NOTTINGHAM TRENT UNIVERSITY

PEPTIDE MODIFICATION BY COMBINING C-H FUNCTIONALIZATION AND SULFUR(VI)-FLUORIDE EXCHANGE; DEVELOPING NEW PEPTIDE CYCLIZATION METHODS

ASHLEY HOLMES

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

September 2022

Declaration

I confirm that this is my own work and the use of all materials from other sources has been properly and fully acknowledged. No part of this thesis has already been, or is being currently submitted for any such degree, diploma or other qualification.

Signed:…….. Date: 29/09/2022

Ashley Holmes

Abstract

Chemically modified peptides often display improved biological activity and pharmacological properties when compared with their native equivalents. In contrast to their linear counterparts, conformationally constrained peptides demonstrate higher structural rigidity and improved pharmacokinetic properties.

Whilst there are many synthetic techniques that could be employed to cyclize peptides, these often rely upon polar side chains, heteroatoms or unnatural amino acids, which can detract from peptide function and contribute to poor atom economy. Methods focussed on alternative strategies to achieve cyclization are therefore crucial for maximising versatility and enabling a broader scope for constrained peptide application. Click chemistry reactions are well suited to cyclization due to their tolerance for other functional groups and high yields. Recently, sulfur(VI)-fluoride exchange (SuFEx) has been described as the “next generation click reaction” however, it is yet to be fully explored as a technique in peptide modification. Methods of introducing SuFEx-able hubs to amino acids and particularly peptide substrates are lacking.

This body of work describes the development of a new cyclization method involving the C-H functionalization of ethenesulfonyl fluorides (ESF) onto linear peptides to install a reactive arylvinylsulfonyl fluoride (aryl VSF) at phenylalanine, an otherwise unreactive residue. The protocol then subjects the modified peptide to sulfur(VI)-fluoride exchange (SuFEx) chemistry in order to facilitate the synthesis of further diversified structures, including cyclic peptides through intramolecular SuFEx transformations. The results show that a broad scope of linear, short-chained peptides containing phenylalanine residues can be modified with ESF to form aryl VSFs in good yields. The modified peptides were then exposed to developed conditions in order to facilitate SuFEx transformations generating diverse new structures. SuFEx reactions were carried out successfully with primary amine, secondary amine and silyl ether nucleophiles. The power of the technique was demonstrated by the generation of cyclic peptides from their linear counterparts.

Acknowledgements

There are several people whom I would like to thank for contributing to this project and for helping me throughout my time at NTU.

Firstly, I would like to express my sincerest gratitude to my supervisor Dr. Warren Cross for allowing me the opportunity to undertake this project. His unwavering support, guidance and positive outlook have been indispensable in its completion. I would also like to thank Prof. Carole Perry, who's perspective and advice has been invaluable in bringing this thesis to fruition.

Furthermore, I would like to say a huge thankyou to Dr. Ray Leslie, who gave me my first opportunity in research and taught me my first ever lesson – nobody likes sugars! I owe a huge debt of gratitude to the ECX and ECZ devotee and one of the most knowledgeable people I have ever met, Dr. Chris Garner. I must also thank Prof. John Wallis, a veritable mine of information, who made me laugh every single time we spoke.

I would also extend my thanks to Mark Sladen, Charlotte Barker and Hannah Beska who made my weekly RFB excursions so thoroughly entertaining. I would also like to express a huge thankyou to every other technician that I have met during my Ph.D. – there are too many to name.

I must also thank everyone I have had the pleasure of sharing iSTeC 105 with, I am indebted to each and every one of you. Particularly Omar Coughlin, Toby Blundell, Elizabeth Rusbridge and Max Winslow who became my extended family for the longest time. A special mention must also go to closet Spireite, Jonny Bristow!

Finally, I would like to thank my parents for their unconditional love, patience and support throughout my Ph.D. I am more grateful than they can ever know.

Publications

Publications associated with this work:

1. M. J. Terrey, A. Holmes, C. C. Perry and W. B. Cross, 'C–H Olefination of Tryptophan Residues in Peptides: Control of Residue Selectivity and Peptide–Amino Acid Cross-linking' *Org. Lett.*, 2019, **21**, 7902–7907.

   DOI: 10.1021/acs.orglett.9b02894

Abbreviations

Å	Angstroms
Ac	Acetyl
BEMP	2-tert-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine
Boc	*tert*-butyl carbonate
br	Broad
COSY	Correlation Spectroscopy
CuAAC	Copper Azide Alkyne Cycloaddition
ºC	Degrees Celsius
d	Doublet
Da	Daltons
DCE	1,2 Dichloroethane
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
Eq.	Equivalents
ESF	Ethenesulfonyl fluoride
EtOAc	Ethyl Acetate
Et_3N	Triethylamine
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
HCTU	Hexafluorophosphate Chlorobenzotriazole Tetramethyl Uronium
HFIP	1,1,1,3,3,3,-Hexafluoroisopropanol
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
m	Multiplet
Me	Methyl

mol	Moles
NMR	Nuclear Magnetic Resonance
PG	Protecting Group
Ph	Phenyl
PhMe	Toluene
ppm	Parts Per Million
q	Quartet
s	Singlet
SuFEx	Sulfur(VI)-Fluoride Exchange
t	Triplet
t-amyl-OH	2-methylbutan-2-ol
TLC	Thin Layer Chromatography
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TBDMS	*tert*-butyldimethylsilyl
VSF	Vinylsulfonyl fluoride

Table of Contents

Table of Schemes

Table of Figures

Table of Tables

1 Introduction

1.1 Peptides as drug candidates

Until late in the 20th century, drug design was limited to small molecules due to the need to produce candidates that could be administered orally.[1] These small compounds typically required a molecular weight below 500 Da. Recent advances in the field of proteomics have led to the understanding that proteins demonstrate exceptionally high selectivity to molecular targets as well as outstanding site potency.[2] This makes them highly desirable drug candidates. *In vivo*, breakdown of proteins to peptides, and subsequently peptides to amino acids, means that a potentially lower toxicity is observed in protein-based drug molecules.

Small molecule drugs can be adapted to traverse quickly over surface membranes and arrive at the target protein easily. However, the target itself must be a protein with a hydrophobic pocket for binding the small molecule. This is a considerable limitation on what is 'druggable' by this class of drug.[3,4] Comparatively, proteins have a plethora of surface area with which to engage another target protein. Ironically, these protein drug candidates are limited to extracellular targets, which according to a bioinformatic study accounts for less than 10% of all human proteins.[3] Collectively, these two main classes of drug candidates are unable to access or interact with an estimated 75-80% of all existing protein structures.[5] This stark statistic has provided the impetus to develop different classes of drug to the well-developed existing families.

Peptides represent a class of drug that occupies the space between small molecule drugs and biologics (e.g., antibodies and proteins). A huge amount of effort is now being invested in the design of peptide-based drugs as a means of mimicking protein structures. This has culminated in a vast number of peptide-based drugs available for the treatment of a wide

variety of conditions. These range from carbetocin, a synthetic analogue of oxytocin for the treatment of amenorrhea,[6] octreotide used to treat tumours in acromegaly patients[7] and degarelix, which has been used in the treatment of hormone responsive prostate cancer[8] among many others, **Figure 1**.[9,10] Indeed, peptide-based drugs now occupy more than 10% of the pharmaceutical market and the increase of market share is rivalled by no other class of drug.[2]

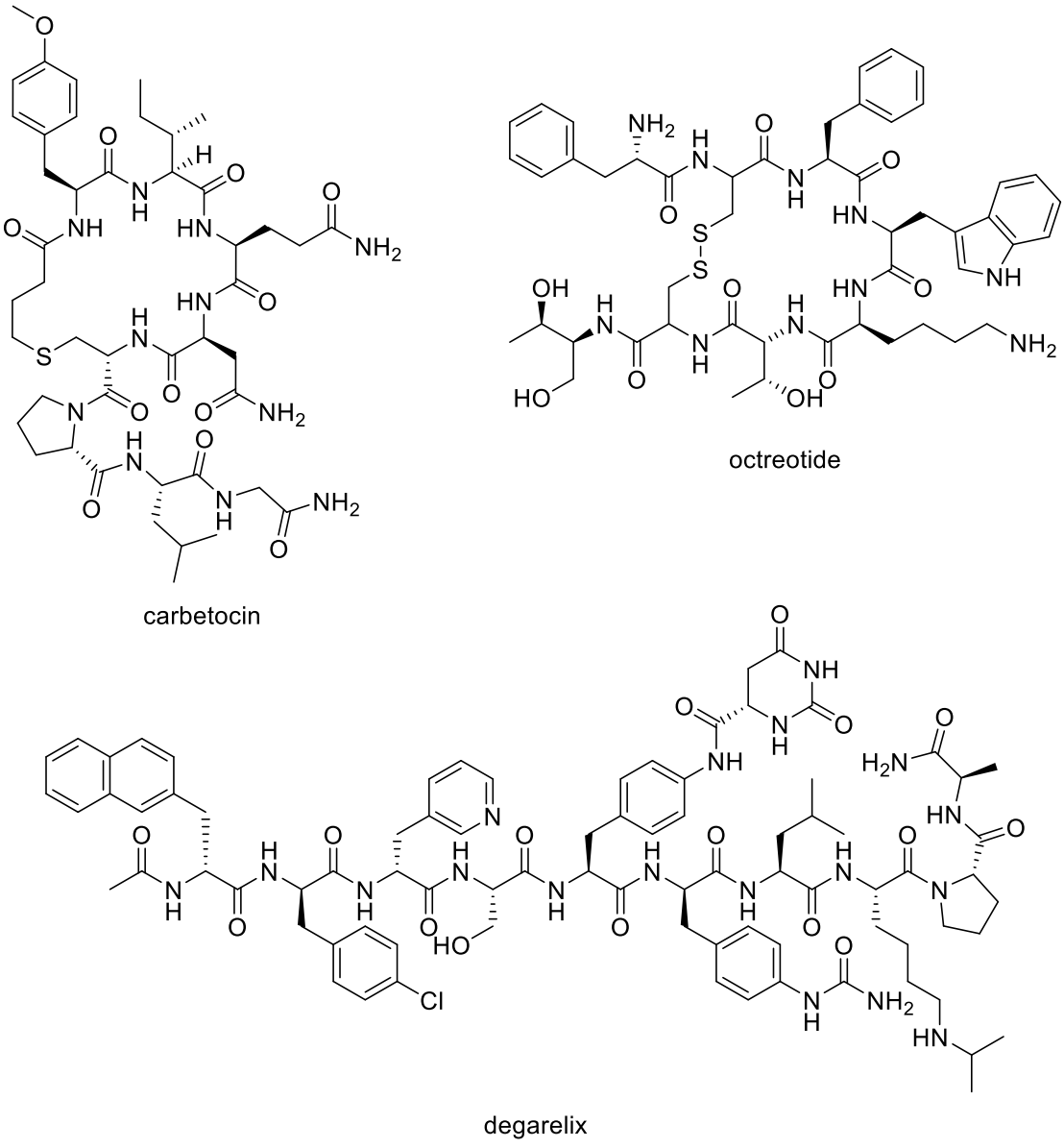


Figure 1 – A selection of FDA-approved, peptide-based drugs.

The use of native peptides as protein mimics has not been without its challenges. Peptides that are not housed in a protein scaffold usually demonstrate a diminished biological activity and low proteolytic stability.[5] The cost associated with the manufacture of peptide-based

drugs is also relatively high. Despite these generic disadvantages, the field of peptide-based drugs continues to receive significant investment. This is attributed to the large number of advantages such as high potency, high selectivity, low toxicity, low accumulation in tissues and high chemical and biological diversity.[11]

The growing interest surrounding peptides is not without merit. Though the highlighted problems associated with peptides as drug candidates are well documented, it can be argued that the benefits of using such molecules vastly outweigh the complications. Calls for advanced medicines that demonstrate increasing potency and selectivity while simultaneously decreasing toxicity continue. Peptides have emerged as a class of molecules that contain enormous potential to address these demands.

1.2 Peptide Modification

Over the last 15 years, the field of peptide modification has received a huge amount of attention from academia and industry alike. The biological activity and pharmacological properties of peptides can be significantly affected by altering the structure, shape or sequence of the native equivalent. Focus on chemical modification, particularly that which can be established 'late-stage', has been remarkably prosperous. A diverse 'toolbox' of modifications are now widely available for overcoming the main drawbacks stipulated in **Section 1.1**.[11,12] It has become common practice to induce a chemical modification in order to tailor peptides to comprise desired properties.

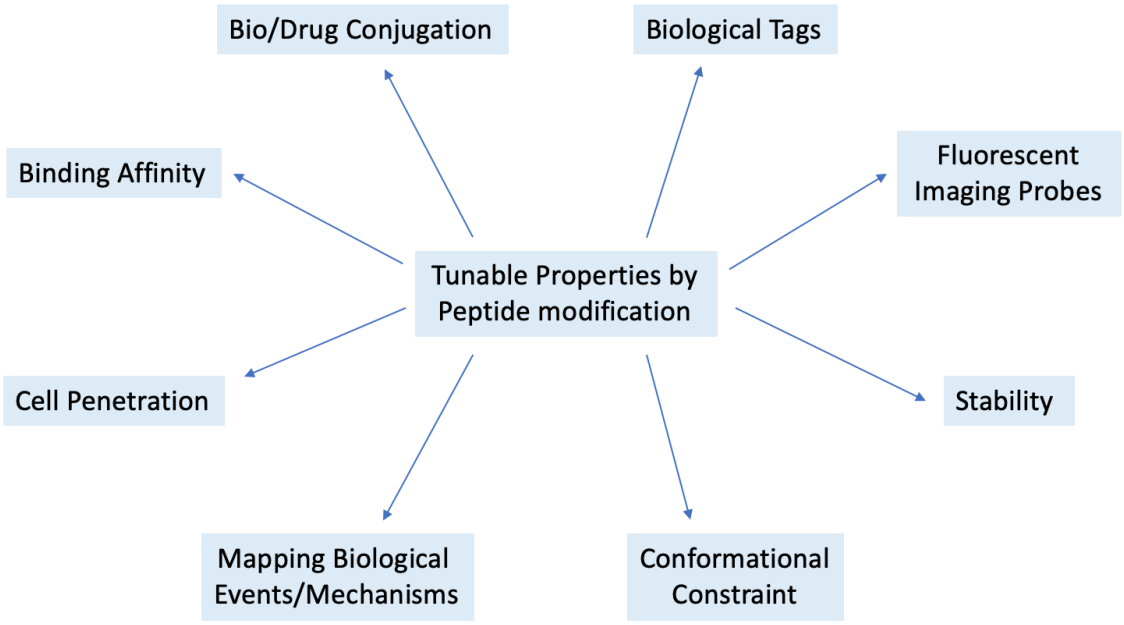


Figure 2 – Properties that can be significantly affected by peptide modification.

The means by which peptides can be modified range from small and subtle, such as deviations from the native peptide amino acid sequence to large more obvious interventions involving the covalent installation of large structures onto the peptide chain. Recently, peptides have been modified to afford derivatives with a whole host of different properties and some of the most biologically significant are highlighted above, **Figure 2**. A selection of key areas in which modification can favourably change a peptides properties will be addressed in the following sections.

1.2.1 Cell Penetration

In the therapeutic treatment of many forms of disease, one of the most significant problems to overcome is the crossing of cellular membranes.[13] If treatment requires the targeting of intracellular proteins, ensuring drug complexes arrive at their intended target across the membrane is not trivial. Small molecules can generally pass over the membrane by diffusion with relative ease. However, much larger and hydrophilic molecules are not able to penetrate the amphiphilic cell membrane without active transport processes.[14]

Oligopeptides do not usually fall into the class of small molecules and often exhibit very poor cell permeability. One technique to enhance cell penetration involves the substitution of amino acids in the sequence with those more likely to facilitate permeation across the cell membrane, such as arginine or lysine residues.[15,16] Substitution must be carried out with care to ensure that amino acids that are crucial for peptide function are not removed or affected. Depending on the nature of the peptide, this is not always possible and in such cases the use of cell penetrating peptides may be required.

Cell-penetrating peptides are small to mid-sized oligopeptides that are able to traverse the cell membrane without the involvement of energy-dependant processes. The structure and conformation are often extremely varied; however, they are frequently cationic, and the only feature that most share is the ability to cross cell membranes using processes that do not require energy.[16,17]

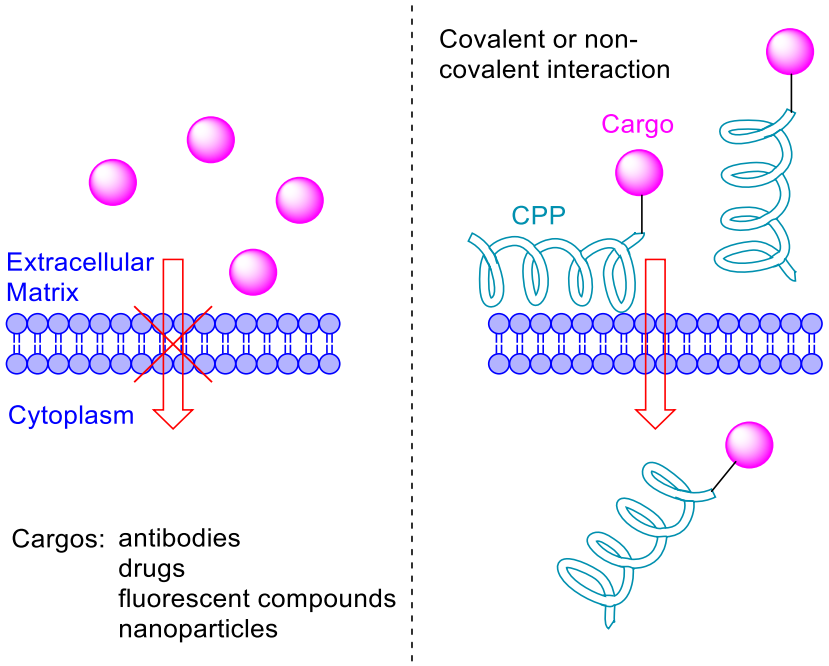


Figure 3 – The delivery of impermeable cargo over cell membranes has been achieved by modification with cell penetrating peptides which are able to draw cargo through when permeating the cell membrane.

Peptides along with other possible cargo that demonstrate poor cell permeability are often conjugated to cell penetrating compounds, harnessing their ability to cross cell membranes and drawing the poorly permeable cross-linked structures with them. The cross-linking of

cell-penetrating peptides and cargo can either be covalent or non-covalent. It is often important in covalent attachment to introduce a spacer group in order to keep the cell-penetrating peptide at a reasonable distance from the cargo, ensuring that optimal distance between the two peptides is maintained.[15,16]

Frequently used cell-penetrating peptides include EB1 (Sequence: LIKLWSHLIHIWF-QNRRLKWKKK) and Transportan (Sequence: GWTLNSAGYLLGKINLKALAALAKKIL).[18] Drugs, fluorescent markers, antibodies and other impermeable compounds can also be delivered over cell membranes in this manner as well as other peptides, **Figure 3**.[17,19–22]

1.2.2 Biological/Drug Conjugation

The conjugation of drug compounds to peptides that demonstrate significant "targeting" ability to certain tissues is a technique used in order to improve selectivity of some therapeutics. Conjugation marries the characteristic high selectivity of the peptide and therapeutic potential of the bound drug to improve efficacy of the treatment.

One significant example of this was the use of peptide conjugates to significantly improve the therapeutic index of chemotherapeutic, Doxorubicin. Phage peptide libraries identified peptide motifs that targeted tumours in mice, the best of which were coupled to doxorubicin molecules, forming peptide-Doxorubicin conjugates. Primary tumour growth and metastasis were inhibited by the peptide-drug conjugate and accumulation of the conjugate in large tumours significantly sequestered the drug, leading to a reduction of toxicity.[23]

1.2.3 Fluorescent Imaging Probes

One of the most of effective modifications of peptides has been the conjugation to a fluorescent tag for use as *in-situ* imaging agents. Labelling peptides in this way is a powerful tool that can be used to chart receptor-ligand binding, discern protein structure and map enzyme activity.[24–27] Fluorescent peptides have also been used as agents to provide real-time imaging of bacterial and fungal infection,[28–30] monitor cell events such as apoptosis,[31]

and as metal sensors.[32–34] The key challenge when designing fluorescent sensors is ensuring that the tag is positioned correctly. The structural characteristics of the peptide must be maintained as much as possible whilst fluorescent change is maximised upon complexation.[27]

1.2.4 Mapping Biological Events/Mechanisms using Fluorophores

Some fluorophores emit different wavelengths of light depending on the nature of the surrounding physiochemical properties. Solvatochromic fluorophores demonstrate a weak fluorescent emission in aqueous solution but become highly fluorescent in non-polar solvents.[27] These types of fluorophores find application in the mapping of binding events in proteins where aromatic residues of peptides can be substituted for fluorogenic unnatural amino acids or modified with fluorophores. If the peptide binds to the target protein, placing the fluorophore into the hydrophobic pocket, a change in fluorescent signal is observed, **Figure 4A**.

Certain fluorophores contain heteroatoms that can coordinate metal ions. Upon binding the metal ion, the fluorophore will display different electronic properties and as such the fluorescent emission will change, **Figure 4B**. This behaviour lends itself to applications in metal ion sensing, although finding the correct geometric and conformational requirements for high affinity to selective metal ions remains a significant challenge.[34,35]

Other fluorophores have been used as molecular beacons, **Figure 4C**. When isolated, the fluorescent moieties arrange themselves close together and emit a certain wavelength. Upon protein binding or interaction, the peptide is no longer free to adopt the conformation that brings the two fluorophores together, causing a change in the emission. The major difficulty with this ‘through space’ effect is that small, isolated peptides have high degrees of freedom and controlling the conformation to allow the FRET or quenching to take place can be problematic.

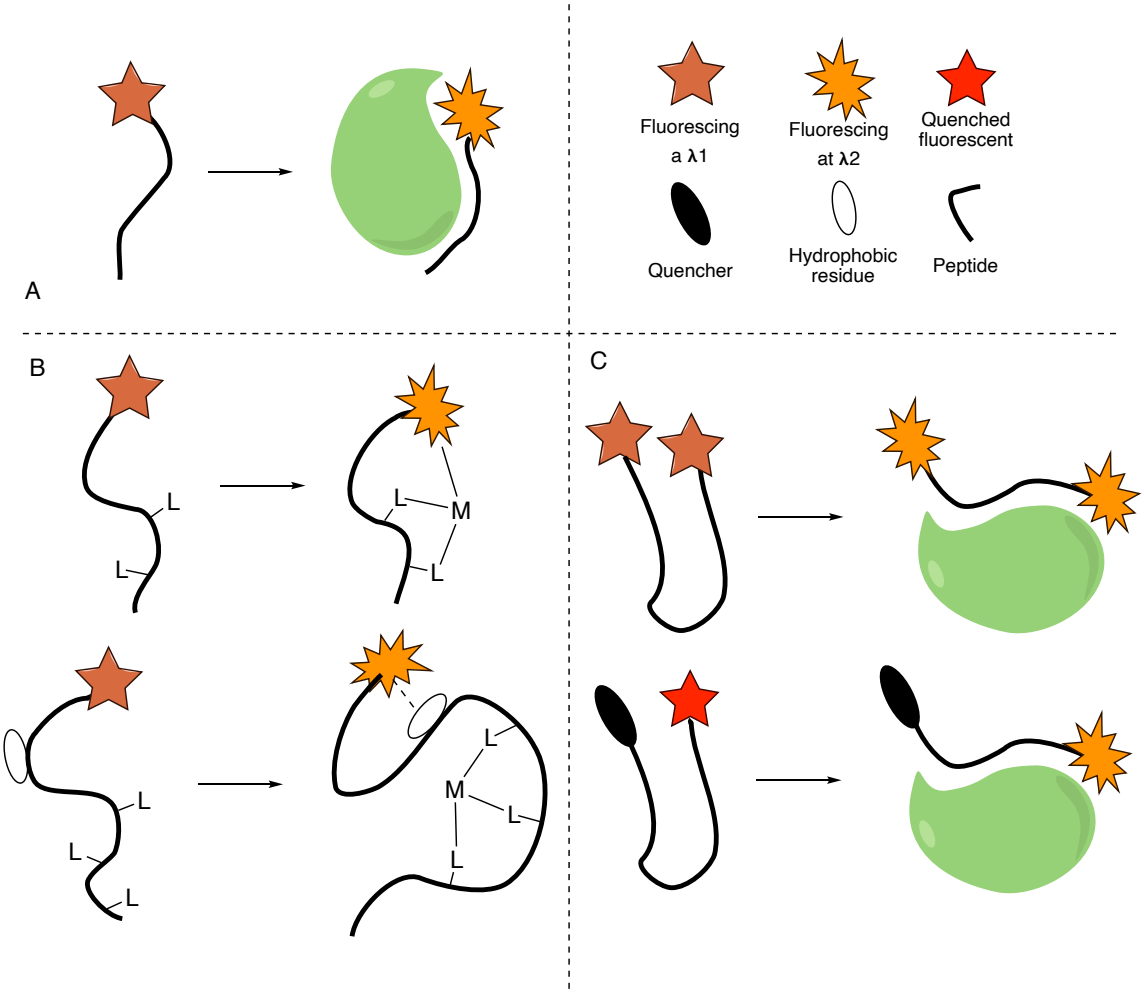


Figure 4 – Mechanisms of fluorescent tags. Figure adapted from reference 26. [A] Fluorescence alters as fluorophore hidden in hydrophobic environment as a result of peptide binding. [B] Fluorophore affected by metal chelation. [C] Fluorophores emit specific wavelength when close together or do not emit when near a fluorescent quencher.

1.2.5 Biological Tags

In addition to tagging peptides with fluorescent labels, cell penetrating peptides and other conjugates of biological importance, they can also be utilised as the tag themselves. Designed in such a way that they will react with specific residues of a specific protein, conjugation allows for the detection or in some cases isolation without significant perturbation of protein function.[36]

Proteins have also been tagged ‘for deletion’ using this technology. A peptide sequence, which is known to be specifically recognised by a protease enzyme was tagged to the -

COOH terminus of a selection of proteins. Rate of degradation of proteins tagged with the peptide sequence was drastically increased.[37]

1.2.6 Stability

One of the most significant obstacles to the use of peptides as therapeutic agents in humans is the rapid proteolytic degradation carried out by endogenous enzymes. Administered peptides are exposed to peptidases in the blood, liver and kidneys[38] and most degrade within minutes. Those peptides that survive are rapidly eliminated by filtration in the kidney, culminating in the characteristic short peptide half-life.[39]

Diminished peptide stability has been addressed in a number of ways, all of which introduce structural modifications to prevent recognition by the protease. Modification of the *C*- or *N*-terminus of peptides can guard significantly against exopeptidases. *C*-terminal amidation or *N*-terminal acetylation are perhaps two of the simplest modifications for stability.[40] Another significant approach is the covalent attachment of PEG to either termini of the peptide, preventing exopeptidases attacking them.[41] The marked increase in molecular mass also reduces the likelihood of complete excretion through the renal route.

Cyclization of peptides by the formation of an amide bond between the *N*-terminus and *C*-terminus of the peptide has generally worked well, however, the chain must be of sufficient length so that the bond does not significantly affect the shape and therefore the activity.[42–45] When carried out effectively, exopeptidase caused degradation can be almost completely supressed.[38]

Recently, β-amino acids have been used as surrogates for α-amino acids as an attempt to increase biological activity, bioavailability and selectivity.[46] They have been known to adopt specific helical conformations and are highly resistant to protease degradation. However, the specific helices that β-amino acids generally adopt do not match those formed by α-amino acids and this mismatch can lead to significant waning of biological activity.[47,48] As a

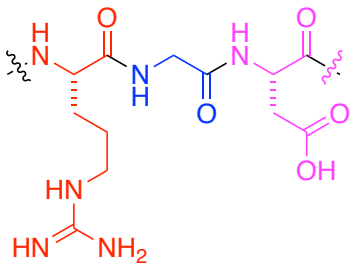
result, recent efforts have been directed at finding a balance between α- and β-amino acids (α/β) in peptides so that a helical conformation can be adopted that closely resembles the canonical α-helix.[49,50]

D-peptides have also been shown to be less susceptible to proteolytic degradation than L-peptides.[51,52] However, use of D-amino acids and D-peptides can significantly alter conformation, in turn severely diminishing activity and selectivity.[48] Whilst they can be a very effective technique for improving peptide stability, caution must be exercised when using different enantiomers due to the significant alteration in conformation they can cause.

Alkylation of the amide has also been a successful stabilising modification. Natural sources of *N*-alkylated peptides can be found in marine or microbial organisms, which generally demonstrate higher proteolytic stability than the isolated natural equivalents.[2] The *N*-alkyl group affects conformational freedom of the backbone and side chain of proximal amino acids whilst eliminating the predominance of *trans* peptide bonds. The removal of the amide proton also results in fewer hydrogen bonds both *inter*- and *intra*molecularly.[53]

1.2.7 Binding Affinity/Selectivity

In order to improve selectivity, specific amino acid sequences can be introduced. Perhaps the most famous example of this is the introduction of RGD sequences, **Figure 5** which are known to specifically target integrins.[23,46,54] One of the most challenging stages of optimisation of peptides to improve binding affinity is the prediction of beneficial amino acid substitutions.[53,55] These decisions can be made based on rational design considerations or assisted by computational methods.[56–59]



Arg-Gly-Asp motif

Figure 5 – The integrin targeting RGD sequence.

1.2.8 Conformational Constraints

Binding affinity is intrinsically linked to peptide conformation. In proteins, which exhibit extraordinary site selectivity, the peptide sequence is held in the biologically active secondary conformation by the rest of the protein structure. Peptide sequences, when isolated from the protein, exhibit diminished biological activity and low proteolytic stability.[5] Simply mimicking the amino acid sequence is not enough to generate the same pharmacokinetic properties demonstrated when the peptide forms part of the protein structure.

The structural rigidity introduced by the protein reduces the number of degrees of freedom a native peptide chain experiences. This conformational constraint, which is inherent to the protein, minimises thermodynamic penalties associated with orientation to the correct binding conformation. The means of introducing conformational constraint to peptides, mimicking protein structures has received huge attention in recent years, the most prevalent of which has been peptide cyclization. Presently, there are in excess of 60 peptides that have been approved for use by the FDA and EMA.[60] Over two thirds of these peptides are conformationally constrained.[61] Unlike techniques such as *N*-alkylation, or the use of peptidomimetics, cyclization does not necessarily alter the structure of peptides, potentially preserving function.[62]

The forming of an intramolecular covalent bond in the synthesis of a peptide macrocycle can have a profound effect on the conformational freedom of the chain. No longer able to

adopt a wide variety of conformers, the peptide assumes a well-defined shape. In order to introduce a conformational constraint, there are four distinct strategies.[63,64] The covalent link can either be generated between the carboxyl 'head' and the amino 'tail', between two side chains, or a combination of side chain and head or side chain and tail, generating four different products accordingly, **Figure 6**.

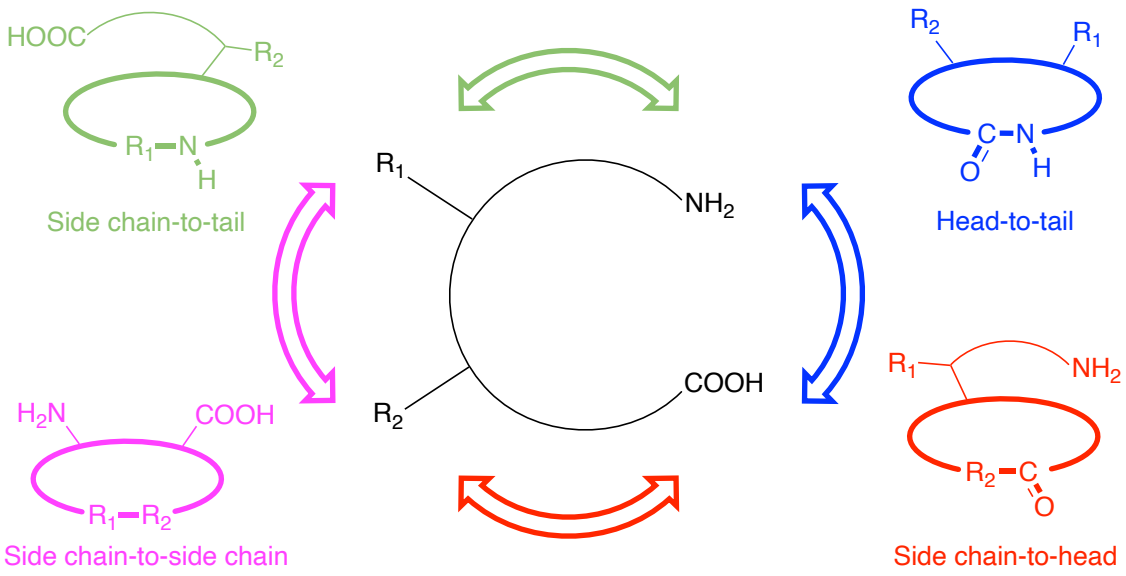


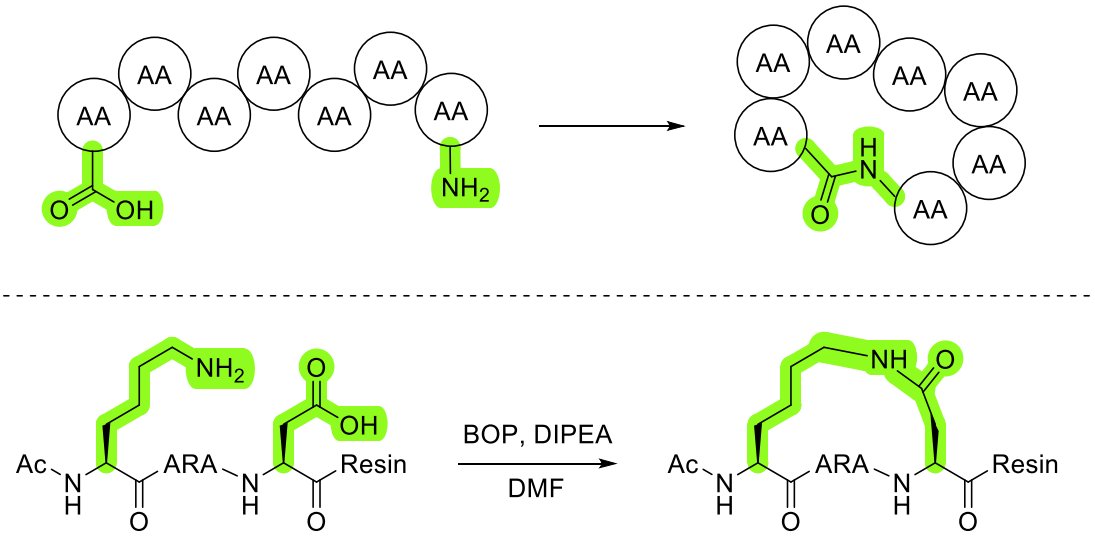
Figure 6 – Strategies to introduce cyclization in peptides. Figure adapted from reference 62.

Peptidase resistance has been demonstrated in all four categories. By linking the head and tail groups, resistance to exopeptidases is significantly improved.[38] However, when forming peptide macrocycles with intent to mimic secondary protein structure, it is likely that the head and tail of the peptide are required for oligomeric peptide bonding and as such, side chain-to-side chain cyclization is often considered the ideal strategy.

In order to afford macrocyclization, peptides have been subjected to a whole host of different chemical reaction conditions with differing target macrocycles and varying degrees of success. The most prevalent methods for cyclic peptide synthesis will be discussed here, with the exception of the immerging method of C-H functionalization, which will be discussed in **Section 1.3**.

1.2.8.1 Lactamization

The means by which peptides are intramolecularly linked are limited in natural peptides to reactivity found in the twenty canonical amino acids. Peptide bonds are amides formed from a free amino group and a carboxylic acid, which are always present at the head and tail of peptide chains. If the chain is long enough, the intramolecular reaction can take place, generating a cyclic peptide via the amide, **Scheme 1**.[45] Peptide macrocyclizations are notoriously tricky, as are most cyclization reactions, due to competing oligomerization pathways and often these steps have to be carried out high dilution or using sophisticated preorganisation strategies.[63,65]



Scheme 1 – Cyclization between carboxyl head and amino tail to form lactam-based macrocycle.[45]

The generation of cyclic amide macrocycles is known as lactamization. As well as utilising the reactivity at the head and tail, amino and carboxyl reactivity is also found on side chains of some amino acids. The lysine side chain consists of a primary amine at the end of a hydrocarbon chain. The amino acids glutamic acid and aspartic acid both contain carboxylic acids, differing in the hydrocarbon chain length linking them to the amino acid backbone, **Figure 7**. These three amino acids can provide the side-chain reactivity needed to form head-to-side chain and side chain-to-tail macrocyclic structures. The presence of a lysine and glutamic acid or aspartic acid also provides the capability for the peptide to undergo sidechain to sidechain macrocyclization.

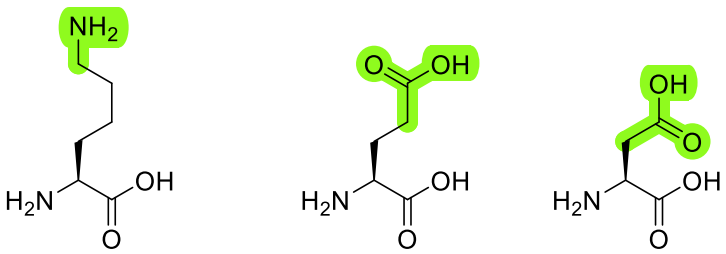
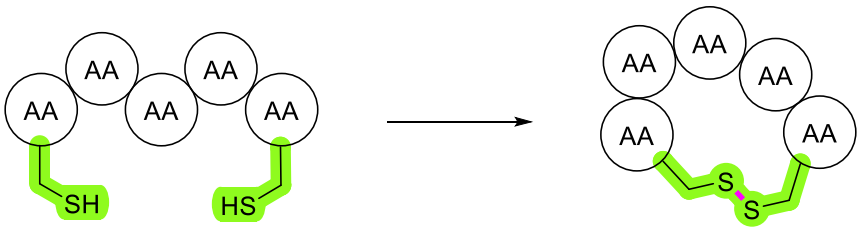


Figure 7 – Reactivity in the twenty proteinogenic amino acids that can be used in lactamization strategies.

Lactamization has been one of the most studied methods of peptide macrocyclization. Introduction of the relevant reactivity into the peptide chain is trivial as generally, the canonical amino acids are sufficient. Diversity in structure can be achieved by using the relevant amino acid at different positions on the peptide chain or by swapping different amino acids to alter cycle size. Importantly, lactamization generates an amide bond which is ubiquitous in human biochemistry. Its biocompatibility means that this cyclization technique will not form toxic by-products upon breakdown One problem is the requirement for an orthogonal protecting group strategy for the amino acids in question in order to specifically deprotect those whilst leaving other protected amino acids intact. These orthogonally protected amino acids are easy to source and are relatively cheap, however they are not routinely used in synthesis and bespoke conditions may be required.

1.2.8.2 Disulfide Bridges

Further reactivity native to the natural amino acids is found in cysteine residues, which contain thiol functionality. Thiols are capable of interacting with other thiols, forming a sulfur-sulfur bond. In peptides, thiols of different cysteine residues can form these bonds, forming a covalent link between two side chains, known as a disulfide bridge, **Scheme 2**.



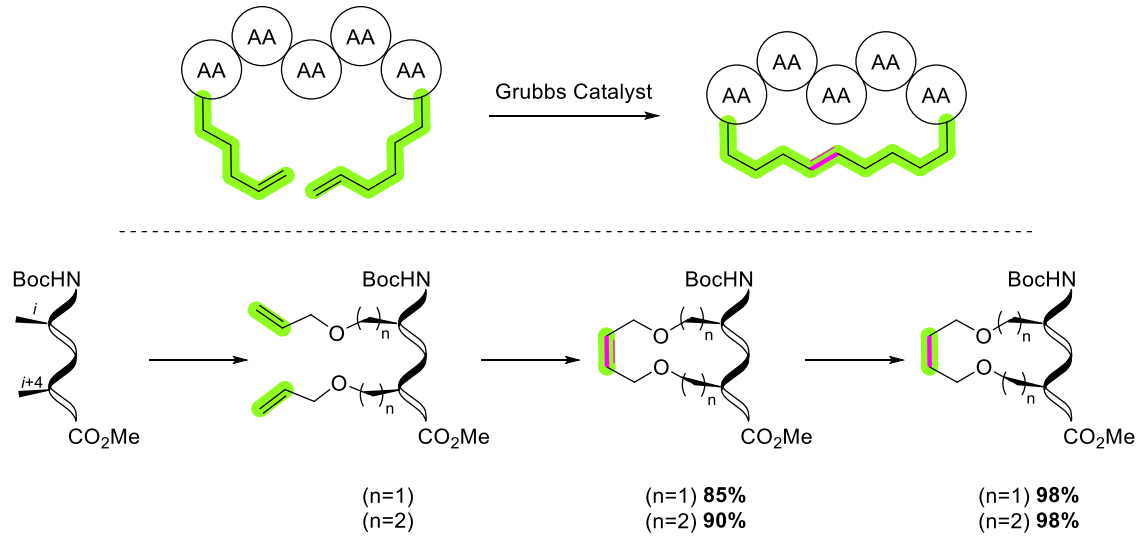
Scheme 2 – The formation of a disulfide bridge between two cysteine residues in a peptide chain.

These are a common structural motif found in secondary protein structures known as β-sheets and are interesting targets for study. However, disulfide bridges are formed under oxidising conditions and are susceptible to the reducing environments repeatedly experienced *in vivo*, yielding them unstable and not always suitable as tools for introducing conformational constraint.[66]

1.2.8.3 Ring Closing Metathesis

Divergence from the natural twenty amino acids significantly increases the means by which cyclization can occur. Still adhering to the four cyclization strategies, non-natural amino acids can provide alternative functionality to peptides meaning that more than just amide and disulfide bridge formation is possible.

One of the most prevalent strategies for introducing macrocyclic character to peptide structures has been the use of Grubbs' catalyst to carry out alkene metathesis. The catalyst works by activating two alkene groups, breaking their double bonds before forming a singular double bond between the two and liberating a molecule of ethylene. Grubbs catalyst demonstrates remarkable selectivity for alkenes and importantly, exhibits high tolerance of other functional groups, **Scheme 3**. All of which makes this technology ideal for use in peptide macrocycle formation.[67,68]



Scheme 3 – Macrocyclization achieved by ring closing metathesis using a ruthenium catalyst.

Grubbs catalyst has been widely used in the conformational constraint of peptides and its use in stabilising secondary protein structures is arguably the most vastly explored methodology.[45] As a result, a variety of Fmoc protected unnatural amino acids with alkene-based side chains are commercially available, meaning synthesis of peptides capable of being cyclised by metathesis is entirely routine. FIG

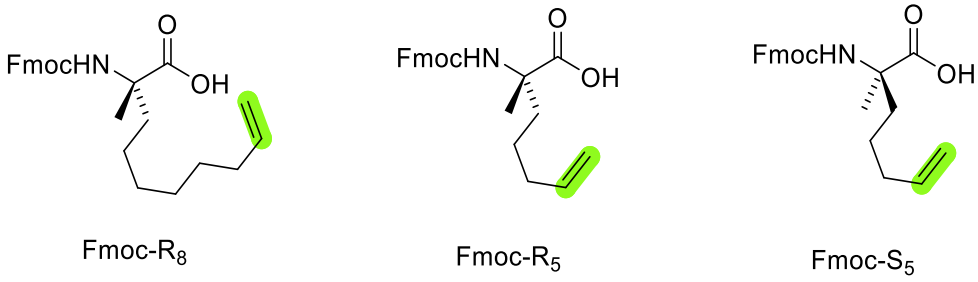


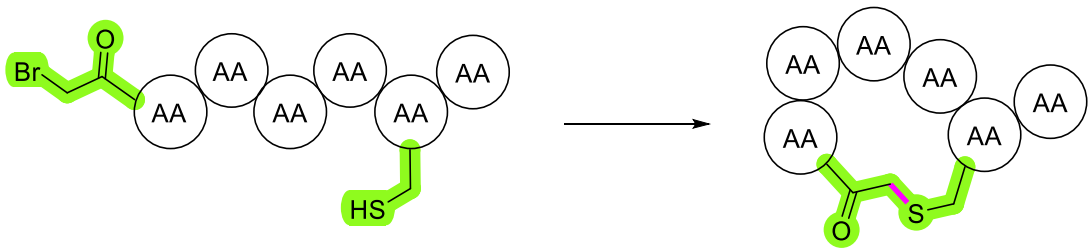
Figure 8 – A selection of commercially available amino acids for RCM cyclization.

This range of amino acids encompasses all-hydrocarbon side chains of differing length, as well as protected polar amino acids such as *O*-allyl-serine and homoserine along with many others. A published protocol for the introduction of allylic amides at any position on the peptide backbone has also increased applicability, ensuring that this type of macrocyclization chemistry can be applied to a wide variety of problems.[69]

1.2.8.4 Thioether formation

Aside from reactions with other thiols, sulfur is capable of other reactivity when non-native functionality is introduced to peptides. Sulfur is an excellent nucleophile, which makes it an ideal species to perform S_N2 reactions. Installation of alkyl halides in unnatural amino acids means that once the peptide has been formed and deprotected, the displacement reaction can take place forming a thioether.

One advantage to using sulfur to form thioether bonds in this manner rather than disulfide bridges is the formation a bond which is stable in cell extracts.[70] However, perhaps the most interesting property is the nonimmunogenic character of the thioether bond which has the potential to be advantageous over other cyclization techniques.[71] Thioether formation has been achieved most commonly by cyclization between a cysteine residue side chain and a protected amino tail, **Scheme 4**, as well as between a cysteine side chain and another non-native side chain.

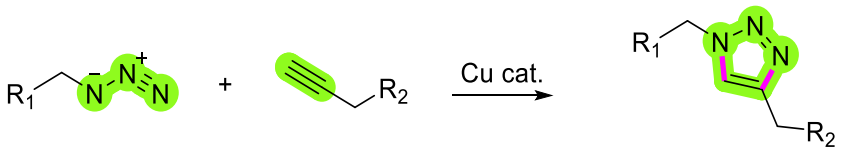


Scheme 4 – Thioether formation as a means of producing a peptide macrocycle.

This methodology lends itself to use in solid phase peptide synthesis due to the ease at which the halo-acetyl group can be installed. A published method describes a strategy for installation on-resin, introducing a modular characteristic to the technique.[72] Due to its impressive nucleophilicity, ligation strategies where a cysteine residue is the site of the covalent link are well developed. This has led to a wide variety of orthogonal protecting groups for cysteine residues in solid phase peptide synthesis, which can be manipulated to increase selectivity of one cysteine residue over another.[73]

1.2.8.5 Copper-catalysed Alkyne-Azide Cycloaddition (CuAAC)

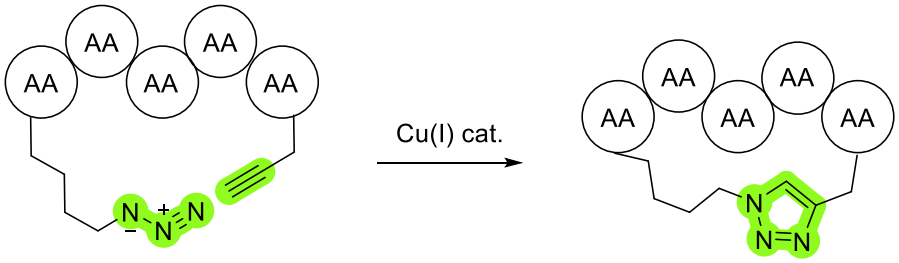
An interesting approach to introducing macrocyclic structure to linear peptides has been the use of click chemistry. In order to be characterised as a click reaction, the transformation must be easy to perform in the presence of oxygen or water, giving consistently high yields without the need for laborious work up and purification. The scope of the reaction should be wide with a variety of starting materials available that demonstrate click capacity consistently.[74] Such demands on reactivity set the bar for this type of reaction extremely high and as a result, real examples are few and far between.



Scheme 5 – A copper catalysed azide-alkyne cycloaddition reaction.

In terms of peptide chemistry, the most successful and relevant of the click reactions is the copper catalysis of Huisgen's 1,3-dipolar cycloaddition of azides and alkynes to generate triazoles, **Scheme 5**.[75] Azides and alkynes are simultaneously extremely energetic species as well as existing amongst the least reactive functional groups in organic chemistry. As a result, these functional groups act as benign spectators when exposed to a variety of reaction conditions until they are deliberately activated.[74] The copper catalysis of this reaction, and the beneficial effects of performing it in water have propelled this reactivity to prevalence in the field of peptide conjugation.[76]

Amino acids containing azides or alkynes are accessible either by purchasing from chemical vendors or synthesis from readily available starting materials.[77,78] Recently, advances using biosynthetic routes to the target starting materials have been determined.[79] They can be easily installed in peptide sequences by standard solid phase methodology and then activated to form triazole-linked macrocycles, **Scheme 6**. This transformation can be achieved either on- or off-resin.[80]



Scheme 6 – A CuAAC reaction used to produce peptide macrocycles.

Aside from the excellent 'clean' reactivity and tolerance of other functional groups, another interesting property is the isosteric nature of the triazole unit when compared with peptide bonds, **Figure 9**. Isosteres are defined as groups that possess approximately the same number of electrons, exhibit the same molecular shape and demonstrate similar physical properties.[81]

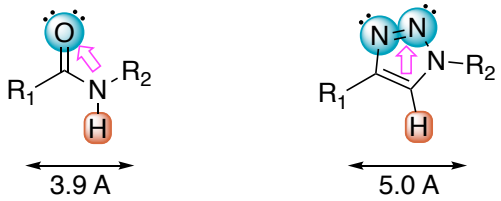


Figure 9 – Amides and tetrazoles are isosteric functional groups. They have lone pairs and protons available for hydrogen bonding, are similar in dimension and even have comparable dipole moments.

Although the triazole is approximately 1 angstrom longer than an amide bond, the hydrogen bonding abilities can be easily mimicked by the lone pairs on N-2 and N-3 and the C-H bond donor.[82] Despite these common features, triazoles are stable to proteolytic degradation, hydrolysis and oxidation, making them more stable than lactam based cyclization techniques *in vivo*.[83] The exhibition of these properties makes the triazole linkage an excellent peptide bond surrogate. Non-natural amino acids containing 1,2,3-triazoles have been demonstrated to replace dipeptides in an α-helix and maintain the conformational secondary structure.[84]

1.2.8.6 Summary

A wide range of macrocyclization techniques have been reported over the recent past. The two most developed methods are arguably the ring closing metathesis and the CuAAC click reaction. The relative ease of the method is what understandably drives the research carried out in these areas. Coupled with reactions that are high yielding, have good functional group tolerance and generate single products, it is easy to see why these methods are prevalent in peptide macrocyclization.

1.3 C-H functionalization of peptides

The use of nucleophilic or electrophilic side chains has been needed to afford cyclization if new, non-canonical amino acids are not used in the peptide synthesis. This covers only a few of the twenty proteinogenic amino acids and substantially limits the diversification of natural peptides. In order to increase peptide cyclization applicability and markedly improve structural diversity, methods of modification of all the natural amino acids are required.[12] A notable portion of these remaining amino acids are non-polar, with side chains consisting in many cases of only hydrocarbon bonds of sp^2 or sp^3 hybridised orbital character, **Figure 10**. As they possess no obvious reactive handle, modification of specific, non-polar amino acids has traditionally not been possible.

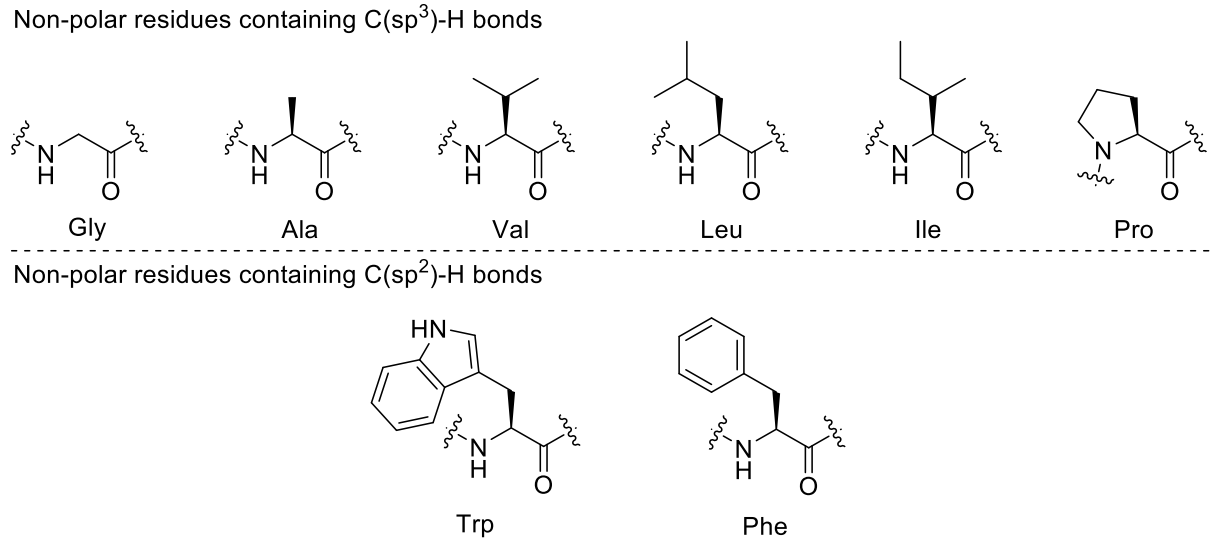


Figure 10 – Non-polar proteinogenic amino acids.

Recently, the field of C-H functionalization has gained significant traction as a tool not just for diversifying peptides in a stepwise and atom economical manner, but for improving the environmental impact of synthetic chemistry.[85] The functionalization of C-H bonds has become a powerful means of achieving transformations originally carried out by cross-coupling technology. The activation of a ubiquitous and native C-H bond rather than a C-X bond means that often, the step economy of a reaction can be vastly improved.

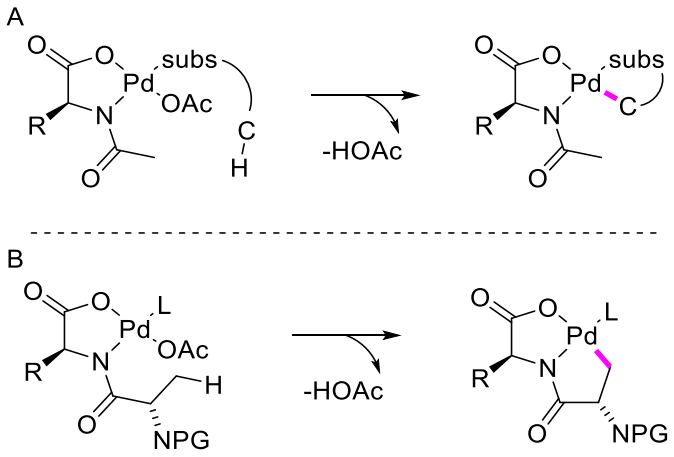
1.3.1 C(sp^3)-H Functionalization

Since the turn of the century, huge interest in the functionalization of peptides by activation of C-H bonds has been shown. With so many potential C-H bonds available in peptides, the issue of selectivity is a significant challenge. Impressive strides forward in the functionalization of activated C(sp^3)-H bonds, where the C-H bond is α- to a carbonyl species have been documented.[86] Since then, the application to amino acids and peptides, mainly Gly derivatives, have been reported.[87]

The more challenging functionalization of C(sp^3)-H bonds which are not activated has also received much attention. As a result, the alkylation[88–90], alkenylation[91], arylation[92–96] and acetoxylation[92,97] of β- and γ- C(sp^3)-H bonds in peptides have been reported. However, in

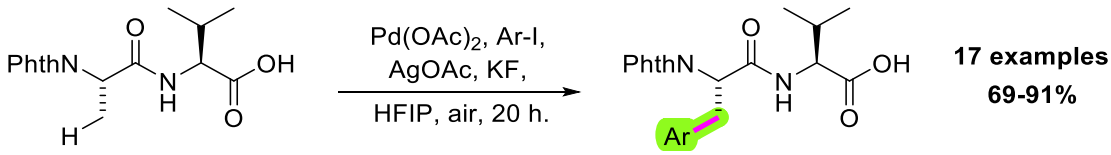
order to achieve this activation, the use of directing groups, in all cases, has been employed, culminating in a loss of atom and step economy.

Previous work in the Yu group had led to the discovery that monoprotected amino acid ligands could accelerate C-H functionalization reactions[98–101] by utilising the chelating power of amides and carboxylates as a means to coordinate the transition metal catalyst, **Scheme 7A**. Subsequently, in 2014, the group provided the next major milestone using the native peptide backbone to direct the catalyst into close proximity of a C-H bond, facilitating activation, **Scheme 7B**.[102]



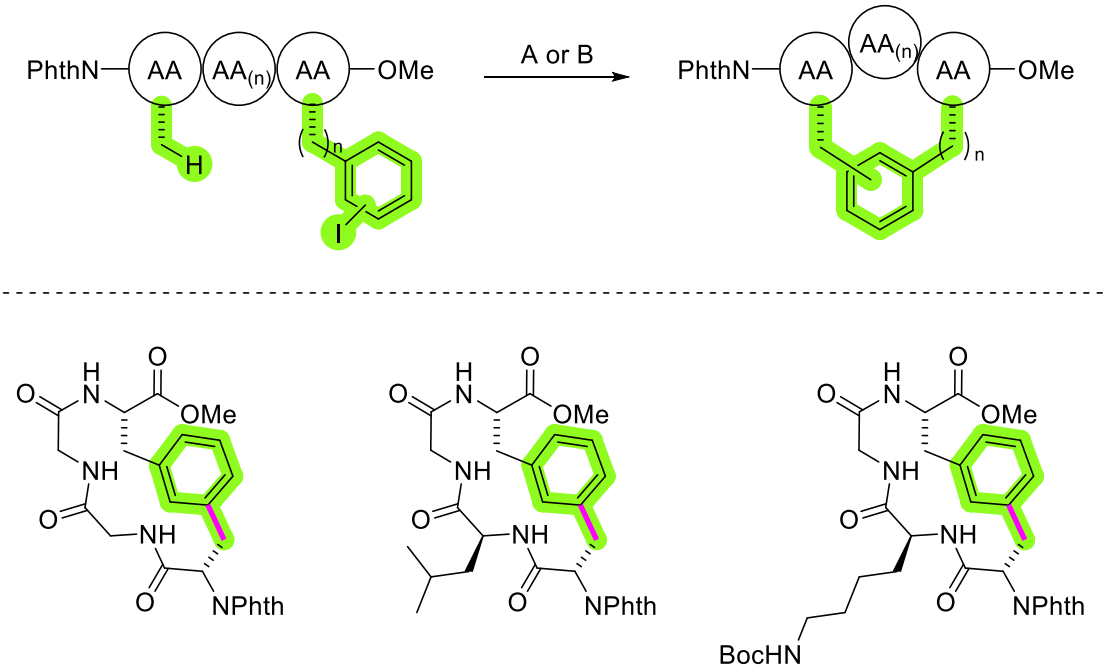
Scheme 7 – Binding to metal catalysts to facilitate C-H functionalization reactions. [A] N-protected amino acid and [B] N-protected peptide binding to a catalyst.

Building on their work modifying amino acids by C-H functionalization,[96] Yu was able to modify residues situated at the phthaloyl-protected *N*-terminus of di-, tri- and tetrapeptides, with large scope of aryl halides, including those decorated with electron donating and electron withdrawing groups, **Scheme 8**.[102] In addition, alkynylation of *N*-terminal alanine residues in oligopeptides has also been achieved, providing the means to modify peptides with special cargo such as drug conjugates or fluorescent markers.[103]



Scheme 8 – Selective arylation of N-terminal residues in dipeptides. Examples of tri- and tetrapeptides are not shown.[102]

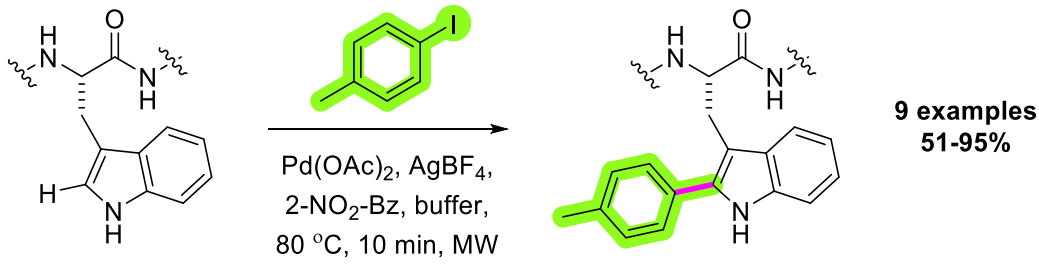
The potent selectivity demonstrated in Yu's work inspired modification as a means to facilitate macrocyclization. Independently, the groups of Albericio and Wang published work that utilised this technology, **Scheme 9**.[104,105] It was reported that C(sp^3)-H activation could be used to generate macrocyclic peptide structures in good yield in a wide variety of peptides and was tolerant to a host of protected functional groups. A procedure was developed to carry out this transformation on-resin in the solid phase[104] and the biological properties and application in the synthesis of natural products were reported.[105]



Scheme 9 – Intramolecular C(sp^3)-H functionalization generating peptide macrocycles. [A] $Pd(OAc)_2$, AgOAc, toluene/^{t}BuOH, 100 °C, 24h. [B] $Pd(OAc)_2$, AgOAc, DCE, air, 100 °C, 12h.[104,105]

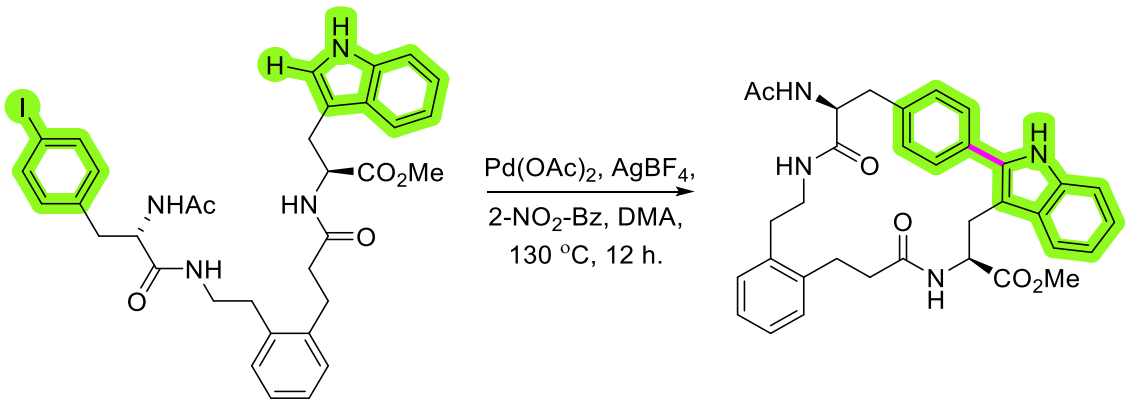
1.3.2 C(sp^2)-H Functionalization

Early examples of activation and functionalization of C(sp^2)-H bonds in peptides date back a decade to the arylation of tryptophan residues.[106] The report utilised the reactivity of the C(sp^2)-H bond at the C-2 position on the indole and aryl iodide coupling partners to ensure a highly selective transformation in peptides, **Scheme 10**. A range of examples were reported, including both polar and non-polar unprotected residues at differing positions in the tri- or tetrapeptide chain in moderate to excellent yield.



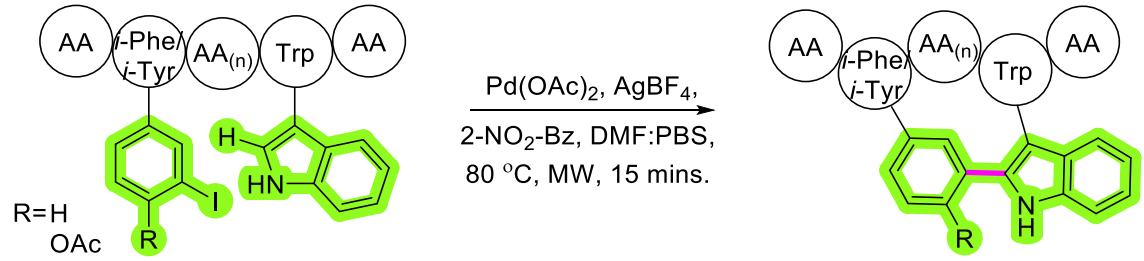
Scheme 10 – Arylation of Trp residues in tri- and tetrapeptides.

The first use of this technology to generate macrocyclic structures was described soon after, where tryptophan and halophenyl-containing amino acids, at either end of a linker were cyclized, **Scheme 11**.[107] Using adapted conditions, 15-25 membered macrocyclic structures were synthesised in moderate yield by intermolecular arylation at C-2 on Trp residues.



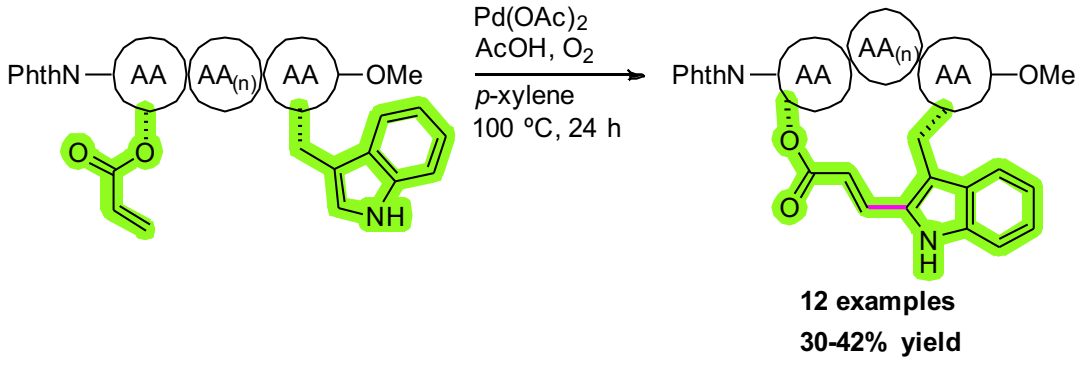
Scheme 11 – Cyclization of structures by intramolecular arylation.[107]

More recently, by linking aryl iodide amino acid derivatives, iodo-Phe and iodo-Tyr with unprotected Trp residues on the same peptide chain, the first transformations by C(sp^2)-H functionalization to conformationally constrained macrocyclic peptides were reported, **Scheme 12**.[108] The macrocyclization was found to occur between amino acids that were separated by one residue. In the case where the coupling partners were next to each other in the peptide chain, the cyclo-dimeric species was observed rather than the macrocyclic peptide.[109]



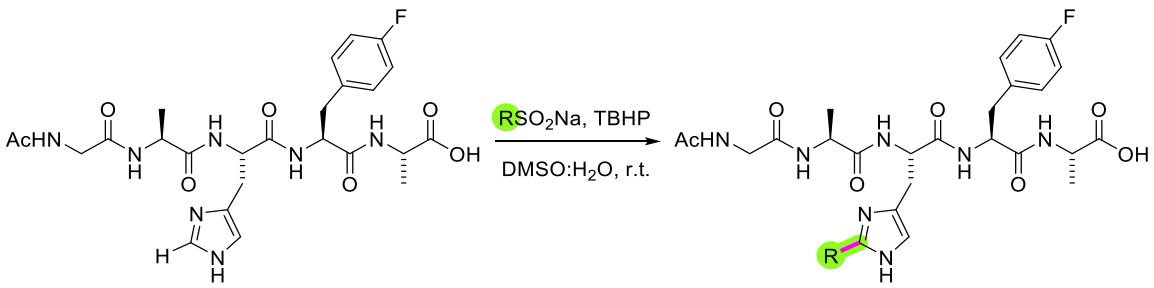
Scheme 12 – Conformational constraint of oligopeptides by intramolecular arylation.

Primarily, functionalization of C(sp^2)-H bonds in amino acids and peptides has focused on tryptophan residues, which contain the relatively labile C(sp^2)-H bond at the C-2 position, aiding in the selectivity of the transformations reported.[12,110] Comparatively, the modification of Trp residues in peptides is much more developed than other aromatic residues. Since the initial report in 2010,[106] C-H functionalization has been used as a tool to perform arylation,[107–109] alkylation,[111,112] alkynylation[113] and olefination[114,115] at the C-2 position on tryptophan residues in peptides. This technology has been further utilised as a cyclization strategy, culminating in the cyclization of peptides containing tryptophan residues and an alkene based on the same linear peptide, **Scheme 13**.[116]



Scheme 13 – Synthesis of cyclic peptides via C(sp^2)-H olefination of tryptophan residues.

The functionalization of other aromatic residues besides tryptophan has also been reported. In contrast with Trp residues in peptides, examples of modification of His residues are rare. Arylation of the protected amino acid has been documented,[117,118] as well as an example of late stage alkylation, which allows for selective modification and bioconjugation of His residues in peptides, **Scheme 14**.[119]



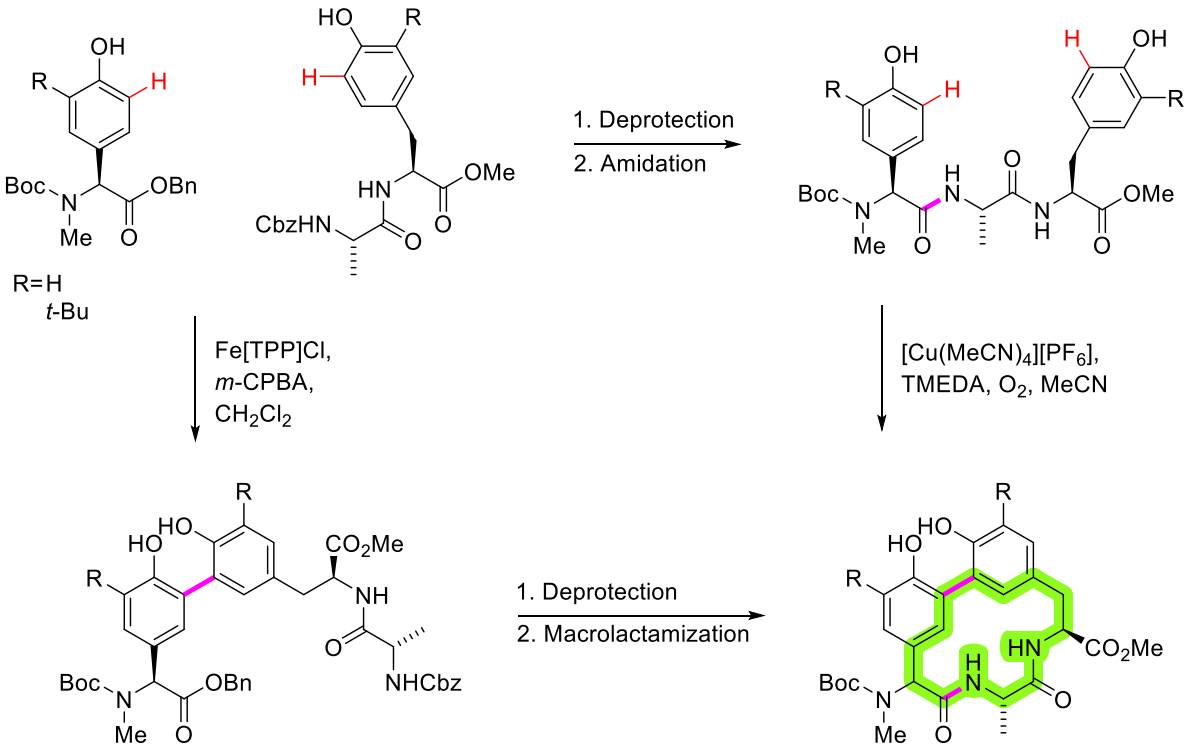
Scheme 14 – Alkylation of N-terminal protected histidine residues in peptides.[119]

Similarly, Tyr modification has received much less attention than Trp. Olefination of the amino acid has been documented,[120,121] followed more recently by olefination of silanol protected tyrosine residues in peptides, **Scheme 15**.[122] The silanol is reported to contain bifunctionality, acting as an orthogonal protecting group for use in SPPS as well as a directing group for the catalytic C-H olefination.

Scheme 15 – Olefination of protected tyrosine residues in peptides.[122]

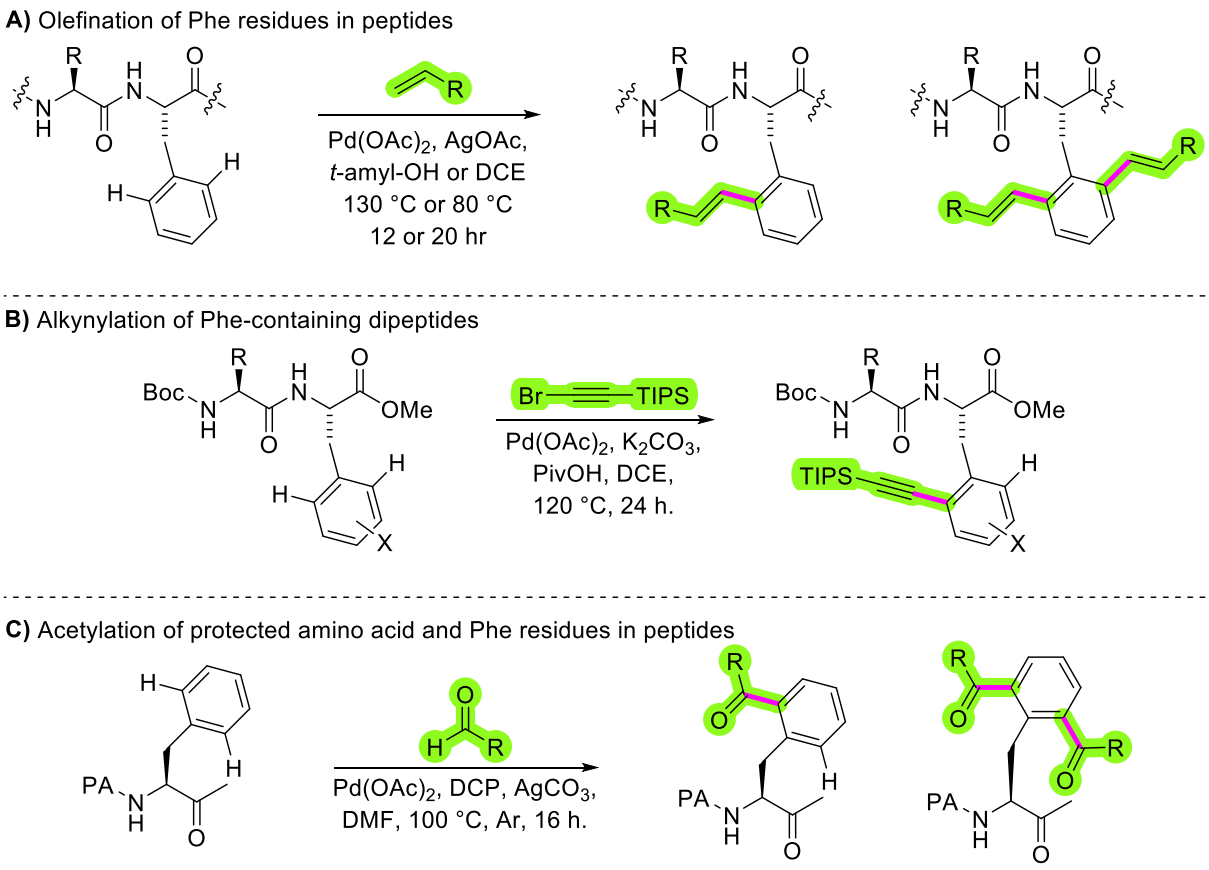
Self-arylation of two tyrosine residues to form biaryl-bridged cyclic peptides has recently been investigated independently by different research groups.[123,124] The tripeptide backbone linked by a biaryl-bridge forms the core motif of arylomycin, a bioactive class of

natural and unnatural cyclic peptides that exhibit potent antibiotic activity against gram negative bacteria. Two main synthetic routes have been taken in order to access the arylomycin cyclic core using C-H functionalization, **Scheme 16**. The first approach involved forming the linear tripeptide before carrying out C-H functionalization as a cyclization step whereas more recently, bi-aryl bridge has been formed before the cyclic lactamization was carried out. This second approach has been reported to be better yielding overall.[124]



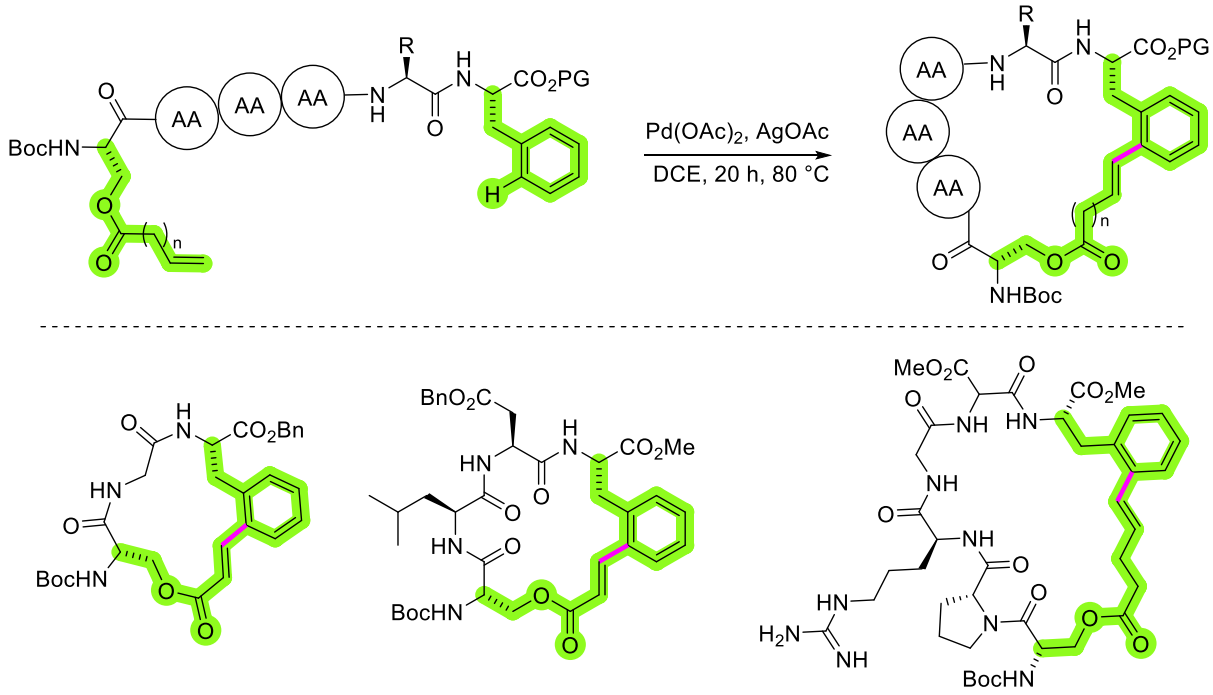
Scheme 16 – Reported access to arylomycin cyclic core by C-H functionalization methods.[123,124]

Like His and Tyr, modification of Phe residues is still in its infancy. Examples of olefination of the amino acid appeared first in the literature,[120] followed much more recently by olefination of peptides containing Phe residues[125,126] as well as reports of alkynylation[127] and acylation,[128] **Scheme 17**.



Scheme 17 – Modification of Phe residues in peptides.

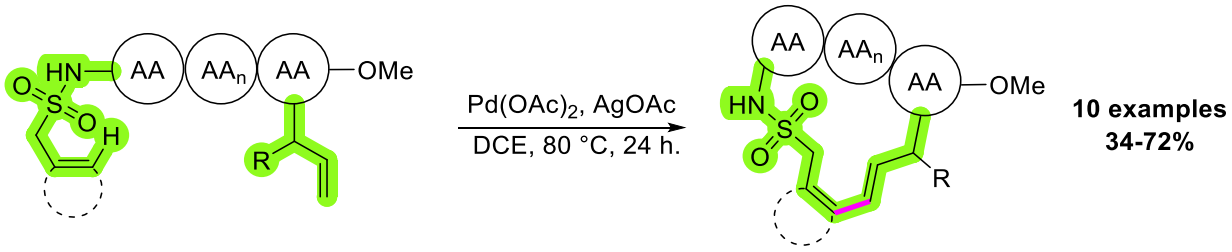
The first documented case of macrocyclic peptides generated by C(sp^2)-H activation of Phe residues, **Scheme 18** was published by the Wang group.[125] In this example, the group used the peptide backbone as endogenous directing groups to present the palladium catalyst to the *ortho*-aryl C-H bonds on phenylalanine residues. After activation of both the phenylalanine residue and the alkene functionality installed on the peptide chain, an intramolecular arene-alkene bond formed the macrocyclic peptides.



Scheme 18 – Peptide macrocyclization by formation of arene-alkene bond between phenylalanine residues and preinstalled alkene functionality.[125]

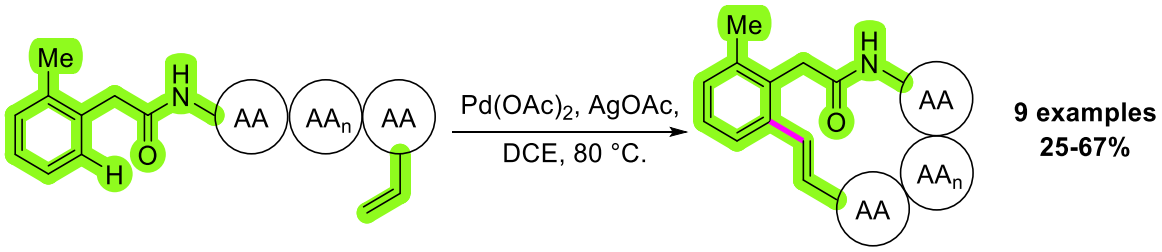
The main limitation to using this method is the requirement for the phenylalanine residue to be away from the *N*-terminus of the peptide. The technique utilises the peptide backbone for catalyst coordination and it has been postulated that two amides are required in the *N*-terminal direction in order to facilitate the transformation.[125,126] Wang and co-workers were then able to expedite C(sp^2)-H functionalization on aryl groups at the *N*-terminus of peptides using preinstalled directing groups at this site.

The use of aryl sulfonamides as an *N*-terminal directing group has been introduced as one way to modify aryl moieties at the *N*-terminus. Indeed, the Wang group was able to show that cyclization using this technique produces a varied library of cyclic peptides containing aryl sulfonamides linked to a preinstalled terminal alkene, **Scheme 19**.[129] Moreover, the installation of an arylacetamide handle at the *N*-terminus was also used as a method to modify *N*-terminal aryl substituents.[130] The cyclization was carried out on a range of different linear peptides possessing the handle forming peptide macrocycles in good yield linked by the arene-alkene C-C bond.



Scheme 19 - Sulfonamide directed C(sp^2)-H olefination to facilitate macrocyclic peptide structures.[129]

Although the transformations described do not take place on canonical amino acids and may still require the backbone amides as directing groups in some cases, they represent macrocyclizations that takes place in a different 'direction' to previously reported, broadening the applicability of the method.



Scheme 20 – C(sp^2)-H olefination of arylacetamide handles to generate macrocyclic peptide structures.[130]

1.3.3 Residue Selectivity

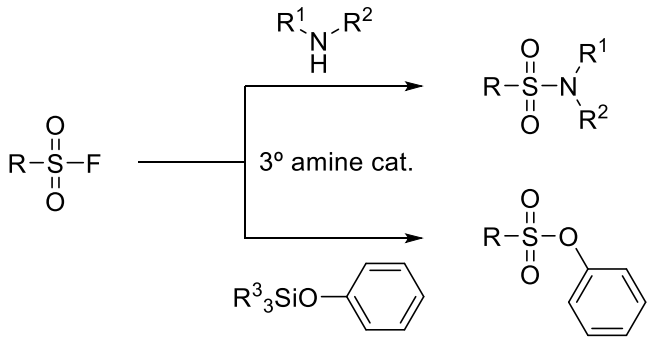
The use of C-H functionalization to modify peptides post-synthetically has gained enormous traction in the past 10 years. The Cross group has contributed to this field by developing separate methods for the olefination of peptides containing phenylalanine or tryptophan residues.[114,126] The peptides used in these studies contained non-polar amino acids as well as the phenylalanine or tryptophan residues required for olefination. The C-H functionalization reaction is selective for C(sp^2)-H over C(sp^3)-H, which is expected. However, the olefination of one C(sp^2)-H in the presence of another C(sp^2)-H, in this case phenylalanine and tryptophan residues in the presence of each other had not been reported.

1.3.4 Summary

In summary, the development of C-H functionalization methods for peptides has contributed to an expanding toolbox for post-synthetic manipulation of peptide properties. Modification by this means lends itself to applications in peptide chemistry due to the ability to target ubiquitous C-H bonds in contrast with other cyclization techniques. Huge research efforts in the area have significantly improved the scope of the technique yet some challenges remain. These include selectivity and decreased yields when modifying complex structures compared to ring closing metathesis and click chemistry.

1.4 Click Chemistry – Sulfur(VI)-Fluoride Exchange (SuFEx)

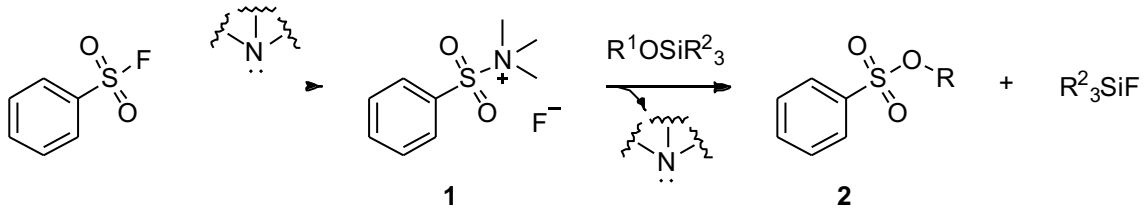
The copper catalysed azide-alkyne cycloaddition transformation is considered to be the quintessential click reaction although it is one a few reactions that qualify as a click chemistry.[131] Its applications range from drug discovery[74] and bioconjugation[132] to materials science.[133] In 2014, a major milestone in the development of click chemistry was reported: sulfur-fluoride exchange, known as SuFEx.[134] Described as the "next generation of click chemistry" the sulfur-fluoride exchange reaction involves the transformation of a sulfur(VI)-fluoride bond into a sulfonamide or sulfonate ester, **Scheme 21**.[131]



Scheme 21 – Sulfur fluoride exchange transformations generate sulfonamides or sulfonate esters depending on selected coupling partner.

Unlike in CuAAC click chemistry, the catalysis of the reaction is metal free, which significantly increases its appeal in drug discovery and other biological applications. Traditionally these types of reactions have been catalysed by DBU[135–137] and more recently

have been expanded to different tertiary amine[138,139] as well as phosphazine bases[140] and even Lewis acids such as $Ca(NTf_2)_2$.[141]



Scheme 22 – General suggested mechanistic pathway for SuFEx transformations.

Although the reaction was identified as a click reaction over 10 years ago, and SuFEx chemistry has since received an enormous amount of attention, a fully determined mechanism is yet to be reported. However, the general consensus is that a basic tertiary amine forms the corresponding arylsulfonyl fluoride salt **1**. This is then believed to activate the silyl ether forming the Si-F bond and releasing the *O*-aryl to form the sulfonate ester product **2** as in **Scheme 22**.

1.4.1 SuFEx-able hubs

A SuFEx-able hub is defined as a functional group capable of undergoing sulfur(vi)-fluoride exchange. Contrastingly with CuAAC, where the functional groups required are an alkyne and an azide, a sulfur-fluorine bond can be present in a few distinct functional groups. These discrete classes of SuFEx-able hub which generate differing functionality upon sulfur(vi)-fluoride exchange, are highlighted in **Figure 11** below.

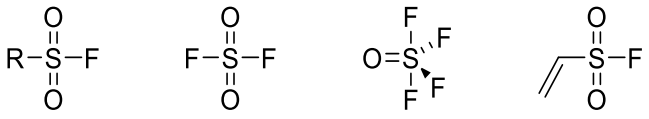
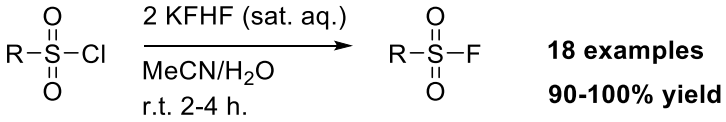


Figure 11 – Different types of SuFEx-able hubs.

The phrase “molecular plugins” has been coined for these compounds. They have the ability to allow multiple bonds or links to be formed around the central sulfur atom. The generation of these different connecters contributes to the continually growing SuFEx toolbox.

1.4.1.1 Arylsulfonyl fluorides

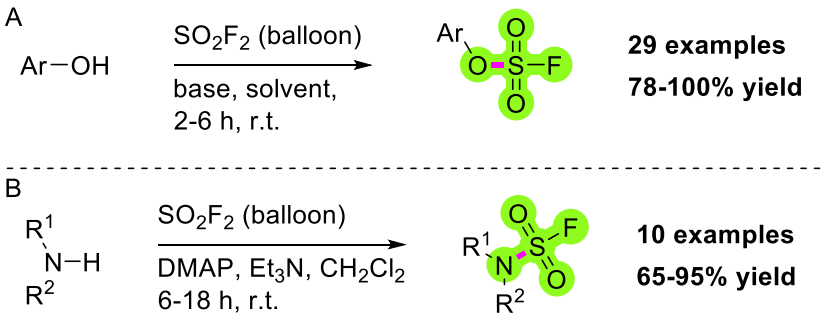
Aryl sulfonyl fluorides represent an example of a SuFEx-able hub in which the C-S bond has to be previously installed. They are relatively rare in organic chemistry when compared to aryl sulfonyl chlorides meaning synthetic routes to these compounds are usually required. One practical synthesis is direct halogen exchange reaction, however a more convenient method was described by Sharpless which involves the conversion of a sulfonyl chloride to its corresponding sulfonyl fluoride, **Scheme 23**.[134]



Scheme 23 – The synthesis of aryl-sulfonyl fluorides from aryl sulfonyl chlorides using a biphasic acetonitrile mixture and saturated potassium hydrogen bifluoride.

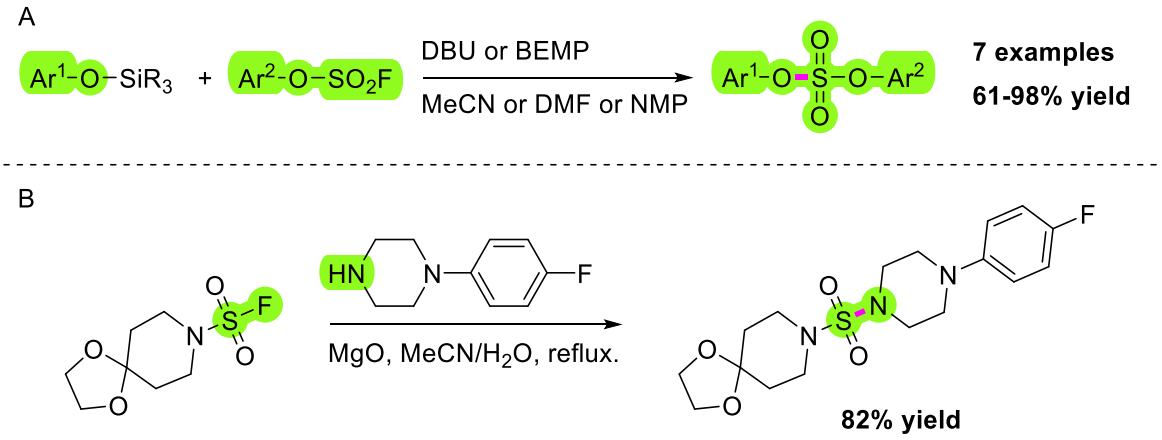
1.4.1.2 Sulfuryl fluorides and Surrogates

Sulfuryl fluoride (SO_2F_2) is a colourless and odourless gas which has been used widely as a pesticide[142] due to its ability to saturate air at concentrations lethal to pests and rodents. The gas can be thought of itself as a SuFEx-able hub due to its two S-F bonds. One of these bonds is firstly transformed in order to install the SO_2F moiety onto a target compound. This reactivity is well documented on phenols, generating fluorosulfonates by exposure of the phenol to gaseous SO_2F_2 introduced by balloon and Et_3N in CH_2Cl_2, **Scheme 24A**. Furthermore, sulfamoyl fluorides are generated by exposure of secondary amines to gaseous SO_2F_2, DMAP and Et_3N in CH_2Cl_2, **Scheme 24B**.[131,134]



Scheme 24 – Synthesis of [A] fluorosulfonates and [B] sulfamoyl fluorides from sulfuryl fluoride.

Upon installation of the sulfuryl fluoride to phenol or a secondary amine, a further SuFEx transformation can be facilitated. Fluorosulfates react with silyl ethers using DBU or BEMP as base to afford sulfate linked aromatic products in excellent yields, **Scheme 25A**. In the case of sulfamoyl fluorides, the subsequent SuFEx reaction is achievable only at high temperatures with a hydrogen bonding solvent, **Scheme 25B**.



Scheme 25 – Second SuFEx reaction taking place on [A] fluorosulfates and [B] sulfamoyl fluorides.[131,134]

Despite the usefulness of SO_2F_2 as a SuFEx reagent, its acquisition and use are difficult for laboratories without sufficient apparatus to handle harmful gas. The reaction with secondary amines to generate sulfamoyl fluorides also exhibited a limited substrate scope. Moreover, when primary amines are used, the expected *NH*-sulfamoyl fluorides are not isolated. In order to avoid the use of sulfuryl fluoride, surrogates that afford the same reactivity by acting as a F-SO_2^+ donor have been developed, **Figure 12**.[143,144]

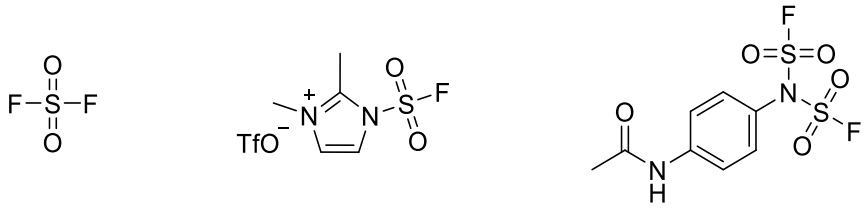
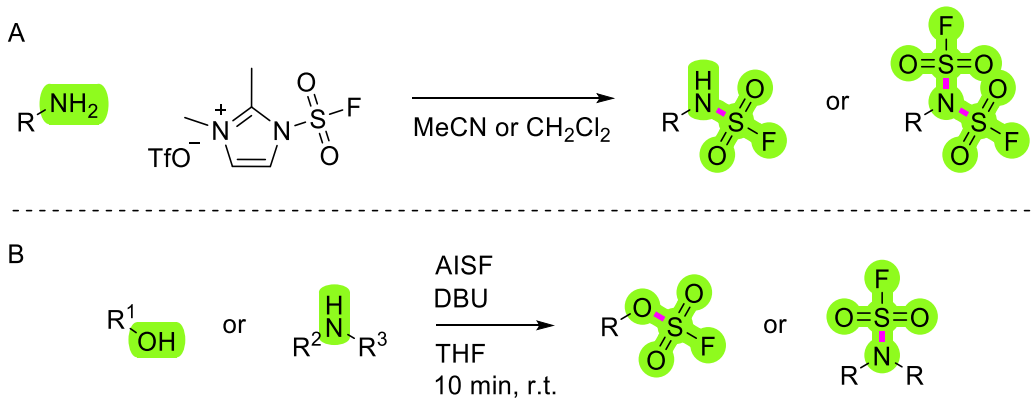


Figure 12 – Sulfuryl fluoride and its surrogates.

The fluorosulfuryl imidazolium salt displayed enhanced reactivity with phenols to generate fluorosulfates when compared to the parent gas and is now used to generate sulfamoyl fluorides on primary amines as well as secondary amines, **Scheme 26A**.[143]



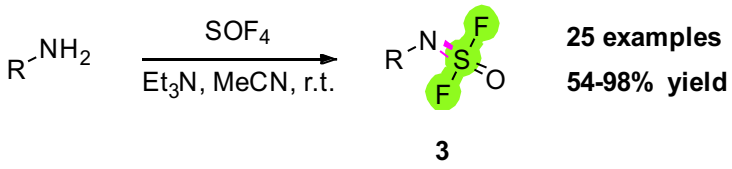
Scheme 26 – Synthesis of [A] NH-sulfamoyl fluorides using a fluorosulfuryl imidazolium salt surrogate and [B] fluorosulfates or sulfamoyl fluorides using the AISF surrogate.

The surrogate (acetylamino-phenyl)imidodisulfuryl difluoride (AISF) has been reported to facilitate the synthesis of fluorosulfates and sulfamoyl fluorides similarly to sulfuryl fluoride in excellent yields, **Scheme 26B**.[144]

1.4.1.3 Thionyl tetrafluoride

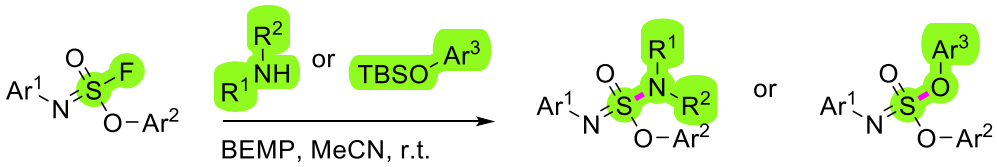
Early examples of fluoride substitution on thionyl tetrafluoride have been present in literature for 50-60 years,[145–147] however these only gave products in moderate yields. Until the advent of SuFEx click chemistry decades later there appears to have been no practical application of this chemistry.[148] In 2017, when using the gas in tandem with a primary amine

and a tertiary base such as triethylamine, iminosulfur oxydifluorides **3** were synthesised once again and in excellent yields, **Scheme 27**.



Scheme 27 – Reaction of primary amines with sulfonyl tetrafluoride in the presence of triethylamine.

One of the most exciting characteristics of SuFEx click chemistry can be seen in thionyl tetrafluoride and is the ability to build in multiple planes or direction. CuAAC click chemistry always produces a planar 1,2,3-triazole and as such the reaction cannot build into the 3rd dimension. Contrastingly, the number of S-F bonds present in **3** allows for multidimensional building in which more molecular space can theoretically be targeted.[148,149]

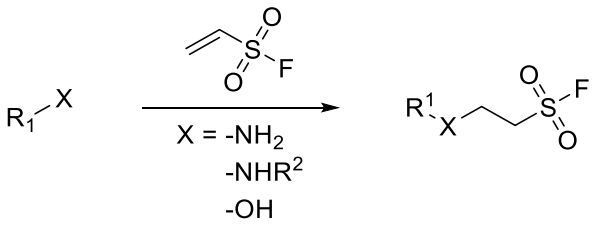


Scheme 28 – Multidimensional click chemistry by sequential SuFEx transformations.

As in all SuFEx-able hubs highlighted so far, transformations involving silyl ethers or secondary amines are tolerated. Impressively, a second SuFEx transformation can then be facilitated as in **Scheme 28**. The sulfur atom retains only the S=O bond from the gaseous starting material demonstrating the power of this particular molecular plugin and in the product adopts a tetrahedral geometry. The four starting S-F bonds have been converted by three separate SuFEx reactions generating three separate vectors from the tetrahedral sulfur hub in which click chemistry has been facilitated.[148]

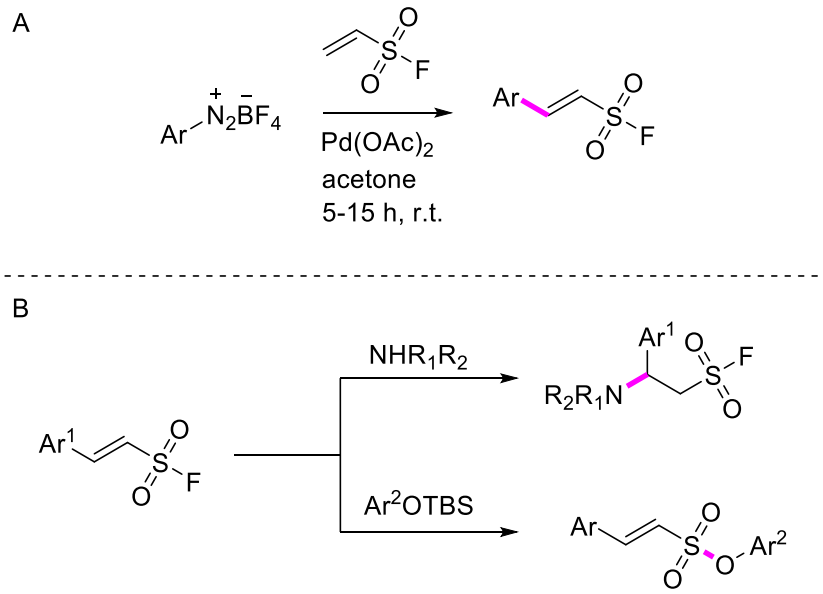
1.4.1.4 Ethenesulfonyl Fluoride

Ethenesulfonyl fluoride (ESF) represents an interesting example due to the activated double bond present in the structure. It is possible to generate stable products through the Michael addition, leaving the sulfonyl fluoride untouched. The two distinct trajectories present on ESF are both considered clickable hubs. The sulfonyl fluoride is SuFEx click-ready whilst the alkene moiety has been described as the most perfect Michael acceptor ever found. The reaction is so highly efficient that the Michael addition reaction can be thought of as orthogonal to the SuFEx reaction, **Scheme 29**.[134,150] This reactivity allows a wide variety of different molecules to be decorated with a pendant alkyl sulfonyl fluoride group. This Michael addition has been explored in industry, leading to a host of medicinal chemistry applications.[151–153]



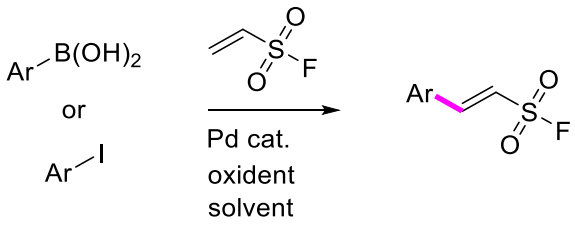
Scheme 29 – Reactivity of ESF towards nucleophiles.

Michael addition to ESF is not the only way to install this reactivity to molecules. Upon identification of the SuFEx-able hub, it became paramount to find methods of installation of ESFs onto substrates whilst retaining the activated alkene in the structure. The first noteworthy report adopted a cross-coupling approach to install arylvinylsulfonyl fluorides (aryl VSFs) using ESF and tetrafluoroborate salts, **Scheme 30A**.[154]



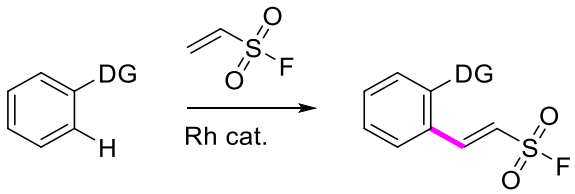
Scheme 30 – [A] Installation of aryvinylsulfonyl fluorides and [B] post-synthetic SuFEx transformations

By utilising this method, the installed vinylsulfonyl fluoride retained its desirable SuFEx-able hub and the native Michael addition centre. Both these reactive centres were then proven to carry out SuFEx transformations as well as Michael addition reactions with different nucleophiles **Scheme 30B**.[131,154]



Scheme 31 – Cross-coupling reactions to generate aryl VSFs.

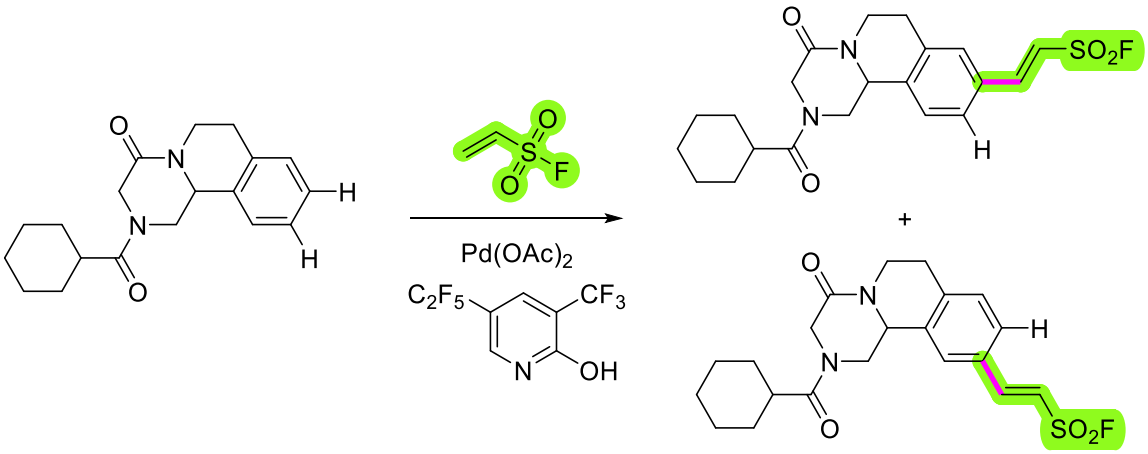
Building on this success, an oxidative Heck coupling reaction between boronic acids[155,156] and ESF has been explored as well as a reaction involving alkenyl iodides[157] and ESF to form aryl VSFs (**Scheme 31**). In each case, the aryl VSF was proven to undergo SuFEx transformations post-synthetically.



DG = COR, CHO, CO_2R, CONMeR

Scheme 32 – Rhodium catalysed C-H functionalization for the installation of aryl VSFs using directing groups.

Most recently, attempts to install the ESF functionality using C-H functionalization have been reported. A rhodium catalysed reaction using native directing groups was applied to aryl compounds to successfully install the vinylsulfonyl fluoride functionality, **Scheme 32**.[158–160] The installation of vinyl sulfonyl fluorides by non-directed C-H functionalization has also been achieved, **Scheme 33**. Once again, post synthetic modification by SuFEx transformations was carried out.[161]



Scheme 33 – Synthesis aryl VSFs by non-directed C-H functionalization.[161]

1.4.2 Vinyl sulfonyl fluorides in peptide science

Although there have been several methods developed for the introduction of aryl VSFs onto substrates as discussed in **Section 1.4.1.4**, remarkably few have been introduced to amino acid substrates, **Figure 13**.

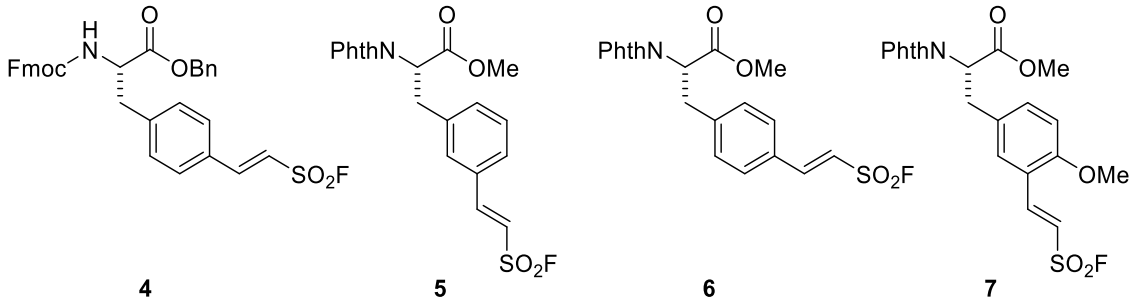


Figure 13 – aryl VSFs introduced to aromatic amino acids.[157,161]

Compound **4** was formed by the conversion of the aryl iodide to the corresponding aryl VSF and requires pre-functionalized amino acids to facilitate the reaction.[157] Modified amino acids **5-7** were formed by the C-H functionalization technique outlined in **Scheme 33** above.[161] In order to carry out this transformation there is a requirement for a developed electron-deficient 2-pyridone ligand which limits the atom economy of the reaction. Compounds **5** and **6** were isolated from the same reaction in a 1:1 mixture, demonstrating poor selectivity at different sites on the phenyl ring.

The introduction of a SuFEx-able hub to an amino acid in peptides with no obvious reactive handle is an unexplored way of generating diverse peptide structures. However, methods to facilitate this installation of vinylsulfonyl fluorides on peptides have not been reported. The development of a methodology which introduces vinyl sulfonyl fluorides to non-polar amino acids is required to broaden the scope of the SuFEx-able hub. These substrates could then be further manipulated to form complex molecules with enhanced properties. Installation of these residues on peptides whilst keeping both reaction pathways of ESF possible generates even more potential for diversification.

1.5 Aims and Objectives

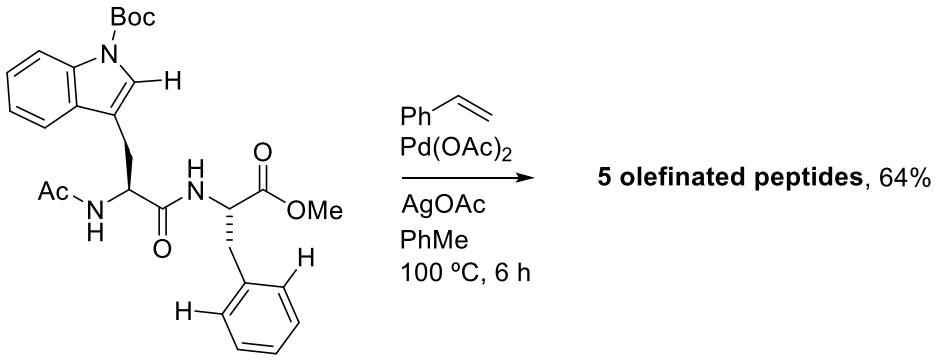
1.5.1 Project aims overview

As discussed in **Section 1.2.8**, the modification of peptides post-synthetically is an attractive approach to produce diverse structures with non-canonical reactivity. Moreover, peptide macrocyclization is an effective modification technique to generate compounds with

improved pharmacokinetic products. CuAAC click chemistry is one of the most prevalent techniques used to achieve cyclization in peptides, however the development of SuFEx click chemistry poses an exciting new prospect in this field. The primary aim of this project was to develop a new protocol that could facilitate the installation of a SuFEx-able hub onto phenylalanine residues and carry out SuFEx click transformations on peptides that had been modified post synthetically. The developed method will then be applied to short-chain peptide structures in order to facilitate an intramolecular SuFEx reaction and fashion peptide macrocycles from their natural linear counterparts. The method developed will be useful in expanding the toolbox of macrocyclization strategies for synthetic peptide chemists.

1.5.2 Residue Selectivity

The modification of amino acid residues in peptides by C-H functionalization is a research area that has achieved huge attention in the recent past.[65,87,162,163] Due to the pervasiveness of each amino acid, high control of residue selectivity is required for broad applicability and viability of developed methodologies. As discussed in **Section 1.3.3**, the Cross research group at NTU has already contributed two protocols to the olefination of native aromatic residues in peptides.[114,126] The two protocols are not significantly different enough to demonstrate residue selectivity in peptides containing both tryptophan and phenylalanine residues, **Scheme 34**.



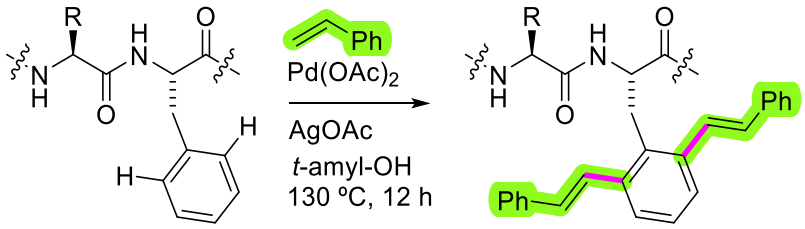
Scheme 34 – Lack of residue selectivity in peptides containing tryptophan and phenylalanine.

The first section of this work will attempt to solve this residue selectivity issue in peptides which contain both phenylalanine and tryptophan. As a starting point, observations made

about the mechanism of the reaction will be utilized in the hope of supressing the reactivity of residues.

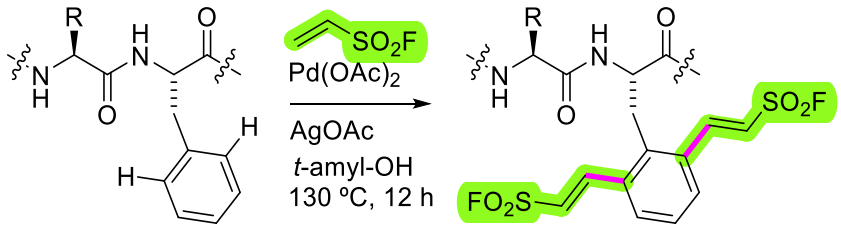
1.5.3 C-H functionalization of phenylalanine residues in peptides with ESF

Presently a small number of protocols have been developed in order to olefinate aromatic amino acids with ESF.[157,161] To date, there are no reported instances where phenylalanine residues in peptides are modified with ESF to generate peptide-based aryl VSF. Modification of non-polar residues to form diversified peptide substrates containing SuFEx-able hubs are required to introduce otherwise unachievable reactivity to unreactive amino acids in peptides.



Scheme 35 – Conditions developed by the Cross group for the modification of phenylalanine residues in peptides.

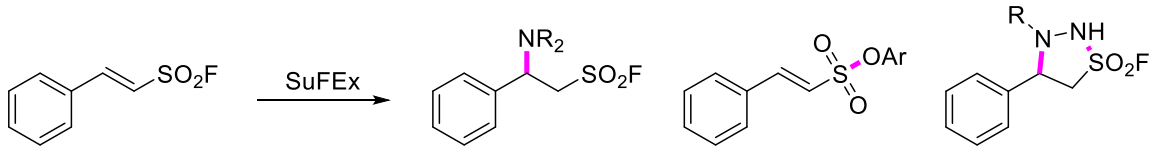
Initially, the Cross group developed conditions for the olefination of styrene-based coupling partners onto phenylalanine residues in short-chained peptides, **Scheme 35**.[126] The first main body of work will target the development of a new protocol that can be used to facilitate the olefination of ESF onto phenylalanine residues in peptides. As a starting point, a model dipeptide will be exposed to similar reaction conditions, with ESF replacing styrene in the reaction, **Scheme 36**.



Scheme 36 – Proposed C-H functionalization of phenylalanine residues in peptides with ESF.

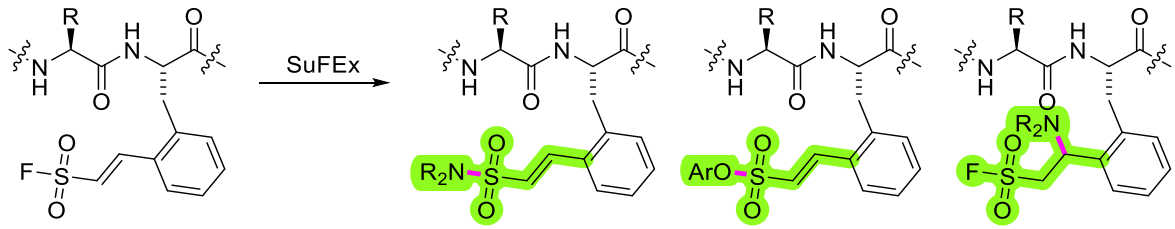
1.5.4 SuFEx transformations of peptides previously modified to include vinylsulfonyl fluorides

Substances containing SuFEx-able hubs are currently attracting huge attention in synthetic organic chemistry.[131,164] The unique reactivities of the compounds that undergo the "next generation click reaction" has enormous potential to afford new compounds that occupy new molecular space. As discussed in **Section 1.4**, SuFEx transformations are now well documented on a wide variety of aryl VSF substrates, **Scheme 37**. However, the manipulation of the reactions involving aryl VSFs upon peptide-based compounds, forming sulfonamides, sulfonate esters or aza-Michael addition products appear to be lacking.



Scheme 37 – Documented SuFEx transformations of aryl VSFs.

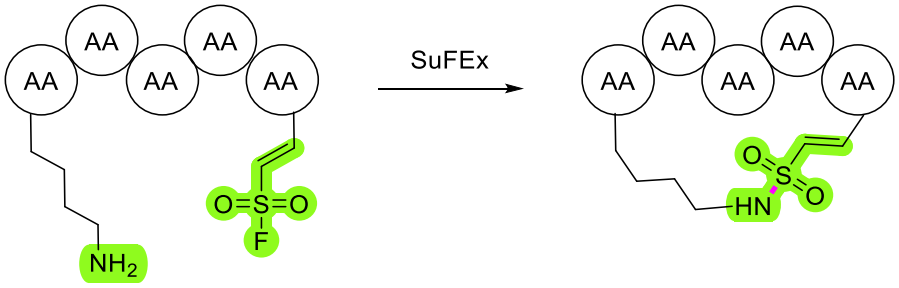
The aim of this body of work is to introduce SuFEx transformations already known to take place on substrates containing aryl VSFs, onto phenylalanine residues modified with ESF. The generation of sulfonamides, sulfonate esters or pendant sulfonyl fluorides from newly formed aryl VSFs and nucleophiles, **Scheme 38** will demonstrate the power of the techniques when used in tandem.



Scheme 38 – Targeted SuFEx transformations to carry out on peptides containing aryl VSFs.

1.5.5 Generation of peptide macrocycles using C-H functionalization protocols followed by SuFEx transformations

As discussed in **Section 1.2.8**, new methodologies for the synthesis of macrocyclic peptide structures have received an enormous amount of attention in the last 30 years.[65] Notably, reactions involving click chemistry to form macrocyclic bonds are well developed and ideally poised due to their high yielding reactions and tolerance of other functional groups. The final investigation will involve the application of the developed C-H functionalization and SuFEx methodology in order to generate peptide macrocycles with sulfonamide linkers, **Scheme 39**.



Scheme 39 – Peptide macrocyclization using C-H functionalization followed by SuFEx click chemistry.

1.5.6 Project Aims Summary

The primary aims of this project are outlined as follows:

(i) To develop a protocol for the selective olefination of tryptophan and/or phenylalanine residues in peptides that contain both tryptophan and phenylalanine.

(ii) To develop a new C-H functionalization methodology for the post-synthetic modification of phenylalanine residues in peptides, generating an aryl VSF motif by using ESF as a coupling partner.

(iii) To carry out SuFEx transformations on SuFEx hubs, previously installed by the modification of natural, linear peptide substrates.

(iv) To apply the developed techniques on synthesised linear peptides in order to generate macrocyclic structures linked by the newly formed sulfonamide functionality.

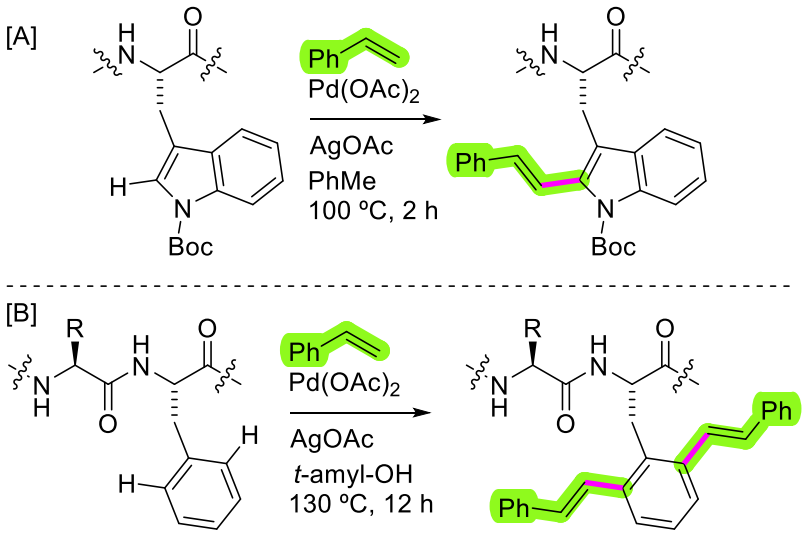
The developed technique will provide a procedure to modify an otherwise unreactive phenylalanine residue. The peptide will be converted post-synthetically to a SuFEx-able substrate which will then allow further diversification upon the facilitation of the click reaction. The method will contribute to an expanding list of protocols for the cyclization of peptides.

2 Residue selectivity and peptide-amino acid cross-linking

2.1 Introduction

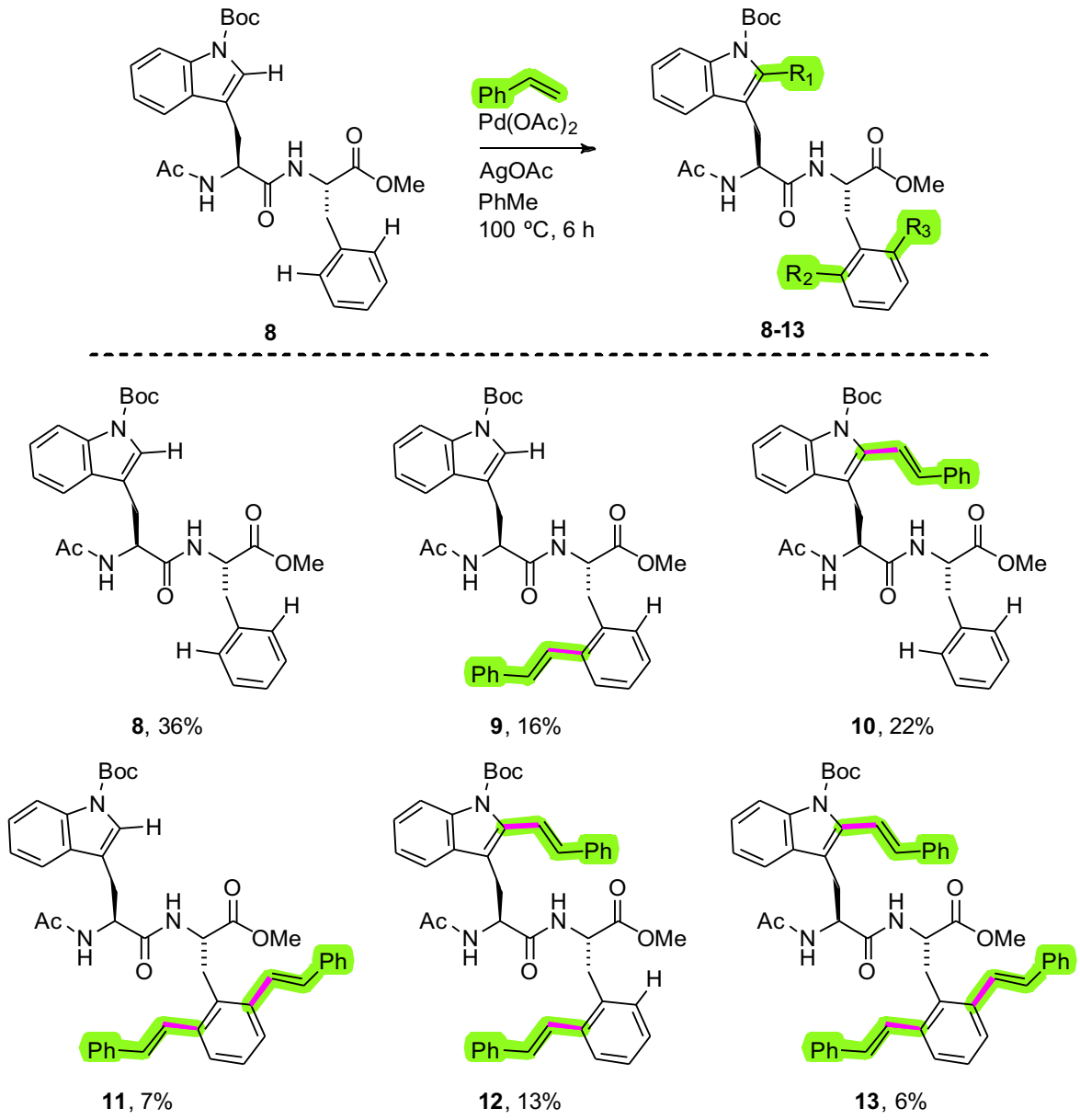
2.1.1 Residue Selectivity

The Cross research group had established separate methods for the olefination of phenylalanine and tryptophan residues in peptides, **Scheme 40**. However, these procedures were not significantly different to each other and selectivity of the method for one residue over the other was unlikely to be achieved.



Scheme 40 – Developed conditions for the olefination of [A] tryptophan and [B] phenylalanine residues in peptides.

The optimised protocols use different solvents. In each example, both solvents facilitated the olefination reaction to some degree, indicating that solvent control was unlikely to give satisfactory selectivity in peptides containing both residues. When dipeptide Ac-Trp(Boc)-Phe-OMe **8** was exposed to olefination conditions in toluene, a mixture of products was obtained, comprising of starting material **8** and every possible combination of olefinated residues **9-13**, **Scheme 41**.[114]



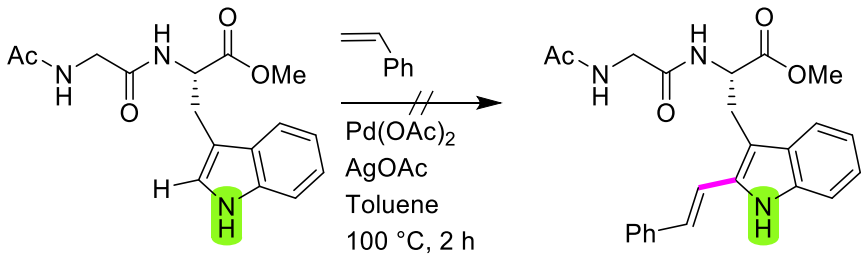
Scheme 41 – Unselective olefination of tryptophan and phenylalanine residues on the same dipeptide model.

It cannot be disputed that this reaction lacks selectivity and is destined to be poorly yielding as a result. The dipeptide **8** was selected as the simplest peptide possible in which reactions at both aromatic residues can be carried out. A best olefinated yield of 22% is likely to fall even further once the complexity of the model substrate is amplified.

2.1.2 Directing Group Manipulation

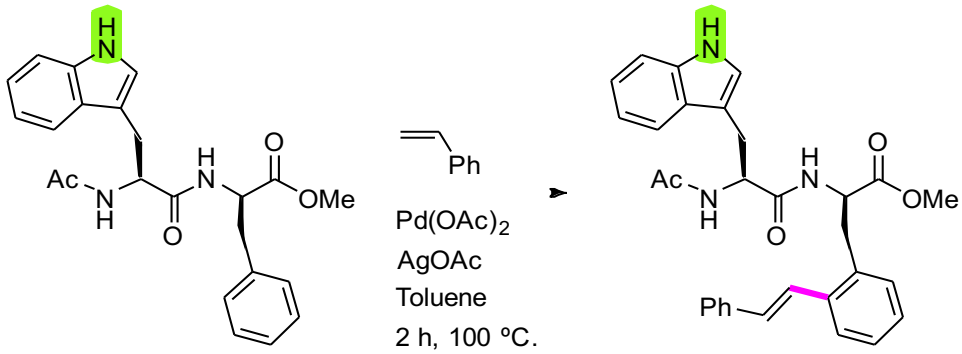
The procedure used to facilitate late-stage functionalization on tryptophan residues in peptides requires the indole nitrogen to be masked by a protecting group. The Boc

functional group was chosen due to its routine employment in solid-phase peptide synthesis protocols. Its chelating power is also required to facilitate the reaction. Without this functional group protecting the indole nitrogen atom, the reaction did not proceed, **Scheme 42**.



Scheme 42 – An unprotected tryptophan containing peptide does not undergo olefination.

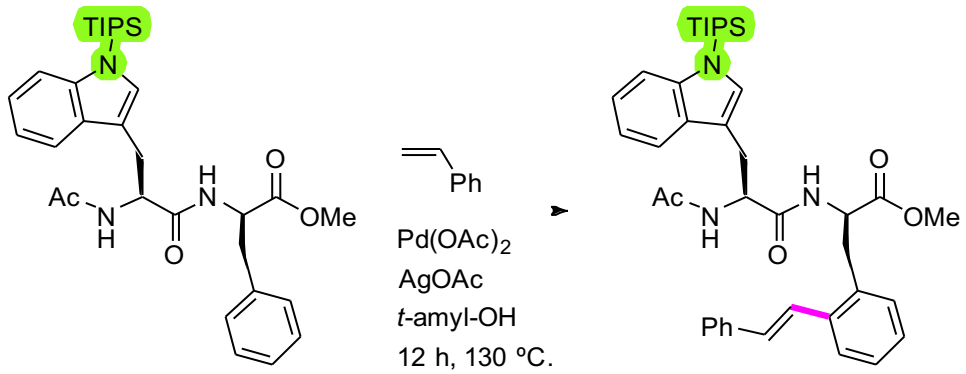
However, when olefination of peptide **14** containing a phenylalanine and tryptophan residue was undertaken, the reaction yield of **15** (**Scheme 43**) was poor (21%). It is likely that the polar handles of the peptide substrate need to be protected in order for the olefination to be facilitated efficiently.



14 **15**, 21%

Scheme 43 – Reactions with unprotected tryptophan residues are poor yielding.

Indeed, when the indole nitrogen was protected by a TIPS protecting group as in peptide **16**, which lacks the ability to chelate the palladium catalyst, the reaction at tryptophan was successfully supressed, yielding modified peptide **17** (**Scheme 44**).



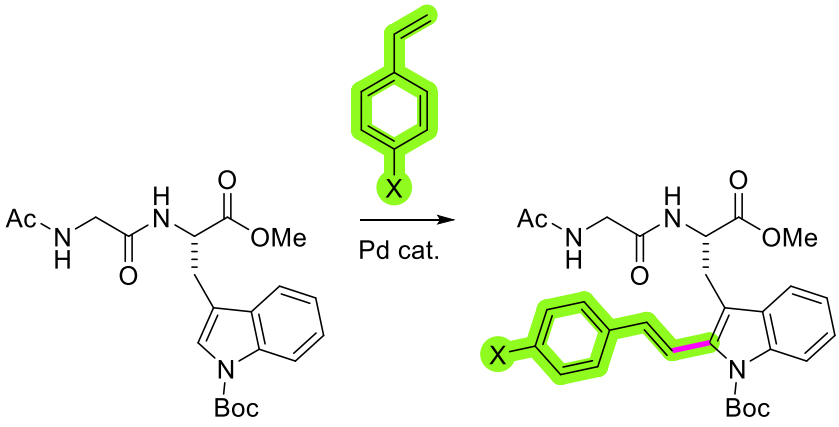
16 **17**, 49%

*Scheme 44 – Protected indole on tryptophan allows for increased yield of **17**.*

No method of supressing reactivity at phenylalanine effectively had previously been established. However, as demonstrated above, effective manipulation of the directing groups which ultimately reduces their chelating power has the potential to provide a way doing so.

2.1.3 Peptide-Amino acid cross-linking

The olefination of tryptophan residues with styrene derivatives had been previously carried out by the Cross research group, **Scheme 45**.[165] These derivatives range from electron donating to electron withdrawing with differing levels of chemical complexity. However, none of the reported styrene derivatives contained an amide or ester.



X = -H, -CH_3, -Cl, -OMe, -CF_3, -C≡N

Scheme 45 – Olefination of tryptophan residues with styrene derivatives already reported by the Cross group.

As the backbone amides are required for the successful olefination by this protocol it was important that these motifs be investigated to ensure that they could be tolerated by the reaction. Confirmation that these substrates can be tolerated would significantly increase the applicability of the method.

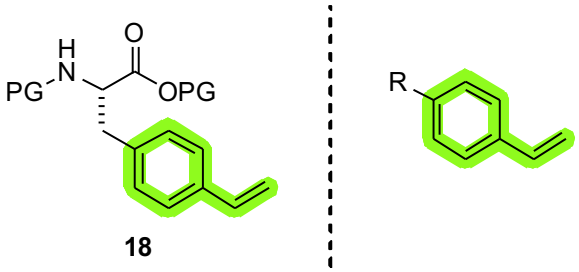


*Figure 14 – The styrene motif is present in theoretical protected amino acid **18**.*

If the theoretical unnatural amino acid **18** was designed to contain both an amide and an ester as well as the styrene motif, a lot of information could be collected from its investigation, **Figure 14**.

The main aim of this study was to determine if it was possible to olefinate at one available reaction site on a peptide chain where another potential site for olefination was present. Control of selectivity of the reaction is crucial for broader applicability of the method. Additionally, in order to prove that the method was well adapted to peptide-amino acid or peptide-peptide cross-linking, this section aimed to prove that olefination of styrene-based amino acid derivatives to peptides was possible.

2.2 Selectivity – Promoting the reactivity of tryptophan

The published protocol[114] for olefinating tryptophan residues generally produced modified peptides in higher yields than the one used to transform phenylalanine residues. However, this higher yielding reaction is not noticeable in the olefination of the dipeptide compound **8** discussed in **Section 2.1.1**. Considering the location of the residue on the peptide, it was proposed that the diminished selectivity may be caused by the positioning of the tryptophan

residue at the *N*-terminus. Whilst reaction at the *N*-terminus had been documented, the yields were not as high as when tryptophan was placed in the middle of the chain or at the *C*-terminus.[114]

Initially, peptide **19** was designed as a means of moving the tryptophan residue away from the *N*- terminus of the peptide. However, attempts to synthesise and subsequently clean this peptide ready for olefination failed several times. This was attributed to a Phe-Trp link which proved difficult to synthesise by solution-phase methods.

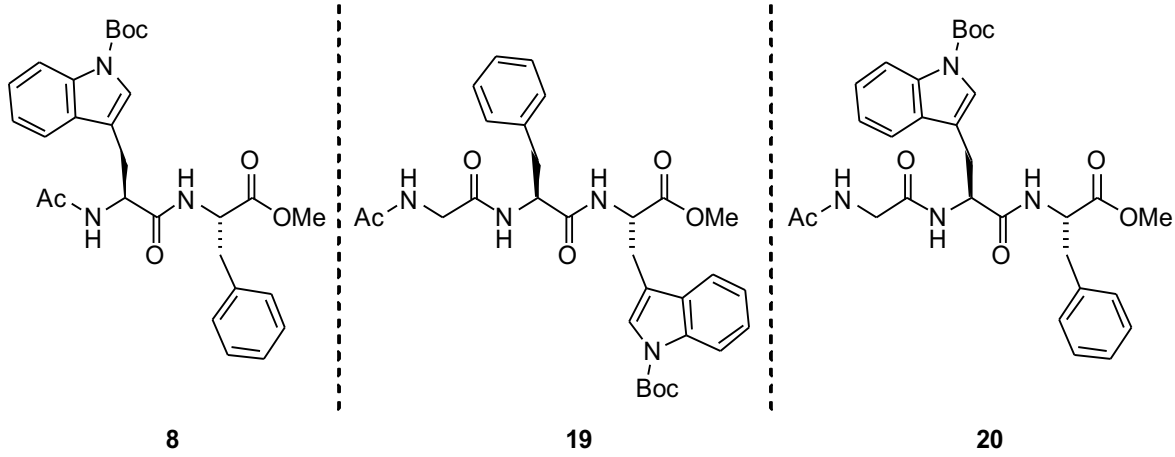
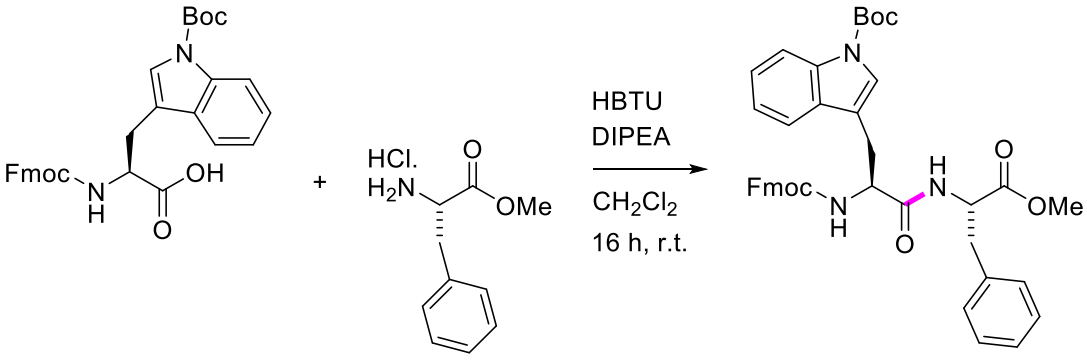


*Figure 15 – Tripeptides **19** and **20** contain an extra residue towards the N-terminus than dipeptide **8** which could promote reactivity of the tryptophan residue. Unfortunately, the synthesis of tripeptide **19** proved problematic and tripeptide **20** was synthesised instead.*

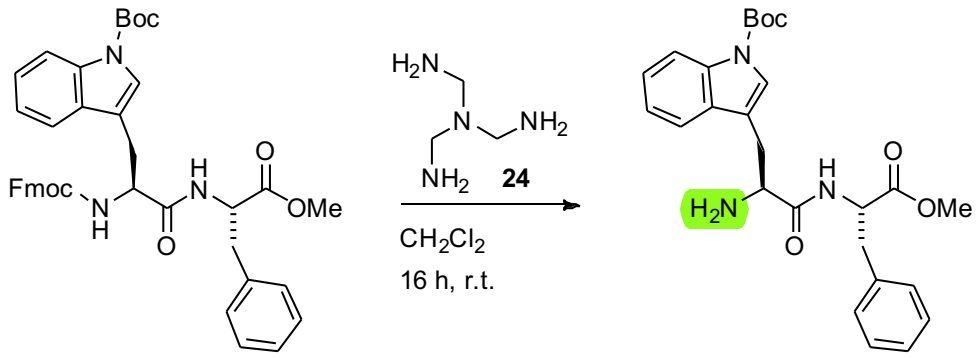
To that end, peptide **20** was designed containing an extra residue towards the *N*-terminus in the hope that the reactivity of the tryptophan olefination pathway was increased, **Figure 15**. This peptide was designed to ensure that the tryptophan residue is no longer placed at the *N*-terminus while ensuring that the phenylalanine residue is not placed at the *N*-terminus either. In order to synthesise the target tripeptide **20**, Fmoc-Trp(Boc)-OH **21** and phenylalanine methyl ester hydrochloride **22** were exposed to solution phase reaction conditions, **Scheme 46** to generate protected dipeptide intermediate **23**.



21 **22** **23**, 61%

*Scheme 46 – Synthesis of protected dipeptide intermediate **23**.*

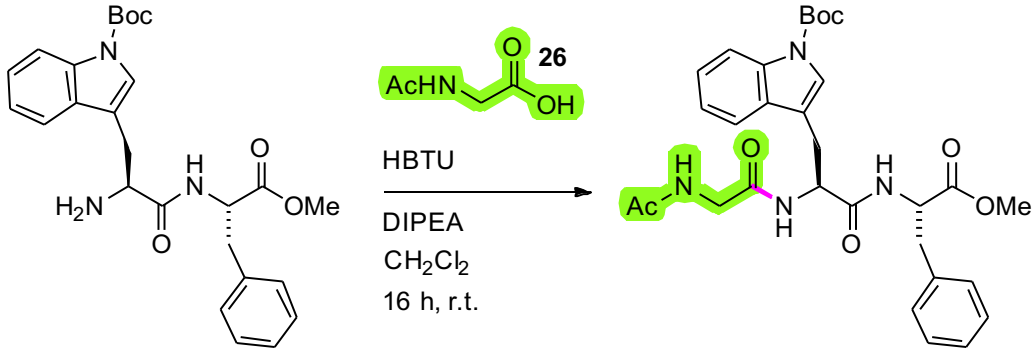
The protected peptide intermediate **23** was synthesised in good yield. Analysis of the ^{1}H NMR spectrum of the purified product confirmed the synthesis had taken place by the appearance of a doublet at 6.10 ppm corresponding to the newly formed amide proton.



23 **25**, 78%

*Scheme 47 – Solution-phase Fmoc deprotection to yield the N-terminal free amine in dipeptide **25**.*

In order to extend the peptide sequence further, a solution-phase Fmoc deprotection had to be carried out (**Scheme 47**). Tris(2-aminoethyl)amine **24** was used as a DCM soluble alternative to piperidine meaning that the deprotection could be carried out easily in solution. Once again, the reaction was carried out in excellent yield. The success of the synthesis was judged by ^{1}H NMR spectroscopy. The disappearance of the signals corresponding to the Fmoc protons as well as the emergence a signal relating to the primary amine at 1.38 ppm provided evidence that the target peptide **25** had been synthesised. With the free amine revealed, it was possible to carry out a solution-phase peptide synthesis in order to generate the target tripeptide **20**.

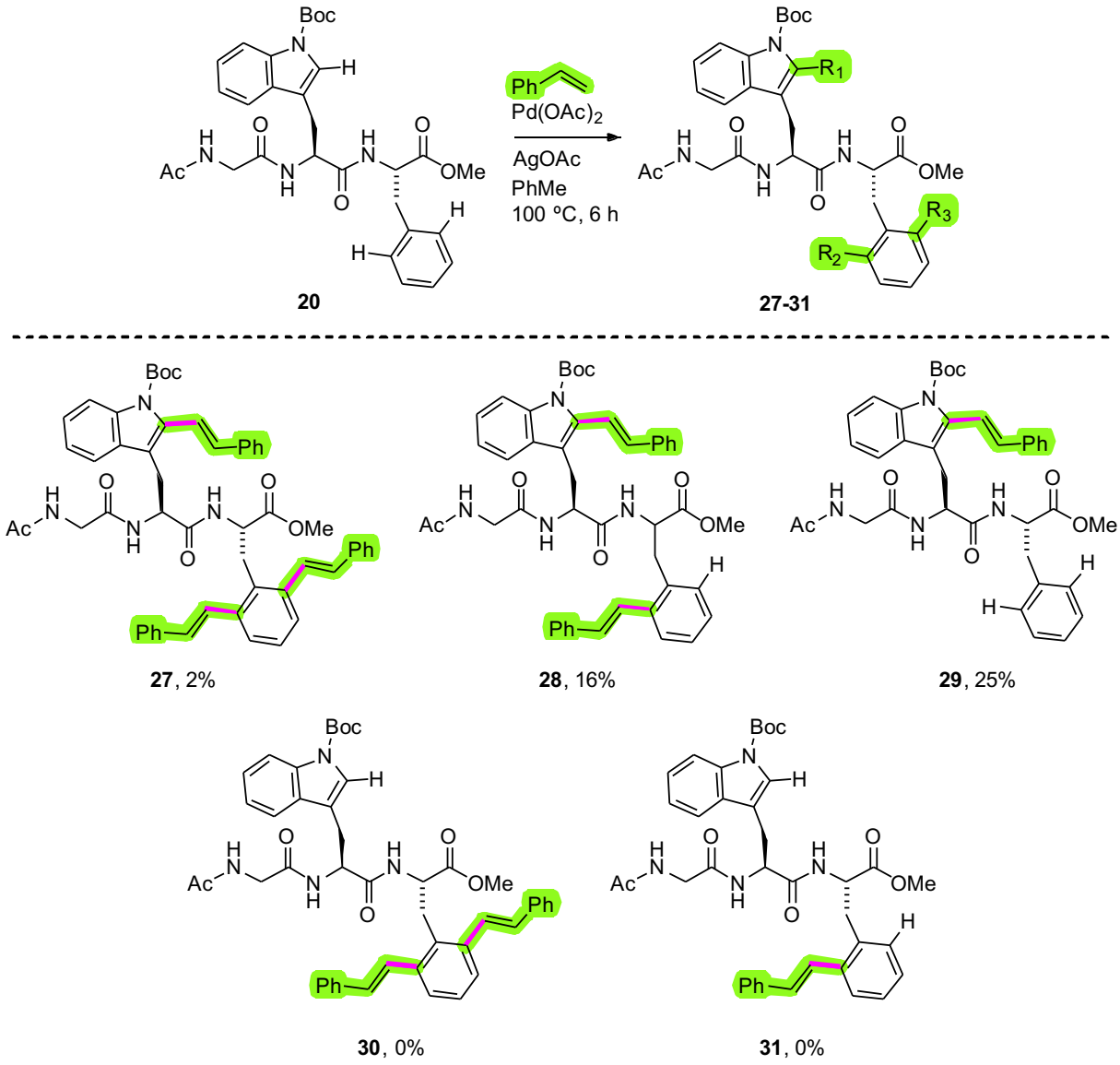


25 **20**, 76%

*Scheme 48 – Synthesis of target tripeptide **20**.*

The peptide intermediate **25** and *N*-acetyl glycine **26** were exposed to the solution phase coupling reaction conditions as in **Scheme 48** above. The reaction was completed in good yield and the synthetic yield for the synthesis of tripeptide **20** was determined to be 36%. After purification by flash column chromatography and recrystallization from CH_2Cl_2 and hexanes, the compound was exposed to the olefination conditions outlined in **Scheme 49**.

