Towards the Synthesis of a Protein β-Turn Mimetic Based on the Opioid Pentapeptide Leu-Enkephalin

Derek Strowes

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School of Chemistry
College of Science and Engineering
University of Glasgow
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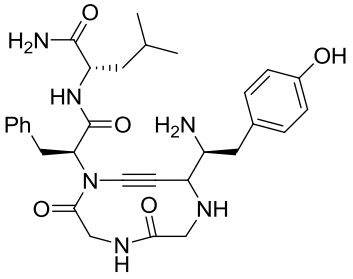
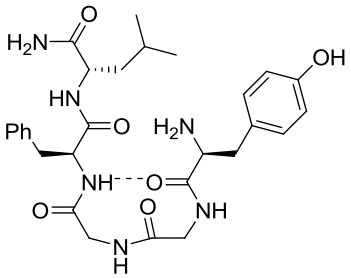
Abstract

Synthetic targets that mimic key structural and conformational features in proteins are of intense scientific interest. These structures can reproduce the biological activity of their naturally occurring counterparts, while improving their properties *in vivo*. This project focuses on the synthesis of β-turn mimetic structures, based on the opioid pentapeptide leu-enkephalin. More specifically, the target mimetics in this project are based on the enkephalin derivative, leu-enkephalinamide.



Peptidomimetic Structure

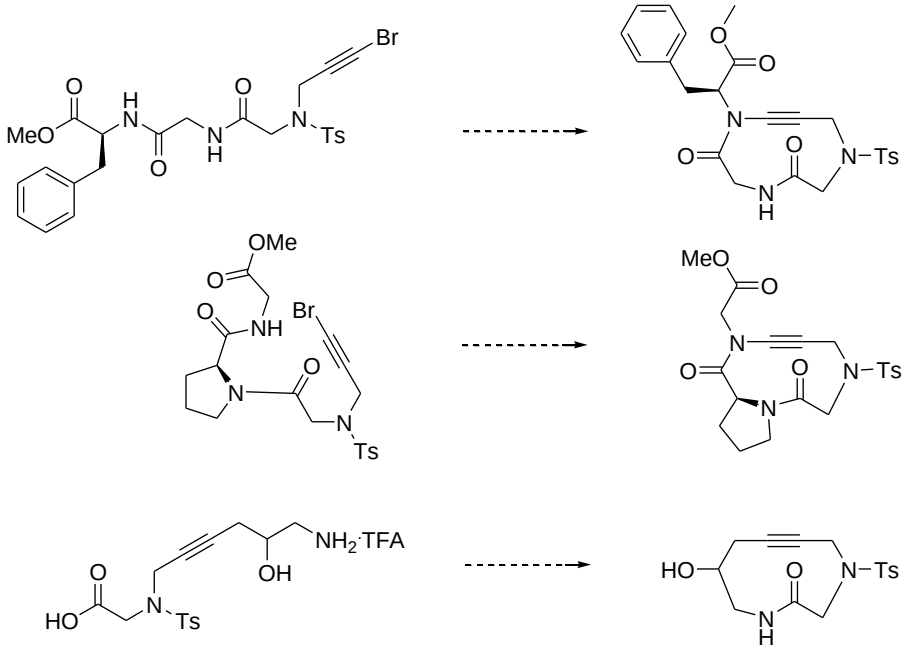


Leu-Enkephalinamide Turn Structure

Leu-enkephalin is conformationally very flexible, giving it the ability to bind to different opioid receptors. This results not only in the physiological relief of pain, but also in various potential side effects such as miosis or physical dependency. Natural peptide structures also have limited suitability for drug applications because of their poor transport properties and their tendency to undergo proteolytic degradation. The synthesis of macrocyclic mimetics can overcome these problems and give a conformationally rigid molecule that exhibits a desired biological activity.

This thesis will discuss some published examples of β-turn mimetic structures that have been designed and synthesised by research groups working in this field. The examples that are shown have been chosen to illustrate some of the work that has taken place in this area over recent years. Advances in the synthesis of ynamides and other ynamine analogues is also discussed, due to the importance of this type of chemistry in the research project.

Efforts towards the synthesis of several 10-membered macrocycles are described herein. The target structures are model compounds that are designed to explore varied chemical approaches towards such ring systems. This thesis will discuss the synthesis of several linear precursors to the proposed macrocycles and efforts to cyclise these structures to form the desired targets.



The targets that were synthesized employed varied approaches to the key cyclisation reaction. Initially, a metal-mediated coupling approach to an ynamide target was chosen. This was subsequently refined by including a proline residue in the linear precursor to bring the reacting groups into closer proximity. Finally, several approaches were investigated to close an alkyne bonded macrocycle by coupling the amide functionality at the opposite side of the ring.

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Finally, my thanks go to Kirsty Cleland. You have supported and encouraged me throughout my PhD. I could not have done it without you.

Declaration

I hereby declare that the substance of this thesis has not been submitted, nor is concurrently submitted, in candidature for any other degree.

I further declare that all of the work presented in this manuscript is the result of my own investigations. Where the work of other investigators has been used, this has been acknowledged in an appropriate manner.

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Derek Strowes

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Prof. J. Stephen Clark

Abbreviations

Ac	acetyl
acac	acetylacetonate
aq	aqueous
Arom	aromatic
atm	atmosphere
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
Cbz	benzyloxycarbonyl
DABCO	1,4-diazobicyclo[2.2.2]octane
DAMGO	[D-Ala^2,N-MePhe^4,Gly-ol]-enkephalin
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIC	*N,N'*-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DKP	diketopiperazine
DMAP	4-(dimethylamino)pyridine
DMEDA	*N*,*N*-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
DMP	dimethylpyrrole
DMTMM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
EDCI	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
Et	ethyl
eq	equivalents
EWG	electron withdrawing group
FDPP	pentafluorophenyl diphenylphosphinate
Fm	9-fluorenylmethyl
Fmoc	fluorenylmethyloxycarbonyl
GPCR	G-protein coupled receptor

HBTU	*O*-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyluroniumhexafluorophosphate
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration
i-Pr	*iso*-propyl
IR	infrared
KHMDS	potassium hexamethyldisilazide
Leu	leucine
LiHMDS	lithium hexamethyldisilazide
LRMS	low resolution mass spectrometry
Me	methyl
Ms	methanesulfonyl
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
Ns	(4-nitrophenyl)sulfonyl
PAM	4-hydroxymethyl-phenylacetamidomethyl
PCC	pyridinium chlorochromate
Pfp	pentafluorophenyl
PMB	*para*-methoxybenzyl
PTSA	*para*-toluenesulfonic acid
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
RCM	ring closing metathesis
SAR	structure activity relationship
Su	succinimide
t-Bu	*tert*-butyl
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TC	thiophenecarboxylate
TEA	triethylamine
Tf	trifluoromethanesulfonate

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Troc	2,2,2-trichloroethoxycarbonyl
TRH	thyrotropin releasing hormone
Ts	*para*-toluenesulfonyl
UV	ultra violet

Chapter 1

Introduction

1.1 Proteins, Peptides and their Folding

1.1.1 Amino Acids: Protein Building Blocks

In nature there are thousands of different peptides and proteins all with their own unique properties. These structures can be accurately described as chains, or polymers, of amino acids held together using amide bonding units. These structures are all synthesised from a group of 20 naturally occurring amino acids that are displayed in Figure 1.[1]

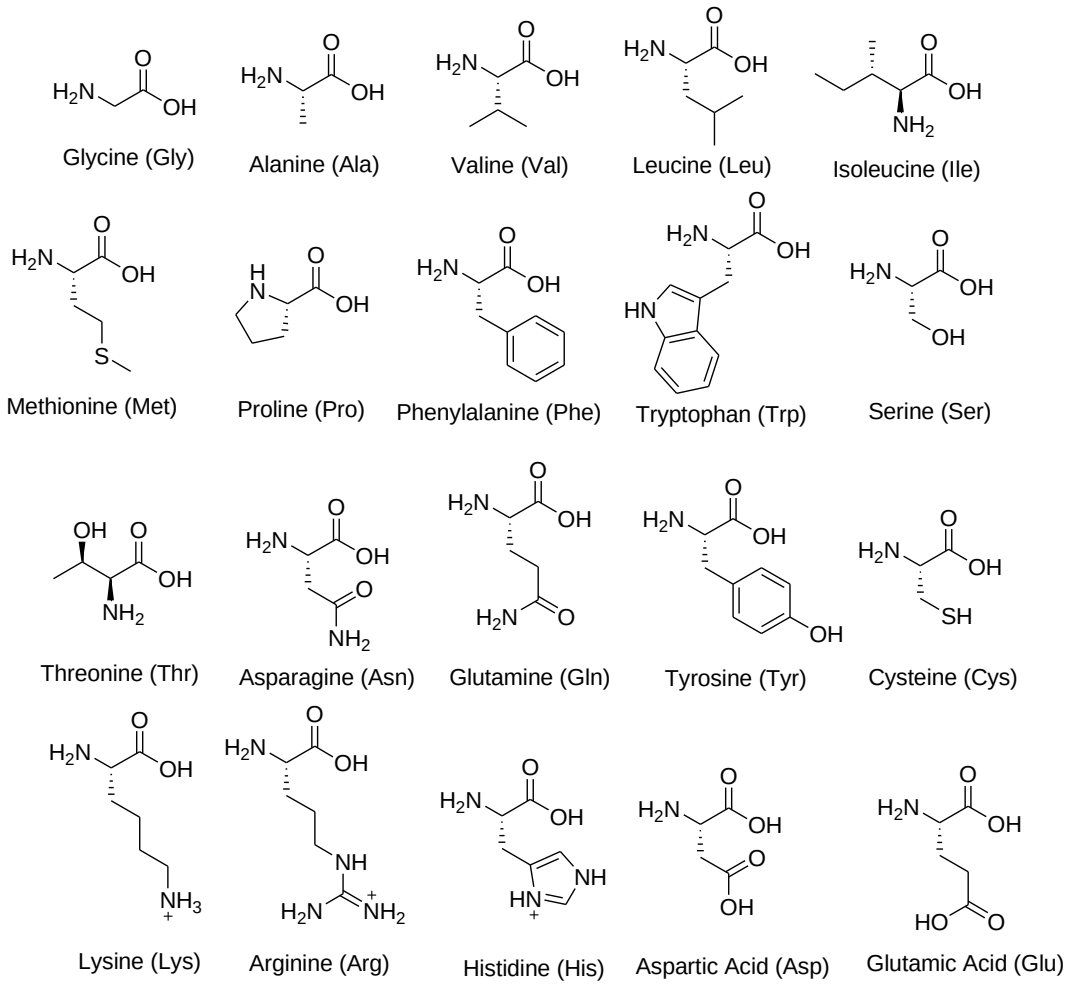


Figure 1: 20 Naturally Occurring α-Amino Acids

An amino acid is a structure consisting of a carboxylic acid and a primary amine located on either terminus, connected by an alkyl carbon unit with a functionalised side chain. The naturally occurring amino acids all have their unique functionality located on the carbon α to the carbonyl group, hence are referred to as α-amino acids.

Any peptide or protein that is isolated in nature can be attributed to some combination of these structures held together by amide bonds. These can be as small as a dipeptide, containing just two amino acid residues, or as large as a polypeptide, containing hundreds, if not thousands, of residues. Due to the variation in chain length and the number of possible combinations of residues, the diversity of protein and peptide structures is staggering. Similarly, the applications of these proteins are just as varied. Figure 2 shows two naturally occurring proteins that illustrate the complexity and variation in protein structure, which translates directly into the wide scope of protein functions. On the left is immunoglobulin,[2] a protein involved in immune response, and on the right is actin,[3] an abundant protein largely involved in cell infrastructure.

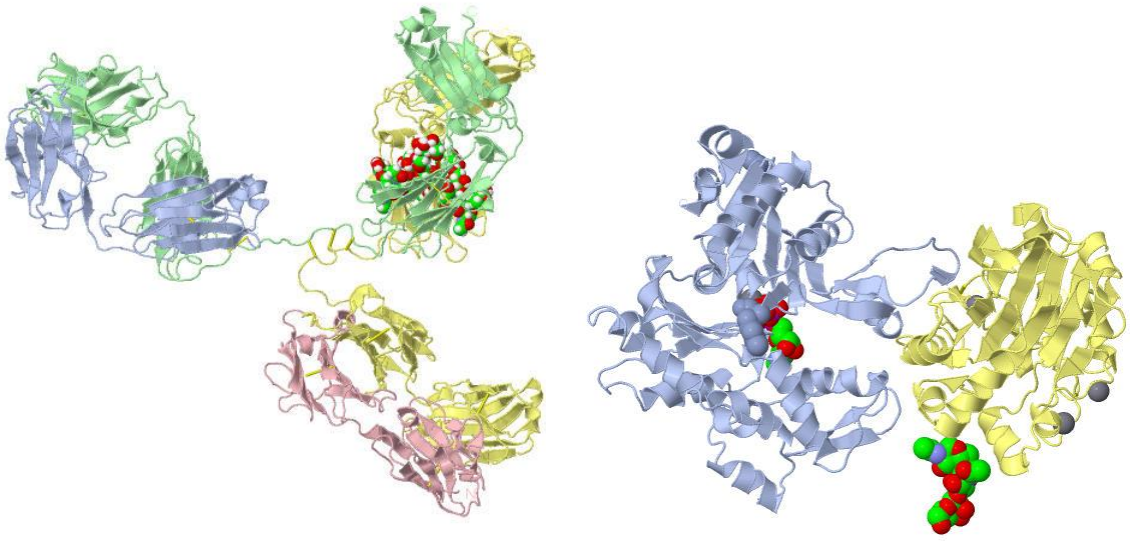


Figure 2: Examples of Proteins Occurring in Nature, Left: Immunoglobulin, Right: Actin[4,5]

The difference in definition between peptides and proteins lies only in the length of the peptide chain that is being described. There is not a strict cut-off point that marks the

boundary between proteins and peptides, but conventionally peptides are described as containing less than 50 monomer units.

Proteins are among the most important compounds found in nature due to their diverse uses and biological properties. The word protein is derived from the Greek word prōtos, which means ‘of prime importance’. This is an apt term to apply to proteins and accurately reflects the necessity of these compounds in life sustaining processes. Their structures are often remarkably complex and their functions are incredibly diverse, ranging from acting as enzymes to participating in immune response and cell signaling.

1.1.2 Protein Structure and Biological Activity

A sequence of amino acid residues will not retain a straightforward open-chain conformation once it has formed. It will instead fold into a complex three-dimensional structure of lower energy, stabilised by non-covalent interactions between the residues. This is known as the protein’s native structure. The overall three-dimensional structure of a protein, and its functional groups, is extremely important in dictating biological activity. It is these folded structures that lend proteins their high selectivity and makes them perfect for use in natural systems. Protein folding is one of the most fundamental processes in naturally occurring systems.[6]

A protein will fold into its native conformation in response to a variety of physiological conditions. Solvent, pH, ionic strength and the presence of metal ions or prosthetic groups (groups other than amino acids) are several factors that will directly influence the folding of a protein in a biological system.[7]

Figure 3 displays a diagrammatical representation of the enzyme lysozyme. This enzyme consists of 129 amino acid residues and is useful in breaking down bacterial cell walls. The structure was determined in 1965 using X-ray analysis.[8,9] This representation contains several units of secondary structure and effectively demonstrates the complexities of a folded protein structure. The long chain represents the amino acid sequence, with various recognisable secondary structure motifs represented by arrows and coils. These secondary structures will be discussed in more detail later on.

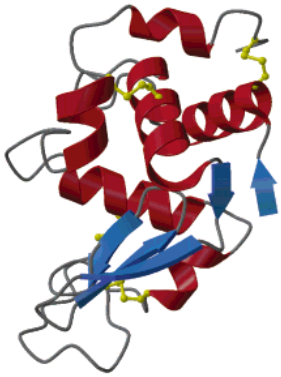


Figure 3: Folded Representation of the Enzyme Lysozyme[10,11]

In biology, for a molecule to exhibit any kind of activity it must be able to interact with a particular site or receptor. Natural systems are notable for their extremely high selectivity, which is necessary to avoid unwanted interactions as well as competition at, or inhibition of, active sites. This is of course where the 'lock and key' principle is important. A receptor will only be able to interact with a molecule of appropriate size, structure and functionality, much like a key fitting into a lock. A protein must be of an appropriate size and shape to fit into a binding pocket, and it must have the appropriate functional groups to interact non-covalently with the groups on the inner surface of that pocket. The importance of proteins and peptides in metabolic processes is without doubt. The failure of a protein to fold correctly is the cause of a variety of physiological conditions.[12]

Figure 4 shows a basic representation of bovine pancreatic ribonuclease, a protein that consists of 124 amino acid residues. Each amino acid residue is represented in the chain by its three-letter abbreviation and the chain is folded into its native conformation. The folded structure is stabilised in this case by four disulfide bonds, which are marked on the chain. These are covalent bonds that form between the thiol side chains in cysteine residues. The diagram does not show the complexity of non-covalent interactions that are at work in the structure nor does it indicate the recognisable secondary structure features. However, this image does give a good representation of both the length of the protein and how this folds into a more complex native structure. It also illustrates another possibility in representing a complex protein diagrammatically.

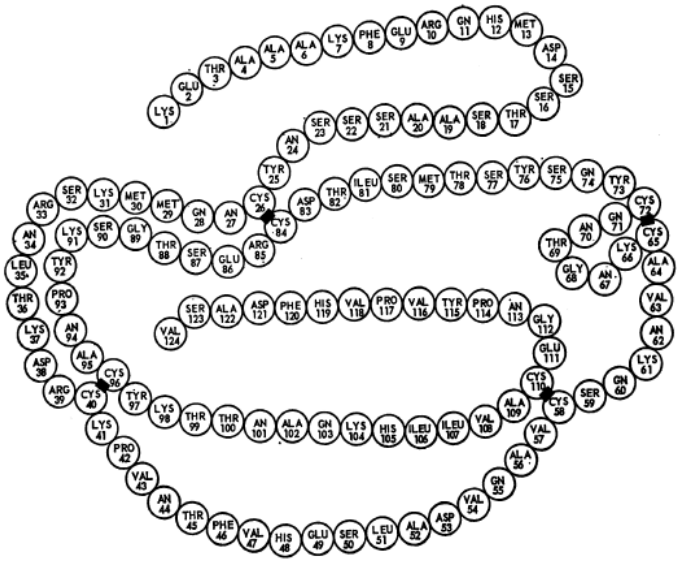


Figure 4: Amino Acid Chain of Bovine Pancreatic Ribonuclease[13]

The structure of a protein can be classified in four broad terms, each representing a different level of complexity. The most basic representation is simply the order of residues in the polypeptide chain and this is referred to as the primary structure. Traditionally, the sequence of amino acids is reported starting from the amino terminus to the carboxy terminus. The secondary structure describes several recognisable structural sub-units that are present in the folded state, such as an α-helix or a β-sheet. The tertiary structure includes the overall structure of the folded protein, including the various secondary units. Finally, the quaternary structure applies in cases where a protein combines multiple individual units in one overall structure, i.e. haemoglobin, the oxygen carrier in the blood, which exists as a tetramer.[14] The structure of the deoxygenated form of haemoglobin is illustrated in Figure 5.

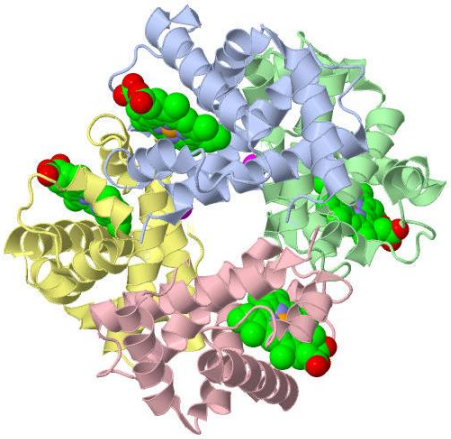


Figure 5: Human Deoxyhaemoglobin Structure[4,5]

Within the secondary structure of a protein, chemists can glean valuable information about structure activity relationships (SAR's) revealing important factors that are required for recognition and binding to take place. There are several known secondary structure units that repeat within a folded conformation such as the aforementioned α-helix and β-sheet, and these have been studied extensively in recent decades.

The mechanism by which a chain of amino acids folds into its native conformation is not completely understood. A well known thought experiment was developed in 1969 by Cyrus Levinthal.[15] It has since become known as the Levinthal paradox and considers the vast number of possible conformations an extended chain of amino acids can theoretically adopt. Taking a simple example, a chain of 100 amino acids is bonded together by a total of 99 amide bonds. This in turn means that there are 198 different φ and ψ angles associated with the structure and, assuming each of these can exist in 3 distinct stable conformations, there will be 3^{198} different folded conformations to explore. If the protein reached its native conformation by sequentially fulfilling each of these conformations it would take longer than the universe has existed to reach the appropriate one. This is true even if the random search for the correct conformation is carried out on a picosecond timescale. Of course, proteins fold very rapidly on the order of minutes, therefore it was suggested that protein folding is based on a funnel-like energy model with the protein 'falling' into a lower energy state. The protein will achieve its thermodynamically most favourable state with lowest Gibbs' free energy depending on the order of residues and the interactions taking place between them.

The process of protein folding is very accurate with specific sequences of amino acids giving the same native conformation every time. It has been suggested that folding begins with local interactions between residues, which then facilitate interactions between more remote groups as the folding brings them into proximity with each other.[1] Although a precise relationship between amino acid sequence and three-dimensional structure remains elusive, extensive work in the field is providing new insights into protein folding.[16] With enough understanding, scientists should hopefully be able to predict the native conformation of a protein simply by knowing the order and number of residues.

Proteins developed in natural systems specifically to function under certain physiological conditions. As previously mentioned, factors such as pH have a direct effect on the folding of a protein, and so these structures often have a very narrow band of conditions in which they can operate. This explains why a protein will denature at extremes of pH or

temperature. A denatured protein will have lost its native structural features and be unable to undertake its designed biological function, thus leaving it inert. The number of factors involved in directing a protein into its native conformation makes it a challenging area of research with many discoveries still to be made.

1.1.3 Secondary Structure Units

The various secondary structure units that a protein can form have been studied extensively in recent decades in an attempt to develop an understanding of all aspects of protein structure and function. When studying a folded protein or peptide several units of secondary structure quickly become apparent such as strands, coils, helices and turns. These conformations are stabilised by multiple non-covalent interactions taking place between various sections of the peptide chain.

One of the most prevalent and fundamental stabilising interactions is hydrogen bonding[17] which can take place between NH groups and CO groups in the peptide backbone or by interactions between polar groups in the functionalised side chain. There are also opportunities for alternative types of interaction such as π-stacking interactions or ionic interactions. This latter situation will be observed in cases where amino acid residues contain NH_2 (lysine) or CO_2H (glutamic acid) groups in the side chain that will form charged zwitterionic species through proton transfer to give NH_3^+ and COO^-.

α-Helix

One of the most frequently encountered units of secondary structure is the α-helix. This is a repeating unit that is characterised by a coil or spiral formation, which is stabilised by repeated hydrogen bonding. Each NH group forms a hydrogen bond with a carbonyl group located on an amino acid residue that is further along the chain. The two functional groups are described as having a 1 to 4 relationship as the hydrogen bond always occurs between groups that are 4 residues apart in the amino acid sequence.

Two prominent examples of proteins that extensively utilise the α-helix are myoglobin[18] and haemoglobin, which are both essential proteins in oxygen transport in the body. Myoglobin contains eight α-helices and is structurally similar to a single subunit of haemoglobin. This structure is illustrated in Figure 6 with the α-helix structures represented by coil formations.

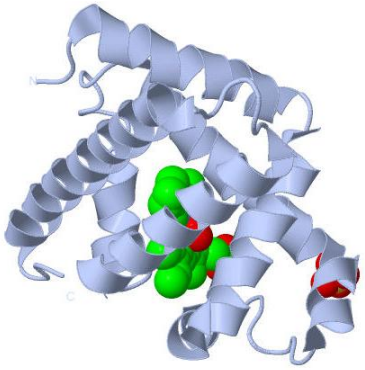


Figure 6: Myoglobin Structure[4,5]

The existence of the α-helix structure was originally postulated by Linus Pauling[19] and this was confirmed by the first three-dimensional structure determination of myoglobin using X-ray crystallography in 1958.[20]

β-Pleated Sheet

Another prominent secondary structure unit is the well-known *β*-sheet or *β*-pleated sheet. This consists of several *β*-strands lining up alongside each other and subsequent stabilisation with hydrogen bonding. A *β*-strand is a chain of amino acids, typically 3 to 10 residues long, arranged largely in their extended conformation. The *β*-strands can align themselves either parallel or anti-parallel, meaning that they are either arranged with the amino acid chain running in the same direction or in opposite directions respectively. The direction of the chain is indicated on diagrammatic representations with an arrow (Figure 7). The hydrogen bonding can also form between chains that are not necessarily adjacent to each other. This means that a *β*-sheet can consists of several chains of amino acids from portions of the protein's extended conformation that are remote in the primary sequence.

Figure 7: Example of 2 Layered Orthogonal Antiparallel *β*-Sheets[11,21]

The pleated appearance of a *β*-sheet is derived from the tetrahedral geometry of the *α*-carbon centres. If the two *β*-strands of the sheet are arranged so that the C^{α} functional groups on the strands are in the same plane, then the bonds from the C^{α} leading into the rest of the chain will lead backward or forward from that plane, meaning that the bonding will alternate above and below the plane of the sheet giving it a pleated appearance.

1.1.4 The *β*-Turn Structure

Turns are particularly important units of peptide secondary structure. There are several distinct classes of turn and they are defined by the extended peptide chain folding back on itself in a U-turn conformation. This feature is stabilised by non-covalent interactions. The turn type is classified by the number of residues involved in the turn and hence the size of the pseudo-ring system that is formed.[22] Figure 8 shows a concise representation of the difference between three distinct turn types. The amino acids are coloured differently to clearly illustrate the number of residues that are involved in each turn. The *α*-turn (5 residues), *β*-turn (4 residues) and *γ*-turn (3 residues) form 13-, 10- and 7- membered hydrogen bonded rings respectively.[23]

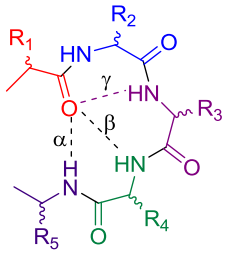


Figure 8: Three Distinct Turn Types[24]

The most common turn-type found in nature is the *β*-turn. The *β*-turn was first recognised in the 1960s by Venkatachalam.[25] This structural unit is now known to be found commonly in peptides and proteins, comprising up to 25% of all residues.[26] The *β*-turn is also present in the active conformation of many biologically active peptides and proteins

These units populate a folded structure between regions of regular secondary structure such as helices and sheets.[27] A *β*-turn consists of four residues that are labelled *i*, *i*+1, *i*+2 and *i*+3. The hydrogen bonding typically takes place between NH and carbonyl groups located on the *i* and *i*+3 residues. The less common *α*- and *γ*-turns will utilise hydrogen bonding interactions between the *i* and *i*+4 or the *i* and *i*+2 positions respectively.

Figure 9 shows a general representation of a *β*-turn that highlights the four residues that are involved in the turn. There are generally four opportunities for functionalisation at the 4 R groups around the turn. The hydrogen bonding that stabilises the turn is formed between the carbonyl group and NH proton of residues *i* and *i*+4 respectively and this can be further stabilised down the peptide chain by further hydrogen bonding between proximal groups.

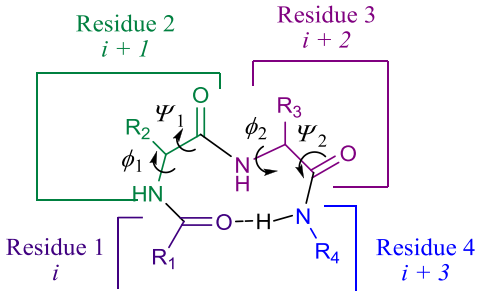


Figure 9: A General Representation of a *β*-Turn Structure[28]

β-Turns can be further sub-categorised into types. These are labelled, for example, as type I, type II, type II' etc. There are a total of more than 10 different types of *β*-turn known today.[29] These types are distinguished by the dihedral angles, represented as *ψ*-angles and *ϕ*-angles in Figure 9. Table 1 shows a selection of some different *β*-turn types as defined by their characteristic bond angles.[28]

Table 1: *β*-Turn Types and Their Ideal Angles

Angle	Type I	Type I'	Type II'	Type III
ϕ_1	–60	60	60	–60
ψ_1	–30	30	–120	–30
ϕ_2	–90	90	–80	–60
ψ_2	0	0	0	–30

A *β*-turn can also be involved in a *β*-sheet if two of the *β*-strands are adjacent to each other on the extended chain. The turn that brings the two strands into close proximity for hydrogen bonding is referred to as a *β*-hairpin.[21] It is important to note that the term *β*-hairpin is a generic term that is used to describe the turn motif that is bringing the two *β*-strands into proximity and can mean any type of turn, be it a *γ*-, *α*- or *β*-turn i.e. it can include, but is not restricted to, a *β*-turn.

β-Turns in a protein's native conformation can be a contributing factor to the overall tertiary structure. The formation of a *β*-turn does not involve a large decrease in entropy because the interactions that are involved are largely local. A turn does however bring other groups in the amino acid chain into closer spatial proximity to each other thus improving the likelihood of their interaction. Thus, a turn can make the formation of further secondary structure units more energetically favourable and can contribute to a hierarchical folding model where the formation of a turn induces further protein folding in the extended chain.

Alternatively, *β*-turns can play a passive role in the overall process of protein folding. In this case, they are themselves induced by the other secondary structures in the native conformation in a process whereby a section of the chain is simply forced into a turn, which further stabilises itself through hydrogen bonding. In either event, the *β*-turns are important secondary structure units and they are often found in the active conformation of peptides and proteins that exhibit biological activity.

Bearing in mind that the three-dimensional structure of a protein is critical for its biological interactions, a *β*-turn that is located on the surface of the protein can be a significant feature as it exhibits a distinctive structure that may be essential for recognition at a receptor site. Due to the abundance of *β*-turns in biologically active proteins and peptides there has been a surge of interest towards the study of *β*-turns in recent years. It is hoped that the study of naturally occurring proteins with biological activity could inspire research into synthetic drug molecules with similar, desirable biological activities (*vide infra*).

1.2 Opioid Peptides and Peptidomimetics

1.2.1 Opioid Peptides and Receptors

Opioids are compounds that will bind to opioid receptor sites resulting in the physiological relief of pain. Opioid receptors are found principally in the central nervous system and the gastrointestinal tract. There are several different classes of endogenous opioid peptides in humans such as the enkephalins, the dynorphins, and the endomorphins, examples of which are show in Figure 10.

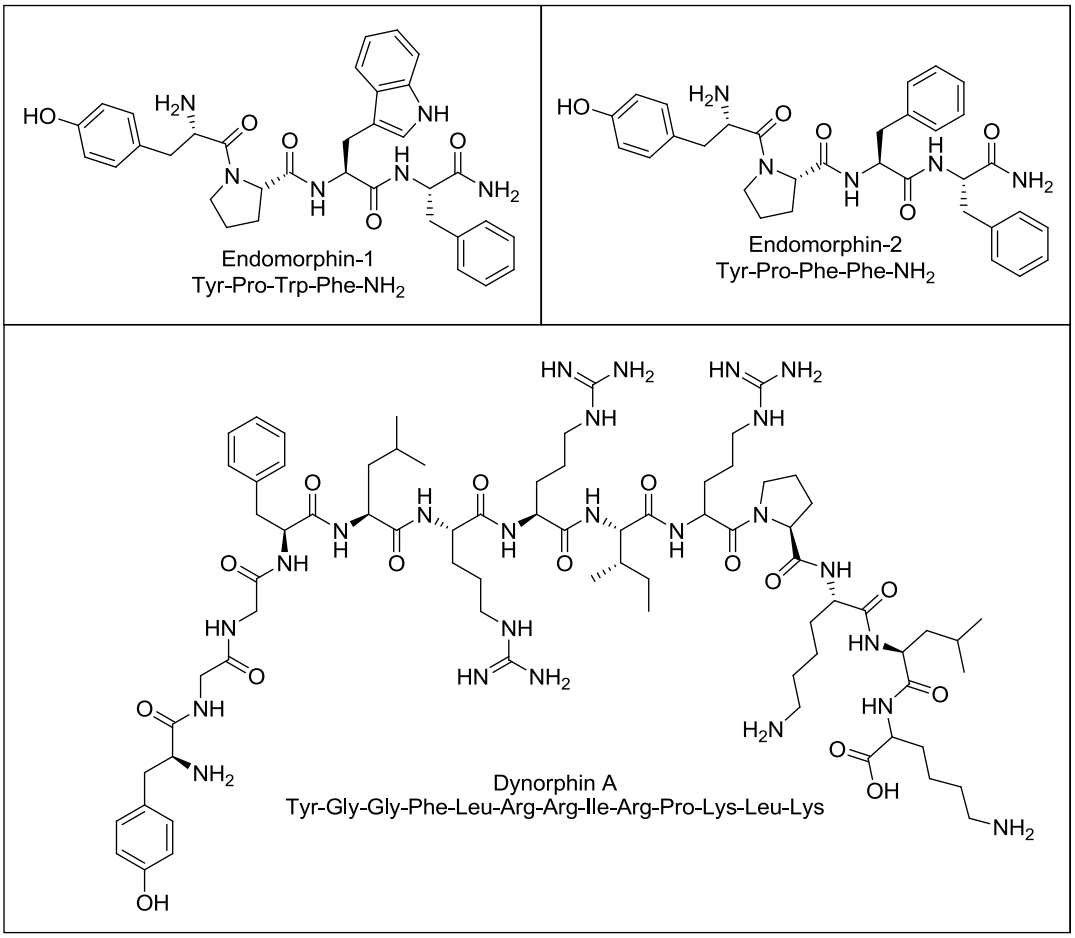


Figure 10: A Selection of Endogenous Opioid Peptides

There are three types of opioid receptors that are widely accepted today. These are labelled as the δ-, κ- and μ-sites and can be further sub-categorized. The existence of opioid receptors was first suggested in the 1950s,[30] and this was followed in the 1960s and 1970s by the proposal that there were several different classes of opioid receptor. This latter proposal was based upon a series of opioid peptides each displaying different profiles of pharmacokinetic activity *in vivo*. Three opioid receptor types were proposed and labelled μ, κ and σ. The δ-receptors were identified shortly after the discovery of the enkephalins in 1975.[31] The σ-receptor is no longer regarded as an opioid receptor as it does not exhibit the stereoselectivity characteristics of opioid receptors or antagonism by opioid antagonists.[32]

Each of the three types of opioid receptor have a series of side effects that are associated with their binding, for example physical dependence, depression, miosis or euphoria. The μ-opioid receptor is a well known target for pain relief and is the subject of many current opiate analgesics such as morphine. Having said this, the use of opiates is limited by their side effects which include respiratory depression, desensitisation with chronic

use and the development of dependence.[33] The δ-opioid receptors are known to exhibit side effects that include convulsions and the κ-opioid receptors are accompanied by dysphoria (depression), hallucinations and sedation.

These issues can be problematic if an opiate binds to multiple receptors, as this will not only result in the analgesic effects, but will also ensure the full range of unwanted side effects is experienced by the patient. These effects are where the addictive properties of morphine (Figure 11) and other related opiates originate. Understanding these receptor sites is key to developing new drugs with increased analgesic potency and reduced side effects.

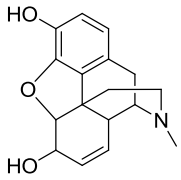


Figure 11: Structure of Morphine

The endogenous opioid peptides have varying structures and their own individual binding characteristics. It is important to consider this fact when designing a molecule to exhibit opioid activity. Selectivity is an important issue as illustrated by the diversity of side effects associated with each receptor type. Both endomorphin-1 and endomorphin-2 show a high affinity for binding to the μ-receptors. leu- and met-enkephalin display affinity for μ-receptors but they also bind with the δ-receptors.[34] The β-endorphins, dynorphin and neoendorphin all bind to the κ-receptors. Clearly the ability of each of these to bind to opioid receptors is determined by its amino acid sequence and its secondary structure characteristics. Furthermore, being that the β-turn secondary structure unit has been identified as the most likely conformation for ligand binding to opioid receptors, it must only require very subtle variations between turn types to affect binding affinity to specific receptor types.

1.2.2 Leu-Enkephalin

In 1975, two endogenous opioid pentapeptides were discovered.[35] These two peptides were named leu-enkephalin and met-enkephalin and were isolated from pig brain (Figure 12). The two peptides have very similar structures, only differing in the identity of the terminal residue. The two peptides are shown below with their extended chain conformations and the different terminal residues highlighted. The ratio of met-enkephalin to leu-enkephalin in

biological systems appears to be species-specific with four times more of the former than the latter in pig brain. Both types of enkephalin show an affinity for δ- and μ-opioid receptors, with a strong preference for the δ-receptors in particular.[36]

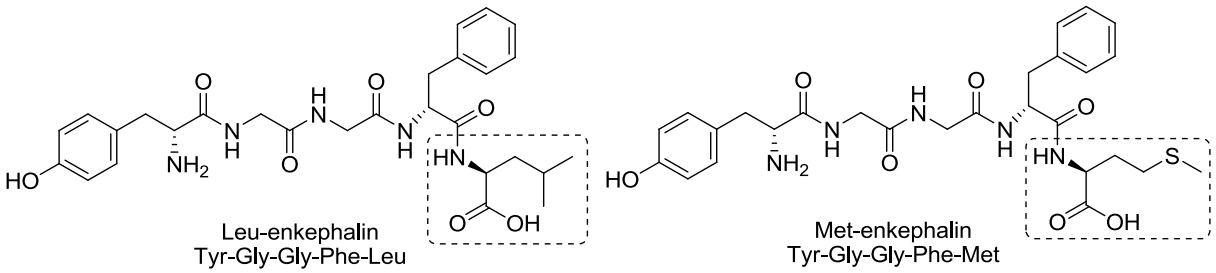


Figure 12: Chain Structure of Both Enkephalin Pentapeptides.

Three years after the elucidation of the enkephalins the three dimensional structure of leu-enkephalin was revealed using X-ray diffraction.[37] It was apparent that the pentapeptide adopted a β-turn conformation in the solid state. Based on this finding it was proposed that the enkephalins utilised a β-turn in their bioactive conformation. This theory has since been supported by the bioactivity of structural analogues of enkephalin β-turns.[38-40] The turn is stabilised by intramolecular hydrogen bonding between the tyrosine carbonyl oxygen (i) and the phenylalanine NH (i + 3) thus fulfilling the 1 to 4 interaction that is characteristic of the β-turn.

Two representations of the folded conformation of leu-enkephalin are illustrated in Figure 13, one clearly highlighting the four residues involved in the turn, and the other based upon the X-ray structure that was determined in 1978.[37] X-ray analysis indicated that the tyrosine side chain was disordered and occupied two discrete positions with nearly equal frequency. This is shown on the X-ray structure at the lower terminus of the chain by the two possible orientations of the *p*-hydroxyphenyl group. These two orientations are both displayed in the X-ray crystal structure.

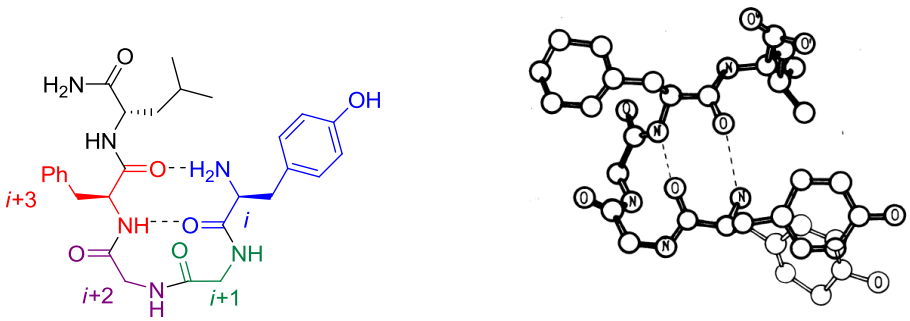


Figure 13: Representations of Leu-Enkephalin in its β-Turn Conformation[13]

Both diagrams show that as well as the hydrogen bond stabilising the turn, there is a second hydrogen bond further along the extended structure. This second H-bond is induced by the conformation of the chain and takes place between the phenylalanine carbonyl oxygen and the primary amine group of tyrosine, thus giving an antiparallel relationship between the two hydrogen bonds.

Whilst the X-ray structure of leu-enkephalin in its solid state is not definitive proof that it will adopt the β-turn conformation in its active form, it is a promising starting point. leu-Enkephalin is, in fact, a highly flexible pentapeptide and has been found to occupy type I, I', II' and III β-turn conformations in solution.[41] This conformationally dynamic structure presents some problems when trying to determine its biologically active state. Further difficulties have arisen from the fact that it is difficult to obtain sufficient quantities of purified opioid receptor of the μ- and δ-type, for which the enkephalins show the highest affinity for binding.[42]

Despite the difficulty inherent in studying the active conformation of the enkephalins, research to further understand these peptides is ongoing. One avenue of investigation involves the development of peptide analogues and peptide mimetic structures that can be used to further probe the biological interactions of opioids with their receptors.[43,44]

1.2.3 Introduction to Peptidomimetics

In some respects a natural protein or peptide should be theoretically ideal as a drug molecule. They exhibit known biological activity and they clearly don't have any appreciable toxicity or serious side effects. However, there are two main practical reasons why naturally occurring peptides and proteins are unsuitable for clinical use.[45]

Firstly, they are prone to undergoing proteolytic degradation and so the peptide chain can be broken down quite easily under physiological conditions. The second reason relates to their poor transport properties which results from the difficulties that peptides have in crossing membranes due to their propensity to form hydrogen bonds.[46] These two factors contribute to peptides and proteins exhibiting generally poor pharamacokinetic properties. These are, of course, beneficial for natural proteins as it means that they can be broken down quickly once they have performed their function and can be localised in the specific region of action. However, if a peptide is introduced into a biological system as a drug molecule (i.e. orally) it cannot reach the site at which it exhibits its activity and it is broken down very rapidly.

It is unfortunate that peptides are such poor candidates for drug applications as there is a great deal of information to be learned from them and how they interact with biological systems. Instead of using the natural structures to induce the biological activity of interest, chemists and biologists have taken inspiration from these structures when considering the development of synthetic molecules for drug use.

Focusing on a particular peptide or protein with a known activity, there are several lessons to be learned about how to reproduce the same activity. Determining the three-dimensional structure of a peptide in its receptor-bound form is important as this develops understanding of exactly which structural features and functional groups are important for activity to take place. A good understanding of structure activity relationships (SAR's) is essential when designing a mimetic with targeted biological activity.

Once a reasonable understanding of the essential elements required for biological activity has been developed, a simplified model can be constructed utilising only these essential features. A small molecule can be designed that will stabilise these functional groups and three-dimensional features in a covalent scaffold.[47] A common approach towards synthesising these mimetic structures involves a generic synthetic pathway that permits inclusion of a variety of side chain functionalities. This produces libraries of similar compounds that can then be screened for biological activity. In this manner, chemists can learn more about the features that will improve or impede desired activity. Through repeated syntheses the structure is further refined to optimise the required activity.

Ultimately, the molecules that are synthesised via this method can have even greater potency than the natural peptides upon which they are based. Furthermore, by synthesising a covalently bonded analogue, the poor pharamacokinetic properties associated with natural peptides and proteins can be bypassed. The new structure can be tailored to make it more robust under physiological conditions. A further benefit lies in the entropic qualities of a small molecule mimetic. A flexible peptide will suffer a large decrease in entropy in order to interact with a receptor site, but a small covalently bonded, and comparatively rigid, mimetic, will not experience such a large decrease in entropy. A mimetic can therefore bind more efficiently to a receptor as it is thermodynamically more favourable to do so.[48]

In this manner, it is ultimately possible to synthesise structures that exhibit high biological activity and selectivity. Ultimately, these studies constitute a pathway to generate medicinally useful compounds.

The applications of peptidomimetics do not stop with drug development. This type of synthesis also finds promising applications in the field of synthetic vaccines. Synthetic systems have several advantages over traditional, whole-killed, live-attenuated vaccines[45] such as simplified preparation, increased safety and the ability to focus the immune response to a particular antigenic determinant of the pathogen. In practice, natural peptides have several drawbacks in this area. They are often unable to elicit a high-titer, high-specificity antibody response to the native protein from which the peptide sequence was chosen. The difficulty lies in the conformational flexibility of the natural peptides and their inability to preserve the specific topological and conformational features that are required for recognition and induction of high-titer, high-affinity antibodies.

Again, natural peptides and proteins can provide a blueprint in developing biologically active structures of defined function. As understanding of the structural features of proteins and peptides improves, so will the efficacy of artificial mimetics based on them.

It is estimated that of all modern drugs that are in use, almost half of them target G-protein coupled receptors (GPCRs) in order to exhibit their effects. The GPCRs are a large family of proteins that share a similar structure composed of seven transmembrane helices[49] as shown in Figure 14. Located on the surface of cells, GPCRs are responsible for the translation of various types of external stimuli into some form of intracellular response. They can exhibit a response to both endogenous chemical signals and exogenous environmental agents, for example, peptides, proteins, metal ions, pharmaceuticals and light.[23]

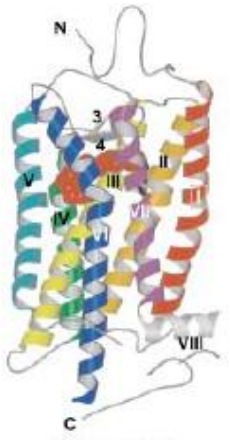


Figure 14: Ribbon Drawing of Rhodopsin, a G-Protein Coupled Receptor[24]

Due to their diverse utility, designing ligands for such receptors is a fertile area of research in the pharmaceutical industry.[50] It has been shown previously that most peptide activated GPCRs bind to their ligands through a turn structure.[23] Furthermore, turns often take

place on the exposed surfaces of proteins. As a result they can be important for molecular recognition events such as interactions between antibodies, antigens and regulatory enzymes and their corresponding substrates. The use of protein and peptide mimetics is an effective method of exploring the interface between secondary structures and receptors using small, robust molecules with reliable and accessible syntheses.

