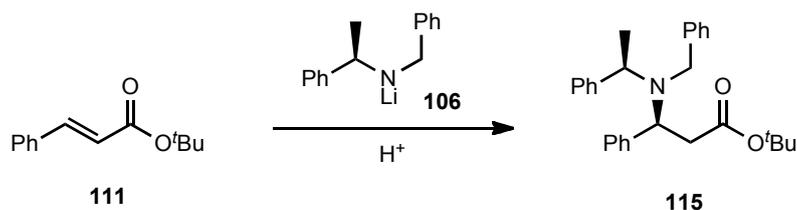
Scheme 8 Addition of lithium (*R*)-*N*-benzyl-*N*-α-methylbenzyl-amide addition to *tert*-butyl cinnamate.

Assuming a tetrahedral approach trajectory and the presence of lithium chelation, it was found that at distances of approximately 3.0-4.5 Å between the nitrogen and β-carbon, the two phenyl rings of the amide adopted a splayed arrangement (*Figure 1*, left). However, at closer range (between 1.7 and 3.0 Å), the phenyl rings were reorientated approximately parallel to each other, and perpendicular to the ester to minimise steric interactions (*Figure 1*, right).
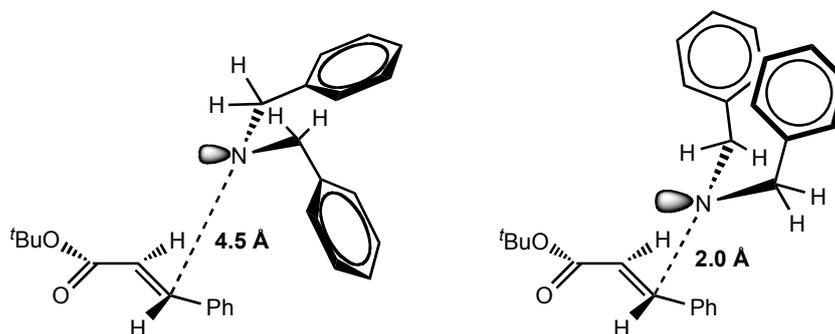
Figure 1 Orientation of the benzyl groups at different distances on approach towards the α,β-unsaturated ester.

Using this parallel 'butterfly conformation' as the minimum energy structure in the transition state, and a distance of 2 Å between nucleophile and electrophile, the protons attached to the benzylic carbons of the amide moiety were sequentially replaced with a methyl group. Rotation of the N—C$_{benzyl}$ dihedral angle through 360° gave the lowest energy conformations shown in *Figure 2*. The calculated energy of structure A was arbitrarily set to 0 kJ/mol to ascertain the relative energies of the conformers. This figure shows that placing the methyl group in position A is the only time the butterfly conformation is retained. This is also the only structure that corresponds to the stereochemistry observed experimentally i.e., gives the (3*S,aR*)-product in *Figure 1*. The methyl group is not well tolerated in any of the other three positions, and rotation about the N—C$_{benzyl}$ bonds must occur to accommodate the extra strain. However these structures still remain higher in energy relative to structure A, even after the rotation. Placement of the methyl group in position B equates to the use of the (*S*) enantiomer of (α-methylbenzyl)benzylamine—with structure B having an energy 11 kJ/mol higher than A (the (*R*)-isomer), the (*S*)-isomer is unlikely to add to the 3-*Si* face of the ester to give a product featuring *anti* stereochemistry.
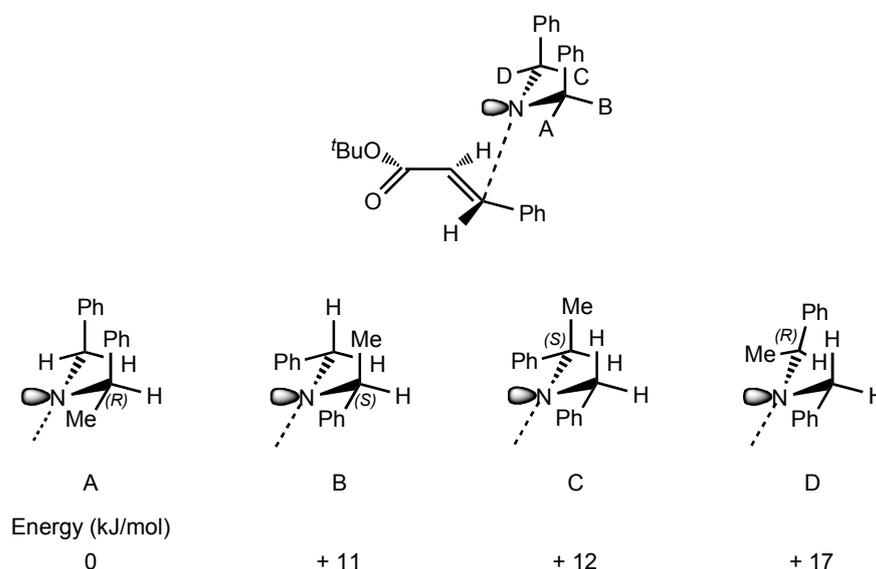
Figure 2 The four possible conformers of the dibenzyl moiety and their relative energies.

2.1.1.1 α-Substituted-β-amino esters

The conjugate addition reaction is a versatile one as it provides a number of routes to both *anti* and *syn* α-substituted-β³-amino esters. As will be seen, both the yield and diastereoselectivity are highly dependent on a number of factors: the nature of the substrate (both the type of carbonyl compound and the β-substituents), the electrophile, and the experimental method. The three approaches that have been devised to achieve the best outcome for any particular set of reagents will be discussed here.

The first method is a one pot, or tandem, approach that involves quenching the enolate with an electrophile (typically a primary alkyl or benzyl halide) and provides access to the *anti* substrates, with the stereoselectivity resulting from steric hindrance by the bulky dibenzylamide. Poor de's are often obtained due to the fact that the enolate alkylations require higher temperatures, resulting in a loss of stereoselectivity; in some cases there is virtually no preference for the *anti* isomer.[31] This is most likely due to a disruption of the lithium chelation between the nitrogen atom and the ester carbonyl. This chelation appears to be an essential factor in maintaining the stereoselectivity of these reactions, as the addition of a small amount of DMPU—a dipolar aprotic solvent that solvates the lithium cation and prevents chelation—to the reaction mixture results in de's of 0%.[32] In contrast to these results, which were obtained with either methyl or *tert*-butyl esters, α,β-unsaturated amides give yields typically above 70% and de's >94% (*Scheme 9*).[24] However, the harsh conditions usually required for conversion of the amide to a carboxylic acid make them less preferable as conjugate addition substrates.

111 R^1 = O^{t}Bu
120 R^1 = N(Me)$_2$

121a R^1 = O^{t}Bu, R^2 = Me: 78%, 15% de
121b R^1 = O^{t}Bu, R^2 = Bn: 46%, 43% de
122a R^1 = N(Me)$_2$, R^2 = Me: 70%, >94% de
122b R^1 = N(Me)$_2$, R^2 = Bn: 89%, >94% de
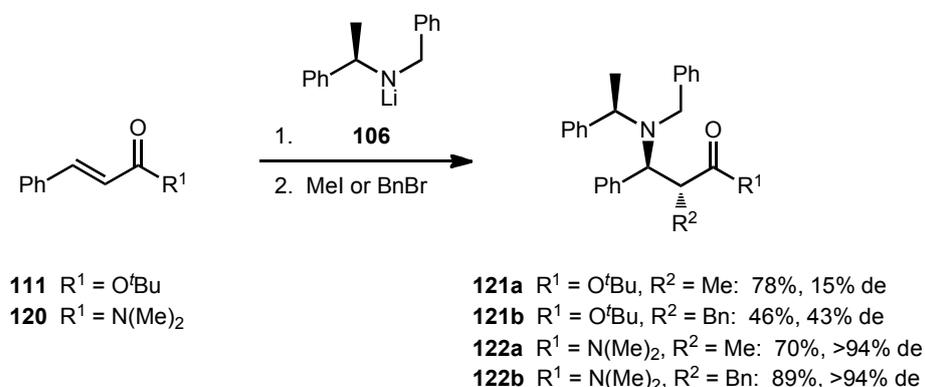
Scheme 9 Tandem conjugate addition-alkylation to tert-butyl esters and amides.

In response to the disappointing results obtained for the alkylations under the tandem method, Davies and co-workers then looked to a stepwise approach, where the unsubstituted conjugate adduct is first isolated, then separately deprotonated and alkylated. The preference for the *anti* isomer remained, but the difference in selectivities between the two methods was considerable in some cases, and is illustrated by the methylation of *tert*-butyl cinnamate in *Scheme 10*. In contrast to the tandem reaction (shown in *Scheme 9*), which showed little preference for the *anti* isomer, the stepwise method was highly selective, giving the desired isomer in 94% de. However, benzylation actually gave poorer results than the tandem reaction; the only synthetically useful benzylation (in terms of selectivity) in either of these methods was to the α,β-unsaturated amides, mentioned above.
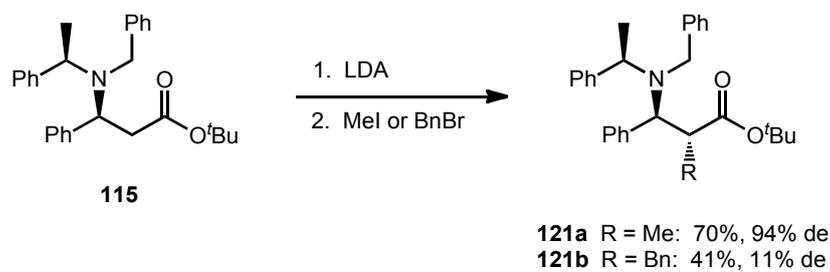
Scheme 10 Stepwise deprotonation-alkyation of conjugate adducts.

The third method provides a route to *syn* α-substituted-β³-amino esters. The conjugate additions are conducted with α-alkyl-α,β-unsaturated esters in toluene and quenched with a bulky proton source, of which 2,6-di-*tert*-butylphenol (**124**) was found to give the best results (*Scheme 11*).[33] As occurs in the tandem method with α-substitution using an alkyl halide, the proton donor approaches from the less hindered face, giving the *syn* isomer instead of the *anti* isomer seen in the tandem conjugate addition-alkylation reaction. Furthermore, no C3 epimers were found to be produced, so the lithium amide continues to attack one face exclusively. Dilution of the reaction mixture with THF just prior to addition of the phenol was found to be essential for good *syn* selectivity, with de's >99% achieved, however yields were only moderate in most cases. In stark contrast to the original tandem method, an α-benzyl-substituted ester could be synthesised in 96% de, although in only 45% yield.

123a R¹ = R² = Me
123b R¹ = Ph, R² = Me
123c R¹ = Ph, R² = Bn
123d R¹ = Et, R² = Me

125a 65%, >98% de
125b 61%, >98% de
125c 45%, 96% de
125d 65%, >98% de
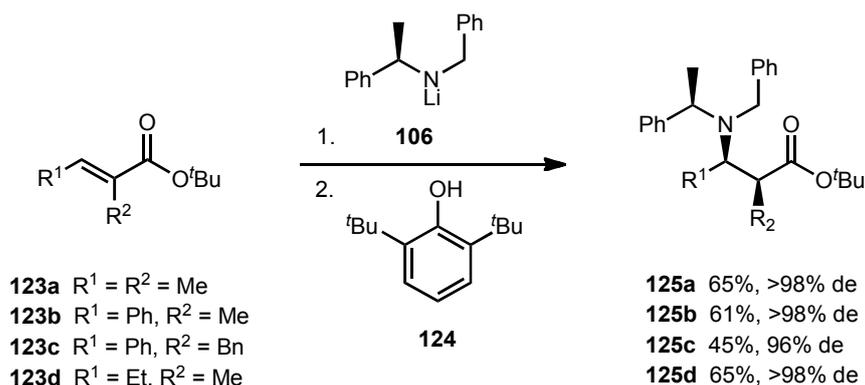
Scheme 11 Synthesis of *syn*-α-alkyl-β-amino esters from α-substituted acrylates.

Quenching the enolates with alkyl halides to generate a quaternary stereocentre was also found to be possible when conducted using the toluene-THF solvent system. Methylation of the enolate with methyl iodide occurred in 50% yield to give the *gem*-dimethyl adduct **124a**, while benzyl bromide gave **124b** as a 10:1 mixture of C2 epimers (*Scheme 12*).

123a

124a R = Me: 50%
124b R = Bn: 56%, 10:1 (*anti:syn*)
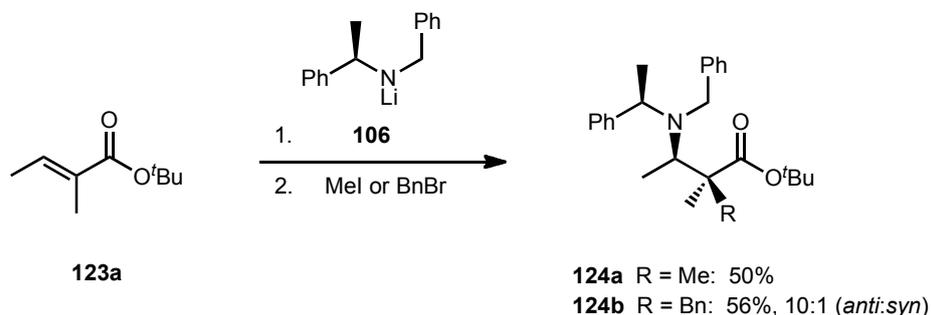
Scheme 12 Generation of quaternary α-carbon via alkyl halide quench.

In order to rationalise the profound differences between the results seen for each method, a study of the enolate geometries was undertaken by trapping the enolates with trimethylsilyl chloride, an electrophile that reacts preferentially at the oxygen atom because of the strong oxygen-silicon bond formed. The tandem and stepwise methods were each found to produce one major enolate of differing geometries. Assignment of their configurations using ¹H NMR nOe difference experiments was largely inconclusive, however the authors concluded from previous studies[34] that the enolate generated during the conjugate addition existed entirely as the (*Z*)-

isomer, while the enolate derived from deprotonation of the conjugate adduct was found to exist primarily in the (*E*)-configuration.[31] Furthermore, it was discovered that, in addition to the C3 chiral centre, the distant *N*-α-methylbenzyl stereocentre also played a role in the diastereoselectivity of the alkylation. Together, these results indicate that many factors influence the facial selectivity in the alkylation reactions, and with no generally applicable rules, stereoselectivities are hard to predict *de novo.* It is therefore impossible to predict which of these methods is most suited to the preparation of α-fluoro-β³-amino esters.

2.1.1.2   α-Hetero-β-amino esters

These tandem and stepwise methodologies have extended beyond alkylations to include α-heteroatom substitution, providing access to a new range of β²-amino substrates. The synthesis of α-hydroxy-β-amino esters involves hydroxylation of the intermediate enolate *in situ* with either (-) or (+)-(camphorsulfonyl)-oxaziridine enantiomers, with the chirality of the oxaziridine found to have little influence on the stereoselectivity of the reaction. In most cases the oxaziridine provided the *anti*-α-hydroxy-β-amino esters in excellent de and yield, giving the hydroxylated derivative **127** in 89% yield and >95% de (*Scheme 13*).[35]
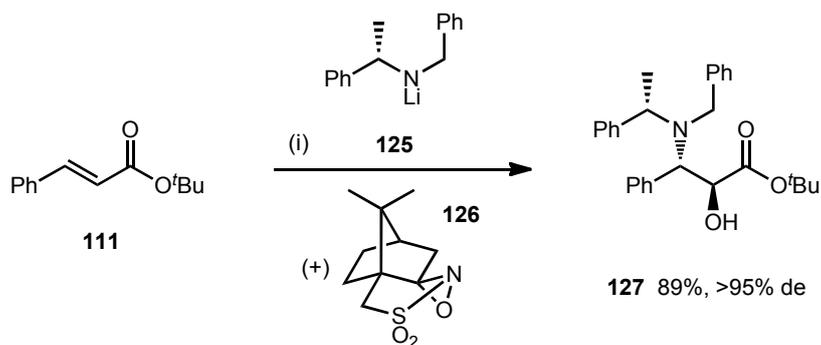
Scheme 13 Reagents and conditions (i) **125**, THF, -78°C, 2 hr, then (1*R*)-(-)-(camphorsulfonyl)oxaziridine **126**.

α-Amino esters can be synthesised in this way via amination of the enolates with electrophilic trisyl azide, with the resultant azide then being reduced to give the primary amine.[36] Although the reaction is highly diastereoselective (>95% de), it is

low-yielding, and the desired products are much more efficiently synthesised via other routes (e.g., via manipulation of the corresponding α-hydroxy esters[37]).

α-Mercapto-β-amino esters have also been successfully synthesised using benzyl or *tert*-butyl thiotosylate as the electrophilic source of sulfur (*Scheme 14*).[38] However, as sulfur compounds are well-known palladium catalyst poisons, hydrogenolysis of the benzyl groups predictably failed, hence the need for allyl and *p*-methoxyphenyl protecting groups on the ligand. These were removed sequentially, enabling isolation of amino acid **132** after hydrolysis of the *tert*-butyl ester (*Scheme 15*).

128 $R^1 = R^2 = Ph$: 78%, 97% de
129 $R^1 = PMB$, $R^2 = allyl$: 99%, >98% de
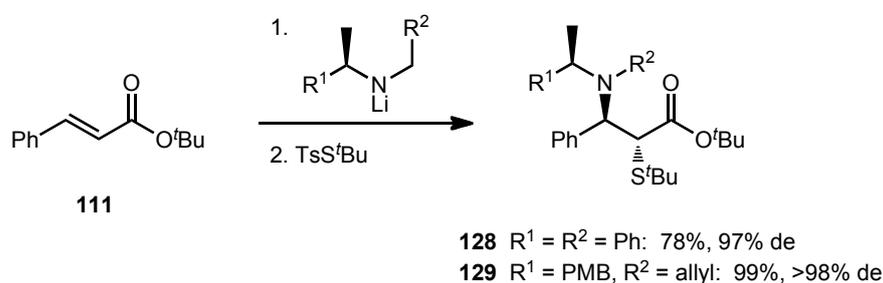
Scheme 14 Quenching of the intermediate enolate with *tert*-butyl thiotosylate produced a series of α-mercapto-β-amino esters.

128 $R^1 = R^2 = Ph$
129 $R^1 = PMB$, $R^2 = allyl$

130 58%
131 19%

132 $R^1 = Ph$; 98%, >98% de
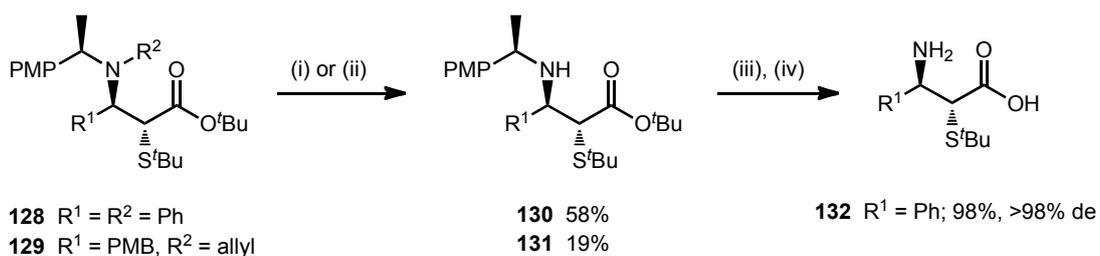
Scheme 15 Reagents and conditions: where R = Bn: (i) CAN, MeCN-H$_2$O (5:1), -7°C, 5 min; where R = allyl: (ii) TolSH, AIBN, benzene, reflux; (iii) TFA-DCM (1:1), 55°C, 24 hr; (iv) Dowex 50WX8-200.

2.1.2 Application of the conjugate addition methodology to the synthesis of β³-lysine and β³-arginine derivatives

To date, two complete syntheses of β-lysine analogues using this conjugate addition methodology have been published, both using amino-functionalised α,β-unsaturated esters in the conjugate addition step. In 2003 Langenham and Gellman published their synthesis of α-methyl amino acid **138** from *tert*-butyl propionate (*Scheme 16*).[39] After preparation of α-methyl-α,β-unsaturated ester **135** via the tosylate **143**, it was subjected to Davies' standard protocol for α-substituted substrates, which furnished *syn*-**136** in 98% de and 20% overall yield from *tert*-butyl propionate. Initial attempts at using an *N*-Boc protected derivative in the conjugate addition resulted in recovered starting material. The two N^β-benzyl groups were removed using transfer hydrogenation and the amine re-protected as the Cbz derivative (**137**). The third benzyl group proved resistant to various hydrogenolysis conditions (a problem also encountered by others[23]), and an alternative strategy involving a dissolving metal reduction had to be employed. This also removed the Cbz group, with the free amine then re-protected as the Fmoc derivative.
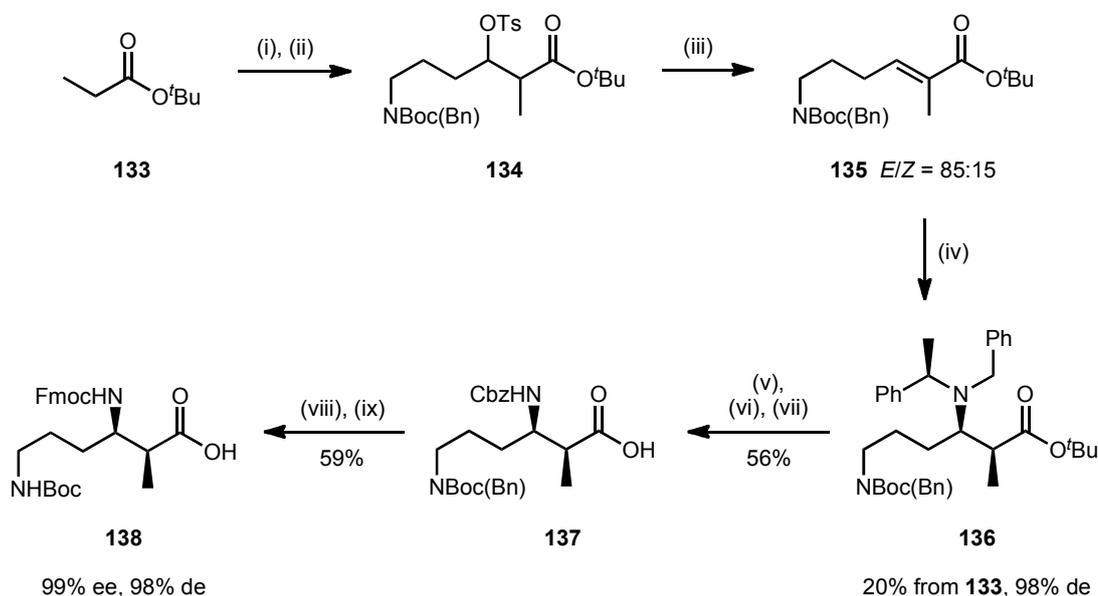
Scheme 16 Reagents and conditions: (i) LDA, THF, 0°C, *N*-Boc-*N*-benzylaminobutyraldehyde; (ii) DMAP, TsCl, Et₃N, DCM; (iii) KO*t*Bu; (iv) lithium (*R*)-*N*-benzyl-*N*-α-methylbenzylamide, THF, -78°C, 2 hr, then 2,6-di-*tert*-butylphenol; (v) 10% Pd/C, HCO₂NH₄, *t*BuOH; (vi) Cbz-OSu, NaHCO₃; (vii) TFA-DCM (4:1), then Boc₂O, Et₃N; (viii) Na, NH₃, THF; (ix) Fmoc-OSu, NaHCO₃.

The second example, published in 2006 by Davies *et al.*, features a relatively successful conjugate addition of the lithium amide to an *N,N*-di-Boc protected α,β-unsaturated ester,[40] with further elaboration furnishing (*R*)-β-lysine (**144**, *Scheme 17*). The requisite ester (**140**) is synthesised in three steps in 56% overall yield from commercially available *N*-Boc pyrolidinone, which is first reduced, then subjected to Wittig olefination and further Boc-protection. Conjugate addition with lithium (*R*)-*N*-benzyl-*N*-α-methyl-benzylamide gave a 77:23 mixture of the desired adduct and the de-conjugated ester **142**, produced as a result of γ-deprotonation by the amide. This by-product proved difficult to separate by chromatography and was thus carried through to the hydrogenation step, where the primary β-amine **143** was isolated in 55% yield from the unsaturated ester. Finally, the *tert*-butyl ester was cleaved with concomitant removal of the *N*-Boc groups by treatment with TFA, followed by ion exchange chromatography to liberate the free β-amino acid.
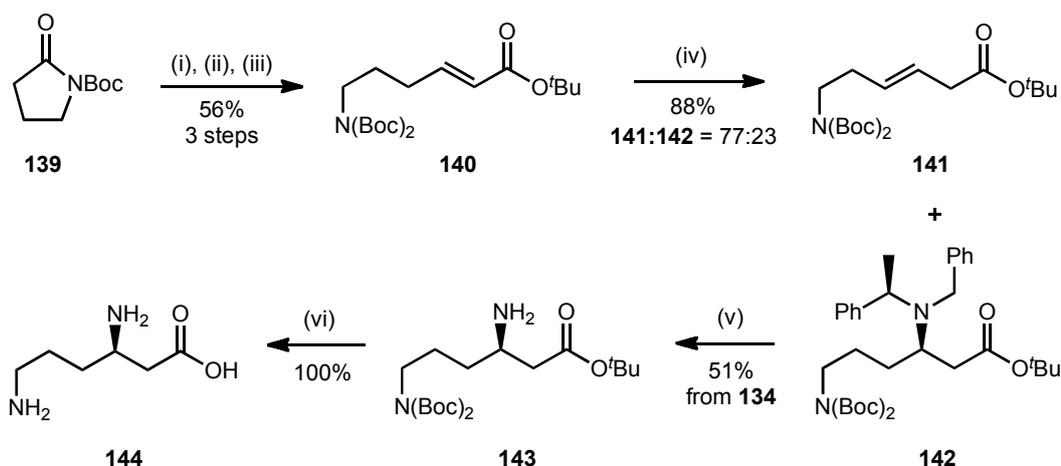
Scheme 17 Reagents and conditions: (i) DIBAL-H, THF, -78°C; (ii) toluene, $Ph_3P=CHCO_2{}^tBu$, 110°C; (iii) Boc_2O, DMAP, MeCN; (iv) lithium (*R*)-*N*-benzyl-*N*-α-methylbenzylamide, THF, -78°C; (v) Pd/C, HCO_2NH_4, MeOH, 65°C; (vi) TFA, DCM, then Dowex 50WX-200.

Lastly, a tosyl derivative (**145**) was found to be suitably stable for the synthesis of β-homolysine (**148**), which has a side-chain elongated by one carbon relative to β-lysine (*Scheme 8*).[23] The crude *O*-tosylated acrylate **145**, prepared in three steps from 3,4-dihydropyran, is treated with (*S*)-*N*-benzyl-*N*-α-methylbenzylamine then hexyllithium in DME to give the conjugate adduct in 75% yield. If allowed to warm to 0°C before quenching, the enolate undergoes an intramolecular cyclisation as a result of tosylate displacement. In contrast, the tosylate resisted several attempts at removal to install the nitrogen functionality (sodium azide, Gabriel conditions), but conversion was eventually achieved with excess ammonia in ethanol in a sealed vessel. Boc-protection and hydrogenolysis of the benzyl groups provided the β-amino ester **148** in 20% overall yield. The authors also investigated the use of a phthaloyl acceptor in the conjugate addition step, but found no trace of product.
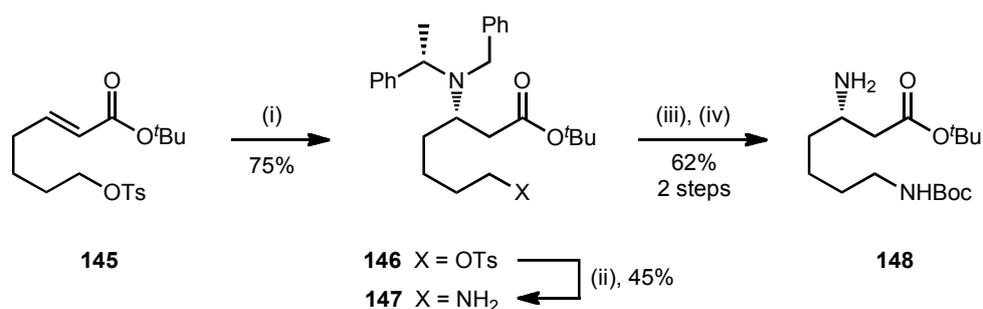
Scheme 18 Reagents and conditions: (i) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide, hexyllithium, DME, -60°C, then AcOH; (ii) NH_3, EtOH, 50°C, sealed vessel; (iii) Boc_2O, K_2CO_3, EtOAc-H_2O; (iv) Pd/C, EtOH-EtOAc, oxalic acid.

A number of other examples pertaining to the use of *O*-protected hexenoates in these conjugate addition reactions exist. Benzyl ethers are stable under these conditions, giving products in high de,[41] while Davies found that both formyl and unprotected hydroxy groups survive the reaction relatively unscathed, however this was dependent on the conditions used.[22]

Only one β³-arginine analogue has previously been synthesised via these methods, with Bischoff *et al.* seeking an efficient route to blastidic acid **155** (*Scheme 19*).[42] Their 12-step synthesis began from β-alanine, which was converted to the Boc-protected α,β-unsaturated ester **149** over 6 steps. Conjugate addition under standard conditions gave **150**, which could potentially be converted directly to the arginine derivative after Boc removal, however the authors also wished to develop a route to the orthogonally protected **154**. In addition, the acidic conditions used in the final carbamate deprotection (trimethylsilyl iodide and acidic methanol) serve to protonate the amino groups as they are formed, preventing unwanted intramolecular cyclisation from occurring.
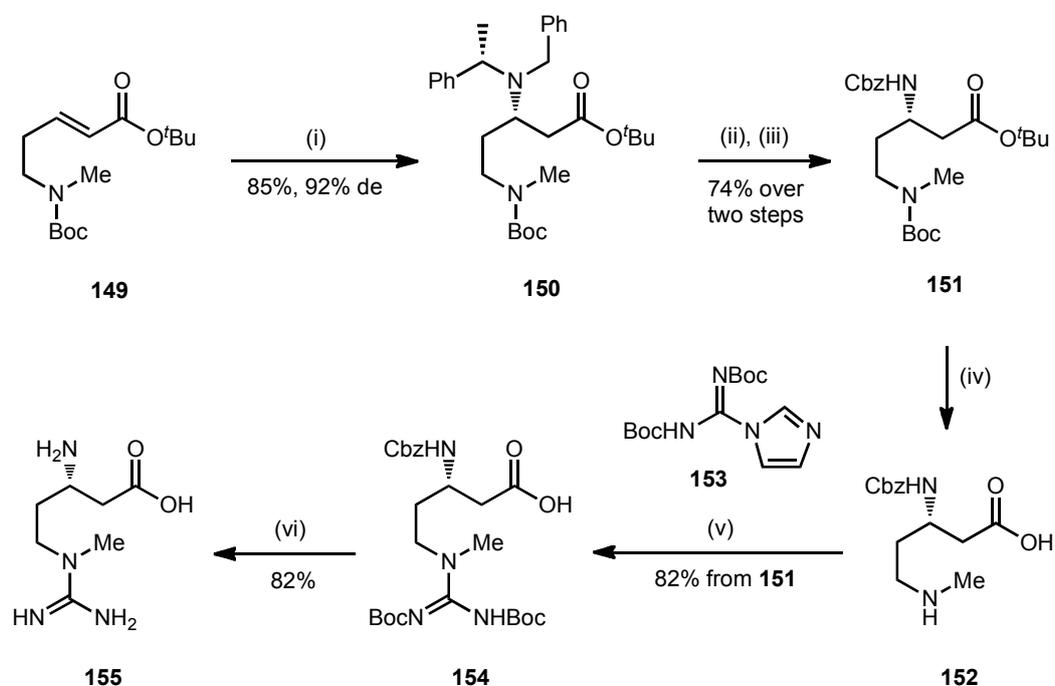
Scheme 19 Reagents and conditions: (i) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide (2.4 eq.), THF, -78°C; (ii) 5% Pd/C, HCO$_2$NH$_4$, ^{t}BuOH, reflux; (iii) Cbz-Cl, NaHCO$_3$, acetone-H$_2$O; (iv) TFA, DCM; (v) **153**, DIPEA, MeOH; (vi) TMSI, CHCl$_3$, then HCl, MeOH.

2.2 Synthetic Strategy

It was envisaged that the desired (2*R*,3*S*) mono-fluorinated amino acids shown in *Figure 3* could be synthesised via conjugate addition of the (*S*)-lithium amide to a suitably protected α,β-unsaturated *tert*-butyl ester, followed by quenching of the enolate with an electrophilic source of fluorine. The chosen fluorinating agent was *N*-fluorobenzenesulfonimide (NFSI) because of its ease of handling and solubility in THF, as described in Chapter 1 (page 17). Two other reagents, *N*-fluoro-2,4,6-trimethylpyridinium triflate and *N*-fluoro-*N*-methyl-*p*-toluene-sulfonamide, were trialled previously in the conjugate addition-fluorination reaction and found to give poor results when compared to NFSI.[32]
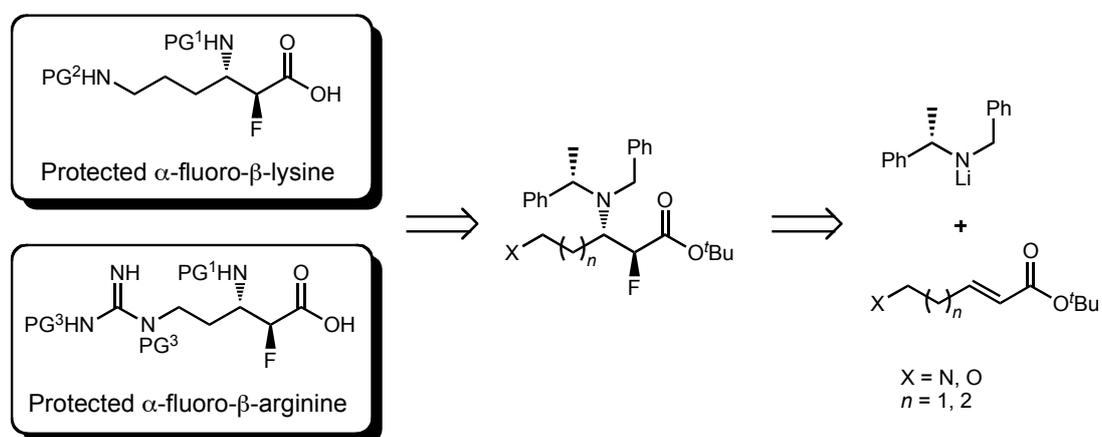
Figure 3 Retrosynthetic analysis for the desired α-fluoro-β-amino acids, where PG = protecting group, and X = protected heteroatom functionality.

Given the unpredictable stereoselectivity of each of the conjugate addition methods (tandem, stepwise and α-substituted) with different Michael acceptors and electrophiles, a comparison of the three methods using *tert*-butyl cinnamate as a model substrate was necessary to determine which was most suitable for the synthesis of the target α-fluoro-β³-amino esters. The tandem procedure that involves quenching the enolate with NFSI was anticipated to provide the β-amino ester **157**, which displays *anti* stereochemistry relative to the C2 and C3 stereocenters (*Scheme 20*). This would occur as a result of the sulfonimide approaching the less hindered face of the enolate i.e., the opposite face from which

Tandem
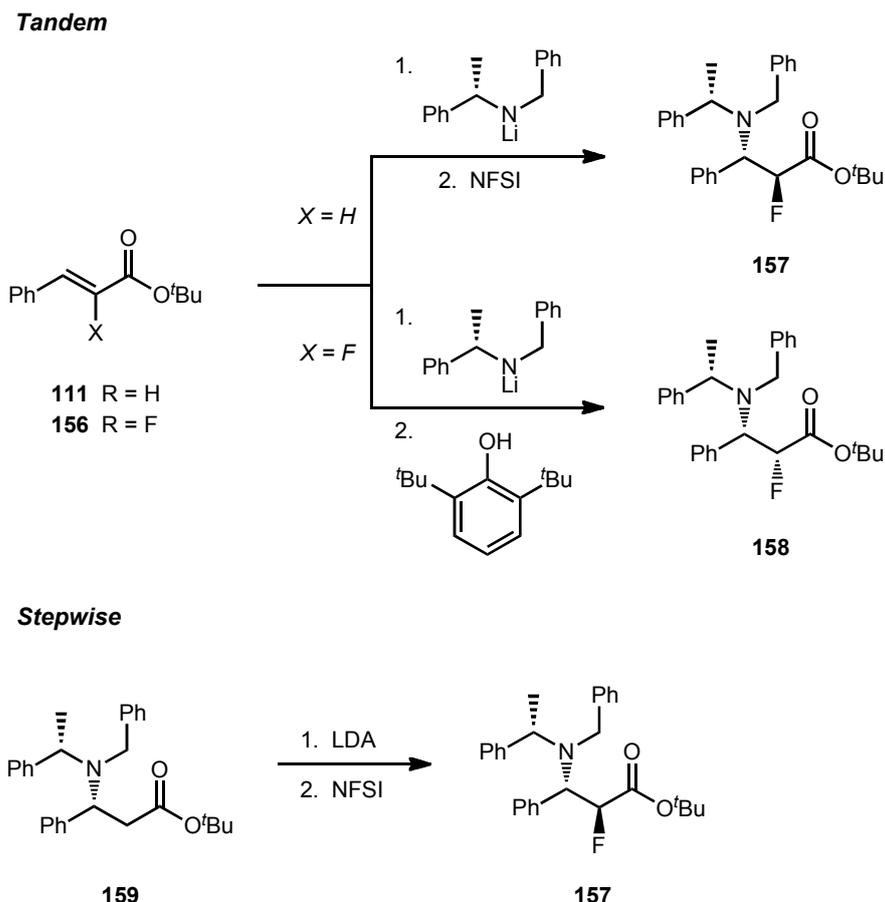
Scheme 20 Predicted outcomes for each of the three conjugate addition strategies in the synthesis of α-fluoro-β-amino esters.

attack of the dibenzylamine had occurred. A reversal of the C2 stereochemistry would be expected for the alternative tandem method, in which the conjugate addition reaction is carried out with an α-fluoro substituted α,β-unsaturated ester (**156**). In this case, as the electrophile approaching from the less hindered face is the proton source, the *syn* stereochemistry seen in β-amino ester **158** should result. The importance of this pathway is two-fold: firstly, it provides a possible route to the *syn* diastereomer of these α-fluoro-β³-amino esters, which could be useful either in studying stereochemical effects in enzyme inhibition or to act as synthons in larger molecules; and secondly, quenching the enolate with electrophilic fluorine has the potential to lead to the desirable α,α-difluoro-β³-amino analogues. Like the first tandem method, the stepwise approach should also provide β-amino ester **157**, as the bulky dibenzyl moiety is expected to exert enough stereochemical control to direct attack on the sulfonimide at the less

hindered face of the enolate. This enolate is generated from the deprotonation of non-fluorinated β-amino ester **159**, which is synthesised via a conjugate addition reaction in which the enolate has been quenched with NH_4Cl.

Once the optimal procedure had been established, this could then be applied to a number of other α,β-unsaturated esters featuring a terminal, suitably protected heteroatom functionality, to give a series of conjugate adducts from which further structural elaboration could take place.

2.3 Results and Discussion

2.3.1 Model study comparing tandem versus stepwise methods

As described in Chapter 1 (page 33), the conjugate addition of lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide to *tert*-butyl cinnamate with a subsequent NFSI quench proceeded to give the fluorinated product in quantitative yield as an 82:18 mixture (64% de) in favour of the *anti* isomer.[32] The known configuration of the α-methylbenzylamine reagent used in the conjugate addition step allowed the configuration of the C3 stereocentre to be assigned based on the large literature precedent for these reactions. X-ray crystallography performed on the fluorinated conjugate adduct then enabled the absolute configuration of the major product to be defined as (2*S*,3*S*), corresponding to the *anti* isomer **157**, which is consistent with Davies' findings that attack on the electrophile occurs predominantly from the least hindered face. Only two diastereomers were seen in this case as the lithium amide adds exclusively to one face of the cinnamate ester. To determine whether the stepwise deprotonation-fluorination approach would be more stereoselective, the β-amino ester **159** was prepared according to the standard procedure.[43] This was then deprotonated with LDA, and under similar conditions to the tandem method, quenched with a solution of NFSI in THF. The product was again isolated in quantitative yield, although integration of the characteristic α-proton signals in the ¹H NMR spectrum of the crude product revealed a disappointing 61:39 mixture of isomers. (*Scheme 21*). The difference in diastereoselectivity between the two methods is likely a result of the different enolate geometries identified by Davies and co-workers, as discussed in section 2.1.1.1.

Tandem
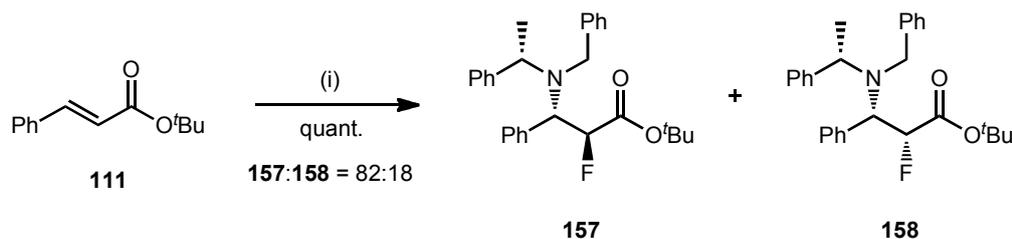
Stepwise
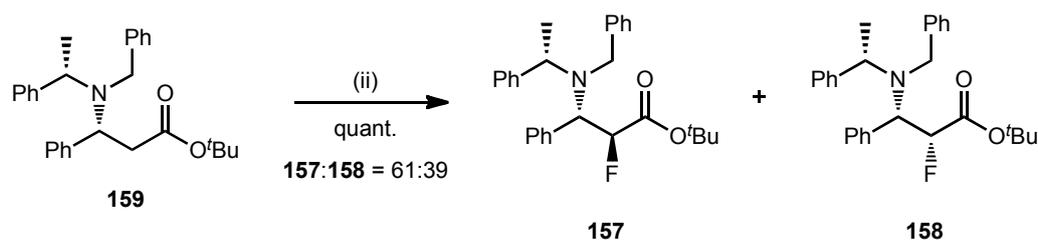
Scheme 21 Reagents and conditions: (i) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide (1.1 eq.), THF, -78°C, 30 min, then NFSI, 5 hr; (ii) LDA, THF, -78°C, 1 hr, then NFSI, -78°C, 4 hr.

With the stepwise approach proving less effective than the tandem method with regard to diastereoselectivity in this model system, attention was turned to the alternative tandem strategy. This involved the conjugate addition of the lithium amide to an α-substituted-α,β-unsaturated ester, which required the synthesis of (*Z*)-*tert*-butyl α-fluorocinnamate (**156**). This was to be accomplished using the Horner-Wadsworth-Emmons (HWE) reaction,[44] which originally employed the use of phosphonate esters in the Wittig-type synthesis of (*E*)-α-fluoro-α,β-unsaturated esters,[45,46] but was later extended to the synthesis of (*Z*)-isomers using phosphonofluoroacetic acids.[47] The reason the acid produces the (*Z*)-isomer can be seen in *Scheme 22*;[47] a comparison of the two oxyanions reveals an unfavourable interaction between the *R* group and the carboxylate anion of the pro-(*E*)-oxyanion, leading to a highly strained cyclic intermediate. The addition of the aldehyde to the dianion is reversible, and at higher temperatures equilibration easily occurs to negate this interaction, with the thermodynamically favoured product thus favoured. An added benefit associated with the use of phosphono compounds is that the by-products can be removed with an acidic aqueous extraction, unlike the

corresponding phosphine oxides, which often require chromatographic separation.
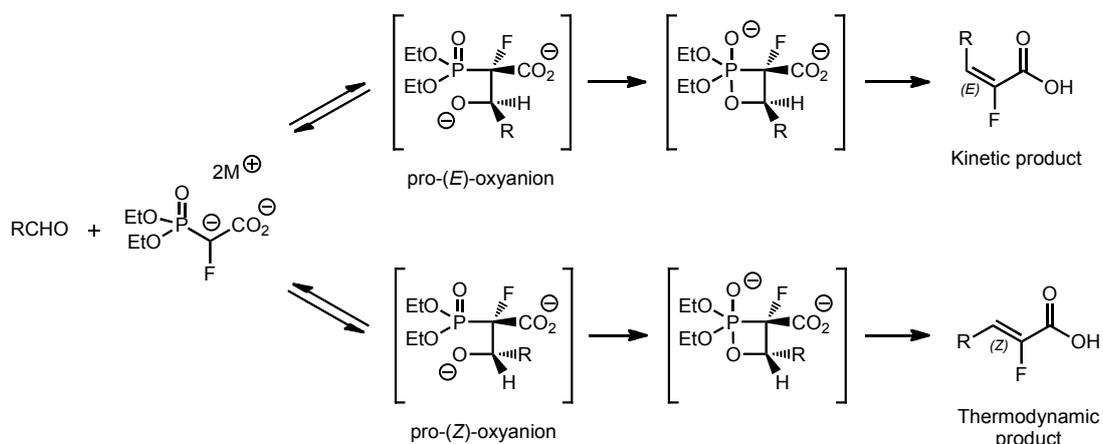
Scheme 22 Rationalisation for the formation of the thermodynamic product in the Horner-Wadsworth-Emmons reaction.

Preparation of diethoxyphosphoryl fluoroacetic acid (**162**) began from ethyl bromofluoroacetate, which undergoes an Arbuzov reaction when heated at reflux with triethyl phosphite; concomitant distillation of ethyl bromide drives the reaction toward completion.[48] Saponification of phosphonate ester **161** with KOH in ethanol at reflux proceeds in almost quantitative yield to give the carboxylic acid (*Scheme 23*).
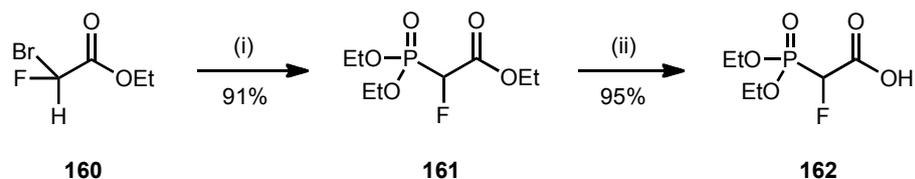
Scheme 23 Reagents and conditions: (i) triethyl phosphite, reflux, 4 hr; (ii) KOH, EtOH, reflux, 1.5 hr.

An initial attempt at conducting the HWE reaction with ^{n}BuLi via a lithium α-lithiocarboxylate anion produced disappointing results. In contrast to the results described in the literature,[47] which claimed complete (*Z*)-selectivity for the reaction with benzaldehyde, in this case the reaction gave an almost 1:1 mixture of isomers under identical conditions (^{n}BuLi (2.1 eq.), THF, -78°C). This result was also

corroborated by Sano *et al.*,[49] who found that the di-lithio species gave a 54:46 *E/Z* ratio at -78°C. In contrast, they found that the corresponding di-magnesium salt, formed from *i*PrMgBr, to be >99% (*Z*)-selective under the same conditions, although the reaction was lower yielding. When they raised the temperature to 0°C, the yield increased from 51 to 81%, with a further increase to 84% obtained upon heating the mixture to reflux before benzaldehyde addition. Complete (*Z*)-selectivity was maintained throughout, which is to be expected given higher temperatures promote the formation of this more stable thermodynamic product.

These reaction conditions involving the use of *i*PrMgBr and high temperatures were thus used for the synthesis of α-fluorocinnamic acid. A crude *E/Z* ratio of 5:95 was obtained, as determined by examination of the ^{1}H NMR spectrum. The magnitude of the $^3J_{(H-F)}$ coupling constant (36.5 Hz) was consistent with the assigned stereochemistry ($^3J_{(H-F)}$ for (*E*)-isomers are typically around 21-22 Hz[47]). (*Z*)-**163** was isolated in 80% yield after work-up and recrystallisation. Esterification to the required *tert*-butyl ester was then achieved via conversion to the acid chloride with oxalyl chloride and a catalytic amount of DMF, followed by reaction with *tert*-butanol in pyridine (*Scheme 24*).

162   **163** *Z/E* = 95:5   **156**
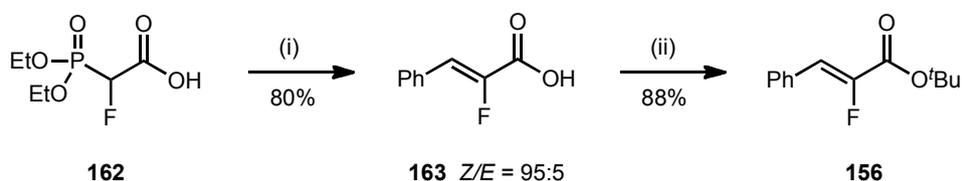
Scheme 24 *i*PrMgCl, THF, 0°C, 1 hr, then reflux, benzaldehyde, 2.5 hr; (iv) (COCl)$_2$, DMF (cat.), DCM, then *t*BuOH, pyridine.

With the α-fluoroalkene in hand, the conjugate addition-acid quench was undertaken in attempt to produce the *syn* α-fluoro-β-amino ester **158**. The α,β-unsaturated ester was added to the lithium amide solution at -78°C, and after stirring for 1 hr, a solution of 2,6-di-*tert*-butylphenol in THF was added. ^{1}H NMR analysis of the crude reaction mixture showed no sign of the conjugate adduct, only the phenol and another product. Subsequent chromatography failed to separate the

two materials, and hence the product remained unidentified. To determine if the phenol was having a deleterious effect on product formation, the experiment was repeated but in this case using saturated NH_4Cl instead of 2,6-*tert*-butylphenol to quench the reaction. Again, there was no sign of the desired product, but surprisingly the unidentified product from the previous reaction had been formed in almost quantitative conversion from the starting material, as determined by ^{1}H NMR spectroscopy. This product was identified as the known alkyne **164**,[50] as shown in *Scheme 25* with the structure established through a combination of NMR spectroscopy and mass spectrometry. The lack of any proton resonances apart from those attributable to the aromatic and *tert*-butyl protons in the ^{1}H NMR spectrum of the product indicated that the centre of the molecule had been affected. The ^{13}C NMR spectrum was the most revealing: firstly, the absence of any C-F coupling suggested that the product no longer contained fluorine. Secondly, the two alkenyl carbons of the starting material were shifted from 148 and 116 ppm to 83 and 82 ppm in the product, coinciding with an upfield shift of the aromatic *ipso* carbon from 131 to 120 ppm. These shifts are indicative of the shielding that arises from the π-electrons of the *sp*-hybridised carbons in alkynes. Finally, strong supporting evidence for the assigned structure was provided by ESI HRMS analysis, which identified a strong ion at m/z = 225.0886, corresponding to [alkyne+Na⁺]. The most likely mechanism for formation of this product is through β-deprotonation by the lithium amide, with subsequent loss of fluoride to form the stable conjugated alkyne. The presence of fluorine on the α-carbon may lead to an increased acidity of the β-proton, resulting in its easy abstraction. Indeed, the chemical shift of the β-proton in *tert*-butyl α-fluorocinnamate is 6.72 ppm, compared to the slightly more upfield 6.37 ppm for the unfluorinated *tert*-butyl cinnamate. This 'dehydrofluorination' of α-fluoroacrylates is previously unreported in the literature.

With the conjugate addition of the lithium amide to α-fluoro acrylates ruled out as a viable alternative for the preparation of α-fluoro-β³-amino esters, the original tandem conjugate addition-fluorination approach was ready to be applied to a series of pro-lysine and pro-arginine α,β-unsaturated esters.
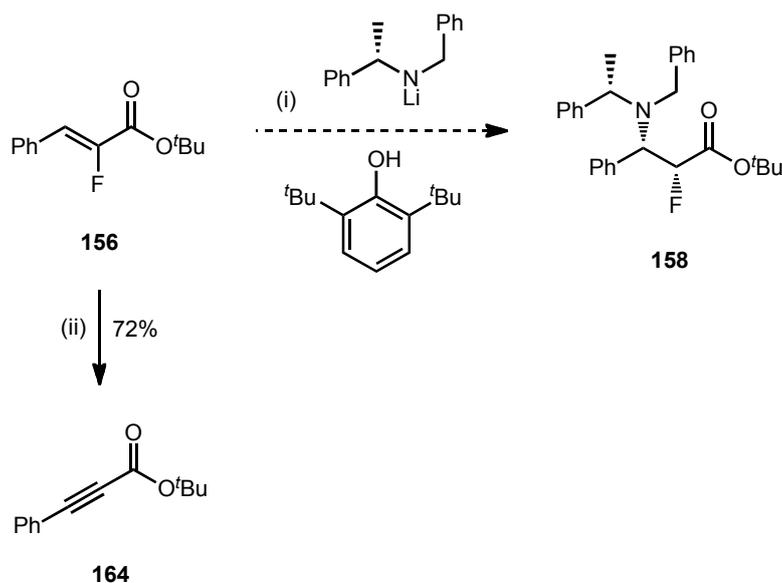
Scheme 25 Reagents and conditions: (i) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzyl-amide, THF, -78°C, 1 hr, then 2,6-di-*tert*-butylphenol; (ii) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide, THF, -78°C, 2 hr, then NH₄Cl.

2.3.2   Synthesis of α,β-unsaturated esters

The α,β-unsaturated esters selected for use in the conjugate addition reactions should ideally be as closely related to the lysine and arginine final products as possible to facilitate the synthesis of the final products and minimise the number of overall steps. Both of these target amino acids possess nitrogen-functionalised side-chains, which if present during the conjugate addition reaction, require suitable protection to mask the amino protons. Both the ease of removal and the ability to achieve orthogonal deprotection are two factors that need to be taken into consideration when selecting an appropriate protecting group. Orthogonal deprotection is especially important where functional groups require deprotection then reprotection with an alternative group e.g., to make them more amenable to the use of solid-phase peptide synthesis (SPPS) techniques. The most important requirement in our case is that they must be stable under the strongly basic and nucleophilic reaction conditions of the conjugate addition to avoid decomposition. This rules out most of the protecting groups used for nitrogen, which are typically quite base sensitive. The Boc group, which is highly resistant to strong base and nucleophilic attack, is the most commonly used nitrogen protecting group, with the

tosyl and benzyl protecting groups also widely used. However tosyl groups can require forcing conditions for removal, while also rendering the remaining proton on nitrogen extremely acidic,[51] while an additional benzyl protecting group on the terminal nitrogen may be difficult to remove independently of the N^β-benzyl groups.

One potentially useful protecting group is diphenylsilyldiethylene (DPSide), which was first described in 1999 by Kim and Cho for the protection of primary amines in cases where both amine hydrogens needed to be masked.[52] These silapiperidine structures have also appeared in the literature previously as components of silicon-based drugs, where they do not serve in any sort of protection capacity.[53,54] This DPSide group can apparently be removed using a combination of TBAF and CsF as the fluoride source, which leaves Cbz, Boc and phthalimide groups untouched, while being resistant to the conditions used to cleave these three common protecting groups.

An alternative approach is the use of oxygen-protected substrates; the range of oxygen protecting groups is much larger, and they tend to be significantly more stable to basic conditions than the corresponding *N*-protected substrates. Furthermore, they are easily deprotected and converted to the required nitrogen functionalities after serving their purpose by surviving the harsh reaction conditions. One of the most popular methods of protecting the oxygen atom is by converting it to a silyl ether, with its susceptibility to acid or base determined by the alkyl or aryl substituents attached to the silicon atom.[55] *tert*-Butyldimethylsilyl (TBS) ethers have found widespread use for their combination of stability and ease of removal under either acidic or basic conditions, or with fluoride ion.

A number of potential α,β-unsaturated esters bearing terminal *O*- and *N*-protected functionalities were identified for use in the conjugate addition reactions, and primarily related to the synthesis of the six-carbon lysine analogues. The majority were nitrogen based as this would ultimately require fewer overall steps to reach the target compound. Two TBS ethers of different side chain length were also included, with α,β-unsaturated esters bearing this moiety already proven to be stable to the conditions in the conjugate addition reaction.[25,56,57] These proposed

acceptors included a substrate bearing a *tert*-butyldiphenylsilyl-masked amine, and another with the amine protected as the azido functionality, which could be reduced to a primary amine after the conjugate addition reaction. However these were both abandoned early on due to synthetic difficulties, and all efforts were thus focussed on the synthesis of the esters shown in *Figure 4*.

143 X = (Boc)$_2$N, *n* = 2   **168** X = (Boc)$_2$N, *n* = 2   **172**

165 X = [structure], *n* = 2   **169** X = [structure], *n* = 2

166 X = OTBS, *n* = 1   **170** X = OTBS, *n* = 1
167 X = OTBS, *n* = 2   **171** X = OTBS, *n* = 2
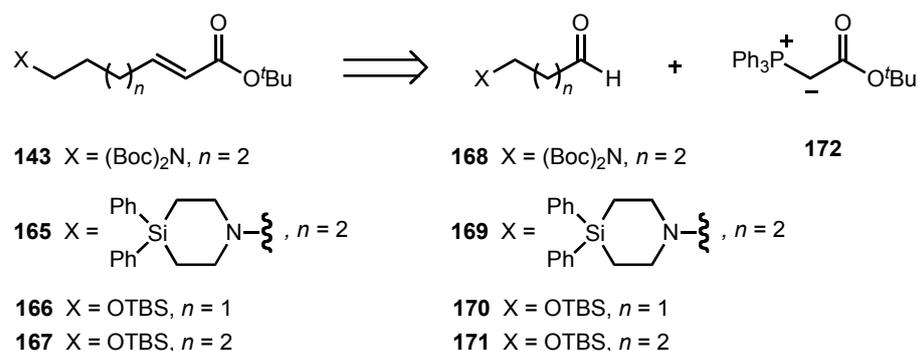
Figure 4 Proposed pathway for the synthesis of a series of *O*- or *N*-protected α,β-unsaturated esters for use in the conjugate addition reaction.