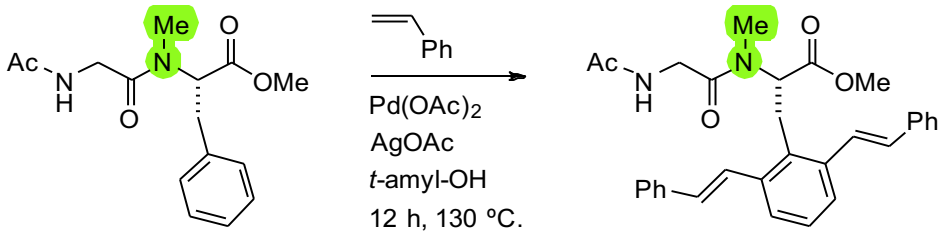
The selectivity of the reaction was not significantly improved by extending the peptide chain length to increase the reactivity of the tryptophan residue. As in the case discussed in **Section 2.1.1**, the main product of the reaction is still the corresponding mono-olefinated species **29** however the reaction is still not selective. Notably, no species was isolated which contained olefinated phenylalanine residues only. This suggests that the tryptophan residue is reactive enough to undergo olefination first. However, the olefination of the phenylalanine residue cannot be controlled or stopped by the installation of more residues towards the *N*-terminus.



*Scheme 49 – Exposure of tripeptide **20** to olefination conditions.*

2.3 Selectivity – Suppression of phenylalanine reactivity in peptides

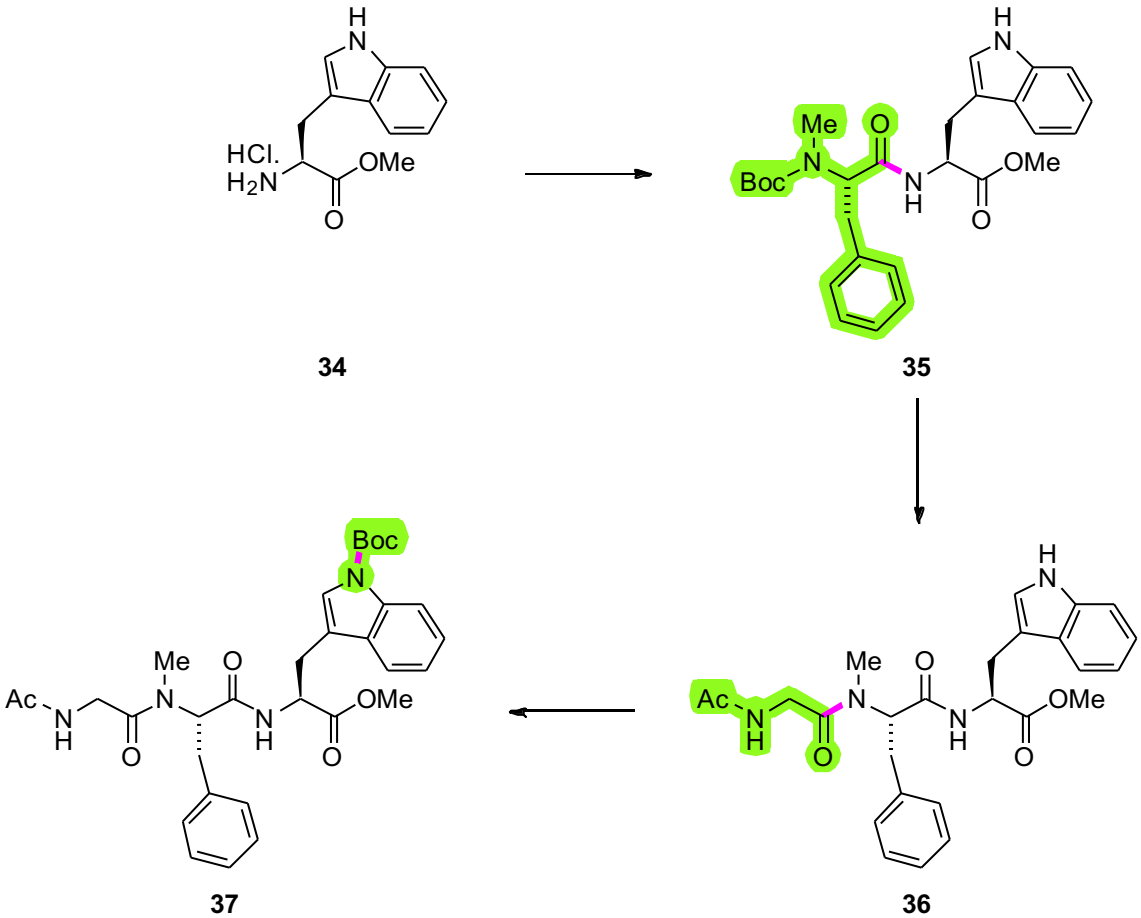
Efforts to improve the reactivity of tryptophan in order to garner more selectivity had proven ineffectual. However, in **Section 2.1.2** the suppression of the reactivity of tryptophan was accomplished by the manipulation of a directing group. It was believed that the reactivity of phenylalanine residues could be inhibited in a similar manner. For example, upon the attempted olefination of *N*-methylated phenylalanine containing dipeptide **32** the formation of modified dipeptide **33** was not observed, **Scheme 50**.[126]



32 **33**, 0%

Scheme 50 – N-methylated phenylalanine residues are not olefinated in peptides when exposed to the developed reaction conditions.

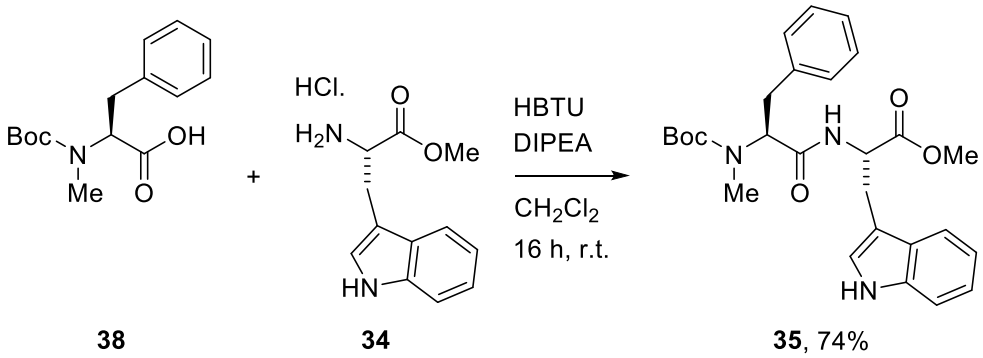
This result shows that when the amide is 'blocked', and the chelating power of the directing group reduced, the reaction cannot be facilitated. It was argued that manipulation of a directing group by *N*-methylation of the phenylalanine amide would effectively supress the reactivity at this residue, similarly to the example involving tryptophan in **Section 2.1.2**. To this end, the target compound **37** was identified and a synthetic route was designed, **Scheme 51**.



34 **35** **36** **37**

*Scheme 51 – Synthetic route to target tripeptide **37**.*

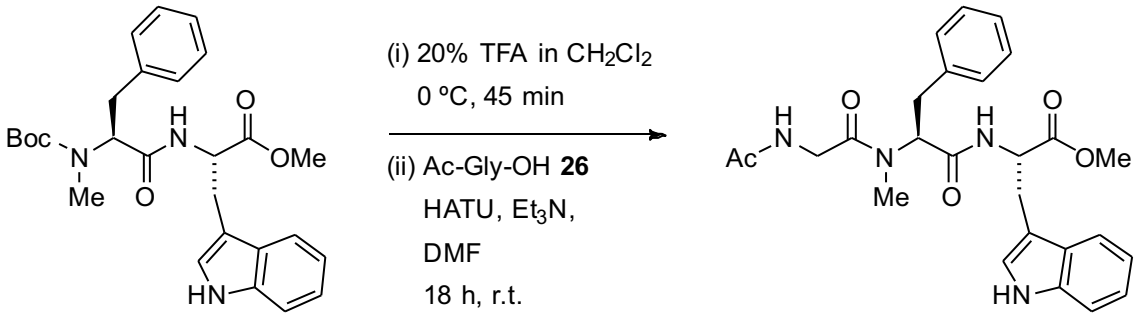
Starting from tryptophan methyl ester hydrochloride **34**, a solution-phase peptide coupling was planned to generate an *N*-methylated dipeptide **35**. Cleavage of the Boc protecting group revealed the secondary amine followed by a further solution-phase peptide coupling to generate unprotected tripeptide **36**. A standard Boc protection reaction would then install the Boc protecting group on the indole and generate protected tripeptide target **37**. It was hoped that when exposed to the reaction conditions, **37** would undergo mono-olefination at the tryptophan site only.



38 **34** **35**, 74%

*Scheme 52 – Synthesis of N-methylated dipeptide **35**.*

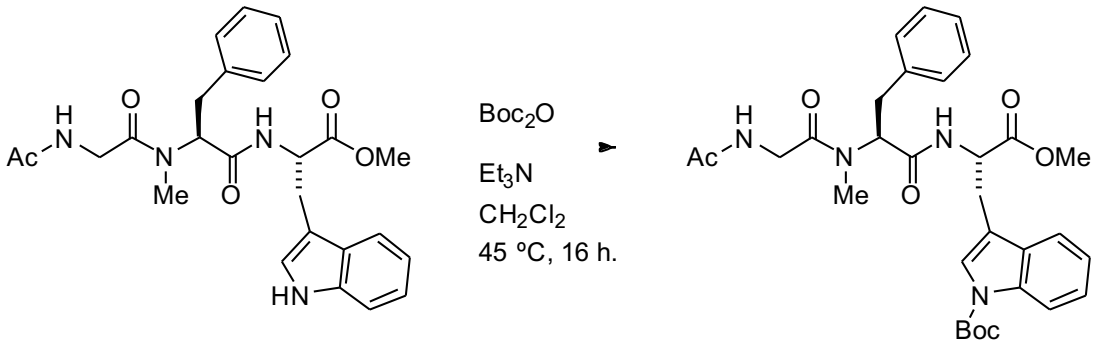
The *N*-methylated amino acid (*N*-Boc)(*N*-Me)-Phe-OH **38** and tryptophan methyl ester hydrochloride **34** were exposed to solution-phase peptide synthesis conditions in order to generate a Boc-protected, *N*-methylated peptide intermediate **35**, **Scheme 52**. The reaction proceeded in good yield and purification by flash column chromatography yielded **35** as a cream solid. Analysis of the ^{1}H NMR spectrum proved tricky due to the presence of two rotamers of compound **35**. The NMR spectra of both the major and minor rotamers are reported in **Section 2.6.9**.



35 **36**, 62%

*Scheme 53 – The Boc deprotection followed by exposure to solution-phase peptide coupling conditions yielded N-methylated tripeptide **36**.*

In order to generate unprotected tripeptide **36**, the Boc group had to be cleaved before a solution-phase peptide coupling could install the *N*-terminal amino acid, **Scheme 53**. Exposure to dilute TFA generated the unprotected secondary amine followed by exposure to *N*-acetyl glycine **26** in order to generate the tripeptide **36**. Analysis of the ^{1}H NMR spectrum revealed the disappearance of the rotameric species which was suggestive that the Boc protecting group had been removed. Once again, the reaction proceeded in good yield and purification by flash column chromatography was required before the intermediate **36** could be used in the next synthetic step.

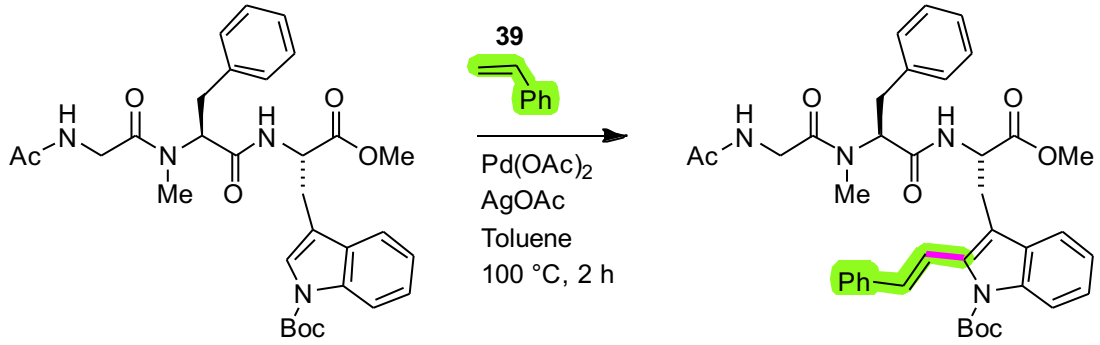


36 **37**, 54%

*Scheme 54 – Boc protection of the indole nitrogen to generate protected tripeptide **37**.*

In order to generate target peptide **37** from intermediate **36**, the indole nitrogen had to be protected as in **Scheme 54**. Analysis of the ^{1}H NMR spectrum of the purified product showed a peak at 1.68 ppm corresponding to the newly installed Boc protecting group. The protection was carried out in fair yield and the overall yield of the synthesis of target **37** from

tryptophan methyl ester hydrochloride **34** was determined to be 25%. The target peptide was then ready to be exposed to olefination conditions outlined in **Scheme 55**.



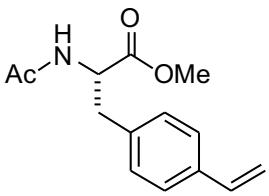
37 **40**, 55%

*Scheme 55 – Exposure of tripeptide **37** to the olefination conditions yielded modified tripeptide **40**.*

Gratifyingly, the reaction with styrene **39** proceeded well giving the singular olefinated tryptophan peptide **40** in a 55% yield. The rest of the reaction mass was accounted for in the starting material **37**. Analysis of the ^{1}H NMR spectrum of the purified product demonstrated that only one styrene equivalent had been added to the peptide. Conclusive proof was found in the HMBC spectrum where the phenylalanine *ortho* protons coupled to the methylene carbon atom suggesting that no C-H functionalization had occurred at this site. Furthermore, the methylene carbon of tryptophan did not couple to any aromatic protons which was indicative that olefination had occurred as expected at tryptophan C-2. This result verifies that the olefination of phenylalanine residues can be supressed by the manipulation of the amide directing groups.

2.4 Peptide-amino acid cross-linking

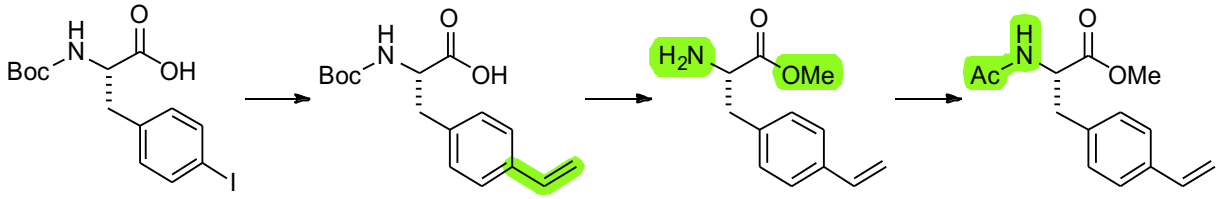
As discussed in **Section 2.1.3,** for broad applicability of the method a styrene derivative with biological relevance was identified, **Figure 16**. As an unnatural amino acid, the successful olefination of **41** to tryptophan residues would highlight the plausibility of the reaction to generate complex peptide-amino acid and peptide-peptide cross-linked structures.



41

*Figure 16 – Ac-Phe(4-vinyl)-OMe **41** is an unnatural amino acid with the potential to undergo olefination to a tryptophan containing peptide.*

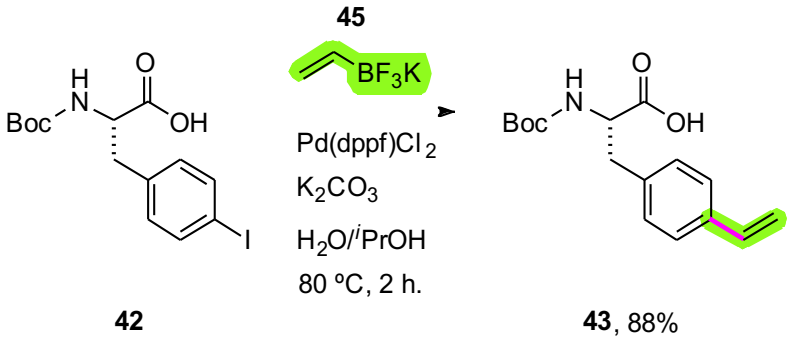
In order to generate **41**, a synthetic route was devised as in **Scheme 56**. Initially the amino acid Boc-Phe(4-iodo)-OH **42** was subjected to a Suzuki-Miyaura cross-coupling reaction in order to access the aryl alkene and generate the styrene motif on the amino acid intermediate **43**. The intermediate would then be exposed to esterification conditions generating the free amino ester **44**. Finally, the free amine would be protected by acetylation to form the target protected amino ester **41**.



42 **43** **44** **41**

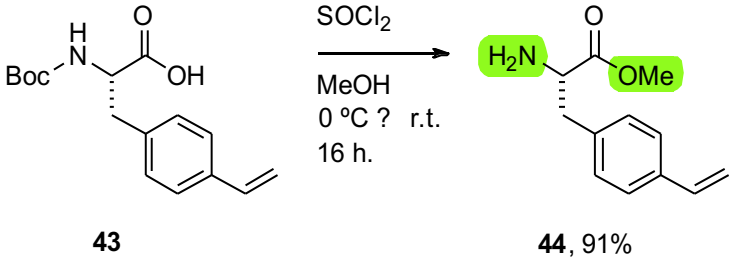
*Scheme 56 – Synthetic route to unnatural amino acid **41**.*

According to a literature procedure, the Boc-Phe(4-iodo)-OH **42**, vinyl trifluoroborate salt **45**, palladium catalyst, and base were stirred in H_2O/^{i}PrOH at 80 ºC, **Scheme 57**.[166] The vinylation proceeded in good yield and upon purification by flash column chromatography yielded intermediate **43** as a brown solid.



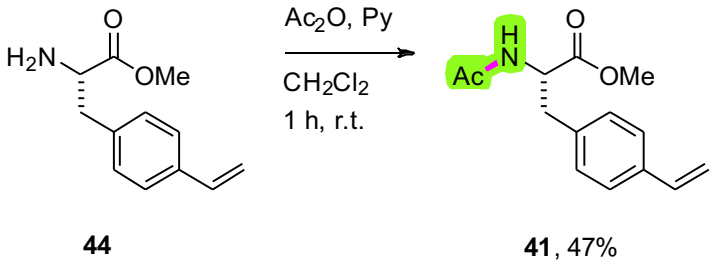
*Scheme 57 – A Suzuki-Miyaura palladium catalysed cross-coupling reaction generated the vinyl amino acid **43**.*

The presence of three new peaks in the ^{1}H NMR spectrum provided evidence that the alkene had been installed. The splitting pattern of the aromatic region remained the same which implied that the alkene had been installed at the *para* position on the phenyl ring. The NMR data for compound **43** matched that previously reported.[166] Intermediate **43** was then used in the next step to generate the amino ester **44**, **Scheme 58**.



*Scheme 58 – Esterification with thionyl chloride to yield the amino ester intermediate **44**.*

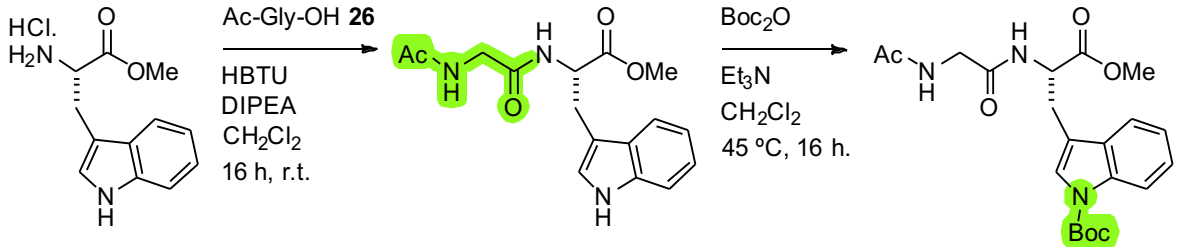
The amino acid **43** was dissolved in MeOH and treated with the dropwise addition of thionyl chloride. The formation of the ester via this method generated hydrochloric acid *in situ* which cleaved the Boc functionality yielding the free amine **44**. Confirmation of the synthesis was found in the ^{1}H NMR spectrum in which a singlet at 3.74 ppm corresponding to the ester protons was found. The signal corresponding to the Boc protons at 1.31 ppm in the starting material had also disappeared. Finally, it was necessary to protect the amine as an amide. An acetylation was undertaken, **Scheme 59**.



44 **41**, 47%

*Scheme 59 – Acetylation of the free amine using acetic anhydride and pyridine in CH_2Cl_2 to generate target amino acid **41**.*

The amino ester **44** was dissolved in CH_2Cl_2 and treated with a solution of acetic anhydride and pyridine. After stirring at room temperature for 12 h the target compound **41** was isolated by flash column chromatography and ready to use in the olefination. The overall synthetic yield of the synthesis of the target unnatural amino acid **41** was determined to be 38%.

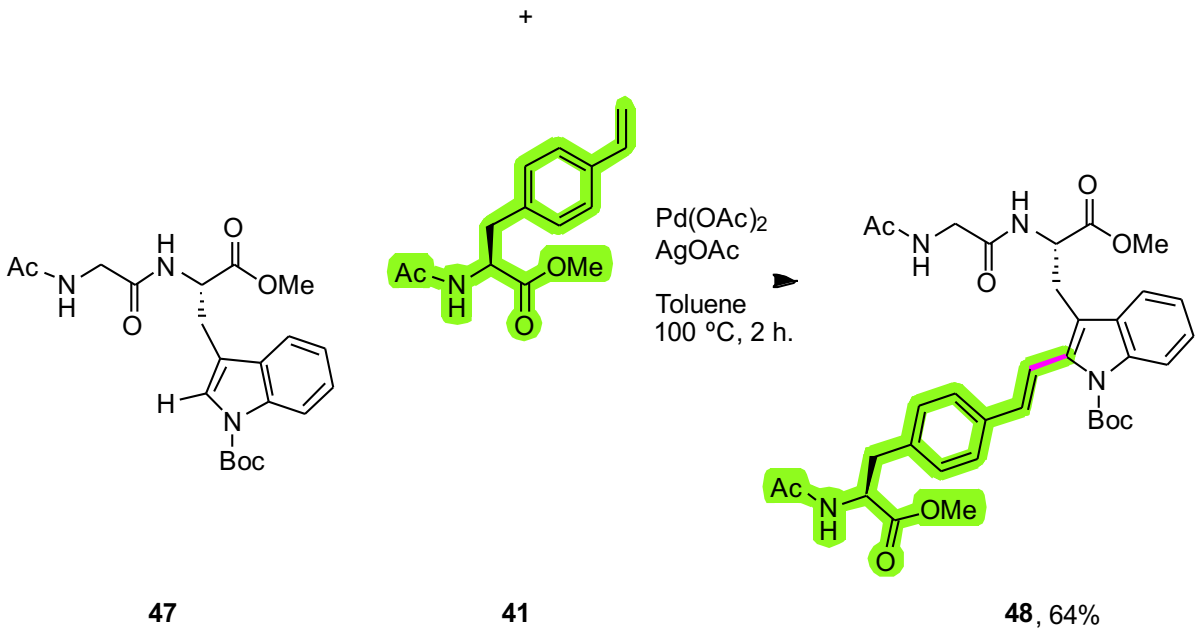


34 **46**, 79% **47**, 27%

*Scheme 60 – Synthesis of intermediate **46** and target protected dipeptide **47**.*

To synthesise the dipeptide Ac-Gly-Trp(Boc)-OMe **47**, a solution phase peptide synthesis was undertaken, **Scheme 60**. The dipeptide intermediate **46** was synthesised in good yield from tryptophan methyl ester hydrochloride **34** and *N*-acetyl glycine **26** before the protection of the indole nitrogen to yield target dipeptide **47** was carried out. The overall yield of this synthetic pathway was 21% and was significantly limited by the poorly yielding Boc protection.

With the dipeptide **47** and styrene derivative **41** in hand, the olefination reaction was carried out as in **Scheme 61**. Exposure of both compounds to palladium catalyst and silver salt in toluene for 2 h generated modified dipeptide **48** in good yield.



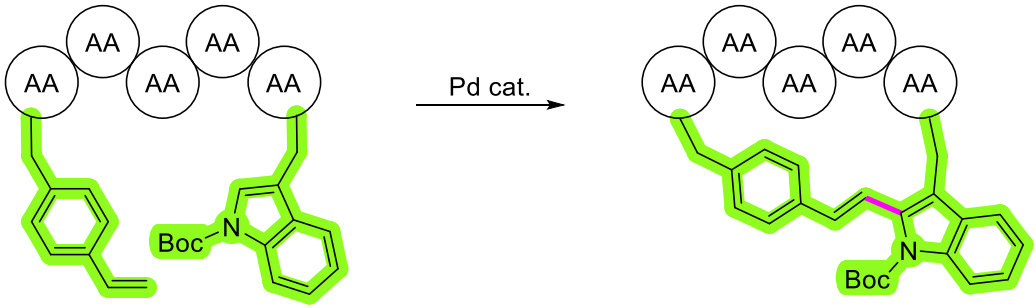
*Scheme 61 – Olefination of dipeptide **47** with unnatural amino acid **41** to yield peptide-amino acid cross-linked structure **48**.*

The modified dipeptide was fully characterised by NMR spectroscopy. The characteristic peaks associated with the olefinated alkene were present confirming that the olefination had taken place. A parent ion peak in the HRMS (ESI) [M+H+] of 663.3068 provided further confirmation that the reaction had been successful. This represents an interesting result as there is an implication that peptide-amino acid and even peptide-peptide cross-linking can be achieved using the developed methodology.

2.5 Conclusion

In conclusion, it has been determined that attempting to promote reactivity does not lead to meaningful residue selectivity. For high selectivity, suppression of the reactivity of a specific residue is required. This has been successfully carried out by directing group manipulation and has been proven to be an effective method. In peptides containing phenylalanine and tryptophan residues, suppression can be achieved by the *N*-methylation of phenylalanine residues or the installation of a TIPS protecting group on the indole nitrogen of tryptophan.

Furthermore, the cross-linking of peptide and amino acids has also been successfully carried out between a tryptophan containing peptide **47** and the styrene derivative **41** to generate modified peptide **48** in good yield. This result indicates that the developed olefination reaction is a plausible route to complex post-synthetically modified peptides.



Scheme 62 – Planned general olefination protocol to generate cyclic peptides.

In the next stage of the study, the synthesis of cyclic peptides using the protocol developed to olefinate phenylalanine and tryptophan was planned as in **Scheme 62** above. However, publications around this research area somewhat diminished the novelty of the idea.[116,125] As a result, a different research path was undertaken which would see the development of a method to install a clickable SuFEx hub onto peptides which could be used for further diversification.

Additional work not captured in this chapter has been published here.[114]

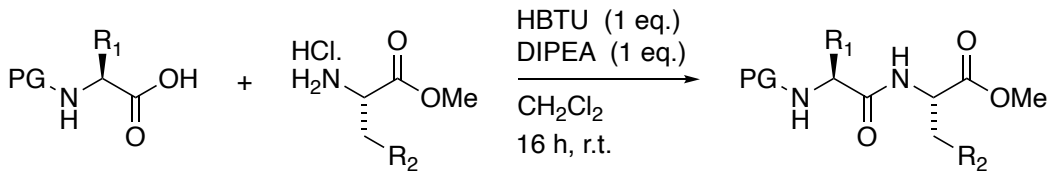
2.6 Experimental

2.6.1 General Experimental information

All reagents and solvents were purchased from Alfa Aesar, Fischer Scientific, Fluorochem or Merck and used as provided. Chemical manipulations were carried out in oven-dried glassware and in an atmosphere of air unless otherwise stated. Flash column chromatography was performed manually on silica gel (Fluorochem silica gel 60 A particle size 40-63 μm) or on a Biotage® Isolera One auto column using Biotage® Sfar silica Duo pre-loaded flash columns. Thin layer chromatography was carried out on glass-backed silica gel plates (2.5 x 7.5 cm; Merck, TLC silica gel 60 Å). Compounds were visualised on TLC plates by exposure to UV light (254 nm). NMR spectra were recorded on a JEOL ECX or ECZ 400 Spectrometer at 298K. Chemical shifts are reported in parts per million and coupling constants are reported in Hz. For some compounds, it was necessary to use ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC 2D NMR experiments in order to aid assignment. FTIR spectra were recorded on an Agilent Cary 630 FTIR spectrometer and wavenumbers are reported to the nearest whole number. Melting points of compounds were obtained using a Stuart SMP10 melting point apparatus. High-resolution mass spectrometry was obtained from the EPSRC UK National Mass Spectrometry Facility at Swansea University on an LTQ Orbitrap XL 1, using positive electrospray ionisation (ESI+).

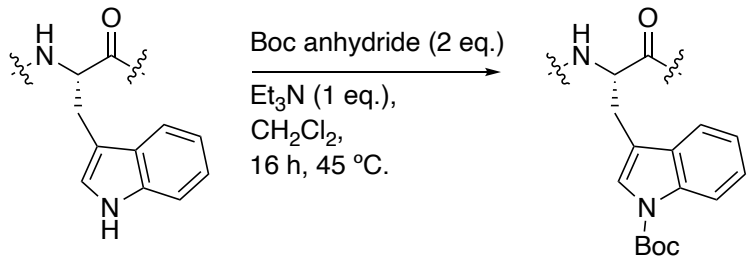
2.6.2 General Experimental Procedures

2.6.2.1 General procedure for the solution-phase synthesis of dipeptides



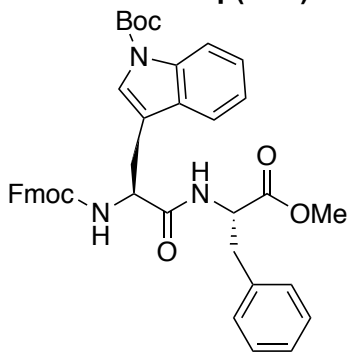
The L-amino ester hydrochloride salt (2.50 mmol) and K_2CO_3 (3.60 mmol) were dissolved in distilled water (30 mL) and stirred at room temperature for 20 min. The free amino ester was then extracted into Et_2O (3 x 20 mL) before the organic layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was dissolved in CH_2Cl_2 (30 mL) and treated with *N*-protected amino acid (1.0 mmol), HBTU (1.00 mmol) and DIPEA (1.00 mmol) before stirring for 12 h. The reaction mixture was then quenched with CH_2Cl_2 (30 mL), then washed with 1M HCl (25 mL), sat. $NaHCO_3$ (3 x 25 mL) and distilled water (25 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was then recrystallised from CH_2Cl_2 / hexanes.

2.6.2.2 General procedure for the Boc protection of Trp residues



The tryptophan containing peptide (0.75 mmol) was dissolved in CH_2Cl_2 (15 mL) and treated with Et_3N (0.105 mL, 0.75 mmol). A solution of Boc anhydride (0.327 g, 1.50 mmol) in CH_2Cl_2 (5 mL) was added dropwise to the peptide solution. The reaction mixture was then stirred at 45 ºC for 16 h. The solution was allowed to cool, before the volatiles were removed *in vacuo*. The crude compound was purified by flash column chromatography and the resulting residue was recrystallised from CH_2Cl_2 / hexanes to yield the Boc-protected peptide.

2.6.3 Fmoc-Trp(Boc)-Phe-OMe (23)



The peptide Fmoc-Trp(Boc)-Phe-OMe **23** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.543 g, 2.50 mmol) and Fmoc-Trp(Boc)-OH **21** (0.527 g, 1.00 mmol), using the procedure in **Section 2.6.2.1**. The crude compound was purified by flash column chromatography (25% EtOAc / pet ether) then recrystallised from CH_2Cl_2 / hexanes to afford **23** as a white solid (0.421 g, 61%); m.p. 100-103 °C, R_f = 0.19 (25% EtOAc/pet. ether).

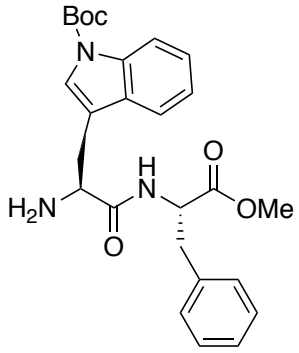
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 2.90-3.15 (3H, m, Trp-C*H*H/Phe-C*H*$_2$), 3.26 (1H, dd, *J* = 14.5, 4.1, Trp-CH*H*), 3.61 (3H, s, ester-C*H*$_3$), 4.21 (1H, t, *J* = 6.8, Fmoc-C*H*), 4.31-4.44 (2H, m, Fmoc-C*H*$_2$), 4.45-4.54 (1H, m, Trp-α-C*H*), 4.68-4.75 (1H, m, Phe-α-C*H*), 5.49 (1H, br d, *J* = 7.0, Trp-N*H*), 6.10 (1H, d, *J* = 7.4, Phe-N*H*), 6.86 (2H, br d, *J* = 7.0, Ar-*H*), 7.07-7.17 (3H, m, Ar-*H*), 7.23-7.35 (4H, m, Ar-*H*), 7.37-7.44 (2H, m, Ar-*H*), 7.47 (1H, s, Ar-*H*), 7.51-7.56 (2H, m, Ar-*H*), 7.63 (1H, d, *J* = 7.3, Ar-*H*), 7.77 (2H, d, *J* = 7.6, Ar-*H*), 8.14 (1H, br d, *J* = 7.1, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1 (Boc-(CH_3)$_3$), 28.3 (Trp-CH_2), 37.8 (Phe-CH_2), 47.0 (Fmoc-CH), 52.3 (ester-CH_3), 53.3 (Phe-α-CH), 54.9 (Trp-α-CH), 67.3 (Fmoc-CH_2), 83.7 (Boc-C), 115.1 (Ar C), 115.3 (Ar C), 119.0 (Ar C), 120.0 (Ar C), 122.8 (Ar C), 124.6 (Ar C), 124.7 (Ar C), 125.1 (Ar C), 127.1 (Ar C), 127.1 (Ar C), 127.7 (Ar C), 128.5 (Ar C), 129.0 (Ar C), 130.1 (Ar C), 135.3 (Ar C), 135.5 (Ar C), 141.3 (Ar C), 143,7 (Ar C), 149.4 (C=O), 155.8 (C=O), 170.2 (C=O), 171.0 (C=O).

IR U_{max} /cm^{-1} (solid) 3302 w (N-H), 3063 w (C-H), 2930 (C-H), 1727 m (ester C=O), 1654 s (amide C=O), 1533 m (C=C), 1366 s (C-O), 1248 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{41}H_{42}N_3O_7$: 688.3023, found: 688.3006.

2.6.4 H-Trp(Boc)-Phe-OMe (25)



The protected peptide Fmoc-Trp(Boc)-Phe-OMe **23** (0.421 g, 0.612 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with tris(2-aminoethyl)amine **24** (0.914 mL, 6.120 mmol). The reaction mixture was stirred for 2 h at room temperature before being quenched with H_2O (20 mL). The organic layer was then washed with 1 M HCl (20 mL), sat. $NaHCO_3$ (20 mL) and H_2O (20 mL), dried ($MsSO_4$) and concentrated *in vacuo* to yield an off-white solid. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes yielded peptide **25** as a white solid (0.223 g, 78%); m.p. 81-83 °C, R_f = 0.22 (EtOAc).

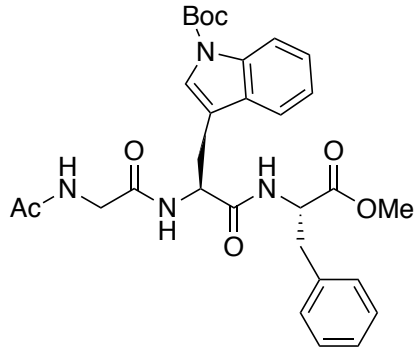
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (2H, br s, N*H*$_2$), 1.65 (9H, s, Boc-(C*H*$_3$)$_3$), 2.76 (1H, dd, *J* = 14.4, 8.9, Trp-C*H*H), 3.04 (2H, d, *J* = 6.1, Phe-C*H*$_2$), 3.22 (1H, dd, *J* = 14.4, 3.7, Trp-CH*H*), 3.66-3.73 (4H, m, ester-C*H*$_3$/Trp-α-C*H*), 4.88 (1H, dt, *J* = 6.2, 6.1, Phe-α-C*H*), 6.94-6.99 (2H, m, Ar-*H*), 7.17-7.28 (4H, m Ar-*H*), 7.33 (1H, t, *J* = Ar-*H*), 7.46 (1H, s, Ar-*H*), 7.61 (1H, d, *J* = 7.7, Ar-*H*), 7.77 (1H, d, *J*= 8.2, Phe-N*H*), 8.13 (1H, br d, *J* = 8.2, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1 (Boc-(CH_3)$_3$), 30.3 (Trp-CH_2), 37.9 (Phe-CH_2), 52.2 (Phe-α-CH), 52.6 (Trp-α-CH), 54.6 (ester-CH_3), 83.6 (Boc-C), 115.2 (Ar C), 116.4 (Ar C), 119.2 (Ar C), 122.6 (Ar C), 124.1 (Ar C), 124.6 (Ar C), 126.9 (Ar C), 128.3 (Ar C), 129.1 (Ar C), 130.2 (Ar C), 135.5 (Ar C), 135.8 (Ar C), 149.5 (C=O), 171.8 (C=O), 173.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3353 w (N-H), 3029 w (C-H), 2930 w (C-H), 1728 s (ester C=O), 1664 s (amide C=O), 1496 (C=C), 1252 (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{26}H_{32}N_3O_5$: 466.2342, found: 466.2341.

2.6.5 Ac-Gly-Trp(Boc)-Phe-OMe (20)



The peptide Ac-Gly-Trp(Boc)-Phe-OMe **20** was synthesised from H-Trp(Boc)-Phe-OMe **25** (0.365 g, 0.784 mmol) and *N*-acetyl glycine **26** (0.037 g, 0.314 mmol), using the procedure outlined in **Section 2.6.2.1**. The crude compound was purified by flash column chromatography (EtOAc) before recrystallisation from CH_2Cl_2 / hexanes to afford peptide **20** as a white solid (0.134 g, 76%); m.p. 186-188 °C, R_f = 0.19 (EtOAc).

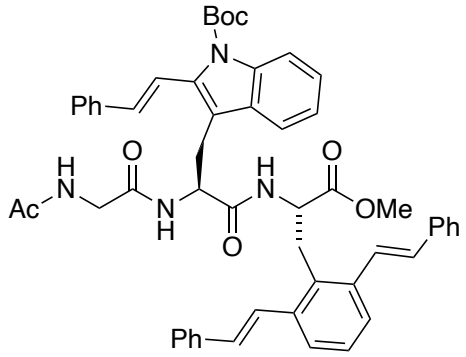
^{1}H NMR (400 MHz, CD_3CN) δ 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.86 (3H, s, acetyl-C*H*$_3$), 2.90-3.01 (2H, m, Phe-C*H*H / Trp-C*H*H), 3.05 (1H, dd, *J* = 13.8, 5.8, Trp-CH*H*), 3.14 (1H, dd, *J* = 14.9, 5.5, Phe-CH*H*), 3.61 (3H, s, ester-C*H*$_3$), 3.65 (2H, dd, *J* = 5.6, 3.5, Gly-C*H*$_2$), 4.54-4.65 – 2H, m, Phe-α-C*H* / Trp-α-C*H*), 6.70 (1H, br t, *J* = 5.3, Gly-N*H*), 6.87 (1H, d, *J* = 8.0, Phe-N*H*), 7.03 (1H, d, *J* = 7.6, Trp-N*H*), 7.10-7.17 (2H, m, Ar-*H*), 7.20-7.35 (5H, m, Ar-*H*), 7.47 (1H, s, Ar-*H*), 7.59 (1H, d, *J* = 7.6, Ar-*H*), 8.09 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C NMR (100 MHz, D_6-DMSO) δ 22.4 (acetyl-CH_3), 27.4 (Phe-CH_2), 27.7 (Boc-$(CH_3)_3$), 36.6 (Trp-CH_2), 41.9 (Gly-CH_2), 51.8 (ester-CH_3), 52.1 (Phe-α-CH), 53.7 (Trp-α-CH), 83.5 (Boc-C), 114.6 (Ar C), 116.2 (Ar C), 119.4 (Ar C), 122.4 (Ar C), 124.1 (Ar C), 124.3 (Ar C), 126.6 (Ar C), 128.3 (Ar C), 129.1 (Ar C), 130.3 (Ar C), 134.6 (Ar C), 137.0 (Ar C), 149.1 (C=O), 168.8 (C=O), 169.5 (C=O), 171.1 (C=O), 171.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3302 m (N-H), 3066 w (C-H), 2982 w (C-H), 1731 s (ester C=O), 1634 s (amide C=O), 1519 s (C=C), 1209 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{30}H_{37}N_4O_7$: 565.2662, found: 565.2673.

2.6.6 Modified peptide (27)



The peptide Ac-Gly-Trp(Boc)-Phe-OMe **20** (0.134 g, 0.237 mmol), $Pd(OAc)_2$ (5 mg, 0.024 mmol), AgOAc (0.100 g, 0.599 mmol) and styrene **39** (0.109 mL, 0.952 mmol) were stirred in toluene (3 mL) at 100 °C for 6 h. The reaction mixture was then allowed to cool to room temperature, filtered through a plug of Celite®, and concentrated to dryness. Purification by flash column chromatography (EtOAc), followed by recrystallisation from CH_2Cl_2 / hexanes, gave **27** as a yellow solid (0.005 g, 2%); m.p. 159-161 °C, R_f = 0.43 (EtOAc).

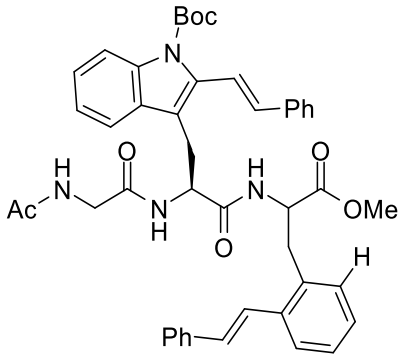
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (9H, s, Boc-(C*H*$_3$)$_3$), 1.98, (3H, s, acetyl-C*H*$_3$), 3.14 (1H, dd, *J* = 13.8, 9.8, Phe-C*H*H), 3.25 (3H, s, ester-CH_3), 3.26-3.40 (3H, m, Phe-CH*H* / Trp-C*H*$_2$), 3.64 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.71 (1H, dd, *J* = 16.6, 5.0, Gly-CH*H*), 4.54-4.62 (1H, m, Trp-α-C*H*), 4.63-4.70 (1H, m, Phe-α-C*H*), 5.90 (1H, t, *J* = 5.9, Gly-N*H*), 5.96 (1H, d, *J* = 7.4, Trp-N*H*), 6.54 (1H, d, *J* = 6.6, Phe-N*H*), 6.74 (1H, d, *J* = 16.7, Trp-alkene-C*H*), 6.93 (2H, d, *J* = 16.0, Phe-alkene-C*H*), 7.07-715 (1H, m, Ar-*H*), 7.20-7.33 (10H, m, Ar-*H* / Trp-alkene-C*H*), 7.34-7.42 (6H, m, Ar-*H* / Phe-alkene-C*H*), 7.47-7.60 (9H, m, Ar-*H*), 8.07 (1H, d, *J* = 8.1, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 23.0 (acetyl-CH_3), 28.2 (Boc-$(CH_3)_3$), 29.7 (Phe-CH_2), 31.7 (Trp-CH_2), 42.8 (Gly-CH_2), 52.5 (ester-CH_3), 53.0 (Phe-α-CH), 53.5 (Trp-α-CH), 83.9 (Boc-C), 114.6 (Ar C), 115.5 (Ar C), 118.7 (Ar C), 119.6 (Trp-alkene-C), 123.1 (Ar C), 124.8 (Ar C), 125.5 (Ar C), 126.1 (Ar C), 126.6 (Ar C), 126.8 (Ar C), 127.5 (Ar C), 127.9 (Ar C), 128.5 (Ar C), 128.7 (Ar C), 128.8 (Phe-alkene-C), 129.6 (Ar C), 131.3 (Phe-alkene-C), 131.4 (Ar C), 132.0 (Trp-alkene-C), 135.9 (Ar C), 136.2 (Ar C), 136.8 (Ar C), 137.3 (Ar C), 137.7 (Ar C), 150.4 (C=O), 168.0 (C=O), 169.6 (C=O), 170.2 (C=O), 170.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3304 m (N-H), 3058 w (C-H), 2980 w (C-H), 1731 s (ester C=O), 1634 s (amide C=O), 1519 s (C=C), 1215 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{54}H_{55}N_4O_7$: 871.4071, found: 871.4060.

2.6.7 Modified peptide (28)



The peptide Ac-Gly-Trp(Boc)-Phe-OMe **20** (0.134 g, 0.237 mmol), $Pd(OAc)_2$ (5 mg, 0.024 mmol), AgOAc (0.100 g, 0.599 mmol) and styrene **39** (0.109 mL, 0.952 mmol) were stirred in toluene (3 mL) at 100 °C for 6 h. The reaction mixture was then allowed to cool to room temperature, filtered through a plug of Celite®, and concentrated to dryness. Purification by flash column chromatography (EtOAc), followed by recrystallisation from CH_2Cl_2 / hexanes, gave **28** as a yellow solid (0.029 g, 16%); m.p. 119-122 °C, R_f = 0.30 (EtOAc).

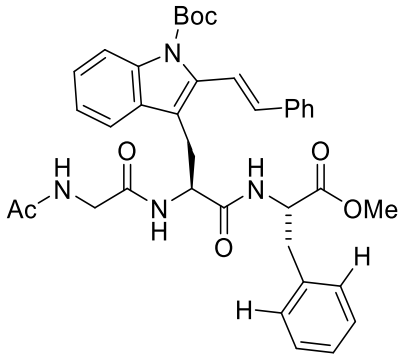
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (9H, s, Boc-(C*H*$_3$)$_3$), 1.96 (3H, s, acetyl-C*H*$_3$), 3.05 (1H, dd, *J* = 14.2, 5.3 Trp-C*H*H), 3.16 (1H, dd, *J* = 14.1, 9.4, Phe-C*H*H), 3.24 (1H, dd, *J* = 14.1, 7.0, Trp-CH*H*), 3.31 (1H, dd, *J* = 14.1, 5.8, Phe-CH*H*), 3.43 (3H, s, ester-C*H*$_3$), 3.60 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.69 (1H, dd, *J* = 16.8, 5.0, Gly-CH*H*), 4.50-4.58 (1H, m, Trp-α-C*H*), 4.62 (1H, m, Phe-α-C*H*), 5.90-5.99 (2H, m, Gly-N*H* / Trp-N*H*), 6.60 (1H, d, *J* = 7.3, Phe-N*H*), 6.76 (1H, d, *J* = 16.6, Trp-alkene-C*H*), 6.83 (1H, d, *J* = 7.4, Ar-*H*), 6.94 (1H, d, *J* = 16.0, Phe-alkene-C*H*), 7.06 (1H, t, *J* = 7.3, Ar-*H*), 7.10-7.22 (2H, m, Ar-*H*), 7.23-7.30 (3H, m, Ar-*H*), 7.31-7.41 (6H, m, Ar-*H* / Phe-alkene-C*H* / Trp-alkene-C*H*), 7.50-7.60 (6H, m, Ar-*H*), 8.09 (1H, d, *J* = 8.0, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 28.2 (Boc-$(CH_3)_3$), 28.5 (Phe-CH_2), 35.1 (Trp-CH_2), 42.7 (Gly-CH_2), 52.3 (ester-CH_3), 53.4 (Phe-CH), 53.5 (Trp-CH), 83.9 (Boc-C), 114.6 (Ar C), 115.5 (Ar C), 118.7 (Ar C), 119.7 (Trp-alkene-C), 123.0 (Ar C), 124.8 (Ar C), 125.3 (Ar C), 125.7 (Ar C), 126.6 (Ar C) 126.7 (Ar C), 127.5 (Ar C), 127.5 (Ar C), 127.8 (Ar C), 127.9 (Ar C), 128.7 (Ar C), 128.8 (Phe-alkene-C), 129.6 (Ar C), 130.2 (Phe-alkene-C), 130.4 (Ar C), 132.1 (Trp-alkene-C), 133.4 (Ar C), 135.9 (Ar C), 136.1 (Ar C), 136.6 (Ar C), 136.8 (Ar C), 137.4 (Ar C), 150.4 (C=O), 168.1 (C=O), 169.8 (C=O), 170.2 (C=O), 170.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3273 w (N-H), 3056 w (C-H), 2976 w (C-H), 1728 s (ester C=O), 1634 s (amide C=O), 1523 s (C=C), 1216 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{46}H_{49}N_4O_7$: 769.3601, found: 769.3625.

2.6.8 Modified peptide (29)



The peptide Ac-Gly-Trp(Boc)-Phe-OMe **20** (0.134 g, 0.237 mmol), $Pd(OAc)_2$ (5 mg, 0.024 mmol), AgOAc (0.100 g, 0.599 mmol) and styrene **39** (0.109 mL, 0.952 mmol) were stirred in toluene (3 mL) at 100 °C for 6 h. The reaction mixture was then allowed to cool to room temperature, filtered through a plug of Celite®, and concentrated to dryness. Purification by flash column chromatography (EtOAc), followed by recrystallisation from CH_2Cl_2 / hexanes, gave **29** as an off-white solid (0.040 g, 25%); m.p. 104-106 °C, R_f = 0.25 (EtOAc).

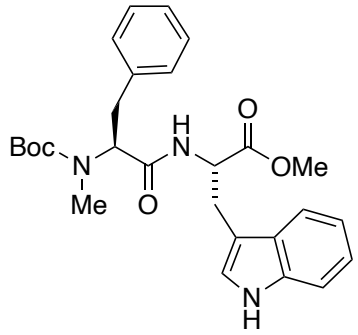
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 2.89 (1H, dd, *J* = 13.8, 5.9, Trp-C*H*H), 2.98 (1H, dd, *J* = 13.6, 6.0, Trp-CH*H*), 3.25 (1H, dd, *J* = 14.2, 8.9, Phe-C*H*H), 3.33 (1H, dd, *J* = 14.0, 6.3, Phe-CH*H*), 3.50 (3H, s, ester-C*H*$_3$), 3.84-3.88 (2H, m, Gly-C*H*$_2$), 4.55-4.62 (1H, m, Trp-α-C*H*), 4.71-4.78 (1H, m, Phe-α-C*H*), 6.06-6.16 (2H, m, Trp-N*H* / Gly-N*H*), 6.74 (1H, d, *J* = 7.6, Phe-N*H*), 6.78 (1H, d, *J* = 16.7, Alkene-C*H*), 6.89-6.93 (1H, m, Ar-*H*), 7.13-7.17 (2H, m, Ar-*H*), 7.19-7.32 (6H, m, Ar-*H* / Alkene-C*H*), 7.33-7.40 (3H, m, Ar-*H*), 7.51-7.58 (2H, m, Ar-*H*), 7.62 (1H, d, *J* = 7.6, Ar-*H*), 8.10 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 28.2 (Boc-$(CH_3)_3$), 28.8 (Phe-CH_2), 38.0 (Trp-CH_2), 43.0 (Gly-CH_2), 52.2 (ester-CH_3), 53.4 (Phe-CH), 53.5 (Trp-CH), 84.0 (Boc-C), 114.6 (Ar C), 115.6 (Ar C), 118.7 (Ar C), 119.8 (Alkene-C), 123.0 (Ar C), 124.8 (Ar C), 126.6 (Ar C), 127.0 (Ar C), 128.0 (Ar C), 128.4 (Ar C), 128.7 (Ar C), 129.1 (Ar C), 129.6 (Ar C), 132.2 (Alkene-C), 135.4 (Ar C), 135.9 (Ar C), 136.2 (Ar C), 136.8 (Ar C), 150.4 (C=O), 168.4 (C=O), 169.8 (C=O), 170.4 (C=O), 170.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3278 m (N-H), 3060 w (C-H), 2968 w (C-H), 1725 s (ester C=O), 1636 s (amide C=O), 1522 s (C=C), 1210 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{38}H_{43}N_4O_7$: 667.3132, found: 667.3127.

2.6.9 (N-Boc)-(N-Me)-Phe-Trp-OMe (35)



Protected dipeptide **35** was synthesised from tryptophan methyl ester hydrochloride **34** (0.637 g, 2.50 mmol) and (N-Boc)-(N-Me)-Phenylalanine **38** (0.279 g, 1.00 mmol), according to the procedure outlined in **Section 2.6.2.1**. Purification by flash column chromatography (80% EtOAc/pet ether) followed by recrystallisation from CH_2Cl_2 / hexanes gave **35** as a cream solid (0.353 g, 74%); m.p. 71-73 °C, R_f = 0.24 (80% EtOAc/pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ (Major Rotamer) 1.20 (9H, s, Boc-(C*H*$_3$)$_3$), 2.41 (3H, s, N-C*H*$_3$), 2.75-2.85 (1H, m, Phe-C*H*H), 3.19-3.40 (3H, m, Phe-CH*H* / Trp-C*H*$_2$), 3.68 (3H, s, ester-C*H*$_3$), 4.69 (1H, br d, *J* = 7.8, Phe-α-C*H*), 4.80-4.90 (1H, m, Trp-α-C*H*), 6.40 (1H, d, *J* = 6.5, Trp-N*H*), 6.88 (1H, s, Ar-*H*), 7.03-7.34 (8H, m, Ar-*H*), 7.52 (1H, d, *J* = 7.8, Ar-*H*), 8.81 (1H, br s, Ar-*H*).

^{1}H NMR (400 MHz, $CDCl_3$) δ (Minor Rotamer) 1.30 (9H, s, Boc-(C*H*$_3$)$_3$), 2.43 (3H, s, N-C*H*$_3$), 2.86-2.97 (1H, m, Phe-C*H*H), 3.19-3.40 (3H, m, Phe-CH*H* / Trp-C*H*$_2$), 3.60 (3H, s, ester-C*H*$_3$), 4.80-4.90 (2H, m, Trp-α-C*H* / Phe-α-C*H*), 6.65 (1H, d, *J* = 6.2, Trp-N*H*), 6.92 (1H, s, Ar-*H*), 7.03-7.34 (8H, m, Ar-*H*), 7.52 (1H, d, *J* = 7.8, Ar-*H*), 8.77 (1H, br s, Ar-*H*).

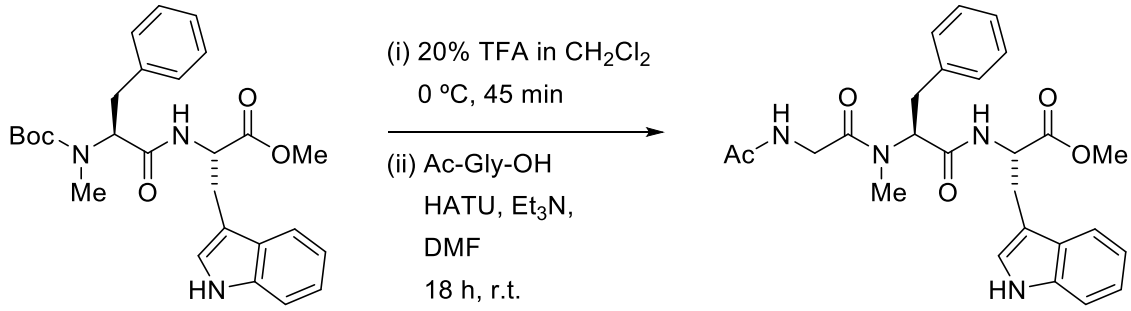
^{13}C NMR (100 MHz, $CDCl_3$) (Major Rotamer) δ 27.3 (Trp-CH_2), 27.8 (Boc-$(CH_3)_3$), 30.8 (N-CH_3), 33.6 (Phe-CH_2), 52.3 (ester-CH_3), 52.6 (Trp-CH), 61.2 (Phe-CH), 80.7 (Boc-C), 109.0 (Ar C), 111.3 (Ar C), 118.0 (Ar C), 119.5 (Ar C), 122.0 (Ar C), 122.7 (Ar C), 126.3 (Ar C), 127.0 (Ar C), 128.3 (Ar C), 128.7 (Ar C), 136.1 (Ar C), 137.6 (Ar C), 154.9 (C=O), 170.0 (C=O), 172.1 (C=O).

^{13}C NMR (100 MHz, $CDCl_3$) (Minor Rotamer) δ 27.3 (Trp-CH_2), 27.9 (Boc-$(CH_3)_3$), 30.2 (N-CH_3), 33.8 (Phe-CH_2), 52.2 (ester-CH_3), 53.0 (Trp-CH), 59.6 (Phe-CH), 80.2 (Boc-C), 109.3 (Ar C), 111.2 (Ar C), 118.1 (Ar C), 119.3 (Ar C), 121.9 (Ar C), 122.7 (Ar C), 126.2 (Ar C), 127.3 (Ar C), 128.2 (Ar C), 128.7 (Ar C), 136.0 (Ar C), 137.2 (Ar C), 156.1 (C=O), 170.5 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3316 w (N-H), 3028 w (C-H), 2954 w (C-H), 1739 m (ester C=O), 1664 s (amide C=O), 1507 m (C=C), 1211 m (C-O).

HRMS (ESI) [$M+H^+$] *m/z* calcd. For $C_{27}H_{34}N_3O_5$: 480.2498, found: 480.2490.

2.6.10 Ac-Gly-(N-Me)-Phe-Trp-OMe (36)



The protected peptide **35** (0.320 g, 0.667 mmol) was stirred in a solution of 20% TFA in CH_2Cl_2 (10 mL) at 0 ºC. The volatiles were removed *in vacuo* and the resulting amine was dissolved in DMF (5 mL). The reaction mixture was then treated with N-acetyl glycine **26** (0.078 g, 0.667 mmol), Et_3N (0.186 mL, 1.334 mmol) and HATU (0.304 g 0.800 mmol) and left to stir at room temperature for 18 h. The reaction was quenched with sat $NaHCO_3$ (5 mL), diluted with H_2O (50 mL) and extracted with EtOAc (2 x 50 mL). The combined organics were dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude, yellow oil was purified by flash column chromatography (EtOAc) and recrystallised from CH_2Cl_2 / hexanes to yield peptide **36** as an off-white solid. (0.198 g, 62%), 92-96 °C, R_f = 0.20 (EtOAc).

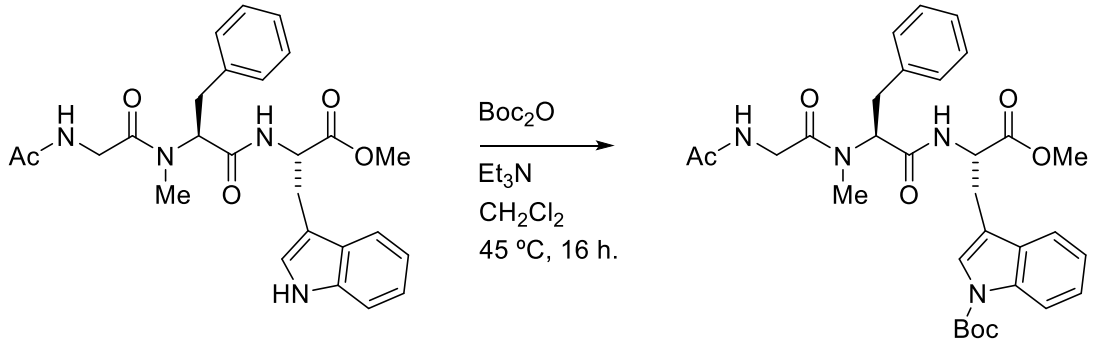
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (3H, s, acetyl-C*H*$_3$), 2.33 (3H, s, N-C*H*$_3$), 2.82-2.87 (1H, m Phe-C*H*H), 3.11 (1H, dd, *J* = 14.6, 8.5, Trp-C*H*H), 3.20 (1H, dd, *J* = 14.6, 6.2, Phe-CH*H*), 3.37 (1H, dd, *J* = 14.6, 5.0, Trp-CH*H*), 3.44 (1H, dd, *J* = 17.8, 3.7, Gly-C*H*H), 3.71 (3H, s, ester-C*H*$_3$), 3.78 (1H, dd, *J* = 17.4, 5.5, Gly-CH*H*), 4.84-4.92 (1H, m, Trp-α-C*H*), 5.29-5.37 (1H, m, Phe-α-C*H*), 6.45 (1H, t, *J* = 4.6, Gly-N*H*), 6.57 (1H, d, *J* = 7.8, Trp-N*H*), 6.87 (1H, d, *J* = 2.3, Ar-*H*), 7.02-7.10 (3H, m, Ar-*H*), 7.12-7.22 (4H, m, Ar-*H*), 7.34 (1H, d, *J* = 8.2, Ar-*H*), 7.51 (1H, d, *J* = 7.8, Ar-*H*), 9.03 (1H, s, Ar-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 27.2 (Trp-CH_2), 29.5 (N-CH_3), 33.4 (Phe-CH_2), 40.9 (Gly-CH_2), 52.1 (Trp-CH), 52.4 (ester-CH_3), 57.2 (Phe-CH), 109.3 (Ar C), 111.3 (Ar C), 118.2 (Ar C), 119.4 (Ar C), 122.1 (Ar C), 123.1 (Ar C), 126.6 (Ar C), 127.0 (Ar C), 128.4 (Ar C), 128.5 (Ar C), 136.0 (Ar C), 136.5 (Ar C), 169.2 (C=O), 169.2 (C=O), 170.5 (C=O), 172.2 (C=O).

IR U_{max} /cm^{-1} (solid) 3299 m (N-H), 3058 w (C-H), 2954 w (C-H), 1735 m (ester C=O), 1636 s (amide C=O), 1522 m (C=C), 1210 m (C-O)

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{26}H_{31}N_4O_5$: 479.2294, found: 479.2300.

2.6.11 Ac-Gly-(N-Me)-Phe-Trp(Boc)-OMe (37)



Peptide **36** (0.198 g, 0.414 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated with Et_3N (0.056 mL, 0.414 mmol). A solution of Boc anhydride (0.181 g, 0.828 mmol) in CH_2Cl_2 (5 mL) was added dropwise to the peptide mixture and heated under reflux for 16 h. The solution was allowed to cool before the volatiles were removed *in vacuo***.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes yielded **37** as a white solid (0.129 g, 54%); m.p. 63-66 °C, R_f = 0.20 (EtOAc).

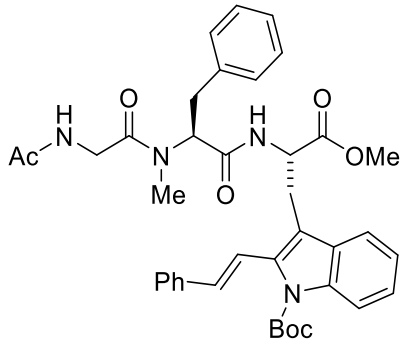
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (9H, s, Boc-(C*H*$_3$)$_3$), 2.03 (3H, s, acetyl-C*H*$_3$), 2.68 (3H, s, N-C*H*$_3$), 2.93 (1H, dd, *J* = 14.6, 9.2, Phe-C*H*H), 3.17 (1H, dd, *J* = 15.1, 6.9, Trp-C*H*H), 3.22-3.31 (2H, m, Phe-CH*H* / Trp-CH*H*), 3.58-3.70 (4H, m, ester-C*H*$_3$ / Gly-C*H*H), 3.83 (1H, dd, *J* = 18.5, 4.4, Gly-CH*H*), 4.84-4.91 (1H, m, Trp-α-C*H*), 5.24 (1H, dd, *J* = 9.2, 6.9, Phe-α-C*H*), 6.50 (1H, br s, Gly-N*H*), 6.58 (1H, d, *J* = 7.8, Trp-N*H*), 7.08-7.27 (6H, m, Ar-*H*), 7.31 (1H, t, *J* = 7.3, Ar-*H*), 7.40 (1H, s, Ar-*H*), 7.45 (1H, d, *J* = 7.8, Ar-*H*), 8.04 (1H, br d, *J* = 7.8, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 27.0 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 30.2 (N-CH_3), 33.5 (Phe-CH_2), 41.4 (Gly-CH_2), 52.1 (Trp-CH), 52.5 (ester-CH_3), 57.9 (Phe-CH), 84.1 (Boc-C), 114.6 (Ar C), 115.3 (Ar C), 118.6 (Ar C), 122.5 (Ar C), 124.3 (Ar C), 124.6 (Ar C), 126.8 (Ar C), 128.6 (Ar C), 128.7 (Ar C) 128.9 (Ar C), 130.4 (Ar C), 136.4 (Ar C), 149.7 (C=O), 169.2 (C=O), 169.4 (C=O), 170.0 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3319 w (N-H), 3060 w (C-H), 2953 m (C-H), 1730 s (ester C=O), 1638 s (amide C=O), 1522 s (C=C), 1254 (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{31}H_{39}N_4O_7$: 579.2819, found: 579.2816.

2.6.12 Modified peptide (40)



The peptide Ac-Gly-(N-Me)-Phe-Trp(Boc)-OMe **37** (0.129 g, 0.223 mmol), $Pd(OAc)_2$ (5 mg, 0.022 mmol), AgOAc (0.093 g, 0.558 mmol) and styrene **39** (0.103 mL, 0.892 mmol) were stirred in toluene (3 mL) at 100 °C for 2 h. The reaction mixture was then allowed to cool to room temperature, filtered through a plug of Celite®, and concentrated to dryness. Purification by flash column chromatography (EtOAc), followed by recrystallisation from CH_2Cl_2 / hexanes, gave **40** as a brown solid (0.084 g, 55%); m.p. 94-98 °C, R_f = 0.28 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 2.41 (3H, s, N-C*H*$_3$), 2.85 (1H, dd, *J* = 14.6, 9.8, Phe-C*H*H), 3.16 (1H, dd, *J* = 14.7, 6.3, Phe-CH*H*), 3.24 (1H, dd, *J* = 14.4, 8.6, Trp-C*H*H), 3.40 (1H, dd, *J* = 14.4, 6.4, Trp-CH*H*), 3.52 (1H, dd, *J* = 17.7, 3.4, Gly-C*H*H), 3.62 (3H, s, ester-C*H*$_3$), 3.67 (1H, dd, *J* = 17.9, 4.5, Gly-CH*H*), 4.93-5.01 (1H, m, Trp-α-C*H*), 5.23 (1H, dd, *J* = 9.6, 6.4, Phe-α-C*H*), 6.29 (1H, br s, Gly-N*H*), 6.45 (1H, d, *J* = 8.0, Trp-N*H*), 6.77 (1H, d, *J* = 16.7, alkene-C*H*), 7.00-7.10 (2H, m, Ar-*H*), 7.12-7.24 (4H, m, Ar-*H*), 7.27-7.41 (5H, m, Ar-*H* / alkene-C*H*), 7.51 (1H, d, *J* = 7.8, Ar-*H*), 7.56 (2H, d, *J* = 7.9, Ar-*H*), 8.11 (1H, d, *J* = 8.8, Ar-*H*).

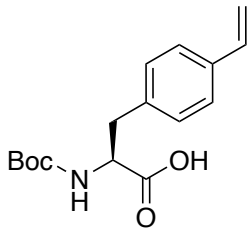
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 27.5 (Trp-CH_2), 28.3 (Boc-$(CH_3)_3$), 29.7 (N-CH_3), 33.5 (Phe-CH_2), 41.4 (Gly-CH_2), 52.0 (Trp-CH), 52.6 (ester-CH_3), 57.6 (Phe-CH), 84.3 (Boc-C), 114.5 (Ar C). 115.6 (Ar C), 118.5 (Ar C), 120.0 (alkene-C), 122.9 (Ar C), 124.9 (Ar C), 126.6 (Ar C), 126.8 (Ar C), 128.1 (Ar C), 128.6 (Ar C), 128.6 (Ar C), 128.8 (Ar C), 130.0 (Ar C), 132.2 (alkene-C), 135.7 (Ar C), 136.2 (Ar C), 136.4 (Ar C), 136.7 (Ar C), 150.4 (C=O), 169.2 (C=O), 169.2 (C=O), 169.8 (C=O), 172.0 (C=O).

IR U_{max} /cm^{-1} (solid) 3275 w (N-H), 3058 w (C-H), 2976 w (C-H), 1728 s (ester C=O), 1634 s (amide C=O), 1541 m (C=C), 1237 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. For $C_{39}H_{45}N_4O_7$: 681.3288, found: 681.3308.

2.6.14 Boc-Phe(4-vinyl)-OH (43)

Compound **43** was prepared using a previously reported procedure.[166]



The unnatural amino acid *N*-Boc-4-iodophenylalanine **42** (0.391 g, 1.00 mmol), potassium vinyl trifluoroborate **45** (0.161 g, 1.20 mmol), K_2CO_3 (0.691 g, 5.00 mmol) and $Pd(dppf)Cl_2$ (0.008 g, 0.01 mmol) were dissolved in H_2O / ^{i}PrOH (10 mL) and refluxed at 80 °C for 1 h. The reaction mixture was allowed to cool to room temperature before the organic solvent was removed *in vacuo*. The remaining aqueous solution was then acidified to pH 3 using 5N HCl (*ca.* 2.0 mL). The resulting aqueous solution was extracted using CH_2Cl_2 (4 x 25 mL) and the combined organics were dried ($MgSO_4$), before the solvent was removed *in vacuo*. Purification by flash column chromatography (CH_2Cl_2 / MeOH / AcOH, 97:1:2) yielded **43** as a brown oil (0.256 g, 88%); R_f = 0.30 (CH_2Cl_2 / MeOH / AcOH 97:1:2).

^{1}H NMR (400 MHz, D_6-DMSO) δ 1.31 (9H, s, Boc-C*H*$_3$), 2.81 (1H, dd, *J* = 13.7, 10.4, Phe-C*H*H), 2.99 (1H, dd, *J* = 13.7, 4.5, Phe-CH*H*), 4.02-4.11 (1H, m, Phe-α-C*H*), 5.21 (1H, d, *J* = 11.1, 0.6, alkene-CH*H*), 5.78 (1H, d, *J* = 17.7, 0.6, alkene-C*H*H), 6.69 (1H, dd, *J* = 17.7, 11.1, alkene-C*H*), 7.10 (1H, d, *J* = 8.5, N*H*), 7.22 (2H, d, *J* = 8.1, Ar-*H*), 7.38 (2H, d, *J* = 8.1, Ar-*H*).

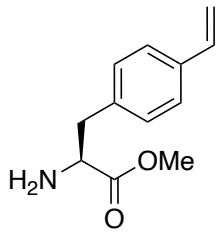
^{13}C NMR (100 MHz, D_6-DMSO) δ 28.2 (Boc-$(CH_3)_3$), 36.2 (Phe-CH_2), 55.2 (Phe-CH), 78.1 (Boc-C), 113.7 (alkene-CH_2), 126.0 (Ar C), 129.4 (Ar C), 135.3 (alkene-CH), 136.5 (4° Ar C), 137.9 (4° Ar C), 155.5 (C=O), 173.6 (C=O).

IR U_{max} /cm^{-1} (oil) 3442 m (N-H), 3027 w (C-H), 2958 w (C-H), 1729 s (C=O), 1534 s (C=C), 1217 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{16}H_{22}NO_4$: 292.1549, found: 292.1551.

The ^{1}H and ^{13}C NMR data match that previously reported.[166]

2.6.15 H-Phe(4-vinyl)-OMe (44)



The unnatural amino acid Boc-Phe(4-vinyl)-OH **43** (0.256 g, 0.88 mmol) was dissolved in ice cold MeOH (10 mL) and treated with the dropwise addition of thionyl chloride (0.290 mL, 4.00 mmol). The solution was allowed to warm to room temperature before refluxing for 4 h. The volatiles were removed *in vacuo*, and the residue was dissolved in toluene and concentrated to dryness three times. The resulting material was slurried in sat. aqueous $NaHCO_3$ solution and then extracted in CH_2Cl_2 (3 x 25 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to yield **44** as a yellow oil. (0.165 g, 91%); R_f = 0.25 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (2H, br s, N*H*$_2$), 2.85 (1H, dd, *J* = 13.5, 8.0, Phe-C*H*H), 3.07 (1H, dd, *J* = 13.5, 5.3, Phe-CH*H*), 3.69-3.74 (4H, m, ester-C*H*$_3$ / Phe-α-C*H*), 5.22 (1H, dd, *J* = 10.9, 0.9, alkene-C*H*H), 5.72 (1H, d, *J* = 17.6, 0.9, alkene-CH*H*), 6.68 (1H, dd, *J* = 17.6, 10.9, alkene-C*H*), 7.15 (2H, d, *J* = 8.2, Ar-*H*), 7.35 (2H, d, *J* = 8.2, Ar-*H*).

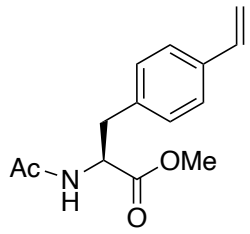
^{13}C NMR (100 MHz, $CDCl_3$) δ 40.6 (Phe-CH_2), 51.9 (ester-CH_3), 55.6 (Phe-α-CH), 113.5 (alkene-CH_2), 126.3 (Ar C), 129.3 (Ar C), 131.2 (Ar C), 136.1 (4° Ar C), 136.3 (alkene-CH), 136.7 (4° Ar C), 175.3 (C=O).

IR U_{max} /cm^{-1} (oil) 3377 m (N-H), 3030 w (C-H), 2793 w (C-H), 1735 s (C=O), 1511 s (C=C), 1172 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{14}H_{16}NO_2$: 206.1181, found: 206.1182.

The ^{1}H NMR data match that previously reported.[167]

2.6.16 Ac-Phe(4-vinyl)-OMe (41)



The unnatural amino ester H-Phe(4-vinyl)-OMe **44** (0.410 g, 2.00 mmol), acetic anhydride (0.945 mL, 10.00 mmol) and pyridine (0.805 mL, 10.00 mmol) were dissolved in CH_2Cl_2 (20 mL) and stirred at room temperature for 12 h. The volatiles were removed *in vacuo* before the resulting residue was re-dissolved in CH_2Cl_2 and washed with 1 M HCl (20 mL), distilled H_2O (20 mL), sat. aqueous $NaHCO_3$ (20 mL) and distilled H_2O (20 mL). The organic layer was then dried ($MgSO_4$) and concentrated to dryness. Purification of the crude residue by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes yielded **41** as a white solid (0.232 g, 47%); m.p. 123-126 °C, R_f = 0.45 (EtOAc).

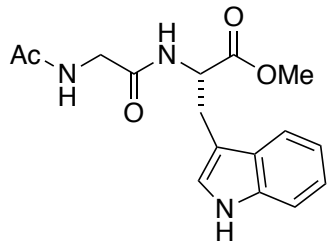
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (3H, s, acetyl-C*H*$_3$), 3.05 (1H, dd, *J* = 13.9, 6.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.9, 5.8, Phe-CH*H*), 3.72 (3H, s, ester-C*H*$_3$), 4.84-4.90 (1H, m, Phe-α-C*H*), 5.23 (1H, dd, *J* = 10.9, 0.8, alkene-C*H*H), 5.72 (1H, dd, *J* = 17.6, 0.8, alkene-CH*H*), 6.23 (1H, d, *J* = 7.8, Phe-N*H*), 6.67 (1H, dd, *J* = 17.6, 10.9, alkene-C*H*), 7.06 (2H, d, *J* = 8.0, Ar-*H*), 7.33 (2H, d, *J* = 8.0, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 37.4 (Phe-CH_2), 52.2 (ester-CH_3), 53.0 (Phe-α-CH), 113.7 (alkene-CH_2), 126.2 (Ar C), 129.3 (Ar C), 135.4 (4° Ar C), 136.2 (alkene-CH) 136.3 (4° Ar C), 169.6 (acetyl C=O), 172.0 (ester C=O).

IR U_{max} /cm^{-1} (solid) 3327 m (N-H), 3027 w (C-H), 2958 w (C-H), 1743 s (ester C=O), 1649 s (amide C=O), 1534 s (C=C), 1217 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{14}H_{18}NO_3$: 248.1287, found: 248.1286.

2.6.17 Ac-Gly-Trp-OMe (46)



Peptide **46** was synthesised from L-tryptophan methyl ester hydrochloride **34** (0.637 g, 2.50 mmol) and *N*-acetyl glycine **26** (0.117 g, 1.00 mmol) using the procedure outlined in **Section 2.6.2.1**. The crude compound was recrystallised from CH_2Cl_2 / hexanes to afford **46** as an off-white solid (0.251 g, 79%); m.p. 177-180 °C. R_f = 0.15 (EtOAc).

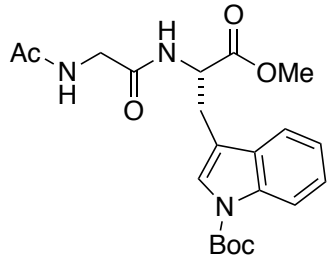
^{1}H NMR (400 MHz, CD_3OD) δ 1.93 (3H, s, acetyl-C*H*$_3$), 3.20 (1H, dd, *J* = 14.6, 7.1, Trp-C*H*H), 3.25-3.32 (1H, m, Trp-CH*H*), 3.64 (3H, s, ester-C*H*$_3$), 3.77-3.86 (2H, m, Gly-C*H*$_2$), 4.72-4.77 (1H, m, Trp-α-C*H*), 7.01 (1H, t, *J* = 7.8, Ar-*H*), 7.05-7.12 (2H, m, Ar-*H*), 7.32 (1H, d, *J* = 8.0, Ar-*H*), 7.49 (1H, d, *J* = 7.8, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 22.5 (acetyl-CH_3), 28.5 (Trp-CH_2), 43.5 (Gly-CH_2), 52.9 (ester-CH_3), 54.9 (Trp-α-CH), 110.5 (4° Ar C), 112.5 (Ar C), 119.2 (Ar C), 120.0 (Ar C), 122.6 (Ar C), 124.7 (Ar C), 128.8 (4° Ar C), 138.1 (4° Ar C), 171.6 (C=O), 173.9 (C=O), 173.9 (C=O).

IR u_{max} /cm^{-1} (solid) 3235 m (N-H), 3060 w (C-H), 2951 w (C-H), 1749 s (ester C=O), 1642 m (amide C=O), 1436 m (C-H).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{16}H_{20}N_3O_4$: 318.1454, found: 318.1456.

2.6.18 Ac-Gly-Trp(Boc)-OMe (47)



Peptide **47** was synthesised from Ac-Gly-(D)Trp-OMe **46** (0.317 g, 1.00 mmol) using the procedure outlined in **Section 2.6.2.2.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **47** as a white solid (0.113 g, 27%); m.p. 93-95 ºC; R_f = 0.20 (EtOAc).

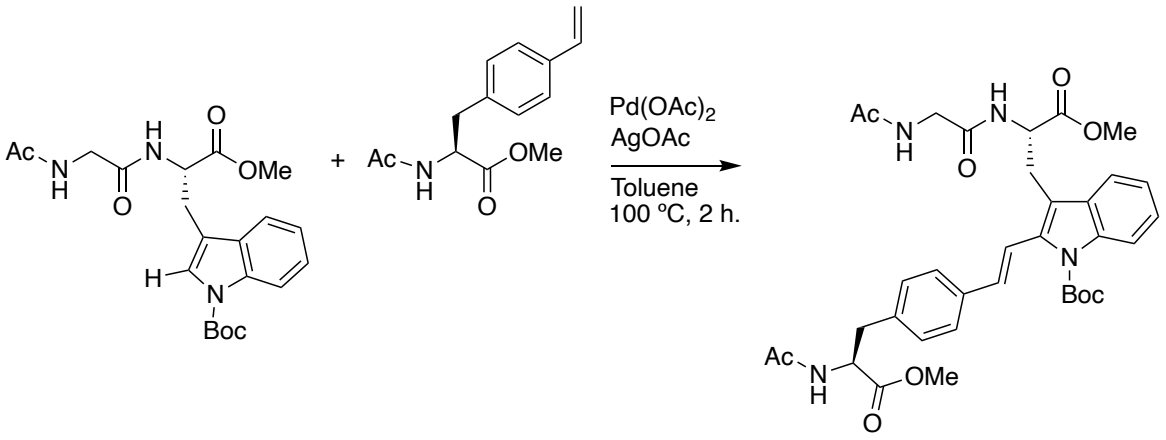
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66 (9H, s, Boc-(C*H*$_3$)$_3$), 1.96 (3H, s, acetyl-C*H*$_3$), 3.19 (1H, dd, *J* = 14.8, 6.1, Trp-C*H*H), 3.27 (1H, dd, *J* = 14.8, 5.7, Trp-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 3.87 (1H, dd, *J* = 16.7, 5.3, Gly-C*H*H), 3.91 (1H, dd, *J* = 16.7, 5.3, Gly-CH*H*), 4.89 (1H, m, Trp-α-C*H*), 6.44 (1H, t, *J* = 4.9, Gly-N*H*), 6.88 (1H, d, *J* = 7.8, Trp-N*H*), 7.23 (1H, t, *J* = 7.8, Ar-*H*), 7.30 (1H, t, *J* = 7.0, Ar-*H*), 7.41 (1H, s, Ar-*H*), 7.47 (1H, d, *J* = 7.8, Ar-*H*), 8.09 (1H, d, *J* = 7.0, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 27.2 (Trp-CH_2), 28.1 (Boc-$(CH_3)_3$), 43.1 (Gly-CH_2), 52.5 (ester-CH_3), 52.6 (Trp-α-CH), 83.8 (Boc-C), 114.7 (4° Ar C), 115.3 (Ar C), 118.6 (Ar C), 122.6 (Ar C), 124.2 (Ar C), 124.6 (Ar C), 130.3 (4° Ar C), 135.2 (4° Ar C), 149.5 (Boc C=O), 168.8 (C=O), 170.6 (C=O), 171.7 (C=O).

IR u_{max} /cm^{-1} (solid) 3273 m (N-H), 3058 w (C-H), 2933 w (C-H), 1740 s (ester C=O), 1658 m (amide C=O), 1428 m (C-H), 1246 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{21}H_{28}N_3O_6$: 418.1978, found: 418.1977.

2.6.19 Modified peptide (48)



The peptide Ac-Gly-Trp(Boc)-OMe **47** (0.030 g, 0.071 mmol), $Pd(OAc)_2$ (1 mg, 0.003 mmol, 10 mol%), AgOAc (0.030 g, 0.180 mmol) and Ac-Phe(4-vinyl)-OMe **41** (0.071 g, 0.287 mmol) were stirred in toluene (3 mL) at 100 °C for 2 h. The reaction mixture was then allowed to cool to room temperature, filtered through a plug of Celite®, and concentrated to dryness. Purification by flash column chromatography (EtOAc), followed by recrystallisation from CH_2Cl_2 / hexanes, gave **48** as a yellow solid (0.032 g, 64%); m.p. 239-242 °C, R_f = 0.10 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s, Boc-(CH_3)$_3$), 1.92 (3H, s, acetyl-CH_3), 2.02 (3H, s, acetyl-CH_3), 3.10 (1H, dd, *J* = 14.0, 5.6, Phe-C*H*H), 3.17 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.35 (1H, dd, *J* = 14.5, 6.5, Trp-C*H*H), 3.42 (1H, dd, *J* = 14.5, 6.6, Trp-CH*H*), 3.54 (3H, s, ester-CH_3), 3.67-3.79 (5H, m, ester-CH_3 / Gly-CH_2), 4.87-4.97 (2H, m, Phe-α-C*H* / Trp-α-C*H*), 6.23 (1H, t, *J* = 4.9, Gly-N*H*), 6.30 (1H, d, *J* = 8.0, Phe-N*H*), 6.68-6.75 (2H, m, alkene-C*H* / Trp-N*H*), 7.09 (2H, d, *J* = 8.1, Ar-*H*), 7.22-7.35 (3H, m, Ar-*H* / alkene-C*H*), 7.45 (2H, d, *J* = 8.1, Ar-*H*), 7.53 (1H, d, *J* = 7.7, Ar-*H*), 8.10 (1H, d, *J* = 8.0, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 23.1 (acetyl-CH_3), 27.4 (Trp-CH_2), 28.2 (Boc-(CH_3)$_3$), 37.6 (Phe-CH_2), 42.9 (Gly-CH_2), 52.4 (ester-CH_3), 52.5 (ester-CH_3), 52.5 (Trp-α-CH), 53.0 (Phe-α-CH), 84.2 (Boc-C), 114.4 (Ar C), 115.6 (Ar C), 118.5 (Ar C), 119.9 (alkene-C), 122.9 (Ar C), 124.8 (Ar C), 126.7 (Ar C), 129.7 (Ar C), 129.8 (Ar C), 131.9 (alkene-C), 135.6 (Ar C), 135.7 (Ar C), 135.8 (Ar C), 136.1 (Ar C) 150.4 (Boc C=O), 160.5 (C=O), 169.9 (C=O), 170.5 (C=O), 172.0 (C=O), 172.0 (C=O).

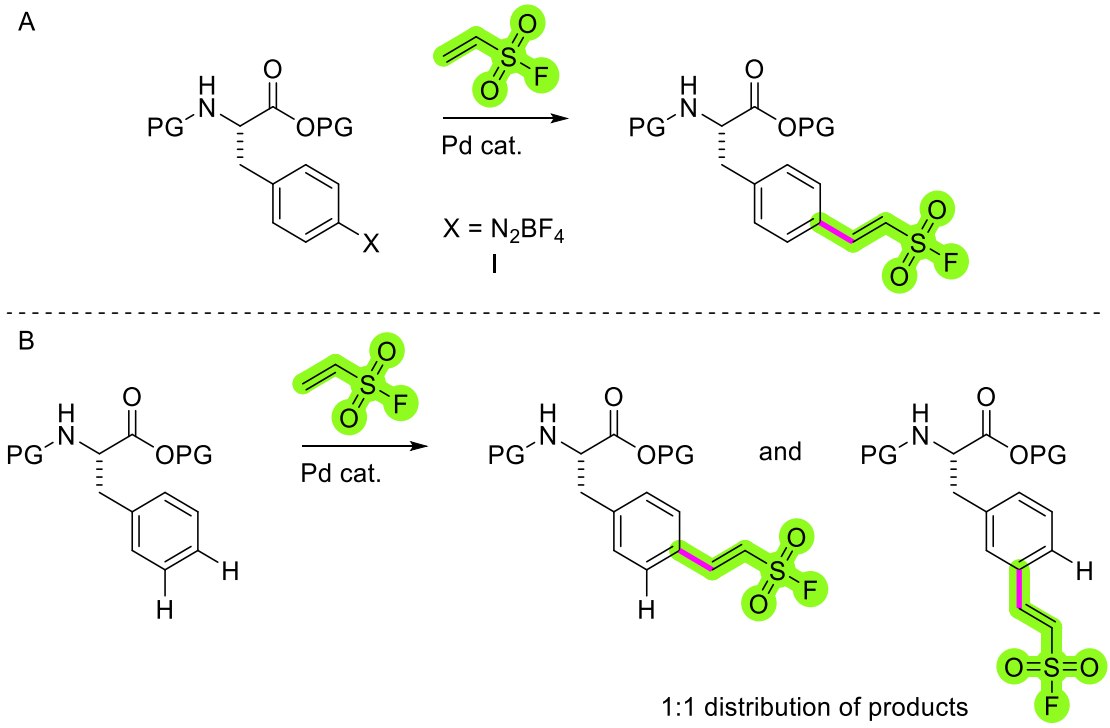
IR U_{max} /cm^{-1} (solid) 3291 w (N-H), 3055 w (C-H), 2952 w (C-H), 1728 s (ester C=O), 1653 s (amide C=O), 1523 s (C=C), 1210 s (C-O).

HRMS (ESI) [M+H$^+$] *m/z* calcd. for $C_{35}H_{43}N_4O_9$: 663.3030, found: 663.3068.

3 Olefination of peptides using ESF as a coupling partner

3.1 Introduction

As discussed in **Section 1.4.2**, protocols for installing SuFEx-able hubs onto peptides are lacking. Methods have been reported that successfully modify protected phenylalanine with ESF, to form aryl VSFs, **Scheme 63**.[154,157,161] However, these methods generally require pre-functionalized starting materials, or display a lack of selectivity at the olefination site on the phenyl ring. Presently, no literature report exists where phenylalanine residues in peptides have been modified with ESF.



Scheme 63 – Existing protocols for the installation of ESF on phenylalanine by [A] cross-coupling reactions and [B] C-H functionalization.

Recently, our group has reported the olefination of phenylalanine residues in peptides with styrene derivatives by C-H functionalization[126] Backbone amides are used as directing groups to facilitate the reaction at the *ortho* position on the aromatic ring system of phenylalanine. Although, styrene and ESF contain very different functionality, there are structural similarities between the two compounds, **Figure 17**. These similarities, in tandem

with the report of unselective palladium catalysed olefination,[161] were indicative that a novel protocol might be devised in order to facilitate olefination of ESF.

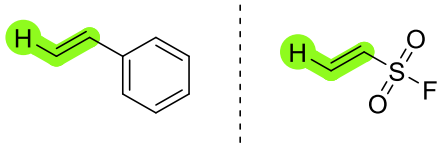
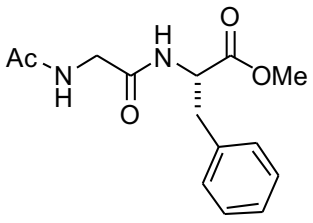


Figure 17 – Structural similarities between styrene and ESF.

The aim of this section of the investigation was to develop a new method for olefination and test the viability of this method against peptides in different contexts. Success would represent a significant advance in the modification of otherwise unreactive phenylalanine residues. This reaction has the potential to introduce click chemistry functionality to phenylalanine residues, which is reactivity complimentary to that found on natural peptides.

3.2 Olefination of model dipeptide Ac-Gly-Phe-OMe (49)

Investigation into the olefination of phenylalanine residues in peptides using ESF as a coupling partner began with the selection of a model dipeptide for the study. The dipeptide Ac-Gly-Phe-OMe **49** was picked as it represents the simplest dipeptide containing all characteristics necessary for olefination to occur, **Figure 18**.

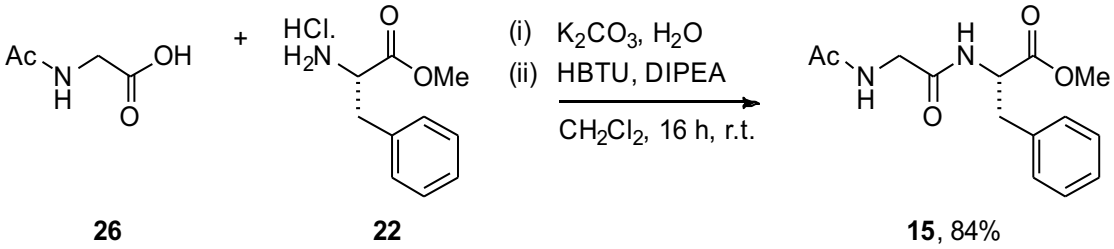


49

*Figure 18 – Model dipeptide Ac-Gly-Phe-OMe **49**.*

Structurally, glycine comprises the simplest sidechain and as a result is the most flexible naturally occurring amino acid. Theoretically, this introduces a further residue to the peptide chain at minimal steric cost. This simplicity also lends itself to characterisation by ^{1}H NMR spectroscopy, as only two extra signals are seen in the spectrum. The glycine residue was

deliberately placed at the *N*-terminus of the dipeptide. Previous research had demonstrated that olefination using styrene as a coupling partner does not occur when phenylalanine residues are positioned at the *N*-terminus.[114,125,126] In order to maximise the chance of success of the C-H functionalization reaction it followed that this residue should be placed at the *C*-terminus of the model dipeptide.

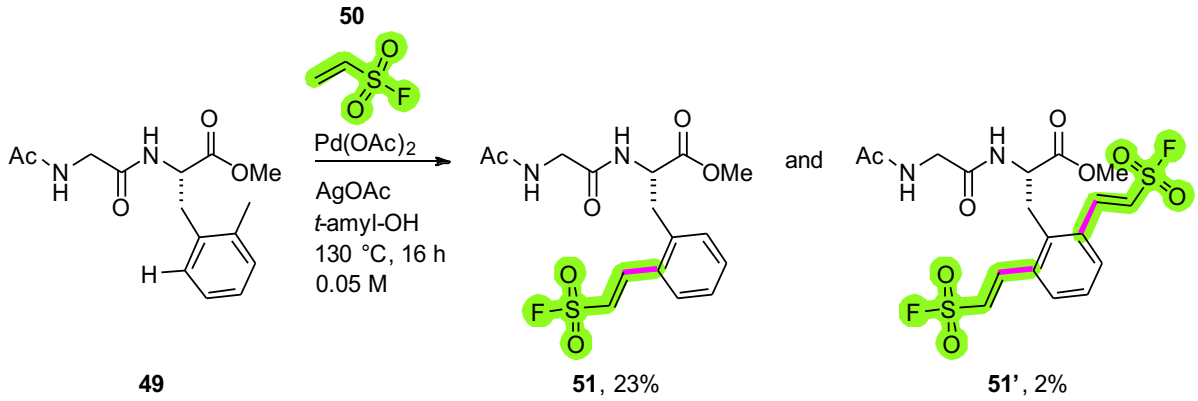


26 **22** **15**, 84%

*Scheme 64 – Synthesis of model dipeptide **49**.*

Protected amino acids *N*-acetyl-glycine **26** and (L)-phenylalanine methyl ester hydrochloride **22** were stirred in the presence of HBTU as coupling reagent and DIPEA as base to generate dipeptide **49** in an 84% yield, **(Scheme 64**). Success of the synthesis was confirmed by the emergence of a broad doublet in the ^{1}H NMR spectrum at 6.46 ppm, corresponding to an N-H bond in the new amide formed in the product.

Consideration was then given to the modification of the peptide with ESF. Previous studies into the olefination of phenylalanine residues in peptides by our group had utilised palladium acetate as a catalyst and silver acetate as oxidant, solvated in *t*-amyl-OH at 130 ºC for 24 h.[126] Therefore, the initial reaction of **49** and ESF **50** was carried out under these conditions, **Scheme 65.** The resulting crude mixture was filtered through a Celite® plug and concentrated *in vacuo* to yield a brown crude compound, which was purified by flash column chromatography and then recrystallised from CH_2Cl_2 and hexanes to yield two distinct products.

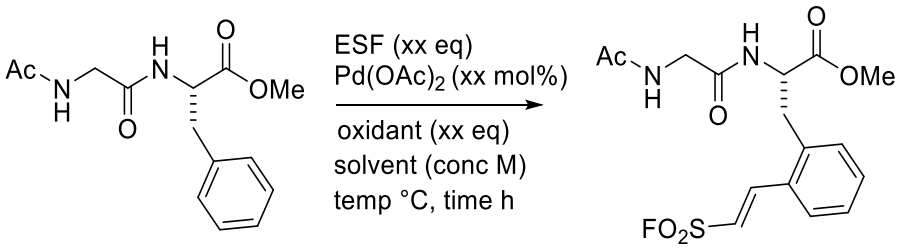


*Scheme 65 – Initial attempt to modify model dipeptide **49** with ESF.*

This initial reaction yielded a mixture of mono-olefinated **51** and di-olefinated **51'** products in 23% and 2% yields respectively along with a significant amount of unreacted starting material. Analysis of the ^{1}H NMR spectrum gave evidence of a successful reaction, showing that an alkene had been installed on the aromatic system of the phenylalanine residue. In the spectra of mono-olefinated product **51**, A doublet of doublets that integrated to 1H at 6.88 ppm (J = 15.3, 2.5 Hz) and a doublet that integrated to 1H at 8.12 ppm (J = 15.3 Hz) were consistent with a *trans* alkene in an aryl VSF.[157,161] The same data was also found in the spectrum corresponding di-olefinated product **51'**. The signals for the protons on the phenyl ring also differentiated **51** from the starting material. The spectrum for **51** displayed four distinct signals: two doublets and two triplets which were indicative that the olefination had occurred at the *ortho*- position. The di-olefinated product **51'** showed a different splitting pattern still, involving a doublet integrated to 2H and a triplet that integrated to 1H. Further reassurance of the reaction being successful could be seen in the ^{19}F NMR spectrum, where a peak present at 62.1 ppm provided evidence of a fluorine environment now present in the peptide structure. NMR data for peptides **51** and **51'** contained data consistent with that present in aryl VSFs synthesised by other synthetic routes such as a peak at ca. 60.0 ppm in the ^{19}F NMR spectrum.[155,157,161] Additionally, strong peaks at wavenumbers at 1405 cm^{-1} and 1189 cm^{-1} in the IR spectrum were attributed to S=O and S-F bonds respectively which implied that ESF had been installed. Parent ion peaks $[M+H]^{+}$ in the HRMS spectrum for **51** of 387.1027 and for **51'** of 495.0703 also supported this conclusion.

3.3 **Method Optimisation**

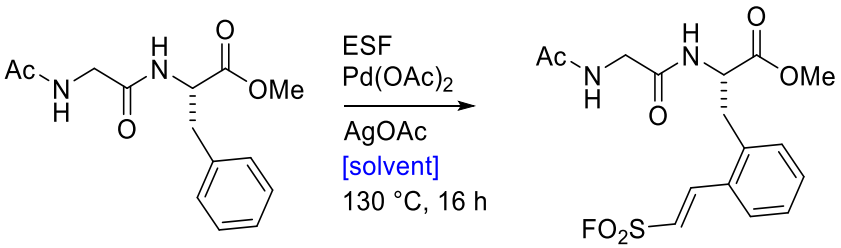
The results documented in **Section 3.2** demonstrated that olefination with ESF of the phenylalanine residue in the dipeptide model was achievable. However, time was taken to further develop and optimise the olefination conditions in order to maximise the yield of mono-olefinated product **51**. It was argued that for peptide modification, introducing a single SuFEx-able hub for further reactivity was preferential to mitigate any selectivity issues that could arise in future synthetic manipulations. A range of variables were altered systematically, and the effect on yield of mono-olefinated product was investigated, **Scheme 66**.



Scheme 66 – Variables investigated in the reaction optimisation.

The concentration of the reaction was varied initially by changing the volume of solvent present, **Table 1**. Changing the concentration from 0.05 M to 0.10 M by decreasing solvent volume provided a promising increase in yield of mono-olefinated product. However, when the reaction was further concentrated (0.20 M) a corresponding rise in yielded peptide was not observed. In this experiment the coupling reagents did not slurry properly in the decreased volume of solvent. It is likely that poor mixing is responsible for the diminished yield in this example. No attempt to overcome this issue was made and a concentration of 0.1 M was used throughout the rest of the investigation.

*Table 1 – Effect of varying concentration of peptide and solvent on yield of **51***



Entry	Concentration	Solvent	% Yield of 51*
A	0.05	*t*-amyl-OH	23
B	0.10	*t*-amyl-OH	40
C	0.20	*t*-amyl-OH	34
D	0.10	MeCN	0[#]
E	0.10	Dioxane	0[#]
F	0.10	DCE	41
G	0.10	DMF	0[#]
H	0.10	THF	15
I	**0.10**	**HFIP**	**45[†]**

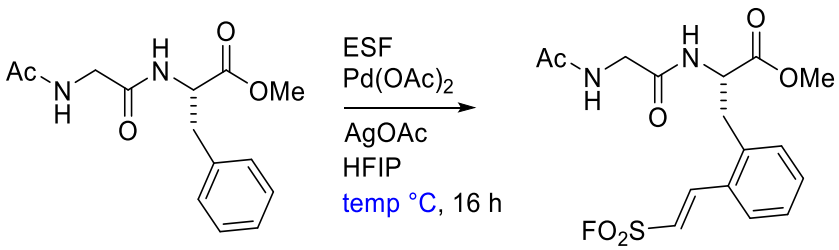
* Isolated yields reported.
[†] Product not isolated due to vial failure.
[#] Only starting materials observed

Whilst *t*-amyl-OH had worked effectively to facilitate the olefination of Phe residues in peptides with styrene derivatives, other solvents commonly used in C-H functionalization reactions were considered. Upon solvent variation, MeCN, dioxane and DMF were found to be incompatible with the olefination reaction whilst THF proved to be a poorer solvent choice than *t*-amyl-OH. Better results were initially garnered using DCE, which facilitated the reaction similarly to the original solvent.

Solvents were initially screened at 130 ºC to ensure temperature did not skew the observed yield. However, the boiling point of HFIP (lit. 58.2 ºC) is significantly lower than the screening temperature. When used as the solvent in these conditions, the vapour pressure became so great that failure of the v-vial occurred after 30 min. Surprisingly, a crude ^{1}H NMR of the remaining solid residue was taken after the 16 h had elapsed, revealing that the composition of the mixture was 45% mono-olefinated product. Despite the short reaction time and vial failure, this solvent system had produced the best yielding reaction. It was anticipated that

the vial failure could be mitigated by lowering the reaction temperature, which was then investigated, **Table 2**.

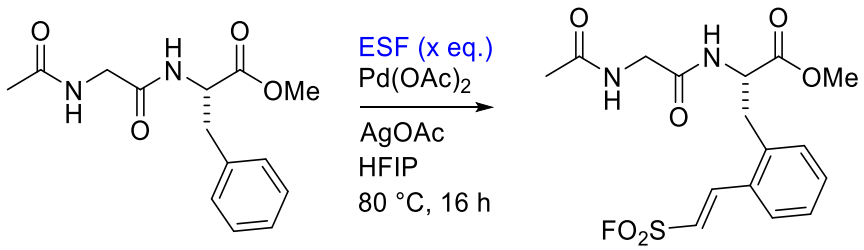
*Table 2 - Effect of varying reaction temperature on yield of **51**.*



Entry	Temperature / ºC	% Yield of 51
A	25	16
B	60	34
C	**80**	**54**
D	100	57

Upon exposure of the peptide to the reaction conditions at room temperature, the isolated product yield was severely diminished. When the reaction was carried out at 60 °C, an increase in the yield was seen. However, this was still diminished compared to the observed yield in the initial experiment. Exposure of the peptide at 80 °C was enough to elevate the yield significantly to approximately 54%. At 100 °C, the elevated temperature had a marginal effect on the yield and as a result, the optimised temperature for the reaction was determined to be 80 °C. In the hope of further increasing the reaction yield, an investigation into the effect of varying the equivalents of ESF was carried out, **Table 3**.

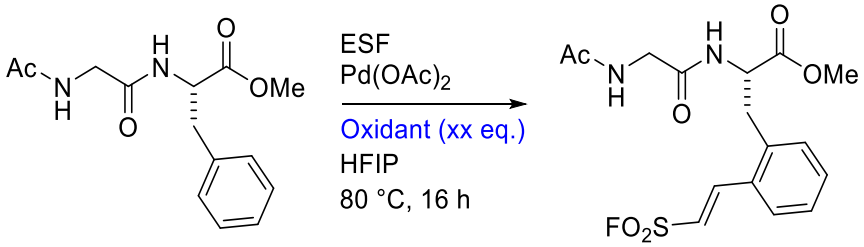
*Table 3 - Effect of varying equivalents of ESF on yield of **51**.*



Entry	Eq. of ESF	% Yield of 51
A	1	40
B	**2**	**63**
C	4	54
D	8	38

The reaction performed best when exposed to either 2 or 4 equivalents of ESF. Entry A and D (**Table 3**) exhibited diminished reaction conditions. Entry D is justified by presence of more ESF molecules to facilitate the generation of the di-olefinated species from the mono-olefinated species. Whilst there is not a lot of difference in yield between entry B and C (**Table 3**), B was chosen for the optimised conditions on environmental grounds due to the use of a smaller quantity of toxic starting material per reaction. A study of the oxidant and number of equivalents required was then undertaken, **Table 4**.

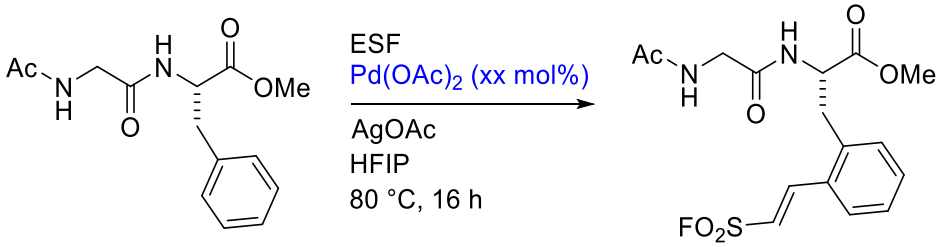
*Table 4 - Effect of varying oxidant and equivalents of oxidant on yield of **51.***



Entry	Oxidant	Eq. of Oxidant	% Yield of 51
A	**AgOAc**	**5**	**63**
B	$AgCO_3$	5	21
C	AgOPiv	5	30
D	$Cu(OAc)_2$	5	60
E	Benzoquinone	5	11
F	AgOAc	2.5	37

In spite of testing a variety of oxidants, entry A (**Table 4**) proved to be the best conditions for the olefination protocol. Disappointingly, reducing the number of equivalents of oxidant was detrimental to yield of the reaction meaning that 5 equivalents were reported in the optimised conditions.

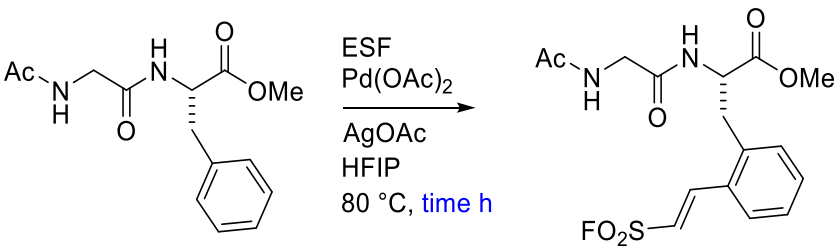
*Table 5 - Effect of varying the catalyst loading on conversion of **49**.*



Entry	Catalyst Load / mol%	% Conversion of 49
A	10	71
B	15	74
C	20	80
D	**35**	**98**
E	50	98

When varying the catalyst loading in the reaction, the largest improvement to yield was observed, **Table 5**. In this example, the yielded peptides were not isolated, and the conversion of starting material was monitored by ^{1}H NMR spectroscopy. Exposure to increasing catalytic mol% generated increasing conversion of the starting material peptide. At 35 mol%, the conversion after 16 hours was the same as that at 50 mol%. Entry D (**Table 5**) shows the optimised conditions for catalytic loading.

*Table 6 - Effect of reaction time on yield of **51**.*



Entry	Time / h	% Yield of 51
A	0.5	36
B	1	48
C	2	55
D	4	63
E	8	70
F	**12**	**77**
G	16	75
H	24	72
I	100	63

Finally, the yield was monitored over time in order to determine the optimal reaction length, **Table 6**. The yield increased with reaction time until 12 h had elapsed. After 12 h the reaction time appeared to have very little bearing on the yield of the reaction. A slight decrease in yield is justified by the conversion of mono-olefinated product to di-olefinated product.

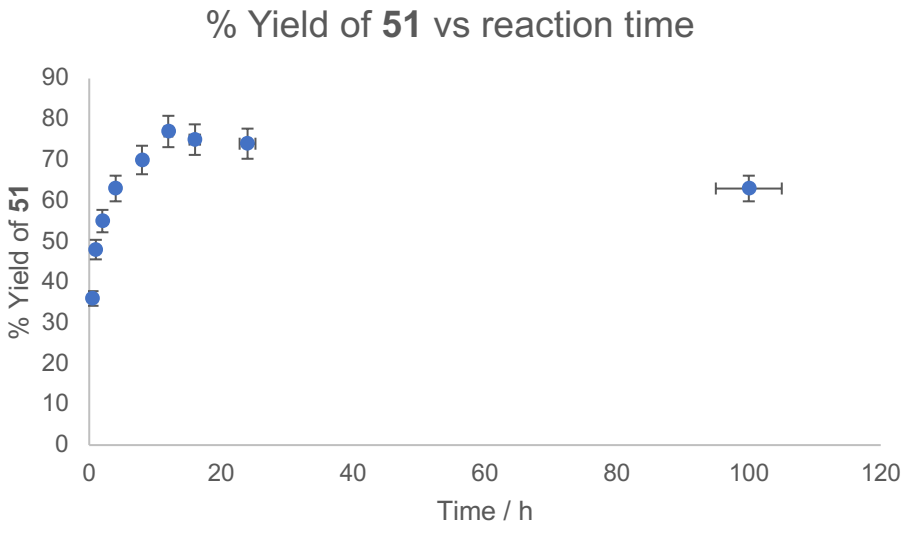
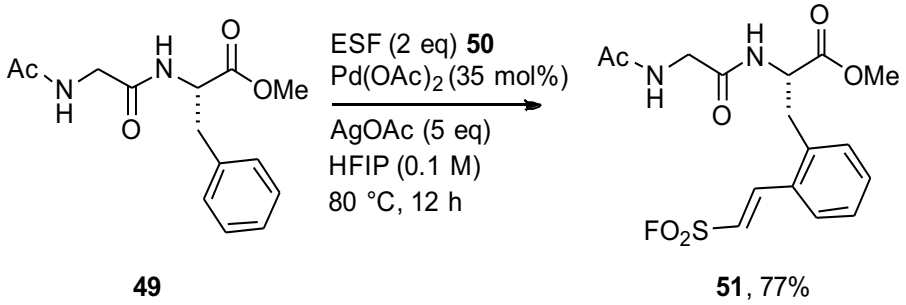


Figure 19 – Progress of the olefination reaction through time.

As seen from **Figure 19**, the progress of the reaction stops after 12 h. As a result, the optimised reaction time of this protocol was determined to be 12 h. Collectively, these results provide an optimised protocol for the olefination of phenylalanine containing peptides with ESF. These conditions are displayed in **Scheme 67** below.



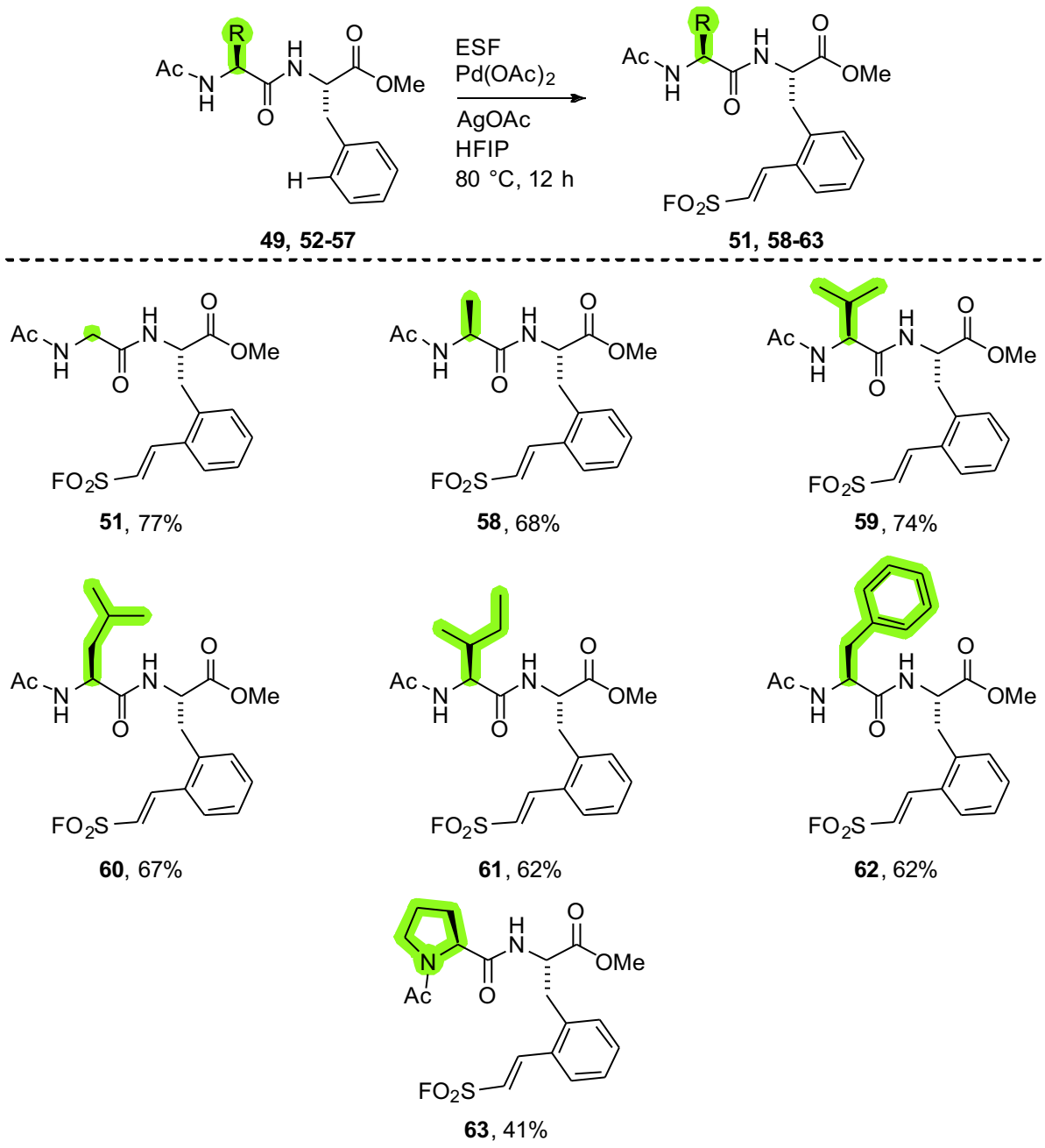
49 **51**, 77%

*Scheme 67 – Optimised conditions for the olefination of dipeptide **49** to generate modified peptide **51**.*

3.4 Varying the neighbouring amino acid residue

3.4.1 Non-polar neighbouring amino acids

For broad applicability, the olefination of phenylalanine residues in peptides needed to transcend peptides with the Gly-Phe motif. To that end, an investigation into tolerable neighbouring amino acids was undertaken. The optimised conditions were applied to a series of dipeptides **52-57** where the amino acid residue at the *N*-terminus was varied to other non-polar amino acids, **Scheme 68**. Non-polar amino acids were chosen due to their inert nature. It was anticipated that these amino acids would increase the complexity of the peptide structure whilst providing no chemical interference with catalyst or coupling partner.

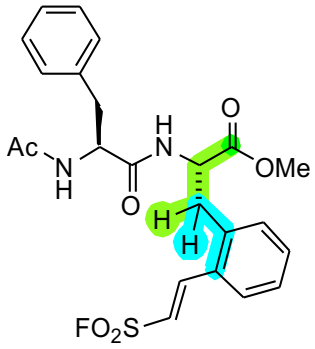


Scheme 68 – Olefination of phenylalanine residues neighbouring non-polar amino acids in dipeptides.

As in **Section 3.2** above, modified peptides were fully characterised by NMR spectroscopy. The appearance of the two doublets in each ^{1}H NMR spectrum associated with *trans* alkenes present confirmed the olefination had taken place. Analysis of the ^{19}F NMR spectrum showed a new peak (ca. 62 ppm) in each case. The olefination of dipeptides **49** and **52-57** proceeded smoothly when exposed to the reaction conditions developed in **Section 3.3**, producing olefinated peptides **51** and **58-63** in 62-77% yields.

The modification of Ac-Phe-Phe-OMe **62** represents an interesting example due to the presence of another phenylalanine residue with the potential for olefination. In this instance,

the olefination was observed only on the residue towards the *C*-terminus and yielded modified peptide **62** in a 62% yield. This result was confirmed by the analysis of the HMBC spectrum, which contained cross-peaks connecting the 4° carbon with CH_2 (blue) and CH_2 with C=O (green) as demonstrated in **Figure 20**.

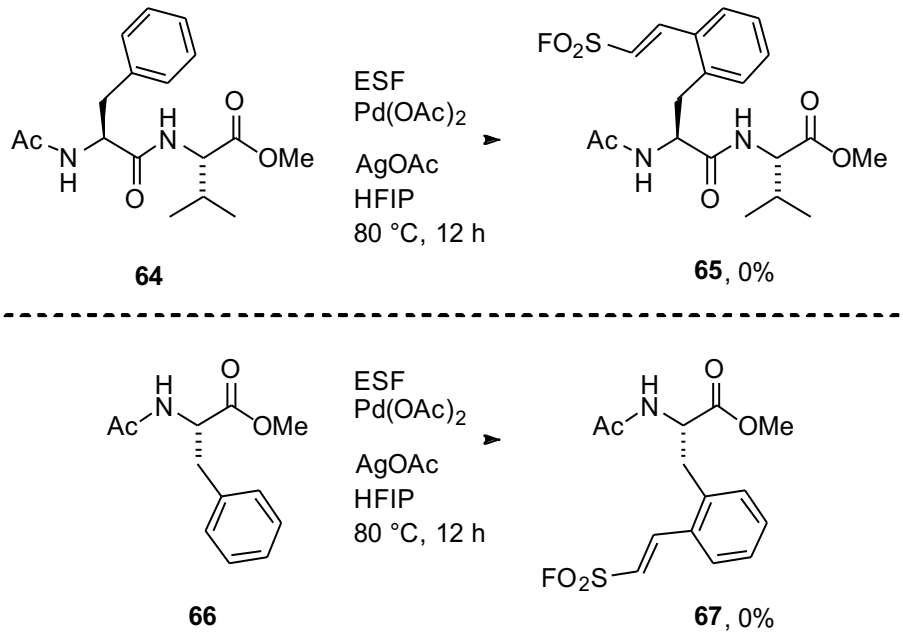


62

*Figure 20 – HMBC spectrum revealed coupling on modified peptide **62**.*

The olefination of dipeptide Ac-Pro-Phe-OMe **57** which generated modified peptide **63** represents another significant example. Our group previously reported the olefination of Ac-Pro-Phe-OMe **57** with styrene in a very low yield of the modified peptide. However, in the reaction with ESF an improved 41% yield of the modified peptide was achieved.

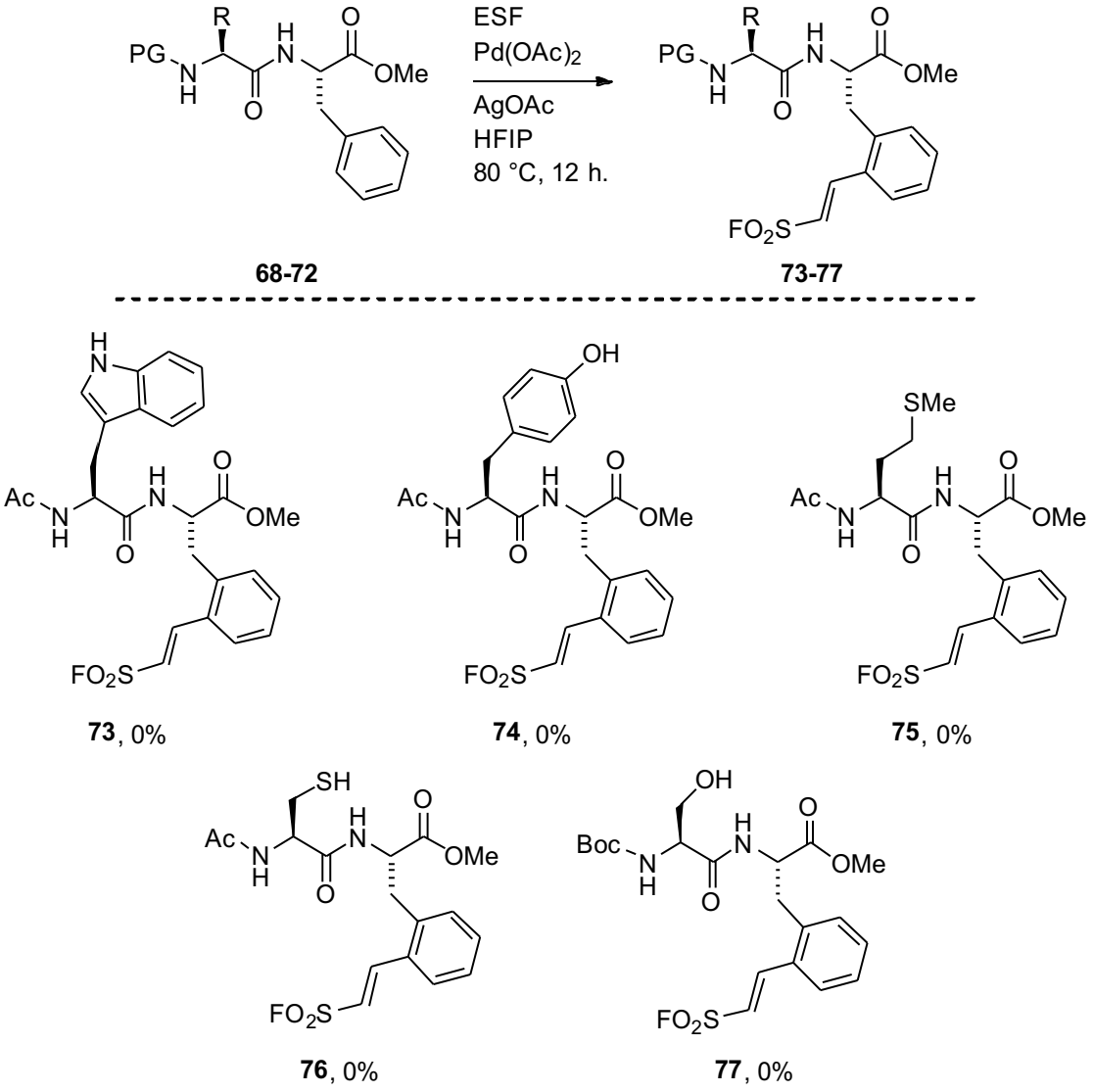
As well as neighbouring amino acids, the effect of moving the phenylalanine residue to the *N*-terminus was also evaluated. The peptide Ac-Val-Phe-OMe **53** was demonstrated above to undergo modification to the mono-olefinated structure effectively. The dipeptide **64**, which displays the reverse sequence, was exposed to the reaction conditions as shown in **Scheme 69**. No olefinated products of the corresponding modifeid peptide **65** were observed in the crude yield. Furthermore, when the amino ester Ac-Phe-OMe **66** was exposed to the reaction conditions, the olefination reaction did not proceed to yield modified amino acid **67** and the starting compound was recovered. In conjunction with modified peptide **62**, where no olefination at the *N*-terminus was observed, this result supports the theory that phenylalanine residues at the *N*-terminus of peptide cannot be olefinated by this protocol.



Scheme 69 – Investigation into olefination at N-terminal phenylalanine residues.

3.4.2 Polar neighbouring amino acids

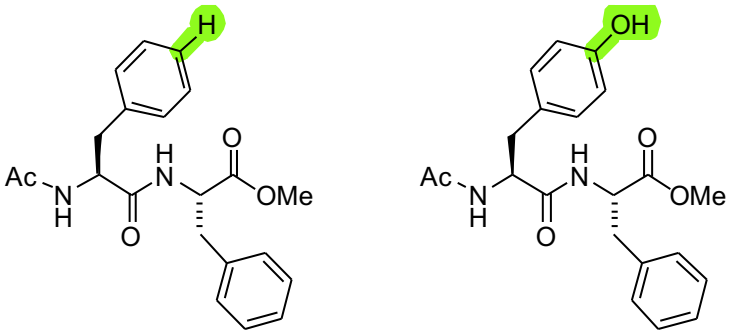
Non-polar amino acids account for less than half of the naturally occurring residues. To further broaden the scope of the method, a series of dipeptides **68-72** containing unprotected polar amino acids were also evaluated, **Scheme 70**.



Scheme 70 – An investigation into the limitations of the neighbouring amino acid in the olefination reaction.

Upon exposure to the reaction conditions, none of the peptides **68-72** were modified with aryl VSF. Peptides **68-72** did not yield expected modified peptides **73-77**. In each case an intractable mixture was produced, and no experimental data could be salvaged. However, information can still be garnered from this result. In some cases, the presence of a heteroatom is the only change in the peptide structure from those peptides modified in **Section 3.4.1** meaning that its presence is likely the reason for a change in outcome. Heteroatoms have the potential to poison the reactive catalytic species or even direct the reactive catalysts away from the intended reaction site. It must also be noted that ESF is an exceptional Michael acceptor,[150] meaning that any good nucleophilic species could react with ESF before the olefination could occur, however no evidence of this was found in the intractable mixture of products.

The extra aromaticity in dipeptides Ac-Trp-Phe-OMe **68** and Ac-Tyr-Phe-OMe **69** was not deemed to be problematic as modified peptide **62** was formed from its corresponding dipeptide without issue. The structural similarity demonstrated in **Figure 21** between Ac-Tyr-Phe-OMe **69** and Ac-Phe-Phe-OMe **62** provides compelling evidence that olefination was not facilitated on peptide **69** due to limitations involving the presence of a heteroatom.



62

Modified and isolated in 62% yield

69

Modified compound not observed

*Figure 21 – Structural similarities between Ac-Phe-Phe-OMe **62** and Ac-Tyr-Phe-OMe **69**.*

The olefination of Ac-Cys-Phe-OMe **71** also did not yield the desired modified peptide. Cysteine contains an extremely good nucleophile in the form of R-SH which may provide some justification for the result. Interestingly, Ac-Met-Phe-OMe **70** did not generate the expected olefination product when subjected to the olefination conditions. The research group had previously reported the olefination of Ac-Met-Phe-OMe **70** with styrene in good yield[126] and it was expected that this modification with ESF could be facilitated. It was not possible to identify the peptide starting material or ESF in the crude NMR spectra and it was concluded that methionine is not a compatible neighbour to phenylalanine in olefination reactions with ESF under the developed protocol.

Boc-Ser-Phe-OMe **72** was also evaluated and yielded no significant result. The change in *N*-protecting group was potentially the reason the absence of reaction. However, similar to the other polar amino acids above, the presence of the free heteroatom in the hydroxyl sidechain was likely a factor. The cases discussed in this section collectively implied that unprotected heteroatoms are beyond the scope of the developed reaction conditions at this

stage. It therefore followed that a protecting group strategy might be employed as a way of increasing the applicability of the method.

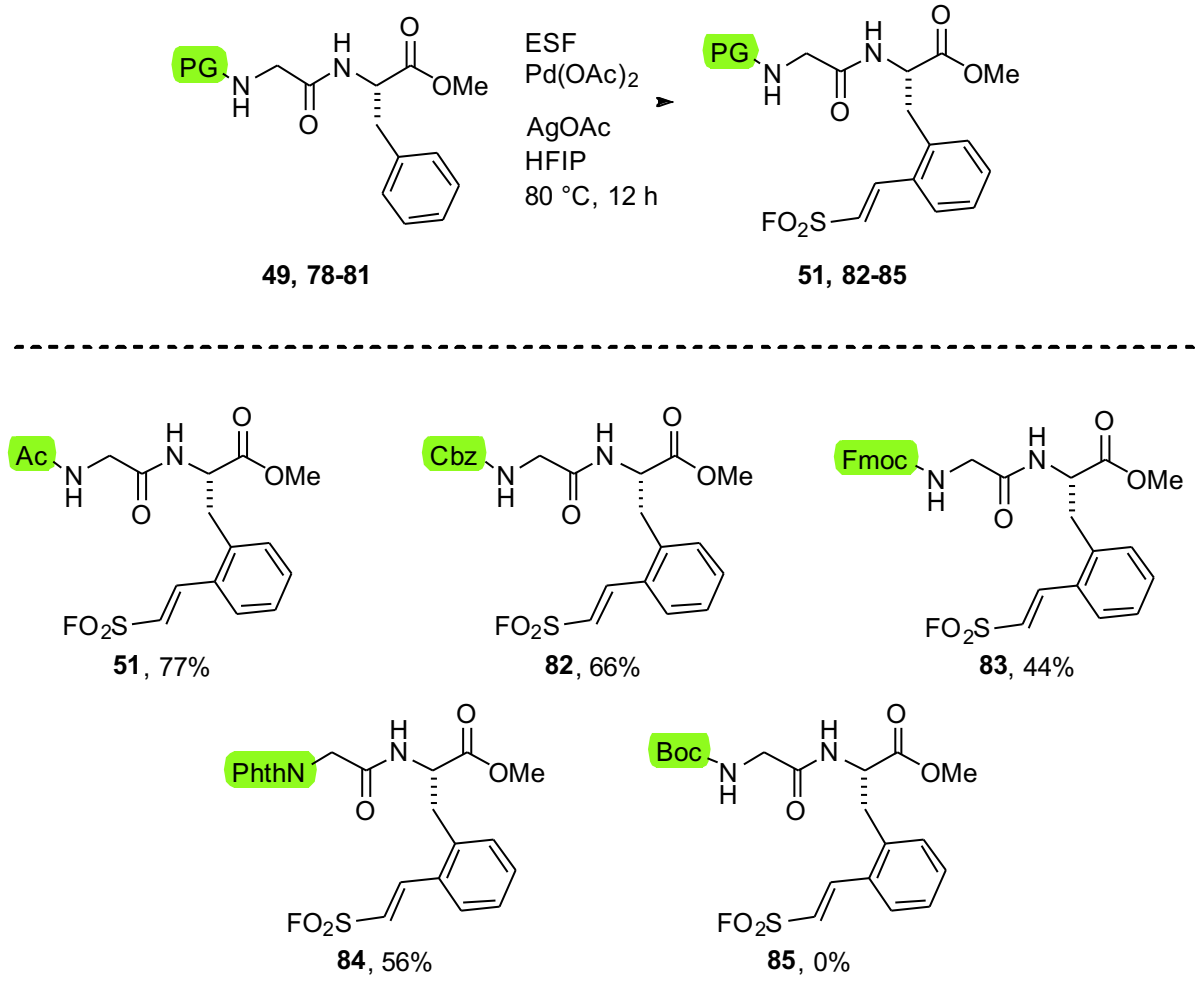
3.5 **Protecting Group Scope**

The model dipeptide in this study contained an *N*-acetyl protecting group. This functionality was an excellent starting point for the investigation due to its ability to withstand a wide range of chemical transformations. The conditions used to carry out the C-H functionalization were therefore unlikely to cause deterioration of the model starting material. However, the amide bond formed upon installation of the *N*-acetyl is difficult to selectively cleave over other present peptide bonds. Furthermore, amide cleavage generally requires refluxing in 10% NaOH overnight or exposing to concentrated HCl in a sealed vessel. Both of these processes are considered harsh conditions for biological molecules. As a result, in most cases of peptide bond formation, protecting acetyl groups are circumvented to avoid complicated cleavage processes. The dipeptide also contained a *C*-terminal methyl ester protecting group. Similarly, this was picked for its stability and subject to the same pitfalls as the acetyl protecting group. It is not often used as a carboxylic acid protecting group when the acid requires revealing post synthesis in complex structures.

Solid phase peptide synthesis, which represents one of the most important examples of protecting group chemistries employed in large scale industrial processes, utilises a variety of other *N*-protecting groups. Standard SPPS uses two orthogonally reactive strategies in the acid labile ^{t}Bu/Boc groups and base labile Fmoc groups. These protecting groups are used in tandem, generally employing the Fmoc group as the *N*-terminal protecting group whilst the ^{t}Bu/Boc or other acid labile groups such as Cbz or Trt are used to protect functionality present on side chains.

In order to broaden the applicability of the method, the conditions were then applied to a series of dipeptides **78-81** where the *N*-terminal protecting acetyl group had been substituted for other protecting groups, **Scheme 71**. Protecting groups Fmoc, Boc, Cbz and

Phth were evaluated as these are generally the most common nucleophile protecting groups used in SPPS. All these protecting groups form carbamates when installed onto the amino functionality in peptides.



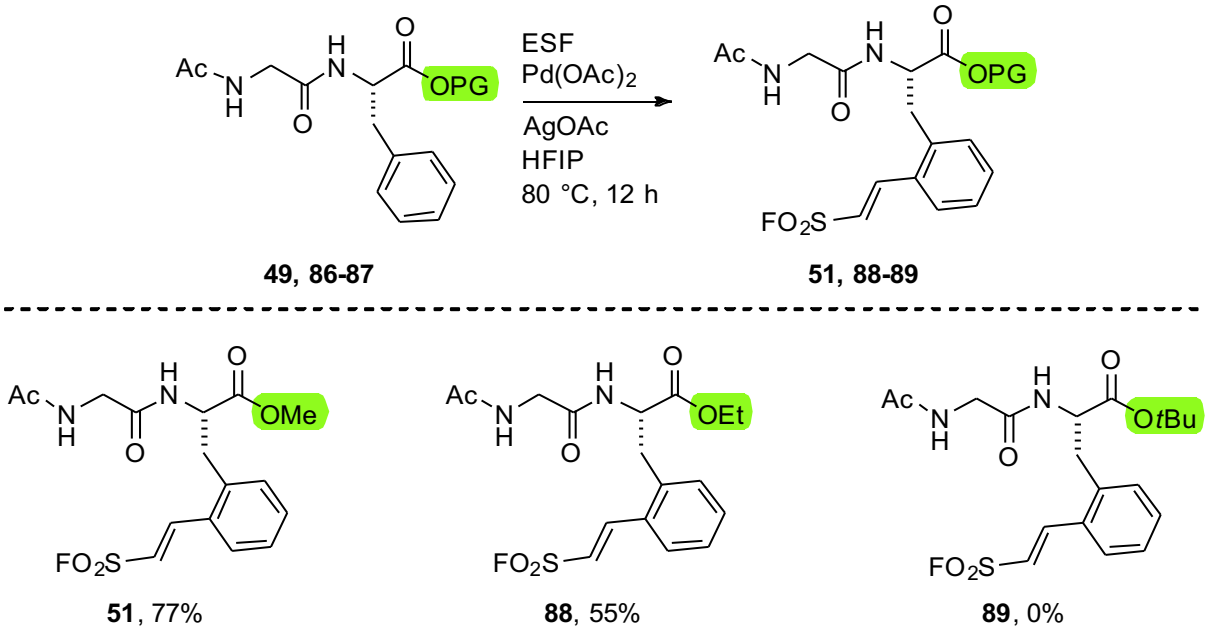
Scheme 71 – Olefination of N-terminally protected dipeptides.

For the attempted reaction of Boc-Gly-Phe-OMe **81** with ESF, a ^{1}H NMR spectrum of the crude residue revealed that no C-H functionalization had taken place and expected peptide **85** had not been synthesised. The usual characteristic doublet of doublets peaks at 6.85 ppm (J = 15.3 Hz) corresponding to the *trans* alkene associated with aryl VSF was not present in the spectrum. Furthermore, the spectrum suggested that the Boc protecting group had been removed during the reaction due to the lack of a 9H singlet at 1.40 ppm.

However, the olefination of Cbz-Gly-Phe-OMe **78** Fmoc-Gly-Phe-OMe **79** and with ESF proceeded well with yields of modified peptide **82** and **83** of 66% and 44% respectively. The

dipeptide Phth-Gly-Phe-OMe **81** was also modified to form peptide **84** and isolated in good yield which was significant as the reaction between dipeptide **81** and styrene in a previous study[126] had yielded no olefinated product.

Dipeptides **86** and **87** with differing ester protecting groups on the *C*-terminus were then exposed to the optimised reaction conditions, **Scheme 72**. Along with the methyl ester present in the model dipeptide, ethyl and *tert*-butyl esters were evaluated.



Scheme 72 – Olefination of C-terminally protected dipeptides.

Olefination of Ac-Gly-Phe-OEt **86** proceeded well, yielding olefinated peptide **88** in a 55% yield. However, when exposed to the olefination conditions, the peptide Ac-Gly-Phe-O*t*Bu **87** did not yield the expected modified peptide **89**. Moreover the starting material was not recovered, with ^{1}H NMR spectroscopy showing that the *tert*-butyl ester had not survived the reaction conditions.

3.6 **Developing a milder olefination protocol**

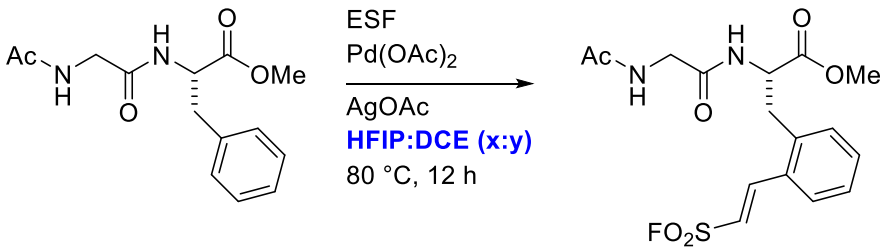
Exploration of protecting group compatability had demonstrated that the reaction conditions were presently unsuitable for the *N*-terminal protecting Boc group and the *C*-terminal protecting *tert*-butyl ester. Both of these protecting groups are acid-labile and the standard

deprotection of such functionality in SPPS involves the exposure to dilute trifluoroacetic acid.

The reaction conditions developed previously involved the use of HFIP as solvent which can be considered acidic, particularly at elevated temperatures.[168] An investigation was therefore devised to determine if HFIP was necessary for the reaction to proceed. If this was the case, the concentration of HFIP could potentially be lowered and used as an additive rather than solvating agent. During the optimisation in **Section 3.3**, DCE had performed well as a solvent, being outperformed only by HFIP. A solvent system incorporating both solvents could provide a system that did not cleave acid labile groups.

Whilst it was important to decrease the acidity of the solvent, it was also imperative that this was not significantly detrimental to reactivity. Demonstrated in **Table 7** below, the model dipeptide **49** was exposed to the same reaction conditions developed in the optimisation, altering only the solvent system in each case.

*Table 7 – Effect of altering solvent composition on % conversion of **49**.*



Run	% HFIP	% DCE	% Conversion
A	0	100	50*
B	100	0	>95*
C	50	50	>95*
D	25	75	>95*
E	**10**	**90**	**>95***

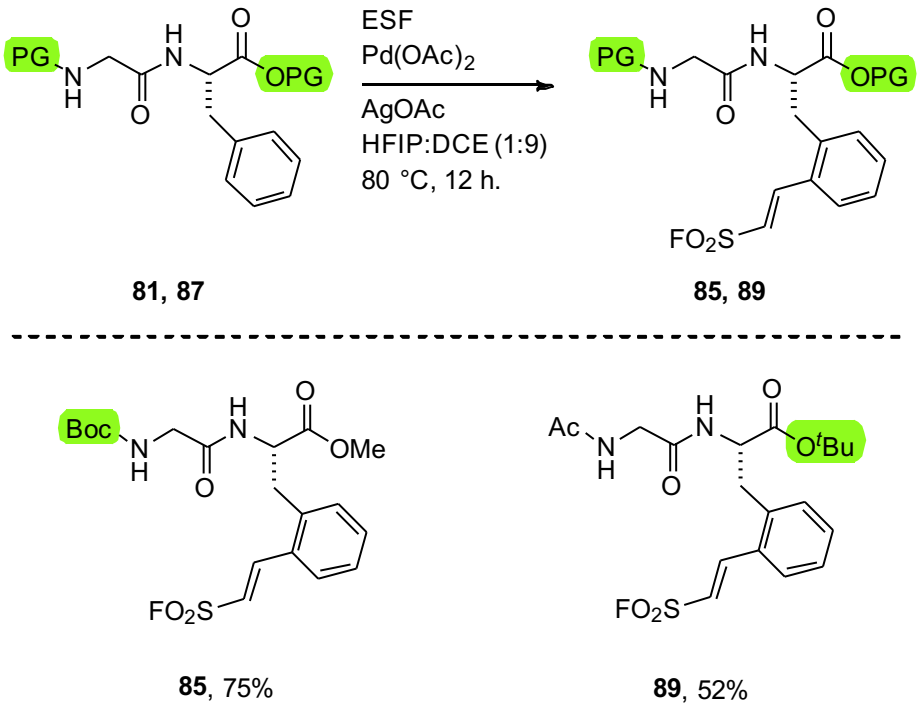
**Conversion determined by crude ^{1}H NMR data.*

Complete elimination of HFIP was attempted first. This led to a conversion of 50% of the starting material to the mono-olefinated product, implying that HFIP was imperative for good conversion. Therefore a mixed solvent system would be required. A 1:1 mixture was then used as solvating agent which proceeded well, yielding the same result that using neat HFIP produced.

As a result, further solvent systems with increasing dilution factors were employed. A dilution factor of 4, and more impressively 10 yielded results on a par with neat HFIP. This indicated that HFIP could be used much more sparingly in DCE without significantly altering the reactivity.

3.7 Re-evaluating the olefination of peptides with acid-labile protecting groups.

Once it had been established that the modification would proceed well with the new solvent system outlined in **Section 3.6**, the experiments which previously did not yield the desired modified peptides were re-examined. The new solvent system was used in these cases whilst controlling all other reaction variables. These results can be seen in **Scheme 73** below.



