NOTTINGHAM TRENT UNIVERSITY

C-H Functionalization for the Post-Synthetic Modification of Peptides

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partial fulfilment of the requirements of
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for the degree of Doctor of Philosophy

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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:…….

Myles Joseph Terrey

Abstract

Peptidic natural products have been the focus of many recent research projects as they exhibit a variety of biological properties due to the large diversity of amino acid building blocks. Modified peptides have found uses in medicinal chemistry as therapeutics and as tools for the analysis of disease. Through sidechain modification, the conformation, physical properties and hence the function of the peptide can be altered, however, traditional modifications target reactive heteroatoms that may be crucial for peptide function. Post-synthetic modification of peptides enables the manipulation of natural amino acids in a peptide after the sequence has been set.

Palladium-catalysed cross-coupling reactions are a relatively new approach to peptide modification, which enable selective functionalization of the aromatic side chains. However, these reactions require the incorporation of non-natural, halogen-containing amino acids into the peptide sequence. In contrast, direct C-H functionalization is a more efficient strategy for peptide modification, which crucially enables post-synthetic modification of the natural peptide.

When this project started, there had been some research on the C-H functionalization of tryptophan residues in peptides. To expand the opportunities for peptide modification, the primary aim of this investigation was to develop methods for the modification of phenylalanine containing peptides.

The work documented in this thesis describes the development of a new C-H functionalization method for the direct modification of phenylalanine residues in peptides. By employing catalytic palladium, phenylalanine aromatic side chains were modified with alkenes, to produce functionalized side chains in a single step. The methodology was applied to di-, tri and tetrapeptides and also accommodated a range of alkenes. The olefination of phenylalanine appears to be selective; targeting mid sequence and C-terminal residues. Bidentate coordination of the metal catalyst to the peptide backbone is critical for the olefination of phenylalanine residues.

The methodology was successfully applied to tryptophan residues, where the amino acid can be modified throughout a peptide sequence. The installation of a Boc directing group appears crucial for the functionalization of tryptophan residues.

The work described for the modification of phenylalanine and tryptophan residues expands the current methodologies for peptide modification.

Acknowledgements

I would like to thank the following people who have helped me throughout my PhD

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Finally, to Lauren who has stuck by me throughout my irritable times and frequent moaning about chemistry. It will all be over soon!

And to anyone that has worked with me over the past couple of years.

Cheers everyone.

Publications

Below is a list of associated publications generated from the work documented in this thesis:

1. Terrey, M. J.; Perry, C. C.; Cross, W. B. *Org. Lett.* **2019**, *21* (1), 104–108.

   DOI: 10.1021/acs.orglett.8b03536

2. Terrey, M. J.; Holmes, A.; Perry, C. C.; Cross, W. B. *Org. Lett.* **2019**, *21* (19), 7902–7907.

   DOI: 10.1021/acs.orglett.9b02894

Abbreviations

Ac	Acetyl
AMLA	Ambiphilic Metal Ligand Activation
8-AQ	8 aminoquinoline
Boc	*tert*-butyl carbonate
Br	Broad
CMD	Concerted Metallation-Deprotonation
COSY	Correlation Spectroscopy
d	Doublet
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DG	Directing group
DMF	Dimethyl formamide
eq.	Equivalents
Hz	Hertz
in vacuo	Under vacuum (reduced pressure)
m	Multiplet
Me	Methyl
mol	Moles
MS	Mass Spectrometry
NaHMDS	Sodium hexamethyldisilazide
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
ppm	Parts per million
q	Quartet
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl

Contents

1. Introduction

1.1 An introduction to peptide modification

Peptides and proteins are key components of carbon-based life on this planet, often governing many biological processes such as cell signalling and metabolism.[1–5] Peptide structure plays a vital role in the biochemistry of peptides, shape and peptide function. Primary peptide structure is determined by the order of amino acids, which in turn gives a peptide sequence a specific shape such as an α-helix, β-sheet or β-turn. Altering the sequence of amino acids or the bonding between amino acid side chains, leads to changes in peptide function. Modified peptides are then used to explore biological function that was not possible with a native peptide.[6] For example, the development of fluorescent peptides through peptide modification, enables further understanding of disease and can be used a fluorescent marker to aid surgical treatment.[7–10]

Modified peptides have also found uses as therapeutic agents, which when compared to small molecule drugs, often possess higher target specificity and afinity.[11] Many peptide therapeutics although highly active, suffer from poor pharmacokinetics when tested. One reason for this is the increased flexibility of peptides compared to small molecule drugs. Generating a more constrained, rigid structure through peptide modification can lead to an increase in potency.

For example, N-methylation of amide residues reduces hydrogen bonding, cyclisation of peptide chains increases the rigidity of a peptide and the incorporation of non-natural amino acids can improve potency.[12] Cyclosporine A is an 11 amino acid peptide sequence, in which all of these features have been included into the drug candidate, improving the potency and pharmacokinetic properties of the drug (**Figure 1**).[13]

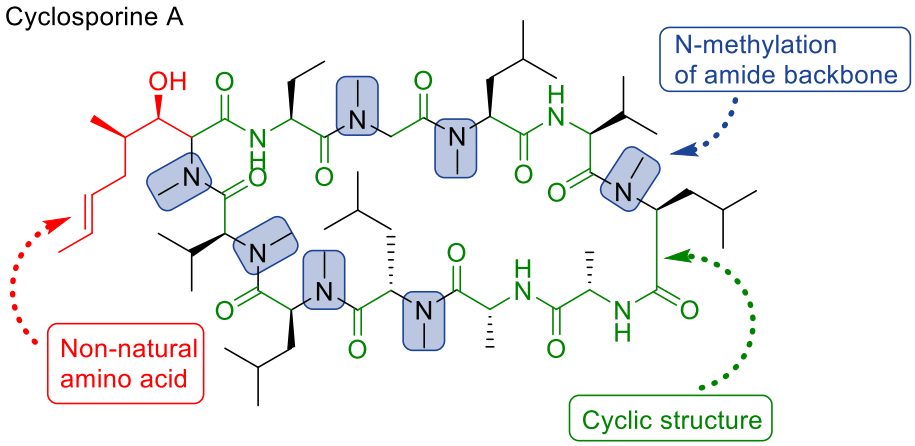


Figure 1: *Structure of Cyclosporine A.*

Site-specific peptide modification is a crucial tool in the development of peptide therapeutics to combat the disadvantages to contemporary peptide therapeutics.[14–18]

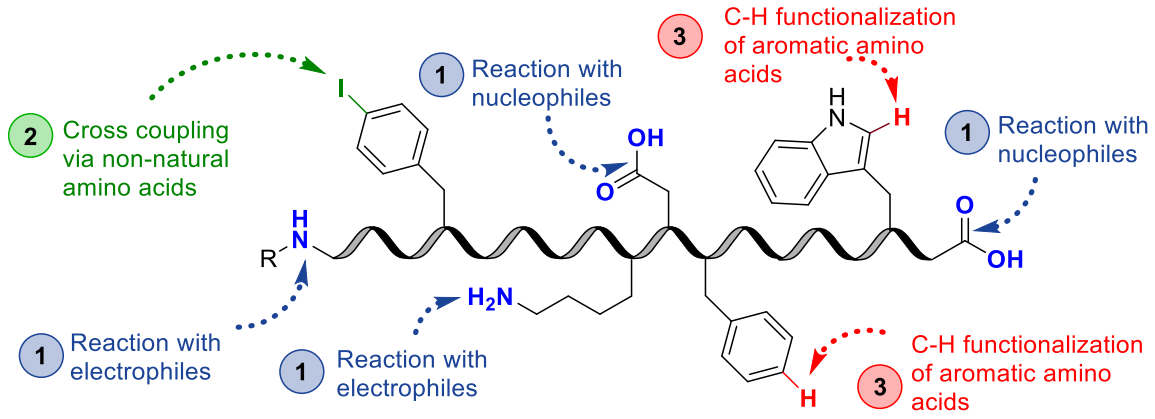


Figure 2: *A display of some of the approaches to peptide modification.*

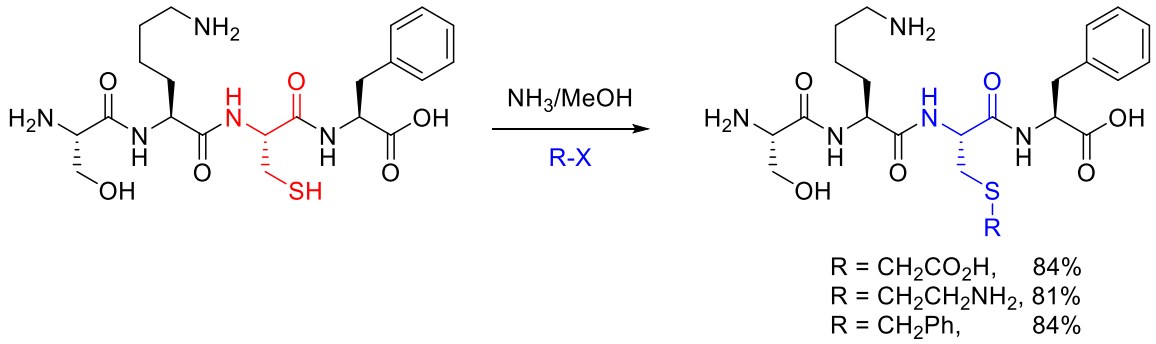
Peptide modification strategies have mainly focussed upon reactions targeting heteroatoms on amino acid side chains and termini, (**Figure 2**, strategy 1). These side chains are often crucial for peptide function and modification of these sites is not always a suitable approach.[19–21] Heteroatom modifications are the most abundant modification as these heteroatoms are generally reactive. The modification of less reactive centres in peptides is troublesome and site selectivity can be a major issue.[22,23]

For reactions at carbon atoms, improving site selectivity can be achieved through targeting unnatural amino acids or adding in pre-functionalized amino acids to a peptide sequence (**Figure 2**, strategy 2). The inclusion of these pre-functionalized amino acids increases the number of synthetic steps and means that native peptides cannot be modified in the same way. Post-synthetic modifications (such as C-H functionalization) are desirable alternatives to pre-functionalization as they enable modifications upon natural peptides in a single step, (**Figure 2**, strategy 3).[24–26]

1.1.1 Modification of heteroatoms on peptide side chains

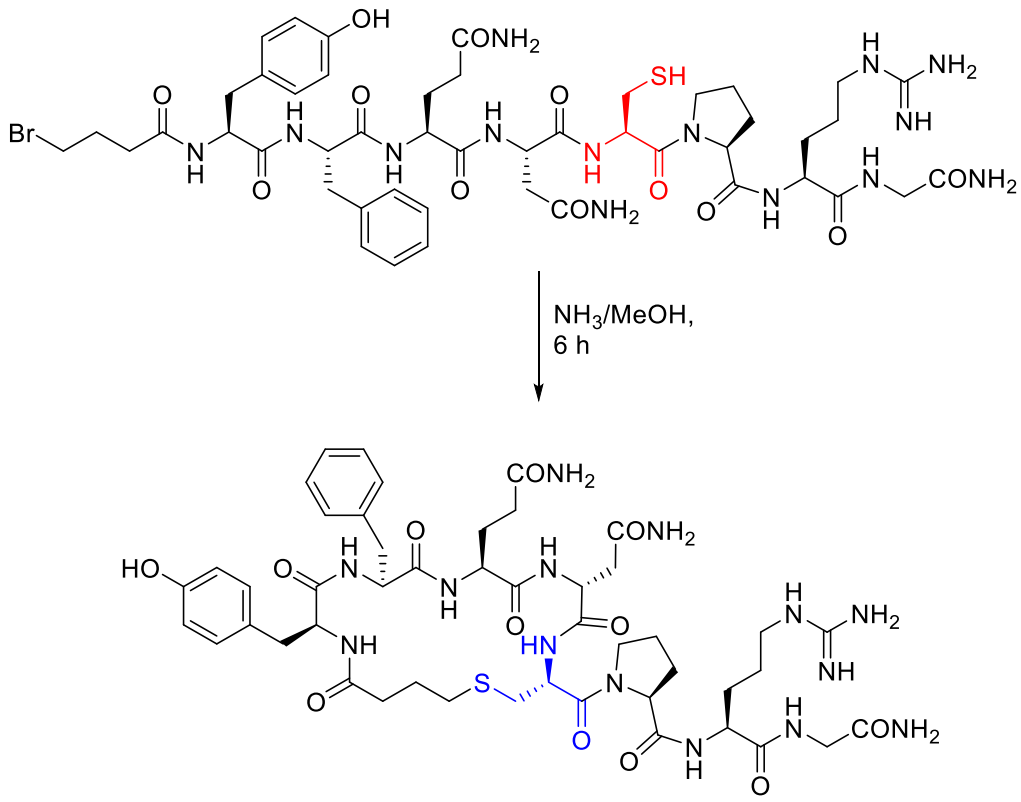
Perhaps the most widely reported method of peptide modification are transformations upon heteroatoms on peptide side chains.[27] Thiol side chains upon cysteine are often the chosen site of modification, due to the increased nucleophilicity when compared to amine side chains of lysine and arginine side chains for example, as well as the lower natural abundance in peptides.[28] Crosslinking of cysteine side chains between peptides is a common feature in natural peptides and proteins. The alkylation of cysteine residues

provides a convenient route to modified peptides, such as those displayed in **Scheme 1**.[29]



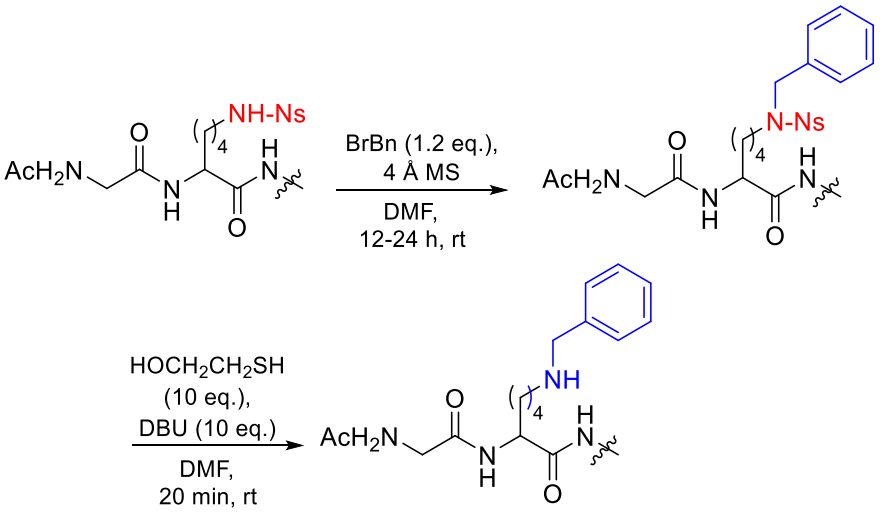
***Scheme 1**: Alkylation of cysteine residues in a peptide sequence.*[29]

The methodology developed for the S-alkylation of cysteine residues has also been demonstrated on the intramolecular cyclisation of a septa-peptide. The alkylation of the cysteine side chain in **Scheme 2**, lead to the formation of a carbavasopressin analogue and is an example of side chain modification to form a natural peptide derivative. The alkylation was performed in the presence of a nucleophilic arginine residue and the acidic tyrosine side chain.[30]



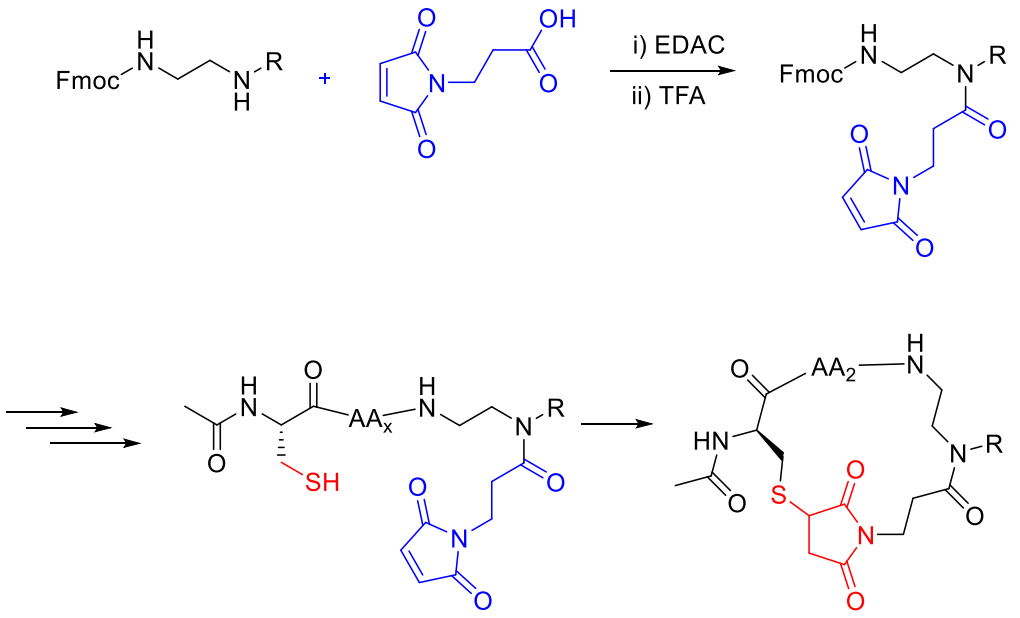
***Scheme 2:** Synthesis of a carbavasopressin analogue by cysteine alkylation.*[30]

As well as reactions involving cysteine residues, the modification of reactive amine residues have been extensively explored. The post-synthetic modification of lysine is one such example in which the side chain of a peptide is modified by selective *N*-alkylation, **Scheme 3**.[31] The free amine is first protected by the addition of a nosyl group before *N*-alkylation using benzyl bromide under mild conditions. The nosyl group was then removed to give the *N*-alkylated peptide. The reaction accommodates a variety of different functionality present within a peptide: from sulphide bridges to histidine and tryptophan residues.



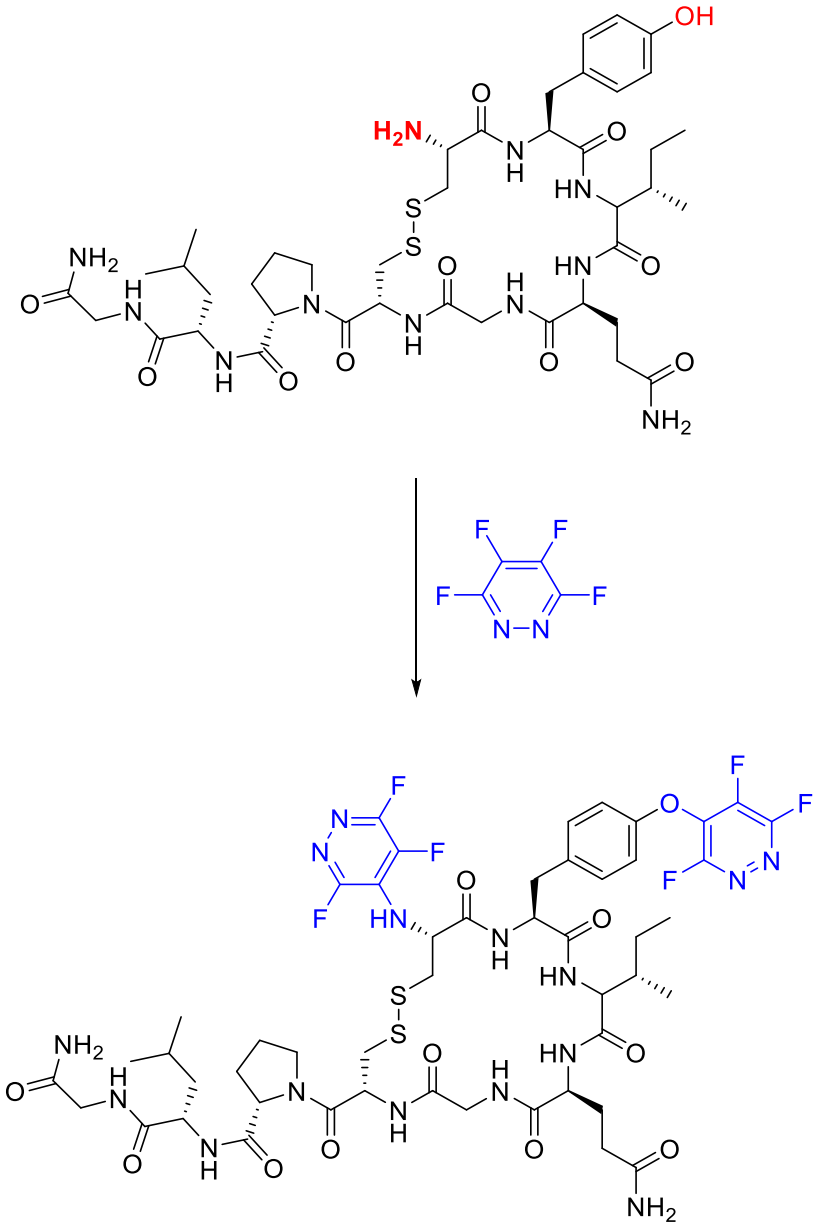
Scheme 3: *Alkylation of a lysine residues in a peptide.*[31]

The development of new amino acids that can undergo simple organic reactions to make peptide derivatives is highly sought after. Koehler *et al.* reported the production and installation of a non-natural amino acid that can undergo Michael additions to give structurally complex peptides, **Scheme 4**.[32] The maleimide functionalized amino acid is incorporated into a peptide sequence during peptide synthesis. The maleimide residue was found to react readily with nucleophiles to create highly functionalized side chains. Moreover, the nucleophilic cysteine side chain was reacted with the maleimide amino acid via a Michael addition to create a modified cyclic peptide.



Scheme 4: *The incorporation of maleimide into a peptide sequence and the cyclisation via Michael addition.*[32]

A recently reported study into the addition of electrophilic pyridazine units to peptides demonstrates this approach, **Scheme 5**.[33] The nucleophilic amino acids (such as serine and tyrosine) are targets for modification, in which pyridizine based electrophiles are added to amino acid side chains in a nucleophilic aromatic substitution reaction. The cyclic peptide oxytocin was also modified by the pyridazine analogues. In the study, two perfluoropyridazine units were added to the peptide at the tyrosine OH and the free amine on the peptide backbone. The investigation aimed to improve the enzymatic stability of the peptide. The modified oxytocin was found to have an increased resistance to enzymatic digestion compared to the unmodified peptide, in which the cyclic structure is easily broken. Heteroatom selectivity becomes increasing more challenging upon larger peptide sequences.

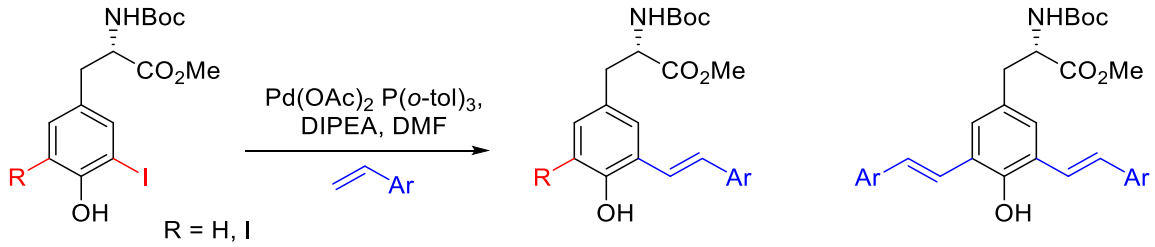


***Scheme 5**: Modification of oxytocin.*[33]

1.1.2 Modification with pre-functionalized amino acids

The challenge for peptide chemists is to develop methodologies for the direct modification of less reactive amino acid side chains. The modification of aliphatic and aromatic amino acid residues has been under explored due to the lack of a functional handle, making residue selectivity difficult. The inclusion of pre-functionalized amino acids into a peptide sequence, allow the direct modification of a specific amino acid, increasing selectivity over natural residues. Draw backs of using pre-functionalized amino acids include an increased cost in materials and the requirement of additional steps to product the non-natural amino acids in question.

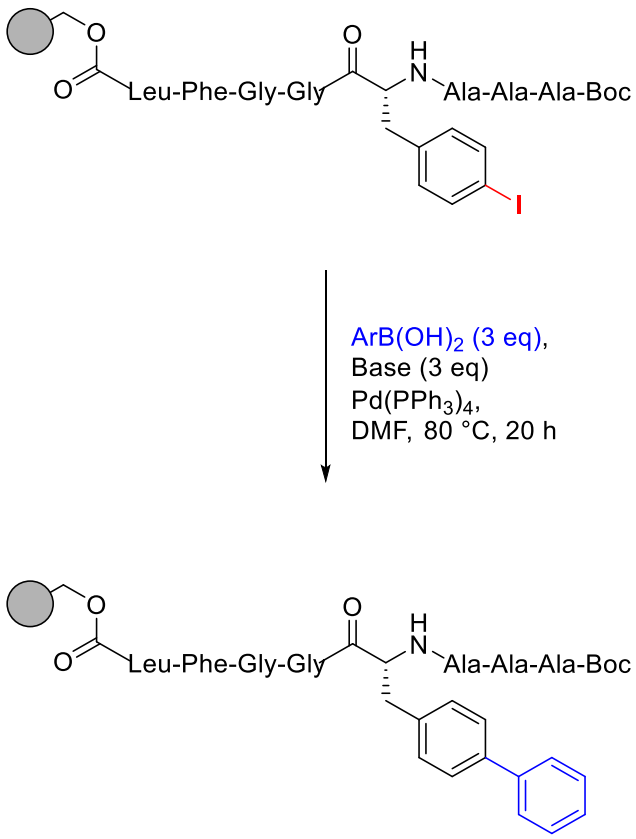
The iodination of tyrosine is one such example of pre-functionalization, **Scheme 6**.[9] The olefination of iodo-tyrosine using catalytic palladium was found to give highly fluorescent modified amino acids. The reaction proceeds via a Heck reaction to give the mono-, di-olefinated amino acid depending on the whether the di-iodo or mono-iodo-tyrosine derivative was used.



Scheme 6: *Olefination of unnatural iodo-tyrosine amino acid.* [9]

The olefination of iodo-tyrosine at the meta-position within a peptide requires the non-natural tyrosine amino acid be placed into the sequence during peptide synthesis. The olefinated peptide displays increased fluorescent characteristics compared to the unmodified starting material, making it a suitable tool for fluorescent imaging. The olefination of tyrosine containing peptides is limited, however to non-natural species, pre-functionalized amino acids. The olefination of natural tyrosine containing peptides has yet to be reported.

The incorporation of pre-functionalize amino acids into a peptide sequence allows further modification of a peptide allowing greater selectivity and control. These non-natural residues are then the targets for selective modifications by C-C cross coupling.[34] One such example of this is demonstrated in **Scheme 7**.



Scheme 7: *Suzuki cross coupling of pre-functionalized phenylalanine.*[35]

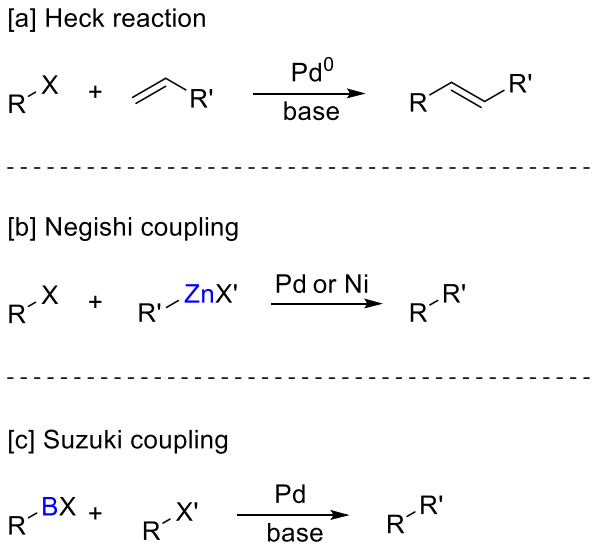
The non-natural amino acid 4-iodophenylalanine has been inserted into a peptide as the sequence was created. An aryl boronic acid derivative was then reacted with the peptide in the presence of a palladium catalyst to create a bi-aryl peptide. The inclusion of the non-natural amino acid allows for selective arylation at the para position of phenylalanine derivative.

The inclusion of pre-functionalized amino acids, however, increases the number of steps in the synthesis. The challenge for synthetic chemists is to develop new methods in which less reactive amino acids are modified without the use of pre-functionalization.

1.2 C-H functionalization

In the search for new C-C bond forming processes, cross coupling reactions have until recently been the primary way of creating new C-C bonds.[36] Cross coupling reactions arose in the early 1970s as new ways of generating previously impossible C-C bonds using a metal catalyst. The early pioneers of C-C cross couplings were Heck, Negishi and Suzuki, who were awarded the Nobel prize for chemistry in 2010 for development of cross coupling reactions.[36] The Negishi and Suzuki reactions require the use of a pre-

functionalized organozinc and organoborane species for the cross coupling reaction to occur, however, Heck reactions do not require the generation of an organometallic reagent prior to catalysis.



Scheme 8: *Examples of [a] Heck reaction, [b] Negishi coupling and [c] Suzuki cross coupling reactions.*

C-H activation has developed into a desirable alternative to cross coupling reactions by manipulating non-acidic C-H bonds.[37] Both C-C cross coupling and C-H activation methods allow the modification inert carbon bonds in the presence of a metal catalyst. C-H functionalization reactions differ to that of cross couplings in that they do not necessarily require the inclusion of organo halides or pre-functionalization prior to the reaction. Furthermore, C-H functionalization allows the generation of new compounds by generating unique disconnections in retrosynthetic challenges.

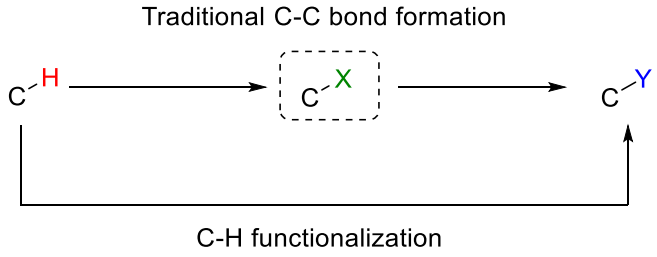


Figure 3: *A representation of C-H functionalization compared to traditional C-C bond formation reactions.*

C-H functionalization unlocks the ability to design molecules using fewer synthetic steps, and cheaper starting materials. The modification of natural amino acids and peptides can be exploited using C-H functionalization. Peptide modifications usually require the

incorporation of non-natural amino acids that are expensive or require many synthetic steps to install. Designing a synthesis using C-H activation allows the generation of a new library of peptides without the incorporation of pre-functionalized materials, thus reducing the number of synthetic steps and the cost of synthesising the desired modified peptide.[38]

1.2.1 Mechanisms of C-H activation

Since the early reports of C-H activation, there has been a dramatic increase in the number of reported transformations by C-H activation. C-H activation can be divided into four mechanisms as illustrated in **Figure 4**.

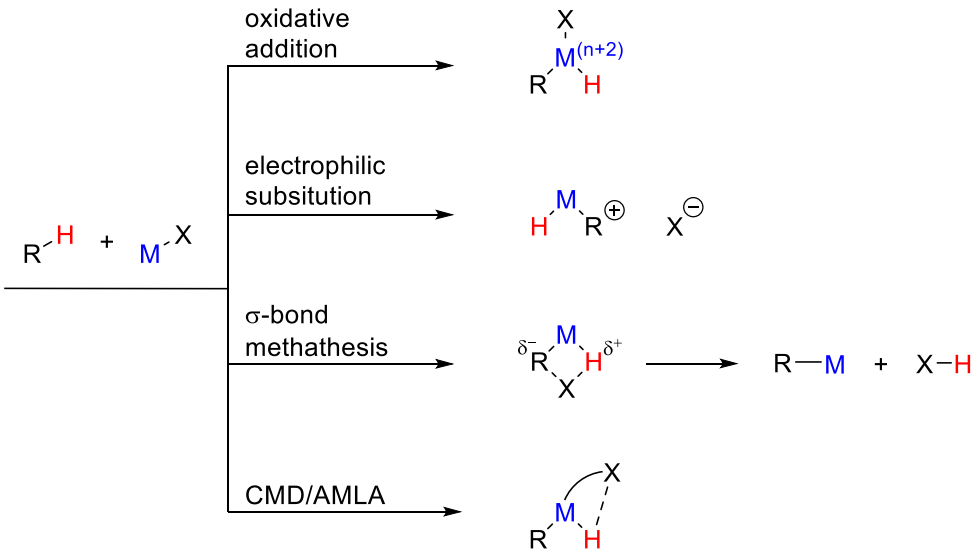
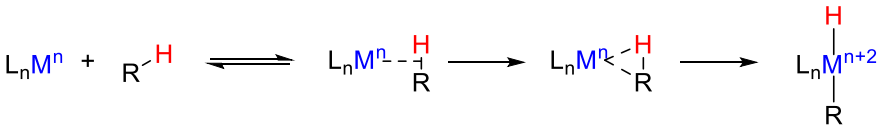


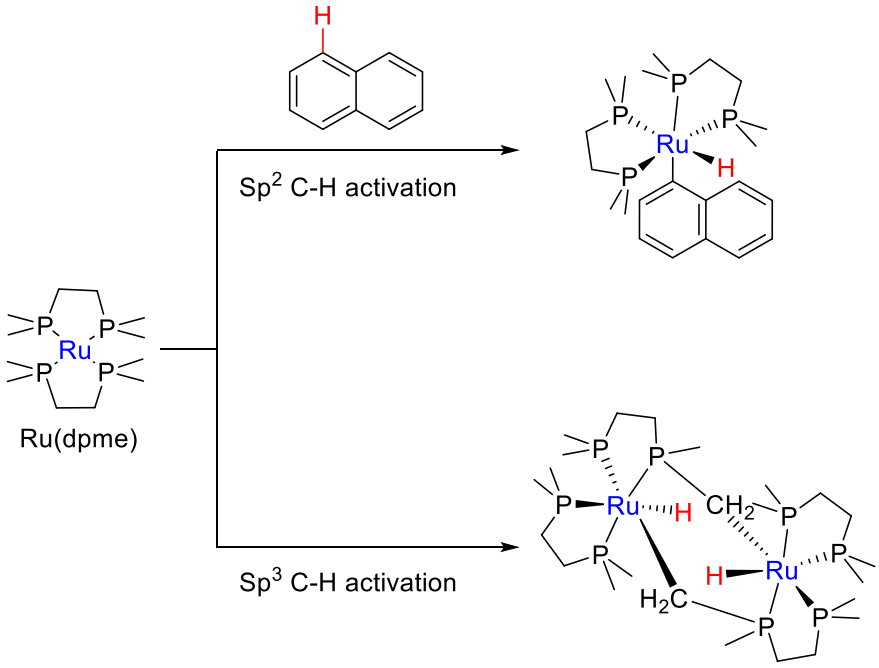
Figure 4: *Mechanisms of C-H activation.*

Oxidative addition is defined by the addition of a C-H bond to an electron rich metal, increasing the oxidation state and coordination number of the complex.[39] The addition results in the formation of a new metal hydride and metal carbon bond. The C-H bond is cleaved as the M-C and M-H bonds are formed through a three-membered transition state, **Scheme 9**. For oxidative addition to occur the metal must be relatively electron-rich and be able to accommodate the new M-C and M-H bonds around the metal centre.[37,40–42]



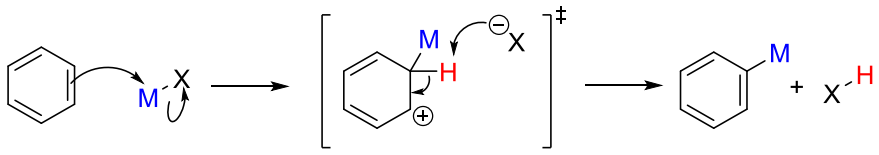
Scheme 9: *Mechanism of oxidative addition by C-H activation.*

Early examples of C-H activation via an oxidative addition mechanism were reported by Chatt, in which the ruthenium complex Ru(dpme)^2 activates the sp^2 naphthalene proton and also the sp^3 C-H bonds on the metal ligand,[43] **Scheme 10**.



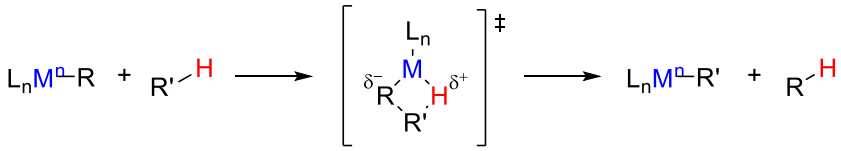
Scheme 10: *C-H activation of Ru(dmpe)$_2$ through oxidative addition.*[43]

Electrophilic substitution mechanisms are defined by the coordination of a C-H bond to an electrophilic metal, forming a cationic metal complex which weakens the C-H bond, allowing deprotonation, **Scheme 11**. Electrophilic C-H activation was first observed with the isotopic H/D change of methane in a solution of D_2O with a metal complex K_2PtCl_4 by Shilov.[44]



Scheme 11: *Mechanism for electrophilic substitution in C-H activation.*

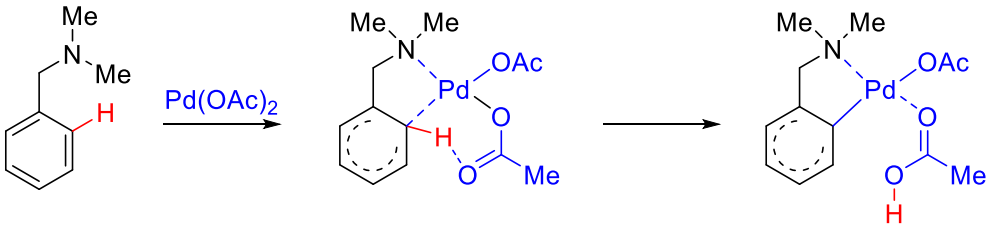
σ-Bond metathesis (**Figure 4**, Pathway 3), is the cleavage of two σ-bonds and the formation of two new σ-bonds, within a four-centre transition state.[45] Notably the formal oxidation state does not change, **Scheme 12**. The most appropriate metals for σ-bond metathesis are electron deficient metals such as the early transition metals and the lanthanides and actinides. The first reported observation of σ-Bond metathesis was observed by Watson for the exchange of the methyl ligand of Cp^*_2LuMe with radiolabelled $^{13}CH_4$.[46] Further examples were reported by Bercaw with alkyl and aryl organo-scandium complexes.[47]



Scheme 12: *Mechanism for σ-bond metathesis in C-H activation.*

CMD (concerted metalation deprotonation) or AMLA (amphiphilic metal ligand activation) as it is also known, is the combined interaction of both the metal and the ligand with the C-H bond, and subsequent deprotonation by the ligand.[48–50] The mechanism comprises of the activation of a Lewis acidic metal followed by deprotonation by an intramolecular base which affords a low energy pathway for C-H activation.

One early example of this mechanism was reported by Ryabov in the cyclometallation of dimethylbenzylamine (DMBA) with $Pd(OAc)_2$.[51] The work was followed up by a computational study by Davies to suggest the mechanism shown in **Scheme 13**.[52] The reaction proceeds by the coordination of the metal catalyst to the substrate through a monodentate amine directing group. The acetate ligand is aligned close to the ortho protons of the substrate and C-H bond cleavage is achieved through the interaction of the ligand and the ortho protons.



Scheme 13: *Proposed C-H activation pathway of the reaction of DMBA with $Pd(OAc)_2$ via a CMD/AMLA mechanism.*

C-H activation using an AMLA/CMD mechanism is the most diverse method for C-H functionalization. The mechanism can accommodate a variety of different metal catalyst and directing groups for C-H activation.

1.2.2 Directing groups

C-H bonds are the most abundant bond present in organic molecules and are generally the least reactive species in a compound and in many species high in abundance. The challenge for chemists has been to develop methods for the selective modification of C-H bonds.[53] The use of metal coordinating species as directing groups for C-H functionalization has become a useful tool to enhance the selectivity of C-H bond activation. Directing groups allow the coordination of the metal to the substrate close to the site of the desired C-H activation, **Figure 5**.[54,55]

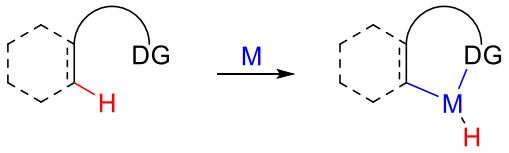


Figure 5: *Representation of directing group assisted C-H activation.*

Although the installation of directing groups are not always essential for C-H functionalization, it increases the selectivity of the reaction and increase the reactivity of the metal ligand complex.[56] Common directing groups for C-H activation include examples such as bipyridyl, amides and carbonyls. In order to function successfully as a directing group, the substrate must be weakly coordinating to the metal atom, so that the metal complex is not rendered inert through binding.[57]

The use of bidentate directing groups allows the formation of metallacycles that would not be possible by coordination through monodentate systems. The 8-aminoquinoline (8-AQ) directing group is among one of the most commonly used directing groups for C-H functionalization.[58] First reported in 2005 by Daugulis, 8-AQ was used as a bidentate directing group for the arylation of C-H sp^3 bonds using $Pd(OAc)_2$, **Figure 6**.[59]

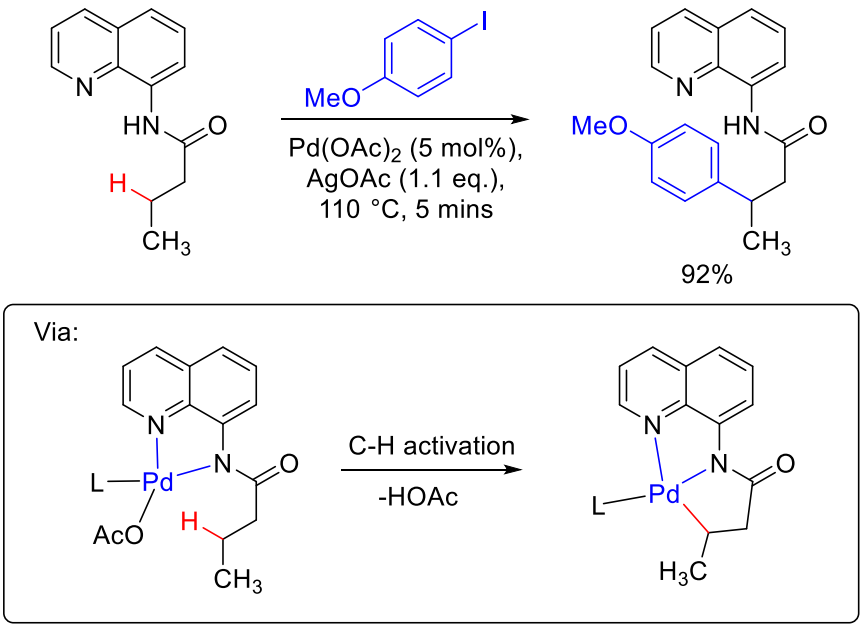
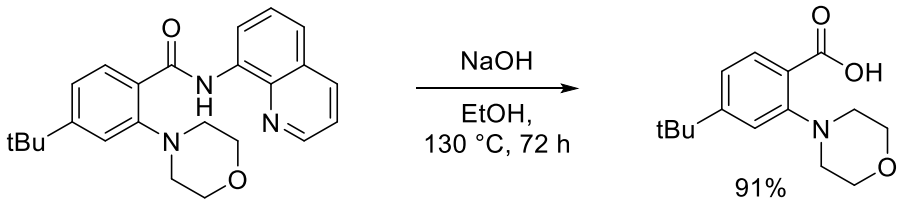


Figure 6: *Palladium catalysed C-H arylation using 8-aminoquinoline as a directing group.*[59]

The bidentate coordination of the metal centre forms a stable five-membered metallacycle. Subsequent C-H activation at the β-carbon occurs through an AMLA mechanism, to form a five-five ring system. The incoming aryl halide oxidatively adds to the metal centre before the formation of a new C-C bond. The pre-installed directing group can then be easily removed thereafter by treatment with base, **Scheme 14**.[58]

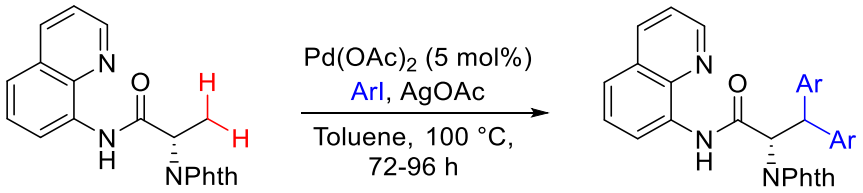


Scheme 14: *Removal of 8-AQ directing group.*[58]

To date, a diverse library of directing groups have been reported including; amides, amines, imines, carboxylic acids, ketones, esters and hydroxyl groups.[60] These directing groups have been allied to modifications such as arylations, alkylations and alkenylations to name but a few.

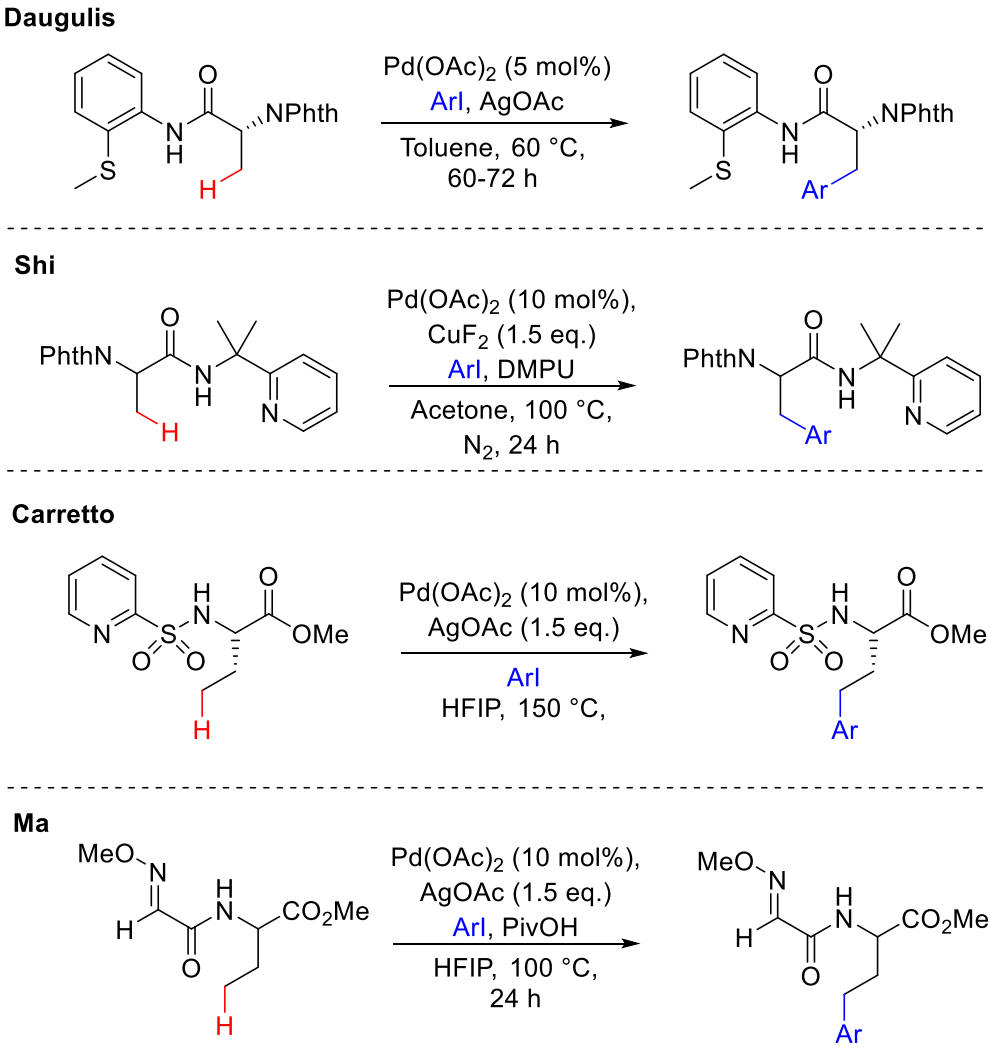
1.2.3 C-H functionalization of amino acids

One of the earliest reported modifications of amino acids was completed by Daugulis.[61] Following on from his previous work using the bidentate 8-AQ directing group, the palladium catalysed sp^3 alkylation of amino acid side chains was then reported, **Scheme 15**. The methodology provided a route to the synthesis of substituted phenylalanine derivatives through C-H functionalization, generating a small library of unnatural aryl containing amino acids. [62]



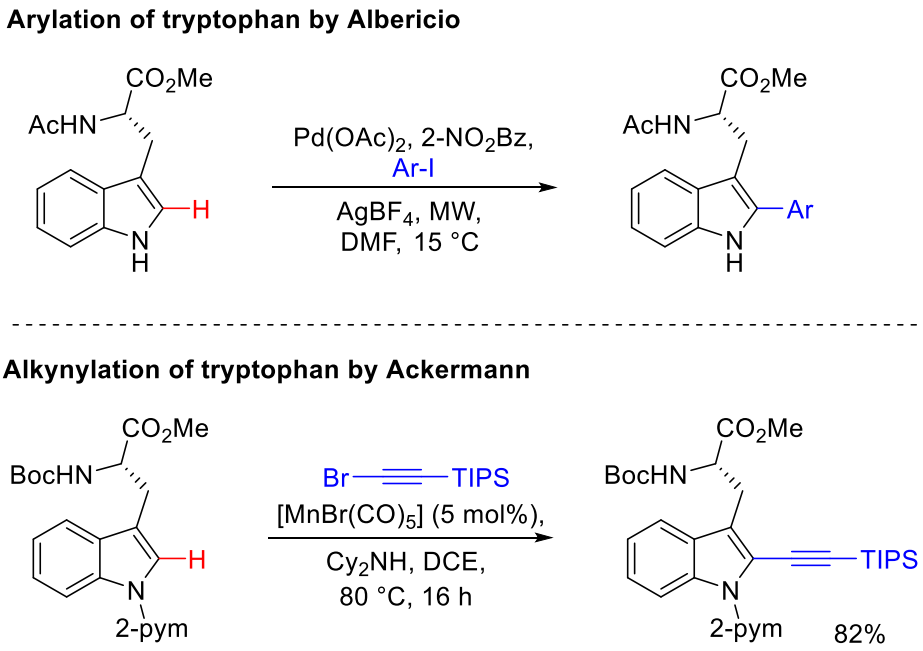
Scheme 15: *Arylation of sp3 amino acid side chains using 8-AQ directing group.*[61]

Interestingly, the arylation of alanine using the 8-AQ directing group, lead to a mixture of mono- and di-arylated products. The synthesis of the mono-arylated product was achieved by changing the directing group to a 2-thiomethylaniline derivative, which gave selective mono-arylation. Inspired by the work of Dauglulis, a number of other groups have reported selective mono-arylation through varied directing groups such as Shi,[63] Caretto,[64] and Ma,[65] **Scheme 16**.



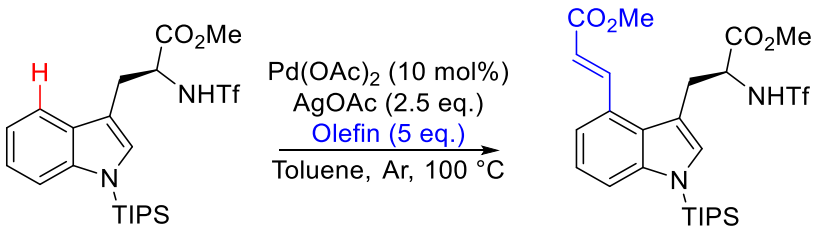
Scheme 16: *Mono-arylation of sp² amino acid side chains by Daugulis,[61] Shi,[63] Caretto,[64] and Ma,[65]*

Many of the C-H functionalization methodologies have focussed upon creating unnatural aromatic amino acid side chains via sp^3 arylations. The appropriate aryl halide is installed via a C-H arylation reaction. Modifications of natural aromatic amino acids have been less explored. Research into the direct C-H functionalization of aromatic amino acids has been largely dominated by studies focussing on tryptophan.[66–69] Key examples of tryptophan modification have been demonstrated by Alberico and Ackermann, **Scheme 17**. [66,70]



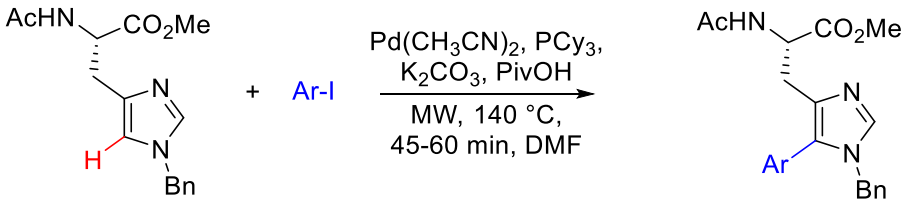
Scheme 17: *C-H arylation of tryptophan by Albericio*[66] *and alkynylation by Ackermann.*[70]

The C-H sp^2 modifications of tryptophan has largely focused on modifying the highly reactive C-2 position of the indole ring. However, olefination at the less reactive C-4 position have been demonstrated by installation of a TIPS protecting group, **Scheme 18**.[71]



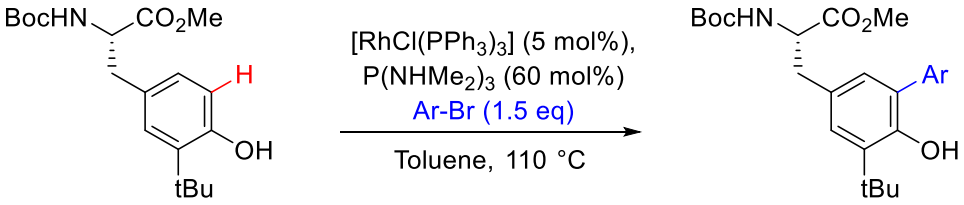
Scheme 18*: C-H Olefination of tryptophan at the C-4 position.*[71]

The imidazole containing amino acid histidine is another common target for modification.[72,73] The arylation of histidine was achieved by using a palladium catalyst under microwave conditions, **Scheme 19**.[72] The reaction was found to tolerate a variety of different aryl halides in a relatively short reaction time to give highly functionalised amino acids that can be readily incorporated into a peptide sequence.



Scheme 19: *Direct C-H arylation of hisitidine.*[72]

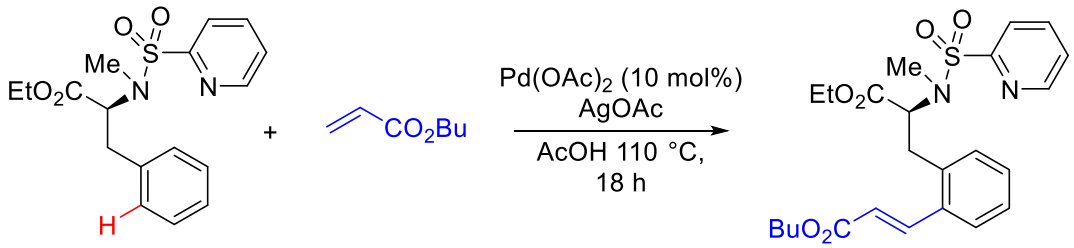
The electron rich phenol side chain in tyrosine permits modifications ortho to the phenolic OH. The C-H arylation of tyrosine was reported by Bedford *et al*., **Scheme 20**.[74] The methodology developed requires pre-functionalization of tyrosine to achieve mono-arylation. The *tert*-butyl group can then be removed so that a second arylation using a different aryl bromide can be used. The use of pre-functionalized amino acid makes the transformation less desirable than direct C-H arylation reactions.



Scheme 20: *Mono-arylation of tyrosine by Bedford and co-workers.*[74]

In the literature, phenylalanine derivatives have been readily modified using pre-functionalization strategies.[75–77] Strategies for phenylalanine modification have focused upon borylation[78] and iodination[79] methodologies. However, only the direct ortho-olefination of the amino acid phenylalanine has been reported.[80] Strategies for phenylalanine modification have focused upon borylation[78] and iodination[79] methodologies.

The olefination of the amino acid phenylalanine required the installation of a pyridyl sulfone directing group at the N-terminus, **Scheme 21**. Applying the methodology reported by Garcia and co-workers to phenylalanine containing peptides would be a problem, as the phenylalanine residue could only be located at the N-terminus of the peptide. To place the phenylalanine residue in other location of a peptide sequence would require the modified amino acid residue to be placed into a sequence when required.



Scheme 21: *The olefination of phenylalanine using a pre-installed sulfonamide directing group.*[80]

The modification of amino acids by C-H functionailzation provides an efficient route to the synthesis of a variety of complex and diverse amino acids. These unnatural amino acids, however, are synthesised prior to peptide coupling and are incorporated into a peptide sequence after modification. This increases the number of steps before the desired modified peptide can be isolated. The aim for chemists is to develop methods for the direct modification of amino acids in peptide sequences, after the synthesis of the peptide.

1.3 C-H functionalization of peptides

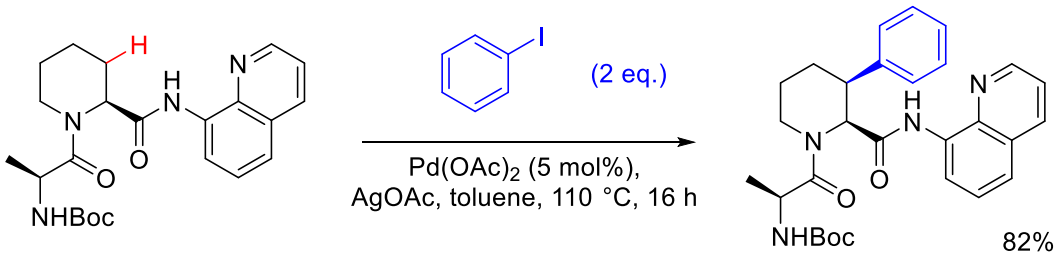
C-H functionalization methods have been developed for many amino acids, however, there are fewer instances in which peptide modification has been achieved by C-H functionalization.[26,81,82] Compared to modifications on amino acids, the C-H functionalization of peptides is noticeably harder due to a number of factors; Peptides contain multiple acidic C-H bonds, making selective C-H functionalization difficult compared to modifications of amino acids, the incorporation of highly functionalized side chains can affect metal binding, and also, peptides contain multiple coordination sites, which complicates metal binding.[83] The combination of these factors makes the C-H functionalization of peptides challenging.

Achieving peptide modification by C-H functionalization allows for the generation of peptide libraries through late-stage diversification. Developing C-H functionailzation methodologies is therefore a useful strategy for the development of peptide based chemical libraries.

1.3.1 C-H functionalization of peptides using pre-installed directing groups

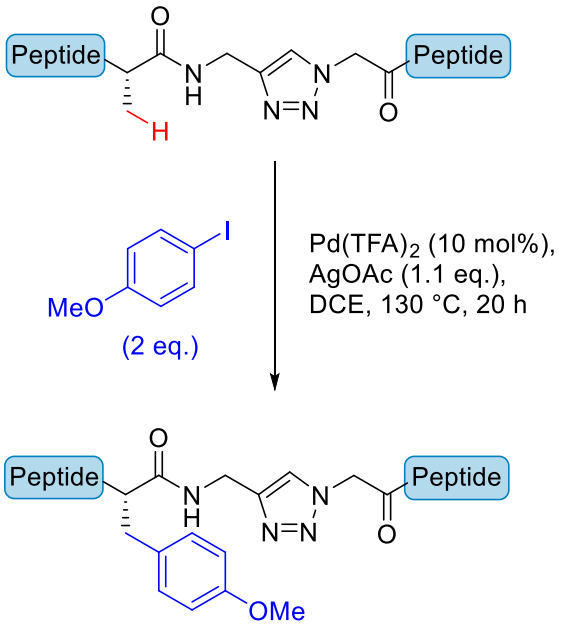
The modification of aliphatic amino acid side chains has been underexplored, as the aliphatic side chains do not contain a functional handle. Applying C-H functionalization strategies to relatively inert bonds, is a way of producing derivatives of these simple

amino acid side chains. However, site selectivity remains a problem. One such way of overcoming the selectivity problems associated with the C-H functionalization of peptides is by installing a directing group for selective modifications. As described in **Section 1.2.2**, 8-AQ is a useful bidentate directing group for C-H functionalization. The arylation of piperidine based amino acids in peptides by Kazmaier and co-workers is an example of the C-H functionalization of an amino acid residue using the 8-AQ directing group,[84] **Scheme 22**. This methodology, however, is limited to modifications at the C-terminal amino acid residues, as the directing group is tethered to the C-terminus of the peptide.



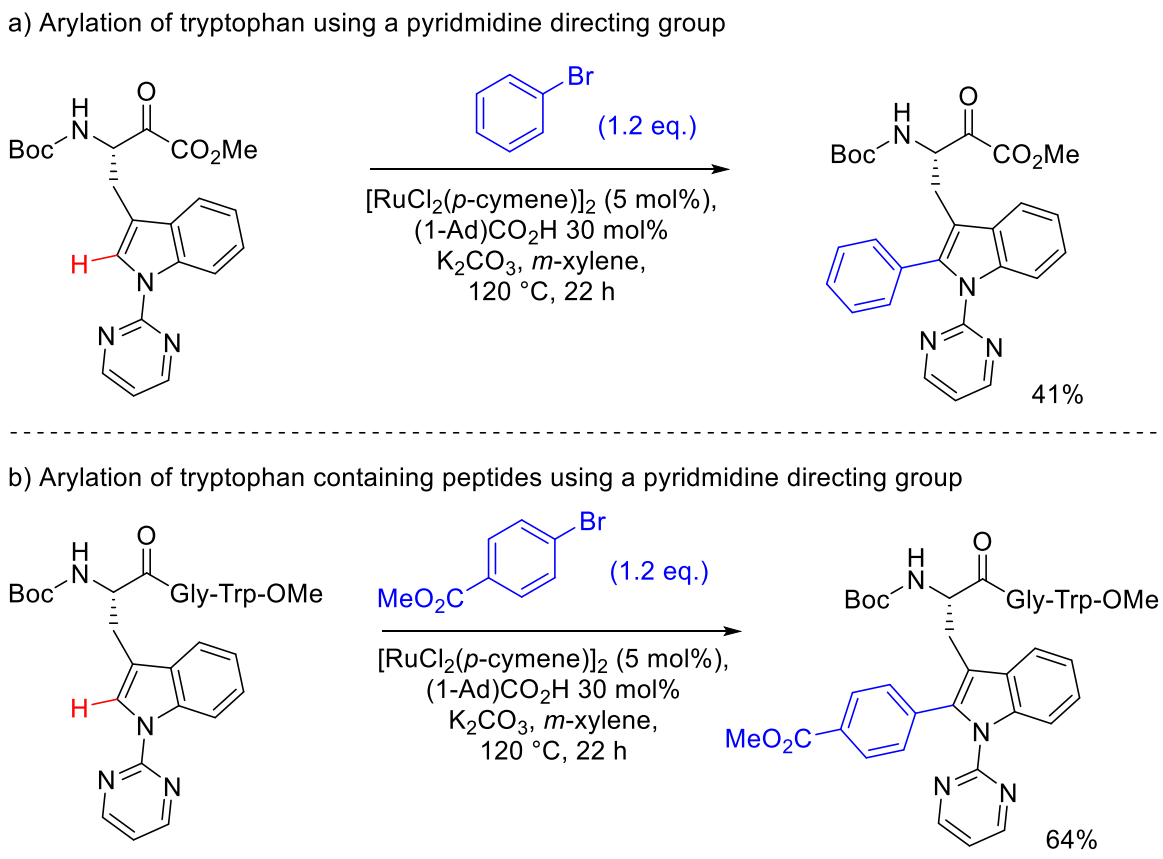
Scheme 22: *Arylation of piperidine based amino acids in a dipeptide using 8-amino quinoline directing group.*[84]

Contrastingly, the reported C-H arylation of peptides by Ackermann utilised a triazole directing group,[85] **Scheme 23**. The method developed by Ackerman generates new unnatural amino acid side chains by the C-H arylation of sp^3 amino acid residues. The triazole directing group was installed within the peptide backbone and allows for the C-H arylation of sp^3 amino acids proximal to the directing group. The methodology was applied to amino acids at the N-terminal and the middle of a peptide sequence.



Scheme 23: *C-H Arylation of alanine residues using a triazole based directing group located in the peptide backbone.*[85]

The C-H arylation of tryptophan residues have flourished using pyridine-based directing groups installed upon the indole nitrogen. The methodology for the arylation of indoles using a pyridine directing group was reported by Ackermann. [86] The methodology was developed on the amino acid tryptophan and then applied to tryptophan residues within peptides,[87] **Scheme 24**. Impressively, the pyrimidine directed arylation of tryptophan accommodated the native tryptophan residue within a peptide sequence. Selectivity had been established through the installation of the pyrimidine directing group.



Scheme 24: *Pyrimidine directed C-H arylation of* ***a)*** *tryptophan* [86] *and* ***b)*** *tryptophan containing peptides.*[87]

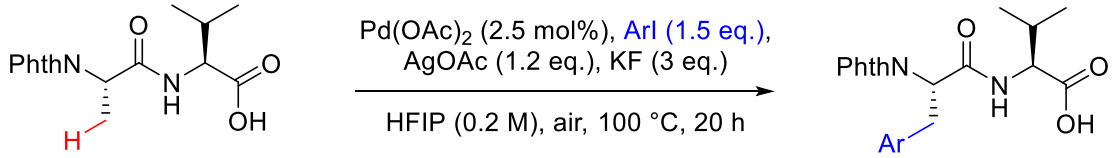
The installed directing groups improve residue selectivity, however, the installation and removal of directing groups increase the number of synthetic steps needed for a given C-H functionalization reaction.

1.3.2 C-H functionalization of peptides using native amide backbone

Despite the development of new peptide modification methodologies by C-H functionalization, the installation of directing groups remains necessary for selective modifications. The pre-installation of directing groups increases the number of synthetic steps needed for C-H functionalization, making the methods less desirable.

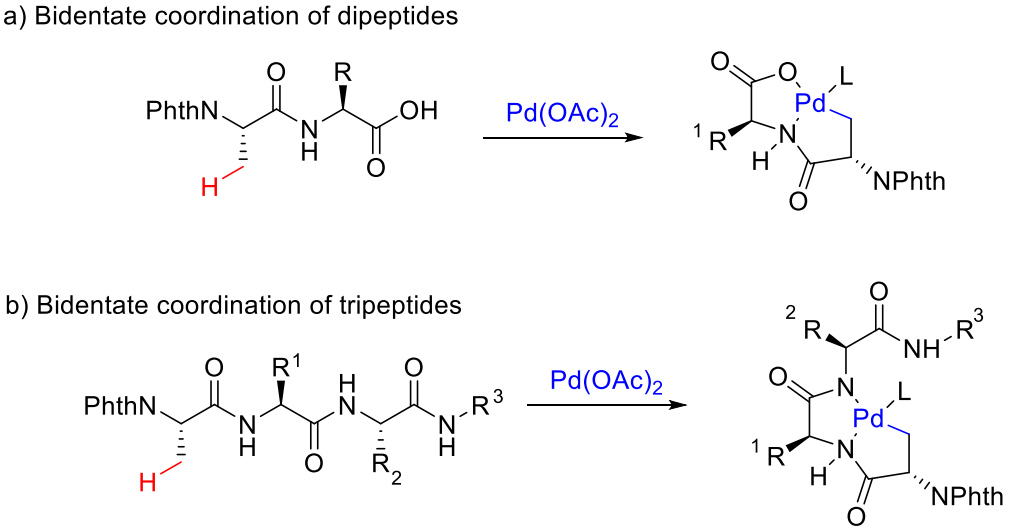
A critical development in the C-H functionalization of peptides was reported by Yu and co-workers on the C-H functionalization of sp^3 amino acid residues in di-, tri- and tetrapeptides,[88] **Scheme 25**. The report demonstrated the C-H arylation of alanine residues upon peptide side chains without the need for the installation of a directing group such as 8-aminoquinoline. Here the peptide backbone acts a directing group for the incoming palladium catalyst, allowing direct alkylation of the alanine residue.

Impressively the methodology has been shown to work well on larger peptide systems such as tri-, and tetrapeptides as well as a variety of aryl substituents.



Scheme 25: *C-H Arylation of sp³ amino acid residues by Yu et al.*[88]

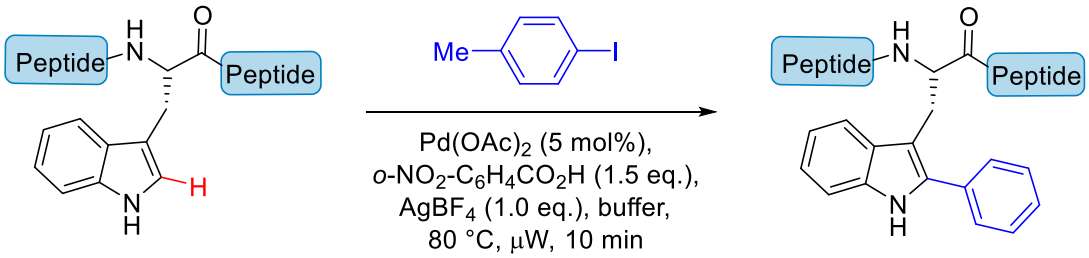
The methodology details the selective N-terminal C-H arylation of peptides without the preinstallation of a directing group. C-H activation of dipeptides was achieved by the formation of a metal-nitrogen bond with the amide nitrogen and free C-terminal carboxylic acid. The modification of tri- and tetrapeptides was achieved through bidentate coordination, only this time through two amide residues rather than the C-terminal carboxylic acid. A peptide backbone consists of multiple amide nitrogen atoms that can act as directing groups for C-H activation.[89,90] Coordination of a metal to a peptide backbone to aid in C-H functionalization has been reported by Yu *et al,* in the C-H arylation of small peptides.[88] Bidentate coordination of a palladium atom to a peptide back bone forms a five-five ring system which is setup for C-H activation, **Scheme 26**.



Scheme 26: *C-H Arylation of peptide side chains by bidentate coordination to the peptide backbone.*[88]

Since Yu proposed the bidentate coordination of a metal catalyst to the peptide backbone, there has been a number of reported C-H modification of peptides through backbone coordination to the metal catalyst.

Table 1: *Arylation of tryptophan residues in tri- and tetrapeptides by Alberico.*[66]



Entry	Sequence	Yield / %
1	Ac-Ala-**Trp**-Ala-OH	57
2	Ac-**Trp**-Leu-Asp-Phe-OH	68
3	Ac-Tyr-Pro-**Trp**-Phe-OH	75
4	Ac-Arg-Gly-**Trp**-Ala-OH	94
5	Ac-His-Gly-**Trp**-Ala-OH	51
6	Ac-Lys-Gly-**Trp**-Ala-OH	95
7	Ac-Ser-Gly-**Trp**-Ala-OH	62
8	Ac-Gln-Phe-Ala-**Trp**-OH	88

Alberico *et al*., developed a method for the direct C-H arylation of tryptophan containing tri-, and tetrapeptides, **Table 1**.[66] The C-H arylation strategy was developed first from the arylation of the native tryptophan amino acids.[91,92] The mild conditions developed for the C-H arylation were found to tolerate a wide variety of amino acid side chains containing; carboxylic acids (aspartic acid (**entry 2**)) amines (arginine and lysine, **entries 4** and **6**), aromatic residues (phenylalanine (**entries 2, 3** and **8**), tyrosine (**entry 3**) and histidine (**entry 5**)), alcohols (serine (**entry 7**)) and amides (glutamine (**entry 8**)). The location of the tryptophan residue does not appear to influence the C-H arylation reaction, as tryptophan residues in the middle (**entries 1, 3-7**) and at the N-terminus (**entry 2**) and C-terminus (**entry 8**) were modified under the reaction conditions.

Since the reported C-H arylation of tryptophan containing peptides by Albericio, a handful of other reported arylations of tryptophan containing peptides have been developed by Fairlamb,[93,94] amongst others,[95] **Figure 7**.

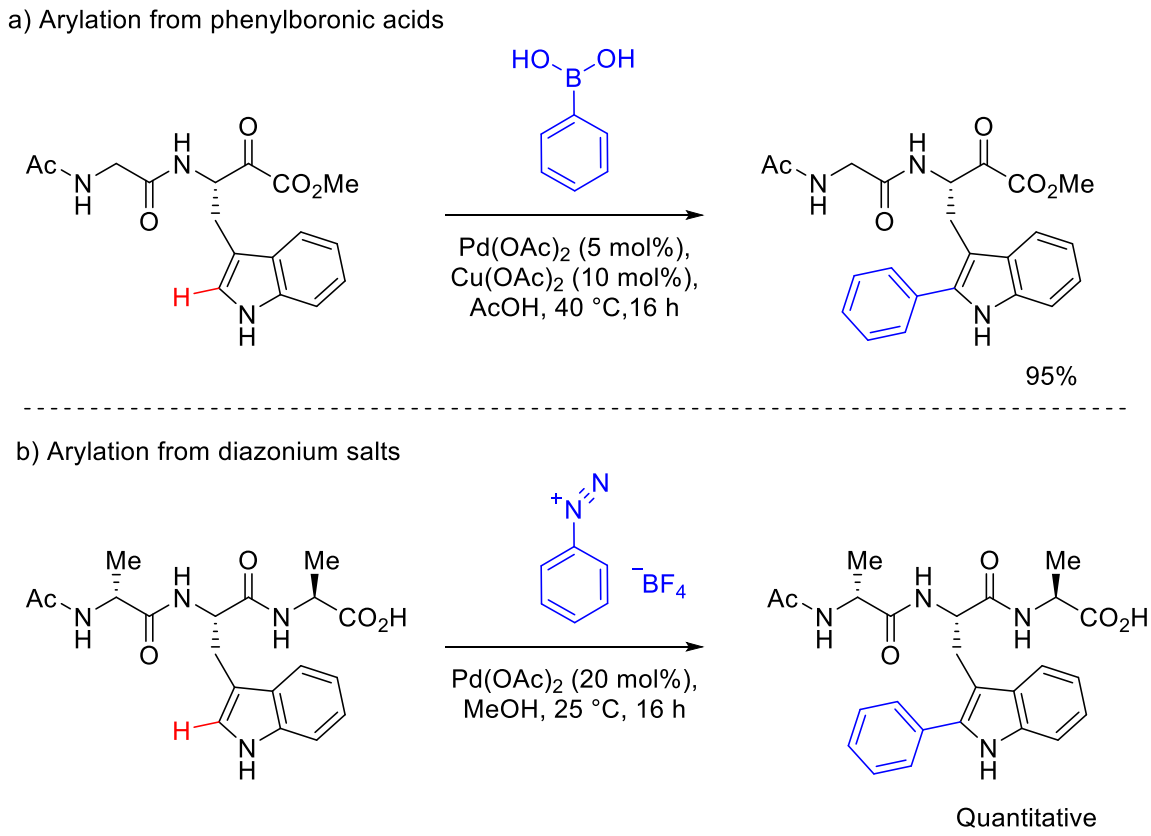


Figure 7: *Arylation of tryptophan containing by peptides by* ***a)*** *phenylboronic acids* [93] *and* ***b)*** *phenyl diazonium salts.*[94]

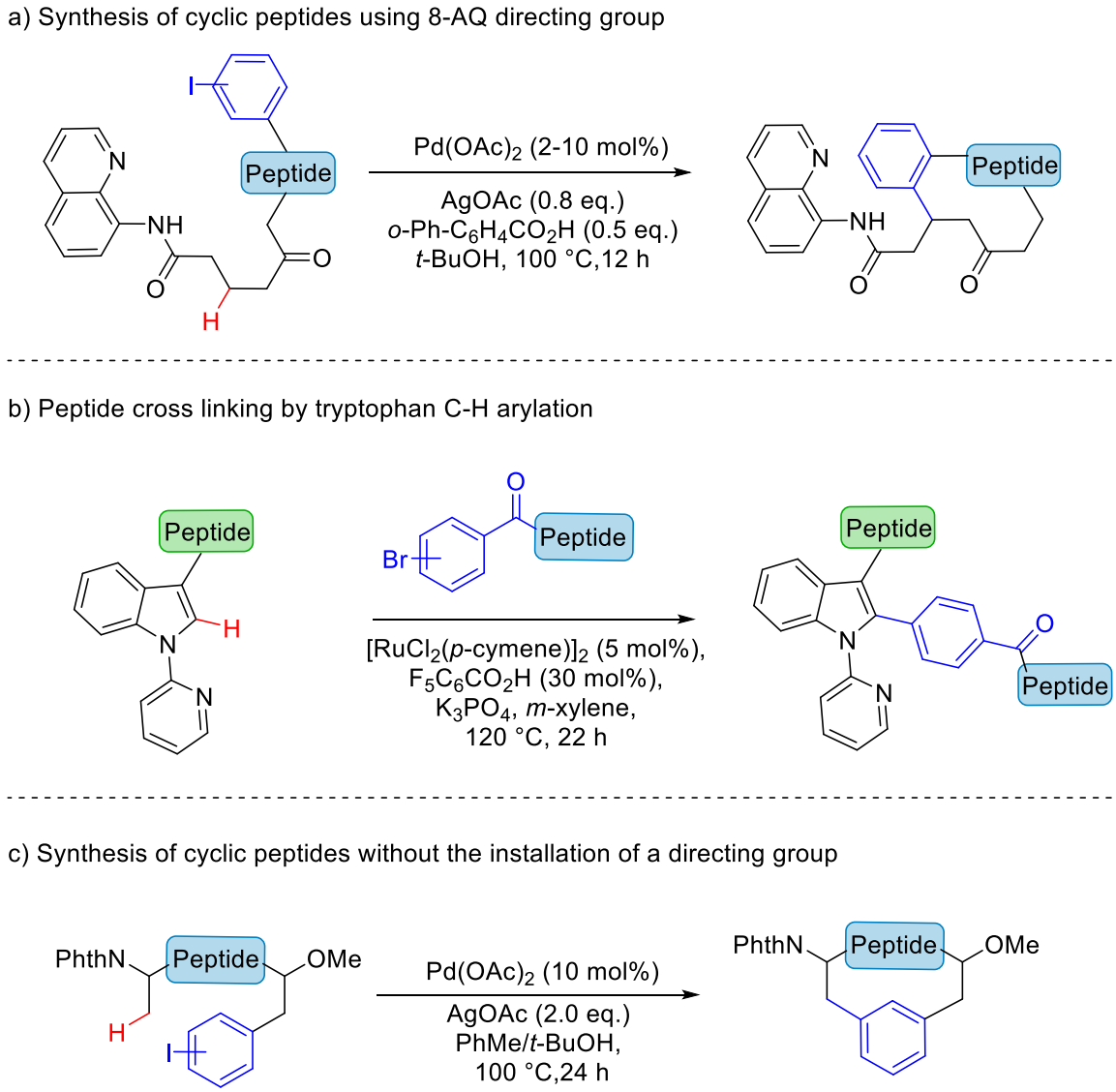
The arylation of tryptophan residues by Fairlamb was achieved from phenylboronic acids at 40 °C, to give arylated tryptophan peptides in excellent yields.[93] Complimentary methods have also been developed by Fairlamb utilizing phenyl diazonium salts for the arylation of tryptophan.[94] Despite the development of the arylation of tryptophan residues, there are however, no examples of the olefination of tryptophan containing peptides in the literature.