2.3.2.1   Synthesis of the di-Boc protected α,β-unsaturated ester

Synthesis of the Boc protected substrate began from 4-aminobutyraldehyde dimethyl acetal (**173**), with di-Boc protection carried out over two steps according to the method of Carretero and Arayás[58] in 91% yield (*Scheme 26*). Acetal hydrolysis was accomplished with AcOH-H$_2$O (2:1), although the isolation of varying amounts of the mono-Boc compound was common using these acidic conditions. The crude aldehyde was subsequently treated with *tert*-butoxycarbonylmethylene triphenylphosphorane **172**[59] in a Wittig reaction, giving the crude ester **143** in a 96:4 *E/Z* ratio, which was then purified to >99% (*E*) in 56% yield. Analysis of the ^{1}H NMR spectrum of the product revealed two proton resonances at 6.83 ppm and 5.75 ppm. The location of these, together with their coupling constants and characteristic splitting patterns, were indicative of an α,β-unsaturated ester. Integration of the peaks in the ^{1}H NMR spectrum confirmed the presence of three *tert*-butoxycarbonyl groups—two attached to the nitrogen atom, and the third pertaining to the *tert*-butyl ester. Both the ^{1}H and the ^{13}C NMR spectra were consistent with the data published in the literature for this compound, which

had been previously synthesised via an alternative method.[40]
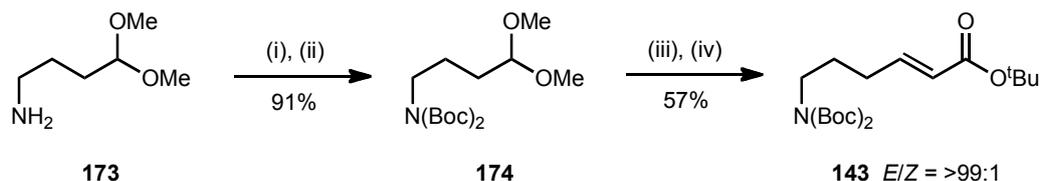
Scheme 26 Reagents and conditions: (i) Boc$_2$O, DCM, 1 hr; (ii) ^{n}BuLi, 0°C, then Boc$_2$O, 1 hr; (iii) AcOH-H$_2$O (2:1), then **172**, DCM.

2.3.2.2   Synthesis of the silapiperidine protected α,β-unsaturated ester

Preparation of the cyclic silapiperidine moiety required diphenyl(divinyl)silane (**176**), which was prepared in 93% yield from the corresponding dichlorosilane via Grignard reaction.[60] The bis-ethoxy silane **177** was then prepared using a hydroboration-oxidation strategy, with the bulky hydroborating reagent 9-BBN used to ensure the reaction was regiospecific. In this case the reaction was quite sluggish, returning a yield of only 56%, in contrast to the reported yield of 88%. Evans had previously found the use of ultrasound in the hydroboration reaction beneficial,[61] and thus application of ultrasound to this reaction followed by oxidation at reflux furnished the diol in 78% yield (*Scheme 27*). Only one regioisomer was observed, with both the ^{1}H and the ^{13}C NMR data consistent with that in the literature.
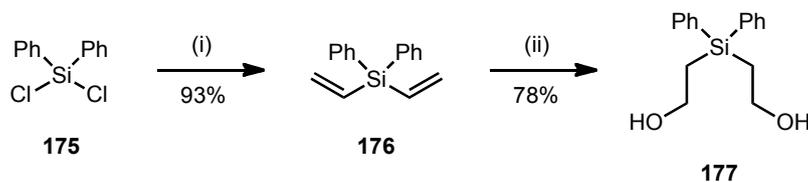
Scheme 27 Reagents and conditions: (i) vinylmagnesium bromide, THF
(ii) 9-BBN, THF, ultrasound, then H$_2$O, 3M NaOH, 50% H$_2$O$_2$.

In the literature examples cited, the diol is tosylated in order to effect cyclisation with the primary amine. After following this route for several attempts and averaging only around 46% yield of the protected amine, it was abandoned in

favour of iodide as the leaving group. A diiodosilane species was prepared via the commonly used PPh₃/I₂/imidazole methodology,[62] with the triphenylphosphine oxide removed over a plug of silica. The crude product was then subjected to the nucleophilic amino cyclisation with 4-aminobutyraldehyde dimethyl acetal in the presence of K₂CO₃ in a procedure adapted from Igarashi *et al.*,[63] with the silapiperidine **178** isolated in 83% yield over two steps (*Scheme 28*). Finally, the acetal was hydrolysed with 3M HCl and the crude aldehyde treated with the phosphorane ylide to give α,β-unsaturated ester **165** in 96% yield over two steps.
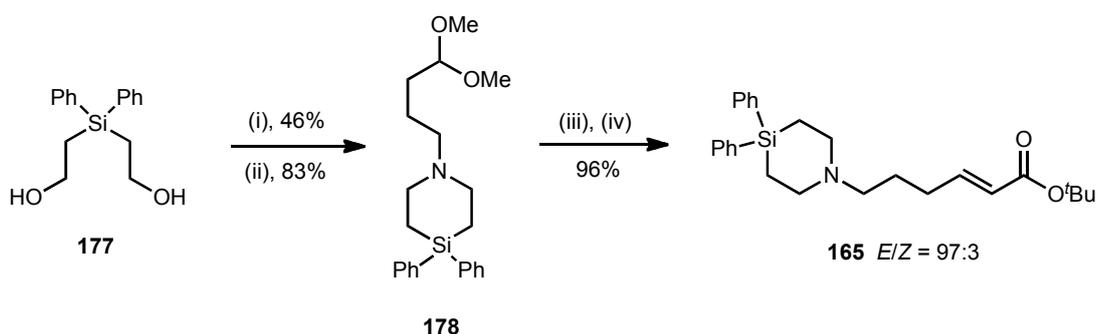
Scheme 28 Reagents and conditions: (i) TsCl, Et₃N, DCM, then **173**, Et₃N, DMF, 45°C, 2 days, then RT, 1 day; (ii) PPh₃, I₂, imidazole, THF, 2hr, then **173**, 1,4-dioxane, K₂CO₃, 95°C, 26 hr; (iii) 3M HCl, THF, reflux, 2.5 hr; (iv) PPh₃=CHCO₂ᵗBu, DCM.

2.3.2.3 Synthesis of the silyl protected α,β-unsaturated esters

Both **166** and **167** were synthesised under identical conditions from TBSO-propanol[64] or the commercially available TBSO-butanol **180** (*Scheme 29*). The alcohols were subjected to Swern oxidation,[65] cleanly producing aldehydes which were used without further purification. Subsequent Wittig reaction with **172** produced the two unsaturated esters in excellent overall yield and (*E*) selectivity.

179 *n* = 1
180 *n* = 2

166 84% *E/Z* = 98:2
167 91% *E/Z* = 98:2
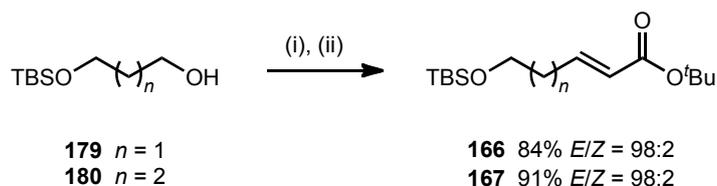
Scheme 29 Reagents and conditions: (i) (COCl)₂, DMSO, -78°C,
then Et₃N; (ii) **172**, DCM.

2.3.3   α-Fluoro-β-amino esters via tandem conjugate addition-fluorination

With the appropriately protected Michael acceptors in hand, attention was now turned to the conjugate addition-fluorination reactions in an attempt to synthesise the desired α-fluoro-β-amino esters (*Scheme 30*). Initially the method was applied to *tert*-butyl crotonate (R = Me) to gauge the performance of simple aliphatic substrates, as compared to the aryl-substituted *tert*-butyl cinnamate.

159 R = Ph
181 R = Me
182 R = TBSO(CH₂)₃
183 R = TBSO(CH₂)₂
184 R = Boc₂N(CH₂)₃
185 R = Ph

157 R = Ph
186 R = Me
187 R = TBSO(CH₂)₃
188 R = TBSO(CH₂)₂
189 R = Boc₂N(CH₂)₃
190 R = Ph
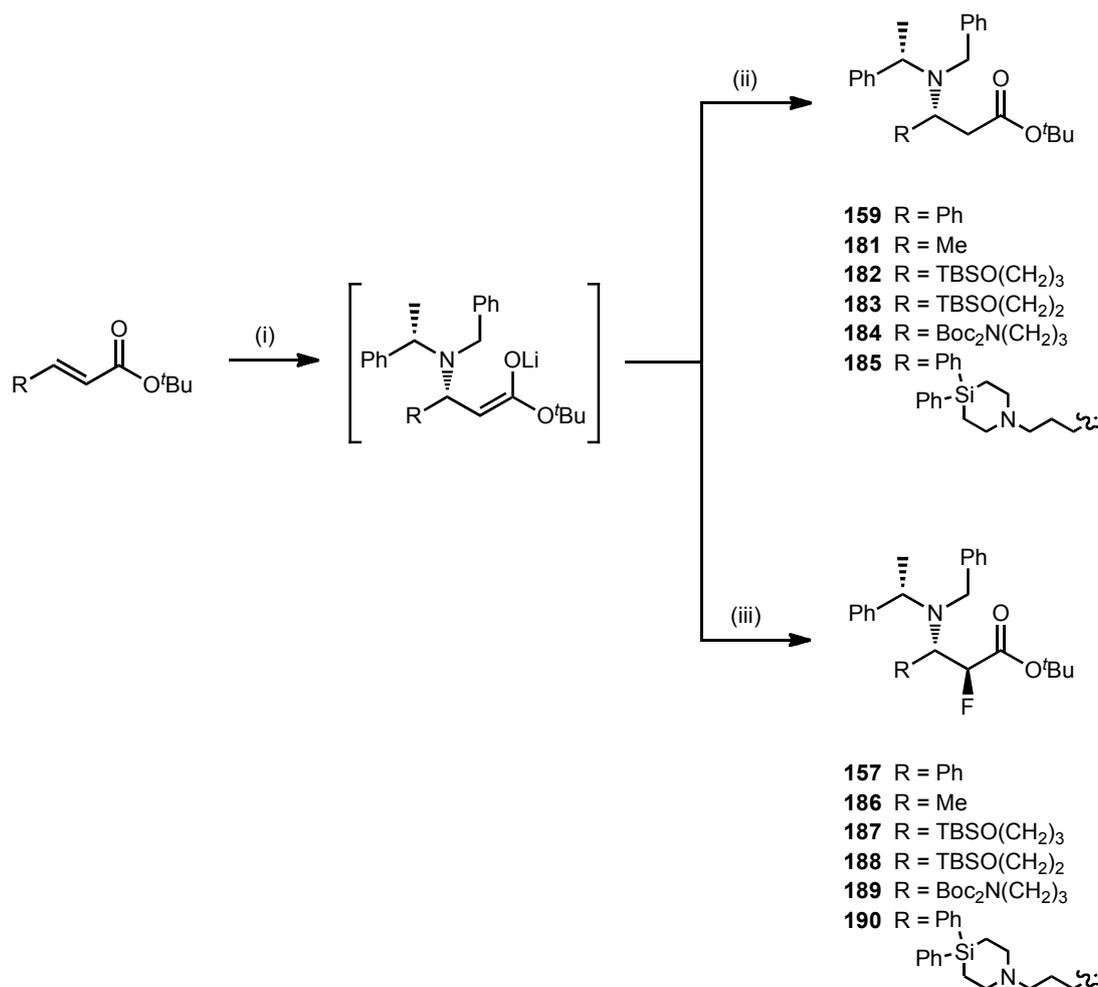
Scheme 30 Reagents and conditions: (i) lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide (1.5 eq.), THF, -78°C; (ii) NH₄Cl; (iii) NFSI (1.5 eq.).

Conjugate addition of lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide to *tert*-butyl crotonate has previously given the β-amino ester **181** in 95% yield and >99% de,[66] a result that was replicated in this case, with **181** obtained in 96% yield with a dr of >99:1. Adapting the method for the conjugate addition-fluorination protocol, a THF

solution of *tert*-butyl crotonate was added to the lithium amide at -78°C and the solution allowed to stir for 1.5 hr before the addition of 1.5 equivalents of NFSI in THF. Stirring was maintained at -78°C for 1 hr, then the mixture was slowly warmed to -30°C over a further 1.5 hr, during which time a white sulfonimide salt precipitated out of solution. ^{1}H NMR analysis of the crude product after work-up revealed a 92:8 ratio of diastereomers. These could not be separated using column chromatography, however the epimeric mixture could be recrystallised to give **186** in 56% yield and >99:1 dr. The major isomer isolated from this experiment was assigned the *anti* configuration based on two factors: the preference for the formation of the *anti* isomer when other electrophiles are used (sections 2.1.1.1 and 2.1.1.2), and the absolute configuration of the major product formed when this experiment had been performed previously with ethyl crotonate, which was assigned from the crystal structure.[32] Supportive evidence for this assignment came from ^{1}H NMR spectroscopy: the $^2J_{\text{H-F}}$ coupling constant of the major isomer was 50.4 Hz, with a small H-F vicinal coupling of 2.0 Hz, resonating at 4.65 ppm. The corresponding peak for the minor isomer was shifted upfield slightly to 4.59 ppm, and possessed a $^2J_{\text{H-F}}$ value of 49.2 Hz and a $^3J_{\text{H-H}}$ value of 5.6 Hz—slightly larger than that of the major isomer. This upfield shift of the minor isomer correlated with the upfield shift observed for the minor isomer of the ethyl ester conjugate adduct, as did the smaller $^2J_{\text{H-F}}$ coupling constant (49 Hz vs. 50 Hz for the major isomer).

One of the by-products of this conjugate addition reaction was benzaldehyde, which was believed to occur as a result of oxidative cleavage of either the amide or addition product's unsubstituted benzyl group. During later attempts to synthesise the difluoro crotonate adduct, the presence of benzaldehyde was also noted after stirring the monofluoro compound with NFSI in the presence of base overnight, indicating it was formed as a result of product—not lithium amide—decomposition. Also, delays in purifying the crude compound after work-up, where the product was left sitting with NFSI and/or other sulfonimide by-products, tended to result in a noticeable benzaldehyde odour. Given that conditions should be close to neutral, this suggests that either NFSI or its protonated by-product is responsible for this oxidation, although the mechanism is unknown. In contrast to results obtained with the other Michael acceptors, benzaldehyde formation during addition to the crotonate was quite substantial, and contributed to the low yield of

addition product. Whether this has anything to do with the presence of a β-methyl group instead of the methylene unit of longer side chains is also unknown.

Conjugate addition of the lithium amide to the TBSO protected ester **167** at -78°C followed by protonation with NH$_4$Cl again occurred with very high stereofacial selectivity, giving the non-fluoro adduct **182** in 81% isolated yield and >99:1 dr, as determined by ^{1}H NMR spectroscopic analysis of the crude material. Quenching the enolate with NFSI at -78°C resulted in a 94:6 mixture of C2 epimers. Crystallisation of the epimeric mixture could not be induced, but fortunately the two diastereomers could be partially separated with column chromatography, giving the **187** in 77% isolated yield with a dr of 98:2. The major product was assigned the *anti* conformation based on correlations between the magnitude of the $^2J_{H-F}$ and $^3J_{H-H}$ couplings and those of the crotonate adducts. These values were 50.0 and 1.0 Hz, respectively, for the major isomer, and 49.0 and 4.6 Hz for the minor isomer, values analogous to those of both the ethyl and *tert*-butyl crotonate diastereomers. There was one major difference however—while the α-proton of the minor isomers of the ethyl and *tert*-butyl crotonate adducts both resonated slightly upfield from the major isomer, the corresponding peak for the minor isomer of **187** appeared further downfield at 4.73 ppm, compared to that of the major isomer at 4.26 ppm. This chemical shift pattern was later found to occur with each of the fluorinated conjugate adducts featuring lengthy side-chains (i.e., **187** to **190**, *Table 1*).

Table 1 Coupling constants and chemical shifts for the α-proton of the fluorinated conjugate adducts.

product	Major Isomer			Minor Isomer		
	$^2J_{H-F}$ (Hz)	$^3J_{H-H}$ (Hz)	δ (ppm)	$^2J_{H-F}$ (Hz)	$^3J_{H-H}$ (Hz)	δ (ppm)
186	50.4	2.0	4.65	49.2	5.6	4.59
187	50.0	1.0	4.45	49.0	4.6	4.73
188	50.4	0.8	4.26	48.4	4.0	4.83
189	50.0	1.0	4.36	48.8	4.4	4.72
190	50.4	1.0	4.50	49.2	5.2	4.74

Synthesis of the unfluorinated adduct **183** under the standard conditions was observed to occur in a highly stereoselective manner, with a crude dr of >99:1 determined by ^{1}H NMR analysis. The reaction was also extremely clean, with the 94% yield obtained demonstrating the high stability of the TBSO group to the basic conditions of the conjugate addition. When the conjugate addition-fluorination was conducted with the unsaturated ester **166** at -78°C, a 69:31 *anti*:*syn* ratio was obtained, with the poor dr responsible for a low 41% isolated yield of **188**. The experiment was repeated but this time the mixture was warmed to -50°C before NFSI addition. The dr was increased to 80:20 in favour of the *anti* isomer, which is slightly better but not ideal for a stereoselective reaction. The combined yield of isomers after chromatography was an impressive 90%, which compensates somewhat for the lack of diastereoselectivity. The mixture was then fractionally crystallised to obtain **188** as a single isomer in 62% isolated yield. As with amino ester **187**, the major isomer $^2J_{H\text{-}F}$ and $^3J_{H\text{-}H}$ coupling constants of 50.4 and 0.8 Hz, respectively, were larger than those of the minor isomer (48.4 and 4.0 Hz). In addition, the α-proton of the major isomer resonated further upfield at 4.26 ppm relative to that of the minor isomer, which resonated at 4.83 ppm. Together, this data lead to the major product being assigned the *anti* configuration. To further confirm the stereochemistry of the product, and by analogy each of the other fluorinated conjugate adducts, X-ray crystallographic analysis was performed on a
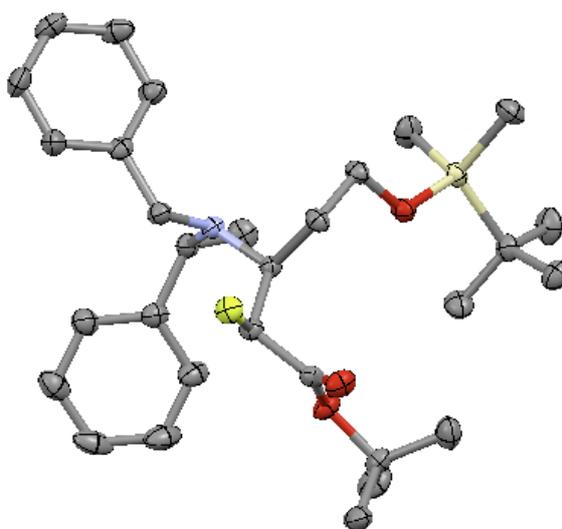
Figure 5 Crystal structure of amino ester **188** showing the
anti relationship between the C2 and C3 stereocentres.

single crystal, with the result displayed in *Figure 5*. Knowing the stereochemistry of the (*S*)-α-methyl group, the configuration of the product was assigned as (2*S*,3*S*). The α- and β-substituents were observed to exist in an *anti* relationship, which supports our stereochemical assignments based on ^{1}H NMR spectroscopic analysis and literature precedent.

^{1}H NMR analysis of the crude reaction mixture for this particular experiment also revealed the presence of a third isomer at 4.84 ppm, with $^2J_{\text{H-F}}$ and $^3J_{\text{H-H}}$ coupling constants of 48.4 and 1.6 Hz, respectively. This is likely to be β-amino ester **192** (*Scheme 31*), which would result from a failure of the dibenzylamine to undergo the conjugate addition in a completely stereoselective manner; a very minor amount of the 3*R*,αS enolate would be formed, which would presumably be fluorinated *anti* to the dibenzylamine, giving **192**. Amino ester **193** would be formed in such small quantities as to be unobservable. However, making up less than 1% of the product mixture, diastereomer **192** was unable to be isolated in sufficient quantities for further stereochemical analysis. In fact, this isomer often failed to be seen in subsequent conjugate addition-fluorination experiments with this substrate, indicating that strict monitoring of the reaction conditions during the conjugate addition step can prevent its formation.

Given the moderate results obtained in the tandem reaction with **166**, the stepwise method was revisited as a possible alternative. Despite the poor facial selectivity obtained with *tert*-butyl cinnamate, the stereoselectivity of these reactions is unpredictable for any given substrate. In a reaction analogous to that of the model stepwise addition, the unfluorinated conjugate adduct **183** was deprotonated with LDA, but the reaction was warmed to -55°C before addition of NFSI. After 3 hr it was allowed to warm to 0°C over approximately 1 hr, then quenched. ^{1}H NMR analysis of the crude reaction mixture revealed a dr of 69:31, with chromatography over a silica plug yielding 93% of the epimeric mixture, recrystallised to obtain the desired *anti* isomer in 59% yield (*Scheme 32*). Repeating the same reaction with NFSI at -78°C only increased the dr to 72:28, proving the tandem method was still preferable to the stepwise method.
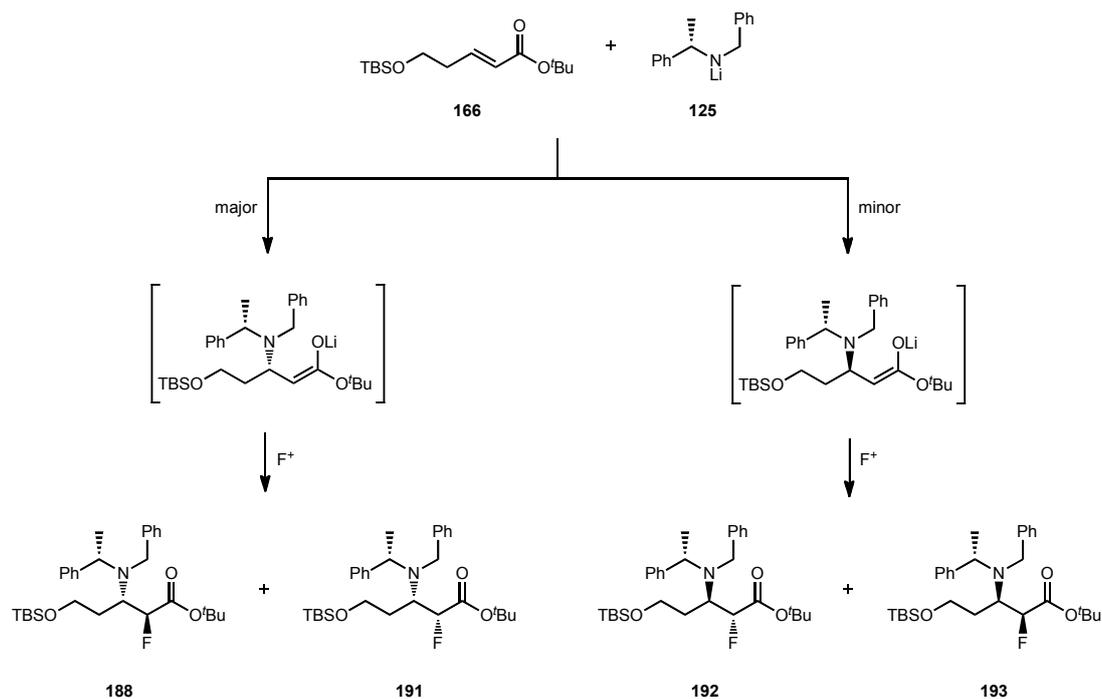
Scheme 31 The observed ratio of the four possible products of the conjugate addition-fluorination reaction when conducted with the **166**.

at -55°C: dr = 69:31
at -78°C: dr = 72:28
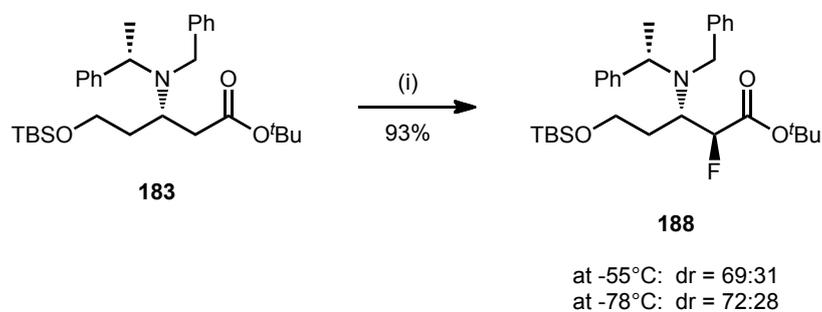
Scheme 32 Reagents and conditions: (i) LDA, THF, -78°C, 1 hr, then NFSI.

When the standard reaction conditions were applied to the di-Boc acceptor **143** disappointing results were obtained when quenching with both NH₄Cl and NFSI. The unfluorinated adduct **184** was obtained in only 42% yield (>99:1 dr) due to a combination of unreacted starting material, decomposition of the carbamate and γ-deprotonation to form the deconjugated alkene, a result also reported by Davies.[40] A slightly improved yield of 48% was achieved when quenching the enolate with NFSI, however the dr was only 88:12. In addition, purification of this experiment

often involved laborious chromatography to obtain the product free of impurities, and the two isomers were also difficult to separate. The major isomer was again assigned as the *anti* product based on similarities between the α-proton chemical shift (4.36 ppm) and coupling constants ($^2J_{H-F}$ = 50.0 Hz; $^3J_{H-H}$ = 1.0 Hz) of this substrate and those of amino ester **188** (**Error! Reference source not found.**). The low yields and difficult purification of the product reflect the di-Boc substrate's poor suitability as a Michael acceptor under these reaction conditions, and thus it was abandoned for further development.

The last acceptor to be examined was the silapiperidine, **165**. The reaction was initially quenched with NH₄Cl to determine the facial selectivity of the lithium amide in the conjugate addition step, and was found to be >99:1 dr by ^{1}H NMR spectroscopic analysis of the crude reaction mixture. The γ-deprotonation that seemed to plague the di-Boc acceptor was absent, although it was found that prolonged reaction times and slight warming of the reaction mixture (to approx. -60°C) were necessary to obtain complete reaction of the starting material. This is presumably due to the steric bulk of the side-chain. Also, purification of the adduct proved troublesome; contrary to the fact that the silapiperidine nitrogen is tri-alkylated, the adduct behaved more like a secondary amine, streaking on the silica during TLC analysis. While this problem could be overcome through the addition of triethylamine to the eluant, the product unfortunately had the same R*f* as the excess dibenzylamine in all of the solvent systems examined, as well as when using alumina as the chromatographic medium. While this excess starting material can usually be extracted during aqueous work-up by washing with 10% citric acid,[25] this tended to affect the yield of product, with possible protonation of the silapiperidine nitrogen also occurring. Dibenzylamine removal was also attempted via Kugelrohr distillation, however this resulted in significant decomposition of the silapiperidine adduct **185**. Therefore, after work-up the crude product was passed through a silica plug to remove the polar silapiperidine by-products, and the adduct and dibenzylamine mixture were then stirred with phenyl isocyanate (1.1 eq. of excess amine) in dry DCM. This converted the secondary amine to a urea (**195**) that was more polar than the conjugate adduct and could thus be separated chromatographically. **185** was eventually obtained in 87% yield (*Scheme 33*).
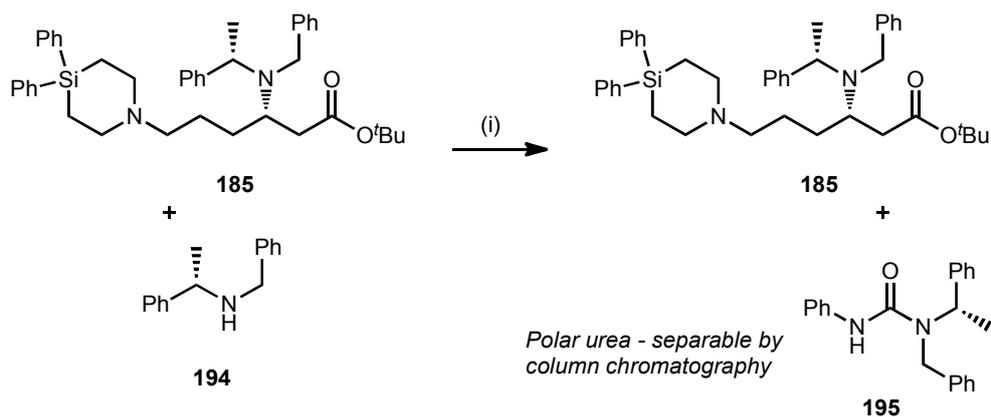
Scheme 33 Reagents and conditions (i) Phenyl isocyanate, DCM.

When fluorinating the lithium enolate at -78°C, the initial reaction afforded a disappointing 77:23 dr. With coupling constants of $^2J_{\text{H-F}}$ = 50.4 Hz and $^3J_{\text{H-H}}$ = 1.0 Hz and a chemical shift of 4.50 ppm, the major product was again identified as the *anti* isomer (**190**). The data for the minor isomer supports this assertion, closely matching the values obtained for the minor isomers of the previous conjugate addition-fluorination reactions (**Error! Reference source not found.**). The relative coupling constants of the major and minor diastereomers within the series of adducts showed a high level of congruence: the $^2J_{\text{H-F}}$ coupling constant was consistently larger for the major isomer, while the $^3J_{\text{H-H}}$ coupling constant was consistently smaller—at ≤1 Hz in most cases, it was virtually non-existent.

In addition to the poor dr, a mixture of both fluorinated (**190**) and unfluorinated (**185**) addition products were commonly present, which were virtually impossible to separate with column chromatography (crystallisation was attempted and ultimately unsuccessful). Raising the temperature to -50°C resulted in almost complete reaction of the unsaturated ester, but it also had another surprising benefit—the dr was increased to >99:1. Given the fact that the reaction is presumably under kinetic control (page 44), with higher temperatures not expected to play a role in selective formation of the *anti* product, this greater selectivity is most likely attributable to a change in the overall conformation of the lithium-chelated enolate. The bulky dibenzylamine must be blocking one face of the enolate more effectively, resulting not only in a higher dr, but also in complete fluorination of the enolate, with the sulfonimide provided easier access to the

substrate. Though this temperature increase did result in only one isomer being formed, it was not without its drawbacks; yields were somewhat lower due to decomposition of the product. The rate of decomposition is presumably greater at higher temperatures, and yields typically averaged around 50%. This is not a particularly acceptable result given the length of the synthetic route to unsaturated ester **165**, despite the excellent selectivity. In an attempt to gauge the level of decomposition, a THF solution of **190** was stirred with NFSI at -50°C for 2.5 hours, then slowly allowed to warm to -10°C over a further 1.5 hours. The transformation of the colourless solution into one that was dark yellow was a preliminary indicator of decomposition, which was later confirmed upon work-up and purification of the product, with only 41% of **190** recovered. The ^{1}H NMR spectrum of the crude mixture also displayed a number of other unidentifiable decomposition products.

The moderate yield was not the only problem, however: deprotection using Kim and Cho's method of TBAF/CsF (1:1) in DMF at RT[52] returned only starting material, despite published yields of 80-90%. More forcing conditions (65°C, 24 hr) also failed to effect cleavage of the DPSide group, as well as microwave irradiation using various temperatures, pressures and solvents. Most experiments returned either starting material or a complex mixture of unidentified polar products that were difficult to isolate with chromatography. With no effective method of deprotection, this acceptor was obviously unsuitable for the future synthesis of our fluorinated β³-lysine and β³-arginine derivatives.

Entry	R	β-amino ester yield (%)[a]	α-fluoro-β-amino ester			
			temp (°C)[b]	crude dr[c]	yield (%)[d]	dr[e]
1	Ph	96[f]	-78	80:20	65[g]	100:0
2	Me	96	-78	92:8	56	100:0
3	TBSO~~~	81	-78	94:6	77	98:2
4	TBSO~~	94	-50	80:20	62	100:0
5	Boc₂N~~~	42	-60	88:12	48	93:7
6	Ph-Si(Ph)~N~~~	87	-50	>99:1	51	100:0

[a] crude dr >99:1 (^{1}H NMR); [b] at NFSI addition; [c] *anti*:*syn* (^{1}H NMR); [d] isolated yield; [e] after purification (^{1}H NMR)
[f] ref. **27**, [g] ref. **31**

Figure 6 Summary of the yields and selectivities obtained during the synthesis of unfluorinated and α-fluoro-β-amino esters via conjugate addition.

2.3.3.1 Further synthetic manipulation of conjugate adducts

Given that both mono and difluoro analogues of our β³-amino esters were desired, it was envisaged that the α-fluoro conjugate adducts could simply be deprotonated then fluorinated again to give the α,α-difluoro compounds (*Scheme 34*). In practice this was a lot more difficult than anticipated: the sterically undemanding base NaH largely failed to deprotonate the crotonate adduct, despite the fact that a number of different batches of NaH were tried. This is evidenced by the fact that ¹H NMR analysis of the crude reaction material revealed that the *anti* isomer predominated, despite the fact that a 1:1 epimeric mixture should result after quenching the reaction with NH₄Cl. To determine whether base strength was an issue, deprotonation with the stronger base LDA was also attempted. This was relatively successful, allowing the difluoro adduct **196** to be obtained in 55% yield. Results were not consistent, however, and this base also often returned a mixture of starting material and difluoro product. The steric bulk of the sulfonimide reagent may contribute to the low yields, and in light of the continually poor results obtained via this method, an alternative route to the desired α,α-difluoro-β³-amino esters was pursued (as discussed in Chapter 4).
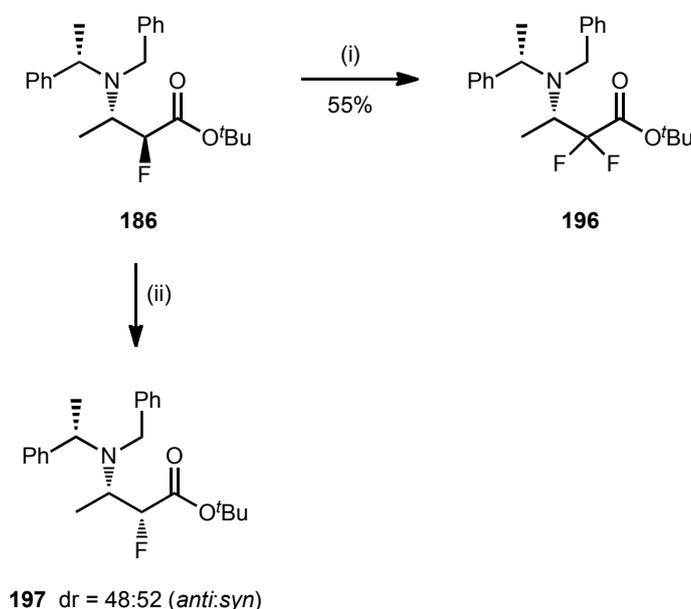
Scheme 34 Reagents and conditions: (i) LDA, THF, -78 to 0°C, 2 hr, then NFSI, -60°C to RT overnight; (ii) LDA, THF, 0°C, then 2,6-di-*tert*-butylphenol, -78°C, 1.5 hr.

As well as synthesising the *anti* α-fluoro-β³-amino esters mentioned in the previous section, access to the *syn* diastereomer was also sought, which would involve epimerisation at C2. A similar process was undertaken by Davies who, upon synthesising a series of cyclic *syn* α-alkyl-β-amino esters via quenching of an α-substituted-α,β-unsaturated enolate with 2,6-di-*tert*-butylphenol, successfully converted the products to the corresponding *anti* isomer via base-catalysed epimerisation with KHMDS in *t*BuOH.[67] After six days, almost complete conversion to the *anti* isomer was observed, suggesting that this is the most thermodynamically favourable product. Following this method, **186** was stirred with LiHMDS in a solution of *t*BuOH/THF for six days. Upon work-up and ¹H NMR analysis of the crude mixture, a dr of 80:20 in favour of the *anti* isomer was observed. Furthermore, only 72% of the product was recovered after chromatographic purification, indicating a general failing of the epimerisation experiment. This result suggests that, as with Davies' cyclic adducts, the *anti* isomer is slightly more stable than the *syn* isomer. A different approach was then taken in an attempt to prepare the *syn* isomer: 2,6-di-*tert*-butylphenol would be used as a bulky proton source instead of the much smaller *t*BuOH in the hope that the α-methylbenzylamine moiety would exert enough stereocontrol to result in protonation from the opposite face. Deprotonation of **186** was achieved with LDA at 0°C, before cooling the reaction down to -78°C and adding the phenol. Work-up and ¹H NMR analysis of the crude material revealed a 48:52 mixture in favour of the desired *syn* isomer. This result mirrors the lack of stereocontrol present in the stepwise deprotonation-fluorination of cinnamate adduct **159** (R = Ph) in the model system (*Scheme 21*), and suggests that the chiral α-methylbenzyl group plays a minor role in directing selective protonation at C2.

2.4 Conclusions

Of the three conjugate addition methods used for the synthesis of α-substituted-β-amino esters first employed by Davies (tandem, stepwise and the alternative tandem method involving α-substituted alkenes), the tandem addition to α,β-unsaturated esters was clearly the most favourable, and the most practical, in synthesising the targeted α-fluoro-β³-amino esters. While the stepwise method did lead to the α-fluoro substrates in good yield, the selectivity of the fluorination was poor. In contrast, the attempted conjugate addition to the α-fluoro-α,β-unsaturated ester failed completely due to a competing dehydrofluorination reaction. However, in being ruled out of the synthetic route, it did prevent the time-consuming need to prepare the series of substituted α-fluoro-α,β-unsaturated esters that would be required for the conjugate addition reactions.

Application of the tandem conjugate addition-fluorination method to nitrogen-functionalised acceptors was generally met with poor results, which is particularly disappointing in the case of the Boc acceptor, which would have provided a short route to the fluorinated β³-lysine analogue. On the other hand, the silyl ether based Michael acceptors **166** and **167** produced generally favourable results, and despite the low dr obtained for the fluorinated adduct **188** (resulting in only a moderate yield), both **187** and **188** could be synthesised efficiently on a large scale. Together with the relative ease of TBSO deprotection, this enables their use as core substrates for the synthesis of our α-fluorinated β³-lysine and β³-arginine analogues, which is described in Chapter 3.

Unfortunately these conjugate adducts failed to provide a reliable, high-yielding route to either the *syn* diastereomers or the α,α-difluoro substrates. While the latter can be obtained via an alternative synthetic method, as discussed in Chapter 4, the problem of obtaining the *syn* isomers selectively remains unsolved. At present, the only way to obtain these via the fluorinated conjugate adducts is through the resolution of racemic mixtures, either by fractional crystallisation (where possible) or exhaustive chromatography. This is obviously undesirable in terms of overall yield, particularly if the *syn* isomer is sought exclusively, however it is a route that remains optional in the absence of any other suitable alternatives.

2.5   References

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CHAPTER 3:

PREPARATION OF α-FLUORO β^3-LYSINE AND β^3-ARGININE DERIVATIVES

3.1   Introduction

As discussed in Chapter 1, the synthesis of peptides containing unnatural fragments is essential in the study of physiological processes and the development of novel bioactive compounds. α-Fluoro-β³-amino acids represent one example of these unnatural residues, and the conjugate addition-fluorination methodology discussed in Chapter 2 provides access to a variety of α-fluoro-β³-amino esters that can be further modified to allow their incorporation into either α- or β-peptides. The peptides used in such biological studies are often lengthy, making traditional solution phase chemistry involving laborious isolation and purification steps highly inefficient; the growing peptide's insolubility increases the problem exponentially. Taking into account the vast number of steps required to synthesise such lengthy peptides, it is easy to understand how the overall yield can fall below 1%. Huge advances in this area were made with the introduction of solid supports, pioneered in 1963 by Robert Merrifield and his polystyrene resin,[1] which led to the technique now commonly known as solid-phase peptide synthesis (SPPS). This involves tethering the *C*-terminus of an *N*α-protected amino acid to an insoluble polymeric resin, enabling simple filtration of the growing peptide after each coupling step, while the by-products and excess reagents are simply washed away. This technique is now used almost universally in peptide and combinatorial synthesis, and with the advent of automated systems, allows extensive peptide libraries to be developed.

The general process is represented in *Figure 1*, with initial attachment of a single amino acid at the *C*-terminus to one of a number of solid supports available. These supports feature a reactive functional group that serves as a covalent linker between the resin and the growing peptide chain, and are often chosen based on the desired terminal functionality on the peptide after cleavage. For example the Wang resin[2] allows the peptide to be released as a carboxylic acid, while the Rink resin[3] releases the peptide as a carboxamide (*Figure 2*). The particular conditions used during the peptide synthesis i.e., acidic or basic/nucleophilic, also dictate the choice of linker as this covalent bond must be stable throughout the synthesis but be easily cleaved in the final step.
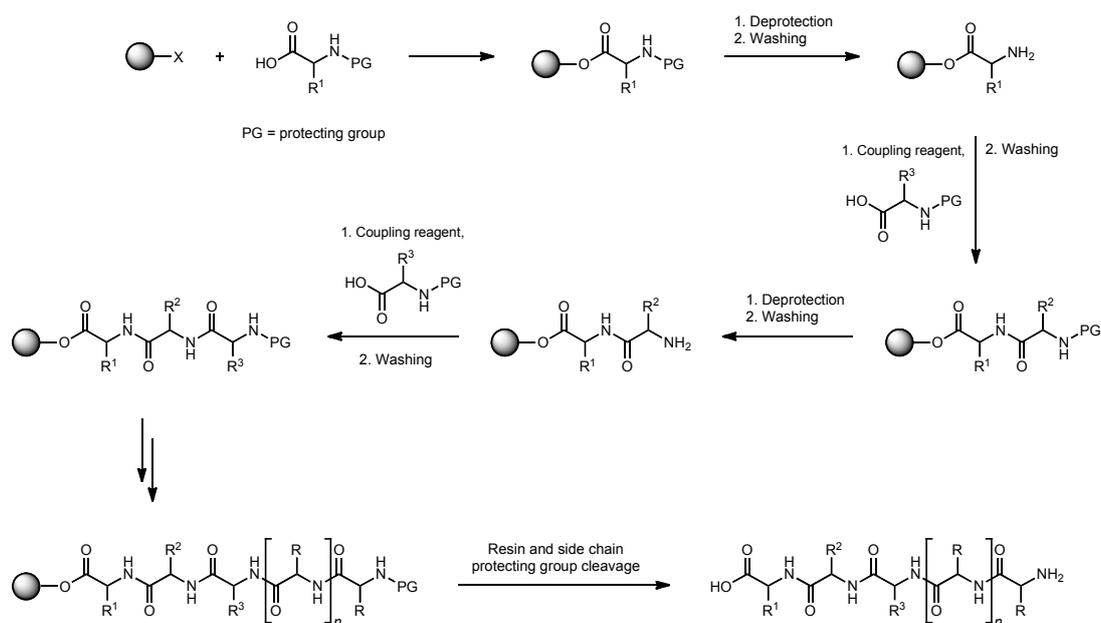
Figure 1 Schematic representation of solid-phase peptide synthesis (SPPS).
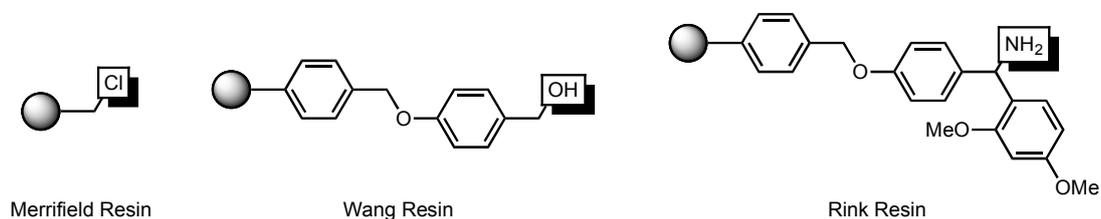
Figure 2 Solid supports commonly used in SPSS.

The next step in the process involves cleavage of the N^α-protecting group to unmask the free amine, with the resin then washed free of reagents and by-products resulting from the deprotection. With the α-amino group now exposed, it is treated with another N^α-protected amino acid, however due to the poor reactivity of the carboxylic acid functional group, a coupling reagent (e.g., DCC, HOBt, BOP) is also required. These reagents facilitate amide bond formation by reacting with the carboxylic acid, providing an activated species that is highly susceptible towards nucleophilic attack by the amine, and also acts as an excellent leaving group. The reagents and conditions have also been tuned to minimize racemisation at the

α-carbon. The resin is again filtered and washed free, and the cycle of N^{α}-deprotection/amino acid coupling is repeated until the desired peptide sequence is produced, at which point the peptide is cleaved from the resin. The reagent used to achieve this depends on the protection strategy used, and often accomplishes simultaneous side chain deprotection of the residues (where acid-labile protecting groups have been used). As the process has developed over time to include the use of standardised reagents and a large excess of the amino acid to be coupled, yields of ≥99% are commonly seen for each step, making the overall process highly efficient.

3.1.1   Protecting groups in peptide synthesis

Many amino acids possess a reactive side chain that needs to be protected in order to prevent unwanted reactions from occurring. Examples of such amino acids are lysine and arginine, derivatives of which are the main targets of this research into the preparation of α-fluoro-β³-amino acids. Before these derivatives can be incorporated into a peptide, their side chain functionalities must be suitably protected. The use of orthogonal deprotection—where a selected protecting group is removed independently of all others, is perhaps the most crucial factor in the successful synthesis of a peptide. These protecting groups can largely be divided into classes based on the conditions required for their removal; ideally, protecting groups from at least three different classes are required to enable a successful orthogonal synthesis. For example, the simplest and most commonly employed strategy would be to include one protecting group sensitive to base, another sensitive to acid, and the third cleavable under neutral conditions, e.g., hydrogenolysis.

As the α-amino functionality must be deprotected many times over the course of a peptide synthesis, the protecting group used here must be very easily and selectively removed in high yield. Reagents that result in carbamate formation are the most popular for a number of reasons: the carbamate's high lability, due in part to irreversible CO_2 liberation upon cleavage, their low electron-withdrawing properties, which help prevent racemisation at the α-carbon,[4] and finally their resistance to commonly used coupling conditions.

There are two main protecting group strategies employed in SPPS, both based on the use of carbamates: the Boc/Bn system developed by Merrifield, which employs acid-labile N^α-Boc-protection with side chain benzyl (Bn) protection, and the Fmoc/^{t}Bu system introduced by Carpino in 1972,[5] which involves masking the α-amino functionality with the base-labile 9-fluorenylmethoxycarbonyl (Fmoc) protecting group. The acid-labile *tert*-butyl group is then used as protection for the side chains. The Fmoc system is generally preferred as it uses TFA for resin cleavage, as opposed to the extremely hazardous HF used in the Boc system, which requires special equipment for handling.

Benzyloxycarbonyl (Cbz or Z), while not ideal for use in SPPS because of its moderate acid-lability and the fact that the solid Pd catalysts needed for its hydrogenolysis can contaminate the polymeric resins,[6] is another widely used carbamate protecting group, particularly in solution-phase peptide synthesis. Its popularity is due to its high overall stability, and the fact that hydrogenolysis over palladium catalysts is remarkably clean, producing only CO_2 and toluene as by-products. However, this method can prove problematic with peptides containing mercapto amino acids, which poison the catalyst, so acidolysis with HBr/AcOH can be employed instead. The Cbz group can be used for N^α-protection, but is most commonly used for side-chains, though it has been somewhat superseded by the more robust 2-chlorobenzyloxycarbonyl (Cl-Cbz), which can withstand the repeated TFA treatments used to cleave Boc groups in the Boc/Bn system.[7]

A range of other α-amino protecting groups have become available over the years, both acid and base sensitive, or cleaved by other mechanisms, e.g., reduction or photolysis. However, the Boc, Fmoc and Cbz groups still remain some of the most easily accessible and efficient options. New groups are continually being developed to improve compatibility with side chain protection and acid or base susceptibility,[8,9] and over time their use in peptide synthesis is expected to increase significantly.

3.1.1.1 Lysine side chain protection

The first synthetic target of the work discussed in this chapter is an α-fluoro-β³-lysine derivative suitably protected for use in SPSS. Like the N^α-functionality, the primary ε-amino group in lysine's side chain also has the potential to be acylated. This presents the problem of branched or cyclic peptides, making side chain protection essential. Those protecting groups commonly used for N^α-protection are also used for the side chain, although given the higher basicity of the terminal ε-amino group (pKa = 10.7 in aqueous solution[10]), they can be harder to cleave at the end of the synthesis. This limits the types of groups that can be used, but has also resulted in the modification of existing reagents to make them more electron-rich e.g., the trityl group is suitable for N^α-protection, whereas the more acid-labile 4-methyltrityl group is preferred for side-chain protection.[11]

The two most common strategies for lysine protection during peptide synthesis are the Fmoc/Boc and the Boc/Cl-Cbz systems, shown in *Figure 3*. The use of these carbamates helps prevent internal cyclisation of the peptide as a result of nucleophilic attack by the terminal amine on the activated carboxylic acid, while also keeping the acidity of the amide proton relatively low. In the first strategy, base-catalysed Fmoc removal is usually accomplished with a secondary amine, typically 20% piperidine in DMF,[12] and the Boc group of the N^ε-functionality removed at the end of the synthesis with 25-50% TFA in DCM.[13] This combination of protecting groups is ideal in that each one is highly inert to the conditions used to cleave the other. In the second strategy, the α-amino moiety is Boc-protected and

198

N^α-Fmoc-N^ε-Boc-lysine

199

N^α-Boc-N^ε-(Cl-Cbz)-lysine
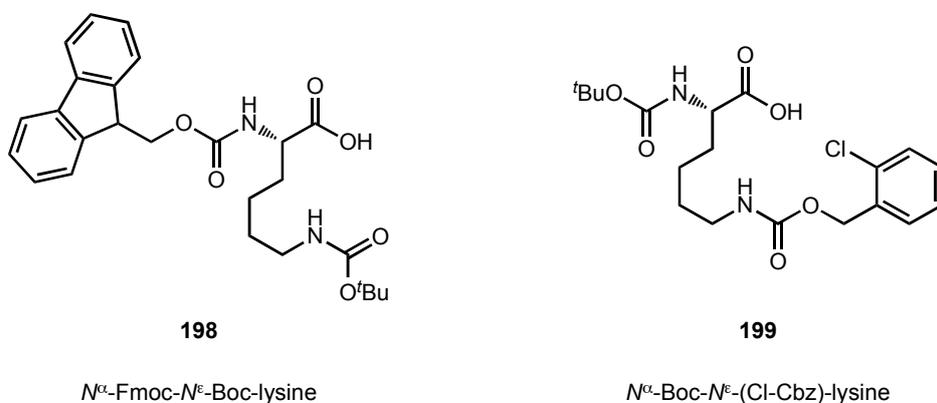
Figure 3 Two common protecting group strategies for lysine.

the 2-chlorobenzyloxycarbonyl group (Cl-Cbz) is used to mask the terminal amine. The Cbz group can also be used, but as mentioned previously it is less stable to TFA than Cl-Cbz. Cl-Cbz is cleaved with either HF or trifluoromethane sulfonic acid (TFMSA),[7] while both Cl-Cbz and Cbz are also removed cleanly via hydrogenolysis.

A number of other alternatives for lysine side chain protection exist, as displayed in *Table 1*, and they can be broadly classified into three categories—acid- or base-labile, or those cleaved under other conditions. Only one other suitable acid-labile group exists (Mtt), but despite this, overall compatibility of the listed lysine protecting groups with the Fmoc strategy is high, even for those in the base-labile category. The trifluoroacetyl (tfa) and 2-(methylsulfonyl)ethoxycarbonyl (Msc) groups can both be cleaved by treatment with weak alkali solutions, which Fmoc is relatively stable to, while the bidentate tetrachlorophthaloyl (TCP)[14] and 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde)[15] groups are able to withstand the piperidine treatment used to cleave Fmoc. The cleavage of these latter two groups is accomplished with ethylenediamine and hydrazinolysis, respectively.

The most useful protecting groups, however, are those that are selectively cleaved by other mechanisms, making them highly compatible with both the Fmoc and Boc strategies. Among them, the allyloxycarbonyl (Alloc) group has found the widest use in peptide chemistry, both for the temporary protection of the α-amino moiety and the semi-permanent protection of the reactive functionalities found in the side chains of various amino acids.[16,17] Alloc removal occurs under essentially neutral conditions with a catalytic amount of $Pd(PPh_3)_4$ in the presence of a nucleophilic allyl group scavenger, typically phenyltrihydrosilane ($PhSiH_3$),[18] which prevents transfer of the newly cleaved allyl group to any other amino functionalities in the peptide. Another benefit is the ability to perform tandem deprotection/acylations, where Alloc cleavage in the presence of suitable reagents, e.g., anhydrides, dicarbonates, activated amino acids etc. allows almost quantitative acylation of the functionality without having to isolate the deprotected intermediate.[19] This is particularly helpful in minimising the intramolecular reactions of the free amine.

Table 1 Protecting groups for the ε-amino moiety of lysine.

ACID-LABILE	BASE-LABILE	OTHER
tert-Butyloxycarbonyl (Boc)	Trifluoroacetyl (tfa)	Allyloxycarbonyl (Alloc)
2-Chlorobenzyloxycarbonyl (Cl-Cbz)	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde)	*p*-Nitrobenzyloxycarbonyl (*p*NZ)
4-Methyltrityl (Mtt)	2-(Methylsulfonyl)ethoxycarbonyl (Msc)	*o*-Nitrobenzyloxycarbonyl (*o*NZ)
	Tetrachlorophthaloyl (TCP)	3-Nitro-2-pyridinesulfenyl (Npys)
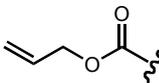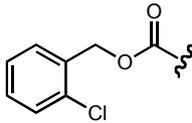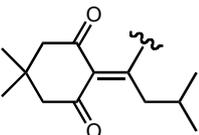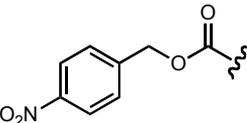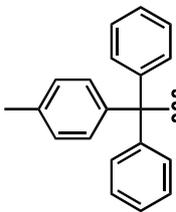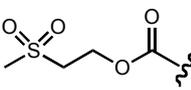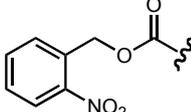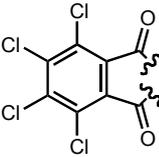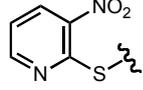
Like Alloc, *p*-nitrobenzyloxycarbonyl (*p*NZ) is orthogonal to most other commonly used amino acid protecting groups, with the nitro substituent conferring a high degree of stability in acidic conditions. Its reductive cleavage with $SnCl_2$ is carried out in near neutral conditions (1.6mM HCl in dioxane).[20] Shifting the nitro substituent into the *ortho* position gives another protecting group, *o*NZ, which displays a totally different reactivity profile - it can be cleaved photolytically at wavelengths of >300 nm in the presence of additives like N_2H_4 or $NH_2OH \cdot HCl$,[21] which leaves all other protecting groups and sensitive amino acid residues untouched. Like these two, the 3-nitro-2-pyridinesulfenyl (Npys) group has also been used in ε-amino lysine protection because of its high orthogonality. While it is easily removed in dilute HCl, it is resistant to TFA. It can also be cleaved with either PPh_3 or 2-mercaptopyridine-*N*-oxide, again under neutral conditions.[22,23]

3.1.1.2 Protection of the arginine guanidino functionality

The second synthetic target of this chapter is based on arginine, an amino acid that possesses a guanidino side chain. This functional group presents a particular problem in peptide synthesis because of its high basicity—with a pK_a of 12.5,[24] the guanidino moiety is protonated even in neutral conditions, and certainly under most conditions used in peptide synthesis. Sometimes this protonation alone is enough to render the guanidine functional group less nucleophilic and prevent side reactions, however this is a very unreliable method of protection, and often leads to one of two undesirable reactions: lactamisation, where an intramolecular peptide bond is formed as a result of attack by the δ-nitrogen (*Figure 4*), or deguanidination (*Figure 5*), where the guanidine moiety is acylated with an activated amino acid and then lost under basic conditions in an intramolecular reaction, transforming the arginine residue into ornithine.[25]
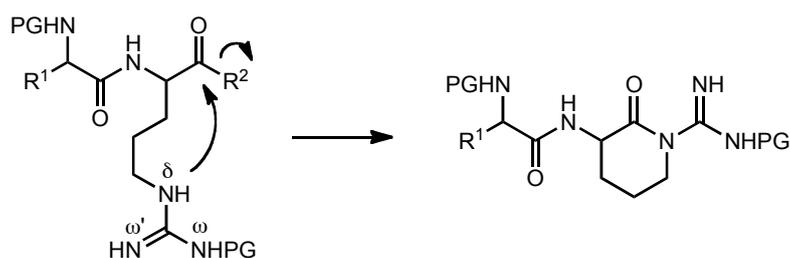
Figure 4 δ-Lactamisation of an arginine residue .
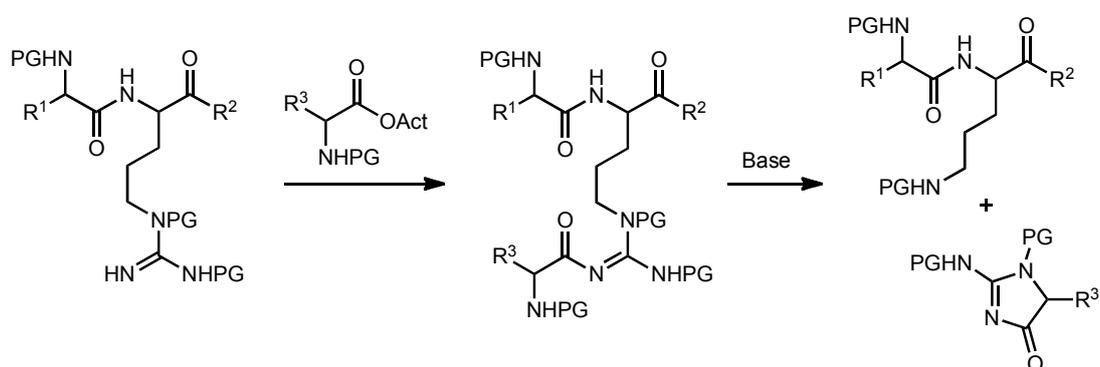
Figure 5 Deguanidination of an arginine residue during peptide synthesis.

Ideally, protection of all three of the guanidino nitrogens is required to completely suppress these types of reactions, but in practical terms this can be quite difficult; mono- or di-protection is more easily achieved and often sufficient, especially with bulky groups. No arginine protecting group developed thus far is perfect for use in peptide synthesis, with problems mainly associated with their cleavage, which generally requires either strongly acidic conditions or prolonged reaction times. This ensures that research into new and improved reagents is ongoing. The most common reagents are those based on the benzenesulfonyl structure (*Table 2*), with the simplest—*p*-toluenesulfonyl (tosyl)—still the most widely used in the Boc/Bn strategy, though its removal requires the use of either HF or TFMSA.

Table 2 Protecting groups for the guanidino moiety of arginine.