1.3 β-Turn Peptidomimetic Structures and Synthesis

1.3.1 Previously Reported β-Turn Mimetics

Of the various secondary structure units found in proteins, β-turns are among the most widely utilised for peptidomimetic synthesis.[28] This is due to the fact that this structural feature is used for biological recognition by many different types of protein e.g. antibodies and T-cell receptors.[51] Additionally, the β-turn, as a unit, is readily amenable to mimetic design.

The use of peptidomimetics in developing therapeutic structures was anticipated by Farmer[52] who proposed the idea of replacing a peptide scaffold with a cyclohexane ring prior to functionalisation with relevant amino acids.[53] The cyclohexyl ring would effectively act as a tether that replaces the U-turn in the chain structure. This forces the extended chain into a similar conformation to the natural turn structure. This was explored some time later, when Bélanger and Dufresne developed a nonpeptidic mimetic of the enkephalin opioid peptides, shown in Figure 15.[54]

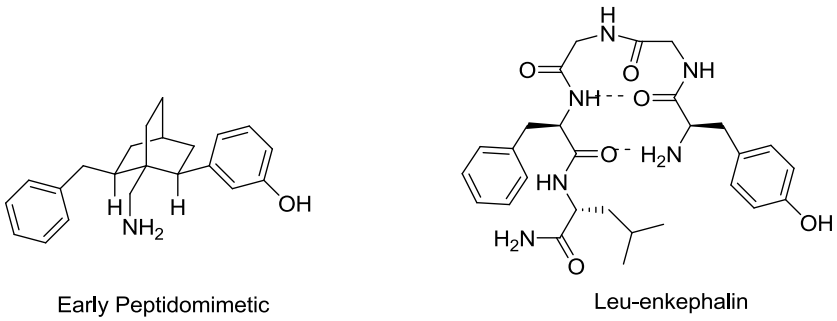


Figure 15: Early Peptidomimetic Based Upon Leu-Enkephalin

Some time later, Olson and co-workers[55] reported a similar approach to thyrotropin releasing hormone (TRH), effectively installing all of the side chains from the natural tripeptide onto a cyclohexane ring, similar to the approach suggested by Farmer. In this case, the peptide mimetic did not bind to the endocrine receptor for which it was designed. Having

said this, it did show oral activity in animal models of cognitive dysfunction. The structure of the mimetic and its natural counterpart are shown in Figure 16.

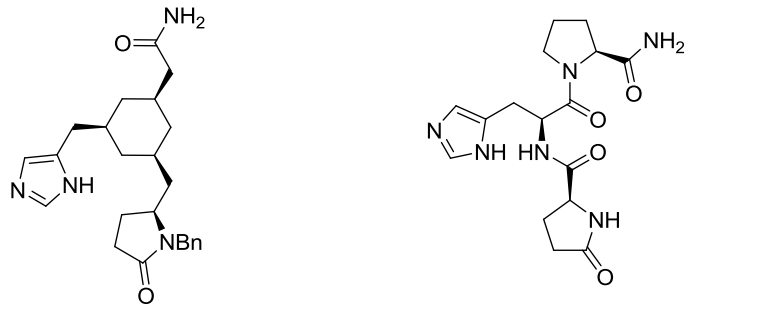


Figure 16: Peptidomimetic Based Upon TRH

In recent decades, the field of peptidomimetic chemistry has received a significant amount of attention. The possibilities for synthesising libraries of bioactive compounds are astounding and as a result countless structures have been developed to mimic a range of known peptides and their secondary structure conformations. Some compounds have displayed activity whereas some have not lived up to their promise. As a result, the scientific literature is peppered with publications containing hundreds of mimetic structures. This area of chemistry lies at the interface between several different scientific disciplines i.e. biology, peptide chemistry, medicinal chemistry etc. The following sections will discuss some notable examples from the past decade that have been published by accomplished and experienced groups in the field. These examples were chosen to highlight some of the diversity that can be considered in a mimetic structure and also some of the difficulty inherent in identifying an appropriate structure. All of these cases were published in the past decade and, although not all of them have been tested biologically, or were able to achieve a truly general synthetic approach conducive to library synthesis, they illustrate a broad range of approaches to β-turns as well as some structural diversity. This diversity highlights the varied possibilities for using artificial structures to induce covalently bonded turn mimetics.

1.3.2 Kahn's Synthesis of an Opioid Peptidomimetic

In 2002, Kahn and co-workers proposed a rigid bicyclic structure, which enforced a conformation similar to that found in the endogenous opioid peptides enkephalin and endomorphin.[56] The general structure of mimetic **1** is shown in Figure 17.

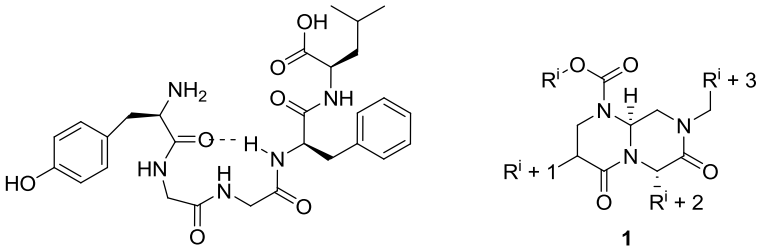


Figure 17: Left: Leu-enkephalin Molecule, Right: Kahn's Bicyclic Mimetic Structure

The bicyclic core of this structure incorporates several sites for potential functionalisation. Kahn and co-workers proposed the use of a library synthesis approach that allowed the development of varied similar structures for biological testing. In this manner, potent and selective ligands were revealed that bind selectively to opioid receptors, thus providing new insights into the SAR's of the opioid peptides.

Kahn and co-workers demonstrated, using X-ray crystallography and 2D NMR spectroscopy, that their designed molecule mimics a type I β-turn conformation. The turn is successfully maintained by the rigid 6,6-bicyclic structure. Further investigation by the group also showed that their β-turn mimic shares similarities between its 3D structure and the receptor selectivity profile of the opioid peptides endomorphin-1 and endomorphin-2, thus opening further avenues of investigation. The bicyclic structure can be used to study several different conformations of the β-turn which are shown in Figure 18 as enkephalin 4→1 (A), enkephalin 5→2 (B) and endomorphin 4→1 (C). To this end, the group synthesised a library of compounds utilising the mimetic structure in conjunction with a variety of functionality at the *i* to *i*+3 positions *via* their established solid phase synthesis procedure shown in Scheme 1.

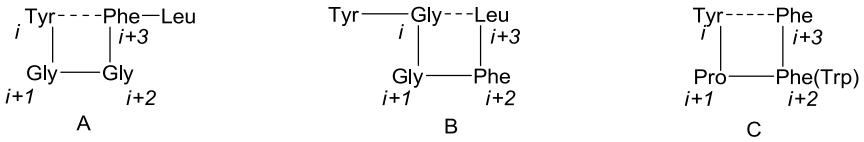
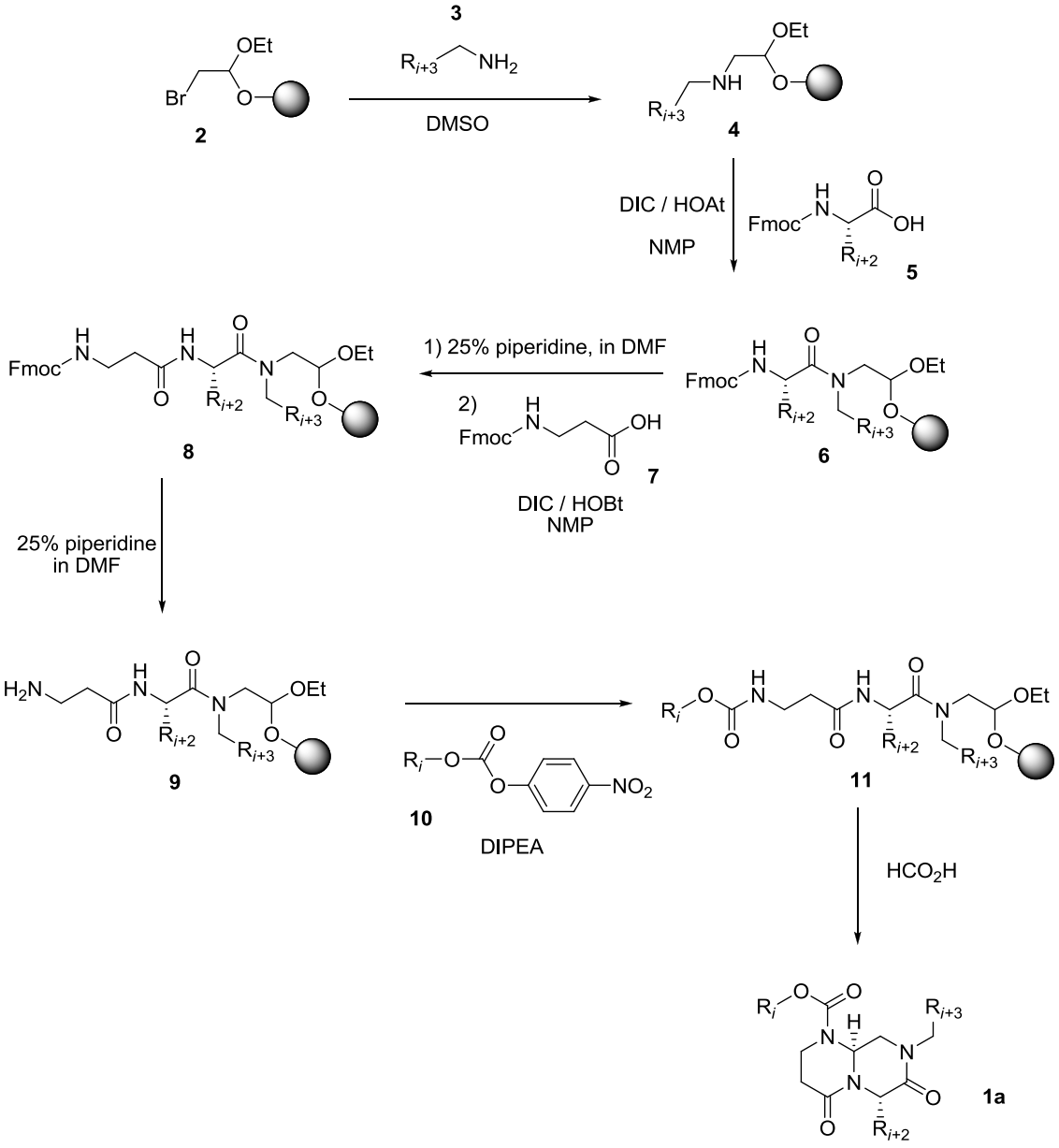


Figure 18: Representation of Enkephalin 4→1 (A), Enkephalin 5→2 (B) and Endomorphin 4→1 (C)[57]

The solid phase synthesis procedure utilised the commercially available bromoacetal resin **2**. Nucleophilic displacement of the bromide with a primary amine, **3**, delivered secondary amine intermediates, **4**. These were then coupled with Fmoc-protected α-amino acids, **5**, using HOAt/DIC to provide intermediates **6**. Treatment with 25% piperidine in DMF was carried out to cleave the Fmoc group, followed by a second coupling with

Fmoc-β-alanine, **7**, to give compounds **8**. Again, the Fmoc group was removed using piperidine in DMF towards intermediates **9**. The deprotected amines were then coupled with a corresponding alkyl *p*-nitrophenyl carbonate of type **10** to form compounds **11**. The final step involved cleavage from the acetal resin, followed by a stereoselective tandem acyliminium cyclisation and this was accomplished by the straightforward addition of formic acid. This gave access to a variety of 6,6-bicyclic β-turn mimetics **1a**, with a choice of functionality at the *i* to *i*+3 positions.



Scheme 1: Synthesis of Bicyclic Mimetic Unit 1a

The synthetic procedure described above resulted in **1a** giving good overall yields, ranging from 11% to 37% for the entire synthesis. Figure 19 displays the range of different side chain functionalities that were incorporated into the general structure **1a** and highlights the utility of this type of library synthesis for the efficient preparation of multiple analogous compounds. One synthetic pathway is designed and optimised, which then leads to a diverse array of structures that can be tested for the desired biological activity.

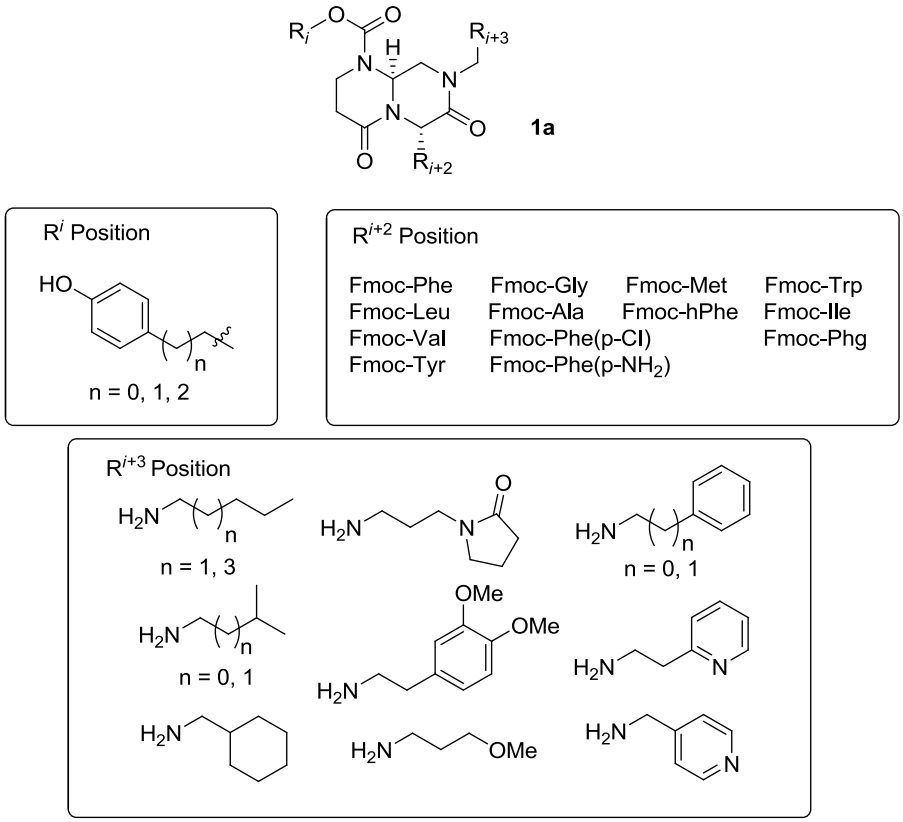


Figure 19: Range of Side Chains Employed in Kahn's Mimetic Structure

Once Kahn and co-workers had achieved their objective of optimising the synthetic route, they turned their attention towards studying the binding of the synthesised structures to opioid receptors. This was studied by looking at competitive binding of radioligand [^{3}H]naloxone with relatively non-selective opioid receptors in rat cerebral cortex.[56] This gave some insight into the relative ability of the mimetics to bind in comparison to each another. Further investigation highlighted structures **1b** to **1e** as having the highest binding affinities of all compounds that had been prepared (Figure 20).

Compounds **1b** and **1d** are representative of the enkephalin 4→1 type β-turn model shown in Figure 18, based on the functional groups at the *i* and *i*+4 positions. Compounds **1c** and **1e** are analogous to the endomorphin 4→1 type β-turn model.

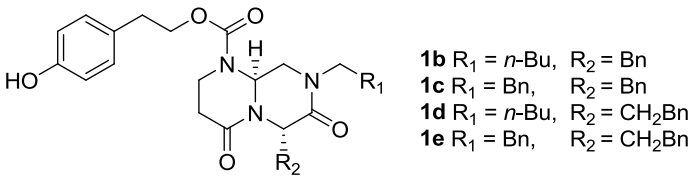


Figure 20: Four Mimetics Exhibiting High Binding Affinities to Opioid Receptors

The four structures described above were tested to determine their IC_{50} values. It was found that there was an increasing ability to bind to nonselective opioid receptors in rat cerebral cortex for the four structures, with structure **1b** showing the least binding affinity and compound **1e** the highest. In fact, compounds **1d** and **1e** were 5- and 15-fold more active respectively towards opioid receptors than compound **1b**. Structures **1d** and **1e** were further studied for activity against cloned human μ-, δ- and κ-receptors and it was found that they both exhibited particularly high selectivity for the μ-receptors.

As a comparison, another novel structure was synthesised containing all of the relevant functionality from compound **1d** in a linear peptide sequence (Figure 21).

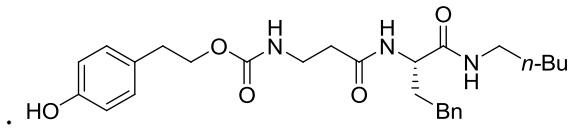


Figure 21: Linear Control for Compound 1d

This linear peptide was tested for the same biological activity as the mimetic structures. It was found that at 1 μM concentration, no binding activity was observed. This strongly suggests a specific three-dimensional structure is essential for receptor binding affinity. The linear peptide, having all of the necessary functional groups, was unable to bind to the opioid receptors as it lacked the conformational rigidity of the bicyclic mimetics. This is further evidence of the nature of the active conformation of the endogenous opioid peptides. The β-turn conformation is seemingly essential for biological activity.

In addition, structure **1d** was used for an *in vivo* evaluation of its analgesic effect on mice. It was found that, while it had a similar initial activity level to morphine, its half-life *in vivo* was 2-fold less.

Overall, Kahn and co-workers were able to synthesise a library of β-turn mimetic compounds that were successfully tested in biological systems. From these studies, they were able to identify the most promising functional groups with regard to opioid receptor binding. Further examination proved that they had synthesised a turn mimetic system that was highly selective towards μ-receptor sites at nanomolar concentration. This research also suggested that the bioactive conformation of endomorphin is a type III 4→1 β-turn and the μ-active conformation of enkephalin is a 5→2 β-turn.

1.3.3 Kihlberg's Mimetic Structures

β-Turn structures have been developed that utilise several different ring types and sizes to induce the extended structure to fold back upon itself. This diversity is readily apparent when examining a selection of reported mimetic structures. Where Kahn and co-workers utilised a bicyclic mimetic, other groups developed structures that induced a turn conformation using a single medium-sized ring.

Figure 22 illustrates two cyclic mimetics, **12** and **13**, that were developed by Kihlberg and co-workers in 2004 and 2006 respectively.[58,59] The side chains are positioned slightly differently in the two structures because of the different ring sizes. Structure **12** contains a 10-membered ring system, whilst molecule **13** contains a smaller 7-membered ring.

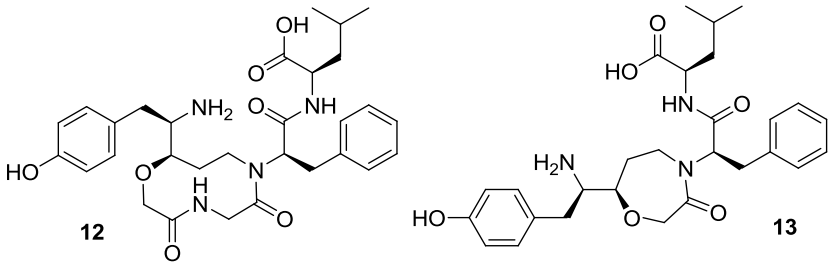


Figure 22: Two Macrocyclic β-Turn Mimetic Structures Developed by Kihlberg

Both of these turn mimetics have similar features in so far as they both have an ethylene bridge in the ring system and a methylene ether isostere. This type of system was deliberately chosen as it has been shown in previous work that replacement of the

tyrosine-glycine amide bond in leu-enkephalin with a methylene ether isostere is well tolerated by the opioid receptors.[60]

The 10-membered cyclic structure **12** was introduced first in 2004 and was synthesised in the solution phase. Conformational studies based on ^{1}H NMR data indicated that this mimetic was conformationally flexible, but that at low temperatures adopted a similar conformation to a type-II β-turn.[58] This conformation resembled that of crystalline leu-enkephalin. In an effort to further explore these structures, and aware that there would be significant scope to explore turn conformations, Kihlberg and co-workers embarked on the synthesis of the 7-membered cyclic mimetic **13** as a natural extension of the work that had already been reported. This structure was synthesised as illustrated in Scheme 2.

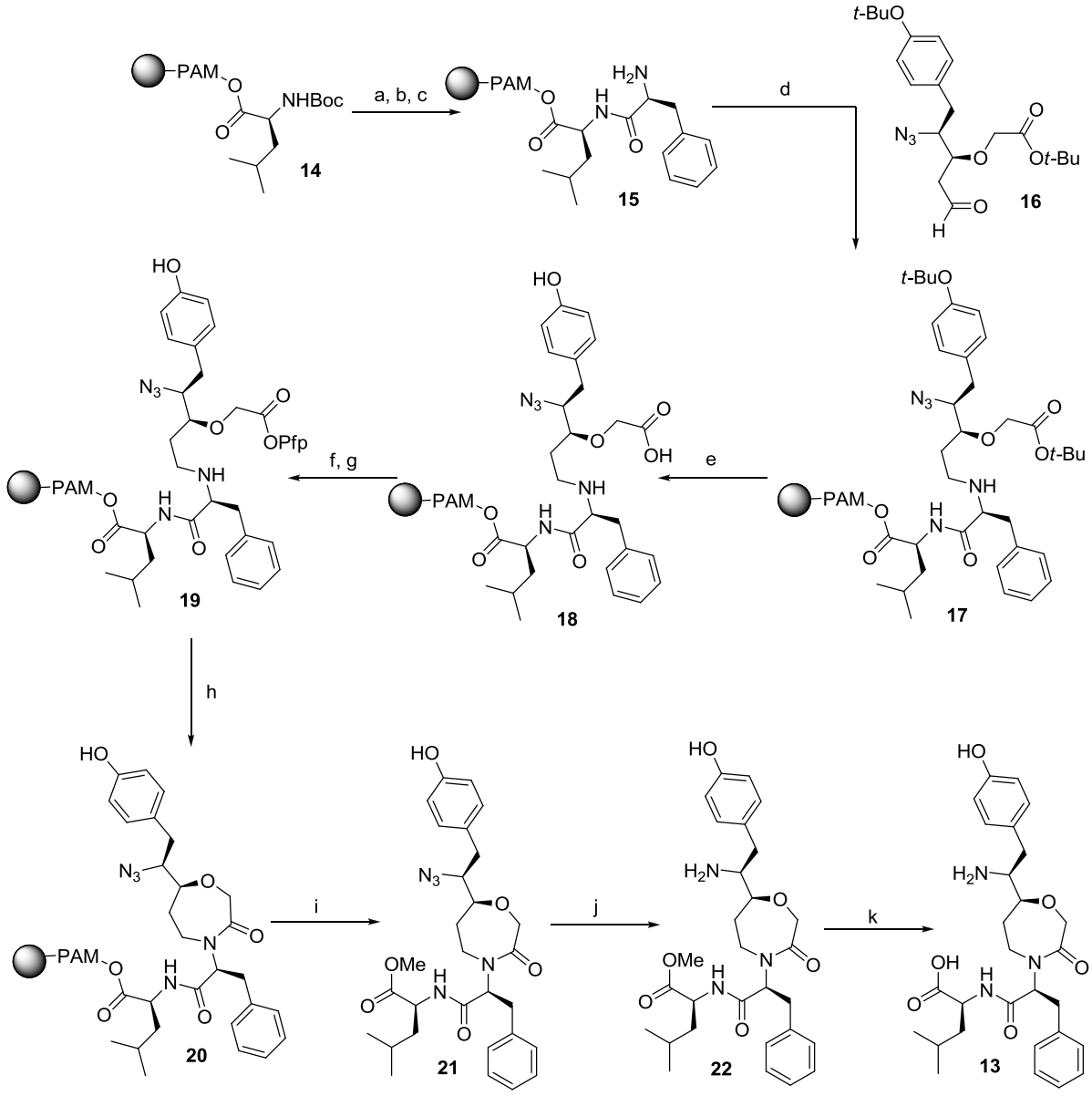
The synthesis of the mimetic was performed on a solid support so as to minimise oligomerisation during formation of the 7-membered ring and also to establish conditions that would allow the development of a library of turn mimetics. The first step in the synthesis was deprotection of Boc-leucine attached to a Tentagel resin under acidic conditions. The deprotected amine was then coupled with Fmoc-protected phenylalanine using DIC and HOBt. Once the coupling was successfully achieved, the Fmoc group was removed using piperidine in DMF to furnish the solid phase bound dipeptide, **15**.

Reductive amination was carried out using aldehyde **16** to provide the desired secondary amine using sodium triacetoxyborohydride as the reducing agent. Following this, the phenolic *t*-butyl ether and the *t*-butyl ester were removed simultaneously under acidic conditions. The authors were pleased to carry this out successfully while not degrading any other functionality in the molecule.

Subsequently, activation of the carboxylic acid in the resultant compound **18** was achieved using DIC and pentafluorophenol to give the pentafluorophenyl (Pfp) ester, **19**. The cyclisation reaction to deliver the 7-membered ring was performed using TEA in refluxing dioxane, giving **20**. This is the stage at which problems were encountered when carrying out this synthesis in the solution phase. In solution, this reaction often resulted in polymerisation, which competed with the desired cyclisation reaction. As a result, this stage can only be carried out at low concentrations in solution phase. The use of a solid support was Kihlbergs solution to this problem.

Once the ring closure was achieved it was possible to remove the solid phase support. This was accomplished *via* treatment with sodium methoxide resulting in **21**. Over the course of all of the steps carried out in solid phase, Kihlberg and co-workers were able to achieve an

appreciable overall yield of 25%. The final two steps involved the deprotection of the carboxylic acid and amine units on the mimetic. Firstly, the azide was reduced to give amine **22** and finally the methyl ester was hydrolysed using lithium hydroxide to give the target β-turn mimetic, **13**. These last, solution phase, steps gave an overall yield of 56%. This resulted in a synthesis towards the desired mimetic structure that was performed in 11 steps, nine of which were carried out in the solid phase, with a total overall yield of 14%.



a) TFA (30%) in DCM, b) Fmoc-Phe-OH, HOBt, DIC, c) piperidine (20%) in DMF, d) **16**, $Na(OAc)_3BH$, NEt_3, 1,2-DCE, e) TFA in DCM, f) HCl (1 M) in 1,4-dioxane, g) PfpOH, DIC, EtOAc, h) NEt_3, 1,4-dioxane, i) Na (0.22 M) in MeOH (Overall yield 25%), j) $SnCl_2$, PhSH, NEt_3, THF, k) LiOH (0.1 M), THF (56% over 2 steps).

Scheme 2: Synthesis of 7-Membered Cyclic Mimetic 13

Further study of these two mimetic structures was undertaken using a similar approach as described by Kahn. The binding affinity of the compounds was tested in rat brain using a competitor to probe the selectivities of the two structures. Kihlberg also used two linear analogues of the mimetic structures to investigate the effect of the cyclisation on the binding affinity of the mimetic (Figure 23). Structures **23** and **24** correspond to structures **12** and **13** respectively.

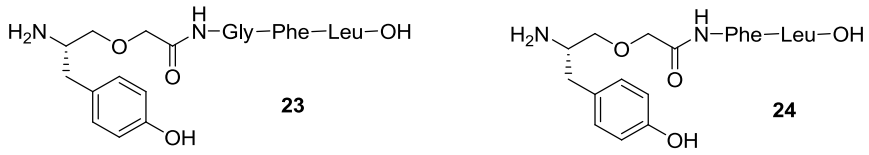


Figure 23: Linear Analogues 23 and 24

The 10-membered ring structure **12**, showed no affinity for either μ- or δ-opioid receptors, whereas compound **13**, containing the 7-membered ring, binds to both receptor sites. The inability of the 10-membered ring to bind to either receptor site is not a factor of having replaced the glycine-tyrosine amide bond with an ethylene ether isostere. The linear analogue **23**, which corresponds to structure **12**, exhibits a very high binding affinity for both sites despite the fact that it shares this feature.

It has been suggested that the cyclic conformation of the 10-membered ring in **12** renders the structure very rigid and unable to adopt the appropriate conformation for binding. In addition, it is possible that loss of a hydrogen bond involving the phenylalanine amide proton is a factor. Both factors may contribute, but neither of them explains fully why compound **12** does not bind whereas 7-membered lactam ring **13**, which shares these features, is able to bind effectively. On the other hand, structure **24**, which is the linear analogue of **13**, does not bind, most likely because of the entropic cost of folding around into a turn conformation.

1.3.4 Hruby's Mimetic Structures

Hruby and co-workers have been working at the forefront of peptide mimetic design and synthesis for many years and have developed several mimetic systems including structures based on β-turn conformations.[61,62] This group has studied several conformationally restricted bicyclic structures as potential β-turn mimetic units.[63] The bicyclic units that they have

developed restrict the conformation due to the stereochemistry of the scaffold, the presence of different substituents and the frame size. The group developed a bicycle leu-enkephalin analogue built from four subunits, shown in Figure 24. Of the fragments that were proposed (**25**–**28**), two were protected α-amino acids that are commercially available (**25** and **28**) and two are novel amino acid analogues that needed to be synthesised (**26** and **27**).

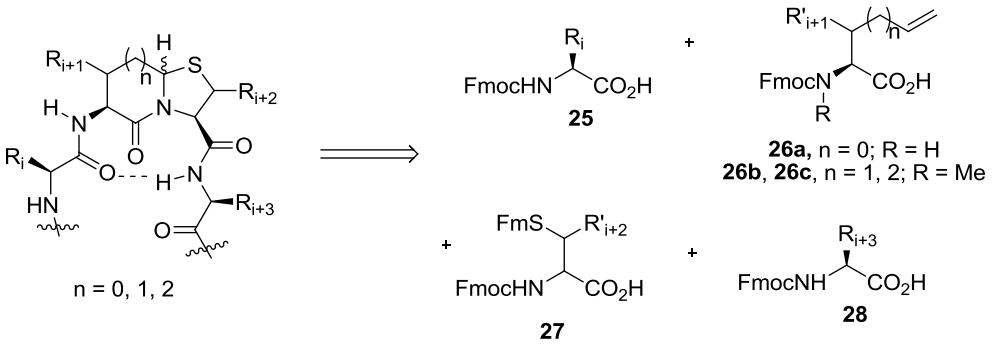
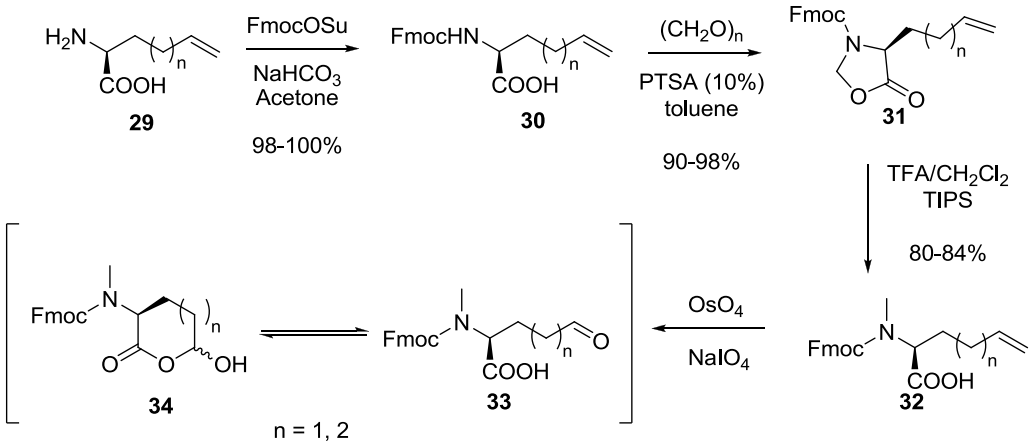


Figure 24: A Series of β-Turn Mimetics with Opportunities for Variation of Functionalisation and Ring Size Designed by Hruby and Co-workers

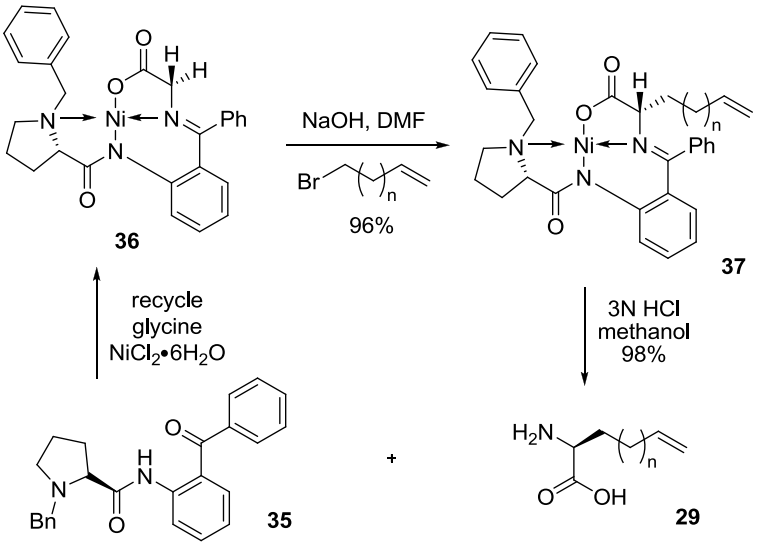
The goal of this research programme was to develop a range of different sized bicycles with the opportunity to incorporate various functional groups around the chain. This type of flexibility allows for a wide range of structures and functionalities to be studied for biological activity, particularly for the effects of varying side chain topography. By varying 'n' Hruby and co-workers were able to construct bicycles containing 5-, 6- and 7-membered rings. This allowed the group to synthesise a library of compounds with broad diversity for biological testing.

Scheme 3 illustrates the synthesis of analogues of fragments **26b** and **26c** in the form of lactone **34**, which was formed by spontaneous cyclisation of **33**. While this fragment is visually different from **26**, previous research by this group had shown that this type of lactone can act as a masked aldehyde and is electrophilic enough towards nitrogen and sulfur for thiazolidine formation, as required in the proposed synthesis.[64]



Scheme 3: Hruby Synthesis of Bicylic Dipeptide Turn Mimetic

Initially, the δ-,ε- unsaturated amino acid starting material **29** had to be synthesised for use in this section of the synthesis. This transformation was carried out using a nickel catalyst with glycine and the appropriate alkyl bromide as shown in Scheme 4,[65,66] and the reaction was optimised to give the alkylated product in overall 94 % yield.

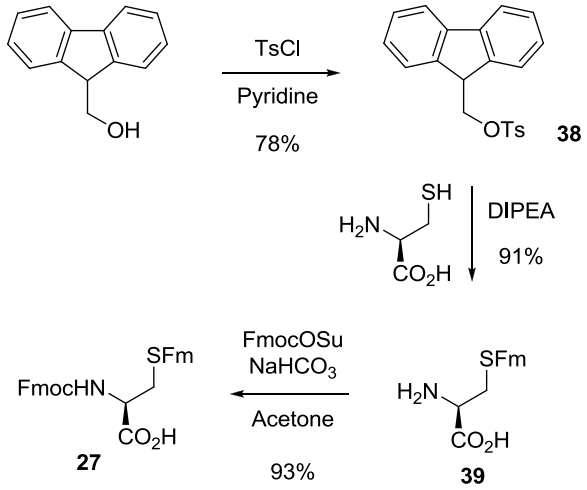


Scheme 4: Synthesis of δ,ε- Unsaturated Amino Acid Starting Material, 29

Once this material had been isolated, the amino group was protected by reaction with *N*-(9-fluoroenylmethoxycarbonyloxy)succinimide (FmocOSu). The reaction took one day or three days when reacting with the homoallylglycine and bishomoallylglycine respectively. Fmoc protected **30** was then cyclised to furnish the oxazolidinone **31**, by refluxing in the presence of paraformaldehyde. The heterocycle was cleaved under acidic conditions, affording **32**. Finally reaction with osmium tetroxide afforded aldehyde **33**, which spontaneously

cyclised to give **34**. This fragment was now ready to be coupled with the other fragments to synthesise the desired bicyclic turn mimetics.

The second novel amino acid fragment **27** was synthesised in a relatively straightforward manner (Scheme 5).[67] 9-Fluorenemethanol was treated with *p*-toluenesulfonyl chloride to give **38**. The OTs group was then displaced with sulphur from the cysteine side chain, giving the amino acid **39** in excellent yield. Finally the NH terminus of the amino acid **39** was protected using Fmoc, again in excellent yield, affording protected fragment **27**. It is important to note that in this synthesis, as well as in previously reported work from the same group concerning the formation of 5,5-bicycles, the R'_{i+1} and R'_{i+2} positions were both unfunctionalised. In these syntheses, only the R_i and R_{i+3} positions are open to modification by selection of an appropriate initial amino acid residue for reaction.



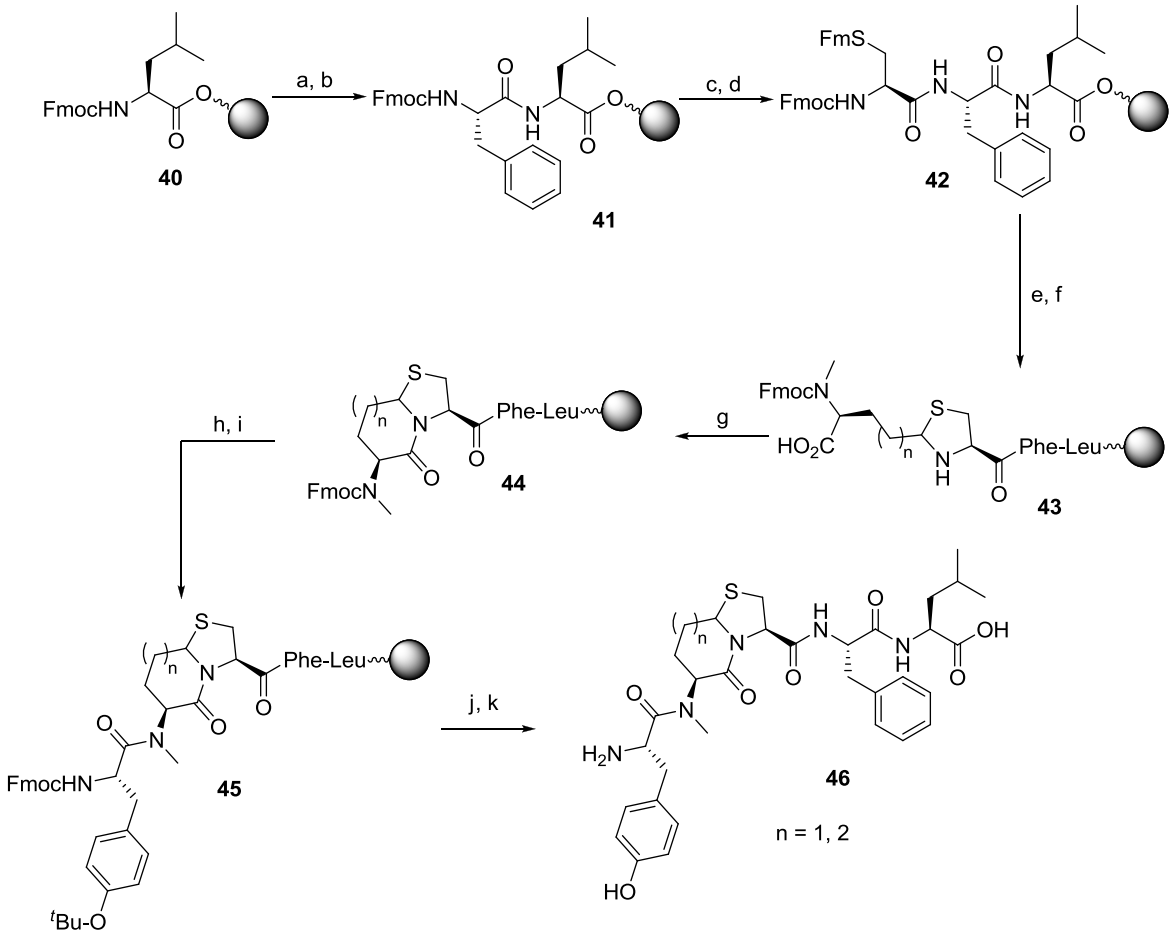
Scheme 5: Synthesis of Protected Cysteine Fragment

Following preparation of all four fragments necessary for mimetic synthesis, the target was prepared using solid phase synthesis. Fragment **34** was synthesised with two ring sizes, translating into the synthesis of both a 6,5 and a 7,5-bicyclic system.

This stage in the synthesis was performed on a solid support, beginning with a N^{α}-Fmoc-Leu-Wang resin **40** (Scheme 6). Removal of the Fmoc protecting group with piperidine in DMF was followed by a standard coupling of Fmoc protected phenylalanine, yielding dipeptide **41**. This was again deprotected and the resulting amine was subjected to further peptide coupling, this time with the Fmoc protected cysteine to give the tripeptide **42**. Simultaneous removal of Fmoc and Fm protecting groups was achieved using 50% piperidine in DMF. Coupling with the previously synthesised fragment **33**/**34** was performed using the

unpurified mixture. This afforded thiazolidine **43** as required, thus installing the 5-membered ring of the bicycle.

The lactam ring in **44** was easily constructed by reaction of carboxylic acid **43** with HBTU in just 1 hour. This procedure was equally effective for formation of both the 6- and 7-membered lactams. The Fmoc group was removed from the secondary amine before coupling with Fmoc protected tyrosine derivative giving lactams **45**, the penultimate compound in the route. The last two steps simply require removal of the final Fmoc group and cleavage of the mimetics from the resin using a mixture of TFA with H_2O and TIPS. This last stage also cleaved the phenolic *t*-butyl ether from the tyrosine residue and afforded both the [6,5] and [7,5] bicyclic mimetics **46**.



a) 25% piperidine, DMF, b) Fmoc-Phe-OH, HBTU, HOBt, DIPEA, c) 25% piperidine, DMF, d) Fmoc-Cys-S(Fm)-OH, HBTU, HOBt, DIPEA, DCM/DMF, e) 50% piperidine, 5% TIPS, DMF, f) **33**, DIPEA, DMF, g) HBTU, HOBt, DIPEA, h) 25% piperidine, DMF, i) Fmoc-Tyr-(O^{t}Bu)-OH, HBTU, HOBt, DIPEA, j) 25% piperidine, DMF, k) 90% TFA, 5% H_2O, 5% TIPS

Scheme 6: Completion of Hruby Synthesis

Hruby and co-workers used this general approach to synthesise various bicyclic mimetics possessing variation in the stereochemistry around the rings (Figure 25). These compounds were tested for binding affinity against δ- and μ-receptor sites by assessing the competition with [^{3}H] deltorphin II for the δ-sites and [^{3}H] DAMGO for μ-sites. Most of the analogues showed binding affinity for the δ-receptors and it was found that the stereochemistry of the bridgehead hydrogen had a limited effect on the outcome, most likely due to conformational flexibility of the systems. The L-, L- analogues with both ring sizes showed the greatest potency, however the D-, L- and L-, D- analogues also showed some activity.

It was also found that the 6,5-bicycles showed good selectivity for the δ-receptors, whilst not showing any binding affinity for the μ-receptors, This differed from the 7,5-bicycles, which showed far less selectivity, binding to both μ- and δ-receptors. This is most likely due to the greater conformational flexibility afforded by the larger ring size.

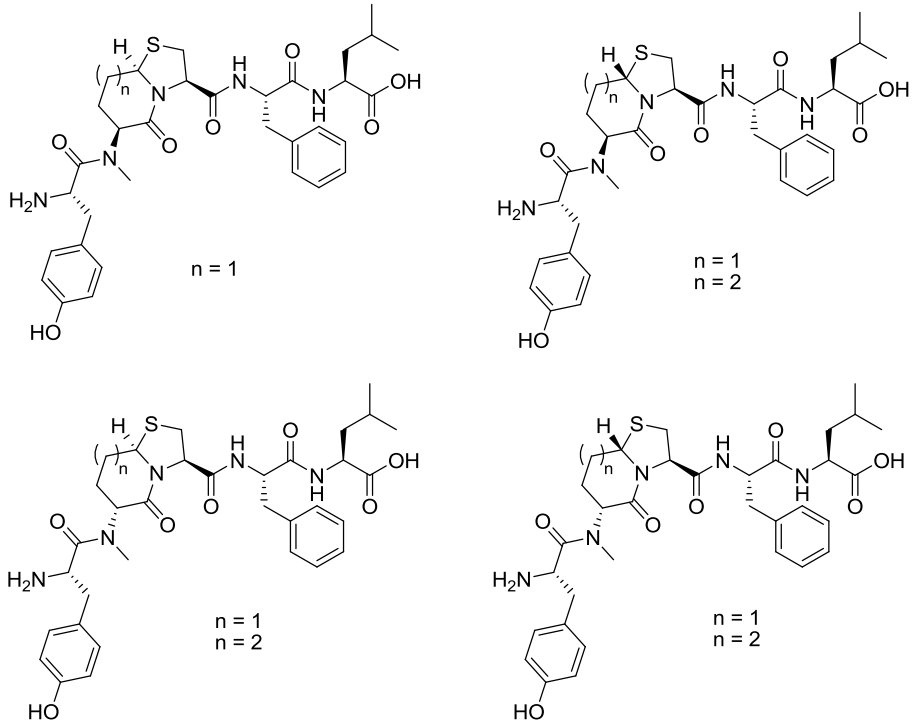


Figure 25: Various Turn Mimetics Utilising Different Stereochemistry Around the Bicyclic Core

Victor Hruby has carried out a great deal of research into the field of peptidomimetic structures. In this case, he was able to replace a section of the peptide chain of leu-enkephalin with bicyclic systems of varying ring size. This example highlights quite effectively the

benefits of a library-based approach to these compounds. Testing similar compounds with distinct structural differences can give new insights into important features for biological activity and contributes to a further understanding of peptide interactions at sites with high selectivity.

1.3.5 Jurczak's 2,5-Diketopiperazine Based Mimetics

In recent literature, 2,5-diketopiperazines (2,5-DKPs) have been pursued as interesting targets for incorporation into β-turn mimetic structures. 2,5-DKPs are found in many natural products, for example the *Asperigillus ustus* metabolite phenylahistin (**47**),[68] which exhibits promising antitumor activity, and the weakly cytotoxic tetracyclic compound roquefortine E (**48**),[69] isolated from *Penicillium rocquefortii* (Figure 26).