1.3.3 Generating conformational constraints in peptides by C-H functionalization

Small peptides with conformational flexibility are easily synthesised by current methodologies, however, chemists are looking to produce peptides with unique conformations.[96] Therapeutics often suffer from a lack of potency when the molecule can adopt multiple conformations. Linear peptide therapeutics tend to adopt many more conformations than a standard small drug molecule, and therefore often suffer from a lack of potency. Reducing the flexibility of a peptide therapeutic could lead to an

increase in the potency of the peptide, making constrained peptides a more desirable drug candidate than their linear precursors .[97,98]

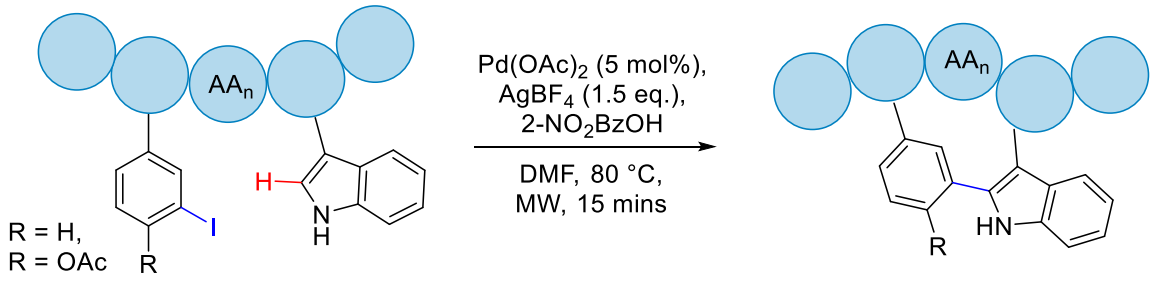
One way of generating new constrained peptides is through the cross linking of amino acid side chains.[99,100] Many of the reported peptide C-H functionalization methodologies have been developed to cross link amino acid side chains together, **Scheme 27**.[87,100,101]



Scheme 27: *Peptide cross linking between amino acids side chains through* ***a)*** *8-AQ directing group,* [88] ***b)*** *pyridine directing group,* [76] ***c)*** *using the native peptide backbone.* [87]

García *et al*. reported the stapling of a pre-functionalized phenylalanine/tyrosine side chains with tryptophan residues.[99] The initial peptide was synthesised using SPPS with the installation of the iodo-aromatic amino acid during the synthesis. The C-H arylation reaction between the aromatic residues is performed whilst the peptide remains on the resin. Microwave irradiation results in the formation of a new C-C bond between the

phenylalanine and tyrosine side chains and tryptophan residue, changing the conformation of the molecule by changing the linear structure to a stapled cyclic peptide, **Scheme 28**.



***Scheme 28**: Peptide stapling between 3-iodo-phenylalanine and 3-iodo-tyrosine residues and tryptophan residues.*[99]

C-H functionalization is a useful tool for the cross linking of amino acid residues and could provide chemists with more tools to generate new conformations for existing peptides. There are, however, no examples of stapling between two residues without a pre-functionalized amino acid. It is essential that a method for the stapling of natural amino acids be investigated to make peptide stapling available to natural peptides such as those found in the body, to reduce the cost of synthesis by avoiding the need for pre-functionalized amino acids and reduce the number of steps.

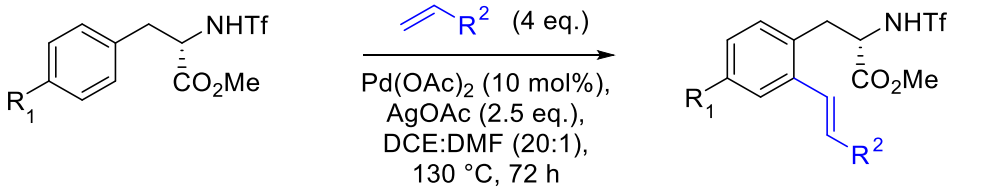
1.4 Aims

1.4.1 Project aims overview

As described in **Section 1.2.3**, the transition metal catalysed, C-H functionalization of peptides is an attractive approach to the generation of modified peptides. C-H functionalization allows for the direct modification of natural amino acids, without the need for potentially expensive pre-functionalized materials. The primary aims of this project was to develop methods for the C-H functionalization of phenylalanine residues in peptides. The methods for phenylalanine modification were then applied to other aromatic amino acids, to investigate site selectivity. The methods explored will be useful in expanding the number of modifications available to peptide chemists.

1.4.2 C-H functionalization of phenylalanine containing peptides

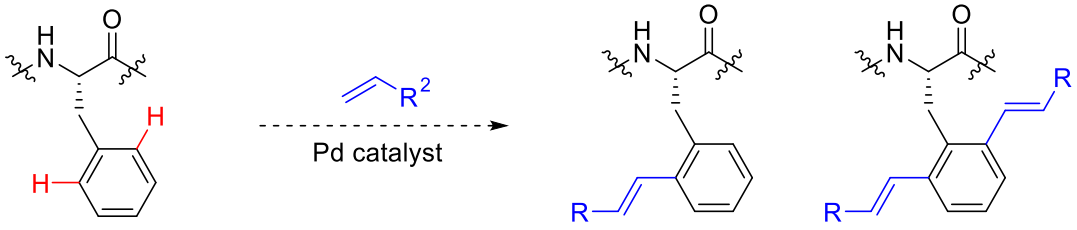
To date, there are very few reported examples of phenylalanine modifications in the literature.[27,80,102] There is a need for chemists to develop and apply methodologies for the modification of phenylalanine to increase the number of synthetic methods available for peptide scientists. The initial reaction conditions were taken from the reported olefination of various aryl amines as potential tetrahydroquinoline precursors. In the study, Yu *et al.* screened a variety of substrates including protected phenylalanine, tyrosine and tryptophan residues, **Scheme 29**.[79]



R_1 = H, R_2 = CO_2Me, 61%
R_1 = H, R_2 = Ph, 51%
R_1 = H, R_2 = C_6H_5Cl, 56%
R_1 = OTf, R_2 = CO_2Me, 65%

Scheme 29: *Olefination of phenylalanine and tyrosine by C-H functionalization.*[79]

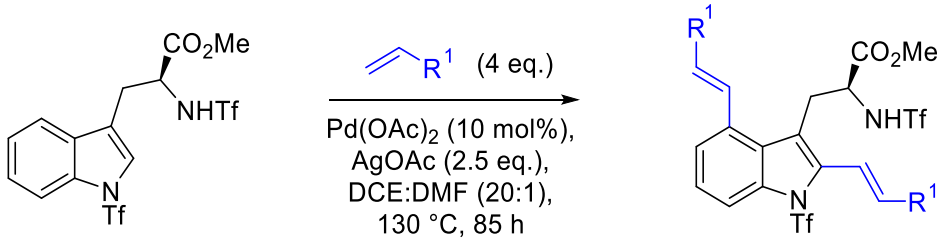
The methodology reported by Yu et al., is an example of an oxidative Heck (Fujiwara-Moritani) reaction and an early example of phenylalanine modification by C-H functionalization.[103] The first body of work will look at developing a method for the direct C-H functionalization of phenylalanine in peptides using conditions developed by Yu for the olefination of amino aryl species[79] as a starting point, **Scheme 30**.



Scheme 30: *Proposed C-H olefination of phenylalanine containing peptides.*

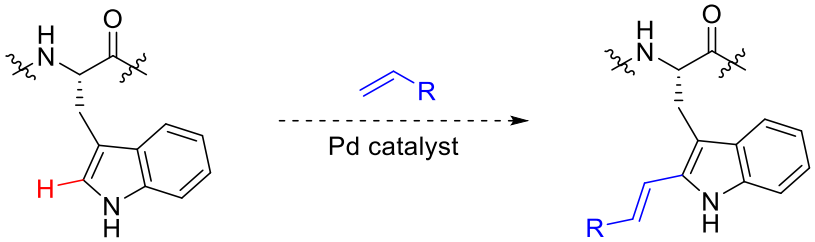
1.4.3 C-H functionalization of tryptophan containing peptides

Tryptophan residues are less prevalent than phenylalanine residues in natural peptides yet the modification of tryptophan by C-H functionalization has attracted significant study by the community.[66,68,94,104–106] The olefination of tryptophan residues in peptides has yet to be reported, whereas arylation reactions have been extensively studied. As shown in **Scheme 31**, Yu *et al.*, successfully olefinated tryptophan by C-H functionalization using the same reaction conditions for the olefination of phenylalanine.[79]



Scheme 31: *C-H olefination of tryptophan by Yu et al.*[79]

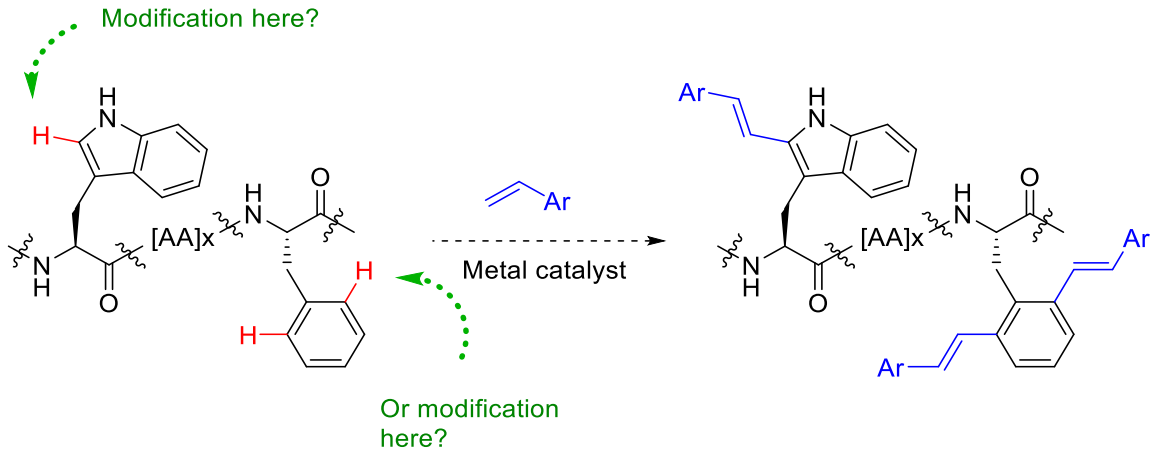
The aim of this investigation is to develop a method for the C-H olefination of tryptophan residues in peptides using the C-H olefination study established for the modification of phenylalanine containing peptides and apply the methodology to tryptophan containing peptides. **Scheme 32** depicts the proposed starting point for the olefination of tryptophan containing peptides.



Scheme 32: *Proposed C-H olefination of tryptophan containing peptides.*

1.4.4 C-H functionalization of peptides containing multiple aromatic amino acids

The methods developed for the modification of aromatic amino acids are important tools that open up more strategies for peptide modification. The model peptides in which the C-H olefination methodologies are investigated are however, limited in scope as many natural peptides contain multiple aromatic residues.[107] The final investigations will focus on applying the methodology used for the C-H olefination of phenylalanine and tryptophan containing peptides to peptides with more than one aromatic amino acid, **Scheme 33**. The aim is to explore the selectivity of the C-H functionalization reactions on complex peptide containing more than one aromatic amino acid.



Scheme 33: *C-H functionalization of peptides bearing multiple aromatic residues.*

1.4.5 Project aims summary

The aims of the project are summarised as:

- To develop and optimise a method for the C-H functionalization of phenylalanine residues within peptides.
- To expand upon the methodology developed for the functionailzation of phenylalanine containing peptides by applying the conditions to tryptophan containing peptides.
- To investigate the selectivity of C-H functionalization to peptides containing multiple aromatic amino acids.

The methods developed and documented will provide peptide chemists with more synthetic methods for the direct functionalization of aromatic residues in peptides and therefore maximise the options available for peptide modification.

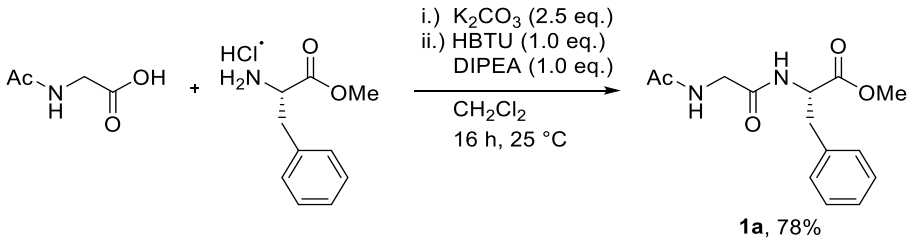
2. Results and discussion

2.1 C-H functionalization of phenylalanine containing peptides

As described in **Section 1.4**, the aim of this investigation was to develop a method for the C-H functionailzation of phenylalanine containing peptides. The olefination methodology developed by Yu *et al.,* for the modification of the amino acid phenylalanine,[79] was applied to phenylalanine residues in peptides. The following section describes the investigations performed on the C-H olefination of phenylalanine containing peptides.

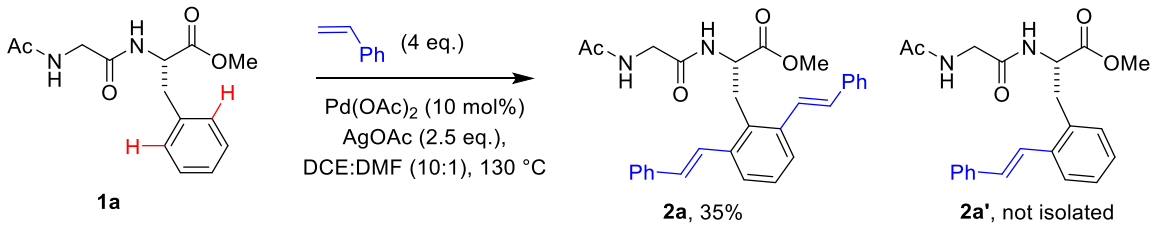
2.1.1 Olefination of model peptide Ac-Gly-Phe-OMe

Investigating the C-H olefination of phenylalanine residues required the synthesis of a model dipeptide. Dipeptide **1a** (Ac-Gly-Phe-OMe) was chosen as a suitable model substrate for study. Glycine was selected as the neighbouring amino acid as the incorporation of glycine unit increases the flexibility of the peptide and will not interfere with the reaction. **Scheme 34** depicts the synthesis of the dipeptide Ac-Gly-Phe-OMe (**1a**). The reaction of *N*-acetyl glycine and L-phenylalanine methyl ester hydrochloride in the presence of a coupling reagent (HBTU) and base (DIPEA), gave peptide **1a** in a 78% yield.



Scheme 34: *Synthesis of Ac-Gly-Phe-OMe (**1a**).*

Initial studies involved the reaction of dipeptide **1a** with styrene, in the presence of $Pd(OAc)_2$ and AgOAc, **Scheme 35**. The reaction was performed at 130 °C for 48 hours. The crude material was then purified by flash column chromatography to afford the di-olefinated peptide **2a** as a yellow solid (35% yield). Peptide **1a** was also recovered along with mono-olefinated peptide **2a'**. Both the dipeptide and the mono olefinated peptide co-eluted during the purification and were not isolated.



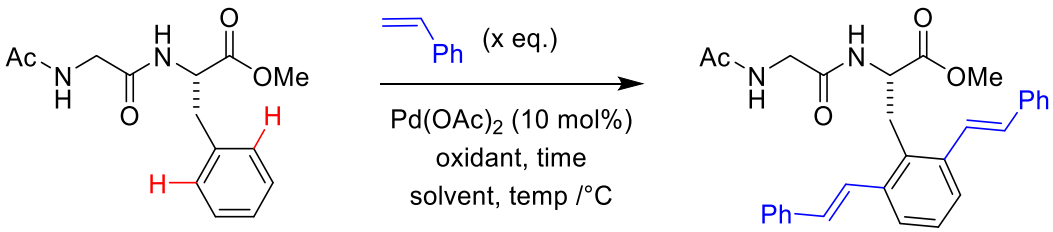
Scheme 35: *Palladium catalysed C-H olefination of model dipeptide (**1a**).*

The ^{1}H NMR spectrum of peptide **1a** contained a 2H doublet at 7.08 ppm from the *ortho* protons on the phenyl ring. In the ^{1}H NMR spectrum for **2a**, the *ortho* protons are not present, indicating substitution at the *ortho* site on the phenylalanine ring.
The ^{1}H NMR spectrum of **2a** contained a pair of 2H doublets at 7.01 and 7.47 ppm, with a coupling constant of 16.0 Hz, that is consistent with the presence of a *trans* alkene which is consistent with a Heck reaction.

2.1.2 Optimisation

Following the discovery that model dipeptide **1a** could be modified to afford peptide **2a** by C-H functionalization, optimisation of the reaction was carried out, **Table 2**.

Table 2: *Optimisation of the C-H functionalization of peptide* ***1a.***



1a **2a**

Entry	Oxidant	Equiv. oxidant	Solvent	Equiv. styrene	Temp / °C	Time / h	Yield / %
1	AgOAc	2.5	DCE/DMF[a]	4.0	130	48	35
2	AgOAc	2.5	DCE/DMF[a]	1.0	130	48	19
3	AgOAc	2.5	DCE/DMF[a]	2.0	130	48	31
4	AgOAc	2.5	DCE/DMF[a]	10.0	130	48	39
5	AgOAc	2.5	DCE/DMF[a]	4.0	100	48	20
6	AgOAc	2.5	DCE/DMF[a]	4.0	80	48	8
7	AgOAc	2.5	DCE/DMF[a]	4.0	25	48	Trace
8	AgOAc	2.5	DCE/DMF[a]	4.0	130	96	38
9	AgOAc	2.5	DCE/DMF[a]	4.0	80	96	12
10	AgOAc	2.5	DCE/DMF[a]	4.0	130	24	8
11	AgOAc	2.5	Toluene	4.0	130	48	26
12	AgOAc	2.5	DMF	4.0	130	48	26
13	AgOAc	2.5	MeCN	4.0	130	48	23
14	AgOAc	2.5	HFIP	4.0	130	48	Trace
15	AgOAc	2.5	*t*-amyl-OH	4.0	130	48	67
16	AgOAc	2.5	*t*-amyl-OH/DMF[b]	4.0	130	48	48
17	$Cu(OAc)_2$	2.5	*t*-amyl-OH	4.0	130	48	34
18	$AgCO_3$	2.5	*t*-amyl-OH	4.0	130	48	11
19	$Ag(OPiv)_2$	2.5	*t*-amyl-OH	4.0	130	48	16
20	Benzoquinone[c]	2.5	*t*-amyl-OH	4.0	130	48	24
21	AgOAc	5.0	*t*-amyl-OH	4.0	130	24	83
22	**AgOAc**	**5.0**	***t*-amyl-OH**	**4.0**	**130**	**12**	**81**[d]
23	AgOAc	5.0	*t*-amyl-OH	4.0	130	6	70
24	AgOAc	5.0	*t*-amyl-OH	4.0	100	12	76
25	AgOAc	5.0	*t*-amyl-OH	4.0	80	12	68

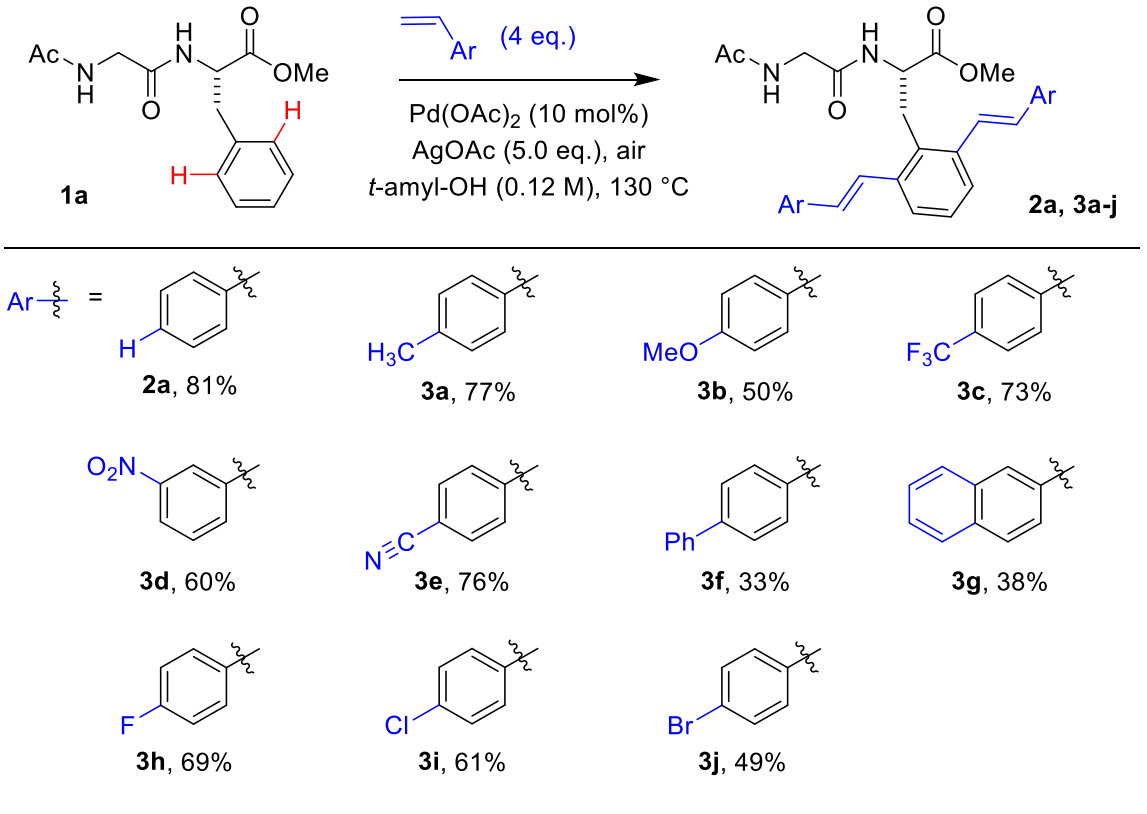
[a] DCE/DMF = 20:1;
[b] *t*-amyl-OH/DMF = 20:1;
[c] 5 eq. of NaOAc were also included in the reaction mixture;
[d] The mono-olefinated peptide **2a'** was also isolated in 8% yield.

The olefination of peptide **1a** (0.359 mmol) was carried out in a sealed vessel in the appropriate solvent (0.12 M). After the allotted time, the reaction mixture was filtered, and purified by flash column chromatography, eluting with EtOAc. The yields quoted in **Table 2** are isolated yields after purification. Firstly, the equivalents of styrene were investigated. Using 1.0 equivalent gave modified peptide **2a** in a 19% yield, whereas 2.0 equivalents gave the peptide in a 31% yield. Employing 10.0 equivalents of styrene produced little change in the yield of **2a** (39%, **entry 4**). Lowering the reaction temperature from 130 °C to 100 °C and 80 °C gave the diolefinated peptide **2a** in yields of 20% and 8% (**entry 5 and 6**). When the reaction was performed at 25 °C, the olefinated peptide was observed by ^{1}H NMR spectroscopy, however, isolated as a trace amount. Increasing the reaction duration from 48 h to 96 h had very little effect on the isolated yield of **2a** (38% yield, **entry 8**). Likewise running the reaction at 80 °C for 96 h afforded **2a** in a 12% yield. Changing the solvent however, gave a larger variety of yields. Running the reaction in toluene, DMF or MeCN gave **2a** in lower yields than using a mixture of DCE/DMF (26%, 26% and 23%, **entries 11, 12** and **13**). Hexafluoroisoproanol (HFIP) is commonly used in peptide chemistry and more recently as a solvent in C-H functionalization.[108] When using HFIP, the reaction did not proceed (**entry 14**). The use of another commonly used reaction solvent for C-H functionalization (*t*-amyl-OH) was studied and found to give **2a**, in a 67% yield (**entry 15**), much higher than the previous reactions conditions investigated. Using a combination of *t*-amyl-OH and DMF as the solvent gave a reduced yield from 67% to 48% (**entry 16**). Changing the oxidant proved to be detrimental to the reaction, with isolated yields of 34%, 11% and 16% when $Cu(OAc)_2$, $AgCO_3$ and $Ag(OPiv)_2$ were used (**entries 17, 18** and **19** respectively). Employing a combination of benzoquinone and NaOAc gave **2a** in a 24% yield. It was discovered that increasing the equivalents of AgOAc from 2.5 to 5.0, gave modified peptide **2a** in a remarkably high yield (83%) in just 12 hours (**entry 22**). The mono-olefinated peptide **2a'** was also recovered in an 8% yield as the minor product of the reaction. Running the reaction at lower temperatures with 5.0 equivalents of AgOAc also gave satisfactory yields of **2a** (76% and 68% at 100 °C and 80 °C, **entries 24** and **25** respectively).

2.1.3 Scope of the alkene

With an optimised method, the next stage of the investigation involved screening a variety of olefins against peptide **1a**, **Scheme 36.** The reaction was found to accommodate a range of substituted styrenes. Olefins containing electron donating groups (CH_3, OMe) (**3a**, **3b**), were well tolerated (77% and 50%). Likewise, alkenes bearing electron withdrawing groups (F_3C-, N_2O- and NC-), gave the corresponding

olefinated peptide (**3c**-**3e**) in excellent yields (73%, 60% and 76%). The incorporation of styrenes with extended conjugation (phenyl- and naphthyl-) (**3f**, **3g**) were also tolerated (33% and 38% yields). The incorporation of further conjugation to the phenylalanine residue could be used as an introduction of new fluorophores in a single step.[9] The olefination of model peptide **1a** with a variety of halogen containing styrenes (F-, Cl-, and Br-), were successful, affording the corresponding modified peptide in good yields (69%, 61% and 49%) (**3h**-**3j**).



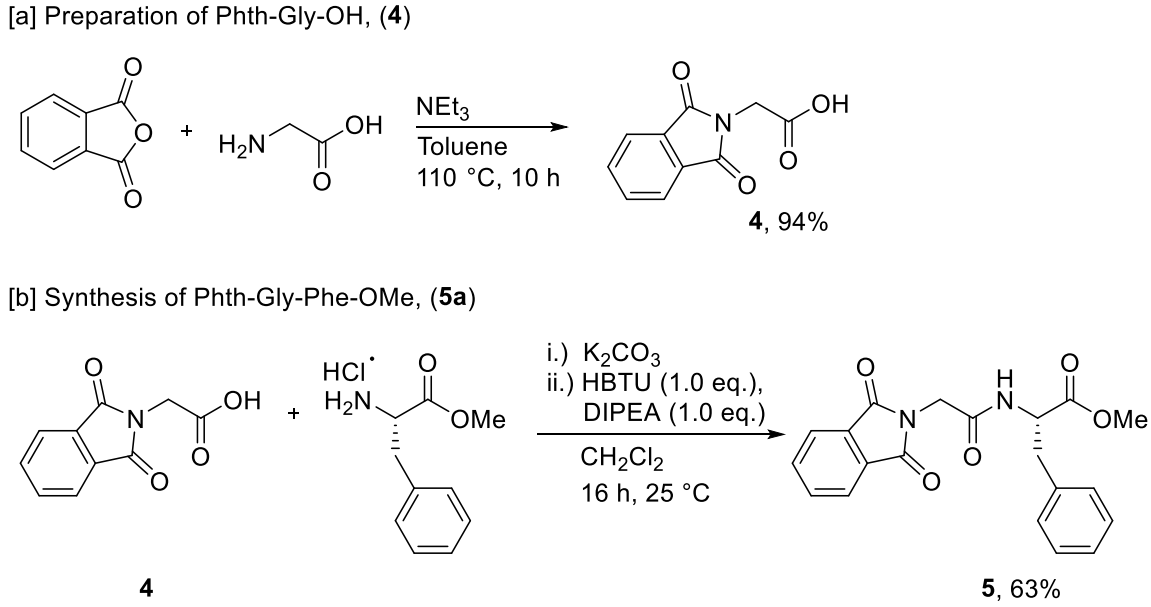
Scheme 36: *Investigating the scope of the olefin with peptide **1a**.*

The incorporation of the bromo-substituted styrene moiety **3j** into a peptide sequence could lead to a variety of different post-synthetic transformations. A second transformation after the C-H olefination could be used together with a C-C cross coupling reaction to increasingly complex functionalized peptides. The potential for further functionalization after C-H olefination of the phenylalanine residues, makes the palladium catalysed olefination reaction a highly desirable method to produce functionalised peptides.

2.1.4 Investigating N-Protecting groups

Model dipeptide **1a** contains an acetyl protecting group at the N-terminus. However, in SPPS acetyl protected amino acids are discouraged, as the acetyl N-protecting group cannot be easily removed without destroying the peptide in the process.[109] The most common nitrogen protecting group used in SPPS is the Fmoc protecting group, followed closely by Boc and Cbz groups. Phthaloyl protected amino acids are an alternative to traditional amine protecting groups (Fmoc, Boc, Cbz), that differ in functionality as the nitrogen lone pair is part of the π-system, reducing the basicity of the lone pair dramatically compared to Fmoc.[110] Investigations then focused on the modification of phenylalanine residues on peptides bearing different N-protecting groups.

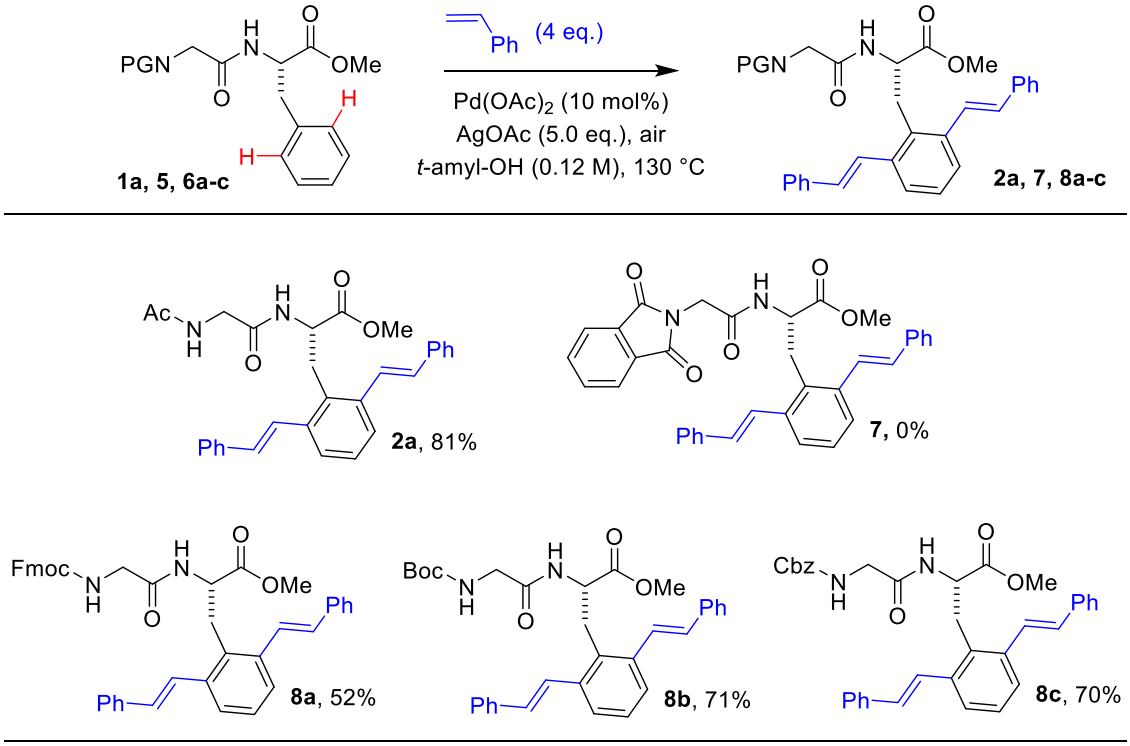
The phthaloyl protected amino acid **4** was prepared by refluxing glycine with phthalic anhydride and NEt_3 under Dean-Stark conditions. The N-protected amino acid was then precipitated with concentrated HCl to give a **4** as a white solid. N-protected amino acid **4**, was then coupled using HBTU and DIPEA, with L-phenylalanine methyl ester to give dipeptide **5**, **Scheme 37**.



Scheme 37: *[a] Preparation of Phth-Gly-OH (**4**) and [b] peptide coupling to give **5**.*

A series of dipeptides bearing different amine protecting groups were prepared and subjected to the optimised olefination reaction conditions, **Scheme 38**. The C-H olefination reaction proceeded well in the presence of a variety of peptides bearing different N-protecting groups, affording the modification of peptides with Fmoc **8a**, Boc **8b** and Cbz **8c** N-protecting groups (yields of 52%, 71% and 70% respectively). When the phthaloyl protected peptide **5** was subjected to the olefination conditions, the

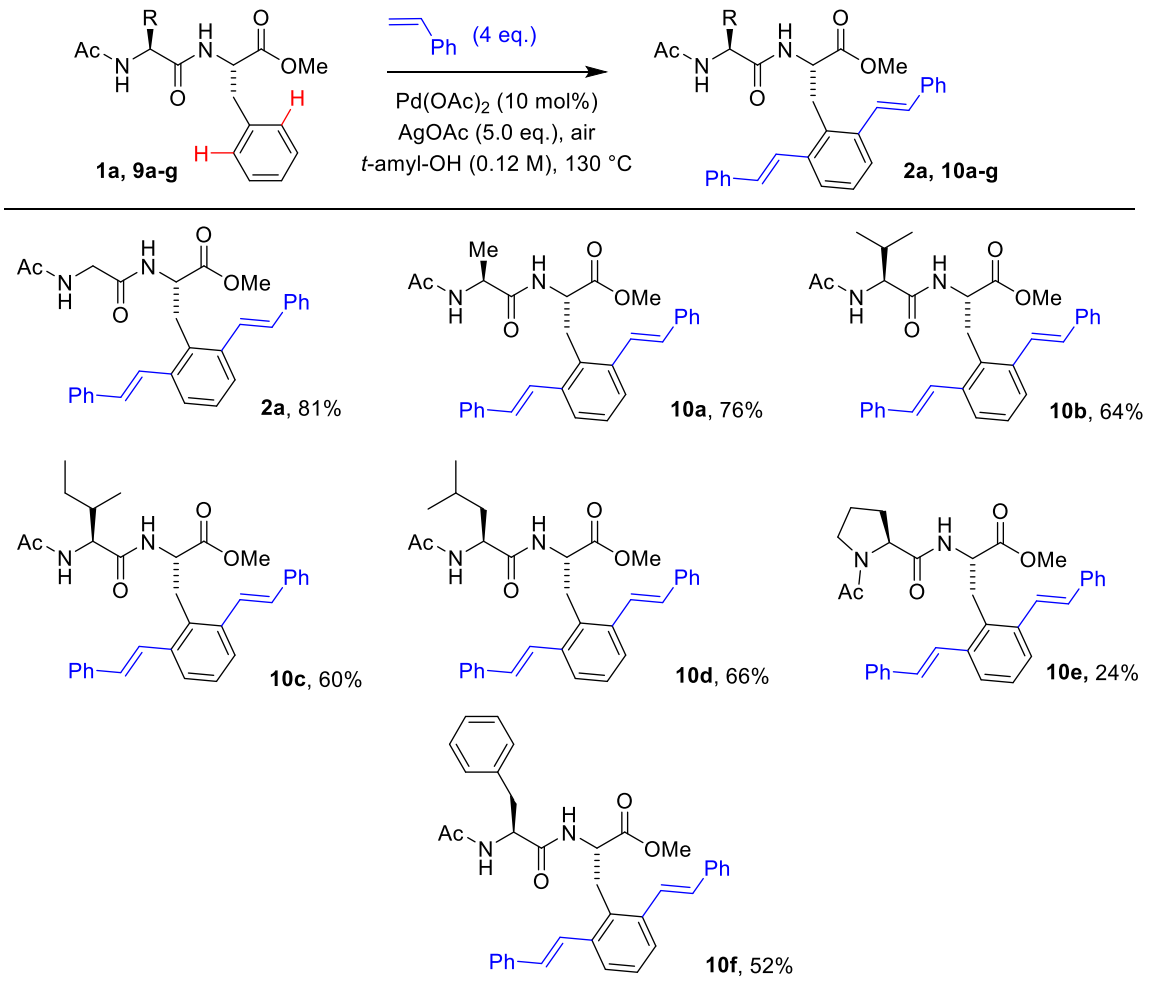
reaction did not proceed and the unmodified peptide **5** was recovered exclusively. A theory as to why peptide **5** was not modified in the olefination reaction is proposed in **Section 2.2.7.**



***Scheme 38**: Scope of the N-protecting group.*

2.1.5 Amino acid sequence

The choice of the neighbouring amino acid in the model dipeptide **1a** and the peptides studied in **Section 2.1.4**, was limited to glycine. With a view to investigate the olefination of phenylalanine residues on long chain peptides containing multiple neighbouring amino acids, a series of dipeptides were synthesised with phenylalanine residues at the C-terminus. The neighbouring N-terminal amino acid was changed each time. The olefination reaction of dipeptides **9a**-**f** were then performed, and the results shown in **Scheme 39**.



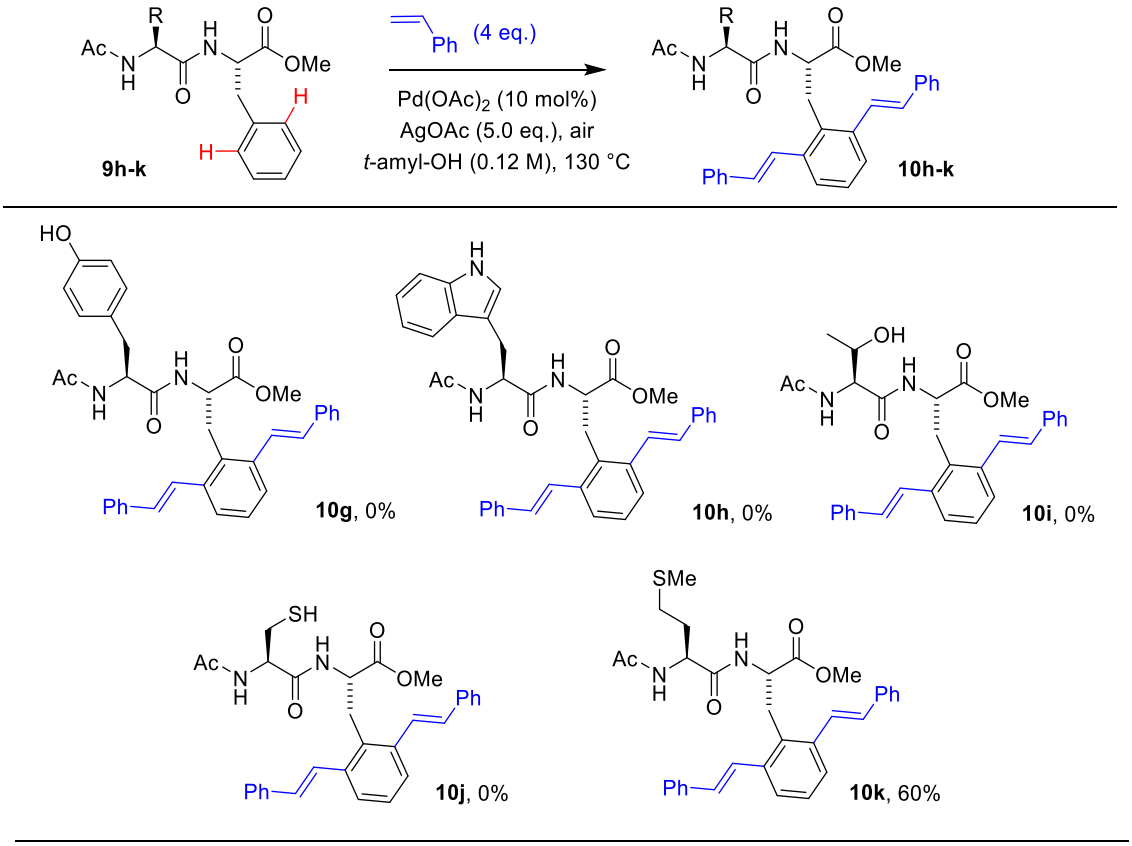
***Scheme 39**: Olefination of phenylalanine containing peptide **9a-f**.*

Dipeptides with neighbouring alkyl amino acid side chains (**2a**, **10a**-**d**) were modified in good yields (60-76% yield). The olefination of Ac-Pro-Phe-OMe (**9e**) gave modified peptide **10e** in a lower yield than the other examples displayed in **Scheme 39** (24%). A reason for the observed reduction in the isolated yield of **10e** is discussed in **Section 2.2.5.**

The reaction of the diphenylalanine containing peptide Ac-Phe-Phe-OMe (**10f**) with styrene was of interest to see whether both phenylalanine residues could be modified by the olefination reaction. After purification by flash column chromatography, modified peptide **10f** was obtained, in which only the C-terminal phenylalanine had been functionalized. There was no evidence of the modification of the N-terminal phenylalanine residue. In the previous investigations up to this point, the phenylalanine residue was located at the C-terminus, and the olefination reaction gave the corresponding modified peptide. The modification of peptide **9f** displays the first

example of site selectivity of the olefination reaction. The olefination of N-terminal residues was investigated further, and the results documented in **Section 2.1.6.**

Following the modification of dipeptides bearing simple aliphatic neighbouring amino acids (**Scheme 40**), the subsequent reactions focused on the olefination of phenylalanine residues in the presence of amino acids containing heteroatoms, **Scheme 39**.



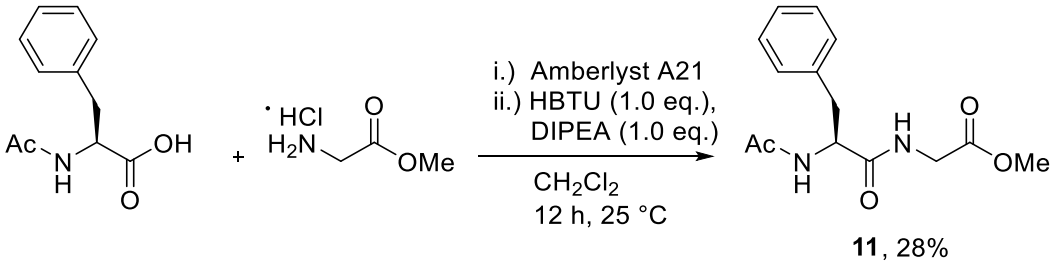
Scheme 40: *Olefination of heteroatom containing dipeptides* ***9g-k.***

The incorporation of the aromatic side chains of tryptophan (**9j**) and tyrosine (**9k**), afforded the peptide starting material only. Similarly, the olefination of phenylalanine residues in the presence of threonine and cysteine residues (**9h** and **9i**), were unsuccessful: yielding only the unmodified peptide. In SPPS, amino acids that contain highly reactive side chains are protected prior to peptide coupling.[111] The thioether containing amino acid methionine is an example of a pseudo protected cysteine residue: albeit methionine side chain is one carbon longer in length. Peptide **9k**, was compatible with the reaction conditions, and gave the corresponding olefinated peptide in a good yield (**10k**, 60%). A potential strategy to olefinate phenylalanine residues within peptides

in the presence of amino acid side chains that contain heteroatoms, might be to protect the side chains prior to C-H functionalization.

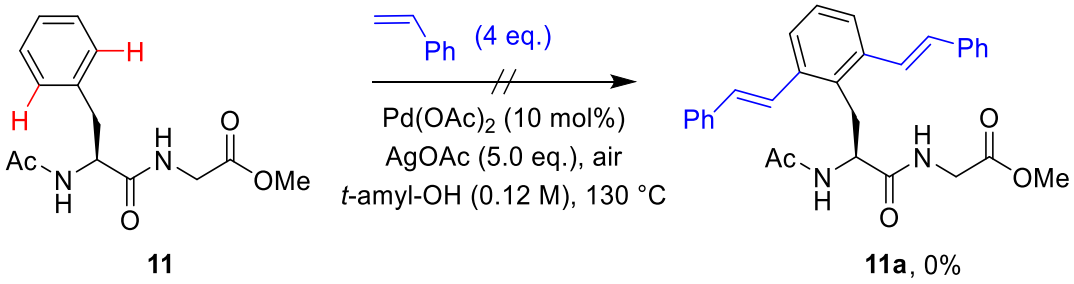
2.1.6 Olefination of phenylalanine at the N-terminus

In **Scheme 39**, only the C-terminal phenylalanine residue of dipeptide Ac-Phe-Phe-OMe (**9f**) had been modified by the olefination reaction. The N-terminal phenylalanine was unaffected. The C-H olefination reaction appeared to select the C-terminal residue only. To investigate the olefination of N-terminal phenylalanine residues, the dipeptide Ac-Phe-Gly-OMe (**11**) was synthesised by first removing the hydrochloride salt from the amino ester. Treating glycine methyl ester hydrochloride with K_2CO_3 and extracting the free amine into an organic solvent proved unsuccessful. Instead, dissolving the C-protected amino acid in water and adding a basic ionic exchange resin (Amberlyst A21) gave the free amine. The resulting residue was slurried in DCM along with HBTU and DIPEA to give **11** in a satisfactory yield (28%), **Scheme 41**.



Scheme 41: *Synthesis of Ac-Phe-Gly-OMe (**11**).*

Diolefinated peptide **11a** was not observed from the reaction of **11** in **Scheme 42**. The unsuccessful olefination of peptide **11** posed the question: does the position of the phenylalanine residue within a peptide sequence influence the outcome of the reaction? The unsuccessful olefination of N-terminal phenylalanine residues is discussed in **Section 2.2.3.**

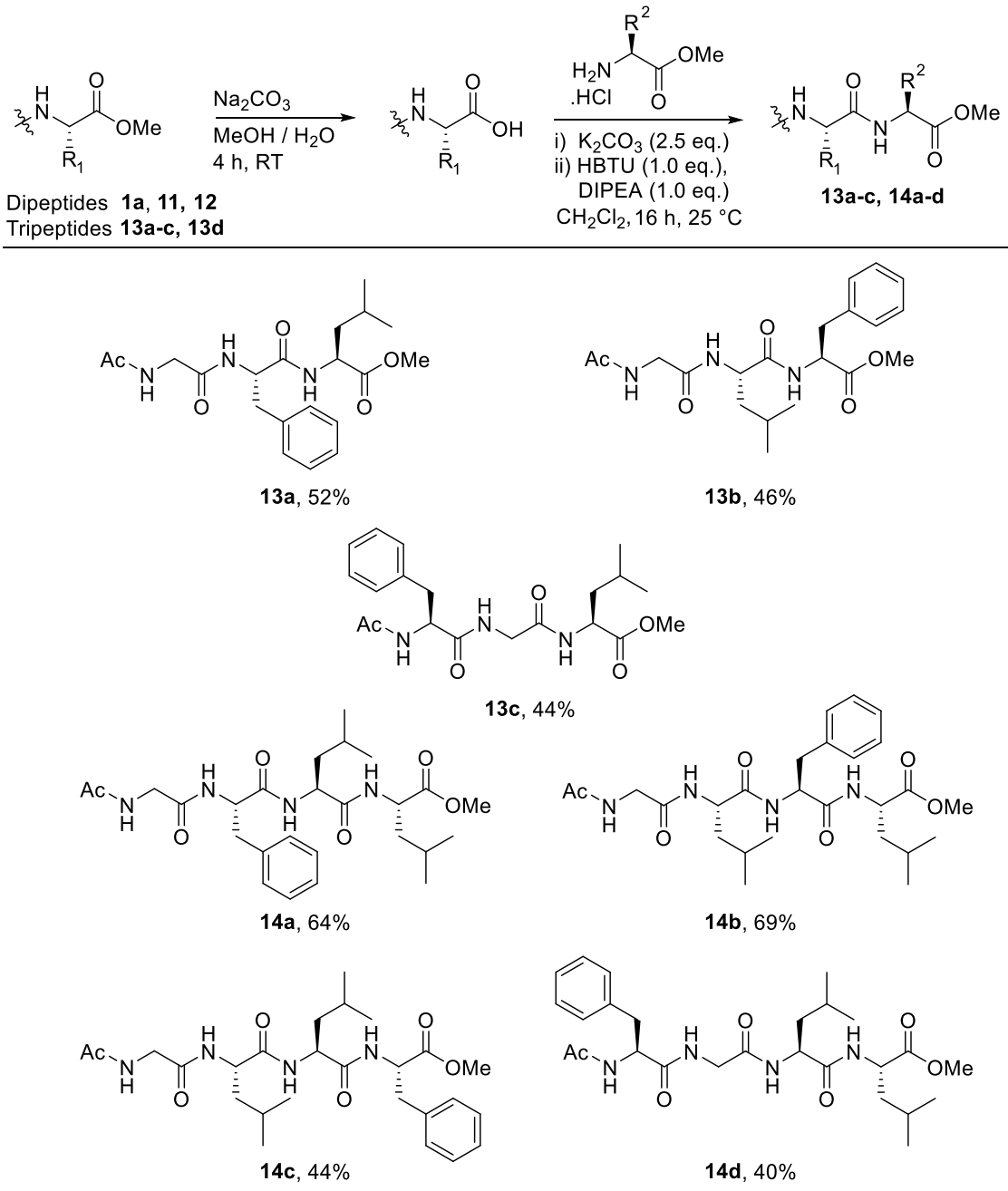


Scheme 42: *Attempted olefination of Ac-Phe-Gly-OMe (**11**).*

2.1.7 Olefination of tri-, and tetrapeptides

Investigations thus far had focussed on the olefination of phenylalanine containing dipeptides. In **Section 2.1.6**, the position of the phenylalanine residue within the peptide sequence dictated the success of the olefination reaction. This may suggest that the reaction is site selective as modification was not observed in peptide **11**, when phenylalanine was located at the N-terminus. The position of phenylalanine residues in dipeptides are limited to either N-, or C-termini. In larger peptides however, phenylalanine residues can lie at the middle of the sequence and not necessarily limited to the termini. To evaluate the viability of the olefination reaction to phenylalanine residues on larger peptide chains, a series of tri-, and tetrapeptides were synthesised from the corresponding dipeptide.

The C-terminus of the appropriate dipeptide or tripeptide was first deprotected by treating the methyl ester with a base to give the free carboxylic acid. The peptide acid was then coupled with a C-protected amino acid with HBTU to give the appropriate peptide, **Scheme 43**. The phenylalanine residues in peptides **13a**-**c**, **14a**-**d** were located at either the N-terminus, the C terminus or in the middle of the peptide sequence.

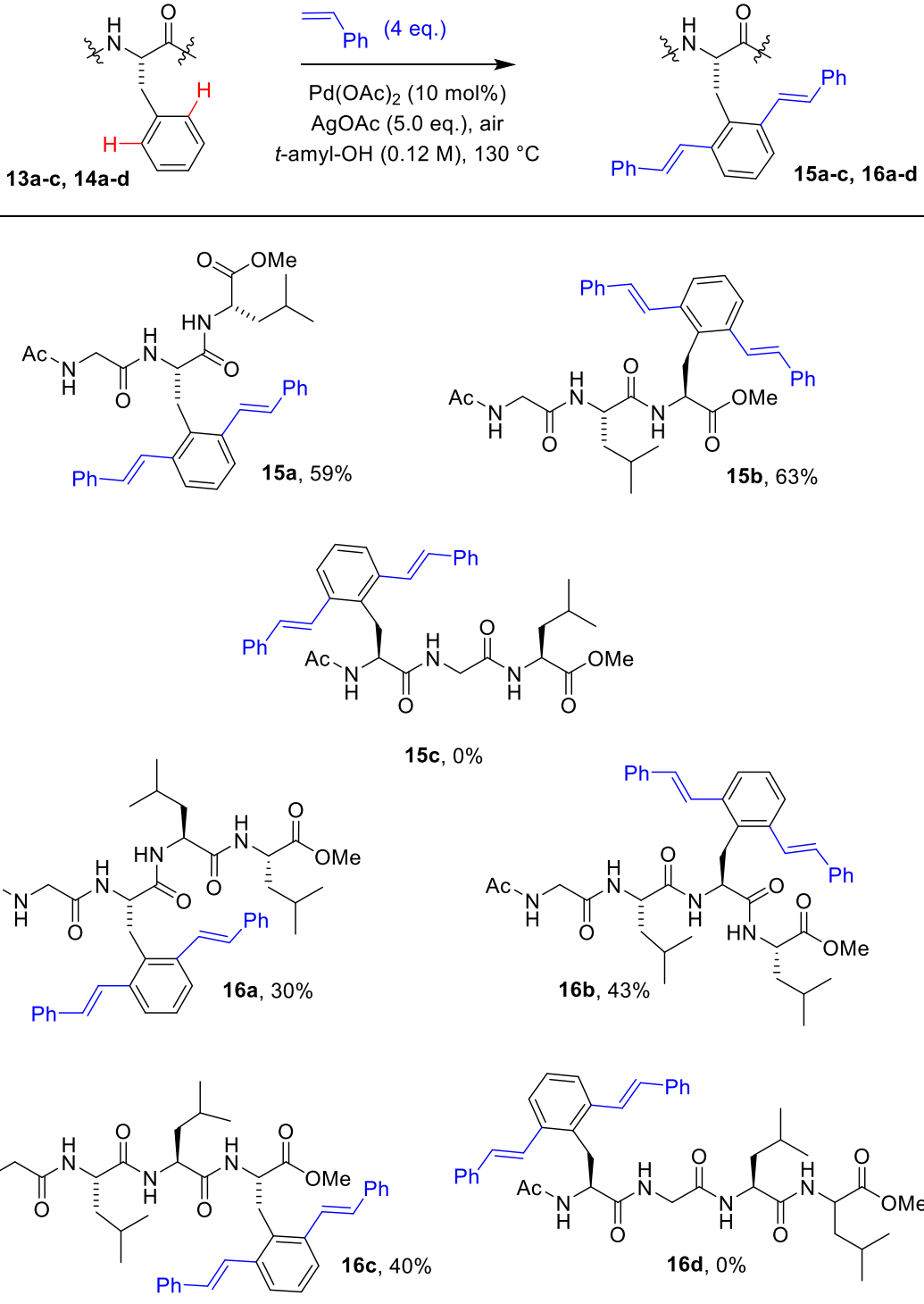


Scheme 43: *Preparation of tripeptides **13a-c**, and tetrapeptides **14a-d**.*

Tripeptides **13a**-**c** were characterised by ^{1}H and ^{13}C NMR spectroscopy. Analysis of tetrapeptides **14a**-**d** by ^{1}H NMR spectroscopy proved challenging at first as the ^{1}H NMR spectra is not very well defined, with areas of indistinguishable multiplets. The messy ^{1}H NMR spectra may be due to the longer chain length of tetrapeptides. Peptides **14a**-**d** can adopt more confirmations in solution compared to tripeptides **13a**-**c**.

Peptides **14a**-**d** were dissolved in many different NMR solvents. Polar NMR solvents (Acetonitrile-d_3 and DMSO-d_6) had little effect on the resolution, while NMR solvents that

promote hydrogen bonding in solution (Methanol-d_4 and Acetone-d_6) did not improve the ^{1}H NMR spectra either. Mass spectrometry was used to aid in the characterisation. HRMS data was used to identify the exact mass of each peptide. From the fragmentation pattern in the mass spectrum of peptides **14a-d**, there was no evidence of any unreacted starting material in the sample. Confident that the samples were suitably pure, the modification of peptides in **Scheme 43** was then studied, **Scheme 44**.



Scheme 44: *Olefination of peptides (**13a-c, 14a-d**).*

The C-H olefination methodology worked well on phenylalanine residues at the C-terminus, and in the middle of tripeptides, giving the modified peptides **15a** and **15b** in good yields (59% and 63%), **Scheme 44**. As was the case with the modification of Ac-Phe-Gly-OMe (**11**) in **Scheme 42**, tripeptide **13c** was not modified by the reaction conditions. Tetrapeptides **14a**-**c** were found to tolerate the C-H olefination, giving modified peptides **16a**-**c** in moderate yields (30%, 43% and 40%). Unsurprisingly, when the phenylalanine residue was located at the N-terminus (peptides **13c** and **14d**), there was no reaction. Analysis of the olefinated tetrapeptides **16a**-**c** in **Scheme 44**, proved challenging compared to the analysis of modified tripeptides **15a** and **15b**. The *trans*-alkenes were easily identified in the ^{1}H NMR spectra, however, the signals correlating to the aromatic system and the peptide backbone were not as easily identifiable. Compared to tripeptides, tetrapeptides can adopt more conformations in solution, which could account for the difficulties in interpreting the NMR spectra. HRMS spectra of modified peptides **16a**-**c** confirmed the diolefinated product had been formed. The olefination of larger peptides demonstrates the olefination method is robust and has the potential for the modification of phenylalanine residues in much larger peptide systems.

2.2 Investigating the reaction mechanism

C-H functionalization of phenylalanine has been shown in **Section 2.1** on peptides with phenylalanine at the C-terminus or in the middle of a peptide. In **Section 2.1.5**, peptide **9h** contained two phenylalanine residues that could be modified. Olefination of the C-terminal phenylalanine residue proceeded well, whilst leaving the N-terminal residue unmodified. This led to the question; why are N-terminal residues unaffected by the reaction? Investigations looked at the C-H activation of the peptide, and how C-H activation was achieved.

2.2.1 C-H activation of C-terminal phenylalanine residues

Investigations started by examining the coordination of palladium to the model peptide **1a**. Coordination of a metal catalyst to a substrate for C-H activation is usually achieved via directing groups. In **Section 1.2.2** directing groups have been shown to be crucial for most examples of C-H activation. Directing groups allow the selective activation of specific C-H bonds.[54,57,112–114] Both mono- and bidentate directing groups allow the coordination of the metal catalyst to the C-H bond, however, bidentate systems can provide a lower energy pathway for C-H activation over monodentate directing

groups.[115] Coordination of a metal to a bidentate directing group forms a thermodynamically stable metalacyclic intermediate, **Figure 8**.[116]

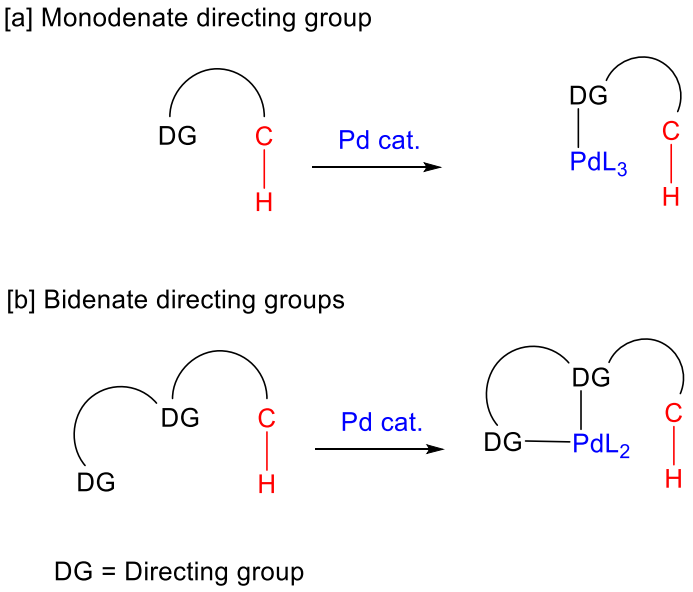
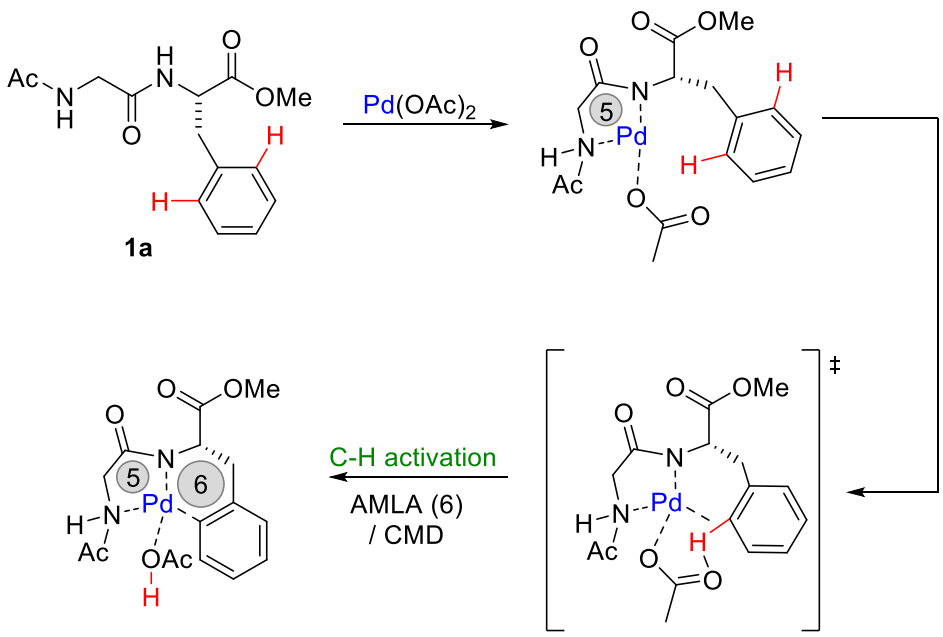


Figure 8: *Examples of palladation via [a] monodentate and [b] bidentate directing groups.*

Usually, directing groups must be installed onto a molecule before C-H activation. However, in all the peptides studies in **Section 2.1**, a directing group was not specifically installed. Instead the reaction uses the native amide backbone to coordinate to a metal for C-H activation. As documented in **Section 1.2.2,** amides can be used as directing groups for C-H activation. Dipeptide **1a** contains two secondary amides, that make up the peptide backbone. The mechanism of C-H activation occurs first through bidentate coordination to the amide nitrogen atoms, before C-H activation by an AMLA / CMD type mechanism, **Scheme 45**.



Scheme 45: *Proposed bidentate coordination of peptide* ***1a*** *with* $Pd(OAc)_2$.

Coordination of palladium to the peptide backbone forms the stable five-, six-membered palladacycle. This bicyclic ring system is key, as it stabilises the energy of the metalated complex. Pd^{II} must adopt square planar geometry for C-H functionalization. Peptide **1a** must be able to adopt the required conformation, so that the amide nitrogen atoms can sit in a square planar conformation. The proximal and distal coordinating atoms must be able to adopt this conformation for C-H activation to occur.

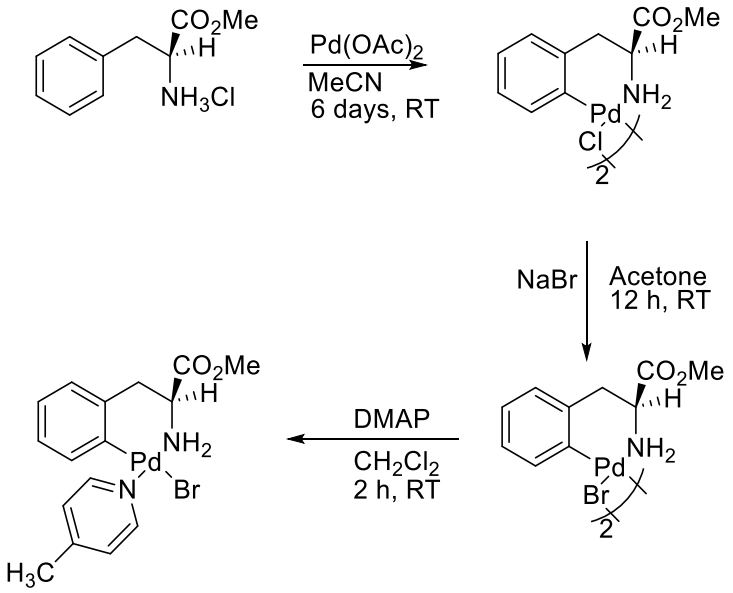
The C-H activation of larger peptides must go through the same reaction pathway as peptide **1a**. Tripeptides **13a** and **13b** contain three amide residues for coordination and tetrapeptides **14a**-**c** contain four potential coordination sites. Despite these multiple coordination sites, phenylalanine residues in larger peptides are modified by the reaction, **Scheme 44**. Coordination through the amide residues in peptides acts as a discrete directing group that does not need to be removed after C-H functionalization. This makes peptide modification by C-H functionalization through the backbone an extremely attractive process.

2.2.2 Palladation of Ac-Gly-Phe-OMe (1a)

In literature there have been a handful of examples of the palladation of phenylalanine[117,118] and phenylglycine.[119–121] However, the palladation of a phenylalanine containing dipeptides has not been reported. It was envisioned that by treating peptide **1a** with stoichiometric $Pd(OAc)_2$, the palladated peptide could be

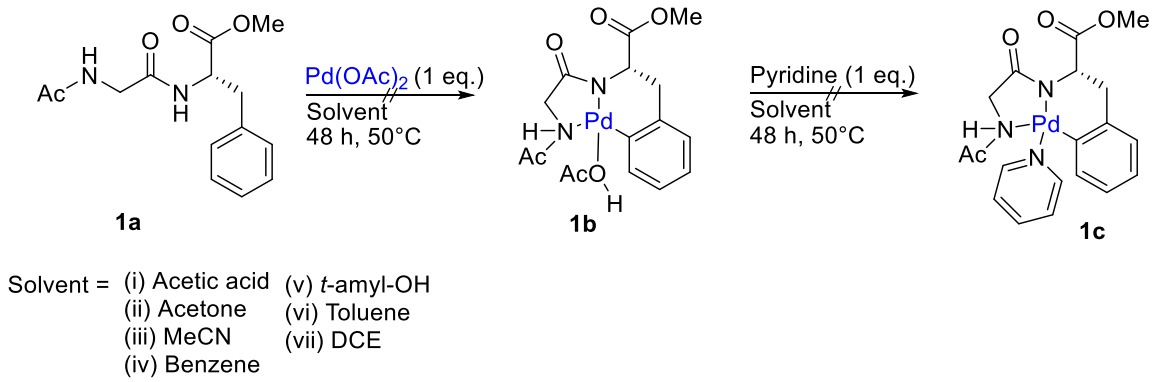
synthesised. The palladated peptide could confirm the proposed bidentate coordination through amide residues in **Scheme 45**.

The palladation of phenylalanine was reported Bautista et al.[117] In the study, the palladated amino acid was isolated with a bridged chloride ligand. Bautista demonstrated that the mono-nuclear complex could be isolated by first treated with NaBr and then DMAP. The metallacycle contains a six-membered ring with coordination through the free amine group. This is consistent with the proposed coordination of palladium in **Scheme 45**.



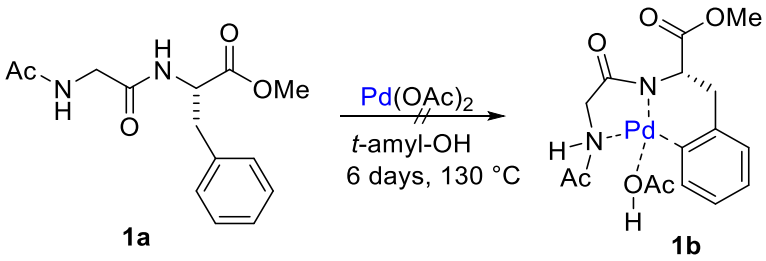
***Scheme 46**: Ortho palladation of phenylalanine by Bautista et a.*[117]

Using the reaction in **Scheme 46** as inspiration, work commenced on synthesising a palladated peptide. Peptide **1a** with treated with stoichiometric $Pd(OAc)_2$ and heated to 50 ˚C for 48 h in a variety of solvents, **Scheme 47**. As with Bautista's example, decomposition of Pd^{II} to Pd^0 was witnessed by the appearance of a black solid in the reaction. The solid was filtered before the addition the addition of pyridine. The solution was again heated to 50 ˚C for a further 48 hours. Analysis of the final mixture by ^1H NMR spectroscopy showed peptide **1a** only. There was no evidence of the palladated peptide. The crude mixture was also analysed by mass spectrometry, which displayed peptide **1a**, pyridine and $Pd(OAc)_2$. The reaction had not taken place.



Scheme 47: *Attempted palladation of peptide* ***1a*** *at 50 ˚C and ligand exchange to* ***1c.***

The reaction in **Scheme 47** to give peptide **1b** may not have worked due to the low temperature compared to the olefination reaction. Instead the reaction of **1a** with $Pd(OAc)_2$ was operated at 130 ˚C for 6 days, **Scheme 48**. The crude mixture was left to evaporate slowly in DCM, to afford a crystalline solid. Analysis by x-ray crystallography showed the solid to be peptide **1a**. There was no evidence of the palladate compound by ^1H NMR spectroscopy or x-ray crystallography.



Scheme 48: *Attempted palladation of peptide* ***1a*** *at 130 ˚C.*

All these endeavours were unsuccessful. This is not to say that the reaction does not proceed via the proposed pathway, it may mean that the intermediate may not be isolatable or stable at room temperature.

2.2.3 C-H activation of N-terminal phenylalanine residues

In the previous section, C-H olefination of phenylalanine is achieved by bidentate coordination of the catalyst to the amide backbone. In **Section 2.1.6** however, the N-terminal phenylalanine residue was unaffected by the reaction. This is even the case in peptide bearing multiple coordination site, such as tripeptide **13c** and tetrapeptide **14d**.

Bidentate coordination of peptide **11** does not lead to the formation of a palladacycle that's is capable of C-H activation, **Figure 9**.

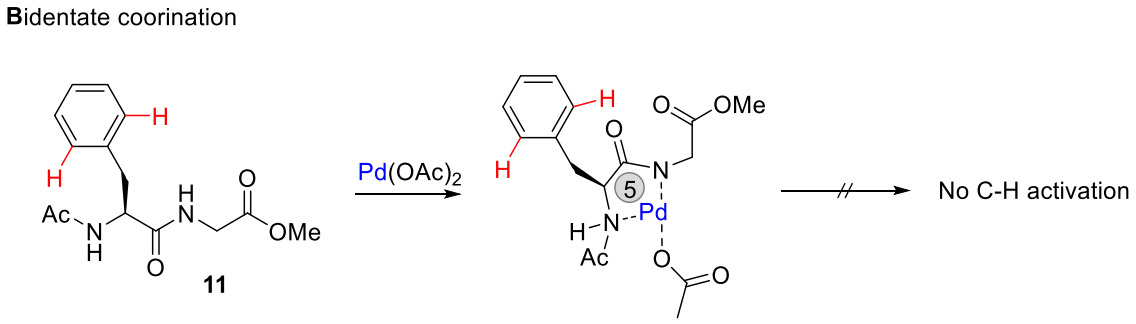


Figure 9: *Bidentate coordination of peptide* ***11*** *to* $Pd(OAc)_2$.

Monodentate coordination in peptides bearing N-terminal phenylalanine residues is still possible, **Figure 10**. The monodentate complex is not as stable as the chelated complex in **Figure 9**. Despite monodentate complexation affording the correct square planar geometry for Pd^{II} C-H activation, the reaction does not proceed.

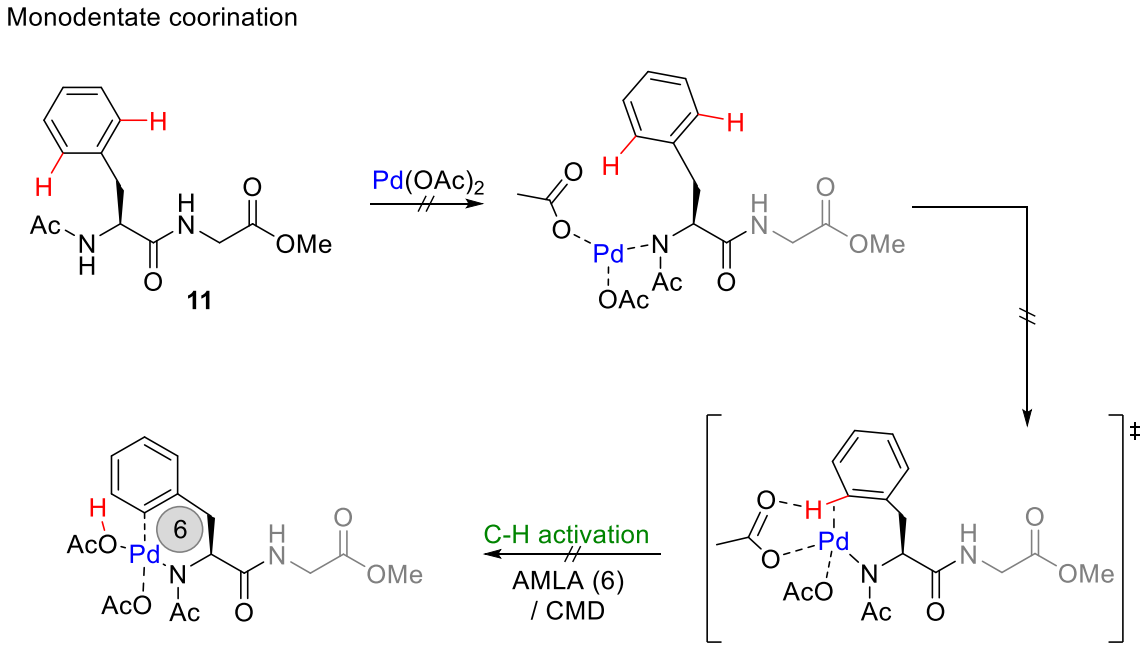


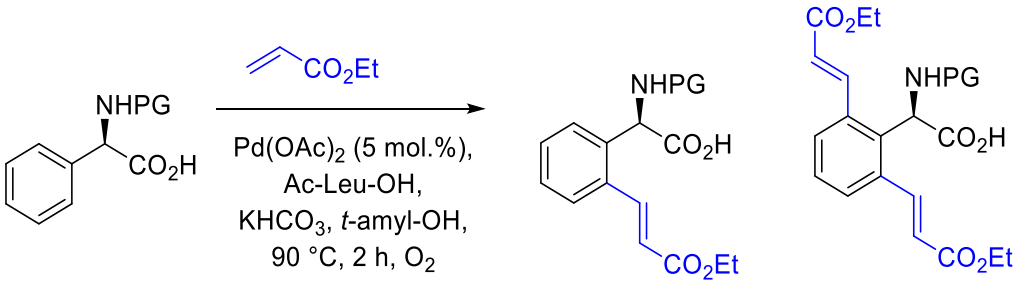
Figure 10: *Monodentate of* $Pd(OAc)_2$ *to peptide* ***11.***

The proposed C-H activation of **11** through monodentate or bidentate coordination does not afford the olefinated peptide. This is consistent with the reaction of other N-terminal phenylalanine residues. In **Section 2.1.5**, only the C-terminal phenylalanine residue was

modified in peptide **9h** (Ac-Phe-Phe-OMe). The N-terminal residue was unaffected. This was also the case with tripeptide **13c** and tetrapeptide **14d**, **Scheme 44**.

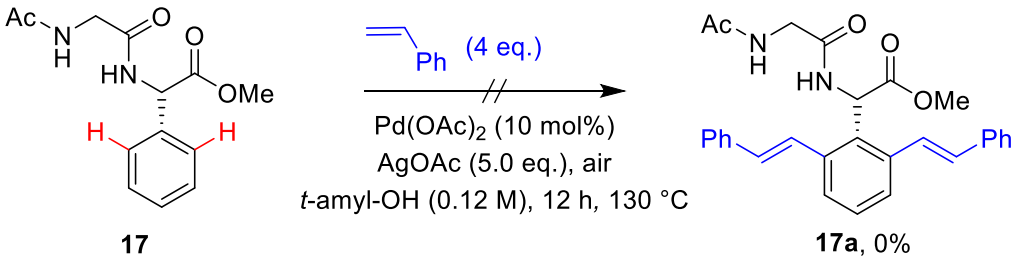
2.2.4 Olefination of phenylglycine residues

Phenylglycine is an unnatural amino acid, but a common feature in some peptide-based therapeutics such as virginiamycin S and streptogramin B.[122,123] The modification of phenylglycine by C-H functionalization has been investigated by Yu et al.[124]



Scheme 49: *Olefination of Phenylglycine by Yu et al.*

The C-H olefination reaction developed in this study was the applied to the phenylglycine containing peptide **17**, to see if the methodology could be extended to unnatural aromatic residues. There was no evidence of the modified peptide **17a** when the reaction was performed using the optimised conditions for the modification of phenylalanine residues within peptides. **Scheme 50**.