ACID-LABILE		BASE-LABILE
p-Toluenesulfonyl (Tos)	1,2-Dimethylindole-3-sulfonyl (MIS)	Trifluoroacetyl (tfa)
4-Methoxy-2,3,6-trimethylphenyl-sulfonyl (Mtr)	Nitro (NO₂)	**OTHER**
2,2,7,7,8-Pentamethylchroman-6-sulfonyl (Pmc)	ω,ω'-*bis-tert*-Butyloxycarbonyl (*bis*-Boc)	ω,ω'-*bis*-Benzyloxycarbonyl (Cbz, Z)
2,2,4,6,7-Pentamethyl-2,3-dihydro-benzofuran-5-sulfonyl (Pbf)	5-Dibenzosuberenyl (Suben)	ω,ω'-*bis*-Allyloxycarbonyl (Alloc)
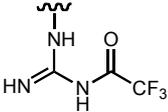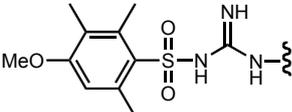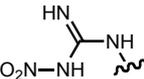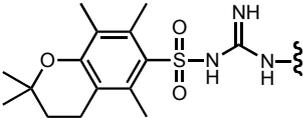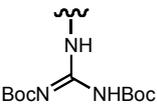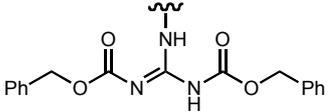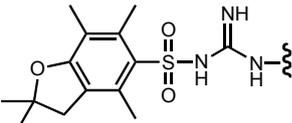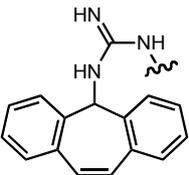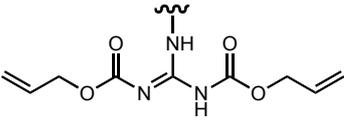
There has been a strategic element to the development of these sulfonyl derivatives: the severity of reagents required to cleave them is highly dependent on the substitution of the aromatic ring e.g., the electron-donating *para*-methoxy substituent in the 4-methoxy-2,3,6-trimethylphenyl-sulfonyl (Mtr) group promotes the formation of the $ArSO_2^+$ cation in acidic conditions, and the extra methyl groups are thought to enhance acid-lability by providing greater interaction between the aromatic π-electrons and the vacant *d*-orbitals of sulfur.[26] The combination of these effects make the Mtr protecting group much more acid-labile than the tosyl group, requiring only TFA and a thioanisole scavenger for removal. Despite this, it is still not acid-labile enough and has generally been superseded by the more electron-rich 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) and 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) groups where the Fmoc strategy is used. These two sulfonyl protecting groups have had their methoxy substituent incorporated into a contiguous ring which fixes the position of the oxygen atom so that a maximum delocalisation of the lone pair electrons into the π-system occurs—the five-membered ether ring is somewhat more electron-donating than the six-membered ring, making the Pbf group slightly more sensitive to TFA cleavage than Pmc.[27] Even then, the Pbf protecting group is still too stable to TFA and lengthy deprotections are often required, which can harm acid-sensitive amino acids.

A very recently developed and promising alternative is the electron-rich 1,2-dimethoxyindole-3-sulfonyl (MIS) group, which appears to be considerably more acid-labile than the Pbf group.[28] In addition, the by-product resulting from its cleavage is stable, unlike those of Pmc and Pbf, which can arylate or sulfonate sensitive amino acid residues.

Other acid-labile guanidino protecting groups do exist but are used much less frequently. ω,ω'-bis-Boc protection, which has a similar acid lability to TFA as Pbf, prevents deguanidination although is not entirely effective at preventing δ-lactamisation.[29] Coupling rates during peptide synthesis are also reported to be lower with di-Boc protected arginine.[29] Another option is ω-nitration, which is one of the earliest forms of arginine protection. It is still used commonly in solution-phase synthesis because of its clean removal using HF and its ability to prevent

both lactamisation and deguanidination. It can also be cleaved under reductive conditions, however the long reaction times required can cause partial hydrogenation of tryptophan or phenylalanine residues.[30] Bulky suberyl and suberenyl derivatives (*Table 2*), introduced in 1997 by Noda and Kiffe,[31] are even more acid-labile than the arylsulfonyl protecting groups, requiring only 25-50% TFA, but their uptake in peptide synthesis has been surprisingly slow.

With regard to base-labile protection, only the trifluoroacetyl group has been effectively used to protect the guanidino functionality of arginine derivatives,[32] although not to a great extent in peptide synthesis. It is orthogonal to Alloc, Cbz, Boc and Fmoc deprotection, although it must be introduced before the N^{α}-Fmoc group, which does not survive the basic conditions used to introduce the guanidino group.[33] For protective groups cleaved by other means, ω,ω'-bis-Cbz protection can be used with the Boc strategy, and occasionally also in the Fmoc system, although it can reportedly require long hydrogenation times for removal.[8] Likewise, ω,ω'-bis-Alloc protection is also used with the Boc strategy, but the basic conditions used for Fmoc cleavage also typically remove one of the Alloc groups,[8] precluding its use as an effective method of protection in this system.

As shown, there is an array of different groups available for the protection of lysine and arginine side chains, each catering to different strategies and types of peptide synthesis. The most suitable group for any given method is dependent on several factors, but namely, ease of removal and orthogonality to other protecting groups— particularly those protecting the α-amino functionality. As no protecting group is perfect, the successful synthesis of a peptide often involves some compromise with respect to efficiency and orthogonality, at least until the time comes that the perfect protecting group does exist!

3.2   Synthetic strategy

As the silyl protected esters **166** and **167** gave the best results in the tandem conjugate addition-fluorination reaction, the corresponding conjugate adducts were chosen as the substrates on which to base the synthesis of the target orthogonally protected amino acids—**187** for the α-fluoro-β³-lysine derivative, and **188**, which features a shorter side chain, for the α-fluoro-β³-arginine derivative.
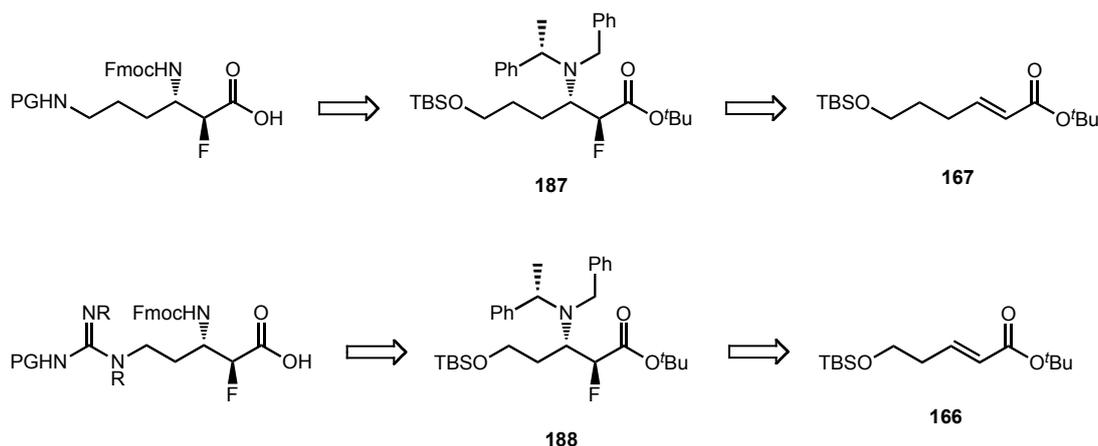
Figure 6 Retrosynthetic analysis for protected α-fluoro-β³-lysine derivatives (*top*) and α-fluoro-β³-arginine derivatives (*bottom*), where R = H or PG (undefined protecting group).

One of the key tasks associated with preparing the desired β³-amino acids from the conjugate adducts is converting the terminal silyloxy groups to the requisite nitrogen-based functionalities. There are several ways to achieve this transformation, although the classic method is the Gabriel synthesis,[34] where an alcohol is converted to the alkyl halide then reacted with potassium phthalimide. This displaces the halide to give the *N*-alkylphthalimide, with phthaloyl cleavage liberating a primary amine. Alternatively, the Mitsunobu reaction, most commonly associated with the use of oxygen nucleophiles, has seen a huge rise in popularity over the last 20 years for the synthesis of amino compounds.[35] It was first reported by Mitsunobu in 1967 as the condensation of a carboxylic acid with an alcohol in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine,[36] and then extended to various other nucleophiles over the years, including phthalimide.[37,38] This method of amine synthesis removes the need for a primary alkyl halide, thereby extending the methodology to secondary alcohols.

To make our fluorinated β³-lysine and β³-arginine derivatives amenable to peptide synthesis, both the side chain and β-amino moieties must be suitably protected according to the conventions just discussed, while ideally providing the free carboxylic acid in preparation for coupling. Given the general preference for the Fmoc system over the Boc system, the N^{α}-functionality was to be masked as the Fmoc derivative, which then dictated the type of protecting group used on the terminal amino functionalities. However, the compatibilities of the existing functional groups, i.e., the dibenzylamine and *tert*-butyl ester of the conjugate adducts, with the synthetic route chosen also has to be taken into consideration, particularly as their modes of cleavage are not completely orthogonal to those of many common protecting groups used in peptide synthesis.

As discussed in Section 3.1.1.1, lysine is particularly well suited to the N^{β}-Fmoc/N^{ϵ}-Boc strategy, with the synthesis of this derivative from conjugate adduct **187** predicted to be relatively straightforward. However, the preparation of a protected arginine analogue was expected to be more problematic given the complexity of the side chain. The option of mono-, bis- or tris-protection of the guanidino functionality was an important consideration, and dependent upon both the protecting group used and its method of introduction. At this stage the main focus was on demonstrating the general applicability of our fluorinated conjugate adducts to the synthesis of orthogonally protected β-amino acids, therefore the choice of guanidino protecting group was ultimately based on convenience and ease of introduction, and not that most appropriate for use in SPPS.

The synthetic approach for the arginine derivative was a little different to that employed for the lysine derivative due to the complications introduced by the guanidino moiety. Installation of this functional group has classically been achieved via the use of thioureas, isothioureas, triflylguanidines or pyrazol-1-carboxamides,[39] however these reagents require an amine nucleophile to be present in the substrate. Alternatively, the Mitsunobu reaction has been successfully used to introduce both Boc and Cbz protected guanidines to molecules where the starting material is an alcohol; both bis-[48] and tris-protected[49] guanidines appear to work equally well as the nucleophile. With regard to the tris-protected guanidines, Feichtinger *et al*. found that reactions with Cbz protected

guanidine somewhat more facile than with a Boc derivative: yields of between 86-100% were obtained with the Cbz derivative when conducting the reaction in THF at RT, while a maximum yield of only 72% was obtained with tri-Boc guanidine, even when heating at reflux in THF or toluene. However, both the Cbz and Boc protecting groups present compatibility problems with the dibenzylamine and *tert*-butyl ester, respectively, of the conjugate adduct. Work-up and purification problems were foreseen should a concomitant ester hydrolysis/guanidino Boc cleavage strategy be employed, especially taking into account the extra step required to reprotect the guanidine. Furthermore, Feichtinger *et al.* also noted a tendency for the N^δ-Boc group to migrate to the α-amino moiety of an arginine ester upon hydrogenolysis of a N^α-Cbz group; the same process was likely to occur upon cleavage of the two benzyl groups during this work (*Figure 7*, top*)*, leaving the two amino functionalities undifferentiated. Unfortunately it was not a matter of just hydrogenating the benzyl groups and installing the Fmoc group before guanidine introduction, as Fmoc is known to be unstable to the basic betaine formed in the Mitsunobu reaction.[40,41] Its cleavage would leave the β-amine exposed (**203**), potentially acting as a competing nucleophile and resulting in intramolecular attack to form an azetidine (**204**, *Figure 7*).
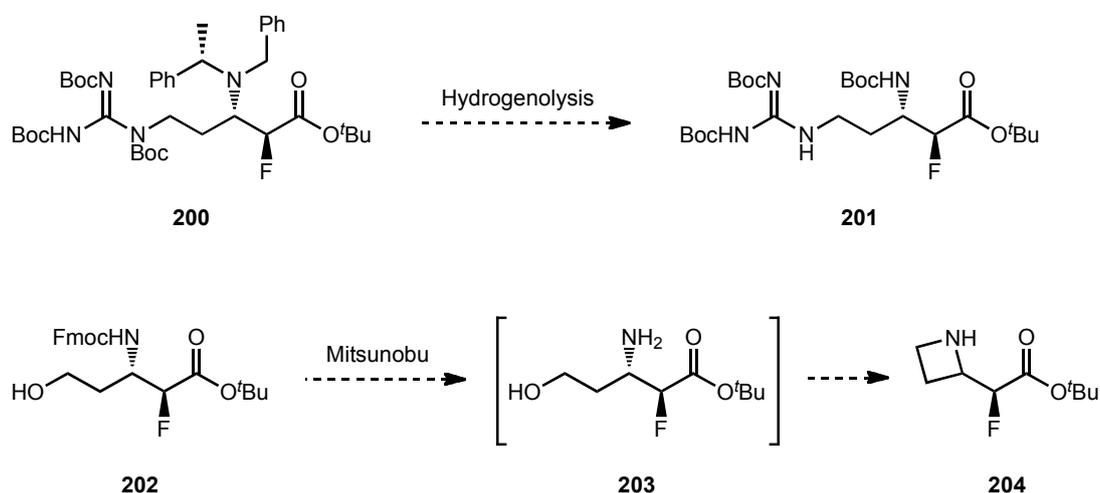
Figure 7 Possible outcomes when attempting hydrogenolysis of the dibenzylamine in **200** in the presence of the tris-Boc protected guanidino moiety (*top*) and Mitsunobu reaction with an Fmoc-protected substrate (*bottom*).

Examining the Cbz guanidine option, this protecting group is stable to both *tert*-butyl ester cleavage and Fmoc deprotection during peptide synthesis, making it more favourable than the Boc derivative. The fact that it is not compatible with the dibenzylamine can be overcome by hydrogenolysis of the benzyl groups prior to the Mitsunobu reaction, with the β-amine reprotected with a group that would withstand the basic conditions. The obvious choice was *N*-Boc, which could be conveniently cleaved concomitant to the *tert*-butyl ester in preparation for Fmoc introduction.

3.3 Results and Discussion

3.3.1 Synthesis of protected α-fluoro-β³-lysine

The success of the Mitsunobu reaction depends on the amine nucleophile having a pK_a below 11 to enable deprotonation by the betaine intermediate, formed from the reaction of DEAD with PPh₃. As a result, primary amines are often precluded from use, with the more acidic amides, imides, sulfonimides or azides typically chosen as the nucleophile.[42] Rather than installing the amine via a Boc-protected sulfonamide with subsequent removal of the tosyl group,[43] we decided to introduce the NH_2 synthon via phthalimide, which could then be cleaved and Boc protected in separate steps. It was envisaged that the β-amino moiety would remain protected during the early stages of the synthesis as the dibenzylamine derivative, a highly stable moiety that would allow orthogonal cleavage in the final steps of the synthesis.

The first task was cleavage of the silyloxy group of **187**. This could be achieved in high yield with TBAF in the presence of an equimolar amount of AcOH to neutralise the strongly basic conditions, which would help prevent the possibility of racemisation at the α-carbon. Conducting the reaction at 40°C allowed the reaction to be completed in 4-5 hours in 93% yield (*Scheme 1*). Alternatively, stirring the substrate with a 3:1:1 AcOH/H_2O/THF mixture overnight was also effective but resulted in slightly lower yields of 73-83%. The loss of the *tert*-butyldimethylsilyl group was clearly evident upon examination of both the ¹H and ¹³C NMR spectra of the product, and was supported by ESI HRMS analysis, which produced a molecular

ion of m/z = 416.2601, corresponding to $[M+H]^+$ for **205**.
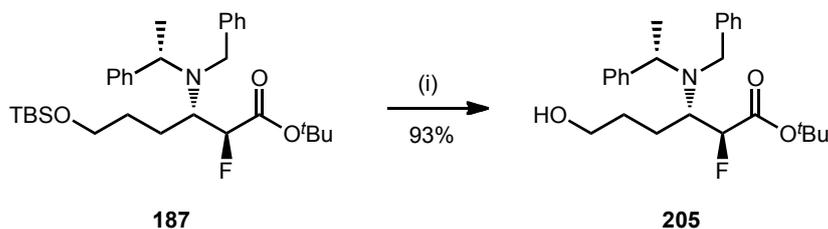
Scheme 1 Reagents and conditions: (i) TBAF, AcOH, THF, 40°C.

Hydroxy ester **205** was then subjected to Mitsunobu conditions using the less-hazardous diisopropyl azodicarboxylate (DIAD) in place of the toxic and potentially explosive DEAD (*Scheme 2*). The order of reagent addition can be important as the betaine has the potential to deprotonate the alcohol in the absence of a nucleophile. Thus, 1.5 equivalents of PPh₃ and phthalimide were added to a solution of the substrate before the dropwise addition of DIAD. Conducting the reaction at ambient temperatures resulted in yields of 79-86%, however cooling the mixture to 0°C before DIAD addition resulted in a virtually quantitative conversion to the phthalimide, which was obtained in 97% yield after chromatography. The successful introduction of phthalimide was supported by the presence of two distinct resonances in the ¹H NMR spectrum of the product at 7.85 and 7.71 ppm, which represented the two sets of equivalent protons in the aromatic ring of the phthalimide. Furthermore, an additional peak in the ¹³C NMR spectrum at 168.2 ppm could be attributed to the two identical imide carbonyl groups. Substitution of the alcohol by the phthalimide was also reflected in a shift of the methylene carbon adjacent to the hydroxy group in **205** from 62.7 ppm to 38.0 ppm for the phthalimide **206**. This is due to the increased electron density around the imide nitrogen atom, which is much less electronegative than the oxygen atom of the alcohol. ESI HRMS analysis of the product supported this result, providing a molecular ion with m/z = 545.2815.
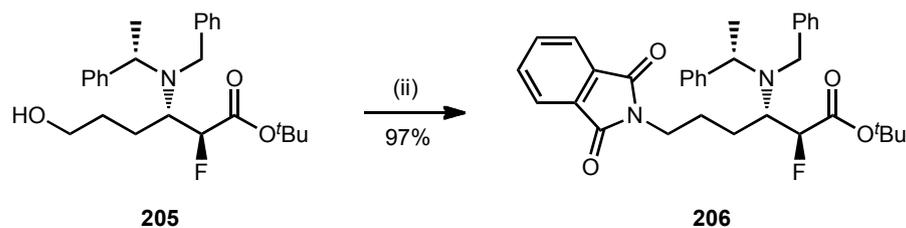
Scheme 2 Reagents and conditions: (i) PPh$_3$, DIAD, phthalimide, THF, 0°C, then RT overnight.

With lysine substrate now bearing the phthaloyl protected amino functionality, the next task was conversion to the free amine then reprotection as the Boc derivative. The phthalimide group is traditionally removed via hydrazinolysis, a reaction facilitated by intramolecular attack of hydrazine's second NH$_2$ group on the normally unreactive amide of the ring-opened intermediate. However, the use of hydrazine can tend to have limited success in phthalimide cleavage, thus the milder method of methylaminolysis[44] was investigated. Treatment of **206** with an excess of aqueous MeNH$_2$ in EtOH at RT resulted in complete dephthaloylation, and after an aqueous work-up, the crude amine was treated with Boc$_2$O to afford the orthogonally protected derivative **207** in almost quantitative yield over two steps. No other isomers—easily visible due to the characteristic H-F coupling—were detected during ^{1}H NMR analysis of the crude reaction mixture, indicating no epimerisation at C2 had occurred. Analysis of the integrated ^{1}H NMR spectrum of the product revealed a loss of five proton resonances in the aromatic region, indicating the loss of the phthalimide group. Furthermore, an additional singlet peak attributable to the newly introduced Boc group appeared at 1.49 ppm, with the corresponding peak in the ^{13}C NMR spectrum resonating at 28.4 ppm. ESI HRMS analysis provided further evidence for Boc protected **207**, giving a molecular ion of m/z = 515.3286 for [M+H]$^+$.
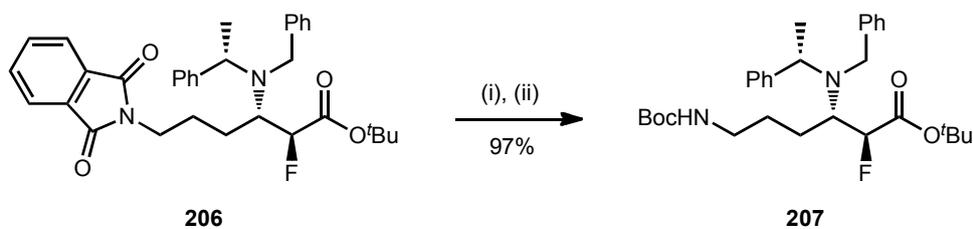
Scheme 4 Reagents and conditions: (i) 41% aq. MeNH₂, EtOH, 2.5 hr; (ii) Boc₂O, THF, Et₃N.

The next step *en route* to the target lysine derivative was N^β-Fmoc protection. While hydrogenolysis of the two benzyl groups has occasionally proven problematic in the past, resulting in either over-hydrogenation (cleavage of the amine) or under-hydrogenation (failure to cleave the α-methylbenzyl group),[45] no problems were experienced in this work when conducting the reaction under 1 atm of H₂ in a mixed solvent system of MeOH-H₂O-AcOH (45:4:1).[46] Acetic acid is a common additive in the hydrogenolysis of amino compounds as it protonates the free amine as it is formed, preventing the metal catalyst from being poisoned and rendered ineffective. The catalyst typically used in these types of *N*-debenzylation reactions is Pearlman's catalyst (Pd(OH)₂/C), a highly selective and efficient catalyst for cleaving benzylamine groups.[47] In addition, as the catalyst is supplied as a wet powder it does not pose the same hazard as Pd/C, a fine, dry powder prone to spontaneous combustion, especially in the presence of MeOH. After stirring dibenzyl ester **207** in the aforementioned solvent system with Pearlman's catalyst under H₂ for 7 hours, the primary amine was obtained cleanly with no sign of by-
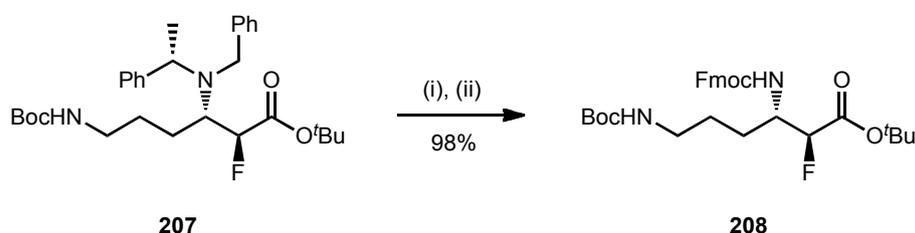
Scheme 5 Reagents and conditions: (i) Pd(OH)₂/C, H₂ (1 atm), MeOH-H₂O-AcOH (45:4:1); (ii) Fmoc-OSu, Na₂CO₃, THF-H₂O (4:1);

products (*Scheme 5*). Formation of the mono-substituted α-methylbenzyl substrate could be observed by TLC over the course of the reaction, but promptly reacted *in situ*. Despite the high polarity of the amine, it was easily extracted with EtOAc during aqueous work-up to remove AcOH. The crude material was then redissolved in a THF-H$_2$O solution, and Na$_2$CO$_3$ and Fmoc succinimide (Fmoc-OSu) were added. Work-up and chromatography afforded the orthogonally protected β^3-lysine ester **208** in 98% yield over the two steps. The α-methylbenzyl and methylene proton resonances of the two benzyl groups were absent from the ^{1}H NMR spectrum of the product. Integration of the resonances in the aromatic region revealed the presence of eight protons rather than the ten attributable to the two benzyl groups. These eight protons, resonating as two doublets at 7.76 and 7.60 ppm, and two triplets at 7.46 and 7.42 ppm, correspond to the four sets of equivalent protons in the Fmoc moiety. Two additional peaks appear at 4.45 and 4.21 ppm, accounting for the three aliphatic protons in Fmoc. The $N^β$ proton appears as a broad doublet at 5.23 ppm, showing a correlation to the β-proton in the 2D COSY spectrum; the $N^δ$ proton appears further upfield at 4.59 ppm. The presence of the Fmoc group was supported by ^{13}C NMR analysis, which reveals an extra carbonyl peak at 155.9 ppm and an additional two peaks at 66.8 and 47.2 ppm, corresponding to the two aliphatic carbon atoms in Fmoc. EI-HRMS analysis provided further evidence, generating a molecular ion with m/z = 565.2683, corresponding to [M+Na]$^+$ for **208**.

The final task was to convert *tert*-butyl ester **208** to the carboxylic acid **209**. As *N*-Boc protecting groups are typically removed under similar (but generally milder) conditions than those required for *tert*-butyl ester hydrolysis, it was almost certain that the terminal amino functionality would require Boc reprotection as a second step. There are various methods for the selective removal of *N*-Boc groups in the presence of *tert*-butyl esters,[48,49] but few exist that possess the opposite selectivity. Those that do feature the Lewis acids ZnBr$_2$[50] CeCl$_3$·H$_2$O-NaI[51] or I$_2$.[52] Initially attempting to avoid the reprotection, the I$_2$ method was identified as the most straightforward, with the reagents easily obtained and requiring no special handling or purification. Heating an acetonitrile solution of **208** at reflux in the presence of 30 mol % of I$_2$ and a catalytic amount of H$_2$O for 5 hours resulted only in starting material, even after continuing the experiment overnight. With that

failing, it was then decided to try the classic method of TFA cleavage, hoping a good yield could be obtained over the two consecutive deprotection and reprotection steps. The ester was stirred in a TFA-DCM mixture at RT for 3 hours, after which time the solution was concentrated, redissolved in THF-H_2O and treated with Na_2CO_3 and Boc_2O. After an aqueous work-up involving back extraction and precipitation of the acid with 6M HCl, the crude **209** was obtained as a white powder (*Scheme 6*). After numerous unsuccessful attempts at recrystallising the solid, the crude product was instead purified by passage over a plug of silica and obtained in a good 81% yield. The structure of **209** was established through a combination of NMR spectroscopy and mass spectrometry. The ^{1}H NMR spectrum of the compound confirmed the presence of the *N*-Boc group, easily identifiable as a large singlet at 1.37 ppm. While the carboxylic acid proton was not visible, the lack of a *tert*-butyl peak in both the ^{1}H and ^{13}C NMR spectra supported the carboxylic acid structure; the β-NH proton resonated as a doublet at 7.62 ppm (identified by COSY), indicating that no cyclisation to form the lactam had occurred. The carboxylic acid carbon atom resonated as a doublet at 169.5 ppm, which is slightly downfield relative to that of the *tert*-butyl ester (166.4 ppm), although solvent effects may also play a role in the shift: the ^{13}C NMR spectrum of the acid was recorded in acetone-d_6, while that of the ester was recorded in the less polar $CDCl_3$. ESI HRMS analysis was used to support the free acid structure, and with a molecular ion of m/z = 509.2059 corresponding to [M+Na]⁺ for **209**, the product was assigned as α-fluoro-$N^β$-Fmoc-$N^ε$-Boc-β-lysine.

208   **209**
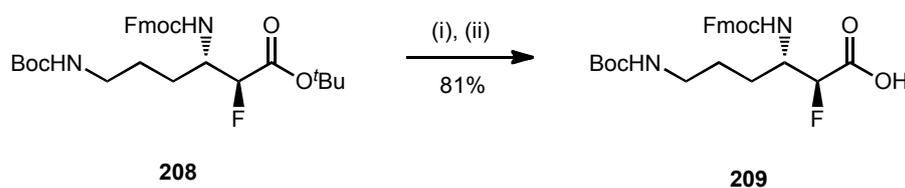
Scheme 6 Reagents and conditions: (i) TFA-DCM (1:2), 3 hr; (ii) Boc_2O, Na_2CO_3, THF-H_2O (1:1).

3.3.2 Synthesis of protected α-fluoro-β³-arginine

The first step involved cleavage of the silyloxy group of **188** to expose the terminal hydroxy group, a reaction that was accomplished using identical conditions to those employed for the lysine derivative (*Scheme 7*). Hydroxy ester **210** was obtained in 93% yield, with the loss of the TBS group observable by ¹H NMR and ¹³C NMR spectroscopy. In addition, the OH proton resonance was visible as a broad multiplet at 2.52 ppm, coupling to the methylene protons on the adjacent carbon. These two protons were diastereotopic in nature, appearing as separate multiplets, unlike those of the lysine derivative, which appeared as a triplet. ESI HRMS analysis produced a molecular ion of m/z = 402.2445, corresponding to [M+H]⁺ for **210** and supporting the assigned structure.

188   **210**
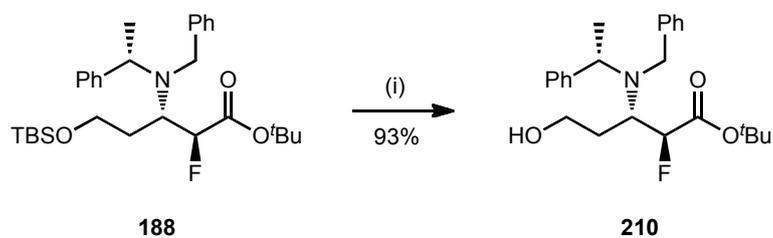
Scheme 7 Reagents and conditions: (i) TBAF, AcOH, THF, RT.

Hydrogenolysis of the benzyl groups and N^{β}-Boc protection of **210** was initially carried out over two steps, with isolation of the crude amine and subsequent treatment with Boc₂O in the presence of Na₂CO₃ resulting in a moderate 71% yield. This yield was improved upon significantly with the implementation of a one-pot method, where Boc₂O is instead added to the hydrogenolysis reaction mixture. As well as being more convenient in avoiding the need to isolate the very polar hydroxy primary amine, it resulted in an almost quantitative conversion to the Boc-protected alcohol **211** (*Scheme 8*). Aromatic resonances were absent from both the ¹H and ¹³C NMR spectra, indicating the successful removal of the two benzyl groups. As expected, an additional singlet peak integrating for the nine protons of the Boc group appeared in the ¹H NMR spectrum adjacent to that of the *tert*-butyl ester, with the primary and quaternary carbons of the Boc group also visible in the ¹³C NMR spectrum. The *N*-Boc carbonyl carbon resonated at 156.5 ppm, further upfield

than that of the ester, which appeared as a doublet at 166.2 ppm as a result of C-F coupling. A broad NH resonance appeared at 5.01 ppm in the ¹H NMR spectrum, and was identified by the correlation to the β-proton in the COSY spectrum. Finally, ESI HRMS analysis produced a molecular ion of m/z = 330.1689, corresponding to [M+Na]⁺ for **211.**
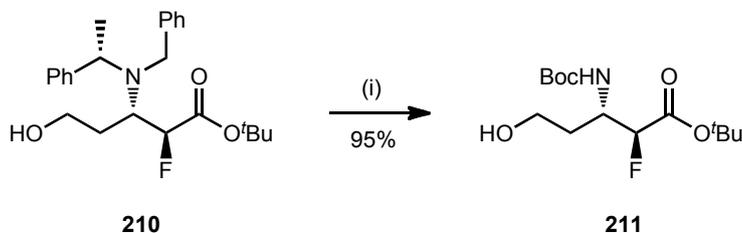
Scheme 8 Reagents and conditions: (i) Pd(OH)$_2$/C, H$_2$, Boc$_2$O, MeOH-EtOAc (1:1), 7 hr.

With the substrate now ready for transformation into an arginine-type derivative, the Cbz-protected guanidine reagent used in the Mitsunobu reaction also had to be prepared. Following the literature procedure,[53] a solution of guanidine hydrochloride was treated with hydroxide, followed by 3 equivalents of Cbz-Cl to produce *N,N'*-bis(benzyloxycarbonyl)guanidine **213** in 84% yield (*Scheme 9*). Integration of the ¹H NMR spectra confirmed the presence of two Cbz groups, with the data closely matching that reported in the literature, while the mp of 151-152°C was in close agreement with the reported range of 149-150°C. Synthesis of the tri-acylated guanidine required a separate deprotonation step with the strong base NaH, which was undertaken at -45°C. Subsequent treatment of the anion with Cbz-Cl afforded the tri-Cbz derivative **214** in 81% yield, with the ¹H NMR and mp data again in agreement with those in the literature.
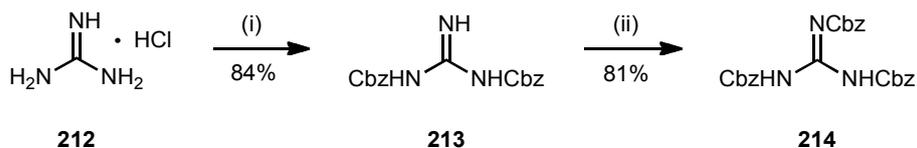
Scheme 9 Reagents and conditions: (i) Cbz-Cl, NaOH, THF-H$_2$O (2:1), 0°C to RT overnight; (ii) NaH, Cbz-Cl, -45°C to RT overnight.

While tris-protection of arginine's guanidino functionality is preferable to ensure it is as unreactive as possible, bis-protected derivatives are also quite commonly encountered in peptide synthesis as the reactivity of the free NH is suppressed somewhat by the steric bulk of the two protecting groups. The electron withdrawing nature of many of the protecting groups used also helps to reduce NH reactivity. As such, we decided to synthesise both the bis- and tris-protected analogues using **213** and **214**, respectively, in order to introduce the guanidino moiety during the Mitsunobu reaction.

Given the presence of the secondary amino functionality in the β-position, three equivalents of the guanidine nucleophile were used in this Mitsunobu reaction to help prevent competing intramolecular attack by the β-amine. Condensation of hydroxy ester **211** with the tris-protected guanidine **214** in the presence of PPh₃ and DIAD produced arginine derivative **215** in 87% yield (*Scheme 10*). Given the large excess of guanidinylating reagent, column chromatography occasionally failed to completely separate it from the product. In these cases residual guanidine in the chromatographed mixture could easily be crystallised out with MeOH and filtered off. Also beneficial was the partial precipitation of the excess guanidinylating agent from the crude reaction mixture with diethyl ether. This could then be filtered off, which decreased the column loading and made the separation more effective. Upon spectroscopic analysis of the product, the hydroxyl proton resonance of the starting material was no longer visible in the ¹H NMR spectrum, and the two methylene protons on the δ-carbon had been resolved into one triplet. Introduction of the tri-Cbz guanidine was evident from three additional features of the ¹H NMR spectrum: the new peaks resonating in the aromatic region (7-7.5 ppm), which integrated for the 15 protons of the Cbz groups; the broad singlet at 11.0 ppm, attributable to the highly deshielded guanidino NH proton; and a multiplet from 5.14-5.06 ppm that integrated for six protons, accounting for the pair of methylene protons in each of the three Cbz groups. Interestingly, while the protons from one of these Cbz groups were well defined as a pair of doublets (being diastereotopic protons), the other four protons appeared together as a very broad singlet. This phenomenon was also observed in the ¹³C NMR spectrum: the *ipso* and methylene carbon resonances of one Cbz group were well resolved peaks at 134.4 and 69.1 ppm, respectively, however the corresponding carbon atoms of the other two Cbz groups, as well as

the carbonyl signals, were broad, poorly resolved peaks. Two small peaks were present at 152.2 and 150.8 ppm, which were assigned as the carbonyl peaks based on 2D HMBC long-range correlation experiments, and at 135.7 and 134.7 ppm, which represented the *ipso* carbon atoms. A single broad peak at 68.1 ppm accounted for the two methylene carbons. Upon switching to the more polar solvent acetone-d_6, the two carbonyl signals merged into one peak at 151.8 ppm, which suggests the phenomenon is related to molecular dynamics (i.e., hydrogen bonding within the guanidino moiety) rather than relaxation issues as a result of quadrupole line broadening. The latter often occurs in molecules containing nitrogen, where the quadrupole moment of ^{14}N causes fast relaxation of the adjacent nuclei, resulting in poorly resolved peaks. While the methylene carbon peak of the Cbz group appeared much sharper as well as doubled in height when using acetone-d_6, each of the peaks from the aromatic carbons remained poorly resolved. Finally, the presence of the tri-Cbz protected guanidino moiety was supported by ESI HRMS analysis of the product, which returned a molecular ion of m/z = 751.3353, corresponding to [M+H]$^+$ for **215**.
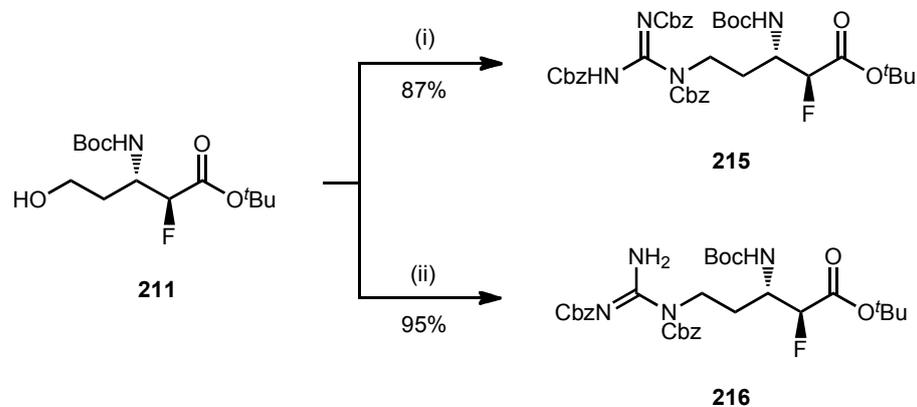
Scheme 10 Reagents and conditions: (i) PPh$_3$, DIAD, **214**, THF, 0°C; (ii) PPh$_3$, DIAD, **213**, THF, 0°C.

Repeating the Mitsunobu reaction with di-Cbz guanidine **213** furnished β³-arginine derivative **216** in 95% yield, and was obtained whilst using only two equivalents of the guanidinylating reagent. The fact that only two of the three nitrogen atoms in the guanidine are protected presents the possibility that either of the structural isomers **216** or **217** may be formed (*Figure 8*). The substitution pattern seen in

217 is the one most commonly encountered for substituted guanidines, which are typically synthesised using electrophilic pyrazole or thio-substituted guanidines (page 99), rather than the nucleophilic guanidine species employed in the Mitsunobu reaction. With few examples of the Mitsunobu reaction involving protected guanidines appearing in the literature, further investigation to determine the correct structure was undertaken.
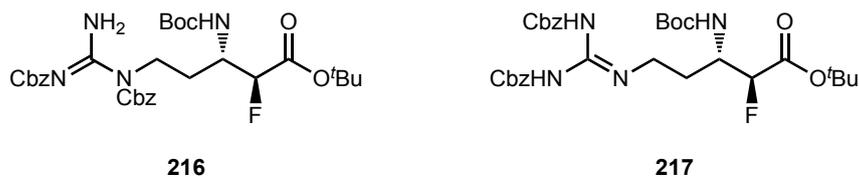
Figure 8 Two possible products resulting from the Mitsunobu reaction.

The ^{13}C NMR spectrum of **216** was ambiguous and thus could not be used to identify the correct structure of the guanidinium moiety, largely because of broadened coincident signals in the 155-165 ppm region of the spectrum, as occurred with the tri-Cbz derivative. However, the analogous di-Cbz *N*-acetylated compound **308** (Chapter 5, page 183), showed correlations between the methylene protons on the δ-carbon and both the quaternary guanidine carbon and one of the two Cbz carbonyl carbons in the HMBC spectrum. Furthermore, two examples in the literature detail the synthesis of compounds featuring the di-Cbz substituted guanidino moiety via the Mitsunobu reaction, both of which identify the substitution pattern seen in **216** as the correct structure. In the first example, Dodd and Kozikowski prepared di-Boc substituted guanidines via both Mitsunobu reaction and using the reagent *N*,*N'*-bis(*tert*-butyloxycarbonyl)-*S*-methyl-isothiourea (*Scheme 11*).[54] The latter method is known to afford products with the N^{ω},$N^{\omega'}$-substitution pattern (**219**), however the product of the Mitsunobu reaction had a different structure, which was identified as the N^{δ},N^{ω}-di-Boc substituted derivative **221**. An analogous result would occur with the di-Cbz protected guanidines.
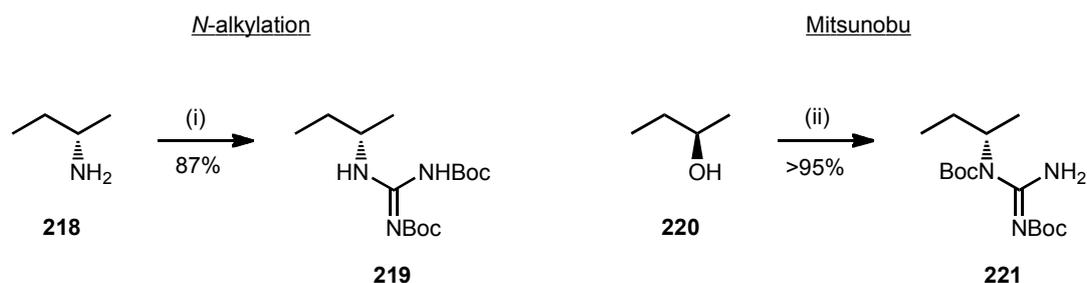
Scheme 11 Reagents and conditions: (i) *N,N'*-bis(*tert*-butyloxycarbonyl)-*S*-methyl-isothiourea, Et₃N, DMF; (ii) di-Boc guanidine, PPh₃, DIAD, THF.

Ohshima *et al.* reported the synthesis of a protease inhibitor featuring di-Cbz protected guanidino intermediate **222** (*Figure 9*), which was prepared using Mitsunobu conditions.[54] The ¹H NMR structure of this intermediate features two broad singlets at 9.45 and 9.24 ppm, which the authors assigned to each of the guanidino NH₂ protons. Similar peaks were observed in the ¹H NMR spectrum of **216**, with two broad singlets at 9.37 and 9.20 ppm, suggesting the same substitution pattern was present. Together with the structural analysis of **306**, it was likely that **216** (not **215**) was the product of the Mitsunobu reaction.
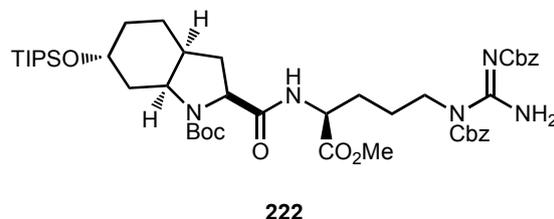
Figure 9 A di-Cbz protected guanidino derivative
prepared by Ohshima *et al.*.

Further to this, tautomerisation of the guanidino moiety is also possible, leading to either the amino or imino forms displayed in *Figure 10*. Guanidines featuring electron-withdrawing substituents are believed to exist primarily in the amino form, as determined through a combination of X-ray crystallography and ¹⁵N NMR spectroscopic analysis of various compounds.[55,56] No evidence for interconversion of the two tautomers was obtained during NMR analyses performed in this work.
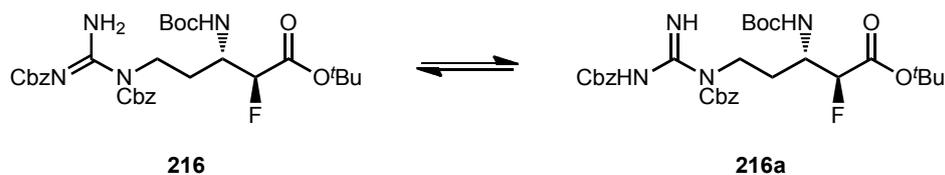
Figure 10 Amino (**216**) and imino (**216a**) tautomers of the guanidine derivative.

The final steps in the synthesis of the orthogonally protected β³-arginine derivatives were exchange of the Boc protecting group for Fmoc, and cleavage of the *tert*-butyl ester. The simultaneous Boc/*tert*-butyl deprotection using TFA had been relatively successful for the lysine analogue, so it was envisaged that similar conditions could be employed here. While Cbz groups are known to be somewhat susceptible to acidolysis in TFA solutions, the method generally allows selective deprotection of Boc and *tert*-butyl esters in the presence of Cbz groups. Another point to consider is that guanidines bearing two electron-withdrawing groups are reported to be less stable than those bearing only one; removal of the first substituent appears to confer extra stability on the remaining protecting group to the reaction conditions used.[57]

Initial attempts involved treatment of the di-Cbz derivative **216** with a 2:1 DCM-TFA solution at RT for 3.5 hrs, after which time the solvent was evaporated and the crude residue treated with a solution of Fmoc-OSu in H_2O-THF in the presence of base. Acidification of the aqueous phase during work-up produced a white solid that was sparingly soluble in most organic solvents; trituration with acetone yielded 32% of what was suspected to be the mono-Cbz protected acid **223** (*Scheme 12*), with attempts at isolating any remaining product from the residue via chromatography failing. ^{1}H NMR analysis of the solid indicated that Fmoc had been incorporated into the molecule, however only one Cbz group remained. The *tert*-butyl peak was no longer present, although the carboxylic acid proton resonance was difficult to distinguish from the broad NH resonances in the spectrum, if it was in fact present at all. Formation of the carboxylic acid was supported by ^{13}C NMR spectroscopy, in which the carbonyl peak was located at 169.3 ppm in DMSO-d_6,

easily identifiable by the C-F splitting. This chemical shift was similar to that observed for the α-fluoro-β³-lysine derivative **209**, in which the carboxylic acid carbon resonated at 169.5 ppm in acetone-d_6. Despite the fact that different solvents were used, the high polarity of both meant that differences in the chemical shifts of the other common functionalities (i.e., the Fmoc and α-protons) were negligible, allowing the general comparison to be made. Unfortunately the product suffered from substantial decomposition before HRMS analysis could be performed, leaving the exact structure unconfirmed.
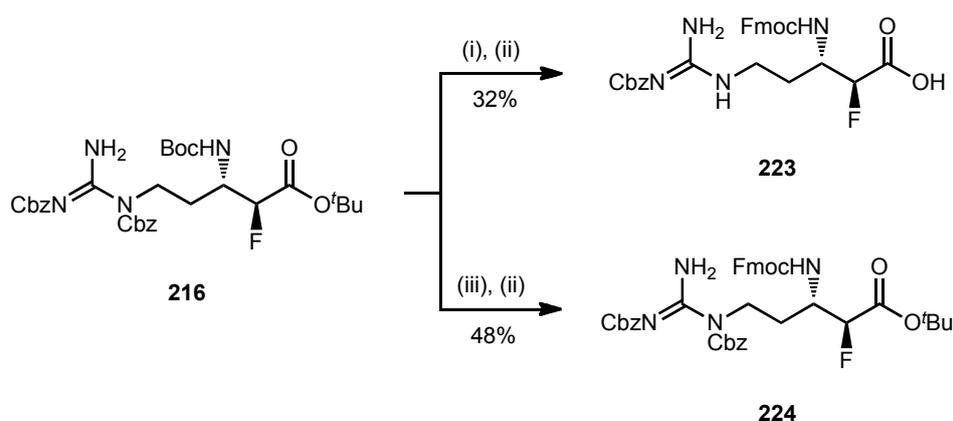
Scheme 12 Reagents and conditions: (i) TFA-DCM (1:2), RT, 3.5 hr; (ii) THF-H_2O (1:1), Fmoc-OSu, Na_2CO_3, 0°C to RT overnight; (iii) TFA-DCM (1:2), 0°C, 3 hr.

In an attempt to prevent cleavage of the second Cbz group, the experiment was repeated at 0°C instead of at RT. While these reaction conditions were mild enough to prevent cleavage of the Cbz groups, the *tert*-butyl ester was largely untouched, and thus **224** was obtained in 48% yield. Two guanidino proton resonances were visible in the ^1H NMR spectrum of the product at 9.41 and 9.19 ppm, as well as the four diastereotopic Cbz methylene proton resonances, which appeared as doublets between 5.03-5.28 ppm. The easily identifiable Boc resonance was replaced by peaks at 4.28 and 4.06 ppm in the ^1H NMR spectrum, and at 66.7 and 47.1 ppm in the ^{13}C NMR spectra, attributable to the aliphatic carbons in Fmoc. Together with integration of the peaks in the aromatic region (18 protons), these features confirm that neither of the Cbz protecting groups were lost, and that the *N*-Boc group was

successfully removed and replaced by Fmoc. Further evidence was provided by ESI HRMS, which produced a molecular ion of m/z = 761.2995, corresponding to [M+Na]$^+$ for **224**. Unfortunately, due to the small amount of material available and the likelihood of decomposition, further reaction to achieve the removal of the *tert*-butyl ester was not attempted.

Traces of the desired carboxylic acid appeared to be visible in the ^{1}H NMR spectrum of the crude residue obtained from acidification and extraction of the aqueous phase during work-up, however separation of the complex mixture using chromatography on silica gel failed, with no carboxylic acid eluted. An alternative deprotection method using 2.5 equivalents of H_2SO_4 in DCM at 0°C for 7 hours to selectively cleave the Boc and *tert*-butyl groups also failed, resulting only in a mixture of highly polar unidentified products that could also not be purified via chromatography.

Attempts at synthesising the orthogonally protected carboxylic acid from tri-Cbz derivative **215** were also unsuccessful: treatment of **215** with a 2:1 DCM-TFA solution containing 1% H_2O, followed by Fmoc-OSu and Na_2CO_3 in THF-H_2O gave a mixture of products. The major product isolated after chromatography appeared to contain the *tert*-butyl ester and two Cbz groups, but also two Fmoc groups. Unfortunately this product could not be isolated pure even after repeated chromatography, and further characterisation was not obtained.

It was then rationalised that bubbling HCl gas through a solution of **215** should provide milder deprotection conditions, with a similar strategy previously proving effective for Gosselin and Lubell, who sought to achieve concomitant Boc and *tert*-butyl cleavage with subsequent Fmoc reprotection of an amino group.[58] Thus, a stream of HCl gas was bubbled through a solution of **215** in DCM at 0°C for 3 hours, by which time all of the starting material had disappeared (TLC analysis). Treatment of the crude product with Fmoc-OSu using conditions similar to those of Gosselin and Lubell (acetone-H_2O, NaHCO$_3$) again resulted in a complex mixture of products. A product suspected to be urea **225** was isolated in 23% yield after two chromatographic purifications. Integration of the ^{1}H NMR spectrum confirmed the presence of only two Cbz groups, and the spectrum was almost identical to that of

224, except for the fact that only a single guanidino proton resonance was present at 11.10 ppm. The ^{13}C NMR spectrum was also telling: it appeared that the Cbz carbonyl peaks (identified from the long-range correlations in the HMBC spectrum) had shifted from 163.6 and 155.5 ppm in **224**, to 155.4 and 150.5 ppm for **225**. Most notable was the shift of the quaternary guanidino carbon from 160.1 to 149.4 ppm, supporting the theory that the product now bore a urea functionality, a problem that was also experienced later during acid-catalysed reactions of a di-Cbz protected difluoro derivative (Chapter 5, page 184). Again, due to a lack of material this last synthetic step could not be further pursued and thus the desired carboxylic acid could not be obtained.
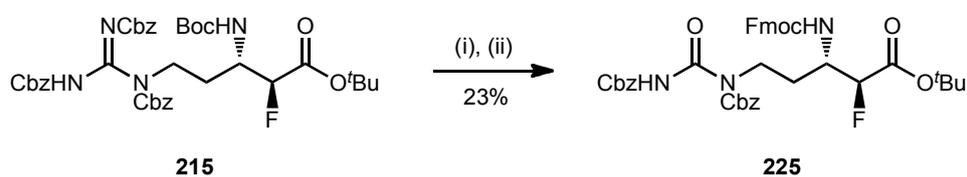
Scheme 13 Reagents and conditions: (i) HCl$_{(g)}$, 0°C, 3 hr; (ii) acetone-H$_2$O, Fmoc-OSu, NaHCO$_3$, 0°C to RT overnight.

3.4 Conclusions

This work has established the applicability of the conjugate adducts to the synthesis of orthogonally protected α-fluoro-β³-amino acids and esters. The chiral α-fluoro-β³-lysine **209** was synthesised in a form suitable for SPPS in nine steps and 53% overall yield from α,β-unsaturated ester **167**, with the integrity of both the α- and β-stereocentres maintained throughout. In most of the steps yields of over 90% were obtained, demonstrating the efficiency of the route. Furthermore, the fact that the core structure of the final product is installed during the initial conjugate addition step means that the subsequent synthetic pathway can be adapted to incorporate alternative protecting groups to accommodate the particular SPPS strategy in use: the orthogonality of the dibenzylamine and TBSO functionalities allows for deprotection at various points along the route.

Synthesis of the α-fluoro-β³-arginine derivatives **223** and **224** was a little less successful given the synthetic complexities introduced by the reactive guanidino moiety, however the orthogonally protected *tert*-butyl ester **224** was successfully synthesised in 6 steps and 25% overall yield from α,β-unsaturated ester **166**. The mono-Cbz protected acid **223** was also isolated in 18% overall yield. As with the synthesis of the lysine analogue, most steps produced yields of over 90%, with disappointing results only obtained in the sequential *tert*-butyl ester/Boc cleavage and Fmoc introduction steps, which resulted in a halving of the overall yield. Further optimisation is necessary to improve the outcome, but given time constraints, was not able to be further pursued. However, it has been shown that these types of α-fluoro-β³-arginine derivatives can be synthesised via a relatively short synthetic pathway from the conjugate adducts prepared in Chapter 2.

Application of these methods to the synthesis of other α-fluoro-β³-amino acids is predicted to be relatively straightforward, especially given the aliphatic, unsubstituted nature of many of the amino acid side chains. It is foreseen that these simple aliphatic Fmoc protected α-fluoro-β³-amino acids could be prepared in as little as four steps from the appropriate α,β-unsaturated ester. This has the potential to open up the field of proteolytic stability studies significantly, removing the need to rely on naturally occurring α-amino acids as starting materials for these types of fluorinated β-amino acids.

3.5   Future directions

Given the outcome of the β-arginine synthesis, which produced less than ideal results in the final step, the synthetic route could be revised to include side chain protecting groups more compatible with the deprotection of the carboxylic acid. Although most protecting groups are generally designed to allow orthogonal deprotection in the presence of common functional groups, many present some difficulties in selective removal e.g., Fmoc is unstable to hydrogenolysis conditions and amine bases, while many of the guanidino protecting groups are susceptible to TFA. Some thought is therefore required when designing a new route, taking into account the stability of each protecting group to the various conditions used throughout the synthetic route. At the same time, masking the guanidino moiety with a protecting group more suited to the process of SPPS, for example Pbf or MIS, is highly desirable. The main challenge with this substrate is thus finding a carboxyl protecting group that can be removed in the presence of the acid-sensitive Pbf or MIS protecting groups—groups which must be introduced early in the synthesis i.e., before Fmoc, which as mentioned previously, is unstable under Mitsunobu conditions and so must be introduced last. One such functional group is the benzyl ester, which can be cleaved in a global deprotection together with the dibenzylamine. An added advantage is that it is stable to the conditions used in the conjugate addition,[59,60] meaning it can therefore be present in the α,β-unsaturated ester, removing the need to transesterify the *tert*-butyl ester after the conjugate addition. Based on this theory, a new route to α-fluoro-β³-arginine derivatives was devised for future attempts, and is shown in *Scheme 14*. The TBSO group is cleaved from conjugate adduct **227** to give hydroxy ester **228**, which is then used to alkylate the mono Pbf or MIS protected guanidine (**229**). These two guanidines are unknown in the literature, but their preparation should be relatively straightforward if the reaction stoichiometry is closely monitored. The Mitsunobu reaction itself is the most unpredictable step of the pathway, as the few reactions of this type that appear in the literature are typically carried out with di- or tri-protected guanidines. Given the ease of alkylation of the second guanidino nitrogen during the synthesis of the di-Boc or di-Cbz guanidines (*Scheme 9*), this nitrogen atom should be basic enough for deprotonation to occur during the Mitsunobu reaction. With the guanidino moiety in place, hydrogenolysis would be used to cleave both the dibenzylamine and benzyl ester moieties of **230**, a reaction that

should require no purification of the deprotected product. The free amine would then be treated with Fmoc-OSu in an attempt to prepare β-amino acid **231**. This pathway excludes the use of strongly acidic or basic conditions after the conjugate addition reaction, thus minimising the likelihood of product decomposition, and should enable the synthesis of the orthogonally protected β-amino acids in only five steps.
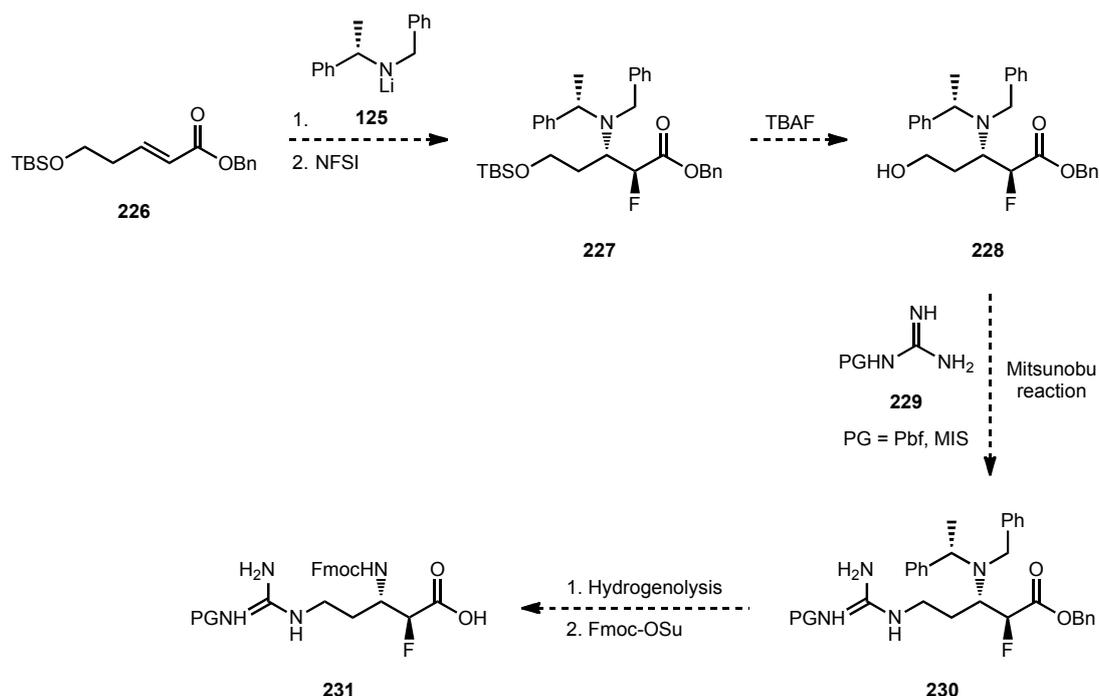
Scheme 14 Proposed alternative route to α-fluoro-β³-arginine derivative **231**, which features acid-labile guanidino protecting groups.