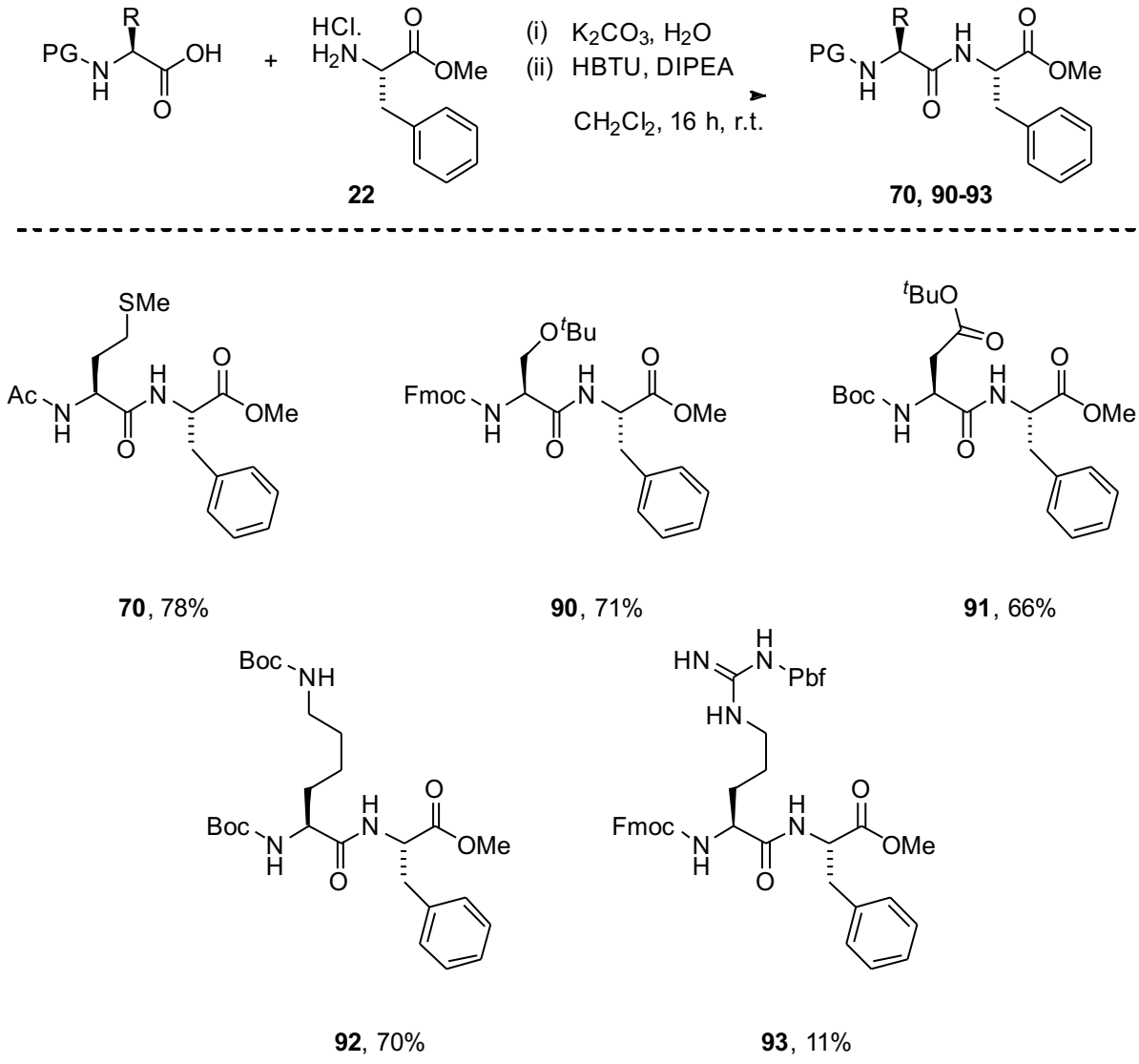
Scheme 73 – Olefination of peptides containing acid-labile groups using the co-solvent system.

Exposure to the new solvent system allowed the modification of both protected dipeptides to occur in good yields. In each case the reaction appeared to be clean, producing only di- and mono-olefinated peptides along with unreacted starting material. Modified dipeptides **85** and **89** were charaterised as in other successfully modified examples above. There was no evidence of breakdown of starting material peptide and the protecting group was observed to be intact in both cases.

3.8 Olefination of phenylalanine residues neighbouring protected, polar amino acids in peptides

The above result represents a significant forward step in the project. It had been evidenced that polar amino acids were problematic substrates in this manipulation and masking this polar reactivity could be a way of overcoming this difficulty. With proof that acid and base labile protecting groups were compatible with the olefination conditions, it was possible that more, and likely all, naturally occuring amino acids could be compatibile as a neighbouring residue on a peptide.

To this end, a series of polar peptides **90-93**, **Scheme 74**, encompassing different types of polar amino acids was synthesised as a means to fully evaluate the theory that all amino acids would tolerate the olefination conditions. The dipeptides were synthesised by solution-phase peptide sythesis as in **Section 3.2**.



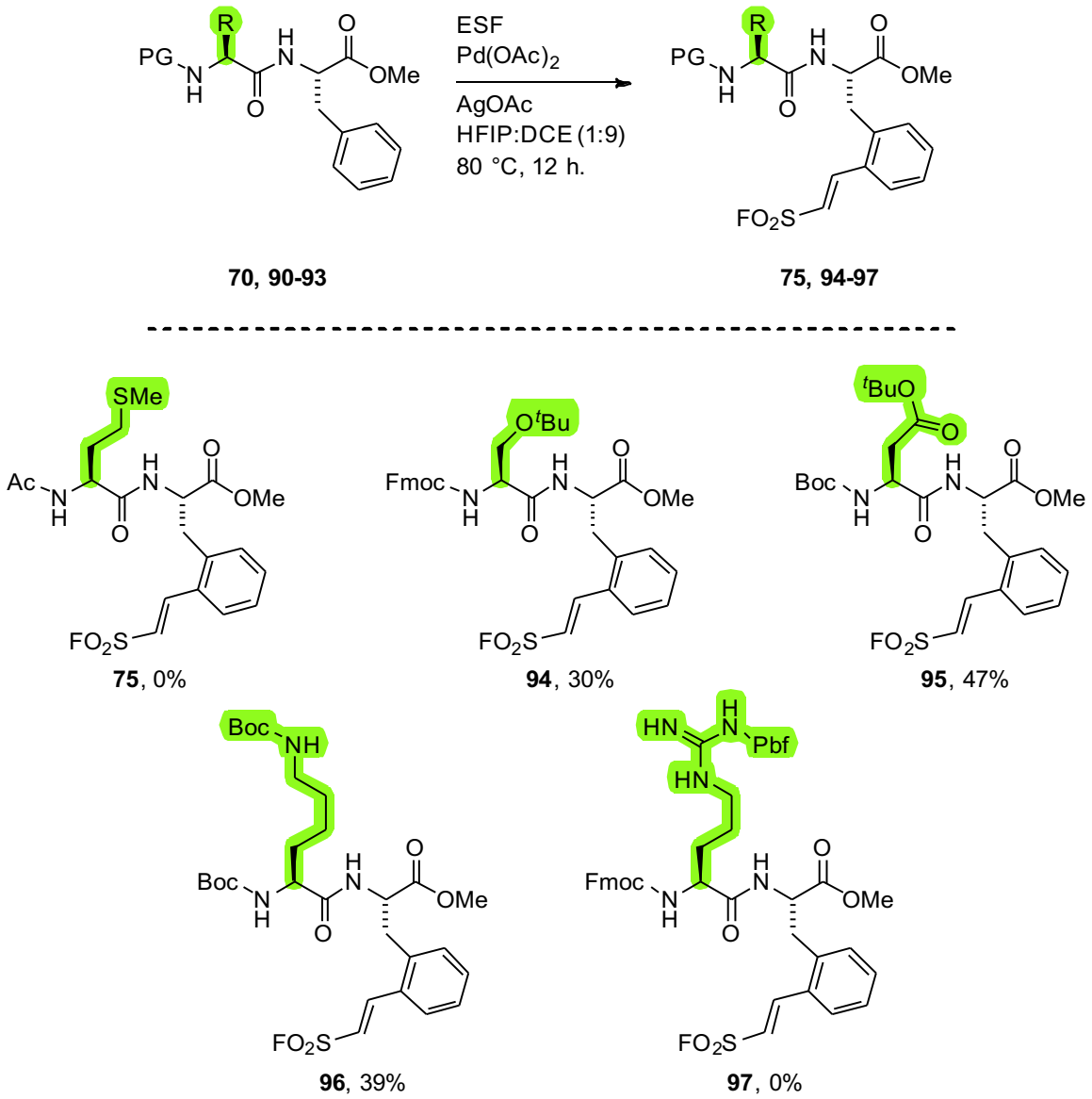
Scheme 74 – Synthesis of polar amino acid containing dipeptides.

The first peptide chosen was Ac-Met-Phe-OMe **70** as it had failed to undergo modification previously under harsher conditions using HFIP as a solvent. It was postulated that the milder reaction conditions may favourably affect the result. Other peptides containing polar heteroatoms were also chosen. Fmoc-Ser(^{t}Bu)-Phe-OMe **90** represented an example of a peptide with a protected hydroxyl group as an ether and Boc-Asp(O^{t}Bu)-Phe-OMe **91** contains a carboxyl group protected as a *tert*-butyl ester. Boc-Lys(Boc)-Phe-OMe **92**

contains a primary amine protected as a carbamate. Additionally, the perceived problematic modification of a peptide with the neighbouring amino acid, arginine in Fmoc-Arg(Pbf)-Phe-OMe **93** was investigated.

The dipeptides were then exposed to the reaction conditions as outlined in **Scheme 75**. Modified dipeptide charaterisation was carried out in accordance with previous dipeptides. The reaction between Ac-Met-Phe-OMe **70** and ESF once again yielded an intractable mixture of products. Analysis of the crude ^{1}H NMR and ^{19}F NMR spectra provided no evidence that the olefination reaction had been successful. This conclusion was supported by the lack of the characteristic peak indicative of an installed sulfonyl fluoirde at approximately 60 ppm in the ^{19}F NMR. However, when exposed to the developed reaction conditions, olefination of dipeptides **90-92** proceeded well to yield modified peptides **94-96**. Although the yields were lower than those of the non-polar peptides, they were regarded favourably when considering the increased complexity of the reaction that is introduced by adding polar, reactive handles.

Disappointly, exposure of the arginine containing peptide **93** to the reaction conditions did not geneate the desired olefinated product **97**. An intractable mixture of products was obtained from the reaction mixture. The guanidine functionality present on the arginine side-chain is problematic even when protected due to its instability to relatively mild reaction conditions. Chelation or poisoning of the catalyst is also still a real possibility on the protected residue which could decrease the catalytic acivity.

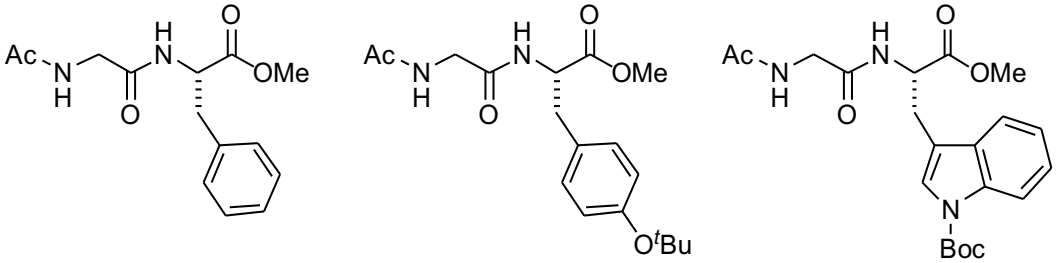


Scheme 75 – Olefination of dipeptides containing protected polar amino acids.

Modified peptide **94**, synthesised from Fmoc-Ser(^{t}Bu)-Phe-OMe **90** was a particularly satisfying result considering previous findings that Boc-Ser-Phe-OMe **72** had not been succesfully olefinated. This represents a clear step forward in broadening applicability acheieved by the introduction of a modified solvent system. Another noteworthy result was the successful modification of phenylalanine in the presence of lysine residues to form modified peptide **96**. The nucleophilicity of the amine on a side-chain is a reactive handle that we were interested in exploiting post-modification. This will be discussed in detail *vida infra*.

3.9 Exposure of other aromatic residues to the developed conditions

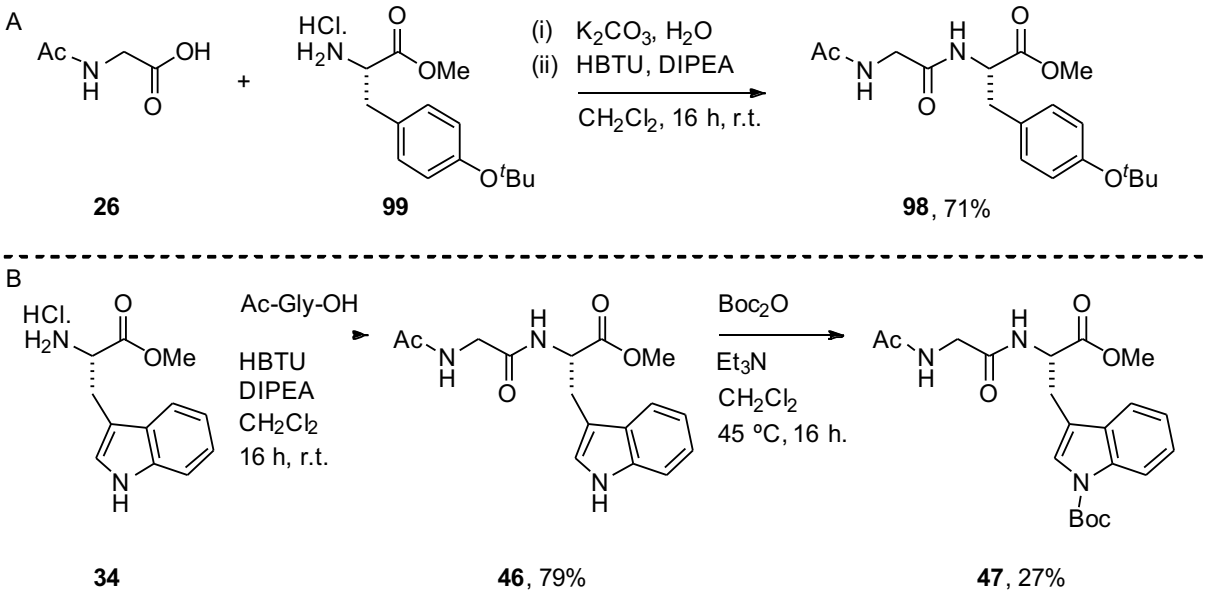
The olefination of other aromatic residues was then attempted using the developed reaction conditions. Like model dipeptide **49**, dipeptides **98** and **47** were identified as synthetic targets to determine if the scope of the developed reaction extended to these residues, **Figure 22**.



49 **98** **47**

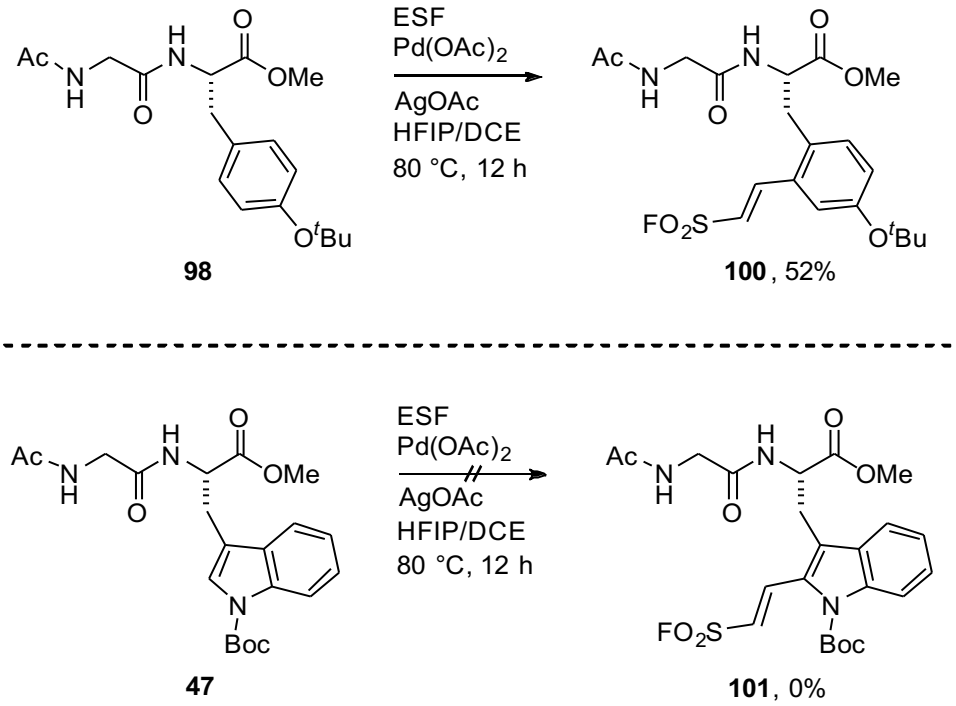
Figure 22 – Corresponding dipeptide models targeted for the investigation into the scope of the aromatic residue.

In the case of tyrosine containing peptide **98**, the phenol functionality was protected as an ether whilst in the example of tryptophan containing peptide **47**, the indole was protected by a Boc protecting group. The synthesis of tyrosine containing compound **98**, **Scheme 76A**, was facilitated by a routine solution-phase peptide synthesis of commercially sourced protected tyrosine methyl ester **99**. The dipeptide Ac-Gly-Trp(Boc)-OMe **47** was synthesised as in **Scheme 76B** in the same manner as described in **Section 2.4**. The dipeptide intermediate **46** was synthesised in good yield before the protection of the indole nitrogen to yield target dipeptide **47** was carried out.



Scheme 76 – Synthesis of peptides containing other aromatic residues [A] tyrosine and [B] tryptophan.

With the target peptides **98** and **47** in hand, both were exposed to the reaction conditions developed for the modification of phenylalanine residues, **Scheme 77**. The amended solvent system was used to nullify the potential cleavage of the protecting groups on the aromatic systems. When the tyrosine containing peptide **98** was exposed to the conditions, the reaction proceeded smoothly to generate modified dipeptide **100** in good yield. The olefination was determined to take place at the ortho site on tyrosine by the analysis of the HMBC spectrum of **100**. This result is significant as it broadens the scope of the reaction to include another residue which can be modified. Upon exposure to the reaction conditions, tryptophan containing peptide **47** did not yield the anticipated modified dipeptide **101**. An intractable mixture of products was obtained, and the starting material peptide could not be recovered.



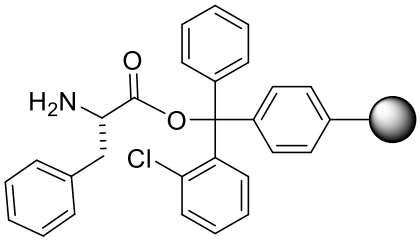
*Scheme 77 – Peptides **98** and **47** containing different aromatic residues exposed to the developed olefination conditions.*

3.10 Tripeptides and tetrapeptides

The olefination of dipeptides containing phenylalanine residues with ESF has been rigorously explored above. It was then appropriate to improve the viability of the method by moving towards *tri*- and *tetra*-peptides, the next logical step when increasing the complexity. Modification on longer peptides significantly increases the difficulty as each new residue installed introduces a further amide as a minimum. Depending on the residue added, more reactivity is potentially introduced via the side chain.

3.10.1 Synthetic routes to *tri*- and *tetra*-peptides

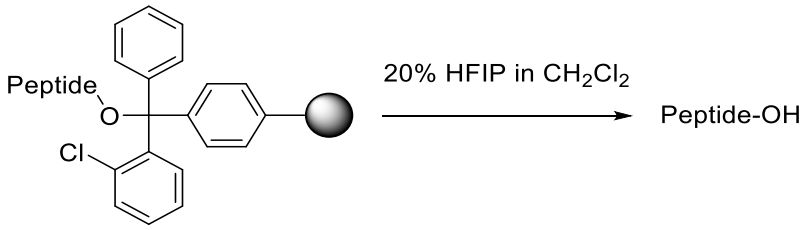
The synthesis of dipeptides is straightforward and arguably time cost effective to synthesise in solution phase. However, increasing chain length is significantly more challenging with every step. The difficulties arise in solution-phase peptide bond forming reactions as the molecular weight of the reactants increases. The work-up and purification processes also become problematic. To avoid these complications, solid-phase peptide synthesis was employed.



H-Phe-2Cl-Trt resin

Figure 23 – An example of a commercially available resin loaded with an amino acid.

From **Section 3.8**, it was identified that a fully protected peptide was necessary in order to facilitate olefination on phenylalanine residues neighbouring polar amino acids. This meant that a resin and linker combination that could be cleaved from the peptide using a base, or a weak acid was required. The identity of the desired product of the cleavage step was then considered. If the peptide could be isolated as a protected peptide-acid, an esterification or final solution-phase coupling would give the required protected peptide. 2-Cl-Trt resins, each pre-loaded with one of the naturally occurring amino acids were readily available and cheap to source from a chemical vendor, **Figure 23**. After assembly on-resin, when exposed to 25% HFIP in DCM, the peptide resin bond is cleaved and removal of volatiles *in vacuo* yields the peptide as a side-chain protected peptide-acid, **Scheme 78**.

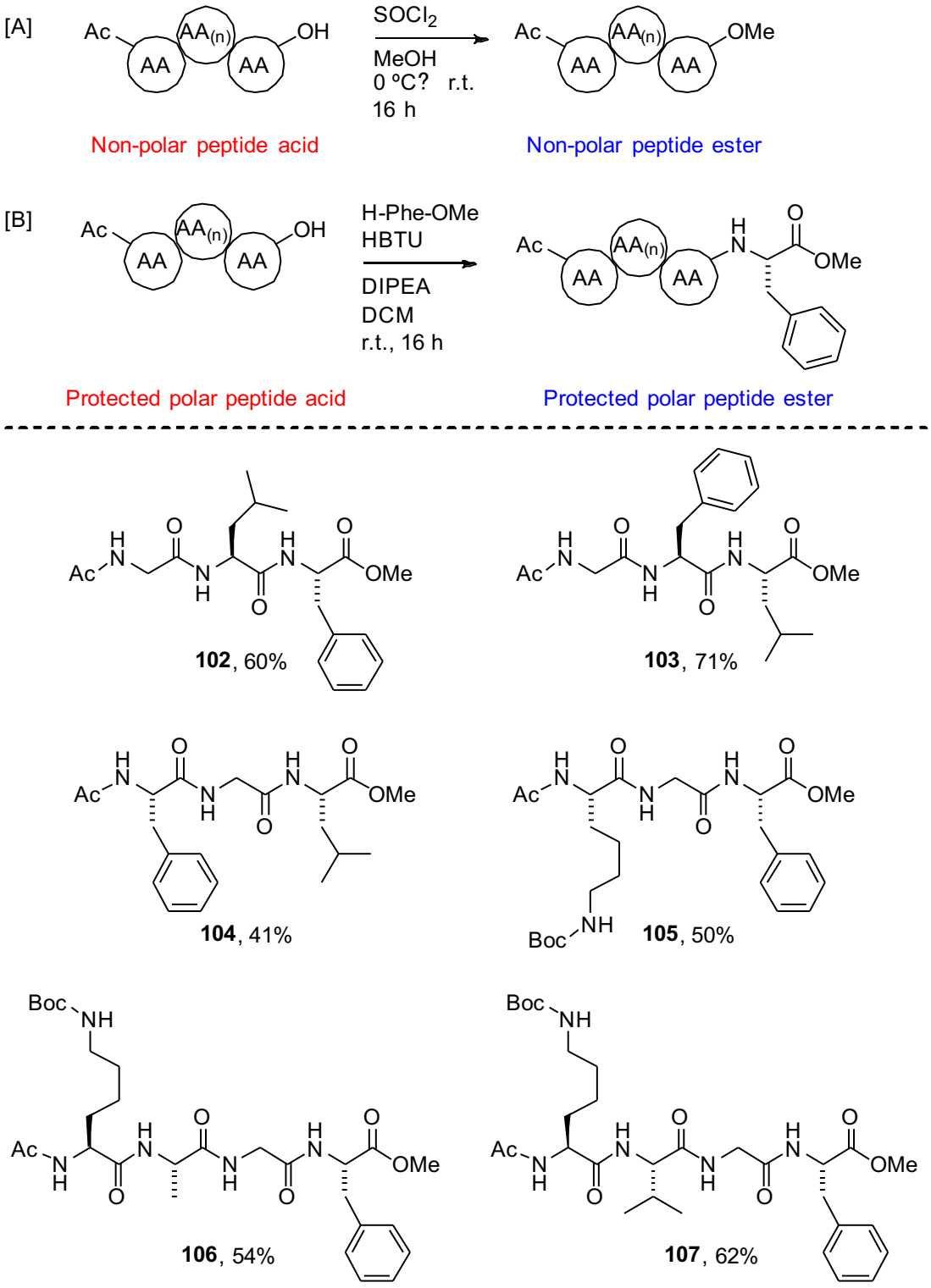


Scheme 78 – When exposed to the cleavage cocktail, the peptide-resin link is cleaved generating a protected peptide-acid.

Once this compound is isolated, a final manipulation must take place, the nature of which depends on the polarity of the residues in the peptide. If there are no protecting groups present i.e., in peptides with non-polar residues only, esterification of the carboxylic acid at the *C*-terminus can be carried out by exposure to thionyl chloride in methanol. However, if polar amino acids with acid labile protecting groups are present, the *C*-terminal residue must be installed by solution-phase peptide synthesis.

3.10.2 Synthesis of *tri-* and *tetra*-peptides

Tri- and tetrapeptides were selected for this study in order to gain information about the method viability of the olefination protocol. Initially non-polar tripeptides **102-104** were chosen as these were deemed to be the simplest substrates upon which to test the method. A tripeptide **105** and two tetrapeptides, **106** and **107**, containing lysine were also chosen to further broaden applicability. If the successful modification of **103-107** could be proven, these compounds would be used in a future experiment *vide infra*. The synthesis of peptides **102-107** can be seen in **Scheme 79**.



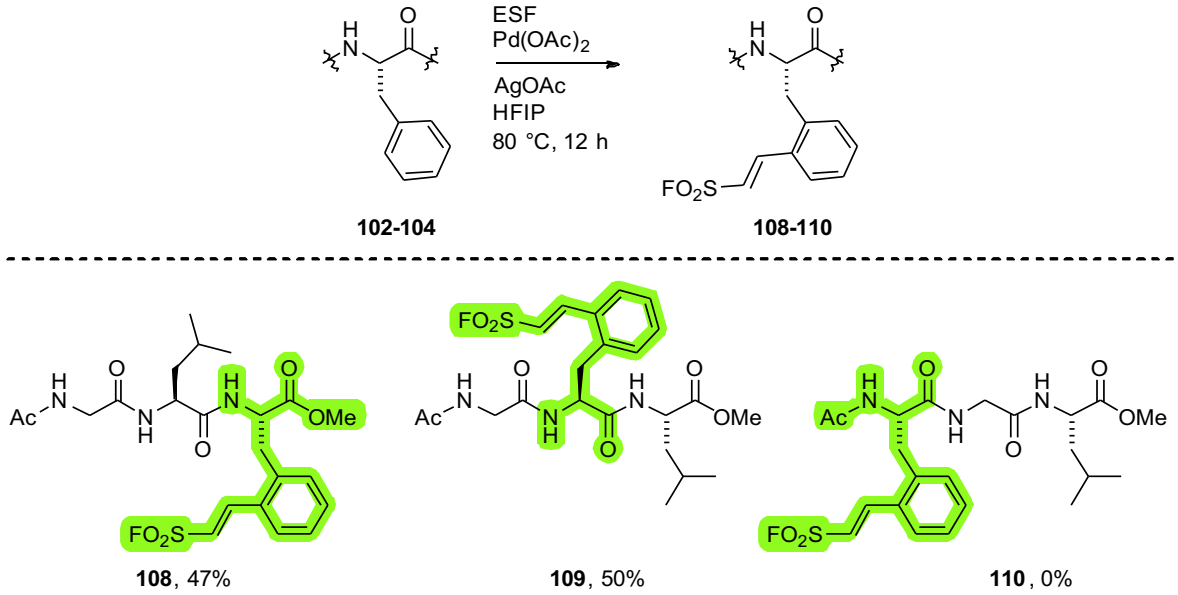
*Scheme 79 – Peptides synthesised in this study using SPPS. [A] highlights the capping step required for non-polar peptides **102-104** to install a methyl ester at the C-terminus. [B] demonstrates the capping step required for polar peptides **105-107** by the installation of an extra amino ester at the C-terminus. Yields are reported for the overall synthesis and capping of the peptide.*

Non-polar peptides **102-104** were synthesised from preloaded 2-Cl-Trt resin and elongated until the desired peptide chain had been assembled. The peptide was cleaved from the resin yielding a non-polar peptide acid which was then esterified with thionyl chloride in MeOH, **Scheme 79A**. Protected polar peptides **105-107** were assembled in the same

fashion. However, upon peptide cleavage, the final residue was installed on the *C*-terminus by solution phase peptide synthesis, **Scheme 79B**. All peptides where fully characterised by NMR spectroscopy and HRMS was used to corroborate successful syntheses.

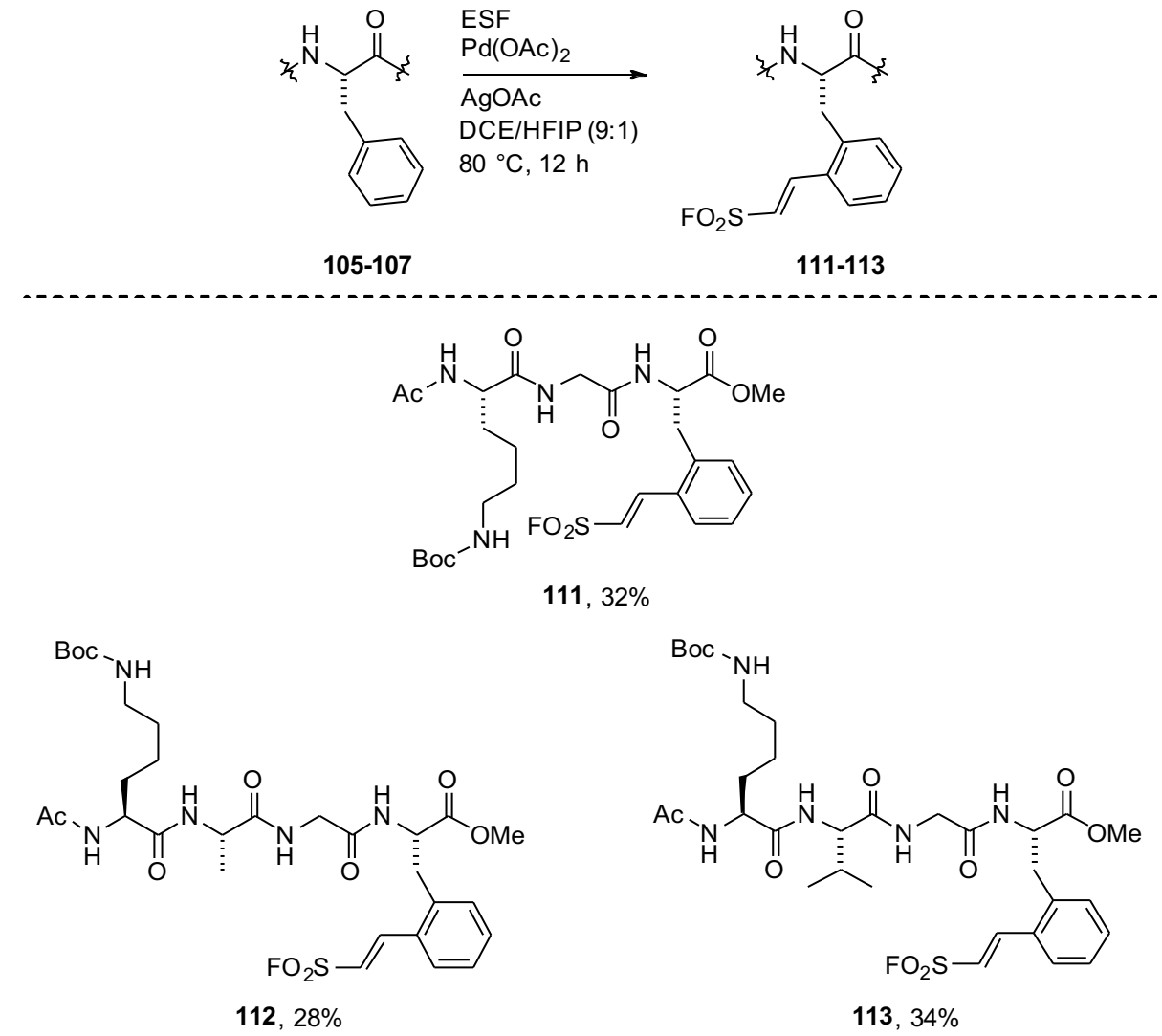
3.10.3 Olefination of *tri*- and *tetra*-peptides

Olefination of non-polar tripeptides **102-104** was carried out using conditions developed earlier in the study as in **Scheme 80**. The modified peptides were fully characterised as in accordance with peptide **51** in **Section 3.2**.



Scheme 80 – Olefination of phenylalanine residues at different positions on non-polar tripeptides.

When the residue was placed in the middle of the peptide or at the *C*-terminus, olefination proceeded well producing modified peptides **108** and **109** in good yield. However, where the phenylalanine residue was situated at the *N*-terminus no olefination reaction was observed to have taken place and peptide **110** was not synthesised. This result provides further evidence that the phenylalanine residue can potentially be situated anywhere except for the *N*-terminus in a peptide chain and modified using the developed method. Significantly, the result proves that modification on longer chained peptides is achievable and broadens method applicability.



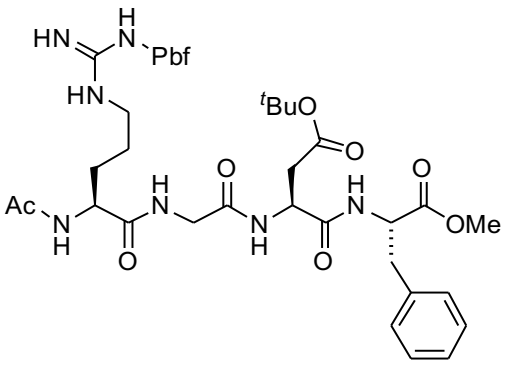
Scheme 81 – Olefination of protected polar tri- and tetra-peptides

Moreover, polar tri- and tetra-peptides were also modified with ESF using the C-H olefination reaction, **Scheme 81**. The polar peptides **105-107** were found to react cleanly to give modified peptides **111-113** in fair yields. No breakdown of starting material to unprotected peptide was observed. The successful olefination of tri-and tetra-peptides suggests that the method developed in this section is robust and likely viable for the modification of penta-peptides and further oligopeptides beyond.

3.10.4 Olefination of Ac-Arg(Pbf)-Gly-Asp(O^{t}Bu)-Phe-OMe (114)

In order to demonstrate the power of the developed methodology, it was applied post-synthetically to a peptide containing the biologically relevant RGD sequence **114**, **Figure 24**. Peptide **114** was synthesised by SPPS similarly to those in **Section 3.10.2**. Although

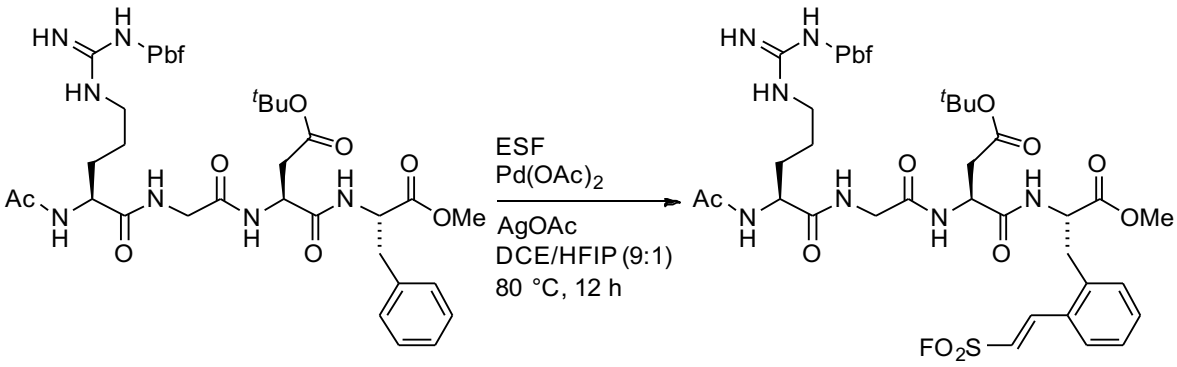
the olefination of phenylalanine in peptides containing arginine residues had already been proven to be problematic, it was hoped that by locating the residues on each end of the peptide chain, any issues caused by the arginine residue would be diluted.



114, 64%

Figure 24 – Peptide containing biologically relevant RGD motif for modification by developed protocol.

Protected RGD peptide **114** was exposed to the reaction conditions developed in the study, **Scheme 82**. The DCE/HFIP solvent system was used due to the presence of polar residues in the peptide sequence. Upon removal of the metal salts and volatiles from the reaction mixture, a crude HRMS was taken. The NMR spectra for this compound appeared to show little or no olefination had taken place and consisted of mainly starting material. However, a parent peak $[M+H]^+$ in the spectrum for **115** of 966.3756 was found, providing evidence that the modification attempt had likely been successful. Due to the time constraints of the project, an effort to purify the modified peptide was not attempted.



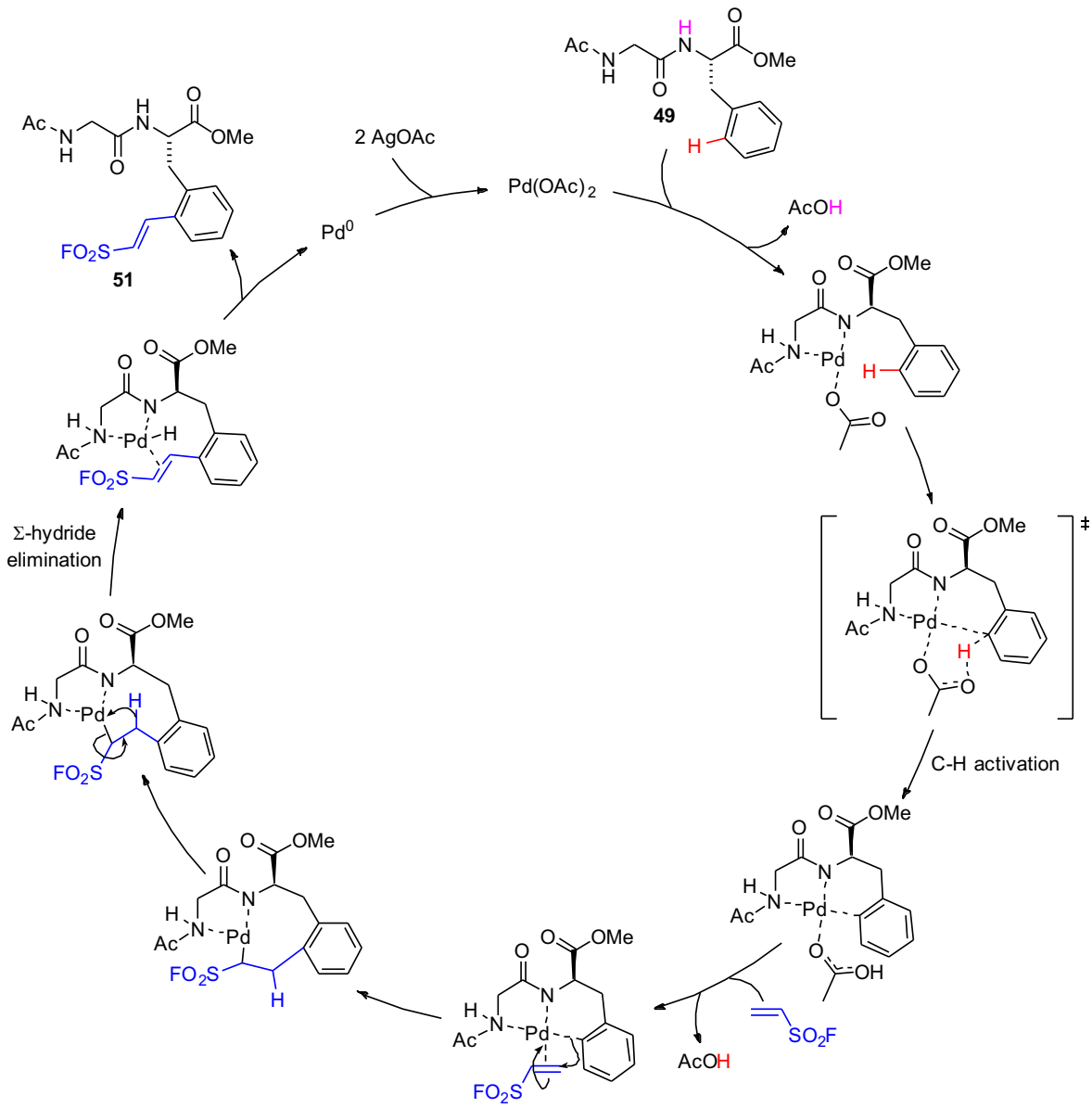
114 **115**

*Scheme 82 – The olefination of RGD peptide **114** containing a C-terminal phenylalanine residue.*

3.11 Mechanism

A significant proportion of the mechanistic pathway of the olefination of phenylalanine containing peptides is still not completely understood. However from the experimental findings, it is possible to suggest a plausable catalytic cycle for the olefination of **49** with ESF, **Scheme 83**.

Upon addition of peptide **49**, the palladium catalyst is coordinated via the amide nitrogen atoms. Deprotonation of the first amide is facilitated by the presence of an acetate ligand which dissociates. The *ortho* C(sp^2)-H bond is positioned in close proximity to the metal centre as a result of the chelation. The C-H activation then takes place via a concerted metallation deprotonation mechanism. The final protonated acetate ligand dissociates as ESF approaches. A formal bond to palladium is made along with a new C-C bond between the phenyl ring and the alkene. The alkene is then reformed as a result of β-hydride elimination which dissociates from the catalyst to give the desired product **51**. The catalyst is regenerated by oxidation using AgOAc. From here the catalytic cycle is repeated on new starting material or mono-olefinated peptide to yield the diolefinated product.



*Scheme 83 – Proposed catalytic cycle of the olefination of dipeptide **49**.*

3.12 Conclusion

In summary, a novel protocol has been described which installs the SuFEx-able hub, aryl VSF onto phenylalanine residues in peptides by C-H functionalization. The nature of the neighbouring amino acids was investigated and the vast majority of amino acids tolerated the reaction conditions (**Scheme 68** and **Scheme 75)**. Commonplace peptide protecting groups (**Scheme 71** and **Scheme 72**), position of residue (**Scheme 80)** and length of peptide (**Scheme 81**) have been extensively evaluated. Modification occurred in good yield on a range of different peptide substrates. The modification of tyrosine residues using the developed method has also been reported as in **Scheme 76**.

The procedure has been used to modify peptides at phenylalanine residues which are otherwise unreactive. The introduction of a new reactive handle, which can undergo SuFEx click chemistry transformations to generate further diverse structures is a significant step forward in the post-synthetic modification of peptides by C-H functionalization. Some of the structures synthesised in this section will be used in future chapters to achieve other aims outlined in **Section 1.5**.

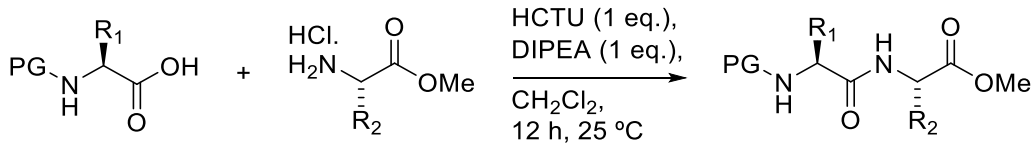
3.13 Experimental

3.13.1 General Information

All reagents and solvents were purchased from Alfa Aesar, Fischer Scientific, Fluorochem or Merck and used as provided. Chemical manipulations were carried out in oven-dried glassware and in an atmosphere of air unless otherwise stated. Flash column chromatography was performed manually on silica gel (Fluorochem silica gel 60 A particle size 40-63 μm) or on a Biotage® Isolera One auto column using Biotage® Sfar silica Duo pre-loaded flash columns. Thin layer chromatography was carried out on glass-backed silica gel plates (2.5 x 7.5 cm; Merck, TLC silica gel 60 Å). Compounds were visualised on TLC plates by exposure to UV light (254 nm). NMR spectra were recorded on a JEOL ECX or ECZ 400 Spectrometer at 298K. Chemical shifts are reported in parts per million and coupling constants are reported in Hz. For some compounds, it was necessary to use ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC 2D NMR experiments in order to aid assignment. FTIR spectra were recorded on an Agilent Cary 630 FTIR spectrometer and wavenumbers are reported to the nearest whole number. Melting points of compounds were obtained using a Stuart SMP10 melting point apparatus. High-resolution mass spectrometry was obtained from the EPSRC UK National Mass Spectrometry Facility at Swansea University on an LTQ Orbitrap XL 1, using positive electrospray ionisation (ESI+).

3.13.2 Synthesis of peptides

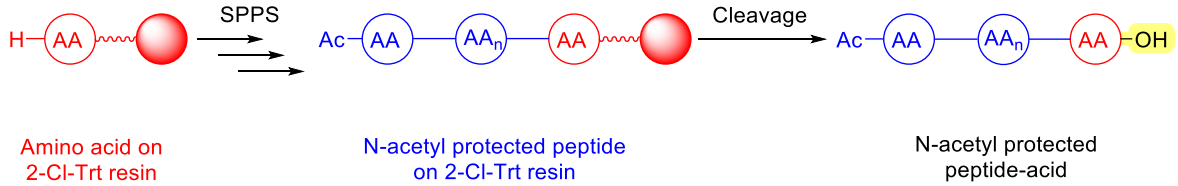
3.13.2.1 General procedure for the solution-phase synthesis of dipeptides



The L-amino ester hydrochloride salt (2.50 mmol) and K_2CO_3 (3.60 mmol) were dissolved in distilled water (30 mL) and stirred at room temperature for 20 min. The free amino ester was then extracted into Et_2O (3 x 20 mL) before the organic layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was dissolved in CH_2Cl_2 (30 mL) and treated with *N*-protected amino acid (1.0 mmol), HCTU (1.00 mmol) and DIPEA (1.00 mmol) before stirring for 12 h. The reaction mixture was then quenched with CH_2Cl_2 (30 mL), then washed with 1M HCl (25 mL), sat. $NaHCO_3$ (3 x 25 mL) and distilled water (25 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* before the resulting oil was recrystallised from CH_2Cl_2 / hexanes.

3.13.2.2 General procedure for the solid-phase synthesis of phenylalanine containing tri- or tetrapeptides

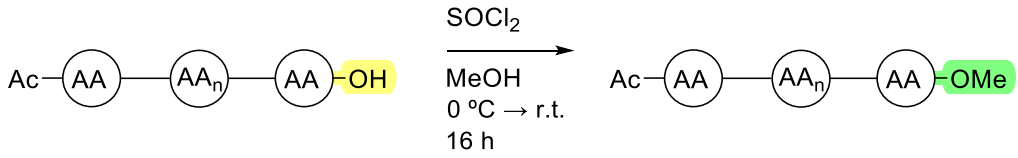
Solid-phase peptide synthesis was carried out on a Biotage® Initiator+ SP wave semi-automated peptide synthesiser. All syntheses were performed on a 0.2 mmol scale using standard Fmoc/^{t}Bu chemistry. Mixing was performed in the microwave cavity by vortexing the reaction vessel at 500 rpm. 2-chlorotrityl resin, preloaded with the relevant amino acids were swelled in DMF for 30 mins prior to reaction. Fmoc-protected amino acids (0.2 M) were activated using HBTU (0.228 g, 0.600 mmol) and DIPEA (0.209 mL, 1.20 mmol) in DMF (3 mL) for 2 min before microwave-assisted synthesis at 75 ºC for 5 min. *N*-terminal Fmoc deprotection was achieved by exposing the resin to 20% piperidine in DMF for 3 min before draining, followed by a further 10 min at room temperature then draining. *N*-acetyl capping was performed by introduction of 3:2 acetic acid / pyridine capping solution to the resin for 30 min. After every step the resin was washed with DMF (4 x 5 mL). The target elongated peptide chain on resin was washed with CH_2Cl_2 (2 x 5 mL) prior to cleavage.



Cleavage of the elongated peptide from the resin was carried out by stirring the resin in cleavage cocktail 25%(w/w) HFIP in CH_2Cl_2 (7.5 mL) for 30 mins. The cocktail was then

filtered, and the resin washed with MeOH (3 x 10 mL) and CH_2Cl_2 (3 x 10 mL). The resin was then exposed to fresh cleavage cocktail (7.5 mL) for a further 1 hr before the solution was filtered and the resin washed with MeOH (3 x 10 mL) and CH_2Cl_2 (3 x 10 mL). The filtrates were then combined, and solvent removed *in vacuo* yielding the peptide acid.

3.13.2.3 Peptides containing non-polar amino acids

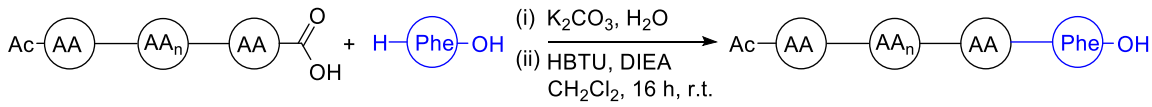


N-acetyl protected peptide-acid

Esterified N-acetyl protected non-polar amino acid

Esterification of non-polar tri-/tetrapeptides was achieved by dissolution of the peptide acid in MeOH (10 mL) at 0 °C. The solution was then treated with the dropwise addition of $SOCl_2$ (0.087 mL, 1.20 mmol) and stirred for 1 hr before allowing the reaction to warm to room temperature and stirring for a further 16 hr. The solvents were then removed *in vacuo* and the crude white solid residue was dissolved in CH_2Cl_2 (20 mL), washed with sat. $NaHCO_3$ solution (3 x 5 mL) and distilled water (1 x 5 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo* to yield the non-polar tri-/tetrapeptide and a white solid.

3.13.2.4 Peptides containing polar protected amino acids



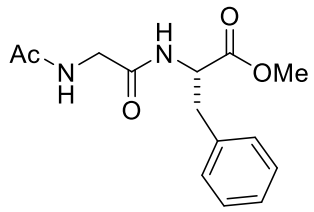
N-acetyl protected peptide-acid

Amino ester

Fully protected polar peptide

The L-phenylalanine methyl ester hydrochloride salt (0.100 g, 0.50 mmol) and K_2CO_3 (0.108 g, 0.720 mmol) were dissolved in distilled water (20 mL) and stirred at room temperature for 20 min. The free amino ester was then extracted into Et_2O (3 x 10 mL) before the organic layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was dissolved in CH_2Cl_2 (30 mL) and treated with *N*-acetyl protected peptide-acid, HBTU (0.076 g, 0.20 mmol) and DIPEA (0.035 mL, 0.20 mmol) before stirring for 16 h. The reaction mixture was then quenched with CH_2Cl_2 (30 mL) and washed with 1M HCl (15 mL), sat. $NaHCO_3$ (3 x 15 mL) and distilled water (15 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was then recrystallised from CH_2Cl_2 / hexanes.

3.13.2.5 Ac-Gly-Phe-OMe (49)



Known compound. Peptide **49** was synthesised from L-phenylalanine methyl ester **22** hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl glycine **26** (0.117 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. The crude compound was recrystallised from CH_2Cl_2 / hexanes to afford **49** as a white solid (0.234 g, 84%); m.p. 95-97 °C. R_f = 0.15 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (3H, s, acetyl-C*H*$_3$), 3.08 (1H, dd, *J* = 14.0, 6.2, Phe-C*H*H), 3.15 (1H, dd, *J* = 14.0, 5.7, Phe-CH*H*), 3.74 (3H, s, ester-C*H*$_3$), 3.84-3.96 (2H, m, Gly-C*H*$_2$), 4.87 (1H, dt, *J* = 7.8, 6.0, Phe-α-C*H*), 6.24 (1H, br t, *J* = 5.4, Gly-N*H*), 6.46 (1H, br d, *J* = 7.8, Phe-N*H*), 7.07-7.13 (2H, m, Ar-*H*), 7.22-7.34 (3H, Ar-*H*).

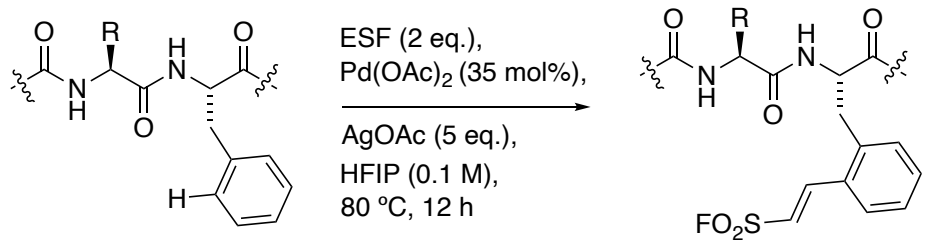
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 37.8 (Phe-CH_2), 43.1 (Gly-CH_2), 52.5 (ester-CH_3), 53.2 (Phe-α-CH), 127.3 (Ar C), 128.7 (Ar C), 129.2 (Ar C), 135.5 (4° Ar C), 168.4 (C=O), 170.5 (C=O), 171.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3299 w (N-H), 3068 w (C-H), 2952 w (C-H), 1711 s (ester C=O), 1655 s (amide C=O), 1543 s (C=C), 1260 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{14}H_{19}N_2O_4$: 279.1345, found: 279.1345.

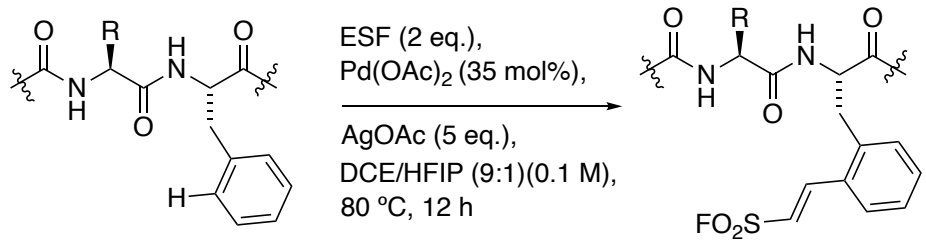
3.13.3 Olefination of peptides

3.13.3.1 General procedure for the olefination of phenylalanine containing 'non-polar' peptides using ethene sulfonyl fluoride as a coupling partner



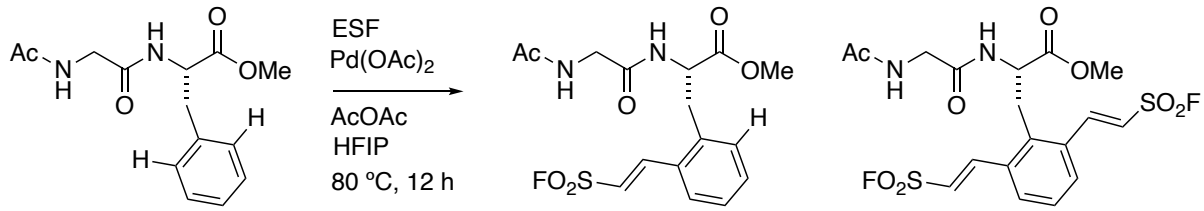
Non-polar amino acid containing peptide (0.30 mmol), $Pd(OAc)_2$ (0.024 g, 0.11 mmol, 35 mol%), AgOAc (0.250 g, 1.50 mmol) and ESF (0.049 mL, 0.60 mmol) were dissolved in HFIP (3 mL) and stirred at 80 ºC in a sealed vial for 12 h. The resulting mixture was filtered through a Celite plug and the filtrate was concentrated *in vacuo*. The crude product was then purified by flash column chromatography before recrystallised from CH_2Cl_2 / hexanes.

3.13.3.2 General procedure for the olefination of phenylalanine containing 'polar' peptides using ethene sulfonyl fluoride as a coupling partner



Polar amino acid containing peptide (0.20 mmol), $Pd(OAc)_2$ (0.016 g, 0.07 mmol, 35 mol%), AgOAc (0.167 g, 1.00 mmol) and ESF (0.034 mL, 0.40 mmol) were dissolved in 9:1 DCE/HFIP (2 mL) and stirred at 80 ºC in a sealed vial for 12 h. The resulting mixture was filtered through a Celite plug and the filtrate was concentrated *in vacuo*. The crude product was then purified by flash column chromatography before being recrystallised from CH_2Cl_2 / hexanes.

3.13.3.3 Olefination of model dipeptide 49; synthesis of modified peptides 51 and 51'



Modified dipeptide **51** was synthesised from Ac-Gly-Phe-OMe **49** (0.084 g, 0.300 mmol) and ESF **50** (0.050 mL, 0.600 mmol) according to the procedure outlined in **Section 3.13.3.1**. The resulting crude product contained a mixture of mono-olefinated **51** and di-olefinated **51'** peptides in an 8:1 ratio. Purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **51** as an off-white solid (0.089 g, 77%) m.p. 138-140 ºC, R_f = 0.30 (8:2 EtOAc / Pet. Ether 40-60 °C).

Data for 51:

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (3H, s, acetyl-C*H*$_3$), 3.22 (1H, dd, *J* = 14.4, 6.5, Phe-C*H*H), 3.36 (1H, dd, 14.4, 6.0, Phe-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 3.84 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.96 (1H, dd, *J* = 16.8, 5.5, Gly-CH*H*), 4.79 (1H, dt, *J* = 7.3, 6.5, Phe-α-C*H*), 6.63 (1H, br t, *J* = 5.0, Gly-N*H*), 6.88 (1H, dd, *J* = 15.3, 2.5, alkene-C*H*), 7.20 (1H, d, *J* = 7.8, Phe-N*H*), 7.25 (1H, dd, *J* = 7.7, 1.0, Ar-*H*), 7.35 (1H, dt, *J* = 7.6, 1.0, Ar-*H*), 7.45 (1H, dt, *J* = 7.6, 1.4, Ar-*H*), 7.59 (1H, dd, *J* = 8.0, 0.9, Ar-*H*), 8.12 (1H, d, *J* = 15.3, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 34.7 (Phe-CH_2), 43.0 (Gly-CH_2), 52.7 (ester-CH_3), 53.4 (Phe-α-CH), 119.4 (d, $^3J_{CF}$ = 27.9, alkene-CH),127.4 (Ar C), 128.1 (Ar C), 130.2 (4º Ar C), 131.5 (Ar C), 132.4 (Ar C). 137.3 (4º Ar C), 145.7 (d, $^4J_{CF}$ = 1.9, alkene-CH), 169.0 (C=O), 170.9 (C=O), 171.1 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.0 (s).

IR υ_{max}/cm^{-1} (solid) 3314 w (N-H), 3071 w (C-H), 2952 w (C-H), 1730 m (ester C=O), 1640 s (amide C=O), 1531 s (C=C), 1405 s (S=O), 1259 s (C-O), 1189 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{16}H_{20}N_2O_6SF$: 387.1026, found: 387.1027.

Data for 51':

Purification by flash column chromatography (70% EtOAc in pet. ether) followed by recrystallisation from CH_2Cl_2 / hexanes gave modified peptide **51'** as an off-white solid (0.015 g, 10%) m.p. 194-196 ºC, R_f = 0.45 (85% EtOAc in pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (3H, s, acetyl-CH_3), 3.34 (1H, dd, *J* = 14.9, 8.0, Phe-C*H*H), 3.48 (1H, dd, *J* = 14.9, 5.5, Phe-CH*H*), 3.77 (3H, s, ester-CH_3), 3.88 (2H, d, *J* = 5.5, Gly-CH_2), 4.68 (1H, dt, *J* = 8.0, 5.5, Phe-α-C*H*), 6.30 (1H, br t, *J* = 5.0, Gly-N*H*), 6.94 (2H, dd, *J* = 15.2, 2.4, alkene-C*H*), 7.29 (1H, d, *J* = 8.0, Phe-N*H*), 7.49 (1H, t, *J* = 7.8, Ar-*H*), 7.72 (2H, d, *J* = 7.8, Ar-*H*), 8.25 (2H, d, *J* = 15.2, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 32.1 (Phe-CH_2), 43.1 (Gly-CH_2), 53.2 (ester-CH_3), 53.2 (Phe-α-CH), 122.0 (d, $^3J_{CF}$ = 27.9, alkene-C), 128.7 (Ar C), 131.0 (2C, Ar C), 132.5 (2C, 4º Ar C), 137.8 (4º Ar C), 145.3 (d, $^4J_{CF}$ = 2.9, alkene-C), 169.1 (C=O), 170.5 (C=O), 171.0 (C=O).

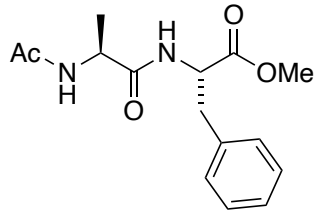
^{19}F NMR (376 MHz, $CDCl_3$) δ 61.7 (s).

IR υ_{max}/cm^{-1} (solid) 3302 w (N-H), 3086 w (C-H), 2956 w (C-H), 1744 m (ester C=O), 1655 (amide C=O), 1536 m (C=C), 1402 s (S=O), 1290 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{21}N_2O_8S_2F_2$: 495.0707, found: 495.0703.

3.13.4 Synthesis of dipeptides (52-57)

3.13.4.1 Ac-Ala-Phe-OMe (52)



Known compound. Peptide **52** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-alanine (0.131 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **52** as a white solid (0.222 g, 76%); m.p. 124-126 ºC; R_f = 0.30 (EtOAc).

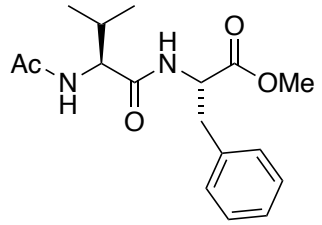
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, d, *J* = 7.1, Ala-C*H*$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 3.07 (1H, dd, *J* = 14.0, 6.4, Phe-C*H*H), 3.17 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.74 (3H, s, ester-C*H*$_3$), 4.44 (1H, quin, *J* = 7.1, Ala-α-C*H*), 4.84 (1H, dt, *J* = 7.9, 6.4, Phe-α-C*H*), 5.96 (1H, br d, *J* = 6.9, Ala-N*H*), 6.43 (1H, br d, *J* = 7.9, Phe-N*H*), 7.08-7.14 (2H, m, Ar-*H*), 7.23-7.33 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (Ala-CH_3), 23.2 (acetyl-CH_3), 37.7 (Phe-CH_2), 48.7 (Ala-α-CH), 52.5 (ester-CH_3), 53.2 (Phe-α-CH), 127.2 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 135.6 (4° Ar C), 169.9 (C=O), 171.6 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3284 w (N-H), 3064 w (C-H), 2974 w (C-H), 1748 m (ester C=O), 1640 s (amide C=O), 1543 s (C=C), 1215 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4$: 293.1501, found: 293.1498.

3.13.4.2 Ac-Val-Phe-OMe (53)



Known compound. Peptide **53** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-valine (0.159 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **53** as a white solid (0.163 g, 51%); m.p. 171-173 ºC; R_f = 0.40 (EtOAc).

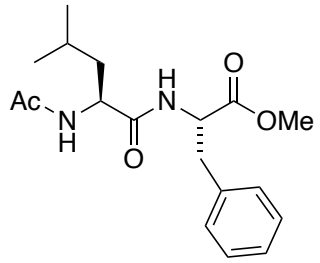
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, d, *J* = 6.3, Val-C*H*$_3$), 0.93 (3H, d, *J* = 6.3, Val-C*H*$_3$), 1.96-2.08 (4H, m, acetyl-C*H*$_3$ / Val-C*H*$(CH_3)_2$), 3.08 (1H, dd, *J* = 14.0, 6.4, Phe-C*H*H), 3.13 (1H, dd, *J* = 14.0, 5.8, Phe-CH*H*), 3.72 (3H,s, ester-C*H*$_3$), 4.27 (1H, dd, *J* = 8.6, 6.9, Val-α-C*H*), 4.86 (1H, dt, *J* = 7.7, 6.1, Phe-α-C*H*), 6.12 (1H, br d, *J* = 8.5, Val-N*H*), 6.38 (1H, br d, *J* = 7.6, Phe-N*H*), 7.07-7.13 (2H, m, Ar-*H*), 7.21-7.32 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (Val-CH_3), 19.0 (Val-CH_3), 23.2 (acetyl-CH_3), 31.2 (Val-CH), 37.8 (Phe-CH_2), 52.3 (ester-CH_3), 53.1 (Phe-α-CH), 58.2 (Val-α-CH), 127.2 (4° Ar C), 128.7 (Ar C), 129.2 (Ar C), 135.5 (4° Ar C), 169.9 (C=O), 170.9 (C=O), 171.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3306 w (N-H), 3064 w (C-H), 2960 w (C-H), 1733 s (ester C=O), 1633 s (amide C=O), 1536 s (C=C), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4$: 321.1814, found: 321.1813.

3.13.4.3 Ac-Leu-Phe-OMe (54)



Known compound. Peptide **54** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-leucine (0.173 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **54** as a white solid (0.197 g, 59%); m.p. 110-112 ºC. R_f = 0.50 (EtOAc).

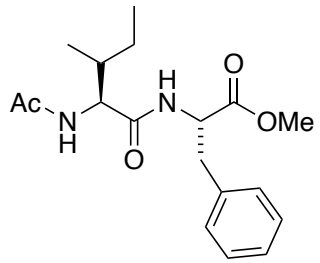
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.94 (6H, m, Leu-(C*H*$_3$)$_2$), 1.42-1.53 (1H, m, Leu-C*H*), 1.57-1.68 (2H, m, Leu-C*H*$_2$), 1.97 (3H, s, acetyl-C*H*$_3$), 3.06 (1H, dd, *J* = 14.0, 6.6, Phe-C*H*H), 3.16 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.73 (3H, s ester-C*H*$_3$), 4.42 (1H, dt, *J* = 8.4, 5.5, Leu-α-C*H*), 4.84 (1H, dt, *J* = 7.7, 6.2, Phe-α-C*H*), 5.81 (1H, br d, *J* = 6.9, Leu-N*H*), 6.47 (1H, br d, *J* = 6.9, Phe-N*H*), 7.08-7.16 (2H, m, Ar-*H*), 7.21-7.32 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1 (Leu-CH_3), 22.8 (Leu-CH_3), 23.1 (acetyl-CH_3), 24.7 (Leu-CH), 37.8 (Phe-CH_2), 40.9 (Leu-CH_2), 51.5 (Leu-α-CH), 52.4 (ester-CH_3), 53.2 (Phe-α-CH), 127.1 (4º Ar C), 128.6 (Ar C), 129.3 (Ar C), 135.7 (4º Ar C), 170.0 (C=O), 171.6 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3265 w (N-H), 3068 w (C-H), 2952 w (C-H), 1756 s (ester C=O), 1633 s (amide C=O), 1543 s (C=C), 1200 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{27}N_2O_4$: 335.1971, found: 335.1965.

3.13.4.4 Ac-Ile-Phe-OMe (55)



Known compound. Peptide **55** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-isoleucine (0.173 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **55** as a white solid (0.161 g, 48%); m.p. 190-192 ºC; R_f = 0.50 (EtOAc).

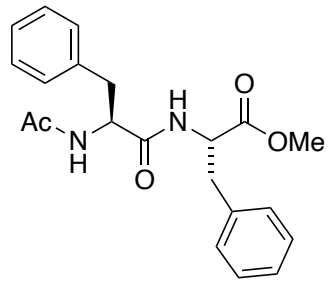
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.91 (6H, m, Ile-C*H*$_3$ / Ile-C*H*$_3$), 1.05-1.18 (1H, m, Ile-C*H*H), 1.42-1.51 (1H, m, Ile-CH*H*), 1.74-1.84 (1H, m, Ile-C*H*), 2.00 (3H, s, acetyl-C*H*$_3$), 3.08 (1H, dd, *J* = 14.0, 6.1, Phe-C*H*H), 3.14 (1H, dd, *J* = 13.7, 7.7, Phe-CH*H*), 3.73 (1H, s, ester-C*H*$_3$), 4.25 (1H, dd, *J* = 8.6, 6.9, Ile-α-C*H*), 4.87 (1H, dt, *J* = 7.9, 6.1, Phe-α-C*H*), 5.98 (1H, d, *J* = 8.6, Ile-N*H*), 6.15 (1H, d, *J* = 7.9, Phe-N*H*), 7.08-7.13 (2H, m, Ar-*H*), 7.22-7.33 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3 (Ile-CH_3), 15.2 (Ile-CH_3), 23.3 (acetyl-CH_3), 24.9 (Ile-CH), 37.4 (Phe-CH_2), 37.9 (Ile-CH_2), 52.4 (ester-CH_3), 53.1 (Phe-α-CH), 57.5 (Ile-α-CH), 127.2 (Ar C), 128.7 (Ar C), 129.2 (Ar C), 135.5 (Ar C), 169.8 (C=O), 170.8 (C=O), 171.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3261 w (N-H), 3026 w (C-H), 2952 w (C-H), 1741 s (ester C=O), 1648 s (amide C=O), 1539 s (C=C), 1215 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{27}N_2O_4$: 335.1971, found: 335.1965.

3.13.4.5 Ac-Phe-Phe-OMe (56)



Known compound. Peptide **56** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-phenylalanine (0.207 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **56** as a white solid (0.265 g, 72%); m.p. 178-179 ºC. R_f = 0.30 (EtOAc).

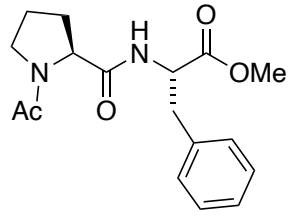
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (3H, s, acetyl-C*H*$_3$), 2.93-3.13 (4H, m, Phe-C*H*$_2$ / Phe-C*H*$_2$), 3.69 (3H, s, ester-C*H*$_3$), 4.62 (1H, dt, *J* = 7.3, 6.9, Phe-α-C*H*), 4.75 (1H, dt, *J* = 7.1, 6.4, Phe-α-C*H*), 6.03 (1H, d, *J* = 7.1, Phe-N*H*), 6.17 (1H, d, *J* = 6.9, Phe-N*H*), 6.98-7.04 (2H, m, Ar-*H*), 7.17-7.32 (8H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1 (acetyl-CH_3), 37.8 (Phe-CH_2), 38.1 (Phe-CH_2), 52.3 (Phe-α-CH), 53.3 (ester-CH_3), 54.2 (Phe-α-CH), 127.0 (Ar C), 127.1 (Ar C), 128.6 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 129.3 (Ar C), 135.5 (4º Ar C), 136.3 (4º Ar C), 169.9 (C=O), 170.3 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3306 w (N-H), 3034 w (C-H), 2974 w (C-H), 1733 s (ester C=O), 1648 s (amide C=O), 1536 s (C=C), 1282 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{21}H_{25}N_2O_4$: 369.1814, found: 369.1814.

3.13.4.6 Ac-Pro-Phe-OMe (57)



Known compound. Peptide **57** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-proline (0.157 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **57** as a white solid (0.162 g, 51%); m.p. 104-105 ºC. R_f = 0.15 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70-1.81 (1H, m, Pro-C*H*$_2$), 1.86-1.97 (2H, m, Pro-C*H*$_2$), 2.01 (3H, s, acetyl-C*H*$_3$), 2.33-2.41 (1H, m, Pro-C*H*$_2$), 2.97 (1H, dd, *J* = 14.0, 7.8, Phe-C*H*H), 3.21 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.32 (2H, t, *J* = 7.0, Pro-C*H*$_2$), 3.73 (3H, s, ester-C*H*$_3$), 4.56 (1H, d, *J* = 7.8, Pro-α-C*H*), 4.83 (1H, dt, *J* = 7.8, 5.5, Phe-α-C*H*), 7.11-7.16 (2H, m, Ar-*H*), 7.19-7.34 (3H, m, Ar-*H*), 7.55 (1H, d, *J* = 7.8, Phe-N*H*).

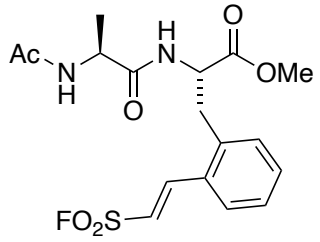
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4 (acetyl-CH_3), 24.8 (Pro-CH_2), 27.0 (Pro-CH_2), 37.8 (Phe-CH_2), 48.0 (Pro-CH_2), 52.3 (ester-CH_3), 53.2 (Phe-α-CH), 59.3 (Pro-α-CH), 126.8 (Ar C), 128.3 (Ar C), 129.3 (Ar C), 136.3 (Ar C), 170.8 (C=O), 170.8 (C=O), 171.9 (Ar C).

IR υ_{max}/cm^{-1} (solid) 3302 w (N-H), 3034 w (C-H), 2952 w (C-H), 1737 s (ester C=O), 1632 s (amide C=O), 1531 s (C=C), 1215 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{23}N_2O_4$: 319.1658, found: 319.1654.

3.13.5 Synthesis of modified dipeptides (58-63)

3.13.5.1 Ac-Ala-Phe(2-VSF)-OMe (58)



Modified dipeptide **58** was synthesised from Ac-Ala-Phe-OMe **52** (0.088 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **58** as a light brown solid (0.082 g, 68%); m.p. 172-174 °C; R_f = 0.25 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, d, *J* = 7.0, Ala-C*H*$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 3.22 (1H, dd, *J* = 14.4 , 6.4, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, 6.2, Phe-CH*H*), 3.72 (3H, s, ester-C*H*$_3$), 4.51 (1H, quin, *J* = 7.0, Ala-α-C*H*), 4.77 (1H, dt, *J* = 7.8, 6.2, Phe-α-C*H*), 6.32 (1H, d, *J* = 7.6, Ala-N*H*), 6.87 (1H, dd, *J* = 15.3, 2.5, alkene-C*H*), 7.07 (1H, d, *J* = 7.8, Phe-N*H*), 7.26 (1H, dd, *J* = 7.7, 1.1, Ar-*H*), 7.34 (1H, dt, *J* = 7.6, 0.9, Ar-*H*), 7.43 (1H, dt, *J* = 7.4, 1.4, Ar-*H*), 7.58 (1H, dd, *J* = 7.8, 0.9, Ar-*H*), 8.14 (1H, d, *J* = 15.3, alkene-C*H*).

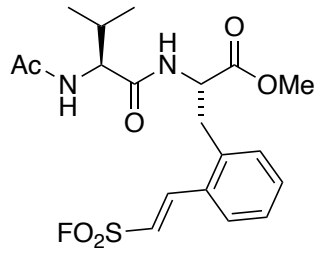
^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (Ala-CH_3), 23.0 (acetyl-CH_3), 34.7 (Phe-CH_2), 48.7 (Ala-α-CH), 52.7 (ester-CH_3), 53.3 (Phe-α-CH), 119.5 (d, $^3J_{CF}$ = 27.9, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.2 (4° Ar C), 131.6 (Ar C), 132.3 (Ar C), 137.3 (4° Ar C), 145.7 (d, $^4J_{CF}$ = 2.9, alkene-C) 170.1 (C=O), 171.1 (C=O), 172.3 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3288 m (N-H), 3030 w (C-H), 2974 w (C-H), 1737 s (ester C=O), 1633 s (amide C=O), 1521 s (C=C), 1405 m (S=O), 1260 m (C-O), 1197 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{22}N_2O_6SF$: 401.1183, found: 401.1179.

3.13.5.2 Ac-Val-Phe(2-VSF)-OMe (59)



Modified dipeptide **59** was synthesised from Ac-Val-Phe-OMe **53** (0.096 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (80% EtOAc in Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **59** as a light brown solid (0.095 g, 74%); m.p. 210-212 °C; R_f = 0.40 (80% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90-0.95 (6H, m, Val-(C*H*$_3$)$_2$), 1.97-2.08 (4H, m, acetyl-C*H*$_3$ / Val-C*H*, 3.20 (1H, dd, *J* = 14.4, 6.4, Phe-C*H*H), 3.34 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.28 (1H, dd, *J* = 8.7, 7.1, Val-α-C*H*), 4.81 (1H, dt, *J* = 7.8, 6.4, Phe-α-C*H*), 6.15 (1H, d, *J* = 8.7, Val-N*H*), 6.74 (1H, d, 7.8, Phe-N*H*), 6.87 (1H, dd, *J* = 15.3, 2.3, alkene-C*H*), 7.25 (1H, dd, *J* = 7.8, 0.9, Ar-*H*), 7.35 (1H, dt, *J* = 7.6, 0.7, Ar-*H*), 7.43 (1H, dt, *J* = 7.6, 1.4, Ar-*H*), 7.58 (1H, dd, *J* = 7.8, 0.7, Ar-*H*), 8.15 (1H, d, *J* = 15.3, alkene-C*H*).

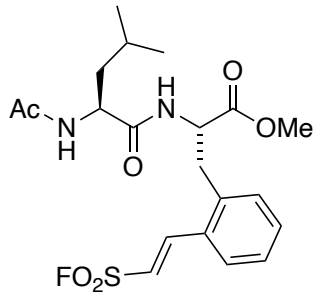
^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.0 (Val-CH_3), 23.2 (acetyl-CH_3), 31.1 (Val-CH), 34.9 (Phe-CH_2), 52.7 (ester-CH_3), 53.0 (Phe-α-CH), 58.4 (Val-α-CH), 119.7 (d, $^3J_{CF}$ = 27.9, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.2 (4° Ar C), 131.6 (Ar C), 132.3 (Ar C), 137.0 (4° Ar C),145.6 (d, $^4J_{CF}$ = 1.9, alkene-C), 170.1 (C=O), 171.1 (C=O), 171.2 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3288 m (N-H), 3083 w (C-H), 2960 w (C-H), 1759 s (ester C=O), 1640 s (amide C=O), 1543 s (C=C), 1402 s (S=O), 1215 s (C-O), 1191 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_2O_6SF$: 429.1496, found: 429.1484.

3.13.5.3 Ac-Leu-Phe(2-VSF)-OMe (60)



Modified dipeptide **60** was synthesised from Ac-Leu-Phe-OMe **54** (0.096 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (60% EtOAc in Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **60** as a light brown solid (0.089 g, 67%); m.p. 165-167 °C; R_f = 0.20 (60% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.92 (3H, d, *J* = 6.4, Leu-C*H*$_3$), 1.44-1.53 (1H, m, Leu-C*H*), 1.55-1.67 (2H, m, Leu-C*H*$_2$), 1.99 (3H, s, acetyl-C*H*$_3$), 3.20 (1H, dd, *J* = 14.4, 6.3, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.42 (1H, dt, *J* = 8.3, 5.6, Leu-α-C*H*), 4.78 (1H, dt, *J* = 7.7, 6.3, Phe-α-C*H*), 5.88 (1H, d, *J* = 8.3, Leu -N*H*), 6.68 (1H, d, *J* = 7.7, Phe-N*H*), 6.86 (1H, 15.3, 2.4, alkene-C*H*), 7.26 (1H, m, Ar-*H*), 7.35 (1H, dt, *J* = 7.6, 0.7, Ar-*H*), 7.44 (1H, dt, *J* = 7.6, 1.1, Ar-*H*), 7.57 (1H, d, *J* = 7.6, Ar-*H*), 8.14 (1H, d, *J* = 15.3, alkene-C*H*).

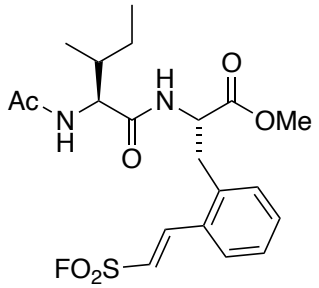
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1 (Leu-CH_3), 22.8 (Leu-CH_3), 23.1 (acetyl-CH_3), 24.7 (Leu-CH), 34.9 (Phe-CH_2), 40.8 (Leu-CH_2), 51.6 (Leu-α-CH), 52.7 (ester-CH_3), 53.1 (Phe-α-CH) 119.6 ($^3J_{CF}$ = 27.9, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.2 (Ar C), 131.7 (Ar C), 132.3 (Ar C), 137.2 (Ar C), 145.74 ($^4J_{CF}$ = 1.9, alkene-C), 170.2 (C=O), 171.0 (C=O), 171.8 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3291 m (N-H), 3071 w (C-H), 2956 w (C-H), 1741 s (ester C=O), 1648 s (amide C=O), 1532 s (C=C), 1409 s (S=O), 1215 m (C-O), 1197 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{28}N_2O_6SF$: 443.1652, found: 443.1645.

3.13.5.4 Ac-Ile-Phe(2-VSF)-OMe (61)



Modified dipeptide **61** was synthesised from Ac-Ile-Phe-OMe **55** (0.100 g, 0.300 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (60% EtOAc in Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **61** as a light brown solid (0.082 g, 62%); m.p. 199-202 °C; R_f = 0.14 (60% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.93 (6H, m, Ile-(C*H*$_3$)$_2$), 1.05-1.18 (1H, m, Ile-C*H*H), 1.41-1.55 (1H, m, Ile-CH*H*), 1.73-1.85 (1H, m, Ile-CH$_3$C*H*CH$_2$), 2.00 (3H, s, acetyl-C*H*$_3$), 3.19 (1H, dd, *J* = 14.4, 6.6, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, 6.2, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.28 (1H, dd, *J* = 8.6, 7.6, Ile-α-C*H*), 4.82 (1H, dt, *J* = 7.8, 6.4, Phe-α-C*H*), 6.10 (1H, d, *J* = 8.6, Ile-N*H*), 6.61 (1H, d, *J* = 7.8, Phe-N*H*), 6.87 (1H, dd, *J* = 15.3, 2.5, alkene-C*H*), 7.25 (1H, d, *J* = 7.6, Ar-*H*), 7.35 (1H, t, *J* = 7.6, Ar-*H*), 7.44 (1H, t, *J*= 7.6, Ar-*H*), 7.57 (1H, d, *J* = 7.6,Ar-*H*), 8.15 (1H, d, *J* = 15.3, alkene-C*H*).

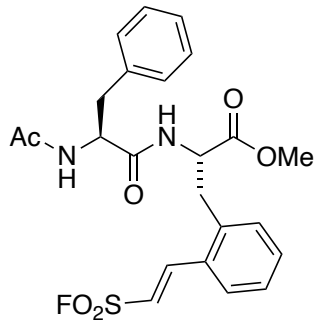
^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2 (Ile-CH_3), 15.2 (Ile-CH_3), 23.2 (acetyl-CH_3), 25.0 (Ile-CH), 34.9 (Phe-CH_2), 37.3 (Ile-CH_2), 52.8 (ester-CH_3), 53.0 (Phe-α-CH), 57.6 (Ile-α-CH), 119.7 (d, $^3J_{CF}$ = 27.9 alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.2 (4° Ar C), 131.6 (Ar C), 132.3 (Ar C), 137.0 (4° Ar C), 145.6 (d, $^4J_{CF}$ = 1.8, alkene-C), 170.0 (C=O), 171.0 (C=O), 171.1 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3299 m (N-H), 3064 w (C-H), 2971 w (C-H), 1737 s (ester C=O), 1628 s (amide C=O), 1528 s (C=C), 1394 m (S=O), 1252 m (C-O), 1170 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{28}N_2O_6SF$: 443.1652, found: 443.1645.

3.13.5.5 Ac-Phe-Phe(2-VSF)-OMe (62)



Modified dipeptide **62** was synthesised from Ac-Phe-Phe-OMe **56** (0.111 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **62** as an off-white solid (0.089 g, 62%); m.p. 214-217 °C; R_f = 0.30 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (3H, s, acetyl-C*H*$_3$), 2.98 (1H, dd, *J* = 13.8, 7.7, Phe-C*H*H), 3.06 (1H, dd, *J* = 13.8, 6.7, Phe-CH*H*), 3.14 (1H, dd, *J* = 14.4, 6.2, Phe-C*H*H), 3.30 (1H, dd, *J* = 14.4, 6.2, Phe-CH*H*), 3.67 (3H, s, ester-C*H*$_3$), 4.59 (1H, dt, *J* = 7.7, 6.7, Phe-α-C*H*), 4.68 (1H, dt, *J* = 7.2, 6.2, Phe-α-C*H*), 6.08 (1H, d, *J* = 7.6, Phe-N*H*), 6.27 (1H, d, *J* = 7.4, Phe-N*H*), 6.82 (1H, dd, *J* = 15.2, 2.5, alkene-C*H*), 7.11-7.19 (3H, m, Ar-*H*), 7.21-7.30 (3H, m, Ar-*H*), 7.34 (1H, dt, *J* = 7.5, 1.2, Ar-*H*), 7.41 (1H, dt, *J* = 7.5, 1.4, Ar-*H*), 7.55 (1H, dd, *J* = 7.8, 1.3, Ar-*H*), 8.08 (1H, d, *J* = 15.2, alkene-C*H*).

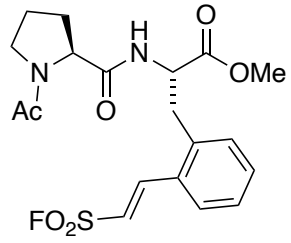
^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1 (acetyl-CH_3), 34.8 (Phe-CH_2), 38.1 (Phe-CH_2), 52.7 (ester-CH_3), 53.3 (Phe-α-CH), 54.6 (Phe-α-CH), 119.5 ($^3J_{CF}$ = 27.8, alkene-C), 127.1 (Ar C), 127.4 (Ar C), 128.1 (Ar C), 128.7 (Ar C), 129.1 (Ar C), 130.2 (Ar C), 131.6 (Ar C), 132.3 (Ar C), 136.2 (Ar C), 137.0 (Ar C), 145.7 ($^4J_{CF}$ = 2.9, alkene-C), 170.1 (C=O), 170.5 (C=O), 170.7 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3280 w (N-H), 3064 w (C-H), 2960 w (C-H), 1737 s (ester C=O), 1648 s (amide C=O), 1536 s (C=C), 1409 s (S=O), 1218 m (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{26}N_2O_6SF$: 477.1496, found: 477.1497.

3.13.5.6 Ac-Pro-Phe(2-VSF)-OMe (63)



Modified dipeptide **63** was synthesised from Ac-Pro-Phe-OMe **57** (0.096 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **63** as a brown solid (0.052 g, 41%); m.p. 80-82 °C; R_f = 0.15 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75-1.87 (1H, m, Pro-C*H*H), 1.92-2.00 (2H, m, Pro-CH_2), 2.08 (3H, s, acetyl-CH_3), 231-2.39 (1H, m, Pro-CH*H*), 3.16 (1H, dd, *J* = 14.4, 7.3, Phe-C*H*H), 3.31-3.56 (3H, m, Phe-CH*H* / Pro-CH_2), 3.72 (3H, s, ester-CH_3), 4.55 (1H, dd, *J* = 8.1, 2.0, Pro-α-C*H*), 4.71 (1H, dt, *J* = 7.4, 6.0, Phe-α-C*H*), 6.86 (1H, dd, *J* = 15.3, 2.5, alkene-C*H*), 7.28-7.38 (2H, m , Ar-*H*), 7.43 (1H, dt, *J* = 7.5, 1.3, Ar-*H*), 7.57 (1H, dd, *J* = 7.8, 0.9, Ar-*H*), 7.66 (1H, d, *J* = 7.4, Phe-N*H*), 8.15 (1H, d, *J* = 15.3, alkene-C*H*).

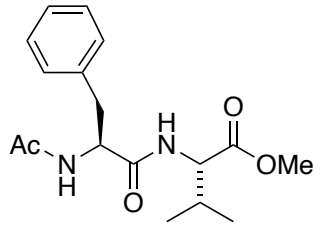
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4 (acetyl-CH_3), 24.9 (Pro-CH_2), 27.1 (Pro-CH_2), 34.8 (Phe-CH_2), 48.2 (Pro-CH_2), 52.6 (ester-CH_3), 53.2 (Phe-α-CH), 59.4 (Pro-α-CH), 119.6 ($^3J_{CF}$ = 27.0, alkene-C), 127.3 (Ar C), 127.9 (Ar C), 130.2 (Ar C), 131.7 (Ar C), 131.9 (Ar C), 137.5 (Ar C), 145.7 ($^4J_{CF}$ = 2.9, alkene-C), 170.9 (C=O), 171.0 (C=O), 171.1 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3243 w (N-H), 3060 w (C-H), 2952 w (C-H), 1737 s (ester C=O), 1625 s (amide C=O), 1528 s (C=C), 1394 s (S=O), 1219 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{24}N_2O_6SF$: 427.1339, found: 427.1337.

3.13.6 Synthesis of Ac-Phe-Val-OMe (64)



Peptide **64** was synthesised from L-valine methyl ester hydrochloride (0.719 g, 2.50 mmol) and *N*-acetyl-L-phenylalanine (0.117 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **64** as a white solid (0.176 g, 55%); m.p. 142-144 ºC; R_f = 0.40 (EtOAc).

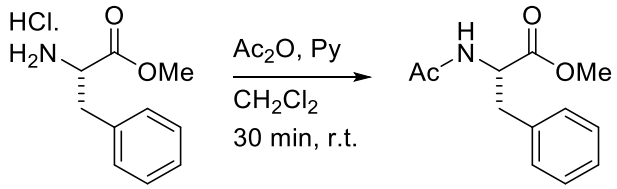
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.86 (3H, d, *J* = 6.9, Val-C*H*$_3$), 1.98 (3H, s, acetyl-C*H*$_3$), 2.05-2.15 (1H, m, Val-C*H*), 3.04 (1H, dd, *J* = 13.8, 7.4, Phe-C*H*H), 3.09 (1H, dd, *J* = 13.8, 6.5, Phe-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 4.42 (1H, dd, *J* = 8.5, 5.1, Val-α-C*H*), 4.71 (1H, dt, *J* = 7.6, 7.2, Phe-α-C*H*), 6.23 (1H, d, *J* = 7.2, Phe-N*H*), 6.31 (1H, d, *J* = 8.5, Val-N*H*), 7.20-7.33 (5H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 17.7 (Val-CH_3), 18.8 (Val-CH_3), 23.1 (acetyl-CH_3), 31.1 (Val-CH), 38.2 (Phe-CH_2), 52.1 (ester-CH_3), 54.5 (Phe-α-CH), 57.4 (Val-α-CH), 127.0 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 136.4 (Ar C), 170.0 (C=O), 170.8 (C=O), 171.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3283 w (N-H), 3026 w (C-H), 2960 w (C-H), 1737 s (ester C=O), 1636 s (amide C=O), 1539 s (C=C), 1200 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4$: 321.1814, found: 321.1812.

3.13.7 Synthesis of amino acid (66)



Known compound. Protected amino acid **66** was synthesised by suspending L-phenylalanine methyl ester hydrochloride (0.300 g, 1.39 mmol) **22** in CH_2Cl_2 (20 mL). Separately, acetic anhydride (0.657 mL, 6.96 mmol) and pyridine (0.560 mL, 6.96 mmol) dissolved in CH_2Cl_2 (5 mL) then added to the suspension and allowed to stir for 30 min. Once complete, the solvents were removed *in vacuo.* The crude residue was dissolved in CH_2Cl_2 (50 mL) and washed with 1M HCl (1 x 20 mL), sat. $NaHCO_3$ solution (3x 20 mL), distilled water (1 x 20 mL) and brine (1 20 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo* yielding protected amino acid **66** as a white solid (0.236 g, 77%); m.p. 176-177 ºC; R_f = 0.44 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (3H, s, acetyl-C*H*$_3$), 3.09 (1H, dd, *J* = 13.7, 5.7, Phe-C*H*H), 3.15 (1H, dd, *J* = 13.7, 5.8, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.89 (1H, dt, *J* = 7.4, 5.7, Phe-α-C*H*), 6.00 (1H, br d, *J* = 7.4, Phe-N*H*), 7.06-7.12 (2H, m, Ar-*H*), 7.22-7.33 (3H, m, Ar-*H*).

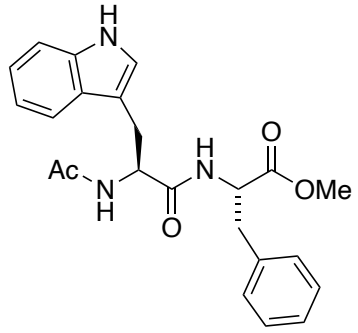
^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1 (acetyl-CH_3), 37.8 (Phe-CH_2), 52.3 (ester-CH_3), 53.0 (Phe-α-CH), 127.1 (Ar C), 128.5 (Ar C), 129.2 (Ar C), 135.8 (4° Ar C), 169.6 (C=O), 172.1 (C=O).

IR υ_{max}/cm^{-1} (solid) 3332 m (N-H), 3030 w (C-H), 2963 w (C-H), 1748 s (ester C=O), 1648 s (amide C=O), 1531 s (C=C), 1219 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{12}H_{16}NO_3$: 222.1130, found: 222.1124.

3.13.8 Synthesis of dipeptides (68-72)

3.13.8.1 Ac-Trp-Phe-OMe (68)



Known compound. Peptide **68** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-tryptophan (0.146 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **68** as an off-white solid (0.347 g, 85%); m.p. 73-74 ºC.

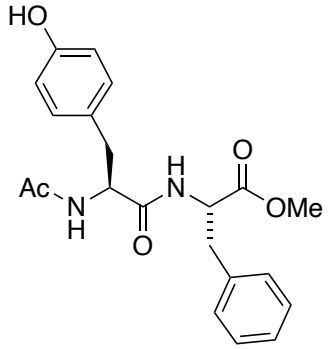
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (3H, s, acetyl-C*H*$_3$), 2.92 (1H, dd, *J* = 13.8, 6.3, Phe-C*H*H), 3.00 (1H, dd, *J* = 13.8, 5.6, Phe-CH*H*), 3.09 (1H, dd, *J* = 14.5, 8.3, Trp-C*H*H), 3.32 (1H, dd, *J* = 14.5, 5.0, Trp-CH*H*), 3.64 (3H, s, ester-C*H*$_3$), 4.65-4.75 (2H, m, Trp-α-C*H* / Phe-α-C*H*), 6.02 (1H, d, *J* = 7.4, Phe-N*H*), 6.23 (1H, d, *J* = 7.4, Trp-N*H*), 6.83-6.89 (2H, m, Ar-*H*), 7.05 (1H, d, *J* = 2.4, Ar-*H*), 7.11-7.24 (5H, m, Ar-*H*), 7.36 (1H, d, *J* = 8.1, Ar-*H*), 7.72 (1H, d, *J* = 7.9, Ar-*H*), 8.03 (1H, br s, Ar-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 23.3 (acetyl-CH_3), 28.3 (Trp-CH_2), 37.8 (Phe-CH_2), 52.3 (ester-CH_3), 53.3 (Phe-α-CH), 53.7 (Trp-α-CH), 110.6 (Ar C), 111.2 (Ar C), 118.9 (Ar C), 119.9 (Ar C), 122.3 (Ar C), 123.4 (Ar C), 127.1 (Ar C), 127.4 (Ar C), 128.5 (Ar C), 129.1 (Ar C), 135.5 (Ar C), 136.1 (Ar C), 169.9 (C=O), 170.8 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3309 w (N-H), 3027 w (C-H), 2978 w (C-H), 1752 s (ester C=O), 1640 s (amide C=O), 1543 s (C=C), 1208 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{26}N_3O_4$: 408.1923, found: 408.1917.

3.13.8.2 Ac-Tyr-Phe-OMe (69)



Known compound. Peptide **69** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-tyrosine (0.223 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **69** as a white solid (0.289 g, 75%); m.p. 57-59 ºC.

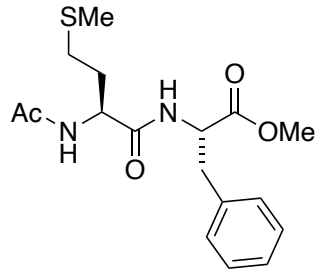
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (3H, s, acetyl-C*H*$_3$), 2.93 (2H, d, *J* = 7.1, Tyr-C*H*$_2$), 2.98 (1H, dd, *J* = 14.0, 6.5, Phe-C*H*H), 3.09 (1H, dd, *J* = 14.0, 5.8, Phe-CH*H*), 3.69 (3H, s, ester-C*H*$_3$), 4.59 (1H, dt, *J* = 7.8, 7.1, Tyr-α-C*H*), 4.75 (1H, dt, *J* = 7.5, 6.5, Phe-α-C*H*), 6.18 (1H, d, *J* = 7.8, Tyr-N*H*), 6.30 (1H, d, *J* = 7.5, Phe-N*H*), 6.57 (1H,s, Tyr-OH), 6.67-6.73 (2H, m, Ar-*H*), 6.97-7.06 (4H, m, Ar-*H*), 7.21-7.28 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1 (acetyl-CH_3), 37.5 (Tyr-CH_2), 37.8 (Phe-CH_2), 52.4 (ester-CH_3), 53.4 (Phe-α-CH), 54.5 (Tyr-α-CH), 115.6 (Ar C), 127.2 (Ar C), 127.7 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 130.4 (Ar C), 135.5 (Ar C), 155.2 (Ar C), 170.3 (C=O),170.7 (C=O). 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3414 w (O-H), 3314 w (N-H), 3027 w (C-H), 2945 w (C-H), 1725 s (ester C=O), 1648 s (amide C=O), 1536 s (C=C), 1227 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{21}H_{25}N_2O_5$: 385.1763, found: 385.1765.

3.13.8.3 Ac-Met-Phe-OMe (70)



Known compound. Peptide **70** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-methionine (0.131 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **70** as an off-white solid (0.275 g, 78%); m.p. 111-113 ºC.

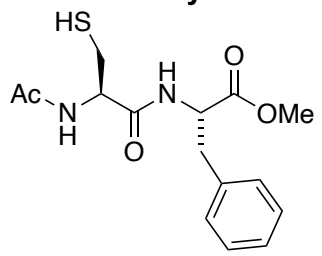
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90-2.03 (5H, m, acetyl-C*H*$_3$ / Met-C*H*$_2$), 2.05 (3H, s, S-C*H*$_3$), 2.48-2.63 (2H, m, Met-C*H*$_2$), 3.07 (1H, dd, *J* = 14.0, 6.6, Phe-C*H*H), 3.14 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.59 (1H, dt, *J* = 7.5, 7.1, Met-α-C*H*), 4.84 (1H, dt, *J* = 7.7, 6.6, Phe-α-C*H*), 6.30 (1H, br d, *J* = 7.8, Met-N*H*), 6.73 (1H, br d, *J* = 8.0, Phe-N*H*), 7.09-7.15 (2H, m, Ar-*H*), 7.22-7.33 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 15.0 (S-CH_3), 23.2 (acetyl-CH_3), 29.9 (Met-CH_2), 31.3 (Met-SCH_2), 37.7 (Phe-CH_2), 51.8 (Met-α-CH), 52.4 (ester-CH_3), 53.2 (Phe-α-CH), 127.2 (Ar C), 128.7 (Ar C), 129.2 (Ar C), 135.5 (4º Ar C), 169.9 (C=O), 170.8 (C=O), 171.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3288 w (N-H), 3064 w (C-H), 2952 w (C-H), 1752 s (ester C=O), 1640 s (amide C=O), 1539 s (C=C), 1289 (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4S$: 353.1535, found: 353.1535.

3.13.8.4 Ac-Cys-Phe-OMe (71)



Known compound. Peptide **71** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-acetyl-L-cysteine (0.163 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **71** as a white solid (0.214 g, 66%); m.p. 120-122 ºC.

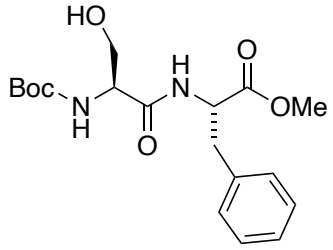
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (3H, s, acetyl-C*H*$_3$), 2.32 (1H, br s, Cys-S*H*), 2.85 (1H, dd, *J* = 14.6, 10.4, Cys-C*H*H), 3.01-3.10 (2H, m, Phe-C*H*H / Cys-CH*H*), 3.18 (1H, dd, *J* = 13.8, 5.6, Phe-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 4.73 (1H, dt, *J* = 7.8, 5.6, Phe-α-C*H*), 5.34 (1H, td, *J* = 10.4, 4.1, Cys-α-C*H*), 6.55 (1H, d, *J* = 9.2, Cys-N*H*), 7.18-7.26 (5H, m, Ar-*H*), 8.50 (1H, d, *J* = 7.8, Phe-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 23.3 (acetyl-CH_3), 37.7 (Phe-CH_2), 45.8 (Cys-CH_2), 52.3 (ester-CH_3), 52.9 (Cys-α-CH), 54.2 (Phe-α-CH), 126.9 (Ar C), 128.5 (Ar C), 129.2 (Ar C), 136.4 (Ar C), 170.3 (C=O), 170.4 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3283 w (N-H), 3030 w (C-H), 2952 w (C-H), 2555 w (S-H), 1737 s (ester C=O), 1640 (amide C=O), 1528 s (C=C), 1215 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4S$: 325.1222, found: 325.1213.

3.13.8.5 Boc-Ser-Phe-OMe (72)



Peptide **72** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N-tert*-butyloxycarbonyl-L-serine (0.205 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Purification by flash column chromatography (EtOAc) yielded **72** as a colourless oil (0.278 g, 76%); R_f = 0.10 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (9H, s, Boc-(C*H*$_3$)$_3$), 3.05 (1H, dd, *J* = 14.0, 6.9, Phe-C*H*H), 3.16 (1H, dd, *J* = 14.0, 5.7, Phe-CH*H*), 3.38 (1H, br t, *J* = 5.5, Ser-O*H*), 3.56-3.64 (1H, m, Ser-C*H*H), 3.71 (3H, s, ester-C*H*$_3$), 3.91-4.01 (1H, m, Ser-CH*H*), 4.14-4.22 (1H, m, Ser-α-C*H*), 4.84 (1H, dt, *J* = 6.6, 6.4 (Phe-α-C*H*), 5.59 (1H, d, *J* = 7.3, Ser-N*H*), 7.10-7.32 (6H, Ar-*H* / Phe-N*H*).

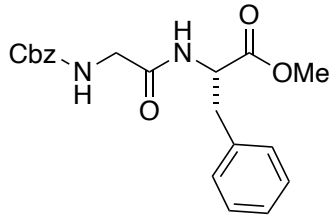
^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (Boc-(CH_3)$_3$), 37.6 (Phe-CH_2), 52.4 (ester-CH_3), 53.4 (Ser-α-CH), 55.1 (Phe-α-CH), 62.8 (Ser-CH_2), 80.3 (Boc-C), 127.1 (Ar C), 128.5 (Ar C), 129.1Ar C), 135.7 (Ar C), 155.8 (C=O), 171.0 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3299 w (N-H), 3063 w (C-H), 2952 w (C-H), 1744 s (ester C=O), 1654 s (amide C=O), 1520 s (C=C), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{27}N_2O_6$: 367.1869, found: 367.1865.

3.13.9 Scope of the *N*-protecting group (78-81)

3.13.9.1 Cbz-Gly-Phe-OMe (78)



Known compound. Protected peptide **78** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Cbz-glycine (0.370 g, 1.00 mmol) according to the procedure outlined in **Section 3.13.2.1**. Purification by flash column chromatography (EtOAc) afforded **78** as a colourless oil (0.274 g, 74%); R_f = 0.30 (4:6 EtOAc / Pet. Ether 40-60 °C).

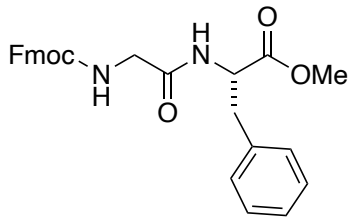
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96-3.10 (2H, m, Phe-C*H*$_2$), 3.64 (3H, s, ester-C*H*$_3$), 3.74 (1H, dd, *J* = 16.9, 5.5, Gly-C*H*H), 3.80 (1H, dd, *J* = 16.9, 5.5, Gly-CH*H*), 4.81 dt, *J* = 7.6, 6.0, Phe-α-C*H*), 5.04 (2H, s, Cbz-C*H*$_2$), 5.34 (1H, br t, *J* = 5.5, Gly-N*H*), 6.41 (1H, br d, *J* = 7.1, Phe-N*H*), 7.00 (2H, d, *J* = 6.6, Ar-*H*), 7.12-7.35 (8H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 37.8 (Phe-CH_2), 44.4 (Gly-CH_2), 52.4 (Phe-α-CH), 53.0 (ester-CH_3), 67.2 (Cbz-CH_2), 127.2 (Ar C), 128.1 (Ar C), 128.3 (Ar C), 128.5 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 135.5 (Ar C), 136.0 (Ar C), 156.5 (C=O), 168.5 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3317 m (N-H), 3030 w (C-H), 2952 w (C-H), 1722 s (ester C=O), 1662 s (amide C=O), 1513 s (C=C), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{23}N_2O_5$: 371.1607, found: 371.1601.

3.13.9.2 Fmoc-Gly-Phe-OMe (79)



Known compound. The protected peptide Fmoc-Gly-Phe-OMe **79** was prepared from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Fmoc-glycine (0.297 g, 1.00 mmol) according to the procedure outlined in **Section 3.13.2.1**. Recrystallisation of the crude product from CH_2Cl_2 / hexanes yielded peptide **79** as a white solid. (0.303 g, 66%); m.p. 128-129 ºC. R_f = 0.42 (EtOAc).

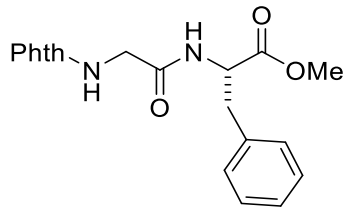
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.09 (1H, dd, *J* = 13.7, 6.2, Phe-C*H*H), 3.16 (1H, dd, *J* = 13.7, 5.7, Phe-CH*H*), 3.71 (3H, s, ester-CH_3), 3.89 (2H, m, Gly-CH_2), 4.22 (1H, t, *J* = 7.1, Fmoc-C*H*), 4.35-4.45 (2H, m, Fmoc-CH_2), 4.92 (1H, dt, *J* = 7.9, 6.1, Phe-α-C*H*), 5.75 (1H, t, *J* = 5.3, Gly-N*H*), 6.81 (1H, d, *J* = 8.0, Phe-N*H*), 7.05-7.13 (2H, m, Ar-*H*), 7.17-7.28 (3H, m, Ar-*H*), 7.32 (2H, t, *J* = 7.6, Ar-*H*), 7.42 (2H, t, *J* = 7.4, Ar-*H*), 7.61 (2H, d, *J* = 7.4, Ar-*H*), 7.79 (2H, d, *J* = 7.6, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 37.7 (Phe-CH_2), 44.2 (Gly-CH_2), 46.9 (Fmoc-CH_2), 52.3 (Phe-α-CH), 53.1 (ester-CH_3), 67.1 (Fmoc-CH), 119.9 (Ar C), 125.0 (Ar C), 127.0 (Ar C), 127.1 (Ar C), 127.6 (Ar C), 128.5 (Ar C), 129.1 (Ar C), 135.5 (Ar C), 141.2 (Ar C), 143.7 (Ar C), 156.4 (Fmoc C=O), 168.6 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3317 w (N-H), 3034 w (C-H), 2978 w (C-H), 1759 m (ester C=O), 1666 s (amide C=O), 1524 m (C=C), 1215 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{27}H_{27}N_2O_5$: 459.1920, found: 459.1916.

3.13.9.3 Phth-Gly-Phe-OMe (80)



Known compound. Phthaloyl-protected peptide, Phth-Gly-Phe-OMe **80** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Phth-Gly-OH (0.366, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. The crude product was recrystallised from CH_2Cl_2 / hexanes to afford **80** as a white solid (0.234 g, 64%); m.p. 172-174 ºC; R_f = 0.30 (45% EtOAc in Pet. Ether 40-60 °C).

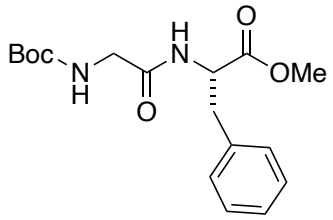
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.12 (1H, dd, *J* = 13.8, 5.3, Phe-C*H*H), 3.19 (1H, dd, *J* = 13.8, 5.8, Phe-CH*H*), 3.75 (3H, s, ester-CH_3), 4.36 (2H, m, Gly-CH_2), 4.88 (1H, dt, *J* = 7.5, 5.4, Phe-α-C*H*), 6.25 (1H, d, *J* = 7.5, Phe-N*H*), 7.05-7.11 (2H, m, Ar-*H*), 7.17-7.27 (3H, Ar-*H*), 7.74-7.79 (2H, m, Ar-*H*), 7.86-7.93 (2H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 37.6 (Phe-CH_2), 40.6 (Gly-CH_2), 52.5 (ester-CH_3), 53.4 (Phe-α-CH), 123.6 (Ar C), 127.2 (Ar C), 128.6 (Ar C), 129.3 (Ar C), 132.0 (Ar C), 134.2 (Ar C), 135.4 (4º Ar C), 165.5 (C=O), 167.6 (C=O), 171.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3310 w (N-H), 3030 w (C-H), 2952 w (C-H), 1774 m (Phth C=O), 1715 s (ester C=O), 1659 s (amide C=O), 1543 m (C=C), 1208 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{19}N_2O_5$: 367.1294, found: 367.1293.

3.13.9.4 Boc-Gly-Phe-OMe (81)



Known compound. Boc-protected peptide, Boc-Gly-Phe-OMe **81** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Boc-glycine (0.175 g, 1.00 mmol) according to the procedure outlined in **Section 3.13.2.1** Purification by flash column chromatography (EtOAc) afforded **81** as a colourless oil (0.239 g, 71%); R_f = 0.30 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, Boc-(CH_3)$_3$), 3.08 (1H, dd, *J* = 13.8, 6.1, Phe-C*H*H), 3.14 (1H, dd, *J* = 13.8, 5.8, Phe-CH*H*), 3.68-3.88 (5H, m, Gly-CH_2 / ester-CH_3), 4.88 (1H, dt, *J* = 7.8, 6.0, Phe-α-C*H*), 5.26 (1H, br t, *J* = 5.5, Gly-N*H*), 6.71 (1H, d, *J* = 7.3, Phe-N*H*), 7.08-7.13 (2H, m, Ar-*H*), 7.21-7.31 (3H, m, Ar-*H*).

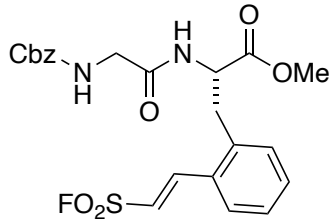
^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (Boc-(CH_3)$_3$), 37.8 (Phe-CH_2), 44.1 (Gly-CH_2), 52.3 (Phe-α-CH), 53.0 (ester-CH_3), 80.1 (Boc-C), 127.1 (Ar C), 128.5 (Ar C), 129.2 (Ar C), 135.6 (Ar C), 155.9 (Boc C=O), 169.1 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (oil) 3317 w (N-H), 3022 w (C-H), 2978 w (C-H), 1759 m (ester C=O), 1662 s (amide C=O), 1498 s (C=C), 1244 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_5$: 337.1763, found: 337.1757.

3.13.10 Synthesis of modified dipeptides (82-85)

3.13.10.1 Synthesis of modified peptide (82)



Modified dipeptide **82** was synthesised from Cbz-Gly-Phe-OMe **78** (0.111 g, 0.300 mmol) and ESF **50** (0.050 mL, 0.600 mmol) according to the procedure outlined in **Section 3.13.2.3**. Purification by flash column chromatography (4:6 EtOAc / Pet. Ether 40-60 °C) gave **82** as a brown oil (0.095 g, 66%); R_f = 0.30 (4:6 EtOAc / Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.22 (1H, dd, *J* = 14.4, 5.9, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, 5.7, Phe-CH*H*), 3.71 (3H, s, ester-CH_3), 3.81 (1H, dd, *J* = 16.9, 5.7, Gly-C*H*H), 3.90 (1H, dd, *J* = 16.9, 6.0, Gly-CH*H*), 4.85 (1H, dt, *J* = 7.3, 6.0, Phe-α-C*H*), 5.11 (2H, s, Cbz-CH_2), 5.41 (1H, br t, *J* = 6.0, Gly-N*H*), 6.65 (1H, d, *J* = 7.6, Phe-N*H*), 6.85 (1H, dd, *J* = 15.3, 2.3, alkene-C*H*), 7.21 (1H, d, *J* = 7.6, Ar-*H*), 7.29-7.38 (6H, m, Ar-*H*), 7.42 (1H, t, *J* = 7.3, Ar-*H*), 7.56 (1H, d, *J* = 7.8, Ar-*H*), 8.11 (1H, d, *J* = 15.2, alkene-C*H*).

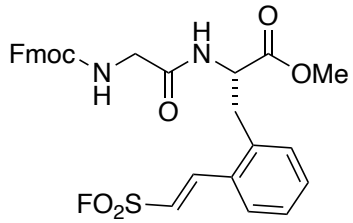
^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8 (Phe-CH_2), 44.4 (Gly-CH_2), 52.8 (ester-CH_3), 53.1 (Phe-α-CH), 67.2 (Cbz-CH_2), 119.4 ($^3J_{CF}$ = 27.8, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 128.2 (Ar C), 128.3 (Ar C), 128.5 (Ar C), 130.2 (Ar C), 131.7 (Ar C), 132.4 (Ar C), 136.0 (Ar C), 137.1 (Ar C), 145.6 ($^4J_{CF}$ = 1.9, alkene-C), 156.6 (C=O), 168.8 (C=O), 171.0 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (oil) 3317 w (N-H), 3071 w (C-H), 2952 w (C-H), 1733 m (ester C=O), 1655 m (amide C=O), 1528 m (C=C), 1402 s (S=O), 1211 m (C-O), 1189 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{24}N_2O_7SF$: 479.1288, found: 479.1284.

3.13.10.2 Synthesis of modified peptide (83)



Modified dipeptide **83** was synthesised from Fmoc-Gly-Phe-OMe **79** (0.138 g, 0.300 mmol) and ESF **50** (0.049 mL, 0.60 mmol) according to the procedure outlined in **Section 3.13.2.3**. Purification by flash column chromatography (40% EtOAc in Pet Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **83** as a brown oil (0.075 g, 44%); R_f = 0.35 (40% EtOAc in Pet Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (1H, dd, *J* = 14.4, 6.0, Phe-C*H*H), 3.36 (1H, dd, *J* = 14.4, 5.7, Phe-CH*H*), 3.70 (3H, s, ester-CH_3), 3.84 (1H, dd, *J* = 16.8, 5.4, Gly-C*H*H), 3.92 (1H, dd, *J* = 16.8, 5.4, Gly-CH*H*), 4.19 (1H, t, *J* = 7.1, Fmoc-C*H*), 4.27-4.42 (2H, m, Fmoc-CH_2), 4.87 (1H, dt, *J* = 7.3, 6.0, Phe-α-C*H*), 5.47 (1H, br t, *J* = 5.4, Gly-N*H*), 6.66 (1H, d, *J* = 7.3, Phe-N*H*), 6.83 (1H, dd, *J* = 15.1, 2.4, alkene-C*H*), 7.19 (1H, d, *J* = 7.8, Ar-*H*), 7.27-7.65 (9H, m, Ar-*H*), 7.77 (2H, d, *J* = 7.3, Ar-*H*), 8.10 (1H, d, *J* = 15.1, alkene-C*H*).

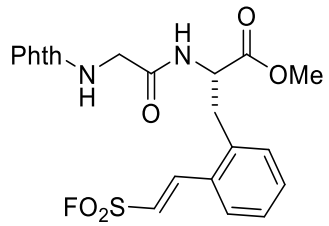
^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8 (Phe-CH_2), 44.4 (Gly-CH_2), 47.0 (Fmoc-CH), 52.8 (Phe-α-CH), 53.2 (ester-CH_3), 67.4 (Fmoc-CH_2), 119.4 ($^3J_{CF}$ = 27.9, alkene-C), 120.0 (Ar C), 125.1 (Ar C), 127.1 (Ar C), 127.8 (Ar C), 128.2 (Ar C), 130.2 (Ar C), 131.6 (Ar C), 132.4 (Ar c), 137.1 (Ar C), 141.2 (Ar C), 143.7 (Ar C), 145.6 ($^4J_{CF}$ = 2.4, alkene-C), 156.6 (Fmoc C=O), 168.7 (C=O), 171.0 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.0 (s).

IR υ_{max}/cm^{-1} (oil) 3314 w (N-H), 3064 w (C-H), 2960 w (C-H), 1718 s (ester C=O), 1674 s (amide C=O), 1513 m (C=C), 1397 s (S=O), 1260 m (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{29}H_{28}N_2O_7SF$: 567.1601, found: 567.1595.

3.13.10.3 Synthesis of modified peptide (84)



Modified peptide **84** was synthesised from phthaloyl-protected peptide **80** (0.110 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) using the procedure outlined in **Section 3.13.2.3**. Purification by flash column chromatography followed by recrystallisation from CH_2Cl_2 / hexanes yielded **84** as an off-white solid (0.080 g, 56%) m.p. 207-209 ºC; R_f = 0.30 (45% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.26 (1H, dd, *J* = 14.4, 6.0, Phe-C*H*H), 3.37 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.74 (3H, s, ester-CH_3), 4.34 (2H, m, Gly-CH_2), 4.85 (1H, dt, *J* = 7.4, 6.0, Phe-α-C*H*), 6.32 (1H, d, *J* = 7.4, Phe-N*H*), 6.89 (1H, dd, *J* = 15.2, 2.4, alkene-C*H*), 7.27-7.36 (2H, m, Ar-*H*), 7.46 (1H, td, *J* = 7.6, 1.1, Ar-*H*), 7.57 (1H, br d, *J* = 8.0, Ar-*H*), 7.73-7.78 (2H, m, Ar-*H*), 7.86-7.91 (2H, m, Ar-*H*), 8.10 (1H, d, *J* = 15.2, alkene-C*H*).

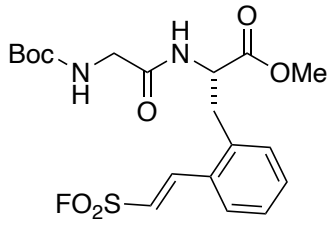
^{13}C NMR (100 MHz, $CDCl_3$) δ 34.6 (Phe-CH_2), 40.6 (Gly-CH), 53.0 (ester-CH_3), 53.3 (Phe-α-CH), 119.5 ($^3J_{CF}$ = 27.9, alkene-CH), 123.7 (Ar C), 127.4 (Ar C), 128.2 (Ar C), 130.1 (Ar C), 131.9 (Ar C), 132.0 (Ar C), 132.4 (Ar C), 134.3 (Ar C), 136.9 (Ar C), 145.5 ($^4J_{CF}$ = 1.9, alkene-CH), 165.7 (C=O), 167.6 (C=O), 171.0 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.2 (s).

IR υ_{max}/cm^{-1} (solid) 3325 w (N-H), 3083 w (C-H), 2960 w (C-H), 1774 m (Phth C=O), 1722 s (ester C=O), 1655 m (amide C=O), 1536 m (C=C), 1394 s (S=O), 1223 m (C-O), 1189 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{20}N_2O_7SF$: 475.0975, found: 475.0971.

3.13.10.4 Synthesis of modified peptide (85)



Modified peptide **85** was prepared from Boc-protected peptide **81** (0.101 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.600 mmol) using the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (65% EtOAc in Pet. Ether 40-60 °C) yielded modified peptide **85** as a light brown oil. (0.100 g, 75%); R_f = 0.30 (65% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, Boc-(CH_3)$_3$), 3.25 (1H, dd, *J* = 14.4, 6.1, Phe-C*H*H), 3.36 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.65-3.90 (5H, m, Gly-CH_2 / ester-CH_3), 4.85 (1H, dt, *J* = 7.1, 6.1, Phe-α-C*H*), 5.14 (1H, br s, Gly-N*H*), 6.74 (1H, br s, Phe-N*H*), 6.88 (1H, dd, *J* = 15.3, 2.4, alkene-C*H*), 7.24 (1H, dd, *J* = 7.8, 1.1, Ar-*H*), 7.36 (1H, t, *J* = 7.3, Ar-*H*), 7.46 (1H, td, *J* = 7.6, 1.4, Ar-*H*), 7.59 (1H, dd, *J* = 7.8, 0.9, Ar-*H*), 8.13 (1H, d, *J* = 15.3, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (acetyl-CH_3), 34.9 (Phe-CH_2), 44.2 (Gly-CH_2), 52.7 (ester-CH_3), 53.1 (Phe-α-CH), 80.4 (Boc-C), 119.4 ($^3J_{CF}$ = 27.8, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.2 (Ar C), 131.7 (Ar C), 132.4 (Ar C), 137.2 (Ar C), 145.7 ($^4J_{CF}$ = 1.9, alkene-C), 156.0 (C=O), 169.4 (C=O), 171.1 (C=O).

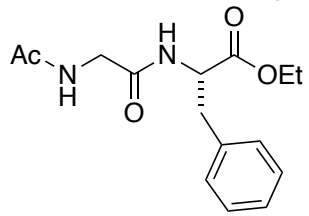
^{19}F NMR (376 MHz, $CDCl_3$) δ 62.0 (s).

IR υ_{max}/cm^{-1} (oil) 3317 w (N-H), 3064 w (C-H), 2974 w (C-H), 1736 s (ester C=O), 1670 s (amide C=O), 1510 s (C=C), 1402 s (S=O), 1252 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_2O_7SF$: 445.1445, found: 445.1458.

3.13.11 Scope of the *C*-protecting group (86-89)

3.13.11.1 Ac-Gly-Phe-OEt (86)



Protected peptide **86** was synthesised from L-phenylalanine ethyl ester hydrochloride (0.574 g, 2.50 mmol) and *N*-acetyl glycine **26** (0.117 g, 1.00 mmol) according to the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes afforded **86** as an off-white solid (0.216 g, 74%); m.p. 80-82 °C; R_f = 0.25 (EtOAc).

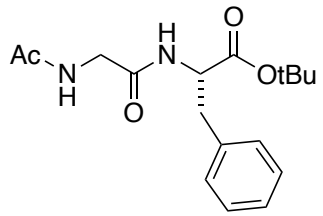
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (3H, t, *J* = 7.2, ester-C*H*$_3$), 1.98 (3H, s, acetyl-C*H*$_3$), 3.05 (1H, dd, *J* = 13.8, 7.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.8, 5.8, Phe-CH*H*) 3.87 (1H, dd, *J* = 16.7, 5.5, Gly-C*H*H), 3.92 (1H, dd, *J* = 16.7, 5.3, Gly-CH*H*), 4.14 (2H, q, *J* = 7.2, ester-C*H*$_2$), 4.81 (1H, dt, *J* = 7.0, 5.8, Phe-α-C*H*), 7.10 (1H, t, *J* = 5.3, Gly-N*H*), 7.13-7.18 (2H, m, Ar-*H*), 7.19-7.35 (4H, m, Ar-*H* / Phe-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9 (ester-CH_3), 22.6 (acetyl-CH_3), 37.6 (Phe-CH_2), 42.9 (Gly-CH_2), 53.3 (Phe-α-CH), 61.3 (ester-CH_2), 126.8 (Ar C), 128.3 (Ar C), 129.1 (Ar C), 135.8 (4º Ar C), 169.0 (C=O), 170.8 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3295 w (N-H), 3060 w (C-H), 2978 w (C-H), 1737 s (ester C=O), 1636 m (amide C=O), 1539 m (C=C), 1252 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4$: 293.1501, found: 293.1498.

3.13.11.2 Ac-Gly-Phe-O*t*Bu (87)



Protected peptide **87** was synthesised from L-phenylalanine *tert* butyl ester hydrochloride (1.289 g, 5.00 mmol) and *N*-acetyl glycine **22** (0.234 g, 2.00 mmol) according to the procedure outlined in **Section 2.6.2**, Purification by flash column chromatography (EtOAc) afforded **87** as a colourless oil (0.391 g, 61%); R_f = 0.35 (EtOAc).

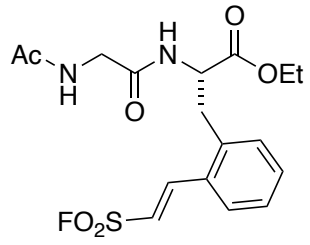
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (9H, s, t-Bu-(C*H*$_3$)$_3$), 1.93 (3H, s, acetyl-C*H*$_3$), 2.97 (1H, dd, *J* = 13.7, 6.1, Phe-C*H*H), 3.02 (1H, dd, *J* = 13.7, 6.1, Phe-CH*H*), 3.79 (1H, dd, *J* = 16.8, 5.3, Gly-C*H*H), 3.84 (1H, dd, *J* = 16.08, 5.3, Gly-CH*H*), 4.65 (1H, dt, *J* = 7.2, 5.6, Phe-α-C*H*), 6.57 (1H, t, *J* = 4.6, Gly-N*H*), 6.72 (1H, d, *J* = 7.8, Phe-N*H*), 7.05-7.10 (2H, m, Ar-*H*), 7.13-7.25 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 27.9 (t-Bu-$(CH_3)_3$), 37.9 (Phe-CH_2), 43.1 (Gly-CH_2), 53.7 (Phe-α-CH), 82.7 (t-Bu 4° C), 127.0 (Ar C), 128.3 (Ar C), 129.4 (Ar C), 135.9 (4° Ar C), 168.5 (C=O), 170.3 (C=O), 170.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3254 w (N-H), 3071 w (C-H), 2978 w (C-H), 1737 s (ester C=O), 1677 s (amide C=O), 1558 m (C=C), 1256 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4$: 321.1814, found: 321.1813.

3.13.11.3 Modified peptide (88)



Modified peptide **88** was synthesised from Ac-Gly-Phe-OEt **86** (0.088 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) using the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes yielded **88** as a light brown solid (0.066 g, 55%); m.p. 147-150 ºC, R_f = 0.25 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, t, *J* = 7.2, ester-C*H*$_3$), 2.03 (3H, s, acetyl-C*H*$_3$), 3.23 (1H, dd, *J* = 14.4, 6.2, Phe-C*H*H), 3.38 (1H, dd, *J* = 14.4, 6.2, PheH*H*), 5.85 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.98 (1H, dd, *J* = 16.8, 5.5, Gly-CH*H*), 4.10-4.25 (2H, m , ester-C*H*$_2$), 4.79 (1H, dt, *J* = 7.4, 6.2, Phe-α-C*H*), 6.25 (1H, br t, *J* = 5.0, Gly-N*H*), 6.67 (1H, d, *J* = 7.4, Phe-N*H*), 6.88 (1H, dd, *J* = 15.3, 2.5, alkene-C*H*), 7.24 (1H, dd, *J* = 7.7, 1.0, Ar-*H*), 7.37 (1H, dt, *J* = 7.7, 1.0, Ar-*H*), 7.45 (1H, dt, *J* = 7.6, 1.4, Ar-*H*), 7.60 (1H, d, *J* = 7.8, 1.0, Ar-*H*), 8.13 (1H, d, *J* = 15.2, alkene-C*H*).

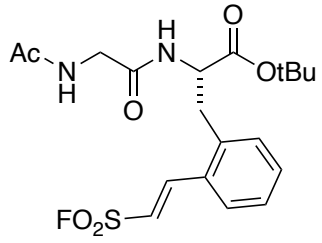
^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0 (ester-CH_3), 22.9 (acetyl-CH_2), 35.0 (Phe-CH_2), 43.1 (Gly-CH_2), 53.4 (Phe-α-CH), 62.1 (ester-CH_2), 119.4 ($^3J_{CF}$ = 27.9, alkene-C), 127.4 (Ar C), 128.2 (Ar C), 130.3 (Ar C), 131.6 (Ar C), 132.4 (Ar C), 137.3 (Ar C), 145.7 ($^4J_{CF}$ = 2.0, alkene-C), 168.7 (C=O), 170.6 (C=O), 170.7 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.0 (s).

IR υ_{max}/cm^{-1} (solid) 3299 w (N-H), 3071 w (C-H), 2989 w (C-H), 1740 s (ester C=O), 1647 s (amide C=O), 1539 m (C=C), 1402 s (S=O), 1263 m (C-O), 1185 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{22}N_2O_6SF$: 401.1183, found: 401.1181.

3.13.11.4 Modified peptide (89)



Modified peptide **89** was prepared from Ac-Gly-Phe-O^{t}Bu **87** (0.096 g, 0.30 mmol) and ESF **50** (0.049 mL, 0.60 mmol) using the procedure outlined in **Section3.13.3.2**. Purification by flash column chromatography (EtOAc) yielded modified peptide **89** as a light brown solid (0.089 g, 69%); m.p. 130-133 ºC, R_f = 0.35 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s, OtBu-(C*H*$_3$)$_3$), 2.01 (3H, s, acetyl-C*H*$_3$), 3.17 (1H, dd, *J* = 14.3, 7.0, Phe-C*H*H), 3.31 (1H, dd, *J* = 14.3, 6.2, Phe-CH*H*), 3.85 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.95 (1H, dd, *J* = 16.8, 5.5, Gly-CH*H*), 4.65 (1H, dt, *J* = 7.0, 6.9, Phe-α-C*H*), 6.52 (1H, t, *J* = 5.0, Gly-N*H*), 6.89 (1H, dd, *J* = 15.3, 2.4, alkene-C*H*), 6.98 (1H, d, *J* = 7.6, Phe-N*H*), 7.30 (1H, dd, *J* = 7.8, 1.0, Ar-*H*), 7.35 (1H, dt, *J* = 7.7, 1.3, Ar-*H*), 7.44 (1H, dt, *J* = 7.6, 1.3, Ar-*H*), 7.59 (1H, dd, *J* = 7.8, 0.9, Ar-*H*), 8.17 (1H, d, *J* = 15.3, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 27.8 (OtBu-$(CH_3)_3$), 35.4 (Phe-CH_2), 43.0 (Gly-CH_2), 54.0 (Phe-α-CH), 83.2 (OtBu-C), 119.4 ($^3J_{CF}$ = 27.8, alkene-C), 127.4 (Ar C), 128.4 (Ar C), 129.4 (Ar C), 131.8 (Ar C), 132.2 (Ar C), 137.6 (Ar C), 145.9 ($^4J_{CF}$ = 1.9, alkene-C), 168.8 (C=O), 169.7 (C=O), 170.9 (C=O).

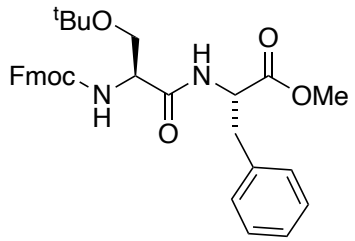
^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3284 w (N-H), 3064 w (C-H), 2967 w (C-H), 1730 s (ester C=O), 1651 s (amide C=O), 1525 m (C=C), 1402 s (S=O), 1260 m (C-O), 1191 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_2O_6SF$: 429.1496, found: 429.1495.

3.13.12 Synthesis of polar dipeptides 90-93

3.13.12.1 Fmoc-Ser(tBu)-Phe-OMe (90)



Peptide **90** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Fmoc-*O*-*tert*-butyl-ether-L-serine (0.383 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **90** as a white solid (0.387 g, 71%); m.p. 142-144 ºC; R_f = 0.31 (8:2 EtOAc / Pet. Ether 40-60 °C).

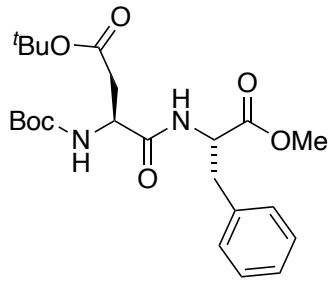
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (9H, s, *t*Bu-(C*H*$_3$)$_3$) 3.10 (1H, dd, *J* = 13.7, 5.7, Phe-C*H*H), 3.15 (1H, dd, *J* = 13.7, 5.5, Phe-CH*H*), 3.33-3.42 (1H, m, Ser-C*H*H), 3.71 (3H, s, ester-C*H*$_3$), 3.82 (1H, dd, *J* = 8.2, 3.7, Ser-CH*H*), 4.16-4.28 (2H, Ser-α-C*H* / Fmoc-C*H*), 4.34-4.43 (2H, m, Fmoc-C*H*$_2$), 4.90 (1H, dt, *J* = 7.8, 5.9, Phe-α-C*H*), 5.74 (1H, d, *J* = 5.3, Ser-N*H*), 7.09-7.15 (2H, m, Ar-*H*), 7.21-7.33 (5H, Ar-*H*), 7.35-7.44 (3H, m, Ar-*H* / Phe-N*H*), 7.58 (2H, d, *J* = 7.3, Ar-*H*), 7.76 (2H, d, *J* = 7.4, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3 (*t*Bu-(CH_3)$_3$) 38.0 (Phe-CH_2), 47.1 (Fmoc-CH), 52.3 (ester-CH_3), 53.4 (Phe-α-CH), 54.1 (Ser-α-CH), 61.6 (Ser-CH_2), 67.2 (Fmoc-CH_2), 74.4 (*t*Bu-C), 120.0 (Ar C), 125.1 (Ar C), 127.1 (Ar C), 127.1 (Ar C), 127.7 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 135.7 (Ar C), 143.7 (Ar C), 157.6 (Fmoc C=O), 169.9 (C=O), 170.0 (C=O),

IR υ_{max}/cm^{-1} (solid) 3243 w (N-H), 3027 w (C-H), 2982 w (C-H), 1722 s (ester C=O), 1651 s (amide C=O), 1510 m (C=C), 1223 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{37}N_2O_6$: 545.2652, found: 545.2657.

3.13.12.2 Boc-Asp(O^{t}Bu)-Phe-OMe (91)



Peptide **91** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and *N*-Boc-L-aspartic acid-4-*tert*-buyl ester (0.289 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **91** as a white solid (0.298 g, 66%); m.p. 88-90 ºC; R_f = 0.30 (8:2 EtOAc / Pet. Ether 40-60 °C).

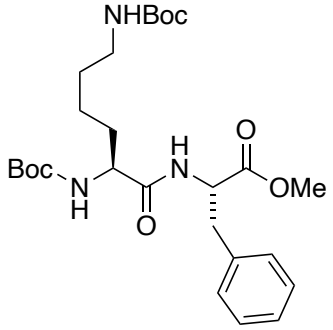
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s, Boc-(C*H*$_3$)$_3$), 1.43 (9H, s, O^{t}Bu-(C*H*$_3$)$_3$), 2.58 (1H, dd, *J* = 17.0, 6.3, Asp-C*H*H), 2.86 (1H, dd, *J* = 17.0, 4.3, Asp-CH*H*), 3.08 (1H, dd, *J* = 13.8, 6.0, Phe-C*H*H), 3.12 (1H, dd, *J* = 13.8, 5.8, Phe-CH*H*), 3.68 (3H, s, ester-C*H*$_3$), 4.47 (1H, dt, *J* = 6.3, 5.5, Asp-α-C*H*), 4.80 (1H, dt, *J* = 7.4, 6.0, Phe-α-C*H*), 5.70 (1H, d, *J* = 8.2, Asp-N*H*), 7.03 (1H, d, *J* = 7.4, Phe-N*H*), 7.11-7.18 (2H, m, Ar-*H*), 7.21-7.33 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9 (OtBu-(CH_3)$_3$), 28.1 (Boc-(CH_3)$_3$), 37.1 (Asp-CH_2), 37.7 (Phe-CH_2), 50.4 (Asp-α-CH), 52.1 (ester-CH_3), 53.4 (Phe-α-CH), 80.1 (Boc-C), 81.5 (OtBu-C), 127.0 (Ar C), 128.4 (Ar C), 129.2 (Ar C), 135.6 (Ar C), 155.3 (Boc C=O), 170.5 (C=O), 171.1 (C=O), 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3299 w (N-H), 3030 w (C-H), 2981 w (C-H), 1715 s (ester C=O), 1662 s (amide C=O), 1506 s (C=C), 1245 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{35}N_2O_7$: 451.2444, found: 451.2439.

3.13.12.3 Boc-Lys(Boc)-Phe-OMe (92)



Peptide **92** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and N_2-Boc-N_6-Boc-L-lysine (0.346 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **92** as a white solid (0.355 g, 70%); m.p. 101-103 ºC. R_f = 0.25 (8:2 EtOAc / Pet. Ether 40-60 °C)

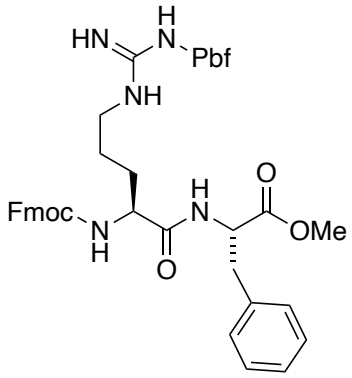
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25-1.65 (24H, m, Boc-(C*H*$_3$)$_3$ / Boc-(C*H*$_3$)$_3$ / Lys-C*H*$_2$ / Lys-C*H*$_2$ / Lys-C*H*H), 1.70-1.86 (1H, m, Lys-CH*H*), 3.04-3.19 (4H, m, Phe-C*H*$_2$ / Lys-C*H*$_2$), 3.72 (3H, s, ester-C*H*$_3$), 3.99-4.11 (1H, m, Lys-α-C*H*), 4.64 (1H, br t, *J* = 5.5, Lys-N*H*), 4.86 (1H, dt, *J* = 7.7, 6.1, Phe-α-C*H*), 5.08 (1H, br d, *J* = 4.8, Lys-N*H*), 6.47 (1H, d, *J* = 7.7, Phe-N*H*), 7.09-7.14 (2H, m, Ar-*H*), 7.20-7.32 (3H, m, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4 (Lys-CH_2), 28.3 (Boc-$(CH_3)_3$), 28.4 (Boc-$(CH_3)_3$), 29.6 (Lys-CH_2), 31.9, 37.9 (Phe-CH_2), 39.8 (Lys-CH_2), 52.3 (ester-CH_3), 53.1 (Phe-α-CH), 54.4 (Lys-α-CH), 79.1 (Boc-C), 80.0 (Boc-C), 127.1 (Ar C), 128.6 (Ar C), 129.2 (Ar C), 135.7 (Ar C), 155.6 (C=O), 156.1 (C=O), 171.6 (C=O), 171.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3340 w (N-H), 3060 w (C-H), 2974 w (N-H), 1741 s (ester C=O), 1692 s (amide C=O), 1517 s (C=C), 1271 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{42}N_3O_7$: 508.3023, found: 508.3021.

3.13.12.4 Fmoc-Arg(Pbf)-Phe-OMe (93)



Peptide **93** was synthesised from L-phenylalanine methyl ester hydrochloride **22** (0.539 g, 2.50 mmol) and N_2-Fmoc-N_5-Pbf-L-arginine (0.649 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **93** as a light brown solid (0.089 g, 11%); m.p. 108-110 ºC; R_f = 0.18 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 1.37-1.73 (10H, m, Pbf-(C*H*$_3$)$_2$ / Arg-C*H*$_2$ / Arg-C*H*$_2$), 2.06 (3H, s, Pbf-C*H*$_3$), 2.51 (3H, s, Pbf-C*H*$_3$), 2.58 (3H, s, Pbf-C*H*$_3$), 2.93-3.02 (3H, m, Pbf-C*H*$_2$ / Phe-C*H*H), 3.08-3.18 (3H, m, Phe-CH*H* / Arg-C*H*$_2$), 3.66 (3H, s, ester-C*H*$_3$), 4.07 (1H, dd, *J* = 8.1, 5.7, Arg-α-C*H*), 4.17 (1H, t, *J* = 6.6, Fmoc-C*H*), 4.30-4.39 (2H, m, Fmoc-C*H*$_2$), 4.66 (1H, dd, *J* = 8.1, 5.7, Phe-α-C*H*), 7.08-7.23 (5H, m, Ar-*H*), 7.28 (2H, t, *J* = 7.2, Ar-*H*), 7.37 (2H, t, *J* = 7.4, Ar-*H*), 7.62 (2H, d, *J* = 7.2, Ar-*H*), 7.78 (2H, d, *J* = 7.4, Ar-*H*).

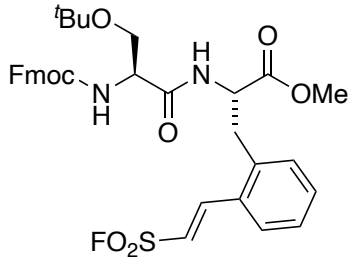
^{13}C NMR (100 MHz, CD_3OD) δ 12.7 (Pbf-CH_3), 18.6 (Pbf-CH_3), 19.8 (Pbf-CH_3), 27.1 (Arg-CH_2), 28.8 (Pbf-$(CH_3)_2$), 30.6 (Arg-CH_2), 38.4 (Phe-CH_2), 41.5 (Arg-CH_2), 44.1 (Pbf-CH_2), 48.2 (Fmoc-CH), 52.9 (ester-CH_3), 55.2 (Phe-α-CH), 56.0 (Arg-α-CH), 68.0 (Fmoc-CH_2), 87.8 (4º Pbf-C), 118.6 (Ar C), 121.1 (Ar C), 126.2 (Ar C), 126.4 (Ar C), 128.0 (Ar C), 128.3 (Ar C), 129.0 (Ar C), 129.6 (Ar C), 130.4 (Ar C), 133.6 (Ar C), 134.5 (Ar C), 138.1 (Ar C), 139.6 (Ar C) 142.7 (Ar C), 145.2 (Ar C), 145.5 (Ar C), 158.4 (C=O), 160.0 (C=N), 173.4 (C=O), 174.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3329 m (N-H), 3030 w C-H, 2930 w (C-H), 1722 s (ester C-O), 1658 s (amide C=O), 1543 m (C=C), 1245 m (C-O), 1156 s (S=O), 1088 s (S-N).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{44}H_{52}N_5O_8S$: 810.3537, found: 810.3532.

3.13.13 Synthesis of modified dipeptide (94-96)

3.13.13.1 Fmoc-Ser(*t*Bu)-Phe(2-VSF)-OMe (94)



Modified dipeptide **94** was synthesised from Fmoc-Ser(*t*Bu)-Phe-OMe **90** (0.094 g, 0.20 mmol) and ESF **50** (0.034 mL, 0.40 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **94** as a brown oil (0.039 g, 30%); R_f = 0.31 (8:2 EtOAc / Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (9H, s, ^{t}Bu-(C*H*$_3$)$_3$), 3.22-3.43 (3H, m, Phe-C*H*$_2$ / Ser-C*H*H), 3.71 (3H, s, ester-C*H*$_3$), 3.78-3.87 (1H, m, Ser-CH*H*), 4.17-4.28 (2H, m, Fmoc-C*H* / Ser-α-C*H*), 4.39 (2H, d, *J* = 6.7, Fmoc-C*H*$_2$), 4.87 (1H, dt, *J* = 7.6, 6.2, Phe-α-C*H*), 5.72 (1H, br d, *J* = 4.9, Ser-N*H*), 6.86 (1H, dd, *J* = 15.4, 2.5, alkene-C*H*), 7.24-7.70 (11H, m, Ar-*H* / Phe-N*H*), 7.77 (2H, d, *J* = 7.6, Ar-*H*), 8.15 (1H, d, *J* = 15.4, alkene-C*H*).