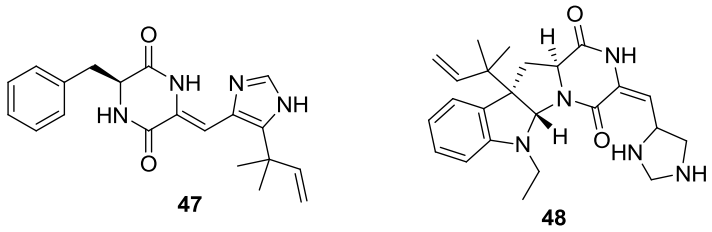


Figure 26: Naturally occurring 2,5-DKPs

However, beyond their significance as constituents of natural products, 2,5-DKP structures have potential applications as peptidomimetics. The 2,5-DKP structure possesses structural similarities to peptides, but without some of the limitations that are inherent in peptide systems such as poor sub-receptor stability and poor biostability. The replacement of biologically active peptide compounds with 2,5-DKP derivatives leads to improved resistance of the amide bond to metabolic cleavage and reduced conformational flexibility thus improving its ability to interact with macromolecules. Previous structures incorporating 2,5-DKPs have been synthesised during the drug discovery process and have shown diverse biological activities.[70-72] These structures are of potential interest in the continuous search for useful structural motifs to emulate the β-turn.

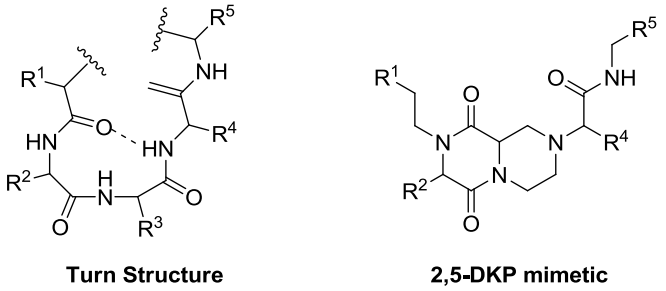


Figure 27: Bicyclic Mimetic Structure Utilising 2,5-DKP Unit

This type of β-turn mimetic was previously reported by Golebiowski and co-workers in 2000,[73-75] an example of which is shown in Figure 28. Despite the widespread interest in developing 2,5-DKP analogues for biological applications, only a few structures analogous to Golebiowski's proposed structure have been reported, most of which are derived from racemic substrates and include little or no spectroscopic data. The Jurczak group took it upon themselves to develop a library of mimetics of this type using a general approach and beginning from optically pure (*S*)-piperazine-2-carboxylic acid.[76]

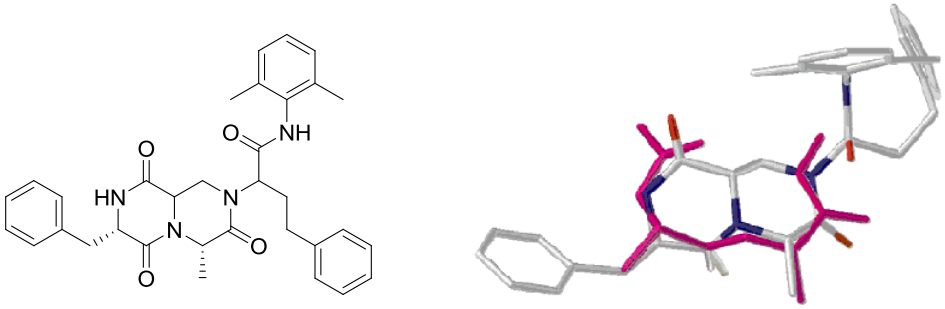
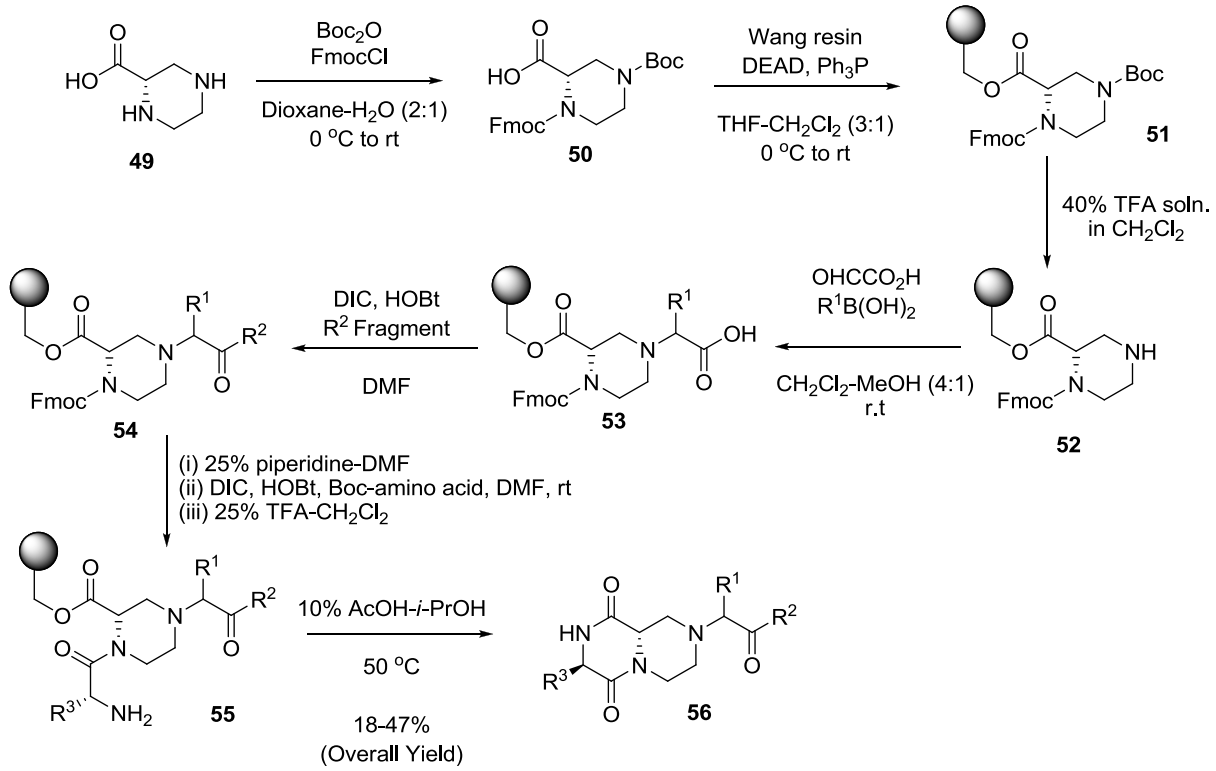


Figure 28: Golebiowski's 2,5-DKP Structure and X-Ray Structure Comparison With Type II β-turn Unit[77]

The library synthesis of 2,5-DKPs is shown in Scheme 7. The first step in the synthesis is the protection of both amines from the optically pure starting material, (*S*)-piperazine-2-carboxylic acid dihydrochloride. This is accomplished with orthogonal protecting groups in order to selectively deprotect one over the other in a subsequent step. In this case, the Boc and Fmoc groups were chosen to form the protected intermediate **50**. The carboxylic acid was then bound to the Wang resin under Mitsunobu conditions, resulting in formation of **51**. Treatment of **51** with a solution of TFA in CH_2Cl_2 cleaved the Boc protecting group, furnishing structure **52**. The next step involved a Petasis reaction with glyoxylic acid and an appropriate boronic

acid to give amine intermediates **53**. These intermediates were then coupled at the carboxylic acid to the appropriate primary or secondary amine coupling fragment using standard DIC/HOBt conditions to give functionalised compounds **54**. This was determined to be the best approach after testing several different coupling conditions.

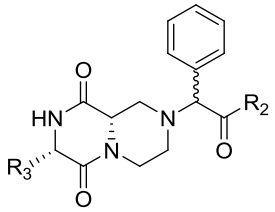
The next step was the removal of the Fmoc group, which was accomplished using a solution of piperidine in DMF. The free amines were then coupled with an appropriate Boc-protected α-amino acid, again using DIC/HOBt conditions. Treatment of the newly coupled intermediates with a solution of TFA removed the Boc group leading to formation of structures **55**. Finally the resin was removed using 10% acetic acid in isopropyl alcohol. Upon removal of the substrate from the resin, the final cyclisation reaction took place spontaneously resulting in the target functionalised bicyclic structures **56**.



Scheme 7: Solid Supported Synthesis of 2,5-DKP β-Turn Mimetic Library

Jurczak and co-workers synthesised a library of compounds with an overall yield ranging from 18% to 47% for the full synthetic sequence. The targets contain up to 3 stereogenic centres. The first is present in the optically pure starting material, the second is introduced by the Petasis reaction and the third is present after the coupling with an optically

pure amino acid to form **55**. As a result of the non-stereoselective addition of R^1, all of the final compounds were isolated as 1:1 mixtures of two diastereomers. Unfortunately, the research group was unable to separate the diastereomers using chromatographic methods, and as a result was only able to report the targets as mixtures. A selection of the synthesised structures is displayed in Figure 29.



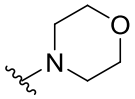
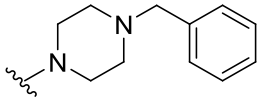
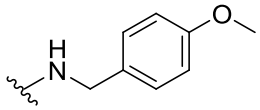
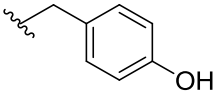
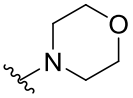
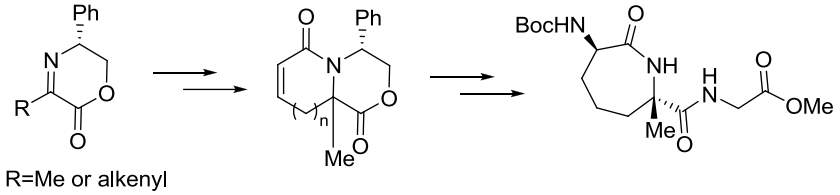
Compound Number	R_2	R_3
56a		H
56b		Me
56c		Ph
56d		

Figure 29: 4 Structures Synthesised Using Jurczak's Synthetic Pathway

Although it was not possible for Jurczak and co-workers to isolate single optically pure 2,5-DKP's for use in biological studies, this research does demonstrate a possible approach to the synthesis of similar, potentially useful, peptidomimetic structures. At the time of publication of this research, no biological studies had been performed and such studies have yet to be published. This research represents a potentially useful approach to the use of 2,5-DKP's as effective *β*-turn mimetics and holds promise for future beneficial results.

1.3.6 Aceña's Cyclic Peptidomimetics

Another example from recent literature demonstrates an approach to the synthesis of cyclic and bicyclic lactam rings of varying size.[78] This research involves the use of chiral imino lactones as starting materials: Scheme 8 shows a brief representation of the route by which Aceña and co-workers intended to prepare their targets.

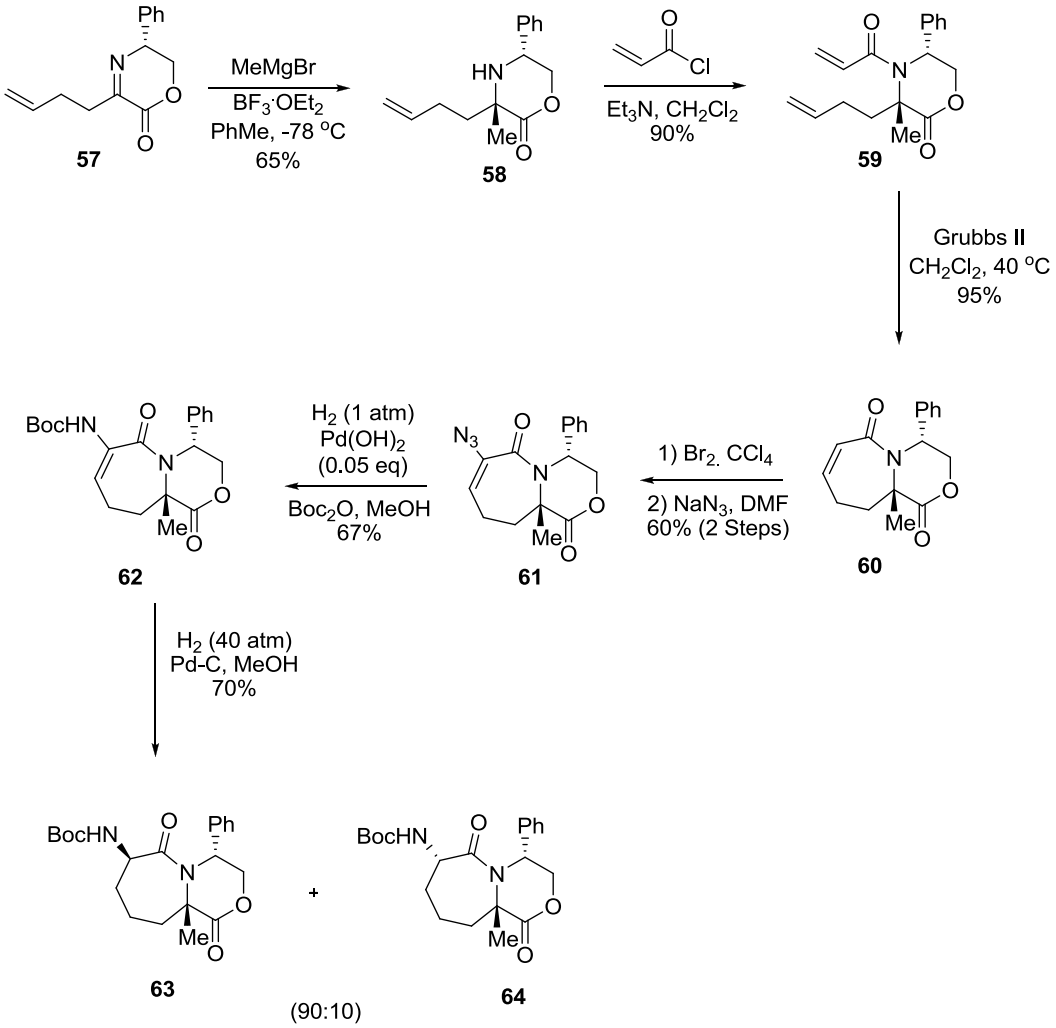


Scheme 8: General Forward Synthesis of Cyclic Mimetic

The initial bicyclic system was constructed using two similar protocols, both involving the application of ring-closing metathesis reactions of appropriately functionalised dienes, which were readily obtained from chiral imino lactones. A straightforward procedure for the synthesis of the starting chiral imino lactones is the condensation of the corresponding α-keto esters with (*R*)-phenyl glycinol.[79]

The first of the two syntheses involved the preparation of a 7,6-bicyclic system and started with a chemo- and stereoselective addition of Grignard reagent MeMgBr to imine **57** mediated by $BF_3 \cdot OEt_2$ to give **58** (Scheme 9). Compound **58** was isolated as a single isomer with the phenyl and methyl groups in a *trans* relationship. Reaction of **58** with acryloyl chloride then generated the diene **59** in 90% yield. Ring-closing metathesis was very successful, resulting in formation of the bicyclic compound **60**, containing a 7-membered ring in 95%.

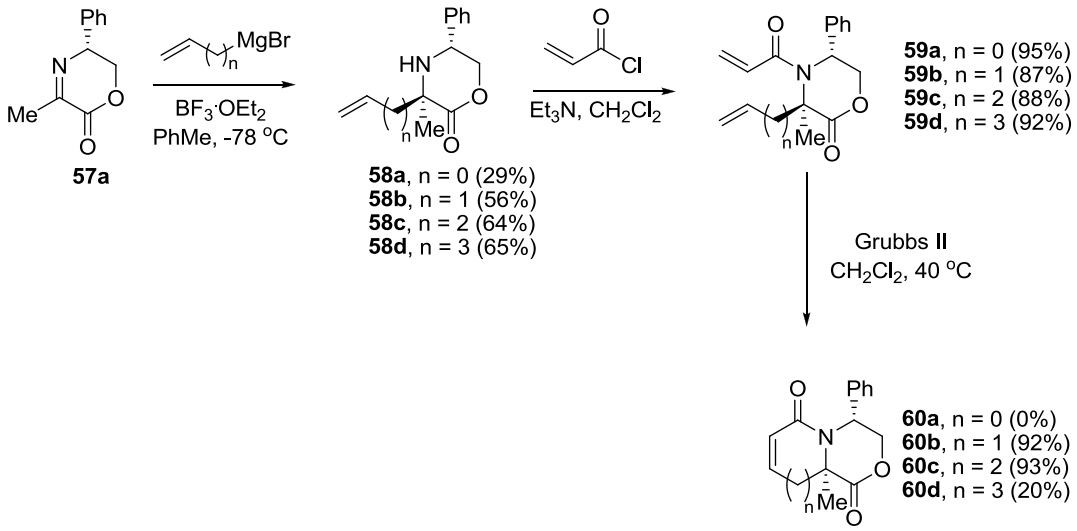
The next step was the functionalisation of the double bond in **60**, firstly by reaction with Br_2 creating a mixture of diastereomeric dibromides, which were then converted to the vinyl azide **61** using NaN_3, in an overall yield of 60%. Hydrogenation of the azide and subsequent protection of the resulting amine to give the carbamate **62** was swiftly followed by hydrogenation of the double bond to give a 90:10 mixture of lactams **63** and **64**. These diastereomeric compounds were easily separated *via* column chromatography



Scheme 9: Synthesis of Bicyclic Lactam

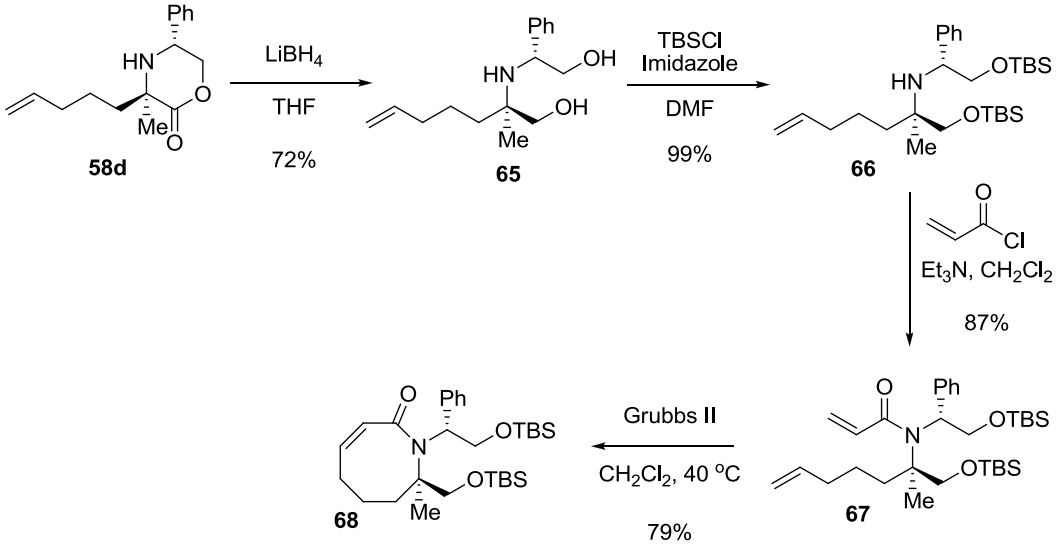
At this stage, Aceña and co-workers explored the development of a general method for the installation of rings of varying sized that would complement library synthesis techniques (Scheme 10). Imino lactones functionalised with vinyl or allyl groups similar to **57**, are not simple to prepare, which prevents them from being used to introduce 5-, or 6-membered rings into the target. Instead, Aceña decided to use a starting material, such as **57a**, in which the methyl group was in place prior to incorporation of rings of varying size *via* the use of vinyl-allyl-, but-3-enyl- and pent-4-enylmagnesium bromides. This afforded compounds **58a-d** with variable yields. Subsequently, these compounds were converted into acrylamides **59a-d** with variable yields, before performing the key ring-closing metathesis reaction using the second generation Grubbs catalyst. Unfortunately, this approach was only successful when applied to the preparation of compounds possessing 6- and 7-membered rings e.g. compounds **60b** and

60c respectively. There was no reaction when attempting to form a 5-membered ring (**60a**) and poor yields were obtained upon formation of an 8-membered ring (**60d**).



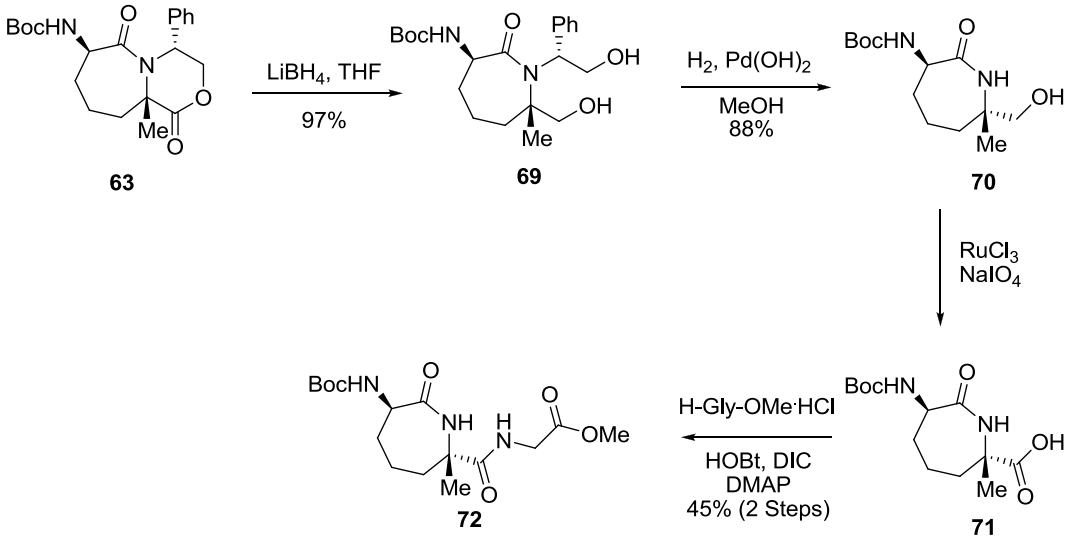
Scheme 10: General Approach to Fused Bicyclic Lactams of Varying Size

Alternatively, the same ring-closing metathesis was employed to prepare systems containing problematic ring sizes by first opening the lactone on **58d** and protecting the resulting diol **65** as a TBS ether (Scheme 11). The acrylamide **67** was then synthesised and underwent a ring closing metathesis reaction to give the desired 8-membered ring **68** in good yield. It was anticipated that this method would relieve any element of constraint that might be retarding the cyclisation reaction, but in the case of the 5-membered ring this approach was not successful and none of the desired RCM product was obtained.



Scheme 11: Alternative Approach to 8-Membered Ring

Having achieved a reasonably general approach to the synthesis of systems containing various ring sizes, Aceña and co-workers focused their efforts on completing the synthesis for the previously synthesised bicycle **63**. This ring system was subjected to ester reduction to give the diol **69**. Hydrogenolysis yielded lactam **70**, and this was followed by reoxidation to give the carboxylic acid **71**. The final step required installation of a new amino acid residue using HOBt and DIC with glycine methyl ester hydrochloride to give tripeptide mimetic **72**. This is as far as this recently published research extends, but the cyclic structure that has been developed has good prospects for further functionalisation so that it can serve as the central segment in a *β*-turn mimetic.



Scheme 12: Final Synthesis of *β*-Turn Mimetic

Indeed, the synthesis of lactam **71** is in itself potentially useful because both ends of the structure can be easily functionalised with a variety of amino acid residues to create an extended peptide mimetic. Having effectively established a method to synthesise structures containing 6-, 7- and 8-membered rings these various rings can be carried through the same synthetic pathway as 7,6-bicycle **63** to create systems with varying ring sizes and, importantly, different enantiomers. Compounds **60b, 60c** and **68** are equivalent to compound **60** in Scheme 9 and ideally they could be used to create a small library of *β*-turn mimetics. As of their last report, Aceña and co-workers were continuing to investigate this series of targets. This work is being performed to establish how the conformational features of the peptidomimetics relate in three dimensional space when compared to *β*-turns of various types and also to explore the viability of the synthetic pathway.

1.3.7 Boger's *β*-Turn Mimetic Library

In the past decade, Boger and co-workers have explored the synthesis of libraries of small molecule mimetic structures. On the premise that the majority of protein-protein and peptide-receptor interactions are mediated by three main recognition motifs (*α*-helix, *β*-turn and *β*-strand), they have focused their efforts on small molecules that reflect these motifs. This research has utilised simple solution-phase protocols that complement more conventional solid-phase techniques. The group is tackling the three motifs sequentially and has already published an extensive library of 8000 *α*-helical mimetics.[80]

In subsequent work, Boger and co-workers began their studies into the *β*-turn structure.[33] Initially, they calculated the mean distances between the *α*-carbon centres in a series of over 10,000 *β*-turns that are reported in the protein databank. Furthermore, they recognised that one of the amino acid residues in a *β*-turn often performed a structural rather than recognition role. This was often a proline or a glycine residue at either the *i*+1 or the *i*+2 position. Consequently, it was decided that Cα_{i+1} or Cα_{i+2} functionality could be omitted safely from the mimetic structures. Based on their analysis, and in order to achieve similar geometry for the extended functionality leading away from the turn, *trans*-pyrrolidine-3,4-dicarboxamide was proposed as a synthetically accessible template for the construction of *β*-turn mimetic structures (Figure 30). This scaffold has the further feature of being rigidified by a stabilising H-bond between two of the substituted side chains.

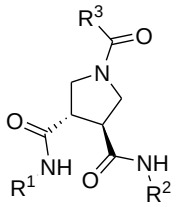
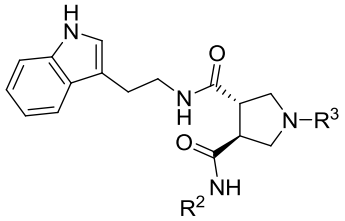


Figure 30: *trans*-Pyrrolidine-3,4-dicarboxamide Mimetic Template

Before proceeding with full synthesis of this library of compounds, Boger and co-workers performed some exploratory studies on a small group of pyrrolidine-3,4-dicarboximide derivatives. These structures were tested against *μ*-, *δ*- and *κ*-opioid receptors for their binding affinity and three structures were found to be very effective binders (Figure 31).



73 R^2 = Phe, R^3 = CO(HoTyr)
74 R^2 = HoPhe, R^3 = CO(Tyr)
75 R^2 = Phe, R^3 = CH_2(Tyr)

Figure 31: Most Effective Test Analogues

The two most effective ligands for μ-receptor binding were **73** and **74**. Compound **75** was somewhat less effective for μ-binding (Table 2). These structures have the distinction of sharing their combination of side chains with the endogenous endorphin structures. Furthermore, it was realised that the best affinities for μ-receptor binding were obtained when single carbon extensions were introduced into certain side chains. In this case, this means using homophenylalanine (HoPhe) and homotyrosine (HoTyr). This concept was employed into the library synthesis approach adopted by the group later.

Table 2: Binding Affinities of Mimetic Structures 73, 74 and 75

Compound	**μ binding affinity K_i (nM)**	**κ binding affinity K_i (nM)**
73	930	390
74	820	420
75	2,700	23

Interestingly, all of these compounds exhibited a much higher binding affinity for the κ-receptors, particularly in the case of compound **75**. The origin of this affinity is as yet undefined, but the binding data suggests that some intrinsic properties to the *trans*-pyrrolidine-3,4-dicarboxamide template favour κ binding in particular. Despite this unexpected finding, the high affinity of compounds **73** and **74** suggest that the template can effectively mimic the active turn conformation of the endomorphins. Having confirmed the viability of these structures, and revealing some useful structural details, work progressed towards the synthesis of a library of 4200 analogous compounds with various combinations of 20 side chains, all of which are illustrated alongside their appropriate labels in Figure 32.

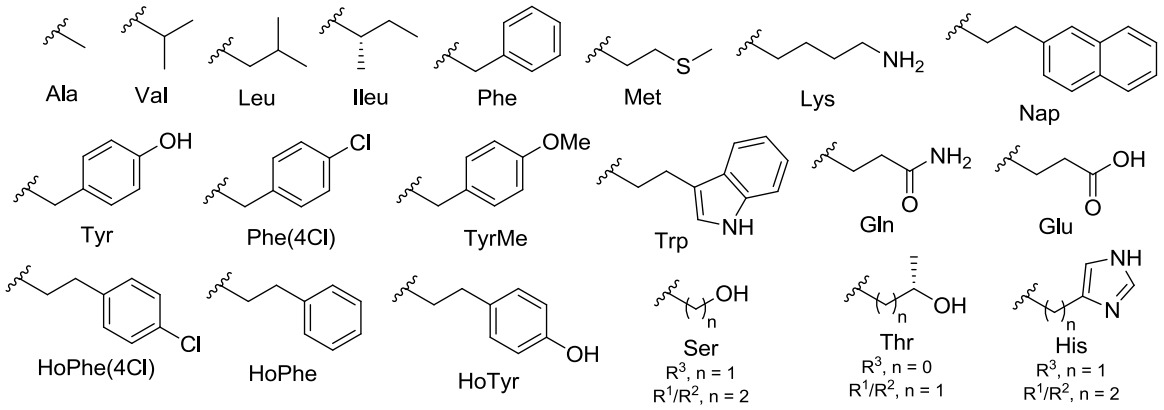
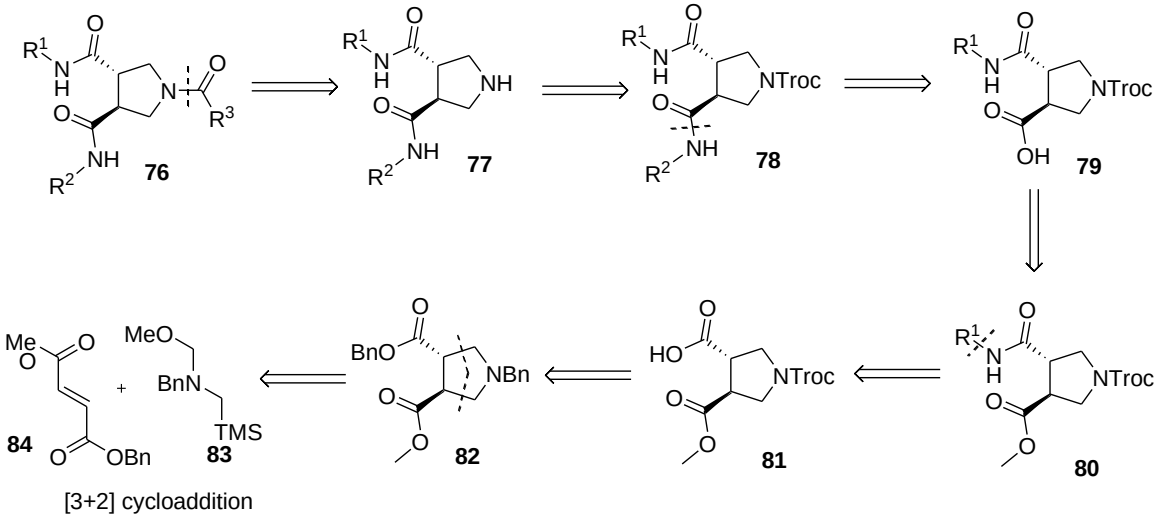


Figure 32: 20 Side Chain Functionalities Used in Library

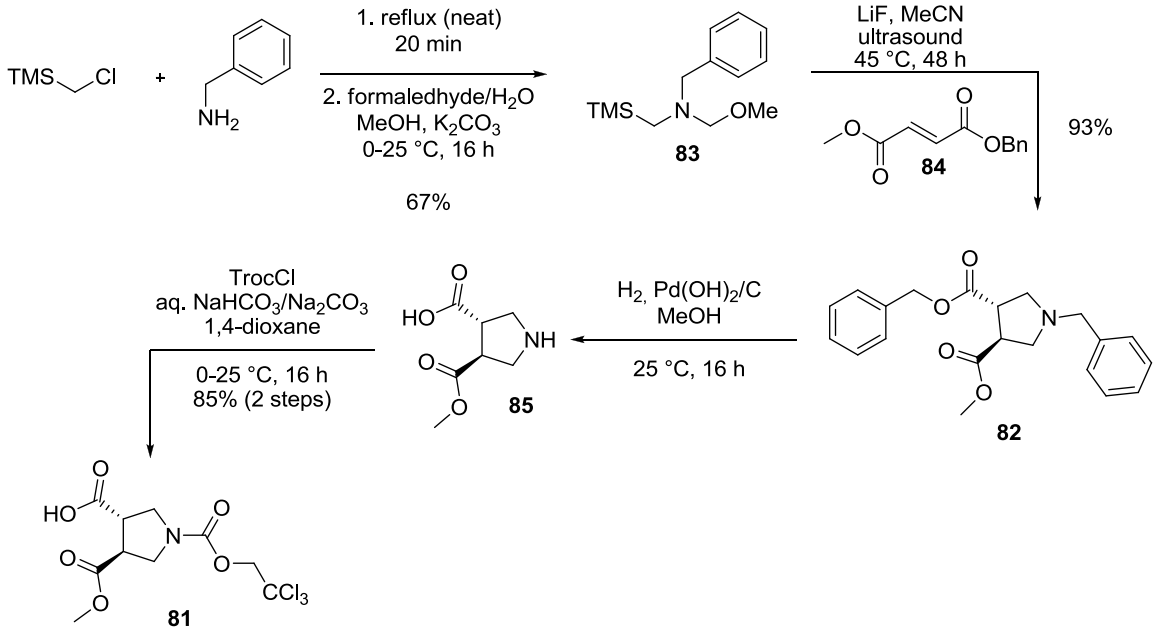
By exploring each possible combination of the 20 residues around the 3 functional positions on the template, a library of 4200 compounds can be established. Due to the perceived degeneracy of incorporating side chains of asparagine and aspartic acid compared to glutamine and glutamic acid, it was decided only to include Glu and Gln in the library. For this same reason, only lysine was incorporated in the library where arginine was omitted. Cysteine was dismissed due to anticipated stability and storage issues and finally glycine and proline were not used due to their proposed minor role in recognition events. The six natural amino acids that were removed from the library were replaced by various unnatural side chains with a proven ability to bind in protein – protein and protein – peptide interactions. These were *O*-methyl tyrosine (TyrMe), naphthyl (Nap) and 4-chlorophenylalanine [Phe(4Cl)] as well as the aforementioned one carbon extension residues, namely homophenylalanine (HoPhe), homotyrosine (HoTyr) and 4-chlorohomophenylalanine [HoPhe(4Cl)]. The 20 residues were incorporated into the library in their terminal amine form at the R^1 and R^2 positions and in their terminal carboxylic acid form at the R^3 position.

The proposed retrosynthesis of the library is shown in Scheme 13. It was suggested that the 4200 target compounds could be reached by acylation of the pyrrolidine nitrogen in compound **77** using the carboxylic acids of the 20 selected side chains. The 210 compounds that comprise **77** were derived from the Troc-protected precursor **78**. The second side chain was then removed to give carboxylic acid **79**, which was obtained from methyl ester **80**. The final side chain was removed to give another carboxylic acid precursor **81**, which was derived from the benzyl functionalised compound **82**. Finally, **82** was prepared *via* a [3+2] dipolar cycloaddition leading to starting materials **83** and **84**, which are both synthetically accessible.



Scheme 13: Retrosynthetic Analysis of Mimetic Library

With a reasonable retrosynthesis in place, Boger and co-workers were confident to begin work on the forward synthesis. The first step involved the reaction of benzylamine with chloromethyltrimethylsilane to yield *N*-benzyl-*N*-(trimethylsilyl)methylamine (Scheme 14). This was then treated with formaldehyde under basic conditions to produce compound **83**. This material was utilised in a [3+2] cycloaddition reaction with benzyl methyl fumarate **84** to furnish substituted heterocycle **82**.[81] The benzyl groups were removed by hydrogenolysis catalysed by $Pd(OH)_2/C$ and the resulting free amine was then Troc-protected by reaction with 2,2,2-trichloroethyl chloroformate to give core template **81** for subsequent functionalisation.

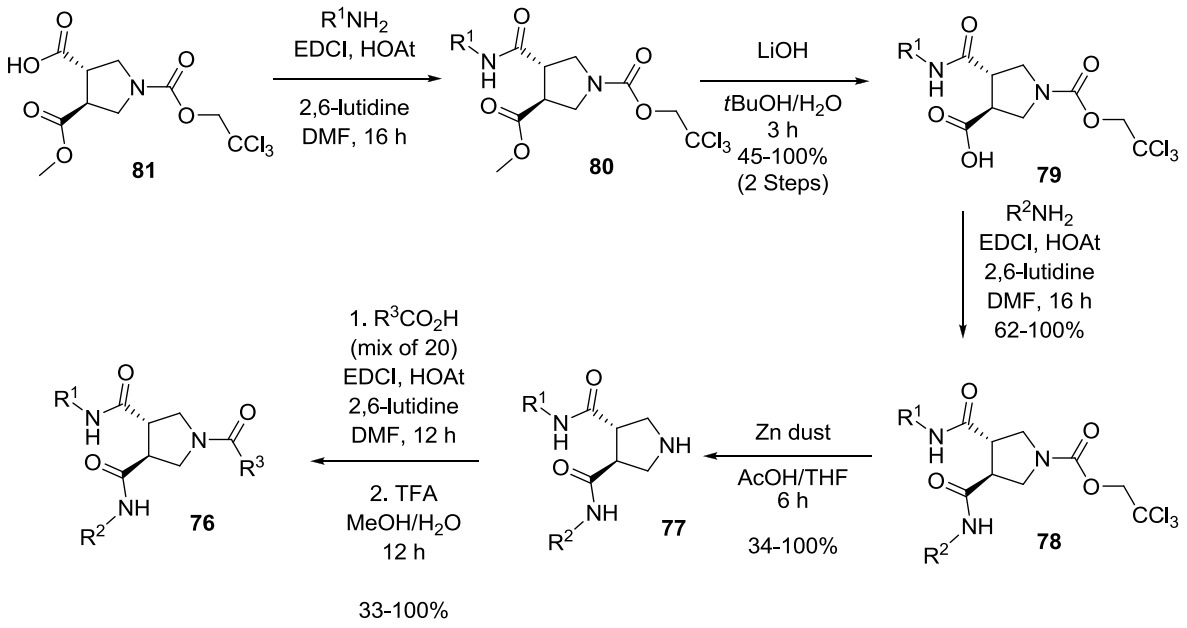


Scheme 14: Synthesis of Core Fragment 81

With this structure in hand, synthetic efforts diversified to include the various side chain groups that had been selected earlier (Scheme 15). The first peptide coupling was carried out to install R^1 using EDCI/HOAt-mediated coupling conditions. This coupling protocol was successful in all cases, giving the 20 distinct amide compounds, **80** in variable yield. The next stage involved removal of the methyl ester in preparation for the subsequent peptide coupling reaction. This transformation was accomplished by treatment of the ester with LiOH giving carboxylic acids **79**.

The EDCI/HOAt conditions were also utilised in the second peptide coupling to form a group of 210 *trans*-pyrrolidine-3,4-dicarboxamides (**78**). Purification was performed at this stage using standard acid-base extraction techniques to remove unwanted starting material, byproducts and reagents. The Troc protecting group was removed using activated zinc nanopowder in a 2:1 mixture of THF/AcOH[82] to yield secondary amine **77**. The material obtained at this stage was purified by filtration through a pad of Celite® to remove traces of zinc, followed by concentration *in vacuo* with a toluene azeotrope. Traces of acetic acid were removed by dissolving the residue in methanol and passing it through a short column of basic silica gel. These steps ensured the isolation of high purity amines. Aqueous base extraction was unsuitable for use at this stage because there was low product recovery in some instances due to aqueous solubility of the product.

The final peptide coupling was performed by reaction of an excess of the free amines **77** with equimolar mixtures of the 20 carboxylic acids containing the R^3 side-chains. The EDCI/HOAt coupling conditions were used once again and excess base was removed at the end of the reaction by extraction with aqueous acid. Global deprotection was carried out using a 3:1:1 mixture of TFA/MeOH/H_2O. This removed Boc, *tert*-butyl ester, TIPS and trityl groups within 12-16 h to give 210 mixtures of 20 functionalised *trans*-pyrrolidine-3,4-dicarboxamides (**76**). Time consuming chromatographic techniques were avoided in the latter stages of the synthesis, which is extremely useful when synthesising such a large number of different compounds.



Scheme 15: Library Synthesis of 4200 Mimetic Structures

Boger and co-workers synthesised the final targets as mixtures in order to allow identification of the most potent compound families in opioid receptor binding studies. Once the most effective families had been identified, the individual compounds from each family could be isolated, thus avoiding the unnecessary purification of thousands of individual compounds. The end result of the synthetic scheme described above was 210 mixtures of 20 compounds, with different R^3 functionality. The successful coupling of **77** to the mixture of carboxylic acids was confirmed using mass spectrometry techniques to analyse a selection of 20 of the mixtures. Furthermore, the quality of the mixtures was demonstrated by HPLC analysis of one of the mixture samples. A control sample of this mixture was produced by individual synthesis of the 20 compounds and equimolar mixing of the products. Although separation of all 20 compounds is not possible by HPLC, both the control and the originally synthesised compound mixture were found to have nearly identical detection profiles. This confirms, not only that the 20 compounds are in the mixture, but also that they are present in approximately equimolar amounts.

Following the preparation of 210 final mixtures it was possible for Boger and co-workers to undertake screening to determine activity against multiple opioid receptors. The mixtures were screened at 10 μM concentration (0.5 μM per compound) in radioligand binding studies with cloned human receptors. The compounds showed improved inhibition of κ-receptors over μ-receptors and least inhibition of δ-receptors. The initial screening of

mixtures revealed some additional important data. It was apparent that there was a strong correlation between the presence of a hydrophobic aromatic side chain and the binding affinity of the substrate. The Phe(4Cl), Trp and Nap showed strong inhibition of the κ-receptors, where HoTyr, Trp and HoPhe(4Cl) showed strong inhibition of the μ-receptors. One exception to this finding was the potent inhibition observed with the presence of a basic His side chain, when combined with a bulky aliphatic side chain such as Val or Leu.

Based on these findings, three compound mixtures were chosen for deconvolution so that individual binding studies could be performed whilst varying each of the substituents. The mixtures, and their initial results, are shown in Table 3, alongside structural representations of the mimetic and the chosen side chains. The mixture with Trp/Phe at R^1/R^2 was chosen due to its close resemblance to the endomorphin-1 sequence. This sequence also contains compound **73**, which was used in the initial studies carried out before library synthesis. The Trp/Phe(4Cl) mixture is structurally similar to the Trp/Phe mixture with the addition of a 4-Cl substituent. This appeared to enhance the binding affinity for both μ- and κ-receptors, hopefully meaning that some potent compounds can be found in this mixture. The final mixture that was chosen included Phe(4Cl)/Phe(4Cl) at R^1/R^2. This mixture showed the highest affinity for κ-receptors and also the greatest difference between the affinity for κ-receptors and μ-receptors. These mixtures were deconvoluted by resynthesising separate constituents from precursor **77**, giving 60 distinct compounds for screening.

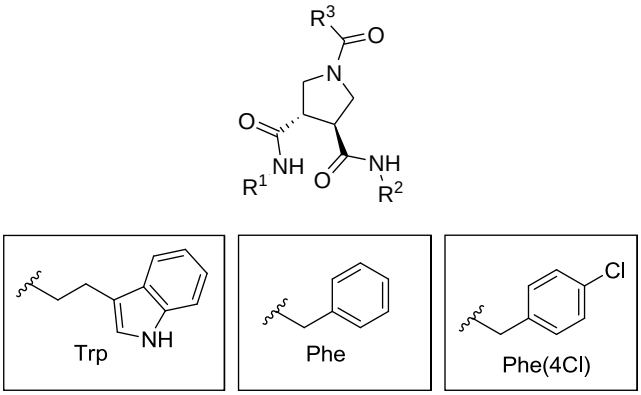


Table 3: Families of Compounds Selected for Deconvolution

R^1/R^2 Sidechains	**% Inhibition**		
	κ-receptors	**μ-receptors**	**δ-receptors**
Trp/Phe	73	56	25
Trp/Phe(4Cl)	86	66	26
Phe(4Cl)/Phe(4Cl)	91	48	18

The 60 compounds were screened at 10 μM concentration for activity at μ- and κ-receptor sites, again using radioligand binding assays. Focusing initially on the μ-receptor selectivity, it was expected that Tyr and HoTyr would be the most effective side chains at the R^3 position based on the proposed mimicry of the endomorphin-1 peptides. As a brief reminder, these two side chains are illustrated in Figure 33.

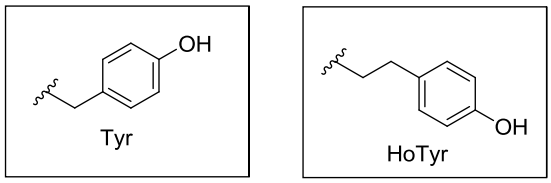
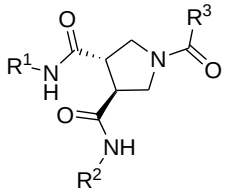


Figure 33: Most Effective R^3 Side Chains

The most effective combinations of the three side chains are clearly illustrated in Figure 34. The results were encouraging and they confirmed the effectiveness of the Tyr and HoTyr groups at R^3. This suggests that the presence of the free phenol can play a role in recognition of the structure at μ-receptor sites It was found that the most effective combinations were with HoTyr at the R^3 position when Trp/Phe and Trp/Phe(4Cl) were located at R^1/R^2 (structures **73** and **86** respectively) and with Tyr at R^3 with Phe(4Cl)/Phe(4Cl) at R^1/R^2 (structure **87**). Substrates possessing other aromatic side chains at R^3 were found to be consistently less effective, with aliphatic or charged side chains exhibiting even lower activity.