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CHAPTER 4:

PREPARATION OF α,α-DIFLUORO-β^3-AMINO ESTERS VIA REFORMATSKY REACTION

4.1   Introduction

While synthetic efforts towards the preparation of monofluorinated β³-amino acid derivatives are continually being pursued, the presence of these compounds in the literature is far outweighed by those β-amino derivatives possessing difluorination of the α-carbon. This can be linked to several factors: removal of the C2 stereocentre prevents the troublesome problem of racemisation of the α-carbon; the presence of the second electronegative fluorine atom greatly enhances the electronic effects on the adjacent carbonyl group; and from a synthetic point of view, introduction of an achiral CF_2 synthon is generally far easier than the asymmetric introduction of a single fluorine atom. In order to have ready access to both mono- and difluorinated β³-amino analogues, a reliable technique was sought that would allow the synthesis of difluorinated β³-lysine and β³-arginine derivatives. Given the low yielding, irreproducible results obtained when attempting the deprotonation-fluorination of fluorinated conjugate adduct **186** (Chapter 2, page 81), an alternative method complementary to the conjugate addition route was sought. As mentioned in Chapter 1, the Reformatsky (or Reformatsky-type) reaction, which typically involves zinc insertion into a halocarbon bond, is perhaps the most popular way of synthesising such α,α-difluoro compounds.[1] Early synthetic efforts in this particular area were sporadic, with the method only gaining popularity for the preparation of these compounds over the last decade. It has a number of advantages over the aldol reaction—the main alternative for C-C bond-forming reactions via enolates: aldol reactions typically require a strong base to generate the nucleophile, limiting the functional diversity of the substrates, whereas the neutral conditions of the Reformatsky reaction ensure a wide variety of functional groups are tolerated. A range of β³-amino acids can thus be easily synthesised by altering the side chain of the aldehyde component used to prepare the imine starting material. Furthermore, creation of the nucleophile is much more site-specific in complex substrates when using Reformatsky reagents: the generation of the required carbanion depends only on the position of the halogen substituent, in contrast to the mechanism of the aldol reaction, which depends more on the relative pK_a's of the acidic protons and steric properties of the substrate as to where deprotonation occurs. In addition, *O*-alkylation is seldom seen, even with electrophiles like silyl chlorides, whose strong

affinity for oxygen makes them prone to reaction at the carbonyl oxygen in related types of enolisation reactions.[2]

There is now a huge variability in reagents and reaction conditions that are collectively grouped under the term 'Reformatsky reaction', however the classical conditions first described in 1887 involved the zinc-mediated coupling of ethyl α-haloacetates with either aldehydes or ketones (*Scheme 1*). Bromoacetates appear most frequently in the literature due to their high reactivity and are greatly favoured over the corresponding chloroacetates, which generally react much more sluggishly.[2]
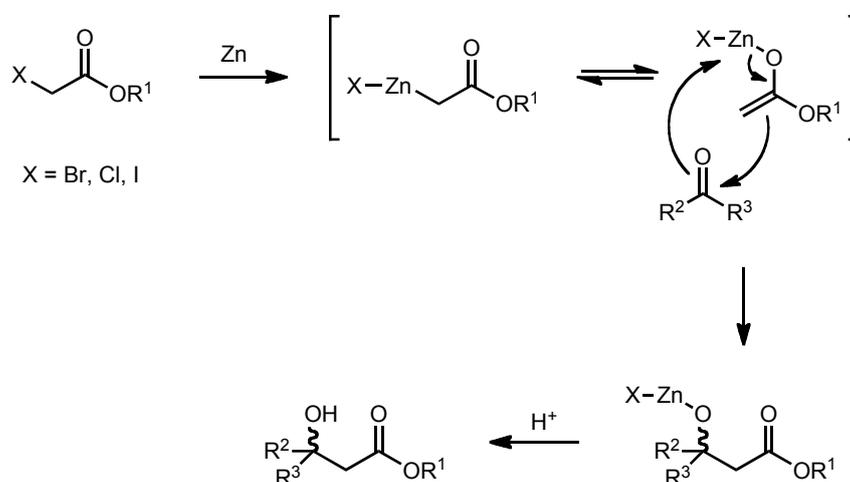
Scheme 1 General mechanism for the classical Reformatsky reaction.

This type of reaction has subsequently come to encompass such a range of alternative electrophiles (e.g., nitriles, phosphonates, imides, acetals, acyl chlorides, anhydrides etc.) and metals (e.g., indium, chromium, SmI₂, etc.) that the term 'Reformatsky reaction' is now loosely used to describe any enolate formation via oxidative insertion of a metal into a carbon-halogen bond, and the subsequent reaction of this enolate with an appropriate electrophile. Extensive reviews encompassing the use of different metals, reaction conditions, electrophiles and wider synthetic applications have appeared over the years,[2-8] however given the sheer scope of the Reformatsky reaction, only those examples in the literature pertaining to the direct synthesis of α,α-difluoro-β³-amino esters will be examined

here. Given the aliphatic nature of the lysine and arginine side chains in our target compounds, particular emphasis will be placed on the application of these existing synthetic techniques to aliphatic imines. As will be seen shortly, the nature of the substituents often makes a considerable difference to the reactivity of the imine, which is already less electrophilic than the corresponding carbonyl compounds because of the lower polarisation of the C=N bond. An otherwise superior synthetic technique that produces β-aryl substituted α,α-difluoro-β³-amino esters in high yield and dr can be quite ineffective for the synthesis of the corresponding β-alkyl analogues. It is a fair observation that because of the difficulties associated with the stability and reactivities of these alkyl imines, their use in Reformatsky or Reformatsky-type reactions has largely been neglected by chemists—recent literature reports of new methods tend to focus almost exclusively on the use of aromatic imines, with generally only two to three token examples of the reaction's application to aliphatic substrates; where they are included, the results are generally poor, hence our need to develop a method more suited to the preparation of β-alkyl substituted α,α-difluoro-β³-amino esters.

4.1.1 Current Reformatsky techniques for the preparation of α,α-difluoro-β³-amino esters

The synthesis of difluorinated β-amino esters under Reformatsky-type conditions requires two essential components: a halodifluoroacetate, of which ethyl bromodifluoracetate is the most common, and an aldimine, which is generally prepared from the requisite aldehyde and primary amine and used crude due to its susceptibility to hydrolysis. These reversible condensation reactions typically proceed in near quantitative yield, particularly when dehydrating methods are employed to facilitate the reaction e.g., using a Dean-Stark apparatus, molecular sieves or other drying agents, and when aromatic or tertiary aliphatic aldehydes are used. These aldehydes react readily with primary amines, with the resulting imines often stable enough to be isolated and purified, particularly in the case of aromatic imines, which are stabilised through conjugation. Secondary aliphatic aldehydes produce imines that are slightly less stable, however those aldimines prepared from primary aliphatic aldehydes are highly unstable, with the imine prone to self-condensation aldol-type reactions which results in polymeric materials.[9] This can be suppressed somewhat by maintaining low temperatures

during the experiment, with sterically hindered amines also appearing to lower the rate of polymerisation.[10]

The use of ethyl bromodifluoroacetate in the Reformatsky reaction was first reported in 1984 by Fried and Hallinan in their synthesis of several β-hydroxy esters.[11] The presence of the electronegative fluorine atoms makes the acetate significantly more reactive than the unfluorinated bromoacetate, and after reacting vigorously with activated zinc dust, the organozinc reagent thus formed is highly unstable—the reagent was found to decompose within 15 minutes in refluxing THF. While the Reformatsky reaction is classically carried out using a one-step method, where the carbonyl compound is already present in the mixture and reacts with the organozinc reagent as it is formed *in situ*, Fried and Hallinan found a two-step procedure in which $BrZnCF_2CO_2Et$ was generated before the addition of the carbonyl compound increased the product yield significantly. For this reason, the $BrZnCF_2CO_2Et$ Reformatsky reagent is now typically generated in refluxing THF separately, followed by the prompt addition of the electrophile. Shortly after this initial report, Burton and Easdon further investigated the properties of this reagent, finding that it existed almost exclusively as the carbon-metallated structure.[12] They also confirmed its instability in solution: in triglyme it was formed in 77% yield from zinc/mercury amalgam, with 8.5% decomposition after 20 hours standing at room temperature, and 23% after 45 hours. In THF solutions, however, total decomposition was observed after 20 hours at RT. The preparation of DMF solutions of the reagent at RT have also been reported, with the yield estimated at ~75%.[13]

In comparison, the properties of the related compound methyl iododifluoroacetate have also been investigated by Kobayashi and co-workers,[14] who found that the reactive organozinc species was formed in 75-85% yield in only 5-10 minutes at 0°C in CH_3CN. In line with the iodo-organozinc reagent's greater reactivity, 80% was found to have decomposed within 2 hours, while it survived only minutes at 80°C. Reaction of this Reformatsky reagent with various aldehydes at RT for between 30 minutes and 4 hours allowed various β-hydroxy esters to be obtained in good yield. While these conditions are much more amenable to the use of sensitive substrates than the reflux conditions employed with the bromo analogue,

the use of iododifluoroacetates has been restricted due to their high cost and limited availability.

The first Reformatsky reaction with imines was reported by Gilman and Speeter in 1943,[15] who used ethyl bromoacetate to synthesise 1,4-diphenyl-2-azetidinone from *N*-phenylbenzaldimine. The field then lay relatively dormant for another 15 years before further studies began to appear in the literature. However, it was not until the 1970s—when the pharmaceutical benefits of β-lactam-based antibiotics were beginning to be fully realised—that synthetic efforts into enolate-imine condensation reactions were stepped up. Most of these experiments focussed on non-enolisable *N*-arylaldimines, with the resultant β-lactams generally obtained in good yield under reflux conditions.[16] While β-amino esters are the initial products formed in these reactions, they can undergo a reversible cyclisation to the corresponding β-lactam. As a result, most Reformatsky reactions performed with imines tend to result in a mixture of cyclic and acyclic products. The relative ratios are largely dependent on the nature of the imine—aromatic *N*-substituents generally facilitate intramolecular cyclisation, with the inductive effects of ring substituents playing a further role by influencing the nucleophilicity of the nitrogen atom. Electron-donating groups such as *p*-MeO favour ring-closure to form the β-lactam, while electron-withdrawing groups like *p*-CF$_3$ draw electron density away from the nitrogen atom, retarding the cyclisation and leaving the β-amino ester as the major or even sole product.[17,18] The effect of an imine's *C*-substituent on cyclisation is much less predictable due to its remoteness from the nitrogen atom, with no strong pattern observable. Higher temperatures and longer reaction times generally promote cyclisation to the β-lactam,[17,19] so by choosing the appropriately *N*-substituted imine, the reaction conditions can generally be tailored to suit the desired outcome. It would be preferable for the purposes of this work to be able to synthesise the desired β-amino esters as the exclusive product. In the event that undesirable cyclisation occurs, the β-lactam can be subjected to a ring-opening reaction, but this of course adds an extra step to the synthesis.

Following on from earlier work with the Reformatsky reagent derived from iododifluoroacetate and its reaction with aldehydes, Kobayashi's group were the first to report the successful synthesis of difluorinated β³-amino compounds via

Reformatsky reaction in the form of 3,3-difluoro-2-azetidinones.[20] Using either a one-step ($BrCF_2CO_2Et$) or two-step (ICF_2CO_2Me) procedure, they were able to prepare a series of β-lactams (**236**) in yields ranging from 35-87%, with the two organozinc reagents giving comparable results (*Scheme 2*). Two of the imines featured the chiral α-methylbenzyl moiety as the *N*-substituent, however the stereoselectivity obtained in the reaction was not reported. Several years later, Angelastro *et al.*,[21] used a similar method to prepare an α,α-difluoro-β-amino propionate from an imine derived from benzylamine and isovaleraldehyde, with the β-lactam obtained in 73% yield.
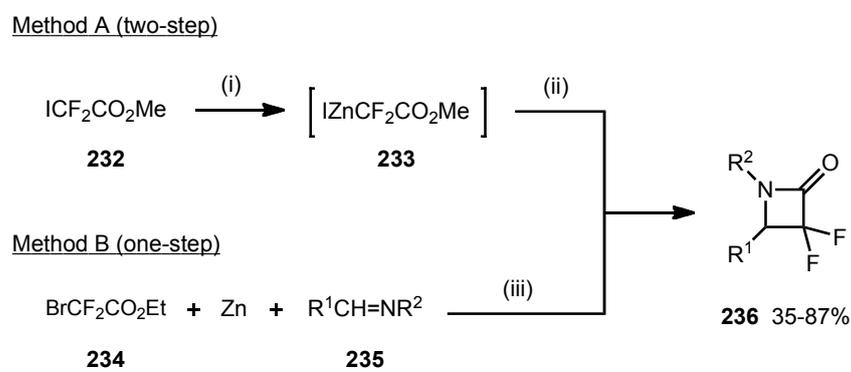
Scheme 2 Reagents and conditions: (i) Zn, THF, 0°C; (ii) $R^1CH=NR^2$, 0°C; (iii) THF, reflux.

It took another seven years for further work in this field to appear, with the Quirion group publishing their investigation of a new enantioselective route to α,α-difluoro-β³-amino acids via the Reformatsky reaction in 1999.[22] One of the drawbacks to the use of the Reformatsky reaction has historically been its lack of stereocontrol. The facial selectivity of attack on the electrophile by the achiral organozinc species is extremely difficult to control, thus producing a mixture of isomers epimeric at C3; in the absence of any stereochemical induction by a chiral imine (or imine equivalent), a 1:1 mixture of enantiomers should result. Quirion inferred from Kobayashi's paper that the diastereoselectivity obtained using α-methylbenzylamine as a chiral auxiliary was poor, so it was therefore decided to use 1,3-oxazolidines derived from chiral amino alcohols as the stereodirecting group. These cyclic compounds are stable imine equivalents that exist in

equilibrium with the corresponding imino alcohol, and had previously been shown to participate in organometallic reactions with high diastereoselectivities.[23] Treating the oxazolidines **237a-e**, derived from either (*R*)-phenylglycinol (when R^1 = Ph) or (*R*)-aminobutanol (when R^1 = Et), with 3.5 equivalents of $BrZnCF_2CO_2Et$ gave the 3,3-difluoroazetidin-2-ones (**238**) in good yield and excellent de (*Scheme 3*). It should be noted that in the case of the two aliphatic oxazolidines (**237c** and **237e**), yields were typically half those obtained when substrates featuring aromatic substituents were used, which is in general agreement with Kobayashi's results. The formation of a small amount (5%) of the non-cyclic product was only observed with **237b**. Subsequent conversion to the β-amino acid was dependent on the nature of R^1: with the β-lactam derived from phenylglycinol, ring-opening was achieved with 6M HCl in 71% yield, with *N*-deprotection occurring under hydrogenolysis conditions to give the acid in 90% yield. When R^1 was an ethyl group, an alternative route had to be found which involved conversion of the glycinol moiety to an enamide, which was then hydrolysed under acidic conditions to give the amino acids as their salts.

237a $R^1 = R^2 = Ph$	**238a** 65%, >99% de
237b $R^1 = Et, R^2 = Ph$	**238b** 69%, 96% de
237c $R^1 = Et, R^2 = {}^nC_5H_{11}$	**238c** 35%, 90% de
237d $R^1 = Et, R^2 = 2\text{-Furyl}$	**238d** 62%, 85% de
237e $R^1 = Et, R^2 = (E)\text{-CH}_3CH=CH$	**238e** 32%, 96% de
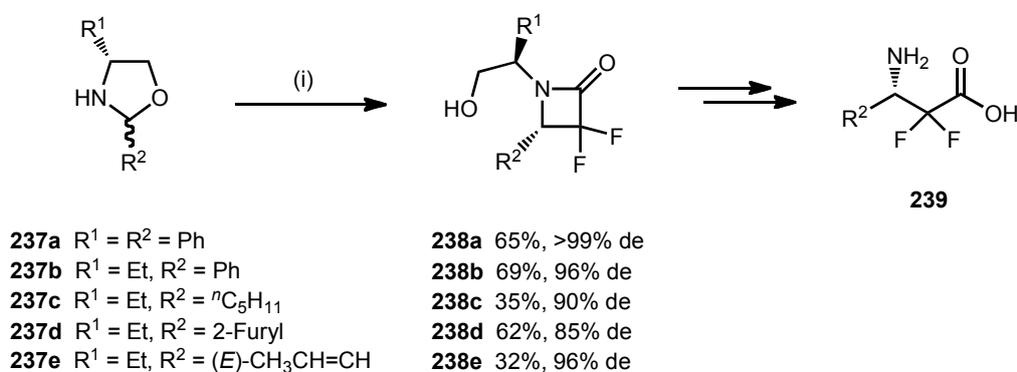
Scheme 3 Reagents and conditions: (i) $BrZnCF_2CO_2Et$ (3.5 eq.), THF, reflux, 1 hr.

The high level of diastereoselectivity observed in the Reformatsky reaction was rationalised using Pridgen's model[24,23] of a highly rigid intermediate in which the zinc of the alcoholate is chelated to the nitrogen atom (*Figure 1*). The steric hindrance created on one side by the phenyl or ethyl substituent results in the organozinc reagent attacking the less hindered side, which in this case gives the (3*S*)-β³-amino ester or lactam as the major product.
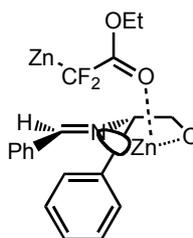
240

Figure 1 Strongly chelated intermediate proposed by Pridgen to be responsible for the high diastereoselectivity observed in the Reformatsky reaction with imines.

Eight years later the same group published a comprehensive study on the scope of this reaction, investigating a number of variables including reaction conditions (e.g., solvents, additives), the stereodirecting ability of several chiral imine auxiliaries, and the influence of the *C*- and *N*-substituents on yield, de, and β-lactam/β-amino ester ratios.[18] Given the fact that the β-lactams were the desired products, efforts were mainly focussed on optimising the reaction conditions in favour of their preparation. The standard solvent used in the Reformatsky reaction, THF, was found to favour β-lactam formation (89:11), as did the relatively non-polar DCM (93:7). Highly polar solvents such as DMF and DMSO had the reverse effect, favouring the acyclic product (34:66 and 11:89, respectively). This is believed to be due to their solvating properties and consequent influence on the aggregation of the organozinc reagent, which is known to be dimeric in THF and DCM solutions, and monomeric in DMSO.[25] Diluting the reaction 15-fold had largely no effect on the ratio. Lewis acids had a negative effect on cyclisation: the presence of the hard Lewis acid $MgCl_2$ resulted in a 38:62 ratio in favour of the β-amino ester, while the softer acid $ZnCl_2$ had a less pronounced effect (86:14). The addition of NaOEt completely inhibited the intramolecular reaction, with no evidence of β-lactam formation. The authors put forward two theories to explain these observed ratios—that the Lewis acid either stabilises the intermediate through chelation, or that it activates the carbonyl of the β-lactam, making it highly susceptible to ring-opening *in situ*.

In order to examine the substituent effects of the imine on both the β-lactam/β-amino ester ratio and the de of the resulting products, a series of experiments were undertaken sequentially varying either the aldehydic or *N*-substituent. Initial investigations sought to identify the most effective chiral auxiliary, as this would have a significant bearing on the stereochemical outcomes of subsequent experiments. A series of imines derived from one of three chiral auxiliaries—(*R*)-phenylglycinol (**241**), (*R*)-methoxyphenylglycinol (**242**), (*R*)-α-methylbenzylamine (**243**)—or an achiral *N*-substituent (4-methoxybenzylamine) were prepared, with each of these bearing phenyl, 4-pyridyl or 3-pyridyl groups as the *C*-substituent.

It was found with each of the R^1 substituents that the diastereoselectivity of the reaction decreased as the chelating power of the chiral auxiliaries decreased; only racemic mixtures were obtained with the achiral 4-methoxybenzylamine. This pattern was explained using the proposed transition state structures shown in *Figure 2*. The use of (*R*)-phenylglycinol creates a highly chelated cyclic transition state between the nitrogen and the zinc alcoholate, with steric hindrance resulting in the formation of a single isomer. Masking the hydroxy proton with a methoxy
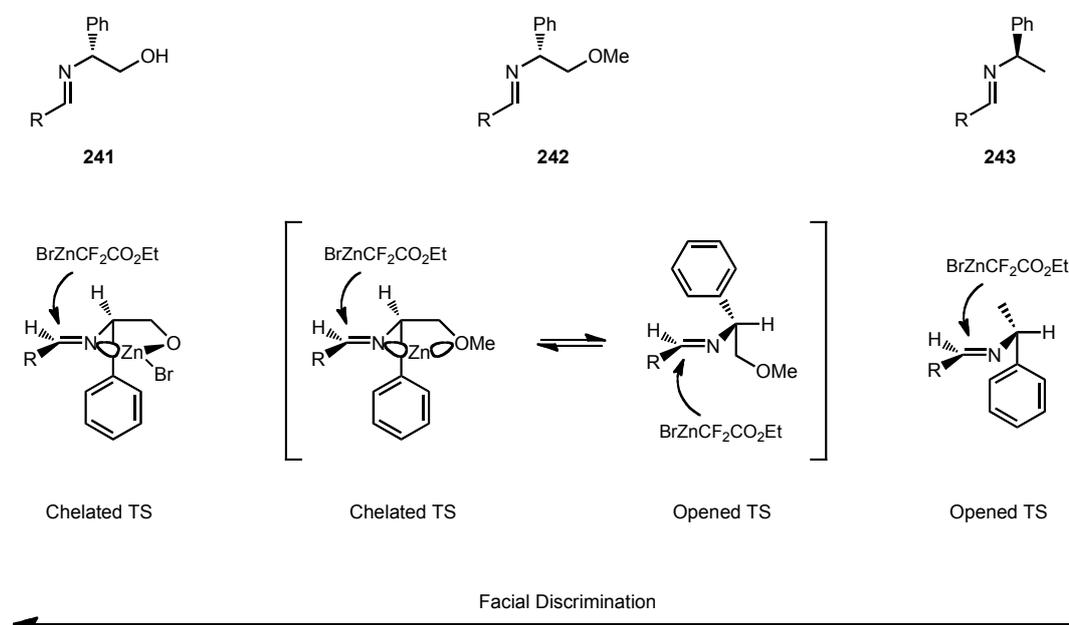
Figure 2 Transition states proposed by Quirion to explain the observed stereoselectivity in the Reformatsky reaction between chiral imines and $BrZnCF_2CO_2Et$.[22]

group results in a slightly diminished de, which is postulated to be a consequence of a less stable transition state that has the potential to open, exposing the chelate to nucleophilic attack from either face. The α-methylbenzylamine auxiliary features no such oxygen atom enabling chelation, with the open transition state responsible for a lack of facial discrimination and the production of de's between 23-55% for both the β-lactam and β-amino ester products.

The wide-ranging study of reaction conditions and parameters undertaken by Quirion *et al.* remains the only in-depth report published on the zinc-catalysed reaction of aldimines with ethyl bromodifluoroacetate under classical conditions, which is perhaps a reflection of the difficulties associated with the reaction. However, a number of variations that either aim to improve the yields and de of the notoriously fickle Reformatsky reaction, or are better suited to different applications like solid-phase synthesis,[26] have been developed over the last decade. These modifications typically involve the use of alternative electrophiles that behave as imine equivalents, for example the *N*-sulfinylimines of Soloshonok[27] and Staas[28] (Chapter 1) or the benzotriazole-mediated methods of Houghten[26] and Quirion.[29] However, these still tend to give poor yields and de's when applied to *C*-alkyl imines. The method attracting the most attention over the last decade has, however, been a rhodium-catalysed reaction using diethylzinc—the so-called Honda-Reformatsky reaction. This method comprises the majority of literature reports on the synthesis of α,α-difluoro-β³-amino esters from aldimines via a Reformatsky-type reaction, and even though it does not use classical conditions, much can be learnt from the imines and chiral auxiliaries employed and thus deserves further discussion.

4.1.1.1 The Honda-Reformatsky reaction

One of the biggest challenges associated with the Reformatsky reaction is the achievement of consistently high yields, a problem that generally relates to the use of zinc, a metal that is less reactive than others commonly used in organometallic reactions. Commercial zinc must be purified, or activated, before use to ensure that it reacts sufficiently with the haloacetate to form the organozinc species. This is typically a two-step process involving an initial acid wash followed by activation *in situ* before the haloacetate is introduced. A range of different methods for the

activation of zinc metal exist, ranging from chemical and electrochemical processes to physical techniques like sonication.[30] Among the popular chemical methods of activation are iodine,[31] 1,2-dibromoethane[32,33] and chlorotrimethylsilane[34], with a small amount (typically 5-10 mol %) of one or a combination[35] of these reagents being added to a suspension of the pre-washed zinc. These activation methods are usually sufficient for the formation of simple organozinc reagents, but more complex and difficult substrates often require the use of highly reactive zinc freshly prepared *in situ* under strictly anhydrous conditions. The process developed by Rieke[36,37] involving the reduction of zinc halides by alkali metals such as potassium or lithium is generally considered to be the supreme method for the production of reactive metals for organometallic syntheses. Wishing to exploit the fact that the use of such activated metal species prepared *in situ* generally allow reactions to be performed under milder conditions, Honda *et al.* examined the use of diethylzinc in a Reformatsky-type reaction mediated by Wilkinson's catalyst (RhCl[PPh3]3). Initially investigated using carbonyl compounds,[38] the mechanism is proposed to involve the oxidative addition of the bromoacetate into the rhodium complex, forming a Rh(III) species (*Scheme 4*). This then undergoes a transmetallation reaction with diethylzinc, regenerating Rh(I) and forming a zinc enolate, the active species responsible for nucleophilic attack on the electrophile.
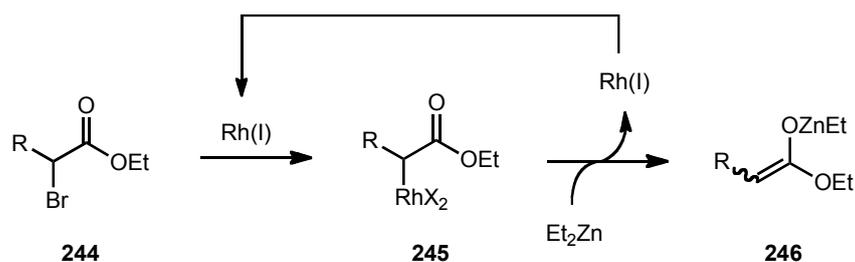
Scheme 4 Proposed mechanism for the Honda-Reformatsky reaction.

These conditions were subsequently applied to chiral imines based on the benzyl ether of (*R*)-phenylglycinol (**248**) for the one-pot preparation of unfluorinated β-amino esters (*Scheme 5*).[39] The β-amino esters were obtained in good yield, especially given the aliphatic nature of the aldehydes, and only one diastereomer was detected. The reaction took barely 10 minutes to go to completion at 0°C; in the absence of RhCl(PPh$_3$)$_3$, yields of only 10-20% were obtained, even after 2 hours.

247a R = Ph   **248**   **249a** 78%, >98% de   **250a** 58%
247b R = ^{i}Pr   **249b** 70%, >98% de   **250b** 46%
247c R = ^{n}Pr   **249c** 85%, >98% de   **250c** 39%
247d R = Cyclohexyl   **249d** 62%, >98% de   **250d** 40%
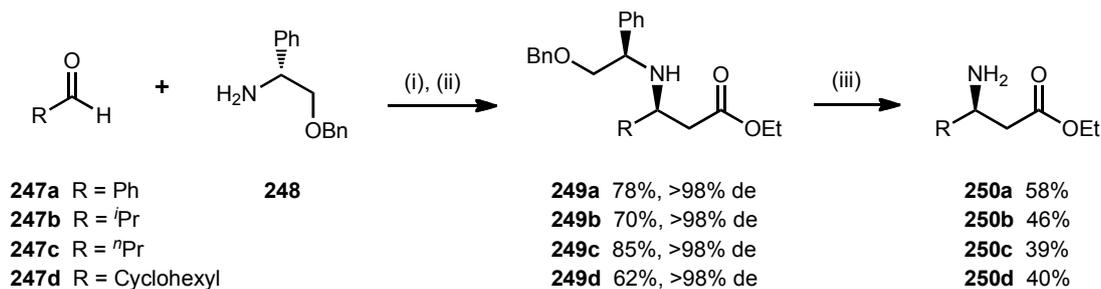
Scheme 5 Reagents and conditions: (i) 3Å MS, THF 0°C, 4 hr; (ii) BrCH$_2$CO$_2$Et (1.1 eq.), RhCl(PPh$_3$)$_3$ (5 mol %), Et$_2$Zn (4 eq.), THF, 0°C, 10 min; (iii) Pd(OH)$_2$/C, H$_2$ (4 atm), AcOH.

Of course, it could be anticipated that replacing ethyl bromoacetate with the difluoro analogue would have an adverse effect on the yield given the instability of the organozinc reagent. This was indeed the case when Fujii *et al*. applied the Honda-Reformatsky conditions to imines prepared *in situ* from (*R*)-methoxyphenylglycinol and isobutyraldehyde (*Scheme 6*) or TBSO-protected butanal (*Scheme 7*).[40] Attracted to Honda's method because the reaction conditions provided the acyclic product exclusively, they were pleased to find the desired β-amino esters were produced as single isomers. While the isobutyl-derived ester **253** was synthesised in a good 58% yield, the TBSO derivative **255** was lower yielding, obtained in 39% overall yield from the alcohol. The (3*S*) configuration of the products was confirmed by X-ray crystallographic analysis.
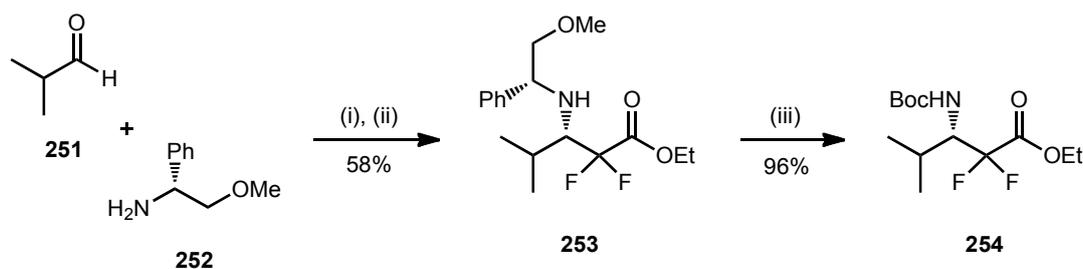
Scheme 6 Reagents and conditions: (i) MS 4Å, THF, 0°C; (ii) RhCl(PPh₃)₃ (5 mol %), Et₂Zn, BrCF₂CO₂Et, 0°C; (iii) Pd(OH)₂/C, H₂, Boc₂O, EtOH.
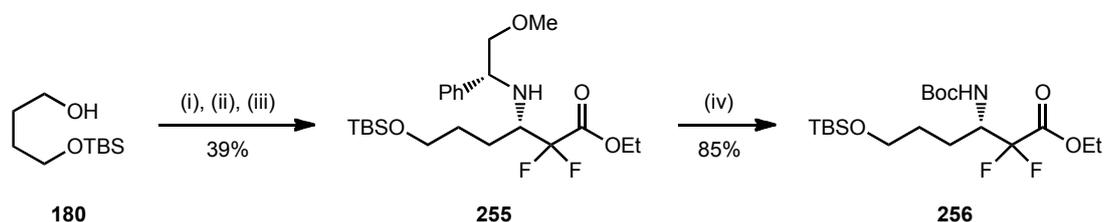
Scheme 7 Reagents and conditions: (i) (COCl)₂, DMSO, Et₃N, -78°C; (ii) MS 4Å, THF, 0°C; (iii) RhCl(PPh₃)₃ (5 mol %), Et₂Zn, BrCF₂CO₂Et, 0°C; (iv) Pd(OH)₂/C, H₂, Boc₂O, EtOH.

Meanwhile, Kumadaki *et al.* were independently conducting their own research into the use of the Honda-Reformatsky reaction with ethyl bromodifluoroacetate and aldimines.[41] However, a major point of difference was that they sought to prepare the difluorinated β-lactams, which they achieved in yields of 80-93% with various *N*-benzyl aromatic imines. These yields decreased substantially upon application of the reaction conditions to aliphatic imines: an *n*-hexyl derived imine gave only 35% after 18 hours, with a *tert*-butyl derivative showing no signs of reaction after 17 hours. Intrigued by the fact that Fujii's experiments resulted in the exclusive formation of the β-amino ester and not the lactam, and noting that the only difference was that Fujii's method was carried out in one pot in the presence of molecular sieves, they undertook a study investigating the presence of various additives on the cyclic to acyclic product ratio. It was found that a small amount of water present in the molecular sieves inhibited the cyclisation reaction, with suggestions that water may act to quench the zinc amide intermediate. The same

group also found that imines derived from unprotected (*R*)-phenylglycinol were suitable for the reaction, giving the (3*S*)-β-lactams in 29-74% yield.[42]

The application of the Honda-Reformatsky conditions to the synthesis of these α,α-difluoro-β³-amino esters demonstrates that good yields and excellent selectivities can be obtained with imine substrates. Fujii's method is particularly favoured for aliphatic imines, where the one-pot experimental conditions eliminate the need to isolate the unstable imine intermediates. However, scrupulously dry conditions generally need to be maintained, and the use of the expensive RhCl(PPh₃)₃ catalyst and the highly hazardous diethylzinc can limit its usefulness, particularly in large scale applications. A straightforward method without the need for special reagents was sought for this work, and as Quirion's approach favoured the preparation of β-lactams instead of the esters, an investigation into a new method for the asymmetric synthesis of α,α-difluoro-β³-amino esters was embarked upon.

4.2   Synthetic Strategy

As demonstrated by the introductory examples, the Reformatsky reaction is an excellent tool for the synthesis of α,α-difluoro-β³-amino esters, but generally only when the substrates are β-aryl substituted. However, the likely outcome is the formation of the β-lactam product, which requires a further ring-opening step to access the β³-amino ester. As the two amino acids that are the target of the work presented in this thesis are lysine and arginine—both of which feature aliphatic side chains—the existing methods needed to be adapted to suit these substrates. The main problem faced was the instability of the fluorinated organozinc reagent, with its rapid decomposition at reflux temperatures allowing a maximum of 15 minutes of reaction time.[12] A solution to this problem was found by Altenburger and Schirlin, who sought to prepare a series of α,α-difluoro-β-hydroxy esters *en route* to peptidic thrombin inhibitors.[43] Using unreactive nitro-substituted aldehydes, they found that pre-preparation of the BrZnCF₂CO₂Et reagent and the use of sonicating conditions increased yields from 0% to as high as 80% (*Scheme 8*). Not only could the organozinc reagent be prepared at room temperature under these conditions, but it was also found to be stable and could even be stored at -18°C for 24 hours.
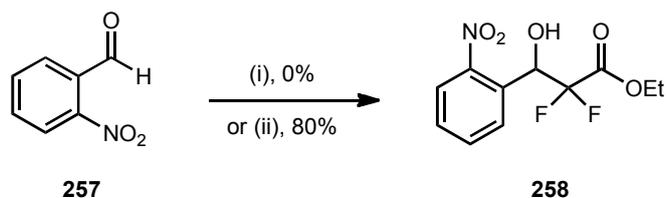
Scheme 8 Reagents and conditions: (i) BrCF₂CO₂Et, Zn, THF, reflux (one step); (ii) BrZnCF₂CO₂Et, THF, ultrasound (two steps).

Despite the fact that this ultrasound method was reported in 1991, it appears to have been seldom used since then. Maffre *et al.*[44] generated the reagent in this way and then treated it with a chiral aldehyde to give the product in high yield, while Coe *et al.*[45] prepared and used the organozinc reagent derived from ethyl 2-bromotetrafluoropropanoate under sonicating conditions, also using aldehydes. Neither of these methods involved fluorinated Reformatsky reagents together with imines, which, as mentioned above, tend to have poorer reactivities when compared to carbonyl compounds.

The use of sonication as an aid in the Reformatsky reaction had already been well documented prior to the publication of Altenburger and Schirlin's paper, with the first report by Boudjouk and Han appearing in 1982.[46] Their investigations into the so-called 'sonocatalysed' Reformatsky reaction of carbonyl compounds with ethyl bromoacetate revealed that zinc activated by ultrasound gave results at least comparable to those obtained when using zinc prepared using the Rieke method, and far exceeded those obtained using conventional reflux conditions. Since that time sonication has found widespread use[47-51] as a means of increasing the yields and reaction rates of the typically low-yielding Reformatsky reaction while bypassing the need to prepare the costly Rieke zinc. While simple laboratory cleaning baths remain the major source of ultrasonic irradiation because of their low cost and availability, specialised high intensity ultrasound (HIU) probes inserted directly into the reaction mixture have been used to further improve yields.[52,17,53] The means by which ultrasound improves the outcome of a reaction has been attributed to two main factors: firstly, it acts as an alternative energy source to thermal heating, which is based on the growth and subsequent violent collapse of cavitation bubbles within the solvent. Each bubble acts as a kind of

microreactor, in which temperatures of several thousand degrees and pressures in excess of one thousand atmospheres can be generated.[54] The second effect is more mechanical in nature and results from both a deaggregation of the metal particles as well as a pitting of the oxide-covered zinc surface, which exposes fresh and highly reactive sites.[55]

Given the well-established benefits of ultrasound on the Reformatsky reaction, it was decided to apply it to the zinc-mediated reaction of ethyl bromodifluoroacetate with aldimines. As the condensation of these two reagents under sonicating conditions was yet to be reported in the literature the outcome was unpredictable, although it was hoped that the lack of thermal energy could prevent cyclisation from occurring. Anything approaching the yields and de's obtained using the other methods would be considered a success, especially if a method requiring no special additives or alternative imine-like electrophiles could be developed.

The second problem was the issue of stereoselectivity, which was almost universally inferior with aliphatic imines than it was with *C*-aryl imines. This was best typified by the *tert*-butyl and tolyl chiral sulfinylamines (Chapter 1, page 25), where de's fell to between 60-76% when the substrates featured aliphatic substituents. As demonstrated throughout Section 4.1.1, the amino alcohol (*R*)-phenylglycinol (or derivatives thereof) has already proven to be an extremely effective chiral auxiliary, particularly under Honda-Reformatsky conditions, where a single isomer was typically observed. However at the time of writing, the cost of (*R*)-phenylglycinol was found to be AUD$447 for 25 grams, with the cost of the (*S*)-isomer even higher at AUD$52 for a single gram.[56] Given that cost was a factor in our efforts to devise a new synthetic technique, and the expense of these reagents may pose significant problems upon scale-up, it was decided to explore other options. It became apparent that the methyl ester hydrochloride salt of (*R*)-phenylglycine could be obtained for only AUD$43 per 25 grams, with the (*S*)-isomer available for AUD$35 per 10 grams. Evidence that it had the potential to be an effective chiral auxiliary is provided by the fact that the freebase had previously been used in organometallic enolate condensation reactions with extremely high stereocontrol (products obtained in >98% de).[57-59] This is despite the propensity of phenylglycines to epimerise at the α-carbon,[60,61] a fact which typically precludes

their use as chiral auxiliaries. Furthermore, with the methyl and benzyl ethers of phenylglycinol difficult to obtain commercially, the use of the phenylglycine ester would remove the need for the extra synthetic step to protect the phenylglycinol hydroxy group. Despite the presence of the ester functional group in the proposed auxiliary, this new method was still expected to give the (3S)-β³-amino ester (as shown in *Scheme 9*) in accordance with the accepted transition state model.[18] It should be noted that, despite the fact that the Reformatsky reaction with the organozinc reagent prepared from ethyl bromodifluoroacetate is relatively fast, should the reaction conditions promote epimerisation of the phenylglycine ester's α-carbon before addition occurs, a mixture of enantiomeric products would result.
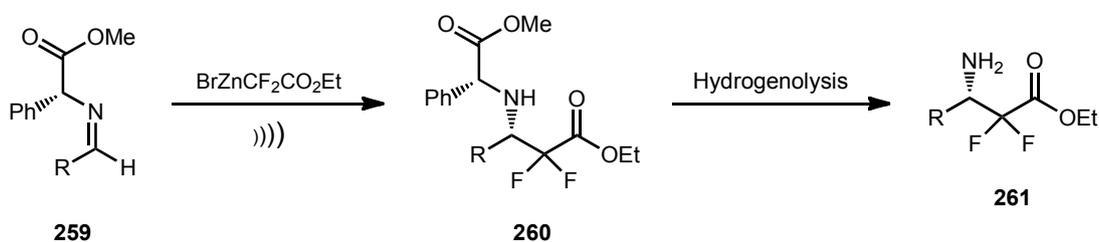
Scheme 9 Proposed route to β-amino esters **261** via sonocatalysed Reformatsky reaction.

Anticipating the successful synthesis of these Reformatsky products, it was then envisaged that the chiral auxiliary could be removed under hydrogenolysis conditions to give the free amines. With the configurationally unstable phenylglycine moiety cleaved immediately following the Reformatsky reaction, the problem of isomerisation under other conditions should be irrelevant. In the event that epimerisation were to occur, the fact that the α-carbon stereocentre does not comprise part of the target structure means that a mixture of the two diastereomers could be reduced to a single enantiomer upon cleavage of the auxiliary.

4.3   Results and Discussion

4.3.1   Optimisation of Reformatsky reaction conditions

The first task in establishing the optimal conditions was to identify the most suitable solvent in which to perform these Reformatsky reactions, with the chosen solvent ideally providing a combination of good yields and high stereoselectivity. To allow a fair comparison of the solvent effects an appropriate model compound had to be selected. The fact that efforts were specifically directed towards the synthesis of β-alkyl-β³-amino esters necessitated the use of an aliphatic aldehyde from which to prepare the aldimines. An ideal reagent was isovaleraldehyde, which featured a number of valuable properties in addition to it being chosen for its aliphatic side chain: it is cheap and readily available, while possessing a sufficiently high boiling point (90°C) to allow for easy handling and purification. Secondly, the isopentyl moiety gives highly characteristic splitting of peaks in the ^{1}H NMR spectrum, which lends itself to easy identification of reaction by-products or diastereomers upon analysis of the mixture after work-up.

With a model substrate identified it was time to prepare the imine and subject it to the Reformatsky reagent in the presence of ultrasound. Rather than the one-pot method of Fujii that involved the use of molecular sieves, which remained in the mixture during the Reformatsky reaction itself, it was decided to isolate the imine before its addition to a pre-prepared solution of $BrZnCF_2CO_2Et$. Addition of the neat aldehyde to a solution of (*R*)-phenylglycine methyl ester in DCM was undertaken at 0°C to help prevent polymerisation of the resulting imine. A seven-fold excess of Na_2SO_4 was used to help drive the reaction forward by absorbing the H_2O formed as a by-product of the condensation reaction. With granular Na_2SO_4 as the dehydrating reagent, filtration of the crude imine solution through a cotton wool-filled pipette was found to be sufficient purification. In light of the low stabilities of aliphatic imines, further purification was not undertaken due to the likelihood of decomposition.

When using imines as the electrophile in asymmetric syntheses, the reaction is further complicated by the fact that imines usually exist as a mixture of geometrical isomers, with the more stable (*E*) geometry generally favoured. As the C=N bond is relatively weak, the energy barrier to interconversion between the (*E*) and (*Z*)-isomers is quite low and, as a result, it is not often possible to isolate isomerically pure materials.[62] This could have a significant effect on the stereoselectivity of the reaction, particularly if nucleophilic attack can occur from either face of the imine, in which case four isomers could result. In the case of these asymmetric Reformatsky reactions, attack by the organozinc reagent occurs predominantly from the *Re* face, with the stereochemical outcome of the reaction demonstrated in *Figure 3.* The geometry of the C=N double bond dictates the configuration of the C3 stereocentre in the product—the *anti* conformation is obtained when the imine exists as the (*E*)-isomer, while addition to the (*Z*)-isomer results in the *syn* conformation.
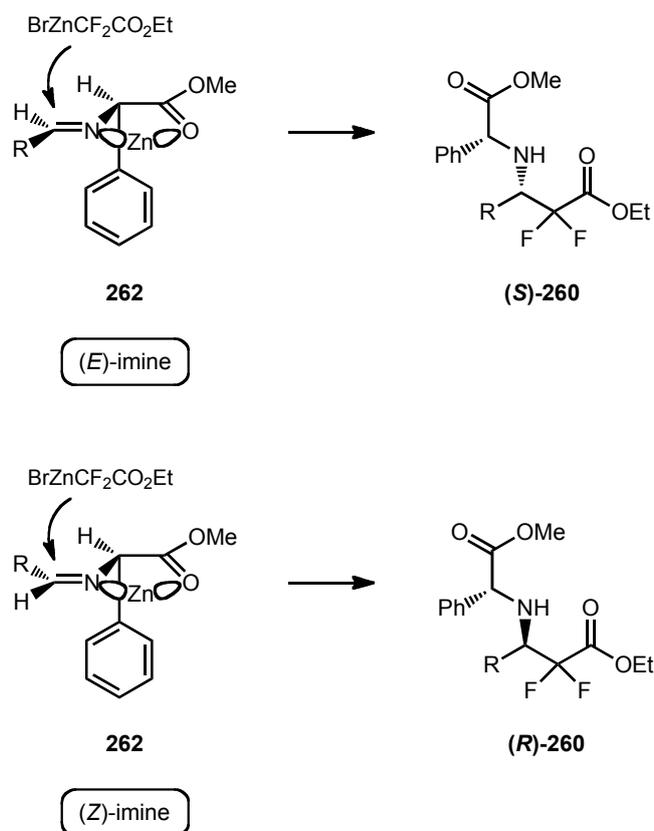
Figure 3 The stereochemical outcome of *Re* face attack
on both (*E*) and (*Z*)-imines in the Reformatsky reaction.

Given the impact that the imine geometry can have on the dr's of the reaction products, it was important to ascertain the E/Z ratio of the imine derived from phenylglycine methyl ester. ^{1}H NMR spectroscopy performed on the crude imine in K_2CO_3-neutralised $CDCl_3$ failed to show any evidence of the minor isomer, indicating it was isomerically pure. Also pleasing was the fact that only minor amounts of decomposition products were present, even after standing for several hours. The geometry of the C=N bond was confirmed as (E) through NOESY experiments. The steric bulk of the phenylglycine auxiliary is likely to be responsible for the high isomeric ratio observed, particularly in conjunction with the branched C-isopentyl substituent. As the isomeric ratio is highly dependent on solvent,[63,64] a solution of the imine in the aromatic solvent benzene-d_6 was also analysed using ^{1}H NMR spectroscopy. Peaks due to the minor isomer were difficult to identify, although the amount of (E)-isomer appeared to be relatively unchanged and was thus estimated to be ≥95%.

Satisfied that the imine was essentially one isomer and was being formed in very high yield, attention was turned to the Reformatsky reaction. When preparing the difluorinated Reformatsky reagent, a moderate excess of zinc (typically 2-3 equivalents with respect to the imine) is used, with the amount of ethyl bromodifluoroacetate generally ranging from 1 to 2 equivalents. These excesses are required to contend with both the low reactivity of the zinc and the instability of the organozinc species once formed. The initial reaction conditions employed in this work used an arbitrary imine:Zn:$BrCF_2CO_2Et$ molar ratio of 1:3:2 in accordance with the general ratios reported in the literature. Another common feature of these literature procedures is the use of iodine as an activator. The positive effect that iodine has on the reaction is well documented,[31,30] with its addition to the reaction mixture now considered somewhat essential for the reaction to proceed in high yield. I_2 is believed to play dual roles by both activating the surface of the zinc as well as suppressing enolisation of the intermediates.[46] Thus 0.24 equivalents of I_2 (8 mol % with respect to Zn) was added to a suspension of acid-washed zinc in dry THF and sonicated for 15 minutes as a means of further activating the zinc surface. The brown colour of the iodine disappeared within a few seconds of ultrasound being applied to the suspension, indicating a prompt reaction. After this initial 15 minute activation period, neat ethyl bromodifluoroacetate was added to the

suspension while maintaining sonication under N_2. A brief induction period of generally between 5-10 seconds was observed before a violent exotherm ensued. Sonication was maintained for a further 2 minutes to ensure complete reaction of the haloacetate, after which time a solution of the crude imine in THF was added. Sonication was continued for 15 minutes, with the development of a green/yellow colour over the course of the reaction in line with the colour changes commonly observed in the Reformatsky reaction. This general procedure, represented in *Scheme 10*, was repeated in each subsequent experiment unless otherwise stated.
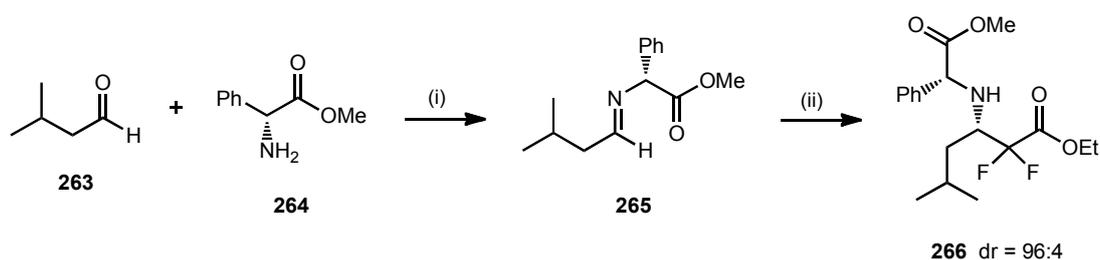
Scheme 10 Reagents and conditions: (i) Na_2SO_4, DCM, 0° to RT, 3 hr; (ii) Zn, I_2, $BrCF_2CO_2Et$, THF, ultrasound.

^{1}H NMR spectroscopic analysis of the crude reaction mixture revealed the desired β-amino ester **266** to be the major product—no resonances indicating the presence of the corresponding β-lactam were visible. Furthermore, a dr of 96:4 was determined through integration of the α-proton resonance of the phenylglycine moiety—the major isomer of **266** exhibited a sharp doublet at 4.67 ppm, while the corresponding peak for the minor isomer resonated slightly further upfield at 4.58 ppm. The excellent dr observed appeared to vindicate our choice of the phenylglycine ester as an effective chiral auxiliary. At this point the absolute stereochemistry of the major product was still unknown, however was tentatively assigned as the (3*S*)-isomer based on literature precedent (later experiments would provide justification for this (Section 4.3.2.1)).

It was assumed that these two diastereomers were epimeric at C3, where the configuration is determined from stereofacial attack of the organozinc reagent. However, there was a possibility that these two diastereomers were instead epimeric at the α-carbon of the phenylglycine moiety. For this to be possible the α-

carbon would have to epimerise after the product has formed from attack by the organozinc species; racemisation before attack on the imine would produce (3*R*,α*S*)-**266** (*Figure 4*), which would be indistinguishable from the enantiomeric (3*S*,α*R*)-**266**—the major product expected from this reaction—in the NMR spectrum. Furthermore, the amount of the minor (3*S*,α*S*)-**266** isomer generated in this way (i.e., from the epimerised imine) would be negligible when compared to the amount of (3*R*,α*S*)-**266**, assuming that the degree of racemisation was minimal. It was therefore unlikely to be visible in the ¹H NMR spectrum. While (3*S*,α*S*)-**266** could also be formed as a result of epimerisation of the phenylglycine moiety after product formation, the carbon atom in question did not appear susceptible to epimerisation under these conditions—as mentioned on the next page, prolonged sonication of the reaction mixture resulted in an unchanged dr, suggesting that the products were configurationally stable under these conditions. It is of course possible that the isolated product was not enantiomerically pure, however chiral chromatography techniques were unavailable at the time and further derivatisation through the use of chiral reagents such as Mosher's acid[65] was not pursued. However, given the apparent configurational stability of the products, the (3*S*,α*R*):(3*R*,α*S*) ratio of enantiomers was assumed to be high.
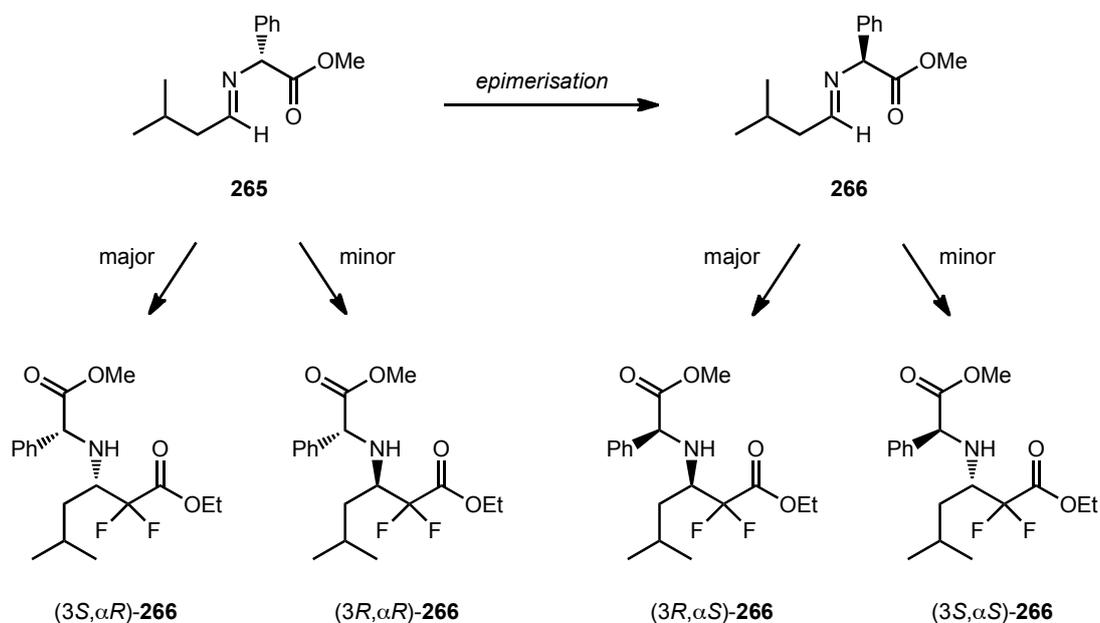
Figure 4 The possible isomers formed as a result of epimerisation in the Reformatsky reaction.

β³-Amino ester **266** was ultimately obtained in 53% yield from the aldehyde, and was isolated as a single diastereomer after column chromatography. Verifying that this was not the cyclic product, the CH_2 and CH_3 proton resonances of the ethyl ester were observed at 4.32 ppm and 1.35 ppm, respectively, while the NH proton resonated as a broad triplet at 2.24 ppm. Finally, HRMS was used to support our spectroscopic analysis, returning a molecular ion of m/z = 358.1830 for $[M+H]^+$.

In an effort to try to improve the 53% yield a number of variations to the method were undertaken, with the results displayed in *Table 1*. Firstly, the experiment was repeated but the sonication time doubled to 30 minutes (entry 2). This had a beneficial effect on the yield, which was increased to 64%, while the dr remained largely unchanged, suggesting that the prolonged exposure to the reaction conditions had little effect on the stereochemical integrity of the product. As the reaction appeared to have ceased after 20 minutes, the reaction time for the following experiments was modified accordingly. In addition, as a large excess of the unreacted bromoacetate appeared to be present in the ^{1}H NMR spectrum of the crude material, the amount of haloacetate used was decreased from 2 to 1.5 equivalents.

Diethyl ether has historically been one of the solvents of choice for the Reformatsky reaction, but surprisingly the yield fell to 45% with a concomitant loss of stereoselectivity (80:20). The use of 1,4-dioxane, another ethereal solvent, resulted in an even sharper drop in yield to only 19%, although the high dr found with THF (entries 1 and 2) was maintained when this solvent was used. The tendency for 1,4-dioxane to promote unwanted enolisation under classical Reformatsky conditions[66] could provide a possible explanation for this low yield.

Other solvents popular in the Reformatsky reaction are the aromatic hydrocarbons. Thus, the procedure was carried out in dry toluene, but again disappointing results were obtained (21%, dr 75:25). As iodine forms a visibly purple charge-transfer complex with toluene, which may have interfered with the reaction, 1,2-dibromoethane was instead used to activate the zinc (entry 6). Interestingly, upon work-up of the reaction mixture and ^{1}H NMR analysis, only unreacted imine was observed, indicating no reaction (including by-product formation) had occurred at

all. This was in contrast to the iodine-catalysed reaction, which despite producing only 21% of the desired product, was sufficiently more activated (as evidenced by the formation of a number of by-products). This result reinforced the notion that iodine is an essential component in these reactions.

Two of the less commonly used solvents, DCM and acetonitrile (entries 7 and 8) both gave similar results, surprisingly outperforming diethyl ether. Both the yields and dr's still fell short of those obtained in THF, however. Finally, the use of dipolar aprotic solvents was examined (DMSO and DMF, entries 9 and 10 respectively). Running the experiment with DMSO resulted in significant decomposition—in fact not even unreacted bromoacetate was visible in the ^{1}H NMR spectrum of the crude material. DMF, which is known to react with the organozinc reagents,[63] gave a slightly better yield of the difluoro β-amino ester **30**, although it was accompanied by very limited stereocontrol. The geometrical instability of imines in certain solvents may play a role in some of the low dr's observed, with addition to both (*E*)- and (*Z*)-isomers resulting in a mixture of diastereomers.

Table 1 Effect of solvent on yield and diastereoselectivity in the Reformatsky reaction.

Entry	Solvent	Yield of **266** (%)	dr[a]
1	THF[b]	53	96:4
2	THF[c]	64	96:4
3	diethyl ether	45	80:20
4	1,4-dioxane	19	95:5
5	toluene	21	75:25
6	toluene[d]	0	-
7	DCM	52	86:14
8	MeCN	47	85:15
9	DMSO	trace	-
10	DMF	25	59:41

[a] crude, determined by ^{1}H NMR; [b] 2 eq. $BrCF_2CO_2Et$; [c] sonicated for 30 min.; [d] Activated with 1,2-dibromoethane

4.3.1.1 Chiral auxiliary optimisation

These results clearly established THF as the optimal solvent in which to perform these sonocatalysed Reformatsky reactions, however one problem had become apparent over the course of the solvent studies: a small quantity (typically 10-15%) of an unknown by-product was continually observed in the ^{1}H NMR spectrum of the crude reaction mixture. Isolation of this product via column chromatography followed by 1D and 2D NMR analysis eventually identified it as the bis-ethyl ester **267** (*Figure 5*), in which the methyl ester of the phenylglycine moiety had been replaced with an ethyl ester. Contributing to the initial difficulties in identifying this product was the fact that the two methylene protons of this new ethyl ester moiety do not resonate as a simple quartet, as is the case for most ethyl esters, but as a complex doublet of doublets. The diastereotopic behaviour of these protons suggests a high degree of intramolecular hydrogen bonding, resulting in restricted rotation. The generation of ethoxide anions as a result of self-condensation between two molecules of BrZnCF$_2$CO$_2$Et, with the anions then attacking the methyl ester, is the most likely mechanism for the formation of **266**. Of course, the ethyl ester adjacent to the CF$_2$ moiety is likely to be highly susceptible to nucleophilic attack given its high electrophilicity, but substitution by the ethoxide species would obviously not change the structure of the final product. Interestingly, formation of the product that would result from attack of the organozinc reagent on this electrophilic ethyl ester was not apparent from spectroscopic analyses of the crude reaction mixture. This suggests that the Reformatsky reagent possesses only moderate nucleophilicity, indicating that some sensitive functional groups may be able to withstand the reaction conditions.