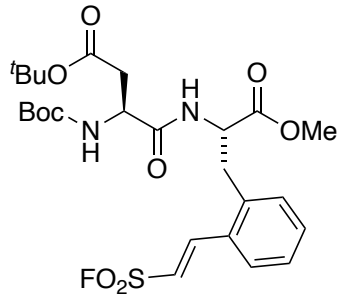
^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3 (^{t}Bu-(CH_3)$_3$), 35.0 (Phe-CH_2), 47.1 (Fmoc-CH), 52.7 (ester-CH_3), 53.2 (Phe-α-CH), 54.1 (Ser-α-CH), 61.6 (Ser-CH_2), 67.2 (Fmoc-CH_2), 74.5 (^{t}Bu-C), 119.5 ($^3J_{CF}$ = 27.8, alkene-C), 120.0 (Ar C), 125.1 (Ar C), 127.1 (Ar C), 127.4 (Ar C), 127.7 (Ar C), 128.1 (Ar C), 130.1 (Ar C), 131.7 (Ar C), 132.3 (Ar C), 137.2 (Ar C), 141.3 (Ar C), 143.7 (Ar C), 145.5 ($^4J_{CF}$ = 2.9, alkene-C), 158.0 (Fmoc C=O), 170.2 (C=O), 170.9 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.2 (s).

IR υ_{max}/cm^{-1} (solid) 3247 w (N-H), 3064 w (C-H), 2971 w (C-H), 1718 s (ester C=O), 1651 s (amide C=O), 1510 s (C=C), 1402 s (S=O), 1215 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{34}H_{38}N_2O_8SF$: 653.2333, found: 653.2330.

3.13.13.2 Boc-Asp(O^{t}Bu)-Phe(2-VSF)-OMe (95)



Modified dipeptide **95** was synthesised from Boc-Asp(O^{t}Bu)-Phe-OMe **91** (0.075 g, 0.20 mmol) and ESF **50** (0.034 mL, 0.40 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 °C).) followed by recrystallisation from CH_2Cl_2 / hexanes gave **95** as a light brown solid (0.053 g, 47%); m.p. 69-71 °C; R_f = 0.30 (8:2 EtOAc / Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (18H, s, Boc-(C*H*$_3$)$_3$ / tBu-(C*H*$_3$)$_3$), 2.58 (1H, dd, *J* = 17.0, 6.2, Asp-C*H*H), 2.89 (1H, dd, *J* = 17.0, 4.4, Asp-CH*H*), 3.23 (1H, dd, *J* = 14.4, 6.0, Phe-C*H*H), 3.30 (1H, dd, *J* = 14.4, 6.1, Phe-CH*H*), 3.69 (3H, s, ester-C*H*$_3$), 4.42-4.50 (1H, m, Asp-α-C*H*), 4.73-4.81 (1H, m, Phe-α-C*H*), 5.65 (1H, d, *J* = 8.0, Asp-N*H*), 6.87 (1H, dd, *J* = 15.3, 2.2, alkene-C*H*), 7.12-7.20 (2H, m, Ar-*H* / Phe-N*H*), 7.36 (1H, t, *J* = 7.6, Ar-*H*), 7.47 (1H, t, *J* = 7.5, Ar-*H*), 7.58 (1H, *J* = 7.9, Ar-*H*), 8.16 (1H, *J* = 15.3, alkene-C*H*).

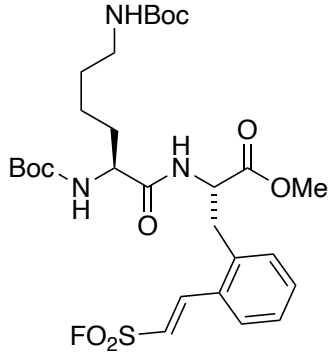
^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0 (Boc-(CH_3)$_3$), 28.2 (tBu-(CH_3)$_3$), 34.8 (Phe-CH_2), 37.1 (Asp-CH_2), 50.5 (Asp-α-CH), 52.7 (ester-CH_3), 53.4 (Phe-α-CH), 80.4 (tBu-C), 81.8 (Boc-C), 119.5 ($^3J_{CF}$ = 28.8, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 129.3 (Ar C), 130.1 (Ar C), 131.8 (Ar C0, 132.4 (Ar C), 145.6 ($^4J_{CF}$ = 2.9, alkene-C), 155.4 (C=O), 155.5 (C=O), 170.9 (C=O), 171. 4 (C=O), 171.4 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.2 (s).

IR υ_{max}/cm^{-1} (solid) 3322 w (N-H), 3030 w (C-H), 2974 w (C-H), 1737 s (ester C=O), 1684 s (amide C=O), 1510 s (C=C), 1394 s (S=O), 1244 s (C-O), 1160 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{36}N_2O_9SF$: 559.2126, found: 559.2122.

3.13.13.3 Boc-Lys(Boc)-Phe(2-VSF)-OMe (96)



Modified dipeptide **96** was synthesised from Boc-Lys(Boc)-Phe-OMe **92** (0.102 g, 0.20 mmol) and ESF **50** (0.034 mL, 0.40 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **96** as a brown solid (0.048 g, 39%); m.p. 78-80 °C; R_f = 0.25 (8:2 EtOAc / Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28-1.38 (2H, m, Lys-CH_2), 1.40-1.50 (20H, m, Boc-(CH_3)$_3$ / Boc-(CH_3)$_3$ / Lys-CH_2), 1.54-1.62 (1H, m, Lys-C*H*H), 1.74-1.83 (1H, m, Lys-CH*H*), 3.06-3.14 (2H, m, Lys-CH_2), 3.21 (1H, dd, *J* = 14.4, 6.7, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, 6.1, Phe-CH*H*), 3.72 (3H, s, ester-CH_3), 3.39-4.08 (1H, m, Lys-α-C*H*), 4.63 (1H, br t, *J* = 5.4, Boc-N*H*), 4.80 (1H, dt, *J* = 7.3, 6.7, Phe-α-C*H*), 5.09 (1H, br d, *J* = 5.7, Lys-N*H*), 6.72 (1H, d, *J* = 7.6, Phe-N*H*), 6.88 (1H, dd, *J* = 15.3, 2.3, alkene-C*H*), 7.28-7.30 (1H, m, Ar-*H*), 7.35 (1H, dt, *J* = 7.4, 0.6, Ar-*H*), 7.45 (1H, dt, *J* = 7.6, 1.3, Ar-*H*), 7.59 (1H, d, *J* = 7.4, Ar-*H*), 8.15 (1H, d, *J* = 15.3, alkene-C*H*).

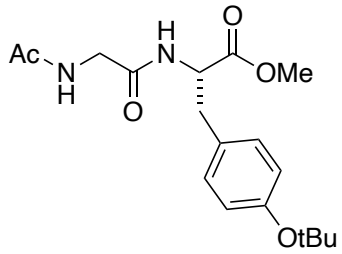
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5 (Lys-CH_2), 28.3 (Boc-$(CH_3)_3$), 28.4 (Boc-$(CH_3)_3$), 29.6 (Lys-CH_2), 31.5 (Lys-CH_2), 34.9 (Phe-CH_2), 39.7 (Lys-CH_2), 52.7 (ester-CH_3), 53.0 (Phe-α-CH), 54.4 (Lys-α-CH), 79.2 (Boc-C), 80.2 (Boc-C), 119.6 ($^3J_{CF}$ = 27.8, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 128.6 (Ar C), 130.1 (Ar C), 131.7 (Ar C), 132.3 (Ar C), 145.6 ($^4J_{CF}$ = 1.9, alkene-C), 156.1 (Boc C=O), 156.2 (Boc C=O), 171.2 (C=O), 171.7 (C=O), 172.0 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.2 (s).

IR υ_{max}/cm^{-1} (solid) 3317 w (N-H), 3056 w (C-H), 2978 w (C-H), 1748 s (ester C=O), 1670 s (amide C=O), 1510 s (C=C), 1402 s (S=O), 1245 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{28}H_{43}N_3O_9SF$: 616.2704, found: 616.2705.

3.13.14 Synthesis of Ac-Gly-Tyr(*t*Bu)-OMe (98)



Peptide **98** was synthesised from L-tyrosine methyl ester **99** (0.719 g, 2.50 mmol) and *N*-acetyl-glycine **26** (0.117 g, 1.00 mmol) using the procedure outlined in **Section 3.13.2.1**. Recrystallisation from CH_2Cl_2 / hexanes yielded **98** as an off-white solid (0.249 g, 71%); m.p. 70-72 ºC; R_f = 0.30 (EtOAc).

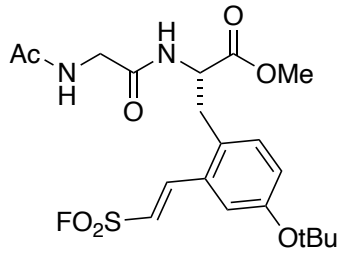
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (9H, s, tBu-(C*H*$_3$)$_3$), 1.98 (3H, s, acetyl-C*H*$_3$), 2.99 (1H, dd, *J* = 14.0, 6.9, Tyr-C*H*H), 3.05 (1H, dd, *J* = 13.7, 6.0, Tyr-CH*H*), 3.66 (3H, s, ester-C*H*$_3$), 3.82 (1H, dd, *J* = 16.7, 5.2, Gly-C*H*H), 3.91 (1H, dd, *J* = 16.7, 5.2, Gly-CH*H*), 4.76 (1H, dt, *J* = 7.8, 6.6, Tyr-α-C*H*), 6.76 (1H, t, *J* = 5.2, Gly-N*H*), 6.88 (2H, d, *J* = 8.5, Ar-*H*), 7.00 (2H, d, *J* = 8.5, Ar-*H*), 7.04 (1H, d, *J* = 7.8, Tyr-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.7 (acetyl-CH_3), 28.7 (*t*Bu-$(CH_3)_3$), 37.1 (Tyr-CH_2), 43.0 (Gly-CH_2), 52.2 (ester-CH_3), 53.4 (Tyr-α-CH), 78.4 (*t*Bu-C), 124.1 (Ar C), 129.6 (Ar C), 130.5 (Ar C), 154.3 (Ar C-O), 168.8 (C=O), 170.8 (C=O), 171.8 (C=O).

IR u_{max} /cm^{-1} (solid) 3265 w (N-H), 3068 w (C-H), 2974 w (C-H), 1752 s (ester C=O), 1640 s (amide C=O), 1566 m (C=C), 1233 m (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{27}N_2O_5$: 351.1920, found: 351.1919.

3.13.15 Synthesis of modified dipeptide Ac-Gly-Tyr(^{t}Bu)(2-VSF)-OMe (100)



Modified dipeptide **100** was synthesised from Ac-Gly-Tyr(^{t}Bu)-OMe **98** (0.070 g, 0.20 mmol) and ESF **50** (0.034 mL, 0.40 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (60% EtOAc in Pet. Ether 40-60 °C) followed by recrystallisation from CH_2Cl_2 / hexanes gave **100** as an off-white solid (0.048 g, 52%); m.p. 180-182 °C; R_f = 0.12 (60% EtOAc in Pet. Ether 40-60 °C).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (9H, s, Boc-(C*H*$_3$)$_3$), 1.95 (3H, s, acetyl-C*H*$_3$), 3.12 (1H, dd, *J* = 14.5, 6.2, Tyr-C*H*H), 3.23 (1H, dd, *J* = 14.5, 6.0, Tyr-CH*H*), 3.63 (3H, s, ester-C*H*$_3$), 3.77 (1H, dd, *J* = 16.8, 5.1, Gly-C*H*H), 3.91 (1H, dd, *J* = 5.4, Gly-CH*H*), 4.71 (1H, dt, *J* = 7.6, 6.1, Tyr-α-C*H*), 6.36 (1H, t, *J* = 4.9, Gly-N*H*), 6.76 (1H, dd, *J* = 15.3, 2.4, alkene-C*H*), 6.83 (1H, d, *J* = 7.6, Tyr-N*H*), 7.02 (1H, dd, *J* = 8.5, 2.3, Ar-*H*), 7.06 (1H, d, *J* = 8.5, Ar-*H*), 7.10 (1H, d, *J* = 2.3, Ar-*H*), 7.99 (1H, d, *J* = 15.3, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (acetyl-CH_3), 28.8 (tBu-$(CH_3)_3$), 34.2 (Tyr-CH_2), 43.1 (Gly-CH_2), 52.7 (ester-CH_3), 53.4 (Tyr-α-CH), 79.5 (tBu-C), 119.3 (J_{CF} = 27.8, alkene-C), 122.2 (Ar C), 127.9 (Ar C), 130.8 (Ar C), 131.9 (Ar C), 132.2 (Ar C), 145.6 (J_{CF} = 1.9, alkene-C), 155.1 (Ar C), 168.8 (C=O), 170.9 (C=O), 171.1 (C=O).

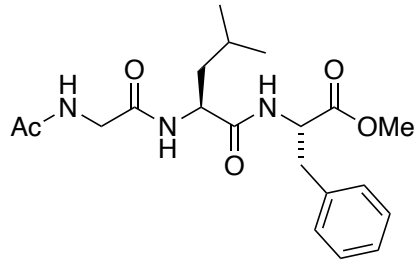
^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3310 w (N-H), 3079 w (C-H), 2960 w (C-H), 1730 s (ester C=O), 1651 s (amide C=O), 1539 s (C=C), 1398 s (S=O), 1223 s (C-O), 1189 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{28}N_2O_7SF$: 459.1601, found: 459.1600.

3.13.16 Synthesis of *tri*- and *tetra*-peptides (102-107)

3.13.16.1 Ac-Gly-Leu-Phe-OMe (102)



Known compound. The tripeptide **102** was elongated from H-Phe-2-Cl-Trtyl resin (0.256 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the esterification process outlined in **Section 3.13.2.3**. Recrystallisation from CH_2Cl_2 / hexanes yielded tripeptide **102** as a white solid (0.047 g, 60%); m.p. 125-127 °C; R_f = 0.10 (EtOAc).

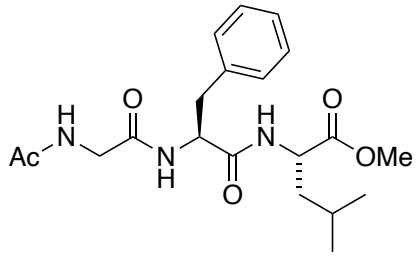
^{1}H NMR (400 MHz, CD_3OD) δ 0.89 (3H, d, *J* = 6.6, Leu-C*H*$_3$), 0.93 (3H, d, *J* = 6.6, Leu-C*H*$_3$), 1.46-1.55 (2H, m, Leu-C*H*$_2$), 1.57-1.67 (1H, m, Leu-C*H*), 2.00 (3H, s, acetyl-C*H*$_3$), 3.01 (1H, dd, *J* = 13.8, 8.7, Phe-C*H*H), 3.15 (1H, dd, *J* = 13.8, 5.8, Phe-CH*H*), 3.67 (3H, s, ester-C*H*$_3$), 3.82 (2H, m, Gly-C*H*$_2$), 4.43 (1H, dd, *J* = 9.0, 6.1, Leu-α-C*H*), 4.64 (1H, dt, *J* = 8.7, 5.8, Phe-α-C*H*), 7.15-7.32 (5H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 22.0 (Leu-CH_3), 22.4, (acetyl-CH_3), 23.4 (Leu-CH_3), 25.7 (Leu-CH), 38.2 (Phe-CH_2), 41.9 (Leu-CH_2), 43.5 (Gly-CH_2), 52.7 (ester-CH_3), 52.8 (Leu-α-CH), 55.2 (Phe-α-CH), 127.9 (Ar C), 129.5 (Ar C), 130.3 (Ar C), 138.1 (4º Ar C), 171.4 (C=O), 173.2 (C=O), 173.8 (C=O), 174.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3288 w (N-H), 3064 w (C-H), 2956 w (C-H), 1744 s (ester C=O), 1647 s (amide C=O), 1528 s (C=C), 1204 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{30}N_3O_5$: 392.2185, found: 392.2181.

3.13.16.2 Ac-Gly-Phe-Leu-OMe (103)



Known compound. The tripeptide **103** was elongated from H-Leu-2-Cl-Trtyl resin (0.250 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the esterification process outlined in **Section 3.13.2.3**. Recrystallisation from CH_2Cl_2 / hexanes yielded tripeptide **103** as a white solid (0.056 g, 71%); m.p. 180-182 °C; R_f = 0.10 (EtOAc).

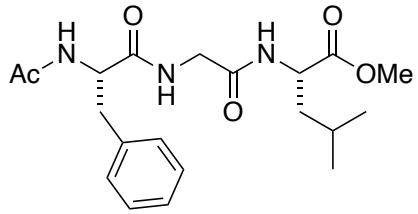
^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.95 (3H, d, *J* = 6.3, Leu-C*H*$_3$), 1.55-1.71 (3H, m, Leu-C*H*$_2$ / Leu-C*H*), 1.96 (3H, s, acetyl-C*H*$_3$), 2.90 (1H, dd, *J* = 14.0, 8.7, Phe-C*H*H), 3.16 (1H, dd, *J* = 14.0, 5.4, Phe-CH*H*), 3.68 (3H, s, ester-C*H*$_3$), 3.71 (1H, d, *J* = 16.6, Gly-C*H*H), 3.81 (1H, d, *J* = 16.6, Gly-CH*H*), 4.45 (1H, dd, *J* = 9.6, 5.3, Leu-α-C*H*), 4.67 (1H, dd, *J* = 8.7, 5.4, Phe-α-C*H*), 7.17-7.31 (5H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 22.0 (Leu-CH_3), 22.5 (acetyl-CH_3), 23.5 (Leu-CH_3), 26.0 (Leu-CH), 38.9 (Phe-CH_2), 41.5 (Leu-CH_2), 43.6 (Gly-CH_2), 52.3 (Leu-α-CH) 52.8 (ester-CH_3), 55.7 (Phe-CH_2), 127.9 (Ar C), 129.6 (Ar C), 130.5 (Ar C), 138.4 (4º Ar C), 171.5 (C=O), 173.7 (C=O), 174.0 (C=O), 174.4 (C=O).

IR υ_{max}/cm^{-1} (solid) 3284 w (N-H), 3030 w (C-H), 2952 w (C-H), 1748 s (ester C=O), 1648 s (amide C=O), 1543 s (C=C), 1208 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{30}N_3O_5$: 392.2185, found: 392.2184.

3.13.16.3 Ac-Phe-Gly-Leu-OMe (104)



Known compound. The tripeptide **104** was elongated from H-Leu-2-Cl-Trtyl resin (0.250 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the esterification process outlined in **Section 3.13.2.3**. Recrystallisation from CH_2Cl_2 / hexanes yielded tripeptide **104** as an off-white solid (0.033 g, 42%); m.p. 142-144 °C; R_f = 0.10 (EtOAc).

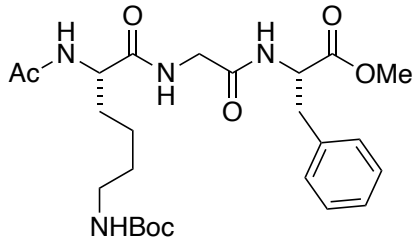
^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.94 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 1.55-1.74 (3H, m , Leu-C*H*$_2$ / Leu-C*H*), 1.91 (3H, s, acetyl-C*H*$_3$), 2.92 (1H, dd, *J* = 13.8, 9.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.8, 6.3, Phe-CH*H*), 3.70 (4H, m, ester-C*H*$_3$ / Gly-C*H*H), 3.93 (1H, d, *J* = 16.9, Gly-CH*H*), 4.43-4.53 (2H, m, Phe-α-C*H* / Leu-α-C*H*), 7.16-7.32 (5H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 22.6 (acetyl-CH_3), 23.6 (Leu-CH_3), 25.9 (Leu-CH), 38.4 (Phe-CH_2), 41.3 (Leu-CH_2), 43.4 (Gly-CH_2), 52.2 (Phe-α-CH), 52.9 (ester-CH_3), 57.1 (Leu-α-CH), 128.0 (Ar C), 129.6 (Ar C), 130.4 (Ar C), 138.6 (4º Ar C), 171.7 (C=O), 173.6 (C=O), 174.6 (C=O), 174.7 (C=O).

IR υ_{max}/cm^{-1} (solid) 3299 w (N-H), 3064 w (C-H), 2956 w (C-H), 1751 s (ester C=O), 1625 s (amide C=O), 1536 s (C=C), 1204 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{30}N_3O_5$: 392.2185, found: 392.2183.

3.13.16.4 Ac-Lys(Boc)-Gly-Phe-OMe (105)



The tripeptide **105** was elongated from H-Gly-2-Cl-Trtyl resin (0.238 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the solution-phase peptide synthesis outlined in **Section 3.13.2.4**. Purification by flash column chromatography (EtOAc) followed by trituration in Et_2O (20 mL) yielded tripeptide **105** as a white solid which was dried on the filter paper (0.051 g, 50%); m.p. 120-121 °C; R_f = 0.15 (EtOAc).

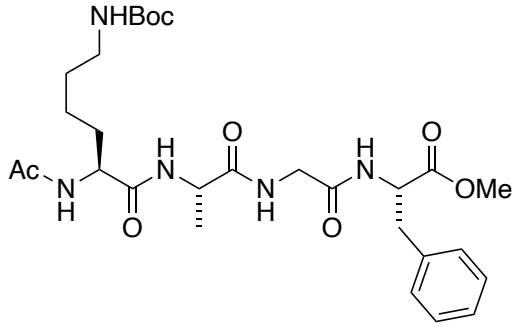
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29-1.53 (13H, m, Boc-(C*H*$_3$)$_3$ / Lys-C*H*$_2$ / Lys-C*H*$_2$), 1.57-1.69 (1H, m, Lys-C*H*H), 1.72-1.83 (1H, m, Lys-CH*H*), 1.96 (3H, s, acetyl-CH_3), 3.00-3.16 (4H, m, Phe-C*H*$_2$ / Lys-C*H*$_2$), 3.68 (3H, s, ester-C*H*$_3$), 3.88 (1H, dd, *J* = 16.8, 5.4, Gly-C*H*H), 4.00 (1H, dd, *J* = 16.9, 5.7, Gly-CH*H*), 4.43 (1H, dt, *J* = 7.1, 6.4, Lys-α-C*H*), 4.83 (1H, dt, *J* = 7.6, 6.5, Phe-α-C*H*), 4.89 (1H, t, *J* = 5.2, Boc-N*H*), 6.85 (1H, d, *J* = 7.4, Lys-N*H*), 7.08-7.15 (2H, m, Ar-*H*), 7.19-7.31 (4H, m, Ar-*H* / Phe-N*H*), 7.43 (1H, t, *J* = 5.5, Gly-N*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5 (Lys-CH_2), 22.9 (acetyl-CH_3), 28.4 (Boc-$(CH_3)_3$), 29.6 (Lys-CH_2), 31.6 (Lys-CH_2), 37.8 (Phe-CH_2), 39.8 (Lys-CH_2), 42.8 (Gly-CH_2), 52.3 (ester-CH_3), 53.2 (Lys-α-CH), 53.4 (Phe-α-CH), 79.0 (Boc-C), 127.0 (Ar C), 128.5 (Ar C), 129.2 (Ar C), 135.9 (Ar C), 156.2 (Boc C=O), 168.6 (C=O), 170.8 (C=O), 172.0 (C=O), 172.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3299 m (N-H), 3064 w (C-H), 2933 w (C-H), 1737 s (ester C=O), 1629 s (amide C=O), 1528 s (C=C), 1218 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{39}N_4O_7$: 507.2819, found: 507.2813.

3.13.16.5 Ac-Lys(Boc)-Ala-Gly-Phe-OMe (106)



The tetrapeptide **106** was elongated from H-Gly-2-Cl-Trtyl resin (0.238 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the solution-phase peptide coupling outlined in **Section 3.13.2.4**. Trituration in Et_2O (20 mL) yielded tetrapeptide **106** as a white solid which was dried on the filter paper (0.062 g, 54%); m.p. 60-62 °C; R_f = 0.08 (EtOAc).

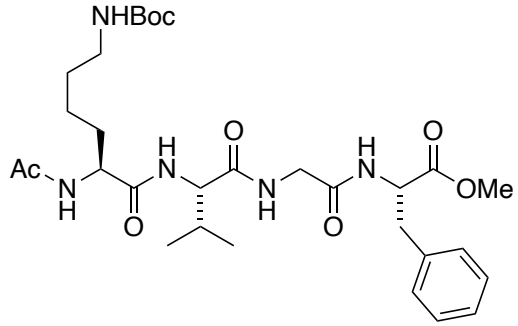
^{1}H NMR (400 MHz, CD_3OD) δ 1.33-1.52 (17H, m, Ala-C*H*$_3$ / Boc-(C*H*$_3$)$_3$ / Lys-C*H*$_2$ / Lys-C*H*$_2$) 1.58-1.70 (1H, m, Lys-C*H*H), 1.74-1.84 (1H, m, Lys-CH*H*), 1.98 (3H, s, acetyl-C*H*$_3$), 2.98-3.08 (3H, Lys-C*H*$_2$ / Phe-CH*H*), 3.15 (1H, dd, *J* = 13.7, 5.8, Phe-CH*H*), 3.67 (3H, s, ester-C*H*$_3$), 3.74 (1H, d, *J* = 16.9, Gly-C*H*H), 3.93 (1H ,d, *J* = 16.9, Gly-CH*H*), 4.21-4.32 (2H, m, Lys-α-C*H* / Ala-α-C*H*), 4.65 (1H, dd, *J* = 8.3, 5.8, Phe-α-C*H*), 7.17-7.23 (3H, m, Ar-*H*), 7.24-7.31 (2H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 17.6 (Ala-CH_3), 22.6 (acetyl-CH_3), 24.3 (Lys-CH_2), 28.9 (Boc-$(CH_3)_3$), 30.7 (Lys-CH_2), 32.7 (Lys-CH_2), 38.6 (Phe-CH_2), 41.2 (Gly-CH_2), 43.3 (Lys-CH_2), 51.0 (Ala-α-CH), 52.9 (ester-CH_3), 55.2 (Phe-α-CH), 55.6 (Lys-α-CH), 80.8 (Boc-C), 128.0 (Ar C), 129.7 (Ar C), 130.5 (Ar C), 138.3 (Ar C), 158.7 (Boc C=O), 171.5 (C=O), 173.5 (C=O), 174.0 (C=O), 174.7 (C=O), 175.4 (C=O).

IR υ_{max}/cm^{-1} (solid) 3284 m (N-H), 3064 w (C-H), 2978 w (C-H), 1741 m (ester C=O), 1629 s (amide C=O), 1528 s (C=C), 1226 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{28}H_{44}N_5O_8$: 578.3190, found: 578.3187.

3.13.16.6 Ac-Lys(Boc)-Val-Gly-Phe-OMe (107)



The tetrapeptide **107** was elongated from H-Gly-2-Cl-Trtyl resin (0.238 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the solution-phase peptide coupling outlined in **Section 3.13.2.4**. Trituration in Et_2O (20 mL) yielded tetrapeptide **107** as a white solid which was dried on the filter paper (0.075 g, 62%); m.p. 203-205 °C; R_f = 0.08 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 0.95 (3H, d, *J* = 6.7, Val-C*H*$_3$), 0.96 (3H, d, *J* = 6.7, Val-C*H*$_3$), 1.32-1.48 (12H, m, Boc-(C*H*$_3$)$_3$ / Lys-C*H*H / Lys-C*H*$_2$), 1.55-1.70 (2H, m, Lys-C*H*$_2$), 1.72-1.81 (1H, m, Lys-CH*H*), 1.97 (3H, s, acetyl-C*H*$_3$), 2.03-2.12 (1H, m, Val-C*H*), 2.97-3.06 (3H, m, Lys-C*H*$_2$ / Phe-C*H*H), 3.14 (1H, dd, *J* = 13.8, 6.0, Phe-CH*H*), 3.67 (3H, s, ester-C*H*$_3$), 3.78 (1H, d, *J* = 16.1, Gly-C*H*H), 3.92 (1H, d, *J* = 16.7, Gly-CH*H*), 4.11 (1H, d, *J* = 7.1, Val-α-C*H*), 4.29-4.35 (1H, m, Lys-α-C*H*), 4.67 (1H, dd, *J* = 8.2, 6.0, Phe-α-C*H*), 7.16-7.24 (3H, m, Ar-*H*), 7.25-7.31 (2H, m, Ar-*H*).

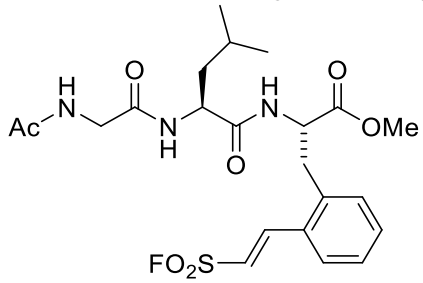
^{13}C NMR (100 MHz, CD_3OD) δ 18.9 (Val-CH_3), 19.9 (Val-CH_3), 22.5 (acetyl-CH_3), 24.4 (Val-CH), 28.9 (Boc-$(CH_3)_3$), 30.8 (Lys-CH_2), 31.8 (Lys-CH_2), 32.7 (Lys-CH_2), 38.7 (Phe-CH_2), 41.2 (Lys-CH_2), 43.2 (Gly-CH_2), 52.8 (ester-CH_3), 55.0 (Phe-α-CH), 55.5 (Lys-α-CH), 60.7 (Val-α-CH), 80.0 (Boc-C), 128.1 (Ar C), 129.7 (Ar C), 130.5 (Ar C), 138.2 (Ar C), 158.7 (Boc C=O), 171.2 (C=O), 173.4 (C=O), 173.6 (C=O), 174.2 (C=O), 174.9 (C=O).

IR υ_{max}/cm^{-1} (solid) 3313 m (N-H), 3060 w (C-H), 2967 w (C-H), 1744 s (ester C=O), 1625 s (amide C=O), 1535 s (C=C), 1226 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{48}N_5O_8$: 606.3503, found: 606.3500.

3.13.17 Modified tri- and tetrapeptides (108-113)

3.13.17.1 Ac-Gly-Leu-Phe(2-VSF)-OMe (108)



Modified tripeptide **108** was synthesised from Ac-Gly-Leu-Phe-OMe **102** (0.039 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **108** as a brown solid (0.023 g, 47%); m.p. 182-184 °C; R_f = 0.09 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (3H, d, *J* = 6.7, Leu-C*H*$_3$), 0.93 (3H, d, *J* = 6.4, Leu-C*H*$_3$), 1.45-1.55 (2H, m, Leu-C*H*$_2$), 1.56-1.68 (1H, m, Leu-C*H*), 2.00 (3H, s, acetyl-C*H*$_3$), 3.17 (1H, dd, *J* = 14.3, 8.7, Phe-C*H*H), 3.43 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.68 (3H, s, ester-C*H*$_3$), 3.81 (2H, s, Gly-C*H*$_2$), 4.42 (1H, dd, *J* = 8.8, Leu-α-C*H*), 4.58 (1H, dd, *J* = 8.7, 6.0, Phe-α-C*H*), 7.33-7.38 (2H, m, Ar-*H*), 7.42 (1H, dd, *J* = 15.1, 2.5, alkene-C*H*), 7.47 (1H, dt, *J* = 7.6, 1.4, Ar-*H*), 7.77 (1H, d, *J* = 7.8, Ar-*H*), 8.23 (1H, d, *J* = 15.1, alkene-C*H*).

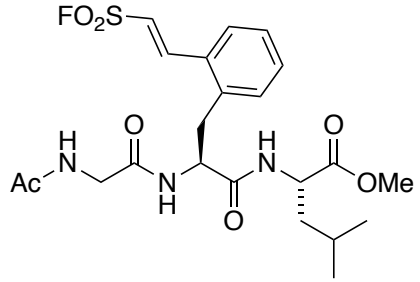
^{13}C NMR (100 MHz, CD_3OD) δ 22.1 (Leu-CH_3), 22.6 (acetyl-CH_3), 23.5 (Leu-CH_3), 25.9 (Leu-CH), 35.3 (Phe-CH_2), 42.0 (Leu-CH_2), 43.7 (Gly-CH_2), 53.0 (Leu-α-CH), 53.1 (ester-CH_3), 55.1 (Phe-α-CH), 121.5 ($^3J_{CF}$ = 27.9, alkene-C), 129.0 (Ar C), 129.2 (Ar C), 131.9 (4º Ar C), 132.7 (Ar C), 133.5 (Ar C), 139.5 (4º Ar C), 147.1 ($^4J_{CF}$ = 2.9, alkene-C), 171.6 (C=O), 172.8 (C=O), 174.1 (C=O), 174.8 (C=O).

^{19}F NMR (376 MHz, CD_3OD) δ 59.7 (s).

IR υ_{max}/cm^{-1} (solid) 3295 w (N-H), 3049 w (C-H), 2960 w (C-H), 1752 s (ester C=O), 1625 s (amide C=O), 1543 m (C=C), 1409 s (S=O), 1267 s (C-O), 1197 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{31}N_3O_7SF$: 500.1867, found: 500.1862.

3.13.17.2 Ac-Gly-Phe(ESF)-Leu-OMe (109)



Modified tripeptide **109** was synthesised from Ac-Gly-Phe-Leu-OMe **103** (0.039 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.1**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **109** as a brown solid (0.025 g, 50%); m.p. 205-208 °C; R_f = 0.10 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 0.89 (3H, d, *J* = 6.3, Leu-C*H*$_3$), 0.92 (3H, d, *J* = 6.3, Leu-C*H*$_3$), 1.52-1.67 (3H, m, Leu-C*H*$_2$ / Leu-C*H*), 1.98 (3H, s, acetyl-C*H*$_3$), 3.11 (1H, dd, *J* = 14.3, 7.4, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.3, 7.1, Phe-CH*H*), 3.64 (3H, s, ester-C*H*$_3$), 3.74-3.84 (2H, m, Gly-C*H*$_2$), 4.42 (1H, dd, *J* = 9.1, 5.8, Leu-α-C*H*), 4.66 (1H, dd, *J* = 7.4, 7.1, Phe-α-C*H*), 7.32-7.41 (3H, m, Ar-*H* / alkene-C*H*), 7.46 (1H, dt, *J* = 7.6, 1.3, Ar-*H*), 7.76 (1H, d, *J* = 7.9, Ar-*H*), 8.22 (1H, d, *J* = 15.3, alkene-C*H*).

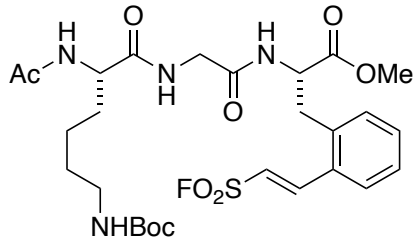
^{13}C NMR (100 MHz, CD_3OD) δ 22.0 (Leu-CH_2), 22.5 (acetyl-CH_3), 23.4 (Leu-CH_3), 26.0 (Leu-CH), 35.6 (Phe-CH_2), 41.6 (Leu-CH_2), 43.7 (Gly-CH_2), 52.4 (ester-CH_3), 52.8 (Leu-α-CH), 55.5 (Phe-α-CH), 121.3 ($^3J_{CF}$ = 27.8, alkene-C), 129.0 (Ar C), 129.0 (Ar C), 132.2 (Ar C), 132.6 (Ar C), 133.4 (Ar C), 139.4 (Ar C), 147.4 ($^4J_{CF}$ = 1.9, alkene-C), 171.5 (C=O), 172.7 (C=O), 174.0 (C=O), 174.2 (C=O).

^{19}F NMR (376 MHz, CD_3OD) δ 59.7 (s).

IR υ_{max}/cm^{-1} (solid) 3283 w (N-H), 3067 w (C-H), 2967 w (C-H), 1744 s (ester C=O), 1625 s (amide C=O), 1528 m (C=C), 1402 s (S=O), 1278 m (C-O), 1197 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{31}N_3O_7SF$: 500.1867, found: 500.1863.

3.13.17.3 Ac-Lys(Boc)-Gly-Phe(2-VSF)-OMe (111)



Modified tripeptide **111** was synthesised from Ac-Lys(Boc)-Gly-Phe-OMe **105** (0.051 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **111** as a brown solid (0.020 g, 32%); m.p. 152-154 °C; R_f = 0.15 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.55 (11H, m, Boc-(C*H*$_3$)$_3$ / Lys-C*H*$_2$), 1.64-1.91 (4H, m, Lys-C*H*$_2$ / Lys-C*H*$_2$), 2.03 (3H, s, acetyl-C*H*$_3$), 3.05-3.17 (2H, m, Lys-C*H*$_2$), 3.24 (1H, dd, *J* = 14.4, 6.9, Phe-C*H*H), 3.38 (1H, dd, *J* = 14.4, 5.5, Phe-CH*H*), 3.72 (3H, s, ester-C*H*$_3$), 3.83 (1H, dd, *J* = 17.0, 5.3, Gly-C*H*H), 4.02 (1H, dd, *J* = 17.0, 6.3, Gly-CH*H*), 4.21-4.29 (1H, m, Lys-α-C*H*), 4.72 (1H, t, *J* = 5.5, Boc-N*H*), 4.78 (1H, dt, *J* = 6.9, 6.1, Phe-α-C*H*), 6.57 (1H, br d, *J* = 4.6, Lys-N*H*), 6.88 (1H, dd, *J* = 15.3, 2.4, alkene-C*H*), 7.18-7.27 (2H, m, Ar-*H* / Phe-N*H*), 7.35 (1H, t, *J* = 7.6, Ar-*H*), 7.46 (1H, t, *J* = 7.6, Ar-*H*), 7.59 (1H, d, *J* = 7.8, Ar-*H*), 8.15 (1H, d, *J* = 15.3, alkene-C*H*).

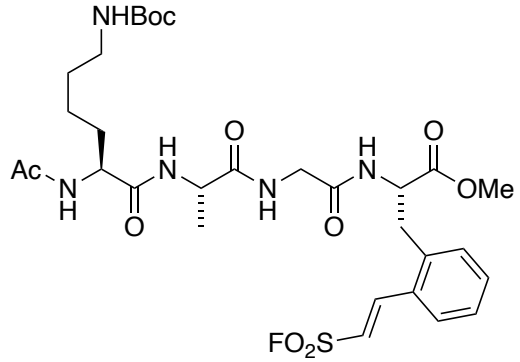
^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2 (Lys-CH_2), 23.0 (acetyl-CH_3), 28.4 (Boc-$(CH_3)_3$), 29.8 (Lys-CH_2), 30.7 (Lys-CH_2), 34.8 (Phe-CH_2), 39.4 (Lys-CH_2), 42.9 (Gly-CH_2), 52.7 (ester-CH_3), 53.4 (Lys-α-CH), 54.0 (Phe-α-CH), 79.3 (Boc-C), 119.2 ($^3J_{CF}$ = 27.9, alkene-C), 127.4 (Ar C), 128.1 (Ar C), 130.1 (Ar C), 131.6 (Ar C), 132.5 (Ar C), 137.8 (Ar C), 146.0 ($^4J_{CF}$ = 2.8, alkene-C), 156.5 (Boc C=O), 168.8 (C=O), 171.3 (C=O), 171.4 (C=O), 172.4 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3302 w (N-H), 3049 w (C-H), 2933 w (C-H), 1718 s (ester C=O), 1633 s (amide C=O), 1524 s (C=C), 1402 s (S=O), 1264 s (C-O), 1193 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{27}H_{40}N_4O_9SF$: 615.2500, found: 615.2505.

3.13.17.4 Ac-Lys(Boc)-Ala-Gly-Phe(ESF)-OMe (112)



Modified tetrapeptide **112** was synthesised from Ac-Lys(Boc)-Ala-Gly-Phe-OMe **106** (0.058 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **112** as a brown solid (0.019 g, 28%); m.p. 150-151 °C; R_f = 0.05 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 1.34-1.50 (16H, m, Boc-(C*H*$_3$)$_3$ / Ala-C*H*$_3$ / Lys-C*H*$_2$ / Lys-C*H*$_2$), 1.58-1.70 (1H, m, Lys-C*H*H), 1.75-1.85 (1H, m, Lys-CH*H*), 1.99 (3H, s, acetyl-C*H*$_3$), 3.02 (2H, app t, *J* = 6.5, Lys-C*H*$_2$), 3.19 (1H, dd, *J* = 14.4, 8.2, Phe-C*H*H), 3.44 (1H, dd, *J* = 14.4, 6.0, Phe-CH*H*), 3.64-3.76 (4H, m, ester-C*H*$_3$ / Gly-C*H*H), 3.94 (1H, d, *J* = 17.0, Gly-CH*H*), 4.21-4.30 (2H, m, Ala-α-C*H* / Lys-α-C*H*), 4.62 (1H, dd, *J* = 8.0, 6.0, Phe-α-C*H*), 7.32-7.50 (4H, m, Ar-*H* / alkene-C*H*), 7.78 (1H, d, *J* = 8.0, Ar-*H*), 8.23 (1H, d, *J* = 15.2, alkene-C*H*).

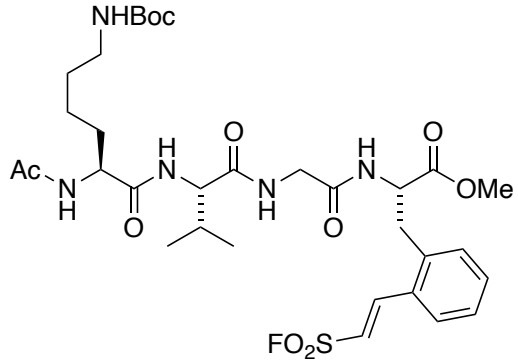
^{13}C NMR (100 MHz, CD_3OD) δ 17.5 (Ala-CH_3), 22.6 (acetyl-CH_3), 24.3 (Lys-CH_2), 28.9 (Boc-$(CH_3)_3$), 30.7 (Lys-CH_2), 32.7 (Lys-CH_2), 35.5 (Phe-CH_2), 41.1 (Gly-CH_2), 43.4 (Lys-CH_2), 51.0 (Ala-α-CH), 53.1 (ester-CH_3), 55.2 (Phe-α-CH), 55.3 (Lys-α-CH), 80.0 (Boc-C), 121.5 ($^3J_{CF}$ = 27.8, alkene-C), 129.0 (Ar C), 129.2 (Ar C), 131.8 (Ar C), 132.9 (Ar C), 133.5 (Ar C), 139.5 (Ar C), 147.0 ($^4J_{CF}$ = 2.9, alkene-C), 158.7 (Boc C=O), 171.6 (C=O), 172.8 (C=O), 174.0 (C=O), 174.8 (C=O), 175.4 (C=O).

^{19}F NMR (376 MHz, CD_3OD) δ 59.7 (s).

IR υ_{max}/cm^{-1} (solid) 3288 w (N-H), 3060 w (C-H), 2930 w (C-H), 1737 s (ester C=O), 1632 s (amide C=O), 1521 m (C=C), 1402 s (S=O), 1215 s (C-O), 1167 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{45}N_5O_{10}SF$: 686.2871, found: 616.2870.

3.13.17.5 Ac-Lys(Boc)-Val-Gly-Phe(ESF)-OMe (113)



Modified tetrapeptide **113** was synthesised from Ac-Lys(Boc)-Val-Gly-Phe-OMe **107** (0.061 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.2**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from CH_2Cl_2 / hexanes gave **113** as a brown solid (0.024 g, 34%); m.p. 160-162 °C; R_f = 0.07 (EtOAc).

^{1}H NMR (400 MHz, CD_3OD) δ 0.95 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.97 (3H, d, *J* = 6.9, Val-C*H*$_3$), 1.33-1.51 (13H, m, Boc-(C*H*$_3$)$_3$ / Lys-C*H*$_2$ / Lys-C*H*$_2$), 1.58-1.68 (1H, m, Lys-C*H*H), 1.72-1.83 (1H, m, Lys-CH*H*), 1.98 (3H, s, acetyl-C*H*$_3$), 2.04-2.14 (1H, m, Val-C*H*), 2.97-3.07 (2H, m, Lys-C*H*$_2$), 3.17 (1H, dd, *J* = 14.4, 7.8, Phe-C*H*H), 3.43 (1H, dd, *J* = 14.2, 6.2, Phe-CH*H*), 3.67 (3H, s, ester-C*H*$_3$), 3.76 (1H, d, *J* = 16.9, Gly-C*H*H), 3.94 (1H, d, *J* = 16.9, Gly-CH*H*), 4.10 (1H, d, *J* = 7.2, Val-α-C*H*), 4.30-4.36 (1H, m, Lys-α-C*H*), 6.63, (1H, dd, *J* = 7.8, 6.2, Phe-α-C*H*), 7.30-7.52 (4H, m, alkene-C*H* / Ar-*H*), 7.78 (1H, d, *J* = 7.9, Ar-*H*), 8.22 (1H, d, *J* = 15.2, alkene-C*H*).

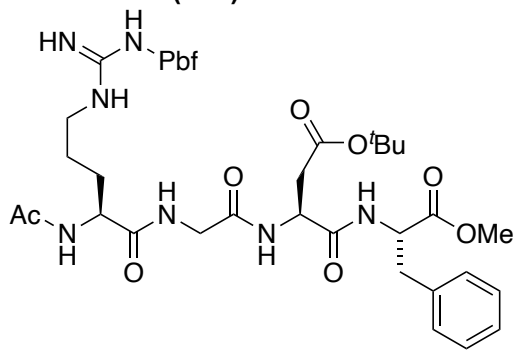
^{13}C NMR (100 MHz, CD_3OD) δ 19.0 (Val-CH_3), 19.9 (Val-CH_3), 22.6 (acetyl-CH_3), 24.3 (Lys-CH_2), 28.9 (Boc-$(CH_3)_3$), 30.7 (Lys-CH_2), 32.7 (Val-CH), 35.5 (Phe-CH_2), 41.2 (Gly-CH_2), 43.2 (Lys-CH_2), 53.1 (ester-CH_3), 55.0 (Phe-α-CH), 55.3 (Lys-α-CH), 60.8 (Val-α-CH), 80.0 (Boc-C), 121.5 ($^3J_{CF}$ = 27.8, alkene-C), 129.0 (Ar C), 129.2 (Ar C), 131.9 (Ar C), 132.8 (Ar C), 133.5 (Ar C), 139.5 (Ar C), 147.0 ($^4J_{CF}$ = 2.5, alkene-C), 158.7 (Boc C=O), 171. 3 (C=O), 172.8 (C=O), 173.6 (C=O), 174.2 (C=O), 174.9 (C=O).

^{19}F NMR (376 MHz, CD_3OD) δ 57.2 (s).

IR υ_{max}/cm^{-1} (solid) 3288 w (N-H), 3071 w (C-H), 2933 w (C-H), 1737 s (ester C=O), 1629 s (amide C=O), 1528 s (C=C), 1402 s (S=O), 1215 s (C-O), 1170 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{49}N_5O_{10}SF$: 714.3184, found: 714.3188.

3.13.18 Synthesis of RGD containing peptide Ac-Arg(Pbf)-Gly-Asp(O^{t}Bu)-Phe-OMe (114)



The tetrapeptide **114** was elongated from H-Asp(*O*^{t}Bu)-2-Cl-Trtyl resin (0.345 g, 0.20 mmol) using the procedure outlined in **Section 3.13.2.2.** Upon cleavage from the resin and isolation, the peptide acid was subjected to the solution-phase peptide coupling outlined in **Section 3.13.2.4**. Trituration in Et_2O (20 mL) yielded tetrapeptide **114** as a pale-yellow solid which was dried on the filter paper (0.110 g, 64%); m.p. 164-167 °C; R_f = 0.05 (EtOAc).

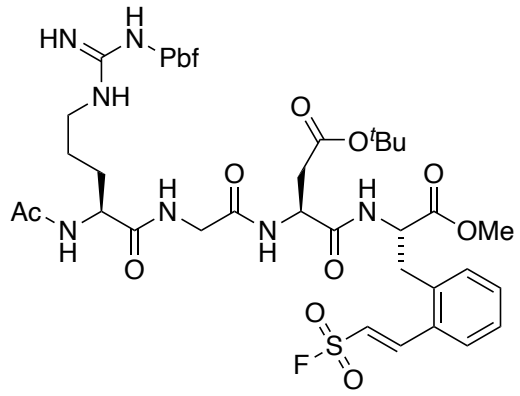
^{1}H NMR (400 MHz, CD_3OD) δ 1.38-1.49 (17H, m, Pbf-(C*H*$_3$)$_2$ / Asp-(C*H*$_3$)$_3$ / Arg-C*H*$_2$), 1.50-1.72 (3H, m, Arg-C*H*$_2$ / Arg-C*H*H), 1.75-1.85 (1H, m, Arg-CH*H*), 1.99 (3H, s, Pbf-C*H*$_3$), 2.07 (3H, s, acetyl-C*H*$_3$), 2.51 (3H, s, Pbf-C*H*$_3$), 2.54-2.64 (4H, m, Pbf-C*H*$_3$ / Asp-C*H*H), 2.73 (1H, dd, *J* = 16.5, 5.5, Asp-CH*H*), 3.02-3.18 (2H, m, Phe-C*H*$_2$), 3.65 (3H, s, ester-C*H*$_3$), 3.79 (1H, d, *J* = 16.7, Gly-C*H*H), 3.90 (1H, d, *J* = 16.7, Gly-CH*H*), 4.28 (1H, dd, *J* = 7.3, 6.0, Arg-α-C*H*), 4.60 (1H, dd, *J* = 8.2, 6.0, Phe-α-C*H*), 4.74 (1H, dd, *J* = 8.2, 5.5, Asp-α-C*H*), 7.12-7.30 (5H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 12.7 (Pbf-CH_3), 18.6 (Pbf-CH_3), 19.8 (Pbf-CH_3), 22.7 (acetyl-CH_3), 27.0 (Arg-CH_2), 28.4 (Asp-$(CH_3)_3$), 28.9 (Pbf-$(CH_3)_2$), 29.8 (Arg-CH_2) 38.2 (Asp-CH_2), 38.4 (Phe-CH_2), 40.2 (Arg-CH_2), 43.7 (Gly-CH_2), 44.1 (Pbf-CH_2), 51.3 (Asp-α-CH), 52.9 (ester-CH_3), 55.1 (Arg-α-CH), 55.6 (Phe-α-CH), 82.6 (Asp-O^{t}Bu-C), 87.8 (4º Pbf-C), 118.6 (Ar C), 126.2 (Ar C), 128.0 (Ar C), 129.7 (Ar C), 130.1 (Ar C), 130.5 (Ar C), 133.7 (Ar C), 134.4 (Ar C), 138.2 (Ar C), 139.6 (Ar C), 158.3 (C=O), 160.0 (C=N), 171.3 (C=O), 172.8 (C=O), 173.2 (C=O), 173.8 (C=O), 175.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3302 w (N-H), 3060 w (C-H), 2974 w (C-H), 1730 m (amide C=O), 1651 s (ester C=O), 1641 m (C=N), 1543 s (C=C), 1405 s (S=O), 1249 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{41}H_{60}N_7O_{11}S$: 858.4072, found: 858.4074.

3.13.19 Modified RGD containing peptide (115)



Modified tetrapeptide **115** was synthesised from Ac-Lys(Boc)-Val-Gly-Phe-OMe **114** (0.061 g, 0.10 mmol) and ESF **50** (0.017 mL, 0.20 mmol) according to the procedure outlined in **Section 3.13.3.2**. The solvents were removed *in vacuo* and the crude compound **115** was collected as a brown solid. (0.032 g). R_f = 0.05 (EtOAc).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{43}H_{61}N_7O_{13}S_2F$: 966.3753, found: 966.3756.

4 Sulfur(vi)-fluoride exchange reactions on modified peptides

4.1 Introduction

SuFEx transformations have gained significant popularity over the last decade and are referred to as '*the next generation click reaction*'.[148] The installation of ESF by C(sp^2)-H olefination on a phenylalanine residue in peptides, as described in **Chapter 3** generates a substituted aryl vinylsulfonyl fluoride (aryl VSF), **Figure 25**.

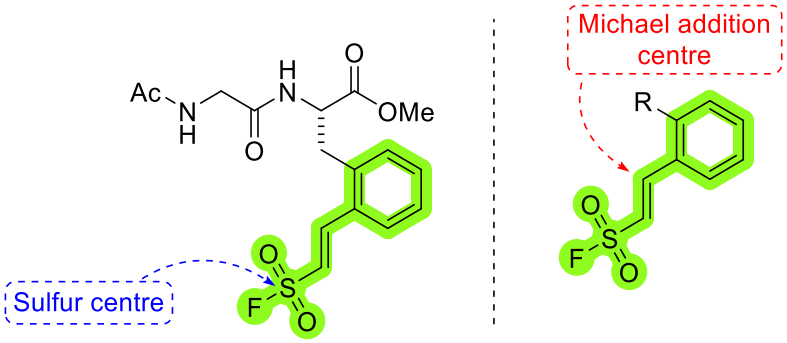
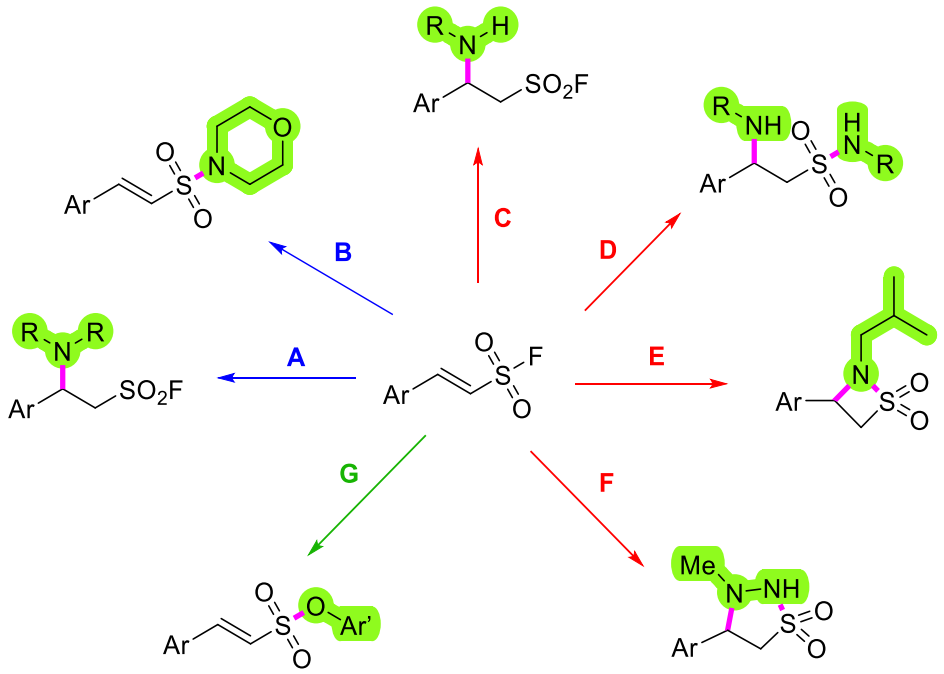


Figure 25 – Structural characteristics of aryl VSFs.

The aryl VSF 'SuFEx-able' motif has been proven to undergo modification with nucleophiles at the sulfur centre to form sulfur-heteroatom bonds or by Michael addition at the C=C double bond to form carbon-heteroatom bonds.[131] Both of these transformations from aryl VSF are well documented on simple aromatic structures, **Scheme 84**. The reactions require nucleophiles from three distinct types: secondary amines, primary amines and silyl ethers. Due to the dual-reactivity possessed by aryl VSF, secondary amines have been reported as a nucleophilic partner with reactions at both the C=C double bond, **Scheme 84A** and sulfur atom, **Scheme 84B** of the molecule.[154,161]



Scheme 84 - Documented SuFEx reactions on aryl VSFs.

Primary amines have been documented to react by Michael addition at the C=C double bond, **Scheme 84C** and by displacement of fluoride at sulfur, **Scheme 84D**.[169,170] A primary amine also possesses a unique ability to react sequentially at both centres on the aryl VSF to form a four membered cyclic sulfonamide, **Scheme 84E**.[155] When exposed to methyl hydrazine, the aryl VSF reacts at both electrophilic centres, once with each amine to form a five membered ring, **Scheme 84F**.[154]

Silyl ethers represent the final distinct type of nucleophile. In each case, they react exclusively at the sulfur centre and generate sulfonate esters from aryl silyl ethers, **Scheme 84G**.[154,161] This is the most significantly reported transformation in the entire SuFEx family and possesses a vast and ever-growing list of applications from carbon fibre surface modification[171] to the synthesis of scarce and under-developed polysulfonate polymers.[131,140]

There are many literature examples of each of these three types of transformations on simple aromatic molecules. However, this chemistry is not well documented when the aryl groups are peptide-based compounds such as the compound highlighted in **Figure 25**. This

study in the project was therefore concerned with the further reactivity of aryl VSFs when housed on phenylalanine containing peptides.

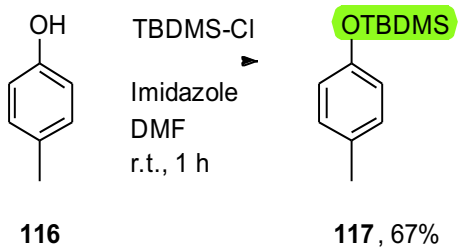
4.2 Practical considerations

Initially Ac-Gly-Phe(2-VSF)-OMe **51** was chosen as the model modified dipeptide for this study. The R_f of this dipeptide in polar solvents such as ethyl acetate is small (R_f = 0.15). Investigations initially began with this model modified dipeptide, however purification by normal-phase chromatography quickly became problematic. It was recognised that modification using SuFEx chemistry in some cases significantly increased the polarity of the peptide, leading to difficulties purifying on normal-phase silica gel. To avoid the need to switch to reversed-phase silica, a less polar peptide was selected as the model. Ac-Val-Phe(2-VSF)-OMe **59** was selected as the new model substrate. It was hoped that the decreased polarity of **59** (R_f = 0.35) vs that of peptide **51** (R_f = 0.15) was enough to make purification easier.

4.3 Silyl Ether SuFEx reactions

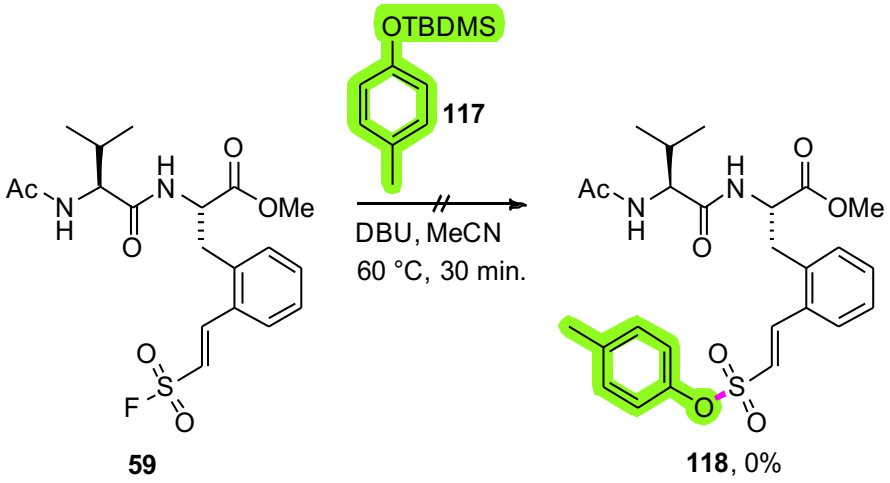
A variety of transformations have been described which activate the S-F bond on an aryl VSF allowing for attack by a nucleophilic species; these were discussed in **Section 4.1**. Having converted chemically inert phenylalanine residues into reactive SuFEx-able hubs, an attempt to perform these documented transformations on peptide-based residues was implemented.

The investigation into silyl ether nucleophiles began with the synthesis of simple silyl ether **117** as shown in **Scheme 85**. The aromatic substrate *p*-cresol **116** was chosen to simplify the ^{1}H NMR spectra of future products. The doublets corresponding to the aromatic protons in *p*-cresol appear at below 7.00 ppm and consequently do not interfere with the aromatic protons on the modified peptide.



*Scheme 85 – Synthesis of silyl ether **117**.*

The synthesised compound **117** was fully characterised by NMR spectroscopy. The ^{1}H NMR of the purified product matched that reported for the compound in literature.[172] A parent ion peak in the HRMS (ESI) $[M+H]^{+}$ of 223.1510 provided further evidence that the silyl ether had been formed. An attempt to carry out SuFEx chemistry upon the modified dipeptide model **59** was then undertaken.



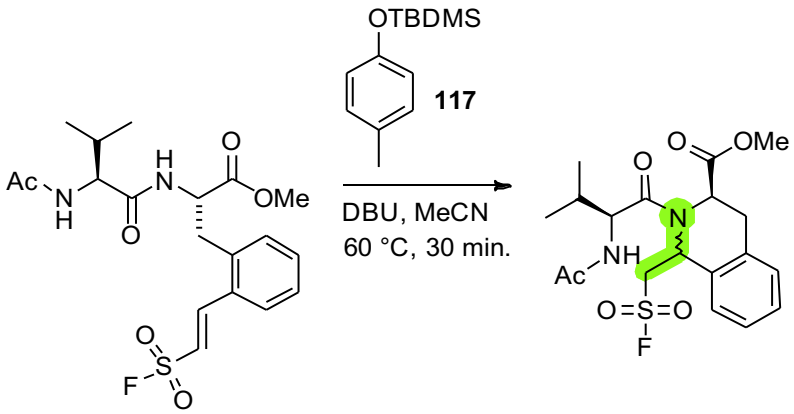
*Scheme 86 – Initial SuFEx attempt with model dipeptide **59** and silyl ether nucleophile **117**.*

Upon exposure to the silyl ether **117** in the presence of sterically hindered tertiary amine base DBU, **Scheme 86**, modified peptide **59** did not undergo any SuFEx transformation to expected diversified peptide **118**. Analysis of the compound's ^{1}H NMR spectrum showed signals corresponding to the alkene protons had disappeared and a significant number of new signals had arisen which were not possible to account for. The disappearance of a signal at 6.74 ppm was suggestive that the amide proton had also significantly changed or

was no longer present. Moreover, the lack of new aromatic signals expected in in the product was suggestive that the attempted intermolecular SuFEx reaction had not occurred.

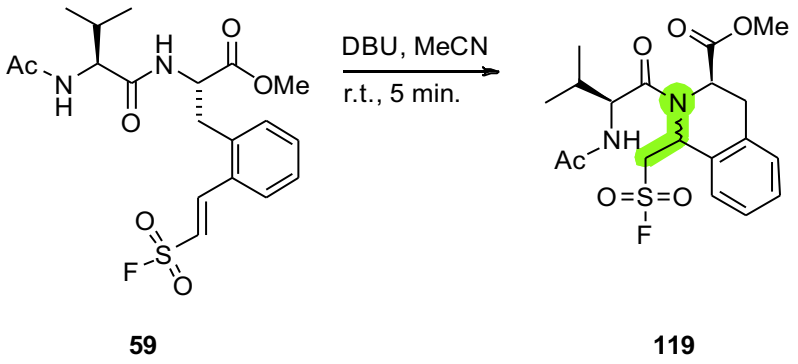
4.3.1 Intramolecular Michael addition

These observations provide strong evidence that an intramolecular reaction had taken place. The absence of signals corresponding to amide and alkene protons can be accounted for if an attack from the nitrogen atom at the Michael addition centre occurs. The significant number of new signals can then be justified by the formation of two products. One of the two products is formed depending on which face of the alkene is attacked. Relative approach of the amide from one side or the other generates a product with either an *R* or *S* stereocentre as shown in **Scheme 87**. Both products could not be separated by flash column chromatography, however they are significantly different to each other in the NMR spectrum.



Scheme 87 – Possible intramolecular reaction between the amide nitrogen and the installed alkene.

The silyl ether was believed to act as a spectator to the above reaction. It was therefore repeated without cresol, **Scheme 88**. So that analysis of the reaction mixture could be taken quickly at short intervals the reaction was carried out in MeCN-d_3. Prior to the addition of base, analysis of the ^{1}H NMR and ^{19}F NMR spectra of the peptide in MeCN-d_3 showed the modified peptide with the alkene and fluorine environments intact.

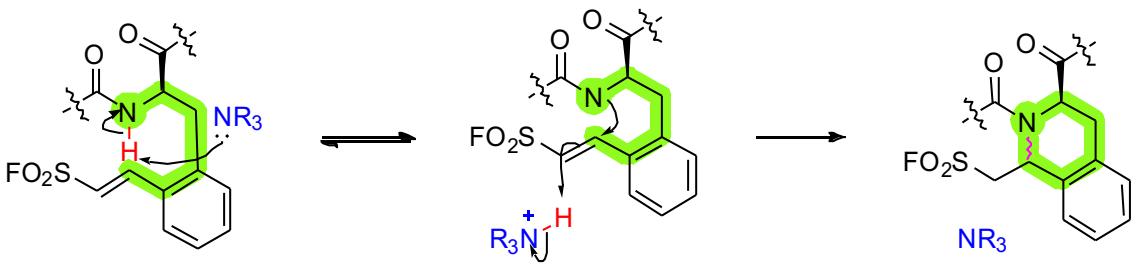


59 **119**

*Scheme 88 – Intramolecular reaction upon addition of DBU to modified peptide **59** after 5 min.*

The DBU was added to the reaction mixture and immediately analysed by NMR at room temperature. During the time it had taken to add the base and collect the ^{1}H NMR spectrum, the peaks corresponding to the alkene had disappeared. The ^{1}H NMR spectrum contained peaks that matched those in the spectrum of compound **119**. Evidence of the fluorine environment in the ^{19}F NMR spectrum remained unchanged. This result implies that the added base causes the breakdown of the starting material **59** to form conformationally restricted peptide **119**.

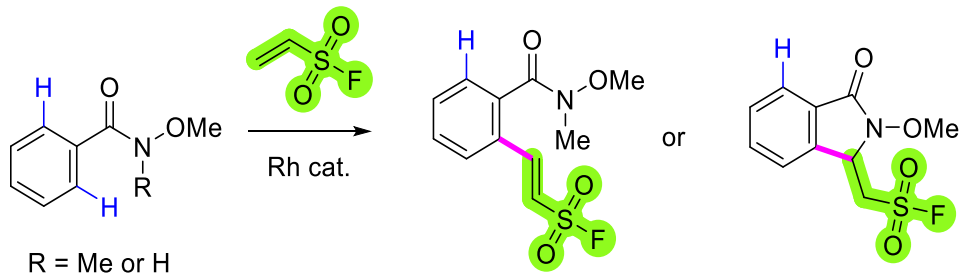
It was speculated that the DBU was catalysing a different reaction which occurred rapidly upon addition. When considering the structure of the peptide, **Scheme 89** it can be seen that the amide nitrogen sits in a six-membered ring with the extended aromatic system of the phenylalanine residue. The neighbouring member of the ring to the amide is the alkene which is susceptible to Michael addition.



Scheme 89 – Proposed Michael addition of native amide upon addition of DBU.

As in **Scheme 89**, deprotonation of the amide by DBU causes it to attack the alkene. The DBU then yields the proton to the other developing sp^3 carbon centre to reform the base catalyst. The attack of the nitrogen lone pair from one face of the alkene generates a chiral centre. Attack of the nitrogen lone pair from the other side generates the opposing chirality. DBU has been reported to function in SuFEx reactions as a nucleophilic catalyst.[131,137] However, here this would liberate the fluoride ion, which is present in the product implying it is unlikely to act in this manner here.

Additionally, an example of similar reactivity between an amide and the C=C double bond centre of an aryl VSF has been reported.[158] A rhodium catalysed olefination reaction involving *N*-methoxybenzamide substrates underwent a further intramolecular aza-Michael addition unless the amide was 'blocked' by a methyl group, **Scheme 90**. This five-membered ring is synthesised spontaneously as soon as the olefination takes place. Excluding one example where extenuating circumstances prevailed, this addition occurred every time there was a labile proton present on the amide. Whilst the compounds created in this project do not undergo spontaneous aza-Michael addition, exposure to a strong tertiary amide base such as DBU facilitates the same reactivity of these compounds. The electron donating *O*-methyl group and the preorganisation of the amide and alkene are likely to be the reasons that the substrates in **Scheme 90** undergo this addition.



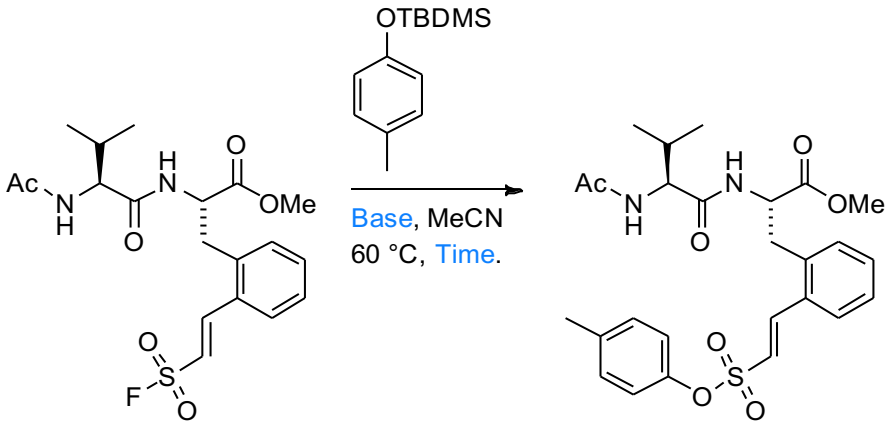
Scheme 90 – Aza-Michael addition of an amide to vinyl sulfonyl fluoride upon olefination.[158]

The *aza*-Michael addition is a particularly interesting result as there is potential to lock the conformation of the peptide at a phenylalanine residue post-synthetically. The joining of the amide backbone to the side chain removes degrees of freedom normally afforded to short

peptide chains *in vitro* and likely has interesting applications if developed. However, the aim was to carry out SuFEx transformations on peptides modified with ESF. Due to time constraints this result was not further investigated.

In an attempt to avoid facilitating the *aza*-Michael addition, bases of differing strengths were exposed to the model peptide and analysed by NMR. It was hoped that a base could be used that would actively participate in the catalysis of the SuFEx reaction but would not facilitate the addition reaction. From **Section 1.4** the base is expected to act as a nucleophilic catalyst at the sulfur centre and liberate the fluoride ion. The bases investigated are displayed in **Table 8** below.

Table 8 – SuFEx reaction carried out on the model dipeptide using different bases.



59 **118**

Base	Time	Alkene signals present	% Yield of SuFEx product
DBU	5 min	No	0
Cs_2CO_3	12 hr	No	0
DMAP	12 hr	Some degradation	0
Et_3N	12 hr	Yes	0

From the table it can be seen that bases such as DBU and Cs_2CO_3 proved too strong to facilitate the SuFEx reaction. Both DMAP and Et_3N were tolerated and in each case a significant amount of the alkene starting material was isolated. However, upon exposure of

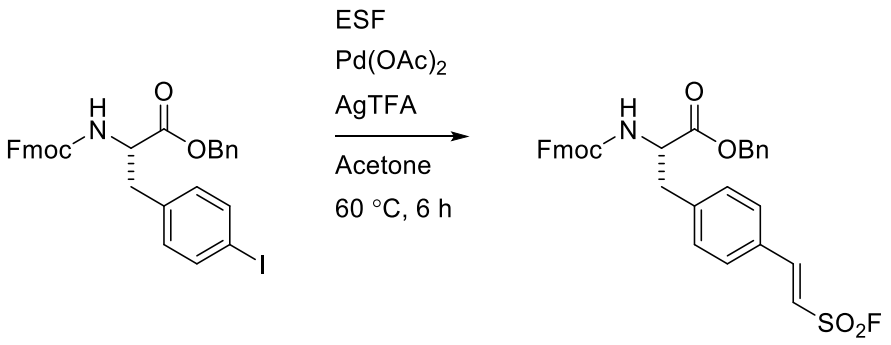
the silyl ether to the modified peptide, no reaction was observed. In both cases the recovery of the starting materials was carried out.

The reaction was then attempted using a non-basic catalyst. The SuFEx reaction of an aryl VSF catalysed by TBAF had been reported.[173] Taking inspiration from this example, the modified peptide and silyl ether were exposed to TBAF. However, this did not facilitate the desired reaction and again the starting material peptide was recovered.

4.3.2 Synthesis of *meta* and *para* isomers

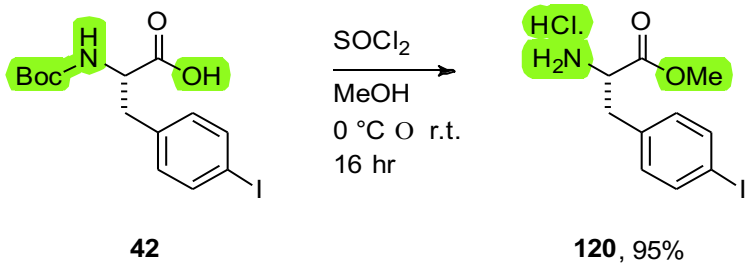
The observed reactivity in **Section 4.3.1** is a direct consequence installing the aryl VSF at the *ortho* position on the phenyl ring. The developed olefination conditions utilise the backbone amide directing groups native to the peptide. This facilitates *ortho* substitution in every case. Unavoidably, the six-membered ring motif is inadvertently generated which provides the platform for the attack of the deprotonated amide. A protocol that enabled modification at the *meta* or *para* position would move the alkene away from the amide and likely be sufficient to stop any reaction.

Methods that produce *meta* or *para* olefinated phenylalanine residues are not well documented. The lack of reactive handle poses a significant challenge when attempting to selectively olefinate. However, the installation of the aryl VSF functionality has been achieved using an aryl iodide in a Heck reaction, in this case protected *para*-iodo-phenylalanine, **Scheme 91**.[157]



Scheme 91 – The installation of vinyl sulfonyl fluorides on a protected amino acid via a Heck reaction.

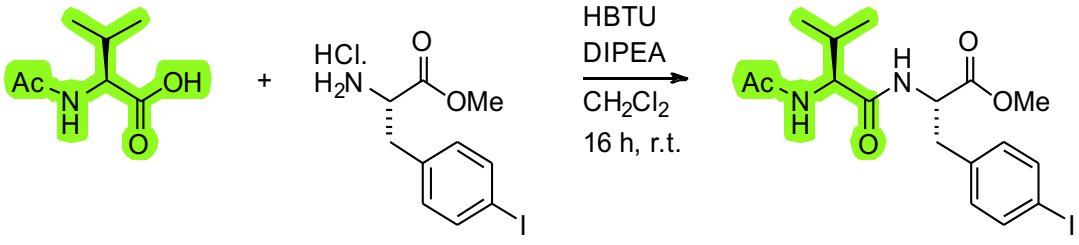
The olefination outlined above is performed on a protected amino acid. It was postulated that if an iodo-phenylalanine-containing peptide could be synthesised, it was likely to be able to be modified in a similar fashion, generating a *para*-vinylsulfonyl fluoride on the phenyl ring. To this end, 4-iodo-phenylalanine methyl ester **120** was synthesised from Boc-Phe(4-iodo)-OH **42** as in **Scheme 92**.



42 **120**, 95%

*Scheme 92 – Synthesis of aryl-iodide containing phenylalanine amino ester **120**.*

The routine esterification using thionyl chloride generated HCl *in situ* which in turn cleaved the Boc protecting group. The amino ester was isolated as its hydrochloride salt. The reaction was carried out in excellent yield. In order to generate a dipeptide from **120**, the hydrochloride salt was removed and the free amine was reacted with *N*-acetyl-L-valine **121** in order to generate the peptide **122** as shown in **Scheme 93**.

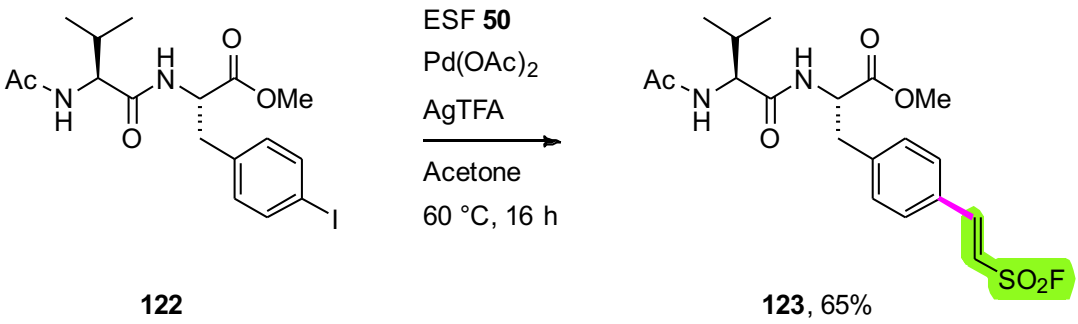


121 **120** **122**, 61%

*Scheme 93 – Solution phase synthesis of 4-iodo-phenylalanine containing peptide **122**.*

The dipeptide **122** was synthesised in good yield and fully characterised by NMR spectroscopy. Next, peptide **122** was subjected to the reaction conditions highlighted in **Scheme 94** in order to generate a peptide containing a 4-VSF **123**. Peptide **122** was

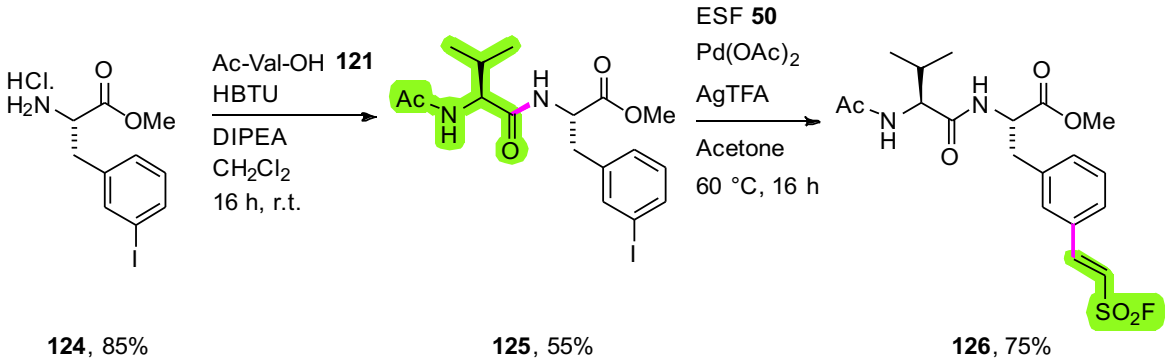
dissolved in acetone and exposed to ESF **50** in the presence of palladium acetate and silver trifluoroacetate before heating to 60 ºC and allowing to stir overnight.



122 **123**, 65%

*Scheme 94 – Synthesis of 4-vinyl sulfonyl fluoride containing peptide **123**.*

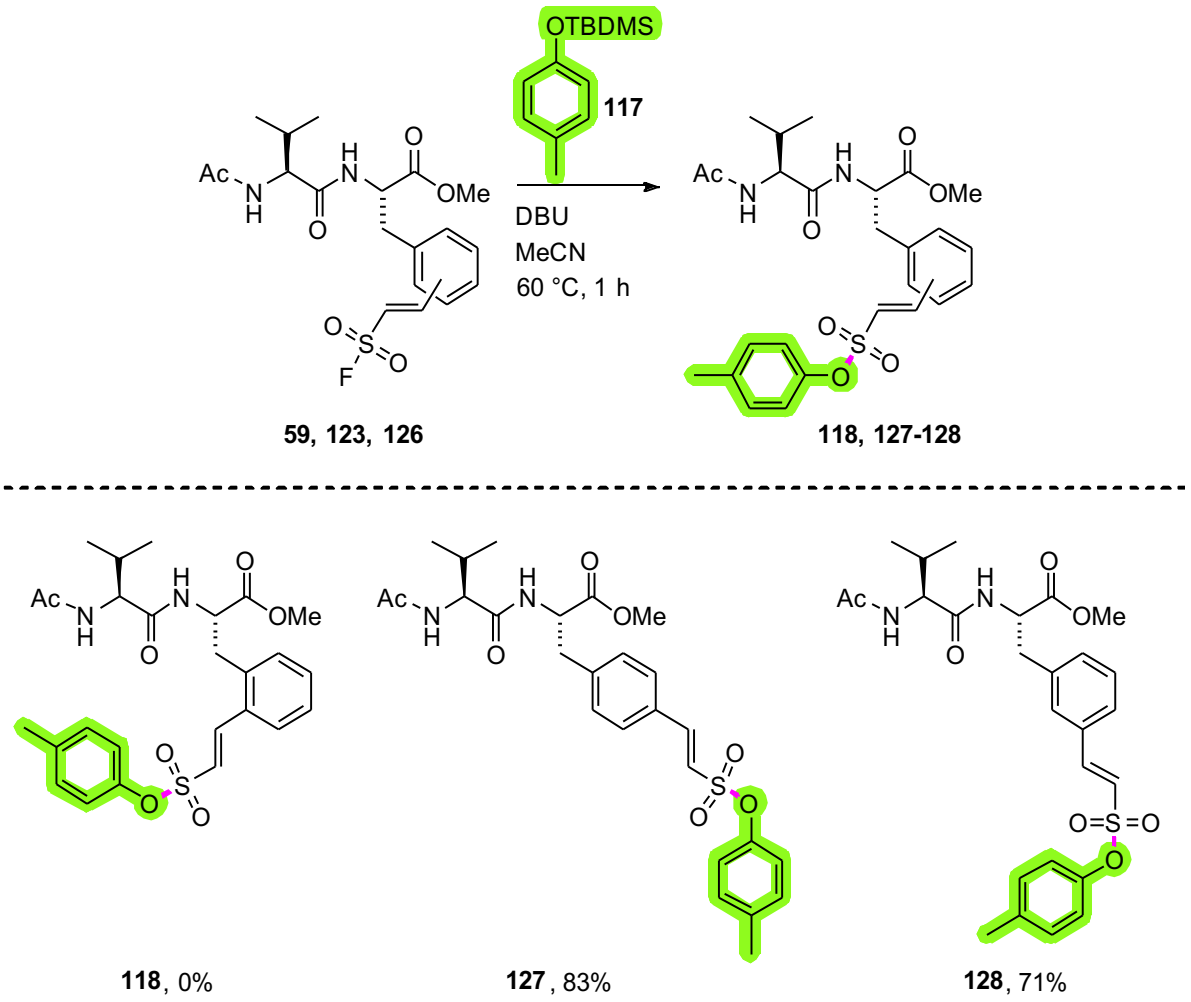
Analysis of the ^{1}H NMR spectrum provided conclusive proof that the 4-VSF had been introduced. The appearance of the usual doublet of doublets at 6.84 ppm (J = 15.6, 2.5 Hz) and a doublet at 7.76 ppm (J = 15.6 Hz) provided evidence that a *trans* alkene had been installed at the *para* position on the phenyl ring. This result gave a clear indication that a Heck reaction was a viable route to olefinated peptides from peptides containing a Phe(4-*iodo*) residue. It was hypothesised that if a vinyl sulfonyl fluoride could be installed at the *meta* position on a phenylalanine residue if the corresponding 3-*iodo*-phenylalanine peptide **125** was sourced. The amino ester hydrochloride salt **124** was synthesised in excellent yield in the same method as outlined in **Scheme 92**. A *meta* substituted vinyl sulfonyl fluoride containing peptide **126** was then generated using the synthetic route described in **Scheme 95** below.



124, 85% **125**, 55% **126**, 75%

*Scheme 95 – Synthetic route to 3-VSF-phenylalanine containing peptide **126**.*

An overall yield of 35% was achieved. As a result of these manipulations, a series of *ortho*, *meta*, and *para* isomers of the modified model dipeptide were obtained. These isomers were then investigated to determine if SuFEx chemistry using silyl ethers could be performed, **Scheme 96**.



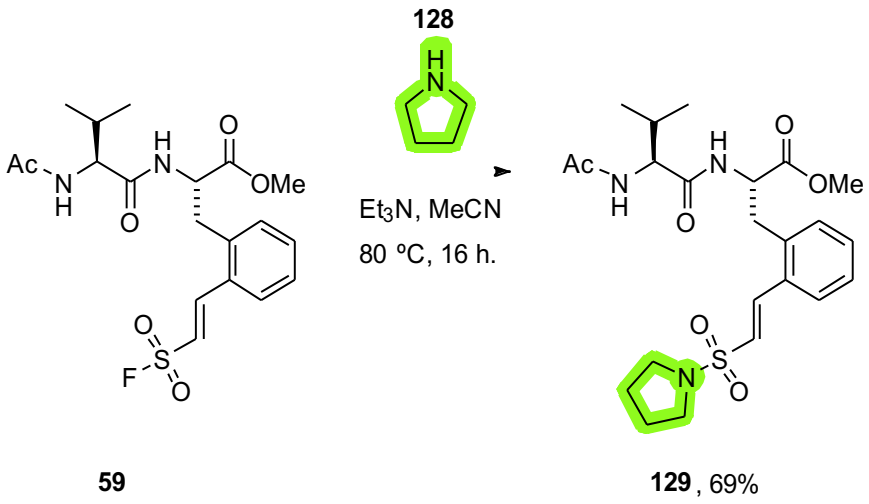
Scheme 96 – Silyl ether SuFEx carried out on 2-, 3- and 4-vinylsulfonyl fluoride containing peptides.

The products were fully characterised by NMR spectroscopy. Evidence of the successful synthesis of **127** and **128** was found in the ^{19}F NMR spectra where both cases lacked the fluorine environment present in the starting material. The alkene peaks present in the ^{1}H NMR spectra had also significantly changed. In the example of modified peptide **127**, the doublet of doublets present in the starting material at 6.83 ppm was no longer present and had been replaced by a doublet (J = 15.6 Hz) at the same chemical shift.

So far it has not been possible to modify an *ortho* aryl VSF containing peptide **59** using a silyl ether nucleophilic species during this study. However, when peptides containing *meta* and *para* aryl VSF **123** and **126** are subjected to the same conditions, sulfur(vi)-fluoride exchange is facilitated quickly and in good yield.

4.4 SuFEx reactions with 2º amines

One of the most commonly documented SuFEx transformations involves the reaction between a sulfonyl fluoride and a secondary amine. As outlined in **Section 4.1**, reactions with aryl VSF and nucleophiles such as amines can occur at two different sites. The target of this section of the project was to facilitate both types of reaction on the modified dipeptide **59**. To this end, the model peptide was subject to the reaction conditions outlined in **Scheme 97**.

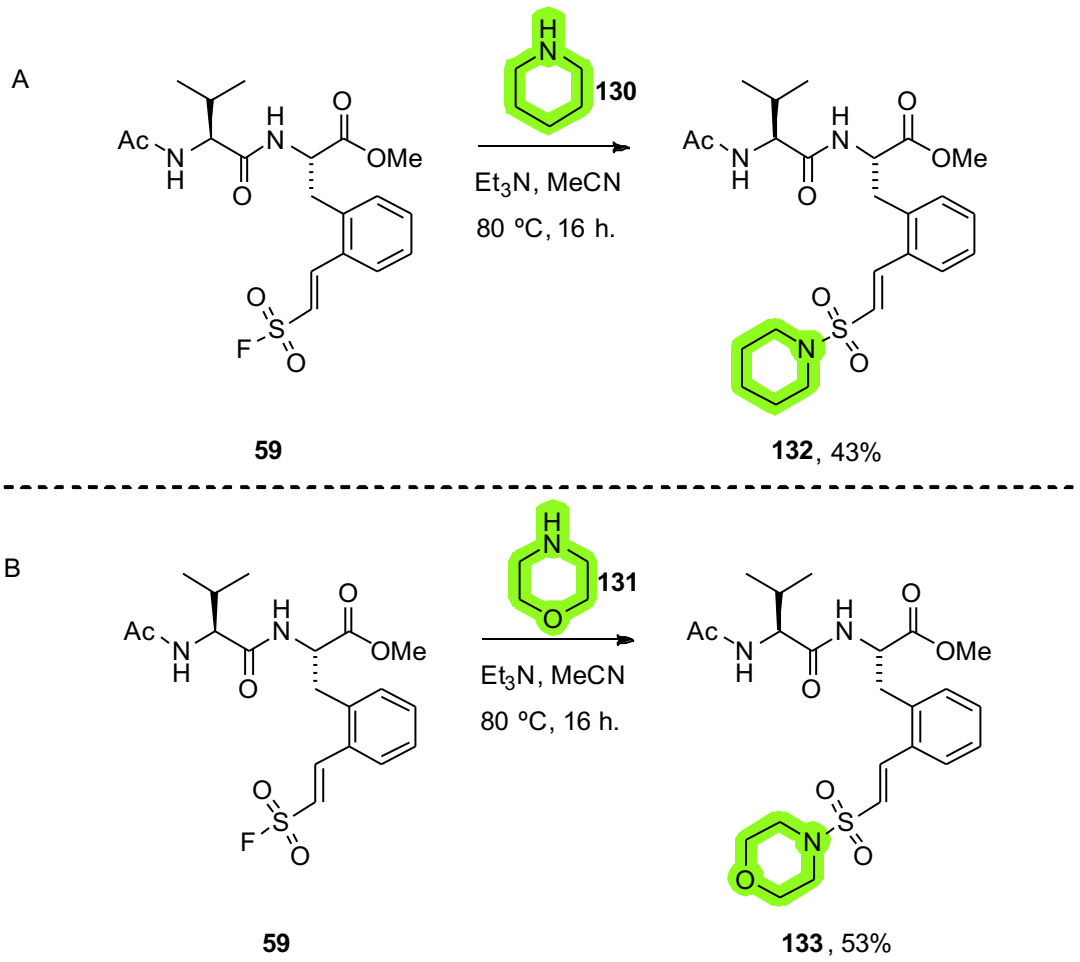


*Scheme 97 – Further complexity installed by SuFEx transformation of modified peptide **59** and pyrrolidine **128**.*

The successful synthesis of diversified peptide **129** was confirmed by the ^{19}F NMR spectrum which showed the fluorine environment in the starting materials was no longer present. In the ^{1}H NMR spectrum, the familiar doublet of doublets had been converted to a doublet, further suggesting that the fluorine atom had been displaced or substituted. There was no

evidence of a Michael addition product in the crude NMR spectra and reactivity was strictly limited to the SuFEx transformation.

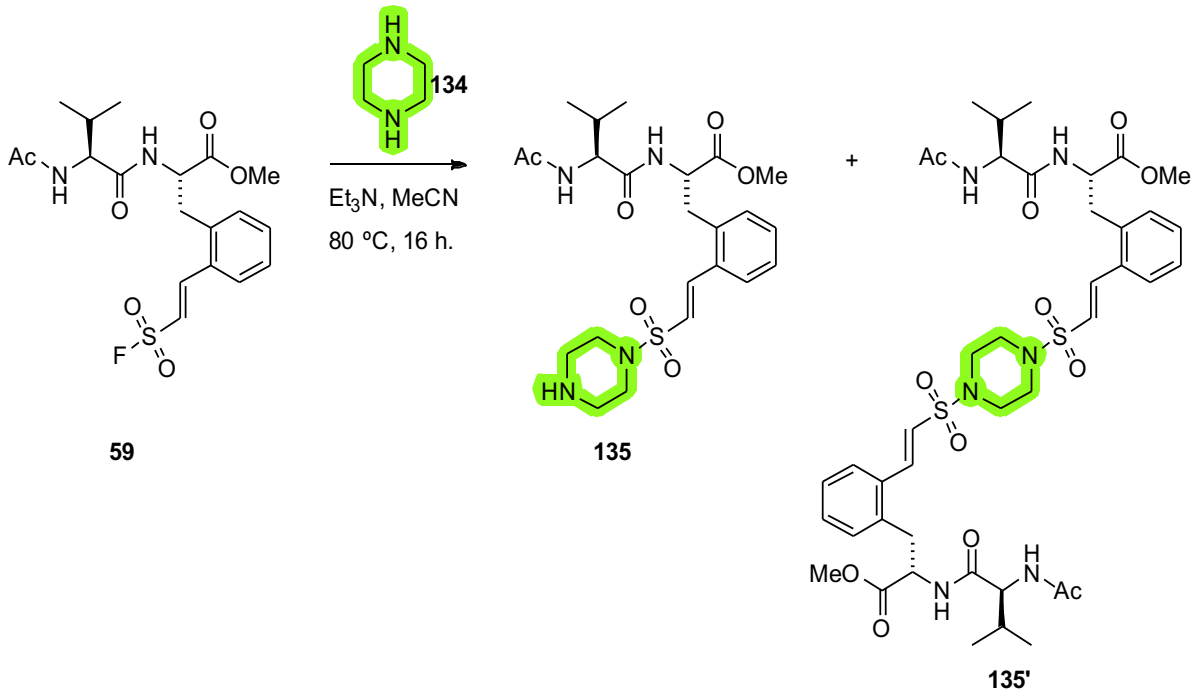
Armed with the successful reaction from **Scheme 97**, further secondary amines were then exposed to the model dipeptide under the same reaction conditions to broaden applicability. Piperidine **130** and morpholine **131** were chosen as in **Scheme 98**.



Scheme 98 – SuFEx transformations using six-membered cyclic amines.

In each case the SuFEx transformation was carried out successfully. Although yields were slightly lower than expected, this was again attributed to the steric hinderance encountered when modifying on the *ortho* position on the phenylalanine ring. The successful modification of the model dipeptide **59** in both examples demonstrated that adding complexity to the coupling partners does not inhibit the SuFEx reaction significantly.

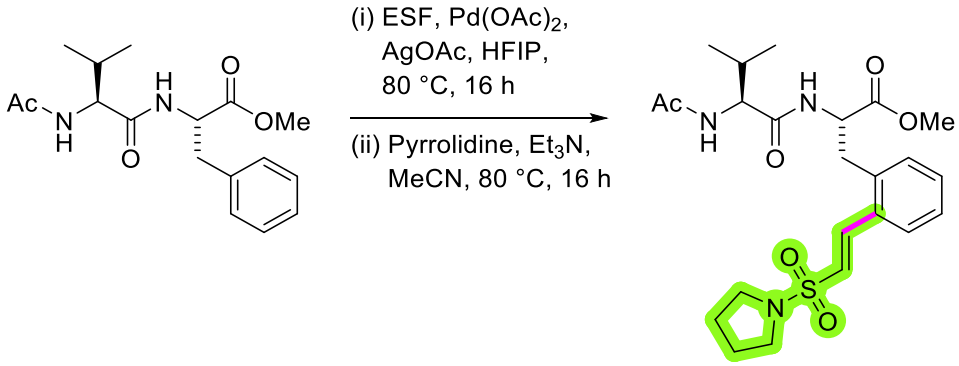
In the six-membered cyclic amine family there is one more candidate to consider. Piperazine **134** is a bifunctional cyclic amine, containing two secondary amine nucleophiles within its structure. The model dipeptide **59** and piperazine **134** were exposed to the same conditions, **Scheme 99**.



*Scheme 99 – Modification with bifunctional cyclic amine piperazine **134**.*

HRMS of the crude reaction mixture identified two products **135** and **135'** within the mixture. Particularly diversified dipeptide **135** represents an interesting example as there is scope for further modification. It contains another secondary amine which can utilised to build higher complexity into the compound and could potentially participate in a further SuFEx reaction to cross-link two peptides. From **Scheme 99** it can be seen that **135** is nucleophilic enough after the first reaction to undergo further SuFEx transformations. It is likely that using half an equivalent of piperazine would ensure that **135'** is the major product of the reaction whilst using an excess of piperazine would ensure that the major product is the modified dipeptide **135**. Due to the time constraints of the project, this avenue was not pursued further.

To investigate the power of the SuFEx transformation, the olefination of dipeptide **53** was carried out in order to generate modified peptide **59** which contained the SuFEx-able hub aryl VSF. Following this modification, the crude product was exposed to pyrrolidine **128** and Et_3N without purification in order to form diversified peptide **129**. The reactions proceeded smoothly to generate the modified compound **129** in 54% overall isolated yield without the need for purification upon olefination, **Scheme 100**. This compared favourably with the overall yield of the two reactions carried out sequentially, in which combined yields of 74% and 69% gave an overall yield of 51%.



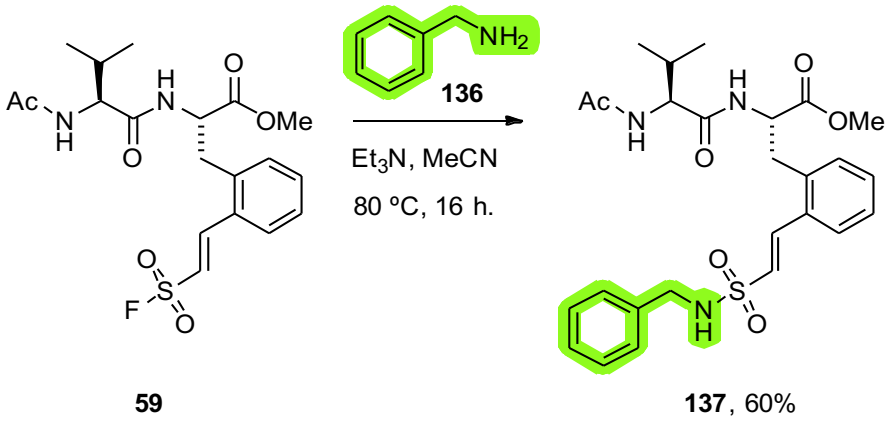
53 **129**, 54%

Scheme 100 – An investigation into the necessity of purification of the olefinated product

The results reported in this section represent an important step towards the project aims and illustrate the significance of the developed technique. A dipeptide with no obvious reactive handle had been modified to generate a reactive SuFEx-able hub at the expense of a ubiquitous C-H bond. The now reactive phenylalanine residue had further complexity installed by the conversion of the sulfonyl fluoride to a sulfonamide. Whilst the secondary amines chosen were relatively simple, it was hoped that the complexity could be increased easily using the technique developed in this section to produce more biologically relevant compounds in good yield.

4.5 SuFEx reactions with 1º amines

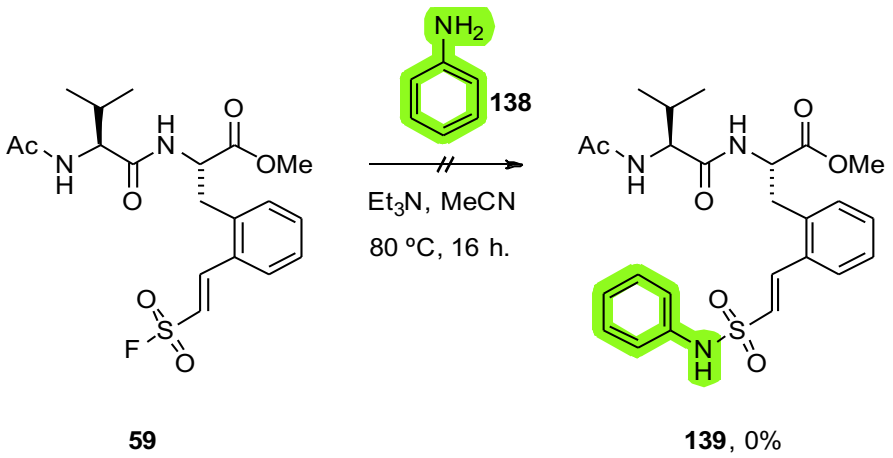
The investigation then proceeded to primary amine nucleophiles. It was expected that these would behave in the same way as secondary amines. However, primary amines are less nucleophilic than secondary amines and it was anticipated that it could be difficult to facilitate a reaction. Due to its structural simplicity, benzylamine **136** was chosen as the first primary amine SuFEx partner, **Scheme 101**.



59 **137**, 60%

*Scheme 101 – SuFEx transformation with model dipeptide **59** and benzylamine **136**.*

The reaction proceeded in the same way the secondary amines had in **Section 4.4**. Diversified peptide **137** was produced in good yield, as the only product of the reaction. No Michael addition product was observed. This successful reaction provided evidence that primary amines can be utilised to further modify vinyl sulfonyl fluoride containing peptides which significantly broadens applicability of the method. Interestingly, a four-membered cyclic sulfonamide was not synthesised. This was once again attributed to steric hinderance at the reaction site due to the native peptide structure.



59 **139**, 0%

Scheme 102 – Investigation into SuFEx chemistry using poorly nucleophilic amines.

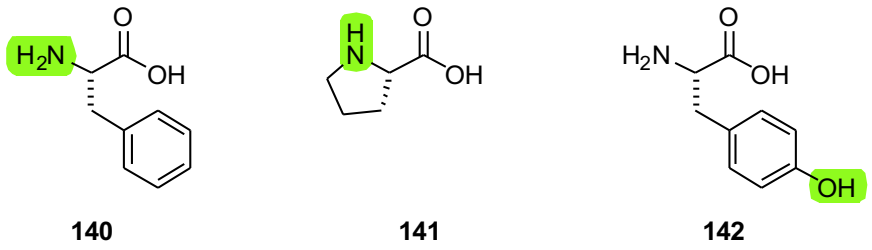
To determine the limit of the reaction, a less nucleophilic amine **138** was exposed to the model peptide **59**, **Scheme 102**. Aniline **138** is structurally similar to benzylamine **136** but lacks the connecting methylene group between the aromatic ring and the amine. The amine lone pair is delocalised significantly over the aromatic system meaning that it is a much less nucleophilic species than benzylamine **136**. In this example the reaction did not proceed. Analysis of the crude ^{1}H NMR spectrum after 16 hours showed that no reaction to generate diversified peptide **139** had taken place and both peptide **59** and aniline **138** were fully recovered from the reaction mixture.

Considering the success of the SuFEx reactions of nucleophiles discussed in this section, attention was turned to significantly increasing the complexity of the nucleophilic species. It was hypothesised that these transformations could eventually be used to synthesise cyclic peptides. In order to attain this goal several questions about the feasibility of the reaction needed addressing. The first of which was if the reaction could be carried out on amino acid based nucleophilic species.

4.6 SuFEx reactions using amino acid nucleophiles

Following the successful further modifications reported *vide supra* attention was turned to the SuFEx transformations with nucleophiles that were contained within amino acids or

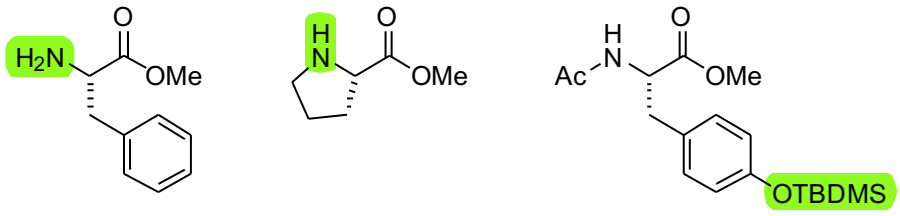
peptides. **Figure 26** below shows the naturally occurring amino acids **140-142** that have a suitable nucleophile for SuFEx transformations.



140 **141** **142**

Figure 26 – Naturally occurring amino acids with nucleophiles compatible with SuFEx transformations.

A number of amino acids contain nucleophilic species that could be compatible with SuFEx transformations. The primary amine highlighted on the phenylalanine residue **140** is present on nineteen of the twenty naturally occurring amino acids. Phenylalanine was chosen in this study due to its commercial availability to the research group. The aryl hydroxyl highlighted on tyrosine **142** could be protected as silyl ether, making it a compatible nucleophile.



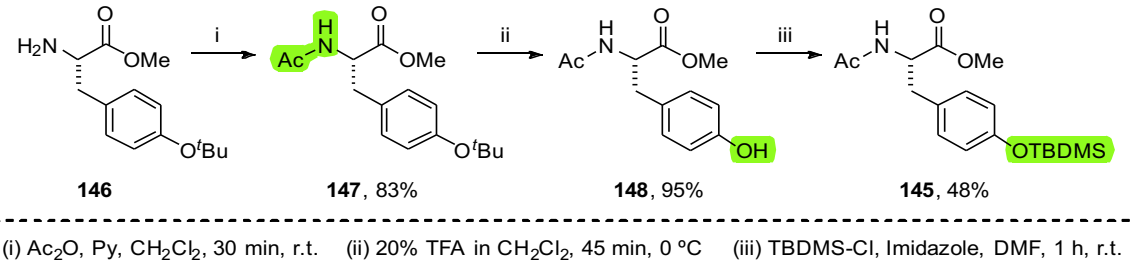
143 **144** **145**

*Figure 27 – Protected amino acids **143-145** for SuFEx transformations.*

The decision was taken to fully protect the amino acids leaving only the relevant nucleophile unprotected in order to simplify the reaction and give the greatest chance of success. To this end the three protected amino acid targets **143-145** were highlighted as in **Figure 27** above.

4.6.1 Synthesis of target protected amino acids

The amino acids phenylalanine and proline methyl ester hydrochloride were commercially sourced. Prior to use in the SuFEx transformations their hydrochloride salts would be removed. To synthesise Ac-Tyr(OTBDMS)-OMe **145** a three-step synthesis was devised, **Scheme 103**. Beginning with the partially protected amino ester H-Tyr(O^{t}Bu)-OMe **146**, an acetylation step was carried out. Upon protection of the amine as an acetyl group, the compound **147** was exposed to TFA in order to cleave the ^{t}Bu group. Finally, **148** was exposed to TBDMS-Cl in order to install the target silyl ether **145**.



*Scheme 103 – Synthetic pathway to Ac-Tyr(OTBDMS)-OMe **145**.*

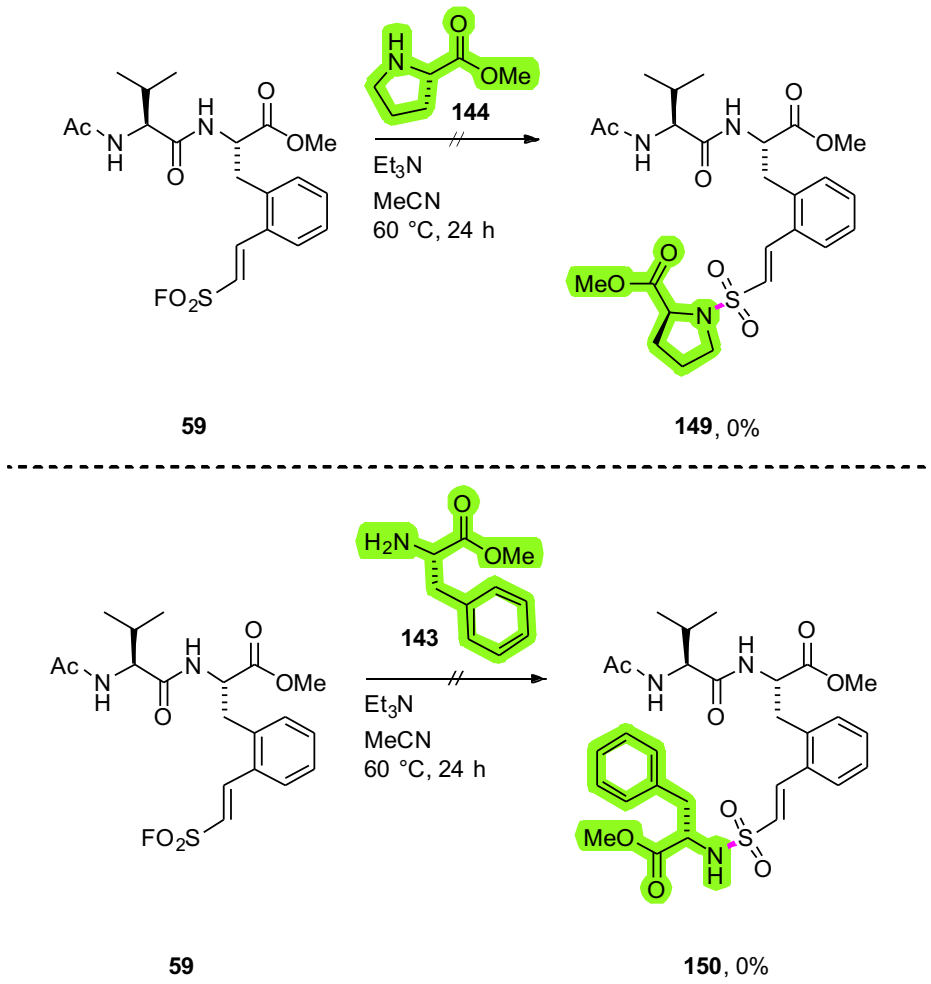
N-acetyl protected tyrosine **147** was characterised fully by ^{1}H NMR spectroscopy. The appearance of an amide N-H proton at 6.32 ppm provided proof that the acetylation had been successful. Evidence was also found in the IR spectrum, in which a strong peak at 1655 cm^{-1} demonstrated that an amide bond had been formed. The reaction proceeded in excellent yield and after washing with saturated $NaHCO_3$ solution to remove excess acetic acid the compound was ready for use in the next step without the need for further purification.

The second synthetic manipulation removed the acid-labile *t*-butyl ether on the aromatic system. In order to achieve this the compound **147** was treated with dilute TFA in CH_2Cl_2. Once again, the reaction proceeded well in near-stoichiometric yield and the compound **148** did not require further purification before the next synthetic step.

Finally compound **148** was dissolved in DMF and exposed to imidazole before treating with TBDMS-Cl. Following the work up and purification by flash column chromatography, the fully protected peptide **145** was isolated as a white solid. The overall yield of the three synthetic steps was determined to be 38%.

4.6.2 SuFEx transformations with nucleophilic amino acids.

After the synthesis of the nucleophiles **143-145**, exposure to the model vinyl sulfonyl fluoride dipeptide was undertaken. The model dipeptide **59** and amino acids **143-145** were exposed to the reaction conditions developed in **Section 4.4** and **Section 4.5** as in **Scheme 104**. In cases where the amino acid had been isolated as the hydrochloride salt, the amino acid was dissolved in deionised water and treated with K_2CO_3 in order to generate the free amine. The aqueous solution was then extracted using Et_2O or CH_2Cl_2 in order to isolate the amino acid. Triethylamine was chosen as the base in this reaction as the use of DBU would likely yield the Michael addition product discussed in **Section 4.3.1**.

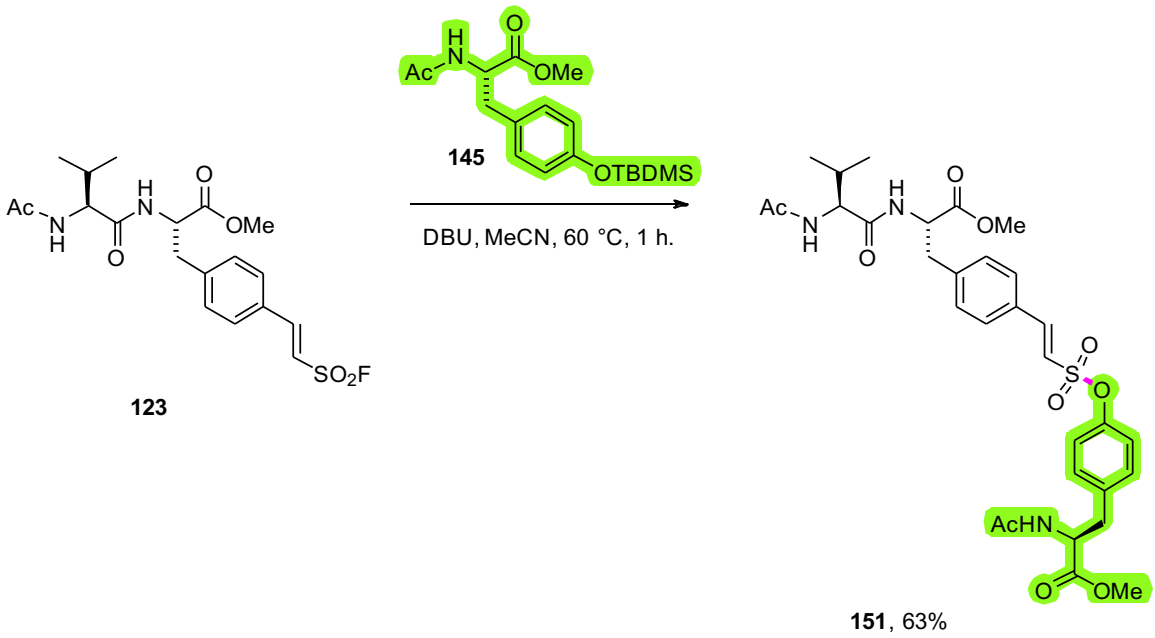


*Scheme 104 – Attempted SuFEx transformations using model dipeptide **59** and nucleophilic amino acids **143** and **144.***

Disappointingly attempted SuFEx transformations with amino acids containing amine nucleophiles **143** and **144** did not yield the intended peptide-amino acid cross-linked compounds **149** and **150**. Analysis of the crude ^{1}H NMR spectra showed both starting materials present in each case. The continued presence of a singlet peak at ca. 60 ppm in the ^{19}F NMR spectra also supported this conclusion. No evidence to suggest that the transformation had taken place was found.

The investigation had already determined that for silyl ether SuFEx reactions to take place, the *ortho* vinyl sulfonyl fluoride was not a suitable candidate owing to the need for the presence of the stronger DBU base. As a result, the modification of the *ortho*-vinylsulfonyl fluoride **59** was not attempted. Instead, to prove the reaction would work on peptide species

to form peptide-amino acid cross-linked compounds, the reaction was attempted on the *para*-vinylsulfonyl fluoride containing peptide **123**, **Scheme 105**.

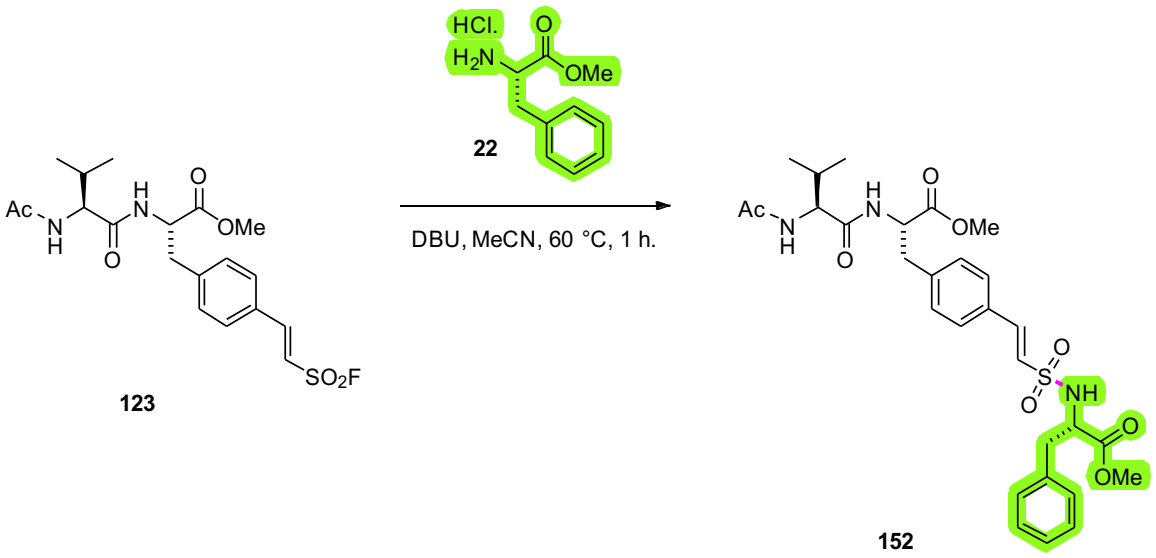


*Scheme 105 – Successful SuFEx transformation between modified peptide **123** and silyl ether nucleophile **145**.*

The cross-linked product **151** was fully characterised by NMR spectroscopy. The characteristic change of the alkene peaks from a doublet and a doublet of doublets to two doublets was observed which was indicative of the activation and removal of the sulfur-fluoride bond. The loss of singlets at 0.18 and 0.96 ppm also provided evidence of the breakdown of the silyl ether. A ^{19}F NMR spectrum of the purified compound was acquired containing only baseline noise which was consistent with the lack of fluorine environment in the product. This result was significant as it represents a successful example of peptide-amino acid cross-linking using the sulfur(vi)-fluoride exchange reaction.

The reactions with amino acids containing amine-based nucleophiles did not yield and successful results when exposed to Ac-Val-Phe(2-VSF)-OMe **59** as in **Scheme 104**, it was hypothesised that altering the base from Et_3N to DBU would produce a more satisfactory outcome. However, DBU had already been discovered to be incompatible with the peptide **59**. To test this theory, the modified peptide Ac-Val-Phe(4-VSF)-OMe **123** was used instead

and exposed to H-Phe-OMe.HCl **22** with base DBU as in **Scheme 106**. The amino ester was used as its hydrochloride salt and enough base was added to account for the removal of this *in situ*.



*Scheme 106 – SuFEx transformation on para-VSF containing peptide **123** using nucleophilic amino acid **22** and DBU as base.*

A ^{19}F NMR of the crude material showed that the S-F bond had been broken. A parent ion peak was found in the HRMS (ESI) $[M+H]^+$ of 588.2376 further evidencing that the reaction had taken place and diversified peptide **152** had been synthesised. Due to the time constraints of the project, purification of the product was not undertaken.

4.7 SuFEx reactions to generate peptide macrocycles

The reactions carried out in **Section 4.6.2** demonstrated that although SuFEx transformations are more complex when carried out using nucleophilic amino acids, they are still possible when the conditions are favourable. The power of an intramolecular reaction versus an intermolecular reaction had already been demonstrated in **Section 4.3** upon the addition of DBU to an *ortho* vinyl sulfonyl fluoride containing peptide **59** generating conformationally restricted peptide **119** (**Scheme 87**). If the sulfonyl fluoride and the amine were part of the same molecule and could move close together in space when exposed to

the conditions, a reaction might still be facilitated. An experiment was devised where these functional groups were on the same peptide and an intramolecular reaction might be established.

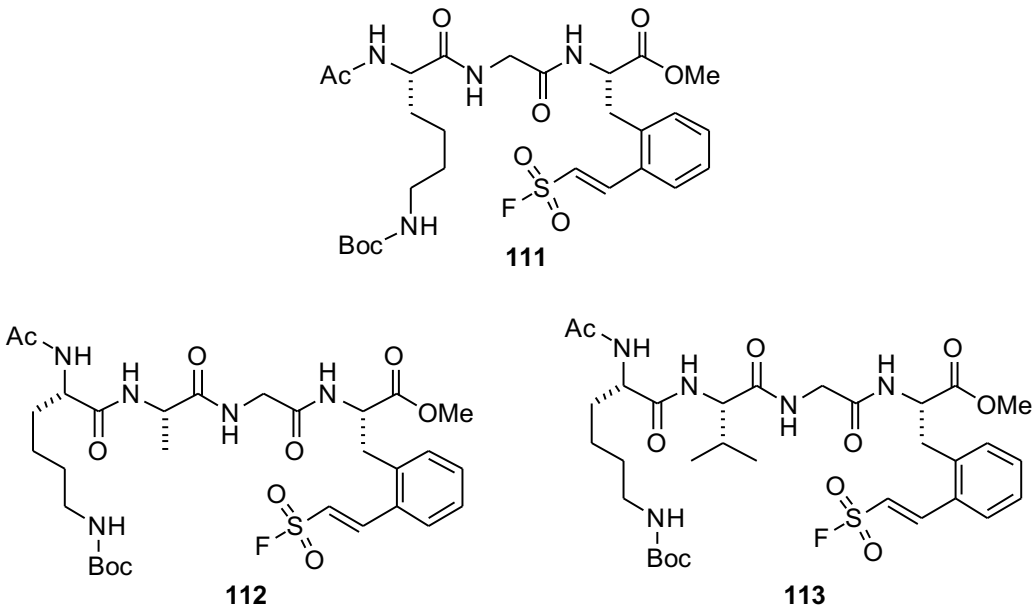
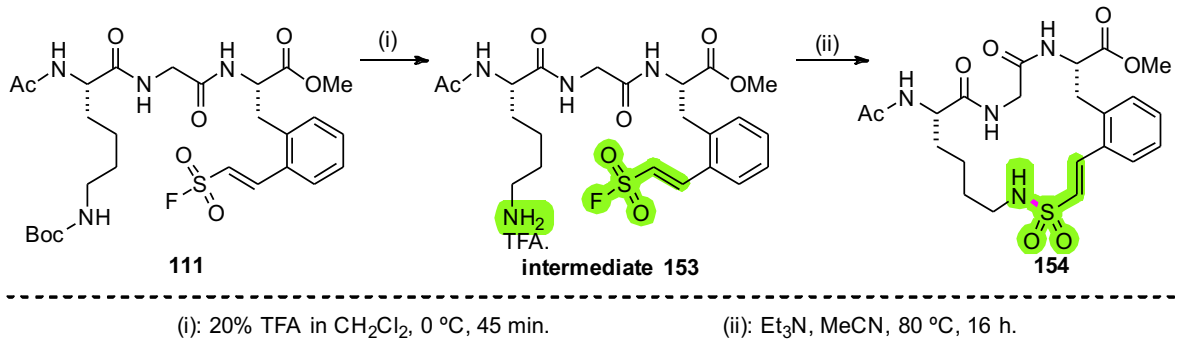


*Figure 28 – Synthesised peptides **111-113** containing protected amines and ortho vinyl sulfonyl fluorides.*

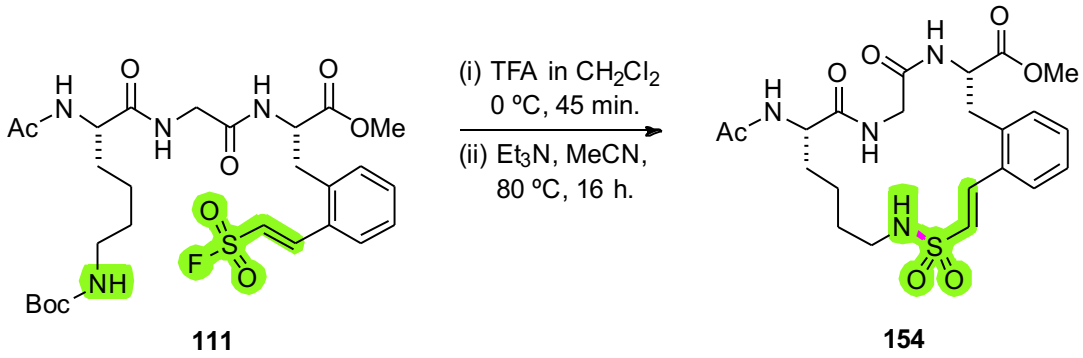
Previously in **Chapter 3**, a modified tripeptide **111** and two modified tetrapeptides **112** and **113** were synthesised, **Figure 28**. These generated peptides contain vinyl sulfonyl fluorides as well as lysine residues with Boc-protected amine side chains. In order to generate cyclic peptides from these compounds, a reaction sequence was devised using tripeptide **111** as an example, **Scheme 107**.



*Scheme 107 – Devised synthetic route to cyclic peptide **154.***

A reaction likely to compete with the cyclisation involved the intermolecular formation of a cross-linked peptide containing either six or eight amino acid residues linked by a sulfonamide generated between a phenylalanine residue and a lysine residue. The number of residues present is dependent on the chain length of the peptide. However, the intermolecular reactions carried out in the previous section proved particularly difficult, which could aid the generation of one cyclic product. The reaction was also to be carried out at high dilution as a precaution to minimise any risk of intermolecular reaction.

The modified tripeptide **111** was first exposed to dilute TFA in CH_2Cl_2 in order to cleave the Boc-protecting group, generating cyclic peptide **154** (**Scheme 108**). Upon cleavage of the Boc protecting group, ^{1}H NMR and ^{19}F NMR analyses were carried out in order to ensure the vinyl sulfonyl fluoride had survived the reaction conditions. The recognisable alkene doublet of doublets in the 1H NMR spectrum and the peak at ca. 60 ppm in the ^{19}F NMR spectrum were indicative that the vinyl sulfonyl fluoride was still intact.

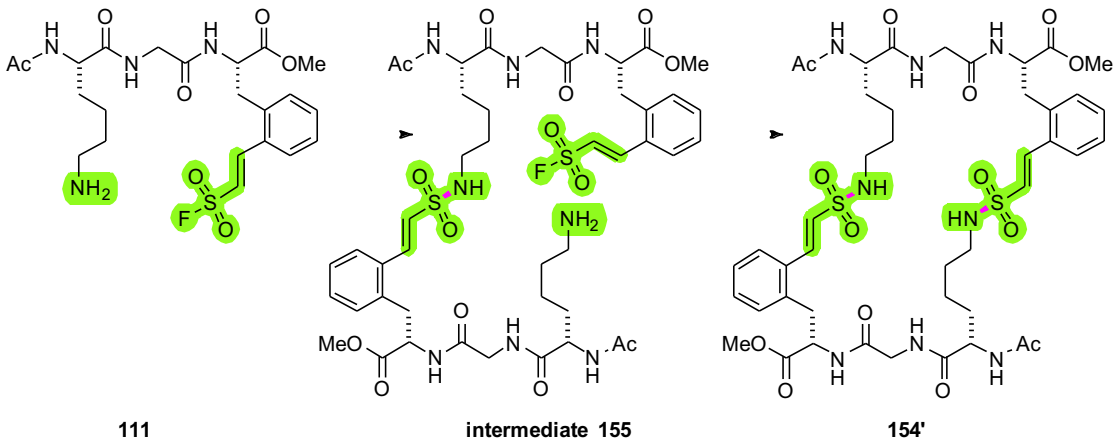


111 **154**

*Scheme 108 – Cyclisation of tripeptide **111** to form cyclic peptide **154**.*

After the Boc cleavage reaction had been completed, the crude compound (0.008 mmol) was then dissolved in excess MeCN (4 mL) to yield a peptidic solution of 0.002 M and treated with Et_3N in order to attempt to cyclise the peptide. Analysis of the crude ^{19}F NMR showed that a reaction had taken place as the usual peak present at ca. 60 ppm disappeared suggesting a SuFEx transformation had taken place. The crude ^{1}H NMR spectrum became incredibly complex and difficult to analyse which could be considered consistent with the generation of complex macrocyclic structures. Further confirmation that

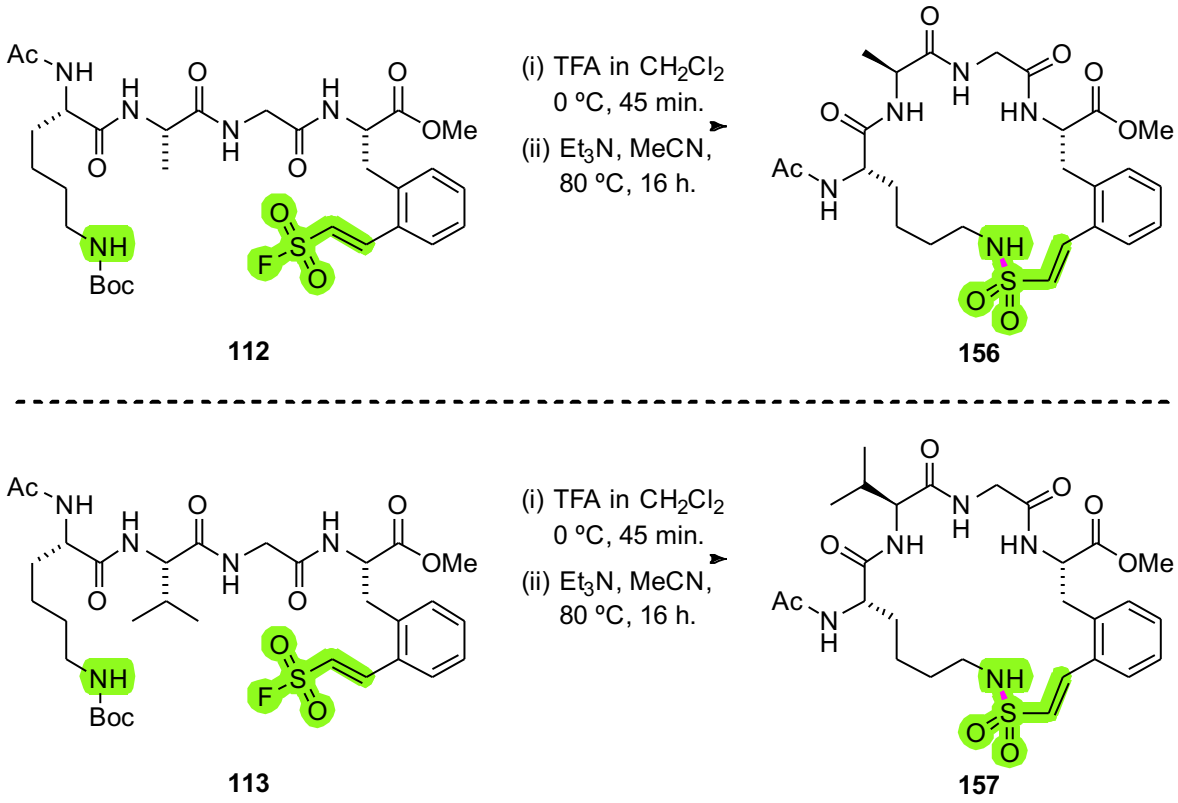
the peptide had undergone cyclisation was found when a parent ion peak in the HRMS (ESI) $[M+H]^+$ of 495.1910 was identified. This compound was synthesised in the very last week of the project and due to strict time constraints, was not purified and no further data gathered. However, this result is extremely significant as it demonstrates that in two synthetic steps, an otherwise unreactive phenylalanine residue can be modified and subsequently used to cyclize a peptide through the installed SuFEx-able hub and a native lysine residue.



*Scheme 109 – An intermolecular followed by an intramolecular SuFEx reaction yielded dimer **154'**.*

Further in-depth analysis of the HRMS data showed a parent ion peak $[M+H]^+$ of 989.3739. This correlates to the exact mass for a dimeric species which has undergone conversion from the linear peptide **111** to a peptide-peptide cross-linked structure **intermediate 155** via an intermolecular SuFEx reaction. This was then followed by a further intramolecular SuFEx reaction to yield dimeric species **154'**, **Scheme 109**.

Gratified by the success of the tripeptide cyclisation, the remaining tetrapeptides **112** and **113** were subjected to the same reaction conditions, **Scheme 110** in the hope of generate cyclic peptides **156** and **157**. Once again, the disappearance of the S-F bond was shown in both ^{19}F NMR spectra and the ^{1}H NMR spectra became difficult to interpret.



*Scheme 110 – The cyclisation of tetrapeptides **112** and **113** using an intramolecular SuFEx transformation.*

These finding are consistent with the successful cyclisation of the tetrapeptide **156** and **157**. The parent ion peaks $[M+H]^+$ in the HRMS of the crude mixtures of 566.2281 and 594.2599 indicated that the cyclic product had been synthesised in both cases. Due to the time constraints at this stage of the investigation, the compounds were not purified, and no further experimental data was gathered. Interestingly, in the cases of the tetrapeptides, no dimeric species like compound **154'** in **Scheme 109** were detected.

4.8 Conclusion

In summary, peptides containing aryl VSF on phenylalanine residues have been successfully further modified using SuFEx transformations. The modification of *ortho* aryl VSF with silyl ether nucleophiles was found be problematic due to the inherent preorganisation that is introduced upon *ortho* olefination of phenylalanine in peptides leading to unavoidable Michael addition. However, after a successful development of the

Mizoroki-Heck reaction to introduce *meta* and *para* aryl VSF functionality to peptides, the SuFEx reaction with silyl ethers proceeded quickly and in excellent yields.

The modification with amine nucleophiles proceeded more smoothly. Simple primary and secondary amines were used to further modify the model dipeptide in fair yields. Intermolecular SuFEx transformations with nucleophilic amino acids were more difficult to facilitate. However, intramolecular SuFEx reactions between *ortho* aryl VSFs and amines situated on lysine residues in the same peptide appeared to be carried out to form complex peptide macrocyclic structures. However, due to the time constrains of the project, these compounds were not purified. The successful isolation of these compounds would prove that the synthesis of these cyclic peptides was possible. This result would contextualise the power of the technique and demonstrate its applicability in the post-synthetic modification of peptides.

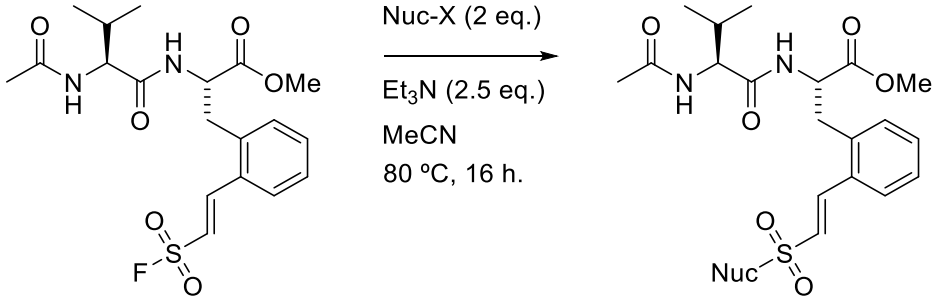
4.9 Experimental

4.9.1 General Information

All reagents and solvents were purchased from Alfa Aesar, Fischer Scientific, Fluorochem or Merck and used as provided. Chemical manipulations were carried out in oven-dried glassware and in an atmosphere of air unless otherwise stated. Flash column chromatography was performed manually on silica gel (Fluorochem silica gel 60 A particle size 40-63 μm) or on a Biotage® Isolera One auto column using Biotage® Sfar silica Duo prepacked flash columns. Thin layer chromatography was carried out on glass-backed silica gel plates (2.5 x 7.5 cm; Merck, TLC silica gel 60 Å). Compounds were visualised on TLC plates by exposure to UV light (254 nm). NMR spectra were recorded on a JEOL ECX or ECZ 400 Spectrometer at 298K. Chemical shifts are reported in parts per million and coupling constants are reported in Hz. For some compounds, it was necessary to use ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC 2D NMR experiments in order to aid assignment. FTIR spectra were recorded on an Agilent Cary 630 FTIR spectrometer and wavenumbers are reported to the nearest whole number. Melting points of compounds were obtained using a Stuart SMP10 melting point apparatus. High-resolution mass spectrometry was obtained from the EPSRC UK National Mass Spectrometry Facility at Swansea University on an LTQ Orbitrap XL 1, using positive electrospray ionisation (ESI+).

4.9.2 General Procedures for SuFEx transformations

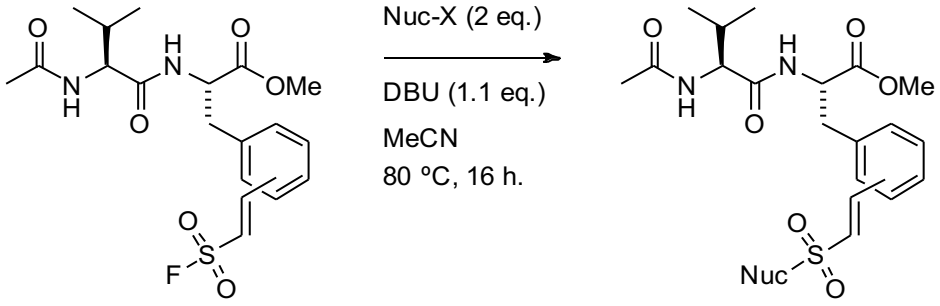
4.9.2.1 SuFEx transformations of 2-VSF containing peptides



X = H
$Si(CH_3)_2C(CH_3)_3$

The modified peptide (0.117 mmol) and nucleophile (2 eq.) were dissolved in MeCN (1 mL) and treated with Et_3N (2.5 eq.). The reaction mixture was heated to 80 ºC and stirred for 16 h. Upon completion, the volatiles were removed *in vacuo* and the crude oil was purified by flash column chromatography. Recrystallisation from CH_2Cl_2 / hexanes yielded the SuFEx product as a solid.

4.9.2.2 SuFEx transformations of 3-/4-VSF containing peptides



X = H
$Si(CH_3)_2C(CH_3)_3$

The modified peptide (0.117 mmol) and nucleophile (2 eq.) were dissolved in MeCN (1 mL) and treated with DBU (1.1 eq.). The reaction mixture was heated to 80 ºC and stirred for 16 h. Upon completion, the volatiles were removed *in vacuo* and the crude oil was purified by flash column chromatography. Recrystallisation from CH_2Cl_2 / hexanes yielded the SuFEx product as a solid

4.9.3 Synthesis of *p*-cresol-OTBDMS (117)

Known compound. The phenol-based compound *p*-cresol **116** (0.300 g, 2.774 mmol) was dissolved in DMF (10 mL) before being treated with Imidazole (0.378 g, 5.548 mmol) and allowed to stir at room temperature for 10 min. After the time had elapsed, TBDMS-Cl (0.502 g, 3.329 mmol) was added, and the reaction was allowed to stir for a further 30 min. The mixture was then quenched with deionised water (30 mL) followed by extraction into Et_2O (3 x 30 mL). The combined organic layers were washed with 5% LiCl solution (30 mL) and brine (30 mL) before being dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (Pet. Ether 40-60 ºC) yielded the silyl ether product **117** as a colourless oil (0.413 g, 67%); R_f = 0.60 (Pet. Ether 40-60 ºC).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.18 (6H, s, Si-(C*H*$_3$)$_2$), 0.97 (9H, s, Si-C(C*H*$_3$)$_3$), 2.27 (3H, s, Ar-C*H*$_3$), 6.73 (2H, d, *J* = 8.5, Ar-*H*), 7.01 (2H, d, *J* = 8.5, Ar-*H*).

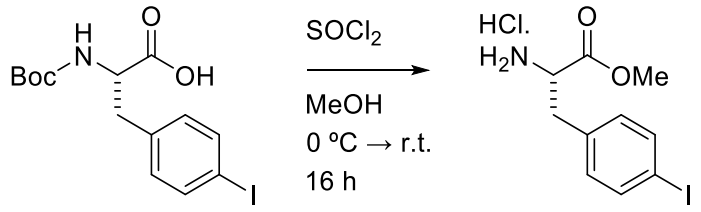
^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5 (Si-$(CH_3)_2$), 18.2 (4° Si-C), 20.6 (Ar-CH_3), 25.7 (*t*Bu-$(CH_3)_3$), 119.8 (Ar C), 129.8 (Ar C), 130.4 (4° Ar C), 153.3 (4° Ar C).

IR υ_{max}/cm^{-1} (oil) 3027 w (C-H), 2956 w (C-H), 1510 s (C=C), 1252 s (Si-O), 1240 s (C-O), 820 s (Si-CH_3).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{13}H_{23}OSi$: 223.1518, found: 223.1510.

4.9.4 Synthesis of *meta-/para-* vinyl sulfonyl fluoride phenylalanine-based peptides (39-44)

4.9.4.1 H-Phe(4-iodo)-OMe.HCl (120)



The protected amino acid Boc-Phe(4-iodo)-OH **42** (1.000 g, 2.556 mmol) was dissolved in MeOH (50 mL) and cooled to 0 ºC. The reaction was then treated with the dropwise addition of $SOCl_2$ (1.112 mL, 15.337 mmol) and stirred for 1 h before being allowed to warm to room temperature. The mixture was stirred for a further 16 h before the volatiles were removed *in vacuo* yielding **120** as an off white solid (0.829 g, 95%); m.p. 199-201 °C. R_f = 0.42 (EtOAc).

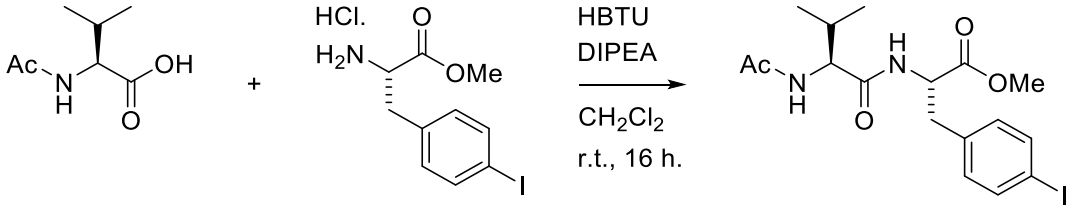
^{1}H NMR (400 MHz, CD_3OD) δ 3.14 (1H, dd, *J* = 14.4, 7.3, Phe-C*H*H), 3.23 (1H, dd, *J* = 14.4, 6.3, Phe-CH*H*), 3.81 (3H, s, ester-CH_3) 4.33 (1H, dd, *J* = 7.3, 6.3, Phe-α-C*H*), 7.06 (2H, d, *J* = 8.4, Ar-*H*), 7.73 (2H, d, *J* = 8.4, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 37.0 (Phe-CH_2), 53.8 (Phe-α-CH), 55.0 (ester-CH_3), 94.3 (Ar C-I), 132.7 (Ar C), 135.3 (4º Ar C), 139.5 (Ar C), 170.5 (C=O).

IR υ_{max}/cm^{-1} (solid) 3012 w (C-H), 2952 w (C-H), 2825 w (N-H salt), 1737 s (ester C=O), 1538 m (C=C), 1207 s (C-O), 600 s (C-I).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_{13}NO_2I$: 305.9991, found: 305.9989.

4.9.4.2 Ac-Val-Phe(4-iodo)-OMe (122)



The amino ester 4-iodo-L-phenylalalnine methyl ester hydrochloride **120** (0.978 g, 2.50 mmol) was dissolved in deionised water and treated with K_2CO_3 (0.498 g, 3.60 mmol) and stirred at room temperature for 20 min. The free amino ester was then extracted into Et_2O (3 x 20 mL) before the organic layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was dissolved in CH_2Cl_2 (30 mL) and treated with *N*-acetyl valine **121** (0.159 g, 1.000 mmol), HBTU (0.379 g, 1.000 mmol) and DIPEA (0.174 mL, 1.00 mmol) before stirring for 12 h. The reaction mixture was then quenched with CH_2Cl_2 (30 mL), then washed with 1M HCl (25 mL), sat. $NaHCO_3$ (3 x 25 mL) and distilled water (25 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was recrystallised from CH_2Cl_2 / hexanes to yield peptide **122** as a white solid (0.270 g, 61%); m.p. 237-240 °C. R_f = 0.35 (EtOAc).