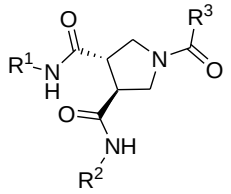


73 R^1 = Trp, R^2 = Phe, R^3 = HoTyr
86 R^1 = Trp, R^2 = Phe(4Cl), R^3 = HoTyr
87 R^1 = Phe(4Cl), R^2 = Phe(4Cl), R^3 = Tyr

Figure 34: Structures with Highest Activity Towards μ-Receptors from Each R^1/R^1 Family

Screening of all 60 of the compounds against all three receptor types clearly showed that this type of structure shows a significantly higher affinity for κ-receptors. Of the three series that were deconvoluted for more detailed examination it was found that in general they followed a trend of activity; Phe(4Cl)/Phe(4Cl) > Trp/Phe(4Cl) > Trp/Phe. The most active compounds from the two most active series are shown in Figure 35. Interestingly, the most active compound **88** not only exhibited high activity, it also had high selectivity for κ-receptors and was >100-fold more selective in comparison to the δ- and μ-receptors. This

result also highlights a difference in interaction between the κ- and μ-receptors as the free phenol functional group is clearly not essential for binding to the κ-receptors as it is for the μ-receptors. The synthesis of this ambitious library of compounds had successfully led to identification of several opioid active structures with potential for future study.



86 R^1 = Trp, R^2 = Phe(4Cl), R^3 = HoTyr
88 R^1 = Phe(4Cl), R^2 = Phe(4Cl), R^3 = Phe(4Cl)

Figure 35: Structures Exhibiting Highest Activity at κ-Receptor Sites

1.3.8 Summary

This selective overview of recent research into β-turn mimetics serves to highlight the diversity that can be incorporated into these structures. Considering how diverse an area heterocyclic chemistry is, there are virtually endless avenues for exploring cyclic and bicylic structures as a means of stabilising a turn conformation. The field of peptidomimetics has been of interest to researchers for many years now and a wide array of structures has been developed by various groups internationally. The examples presented here are merely a selection of those that have been published within the last decade. Efforts are still ongoing, with mimetic structures being published in the literature on a regular basis.[83,84]

The impressive biological activity of many of these mimetic structures demonstrates the utility of this approach. At a time when there is a need to develop novel drugs and medicinal treatments, the design, synthesis and application of peptidomimetics is a potentially important area that can not be ignored. In the examples selected above, various cyclic cores have been used to tether the peptide chain to enforce a turn and there is a common theme of trying to develop generic library approaches for the preparation of these structures. The ability to access large numbers of similar compounds quickly is an important aspect in this chemistry and the work of Boger and co-workers is certainly one of the most ambitious attempts to adopt this approach.

The effort to understand proteins and peptides and their interactions in biological systems is an ongoing endeavour across several scientific disciplines. Research in this area lies at the interface between biology, chemistry and peptide science and has shown great promise

already. In coming years, this field will continue to grow and new insights and understanding will be gained, leading to new drugs, vaccines and medicines.

The following sections introduce some of the chemistry that has been developed in the synthesis of ynamides. This type of functional group is of particular importance in this project.

1.4 Ynamides and their Synthesis

1.4.1 Introduction

Hetero-substituted alkynes have become important intermediates in the development of new synthetic methodology. One of the most prominent classes of heteroatom substituted alkynes is that in which an amino group is located on one terminus of the triple bond.[85] Ynamines in particular have been valuable in synthetic chemistry since a practical synthesis of them was developed in the 1960s. The first recorded attempt to synthesise an ynamine was by Bode in 1892,[86] but this was unsuccessful. It was not until 1958[87] that Zaugg and co-workers isolated the first ynamine and a subsequent synthesis was reported by Wolf and Kowitz in 1960.[88] However, it was not until 1963 that a practically accessible synthesis of ynamines was reported by Viehe and co-workers (Figure 36).[89] In the decades that followed this discovery, ynamines were of significant interest as intermediates and found applications in organic and organometallic chemistry.[90]

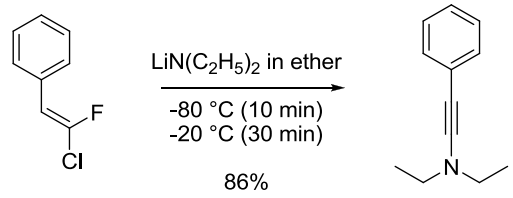


Figure 36: Viehe's Practically Accessible Ynamine Synthesis

Ynamines are of interest to the synthetic community because they exhibit high reactivity and are predictably regioselective in their transformations. Figure 37 illustrates clearly how the nitrogen heteroatom at one end of the alkyne imposes an electronic bias on the triple bond so that any reaction that takes place is dictated by the nucleophilic and electrophilic substituents involved.

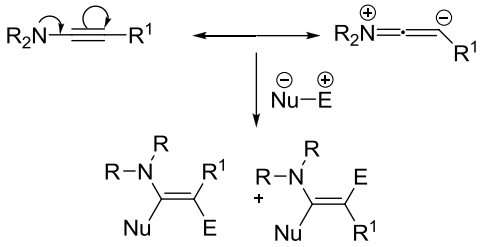
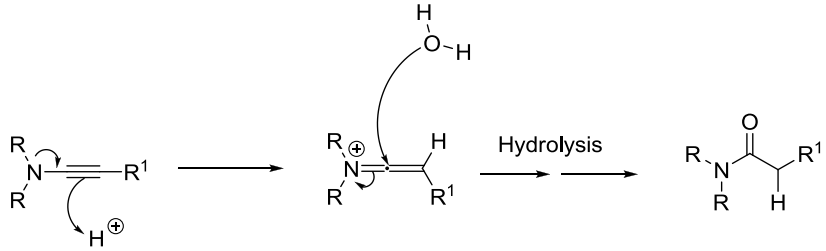


Figure 37: Electronic Bias of Ynamines in Synthetic Reactions

Unfortunately, the high reactivity of ynamines is also a major drawback because it means these compounds are sensitive towards hydrolysis, leading to difficulties in synthesis and handling. For this reason the use of ynamines in organic synthesis has been somewhat limited. Protonation of the alkyne derivative leads to the formation of a keteniminium intermediate, which is quickly trapped by water leading to the formation of an amide as demonstrated in Scheme 16. The synthetic uses of ynamines have been explored thoroughly since their initial synthesis; but they are often regarded as a scientific curiosity by organic chemists due to their high reactivity and difficulties handling them. As a consequence, any potential for synthetic use has declined in recent decades.



Scheme16: Hydrolysis of Reactive Ynamines

Logically, a possible method to stabilise ynamines and make them more synthetically applicable is to reduce electron density by introducing an electronegative substituent onto the nitrogen or the alkyne. There have been many attempts to explore these electron-deficient ynamine systems with some innovative approaches. Figure 38 shows some structures that have been synthesised to this end, utilising various aromatic substituents for example. There have also been investigations into the synthesis of push-pull ynamine systems in which an electron-withdrawing group is positioned at the opposite side of the alkyne, such as those reported by Ishihara[91] and Stang.[92]

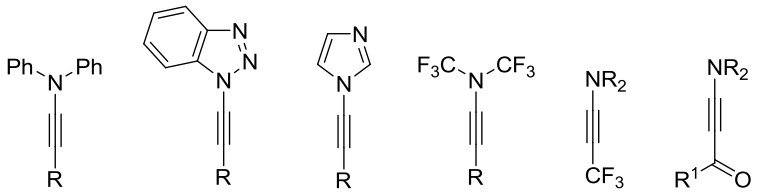


Figure 38: Electron-Deficient Ynamines

Despite the many solutions to the ynamine reactivity problem, one class of compound has emerged as the ideal alternative in the last 15 years. By simple introduction of an electron withdrawing carbonyl group onto the nitrogen, thus converting the amine into an amide or carbamate, the ability of the nitrogen to donate electrons into the alkyne is dramatically curtailed, due to resonance delocalisation into the carbonyl oxygen (Figure 39).

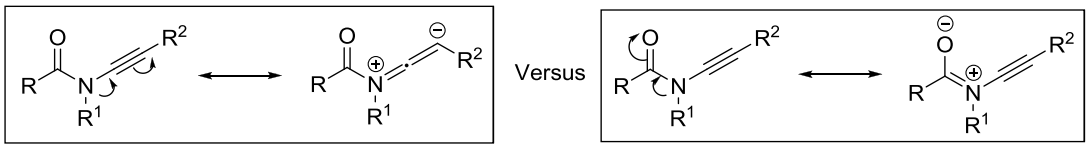


Figure 39: Carbonyl Versus Alkyne Electron Withdrawal

These ynamides offer the perfect balance between reactivity and stability that ynamines cannot provide.[93] The synthesis of the first ynamide was reported by Viehe in 1972,[94] the structure of which is displayed in Figure 40. Despite the apparent synthetic utility of ynamides, there were very few reports of ynamide synthesis over the 25 years that followed Viehe's synthesis, with some literature articles appearing only in the late 1990s.

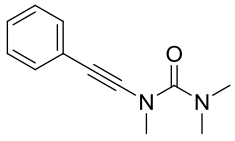


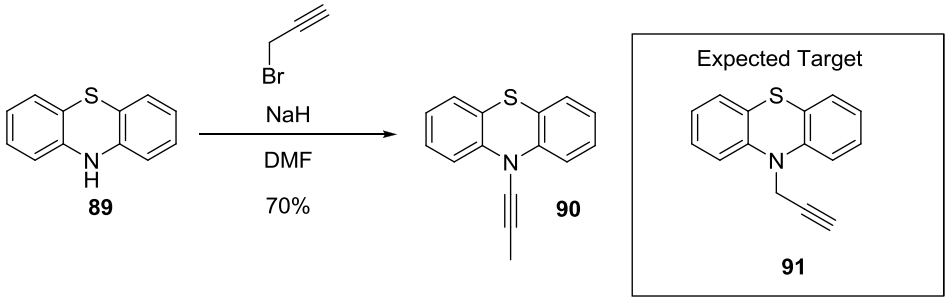
Figure 40: Structure of Viehe's Ynamide, 1972[94]

While other heteroatom bonded alkynes are valuable intermediates, nitrogen bonded alkynes are particularly important for several reasons.[93] The trivalent nature of nitrogen allows for the tethering of a chirality-inducing unit to allow asymmetric induction in subsequent transformations. It also allows for the inclusion of the coordinating unit, which leads to conformational rigidity. There is a great deal more flexibility when designing intramolecular reactions than when using oxygen or sulfur substituted alkynes.

In the past 10 years interest in ynamides has increased rapidly with numerous groups publishing methods for their synthesis and application in synthetic chemistry. The field of ynamine chemistry has essentially been revived in the form of ynamide chemistry. As work progresses towards truly general synthetic methods with broad applicability, the synthetic utility of ynamides will only increase. The following sections will give some insight into the various methods available to access ynamides and the degree to which they are useful as intermediates in organic synthesis. For generality, nitrogen bonded alkynes containing other types of amide functionality, such as carbamates or sulfonamides, will be referred to under the broad term of ynamides.

1.4.2 Isomerisation Methods

The synthesis of ynamides by isomerisation is of limited scope and applicability, but is still appropriate in certain circumstances. Only a handful of successful isomerisation reactions have been used for the preparation of ynamides. The method was originally used to synthesise an ynamine in 1958 as reported by Zaugg (Scheme 17).[87]



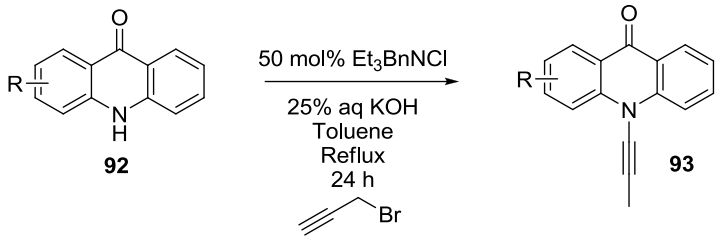
Scheme 17: Zaugg's "Unusual Reaction of Propargyl Bromide"

In this case, Zaugg and co-workers were trying to alkylate phenothiazine using propargyl bromide to give *N*-(2-propynyl)phenothiazine **91** but the unexpected ynamide isomer *N*-(1-propynyl)phenothiazine **90** was obtained instead. The *N*-alkylation reaction took place successfully but the resulting terminal alkyne was isomerised *in situ* to give the ynamine instead of the propargylic amine.

This approach was used by Galy[95,96] and Katritzky[97], some 20 years later, in order to access aromatically functionalised ynamines. Galy and co-workers reported the propargylation of acridones **92** using phase transfer conditions (Scheme 18). These workers were investigating the preparation of functionalised acridanone derivatives because the compounds are known to possess therapeutic properties. The resulting terminal alkyne was isomerised *in*

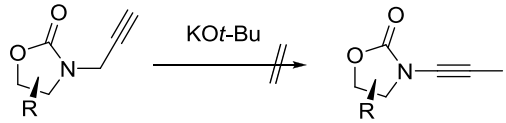
situ to yield the target ynamide **93** in yields ranging from 55-80%. Interestingly, Galy also reported that when only a 12.5% aqueous solution of KOH was used over a shorter reaction time, the corresponding allenamide was isolated instead.[96]

Katritzky and co-workers reported the use of the reaction to prepare ynamine **93** from amine **92** in a two-step process. The procedure involved propargylation of **92** by treatment with NaH and propargyl bromide. The resulting alkyne was isolated and isomerised to give ynamine **93** using KOH.



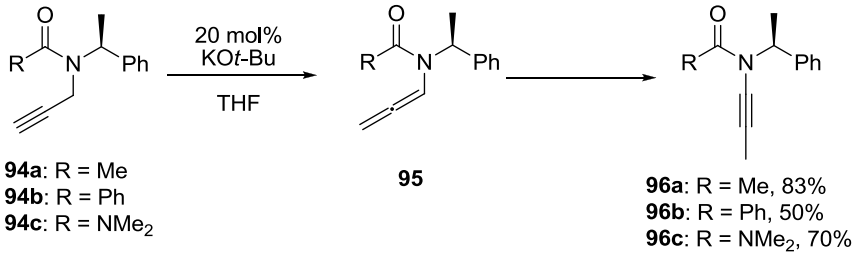
Scheme 18: Galy's Reported *N*-Alkylation/Isomerisation Sequence

Despite early success in using this approach to synthesise ynamines, it was not applied to ynamide synthesis until 2001.[98] During investigations into development of a valid route to ynamides, Hsung and co-workers attempted to synthesise chiral ynamides but with limited success, initially (Scheme 19).



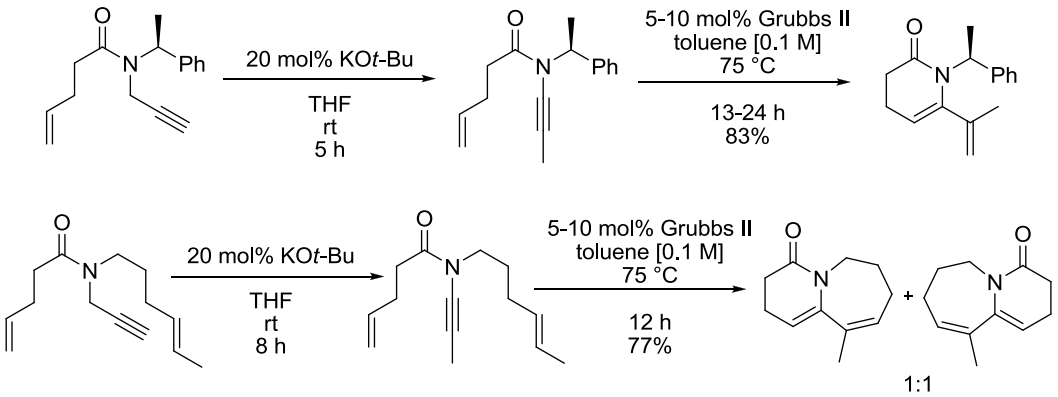
Scheme 19: Hsung's Initial Attempts at Isomerisation Approach

In 2002, Hsung reported isomerisation of propargyl amides **94a-c** to give their ynamide counterparts **96a-c** upon treatment with KO*t*-Bu.[99] The results of these reactions are illustrated in Scheme 20.



Scheme 20: Hsung and Co-Workers Isomerisation

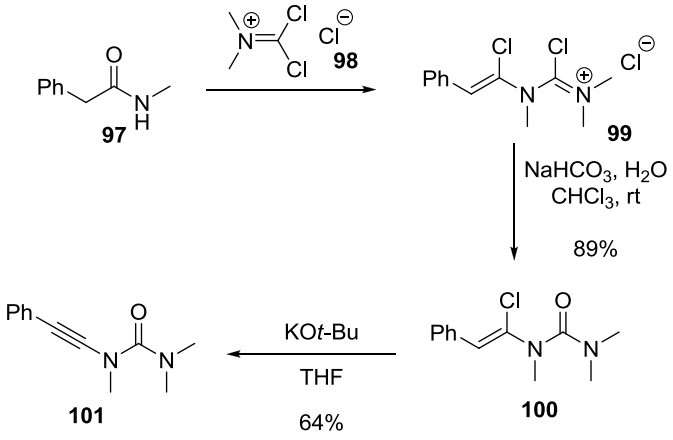
This chemistry was then extended to the preparation of ynamides containing alkene functionality in order to carry out subsequent ring-closing enyne metathesis. Examples of enyne ring-closing metathesis reactions performed on C1-heteroatom-substituted alkynes were only reported very recently, prior to this work (Scheme 21).[100] Hsung and co-workers were successful in demonstrating several examples of enynamide metathesis using the Grubbs second generation catalyst,[101] and reported the first examples of tandem RCM of diene-ynamides. This sequence of reactions allows the synthesis of fused heterocyclic systems in good yield and is indicative of the value of ynamides as synthetic intermediates.



Scheme 21: Hsung's Enynamide Metathesis Products

1.4.3 Elimination

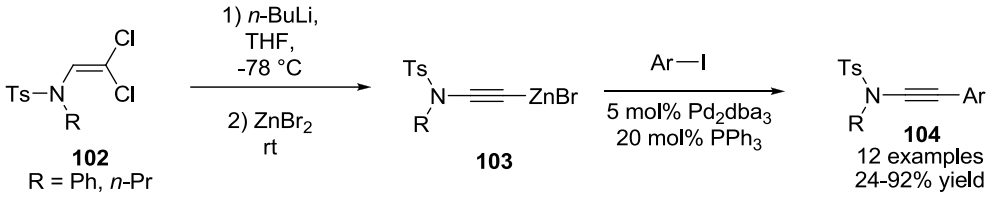
Elimination is an approach that was originally used by Viehe in 1972[94] to generate the first ynamides (Scheme 22). The simple amide **97** was reacted with phosgeneimmonium chloride **98** to deliver the chloroformamidinium salt **99**. This compound then underwent hydrolysis to provide urea **100** in 89% yield. Finally, HCl was eliminated from the double bond to give the desired ynamide **101** in 64 % yield.



Scheme 22: Viehe's Ynamide Synthesis

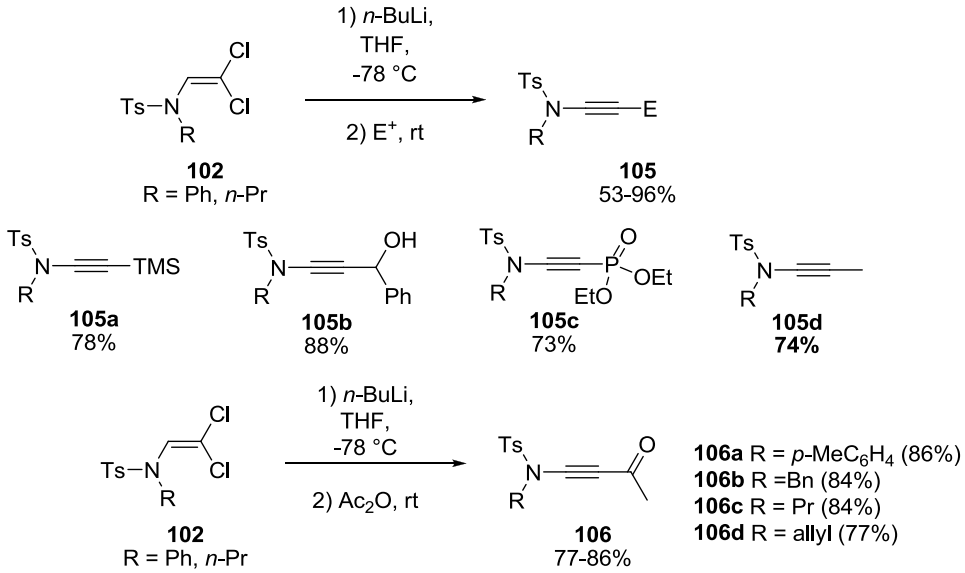
Several groups have attempted the synthesis of ynamides using this approach including Hsung[98,99] and Brückner,[102] with the latter having explored the conversion of formamides to dichlorovinylamides, followed by dehalogenation. An improved protocol for the elimination reaction was reported by Saá and co-workers in 2004. [103] They were inspired to use metal-mediated cross coupling reactions. Initially, Sonogashira conditions were tested resulting in the formation of complex mixtures of compounds of which the most prominent were dimeric alkyne products. Subsequently, Negishi coupling reactions were tested, but these resulted in only partial consumption of starting material, isolation of the alkyne dimer product and in some cases very poor yields of the desired coupled product.

The results described above encouraged Saá and co-workers to focus on the slightly different approach shown in Scheme 23. Starting with *β*,*β*-dichloroenamides **102**, treatment with *n*-BuLi followed by transmetallation with $ZnBr_2$ gave the desired zinc acetylide **103**. Following installation of zinc onto the terminal alkyne to give an alkynyl zinc reagent, it was possible to perform Negishi coupling with various aryl iodides to deliver the required ynamides **104**. Saá demonstrated the utility of this procedure by presenting several examples of the reaction giving excellent yields. It is worth noting that the Sonogashira approach was further perfected by Hsung and co-workers in 2004.[104]



Scheme 23: Saá's Improved Elimination Procedure

Saá and co-workers re-visited this area of chemistry in 2007[105] with a further modified procedure (Scheme 24). Having treated compound **102** with *n*-BuLi, the resulting lithium acetylide could be quenched with various electrophiles to give products possessing a wider variety of functionality adjacent to the alkyne.

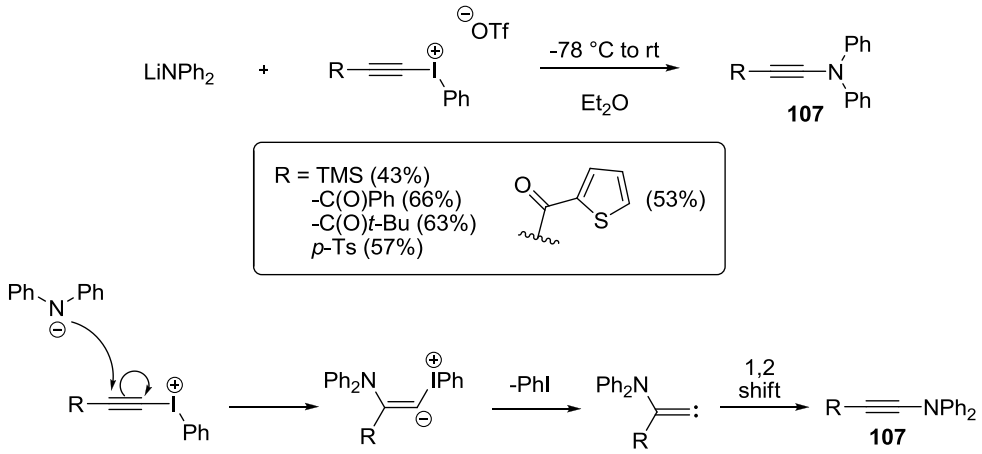


Scheme 24: Saá's Further Modified Approach to Ynamide Synthesis

Both isomerisation and elimination approaches to ynamide synthesis have their merits and are worth considering when designing a synthetic pathway towards these compounds. However, both methods lack the wide scope of applicability that is desirable in any synthetic procedure. In order to make ynamides truly accessible to the synthetic community it was essential to develop more robust methods that would be effective for the preparation of a diverse range of structures with various functional groups.

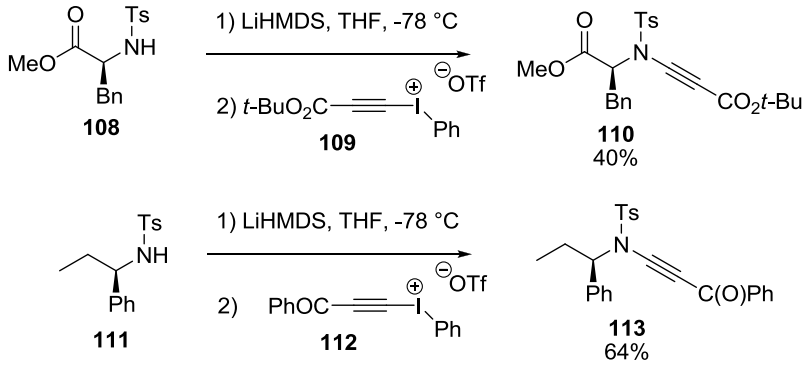
1.4.4 Synthesis Using Alkynyliodonium Salts

The reaction of alkynyliodonium salts with lithiated amines has proven to be an effective means of accessing ynamides, and especially for the preparation of sulfonyl-substituted ynamides. This approach was first reported by Stang in the 1990s to synthesise ynamine **107** (Scheme 25).[92,106] It is believed that this reaction proceeds *via* a vinylcarbene intermediate that is first generated by nucleophilic addition *β* to the iodine and this is followed by a 1,2-shift to form the acetylide.



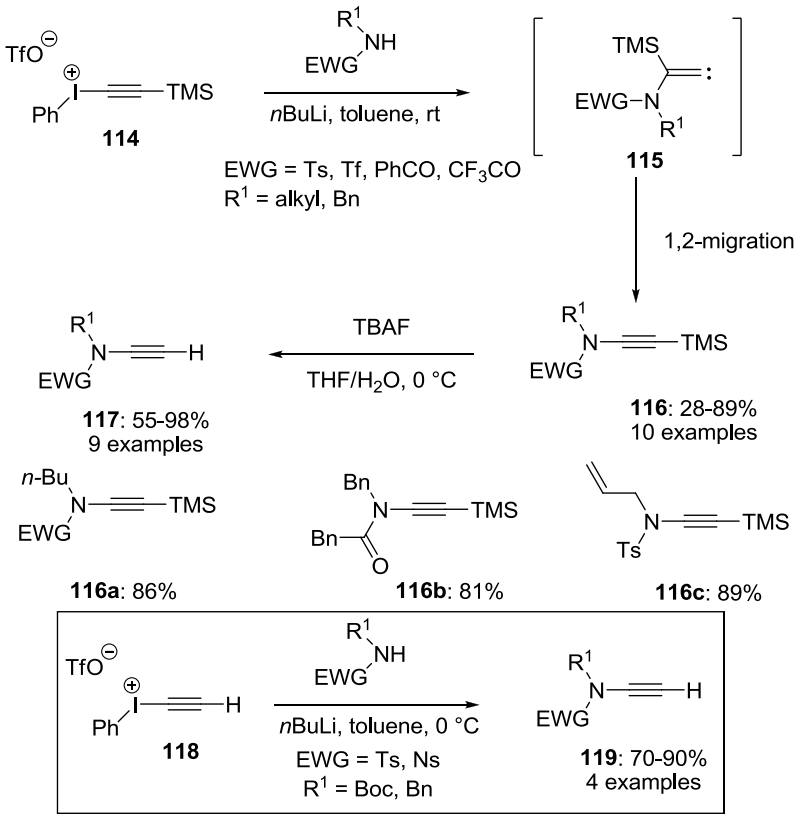
Scheme 25: Stang's Ynamine Synthesis and Proposed Mechanism

It was by this method that the first chiral ynamides were synthesised in 1996. Feldman and co-workers[94,107] reacted electrophilic iodonium triflates **109** and **112** with chiral sulfonamides **108** and **111** (Scheme 26). Feldman performed these reactions while investigating 1,5 C-H insertion reactions. The use of the more electrophilic alkynyliodonium triflate salts was expected to overcome some of the problems of low reactivity and lengthy reaction times. Instead of promoting the reaction of interest, these salts prevented formation of any of the 1,5 C-H insertion products and delivered ynamide products instead. The chirality was preserved during the reaction and the outcome was the successful synthesis of the chiral ynamides **110** and **113**, albeit in moderate yields.



Scheme 26: Chiral Ynamides Synthesised by Feldman

Additional studies have been performed by Witulski and co-workers[108] using this method of ynamide synthesis. The aim of these studies was to explore the proposed 1,2-shift mechanism of the reaction and probe the various steric factors that affect its outcome. Witulski was successful in synthesising a range of ynamides of general structure **116** in up to 89% yield. In cases where α-branched amides were used, it was found that lower yields were observed suggesting that steric factors are an issue in this type of ynamide synthesis. Witulski and co-workers also demonstrated that terminal ynamides could be obtained by treatment of the silylated products **116** with TBAF to remove the TMS group. The products **117** were obtained in moderate to good yields using this procedure. In later studies from Witulski and co-workers a terminal ynamide was synthesised directly using compound **118**.



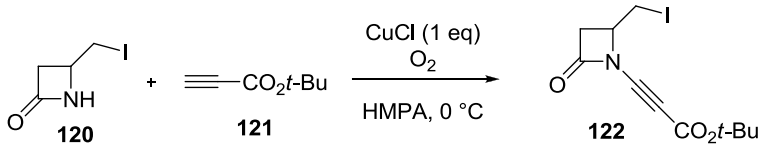
Scheme 27: Witulski's Studies of the Alkynyliodonium Triflate Approach

By the end of the twentieth century the use of alkynyliodonium salts seemed to be the most effective method of producing ynamides. Unfortunately, this method still suffered from some serious limitations that curtailed its widespread use in organic synthesis. The main drawbacks are the lack of availability of the starting iodonium salts and the fact that it is necessary for them to be substituted by a silyl, aromatic or electron-withdrawing group for the reaction to be successful.[109]

There are severe practical limitations for all three of the methods that have been described thus far. Low substrate scope, harsh reaction conditions and lengthy reaction sequences resulted in a lack of generality towards ynamide synthesis and resulted in the very slow growth of this field. It has only been within the past decade that efficient methods have been developed with a sufficient breadth of applicability to allow appropriately functionalised ynamides to be readily accessible to those working in a wide range of research areas.

1.4.5 Amidative Cross Coupling Methods

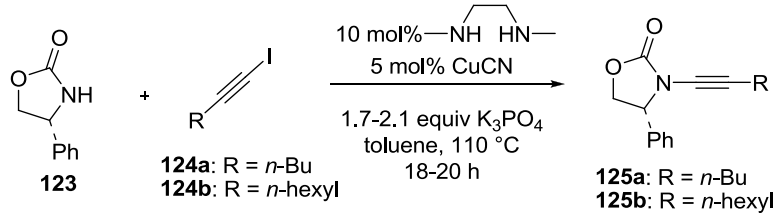
The first example of ynamide synthesis *via* a metal-mediated process was reported by Balsamo and Domiano in 1985.[110] The authors were attempting to carry out a reaction to displace the iodine in **120** with the copper acetylide of **121** but to their surprise, ynamide **122** was the sole product and its structure was confirmed using X-ray crystallography (Scheme 28). In this example, a stoichiometric amount of CuCl was used to promote the reaction.



Scheme 28: Metal-Mediated Amidation of a Terminal Alkyne

This approach had not been widely utilised in chemical synthesis until recent years, after Hsung and co-workers advanced the field in 2003.[111] The authors were inspired by the work of Buchwald and Hartwig[112-114] towards the *N*-arylation of amines. It was their belief that a metal mediated coupling of an alkynyl halide and an amide would have wide scope. To this end Hsung and co-workers began to research this approach initially using palladium-catalysed conditions.[115] Reactions with alkynyl halides and stannylated or other metalated amides were tested with little success. Instead of the desired amidation taking place at the alkyne, the dominant reaction was instead alkyne homocoupling.

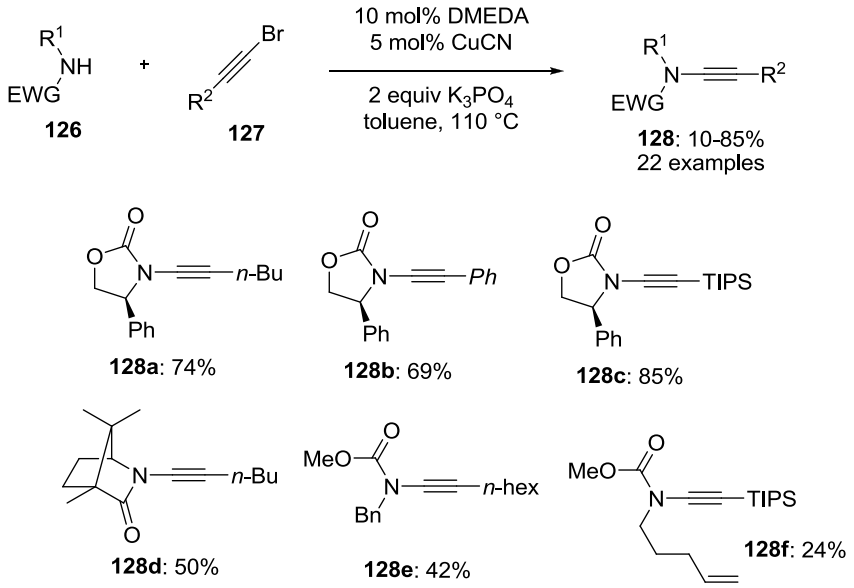
When looking for possible alternative procedures, Hsung and co-workers were encouraged by some recent work in copper catalysed *N*-arylation of amines.[116,117] The reaction between oxazolidinone **123** and alkynyl iodides **124** was used to test and optimise the reaction conditions. The optimal conditions are depicted in Scheme 29, but at this stage the results were still highly variable and homocoupling of the alkynyl iodides continued to be a problem.



Scheme 29: Hsung's Amidation Protocol Using Alkynyl Iodide

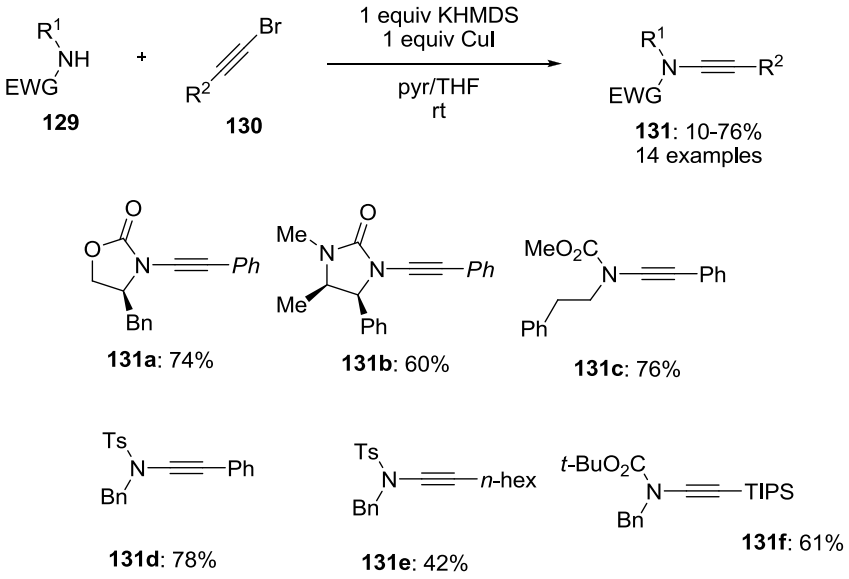
The homocoupling problem was overcome by replacing the iodides with alkynyl bromides. It was also found that whilst CuI was an effective catalyst for the reaction, CuCN provided more consistent results overall. The general protocol involved performing at reflux in

toluene in the presence of K_3PO_4 as a base (Scheme 30). This procedure was particularly effective for the alkynylation of oxazolidinones and lactams, affording the ynamides **128** in good to excellent yield. Unfortunately, when the reaction was applied to imidazolidinones and sulfonamides, very poor yields were obtained. Nevertheless, this type of reaction was a major breakthrough in the search for an atom economical and efficient method to synthesise ynamides. Further research was required in order widen the substrate scope of the reaction.



Scheme 30: Hsung's Optimised 1st Generation Amidation Protocol and Examples

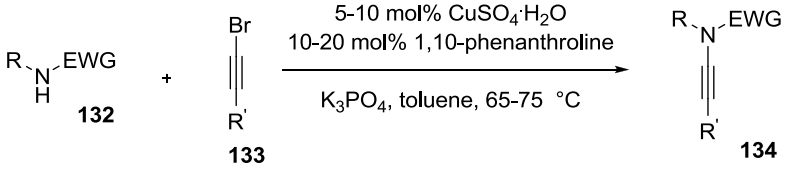
At the same time as Hsung was developing his protocol, Danheiser and co-workers described another procedure for the copper-catalysed amidation of alkynyl bromides,[118] as illustrated in Scheme 31. In this procedure, a stoichiometric amount of CuI was used to generate the copper derivative, which reacts with the deprotonated amide or carbamate.



Scheme 31: Danheiser's Stoichiometric Cu(I) Protocol

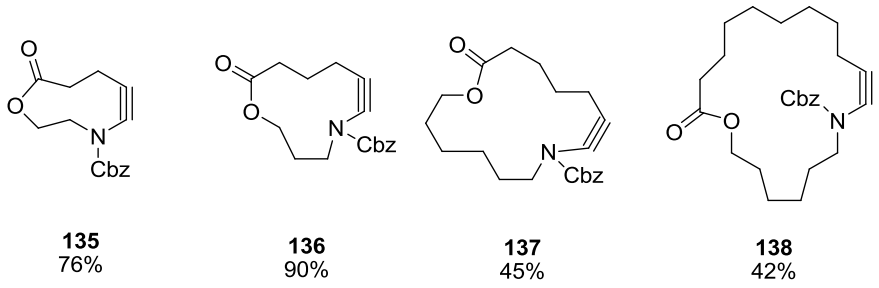
This protocol resulted in good to excellent yields of the product and was shown to be tolerant of a variety of functional groups. Its utility was also successfully demonstrated in several multi-gram scale reactions. Of course this is not a catalytic process, but the metal that is used is relatively cheap and so this is not a serious drawback to the overall efficiency of the reaction. In addition, the reaction was used to alkynylate some sulfonamides for which Hsung's procedure had proved to be unsuccessful. This protocol also had the advantage that it can be performed at room temperature, thus making it a viable route for the preparation of thermally sensitive ynamides.

In 2004, Hsung and co-workers reinvestigated this area and published a modified approach to ynamide synthesis (Scheme 32). [118,119] In this second generation protocol, Hsung and co-workers used copper sulphate pentahydrate as the copper source, 1,10-phenanthroline as a ligand and K_3PO_4 as the base. This proved to be a more efficient and general approach to generating ynamides than the first generation protocol. The reaction was carried out in toluene at temperatures below reflux and in some cases as low as 60 °C. This approach was useful for the reactions of substrates that had proven difficult using the first generation protocol.



Scheme 32: Hsung's 2nd Generation Amidation Protocol

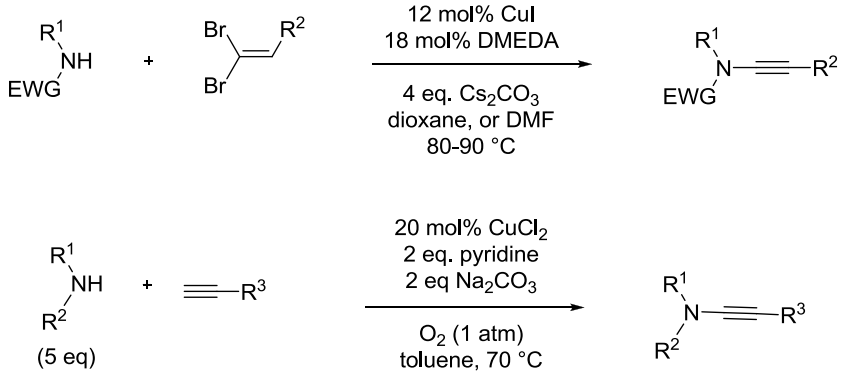
Hsung and co-workers continued to demonstrate the synthetic utility of this protocol by successfully applying it to several intramolecular examples. Nitrogen-containing cyclic systems with rings ranging from 9- to 19-members (compounds **135** to **138**, Figure 41) were successfully prepared. The ability to synthesise nitrogen-containing heterocycles means that this chemistry is of interest from the perspective of natural product synthesis. Having said this, the yields fall dramatically with larger ring sizes, likely due to the entropic cost of cyclising longer chain lengths.



135 76% **136** 90% **137** 45% **138** 42%

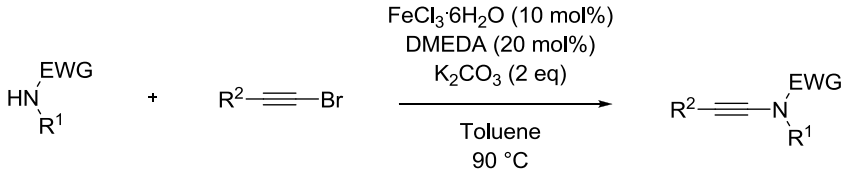
Figure 41: Intramolecular Amidation Products

Progress towards synthetically accessible ynamides in the past 10 years has brought this class of compound into mainstream organic chemistry. The pioneering work of Hsung and Danheiser in advancing this field has been built upon in subsequent years, with several other research groups making their own contributions. Some notable efforts include the synthesis of ynamides from 1,1-dibromo-1-alkenes that was published by Evano and co-workers[120] and the copper catalysed aerobic oxidative amidation of terminal alkynes as published by Stahl and co-workers[121] as shown in Scheme 33.



Scheme 33: Upper: Evano Protocol, Lower: Stahl Protocol

There was also an interesting procedure published by Zhang and co-workers[122] in 2009 in which an iron source ($FeCl_3 \cdot 6H_2O$) was utilised alongside DMEDA to give successful coupling (Scheme 34). This provided access to ynamides by reaction with a selection of oxazolidinone and sulphonamide starting materials. This reaction had the added benefit of utilising an inexpensive and environmentally benign catalyst.



Scheme 34: Zhang's Iron Catalysed Amidation

This field of chemistry methodology is continuing to produce versatile procedures for ynamide synthesis[123,124] and will undoubtedly continue to do so in future. The number of publications describing the use of ynamide synthesis methods to produce useful intermediates is steadily growing, demonstrating the potential synthetic value of these compounds.

1.4.6 Summary

Ynamides are a class of compound that the synthetic community has been aware of for some time. Until recently, however, they have been under-utilised despite their clear value in organic synthesis, largely because of their inaccessibility. In the past 10 years this situation has changed drastically, and while the field of ynamide synthesis will continue to improve through innovation and new approaches, chemists now have practical tools to access ynamides quickly and easily.[125]

As a direct result of the latest metal mediated coupling conditions that are being used in ynamide synthesis, the number of ynamides being used as intermediates in organic syntheses has increased dramatically. Evano and co-workers accurately described this as an "ynamide boom" in their review of synthetic applications of ynamides in 2010.[109]

As has already been discussed, ynamides are excellent substrates for regioselective addition of electrophiles and nucleophiles due to their unique electronic properties (Figure 42). This is a property that they share with their ynamine counterparts.

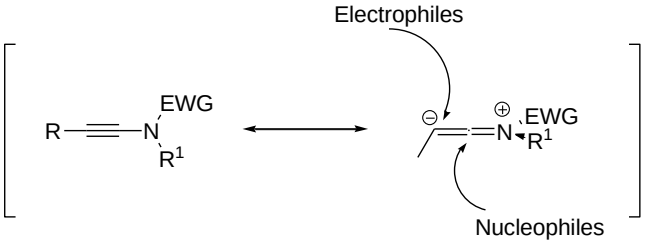


Figure 42: General Reactivity of Ynamides

Ynamides can now be functionalised in a variety of ways, meaning that they can be used to introduce unique structures into larger molecules. They can undergo oxidation and reduction reactions as a novel way of introducing, and then manipulating, functionality. They have also been shown to be competent reactants in several types of cycloaddition[126-128] ring-closing metathesis[100,129] and cycloisomerisation reactions.[130] This allows for the synthesis of nitrogen-containing heterocycles, which are desirable targets and intermediates in many areas of chemistry including natural product synthesis and medicinal chemistry.

The growth that has been demonstrated in this field in the past decade is impressive and there is no sign of it slowing down in the near future. As researchers become more acquainted with ynamides and methods of synthesising them are continually improved, even more novel and exciting uses for them will quickly become apparent.

Chapter 2

Results and Discussion

2.1 Introduction

β-Turn mimetics are promising tools in the fields of drug discovery and synthetic vaccines. The number of mimetic structures that have been reported and tested for biological activity in recent decades has exploded.[131-133] Most of these structures have been based upon macrocyclic or bicyclic systems with functional handles located around the ring allowing incorporation of peptide chains found in the natural structures and the utilisation of library synthesis techniques to generate families of structurally related compounds for biological evaluation.

This type of research is in progress in several areas of medicinal chemistry and is a fertile area in which potential drug targets are abundant.[134-136] Turn-like structures are particularly attractive targets for several reasons. Firstly, *β*-turns are present, or postulated to be present, in the active conformations of many naturally occurring peptides and proteins.[137] Secondly, *β*-turns are readily amenable to mimetic design due to their relative simplicity.[138]

Leu-enkephalin is one of two enkephalin opioid peptides that were discovered in 1975[35] and is a pentapeptide that binds to both *δ*- and *μ*-opioid receptors. Although an X-ray crystal structure of this peptide in its bound conformation has not been obtained, there is strong evidence to suggest that its biologically active conformation contains a *β*-turn.[139] X-ray crystallography of unbound leu-enkephalin shows that it forms a *β*-turn, with a stabilising H-bond between the phenylalanine and tyrosine amino acid residues. As a result, the enkephalins have been a popular choice for validating *β*-turn mimetics. The aim of this project was to synthesise a macrocyclic ynamide unit that mimics the turn conformation of leu-enkephalinamide (Figure 43).