266   **267**
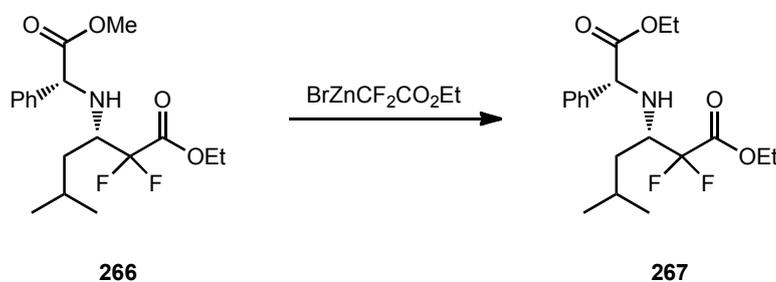
Figure 5 Bis-ethyl ester by-product formed during the reaction.

Although the substitution of the methyl ester made no difference to the core structure of the target compounds, it did reduce yields. To make the carbonyl group less susceptible to nucleophilic attack, the *tert*-butyl ester of phenylglycine was prepared in 35% yield via the dropwise addition of perchloric acid to a solution of (*R*)-phenylglycine (**268**) in *tert*-butyl acetate.[67] Both the ^{1}H and the ^{13}C NMR data were in agreement with literature reports, while the optical rotation of $[\alpha]_D^{20}$ = - 98.1 (c = 1.61, CHCl₃) was identical to that recorded by others.[68] Upon subjecting this ester to the optimal Reformatsky conditions used with the phenylglycine methyl ester, the product **272** was obtained in 48% yield as an 86:14 mixture of isomers, which is a significant loss of selectivity when compared to the methyl ester **266** (*Scheme 12*). Compounding the disappointing result was the fact that ^{1}H NMR analysis of the crude reaction mixture revealed a ~1:1 ratio of β-amino ester **272** to the β-hydroxy ester **273**, which was also prepared independently in a separate Reformatsky reaction with isovaleraldehyde to verify its identity. This unwanted by-product results from incomplete formation of the imine **271**, which may be caused by the additional steric bulk of the *tert*-butyl ester, leaving a significant amount of the aldehyde in the crude mixture. Increasing the reaction time for imine formation from 3 hours to 5 hours did little to improve the ratio of the two products.
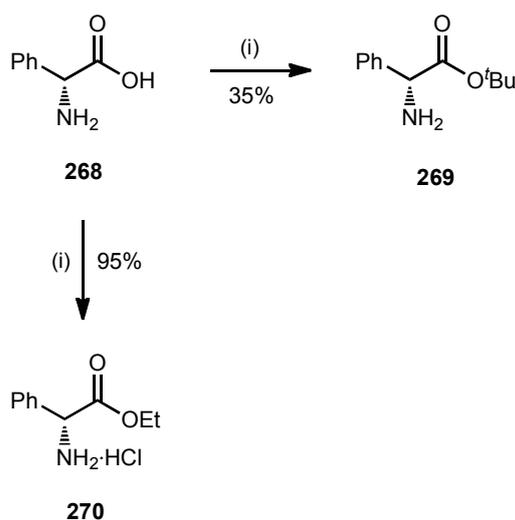
Scheme 11 Reagents and conditions: (i) HClO₄, *tert*-butyl acetate, 0°C; (ii) SOCl₂, EtOH.
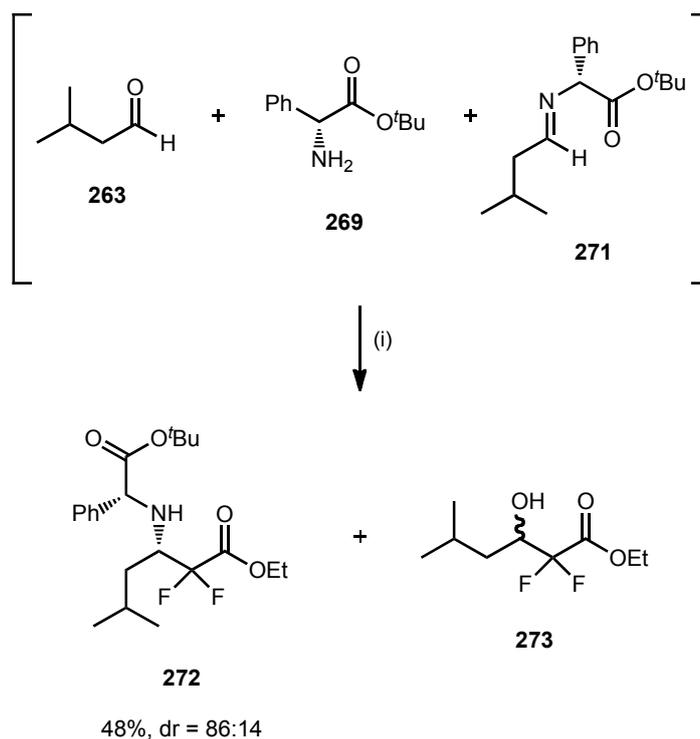
Scheme 12 Reagents and conditions: (i) BrZnCF₂CO₂Et, THF, ultrasound.

With the *tert*-butyl ester failing to improve the yield it was decided to perform the reaction with the ethyl ester of phenylglycine, which would negate the effects of attack by the ethoxide anion. (*R*)-Phenylglycine ethyl ester was prepared in 95% yield from the amino acid using EtOH/SOCl₂ as per standard procedures (*Scheme 11*),[69] and isolated and stored as the HCl salt (**270**). The freebase was obtained as needed by washing with saturated aqueous NaHCO₃. ¹H NMR data and optical rotation measurements both supported the successful synthesis of the enantiopure ethyl ester. On conducting the Reformatsky reaction with the imine **274**, the product was obtained in an improved 72% yield (*Scheme 13*), as compared to the 64% obtained with the methyl ester imine (**265**). Furthermore, the high diastereoselectivity of the reaction was maintained, with a dr of 96:4 observed upon ¹H NMR analysis of the crude material, which was increased to ≥99:1 upon chromatographic purification of the product. As expected, the ¹H NMR spectrum of the product (**267**) exhibited similar features to that of methyl ester **266**, with the exception of the new ethyl ester peaks, which appeared at 4.32 ppm and 1.17 ppm.

The only other major point of difference was the phenylglycine α-proton peak, which now resonated as a singlet at 4.64 ppm rather than the doublet that appeared in the spectrum of **266**. Associated with this was a loss of splitting of the NH peak, which now appeared as a very broad singlet at 2.35 ppm rather than a triplet. Interestingly, the two isopropyl CH$_3$ groups are exposed to different chemical environments, resonating individually at 0.83 ppm and 0.45 ppm, indicating a lack of free rotation within the molecule. The ^{13}C NMR spectrum also displayed characteristic splitting as a result of the CF$_2$ moiety: the α-carbon resonated as a triplet at 117.6 ppm (J = 254 Hz), while the β- and γ-carbon peaks also featured small couplings (22.9 and 3.1 Hz, respectively). Interestingly, the carbonyl carbon adjacent to the CF$_2$ moiety resonated considerably higher upfield than the phenylglycine carbonyl carbon (164.0 ppm versus 172.9 ppm), which is indicative of the shielding effects provided by the electron density surrounding the two fluorine atoms. Finally, a molecular ion of m/z = 394.1800 for [M+Na]$^+$ was obtained upon ESI HRMS, supporting the structural assignment for **267**.
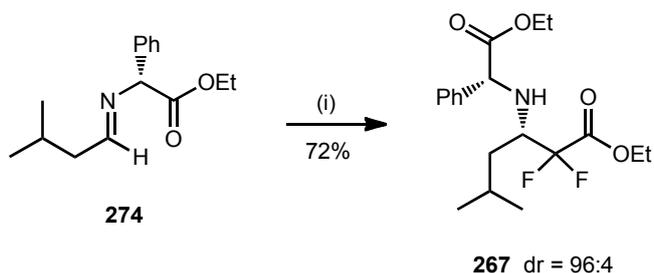
Scheme 13 Reagents and conditions: (i) BrZnCF$_2$CO$_2$Et, THF, ultrasound.

With the ethyl ester of phenylglycine identified as the preferred auxiliary, several modifications to the standard conditions were investigated to try to improve the yield. The use of two equivalents of BrCF$_2$CO$_2$Et increased the yield by only 4% (to 76%), with the associated increase in the difficulty of chromatographic purification offsetting the minor benefit on yield. An increase in the amount of zinc dust to four equivalents also had little impact on yield or dr. At this stage it may be interesting to note that zinc dust that had not been sufficiently purified (e.g., not fully neutralized or still containing traces of water) was found to have quite a negative

impact on the dr of the reaction, the reason for which is unknown. Lastly, a one-pot Reformatsky reaction in which the organozinc reagent is not pre-formed before the imine is introduced was examined. Addition of neat ethyl bromodifluoroacetate to a mixture of zinc dust and the imine in THF made little difference to the yield, with **267** obtained in 71% yield. However, the stereoselectivity of the reaction fell sharply, with the dr of the crude reaction mixture decreasing from the 96:4 obtained in the two-pot procedure to 82:18. One possible explanation may involve isomerisation of the imine: exposing the imine to ultrasound before the reaction takes place may provide enough energy to isomerise the C=N bond and provide minor amounts of the less stable (*Z*)-isomer, which could survive for long enough to react and give (3*R*)-**267**. With the imine added directly to a solution of the organozinc reagent prompt reaction should occur, minimising the time allowed for isomerisation of the imine.

4.3.1.2   The effect of Lewis acids on the reaction

Although the dr of the Reformatsky reaction using (*R*)-phenylglycine ethyl ester was already extremely high, there was still room for some improvement to make the reaction completely stereoselective. As Lewis acids are commonly added to enolate condensation reactions to facilitate high selectivity, it was thought that such reagents might be of benefit to this Reformatsky reaction. The first choice was the soft Lewis acid $ZnBr_2$, with one equivalent added to the imine solution to ensure that the two reagents were introduced to the Reformatsky reagent simultaneously. Sonication for 20 minutes followed by the usual work-up gave a crude reaction mixture that was identified as comprising of a 71:29 mixture of isomers according to ^{1}H NMR analysis. Chromatographic purification allowed the isolation of the major isomer in 34% yield, while the minor isomer was isolated in 16% yield. While the decrease in yield to 50% was disappointing, the most surprising result was the loss of stereoselectivity. Zinc bromide was then replaced with the strong Lewis acid $BF_3 \cdot OEt_2$ and the experiment repeated. However, upon spectroscopic analysis the crude reaction mixture was found to have an even poorer dr of 60:40, although the two diastereomers were isolated in a combined 67% yield. A similar result was observed with the comparably strong Lewis acid $TiCl_4$, which gave a dr of only 57:43. The presence of these additional chelating agents appeared to significantly disrupt the standard chelated transition state generated in the

Reformatsky reaction. If this resulted in a conformational change to a less sterically hindered intermediate, the nucleophile would be less discriminating in its facial attack and a mixture of isomers would result. The theory proposed by Waldmann and Braun[70] during their work on asymmetric tandem Mannich-Michael reactions of amino acid ester imines provides a good rationale for the loss of stereoselectivity observed. In their model (*Figure 6*), which was based on experimental observations, one equivalent of ZnCl₂ effectively chelates the imine nitrogen and the ester carbonyl group (**A**), however the use of two equivalents results in the formation of a complex in which the imine and carbonyl groups are each complexed to a molecule of the Lewis acid, thus breaking the chelation (**B**). In addition to this, they also discovered through NMR experiments that the imine double bond has a tendency to isomerise in the presence of Lewis acids, which as discussed previously, leads to the formation of two diastereomeric products.
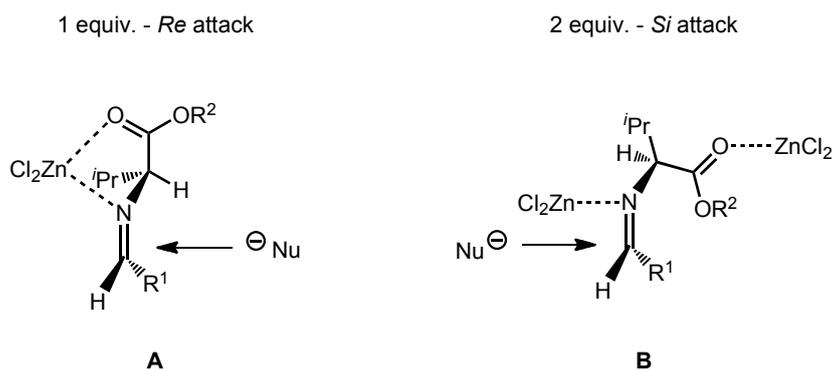
Figure 6 The model proposed by Waldmann and Braun to explain the difference in stereoselectivity observed when using one (**A**) and two (**B**) equivalents of a Lewis acid with amino ester imines.[70]

The effect that a larger excess of a strong Lewis acid had on the reaction was also investigated. Thus three equivalents of BF₃·OEt₂ were added to the imine solution and the experiment conducted according to the standard procedure. The excess acid was found to have little further impact on the diastereomeric ratio of the products—in fact the dr actually increased slightly to 64:36, which may have just resulted from minor variations in reaction conditions. It was surprising to note, however, that the yield increased from 67% with only equivalent of BF₃·OEt₂ to

81% when three equivalents were used. This suggests that while the Lewis acid has a detrimental effect on stereoselectivity, it does help facilitate the reaction, perhaps by bringing the two reacting species into closer proximity. This would enable the desired reaction to occur before decomposition or by-product formation.

Finally, to gauge the merits of the newly developed Reformatsky reaction against those already established in the literature, two additional experiments were performed. The first employed (*R*)-phenylglycinol (**275**)—the widely accepted auxiliary of choice—as the chiral component of the isovaleraldehyde-derived imine. This imine was given the standard 3 hours to form before being isolated in the usual manner. In slightly modified Reformatsky conditions, whereby 2.1 equivalents of $BrCF_2CO_2Et$ were used to account for deprotonation of the glycinol hydroxy group of **276**, disappointing results were obtained. Only trace amounts of the desired β-amino ester **277** were detected upon ^{1}H NMR analysis of the crude reaction mixture, with the main species found to again be the β-hydroxy ester **273**, resulting from addition to isovaleraldehyde. Strangely, the equilibrium in the imine condensation reaction appeared to strongly favour the starting materials. Attempting to overcome this by allowing the aldehyde and amine time to react overnight also failed, with **277** isolated in only 8.5% yield, with **36** again predominating (*Scheme 14*). These results highlight the incompatibility of (*R*)-phenylglycinol with these reaction conditions.
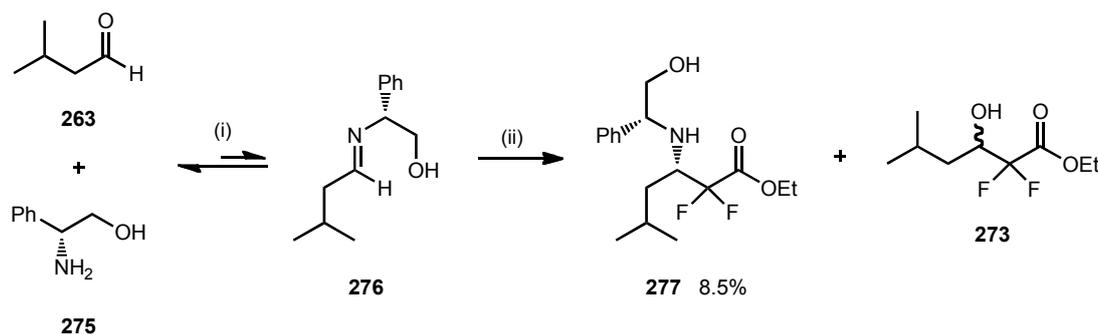
Scheme 14 Reagents and conditions: (i) DCM, Na_2SO_4, 0° to RT, overnight; (ii) $BrZnCF_2CO_2Et$ (2.1 eq.), THF, ultrasound.

The next experiment aimed to compare the effects of ultrasound with the reflux conditions of the classical Reformatsky reaction. Under these conditions, a suspension of acid washed zinc dust in THF was activated by heating at reflux with I_2 for 15 minutes. The mixture was then allowed to cool slightly before the addition of neat ethyl bromodifluoroacetate, which resulted in a visible exotherm almost immediately. After 30 seconds, a solution of the crude imine was added and the mixture stirred at reflux for a further 8 minutes before being removed from the heat and quenched. Analysis of the crude reaction mixture using ^{1}H NMR spectroscopy revealed the presence of a 63:37 mixture of the desired β-amino ester **267** and β-lactam **279** (*Scheme 15*), which were eventually isolated in 57% and 34% yields respectively. Each appeared to be present as a single isomer. In contrast to ultrasound, the application of thermal energy to the substrate clearly favours cyclisation, with the ratio of lactam to ester almost certain to approach 100:0 should the reaction time be extended beyond the short 8-minute time frame. Although high-yielding overall, the reaction requires chromatographic separation of the two products, with an additional ring-opening step necessary to convert the β-lactam to the β-amino ester.
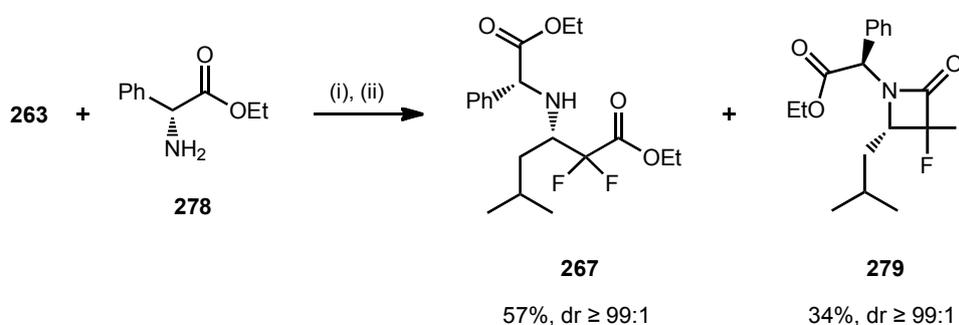
Scheme 15 Reagents and conditions: (i) DCM, Na$_2$SO$_4$, 0° to RT, 3 hr; (ii) BrZnCF$_2$CO$_2$Et, THF, reflux, 8 min.

4.3.2 Application to aliphatic imines

In order to test the generality of the newly developed reaction conditions within the alkyl series, a number of aliphatic aldehydes possessing branched, non-branched and cyclic substituents were obtained either commercially or by oxidation of the corresponding alcohol using standard Swern[71] or PCC[72] techniques. These aldehydes were synthesised or purified immediately before use. In each experiment a 1:3:0.2:1.5 molar ratio of imine:Zn:I$_2$:BrCF$_2$CO$_2$Et was employed, with ultrasound applied to the reaction mixture for 20 minutes. The results are summarised in *Table 2*. Under these conditions, Reformatsky reaction with an isobutyraldehyde-derived aldimine resulted in an isolated yield of 66% (**280**, entry 2). The high stereoselectivity previously observed with isovaleraldehyde was maintained, with a dr of 98:2 observed upon ^{1}H NMR spectroscopic analysis of the crude reaction mixture. Similarly pleasing results were obtained with those imines derived from octanal (**281**) (entry 3; 70% yield, dr = 94:6) and 3-phenylpropanal (**283**) (entry 4; 75% yield, dr = 96:4). The imine prepared from cyclohexanecarboxaldehyde (**285**), which was synthesised from the alcohol in 95% yield via Swern oxidation, displayed somewhat poorer reactivity, most likely due to steric hindrance of the imine carbon. The cyclohexyl-substituted ester **286** was obtained in only 46% yield, although the bulky ring likely contributed to the excellent selectivity of dr = 98:2 that was obtained. Application of the conditions to an alkene, 4-pentenal, (entry 6) gave surprising results: the β-amino ester **288** was obtained in 97% yield, with a crude dr of 97:3. The reason for the exceptional success of this experiment when compared to the other, lower-yielding substrates is difficult to determine. One possible explanation could involve coordination of zinc to the olefin bond, perhaps bringing the reagents together before decomposition or side-reactions can occur. Although zinc-olefin complexes are rare, weak inter- and intramolecular interactions between zinc and carbon-carbon double bonds have been observed in some non-conjugated systems.[73] In particular, NMR studies have revealed the existence of weak interactions between 1-pentene and Zn,[74] supporting the notion that coordination may play a role in facilitating this particular reaction. The structural identity of each of the α,α-difluoro-β³-amino esters prepared in this manner was determined by ESI HRMS and ^{1}H, ^{13}C and ^{19}F NMR spectroscopic analysis, with each displaying similar spectral characteristics to those of the isovaleraldehyde-derived **267** described earlier.

Table 2 Results of the sonocatalysed Reformatsky reaction using imines derived from (*R*)-phenylglycine ethyl ester (**278**) and a series of aliphatic aldehydes.

Entry	Aldehyde	α,α-Fluoro-β-amino ester	Yield (%)[a]	dr[b]
1	**263**	**267**	76	98:2
2	**251**	**280**	66	96:4
3	**281**	**282**	70	94:6
4	**283**	**284**	75	96:4
5	**285**	**286**	46	98:2
6	**287**	**288**	97	97:3
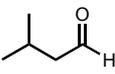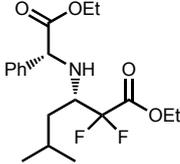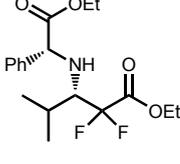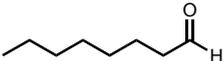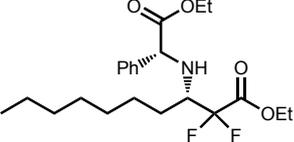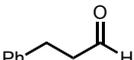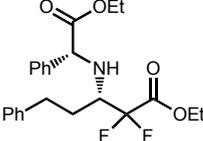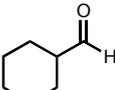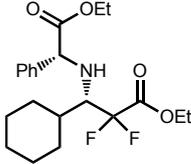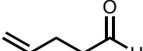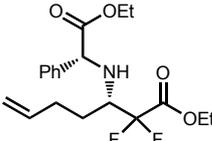
Table 2 (continued)

7		45[c]	96:4
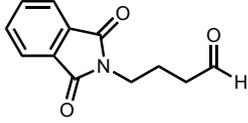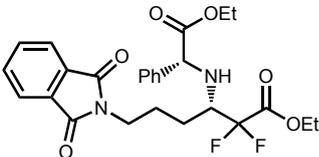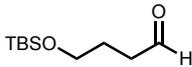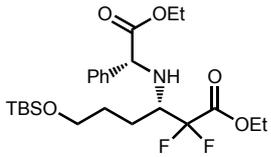
8		53[d]	96:4
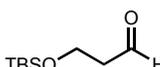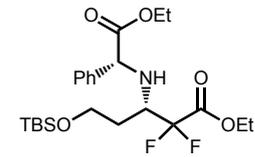
9		52[d]	95:5

[a] Isolated yield; [b] Crude, determined by ^{1}H NMR; [c] 2:1 diethyl ether/THF used as the solvent;
[d] Magnetic stirring used instead of sonication

Confident that these sonocatalysed Reformatsky reactions were generally high yielding and maintained an overall excellent level of stereocontrol, the method could now be applied to more highly functionalised aldimines. This would allow the preparation of α,α-difluoro-β³-amino esters possessing side chains amenable to further synthetic manipulation. The first such compound was based around the phthalimide protecting group, which despite not surviving the highly basic conditions of the conjugate addition reaction, was predicted to be resistant to the somewhat milder Reformatsky conditions. The synthesis of 4-phthalimidobutyraldehyde (**289**) initially involved the preparation of the dimethyl acetal (**295**) in 92% yield from *N*-carbethoxy phthalimide (**294**).[75,76] This stable aldehyde precursor was then hydrolysed in dilute acid as needed to give **296** in ≥97% purity (as determined by ^{1}H NMR analysis) after a simple aqueous work-up. The ^{1}H and ^{13}C NMR spectra were in agreement with literature data.
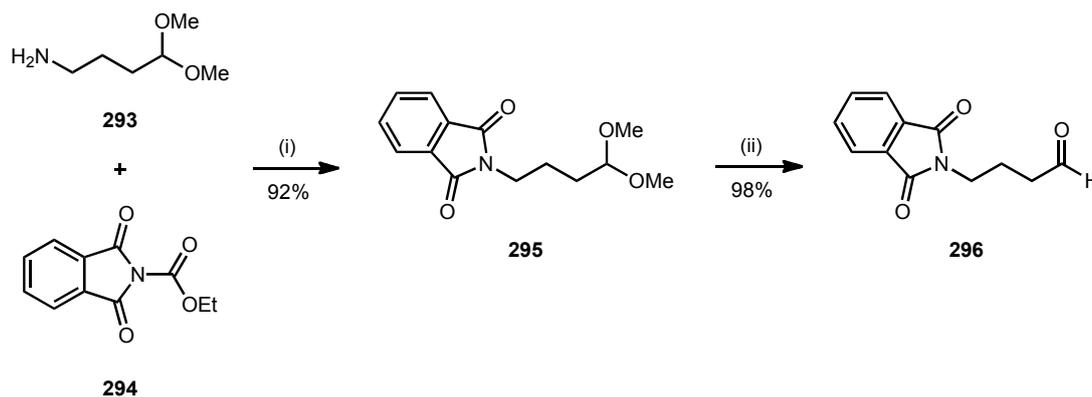
Scheme 16 Reagents and conditions: (i) Et₃N, THF, 0°C, 2.5 hr; (ii) acetone, 1M HCl, reflux, 30 min.

Due to the moderate reactivity of the phthalimide moiety, it was likely that the Reformatsky reaction conditions would need to be even further optimised to prevent degradation. The first experiment was performed under relatively standard conditions, with ultrasound applied to the mixture for 25 minutes. Work-up and chromatographic purification resulted in a mixture of β-amino ester **290** and an inseparable by-product. Although the exact yield of **290** was incalculable because of the presence of this by-product, it was estimated to be around 40% (^{1}H NMR). To determine whether the modest yield was a result of the phthalimide decomposing during the reaction, the experiment was repeated but sonication maintained for only 12 minutes. Comparing the ^{1}H NMR spectrum of the crude material with the corresponding spectrum obtained in the previous experiment, the two mixtures appeared to be nearly identical in composition, both with dr's of 95:5. This indicates that the desired reaction is essentially complete in ≤12 minutes. In this case, however, **290** was isolated in 43% yield as a single isomer with no sign of the by-product observed previously. The by-products observed in the NMR spectra of the crude product mixtures could not be successfully identified, but it was possible that the phthalimide moiety was subject to ring-opening reactions by the excess Reformatsky reagent. In order to decrease the amount of nucleophile present, the molar ratio was modified so that only two equivalents of zinc and 1.2 equivalents of ethyl bromodifluoroacetate were used. In addition, the reaction time was further shortened to 8 minutes to try to prevent the degradation. This modification resulted in a largely unchanged yield, although the ^{1}H NMR

spectrum of the crude mixture did appear to show fewer by-products. Performing the sonication at a lower temperature (0-10°C) for 20 minutes also failed to help, and resulted in a 35% yield. A different strategy was then devised in which the organozinc reagent was prepared using ultrasound, but the flask then removed and magnetic stirring maintained before and after imine addition. The first experiment to use these new conditions employed 30 minutes of stirring time, and resulted in a yield of 40%, with the stereoselectivity of the reaction relatively unchanged (94:6 crude dr). Repeating this experiment at 0°C for 45 minutes gave a similar result (35% yield), however stirring the mixture at -20°C for the same length of time resulted in a poor yield of only 9%. Furthermore, the lower temperature had a negative effect on stereoselectivity, which decreased to 78:22. Finally, a mixed solvent system of 2:1 diethyl ether/THF was trialled, with the mixture sonicated for 15 minutes after imine addition. These conditions resulted in the highest yield of **290** obtained thus far (45%), but still failed to give the substantial improvement sought.

An alternative to the phthaloyl functionalised imine was the TBSO protected analogue. This protecting group had already proven stable under the harsher conditions of the conjugate addition reaction, so it was anticipated that it would also provide good results in the Reformatsky reaction. The requisite TBSO-protected aldehyde **171** was synthesised immediately before use as previously described in Chapter 2 (page 70). The aldehyde was sufficiently pure to be used crude, in which case a slight molar excess was added to phenylglycine ester **278**, with any of the unreacted volatile aldehyde removed in vacuo after imine formation. Initially applying the standard reaction conditions to gauge the stability of this imine relative to the other imines, a significant degree of decomposition was unfortunately observed. Predicting that, like the phthaloyl imine, the TBSO substrate would also behave unfavourably when exposed to different temperatures and reaction times, it was decided to use magnetic stirring in place of sonication as a means of keeping the reaction conditions as mild as possible. This immediately proved beneficial: addition of the imine solution to the sonochemically prepared organozinc reagent followed by 15 minutes of stirring at RT provided β-amino ester **291** in a reasonable 53% yield. This improves upon the 39% yield obtained for the TBSO-protected analogue prepared by Fujii under Honda-Reformatsky

conditions,[40] with ours achieved without the aid of an additional catalyst.

The shorter chain analogue **292**, corresponding to the side chain length present in β-arginine, was also prepared in a similar manner, although on a scale seven times larger. Initially employing 3 equivalents of zinc and 1.5 equivalents of ethyl bromodifluoroacetate, with stirring maintained for 25 minutes, the β-amino ester was isolated in only 36% yield after chromatographic work-up. The molar ratio was then modified slightly to 2.5 equivalents of zinc and 1.25 equivalents of ethyl bromodifluoroacetate in an effort to reduce the amount of nucleophile present in the reaction mixture. This, combined with a reduction in reaction time to only 15 minutes, resulted in a substantially improved yield of 52%. As with **291**, the condensation of the two species was highly stereocontrolled, with a dr of 95:5 determined by ^{1}H NMR analysis of the crude reaction mixture. The results obtained for **291** and **292** demonstrate the scalability of the reaction, with equivalent yields and diastereomeric ratios being obtained for these compounds.

4.3.2.1   Deprotection of the chiral auxiliary

Unfortunately each of the α,α-difluoro-β³-amino esters synthesised were viscous oils and could not be crystallised. Several attempts at preparing crystalline 2,4-DNP or picrate derivatives of the Reformatsky products also failed. This meant that a crystal on which to perform X-ray diffraction analysis could not be obtained to confirm the absolute configuration of the C3 stereocentre. Instead, a comparison of optical rotations with known compounds from the literature had to be used. Of the nine β³-amino esters prepared using this Reformatsky method, two were analogous to substrates prepared by Fujii—the β-isopropyl ester **280** and the TBSO protected ester **291**. Although Fujii used a different chiral auxiliary the core structures of the products are the same, with each featuring the same side chain and the ethyl ester moiety. Thus, removal of the phenylglycine auxiliary from these products and reprotection of the free amine as the Boc derivative, as performed by Fujii (*Scheme 6*,*Scheme 7*),[40] would enable the comparison of optical rotation values between identical compounds.

As discussed in Chapter 3, the removal of *N*-benzyl substituents is frequently carried out with $Pd(OH)_2$/C as a hydrogenolysis catalyst in the presence of H_2. Phenylglycine derivatives are more complex than simple *N*-benzylamines because of the extra substitution but they can still be cleaved using hydrogenolytic techniques.[40,77] However, the steric hindrance around the α-carbon can lead to lengthy reaction times or the need for higher pressures of H_2.[39] Phenylglycinol auxiliaries have also been successfully cleaved in high yield using the alternative catalyst Pd/C.[78,79] Employing a one-pot hydrogenolysis/Boc-protection method analogous to that of Fujii, **291** was stirred in the presence of 1.25 equivalents of Boc_2O and a catalytic amount of $Pd(OH)_2$/C under 1 atm of H_2 (*Scheme 17*). Monitoring the reaction by TLC proved difficult as the starting material and the hydrogenolysis by-product ethyl phenylacetate **294** had nearly identical R_f's, making it difficult to ascertain whether the reaction had gone to completion. After two days the mixture was filtered, with ¹H NMR analysis of the residue indicating that the reaction had proceeded with essentially quantitative conversion. The Boc protected β-amino ester **256** was obtained in 81% yield after chromatographic purification. The effective removal of the phenylglycine auxiliary was evident from ¹H and ¹³C NMR spectroscopic analysis, with no peaks visible in the aromatic region of the spectra and only one ethyl ester moiety now present. These resonances were replaced by a large peak integrating for nine protons at 1.42 ppm in the ¹H spectrum, and at 25.9 ppm in the ¹³C NMR spectrum, which was attributable to the Boc *tert*-butyl group. The carbamate NH peak also shifted downfield accordingly, with the proton resonating as a broad doublet at 4.62 ppm. ESI HRMS analysis was used to support the structural assignment, producing a molecular ion of m/z = 448.2316 which corresponded to [M+Na]⁺ for **256**.

291 R = TBSO(CH₂)₃
292 R = TBSO(CH)₂
280 R = ⁱPr

256 81%
293 85%
254 76%

294
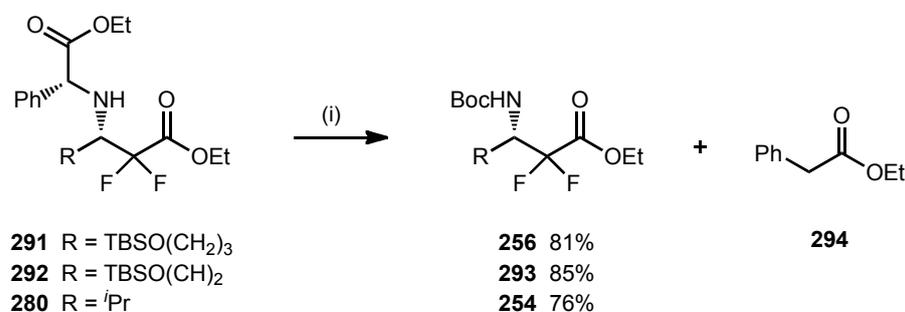
Scheme 17 Reagents and conditions: (i) $Pd(OH)_2$/C, EtOH, Boc_2O, H_2.

With the identity of **256** established, its optical rotation was measured twice using samples prepared in two separate experiments and found both times to be $[\alpha]_D^{20}$ = -5 (c = 1.0, CHCl$_3$). While the magnitude of the rotation is substantially smaller than that measured by Fujii ($[\alpha]_D^{22}$ = -13.9 (c = 1.16, CHCl$_3$)), the negative sign of the rotation supports the assertion that both β³-amino esters possess the same configuration at C3. It is doubtful that the low $[\alpha]_D$ value obtained in our case results from a mixture of enantiomers as the hydrogenolysis process is unlikely to result in epimerisation of the β-carbon. Small differences in concentration and the temperature at which the measurement was recorded may be partly responsible, but it is also impossible to determine the accuracy of the value obtained by Fujii. In an effort to provide further evidence for the (3*S*)-configuration, the isopropyl substituted β-amino ester **280** was also subjected to similar hydrogenolysis conditions, with stirring under H$_2$ maintained for a total of 20 hours. After work-up and chromatography the Boc protected amine **254** was isolated in 76% yield. Again, the successful substitution of the phenylglycine ethyl ester moiety for Boc was clearly evident upon ESI HRMS and NMR spectroscopic analysis, with the *tert*-butyl, amide and ethyl ester protons all resonating where expected, and analogous to the signals observed for the corresponding TBSO protected substrate. The optical rotation was found to be $[\alpha]_D^{20}$ = -3 (c = 1.0, CHCl$_3$), which again is much lower than that obtained by Fujii ($[\alpha]_D^{23}$ = -10.9 (c = 1.01, CHCl$_3$)), although the negative sign of the rotation was maintained. Together with the previous literature reports demonstrating that addition of organozinc reagents to analogous (*R*)-phenylglycine systems occurs almost exclusively with *Re* facial attack,[59,57,22,18,39,40,77] the stereochemistry of the major β-amino esters formed in the sonocatalysed diastereoselective Reformatsky reaction were assigned as (3*S*).

Finally, although not previously reported in the literature, the Boc derivative of the other TBSO protected β-amino ester, **292**, was prepared in a similar manner and obtained in 85% yield. With yields for the hydrogenolysis of the three substrates averaging around 80%, the phenylglycine ethyl ester was deemed sufficiently labile for use as an effective chiral auxiliary.

4.4   Conclusions

The results described here for the sonocatalysed Reformatsky reaction between aliphatic imines and ethyl bromodifluoroacetate demonstrate that such alkyl-substituted α,α-difluoro-β³-amino esters can be prepared in good to very high yield using zinc-mediated ester enolate-imine condensation methodology. Moreover, β³-amino esters bearing side chains with heteroatom functionalities that enable further chemical manipulation can also be prepared in synthetically useful yields. Unlike many literature reports detailing the preparation of such compounds from aliphatic imines, this new technique proceeds with exceedingly high stereocontrol, with each of the products easily isolated as a single isomer after purification. This is in part due to the effectiveness of the (*R*)-phenylglycine ester **278** as a chiral auxiliary—not only was this cost effective reagent highly stereocontrolling and stable to the reaction conditions, but it was also easily removed using standard hydrogenolysis techniques. Its ability to maintain the stable (*E*)-geometry of the imine C=N double bond and thus control the diastereoselectivity of the reaction is also highly advantageous. In contrast, the commonly used (*R*)-phenylglycinol failed under identical reaction conditions, reacting poorly with the aldehydes and thus giving only minor amounts of the desired fluorinated β³-amino esters. Of course, the ester derivatives of phenylglycine are notably prone to epimerisation at the α-carbon under basic conditions—unlike phenylglycinol—however as an auxiliary designed to withstand one key reaction it seems to serve the purpose for which it was intended. However, measuring the ee of the reaction products to determine the extent of any epimerisation of the phenylglycine auxiliary would also be recommended before this auxiliary could find more general use.

Once cleaved, the free amine can easily be reprotected as a more stable derivative or used as a scaffold on which to build more complex molecules. Although the two Boc/TBSO protected substrates **256** and **293** were not further functionalised, their conversion to compounds analogous to the β³-lysine and β³-arginine compounds prepared in Chapter 3 is predicted to be relatively straightforward. As this would involve similar synthetic transformations to those seen previously, the synthesis of similar orthogonally protected amino acids was not pursued further.

Although the benefits of using ultrasound to achieve high-yielding Reformatsky reactions had already been well established with either imine electrophiles or halodifluoroacetates, the technique had yet to be used in experiments featuring these two types of reagents together. Conducting the experiments in a simple laboratory ultrasonic cleaning bath proved invaluable in the selective formation of the desired acyclic β³-amino esters, with no trace of the corresponding β-lactams observed in the crude mixtures. This is in contrast to the classical reflux conditions typically employed in such transformations; under these conditions a mixture of the cyclic and acyclic products was obtained, which is undesirable when taking into account the extra ring-opening step required should the β-lactam be converted to the ester. The fact that ultrasonic waves provide a high amount of energy whilst maintaining relatively mild reaction conditions through the absence of thermal energy most likely plays a part in the high yields obtained here. The lifetimes of the reactive intermediates are prolonged under these conditions, as opposed to the short lifetimes experienced at reflux temperatures, where the substrates readily decompose. The additional reaction time provided to the imine and the enolate ensures a much more successful outcome.

This new technique certainly fills a gap in the current field pertaining to the preparation of β-alkyl α,α-difluoro-β³-amino esters in high yield and dr. Given the common laboratory reagents and equipment used it is hoped that the technique can find wider use amongst the synthetic community.

4.5 References

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CHAPTER 5:

PREPARATION OF

N^{β}-ACETYL-β^3-ARGININE ANALOGUES

5.1   Introduction

As discussed in Chapter 1, the interaction of fluorinated molecules with enzymes, more specifically their binding efficacy, is known to depend on both the number of fluorine atoms present and the configuration of the fluorinated carbon (in the case of monofluorides), with *gem*-difluoro compounds often much more active than the corresponding monofluoro analogues.[1-3] In order to conduct similar enzyme inhibition assays as those performed by Ohba[1] and Bromfield[4,5] with fluorinated phenylalanine derivatives (Chapter 1), three β-arginine derivatives featuring methylene (CH$_2$), fluoromethine (CHF) or difluoromethylene (CF$_2$) groups alpha to the carbonyl were sought. These substrates would help probe the influence that the degree of α-fluorination has on binding within the active site of the serine protease trypsin. Furthermore, the preparation of these compounds would provide an excellent opportunity to further demonstrate the versatility of the newly developed conjugate addition and Reformatsky methodologies.

5.2   Synthetic strategy

The work discussed in Chapter 3 demonstrated how suitably functionalised β^3-amino esters prepared from the conjugate addition of chiral lithium amides to α,β-unsaturated esters could be transformed into α-fluoro-β^3-arginine derivatives. An identical route can be applied to the synthesis of the analogous unfluorinated β^3-arginine, with the only difference being that the intermediate enolate formed in the conjugate addition reaction is quenched with NH$_4$Cl rather than NFSI. Preparation of the desired difluorinated β^3-arginine analogue via the Reformatsky reaction would be expected to take a slightly different route—although the chosen Reformatsky product possesses similar TBSO and *N*-benzyl functionalities to the conjugate adducts, the presence of the ethyl ester moiety, which has a substantially different reactivity profile to *tert*-butyl esters, would require alternative conditions to achieve the desired outcome. In keeping with enzyme inhibition studies mentioned above, the target substrates **295-297** (*Figure 1*) were to feature *N*-acetyl protection of the β-amino group and a methyl ester protecting the carboxyl group. The guanidino moiety was to remain unprotected to mimic the interaction of endogenous arginine-based ligands with the enzyme's binding sites.
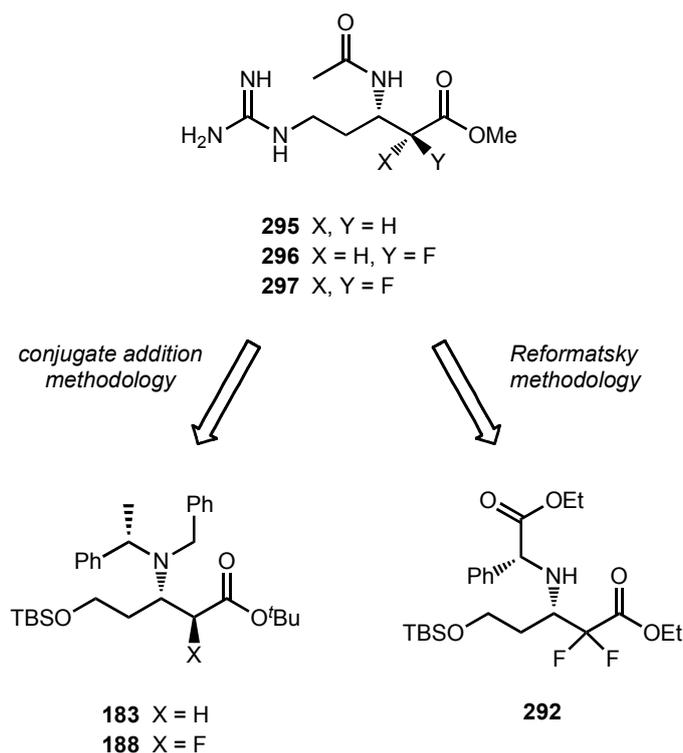
Figure 1 Synthetic routes to *N*-acetyl-β³-arginine derivatives.

Both routes would involve an initial cleavage of the chiral auxiliaries followed by *N*-acetylation. Not only is the acetyl group stable to a range of conditions, including acids and bases, it also provides the orthogonal protection required for the installation of the guanidino group. This group, protected as the di-Cbz derivative, was to be introduced in a Mitsunobu reaction identical to that described in Chapter 3. Transesterification to the methyl ester would be the penultimate step in each route followed by hydrogenolysis of the Cbz protecting groups, an extremely clean reaction that should eliminate the need for purification of the highly polar final product.

5.3   Results and discussion

5.3.1   Synthesis of (*3S*)-*N*-acetyl-β³-arginine methyl ester

With the unfluorinated conjugate adduct **183** prepared as described in Chapter 2, the first task was the deprotection of the β-amino moiety and reprotection as the *N*-acetyl derivative (*Scheme 1*). Pearlman's catalyst again proved highly effective in the hydrogenolysis of the *N*-benzyl groups, with the reaction taking only 2.5 hours at atmospheric pressure in the presence of a catalytic amount of AcOH. The crude primary amine was then used directly in the next step where it was stirred with Et$_3$N and acetic anhydride in the presence of the amidation catalyst 4-dimethylaminopyridine (DMAP) for 1 hour. An aqueous work-up was unnecessary, with column chromatography providing the *N*-acetyl derivative **298** in 98% yield. The successful transformation was clearly evident upon NMR spectroscopic analysis of the product: the appearance of a singlet at 1.91 ppm in the ^{1}H NMR spectrum that integrated for three protons, as well as an additional carbonyl peak at 169.1 ppm in the ^{13}C NMR spectra confirmed the introduction of the acetyl group. In addition, no resonances were present in the aromatic region of either spectrum, indicating a lack of benzyl groups in the molecule. The introduction of the acetyl group was also associated with a shift of the β-proton peak from 3.51 ppm for the starting material to 4.29 ppm for **298**, reflecting the decreased electron density around the nitrogen atom due to delocalisation of electrons onto the carbonyl group. The deshielding effect was also notable for the amide proton, which resonated quite far downfield at 6.44 ppm as a broad doublet. The assigned structure was supported by ESI HRMS analysis, with a molecular ion of *m/z* = 346.2414 for [M+H]$^+$ observed.
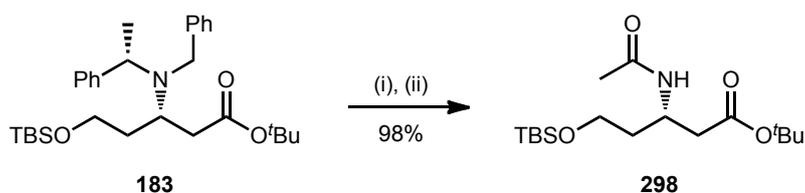
Scheme 1 Reagents and conditions: (i) Pd(OH)$_2$/C, H$_2$, MeOH/EtOAc (2:1), AcOH (cat.), 2.5 hr; (ii) Et$_3$N, Ac$_2$O, DMAP (cat.), DCM, 1 hr.

The next step involved removal of the silyloxy protecting group in preparation for the introduction of the guanidino moiety. This was achieved with a combination of TBAF and AcOH, and the free hydroxy compound **299** was obtained in 92% isolated yield after passage through a plug of silica gel (*Scheme 2*). The OH proton resonance was not visible in the ^{1}H NMR spectrum but the removal of the silyl group was supported by the absence of the large dimethyl and *tert*-butyl peaks— present at 0.03 and 0.88 ppm, respectively, in the spectrum of **298**. Both ^{13}C NMR and ESI HRMS analysis provided further evidence supporting the silyl ether cleavage, with a molecular ion of m/z = 232.1544 obtained, correlating to [M+H]$^+$ for the assigned structure of **299**.
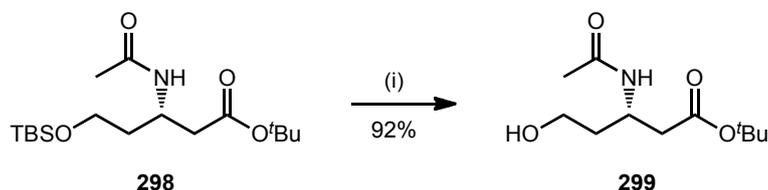
Scheme 2 Reagents and conditions: (i) TBAF, AcOH, THF, 40°C, 4 hr.

Introduction of the di-Cbz protected guanidine was performed using a similar procedure to that employed previously (Chapter 3, page 109). In this case 2.5 equivalents of the guanidinylating reagent were used in an effort to prevent intramolecular cyclisation, with the reduced steric bulk of the acetyl group (as compared to Boc) less likely to hinder attack by the β-amido group on the reactive phosphonium intermediate. Column chromatography failed to completely separate the guanidinylating reagent from the product, however the minor impurity was easily crystallised out of solution overnight using MeOH. β^3-Arginine derivative **300** was thus obtained in 86% yield as a white solid with a melting point of 54-56°C. Two key features present in the ^{1}H NMR spectrum of the product indicated that the Mitsunobu reaction had been successful: a mass of peaks resonating between 7.39-7.30 ppm and integrating for ten protons was attributed to the two Cbz protecting groups, as were the two signals at 5.25 and 5.13 ppm, corresponding to the two sets of methylene protons. These two Cbz groups appeared to be residing in significantly different chemical environments—one pair of methylene

protons resonated as a sharp singlet at 5.13 ppm, while the other was a highly distorted AB quadruplet resonating at 5.25 ppm (J = 16.8 Hz), indicating that the two protons were diastereotopic. The methylene carbon resonances in the ^{13}C NMR spectrum were identified at 69.3 and 67.3 ppm through the aid of 2D HMQC experiments, while the guanidino and Cbz carbonyl signals were generally poorly resolved, most likely as a result of tautomeric effects. ESI HRMS analysis returned a molecular ion of m/z = 541.2651 for [M+H]⁺, supporting the assigned structure.
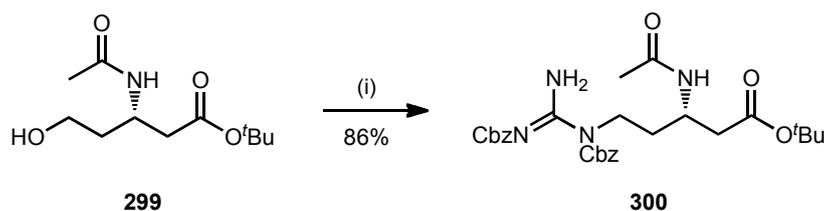
Scheme 3 Reagents and conditions: (i) PPh₃, di-Cbz guanidine, DIAD, THF, 0°C to RT overnight.

Conversion of **300** to the methyl ester **301** was performed as a two-step process involving an initial TFA-mediated cleavage of the *tert*-butyl ester, with the crude carboxylic acid then esterified with SOCl₂ in dry MeOH (*Scheme 4*). This esterification procedure, which proceeds via a highly electrophilic methylchlorosulfinate intermediate,[6] is typically much more efficient than the common Fischer esterification process, which requires reflux conditions and a Brønsted acid. The fact that the reaction is performed at 0°C and only 1.5 equivalents of SOCl₂ are used is highly advantageous—*N*-Cbz groups are known to be unstable in acidic conditions at high temperatures.[7] Given the problems experienced in Chapter 3 when attempting the *tert*-butyl hydrolysis of β³-arginine derivatives with TFA, namely concomitant hydrolysis of one of the Cbz protecting groups, the likely outcome of the experiment was somewhat unpredictable. However, any fears were allayed when the methyl ester was isolated in an excellent 86% overall yield after work-up and flash chromatography; no sign of the mono-Cbz protected substrate was detected by ^1H NMR analysis of the crude reaction mixture. The product was an easily crystallisable solid with a well-defined melting

point of 106°C. NMR spectroscopic analysis was used to establish the success of the transesterification, with the *tert*-butyl ester peak of **300** replaced by a resonance integrating for three protons at 3.59 ppm in the ^{1}H NMR spectrum, and 51.5 ppm in the ^{13}C NMR spectrum. As expected, the spectra of **300** and **301** were otherwise identical, confirming that the reaction conditions had not interfered with any other part of the molecule. This was further supported by ESI HRMS analysis, which returned a molecular ion of 521.1996, corresponding to [M+Na]$^+$ for **301**.

300 (i), (ii) 84% **301**
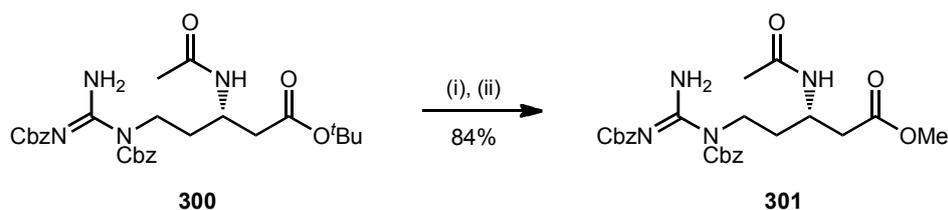
Scheme 4 Reagents and conditions: (i) TFA/DCM (1:2), 3 hr; (ii) SOCl$_2$, MeOH, 0°C to RT overnight.

The final step required to complete the synthesis of the target compound **295** was the hydrogenolysis of the two Cbz protecting groups. Whereas the cleavage of *N*-benzyl substituents typically necessitates the use of Pd(OH)$_2$/C, the carbamate-based Cbz groups are much more labile, thus enabling Pd/C to be used as the catalyst. With the only by-products of the reaction expected to be toluene and CO$_2$, it was anticipated that the final product could be isolated almost pure; the unprotected guanidino moiety is highly polar and difficult to purify by standard chromatographic techniques, while crystallisation of the unknown compound could not be relied upon. After stirring a solution of **301** in MeOH with 10% Pd/C under 1 atm of H$_2$ for 2 hours, the catalyst was filtered off and the filtrate simply concentrated *in vacuo* (*Scheme 5*).
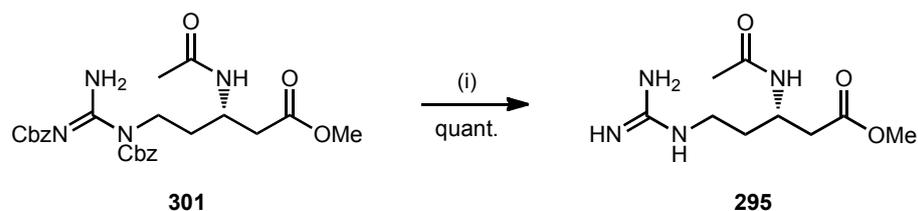
Scheme 5 Reagents and conditions: (i) Pd/C, MeOH, H_2.

The colourless glassy residue had little solubility in organic solvents, therefore ^{1}H NMR spectroscopic analysis was performed in MeOH-d_4. This revealed a purity of >95% with no sign of any unwanted by-products, with the reaction thus having proceeded in quantitative yield. Neither the guanidino nor the β-amino NH proton resonances were visible due to deuterium exchange with the solvent. The C3 proton resonance was identified as a multiplet at 4.27 ppm, while the three sets of methylene protons were observed to be diastereotopic, giving overlapping signals between 3.28-3.12 ppm (δ-protons adjacent to the guanidino NH), 2.62-2.50 ppm (α-protons) and 1.90-1.70 ppm (γ-protons). The three *N*-acetyl protons resonated as a sharp peak at 1.95 ppm, while the more deshielded methyl ester protons resonated further downfield at 3.35 ppm. Each of the carbonyl peaks were visible in the ^{13}C NMR spectrum at 173.3 and 173.0 ppm, while the quaternary guanidino carbon resonated at 158.9 ppm. The six remaining peaks between 49.9 and 22.7 ppm showed correlations to each of the remaining carbon atoms (identified using 2D C-H coupling experiments). The peak belonging to the δ-carbon was extremely small and broad, most likely as a result of quadrupolar line-broadening effects brought about by the adjacent nitrogen atom. An $[\alpha]_D^{20}$ of -41 (c = 1.0, MeOH) was recorded, indicating that a racemic mixture was not present, with ESI HRMS analysis used as a final tool in establishing that the target compound **1** had been successfully synthesised (m/z = 231.1441 for [M+H]⁺).

5.3.2 Synthesis of (2*S*,3*S*)-*N*-acetyl-α-fluoro-β³-arginine methyl ester

The preparation of the monofluoro substrate was expected to follow an identical synthetic route to that applied to the unfluorinated substrate, and again began with the hydrogenolysis of the *N*-benzyl groups. After stirring a solution of conjugate adduct **188** with 20% Pd(OH)$_2$/C under an atmosphere of H$_2$ for 3 hours, the crude amine was filtered and acetylated according to the conditions used previously (*Scheme 6*). This afforded the *N*-acetyl ester **302** in quantitative yield as a single diastereomer, which was determined through ^{1}H NMR analysis. The characteristic H-F coupling was visible as a doublet of doublets resonating at 5.02 ppm, with $^2J_{\text{H-F}}$ = 49.2 Hz and $^3J_{\text{H-H}}$ = 3.2 Hz. The vicinal coupling was also reflected in the appearance of the β-proton peak, which resonated as a doublet of multiplets at 4.50 ppm due to additional coupling to the adjacent methylene protons and the *N*$^\beta$-proton, which itself resonated as a broad doublet at 6.33 ppm (*J* = 7.6 Hz). The three protons of the acetyl group were responsible for a large singlet at 1.98 ppm, with the corresponding carbon atom identified with 2D HMQC experiments and resonating at 23.3 ppm in the ^{13}C NMR spectrum. The new carbonyl carbon appeared at 169.7 ppm, easily distinguishable from the *tert*-butyl ester carbonyl carbon, which was split as a result of C-F coupling (*J* = 24.1 Hz). ESI HRMS analysis supported the assigned structure of **302**, returning a molecular ion of *m/z* = 364.2319 for [M+H]$^+$.
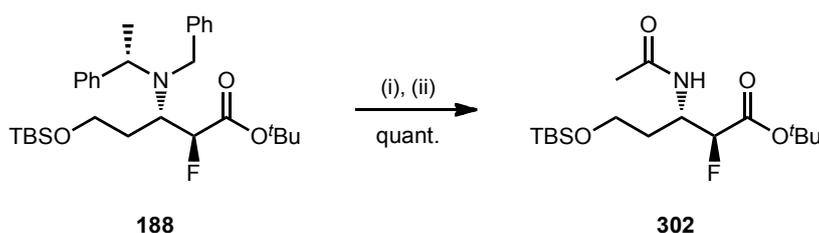
Scheme 6 Reagents and conditions: (i) Pd(OH)$_2$/C, H$_2$, MeOH/EtOAc (1:1), AcOH (cat.), 3 hr; (ii) Et$_3$N, Ac$_2$O, DMAP (cat.), DCM, 1 hr.

The TBSO protecting group was then removed by stirring a solution of the silyl ether with TBAF and AcOH at 40°C for 4 hours. After an aqueous work-up to remove excess TBAF, passage of the residue through a silica gel plug provided the hydroxy compound **303** in 77% yield (*Scheme 7*). Unlike the corresponding unfluorinated analogue, the OH peak was visible as a very broad peak at 3.33 ppm in the ^{1}H NMR spectrum. The resonances attributable to the nine *tert*-butyl and six methyl protons of the TBS group were now absent, which also corresponded to a loss of three peaks in the ^{13}C NMR spectrum (due to equivalent carbon atoms in the TBS group). The large decrease in molecular mass was also evident upon ESI HRMS analysis, with a molecular ion of *m/z* of 272.1270 corresponding to [M+Na]$^+$ obtained, which supported the successful silyloxy cleavage.
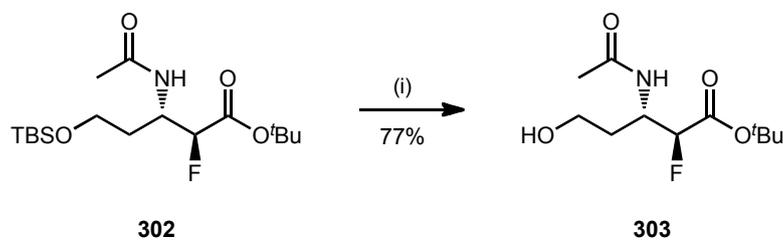
Scheme 7 Reagents and conditions: (i) TBAF, AcOH, THF, 40°C, 4 hr.