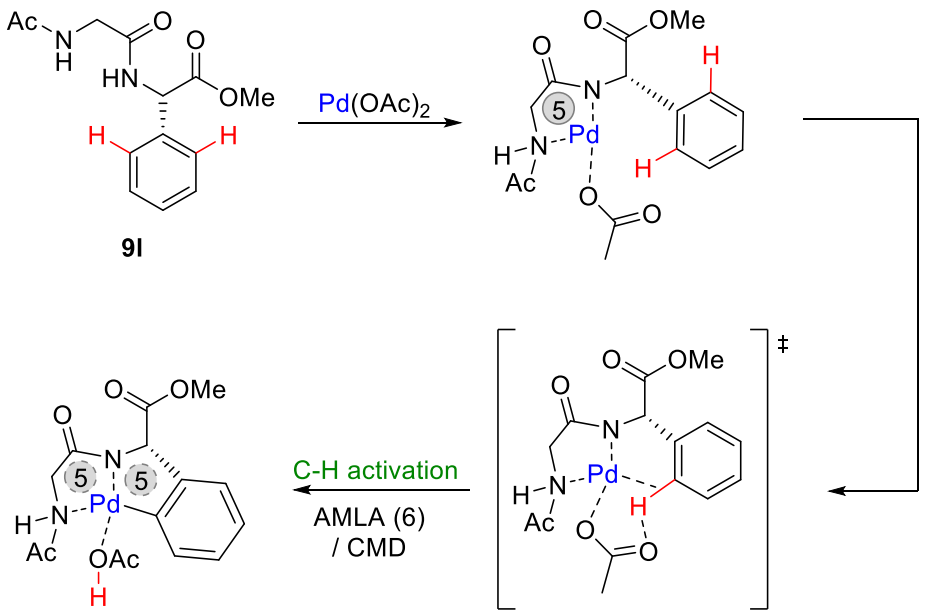


17 **17a**, 0%

Scheme 50: *Attempted olefination of Ac-Gly-Phg-OMe (**17**).*

The absence of the modified peptide in **Scheme 50** was surprising. We had already seen that the C-terminal phenylalanine residues are modified in the olefination reaction. In **Scheme 50** the phenylglycine residue is also located at the C-terminus and under the devised conditions, should be modified by the C-H olefination reaction. Coordination of the metal catalyst to the peptide backbone by the bidentate directing group is still

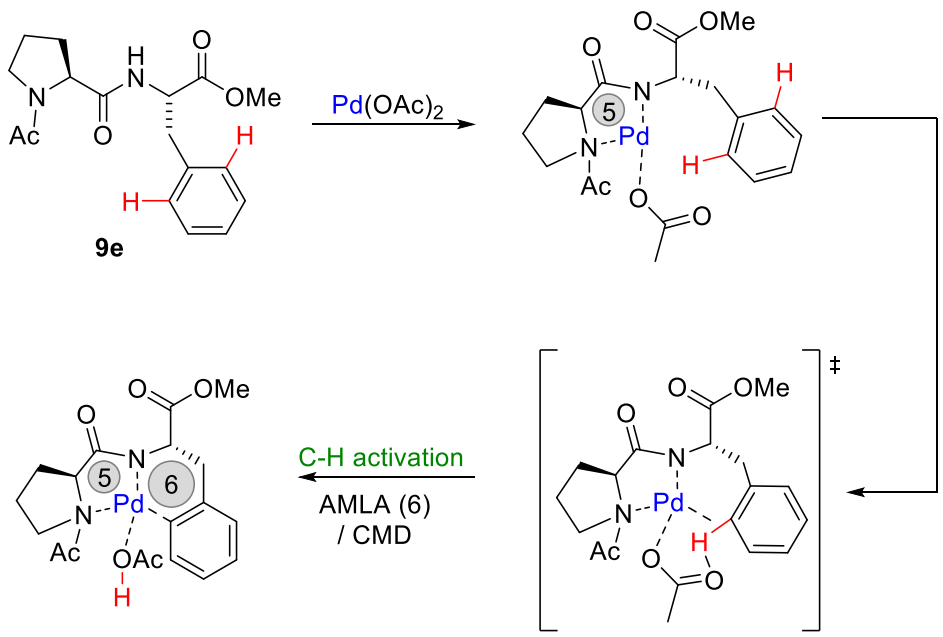
available. The five-, five-ring system is most likely higher in energy than the one formed by peptide **1a** in **Scheme 45**, and is not formed in the reaction.



Scheme 51**: Proposed bidentate coordination of $Pd(OAc)_2$ peptide **17.

2.2.5 Olefination of Ac-Pro-Phe-OMe (9e)

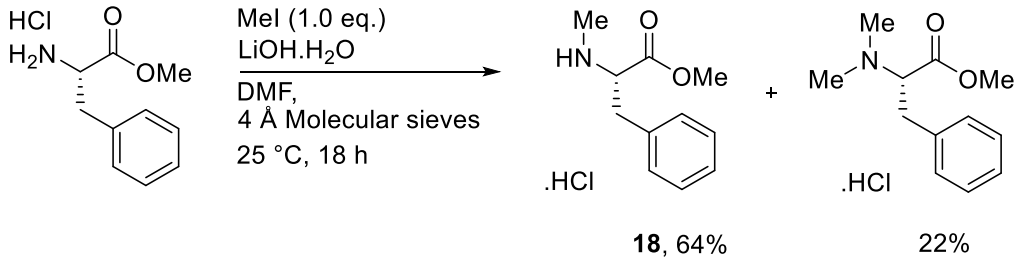
Coordination through the amide residues can be strengthened by deprotonation of the amide nitrogen, creating a more stable metallacycle. But what happens if one of these amide protons is not present: Does the olefination reaction still take place? The amide proton from the peptide backbone can be removed by installation of an imide. In **Section 2.1.5**, the proline containing peptide **9e** was modified by the reaction conditions to afford the modified peptide in a low yield (24%). Despite the lower yield than modified peptide **1a** (81%), the reaction still proceeds. Proline is the only natural amino acid that creates an imide in a peptide sequence. The inclusion of proline causes conformational change to the peptide, creating beta turns in large peptide chains. Peptide **9e** contains an imide at the N-terminus and still afforded the modified peptide **10e**. The side chain of proline residues contains a 5-membered aliphatic ring, **Scheme 52.** When the palladium catalyst chelates to the nitrogen amides in peptide **9e**, the proline side chain produces a greater strain on the ring system. However, the peptide can still adopt the required conformation for C-H activation as modified peptide **10e** was recovered. The strained ring system is less stable than other bidentate system in **Scheme 45**.This could account for the reduction in yield of the modified peptide. None the less, the reaction works with a neighbouring proline residue, and can accommodate an imide at the distal nitrogen.



***Scheme 52**: Proposed coordination and C-H activation of peptide **9e** with Pd(OAc)₂.*

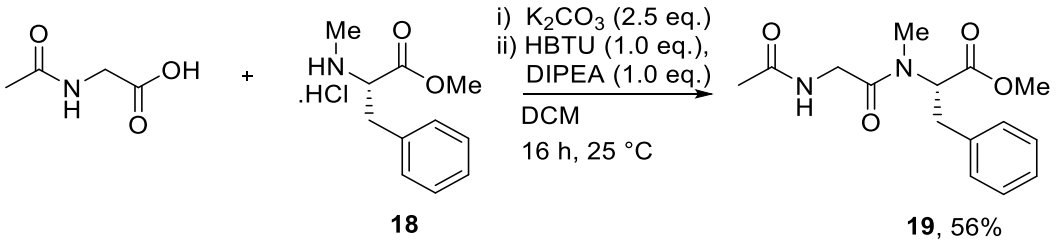
2.2.6 Olefination of *N*-alkylated Phenylalanine residues

The imide residue was then moved to the proximal amide. Peptide **19** was first prepared by the *N*-methylation of phenylalanine. The *N*-alkylated amino acid **18** was prepared by treating phenylalanine methyl ester hydrochloride with LiOH.H_2O in dry DMF, before the addition of iodomethane. After stirring at room temperature for 16 hours, the suspension was filtered and worked up with aqueous washings. Purification by flash column chromatography afforded the mono-alkylated amino acid **18** in a 64% yield, with the di-alkylated phenylalanine in 22%, **Scheme 53**.



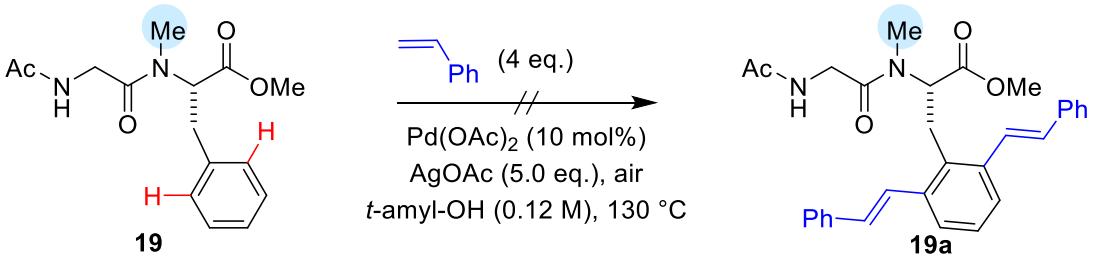
***Scheme 53:** Alkylation of phenylalanine methyl ester hydrochloride to give amino acid **18**.*

The *N*-methylated amino acid **18** was then coupled with *N*-acetyl glycine under the conditions in **Scheme 54**, to give peptide **19** in a good yield (56%).



Scheme 54: *Synthesis of peptide* ***19.***

The *N*-methylated peptide **19** was then subjected to the olefination reaction. There was no evidence of modified peptide **19a** from the reaction. The installation of an imide proximal to the phenylalanine residue does not allow C-H activation. The methyl group may not allow the peptide to coordinate to palladium, despite increasing the nucleophilicity of the proximal amide. Peptide **19** may not be able to adopt the square planar geometry that is required for C-H activation. Presumably, monodentate coordination is formed by N-terminal nitrogen only.



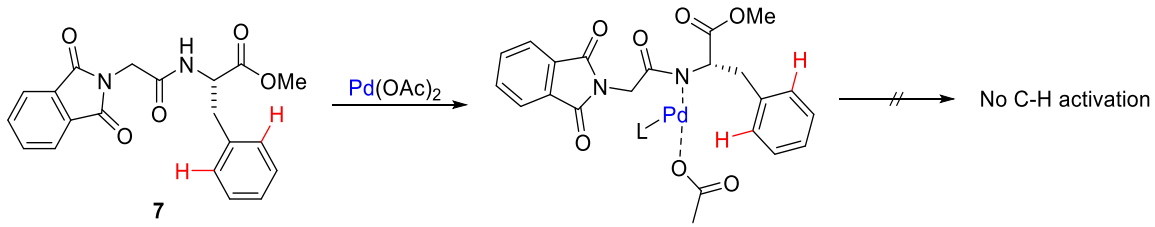
Scheme 55: *Attempted olefination of peptide* ***18.***

Peptides bearing multiple phenylalanine residues can be selectively modified by installing *N*-alkyl phenylalanine residues at sites where modification of the aromatic side chain is not desired. The draw back to this process is that the *N*-alky phenylalanine amino acid must be installed during peptide synthesis and cannot be added post-synthetically.

2.2.7 Olefination of Phth-Gly-Phe-OMe (5)

In **Section 2.1.4**, the incorporation of the phthaloyl protect amino acid at the C-terminus stopped functionalization of the neighbouring phenylalanine residue. Peptide **5** contains an imide at the N-terminus. The nitrogen lone pair in the phthaloyl amide is less nucleophilic than the distal amide of peptide **9e**. The phthaloyl nitrogen lone pair lies in

conjugation with two carbonyls, making it a poor Lewis base. The bidentate complex is not formed in peptide **5**, due to the poor Lewis basicity of the phthaloyl amide. The C-H activation pathway by monodentate coordination is too high in energy (see **Section 2.2.3**). Thus, the olefination reaction does not work with neighbouring phthaloyl protected amino acids.



Scheme 56: *Proposed coordination of $Pd(OAc)_2$ to peptide **7**.*

2.2.8 Oxidative Heck reaction

The direct olefination of peptide **1a** (**Scheme 35**), is an example of an oxidative-Heck reaction (also known as a Fujiwara-Moritana reaction), which unlike classic Heck reactions, does not require an organo-halide to facilitate metal-carbon bond formation.[125] An oxidative-Heck reaction produces a characteristic *trans* alkene. After the alkene insertion to palladium, the C-C bond rotates to the lowest energy confirmation to reduce the tortional strain on the molecule, creating the *trans* geometry. β-H elimination the proceeds to afford the *trans* alkene and the corresponding metal hydride complex, **Figure 11**.

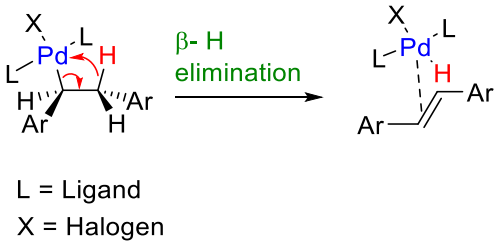


Figure 11: *β-H elimination to form a trans alkene.*

The proposed catalytic cycle for the olefination of peptide **1a** is shown in **Figure 12**. In a traditional Heck reaction, the metal carbon bond is formed by the loss of a halogen on the phenyl ring. Instead the metal coordinates to the peptide via the bidentate amide directing group. The ortho phenylalanine protons are then removed via the AMLA 6 / CMD mechanism. The acetate ligand aids in deprotonation, creating a new metal-

carbon bond. The reaction then proceeds with the insertion of the olefin to the metal by carbopalladation. A new C-C bond is then formed from the alkene to the phenyl ring. The complex, however, is still bound to the metal catalyst and dissociates by β-H elimination, producing a *trans* alkene and at first, the mono-olefinated peptide. The catalyst is regenerated by the oxidation of Pd^0 to Pd^{II} using AgOAc. The catalytic cycle is then repeated using the mono-olefinated peptide **2a'** to give di-olefinated peptide **2a**.

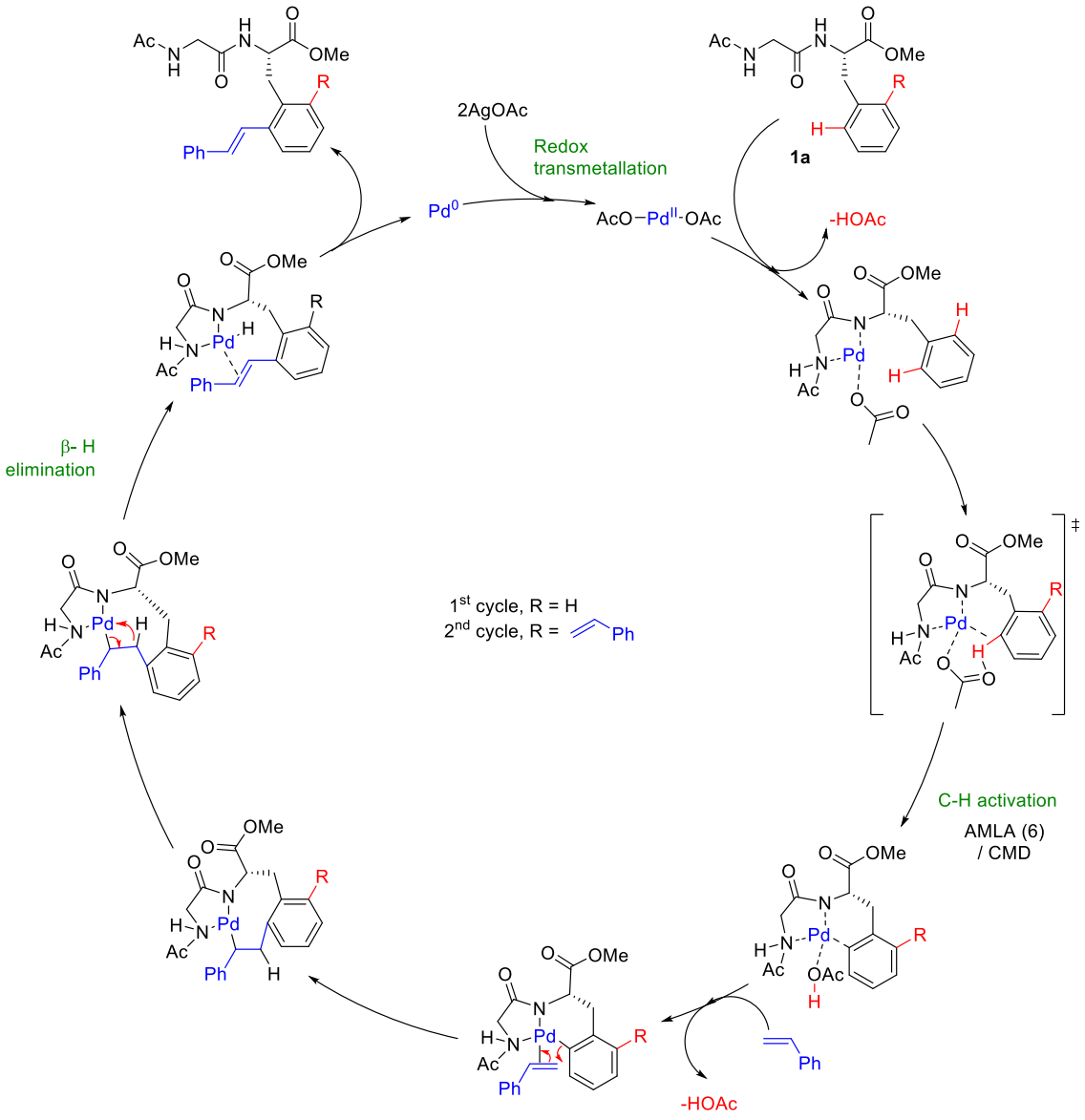


Figure 12: *Proposed catalytic cycle for the olefination of peptide* ***1a****.*

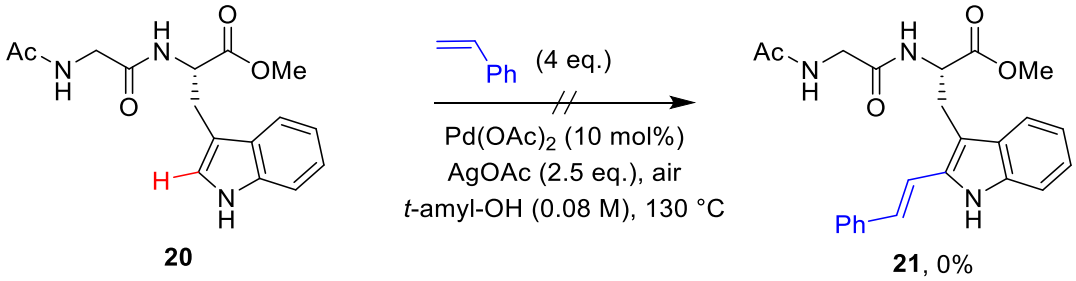
2.3 C-H olefination of tryptophan containing peptides

In **Section 2.1**, methods were devised for the direct C-H functionalization of phenylalanine residues in peptides. However, phenylalanine represents one of four naturally occurring aromatic amino acids; the others being histidine, tyrosine and tryptophan. To extend the methodology developed in **Section 2.1,** the olefination of other aromatic residues must be explored. The work described in this section details investigations into the C-H olefination of tryptophan containing peptides.

2.3.1 Olefination of Ac-Gly-Trp-OMe

Initial investigations focussed on the olefination of a model substrate: Ac-Gly-Trp-OMe (**20**). The dipeptide was synthesised by reacting *N*-acetyl glycine with L-tryptophan methyl ester, using the same methodology for the production of phenylalanine containing peptides, in **Scheme 34**.

The olefination methodology developed for the C-H functionalization of phenylalanine residues was then applied to the model tryptophan containing dipeptide **20**, **Scheme 57**. Under C-H olefination conditions

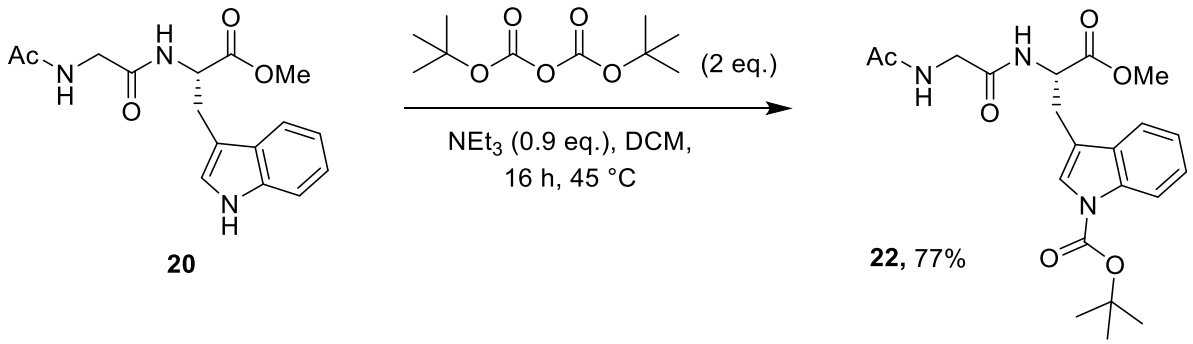


Scheme 57: *Attempted olefination of Ac-Gly-Trp-OMe (**1a**)*

Previously, the olefination of phenylalanine residues in peptides that contained unprotected heteroatoms proved unsuccessful, **Section 2.1.5**. However, the olefination of the thioether containing peptide Ac-Met-Phe-OMe (**9k**), gave the desired di-olefinated peptide (**10k**), **Scheme 40**. The attempted olefination of the thiol containing peptide Ac-Thr-Phe-OMe (**10j**) indicates that heteroatoms on amino acid side chains require protection for the C-H olefination reaction.

2.3.2 Boc protection of the tryptophan side chain

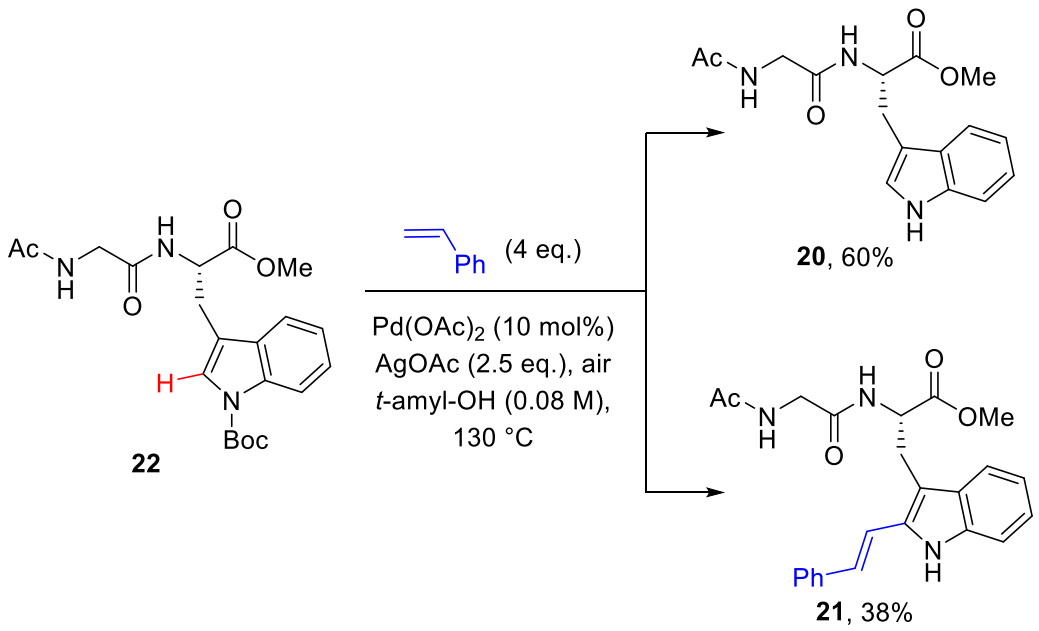
Protection of the nitrogen heteroatom became a priority to negate competitive binding between the side chain and the peptide backbone. In order to investigate the olefination of tryptophan residues, the indole side chain was protected with a Boc protecting group, **Scheme 58**. Boc protecting groups are a common feature in peptide chemistry as they are chemically robust and resistant to cleavage with base. Boc groups are a complimentary alternative to Fmoc protecting groups and can be easily installed from the corresponding anhydride. Peptide **20** was treated with Boc anhydride, in the presence of triethylamine to give the protected product in good yield (77%). The installation of Boc protecting group was confirmed by the ^{1}H NMR spectrum of **21** in which the *tert*-butyl group appears as a 9H singlet at 1.66 ppm.



Scheme 58: *Installation of the Boc protecting group.*

Next, peptide **22** was subjected to the C-H olefination conditions as shown in **Scheme 59**. The olefination of peptide **22** afforded the mono-olefinated peptide **21** without the Boc protecting group, in a satisfactory yield (38%). The ^{1}H NMR spectrum of peptide **21** displayed a pair of doublets at 6.78 ppm and another set of doublets contained within a multiplet at 7.27-7.34 ppm. The *trans* alkene was identified by a coupling constant of 16.6 Hz for the alkene proton at 6.78 ppm.

The other material recovered from the reaction did not contain the Boc protecting group. Peptide **20** accounted for the other species from the reaction. The Boc protecting group had been removed from both isolated products. Nevertheless, the direct olefination of **22** gave the olefination tryptophan residue is the first time that such a transformation had been achieved on a tryptophan containing peptide.



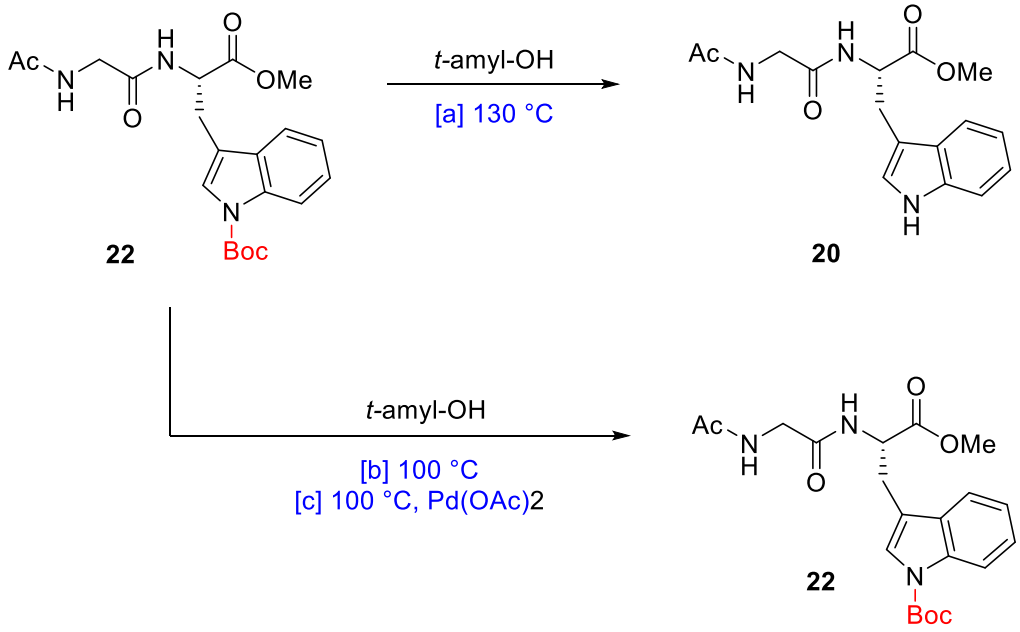
Scheme 59: *C-H Olefination of peptide* ***22*** *at 130 °C.*

Modified peptide **21** was recovered without the Boc protecting group. The in-situ cleavage of the protecting group may be a desirable feature in some synthetic reactions. However, the uncontrolled cleavage of the protecting group poses a problem in this reaction. As the reaction progresses, the concentration of the unprotected material increases, and the unprotected peptide will compete with the protected material in binding to the metal catalyst. The indole nitrogen binds more strongly than the peptide backbone, and poisons the metal catalyst, leading to a reduction in the yield of modified product. The reaction therefore can only progress to form a small amount of the modified material before the reaction stops and all that is produced is the unprotected materials **21** and **20**.

Boc protecting groups are routinely removed in the presence of acid; most commonly trifluoroacetic acid (TFA). In **Scheme 59**, acetic acid is generated as a by-product of the reaction and could cleave the Boc protecting group. A combination of the elevated temperatures and the acid produced by the reaction could contribute to the removal of the protecting group.

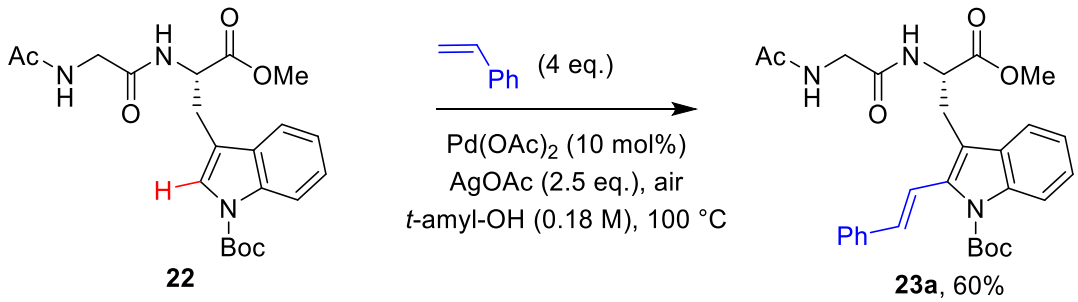
To determine if the N-Boc protecting group was thermally stable, peptide **22** was heated to 130 °C in *t*-amyl-OH, in an NMR tube, **Scheme 60**. After 24 hours, analysis of the crude ^{1}H NMR spectrum, showed the decomposition of peptide **22** to Ac-Gly-Trp-OMe (**20**). Running the reaction at 100 °C, afforded the protected peptide **22** only. Likewise, adding in $Pd(OAc)_2$ to the reaction at 100 °C, gave peptide **22** exclusively, despite the

generation of acetic acid as the catalyst was heated. Operating the olefination reaction (**Scheme 59**) at lower temperatures may yield the olefinated peptide **21** with the Boc protecting group intact.



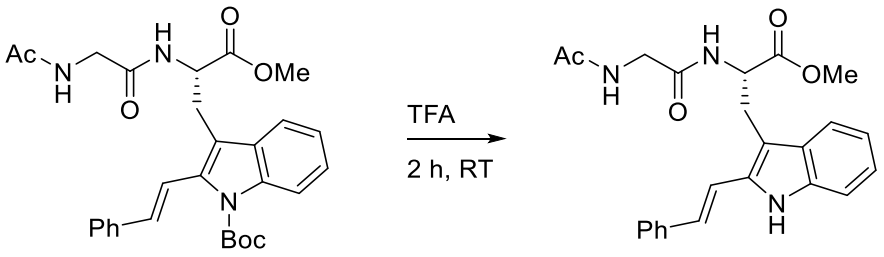
Scheme 60: *Decomposition study of peptide* ***22.***

Following the successful retention of the Boc protecting group at 100 °C (**Scheme 60**), the C-H olefination of tryptophan residues was attempted **Scheme 61**. Peptide **22** was olefinated at 100 °C to give the mono-olefinated peptide **23a** in a 60% yield. Modified peptide **23a** was recovered with the Boc protecting group, along with the protected starting material **22**. The unprotected peptide **21** was not observed in the crude ^{1}H NMR spectrum, making the conditions in **Scheme 61**, a suitable starting point to investigate the olefination reaction further.



Scheme 61: *Olefination of Ac-Gly-Trp(Boc)-OMe (**22**) at 100 °C.*

Protection of the indole side chain appears to be crucial for the olefination of tryptophan residues. In **Scheme 61**, modified peptide **23a** was recovered with the indole protecting group intact. If the protecting group could be easily removed, then the C-H functionalization can lead to the synthesis of a diverse library of modified tryptophan residues. The Boc protecting group was successfully removed by treating peptide **23a** with TFA at room temperature to afford the deprotected modified peptide **21**, **Scheme 62**.



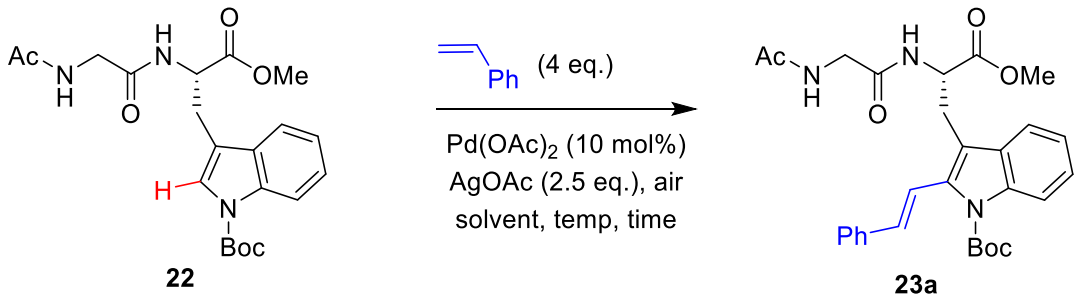
23a **21**, 97%

Scheme 62: *Removal of the Boc protecting group.*

2.3.3 Reaction optimisation

The successful olefination of peptide **22**, provided the basis for future investigations into reaction optimisation, **Table 3**. Running the reaction at 70 °C and 50 °C gave reduced yield (48% and 37%, **entry 2** and **3**), compared to 100 °C (60%, **entry 1**). Increasing the duration of the reaction (**entry 5**) to 96 hours at 100 °C gave very little change in the yield (61%). Next, a series of different solvents that are commonly used in C-H functionalization were screened. In the olefination of phenylalanine containing peptides **Section 2.1.2**, the initial results were obtained using a mixed solvent system of DCE/DMF. As with the olefination of Ac-Gly-Phe-OMe (**1a**) the DCE/DMF solvent system gave the olefin **2a** in a 44% yield, much less than the *t*-amyl-OH solvent system (60%). Running the olefination reaction in toluene was found to give the highest yield of modified peptide **23a** at 100 °C after 48 hours (85%). Reducing the reaction time form 48 hours was shown to have a significant effect on the yield of peptide **23a**, yielding 82% of the modified material in as little as 2 hours (**entry 17**). Surprisingly, running the reaction for just 15 minutes gave a yield of 49% of the mono-olefinated peptide.

Table 3: *Optimisation of the olefination of Ac-Gly-Trp(Boc)-OMe (**22**).*



22 **23a**

Entry	Solvent	Temp / °C	Time / h	Yield / %
1	*t*-amyl-OH	100	48	60
2	*t*-amyl-OH	70	48	48
3	*t*-amyl-OH	50	48	37
4	*t*-amyl-OH	70	96	50
5	*t*-amyl-OH	100	96	61
6	HFIP	100	48	0
7	MeCN	100	48	42
8	Dioxane	100	48	40
9	DCE	100	48	60
10	DMF	100	48	0
11	DCE/DMF	100	48	44
12	**THF**	100	48	36
13	Toluene	100	48	85
14	Toluene	100	24	88[a]
15	Toluene	100	12	87a
16	Toluene	100	6	86 [a]
17	**Toluene**	**100**	**2**	**82[a]**
18	Toluene	100	1	78 [a]
19	Toluene	100	0.75	74 [a]
20	Toluene	100	0.50	69 [a]
21	Toluene	100	0.25	49 [a]

[a] *yields were determined from the crude ^{1}H NMR spectrum of **23a***

Some of the yields in **Table 3** are taken from interpretation of the crude ^{1}H NMR spectrum after filtering the reaction mixture through Celite (entries **14-21**). The integrals from the methylene (CH_2) protons of the tryptophan residue were compared from the crude ^{1}H NMR spectra, as there was very little evidence of any other protons that correspond to other artefacts present in this area. The ^{1}H NMR for the pure modified peptide, shows the methylene protons from the tryptophan side chain, as a set of

doublet of doublets at 3.38 ppm and 3.47 ppm, whereas the methylene protons from the peptide starting material appears at 3.19 ppm and 3.27 ppm. The ratio of modified peptide to starting material from the integrals gave 3.75:1, giving a yield of 78% of olefinated peptide. An example of the crude ^{1}H NMR after 1 hour is shown in **Figure 2**.

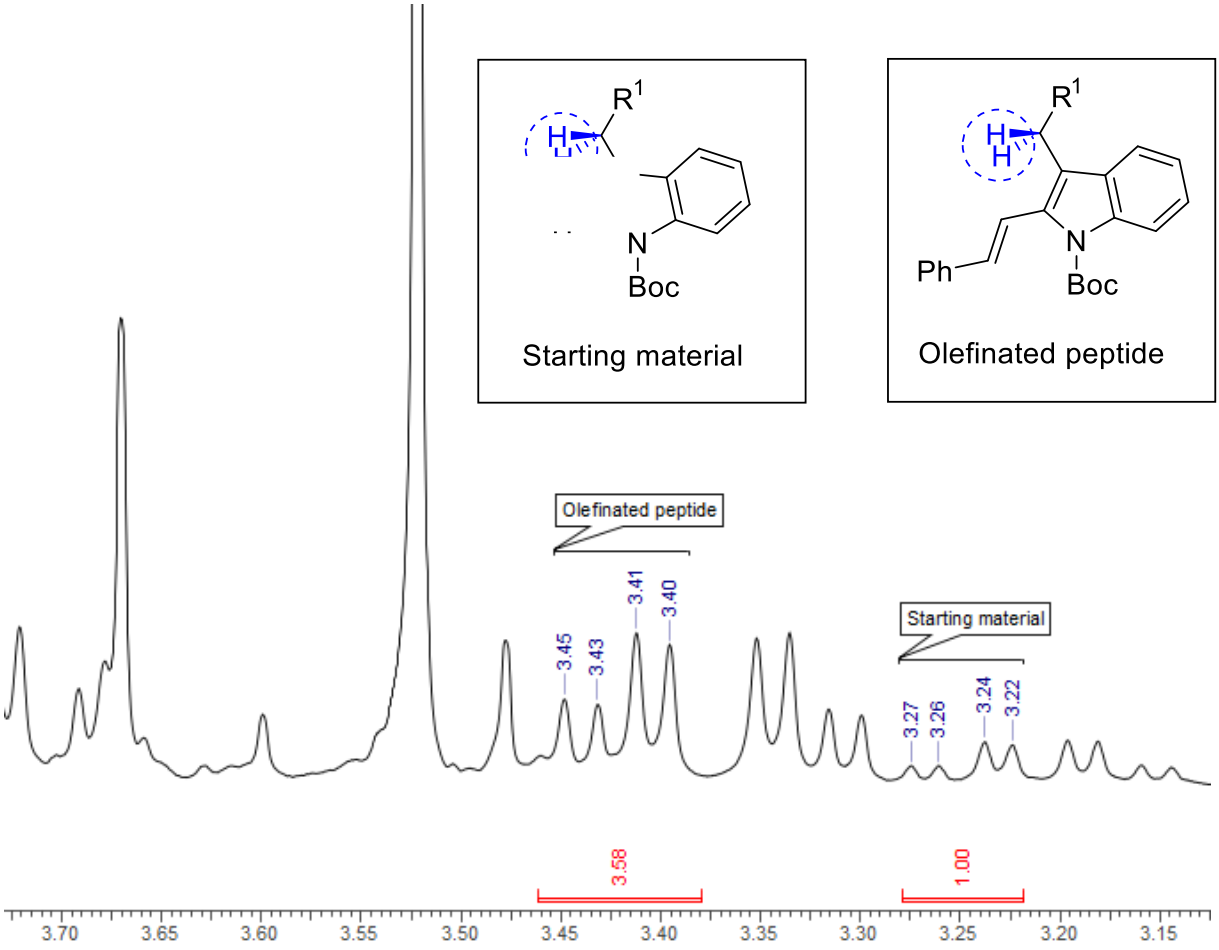


Figure 13: *Crude ^{1}H NMR from a 400 MHz spectrometer, of the olefination of Ac-Gly-Trp(Boc)-OMe after 1 hour.*

Plotting the data from **Table 3** into **Figure 14**, shows there is very little change in the yields produced between the hours of 2 to 24. **Figure 14** displays a large initial surge in the production of olefinated peptide **23a,** however, the rate of production of **23a** slows down after 45 minutes and eventually plateaus as the reaction approaches 6 hours. Consequent olefinations of tryptophan containing peptides were then run for a period of 2 hours.

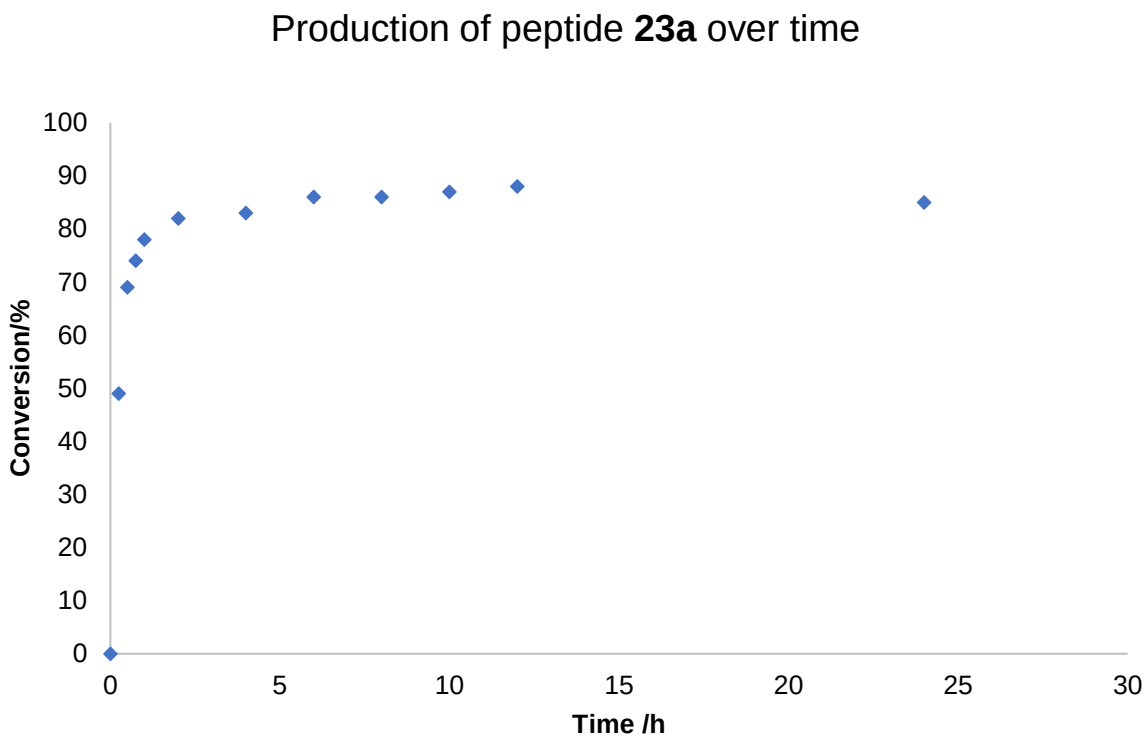
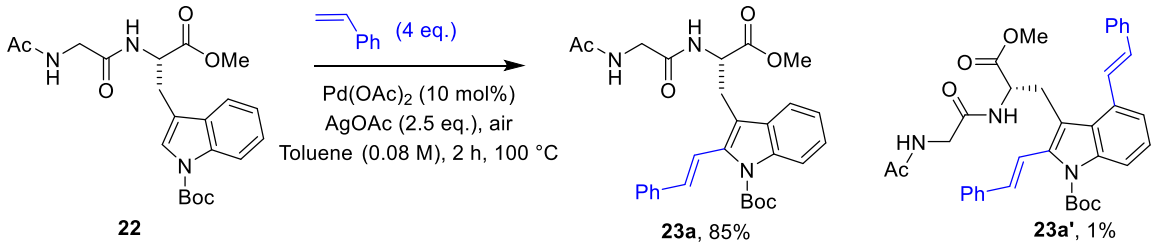


Figure 14: *Conversion of olefinated Ac-Gly-Trp(Boc)-OMe produced over time, determined by integration of methylene protons in the crude ^1H NMR spectrum.*

The model dipeptide **20** was then olefinated using the optimised reaction conditions and the products isolated after purification by column chromatography,

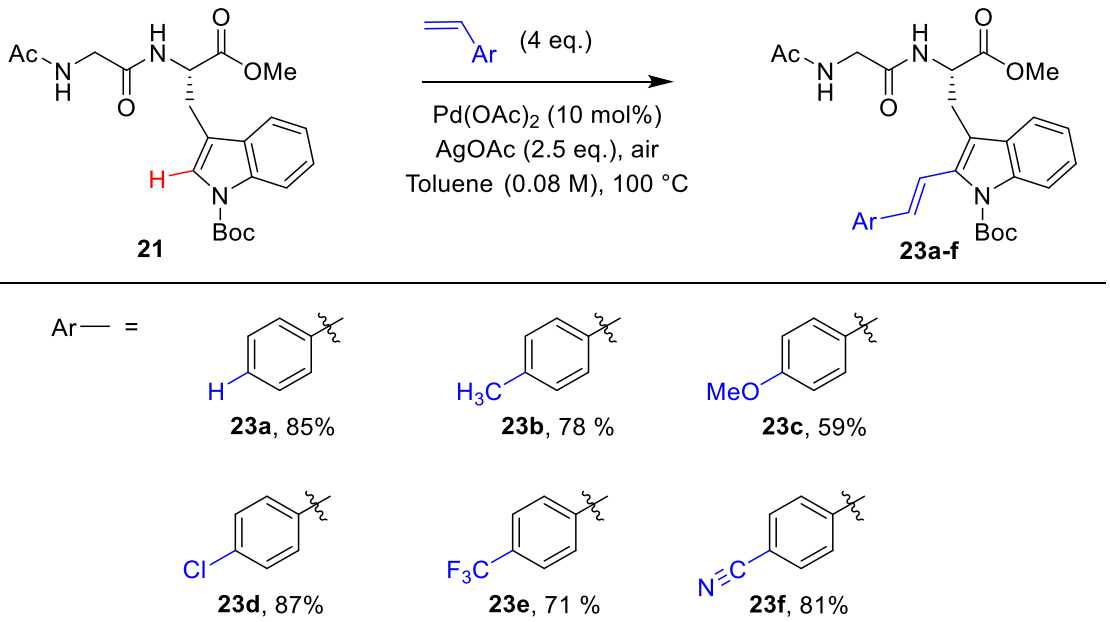


Scheme 63: *Olefination of A-Gly-Trp(N-Boc)-OMe to give the mono-, and di-olefinated tryptophan containing peptides.*

The di-olefinated tryptophan containing peptide **23a'**, was observed and isolated from the olefination reaction. The yield recovered was incredibly small (1% yield) and is the minor product from the reaction.

2.3.4 Scope of the alkene

The diversity of the alkene installed can increase the desirability of the reaction allowing the generation of a series of structurally related chemical libraries, using the same methodology each time. Investigations then focused on the olefination of the model dipeptide with a variety of substituted styrene analogues, **Scheme 64**.

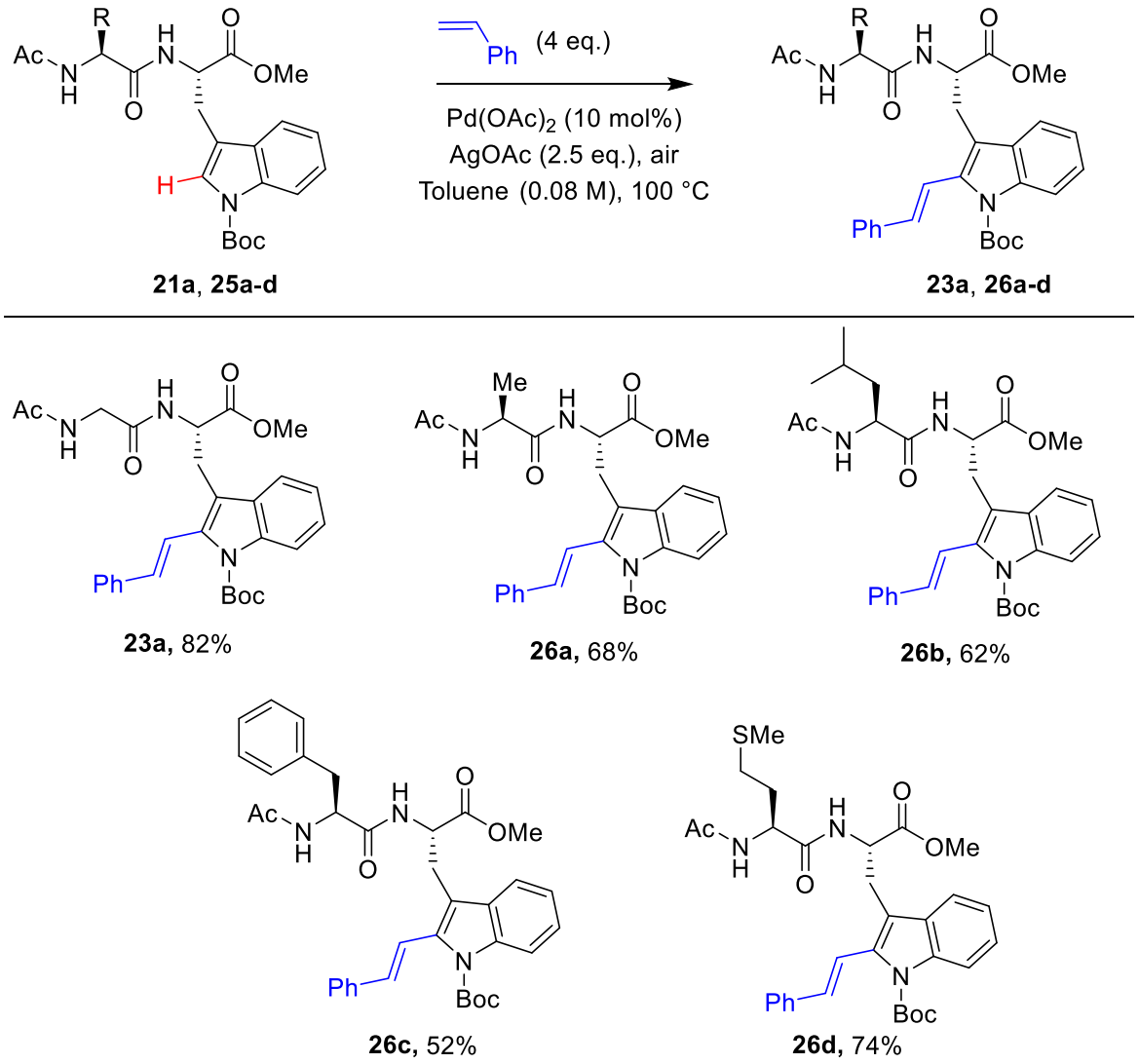


Scheme 64: *Investigating the scope of the alkene used in the olefination of tryptophan residues.*

The olefination reaction was found to tolerate a variety of styrene-based olefins. Similarly, to the reactions conducted in **Section 2.1.3**, olefins bearing electron donating groups (CH_3 and OMe, 23b and 23c) were well tolerated (78% and 59% respectively). Whilst olefins bearing electron donating groups (CF_3 and CN, **23e** and **23f**) were also recovered (71% and 81%).

2.3.5 Changing the neighbouring amino acid

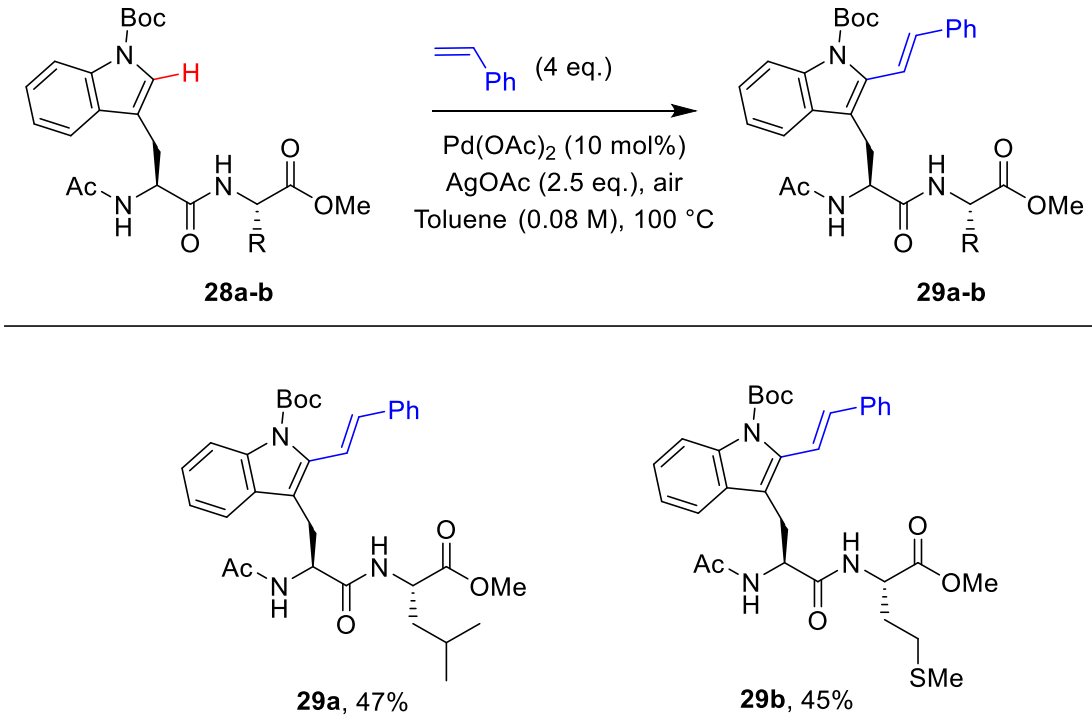
With the optimised conditions in hand, the next stage of the investigation involved screening the reaction against a series of different amino acids within a peptide sequence. The investigation changed the N-terminal amino acids and subjected peptides **21a** and **25a-d** to the olefination conditions, **Scheme 65**.



Scheme 65: *Screening of different amino acids at the N-terminus in tryptophan containing dipeptides.*

The C-H olefination reaction tolerated peptides containing alkyl chains upon neighbouring amino acids such as alanine **26a** and leucine **26b**, to afford modified peptides in good yields (68% and 62%). The addition of methionine at the N-terminus afford the corresponding modified peptide in an excellent yield (**26d**, 74%). The incorporation of phenylalanine at the N-terminus gave rise to olefination at the tryptophan residue exclusively (**26c**). Just as the previous study into the olefination of phenylalanine in **Section 2.1**, the N-terminal phenylalanine residue is unmodified by the reaction conditions.

The peptides investigated in **Scheme 65** each contain tryptophan residues at the C-terminus only. The next stage involved investigating the olefination of N-terminal tryptophan residues in a series of dipeptides, **Scheme 66.**

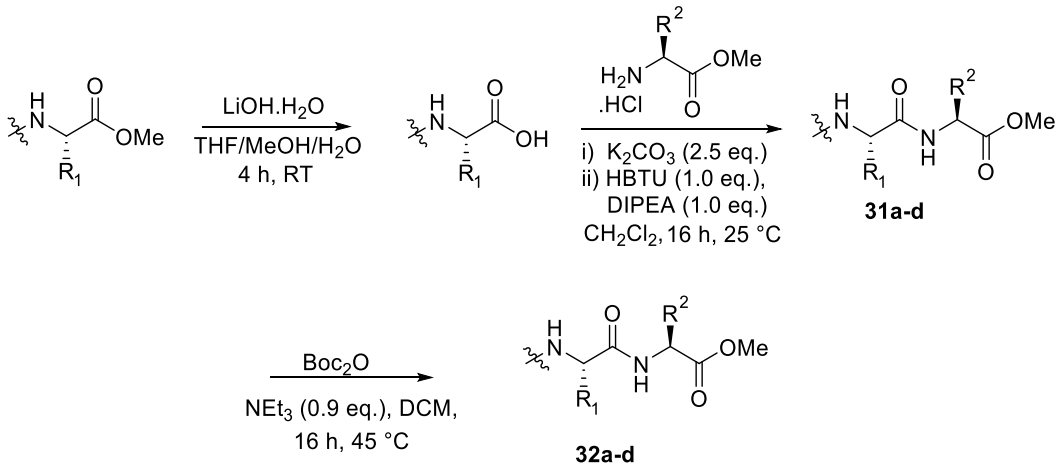


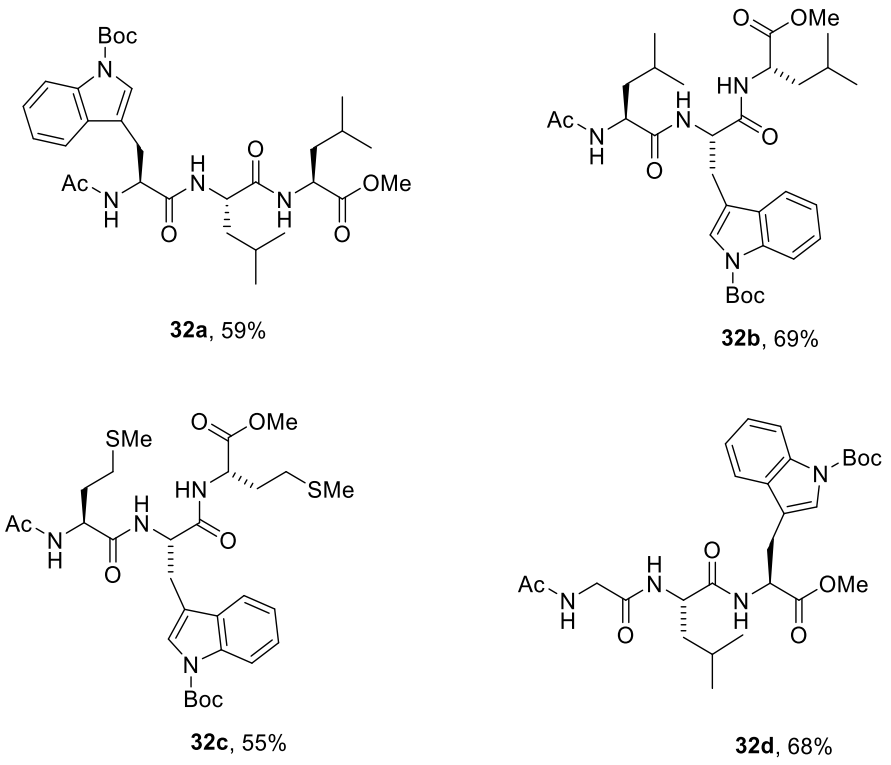
Scheme 66: *Olefination of N-terminal tryptophan residues.*

In contrast to the olefination of C-terminal phenylalanine residues **Section 2.1.6**, N-terminal tryptophan resides were modified by the olefination reaction. Modified peptide **29a** and **29b** were recovered in good yields (47% and 45%), lower than recovered yield of the complimentary the complimentary modified dipeptides **26b** and **26d** (68% and 74%). Nevertheless, the olefination of N-terminal tryptophan residues is significant compared to phenylalanine residues, which were unaffected by the olefination conditions.

2.3.6 Olefination of tryptophan containing tripeptides

With the olefination of a variety of dipeptides accomplished, the next stage involved the modification of tryptophan within longer chain peptides. The model tripeptides were synthesised as shown in **Scheme 67.**

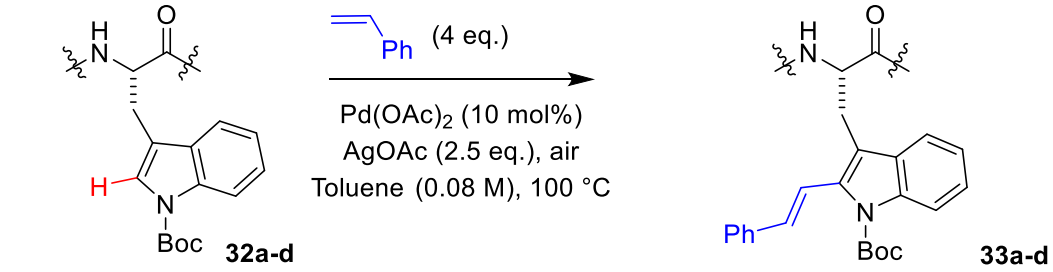


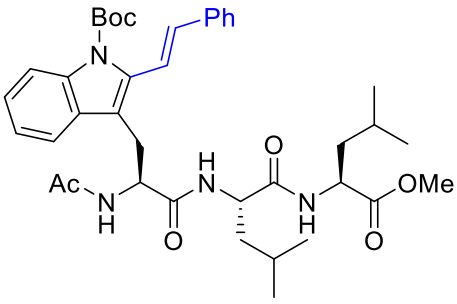


Scheme 67: *Preparation of tryptophan containing tripeptides.*

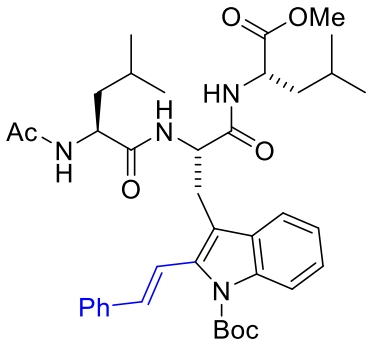
The four model tripeptides in **Scheme 67** were synthesised in solution, building from the corresponding dipeptides. The C-terminal protecting group was cleaved under mild conditions using LiOH in a mixture of THF: MeOH: H_2O to afford the corresponding dipeptide acid. The appropriate C-protected amino acid was then coupled to the peptide acid using HBTU and DIPEA. The sequential building of the peptide allowed for easy purification of the material as the tripeptide could be triturated from Et_2O. The final stage involved the protection of the tryptophan side chain using Boc anhydride in the presence of NEt_3. The protected material was then purified by column chromatography to afford

the desired tripeptide. The amino acids glycine, leucine and methionine were chosen as neighbouring residues as they had shown in **Scheme 65**, that tryptophan residues in dipeptides with these neighbouring amino acids were olefinated in the reaction conditions.

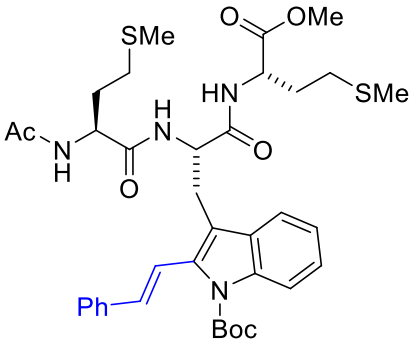




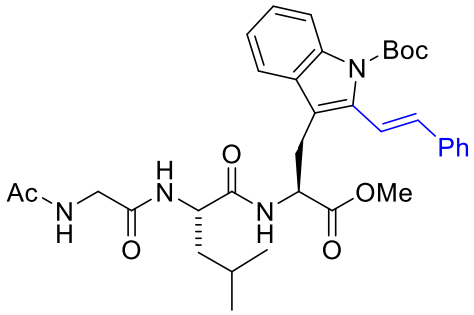
33a, 59%



33b, 69%



33c, 55%



33d, 68%

Scheme 68: *Olefination of tryptophan containing tripeptides.*

The olefination of tripeptide **32a** with an N-terminal tryptophan residue afforded the corresponding modified tripeptide in a good yield (59%). Likewise, tripeptides bearing tryptophan residues in the middle of the peptide sequence (**32b**, **32c**), were successfully olefinated by the reaction conditions, achieving yields of 69% and 55% respectively.

Moving the tryptophan residue to the C-terminus of the peptide afforded the modified peptide in a good yield (68%); analogous to all of the examples in **Scheme 68**.

2.4 Investigating the reaction mechanism for the olefination of tryptophan residues

2.4.1 Olefination of N-terminal tryptophan residues

Modified tripeptide **33a** and dipeptides **29a-c** were isolated with modified N-terminal tryptophan residues. However, in **Section 2.1.6**, the olefination of N-terminal phenylalanine residues was not observed by C-H functionalization. As discussed in **Section 2.2.1**, bidentate chelation of the metal catalyst to the peptide backbone appears crucial for C-H activation, yet olefination of monodentate species is still observed when targeting tryptophan residues. The modification of tryptophan containing peptides does not rely upon bidentate coordination to the peptide backbone. Instead, the Boc protecting group may be directing C-H functionalization in the tryptophan system by chelation to the carbamate functional group. Chelation to the peptide backbone does not appear to be crucial for functionalization. There are two proposed coordination sites: the formation of a metal-peptide species through chelation at the Boc protecting group, or chelation through the amide backbone, **Figure 15**.

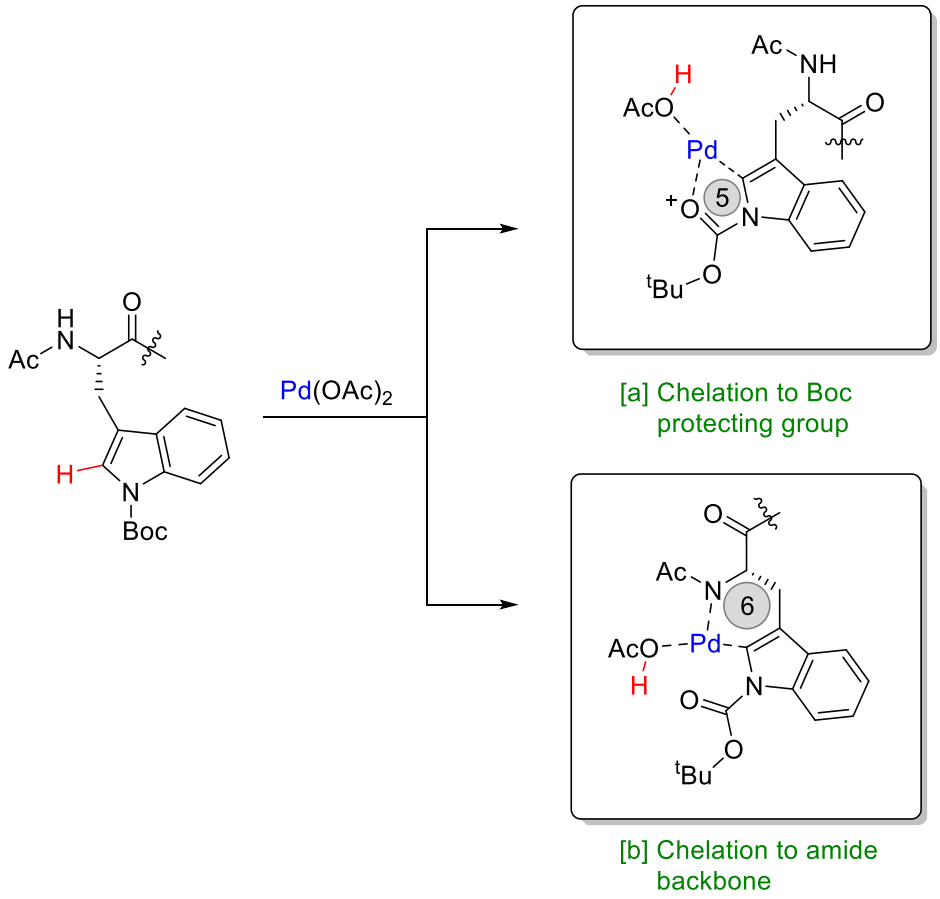
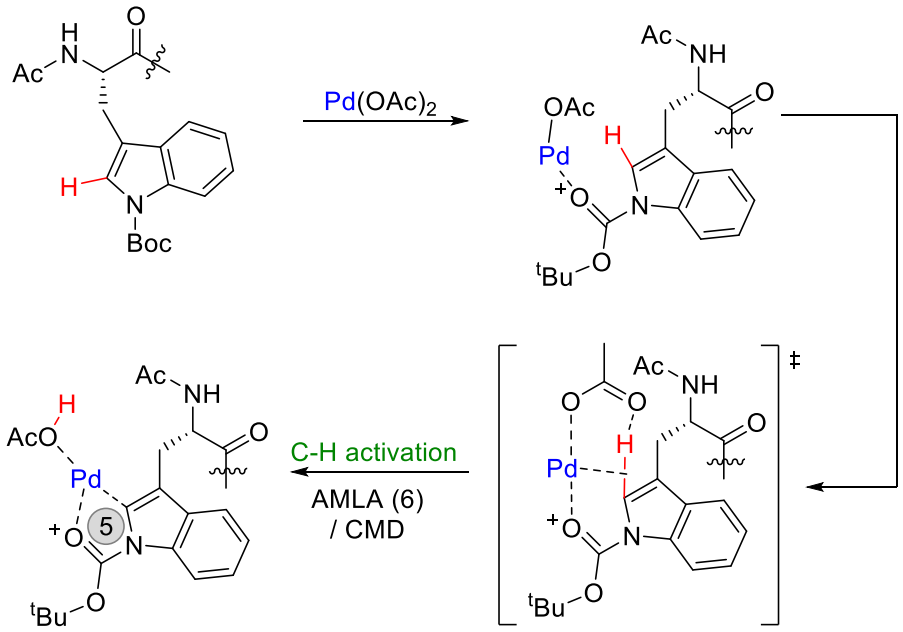


Figure 15: *Proposed chelation of tryptophan residues.*

In **Figure 15**, pathway A shows coordination of the metal through the carbamate amide, forming a 5 membered metallocycle. Pathway B depicts the chelation of the metal to the amide backbone, forming a six-membered ring. Coordination by pathway B is presumably higher in energy than coordination in pathway A. Chelation through a single amide residue was suggested as unfavourable due to the high energy pathway, under the reaction conditions in the olefination of N-terminal phenylalanine residues, (**Section 2.2.3**). This is most likely also the case with Boc protected N-terminal tryptophan residues. It is therefore more likely that the C-H olefination of N-terminal tryptophan proceeds as shown in **Scheme 69**.

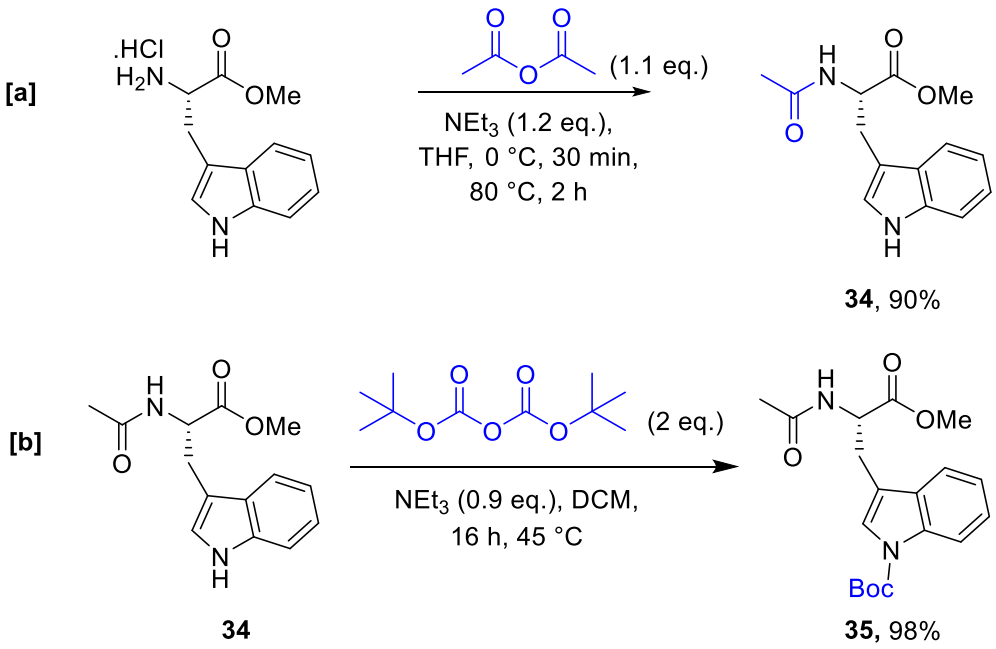


Scheme 69: *Proposed chelation of N-terminal tryptophan residues through the Boc protecting group.*

Scheme 69 displays the proposed C-H activation pathway, to give the monodentate metal-peptide complex. The protons upon the benzene ring of phenylalanine are less acidic than the proton present at the C-2 position of tryptophan. This presumably means there is less energy required to remove the C-2 proton, allowing the reaction to be carried out at lower temperatures, via a monodentate directing group. Activation of the C-2 position of the indole moiety is easily achieved in this example. The C-2 proton is removed by the acetate ligand in an AMLA mechanism, resulting in the formation of a new metal-carbon bond and a 5-membered ring. The resulting metal complex is presumably lower in energy than the complex depicted in pathway B (**Figure 15**).

2.4.2 Modification of the amino acid tryptophan

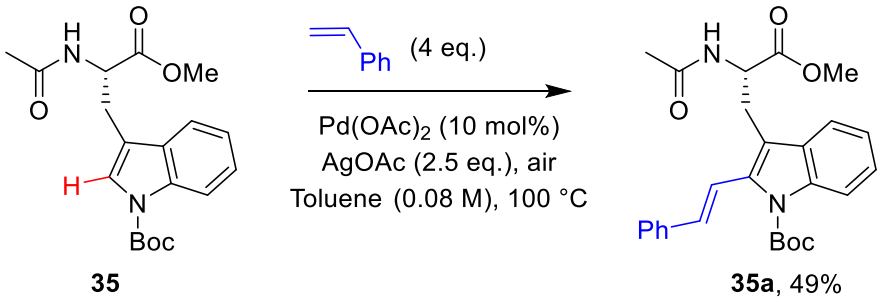
It appears that bidentate coordination is not necessary for the C-H functionalization of tryptophan residues as the Boc group is directing C-H activation. To test this theory, the C-H olefination of the native Boc protected amino acid would be observed if this is the case. Work began upon the synthesis of a di-protected tryptophan fragment, **Scheme 70**.



Scheme 70: *[a] Acylation of Trp-OMe to give compound **34**, and [b] Boc protection of the indole to give amino acid **35**.*

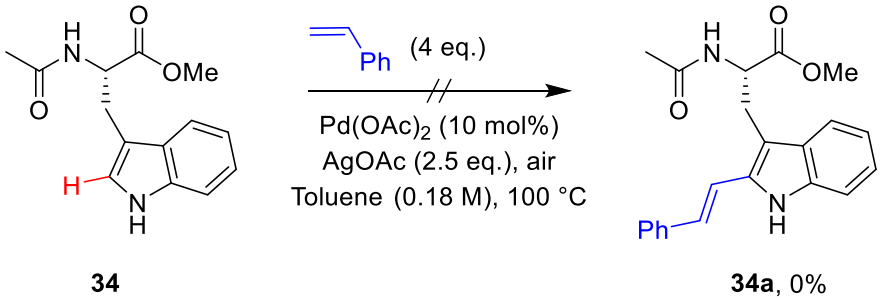
Starting from commercially available tryptophan methyl ester hydrochloride, the amine was protected using acetic anhydride to afford acetyl tryptophan methyl ester **(34)** in an excellent yield (90%). The protection was confirmed by the presence of a singlet at 1.95 ppm in the ^{1}H NMR spectrum, corresponding to the acetate group. The protected amino acid was then reacted with Boc anhydride to give the indole protected compound **35** as a white solid.

Compound **35** was then subjected to the olefination reaction conditions for 2 hours, **Scheme 71**. After purification by column chromatography, the mono olefinated amino acid was obtained in a 49% yield and identified by a characteristic doublet at 6.79 ppm with a coupling constant of 16.6 Hz, which corresponds to the formation of the *trans* alkene.



Scheme 71: *Olefination of Ac-Trp(Boc)-OMe (34) to afford modified amino acid **35a**.*

The olefination of the protected amino acid **35** further demonstrates that bidentate coordination is not necessary for the C-H functionalization of tryptophan, as the metal catalyst can only coordinate to a single directing group. To confirm the necessary indole protection, the free indole containing amino acid **34** was exposed to the C-H olefination conditions, **Scheme 72**.



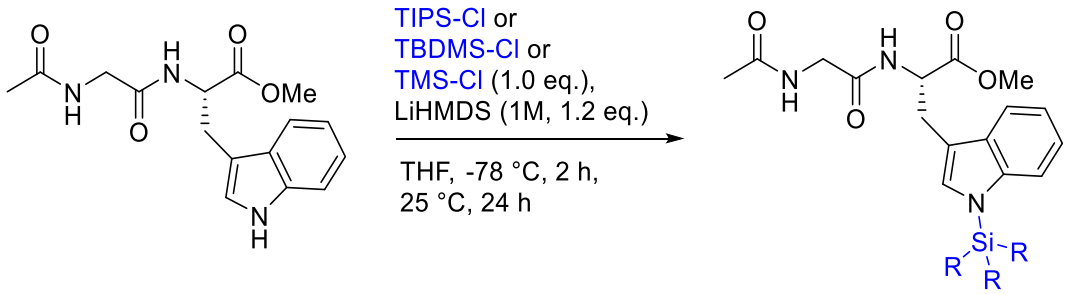
34 **34a**, 0%

Scheme 72: *Attempted olefination of Ac-Trp-OMe (**34**).*

Subjecting compound **34** to the C-H olefination conditions did not give modified amino acid **34a**, **Scheme 72**. The Boc-protecting group appears crucial for C-H olefination of tryptophan residues.

2.4.3 Olefination of silyl protected tryptophan residues

Next, investigations looked at changing the indole protecting group on the tryptophan residue. Silyl protecting groups are used to protect not only amines, but also alcohols, thiols and indole to name but a few. A number of different silyl protecting groups were installed onto peptide **20**, as shown in **Scheme 73**.



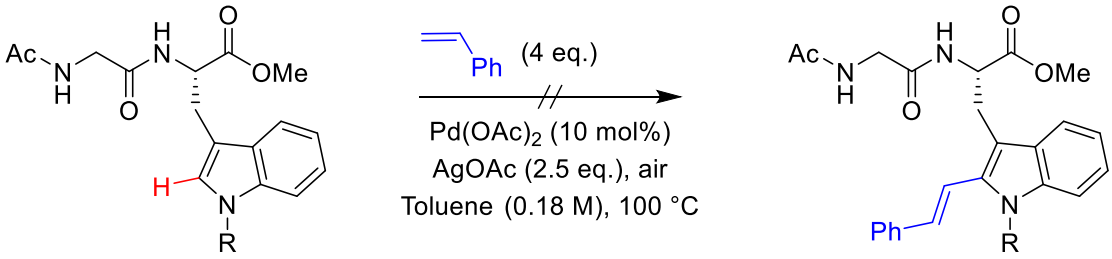
20

36a, R = TIPS, 67%
36b, R = TBDMS, 66%
36c, R = TMS, 0%

Scheme 73: *Installation of a silyl protecting group on model dipeptide **20**.*

Model peptide **20** was deprotonated using a 1M solution of LiHDMS under an inert environment before the addition of the silyl electrophile. After an aqueous work up and isolation by column chromatography, the silyl protected peptide was recovered in decent yields, **Scheme 73**.

The sterically bulky *tert*-butyl dimethyl silyl (TBDMS) and isopropyl silyl (TIPS) groups were installed successfully to give the corresponding peptides **36a** and **36b**. However, the much more labile trimethyl silyl (TMS) protected peptide **36c** was not recovered. The TMS group is much more susceptible to hydrolysis by acidic or basic media than the other silyl protecting groups and could have been removed during the workup of the crude material. The silyl protected tryptophan residues were then subjected to the olefination reaction, **Scheme 74**.