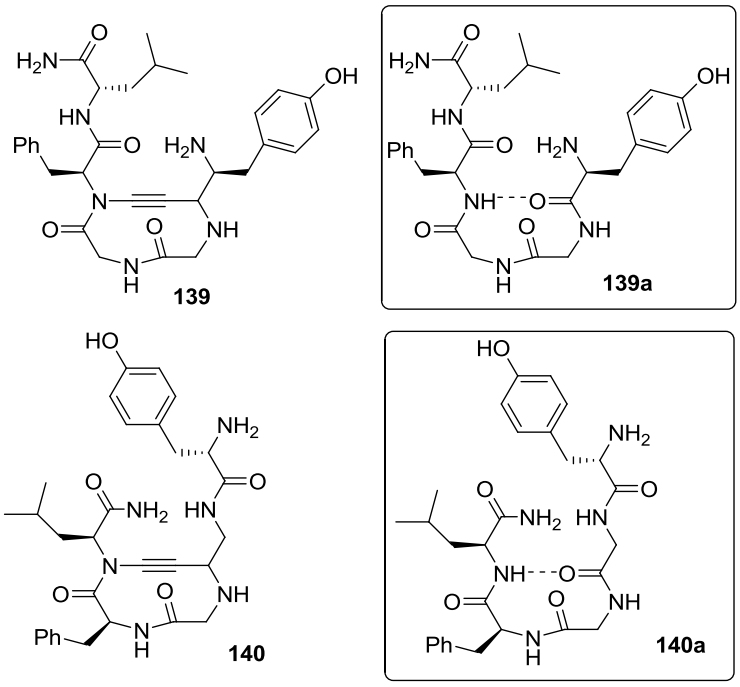


Figure 43: Leu-Enkephalinamide *β*-Turn Structures (139a and 140a) and Corresponding Mimetic Structures (139 and 140)

The ynamide functional group was chosen to act as a covalent tether and was expected to enforce the turn conformation of the extended chain and consequently rigidify it. Leu-enkephalin is conformationally flexible and can conceivably adopt a *β*-turn at several positions.[140] This probably accounts for its ability to bind to different types of opioid receptor. Consequently, two mimetic structures, labelled **139** and **140**, have been proposed as mimics for two corresponding turn structures, **139a** and **140a**, respectively.

The ynamide unit was selected as the covalent linker in the mimetic structure for several reasons. Firstly, the use of an ynamide retains as many of the original functional groups as possible in the mimetic target. The only group that is completely absent from the mimetic structures is the hydrogen bonded carbonyl group. Otherwise, maximum retention of functionality is achieved. Furthermore, the bond distances are appropriately similar between the ynamide unit and the hydrogen bonding in the natural substrate. Using known average bond lengths and calculating the total length of the bonds involved, the two chains of the reverse turn are stabilised at 4.14 Å distance from each other as illustrated in Table 4. The distance between equivalent N and C atoms in the natural peptide substrate is very similar at 4.21 Å. This figure was calculated based on average carbonyl bond distances and measurements published alongside the X-ray crystal structure of leu-enkephalin. Of course the

hydrogen bonding in the natural structure is not as rigid as a covalent bond, and, as a result, the natural system will likely have more variation in distance than the covalent target. However, the ynamide unit should maintain a suitable distance across the turn that approximates that in the average representation of the natural turn structure.

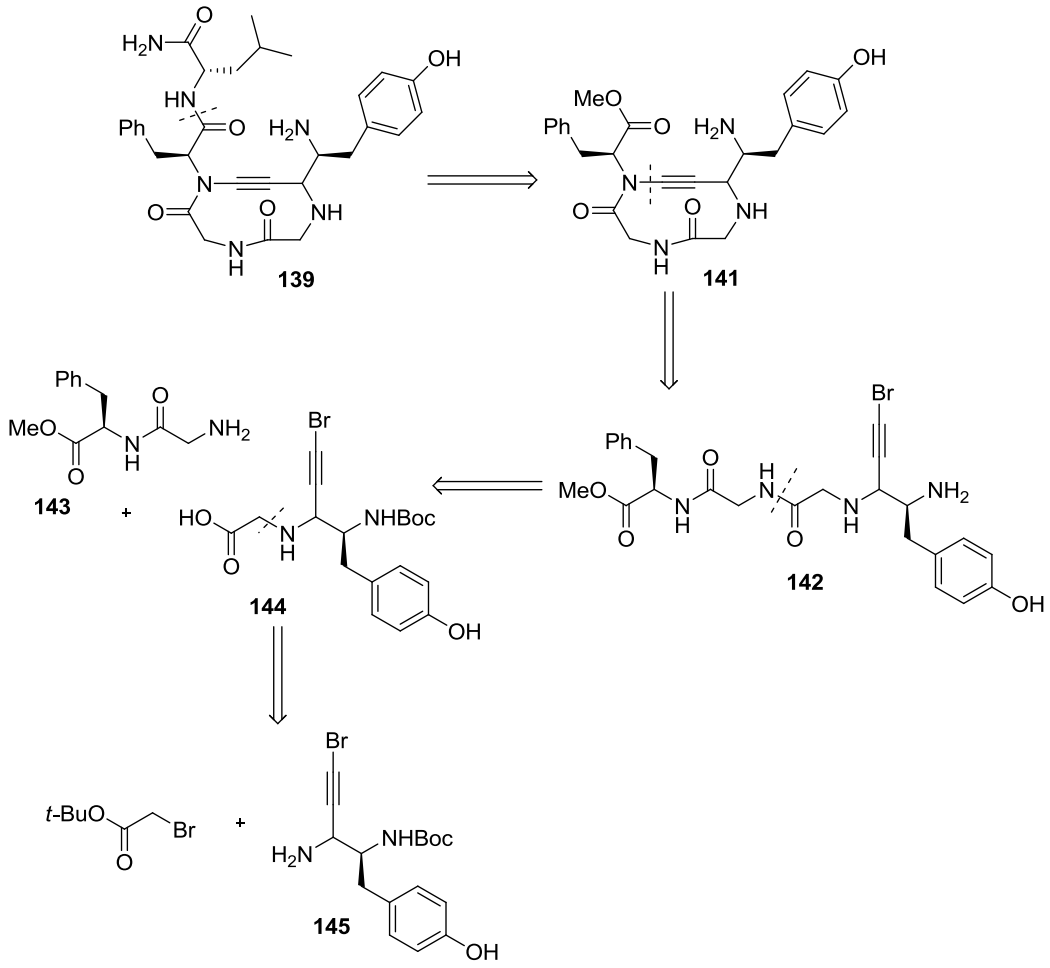
Table 4: Bond Lengths of Mimetic			
Atoms (Bonds)	**Bond Length (Å)**	**Atoms (Bonds)**	**Bond Length (Å)**
N–C	1.47	N to O distance	2.99
C≡C	1.21	O=C	1.22
C^{sp}–C^{sp3}	1.46		
Total	**4.14**	**Total**	**4.21**

The advances that have been made in ynamide synthesis in the past decade are very encouraging and provided a potential route to access the desired structures.[109] It is also worthwhile to note that one side of the ynamide linker incorporates a chiral centre into the structure. This raises the possibility of modifying stereochemistry and testing different stereoisomers for biological activity, thus opening an alternate avenue of investigation.

There are also several sites on the ring that can be modified to incorporate varied functionality, for example amino acid side chains. This versatility is important for peptidomimetics in order to screen numerous related structures.[141] This type of diversity can probably be introduced in a general synthesis using commercially available protected amino acids.

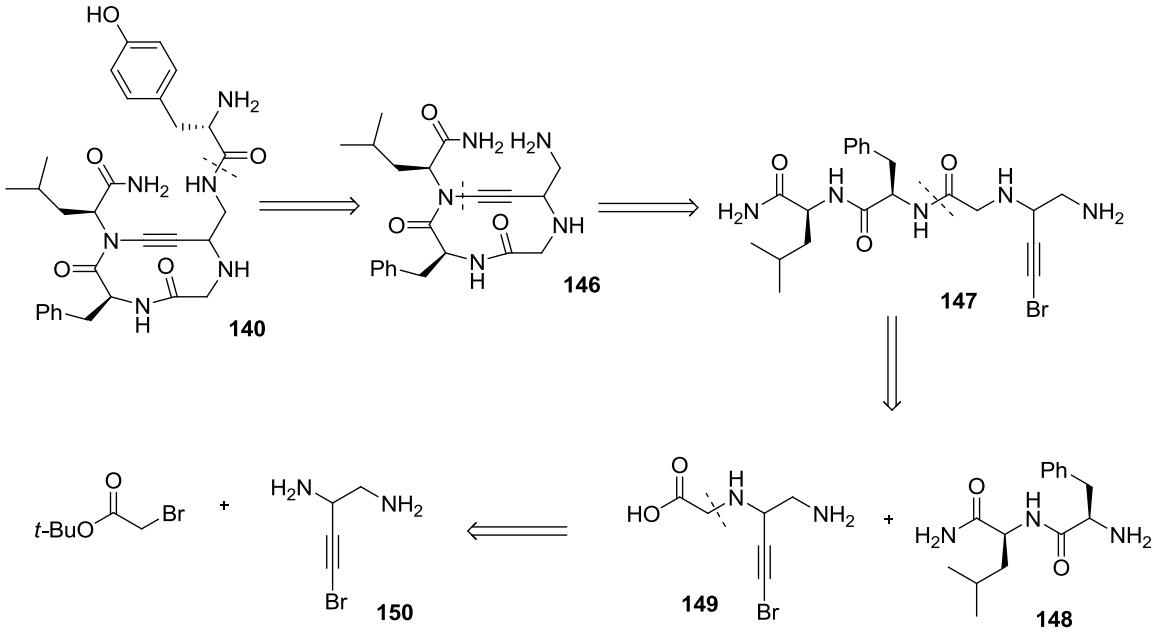
A preliminary retrosynthesis (Scheme 35) suggests that the most efficient way of accessing the target macrocyclic mimetics would involve a combination of peptide coupling methodology and alkyne amidation coupling chemistry to construct the ynamide functional group. Beginning with target **139**, the leucine fragment can be removed taking the structure back to the terminal phenylalanine residue that is protected, for example, as a methyl ester. At this stage, the ynamide ring can be opened giving tripeptide precursor **142**. This can be further simplified, again using peptide disconnection to give dipeptide fragment **143** and alkynyl bromide fragment **144**. Dipeptide **143** could be synthesised by amide coupling between suitably protected phenylalanine and glycine.

Fragment **144** is significantly more complex than the dipeptide **143**, due to the fact that it contains novel, non-peptidic functionality in the form of an alkyne. This fragment is a dipeptide analogue of glycine and tyrosine. This can be simplified by C-N bond disconnection to give the protected diamine **145** along with *t*-butyl bromoacetate.



Scheme 35: Preliminary Retrosynthetic Analysis of Target Mimetic 139

A similar analysis can be utilised to inform the synthetic design of the alternative leu-enkephalinamide mimetic **140** and is illustrated in Scheme 36. The first step involves the removal of tyrosine residue by peptide disconnection. This leaves the cyclic ynamide that is ring-opened to give a linear tripeptide precursor **147**. This can then be simplified using another peptide disconnection, to provide fragments **148** and **149.** Dipeptide **148** could be synthesised from protected leucine and phenylalanine residues using standard coupling conditions. Finally, fragment **149** is simplified by C-N bond disconnection to give the 1,2-diamine **150**.



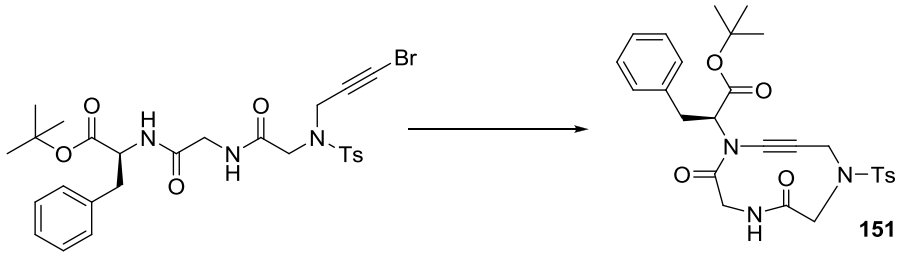
Scheme 36: Retrosynthetic Analysis of Mimetic 140

Both of these retrosyntheses imply that well-known peptide coupling techniques will be used in the forward synthesis. This makes it possible to effectively tailor the syntheses, utilising diverse amino acids to install varied groups at the four possible sites of functionality. While the initial targets are analogues of leu-enkephalin, and the functional groups selected reflect this, further investigation could be carried out to incorporate alternate groups that may be found in other opioid peptides such as the endomorphins.

2.2 Synthesis of Ynamide Model Compounds

2.2.1 Original Model Compound Synthesis

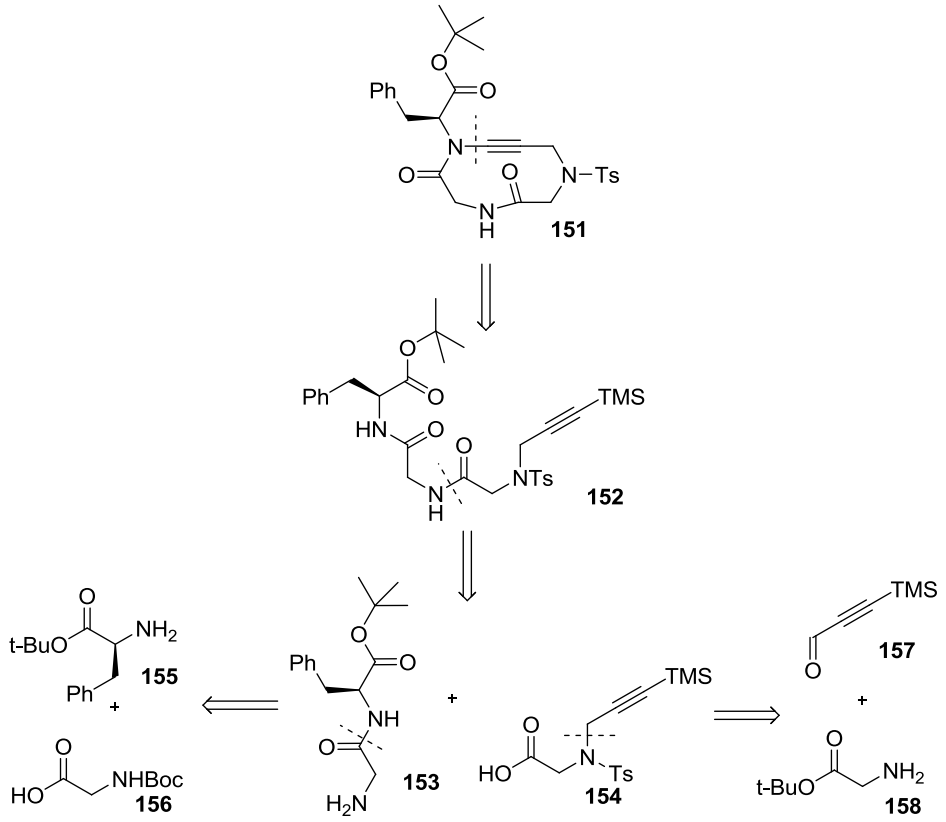
In order to proceed effectively and confidently with the synthesis of complex mimetic structures based on a 10-membered ynamide macrocycle, it was necessary to test the chemistry that would be used to construct the core structure. To this end, a simple model (**151**) of the ynamide macrocycle was selected as a target in order to test the alkyne coupling reaction that would be used to assemble the ring. The proposed key reaction is illustrated in Scheme 37.



Scheme 37: Coupling Protocol Towards Ynamide Core

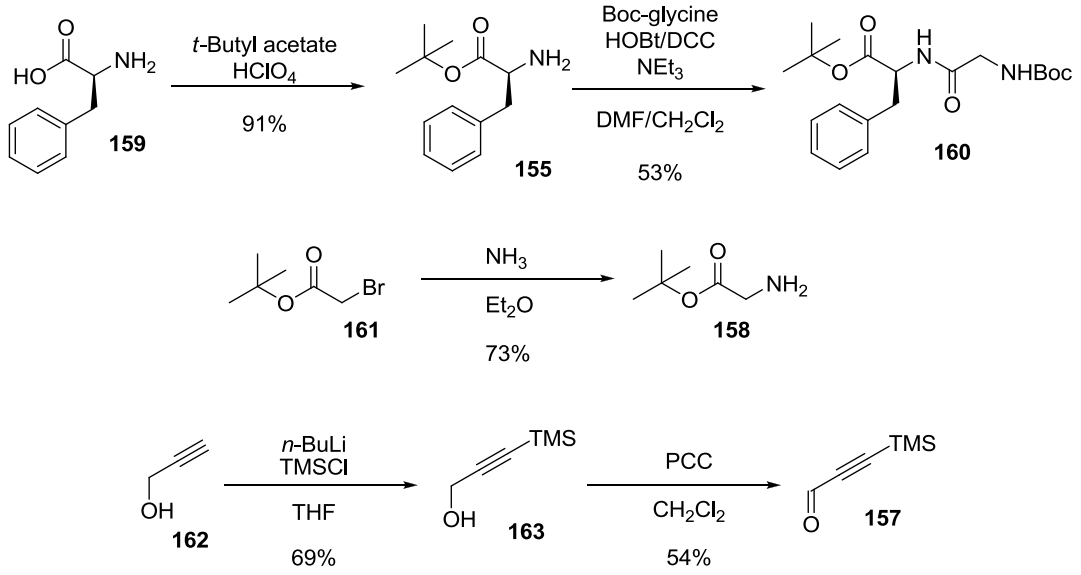
Sections of the mimetic structures could be synthesised using standard amide coupling methods which have been very well explored and for which there are many alternatives.[142-145] Accessible methods for ynamide synthesis are a fairly recent innovation and, although several new approaches to the synthesis of ynamides have been published in recent years, the chemistry is still in its infancy and as a result is not as robust as it should be. It was this chemistry that required further investigation in order to ensure that it was reliable enough to permit construction of the target macrocycles.

A retrosynthetic analysis of the model target **151** is illustrated in Scheme 38. The first step in the retrosynthetic scheme was to disconnect the ynamide bond and open the ring, simplifying the target structure to give the tripeptidic precursor **152**. The alkyne in this retrosynthesis is protected by a TMS group to prevent unwanted reactions either at the terminal alkyne or at the analogous alkynyl bromide. Structure **152** can be disconnected to give the fragments **153** and **154**, implying a standard amide coupling reaction in the forward direction. Dipeptide **153** is a protected dipeptide that can be further simplified, again using an amide bond disconnection. This implies the use of protected phenylalanine *t*-butyl ester (**155**) and Boc-glycine (**156**) as two of the starting materials. The alkyne **154** was disconnected into two fragments (**157** and **158**) using a reductive amination protocol. The amino ester **158** is another protected derivative of glycine and so is easily obtained either commercially or through a simple esterification reaction. Fragment **157** can be synthesised using relatively straightforward procedures starting from propargyl alcohol. The use of derivatives of naturally occurring amino acids meant that the synthesis of the model target is simplified significantly. With the retrosynthesis in place it was possible to focus on developing the forward synthesis.



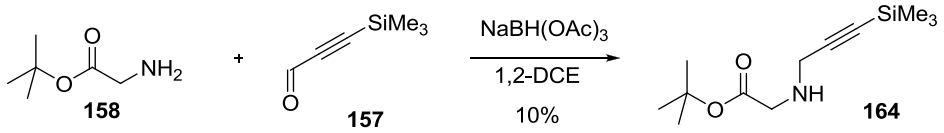
Scheme 38: First Proposed Retrosynthesis of Model Compound

Several of the required fragments were easily synthesised as shown in Scheme 39. L-Phenylalanine was easily protected as its *t*-butyl ester by treatment with *t*-butyl acetate in the presence of perchloric acid.[146] This compound was then coupled to Boc-glycine using standard DCC/HOBt conditions[147] to produce the protected dipeptide **160**. Glycine *t*-butyl ester (**158**) was synthesised by the reaction of *t*-butyl bromoacetate with liquid ammonia diluted in a solution of diethyl ether.[148] The TMS protected alkyne **157** was synthesised from propargyl alcohol. This was accomplished by deprotonation of the terminal alkynyl group and reaction with TMSCl, followed by a PCC oxidation to generate the aldehyde as required.[149] With all of these fragments in place the aforementioned reductive amination of the aldehyde **157** with the amine **158** was performed to obtain a coupling partner analogous to structure **154**.



Scheme 39: Synthesis of Individual Fragments

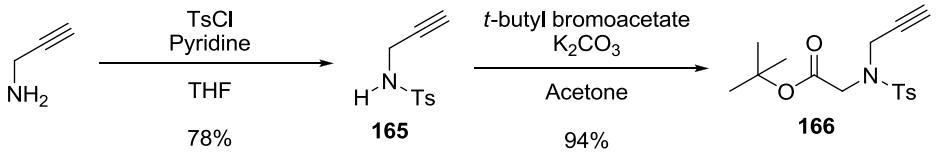
The reductive amination reaction was performed using sodium triacetoxyborohydride (Scheme 40). This reaction was attempted several times but with little or no success. The reaction either did not proceed or produced only a minimal yield of the target amine. Several attempts were made to optimise the reaction but with little success; a yield of just 10% was obtained in the best case. The reaction was also carried out with the intention of isolating the intermediate enamine and then reducing this separately. Although enamine formation appeared to work well under these conditions, the intermediate decomposed during work up and could not be isolated. The amine material that was successfully isolated was promising however the inability to improve the yield to >10% rendered this approach unsuitable.



Scheme 40: Reductive Amination Procedure

In order to achieve a similar fragment, an alternate approach was considered. Pleasingly, using propargylamine as a starting material, treatment with *p*-toluenesulfonic acid in the presence of pyridine furnished the tosylated propargylamine **165** in excellent yield (Scheme 41).[150,151] This compound was then *N*-alkylated using *t*-butyl bromoacetate in the presence of potassium carbonate to produce the required acetylenic fragment **166** in excellent

yield. Both of these reactions were reliable and provided clean material for subsequent transformation.



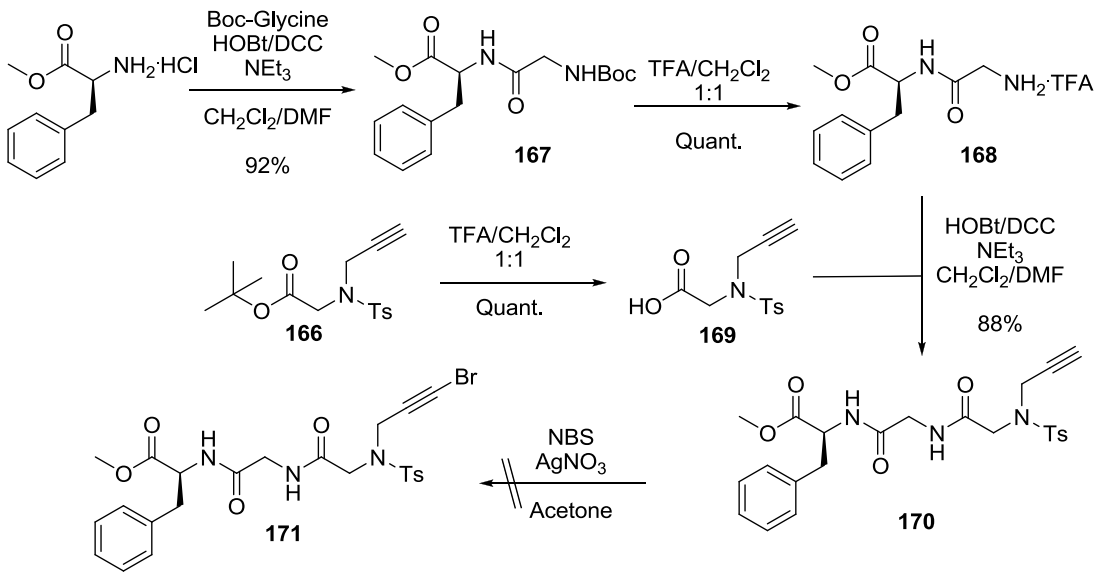
Scheme 41: Alternate Synthesis of Acetylenic Fragment

It quickly became apparent that by using protected dipeptide **160** there were obvious difficulties with subsequent steps in the synthesis. By removing the Boc protecting group under acidic conditions, the *t*-butyl ester would also be removed, meaning that the resulting carboxylic acid would have to be protected once again in order to proceed further. In order to avoid this problem, the dipeptide was synthesised as its methyl ester **167** starting from commercially available Boc-glycine and L-phenylalanine methyl ester hydrochloride (Scheme 42). This was accomplished in good yield using standard DCC/HOBt coupling conditions.

The *N*-alkylated intermediate **166** was deprotected under acidic conditions to give the carboxylic acid **169** in quantitative yield. Dipeptide **166** was deprotected using the same conditions, again giving the product in quantitative yield. Initially, the Boc deprotection reaction was worked up by neutralisation of the remaining TFA, extraction of the free amine of **168** into organic solution, and concentration *in vacuo*. This presented problems for the subsequent coupling step because the free amine was particularly insoluble in the reaction solvent. As a result, only partial reaction of the amine took place and the yields were very poor. As an alternative approach, the TFA salt **168** was isolated by concentration of the crude reaction mixture to remove all of the TFA; trace amounts were completely removed by concentration several times with toluene *in vacuo*, followed by an extended period under high vacuum. This was essential, as traces of TFA interfere with the subsequent coupling reaction.

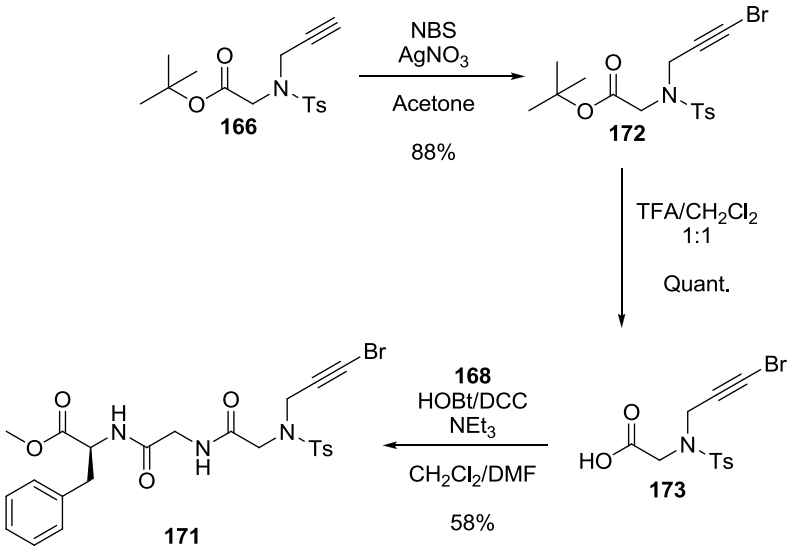
The TFA salt **168** was readily soluble in the CH_2Cl_2/DMF reaction solvent and it was successfully coupled with carboxylic acid **169** to give the acetylenic tripeptide **170**. The next stage involved bromination of the terminal alkyne and the reaction was first attempted using recrystallised NBS and silver nitrate at room temperature. Unfortunately, the desired alkynyl bromide **171** could not be isolated. Instead TLC indicated the formation of a complex mixture of products suggesting that the tripeptide was brominated at several positions, including the desired one. Separation of the mixture of products was not possible as several of them were of

similar polarity. Consequently, it was decided that it would be necessary to introduce the bromine substituent at an earlier stage in the synthesis to bypass this difficulty.



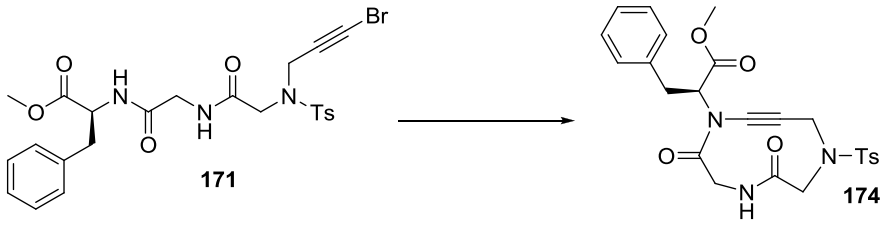
Scheme 42: Synthesis of Acetylene Bromide Precursor

Terminal alkyne **166** was successfully brominated using recrystallised NBS and silver nitrate to give compound **172** in excellent yield (Scheme 43). The ester functionality was removed using a 1:1 mixture of TFA and CH_2Cl_2, giving carboxylic acid **173** in quantitative yield. Finally, this fragment was coupled with dipeptide **168** using DCC/HOBt, to obtain the required cyclisation precursor **171**. Although this final coupling reaction did not give the high yields observed in the previous peptide coupling reactions, it was adequate for the purposes of constructing this model compound and so was not optimised further. Having prepared the cyclisation precursor, the next stage was to test the ynamide coupling chemistry on this system.



Scheme 43: Early Installation of Bromide and Completion of Tripeptide Precursor

The field of ynamide synthesis has taken some dramatic strides forward in the past decade.[109] This is largely due to the improved generality and accessibility that has been afforded by the development of metal mediated coupling reactions to prepare these systems. Much of this pioneering work has been performed by Richard Hsung and co-workers, and his second generation Cu(II) protocol is often regarded as the benchmark in ynamide synthesis.[119] This appeared to be an ideal procedure for the synthesis of cyclic ynamide **174**, particularly because intramolecular variants of this transformation had already proven to be effective for the synthesis of medium-sized ring systems. It was found that cyclisation reactions leading to large ring sizes gave poorer yields with 9- and 11-membered rings showing the optimal results. Thus, target **174** falls into what should be the ideal category of substrates for cyclisation using this chemistry. The desired transformation is illustrated in Scheme 44 and involves coupling of the alkynyl bromide with the relevant amide centre to form the 10-membered ynamide ring.



Scheme 44: Proposed Coupling Reaction Towards Macrocyclic Ynamide

Although there are two possible centres on which this bromide can potentially react, only one centre is really a feasible intramolecuar nucleophile. Reaction at the rightmost amide centre in structure **171** would result in a 7-membered ring **175** as illustrated in Figure 44. The linear alkyne system alone would make this ring size highly strained and most likely impossible, or at the very least highly unfavourable.

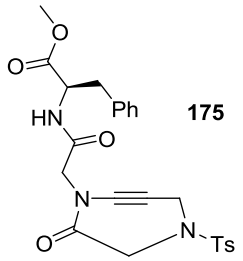


Figure 44: Highly Strained Alternative 7-Membered Cyclisation Product

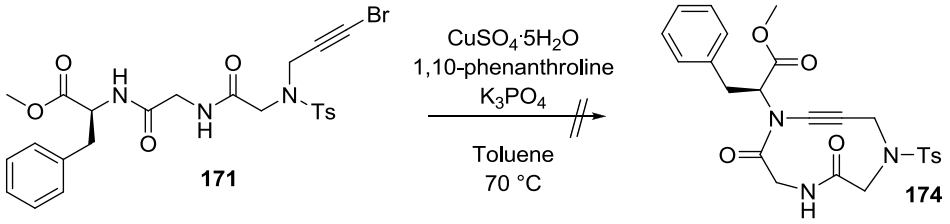
Based on this reasoning, the relevant coupling reaction was approached with confidence and the 10-membered lactam was expected as the only product.

2.2.2 Ynamide Coupling Reactions Towards Macrocyclic Core

Initially, the coupling protocol was performed using standard literature conditions reported by Hsung and co-workers. This chemistry has been utilised extensively by others to synthesise ynamide intermediates that are suitable for further transformations.[119]

This particular reaction required the addition of 10 mol% of $CuSO_4 \cdot 5H_2O$ and 20 mol% of 1,10-phenanthroline in the presence of two equivalents of K_3PO_4. Unfortunately, the precise reaction mechanism has not been elucidated, but it is likely to proceed *via* insertion of the copper into the alkynyl bromide and subsequent activation and coupling with the amide.

Initial attempts to utilise Hsung's second generation amidation protocol were unsuccessful (Scheme 45). In this case the reaction only showed limited progression with traces of possible products or decomposition fragments evident by TLC analysis. A large proportion of the tripeptidic starting material was isolated from the reaction mixture. The new product traces were analysed by NMR spectroscopy, which clearly indicated that they were not the desired structure and were deemed to be decomposition products.



Scheme 45: Attempted Intramolecular Amidation Using Hsung's Conditions

Following the failure to synthesise the required product using standard conditions, further experiments were performed under various conditions, the results of which are illustrated in Table 5. Hsung's intramolecular protocol can also be performed using K_2CO_3 as the base and so the reaction was carried out with this modification as illustrated in entry 1. However, the reaction resulted in almost complete recovery of starting material; subsequent reactions were performed using K_3PO_4 as the base.

In the literature examples, intramolecular reactions were carried out using a higher loading of catalyst than the intermolecular examples. Hsung's procedure began using 10 mol% of copper catalyst and 20 mol% of the ligand, with successive additions to double the catalyst loading during the reaction. The intramolecular reactions were also performed at reflux over a prolonged period, four days in the longest case. In light of this, the coupling reaction was undertaken using progressively harsher conditions over longer reaction times.

In entries 2 and 3 of Table 5, the loading was kept at 10 mol% and 20 mol% for the catalyst and ligand respectively. The temperature was increased slightly in entry 3, but there was no improvement in the reaction outcome – starting material remained and there were no identifiable products. Subsequently, entries 4 and 5 doubled the catalyst and ligand loading and increased the temperature further. Both of these examples showed the formation of new material. Although TLC analysis showed that only very small amounts of the products were present, separation of the constituents by chromatography for attempted characterisation was deemed to be worthwhile. Unfortunately, NMR analysis of the isolated material was inconclusive and the compounds were thought to be decomposition products. Reaction of the substrate at lower concentrations was investigated as a means of avoiding possible dimerisation reactions but unfortunately this did not give better results (entries 3 and 5).

Table 5: Attempted Amidation Using Hsung Protocol						
Entry	**$CuSO_4 \cdot 5H_2O$**	**1,10-Phenanthroline**	**Base**	**Conc.**	**Temp.**	**Result**
1	10 mol%	20 mol%	K_2CO_3	15 mM	70 °C	S.M.
2	10 mol%	20 mol%	K_3PO_4	10 mM	70 °C	S.M.
3	10 mol%	20 mol%	K_3PO_4	5 mM	75 °C	S.M.
4	20 mol%	40 mol%	K_3PO_4	10 mM	80 °C	Slight Decomp.
5	20 mol%	40 mol%	K_3PO_4	3.7 mM	Reflux	Decomp.

As the reaction conditions became progressively harsher there was further evidence of starting material decomposition. Under reflux, and using a double loading of the catalyst, the starting material was completely destroyed. The higher temperature of the reaction further stimulated decomposition and TLC analysis of the reaction showed a complex mixture of products with similar polarity. Efforts to isolate the constituent products did not deliver the desired macrocycle but gave mixtures of decomposition products instead.

Having encountered serious difficulties in using copper-catalysed amination of alkynyl bromides to affect the intramolecular coupling, several alternative procedures were investigated. The field of ynamide synthesis has been a fertile area of chemistry in recent years and so there are protocols that can be used to undertake this type of transformation.

Initially, the reaction was performed using iron-catalysed conditions reported by Zhang and co-workers.[122] Their standard conditions involve the utilisation of $FeCl_3 \cdot 6H_2O$ as the metal source, DMEDA as the ligand and K_2CO_3 as the base. This example involves the reaction between an alkynyl bromide and an amide and is generally performed in toluene at 90 °C. Zhang reported successful coupling when using oxazolidinones, sulfonamides and in one example, a 5-membered lactam. No examples were reported when the reaction was carried out intramolecularly, but the reaction was attempted nevertheless (Table 6, entry 1). In this case there was no indication that the reaction had progressed at all. No new products were evident in the reaction mixture after 22 hours and the starting material was recovered. No by-products or decomposition products were evident either. This negative result meant that the reacton did not merit any further investigation and alternative catalytic systems were explored instead.

It is worthwhile noting that subsequent to the publication of the iron-catalysed system, Buchwald and Bolm reported that in some cases, the outcome of the reactions can be significantly affected by the presence of trace amounts of copper.[152]

Subsequent reaction conditions were tested using protocols that were developed and published independently by Hsung and Danheiser in 2003.[111,153] Both of these protocols were instrumental in developing the field of metal catalysed amidation reactions as a method of preparing ynamides. Danheiser's stoichiometric Cu(I) protocol was carried out using CuI and KHMDS at room temperature as detailed in the literature (Table 6, entry 2).[154] It had shown some impressive versatility when used in the cases of small molecule reactions, but when this protocol was used in our intramolecular case, starting material was not consumed and new products were not isolated.

The first generation Hsung amidation protocol utilised CuCN as a catalyst and the reaction was carried out at 90 °C in toluene. This protocol was not as effective in some cases when compared to the second generation protocol that Hsung published several years later. However, it was still worth attempting the reaction using our substrate because both procedures had produced similar results in the case of couplings involving standard amides (Table 6, entry 3). Unfortunately this reaction was also unsuccessful and there was no consumption of starting material.

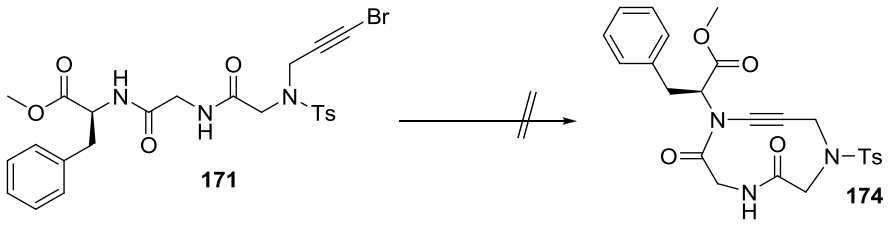
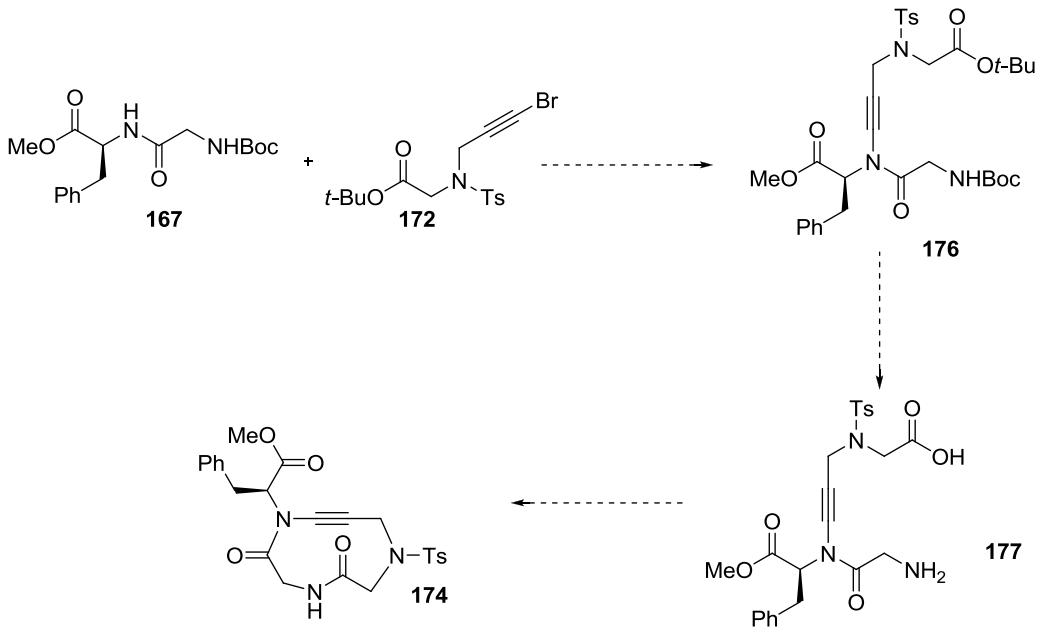


Table 6: Alternative Conditions for Amidation Reaction		
Entry	**Conditions**	**Results**
1	$FeCl_3 \cdot 6H_2O$, DMEDA, K_2CO_3 Toluene, 90 °C	No Reaction
2	CuI, KHMDS, Pyridine THF, r.t.	No Reaction
3	CuCN, K_3PO_4, Pyridine Toluene, 90 °C	No Reaction

Having investigated numerous conditions to affect the cyclisation, but without success, an alternative approach was considered. Rather than trying to perform ring closure by intramolecular reaction to give the ynamide, a group that can sometimes be difficult to produce and uses chemistry that is still in its infancy, it was decided to try and install this group earlier in the reaction sequence and then close the ring by amide bond formation in the last step (Scheme 46). Due to the likely ring strain in the target system, this would appear to be a more robust approach and uses well-established chemistry for the intended cyclisation reaction. The ring closing stage in the synthesis would now involve tried and tested chemistry that has been developed and perfected for decades. In addition, there is an abundance of different coupling reagents that could be used for amide coupling reactions and there are also many less conventional alternatives that could be explored in order to find the optimal approach.

In the new approach, coupling of alkynyl bromide **172** with amide **167** would be carried out, followed by a double deprotection of the required termini to afford the cyclisation precursor **177**. Finally, amide coupling chemistry would furnish the desired ynamide macrocycle **174**.



Scheme 46: Alternate Approach to Cyclic Ynamide Target

Before this approach could be considered, the synthesis of the ynamide unit needed to be tackled. Utilising previously synthesised fragments **167** and **172**, the reaction was carried out using Hsung second generation conditions and Danheisers stoichiometric approach

(Table 7, entries 1 and 2). This system was investigated despite there being two possible amide centres at which the desired coupling could take place. If the alkyne reacted at the carbamate nitrogen instead of the amide as was required, this would still have been an encouraging result because it would prove that the reaction was effective in the case of bromide fragment **172**. Unfortunately, the reactions that were carried out did not proceed and only starting materials could be recovered from the crude mixtures.

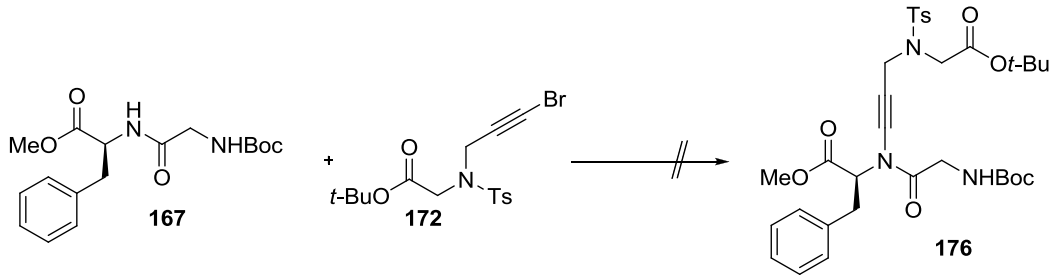
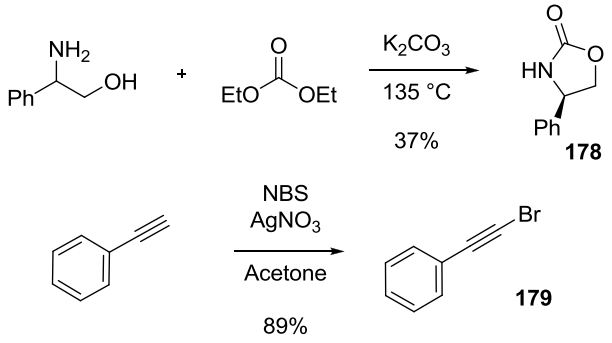


Table 7: Intermolecular Amidation Approach

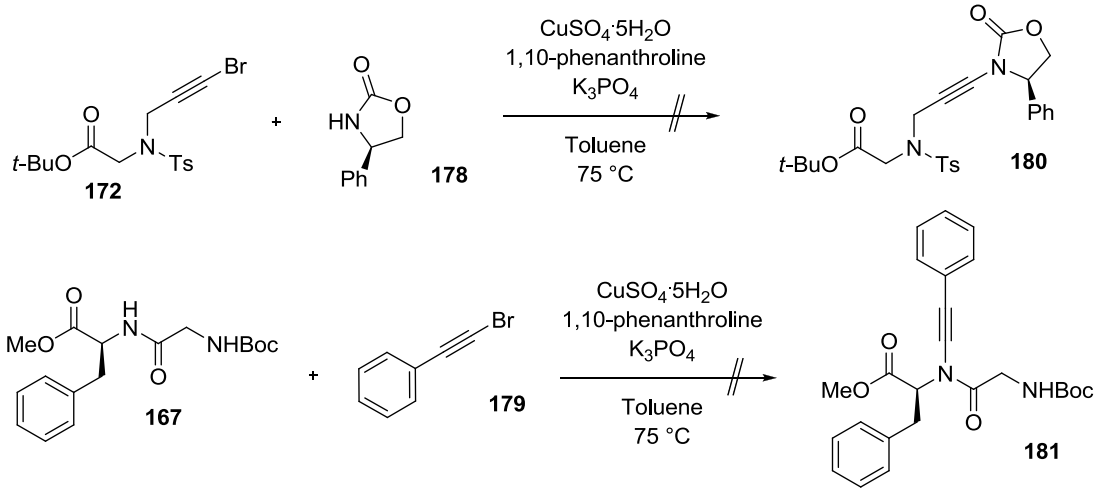
Entry	Conditions	Results
1	$CuSO_4 \cdot 5H_2O$, 1,10-Phenanthroline, K_3PO_4 Toluene, 75 °C	No Reaction
2	CuI, KHMDS, Pyridine THF, r.t.	No Reaction

Following the setback described above, the ynamide coupling chemistry was explored using simpler substrates that had been described in the literature by Hsung and co-workers. The versatility of the procedures had been demonstrated in several intermolecular reactions involving small cyclic and acyclic amide compounds such as oxazolidinone **178** (Scheme 47) and so the required literature substrates were synthesised to allow attempted coupling with the relevant peptide fragments **167** and **172**. Oxazolidinone **178** was synthesised from diethyl carbonate and 2-phenylglycinol in the presence of potassium carbonate. While the yield obtained from the reaction was not particularly good, sufficient quantities of the oxazolidinone were prepared for the subsequent test reactions to be explored. Alkynyl bromide fragment **179** was obtained in excellent yield by reaction of phenylacetylene with NBS.



Scheme 47: Synthesis of Simple Amidation Substrates

With these simple substrates in hand, their coupling to the fragments **167** and **172** was tested first. It was thought that these substrates, which are known to couple effectively, would be useful to help probe the potential reactivity of our substrates in the desired coupling reactions. Due to the fact that fragments **178** and **179** had been coupled successfully using the Hsung second generation protocol, and because the versatility of this approach has been demonstrated in the literature, these conditions were the primary focus for the series of experiments shown in Scheme 48.