The Mitsunobu reaction to introduce the di-Cbz guanidino moiety to **303** employed 1.4 equivalents of PPh$_3$ and DIAD, while two equivalents of di-Cbz guanidine were used. Again, the excess guanidinylating reagent was difficult to completely separate from the product using column chromatography, with the remaining trace amounts crystallised out using MeOH and filtered off from the product **304**, which was subsequently obtained in 68% isolated yield (*Scheme 8*). ^{1}H NMR spectroscopic analysis of the newly synthesised β^3-arginine derivative showed the two guanidine NH protons each resonating as a broad doublet at 9.42 and 9.23 ppm, distinguishable from the *N*$^\beta$-proton, which resonated as a doublet at 6.89 ppm (*J* = 6.4 Hz) as a result of coupling to the β-proton (confirmed through 2D COSY experiments). The spectrum also revealed a similar phenomenon to that present in the unfluorinated analogue **300**, whereby the resonances attributable to the two

Cbz protecting groups were quite different—one Cbz group appeared to have unrestricted rotation, with the two equivalent methylene protons resonating as a singlet, while the other appeared constrained, resulting in diastereotopic methylene protons that each resonated as a doublet. Unlike in the ¹³C NMR spectrum of **300**, however, the two Cbz carbonyl carbon resonances of **304** were clearly visible at 163.4 and 155.5 ppm, identified through long-range correlations to the methylene protons in the HMBC spectrum. This left the remaining quaternary carbon peak at 160.2 ppm attributable to the guanidino carbon. ESI HRMS analysis supported the di-Cbz protected arginine structure of **304**, returning a molecular ion of m/z = 581.2392 corresponding to [M+Na]⁺.
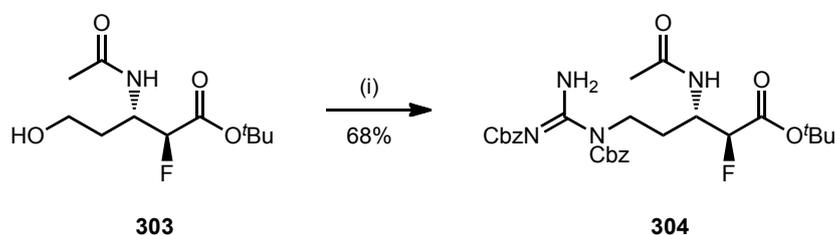
Scheme 8 Reagents and conditions: (i) PPh₃, di-Cbz guanidine, DIAD, THF, 0°C to RT overnight.

A two-step procedure analogous to that employed with the unfluorinated substrate was used to convert *tert*-butyl ester **304** to the methyl ester **305** (*Scheme 9*), affording product in an excellent 94% yield. The crystalline product had a narrow melting point range of 126-127°C, and was determined to be diastereomerically pure by ¹H NMR spectroscopic analysis. Apart from the loss of the *tert*-butyl peak present in the spectra of **304**, which was replaced in the ¹H NMR spectrum of the product by the singlet at 3.67 ppm integrating for three protons, the spectra of the starting material and product were largely identical. The only difference was that both pairs of Cbz methylene protons now resonated as singlets, suggesting that the conformation of the molecule had changed slightly. However, this could also be due to concentration effects experienced while performing the NMR experiments, or the presence of a minor amount of residual MeOH, which could disrupt the hydrogen bonding within the guanidino moiety. Finally, ESI HRMS analysis was used to

support the success of the transesterification reaction, providing a molecular ion of $m/z = 539.1906$, corresponding to $[M+Na]^+$ for **305**.
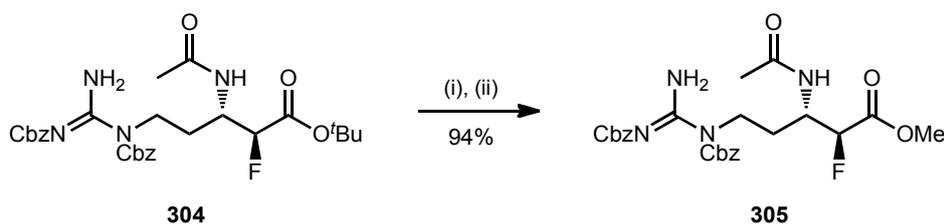
Scheme 9 Reagents and conditions: (i) TFA/DCM (1:2), 3 hr; (ii) SOCl$_2$, MeOH, 0°C to RT overnight.

The monofluoro target **296** was then prepared via hydrogenolysis of the two Cbz protecting groups, as for **301** (*Scheme 5*). After stirring **305** with Pd/C under H$_2$ for 3 hours, the catalyst was filtered off and the filtrate concentrated *in vacuo* to give the deprotected guanidino derivative **296** in quantitative yield (*Scheme 10*). The purity was ascertained by ^{1}H NMR analysis, and estimated to be >95%, with no detectable diastereomers or by-products. The ^{1}H NMR spectrum of the product, which was obtained with MeOH-d_4 as the solvent, appeared as expected: the β-proton resonated at 5.02 ppm as a doublet of doublets due to geminal H-F (J = 48.6 Hz) and vicinal H-H (J = 3.8 Hz) coupling. While the δ-protons again appeared diastereotopic, those attached to the γ-carbon were not, resembling a broad quartet, which is in contrast to the behaviour of each of the precursor compounds. The methyl ester was still present, as evidenced by the large singlet integrating for three protons at 2.99 ppm, and easily distinguishable from the more highly shielded acetyl protons resonating at 1.65 ppm. In contrast, the ^{13}C NMR spectrum showed the carbonyl carbon of the acetyl group resonating further downfield than that of the methyl ester (173.6 vs. 169.6 ppm), which is to be expected given the additional electron density provided by the adjacent oxygen atom of the methyl ester. The long range C-F coupling along the carbon chain was clearly evident from progressively smaller coupling constants for the doublet peaks: the largest was obviously a result of geminal coupling to the α-carbon (J = 187.2 Hz), with coupling of fluorine to the β-carbon (J = 21.2 Hz) and γ-carbon (J = 4.0 Hz) also observed. The decoupled ^{19}F NMR spectrum showed a single peak resonating at -203.1 ppm.

The identity of the target compound was further established through ESI HRMS analysis, which returned a molecular ion of m/z = 249.1341 [M+H]$^+$, corresponding to the assigned structure of **296**. Finally, an optical rotation of $[\alpha]_D^{20}$ = -19 (c = 1.0, MeOH) was recorded for the product.
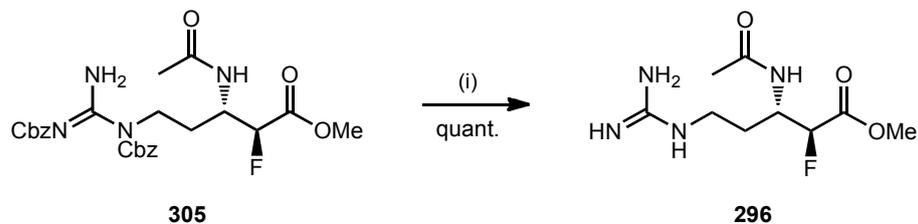
Scheme 10 Reagents and conditions: (i) Pd/C, MeOH, H$_2$.

5.3.3   Synthesis of (3*S*)-*N*-acetyl-α,α-difluoro-β^3-arginine methyl ester

Although the functionalities of the β-amino esters synthesised via the Reformatsky and conjugate adduct methodologies differed somewhat, the overall approach to the synthesis of the difluoro analogue **297** was initially the same—it was envisaged that similar reaction conditions could be employed to achieve many of the functional group transformations that were required. Of course, in synthetic chemistry the best-laid plans often go astray, with the strongly electron-withdrawing fluorine atoms having an unforseen effect on the electrophilicity of the adjacent ethyl ester, as shall be seen shortly.

Given the high yields obtained previously for the concomitant hydrogenolysis/Boc protection of the Reformatsky products (Chapter 4, page 160), the preparation of the analogous *N*-acetyl compound was predicted to be relatively straightforward. While the *in situ* Boc protection of a free amine liberated through hydrogenolysis is common, the corresponding acylation reaction is usually performed as a separate step in the presence of pyridine or Et$_3$N. As such, initial attempts at converting the Reformatsky product **292** to the *N*-acetyl derivative **306** focussed on removal of the phenylglycine auxiliary to obtain the free amine, which would then be acetylated according to standard procedures. However, **292** was found to be almost

completely resistant to hydrogenolysis under similar conditions to those previously employed (Pearlman's catalyst, 1 atm H$_2$)—after 18 hours only starting material was recovered. More forcing conditions involving high-pressure hydrogenation may have accomplished the cleavage, but as the appropriate equipment was not available at the time, alternative methods were required. Transfer hydrogenolysis, in which ammonium formate serves as a proton donor was firstly investigated: using Pd/C as the catalyst, an ethanolic solution of **292** was stirred with 15 equivalents of HCO$_2$NH$_4$ overnight, however no reaction was observed. Heating the same reaction mixture at reflux overnight also had little effect, with starting material again recovered. The stark lack of reaction displayed by **292** was in contrast to that observed for the hydrogenolysis/Boc protection procedure, and could only be attributed to the omission of Boc$_2$O from the reaction mixture. It was thought that a quaternary intermediate in which the β-amine was partially bound to Boc$_2$O may have somehow facilitated the hydrogenolysis of the sterically hindered phenylglycine moiety, and therefore the same might be possible if Ac$_2$O was present in the reaction mixture. Thus a solution of **292** in EtOH/EtOAc (1:1) was stirred with 2.4 equivalents of Ac$_2$O in the presence of Pd(OH)$_2$/C under H$_2$ for 24 hours. Unfortunately, the similar R_f's of the starting material and phenylacetate by-product **294** made monitoring of the reaction's progress difficult by TLC. The reaction was worked-up and the crude material analysed by ^{1}H NMR analysis, however little conversion to the *N*-acetylated product was observed. After redissolving the residue in EtOAc and adding more catalyst and Ac$_2$O (1.2 equiv.), the mixture was stirred under H$_2$ for a further 36 hours then filtered and worked up. ^{1}H NMR analysis revealed the crude mixture to comprise of four main compounds: **292**, the desired *N*-acetyl product **306**, ethyl phenylacetate and an unidentified by-product. This unknown compound was subsequently isolated using column chromatography, with 1D and 2D NMR experiments providing strong evidence for the *N,N*-diethyl compound **307**. While the aromatic, ethyl ester and α-proton resonances of the phenylglycine moiety were absent from the ^{1}H NMR spectrum, no broad NH peak was visible either; the ethyl ester was still present, however, indicating lactamisation had not taken place. Instead, a symmetrical pair of multiplets at 2.62 and 2.46 ppm, each integrating for two protons, was visible. With each pair of methylene protons involved in a four-bond coupling to the other pair through the nitrogen atom, COSY experiments also showed a correlation to a

single triplet at 1.34 ppm. Resonating for six protons, this triplet could be attributable to the two equivalent methyl groups adjacent to the methylene protons. **307** is possibly produced as a result of over-hydrogenation of the *N*-acetyl species, with the resulting ethylamine reacting with a second molecule of Ac$_2$O. This acetyl group is then subject to further over-hydrogenation. Neither a *N,N*-diacetyl nor a mono *N*-ethyl species were observed in the spectra of the crude material. This problem of by-product formation is exacerbated by the sluggish reactivity of **292**, where the long reaction times required to remove the auxiliary allow the production of ever-increasing amounts of **307**. While the yields were not quantified, the ratio of **306**:**307** was estimated to be approximately 1:1 (^{1}H NMR analysis).

The experiment was then repeated using EtOAc as the solvent and only 1.5

292   **306** 78%   **307**
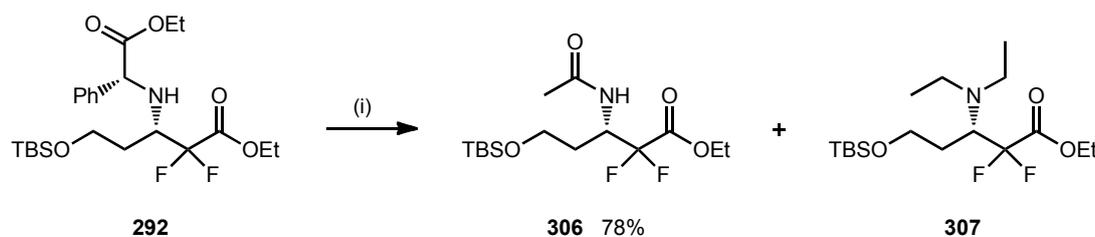
Scheme 11 Reagents and conditions: (i) Pd(OH)$_2$/C, Ac$_2$O, AcOH, EtOAc, H$_2$.

equivalents of Ac$_2$O in an attempt to minimise the formation of **307**. After stirring under H$_2$ for 24 hours and with little sign of progress, 10 drops of AcOH was added to try to speed up the reaction—the presence of the acid catalyst would also result in protonation of the nitrogen and reduce the chances of catalyst poisoning, which was one possible reason for the lack of reactivity. The mixture was stirred under H$_2$ for a further two days, after which time the catalyst was filtered off and the mixture neutralised using an aqueous work-up. Only minor amounts of starting material and **307** were observed upon ^{1}H NMR analysis of the crude mixture, with column chromatography affording **306** in 63% yield. The NH peak was clearly visible as a broad doublet at 6.11 ppm (J = 8.8 Hz), while the methyl protons of the newly introduced acetyl group appeared as a singlet at 1.96 ppm. Despite the fact that hydrogenolysis conditions can cleave silyl ethers,[8,9] the TBSO group was still present, as evidenced by the large singlet at 0.88 ppm and the two smaller singlet

peaks at 0.04 and 0.03 ppm. The ^{13}C NMR spectrum displayed two peaks in the carbonyl region—a singlet at 169.8 ppm attributable to the *N*-acetyl group, and a doublet of doublets at 163.2 ppm (*J* = 33.0, 30.8 Hz) belonging to the ethyl ester carbonyl carbon. As expected, a pair of doublets for each of the fluorine atoms in the proton-decoupled ^{19}F NMR spectrum appeared at -112.9 (d, *J* = 259.8 Hz) and -117.3 (d, *J* = 261.7 Hz) ppm, while ESI HRMS analysis produced a molecular ion of *m/z* = 376.1725, corresponding to [M+Na]$^+$ for **306**.

The fact that **306** was isolated in only 63% yield was puzzling given that virtually complete conversion of the starting material was apparent from the ^{1}H NMR analysis of the crude reaction mixture, with few by-products visible (excluding the expected phenylacetate **294**). It was reasoned that the presence of H_2O in the reaction mixture, primarily as a result of wet AcOH, resulted in partial hydrolysis of the ethyl ester. The resulting carboxylic acid would then be extracted into the aqueous layer upon work-up with a saturated aqueous $NaHCO_3$ solution. This theory was supported when the experiment was repeated using anhydrous AcOH, with the amount used also increased substantially in attempt to speed up the reaction. Both of these factors served to increase the yield of **306** from 63% to 78%, with constant monitoring of the reaction by TLC analysis indicating that the reaction was indeed faster than previous experiments. This contributed to a reduction in the formation of **307**, which in this case was produced in only ~2% yield. Remarkably, under these acidic conditions (10% AcOH/EtOAc) the TBSO group was left totally unscathed—no sign of the hydroxy compound was observed during ^{1}H NMR analysis of the crude reaction mixture.

The next task was to in fact remove this TSBO protecting group, and while TBAF had previously been used to achieve this transformation with other substrates in high yield, performing this experiment with the analogous *N*-Boc protected derivative **254** (the synthesis of which was described in Chapter 4) had failed— while the major isolated product had no TBS group, the ethyl ester peaks were also absent from the ^{1}H NMR spectrum. Four large multiplets attributable to the tetra-butyl ammonium protons were present, suggesting that the quaternary ammonium carboxylate salt may have been formed, however integration of these multiplets indicated otherwise (^{n}Bu$_4$N:product = ~3:1). Rather, the unidentified product may

have just co-eluted with excess TBAF during column chromatography. Given the unavoidable presence of small amounts of water in TBAF solutions the ester may have instead been hydrolysed to the carboxylic acid, a reaction facilitated by the electron withdrawing CF_2 moiety. Using an unprotected acid in the Mitsunobu reaction would lead to unwanted guanidinylation of the carbonyl group, while protection of the carboxylic acid as the methyl ester using the MeOH/SOCl$_2$ method employed previously would also affect the unprotected hydroxy group of the side chain. Thus an alternative, anhydrous method of desilylation was sought.

As well as being susceptible to cleavage by fluoride sources, the TBSO group is labile to varying degrees in both acidic and basic solutions. Given the susceptibility of the ethyl ester to nucleophilic attack, it was decided that non-aqueous acidic conditions would be the most effective method of removing the silyl ether while maintaining the integrity of the rest of the structure. However, the sensitive nature of the ester could also prove useful: while conducting the hydrogenolysis of the chiral auxiliary in MeOH, a high degree of transesterification to the methyl ester was observed, even under essentially neutral conditions. Performing the TBSO cleavage in MeOH with an acid catalyst, which would facilitate the transesterification reaction, should enable the concomitant conversion to the methyl ester. To test this theory, a solution of the ester in dry MeOH was stirred with anhydrous *p*-toluenesulfonic acid for 24 hours, after which time it was neutralised with a couple of drops of Et$_3$N. ^{1}H NMR analysis of the major fractions obtained from flash chromatography of the residue did indicate the presence of the hydroxy methyl ester, however given the similarity in R_f's of the ethyl and methyl esters, complete separation was not achieved. In addition, evidence for a number of other unidentified and inseparable by-products was visible in the NMR spectrum, which caused this one-step method to be discarded in favour of separate silyl ether cleavage and transesterification steps. After repeating the experiment in EtOH, the solution was neutralised with saturated aqueous NaHCO$_3$ before removing the EtOH *in vacuo*. However this resulted only in hydrolysis of the ester, with what was presumed to be the carboxylic acid proving to be unextractable during aqueous work-up.

Attention was then turned to alternative reagents for silyl ether cleavage. The use of catalytic amounts of acetyl chloride in alcohol solvents, resulting in the

formation of HCl *in situ*, had been shown to achieve the transformation in high yield in as little as 5 minutes.[10] Thus, to a solution of the ester in dry EtOH was added 4 drops of freshly distilled AcCl and the mixture stirred for 2.5 hours. Concentration and aqueous work-up led to a 66% crude yield of the product, with ^{1}H NMR analysis showing no sign of either starting material or by-products—no lactonisation appeared to have occurred from attack of the hydroxy group on the ethyl ester. This crude product was then subjected to Mitsunobu reaction without further purification, using 2.5 equivalents of the di-Cbz protected guanidinylating reagent **213**. This two-step procedure led to the isolation of the desired arginine derivative **308** in 40% overall yield. The ethyl ester remained in place, as evidenced by ^{1}H NMR analysis, while the newly introduced di-Cbz guanidino moiety was identifiable from the ten aromatic protons now resonating in the spectrum, the two pairs of methylene protons resonating as two AB quadruplets at 5.14 and 5.11 ppm (J = 14.8 Hz), and the two NH protons resonating as broad doublets at 9.37 and 9.20 ppm. This was supported by the ^{13}C NMR spectrum, which now featured three additional peaks in the downfield region attributable to the guanidino and Cbz carbonyl carbons, the characteristic triplet of the CF_2 carbon at 113.6 ppm (J = 254.2 Hz), and the ethyl ester carbons resonating at 63.1 and 13.8 ppm (identified using HMQC correlations). ESI HRMS analysis provided further evidence of the assigned structure, returning a molecular ion of m/z = 549.2160 for [M+H]$^+$.

The desilylation reaction was the principle cause of the low overall yield, however the reason for this was not entirely clear. Repeating the experiment with variations to both reaction time and AcCl concentration did not increase the consistently low yield of the crude hydroxy compound. In fact, increasing the amount of AcCl to one equivalent resulted in almost complete decomposition of the starting material, despite the fact that both the *N*-acetyl and ethyl ester functionalities should have remained unaffected by ethanolic HCl solutions. Eliminating the aqueous work-up in case extraction of the product into organic solvents was the problem also had little effect on yields. The second step of the reaction sequence—the Mitsunobu reaction—also contributed to the lower than expected yields, although to a much lesser extent. A number of nucleophiles are present that could attack the ethyl ester in addition to the possible side reaction involving intramolecular cyclisation with

the β-amide. Both of these factors could combine to further decrease the overall yield. Despite this, analysis of the ^{1}H NMR spectrum of the crude material recorded before chromatography revealed few other products—aside from the reduced DIAD by-product, **308** was the dominant species present. Repeating the experiment and using 3 equivalents of the guanidinylating reagent in the Mitsunobu reaction did little to increase the yield. It is possible that the use of a chlorinated solvent such as DCM instead of THF might improve the yield of this step, but further attempts to optimise the yield of this reaction were not pursued given the small amount of starting material in hand at the time.

After persisting with AcCl/EtOH with disappointing results, a final approach involving the use of pyridinium *p*-toluenesulfonate (PPTS) in EtOH was explored to cleave the TBSO ether. The reaction was sluggish at RT, and failed to go to completion using a catalytic amount (0.2 equivalents) of PPTS, however upon increasing the amount of PPTS to 1.5 equivalents and the temperature to 40°C, the reaction was complete in 24 hours. Excess PPTS and any by-products formed were removed with an aqueous work-up and concentration under vacuum, with TLC analysis of the resulting mixture indicating that the hydroxy compound was sufficiently pure to be used immediately in the next step. The crude product was thus subjected to Mitsunobu conditions, which afforded the guanidino derivative **308** in a much improved 79% overall yield (*Scheme 12*).
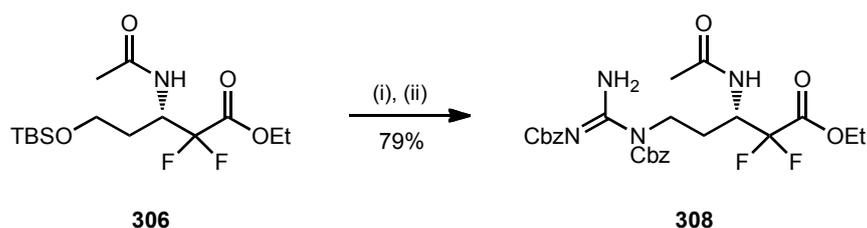
Scheme 12 Reagents and conditions: (i) PPTS, EtOH, 40°C, 24 hr; (ii) PPh$_3$, di-Cbz guanidine, DIAD, THF, 0°C to RT overnight.

Conversion of ethyl ester **308** to the desired methyl ester via a one-step transesterification reaction would be highly preferable, but given the difficulty in

going from a larger ester to a smaller one that is highly susceptible to attack by the displaced species, the likelihood of success would be low. In addition, the somewhat forcing conditions required (i.e., heating at reflux for prolonged periods in the presence of acid or base) to drive the reaction to completion would likely have a detrimental effect on the Cbz protecting groups. It was therefore decided to hydrolyse the ethyl ester to the carboxylic acid, which would then be re-esterified to the methyl ester in a separate step. With the lability of the Cbz groups initially unknown, an acetone solution of the ester was heated at reflux with 1M HCl for one hour. However this afforded a highly polar product that could not be extracted into organic solvents, most likely as a result of Cbz hydrolysis. The procedure was then modified to be much more gentle, with a EtOAc/MeOH solution of the ester stirred with 1M HCl for 24 hours, however the rate of reaction was quite slow according to TLC analysis. Several drops of concentrated HCl were added and the mixture left to stir for a further 48 hours, when the reaction finally appeared to be complete (TLC). Avoiding the use of high temperatures and an aqueous work-up, the solution was then concentrated under high vacuum to remove all traces of H_2O, and the residue subjected to the same MeOH/SOCl$_2$ esterification method used previously. The crude mixture obtained after work-up was analysed using ^{1}H NMR spectroscopy and found to predominantly consist of a 3:1 mixture of an unidentified compound and the desired methyl ester (**309**). This unknown by-product was isolated using column chromatography and found to be almost identical to **309**—it even possessed the methyl ester—except for the fact that only a single guanidino proton peak appeared in the spectrum. Furthermore, this NH proton no longer resonated at around 9.4 ppm, instead shifting significantly downfield to 11.1 ppm, while the $N^β$-proton peak also shifted from 6.62 ppm for **309** to 5.81 ppm for the unknown compound. The ^{13}C NMR spectrum was also telling: while the chemical shift of one Cbz carbonyl carbon remained unchanged at 155 ppm, the carbons of both the second Cbz carbonyl group and the guanidino moiety displayed substantial upfield shifts from 163.3 and 160.0 ppm to 150.5 and 149.5 ppm, indicating that the guanidine moiety had reacted in some way. With this product still unidentified, the experiment was repeated but **308** was only subjected to the hydrolytic conditions overnight; 4M HCl was used in place of 1M HCl to counteract the shorter reaction time. This produced a favourable result by effectively reversing the product ratio, which changed from 3:1 in favour of the by-product to 2.5:1 in favour of **309**. A

further reduction in the hydrolysis time to only 2 hours led to the best outcome, with none of the ethyl ester and only trace amounts of the by-product visible in the ^{1}H NMR spectrum of the crude mixture obtained after esterification. Column chromatography afforded **309** in 78% yield over the two steps (*Scheme 13*), with the newly installed methyl ester resonating in the ^{1}H NMR spectrum as a large singlet peak at 3.79 ppm. This also corresponded to a loss of the ethyl ester quartet and triplet peaks from the spectrum. The acidic conditions seemed to have little effect on the two Cbz protecting groups, with both still visible in the ^{1}H and ^{13}C NMR spectra. ESI HRMS analysis provided supporting evidence for the successful conversion, returning a molecular ion of m/z = 535.2007, which corresponded to [M+H]$^+$ for **309**.
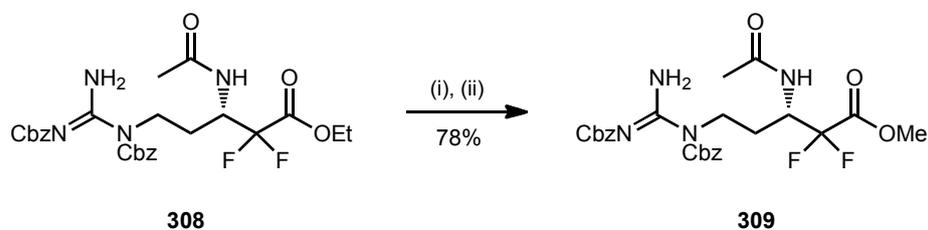
Scheme 13 Reagents and conditions: (i) 4M HCl, MeOH, 2.5 hr; (ii) SOCl$_2$, MeOH, 0°C to RT, overnight.

Mass spectroscopic analysis was also performed on the unidentified by-product, returning a molecular ion two mass units heavier than **309**. The fact that the compound possessed one fewer proton than **309** (determined through integration of the ^{1}H NMR spectrum), together with the upfield shift of the quaternary guanidino carbon observed in the ^{13}C spectrum means that a urea structure is the most likely possibility. This results from the acid-catalysed hydrolysis of the guanidine moiety, most likely proceeding via nucleophilic attack of water on a dication species[11,12] to give the urea **311** (*Scheme 14*). This hydrolysis is reported to be quite slow and a function of both acid strength and time, and therefore can be limited by adjusting the experimental conditions accordingly.
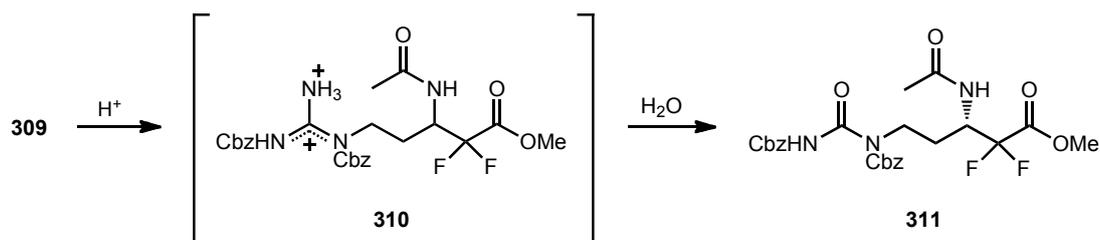
Scheme 14 Urea formation via acid-catalysed guanidine hydrolysis.

The final step in the synthesis of the target difluorinated derivative **297** was the hydrogenolysis of the Cbz protecting groups under experimental conditions analogous to those used for the methylene and fluoromethine analogues. As expected, the Pd/C-catalysed hydrogenolysis reaction proceeded in quantitative yield to give **297** as a colourless glassy solid, which recorded an optical rotation of $[\alpha]_D^{20}$ = -5 (c = 0.57, MeOH). Trituration with MeOH gave a white solid with a melting point of 262-264°C.
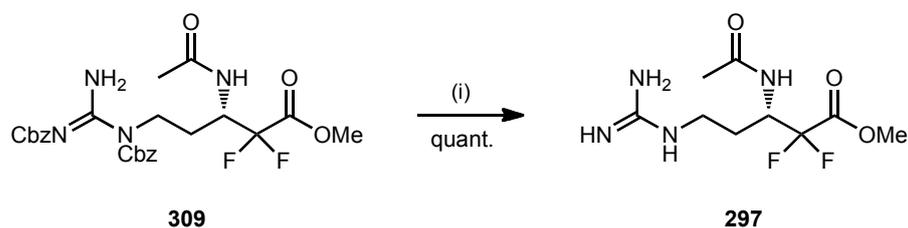
Scheme 15 Reagents and conditions: (i) Pd/C, MeOH, H_2.

The ^{1}H NMR spectrum of **297** in MeOH-d_4 was very similar to that of the monofluoride **296**—the location and appearance of the methyl ester and δ-proton peaks were virtually unchanged, although the highly deshielded β-proton now resonated at 4.52 ppm. There were, however, two singlet peaks present at 2.03 and 2.01 ppm where only one was expected for the three acetyl protons. Despite this, the two peaks—together with one overlapping γ-proton, integrated for only 4 protons. One possible explanation for this observation was the presence of two rotamers, while another involved the formation of an orthoester- or orthoamide-

type species, which would result from nucleophilic attack by either MeOH-d_4 or the unprotected δ-nitrogen of the guanidino moiety, respectively. The latter scenario was a real possibility given the enhanced electrophilicity of the ester carbonyl carbon. The situation was made clearer upon inspection of the ^{13}C NMR spectrum, with two products apparent from the duplication of each resonance. In MeOH-d_4 the ratio of peak heights for the two products was ~2:1, while upon switching to D$_2$O the ratio approached ~6:1. The chemical shifts of some of the carbon atoms varied very little between the two compounds, i.e., the β- and δ-carbons of the side chain, as well as the two acetyl carbons and that of the methyl ester. In general, the peaks for the minor compound resonated slightly higher upfield than those of the major compound. The biggest differences were observed for the α-carbon and the adjacent methyl ester carbonyl carbon, with those of the minor compound resonating further upfield by 2.0 and 4.1 ppm, respectively. Each of these peaks was split into characteristic triplets as a result of the C-F coupling. The location of the two methyl ester carbonyl carbon signals strongly suggested that a rotameric mixture was indeed present—resonating at 169 and 165 ppm, these signals were both indicative of sp^2-hydridised carbon atoms. The sp^3-hydridised carbon atoms of orthoesters, however, generally resonate much higher upfield (~120 ppm). With no sign of an additional triplet peak in this region of the ^{13}C NMR spectrum of the product, the presence of such a compound was unlikely. ESI HRMS analysis returned a molecular ion of m/z = 267.1260, corresponding to the assigned structure of **297**. Hydrogen bonding interactions are the most likely reason for the two rotamers, with a large number of hydrogen bond donors and acceptors present in the molecule, allowing a range of different conformations to exist. However these conformations were not further investigated through either molecular modelling or NMR spectroscopic experiments.

5.4 Conclusions

Each of the desired *N*-acetylated β^3-arginine targets was successfully synthesised using a short series of standard synthetic transformations. The unfluorinated analogue **295** was synthesised in eight steps from α,β-unsaturated ester **166** in 61% overall yield. The synthetic route was extremely efficient with yields of ≥84% obtained for each step; β-amino acids featuring less complex side chains that required fewer chemical transformations would likely be prepared in even higher overall yield. Despite the use of essentially identical reaction conditions, the monofluorinated analogue **295** was prepared in only 31% overall yield from **166**. However, this is largely a consequence of the poor diastereoselectivity obtained in the conjugate addition reaction, leading to a yield of only 62% for the first step. As mentioned above, the α,α-difluoro analogue was the most difficult substrate with which to deal. Substantial optimisation of the reaction conditions employed during the synthetic route was necessary, with an overall yield of 25% eventually obtained over eight steps from TBSO-propanal (**170**). The first step of the synthesis— preparation of the Reformatsky product, **292**, which proceeded in only 52% yield—largely accounts for the low overall yield. Nevertheless, a route to optically active α,α-difluoro-β^3-arginine analogues—compounds as yet unreported in the literature—has now been established using enantiopure phenylglycine ethyl ester as a chiral auxiliary. The Reformatsky reaction demonstrably complements the conjugate addition methodology and allows the preparation of unfluorinated, monofluoro and difluoro analogues of the desired β^3-amino acid.

The greater overall yields obtained for the unfluorinated and monofluorinated analogues is due to a combination of things, namely the well-established techniques for *N*-debenzylation and *tert*-butyl ester hydrolysis that were used, and also because the presence of a single fluorine atom at the α-carbon did not alter the electronic properties of the compounds to the degree that α,α-difluorination does— the combination of inductive and resonance effects from the two fluorine atoms make the compound's behaviour slightly less predictable in otherwise straightforward reactions. The electron withdrawing effects of the difluoromethylene group were particularly evident when performing experiments with the Reformatsky product **292**, which features two ethyl esters—one bearing an adjacent α-amino moiety and the other the CF_2 moiety. The difference in the

reactivities of the two esters was marked, with several different nucleophilic reagents attacking the α,α-difluorocarbonyl moiety while leaving the phenylglycine ester completely untouched. This reflects the greater electrophilicity of the former when compared to standard ethyl esters, and perfectly illustrates one of the key reasons for the use of α,α-difluorocarbonyl species as enzyme inhibitors—that their enhanced electrophilicity can be expected to facilitate nucleophilic attack by active site residues.

5.5   References

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CHAPTER 6:

CONCLUSIONS

6.1 General Conclusions

The introduction of fluorine, an atom that can significantly alter the physicochemical properties of a compound, to β-amino acids has lead to a new class of potentially bioactive compounds. Over recent years the interest in fluorinated β-amino acids has grown considerably, particularly with regard to their potential role in enzyme inhibition. Unfortunately synthetic techniques have barely kept pace with this rising interest, particularly in the case of α-fluoro-β³-amino acids. These compounds represent a greater degree of synthetic difficulty than the corresponding α,α-difluoro-β³-amino acids, with this difficulty further compounded by the need for enantiopure products. Current methods for the synthesis of α-fluoro-β³-amino acids are typically based on the homologation of naturally occurring α-amino acids, which can limit the diversity of the β³-amino products. In contrast, α,α-difluoro-β³-amino acids have received much more attention, with most methods relying on a building block approach to introduce the achiral CF_2 synthon. However, most of these methods tend be limited in their success, particularly when the β-substituent is of an aliphatic nature, as is the case with most naturally occurring amino acids. Amino acids featuring heteroatom-functionalised side chains introduce an even greater level of complexity to the synthesis, and as a result there are few examples in the literature of α-fluoro- or α,α-difluoro-β³-amino acids that extend beyond such simple substrates as alanine or phenylalanine. Thus, versatile new routes to these fluorinated β³-amino acids were desired, with this thesis detailing the extensive investigations undertaken in this area.

It had previously been shown that α-fluoro-β³-phenylalanine derivatives could be prepared via a one-pot tandem conjugate addition-fluorination method involving the use of chiral lithium amides.[1] This project sought to expand upon this earlier work in order to prepare more complex substrates, e.g., derivatives of β³-lysine and β³-arginine, two amino acids that feature highly basic nitrogen-functionalised side chains. As a number of variations on the conjugate addition methodology exist, namely the tandem addition to α,β-unsaturated esters, tandem addition to α-substituted α,β-unsaturated esters, and a stepwise protocol involving separate deprotonation and fluorination steps, a model system was devised in order to

determine the compatibility of our fluorinating reagent, *N*-fluoro-benzenesulfonimide (NFSI), with each of the techniques. It was found that the selectivity of the fluorination step in the tandem reaction was far superior to that obtained in the stepwise protocol, in which the unfluorinated conjugate adduct had been prepared and then subjected to a separate deprotonation-fluorination reaction; yields were comparable, however. Meanwhile, conjugate addition to an α-fluoro-α,β-unsaturated ester, where the intermediate enolate is quenched with a proton source rather than NFSI, failed due to deprotonation of the fluorinated alkene by the lithium amide.

Having demonstrated that the tandem addition-fluorination reaction was the best approach, a series of α,β-unsaturated esters featuring heteroatom functionalised side chains were prepared; the length of these side chains corresponded to the number of carbon atoms in the side chains of lysine and arginine. Two α,β-unsaturated esters bearing protected nitrogen-based moieties generally fared poorly due to decomposition under the reaction conditions, while silyl ether protected α,β-unsaturated esters proved stable. The fluorinated β-amino esters prepared from these latter substrates could be obtained in good to high yield (*Figure 1*).
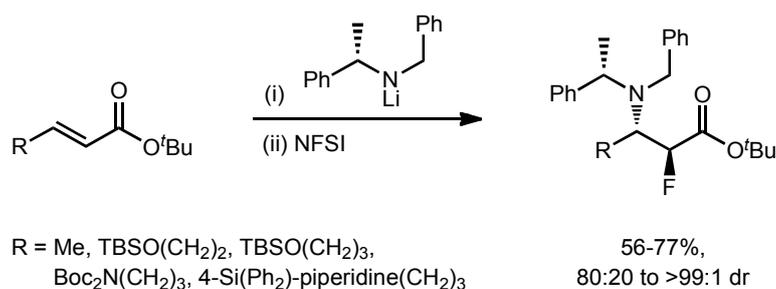
R = Me, TBSO(CH$_2$)$_2$, TBSO(CH$_2$)$_3$,   56-77%,
     Boc$_2$N(CH$_2$)$_3$, 4-Si(Ph$_2$)-piperidine(CH$_2$)$_3$ 80:20 to >99:1 dr

Figure 1 Tandem conjugate addition-fluorination method for the preparation of α-fluoro-β-amino esters.

In an effort to demonstrate the suitability of the conjugate addition reaction to the preparation of α-fluoro-β³-amino acids, the synthesis of orthogonally protected β³-lysine and β³-arginine derivatives was attempted. Conjugate adduct **187** was thus subjected to a series of high-yielding functional group transformations, the most important of which was the Mitsunobu reaction, which was successfully used to convert the oxygen functionality to the desired δ-nitrogen functionality. The Fmoc protected α-fluoro-β³-lysine derivative **209** was eventually prepared in 53% overall yield over nine steps, starting from the α,β-unsaturated ester **167**.

167   **209**

53% over nine steps
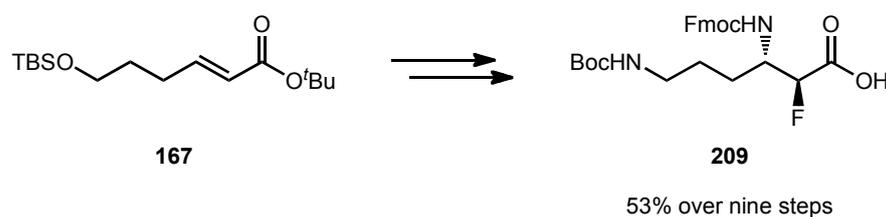
Figure 2 Orthogonally protected α-fluoro-β³-lysine derivative **209**.

The conjugate adduct **188** was converted to an orthogonally protected β³-arginine derivative in a similar manner, although the presence of arginine's guanidino moiety necessitated a slightly different synthetic route. The Mitsunobu reaction was again key to the synthesis, allowing the introduction of the guanidino moiety pre-protected as the di-Cbz derivative. Unfortunately, concomitant cleavage of the *tert*-butyl and *N*-Boc groups proved problematic and somewhat incompatible with the Cbz protecting groups. As a result the desired amino acid could not be obtained, however the α-fluoro-β³-arginine ester derivative **224** was prepared in six steps in 25% overall yield from the α,β-unsaturated ester (*Figure 3*).

166   **224**

25% over six steps
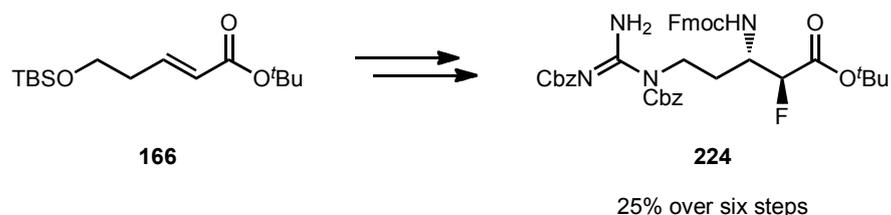
Figure 3 α-Fluoro-β³-arginine ester derivative **224**.

It was envisaged that the corresponding α,α-difluoro-β³-amino acids could be accessed through the deprotonation-fluorination of the α-fluoro conjugate adducts, however such attempts consistently met with poor results. An alternative route was therefore required, and given the popularity of the Reformatsky reaction for the synthesis of such compounds, this methodology was adapted to suit the preparation of aliphatic α,α-difluoro-β³-amino acids. This new method, which involved the application of ultrasound to promote the reaction, substantially improves upon existing techniques with regard to aliphatic imines, which have historically been poor substrates in the Reformatsky reaction, giving low yields and selectivities. Employing the ethyl ester of (*R*)-phenylglycine as a chiral auxiliary and ethyl bromodifluoroacetate as the fluorinated building block, a series of aliphatic β³-amino esters were prepared in good yield and excellent dr (*Figure 4*). While the diastereoselectivity of the reaction is extremely high, further work to determine the ee of the reaction products would be valuable. The phenylglycine auxiliary could be cleaved and the β-amino moiety reprotected as the Boc derivative in a high yielding one-pot reaction using standard hydrogenolysis techniques.

R = ^{i}Pr, ^{i}Bu, ^{n}C$_7$H$_{15}$, ^{c}C$_6$H$_{11}$, Ph(CH$_2$)$_2$, CH$_2$=CH(CH$_2$)$_2$,
TBSO(CH$_2$)$_2$, TBSO(CH$_2$)$_3$, Phth(CH$_2$)$_3$

45-97%,
95:5 to 98:2 dr
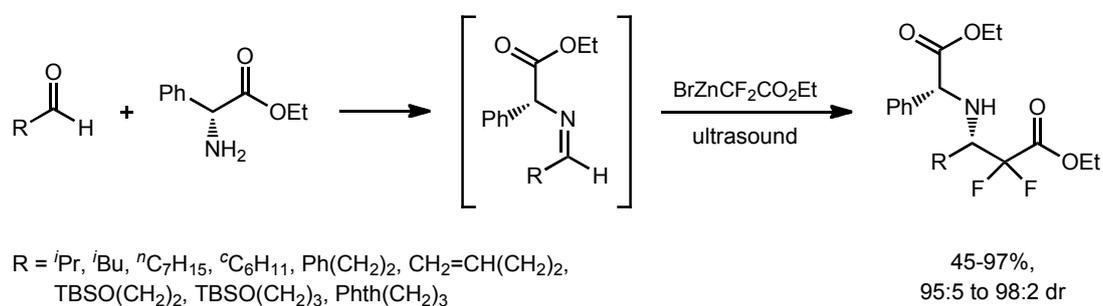
Figure 4 The zinc-mediated Reformatsky reaction of chiral aliphatic imines with ethyl bromodifluoroacetate for the preparation of α,α-difluoro-β³-amino esters.

The final chapter of this thesis detailed the preparation of three N^β-acetyl protected β³-arginine esters from substrates prepared using the conjugate addition and Reformatsky reactions, which demonstrated the complementarity of the two methods (*Figure 5*). An unfluorinated (**295**) and α-fluorinated (**296**) analogue were both prepared from the appropriate conjugate adducts (**183** and **188**, respectively). β³-Arginine derivative **295** was synthesised over nine steps in 51%

overall yield from TBSO-propanal (**170**), while the application of an identical synthetic route to the synthesis of α-fluorinated analogue **296** resulted in an overall yield of only 26%. This was largely a consequence of the poor dr obtained in the conjugate addition-fluorination reaction, which resulted in only a moderate yield of *anti*-**188**.

Preparation of the α,α-difluoro-β³-arginine derivative **297** also employed **170** as the starting material, which was used to generate the requisite Reformatsky product using the experimental conditions described in Chapter 4. The enhanced electrophilicity of the ethyl ester adjacent to the CF_2 moiety meant that the same chemical transformations employed with the conjugate adducts weren't suited to this difluoro substrate—the use of strongly acidic or basic/nucleophilic conditions had to be avoided to avoid unwanted reaction of the ethyl ester. After extensive optimisation of the synthetic route, the target arginine derivative **297** was eventually prepared in 25% yield over eight steps.

295 X = H, 51% over nine steps
296 X = F, 26% over nine steps

25% over eight steps
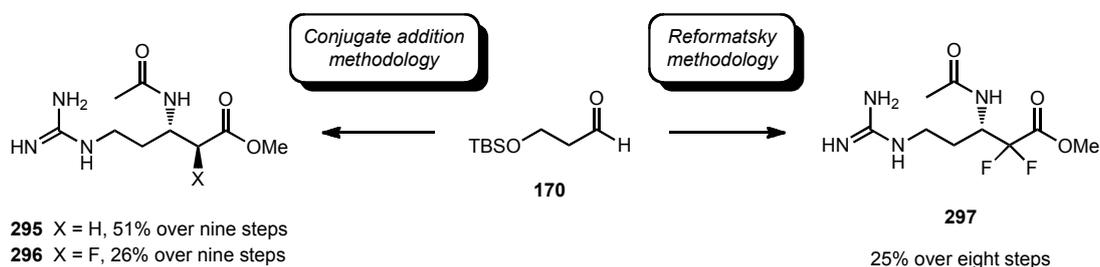
Figure 5 The complementarity of the conjugate addition and Reformatsky methodologies is demonstrated by the preparation of unfluorinated, monofluorinated and difluorinated β³-arginine esters from the same achiral aldehyde.

The two methodolgies that form the basis of the work presented in this thesis represent a significant advance in the methods available for the preparation of fluorinated β³-amino acids. Whilst this work dealt primarily with the synthesis of β³-lysine and β³-arginine derivatives, these new methods are applicable to the synthesis of a broad range of fluorinated β³-amino acids and hence should find wider use amongst the synthetic community.

6.2 References

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CHAPTER 7:

EXPERIMENTAL SECTION

7.1   General experimental procedures

4-Aminobutyraldehyde dimethyl acetal was purchased from Alfa Aesar and distilled before use. Ethyl bromodifluoroacetate was purchased from SynQuest Laboratories, Inc. and distilled from CaH before use. All other products were obtained from Sigma-Aldrich. DCM was distilled from CaH and THF was distilled from sodium benzophenone ketyl immediately before use. All other reagents were purified according to Perrin,[1] with dried solvents stored over molecular sieves. TBAF (1M in THF) was stored over molecular sieves after opening. The temperatures of the conjugate addition and fluorination experiments were controlled using an acetone/dry ice cryobath, with the periodic addition of dry ice used to maintain the temperature between ±3°C of that stated.

Thin layer chromatography (TLC) was performed on pre-coated sheets of Merck silica gel 60 and visualised under UV light (254 nm) or with ninhydrin or $KMnO_4$. Column chromatography was carried out at atmospheric pressure using Davisil silica gel (LC60Å, 40-63 μm). Melting points were obtained on a Reichert hot-stage microscope and are uncorrected. Optical rotations were recorded on a PoIA AR21 polarimeter referenced to the sodium D line (589 nm) at the temperature specified. Sonication was carried out with a Digitech 100W ultrasonic cleaning bath.

^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C), a Bruker AV 600 spectrometer at 600 MHz (^1H) and 150 MHz (^{13}C) and a Varian Gemini spectrometer at 300 MHz (^1H) and 75 MHz (^{13}C). ^{19}F NMR spectra were recorded on a Bruker AV 200 spectrometer at 188 MHz (proton-decoupled) or Varian Gemini spectrometer at 282 MHz (proton-coupled). All spectra were recorded at 293K unless otherwise stated, and in deuterated solvents as indicated. Chemical shifts (δ) are reported as parts per million (ppm) and referenced to residual solvent peaks. Spin multiplicities are indicated by: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets and ddd, doublet of doublets of doublets. Peaks were assigned with the aid of homonuclear (^1H-^1H) correlation spectroscopy (COSY) and heteronuclear (^1H-^{13}C) correlation spectroscopy (HMQC and HMBC) when required.

HRMS data was obtained using positive ion electrospray ionisation (ESI-MS) at Monash University, Melbourne, Australia; CSIRO Molecular and Health Technologies, Melbourne, Australia; and Otago University, Dunedin, New Zealand.

7.2   Experimental procedures

tert-Butyl (_E_)-6-(_tert_-butyldimethylsilyloxy)hex-2-enoate
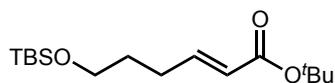
167

To a solution of oxalyl chloride (1.22 ml, 14.2 mmol) in dry DCM (140 ml) at −78°C and under N_2 was added dry DMSO (2.10 ml, 29.7 mmol). After stirring for 45 min, a solution of 4-(*tert*-butyldimethylsilyloxy)butanol (2.53 g, 12.4 mmol) in DCM (40 ml) was added dropwise. After stirring at −78°C for 40 min, Et_3N (8.61 ml, 61.8 mmol) was added dropwise and the solution allowed to warm to 0°C over 1 hr. The mixture was then poured into a saturated aq $NaHCO_3$ solution (150 ml) and the layers separated. The organic layer was washed with H_2O (100 ml) and brine (150 ml), then concentrated *in vacuo*. The crude aldehyde was dissolved in dry DCM (80 ml) and *tert*-butoxycarbonylmethylenetriphenylphosphorane[2] (5.82 g, 15.5 mmol) added in one portion. The mixture was stirred under N_2 overnight then concentrated *in vacuo* and purified by column chromatography (1:19 ether/hexane), yielding **167** as a colourless oil (3.28 g, 91%; *E/Z* = 98:2).

^{1}H NMR (400 MHz, CDCl₃) δ 6.87 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.75 (dt, *J* = 15.6, 1.6 Hz, 1H), 3.62 (t, *J* = 6.4 Hz, 2H), 2.27-2.21 (m, 2H), 1.69-1.62 (m, 2H), 1.48 (s, 9H), 0.89 (s, 9 H), 0.04 (s, 6H); [13]C (100 MHz, CDCl₃) δ 166.0, 147.5, 123.1, 79.9, 62.1, 31.1, 28.4, 28.1, 25.8, 18.2, -5.4.

HRMS calcd for $C_{16}H_{32}O_3SiNa$ 323.2018, found 323.2014 [M+Na]⁺.

tert-Butyl (*E*)-5-(*tert*-butyldimethylsilyloxy)pent-2-enoate

166
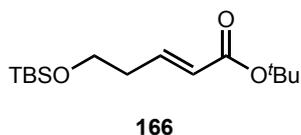
To a solution of oxalyl chloride (0.38 ml, 4.43 mmol) in dry DCM (45 ml) at −78°C and under N_2 was added a solution of DMSO (0.65 ml, 9.12 mmol) in dry DCM (1.5 ml). After stirring for 1 hr, a solution of 3-(*tert*-butyldimethylsilyloxy)propanol[3] (723 mg, 3.80 mmol) in DCM (10 ml), pre-cooled to −78°C, was added slowly. After stirring at −78°C for 30 min, Et_3N (2.65 ml, 19.0 mmol) was added dropwise and the solution allowed to warm to 0°C over 1 hr. This was then poured into a saturated aq $NaHCO_3$ solution (40 ml) and the layers separated. The aqueous layer was extracted with DCM (15 ml) and the combined organic layers washed with H_2O (50 ml), then brine (50 ml). After drying (Na_2SO_4) and concentration *in vacuo*, the crude aldehyde was dissolved in dry DCM (30 ml) and *tert*-butoxycarbonyl-methylenetriphenylphosphorane (2.0 g, 5.32 mmol) was added in one portion. The mixture was stirred under N_2 overnight and concentrated *in vacuo*. Column chromatography (1:19 ether/hexane) afforded **166** as a colourless oil (920 mg, 84%; *E/Z* 98:2).

^1H NMR (400 MHz, $CDCl_3$) δ 6.84 (dt, *J* = 15.6, 7.2 Hz, 1H), 5.74 (dt, *J* = 15.6, 1.6 Hz, 1H), 3.71 (t, *J* = 6.6 Hz, 2H), 2.41-2.35 (m, 2H), 1.47 (s, 9H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 144.5, 124.7, 80.1, 61.7, 35.6, 28.2, 25.9, 18.3, -5.3.

HRMS calcd for $C_{15}H_{30}O_3SiNa$ 309.1862, found 309.1855 [M+Na]$^+$

tert-Butyl (*E*)-6-(*N,N*-di-*tert*-butylcarbonyloxy)aminohex-2-enoate
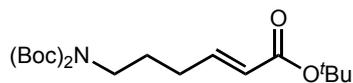
143

4-(*N,N*-di-*tert*-Butoxycarbonyl)aminobutyraldehyde dimethyl acetal (394 mg, 1.18 mmol) was stirred in a mixture of AcOH/H_2O (2:1, 10 ml) for 6 hr, after which time the mixture was poured into a saturated solution of $NaHCO_3$ (10 ml). DCM (15 ml) was added, the layers were separated, and the aqueous layer further extracted with DCM (2 × 15 ml). The combined organic layers were washed with brine (30 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was redissolved in dry DCM (5 ml) and *tert*-butoxycarbonyl-methylenetriphenylphosphorane (536 mg, 1.42 mmol) added, with stirring continued under N_2 overnight. The solution was concentrated then purified with chromatography (1:9 ether/hexane) to afford **143** a colourless oil that solidified on standing (260 mg, 57%; *E/Z* >99:1); mp 32-34°C.

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.83 (dt, *J* = 15.6, 6.8 Hz, 1H), 5.75 (dt, *J* = 15.6, 1.6 Hz, 1H), 3.58 (m, 2H), 2.17 (m, 2H), 1.71 (m, 2H), 1.49 (s, 18H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 165.9, 152.5, 146.7, 123.4, 82.3, 80.0, 45.8, 29.3, 28.11, 28.06, 27.4.

1-(4',4'-dimethoxybutyl)-4,4-diphenyl-4-silapiperidine
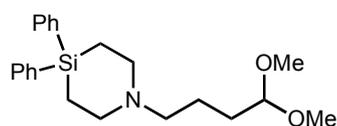
178

A solution of 2,2'-(diphenylsilylene)bis(ethanol) (1.13 g, 4.16 mmol), triphenylphosphine (3.27 g, 12.5 mmol) and imidazole (0.850 g, 12.5 mmol) in THF (25 ml) under N_2 was cooled to 0°C and iodine (3.17 g, 12.5 mmol) added portionwise. The mixture was stirred at 0°C for 1 hr, then at RT for a further hour, after which time 50% H_2O_2 (10 drops) was slowly added. The mixture was poured

into ether (75 ml) and H_2O (75 ml) and the layers were separated. The ether layer was washed with saturated aq $Na_2S_2O_3$ (50 ml) and brine (50 ml), then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was vacuum filtered through silica gel (~3 cm) to remove triphenylphosphine oxide, eluting with a 1:1 ether/hexane. The filtrate was concentrated and redissolved in dry 1,4-dioxane (30 ml), before 4-aminobutyraldehyde dimethyl acetal (0.66 ml, 4.58 mmol) and dry K_2CO_3 (1.72 g, 12.5 mmol) were added. The mixture was heated at reflux in the dark for 42 hr, after which time more 4-aminobutyraldehyde dimethyl acetal (0.08 ml) was added. After stirring for a further 12 hr, the mixture was allowed to cool and then poured into $CHCl_3$ (50 ml). H_2O (50 ml) was added and the layers separated, with the aqueous layer extracted with $CHCl_3$ (2 × 25 ml). The organic layers were combined and washed with brine (80 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (25:75:1 ether/hexane/Et_3N, then 50:50:1 ether/hexane/Et_3N) gave **178** as a colourless oil (1.06 g, 79%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.52 (m, 4H), 7.41-7.33 (m, 6H), 4.38 (t, *J* = 5.6 Hz, 1H), 3.31 (s, 6H), 2.83 (t, *J* = 6.2 Hz, 4H), 2.46 (t, *J* = 7.2 Hz, 2H), 1.62-1.51 (m, 4H), 1.35 (t, *J* = 6.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 134.6, 129.3, 127.8, 104.3, 57.7, 52.6, 52.0, 30.4, 22.0, 11.0.

HRMS calcd for $C_{22}H_{32}NO_2Si$ 370.2202, found 370.2206 $[M+H]^+$.

tert-Butyl (E)-6-(4,4-diphenyl-1-aza-4-silacyclohexy-1-yl)hex-2-enoate
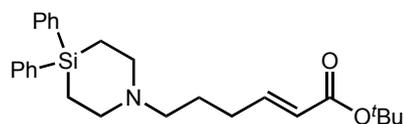
165

Dimethyl acetal **178** (398 mg, 1.08 mmol) was dissolved in a 2:1 THF/3M HCl solution (15 ml) and heated at reflux for 2.5 hr. The THF then removed *in vacuo* and the residue neutralised with a saturated aqueous $NaHCO_3$ solution (25 ml). $CHCl_3$ (25 ml) was added and the layers separated. The aqueous layer was extracted with $CHCl_3$ (2 × 10 ml), then the combined organic phase was washed with brine (25

ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was redissolved in dry DCM (6 ml) and cooled to 0°C, before *tert*-butoxycarbonylmethylenetriphenyl-phosphorane (610 mg, 1.62 mmol) was added. The solution was allowed to warm to RT overnight then concentrated *in vacuo*. Column chromatography (25:75:1 ether/hexane/Et$_3$N) gave **165** as a colourless oil (438 mg, 96%; 97:3 *E/Z*).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.55-7.52 (m, 4H), 7.41-7.33 (m, 6H), 6.86 (dt, *J* = 15.6, 6.8 Hz, 1H), 5.74 (dt, *J* = 15.6, 1.6 Hz, 1H), 2.82 (t, *J* = 6.2 Hz, 4H), 2.47-2.43 (m, 2H), 2.21-2.16 (m, 2H), 1.63 (pentet, *J* = 7.4 Hz, 2H), 1.48 (s, 9H), 1.35 (t, *J* = 6.2 Hz, 4H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.0, 147.6, 135.7, 134.7, 129.4, 127.9, 123.1, 80.0, 57.3, 52.1, 30.1, 28.2, 25.8, 11.2.