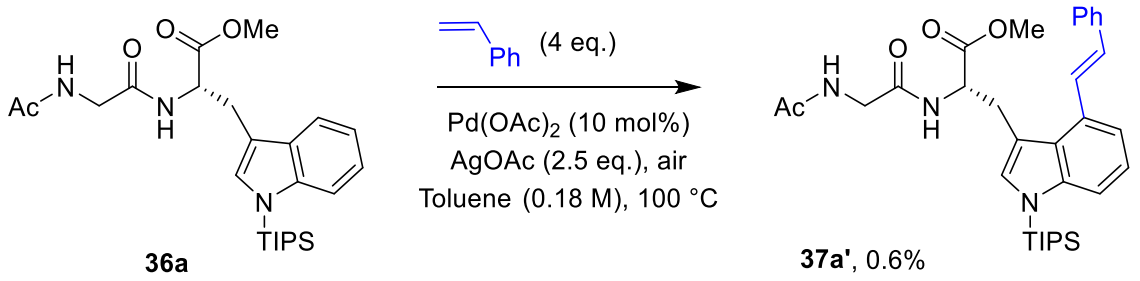


36a, R = TIPS
36b, R = TBDMS

37a, R = TIPS, 0%
37b, R = TBDMS, 0%

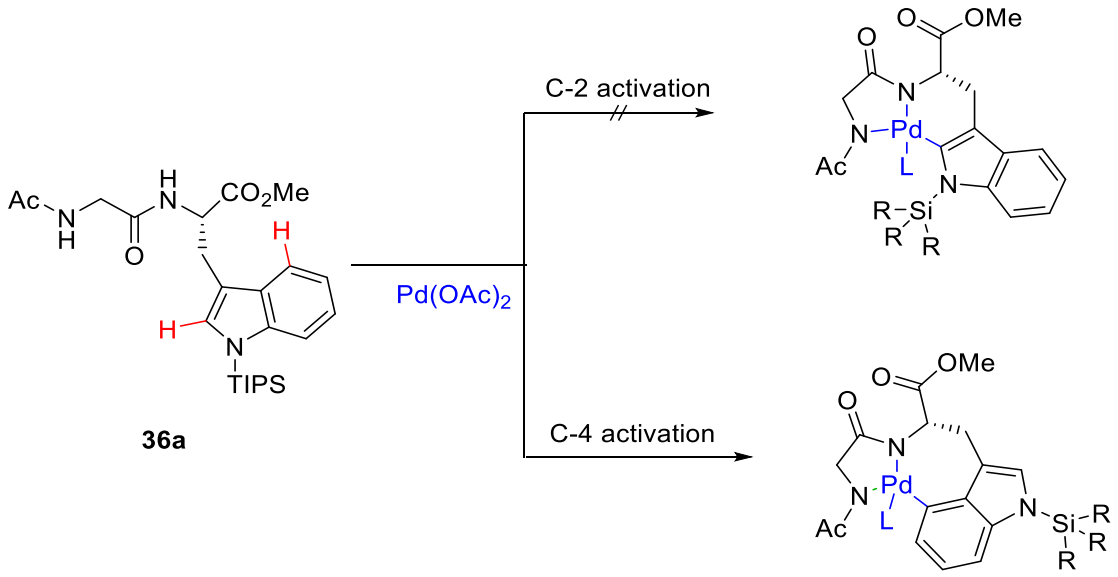
Scheme 74: *The olefination of silyl protected tryptophan containing peptides **37a** and **37b**.*

The olefination of the TIPS protected peptide **36a** was monitored by TLC. After 12 hours, a new spot was observed on the TLC plate, however, there was little evidence in the crude ^{1}H NMR to suggest that any the alkene had added. After purification by column chromatography, a trace amount (approximately 0.6%) of a colourless oil was recovered. The ^{1}H NMR spectrum of the oil showed the compound contained a pair of doublets at 7.08 ppm and 7.85 ppm with *J* values of 15.8 and 16.0 Hz respectively, which are characteristic of *trans* alkene. The proton at the C-2 position of the indole was observed due to the presence of a singlet at 7.07 ppm; likewise, the presence of a doublet at 7.58 ppm, corresponds to a proton at C-7, confirms the olefin has not been added at either of these potions. Olefination has occurred at the C-4 position and has been determined by ^{1}H NMR spectroscopy. The olefination of the TBDMS protected peptide **36a** was not observed however.



Scheme 75: *C-H olefination of TIPS protected tryptophan containing dipeptide to afford olefination at the C-4 position.*

The TIPS protecting group contains a bulky silicon atom, compared to that of the Boc protected peptide **22.** The silicon atom is hindering the coordination the incoming metal, and C-H activation at the C-2 position is not formed due to the steric repulsion, **Scheme 76**. Palladium can however, chelate to the amide backbone, forming a five-, seven-metallacycle by covalently bonding to the C-4 carbon of the indole. The five, seven-system is not very stable as reflected in the low yield observed.

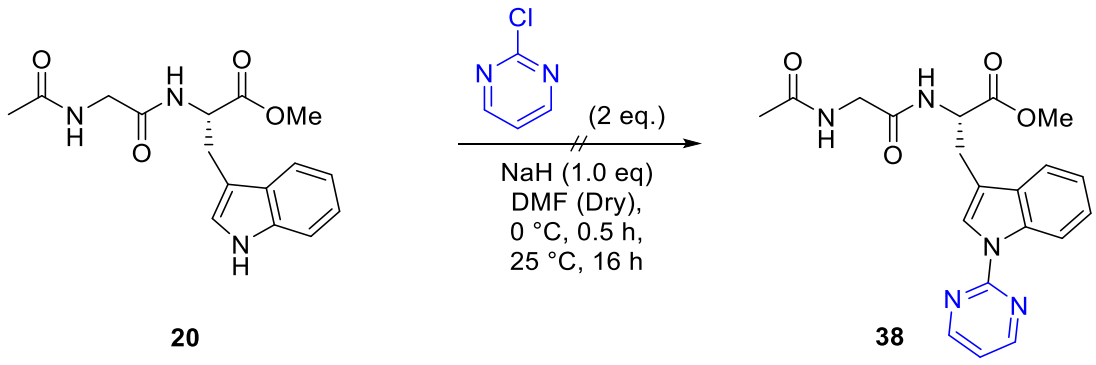


Scheme 76: *Coordination of palladium to the TIPS protected peptide* ***37a.***

2.4.4 Installation of a pyrimidine directing group to tryptophan residues

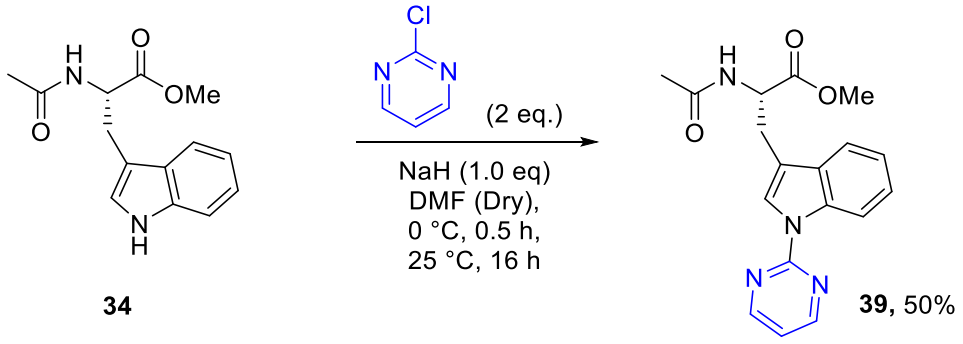
Next, attention turned to incorporating purpose built directing groups onto the peptide side chain. The pyrimidine-protecting group is used in literature as a directing group for indoles in C-H functionalisation reactions.[126–128] The installation of a pyrimidine directing group onto a dipeptide started with peptide **20**, **Scheme 77**.

Under an inert atmosphere, the aromatic N-H was deprotonated using sodium hydride at 0 ˚C, before the electrophile was added to the reaction. After an aqueous workup, the crude mixture was analysed by ^{1}H NMR spectroscopy, however there was no evidence to suggest the pyrimidine group had been installed upon the indole. Instead, there appeared to be fragmentation of the peptide itself from the reaction. Installing the protecting group onto a peptide post synthetically was not suitable in this transformation.



Scheme 77: *Attempted installation of pyrimidine onto peptide* ***(1a)*** *post synthetically.*

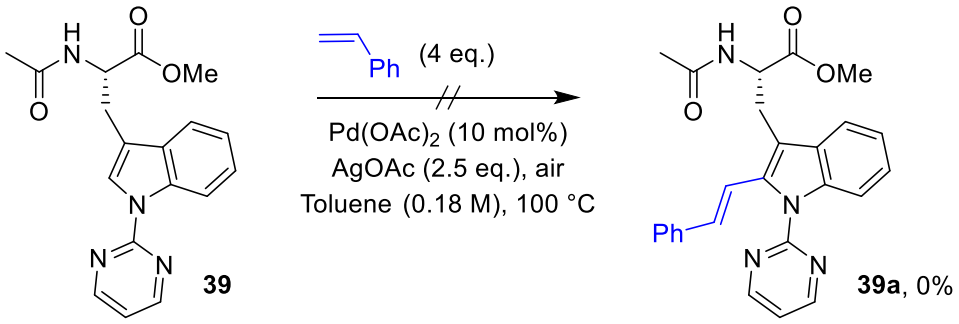
Efforts then changed to install the pyrimidine onto the tryptophan amino acid (**34**). Unnatural amino acid **39** was synthesised using the same conditions that had caused degradation of peptide **20**, **Scheme 78**. The crude material was purified by column chromatography, to give the desired product in a 50% yield. ^{1}H NMR spectroscopy was used to confirm the presence of the pyrimidine group.



Scheme 78: *Installation of the pyrimidine directing group onto amino acid* ***33.***

The unnatural amino acid **39,** was then subjected to the olefination conditions, that had given rise to the modified tryptophan residues. Initially the reaction was heated to 100 ˚C for 2 hours, however, there was no sign of the olefinated peptide, instead only the

unreacted amino acid was recovered. The reaction was then repeated, increasing the duration to 48 hours, but this again yielded only the amino acid starting material, **Scheme 79**.

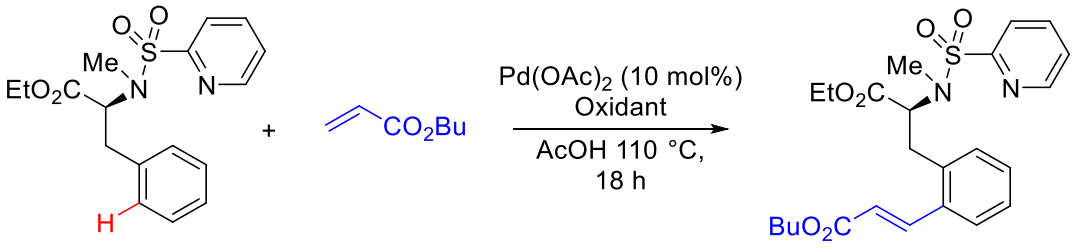


Scheme 79: *Attempted olefination of unnatural amino acid **40**.*

The desired modified amino acid **41**, was not observed in **Scheme 79**. The installed pyrimidine directing group appears to halt the olefination reaction all together. In this case, the installed directing group is not sterically bulky as was the case with the silyl protected tryptophan residues. The pyrimidine group may be poisoning the metal catalyst by irreversibly binding to the catalyst. The pyrimidine instead is not a suitable directing group for the olefination of tryptophan residues.

2.4.5 Installation of sulfonyl directing groups to tryptophan residues

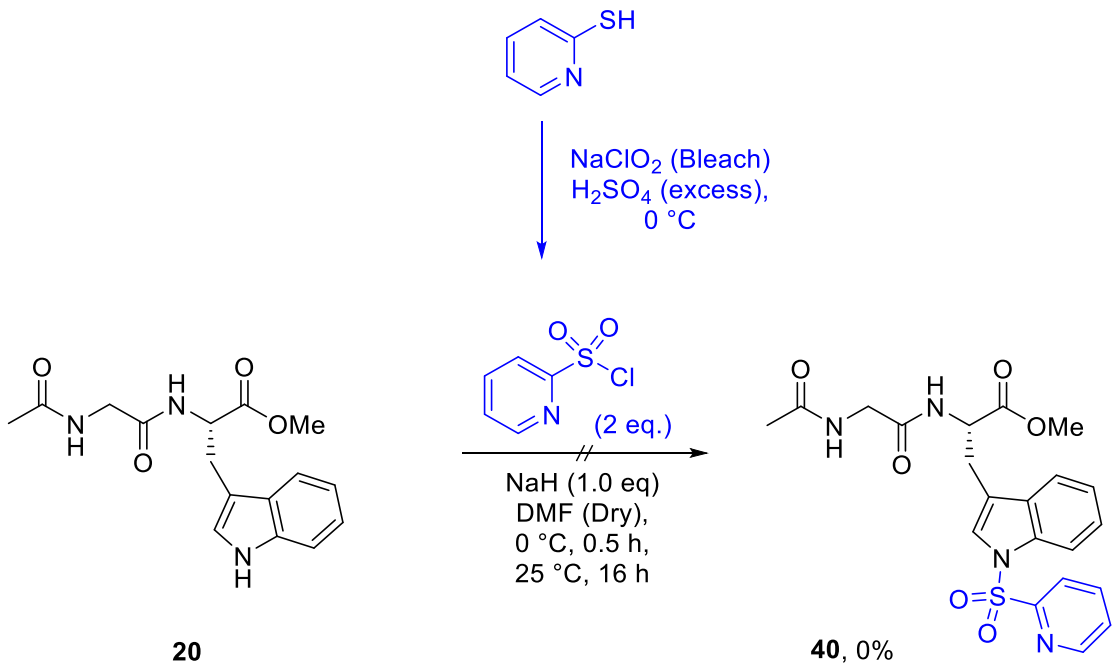
Next, the investigation into tryptophan directing groups turned towards sulfonyl directing groups. As described by García-Rubia and co-workes, the olefination of the phenylalanine amino acid residues has been shown using a sulfonyl directing group. [80]



Scheme 80: *The olefination of phenylalanine using a pre-installed pyridyl sulfone directing group.*[80]

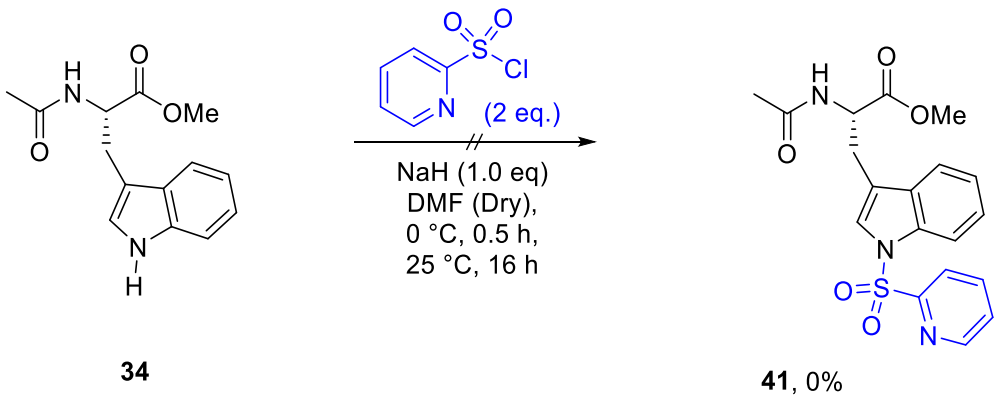
The first step of the reaction involved generating the electrophile, pyridine sulphonyl chloride by reacting mercaptopyridine with a hypochlorite solution. The electrophile is unstable at room temperature and decomposes to give the less reactive sulfonic acid

derivative. Due to the rapid decomposition of the electrophile, the bright yellow mobile oil that was formed was quickly added to peptide **20**, which had been dissolved in dry DMF and deprotonated using sodium hydride. After stirring for 16 hours at room temperature, the solution was worked up to afford a slimy brown oil. Analysis of the oil by ^{1}H NMR spectroscopy afforded a fragmented peptide and a pyridine-based derivative only. The peptide has been destroyed in the reaction.



Scheme 81: *Attempted synthesis of a pyridyl sulphonyl peptide.*

A new strategy was employed for the addition of the sulphonyl directing group. Instead of attempting to functionalise the peptide post synthetically, a second approach sought to functionalize the tryptophan amino acid first, before incorporating the modified tryptophan based amino acid into a peptide sequence. The sulphonyl chloride electrophile was generated as using the procedure outlined in **Scheme 81,** and used without further purification with the di-protected amino acid **34**, **Scheme 82.**



Scheme 82: *Attempted generation of a tryptophan-based sulphonyl pyridine compound.*

The installation of the sulfone directing group onto the protected amino acid was unsuccessful, affording the amino acid only, **Scheme 82**. A reason for the failure of the reaction could be during the preparation of the sulphonyl chloride. If the sulphonyl chloride is not generated before its addition to the amino acid mixture, the reaction would not produce the intended unnatural amino acid. During the synthesis of the electrophile, a small sample was taken for analysis by ^{1}H NMR spectroscopy. The spectrum produced clearly showed a transformation had taken place when compared to the pyridine-based starting material. However, the presence of the chloride or acid could not be confirmed by the ^{1}H NMR spectrum. The in-situ formation of the pyridyl sulfone was not clearly observed.

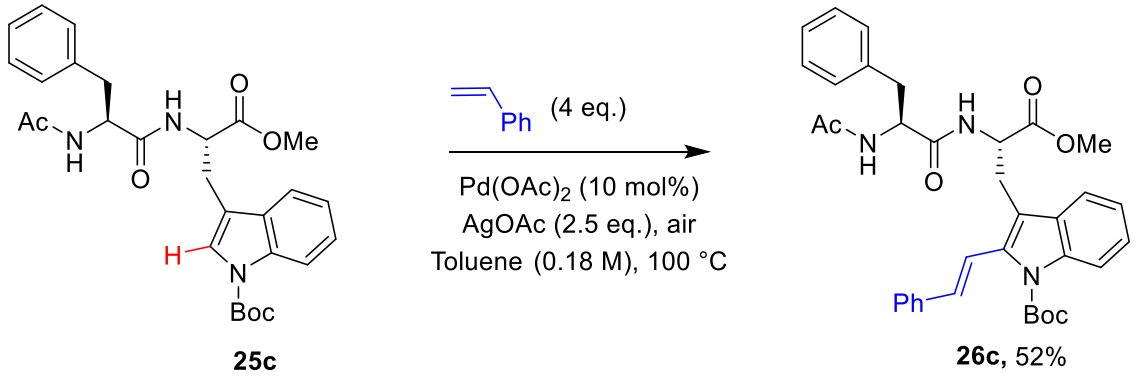
2.5 Olefination of peptides containing multiple aromatic amino acids

2.5.1 Introduction

Site-selective modification of amino acid side chains is a problem in many C-H functionalization reactions. The work undertaken in this study has developed a method for the C-H olefination of both phenylalanine and tryptophan residues in small peptides. The methodology developed for the modification of these aromatic amino acids is similar, making site-selective C-H olefination difficult. The aim was to investigate the C-H olefination reaction on peptides containing both tryptophan and phenylalanine residues.

2.5.2 Olefination of Ac-Trp(Boc)-Phe-OMe

As documented in **Section 2.1.6**, the C-H functionailzation of N-terminal phenylalanine residues is not observed under the developed methodology. With this in mind, the dipeptide Ac-Phe-Trp(Boc)-OMe, with an N-terminal phenylalanine was synthesised, and the subsequent olefination studied, **Scheme 83**.



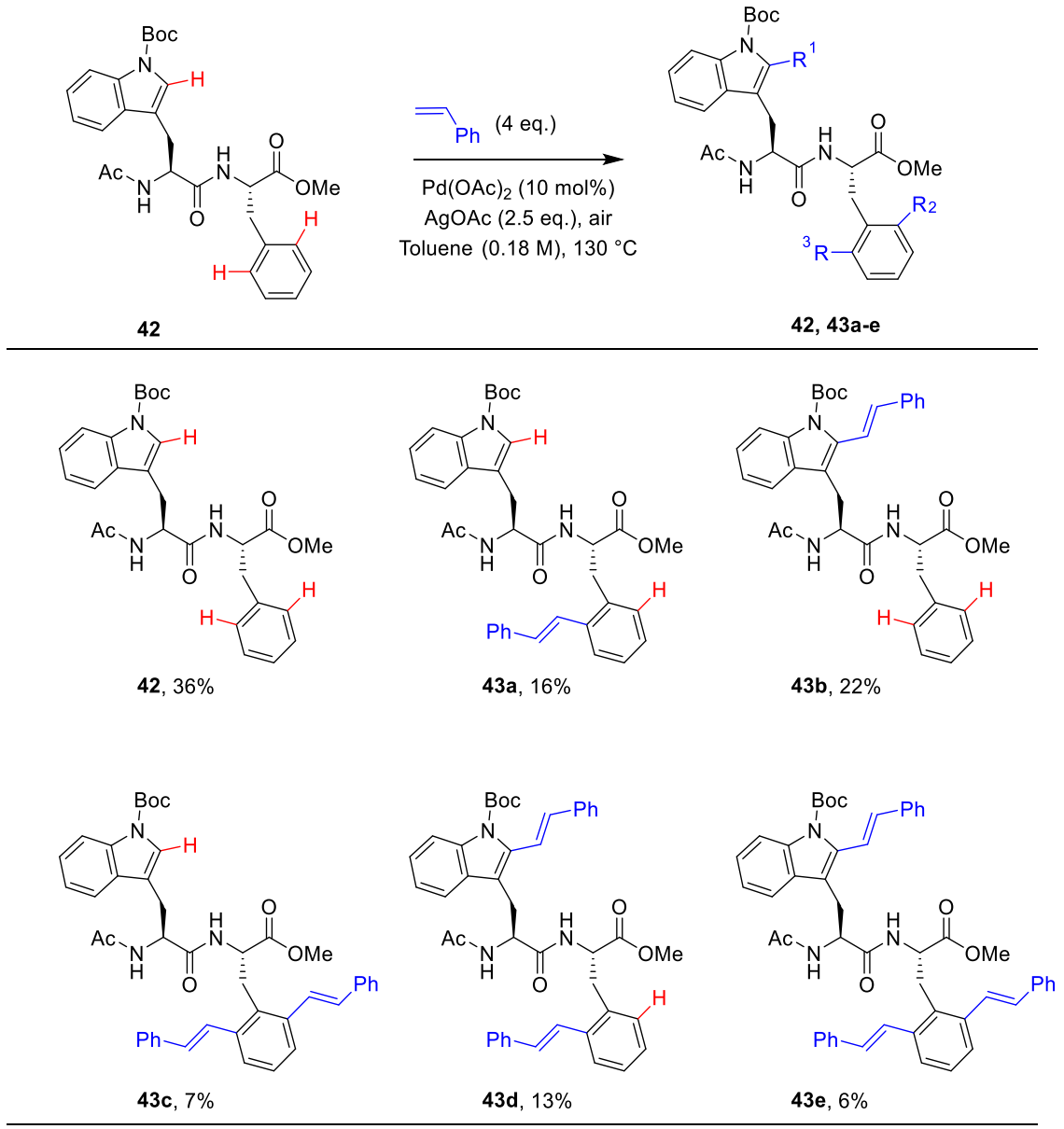
25c

26c, 52%

Scheme 83: *Olefination of Ac-Phe-Trp(Boc)-OMe (**25c**).*

As expected, the C-H olefination of peptide **25c** afforded the corresponding mono-olefinated tryptophan residue. The N-terminal phenylalanine residue was unaffected by the reaction. Site-selectivity had been exploited by positioning the phenylalanine residue at the N-terminus.

Next, the peptide sequence was altered so that the tryptophan residue was located at the N-terminus with phenylalanine at the C-terminus. It has already been established that N-terminal tryptophan residues are modified under the olefination conditions (**Section 2.4.1**). The dipeptide Ac-Trp(Boc)-Phe-OMe (**45**), was subjected to the olefination conditions developed for the C-H olefination of tryptophan residues, **Scheme 84**.

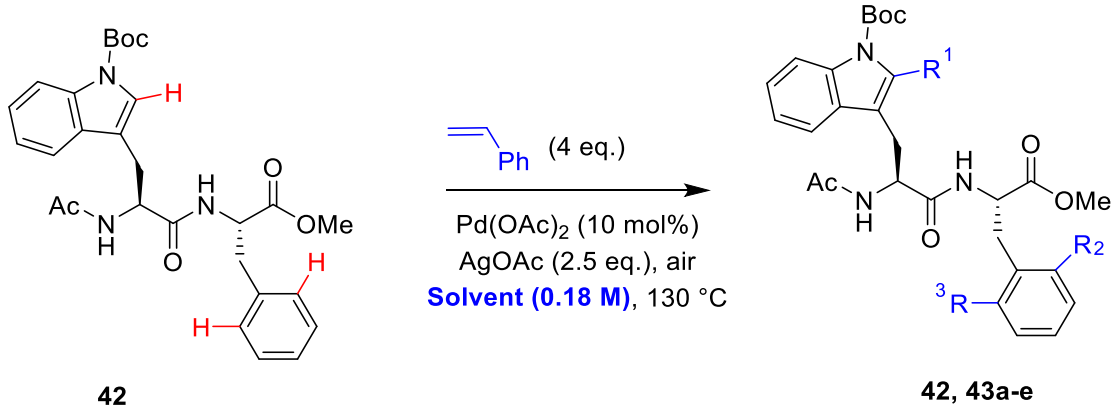


Scheme 84: *Olefination of Ac-Trp(Boc)-Phe-OMe under the optimised conditions for the C-H olefination of tryptophan residues.*

Analysis of the crude material from the olefination of Ac-Trp(Boc)-Phe-OMe was difficult as the crude ^{1}H NMR spectrum was extremely complicated at first glance. The crude compound was then purified by column chromatography to afford the 6 compounds as shown in **Scheme 84**, including the unmodified peptide **43**.

The olefination of dipeptide **43**, gave a mixture of modified products, with olefinations occurring on both tryptophan and phenylalanine residues. The olefination of dipeptide **42** was repeated to see if site-selective modification could be obtained by changing the solvent and duration of the reaction, **Table 4**.

Table 4**: Investigating the solvent used for the olefination of peptide **43.



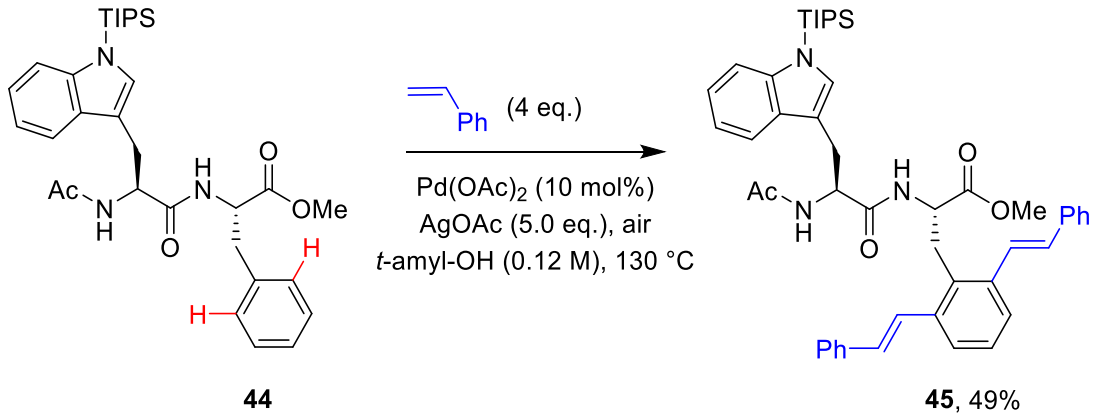
42 **42, 43a-e**

Entry	Time/h	Solvent	Peptide					
			45	46a	46b	46c	46d	46e
1	6	Toluene	36	16	22	7	13	6
2	24	Toluene	32	17	24	7	13	7
3	6	DCE	40	20	24	7	9	0
4	24	DCE	41	20	21	7	11	0
5	6	*t*-amyl-OH	42	20	11	14	6	7
6	24	*t*-amyl-OH	40	22	12	14	6	6
7	6	Toluene	36	18	19	10	13	4
8	6	Toluene	42	25	18	7	8	0
9	1	Toluene	30	20	21	7	15	7

Table 4, displays very little variation in the distribution of the olefinated products when changing the reaction solvent and duration of the reaction. When changing the solvent to *t*-amyl-OH which had been shown to be effective at the olefination of phenylalanine containing peptides, there was a small increase in the yield of peptides **43a**, and **43c** in which only the phenylalanine residue had been modified (**entries 5** and **6**). Overall however, the selective olefination of peptide **42** was not observed.

2.5.3 Selective phenylalanine modification in the presence of tryptophan residues

In **Section 2.4.3**, the modification of tryptophan residues bearing silyl protecting groups was not observed. It was theorised that to achieve selective phenylalanine modification in tryptophan containing peptides, that the tryptophan residue must be silyl protected. The TIPS protected peptide **44** was synthesised using the conditions in **Scheme 73**, **Section 2.4.3**. The silyl protected peptide was then subjected to the olefination conditions as shown in **Scheme 85**.



44 **45**, 49%

Scheme 85: *C-H olefination of Ac-Trp(TIPS)-Phe-OMe* ***44.***

The reaction of Ac-Trp(TIPS)-Phe-OMe was performed using the optimised conditions for the C-H olefination of phenylalanine residues using t-amyl-OH as the reaction solvent. As expected, the olefination of peptide **45** afforded the corresponding phenylalanine modified peptide **48** only. The olefination of tryptophan was not observed.

3. Conclusion

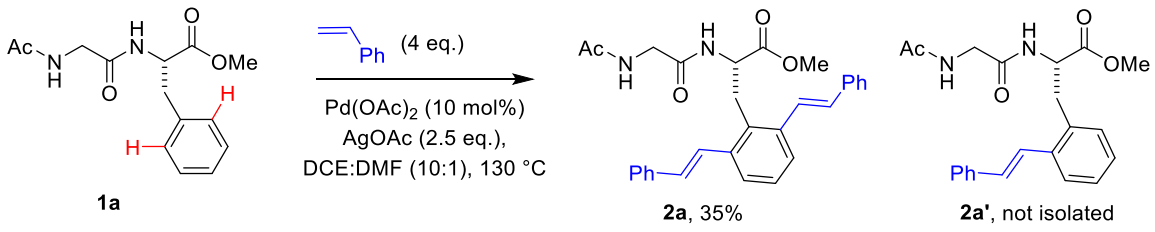
Peptides are playing an ever-increasing role in the pharmaceutical industry; with applications as therapeutics and in the imaging of diseases for example. Peptide modification leads to the generation of more varied libraries of peptides, which have different physical and chemical proprieties compared to their unmodified counterparts. C-H functionalization is an attractive method for the modification of peptides, as this methodology does not necessarily rely on the use of pre-functionalized materials for transformations. Natural amino acids can be transformed using C-H functionalization to produce complex peptides in a single step.

The aim of this investigation was to develop new methodology for the C-H functionalization of peptides, and therefore increase the number of modification strategies for chemists. The work documented in this thesis describes the development of a new C-H functionalization method for the direct modification of phenylalanine and tryptophan residues within peptides.

3.1 Olefination of phenylalanine and tryptophan residues in peptides

The aromatic amino acids phenylalanine and tryptophan were identified as potential targets for modification. As described in **Section 1.4.2,** the direct modification of phenylalanine residues by C-H functionalization has been under explored, despite the relative abundance of phenylalanine residues in natural peptides. The modification of tryptophan residues has been explored in the literature, but the study is still in its infancy. Developing new methodologies for the direct modification of amino acids within peptides sequences remains an area of great importance to maximise the options for peptide modification.

Chapter 2 described the C-H olefination of the aromatic amino acids phenylalanine and tryptophan within a peptide sequence. Investigations started with the olefination of the model peptide Ac-Gly-Phe-OMe **1a**, which gave the mono-, and di-olefinated modified peptides **2a** and **2a'**, **Scheme 86**.



Scheme 86: *Palladium catalysed C-H olefination of model dipeptide* ***1a****.*

Optimising the olefination reaction conditions afford the di-olefinated product **2a** as the major species every time, with dwindling returns of the mono-olefinated peptide **2a'**. Crucially it was found that changing the solvent to *t*-amyl-OH gave the di-olefinated product **2a** in a yield of 81%. The methodology developed for the C-H olefination of phenylalanine residues was extended to peptides of greater chain lengths, different neighbouring amino acids and different styrenes, **Figure 16**.

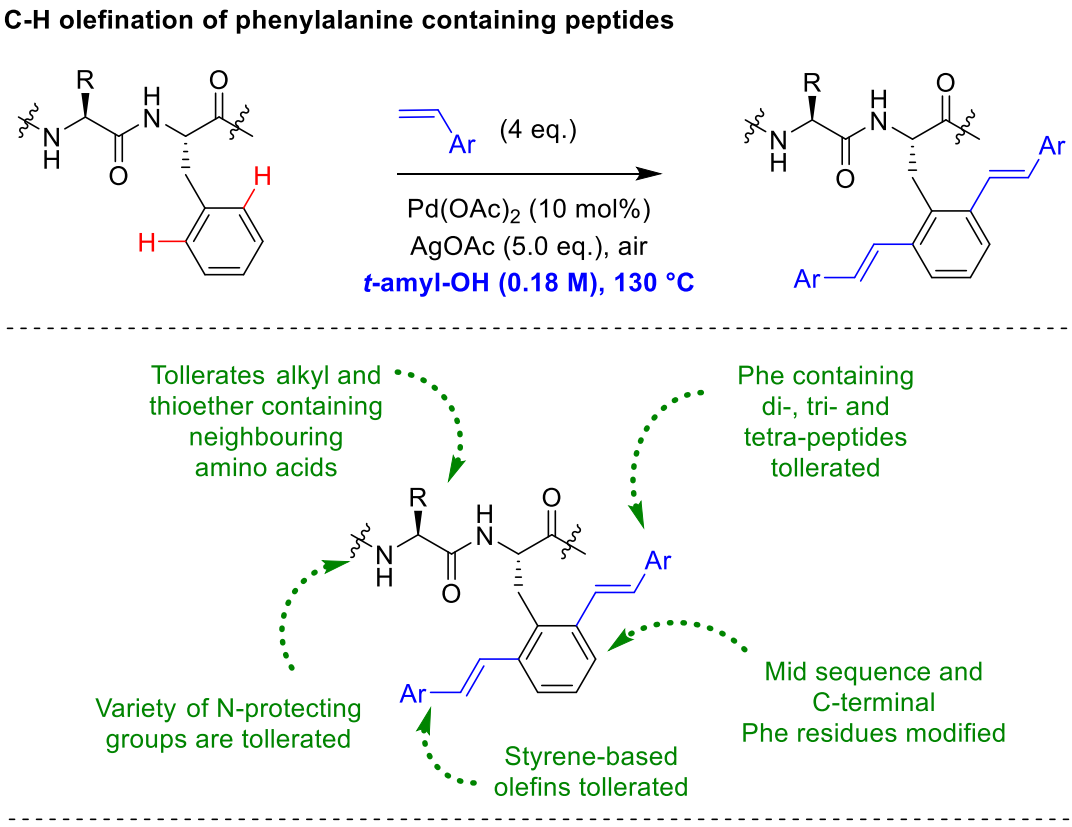


Figure 16: *Summary of the C-H olefination of phenylalanine containing peptides.*

Section 2.3 detailed the application of the methodology for the C-H olefination of phenylalanine to peptides bearing tryptophan side chains. Initial attempts to olefinate tryptophan containing peptides were problematic due to the indole N-H present in tryptophan. The C-H olefination of tryptophan was achieved by first Boc protecting the indole and then applying the olefination methodology developed of the modification of phenylalanine residues. The model dipeptide Ac-Gly-Trp(Boc)-OMe was olefinated to give the mono-olefinated peptide in a 60% yield. In contrast to the studies conducted on the phenylalanine containing model peptide where *t*-amyl-OH had given the biggest

yield of olefinated material, toluene was found to be a more suitable solvent, giving an 82% yield.

The methodology developed for the C-H olefination of tryptophan residues was extended to di- and tripeptides containing a number of different neighbouring amino acids and different styrenes, **Figure 17**. The Boc protecting group appears crucial for C-H functionalization.

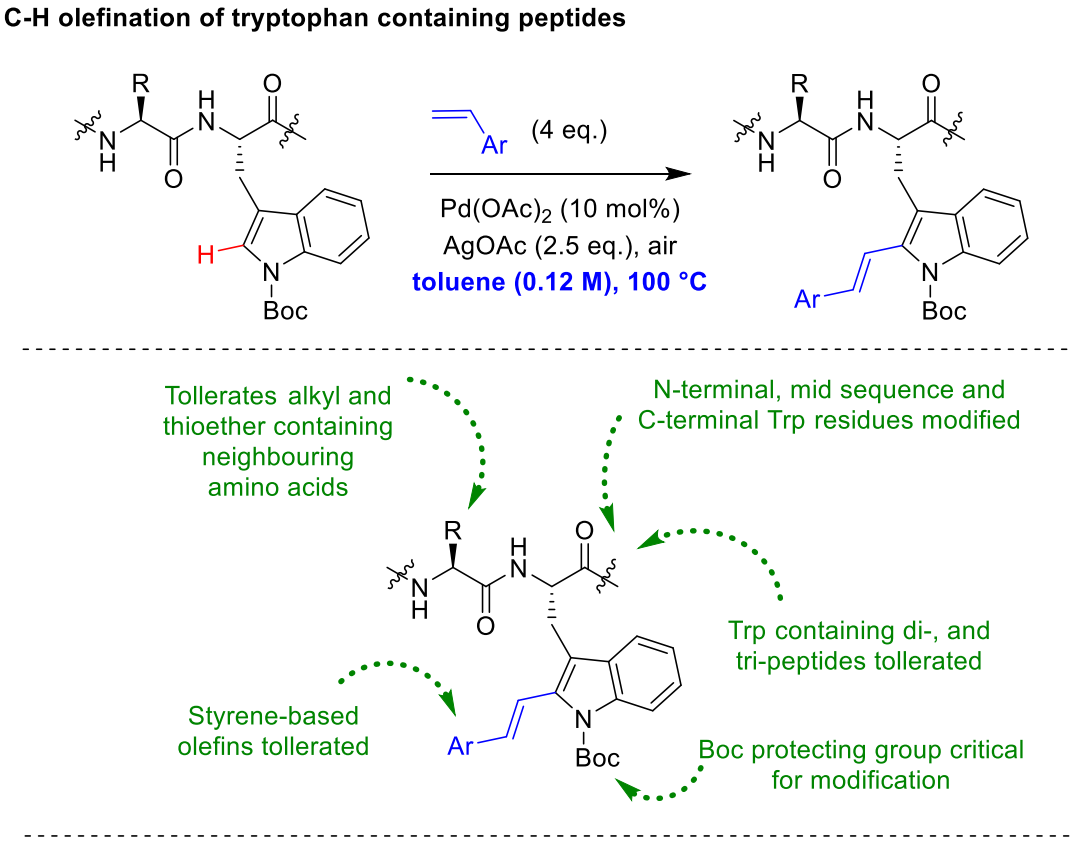


Figure 17: *Summary of the C-H olefination of tryptophan containing peptides.*

Greater differences between the two developed C-H olefination strategies were observed when the position of the aromatic amino acid was changed within a peptide sequence. N-terminal modifications were not observed when a phenylalanine residue was located at the N-terminus, yet tryptophan residues when located at the N-terminus, were modified using the appropriate reaction conditions. The observed differences in site reactivity could be explained due to the coordination of the peptide to the metal, **Figure 18**.

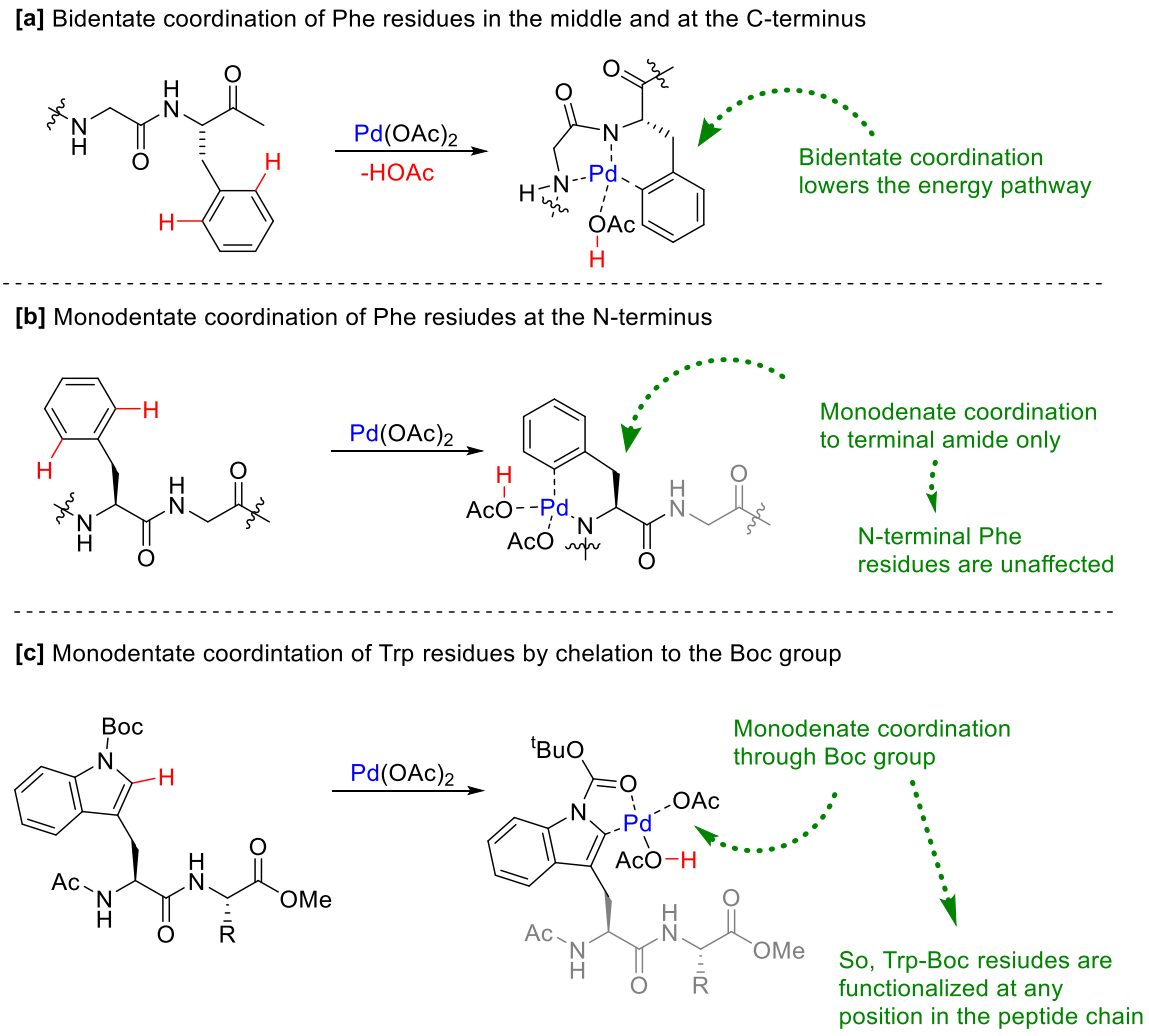
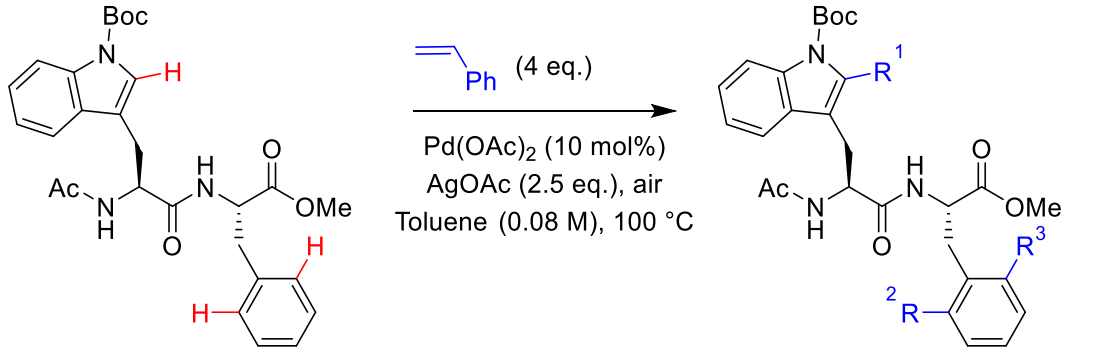


Figure 18: *a) Bidentate coordination of Phe residues in the middle and at the C-terminus of peptide, b) Monodentate coordination of Phe residues at the N-terminus and c) monodentate coordination of Trp residues to the Boc directing group.*

Bidentate coordination of the peptide backbone to the metal centre appears crucial for the C-H activation of phenylalanine residues within peptides. The olefination of phenylalanine containing peptides relies upon bidentate coordination of the metal for the reaction to proceed. N-terminal phenylalanine residues are unaffected by the olefination methodology (Pathway B, **Figure 18**). In contrast, the modification of N-terminal tryptophan residues suggests that bidentate coordination is no longer crucial. Instead, the Boc protecting group is acting as a directing group, and allows for monodentate coordination to the metal centre.

The final investigations looked at applying the olefination conditions to peptides containing both phenylalanine and tryptophan residues. When the olefination reaction

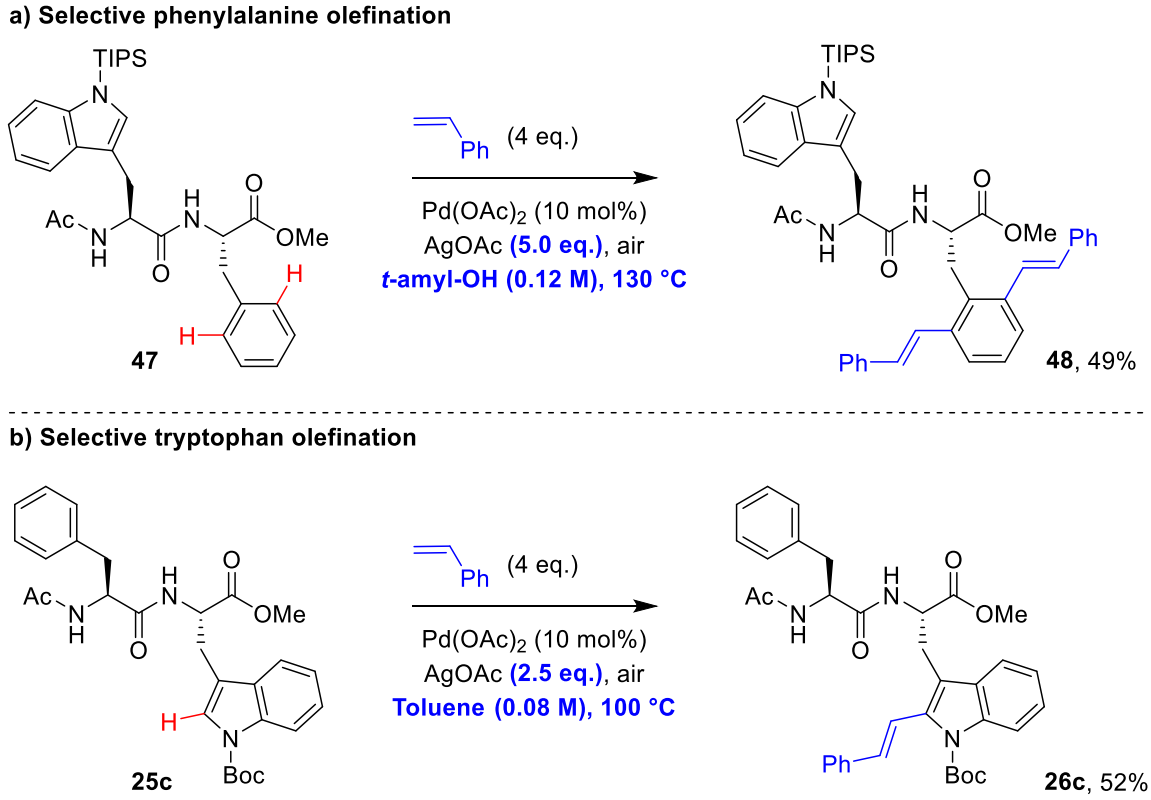
was applied to the model peptide **45**, the reaction gave a mixture of olefinated products with no apparent selectivity, **Scheme 87**.



45 **46a-e** (Mixture of products)

Scheme 87: *Olefination of di-aromatic peptide 45.*

Selective modification of the phenylalanine residue was achieved by removing the Boc group from the tryptophan residue and replacing it with a silyl protecting group. Likewise, positioning the phenylalanine residue to the N-terminus of the sequence afforded modification of the tryptophan residue exclusively, **Scheme 88**.



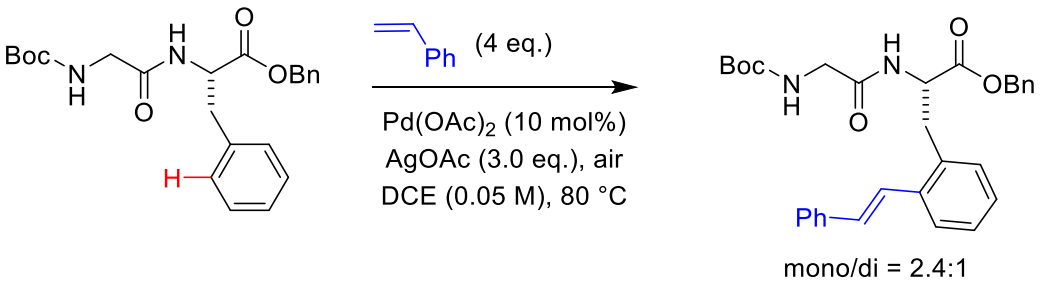
Scheme 88: *Selective olefination of a) phenylalanine and b) tryptophan residues in model dipeptides **47** and **25c**.*

In summary, the C-H olefination of the aromatic residues phenylalanine and tryptophan were successfully modified within a peptide sequence. The methodology developed for the modification of each aromatic residue was found to tolerate peptides bearing different neighbouring amino acids, peptide chain lengths and olefins. Coordination of the metal to the peptide backbone in the olefination phenylalanine appears to be crucial for the success of the reaction. However, the use of a proximal directing group upon the indole nitrogen allows tryptophan residues to be modified through monodentate coordination. Finally, the modification of peptides bearing multiple aromatic residues were investigated. Selective olefination of tryptophan was achieved when placing the phenylalanine residue at the N-terminus, and selective tryptophan olefination was achieved by installing a bulky silyl protecting group upon the indole nitrogen.

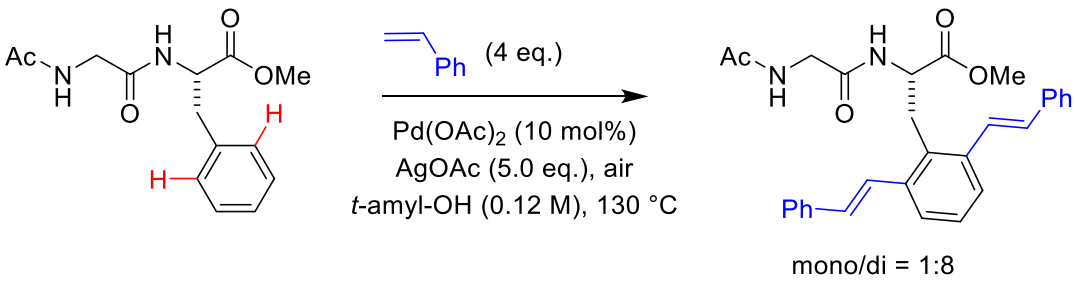
3.2 Current literature

During the writing of this thesis, the first reported direct modification of phenylalanine residues was described by Wang *et al*.[129] In this study, Wang reported the olefination of phenylalanine on di-, and tripeptides, to give the corresponding mono-olefinated peptides, **Scheme 89**.

a) C-H olefination of phenyalanine by Wang *et al.* to give mono-olefinated peptides

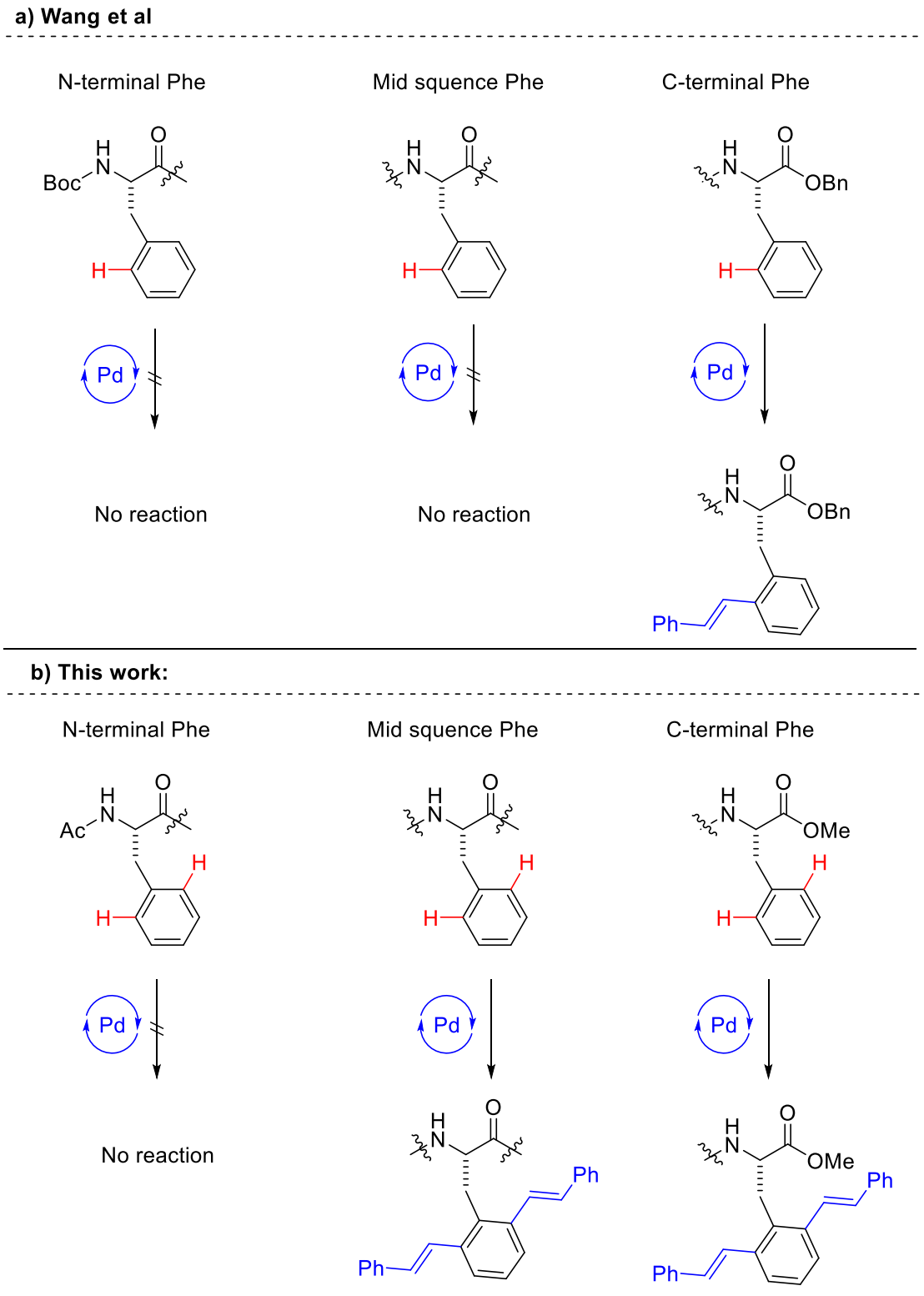


b) This work: C-H olefination of phenyalanine to give di-olefinated peptides



Scheme 89: *The olefination of phenylalanine containing peptides by a) Wang et al.*[129] *and b) this work.*

The methodology developed in this thesis, affords the di-olefinated peptide in higher yields than the mono-olefinated peptide. As described in **Section 1.2.3**, Wang also proposed that coordination of the metal to the peptide backbone is crucial for the C-H activation of phenylalanine residues. Similarly, Wang also found that changing the position of the phenylalanine residue was crucial to the reaction success. C-terminal phenylalanine residues were modified by the reported functionalization methodology and N-terminal phenylalanine residues remained unmodified. In contrast to this report, Wang's methodology when applied to peptides bearing mid sequence phenylalanine residues were unmodified by the reaction conditions. The findings in this thesis provide a route to the modification of phenylalanine residues within the middle of a peptide sequence. Wang reported that the mono-olefinated peptides were the major product of the olefination reaction, which contrasts with the findings of this thesis where the di-olefinated phenylalanine was obtained as the major product each time, **Scheme 90**.



Scheme 90: *The contrasting olefination of phenylalanine residues at the N- and C-termini and in the middle of a peptide sequence by Wang et al., and this work.*

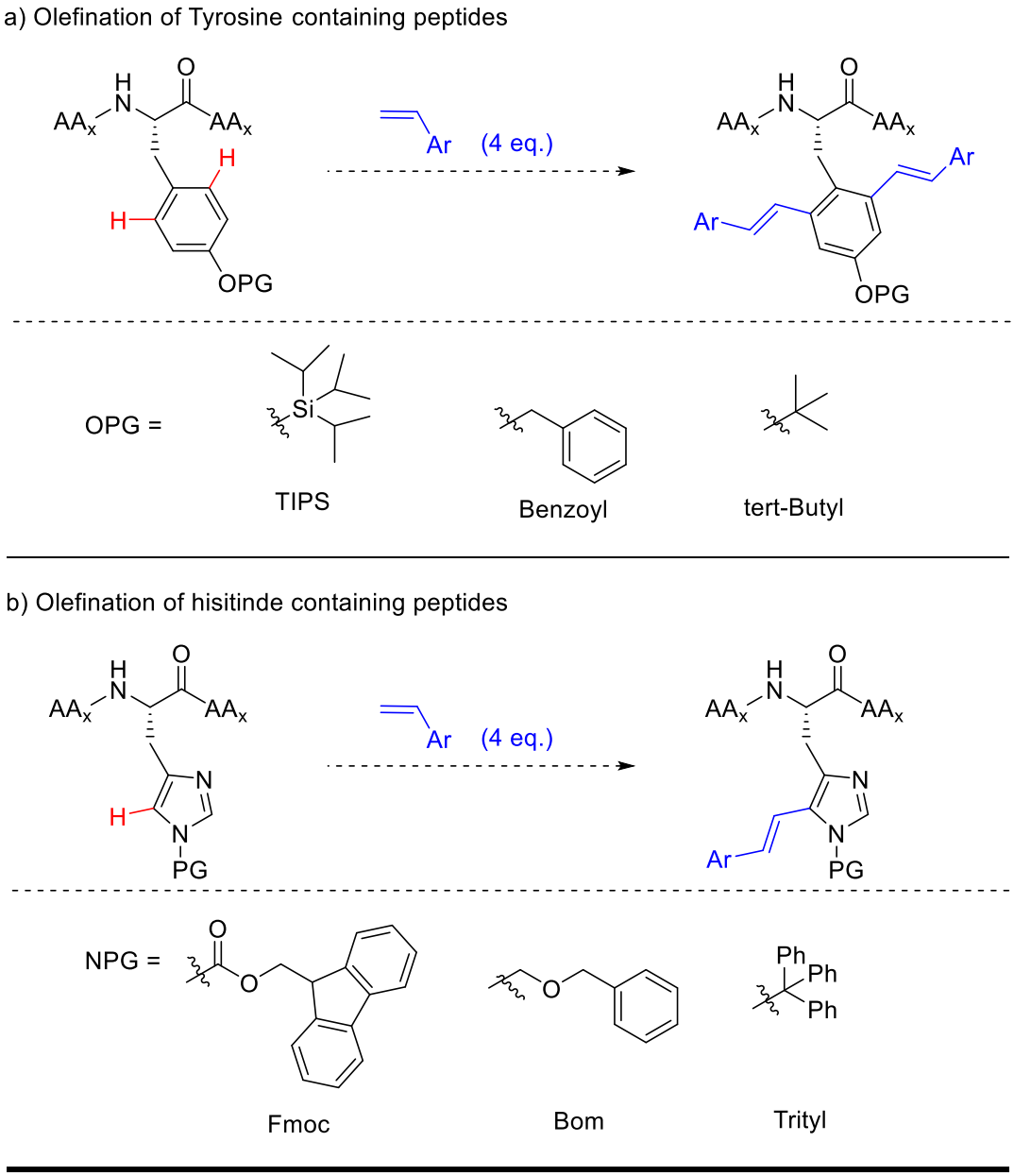
The reactions conditions in **Scheme 89** and **Scheme 90** are similar to the methodology in this report, yet the major products differ. Wang's method and the work presented here

offer two unique methods for the direct modification of phenylalanine residues in peptides. The work undertaken in this thesis is a complementary method for the modification of phenylalanine residues to afford the major product as the corresponding di-olefinated peptide.

3.3 Future work

3.3.1 Olefination of tyrosine and histidine containing peptides

The next steps in this investigation comprise of developing a strategy for the olefination of the aromatic amino acids tyrosine and histidine. Tyrosine is a phenolic derivative of phenylalanine. The methodology developed on the olefination of phenylalanine could then be directly applied to protected tyrosine analogues. As discussed in **Section 2.1.5**, the olefination of phenylalanine residues in the presence of the tyrosine OH did not occur. Protection of the phenol with a suitable protecting group such as benzyl or *tert*-butyl protecting groups may be crucial for C-H olefination, **Scheme 91**. When developing methodology for the olefination of histidine residues, the imidazole side chain may also need to be protected. Common protecting groups for histidine residues are shown in **Scheme 91**.



Scheme 91: *Olefination of a) tyrosine and b) histidine containing peptides.*

In conjunction with the work described in this thesis, the development of the olefination of tyrosine and histidine residues, would generate more methods for peptide modification. The application of this new methodology could lead to the functionalization of more complex peptide sequences, containing a greater variety of aromatic amino acids such as those displayed in **Figure 19**.

The methodology developed thus far in this thesis has been applied on peptides up to 4 amino acids in length. There are few examples in nature of natural peptides with small chain lengths. Chemotactic peptide is an example of a phenylalanine containing therapeutic tripeptide.[5] Pasireotide is a cyclic peptide prescribed for the treatment of

Cushing's disease.[130] The peptide Pasireotide contains 4 aromatic residues; tryptophan phenylalanine, phenylglycine and tyrosine. Each of the aromatic side chains could be modified using the C-H olefination procedure, apart from the phenylglycine unit (as described in **Section 2.2.4**). The modification of peptide therapeutics could lead to the development of more potent drugs for disease.

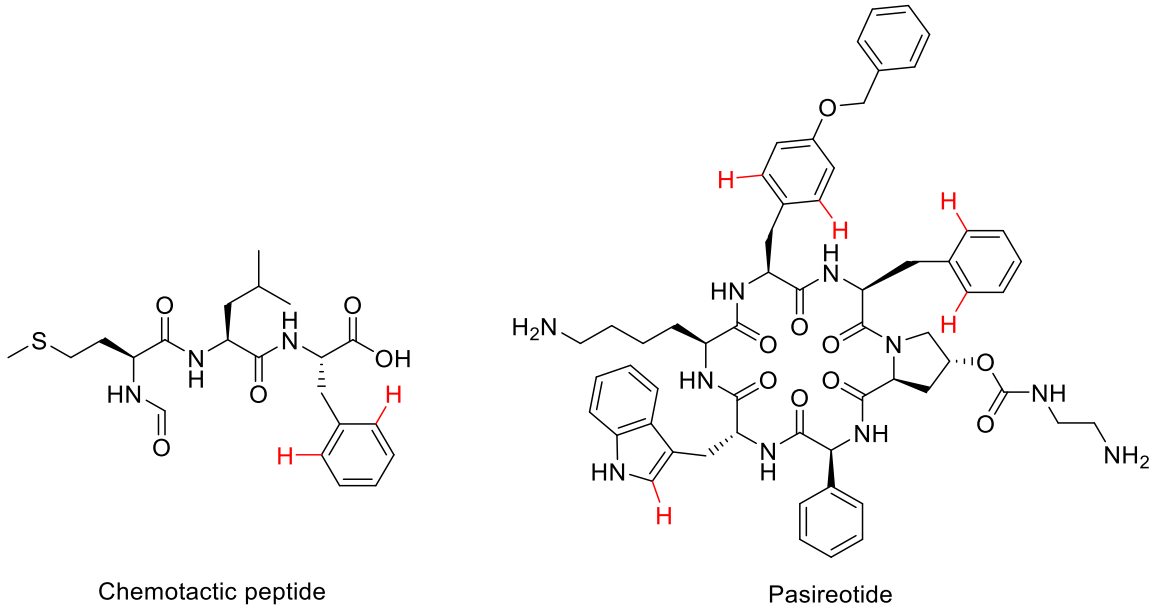


Figure 19: *Natural therapeutic peptides that could be modified by C-H olefination.*

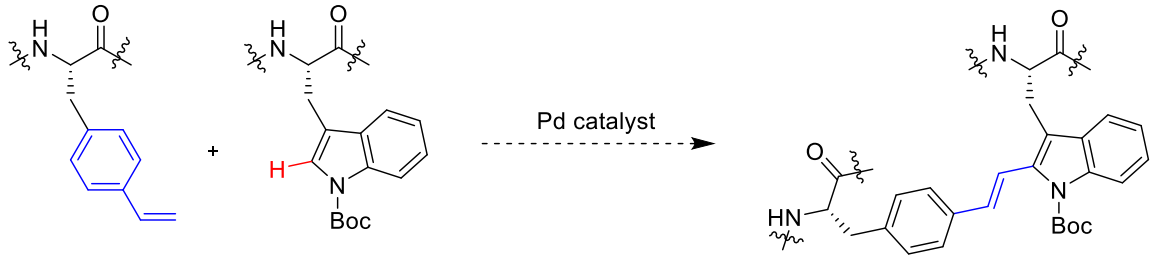
The therapeutics chemotactic peptide and Pasireotide[131] (**Figure 19**), could be modified using the olefination procedures described in this thesis. The modification of these "real world" peptides is an example of how the C-H olefination methodology gives chemists more tools for peptide modification to introduce varied amino acid side chains.

3.3.2 Generating conformational constraints in peptides by C-H olefination reactions

The intramolecular modification of peptide side chains leads to a change in peptide conformation and shape.[99,100] The development of methods to alter peptide conformation are of great importance to chemists in areas such as therapeutics. These changes could also induce changes to the photochemical properties of the peptide too.[69]

Conformational changes could be installed by using the C-H functionalization methodology in two ways. Peptides with non-natural vinylic side chains could be coupled to natural amino acids through C-H olefination. The olefin in question could be attached

to another peptide chain and when coupled by C-H olefination to an aromatic side chain, would form a new C-C bond between two peptide strands, **Scheme 92**.



Scheme 92: *Proposed C-C cross linking between two peptides by C-H olefination of a vinylic amino acid side chain and a tryptophan residue.*

Another transformation could use the C-H olefination procedure to install and olefin containing a secondary handle which would facilitate a second C-H functionalized transformation i.e. C-H arylation reaction.[100] The methods described here would produce new links between peptide sequences without the need for pre-functionalization. These cross linked peptides have been shown to exhibit an increase in biological activity making them suitable for applications such as therapeutics.[99,25]

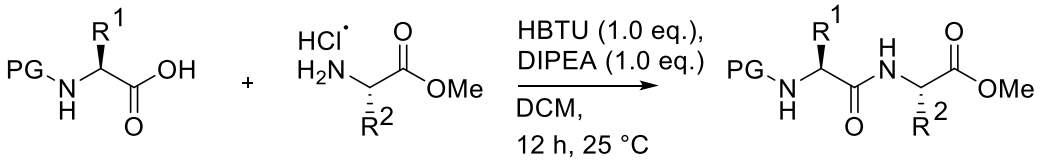
4. Experimental

4.1 General Experimental Information

All manipulations were performed in oven-dried glassware in an atmosphere of air unless stated. Ag_2CO_3 and AgOPiv were prepared according to literature procedures.[132,133] All other reagents and solvents were purchased from either Alfa Aesar, Fisher Scientific or Sigma Aldrich and used as supplied. Flash column chromatography was performed on silica gel (Fluorochem, silica gel 60 Å, particle size 40-63 µm). Thin layer chromatography was performed on glass-backed silica gel plates (2.5 x 7.5 cm; Merck, TLC silica gel 60 Å); compounds were visualised by exposure to UV light (254 nm) or using a permanganate stain. NMR spectra were recorded on a JEOL Eclipse 400 spectrometer at 298 K; chemical shifts are reported in parts per million and coupling constants are reported in Hz. For some compounds, assignments for ^{1}H and ^{13}C NMR peaks were aided by ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY and ^{1}H-^{13}C HMQC 2D NMR experiments. FTIR spectra were recorded in a diamond ATR cell using Perkin-Elmer Spectrum 2 instrument or an Aligent Technologies Cary 630 instrument. Melting points were recorded on a Stuart SMP10 melting point apparatus and are uncorrected. 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+).

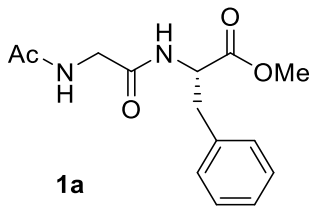
4.2 Synthesis of phenylalanine containing peptides

4.2.1 General procedure for the preparation of phenylalanine containing dipeptides



The L-(amino acid) methyl ester hydrochloride (2.50 mmol) and K_2CO_3 (0.498 g, 3.60 mmol) were dissolved in distilled water (30 mL) and stirred for 10 min at room temperature. The free amine was extracted with Et_2O (3 x 20 mL), dried ($MgSO_4$) and concentrated by rotary evaporation. The resulting colourless oil was dissolved in DCM (20 mL); the *N*-protected amino acid (1.00 mmol), HBTU (0.379 g, 1.00 mmol) and DIPEA (0.174 mL, 1.00 mmol) were then added to the reaction mixture, which was stirred for 12 h. The resulting suspension was filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and H_2O (20 mL). The organic layers were then dried ($MgSO_4$) and concentrated to dryness *in vacuo*. The resulting oil was recrystallised from DCM / hexanes.

4.2.2 Ac-Gly-Phe-OMe (1a)



1a

Peptide **1a** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetylglycine (0.117 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **1a** as a white solid (0.218 g, 78%); m.p. 95-97 °C.

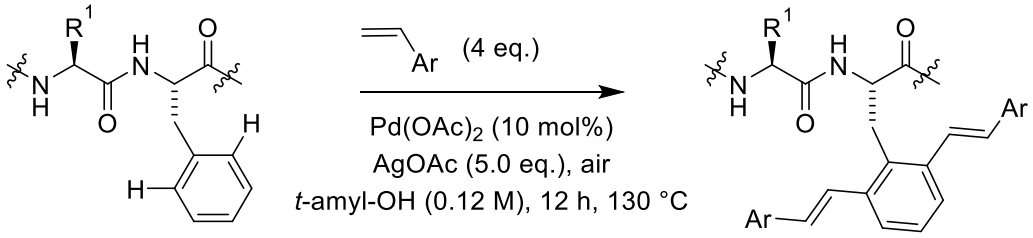
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (3H, s, Acetyl-C*H*$_3$), 3.07 (1H, dd, *J* = 13.9, *J* = 6.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.9, *J* = 6.0, Phe-C*H*H), 3.72 (3H, s, Ester-C*H*$_3$), 3.88 (2H, app dd, *J* = 16.5, *J* = 5.0, Gly-C*H*$_2$), 4.84 (1H, dt, *J* = 7.6, *J* = 6.0, Phe-α-C*H*), 6.33 (1H, m, Gly-N*H*), 6.57 (1H, br d, *J* = 7.6, Phe-N*H*), 7.08 (2H, d, *J* = 6.4, Ar-*H*) 7.23-7.30 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.0 (Acetyl-CH_3), 37.9 (Phe-CH_2), 43.2 (Gly-CH_2), 52.6 (Ester-CH_3), 53.3 (Phe-α-CH), 127.3 (Ar-C), 128.7 (Ar-C), 129.3 (Ar-C), 135.7 (Ar-C), 168.6 (C=O), 170.7 (C=O), 171.8 (C=O).

IR U_{max}/cm^{-1} (solid) 3263 m (N-H), 3072 s (C-H), 2958 s (C-H), 1751 s (Ester C=O), 1709 s (Amide C=O), 1657 m (C=C), 1445 m (C-H).

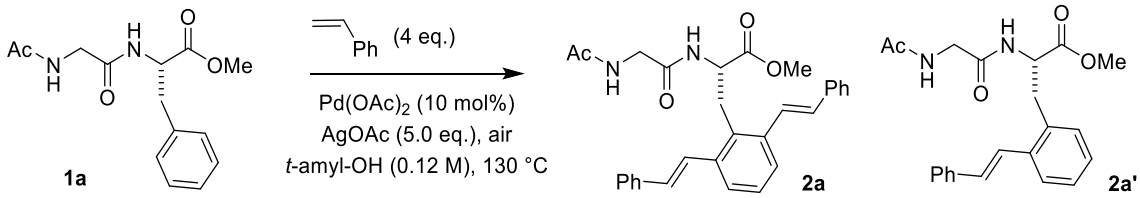
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{14}H_{19}N_2O_4$: 279.1339, found: 279.1339.

4.3 General procedure for the olefination of phenylalanine containing peptides



Peptide (0.359 mmol), $Pd(OAc)_2$ (8 mg, 0.036 mmol, 10 mol%), AgOAc (0.300 g, 1.780 mmol) and styrene (0.166 mL, 1.440 mmol) were stirred together in *t*-amyl-OH (3 mL) at 130 °C for 12 h. The reaction mixture was then allowed to cool to room temperature and filtered through a plug of Celite. The filtrate was then concentrated to dryness. The resulting residue was purified by flash column chromatography and recrystallised from DCM / hexanes.

4.3.1 C-H olefination of model dipeptide 1a; synthesis of modified peptides 2a and 2a'



Following the general procedure in **Section 4.3**, the reaction of Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol), styrene (0.166 mL, 1.440 mmol), $Pd(OAc)_2$ (8 mg, 0.036 mmol, 10 mol%) and AgOAc gave a crude product that was a mixture of the di-olefinated peptide **2a** and the mono-olefinated peptide **2a'** in a ratio of 8:1, as judged by ^{1}H NMR spectroscopy. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **2a** as an off-white solid (0.140 g, 81%); m.p. 198-199 °C, R_f 0.33 (EtOAc), and **2a'** as an off-white solid (0.011 g, 8%); m.p. 129-132 °C, R_f 0.15 (EtOAc).

Data for 2a:

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (3H, s, Acetyl-C*H*$_3$), 3.49 (2H, d, *J* = 7.0, Phe-C*H*$_2$), 3.55 (3H, s, Ester-C*H*$_3$), 3.70 (1H, dd, *J* = 16.9, *J* = 5.0, Gly-C*H*H), 3.79 (1H, dd, *J* = 16.9, *J* = 5.0, Gly-C*H*H), 4.78 (1H, dt, *J* = 7.4, *J* = 6.9, Phe-α-C*H*), 5.82 (1H, m, Gly-N*H*), 6.40 (1H, br d, *J* = 7.7, Phe-N*H*), 7.01 (2H, d, *J* = 16.0, Alkene-C*H*), 7.27-7.33 (3H, m, Ar-*H*), 7.39 (4H, t, *J* = 7.8, Ar-*H*), 7.47 (2H, d, *J* = 16.0, Alkene-C*H*), 7.58 (6H, d, *J* = 7.8, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 31.0 (Phe-CH_2), 42.8 (Gly-CH_2), 52.7 (Ester-CH_3), 53.0 (Phe-α-CH), 125.8 (Ar-C), 126.2 (Alkene-C=C), 126.7 (Ar-C), 127.7 (Ar-C), 128.0 (Ar-C), 128.8 (Ar-C), 131.6 (Ar-C), 131.8 (Alkene-C=C), 137.1 (Ar-C), 137.8 (Ar-C), 168.2 (C=O), 170.2 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3300 m (N-H), 3057 s (C-H), 2954 s (C-H), 1727 s (Ester C=O), 1632 s (Amide C=O), 1535 m (C=C), 1436 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{31}N_2O_4$: 483.2278 found: 483.2275.

Data for 2a':

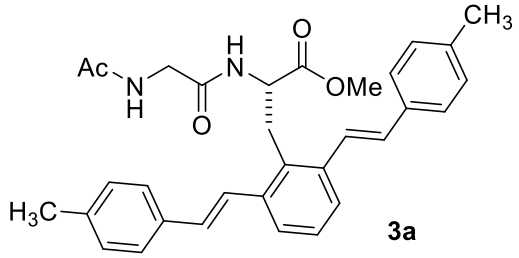
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.87 (3H, s, Acetyl-C*H*$_3$), 3.18 (1H, dd, *J* = 14.2, *J* = 6.2, Phe-C*H*H), 3.41 (1H, dd, *J* = 14.2, *J* = 6.2, Phe-C*H*H), 3.65 (3H, s, Ester-C*H*$_3$), 3.68 (1H, m, Gly-C*H*H), 3.85 (1H, dd, *J* = 16.9, *J* = 5.3, Gly-C*H*H), 4.81 (1H, dt, *J* = 7.3, *J* = 6.2, Phe-α-C*H*), 6.34 (1H, t, *J* = 5.3, Gly-N*H*), 6.94 (1H, d, *J* = 7.3, Phe-N*H*), 7.01 (1H, d, *J* = 16.0, Alkene-C*H*), 7.07-7.11 (1H, m, Ar-*H*), 7.18 (1H, t, *J* = 7.3, Ar-*H*), 7.24-7.29 (2H, m, Ar-H), 7.35-7.40 (3H, m, Ar-*H* / Alkene-C*H*), 7.54 (2H, d, *J* = 7.3, Ar-*H*), 7.63 (1H, d, *J* = 7.3, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.7 (Acetyl-CH_3), 34.8 (Phe-CH_2), 42.9 (Gly-CH_2), 52.4 (Ester-CH_3), 53.3 (Phe-α-CH), 125.4 (Ar-C),125.8 (Ar-C), 126.6 (Alkene-C=C), 127.1 (Ar-C), 127.6 (Ar-C), 127.8 (Ar-C), 128.5 (Ar-C), 128.7 (Ar-C), 130.5 (Ar-C), 133.7 (Alkene-C=C), 136.7 (Ar-C), 137.1 (Ar-C), 168.2 (C=O), 170.2 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3302 m (N-H), 3027 s (C-H), 2950 s (C-H), 1733 s (Ester C=O), 1636 s (Amide C=O), 1511 m (C=C), 1215 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{25}N_2O_4$: 381.1814, found: 381.1810.