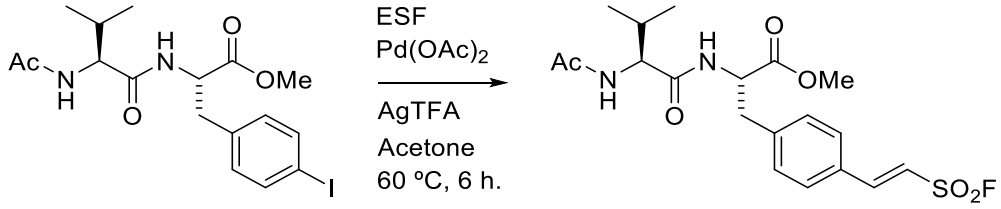
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 6.8, Val-C*H*$_3$), 0.93 (1H, d, *J* = 6.8, Val-C*H*$_3$), 1.97-2.07 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 2.99 (1H, dd, *J* = 13.9, 6.3, Phe-C*H*H), 3.09 (1H, dd, *J* = 13.9, 5.7, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 4.22 (1H, dd, *J* = 8.7, 7.0, Val-α-C*H*), 4.85 (1H, dt, *J* = 8.0, 6.3, Phe-α-C*H*), 6.01 (1H, d, *J* = 8.7, Val-N*H*), 6.38 (1H, d, *J* = 8.0, Phe-N*H*), 6.87 (2H, d, *J* = 8.1, Ar-*H*), 7.60 (2H, d, *J* = 8.1, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.1 (Val-CH_3) 23.3 (acetyl-CH_3), 31.0 (Val-CH), 37.5 (Phe-CH_2), 52.5 (ester-CH_3), 52.8 (Phe-α-CH), 58.4 (Val-α-CH), 92.8 (Ar C-I), 131.3 (Ar C), 135.2 (4º Ar C), 137.7 (Ar C), 170.0 (C=O), 170.9 (C=O), 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3265 m (N-H), 3071 w (C-H), 2956 w (C-H), 1737 s (ester C=O), 1640 s (amide C=O), 1539 s (C=C), 1241 s (C-O), 596 s (C-I).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{24}N_2O_4I$: 447.0781, found: 447.0783.

4.9.4.3 Ac-Val-Phe(4-VSF)-OMe (123)



The 4-iodo-phenylalanine containing compound **122** (0.223 g, 0.500 mmol), palladium acetate (0.011 g, 0.050 mmol) and silver trifluoroacetate (0.185 g, 0.600 mmol) were slurried in acetone (1 mL) and treated with ESF **50** (0.083 mL, 1.000 mmol) before heating to 60 ºC and stirring for 6 h. The reaction mixture was then allowed to cool before filtering through a Celite® plug (EtOAc). The volatiles were then removed *in vacuo* before purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). Recrystallisation from CH_2Cl_2 / hexanes yielded modified dipeptide **123** as a light brown solid (0.138 g, 65%); m.p. 206-208 °C. R_f = 0.35 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90-0.96 (6H, m, Val-(C*H*$_3$)$_2$), 1.97-2.08 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 3.11 (1H, dd, *J* = 13.8, 6.2, Phe-C*H*H), 3.22 (1H, dd, *J* = 13.8, 5.7, Phe-CH*H*), 3.75 (3H, s, ester-C*H*$_3$), 4.20 (1H, dd, *J* = 8.7, 7.1, Val-α-C*H*), 4.90 (1H, dt, *J* = 7.8, 6.2, Phe-α-C*H*), 6.01 (1H, d, *J* = 8.5, Val-N*H*), 6.41 (1H, d, *J* = 7.8, Phe-N*H*), 6.84 (1H, dd, *J* = 15.6, 2.5, alkene-C*H*), 7.24 (2H, d, *J* = 8.3, Ar-*H*), 7.48 (2H, d, *J* = 8.3, Ar-*H*), 7.76 (1H, d, *J* = 15.6, alkene-C*H*).

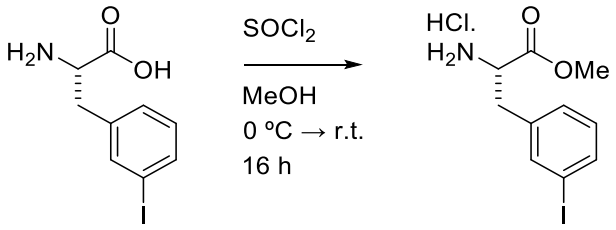
^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.1 (Val-CH_3), 23.2 (acetyl-CH_3), 30.9 (Val-CH), 37.9 (Phe-CH_2), 52.6 (ester-CH_3), 52.9 (Phe-α-CH), 58.6 (Val-α-CH), 117.8 ($^3J_{CF}$ = 28.4, alkene-C), 129.3 (Ar C), 129.9 (4º Ar C), 130.4 (Ar C), 141.0 (Ar C), 148.3 ($^4J_{CF}$ = 2.4, alkene-C), 170.2 (C=O), 171.0 (C=O), 171.2 (C=O).

^{19}F NMR (376 MHz, $CDCl_3$) δ 62.5 (s).

IR υ_{max}/cm^{-1} (solid) 3280 m (N-H), 3083 w (C-H), 2963 w (C-H), 1756 s (ester C=O), 1643 s (amide C=O), 1543 s (C=C), 1402 s (S=O), 1271 s (C-O), 1185 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_2O_6SF$: 429.1496, found: 429.1493.

4.9.4.4 H-Phe(3-iodo)-OMe.HCl (124)



The amino acid H-Phe(3-iodo)-OH (0.500 g, 1.718 mmol) was dissolved in MeOH (30 mL) and cooled to 0 ºC. The reaction was then treated with the dropwise addition of $SOCl_2$ (0.747 mL, 10.308 mmol) and stirred for 1 h before being allowed to warm to room temperature. The mixture was stirred for a further 16 h before the volatiles were removed *in vacuo* yielding **124** as a light-yellow solid (0.495 g, 85%); m.p. 160-163 °C. R_f = 0.52 (EtOAc).

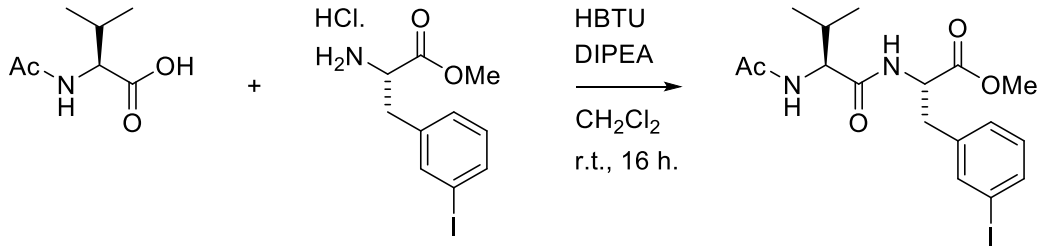
^{1}H NMR (400 MHz, CD_3OD) δ 3.13 (1H, dd, *J* = 14.4, 7.2, Phe-C*H*H), 3.23 (1H, dd, *J* = 14.4, 6.2, Phe-CH*H*), 3.81 (3H, s, ester-CH_3), 4.34 (1H, t, *J* = 7.2, Phe-α-C*H*), 7.16 (1H, t, *J* = 7.8, Ar-*H*), 7.28 (1H, d, *J* = 7.8, Ar-*H*), 7.67-7.73 (2H, m, Ar-*H*).

^{13}C NMR (100 MHz, CD_3OD) δ 36.9, (Phe-CH_2), 53.8 (ester-CH_3), 55.1 (Phe-α-CH), 95.7 (Ar C-I), 130.0 (Ar C), 132.1 (Ar C), 138.1 (4º Ar C), 138.3 (Ar C), 139.7 (Ar C), 170.4 (C=O).

IR υ_{max}/cm^{-1} (solid) 3063 w (C-H), 2958 w (C-H), 2814 m (N-H salt), 1737 s (ester C=O), 1561 s (C=C), 1245 s (C-O), 600 m (C-I).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_{13}NO_2I$: 305.9991, found: 305.9987.

4.9.4.5 Ac-Val-Phe(3-iodo)-OMe (125)



The amino ester 3-iodo-L-phenylalalnine methyl ester hydrochloride **124** (0.184 g, 0.470 mmol) was dissolved in deionised water and treated with K_2CO_3 (0.094 g, 0.677 mmol) and stirred at room temperature for 20 min. The free amino ester was then extracted into Et_2O (3 x 20 mL) before the organic layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was dissolved in CH_2Cl_2 (30 mL) and treated with *N*-acetyl valine **121** (0.30 g, 0.188 mmol), HBTU (0.071 g, 0.188 mmol) and DIPEA (0.033 mL, 0.188 mmol) before stirring for 12 h. The reaction mixture was then quenched with CH_2Cl_2 (30 mL), then washed with 1M HCl (25 mL), sat. $NaHCO_3$ (3 x 25 mL) and distilled water (25 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was recrystallised from CH_2Cl_2 / hexanes to yield peptide **125** as a white solid (0.046 g, 55%); m.p. 194-195 °C. R_f = 0.35 (EtOAc).

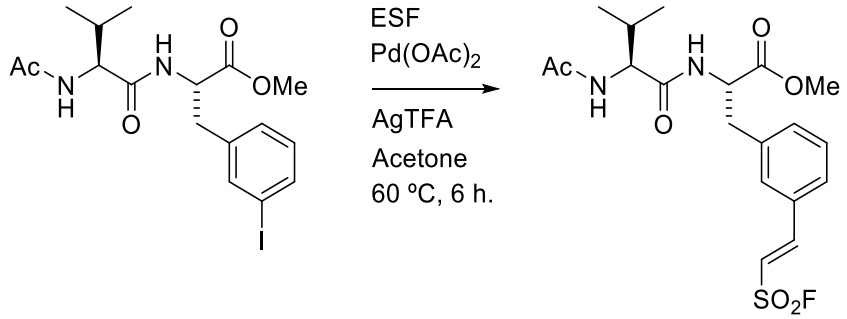
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.94 (3H, d, *J* = 6.8, Val-C*H*$_3$), 1.99-2.10 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 2.99 (1H, dd, *J* = 13.9, 6.3, Phe-C*H*H), 3.08 (1H, dd, *J* = 13.9, 5.9, Phe-CH*H*), 3.72 (3H, s, ester-C*H*$_3$), 4.33 (1H, dd, *J* = 8.8, 6.0, Val-α-C*H*), 4.81 (1H, dt, *J* = 7.7, 6.3, Phe-α-C*H*), 6.28 (1H, d, *J* = 8.8, Val-N*H*), 6.81 (1H, d, *J* = 7.7, Phe-N*H*), 7.00 (1H, t, *J* = 7.7, Ar-*H*), 7.10 (1H, dt, *J* = 7.7, 1.2, Ar-*H*), 7.48 (1H, t, *J* = 1.4, Ar-*H*), 7.56 (1H, dt, *J* = 7.7, 1.4, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.0 (Val-CH_3), 23.3 (acetyl-CH_3), 31.1 (Val-CH), 37.3 (Phe-CH_2), 52.4 (ester-CH_3), 53.1 (Phe-α-CH), 58.3 (Val-α-CH), 94.4 (Ar C-I), 128.5 (Ar C), 130.2 (Ar C), 136.2 (Ar C), 138.1 (Ar C), 138.2 (4º Ar C), 170.1 (C=O), 171.1 (C=O), 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3280 m (N-H), 3058 w (C-H), 2960 w (C-H), 1737 s (ester C=O), 1633 s (amide C=O), 1531 s (C=C), 1215 s (C-O), 600 s (C-I).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{24}N_2O_4I$: 447.0781, found: 447.0779.

4.9.4.6 Ac-Val-Phe(3-VSF)-OMe (126)



The 3-iodo-phenylalanine containing compound **124** (0.029 g, 0.065 mmol), palladium acetate (0.002 g, 0.007 mmol) and silver trifluoroacetate (0.024 g, 0.078 mmol) were slurried in acetone (1 mL) and treated with ESF **50** (0.011 mL, 0.130 mmol) before heating to 60 ºC and stirring for 6 h. The reaction mixture was then allowed to cool before filtering through a Celite® plug (EtOAc). The volatiles were then removed *in vacuo* before purification by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). Recrystallisation from CH_2Cl_2 / hexanes yielded modified dipeptide **126** as a light brown solid (0.021 g, 75%); m.p. 222-225 °C. R_f = 0.34 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, d, *J* = 6.7, Val-C*H*$_3$), 0.95 (3H, d, *J* = 6.7, Val-C*H*$_3$), 1.99-2.06 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 3.11 (1H, dd, *J* = 14.0, 5.9, Phe-C*H*H), 3.22 (1H, dd, *J* = 14.0, 5.3, Phe-CH*H*), 3.75 (3H, s, ester-C*H*$_3$), 4.08 (1H, app t, *J* = 8.0, Val-α-C*H*), 4.92 (1H, dt, *J* = 7.9, 5.9, Phe-α-C*H*), 5.95 (1H, d, *J* = 8.7, Val-N*H*), 6.39 (1H, d, *J* = 7.9, Phe-N*H*), 7.18 (1H, dd, *J* = 15.6, 2.5, alkene-C*H*), 7.23-7.27 (1H, m, Ar-*H*), 7.39 (1H, t, *J* = 7.5, Ar-*H*), 7.42 (1H, dt. *J* = 7.8, 1.6, Ar-*H*), 7.47 (1H, t, *J* = 1.6, Ar-*H*), 7.79 (1H, d, *J* = 15.6, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5 (Val-CH_3), 19.2 (Val-CH_3), 23.1 (acetyl-CH_3), 30.5 (Val-CH), 37.6 (Phe-CH_2), 52.6 (ester-CH_3), 52.8 (Phe-α-CH), 59.2 (Val-α-CH), 118.8 ($^3J_{CF}$ = 27.8, alkene-C), 128.4 (Ar C), 129.5 (Ar C), 129.8 (Ar C), 131.5 (4º Ar C), 133.4 (Ar C), 137.2 (4º Ar C), 148.4 ($^4J_{CF}$ = 1.9, alkene-C), 170.3 (C=O), 171.1 (C=O), 171.1 (C=O).

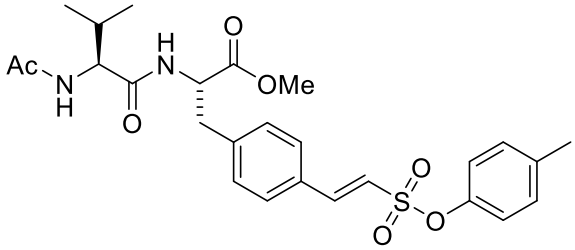
^{19}F NMR (376 MHz, $CDCl_3$) δ 62.1 (s).

IR υ_{max}/cm^{-1} (solid) 3280 m (N-H), 3086 w (C-H), 2960 w (C-H), 1733 m (ester C=O), 1643 s (amide C=O), 1539 s (C=C), 1398 s (S=O), 1215 s (C-O), 1185 s (S-F).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_2O_6SF$: 429.1496, found: 429.1490.

4.9.5 Reactions with silyl ether nucleophiles (127-128)

4.9.5.1 Diversified peptide (127)



The SuFEx transformation was carried out using modified peptide **123** (0.050 g, 0.117 mmol), *p*-cresol-OTBDMS **117** (0.052 g, 0.234 mmol) and DBU (0.019 mL, 0.128 mmol) according to the procedure outlined in **Section 4.9.2**. Upon completion the volatiles were removed *in vacuo* and the crude oil was purified by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). Recrystallisation from CH_2Cl_2 / hexanes yielded the SuFEx product **127** as an off-white solid (0.050 g, 83%); m.p. 167-169 °C. R_f = 0.40 (EtOAc).

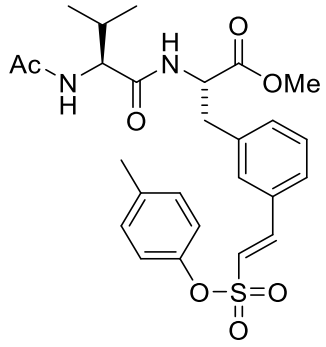
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.93 (3H, d, *J* = 6.8, Val-C*H*$_3$), 1.95-2.08 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 2.33 (3H, s, cresol-C*H*$_3$), 3.08 (1H, dd, *J* = 13.8, 6.4, Phe-C*H*H), 3.18 (1H, dd, *J* = 13.8, 5.6, Phe-CH*H*), 3.74 (3H, s, ester-C*H*$_3$), 4.29 (1H, dd, *J* = 8.8, 7.1, Val-α-C*H*), 4.89 (1H, dt, *J* = 7.9, 6.4, Phe-α-C*H*),6.18 (1H, d, *J* = 8.8, Val-N*H*), 6.72 (1H, d, *J* = 7.9, Phe-N*H*), 6.83 (1H, d, *J* = 15.6, alkene-C*H*), 7.11-7.20 (6H, m, Ar-*H*), 7.37 (2H, d, *J* = 8.2, Ar-*H*), 7.44 (1H, d, *J* = 15.6, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.1 (Val-CH_3), 20.9 (cresol-CH_3), 23.2 (acetyl-CH_3), 31.1 (Val-CH), 37.7 (Phe-CH_2), 52.5 (ester-CH_3), 52.9 (Phe-α-CH), 58.4 (Val-α-CH), 120.5 (alkene-C), 122.1 (Ar C), 128.8 (Ar C), 130.2 (Ar C), 130.3 (Ar C), 130.6 (4º Ar C), 137.1 (4º Ar C), 139.9 (4º Ar C), 145.4 (alkene-C), 147.3 (4º Ar C), 170.1 (C=O), 171.1 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3280 w (N-H), 3083 w (C-H), 2963 w (C-H), 1745 s (ester C=O), 1644 s (amide C=O), 1539 s (C=C), 1371 s (S=O), 1259 s (C-O), 1185 s (S-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{33}N_2O_7S$: 517.2008, found: 517.2010.

4.9.5.2 Diversified peptide (128)



The SuFEx transformation was carried out using modified peptide **126** (0.008 g, 0.019 mmol), *p*-cresol-OTBDMS **117** (0.008 g, 0.037 mmol) and DBU (0.003 mL, 0.021 mmol) according to the procedure outlined in **Section 4.9.2**. Upon completion the volatiles were removed *in vacuo* and the crude oil was purified by preparative thin layer chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). Recrystallisation from CH_2Cl_2 / hexanes yielded the SuFEx product **128** as an off-white solid (0.007 g, 71%); m.p. 182-184 °C. R_f = 0.40 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.82 (3H, d, *J* = 6.9, Val-C*H*$_3$),1.95-2.06 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 2.34 (3H, cresol-C*H*$_3$), 3.06 (1H, dd, *J* = 14.0, 7.6, Phe-C*H*H), 3.20 (1H, dd, *J* = 14.0, 5.5, Phe-CH*H*), 3.72 (3H, s, ester-C*H*$_3$), 4.25 (1H, dd, *J* = 8.7, 6.3, Val-α-C*H*), 4.87 (1H, dt, *J* = 7.6, 5.5, Phe-α-C*H*), 5.98 (1H, d, *J* = 8.7, Val-N*H*), 6.60 (1H, d, *J* = 7.8, Phe-N*H*), 6.90 (1H, d, *J* = 15.6, alkene-C*H*), 7.11-7.19 (4H, m, Ar-*H*), 7.24-7.31 (2H, m, Ar-*H*), 7.33-7.39 (2H, m, Ar-*H*), 7.48 (1H, d, *J* = 15.6, alkene-C*H*).

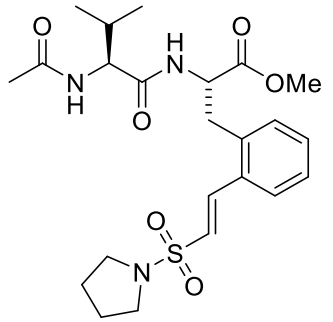
^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4 (Val-CH_3), 19.2 (Val-CH_3), 20.9 (cresol-CH_3), 23.1 (acetyl-CH_3), 30.6 (Val-CH), 37.6 (Phe-CH_2), 52.5 (ester-CH_3), 52.9 (Phe-α-CH), 59.0 (Val-α-CH) 121.4 (alkene-C), 122.2 (Ar C), 127.7 (Ar C), 129.3 (Ar C), 129.4 (Ar C), 130.3 (Ar C), 132.3 (Ar C), 132.5 (Ar C), 137.0 (Ar C), 137.1 (Ar C), 145.5 (alkene-C), 147.4 (Ar C), 170.2 (C=O), 171.0 (C=O), 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3288 m (N-H), 3064 w (C-H), 2960 w (C-H), 1752 m (ester C=O), 1633 s (amide C=O), 1539 s (C=C), 1372 s (S=O), 1208 s (C-O), 1170 (S-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{33}N_2O_7S$: 517.2008, found: 517.2007.

4.9.6 Reactions with 2º amine nucleophiles

4.9.6.1 Diversified peptide (129)



The SuFEx product **129** was synthesised from modified peptide **59** (0.050 g, 0.117 mmol), pyrrolidine **128** (0.019 mL, 0.234 mmol) and Et_3N (0.041 mL, 0.292 mmol) using the procedure outlined in **Section 4.9.2**. The crude product was purified by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC) before recrystallisation from CH_2Cl_2 / hexanes yielded product **129** as a white solid (0.039 g, 69%); m.p. 180-182 °C. R_f = 0.49 (EtOAc).

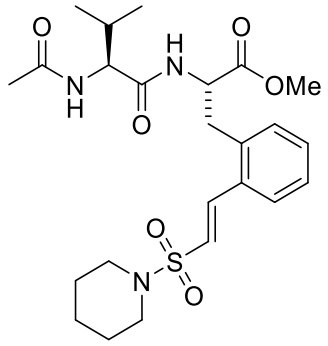
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.91 (3H, d, *J* = 6.9, Val-C*H*$_3$), 1.89-1.94 (4H, m, pyrrolidine-C*H*$_2$), 2.01 (3H, s, acetyl-C*H*$_3$), 2.02-2.13 (1H, m, Val-C*H*), 3.14 (1H, dd, *J* = 14.4, 7.6, Phe-C*H*H), 3.30-3.39 (5H, m, Phe-CH*H* / pyrrolidine-C*H*$_2$), 3.73 (3H, s, ester-C*H*$_3$), 4.28 (1H, dd, *J* = 8.7, 6.9, Val-α-C*H*), 4.81 (1H, dt, *J* = 7.3, 6.0, Phe-α-C*H*), 6.23 (1H, d, *J* = 8.7, Val-N*H*), 6.67-6.73 (2H, m, Phe-N*H* / alkene-C*H*), 7.20 (1H, dd, *J* = 7.6, 1.1, Ar-*H*), 7.28 (1H, dt, *J* = 7.3, 1.1, Ar-*H*), 7.34 (1H, dt, *J* = 7.3, 1.4, Ar-*H*), 7.49 (1H, dd, *J* = 7.6, 1.4, Ar-*H*), 7.77 (1H, d, *J* = 15.3, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (Val-CH_3), 19.1 (Val-CH_3), 23.2 (acetyl-CH_3), 25.7 (pyrrolidine-CH_2), 30.7 (Val-CH), 35.0 (Phe-CH_2), 47.9 (pyrrolidine-CH_2), 52.7 (ester-CH_3), 52.9 (Phe-α-CH), 58.2 (Val-α-CH), 123.3 (alkene-C), 127.0 (Ar C), 127.8 (Ar C), 130.6 (Ar C), 131.2 (Ar C), 132.2 (Ar C), 135.8 (Ar C), 139.6 (alkene-C), 170.3 (C=O), 171.1 (C=O), 171.3 (C=O).

IR υ_{max}/cm^{-1} (solid) 3291 m (N-H), 3064 w (C-H), 2960 w (C-H), 1752 s (ester C=O), 1640 s (amide C=O), 1543 s (C=C), 1334 s (S=O), 1245 m (C-O), 1165 s (S-N).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{34}N_3O_6S$: 480.2168, found: 480.2163.

4.9.6.2 Diversified peptide (132)



SuFEx product **132** was synthesised from modified peptide **59** (0.050 g, 0.117 mmol), piperidine **130** (0.023 mL, 0.234 mmol) and Et_3N (0.041 mL, 0.292 mmol) using the procedure outlined in **Section 4.9.2**. The crude product was purified by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). Before recrystallisation from CH_2Cl_2 / hexanes yielded **132** as a white solid (0.025 g, 43%); m.p. 171-173 °C. R_f = 0.51 (EtOAc).

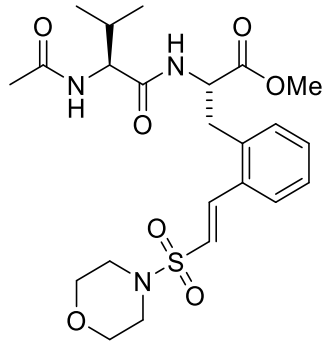
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.91 (3H, d, *J* = 6.9, Val-C*H*$_3$), 1.50-1.58 (2H, m, piperidine-C*H*$_2$), 1.65-1.73 (4H, m, piperidine-C*H*$_2$), 2.00-2.13 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 3.14 (1H, dd, *J* = 14.2, 7.1, Phe-C*H*H), 3.18-3.23 (4H, m, piperidine-C*H*$_2$), 3.33 (1H, dd, *J* = 14.2, 5.5, Phe-CH*H*), 3.74 (3H, s, ester-C*H*$_3$), 4.23 (1H, dd, *J* = 8.9, 6.8, Val-α-C*H*), 4.82 (1H, dt, *J* = 7.3, 6.0, Phe-α-C*H*), 6.16 (1H, d, *J* = 8.9, Val-N*H*), 6.45 (1H, d, *J* = 7.8, Phe-N*H*), 6.63 (1H, d, *J* = 15.2, alkene-C*H*), 7.19 (1H, dd, *J* = 7.6, 1.4, Ar-*H*), 7.28-7.38 (2H, m, Ar-*H*), 7.49 (1H, dd, *J* = 7.6, 1.2, Ar-*H*), 7.71 (1H, d, *J* = 15.2, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (Val-CH_3), 19.1 (Val-CH_3), 23.2 (acetyl-CH_3), 23.7 (piperidine-CH_2), 25.4 (piperidine-CH_2), 30.6 (Val-CH), 35.0 (Phe-CH_2), 46.6 (piperidine-CH_2), 52.8 (ester-CH_3), 53.0 (Phe-α-CH), 58.3 (Val-α-CH), 124.4 (alkene-C), 127.0 (Ar C), 127.9 (Ar C), 130.7 (Ar C), 131.2 (Ar C), 132.2 (Ar C), 135.7 (Ar C0, 140.0 (alkene-C), 170.3 (C=O), 171.0 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3287 w (N-H), 3064 w (C-H), 2937 w (C-H), 1744 m (ester C=O), 1640 s (amide C=O), 1528 s (C=C), 1334 s (S=O), 1215 s (C-O), 1137 s (S-N).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{24}H_{36}N_3O_6S$: 494.2325, found: 494.2320.

4.9.6.3 Diversified peptide (133)



SuFEx product **133** was synthesised from modified peptide **59** (0.050 g, 0.117 mmol), morpholine **131** (0.021 mL, 0.234 mmol) and Et_3N (0.041 mL, 0.292 mmol) using the procedure outlined in **Section 4.9.2**. The crude mixture was purified by flash column chromatography (8:2 EtOAc / Pet. Ether 40-60 ºC). And recrystallised from CH_2Cl_2 / hexanes to yield SuFEx product **133** as a white solid (0.031 g, 53%); m.p. 184-186 °C. R_f = 0.45 (EtOAc).

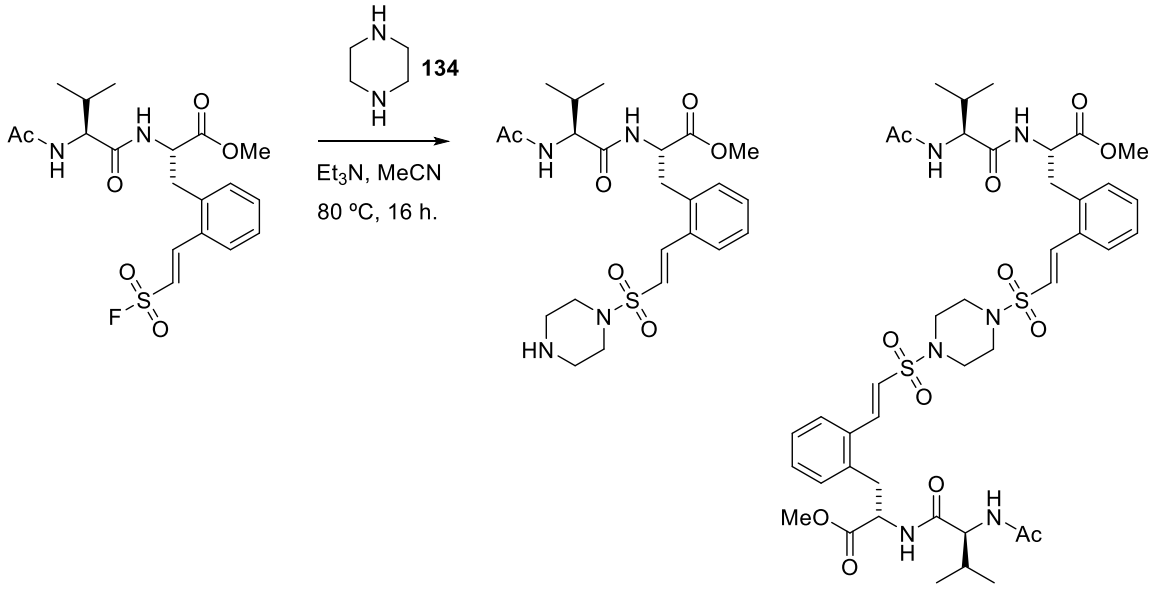
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, d, *J* = 6.9, Val-C*H*$_3$), 0.91 (3H, d, *J* = 6.7, Val-C*H*$_3$), 1.99-2.10 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 3.16 (1H, dd, *J* = 14.2, 7.1, Phe-C*H*H), 3.20-3.26 (4H, m, morpholine-C*H*$_2$), 3.32 (1H, dd, *J* = 14.2, 6.0, Phe-CH*H*), 3.73 (3H, s, ester-C*H*$_3$), 3.79 (4H, t, *J* = 4.7, morpholine-C*H*$_2$), 4.23 (1H, dd, *J* = 8.8, 6.9, Val-α-C*H*) 4.80 (1H, dt, *J* = 7.3, 6.2, Phe-α-C*H*), 6.12 (1H, d, *J* = 8.8, Val-N*H*), 6.51 (1H, d, *J* = 7.8, Phe-N*H*), 6.64 (1H, d, *J* = 15.2, alkene-C*H*), 7.19 (1H, dd, *J* = 7.6, 0.9, Ar-*H*), 7.30 (1H, dt, *J* = 7.3, 0.9, Ar-*H*), 7.36 (1H, dt, *J* = 7.6, 1.4, Ar-*H*), 7.50 (1H, dd, *J* = 7.6, 0.9, Ar-*H*), 7.77 (1H, d, *J* = 15.2, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (Val-CH_3), 19.1 (Val-CH_3), 23.2 (acetyl-CH_3), 30.7 (Val-CH), 35.1 (Phe-CH_2), 45.7 (morpholine-CH_2), 52.7 (ester-CH_3), 53.0 (Phe-α-CH), 58.3 (Val-α-CH), 66.3 (morpholine-CH_2), 123.0 (alkene-C), 127.1 (Ar C), 128.0 (Ar C), 130.9 (Ar C), 131.2 (Ar C), 131.9 (Ar C), 135.8 (Ar C), 141.4 (alkene-C), 170.2 (C=O), 171.0 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3295 m (N-H), 3071 w (C-H), 2967 w (C-H), 1752 m (ester C=O), 1640 s (amide C=O), 1543 s (C=C), 1346 m (S=O), 1264 s (C-O), 1337 s (S-N).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{34}N_3O_7S$: 496.2117, found: 496.2114.

4.9.6.4 Diversified peptide 135



SuFEx product **135** was synthesised from modified peptide **59** (0.050 g, 0.117 mmol), piperazine **134** (0.020 g, 0.234 mmol) and Et_3N (0.041 mL, 0.292 mmol) using the procedure outlined in **Section 4.9.2**. The crude mixture was isolated by removing the volatiles *in vacuo* yielding **135** and **135'** as a brown oil. (0.077 g); R_f = 0.05 (1:9 MeOH / EtOAc).

The ^{1}H and ^{13}C NMR spectra obtained were too complex to assign without purification. The ^{19}F NMR spectrum showed no peak at ca. 60 ppm indicating that the fluorine environment present in the starting material had changed. The HRMS of the crude mixture revealed both diversified product **135** and SuFEx dimer product **135'** had been synthesised by exposure to the reaction conditions.

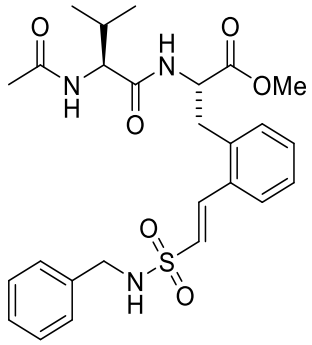
Data for 135

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{35}N_4O_6S$: 495.2277, found: 495.2274.

Data for 135'

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{59}N_6O_{12}S_2$: 903.3632, found: 903.3627.

4.9.7 Reactions with 1º amine nucleophiles to generate diversified peptide (137)



SuFEx product **137** was synthesised from modified peptide **59** (0.050 g, 0.117 mmol), benzylamine **136** (0.026 mL, 0.234 mmol) and Et_3N (0.041 mL, 0.292 mmol) using the procedure outlined in **Section 4.9.2**. The crude mixture was purified by flash column chromatography (7:3 EtOAc / Pet. Ether 40-60 ºC) and recrystallised from CH_2Cl_2 / hexanes yielded SuFEx product **137** as a white solid (0.036 g, 60%); m.p. 132-134 °C. R_f = 0.57 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, d, *J* = 6.7, Val-C*H*$_3$), 0.88 (3H, d, *J* = 6.7, Val-C*H*$_3$), 1.95-2.03 (4H, m, acetyl-C*H*$_3$ / Val-C*H*), 3.13 (1H, dd, *J* = 14.2, 5.7, Phe-C*H*H), 3.22 (1H, dd, *J* = 14.2, 6.7, Phe-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 4.24 (1H, dd, *J* = 8.9, 7.1, Val-α-C*H*), 4.81 (1H, dt, *J* = 8.5, 6.0, Phe-α-C*H*), 6.22-6.30 (2H, Val-N*H* / CSO_2N*H*C), 6.57-6.67 (2H, m, Phe-N*H* / alkene-C*H*), 7.13 (1H, dd, *J* = 7.6, 1.4, Ar-*H*), 7.22-7.40 (8H, m, Ar-*H*), 7.66 (1H, d, *J* = 15.4, Alkene-C*H*).

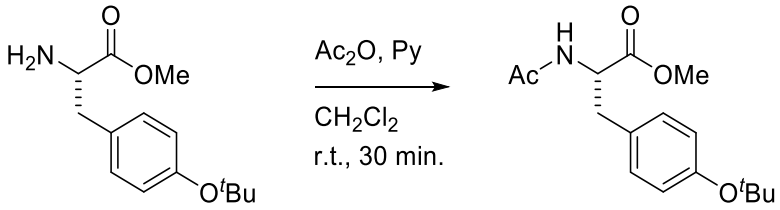
^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (Val-CH_3), 19.1 (Val-CH_3), 23.2 (acetyl-CH_3), 30.9 (Val-CH), 35.3 (Phe-CH_2), 47.2 (benzylamine-CH_2), 52.5 (ester-CH_3), 53.0 (Phe-α-CH), 58.3 (Val-α-CH), 126.8 (alkene-C), 127.7 (Ar C), 128.0 (Ar C), 128.7 (Ar C), 130.4 (Ar C), 131.0 (Ar C), 132.1 (Ar C), 135.8 (Ar C), 137.1 (Ar C), 137.4 (alkene-C), 170.8 (C=O), 171.1 (C=O), 171.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3288 m (N-H), 3063 w (C-H), 2960 w (C-H), 1722 m (ester C=O), 1636 s (amide C=O), 1539 s (C=C), 1326 s (S=O), 1271 s (C-O), 1141 s (S-N).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{34}N_3O_6S$: 516.2168, found: 516.2165.

4.9.8 Synthesis of nucleophilic amino acid (145)

4.9.8.1 Ac-Tyr(O^{t}Bu)-OMe (147)



The protected amino ester H-Tyr(O^{t}Bu)-OMe **146** (0.576 g, 2.000 mmol) was dissolved in CH_2Cl_2 (30 mL). In a separate vessel, Ac_2O (0.945 mL, 10.000 mmol) and pyridine (0.805 mL, 10.000 mmol) were dissolved in CH_2Cl_2 (5 mL) before being added to the amino ester solution. The reaction mixture was allowed to sir for 30 min at room temperature before the volatiles were removed *in vacuo*. The crude oil was then dissolved in CH_2Cl_2 (30 mL) and washed with sat. $NaHCO_3$ solution (3 x 15 mL) before being dried ($MgSO_4$) and concentrated *in vacuo*. The fully protected amino ester **147** was yielded as a colourless oil (0.488 g, 83%); R_f = 0.57 (EtOAc).

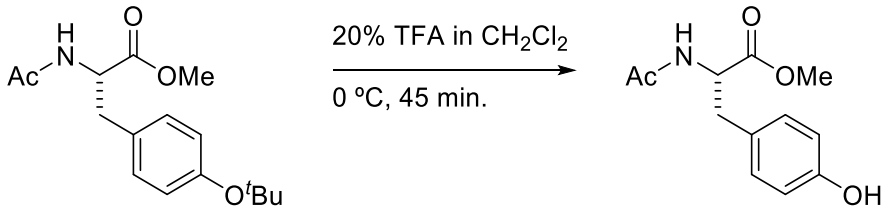
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (9H, s, *t*Bu-(C*H*$_3$)$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 3.02 (1H, dd, *J* = 14.1, 6.3, Tyr-C*H*H), 3.08 (1H, dd, *J* = 14.1, 6.1, Tyr-CH*H*), 3.68 (3H, s, ester-C*H*$_3$), 4.84 (1H, dt, *J* = 7.8, 6.2, Tyr-α-C*H*), 6.32 (1H, br d, *J* = 7.8, Tyr-N*H*), 6.91 (2H, d, *J* = 8.5, Ar-*H*), 7.01 (2H, d, *J* = 8.5, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (acetyl-CH_3), 28.6 (*t*Bu-(CH_3)$_3$), 37.0 (Tyr-CH_2), 52.0 (Tyr-α-CH), 53.1 (ester-CH_3), 78.2 (*t*Bu-C), 124.0 (Ar C), 129.5 (Ar C), 130.6 (4° Ar C), 154.2 (Ar C-O), 169.6 (C=O), 172.1 (C=O).

IR υ_{max}/cm^{-1} (oil) 3280 w (N-H), 3060 w (C-H), 2974 w (C-H), 1744 s (amide C=O), 1655 s (ester C=O), 1510 s (C=C), 1238 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{16}H_{24}NO_4$: 294.1705, found: 294.1701.

4.9.8.2 Ac-Tyr-OMe (148)



The fully protected peptide ester **147** (0.468 g, 1.595 mmol) was dissolved in CH_2Cl_2 (16 mL) and cooled to 0 ºC in an ice bath. The solution was then treated with TFA (4 mL) and stirred at 0 ºC for 45 min. The volatiles were then removed *in vacuo* yielding deprotected product **148** as a white solid (0.359 g, 95%); m.p. 118-120 °C. R_f = 0.50 (EtOAc).

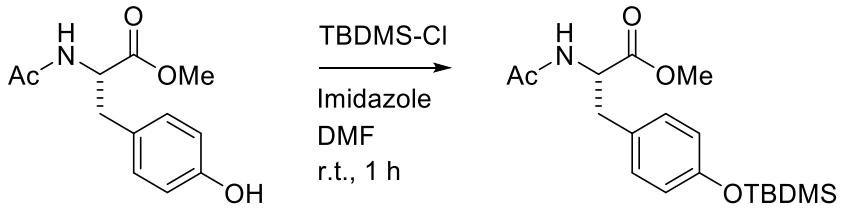
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (3H, s, acetyl-C*H*$_3$), 2.98 (1H, dd, *J* = 14.2, 6.1, Tyr-C*H*H), 3.07 (1H, dd, *J* = 14.2, 5.6, Tyr-CH*H*), 3.75 (3H, s, ester-C*H*$_3$), 4.85 (1H, dt, *J* = 7.9, 6.1, Tyr-α-C*H*), 6.50 (1H, d, *J* = 7.9, Tyr-N*H*), 6.73 (2H, d, *J* = 8.5, Ar-*H*), 6.92 (2H, d, *J* = 8.5, Ar-*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5 (acetyl-CH_3), 36.8 (Tyr-CH_2), 52.6 (ester-CH_3), 53.7 (Tyr-α-CH), 115.6 (Ar C), 126.7 (4° Ar C), 130.2 (Ar C), 155.4 (Ar C-O), 172.0 (C=O), 172.1 (C=O).

IR υ_{max}/cm^{-1} (solid) 3548 w (O-H), 3317 m (N-H), 3019 w (C-H), 2956 w (C-H), 1730 s (ester C=O), 1655 s (amide C=O), 1543 s (C=C), 1230 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{12}H_{16}NO_4$: 238.1079, found: 238.1073.

4.9.8.3 Ac-Tyr(OTBDMS)-OMe (145)



Protected amino ester **148** (0.400 g, 1.685 mmol) was dissolved in DMF (10 mL) before being treated with imidazole (0.344 g, 5.055 mmol) and stirred for 10 min. TBDMS-Cl (0.305 g, 2.022 mmol) was then added and the reaction was stirred for a further 30 min. The reaction mixture was quenched with deionised water (30 mL) and extracted into Et_2O (3 x 30 mL). The combined organics were washed with brine (1 x 30 mL) before being dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (6:4 EtOAc / Pet. Ether 40-60 ºC and recrystallisation from CH_2Cl_2 / hexanes yielded protected silyl ether nucleophile **145** as a white solid (0.282 g, 48%); m.p. 58-60 °C. R_f = 0.61 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.18 (6H, s, Si-(C*H*$_3$)$_2$), 0.97 (9H, s, Si-(C*H*$_3$)$_3$), 1.97 (3H, s, acetyl-C*H*$_3$), 2.99 (1H, dd, *J* = 14.0, 6.2, Tyr-C*H*H), 3.05 (1H, dd, *J* = 14.0, 6.0, Tyr-CH*H*), 3.69 (3H, s, ester-C*H*$_3$), 4.82 (1H, dt, *J* = 7.9, Tyr-α-C*H*), 6.29 (1H, br d, *J* = 6.3, Tyr-N*H*), 6.76 (2H, m, Ar-*H*), 6.96 (2H, m, Ar-*H*).

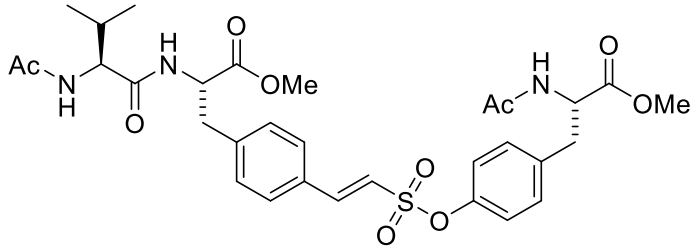
^{13}C NMR (100 MHz, $CDCl_3$) δ -4.6 (Si-(CH_3)$_2$), 18.0 (4° Si-C), 22.8 (acetyl-CH_3), 25.5 (Si-(CH_3)$_3$), 36.9 (Tyr-CH_2), 52.0 (ester-CH_3), 53.2 (Tyr-α-CH), 120.0 (Ar C), 128.4 (4° Ar C), 130.0 (Ar C), 154.4 (Ar C-O), 169.7 (C=O), 172.2 (C=O).

IR υ_{max}/cm^{-1} (solid) 3276 w (N-H), 3068 w (C-H), 2960 w (C-H), 1744 s (amide C=O), 1640 s (ester C=O), 1510 s (C=C), 1248 s (Si-O), 1215 s (C-O), 834 s (Si-CH_3).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{30}NO_4Si$: 352.1944, found: 352.1935.

4.9.9 Peptide-amino acid cross-linking (151-152)

4.9.9.1 Diversified peptide (151)



The SuFEx transformation was carried out using modified peptide **123** (0.050 g, 0.117 mmol), silyl ether nucleophile **145** (0.082 g, 0.234 mmol) and DBU (0.019 mL, 0.128 mmol) according to the procedure outlined in **Section 4.9.2**. Upon completion the volatiles were removed *in vacuo* and the crude oil was purified by flash column chromatography (EtOAc). Recrystallisation from CH_2Cl_2 / hexanes yielded the SuFEx product **151** as a white solid (0.048 g, 63%); m.p. 179-181 °C. R_f = 0.11 (EtOAc).

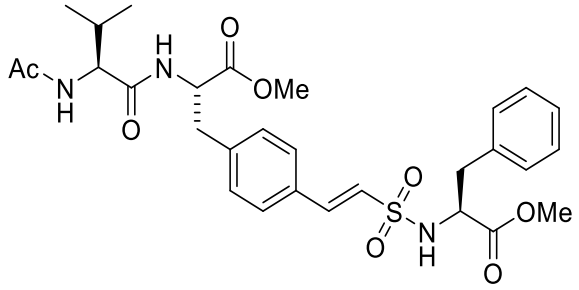
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 8.6, Val-C*H*$_3$), 0.93 (3H, d, *J* = 8.6, Val-C*H*$_3$), 0.94-2.06 (7H, m, acetyl-C*H*$_3$ / acetyl-C*H*$_3$ / Val-C*H*), 3.03-3.12 (2H, m, Tyr-C*H*H / Phe-C*H*H), 3.13-3.23 (2H, m, Tyr-CH*H* / Phe-CH*H*), 3.70 (3H, s, ester-C*H*$_3$), 7.74 (3H, s, ester-C*H*$_3$), 4.24 (1H, dd, *J* = 8.7, 7.2, Val-α-C*H*), 4.83-4.92 (2H, m, Tyr-α-C*H* / Phe-α-C*H*), 6.06 (1H, d, *J* = 7.7, Tyr-N*H*), 6.21 (1H, d, *J* = 8.7, Val-N*H*), 6.59 (1H, d, *J* = Phe-N*H*), 6.83 (1H, d, *J* = 15.6, alkene-C*H*), 7.11 (2H, d, *J* = 8.7, Ar-*H*), 7.16-7.21 (4H, m, Ar-*H*), 7.39 (2H, d, *J* = 8.2, Ar-*H*), 7.45 (1H, d, *J* = 15.6, alkene-C*H*).

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.1 (Val-CH_3), 23.1 (acetyl-CH_3), 23.2 (acetyl-CH_3), 30.9 (Val-CH), 37.2 (Tyr-CH_2), 37.7 (Phe-CH_2), 52.4 (ester-CH_3), 52.5 (ester-CH_3), 52.9 (Tyr-α-CH), 53.0 (Phe-α-CH), 58.5 (Val-α-CH), 120.3 (alkene-C), 122.5 (Ar C), 128.8 (Ar C), 130.2 (Ar C), 130.5 (Ar C), 130.6 (Ar C), 135.2 (Ar C), 140.0 (Ar C), 145.7 (alkene-C), 148.5 (Ar C), 169.7 (C=O), 170.1 (C=O), 171.0 (C=O), 171.2 (C=O), 171.8 (C=O).

IR υ_{max}/cm^{-1} (solid) 3283 m (N-H), 3056 w (C-H), 2956 w (C-H), 1737 s (ester C=O), 1644 s (amide C=O), 1532 s (C=C), 1364 s (S=O), 1215 s (C-O), 1170 s (S-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{31}H_{40}N_3O_{10}S$: 646.2434, found: 646.2438.

4.9.9.2 Diversified peptide (152)



The SuFEx transformation was carried out using modified peptide **123** (0.050 g, 0.117 mmol), H-Phe-OMe.HCl **22** (0.051 g, 0.234 mmol) and DBU (0.079 mL, 0.525 mmol) according to the procedure outlined in **Section 4.9.2**. Upon completion the volatiles were removed *in vacuo* to yield **152** as a brown solid crude mixture (0.064 g); R_f = 0.08 (EtOAc).

The ^{1}H and ^{13}C NMR spectra obtained were too complex to assign without purification. The ^{19}F NMR spectrum showed no peak at ca. 60 ppm indicating that the fluorine environment in the starting material had changed. The HRMS of the crude mixture revealed the diversified product **152** had been synthesised.

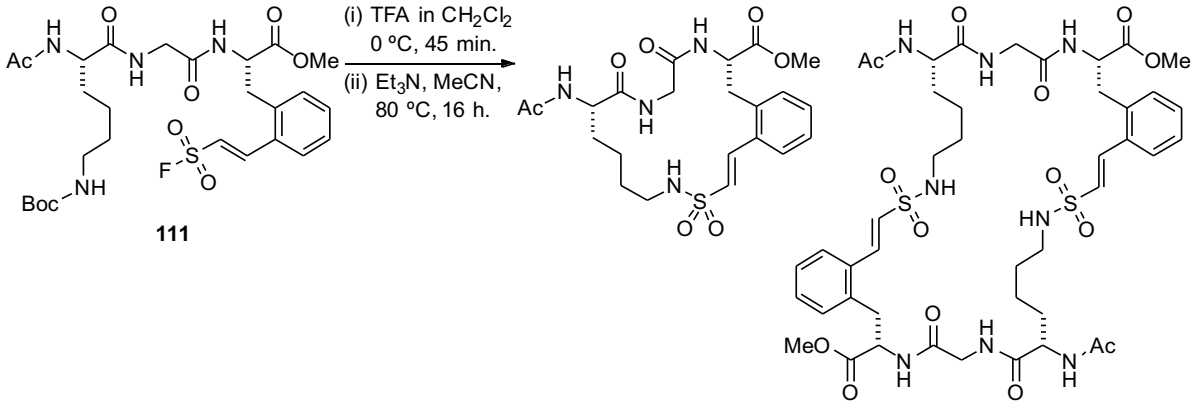
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{29}H_{38}N_3O_8S$: 588.2380, found: 588.2380.

The SuFEx transformation was also carried out using modified peptide **123** (0.050 g, 0.117 mmol), H-Phe-OMe.TFA (0.069 g, 0.234 mmol) and DBU (0.079 mL, 0.525 mmol) according to the procedure outlined in **Section 4.9.2**. Upon completion the volatiles were removed *in vacuo* to yield a crude mixture as a brown solid (0.057 g); R_f = 0.08 (EtOAc).

The HRMS data is consistent with that acquired for the reaction with phenylalanine methyl ester hydrochloride above.

4.9.10 Cyclic Peptides (154, 156-157)

4.9.10.1 Cyclic peptide (154)



111 **154** **154'**

Linear protected 2-VSF containing peptide **111** (0.005 g, 0.008 mmol) was exposed to 20% TFA in CH_2Cl_2 (2 mL) at 0º C for 45 min before the volatiles were removed *in vacuo*. The resulting oil was dissolved in MeCN (4 mL) and treated with Et_3N (0.005 mL, 0.036 mmol). After stirring at 80 ºC for 16 h, the solvents were removed in vacuo. The crude cyclized products **154** and **154'** was isolated as a light brown oil (0.004 g); R_f = 0.05 (EtOAc).

The ^{1}H and ^{13}C NMR spectra obtained were too complex to assign without purification. The ^{19}F NMR spectrum showed no peak at ca. 60 ppm indicating that the fluorine environment present in the starting material had changed.

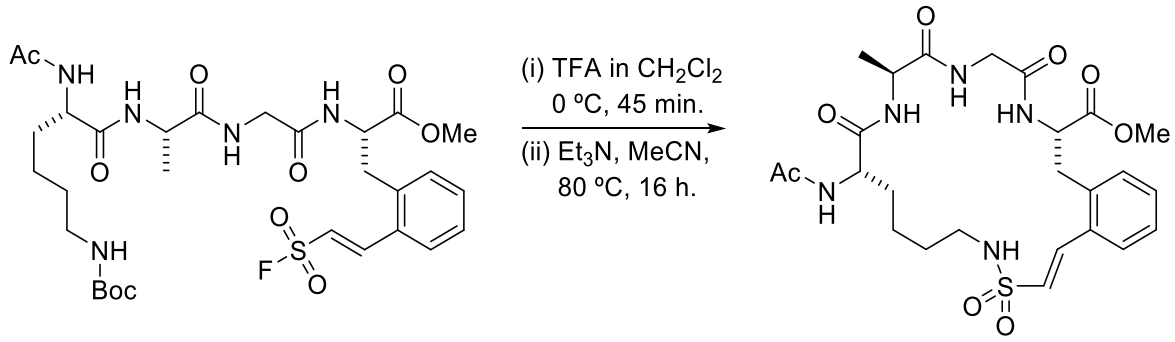
Data for 154

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{31}N_4O_7S$: 495.1913, found: 495.1907.

Data for 154'

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{44}H_{61}N_8O_{14}S_2$: 989.3749, found: 989.3739.

4.9.10.2 Cyclic peptide (156)

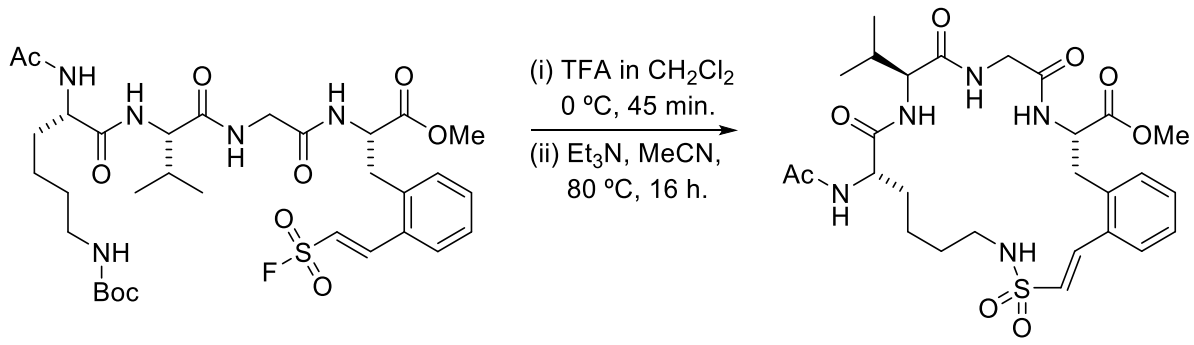


Linear protected 2-VSF containing peptide **112** (0.015 g, 0.022 mmol) was exposed to 20% TFA in CH_2Cl_2 (2 mL) at 0º C for 45 min before the volatiles were removed *in vacuo*. The resulting oil was dissolved in MeCN (4 mL) and treated with Et_3N (0.014 mL, 0.098 mmol). After stirring at 80 ºC for 16 h, the solvents were removed in vacuo. The crude cyclized product **156** was isolated as a brown oil (0.014 g); R_f = 0.06 (EtOAc).

The ^{1}H and ^{13}C NMR spectra obtained were too complex to assign without purification. The ^{19}F NMR spectrum showed no peak at ca. 60 ppm indicating that the fluorine environment present in the starting material had changed.

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{36}N_5O_8S$: 566.2285, found: 566.2282.

4.9.10.3 Cyclic peptide (157)



Linear protected 2-VSF containing peptide **157** (0.011 g, 0.015 mmol) was exposed to 20% TFA in CH_2Cl_2 (2 mL) at 0º C for 45 min before the volatiles were removed *in vacuo*. The resulting oil was dissolved in MeCN (4 mL) and treated with Et_3N (0.010 mL, 0.069mmol). After stirring at 80 ºC for 16 h, the solvents were removed in vacuo. The crude cyclized product **157** was isolated as a light brown oil (0.008 g); R_f = 0.07 (EtOAc).

The ^{1}H and ^{13}C NMR spectra obtained were too complex to assign without purification. The ^{19}F NMR spectrum showed no peak at ca. 60 ppm indicating that the fluorine environment present in the starting material had changed.

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{27}H_{40}N_5O_8S$: 594.2598, found: 594.2599.

5 Conclusions and Future Work

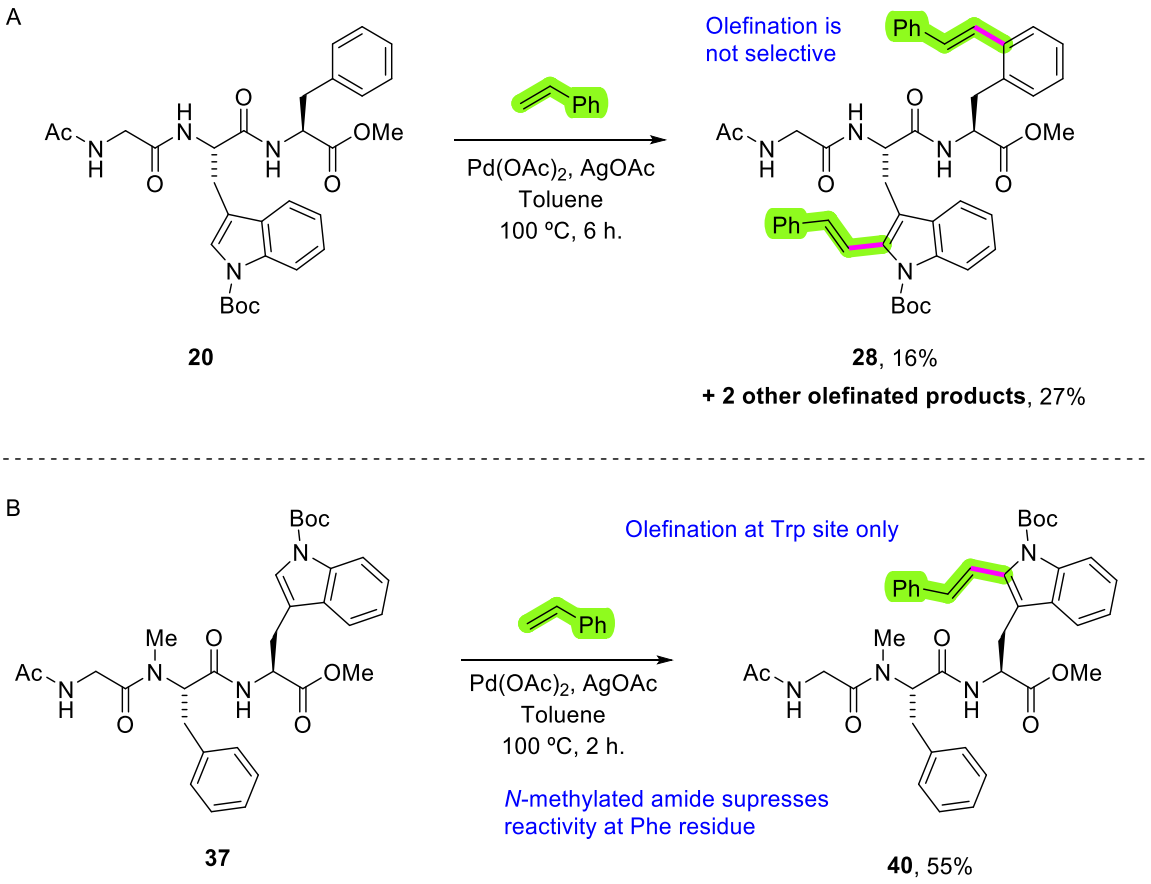
The emergence of peptides as an extremely viable class of therapeutics, alternative to small molecule or protein-based drugs has meant that the field of peptide modification is experiencing a period of huge attention. The modification of peptides can eradicate undesirable characteristics and improve their pharmacokinetic properties, making them better suited to pharmaceutical application. C-H functionalization and click chemistry are tools available to synthetic chemists which represent attractive methods of facilitating peptide modification without the need to rely on pre-functionalized starting materials or reactive handles native to the peptide structure.

The aim of this body of work was to develop a novel protocol which married these two techniques to modify peptides. Increasing the number of methodologies available for the diversification of peptides is critical to broadening the applicability of peptide-based structures for use in the pharmaceutical industry. The work documented *vide supra* describes the development of a novel method for the post-synthetic modification of phenylalanine residues in peptides with ESF. These structures can then be further differentiated by employment of click chemistry procedures to form complex and diversified linear or cyclic peptides.

5.1 Residue selectivity and peptide-amino acid cross-linking

At the beginning of the project there were several research questions that needed to be addressed. Two methods had been separately developed for the olefination of phenylalanine and tryptophan residues in peptides. However, exposure of peptides containing both residues to the developed conditions did not selectively olefinate one residue over the other. For broad applicability of the method, the development of residue selectivity was necessary. The initial attempt to address this issue utilised designed peptide **20** in which the extra residue at the *N*-terminus was installed to promote the reactivity of tryptophan. However, the reaction yielded three main products and selectivity was not achieved via this route, **Scheme 111A**. A previous investigation had determined that the

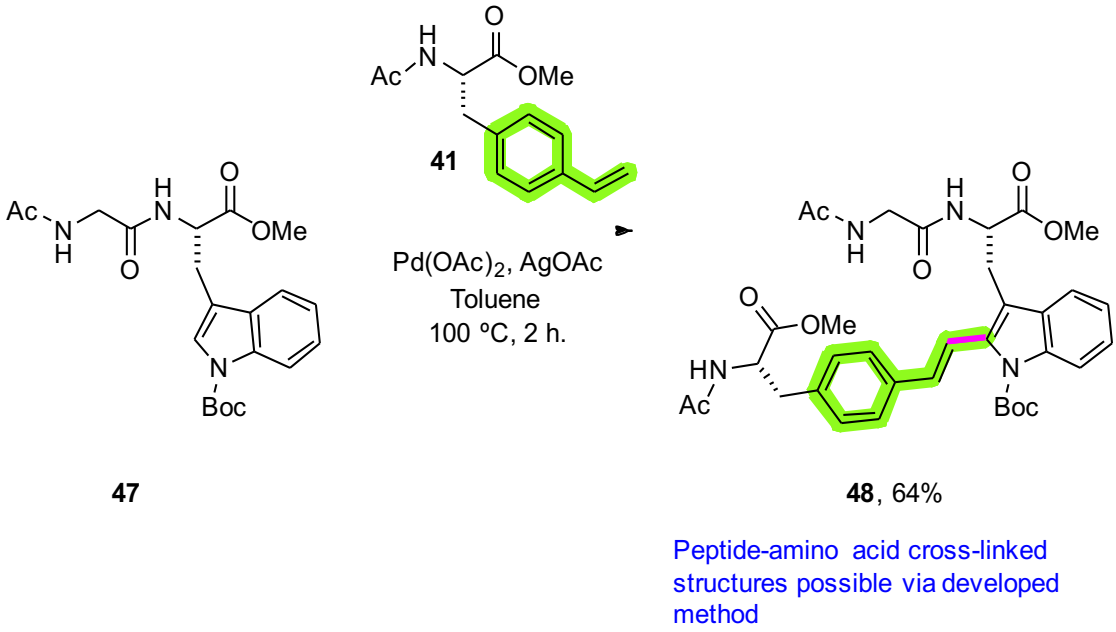
reactivity of phenylalanine residues in peptides can be supressed by the manipulation of the peptide backbone directing group. When an *N*-methyl group is installed on the phenylalanine residue, olefination is not facilitated on that residue. With that in mind, tripeptide **37** was designed in which phenylalanine reactivity should be theoretically supressed. Gratifyingly, upon exposure to the developed olefination conditions, modified peptide **40** was synthesised in good yield and residue selectivity was afforded, **Scheme 111B**.



Scheme 111 – Residue selectivity is not achieved by promoting reactivity of residues as in [A] but by supressing reactivity of residues as in [B].

The scope of the alkene had been evaluated previously by the research group. However, investigations into alkene scope where styrene is a moiety on a more complex system had not been assessed. By increasing the complexity of the styrene derivative to an amino acid-based substrate **47**, an indication about the suitability of the developed protocol for use as a cross-linking method was evaluated. Exposure of amino acid **41** and peptide **47** to the

developed olefination conditions yielded modified dipeptide **48** in good yield, **Scheme 112**. This was a significant result as it proved the method was well adapted to peptide-amino acid cross-linking and could likely be used as a method to develop constrained peptides.



47 **48**, 64%

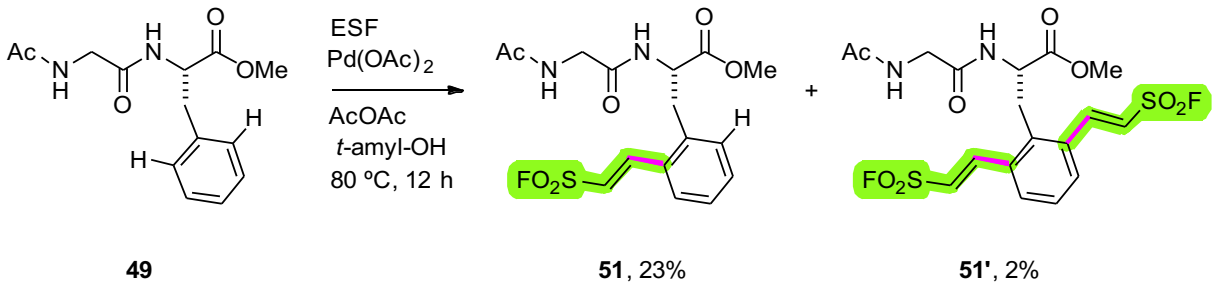
Scheme 112 – Peptide-amino acid cross-linking using C-H functionalization.

Despite this success, a flurry of publications by other groups concurrently with this work somewhat detracted from the originality.[116,125] As a result, the planned investigation into using C-H functionalization as a means to facilitate peptide cyclization was redesigned.

5.2 The modification of phenylalanine residues with ESF.

Phenylalanine residues in peptides, along with other non-polar residues, have traditionally been notoriously difficult amino acids to modify post-synthetically. The natural residue possesses no obvious reactive handle making it difficult to facilitate a selective change using traditional modification methods. More recently, reports have appeared that use C-H functionalization as a means to afford chemical modification of the residue in exchange for the ubiquitous C-H bond. However, the field is still in its relative infancy and although growing rapidly as a research area, only a few protocols exist. Moreover, an extremely limited amount of these methods have been reported to introduce SuFEx-able hubs like ESF onto peptides.

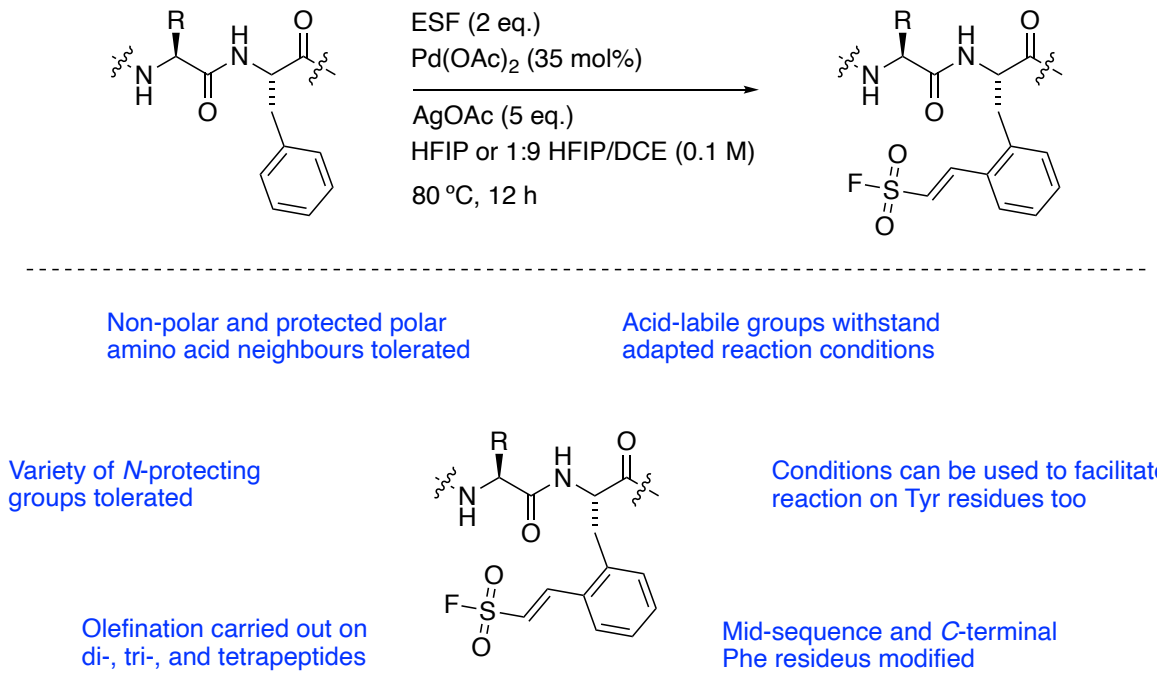
Section 3 of this thesis described the olefination of ESF onto phenylalanine residues in short chain peptides sequences. The investigation began with the exposure of Ac-Gly-Phe-OMe **49** to olefination conditions which yielded modified peptides **51** and **51'**, **Scheme 113**. Modified compound **51** was the highest yielding product of the reaction.



49 **51**, 23% **51'**, 2%

Scheme 113 – The olefination of phenylalanine residues with ESF in peptides to modify at the ortho position has been developed in this project.

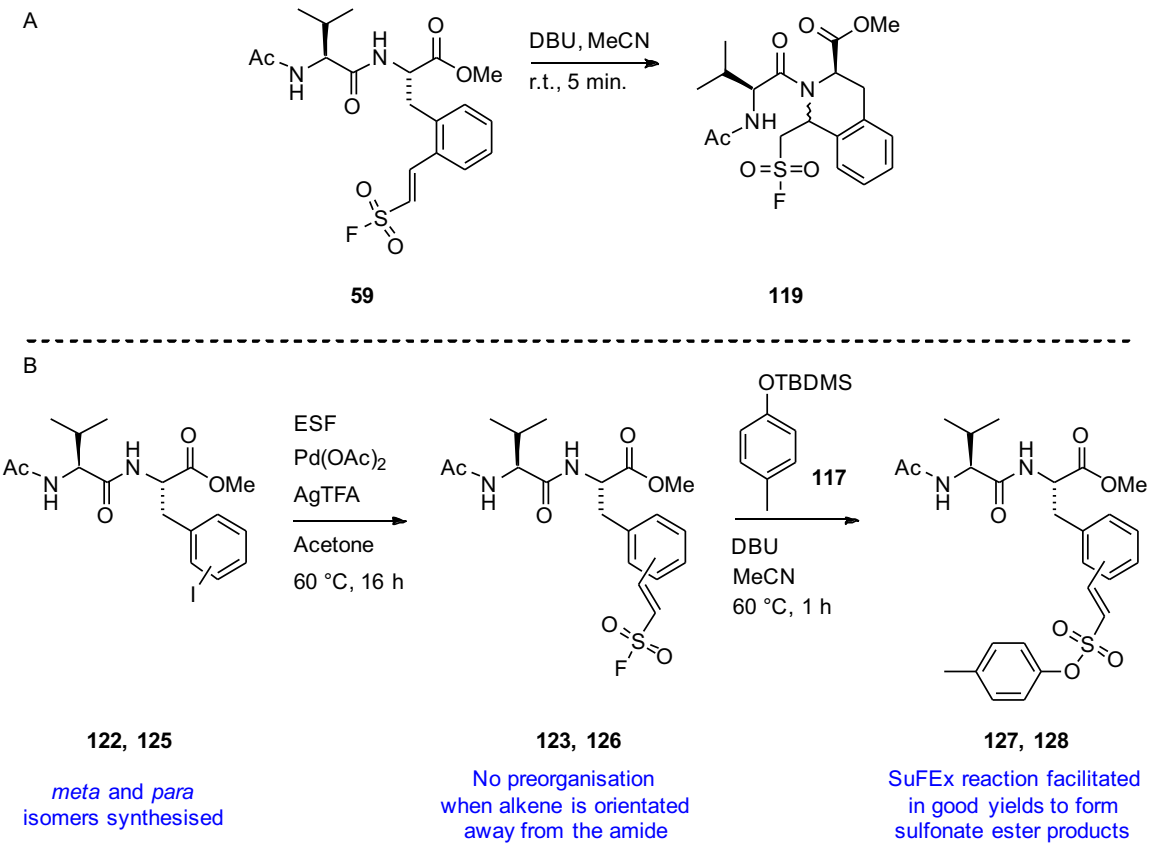
An optimisation process was then undertaken to further maximise the yield of this structure. The mono-olefinated product was considered to be more desirable than the di-olefinated species as it should avoid any selectivity issues arising when carrying out further modification. During the optimisation, the importance of the solvent HFIP was identified. After further optimisation, its inclusion as a solvent in the final optimised conditions was established. The developed method was then used on a wide range of different peptides to test the limitations of the technique. In summary, peptides were chosen for the study to investigate the nature of the neighbouring amino acid, polarity, protecting group, type of aromatic residue, peptide chain length and position of the residue on the chain, **Scheme 114**.



Scheme 114 - A summary of the scope of the olefination reaction developed in ***Section 3****.*

5.3 SuFEx Chemistry on Modified Peptides

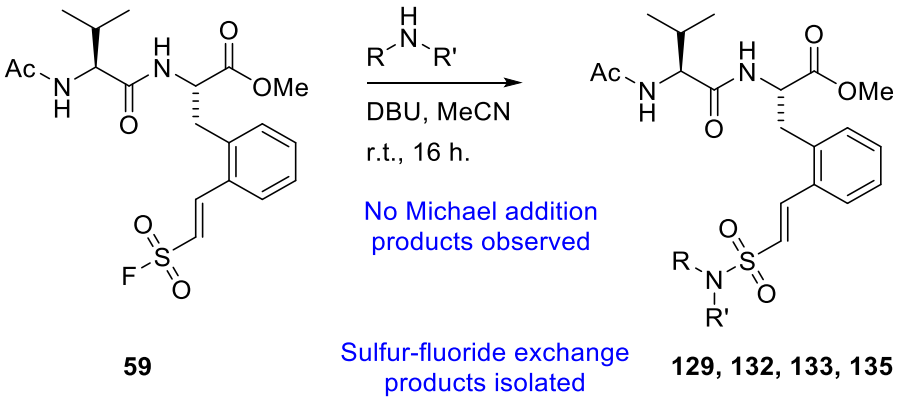
Following the olefination of phenylalanine residues with ESF to generate *ortho* olefinated compounds, further reactions of the installed aryl VSF with different types of nucleophiles were evaluated. Upon exposure of the VSF containing peptide **59** to DBU and silyl ether **117**, the expected sulfonate ester product was not synthesised. Instead, a competing Michael addition between the native amide and alkene prevailed to yield a non-isolatable constrained peptide **119** (**Scheme 115A**) which significantly altered the structural properties of the product. The preorganisation of the key components was adjudged to be the cause of this unexpected side reaction.



Scheme 115 – SuFEx transformations with silyl ethers on peptides.

The corresponding *meta* and *para* isomers of the VSF containing peptides were synthesised by successfully expanding on a documented Heck protocol. The preorganisation that drives the unwanted side reaction was overcome and the Michael reactivity of the compound was supressed. When exposed to a silyl ether, the SuFEx transformation was carried out to form **127** and **128** in high yield and was purified easily by chromatography, **Scheme 115B**.

The modified dipeptide model **59** was then exposed to a series of simple cyclic amines and produced the corresponding sulfonamides **129**, **132**, **133** and **135** in good yield, **Scheme 116**. Surprisingly, no Michael addition products were observed, and this was attributed to the steric penalties required to bring the amine into close proximity of the native peptide backbone. The SuFEx transformation involving piperazine represented an interesting development. A single SuFEx reaction yielded a complex modified peptide containing another secondary amine. This amine could then participate in a further SuFEx reaction. Indeed, this was found to be the case as evidence of a di-SuFEx product was found in the mass spectrum.

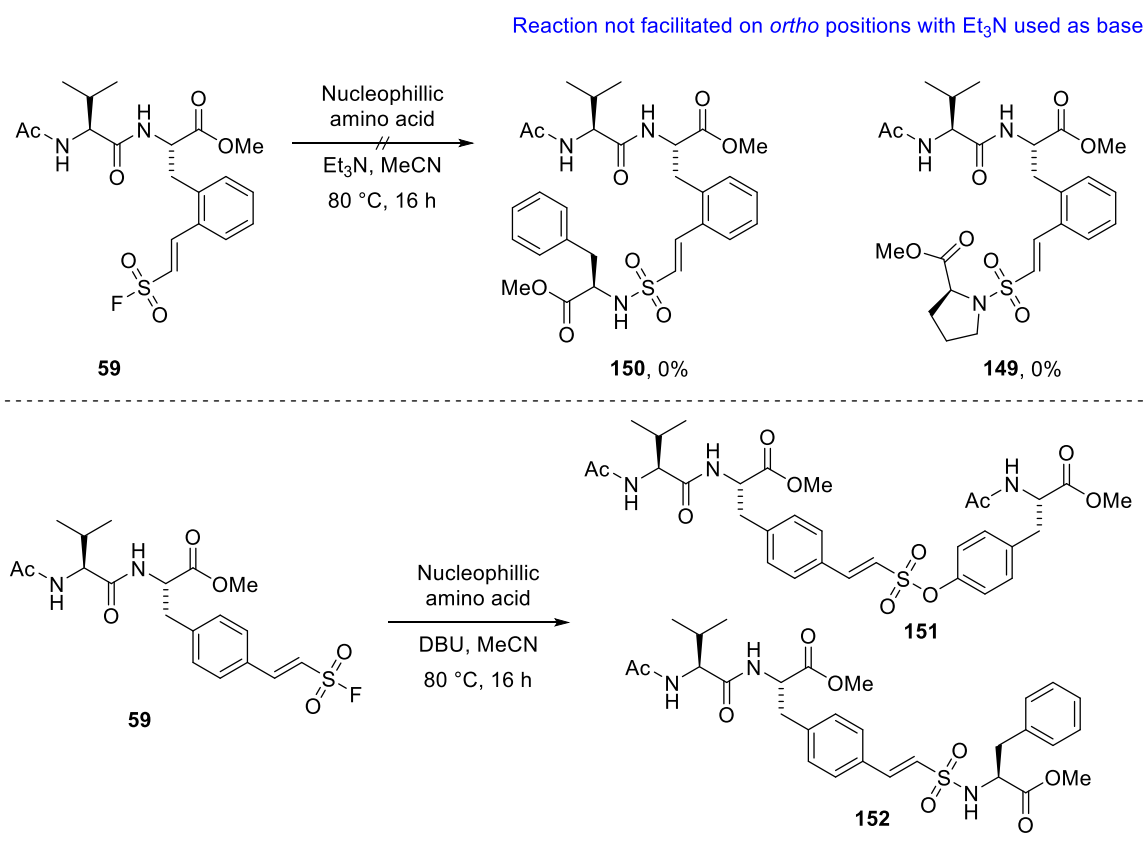


59 **129, 132, 133, 135**

*Scheme 116 – SuFEx transformations on peptide **59** with amine nucleophiles.*

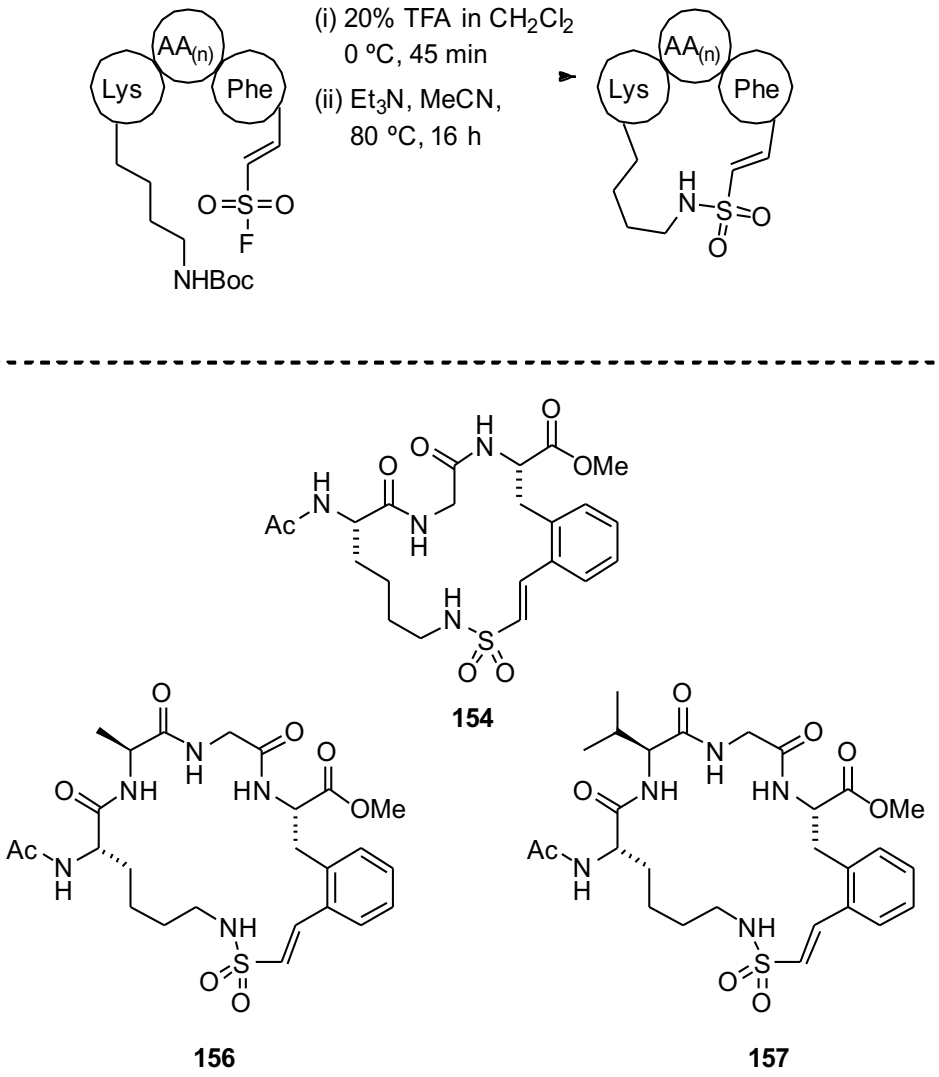
Primary amines were also used to synthesise sulfonamide containing peptide **137** from the modified dipeptide model **59**. Like the secondary amines, they did not undergo Michael addition at the alkene. Moreover no 4-membered cyclic sulfonamides were generated. This was once again attributed to the steric hinderance around the Michael addition centre. A limit on the nucleophilicity of the amine species was also discovered. Poor amine nucleophiles such as aniline did not undergo a SuFEx transformation.

When increasing the complexity of the nucleophiles to amino acids, it was discovered that *ortho* VSF containing peptide **59** did not successfully react with the nucleophile to form sulfonamides or sulfonate esters. When the *para* isomer **123** was exposed to the reaction conditions however, peptide-amino acids cross-linked structures **151** and **152** were obtained, **Scheme 117**.



Scheme 117 – Peptide-amino acid cross linking is facilitated on para VSF containing peptides.

Finally, intramolecular SuFEx reactions were carried out on tri- and tetrapeptide species containing an amine from a lysine residue and an *ortho* VSF on a phenylalanine residue, **Scheme 118**. Analysis of the HRMS of these compounds provided evidence that some dimeric species are formed as well as the cyclic peptides.



Scheme 118 – Cyclic peptides synthesised by the developed method.

In summary, a variety of SuFEx transformations have been carried out on aryl VSF containing peptides. The results accumulated in this section provide compelling evidence that peptides modified as in **Chapter 3** can undergo SuFEx transformations with a variety of nucleophiles. Furthermore, when primary amine nucleophiles and *ortho* VSFs are present on the same peptide structures, a SuFEx transformation can be facilitated to synthesis cyclic peptides in a novel approach.

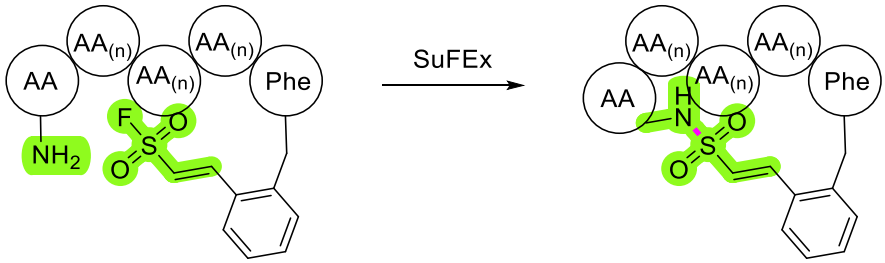
5.4 Future Work

The work undertaken in this project has provided several avenues for future exploration. Notably, the synthesis of cyclic peptides represented a huge step forward in the project. However, time constraints meant that a full characterisation could not be carried out on these compounds. In the immediate future, work should focus on the isolation and characterisation of these compounds and efforts should be geared to generating cyclic peptides.

5.4.1 Catalytic Loading

One potential drawback of the developed method is the comparatively high catalytic loading required to facilitate the olefination reaction. Due to the similarity in R_f of the product and starting material peptide, the catalytic loading was utilised in an attempt to convert all starting material to product in order to remove difficult purification steps. Future efforts should explore the reasons for poor catalytic turnover and seek to address them. One potential method for improving the yield is the use of $Cu(OAc)_2$ as an oxidant. In the optimisation stages in **Section 3.3**, $Cu(OAc)_2$ performed reasonably well as an oxidant but was deemed not as highly yielding as AgOAc. However, $Cu(OAc)_2$ was used more recently and gave a high yielding reaction. Investigations into the reproducibility of this result and an in-depth analysis of $Cu(OAc)_2$ as an oxidant in this reaction could prove fruitful.

5.4.2 Synthesis of conformationally constrained peptides.



Scheme 119 – Peptide cyclization from amino head to phenylalanine side chain

The synthesis of cyclic peptides in this study has been carried out by facilitating cyclization by the formation of a covalent bond between two side chains of amino acids a peptide. However, there are other possible ways of facilitating the cyclization reaction. The amino head of a peptide chain contains an amine that has been left protected during this project. Protection with a temporary protecting group could lead to the amino head being revealed post-synthesis before an intramolecular SuFEx reaction forms the peptide cycle between a phenylalanine side chain and the amino head, **Scheme 119**. Cyclization in this way generates further diverse structures than the class documented in this project.

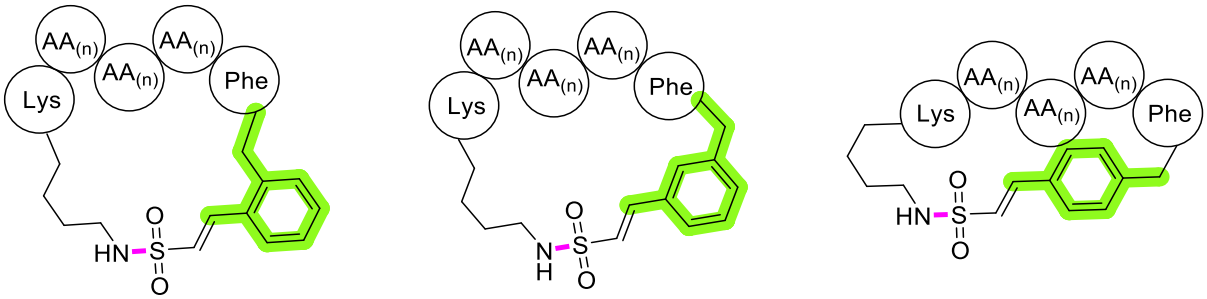


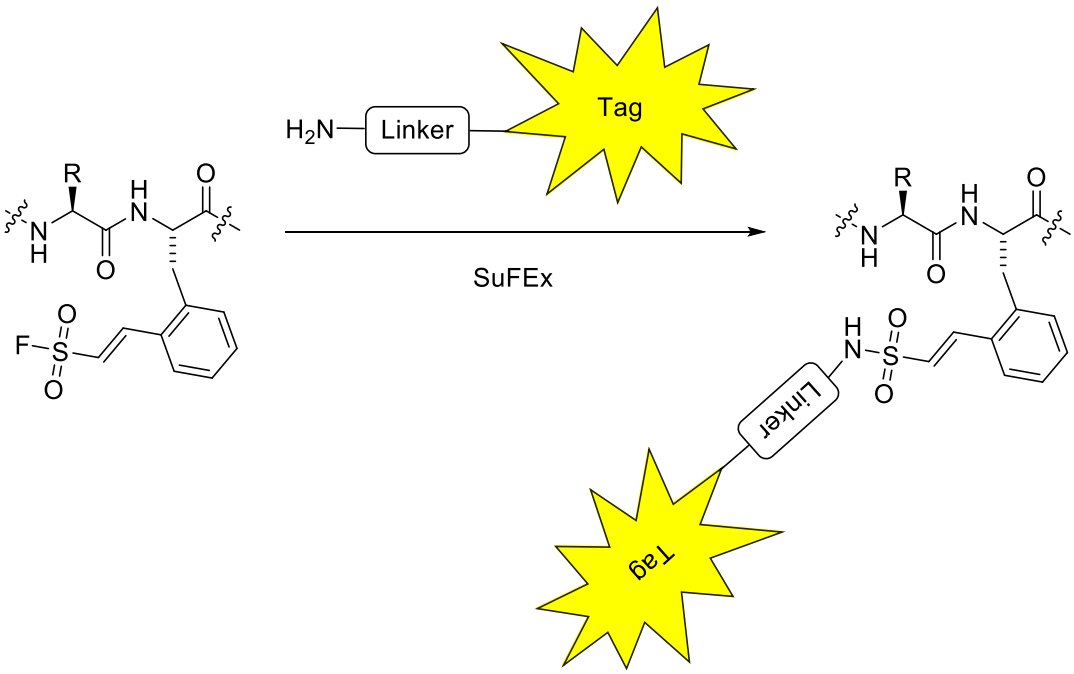
Figure 29 – Substitution patterns on the phenyl ring could have profound effects on the properties of the cyclized peptide.

Further diversification could be introduced by the employment of the Heck reaction used in **Section 4.3.2**. Generating peptides with a 3-aryl VSF or 4-aryl VSF means that the sulfonyl fluoride is projected in different relative directions, **Figure 29**. This could have profound effects on the conformation and rigidity of the cyclic structure, leading to peptides with different properties to those which were olefinated at the *ortho* position.

Another possible avenue of exploration is the olefination of other aromatic resides to diversify cyclic peptide structures. It was determined in **Section 3.9** that olefination of tyrosine residues in peptides is possible. For broad applicability of the developed method, the scope of the reaction should be expanded as much as possible. The olefination of different aromatic residues goes some way to achieve that aim. The study also revealed that tryptophan residues are not well suited to olefination in this manner. This result was surprising due to the relative reactivity of the C-H bond at C-2 and warrants further investigation. Changing the protecting group on the indole is likely to be a good starting point. However, as discussed in **Chapter 1** and **2**, improving the scope of the reaction is likely to introduce selectivity issues which will therefore need to be addressed. The techniques developed in **Chapter 2** may go some way to tackling these potential issues.

5.4.3 Peptide Conjugation

This body of work has demonstrated a proof-of-concept that the olefination of unreactive residues in peptides can be facilitated by the developed method, installing a reactive aryl VSF moiety to the peptide. Efforts to further diversify the peptides structures then focused on simple amine or silyl ethers nucleophiles in order to prove that SuFEx transformations could be carried out following olefination. The nucleophiles could now be expanded to include biologically relevant species such as cell penetrating peptides, fluorescent markers or drug conjugates. As described in **Section 1.2**, there are a wide variety of motivations for peptide modification. The installed aryl VSF is a clickable hub which could be used to further modify the peptide to tailor its properties. Biological tags can be designed in such a way that an amine or silyl ether nucleophile can form a covalent bond between tags or a linker and the modified peptide, **Scheme 120**. This type of modification is an example of how the developed methodology provides chemists with another tool for post-synthetic modification to tune peptide properties.



Scheme 120 – Potential peptide modification with biological tag by SuFEx transformation.

6 References

1 C. A. Lipinski, *J. Pharmacol. Toxicol. Methods*, 2000, **44**, 235–249. DOI: 10.1016/S1056-8719(00)00107-6.

2 D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.*, 2013, **81**, 136–147. DOI: 10.1111/cbdd.12055.

3 A. L. Hopkins and C. R. Groom, *Nat. Rev. Drug Discov.*, 2002, **1**, 727–730. DOI: 10.1038/nrd892.

4 A. P. Russ and S. Lampel, *Drug Discov. Today*, 2005, **10**, 1607–1610. DOI: 10.1016/S1359-6446(05)03666-4.

5 G. L. Verdine and L. D. Walensky, *Clin. Cancer Res.*, 2007, **13**, 7264–7270. DOI: 10.1158/1078-0432.CCR-07-2184.

6 V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *J. Am. Chem. Soc.*, 1954, **76**, 3115–3121. DOI: 10.1021/ja01641a004.

7 M. Pawlikowski and G. Mełeń-Mucha, *Curr. Opin. Pharmacol.*, 2004, **4**, 608–613. DOI: 10.1016/j.coph.2004.06.010.

8 P. Broqua, P. J.-M. Riviere, P. M. Conn, J. E. Rivier, M. L. Aubert and J.-L. Junien, *J. Pharmacol. Exp. Ther.*, 2002, **301**, 95–102. DOI: 10.1124/jpet.301.1.95.

9 L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G. Shao, X. Wang, R. Wang and C. Fu, *Signal Transduct. Target. Ther.*, 2022, **7**, 48. DOI: 10.1038/s41392-022-00904-4.

10 A. Henninot, J. C. Collins and J. M. Nuss, *J. Med. Chem.*, 2018, **61**, 1382–1414. DOI: 10.1021/acs.jmedchem.7b00318.

11 M. Erak, K. Bellmann-sickert, S. Els-heindl and A. G. Beck-sickinger, *Bioorganic Med. Chem.*, 2018, **26**, 2759–2765. DOI: 10.1016/j.bmc.2018.01.012.

12 J. N. DeGruyter, L. R. Malins and P. S. Baran, *Biochemistry*, 2017, **56**, 3863–3873. DOI: 10.1021/acs.biochem.7b00536.

13 S. Deshayes, M. C. Morris, G. Divita and F. Heitz, *Cell. Mol. Life Sci.*, 2005, **62**, 1839–1849. DOI: 10.1007/s00018-005-5109-0.

14 E. Böhmová, D. Machová, M. Pechar, R. Pola, K. Venclíková, O. Janoušková and

T. Etrych, *Physiol. Res.*, 2018, **67**, S267–S279. DOI: 10.33549/physiolres.933975.

15 G. Tünnemann, G. Ter-Avetisyan, R. M. Martin, M. Stöckl, A. Herrmann and M. C. Cardoso, *J. Pept. Sci.*, 2008, **14**, 469–476. DOI: 10.1002/psc.968.

16 D. Kalafatovic and E. Giralt, *Molecules*, 2017, **22**, 1929. DOI: 10.3390/molecules22111929.

17 T. Tashima, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 121–130. DOI: 10.1016/j.bmcl.2016.11.083.

18 S. Reissmann, *J. Pept. Sci.*, 2014, **20**, 760–784. DOI: 10.1002/psc.2672.

19 W. B. Kauffman, T. Fuselier, J. He and W. C. Wimley, *Trends Biochem. Sci.*, 2015, **40**, 749–764. DOI: 10.1016/j.tibs.2015.10.004.

20 D. Raucher and J. S. Ryu, *Trends Mol. Med.*, 2015, **21**, 560–570. DOI: 10.1016/j.molmed.2015.06.005.

21 H. Margus, K. Padari and M. Pooga, *Mol. Ther.*, 2012, **20**, 525–533. DOI: 10.1038/mt.2011.284.

22 D. M. Copolovici, K. Langel, E. Eriste and Ü. Langel, *ACS Nano*, 2014, **8**, 1972–1994. DOI: 10.1021/nn4057269.

23 W. Arap, R. Pasqualini and E. Ruoslahti, *Science (80-.).*, 1998, **279**, 377–380. DOI: 10.1126/science.279.5349.377.

24 A. Hoppe, K. Christensen and J. A. Swanson, *Biophys. J.*, 2002, **83**, 3652–3664. DOI: 10.1016/S0006-3495(02)75365-4.

25 A. K. Kenworthy, *Methods*, 2001, **24**, 289–296. DOI: 10.1006/meth.2001.1189.

26 T. Ozawa and Y. Umezawa, *Supramol. Chem.*, 2002, **14**, 271–280. DOI: 10.1080/10610270290026185.

27 E. Pazos, O. Vázquez, J. L. Mascareñas and M. Eugenio Vázquez, *Chem. Soc. Rev.*, 2009, **38**, 3348. DOI: 10.1039/b908546g.

28 M. van Oosten, T. Schäfer, J. A. C. Gazendam, K. Ohlsen, E. Tsompanidou, M. C. de Goffau, H. J. M. Harmsen, L. M. A. Crane, E. Lim, K. P. Francis, L. Cheung, M. Olive, V. Ntziachristos, J. M. van Dijl and G. M. van Dam, *Nat. Commun.*, 2013, **4**, 2584. DOI: 10.1038/ncomms3584.

29 M. Bradley, A. M. Perez-Lopez, C. Haslett, A. R. Akram, N. McDonald, A.

Lilienkampf, S. V. Chankeshwara, E. Scholefield, N. Avlonitis and K. Dhaliwal, *Chem. Sci.*, 2015, **6**, 6971–6979. DOI: 10.1039/c5sc00960j.

30 L. Mendive-Tapia, C. Zhao, A. R. Akram, S. Preciado, F. Albericio, M. Lee, A. Serrels, N. Kielland, N. D. Read, R. Lavilla and M. Vendrell, *Nat. Commun.*, 2016, **7**, 10940. DOI: 10.1038/ncomms10940.

31 H. Shi, R. T. K. Kwok, J. Liu, B. Xing, B. Z. Tang and B. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 17972–17981. DOI: 10.1021/ja3064588.

32 Z. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996–2006. DOI: 10.1039/b916287a.

33 E. M. Nolan and S. J. Lippard, *Chem. Rev.*, 2008, **108**, 3443–3480. DOI: 10.1021/cr068000q.

34 G. K. Walkup and B. Imperiali, *J. Org. Chem.*, 1998, **63**, 6727–6731. DOI: 10.1021/jo980563h.

35 B. P. Joshi, W.-M. Cho, J. Kim, J. Yoon and K.-H. Lee, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6425–6429. DOI: 10.1016/j.bmcl.2007.10.008.

36 B. Zakeri and M. Howarth, *J. Am. Chem. Soc.*, 2010, **132**, 4526–4527. DOI: 10.1021/ja910795a.

37 K. C. Keiler, P. R. H. Waller and R. T. Sauer, *Science (80-.).*, 1996, **271**, 990–993. DOI: 10.1126/science.271.5251.990.

38 M. Werle and A. Bernkop-Schnürch, *Amino Acids*, 2006, **30**, 351–367. DOI: 10.1007/s00726-005-0289-3.

39 A. Angelini, J. Morales-Sanfrutos, P. Diderich, S. Chen and C. Heinis, *J. Med. Chem.*, 2012, **55**, 10187–10197. DOI: 10.1021/jm301276e.

40 L. H. Brinckerhoff, V. V. Kalashnikov, L. W. Thompson, G. V. Yamshchikov, R. A. Pierce, H. S. Galavotti, V. H. Engelhard and C. L. Slingluff, *Int. J. Cancer*, 1999, **83**, 326–334. DOI: 10.1002/(SICI)1097-0215(19991029)83:3<326::AID-IJC7>3.0.CO;2-X.

41 D. Schumacher and C. P. R. Hackenberger, *Curr. Opin. Chem. Biol.*, 2014, **22**, 62–69. DOI: 10.1016/j.cbpa.2014.09.018.

42 D. Knappe, P. Henklein, R. Hoffmann and K. Hilpert, *Antimicrob. Agents*

Chemother., 2010, **54**, 4003–4005. DOI: 10.1128/AAC.00300-10.

43 F. Vernen, P. J. Harvey, S. A. Dias, A. S. Veiga, Y.-H. Huang, D. J. Craik, N. Lawrence and S. Troeira Henriques, *Int. J. Mol. Sci.*, 2019, **20**, 4184. DOI: 10.3390/ijms20174184.

44 C. J. Armishaw, J. L. Dutton, D. J. Craik and P. F. Alewood, *Biopolymers*, 2010, **94**, 307–313. DOI: 10.1002/bip.21360.

45 Y. H. Lau, P. De Andrade, Y. Wu and D. R. Spring, *Chem. Soc. Rev.*, 2015, **44**, 91–102. DOI: 10.1039/c4cs00246f.

46 R. De Marco, A. Tolomelli, E. Juaristi and L. Gentilucci, *Med. Res. Rev.*, 2016, **36**, 389–424. DOI: 10.1002/med.21383.

47 J. A. Lovshin and D. J. Drucker, *Nat. Rev. Endocrinol.*, 2009, **5**, 262–269. DOI: 10.1038/nrendo.2009.48.

48 J. W. Checco and S. H. Gellman, *Curr. Opin. Struct. Biol.*, 2016, **39**, 96–105. DOI: 10.1016/j.sbi.2016.06.014.

49 L. M. Johnson and S. H. Gellman, in *Methods in Enzymology*, Elsevier Inc., 1st edn., 2013, vol. 523, pp. 407–429. DOI: 10.1016/B978-0-12-394292-0.00019-9.

50 A. Turalić, J. Đeđibegović, Z. Hegedus and T. Martinek, *ChemBioChem*, 2020, cbic.202000050. DOI: 10.1002/cbic.202000050.

51 S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr and W. H. Moos, *Drug Dev. Res.*, 1995, **35**, 20–32. DOI: 10.1002/ddr.430350105.

52 R. Milton, S. Milton and S. Kent, *Science (80-.).*, 1992, **256**, 1445–1448. DOI: 10.1126/science.1604320.

53 L. Gentilucci, R. De Marco and L. Cerisoli, *Curr. Pharm. Des.*, 2010, **16**, 3185–3203. DOI: 10.2174/138161210793292555.

54 T. A. Knappe, F. Manzenrieder, C. Mas-Moruno, U. Linne, F. Sasse, H. Kessler, X. Xie and M. A. Marahiel, *Angew. Chemie Int. Ed.*, 2011, **50**, 8714–8717. DOI: 10.1002/anie.201102190.

55 S. Chen, D. Gfeller, S. A. Buth, O. Michielin, P. G. Leiman and C. Heinis, *ChemBioChem*, 2013, **14**, 1316–1322. DOI: 10.1002/cbic.201300228.

56 T. Kortemme and D. Baker, *Proc. Natl. Acad. Sci.*, 2002, **99**, 14116–14121. DOI:

10.1073/pnas.202485799.

57 D. Gfeller, O. Michielin and V. Zoete, *J. Comput. Chem.*, 2012, **33**, 1525–1535. DOI: 10.1002/jcc.22982.

58 V. Zoete, M. B. Irving and O. Michielin, *J. Mol. Recognit.*, 2010, **23**, 142–152. DOI: 10.1002/jmr.1005.

59 K. K. Jensen, M. Andreatta, P. Marcatili, S. Buus, J. A. Greenbaum, Z. Yan, A. Sette, B. Peters and M. Nielsen, *Immunology*, 2018, **154**, 394–406. DOI: 10.1111/imm.12889.

60 S. S. Usmani, G. Bedi, J. S. Samuel, S. Singh, S. Kalra, P. Kumar, A. A. Ahuja, M. Sharma, A. Gautam and G. P. S. Raghava, *PLoS One*, 2017, **12**, e0181748. DOI: 10.1371/journal.pone.0181748.

61 H. Zhang and S. Chen, *RSC Chem. Biol.*, 2022, **3**, 18–31. DOI: 10.1039/d1cb00154j.

62 J. Iegre, J. S. Gaynord, N. S. Robertson, H. F. Sore, M. Hyvönen and D. R. Spring, *Adv. Ther.*, 2018, **1**, 1800052. DOI: 10.1002/adtp.201800052.

63 C. J. White and A. K. Yudin, *Nat. Chem.*, 2011, **3**, 509–524. DOI: 10.1038/nchem.1062.

64 T. A. Hill, N. E. Shepherd, F. Diness and D. P. Fairlie, *Angew. Chemie - Int. Ed.*, 2014, **53**, 13020–13041. DOI: 10.1002/anie.201401058.

65 D. G. Rivera, G. M. Ojeda-Carralero, L. Reguera and E. V. Van der Eycken, *Chem. Soc. Rev.*, 2020, **49**, 2039–2059. DOI: 10.1039/C9CS00366E.

66 A. Janecka, M. Zubrzycka and T. Janecki, *J. Pept. Res.*, 2001, **58**, 91–107. DOI: 10.1034/j.1399-3011.2001.00873.x.

67 S. J. Miller and R. H. Grubbs, *J. Am. Chem. Soc.*, 1995, **117**, 5855–5856. DOI: 10.1021/ja00126a027.

68 S. J. Miller, H. E. Blackwell and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 9606–9614. DOI: 10.1021/ja961626l.

69 J. F. Reichwein and R. M. J. Liskamp, *Tetrahedron Lett.*, 1998, **39**, 1243–1246. DOI: 10.1016/S0040-4039(97)10766-3.

70 F. A. Robey, *J. Pept. Res.*, 2000, **56**, 115–120. DOI: 10.1034/j.1399-

3011.2000.00734.x.

71 A. Frey, N. Mantis, P. A. Kozlowski, A. J. Quayle, A. Bajardi, J. J. Perdomo, F. A. Robey and M. R. Neutra, *Vaccine*, 1999, **17**, 3007–3019. DOI: 10.1016/S0264-410X(99)00163-2.

72 F. A. Robey and R. L. Fields, *Anal. Biochem.*, 1989, **177**, 373–377. DOI: 10.1016/0003-2697(89)90068-7.

73 P. Li and P. Roller, *Curr. Top. Med. Chem.*, 2005, **2**, 325–341. DOI: 10.2174/1568026023394209.

74 H. C. Kolb and K. B. Sharpless, *Drug Discov. Today*, 2003, **8**, 1128–1137. DOI: 10.1016/S1359-6446(03)02933-7.

75 R. Huisgen, *Angew. Chemie Int. Ed. English*, 1963, **2**, 565–598. DOI: 10.1002/anie.196305651.

76 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chemie Int. Ed.*, 2002, **41**, 2596–2599. DOI: 10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.

77 Y. Lau and D. Spring, *Synlett*, 2011, **2011**, 1917–1919. DOI: 10.1055/s-0030-1260950.

78 H. Johansson and D. S. Pedersen, *European J. Org. Chem.*, 2012, **2012**, 4267–4281. DOI: 10.1002/ejoc.201200496.

79 J. A. Marchand, M. E. Neugebauer, M. C. Ing, C. I. Lin, J. G. Pelton and M. C. Y. Chang, *Nature*, 2019, **567**, 420–424. DOI: 10.1038/s41586-019-1020-y.

80 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064. DOI: 10.1021/jo011148j.

81 A. Burger, in *Progress in Drug Research*, Birkhäuser Basel, Basel, 1991, vol. 37, p. 298. DOI: 10.1007/978-3-0348-7139-6_7.

82 J. Bachl, J. Mayr, F. J. Sayago, C. Cativiela and D. Díaz Díaz, *Chem. Commun.*, 2015, **51**, 5294–5297. DOI: 10.1039/c4cc08593k.

83 A. Ahmad Fuaad, F. Azmi, M. Skwarczynski and I. Toth, *Molecules*, 2013, **18**, 13148–13174. DOI: 10.3390/molecules181113148.

84 W. S. Horne, M. K. Yadav, C. D. Stout and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2004,

126, 15366–15367. DOI: 10.1021/ja0450408.

85 B. M. Trost, *Acc. Chem. Res.*, 2002, **35**, 695–705. DOI: 10.1021/ar010068z.

86 F. Bellina and R. Rossi, *Chem. Rev.*, 2010, **110**, 1082–1146. DOI: 10.1021/cr9000836.

87 A. F. M. Noisier and M. A. Brimble, *Chem. Rev.*, 2014, **114**, 8775–8806. DOI: 10.1021/cr500200x.

88 L. D. Tran and O. Daugulis, *Angew. Chemie - Int. Ed.*, 2012, **51**, 5188–5191. DOI: 10.1002/anie.201200731.

89 K. Chen, F. Hu, S. Q. Zhang and B. F. Shi, *Chem. Sci.*, 2013, **4**, 3906–3911. DOI: 10.1039/c3sc51747k.

90 S. Y. Zhang, Q. Li, G. He, W. A. Nack and G. Chen, *J. Am. Chem. Soc.*, 2013, **135**, 12135–12141. DOI: 10.1021/ja406484v.

91 G. He and G. Chen, *Angew. Chemie Int. Ed.*, 2011, **50**, 5192–5196. DOI: 10.1002/anie.201100984.

92 V. G. Zaitsev, D. Shabashov and O. Daugulis, *J. Am. Chem. Soc.*, 2005, **127**, 13154–13155. DOI: 10.1021/ja054549f.

93 Q. Zhang, K. Chen, W. Rao, Y. Zhang, F. J. Chen and B. F. Shi, *Angew. Chemie - Int. Ed.*, 2013, **52**, 13588–13592. DOI: 10.1002/anie.201306625.

94 N. Rodríguez, J. A. Romero-Revilla, M. Á. Fernández-Ibáñez and J. C. Carretero, *Chem. Sci.*, 2013, **4**, 175–179. DOI: 10.1039/c2sc21162a.

95 M. Fan and D. Ma, *Angew. Chemie - Int. Ed.*, 2013, **52**, 12152–12155. DOI: 10.1002/anie.201306583.

96 L. C. H. Arylation, J. He, S. Li, Y. Deng, H. Fu, B. N. Laforteza, J. E. Spangler, A. Homs and J. Yu, *Science (80-.).*, 2014, **343**, 1216–1221. DOI: 10.1126/science.1249198.

97 B. V. S. Reddy, L. R. Reddy and E. J. Corey, *Org. Lett.*, 2006, **8**, 3391–3394. DOI: 10.1021/ol061389j.

98 K. M. Engle, D. H. Wang and J. Q. Yu, *J. Am. Chem. Soc.*, 2010, **132**, 14137–14151. DOI: 10.1021/ja105044s.

99 D.-H. Wang, K. M. Engle, B.-F. Shi and J.-Q. Yu, *Science (80-.).*, 2010, **327**, 315–

319. DOI: 10.1126/science.1182512.

100 P. S. Thuy-Boun, G. Villa, D. Dang, P. Richardson, S. Su and J. Q. Yu, *J. Am. Chem. Soc.*, 2013, **135**, 17508–17513. DOI: 10.1021/ja409014v.

101 L. Wan, N. Dastbaravardeh, G. Li and J. Q. Yu, *J. Am. Chem. Soc.*, 2013, **135**, 18056–18059. DOI: 10.1021/ja410760f.

102 W. Gong, G. Zhang, T. Liu, R. Giri and J. Q. Yu, *J. Am. Chem. Soc.*, 2014, **136**, 16940–16946. DOI: 10.1021/ja510233h.

103 T. Liu, J. X. Qiao, M. A. Poss and J.-Q. Yu, *Angew. Chemie Int. Ed.*, 2017, **56**, 10924–10927. DOI: 10.1002/anie.201706367.

104 A. F. M. Noisier, J. García, I. A. Ionuţ and F. Albericio, *Angew. Chemie - Int. Ed.*, 2017, **56**, 314–318. DOI: 10.1002/anie.201608648.

105 J. Tang, Y. He, H. Chen, W. Sheng and H. Wang, *Chem. Sci.*, 2017, **8**, 4565–4570. DOI: 10.1039/C6SC05530C.

106 J. Ruiz-Rodríguez, F. Albericio and R. Lavilla, *Chem. - A Eur. J.*, 2010, **16**, 1124–1127. DOI: 10.1002/chem.200902676.

107 H. Dong, C. Limberakis, S. Liras, D. Price and K. James, *Chem. Commun.*, 2012, **48**, 11644. DOI: 10.1039/c2cc36962a.

108 L. Mendive-Tapia, S. Preciado, J. García, R. Ramón, N. Kielland, F. Albericio and R. Lavilla, *Nat. Commun.*, 2015, **6**, 7160. DOI: 10.1038/ncomms8160.

109 L. Mendive-Tapia, A. Bertran, J. García, G. Acosta, F. Albericio and R. Lavilla, *Chem. - A Eur. J.*, 2016, **22**, 13114–13119. DOI: 10.1002/chem.201601832.

110 W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi and L. Ackermann, *Angew. Chemie - Int. Ed.*, 2018, **57**, 14700–14717. DOI: 10.1002/anie.201806250.

111 A. Schischko, N. Kaplaneris, T. Rogge, G. Sirvinskaite, J. Son and L. Ackermann, *Nat. Commun.*, 2019, **10**, 1–9. DOI: 10.1038/s41467-019-11395-3.

112 I. Guerrero and A. Correa, *Org. Lett.*, 2020, **22**, 1754–1759. DOI: 10.1021/acs.orglett.0c00033.

113 Z. Ruan, N. Sauermann, E. Manoni and L. Ackermann, *Angew. Chemie - Int. Ed.*, 2017, **56**, 3172–3176. DOI: 10.1002/anie.201611118.

114 M. J. Terrey, A. Holmes, C. C. Perry and W. B. Cross, *Org. Lett.*, 2019, **21**, 7902–

7907. DOI: 10.1021/acs.orglett.9b02894.

115 J. Peng, C. Li, M. Khamrakulov, J. Wang and H. Liu, *Org. Lett.*, 2020, **22**, 1535–1541. DOI: 10.1021/acs.orglett.0c00086.

116 Z. Bai, C. Cai, W. Sheng, Y. Ren and H. Wang, *Angew. Chemie Int. Ed.*, 2020, anie.202007226. DOI: 10.1002/anie.202007226.

117 A. Mahindra, N. Bagra and R. Jain, *J. Org. Chem.*, 2013, **78**, 10954–10959. DOI: 10.1021/jo401934q.

118 A. Mahindra and R. Jain, *Org. Biomol. Chem.*, 2014, **12**, 3792–3796. DOI: 10.1039/c4ob00430b.

119 A. F. M. Noisier, M. J. Johansson, L. Knerr, M. A. Hayes, W. J. Drury, E. Valeur, L. R. Malins and R. Gopalakrishnan, *Angew. Chemie - Int. Ed.*, 2019, **58**, 19096–19102. DOI: 10.1002/anie.201910888.

120 J. J. Li, T. S. Mei and J. Q. Yu, *Angew. Chemie - Int. Ed.*, 2008, **47**, 6452–6455. DOI: 10.1002/anie.200802187.

121 Y. Dou, Kenry, J. Liu, J. Jiang and Q. Zhu, *Chem. - A Eur. J.*, 2019, **25**, 6896–6901. DOI: 10.1002/chem.201900530.

122 Q. Hu, K. Hou, J. Li, Y. Ge, Z. Song, A. S. C. Chan and X. Xiong, *Chem. Sci.*, , DOI:10.1039/D0SC02439B. DOI: 10.1039/D0SC02439B.

123 D. S. Peters, F. E. Romesberg and P. S. Baran, *J. Am. Chem. Soc.*, 2018, **140**, 2072–2075. DOI: 10.1021/jacs.8b00087.

124 M. Ben-Lulu, E. Gaster, A. Libman and D. Pappo, *Angew. Chemie Int. Ed.*, 2020, 1–6. DOI: 10.1002/anie.201913305.

125 Z. Bai, C. Cai, Z. Yu and H. Wang, *Angew. Chemie - Int. Ed.*, 2018, **57**, 13912–13916. DOI: 10.1002/anie.201807953.

126 M. J. Terrey, C. C. Perry and W. B. Cross, *Org. Lett.*, 2019, **21**, 104–108. DOI: 10.1021/acs.orglett.8b03536.

127 Y. Zheng and W. Song, *Org. Lett.*, 2019, **21**, 3257–3260. DOI: 10.1021/acs.orglett.9b00987.

128 M. San Segundo and A. Correa, *Chem. Sci.*, 2019, **10**, 8872–8879. DOI: 10.1039/c9sc03425k.

129 J. Tang, H. Chen, Y. He, W. Sheng, Q. Bai and H. Wang, *Nat. Commun.*, 2018, **9**, 1–8. DOI: 10.1038/s41467-018-05440-w.

130 J. Tan, J. Wu, S. Liu, H. Yao and H. Wang, *Sci. Adv.*, 2019, **5**, 1–9. DOI: 10.1126/sciadv.aaw0323.

131 A. S. Barrow, C. J. Smedley, Q. Zheng, S. Li, J. Dong and J. E. Moses, *Chem. Soc. Rev.*, 2019, **48**, 4731–4758. DOI: 10.1039/C8CS00960K.

132 R. Breinbauer and M. Köhn, *ChemBioChem*, 2003, **4**, 1147–1149. DOI: 10.1002/cbic.200300705.

133 W. H. Binder and R. Sachsenhofer, *Macromol. Rapid Commun.*, 2008, **29**, 952–981. DOI: 10.1002/marc.200800089.

134 J. Dong, L. Krasnova, M. G. Finn and K. B. Sharpless, *Angew. Chemie Int. Ed.*, 2014, **53**, 9430–9448. DOI: 10.1002/anie.201309399.

135 B. Bennua-Skalmowski and H. Vorbrüggen, *Tetrahedron Lett.*, 1995, **36**, 2611–2614. DOI: 10.1016/0040-4039(95)00355-G.

136 H. Vorbrüggen, *Synthesis (Stuttg).*, 2008, **2008**, 1165–1174. DOI: 10.1055/s-2008-1067006.

137 V. Gembus, F. Marsais and V. Levacher, *Synlett*, 2008, **2008**, 1463–1466. DOI: 10.1055/s-2008-1078407.

138 J. N. Luy and R. Tonner, *ACS Omega*, 2020, **5**, 31432–31439. DOI: 10.1021/acsomega.0c05049.

139 A. Dondoni and A. Marra, *Org. Biomol. Chem.*, 2017, **15**, 1549–1553. DOI: 10.1039/c6ob02458k.

140 J. Dong, K. B. Sharpless, L. Kwisnek, J. S. Oakdale and V. V. Fokin, *Angew. Chemie*, 2014, **126**, 9620–9624. DOI: 10.1002/ange.201403758.

141 P. Mukherjee, C. P. Woroch, L. Cleary, M. Rusznak, R. W. Franzese, M. R. Reese, J. W. Tucker, J. M. Humphrey, S. M. Etuk, S. C. Kwan, C. W. Am Ende and N. D. Ball, *Org. Lett.*, 2018, **20**, 3943–3947. DOI: 10.1021/acs.orglett.8b01520.

142 M. P. S. Andersen, D. R. Blake, F. S. Rowland, M. D. Hurley and T. J. Wallington, *Environ. Sci. Technol.*, 2009, **43**, 1067–1070. DOI: 10.1021/es802439f.

143 T. Guo, G. Meng, X. Zhan, Q. Yang, T. Ma, L. Xu, K. B. Sharpless and J. Dong,

Angew. Chemie - Int. Ed., 2018, **57**, 2605–2610. DOI: 10.1002/anie.201712429.

144 H. Zhou, P. Mukherjee, R. Liu, E. Evrard, D. Wang, J. M. Humphrey, T. W. Butler, L. R. Hoth, J. B. Sperry, S. K. Sakata, C. J. Helal and C. W. Am Ende, *Org. Lett.*, 2018, **20**, 812–815. DOI: 10.1021/acs.orglett.7b03950.

145 R. Cramer and D. D. Coffman, *J. Org. Chem.*, 1961, **26**, 4010–4014. DOI: 10.1021/jo01068a088.

146 K. Seppelt and W. Sundermeyer, *Angew. Chemie Int. Ed. English*, 1970, **9**, 905–905. DOI: 10.1002/anie.197009051.

147 W. Sundermeyer, A. Roland and K. Seppelt, *Angew. Chemie Int. Ed. English*, 1971, **10**, 419–420. DOI: 10.1002/anie.197104191.

148 S. Li, P. Wu, J. E. Moses and K. B. Sharpless, *Angew. Chemie Int. Ed.*, 2017, **56**, 2903–2908. DOI: 10.1002/anie.201611048.

149 B. Gao, S. Li, P. Wu, J. E. Moses and K. B. Sharpless, *Angew. Chemie - Int. Ed.*, 2018, **57**, 1939–1943. DOI: 10.1002/anie.201712145.

150 Q. Chen, P. Mayer and H. Mayr, *Angew. Chemie Int. Ed.*, 2016, **55**, 12664–12667. DOI: 10.1002/anie.201601875.

151 B. Aguilar, F. Amissah, R. Duverna and N. S. Lamango, *Curr. Cancer Drug Targets*, 2011, **11**, 752–762. DOI: 10.2174/156800911796191015.

152 P. Jimonet, F. Audiau, M. Barreau, J. C. Blanchard, A. Boireau, Y. Bour, M. A. Coléno, A. Doble, G. Doerflinger, C. Do Huu, M. H. Donat, J. M. Duchesne, P. Ganil, C. Guérémy, E. Honoré, B. Just, R. Kerphirique, S. Gontier, P. Hubert, P. M. Laduron, J. Le Blevec, M. Meunier, J. M. Miquet, C. Nemecek, M. Pasquet, O. Piot, J. Pratt, J. Rataud, M. Reibaud, J. M. Stutzmann and S. Mignani, *J. Med. Chem.*, 1999, **42**, 2828–2843. DOI: 10.1021/jm980202u.

153 A. Kreimeyer, B. Laube, M. Sturgess, M. Goeldner and B. Foucaud, *J. Med. Chem.*, 1999, **42**, 4394–4404. DOI: 10.1021/jm9910730.

154 H.-L. Qin, Q. Zheng, G. A. L. Bare, P. Wu and K. B. Sharpless, *Angew. Chemie Int. Ed.*, 2016, **55**, 14155–14158. DOI: 10.1002/anie.201608807.

155 P. K. Chinthakindi, K. B. Govender, A. S. Kumar, H. G. Kruger, T. Govender, T. Naicker and P. I. Arvidsson, *Org. Lett.*, 2017, **19**, 480–483. DOI:

10.1021/acs.orglett.6b03634.

156 G. F. Zha, G. A. L. Bare, J. Leng, Z. P. Shang, Z. Luo and H. L. Qin, *Adv. Synth. Catal.*, 2017, **359**, 3237–3242. DOI: 10.1002/adsc.201700688.

157 G. F. Zha, Q. Zheng, J. Leng, P. Wu, H. L. Qin and K. B. Sharpless, *Angew. Chemie - Int. Ed.*, 2017, **56**, 4849–4852. DOI: 10.1002/anie.201701162.

158 S. M. Wang, C. Li, J. Leng, S. N. A. Bukhari and H. L. Qin, *Org. Chem. Front.*, 2018, **5**, 1411–1415. DOI: 10.1039/c7qo01128h.

159 S. M. Wang, B. Moku, J. Leng and H. L. Qin, *European J. Org. Chem.*, 2018, **2018**, 4407–4410. DOI: 10.1002/ejoc.201800762.

160 C. Li, S. M. Wang and H. L. Qin, *Org. Lett.*, 2018, **20**, 4699–4703. DOI: 10.1021/acs.orglett.8b02037.

161 X.-Y. Chen, Y. Wu, J. Zhou, P. Wang and J.-Q. Yu, *Org. Lett.*, 2019, **21**, 1426–1429. DOI: 10.1021/acs.orglett.9b00165.

162 B. B. Zhan, M. X. Jiang and B. F. Shi, *Chem. Commun.*, 2020, **56**, 13950–13958. DOI: 10.1039/d0cc06133f.

163 S. Sengupta and G. Mehta, *Org. Biomol. Chem.*, 2020, **18**, 1851–1876. DOI: 10.1039/c9ob02765c.

164 P. K. Chinthakindi and P. I. Arvidsson, *European J. Org. Chem.*, 2018, **2018**, 3648–3666. DOI: 10.1002/ejoc.201800464.

165 M. J. Terrey, PhD thesis, Nottingham Trent University, 2019.

166 T. Willemse, K. Van Imp, R. J. M. Goss, H. W. T. Van Vlijmen, W. Schepens, B. U. W. Maes and S. Ballet, *ChemCatChem*, 2015, **7**, 2055–2070. DOI: 10.1002/cctc.201500190.

167 J. D. Rudolf and C. D. Poulter, *ACS Chem. Biol.*, 2013, **8**, 2707–2714. DOI: 10.1021/cb400691z.

168 R. Bollhagen, M. Schmiedberger, K. Barlos and E. Grell, *J. Chem. Soc. Chem. Commun.*, 1994, 2559. DOI: 10.1039/c39940002559.

169 M. Lin, Ji. Luo, Y. Xie, G. Du, Z. Cai, B. Dai and L. He, 2021, 1–21.

170 M. F. Khumalo, E. D. Akpan, P. K. Chinthakindi, E. M. Brasil, K. K. Rajbongshi, M. M. Makatini, T. Govender, H. G. Kruger, T. Naicker and P. I. Arvidsson, *RSC Adv.*,

2018, **8**, 37503–37507. DOI: 10.1039/C8RA07627H.

171 J. D. Randall, D. J. Eyckens, F. Stojcevski, P. S. Francis, E. H. Doeven, A. J. Barlow, A. S. Barrow, C. L. Arnold, J. E. Moses and L. C. Henderson, *ChemPhysChem*, 2018, **19**, 3176–3181. DOI: 10.1002/cphc.201800789.

172 J. Liu, S. Krajangsri, T. Singh, G. De Seriis, N. Chumnanvej, H. Wu and P. G. Andersson, *J. Am. Chem. Soc.*, 2017, **139**, 14470–14475. DOI: 10.1021/jacs.7b06829.