Scheme 48: Amidation Coupling Experiments

The oxazolidinone **178** was reacted with alkynyl bromide fragment **172**. The reaction was carried out over a prolonged period of time, but even after several days the reaction had not progressed. During this time, the reaction was supplemented with further catalyst and gentle increases in temperature, but to no avail. A second amidation reaction was carried out in parallel utilising phenylacetylene bromide fragment **179** with dipeptide fragment **167**. The

progression of the reaction was monitored by TLC, which clearly showed newly developed product material. Following workup and isolation, NMR analysis of the new product showed that it was very similar to the alkynyl bromide starting material, suggesting that it was in fact the dimeric product **182** (Figure 45). In any event, it was clearly not the desired ynamide and thus the reaction was abandoned.

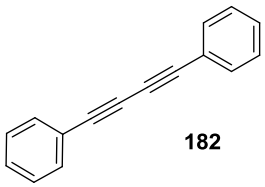
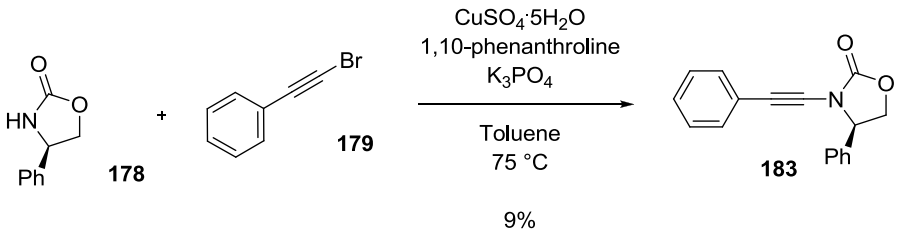


Figure 45: Alkyne Dimer Product

A subsequent variant of this reaction was performed at a higher dilution. The first reaction was carried out at ~50 mM concentration, whilst a subsequent reaction was carried out at ~10 mM. This alteration in concentration prevented formation of the putative dimerisation product, but, the desired product was not produced even after prolonged reaction times.

The failure of the experiments above led us to investigate the reaction using the specific conditions and substrates – compounds **178** and **179** – reported in the literature (Scheme 49). The reaction was attempted in an effort to compare laboratory yields with those reported in the literature. It was hoped that by investigating a specific reaction that was known to be successful, any intrinsic difficulties with the reaction procedure could be identified and understood.



Scheme 49: Attempted Reproduction of Literature Example

Initially, when the reaction was attempted following the literature procedure, the best yield obtained was only 9%. In the literature it had been reported that the reaction could be carried out in greater than 80% yield. It quickly became apparent from the literature that there

were some reproducibility issues relating to the Hsung second generation protocol.[128] In light of this disappointing preliminary result, it was decided that the best course of action was to optimise reaction conditions towards literature yields. These new conditions could then be applied to the desired intramolecular variant of the reaction.

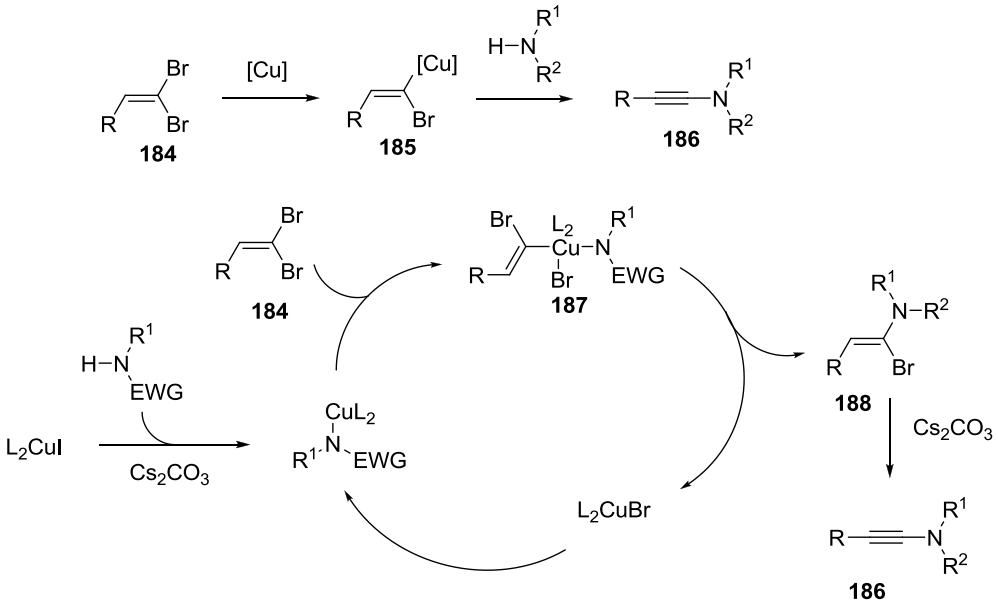
2.2.3 Optimisation of Amidation Reactions

When performing the reaction in the manner described in the literature a high degree of variability in the yields was observed. Initially, the reaction failed to give any product, but on a subsequent attempt, the target compound was isolated in 9% yield (Table 8, entries 1 and 2). Both reactions were performed at almost the same concentration. The reaction seemed to be somewhat erratic, which pointed to some, as yet, undiscovered cause that affects its outcome. In the literature a yield of 88% was reported when this specific reaction was performed on a small scale[118] and a yield of 71% was recorded when the reaction was performed on gram scale.[119]

In a subsequent run, the reaction was carried out at a higher concentration but maintaining the temperature and catalyst loading (Table 8, entry 3). In this case, a sample of K_3PO_4 that had been dried for 12 hours in a Kugelrohr distillation apparatus was used. This gave a marked improvement in yield (43%), which was very encouraging. Unfortunately, a subsequent attempt using exactly the same reaction conditions on slightly larger scale gave a yield of only 22% (Table 8, entry 4). In further experiments, the reaction was performed using a stoichiometric amount of the copper catalyst, an approach previously adopted by Danheiser and co-workers, but this reaction also gave a yield of just 26 % (Table 8, entry 5). Finally, this reaction was attempted at higher temperature, but the yield was largely unaffected (Table 8, entry 6). The approach of drying K_3PO_4 immediately prior to reaction seemed to be somewhat unreliable in this reaction, but the approach was adopted further later in the project.

Table 8: Conditions Tested on Literature Reaction					
Entry	**$CuSO_4 \cdot 5H_2O$**	**1,10-Phenanthroline**	**Conc.**	**Temp.**	**Yield**
1	10 mol%	20 mol%	0.017 M	75 °C	S. M.
2	10 mol%	20 mol%	0.015 M	75 °C	9%
3	10 mol%	20 mol%	0.85 M	75 °C	43%
4	10 mol%	20 mol%	0.85 M	75 °C	22%
5	1 eq.	2 eq.	0.34 M	75 °C	26%
6	1 eq.	2 eq.	0.34 M	100 °C	24%

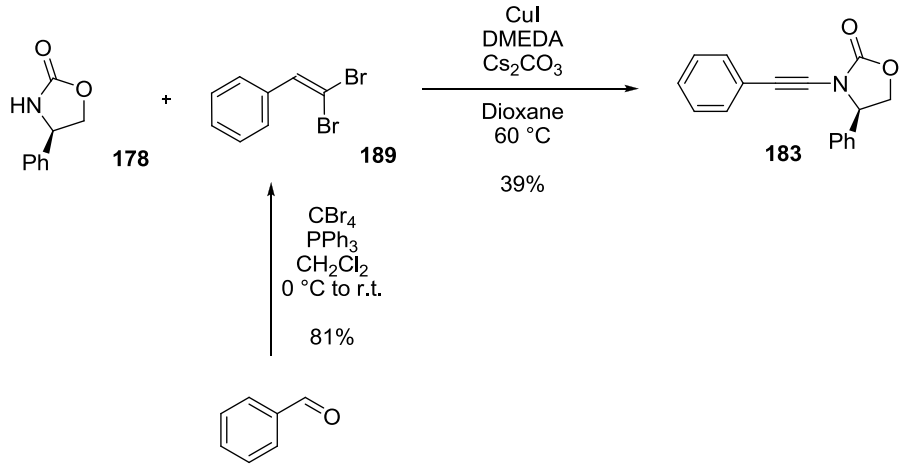
In conjunction with these test reactions, the alternative approach pioneered by Evano and co-workers involving the reaction of a 1,1-dibromoalkene and an amide centre to yield the ynamide, was investigated (Scheme 50).[155] This procedure was reported in 2009 and was demonstrated to be successful using a range of sulfonamides, cyclic carbamates and cyclic amides. The general reaction was reported alongside its proposed mechanism, which is based on the known reactivity of *trans* C-Br bonds in dibromides, such as **184**, towards oxidative insertion. This substrate is believed to undergo reaction to give the organocopper intermediate **187** which then participates in regioselective coupling to produce the bromo enamine **188**. Finally dehydrobromination in the presence of Cs_2CO_3 produces the target ynamide **186**. This final step was proven by isolation of an intermediate of type **188** and its subsequent transformation into ynamide **186** by treatment with Cs_2CO_3 in a separate reaction.



Scheme 50: Evano's General Protocol and Proposed Mechanism

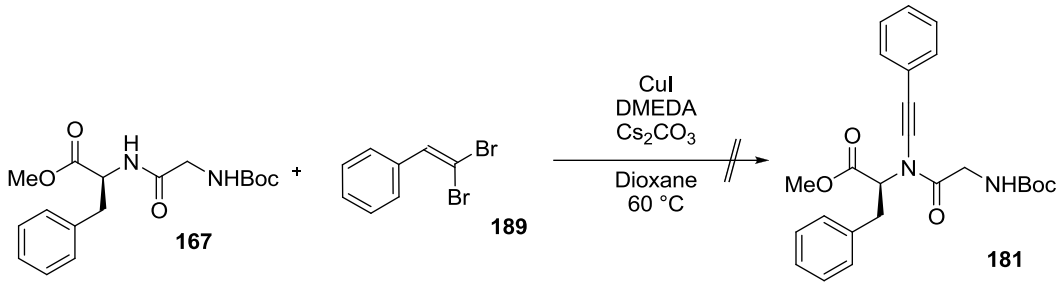
This type of reaction was attempted using analogous starting substrates in an effort to prepare the ynamide **183** (Scheme 51). This approach was tested before investigating the optimisation of Hsung's protocol in the hope that these conditions would be more reliable and could be applied to the target system. The main difficulty with this approach would be the installation of the the 1,1-dibromoalkene group on the tripeptide fragment prior to cyclisation, which would most likely be achieved from an aldehyde.

The 1,1-dibromoalkene **189** was synthesised by reaction of benzaldehyde with CBr_4, using the Corey-Fuchs procedure:[156,157] the compound was isolated in good yield after column chromatography. The 1,1-dibromoalkene **189** was then reacted with oxazolidinone **178** using CuI and DMEDA as catalyst and ligand respectively, at 60 °C in dioxane, to give the coupled product **183** in a yield of 39%.



Scheme 51: Employing Evano's Approach

It was found that yields are reduced if the reaction is performed at higher temperatures – for example, when the reaction was performed at 75 °C, a yield of 29% was obtained. This approach did not yield results that were significantly better than those obtained using the Hsung protocol. Even so, it was decided to test the reactivity of the simple 1,1-dibromoalkene with the dipeptide fragment **167** under the Evano conditions to determine if reaction would take place at the desired centre (Scheme 52).



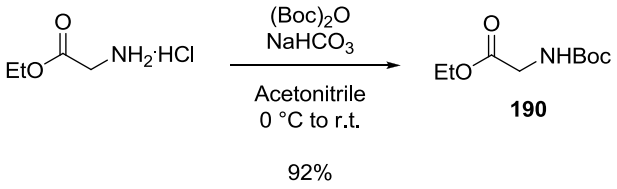
Scheme 52: Application of Evano's Approach to Target Dipeptide

The reaction was attempted several times but, as with other methods, it failed to deliver the required product. When the reaction was performed at 60 °C there was no reaction and at higher temperatures it appeared that the dibromoalkene starting material decomposed – partial decomposition was observed at 70 °C and complete decomposition took place at 100 °C.

Having found that this approach was unlikely to be successful, efforts were once again focused on optimising the Hsung protocol. A further search of the literature revealed an example where this type of alkyne amide coupling had been performed on a protected glycine analogue **190** in reasonably high yields.[158] This system was then chosen for all future

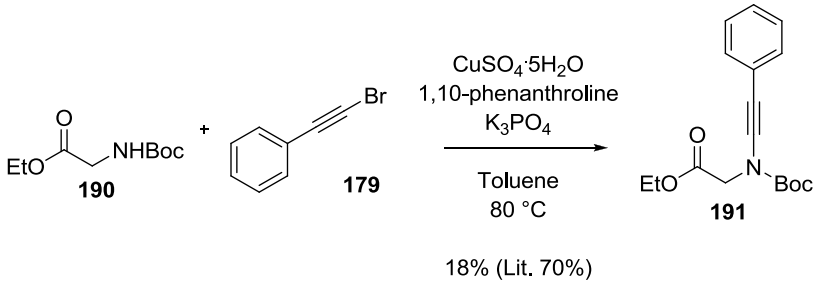
optimisation work as it bore a close resemblance to the polypeptide systems that were required for the actual target.

The glycine analogue was synthesised, very simply, by reacting glycine ethyl ester hydrochloride with di-*tert*-butyl dicarbonate in the presence of $NaHCO_3$.[159] This reaction gave the target carbamate **190** in 92% yield and so provided plenty of material for optimisation reactions (Scheme 53).



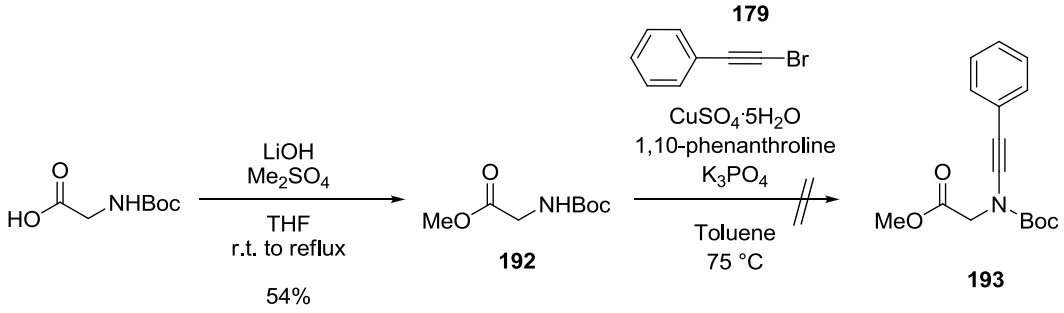
Scheme 53: Synthesis of Glycine Analogue

In the literature example, **190** had been coupled with phenylacetylene bromide **179** to give the desired ynamide **191** in 70% yield. The first attempt with this reaction gave a very disappointing yield of just 18% (Scheme 54). This result was achieved following the standard Hsung protocol that had been utilised previously. The substrates were treated with $CuSO_4 \cdot 5H_2O$ (10 mol%), 1,10-phenanthroline (20 mol%) and K_3PO_4 (2 eq.) in toluene at 80 °C. The reaction was performed at reasonably high concentration, and the mass of catalyst and ligand was doubled mid way through the reaction because it was apparent that it was not proceeding efficiently towards completion. Increasing the concentration appeared to have no effect on the outcome of the reaction.



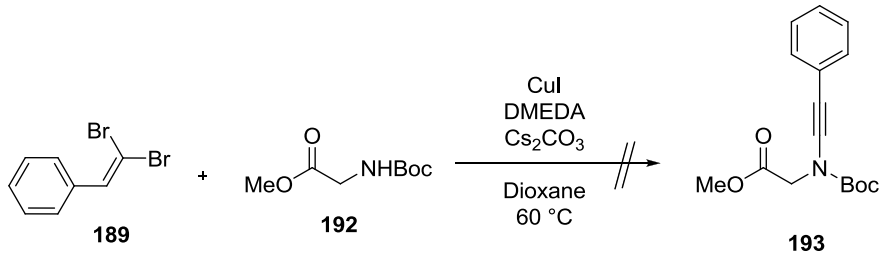
Scheme 54: Amidation of Glycine Derivative

It is interesting to note that the analogous reaction was carried out in the laboratory using the methyl ester analogue (Scheme 55). The methyl ester **192** was prepared[160] and reacted with alkynyl bromide **179** using similar conditions to those previously mentioned. Curiously, the reaction failed and there was no apparent progression at all. The reaction was also repeated at 80 °C but again the substrate appeared to be completely unreactive.



Scheme 55: Attempted Amidation With Methyl Ester of Glycine

While this system was being tested, it also seemed prudent to explore the reaction of the 1,1-dibromoalkene in order to gain a more complete picture of the methyl esters reactivity. This reaction was carried out under standard Evano conditions, but once again no reaction took place after 24 hours (Scheme 56). There is no satisfactory explanation for the failure of both of these reactions and the use of the methyl ester was abandoned in favour of the ethyl ester.

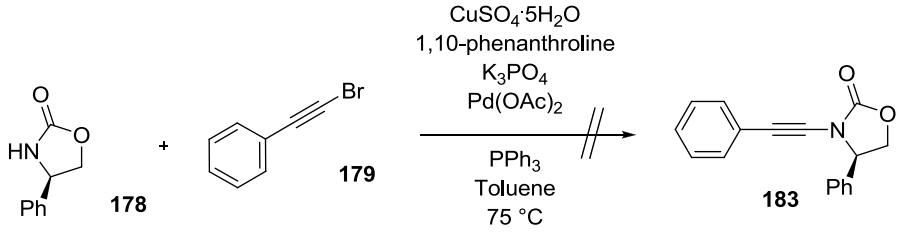


Scheme 56: Evano Approach Towards Ynamide 187

At this stage, studies were performed in an attempt to improve the reaction and identify the optimum conditions for application to the desired system. Initially, it was proposed that the use of an additional metal additive may have a reinforcing effect on the reaction. The basis for this was the possibility that cross contamination of metal sources can often occur in small amounts. It was postulated that perhaps another metal might be at work in addition to the copper in Hsung's published reactions.

To this end, several available metals were screened at 10 mol% alongside standard Hsung coupling conditions. The metals included cobalt, nickel, manganese, zinc, rhodium and copper (I). Palladium had been tested previously, in the reaction of **178** and **179**, during some preliminary work and had been found to completely inhibit the coupling reaction, with no consumption of starting material (Scheme 57). For this reason no further reactions were

carried out using palladium compounds. The results from this series of reactions are shown in Table 8 and in some cases the yields obtained were very encouraging.



Scheme 57: Use of Palladium Additive

In several of the reactions that were carried out the yields showed significant improvement over the initial yield of 18%. This appeared to support the proposal that additional metal additives were of appreciable synergistic benefit to the reaction. The presence of zinc appeared to inhibit the reaction entirely (Table 9, entry 1), whereas iron had only a slight effect (entries 2 and 3). The two additives that delivered the most significant improvements were the Co(II) and Ni(II) salts, therefore these two metals received further attention (entries 6 and 7). Mn(II) also showed some promise (entries 4 and 5), but was not investigated further because it did not show as much improvement as Ni or Co. The addition of Rh (III) resulted in comparable yields to those obtained without metal additives (entry 8).

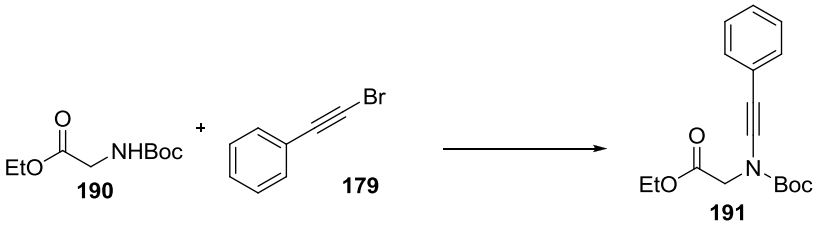


Table 9: Screening of metal additives		
Entry	**Metal Salt**	**Isolated Yield**
1	$ZnCl_2$	0%
2	$FeCl_2$	26%
3	$FeCl_3$	16%
4	$Mn(OAc)_2$	31%
5	$Mn(acac)_3$	23%
6	$Co(acac)_2$	37%
7	$NiBr_2$	36%
8	$Rh(acac)_3$	20%

Further investigation of the cobalt additive revealed some interesting results. TLC analysis of the reaction mixture showed almost complete consumption of the alkynyl bromide. The bromide being the limiting reagent in the reaction, this appeared to be a positive outcome at first glance but it was quickly apparent from the yields that all of the bromide had not been utilised to give the ynamide target. Instead, a second product was isolated that appeared to be the alkynyl dimer **182** and this was confirmed by mass spectrometry (Figure 45).

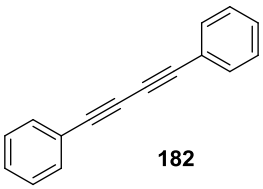
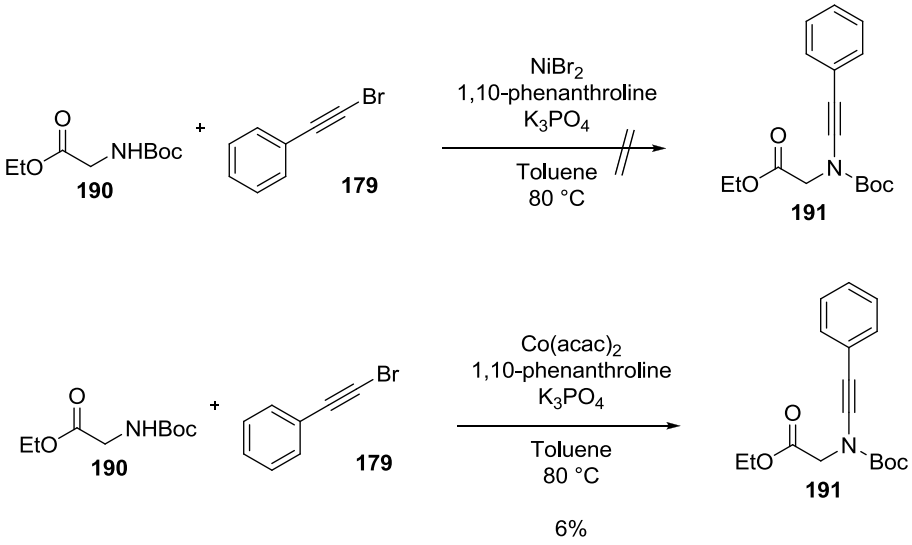


Figure 46: Alkynyl Dimer Product

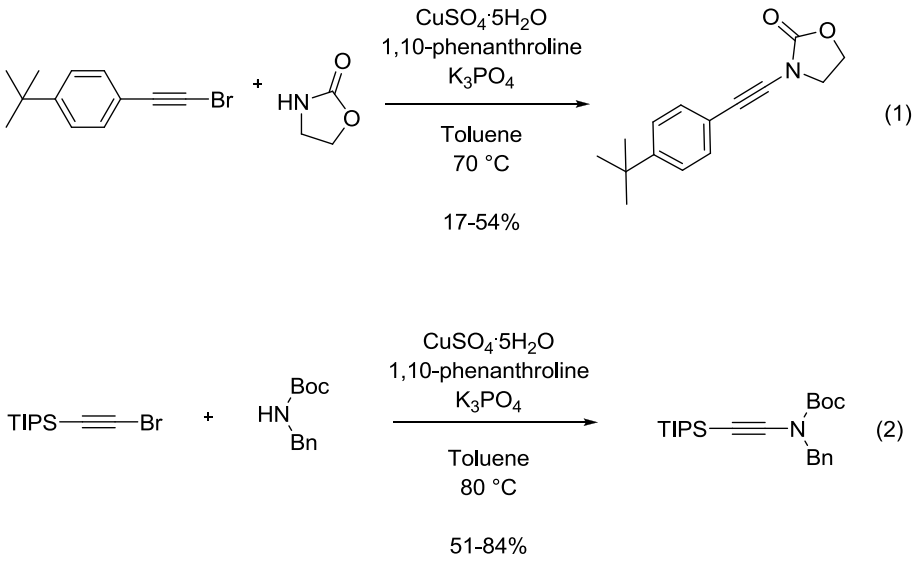
From the revelation that Co(II) was an efficient dimerisation catalyst, more so than an amidation catalyst, it was decided to focus attention exclusively on Ni(II) as an additive. In addition, a test reaction was carried out using the best Co(II) and Ni(II) sources as a catalyst in place of Cu(II) to determine if these metals could catalyse the coupling reaction in the absence of copper. In the nickel case, no formation of the ynamide was detected. Instead, only a small quantity of the alkynyl dimer was isolated. In the cobalt case, the target ynamide was isolated

in 6% yield and more of the dimer was obtained. These results indicate that these metals were largely ineffective as catalysts in this type of reaction, in the absence of copper.



Scheme 58: Test Reactions of Metal Additives in the Absence of Copper

At this time several reports appeared in the literature suggesting that it was difficult to reproduce the high yields reported by Richard Hsung and his co-workers in their original publications. A study was performed by Skrydstrup and co-workers in which batches of K_3PO_4 from various well-known chemical suppliers were tested.[161] A correlation was found between yield of reaction and quantity of K_3PO_4 hydrates. K_3PO_4 is particularly hygroscopic and can often be found as a lumpy white solid. Using two simple test reactions (Scheme 59), Skrydstrup and co-workers found that the yields were highly dependent on the commercial source of K_3PO_4. Depending on the supplier, the yields ranged from 17- 54% (reaction 1) and 51-84% (reaction 2). It was found that in cases where low yields were obtained, the K_3PO_4 source had very high quantities of $K_3PO_4 \cdot 7H_2O$ and $K_3PO_4 \cdot 1.5H_2O$ hydrates. When the highest yields were obtained the batch of K_3PO_4 contained very low quantities of hydrates.



Scheme 59: Skrydstrup's K_3PO_4 Screening Reactions

In order to investigate this in the laboratory on the chosen model system, the K_3PO_4 was first dried in a Kugelrohr distillation apparatus over a prolonged period of time. This approach had been investigated briefly in a previous ynamide formation reaction (*cf.* Table 8, entries 3 and 4). In the previous case, the dried K_3PO_4 showed some improvement on one occasion, but the results could not be replicated on a second attempt. The freshly dried K_3PO_4 was tested in the reaction of a glycine derivative (*cf.* Scheme 54) and was found to give only marginal improvement of 3-5% yield. This was an extremely disappointing result but did not mark the end of this second avenue of investigation. It was also found that the small improvement was quickly lost as the K_3PO_4 reabsorbed water vapour from the air.

In an alternative approach, the K_3PO_4 was ground into a fine powder using a mortar and pestle immediately prior to addition. Furthermore, the toluene that was used as solvent was thoroughly degassed by purging with argon before use in the reaction.

Previous studies had found that scrupulously dried $CuSO_4$ was detrimental to the reaction outcome so this was never pursued as a possible avenue for investigation. At this stage, several reactions were carried out using different sources of Ni(II) in an effort to determine the best additive. These reactions tested the new approach using ground K_3PO_4 alongside the various Ni sources (Table 10). The metal additives were used at 10 mol% in conjunction with $CuSO_4 \cdot 5H_2O$.

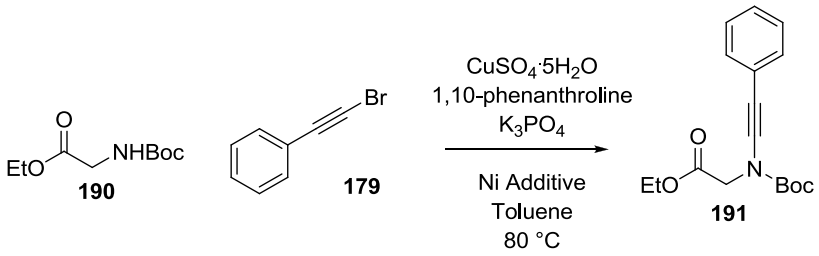


Table 10: Screening of Ni(II) Additives		
Entry	**Metal Salt**	**Isolated Yield**
1	$NiCl_2$	72%
2	$Ni(acac)_2$	73%
3	$NiBr_2$	49%

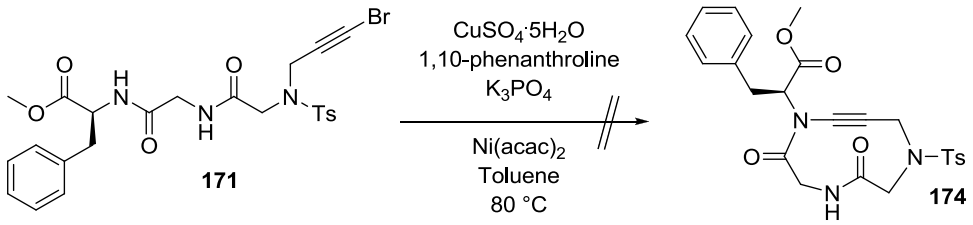
Results from this series of screening reactions were very promising and showed that the previously reported literature yield had been surpassed by 3% when using $Ni(acac)_2$ as an additive (Table 10, entry 2). $NiCl_2$ also showed a comparable improvement (entry 1) where $NiBr_2$ was not as impressive (entry 3).

When taken straight from the bottle, K_3PO_4 is a white solid with a very lumpy consistency. By grinding the K_3PO_4 to a fine white powder immediately prior to reaction, the dry inner surfaces were exposed to the reactant material. While an improvement in yield was the goal of this latest series of reactions, such a large increase was unexpected. It was important at this stage to ascertain the contribution of both of the altered conditions i.e. the nickel additive and the finely ground K_3PO_4.

In order to do this, two subsequent reactions were carried out to probe the effects of the individual conditions. One reaction was carried out using ground K_3PO_4 and degassed toluene in the presence of $Ni(acac)_2$, where one was carried out in the absence of $Ni(acac)_2$. These reactions gave 55% and 62% respectively. While it seems that the greatest improvement in yield comes from the dry K_3PO_4, the addition of the $Ni(acac)_2$ was also improving the reaction yield. As a result, both alterations to the procedure were maintained in subsequent reactions. Having achieved such an improved yield, it was decided that the best conditions had been found.

When the optimised conditions were applied to the desired model system, the reaction was still unsuccessful (Scheme 60). Analysis of the crude mixture after prolonged reaction times showed that significant amounts of starting material were still present and no new products were obtained except very small quantities of decomposition products. This was

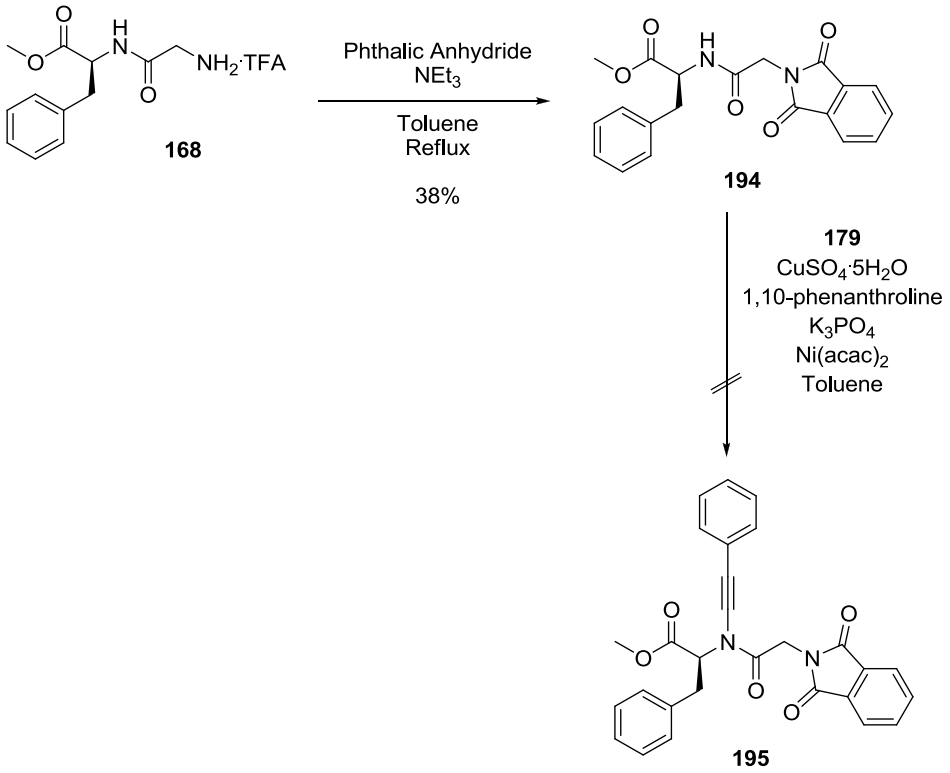
extremely disappointing as it was hoped that the modified approach would produce at least some of the cyclisation product. The isolation of even a small quantity of target material would have shown that the cyclisation reaction could take place to some degree and that it had potential for optimisation.



Scheme 60: Application of Optimised Conditions to Target Synthesis

The newly developed reaction conditions were applied to an alternative intermolecular coupling reaction to determine if they were effective in achieving an ynamide synthesis using the dipeptide fragment. For this experiment, the dipeptide was protected at the terminal amine, so that no N-H was present at this position and the reaction would only take place at the desired amide centre (Scheme 61).

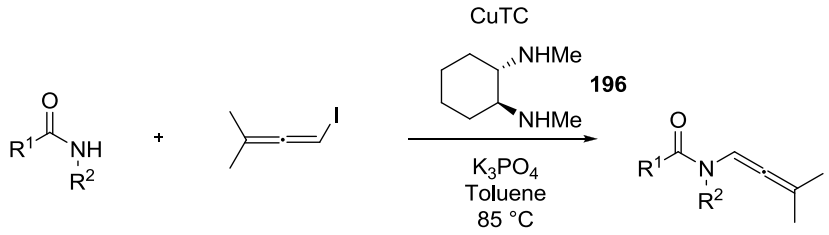
The TFA salt of dipeptide **168** was protected at the amine terminus using a phthalimide group.[162] The protection reaction was carried out using phthalic anhydride at reflux and a very modest yield (38%) was obtained. This was not a serious drawback, because only relatively small quantities of **194** were required for these test reactions. Unfortunately, the intermolecular coupling reaction of the substrate **194** was also unsuccessful. This may have been due to steric factors, perhaps with regards to the two bulky aromatic substituents flanking either side of the amide centre. Analysis of the reaction mixture again gave no indication that the starting materials had reacted or that there were any new products.



Scheme 61: Intermolecular Amidation Using Modified Conditions

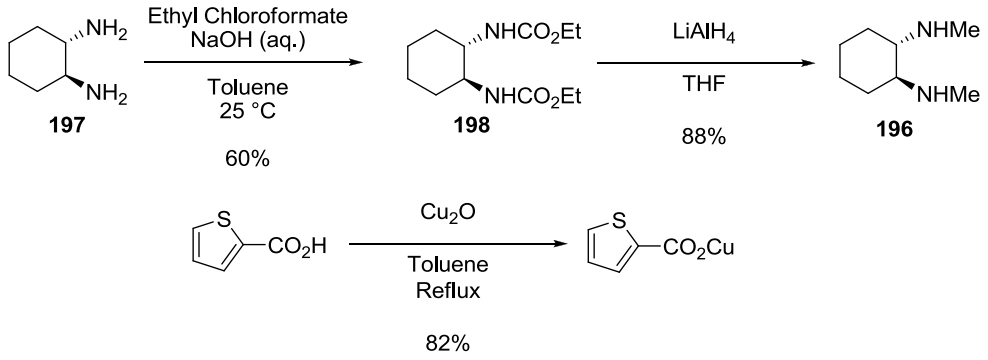
It was clear at this point that the Hsung second generation protocol was not delivering useful results in spite of all attempts to improve it. Consequently, an alternate set of reaction conditions was tested.

In recent literature, a catalytic system for the synthesis of allenamides had been reported (Scheme 62). This chemistry had also been demonstrated peripherally with regard to the synthesis of ynamides.[163] This approach appeared to be promising and so it was worthy of further investigation. Again, this chemistry was to be carried out with a halogenated alkyne and an amide, in this case utilising copper thiophenecarboxylate as a catalyst and a 1,2-dimethylaminocyclohexane ligand **196**. The literature procedure stated that the alkynyl bromide or alkynyl iodide were both suitable for this reaction.



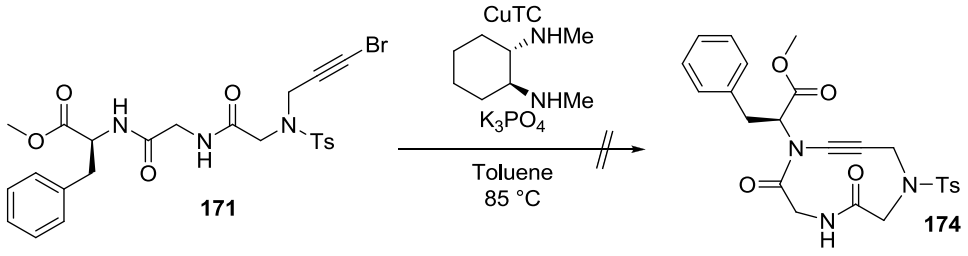
Scheme 62: Allenamide Synthesis Catalytic System

The required ligand and copper complex were easily synthesised by methods shown in Scheme 63. The dimethylaminocyclohexane ligand was synthesised from racemic *trans*-1,2-diaminocyclohexane **197** by reacting with ethyl chloroformate to give the bis-carbamate **198**, followed by reduction using $LiAlH_4$ to furnish **196**.[164] The copper complex was synthesised in 82% yield, by reaction of 2-thiophenecarboxylic acid with copper (I) oxide in toluene at reflux.[165]



Scheme 63: Synthesis of Ligand and Catalyst

Having successfully isolated both the ligand and the copper complex, the literature conditions were applied to the system of interest (Scheme 64). The reaction was carried out over the course of 24 hours, during which time a new product was clearly visible by TLC analysis. Although the reaction did not progress to completion, it was worked up and the new product was isolated. Unfortunately, the product isolated was not the desired macrocycle.



Scheme 64: Alternative Approach to Intramolecular Amidation

Closer investigation of the product identified it as the dimeric product **199** formed by coupling at the terminus of the alkyne. The proposed structure of this by-product is illustrated in Figure 47. This structure was confirmed using mass spectrometry analysis and NMR methods. This result was in itself encouraging because, until this point, there was no evidence

for copper complex formation at the alkynyl position of tripeptide **171**. This result proved that this complex was in fact being formed and that, in theory, it should be possible to couple this to an amide.

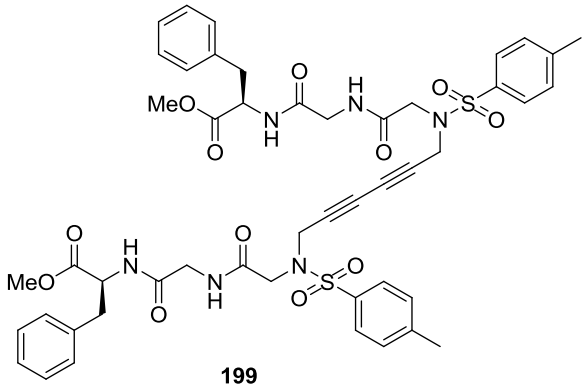
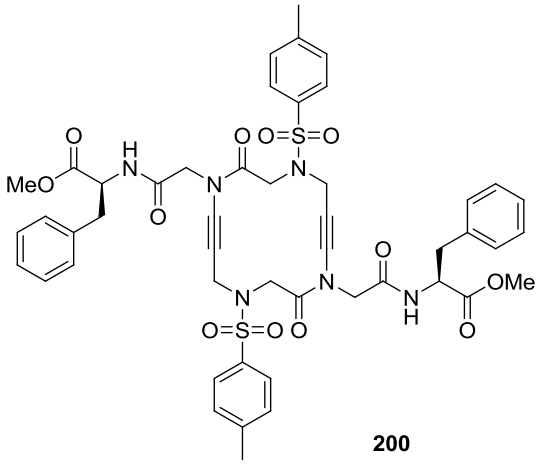


Figure 47: Isolated Dimer Product

Following this encouraging result the reaction was carried out once again, this time allowing it to take place over a longer period of time and with close monitoring of its progress by TLC. This second attempt was also carried out in a significantly more dilute solution compared to the first attempt, the tripeptide substrate was dissolved in toluene to produce a 4.5 mM solution. A very small amount of another new product became apparent by TLC analysis and this was isolated for the purposes of characterisation. Initially, there was cause for excitement when the ^{1}H NMR spectrum showed that one of the two amide hydrogens had been removed. However, after comparing with NMR spectra of the precursor it was quickly realised that the wrong hydrogen had been displaced. This suggested that the alkynyl bromide had in fact coupled onto the wrong amide centre and formed a 7-membered ring. As was discussed previously, this is is a highly unlikely outcome because the ring strain would be far too great due to the linear alkyne system imposed by the triple bond. The only reasonable answer was that another dimerisation reaction had taken place to give the 14-membered macrocycle **200** shown in Figure 48 instead of the required 10-membered lactam.



200

Figure 48: Macrocyclic Dimer Product

It was now quite clear that the intramolecular coupling reaction to make a 10-membered ring was severely disfavoured in this case. This may be due to the propensity for amide groups to exist in a *trans* configuration thus preventing the chain from turning back upon itself. As a result, the alkynyl bromide may not be able to orient itself in a suitable proximity to the amide for reaction to take place. This is unfortunate, and somewhat unexpected, as this type of chain must, theoretically, be able to fold back on itself in such a way, otherwise it would not be viable for a β-turn in the natural system. In light of these results, the next objective was to consider alternative structures that could be synthesized and would not be subject to the same problem.

2.2.4 Proline Model Structure

The solution to the intramolecular amidation problem was considered carefully and it was realised that the best approach might be to alter one of the residues in the tripeptide chain. In naturally occurring β-turns, a proline residue is often located at the i+1 or i+2 position.[166-168] As was discussed previously in the research of Boger and co-workers, glycine and proline are often located in the turn and perform a structural role. The proline residue can effectively enforce the conformation required for turn formation. Therefore using this logic, it was anticipated that a proline residue could help position the two reacting functional groups in close proximity, in the target model system. Figure 49 displays the envisioned model system **201** as well as its alkynyl bromide precursor **202**. In theory, it should be far easier for the alkynyl bromide in **202** to approach the desired amide, resulting in an increased likelihood of reaction.

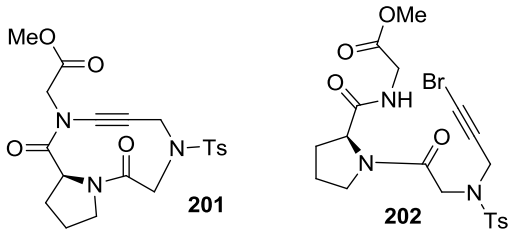
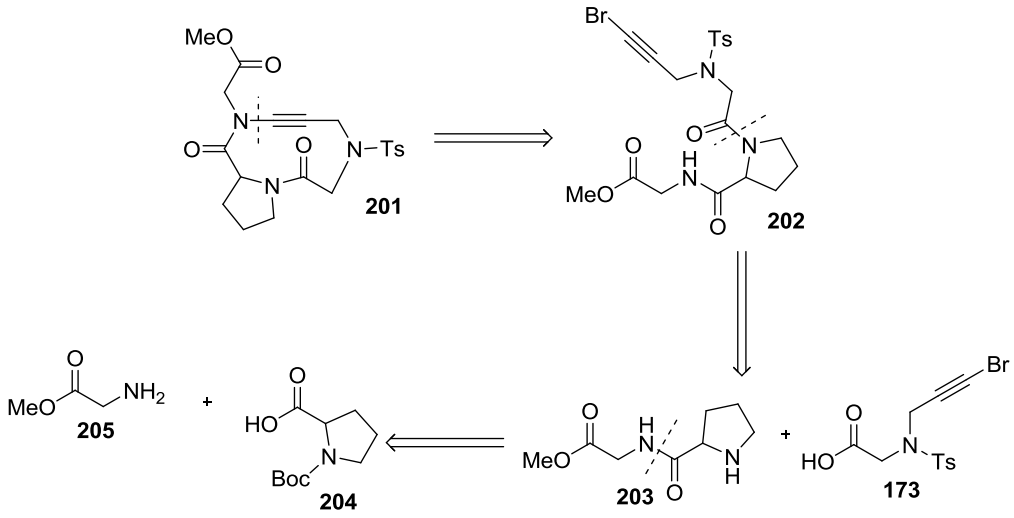


Figure 49: Proposed Proline Analogue and Alkynyl Bromide Precursor

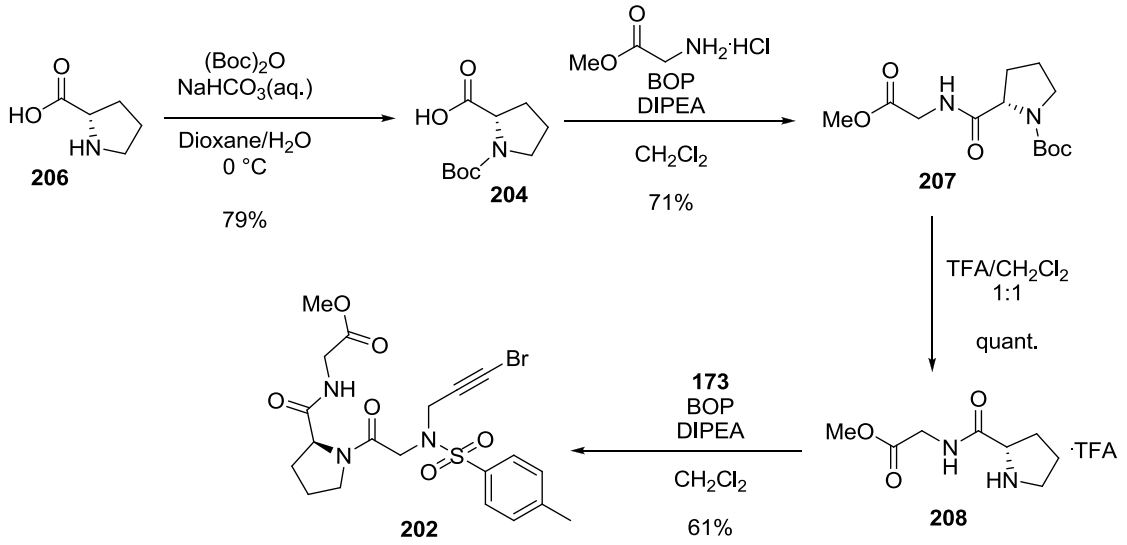
This alternative target can be synthesised utilising some of the fragments previously used in the attempt to synthesise the macrocycle **174**; the retrosynthesis is illustrated in Scheme 65. Cyclic target **201** is opened in a way that implies the type of ynamide coupling reactions explored previously would be employed to cyclise the tripeptidic alkynyl bromide **202**. This open-chain tripeptide fragment incorporates glycine and proline amino acids residues. This is then easily disconnected into two fragments: the dipeptide fragment **203** and the previously synthesised carboxylic acid **173**. This carboxylic acid is easily synthesised from propargylamine, using the same procedure of *N*-alkylation and alkynyl bromination followed by removal of the *t*-butyl ester as was described earlier. Proline dipeptide **203** was further simplified to give the two suitably protected amino acid derivatives **204** and **205**. These compounds are either commercially available or easily synthesised in one step. This provided access to the alkynyl bromide **202** in the simplest manner using familiar chemistry. The use of the proline residue should not only bring the two reacting groups into close proximity, it should also provide a dipeptide fragment, in the form of **203**, for testing in intermolecular ynamide couplings. By protecting the proline nitrogen, there is only one suitable amide centre at which a corresponding alkynyl group can couple.