4.3.2 Synthesis of modified peptide 3a



3a

Modified peptide **3a** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-methylstyrene (0.190 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3a** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3a** as an off-white solid (0.141 g, 77%); m.p. 239-242 °C, R_f 0.39 (EtOAc).

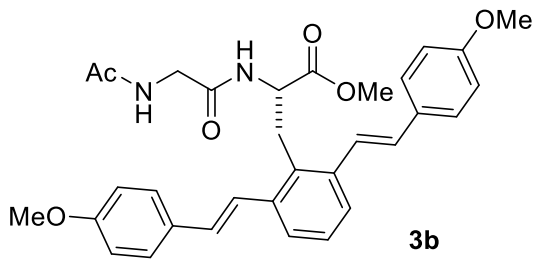
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (3H, s, Acetyl-C*H*$_3$), 2.37 (6H, s, Ar-C*H*$_3$), 3.45-3.53 (2H, m, Phe-C*H*$_2$), 3.55 (3H, s, Ester-C*H*$_3$), 3.68 (1H, dd, *J* = 16.9, *J* = 5.1, Gly-C*H*H), 3.77 (1H, dd, *J* = 16.9, *J* = 5.1, Gly-C*H*H), 4.75 (1H, dt, *J* = 7.5, *J* = 6.9, Phe-α-C*H*), 5.71 (1H, m, Gly-N*H*), 6.24 (1H, br d, *J* = 7.5, Phe-N*H*), 6.98 (2H, d, *J* = 15.9, Alkene-C*H*), 7.20 (4H, d, *J* = 7.9, Ar-*H*), 7.30 (1H, t, *J* = 7.9, Ar-*H*), 7.40 (2H, d, *J* = 15.9, Alkene-C*H*), 7.47 (4H, d, *J* = 7.9, Ar-*H*), 7.55 (2H, d, *J* = 7.9, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.3 (Ar-CH_3), 22.7 (Acetyl-CH_3), 30.8 (Phe-CH_2), 42.8 (Gly-CH_2), 52.7 (Ester-CH_3), 53.1 (Phe-α-CH), 125.2 (Ar-C), 125.3 (Ar-C), 126.6 (Alkene C=C), 127.6 (Ar-C), 129.5 (Ar-C), 131.4 (Ar-C), 131.6 (Alkene C=C), 134.4 (Ar-C), 137.9 (Ar-C), 137.9 (Ar-C), 168.3 (C=O), 170.3 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3264 m (N-H), 3056 w (C-H), 2917 w (C-H), 1740 s (Ester C=O), 1696 s (Amide C=O), 1652 m (C=C), 1510 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{35}N_2O_4$: 511.2591, found: 511.2582.

4.3.3 Synthesis of modified peptide 3b



3b

Modified peptide **3b** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-methoxystyrene (0.194 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3b** and the mono-olefinated peptide in a ratio of 3:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3b** as an off-white solid (0.097 g, 50%); m.p. 260-262 °C, R_f 0.34 (EtOAc).

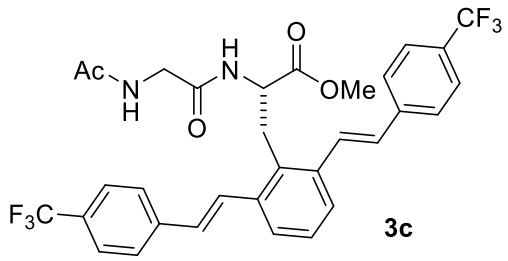
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (3H, s, Acetyl-C*H*$_3$), 3.48 (2H, br d, *J* = 7.1, Phe-C*H*$_2$), 3.54 (3H, s, Ester-C*H*$_3$), 3.69 (1H, dd, *J* = 16.9, *J* = 4.9, Gly-C*H*H), 3.76 (1H, dd, *J* = 16.9, *J* = 4.9, Gly-C*H*H), 3.83 (6H, s, OC*H*$_3$), 4.77 (1H, dt, *J* = 7.4, *J* = 7.0, Phe-α-C*H*), 5.76 (1H, m, Gly-N*H*), 6.25 (1H, br d, *J* = 7.4, Phe-N*H*), 6.92-6.98 (6H, m, Alkene-C*H* / Ar-*H*), 7.28-7.33 (4H, m, Alkene-C*H* / Ar-*H*), 7.50-7.54 (5H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 30.9 (Phe-CH_2), 42.8 (Gly-CH_2), 52.7 (Ester-CH_3), 53.1 (Phe-α-CH), 55.3 (OCH_3), 114.2 (Ar-C), 124.1 (Ar-C), 125.3 (Alkene-C=C), 127.6 (Ar-C), 128.0 (Ar-C), 130.0 (Ar-C), 131.0 (Ar-C), 131.3 (Alkene-C=C), 138.1 (Ar-C), 159.5 (Ar-C), 168.3 (C=O), 170.2 (C=O), 171.7(C=O).

IR U_{max} /cm^{-1} (solid) 3265 m (N-H), 3062 w (C-H), 2943 w (C-H), 1738 s (Ester C=O), 1694 s (Amide C=O), 1651 m (C=C), 1538 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{35}N_2O_6$: 543.2490, found: 543.2486.

4.3.4 Synthesis of modified peptide 3c



3c

Modified peptide **3c** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-trifluoromethylstyrene (0.213 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3c** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **3c** as a white solid (0.162 g, 73%); m.p. 218-220 °C, R_f 0.40 (50% EtOAc / pet. ether).

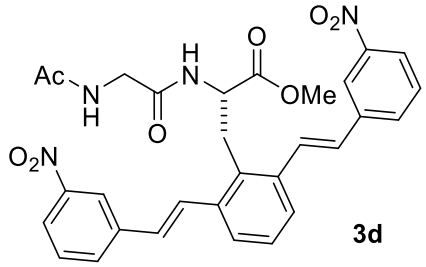
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (3H, s, Acetyl-C*H*$_3$), 3.43 (1H, dd, *J* = 14.2, *J* = 7.8, Phe-C*H*H), 3.50-3.56 (4H, m, Phe-C*H*H / Ester-C*H*$_3$), 3.78 (1H, dd, *J* = 16.5, *J* = 5.0, Gly-C*H*H), 3.87 (1H, dd, *J* = 16.5, *J* = 5.0, Gly-C*H*H), 4.78 (1H, dt, *J* = 7.3, *J* = 6.9, Phe-α-C*H*), 5.92-5.95 (1H, m, Gly-N*H*), 6.51 (1H, br d, *J* = 7.3, Phe-N*H*), 7.05 (2H, *J* = 16.0, Alkene-C*H*), 7.35 (1H, t, *J* = 7.8, Ar-*H*), 7.58-7.71 (12H, m, Ar-*H* / Alkene-CH).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 31.6 (Phe-CH_2), 43.0 (Gly-CH_2), 52.8 (Ester-CH_3), 53.1 (Phe-α-CH), 125.7 (Ar-C), 126.3 (Ar-C), 126.9 (Ar-C), 127.8 (Ar-C), 128.4 (Ar-C), 129.4 (Ar-C), 129.7 (Alkene-C=C), 130.2 (Alkene-C=C), 132.2 (Ar-C), 137.3 (Ar-C), 140.5 (Ar-C), 168.3 (C=O), 170.4 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3318 m (N-H), 3055 w (C-H), 2956 w (C-H), 1720 s (Ester C=O), 1612 m (C=C), 1507 m (C=C), 1321 s (C-F), 1109 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{30}F_6N_2O_4$: 619.2026, found 619.2028.

4.3.5 Synthesis of modified peptide 3d



3d

Modified peptide **3d** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 3-nitrostyrene (0.201 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3d** and the mono-olefinated peptide in a ratio of 8:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3d** as a white solid (0.123 g, 60%); m.p. 172-174 °C, R_f 0.31 (EtOAc).

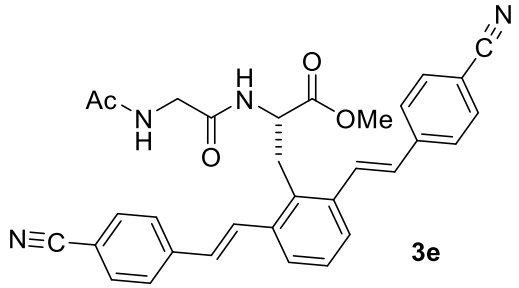
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (3H, s, Acetyl-C*H*$_3$), 3.36 (1H, dd, *J* = 14.2, *J* = 9.2, Phe-C*H*H), 3.50-3.55 (4H, m, Ester-C*H*$_3$ / Phe-C*H*H), 3.91-4.02 (2H, m, Gly-C*H*$_2$), 4.75 (1H, m, Phe-α-C*H*), 6.63 (1H, m, Gly-N*H*), 7.05 (2H, d, *J* = 16.0, Alkene-C*H*), 7.14 (1H, d, *J* = 7.3, Phe-N*H*), 7.33 (1H, t, *J* = 7.8, Ar-*H*), 7.53 (2H, t, *J* = 8.2, Ar-*H*), 7.62 (2H, d, *J* = 7.8, Ar-*H*), 7.74 (2H, d, *J* = 16.0, Alkene-C*H*), 7.82 (2H, d, *J* = 7.8, Ar-*H*), 8.09 (2H, d, *J* = 8.2, Ar-*H*) 8.61 (2H, s, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 32.4 (Phe-CH_2), 43.1 (Gly-CH_2), 52.9 (Ester-CH_3), 53.8 (Phe-α-CH), 121.2 (Ar-C), 122.4 (Ar-C), 126.3 (Ar-C), 127.9 (Ar-C), 128.9 (Ar-C), 129.4 (Alkene-C=C), 129.8 (Alkene-C=C), 132.7 (Ar-C), 133.1 (Ar-C), 137.0 (Ar-C), 139.1 (Ar-C), 149.0 (Ar-C), 168.7 9 (C=O), 171.0 (C=O), 171.9 (C=O).

IR υ_{max} /cm^{-1} (solid) 3301 m (N-H), 3070 w (C-H), 2957 w (C-H), 2222 m (C≡N), 1727 s (Ester C=O), 1653 m (C=C), 1519 m (C=C), 1349 s (N=O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{30}N_4O_8$: 573.1980, found 573.1984.

4.3.6 Synthesis of modified peptide 3e



3e

Modified peptide **3e** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-cyanostyrene (0.173 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3e** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3e** as a yellow solid (0.145 g, 76%); m.p. 209-211 °C, R_f 0.40 (EtOAc).

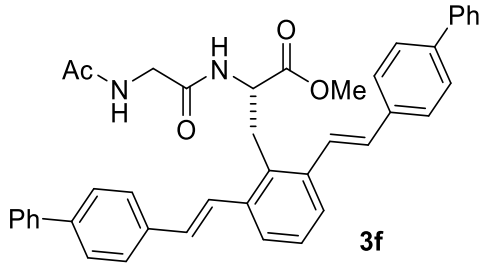
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (3H, s, Acetyl-C*H*$_3$), 3.36 (1H, dd, *J* = 14.4, *J* = 8.3, Phe-C*H*H), 3.48-3.54 (4H, m, Phe-C*H*H / Ester-C*H*$_3$), 3.82 (1H, dd, *J* = 16.6, *J* = 5.3, Gly-C*H*H), 3.90 (1H, dd, *J* = 16.6, *J* = 5.3, Gly-C*H*H), 4.75 (1H, dt, *J* = 8.0, *J* = 6.5, Phe-α-C*H*), 6.08 (1H, br t, *J* = 5.3, Gly-N*H*), 6.75 (1H, d, *J* = 8.0, Phe-N*H*), 7.02 (2H, d, *J* = 15.9, Alkene-C*H*), 7.35 (1H, t, *J* = 7.8, Ar-*H*), 7.62-7.64 (3H, m, Ar-*H*), 7.66-7.71 (9H, m, Ar-*H* / Alkene-C*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 31.9 (Phe-CH_2), 43.2 (Gly-CH_2), 52.8 (Ester-CH_3), 53.2 (Phe-α-CH), 110.9 (Ar-C), 119.0 (Ar-C), 126.5 (Ar-C), 127.2 (Ar-C), 127.9 (Ar-C), 129.4 (Alkene-C=C), 130.0 (Alkene-C=C), 132.5 (Ar-C), 132.6 (Ar-C), 137.0 (Ar-C), 141.5 (Ar-C), 168.5 (C=O), 170.6 (C=O), 171.7 (C=O).

IR υ_{max} /cm^{-1} (solid) 3301 m (N-H), 3090 w (C-H), 2958 w (C-H), 1728 s (Ester C=O), 1653 m (C=C), 1518 m (C=C), 1262 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{29}N_4O_4$: 533.2183, found 533.2181.

4.3.7 Synthesis of modified peptide 3f



3f

Modified peptide **3f** was prepared from Ac-Gly-Phe-OMe (0.100 g, 0.359 mmol) and 4-vinylbiphenyl (0.260 g, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3f** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3f** as an off-white solid (0.075 g, 33%); m.p. 246-249 °C, R_f 0.52 (EtOAc).

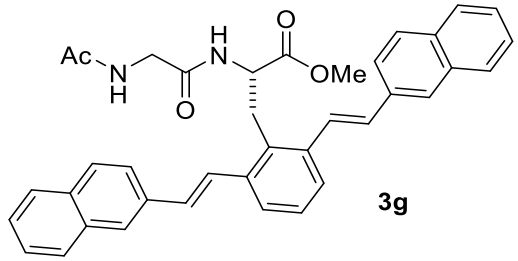
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (3H, s, Acetyl-C*H*$_3$), 3.55 (2H, d, *J* = 6.9, Phe-C*H*$_2$), 3.57 (3H, s, Ester-C*H*$_3$), 3.75 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 3.82 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 4.82 (1H, dt, *J* = 7.5, *J* = 6.9, Phe-α-C*H*), 5.81 (1H, br t, *J* = 5.0, Gly-N*H*), 6.34 (1H, br d, *J* = 7.5, Phe-N*H*), 7.07 (2H, d, *J* = 15.9, Alkene-C*H*), 7.32-7.39 (4H, m, Ar-*H*), 7.45-7.48 (4H, m, Ar-*H*), 7.52 (2H, d, *J* = 15.9, Alkene-C*H*), 7.60-7.68 (13H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 22.4 (Acetyl-CH_3), 30.7 (Phe-CH_2), 41.7 (Gly-CH_2), 52.1 (Ester-CH_3), 53.2 (Phe-α-CH), 126.0 (Ar-C), 126.5 (Ar-C), 126.6 (Ar-C), 126.9 (Alkene-C=C), 127.0 (Ar-C), 127.4 (Ar-C), 127.5 (Ar-C), 129.0 (Ar-C), 129.1 (Ar-C), 130.0 (Ar-C), 130.6 (Ar-C), 132.9 (Alkene-C=C), 136.5 (Ar-C), 139.3 (Ar-C), 139.7 (Ar-C), 169.2 (C=O), 169.5 (C=O), 171.8 (C=O).

IR υ_{max} /cm^{-1} (solid) 3266 m (N-H), 3029 w (C-H), 2924 w (C-H), 1725 s (Ester C=O), 1633 s (Amide C=O), 1601 m (C=C), 1518 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{39}N_2O_4$: 635.2904 found: 635.2895.

4.3.8 Synthesis of modified peptide 3g



3g

Modified peptide **3g** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 2-vinylnapthalene (0.222 g, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3g** and the mono-olefinated peptide in a ratio of 3:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **3g** as an off-white solid (0.079 g, 38%); m.p. 224-227 °C, R_f 0.54 (50% EtOAc / pet. ether).

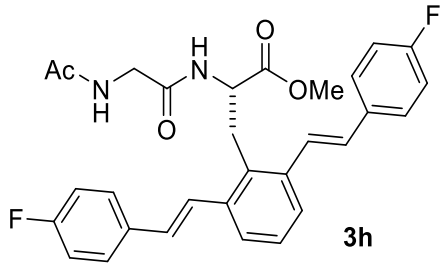
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.77 (3H, s, Acetyl-C*H*$_3$), 3.48-3.52 (5H, m, Ester-C*H*$_3$ / Phe-C*H*$_2$), 3.61 (1H, dd, *J* = 16.5, *J* = 5.7, Gly-C*H*H), 3.65 (1H, dd, *J* = 16.5, *J* = 5.7, Gly-C*H*H), 4.57 (1H, dt, *J* = 7.9, *J* = 6.5, Phe-α-C*H*), 7.28 (2H, d, *J* = 16.0, Alkene-C*H*$_2$), 7.33 (1H, t, *J* = 7.8, Ar-*H*), 7.45-7.52 (4H, m, Ar-*H*), 7.69 (2H, d, *J* = 7.8, Ar-*H*), 7.72 (2H, d, *J* = 16.0, Alkene-C*H*), 7.88-7.90 (4H, m, Ar-*H*), 7.92-7.96 (4H, m, Ar-*H*), 7.99 (1H, br t, *J* = 6.5, Phe-N*H*), 8.05 (2H, s, Ar-*H*), 8.64 (1H, br d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 22.9 (Acetyl-CH_3), 31.4 (Phe-CH_2), 42.4 (Gly-CH_2), 52.7 (Ester-CH_3), 53.7 (Phe-α-CH), 124.0 (Ar-C), 125.5 (Ar-C), 126.1 (Ar-C), 126.5 (Ar-C), 126.7 (Alkene-C=C), 127.4 (Ar-C), 127.6 (Ar-C), 128.0 (Ar-C), 128.2 (Ar-C), 131.2 (Alkene-C=C), 132.7 (Ar-C), 133.0 (Ar-C), 133.3 (Ar-C), 134.9 (Ar-C), 137.4 (Ar-C), 169.2 (C=O), 169.5 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3289 m (N-H), 2958 w (C-H), 1726 s (Ester C=O), 1636 m (C=C), 1508 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{38}H_{35}N_2O_4$: 583.2591 found 583.2579.

4.3.9 Synthesis of modified peptide 3h



3h

Modified peptide **3h** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-fluorostyrene (0.172 mL, 1.440 mmol), using the general procedure in **Section 4.3.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3h** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **3h** as an off-white solid (0.128 g, 69%); m.p. 190-193 °C, R_f 0.25 (50% EtOAc / pet. ether).

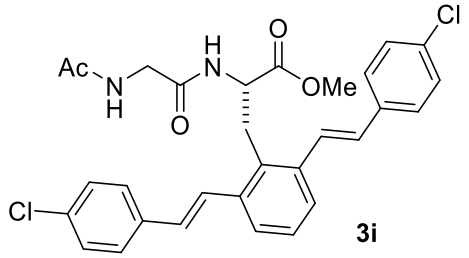
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (3H, s, Acetyl-C*H*$_3$), 3.42 (1H, dd, *J* = 14.5, *J* = 7.3, Phe-C*H*H), 3.45 (1H, dd, *J* = 14.5, *J* = 6.4, Phe-C*H*H), 3.53 (3H, s, Ester-C*H*$_3$), 3.72-3.76 (1H, m, Gly-C*H*H), 3.82 (1H, dd, *J* = 16.5, *J* = 5.0, Gly-C*H*H), 4.77 (1H, dt, *J* = 7.5, *J* = 7.3, Phe-α-C*H*), 6.03 (1H, br t, *J* = 5.0, Gly-N*H*), 6.59 (1H, br d, *J* = 7.5, Phe-N*H*), 6.96 (2H, d, *J* = 16.0, Alkene-C*H*$_3$), 7.07 (4H, t, *J* = 8.7, Ar-*H*), 7.30 (1H, t, *J* = 7.8, Ar-*H*), 7.38 (2H, d, *J* = 16.0, Alkene-C*H*$_3$), 7.54-7.57 (6H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 31.3 (Phe-CH_2), 42.9 (Gly-CH_2), 52.7 (Ester-CH_3), 53.1 (Phe-α-C), 115.6 (Ar-C), 115.8 (Ar-C), 125.7 (Ar-C), 125.8 (Alkene-C=C), 127.7 (Ar-C), 128.3 (Ar-C), 128.3 (Ar-C), 130.5 (Alkene-C=C), 131.6 (Ar-C), 133.4 (Ar-C), 137.7 (Ar-C), 161.2 (Ar-C-F), 163.7 (Ar-C-F), 168.3 (C=O), 170.4 (C=O), 171.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3298 m (N-H), 3066 w (C-H), 2930 w (C-H), 1726 s (Ester C=O), 1630 m (Amide C=O), 1507 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{29}F_2N_2O_4$: 519.2090 found: 519.2086.

4.3.10 Synthesis of modified peptide 3i



3i

Modified peptide **3i** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-chlorostyrene (0.173 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3i** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **3i** as a yellow solid (0.121 g, 61%); m.p. 234-238 °C, R_f 0.53 (EtOAc).

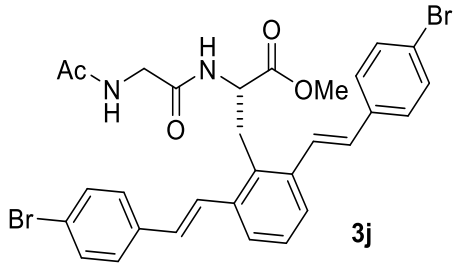
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (3H, s, Acetyl-C*H*$_3$), 3.41-3.51 (2H, m, Phe-C*H*$_2$), 3.52 (3H, s, Ester-C*H*$_3$), 3.74 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 3.82 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 4.76 (1H, dt, *J* = 7.4, *J* = 7.1, Phe-α-C*H*), 5.87 (1H, m, Gly-N*H*), 6.39 (1H, br d, *J* = 7.4, Phe-N*H*), 6.96 (2H, d, *J* = 16.0, Alkene-C*H*), 7.31 (1H, t, *J* = 8.2, Ar-*H*), 7.36 (4H, d, *J* = 8.5, Ar-*H*), 7.45 (2H, d, *J* = 16.0, Alkene-C*H*), 7.52 (4H, d, *J* = 8.2, Ar-*H*), 7.57 (2H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 22.4 (Acetyl-CH_3), 30.6 (Phe-CH_2), 41.6 (Gly-CH_2), 52.1 (Ester-CH_3), 53.1 (Phe-α-CH), 125.6 (Ar-C), 126.8 (Alkene-C=C), 127.3 (Ar-C), 128.4 (Ar-C), 128.7 (Ar-C), 129.8 (Ar-C), 132.1 (Alkene-C=C), 133.2 (Ar-C), 136.2 (Ar-C), 137.0 (Ar-C), 169.2 (C=O), 169.5 (C=O), 171.7 (C=O).

IR υ_{max} /cm^{-1} (solid) 3344 m (N-H), 3063 w (C-H), 2949 w (C-H), 1725 s (Ester C=O), 1659 s (C=C), 1630 s (Amide C=O), 1514 m (C=C), 1437 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{29}Cl_2N_2O_4$: 551.1499, found: 551.1492.

4.3.11 Synthesis of modified peptide 3j



3j

Modified peptide **3j** was prepared from Ac-Gly-Phe-OMe (**1a**) (0.100 g, 0.359 mmol) and 4-bromostyrene (0.188 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **3j** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (65% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **3j** as a yellow solid (0.113 g, 49%); m.p. 177-180 °C, R_f 0.31 (65% EtOAc / pet. ether).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.76 (3H, s, Acetyl-C*H*$_3$), 3.24-3.26 (1H, m, Phe-C*H*H), 3.36-3.42 (1H, m, Phe-C*H*H), 3.45 (3H, s, Ester-C*H*$_3$), 3.54-3.59 (1H, m, Gly-C*H*H), 3.64-3.70 (1H, m, Gly-C*H*H), 4.41-4.47 (1H, m, Phe-α-C*H*), 7.07 (2H, d, *J* = 16.0, Alkene-C*H*$_3$), 7.25-7.29 (2H, m, Ar-*H*), 7.53-7.62 (11H, m, Alkene-C*H* / Ar-*H*), 7.94-7.96 (1H, m, Gly-N*H*), 8.55-8.57 (1H, m, Phe-N*H*).

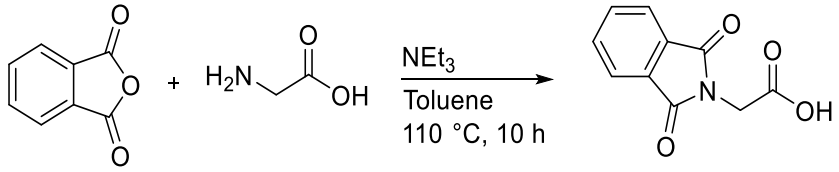
^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 22.4 (Acetyl-C*H*$_3$), 30.6 (Phe-CH_2), 41.6 (Gly-CH_2), 52.1 (Ester-CH_3), 53.1 (Phe-α-CH), 120.7 (Ar-C), 125.6 (Ar-C), 126.9 (Alkene-C=C), 127.3 (Ar-C), 128.8 (Ar-C), 129.9 (Ar-C), 131.6 (Alkene-C=C), 133.2 (Ar-C), 136.5 (Ar-C), 137.0 (Ar-C), 169.2 (C=O), 169.5 (C=O), 171.7 (C=O).

IR υ_{max} /cm^{-1} (solid) 3309 m (N-H), 2924 w (C-H), 1725 s (Ester C=O), 1639 m (C=C), 1488 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{29}Br_2N_2O_4$: 641.0471, found: 641.0465.

4.4 Scope of the N-protecting group

4.4.1 Synthesis of N-Phthaloyl glycine (Phth-Gly-OH) (4)



4

Phthalic anhydride (2.000 g, 13.50 mmol) and glycine (0.780 g, 10.39 mmol) were slurried in toluene before the addition of triethylamine (1.88 mL, 13.50 mmol). The resulting suspension was refluxed under Dean-Stark conditions for 10 h. After allowing the mixture to cool to room temperature, the toluene was removed by rotary evaporation to give a crude oil. The oil was suspended in water and acidified using conc. HCl(aq.) (3 mL) to give a white solid that was isolated by filtration and dried on the filter pad to afford off-white needles of Phth-Gly-OH (**4**) (2.005 g, 94%), m.p. 190-192 °C.

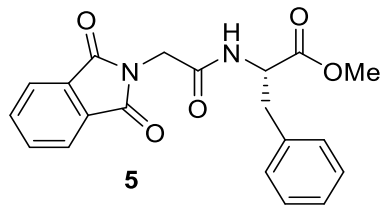
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (2H, s, Gly-C*H*$_3$), 7.76 (2H, dd, *J* = 5.5, *J* = 3.1, Ar-*H*), 7.90 (2H, dd, *J* = 5.5, *J* = 3.1, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 38.5 (Gly-CH_2), 123.7 (Phth-CH), 131.9 (Phth-C), 134.3 (Phth-CH), 167.4 (C=O), 171.6 (C=O).

IR υ_{max}/cm^{-1} (solid) 3559 m (O-H), 3051 w (C-H), 2988 w (C-H), 2937 w (C-H), 1773 s (Phth C=O), 1710 s (carboxylic acid C=O), 1610 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_8NO_2$: 206.0448, found: 206.0448.

4.4.2 Phth-Gly-Phe-OMe (5)



5

Peptide **5** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and Phth-glycine (**4**) (0.205 g, 1.00 mmol), using the procedure in **Section 4.2.1.** The crude compound was recrystallised from DCM / hexanes to afford **5** as a white solid (0.231 g, 63%); m.p. 172-173 °C.

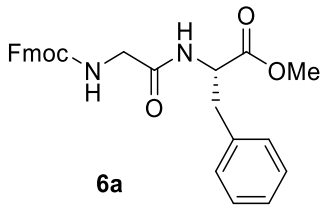
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.09 (1H, dd, *J* = 13.7, *J* = 5.5, Phe-C*H*H), 3.16 (1H, dd, *J* = 13.7, *J* = 5.5, Phe-C*H*H), 3.73 (3H, s, Ester-CH_3) 4.35 (2H, dt, *J* = 17.4, *J* = 16.5, Gly-CH_2), 4.87 (1H, dt, *J* = 7.3, *J* = 5.5, Phe-α-C*H*), 6.36 (1H, m, Phe-N*H*), 7.07-7.09 (2H, m, Ar-*H*), 7.17-7.27 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 37.5 (Phe-CH_2), 40.5 (Gly-CH_2), 52.5 (Ester-CH_3), 53.3 (Phe-α-CH), 123.6 (Ar-C), 127.1 (Ar-C), 128.5 (Ar-C), 129.3 (Ar-C), 131.9 (Ar-C), 134.2 (Ar-C), 135.4 (Ar-C), 165.6 (C=O), 167.6 (C=O), 171.5 (C=O).

IR υ_{max} /cm^{-1} (solid) 3308 m (N-H), 3032 s (C-H), 2950 s (C-H), 1776 s (Ester C=O), 1714 s (C=O), 1659 s (Amide C=O), 1543 m (C=C), 1417 m (C=C), 1206 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{19}N_2O_5$: 367.1288, found: 367.1289.

4.4.3 Fmoc-Gly-Phe-OMe (6a)



6a

Peptide **6a** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and Fmoc-glycine (0.297 g, 1.00 mmol), using the procedure in **Section 4.2.1.** The crude compound was recrystallised from DCM / hexanes to afford **6a** as a white solid (0.307 g, 67%); m.p. 127-128 °C.

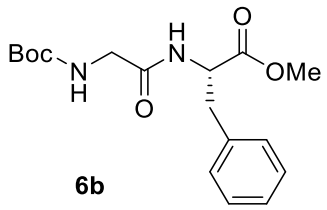
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.08-3.18 (2H, m, Phe-CH_2), 3.74 (3H, s, Ester-CH_3), 3.82-3.94 (2H, m, Gly-CH_2), 4.22 (1H, br dd, *J* = 7.2, *J* = 7.0, Fmoc-C*H*), 4.41 (2H, br d, *J* = 7.2, Fmoc-CH_3), 4.90 (1H, dt, *J* = 7.6, *J* = 6.0, Phe-α-C*H*), 5.34-5.36 (1H, m, Gly-N*H*), 6.31 (1H, br d, *J* = 7.6, Phe-N*H*), 7.07 (2H, br d, *J* = 6.4, Ar-*H*), 7.21-7.28 (3H, m, Ar-*H*), 7.32 (2H, t, *J* = 7.3, Fmoc-Ar-*H*), 7.41 (2H, t, *J* = 7.3, Fmoc-Ar-*H*), 7.59 (2H, br d, *J* = 7.3, Fmoc-Ar-*H*), 7.78 (2H, d, *J* = 7.3, Fmoc-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 37.8 (Phe-CH_2), 44.3 (Gly-CH_2), 47.0 (Fmoc-CH), 52.4 (Ester-CH_3), 53.1 (Phe-α-CH), 67.3 (Fmoc-CH_2), 120.0 (Ar-C), 125.0 (Ar-C), 127.1 (Ar-C), 127.2 (Ar-C), 127.7 (Ar-C), 128.6 (Ar-C), 129.2 (Ar-C), 135.5 (Ar-C), 141.2 (Ar-C), 143.7 (Ar-C), 156.4 (C=O), 168.5 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3302 m (N-H), 3029 s (C-H), 2950 s (C-H), 1724 s (Ester C=O), 1665 s (Amide C=O), 1515 m (C=C), 1479 m (C=C).

HRMS (ESI) [M+H^{+}] *m/z* calcd. for $C_{27}H_{27}N_2O_5$: 459.1914, found: 459.1908.

4.4.4 Boc-Gly-Phe-OMe (6b)



6b

Peptide **6b** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and Boc-glycine (0.175 g, 1.00 mmol), using the procedure in **Section 4.2.1.**, to afford **6b** as a colourless oil (0.205 g, 61%).

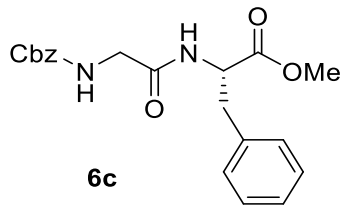
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, Boc-(C*H*$_3$)$_3$), 3.08 (1H, dd, *J* = 13.8, *J* = 6.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.8, *J* = 6.0, Phe-C*H*H), 3.70-3.84 (5H, m, Ester-C*H*$_3$ / Gly-C*H*$_2$), 4.87 (1H, dt, *J* = 7.5, *J* = 6.0, Phe-α-C*H*), 5.29 (1H, m, Gly-N*H*), 6.74 (1H, br d, *J* = 7.5, Phe-N*H*), 7.09-7.11 (2H, m, Ar-*H*) 7.22-7.30 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 28.2 (Boc-$(CH_3)_3$), 37.8 (Phe-CH_2), 44.0 (Gly-CH_2), 52.3 (Ester-CH_3), 53.0 (Phe-α-CH), 80.0 (Boc-C), 127.0 (Ar-C), 128.5 (Ar-C), 129.1 (Ar-C), 135.6 (Ar-C), 155.9 (C=O), 169.2 (C=O), 171.7 (C=O).

IR υ_{max} /cm^{-1} (oil) 3251 m (N-H), 3071 s (C-H), 2979 s (C-H), 1753 s (Ester C=O), 1741 s (Carbamate C=O), 1536 s (C=C), 1497 m (C=C), 1436 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{24}N_2O_5$: 337.1758, found: 337.1756.

4.4.5 Cbz-Gly-Phe-OMe (6c)



6c

Peptide **6c** was synthesised from L-Phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and Cbz-glycine (0.209 g, 1.00 mmol), using the procedure in **Section 4.2.1.,** to afford **6c** as a colourless oil (0.193 g, 52%).

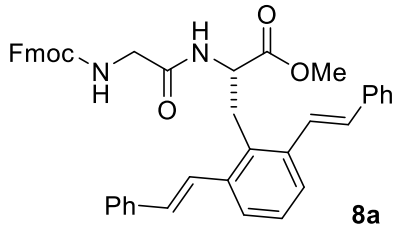
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.10 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.67 (3H, s, Ester-CH_3), 3.75-3.87 (2H, m, Gly-CH_2), 4.86 (1H, dt, *J* = 7.8, *J* = 6.0, Phe-α-C*H*), 5.08 (2H, s, Cbz-CH_2) 5.71 (1H, m, Gly-N*H*), 6.82 (1H, m, Phe-N*H*), 7.07 (2H, br d, *J* = 6.4, Ar-*H*) 7.18-7.26 (3H, m, Ar-*H*), 7.29- 7.33 (5H, m, Cbz-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 37.7 (Phe-CH_2), 44.2 (Gly-CH_2), 52.3 (Ester-CH_3), 53.0 (Phe-α-CH), 67.0 (Z-CH_2), 127.0 (Ar-C), 127.9 (Ar-C), 128.1 (Ar-C), 128.4 (Ar-C), 128.9 (Ar-C), 129.1 (Ar-C), 135.5 (Ar-C), 136.0 (Ar-C), 156.5 (C=O), 168.8 (C=O), 171.7 (C=O).

IR U_{max} /cm^{-1} (oil) 3317 m (N-H), 3030 s (C-H), 2952 s (C-H), 1723 s (Carbamate C=O), 1656 s (Amide C=O), 1604 m (C=C), 1518 m (C=C), 1215 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{23}N_2O_5$: 371.1601, found: 371.1602.

4.4.6 Synthesis of modified peptide 8a



8a

Modified peptide **8a** was prepared from Fmoc-Gly-Phe-OMe (**6a**) (0.165 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **8a** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **8a** as an off-white solid (0.124 g, 52%); m.p. 145-148 °C, R_f 0.34 (25% EtOAc / pet. ether).

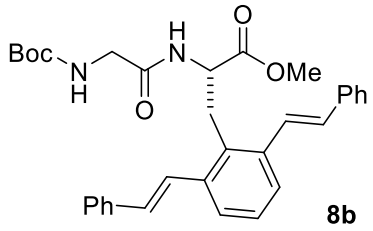
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.48 (2H, br d, *J* = 6.9, Phe-C*H*$_2$), 3.53 (3H, s, Ester-C*H*$_3$), 3.70-3.74 (2H, m, Gly-C*H*$_2$), 4.16-4.19 (1H, m, Fmoc-C*H*), 4.31-4.33 (2H, m, Fmoc-C*H*$_2$), 4.81 (1H, dt, *J* = 7.3, *J* = 6.9, Phe-α-C*H*), 5.10 (1H, m, Gly-N*H*), 6.51 (1H, br d, *J* = 7.3 Phe-N*H*), 7.00 (2H, d, *J* = 15.8, Alkene-C*H*), 7.26-7.32 (6H, m, Ar-*H*), 7.35-7.42 (6H, m, Ar-*H*), 7.48 (2H, d, *J* = 15.8, Alkene-C*H*), 7.55-7.58 (7H, m, Ar-*H*), 7.75-7.77 (2H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 31.4 (Phe-CH_2), 44.3 (Gly-CH_2), 47.1 (Fmoc-CH), 52.8 Ester-CH_3), 53.2 (Phe-α-CH), 67.2 (Fmoc-CH_2), 120.1 (Ar-C), 124.8 (Ar-C), 125.2 (Ar-C), 125.9 (Ar-C), 126.3 (Alkene-C=C), 126.8 (Ar-C), 127.2 (Ar-C), 127.8 (Ar-C), 128.1 (Ar-C), 128.9 (Ar-C), 131.8 (Alkene-C=C), 131.9 (Ar-C), 137.3 (Ar-C), 137.9 (Ar-C), 141.4 (Ar-C), 143.9 (Ar-C), 156.4 (C=O), 168.4 (C=O), 171.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3300 m (N-H), 3057 s (C-H), 2949 s (C-H), 1725 s (Ester C=O), 1691 s (C=O), 1645 s (Amide C=O), 1530 m (C=C), 1448 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{43}H_{39}N_2O_5$: 663.2853 found: 663.2851.

4.4.7 Synthesis of modified peptide 8b



8b

Modified peptide **8b** was prepared from Boc-Gly-Phe-OMe (**6b**) (0.121 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **8b** and the mono-olefinated peptide in a ratio of 8:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **8b** as an off-white solid (0.138 g, 71%); m.p. 164-166 °C, R_f 0.45 (EtOAc).

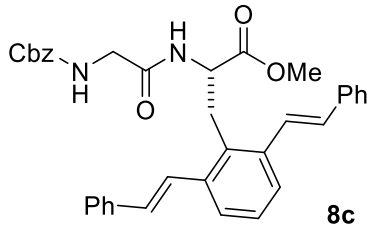
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (9H, s, Boc-(C*H*$_3$)$_3$), 3.39 (1H, dd, *J* = 14.4, *J* = 7.1, Phe-C*H*H), 3.48 (1H, d, *J* = 14.4, *J* = 7.1, Phe-C*H*H), 3.51 (3H, s, Ester-C*H*$_3$), 3.62-3.70 (2H, m, Gly-C*H*$_2$), 4.80 (1H, app q, *J* = 7.1, Phe-α-C*H*), 4.88-4.93 (1H, m, Gly-N*H*), 6.68 (1H, br d, *J* = 7.1, Phe-N*H*), 7.01 (2H, d, *J* = 16.0, Alkene-C*H*), 7.26-7.31 (3H, m, Ar-*H*), 7.38 (4H, t, *J* = 7.4, Ar-*H*), 7.49 (2H, d, *J* = 16.0, Alkene-C*H*), 7.56-7.59 (6H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 28.2 (Boc-(CH_3)$_3$), 31.5 (Phe-CH_2), 44.0 (Gly-CH_2), 52.6 (Ester-CH_3), 53.0 (Phe-α-CH), 80.1 (Boc-C), 125.7 (Ar-C), 126.1 (Ar-C), 126.7 (Alkene-C=C), 127.6 (Ar-C), 127.9 (Ar-C), 128.8 (Ar-C), 131.6 (Alkene-C=C), 131.8 (Ar-C), 137.2 (Ar-C), 137.2 (Ar-C), 137.7 (Ar-C), 155.8 (C=O), 169.8(C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3324 m (N-H), 2977 s (C-H), 1725 s (Ester C=O), 1647 s (Amide C=O), 1598 m (C=C), 1449 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{37}N_2O_5$: 541.2697, found: 541.2692.

4.4.8 Synthesis of modified peptide 8c



8c

Modified peptide **8c** was prepared from Cbz-Gly-Phe-OMe (**6c**) (0.133 g, 0.359 mmol) styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **8c** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **8c** as an off-white solid (0.144 g, 70%); m.p. 177-179 °C, R_f 0.55 (25% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41-3.45 (2H, m, Phe-C*H*$_2$), 3.51 (3H, s, Ester-C*H*$_3$), 3.65-3.76 (2H, m, Gly-C*H*$_2$), 4.80 (1H, app q, *J* = 7.3, Phe-α-C*H*), 5.03 (2H, s, Cbz-C*H*$_2$), 5.71 (1H, m, Gly-N*H*), 6.60 (1H, br d, *J* = 7.3, Phe-N*H*), 6.99 (2H, d, *J* = 16.0, Alkene-C*H*), 7.24-7.31 (7H, m, Ar-*H*), 7.36 (5H, t, *J* = 7.6, Ar-*H*), 7.47 (2H, d, *J* = 16.0, Alkene-C*H*), 7.54-7.57 (6H, m, Ar-*H*).

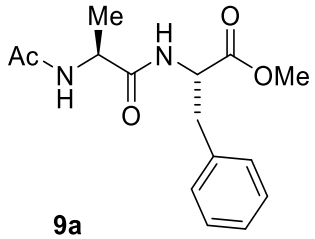
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 31.3 (Phe-CH_2), 44.2 (Gly-CH_2), 52.7 (Ester-CH_3), 53.0 (Phe-α-CH), 67.1 (Cbz-CH_2), 125.7 (Ar-C), 126.1 (Ar-C), 126.7 (Alkene-C=C), 127.7 (Ar-C), 128.0 (Ar-C), 128.0 (Ar-C), 128.2 (Ar-C), 125.5 (Ar-C), 128.8 (Ar-C), 131.7 (Alkene-C=C), 131.7 (Ar-C), 136.1 (Ar-C), 137.1 (Ar-C), 137.8 (Ar-C), 156.2 (C=O), 168.3 (C=O), 171.8 (C=O).

IR υ_{max} /cm^{-1} (solid) 3317 m (N-H), 3029 s (C-H), 2954 s (C-H), 1726 s (Ester C=O), 1688 s (Amide C=O), 1644 m (C=C), 1533 m (C=C), 1441 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{35}N_2O_5$: 575.2540 found: 575.2534.

4.5 Synthesis of dipeptides 9a-k

4.5.1 Ac-Ala-Phe-OMe (9a)



9a

Peptide **9a** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-alanine (0.131 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9a** as a white solid (0.261 g, 89%); m.p. 125-126 °C.

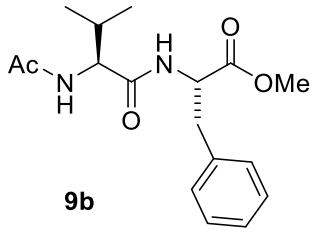
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, d, *J* = 7.0, Ala-C*H*$_3$), 1.93 (3H, s, Acetyl-C*H*$_3$), 3.03 (1H, dd, *J* = 13.7, *J* = 6.4, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.7, *J* = 6.4, Phe-C*H*H), 3.70 (3H, s, Ester-C*H*$_3$), 4.48 (1H, dq, *J* = 7.4, *J* = 6.9, Ala-α-C*H*), 4.80-4.85 (1H, m, Phe-α-C*H*), 6.33 (1H, br d, *J* = 7.4, Ala-N*H*), 6.81 (1H, br d, *J* = 7.9, Phe-N*H*), 7.08-7.10 (2H, m, Ar-*H*), 7.18-7.27 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.5 (Ala-CH_3), 23.1 (Acetyl-CH_3), 38.3 (Phe-CH_2), 48.1 (Ala-α-CH), 52.7 (Ester-CH_3), 53.8 (Phe-α-CH), 127.1 (Ar-C), 128.5 (Ar-C), 129.2 (Ar-C), 135.7 (Ar-C), 169.9 (C=O), 171.1 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3252 m (N-H), 3075 w (C-H), 2988 w (C-H), 2938 w (C-H), 1753 s (Ester C=O), 1637 s (Amide C=O), 1537 m (C=C), 1436 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4$: 293.1496, found: 293.1492.

4.5.2 Ac-Val-Phe-OMe (9b)



9b

Peptide **9b** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-valine (0.159 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9b** as a white solid (0.224 g, 70%); m.p 176-177 °C.

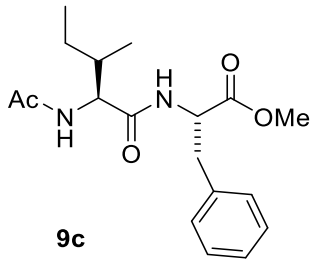
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, app t, *J* = 6.4, Val-C*H*$_3$), 2.00-2.07 (4H, m, Acetyl-C*H*$_3$ / Val-CH$_3$C*H*CH$_3$) 3.07 (1H, dd, *J* = 14.0, *J* = 6.2, Phe-C*H*H), 3.12 (1H, dd, *J* = 14.0, *J* = 6.2, Phe-C*H*H), 3.72 (3H, s, Ester-C*H*$_3$), 4.28 (1H, dd, *J* = 9.2, *J* = 6.9, Val-α-C*H*), 4.85 (1H, dd, *J* = 7.8, *J* = 6.2, Phe-α-C*H*), 6.19 (1H, br d, *J* = 9.2, Val-N*H*), 6.49 (1H, br d, *J* = 7.8, Phe-N*H*), 7.10-7.12 (2H, m, Ar-*H*), 7.22-7.30 (3H, m, Ar-*H*).

^{13}C{^{1}H} 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 (Ar-C), 128.6 (Ar-C), 129.2 (Ar-C), 135.5 (Ar-C), 170.0 (C=O), 170.9 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3288 m (N-H), 3030 w (C-H), 2954 w (C-H), 1743 s (Ester C=O), 1643 s (Amide C=O), 1538 m (C=C), 1383 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4$: 321.1809, found: 321.1810.

4.5.3 Ac-Ile-Phe-OMe (9c)



9c

Peptide **9c** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-isoleucine (0.173 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9c** as a white solid (0.234 g, 70%); m.p. 194-195 °C.

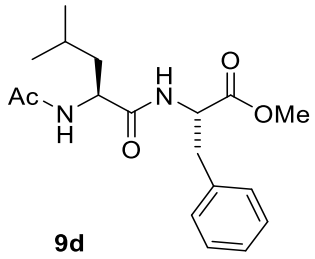
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.88 (6H, m, Ile-(C*H*$_3$)$_2$), 1.04-1.14 (1H, m, Ile-C*H*HCH$_3$), 1.41-1.51 (1H, m, Ile-C*H*HCH$_3$), 1.72-1.82 (1H, m, Ile-CH$_2$C*H*CH$_3$), 1.98 (3H, s, Acetyl-C*H*$_3$), 3.07 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H) 3.12 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.71 (3H, s, Ester-C*H*$_3$), 4.26 (1H, dd, *J* = 8.7, *J* = 6.5, Ile-α-C*H*), 4.85 (1H, dt, *J* = 7.8, *J* = 6.0, Phe-α-C*H*), 6.07 (1H, br d, *J* = 6.5, Ile-N*H*), 6.29 (1H, br d, *J* = 7.8, Phe-N*H*), 7.08-7.10 (2H, m, Ar-*H*), 7.21-7.30 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 11.3 (Ile-CH$_2$*C*H$_3$), 15.2 (Ile-CH*C*H$_3$), 23.3 (Acetyl-CH$_3$), 24.9 (Ile-CH), 37.4 (Ile-CH$_2$), 37.8 (Phe-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 (Ar-C), 171.5 (C=O).

IR υ_{max} /cm^{-1} (solid) 3296 m (N-H), 2971 w (C-H), 1741 w (Ester C=O), 1663 s (Amide C=O), 1647 m (C=C), 1541 m (C=C), 1384 m (C-H).

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

4.5.4 Ac-Leu-Phe-OMe (9d)



9d

Peptide **9d** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-leucine (0.173 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9d** as a white solid (0.261 g, 78%); m.p. 110-111 °C.

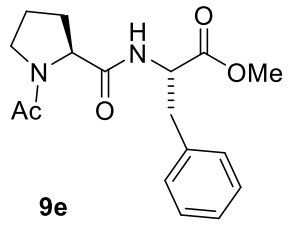
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, d, *J* = 5.5, Leu-(C*H*$_3$)$_2$), 1.43-1.50 (1H, m, Leu-CH$_2$C*H*(CH$_3$)$_2$), 1.55-1.64 (2H, m, Leu-C*H*$_2$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.03 (1H, dd, *J* = 13.9, *J* = 6.4, Phe-C*H*H), 3.10 (1H, dd, *J* = 13.9, *J* = 6.4, Phe-C*H*H), 3.68 (3H, s, Ester-C*H*$_3$), 4.45-4.50 (1H, m, Leu-α-C*H*), 4.79 (1H, dt, *J* = 7.8, *J* = 6.4, Phe-α-C*H*), 6.34 (1H, br d, *J* = 8.2, Leu-N*H*), 6.83 (1H, br d, *J* = 7.8, Phe-N*H*), 7.09-7.11 (2H, m, Ar-*H*) 7.18-7.27 (3H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.3 (Leu-CH_3), 22.9 (Leu-CH_3), 23.1 (Acetyl-CH_3), 24.8 (Leu-CH), 38.0 (Phe-CH_2), 41.2 (Leu-CH_2), 51.6 (Leu-α-CH), 52.4 (Ester-CH_3), 53.4 (Phe-α-CH), 127.0 (Ar-C), 128.5 (Ar-C), 129.2 (Ar-C), 135.9 (Ar-C), 170.3 (C=O), 171.9 (C=O), 172.3 (C=O)**.**

IR υ_{max} /cm^{-1} (solid) 3265 m (N-H), 3055 w (C-H), 2896 w (C-H), 1753 s (Ester C=O), 1687 s (Amide C=O), 1601 m (C=C), 1566 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{27}N_2O_4$: 335.1965, found: 335.1963.

4.5.5 Ac-Pro-Phe-OMe (9e)



9e

Peptide **9e** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-proline (0.157 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9e** as a white solid (0.213 g, 67%); m.p. 106-108 °C.

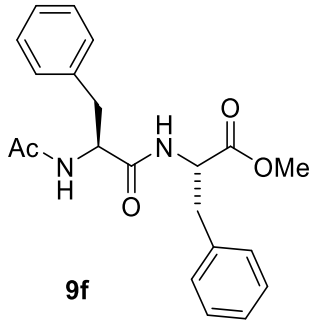
^{1}H NMR (400 MHz, CD_3OD) δ 1.72-1.80 (1H, m, Pro-CHC*H*HCH$_2$), 1.88-1.95 (2H, m, Pro-CH$_2$C*H*$_2$CH$_2$), 2.01 (3H, s, Acetyl-C*H*$_3$), 2.34-2.40 (1H, m, Pro-CHC*H*HCH$_2$), 2.98 (1H, dd, *J* = 14.0, *J* = 7.8, Phe-C*H*H), 3.21 (1H, dd, *J* = 14.0, *J* = 5.5, Phe-C*H*H), 3.33 (2H, t, *J* = 7.0, Pro-NHC*H*$_2$CH$_2$), 3.73 (3H, s, Ester-C*H*$_3$), 4.56 (1H, dd, *J* = 8.0, *J* = 7.0, Pro-α-C*H*), 4.83 (1H, dt, *J* = 7.8, *J* = 5.5, Phe-α-C*H*), 7.10-7.15 (2H, m, Ar-*H*), 7.20-7.30 (3H, m, Ar-*H*), 7.54 (1H, br d, *J* = 5.5, Phe-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.4 (Acetyl-CH_3), 24.8 (Pro-CH_2), 26.9 (Pro-CH_2), 37.9 (Phe-CH_2), 48.0 (Pro-CH_2), 52.3 (Ester-CH_3), 53.1 (Phe-α-CH), 59.3 (Pro-α-CH_3), 126.8 (Ar-C), 128.2 (Ar-C), 129.3 (Ar-C), 136.3 (Ar-C), 170.8 (C=O), 170.8 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3524 m (N-H), 3065 w (C-H), 2900 w (C-H), 1732 s (Ester C=O), 1644 s (Amide C=O), 1497 m (C=C), 1292 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{23}N_2O_4$: 319.1652, found: 319.1652.

4.5.6 Ac-Phe-Phe-OMe (9f)



9f

Peptide **9f** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-phenylalanine (0.207 g, 1.00 mmol), using the procedure in **Section 4.2.1**, and isolated as a white solid (0.195 g, 53%); m.p. 170-171 °C.

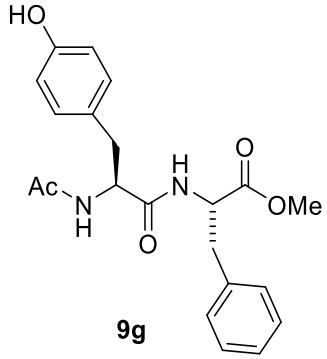
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (3H, s, Acetyl-C*H*$_3$), 2.94-3.12 (4H, m, Phe-C*H*$_2$), 3.65 (3H, s, Ester-C*H*$_3$), 4.74 (2H, m, Phe-α-C*H* (x2)), 6.57 (1H, br d, *J* = 8.2, Phe-N*H*), 6.72 (1H, d, *J* = 7.8, Phe-N*H*), 7.02-7.04 (2H, m, Ar-*H*) 7.15-7.26 (8H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 37.8 (Phe-CH_2), 38.1 (Phe-CH_2), 52.2 (Ester-CH_3), 53.4 (Phe-α-CH), 54.2 (Phe-α-CH), 126.8 (Ar-C), 127.0 (Ar-C), 128.4 (Ar-C), 129.1 (Ar-C), 129.2 (Ar-C), 135.6 (Ar-C), 136.3 (Ar-C), 170.1 (C=O), 170.8 (C=O), 171.2 (C=O).

IR υ_{max} /cm^{-1} (solid) 3308 m (N-H), 3066 w (C-H), 2973 w (C-H), 1733 s (Ester C=O), 1646 s (Amide C=O), 1538 m (C=C), 1435 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{21}H_{25}N_2O_4$: 369.1809, found: 369.1813.

4.5.7 Ac-Tyr-Phe-OMe (9g)



9g

Peptide **9g** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-tyrosine (0.223 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9g** as a cream solid (0.311 g, 81%); m.p. 57-59 °C.

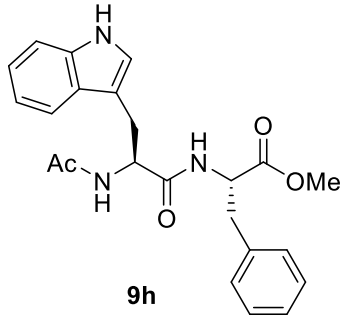
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (3H, s, Acetyl-C*H*$_3$), 2.79-2.81 (1H, m, Tyr-C*H*H), 2.89-2.93 (1H, m, Tyr-C*H*H), 2.95-3.00 (1H, m, Phe-C*H*H), 3.06-3.11 (1H, m, Phe-C*H*H), 3.67 (3H, s, Ester-C*H*$_3$), 4.58-4.64 (1H, m, Tyr-α-C*H*), 4.73-4.48 (1H, m, Phe-α-C*H*), 6.30 (1H, d, *J* = 8.0, Tyr-N*H*), 6.45 (1H, d, *J* = 7.8, Phe-N*H*), 6.70 (2H, d, *J* = 8.9, Phe-Ar-*H*), 6.98-7.04 (4H, m, Tyr-Ar-*H*), 7.21-7.26 (3H, m, Phe-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.0 (Acetyl-CH_3), 37.5 (Tyr-CH_2), 37.8 (Phe-CH_2), 52.4 (Ester-CH_3), 53.5 (Phe-α-CH), 54.5 (Tyr-α-CH), 115.6 (Ar-C), 127.1 (Ar-C), 127.5 (Ar-C), 128.6 (Ar-C), 129.2 (Ar-C), 130.3 (Ar-C), 135.5 (Ar-C), 155.4 (Ar-C), 170.4 (C=O), 170.8 (C=O), 171.4 (C=O).

IR U_{max} /cm^{-1} (solid) 3274 m (N-H), 2947 s (C-H), 1726 s (Ester C=O), 1647 m (C=C), 1515 m (C=C), 1435 m (C-H), 1220 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{21}H_{25}N_2O_5$: 385.1763, found: 385.1762.

4.5.8 Ac-Trp-Phe-OMe (9h)



9h

Peptide **9h** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-tryptophan (0.246 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9h** as a cream solid (0.371 g, 91%); m.p. 73-76 °C.

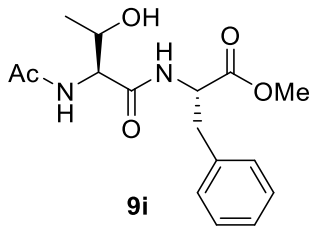
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (Acetyl-C*H*$_3$), 2.89 (1H, dd, *J* = 13.7, *J* = 6.2, Phe-C*H*H), 2.99 (1H, dd, *J* = 13.7, *J* = 6.2, Phe-C*H*H), 3.10 (1H, dd, *J* = 14.4, *J* = 7.8, Trp-C*H*H), 3.27 (1H, dd, *J* = 14.4, *J* = 5.0, Trp-C*H*H), 3.61 (3H, s, Ester-C*H*$_3$), 4.65-4.74 (2H, m, Trp-α-C*H*, Phe-α-C*H*), 6.23 (1H, br d, *J* = 7.3, Trp-N*H*), 6.33 (1H, br d, *J* = 7.8, Phe-N*H*), 6.85-6.87(2H, m, Phe-Ar-*H*), 7.01 (1H, d, *J* = 2.3, Trp-Ar-*H*), 7.08-7.19 (5H, m, Phe-Ar-*H* / Trp-Ar-*H*), 7.33 (1H, d, *J* = 8.0, Trp-Ar-*H*), 7.67 (1H, d, Trp-Ar-*H*), 8.30 (1H, br s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.2 (Acetyl-CH_3), 28.2 (Trp-CH_2), 37.1 (Phe-CH_2), 52.2 (Ester-CH_3), 53.3 (Phe-α-CH), 53.7 (Trp-α-CH), 110.4 (Ar-C), 111.2 (Ar-C), 118.7 (Ar-C), 119.7 (Ar-C), 122.1 (Ar-C), 123.4 (Ar-C), 127.0 (Ar-C), 128.5 (Ar-C), 129.1 (Ar-C), 135.5 (Ar-C), 136.1 (Ar-C), 170.0 (C=O), 170.9 (C=O), 171.3 (C=O).

IR υ_{max} /cm^{-1} (solid) 3291 m (O-H), 3064 s (C-H), 2969 s (C-H), 1737 s (Ester C=O), 1637 m (C=C), 1541 m (C=C), 1445 m (C-H), 1216 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{26}N_3O_4$: 408.1918, found: 408.1923.

4.5.9 Ac-Thr-Phe-OMe (9i)



9i

Peptide **9i** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-threonine (0.162 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9i** as a cream solid (0.259 g, 78%); m.p. 127-129 °C.

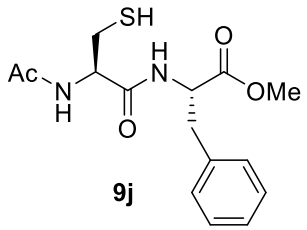
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, d, *J* = 6.5, Thr-C*H*$_3$), 1.98 (3H, Acetyl-C*H*$_3$), 3.00 (1H, dd, *J* = 14.0, *J* = 7.8, Phe-C*H*H), 3.18 (1H, dd, *J* = 14.0, *J* = 5.4, Phe-C*H*H), 3.74 (3H, s, Ester-C*H*$_3$), 4.29 (1H, ddd, *J* = 13.0, *J* = 6.5, *J* = 2.5, Thr-C*H*), 4.36 (1H, dd, *J* = 7.7, *J* = 2.5, Thr-α-C*H*), 4.81 (1H, dt, *J* = 7.9, *J* = 5.4, Phe-α-C*H*), 6.49 (1H, br d, *J* = 7.7, Thr-N*H*), 7.12-7.14 (2H, m, Phe-Ar-*H*), 7.21-7.30 (4H, m, Phe-Ar-*H* /Phe-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 17.8 (Thr-CH_3), 23.0 (Acetyl-CH_3), 37.6 (Phe-CH_2), 52.5 (Ester-CH_3), 53.3 (Phe-α-CH), 56.3 (Thr-α-CH), 66.3 (Thr-CH), 127.1 (Ar-C), 128.6 (Ar-C), 129.1 (Ar-C), 135.7 (Ar-C), 171.0 (C=O), 171.0 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3273 m (N-H), 3062 s (C-H), 2928 s (C-H), 1737 s (Ester C=O), 1640 m (C=C), 1517 m (C=C), 1435 m (C-H), 1214 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{16}H_{23}N_2O_5$: 323.1607, found: 323.1605.

4.5.10 Ac-Cys-Phe-OMe (9j)



9j

Peptide **9j** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-cysteine (0.163 g, 1.00 mmol), using the procedure in **Section 4.2.1**. The crude compound was recrystallised from DCM / hexanes to afford **9j** as a cream solid (0.272 g, 84%); m.p. 121-123 °C.

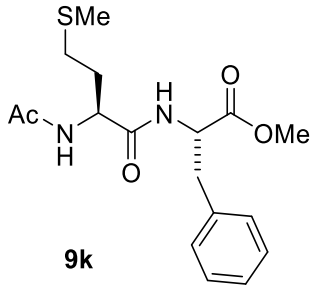
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66 (1H, dd, *J* = 10.1, *J* = 7.8, Cys-S*H*), 2.00 (3H, s, Acetyl-CH_3), 2.66 (1H, ddd, *J* = 14.2, *J* = 10.1, *J* = 6.9, Cys-C*H*H), 2.97-3.07 (2H, m, Cys-C*H*H / Phe-C*H*H), 3.19 (1H, dd, *J* = 13.7, *J* = 5.5, Phe-C*H*H), 3.76 (Ester-CH_3), 4.55-4.59 (1H, m, Cys-α-C*H*), 4.81-4.86 (1H, m, Phe-α-C*H*), 6.37 (1H, d, *J* = 7.8, Cys-N*H*), 6.69 (1H, d, *J* = 7.8, Phe-N*H*), 7.11-7.13 (2H, m, Phe-Ar-*H*), 7.23-7.32 (3H, m, Phe-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.1 (Acetyl-CH_3), 26.4 (Cys-CH_2), 37.6 (Phe-CH_2), 52.5 (Ester-CH_3), 53.4 (Phe-α-CH), 53.9 (Cys-α-CH), 127.3 (Ar-C), 128.7 (Ar-C), 129.2 (Ar-C), 135.5 (Ar-C), 169.4 (C=O), 170.1 (C=O), 171.5 (C=O).

IR U_{max} /cm^{-1} (solid) 3266 m (N-H), 3069 s (C-H), 2939 s (C-H), 1736 s (Ester C=O), 1635 m (C=C), 1541 m (C=C), 1481 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4S$: 325.1222, found: 325.1218.

4.5.11 Ac-Met-Phe-OMe (9k)



9k

Peptide **9k** was synthesised from L-phenylalanine methyl ester hydrochloride (0.539 g, 2.50 mmol) and *N*-acetyl-L-methionine (0.191 g, 1.00 mmol), using the procedure in **Section 4.2.1.** The crude compound was recrystallised from DCM / hexanes to afford **9k** as a white solid (0.268 g, 76%); m.p. 110-111 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89-2.03 (5H, m, Met-SC*H*$_3$ / Met-CH_2C*H*$_2$S), 2.04 (3H, s, Acetyl-C*H*$_3$), 2.47-2.60 (2H, m, Met-CHC*H*$_2$$CH_2$), 3.06 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.72 (3H, s, Ester-C*H*$_3$), 4.58 (1H, dt, *J* = 7.8, *J* = 6.9, Met-α-C*H*), 4.80-4.85 (1H, m, Phe-α-C*H*), 6.26 (1H, br d, *J* = 7.8, Met-N*H*), 6.68 (1H, br d, *J* = 8.2, Phe-N*H*), 7.10-7.12 (2H, m, Ar-*H*), 7.22-7.31 (3H, m, Ar-*H*).

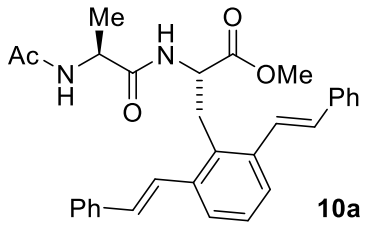
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 14.9 (Met-CH_3), 23.2 (Acetyl-CH_3), 29.9 (Met-SCH_2), 31.3 (Met-CH_2), 37.7 (Phe-CH_2), 51.8 (Met-α-CH), 52.4 (Ester-CH_3), 53.2 (Phe-α-CH), 127.2 (Ar-C), 128.7 (Ar-C), 129.2 (Ar-C), 135.5 (Ar-C), 169.9 (C=O), 170.8 (C=O), 171.5 (C=O).

IR υ_{max} /cm^{-1} (solid) 3287 m (N-H), 3061 w (C-H), 2952 w (C-H), 2914 w (C-H), 1750 s (Ester C=O), 1641 s (Amide C=O). 1537 m (C=C), 1367 m (C=C), 1158 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{25}N_2O_4S$: 353.1530, found: 353.1529.

4.6 Modification of Phe dipeptides 9a-f, 9k

4.6.1 Synthesis of modified peptide10a



10a

Modified peptide **10a** was prepared from Ac-Ala-Phe-OMe (**9a**) (0.105 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **10a** and the mono-olefinated peptide in a ratio of 8:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **10a** as an off-white solid (0.135 g, 76%); m.p. 193-194 °C, R_f 0.48 (EtOAc).

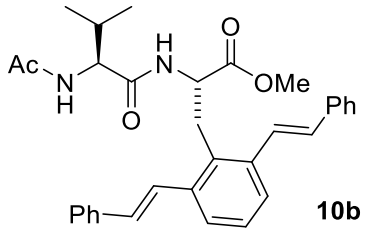
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (3H, d, *J* = 7.3, Ala-C*H*$_3$), 1.83 (3H, s, Acetyl-C*H*$_3$), 3.44 (2H, d, *J* = 7.3, Phe-C*H*$_2$), 3.56 (3H, s, CO_2C*H*$_3$), 4.38 (1H, dq, *J* = 7.3, *J* = 6.8, Ala-α-C*H*), 4.76 (1H, dq, *J* = 7.8, *J* = 7.3, Phe-α-C*H*), 6.07 (1H, br d, *J* = 7.3, Ala-N*H*), 6.65 (1H, br d, *J* = 7.8, Phe-N*H*), 7.01 (2H, d, *J* = 15.8, Alkene-C*H*), 7.26 - 7.31 (3H, m, Ar-*H*), 7.38 (4H, dt, *J* = 7.8, *J* = 7.3, Ar-*H*), 7.47 (2H, d, *J* = 15.8, Alkene-C*H*), 7.56 (2H, d, *J* = 7.8, Ar-*H*), 7.58 (4H, br d, *J* = 7.3, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.4 (Ala-CH_3), 22.9 (Acetyl-CH_3), 31.3 (Phe-CH_2), 48.6 (Ala-α-CH), 52.6 (Ester-CH_3), 53.1 (Phe-α-CH), 125.8 (Ar-C), 126.1 (Alkene-C=C), 126.7 (Ar-C), 127.6 (Ar-C), 127.9 (Ar-C), 128.7 (Ar-C), 131.8 (Ar-C), 131.9 (Alkene-C=C), 137.1 (Ar-C), 137.8 (Ar-C), 169.7 (C=O), 171.8 (C=O), 171.8 (C=O);

IR υ_{max} /cm^{-1} (solid) 3290 m (N-H), 3057 w (C-H), 2979 w (C-H), 1732 s (Ester C=O), 1632 m (C=C), 1532 m (C=C), 1260 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{31}H_{33}N_2O_4$: 497.2435 found: 497.2428.

4.6.2 Synthesis of modified peptide 10b



10b

Modified peptide **10b** was prepared from Ac-Val-Phe-OMe (**9b**) (0.115 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **10b** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10b** as an off-white solid (0.121 g, 64%); m.p. 210-213 °C; R_f 0.36 (25% EtOAc / pet. ether).

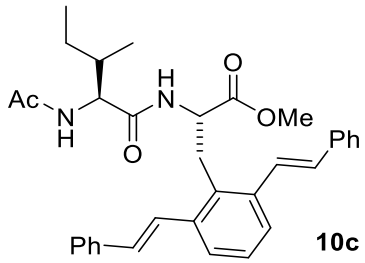
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (6H, dd, *J* = 6.8, *J* = 6.3, Val-(C*H*$_3$)$_2$), 1.88-1.96 (4H, m, Acetyl-C*H*$_3$ / Val-CH$_3$C*H*CH$_3$), 3.37 (1H, dd, *J* = 14.3, *J* = 8.5, Phe-C*H*H), 3.45 (1H, dd, *J* = 14.3, *J* = 6.5, Phe-C*H*H), 3.63 (3H, s, Ester-C*H*$_3$), 4.15 (1H, dd, *J* = 8.5, *J* = 6.5, Val-α-C*H*), 4.72 (1H, dt, *J* = 8.5, *J* = 6.5, Phe-α-C*H*), 5.99 (1H, br d, *J* = 6.5, Val-N*H*), 6.34 (1H, br d, *J* = 6.5, Phe-N*H*), 7.04 (2H, d, *J* = 15.9, Alkene-C*H*), 7.28-7.33 (3H, m, Ar-*H*), 7.39 (4H, t, *J* = 7.8, Ar-*H*), 7.49 (2H, d, *J* = 15.9, Alkene-C*H*), 7.55 (2H, d, *J* = 7.8, Ar-*H*), 7.61 (4H, d, *J* = 7.8, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.2 (Val-CH_3), 19.0 (Val-CH_3), 23.2 (Acetyl-CH_3), 31.4 (Val-CH), 35.4 (Phe-CH_2), 52.6 (Ester-CH_3), 53.3 (Phe-α-CH), 58.2 (Val-α-CH), 125.4 (Ar-C), 126.2 (Alkene-C=C), 126.6 (Ar-C), 127.8 (Ar-C), 128.0 (Ar-C), 128.9 (Ar-C), 130.6 (Ar-C), 131.3 (Alkene-C=C), 133.7 (Ar-C), 136.8 (Ar-C), 137.4 (Ar-C), 170.0 (C=O), 171.1 (C=O), 171.9 (C=O);

IR U_{max} /cm^{-1} (solid) 3268 m (N-H), 3060 w (C-H), 2926 w (C-H), 1736 s (Ester C=O), 1629 m (C=C), 1538 m (C=C), 1256 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{37}N_2O_4$: 525.2743, found: 525.2743.

4.6.3 Synthesis of modified peptide 10c



10c

Modified peptide **10c** was prepared from Ac-Ile-Phe-OMe (**9c**) (0.120 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **10c** and the mono-olefinated peptide in a ratio of 10:3. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10c** as an off-white solid (0.116 g, 60%); m.p. 198-200 °C, R_f = 0.34 (25% EtOAc / pet. ether).

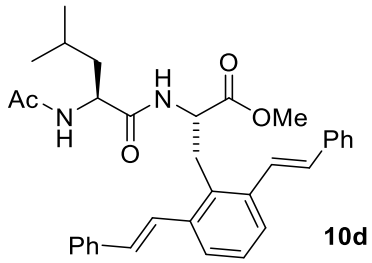
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73-0.77 (6H, m, Ile-(C*H*$_3$)$_2$), 0.96-1.04 (1H, m, Ile-C*H*HCH$_3$), 1.27-1.35 (1H, m, Ile-C*H*HCH$_3$), 1.63-1.70 (1H, m, Ile-CH$_2$C*H*CH$_3$), 1.87 (3H, s, Acetyl-C*H*$_3$), 3.37 (1H, dd, *J* = 14.5, *J* = 8.6, Phe-C*H*H), 3.45 (1H, dd, *J* = 14.5, *J* = 6.8, Phe-C*H*H), 3.63 (3H, s, Ester-C*H*$_3$), 4.16 (1H, dd, *J* = 8.6 Ile-α-C*H*), 4.70-4.75 (1H, m, Phe-α-C*H*), 5.95 (1H, br d, *J* = 8.6, Ile-N*H*), 6.28 (1H, br d, *J* = 6.8, Phe-N*H*), 7.05 (2H, d, *J* = 16.0, Alkene-C*H*), 7.28-7.33 (3H, m, Ar-*H*), 7.38-7.41 (4H, m, Ar-*H*), 7.49 (2H, d, *J* = 16.0, Alkene-C*H*), 7.56 (2H, d, *J* = 7.7, Ar-*H*), 7.61 (4H, br d, *J* = 7.7, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 11.4 (Ile-CH_3), 14.8 (Ile-$CHCH_3$), 23.2 (Acetyl-CH_3), 24.9 (Ile-CH_2), 31.3 (Phe-CH_2), 37.9 (Ile-CH), 52.6 (Ester-CH_3), 53.4 (Phe-α-CH), 57.4 (Ile-α-CH), 126.0 (Ar-C), 126.1 (Alkene-C=C), 126.8 (Ar-C), 127.8 (Ar-C), 128.0 (Ar-C), 128.8 (Ar-C), 131.7 (Alkene-C=C), 132.5 (Ar-H), 137.1 (Ar-C), 137.8 (Ar-C), 169.6 (C=O), 170.7 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3278 m (N-H), 3061 w (C-H), 2923 w (C-H), 1736 s (Ester C=O), 1632 m (C=C), 1538 m (C=C), 1256 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{34}H_{39}N_2O_4$: 539.2904, found: 539.2900.

4.6.4 Synthesis of modified peptide 10d



10d

Modified peptide **10d** was prepared from Ac-Leu-Phe-OMe (**9d**) (0.120 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **9d** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10d** as an off-white solid (0.128 g, 66%); m.p. 198-202 °C, R_f = 0.30 (50% EtOAc / pet. ether).

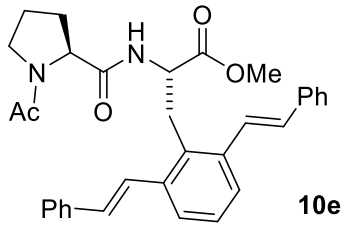
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (6H, dd, *J* = 6.1, *J* = 5.7, Leu-(C*H*$_3$)$_2$), 1.36-1.43 (1H, m, Leu-CH$_2$C*H*(CH$_3$)$_2$), 1.46-1.52 (2H, m, Leu-C*H*$_2$CH), 1.87 (3H, s, Acetyl-C*H*$_3$), 3.41 (1H, dd, *J* = 14.4, *J* = 7.6, Phe-C*H*H), 3.46 (1H, dd, *J* = 14.4, *J* = 7.6, Phe-C*H*H), 3.60 (3H, s, Ester-C*H*$_3$), 4.36 (1H, dt, *J* = 8.2, *J* = 5.4, Leu-α-C*H*), 4.76 (1H, dt, *J* = 7.7, *J* = 7.5, Phe-α-C*H*), 6.30 (1H, br d, *J* = 8.2, Leu-N*H*), 6.57 (1H, br d, *J* = 7.6, Phe-N*H*), 7.03 (2H, d, *J* = 16.0, Alkene-C*H*), 7.27-7.32 (3H, m, Ar-*H*), 7.39 (4H, t, *J* = 7.8, *J* = 7.3, Ar-*H*), 7.48 (2H, d, *J* = 16.0, Alkene-C*H*), 7.56 (2H, d, *J* = 7.8, Ar-*H*), 7.59 (4H, d, *J* = 7.3, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Acetyl-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 24.5 (Leu-CH), 31.4 (Phe-CH_2), 41.4 (Leu-CH_2), 51.6 (Leu-α-CH_3), 52.7 (Ester-CH_3), 53.3 (Phe-α-CH), 125.9 (Ar-C), 126.0 (Ar-C), 126.8 (Alkene-C=C), 127.7 (Ar-C), 128.0 (Ar-C), 128.8 (Ar-C), 131.7 (Alkene-C=C), 132.2 (Ar-C), 137.1 (Ar-C), 137.8 (Ar-C), 170.7 (C=O), 171.7 (C=O), 171.8 (C=O);

IR U_{max} /cm^{-1} (solid) 3260 m (N-H), 3021 w (C-H), 2964 w (C-H), 1728 s (Ester C=O), 1671 s (Amide C=O), 1650 m (C=C), 1555 m (C=C), 1207 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{34}H_{39}N_2O_4$: 539.2904, found: 539.2897.

4.6.5 Synthesis of modified peptide 10e



10e

Modified peptide **10e** was prepared from Ac-Pro-Phe-OMe (**9e**) (0.114 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **10e** and the mono-olefinated peptide in a ratio of 5:4. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10e** as an off-white solid (0.045 g, 24%); m.p. 194-197 °C, R_f 0.21 (50% EtOAc / pet. ether).