173 J. C. Brendel, L. Martin, J. Zhang and S. Perrier, *Polym. Chem.*, 2017, **8**, 7475–7485. DOI: 10.1039/c7py01636k.

Appendix

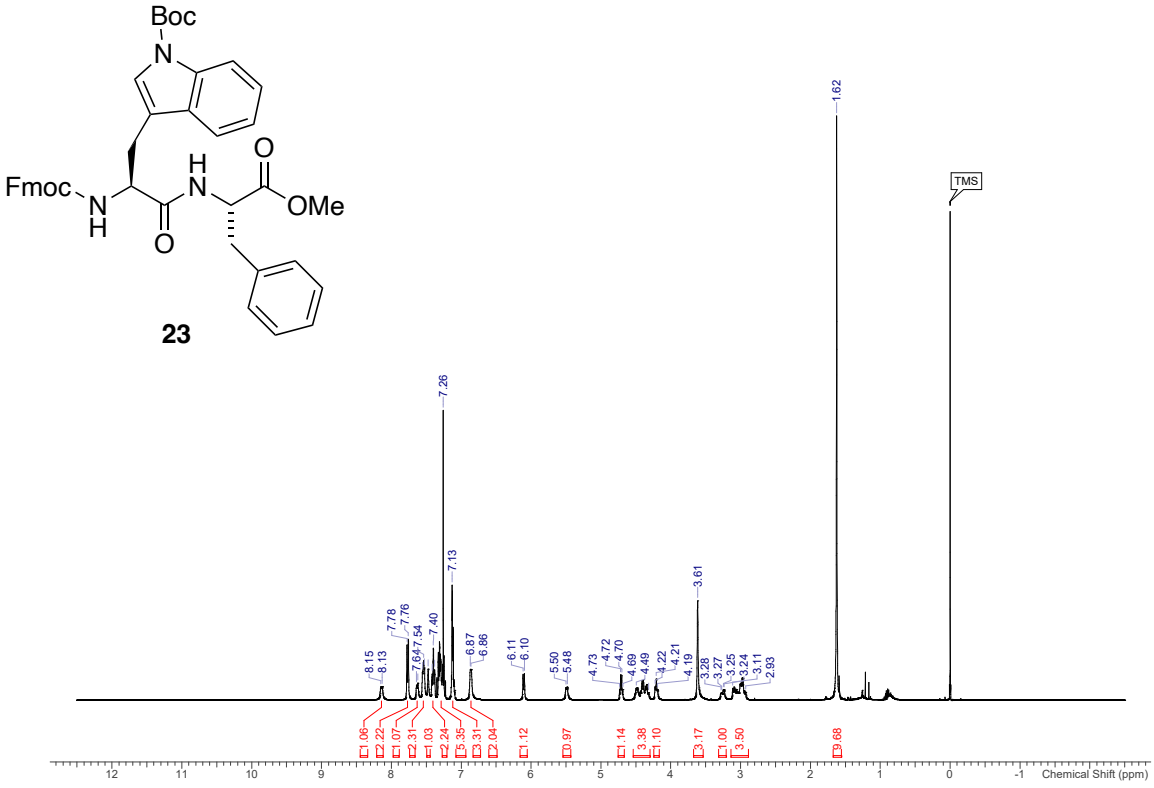
NMR data for compounds present in

experimental sections

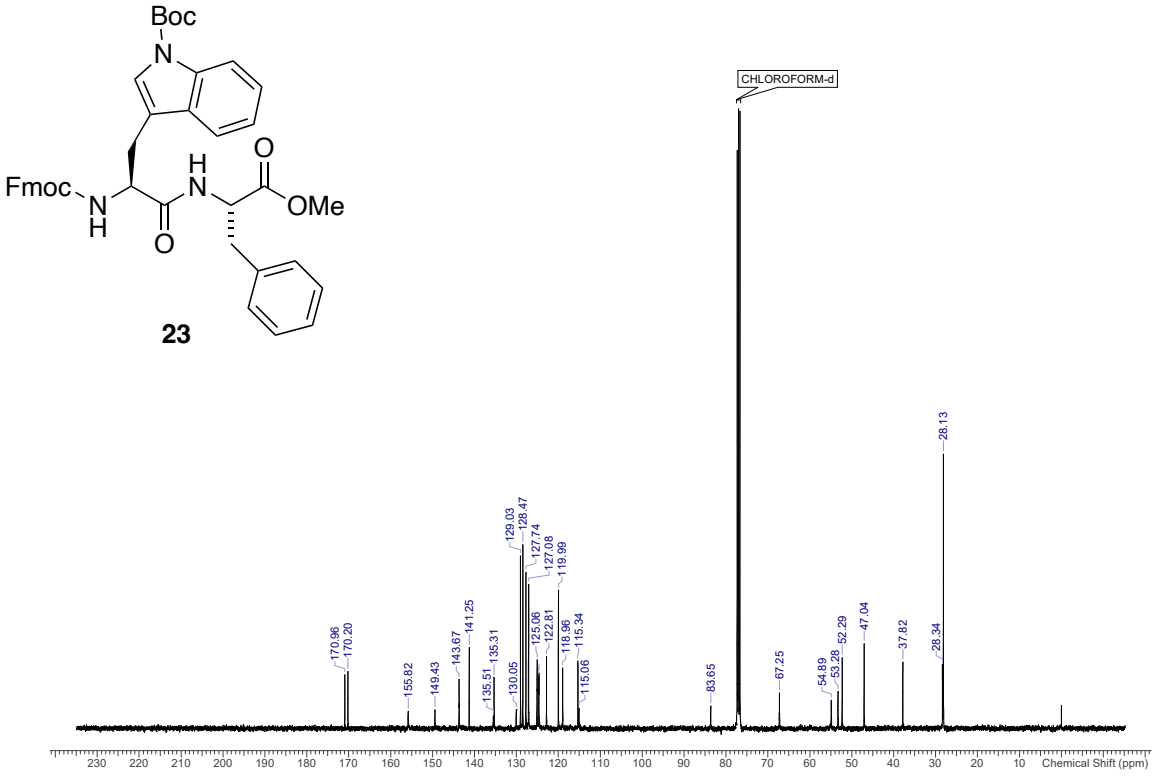
&

important HRMS data

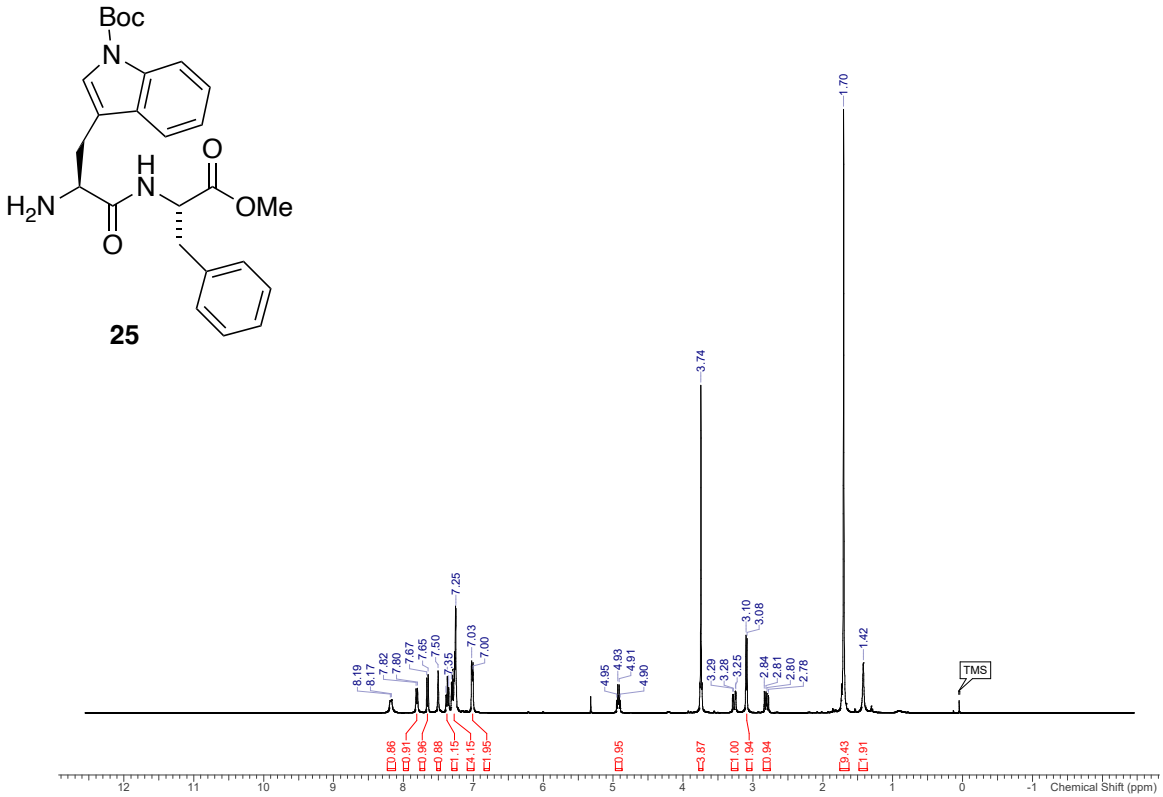
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **23**



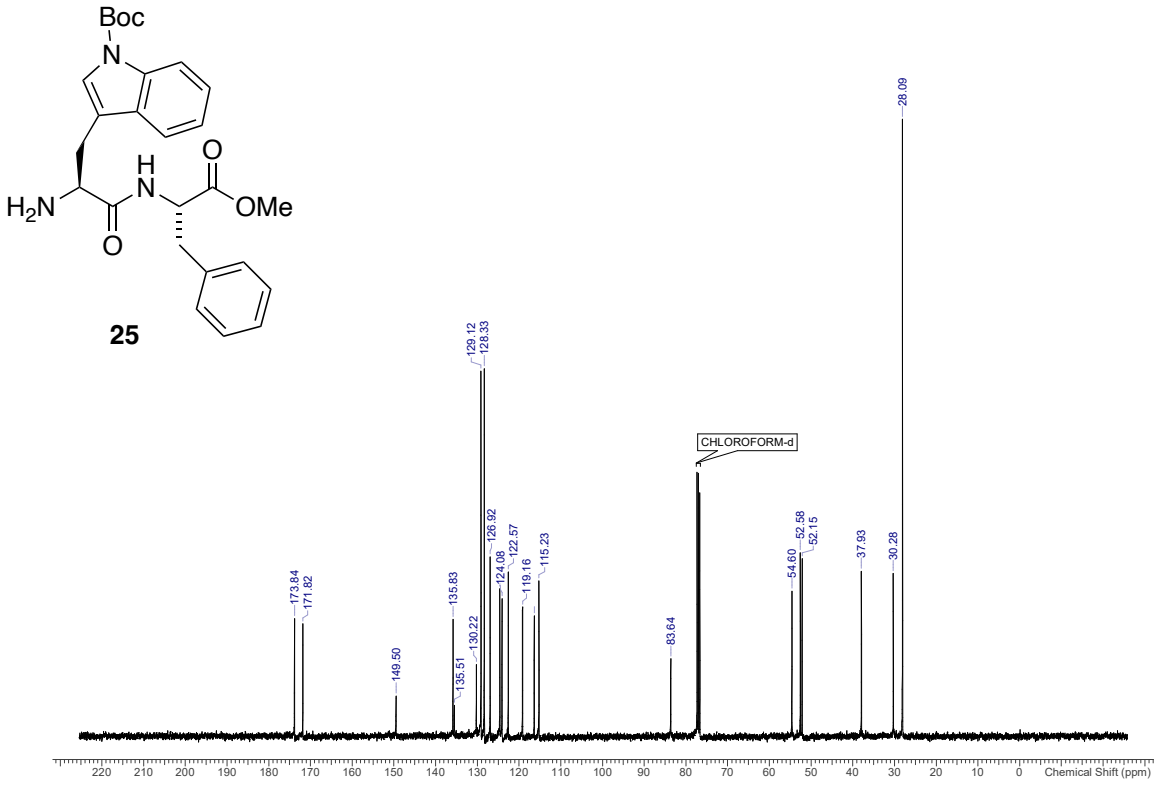
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **23**



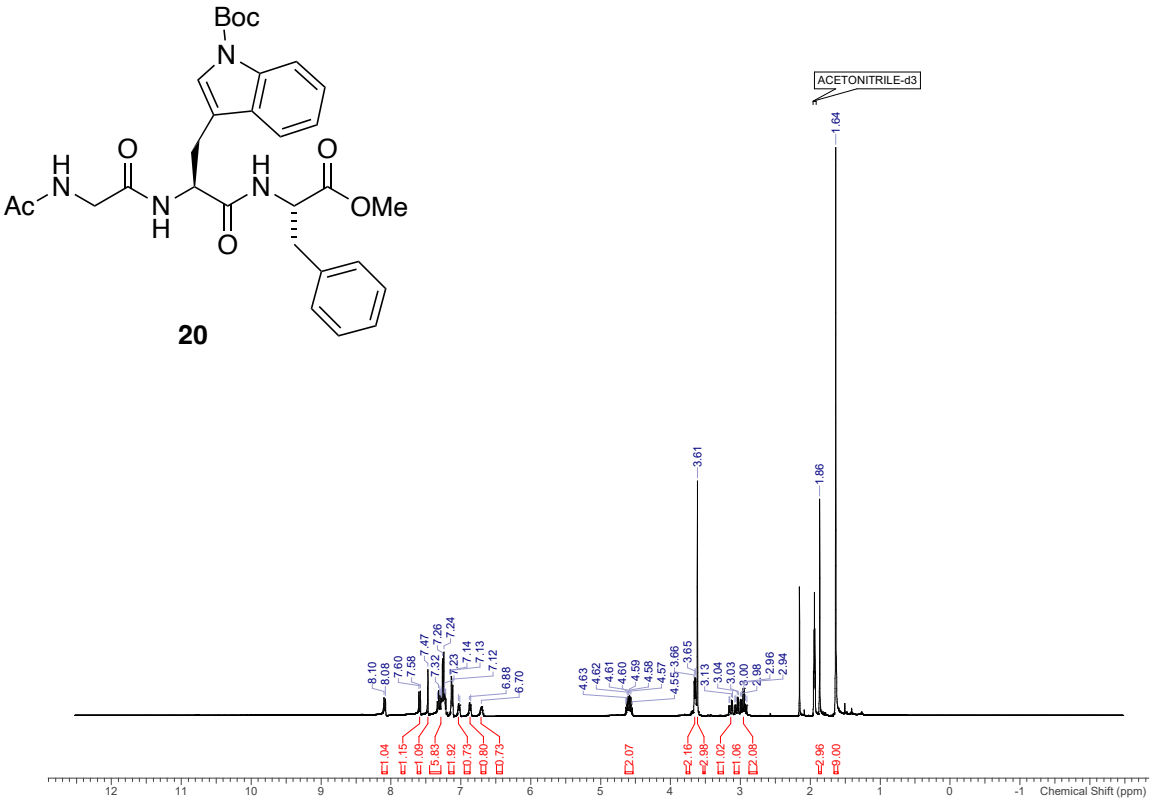
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **25**



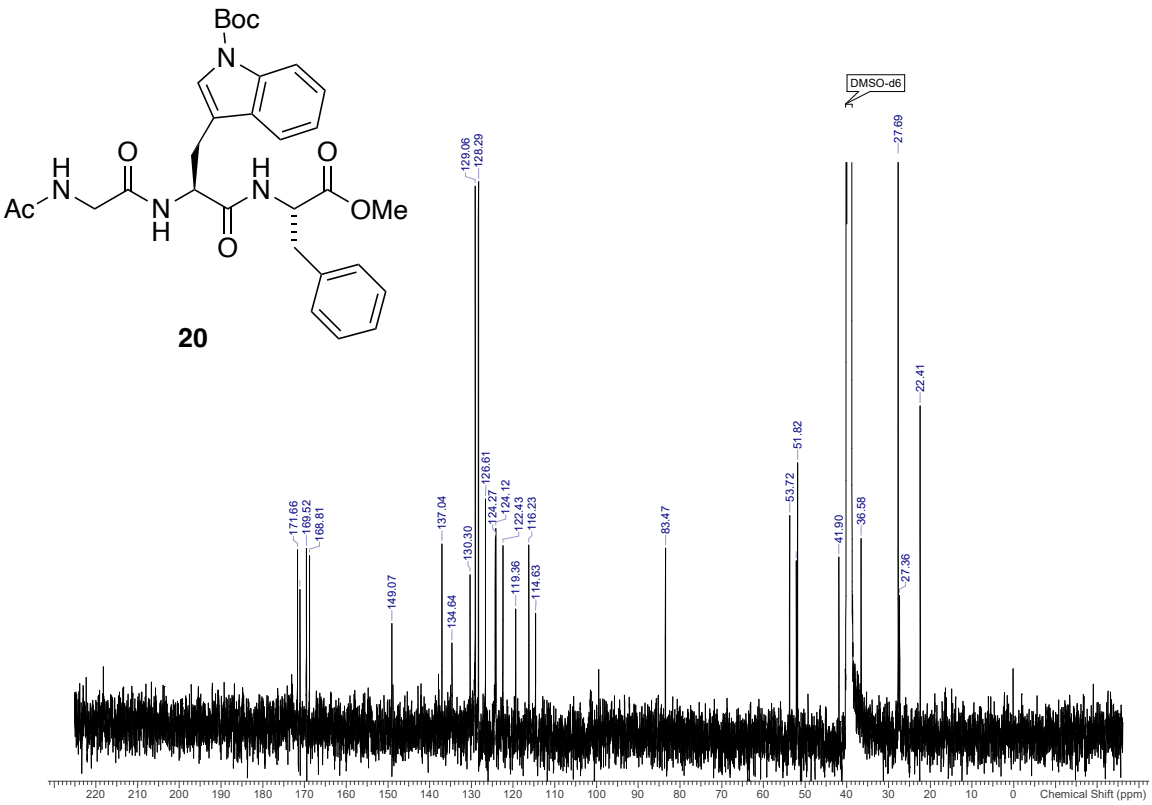
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **25**



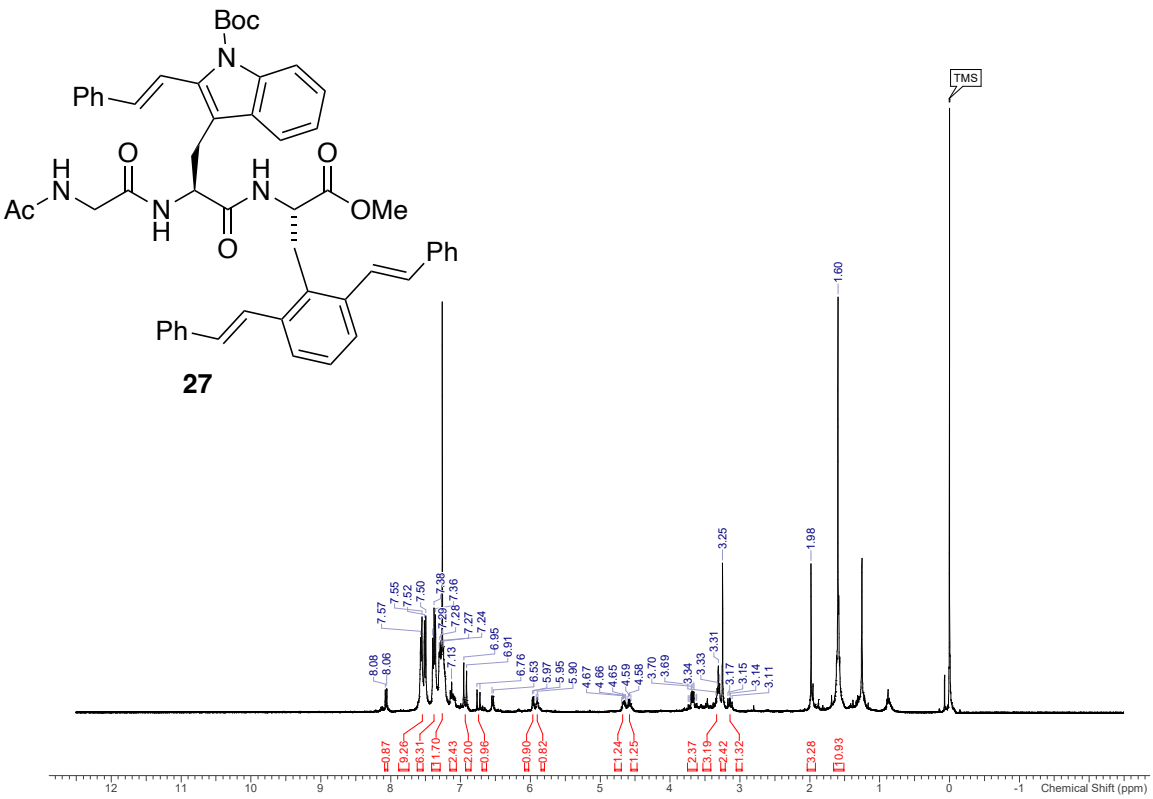
^{1}H NMR spectrum (400 MHz, CD_3CN) of **20**



^{13}C{^{1}H} NMR spectrum (100 MHz, D_6-DMSO) of **20**

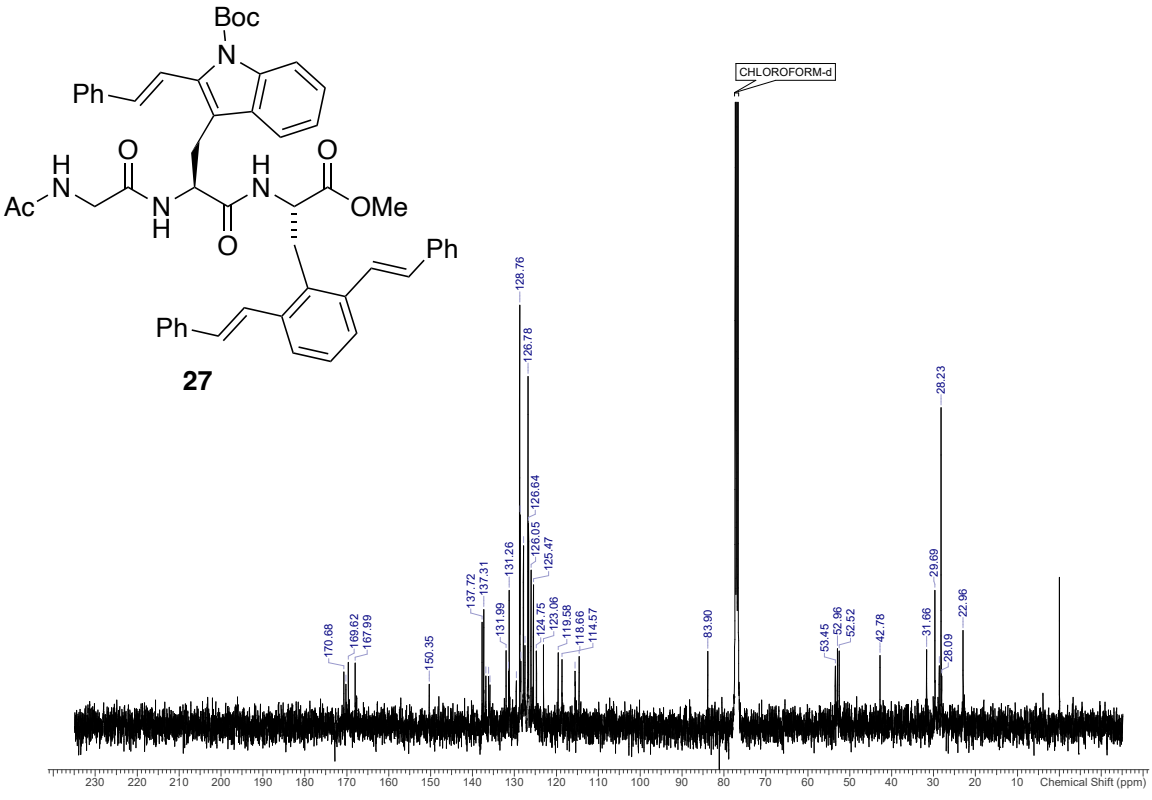


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **27**



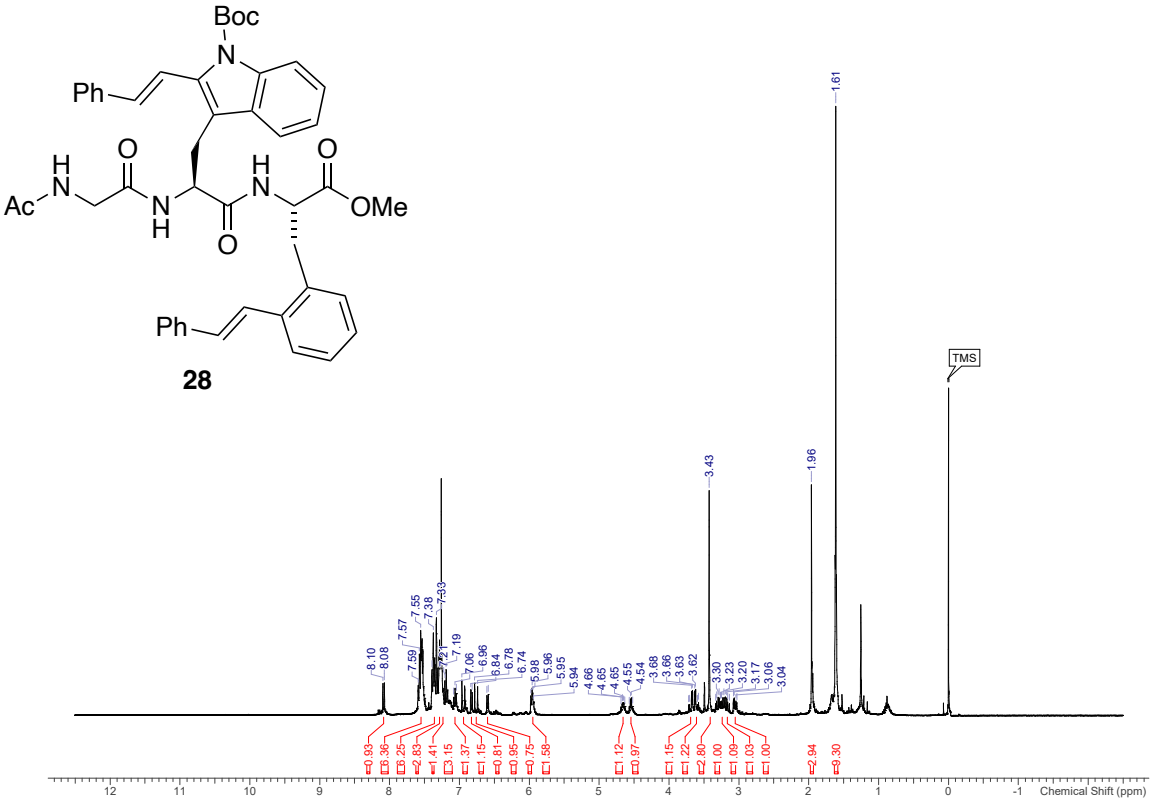
27

^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **27**

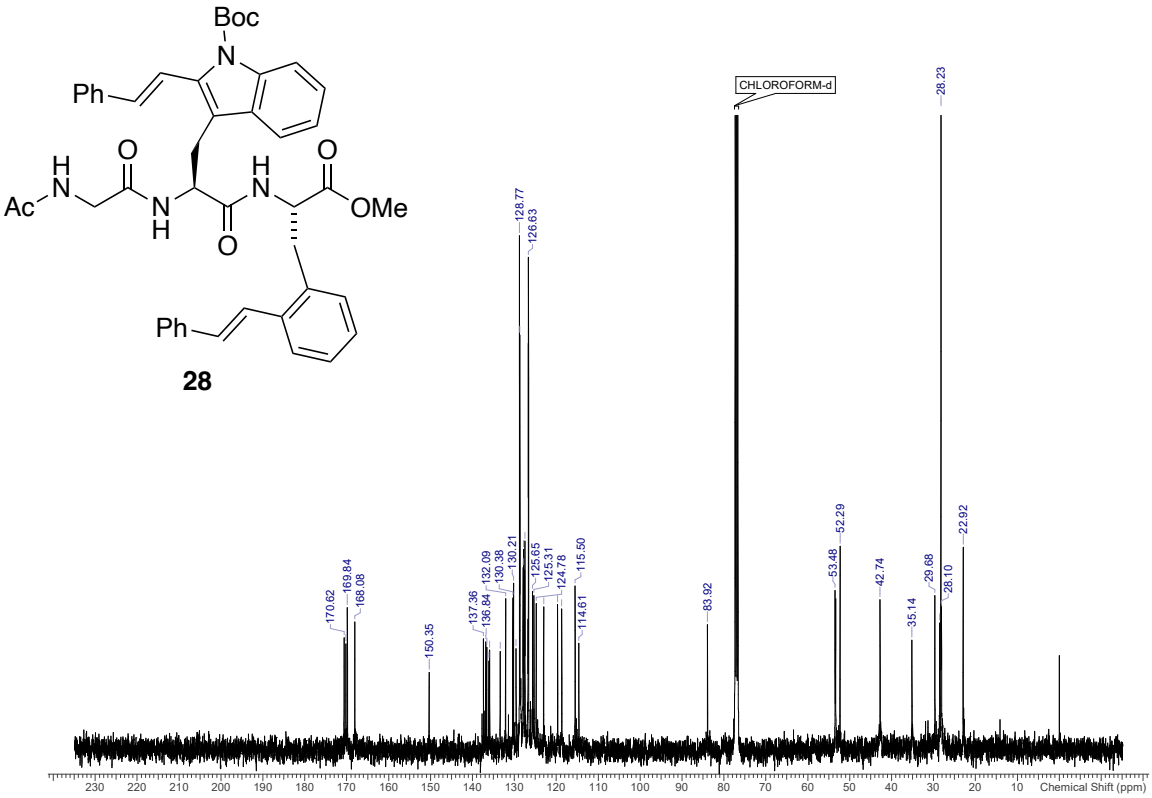


27

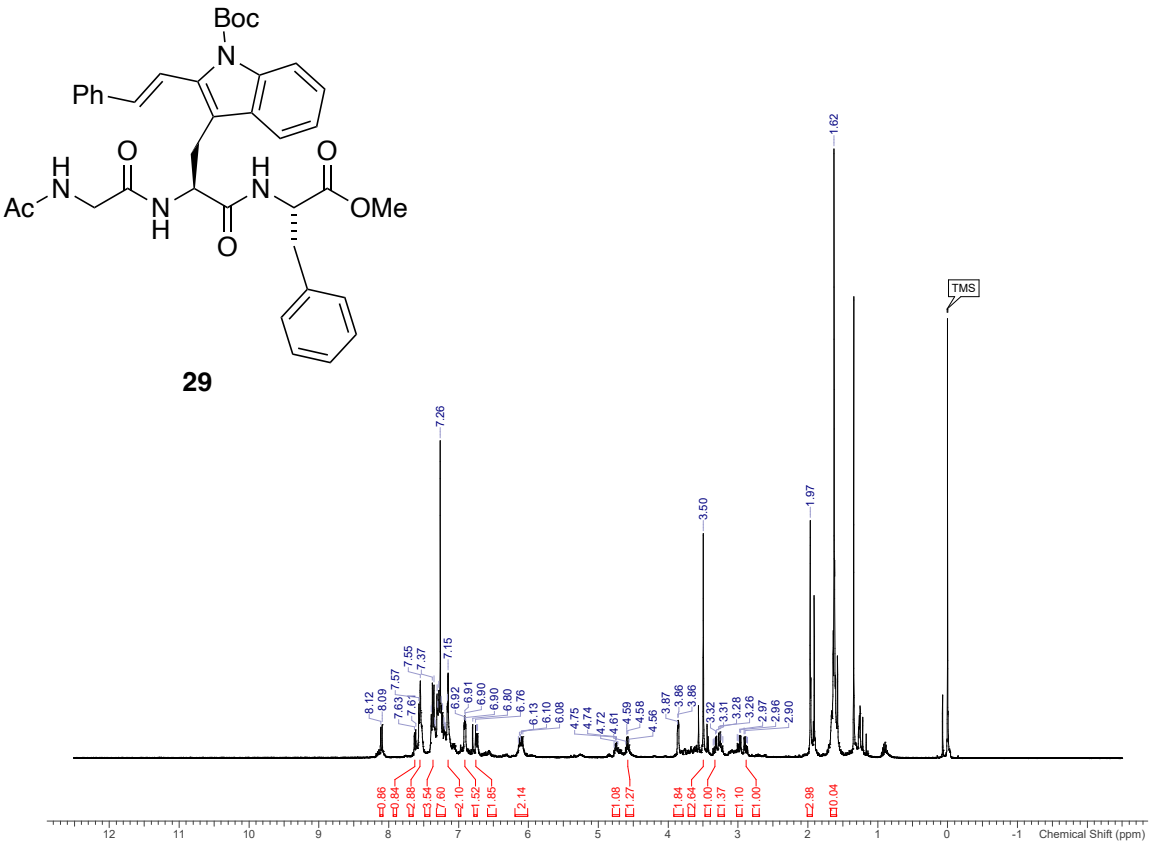
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **28**



^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **28**

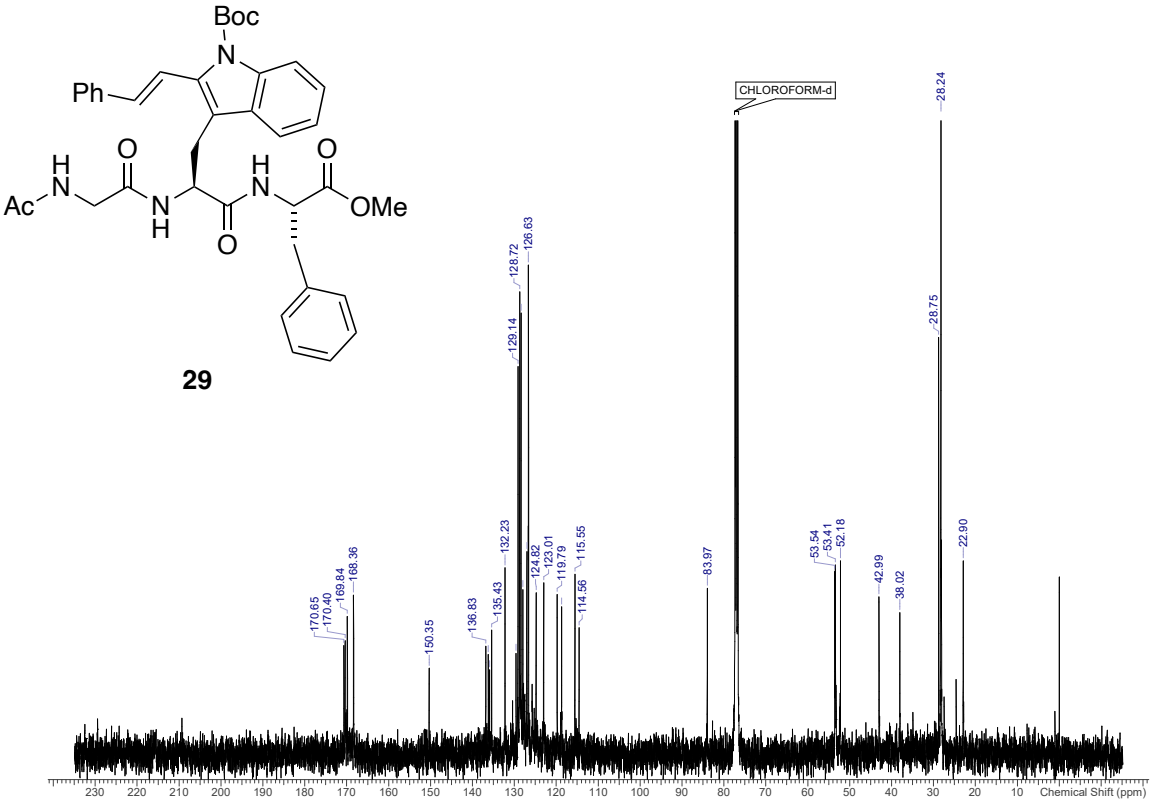


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **29**



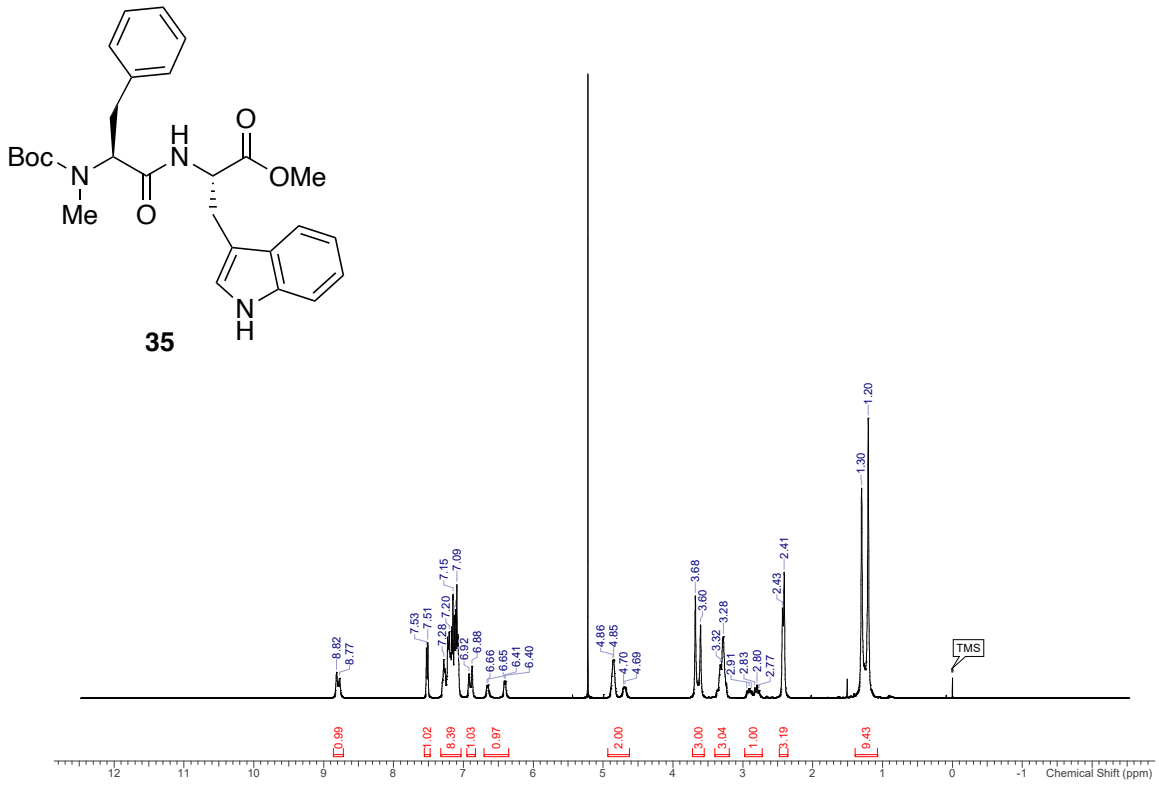
29

^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **29**

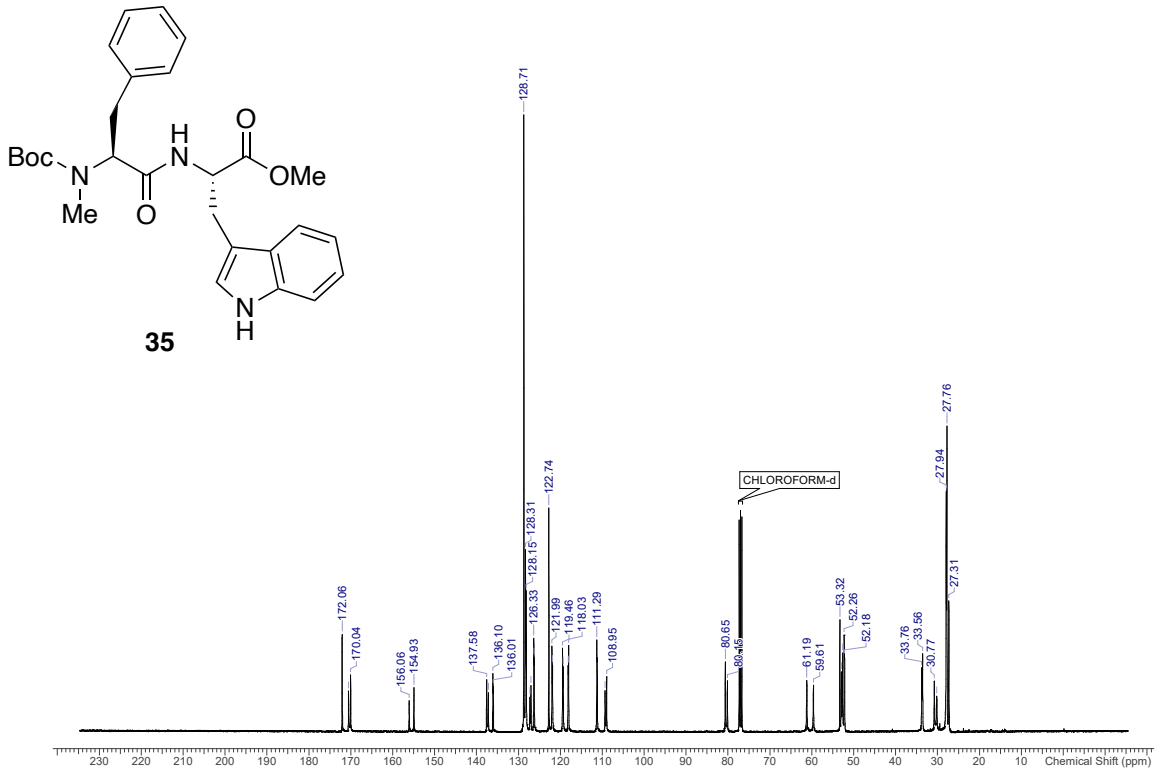


29

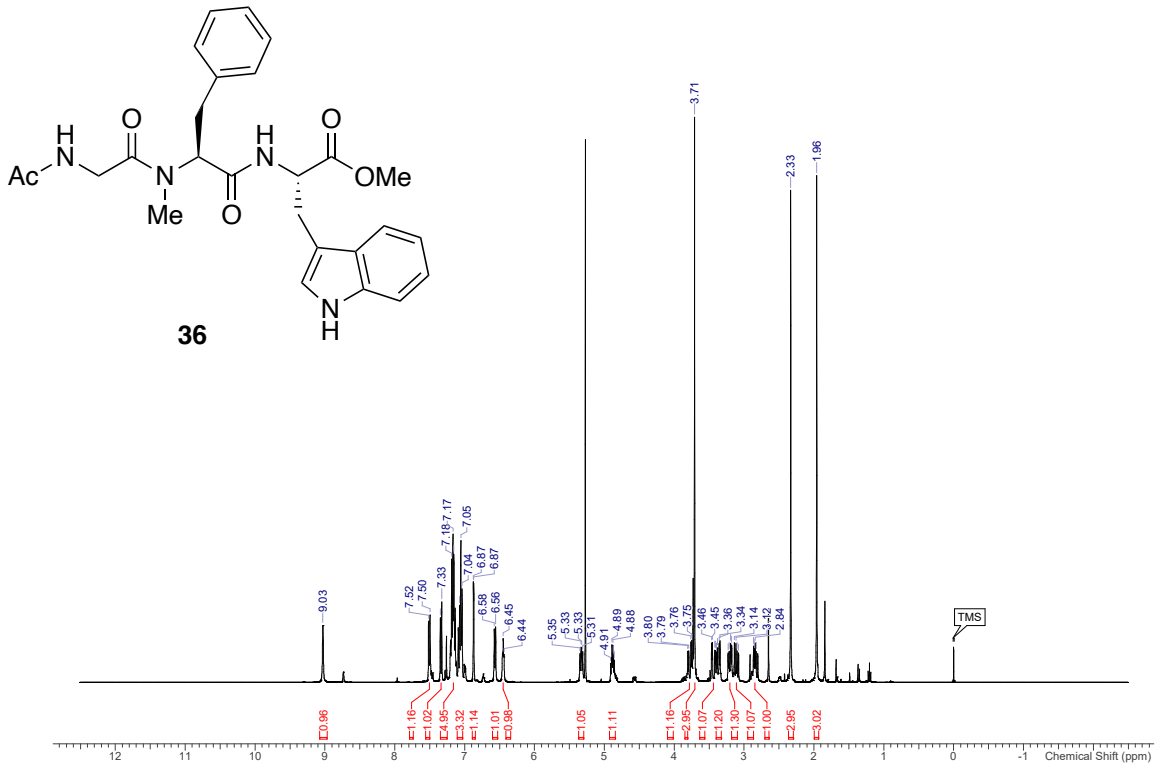
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **35**



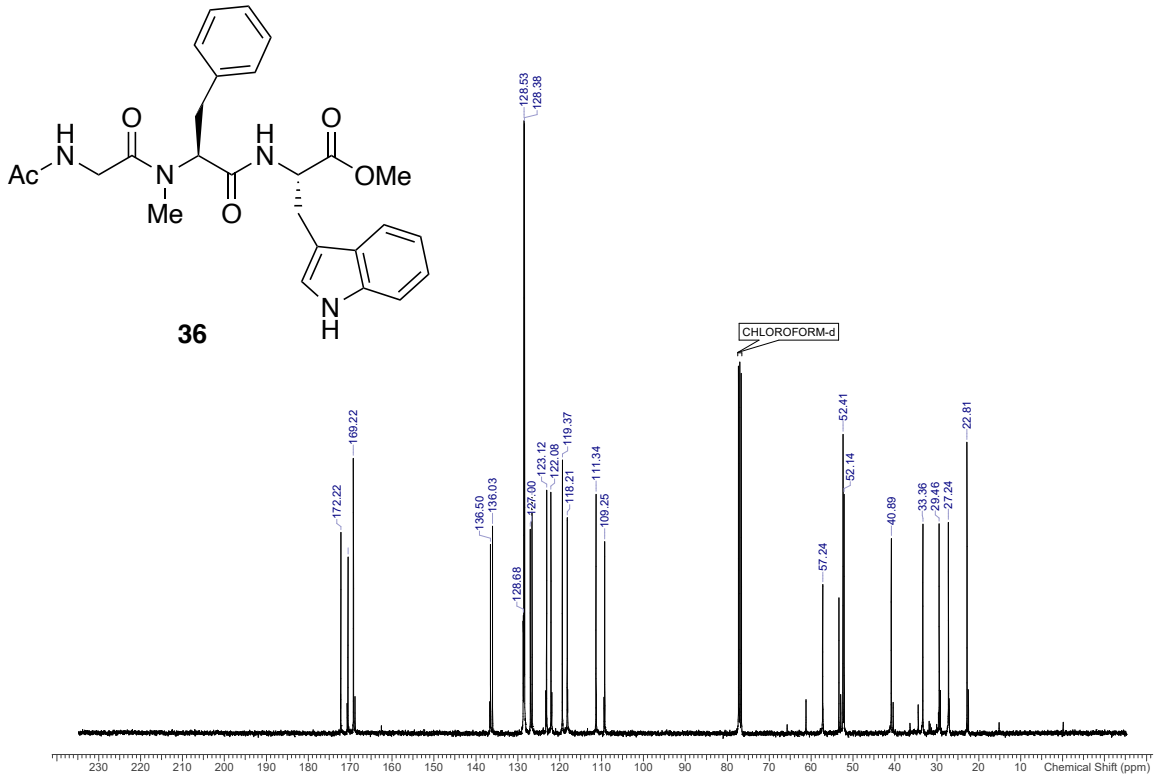
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **35**



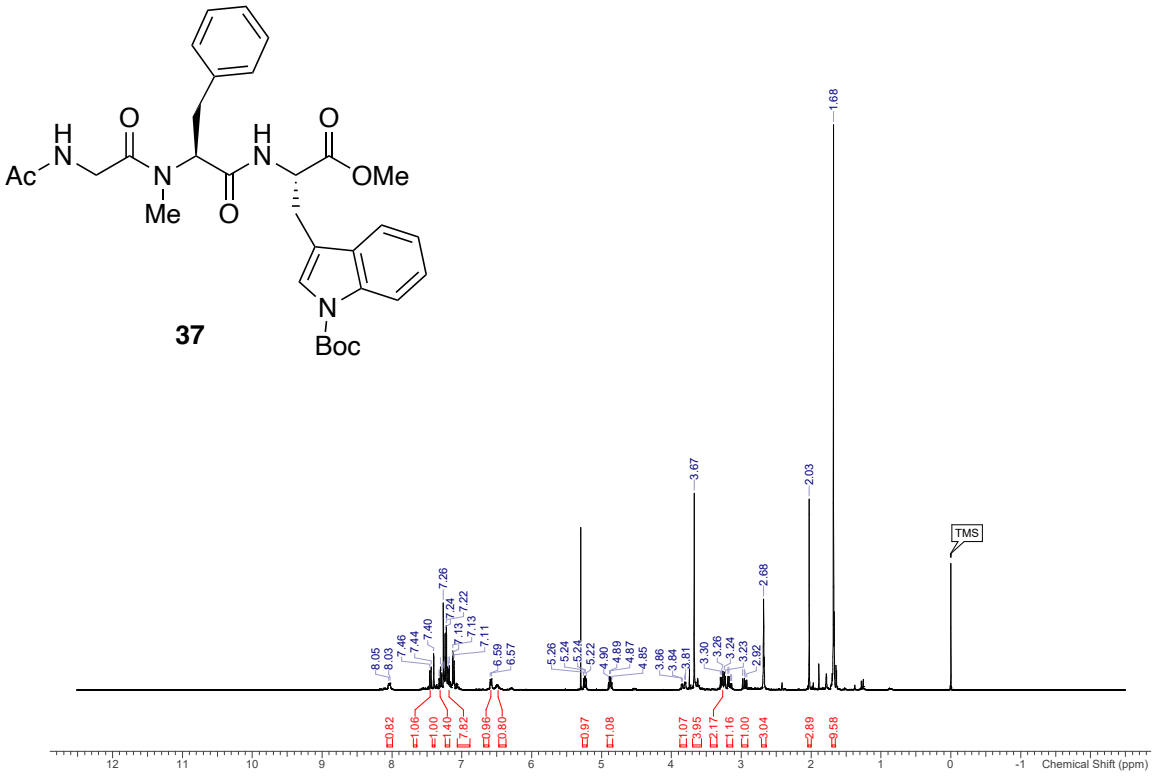
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **36**



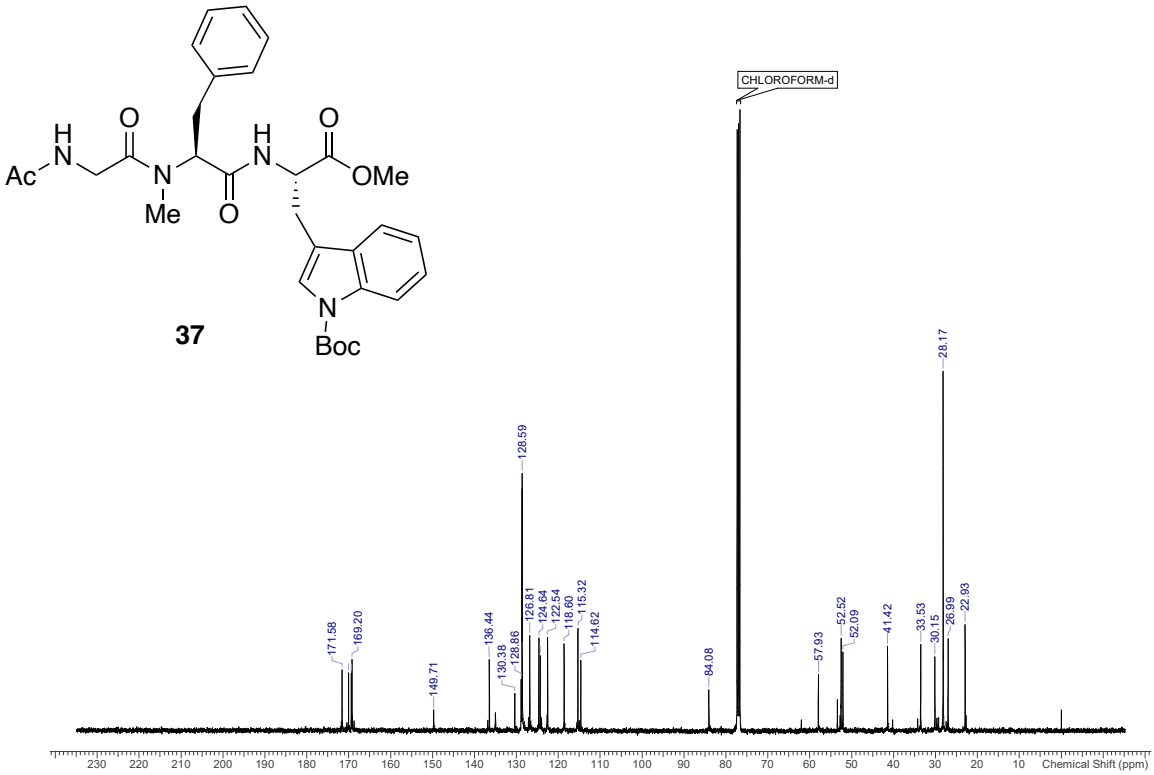
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **36**



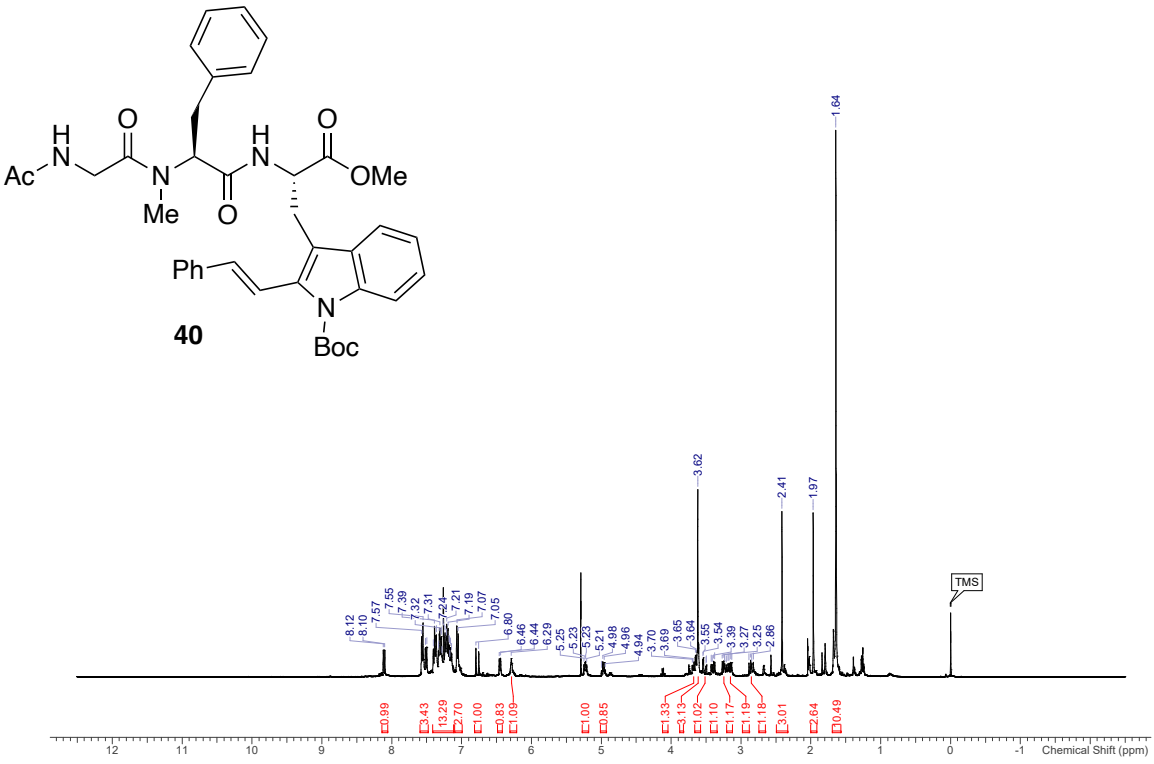
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **37**



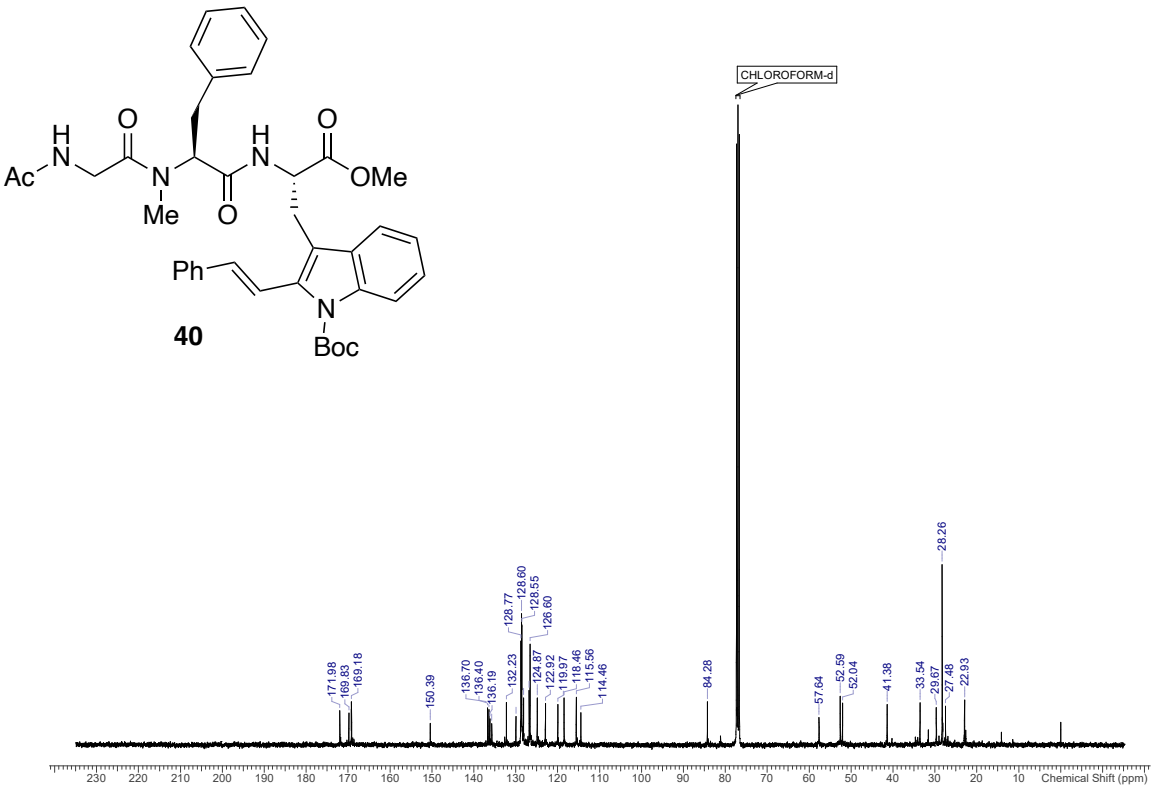
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **37**



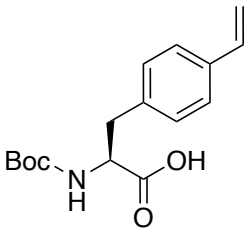
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **40**



^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **40**

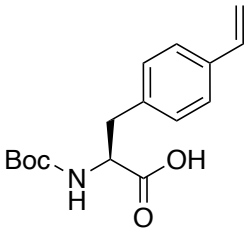


^{1}H NMR spectrum (400 MHz, D_6-DMSO) of **43**



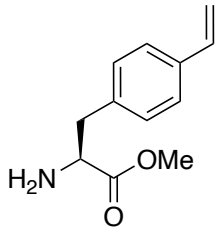
43

^{13}C NMR spectrum (100 MHz, D_6-DMSO) of **43**



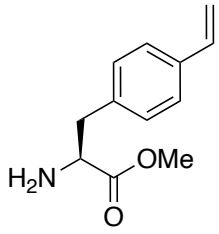
43

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **44**

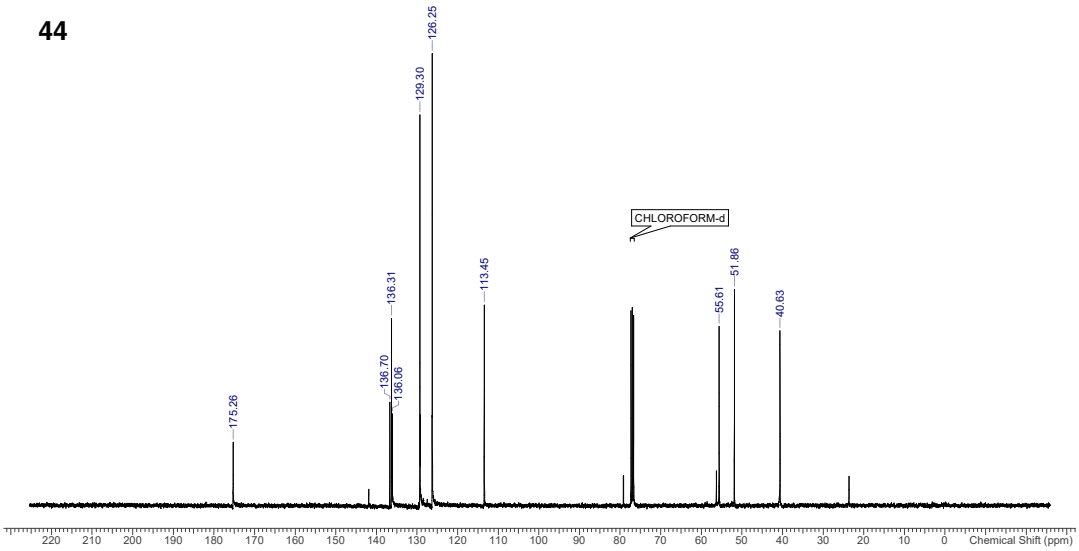


44

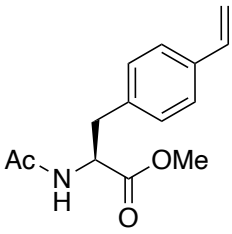
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **44**



44

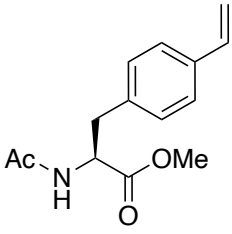


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **41**



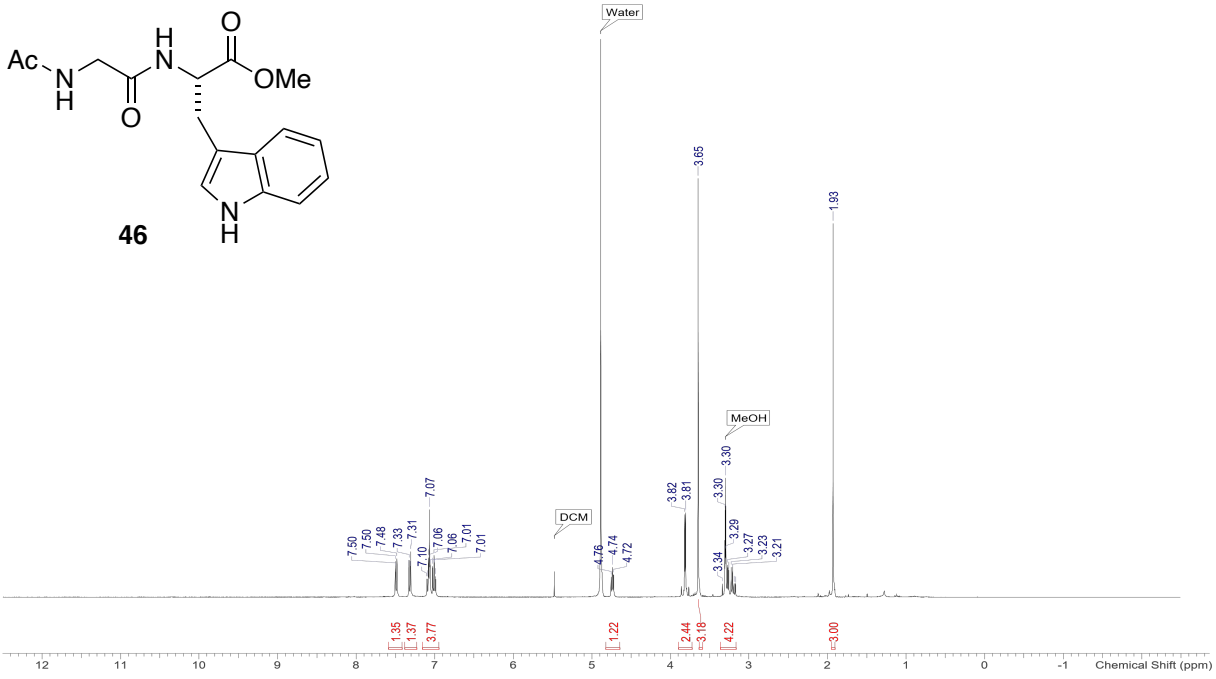
41

^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **41**

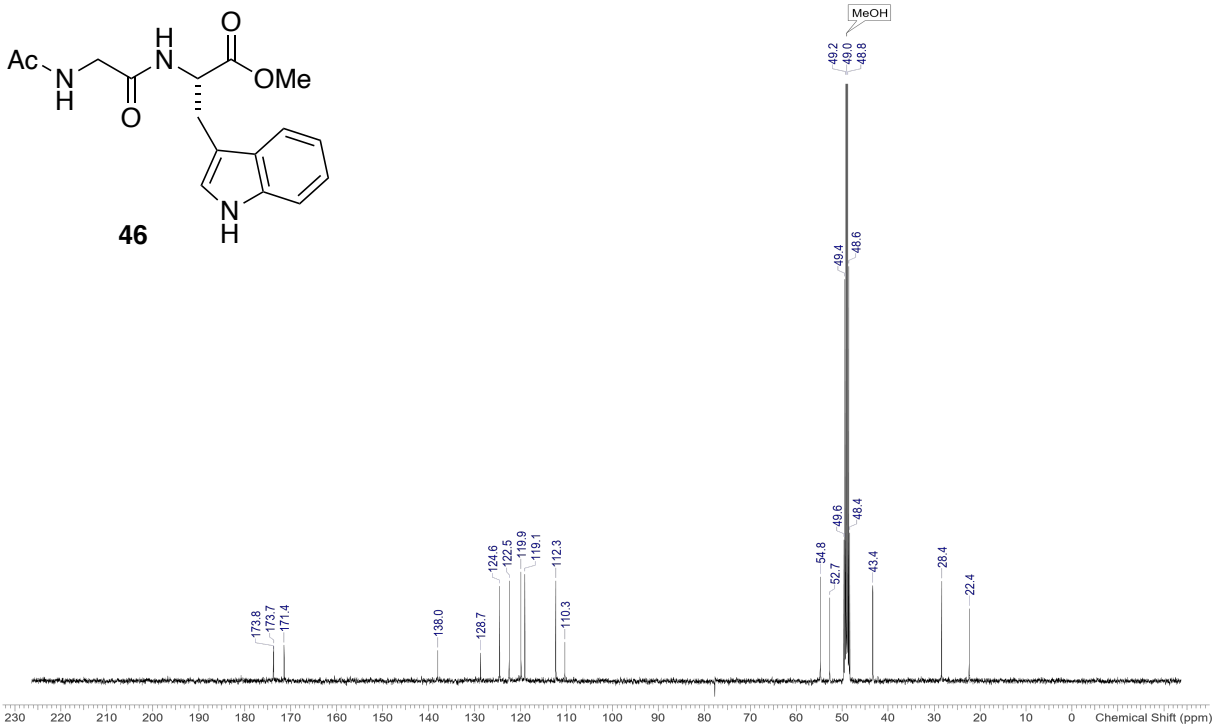


41

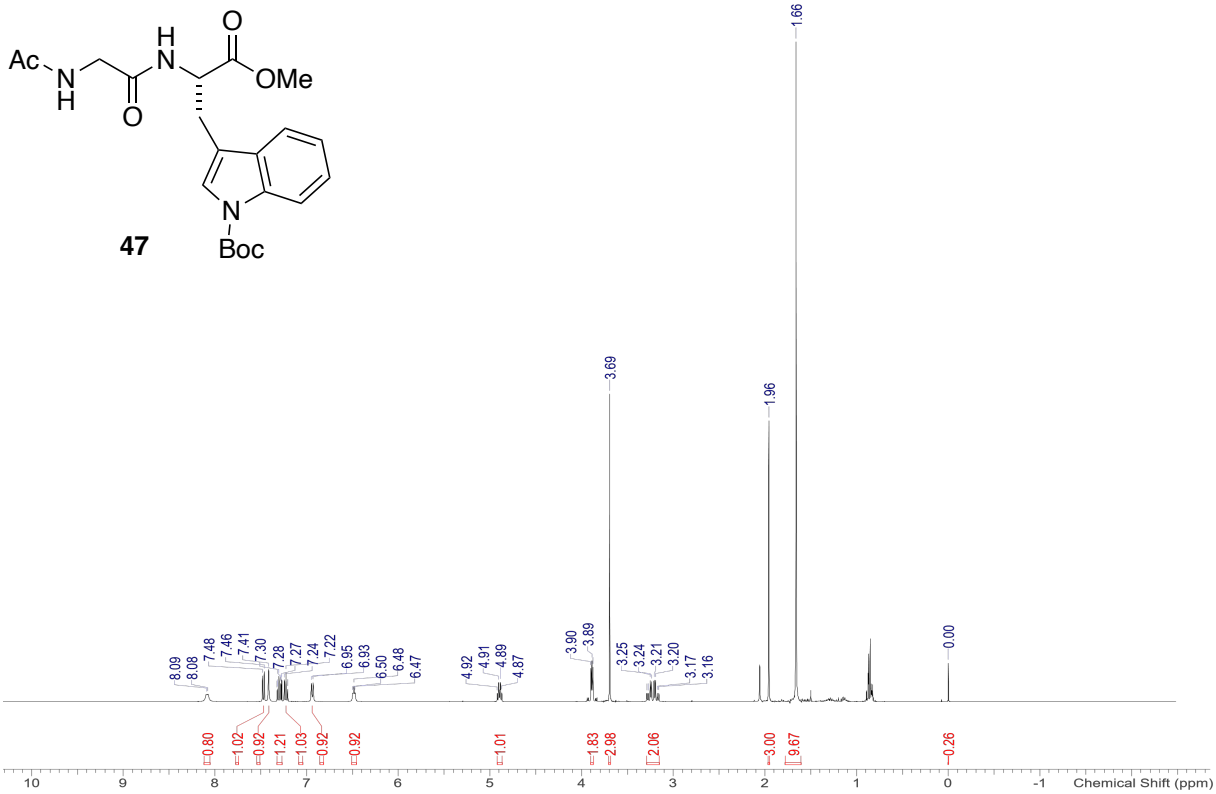
^{1}H NMR spectrum (400 MHz, CD_3OD) of **46**



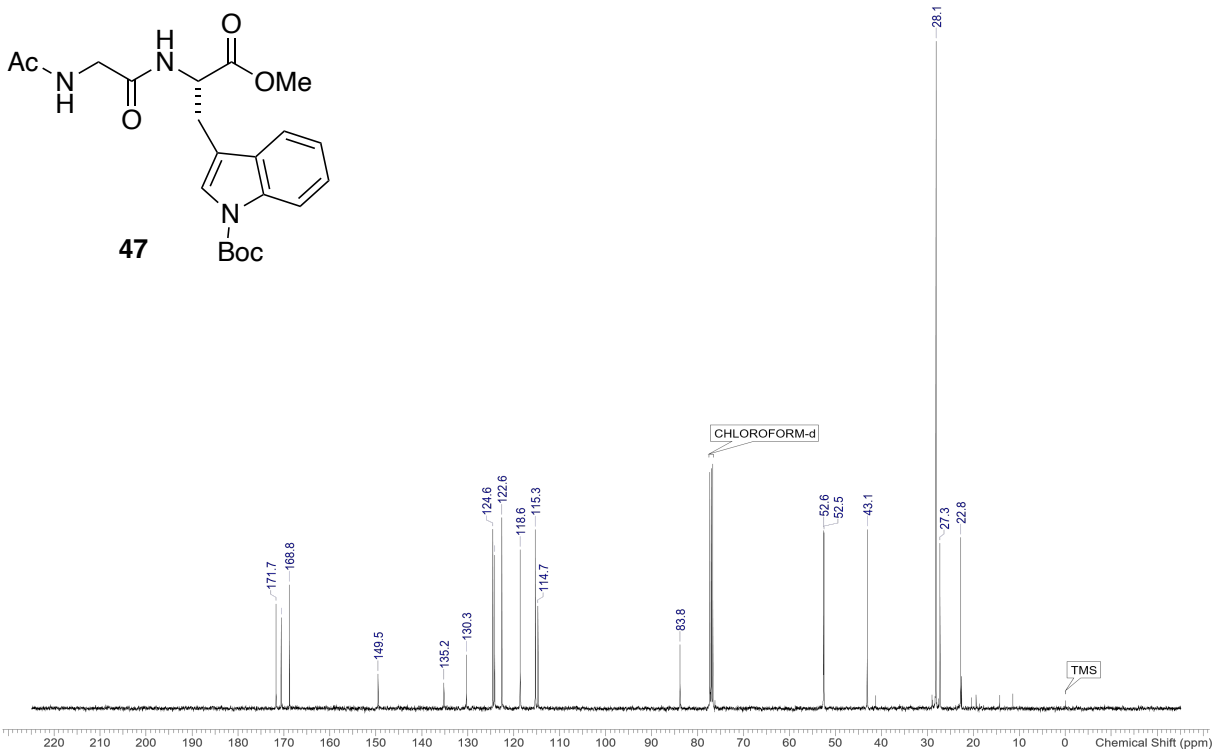
^{13}C{^{1}H} NMR spectrum (100 MHz, CD_3OD) of **46**



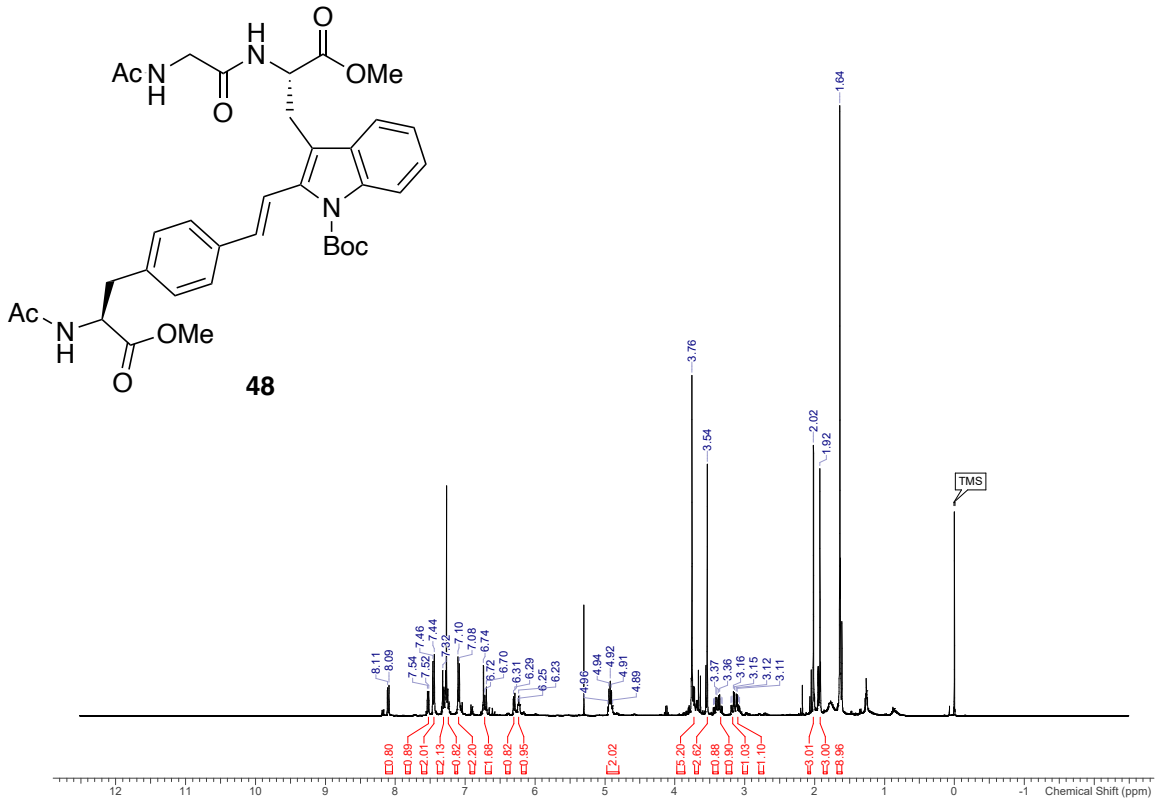
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **47**



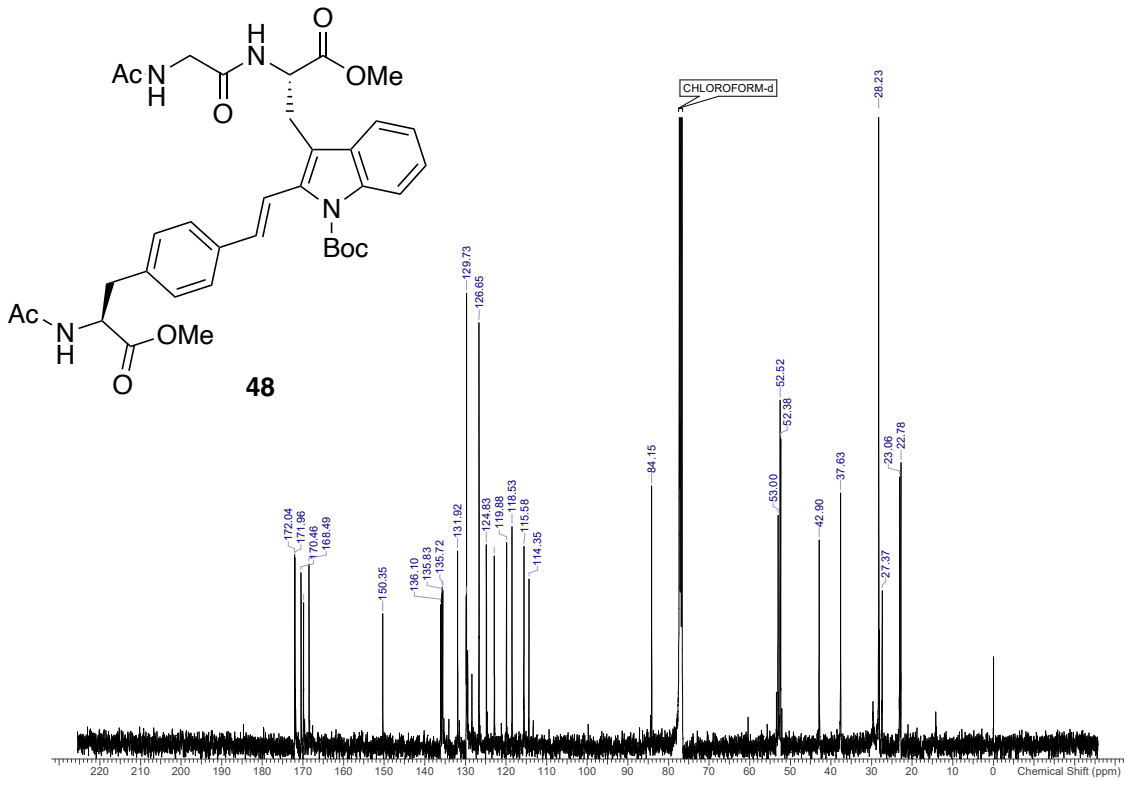
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **47**



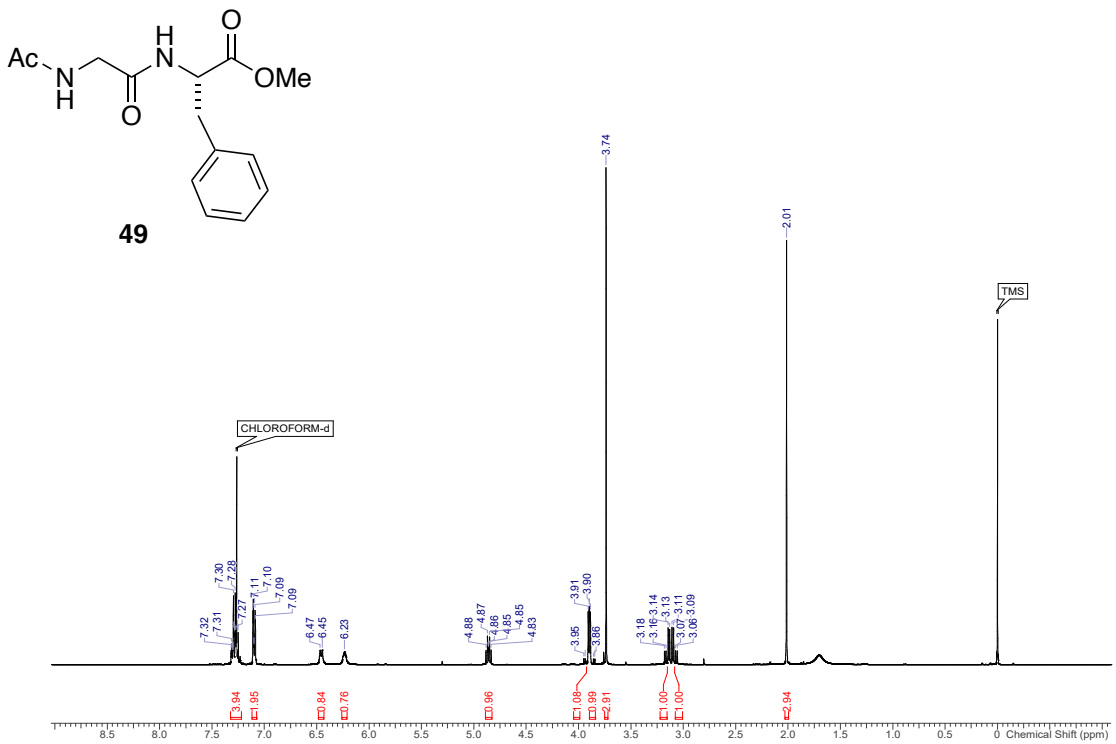
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **48**



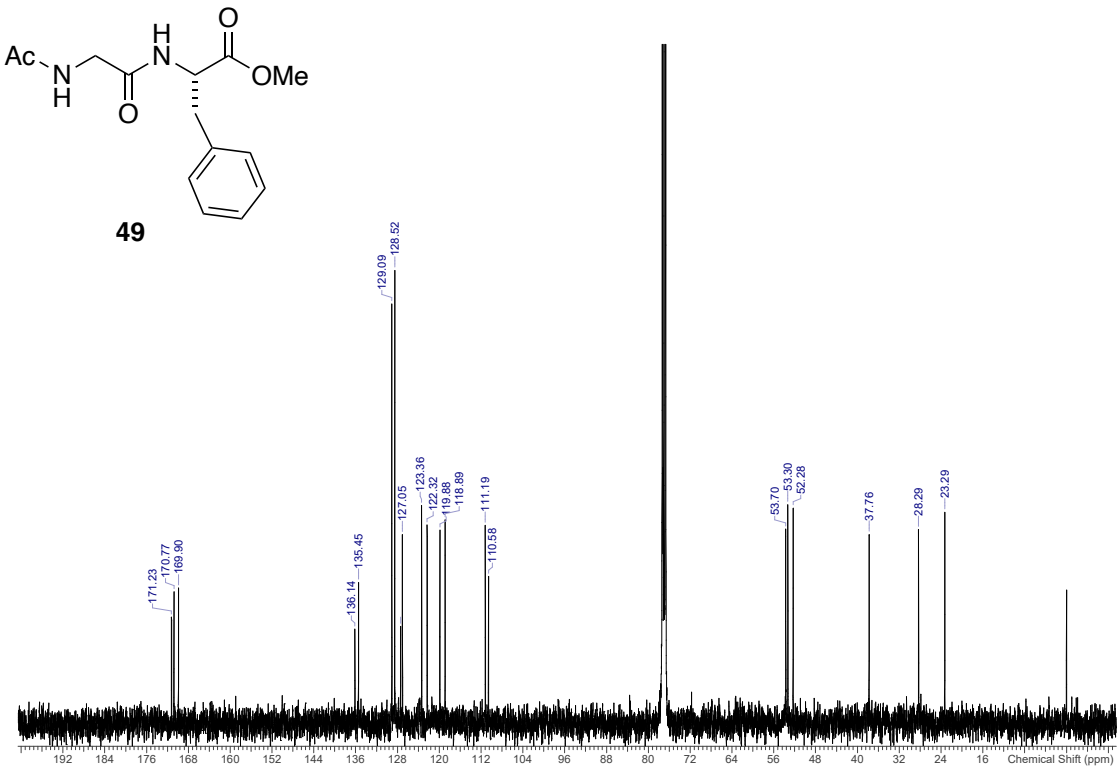
^{13}C{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of **48**



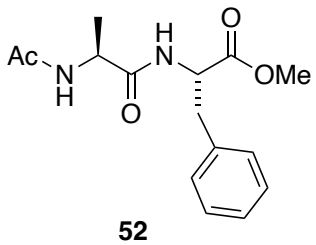
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **49**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **49**

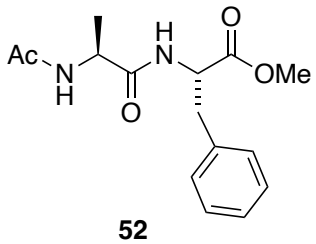


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **52**



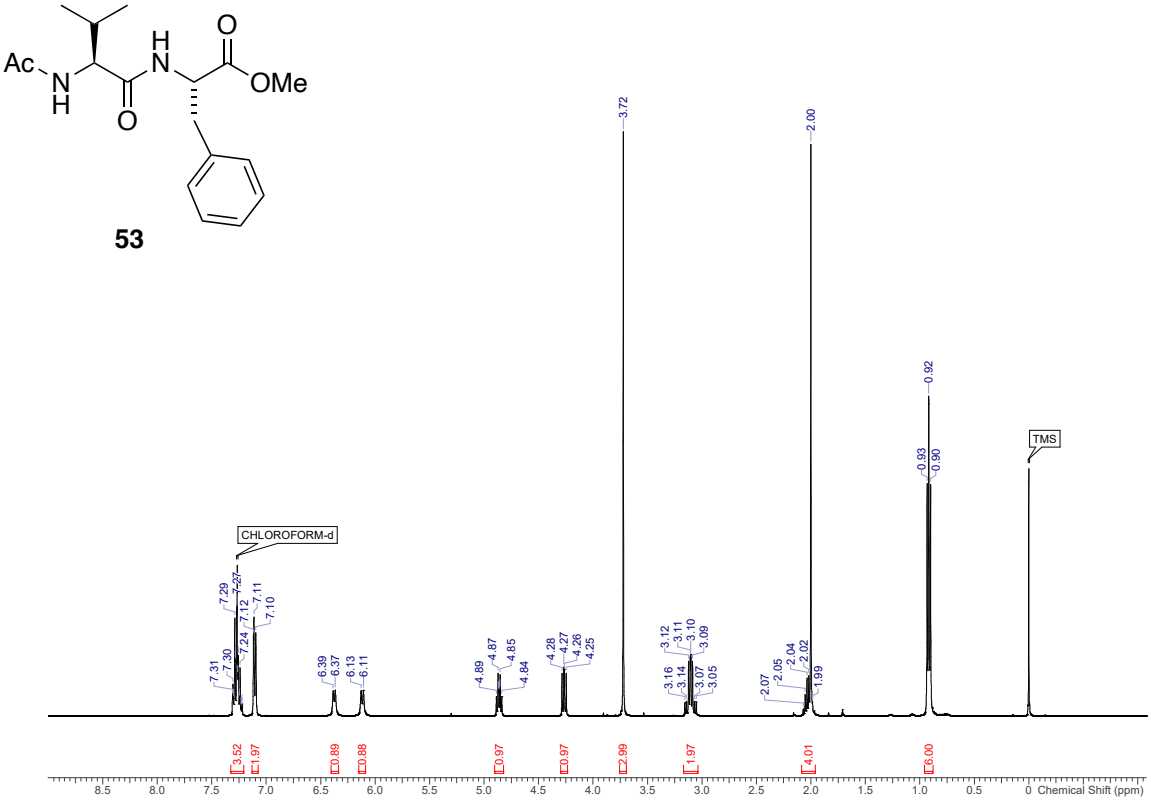
52

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **52**

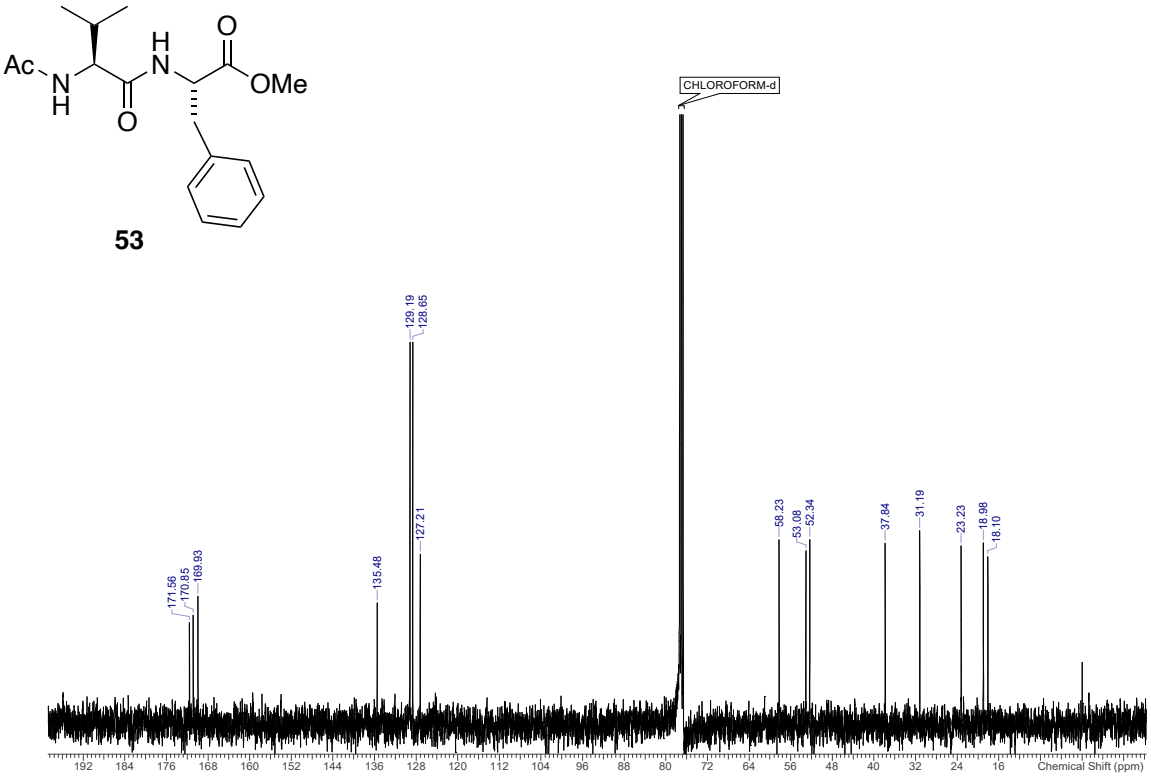


52

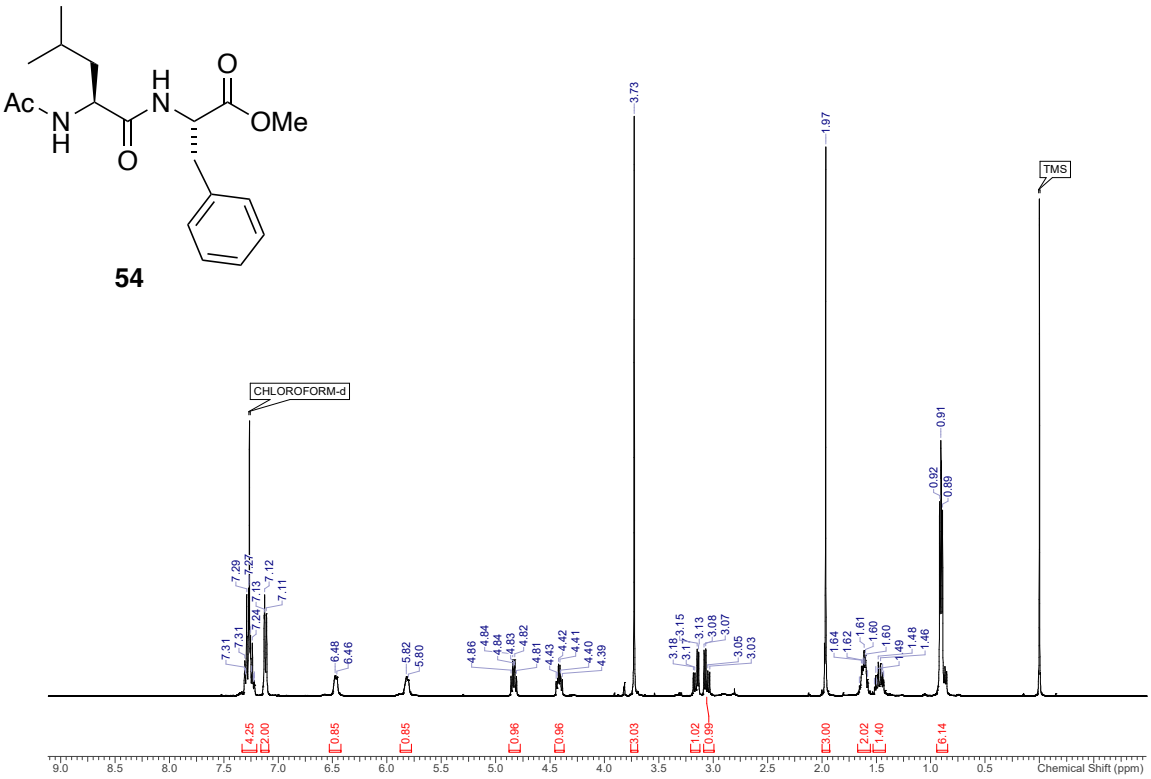
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **53**



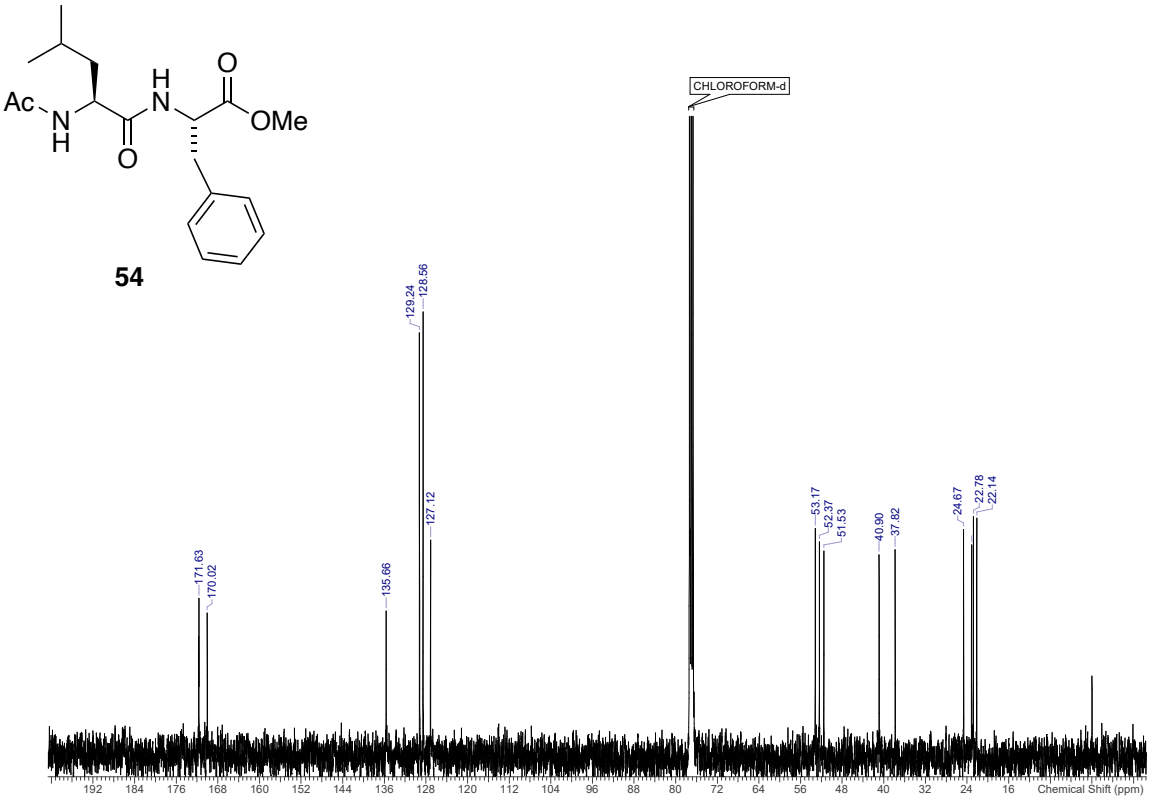
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **53**



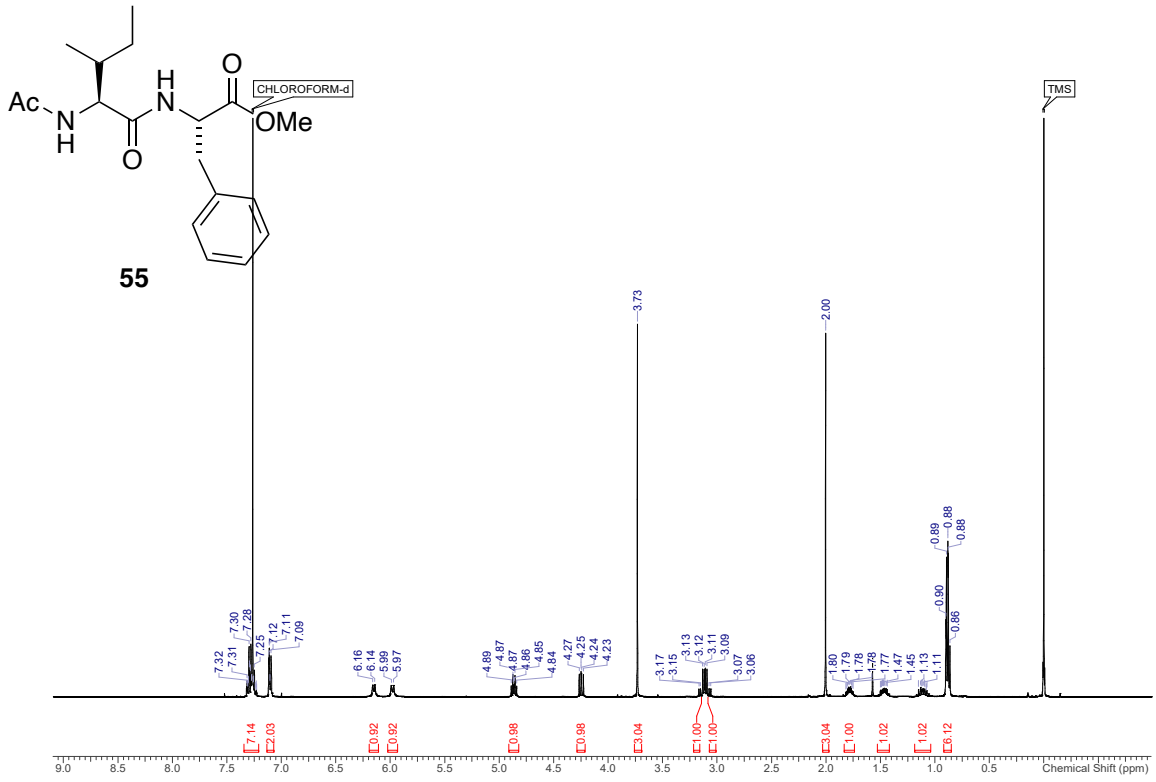
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **54**



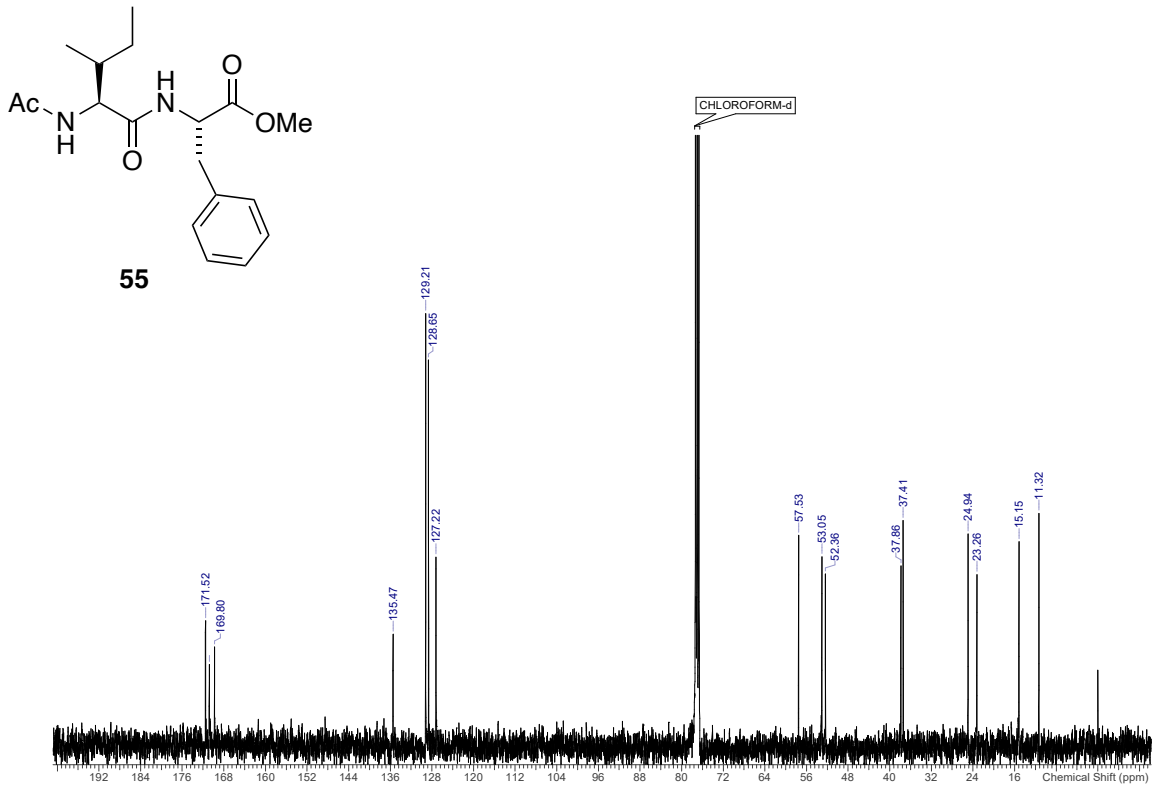
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **54**



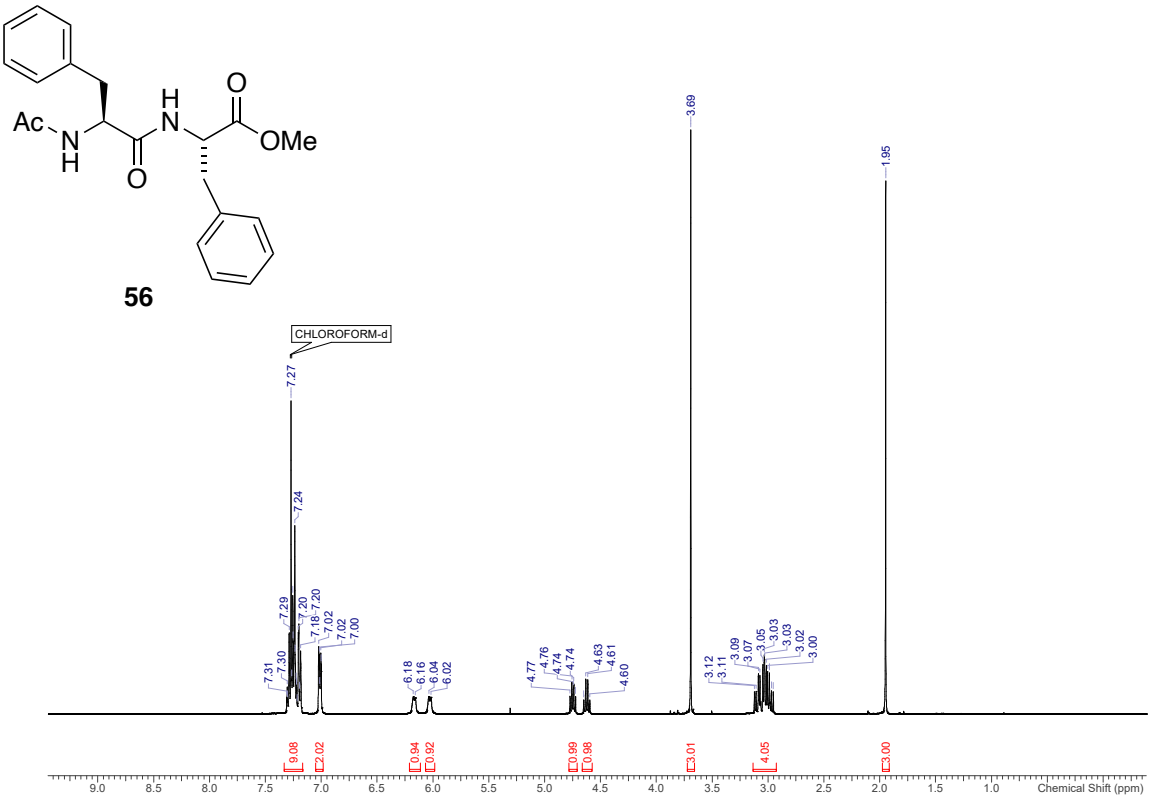
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **55**



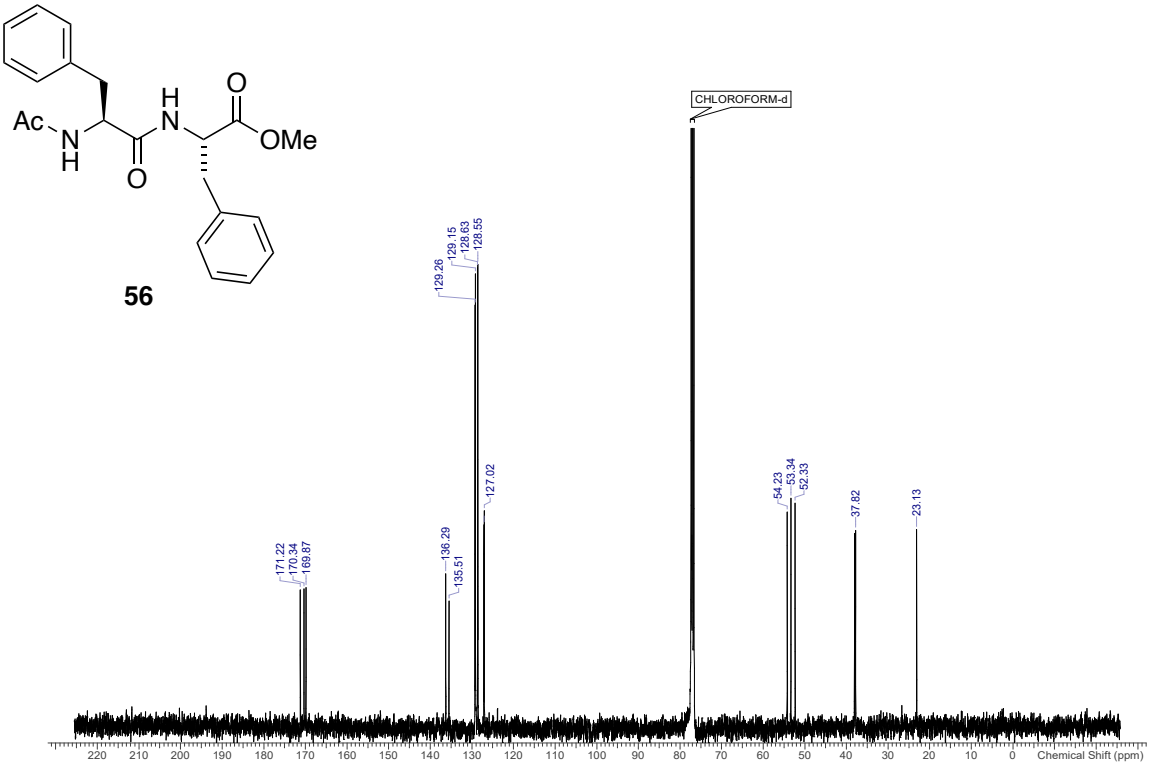
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **55**



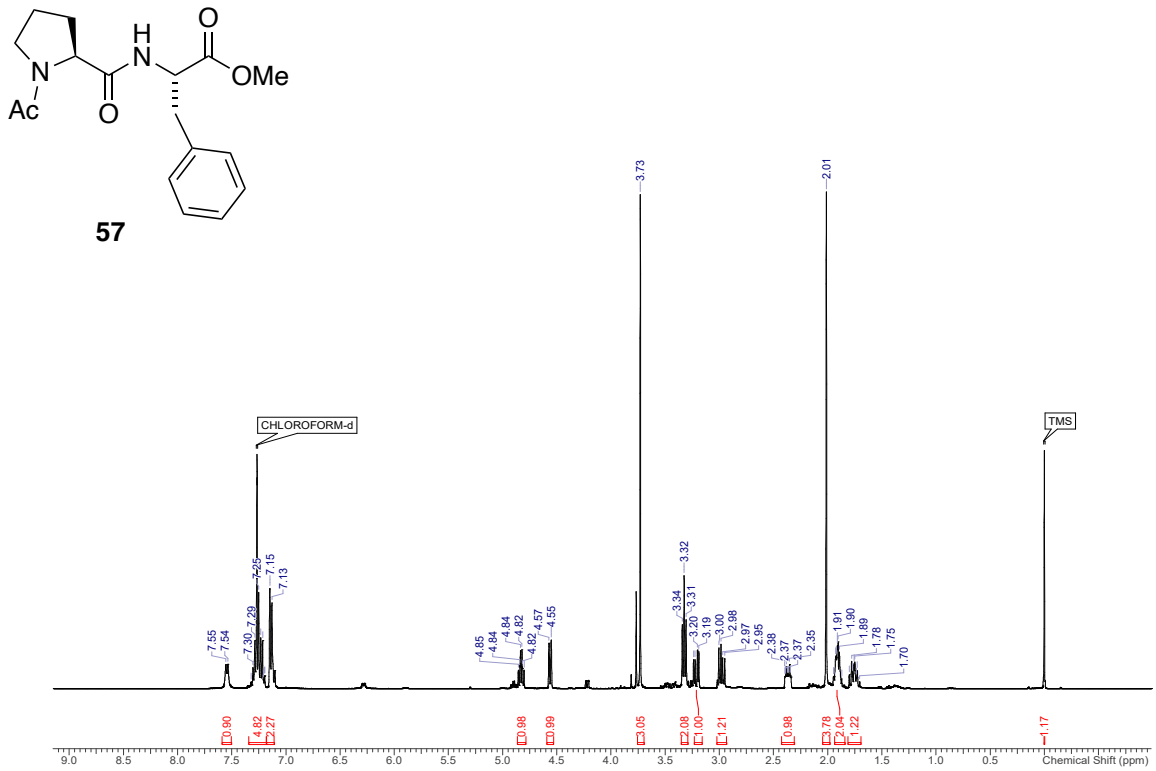
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **56**



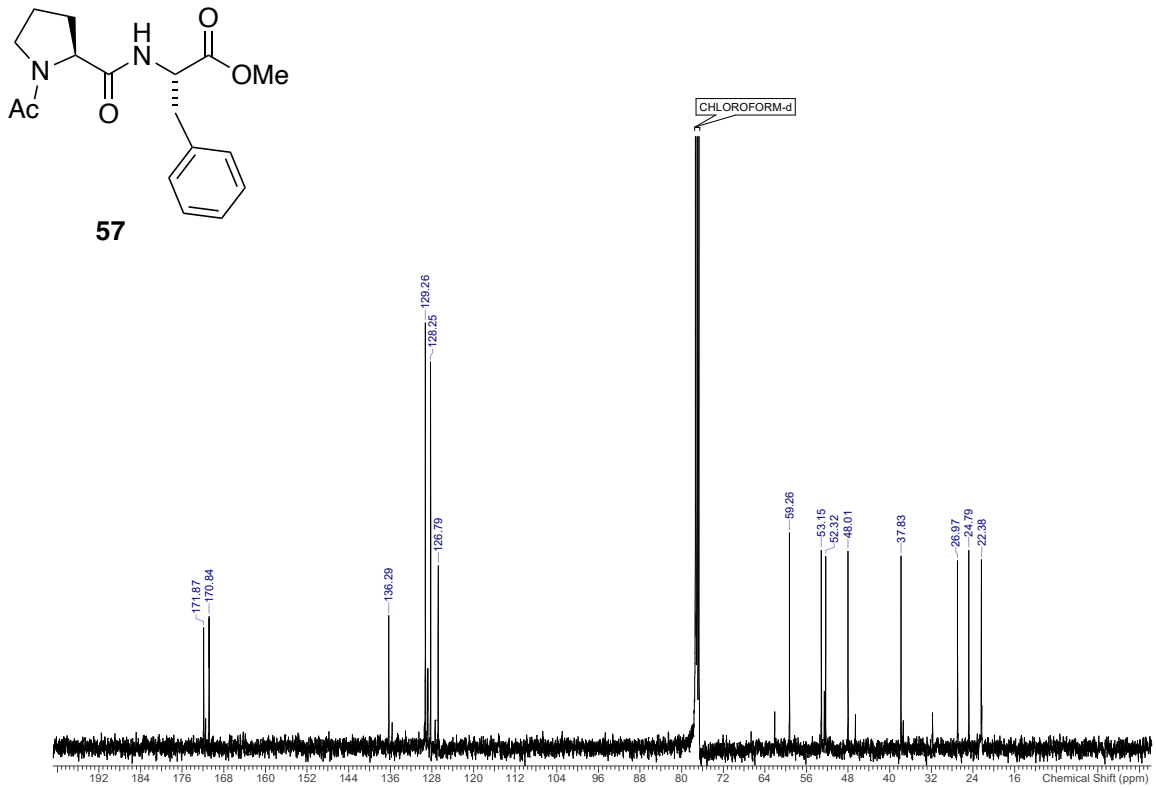
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **56**



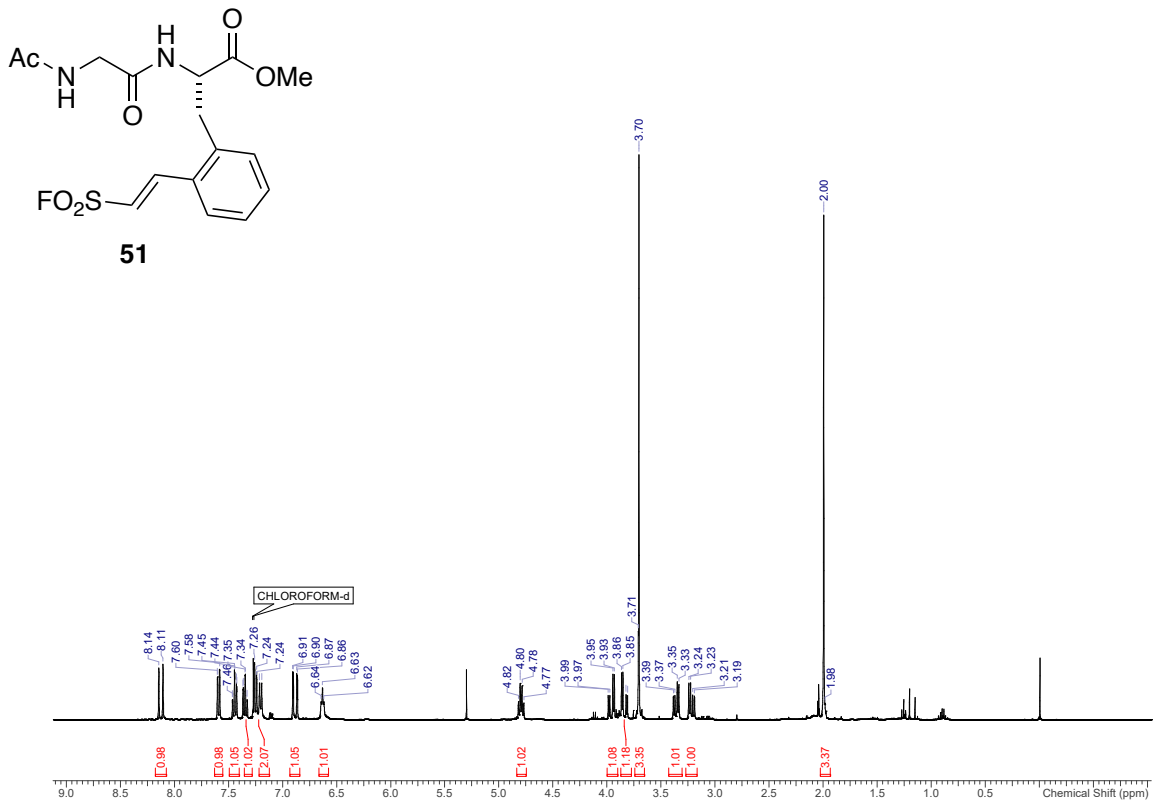
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **57**



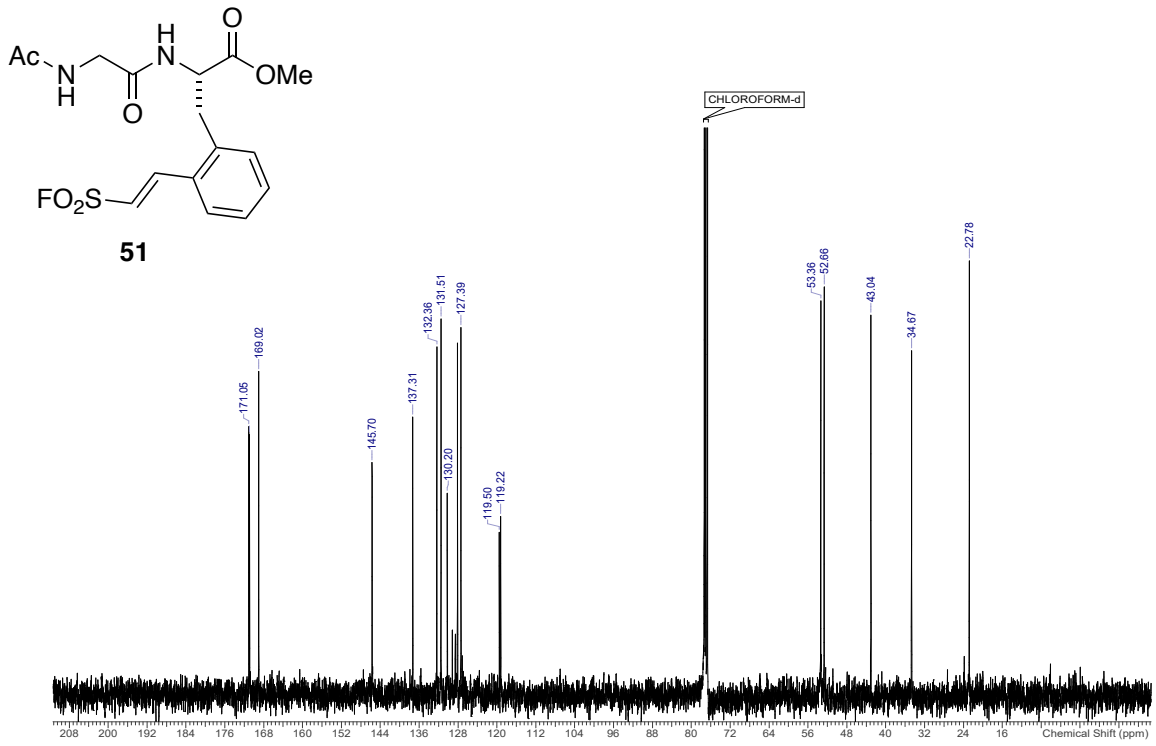
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **57**



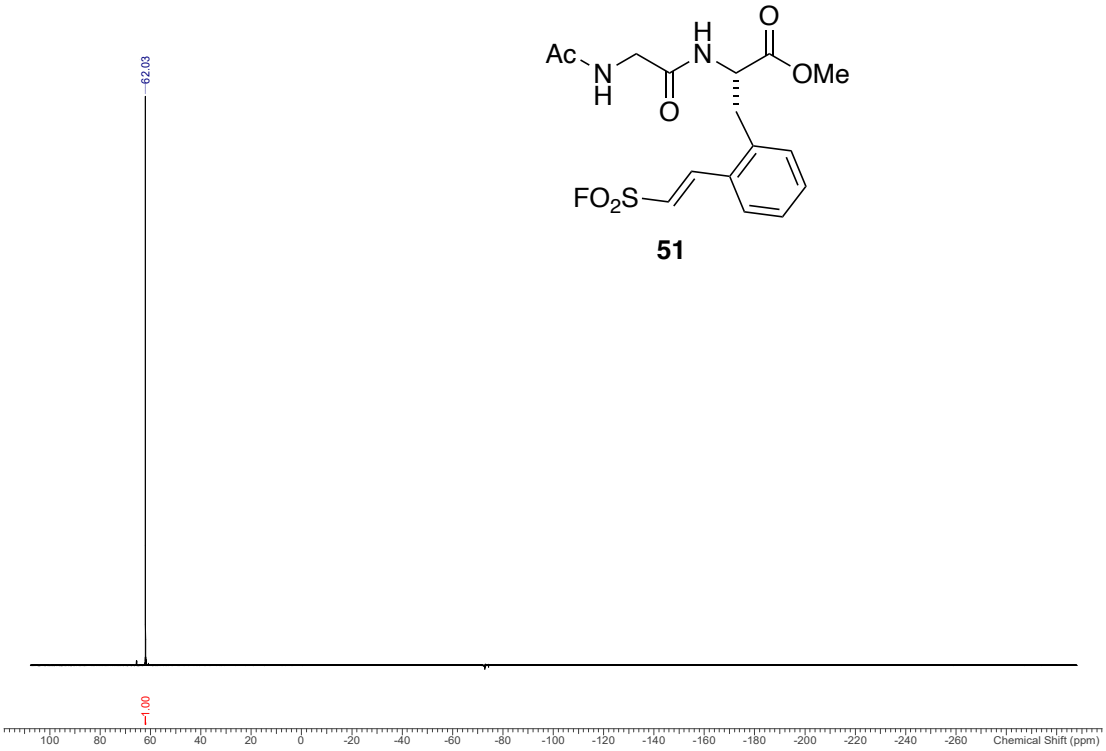
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **51**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **51**

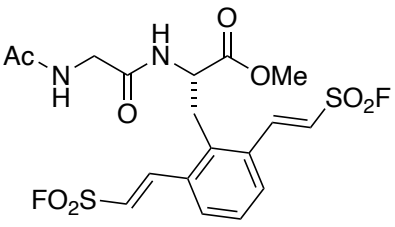


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **51**

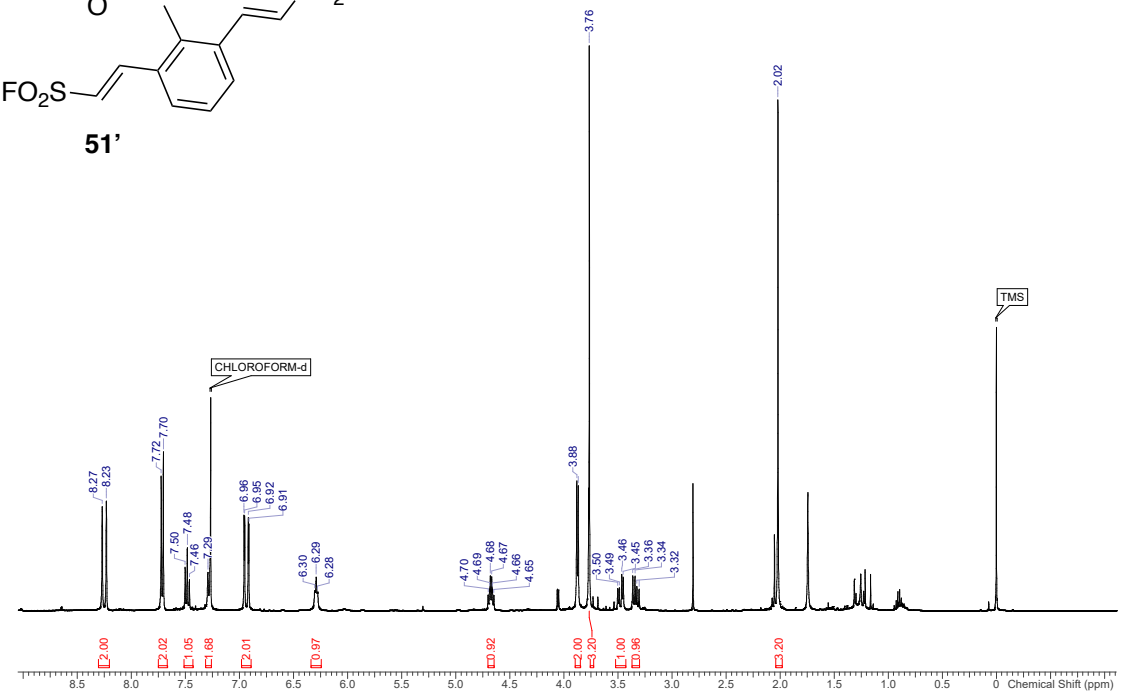


51

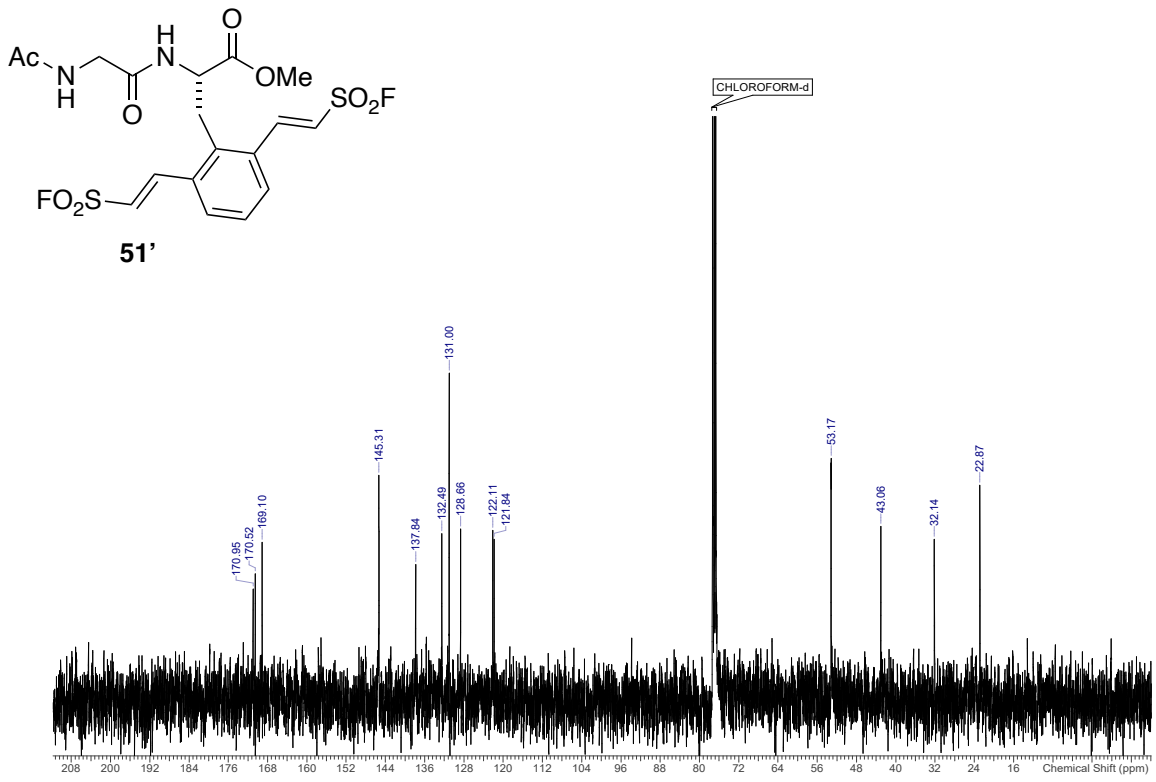
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **51'**



51'

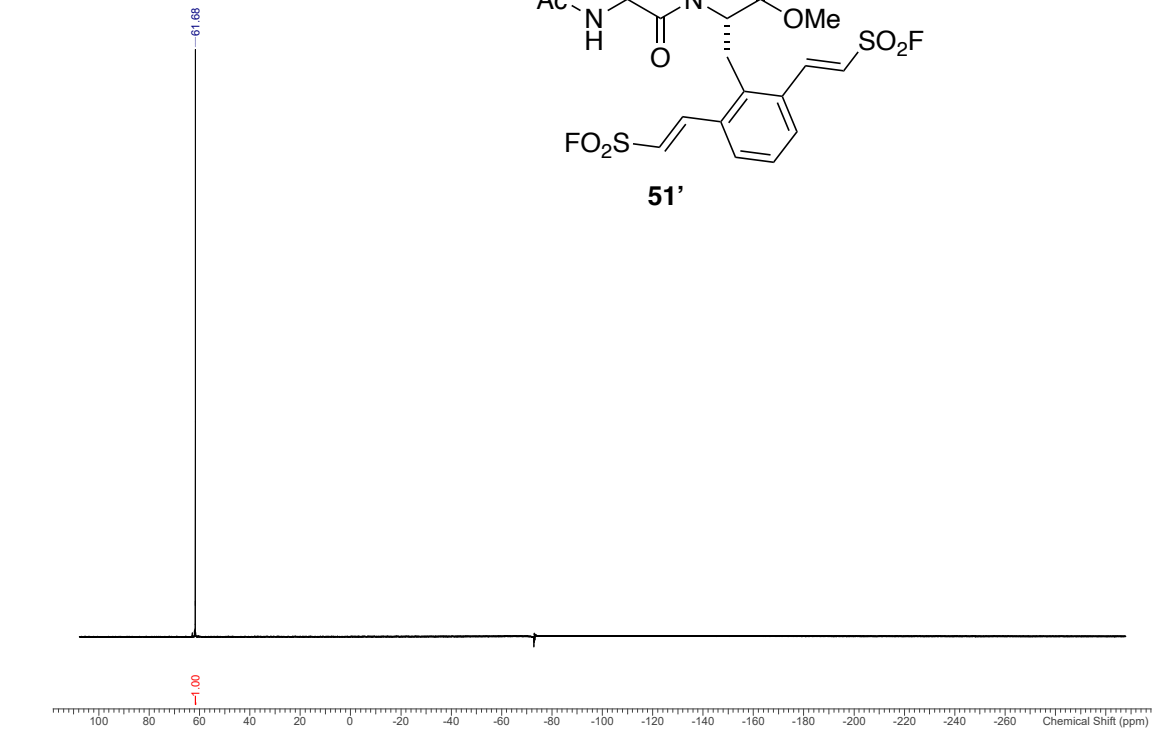


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **51'**



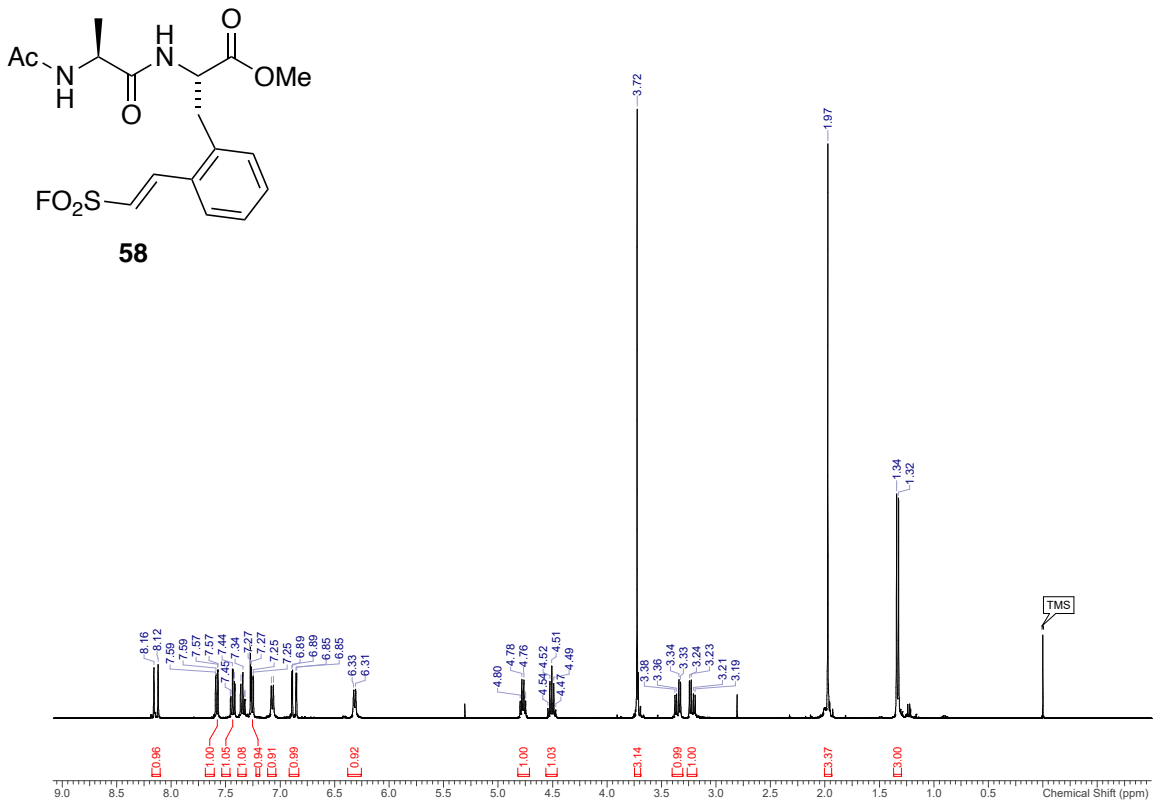
51'

^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **51'**

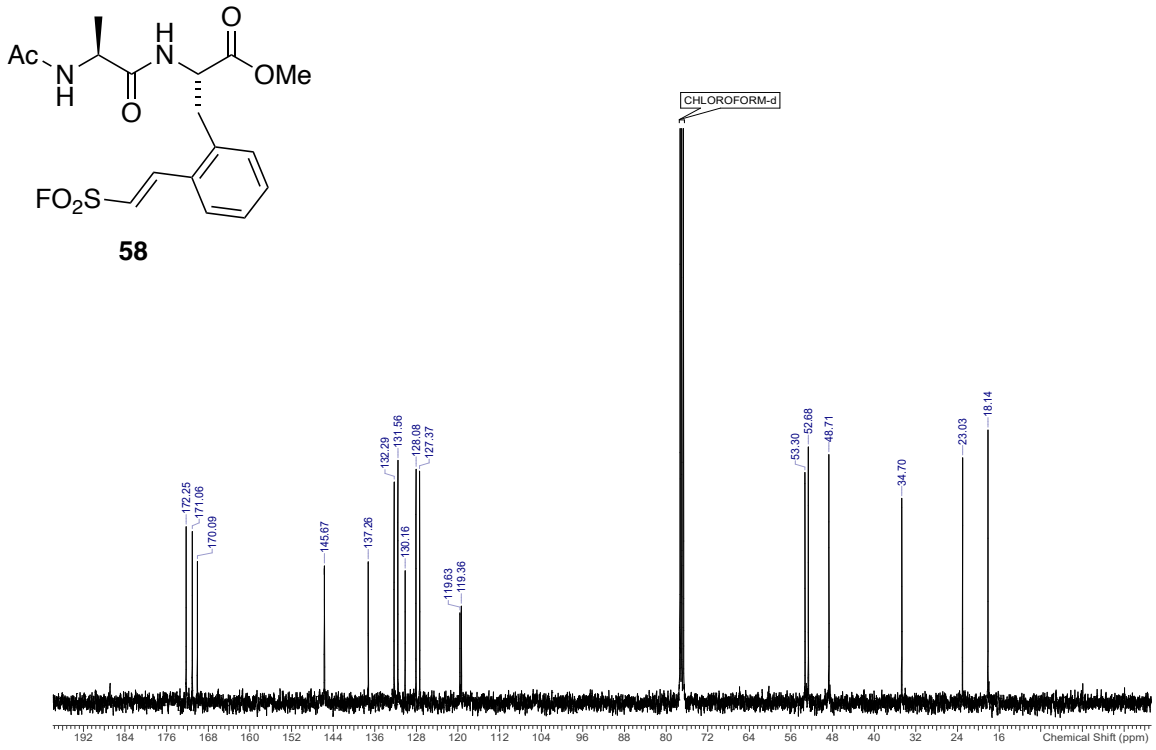


51'

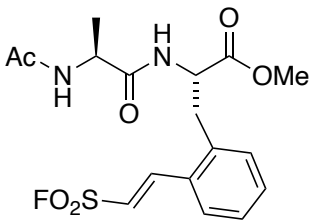
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **58**



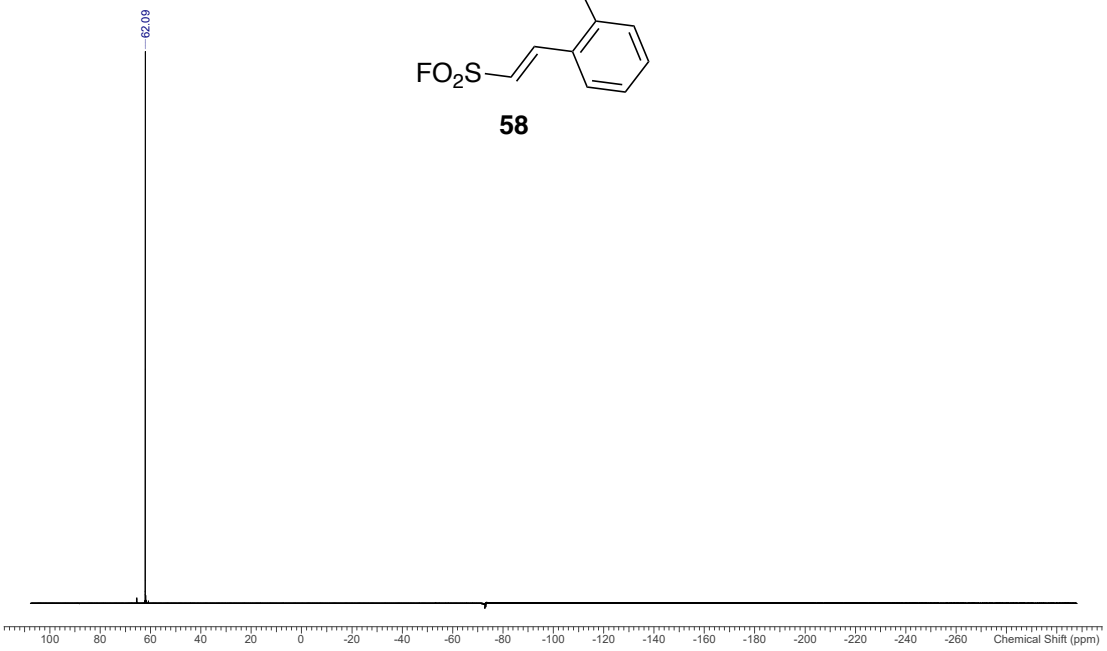
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **58**



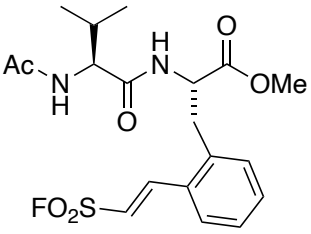
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **58**



58

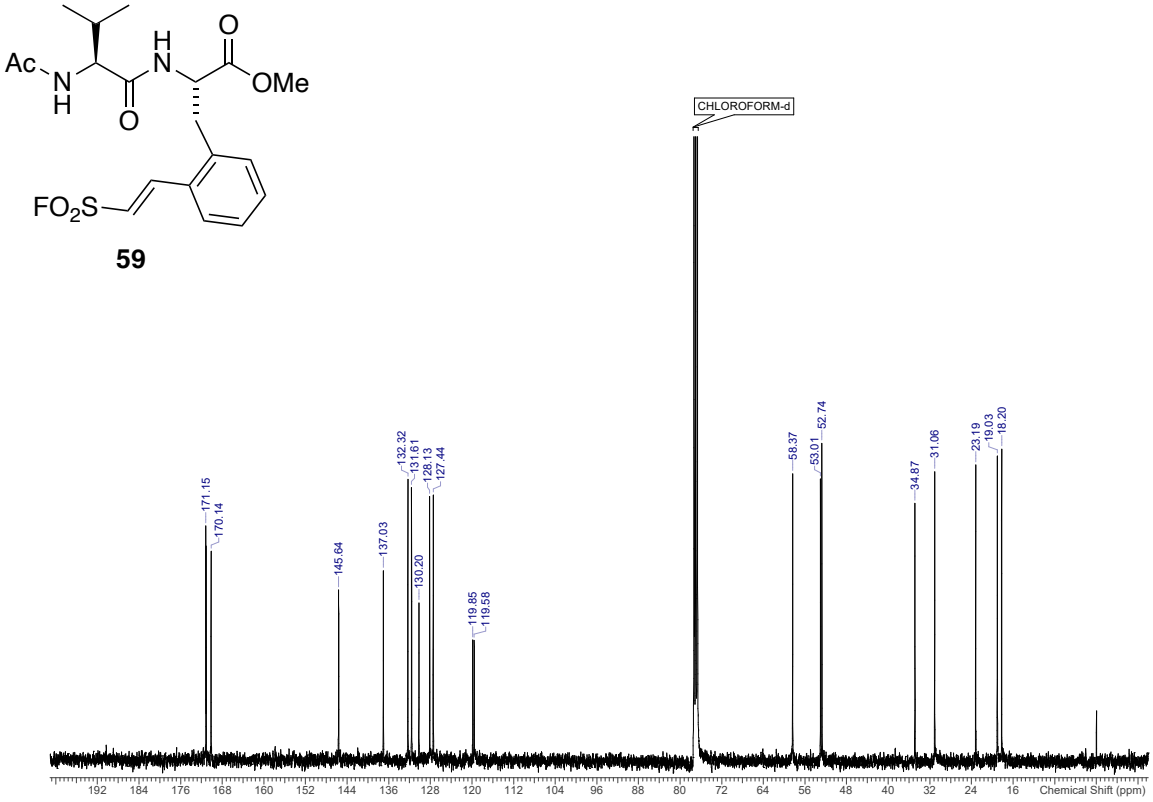


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **59**

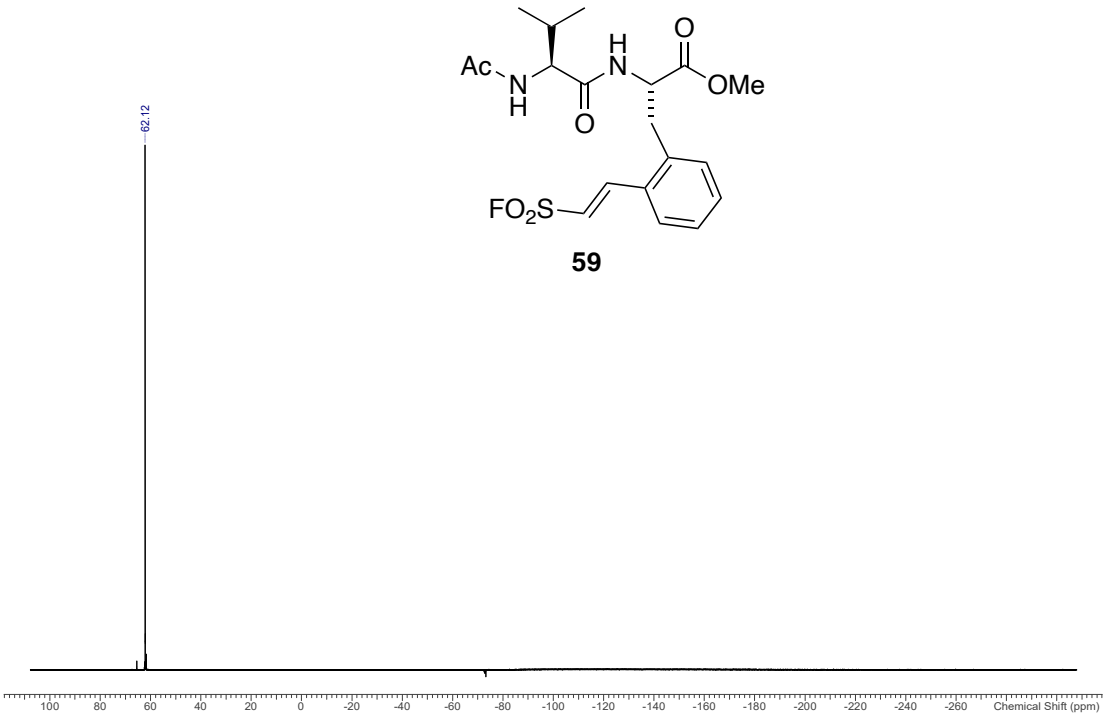


59

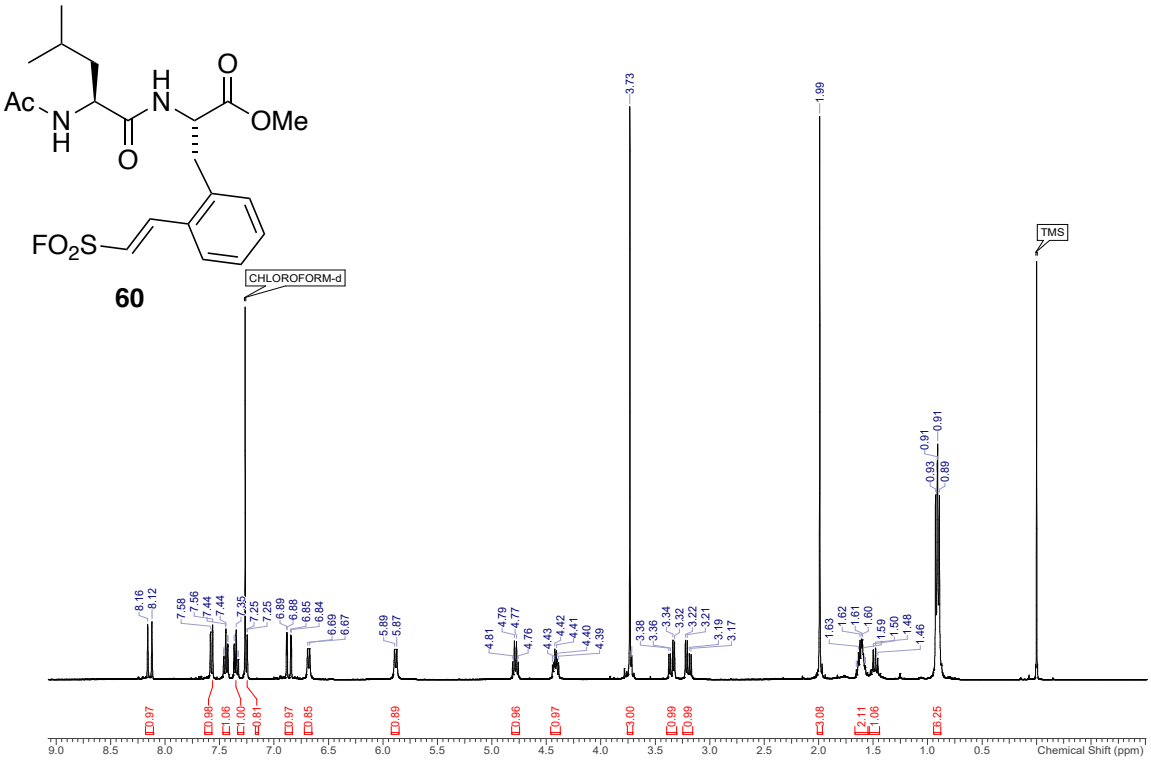
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **59**



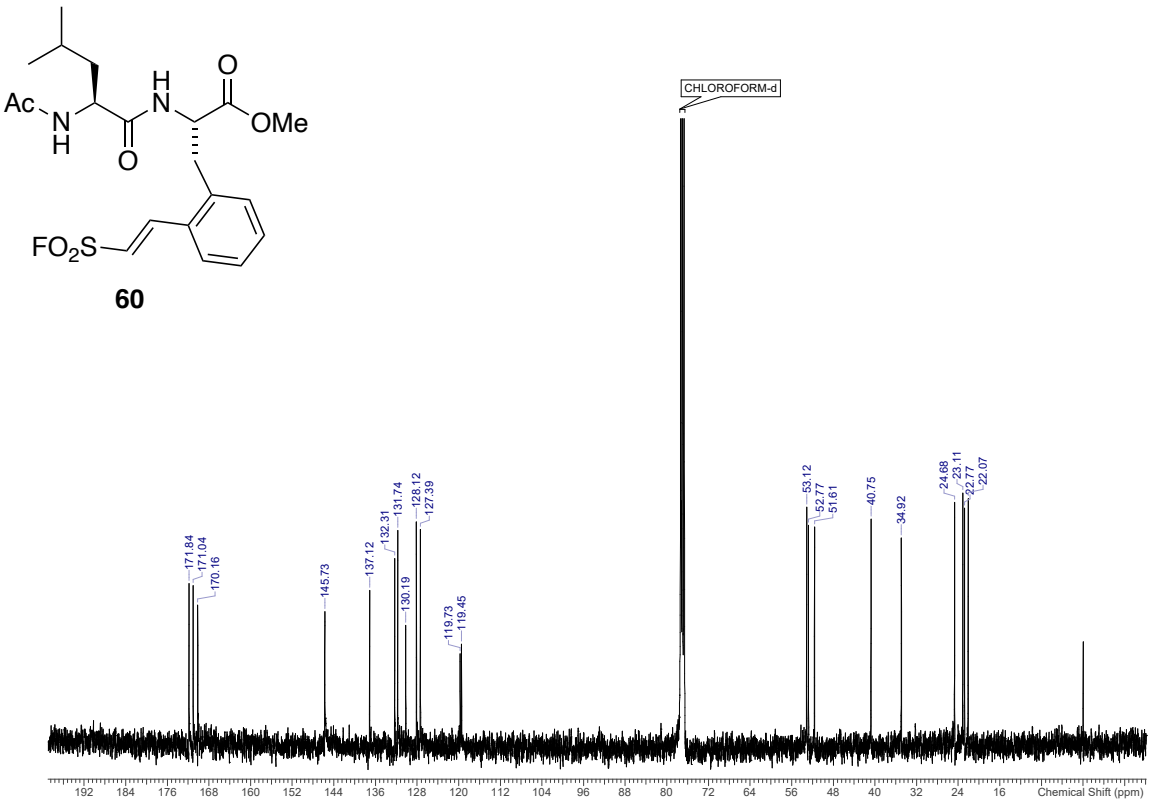
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **59**



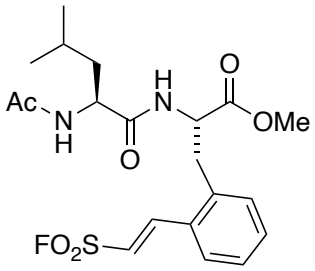
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **60**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **60**

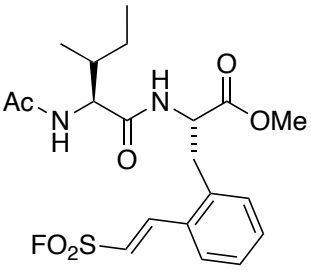


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **60**



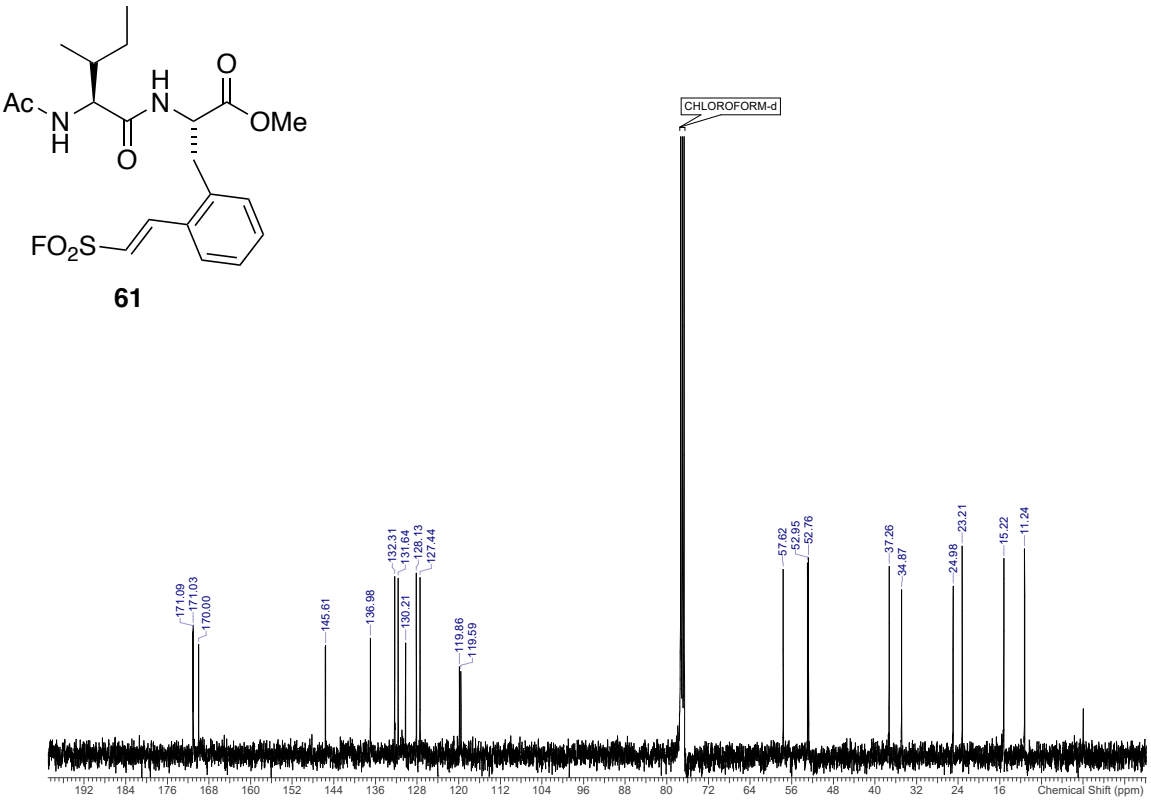
60

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **61**

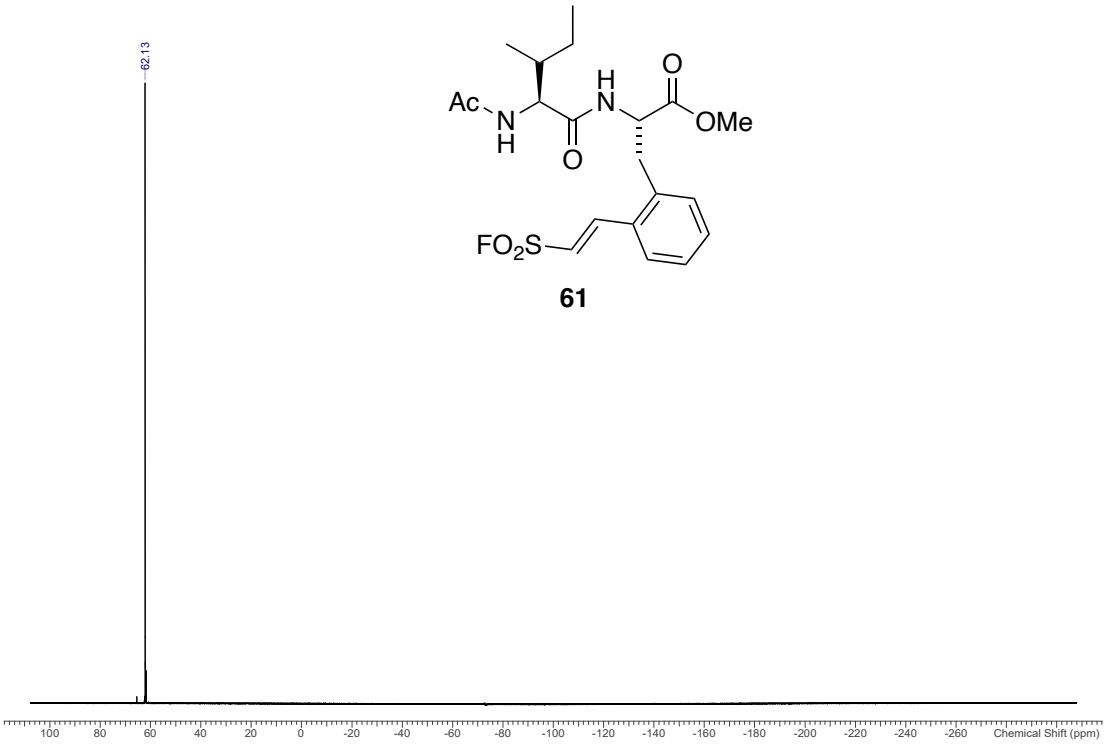


61

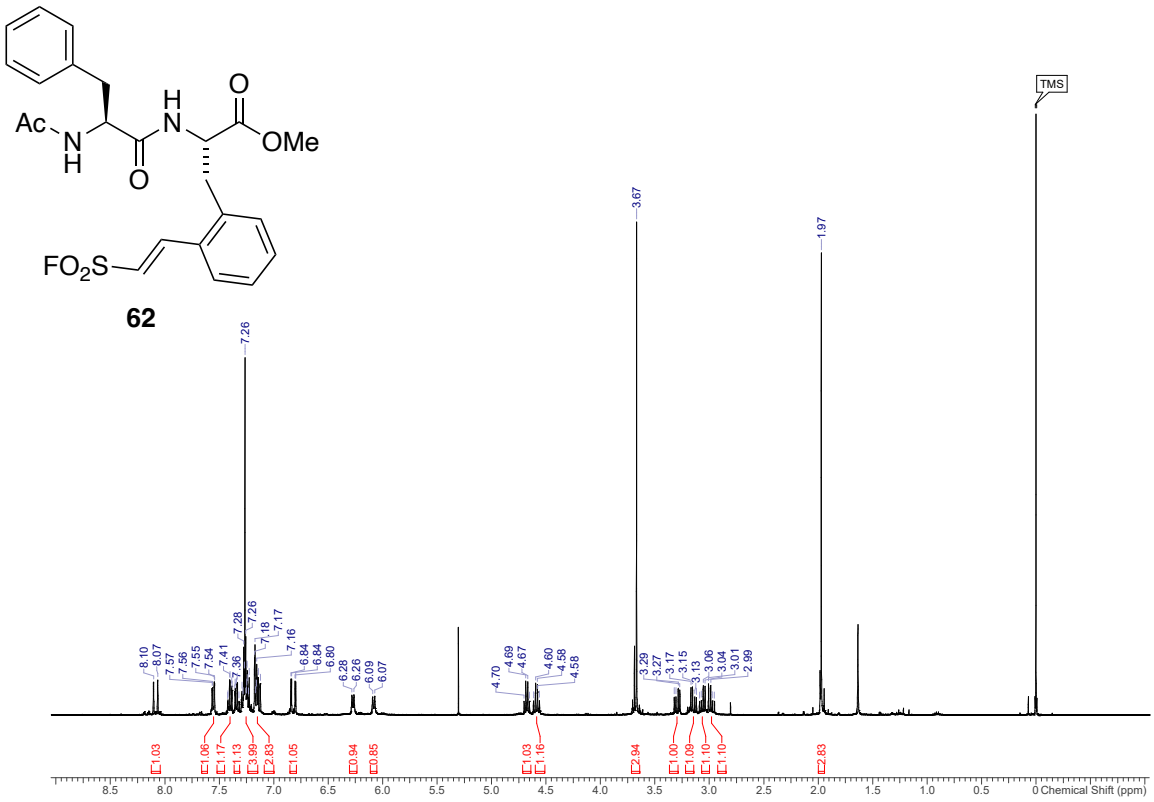
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **61**