Scheme 65: Retrosynthetic Analysis of Proline Tripeptide

With a feasible retrosynthesis in place, the process of synthesising the tripeptide precursor commenced (Scheme 66). Firstly, L-proline was Boc-protected using di-*tert*-butyl dicarbonate to give **204** in good yield. This compound was then coupled to glycine methyl ester hydrochloride using standard coupling conditions, utilising BOP and DIPEA. Initially, the reaction was carried out under the previously utilised HOBt/DCC coupling conditions as they were the most familiar at this stage. Unfortunately, the reaction delivered only 12% yield, which was not adequate for the further steps in the synthesis. Utilising different conditions, BOP was found to be an effective reagent when coupling to a proline residue and so was subsequently adopted for all of the couplings in this route.[169]

The Boc-protected dipeptide fragment was easily deprotected using a 1:1 mixture of TFA in CH_2Cl_2 giving the TFA salt **208** in quantitative yield. Following the discovery that the TFA salt was far more effective at the analogous stage in the previous model synthesis, it was decided to, once again, scrupulously remove all traces of excess TFA and proceed using the salt as before. This strategy proved to be effective and the dipeptide was coupled to the previously synthesised alkynyl bromide fragment **173** in a straightforward manner and in reasonably good yield. This resulted in the proline derived tripeptide fragment **202**, which was now ready to undergo testing in an intramolecular alkyne amidation reaction.



Scheme 66: Synthesis of Proline Tripeptide

Several reactions were performed, much as before, to test the capability of this tripeptide to react at the desired centre (Table 11). The reaction was carried out using both the CuTC catalyst system and Hsung's second generation protocol. When the $CuSO_4$ catalysed reaction was performed under standard conditions it was unsuccessful (entry 1); only minor decomposition products were found by TLC analysis, and most of the starting material was retained. All of the reaction constituents were separated by chromatography to confirm that none of the products were the target cycle. Subsequently, the reaction was repeated at reflux temperatures (entry 2) and under these conditions greater amounts of the same decomposition products were isolated along with significant amounts of starting material. Both of these reactions were carried out at 5 mM concentration to minimise the possibility of dimerisation and they were allowed to proceed for a period of between 18 and 24 hours.

The use of the CuTC standard conditions resulted in what appeared to be very small amounts of a new material by TLC (entry 3). This was isolated and characterised using NMR techniques. Only a small amount of this new product could be isolated and so results were somewhat ambiguous, but it appeared to be the product arising from dimerisation at the alkynyl position. Carrying out the reaction with higher concentrations of the ligand and the metal only yielded the same disappointing results (entry 4). It was apparent that the cyclisation reaction was just as ineffective when using a proline residue in the tripeptide chain as it had been in the previous model compound.

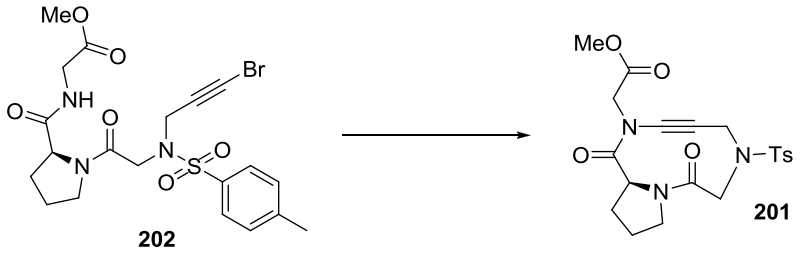
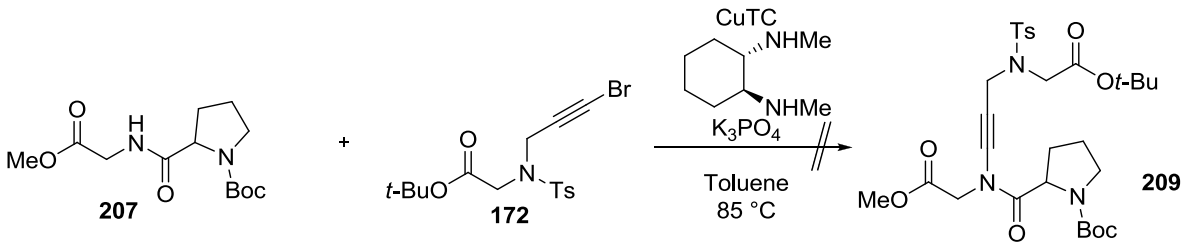


Table 11: Conditions For Cyclisation		
Entry	**Conditions**	**Result**
1	$CuSO_4 \cdot 5H_2O$ (20 mol%), 1,10-phenanthroline (40 mol%), K_3PO_4 (2 eq.), Toluene (5 mM), 75 °C	Slight Decomposition
2	$CuSO_4 \cdot 5H_2O$ (20 mol%), 1,10-phenanthroline (40 mol%), K_3PO_4 (2 eq.), Toluene (5 mM), Reflux	Significant Decomposition
3	CuTC (7 mol%), Diamine ligand **43** (15 mol%), K_3PO_4 (2 eq.), Toluene (4 mM), 85 °C	Possible Dimer
4	CuTC (15 mol%), Diamine ligand **43** (30 mol%), K_3PO_4 (2.5 eq.), Toluene (4 mM), 85 °C	Possible Dimer

Once the problems with the cyclisation reaction became clear, the intermolecular variant of the reaction was performed using the protected proline dipeptide **207** and bromide **172**. The reaction was carried out using the same CuTC promoted reaction conditions that had been utilised previously (Scheme 67). The reaction was performed over the course of 24 hours and TLC analysis of the reaction mixture revealed products along with large quantities of unreacted starting material. After work up and purification, it was found that all of the product compounds were undesired by-products. This was an extremely disappointing result and the reaction was performed again over a longer period to determine whether any of the products would develop further and allow a more conclusive NMR analysis to be performed. Unfortunately, after 48 hours the reaction gave the same poor results as in the first instance.



Scheme 67: Intermolecular Reaction of Proline Tripeptide

The use of copper-mediated coupling chemistry to form an ynamide had appeared to be a promising approach at the inception of this project. The development of ynamide synthesis chemistry has opened up a number of avenues for investigation in organic synthesis and it was hoped that it could allow us to synthesise the peptidomimetic system of interest. Unfortunately, the chemistry proved to be unsuitable for the preparation of these systems, despite many different attempts with varying reaction parameters and catalytic systems.

The various reactions performed often had similar outcomes, with large amounts of unreacted starting material being recovered and small quantities of decomposition or dimerisation products being formed in some cases. It was clear at this stage that the use of amidative coupling was ineffective when applied to the synthesis of the target ynamides. Perhaps the development of more general methods for the synthesis of ynamides might allow this approach to be revisited in future, but at this point an alternative approach to the synthesis of mimetics of leu-enkephalin had to be considered.

2.3 Synthesis of Alkyne Model Compounds

2.3.1 Alkyne Targets and Retrosynthesis

Due to the failure of the amidative cross coupling approach to ynamide synthesis to deliver the desired systems, the project targets were revised. The complexity of the target systems appeared to be unfavourable in conjunction with this type of chemistry. Previous literature examples were often successful when simpler substrates were employed and in some cases it was found that when using a simple amide as opposed to a carbamate or sulfonamide, the yields often ranged from moderate to poor. Although the chemistry had already been proven on intramolecular examples to form medium-sized rings, none of these compounds possessed an amide bond in the ring system or incorporated extended functionality leading away from the ring.

The cyclisation reaction may have failed because the amide bond prevented the chain from folding back in the desired conformation. This problem should have been solved by the inclusion of a proline residue in the structure, but this approach delivered similarly poor results. Alternatively, the combination of amide bond and triple bond in the same ring may simply have made the product too strained. However, this would seem to be discounted as the

natural peptide adopts a very similar conformation without difficulty. It is perhaps more likely that there is some inherent reactivity problem with one or both of the reacting centres. The peptidic systems that are involved in these transformations may be too sterically crowded on either side of the amide for the reaction to take place, which would account for the lack of success in both the intramolecular and intermolecular examples. None of the reactions used to perform the intermolecular coupling were successful and these tested the reactivity of both the alkynyl bromide and the amide centre using varied reaction conditions.

As a consequence of the serious difficulties encountered when using the amidative cross coupling reaction, a different target was selected for the mimetic. This new structure still incorporates a 10-membered ring system and is illustrated in Figure 50.

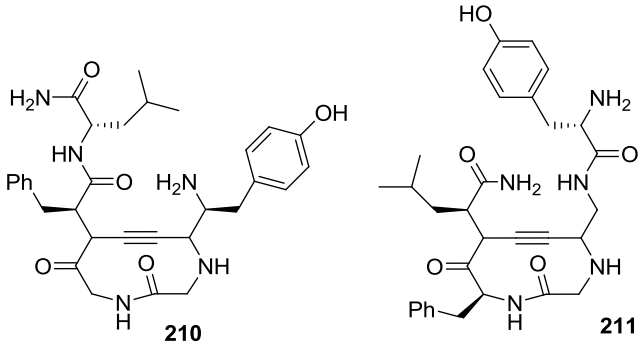


Figure 50: Alkyne Mimetic Targets

The revised mimetics were similar to the original target structures, but a simple alkyne was used instead of an ynamide in order to deliver the required conformation. This means that the structures deviate more from the actual peptide system than in the case of the ynamide targets, but that most of the functionality is still in place. The new targets have the added complication that they contain an additional chiral centre, but provided the stereochemistry at this centre can be controlled, it opens up some very interesting possibilities for different diastereomers to have different activities at opioid receptor sites.

In order to make a brief comparison, the average bond lengths of the alkyne system were investigated (Table 12). This method of comparing the bond lengths may only give an approximation, however it is sufficient to show that the covalent linker will provide a reasonably similar distance when compared to the hydrogen-bonded system. The alkyne unit is slightly longer than the ynamide, meaning that it bears a closer resemblance to the natural H-bonded system, at least in terms of the distance between the *i* and *i*+4 turn units.

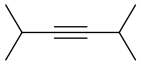
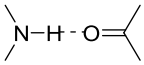



Table 12: Bond Lengths of All Carbon Mimetic			
Atoms (Bonds)	**Bond Length (Å)**	**Atoms (Bonds)**	**Bond Length (Å)**
C−C	1.54	N to O distance	2.99
C≡C	1.21	O=C	1.22
$C^{sp}-C^{sp3}$	1.46		
Total	**4.21**	**Total**	**4.21**

The first step towards these targets was their investigation to ensure the validity of synthesising the cyclic core. To this end another simple model system was selected as the target in an attempt to prove that this type of ring can be accessed. A structure with no extraneous functionality was proposed (target **212**, Figure 51). This structure differs from the previous model compound, not only because it utilises an alkyne instead of an ynamide to close the ring, but also because the phenylalanine functionality that resided on the ynamide nitrogen has been removed. This simplifies the model significantly and removes the added concern of having another chiral centre at this stage - the secondary hydroxyl group is located on a chiral carbon and so this avoids the distraction of working with complex mixtures of diastereomers when the priority is closure of this type of ring system.

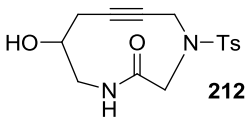
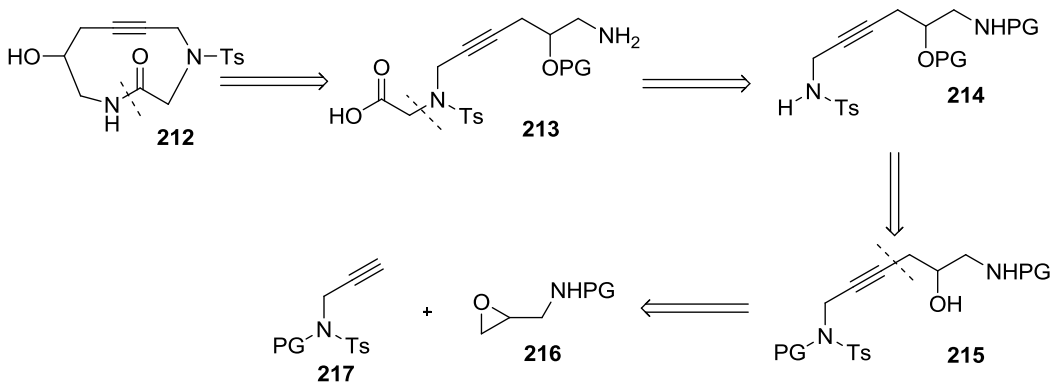


Figure 51: Proposed Model Compound

A relatively straightforward retrosynthesis was suggested for the preparation of the medium-sized ring system (Scheme 68). It was decided that the most effective approach to the ring closure was by amide coupling to form a lactam. This approach makes use of well-established chemistry, with many possible reagents to choose from and so it was felt that this would be the most robust approach to the synthesis of this system. This also provided more freedom to install the alkyne in the more complex target systems, bearing in mind that it must be coupled to a secondary carbon centre. This took the retrosynthesis back to the protected system **213**. At this stage the nitrogen could be functionalised starting from the sulphonamide **214**. This was taken back to the alcohol with a protected primary amine **215**,

which in turn was disconnected to give the terminal alkyne **217** and epoxide **216**. The retrosynthetic analysis seemed fairly robust and it was decided to proceed with the forward synthesis and identify the appropriate protecting groups as this synthesis progressed.

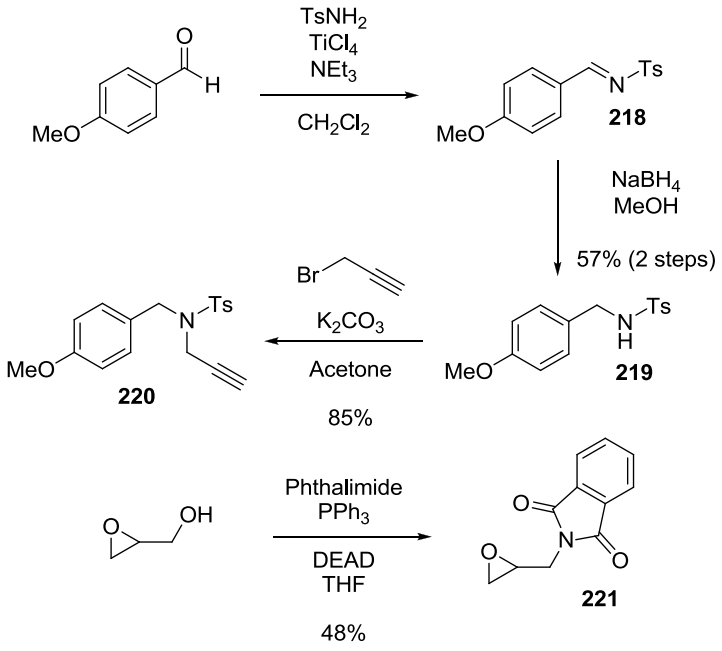


Scheme 68: Initial Retrosynthesis of Lactam Model

2.3.2 Towards the Synthesis of the Alkyne Model Structure

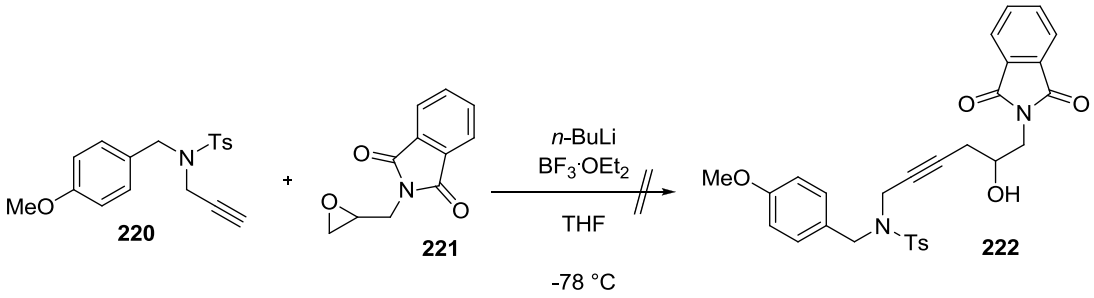
Initially, two fragments were synthesised for the intended epoxide opening reaction. The first of these was the sulphonamide **220** incorporating a PMB protecting group (Scheme 69). The fragment was synthesised using a three-step process starting from *p*-methoxybenzaldehyde. Reaction of this aldehyde with *p*-toluenesulfonamide in the presence of $TiCl_4$ and TEA gave the imine **218**. This intermediate was extracted from the quenched reaction mixture and concentrated, before using in the next stage without further purification. The imine was reduced using $NaBH_4$ to give sulphonamide **219** in 57% yield for the two steps.[170] The amine could then be alkylated easily using propargyl bromide in the presence of K_2CO_3 to give terminal alkyne **220** in 85% yield.

The second fragment was synthesised easily from racemic glycidol. It was decided that the amine in fragment **216** should be masked in such a way that there would be no N-H available so as to avoid a double alkylation in subsequent steps. Phthalimide seemed to be a perfect protecting group in this respect because it fully substitutes the amine thus preventing any reaction at this centre, and could be removed easily once it was no longer required.[171] The protected amine **221** was synthesised by reacting glycidol with phthalimide using standard Mitsunobu conditions to displace the alcohol. This was achieved in a moderate 48% yield, but further optimisation was not pursued as sufficient quantities of material were obtained to satisfy the requirement of this model synthesis.



Scheme 69: Synthesis of Coupling Partners

The attempted coupling of fragments **220** and **221** was carried out, as shown in Scheme 70. *n*-BuLi was used to deprotonate terminal alkyne **220** at –78 °C, and $BF_3 \cdot OEt_2$ was added followed by the epoxide **221**. A temperature of –78 °C was maintained throughout and the reaction was monitored by TLC. Unfortunately, the reaction did not proceed and starting materials were recovered following reaction quench.



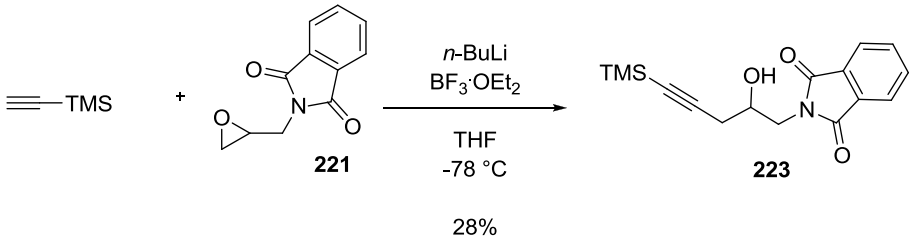
Scheme 70: Epoxide Opening Reaction

The reaction was attempted several more times under varied conditions (Table 13). Using *n*-BuLi and Et_2AlCl in toluene at 0 °C resulted in the formation of a new product which was visible by TLC. However, it was found that the new product could not be isolated as a pure sample because it co-eluted with starting material (entry 1). ^1H NMR analysis showed clearly that it was not the target product and so it was not purified further. Most of the starting

materials could be recovered from this reaction. The next attempt, using Me_2AlCl in toluene at temperatures ranging from –25 °C up to 0 °C (entry 2), showed significant decomposition. The products were separated by column chromatography but none of them was identified as the desired product. When this reaction was repeated in THF (entry 3) it did not progress at all and starting materials could be cleanly isolated from this reaction mixture. In a final attempt, *n*-BuLi and DMPU were used in toluene and the reaction was allowed to warm from –25 °C up to room temperature (entry 4). Under these conditions the outcome was similar to that obtained under the initial conditions utilising Et_2AlCl; slight decomposition was noted along with recovery of most of the starting material.

Table 13: Testing of Conditions for Epoxide Opening		
Entry	**Conditions**	**Result**
1	*n*-BuLi, Et_2AlCl, Toluene, 0 °C	Slight Decomposition
2	*n*-BuLi, Me_2AlCl, Toluene, –25 °C to 0 °C	Decomposition Products
3	*n*-BuLi, Me_2AlCl, THF, –25 °C to 0 °C	Starting Material Recovered
4	*n*-BuLi, DMPU, Toluene, –25 °C to 20 °C	Slight Decomposition

The difficulties encountered when attempting to open the epoxide were somewhat unexpected, so a brief test reaction was performed using TMS acetylene and the phthalimide protected epoxide **221** (Scheme 71). The initially employed conditions were used in this case and it was found that the epoxide was opened, giving the secondary alcohol, albeit in a fairly modest yield.

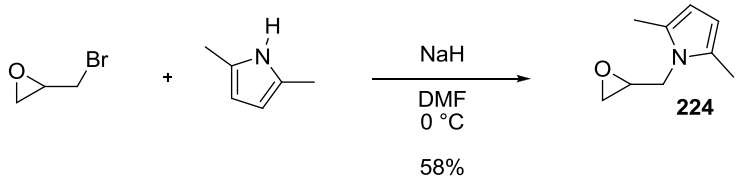


Scheme 71: Epoxide Opening Test Reaction

It was clear that in this instance, the epoxide exhibited limited reactivity towards a deprotonated alkyne. The next logical step was to modify one of the reacting substrates and attempt a similar coupling reaction. The most obvious way in which to do this was to alter the protecting group of the nitrogen on the epoxide fragment. The phthalimide group is rather

bulky with plenty of freedom to rotate which could block the approach of the deprotonated acetylene to the epoxide.

As an alternative, it was decided to protect the epoxide using a 2,5-dimethylpyrrole group. This has been used successfully in the past as a protecting group and can be removed using very mild conditions.[172-174] Its use means that potential reaction at the terminal amine is avoided, a feature it shares with the phthalimide group. The epoxide was easily prepared by deprotonating 2,5-dimethylpyrrole at 0 °C and then displacing the bromide from epibromohydrin.[174] Protected amine **224** was afforded in respectable yield, (Scheme 72).



Scheme72: Synthesis of 2,5-Dimethylpyrrole Protected Epoxide

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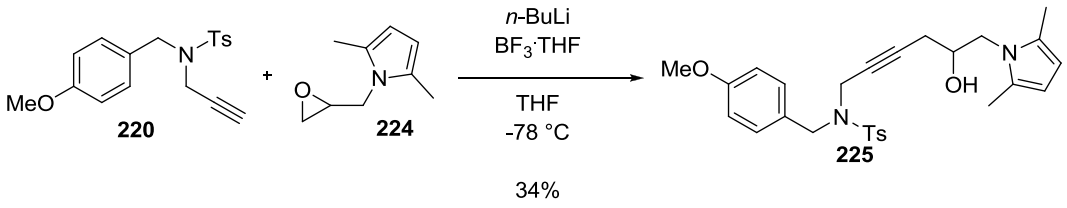
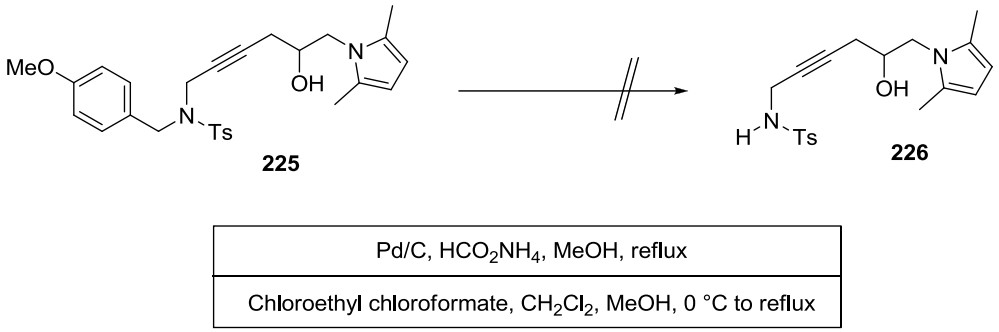
The newly protected epoxide **224** was immediately used in a reaction with previously synthesised alkyne **220**. The same conditions that have been tested previously were utilised initially; *n*-BuLi was used to deprotonate the alkyne at –78 °C and $BF_3{\cdot}OEt_2$ to activate the epoxide. This first attempt at the reaction resulted in a very disappointing 7% yield. In the literature it was suggested that using $BF_3{\cdot}THF$ gave higher yields for this type of reaction.[175] A second attempt, using $BF_3{\cdot}THF$ instead, resulted in a 34% yield of the product **225**, a significant improvement (Scheme 73). The yield was still a fairly modest one but enough material was isolated for the next stage of the synthesis to be attempted.



Scheme 73: Successful Epoxide Opening

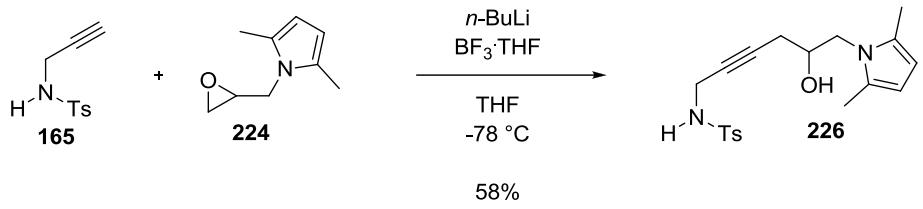
The subsequent step required the removal of the PMB protecting group, which was attempted using two sets of conditions (Scheme 74).[176,177] Firstly, the PMB-protected amine was reacted with Pd/C in the presence of ammonium formate in methanol at reflux. This reaction showed no progression after 2 hours and so it was abandoned in favour of a reaction

in which the substrate was treated with *α*-chloroethyl chloroformate starting at 0 °C and heating gradually up to reflux. This second set of conditions also resulted in no reaction and the recovery of clean starting material. Rather than carry out further reactions to try and remove the PMB protection, it was decided to synthesise the unprotected amine and try to perform the epoxide opening directly on this substrate.



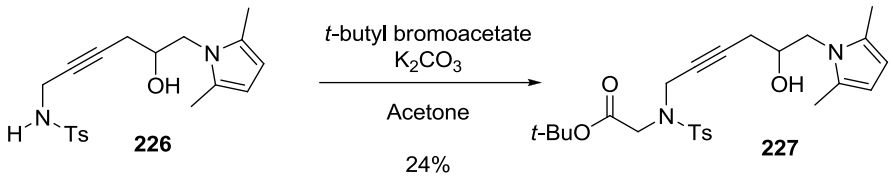
Scheme 74: Attempted PMB Deprotection

Tosylated propargylamine **165** had been prepared previously during the synthesis of the tripeptidic alkynyl bromide target **171**. This substrate was reacted with the 2,5-dimethylpyrrole protected epoxide **224**, again using *n*-BuLi and BF_3·THF to generate the reactive species (Scheme 75). It was, of course, important to use more than two equivalents of *n*-BuLi in order to completely deprotonate both the terminal alkyne and the amine. Attack of the epoxide originated solely from the acetylenic position due to its relative instability in comparison with the nitrogen centre. Initially, this reaction gave disappointing yields of approximately 25% when performed on a 100-200 mg scale. It was found very quickly that the yield could be improved significantly (up to 58%) by performing the reaction on a larger scale (1-2 g). The same reaction was also carried out in the presence of BF_3·OEt_2 in order to compare yields. In this case, the reaction gave a very disappointing yield of 16%. It is important to note that, at this stage in the synthesis, the product was prone to degradation even when stored under argon in the freezer. Immediately after isolation the product had the appearance of a clear oil, but turned viscous and brown within 24 hours, requiring purification to be performed once again before use. This made it somewhat difficult to handle the alcohol **226** and to obtain good characterisation data.



Scheme 75: Epoxide Opening with Unprotected Amine

With the secondary alcohol in place and the free sulphonamide available for *N*-alkylation, the next synthetic transformation was attempted. This step in the reaction sequence required alkylation of the nitrogen and was performed using *t*-butyl bromoacetate and K_2CO_3 as a source of base (Scheme 76).



Scheme 76: *N*-Alkylation Before Alcohol Protection

When *N*-alkylation was performed it was found that the yields were particularly low and did not rise above 24%. This is most likely because of the possibility of alkylation of the unprotected oxygen as well as the desired NH. On TLC analysis, there were products other than the desired *N*-alkylated target **227**. To remedy this it was decided that the secondary alcohol should be protected prior to alkylation. The protecting group would not need to be removed later in the synthesis, as the alcohol does not have any further role in this model study. Thus, the only requirement for this protecting group was that it would survive intact during the remaining synthetic steps.

The protecting group that was selected was a TBDPS ether. The reason that such a bulky silyl ether was chosen was that it needed to survive the acidic conditions that are required to remove the *t*-butyl ester in the later stages of the synthesis. Of all the silyl ethers, the TBDPS group is the most likely to survive under these conditions.

The conditions for successful protection of the hydroxyl group required some exploration. Due to the bulk of the silyl group that is being attached in this case, coupled with the fact that it is reacting at a secondary alcohol with reasonably large groups on either side, this is a problematic reaction to carry out. Table 14 details the reaction conditions that were

applied to this system. Initially, the substrate was treated with TBDPSCl with imidazole in DMF (entry 1), but this reaction did not progress at all and the starting material could be recovered. The subsequent two reactions utilised TBDPSCl again, this time in the presence of DMAP and TEA. Firstly the reaction was performed at room temperature, giving a modest 35% yield of the protected alcohol (entry 2). The second attempt under these conditions was carried out at reflux to try and drive the reaction further towards completion (entry 3). Unfortunately, this resulted in the decomposition of the starting material. Finally, the alcohol was treated with the more reactive TBDPSOTf in the presence of 2,6-lutidine giving the best yield of 47% (entry 4). This last reaction was attempted in parallel using purchased TBDPSOTf and laboratory synthesised TBDPSOTf. It was found that the best yields were obtained when using the commercial material.

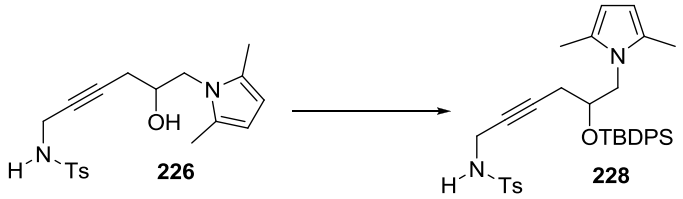
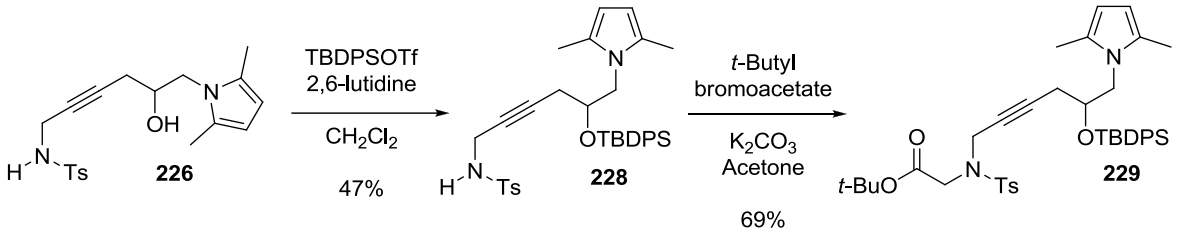


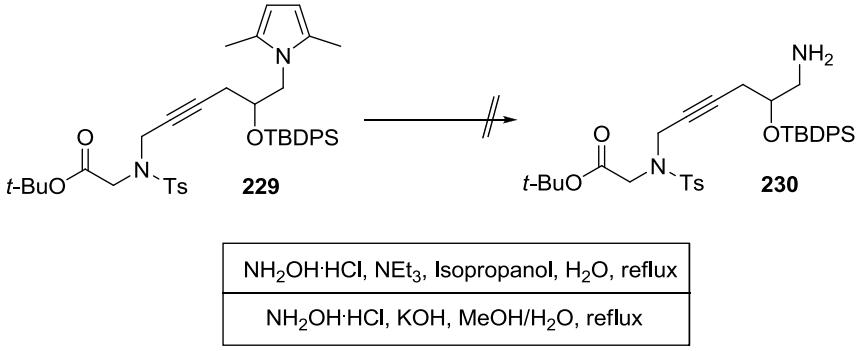
Table 14: TBDPS Protection Reactions		
Entry	**Reaction Conditions**	**Reaction Outcome**
1	TBDPSCl, imidazole, DMF, 90 °C	No Reaction
2	TBDPSCl, DMAP, TEA, CH_2Cl_2, r.t.	35%
3	TBDPSCl, DMAP, TEA, CH_2Cl_2, reflux	Decomposition
4	TBDPSOTf, 2,6-lutidine, CH_2Cl_2	47%

Having protected the alcohol, albeit in moderate yield, it was found that the *N*-alkylation, towards **229**, took place far more effectively. When standard basic conditions were employed, as described previously, the desired product was obtained in a much improved yield of 69% (Scheme 77). This took the structure to within a few steps of the key intramolecular lactamisation reaction. All that was required was removal of the *t*-butyl ester and the 2,5-dimethylpyrrole before the two ends of the molecule could be coupled together.



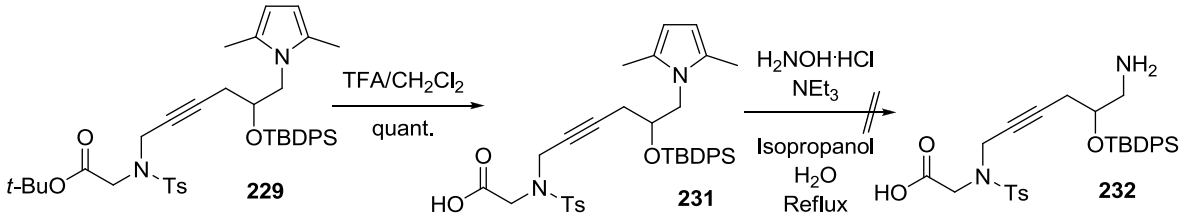
Scheme 77: TBDPS Protection and *N*-Alkylation

In the first instance, the removal of the 2,5-DMP group was attempted using standard conditions with hydroxylamine and TEA, as reported in the literature (Scheme 78).[172] Unfortunately, this reaction did not occur and the starting material **229** was recovered. The reaction was performed using slightly different conditions – treating with hydroxylamine and KOH in a mixture of methanol and water – but to no avail.[178]



Scheme 78: Attempted Removal of 2,5-DMP Group

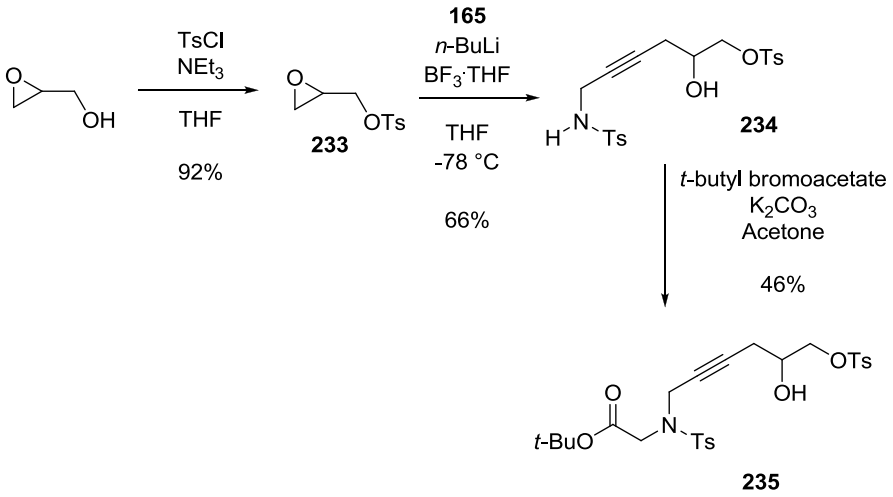
As an alternative approach, it was decided to deprotect the *t*-butyl ester and then remove the 2,5-dimethylpyrrole group (Scheme 79). The *t*-butyl ester was removed under acidic conditions without incident in quantitative yield, furnishing **231**. The TBDPS ether survived this transition without any problems, proving the validity of its selection as the protecting group in this case. Subsequently, removal of the 2,5-dimethylpyrrole was attempted using the same series of conditions as before. Again, there was no indication of any reaction and when the reaction constituents were separated after work up, the target amine **232** was not isolated.



Scheme 79: Alternative Approach to 2,5-DMP Removal

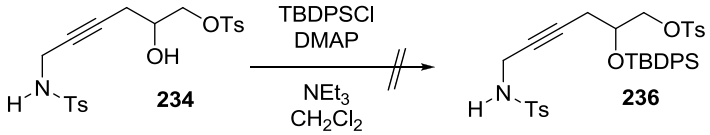
This was an unexpected difficulty in the synthesis and unfortunately it was one that was difficult to circumvent. The most obvious solution was to replace the 2,5-DMP protected amine with a more appropriate group The amine either had to be protected in a different manner, or replaced with a functional group that could be converted into the amine later in the synthesis. The latter approach was chosen because of the difficulties that had been experienced with two different protecting groups already. The approach that was chosen was one in which the amine was masked as a tosylate, which would be displaced with an azide at an appropriate stage in the synthesis followed by reduction to the primary amine.

Starting once again with racemic glycidol, the tosylated epoxide **233** was synthesised in high yield by reaction with *p*-toluenesulfonyl chloride in the presence of TEA (Scheme 80). The epoxide was opened with the alkyne to give the secondary alcohol, again using *n*-BuLi with BF_3·THF at –78 °C affording the product **234** in a moderate 66% yield. This particular yield is dependent on the quantity of recovered starting material. The reaction is best carried out by recycling unused starting material as it never progresses to full conversion. After allowing the reaction to progress as far as possible, it was then fairly simple to purify both the unreacted starting material and the product by chromatography. The starting material is then recycled in a repeat reaction. This made maximum use of the available material and gave a respectable overall yield. The nitrogen was then alkylated using *t*-butyl bromoacetate in the presence of K_2CO_3 to furnish **235** in a modest yield.



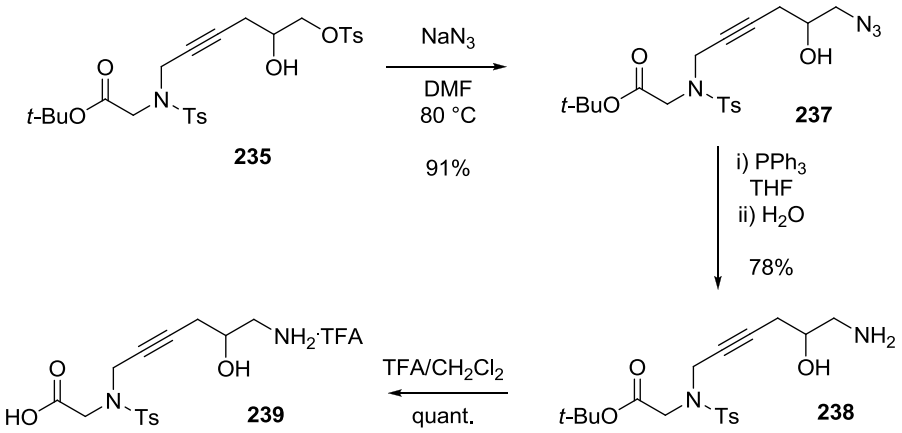
Scheme 80: Towards the Synthesis of the Cyclisation Precursor

At this stage, efforts were made to protect the secondary alcohol as before (Scheme 81) with the expectation that the yield of the alkylation could be improved. Several attempts were made to install the TBDPS ether at this position but they were unsuccessful and returned only starting materials. This may be caused by the presence of the bulky tosyl group, located α to the secondary alcohol, blocking the approach of the similarly bulky silylating reagent. Having been unsuccessful in protecting the hydroxyl group, the synthesis was continued with the free alcohol. It was felt that a 46% yield was sufficient to obtain reasonable quantities of the final product for testing.



Scheme 81: Attempted Protection of Secondary Alcohol

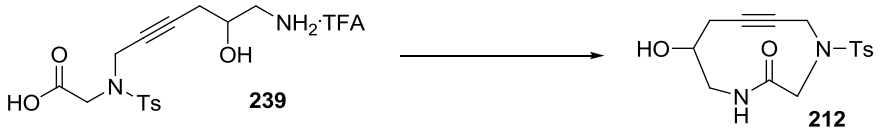
The final two stages in this section of the synthesis were accomplished without incident. The tosyl group was displaced successfully using sodium azide, giving the desired target **237** in excellent yield (91%). The azide was then reduced using Staudinger conditions, giving the amine **238** in a good 78% yield. The final stage was the cleavage of the *t*-Bu ester which was performed under acidic conditions using TFA in CH_2Cl_2 as a 1:1 mixture. This gave the amino alcohol salt **239** in quantitative yield. This compound was very insoluble in $CHCl_3$ and so dissolution in deuterated methanol was required for characterisation by NMR spectroscopy.



Scheme 82: Final Steps in Cyclisation Precursor Synthesis

2.3.3 Testing of Amide Coupling Conditions

The next stage required the coupling of the amine and carboxylic acid groups to give 10-membered lactam **212** required for the model target (Scheme 83). There are various coupling agents in the literature for formation of amide bonds so there was no shortage of options.



Scheme 83: Towards the Synthesis of 10-Membered Lactam Model

The reaction conditions that were tested are displayed in Table 15. This was a difficult reaction to monitor by TLC because the TFA salt **239** is very polar and occupies the base line of a chromatography plate, regardless of eluent polarity. This made it very difficult to ascertain whether any of the starting material had been consumed. In the first attempt to perform lactamisation BOP was utilised as the coupling reagent and the reaction was carried out over the course of 20 hours in the presence of HOBt and DIPEA (entry 1), but the reaction showed no progression at all. EDCI was then used as an alternative coupling reagent, this time with DMAP and reacting for 20 hours, with similarly poor results (entry 2). At this stage a report in the literature was found showing that pentafluorophenyl diphenylphosphinate and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (FDPP and DMTMM respectively) are useful amide coupling reagents for the synthesis of peptide containing macrolactams,[179] therefore these reagents were utilised for the subsequent test reactions (entries 3 and 4). In both of these cases, reactions were carried out over periods of 3-4 days

and there was formation of a new product as detected by TLC analysis. The new product was formed in greater amounts when using FDPP and it was isolated in both cases. Unfortunately, NMR analysis of the product proved to be inconclusive and it was clear that it was not the desired ring system. In a final attempt to close the ring, PyBOP was utilised alongside DIPEA (entry 5). This reaction was carried out over 3 days and in this case a new product was detected by TLC analysis. This product was again isolated for NMR studies, but was found to be an undesired side product. The NMR spectra for all three of these decomposition products were complex and showed large broad peaks in the upfield regions. The structures of these products could not be determined but it was clear that the cyclisation reactions were not producing the desired target.

Table 15: Screening of Coupling Conditions for Lactamisation

Entry	Reaction Conditions	Reaction Outcome
1	BOP, HOBt, DIPEA CH_2Cl_2/DMF	No Reaction
2	EDCI, DMAP CH_2Cl_2/DMF	No Reaction
3	FDPP, DIPEA DMF	Decomposition
4	DMTMM, DIPEA DMF	Slight Decomposition
5	PyBOP, DIPEA DMF	Decomposition

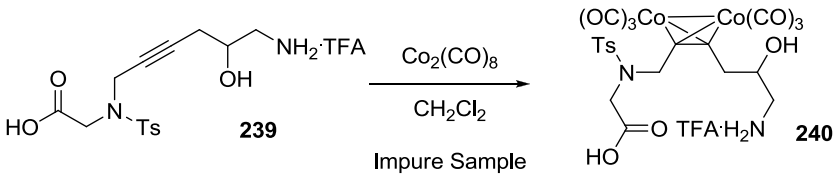
Due to the high polarity of compound **239**, the starting material could not be reisolated at the end of each test reaction and so it had to be synthesised several times as these reactions were carried out. All of the test reactions were performed at 0.25 mM concentration to prevent dimerisation couplings.

Having explored the use of a variety of standard coupling reagents to perform cyclisation to give the medium-sized lactam, it was decided that an alternative approach might be more useful. One possible reason for the low reactivity is the strain induced by the triple bond in the ring. In the linear precursor **239**, the alkyne is most likely making it more difficult for the two reactive centres to approach one another. Unfortunately, none of the attempts to

perform the cyclisation reaction revealed any of the desired product. Even trace yields of product were elusive. A possible solution for this problem would be to find a way to lower the transition state energy for the cyclisation reaction.

2.3.4 Cobalt Complex Approach to Lactam

In the literature it has been reported that complexation of dicobalt octabarbonyl to an alkyne in a chiral compound allows diastereomers to be separated.[180] The cobalt was easy to install, robust under a variety of reaction conditions and straightforward to remove. It also had the effect of giving the alkyne triple bond more double bond character. This was a perfect avenue for exploration in this system and could provide the solution to the difficulties mentioned previously. To this end, the synthesis of cobalt complex **240** was accomplished by direct treatment of the alkyne with dicobalt octacarbonyl (Scheme 84). Initially, this reaction was attempted using the deprotected salt **239**. However, it was quickly realised that this approach was not appropriate because there was no readily apparent method to purify the resulting complex. The yields of the complex were greater than 100% due to the slight excess of dicobalt octacarbonyl complex that was still present after reaction workup. In addition, due to the charged nature of both the starting material and the target product, there was no conclusive way to prove that all of the starting material had been consumed.