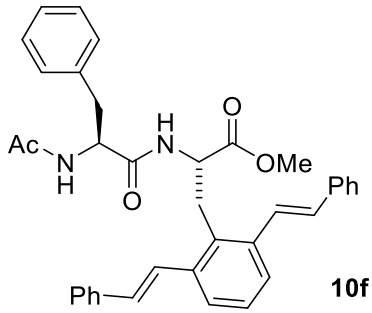
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63-1.68 (1H, m, Pro-CHC*H*HCH$_2$), 1.81-1.86 (2H, m, Pro-CH$_2$C*H*$_2$CH$_2$), 1.95 (3H, s, Acetyl-C*H*$_3$), 2.29-2.32 (1H, m, Pro-CHC*H*HCH$_2$), 3.24-3.36 (2H, m, Pro-NHC*H*$_2$CH$_2$), 3.42 (2H, d, *J* = 7.5, Phe-C*H*$_2$), 3.59 (3H, s, Ester-C*H*$_3$), 4.49-4.51 (1H, m, Pro-α-C*H*), 4.77 (1H, app q, *J* = 7.8, Phe-α-C*H*), 6.98 (2H, d, *J* = 15.8, Alkene-C*H*), 7.27-7.29 (3H, m, Ar-*H*), 7.35-7.39 (4H, m, Ar-*H*), 7.50 (2H, d, *J* = 15.8, Alkene-C*H*); 7.54-7.66 (6H, m, Ar-*H*), 7.71 (1H,d, *J* = 7.8, Phe-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.4 (Acetyl-CH_3), 24.9 (Pro-CH_2), 27.2 (Pro-CH_2), 31.7 (Phe-CH_2), 48.3 (Pro-CH_2), 52.7 (Ester-CH_3), 53.2 (Phe-α-CH), 59.6 (Pro-α-CH_3), 125.6 (Ar-C), 126.4 (Ar-C), 127.0 (Alkene-C=C), 127.5 (Ar-C), 127.9 (Ar-C), 128.8 (Ar-C), 131.8 (Ar-C), 132.4 (Ar-C), 137.5 (Alkene-C=C), 138.0 (Ar-C), 171.1 (C=O), 171.2 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3275 m (N-H), 2921 w (C-H), 1735 s (Ester C=O), 1630 m (C=C), 1536 m (C=C), 1255 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{35}N_2O_4$: 523.2591, found: 523.2589.

4.6.6 Synthesis of modified peptide 10f



10f

Modified peptide **10f** was prepared from Ac-Phe-Phe-OMe (**9f**) (0.132 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **9f** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10f** as an off-white solid (0.107 g, 52%); m.p. 202-204 °C; R_f 0.52 (50% EtOAc / pet. ether).

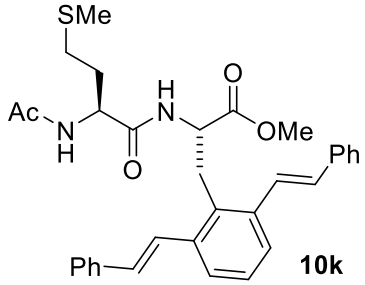
^{1}H NMR (400 MHz, $CDCl_3$) (* denotes the modified phenylalanine residue) δ 1.78 (3H, s, Acetyl-C*H*$_3$), 2.89 (1H, dd, *J* = 13.7, *J* = 7.3, Phe-C*H*H), 2.97 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.38* (2H, d, *J* = 7.3, Phe-C*H*$_2$), 3.52 (3H, s, Ester-C*H*$_3$), 4.54 (1H, dt, *J* = 7.7, *J* = 7.3, Phe-α-C*H*), 4.71* (1H, dt, *J* = 7.8, *J* = 7.3, Phe-α-C*H*), 5.88 (1H, br d, *J* = 7.7, Phe-N*H*), 6.18* (1H, br d, *J* = 7.8, Phe-N*H*), 6.99 (2H, d, *J* = 16.0, Alkene-C*H*), 7.03-7.05 (2H, m, Phe-Ar-*H*), 7.15-7.20 (3H, m, Phe-Ar-*H*), 7.27-7.32* (3H, m, Ar-*H*), 7.40* (4H, t, *J* = 7.8, Ar-*H*), 7.46 (2H, d, *J* = 16.0, Alkene-C*H*), 7.55* (2H, d, *J* = 7.8, Ar-*H*), 7.59* (4H, d, *J* = 7.8, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.0 (Acteyl-CH_3), 31.5 (Phe-CH_2), 38.3 (Phe-CH_2), 52.6 (Ester-CH_3), 53.1 (Phe-α-CH), 54.2 (Phe-α-CH), 125.8 (Ar-C), 126.1 (Alkene-C=C), 126.8 (Ar-C), 126.9 (Ar), 127.6 (Ar-C), 127.9 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 129.2 (Ar-C), 131.7 (Ar-C), 131.9 (Alkene-C=C), 136.6 (Ar-C), 137.2 (Ar-C), 137.8 (Ar-C), 169.7 (C=O), 170.3 (C=O), 171.4 (C=O);

IR U_{max} /cm^{-1} (solid) 3221 m (N-H), 3058 w (C-H), 2927 w (C-H), 1736 s (Ester C=O), 1636 m (C=C), 1537 m (C=C), 1214 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{37}H_{37}N_2O_4$: 573.2748, found: 573.2744.

4.6.7 Synthesis of modified peptide 10k



10k

Modified peptide **10k** was prepared from Ac-Met-Phe-OMe (**9k**) (0.127 g, 0.359 mmol) and styrene (0.166 mL, 1.440 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **10k** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (25% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **10k** as an off-white solid (0.120 g, 60%); m.p. 186-190 °C; R_f 0.38 (50% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81-1.90 (5H, m, Met-SC*H*$_3$ / Met-CH_2C*H*$_2$S), 1.93 (3H, s, Acetyl-C*H*$_3$), 2.38-2.48 (2H, m, Met-CHC*H*$_2$CH$_2$), 3.39 (1H, dd, *J* = 14.4, *J* = 8.2, Phe-C*H*H), 3.47 (1H, dd, *J* = 14.4, *J* = 6.6, Phe-C*H*H), 3.61 (3H, s, Ester-C*H*$_3$), 4.47 (1H, dt, *J* = 7.7, *J* = 6.9, Met-α-C*H*), 4.77 (1H, dt, *J* = 7.8, *J* = 6.6, Phe-α-C*H*), 6.13 (1H, br d, *J* = 7.7, Met-N*H*), 6.73 (1H, br d, *J* = 7.8, Phe-N*H*), 7.03 (2H, d, *J* = 16.0, Alkene-C*H*), 7.27-7.32 (3H, m, Ar-*H*), 7.39 (4H, app t, *J* = 7.8, *J* = 7.4, Ar-*H*), 7.48 (2H, d, *J* = 16.0, Alkene-C*H*), 7.56 (2H, d, *J* = 7.8, Ar-*H*), 7.60 (4H, br d, *J* = 7.4, Ar-*H*).

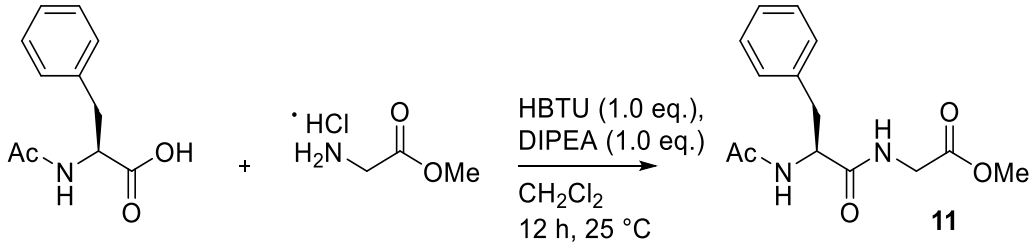
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 14.7 (Met-SCH_3), 23.0 (Acetyl-CH_3), 29.6 (Met-CH_2S), 31.3 (Met-CH_2), 31.5 (Phe-CH_2), 51.7 (Met-α-CH), 52.7 (Ester-CH_3), 53.2 (Phe-α-CH), 126.0 (Ar-C), 126.0 (Alkene-C=C), 126.8 (Ar-C), 127.7 (Ar-C), 128.8 (Ar-C), 131.7 (Ar-C), 132.2 (Alkene-C=C), 137.1 (Ar-C), 137.8 (Ar-C), 169.9 (C=O), 170.6 (C=O), 171.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3286 s (N-H), 3027 w (C-H), 2924 w (C-H), 1738 s (Ester C=O), 1679 s (Amide C=O), 1649 m (C=C), 1511 m (C=C), 1200 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{37}N_2O_4S$: 557.2469, found: 557.2464.

4.7 Synthesis of peptides bearing C-terminal glycine residues

4.7.1 Ac-Phe-Gly-OMe (11)



Glycine methyl ester hydrochloride (0.314 g, 2.50 mmol) was dissolved in H_2O (20 mL) before the addition of Amberlyst A21 ion exchange resin (3.140 g, 10.0 w/w). The suspension was stirred for 20 min at room temperature before filtration and concentration to dryness *in vacuo*. The resulting residue was slurried in DCM (30 mL), along with *N*-acetyl-L-phenylalanine (0.207 g, 1.00 mmol), HBTU (0.379 g, 1.00 mmol) and DIPEA (0.174 mL, 1.00 mmol) and stirred for 12 h. The resulting suspension was filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and H_2O (20 mL). The organic layers were then dried ($MgSO_4$) and concentration to dryness *in vacuo*. The resulting oil was recrystallised from DCM / hexanes to afford **4h** as a white solid (0.078 g, 28%); m.p. 129-130 °C.

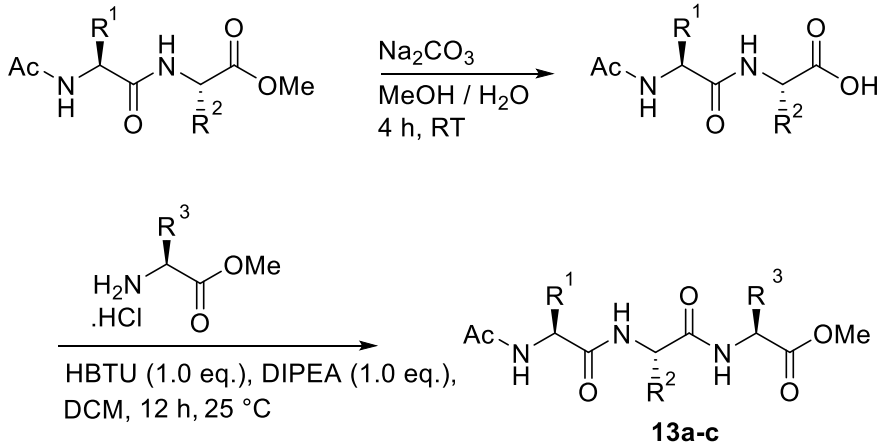
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (3H, s Acetyl-C*H*$_3$), 3.03-3.13 (2H, m, Phe-C*H*$_2$), 3.72 (3H, s, Ester-C*H*$_3$), 3.91 (1H, dd, *J* = 18.0, *J* = 5.3, Gly-C*H*H), 4.00 (1H, dd, *J* = 18.0, *J* = 5.3 Gly-C*H*H), 4.77 (1H, dt, *J* = 8.0, *J* = 7.3, Phe-α-C*H*), 6.49 (1H, br d, *J* = 8.0, Phe-N*H*), 6.80 (1H, br t, *J* = 5.3, Gly-N*H*), 7.20-7.31 (5H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.0 (Acetyl-CH_3), 38.1 (Phe-CH_2), 41.1 (Gly-CH_2), 52.3 (Ester-CH_3), 54.2 (Phe-α-CH), 126.9 (Ar-C), 128.6 (Ar-C), 129.2 (Ar-C), 136.4 (Ar-C), 169.8 (C=O), 170.3 (C=O), 171.4 (C=O).

IR U_{max} /cm^{-1} (solid) 3278 m (N-H), 3070 w (C-C), 2976 w (C-C), 2923 s (C-C), 1748 s (Ester C=O), 1657 s (Amide C=O), 1548 m (C=C), 1498 m (C=C), 1433 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{14}H_{19}N_2O_4$: 279.1339 found: 279.1334.

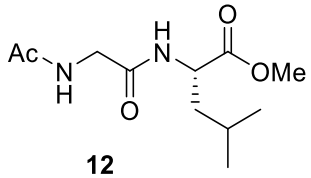
4.8 General procedure for the synthesis of phenylalanine containing tripeptides



13a-c

Sodium carbonate (0.206 g, 1.940 mmol) was dissolved in H_2O (15 mL) and added to a solution of the appropriate dipeptide ester (0.970 mmol) in MeOH (15 mL). The resulting suspension was stirred at room temperature for 4 h before acidification by the dropwise addition of 1M HCl (~3 mL). The aqueous solvent was then removed and the resulting solid washed with ethanol (3 x 10 mL), before the filtrate was evaporated to dryness. The crude dipeptide acid was then used without further purification. L-(amino acid) methyl ester hydrochloride (2.280 mmol) and K_2CO_3 (0.454 g, 3.283 mmol) were dissolved in distilled water (30 mL) and stirred for 10 min at room temperature. The free amine was extracted with Et_2O (3 x 20 mL), dried ($MgSO_4$) and concentrated to dryness. The resulting residue was slurried in DCM (20 mL) before the N-protected dipeptide (0.912 mmol), HBTU (0.346 g, 0.912 mmol) and DIPEA (0.158 mL, 0.912 mmol) were added. The suspension was then stirred for 16 h at room temperature. The suspension was then filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and water (20 mL). The organic layers were then dried ($MgSO_4$) and concentration to dryness *in vacuo*. The resulting residue was purified by column chromatography using EtOAc as eluent, before being recrystallised using DCM / hexanes.

4.8.1 Ac-Gly-Leu-OMe (12)



12

Peptide **12** was synthesised from L-leucine methyl ester hydrochloride (0.454 g, 2.50 mmol) and *N*-acetylglycine (0.117 g, 1.00 mmol), using the procedure in **Section 4.2.1**, to afford **12** as a yellow oil (0.171 g, 70%);

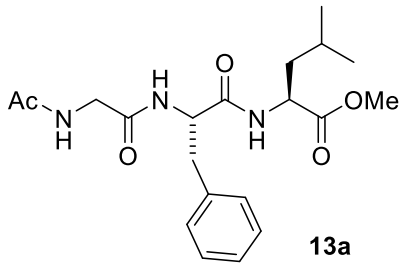
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (6H, app t, *J* = 6.2, Leu-(C*H*$_3$)$_3$), 1.47-1.63 (3H, m, Leu-C*H*C*H*$_2$), 1.95 (3H, s, Acetyl-C*H*$_3$), 3.64 (3H, s, Ester-C*H*$_3$), 3.89-3.99 (2H, m, Gly-C*H*$_2$), 4.44-4.49 (1H, m, Leu-α-C*H*), 7.36 (1H, br t, *J* = 5.3, Gly-N*H*), 7.67 (1H, d, *J* = 7.8, Leu-N*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.4 (Leu-$(CH_3)_3$), 22.5 (Ester-CH_3), 24.5 (Leu-CH), 40.5 (Leu-CH_2), 42.7 (Gly-CH_2), 50.7 (Leu-α-CH), 52.1 (Ester-CH_3), 169.4 (C=O), 171.1 (C=O), 173.2 (C=O);

IR U_{max} /cm^{-1} (oil) 3283 m (N-H), 3073 w (C-H), 2957 w (C-H), 2871 w (C-H), 1739 s (Ester C=O), 1646 s (Amide C=O), 1533 m (C=C), 1438 m (C-H), 1206 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{11}H_{21}N_2O_4$: 245.1496, found: 245.1496.

4.8.2 Ac-Gly-Phe-Leu-OMe (13a)



13a

Peptide **13a** was synthesised from Ac-Gly-Phe-OMe (**1a**) (0.270 g, 0.970 mmol) and L-leucine methyl ester hydrochloride (0.414 g, 2.280 mmol), using the procedure in **Section 4.8**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **13a** as a white solid (0.186 g, 52% over 2 steps); m.p. 180-181 °C**,** R_f 0.11 (EtOAc).

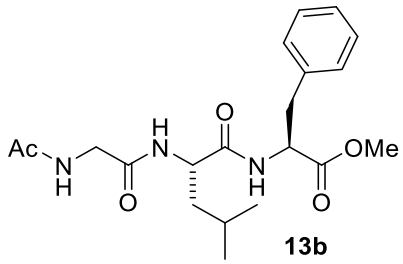
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (6H, m, Leu-(C*H*$_3$)$_2$), 1.49-1.56 (3H, m, Leu-CH_2C*H*CH_3)$_2$), 2.01 (3H, s, Acetyl-C*H*$_3$), 3.03-3.14 (2H, m, Phe-C*H*$_2$), 3.71 (3H, s, Ester-C*H*$_3$), 3.89 (2H, d, *J* = 5.5, Gly-C*H*$_2$), 4.49-4.55 (1H, m, Phe-α-C*H*), 4.68-4.74 (1H, dt, *J* = 7.8, *J* = 6.9, Leu-α-C*H*), 6.30 (1H, m, Gly-N*H*), 6.37 (1H, br d, *J* = 7.8, Phe-N*H*), 6.70 (1H, br d, *J* = 7.8, Leu-N*H*), 7.20-7.22 (2H, m, Ar-*H*), 7.24-7.31 (3H, m, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 22.4 (Leu-CH_3), 23.3 (Acetyl-CH_3), 25.8 (Leu-CH), 38.8 (Phe-CH_2), 41.4 (Leu-CH_2), 43.5 (Gly-CH_2), 52.2 (Ester-CH_3), 52.7 (Phe-α-CH), 55.6 (Leu-α-CH), 127.8 (Ar-C), 129.4 (Ar-C), 130.4 (Ar-C), 138.2 (Ar-C), 171.4 (C=O), 173.5 (C=O), 173.8 (C=O), 174.3 (C=O);

IR U_{max} /cm^{-1} (solid) 3278 m (N-H), 2975 w (C-H), 1748 s (Ester C=O), 1694 s (Amide C=O), 1530 m (C=C), 1435 m (C-H), 1224 s (C-O);

HRMS (ESI) $[M+H]^+$ m/z calcd. for $C_{20}H_{30}N_3O_5$: 392.2180, found: 392.2178.

4.8.3 Ac-Gly-Leu-Phe-OMe (13b)



13b

Peptide **13b** was synthesised from Ac-Gly-Leu-OMe (**12**) (0.237 g, 0.970 mmol) and L-phenylalanine methyl ester hydrochloride (0.499 g, 2.280 mmol), using the procedure in **Section 4.8**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **13b** as a white solid (0.164 g, 46% over 2 steps); m.p. 125-128 °C, R_f 0.11 (EtOAc).

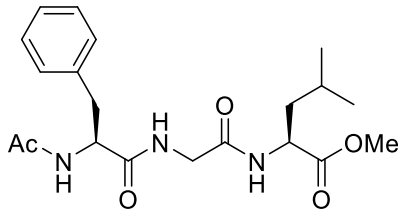
^{1}H NMR (400 MHz, CD_3OD) δ 0.89 (3H, d, *J* = 6.4, Leu-C*H*$_3$), 0.93 (3H, d, *J* = 6.4, Leu-C*H*$_3$), 1.47-1.51 (2H, m, Leu-C*H*$_2$), 1.59-1.63 (1H, m, Leu-CH_2C*H*$(CH_3)_2$), 2.00 (3H, s, Acetyl-C*H*$_3$), 2.97-3.03 (1H, m, Phe-C*H*H), 3.14 (1H, dd, *J* = 14.2, *J* = 6.0, Phe-C*H*H), 3.67 (3H, s, Ester-C*H*$_3$), 3.82 (2H, s, Gly-C*H*$_2$), 4.42 (1H, dd, *J* = 8.7, *J* = 6.4, Leu-α-C*H*), 4.62 (1H, dd, *J* = 8.7, *J* = 6.0, Phe-α-C*H*), 7.18-7.21 (2H,m, Ar-*H*), 7.25-7.29 (3H, m, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 22.0 (Leu-CH_3), 22.4 (Leu-CH_3), 23.4 (Acetyl-CH_3), 25.8 (Leu-CH), 38.2 (Phe-CH_2), 41.9 (Leu-CH_2), 43.5 (Gly-CH_2), 52.7 (Phe-α-CH), 52.9 (Ester-CH_3), 55.2 (Leu-α-CH), 127.9 (Ar-C), 129.5 (Ar-C), 130.3 (Ar-C), 138.1 (Ar-C), 171.5 (C=O), 173.2 (C=O), 173.9 (C=O), 174.6 (C=O);

IR U_{max} /cm^{-1} (solid) 3285 m (N-H), 2956 w (C-H), 1745 s (Ester C=O), 1694 s (Amide C=O), 1659 s (Amide C=O), 1531 m (C=C), 1435 m (C-H), 1210 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{30}N_3O_5$: 392.2180, found: 392.2181.

4.8.4 Ac-Phe-Gly-Leu-OMe (13c)



13c

Peptide **13c** was synthesised from Ac-Phe-Gly-OMe (**11**) (0.270 g, 0.970 mmol) and L-leucine methyl ester hydrochloride (0.414 g, 2.280 mmol), using the procedure in **Section 4.8**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **13c** as a white solid (0.157 g, 44% over 2 steps); m.p. 144-145 °C, R_f 0.14 (EtOAc).

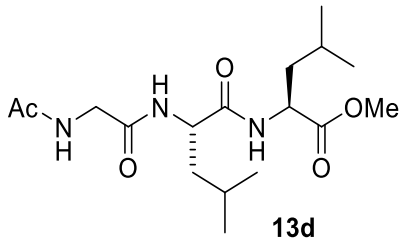
^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.94 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 1.56-1.67 (3H, m, Leu-C*H*$_2$C*H*$(CH_3)_2$), 1.91 (3H, s, Ester-C*H*$_3$), 2.92 (1H, dd, *J* = 13.7, *J* = 8.9, Phe-C*H*H), 3.13 (1H, dd, *J* = 13.7, *J* = 6.2, Phe-C*H*H), 3.65-3.70 (4H, m, Ester-C*H*$_3$ / Gly C*H*H), 3.93 (1H, d, *J* = 16.9, Gly-C*H*H), 4.44-4.50 (1H, m, Phe-α-C*H*), 7.19-7.30 (5H, m, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.6 (Acetyl-CH_3), 22.4 (Leu-CH_3), 23.4 (Leu-CH_3), 25.8 (Leu-CH), 38.2 (Phe-CH_2), 41.2 (Gly-CH_2), 43.3 (Leu-CH_2), 52.1 (Ester-CH_3), 52.7 (Leu-α-CH), 57.0 (Phe-α-CH), 127.8 (Ar-C), 129.5 (Ar-C), 130.2 (Ar-C), 138.4 (Ar-C), 171.6 (C=O), 173.6 (C=O), 174.4 (C=O), 174.6 (C=O);

IR U_{max} /cm^{-1} (solid) 3285 m (N-H), 2959 w (C-H), 1750 s (Ester C=O), 1692 s (Amide C=O), 1631 s (Amide C=O), 1436 m (C-H), 1228 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{29}N_3O_5$: 392.2180, found: 392.2181.

4.8.5 Ac-Gly-Leu-Leu-OMe (13d)



13d

Peptide **13d** was synthesised from Ac-Gly-Leu-OMe (**12**) (0.237 g, 0.970 mmol) and L-leucine methyl ester hydrochloride (0.414 g, 2.280 mmol), using the procedure in **Section 4.8**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **13d** as a white solid (0.235 g, 72% over 2 steps); m.p. 125-128 °C; Rf 0.19 (EtOAc).

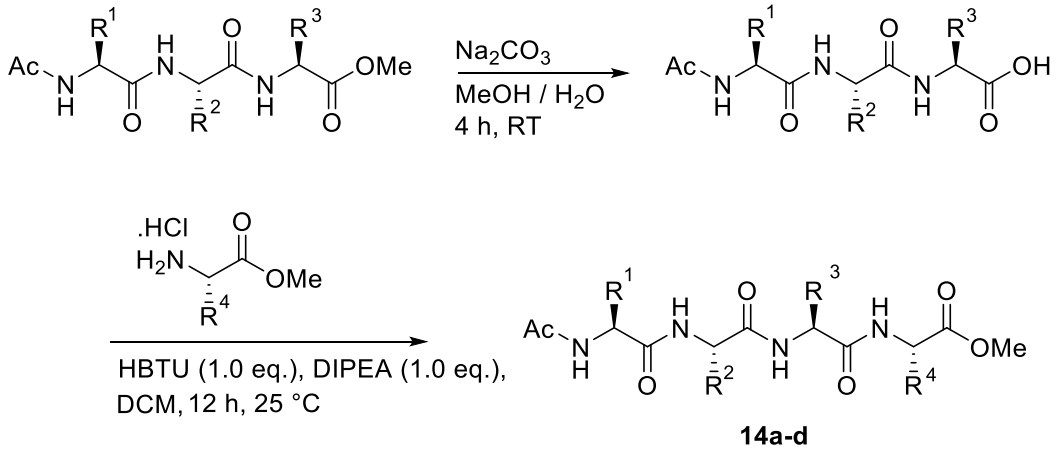
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91-0.95 (12H, m, (Leu-(C*H*$_3$)$_2$) x2), 1.52-1.69 (6H, m, (Leu-C*H*$_2$C*H*(CH$_3$)$_2$) x2), 2.04 (3H, s, Acetyl-C*H*$_3$), 3.73 (3H, s, Ester-C*H*$_3$), 3.93-4.03 (2H, m, Gly-C*H*$_2$), 4.52-4.58 (2H, m, (Leu-α-C*H*) x2), 6.62 (1H, br t, *J* = 5.0, Gly-N*H*), 6.72 (1H, br d, *J* = 8.2, Leu-N*H*), 6.86 (1H, br d, *J* = 8.7, Leu-N*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Acetyl-CH_3), 22.2 (Leu-CH_3), 22.7 (Leu-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 22.8 (Leu-CH_3), 24.6 (Leu-CH, 24.8 (Leu-CH), 40.9 (Leu-CH_2), 41.7 (Leu-CH_2), 43.2 (Gly-CH_2), 50.9 (Leu-α-CH), 51.7 (Leu-α-CH), 52.2 (Ester-CH_3), 169.0 (C=O), 170.9 (C=O), 172.1 (C=O), 173.2 (C=O);

IR U_{max} /cm^{-1} (solid) 3275 m (N-H), 3075 w (C-H), 2957 w (C-H), 1750 s (Ester C=O), 1691 s (Amide C=O), 1630 s (Amide C=O), 1532 m (C=C), 1438 m (C-H), 1202 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{41}N_4O_6$: 505.3021 found: 505.3020.

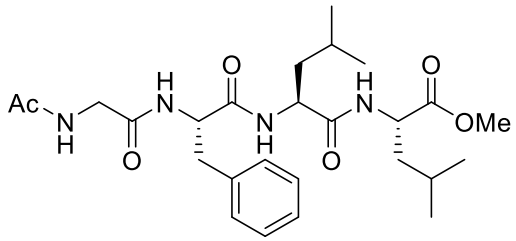
4.9 General procedure for the synthesis of phenylalanine containing tetrapeptides



14a-d

Sodium carbonate (0.068 g, 0.646 mmol) was dissolved in H_2O (10 mL) and added to a solution of the appropriate tripeptide ester (0.323 mmol) in MeOH (10 mL). The resulting suspension was stirred at room temperature for 4 h before acidification by the dropwise addition of 1M HCl (~3 mL). The aqueous solvent was then removed and the resulting solid washed with ethanol (3 x 10 mL), before the filtrate was evaporated to dryness. The crude tripeptide acid was carried through without further purification. The appropriate L-(amino acid) methyl ester hydrochloride (0.808 mmol) and K_2CO_3 (0.161 g, 1.162 mmol) were dissolved in distilled water (30 mL) and stirred for 10 mins at room temperature. The free amine was extracted with Et_2O (3 x 20 mL), dried ($MgSO_4$) and concentrated to dryness. The resulting residue was slurried in DCM (20 mL), before N-protected dipeptide (0.323 mmol), HBTU (0.122 g, 0.323 mmol) and DIPEA (0.056 mL, 0.323 mmol) were added. The suspension was then stirred for 16 h at room temperature. The suspension was then filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and water (20 mL). The organic layers were then dried ($MgSO_4$) and concentrated to dryness *in vacuo*. The resulting residue was purified by column chromatography using EtOAc as eluent, before being recrystallised using DCM / hexanes.

4.9.1 Ac-Gly-Phe-Leu-Leu-OMe (14a)



14a

Peptide **14a** was synthesised from Ac-Gly-Phe-Leu-OMe (**13a**) (0.126 g, 0.323 mmol) and L-leucine methyl ester hydrochloride (0.147 g, 0.808 mmol), using the general procedure in **Section 4.9**. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **14a** as a white solid (0.104 g, 64%); m.p. 179-181 °C, R_f 0.13 (10% MeOH / DCM).

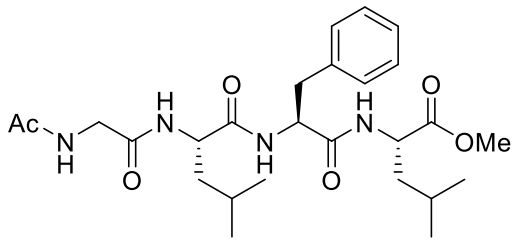
^{1}H NMR (400 MHz, CD_3CN) δ 0.80-0.87 (12H, m, Leu-(C*H*$_3$)$_2$ (x2)), 1.42-1.61 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.81 (3H, s, Acetyl-C*H*$_3$), 2.96 (1H, dd, *J* = 14.2, *J* = 8.2, Phe-C*H*H), 3.10 (1H, dd, *J* = 14.2, *J* = 5.0, Phe-C*H*H), 3.56 (1H, dd, *J* = 5.3, *J* = 2.6, Gly-C*H*H), 3.59 (4H, m, Ester-C*H*$_3$ / Gly-C*H*H), 4.19-4.29 (1H, m, Phe-α-C*H* / Leu-α-C*H*), 4.38-4.43 (1H, m, Leu-α-C*H*), 6.78 (1H, br d, *J* = 6.0, N*H*), 6.88 (2H, m, N*H*), 7.02 (1H, d, *J* = 8.7, N*H*), 7.16-7.28 (5H, m, Phe-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 22.0 (Leu-CH_3), 22.4 (Acetyl-CH_3), 23.4 (Leu-CH_3), 23.5 (Leu-CH_3), 25.7 (Leu-CH), 25.8 (Leu-CH), 38.3 (Phe-CH_2), 41.4 (Leu-CH_2), 41.8 (Leu-CH_2), 43.6 (Gly-CH_2), 52.6 (Ester-CH_3), 53.0 (Leu-α-CH), 53.2 (Leu-α-CH), 55.2 (Phe-α-CH), 127.9 (Ar-C), 129.5 (Ar-C), 130.3 (Ar-C), 138.0 (Ar-C), 171.9 (C=O), 173.2 (C=O), 173.9 (C=O), 174.6 (C=O), 174.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3279 m (N-H), 3067 w (C-H), 2956 w (C-H), 1748 s (Ester C=O), 1692 s (Amide C=O), 1630 m (C=C), 1526 m (C=C), 1439 m (C-H), 1203 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{41}N_4O_6$: 505.3021 found: 505.3013.

4.9.2 Ac-Gly-Leu-Phe-Leu-OMe (14b)



14b

Peptide **14b** was synthesised from Ac-Gly-Leu-Phe-OMe (**13a**) (0.126 g, 0.323 mmol) and L-leucine methyl ester hydrochloride (0.147 g, 0.808 mmol), using the general procedure in **Section 4.9**. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **14b** as a white solid (0.112 g, 69%); m.p. 190-193 °C, R_f 0.10 (10% MeOH / DCM).

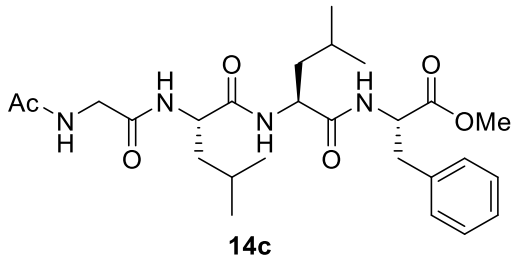
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86-0.98 (12H, m, Leu-(C*H*$_3$)$_2$ (x2)), 1.54-1.65 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.99 (3H, s, Acetyl-C*H*$_3$), 3.02-3.11 (2H, m, Phe-C*H*$_2$), 3.71 (3H, s, Ester-C*H*$_3$), 3.89-3.94 (1H, m, Gly-C*H*H), 4.06-4.13 (1H, m, Gly-C*H*H), 4.47-4.54 (1H, α-C*H*), 4.77-4.78 (1H, m, α-C*H*), 4.96-5.01 (1H, m, α-C*H*), 7.16-7.28 (7H, m, Phe-Ar-*H* / N*H* / N*H*), 7.54-7.59 (2H, m, N*H* (x2)).

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 21.9 (Leu-CH_3), 22.5 (Acetyl-CH_3), 23.1 (Leu-CH_3), 23.4 (Leu-CH_3), 25.5 (Leu-CH), 25.8 (Leu-CH), 38.4 (Phe-CH_2), 41.4 (Leu-CH_2), 41.7 (Leu-CH_2), 43.7 (Gly-CH_2), 52.2 (α-CH), 52.7 (Ester-CH_3), 53.3 (α-CH), 55.8 (α-CH), 127.7 (Ar-C), 129.4 (Ar-C), 130.4 (Ar-C), 138.5 (Ar-C), 171.9 (C=O), 173.4 (C=O), 174.2 (C=O), 174.4 (C=O), 174.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3277 m (N-H), 3073 w (C-H), 2956 w (C-H), 1745 s (Ester C=O), 1694 s (Amide C=O), 1630 m (C=C), 1526 m (C=C), 1438 m (C-H), 1205 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{41}N_4O_6$: 505.3021 found: 505.3017.

4.9.3 Ac-Gly-Leu-Leu-Phe-OMe (14c)



14c

Peptide **14c** was synthesised from Ac-Gly-Leu-Leu-OMe (**13d**) (0.155 g, 0.323 mmol) and L-phenylalanine methyl ester hydrochloride (0.174 g, 0.808 mmol), using the procedure in **Section 4.9**. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **14c** as a white solid (0.072 g, 44%); m.p. 181-184 °C, R_f 0.09 (10% MeOH / DCM).

^{1}H NMR (400 MHz, CD_3OD) δ 0.88-0.97 (12H, m, Leu-C*H*$_3$ (x2)), 1.46-1.65 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.98 (3H, s, Acetyl-C*H*$_3$), 2.98-3.03 (1H, m, Phe-C*H*H), 3.11-3.19 (1H, m, Phe-C*H*H), 3.66 (3H, s, Ester-C*H*$_3$), 3.83-3.86 (2H, m, Gly-C*H*$_2$), 4.29-4.40 (3H, m, Leu-α-C*H* / Leu-α-C*H*), 4.60-4.65 (1H, m, Phe-α-C*H*) 7.18-7.28 (5H, m, Phe-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 22.0 (Leu-CH_3), 22.4 (Acetyl-CH_3), 23.4 (Leu-CH_3), 23.5 (Leu-CH_3), 25.8 (Leu-CH), 25.8 (Leu-CH), 38.3 (Phe-CH_2), 41.4 (Leu-CH_2), 41.7 (Leu-CH_2), 43.7 (Gly-CH_2), 52.6 (Ester-CH_3), 53.2 (α-CH), 53.8 (α-CH), 55.2 (α-CH), 127.9 (Ar-C), 129.5 (Ar-C), 130.3 (Ar-C), 138.0 (Ar-C), 171.9 (C=O), 173.2 (C=O), 173.9 (C=O), 174.5 (C=O), 174.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3270 m (N-H), 3070 w (C-H), 2955 w (C-H), 1744 s (Ester C=O), 1692 s (Amide C=O), 1630 m (C=C), 1532 m (C=C), 1436 m (C-H), 1210 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{41}N_4O_6$: 505.3021 found: 505.3015

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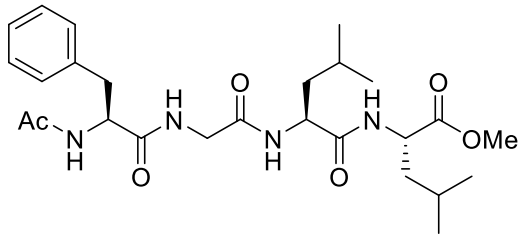
4.9.4 Ac-Phe-Gly-Leu-Leu-OMe (14d)



14d

Peptide **14d** was synthesised from Ac-Phe-Gly-Leu-OMe (**13c**) (0.126 g, 0.323 mmol) and L-leucine methyl ester hydrochloride (0.147 g, 0.808 mmol), using the procedure in **Section 4.9**. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **14d** as a white solid (0.065 g, 40%); m.p. 183-186 °C, R_f 0.09 (10% MeOH / DCM).

^{1}H NMR (400 MHz, CD_3OD) δ 0.89-0.97 (12H, m, Leu-C*H*$_3$ (x2)), 1.57-1.75 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.91 (3H, s, Acetyl-C*H*$_3$), 2.92 (1H, dd, *J* = 13.7, *J* = 8.8, Phe-C*H*H), 3.14 (1H, dd, *J* = 13.7, *J* = 6.2, Phe-C*H*H), 3.64-3.68 (4H, m, Ester-C*H*$_3$ / Gly-C*H*H), 3.91 (1H, d, *J* = 16.0, Gly-C*H*H), 4.37-4.48 (3H, m, Phe-α-C*H* / Leu-α-C*H* / Leu-α-C*H*), 7.19-7.32 (5H, m, Phe-Ar-*H*).

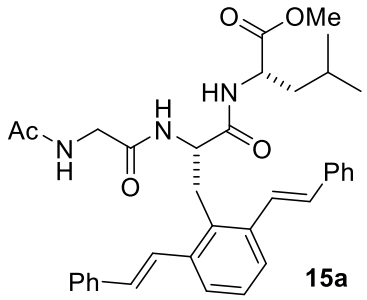
^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ 21.7 (Leu-CH_3), 21.8 (Leu-CH_3), 22.4 (Acetyl-CH_3), 23.4 (Leu-CH_3), 23.5 (Leu-CH_3), 25.7 (Leu-CH), 25.8 (Leu-CH), 38.3 (Phe-CH_2), 41.4 (Leu-CH_2), 41.8 (Leu-CH_2), 43.6 (Gly-CH_2), 52.1 (Leu-α-CH), 52.6 (Ester-CH_3), 53.0 (Leu-α-CH), 57.0 (Phe-α-CH), 127.8 (Ar-C), 129.5 (Ar-C), 130.2 (Ar-C), 138.4 (Ar-C), 171.4 (C=O), 173.4 (C=O), 174.5 (C=O), 174.9 (C=O), 175.2 (C=O).

IR υ_{max} /cm^{-1} (solid) 3280 m (N-H), 2956 w (C-H), 1747 s (Ester C=O), 1694 s (Amide C=O), 1630 m (C=C), 1532 m (C=C), 1436 m (C-H), 1222 s (C-O);

HRMS (ESI) $[M+H]^+$ m/z calcd. for $C_{26}H_{41}N_4O_6$: 505.3021 found: 505.3015.

4.10 Modification of phenylalanine containing tri- and tetra-peptides

4.10.1 Synthesis of modified peptide 15a



15a

Modified peptide **15a** was prepared from Ac-Gly-Phe-Leu-OMe (**13a**) (0.100 g, 0.255 mmol) and styrene (0.118 mL, 1.022 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **15a** and the mono-olefinated peptide in a ratio of 4:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **15a** as an off-white solid (0.090 g, 59%); m.p. 206-207 °C, R_f 0.24 (EtOAc).

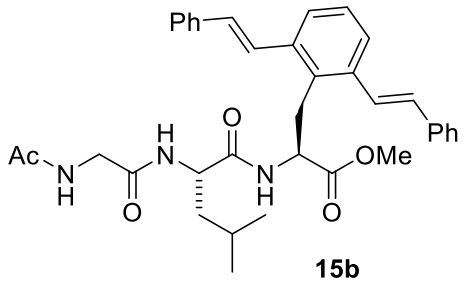
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.83 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 1.32-1.37 (1H, m, Leu-CH$_2$C*H*(CH$_3$)$_3$), 1.42-1.48 (2H, m, Leu-C*H*$_2$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.34-3.46 (2H, m, Phe-C*H*$_2$), 3.76 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 3.85 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 4.43 (1H, dt, *J* = 7.4, *J* = 5.5, Leu-α-C*H*), 4.60 (1H, dt, *J* = 7.9, *J* = 7.4, Phe-α-C*H*), 6.05 (1H, br d, *J* = 7.9, Phe-N*H*), 6.13 (1H, br t, *J* = 5.0, Gly-N*H*), 6.79 (1H, br d, *J* = 7.4, Leu-N*H*), 6.98 (2H, d, *J* = 16.0, Alkene-C*H*), 7.26-7.32 (3H, m, Ar-*H*), 7.37-7.41 (4H, m, Ar-*H*), 7.54-7.59 (4H, m, Alkene-C*H* / Ar-*H*), 7.61 (4H, d, *J* = 7.4, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.0 (Leu-CH_3), 22.4 (Leu-CH_2), 22.8 (Acetyl-CH_3), 24.6 (Leu-CH), 32.1 (Phe-CH_2), 41.5 (Gly-CH_2), 42.9 (Leu-CH_2), 51.1 (Phe-α-CH), 52.2 (Ester-CH_3), 54.4 (Leu-α-CH) 125.9 (Ar-C), 126.3 (Alkene-C=C), 126.8 (Ar-C), 127.6 (Ar-C), 128.0 (Ar-C), 128.8 (Ar-C), 131.9 (Alkene-C=C), 132.1 (Ar-C), 137.1 (Ar-C), 137.9 (Ar-C), 168.2 (C=O), 170.0 (C=O), 170.4 (C=O), 127.3 (C=O);

IR U_{max} /cm^{-1} (solid) 3276 m (N-H), 3062 w (C-H), 2928 w (C-H), 1741 s (Ester C=O), 1632 m (C=C), 1533 m (C=C), 1448 m (C-H), 1150 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{42}N_3O_5$: 596.3119 found: 596.3113.

4.10.2 Synthesis of modified peptide 15b



15b

Modified peptide **15b** was prepared from Ac-Gly-Leu-Phe-OMe (**13b**) (0.100 g, 0.255 mmol) and styrene (0.118 mL, 1.022 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **15b** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **15b** as an off-white solid (0.096 g, 63%); m.p. 204-207 °C, R_f 0.22 (EtOAc)

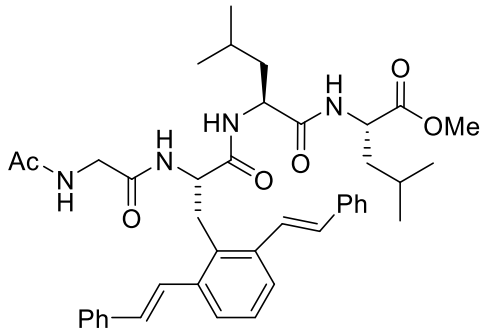
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.80 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 1.34-1.40 (1H, m, Leu-CH$_2$C*H*(CH$_3$)$_3$), 1.44-1.56 (2H, m, Leu-C*H*$_2$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.42 (2H, d, *J* = 7.7, Phe-C*H*$_2$), 3.57 (3H, s, Ester-C*H*$_3$), 3.63 (1H, dd, *J* = 16.5, *J* = 5.0, Gly-C*H*H), 3.71 (1H, dd, *J* = 16.5, *J* = 5.0, Gly-C*H*H), 4.39 (1H, dt, *J* = 5.5, *J* = 4.6, Leu-α-C*H*), 4.76 (1H, app q, *J* = 7.7, Phe-α-C*H*), 6.43 (1H, br d, *J* = 8.3, Leu-N*H*), 6.48 (1H, m, Gly-N*H*), 6.92 (1H, br d, *J* = 7.7, Phe-N*H*), 6.99 (2H, d, *J* = 16.0, Alkene-C*H*), 7.25-7.29 (3H, m, Ar-*H*), 7.35-7.39 (4H, m, Ar-*H*), 7.48-7.55 (4H, m, Alkene-C*H* / Ar-*H*), 7.58 (4H, d, *J* = 7.7, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.7 (Acetyl-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 24.6 (Leu-CH), 31.4 (Phe-CH_2), 41.0 (Gly-CH_2), 43.2 (Leu-CH_2), 51.7 (Leu-α-CH), 52.6 (Ester-CH_3), 53.1 (Phe-α-CH), 125.8 (Ar-C), 126.3 (Alkene-C=C), 126.8 (Ar-C), 127.6 (Ar-C), 127.9 (Ar-C), 128.8 (Ar-C), 131.8 (Alkene-C=C), 132.1 (Ar-C), 137.2 (Ar-C), 137.7 (Ar-C), 168.8 (C=O), 170.8 (C=O), 171.4 (C=O), 171.9 (C=O);

IR U_{max} /cm^{-1} (solid) 3275 s (N-H), 3062 w (C-H), 2954 w (C-H), 1743 s (Ester C=O), 1634 m (C=C), 1527 m (C=C), 1207 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{42}N_3O_5$: 596.3119 found: 596.3114.

4.10.3 Synthesis of modified peptide 16a



16a

Modified peptide **16a** was prepared from Ac-Gly-Phe-Leu-Leu-OMe (**14a**) (0.060 g, 0.119 mmol) and styrene (0.055 mL, 0.476 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **16a** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **16a** as an off-white solid (0.025 g, 30%); m.p. 206-209 °C, R_f 0.15 (10% MeOH / DCM)

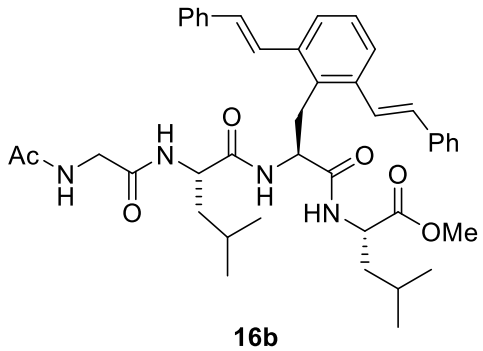
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76-0.83 (12H, m, Leu-(C*H*$_3$)$_2$ (x2)), 1.42-1.52 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.35-3.40 (1H, m, Phe-C*H*H), 3.45-3.56 (2H, m, Phe-C*H*H / Gly-C*H*H), 3.59 (3H, s, Ester-C*H*$_3$), 3.66 (1H, d, *J* = 15.6, Gly-C*H*H), 4.37-4.46 (2H, m, α-C*H* (x2)), 4.73 (1H, m, α-C*H*), 6.94 (2H, d, *J* = 15.6, Alkene-C*H*), 7.22-7.25 (2H, m, Ar-*H*), 7.35 (5H, m, Ar-*H*), 7.50 (2H, d, *J* = 7.3, Ar-*H*), 7.58-7.63 (6H, m, Alkene-C*H* / Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 22.0 (Leu-CH_3), 22.6 (Acetyl-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 24.7 (Leu-CH), 24.7 (Leu-CH), 31.9 (Phe-CH_2), 41.1 (Leu-CH_2), 41.6 (Leu-CH_2), 43.3 (Gly-CH_2), 51.1 (Ester-CH_3), 52.0 (Leu-α-CH), 52.1 (Leu-α-CH), 54.5 (Phe-α-CH), 125.9 (Ar-C), 126.8 (Ar-C), 126.9 (Ar-C), 127.5 (Ar-C),127.9 (Ar-C), 128.8 (Ar-C), 131.8 (Ar-C), 132.7 (Ar-C), 137.2 (Ar-C), 138.0 (Ar-C), 168.8 (C=O), 170.6 (C=O), 170.6 (C=O), 171.4 (C=O), 172.4 (C=O).

IR U_{max} /cm^{-1} (solid) 3272 m (N-H), 3068 w (C-H), 2957 w (C-H), 1747 s (Ester C=O), 1693 s (Amide C=O), 1626 m (amide C=O), 1521 m (C=C), 1438 m (C-H), 1203 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{53}N_4O_6Na$: 731.3779 found: 731.3782.

4.10.4 Synthesis of modified peptide 16b



16b

Modified peptide **16b** was prepared from Ac-Gly-Leu-Phe-Leu-OMe (**14b**) (0.060 g, 0.119 mmol) and styrene (0.055 mL, 0.476 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **16b** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **16b** as an off-white solid (0.036 g, 43%); m.p. 208-211 °C, R_f 0.15 (10% MeOH / DCM)

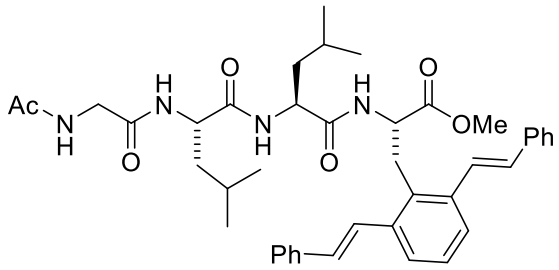
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77-0.85 (12H, m, Leu-(C*H*$_3$)$_2$ (x2)), 1.45-1.52 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.92 (3H, s, Acetyl-C*H*$_3$), 3.36-3.42 (1H, m, Phe-C*H*H), 3.46-3.56 (2H, m, Phe-C*H*H / Gly-C*H*H), 3.60 (3H, s, Ester-C*H*$_3$), 3.74-3.79 (1H, m, Gly-C*H*H), 4.37 (1H, m, α-C*H*), 4.43-4.47 (1H, m, α-C*H*) 4.74 (1H, m, α-C*H*), 6.95 (2H, d, *J* = 15.9, Alkene-C*H*), 7.23-7.25 (2H, m, Ar-*H*), 7.35 (5H, t, *J* = 7.4, Ar-*H*), 7.51 (2H, d, *J* = 7.8, Ar-*H*), 7.59-7.65 (6H, m, Alkene-C*H* / Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 22.0 (Leu-CH_3), 22.6 (Acetyl-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 24.6 (Leu-CH), 24.7 (Leu-CH), 31.9 (Phe-CH_2), 41.1 (Leu-CH_2), 41.5 (Leu-CH_2), 43.3 (Gly-CH_2), 51.1 (Ester-CH_3), 52.0 (Leu-α-CH), 52.1 (Leu-α-CH), 54.5 (Phe-α-CH), 125.9 (Ar-C), 126.7 (Ar-C), 126.9 (Ar-C), 127.5 (Ar-C),127.9 (Ar-C), 128.8 (Ar-C), 131.8 (Ar-C), 132.7 (Ar-C), 137.2 (Ar-C), 137.9 (Ar-C), 168.9 (C=O), 170.6 (C=O), 170.8 (C=O), 171.5 (C=O), 172.4 (C=O).

IR U_{max} /cm^{-1} (solid) 3275 m (N-H), 3062 w (C-H), 2957 w (C-H), 2923 w (C-H), 1742 s (Ester C=O), 1628 m (amide C=O), 1532 m (C=C), 1449 m (C-H), 1259 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{53}N_4O_6$: 709.3965 found: 709.3964.

4.10.5 Synthesis of modified peptide 16c



16c

Modified peptide **16c** was prepared from Ac-Gly-Leu-Leu-Phe-OMe (**14c**) (0.060 g, 0.119 mmol) and styrene (0.055 mL, 0.476 mmol), using the general procedure in **Section 4.3**. The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **16c** and the mono-olefinated peptide in a ratio of 5:1. Purification by flash column chromatography (10% MeOH / DCM) followed by recrystallisation from DCM / hexanes gave **16**c as an off-white solid (0.034 g, 40%); m.p. 205-209 °C, R_f 0.19 (10% MeOH / DCM).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.87 (12H, m, Leu-(C*H*$_3$)$_2$ (x2)), 1.42-1.54 (6H, m, Leu-C*H*$_2$ / Leu-CH$_2$C*H*(CH$_3$)$_2$ (x2)), 1.93 (3H, s, Acetyl-C*H*$_3$), 3.34-3.41 (1H, m, Phe-C*H*H), 3.43-3.49 (1H, m, Phe-C*H*H), 3.59 (3H, s, Ester-C*H*$_3$), 3.64-3.68 (2H, m, Gly-C*H*$_2$), 4.36 (1H, m, α-C*H*), 4.43-4.44 (1H, m, α-C*H*), 4.69-4.71 (1H, m, α-C*H*), 6.94 (2H, d, *J* = 16.0, Alkene-C*H*), 7.09-7.06 (2H, m, Ar-*H*), 7.21-7.23 (2H, m, Ar-*H*), 7.34 (5H, t, *J* = 7.4, Ar-*H*), 7515 (2H, d, *J* = 7.8, Ar-*H*), 7.58-7.63 (6H, m, Alkene-C*H* / Ar-*H*).

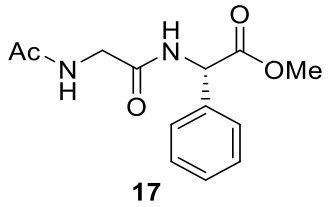
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 21.9 (Leu-CH_3), 22.6 (Acetyl-CH_3), 22.8 (Leu-CH_3), 23.0 (Leu-CH_3), 24.5 (Leu-CH), 24.6 (Leu-CH), 31.7 (Phe-CH_2), 40.7 (Leu-CH_2), 41.3 (Leu-CH_2), 43.3 (Gly-CH_2), 50.9 (Ester-CH_3), 51.8 (Leu-α-CH), 52.3 (Leu-α-CH), 53.9 (Phe-α-CH), 125.7 (Ar-C), 126.4 (Ar-C), 126.9 (Ar-C), 127.4 (Ar-C),127.9 (Ar-C), 128.7 (Ar-C), 131.9 (Ar-C), 132.3 (Ar-C), 137.2 (Ar-C), 137.9 (Ar-C), 169.2 (C=O), 170.6 (C=O), 170.8 (C=O), 171.6 (C=O), 173.3 (C=O).

IR U_{max} /cm^{-1} (solid) 3274 m (N-H), 3062 w (C-H), 2956 w (C-H), 2920 w (C-H), 1742 s (Ester C=O), 1630 m (amide C=O), 1533 m (C=C), 1450 m (C-H), 1203 s (C-O);

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{53}N_4O_6$: 709.3965 found: 709.3958.

4.11 Preparation of unnatural phenylalanine derived peptides

4.11.1 Ac-Gly-Phg-OMe (17)



17

Peptide **17** was synthesised from (*S*)-(+)-2-phenylglycine methyl ester hydrochloride (0.504 g, 2.50 mmol) and *N*-acetylglycine (0.117 g, 1.00 mmol), using the procedure in **Section 4.2.1.** The crude compound was recrystallised from DCM / hexanes to afford **17** as a yellow solid (0.159 g, 60%); m.p. 145-147 °C.

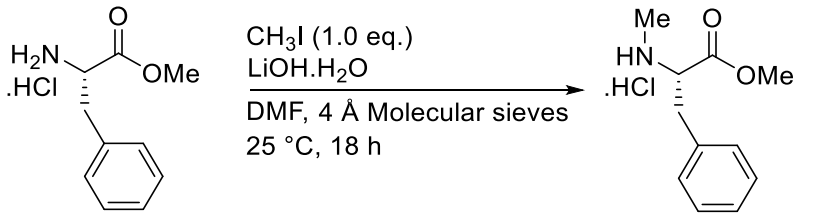
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (3H, s, Acetyl-C*H*$_3$), 3.72 (3H, s, Ester-C*H*$_3$), 3.96 (1H, dd, *J* = 16.7, *J* = 5.2, Gly-C*H*H), 4.05 (1H, dd, *J* = 16.7, *J* = 5.2, Gly-C*H*H), 5.53 (1H, d, *J* = 6.9, Phg-α-C*H*), 6.56 (1H, m, Gly-N*H*), 7.32-7.36 (5H, m, Phg-Ar-*H*), 7.48 (1H, br d, *J* = 6.9, Phg-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 43.0 (Gly-CH_2), 52.8 (Ester-CH_3), 56.6 (Phg-α-CH), 127.3 (Ar-C), 128.6 (Ar-C), 128.9 (Ar-C), 135.8 (Ar-C), 168.6 (C=O), 170.8 (C=O), 171.0 (C=O).

IR υ_{max} /cm^{-1} (solid) 3239 m (N-H), 3096 s (C-H), 2958 s (C-H), 1731 s (Ester C=O), 1627 m (C=C), 1543 m (C=C), 1228 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{13}H_{17}N_2O_4$: 265.1183, found: 265.1193.

4.11.2 N-methyl-l-phenylalanine methyl Ester hydrochloride (Me-Phe-OMe.HCl) (18)



18

Under an atmosphere of dry nitrogen, $LiOH.H_2O$ (0.418 g, 9.967 mmol) and 4 Å molecular sieves were stirred in anhydrous DMF (15 mL) for 20 min. L-phenylalanine methyl ester hydrochloride (1.000 g, 4.636 mmol) was added to the solution and stirred for a further 45 min before the addition of iodomethane (0.317 mL, 5.100 mmol). The resulting suspension was left to stir at room temperature for 18 h. The brown suspension was then filtered and the filter cake washed with EtOAc (50 mL). The filtrate was then washed with water (3 x 30 mL), dried ($MgSO_4$) and concentrated to dryness *in vacuo*. to give a yellow oil. The oil was purified by flash column chromatography using EtOAc as eluent to give Me-Phe-OMe.HCl (**18**) as a pale-yellow oil (0.682 g, 64%), R_f 0.33 (EtOAc).

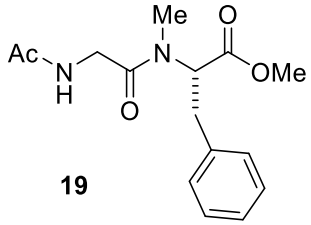
^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (3H, s, C*H*$_3$), 2.90 (2H, d, *J* = 6.7, Phe-C*H*$_2$) 3.39 (1H, t, *J* = 6.7, Phe-α-C*H*), 3.60 (3H, s, Ester-C*H*$_3$), 7.11-7.13 (2H, m, Ar-*H*), 7.16-7.19 (1H, m, Ar-*H*), 7.21-7.25 (2H, m, Ar-*H*).

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 34.4 (NCH_3), 39.2 (Phe-CH_2), 51.3 (ster-CH_3), 64.4 (Phe-α-CH), 126.4 (Ar-C), 128.2 (Ar-C), 128.8 (Ar-C), 136.9 (Ar-C), 174.5 (C=O).

IR U_{max}/cm^{-1} (oil) 3028 s (C-H), 2949 s (C-H), 1732 s (Ester C=O), 1454 m (C-H).

HRMS (ESI) $[M+H]^+$[-HCl] *m/z* calcd. for $C_{11}H_{16}NO_2$: 194.1176 found: 194.1174.

4.11.3 Ac-Gly-(*N*-Me)Phe-OMe (19)



19

Peptide **19** was synthesised from *N*-methyl-L-phenylalanine methyl ester hydrochloride (0.574 g, 2.50 mmol) and *N*-acetylglycine (0.173 g, 1.00 mmol), using the procedure in **Section 4.2.1**. Purification by flash column chromatography (EtOAc) afforded peptide **19** as a yellow oil (0.164 g, 56%), R_f 0.18 (EtOAc).

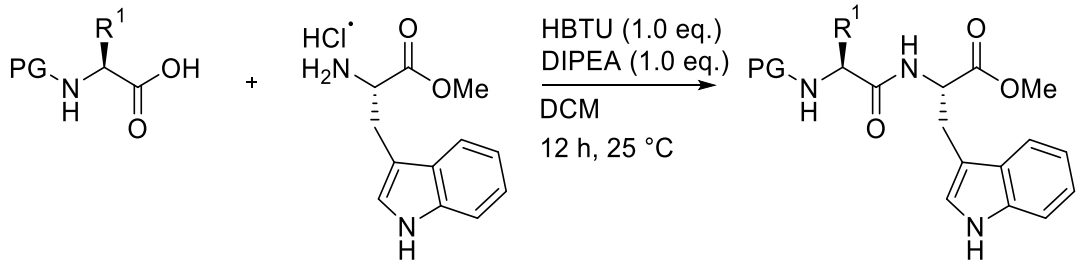
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (3H, s, Acetyl-C*H*$_3$), 2.80 (3H, s, NC*H*$_3$), 3.04 (1H, dd, *J* = 14.4, *J* = 11.0, Phe-C*H*H), 3.35 (1H, dd, *J* = 14.4, *J* = 5.3, Phe-C*H*H), 3.75 (3H, s, Ester-C*H*$_3$), 3.86 (1H, dd, *J* = 17.7, *J* = 4.0, Gly-C*H*H), 4.02 (1H, dd, *J* = 17.7, *J* = 4.0, Gly-C*H*H), 5.22 (1H, dd, *J* = 11.0, *J* = 5.3, Phe-α-C*H*), 6.55 (1H, m, Gly-N*H*), 7.16 (2H, br d, *J* = 7.3, Ar-*H*), 7.20-7.32 (3H, m, Ar-*H*);

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 31.9 (NCH_3), 34.4 (Phe-CH_2), 41.4 (Gly-CH_2), 52.4 (Ester-CH_3), 58.9 (Phe-α-CH), 126.9 (Ar-C), 128.5 (Ar-C), 128.6 (Ar-C), 136.5 (Ar-C), 168.8 (C=O), 170.0 (C=O), 170.6 (C=O);

IR U_{max} /cm^{-1} (oil) 3327 m (N-H), 2952 w (C-H), 1737 s (Ester C=O), 1636 s (Amide C=O), 1496 m (C=C), 1260 s (C-O);

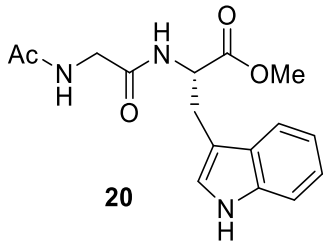
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{15}H_{21}N_2O_4$: 293.1496 found: 293.1496.

4.12 General procedure for the preparation of tryptophan containing peptides



L-Tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and K_2CO_3 (0.498 g, 3.60 mmol) were dissolved in distilled water (30 mL) and stirred for 10 min at room temperature. The free amine was extracted with DCM (3 x 20 mL), dried ($MgSO_4$) and concentrated by rotary evaporation. The resulting oil was dissolved in DCM (20 mL); the appropriate *N*-protected amino acid (1.00 mmol), HBTU (0.379 g, 1.00 mmol) and DIPEA (0.174 mL, 1.00 mmol) were then added to the reaction mixture, which was stirred for 12 h. The resulting suspension was filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and H_2O (20 mL). The organic layers were then dried ($MgSO_4$) and concentrated to dryness *in vacuo*. The resulting oil was recrystallized from DCM / hexanes.

4.12.1 Ac-Gly-Trp-OMe (20)



20

Peptide **20** was synthesised from L-tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and *N*-acetylglycine (0.117 g, 1.00 mmol), using the procedure in **Section 4.12.** The crude compound was recrystallised from DCM / hexanes to afford **20** as an orange solid (0.279 g, 88%); m.p. 177-180 °C.

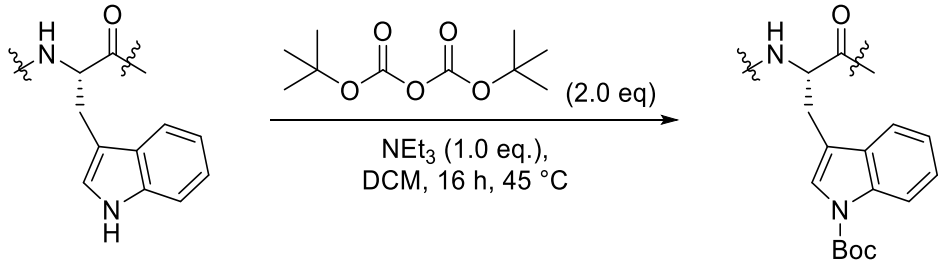
^{1}H NMR (400 MHz, CD_3OD) δ 1.93 (3H, s, Acetyl-C*H*$_3$), 3.21 (1H, dd, *J* = 14.6, *J* = 7.4, Trp-CH*H*), 3.26-3.31 (1H, m, Trp-CH*H*), 3.65 (3H, s, Ester-C*H*$_3$), 3.81 (2H, d, *J* = 4.1, Gly-C*H*$_2$), 4.74 (1H, app q, *J* = 7.4, Trp-α-C*H*), 6.99-7.03 (1H, m, Trp-Ar-*H*), 7.06-7.10 (2H, m, Trp-Ar-*H*), 7.30-7.33 (1H, m, Trp-Ar-*H*), 7.48-7.50 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 22.4 (Acetyl-CH_3), 28.4 (Trp-CH_2), 43.4 (Gly-CH_2), 52.7 (Ester-CH_3), 54.8 (Trp-α-CH), 110.3 (Ar-C), 112.3 (Ar-CH), 119.1 (Ar-CH), 119.9 (Ar-CH), 122.5 (Ar-CH), 124.6 (Ar-CH), 128.7 (Ar-C), 138.0 (Ar-C), 171.4 (C=O), 173.7 (C=O), 173.8 (C=O).

IR υ_{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).

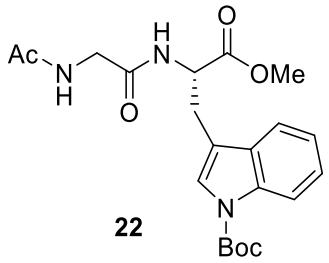
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{16}H_{20}N_3O_4$: 318.1448, found: 318.1451.

4.13 General procedure for the Boc protection of tryptophan residues



Tryptophan containing peptides (0.75 mmol) was dissolved in DCM (20 mL) and treated with NEt_3 (0.105 mL, 0.75 mmol). A solution of Boc anhydride (0.327 g, 1.50 mmol) in DCM (5 mL) was added dropwise to the peptide solution, and heated under reflux for 16 h. The solution was allowed to cool to room temperature, before concentration to dryness by rotary evaporation. The crude compound was purified by flash column chromatography and the resulting residue was recrystallised from DCM / hexanes to afford the Boc protected peptide.

4.13.1 Ac-Gly-Trp(Boc)-OMe (22)



22

Peptide **22** was synthesised from Ac-Gly-Trp-OMe (**20**) (0.238 g, 0.75 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **20** as an off-white solid (0.276 g, 88%); m.p. 92-95 °C, R_f 0.16 (EtOAc).

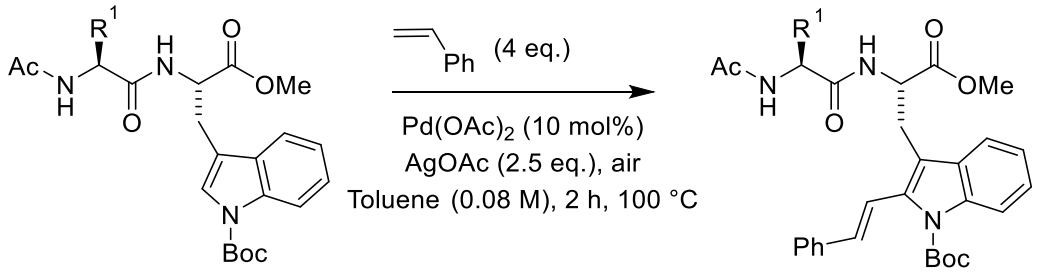
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66 (9H, s, Boc-(C*H*$_3$)$_3$), 1.96 (3H, s, Acetyl-C*H*$_3$), 3.19 (1H, dd, *J* = 14.6, *J* = 6.0, Trp-CH*H*), 3.27 (1H, dd, *J* = 14.6, *J* = 6.0, Trp-CH*H*), 3.69 (3H, s, Ester-C*H*$_3$), 3.83-3.94 (2H, m, Gly-C*H*$_2$), 4.90 (1H, dd, *J* = 7.8, *J* = 6.0, Trp-α-C*H*), 6.48 (1H, br t, *J* = 5.0, Gly-N*H*), 6.94 (1H, br d, *J* = 7.8, Trp-N*H*), 7.22 (1H, t, *J* = 7.7, Trp-Ar-*H*), 7.30 (1H, t, *J* = 6.9, Trp-Ar-*H*), 7.41 (1H, s, Trp-Ar-*H*), 7.47 (1H, d, *J* = 7.7, Trp-Ar-*H*), 8.08 (1H, br d, *J* = 6.9, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 27.3 (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 (Ar-C), 115.3 (Ar-C), 118.6 (Ar-C), 122.6 (Ar-C), 124.2 (Ar-C), 124.6 (Ar-C), 130.3 (Ar-C), 135.2 (Ar-C), 149.5 (C=O), 168.8 (C=O), 170.6 (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).

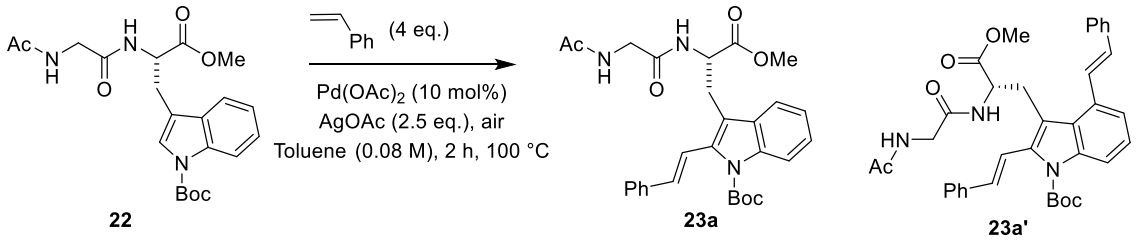
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{21}H_{28}N_3O_6$: 418.1973, found: 418.1973.

4.14 General procedure for the olefination of tryptophan containing peptides



Tryptophan containing peptides (0.238 mmol), $Pd(OAc)_2$ (5 mg, 0.024 mmol, 10 mol%), AgOAc (0.100 g, 0.599 mmol) and styrene (0.109 mL, 0.952 mmol) were stirred together in toluene (3 mL) at 100 °C for 2 h. The reaction was then allowed to cool to room temperature and filtered through a plug of Celite© before the filtrate was concentrated to dryness. The resulting residue was purified by column chromatography and recrystallized in DCM / hexanes.

4.14.1 Synthesis of modified peptide 23a



Following the general procedure in **Section 4.14,** the reaction of Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol), $Pd(OAc)_2$ (5 mg, 0.024 mmol, 10 mol%), AgOAc (0.100 g, 0.599 mmol) and styrene (0.109 mL, 0.952 mmol) gave a crude product that was a mixture of the mono-olefinated peptide **23a** and the di-olefinated peptide **23a'** in a ratio of 20:1, as judged by ^{1}H NMR spectroscopy. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23a** as an off-white solid (0.101 g, 82); m.p. 116-118 °C, R_f 0.26 (EtOAc) and **23a''** as an off-white solid (0.002 g, 1%); m.p. 98-99 °C, R_f 0.42 (EtOAc).

Data for **23a**:

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.92 (3H, s, Acetyl-C*H*$_3$), 3.38 (1H, dd, *J* = 14.5, *J* = 6.6, Trp-C*H*H), 3.47 (1H, dd, *J* = 14.5, *J* = 6.6, Trp-C*H*H), 3.55 (3H, s, Ester-C*H*$_3$), 3.74 (1H, dd, *J* = 16.7, *J* = 5.0, Gly-C*H*H), 3.84 (1H, dd, *J* = 16.7, *J* = 5.0, Gly-C*H*$_2$), 4.89 (1H, dt, *J* = 7.6, *J* = 6.6, Trp-α-C*H*), 5.89 (1H, br t, *J* = 5.0, Gly-N*H*), 6.41 (1H, br d, *J* = 7.6, Trp-N*H*), 6.78 (1H, d, *J* = 16.6, alkene-C*H*), 7.27-7.34 (4H, m, alkene-C*H* / Ar-*H*), 7.39 (2H, t, *J* = 7.6, Ar-*H*), 7.51-7.56 (3H, m, Ar-*H*), 8.13 (1H, d, *J* = 8.1, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 27.3 (Trp-CH_2), 28.3 (Boc-(CH_3)$_3$), 42.9 (Gly-CH_2), 52.6 (Ester-CH_3), 52.6 (Trp-α-CH), 84.2 (Boc-C), 114.3 (Ar-C), 115.6 (Ar-C), 118.5 (Ar-C), 119.9 (alkene-CH), 122.9 (Ar-C), 124.8 (Ar-C), 126.6 (Ar-C), 128.1 (Ar-C), 128.8 (Ar-C), 129.9 (Ar-C), 132.5 (alkene-CH), 135.8 (Ar-C), 136.2 (Ar-C), 136.7 (Ar-C), 150.4 (C=O), 168.3 (C=O), 170.3 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3281 m (N-H), 2955 w (C-H), 1726 s (Ester C=O), 1647 m (amide C=O), 1523 m (C=C), 1455 m (C-H), 1205 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{29}H_{34}N_3O_6$: 520.2442, found: 520.2436.

Data for **23a'**:

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (9H, s, Boc-(CH_3)$_3$), 1.84 (3H, s, Acetyl-CH_3), 3.43-3.46 (1H, m, Trp-C*H*H), 3.50 (3H, s, Ester-CH_3), 3.63-3.68 (3H, m, Gly-CH_2 / Trp-C*H*H), 4.78-4.84 (1H, m, Trp-α-C*H*), 5.92 (1H, br t, *J* = 4.6, Gly-N*H*), 6.26 (1H, br d, *J* = 7.7, Trp-N*H*), 6.67 (1H, d, *J* = 16.6, alkene-C*H*), 7.04 (1H, d, *J* = 16.6, alkene-C*H*), 7.21-7.34 (5H, m, alkene-C*H* / Ar-*H*), 7.35-7.44 (5H, m, Ar-*H*), 7.53 (2H, d, *J* = 7.4, Ar-*H*), 7.57 (2H, d, *J* = 7.4, Ar-*H*), 7.84 (1H, d, J = 16.2, alkene-C*H*), 8.15 (1H, d, *J* = 8.1, Ar-*H*).

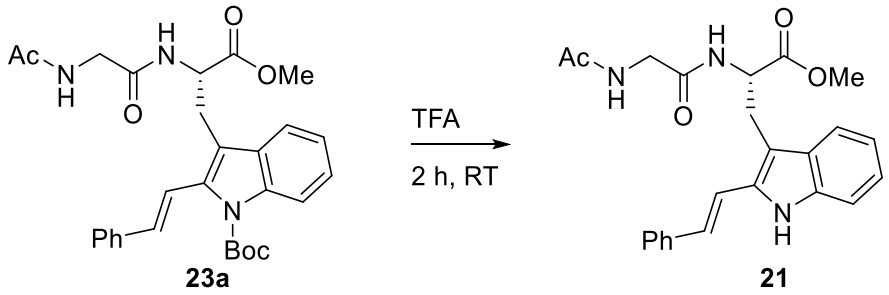
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.7 (Acetyl-CH_3), 28.3 (Boc-(CH_3)$_3$), 28.5 (Trp-CH_2), 42.7 (Gly-CH_2), 52.4 (Ester-CH_3), 53.1 (Trp-α-CH), 84.2 (Boc-C), 114.7 (Ar-C),114.9 (Ar-C), 119.6 (Ar-C), 121.8 (Ar-C), 124.8 (Ar-C), 126.6 (Ar-C), 126.6 (Ar-C), 126.8 (Ar-C), 127.8 (Ar-C), 128.2 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 131.4 (Ar-C), 132.3 (Ar-C), 133.7 (Ar-C), 136.4 (Ar-C), 136.9 (Ar-C), 137.1 (Ar-C), 137.2 (Ar-C), 150.1 (C=O), 168.4 (C=O), 170.1 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3226 m (N-H), 2978 w (C-H), 1720 s (Ester C=O), 1638 m (amide C=O), 1424 m (C-H), 1250 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{37}H_{40}N_3O_6$: 622.2912, found: 622.2910.

4.15 Removal of the Boc protecting group from tryptophan residues

4.15.1 Synthesis of modified peptide 21



23a **21**

Peptide **23a** (0.060 g, 0.115 mmol) was dissolved in trifluoroacetic acid (3 mL, 39.2 mmol) and left to stir at room temperature for 2 h. The resulting solution was then concentrated to by rotary evaporation to give peptide **21** as a dark brown solid (0.047 g, 97%); m.p. 132-134 ˚C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (3H, s, Acetyl-C*H*$_3$), 3.43-3.54 (2H, m, Trp-C*H*$_2$), 3.65 (3H, s, Ester-C*H*$_3$), 3.74 (1H, dd, *J* = 17.0, *J* = 5.0, Gly-C*H*H), 3.81 (1H, dd, *J* = 17.0, *J* = 5.0, Gly-C*H*H), 4.90 (1H, dt, *J* = 7.5, *J* = 5.0, Trp-α-C*H*), 6.44 (1H, br t, *J* = 5.0, Gly-N*H*), 6.58 (1H, br d, *J* = 7.5, Trp-N*H*), 6.87 (1H, d, *J* = 16.5, Alkene-C*H*), 7.04-7.10 (2H, m, Alkene-C*H* / Ar-*H*), 7.19 (1H, t, *J* = 6.9, Trp-Ar-*H*), 7.29 (2H, t, *J* = 8.2, Ar-*H*), 7.35-7.40 (3H, m, Ar-*H*), 7.49 (2H, m, Ar-*H*), 8.41 (1H, br s, Trp-Ar-N*H*).

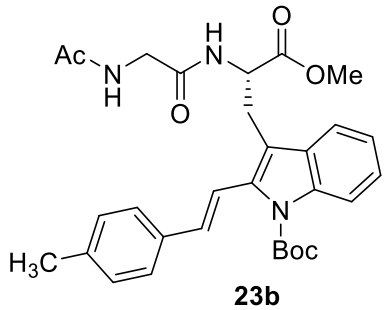
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.1 (Acetyl-CH_3), 26.3 (Trp-CH_2), 42.9 (Gly-CH_2), 52.9 (Ester-CH_3), 53.4 (Trp-α-C*H*), 109.8 (Ar-C), 110.9 (Ar-C), 116.2 (Alkene-C=C), 118.3 (Ar-C), 120.2 (Ar-C), 126.2 (Ar-C), 127.1 (Alkene-C=C), 128.1 (Ar-C), 128.8 (Ar-C), 129.0 (Ar-C), 134.2 (Ar-C), 136.5 (Ar-C), 136.5 (Ar-C), 168.5 (C=O), 171.5 (C=O), 172.5 (C=O).

IR υ_{max} /cm^{-1} (solid) 3312 m (N-H), 3058 s (C-H), 2952 s (C-H), 1728 s (Ester C=O), 1654 m (C=C), 1541 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{24}H_{26}N_3O_4$: 420.1918, found: 420.1918.