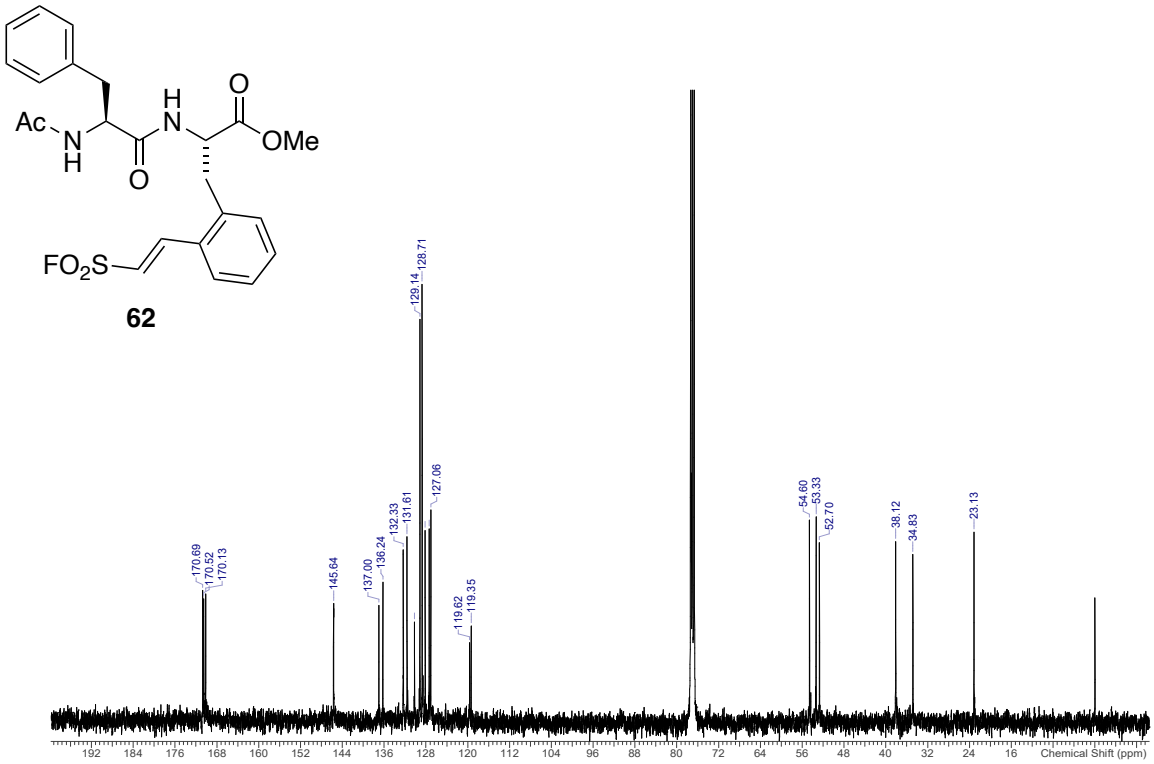
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **61**



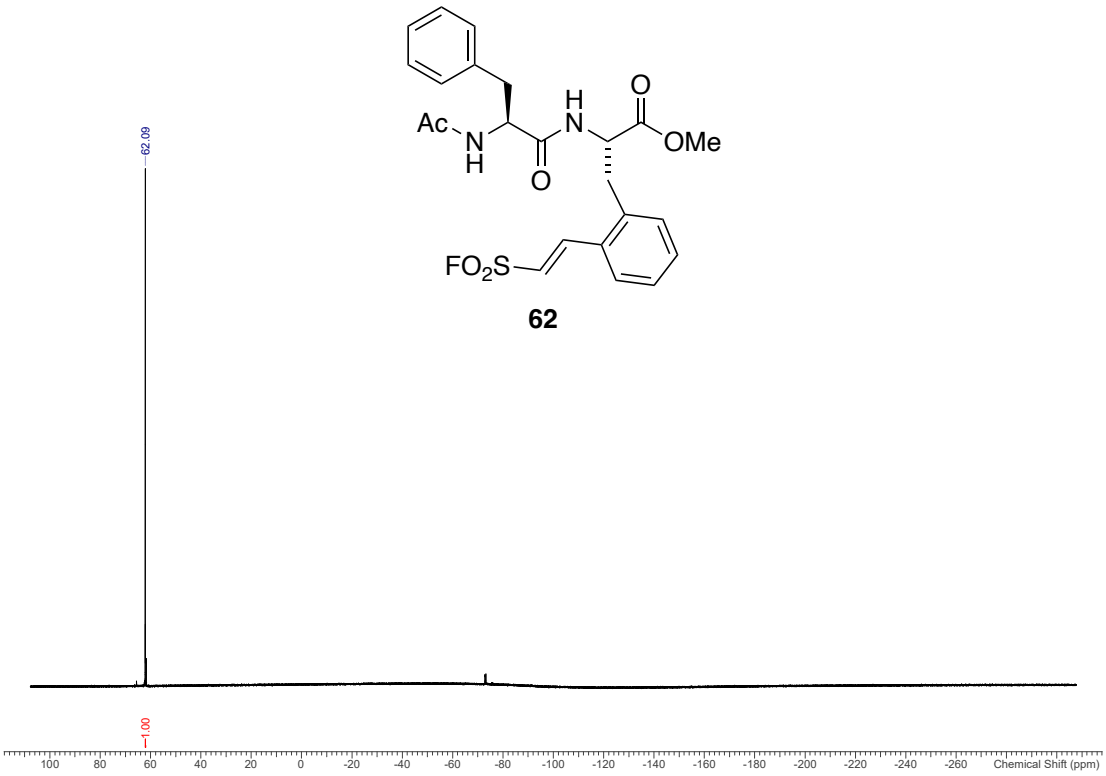
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **62**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **62**

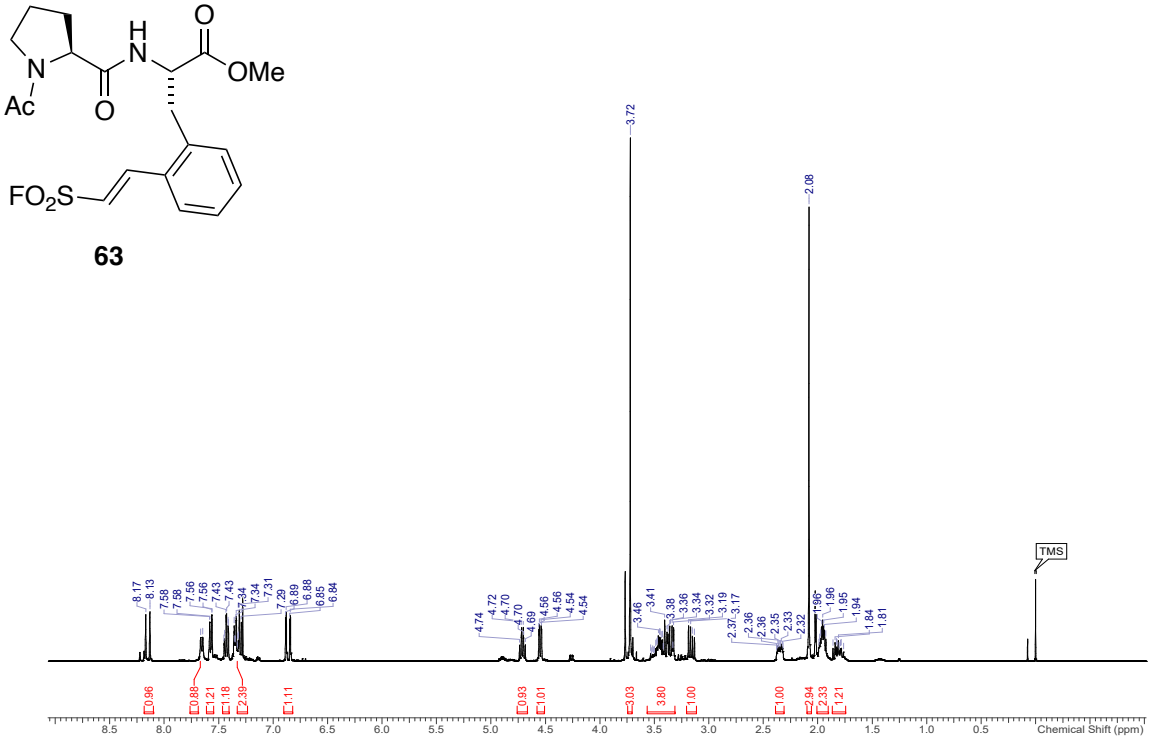


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **62**



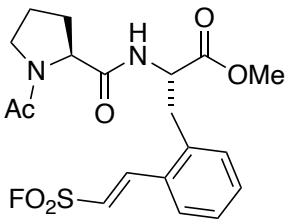
62

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **63**

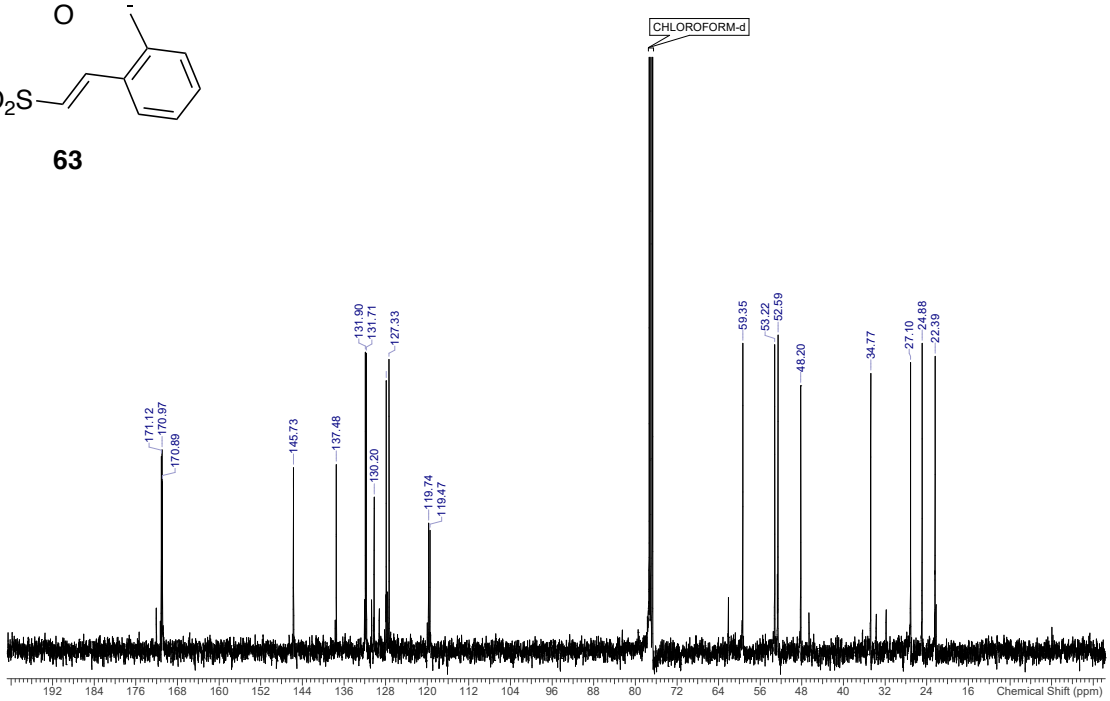


63

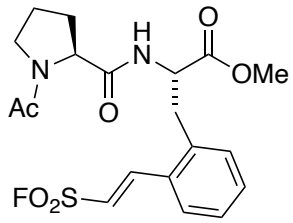
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **63**



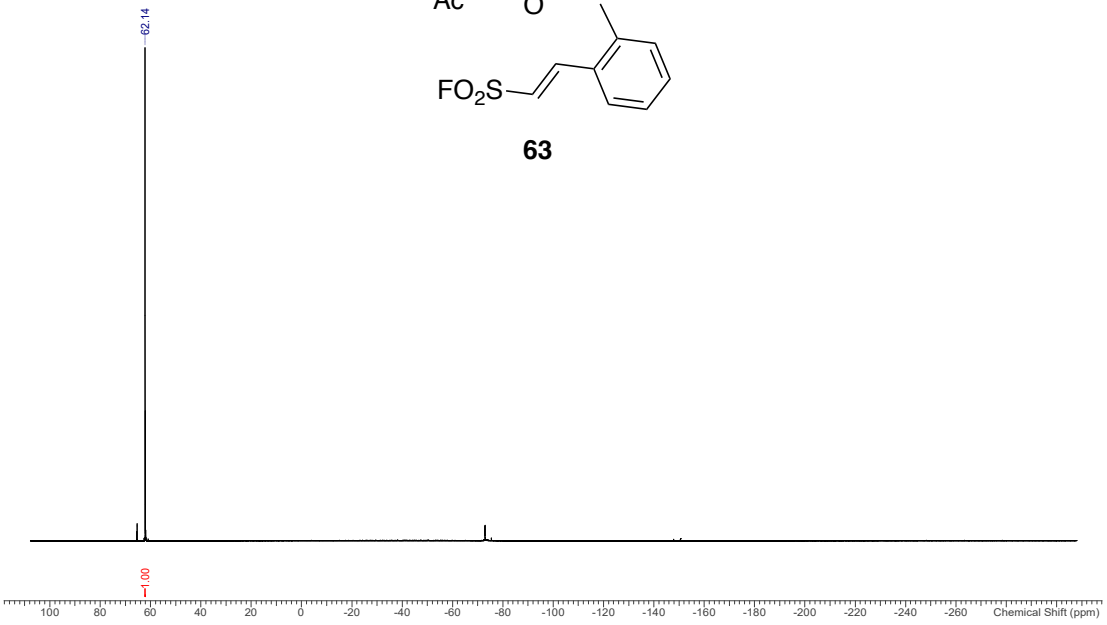
63



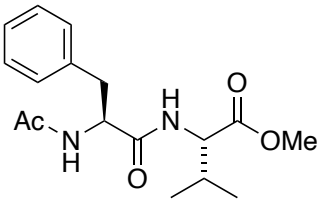
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **63**



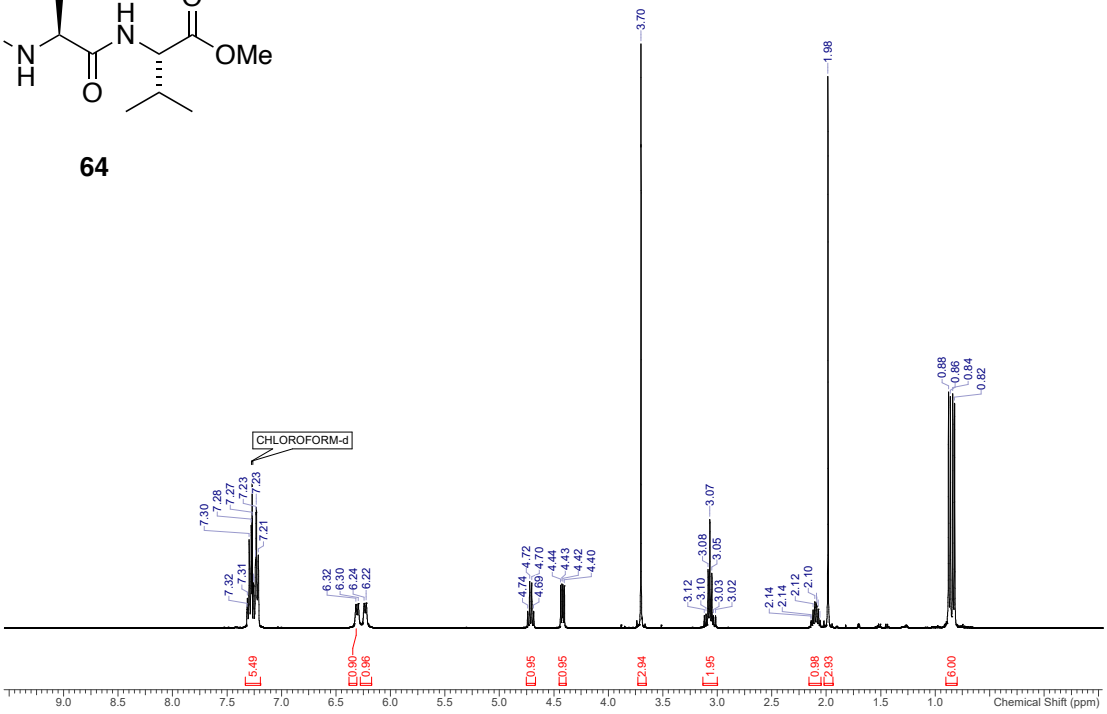
63



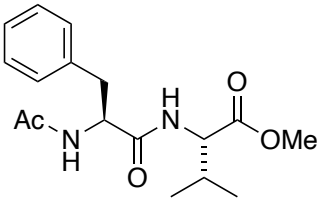
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **64**



64

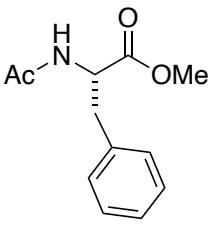


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **64**

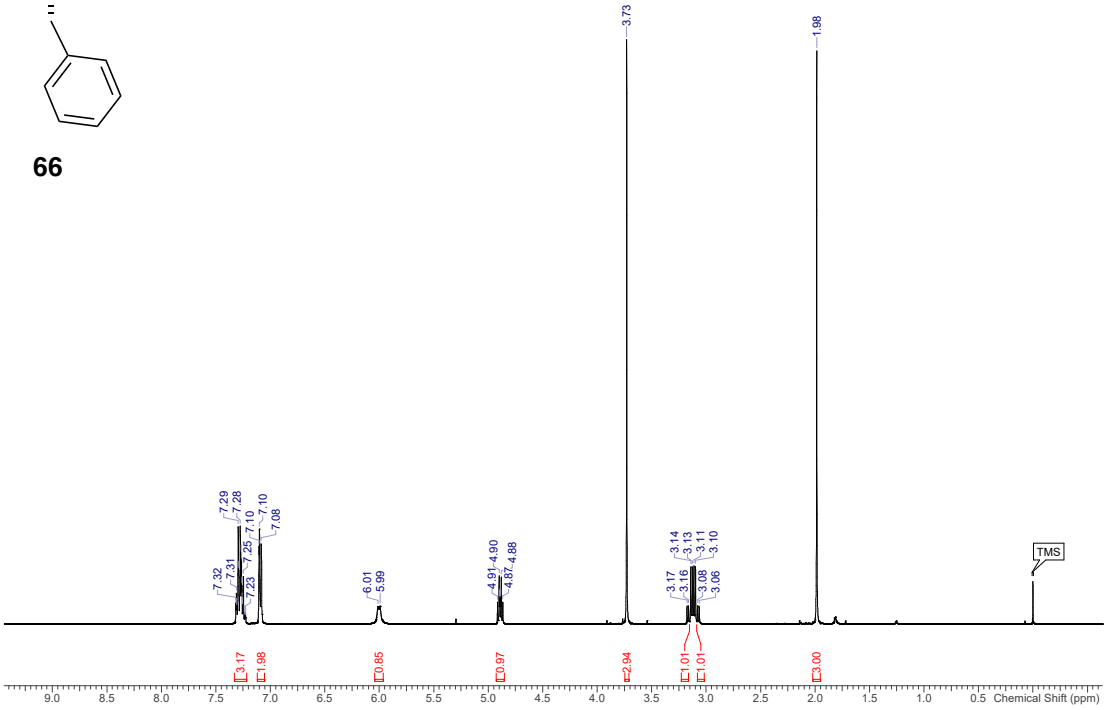


64

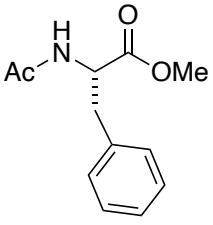
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **66**



66

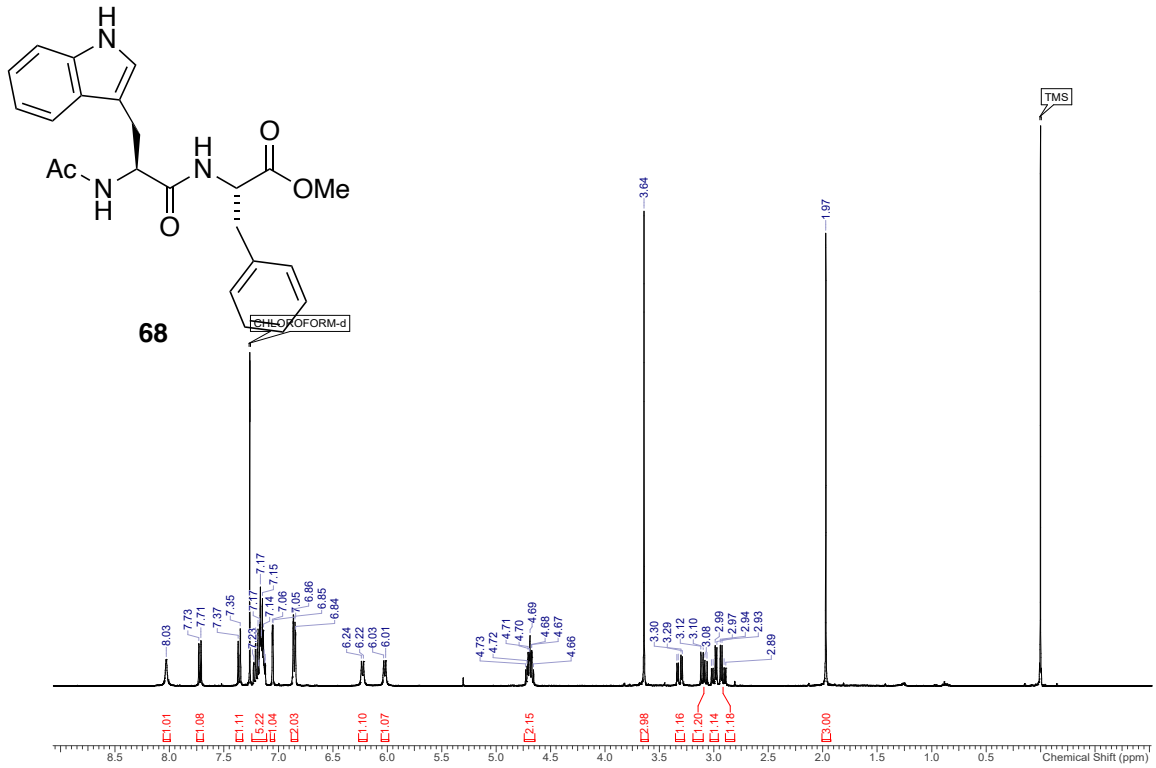


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **66**

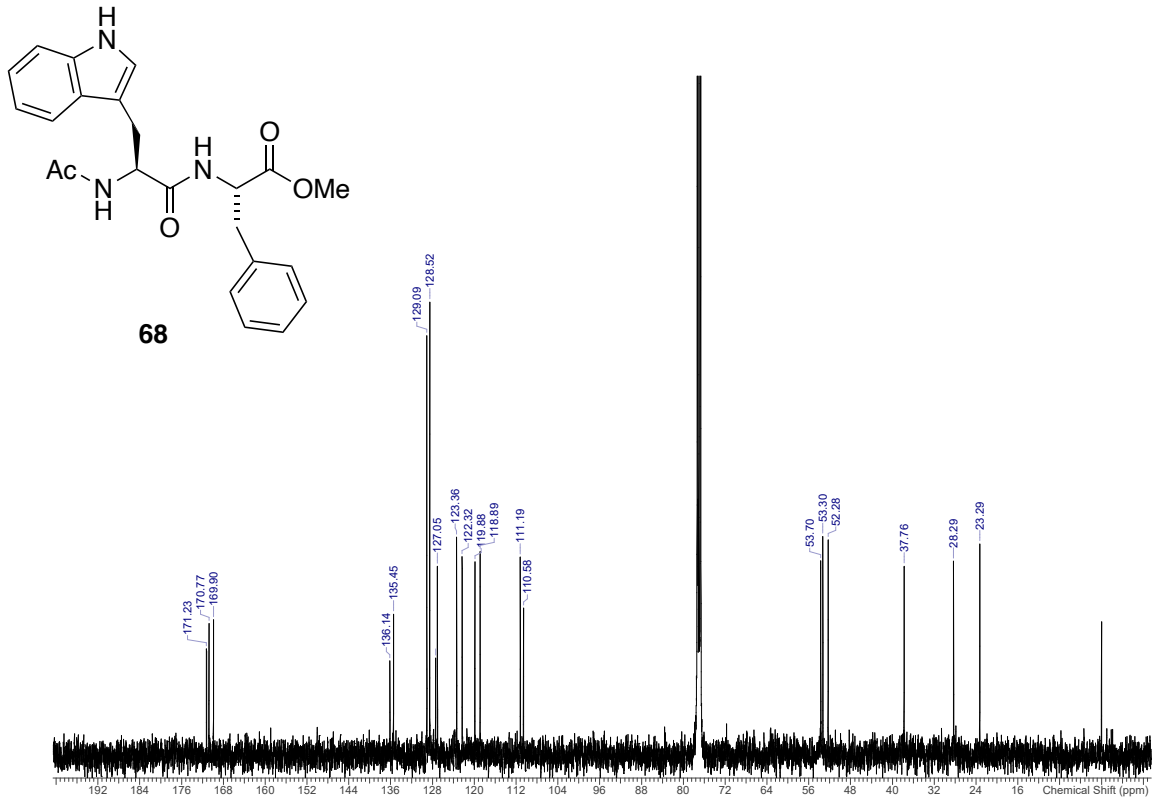


66

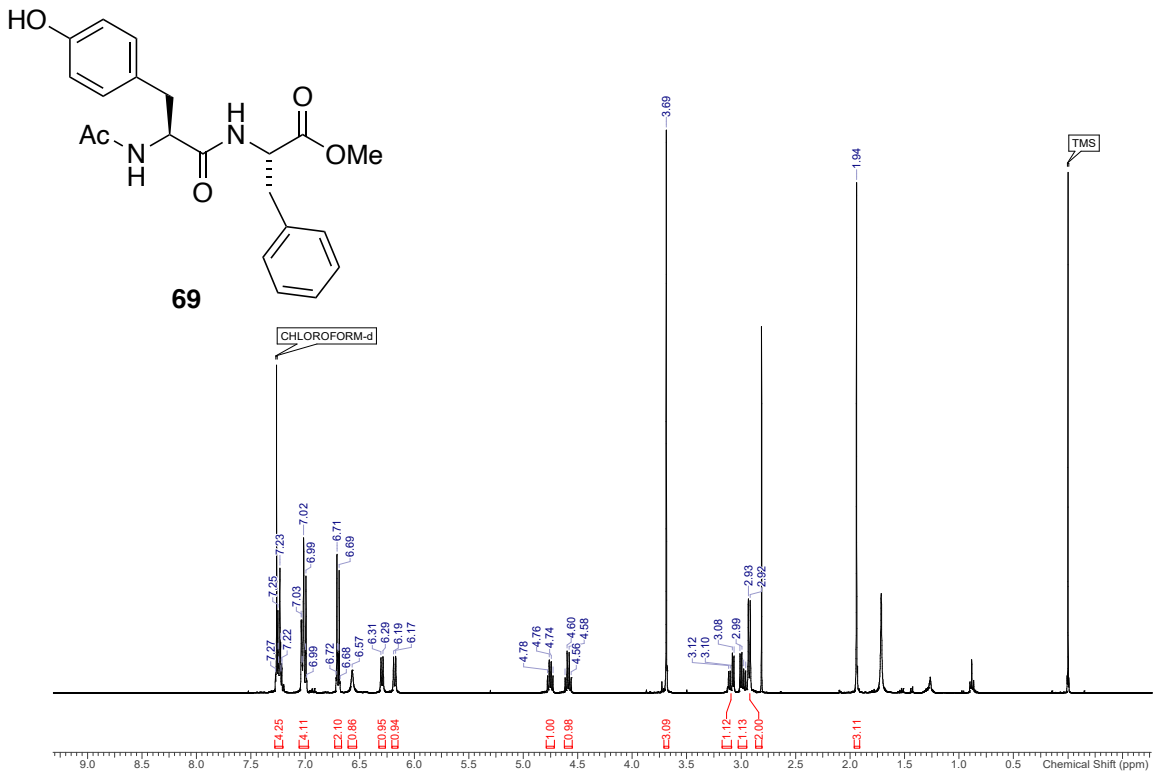
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **68**



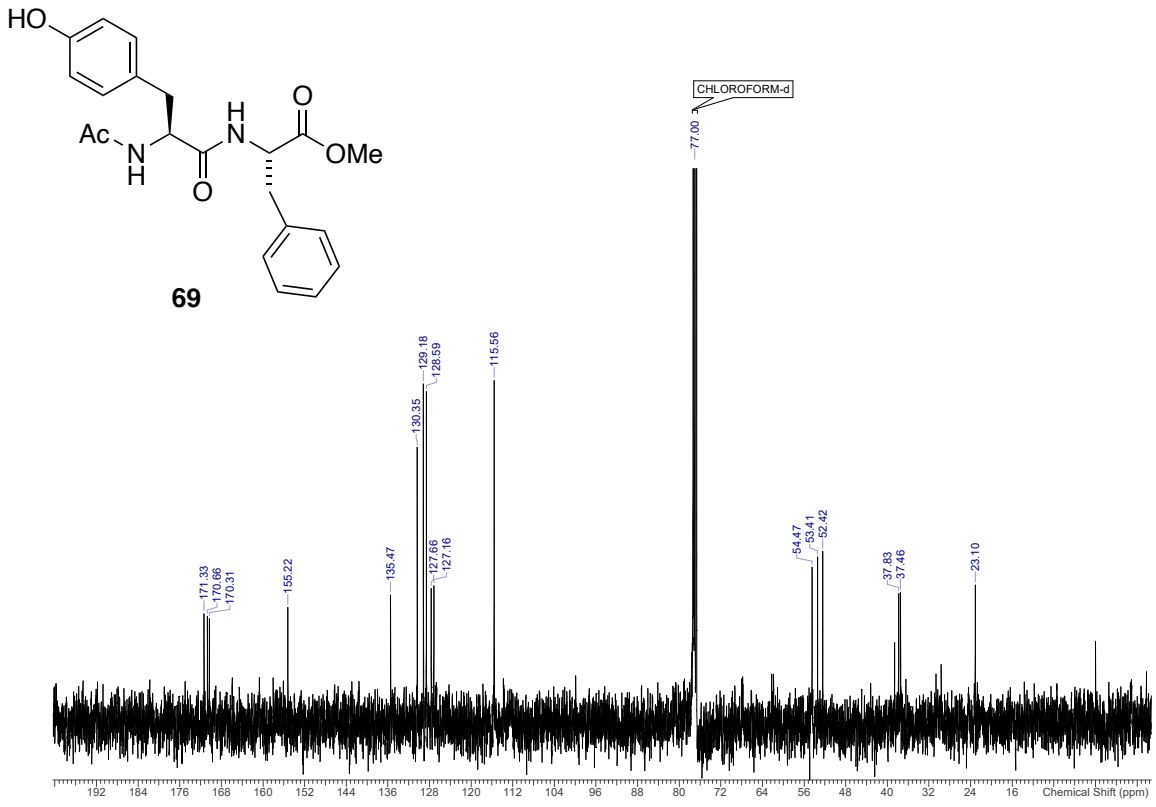
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **68**



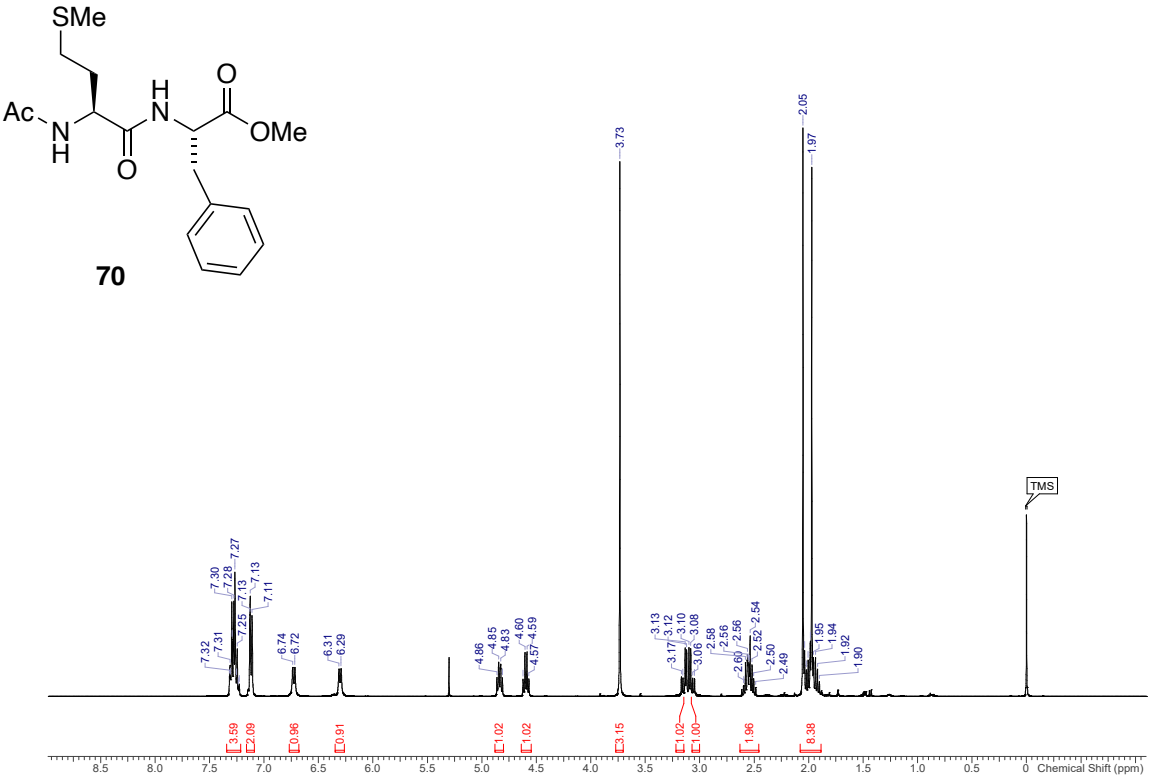
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **69**



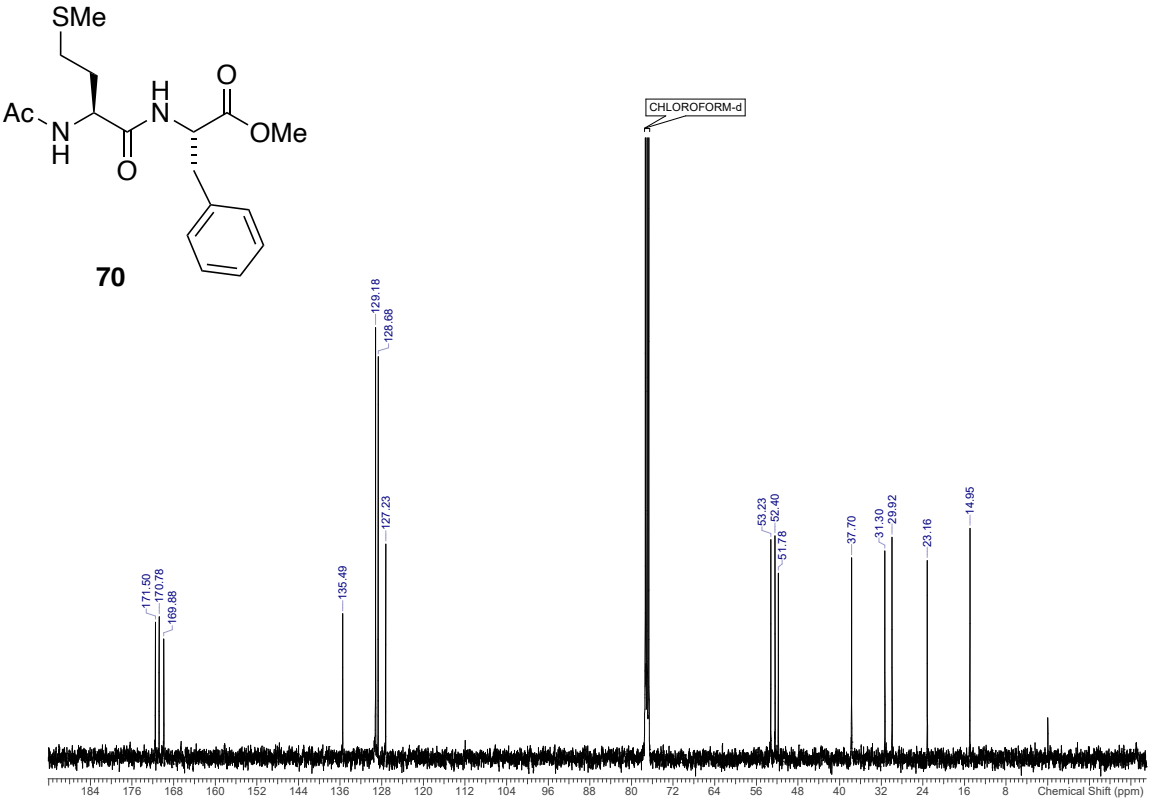
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **69**



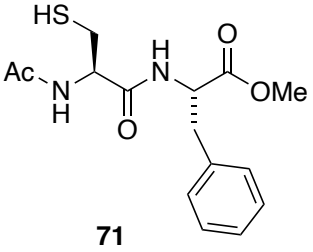
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **70**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **70**

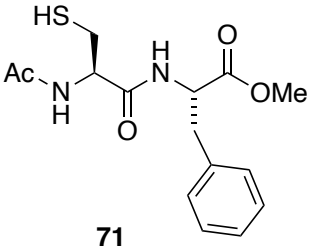


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **71**



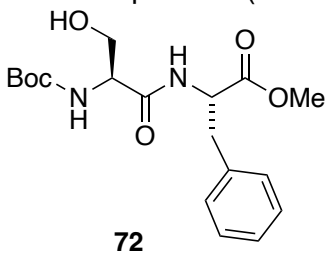
71

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **71**



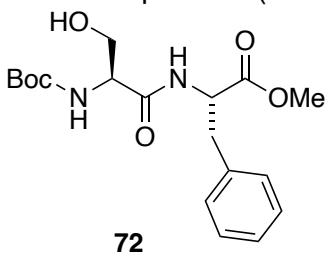
71

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **72**



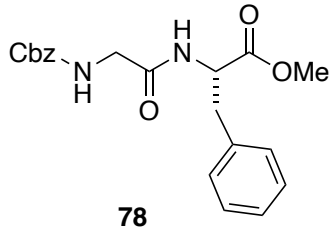
72

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **72**

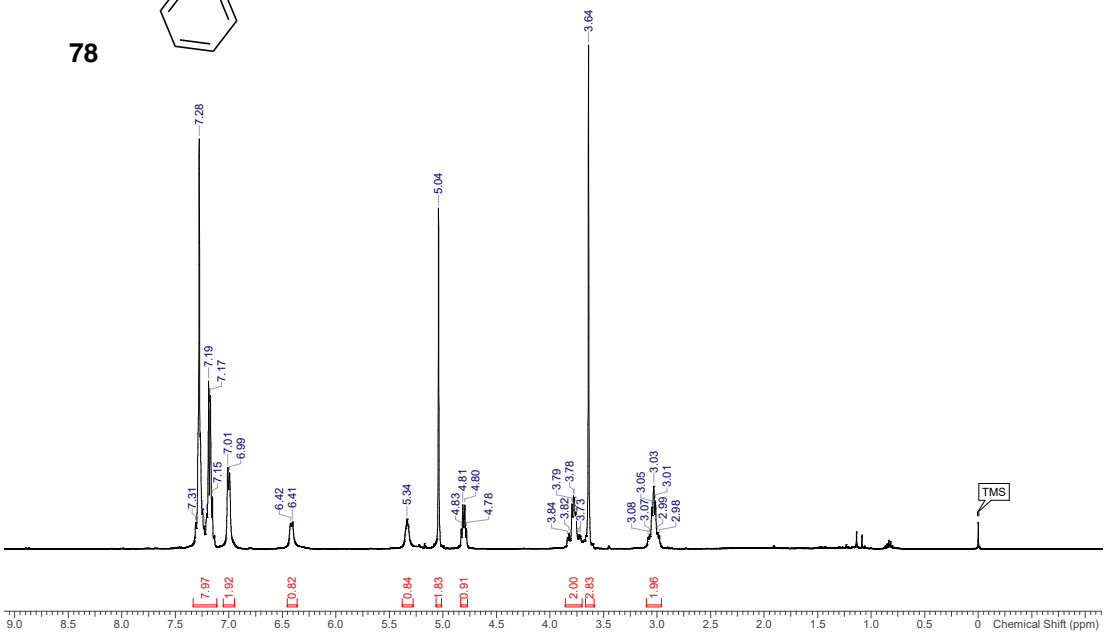


72

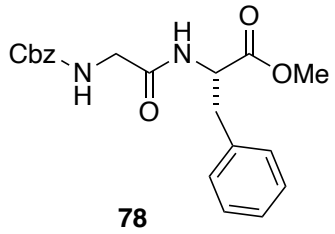
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **78**



78

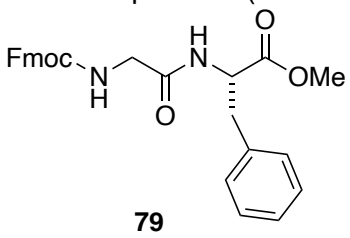


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **78**

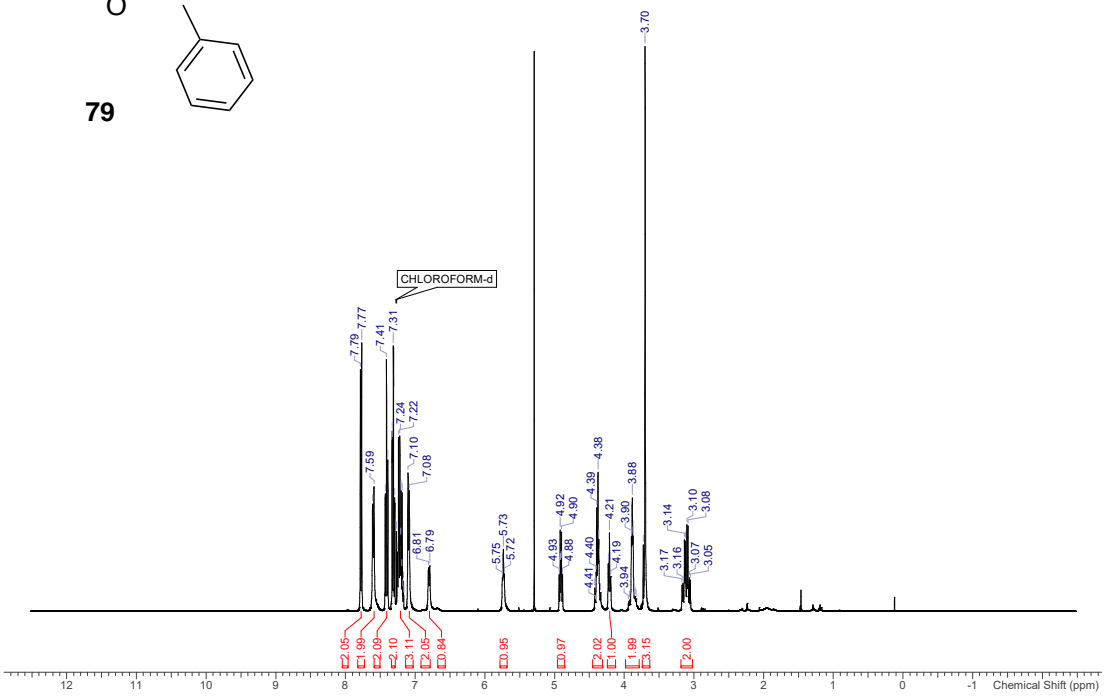


78

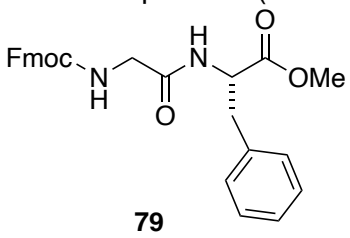
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **79**



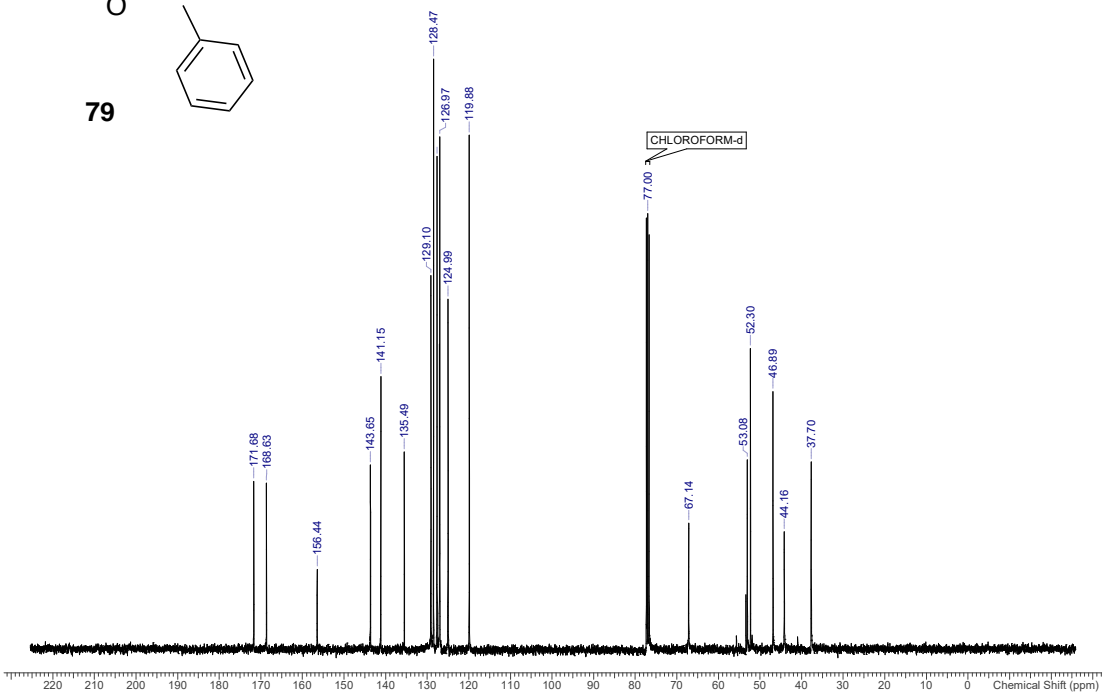
79



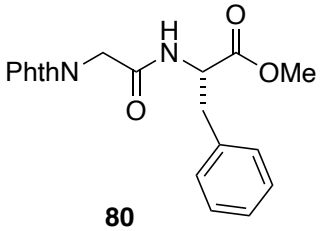
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **79**



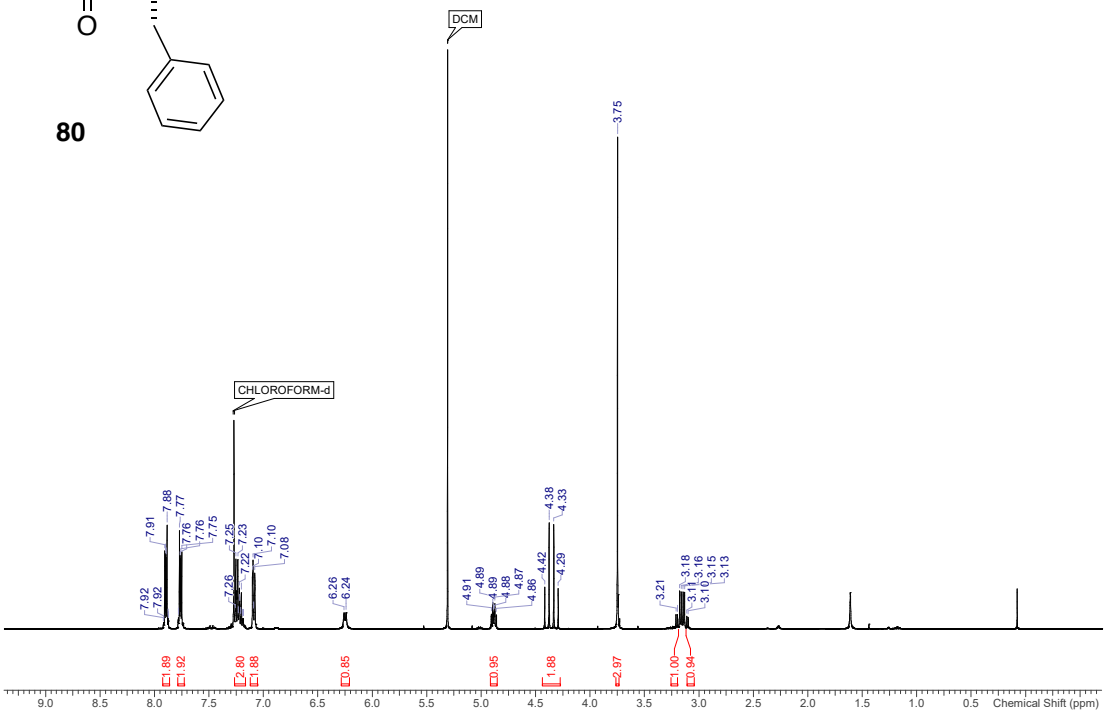
79



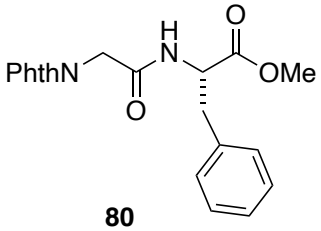
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **80**



80

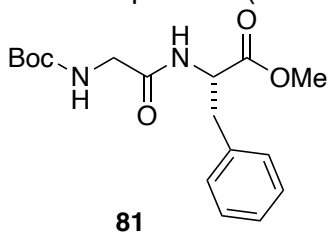


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **80**



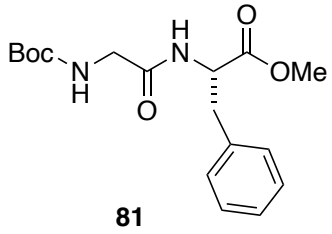
80

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **81**



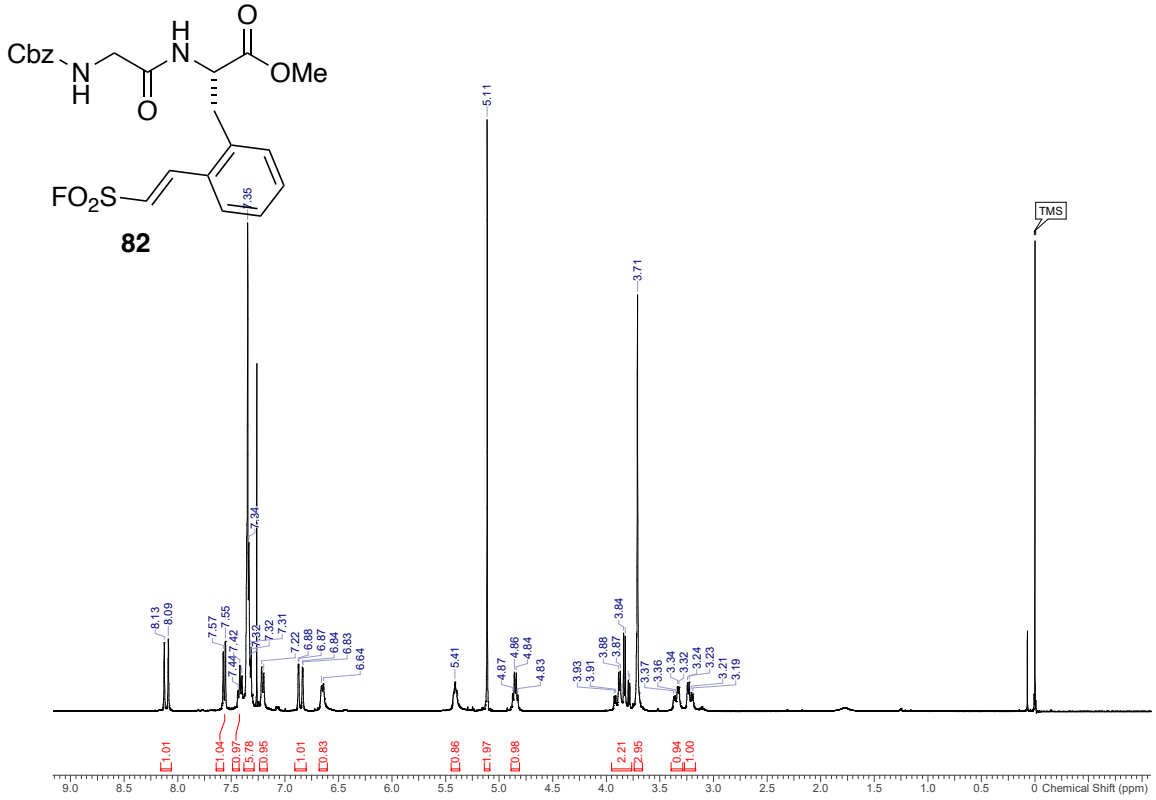
81

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **81**

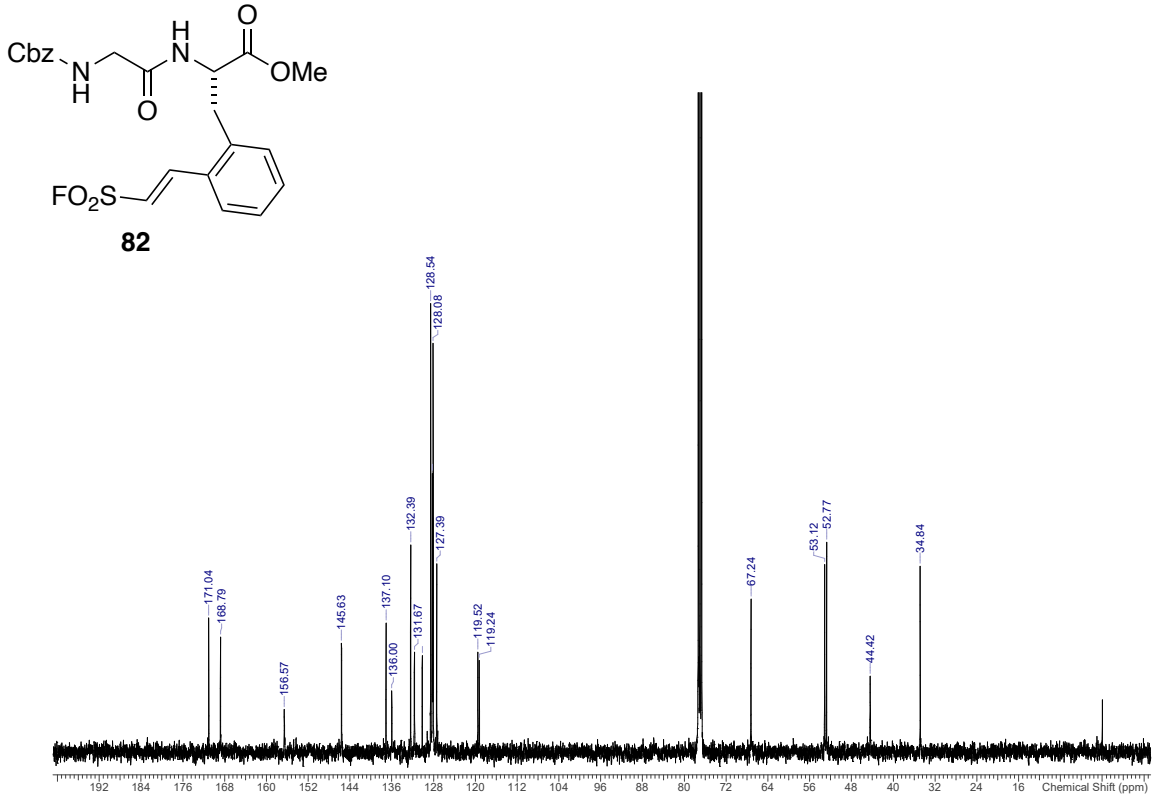


81

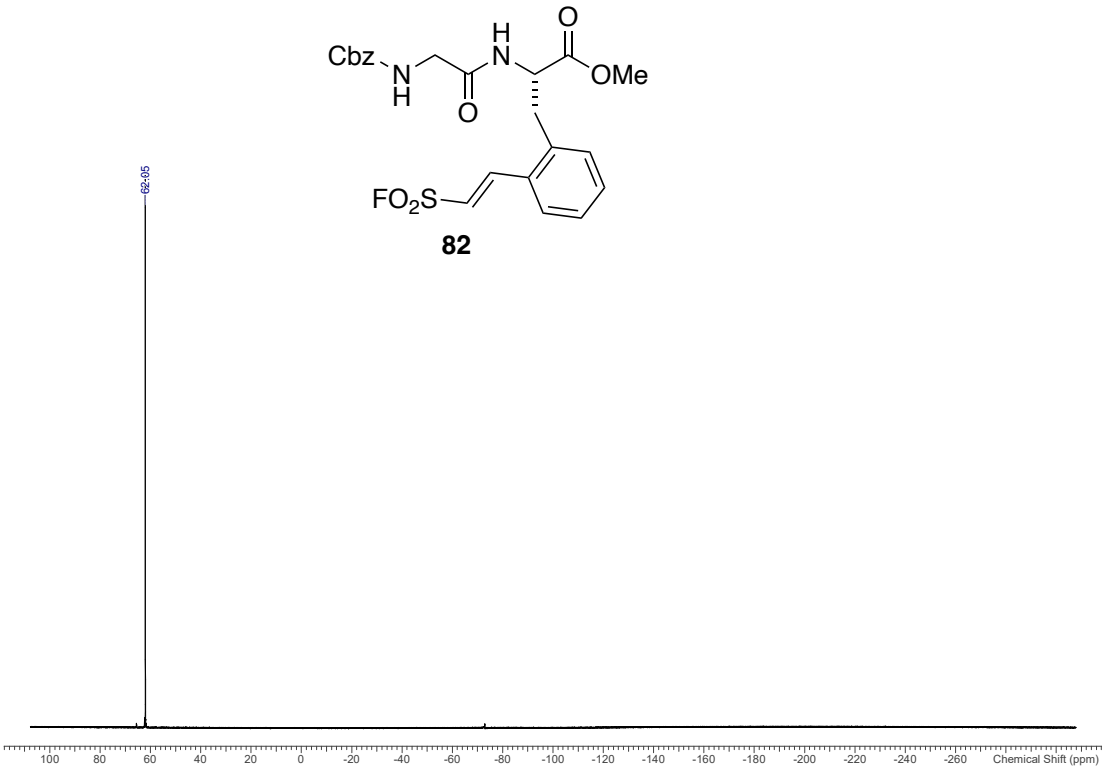
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **82**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **82**

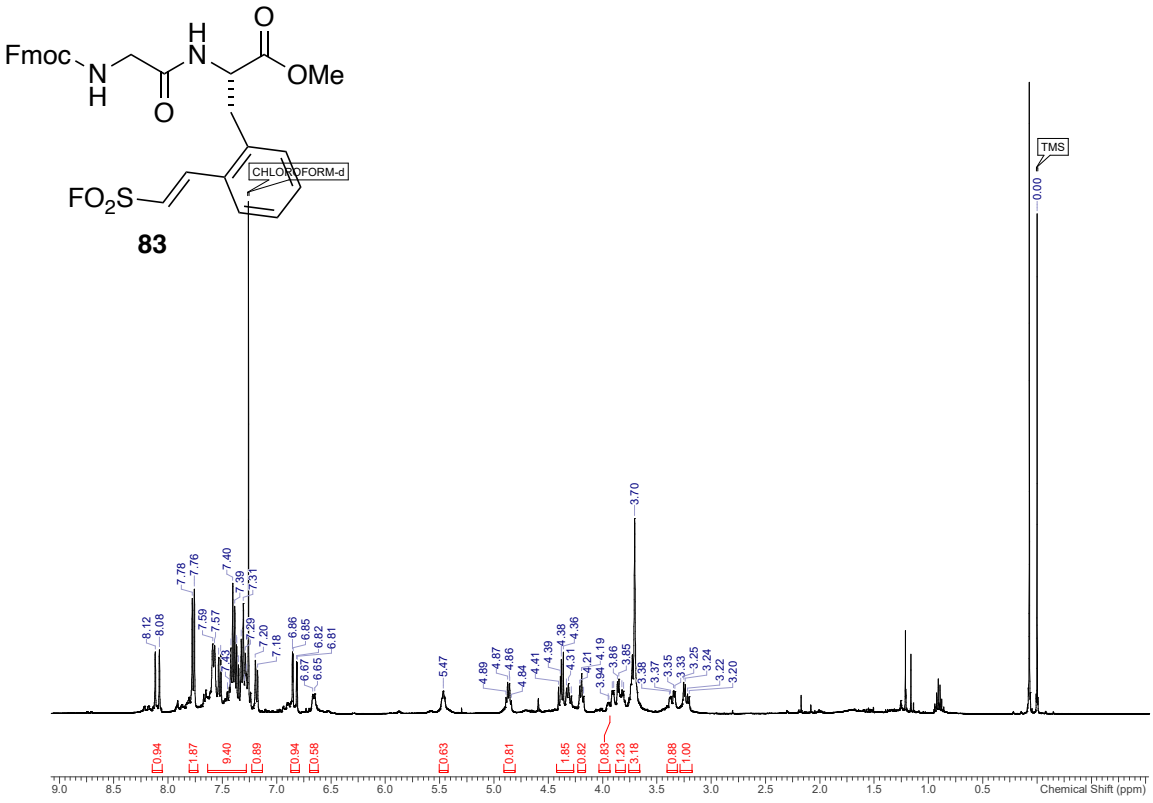


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **82**

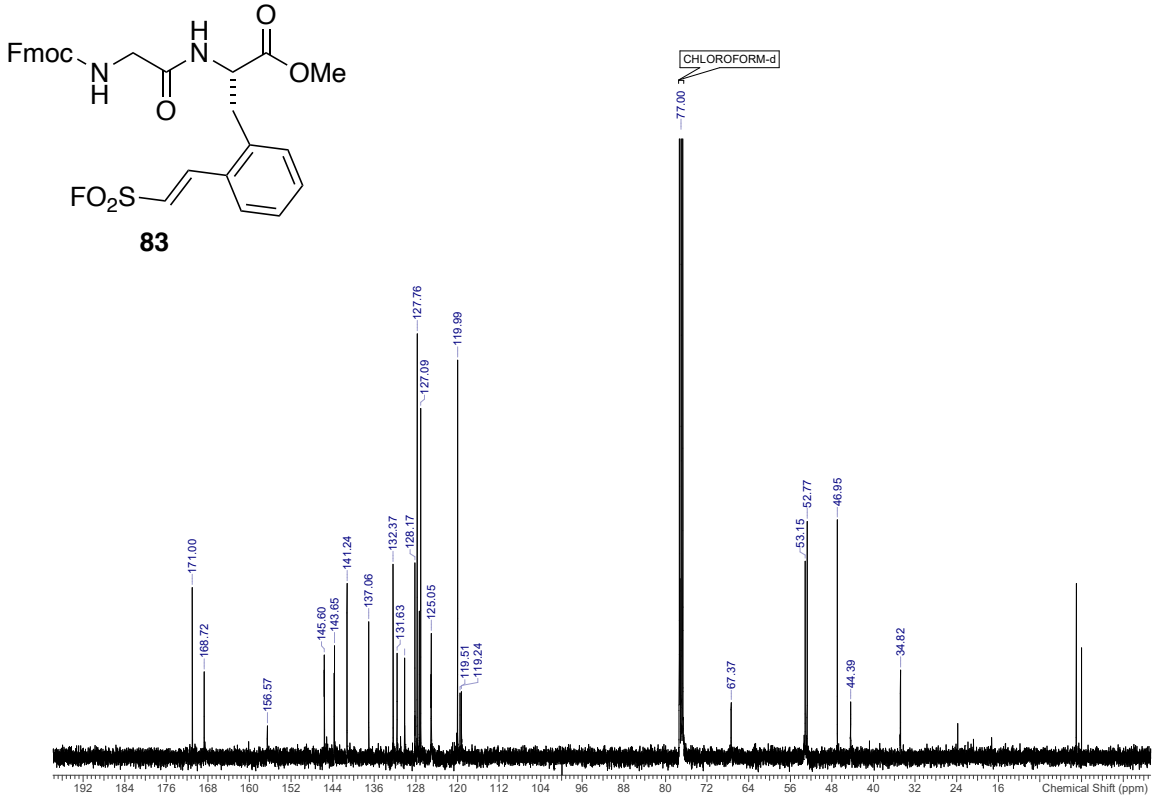


82

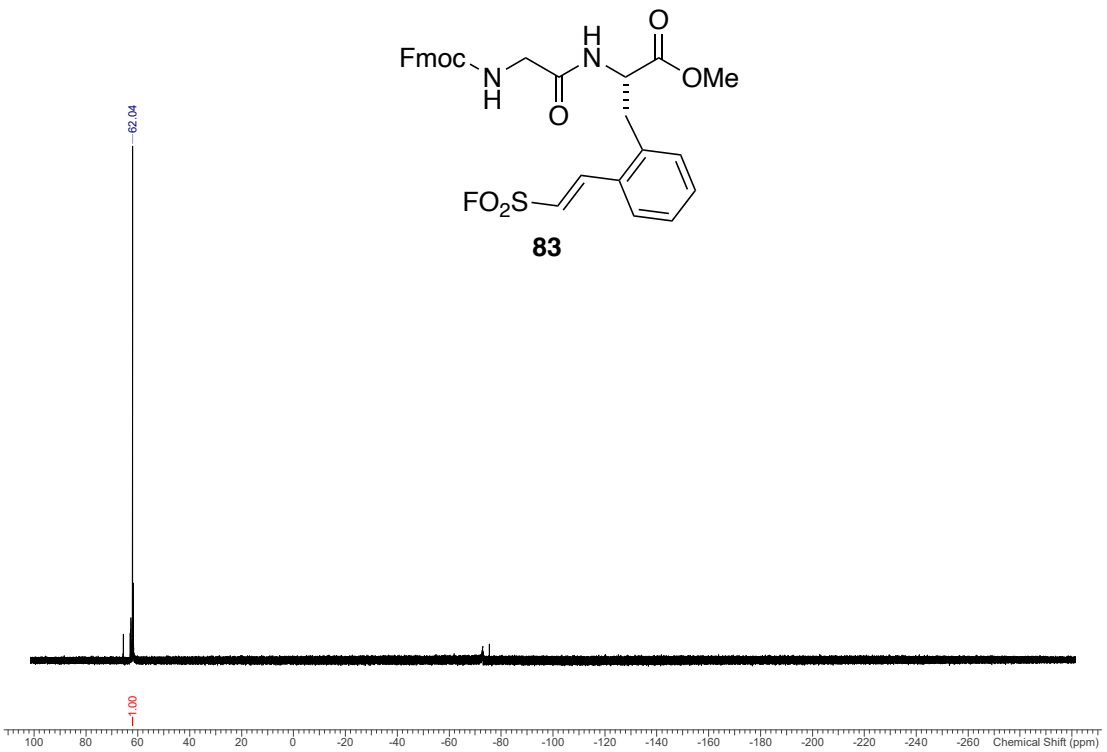
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **83**



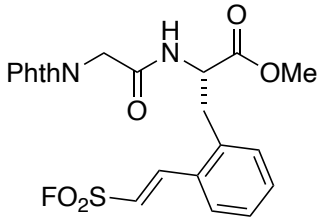
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **83**



^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **83**

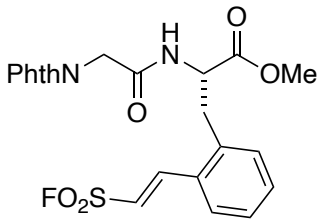


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **84**



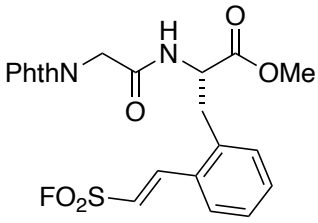
84

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **84**

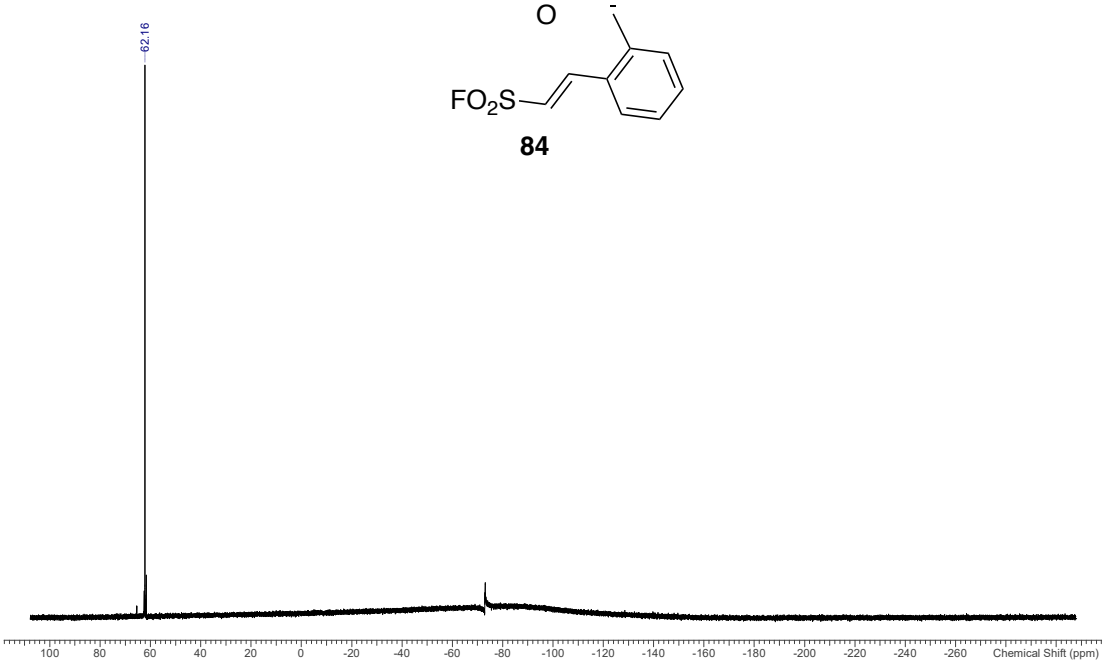


84

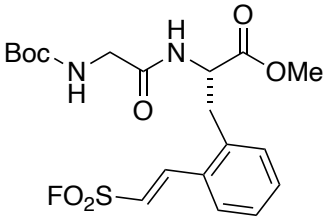
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **84**



84

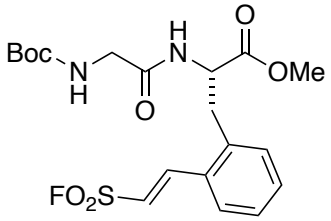


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **85**



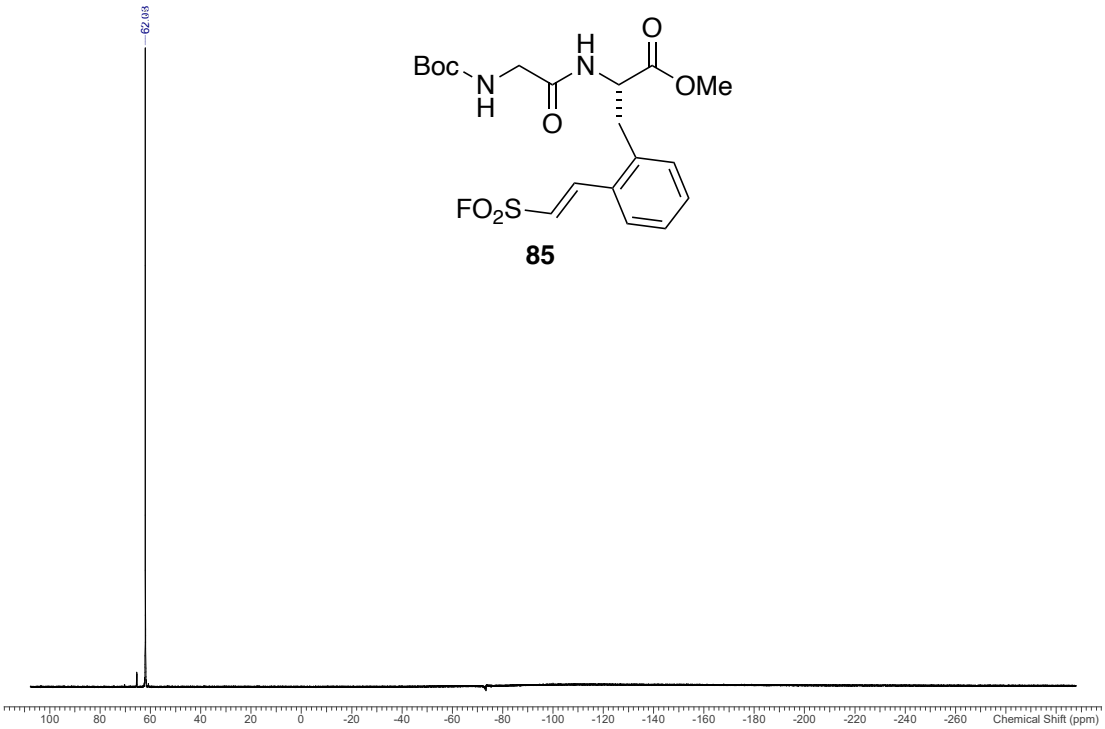
85

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **85**



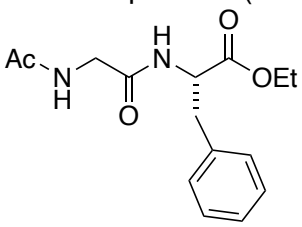
85

^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **85**

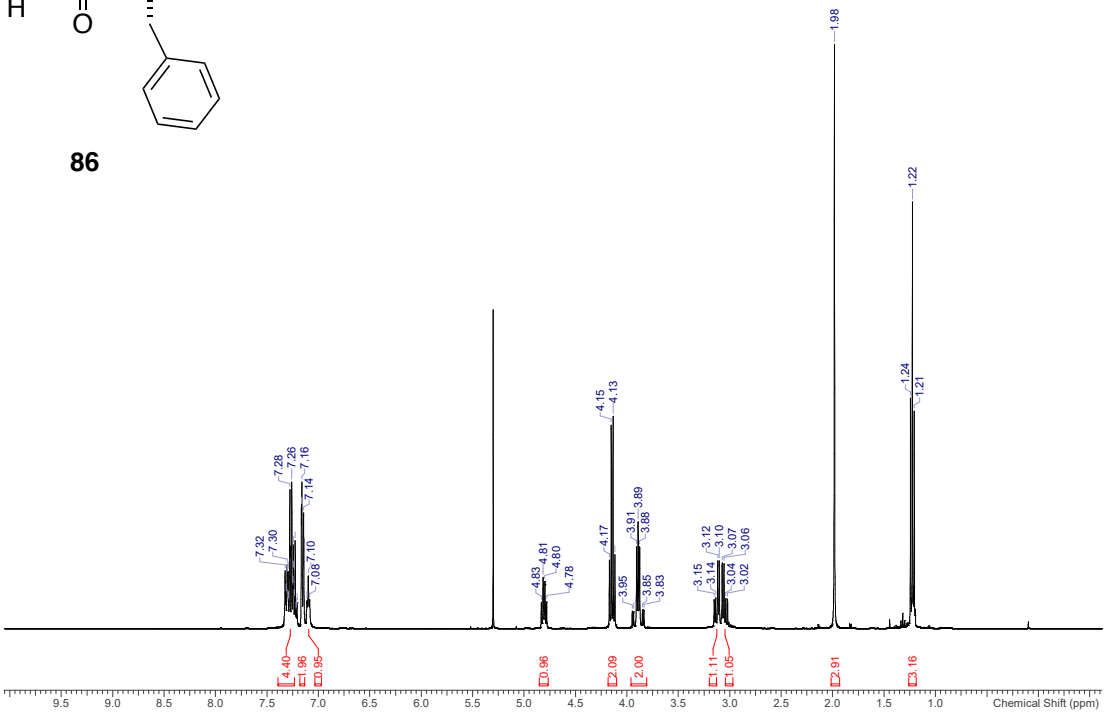


85

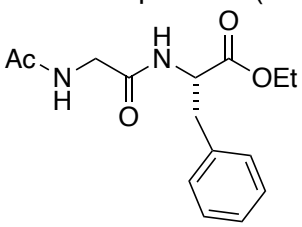
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **86**



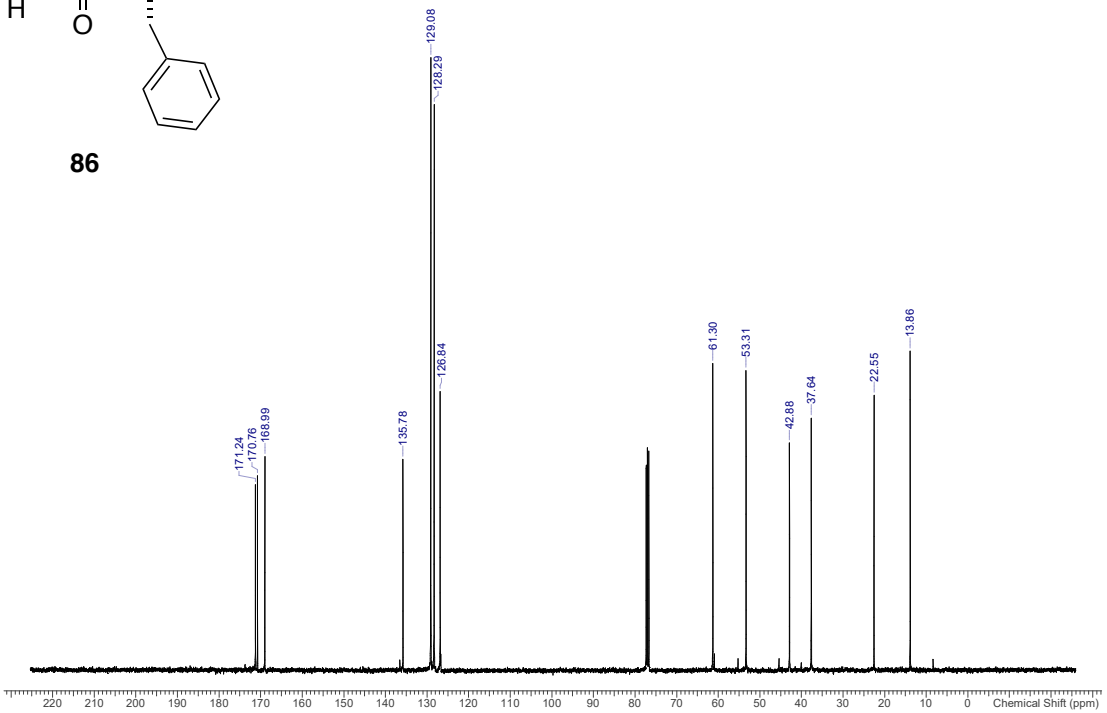
86



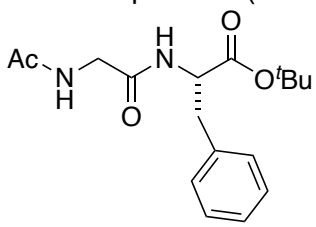
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **86**



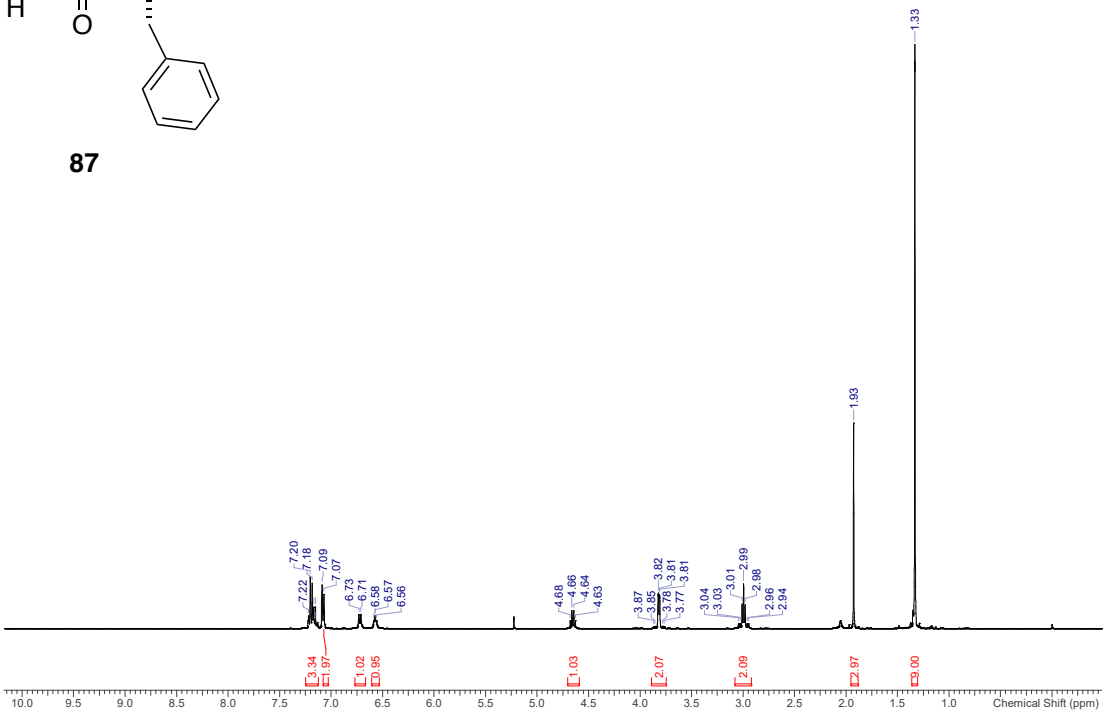
86



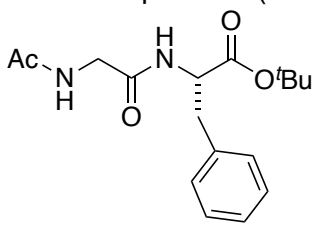
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **87**



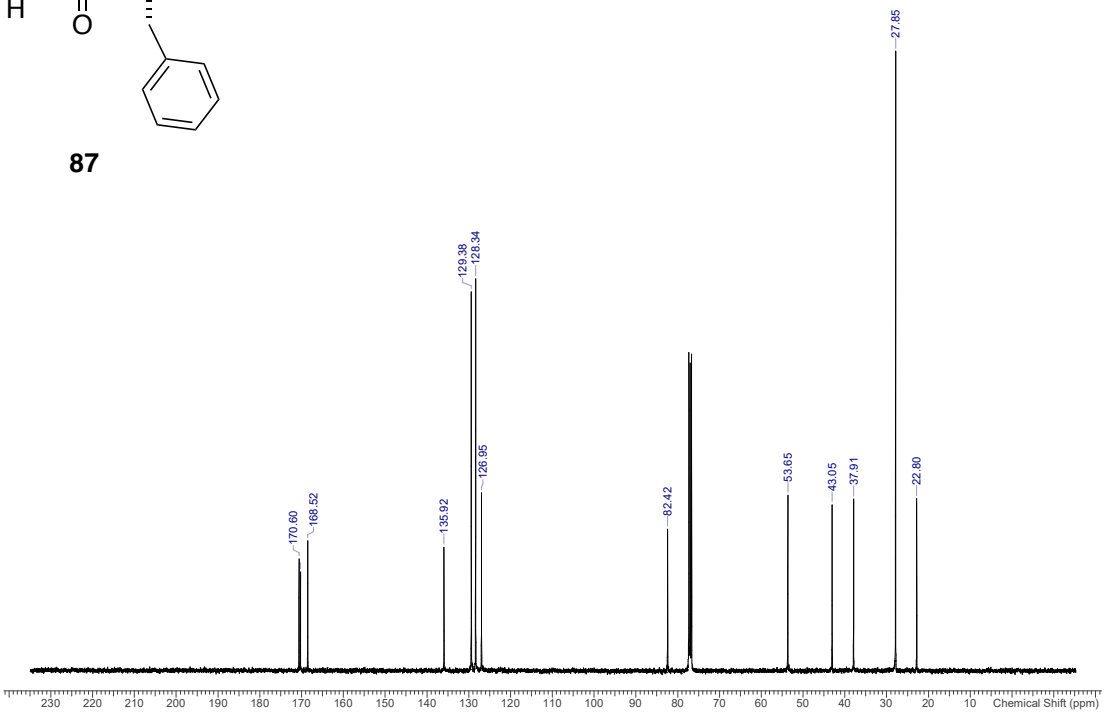
87



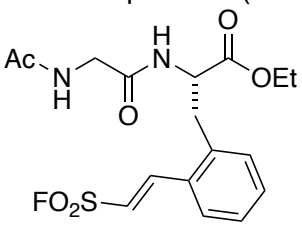
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **87**



87

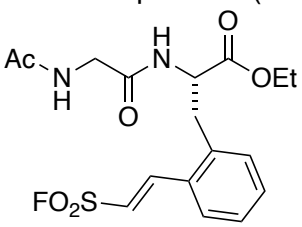


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **88**



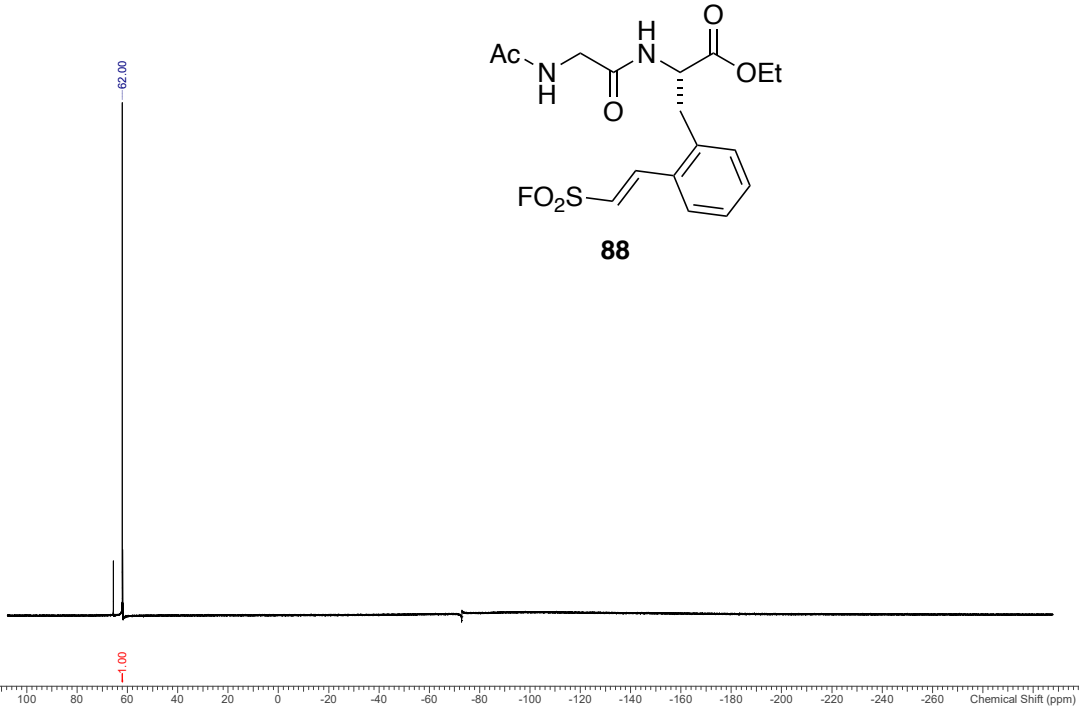
88

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **88**



88

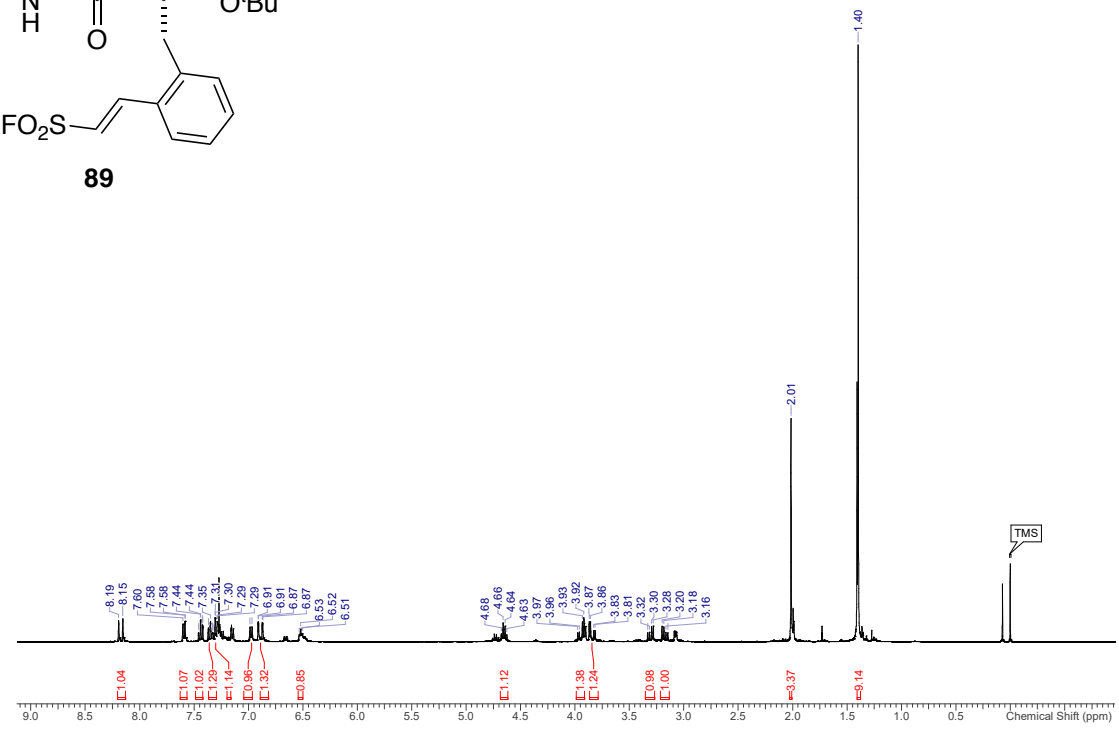
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **88**



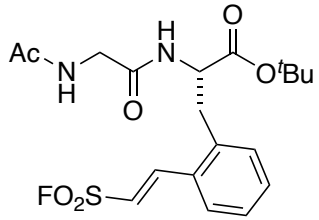
88

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **89**

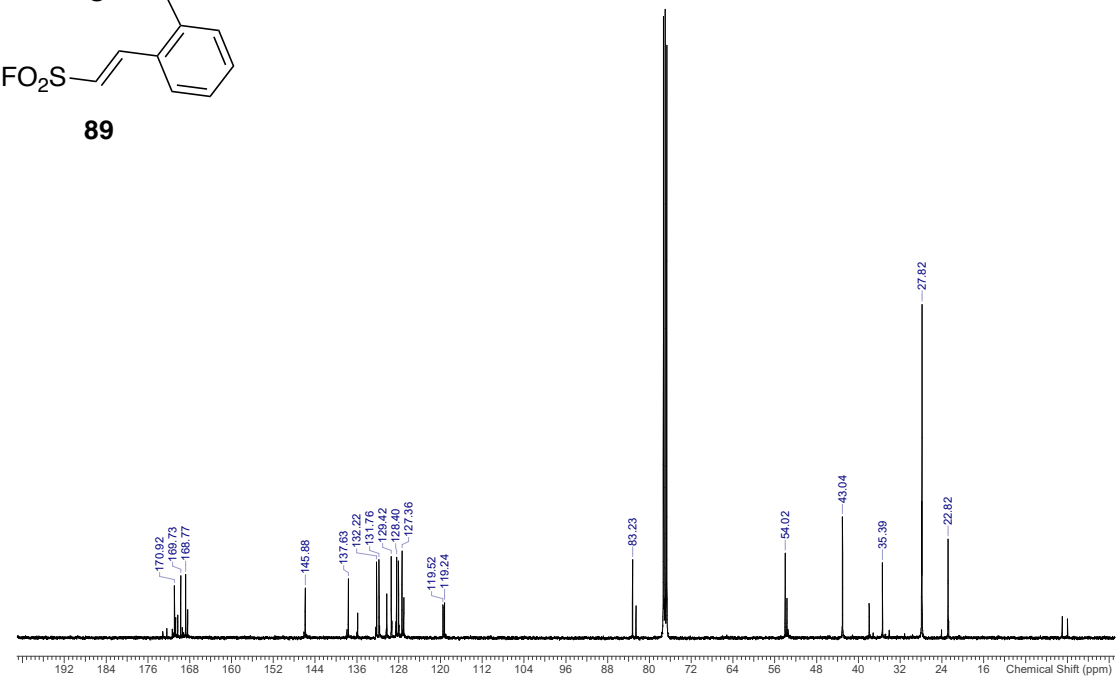
89



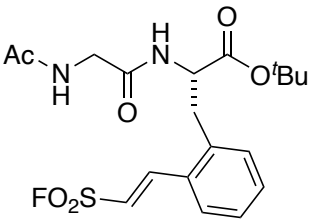
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **89**



89

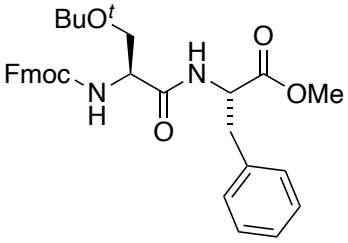


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **89**

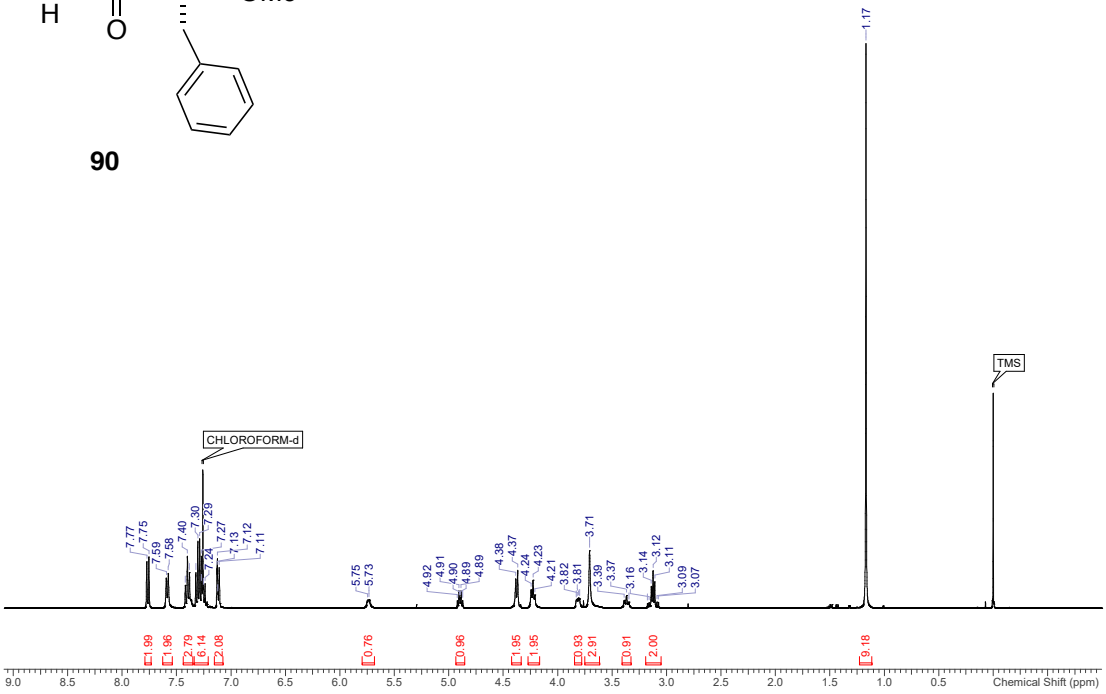


89

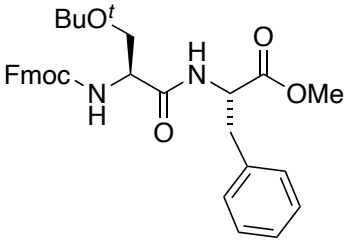
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **90**



90

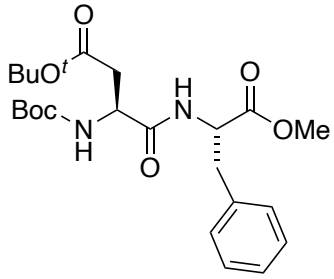


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **90**



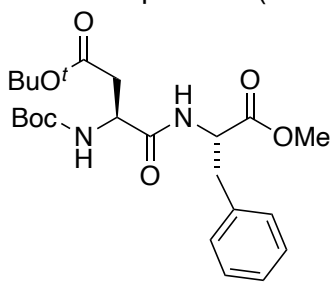
90

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **91**

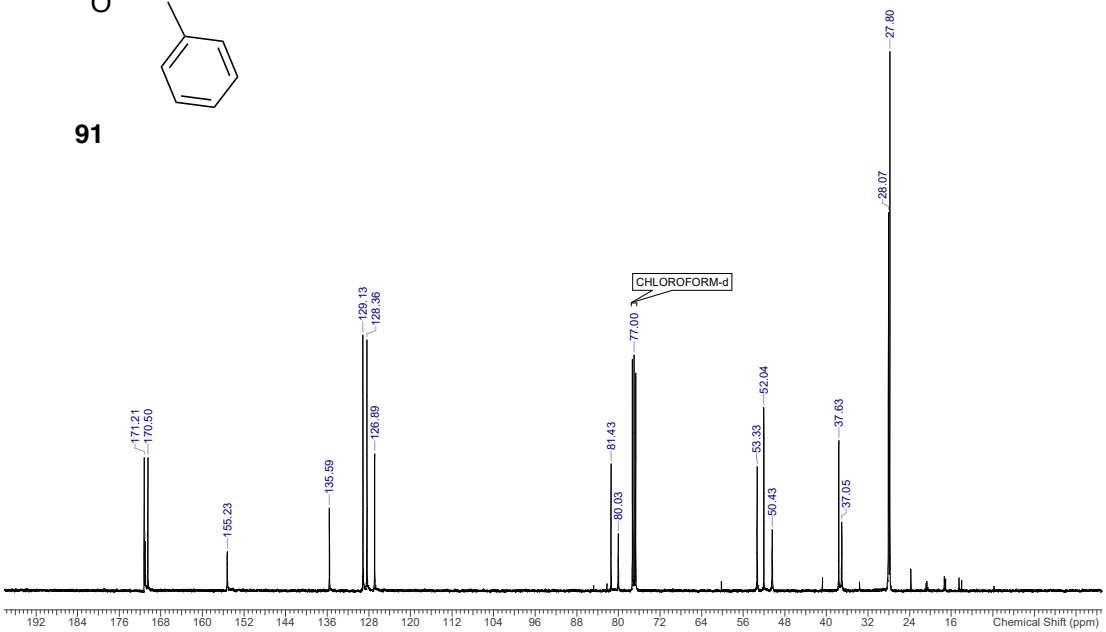


91

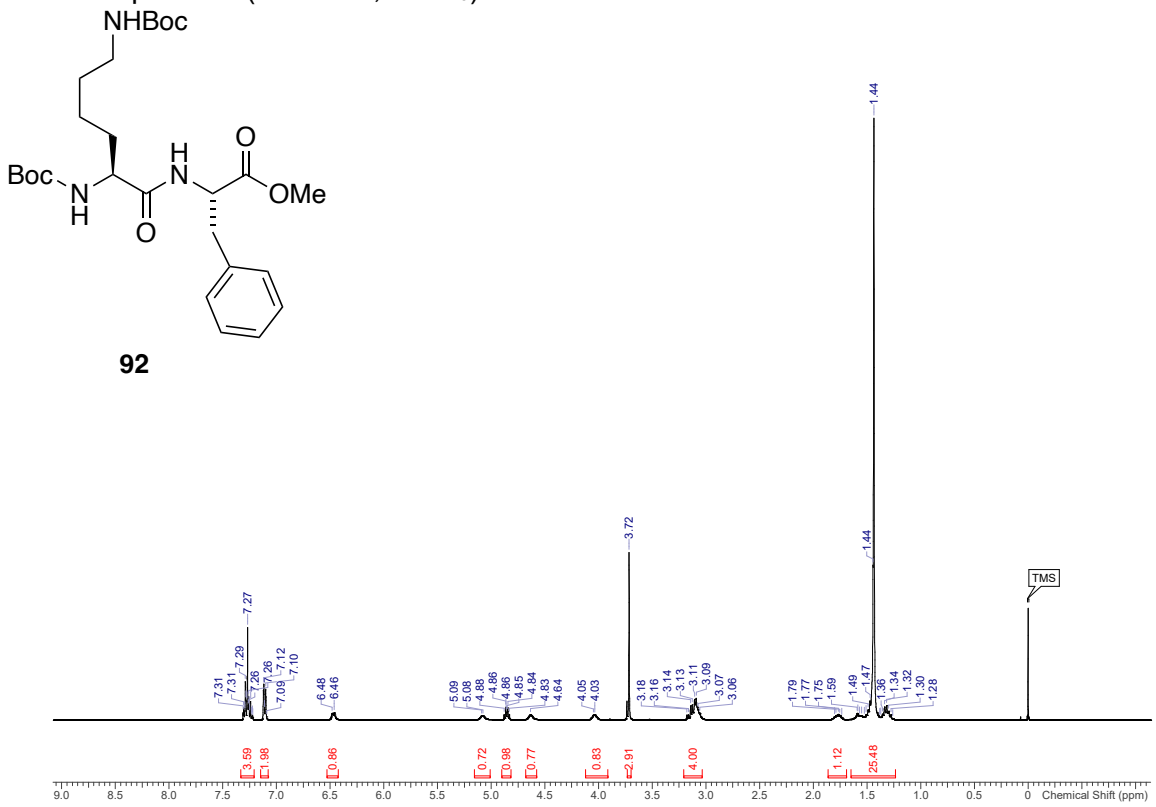
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **91**



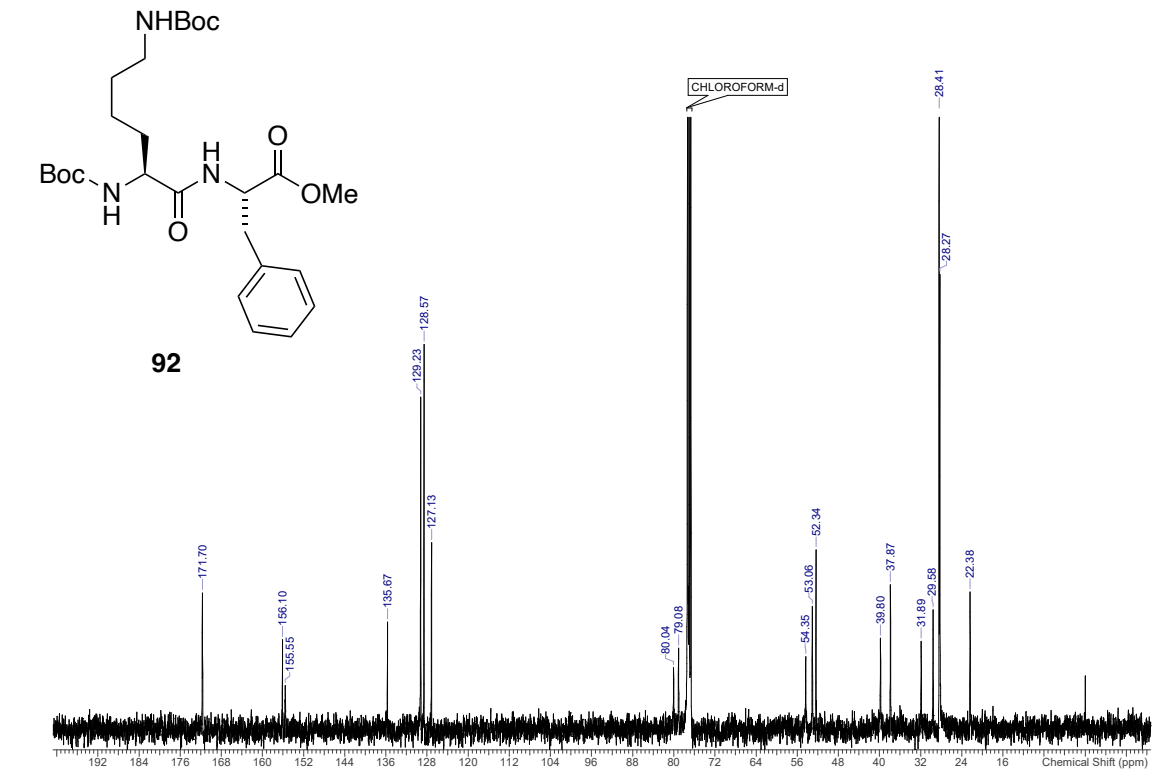
91



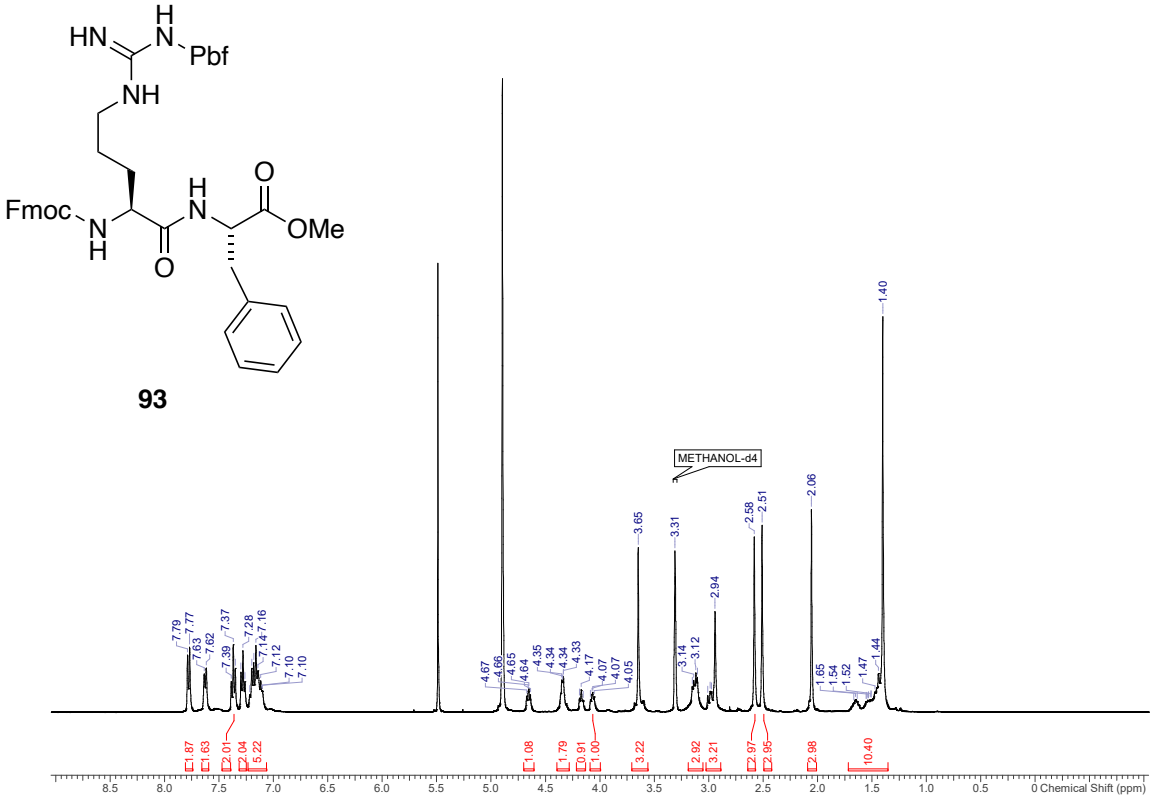
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **92**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **92**

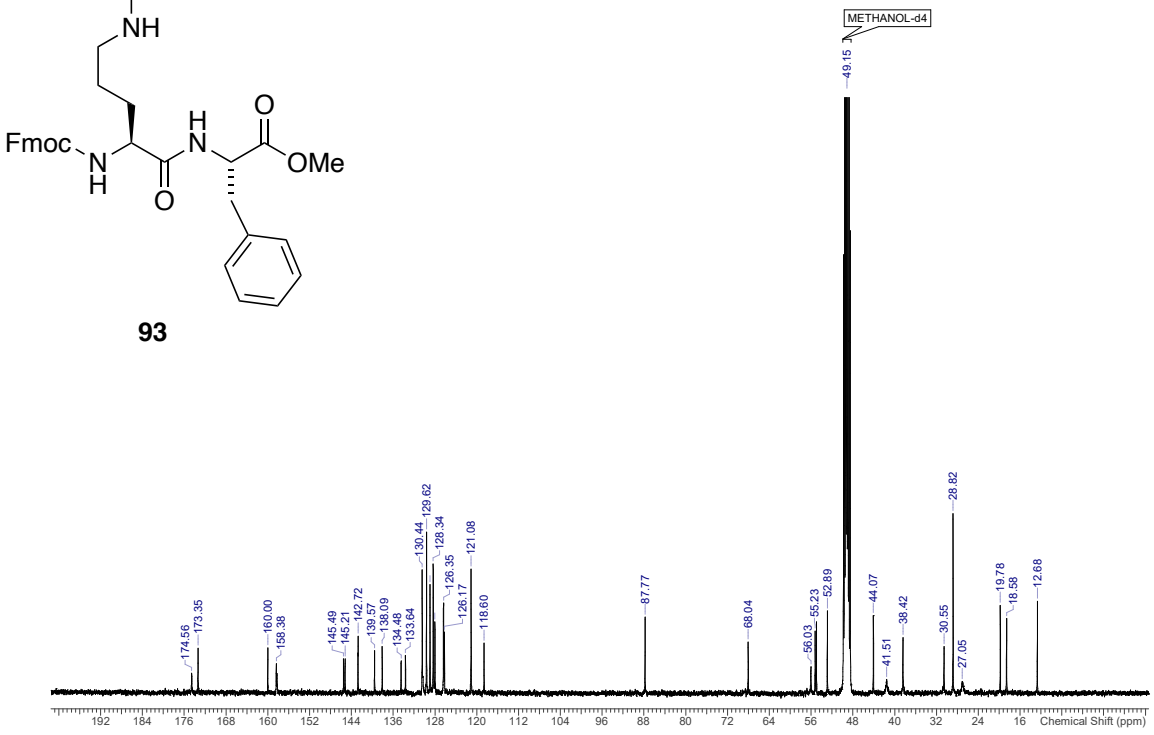


^{1}H NMR spectrum (400 MHz, CD_3OD) of **93**



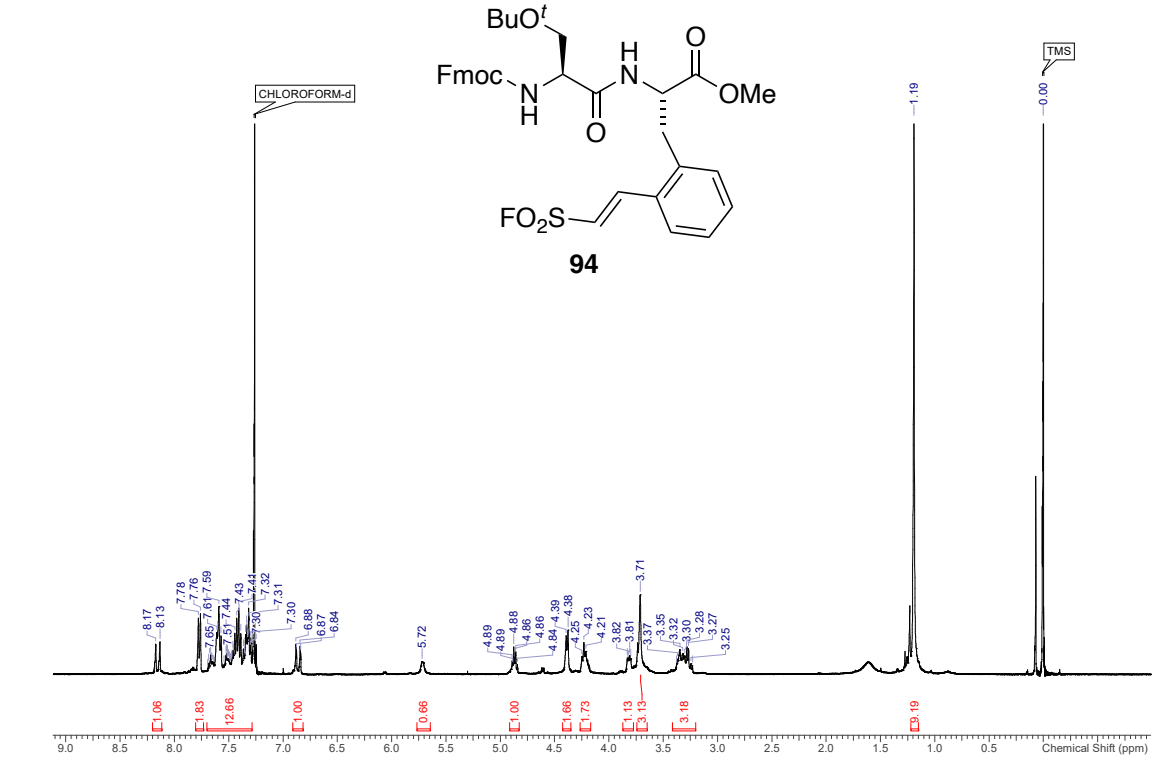
93

^{13}C NMR spectrum (100 MHz, CD_3OD) of **93**

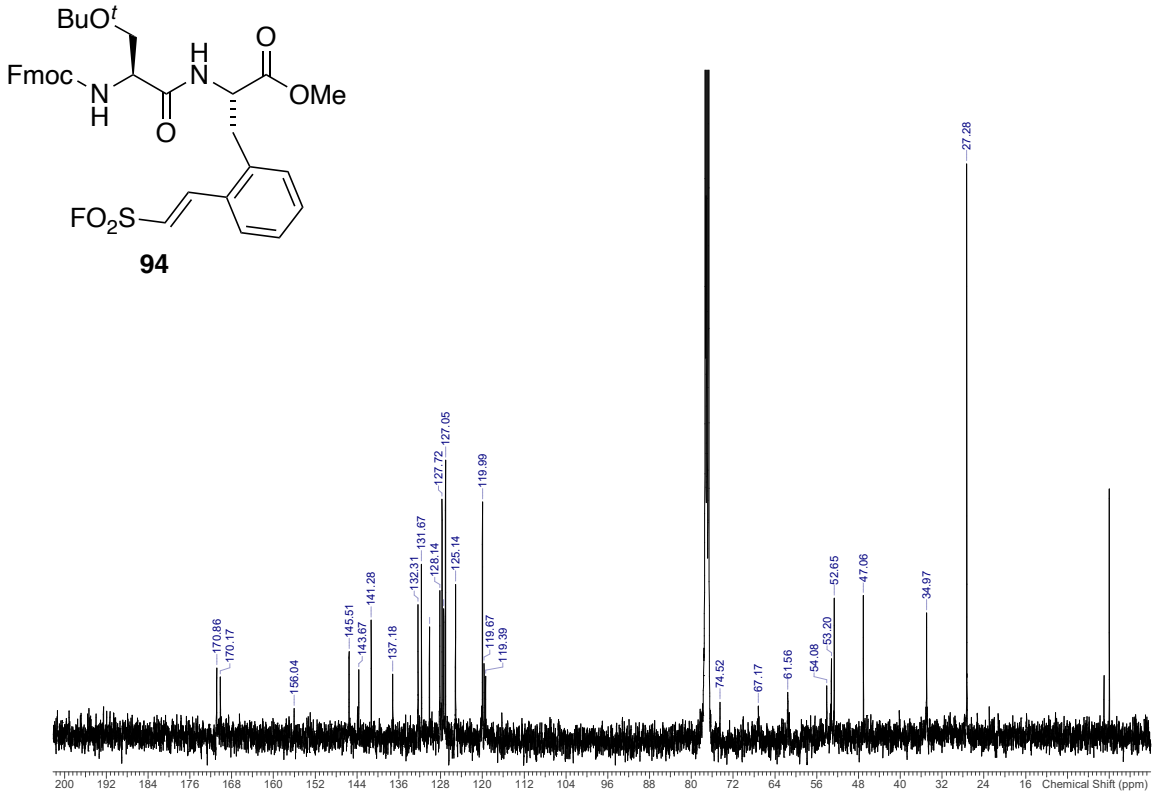


93

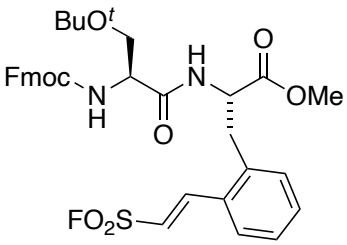
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **94**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **94**

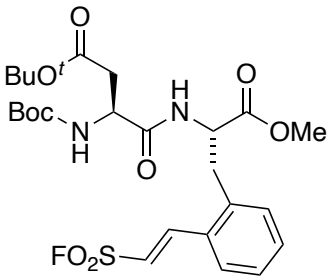


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **94**



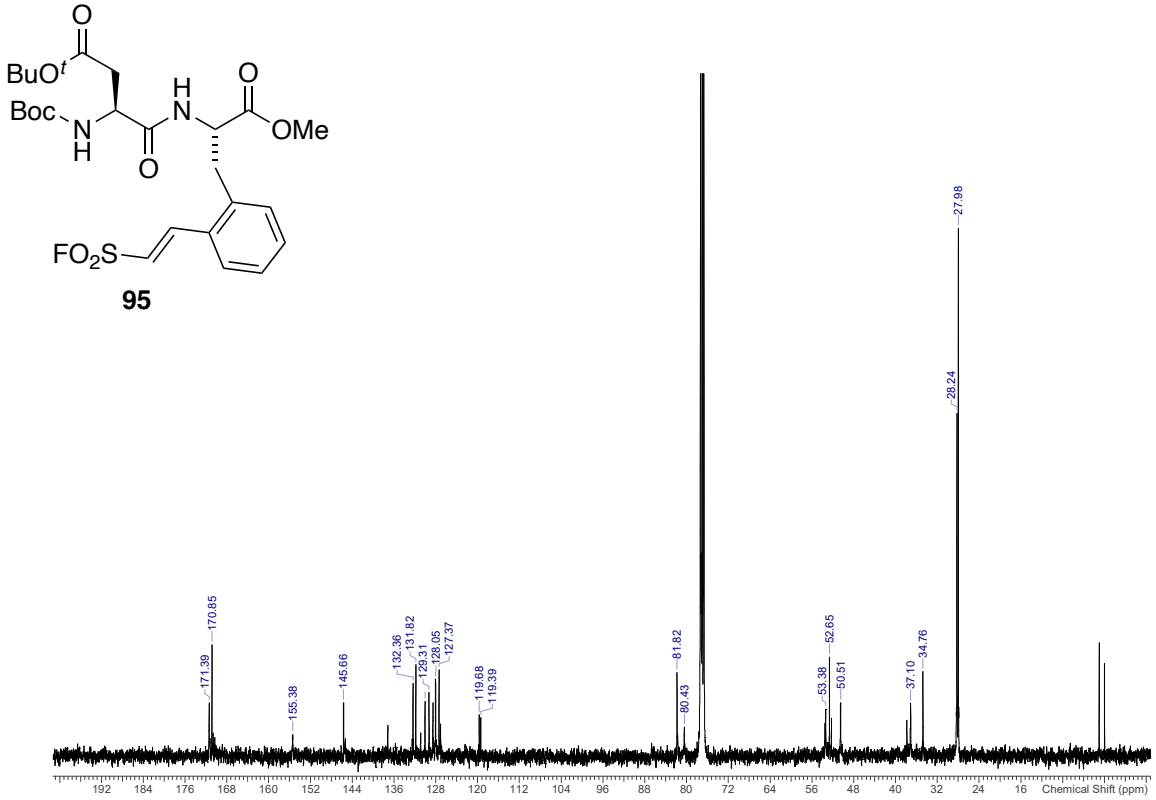
94

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **95**

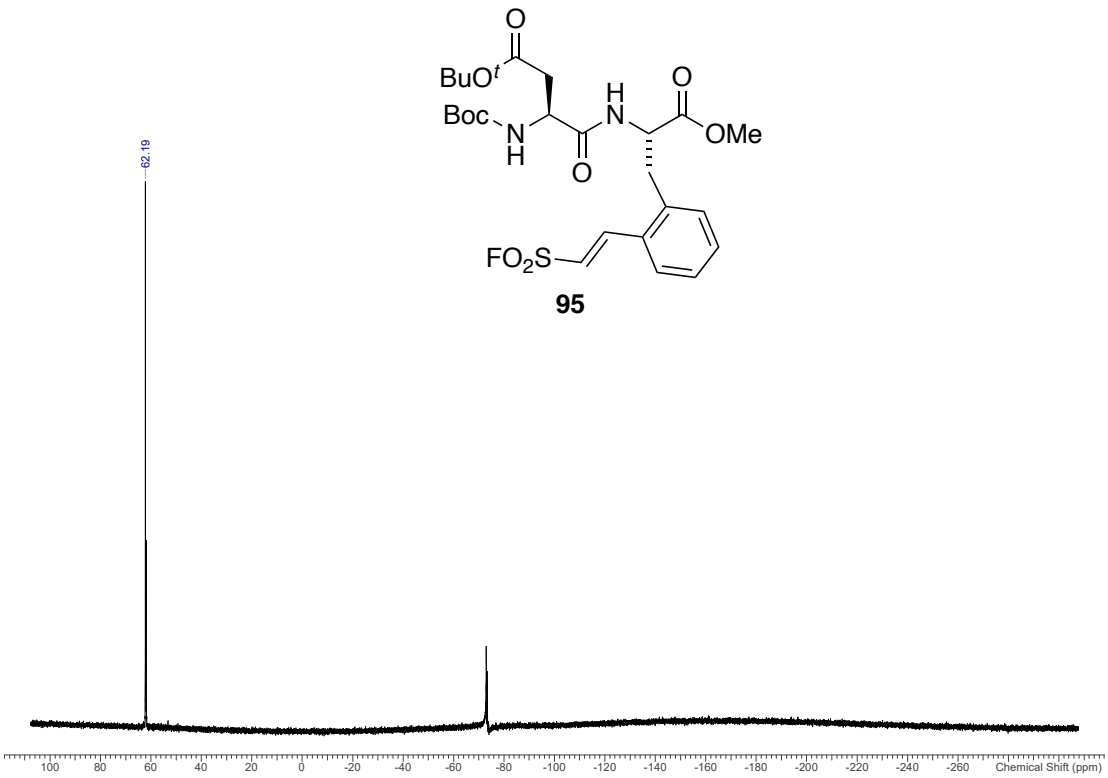


95

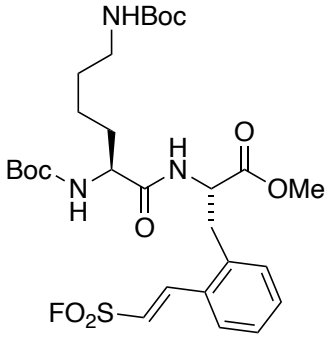
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **95**



^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **95**

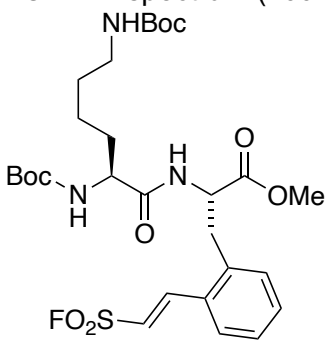


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **96**



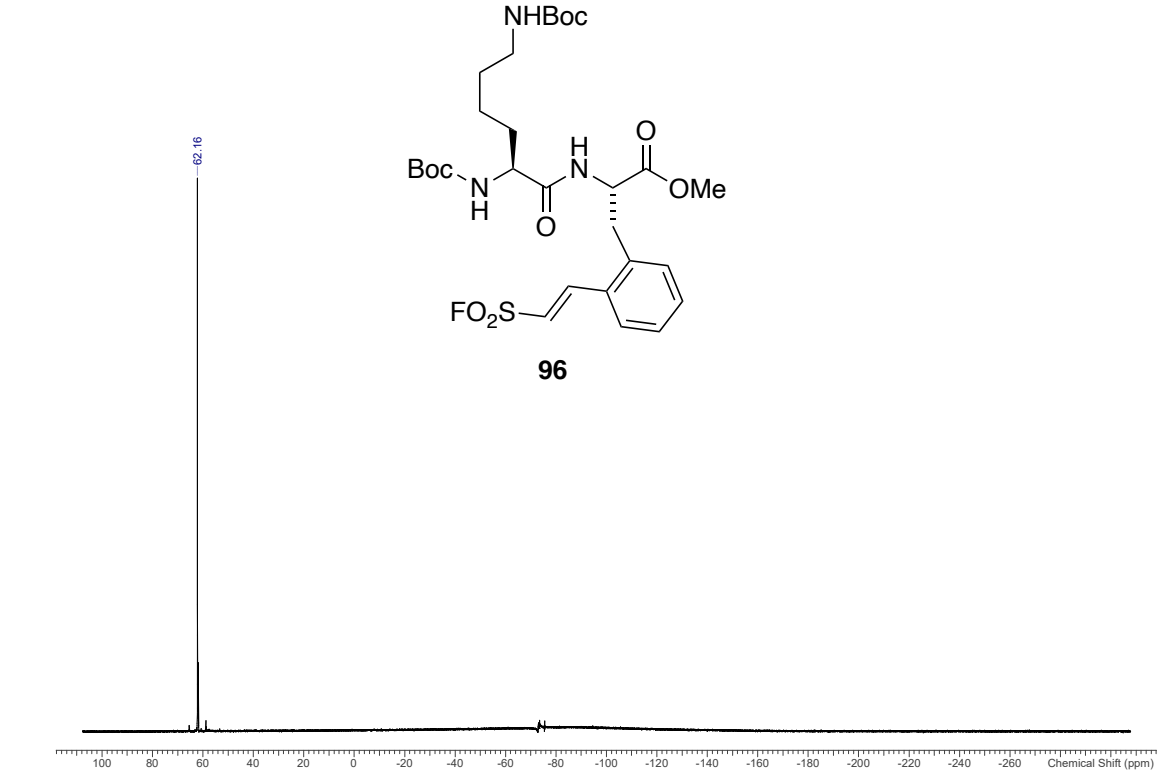
96

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **96**

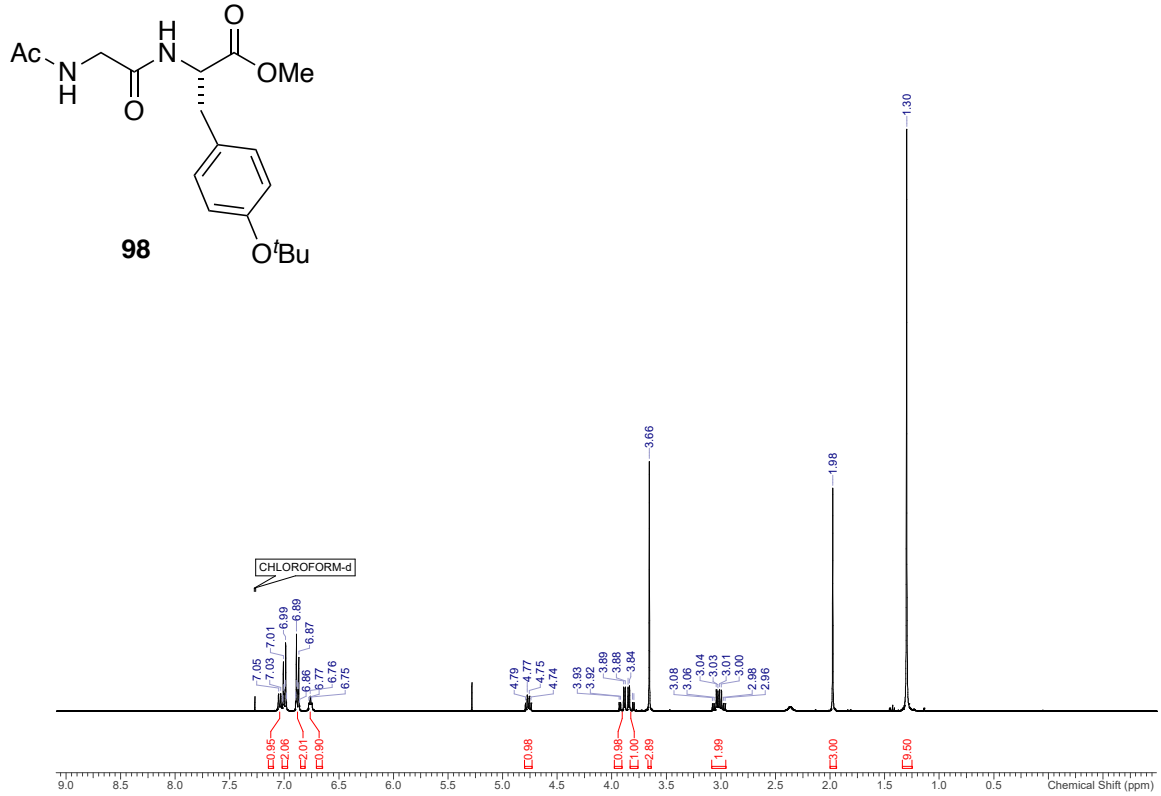


96

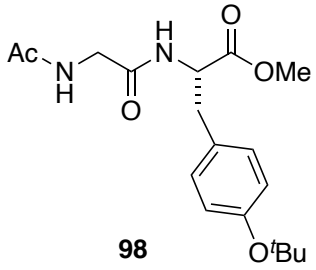
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **96**



^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **98**

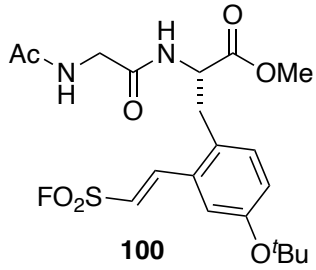


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **98**



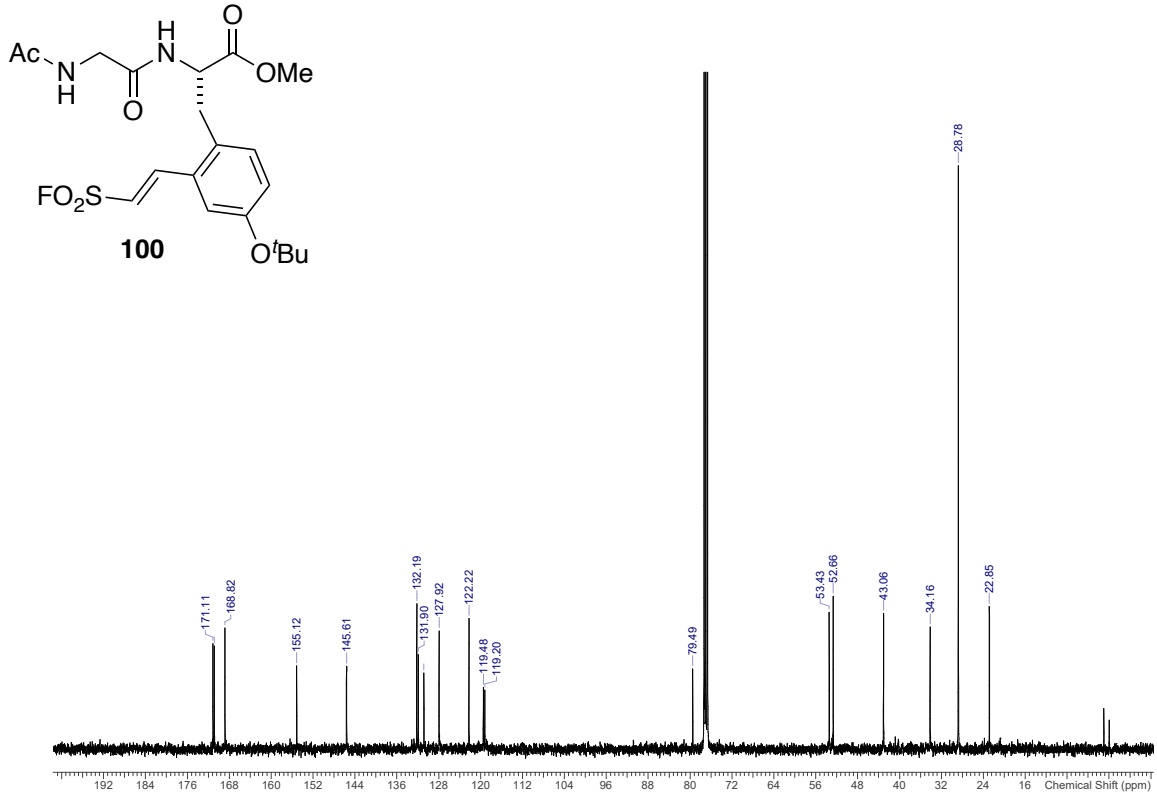
98

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **100**

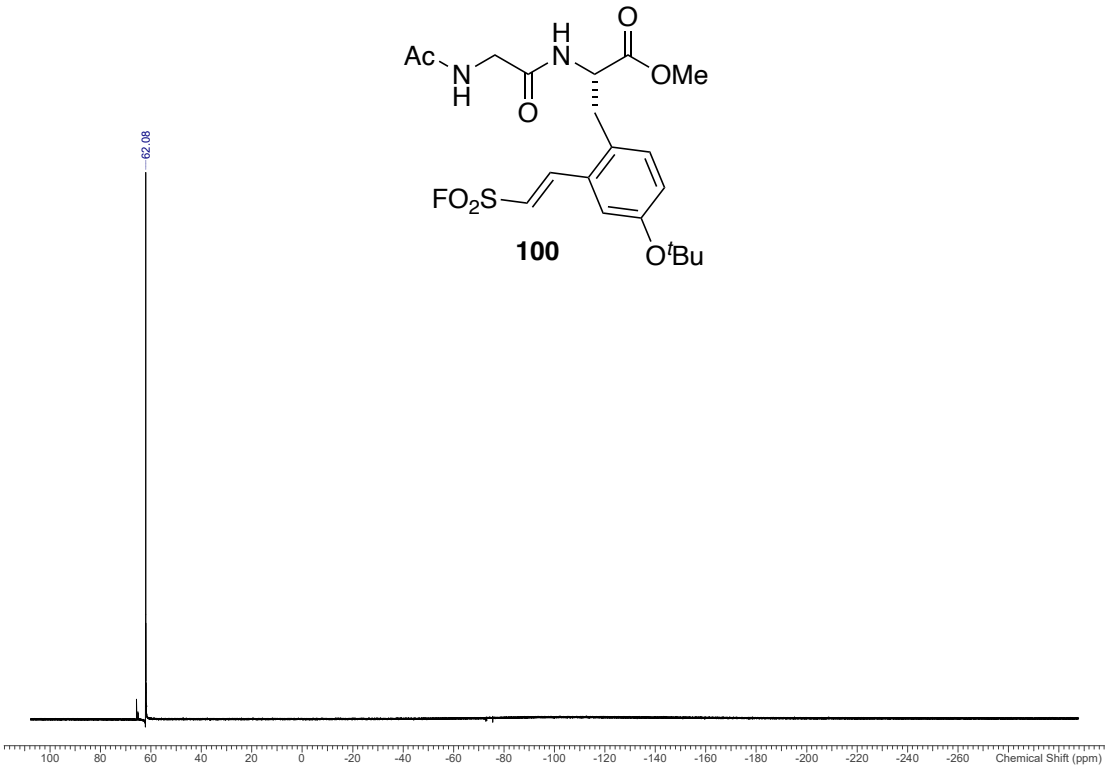


100

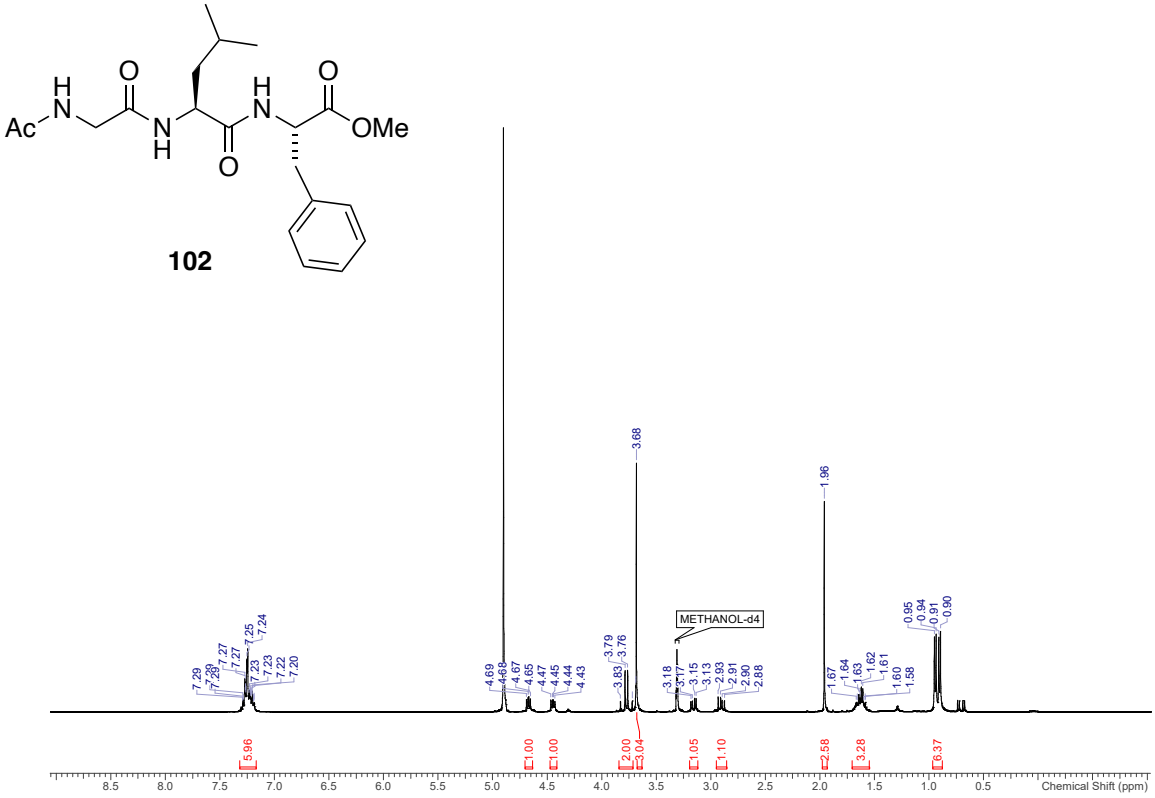
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **100**



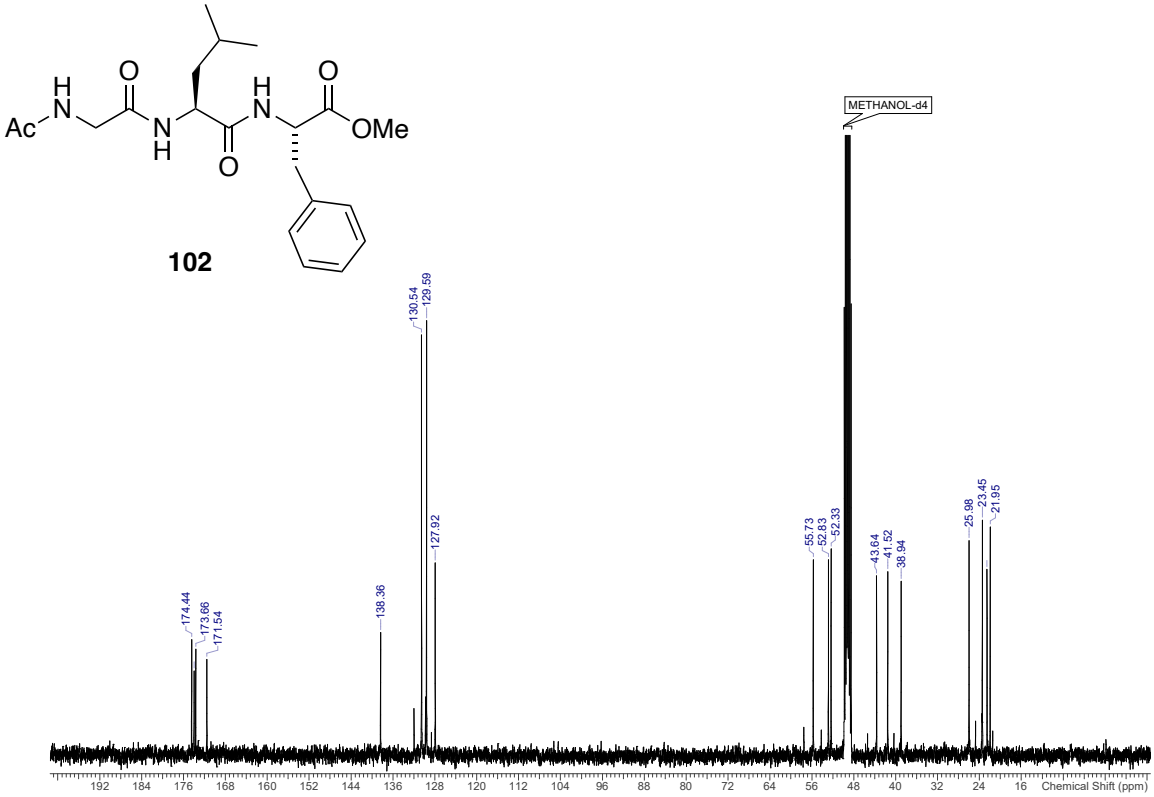
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **100**



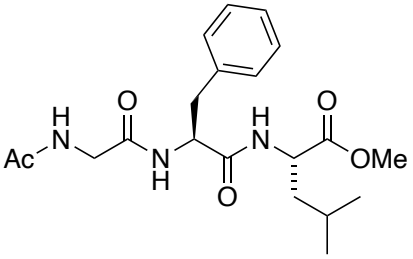
^{1}H NMR spectrum (400 MHz, CD_3OD) of **102**



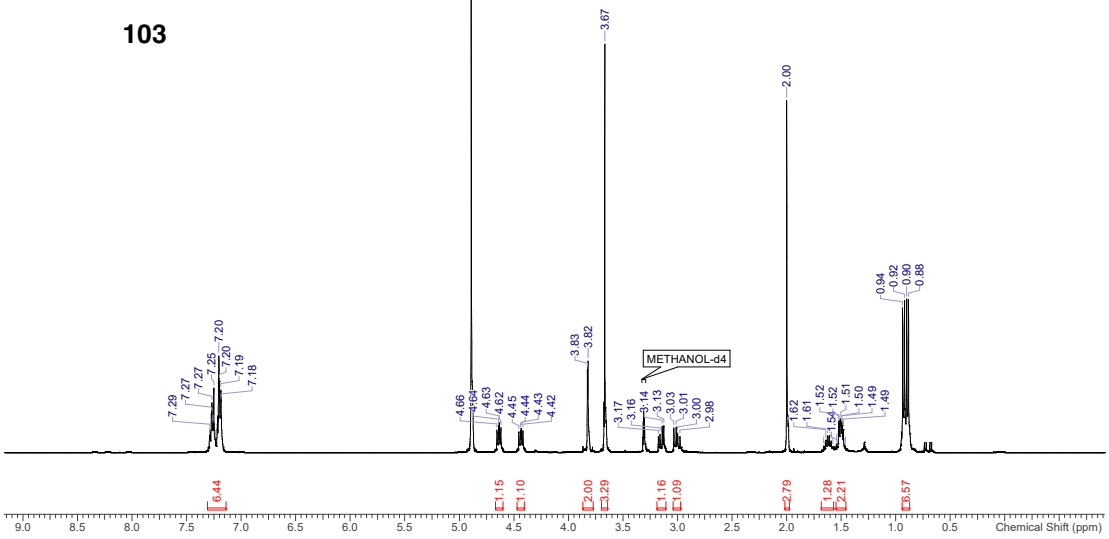
^{13}C NMR spectrum (100 MHz, CD_3OD) of **102**