HRMS calcd for C$_{26}$H$_{36}$NO$_2$Si 422.2515, found 422.2515 [M+H]$^+$.

Representative Procedure A

tert-Butyl (3S,αS)-6-(tert-butyldimethylsilyloxy)-3-(N-benzyl-α-methylbenzylamino)hexanoate
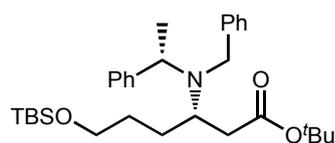
182

To a solution of (*S*)-α-methylbenzylamine (0.11 ml, 0.55 mmol) in THF (2 ml) under N$_2$ at –78°C was added ^{n}BuLi (1.6 M in hexanes, 0.34 ml, 0.55 mmol). After 30 min, a solution of (*E*)-*tert*-butyl 6-(*tert*-butyldimethylsilyloxy)hex-2-enoate **167** (110 mg, 0.37 mmol) in THF (1.5 ml) was added dropwise and the mixture stirred at –78°C for 1.5 hr. Saturated aq NH$_4$Cl (5 ml) was added and the mixture allowed to warm to 0°C. It was then diluted with ether (20 ml), H$_2$O (20 ml) was added, and the layers separated. The aqueous layer was extracted with ether (2 × 10 ml), then the combined organic layers were washed with brine (30 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:19 ether/hexane) gave **182** as a

colourless oil (152 mg, 81%, dr > 99:1).

$[\alpha]_D^{20}$ = -5 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37 (d, *J* = 7.2 Hz, 2H), 7.29-7.22 (m, 6H), 7.21-7.15 (m, 2H), 3.76 (q, *J* = 7.2 Hz, 1H), 3.73 (d, *J* = 14.8 Hz, 1H), 3.52 (t, *J* = 6.4 Hz, 2H), 3.43 (d, *J* = 14.8 Hz, 1H), 3.25 (nonet, *J* = 4.4 Hz, 1H), 1.90-1.79 (m, 3H), 1.51-1.38 (m, 3H), 1.34 (s, 9H), 1.28 (d, *J* = 6.8), 3H), 0.83 (s, 9H), −0.01 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.1, 143.0, 141.8, 128.24, 128.21, 128.1, 128.0, 126.9, 126.5, 79.9, 63.3, 58.1, 54.0, 50.1, 38.0, 30.7, 29.8, 28.0, 26.0, 20.3, −5.2.

HRMS calcd for C$_{31}$H$_{50}$NO$_3$Si 512.3554, found 512.3562 [M+H]$^+$.

tert-Butyl (3*S*,α*S*)-5-(*tert*-butyldimethylsilyloxy)-3-(*N*-benzyl-α-methylbenzylamino)pentanoate
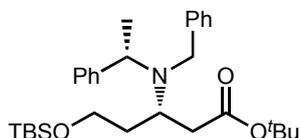
183

The silyloxyaminopentanoate **183** was prepared from (*E*)-*tert*-butyl 5-(*tert*-butyldimethylsilyloxy)pent-2-enoate **166** (354 mg, 1.24 mmol) according to Representative Procedure A, with the following variation: after stirring at −78°C for 2.5 hr, the mixture was allowed to warm to −40°C over 1 hr before quenching. The product was obtained as a colourless oil that crystallised on standing (518 mg, 94%; dr > 99:1). The product could be recrystallised from 95% EtOH to give white needles, mp 77°C.

$[\alpha]_D^{20}$ = -6 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 (d, *J* = 7.2 Hz, 2H), 7.35-7.22 (m, 8H), 3.85-3.71 (m, 4H), 3.56-3.49 (m, 2H), 1.96-1.88 (m, 2H), 1.77-1.68 (m, 1H), 1.58-1.50 (m, 1H),

1.41 (s, 9H), 1.36 (d J = 7.2 Hz, 3H), 0.91 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.8, 142.7, 141.5, 128.2, 128.1, 128.0, 126.9, 126.6, 79.9, 61.1, 58.0, 50.8, 50.1, 38.2, 36.4, 28.1, 26.0, 19.9, 18.3, -5.2, -5.3.

HRMS calcd for C$_{30}$H$_{48}$NO$_3$Si 498.3395, found 498.3395 [M+H]$^+$

tert-Butyl (3_S_,α_S_)-6-(_N_,_N_-di-_tert_-butylcarbonyloxy)amino-(_N_-benzyl-α-methylbenzylamino)hexanoate
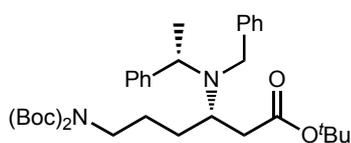
184

The di-Boc protected aminohexanoate **184** was prepared from (_E_)-_tert_-butyl 6-(_N_,_N_-di-_tert_-butylcarbonyloxy)-aminohex-2-enoate **143** (156 mg, 0.40 mmol) according to Representative Procedure A. The product was obtained after column chromatography (1:4 diether/hexane) as a colourless oil (106 mg, 44%; dr > 99:1).

$[\alpha]_D^{20}$ = -7 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41 (d, J = 7.2 Hz, 2H), 7.34-7.29 (m, 6H), 7.25-7.20 (m, 2H), 3.80 (q, J = 6.8 Hz, 1H), 3.76 (d, J = 14.8 Hz, 1H), 3.60-3.52 (m, 2H), 3.47 (d, J = 15.2 Hz, 1H), 3.35-3.28 (m, 1H), 2.01-1.90 (m, 1H), 1.89-1.82 (m, 2H), 1.60-1.44 (m, 20H), 1.39 (s, 9H), 1.37-1.23 (m, 2H), 1.34 (d, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.9, 152.7, 142.7, 141.5, 128.3, 128.1, 127.9, 126.9, 126.6, 81.9, 79.9, 57.8, 53.9, 50.1, 46.6, 37.9, 30.9, 28.1, 28.0, 27.2, 20.1.

HRMS calcd for C$_{35}$H$_{53}$N$_2$O$_6$ 597.3898, found 597.3909 [M+H]$^+$.

tert-Butyl (3*S*,α*S*)-6-(4,4-diphenyl-1-aza-4-silacyclohex-1-yl)-3-(*N*-benzyl-α-methylbenzylamino)hexanoate
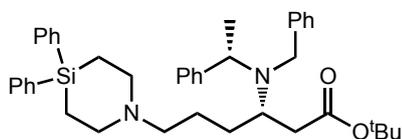
185

The silylpiperazine aminohexanoate **185** was prepared from **178** (138 mg, 0.33 mmol) according to Representative Procedure A, with the following variation: after stirring at −78°C for 2.5 hr, the mixture was allowed to warm to −60°C over 30 min before quenching. After standard workup, the crude residue was redissolved in THF (3 ml) and phenyl isocyanate (0.016 ml, 0.144 mmol) was added. After stirring under N_2 for 4 hr, the solution was concentrated *in vacuo* and purified using column chromatography (5:44:1 ether/hexane/Et_3N) to give 180 mg (87%, dr > 99:1) of the product as a colourless gum.

$[\alpha]_D^{20}$ = +2 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.57-7.54 (m, 4H), 7.43-7.20 (m, 16 H), 3.83 (q, *J* = 6.8 Hz, 1H), 3.80 (d, *J* = 15.2 Hz, 1H), 3.50 (d, *J* = 15.2 Hz, 1H), 3.33-3.27 (m, 1H), 2.82 (t, *J* = 6.2 Hz, 4H), 2.43-2.31 (m, 2H), 1.98 (dd, *J* = 14.8, 3.6 Hz, 1H), 1.88 (dd, *J* = 14.6, 9.4 Hz, 1H), 1.84-1.76 (m, 1H), 1.57-1.42 (m, 2H), 1.39 (s, 9H), 1.38-1.34 (m, 7H), 1.31-1.23 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.2, 143.2, 142.0, 134.8, 129.5, 128.3, 128.20, 128.18, 128.1, 128.0, 127.0, 126.6, 80.1, 58.6, 57.9, 54.1, 52.2, 50.1, 37.7, 31.6, 28.1, 24.1, 20.7, 10.8.

HRMS calcd for $C_{41}H_{53}N_2O_2Si$ 633.3871, found 633.3871 [M+H]$^+$.

<u>*Representative Procedure B*</u>

tert-Butyl (2*S*,3*S*,α*S*)-6-(*tert*-butyldimethylsilyloxy)-3-(*N*-benzyl-α-methylbenzylamino)-2-fluorohexanoate
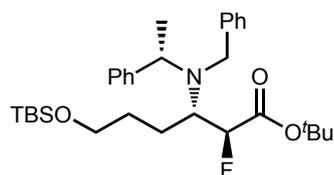
187

To a stirred solution of (*S*)-α-methylbenzylamine (0.135 ml, 0.65 mmol) in THF (2 ml) under N_2 at –78°C was added ^{n}BuLi (1.6 M in hexanes, 0.41 ml, 0.65 mmol). After 30 min, a solution of (*E*)-*tert*-butyl 6-(*tert*-butyldimethylsilyloxy)-hex-2-enoate **167** (130 mg, 0.43 mmol) in THF (1.5 ml) was added dropwise and the mixture stirred at –78°C for 2.5 hr, then warmed to –30°C over 30 min. The solution was recooled to –78°C and a solution of NFSI (205 mg, 0.65 mmol) in THF (1 ml) added dropwise. After stirring at –78°C for 1 hr, the solution was warmed to 0°C over 30 min then quenched with saturated aq NH_4Cl (4 ml). The mixture was diluted with H_2O (20 ml) and poured into hexane (20 ml). The organic layer was washed with H_2O (2 × 10 ml) and brine (20 ml), then dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (1:19 ether/hexane) gave **187** as a colourless oil (177 mg, 77%; dr 98:2).

$[\alpha]_D^{20}$ = +7 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.6 Hz, 2H), 7.37-7.25 (m, 8H), 4.45 (d, *J* = 50.0 Hz, 1H), 4.16 (d, *J* = 15.2 Hz, 1H), 3.94 (q, *J* = 6.8 Hz, 1H), 3.67 (d, *J* = 15.2 Hz, 1H), 3.56 (t, *J* = 6.4 Hz), 3.39 (ddd, *J* = 31.2, 9.6, 3.6 Hz), 1.89 (m, 1H), 1.66 (m, 1H), 1.54-1.35 (m, 2H), 1.45 (s, 9H), 1.34 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.048 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4 (d, *J* = 24.4 Hz), 142.1, 141.7, 128.2, 128.1, 128.0, 127.9, 127.0, 126.5, 90.0 (d, *J* = 188.8 Hz), 82.2, 63.0, 58.1, 57.9, 50.7 (d, *J* = 4.5 Hz), 30.6, 27.9, 25.9, 23.4 (d, *J* = 5.2 Hz), 20.0, 18.2, -5.36, -5.38; ^{19}F NMR (282 MHz, $CDCl_3$) δ -200.0 (dd, *J* = 50.7, 31.2 Hz).

HRMS calcd for $C_{31}H_{49}FNO_3Si$ 530.3460, found 530.3465 [M+H]⁺.

tert-Butyl (2*S*,3*S*,α*S*)-3-(*N*-benzyl-α-methylbenzylamino)-2-fluorobutanoate

186
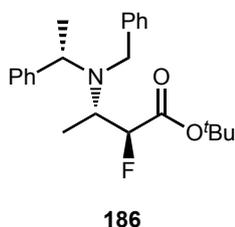
The aminobutanoate **186** was prepared from (*E*)-*tert*-butyl crotonate (134 mg, 0.94 mmol) according to Representative Procedure B, with the following variation: after NFSI addition stirring was continued at −78°C for 1 hr, then the solution was warmed to −30°C over 1.5 hr before quenching. Column chromatography (3:7 ether/hexane) gave a mixture of the C2 epimers as a colourless oil (217 mg, 62%). Recrystallisation from min. hexane gave the *anti* isomer as white needles (195 mg, 56%; dr > 99:1), mp 82.5-84°C.

$[\alpha]_D^{20}$ = +22 (c = 1.0, CHCl₃).

¹H NMR (400 MHz, CDCl₃) δ 7.45 (d, *J* = 7.6 Hz, 2H), 7.36-7.20 (m, 8 H), 4.65 (dd, *J* = 50.4, 2.0 Hz, 1H), 3.99 (q, *J* = 6.8 Hz, 1H), 3.98 (d, *J* = 14.8 Hz, 1H), 3.84 (d, *J* = 14.8 Hz, 1H), 3.40 (m, 1H), 1.38 (s, 9H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.15 (dd, *J* = 7.2, 0.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 168.4 (d, *J* = 24.8 Hz), 143.6, 141.9, 128.23, 128.18, 128.1, 127.7, 126.8, 126.6, 92.3 (d, *J* = 188.0 Hz), 82.1, 58.1, 53.4 (d, *J* = 18.8 Hz), 50.6 (d, *J* = 4.0 Hz), 27.9, 17.2, 12.2 (d, *J* = 5.7 Hz); ¹⁹F NMR (282 MHz, CDCl₃) δ -202.5 (dd, *J* = 49.7, 30.2 Hz).

HRMS calcd for $C_{23}H_{31}FNO_2$ 372.2333, found 372.2336 [M+H]⁺.

tert-Butyl (2*S*,3*S*,α*S*)-5-(*tert*-butyldimethylsilyloxy)-3-(*N*-benzyl-α-methylbenzylamino)-2-fluoropentanoate
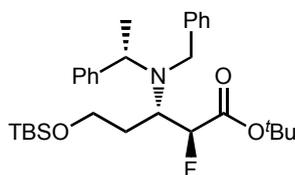
188

Tandem method:

The silyloxyaminohexanoate **188** was prepared from (*E*)-*tert*-butyl 5-(*tert*-butyldimethylsilyloxy)pent-2-enoate **166** (105 mg, 0.37 mmol) according to Representative Procedure B, with the following variation: NFSI was added at –50°C and stirring continued for 1 hr, after which time the solution was quenched and allowed to warm to 0°C. The product was obtained as a colourless oil that crystallised on standing (171 mg, 90%; dr 79:20:1). Recrystallisation from acetone (slow evaporation) gave the major isomer as white needles (118 mg, 62%), mp 109-110°C.

$[\alpha]_D^{20}$ = +4 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43-7.24 (m, 10H), 4.26 (dd, *J* = 50.4, 0.8 Hz, 1H), 4.12 (d, *J* = 14.4 Hz, 1H), 3.94-3.86 (m, 2H), 3.83-3.71 (m, 2H), 3.65 (dd, *J* = 15.2, 1.6 Hz, 1H), 1.86-1.78 (m, 1H), 1.55-1.48 (m, 1H), 1.49 (s, 9H), 1.38 (d, *J* = 7.2 Hz, 3H), 0.93 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.4 (d, *J* = 24.7 Hz), 141.3, 141.2, 128.4, 128.3, 128.2, 128.1, 127.2, 126.7, 90.7 (d, *J* = 187.3 Hz), 82.3, 59.7, 57.0, 53.6 (d, *J* = 18.7 Hz), 50.7, 30.3 (d, *J* = 4.9 Hz), 28.0, 26.1, 19.4, 18.4, -5.2, -5.3; ^{19}F NMR (282 MHz, CDCl$_3$) δ -199.1 (dd, *J* = 51.8, 32.3 Hz).

HRMS calcd for C$_{30}$H$_{47}$FNO$_3$Si 516.3304, found 516.3306 [M+H]$^+$.

Stepwise method:

Fluorination at -50°C

To a solution of diisopropylamine (0.034 ml, 0.24 mmol) in dry THF (2 ml) at 0°C was added ^{n}BuLi (1.6 M in hexanes, 0.16 ml, 0.24 mmol). The solution was stirred for 20 min then cooled to -78°C. A solution of the amino ester **183** (96 mg, 0.19 mmol) in THF (1.5 ml) was added dropwise, with the mixture stirred for 1 hr before being warmed to -55°C. A solution of NFSI (89 mg, 0.28 mmol) in THF (1.5 ml) was then added, and stirring continued at -55°C for 3 hr, before warming to 0°C over a further hour. Saturated aq NH_4Cl (4 ml) was added, followed by ether (25 ml) and H_2O (25 ml). The layers were separated, with the ether layer washed with H_2O (25 ml) and brine (25 ml), then dried (Na_2SO_4) and concentrated *in vacuo*. Passing the residue through a plug of silica gel (1:9 ether/hexane) gave 93 mg (93%, dr 69:31 *anti*:*syn*) of a white solid, which was recrystallised from acetone to give 59 mg (59%) of the *anti* isomer as white needles.

Fluorination at -78°C

To a solution of diisopropylamine (0.033 mL, 0.23 mmol) in dry THF (2 mL) at 0°C was added ^{n}BuLi (1.5 M in hexanes, 0.15 mL, 0.23 mmol) and the solution stirred for 20 min, after which it was cooled to -78°C. The amino ester **183** (94 mg, 0.19 mmol) in THF (1.5 mL) was added dropwise, and the solution stirred for another hour. A solution of NFSI (86 mg, 0.27 mmol) in THF (1.5 ml) was added dropwise with stirring continued at -78°C for 3 hr, after which the solution was allowed to warm to -35°C over 1.5 hr, then quenched with saturated aq. NH_4Cl (4 ml). Ether (25 mL) and H_2O (25 mL) were added and the layers separated. The ether layer was washed with H_2O (25 mL) and brine (25 mL), then dried (Na_2SO_4) and concentrated in vacuo. Crude ^{1}H NMR showed a 72:28 mixture of *anti*:*syn* isomers. The product was not further purified.

tert-Butyl (2*S*,3*S*,α*S*)-6-(*N*,*N*-di-*tert*-butylcarbonyloxy)amino-(*N*-benzyl-α-methylbenzylamino)-2-fluorohexanoate
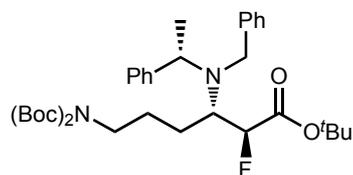
189

The di-Boc protected aminohexanoate **189** was prepared from (*E*)-*tert*-butyl 6-(*N*,*N*-di-*tert*-butylcarbonyloxy)-aminohex-2-enoate **143** (130 mg, 0.34 mmol) according to Representative Procedure B, with the following variation: NFSI was added at –60°C, and after 1.5 hr the reaction was allowed to warm to -35°C over 30 min before quenching. Column chromatography (1:9 to 1:3 ether/hexane) gave the product as a colourless oil (277 mg, 48%; *dr* 93:7). A portion of this was further purified with chromatography to give a sample with dr = 98:2.

$[\alpha]_D^{20}$ = +5 (c = 1.0, CHCl$_3$);

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43 (d, *J* = 7.6 Hz, 2H), 7.36-7.23 (m, 8H), 4.36 (d, *J* = 50.0 Hz, 1H), 4.12 (d, *J* = 15.2 Hz, 1H), 3.92 (q, *J* = 7.2 Hz, 1H), 3.64 (d, *J* = 15.6 Hz, 1H), 3.63-3.51 (m, 2H), 3.40 (ddd, *J* = 31.2, 9.8, 3.4 Hz, 1H), 2.02-1.92 (m, 1H), 1.70-1.60 (m, 1H), 1.55-1.41 (m, 1H), 1.50 (s, 18H), 1.44 (s, 9H), 1.35-1.26 (m, 1H), 1.33 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 168.4 (d, *J* = 24.3 Hz), 152.5, 141.8, 141.4, 128.3, 128.2, 128.1, 128.0, 127.2, 126.6, 89.9 (d, *J* = 188.9 Hz), 82.3, 82.0, 58.0 (d, *J* = 18.5 Hz), 57.5, 50.8 (d, *J* = 4.7 Hz), 46.5, 28.0, 27.9, 27.1, 24.5 (d, *J* = 5.4 Hz), 19.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -199.1 (dd, *J* = 51.8, 32.3 Hz).

HRMS calcd for C$_{35}$H$_{51}$FN$_2$NaO$_6$ 637.3623, found 637.3645 [M+Na]$^+$.

tert-Butyl (2*S*,3*S*,α*S*)-6-(4,4-diphenyl-1-aza-4-silacyclohex-1-yl)-3-(*N*-benzyl-α-methylbenzylamino)-2-fluorohexanoate
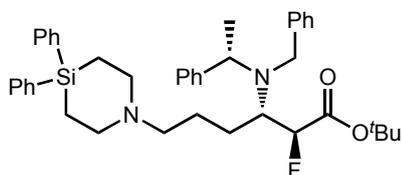
190

The silylpiperazine aminohexanoate **190** was prepared from **165** (136 mg, 0.32 mmol) according to Representative Procedure B, with the following variation: NFSI was added at –45°C and after 2 hr the reaction was allowed to warm to -10°C over 45 min before quenching. The sulfonimide by-products were then filtered off through a plug of silica (1:2 ether/hexane) and the filtrate concentrated *in vacuo*. The residue was redissolved in dry DCM (2 ml) and phenyl isocyanate (22 μL, 0.20 mmol) added to react with the excess dibenzylamine. After stirring under N_2 for 3.5 hr, the solution was concentrated *in vacuo* and purified using column chromatography (5:44:1 ether/hexane/Et₃N) to give a colourless gum (107 mg, 51%; dr 99:1).

$[\alpha]_D^{20}$ = +13 (c = 1.0, CHCl₃).

^{1}H NMR (400 MHz, CDCl₃) δ 7.55-7.54 (m, 4H), 7.45-7.23 (m, 16H), 4.50 (d, *J* = 50.4 Hz, 1H), 4.16 (d, *J* = 15.8 Hz, 1H), 3.94 (q, *J* = 6.8 Hz, 1H), 3.66 (d, *J* = 15.8 Hz, 1H), 3.37 (ddd, *J* = 30.8, 10.0, 3.6 Hz, 1H), 2.81 (t, *J* = 6.0 Hz, 4H), 2.36 (m, 2H), 1.80 (m, 1H), 1.64 (m, 1H), 1.52-1.30 (m, 3H), 1.47 (s, 9H), 1.34 (m, 6H); ^{13}C NMR (100 MHz, CDCl₃) δ 168.4 (d, *J* = 25.0 Hz), 142.4, 141.8, 135.6, 134.6, 129.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 126.5, 89.9 (d, *J* = 189.0 Hz), 82.2, 58.3, 58.1 (d, *J* = 19.0 Hz), 57.9, 52.0, 50.7 (d, *J* = 5.0 Hz), 27.9, 25.2 (d, *J* = 5.0 Hz), 24.4, 20.3, 11.0; ^{19}F NMR (282 MHz, CDCl₃) δ -200 (bs).

HRMS calcd for $C_{41}H_{52}FN_2O_2Si$ 651.3777, found 651.3777 [M+H]$^+$

tert-Butyl (2*S*,3*S*,α*S*)-3-(*N*-benzyl-α-methylbenzylamino)-3-phenyl-2-fluoropropanoate
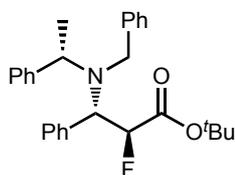
157

To a solution of diisopropylamine (0.053 ml, 0.38 mmol) in dry THF (2 ml) under N_2 at −78°C was added *n*BuLi (1.6 M in hexanes, 0.24 ml, 0.38 mmol) and the solution stirred for 30 min. A solution of (3*S*,α*S*)-*tert*-Butyl-3-(*N*-benzyl-α-methylbenzylamino)-3-phenylpropanoate **159**[4] (143 mg, 0.34 mmol) in THF (2 ml) was then added dropwise. After stirring for 1 hr a solution of NFSI (165 mg, 0.52 mmol) in THF (2 ml) was slowly added. Stirring was continued at −78°C for 4 hr, then warmed to RT overnight before quenching with saturated aq NH_4Cl (10 ml). The mixture was diluted with H_2O (10 ml) and extracted with DCM (2 × 15 ml), with the combined organic layers washed with brine (30 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was passed through a plug of silica (1:9 ether/hexane) to give 149 mg (100%) of an oil consisting of a 61:39 *anti:syn* mixture of **157** isomers (^{1}H NMR), which were not resolved.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.55 (d, *J* = 7.2 Hz, 4H), 7.43-7.32 (m, 11H), 5.08 (dd, *J* = 49.8, 3.0 Hz, 1H), 4.42 (dd, *J* = 33.0, 3.0 Hz, 1H), 4.26 (q, *J* = 4.4 Hz, 1H), 4.03 (d, *J* = 15.0 Hz, 1H), 3.94 (d, *J* = 15.0 Hz, 1H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.24 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 167.7 (d, *J* = 22.8 Hz), 144.1, 141.6, 137.3, 130.0 (d, *J* = 2.85 Hz), 128.6, 128.4, 128.3, 128.2, 127.8, 127.1, 126.8, 91.0 (d, *J* = 194.1 Hz), 82.4, 63.5 (d, *J* = 17.3 Hz), 57.9, 52.1 (d, *J* = 2.25 Hz), 27.7, 16.0.

tert-Butyl (*Z*)-α-fluorocinnamate
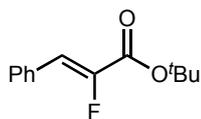
156

To a solution of (*Z*)-α-fluorocinnamic acid (248 mg, 1.49 mmol) in dry DCM (7 ml) was added oxalyl chloride (0.15 ml, 1.79 mmol) and DMF (5 drops). After stirring for 2 hr under N_2, the solution was concentrated *in vacuo*, co-evaporating the excess oxalyl chloride with $CHCl_3$ (10 ml). The residue was redissolved in dry DCM (2 ml), *tert*-butanol (0.43 ml, 4.47 mmol) and pyridine (0.36 ml, 4.47 mmol) were added, and the mixture was left to stir overnight. After removal of the solvent, ether (20 ml) was added and the solution washed with saturated aq $NaHCO_3$ (20 ml) and brine (20 ml). Drying (Na_2SO_4) and concentration *in vacuo* were followed by column chromatography (1:19 ether/hexane), which gave fluoroalkene **156** as a colourless oil (290 mg, 88%).

^1H NMR (300 MHz, $CDCl_3$) δ 7.64-7.62 (m, 2H), 7.42-7.35 (m, 3H), 6.82 (d, J = 35.1 Hz, 1H), 1.58 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4 (d, J = 33.5 Hz), 147.8 (d, J = 266.9 Hz), 131.4 (d, J = 4.6 Hz), 130.1 (d, J = 8.0 Hz), 129.4 (d, J = 2.9 Hz), 128.7, 116.5 (d, J = 5.1 Hz), 82.8, 28.0.

tert-Butyl 3-phenylprop-2-ynoate
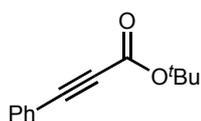
164

To a stirred solution of (*S*)-α-methylbenzylamine (0.11 ml, 0.54 mmol) in THF (2 ml) under N_2 at –78°C was added ^nBuLi (1.5 M in hexanes, 0.36 ml, 0.54 mmol). After 20 min a solution of (*Z*)-*tert*-butyl-α-fluorocinnamate (**156**) (85 mg, 0.38 mmol) in THF (1.5 ml) was added and the dark yellow solution stirred at –78°C for 2 hr. Saturated aq NH_4Cl (2 ml) was added and the mixture warmed to RT, then

diluted with DCM (10 ml). H_2O (10 ml) was added and the aqueous layer was extracted with DCM (2 × 10 ml). The combined organic extracts were washed with brine (20 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Some decomposition of the crude reaction mixture appeared to occur, and subsequent column chromatography (1:2 DCM/hexane) yielded 55 mg (72%) of the known phenylacetylene derivative **164**[5] as a colourless oil.

^{1}H NMR (300 MHz, CDCl$_3$) δ 7.58-7.55 (d, *J* = 6.9 Hz, 2H), 7.45-7.32 (m, 3H), 1.55 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.0, 132.7, 130.1, 129.3, 128.3, 119.9, 83.6, 83.3, 81.9, 27,9.

HRMS calcd for $C_{13}H_{14}O_2Na$ 225.0891, found 225.0886 [M+Na]$^+$.

Attempted epimerisation of fluoroamino ester 186

kinetic control:

To a solution of diisopropylamine (0.047 mL, 0.33 mmol) in dry THF (1.5 mL) at 0°C was added *n*BuLi (1.5 M in hexanes, 0.22 mL, 0.33 mmol) and the solution stirred for 20 min. A solution of fluoroamino ester **186** (89 mg, 0.24 mmol) in THF (1.5 mL) was added dropwise and the solution stirred at 0°C for 30 minutes, before cooling to -78°C. A solution of 2,6-di-*tert*-butylphenol (68 mg, 0.33 mmol) in THF (1.5 ml) was added dropwise and stirring continued at -78°C for 2 hr. The solution was then quenched with saturated aq NH_4Cl (5 ml) and allowed to warm to RT. Ether (20 mL) and H_2O (20 mL) were added and the layers separated. The aqueous layer was extracted with ether (10 mL) and the combined organic layers washed with saturated aq $NaHCO_3$ (20 ml) and brine (20 mL), then dried (Na_2SO_4) and concentrated *in vacuo*. ^{1}H NMR analysis of the crude reaction mixture showed a 48:52 mixture of *anti*:*syn* isomers, which were not isolated or resolved.

thermodynamic control:

A solution of dry *t*BuOH (0.12 ml, 1.61 mmol) in THF (1.5 ml) was cooled to 0°C and LiHMDS (1M in THF; 0.74 ml, 0.74 mmol) was added. After stirring for 15 minutes, a solution of **186** (71 mg, 0.19 mmol) in THF (1 ml) was added dropwise and the

mixture allowed to stir under N_2 at RT for 6 days. The solvent was removed *in vacuo*, brine (25 ml) was added and the mixture poured into ether (25 ml). After separating the ether layer it was dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (1:9 ether/hexane) afforded 51 mg (72%) of **186** as an 80:20 *anti*:*syn* mixture of C2 epimers.

tert-Butyl (3*S*,α*S*)-3-(*N*-benzyl-α-methylbenzylamino)-2,2-difluorobutanoate
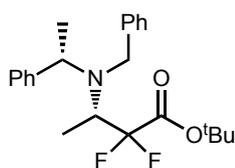
196

*n*BuLi (1.6 M in hexanes, 0.13 ml, 0.21 mmol) was added to a solution of diisopropylamine (30 μL, 0.21 mmol) in THF (2 ml) and stirred for 20 min. After cooling to −78°C, a solution of fluoroamino ester **186** (52 mg, 0.14 mmol) in THF (1 ml) was added dropwise. After warming to 0°C over 2 hr, the solution was re-cooled to −60°C and a solution of NFSI (53 mg, 0.17 mmol) in THF (1 ml) was added dropwise. The mixture was allowed to warm to RT overnight then diluted with ether (20 ml). H_2O (20 ml) was added and the layers separated. After extraction of the aqueous layer with ether (20 ml), combined organic layers were washed with brine (25 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (1:1 DCM/hexane) afforded 30 mg (55%) of the difluoro ester **196** as a colourless oil that could be crystallised from cold hexane, mp 61-63°C.

$[\alpha]_D^{20}$ = -8 (c = 1.0, CHCl$_3$).

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.41 (d, *J* = 7.2 Hz, 2H), 7.33-7.21 (m, 8H), 4.06 (q, *J* = 7.2 Hz, 1H), 3.97 (d, *J* = 14.4 Hz, 1H), 3.75 (d, *J* = 14.4 Hz, 1H), 3.64-3.56 (m, 1H), 1.46 (s, 9H), 1.30 (d, *J* = 6.6 Hz, 3H), 1.19 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.5 (t, *J* = 31.3 Hz), 142.8, 141.1, 128.7, 128.2, 128.04, 127.96, 126.9, 126.7, 116.8 (dd, *J* = 258.8, 252.9 Hz), 83.9, 59.3, 54.7 (dd, *J* = 25.8, 20.8 Hz), 50.3, 27.7, 16.3, 10.3; ^{19}F NMR (282 MHz, CDCl$_3$) δ -108.0 (dd, *J* = 254.5, 11.6 Hz), -117.0

(dd, J = 253.1, 18.1).

HRMS calcd for $C_{23}H_{30}F_2NO_2$ 390.2239, found 390.2241 [M+H]$^+$.

tert-Butyl (2_S_,3_S_,α_S_)-6-hydroxy-3-(_N_-benzyl-α-methylbenzylamino)-2-fluorohexanoate
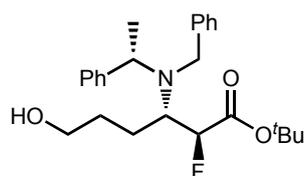
205

Using a fluoride source:

To a solution of **187** (842 mg, 1.59 mmol) in dry THF (8 ml) was added TBAF (1 M in THF, 3.33 ml, 3.33 mmol) and glacial AcOH (0.29 ml, 4.99 mmol) and the solution stirred at 40°C for 6 hr. The reaction mixture was then concentrated and the residue redissolved in diethyl ether (50 ml). This was washed with saturated aq NaHCO$_3$ (50 ml), H$_2$O (2 × 30 ml) and brine (50 ml), then dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:4 EtOAc/hexane) afforded 614 mg (93%) of hydroxy amino ester **205** as a colourless oil.

$[\alpha]_D^{20}$ = +6 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.46 (d, J = 7.2 Hz, 2H), 7.31 (m, 8H), 4.51 (dd, J = 50.0, 0.8 Hz, 1H), 4.18 (d, J = 15.2, 1H), 3.96 (q, J = 6.8 Hz, 1H), 3.68 (d, J = 15.6 Hz, 1H), 3.55 (t, J = 6.4 Hz, 2H), 3.41 (ddd, J = 31.2, 9.6, 3.2 Hz, 1H), 1.93-1.83 (m, 1H), 1.75-1.56 (m, 2H), 1.47-1.37 (m, 1H), 1.46 (s, 9H), 1.35 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.6 (d, J = 24.0 Hz), 142.4, 141.8, 128.4, 128.3, 128.1, 128.0, 127.3, 126.6, 89.9 (d, J = 188.8 Hz), 82.5, 62.7, 58.4, 58.0 (d, J = 18.8 Hz), 50.8 (d, J = 4.4 Hz), 30.0, 28.0, 23.3 (d, J = 5.6 Hz), 20.4; ^{19}F NMR (282 MHz, CDCl$_3$) δ -199.9 (dd, J = 50.7, 31.2 Hz).

HRMS calcd for $C_{25}H_{35}FNO_3$ 416.2595, found 416.2601 [M+H]$^+$.

Using acidic conditions:

187 (256 mg, 0.48 mmol) was dissolved in an AcOH/H$_2$O/THF (6:2:1) solution (5 ml) and allowed to stir at RT overnight, after which time it was poured into saturated aq NaHCO$_3$ (20 ml). DCM (15 ml) was added and the aqueous phase was extracted with DCM (15 ml). The combined organic layers washed with brine (20 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:3 EtOAc/hexane) afforded 165 mg (83%) of **205** as a colourless oil.

tert-Butyl (2*S*,3*S*,α*S*)-6-(isoindole-1,3-dione)-3-(*N*-benzyl-α-methyl-benzylamino)-2-fluorohexanoate
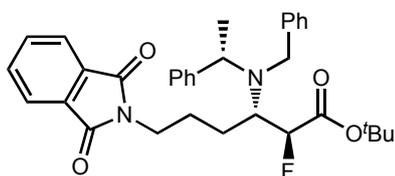
206

A solution of **205** (240 mg, 0.58 mmol), PPh$_3$ (227 mg, 0.87 mmol) and phthalimide (128 mg, 0.87 mmol) in THF (6 ml) was cooled to 0°C and DIAD (0.18 ml, 0.87 mmol) added dropwise. The mixture was allowed to warm to RT overnight, then concentrated *in vacuo* and subjected to column chromatography (1:4 EtOAc/hexane) to give the phthalimide **206** as a colourless oil (307 mg, 97%).

[α]$_D^{20}$ = +9 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.86-7.83 (m, 2H), 7.73-7.70 (m, 2H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.36-7.21 (m, 8H), 4.35 (d, *J* = 50.0 Hz, 1H), 4.13 (d, *J* = 15.2 Hz, 1H), 3.93 (d, *J* = 6.8 Hz, 1H), 3.74-3.60 (m, 3H), 3.44 (ddd, *J* = 15.6, 9.2, 2.2 Hz, 1H), 2.14-2.04 (m, 1H), 1.78-1.65 (m, 2H), 1.42 (s, 9H), 1.37-1.27 (m, 1H), 1.35 (d, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.3 (d, *J* = 24.2 Hz), 168.2, 141.7, 141.3, 133.8, 132.2, 128.4, 128.3, 128.1, 128.0, 127.2, 126.6, 123.1, 90.0 (d, *J* = 189.4 Hz), 82.5, 57.8, 57.6 (d, *J* = 18.6 Hz), 50.8 (d, *J* = 4.7 Hz), 38.0, 27.9, 26.1, 24.5 (d, *J* = 5.3 Hz), 20.0; ^{19}F NMR (282 MHz, CDCl$_3$) δ -199.7 (dd, *J* = 49.7, 30.2 Hz).

HRMS calcd for C$_{33}$H$_{38}$FN$_2$O$_4$ 545.2810, found 545.2815 [M+H]$^+$

tert-Butyl (2*S*,3*S*,α*S*)-6-(*N*-*tert*-butylcarbonyloxy)amino-3-(*N*-benzyl-α-methylbenzylamino)-2-fluorohexanoate

207
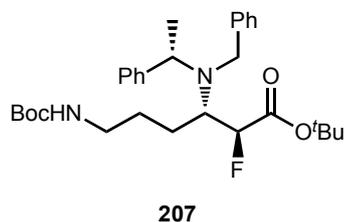
Phthalimide **206** (139 mg, 0.26 mg) was dissolved in EtOH (6 ml) and a solution of MeNH$_2$ in H$_2$O (41% w/w, 1.25 ml) was added. After stirring for 2.5 hr, the mixture was concentrated *in vacuo* and H$_2$O (20 ml) and diethyl ether (20 ml) were added. The aqueous layer was extracted with diethyl ether (2 × 10 ml), then the combined organic layers were washed with 2M NaOH (40 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was redissolved in THF/H$_2$O (4:1, 2 ml), then Na$_2$CO$_3$ (54 mg, 0.51 mmol) and Boc$_2$O (0.062 ml, 0.27 mmol) were added. After 3 hr saturated aq NH$_4$Cl (10 ml) and diethyl ether (20 ml) were added. The layers were separated and the ether layer washed with H$_2$O (2 × 10 ml) and brine (20 ml). The organic layer was dried (Na$_2$SO$_4$) and concentrated, with column chromatography (1:4 diethyl ether/hexane) performed on the residue affording Boc-protected **207** as a colourless gum (130 mg, 99%).

$[\alpha]_D^{20}$ = +10 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42 (d, *J* = 8.4 Hz, 2H), 7.38-7.23 (m, 8H), 4.48 (d, *J* = 50.0 Hz, 1H), 4.49 (bs, 1H), 4.15 (d, *J* = 15.2 Hz, 1H), 3.94 (q, *J* = 6.8 Hz, 1H), 3.66 (d, *J* = 15.6 Hz, 1H), 3.38 (ddd, *J* = 31.0, 9.8, 3.2 Hz, 1H), 3.07-2.99 (m, 2H), 1.86-1.76 (m, 1H), 1.71-1.60 (m, 1H), 1.54-1.44 (m, 1H), 1.49 (s, 9H), 1.45 (s, 9H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.35-1.28 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.4 (d, *J* = 24.1 Hz), 155.8, 142.2, 141.6, 128.3, 128.2, 128.01, 127.98, 127.3, 126.6, 89.9 (d, *J* = 189.3 Hz), 82.5, 79.0, 58.2, 57.8 (d, *J* = 18.5 Hz), 50.8 (d, *J* = 4.5 Hz), 40.4, 28.4, 28.0, 27.2, 24.4 (d, *J* = 5.4 Hz), 20.2; ^{19}F NMR (282 MHz, CDCl$_3$) δ -200.7 (dd, *J* = 49.1, 31.0 Hz). HRMS calcd for C$_{30}$H$_{44}$FN$_2$O$_4$ 515.3280, found 515.3286 [M+H]$^+$.

tert-Butyl (2_S_,3_S_)-6-(_N_-_tert_-butylcarbonyloxy)amino-3-(9_H_-fluoren-9-yl-methoxycarbonylamino)-2-fluorohexanoate

208
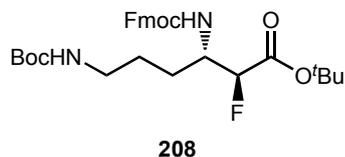
Boc-protected ester **207** (138 mg, 0.26 mmol) was dissolved in MeOH/H_2O/AcOH (45:4:1, 3 ml) and Pd(OH)$_2$/C (20 wt %) (53 mg) was added. After stirring under an atmosphere of H_2 for 7 hr, the mixture was filtered over Celite and concentrated *in vacuo*. The residue was diluted with EtOAc (20 ml) and saturated aq NaHCO$_3$ (20 ml) added. The layers were separated and the organic layer washed with H_2O (20 ml). The aqueous layer was extracted with EtOAc (2 × 20 ml) and the combined organic layers washed with brine (20 ml), dried (Na$_2$SO$_4$), then concentrated *in vacuo*. The residue was dissolved in THF/H_2O (4:1, 2 ml) and Na$_2$CO$_3$ (54 mg, 0.51 mmol) was added. The mixture was cooled to 0°C and Fmoc-OSu (91 mg, 0.27 mmol) was added portionwise, with stirring continued at 0°C for 1 hr then at RT for 1.5 hr. DCM (25 ml) and H_2O (25 ml) were added, the layers separated, and the aqueous layer extracted with DCM (2 × 10 ml). The combined organic layers were washed with brine (30 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:1 diethyl ether/hexane) gave Fmoc derivative **208** as a white solid (144 mg, 98%), mp = 89-91°C.

$[\alpha]_D^{20}$ = -6 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.46 (t, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 5.21 (bs, 1H), 4.84 (dd, *J* = 49.2, 2.4 Hz, 1H), 4.58 (bs, 1H), 4.50-4.40 (m, 2H), 4.42-4.10 (m, 1H), 4.21 (t, *J* = 6.8 Hz, 1H), 3.16-3.06 (m, 2H), 1.60-1.45 (m, 4H), 1.50 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.4 (d, *J* = 23.5 Hz), 155.96, 155.92, 143.7, 141.3, 127.7, 127.0, 125.01, 124.95, 119.97, 119.94, 90.1 (d, *J* = 186.8 Hz), 83.4, 79.2, 66.8, 52.2 (d, *J* = 19.3 Hz), 47.2, 40.0, 28.4, 28.0, 26.4, 25.8; ^{19}F NMR (282 MHz, CDCl$_3$) δ -203.6 (dd, *J* = 49.4, 26.0 Hz).

HRMS calcd for $C_{30}H_{39}FN_2O_6Na$ 565.2690, found 565.2683 [M+Na]$^+$

(2*S*,3*S*)-6-(*N*-*tert*-butylcarbonyloxy)amino-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-2-fluorohexanoic acid
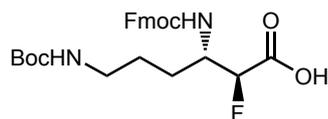
209

The Fmoc amino ester **208** (47 mg, 0.087 mmol) was dissolved in dry DCM (2 ml) and TFA (1 ml) was added. After stirring for 3 hr the solution was concentrated *in vacuo*, and the excess TFA removed via co-evaporation with CHCl$_3$ (2 × 5 ml). The residue was redissolved in THF/H$_2$O (1:1, 3 ml), then Na$_2$CO$_3$ (37 mg, 0.35 mmol) and Boc$_2$O (0.024 ml, 0.10 mmol) were added. The mixture was allowed to stir overnight, with the THF then removed *in vacuo*. The reaction mixture was diluted with saturated aq NaHCO$_3$ (10 ml), then washed with diethyl ether (10 ml). The aqueous layer was acidified to pH 2 with 6M HCl, which resulted in the formation of a white precipitate. The whole mixture was then extracted with CHCl$_3$ (3 × 10 ml) and the combined organic layers were washed with brine (20 ml), dried (Na$_2$SO$_4$) and concentrated to give a white foam. This was passed through a plug of silica (1:1 EtOAc/hexane to 100% EtOAc) to give the Fmoc-protected (2*S*)-fluoro-β^3-amino acid **209** as a white powder (34 mg, 81%), mp = 152-155°C.

$[\alpha]_D^{20}$ = -5 (c = 0.65, CHCl$_3$).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* = 7.6 Hz, 2H), 7.71 (d. *J* = 7.2 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 6.78 (t, *J* = 5.0 Hz, 1H), 4.90 (dd, *J* = 49.0, 3.0 Hz, 1H), 4.34-4.21 (m, 3H), 3.91-3.82 (m, 1H), 3.35 (bs, 1H), 2.89 (q, *J* = 6.0 Hz, 2H), 1.55-1.42 (m, 2H), 1.41-1.27 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (150 MHz, (CD$_3$)$_2$CO) δ 169.5 (d, *J* = 23.0 Hz), 156.9, 156.7, 145.1, 144.0, 142.1, 128.5, 127.9, 126.2, 126.1, 120.8, 91.1 (d, *J* = 188.1 Hz), 79.2, 78.5, 68.0, 67.0, 53.6 (d, *J* = 20.4 Hz), 48.0, 40.6, 28.6, 27.5, 26.2 (d, *J* = 3.84 Hz); ^{19}F NMR (282 MHz, CDCl$_3$) δ -204.8 (dd, *J* = 49.1, 25.7 Hz).

HRMS calcd for $C_{26}H_{31}FN_2O_6Na$ 509.2064, found 509.2059 $[M+Na]^+$.

tert-Butyl (2_S_,3_S_,α_S_)-5-hydroxy-3-(_N_-benzyl-α-methylbenzylamino)-2-fluoropentanoate
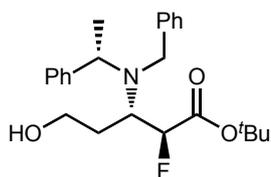
210

To a solution of **188** (204 mg, 0.395 mmol) in THF (2 ml) was added TBAF (1 M in THF, 0.79 ml, 0.79 mmol) and glacial AcOH (0.068 ml, 1.19 mmol), and the resulting mixture stirred at 40°C for 4.5 hr. The reaction mixture was poured into sat NaHCO$_3$ (30 ml) and diethyl ether (30 ml) was added. The separated ether layer was washed with H$_2$O (3 × 25 ml) and brine (25 ml), then dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:3 EtOAc/hexane) performed on the residue afforded **210** as a colourless oil (148 mg, 93%).

$[\alpha]_D^{20}$ = -2 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44 (d, J = 7.2 Hz, 2H), 7.40-7.28 (m, 6H), 4.39 (d, J = 50.4 Hz, 1H), 4.22 (d, J = 14.4, 1H), 3.99 (q, J = 6.8 Hz, 1H), 3.92-3.83 (m, 1H), 3.81-3.74 (m, 1H), 3.72 (ddd, J = 32.0, 10.4, 2.4 Hz, 1H), 2.53 (dd, J = 6.8, 2.5 Hz, 1H), 2.02-1.93 (m, 1H), 1.52-1.45 (m, 1H), 1.48 (s, 9H), 1.41 (d, J = 7.2 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.2 (d, J = 24.4 Hz), 141.36, 140.7, 128.6, 128.4, 128.2, 127.5, 126.9, 89.6 (d, J = 188.7 Hz), 82.7, 60.9, 57.8, 56.2 (d, J = 19.0 Hz), 51.0, 29.2 (d, J = 5.0 Hz), 28.0, 20.1; ^{19}F NMR (282 MHz, CDCl$_3$) δ -198.7 (bs).

HRMS calcd for $C_{24}H_{33}FNO_3$ 402.2439, found 402.2445 $[M+H]^+$.

***tert*-Butyl (2*S*,3*S*)-5-hydroxy-3-(*N-tert*-butylcarbonyloxy)amino-2-fluoropentanoate**
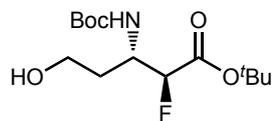
211

To a solution of **210** (303 mg, 0.75 mmol) in MeOH/EtOAc (1:1, 4 ml) was added Pd(OH)$_2$/C (20 wt %) (125 mg) and Boc$_2$O (0.197 ml, 0.86 mmol), and the mixture stirred under an atmosphere of H$_2$ for 7 hr. The reaction mixture was filtered over Celite and the filtrate concentrate *in vacuo*, with column chromatography (1:1 to 3:1 diethyl ether/hexane) performed on the residue Boc-protected **211** as a colourless oil (220 mg, 95%).

$[\alpha]_D^{20}$ = +5 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 5.00 (s, 1H), 4.93 (dd, J = 49.6, 2.8 Hz, 1H), 4.36-4.24 (m, 1H), 3.74-3.58 (m, 2H), 2.97 (dd, J = 8.4, 4.8 Hz, 1H), 1.75-1.67 (m, 1H), 1.62-1.53 (m, 1H), 1.51 (s, 9H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.2 (d, J = 23.5 Hz), 156.5, 90.5 (d, J = 185.6 Hz), 83.6, 80.6, 58.3, 48.7 (d, J = 19.2 Hz), 32.0 (d, J = 3.0 Hz), 28.3, 28.0; ^{19}F NMR (282 MHz, CDCl$_3$) δ -202.6 (dd, J = 49.5, 28.1 Hz).

HRMS calcd for C$_{14}$H$_{26}$FNO$_5$Na 330.1693, found 330.1689 [M+Na]$^+$.

tert-Butyl (2*S*,3*S*)-5-(*N*$^\delta$,*N*$^\omega$-dibenzyloxycarbonylguanidino)-3-(*N-tert*-butylcarbonyloxy)amino-2-fluoropentanoate
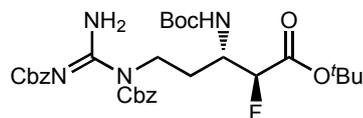
216

A solution of the hydroxy amino ester **211** (150 mg, 0.49 mmol), PPh$_3$ (167 mg, 0.64 mmol) and *N*,*N*'-di-Cbz guanidine[6] (321 mg, 0.98 mmol) in dry THF (8 ml) was cooled to 0°C and DIAD (0.12 ml, 0.64 mmol) added dropwise. The solution was allowed to warm to RT overnight, after which time several drops of H$_2$O were added and the mixture concentrated *in vacuo*. Upon standing for ~ 15 min, excess guanidinylating reagent precipitated out and was filtered off, washing with diethyl ether. The residue was purified with column chromatography (1:3 EtOAc/hexane), which afforded 288 mg (95%) of the arginine derivative **216** as a colourless gum.

$[\alpha]_D^{20}$ = +15 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.37 (bs, 1H), 9.20 (bs, 1H), 7.33 (m, 10H), 5.27 (d, *J* = 12.4 Hz, 1H), 5.26 (bs, 1H), 5.19 (d, *J* = 12.0 Hz), 5.16 (d, *J* = 11.6 Hz, 1H), 5.10 (d, *J* = 12.8 Hz, 1H), 4.94 (dd, *J* = 49.4, 2.6 Hz, 1H), 4.13-3.93 (m, 3H), 1.83-1.76 (m, 2H), 1.39 (s, 9H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.6 (d, *J* = 24.1 Hz), 163.5, 160.0, 155.5, 136.8, 134.4, 128.9, 128.8, 128.4, 128.3, 127.74, 127.73, 89.4 (d, *J* = 186.1 Hz), 83.1, 79.7, 77.2, 69.1, 66.8, 50.3 (d, *J* = 20.2 Hz), 42.1, 28.3, 27.8, 27.2 (d, *J* = 3.2 Hz); ^{19}F NMR (282 MHz, CDCl$_3$) δ -204.6 (bs).

HRMS calcd for C$_{31}$H$_{42}$FN$_4$O$_8$ 617.2981, found 617.2982 [M+H]$^+$

(2*S*,3*S*)-5-(*N*'benzyloxycarbonylguanidino)-3-(9*H*-fluoren-9-ylmethoxy-carbonylamino)amino-2-fluoropentanoic acid
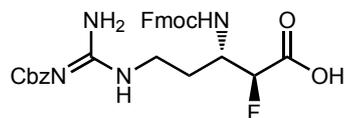
223

TFA (0.5 ml) was added to a solution of **216** (78 mg, 0.13 mmol) in dry DCM (1 ml) and the solution stirred at RT for 3.5 hr. The TFA was removed *in vacuo*, co-evaporating with $CHCl_3$ (2 × 5 ml), and the residue redissolved in 1:1 THF-H_2O (4 ml). Na_2CO_3 (57 mg, 0.51 mmol) was added, the mixture was cooled to 0°C, and Fmoc-OSu (53 mg, 0.16 mmol) was added portionwise. After warming to RT overnight the THF was removed *in vacuo*, and ether (10 ml) and H_2O (5 ml) were added. The layers were separated and the aqueous phase acidified to pH 2, which resulted in the formation of a white precipitate. The whole mixture was extracted with $CHCl_3$ (3 × 10 ml) and the combined organic layers washed with brine (20 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The resulting white solid was triturated with hot acetone, allowed to cool, then filtered to give 23 mg of a compound highly suspected to be β^3-arginine derivative **223**, mp = 198-192°C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (bs, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 7.2 Hz, 2H), 7.43-7.27 (m, 9H), 6.55 (bs, 2H), 5.07 (m, 2H), 4.94 (dd, *J* = 49.4, 4.2 Hz, 1H), 4.27-4.19 (m, 4H), 3.93-3.83 (m, 1H), 3.39-3.24 (m, 2H), 1.94-1.85 (m, 1H), 1.84-1.73 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.3 (d, *J* = 26.4 Hz), 158.8, 156.4, 155.5, 143.8, 140.6, 137.0, 128.1, 127.5, 127.4, 127.3, 126.8, 124.9, 119.8, 89.1 (d, *J* = 195.1 Hz), 65.7, 64.9, 62.8, 49.8 (d, *J* = 22.1 Hz), 46.7, 36.6, 20.4.

tert-Butyl (2*S*,3*S*)-5-(*N*δ,*N*ω-dibenzyloxycarbonylguanidino)-3-(*N*-tert-butylcarbonyloxy)amino-2-fluoropentanoate
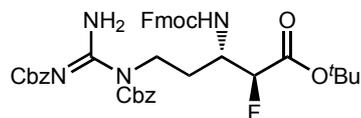
224

A solution of **216** (96 mg, 0.16 mmol) in dry DCM (1 ml) was cooled to 0°C and TFA (0.5 ml) was added. Stirring was continued at 0°C for 3 hr, after which time the TFA was removed under a stream of N_2 and the residue further concentrated *in vacuo*. This was then redissolved in THF (1.5 ml) and a solution of Na_2CO_3 (68 mg, 0.64 mmol) in H_2O (1.5 ml) was added. After cooling to 0°C, Fmoc-OSu (67 mg, 0.20 mmol) was added portionwise and the mixture allowed to warm to RT overnight. The THF was removed *in vacuo*, and ether (10 ml) and H_2O (5 ml) were added. The layers were separated and the ether layer was washed with brine (10 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (1:1:1 ether/DCM/hexane) afforded 38 mg (48%) of the Cbz-protected ester **224** as a colourless oil.

$[\alpha]_D^{25}$ = +12 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.41 (bs, 1H), 9.19 (bs, 1H), 7.76-7.74 (m, 2H), 7.56 (t, *J* = 7.0 Hz, 2H), 7.40-7.20 (m, 14H), 5.88 (d, *J* = 5.2 Hz, 1H), 5.27 (d, *J* = 12.4 Hz, 1H), 5.16 (d, *J* = 12.4 Hz, 1H), 5.11 (d, *J* = 12.8 Hz, 1H), 5.05 (d, *J* = 12.8 Hz, 1H), 4.96 (dd, *J* = 49.6, 2.8 Hz, 1H), 4.33-4.24 (m, 2H), 4.06-3.90 (m, 4H), 1.93-1.79 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5 (d, *J* = 24.04 Hz), 163.6, 160.1, 156.1, 155.5, 143.9, 141.3, 136.7, 134.4, 128.94, 128.87, 128.30, 128.27, 127.74, 127.67, 127.64, 127.61, 127.0, 125.1, 119.92, 119.99, 89.2 (d, *J* = 186.6 Hz), 83.2, 69.2, 66.8, 66.7, 50.9 (d, *J* = 20.5 Hz), 47.1, 41.8, 36.6, 27.8, 27.0 (d, *J* = 3.2 Hz).

HRMS calcd for $C_{41}H_{43}FN_4O_8Na$ 761.2957, found 761.2995 [M+Na]$^+$.

Ethyl (3*S*)-5-methyl-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluorohexanoate
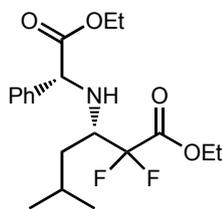
267

To a solution of isovaleraldehyde (0.082 ml, 0.76 mmol) in dry DCM (2 ml) was added freshly dried Na_2SO_4 (0.75g, 5.25 mmol), with the mixture then cooled to 0°C. Neat (*R*)-phenylglycine ethyl ester **278** (0.12 ml, 0.75 mmol) was added dropwise, with the mixture stirred under N_2 at 0°C for 30 min, then RT for 2.5 hr. The Na_2SO_4 was then filtered off through a plug of cotton wool, and the filtrate was concentrated *in vacuo.* This crude imine mixture was not further purified. Meanwhile, freshly acid-washed zinc dust (147 mg, 2.25 mmol) was suspended in dry THF (2.5 ml), then I_2 (46 mg, 0.18 mmol) was added and the mixture was sonicated under N_2 for 15 min. While still sonicating, ethyl bromodifluoroacetate (0.14 ml, 1.12 mmol) was added, which resulted in a vigorous reaction. Once this had fully ceased (~1 min), a solution of the imine in THF (1.5 ml) was added dropwise and the mixture sonicated for a further 20 min, which resulted in the formation of a dark yellow mixture. The reaction flask was then removed from the sonicator and the mixture quenched with sat NH_4Cl (5 ml), with stirring continued for ~10 min. Diethyl ether (30 ml) was added and the layers were separated. The organic layer was washed with H_2O (30 ml), sat $NaHCO_3$ (30 ml) and brine (30 ml), then dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (1:9 diethyl ether/hexane) performed on the residue gave the difluorinated amino ester **267** as a colourless oil (200 mg, 72%).