4.16 Synthesis of modified peptide 23b-f

4.16.1 Synthesis of modified peptide 23b



23b

Modified peptide **23b** was prepared from Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **23b** and the di-olefinated peptide in a ratio of 24:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23b** as a yellow oil (0.099 g, 78%); R_f 0.25 (EtOAc).

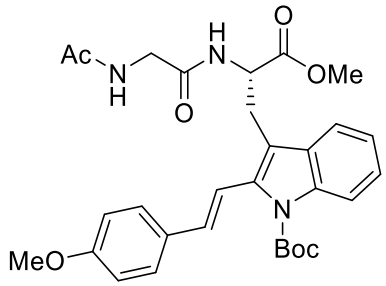
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.91 (3H, s, Acetyl-C*H*$_3$), 2.37 (3H, s, Ar-C*H*$_3$), 3.37 (1H, dd, *J* = 14.4, *J* = 6.4, Trp-C*H*H), 3.46 (1H, dd, *J* = 14.4, *J* = 6.4, Trp-C*H*H), 3.55 (Ester-C*H*$_3$), 3.72 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 3.83 (1H, dd, *J* = 16.8, *J* = 5.0, Gly-C*H*H), 4.88 (1H, dt, *J* = 7.6, *J* = 6.4, Trp-α-C*H*), 5.86 (1H, m, Gly-N*H*), 6.38 (1H, br d, *J* = 7.6, Trp-N*H*), 6.73 (1H, d, *J* = 16.7, alkene-C*H*), 7.18-7.24 (2H, m, alkene-C*H* / Ar-*H*), 7.27-7.32 (2H, m, Ar-*H*), 7.38 (1H, d, *J* = 4.6, Ar-*H*), 7.44 (2H, d, *J* = 8.0, Ar-*H*), 7.51 (1H, br d, *J* = 7.0, Ar-*H*), 8.13 (1H, d, *J* = 8.0, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.3 (Ar-CH_3), 22.8 (Acetyl-CH_3), 27.2 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 42.9 (Gly-CH_2), 52.5 (Ester-CH_3), 52.6 (Trp-α-CH), 84.0 (Boc-C), 114.2 (Ar-C), 115.5 (Ar-C), 118.4 (Ar-C), 118.8 (alkene-C), 122.8 (Ar-C), 124.7 (Ar-C), 126.4 (Ar-C), 127.0 (Ar-C), 128.5 (Ar-C), 129.4 (Ar-C), 129.9 (Ar-C), 132.4 (alkene-C), 133.9 (Ar-C), 135.8 (Ar-C), 136.3 (Ar-C), 138.0 (Ar-C), 150.4 (C=O), 168.4 (C=O), 170.3 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (oil) 3286 m (N-H), 3053 w (C-H), 2978 w (C-H), 1728 s (Ester C=O), 1648 m (amide C=O), 1235 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{36}N_3O_6$: 534.2599, found: 534.2602

4.16.2 Synthesis of modified peptide 23c



23c

Modified peptide **23c** was prepared from Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **23c** and the di-olefinated peptide in a ratio of 14:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23c** as yellow solid (0.077 g, 59%); m.p. 150-152 °C, R_f 0.18 (EtOAc).

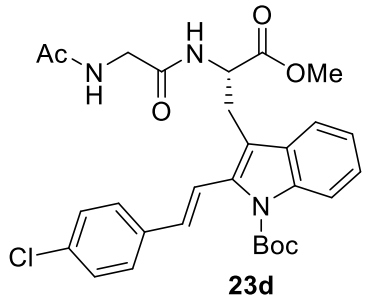
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 1.90 (3H, s, Acetyl-C*H*$_3$), 3.35 (1H, dd, *J* = 14.2, *J* = 6.6, Trp-C*H*H), 3.44 (1H, dd, *J* = 14.2, *J* = 6.6, Trp-C*H*H), 3.54 (3H, s, Ester-C*H*$_3$), 3.71 (1H, dd, *J* = 16.9, *J* = 5.0, Gly-C*H*H), 3.80-3.85 (4H, m, Ph-OC*H*$_3$ / Gly-C*H*H), 4.87 (1H, dt, *J* = 7.4, *J* = 6.6, Trp-α-C*H*), 5.92 (1H, br t, *J* = 5.0, Gly-N*H*), 6.44 (1H, d, *J* = 7.4, Trp-N*H*), 6.70 (1H, d, *J* = 16.4, alkene-C*H*), 6.91 (2H, d, *J* = 8.9, Ar-*H*), 7.15 (1H, d, *J* = 16.4, alkene-C*H*), 7.21-7.24 (1H, m, Trp-Ar-*H*), 7.26-7.30 (1H, m, Trp-Ar-*H*), 7.36 (1H, d, *J* = 4.6, Ar-*H*), 7.46-7.50 (3H, m, Ar-*H*), 8.11 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (acety-CH_3), 27.2 (Trp-CH_2), 28.3 (Boc-(CH_3)$_3$), 42.9 (Gly-CH_2), 52.6 (Ester-CH_3), 55.3 (Trp-α-CH), 84.0 (Boc-C), 113.8 (OMe), 114.2 (Ar-C), 115.6 (Ar-C), 117.7 (Ar-C), 118.4 (Ar-C), 122.8 (Ar-C), 124.6 (Ar-C), 127.0 (Ar-C), 127.8 (Ar-C), 128.5 (Ar-C), 129.5 (Ar-C), 130.0 (Ar-C), 132.1 (Ar-C), 135.8 (Ar-C), 136.5 (Ar-C), 150.4 (C=O), 168.3 (C=O), 170.3 (C=O), 171.8 (C=O).

IR υ_{max} /cm^{-1} (solid) 3296 m (N-H), 3051 w (C-H), 2963 w (C-H), 1727 s (Ester C=O), 1658 m (amide C=O), 1510 m (C=C), 1260 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{36}N_3O_7$: 550.2548, found: 550.2549

4.16.3 Synthesis of modified peptide 23d



23d

Modified peptide **23d** was prepared from Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained only the mono-olefinated peptide **23d**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23d** as a yellow oil (0.113 g, 87%); R_f 0.27 (EtOAc).

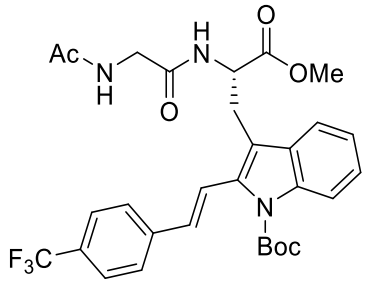
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.92 (3H, s, Acetyl-C*H*$_3$), 3.33 (1H, dd, *J* = 14.4, *J* = 6.6, Trp-C*H*H), 3.41 (1H, dd, *J* = 14.4, *J* = 6.6, Trp-C*H*H), 3.53 (3H, s, Ester-C*H*$_3$), 3.72 (1H, dd, *J* = 16.7, *J* = 5.0, Gly-C*H*H), 3.83 (1H, dd, *J* = 16.7, *J* = 5.0, Gly-C*H*H), 4.87 (1H, dt, *J* = 7.4, *J* = 6.6, Trp-α-C*H*), 6.01 (1H, br t, *J* = 5.0, Gly-N*H*), 6.73 (1H, d, *J* = 16.9, alkene-C*H*), 7.22-7.24 (6H, m, alkene-C*H* / Ar-*H*), 7.46-7.52 (3H, m, Ar-*H*), 8.10 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 27.4 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 43.0 (Gly-CH_2), 52.6 (Ester-CH_3), 52.6 (Trp-α-CH), 84.2 (Boc-C), 114.6 (Ar-C), 115.6 (Ar-C), 118.5 (Ar-C), 122.9 (Ar-C), 124.9 (Ar-C), 127.0 (Ar-C), 127.7 (Ar-C), 128.5 (Ar-C), 128.9 (Ar-C), 129.9 (Ar-C), 130.9 (Ar-C), 133.6 (Ar-C), 135.3 (Ar-C), 135.8 (Ar-C), 150.3 (C=O), 168.4 (C=O), 170.4 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (oil) 3281 m (N-H), 2950 w (C-H), 1728 s (Ester C=O), 1648 m (amide C=O), 1455 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{29}H_{33}N_3O_6Cl$: 554.2052, found: 554.2056.

4.16.4 Synthesis of modified peptide 23e



23e

Modified peptide **23e** was prepared from Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **23e** and the di-olefinated peptide in a ratio of 13:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23e** as an off-white solid (0.099 g, 71%); m.p. 169-172 °C, R_f 0.36 (EtOAc).

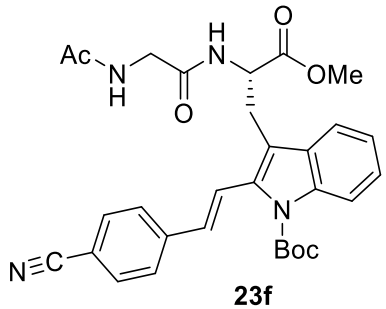
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.33 (1H, dd, *J* = 14.6, *J* = 6.4, Trp-C*H*H), 3.41 (1H, dd, *J* = 14.6, *J* = 6.4, Trp-C*H*H), 3.53 (3H, s, Ester-C*H*$_3$), 3.74 (1H, dd, *J* = 16.9, *J* = 5.0, Gly-C*H*H), 3.85 (1H, dd, *J* = 16.9, *J* = 5.0, Gly-C*H*H), 4.89 (1H, dt, *J* = 7.8, *J* = 6.6, Trp-α-C*H*), 6.10 (1H, br t, *J* = 5.0, Gly-N*H*), 6.72 (1H, d, *J* = 7.8, Trp-N*H*), 6.81 (1H, d, *J* = 16.6, alkene-C*H*), 7.22-7.26 (1H, m, Ar-*H*), 7.29-7.35 (2H, m, Ar-*H*), 7.45 (1H, d, *J* = 16.6, alkene-C*H*), 7.52 (1H, d, *J* = 7.3, Ar-*H*), 7.63 (4H, dt, *J* = 8.2, *J* = 7.3, Ar-*H*), 8.11 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.8 (Acetyl-CH_3), 27.6 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 43.0 (Gly-CH_2), 52.6 (Ester-CH_3), 52.6 (Trp-α-CH), 84.3 (Boc-C), 115.1 (Ar-C), 155.6 (Ar-C), 118.6 (Ar-C), 122.5 (Ar-C), 123.0 (Ar-C), 125.1 (Ar-C), 125.6 (Ar-C), 126.6 (Ar-C), 127.6 (Ar-C), 128.5 (Ar-C), 129.8 (Ar-C), 130.5 (Ar-C), 135.4 (Ar-C), 135.8 (Ar-C), 140.3 (Ar-C), 150.3 (C=O), 168.4 (C=O), 170.5 (C=O), 171.9 (C=O).

IR υ_{max} /cm^{-1} (solid) 3282 m (N-H), 3070 w (C-H), 2980 w (C-H), 1728 s (Ester C=O), 1647 m (amide C=O), 1541 m (C=C), 1237 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{33}N_3O_6F_3$: 588.2316, found: 588.2312.

4.16.5 Synthesis of modified peptide 23f



23f

Modified peptide **23f** was prepared from Ac-Gly-Trp(Boc)-OMe (**22**) (0.100 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained only the mono-olefinated peptide **23f**. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **23f** as a yellow solid (0.105 g, 81%); m.p. 144-147 °C, R_f 0.18 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.95 (3H, s, Acetyl-C*H*$_3$), 3.31-3.41 (2H, m, Trp-C*H*$_2$), 3.52 (3H, s, Ester-C*H*$_3$), 3.76 (1H, dd, *J* = 16.6, *J* = 5.0, Gly-N*H*), 3.86 (1H, dd, *J* = 16.6, *J* = 5.0, Gly-C*H*H), 4.88 (1H, app q, *J* = 8.2, Trp-α-C*H*), 6.04 (1H, br t, *J* = 5.0, Gly-N*H*), 6.65 (1H, d, *J* = 8.2, Trp-N*H*), 6.83 (1H, d, *J* = 16.4, alkene-C*H*), 7.23-7.27 (1H, m, Ar-*H*), 7.30-7.36 (2H, m, Ar-*H*), 7.49-7.53 (2H, m, alkene-C*H* / Ar-*H*), 7.65 (4H, s, Ar-*H*), 8.09 (1H, d, *J* = 8.2, Ar-*H*).

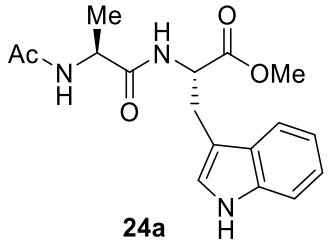
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 27.8 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 43.1 (Gly-CH_2), 52.6 (Ester-CH_3), 52.6 (Trp-α-CH), 84.5 (Boc-C), 110.9 (Ar-C), 115.5 (Ar-C), 115.7 (Ar-C), 118.7 (Ar-C), 119.0 (Ar-C), 123.1 (Ar-C), 123.7 (Ar-C), 125.33 (Ar-C), 127.0 (Ar-C), 129.8 (Ar-C), 130.0 (Ar-C), 132.5 (Ar-C), 135.8 (Ar-C), 141.4 (Ar-C), 150.3 (C=O), 168.4 (C=O), 170.5 (C=O), 171.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3283 m (N-H), 3050 w (C-H), 2979 w (C-H), 2225 s (C≡N), 1728 s (Ester C=O), 1649 m (amide C=O), 1239 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{33}N_4O_6$: 545.2395, found: 545.2398.

4.17 Synthesis of dipeptides 25a-d

4.17.1 Ac-Ala-Trp-OMe (24a)



24a

The peptide Ac-Ala-Trp-OMe (**24a**) was synthesised from L-tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and *N*-acetyl-L-alanine (0.131 g, 1.00 mmol), using the procedure in **Section 4.12.** The crude compound was recrystallised from DCM / hexanes to afford **24a** as an off-white solid (0.315 g, 95%); m.p. 77-79 °C.

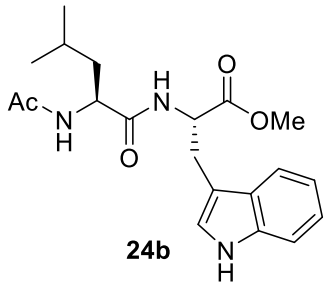
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, d, *J* = 6.9, Ala-C*H*$_3$), 1.86 (3H, s, Acetyl-C*H*$_3$), 3.27-3.36 (2H, m, Trp-C*H*$_2$), 4.48 (1H, dq, *J* = 7.3, *J* = 6.9, Ala-α-C*H*), 4.88 (1H, dt, *J* = 7.8, *J* = 5.5, Trp-α-C*H*), 5.57 (1H, br d, *J* = 7.3, Ala-N*H*), 6.49 (1H, br d, *J* = 7.8, Trp-N*H*), 7.03 (1H, d, Trp-Ar-*H*), 7.09-7.19 (1H, m, Trp-Ar-*H*), 7.16-7.20 (1H, m, Trp-Ar-*H*), 7.33-7.36 (1H, m, Trp-Ar-*H*), 7.50-7.51 (1H, m, Trp-Ar-*H*), 8.18 (1H, br s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.3 (Ala-CH_3), 23.0 (Acetyl-CH_3), 27.3 (Trp-CH_2), 48.7 (Ala-α-CH), 52.5 (Ester-CH_3), 52.9 (Trp-α-CH), 109.6 (Ar-C), 111.3 (Ar-C), 118.5 (Ar-C), 119.7 (Ar-C), 122.3 (Ar-C), 123.1 (Ar-C), 127.5 (Ar-C), 136.0 (Ar-C), 169.8 (C=O), 171.8 (C=O), 172.0 (C=O).

IR υ_{max} /cm^{-1} (solid) 3276 m (N-H), 3060 w (C-H), 2976 w (C-H), 1723 s (Ester C=O), 1631 m (amide C=O), 1550 m (C=C), 1405 m (C-H), 1256 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{17}H_{22}N_3O_4$: 332.1605, found: 332.1605.

4.17.2 Ac-Leu-Trp-OMe (24b)



24b

The peptide Ac-Leu-Trp-OMe (**24b**) was synthesised from L-tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and *N*-acetyl-L-leucine (0.173 g, 1.00 mmol), using the procedure in **Section 4.12**. The crude compound was recrystallised from DCM / hexanes to afford **24b** as a white solid (0.261 g, 78%); m.p. 79-81 °C.

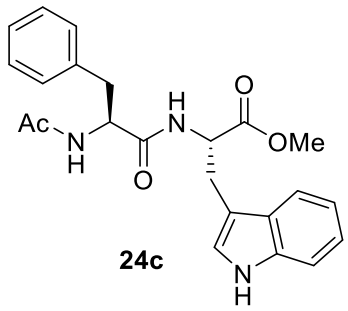
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-0.86 (6H, m, Leu-C*H*$_3$ (x2)), 1.48-1.64 (3H, m, (Leu-C*H*/Leu-C*H*$_2$), 1.80 (3H, s, Acetyl-C*H*$_3$), 3.25-3.37 (2H, m, Trp-C*H*$_2$), 3.64 (3H, s, Ester-C*H*$_3$), 4.56 (1H, dt, *J* = 8.0, *J* = 5.6, Leu-α-C*H*), 4.86 (1H, dt, *J* = 7.7, *J* = 5.4, Trp-α-C*H*), 6.65 (1H, br d, *J* = 8.0, Leu-N*H*), 6.99 (1H, d, *J* = 2.1, Trp-Ar-*H*), 7.03-7.14 (2H, m, Trp-Ar-*H* / Trp-N*H*), 7.10-7.16 (1H, br d, *J* = 7.7, Trp-Ar-*H*), 7.26-7.28 (1H, m, Trp-Ar-*H*), 7.49 (1H, d, *J* = 7.7, Trp-Ar-*H*), 8.73 (1H, s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.2 (Leu-CH_3 x2), 22.9 (Leu-CH_2), 23.1 (Acetyl-CH_3), 24.8 (Leu-C*H*$(CH_3)_2$), 27.5 (Trp-CH_2), 41.3 (Leu-CH_2), 51.7 (Leu-α-CH), 52.6 (Ester-CH_3), 52.9 (Trp-α-CH), 109.5 (Ar-C), 111.4 (Ar-C), 118.6 (Ar-C), 119.7 (Ar-C), 122.2 (Ar-C), 123.5 (Ar-C), 127.6 (Ar-C), 136.1 (Ar-C), 170.2 (C=O), 172.0 (C=O), 172.1 (C=O).

IR υ_{max} /cm^{-1} (solid) 3226 m (N-H), 3058 w (C-H), 2954 w (C-H), 1733 s (Ester C=O), 1638 m (amide C=O), 1541 m (C=C), 1452 m (C-H), 1254 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{28}N_3O_4$: 374.2074, found: 374.2069.

4.17.3 Ac-Phe-Trp-OMe (24c)



24c

The peptide Ac-Phe-Trp-OMe (**24c**) was synthesised from L-tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and *N*-acetyl-L-phenylalanine (0.207 g, 1.00 mmol), using the procedure in **Section 4.12**. The crude compound was recrystallised from DCM / hexanes to afford **24c** as an off-white solid (0.363 g, 89%); m.p. 75-76 °C.

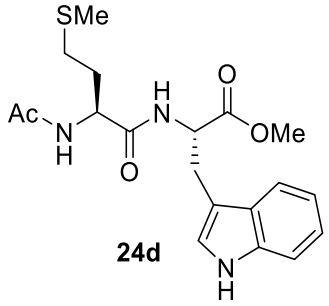
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70 (3H, s, Acetyl-C*H*$_3$), 2.95 (1H, dd, *J* = 14.0, *J* = 6.7, Phe-C*H*H), 3.03 (1H, dd, *J* = 14.0, *J* = 6.7, Phe-C*H*H), 3.22 (2H, d, *J* = 5.5, Trp-C*H*$_2$), 3.62 (3H, s, Ester-C*H*$_3$), 4.76 (1H, app q, *J* = 7.6, Phe-α-C*H*), 4.81-4.86 (1H, m, Trp-α-C*H*), 6.41 (1H, d, *J* = 7.6, Phe-N*H*), 6.83 (1H, d, *J* = 7.8, Trp-N*H*), 6.86 (1H, d, *J* = 1.8, Ar-*H*), 7.02 (1H, t, *J* = 7.8, Ar-*H*), 7.09-7.14 (3H, m, Ar-*H*), 7.19-7.26 (4H, m, Ar-*H*), 7.37 (1H, d, *J* = 7.8, Ar-*H*), 8.54 (1H, m, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.0 (Acetyl-CH_3), 27.4 (Trp-CH_2), 38.1 (Phe-CH_2), 52.4 (Ester-CH_3), 52.9 (Trp-α-CH), 54.1 (Phe-α-CH), 109.5 (Ar-C), 111.4 (Ar-C), 118.4 (Ar-C), 119.7 (Ar-C), 122.3 (Ar-C), 123.1 (Ar-C), 127.0 (Ar-C), 127.4 (Ar-C), 128.6 (Ar-C), 129.4 (Ar-C), 136.0 (Ar-C), 136.4 (Ar-C), 169.9 (C=O), 170.3 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3275 m (N-H), 3024 w (C-H), 2989 w (C-H), 1727 s (Ester C=O), 1646 m (amide C=O), 1550 m (C=C), 1401 m (C-H), 1220 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{23}H_{26}N_3O_4$: 408.1918, found: 408.1914.

4.17.4 Ac-Met-Trp-OMe (24d)



24d

The peptide Ac-Met-Trp-OMe (**24d**) was synthesised from L-tryptophan methyl ester hydrochloride (0.637 g, 2.50 mmol) and *N*-acetyl-L-methionine (0.191 g, 1.00 mmol), using the procedure in **Section 4.12.** The crude compound was recrystallised from DCM / hexanes to afford **24d** as a white solid (0.376 g, 96%); m.p. 70-72 °C.

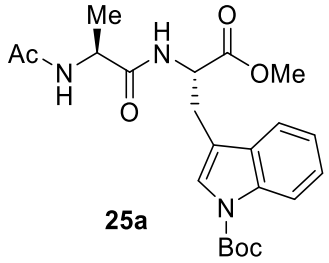
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82-1.90 (4H, m, Acetyl-C*H*$_3$ / Met-C*H*H), 1.96-2.02 (4H, m, Met-C*H*$_3$ / Met-C*H*H), 2.48 (2H, br t, *J* = 7.4, Met-C*H*$_2$), 3.26 (2H, br d, *J* = 5.6, Trp-C*H*$_2$), 3.65 (3H, s, Ester-C*H*$_3$), 4.65-4.71 (1H, m, Met-α-C*H*), 4.82-4.87 (1H, m, Trp-α-C*H*), 6.94 (1H, s, Trp-Ar-*H*), 7.01-7.08 (2H, m, Trp-Ar-*H* / Met-N*H*), 7.12 (1H, t, *J* = 7.6, Trp-Ar-*H*), 7.26 (1H, br d, *J* = 7.6, Trp-Ar-*H*), 7.32 (1H, br d, *J* = 7.6, Trp-N*H*), 7.49 (1H, br d, *J* = 7.6, Trp-Ar-*H*), 8.82 (1H, m, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 14.9 (Met-CH_3), 23.1 (Acetyl-CH_3), 27.3 (Trp-CH_2), 29.9 (Met-CH_2), 31.5 (Met-CH_2), 51.9 (Met-α-CH), 52.5 (Ester-CH_3), 52.8 (Trp-α-CH), 109.5 (Ar-C), 111.4 (Ar-C), 118.4 (Ar-C), 119.7 (Ar-C), 122.3 (Ar-C), 123.1 (Ar-C), 127.4 (Ar-C), 136.1 (Ar-C), 169.9 (C=O), 170.8 (C=O), 171.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3275 m (N-H), 3058 w (C-H), 2976 w (C-H), 1731 s (Ester C=O), 1640 m (amide C=O), 1451 m (C-H), 1254 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_3O_4S$: 392.1639, found: 392.1640.

4.17.5 Ac-Ala-Trp(Boc)-OMe (25a)



25a

Peptide **25a** was synthesised from Ac-Ala-Trp-OMe (**24a**) (0.249 g, 0.75 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **25a** as an off-white solid (0.291 g, 90%); m.p. 83-84 °C, R_f 0.19 (EtOAc).

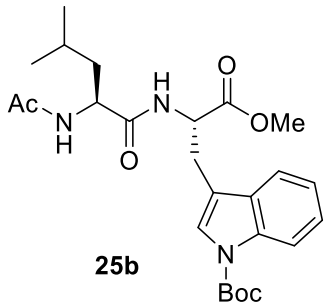
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, d, *J* = 6.9, Ala-C*H*$_3$), 1.66 (9H, s, Boc-(C*H*)$_3$)$_3$), 1.90 (3H, s, Acetyl-C*H*$_3$), 3.20 (1H, dd, *J* = 14.6, *J* = 5.5, Trp-C*H*H), 3.30 (1H, dd, *J* = 14.6, *J* = 5.5, Trp-C*H*H), 3.71 (3H, s, Ester-C*H*$_3$), 4.39-4.47 (1H, m, Ala-α-C*H*), 4.85-4.90 (1H, m, Trp-α-C*H*), 5.91 (1H, br d, *J* = 7.3, Ala-N*H*), 6.59 (1H, br d, *J* = 7.3, Trp-N*H*), 7.22 (1H, t, *J* = 7.3, Trp-Ar-*H*), 7.30 (1H, t, *J* = 7.3, Trp-Ar-*H*), 7.39 (1H, s, Trp-Ar-*H*), 7.47 (1H, d, *J* = 7.3, Trp-Ar-*H*), 8.08-8.10 (1H, m, Trp-Ar-*H*),

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.1 (Ala-CH_3), 22.8 (Acetyl-CH_3), 27.2 (Trp-CH_2), 28.1 (Boc(CH_3)$_3$), 48.6 (Ala-α-CH), 52.4 (Ester-CH_3), 52.6 (Trp-α-CH), 83.7 (Boc-C), 114.8 (Ar-C), 115.2 (Ar-C), 118.7 (Ar-C),122.5 (Ar-C), 124.1 (Ar-C), 124.4 (Ar-C), 130.3 (Ar-C), 135.1 (Ar-C), 149.5 (C=O), 170.0 (C=O), 171.7 (C=O), 172.3 (C=O).

IR U_{max} /cm^{-1} (solid) 3286 m (N-H), 2933 w (C-H), 1728 s (Ester C=O), 1648 m (amide C=O), 1452 m (C-H), 1213 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{30}N_3O_6$: 432.2129, found: 432.2130.

4.17.6 Ac-Leu-Trp(Boc)-OMe (25b)



25b

Peptide **25b** was synthesised from Ac-Leu-Trp-OMe (**24b**) (0.280 g, 0.75 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **25b** as a white solid (0.337 g, 95%); m.p. 84-86 °C, R_f 0.20 (75% EtOAc / pet. ether).

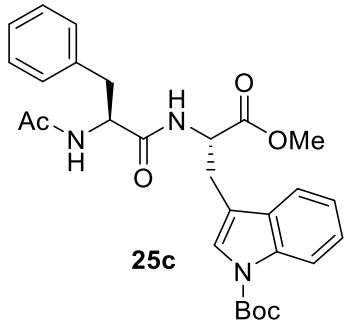
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86-0.91 (6H, m, Leu-C*H*$_3$ (x2)), 1.46-1.50 (1H, m, Leu-C*H*), 1.59-1.63 (2H, m, Leu-C*H*$_2$), 1.66 (9H, s, Boc-(C*H*$_3$)$_3$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.19 (1H, dd, *J* = 14.7, *J* = 5.8, Trp-C*H*H), 3.26 (1H, dd, *J* = 14.7, *J* = 5.8, Trp-C*H*H), 3.68 (3H, s, Ester-C*H*$_3$), 4.49 (1H, dt, *J* = 8.4, *J* = 5.4, Leu-α-C*H*), 4.83-4.88 (1H, m, Trp-α-C*H*), 6.24 (1H, br d, *J* = 8.4, Leu-N*H*), 6.93 (1H, br d, *J* = 7.3, Trp-N*H*), 7.20-7.24 (1H, m, Trp-Ar-*H*), 7.28-7.32 (1H, m, Trp-Ar-*H*), 7.46 (1H, s, Trp-Ar-*H*), 7.49 (1H, d, *J* = 7.7, Trp-Ar-*H*), 8.06-8.10 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.0 (Leu-CH_3), 22.9 (Leu-CH_3), 23.0 (Acetyl-CH_3), 24.7 (Leu-CH), 27.3 (Trp-CH_2), 28.2 (Boc-(CH_3)$_3$), 41.0 (Leu-CH_2), 51.6 (Leu-α-CH), 52.5 (Ester-CH_3), 52.6 (Trp-α-CH), 83.8 (Boc-C), 114.8 (Ar-C), 115.3 (Ar-C), 118.8 (Ar-C), 122.6 (Ar-C), 124.3 (Ar-C), 124.5 (Ar-C), 130.4 (Ar-C), 135.2 (Ar-C), 149.6 (C=O), 170.2 (C=O), 171.8 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3284 m (N-H), 2932 w (C-H), 1731 s (Ester C=O), 1647 m (amide C=O), 1526 m (C=C), 1452 m (C-H), 1254 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{36}N_3O_6$: 474.2599, found: 474.2595.

4.17.7 Ac-Phe-Trp(Boc)-OMe (25c)



25c

Peptide **25c** was synthesised from Ac-Phe-Trp-OMe (**24c**) (0.306 g, 0.75 mmol) using the procedure in **Section 4.13**. Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **25c** as a white solid (0.305 g, 80%); m.p. 79-81 °C, R_f 0.28 (75% EtOAc / pet. ether).

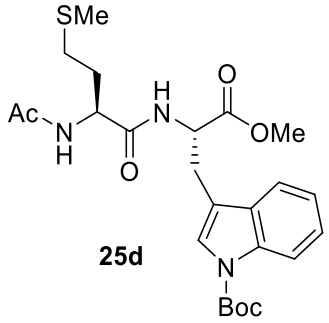
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (9H, s, Boc-(C*H*$_3$)$_3$), 1.79 (3H, s, Acetyl-C*H*$_3$), 2.93 (1H, dd, *J* = 13.8, *J* = 7.4, Phe-C*H*H), 2.97-3.18 (2H, m, Phe-C*H*H / Trp-C*H*H), 3.13 (1H, dd, *J* = 14.8, *J* = 5.8, Trp-C*H*H), 3.59 (3H, s, Ester-C*H*$_3$), 4.57 (1H, app q, *J* = 7.3, Phe-α-C*H*), 4.75 (1H, dt, *J* = 7.6, *J* = 5.8, Trp-α-C*H*), 6.03 (1H, br d, *J* = 7.6, Trp-N*H*), 6.31 (1H, br d, *J* = 7.4, Phe-N*H*), 7.07-7.12 (3H, m, Ar-*H*), 7.13-7.22 (4H, m, Ar-*H*), 7.24-7.28 (2H, m, Ar-*H*), 8.01 (1H, m Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 27.3 (Trp-CH_2), 28.1 (Boc-$(CH_3)_3$), 38.0 (Phe-CH_2), 52.4 (Ester-CH_3), 52.6 (Trp-α-CH), 54.2 (Phe-α-CH), 83.7 (Boc-C), 114.5 (Ar-C), 115.3 (Ar-C), 118.6 (Ar-C), 122.5 (Ar-C), 124.2 (Ar-C), 124.5 (Ar-C), 126.9 (Ar-C), 128.5 (Ar-C), 129.2 (Ar-C), 130.2 (Ar-C), 135.2 (Ar-C), 136.3 (Ar-C), 149.5 (C=O), 169.9 (C=O), 170.6 (C=O), 171.3 (C=O).

IR U_{max} /cm^{-1} (solid) 3278 m (N-H), 3033 w (C-H), 2934 w (C-H), 1736 s (Ester C=O), 1642 m (amide C=O), 1441 m (C-H), 1235 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{28}H_{34}N_3O_6$: 508.2442, found: 508.2436.

4.17.8 Ac-Met-Trp(Boc)-OMe (25d)



25d

Peptide **25d** was synthesised from Ac-Met-Trp-OMe (**24d**) (0.294 g, 0.75 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **25d** as a white solid (0.262 g, 71%); m.p. 80-81 °C, R_f 0.17 (75% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (9H, s, Boc-(C*H*$_3$)$_3$), 1.84-1.88 (4H, m, Acetyl-C*H*$_3$ / Met-C*H*H), 1.94-1.97 (4H, m, Met-C*H*H / Met-C*H*$_3$), 2.42-2.52 (2H, m, Met-C*H*$_2$), 3.13 (1H, dd, *J* = 14.8, *J* = 6.2, Trp-C*H*H), 3.18 (1H, dd, *J* = 14.8, *J* = 6.2, Trp-C*H*H), 3.62 (3H, s, Ester-C*H*$_3$), 4.54 (1H, app q, *J* = 7.1, Met-α-C*H*), 4.78-4.82 (1H, m, Trp-α-C*H*), 6.42 (1H, br d, *J* = 7.1, Met-N*H*), 6.98 (1H, br d, *J* = 7.7, Trp-N*H*), 7.12-7.16 (1H, m, Trp-Ar-*H*), 7.20-7.24 (1H, m, Trp-Ar-*H*), 7.36 (1H, br s, Trp-Ar-*H*), 7.40 (1H, d, *J* = 7.9, Trp-Ar-*H*), 8.01 (1H, m, Trp-Ar-*H*).

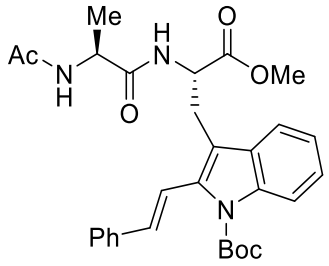
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 14.9 (Met-CH_3), 22.9 (Acetyl-CH_3), 27.2 (Trp-CH_2), 28.1 (Boc-$(CH_3)_3$), 29.8 (Met-CH_2), 31.4 (Met-CH_2), 51.8 (Met-α-CH), 52.4 (Ester-CH_3), 52.5 (Trp-α-CH), 83.7 (Boc-C), 114.6 (Ar-C), 115.2 (Ar-C), 118.6 (Ar-C),122.5 (Ar-C),124.1 (Ar-C), 124.5 (Ar-C), 130.1 (Ar-C), 135.2 (Ar-C), 148.4 (C=O), 170.0 (C=O), 171.1 (C=O), 171.5 (C=O)

IR υ_{max} /cm^{-1} (solid) 3287 m (N-H), 3050 w (C-H), 2978 w (C-H), 1739 s (Ester C=O), 1636 m (amide C=O), 1445 m (C-H), 1220 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{24}H_{34}N_3O_6S$: 492.2163, found: 492.2156.

4.18 Synthesis of modifed dipeptides 26a-d

4.18.1 Synthesis of modified peptide 26a



26a

Modified peptide **26a** was prepared from Ac-Ala-Trp(Boc)-OMe (**25a**) (0.103 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **26a** and the di-olefinated peptide in a ratio of 14:1. Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **26a** as an off-white solid (0.086 g, 68%); m.p. 93-95 °C, R_f 0.26 (75% EtOAc / pet. ether).

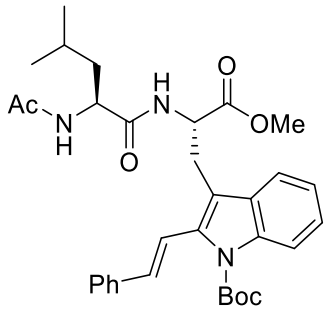
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (3H, d, *J* = 7.0, Ala-C*H*$_3$), 1.56 (9H, s, Boc-(C*H*$_3$)$_3$), 1.79 (3H, s, Acetyl-C*H*$_3$), 3.25-3.36 (2H, m, Trp-C*H*$_2$), 3.48 (3H, s, Ester-C*H*$_3$), 4.33 (1H, dq, *J* = 7.4, *J* = 7.0, Ala-α-C*H*), 4.81 (1H, dt, *J* = 7.8, *J* = 7.3, Trp-α-C*H*), 5.82 (1H, br d, *J* = 7.4, Ala-N*H*), 6.51 (1H, br d, *J* = 7.8, Trp-N*H*), 6.69 (1H, d, *J* = 16.7, alkene-C*H*), 7.15-7.27 (4H, m, Ar-*H*/alkene-C*H*), 7.31 (2H, t, *J* = 7.4, Ar-*H*), 7.48 (3H, d, *J* = 7.4, Ar-*H*), 8.06 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 18.3 (Ala-CH_3), 23.1 (Acetyl-CH_3), 27.5 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 48.5 (Ala-α-CH), 52.5 (Ester-CH_3), 52.6 (Trp-α-CH), 84.1 (Boc-C), 114.4 (Ar-C), 115.6 (Ar-C), 118.7 (Ar-C), 119.3 (alkene-CH), 122.8 (Ar-C), 124.7 (Ar-C), 126.5 (Ar-C), 128.0 (Ar-C), 128.7 (Ar-C), 129.9 (Ar-C), 132.4 (alkene-CH), 135.8 (Ar-C), 136.2 (Ar-C), 136.7 (Ar-C), 150.3 (C=O), 169.7 (C=O), 171.8 (C=O), 171.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3281 m (N-H), 3036 w (C-H), 2928 w (C-H), 1725 s (Ester C=O), 1631 m (amide C=O), 1541 m (C=C), 1448 m (C-H), 1221 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{30}H_{36}N_3O_6$: 534.2599, found: 534.2600.

4.18.2 Synthesis of modified peptide 26b



26b

Modified peptide **26b** was prepared from Ac-Leu-Trp(Boc)-OMe (**25b**) (0.113 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **26b** and the di-olefinated peptide in a ratio of 19:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **26b** as an off-white solid (0.085 g, 62%); m.p. 97-98°C, R_f 0.22 (50% EtOAc / pet. ether).

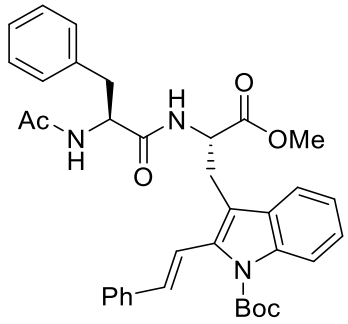
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86-0.88 (6H, m, Leu-C*H*$_3$ (x2)), 1.37-1.41 (1H, m, Leu-C*H*), 1.54-1.59 (2H, m, Leu-C*H*$_2$), 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.87 (3H, s, Acetyl-C*H*$_3$), 3.31-3.43 (2H, m, Trp-C*H*$_2$), 3.57 (3H, s, Ester-C*H*$_3$), 4.41 (1H, dt, *J* = 8.6, *J* = 5.4, Leu-α-C*H*), 4.87 (1H, app q, *J* = 7.2, Trp-α-C*H*), 5.66 (1H, br d, *J* = 8.6, Leu-N*H*), 6.51 (1H, br d, *J* = 7.7, Trp-N*H*), 6.75 (1H, d, *J* = 16.6, alkene-C*H*), 7.24-7.33 (5H, m, Ar-*H* / alkene-C*H*), 7.39 (2H, t, *J* = 7.4, Ar-*H*), 7.56-7.58 (2H, m, Ar-*H*), 8.13 (1H, d, *J* = 8.3, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.0 (Leu-CH_3), 22.8 (Leu-CH_3), 23.1 (Acetyl-CH_3), 24.6 (Leu-CH), 27.7 (Trp-CH_2), 28.3 (Boc-(CH_3)$_3$), 41.2 (Leu-CH_2), 51.3 (Leu-α-CH), 52.5 (Ester-CH_3), 52.5 (Trp-α-CH), 84.0 (Boc-C), 114.5 (Ar-C), 115.6 (Ar-C), 118.7 (Ar-C), 120.0 (alkene), 122.9 (Ar-C), 124.7 (Ar-C), 126.6 (Ar-C), 128.0 (Ar-C), 128.7 (Ar-C), 129.9 (Ar-C), 132.4 (alkene), 135.8 (Ar-C), 136.2 (Ar-C), 136.8 (Ar-C), 150.4 (C=O), 169.8 (C=O), 171.7 (C=O), 172.0 (C=O).

IR U_{max} /cm^{-1} (solid) 3268 m (N-H), 2927 w (C-H), 1728 s (Ester C=O), 1647 m (amide C=O), 1451 m (C-H), 1255 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{42}N_3O_6$ 576.3068, found: 576.3062.

4.18.3 Synthesis of modified peptide 26c



26c

Modified peptide **26c** was prepared from Ac-Phe-Trp(Boc)-OMe (**25c**) (0.121 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **26c** and the di-olefinated tryptophan residue in a ratio of 12:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **26c** as an off-white solid (0.075 g, 52%); m.p. 150-151 °C, R_f 0.31 (50% EtOAc / pet. ether).

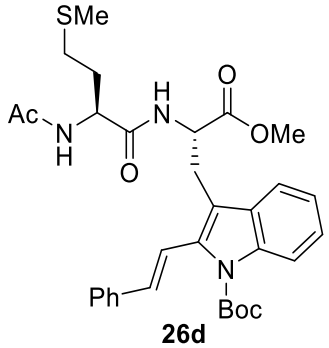
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 1.82 (3H, s, Acetyl-C*H*$_3$), 2.92 (1H, dd, *J* = 13.7, *J* = 7.8, Phe-C*H*H), 3.00 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.23-3.33 (2H, m, Trp-C*H*$_2$), 3.50 (3H, s, Ester-C*H*$_3$), 4.53-4.59 (1H, m, Phe-α-C*H*), 4.80 (1H, app q, *J* = 7.3, Trp-α-C*H*), 5.59-5.62 (1H, m, Phe-N*H*), 6.19-6.22 (1H, m, Trp-N*H*), 6.71 (1H, d, *J* = 16.5, alkene-C*H*), 7.13-7.25 (6H, m, Ar-*H*), 7.26-7.30 (3H, m, Ar-*H* / alkene-C*H*), 7.35-7.42 (3H, m, Ar-*H*), 7.53 (2H, d, *J* = 7.3, Ar-*H*), 8.11 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.2 (Acetyl-CH_3), 27.7 (Trp-CH_2), 28.4 (Boc-$(CH_3)_3$), 38.4 (Phe-CH_2), 52.6 (Ester-CH_3), 52.7 (Trp-α-CH), 54.4 (Phe-α-CH), 84.2 (Boc-C), 114.4 (Ar-C), 115.7 (Ar-C), 118.7 (Ar-C), 120.0 (alkene-CH), 123.0 (Ar-C), 124.9 (Ar-C), 126.7 (Ar-C), 127.1 (Ar-C), 128.1 (Ar-C), 128.7 (Ar-C), 128.9 (Ar-C), 129.4 (Ar-C), 130.0 (Ar-C), 132.5 (alkene-CH), 135.9 (Ar-C), 136.2 (Ar-C), 136.5 (Ar-C), 136.9 (Ar-C), 150.5 (C=O), 169.9 (C=O), 170.5 (C=O), 171.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3238 m (N-H), 3058 w (C-H), 2950 w (C-H), 1749 s (Ester C=O), 1638 m (amide C=O), 1523 m (C=C), 1213 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{40}N_3O_6$: 610.2912, found: 610.2912.

4.18.4 Synthesis of modified peptide 26d



26d

Modified peptide **26d** was prepared from Ac-Met-Trp(Boc)-OMe (**25d**) (0.117 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **26d** and the di-olefinated peptide in a ratio of 8.1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **26d** as an off-white solid (0.105 g, 74%); m.p. 94-96°C, R_f 0.15 (50% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.86-1.90 (4H, m, Met-C*H*H / Acetyl-C*H*$_3$), 1.93-1.97 (1H, m, Met-C*H*), 2.00 (3H, s, Met-C*H*$_3$), 2.47-2.53 (2H, m, Met-C*H*$_2$), 3.35 (1H, dd, *J* = 14.5, *J* = 6.9, Trp-C*H*H), 3.41 (1H, dd, *J* = 14.5, *J* = 6.9, Trp-C*H*H), 3.59 (3H, s, Ester-C*H*$_3$), 4.54 (1H, dt, *J* = 7.3, *J* = 7.0, Met-α-C*H*), 4.87 (1H, dt, *J* = 7.3, *J* = 6.9, Trp-α-C*H*), 6.11 (1H, d, *J* = 7.3, Met-N*H*), 6.73-6.78 (2H, m, alkene-C*H* / Ar-*H*), 7.25-7.32 (3H, m, alkene-C*H* / Ar-*H*), 7.36-7.40 (2H, m, Ar-*H*), 7.54-7.57 (3H, m, Ar-*H*), 8.14 (1H, d, *J* = 8.4, Ar-*H*).

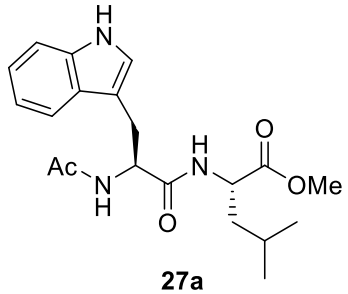
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 14.8 (Met-CH_3), 23.1 (Acetyl-CH_3), 27.4 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 29.8 (Met-CH_2), 31.4 (Met-CH_2), 51.6 (Met-α-CH), 52.5 (Ester-CH_3), 52.6 (Trp-α-CH), 84.1 (Boc-C), 114.2 (Ar-C), 115.6 (Ar-C), 118.6 (Ar-C), 120.0 (alkene-CH), 122.9 (Ar-C), 124.8 (Ar-C), 126.6 (Ar-C), 128.1 (Ar-C), 128.7 (Ar-C), 129.7 (Ar-C), 132.6 (alkene-CH), 135.8 (Ar-C), 136.2 (Ar-C), 136.7 (Ar-C), 150.3 (C=O), 169.7 (C=O), 170.7 (C=O), 171.8 (C=O).

IR U_{max} /cm^{-1} (solid) 3282 m (N-H), 3060 w (C-H), 2927 w (C-H), 1743 s (Ester C=O), 1646 m (amide C=O), 1459 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{40}N_3O_6S$: 594.2632 found: 594.2631.

4.19 Synthesis of dipeptides 28a-d

4.19.1 Ac-Trp-Leu-OMe (27a)



27a

The peptide Ac-Trp-Leu-OMe (**27a**) was synthesised from L-leucine methyl ester hydrochloride (0.454 g, 2.50 mmol) and *N*-acetyl-L-tryptophan (0.246 g, 1.00 mmol), using the procedure in **Section 4.12**. The crude compound was recrystallised from DCM / hexanes to afford **27a** as a yellow solid (0.295 g, 79%); m.p. 74-76 °C.

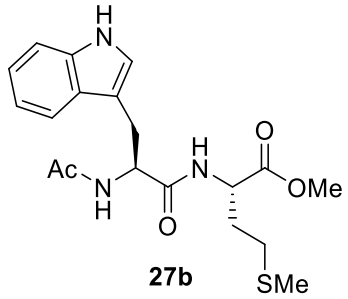
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-0.89 (6H, m, Leu-C*H*$_3$ (x2)), 1.09-1.17 (1H, m, Leu-C*H*), 1.46-1.53 (2H, m, Leu-C*H*$_2$), 2.00 (3H, s, Acetyl-C*H*$_3$), 3.14 (1H, dd, *J* = 14.5, *J* = 8.3, Trp-C*H*H), 3.29-3.35 (1H, m, Trp-C*H*H), 3.66 (3H, s, Ester-C*H*$_3$), 4.48 (1H, dt, *J* = 8.3, *J* = 5.2, Trp-α-C*H*), 4.73-4.78 (1H, m, Leu-N*H*), 6.04-6.06 (1H, m, Trp-N*H*), 6.34-6.35 (1H, m, Leu-N*H*), 7.12-7.16 (2H, m, Trp-Ar-*H*), 7.21 (1H, t, *J* = 7.6, Trp-Ar-*H*), 7.37 (1H, d, *J* = 7.6, Trp-Ar-*H*), 7.75 (1H, d, *J* = 7.6, Trp-Ar-*H*), 8.17 (1H, m, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 22.6 (Leu-CH_3), 23.3 (Acetyl-CH_3), 24.6 (Leu-CH), 28.5 (Trp-CH_2), 41.4 (Leu-CH_2), 50.9 (Leu-α-CH), 52.2 (Ester-CH_3), 53.7 (Trp-α-CH), 110.6 (Ar-C), 111.1 (Ar-C), 118.9 (Ar-C), 119.8 (Ar-C), 122.2 (Ar-C), 123.4 (Ar-C), 127.4 (Ar-C), 136.1 (Ar-C), 169.9 (C=O), 171.0 (C=O), 172.8 (C=O).

IR υ_{max} /cm^{-1} (solid) 3274 m (N-H), 3058 w (C-H), 2918 w (C-H), 1735 s (Ester C=O), 1640 m (amide C=O), 1433 m (C-H), 1340 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{20}H_{28}N_3O_4$: 374.2074, found: 374.2077.

4.19.2 Ac-Trp-Met-OMe (27b)



27b

The peptide Ac-Trp-Met-OMe (**27b**) was synthesised from L-methionine methyl ester hydrochloride (0.499 g, 2.50 mmol) and *N*-acetyl-L-tryptophan (0.246 g, 1.00 mmol), using the procedure in **Section 4.12**. The crude compound was recrystallised from DCM / hexanes to afford **27b** as a yellow solid (0.348 g, 89%); m.p. 126-127 °C.

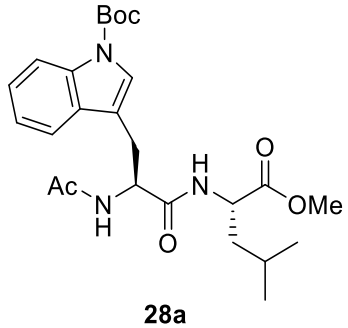
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76-1.81 (1H, m, Met-C*H*H), 1.87 (3H, s, Met-CH_3), 1.92-1.98 (4H, m, Acetyl-CH_3 / Met-C*H*H), 2.27 (2H, d, *J* = 7.7, Met-CH_2), 3.10 (1H, dd, *J* = 14.6, *J* = 7.6, Trp-C*H*H), 3.20 (1H, dd, *J* = 14.6, *J* = 5.4, Trp-C*H*H), 3.56 (3H, s, Ester-CH_3), 4.48 (1H, dt, *J* = 7.6, *J* = 5.4, Met-α-C*H*), 4.73 (1H, dt, *J* = 7.4, *J* = 5.4, Trp-α-C*H*), 6.50 (1H, d, *J* = 7.7, Met-N*H*), 6.71 (1H, d, *J* = 7.4, Trp-N*H*), 7.00-7.03 (2H, m, Trp-Ar-*H*), 7.09 (1H, t, *J* = 7.5, Trp-Ar-*H*), 7.26 (1H, d, *J* = 7.5, Trp-Ar-*H*), 7.56 (1H, d, *J* = 7.5, Trp-Ar-*H*), 8.47 (1H, br s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.2 (Met-CH_3), 23.1 (Acetyl-CH_3), 28.3 (Met-CH_2), 29.6 (Met-CH_2), 31.2 (Trp-CH_2), 51.5 (Trp-α-CH), 52.4 (Ester-CH_3), 53.9 (Met-α-CH), 110.1 (Ar-C), 111.2 (Ar-C), 118.5 (Ar-C), 119.5 (Ar-C), 122.0 (Ar-C), 123.4 (Ar-C), 124.4 (Ar-C), 136.1 (Ar-C), 170.2 (C=O), 171.4 (C=O), 171.7 (C=O).

IR U_{max} /cm^{-1} (solid) 3291 m (N-H), 3054 w (C-H), 2914 w (C-H), 1743 s (Ester C=O), 1631 m (amide C=O), 1431 m (C-H), 1224 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{26}N_3O_4S$: 392.1639, found: 392.1637.

4.19.3 Ac-Trp(Boc)-Leu-OMe (28a)



28a

Peptide **28a** was synthesised from Ac-Trp-Leu-OMe (**27a**) (0.280 g, 0.75 mmol) using the procedure in **Section 4.13**. Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **28a** as a white solid (0.464 g, 98%); m.p. 80-81 °C, R_f 0.21 (75% EtOAc / pet. ether).

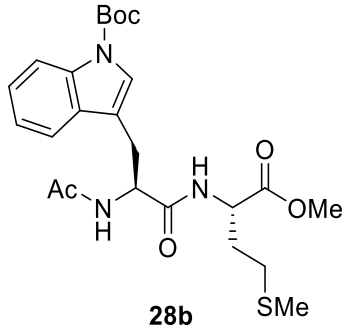
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.88 (6H, m, Leu-C*H*$_3$ (x2)), 1.49-1.55 (3H, m, Leu-C*H*$_2$ / Leu-C*H*), 1.65 (9H, s, Boc-(C*H*$_3$)$_3$), 1.99 (3H, s, Acetyl-C*H*$_3$), 3.11 (1H, dd, *J* = 14.5, *J* = 7.9, Trp-C*H*H), 3.20 (1H, dd, *J* = 14.5, *J* = 5.6, Trp-C*H*H), 3.66 (3H, s, Ester-C*H*$_3$), 4.46-4.51 (1H, m, Trp-α-C*H*), 4.79 (1H, dt, *J* = 7.6, *J* = 5.7, Leu-α-C*H*), 6.36 (1H, d, *J* = 7.9, Trp-N*H*), 6.53 (1H, d, *J* = 7.6, Leu-N*H*), 7.23 (1H, t, *J* = 7.1, Trp-Ar-*H*), 7.31 (1H, t, *J* = 7.1, Trp-Ar-*H*), 7.50 (1H, br s, Trp-Ar-*H*), 7.67 (1H, d, *J* = 7.8, Trp-Ar-*H*), 8.12 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Leu-CH_3), 22.7 (Leu-CH_3), 23.2 (Acetyl-CH_3), 24.7 (Leu-CH), 28.2 (Boc-(CH_3)$_3$), 28.2 (Trp-CH_2), 41.4 (Leu-CH_2), 50.9 (Leu-α-CH), 52.3 (Ester-CH_3), 53.2 (Trp-α-CH), 83.6 (Boc-C), 115.2 (Ar-C), 115.3 (Ar-C), 119.1 (Ar-C), 122.7 (Ar-C), 124.5 (Ar-C), 124.6 (Ar-C), 130.3 (Ar-C), 135.4 (Ar-C), 149.6 (C=O), 170.0 (C=O), 170.7 (C=O), 172.5 (C=O).

IR υ_{max} /cm^{-1} (solid) 3263 m (N-H), 3060 w (C-H), 2955 w (C-H), 1741 s (Ester C=O), 1622 m (amide C=O), 1541 m (C=C), 1430 m (C-H), 1252 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{36}N_3O_6$: 474.2599, found: 474.2595.

4.19.4 Ac-Trp(Boc)-Met-OMe (28b)



28b

Peptide **28b** was synthesised from Ac-Trp-Met-OMe (**27b**) (0.294 g, 0.75 mmol) using the procedure in **Section 4.13**. Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **28b** as a white solid (0.398 g, 81%); m.p. 154-155 °C, R_f 0.19 (75% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66 (9H, s, Boc-(C*H*$_3$)$_3$), 1.87-1.94 (1H, m, Met-C*H*H), 2.00-2.09 (7H, m, Met-C*H*H / Met-C*H*$_3$ / Acetyl-C*H*$_3$), 2.38 (1H, br t, *J* = 7.3, Met-C*H*$_2$), 3.10 (1H, dd, *J* = 14.4, *J* = 8.0, Trp-C*H*H), 3.22 (1H, dd, *J* = 14.4, *J* = 5.5, Trp-C*H*H), 3.66 (3H, s, Ester-C*H*$_3$), 4.55 (1H, dt, *J* = 7.3, *J* = 5.0, Met-α-C*H*), 4.77 (1H, dt, *J* = 8.0, *J* = 5.5, Trp-α-C*H*), 6.49 (1H, d, *J* = 7.3, Met-N*H*), 6.57 (1H, d, *J* = 8.0, Trp-N*H*), 7.23 (1H, t, *J* = 7.5, Trp-Ar-*H*), 7.31 (1H, t, *J* = 7.5, Trp-Ar-*H*), 7.49 (1H, s, Trp-Ar-*H*), 7.65 (1H, d, *J* = 7.5, Trp-Ar-*H*), 8.10 (1H, m, Trp-Ar-*H*),

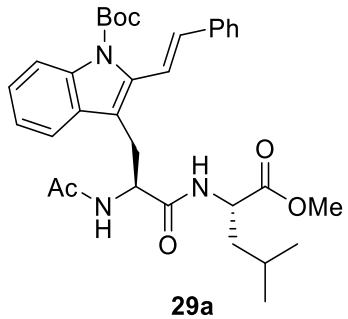
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.3 (Met-CH_3), 23.2 (Acetyl-CH_3), 28.1 (Boc-(CH_3)$_3$), 28.2 (Trp-CH_2), 29.7 (Met-CH_2), 31.3 (Met-CH_2), 51.6 (Trp-α-CH), 52.5 (Ester-CH_3), 53.3 (Met-α-CH), 83.6 (Boc-C), 115.1 (Ar-C), 115.2 (Ar-C), 119.0 (Ar-C), 122.7 (Ar-C), 124.5 (Ar-C), 124.6 (Ar-C), 130.1 (Ar-C), 135.4 (Ar-C), 149.5 (C=O), 170.0 (C=O), 170.7 (C=O), 171.4 (C=O).

IR υ_{max} /cm^{-1} (solid) 3291 m (N-H), 3055 w (C-H), 2915 w (C-H), 1743 s (Ester C=O), 1631 m (amide C=O), 1431 m (C-H), 1224 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{24}H_{34}N_3O_6S$: 492.2163, found: 492.2160.

4.20 Synthesis of modified peptides 29a-b

4.20.1 Synthesis of modified peptide 29a



29a

Modified peptide **29a** was prepared from Ac-Trp(Boc)-Leu-OMe (**28a**) (0.113 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **29a** and the di-olefinated peptide in a ratio of 14:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **29a** as an off-white solid (0.064 g, 47%); m.p. 94-97 °C, R_f 0.38 (50% EtOAc / pet. ether).

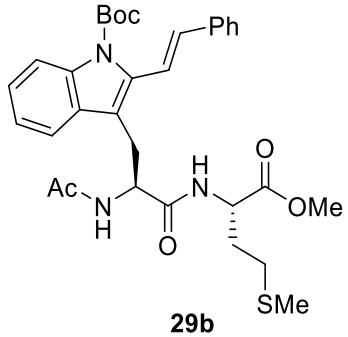
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81-0.86 (6H, m, Leu-C*H*$_3$ (x2)), 1.31-1.41 (1H, m, Leu-C*H*), 1.43-1.47 (2H, m, Leu-C*H*$_2$), 1.65 (9H, s, Boc-(C*H*$_3$)$_3$), 2.00 (3H, s, Acetyl-C*H*$_3$), 3.20 (1H, dd, *J* = 14.0, *J* = 9.7, Trp-C*H*H), 3.33 (1H, dd, *J* = 14.0, *J* = 5.6, Trp-C*H*H), 3.50 (3H, s, Ester-C*H*$_3$), 4.42 (1H, dt, *J* = 8.3, *J* = 5.6, Trp-α-C*H*), 4.77-4.83 (1H, m, Leu-α-C*H*), 5.78 (1H, d, *J* = 8.3, Trp-N*H*), 6.55 (1H, d, *J* = 7.6, Leu-N*H*), 6.79 (1H, d, *J*= 16.6, alkene-C*H*), 7.25-7.34 (4H, m, alkene-C*H* / Ar-*H*), 7.35-7.39 (2H, m, Ar-*H*), 7.58 (2H, d, *J* = 7.1, Ar-*H*), 7.74 (1H, d, *J* = 7.1, Ar-*H*), 8.13 (1H, d, *J* = 7.7, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.0 (Leu-CH_3), 22.5 (Leu-CH_3), 23.2 (Acetyl-CH_3), 24.5 (Leu-CH), 28.2 (Boc-(CH_3)$_3$), 28.8 (Trp-CH_2), 42.0 (Leu-CH_2), 50.8 (Leu-α-CH), 52.1 (Ester-CH_3), 53.4 (Trp-α-CH), 83.8 (Boc-C), 114.8 (Ar-C), 115.5 (Ar-C), 118.9 (Ar-C), 119.8 (alkene-CH), 123.1 (Ar-C), 124.8 (Ar-C), 126.6 (Ar-C), 127.9 (Ar-C), 128.7 (Ar-C), 129.6 (Ar-C), 132.0 (alkene-CH), 135.9 (Ar-C), 136.2 (Ar-C), 136.9 (Ar-C), 150.4 (C=O), 169.7 (C=O), 170.3 (C=O), 171.9 (C=O).

IR U_{max} /cm^{-1} (solid) 3282 m (N-H), 3058 w (C-H), 2955 w (C-H), 1728 s (Ester C=O), 1637 m (amide C=O), 1325 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{33}H_{42}N_3O_6$: 576.3068, found: 576.3062.

4.20.2 Synthesis of modified peptide 29b



29b

Modified peptide **29b** was prepared from Ac-Trp(Boc)-Met-OMe (**28b**) (0.117 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the mono-olefinated peptide **29b** and the di-olefinated peptide in a ratio of 10:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **29b** as an off-white solid (0.064 g, 45%); m.p. 100-103 °C, R_f 0.18 (50% EtOAc / pet. ether).

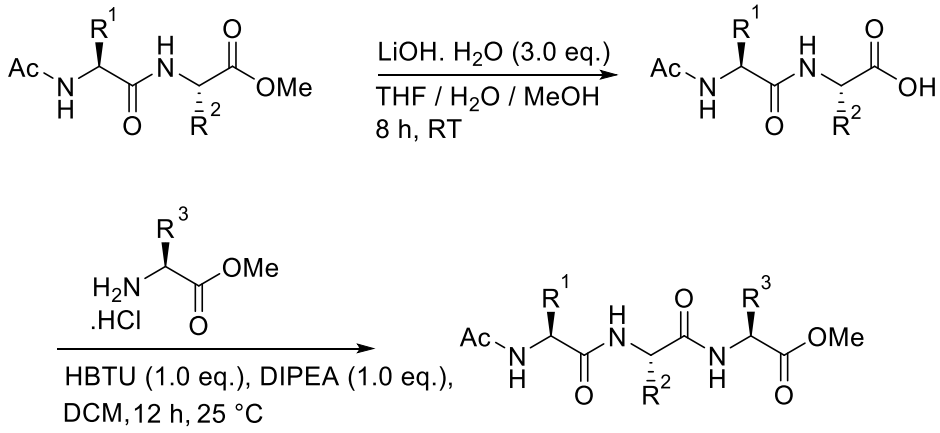
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (9H, s, Boc-(C*H*$_3$)$_3$), 1.78-1.85 (1H, m, Met-C*H*H), 1.94-2.01 (7H, m, Met-C*H*H / Met-C*H*$_3$ / Acetyl-C*H*$_3$), 2.28-2.35 (2H, m, Met-C*H*$_2$), 3.19 (1H, dd, *J* = 13.8, *J* = 9.8, Trp-C*H*H), 3.38 (1H, dd, *J* = 13.8, *J* = 5.5, Trp-C*H*H), 3.52 (3H, s, Ester-C*H*$_3$), 4.40 (1H, m, Trp-α-C*H*), 4.79 (1H, m, Met-α-C*H*), 6.00 (1H, br d, *J* = 7.4, Trp-N*H*), 6.47 (1H, *J* = 7.4, Met-N*H*), 6.84 (1H, dd, *J* = 16.7, alkene-C*H*), 7.28-7.32 (3H, m, alkene-C*H* / Ar-*H*), 7.35-7.40 (3H, m, Ar-*H*), 7.58-7.60 (2H, m, Ar-*H*), 7.69 (1H, d, J = 7.6, Ar-*H*), 8.12 (1H, d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.3 (Met-CH_3), 23.2 (Acetyl-CH_3), 28.3 (Boc-(CH_3)$_3$), 28.8 (Trp-CH_2), 29.5 (Met-CH_2), 31.9 (Met-CH_2), 51.6 (Met-α-CH), 52.4 (Ester-CH_3), 53.5 (Trp-α-CH), 83.9 (Boc-C), 114.8 (Ar-C), 115.6 (Ar-C), 118.8 (Ar-C), 119.7 (alkene-CH), 123.1 (Ar-C), 124.8 (Ar-C), 126.6 (Ar-C), 127.9 (Ar-C), 128.7 (Ar-C), 129.6 (Ar-C), 132.0 (alkene-CH), 136.0 (Ar-C), 136.1 (Ar-C), 136.9 (Ar-C), 150.4 (C=O), 169.7 (C=O), 170.6 (C=O), 170.9 (C=O).

IR υ_{max} /cm^{-1} (solid) 3282 m (N-H), 3053 w (C-H), 2965 w (C-H), 1726 s (Ester C=O), 1642 m (amide C=O), 1455 m (C-H), 1260 s (C-O).

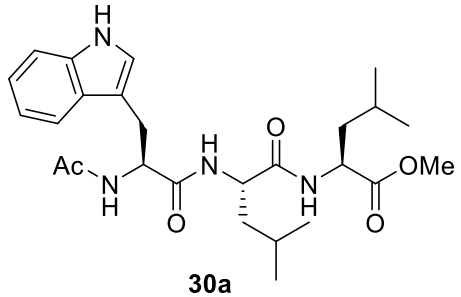
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{40}N_3O_6S$: 594.2632, found: 594.2635.

4.21 General procedure for the synthesis of tryptophan containing tripeptides



Lithium hydroxide monohydrate (0.101 g, 2.40 mmol) was dissolved in Water (2 mL) and added to a solution of the dipeptide ester (0.80 mmol) in a 3:1 mixture of THF / MeOH (8 mL). The solution was then stirred at room temperature for 8 h, followed by concentration to dryness *in vacuo*. The resulting residue was then acidified by the dropwise addition of 1M HCl (~2 mL), before extracting with EtOAc (4 x 10 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to give a colourless oil. The crude dipeptide acid was carried through without further purification. The appropriate amino acid methyl ester hydrochloride (2.00 mmol) and K_2CO_3 (0.398 g, 2.88 mmol) were dissolved in distilled water (30 mL) and stirred for 10 min at room temperature. The free amine was extracted with Et_2O (3 x 20 mL), dried ($MgSO_4$) and concentrated to dryness. The resulting residue was slurried in DCM (20 mL) before the dipeptide acid (0.80 mmol), HBTU (0.303 g, 0.80 mmol) and DIPEA (0.139 mL, 0.80 mmol) were added. The suspension was then stirred for 16 h at room temperature. The suspension was then filtered and washed with 1M HCl (20 mL), sat. $NaHCO_3$ (3 x 20 mL) and water (20 mL). The organic layers were then dried ($MgSO_4$) and concentration to dryness *in vacuo*. The resulting solid was triturated in Et_2O (15 mL) and filtered to afford the corresponding tripeptide as a white solid.

4.21.1 Ac-Trp-Leu-Leu-OMe (30a)



30a

Peptide **30a** was synthesised from Ac-Trp-Leu-OMe (**27a**) (0.299 g, 0.80 mmol) and L-leucine methyl ester hydrochloride (0.363 g, 2.00 mmol), using the procedure in **Section 4.21.** Purification by trituration (Et_2O) afforded **30a** as a white solid (0.370 g, 95%); m.p.187-188 °C.

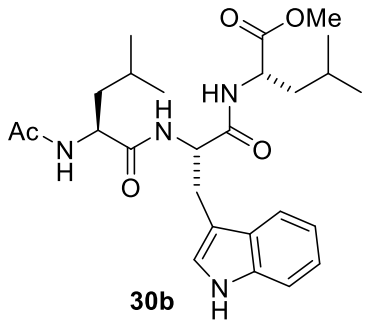
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-0.86 (6H, m, Leu-(C*H*$_3$)$_2$), 0.92-0.94 (6H, m, Leu-(C*H*$_3$)$_2$), 1.36-1.43 (1H, m, Leu-C*H*H), 1.48-1.55 (2H, m, Leu-C*H*H / Leu-C*H*(CH$_3$)$_2$), 1.58-1.65 (3H, m, Leu-C*H*$_2$ / Leu-C*H*(CH$_3$)$_2$), 1.97 (3H, s, Acetyl-C*H*$_3$), 3.15 (1H, dd, *J* = 14.6, *J* = 7.4, Trp-C*H*H), 3.28 (1H, dd, *J* = 14.6, *J* = 6.0, Trp-C*H*H), 3.73 (3H, s, Ester-C*H*$_3$), 4.40 (1H, m, Leu-α-C*H*), 4.56 (1H, dt, *J* = 8.4, *J* = 5.0, Leu-α-C*H*), 4.78 (1H, app q, *J* = 6.0, Trp-α-C*H*), 6.35-6.41 (2H, m, Leu-N*H* / Trp-N*H*), 6.54 (1H, d, *J* = 8.4, Leu-N*H*), 7.08 (1H, m, Trp-Ar-*H*), 7.14 (1H, t, *J* = 7.9, Trp-Ar-*H*), 7.21 (1H, t, *J* = 7.9, Trp-Ar-*H*), 7.36 (1H, d, *J* = 7.9, Trp-Ar-*H*), 7.72 (1H, d, *J* = 7.9, Ar-*H*), 8.23 (1H, br s, Trp-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Leu-CH_3), 22.0 (Leu-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 23.2 (Acetyl-CH_3), 24.6 (Leu-CH_2), 24.8 (Leu-CH_2), 28.1 (Trp-CH_2), 40.8 (Leu-CH), 41.2 (Leu-CH), 50.7 (Leu-α-CH), 52.0 (Trp-α-CH), 52.3 (Ester-CH_3), 53.8 (Leu-α-CH), 110.4 (Ar-C), 111.3 (Ar-C), 118.8 (Ar-C), 119.8 (Ar-C), 122.4 (Ar-C), 123.3 (Ar-C), 127.4 (Ar-C), 136.2 (Ar-C), 170.2 (C=O), 171.4 (C=O), 171.4 (C=O), 173.2 (C-O).

IR U_{max} /cm^{-1} (solid) 382 m (N-H), 3029 w (C-H), 2988 w (C-H), 1739 s (Ester C=O), 1632 m (amide C=O), 1221 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{39}N_4O_5$: 487.2915, found: 487.2914.

4.21.2 Ac-Leu-Trp-Leu-OMe (30b)



30b

Peptide **30b** was synthesised from Ac-Leu-Trp-OMe (**25b**) (0.299 g, 0.80 mmol) and L-leucine methyl ester hydrochloride (0.363 g, 2.00 mmol), using the procedure in **Section 4.21.** Purification by trituration (Et_2O) afforded **30b** as a white solid (0.343 g, 88%); m.p. 212-215 °C.

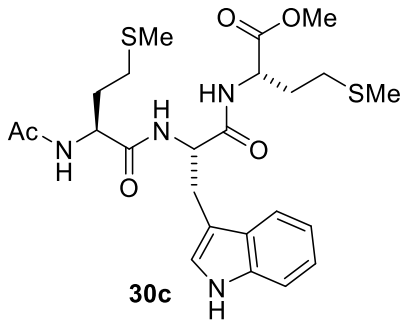
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-0.85 (6H, m, Leu-C*H*$_3$ (x2)), 0.91 (6H, m, Leu-C*H*$_3$ (x2)), 1.37-1.69 (6H, m, Leu-C*H* (x2) / Leu-C*H*$_2$ (x2)), 1.79 (3H, s, Acetyl-C*H*$_3$), 3.17 (1H, dd, *J* = 14.6, *J* = 7.6, Trp-C*H*H), 3.36 (1H, dd, *J* = 14.6, *J* = 5.4, Trp-C*H*H), 3.67 (3H, s, Ester-C*H*$_3$), 4.36-4.42 (1H, m, Leu-α-C*H*), 4.47 (1H, dt, *J* = 8.4, *J* = 5.0, Leu-α-C*H*), 4.73 (1H, dt, *J* = 7.6, *J* = 5.4, Trp-α-C*H*), 5.69 (1H, d, *J* = 7.8, Leu-N*H*), 6.22 (1H, d, *J* = 8.4, Leu-N*H*), 6.70 (1H, d, *J* = 7.6, Trp-N*H*), 7.13-7.16 (2H, m, Trp-Ar-*H*), 7.21 (1H, *J* = 8.0, Trp-Ar-*H*), 7.38 (1H, d, *J* = 8.0, Trp-Ar-*H*), 7.72 (1H, d, *J* = 8.0, Trp-Ar-*H*), 8.14 (1H, br s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 21.8 (Leu-CH_3), 21.9 (Leu-CH_3), 22.3 (Acetyl-CH_3), 23.3 (Leu-CH_3), 23.3 (Leu-CH_3), 25.7 (Leu-CH), 25.8 (Leu-CH), 28.4 (Trp-CH_2), 41.4 (Leu-CH_2), 41.5 (Leu-CH_2), 52.1 (Leu-α-CH), 52.6 (Ester-CH_3), 53.4 (Trp-α-CH), 55.2 (Leu-α-CH), 110.6 (Ar-C), 112.2 (Ar-C), 119.3 (Ar-C), 119.8 (Ar-C), 122.3 (Ar-C), 124.7 (Ar-C), 128.9 (Ar-C), 138.0 (Ar-C), 173.5 (Ar-C), 173.8 (Ar-C), 174.3 (Ar-C), 147.5 (Ar-C).

IR υ_{max} /cm^{-1} (solid) 3290 m (N-H), 3068 w (C-H), 2956 w (C-H), 1741 s (Ester C=O), 1629 m (amide C=O), 1430 m (C-H), 1220 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{26}H_{39}N_4O_5$: 487.2915, found: 487.2915.

4.21.3 Ac-Met-Trp-Met-OMe (30c)



30c

Peptide **30c** was synthesised from Ac-Met-Trp-OMe (**24d**) (0.313 g, 0.80 mmol) and L-methionine methyl ester hydrochloride (0.399 g, 0.80 mmol), using the procedure in **Section 4.21.** Purification by trituration (Et_2O) afforded **30c** as a white solid (0.335 g, 80%); m.p. 191-193 °C.

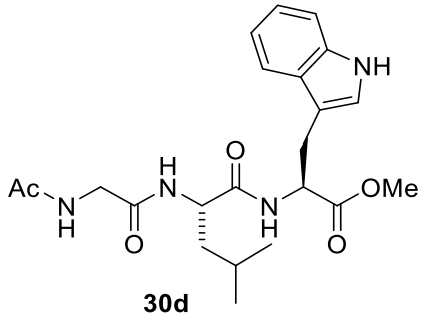
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77 (3H, s, Met-C*H*$_3$), 1.89-1.97 (2H, m, Met-C*H*$_2$), 2.00 (3H, s, Acetyl-C*H*$_3$), 2.03-2.06 (2H, m, Met-C*H*$_2$), 2.09 (3H, s, Met-C*H*$_3$), 2.23-2.29 (2H, m, Met-C*H*$_2$), 2.49-2.61 (2H, m, Met-C*H*$_2$), 3.17 (1H, dd, *J* = 14.5, *J* = 7.0, Trp-C*H*H), 3.39 (1H, dd, *J* = 14.5, *J* = 5.5, Trp-C*H*H), 3.68 (3H, s, Ester-C*H*$_3$), 4.51-4.60 (2H, m, Met-α-C*H* (x2)), 4.73-4.78 (1H, m, Trp-α-C*H*), 6.40 (1H, d, *J* = 7.3, Met-N*H*), 6.68 (1H, d, *J* = 7.8, Met-N*H*), 6.92 (1H, d, *J* = 7.7, Trp-N*H*), 7.10 (1H, d, *J* = 2.3, Trp-Ar-*H*), 7.12-7.16 (1H, m, Trp-Ar-*H*), 7.18-7.22 (1H, m, Trp-Ar-*H*), 7.37 (1H, d, *J* = 7.6, Trp-Ar-*H*), 7.64 (1H, d, *J* = 7.6, Trp-Ar-*H*), 8.27 (1H, br s, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.2 (Met-CH_3), 15.3 (Met-CH_3), 22.8 (Acetyl-CH_3), 27.7 (Trp-CH_2), 29.6 (Met-CH_2), 30.2 (Met-CH_2), 30.8 (Met-CH_2), 31.0 (Met-CH_2), 51.5 (Met-α-CH), 52.5 (Ester-CH_3), 52.9 (Met-α-CH), 54.0 (Trp-α-CH), 109.8 (Ar-C), 111.4 (Ar-C), 118.6 (Ar-C), 119.8 (Ar-C), 122.3 (Ar-C), 123.8 (Ar-C), 127.4 (Ar-C), 136.2 (Ar-C), 170.5 (C=O), 170.8 (C=O), 171.8 (C=O), 172.3 (C=O).

IR U_{max} /cm^{-1} (solid) 3254 m (N-H), 3070 w (C-H), 2915 w (C-H), 17437 s (Ester C=O), 1627 m (amide C=O), 1436 m (C-H), 1224 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{24}H_{35}N_4O_5S_2$: 523.2043, found: 523.2037.

4.21.4 Ac-Gly-Leu-Trp-OMe (30d)



30d

Peptide **30d** was synthesised from Ac-Gly-Leu-OMe **(12**) (0.241 g, 0.80 mmol) and L-tryptophan methyl ester hydrochloride (0.509 g, 2.00 mmol), using the procedure in **Section 4.21.** Purification by trituration (Et_2O) afforded **30d** as a white solid (0.255 g, 74%); m.p. 144-146 °C.