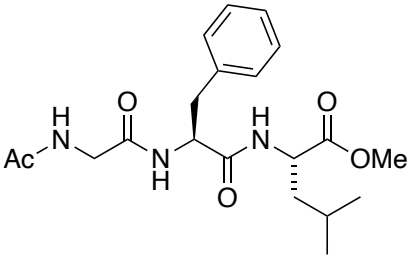
^{1}H NMR spectrum (400 MHz, CD_3OD) of **103**



103

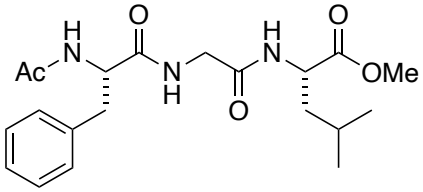


^{13}C NMR spectrum (100 MHz, CD_3OD) of **103**

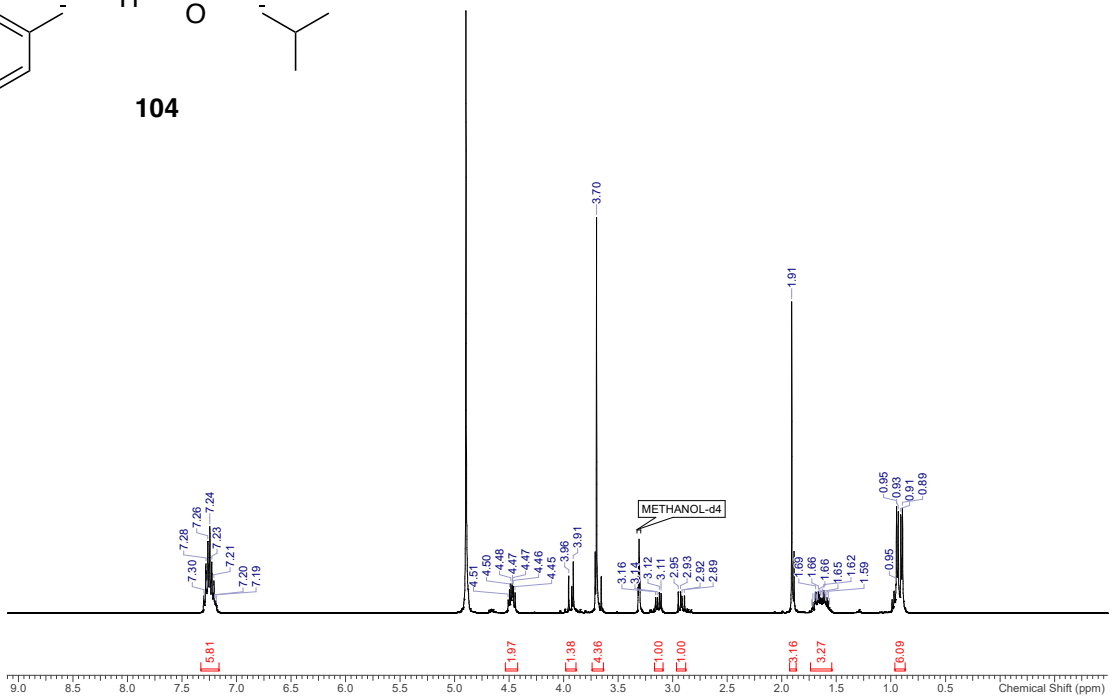


103

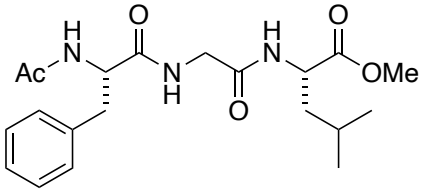
^{1}H NMR spectrum (400 MHz, CD_3OD) of **104**



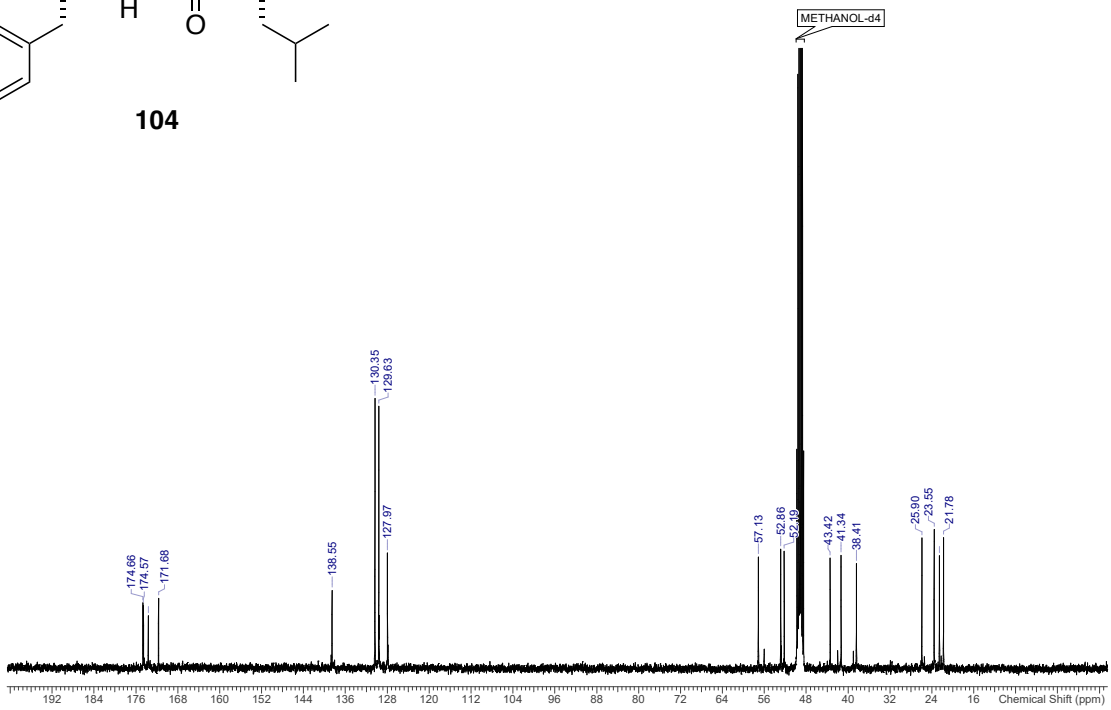
104



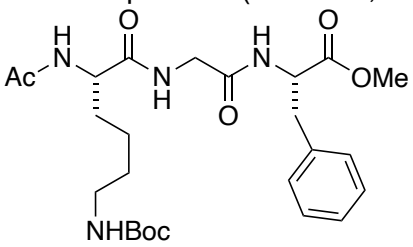
^{13}C NMR spectrum (100 MHz, CD_3OD) of **104**



104

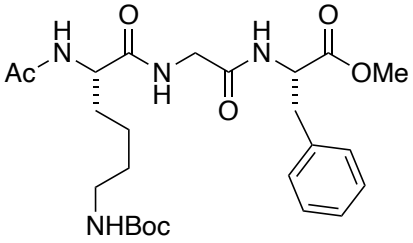


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **105**



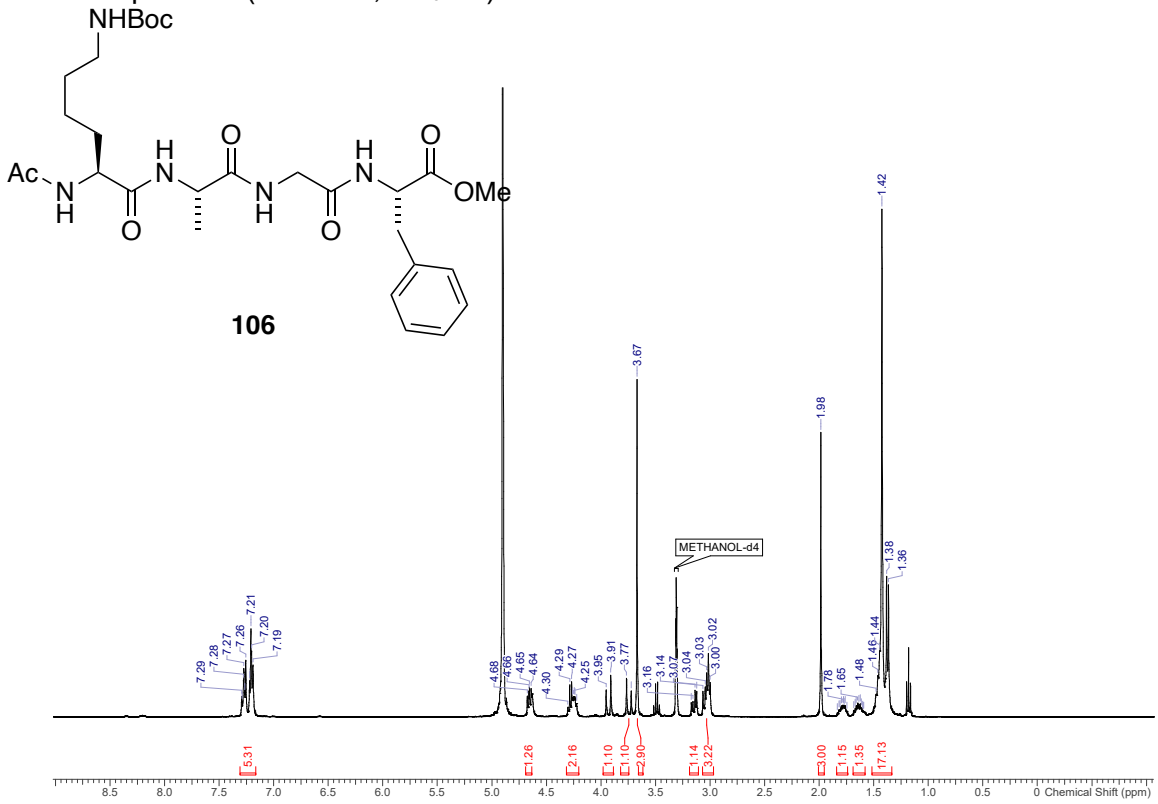
105

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **105**

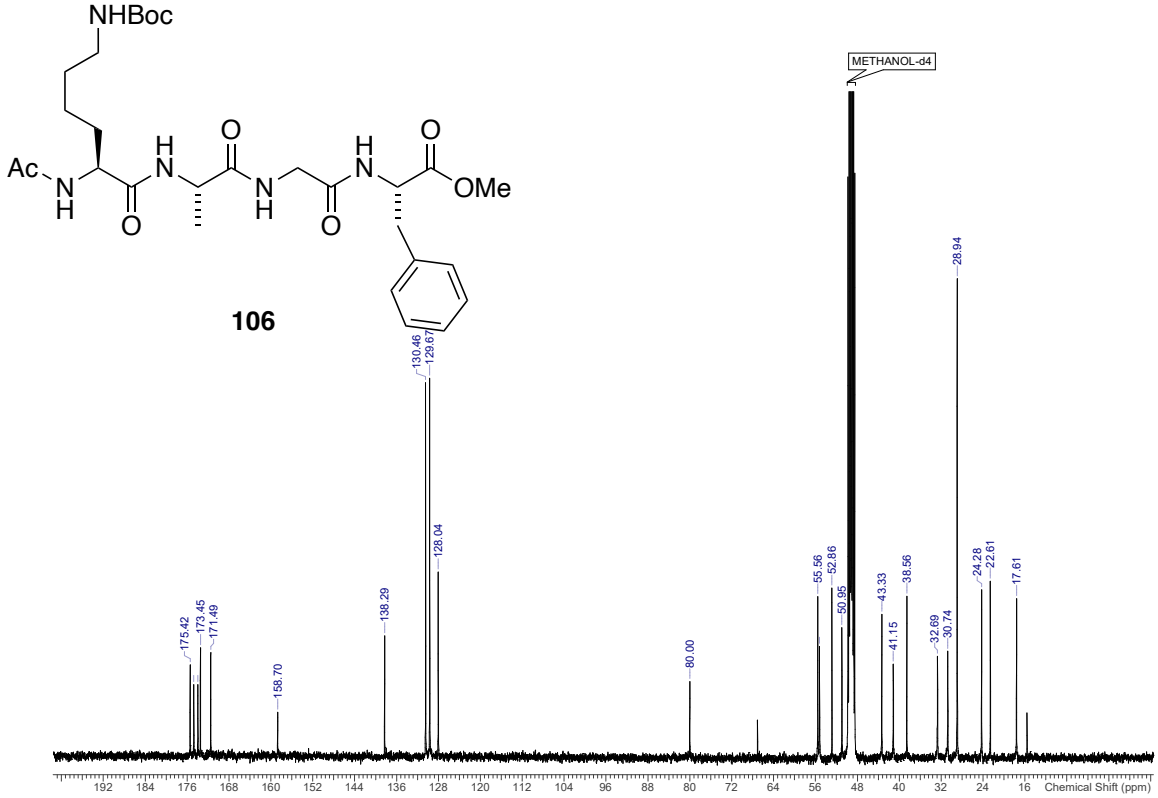


105

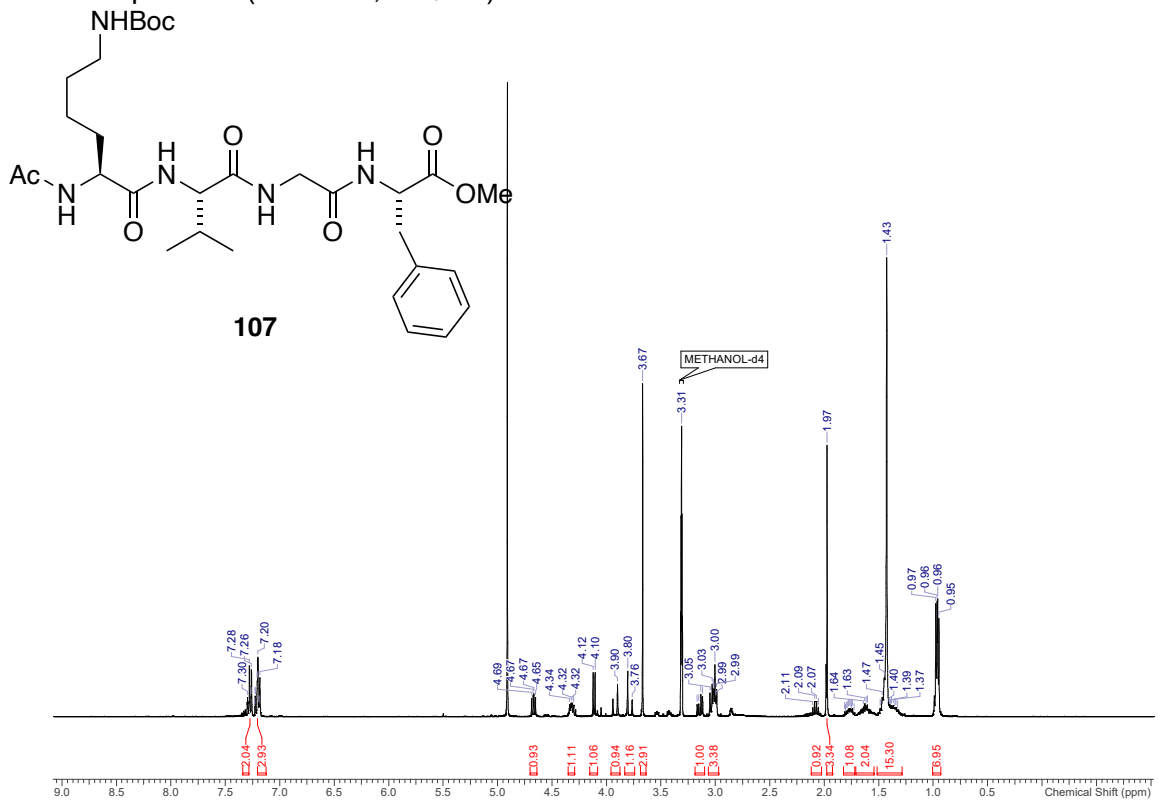
^{1}H NMR spectrum (400 MHz, CD_3OD) of **106**



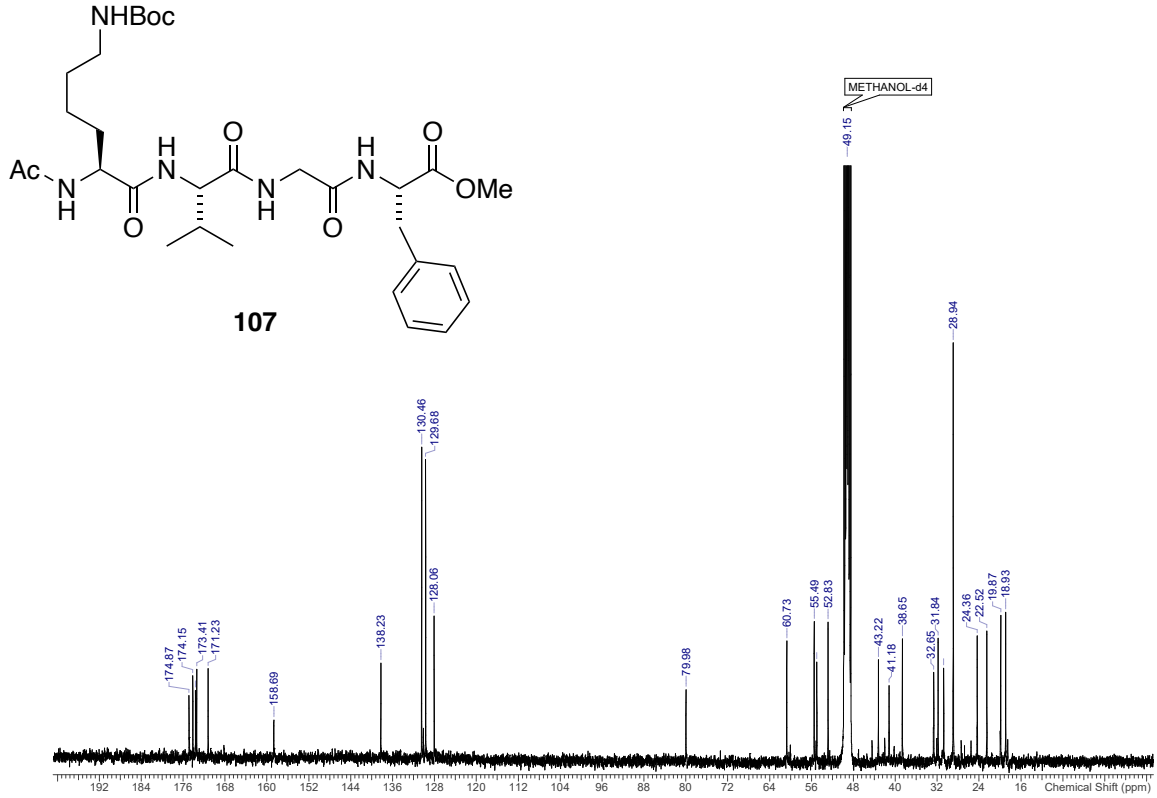
^{13}C NMR spectrum (100 MHz, CD_3OD) of **106**



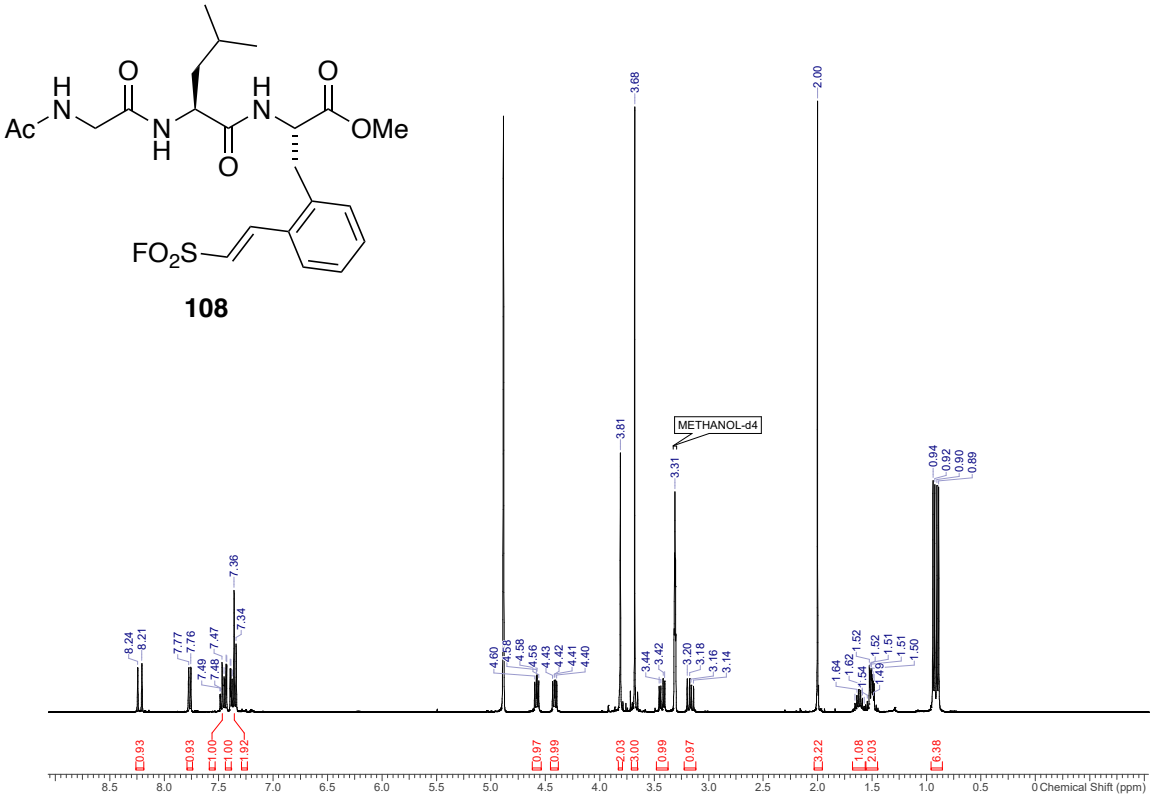
^{1}H NMR spectrum (400 MHz, CD_3OD) of **107**



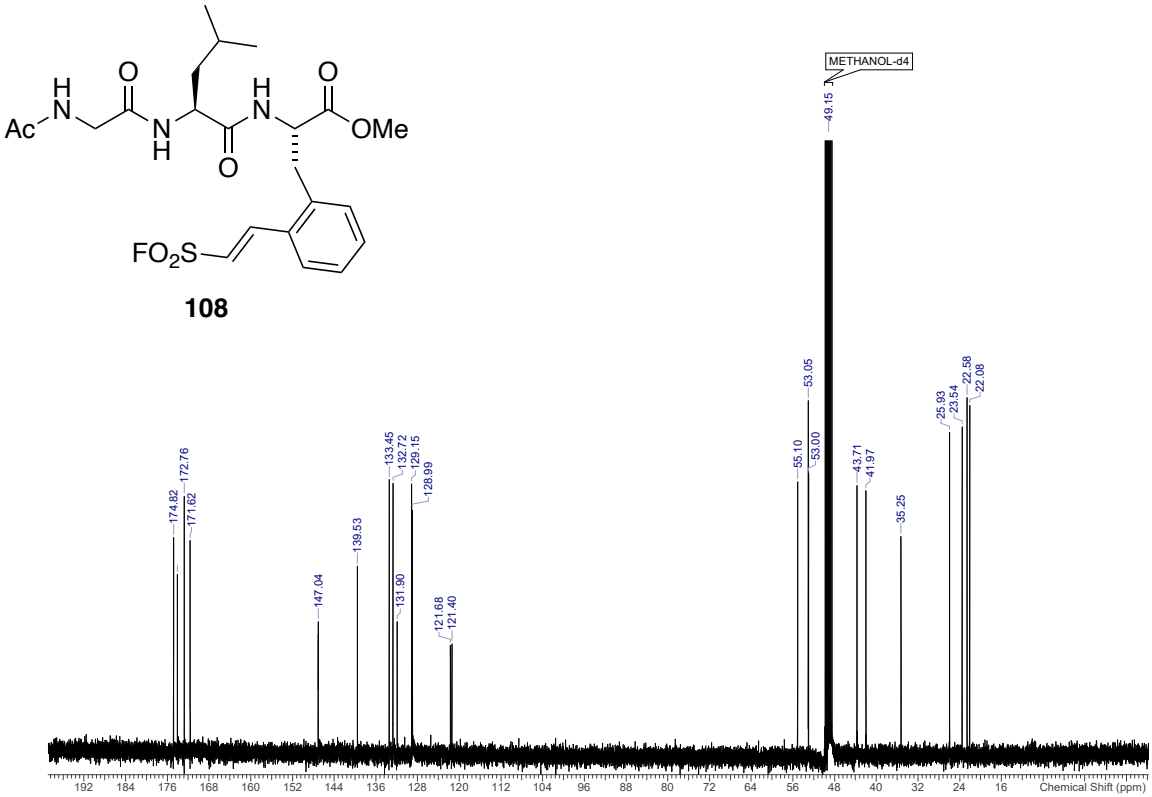
^{13}C NMR spectrum (100 MHz, CD_3OD) of **107**



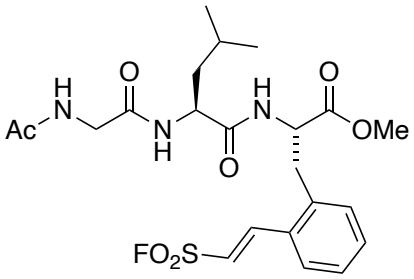
^{1}H NMR spectrum (400 MHz, CD_3OD) of **108**



^{13}C NMR spectrum (100 MHz, CD_3OD) of **108**

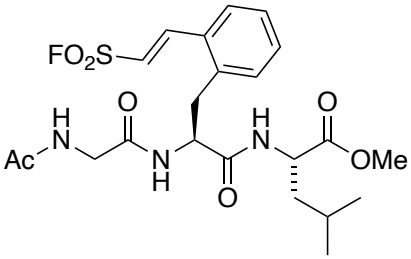


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **108**



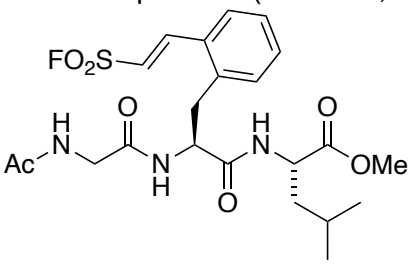
108

^{1}H NMR spectrum (400 MHz, CD_3OD) of **109**



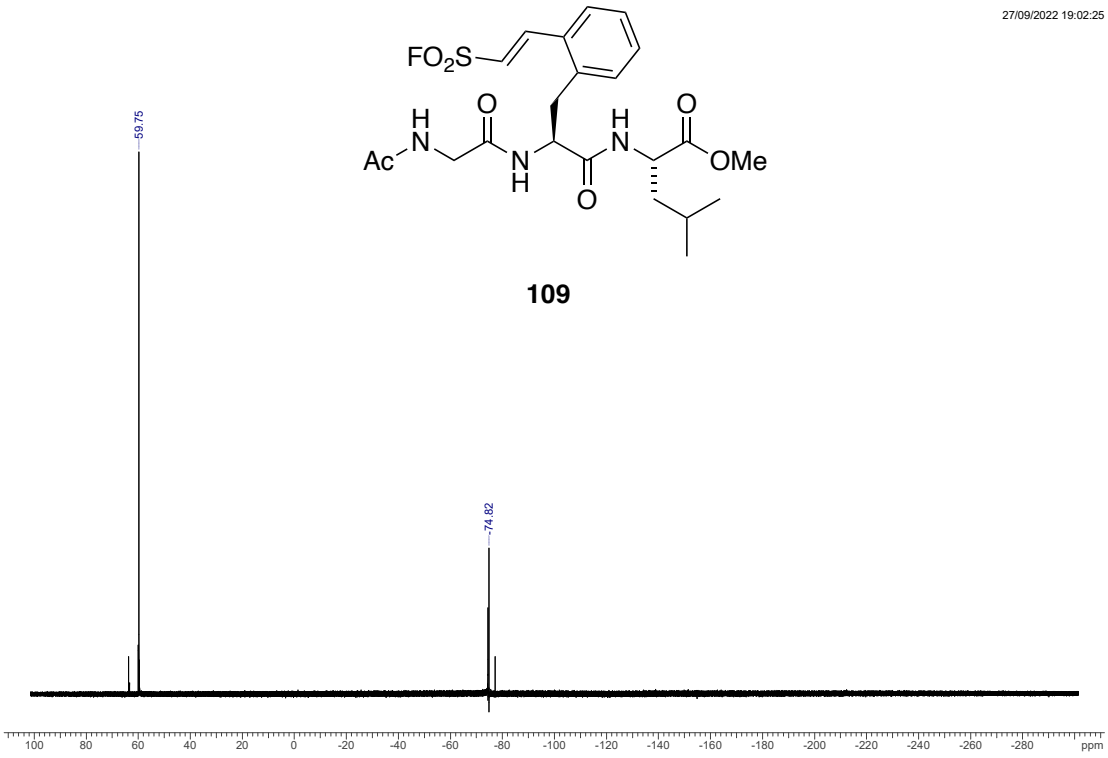
109

^{13}C NMR spectrum (100 MHz, CD_3OD) of **109**



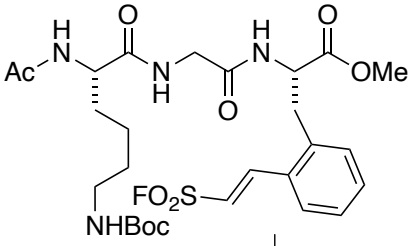
109

^{19}F NMR spectrum (376 MHz, CD_3OD) of **109**



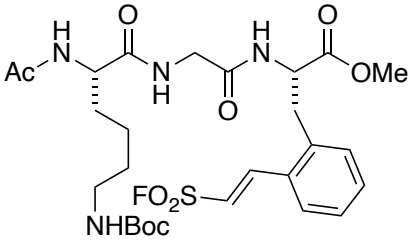
109

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **111**



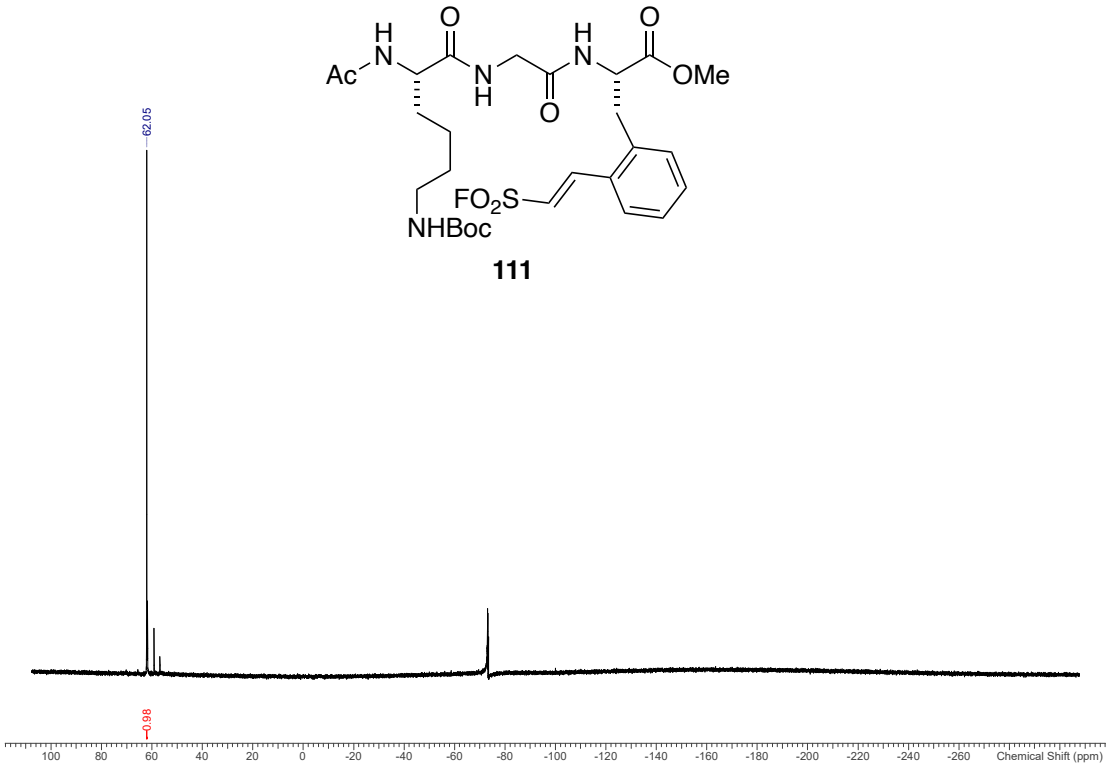
111

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **111**



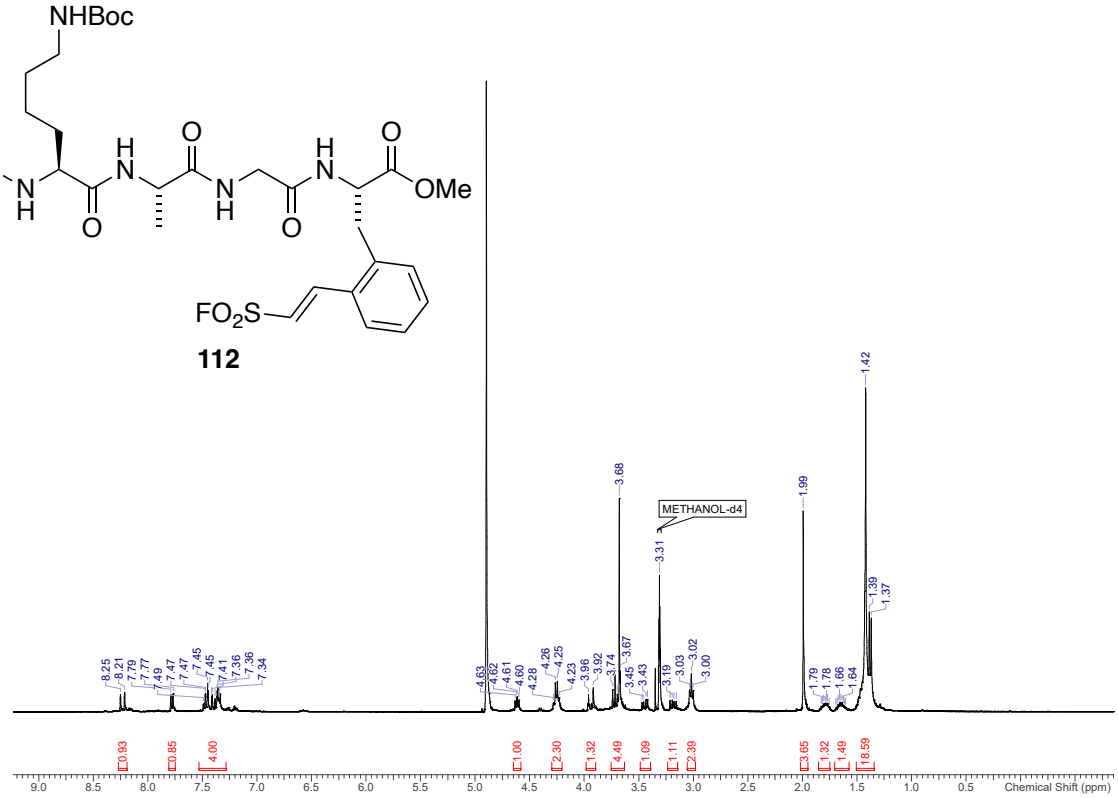
111

^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **111**



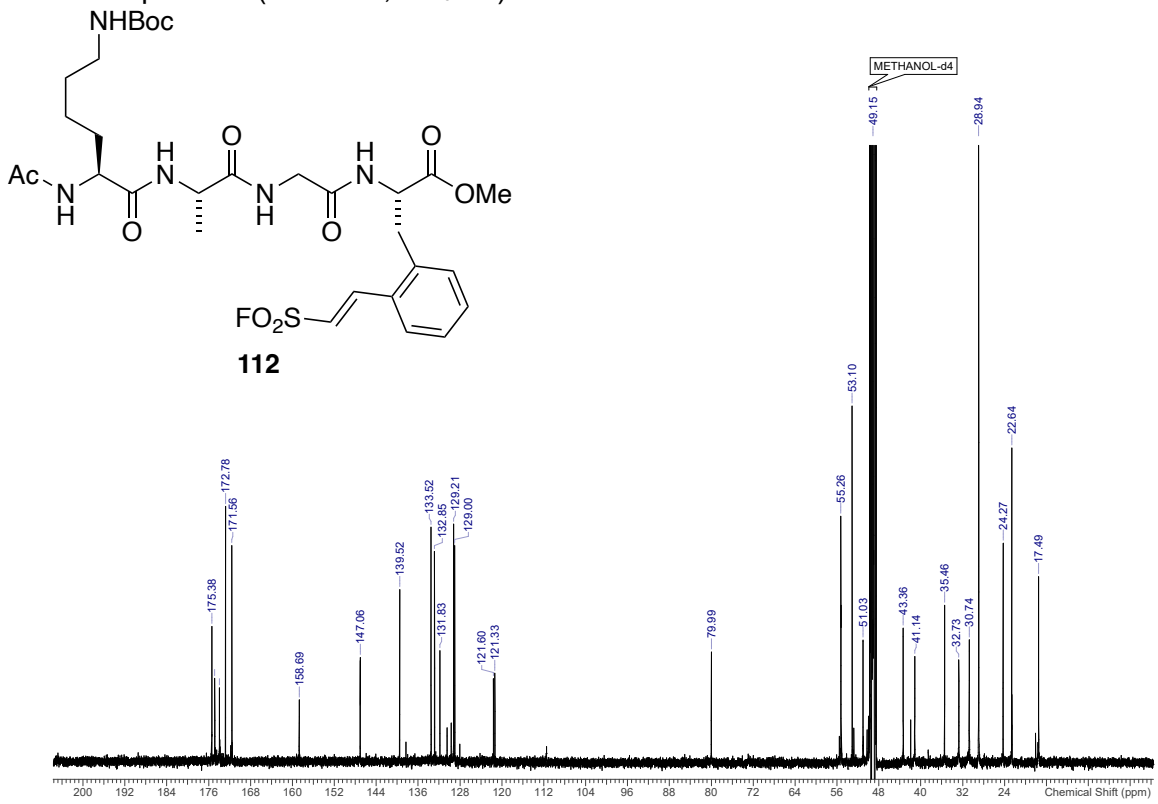
111

^{1}H NMR spectrum (400 MHz, CD_3OD) of **112**



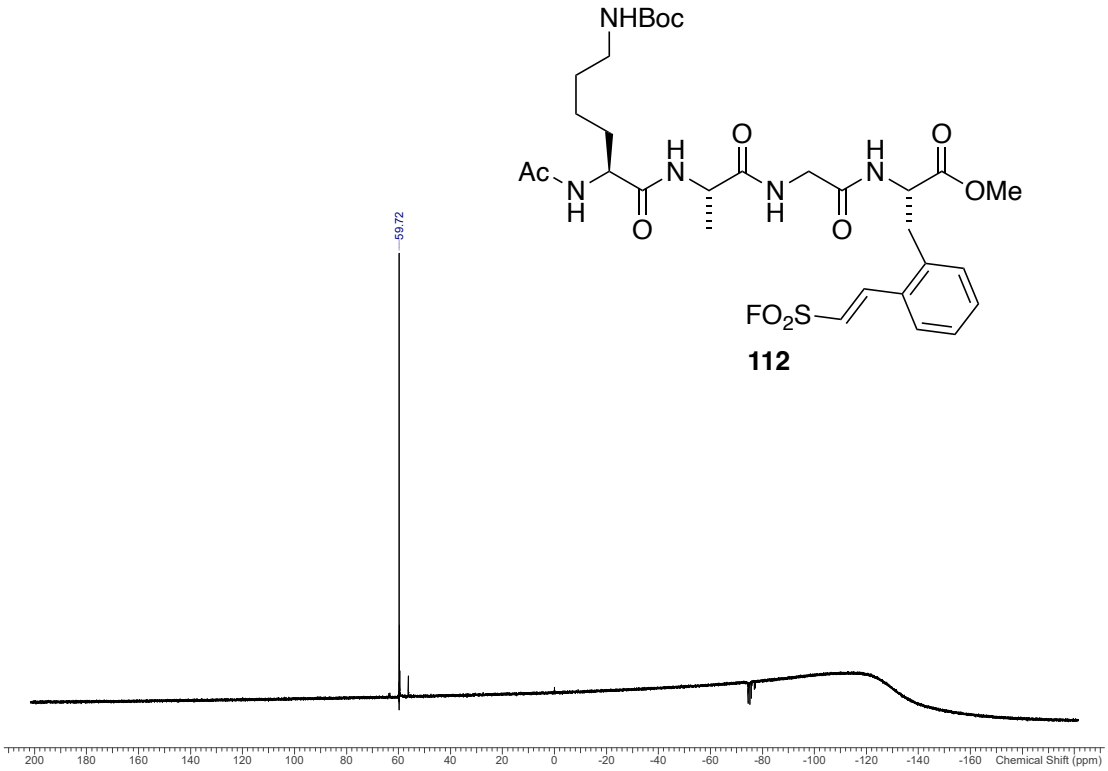
112

^{13}C NMR spectrum (100 MHz, CD_3OD) of **112**



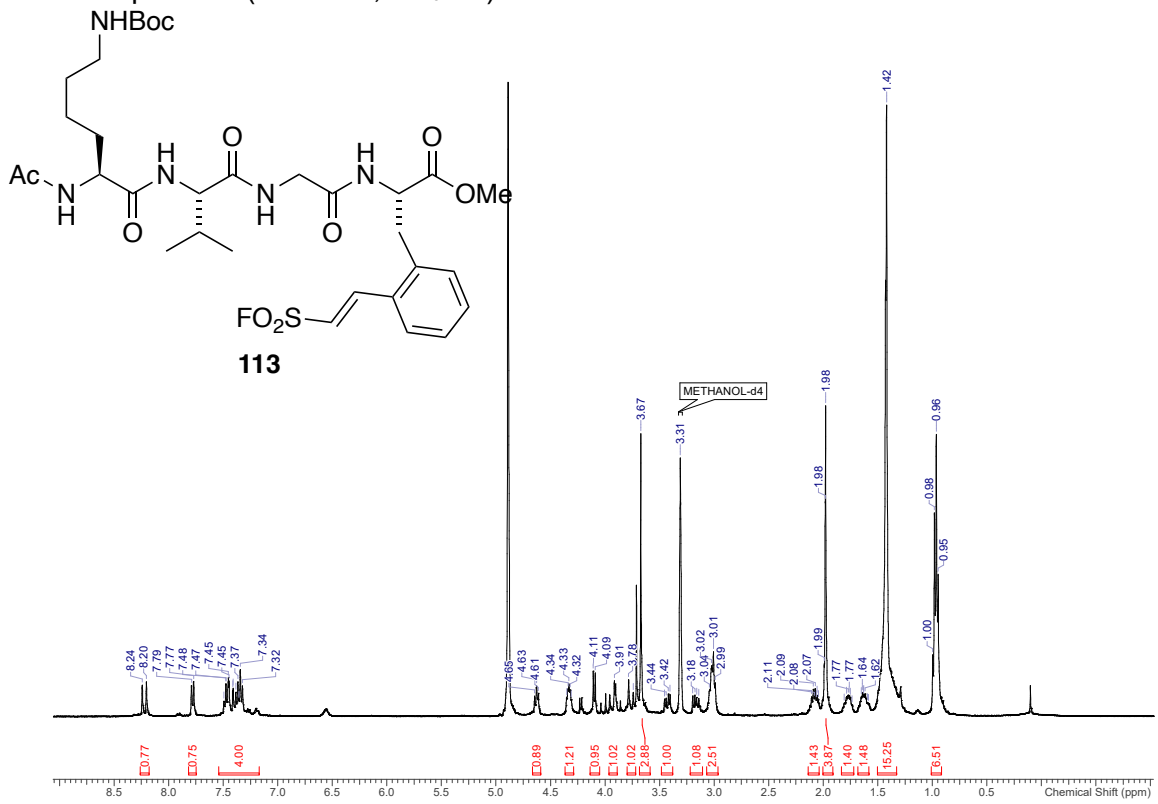
112

^{19}F NMR spectrum (376 MHz, CD_3OD) of **112**

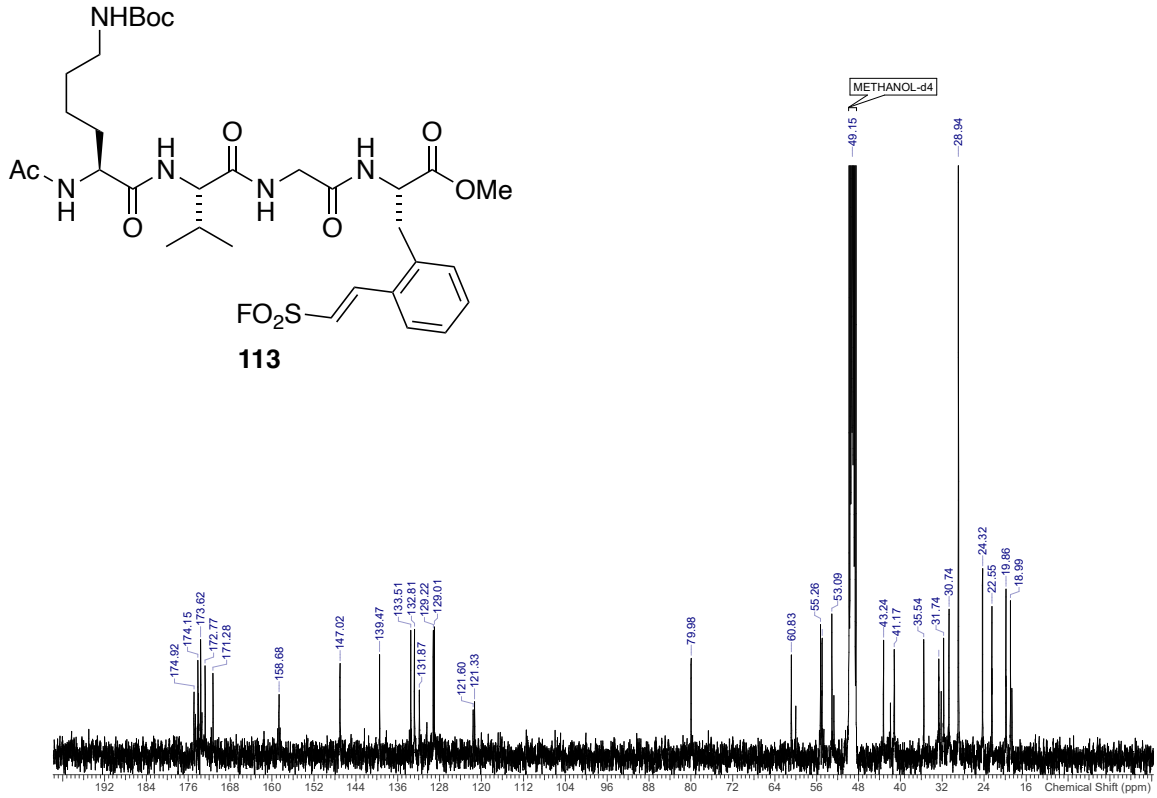


112

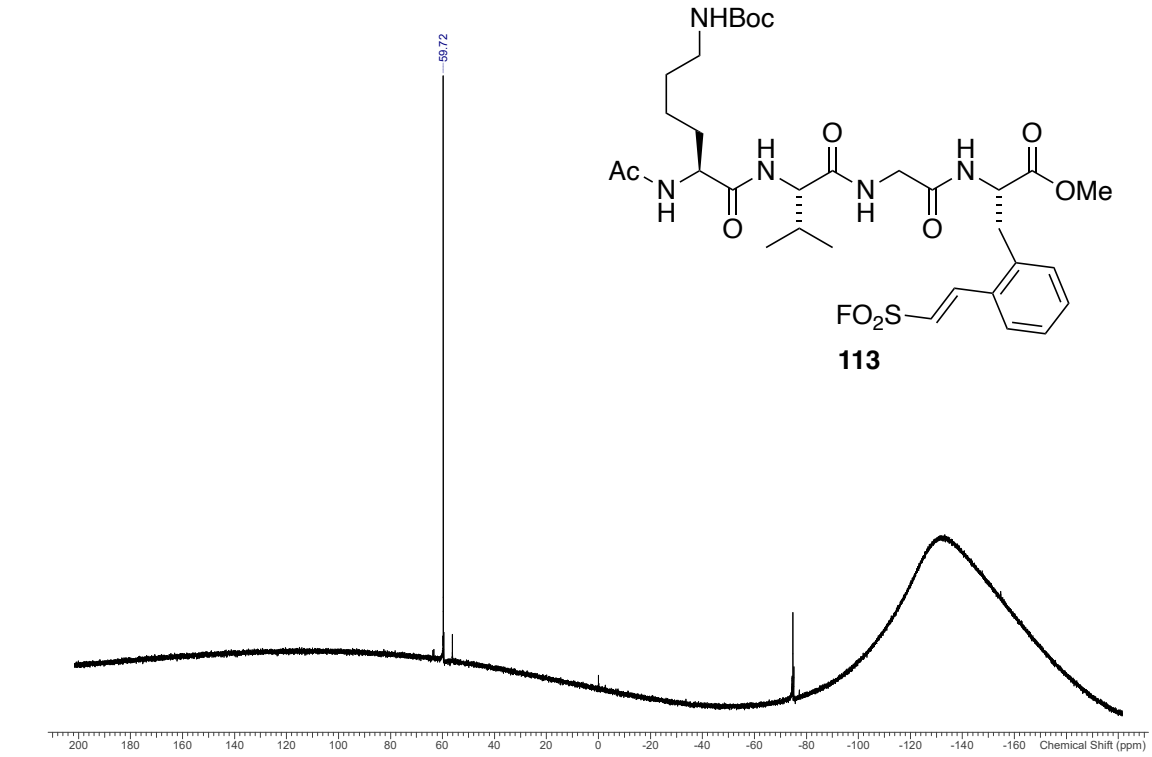
^{1}H NMR spectrum (400 MHz, CD_3OD) of **113**



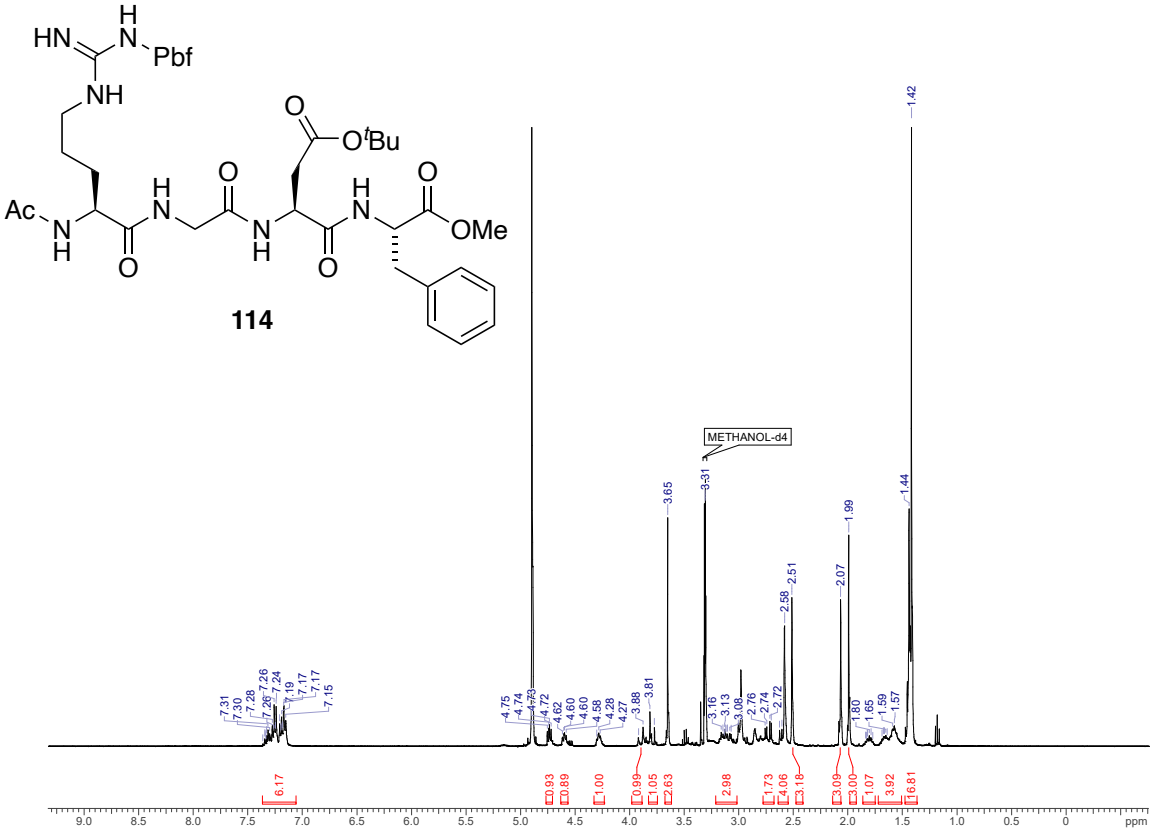
^{13}C NMR spectrum (100 MHz, CD_3OD) of **113**



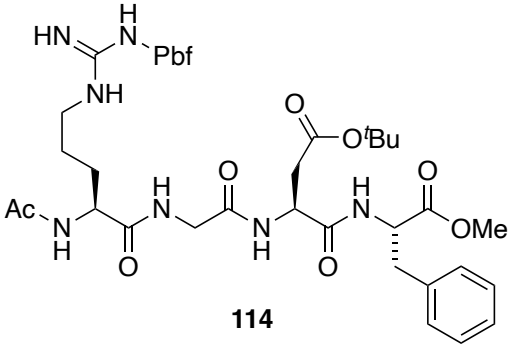
^{19}F NMR spectrum (376 MHz, CD_3OD) of **113**



^{1}H NMR spectrum (400 MHz, CD_3OD) of **114**



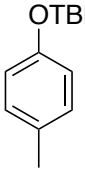
^{13}C NMR spectrum (100 MHz, CD_3OD) of **114**



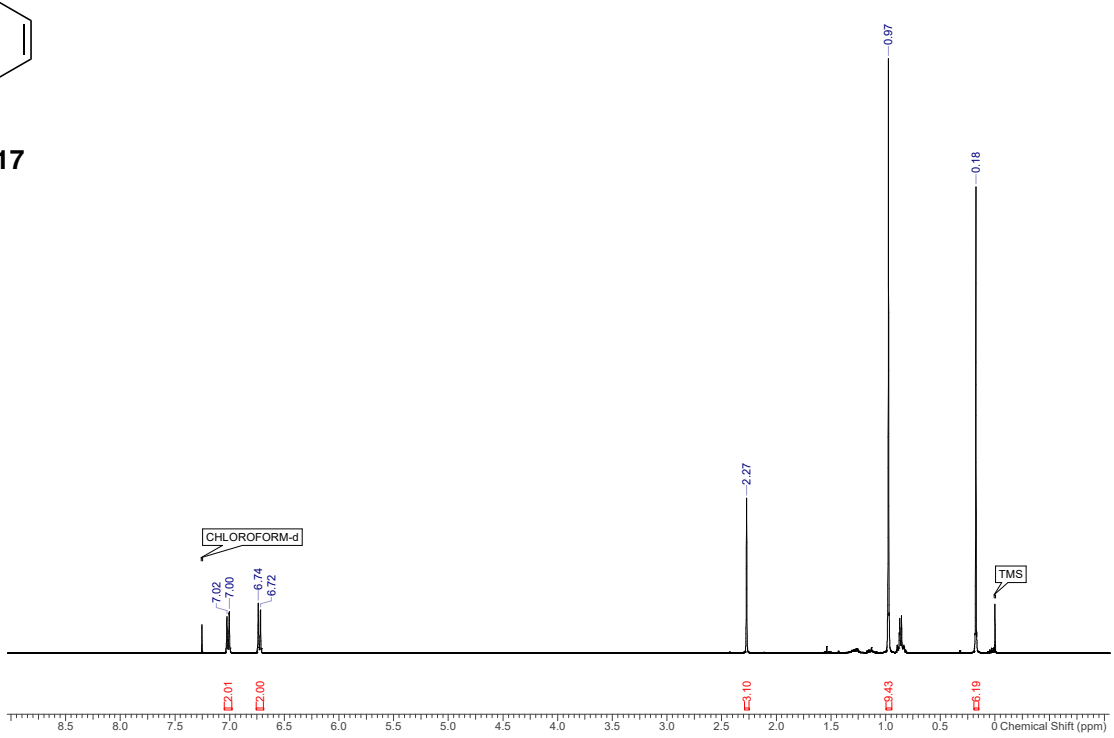
114

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **117**

OTBDMS

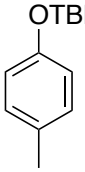


117

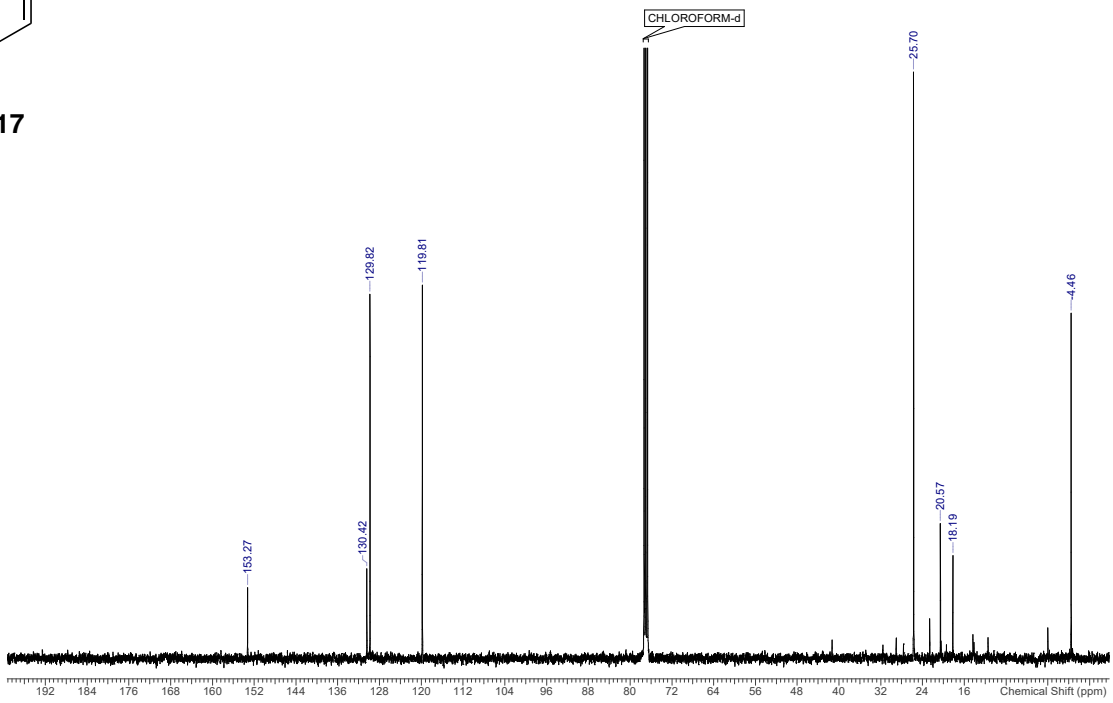


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **117**

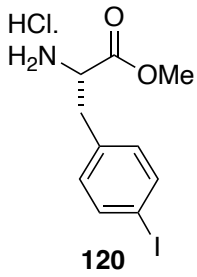
OTBDMS



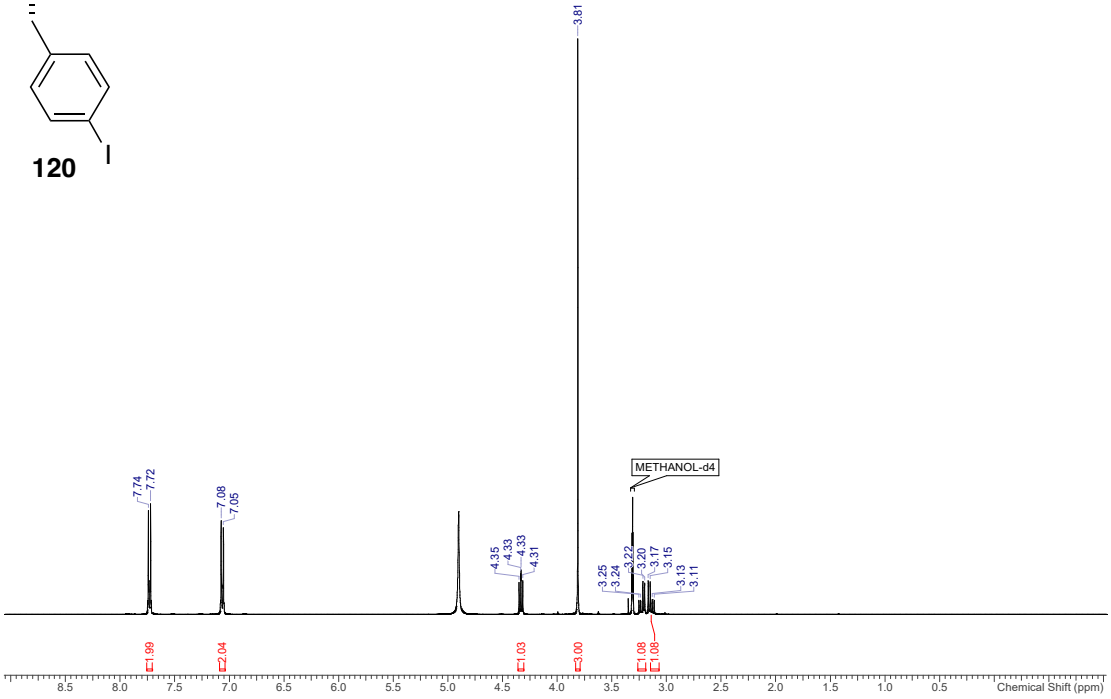
117



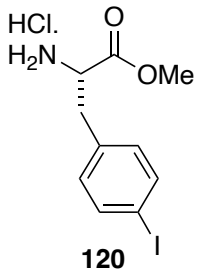
^{1}H NMR spectrum (400 MHz, CD_3OD) of **120**



120

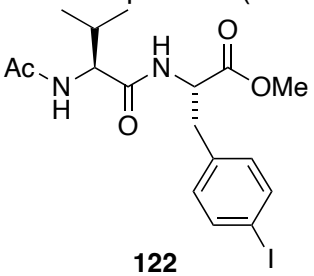


^{13}C NMR spectrum (100 MHz, CD_3OD) of **120**



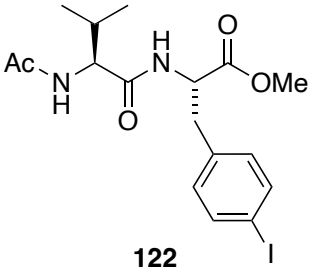
120

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **122**



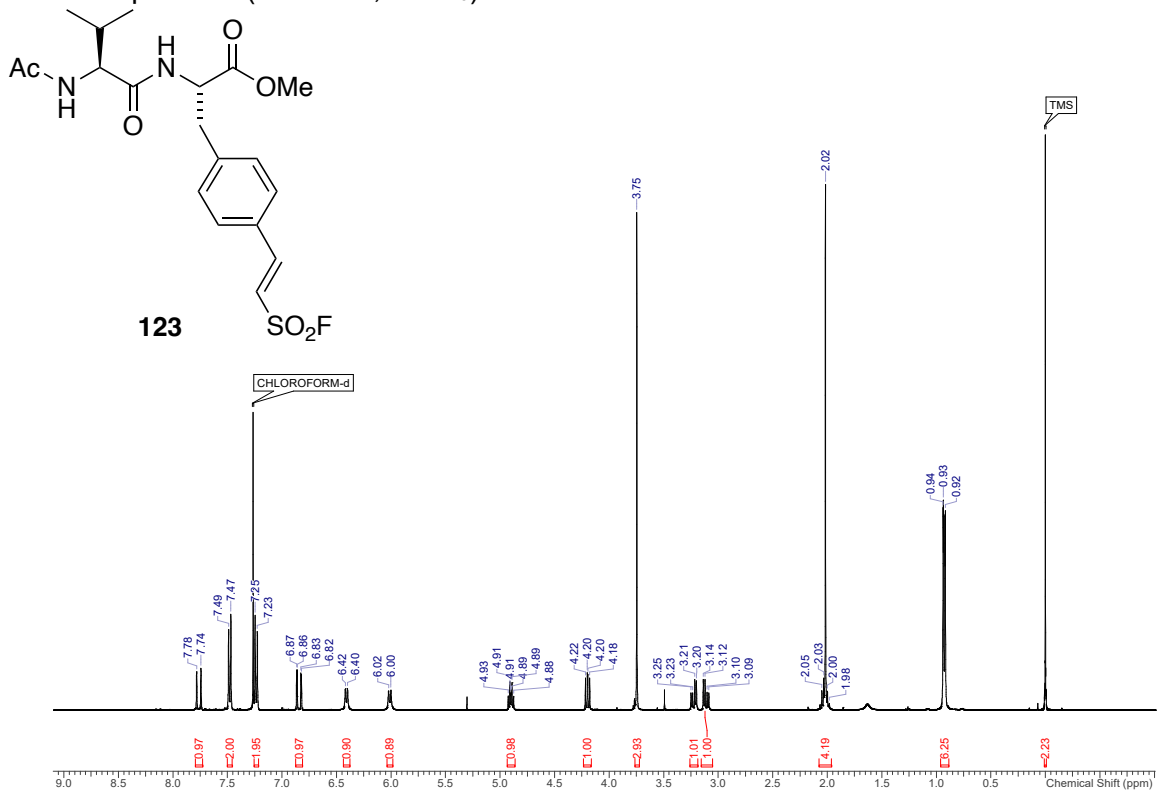
122

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **122**

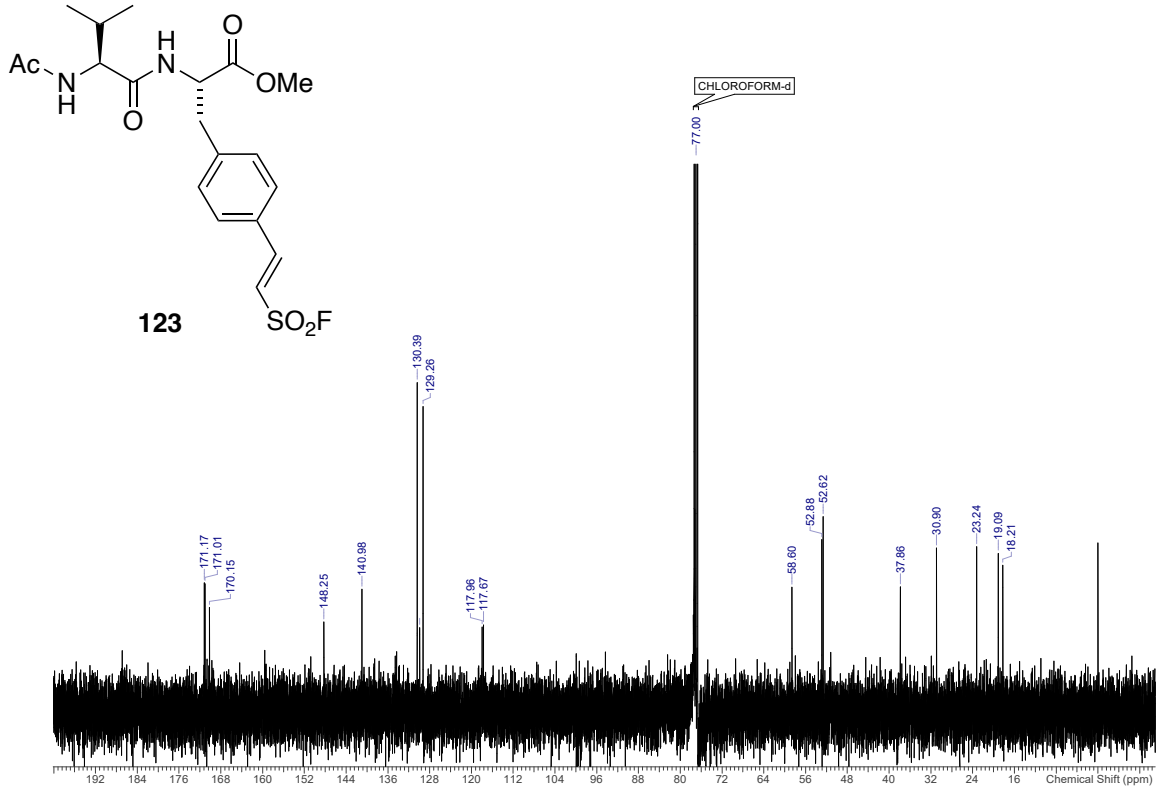


122

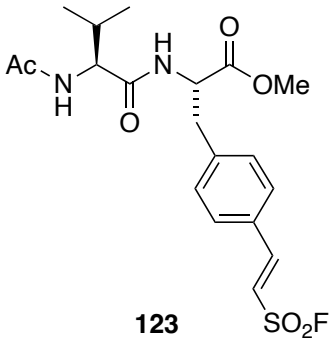
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **123**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **123**

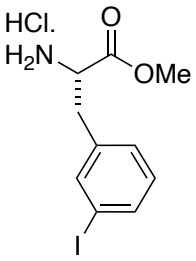


^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **123**

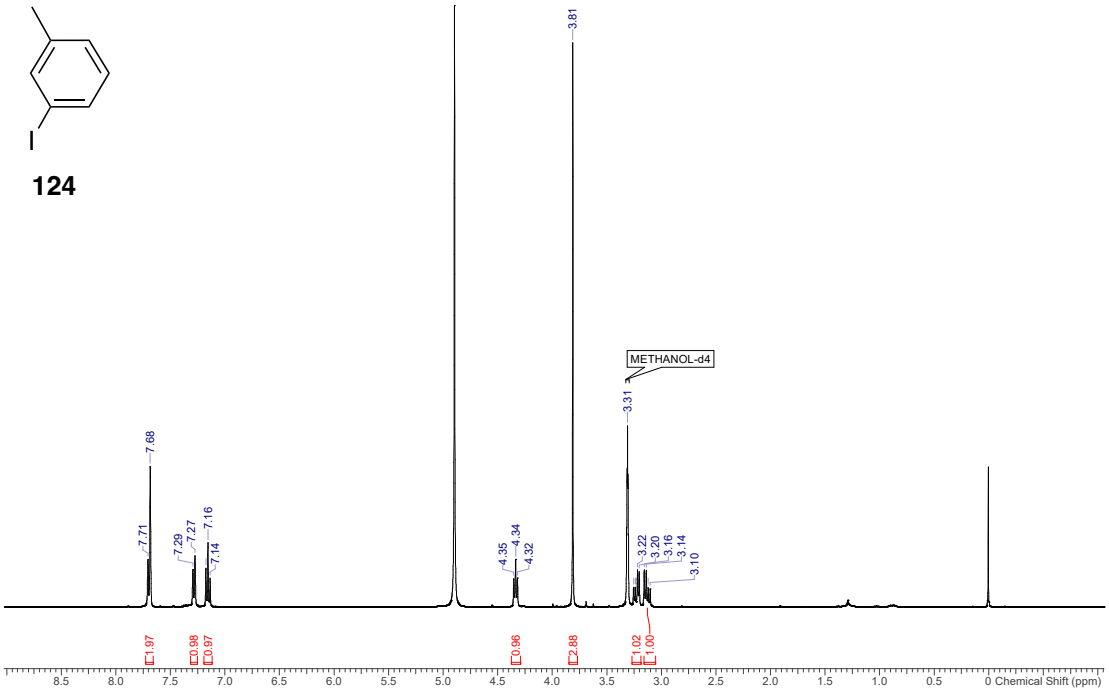


123

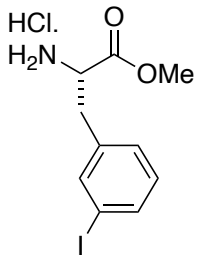
^{1}H NMR spectrum (400 MHz, CD_3OD) of **124**



124

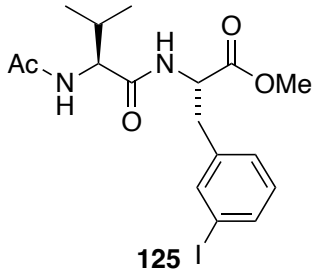


^{13}C NMR spectrum (100 MHz, CD_3OD) of **124**



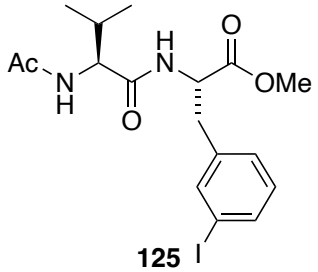
124

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **125**



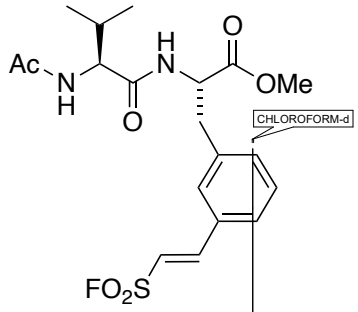
125

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **125**



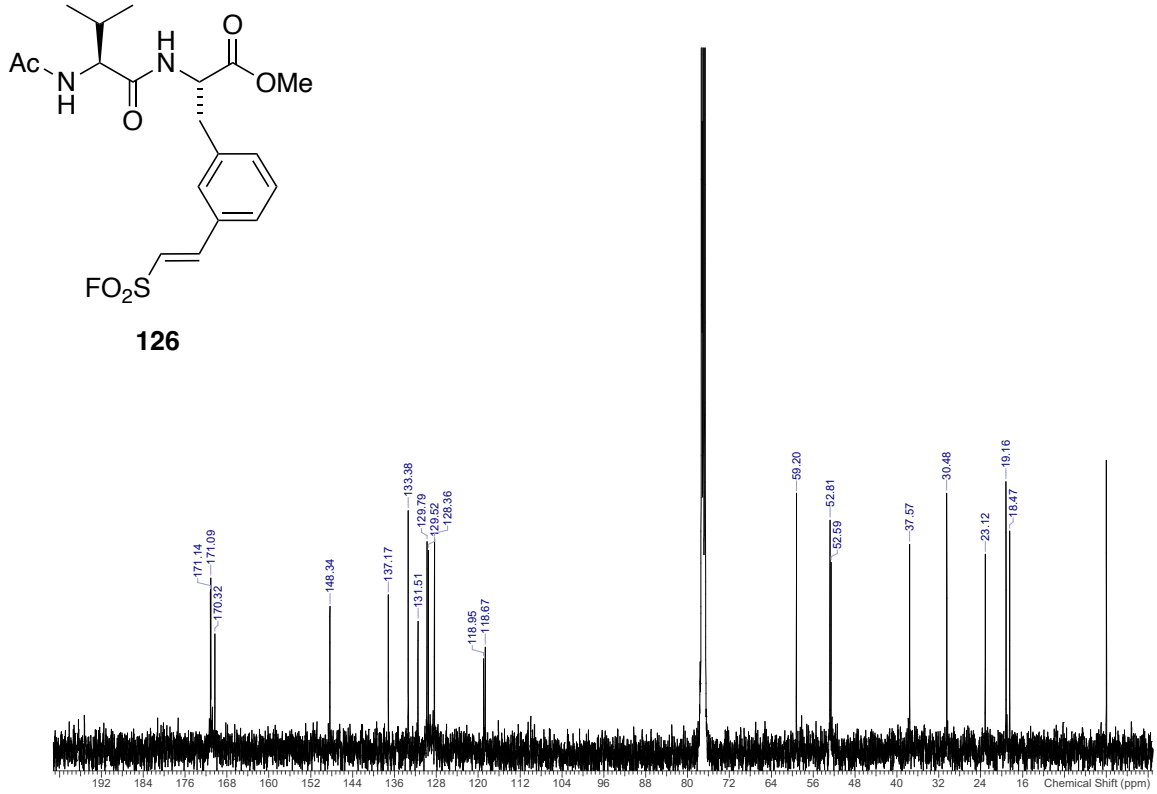
125

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **126**



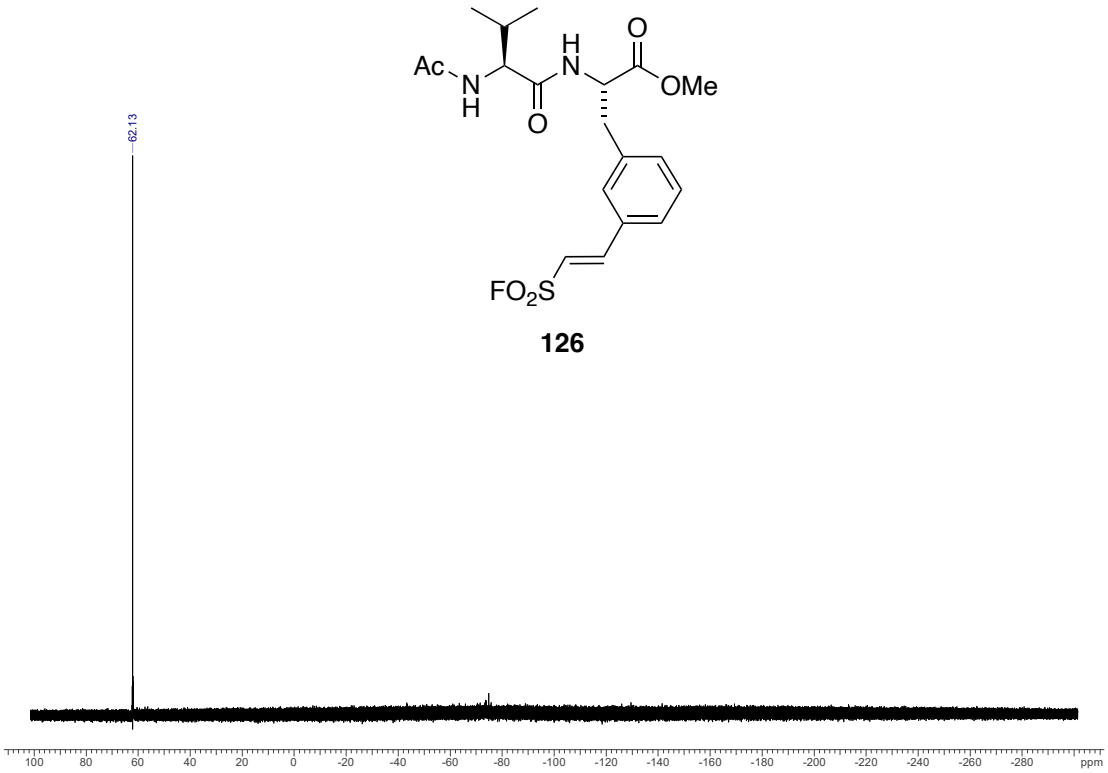
126

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **126**



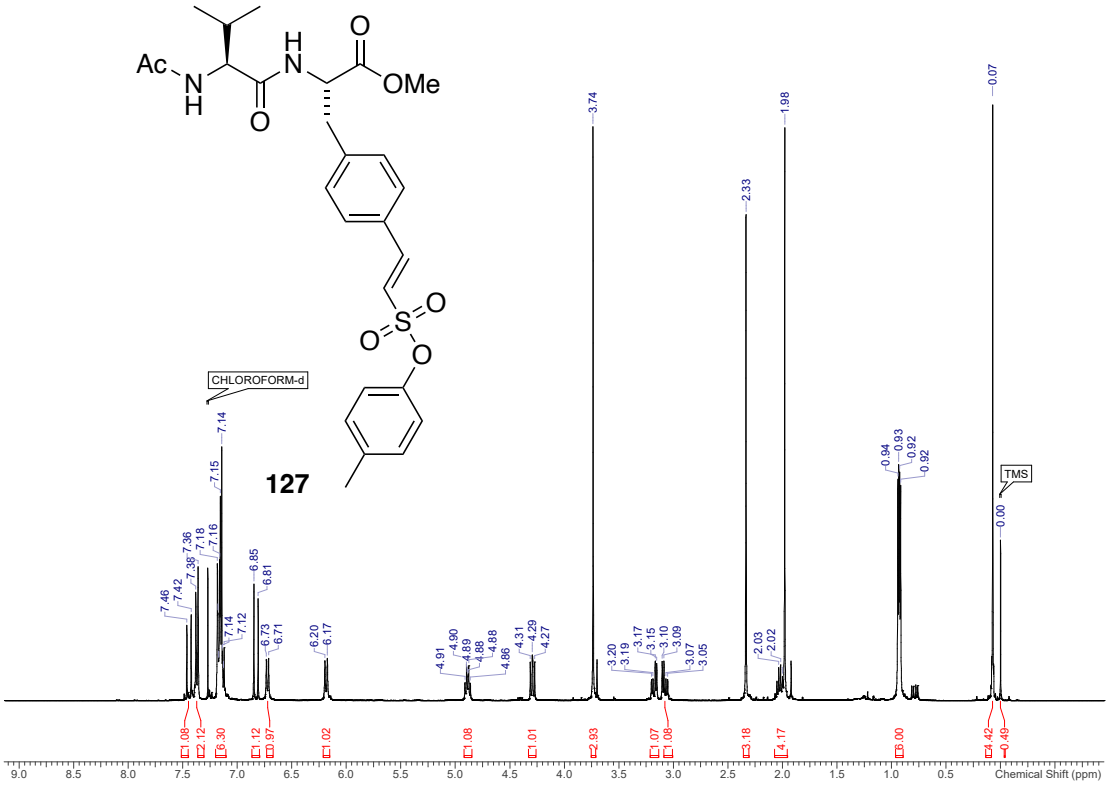
126

^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **126**

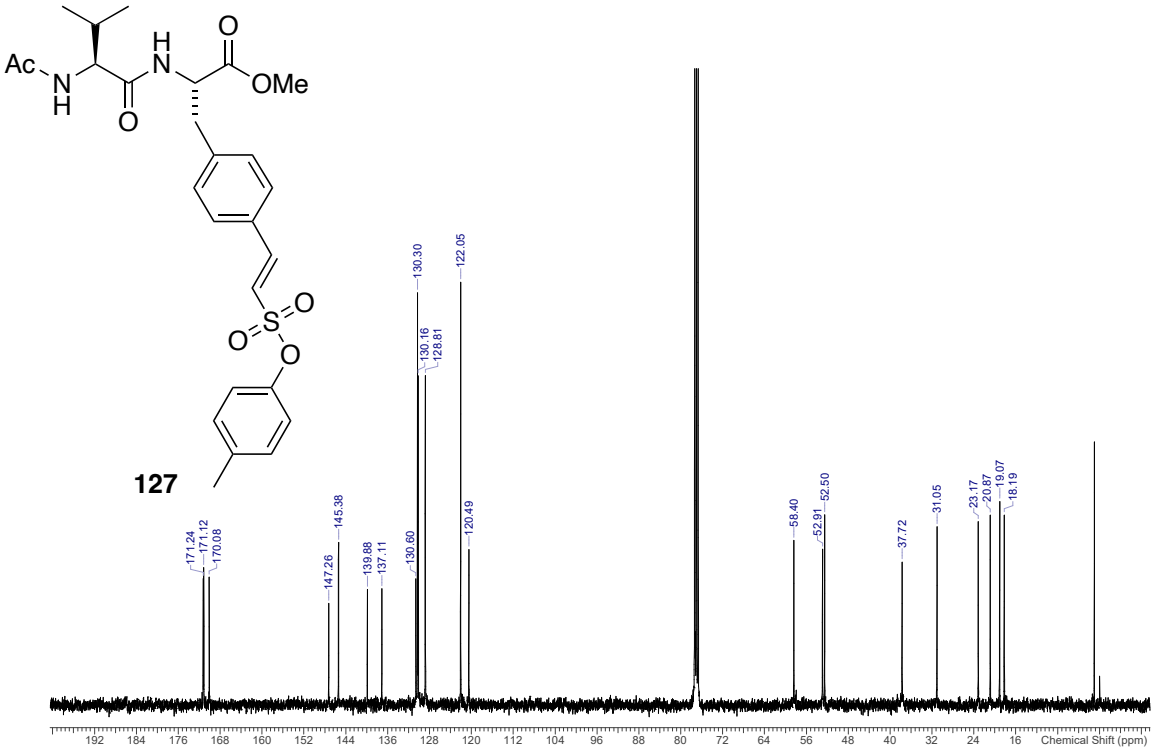


126

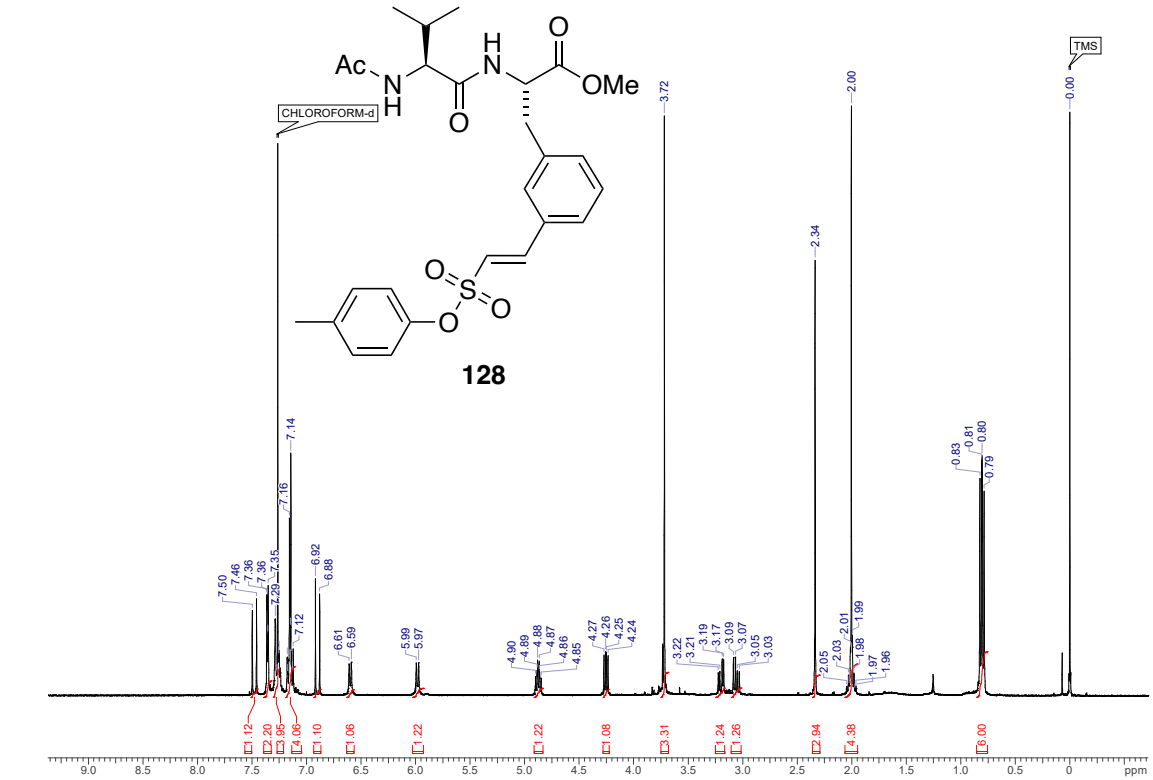
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **127**



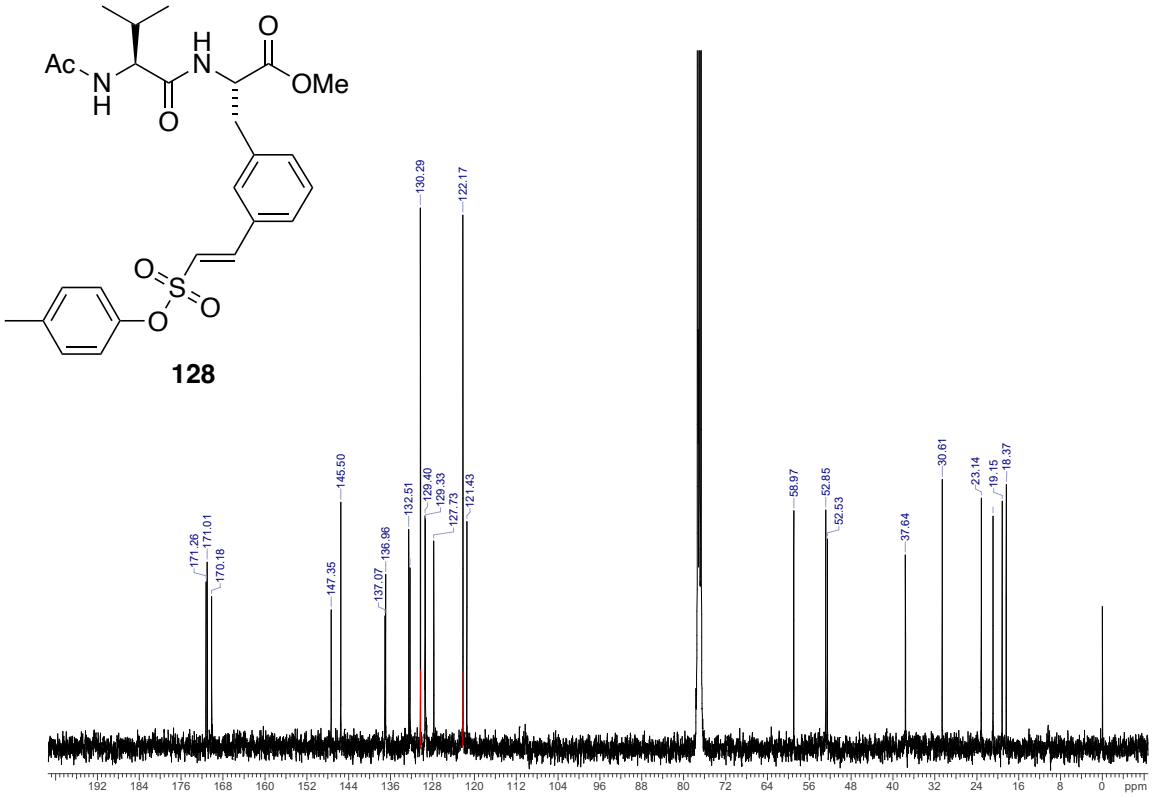
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **127**



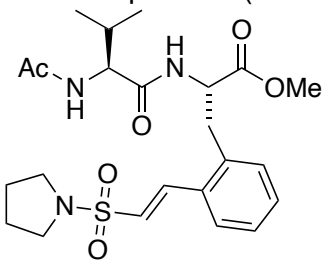
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **128**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **128**

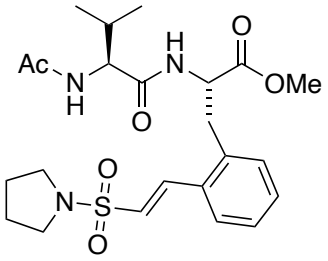


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **129**



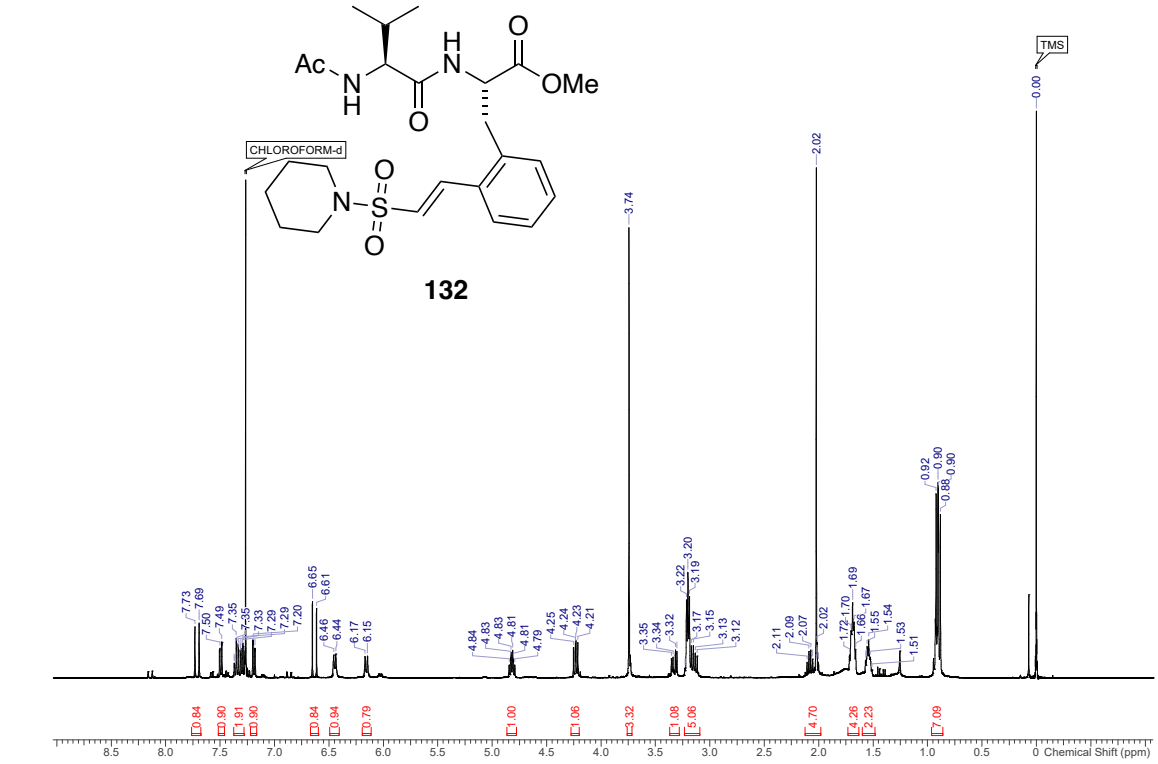
129

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **129**

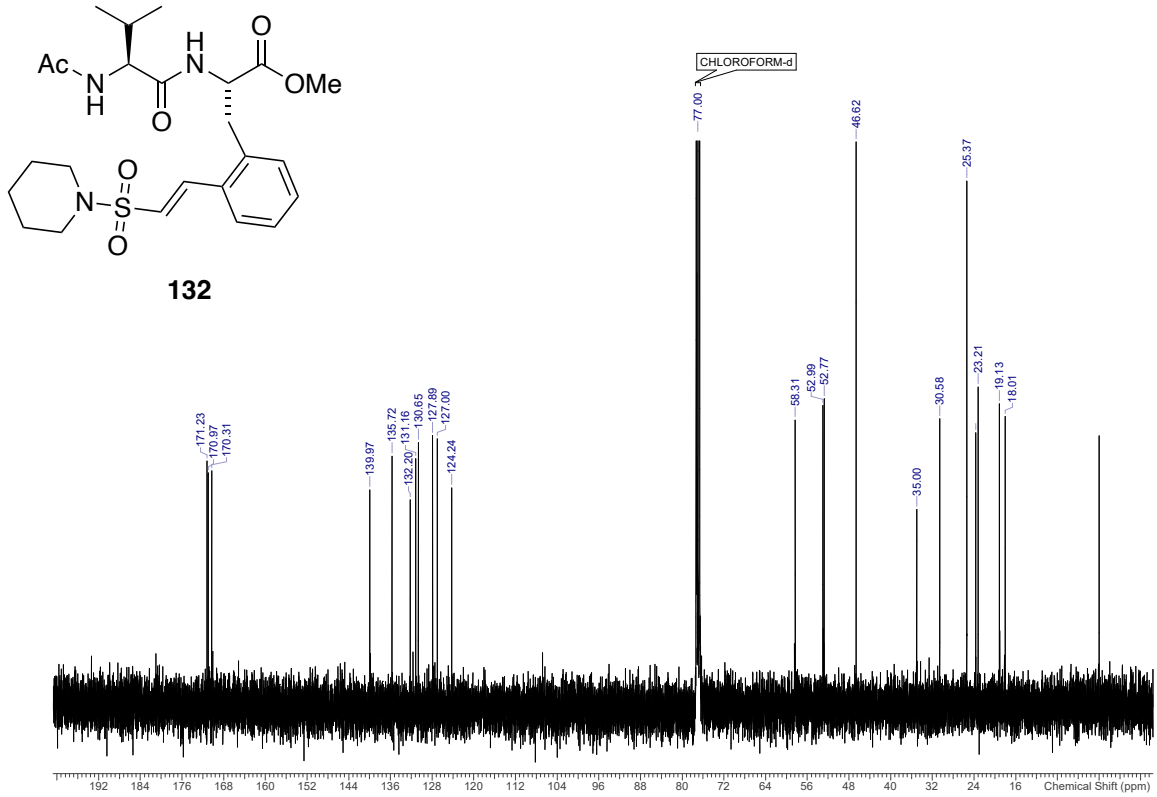


129

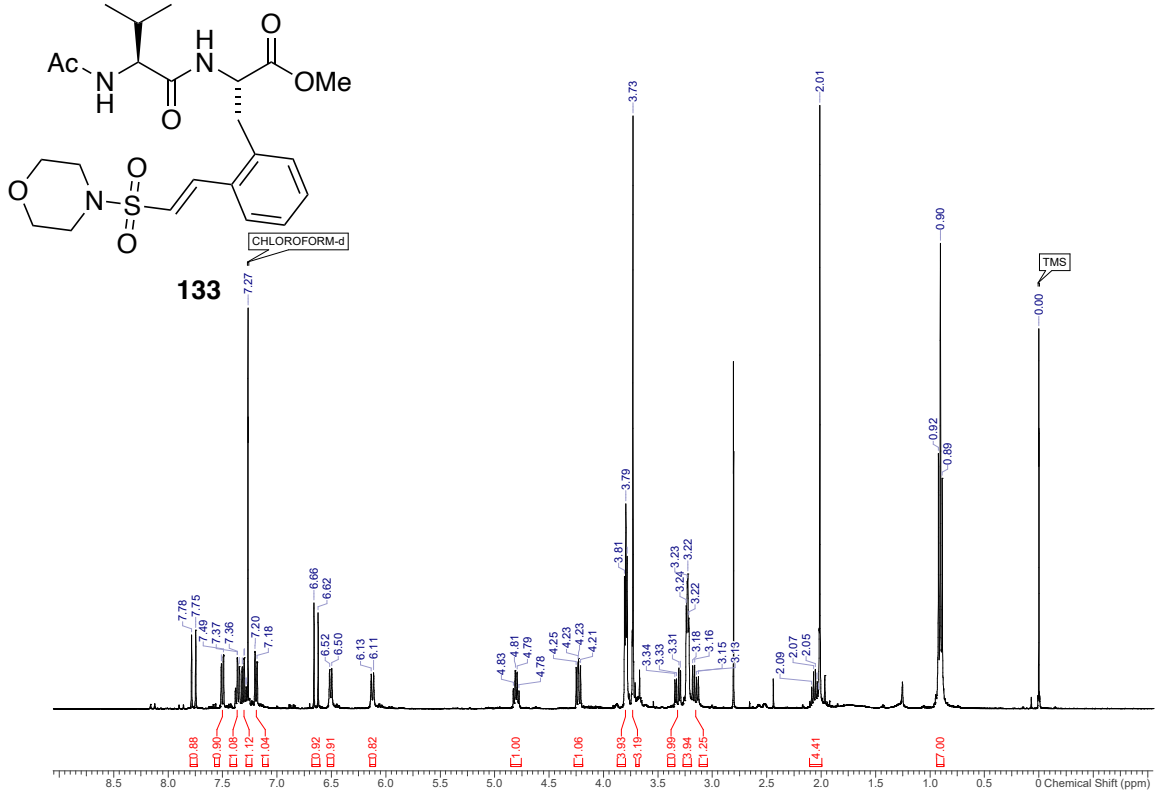
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **132**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **132**

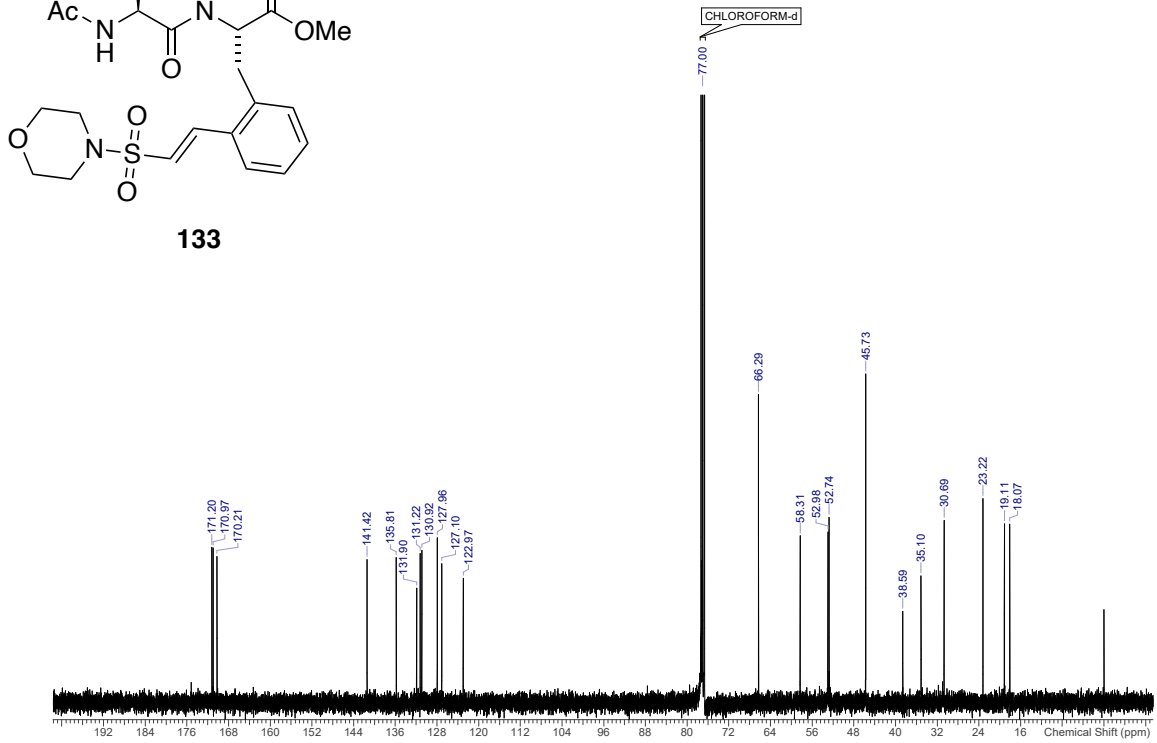


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **133**



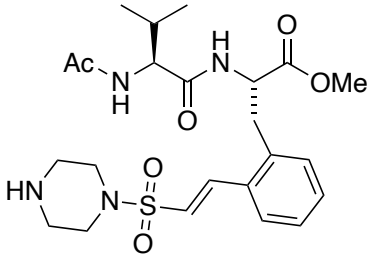
133

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **133**



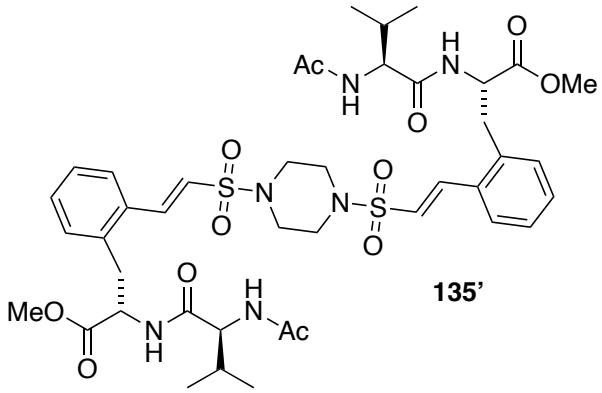
133

HRMS spectrum of **135**



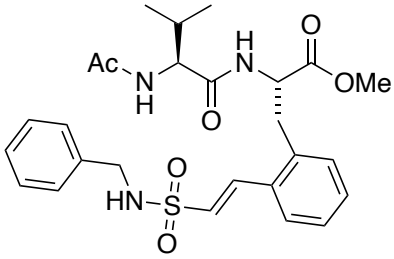
135

HRMS spectrum of **135'**

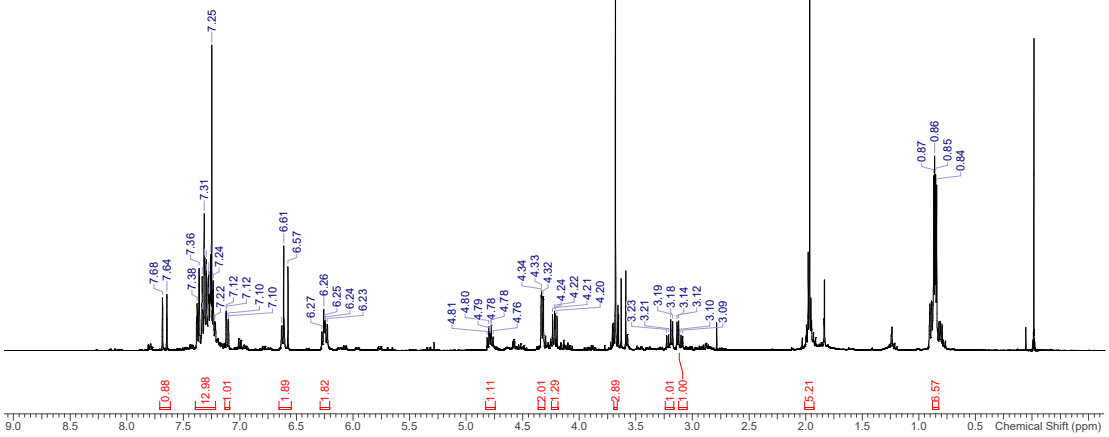


135'

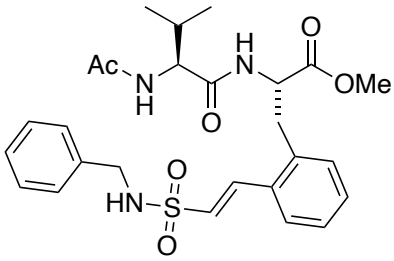
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **137**



137

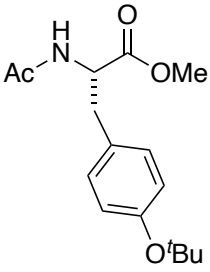


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **137**

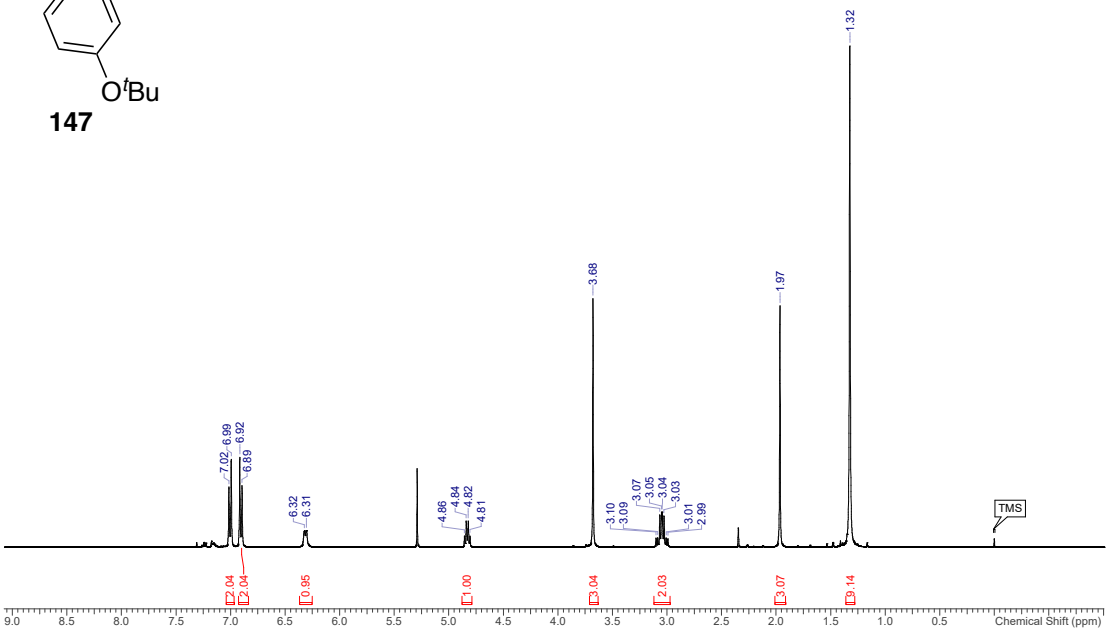


137

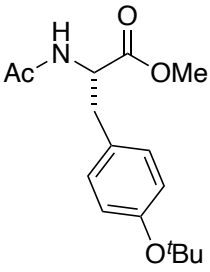
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **147**



147

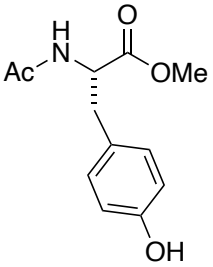


^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **147**



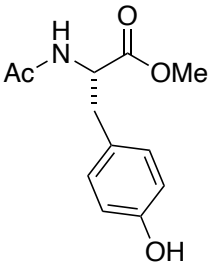
147

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **148**



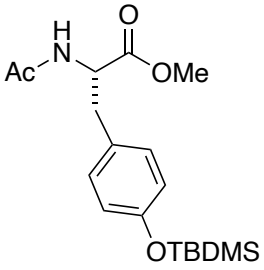
148

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **148**



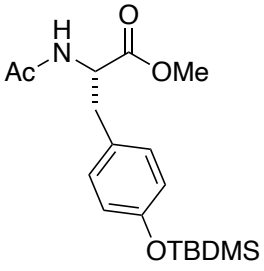
148

^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **145**



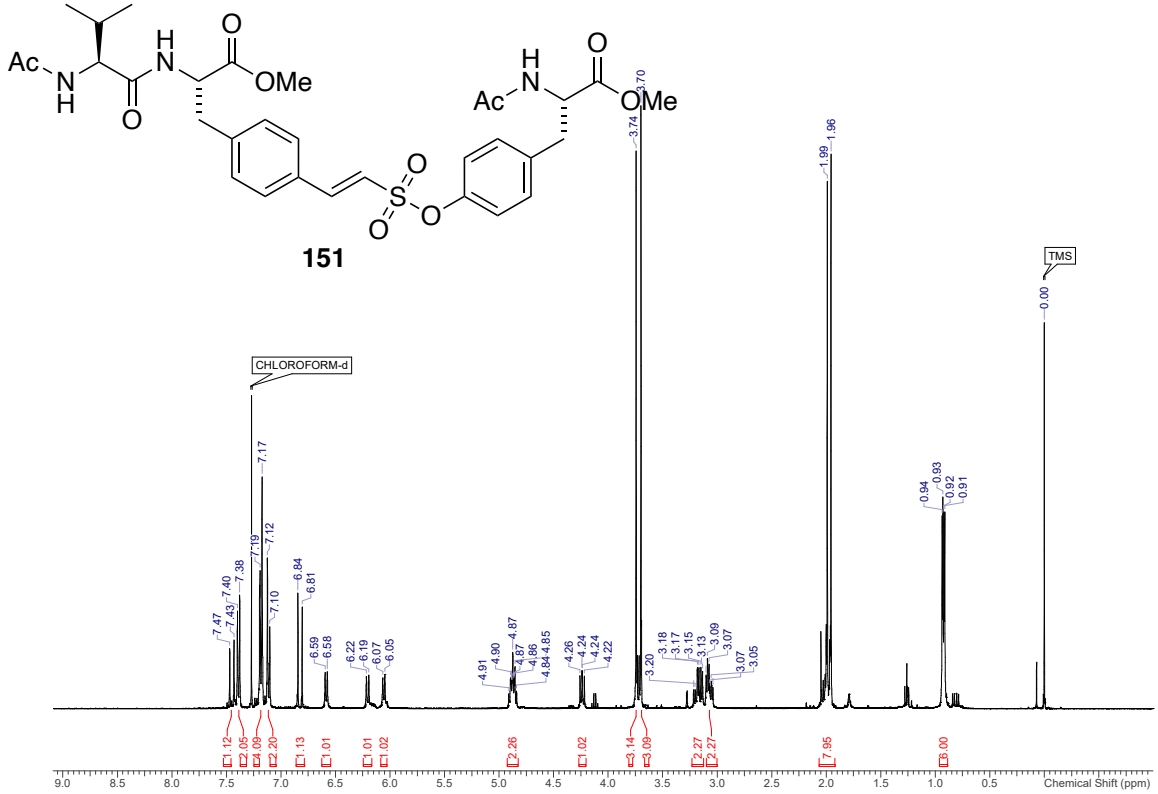
145

^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **145**

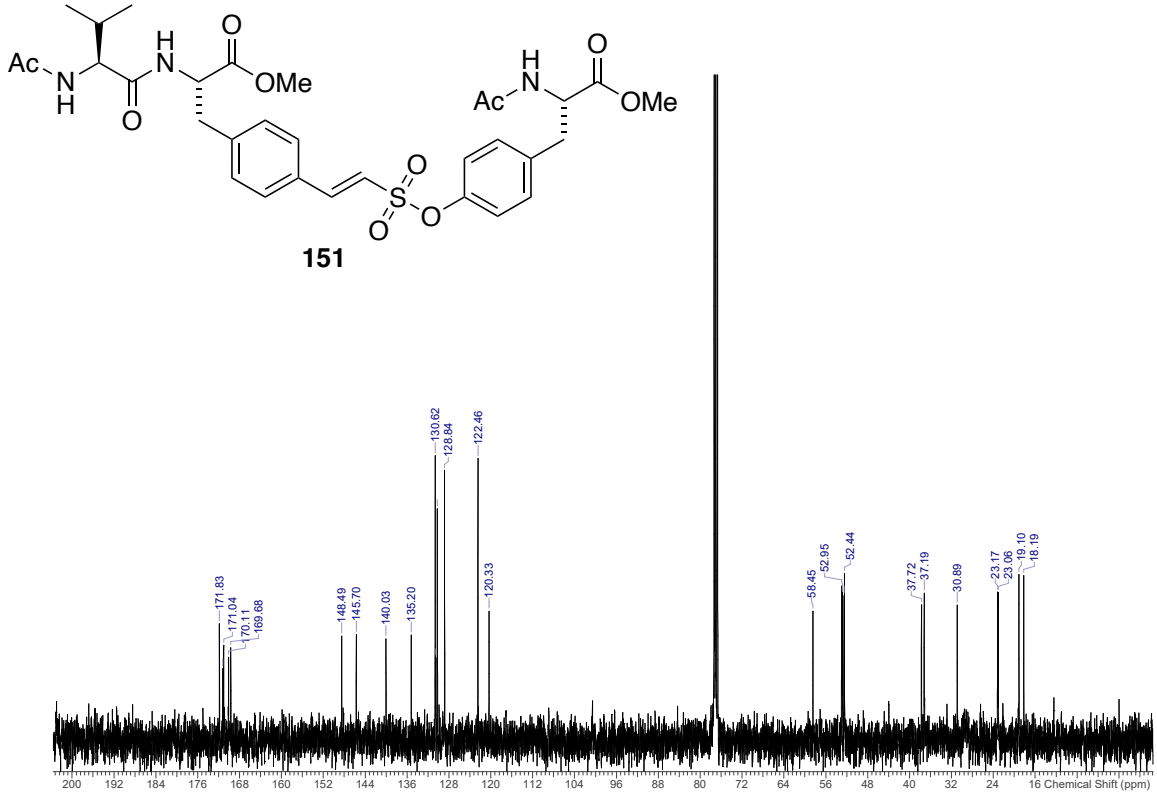


145

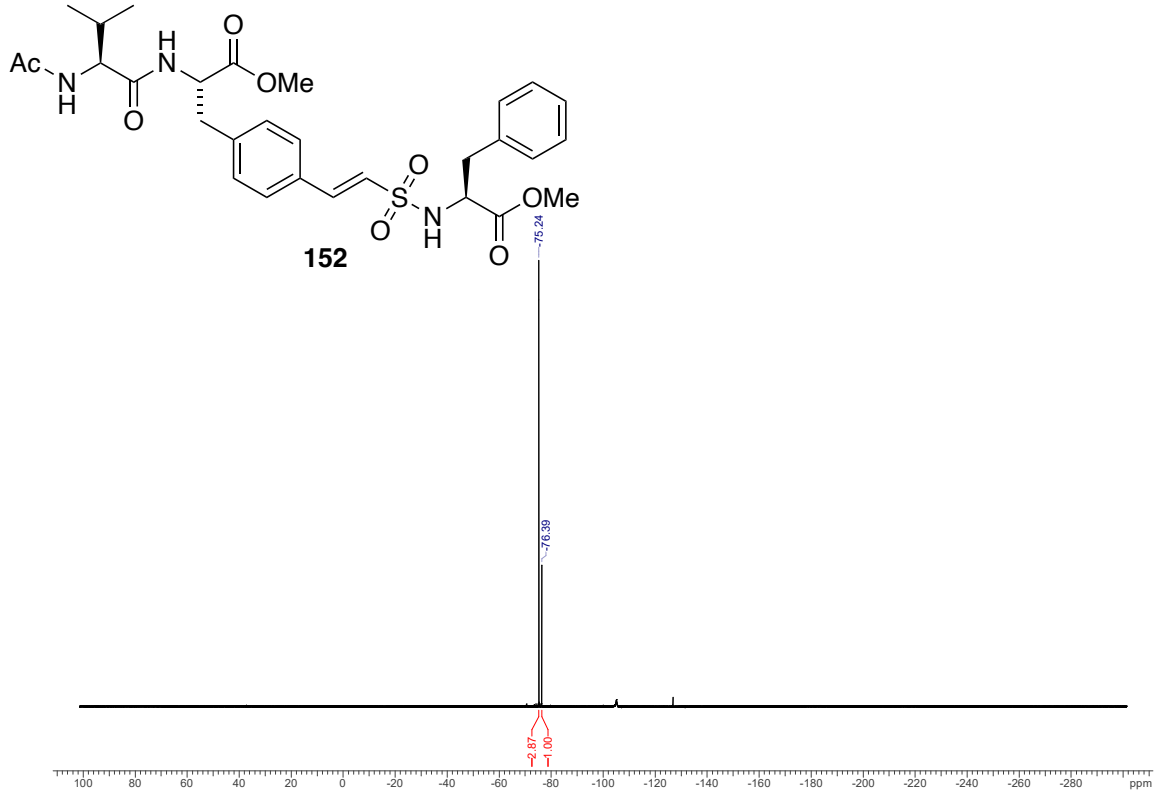
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **151**



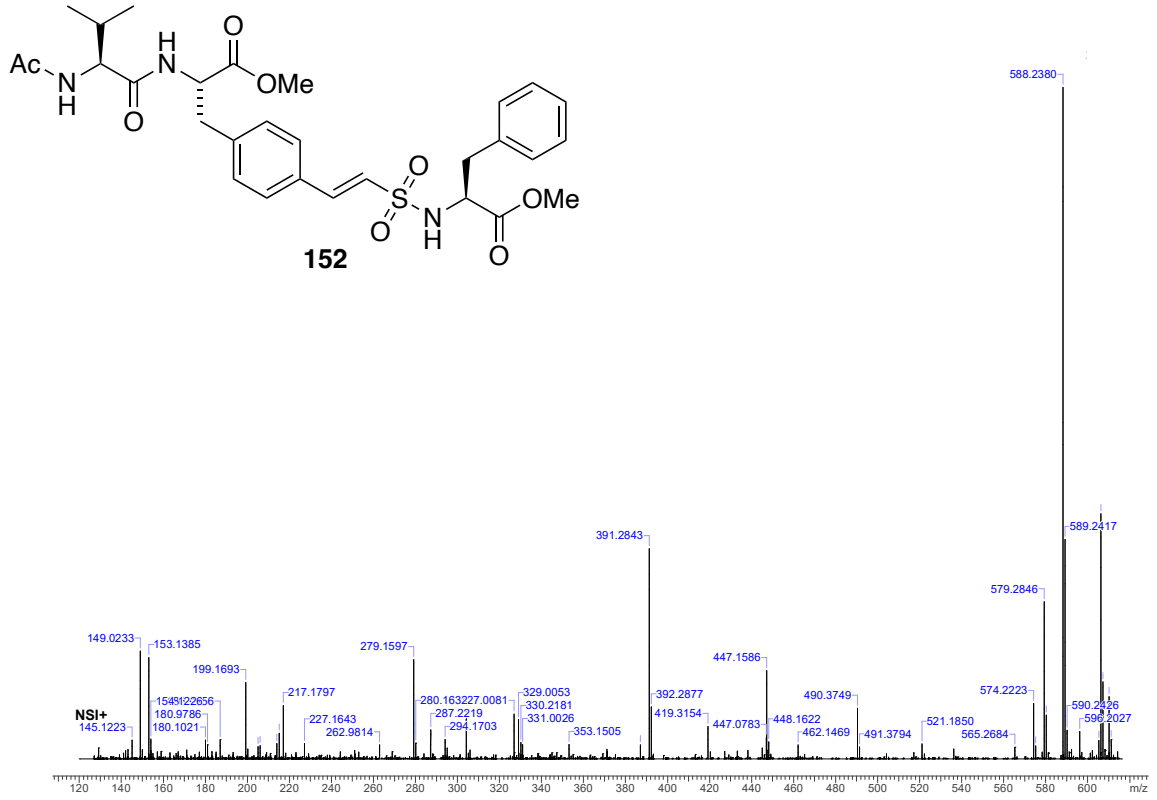
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **151**



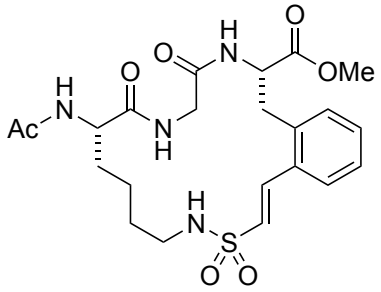
^{19}F NMR spectrum (376 MHz, CD_3CN) of **152**



HRMS spectrum of **152**

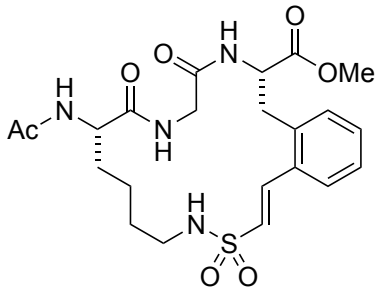


^{1}H NMR spectrum (400 MHz, CD_3CN) of **154**



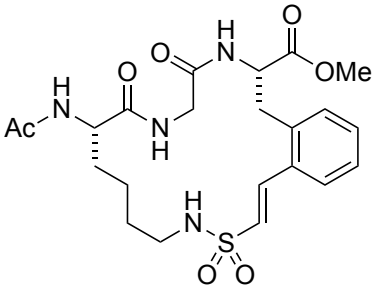
154

^{19}F NMR spectrum (376 MHz, CD_3CN) of **154**



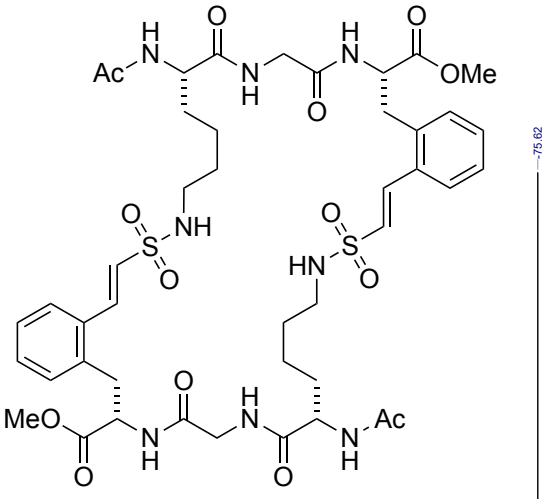
154

HRMS spectrum of **154**



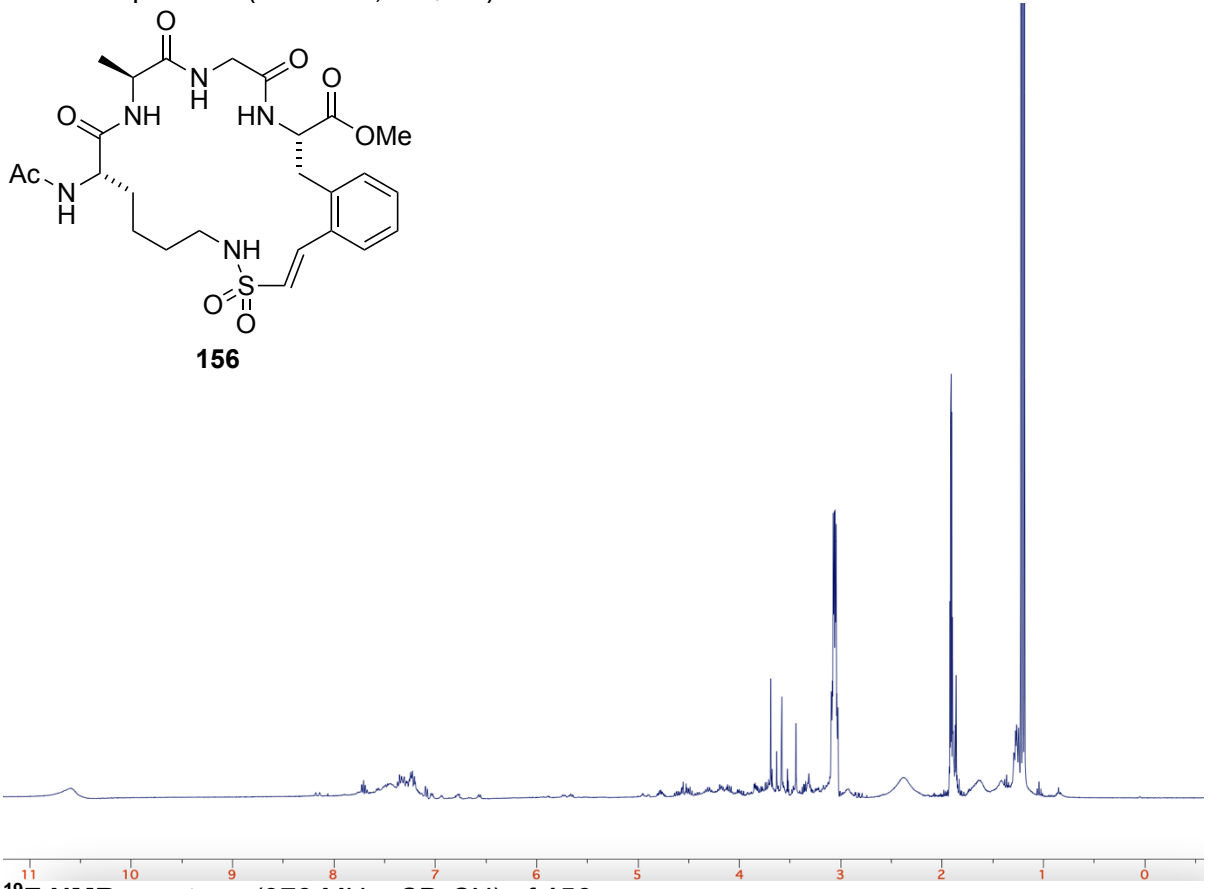
154

^{19}F NMR spectrum (376 MHz, CD_3CN) of **154'**



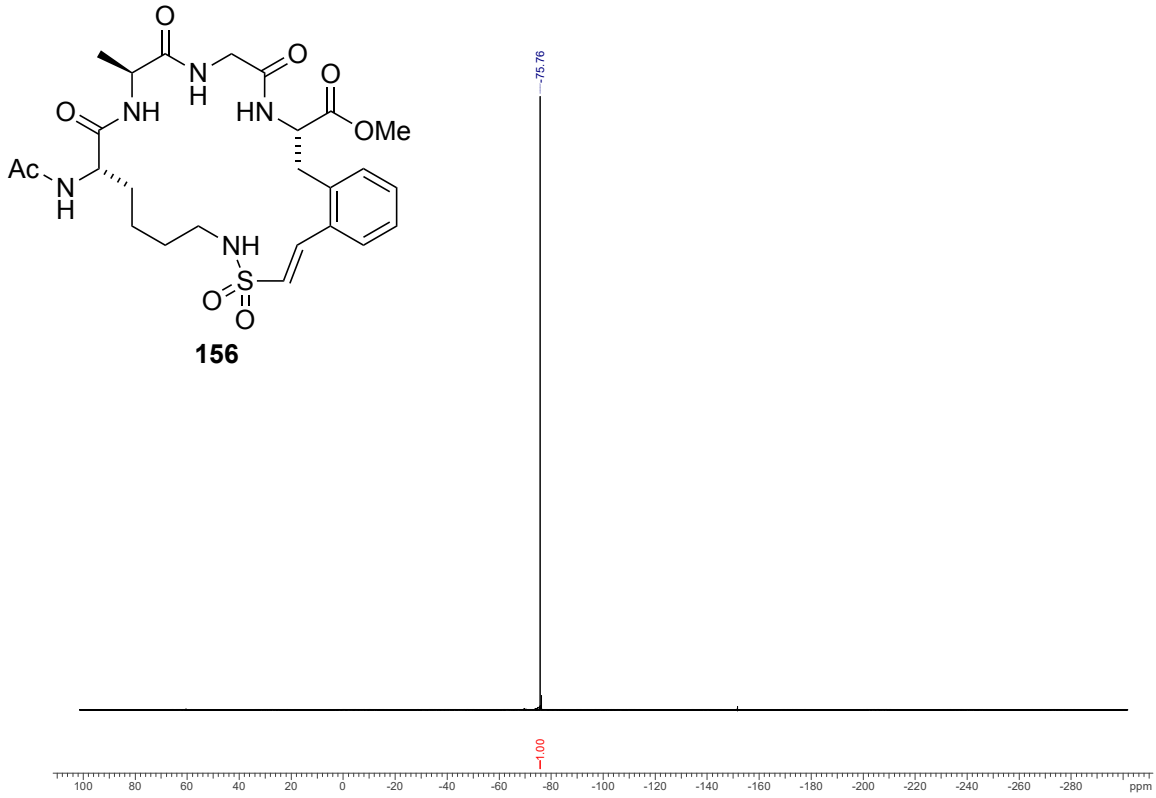
154'

^{1}H NMR spectrum (400 MHz, CD_3CN) of **156**



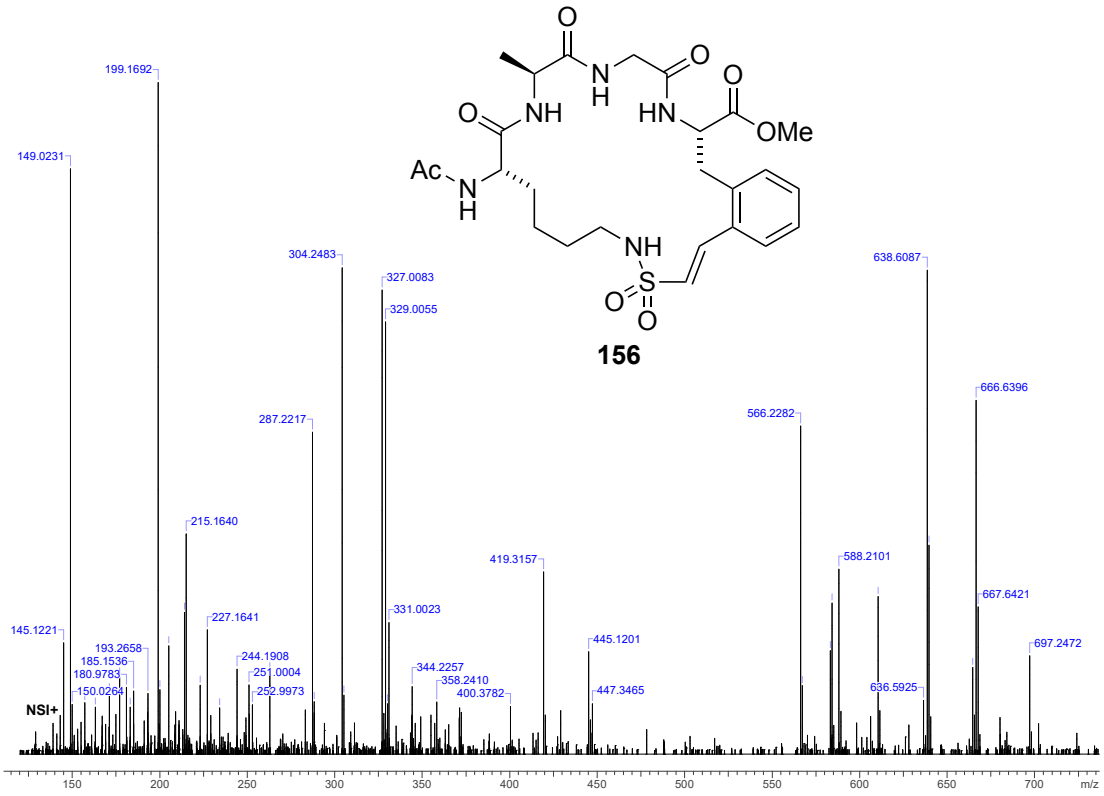
156

^{19}F NMR spectrum (376 MHz, CD_3CN) of **156**



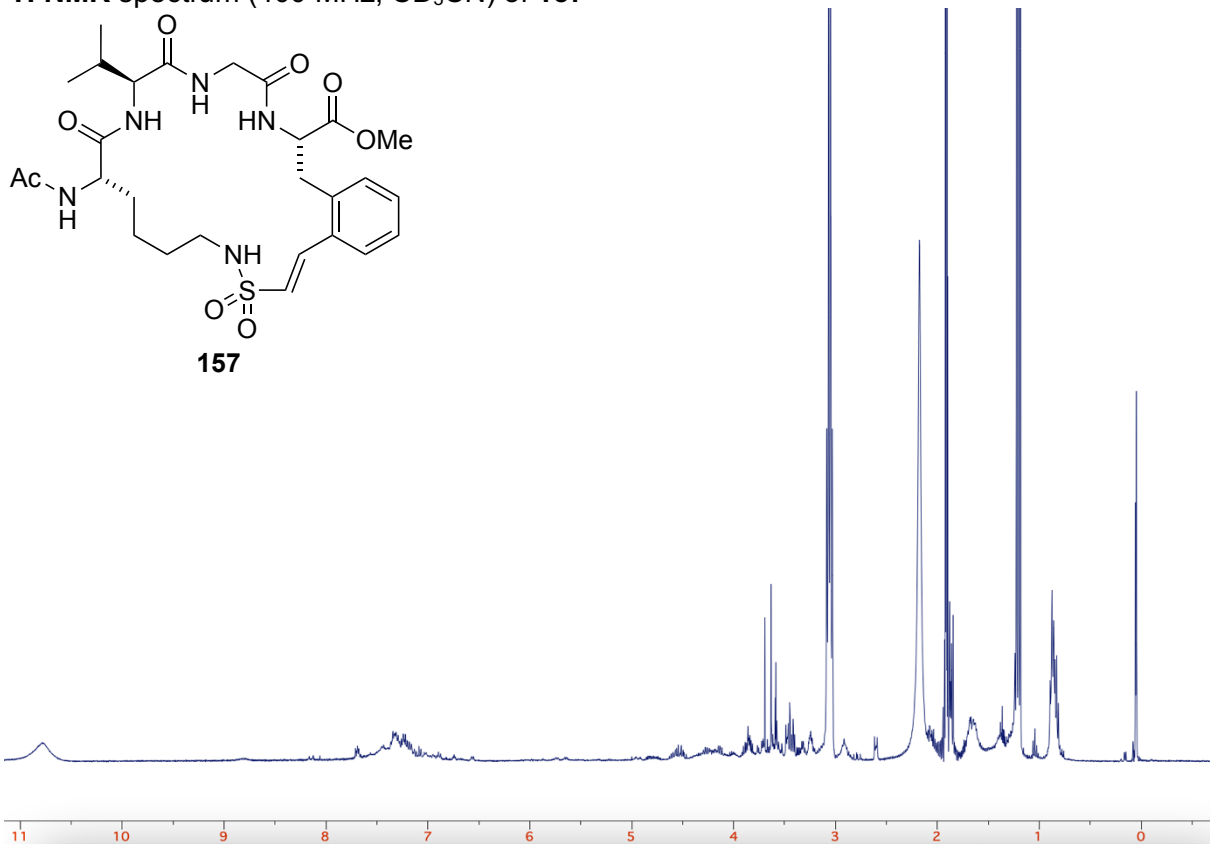
156

HRMS spectrum of **156**



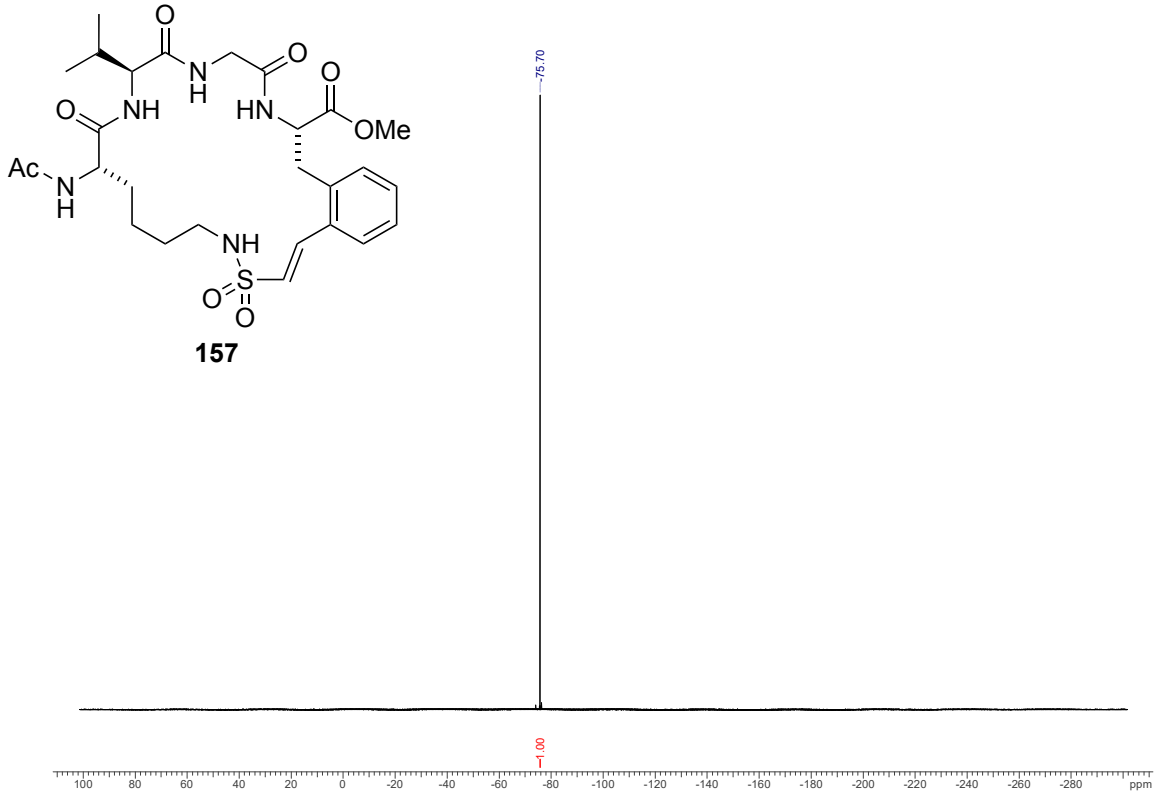
156

^{1}**H NMR** spectrum (400 MHz, CD_3CN) of **157**



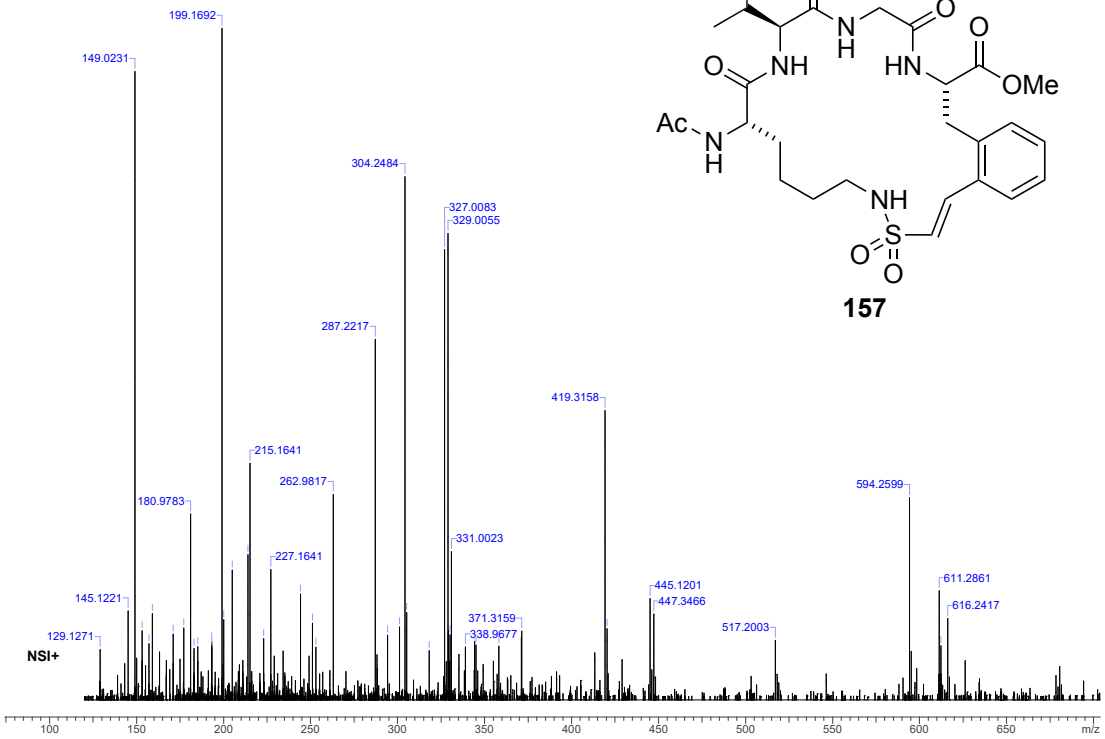
157

^{19}F NMR spectrum (376 MHz, CD_3CN) of **157**



157

HRMS spectrum of **157**



157