$[\alpha]_D^{20}$ = -19 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38-7.29 (m, 5H), 4.64 (s, 1H), 4.32 (q, *J* = 4.8 Hz, 2H), 4.17 (m, 1H), 4.08 (m, 1H), 3.05-2.99 (m, 1H), 2.35 (bs, 1H), 1.67-1.60 (m, 1H), 1.35-1.29 (m, 1H), 1.33 (t, *J* = 4.8 Hz, 3H), 1.23-1.17 (m, 1H), 1.17 (t, *J* = 4.8 Hz, 3H), 0.82 (d, *J* = 4.8 Hz, 3H), 0.45 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 164.0 (t, *J* = 32.0 Hz), 138.2, 128.6, 128.2, 128.1, 117.5 (t, *J* = 254.5 Hz), 63.2 (t, *J* = 1.8 Hz), 62.6, 61.2, 55.1 (t, *J* = 22.9 Hz), 38.6 (t, *J* = 3.1 Hz), 23.9, 23.5, 20.9, 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.1 (dd, *J* = 257.2, 9.2 Hz), -112.5 (dd, *J* = 257.1, 11.6 Hz).

HRMS calcd for C$_{19}$H$_{27}$F$_2$NO$_4$Na 394.1806, found 394.1800 [M+Na]$^+$.

Ethyl (3*S*)-5-methyl-3-(methyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoro hexanoate
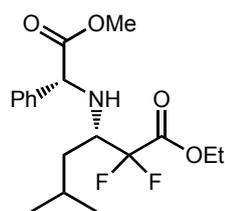
266

The title compound **266** was prepared from (*R*)-phenylglycine methyl ester **264** (0.11 ml, 0.75 mmol) according to the Representative Procedure, giving 170 mg (64%) of the product as a colourless oil.

[α]$_D^{20}$ = -33 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38-7.27 (m, 5H), 4.67 (d, *J* = 6.4 Hz, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.66 (s, 3H), 3.06-2.95 (m, 1H), 2.24 (t, *J* = 7.0 Hz, 1H), 1.67-1.60 (m, 1H), 1.35 (t, *J* = 7.2 Hz, 3H), 1.35-1.38 (m, 1H), 1.22-1.14 (m, 1H), 0.83 (d, *J* = 6.7 Hz, 3H), 0.45 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.9, 164.0 (t, *J* = 32.2 Hz), 138.1, 128.6, 128.3, 128.1, 117.6 (t, *J* = 254.5 Hz), 63.1 (t, *J* = 1.8 Hz), 62.6, 55.0 (t, *J* = 22.8 Hz), 52.3, 38.6 (t, *J* = 3.2 Hz), 23.9, 23.5, 20.9, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.1 (dd, *J* = 257.2, 9.2 Hz), -112.6 (dd, *J* = 256.0, 13.0 Hz).

HRMS calcd for $C_{18}H_{27}F_2NO_4$ 358.1824, found 358.1830 $[M+H]^+$.

Ethyl (3*S*)-5-methyl-3-(*tert*-butyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoro hexanoate
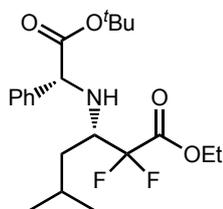
272

The title compound **272** was prepared from (*R*)-phenylglycine *tert*-butyl ester **269** (155 mg, 0.75 mmol) according to the Representative Procedure, giving 144 mg (48%) of the product as a colourless oil.

$[\alpha]_D^{20}$ = -39 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37-7.27 (m, 5H), 4.53 (s, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 3.08-2.99 (m, 1H), 2.41 (bs, 1H), 1.69-1.60 (m, 1H), 1.40-1.31 (m, 13H), 1.24-1.17 (m, 1H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.48 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.5, 164.0 (t, *J* = 32.1 Hz), 138.5, 128.4, 127.9, 117.5 (t, *J* = 254.9 Hz), 81.6, 63.6 (t, *J* = 1.7 Hz), 62.6, 55.2 (t, *J* = 23.0 Hz), 38.5, 27.8, 24.0, 23.5, 21.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -110.8 (dd, *J* = 257.2, 1.3 Hz), -112.2 (dd, *J* = 258.5, 13.0 Hz).

HRMS calcd for $C_{21}H_{32}F_2NO_4$ 400.2294, found 400.2299 $[M+H]^+$.

Ethyl (3*S*)-5-phenyl-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoro pentanoate
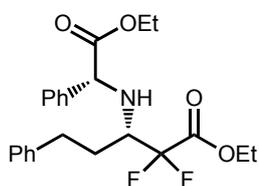
284

The title compound **284** was prepared from 3-phenylpropanal (127 mg, 0.95 mmol) according to the Representative Procedure, giving 300 mg (75%) of the product as a colourless oil.

$[\alpha]_D^{20}$ = -16 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43-7.34 (m, 5H), 7.22-7.12 (m, 3H), 6.94-6.92 (m, 2H), 4.62 (s, 1H), 4.35-4.23 (m, 2H), 4.22-4.06 (m, 2H), 3.08-2.99 (m, 2H), 2.76-2.69 (m, 1H), 2.43-2.36 (m, 2H), 1.87-1.78 (m, 1H), 1.71-1.62 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 163.8 (t, *J* = 32.0 Hz), 141.8, 138.5, 128.7, 128.3, 128.2, 128.0, 125.9, 117.2 (t, *J* = 254.8 Hz), 63.4 (t, *J* = 1.7 Hz), 62.7, 61.3, 56.9 (t, *J* = 23.0 Hz), 31.8, 31.3 (t, *J* = 3.1 Hz), 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.6 (bs).

HRMS calcd for C$_{23}$H$_{28}$F$_2$NO$_4$ 420.1981, found 420.1976 [M+H]$^+$.

Ethyl (3*S*)-4-methyl-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoro pentanoate
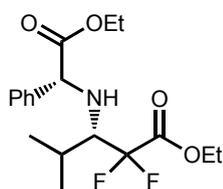
280

The title compound **280** was prepared from isobutyraldehyde (0.068 ml, 0.75 mmol) according to the Representative Procedure, giving 176 mg (66%) of the product as a colourless oil.

$[\alpha]_D^{20}$ = -20 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39-7.38 (m, 2H), 7.36-7.28 (m, 3H), 4.63 (s, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 4.21-4.13 (m, 1H), 4.13-4.05 (m, 1H), 2.97-2.89 (m, 1H), 2.33 (bs, 1H), 1.82-1.75 (m, 1H), 1.35 (t, *J* = 7.2 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.5, 164.4 (t, *J* = 32.1 Hz), 138.5, 128.4, 128.1, 128.0, 117.8 (dd, *J* = 255.5, 254.3 Hz), 64.1 (t, *J* = 1.6 Hz), 62.8, 61.6 (dd, *J* = 21.8, 20.1 Hz), 61.2, 28.0 (t, *J* = 2.3 Hz), 21.0 (t, *J* = 1.3 Hz), 16.5 (t, *J* = 1.5 Hz), 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -107.8 (dd, *J* = 256.4, 12.8 Hz), -111.9 (dd, *J* = 256.4, 15.1 Hz).

HRMS calcd for C$_{18}$H$_{26}$F$_2$NO$_4$ 358.1824, found 358.1834 [M+H]$^+$.

Ethyl (3*S*)-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluorodecanoate
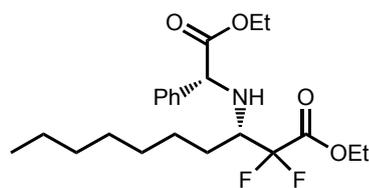
282

The title compound **282** was prepared from 1-octanal (0.12 ml, 0.75 mmol) according to the Representative Procedure, giving 216 mg (70%) of the product as a colourless oil.

$[\alpha]_D^{20}$ = -24 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38-7.28 (m, 5H), 4.61 (s, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 4.21-4.12 (m, 1H), 4.11-4.03 (m, 1H), 3.00-2.91 (m, 1H), 2.20 (bs, 1H), 1.51-1.45 (m, 1H), 1.39-1.30 (m, 5H), 1.27-1.20 (m, 2H), 1.18-1.11 (m, 7H), 1.10-1.01 (m, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 164.0 (t, *J* = 32.0 Hz), 138.1, 128.5, 128.2, 128.0, 117.4 (t, *J* = 254.6 Hz), 63.3 (t, *J* = 1.5 Hz), 62.6, 61.2, 56.9 (t, *J* = 23.1 Hz), 31.7, 29.1 (t, *J* = 2.6 Hz), 29.0, 25.5, 22.6, 14.0, 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -110.8 (dd, *J* = 256.2, 13.0 Hz), -112.1 (dd, *J* = 255.3, 9.7 Hz).

HRMS calcd for C$_{22}$H$_{34}$F$_2$NO$_4$ 414.2450, found 414.2447 [M+H]$^+$.

Ethyl (3*S*)-3-cyclohexyl-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoro propanoate
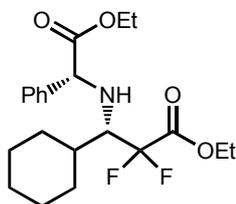
286

The title compound **286** was prepared from cyclohexanecarboxaldehyde (0.091 ml, 0.75 mmol) according to the Representative Procedure, giving 138 mg (46%) of the product as a colourless oil.

$[\alpha]_D^{20}$ = -14 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42-7.28 (m, 5H), 4.63 (d, *J* = 4.8 Hz, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 4.20-4.12 (m, 1H), 4.12-4.04 (m, 1H), 2.95-2.84 (m, 1H), 2.43 (dd, *J* = 9.4, 4.8 Hz, 1H), 1.75-1.58 (m, 4H), 1.45-1.34 (m, 1H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.29-1.02 (m, 6H), 1.17 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.5, 164.4 (t, *J* = 32.1 Hz), 138.4, 128.4, 128.2, 128.0, 117.8 (dd, *J* = 255.5, 254.4 Hz), 64.1, 62.7, 61.3 (t, *J* = 21.4 Hz), 61.2, 38.5 (t, *J* = 2.0 Hz), 30.9, 27.0, 26.9, 26.5, 26.2, 26.0, 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -107.8 (dd, *J* = 256.9, 11.7 Hz), -111.3 (dd, *J* = 256.0, 15.5 Hz).

HRMS calcd for C$_{21}$H$_{30}$F$_2$NO$_4$ 398.2137, found 398.2145 [M+H]$^+$.

Ethyl (3*S*)-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoropent-6-enoate
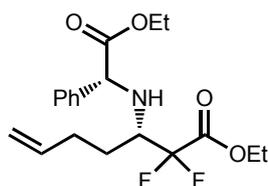
288

PCC (5.39 g, 25 mmol) and silica gel (5.4 g) were ground together using a mortar and pestle, with the mixture then suspended in dry DCM (15 ml) under N_2 and sonicated for 30 seconds. 4-Pentenol (1.02 ml, 10 mmol) was added and the mixture sonicated for a further 25 min. The solids were then removed via vacuum filtration through a silica plug, which was washed with diethyl ether, and the filtrate was gently concentrated *in vacuo*. The residue was distilled to give the aldehyde, which contained some residual DCM (^{1}H NMR analysis). A portion of this solution (0.11 ml) was treated according to the Representative Procedure, with the excess volatile aldehyde removed *in vacuo* after isolation of the crude imine mixture. The amino ester **288** was obtained as a colourless oil (277 mg, 97%).

$[\alpha]_D^{20}$ = -16 (c = 1.0, CHCl$_3$).

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.38-7.29 (m, 5H), 5.60-5.53 (m, 1H), 4.86 (dq, *J* = 10.2, 1.2 Hz, 1H), 4.78 (dq, *J* = 16.8, 1.2 Hz, 1H), 4.61 (s, 1H), 4.34-4.30 (m, 2H), 4.18-4.13 (m, 1H), 4.10-4.06 (m, 1H), 3.03-2.97 (m, 1H), 2.32 (bs, 1H), 2.17-2.11 (m, 1H), 1.89-1.83 (m, 1H), 1.61-1.55 (m, 1H), 1.47-1.40 (m, 1H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.16 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.4, 163.9 (t, *J* = 32.0 Hz), 138.3, 137.3, 128.6, 128.2, 128.0, 117.3 (t, *J* = 254.5 Hz), 115.4, 63.2, 62.7, 61.3, 56.5 (t, *J* = 23.2 Hz), 29.6, 28.6 (t, *J* = 3.1 Hz), 14.0, 13.9; ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.9 (t, *J* = 11.6 Hz).

HRMS calcd for C$_{19}$H$_{25}$F$_2$NO$_3$Na 392.1649, found 392.1656 [M+Na]$^+$.

Ethyl (3*S*)-6-(isoindole-1,3-dione)-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluorohexanoate

290
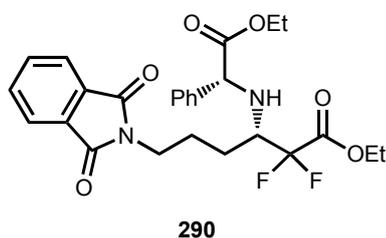
The title compound **290** was prepared from 4-phthalimidobutyraldehyde **289** (193 mg, 0.89 mmol) according to the Representative Procedure, with the following variations: the bromozinc reagent was prepared from Zn (116 mg, 1.78 mmol), I$_2$ (45 mg, 0.18 mmol) and ethyl bromodifluoroacetate (0.14 ml, 1.07 mmol) in diethyl ether/THF (2:1, 3 ml), with the reaction quenched after 15 min. Column chromatography (1:3 EtOAc/hexane) gave the product as a colourless oil (200 mg, 45%).

$[\alpha]_D^{20}$ = -20 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.85-7.79 (m, 2H), 7.72-7.67 (m, 2H), 7.37-7.34 (m, 2H), 7.31-7.27 (m, 2H), 7.24-7.19 (m, 1H), 4.59 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 4.18-4.10 (m, 1H), 4.11-4.03 (m, 1H), 3.56-3.44 (m, 2H), 3.10-3.01 (m, 1H), 2.21 (bs, 1H), 1.82-1.71 (m, 1H), 1.59-1.48 (m, 2H), 1.43-1.35 (m, 1H), 1.32 (t, *J* = 7.2 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.1, 168.1, 163.8 (t, *J* = 32.0 Hz), 138.3, 133.8, 132.2, 128.6, 128.1, 128.0, 123.1, 117.0 (t, *J* = 255.3 Hz), 63.5 (t, *J* = 1.5 Hz), 62.7, 61.2, 57.1 (t, *J* = 23.2 Hz), 37.4, 26.6 (t, *J* = 3.4 Hz), 24.9, 13.9, 13.8; ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.1 (dd, *J* = 258.4, 12.8 Hz), -112.7 (dd, *J* = 258.5, 10.4 Hz).

HRMS calcd for C$_{26}$H$_{28}$F$_2$N$_2$O$_6$Na 525.1813, found 525.1812 [M+Na]$^+$.

Ethyl (3*S*)-6-(*tert*-butyldimethylsilyloxy)-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluorohexanoate
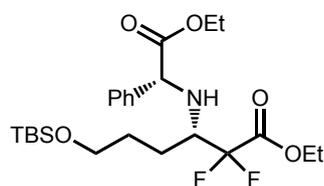
291

Under N_2, a solution of oxalyl chloride (0.09ml, 1.05 mmol) in dry DCM (10 ml) was cooled to -78°C and DMSO (0.15 ml, 2.16 mmol) was added dropwise. After stirring for 30 min, 4-(*tert*-butyldimethylsilyloxy)butanal (**171**) (184 mg, 0.90 mmol) in dry DCM (2.5 ml) was added dropwise. The solution was stirred for 30 min before Et_3N (0.63 ml, 4.50 mmol) was slowly added, then allowed to warm to 0°C over 1 hr. The reaction mixture was poured into saturated aq $NaHCO_3$ (40 ml) and the layers were separated. The aqueous layer was extracted with DCM (15 ml), then the organic layers were combined and washed with H_2O (50 ml) and brine (50 ml). Drying (Na_2SO_4) and concentration *in vacuo* gave a solution of the aldehyde in minimal DCM (1-2 ml) that was sufficiently pure to be used immediately (TLC analysis). Dry DCM (2 ml) and Na_2SO_4 (0.79g, 5.60 mmol) were subsequently added and the mixture was cooled to 0°C. Neat (*R*)-phenylglycine ethyl ester (0.13 ml, 0.80 mmol) was added dropwise and the mixture was stirred at 0°C for 30 min, then RT for 2 hr. The Na_2SO_4 was filtered off through a plug of cotton wool and the filtrate concentrated *in vacuo*. The Reformatsky reaction was then carried out according to the Representative Procedure, with the following variations: the bromozinc reagent was prepared from Zn (105 mg, 2.25 mmol), I_2 (40 mg, 0.15 mmol) and ethyl bromodifluoroacetate (0.13 ml, 1.00 mmol) in THF (2.5 ml). The reaction flask was then removed from the sonicator and stirred under N_2. A solution of the crude imine in THF (1.5 ml) then added slowly, with the reaction mixture left to stir for 15 minutes before quenching. The difluoro amino ester **291** was obtained as a colourless oil (207 mg, 53%).

$[\alpha]_D^{20}$ = -19 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.30 (m, 5H), 4.62 (s, 1H), 4.32 (q, *J* = 7.2 Hz, 2H),

4.21-4.12 (m, 1H), 4.12-4.04 (m, 1H), 3.49-3.38 (m, 2H), 3.04-2.96 (m, 1H), 2.23 (bs, 1H), 1.68-1.55 (m, 2H), 1.43-1.26 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H), 0.86 (s, 9H), -0.008 (s, 3H), -0.01 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 164.0 (t, *J* = 31.9 Hz), 138.2, 128.6, 128.2, 128.0, 117.3 (t, *J* = 254.7 Hz), 63.3 (t, *J* = 1.4 Hz), 62.7, 62.3, 61.2, 57.1 (t, *J* = 23.0 Hz), 28.9, 25.9, 25.6 (t, *J* = 3.4 Hz), 18.2, 14.0, 13.9, -5.4.

HRMS calcd for C$_{24}$H$_{39}$F$_2$NO$_5$SiNa 510.2463, found 510.2437 [M+Na]$^+$.

Ethyl (3*S*)-5-(*tert*-butyldimethylsilyloxy)-3-(ethyl (*R*)-2-amino-2-phenylacetate)-2,2-difluoropentanoate
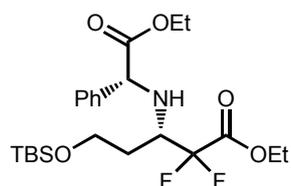
292

The title compound **292** was prepared from 3-(*tert*-butyldimethylsilyloxy)propanal (**172**) (1.21 g, 6.43 mmol) according to the Representative Procedure, with the following variations: the bromozinc reagent was prepared from Zn (1.05 g, 16.1 mmol), I$_2$ (326 mg, 1.29 mmol) and ethyl bromodifluoroacetate (1.03 ml, 8.04 mmol) in THF (20 ml). The reaction flask was then removed from the sonicator and a solution of the crude imine added to the mixture, with stirring continued for 15 minutes before the reaction was quenched. The product **292** was obtained as a colourless oil (1.59 g, 52%).

[α]$_D^{20}$ = -11 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40-7.37 (m, 2H), 7.35-7.28 (m, 3H), 4.61 (d, *J* = 6.8 Hz, 1H), 4.37-4.29 (m, 2H), 4.19-4.11 (m, 1H), 4.10-4.02 (m, 1H), 3.63-3.54 (m, 2H), 3.27-3.17 (m, 1H), 2.55 (t, *J* = 6.8 Hz, 1H), 1.81-1.73 (m, 1H), 1.60-1.53 (m, 1H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.16 (t, *J* = 7.2 Hz, 3H), 0.84 (s, 9H), -0.01 (s, 3H), -0.04 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.4, 163.9 (t, *J* = 32.0 Hz), 138.4, 128.6, 128.1, 127.8,

117.3 (t, J = 254.2 Hz), 63.5 (t, J = 1.7 Hz), 62.7, 61.2, 59.7, 55.4 (t, J = 23.4 Hz), 32.6 (t, J = 3.2 Hz), 25.9, 18.2, 14.0, 13.9, -5.5; ^{19}F NMR (282 MHz, CDCl$_3$) δ -110.9 (dd, J = 256.4, 12.8 Hz), -112.0 (dd, J = 257.9, 12.4 Hz).

HRMS calcd for C$_{23}$H$_{37}$F$_2$NO$_5$SiNa 496.2307, found 496.2288 [M+Na]$^+$.

(3*S*)-4-Isopropyl-2,2-difluoro-1-(ethyl (*R*)-2-amino-2-phenylacetate)-azetidin-2-one
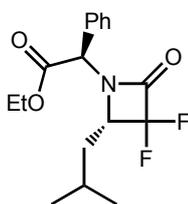
279

The title compound **279** was prepared from isovaleraldehyde (0.082 ml, 0.76 mmol) according to the Representative Procedure, with the following variations: the bromozinc reagent was prepared via the dropwise addition of ethyl bromodifluoroacetate (0.19 mmol, 1.50 mmol) to a refluxing suspension of zinc dust. A solution of the crude imine was then added and the mixture was heated at reflux for 8 minutes before quenching. ^{1}H NMR analysis of the crude reaction mixture revealed the presence of both the β-amino ester and the β-lactam in a 63:37 ratio. They were separated with column chromatography (15% diethyl ether/hexane) to give 160 mg (57%) of amino ester **267** and 83 mg (34%) of β-lactam **279** as colourless oils.

Characteristic data for the β-lactam:

[α]$_D^{20}$ = -5 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43-7.40 (m, 3H), 7.29-7.26 (m, 2H), 5.59 (s, 1H), 4.34-4.22 (m, 2H), 3.67-3.61 (m, 1H), 1.77-1.62 (m, 2H), 1.58-1.51 (m, 1H), 1.28 (t, J = 7.2 Hz, 3H), 0.85 (d, J = 6.4 Hz, 3H), 0.79 (d, J = 6.4 Hz); ^{13}C NMR (100 MHz, CDCl$_3$)

δ 168.3, 160.6 (t, J = 31.1 Hz), 131.6, 129.33, 129.25, 129.2, 128.6, 128.3, 120.3 (t, J = 285.5 Hz), 64.8 (dd, J = 99.6, 93.6 Hz), 62.2, 58.7 (t, J = 3.3 Hz), 36.1, 25.2, 22.5, 21.9, 14.0; ^{19}F NMR (282 MHz, CDCl$_3$) δ -115.3 (dd, J = 229.1, 9.6 Hz), -124.7 (d, J = 230.6 Hz).

HRMS calcd for C$_{17}$H$_{21}$F$_2$NO$_3$Na 348.1387, found 348.1386 [M+Na]$^+$.

Ethyl (3*S*)-6-(*tert*-butyldimethylsilyloxy)-3-(*N-tert*-butylcarbonyloxy)amino-2,2-difluorohexanoate

256
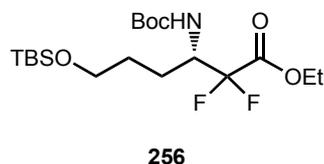
To a solution of **291** (190 mg, 0.39 mmol) in dry EtOH (2 ml) was added Boc$_2$O (0.13 ml, 0.58 mmol) and Pd(OH)$_2$/C (20 wt %, 48 mg). After stirring under an atmosphere of H$_2$ for 2 days, the mixture was filtered over Celite and the filtrate concentrated *in vacuo.* The residue was purified via column chromatography (1:3 to 2:3 EtOAc/hexane) to give the Boc-protected amino ester **256** as a colourless oil (134 mg, 81%).

$[\alpha]_D^{20}$ = -5 (c = 1.0, CHCl$_3$).

^{1}H NMR (600 MHz, CDCl$_3$) δ 4.62 (d, J = 10.3 H, 1H), 4.33-4.23 (m, 3H), 3.63 (t, J = 6.0 Hz, 2H), 1.87-1.80 (m, 1H), 1.68-1.63 (m, 1H), 1.60-1.55 (m, 1H), 1.49-1.43 (m, 1H), 1.42 (s, 9H), 1.34 (t, J = 7.2 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.4 (dd, J = 33.0, 30.8 Hz), 155.1, 114.5 (t, J = 253.7 Hz), 80.1, 63.0, 62.1, 52.6 (dd, J = 27.4, 23.2 Hz), 28.4, 28.2, 25.9, 24.1, 18.3, 13.9, -5.4; ^{19}F NMR (282 MHz, CDCl$_3$) δ -113.6 (dd, J = 257.1, 6.5 Hz), -120.0 (dd, J = 258.2, 18.1).

HRMS calcd for C$_{19}$H$_{37}$F$_2$NO$_5$SiNa 448.2307, found 448.2316 [M+Na]$^+$.

Ethyl (3*S*)-4-methyl-3-(*N-tert*-butylcarbonyloxy)amino-2,2-difluoropentanoate
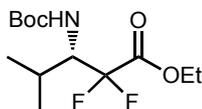
254

To a solution of **280** (225 mg, 0.63 mmol) in dry EtOH (3 ml) was added Boc_2O (0.18 ml, 0.79 mmol) and $Pd(OH)_2/C$ (20 wt %, 67 mg). After stirring under an atmosphere of H_2 for 15 hr, further portions of $Pd(OH)_2/C$ (67 mg) and Boc_2O (0.036 ml, 0.16 mmol) were added. After stirring for a further 5 hr under H_2 the mixture was filtered over Celite, concentrated *in vacuo*, and the residue purified using column chromatography (1:19 EtOAc/hexane). This afforded the Boc-protected amino ester **254** as colourless oil (142 mg, 76%).

$[\alpha]_D^{20}$ = -3 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 4.68 (d, *J* = 10.8 Hz, 1H), 4.33-4.27 (dq, *J* = 7.2, 2.8 Hz, 2H), 4.26-4.14 (m, 1H), 2.14-2.06 (m, 1H), 1.43 (s, 9H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.5 (dd, *J* = 33.1, 30.9 Hz), 155.4, 115.3 (t, *J* = 254.5 Hz), 80.1, 63.0, 56.4 (dd, *J* = 26.2, 21.6 Hz), 28.2, 27.3, 20.4, 17.1, 13.9. ^{19}F NMR (282 MHz, CDCl$_3$) δ -111.7 (dd, *J* = 257.1, 9.0 Hz), -116.7 (dd, *J* = 258.2, 20.6 Hz).

HRMS calcd for $C_{13}H_{23}F_2NO_4Na$ 318.1493, found 31.1494 [M+Na]$^+$.

Ethyl (3*S*)-5-(*tert*-butyldimethylsilyloxy)-3-(*N-tert*-butylcarbonyloxy)amino-2,2-difluoropentanoate
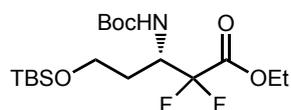
293

To a solution of **292** (229 mg, 0.48 mmol) in dry EtOH (4 ml) was added Boc_2O (0.155 ml, 0.68 mmol) and $Pd(OH)_2/C$ (20 wt %, 69 mg). After stirring under H_2 for 24 hr, a further portion of $Pd(OH)_2/C$ (69 mg) was added and stirring was continued under H_2 for a further 24 hr. The reaction mixture was then filtered over Celite, concentrated *in vacuo* and subjected to column chromatography (1:9 diethyl ether/hexane) to give the Boc-protected amino ester **293** as a colourless oil (169 mg, 85%).

$[\alpha]_D^{20}$ = -3 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (d, *J* = 9.6 Hz, 1H), 4.47-4.36 (m, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 3.79-3.68 (m, 2H), 2.03-1.63 (m, 1H), 1.68-1.60 (m, 1H), 1.41 (s, 9H), 1.34 (t, *J* = 7.2 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4 (dd, *J* = 32.8, 30.9 Hz), 155.0, 114.6 (t, *J* = 254.0 Hz), 79.9, 63.1, 59.2, 50.5 (t, *J* = 25.3 Hz), 30.5, 28.2, 25.8, 18.1, 13.9, -5.58, -5.61; ^{19}F NMR (188 MHz, $CDCl_3$) δ -113.0 (d, *J* = 254.2 Hz), -119.9 (d, *J* = 252.3 Hz).

HRMS calcd for $C_{18}H_{35}F_2NO_5SiNa$ 434.2150, found 434.2132 $[M+Na]^+$.

***tert*-Butyl (3*S*)-5-(*tert*-butyldimethylsilyloxy)-3-*N*-acetylpentanoate**

298
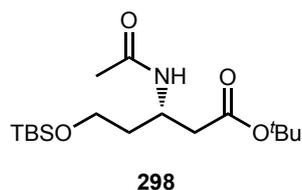
To a solution of **183** (244 mg, 0.49 mmol) in MeOH/EtOAc (2:1, 5 ml) was added Pd(OH)$_2$/C (20 wt %) (98 mg) and glacial AcOH (5 drops). The mixture was stirred under an atmosphere of H$_2$ for 2.5 hr, then filtered over Celite and concentrated *in vacuo*. The residue was redissolved in dry DCM (4 ml) before Et$_3$N (0.14 ml, 0.98 mmol), Ac$_2$O (0.055 ml, 0.59 mmol) and DMAP (6 mg) were added. After stirring for 1 hr the solution was concentrated and the residue purified using column chromatography (1:1 EtOAc/hexane), which yielded 166 mg (98%) of the *N*-acetyl amino ester **298**.

[α]$_D^{20}$ = -22 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.44 (d, *J* = 7.9 Hz, 1H), 4.34-4.26 (m, 1H), 3.77-3.64 (m, 2H), 2.55 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.42 (dd, *J* = 15.6, 6.4 Hz, 1H), 1.91 (s, 3H), 1.79-1.76 (m, 2H), 1.42 (s, 9H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.1, 169.1, 80.8, 60.4, 44.9, 39.3, 35.7, 28.0, 25.9, 23.4, 18.1, -5.5.

HRMS calcd for C$_{17}$H$_{36}$NO$_4$Si 346.2408, found 346.2414 [M+H]$^+$.

tert-Butyl (3*S*)-5-hydroxy-3-*N*-acetylpentanoate

299
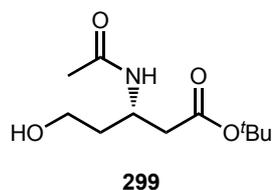
TBAF (1.0M in THF, 1.26 ml, 1.26 mmol) and glacial AcOH (0.072 ml, 1.26 mmol) were added to a solution of silyloxy amino ester **298** (217 mg, 0.63 mmol) in THF (2 ml). After stirring at 40°C for 6 hr the solvent was removed *in vacuo* and the residue redissolved in CHCl$_3$ (50 ml). This solution was poured into saturated aq NaHCO$_3$ (20 ml) and the layers were separated. The organic layer was washed with brine (20 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Passage through a plug of silica with 100% EtOAc afforded 134 mg (92%) of the hydroxy amino ester **299** as a colourless gum.

$[\alpha]_D^{20}$ = -55 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.69 (d, *J* = 8.4 Hz, 1H), 4.40-4.32 (m, 1H), 3.63-3.58 (m, 1H), 3.55-3.48 (m, 1H), 2.51(dd, *J* = 15.6, 5.2 Hz, 1H), 2.40 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.00 (s, 3H), 1.77-1.72 (m, 1H), 1.54-1.45 (m, 1H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.5, 161.0, 81.6, 58.4, 43.3, 39.7, 37.0, 31.1, 28.0, 23.1.

HRMS calcd for C$_{11}$H$_{22}$NO$_4$ 232.1543, found 232.1544 [M+H]$^+$.

tert-Butyl (3*S*)-5-(*N*^δ,*N*^ω-dibenzyloxycarbonylguanidino)-3-*N*-acetylpentanoate

300
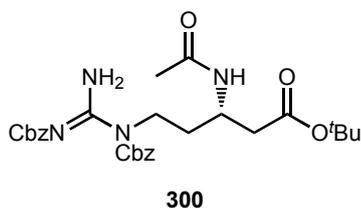
A solution of **299** (55 mg, 0.24 mmol), PPh$_3$ (81 mg, 0.31 mmol) and di-Cbz guanidine (195 mg, 0.59 mmol) in THF (3 ml) was cooled to 0°C and DIAD (0.060 ml, 0.31 mmol) added dropwise. The mixture was allowed to warm to RT overnight, then concentrated *in vacuo*. Column chromatography (1:1 to 3:1 EtOAc/hexane) performed on the residue afforded a mixture consisting of **300** and a minor amount of di-Cbz guanidine. The mixture was redissolved in hot MeOH and allowed to cool overnight to crystallise out the unwanted guanidine, which was then filtered off to leave pure arginine derivative **300** as a white solid (112 mg, 86%), mp = 54-56°C.

$[\alpha]_D^{20}$ = +5 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.42 (bs, 2H), 7.39-7.30 (m, 10H), 6.82 (bs, 1H), 5.26 (d, *J* = 16.8 Hz, 1H), 5.23 (d, *J* = 16.8 Hz, 1H), 5.13 (s, 2H), 4.08-4.00 (m, 1H), 4.00-3.89 (m, 2H), 2.56 (dd, *J* = 15.6, 4.8 Hz, 1H), 2.32 (dd, *J* = 15.2, 7.6 Hz, 1H), 1.95-1.78 (m, 2H), 1.70 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.6, 170.5, 163.0, 160.1, 155.6, 136.2, 134.4, 129.0, 128.9, 128.5, 128.41, 128.38, 128.2, 80.6, 69.3, 67.3, 45.0, 42.3, 40.2, 32.0, 28.0, 22.9.

HRMS calcd for C$_{28}$H$_{37}$N$_4$O$_7$ 541.2657, found 541.2651 [M+H]$^+$.

Methyl (3*S*)-5-(*N*δ,*N*ω-dibenzyloxycarbonylguanidino)-3-*N*-acetylpentanoate

301
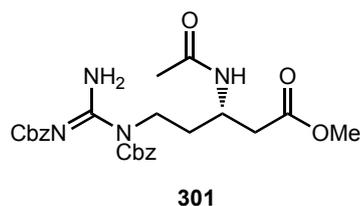
The *tert*-butyl ester **300** (121 mg, 0.22 mmol) was stirred in a solution of DCM/TFA (2:1, 1.5 ml) for 3 hours then concentrated *in vacuo*. Excess TFA was removed via co-evaporation with $CHCl_3$ (5 ml). The residue was redissolved in dry MeOH (2.5 ml) and cooled to 0°C, $SOCl_2$ (0.024 ml, 0.34 mmol) was added dropwise and the solution was allowed to warm to RT overnight. After removal of the solvent, $CHCl_3$ (20 ml) was added and the solution washed with saturated aq $NaHCO_3$ (20 ml) and H_2O (20 ml). The aqueous layer was extracted with $CHCl_3$ (10 ml) and the combined organic layers were washed with brine (20 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography over a plug of silica gel (1:1 EtOAc/hexane to 100% EtOAc) gave the methyl ester **301** as a colourless oil that solidified on standing (92 mg, 84%), mp = 106°C.

$[\alpha]_D^{20}$ = +8 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (bs, 1H), 9.24 (bs, 1H), 7.40-7.29 (m, 10H), 6.89 (d, *J* = 6.4 Hz, 1H), 5.28-5.20 (m, 2H), 5.12 (s, 2H), 4.09-4.00 (m, 1H), 3.95-3.91 (m, 2H), 3.59 (s, 3H), 2.69 (dd, *J* = 15.6, 4.8 Hz, 1H), 2.42 (dd, *J* = 15.6, 7.6 Hz, 1H), 1.95-1.79 (m, 2H), 1.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 170.6, 163.3, 160.3, 155.5, 136.3, 134.4, 128.84, 128.77, 128.4, 128.27, 128.26, 128.1, 69.1, 67.1, 51.5, 44.9, 41.9, 38.6, 32.0, 22.7.

HRMS calcd for $C_{25}H_{30}N_4O_7Na$ 521.2012, found 521.1996 [M+Na]$^+$.

Methyl (3*S*)-5-guanidino-3-*N*-acetylpentanoate
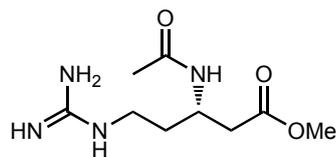
295

The Cbz-protected amino ester **301** (42 mg, 0.084 mmol) was dissolved in dry MeOH (1.5 ml) and Pd/C (10 wt %) (6 mg) was added. After stirred under an atmosphere of H_2 for 2 hr the catalyst was filtered off over Celite. The Celite pad was washed with dry MeOH and the filtrate concentrated *in vacuo* to give the β^3-arginine derivative **295** as a glassy solid (19 mg, 100%).

$[\alpha]_D^{20}$ = -41 (c = 1.0, MeOH).

^{1}H NMR (400 MHz, CD$_3$OD) δ 4.31-4.23 (m, 1H), 3.35 (s, 3H), 3.25-3.12 (m, 2H), 2.61-2.50 (m, 2H), 1.95 (s, 3H), 1.90-1.80 (m, 1H), 1.77-1.67 (m, 1H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 173.3, 173.0, 158.9, 49.9, 45.4, 39.5, 39.4, 34.7, 22.7.

HRMS calc for $C_9H_{19}N_4O_3$ 231.1452, found 231.1441 [M+H]$^+$.

tert-Butyl (2*S*,3*S*)-5-(*tert*-butyldimethylsilyloxy)-3-*N*-acetyl-2-fluoropentanoate
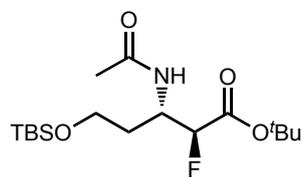
302

To a solution of **188** (240 mg, 0.46 mmol) in MeOH/EtOAc (1:1, 4 ml) was added Pd(OH)$_2$/C (20 wt %) (50 mg) and glacial AcOH (10 drops). The mixture was stirred under an atmosphere of H_2 for 3 hr, then filtered over Celite and concentrated *in vacuo*. The residue was redissolved in dry DCM (2 ml), then Ac$_2$O

(0.066 ml, 0.70 mmol) and DMAP (5 mg) were added. After cooling to 0°C, Et$_3$N (0.065 ml, 0.46 mmol) was added and stirring continued for 1 hr before the solution was concentrated *in vacuo*. Column chromatography (2:3 EtOAc/hexane) afforded 169 mg (100%) of the hydroxy amino ester **302** as a colourless oil.

$[\alpha]_D^{20}$ = -2 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.33 (d, J = 7.6 Hz, 1H), 5.02 (dd, J = 49.2, 3.2 Hz, 1H), 4.56-4.43 (m, 1H), 4.83-3.78 (m, 1H), 3.71-3.67 (m, 1H), 1.98 (s, 3H), 1.75-1.71 (m, 2H), 1.49 (s, 9H), 0.89 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 169.7, 166.8 (d, J = 24.1 Hz), 89.4 (d, J = 185.6), 83.2, 60.2, 49.1 (d, J = 19.7 Hz), 30.8 (d, J = 2.9 Hz), 28.0, 25.8, 23.3, 18.1, -5.56, -5.60; ^{19}F NMR (282 MHz, CDCl$_3$) δ -204.4 (dd, J = 50.4, 27.2 Hz).

HRMS calcd for C$_{17}$H$_{35}$FNO$_4$Si 364.2314, found 364.2319 [M+H]$^+$.

tert-Butyl (2_S_,3_S_)-5-hydroxy-3-_N_-acetyl-2-fluoropentanoate
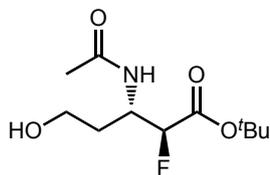
303

To a solution of the silyloxy amino ester **302** (50 mg, 0.14 mmol) in THF (1.5 ml) was added TBAF (1.0M in THF, 0.27 ml, 0.27 mmol) and glacial AcOH (0.016 ml, 0.27 mmol). After stirring at 40°C for 4 hr, the mixture was diluted with CHCl$_3$ (15 ml) then washed with saturated aq NaHCO$_3$ (15 ml) and brine (15 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was passed through a plug of silica gel with 100% EtOAc to give 27 mg (77%) of the hydroxy ester **303** as a colourless gum.

$[\alpha]_D^{20}$ = +2 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.25 (d, J = 8.8 Hz, 1H), 4.91(dd, J = 49.4, 3.0 Hz, 1H), 4.65-4.51 (m, 1H), 3.70-3.65 (m, 1H), 3.58-3.52 (m, 1H), 3.33 (bs, 1H), 2.06 (s, 3H), 1.75-1.66 (m, 1H), 1.59-1.51 (m, 1H), 1.50 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.3, 166.2 (d, J = 23.9 Hz), 90.0 (d, J = 185.4 Hz), 83.9, 58.1, 47.8 (d, J = 19.3 Hz), 31.4 (d, J = 3.1 Hz), 27.9, 23.1; ^{19}F NMR (282 MHz, CDCl$_3$) δ -201.5 (dd, J = 48.4, 27.0 Hz).

HRMS calcd for C$_{11}$H$_{20}$FNO$_4$Na 272.1274, found 272.1270 [M+Na]$^+$.

tert-Butyl (2S,3S)-5-(N^δ,N^ω-dibenzyloxycarbonylguanidino)-3-N-acetyl-2-fluoropentanoate
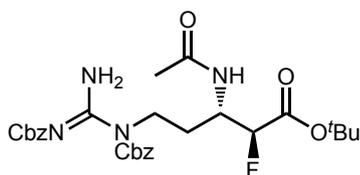
304

The hydroxy ester **303** (36 mg, 0.14 mmol), PPh$_3$ (49 mg, 0.19 mmol) and di-Cbz guanidine (95 mg, 0.29 mmol) were dissolved in THF (3 ml) and cooled to 0°C. DIAD (0.036 ml, 0.19 mmol) was added dropwise and the solution was allowed to warm to RT overnight. After concentrating *in vacuo*, the residue was purified using column chromatography (1:3 to 1:2 EtOAc/hexane), which failed to completely separate the desired product and excess di-Cbz guanidine. The mixture was then dissolved in hot MeOH and allowed to cool overnight, causing the guanidine to crystallise out of solution. This was filtered off, leaving the arginine derivative **304** as a colourless oil (53 mg, 68%).

$[\alpha]_D^{20}$ = +18 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.42 (bs, 1H), 9.23 (bs, 1H), 7.28-7.33 (m, 10H), 6.89 (d, J = 6.4 Hz, 1H), 5.24 (d, J = 18.0 Hz, 1H), 5.21 (d, J = 18.0 Hz, 1H), 5.13 (s, 2H), 5.05 (dd, J = 49.6, 3.2 Hz, 1H), 4.27-4.14 (m, 1H), 4.00-3.88 (m, 2H), 1.92-1.75 (m, 2H), 1.71 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.1, 166.8 (d, J = 23.7 Hz),

163.4, 160.2, 155.5, 136.3, 134.3, 129.0, 128.9, 128.5, 128.44, 128.36, 128.2, 88.6 (d, J = 186.2 Hz), 83.0, 69.3, 67.2, 49.3 (d, J = 19.9 Hz), 41.6, 27.9, 26.7, 22.6; ^{19}F NMR (188 MHz, CDCl$_3$) δ -205.7 (bs).

HRMS calcd for C$_{28}$H$_{35}$FN$_4$O$_7$Na 581.2382, found 581.2392 [M+Na]$^+$.

tert-Butyl (2*S*,3*S*)-5-(N^δ,N^ω-dibenzyloxycarbonylguanidino)-3-*N*-acetyl-2-fluoropentanoate
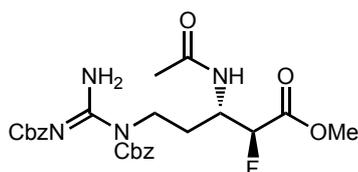
305

The *tert*-butyl ester **304** (52 mg, 0.093 mmol) was dissolved in dry DCM (1 ml) and TFA (0.5 ml) was added. After stirring for 3 hr the solution was concentrated *in vacuo*, removing excess TFA via co-evaporation with CHCl$_3$ (2 × 5 ml). The residue was redissolved in dry MeOH (2 ml), then SOCl$_2$ (0.01 ml, 0.14 mmol) was added dropwise and the solution stirred overnight. The mixture was concentrated, then CHCl$_3$ (20 ml) was added and the solution was washed with NaHCO$_3$ (20 ml) and brine (20 ml), then dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was passed through a plug of silica gel (1:1 to 3:1 EtOAc/hexane) to afford 45 mg (94%) of methyl ester **305** as a colourless oil that solidified on standing, mp = 126-127°C.

$[\alpha]_D^{20}$ = +22 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.41 (bs, 1H), 9.20 (bs, 1H), 7.42-7.30 (m, 10H), 7.00 (d, J = 6.4 Hz, 1H), 5.25-5.10 (m, 5H), 4.27-4.14 (m, 1H), 4.00-3.83 (m, 2H), 3.67 (s, 3H), 1.90-1.74 (m, 2H), 1.71 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.2, 168.2 (d, J = 23.6 Hz), 163.3, 160.2, 155.4, 136.2, 134.4, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 88.8 (d, J = 187.4 Hz), 69.2, 67.1, 52.3, 49.3 (d, J = 20.3 Hz), 41.5, 26.8 (d, J = 3.5 Hz), 22.5; ^{19}F NMR (188 MHz, CDCl$_3$) δ -206.3 (bs).

HRMS calcd for C$_{25}$H$_{29}$FN$_4$O$_7$Na 539.1918, found 539.1906 [M+Na]$^+$.

Methyl (2*S*,3*S*)-5-guanidino-3-*N*-acetyl-2-fluoropentanoate
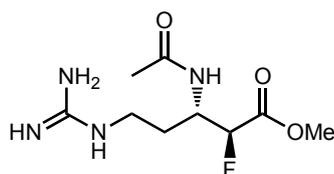
296

The Cbz-protected amino ester **305** (32 mg, 0.062 mmol) was dissolved in dry MeOH (2 ml) and Pd/C (10 wt %) (4 mg) was added. After stirring under an atmosphere of H_2 for 3 hr, the catalyst was filtered off over Celite. The Celite pad was washed with dry MeOH and the filtrate was concentrated *in vacuo* gave 15 mg (100%) of the β-arginine derivative **296** a glassy solid.

$[\alpha]_D^{20}$ = -19 (c = 1.0, MeOH).

^{1}H NMR (400 MHz, CD$_3$OD) δ 5.02 (dd, *J* = 48.6, 3.8 Hz, 1H), 4.47-4.36 (m, 1H), 3.35 (s, 3H), 3.33-3.27 (m, 1H), 3.21-3.14 (m, 1H), 2.01 (s, 3H), 1.81 (m, 2H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 173.6, 169.6 (d, *J* = 23.8 Hz), 158.7, 91.1 (d, *J* = 187.2 Hz), 49.8, 49.4 (d, *J* = 21.2 Hz), 39.1, 28.2 (d, *J* = 4.0 Hz), 22.5; ^{19}F NMR (188 MHz, CD$_3$OD-CDCl$_3$) δ -203.13 (s).

HRMS calcd for C$_9$H$_{18}$FN$_4$O$_3$ 249.1357, found 249.1341 [M+H]$^+$.

Ethyl (3*S*)-5-(*tert*-butyldimethylsilyloxy)-3-*N*-acetyl-2,2-difluoropentanoate
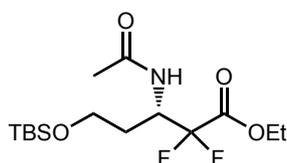
306

292 (286 mg, 0.60 mmol) was dissolved in a solution of EtOAc (4 ml), Ac$_2$O (0.085 ml, 0.91 mmol) and anhydrous AcOH (0.4 ml) before Pd(OH)$_2$/C (144 mg) was added. After stirring under an atmosphere of H_2 for 24 hr, the reaction mixture was

filtered over Celite. The filtrate was diluted with EtOAc (20 ml) then washed with saturated aq NaHCO$_3$ (2 × 20 ml) and brine (20 ml), then dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Purification of the residue using column chromatography (1:9 to 2:1 EtOAc/hexane) afforded the *N*-acetyl amino ester **306** as a colourless oil that crystallised on standing 166 mg (78%), mp = 50-52°C.

$[\alpha]_D^{20}$ = -3 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 6.11 (d, *J* = 8.8 Hz, 1H), 4.81-4.68 (m, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 3.80-3.66 (m, 2H), 2.03-1.94 (m, 1H), 1.96 (s, 3H), 1.75-1.67 (m, 1H), 1.32 (t, *J* = 7.2 Hz, 3), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 169.8, 163.2 (dd, *J* = 33.0, 30.8 Hz), 114.3 (t, *J* = 253.9 Hz), 63.2, 59.6, 49.0 (dd, *J* = 27.7, 23.2 Hz), 30.4 (t, *J* = 1.7 Hz), 25.8, 23.0, 18.1, 13.8, -5.6; ^{19}F NMR (188 MHz, CDCl$_3$) δ -112.9 (d, *J* = 259.8 Hz), -117.3 (d, *J* = 261.7 Hz).

HRMS calcd for C$_{15}$H$_{29}$F$_2$NO$_4$SiNa 376.1726, found 376.1725 [M+Na]$^+$.

Ethyl (3*S*)-5-(*N$^\delta$*,*N$^\omega$*-dibenzyloxycarbonylguanidino)-3-*N*-acetyl-2,2-difluoropentanoate

309
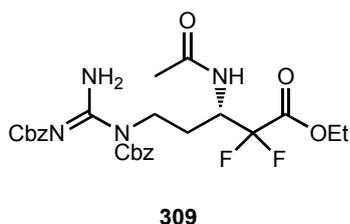
Method 1:

To a solution of silyloxy ether **308** (148 mg, 0.42 mmol) in dry EtOH (2.5 ml) was added freshly distilled acetyl chloride (3 drops). After stirring for 5 hr under N$_2$ the solution was concentrated *in vacuo,* and H$_2$O (5 ml) and saturated aq NaHCO$_3$ (1 ml) added. This mixture was extracted with CHCl$_3$ (3 × 15 ml) and the combined organic layers were washed with brine (40 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was redissolved in THF (8ml), then di-Cbz guanidine (412 mg, 1.26 mmol) and PPh$_3$ (143 mg, 0.54 mmol) were added. The solution was cooled to

0°C and DIAD (0.11 ml, 0.54 mmol) added dropwise, with the solution allowed to warm to RT overnight. The reaction mixture was concentrated to half its volume , which allowed most of the excess di-Cbz guanidine to precipitate out. This was filtered off, with column chromatography (1:1 to 2:1 EtOAc/hexane) performed on the residue yielding 103 mg (45%) of the arginine derivative **309** as a viscous oil.

$[\alpha]_D^{20}$ = +4 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.37 (bs, 1H), 9.20 (bs, 1H), 7.43-7.28 (m, 10H), 6.53 (d, J = 8.4 Hz, 1H), 5.26 (d, J = 18.0 Hz, 1H), 5.23 (d, J = 18.0 Hz, 1H), 5.14 (d, J = 14.8 Hz, 1H), 5.11 (d, J = 14.8 Hz, 1H), 4.57-4.45 (m, 1H), 4.24 (q, J = 7.2 Hz, 2H), 3.40-3.87 (m, 2H), 2.16-2.07 (m, 1H), 1.89-1.79 (m, 1H), 1.72 (s, 3H), 1.28 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.1, 163.3, 163.0 (dd, J = 32.8, 30.8 Hz), 160.0, 155.4, 136.3, 134.4, 129.0, 128.9, 128.5, 128.32, 128.26, 128.1, 113.7 (t, J = 254.2 Hz), 69.2, 67.1, 63.1, 49.4 (dd, J = 28.3, 23.5 Hz), 41.2, 25.6 (t, J = 2.2 Hz), 22.4, 13.8; ^{19}F NMR (188 MHz, CDCl$_3$) δ -113.0 (d, J = 253.8 Hz), -119.9 (d, J = 251.9 Hz).

HRMS calcd for C$_{26}$H$_{31}$F$_2$N$_4$O$_7$ 549.2155, found 549.2160 [M+H]$^+$.

Method 2:

308 (84 mg, 0.24 mmol) was dissolved in dry EtOH (1 ml) and PPTS (90 mg, 0.36 mmol) was added. After stirring at 40°C for 12 hr, the solution was diluted with EtOAc (30 ml) then washed with saturated aq NaHCO$_3$ (10 ml), H$_2$O (10 ml) and brine (20 ml). After drying (Na$_2$SO$_4$) and concentration *in vacuo* the residue was redissolved in THF (6 ml). PPh$_3$ (81 mg, 0.31 mmol) and di-Cbz guanidine (234 mg, 0.71 mmol) were added, the solution was cooled to 0°C, and DIAD (0.06 ml, 0.31 mmol) was added dropwise. After warming to RT overnight, the reaction mixture was concentrated and the residue purified using column chromatography (1:1 to 2:1 EtOAc/hexane), which afforded 103 mg (79%) of **309**.

Methyl (3*S*)-5-(*N*$^\delta$,*N*$^\omega$-dibenzyloxycarbonylguanidino)-3-*N*-acetyl-2,2-difluoropentanoate
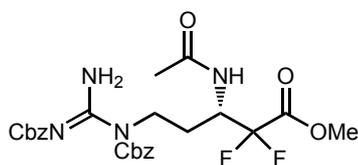
310

The ethyl amino ester **309** (103 mg, 0.19 mmol) was dissolved in MeOH (2 ml) and 4M HCl (0.5 ml) was then added. After stirring for 2 hr the solvent was evaporated and the residue concentrated under high vacuum to remove residual water. The foamy residue was redissolved in dry MeOH (2 ml) and cooled to 0°C before SOCl$_2$ (0.021 ml, 0.28 mmol) was added dropwise. After warming to RT overnight, the MeOH was removed *in vacuo*, EtOAc (20 ml) was added, and the solution was poured into saturated aq NaHCO$_3$ (20 ml). The layers were separated and the organic layer was washed with brine (20ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Column chromatography (1:1 EtOAc/hexane) gave the methyl ester **310** as a colourless oil that solidified on standing (78 mg, 78%), mp = 119-120°C.

$[\alpha]_D^{20}$ = +5 (c = 1.0, CHCl$_3$).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.38 (bs, 1H), 9.20 (bs, 1H), 7.44-7.29 (m, 10H), 6.62 (d, *J* = 8.0 Hz, 1H), 5.27 (d, *J* = 16.6 Hz, 1H), 5.24 (d, *J* = 16.6 Hz, 1H), 5.14 (d, *J* = 14.4 Hz, 1H), 5.11 (d, *J* = 14.4 Hz, 1H), 4.54-4.42 (m, 1H), 4.00-3.86 (m, 2H), 3.79 (s, 3H), 2.17-2.07 (m, 1H), 1.90-1.79 (m, 1H), 1.72 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.3, 163.5 (dd, *J* = 33.6, 30.1 Hz), 163.3, 160.0, 155.4, 136.3, 134.4, 129.0, 128.9, 128.5, 128.33, 128.29, 128.2, 113.6 (t, *J* = 254.0 Hz), 69.3, 67.1, 53.5, 49.6 (t, *J* = 28.6, 23.6 Hz), 41.2, 25.4, 22.3; ^{19}F NMR (282 MHz, CDCl$_3$) δ -113.6 (d, *J* = 254.2 Hz), -120.0 (d, *J* = 254.2 Hz).

HRMS calcd for C$_{25}$H$_{29}$F$_2$N$_4$O$_7$ 535.1999, found 535.2007 [M+H]$^+$.

Methyl (3*S*)-5-guanidino-3-*N*-acetyl-2,2-difluoropentanoate
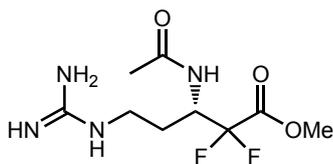
297

To a solution of Cbz-protected amino ester **310** (59 mg, 0.11 mmol) in dry MeOH (2.5 ml) was added Pd/C (10 wt %) (9 mg), and the solution stirred under an atmosphere of H_2 for 1.5 hr. The reaction mixture was then filtered over Celite, washing through with dry MeOH. Concentration of the filtrate *in vacuo* yielded 29 mg (100%) of the β^3-arginine derivative **297** as a white solid, mp = 262-264°C.

$[\alpha]_D^{20}$ = -5 (c = 1.0, MeOH).

^{1}H NMR (600 MHz, D_2O, 308K) two rotamers δ 4.66, (bs, 4H), 4.59-4.49 (m, 1H), 3.90 (s, 1H), 3.36 (s, 3H), 3.34-3.20 (m, 2H), 2.14-2.06 and 2.03-1.94 (m, 1H), 2.06 and 2.05 (s, 3H), 1.87-1.81 and 1.78-1.72 (m, 1H); ^{13}C NMR (150 MHz, D_2O, 308K) major rotamer δ 175.09, 169.3 (t, *J* = 27.1 Hz), 157.5, 116.6 (t, *J* = 253.7 Hz), 54.97, 49.6 (t, *J* = 11.7 Hz), 38.0, 27.2, 22.5; minor rotamer δ 175.12, 169.3 (t, *J* = 31.7 Hz), 160.7, 114.6 (t, *J* = 253.9 Hz), 54.95, 49.8 (t, *J* = 12.3 Hz), 37.7, 25.7, 22.2; ^{19}F NMR (188 MHz, D_2O) δ -112.2 (d, *J* = 242.9 Hz), -115.2 (d, *J* = 242.9 Hz).

HRMS calcd for $C_9H_{17}F_2N_4O_3$ 267.1263, found 267.1260 [M+H]$^+$.

7.3   References

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A.1 List of publications

Duggan, P. J.; Johnston, M. R.; March, T. L. Enantioselective Synthesis of α-Fluoro-β³-amino Esters: Synthesis of Enantiopure, Orthogonally Protected α-Fluoro-β³-lysine, *J. Org. Chem.* **2010**, *75*, 7365. doi: 10.1021/jo101600c

A.2 X-ray crystallographic data for 188:
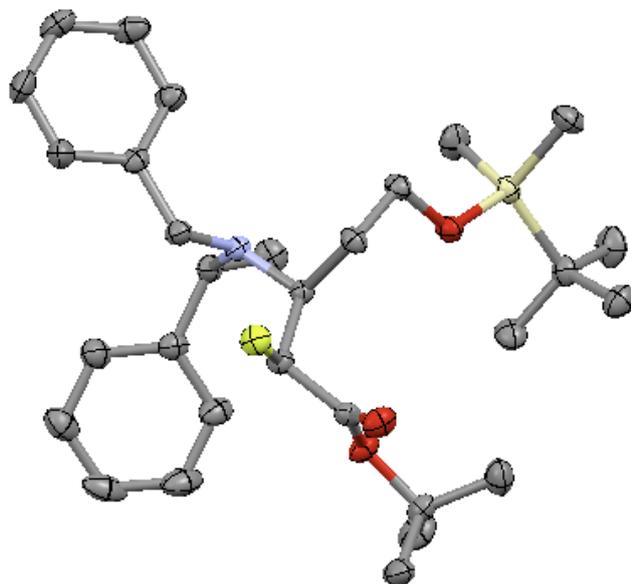
Formula:	$(C_{30}H_{46}FNO_3Si)$
Space group:	P 2_1 2_1 2_1
Cell lengths:	**a** 7.91840(10); **b** 18.5725(2); **c** 20.6359(3)
Cell angles:	**a** 90.00; **b** 90.00; **g** 90.00
Cell volume:	3034.81
Z, Z':	**Z**: 4 **Z'**: 0
R-factor (%):	3.26