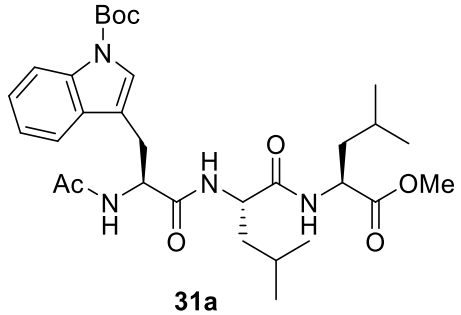
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, d, *J* = 9.5, Leu-C*H*$_3$), 0.88 (3H, d, *J* = 9.5, Leu-C*H*$_3$), 1.48-1.50 (1H, m, Leu-C*H*H), 1.57-1.63 (1H, m, Leu-C*H*H), 1.70-1.76 (1H, m, Leu-C*H*$(CH_3)_2$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.24 (1H, dd, *J* = 14.9, *J* = 6.3, Trp-C*H*H), 3.31 (1H, dd, *J* = 14.9, *J* = 5.0, Trp-C*H*H), 3.66-3.72 (1H, m, Gly-C*H*H), 3.70 (3H, s, Ester-C*H*$_3$), 3.80 (1H, dd, *J* = 16.8, *J* = 5.6, Gly-C*H*H), 4.58-4.60 (1H, m, Leu-α-C*H*), 4.88-4.93 (1H, m, Trp-α-C*H*), 6.28 (1H, br t, *J* = 5.3, Gly-N*H*), 6.70 (1H, br d, *J* = 8.6, Leu-N*H*), 6.98 (1H, m, Trp-Ar-*H*), 7.05-7.10 (2H, m, Trp-Ar-*H* / Trp-N*H*), 7.16 (1H, t, *J* = 7.4, Trp-Ar-*H*), 7.32 (1H, d, *J* = 7.4, Trp-Ar-*H*), 7.50 (1H, d, *J* = 7.4, Trp-Ar-*H*), 8.66 (1H, s, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Leu-CH_3 (x2)), 22.8 (Acetyl-CH_3), 24.7 (Leu-CH), 27.5 (Trp-CH_2), 41.0 (Leu-CH_2), 42.8 (Gly-CH_2), 51.6 (Leu-α-CH), 52.5 (Ester-CH_3), 52.7 (Trp-α-CH), 109.3 (Ar-C), 111.4 (Ar-C), 118.3 (Ar-C), 119.4 (Ar-C), 122.1 (Ar-C), 123.4 (Ar-C), 127.4 (Ar-C), 136.0 (Ar-C),169.0 (C=O), 171.0 (C=O), 171.9 (C=O), 172.2 (C=O).

IR U_{max} /cm^{-1} (solid) 3282 m (N-H), 3055 w (C-H), 2928 w (C-H), 1739 s (Ester C=O), 1631 m (amide C=O), 1431 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{31}N_4O_5$: 431.2289, found: 431.2286.

4.22.1 Ac-Trp(Boc)-Leu-Leu-OMe (31a)



31a

Peptide **31a** was synthesised from Ac-Trp-Leu-Leu-OMe (**30a**) (0.268 g, 0.55 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **31a** as a white solid. (0.284 g, 88%); m.p. 198-199 °C, R_f 0.16 (EtOAc).

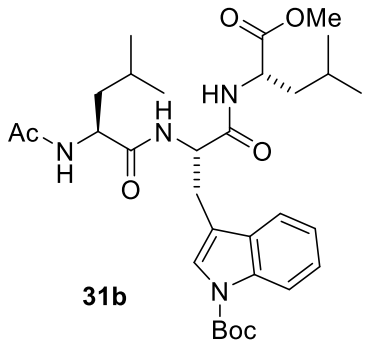
^{1}H NMR (400 MHz, CD_3OD) δ 0.88-0.95 (12H, m, Leu-C*H*$_3$ (x4)), 1.52-1.63 (6H, m, Leu-C*H* (x2) / Leu-C*H*$_2$ (x2)), 1.67 (9H, s, Boc-(C*H*$_3$)$_3$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.00 (1H, dd, *J* = 15.0, *J* = 8.2, Trp-C*H*H), 3.22 (1H, dd, *J* = 15.0, *J* = 5.1, Trp-C*H*H), 3.69 (3H, s, Ester-C*H*$_3$), 4.39-4.45 (2H, m, Leu-α-C*H* (x2)), 4.71-4.75 (1H, m, Trp-α-C*H*), 7.21-7.24 (1H, m, Trp-Ar-*H*), 7.26-7.30 (1H, m, Trp-Ar-*H*), 7.51 (1H, s, Trp-Ar-*H*), 7.65 (1H, d, *J* = 8.0, Trp-Ar-*H*), 8.07 (1H, br d, *J* = 8.0, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Leu-CH_3), 22.1 (Leu-CH_3), 22.6 (Leu-CH_3), 22.7 (Leu-CH_3), 23.0 (Acetyl-CH_3), 24.5 (Leu-CH), 24.9 (Leu-CH), 27.6 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 41.0 (Leu-CH_2), 41.1 (Leu-CH_2), 50.8 (Leu-α-CH), 51.9 (Trp-α-CH), 52.2 (Ester-CH_3), 53.1 (Leu-α-CH), 83.8 (Boc-C), 115.2 (Ar-C), 115.3 (Ar-C), 118.9 (Ar-C), 122.7 (Ar-C), 124.3 (Ar-C), 124.5 (Ar-C), 130.3 (Ar-C), 135.3 (Ar-C), 149.7 (C=O), 170.4 (C=O), 171.3 (C=O), 171.5 (C=O), 173.1 (C=O).

IR υ_{max} /cm^{-1} (solid) 3297 m (N-H), 2960 w (C-H), 1750 s (Ester C=O), 1628 m (amide C=O), 1246 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{31}H_{47}N_4O_7$: 587.3439, found: 587.3441.

4.22.2 Ac-Leu-Trp(Boc)-Leu-OMe (31b)



31b

Peptide **31b** was synthesised from Ac-Leu-Trp-Leu-OMe (**30b**) (0.268 g, 0.55 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **31b** as a white solid. (0.287 g, 89%); m.p. 180-184 °C, R_f 0.14 (EtOAc).

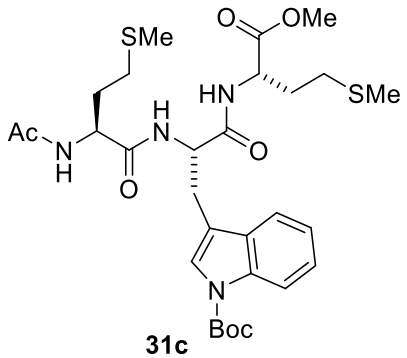
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-0.91 (12H, m, Leu-C*H*$_3$ (x4)), 1.42-1.61 (6H, m, Leu-C*H* (x2) / Leu-C*H*$_2$ (x2)), 1.66 (9H, s, Boc-(C*H*$_3$)$_3$), 1.90 (3H, s, Acetyl-C*H*$_3$), 3.11-3.24 (2H, m, Trp-C*H*$_2$), 3.65 (3H, s, Ester-C*H*$_3$), 4.43-4.52 (2H, m, Leu-α-C*H* (x2)), 4.73-4.79 (1H, m, Trp-α-C*H*), 5.95 (1H, br d, *J* = 7.3, Leu-N*H*), 6.43 (1H, br d, *J* = 8.7, Trp-N*H*), 6.90 (1H, br d, *J* = 7.8, Leu-N*H*), 7.24-7.34 (2H, m, Trp-Ar-*H*), 7.49 (1H, s, Trp-Ar-*H*), 7.64 (1H, d, *J* = 7.8, Trp-Ar-*H*), 8.11 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 22.1 (Leu-CH_3), 22.7 (Leu-CH_3), 22.8 (Leu-CH_3), 22.9 (Acetyl-CH_3), 24.7 (Leu-CH), 24.7 (Leu-CH), 27.7 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 41.1 (Leu-CH_2), 41.2 (Leu-CH_2), 50.8 (Leu-α-CH), 51.8 (Trp-α-CH), 52.2 (Ester-CH_3), 53.0 (Leu-α-CH), 83.7 (Boc-C), 115.2 (Ar-C), 115.3 (Ar-C), 119.1 (Ar-C), 122.7 (Ar-C), 124.5 (Ar-C), 124.6 (Ar-C), 130.1 (Ar-C), 135.4 (Ar-C), 149.6 (C=O), 170.2 (C=O), 170.3 (C=O), 172.0 (C=O), 172.6 (C=O).

IR U_{max} /cm^{-1} (solid) 3254 m (N-H), 3073 w (C-H), 2957 w (C-H), 1720 s (Ester C=O), 1631 m (amide C=O), 1545 m (C=C), 1439 m (C-H), 1291 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{31}H_{47}N_4O_7$: 587.3439, found: 587.3441.

4.22.3 Ac-Met-Trp(Boc)-Met-OMe (31c)



31c

Peptide **31c** was synthesised from Ac-Met-Trp-Met-OMe (**30c**) (0.268 g, 0.55 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **31c** as a white solid. (0.287 g, 87%); m.p. 180-184 °C, R_f 0.35 (EtOAc).

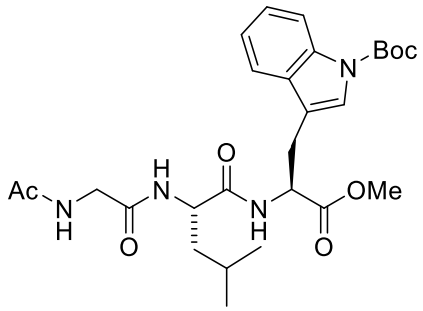
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s, Boc-(C*H*$_3$)$_3$), 1.88 (1H, dd, *J* = 14.6, *J* = 7.3 Met-C*H*H), 1.92-1.94 (4H, m, Met-C*H*$_3$ / Met-C*H*H), 1.96-1.99 (4H, m, Met-C*H*$_3$ / Met-C*H*H), 2.01-2.04 (4H, m, Acetyl-C*H*$_3$ / Met-C*H*H), 2.33 (2H, t, *J* = 7.3, Met-C*H*$_2$), 2.46-2.51 (2H, m, Met-C*H*$_2$), 3.09-3.20 (2H, m, Trp-C*H*$_2$), 3.64 (3H, s, Ester-C*H*$_3$), 4.54 (1H, dt, *J* = 7.3, *J* = 5.0, Met-α-C*H*), 4.66 (1H, dt, *J* = 7.3, *J* = 6.8, Met-α-C*H*), 4.82 (1H, dt, *J* = 7.5, *J* = 6.8, Trp-α-C*H*), 6.68 (1H, d, *J* = 7.8, Met-N*H*), 6.99 (1H, d, *J* = 7.8, Met-N*H*), 7.18 (1H, t, *J* = 7.8, Trp-Ar-*H*), 7.25-7.30 (2H, m, Trp-Ar-*H* / Trp-N*H*), 7.48 (1H, s, trp-Ar-*H*), 7.53 (1H, d, *J* = 7.8, Trp-Ar-*H*), 8.06 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.2 (Met-CH_3), 15.3 (Met-CH_3), 23.0 (Acetyl-CH_3), 28.0 (Trp-CH_2), 28.1 (Boc-$(CH_3)_3$), 29.7 (Met-CH_2), 30.0 (Met-CH_2), 31.2 (Met-CH_2), 31.5 (Met-CH_2), 51.5 (Met-α-CH), 52.3 (Ester-CH_3), 52.4 (Met-α-CH), 53.3 (Trp-α-CH), 83.6 (Boc-C), 115.0 (Ar-C), 115.2 (Ar-C), 118.9 (Ar-C), 122.6 (Ar-C), 124.5 (Ar-C), 124.6 (Ar-C), 130.1 (Ar-C), 135.4 (Ar-C), 149.5 (C=O), 170.3 (C=O), 170.5 (C=O), 171.1 (C=O), 171.6 (C=O).

IR υ_{max} /cm^{-1} (solid) 3260 m (N-H), 3069 w (C-H), 2919 w (C-H), 1728 s (Ester C=O), 1631 m (amide C=O), 1453 m (C-H), 1224 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{29}H_{43}N_4O_7S_2$: 623.2568, found: 623.2571.

4.22.4 Ac-Gly-Leu-Trp(Boc)-OMe (31d)



31d

Peptide **31d** was synthesised from Ac-Gly-Leu-Trp-OMe (**30d**) (0.237 g, 0.55 mmol) using the procedure in **Section 4.13.** Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **31d** as a white solid. (0.181 g, 62%); m.p. 116-118 °C, R_f 0.10 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, d, *J* = 10.0, Leu-C*H*$_3$), 0.91 (3H, d, *J* = 10.0, Leu-C*H*$_3$), 1.46-1.52 (1H, m, Leu-C*H*), 1.55-1.64 (2H, m, Leu-C*H*$_2$), 1.68 (9H, s, Boc-(C*H*$_3$)$_3$), 2.01 (3H, s, Acetyl-C*H*$_3$), 3.19 (1H, dd, *J* = 14.8, *J* = 6.3, Trp-C*H*H), 3.27 (1H, dd, *J* = 14.8, *J* = 6.3, Trp-C*H*H), 3.68 (3H, s, Ester-C*H*$_3$), 3.80-3.92 (2H, m, Gly-C*H*$_2$), 4.42-4.48 (1H, m, Leu-α-C*H*), 4.88 (1H, dt, *J* = 7.4, *J* = 6.3, Trp-α-C*H*), 6.86-6.71 (2H, m, Gly-N*H* / Leu-N*H*), 6.94 (1H, d, *J* = 7.4, Trp-N*H*), 7.22 (1H, t, *J* = 7.4, Trp-Ar-*H*), 7.30 (1H, t, *J* = 7.4, Trp-Ar-*H*), 7.42 (1H, s, Trp-Ar-*H*), 7.48 (1H, d, *J* = 7.4, Trp-Ar-*H*), 8.05 (1H, br d, Trp-Ar-*H*).

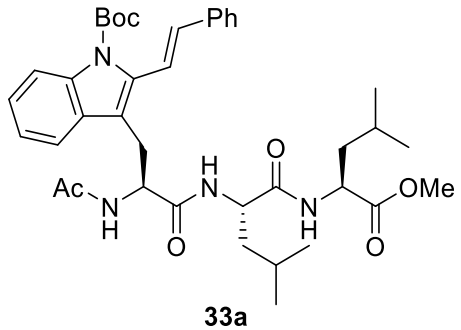
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.9 (Leu-CH_3), 22.8 (Acetyl-CH_3), 24.6 (Leu-CH), 27.2 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 40.4 (Leu-CH_2), 43.2 (Gly-CH_2), 51.7 (Leu-α-CH), 52.4 (Ester-CH_3), 52.5 (Trp-α-CH), 84.0 (Boc-C), 114.9 (Ar-C), 115.3 (Ar-C), 118.8 (Ar-C), 122.6 (Ar-C), 124.3 (Ar-C), 124.5 (Ar-C), 130.4 (Ar-C), 135.1 (Ar-C), 149.9 (C=O), 169.2 (C=O), 170.9 (C=O), 171.5 (C=O), 172.7 (C=O).

IR υ_{max} /cm^{-1} (solid) 3278 m (N-H), 3060 w (C-H), 2954 w (C-H), 1731 s (Ester C=O), 1631 m (amide C=O), 1451 m (C-H), 1226 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{27}H_{39}N_4O_7$: 531.2813, found: 531.2808.

4.23 Synthesis of modified tryptophan containing tripeptides 33a-d

4.23.1 Synthesis of modified peptide 33a



33a

Modified peptide **33a** was prepared from Ac-Trp(Boc)-Leu-Leu-OMe (**32a**) (0.140 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **33a** and the mono-olefinated peptide in a ratio of 11:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **33a** as an off-white solid (0.097 g, 59%); m.p. 165-167 °C, R_f 0.26 (50% EtOAc / pet. ether).

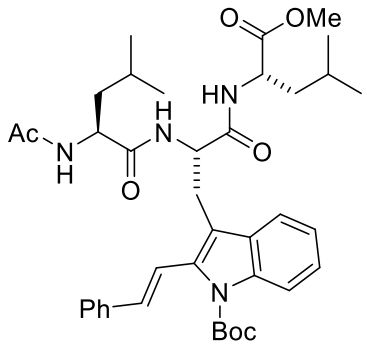
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74-0.80 (12H, Leu-C*H*$_3$ (x4)), 1.24-1.50 (6H, m, Leu-C*H*$_2$ (x2) / Leu-C*H* (x2)), 1.58 (Boc-(C*H*$_3$)$_3$), 1.85 (Acetyl-C*H*$_3$), 3.21 (1H, dd, *J* = 14.3, *J* = 7.1, Trp-C*H*H), 3.28 (1H, dd, *J* = 14.3, *J* = 7.1, Trp-C*H*H), 3.60 (3H, s, Ester-C*H*$_3$), 4.24 (1H, dt, *J* = 8.1, *J* = 5.7, Leu-α-C*H*), 4.33-4.39 (1H, m, Leu-α-C*H*), 4.72 (1H, app q, *J* = 7.3, Trp-α-C*H*), 6.12 (1H, br d, *J* = 8.1, Leu-N*H*), 6.20 (1H, br d, *J* = 7.9, Leu-N*H*), 6.25 (1H, br d, *J* = 7.3, Trp-N*H*), 6.69 (1H, d, *J* = 16.6, alkene-C*H*), 7.16-7.25 (4H, m, Ar-*H*), 7.28-7.35 (3H, m, alkene-C*H* / Ar-*H*), 7.49 (2H, d, *J* = 7.5, Ar-*H*), 7.64 (1H, br d, *J* = 7.5, Ar-*H*), 8.04 (1H, br d, *J* = 8.4, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.0 (Leu-CH_3), 21.0 (Leu-CH_3), 21.6 (Leu-CH_3), 21.7 (Leu-CH_3), 22.1 (Acetyl-CH_3), 23.5 (Leu-CH), 23.8 (Leu-CH), 27.3 (Boc-$(CH_3)_3$), 28.7 (Trp-CH_2), 40.0 (Leu-CH_2), 40.3 (Leu-CH_2), 49.8 (Trp-α-CH), 50.8 (Leu-α-CH), 51.2 (Leu-α-CH), 52.6 (Ester-CH_3), 83.0 (Boc-C), 113.8 (Ar-C), 114.7 (Ar-C), 117.9 (Ar-C), 119.1 (alkene-C), 122.0 (Ar-C), 123.9 (Ar-C), 125.6 (Ar-C), 127.0 (Ar-C), 127.8 (Ar-C), 128.6 (Ar-C), 131.3 (alkene-C), 134.8 (Ar-C), 135.2 (Ar-C),135.8 (Ar-C), 149.4 (C=O), 169.2 (C=O), 169.7 (C=O), 169.9 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3276 m (N-H), 2961 w (C-H), 1728 s (Ester C=O), 1631 m (amide C=O), 1541 m (C=C), 1455 m (C-H), 1258 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{39}H_{52}N_4O_7$: 689.3909, found: 689.3908.

4.23.2 Synthesis of modified peptide 33b



33b

Modified peptide **33b** was prepared from Ac-Leu-Trp(Boc)-Leu-OMe (**32b**) (0.140 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **33b** and the mono-olefinated peptide in a ratio of 12:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **33b** as an off-white solid (0.113 g, 69%); m.p. 100-104 °C, R_f 0.24 (50% EtOAc / pet. ether).

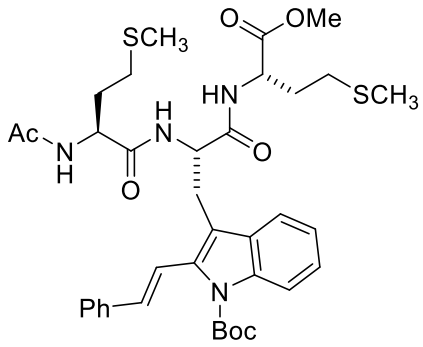
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, t, *J* = 6.2, Leu-C*H*$_3$), 0.83 (3H, t, *J* = 6.2, Leu-C*H*$_3$), 0.87 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 0.88 (3H, d, *J* = 6.2, Leu-C*H*$_3$), 1.36-1.47 (4H, m, Leu-C*H*H (x2) / Leu-CH (x2)), 1.51-1.59 (2H, m, Leu-C*H*H (x2)), 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.91 (3H, s, Acetyl-C*H*$_3$), 3.27-3.37 (2H, m, Trp-C*H*$_2$), 3.55 (3H, s, Ester-C*H*$_3$), 4.39-4.48 (2H, m, Leu-α-C*H* / Trp-α-C*H*), 4.77 (1H, app q, *J* = 7.7, Leu-α-C*H*), 5.75 (1H, br d, *J* = 8.0, Leu-N*H*), 5.99 (1H, br d, *J* = 8.0, Trp-N*H*), 6.73-6.77 (2H, m, alkene-C*H* / Leu-N*H*), 7.25-7.32 (3H, m, alkene-C*H* / Ar-*H*), 7.36 (1H, d, *J* = 7.4, Ar-*H*), 7.38-7.40 (2H, m, Ar-*H*), 7.58 (2H, d, *J* = 7.4, Ar-*H*), 7.71 (1H, br d, *J* = 7.4, Ar-*H*), 8.13 (1H, d, *J* = 7.8, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.0 (Leu-CH_3), 22.0 (Leu-CH_3), 22.6 (Leu-CH_3), 22.8 (Leu-CH_3), 23.1 (Acetyl-CH_3), 24.6 (Leu-CH), 24.7 (Leu-CH), 28.0 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 41.3 (Leu-CH_2), 41.6 (Leu-CH_2), 50.8 (Leu-α-CH), 51.7 (Trp-α-CH), 52.2 (Ester-CH_3), 53.3 (Leu-α-CH), 83.9 (Boc-C), 114.8 (Ar-C), 115.6 (Ar-C), 119.0 (alkene-C), 120.0 (Ar-C), 123.1 (Ar-C), 124.8 (Ar-C), 126.7 (Ar-C), 128.0 (Ar-C),128.7 (Ar-C), 129.5 (Ar-C), 132.2 (alkene-C), 135.9 (Ar-C), 136.3 (Ar-C), 136.8 (Ar-C), 150.4 (C=O), 169.9 (C=O), 170.0 (C=O), 171.8 (C=O), 172.2 (C=O).

IR U_{max} /cm^{-1} (solid) 3273 m (N-H), 3056 w (C-H), 2957 w (C-H), 1730 s (Ester C=O), 1627 m (amide C=O), 1541 m (C=C).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{39}H_{53}N_4O_7$: 689.3909, found: 689.3908.

4.23.3 Synthesis of modified peptide 33c



33c

Modified peptide **33c** was prepared from Ac-Met-Trp(Boc)-Met-OMe (**32c**) (0.148 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **33c** and the mono-olefinated peptide in a ratio of 19:1. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **33c** as an off-white solid (0.095 g, 55%); m.p. 144-146 °C, R_f 0.39 (50% EtOAc / pet. ether).

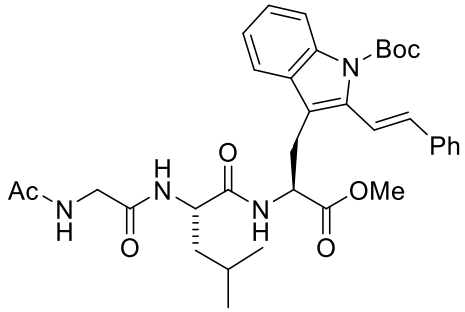
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (9H, s, Boc-(CH_3)$_3$), 1.76-1.84 (2H, Met-CH_2), 1.86 (3H, s, Met-CH_3), 1.92 (3H, s, Met-CH_3), 1.95-1.97 (4H, s, Acetyl-CH_3 / Met-CHH), 2.00-2.08 (1H, m, Met-CHH), 2.19-2.28 (2H, m, Met-CH_2), 2.35-2.45 (2H, m, Met-CH_2), 3.22 (1H, dd, *J* = 14.3, *J* = 8.6, Trp-CHH), 3.29 (1H, dd, *J* = 14.3, *J* = 6.8, Trp-CHH), 3.50 (3H, s, Ester-CH_3), 4.40 (1H, dt, *J* = 7.3, *J* = 5.7, Met-α-CH), 4.50 (1H, app q, *J* = 7.8, Trp-α-CH), 4.69 (1H, app q, *J* = 7.4, Met-α-CH), 6.18 (1H, d, *J* = 7.0, Met-NH), 6.20 (1H, d, *J* = 7.0, Met-NH), 6.71 (1H, d, *J* = 16.8, alkene-CH), 6.91 (1H, br d, *J* = 7.8, Trp-NH), 7.16-7.33 (7H, m, alkene-CH / Ar-H), 7.51 (2H, d, *J* = 7.4, Ar-H), 7.59 (1H, d, *J* = 7.4, Ar-H), 8.06 (1H, d, *J* = 7.4, Ar-H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 15.1 (Met-CH_3), 15.3 (Met-CH_3), 23.1 (Acetyl-CH_3), 28.1 (Trp-CH_2), 28.2 (Boc-(CH_3)$_3$), 29.6 (Met-CH_2), 30.0 (Met-CH_2), 31.4 (Met-CH_2), 31.6 (Met-CH_2), 51.7 (Met-α-CH), 52.3 (Ester-CH_3), 52.4 (Trp-α-CH), 53.6 (Met-α-CH), 84.0 (Boc-C), 114.6 (Ar-C), 115.6 (Ar-C), 118.8 (Ar-C), 119.9 (alkene-C), 123.0 (Ar-C), 124.8 (Ar-C), 126.7 (Ar-C), 128.0 (Ar-C), 128.7 (Ar-C), 129.5 (Ar-C), 132.3 (alkene-C), 136.0 (Ar-C), 136.3 (Ar-C), 136.8 (Ar-C), 150.4 (C=O), 170.0 (C=O), 170.2 (C=O), 170.8 (C=O), 171.2 (C=O).

IR υ_{max} /cm^{-1} (solid) 3275 m (N-H), 3060w (C-H), 2920 w (C-H), 1728 s (Ester C=O), 1634 m (amide C=O), 1453 m (C-H), 1258 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{37}H_{49}N_4O_7S_2$: 725.3037, found: 725.3040.

4.23.4 Synthesis of modified peptide 33d



33d

Modified peptide **33d** was prepared from Ac-Gly-Leu-Trp(Boc)-OMe (**32d**) (0.126 g, 0.238 mmol) and styrene (0.109 mL, 0.952 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained a mixture of the di-olefinated peptide **33d** and the mono-olefinated peptide in a ratio of 20:1. Purification by flash column chromatography (EtOAc) followed by recrystallisation from DCM / hexanes gave **33d** as an off-white solid (0.102 g, 68%); m.p. 165-167 °C, R_f 0.20 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (3H, d, *J* = 9.0, Leu-C*H*$_3$), 0.86 (3H, d, *J* = 9.0, Leu-C*H*$_3$), 1.49-1.57 (3H, m, Leu-C*H* / Leu-C*H*$_2$), 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.97 (3H, s, Acetyl-C*H*$_3$), 3.32 (1H, dd, *J* = 14.4, *J* = 7.3, Trp-C*H*H), 3.39 (1H, dd, *J* = 14.4, *J* = 7.3, Trp-C*H*H), 3.59 (3H, s, Ester-C*H*$_3$), 3.69-3.83 (2H, m, Gly-C*H*$_2$), 4.34-4.39 (1H, m, Leu-α-C*H*), 4.88-4.93 (1H, m, Trp-α-C*H*), 6.31 (2H, br m, N*H* (x2)), 6.77 (1H, d, *J* = 16.6, alkene-C*H*), 7.15-7.23 (1H, m, Ar-*H*), 7.25-7.40 (6H, m, Ar-*H* / alkene-C*H* / N*H*), 7.56 (2H, d, *J* = 8.0, Ar-*H*), 8.11 (1H, d, *J* = 8.0, Ar-*H*).

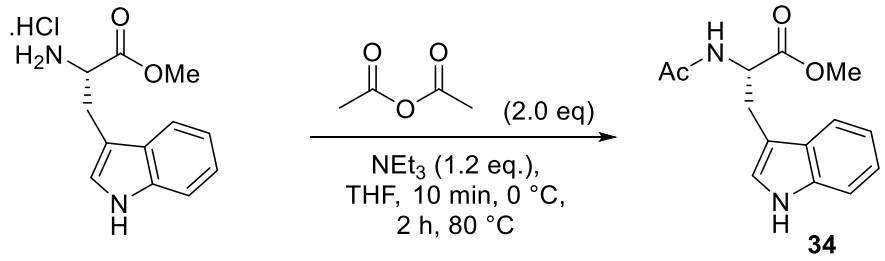
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.8 (Leu-CH_3), 22.8 (Leu-CH_3), 22.8 (Acetyl-C*H*$_3$), 24.6 (Leu-CH), 27.6 (Trp-CH_2), 28.3 (Boc-$(CH_3)_3$), 40.6 (Leu-CH_2), 43.1 (Gly-CH_2), 51.5 (Leu-α-CH), 52.4 (Ester-CH_3), 52.5 (Trp-α-CH), 84.2 (Boc-C), 114.6 (Ar-C), 115.4 (Ar-C), 118.8 (Ar-C), 119.9 (alkene-C), 122.9 (Ar-C), 124.7 (Ar-C), 126.6 (Ar-C), 128.1 (Ar-C), 128.8 (Ar-C), 129.9 (Ar-C), 132.4 (alkene-C), 135.8 (Ar-C), 136.2 (Ar-C), 136.7 (Ar-C), 150.5 (C=O), 168.9 (C=O), 170.9 (C=O), 171.4 (C=O), 172.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3273 m (N-H), 3060 w (C-H), 2958 w (C-H), 1730 s (Ester C=O), 1631 m (amide C=O), 1541 m (C=C), 1224 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{35}H_{45}N_4O_7$: 633.3283, found: 633.3286.

4.24 Synthesis of unnatural tryptophan derived amino acids

4.24.1 Synthesis of Ac-Trp-OMe (34)



34

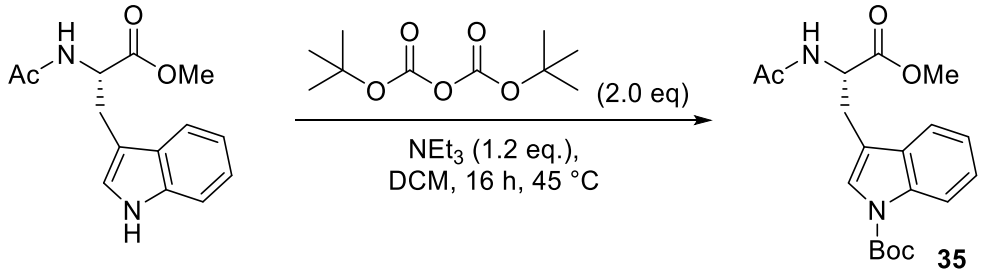
L-tryptophan methyl ester hydrochloride (0.300 g, 1.178 mmol) was slurried in THF (15 mL) and cooled to 0 ˚C in an ice bath before the addition of NEt_3 (0.197 mL, 1.414 mmol). Acetic anhydride (0.223 mL, 2.356 mmol) was then added and heated to 80 ˚C for 2 h. The resulting solution was allowed to cool to room temperature before it was added to water (25 mL), and extracted with EtOAc (3 x 30 mL). The organic layers were combined and washed with 1M HCl (20 mL), sat. $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was then dried ($MgSO_4$), and reduced to a brown solid by rotary evaporation. The solid was then triturated (Et_2O) and filtered to give a sandy brown solid (0.240 g, 78%), m.p. 154-155 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (3H, s, Acetyl-C*H*$_3$), 3.27-3.37 (2H, m, Trp-C*H*$_2$), 3.70 (3H, s, Ester-CH_3), 4.95 (1H, dt, *J* = 7.8, *J* = 5.0, Trp-α-C*H*), 5.98 (1H, br d, *J* = 7.8, Trp-N*H*), 6.97 (1H, d, *J* = 2.3, Trp-Ar-*H*), 7.10-7.13 (1H, m, Trp-Ar-*H*), 7.17-7.21 (1H, m, Ar-*H*), 7.36 (1H, d, *J* = 7.5, Trp-Ar-*H*), 7.52 (1H, d, *J* = 7.5, Trp-Ar-*H*) 8.14 (1H, m, Trp-Ar-N*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.3 (Acetyl-CH_3), 27.6 (Trp-CH_2), 52.4 (Ester-CH_3), 53.0 (Trp-α-CH), 110.1(Ar-C), 113.3 (Ar-C), 118.5 (Ar-C), 119.7 (Ar-C), 122.3 (Ar-C), 122.6 (Ar-C), 127.7 (Ar-C), 136.0 (Ar-C), 169.7 (C=O), 172.4 (C=O).

The data matches that previously reported.[69]

4.24.2 Preparation of Ac-Trp(Boc)-OMe (35)



35

N-Acetyl-L-tryptophan methyl ester (0.200 g, 0.768 mmol) was dissolved in DCM (20 mL) and treated with NEt_3 (0.129 mL, 0.922 mmol). A solution of Boc anhydride (0.335 g, 1.537 mmol) in DCM (5 mL) was added dropwise to the peptide solution, and heated under reflux for 16 h. The solution was allowed to cool to room temperature, and concentrated by rotary evaporation. The crude compound was purified by flash column chromatography (50% EtOAc / pet. ether) to afforded **35** as a yellow oil (0.271 g, 98%); m.p. 57-59 ˚C, R_f 0.19 (50% EtOAc / pet. ether).

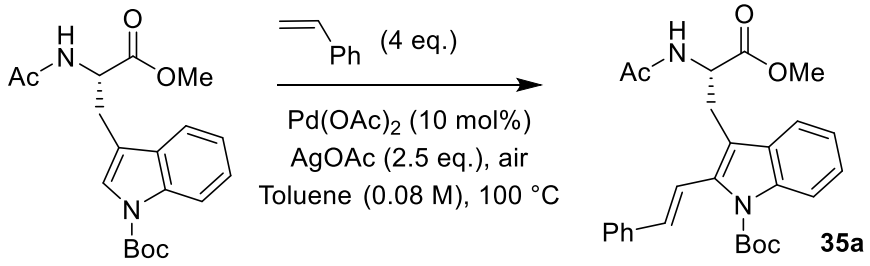
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (Boc-(C*H*$_3$)$_3$), 1.88 (3H, s, Acetyl-C*H*$_3$), 3.10 (1H, dd, *J* = 14.8, *J* = 5.5, Trp-C*H*H), 3.18 (1H, dd, *J* = 14.8, *J* = 5.5, Trp-C*H*H), 3.60 (3H, s, Ester-C*H*$_3$), 4.84 (1H, dt, *J* = 7.7, *J* = 5.5, Trp-α-C*H*), 6.27 (1H, br d, *J* = 7.7, Trp-N*H*), 7.13 (1H, t, *J* = 7.7, Trp-Ar-*H*), 7.21 (1H, t, *J* = 7.7, Trp-Ar-*H*), 7.29 (1H, br s, Trp-Ar-*H*), 7.38 (1H, d, *J* = 7.7, Trp-Ar-*H*), 8.01 (1H, br d, *J* = 6.3, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 27.1 (Trp-CH_2), 28.0 (Boc-$(CH_3)_3$), 52.3 (Ester-CH_3), 52.5 (Trp-α-CH), 83.6 (Boc-C), 114.8 (Ar-C), 115.1 (Ar-C), 118.6 (Ar-C), 122.4 (Ar-C), 123.8 (Ar-C), 124.4 (Ar-C), 130.4 (Ar-C), 135.1 (Ar-C), 149.4 (C=O), 169.7 (C=O), 172.0 (C=O).

IR U_{max} /cm^{-1} (solid) 3286 m (N-H), 2978 w (C-H), 1727 s (Ester C=O), 1654 m (amide C=O), 1452 m (C-H), 1227 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{19}H_{25}N_2O_5$: 361.1758, found: 361.1755.

4.24.3 Synthesis of amino acid (35a)



Modified amino acid **35a** was prepared from Ac-Trp(Boc)-OMe (**35**) (0.058 g, 0.161 mmol) and styrene (0.074 mL, 0.644 mmol), using the general procedure in **Section 4.14.** The ^{1}H NMR spectrum for the crude product contained only mono-olefinated peptide **35a** and the peptide starting material only. There was no evidence of the di-olefinated product. Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **35a** as an off-white solid (0.029 g, 39%); m.p. 55-56 °C, R_f 0.37 (50% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s, Boc-(C*H*$_3$)$_3$), 1.88 (3H, s, Acetyl-C*H*$_3$), 3.38 (2H, d, *J* = 6.4, Trp-C*H*$_2$), 3.51 (3H, s, Ester-CH$_3$), 4.93 (1H, dt, *J* = 7.6, *J* = 6.4, Trp-α-C*H*), 6.02 (1H, br d, *J* = 7.6, Trp-N*H*), 6.79 (1H, d, *J* = 16.6, alkene-C*H*), 7.23-7.33 (4H, m, Ar-*H* /alkene-C*H*), 7.38 (2H, m, Ar-*H*), 7.55 (3H, m, Ar-*H*), 8.12 (1H, d, *J* = 8.4, Ar-*H*).

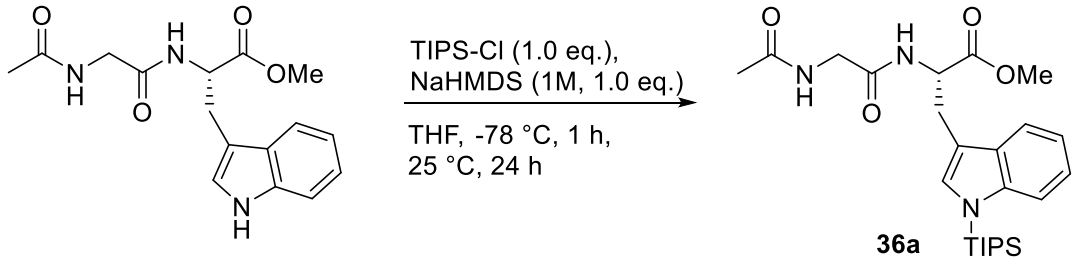
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.1 (Acetyl-CH$_3$), 27.6 (Trp-CH$_2$), 28.3 (Boc-(CH$_3$)$_3$), 52.4 (Ester-CH$_3$), 52.5 (Trp-α-CH), 84.0 (Boc-C), 114.6 (Ar-C), 115.5 (Ar-C), 118.7 (Ar-C), 119.8 (alkene-CH), 122.8 (Ar-C), 124.7 (Ar-C), 126.5 (Ar-C), 128.0 (Ar-C), 128.8 (Ar-C), 130.1 (Ar-C), 132.4 (alkene-CH), 135.8 (Ar-C), 136.0 (Ar-C), 136.8 (Ar-C), 150.4 (C=O), 169.6 (C=O), 172.4 (C=O).

IR υ_{max} /cm^{-1} (solid) 378 m (N-H), 2976 w (C-H), 1726 s (Ester C=O), 1655 m (amide C=O), 1359 m (C-H), 1206 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{27}H_{31}N_2O_5$: 463.2227, found: 463.2225.

4.25 Preparation of silyl protected peptides 36a-b

4.25.1 Ac-Gly-Trp(TIPS)-OMe (36a)



36a

Ac-Gly-Trp-OMe (**20**) (0.100 g, 0.359 mmol) was dissolved in dry THF (10 mL) and cooled in a dry ice/acetone bath. NaHMDS (1M in THF, 0.359 mL, 0.359 mmol) was added dropwise at -78 ˚C and left to stir for 1 h. Triisopropylsilyl chloride (0.092 mL, 0.431 mmol) was added and left to gradually warm to room temperature for 24 h. The solution was added to water (30 mL) and extracted with EtOAc (4 x 30 mL), dried ($MgSO_4$) and concentrated to a brown oil in *vacuuo.* The oil was purified by flash column chromatography (EtOAc) and reduced down to give **36a** as a yellow oil (0.114 g, 67%), R_f 0.27 (EtOAc).

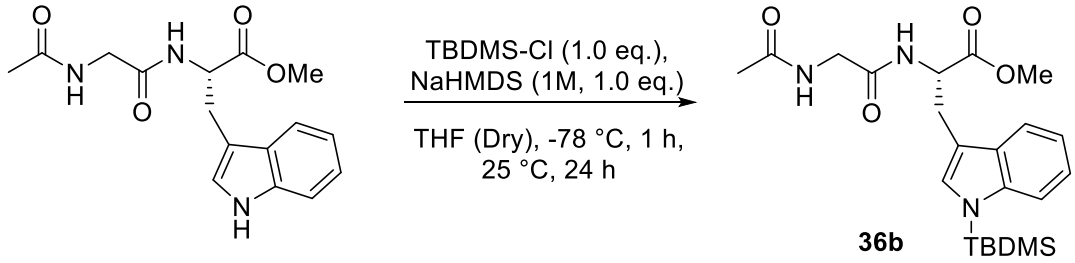
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (18H, dd, *J* = 7.3, *J* = 1.6, TIPS-(C*H*$_3$)$_6$), 1.67 (3H, septet, *J* = 7.3, TIPS-C*H* x3), 1.94 (3H, s, Acetyl-C*H*$_3$), 3.25-3.35 (2H, m, Trp-C*H*$_3$), 3.66 (3H, s, Ester-C*H*$_3$), 3.83-3.94 (2H, m, Gly-C*H*$_2$), 4.94 (1H, dt, *J* = 7.8, *J* = 5.7, Trp-α-C*H*), 6.30 (1H, br t, *J* = 4.3, Gly-N*H*), 6.57 (1H, br d, *J* = 7.8, Trp-N*H*), 7.02 (1H, s, Trp-Ar-*H*), 7.10-7.17 (2H, m, Trp-Ar-*H*), 7.46-7.52 (2H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 12.7 (TIPS-CH x3), 18.0 (TIPS-CH_3), 22.8 (Acetyl-CH_3), 27.6 (Trp-CH_2), 42.8 (Gly-CH_2), 52.3 (Ester-CH_3), 53.2 (Trp-α-CH), 111.6 (Ar-C), 114.1 (Ar-C), 118.2 (Ar-C), 119.8 (Ar-C), 121.7 (Ar-C), 129.7 (Ar-C), 130.9 (Ar-C), 141.1 (Ar-C), 168.4 (C=O), 170.3 (C=O), 172.0 (C=O).

IR U_{max} /cm^{-1} (oil) 3286 m (N-H), 2946 s (C-H), 2868 s (C-H), 1743 s (Ester C=O), 1647 m (C=C), 1450 m (C-H), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{25}H_{40}N_3O_4Si$: 474.2783, found: 474.2777.

4.25.2 Ac-Gly-Trp(TBDMS)-OMe (36b)



Ac-Gly-Phe-OMe (**20**) (0.100 g, 0.359 mmol) was dissolved in dry THF (10 mL) and cooled in a dry ice/acetone bath. NaHMDS (1M in THF, 0.359 mL, 0.359 mmol) was added dropwise to the solution and stirred for 1 h at -78 ˚C. *tert*-butyldimethylsilyl chloride (0.065 g, 0.431 mmol) was added and left to gradually warm to room temperature for 24 h. The solution was added to water (30 mL) and extracted with EtOAc (4 x 30 mL), dried ($MgSO_4$) and concentrated to a brown oil in *vacuuo.* The oil was purified by flash column chromatography (50% EtOAc / pet. ether) and reduced down to afford **36b** as a yellow oil (0.102 g, 66%), R_f 0.41 (50% EtOAc / pet. ether).

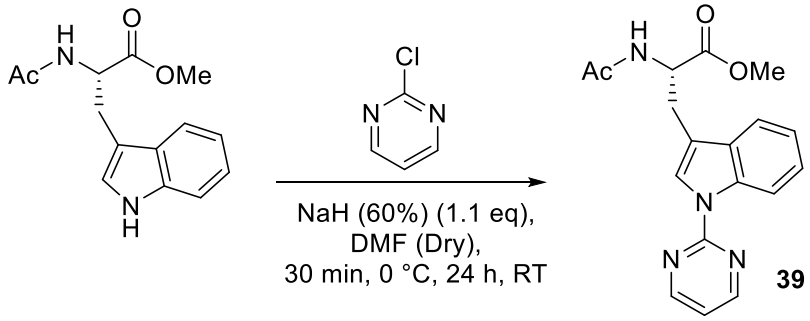
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.58 (6H, s, TBDMS-C*H*$_3$ (x2)), 0.90 (9H, s, TBDMS-C(C*H*$_3$)$_3$), 1.94 (3H, s, Acetyl-C*H*$_3$), 3.23-3.34 (2H, m, Trp-C*H*$_2$), 3.67 (3H, s, Ester-C*H*$_3$), 3.85 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 3.91 (1H, dd, *J* = 16.8, 5.0, Gly-C*H*H), 4.90 (1H, dt, *J* = 7.8, 5.7, Trp-α-C*H*), 6.46 (1H, br t, *J* = 5.0, Gly-N*H*), 6.73 (br d, *J* = 7.8, Trp-N*H*), 6.97 (1H, s, Trp-Ar-*H*), 7.09-7.17 (2H, m, Trp-Ar-*H*), 7.46-7.51 (2H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ -4.06 (TBDMS-CH_3 x3), 19.4 (TBDMS-C), 22.8 (Acetyl-CH_3), 26.2 (TBDMS-$(CH_3)_3$), 27.5 (Trp-CH_2), 42.9 (Gly-CH_2), 52.3 (Ester-CH_2), 53.2 (Trp-α-CH), 111.7 (Ar-C), 114.0 (Ar-C), 118.2 (Ar-C), 119.7 (Ar-C), 121.6 (Ar-C), 129.6 (Ar-C), 130.8 (Ar-C), 141.2 (Ar-C), 168.6 (C=O), 170.4 (C=O), 172.0 (C=O).

IR υ_{max} /cm^{-1} (oil) 3050 s (C-H), 2935 s (C-H), 2835 s (C-H), 1743 s (Ester C=O), 1657 m (C=C), 1433 m (C-H), 1255 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{22}H_{34}N_3O_4Si$: 432.2313, found: 432.2319.

4.26 Synthesis of Ac-Trp(Pym)-OMe (39)



N-Acetyl-L-tryptophan methyl ester (0.200 g, 0.768 mmol) was dissolved in dry DMF (10 mL) and cooled to 0 °C in an ice bath. NaH (60% in oil) (0.034 mg, 0.768 mmol) was added in portions to the solution, and left to stir for 1 h at 0 ˚C. 2-chloropyridine (0.106 g, 0.922 mmol) was added and the resulting suspension was left to gradually warm to room temperature for 24 h. The solution was added to water (30 mL) and extracted with EtOAc (4 x 30 mL), dried ($MgSO_4$) and concentrated to a brown solid in *vacuo.* The solid was purified by flash column chromatography (50% EtOAc / pet. ether), to afford modified amino acid **39a** as a yellow solid (0.148 g, 57%), m.p. 154-155 °C; R_f 0.41 (50% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (3H, s, Acetyl-C*H*$_3$), 3.23 (1H, dd, *J* = 14.3, *J* = 5.5, Trp-C*H*H), 3.29 (1H, dd, *J* = 14.3, *J* = 5.5, Trp-C*H*H), 3.65 (3H, s, Ester-C*H*$_3$), 4.92 (1H, dt, *J* = 7.8, *J* = 5.5, Trp-α-C*H*), 6.07 (1H, br d, *J* = 7.8, Trp-N*H*), 6.96 (1H, t, *J* = 4.8, Ar-*H*), 7.15-7.19 (1H, m, Trp-Ar-*H*), 7.25-7.29 (1H, m, Trp-Ar-*H*), 7.45 (1H, br d, *J* = 7.7, Trp-Ar-*H*), 7.99 (1H, s, Trp-Ar-*H*), 8.59 (2H, d, *J* = 4.8, Ar-*H*), 8.71 (1H, d, *J* = 8.4 , Trp-Ar-*H*).

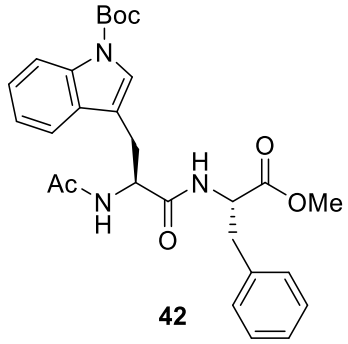
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.2 (Acetyl-CH_3), 27.4 (Trp-CH_2), 52.4 (Ester-CH_3), 52.7 (Trp-α-CH), 114.3 (Ar-C), 116.1 (Ar-C), 116.4 (Ar-C), 118.5 (Ar-C), 122.0 (Ar-C), 124.0 (Ar-C), 124.1 (Ar-C), 131.1 (Ar-C), 135.5 (Ar-C), 157.4 (Ar-C), 158.1 (Ar-C), 169.8 (C=O), 172.2 (C=O).

IR υ_{max} /cm^{-1} (solid) 3291 m (N-H), 3050 s (C-H), 2955 s (C-H), 1735 s (Ester C=O), 1660 m (C=C), 1456 m (C-H), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{18}H_{19}N_4O_3$: 339.1452, found: 339.1455.

4.27 Synthesis of modified peptides 43a-e

4.27.1 Synthesis of Ac-Trp(Boc)-Phe-OMe (42)



42

Peptide **42** was synthesised from Ac-Trp-Phe-OMe (**9f**) (0.306 g, 0.75 mmol) using the general procedure described in **Section 4.13.** Purification by flash column chromatography (75% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **42** as a white solid (0.381 g, 94%); m.p. 89-93 °C, R_f 0.25 (75% EtOAc / pet. ether).

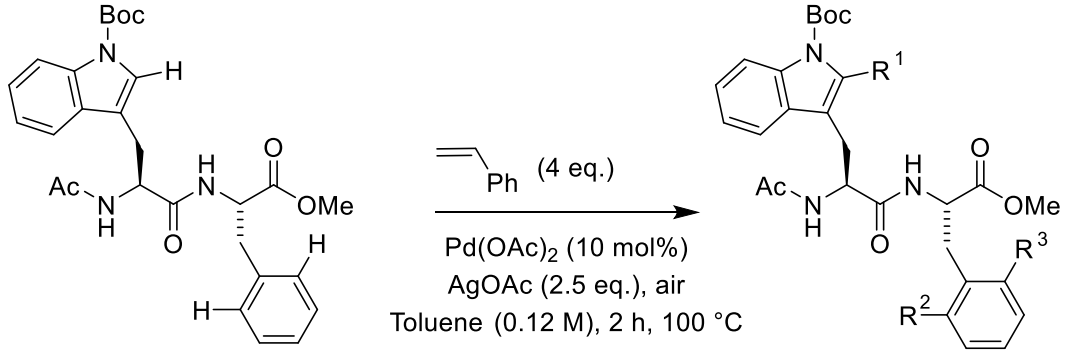
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (9H, s, Boc-(C*H*$_3$)$_3$), 1.98 (3H, s, Acetyl-C*H*$_3$), 2.94 (1H, dd, *J* = 13.7, *J* = 6.0, Phe-C*H*H), 3.01-3.08 (2H, m, Phe-CH*H*/Trp-CH*H*), 3.20 (1H, dd, *J* = 14.6, *J* = 5.5, Trp-C*H*H), 3.63 (3H, s, Ester-C*H*$_3$), 4.66-4.71 (2H, m, Ph-α-C*H*/Trp-α-C*H*), 6.09 (1H, br d, *J* = 7.3, Trp-N*H*), 6.21 (1H, br d, *J* = 7.3, Phe-N*H*), 6.90-6.92 (2H, m, Phe-Ar-*H*), 7.15-7.18 (3H, m, Phe-Ar-*H*), 7.22-7.26 (1H, m, Trp-Ar-*H*), 7.32 (1H, t, *J* = 8.2, Trp-Ar-*H*), 7.45 (1H, s, Trp-Ar-*H*), 7.64 (1H, d, *J* = 7.8¸ Trp-Ar-*H*), 8.11-8.13 (1H, m, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.2 (Acetyl-CH_3), 28.1 (Trp-CH_2), 28.2 (Boc-$(CH_3)_3$), 37.8 (Phe-CH_2), 52.3 (Ester-CH_3), 53.3 (Trp-α-CH), 53.4 (Phe-α-CH), 83.6 (Boc-C), 115.2 (Ar-C), 115.3 (Ar-C), 119.0 (Ar-C), 122.7 (Ar-C), 124.5 (Ar-C), 124.6 (Ar-C), 127.1 (Ar-C), 128.5 (Ar-C), 129.1 (Ar-C), 130.1 (Ar-C), 135.3 (Ar-C), 135.4 (Ar-C), 149.5 (C=O), 169.9 (C=O), 170.3 (C=O), 171.0 (C=O).

IR υ_{max} /cm^{-1} (solid) 3276 m (N-H), 3056 w (C-H), 2986 w (C-H), 1735 s (Ester C=O), 1639 m (amide C=O), 1457 m (C-H), 1341 s (C-O).

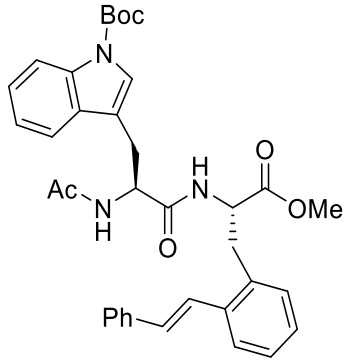
HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{28}H_{34}N_3O_6$: 508.2442, found: 508.2446.

4.27.2 Olefination of Ac-Trp(Boc)-Phe-OMe 43a-e



Ac-Trp(Boc)-Phe-OMe (**42**) (0.182 g, 0.359 mmol), $Pd(OAc)_2$ (8 mg, 0.036 mmol, 10 mol%), AgOAc (0.150 g, 0.898 mmol) and styrene (0.165 mL, 1.436 mmol) were stirred together in toluene (3 mL) at 100 °C for 6 h. The reaction was then allowed to cool to room temperature and filtered through a plug of Celite© before the filtrate was concentrated to dryness. The resulting residue was purified by column chromatography and recrystallized in DCM / hexanes to afford products **43a-e**.

4.27.3 Synthesis of modified peptide 43a



43a

Modified peptide **43a** was prepared from Ac-Trp(Boc)-Phe-OMe (**42**) (0.100 g, 0.197 mmol) and styrene (0.087 mL, 0.788 mmol), using the general procedure in **Section 4.27.2.** Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **43a** as an off-white solid (0.035 g, 16%); m.p. 117-118 °C, R_f 0.22 (50% EtOAc / pet. ether).

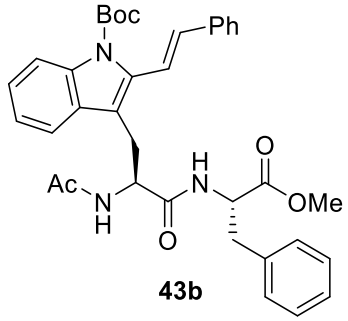
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (9H, s, Boc-(C*H*$_3$)$_3$), 1.74 (3H, s, Acetyl-C*H*$_3$), 2.94 (1H, dd, *J* = 14.4, *J* = 8.2, Phe-C*H*H), 2.98-3.09 (2H, m, Phe-C*H*H / Trp-C*H*H), 3.20 (1H, dd, *J* = 14.0, *J* = 6.4, Trp-C*H*H), 3.47 (3H, s, Ester-C*H*$_3$), 4.52-4.60 (2H, m, Phe-α-C*H* / Trp-α-C*H*), 6.14-6.20 (2H, m, Phe-N*H* / Trp-N*H*), 6.79 (1H, d, *J* = 7.6, Ar-*H*), 6.87 (1H, d, *J* = 16.1, alkene-C*H*), 7.00 (1H, t, *J* = 7.3, Ar-*H*), 7.13 (2H, app q, *J* = 7.6, Ar-*H*), 7.19-7.23 (3H, m, Ar-*H* / alkene-C*H*), 7.26-7.32 (3H, m, Ar-*H*), 7.45-7.52 (4H, m, Ar-*H*), 8.02 (1H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 28.1 (Trp-CH_2), 28.1 (Boc-$(CH_3)_3$), 35.0 (Phe-CH_2), 52.3 (Ester-CH_3), 53.2 (α-CH), 53.3 (α-CH), 83.5 (Boc-C), 115.1 (Ar-C), 115.2 (Ar-C), 119.0 (Ar-C), 122.6 (Ar-C), 124.4 (Ar-C), 124.5 (Ar-C), 125.1 (Ar-C), 125.7 (alkene-C), 126.5 (Ar-C), 126.6 (Ar-C), 127.5 (Ar-C), 127.6 (Ar-C), 127.8 (Ar-C), 128.7 (Ar-C), 130.1 (Ar-C), 130.4 (Ar-C), 130.5 (Ar-C), 133.3 (alkene-C), 136.5 (Ar-C), 137.2 (Ar-C), 149.4 (C=O), 169.9 (C=O), 170.4 (C=O), 171.1 (C=O).

IR U_{max} /cm^{-1} (solid) 3280 m (N-H), 2963 w (C-H), 1731 s (Ester C=O), 1638 m (amide C=O), 1452 m (C-H), 1258 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{40}N_3O_6$: 610.2912, found: 610.2912.

4.27.4 Synthesis of modified peptide 43b



43b

Modified peptide **43b** was prepared from Ac-Trp(Boc)-Phe-OMe (**42**) (0.100 g, 0.197 mmol) and styrene (0.087 mL, 0.788 mmol), using the general procedure in **Section 4.27.2.** Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **43b** as an off-white solid (0.048 g, 22%); m.p. 152-153 °C, R_f 0.29 (50% EtOAc / pet. ether).

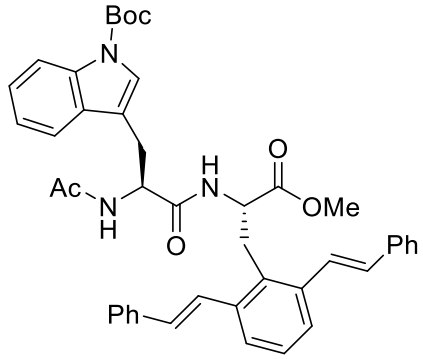
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (9H, s, Boc-(C*H*$_3$)$_3$), 1.90 (3H, s, Acetyl-C*H*$_3$), 2.79 (1H, dd, *J* = 13.8, *J* = 6.0, Phe-C*H*H), 2.90 (1H, dd, *J* = 13.8, *J* = 6.0, Phe-C*H*H), 3.12 (1H, dd, *J* = 14.1, *J* = 9.7, Trp-C*H*H), 3.25 (1H, dd, *J* = 14.0, *J* = 5.6, Trp-C*H*H), 3.37 (3H, s, Ester-C*H*$_3$), 4.45-4.50 (1H, m, Trp-α-C*H*), 4.63-4.68 (1H, m, Phe-α-C*H*), 5.87 (1H, br d, *J* = 7.8, Trp-N*H*), 6.42 (1H, br d, *J* = 7.4, Phe-N*H*), 6.75 (1H, d, *J* = 16.7, alkene-C*H*), 6.83-6.85 (2H, m, Ar-*H*), 7.08-7.10 (2H, m, Ar-*H*), 7.16-7.22 (4H, m, Ar-*H*), 7.28-7.31 (3H, m, Ar-*H*), 7.48-7.58 (3H, m, Ar-*H*), 8.02 (1H, br d, *J* = 8.2, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.1 (Acetyl-CH_3), 28.2 (Boc-$(CH_3)_3$), 28.6 (Trp-CH_2), 38.1 (Phe-CH_2), 52.1 (Ester-CH_3), 53.5 (α-CH), 53.5 (α-CH), 83.9 (Boc-C), 114.9 (Ar-C), 115.4 (Ar-C), 118.8 (Ar-C), 119.7 (alkene-C), 123.0 (Ar-C), 124.7 (Ar-C), 126.6 (alkene-C), 127.0 (Ar-C), 127.9 (Ar-C), 128.4 (Ar-C), 128.7 (Ar-C), 129.1 (Ar-C), 129.7 (Ar-C), 132.1 (alkene-C), 135.4 (Ar-C), 135.9 (Ar-C), 136.0 (Ar-C), 136.9 (Ar-C), 150.4 (C=O), 169.7 (C=O), 170.4 (C=O), 170.4 (C=O).

IR υ_{max} /cm^{-1} (solid) 3282 m (N-H), 3060 w (C-H), 2963 w (C-H), 1726 s (Ester C=O), 1642 m (amide C=O), 1522 m (C=C), 1258 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{36}H_{40}N_3O_6$: 610.2912, found: 610.2912.

4.27.5 Synthesis of modified peptide 43c



43c

Modified peptide **43c** was prepared from Ac-Trp(Boc)-Phe-OMe (**42**) (0.100 g, 0.197 mmol) and styrene (0.087 mL, 0.788 mmol), using the general procedure in **Section 4.27.2.** Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **43c** as an off-white solid (0.018 g, 7%); m.p. 120-122 °C, R_f 0.39 (50% EtOAc / pet. ether).

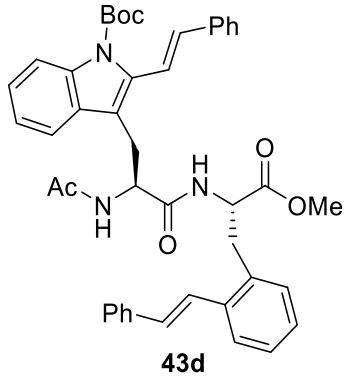
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 1.80 (3H, s, Acetyl-C*H*$_3$), 2.96 (1H, dd, *J* = 14.5, *J* = 8.2, Trp-C*H*H), 3.12 (1H, dd, *J* = 14.5, *J* = 8.2, Trp-C*H*H), 3.34-3.37 (2H, m, Phe-C*H*$_2$), 3.41 (3H, s, Ester-C*H*$_3$), 4.57-4.68 (2H, m, Trp-α-C*H* / Phe-α-C*H*), 6.12 (1H, br d, *J* = 7.3, Trp-N*H*), 6.17 (1H, br d, *J* = 7.7, Phe-N*H*), 6.96 (2H, d, *J* = 16.0, alkene-C*H*), 7.12 (1H, t, *J* = 7.4, Ar-*H*), 7.23-7.31 (4H, m, Ar-*H* / alkene-C*H*), 7.33-7.42 (6H, m, Ar-*H* /alkene-C*H*), 7.50-7.57 (8H, m, Ar-*H*), 8.08 (1H, d, *J* = 8.1, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.1 (Acetyl-CH_3), 28.3 (Boc-$(CH_3)_3$), 28.4 (Trp-CH_2), 31.6 (Phe-CH_2), 52.7 (Phe-α-CH), 53.1 (Ester-CH_3), 53.5 (Trp-α-CH), 83.7 (Boc-C), 115.3 (Ar-C), 119.1 (Ar-C), 119.7 (Ar-C), 122.8 (Ar-C), 123.1 (Ar-C), 124.6 (Ar-C), 125.7 (Ar-C), 126.1 (alkene-C), 126.9 (Ar-C), 127.7 (Ar-C), 128.0 (Ar-C), 128.9 (Ar-C), 130.3 (Ar-C), 130.4 (Ar-C), 131.7 (alkene-C), 132.1 (Ar-C), 137.3 (Ar-C), 137.9 (Ar-C), 149.6 (C=O), 169.9 (C=O), 170.4 (C=O), 171.3 (C=O).

IR υ_{max} /cm^{-1} (solid) 3282 m (N-H), 3055 w (C-H), 2965 w (C-H), 1728 s (Ester C=O), 1638 m (amide C=O), 1451 m (C-H), 1258 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{44}H_{46}N_3O_6$: 712.3381, found: 712.3375.

4.27.6 Synthesis of modified peptide 43d



43d

Modified peptide **43d** was prepared from Ac-Trp(Boc)-Phe-OMe (**42**) (0.100 g, 0.197 mmol) and styrene (0.087 mL, 0.788 mmol), using the general procedure in **Section 4.27.2.** Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **43d** as an off-white solid (0.033 g, 13%); m.p. 100-102 °C, R_f 0.45 (50% EtOAc / pet. ether).

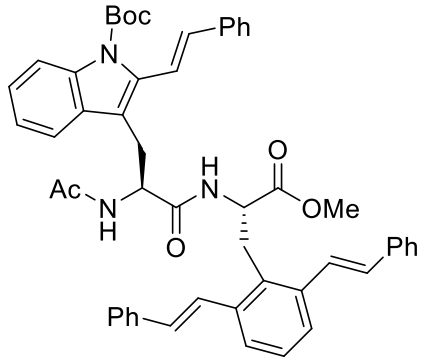
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (9H, s, Boc-(C*H*$_3$)$_3$), 1.77 (3H, s, Acetyl-C*H*$_3$), 3.04 (1H, dd, *J* = 14.2, *J* = 5.2, Phe-C*H*H), 3.11 (1H, dd, *J* = 14.0, *J* = 10.0, Trp-C*H*H), 3.23 (1H, dd, *J* = 14.2, *J* = 7.2, Phe-C*H*H), 3.32-3.41 (1H, m, Trp-C*H*H), 3.38 (3H, s, Ester-C*H*$_3$), 4.48-4.52 (1H, m, Trp-α-C*H*), 4.61-4.67 (1H, m, Phe-α-C*H*), 5.82 (1H, br d, *J* = 7.2, Trp-N*H*), 6.33 (1H, br d, *J* = 7.2, Phe-N*H*), 6.61-6.85 (2H, m, alkene-C*H* / Ar-*H*), 6.98 (1H, d, *J* = 16.0, alkene-C*H*), 7.17-7.40 (13H, m, Ar-*H* / alkene-C*H*), 7.55-7.63 (5H, m, Ar-*H*), 8.08 (1H, d, *J* = 8.4, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 22.9 (Acetyl-CH_3), 28.2 (Boc-$(CH_3)_3$), 28.8 (Trp-CH_2), 35.2 (Phe-CH_2), 52.2 (Ester-CH_3), 53.5 (α-CH), 53.5 (α-CH), 83.8 (Boc-C), 115.0 (Ar-C), 115.4 (Ar-C), 118.8 (Ar-C), 119.6 (alkene-C), 123.0 (Ar-C), 124.7 (Ar-C), 125.2 (Ar-C), 125.6 (Ar-C), 126.6 (Ar-C), 126.7 (Ar-C), 127.4 (Ar-C), 127.5 (Ar-C), 127.7 (Ar-C), 127.8 (Ar-C), 128.7 (Ar-C), 130.3 (Ar-C), 130.4 (Ar-C), 131.9 (Ar-C), 133.3 (alkene-C), 135.9 (Ar-C), 135.9 (Ar-C), 136.6 (Ar-C), 136.9 (Ar-C), 137.4 (Ar-C), 150.4 (C=O), 169.6 (C=O), 170.3 (C=O), 170.5 (C=O).

IR U_{max} /cm^{-1} (solid) 3282 m (N-H), 2976 w (C-H), 1726 s (Ester C=O), 1645 m (amide C=O), 1541 m (C=C), 1233 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{44}H_{46}N_3O_6$: 712.3381, found: 712.3382.

4.27.7 Synthesis of modified peptide 43e



43e

Modified peptide **43e** was prepared from Ac-Trp(Boc)-Phe-OMe (**42**) (0.100 g, 0.197 mmol) and styrene (0.087 mL, 0.788 mmol), using the general procedure in **Section 4.27.2.** Purification by flash column chromatography (50% EtOAc / pet. ether) followed by recrystallisation from DCM / hexanes gave **43e** as an off-white solid (0.018 g, 6%); m.p.106-108 °C, R_f 0.61 (50% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (9H, s, Boc-(C*H*$_3$)$_3$), 1.82 (3H, s, Acetyl-C*H*$_3$), 3.10 (1H, dd, *J* = 13.8, *J* = 10.0, Trp-C*H*H), 3.21 (3H, s, Ester-C*H*$_3$), 3.26-3.44 (3H, m, Trp-C*H*H / Phe-C*H*$_2$), 4.55 (1H, dt, *J* = 7.8, *J* = 6.4, Trp-α-C*H*), 4.65-4.71 (1H, m, Phe-α-C*H*), 5.90 (1H, br d, *J* = 7.8, Trp-N*H*), 6.36 (1H, br d, *J* = 7.3, Phe-N*H*), 6.81 (1H, d, *J* = 16.7, Trp-alkene-C*H*), 6.95 (2H, d, *J* = 16.0, Phe-alkene-C*H*), 7.10 (2H, t, *J* = 7.8, Ar-*H*), 7.20-7.31 (7H, m, Ar-*H* / alkene-C*H*), 7.36-7.41 (7H, m, Ar-*H* / alkene-C*H*), 7.50-7.61 (8H, m, Ar-*H*), 8.05 (1H, d, *J* = 7.8, Ar-*H*).

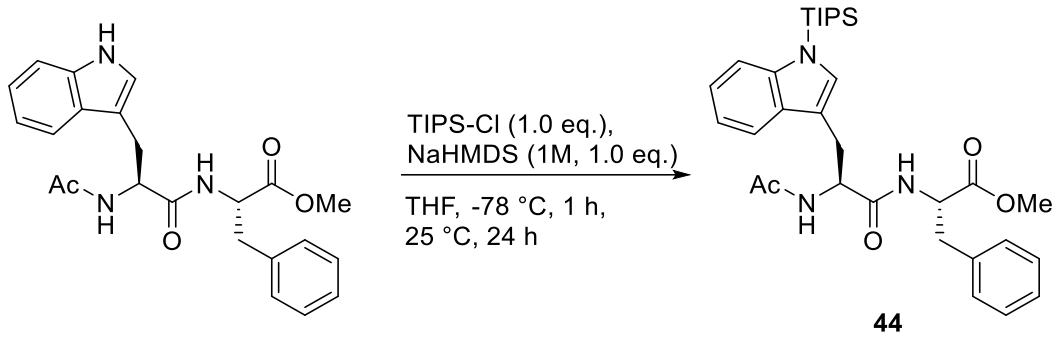
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 23.1 (Acetyl-CH_3), 28.4 (Boc-$(CH_3)_3$), 29.8 (Trp-CH_2), 31.9 (Phe-CH_2), 52.6 (Ester-CH_3), 53.1 (α-CH), 53.8 (α-CH), 84.0 (Boc-C), 115.1 (Ar-C), 115.4 (Ar-C), 118.9 (alkene-C), 119.7 (Ar-C), 123.1 (Ar-C), 123.2 (Ar-C), 124.8 (Ar-C), 125.6 (Ar-C), 126.1 (alkene-C), 126.8 (Ar-C), 126.9 (Ar-C), 127.5 (Ar-C), 127.9 (Ar-C), 128.8 (Ar-C), 128.9 (Ar-C), 128.9 (Ar-C), 129.6 (Ar-C), 131.4 (alkene-C), 131.8 (alkene-C), 136.0 (Ar-C), 137.5 (Ar-C), 137.9 (Ar-C), 150.4 (C=O), 169.7 (C=O), 170.3 (C=O), 170.6 (C=O). There are two Ar-C signals missing; presumably these overlap with other signals in the spectrum.

IR U_{max} /cm^{-1} (solid) 3308 m (N-H), 3026 w (C-H), 2963 w (C-H), 1726 s (Ester C=O), 1655 m (amide C=O), 1508 m (C=C), 1450 m (C-H), 1237 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{52}H_{52}N_3O_6$: 814.3851, found: 814.3852.

4.28 Synthesis of modified peptide 45

4.28.1 Synthesis of Ac-Trp(TIPS)-Phe-OMe (44)



44

Ac-Trp-Phe-OMe (**9h**) (0.200 g, 0.394 mmol) was dissolved in dry THF (10 mL) and cooled in a dry ice/acetone bath. NaHMDS (1M in THF, 0.394 mL, 0.394 mmol) was added dropwise at -78 ˚C and left to stir for 1 h. Triisopropylsilyl chloride (0.084 mL, 0.394 mmol) was added and left to gradually warm to room temperature for 24 h. The solution was added to water (30 mL) and extracted with EtOAc (4 x 30 mL), dried ($MgSO_4$) and concentrated to a brown oil in *vacuuo.* The oil was purified by flash column chromatography (50% EtOAc / pet. ether) and reduced down to afford **44** a yellow oil (0.162 g, 73%), R_f 0.31 (50% EtOAc / pet. ether).

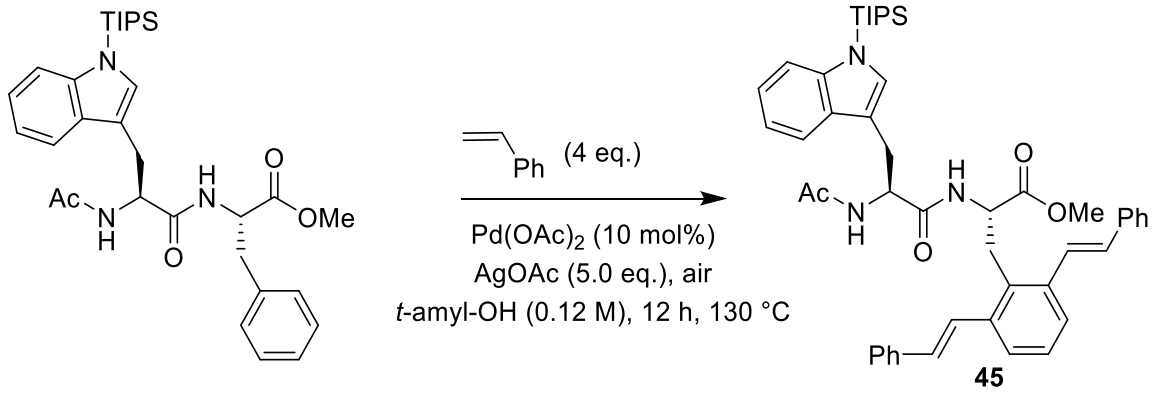
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (18H, d, *J* = 7.3, TIPS-(CH_3)$_2$ (x3)), 1.86 (3H, septet, *J* = 7.5, TIPS-C*H* (x3)), 1.89 (3H, s, Acetyl-CH_3), 2.95 (1H, dd, *J* = 13.7, *J* = 5.9, Phe-C*H*H), 3.05 (1H, dd, *J* = 13.7, *J* = 5.9, Phe-C*H*H), 3.20 (2H, d, *J* = 6.9, Trp-CH_2), 3.62 (3H, s, Ester-CH_3), 4.67-4.78 (2H, m, Phe-α-C*H* / Trp-α-C*H*), 6.26 (1H, d, *J* = 7.3, Phe-N*H*), 6.47 (1H, d, *J* = 7.8, Trp-N*H*), 6.94-6.97 (2H, m, Phe-Ar-*H*), 7.08-7.13 (3H, m, Ar-*H*), 7.18-7.20 (3H, m, Ar-*H*), 7.46 (1H, d, *J* = 8.2, Trp-Ar-*H*), 7.58 (1H, d, *J* = 8.2, Trp-Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 12.8 (TIPS-CH), 18.2 (TIPS-CH_3), 23.2 (Acetyl-CH_3), 28.0 (Trp-CH_2), 37.9 (Phe-CH_2), 52.3 (Ester-CH_3), 53.5 (α-CH), 53.6 (α-CH), 112.4 (Ar-C), 114.1 (Ar-C), 118.7 (Ar-C), 119.9 (Ar-C), 121.7 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 129.3 (Ar-C), 130.1 (Ar-C), 131.1 (Ar-C), 135.8 (Ar-C), 141.3 (Ar-C), 170.2 (C=O), 171.1 (C=O), 171.4 (C=O).

IR U_{max} /cm^{-1} (oil) 3280 m (N-H), 3062 w (C-H), 2946 w (C-H), 2866 w (C-H), 1746 s (Ester C=O), 1638 m (amide C=O), 1541 m (C=C), 1452 m (C-H).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{32}H_{46}N_3O_4Si$: 564.3252, found: 564.3250.

4.28.2 Synthesis of modified peptide 45



Ac-Trp(TIPS)-Phe-OMe (**44**) (0.065 g, 0.115 mmol), $Pd(OAc)_2$ (2.6 mg, 0.012 mmol, 10 mol%), AgOAc (0.096 g, 0.576 mmol) and styrene (0.063 mL, 0.461 mmol) were stirred together in *t*-amyl-OH (1 mL) at 130 °C for 12 h. The reaction was then allowed to cool to room temperature and filtered through a plug of Celite© before the filtrate was concentrated to dryness. The ^{1}H NMR spectrum for the crude product showed only the di-olefinated peptide. Purification by flash column chromatography (30% EtOAc / pet. ether) to afford **45** as a yellow oil (0.043 g, 49%); R_f 0.35 (30% EtOAc / pet. ether).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.06 (18H, d, *J* = 7.6, TIPS-(C*H*$_3$)$_2$ (x3)), 1.55-1.63 (3H, m, TIPS-C*H* (x3)), 1.83 (3H, s, Acetyl-C*H*$_3$), 3.16 (2H, br d, *J* = 6.0, Phe-C*H*$_2$), 3.28 (1H, dd, *J* = 14.2, *J* = 8.1, Trp-C*H*H), 3.39 (3H, s, Ester-C*H*$_3$), 3.46 (1H, dd, *J* = 14.2, *J* = 6.3, Trp-C*H*H), 4.61-4.72 (2H, m, Phe-α-C*H* / Trp-α-C*H*), 6.05 (1H, br d, *J* = 8.1, Trp-N*H*), 6.44 (1H, br d, *J* = 7.2, Phe-N*H*), 6.99-7.10 (5H, m, alkene-C*H* / Ar-*H*), 7.26-7.32 (2H, m, Ar-*H*), 7.36-7.42 (5H, m, Ar-*H*), 7.46-7.54 (4H, m, alkene-C*H* / Ar-*H*), 7.56-7.62 (6H, m, Ar-*H*).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 12.6 (TIPS-CH), 18.0 (TIPS-CH_3), 23.1 (Acetyl-CH_3), 27.9 (Trp-CH_2), 31.7 (Phe-CH_2), 52.5 (Ester-CH_3), 53.1 (Trp-α-CH), 53.8 (Phe-α-CH), 112.3 (Ar-C), 113.9 (Ar-C), 118.4 (Ar-C), 119.7 (Ar-C), 121.5 (Ar-C), 125.5 (Ar-C), 126.0 (alkene-C), 126.8 (Ar-C), 127.5 (Ar-C), 127.8 (Ar-C), 128.7 (Ar-C), 129.8 (Ar-C), 131.0 (Ar-C), 131.6 (Ar-C), 131.8 (alkene-C), 137.2 (Ar-C), 137.8 (Ar-C), 141.1 (Ar-C), 169.9 (C=O), 170.8 (C=O), 171.4 (C=O).

IR υ_{max} /cm^{-1} (oil) 3249 m (N-H), 3058 w (C-H), 2948 w (C-H), 2866 w (C-H), 1743 s (Ester C=O), 1629 m (amide C=O), 1508 m (C=C), 1211 s (C-O).

HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{48}H_{58}N_3O_4Si$: 768.4191, found: 768.4190.

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