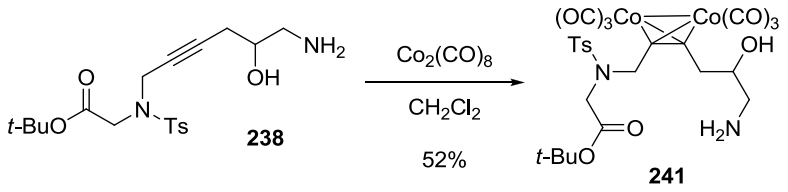


Scheme 84: Attempt to Complex Deprotected Precursor

It was decided that an alternative approach to the preparation of the cobalt complex was more appropriate in order to obtain better control over its purity. It was believed that better results would be obtained during the subsequent cyclisation reactions if the cobalt complex was of higher purity. It was entirely possible that excess cobalt may interfere with the reaction outcome or that only a partial conversion to the complex had in fact taken place.

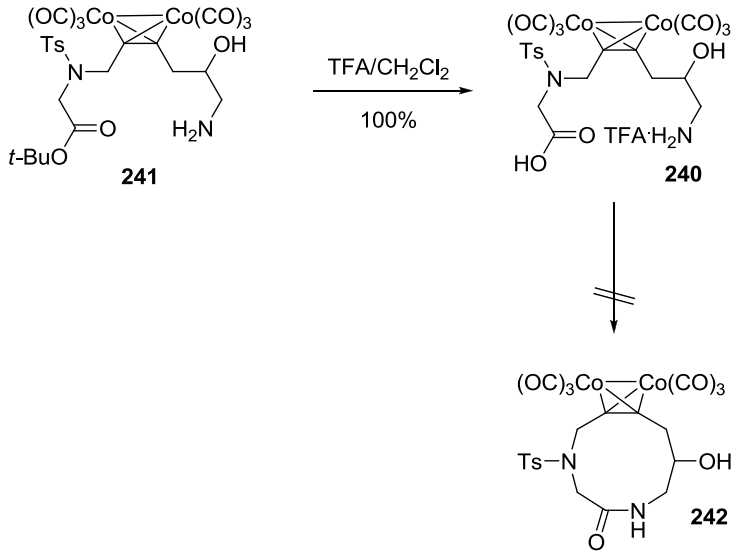
By carrying out the reaction with dicobalt octacarbonyl complex prior to removal of the *t*-butyl ester, purification of the resulting complex **241** was easier (Scheme 85). It was not possible to characterise the metal complexes using NMR techniques, possibly due to the

paramagnetic nature of cobalt. Upon obtaining a sample of the cobalt complex, it was filtered through a layer of Celite® in an effort to remove excess cobalt and prepare the sample for NMR spectroscopy. Unfortunately, in the case of complex **241** this was unsuccessful and NMR data was inconclusive. The presence of the cobalt complex was suggested instead by TLC analysis in conjunction with IR studies to determine the abundance of carbonyl functionality. Curiously, the reaction to install the cobalt complex gave relatively low yield when compared to the high yield reported by the literature. Initial attempts to perform this reaction gave on average 30% yield after purification. These reactions were performed on the scale of 20-30 mg of alkyne. It was then found that when carrying the reaction out on a slightly larger scale, the yields were significantly improved and when the reaction was performed on a 700 mg scale a yield of 52% was obtained. This yield still does not approach those reported in the literature but it was good enough for us to proceed with the subsequent lactamisation reactions.



Scheme 85: Synthesis of Cobalt Complex Before Deprotection

Once the cobalt complex had been installed and the material had been purified, it was a straightforward matter to cleave the *t*-butyl ester under acidic conditions (Scheme 86). This deprotection reaction provided the carboxylic acid **240** in quantitative yield. The final step involved cyclisation of the linear precursor using amide coupling chemistry. Initial attempts at this transformation were unsuccessful and so a series of reaction conditions had to be screened.



Scheme 86: Use of Cobalt Complex in Cyclisation Reaction

The use of reagents such as PyBOP, EDCI and FDPP all gave no reaction as judged by TLC analysis (Table 16, entries 1-3). Even after extended reaction times of 2 to 3 days, there was no indication of product formation. This was a disheartening result, but the screening of reaction conditions was continued with DMTMM as the coupling reagent (entry 4). This set of conditions does not deliver the required product either but instead gave small traces of decomposition products. In an attempt to identity these trace products they were purified and analysed by NMR spectroscopy. However, the resulting spectra showed large overlapping peaks in the alkyl region and could not be adequately interpreted. The process was further complicated by the limited quantities of the decomposition products that were isolated from the reaction.

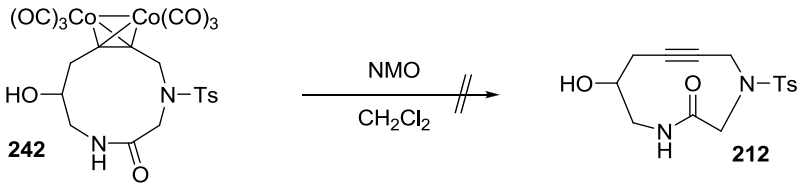
All of the test reactions were performed in DMF, which necessitated the work up of any TLC samples by dilution with water and organic extraction. The DMF solvent blanketed the region on the TLC plate that coincided with the starting materials making it impossible to determine if they had been consumed at all, while the solvent was present.

Finally, the reaction was attempted using BOP in CH_2Cl_2, and this led to a slightly more promising outcome (entry 5). A new product was isolated in very small quantities. The identity of the product could not be accurately verified by NMR analysis because of the same difficulties in removing cobalt contaminants that were encountered previously. The cobalt complex also complicated analysis by mass spectrometric methods. If the material was indeed the cyclised target, the yield would have been 35%, a very respectable outcome considering the difficulties that had been encountered thus far in forming the lactam.

Table 16: Screening of Coupling Conditions for Cobalt Complex Lactamisation		
Entry	**Reaction Conditions**	**Reaction Outcome**
1	PyBOP, DIPEA DMF	No Reaction
2	EDCI, DIPEA DMF	No Reaction
3	FDPP, DIPEA DMF	No Reaction
4	DMTMM, DIPEA DMF	Slight Decomposition
5	BOP, DIPEA CH_2Cl_2	Possible Product

It was decided that the most effective approach to verify the identity of the product was to remove the cobalt complex and regenerate the alkyne functionality. This process has been well described in the literature and assuming it was the desired product that was isolated from the BOP coupling reaction there should have been no difficulty in removing the cobalt.

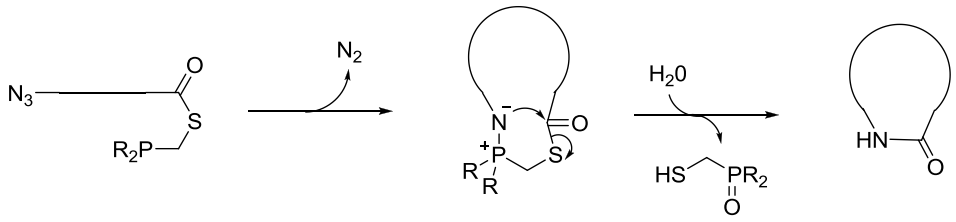
Published procedures required the use of *N*-methylmorpholine-*N*-oxide (NMO) to decomplex cobalt and restore the triple bond. This standard approach was applied to the suspected lactam product **242** (Scheme 87). Literature examples suggested that, by carrying the reaction out in CH_2Cl_2 at room temperature, it should take about one hour to completely remove the cobalt. However, when the reaction was performed under these conditions, no progress was observed at all: starting material remained and there was no sign of reaction or decomposition. The mixture was stirred for several more hours, but to no avail. The mixture was worked up and analysed by NMR spectroscopy to confirm that no reaction had taken place. Unfortunately, the only conclusion that could be drawn is that the required lactam complex was not the product that had been isolated from the previous reaction. This rendered all attempts to cyclise the cobalt complex **240** unsuccessful.



Scheme 87: Attempt to Remove Cobalt From Suspected Lactam Product

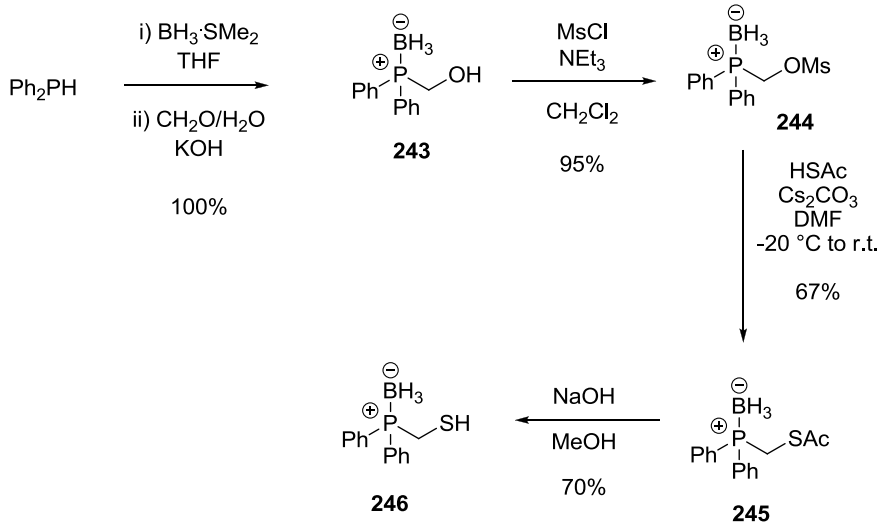
2.3.5 Diphenylphosphine Tether Approach to Lactam

The failure of the approach described above was disappointing and attention was focused on an alternative method to synthesise the desired model compound. Further study of the literature in this area revealed a novel approach to the synthesis of medium-sized lactams.[181,182] A procedure had been reported in which a diphenylphosphine tether had been attached to the carboxylic acid end of the structure. This was then coupled with an azide which resulted in ring-closure by a Staudinger reaction. The unwanted atoms were excised during this process to give nitrogen gas and the phosphine oxide derivative of the tether as by-products alongside the desired lactam product (Scheme 88).



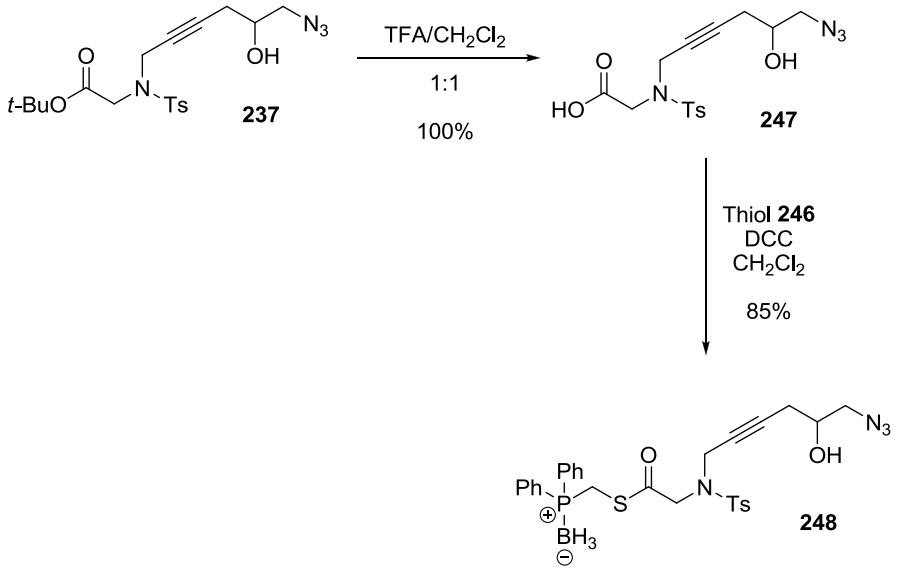
Scheme 88: Staudinger Approach to Lactamisation

The first step required the synthesis of the relevant diphenylphosphine tether before it could be reacted with the desired carboxylic acid centre. This was accomplished using a reported literature procedure (Scheme 89).[183] Initially, diphenylphosphine was treated with borane dimethylsulfide, to provide diphenylphosphine borane. This was then treated with formaldehyde to install the hydroxymethyl group and furnish **243** in quantitative yield. The primary alcohol was then mesylated in the presence of TEA, providing **244**, followed by displacement using thioacetic acid to produce thioacetate **245** in good yield. Finally, the thioester group was cleaved, leaving the thiol group ready for reaction in the next stages of the synthesis; compound **246** was isolated in 70% yield.



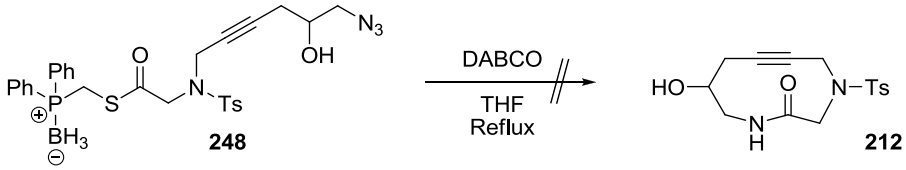
Scheme 89: Synthesis of Diphenylphosphine Tether

With the thiol available, the alkyne fragment had to be prepared for coupling and cyclisation. Due to the requirement of an azide coupling partner, fragment **237** was chosen as the ideal starting point for this procedure (Scheme 90). The *t*-butyl ester was cleaved under acidic conditions to provide carboxylic acid **247** in quantitative yield, while retaining the azide functionality. The next step required the coupling of **247** with thiol fragment **246**. Initially, EDCI was used as the coupling agent, as suggested in the literature, giving the required product in a reasonable 63% yield. It was felt that this yield could be improved and so DCC was used as an alternative coupling reagent. Under these conditions compound **248** was isolated with a markedly improved yield (85%).



Scheme 90: Formation of Cyclisation Precursor

Following installation of the diphenylphosphine tether, the Staudinger mediated cyclisation reaction was attempted using standard literature conditions. This involved treatment of precursor **248** with DABCO in THF at reflux. The reaction was performed over the course of 20 hours and $MgCl_2$ and $HgCl_2$ were then added to scavenge triphenylphosphine oxide by-product. After completion of the work-up, the product mixture was carefully separated by column chromatography. NMR analysis was used to determine if any of the component products was the desired product **212**. Unfortunately, the reaction was not successful. TLC analysis of the crude mixture indicated that a significant amount of starting material remained but several potential products were also visible. These appeared to be trace decomposition products. A subsequent attempt to perform the reaction over a more prolonged period (48 hours), gave similarly negative results.



Scheme 91: Attempted Cyclisation Reaction

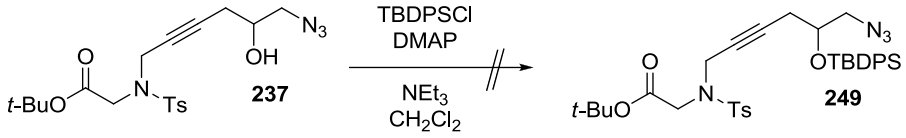
2.3.6 Further Cobalt Complex Experiments

Having exhausted several avenues of investigation for the cyclisation, attention returned to the cyclisation of the cobalt complexed substrates. It was postulated that the linear precursor had the potential to cyclise onto the alcohol, which would have resulted in the lactone product. This 9-membered ring would lack the strong *trans* preference that is associated with amide bonding units. Most of the reaction towards the lactam product showed either no progress or resulted in small amounts of decomposition by-products. It was possible that if the lactamisation was competing with lactone formation, then the lactone intermediate might be decomposing to give the by-products that were seen in the reaction. To this end, it was decided that it might be worthwhile protecting the secondary alcohol prior to cyclisation using standard amide coupling conditions.

Having already attempted to protect the secondary alcohol with no success (*cf.* Scheme 81), attention was focused on alternative approaches. The first attempt to perform protection had been carried out with the tosylate group in the α position to the alcohol and this was believed to be a factor that contributed to its failure. The tosylate is in close proximity to the

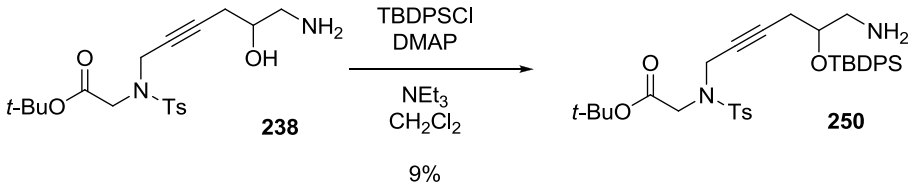
reacting hydroxyl group and it has a great deal of freedom to rotate, so it was suggested that this was blocking the approach of reagents.

Of course, during our proposed synthetic sequence, the tosylate group is displaced by an azide, which is then reduced to give a primary amine. This provides two further opportunities to protect the alcohol but with less bulky groups in close proximity. The first attempt was carried out after the azide was installed and is illustrated in Scheme 92. This reaction was ineffective and did not progress at all. It appeared that the azide in the *α* position was unsuitable for approach by the silylating reagent. The fact that no product was isolated at all discouraged further attempts using the more reactive TBDPSOTf and 2,6-lutidine. Previous experience had shown that in cases where TBDPSCl gave a low yield of the silyl ether, TBDPSOTF gave much improved yields. The fact that in this case there was no reaction at all did not bode well for the success of the triflate in carrying out the same transformation. Due to the difficulty in obtaining the triflate, it was decided that it would be better to use it to silylate the subsequent primary amine substrate, pending the success with the silyl chloride.



Scheme 92: Attempted Protection of Secondary Alcohol

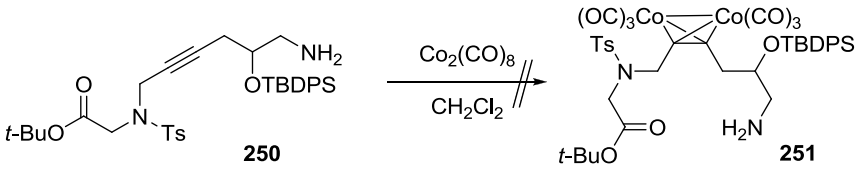
With this approach in mind, the same protection reaction was performed on the primary amine alternative **238**, towards **250**. The reaction was carried out as before using TBDPSCl as shown in Scheme 93. In this instance, the reaction was somewhat more successful, giving a very low yield (9%). Unfortunately, both the starting material and the product in this reaction were very polar and it was very difficult to purify the material using silica gel chromatography. Thus, when purifying the material, especially when the reaction was performed on small scale and with such a low yield, it was very difficult to obtain a pure sample of the product.



Scheme 93: Protection of Alcohol Adjacent to Primary Amine

Following the successful reaction with the silyl chloride, and despite the low yield obtained, an alternative silylation reaction was performed involving treatment of the alcohol with TBDPSOTf in the presence of 2,6-lutidine. Unfortunately, this reaction was not as successful as hoped – a similarly poor yield of the product was obtained and large quantities of starting material were recovered.

As a consequence of the disappointing results obtained, there was no choice but to attempt the following reaction to prepare the protected cobalt complex **251**. The reaction was carried out under the same conditions as before, using dicobalt octacarbonyl in CH_2Cl_2 (Scheme 94). The reaction was unsuccessful and there was no evidence of any reaction upon TLC analysis. This result was very disappointing and a little surprising as the reaction to generate the cobalt is usually very straightforward and tolerates a wide variety of functional groups as reported in the literature.



Scheme 94: Cobalt Complexation Reaction with Protected Alcohol

Having explored several routes to prepare the desired lactam, there were no positive results. The cyclisation reaction refused to take place regardless of the conditions used and the model compounds based on the desired peptidomimetic targets remain elusive. Due to the lack of progress made towards these model structures, the more complex targets remain out of reach. Of course there are multiple avenues for further investigation that should be considered for future research. (See Future Work Chapter 3.2)

In theory, the ynamide and alkyne macrocycles are feasible structures that do not differ a great deal from the actual peptide systems. The distances involved between the two H-bonding groups in the natural systems are approximately the same as the average bond distances along the covalent linker that would close the mimetic macrocycle. Thus, it was reasonable to expect that the ring could be effectively synthesised. This is the case even although there is an amide bonding unit involved, as this factor does not discourage the turn that is found naturally in leu-enkephalin. Hopefully, future investigation can overcome low propensity for the substrates to undergo cyclisation that has been encountered during the project.

Chapter 3

Conclusions and Future Work

3.1 Conclusions

In conclusion, several approaches to the synthesis of β-turn mimetics based on leu-enkephalinamide have been investigated. The use of an ynamide unit as a covalent linker was explored first. Intramolecular coupling of an amide with an appropriate alkyne unit and intermolecular coupling with subsequent ring closure by lactamisation were investigated. In both approaches the synthesis of the ynamide was the problematic step and this could not be overcome, despite the use of numerous different reaction conditions.

An alternative system was investigated in which a proline residue was used to bring the reacting functional groups into close proximity during intramolecular coupling, but this was similarly unsuccessful. The intermolecular approach to ynamide formation did not provide any of the ynamide product either.

Studies were carried out to optimise the Hsung second generation protocol for ynamide synthesis. This was demonstrated on a glycine-containing system and a yield of 72% was achieved following the addition of Ni(II), compared to a literature yield of 70%. The application of the newly optimised conditions to the target system did not yield any of the target ynamide, prompting an alteration to the proposed mimetic structures, namely the replacement of the ynamide with an alkyne.

Research progressed onto the alkyne tethered mimetic system. This structure was approached with the intention of using amide coupling chemistry to close the 10-membered lactam. The penultimate structure was prepared but the ring system could not be constructed even after several attempts.

Further methods utilised a cobalt complex to ease the strain induced by the alkyne unit on the transition state, as well as an alternative approach involving a diphenylphosphine tether to close the ring *via* Staudinger chemistry. Neither of these approaches was successful. Unfortunately, the core ring systems for both the alkyne and ynamide mimetics could not be prepared and their synthesis remains unsolved.

Having attempted a number of approaches to the target ring system, some simple 3D model structures were constructed using computational methods. The purpose of this was to evaluate how closely the covalent linker resembles the hydrogen bond from the natural system. The hydrogen bond that holds the natural β-turn together will not be linear as the interaction between the amine hydrogen and the carbonyl oxygen is directed towards one of the oxygen lone pairs. This has the potential to give the pseudo-ring structure a more open, less strained conformation. As a result, it is possible that the hydrogen bonded system is slightly less strained than the 10-membered ynamide or alkyne ring. The linear conformation that is imposed by the triple bond could increase the strain in the ring system sufficiently to render the structure highly unfavourable.

It was hoped that construction of a 3D model at this stage would provide a definitive explanation for the inability of the target structures to cyclise. The four model structures are illustrated in figure **52** alongside the X-ray structure of leu-enkephalin originally published in 1978.[37,184,185] Visual comparisons of the models and the X-ray structure show that they are similar in terms of the ring systems that are at their core.

In each of the 3D structures, the alkyne and ynamide units are not entirely linear. Instead they show a slight bend that is induced by the overall ring system. This may more closely resemble the hydrogen bond that is in the natural system, but it also suggests that the ring is very strained. Clearly the triple bonded system would prefer to adopt a linear conformation. It is possible that the mimetic system is simply too strained to be formed in this case and that the natural β-turn system has more freedom to relieve this strain.

However, the intermolecular reactions that were carried out in Scheme 48 suggest that there is a reactivity problem at both the amide and the alkynyl bromide centre. This would imply that the problems encountered in forming the ynamide targets are more complex than simple ring strain of the product.

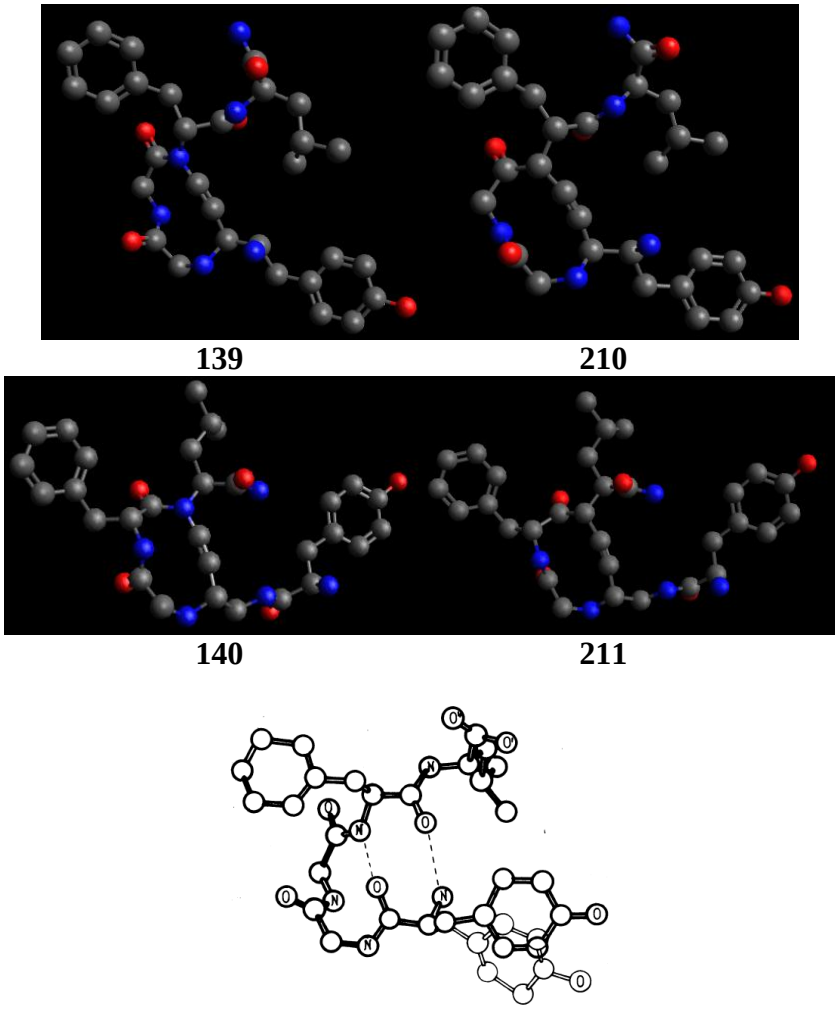


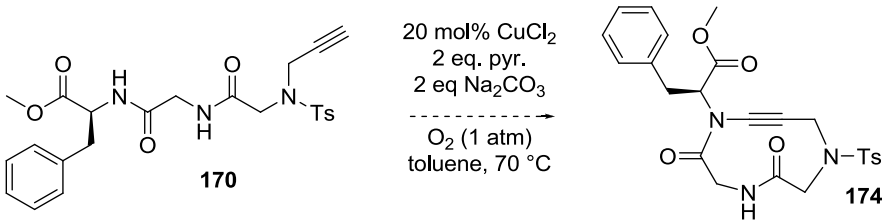
Figure 52: 3D Model Structures of Target Compounds and X-Ray Structure of Leu-Enkephalin

These models appear to support the goals of the project, by suggesting that the ring system would resemble the natural turn fairly closely. There is some variation in the orientation of the side chains, but the core of the structure is very similar and this helps to validate the initial targets that were designed as leu-enkephalin mimetics. This also suggests that continued investigation of these targets, or similar structures, is worthwhile for future research.

Future Work

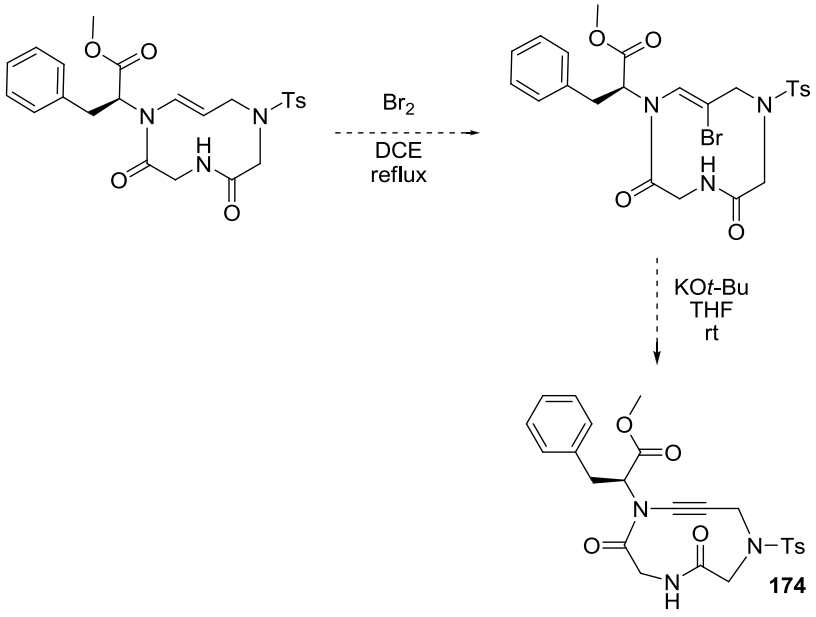
Throughout this project there has been immeasurable difficulty in achieving the cyclic cores of the various mimetic structures that have been proposed. Despite numerous attempts and various different approaches to the issue, none of the reactions met with any success. There are some approaches that may be worth trying in future however and if these are unsuccessful, alternative structures must then be considered.

Returning to the ynamide core model, there are several approaches to this synthesis that were not utilised, including the use of triiodonium triflate salts that was discussed earlier. Alternatively, there is a procedure that was published recently that uses CuI_2 in an oxygen atmosphere to couple a terminal alkyne with an amide (Scheme 95). This approach may have more success than the various amidation protocols utilising an alkynyl bromide.



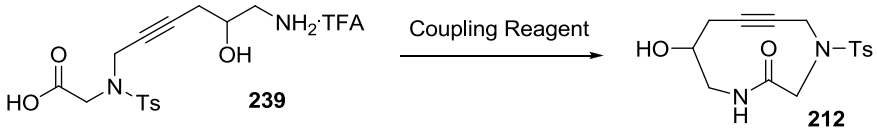
Scheme 95: Stahl Oxidative Amidation of Terminal Alkyne

It may also be worthwhile to investigate other approaches to ynamide synthesis, such as the elimination approach. This type of reaction has been reported previously by several groups, however, the most appropriate for this system has also been suggested by Hsung and co-workers. This group has shown that by refluxing the corresponding enamide with bromine in 1,2-DCE the desired *β*-bromoenamide can be isolated. HBr is then eliminated by treatment with KO*t*-Bu at room temperature to give the desired ynamide. This approach has the added benefit of installing the 10-membered ring before introducing the problematic triple bond system. Unfortunately, the use of the olefin bond itself as the covalent tether in the actual mimetic would introduce too much freedom to the ring and would most likely render the structure unsuitable for its intended function.



Scheme 96: Elimination Approach to Ynamide

Alternatively, the alkyne system could be probed further with the screening of a diverse array of peptide coupling reagents (Scheme 97). The number of compounds that can be used in this transformation is extensive and screening of these may be the best way to approach the difficult lactamisation reaction that was attempted in this project. Of course, if this approach is taken towards the lactam ring, the same variety of coupling reagents can be applied to the cobalt complex system that was investigated earlier as well. While this approach did not yield any positive results, the principles behind it are still valid and should not be dismissed.



Scheme 97: Screening of Lactamisation Conditions

Chapter 4

Experimental

4.1 General Conditions

All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Reactions involving air-sensitive agents and dry solvents were performed in glassware that had been flame dried prior to use and were carried out under an argon atmosphere. Organic solvents were dried using a Pure Solv^{TM} solvent purification system. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 covered aluminium plates F_{254}. TLC plates were developed under UV light and/or with a $KMnO_4$ solution (3 g of $KMnO_4$, 20 g K_2CO_3, 5 mL 5% NaOH (aq) and 300 mL H_2O) or acidic ethanolic anisaldehyde solution (formed by dissolving 15 g of anisaldehyde in 250 mL ethanol and 2.5 mL conc. sulfuric acid). Liquid reagents were distilled prior to use if necessary. Column chromatography was performed under pressure (forced flow) using silica gel (Fluorochem LC60A, 35-70 micron, 60A) as solid support and HPLC-grade solvents as eluent. Petroleum ether used for column chromatography was 40-60 °C fraction.

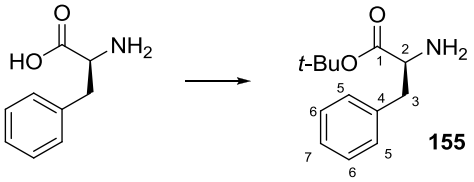
IR spectra were recorded using a type IIa diamond single reflection element on a Shimadzu FTIR-8400 instrument. The IR spectrum of the compound (solid or liquid) was directly detected as a thin layer at ambient temperature.

^{1}H NMR spectra were recorded on a Bruker 400 MHz or 500 MHz Spectrospin spectrometer (^{1}H NMR at 400 MHz and 500 MHz and ^{13}C NMR at 100 MHz). Chemical shifts are reported in ppm. ^{1}H NMR spectra were recorded with $CDCl_3$ as solvent using (δ = 7.26) as internal standard or CD_3OD using (δ = 4.87, 3.31) as internal standard. For ^{13}C NMR spectra, the chemical shifts are reported relative to the central resonance of $CDCl_3$ (δ = 77.2) or CD_3OD (δ = 49.1). Signals in NMR spectra are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination of these, which refers to the spin-spin coupling pattern observed. DEPT 135 and two-dimensional (COSY, HSQC) NMR spectroscopy were used where appropriate to assist the assignment of signals in the ^{1}H and ^{13}C NMR spectra.

High resolution mass spectra (HRMS) were recorded under EI, CI and FAB conditions by the analytical services at the University of Glasgow. Elemental analyses were carried out using an Exeter Analytical Elemental Analyser EA 440. Melting points were recorded with an Electrothermal IA 9100 apparatus. Optical rotations were determined on solutions of samples and irradiating with the sodium D line (λ = 589 nm) using an Autopol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}.

4.2 Experimental Procedures and Characterisation

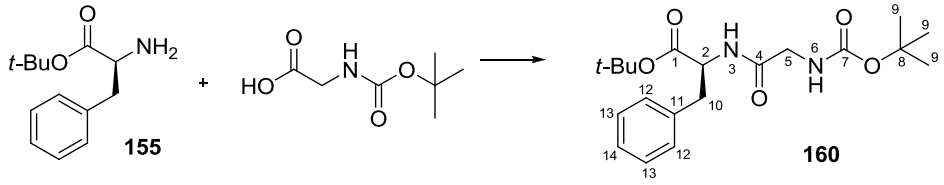
L-Phenylalanine *tert*-butyl ester 155



To a stirred solution of L-phenylalanine (500 mg, 3.03 mmol) in *tert*-butyl acetate (20 mL) at 0 °C was added $HClO_4$ (70% w/w, 0.640 mL, 4.46 mmol) dropwise. The resulting solution was allowed to warm to room temperature and stirred continuously for 16 hours. The reaction mixture was then washed with water (30 mL) and 1M HCl (30 mL). The aqueous layer was adjusted to pH 9 using a saturated aqueous solution of K_2CO_3 before extracting with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash column chromatography on silica gel (petroleum ether-ethyl acetate, 1:1) afforded *tert*-butyl ester **155** as a colourless oil (608 mg, 91%).

R_f = 0.12 (petroleum ether-ethyl acetate, 1:1); $[\alpha]^{28}_D$ +8.0 (*c* = 0.91, $CHCl_3$) {Lit. $[\alpha]^{27}_D$ +10.1 (*c* = 1.01, $CHCl_3$)}[186]; ν_{max} 3377, 2978, 1725, 846, 740, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.13 (5H, m, CH-Arom), 3.53 (1H, dd, *J* = 7.8, 5.4 Hz, CH-C2), 2.96 (1H, dd, *J* = 13.4, 5.4 Hz, CH_2-C3a), 2.76 (1H, dd, *J* = 13.4, 7.8 Hz, CH_2-C3b), 1.40 (2H, s, NH_2), 1.35 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3 (C-C1), 137.5 (C-C4), 129.4 (CH-Arom), 128.5 (CH-Arom), 126.7 (CH-Arom), 81.2 (C-*t*Bu), 56.2 (CH-C2), 41.2 (CH_2-C3), 28.0 (CH_3-*t*Bu); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{13}H_{20}NO_2$ 222.1494, found 222.1493, [Δ –0.6 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 223.1 (16%), 222.1 (100%), 209.1 (12%).

(S)-tert-Butyl 2-(2-(tert-butoxycarbonylamino)acetamido)-3-phenylpropanoate 160

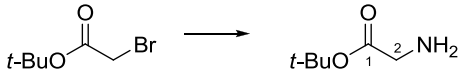


A solution of Boc-glycine (174 mg, 0.993 mmol) and HOBt (135 mg, 0.999 mmol) in CH_2Cl_2 (2 mL) and DMF (0.5 mL) was prepared and cooled to 0 °C. DCC (308 mg, 1.49 mmol) was

added and the mixture stirred for 1 hour at 0 °C. A solution of L-phenylalanine *tert*-butyl ester **155** (221 mg, 0.999 mmol) and TEA (140 μL, 1.00 mmol) in CH_2Cl_2 (1 mL) was added to the reaction. The resulting mixture was stirred for 1 hour at 0 °C, warmed to room temperature and stirred for a further 16 hours. The solution was concentrated *in vacuo* and the residue was suspended in ethyl acetate (15 mL). This solution was filtered and the resulting filtrate was washed with 5% aqueous citric acid (15 mL), 5% aqueous $NaHCO_3$ (15 mL) and brine (15 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Protected dipeptide **160** was isolated by flash column chromatography (petroleum ether-ethyl acetate 3:1) as a clear oil (199 mg, 53%).

R_f = 0.30 (petroleum ether-ethyl acetate 3:1); $[\alpha]^{27}_D$ +34.6 (*c* = 1.01, $CHCl_3$) {Lit. $[\alpha]^{27}_D$ +47.2 (*c* = 1.10, $CHCl_3$)}[187]; ν_{max} 3321, 2978, 1735, 1671, 1508, 741, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.21–7.12 (3H, m, CH-Arom), 7.09–7.07 (2H, m, CH-Arom), 6.68 (1H, d, *J* = 7.0 Hz, NH-N3), 5.22 (1H, br s, NH-N6), 4.68 (1H, dt, *J* = 7.0, 6.1 Hz, CH-C2), 3.75 (1H, dd, J = 16.5, 5.0 Hz, CH_2-C5a), 3.67 (1H, dd, *J* = 16.5, 5.2 Hz, CH_2-C5b) 3.01 (2H, d, *J* = 6.1 Hz, CH_2-C10), 1.37 (9H, s, CH_3-*t*Bu), 1.32 (9H, s, CH_3-C9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1 (C-C1), 167.8 (C-C4), 154.7 (C-C7), 134.8 (C-C11), 128.3 (C-Arom), 127.1 (C-Arom), 125.7 (C-Arom), 81.2 (C-C8), 78.8 (C-*t*Bu), 52.3 (CH-C2), 42.9 (CH_2-C6), 36.8 (CH_2-C10), 27.0 (CH_3-C9), 26.7 (CH_3-*t*Bu); HRMS (EI+) $[M]^+$ calcd for $C_{20}H_{30}N_2O_5$ 378.2155, found 378.2158, [Δ +0.8 ppm]; LRMS (EI+) *m/z* (intensity) 379.2 (28%), 378.2 (100%).

Glycine tert-butyl ester 158

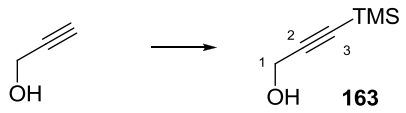


158

Ammonia (20 mL) was condensed into a flask cooled to –40 °C and diluted with dry Et_2O (15 mL). A solution of *tert*-butyl bromoacetate (3.20 mL, 21.7 mmol) in dry Et_2O (5 mL) was added slowly, maintaining the temperature at –40 °C. The reaction was stirred at this temperature for 2 hours before warming to room temperature and stirring, open to the air, for 16 hours. The solution was filtered and concentrated *in vacuo*. Flash column chromatography on silica gel (petroleum ether- ethyl acetate 1:1) afforded *tert*-butyl ester **158** as a clear oil (2.08 g, 73%).

R_f = 0.28 (petroleum ether-ethyl acetate 1:1); ν_{max} 3336, 2983, 2923, 1731, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.27 (2H, s, CH_2-C2), 1.89 (2H, s, NH_2), 1.40 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (C-C1), 81.3 (C-*t*Bu), 50.9 (CH_2-C2), 28.1 (CH_3-*t*Bu).

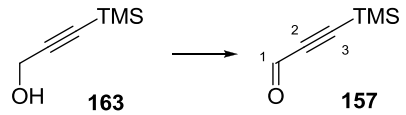
3-(Trimethylsilyl)prop-2-yn-1-ol 163



A solution of propargyl alcohol (570 μL, 10.0 mmol) in THF (50 mL) was prepared and cooled to –78 °C. *n*-BuLi (8.80 mL of a 2.5M solution in hexane, 22.0 mmol) was added to the solution dropwise and the mixture stirred for 30 minutes. Finally, TMSCl (2.86 mL, 22.5 mmol) was added and the mixture was warmed to room temperature. 1M HCl (50 mL) was added and the reaction was stirred for 1 hour. After this time, the aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The extracts were then dried ($MgSO_4$), filtered and concentrated *in vacuo*. The TMS acetylene **163** was isolated after flash column chromatography on silica gel (petroleum ether-ethyl acetate, 19:1) as a colourless oil (880 mg, 69%).

R_f = 0.31 (petroleum ether-ethyl acetate, 9:1); ν_{max} 3333, 2963, 2176, 756, 648 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.09 (2H, s, CH_2-C1), 2.04 (1H, s, OH), 0.00 (9H, s, TMS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.1 (C-C2), 90.8 (C-C3), 51.7 (CH_2-C1), 0.0 (CH_3-TMS); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_6H_{13}OSi$ 129.0736, found 129.0733, [Δ –0.3 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 129.1 (18%), 95.2 (100%); Anal. calcd for $C_6H_{12}OSi$: C, 56.19%; H, 9.43%. Found: C, 55.81%; H, 9.62%.

3-(Trimethylsilyl)prop-2-yn-1-al 157

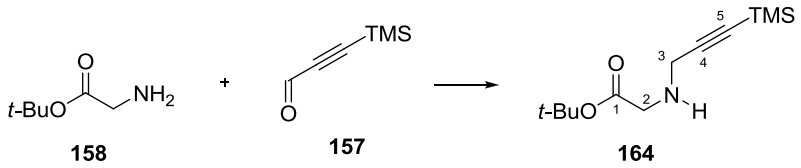


A suspension of PCC (3.51 g, 16.3 mmol) in CH_2Cl_2 (30 mL) was prepared and cooled to 0 °C. 3-(Trimethylsilyl)prop-2-yn-1-ol, **163**, (1.28g, 10.0 mmol) was added dropwise over a period of 10 minutes and the resulting mixture was stirred for 2 hours at room temperature. The reaction mixture was then filtered through a pad of silica washing with dichloromethane,

simultaneously removing the chromium residues and purifying the mixture, to give the target aldehyde **157** as a clear, colourless oil (675 mg, 54%).

R_f = 0.69 (petroleum ether-ethyl acetate, 9:1); ν_{max} 3317, 2965, 2858, 2154, 1667, 625cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (1H, s, CH-C1), 0.08 (9H, s, CH_3-TMS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7 (CH-C1), 104.0 (C-C2), 103.1 (C-C3), 0.0 (CH_3-TMS).

tert-Butyl 2-(3-(trimethylsilyl)prop-2-ynylamino)acetate 164

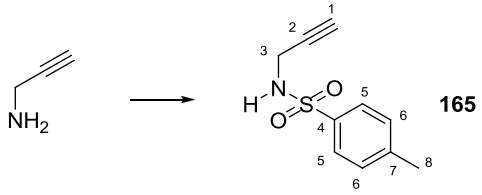


158 **157** **164**

A solution was prepared containing glycine *tert*-butyl ester **158** (310 mg, 2.36 mmol) and 3-(trimethylsilyl)prop-2-yn-1-al **157** (298 mg, 2.36 mmol) in dry THF (20 mL) at room temperature. Sodium triacetoxyborohydride (763 mg, 3.60 mmol) was then added and the reaction mixture was stirred for 16 hours. A further 0.5 equivalents of glycine *tert*-butyl ester (155 mg, 1.18 mmol) was added to assist in driving the reaction to completion. After a further 2 hours reaction time, the mixture was quenched by the addition of aqueous 3M NaOH solution (10 mL) and the aqueous layer was extracted with Et_2O (3 × 10 mL). The organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Amine **164** was isolated by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:1) as a clear colourless oil (56 mg, 10%).

R_f = 0.43 (petroleum ether-ethyl acetate, 1:1); ν_{max} 3338, 2962, 2931, 2868, 2167, 1733, 840, 759, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.31 (2H, s, CH_2-C2), 3.22 (2H, s, CH_2-C3), 1.61 (1H, s, NH), 1.31 (9H, s, CH_3-*t*Bu), 0.00 (9H, s, CH_3-TMS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C-C1), 103.5 (C-C4), 88.4 (C-C5), 81.4 (C-*t*Bu), 50.2 (CH_2-C2), 38.7 (CH_2-C3), 28.1 (CH_3-*t*Bu), 0.0 (CH_3-TMS); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{12}H_{24}NO_2Si$ 242.1576, found 242.1580, [Δ +0.3 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 243.2 (19%), 242.2 (100%).

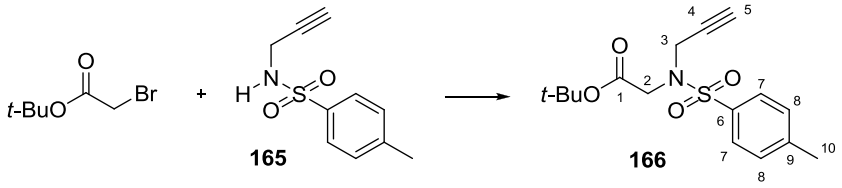
4-Methyl-*N*-(prop-2-ynyl)benzenesulfonamide 165



A flask was charged with TsCl (9.70 g, 51.0 mmol) before the addition of THF (40 mL) and pyridine (4.30 mL, 53.2 mmol). The solution was cooled to 0 °C and propargylamine (3.20 mL, 50.0 mmol) was added dropwise. The reaction mixture was stirred for 18 hours, allowing it to reach room temperature slowly. Aqueous 2M NaOH solution (10 mL) was added and the resulting solution was stirred for a further 45 minutes. The aqueous layer was separated and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Sulfonamide **165** was isolated after flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:1) to give a white solid (8.2 g, 78%).

R_f = 0.41 (petroleum ether-ethyl acetate, 2:1); m.p. = 73.5–74 °C {Lit. m.p. 73–74 °C}[188]; ν_{max} 3271, 2924, 2863, 2129, 810, 664 cm $^{-1}$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (2H, d, *J* = 8.1 Hz, CH-C5), 7.35 (2H, d, *J* = 8.1 Hz, CH-C6), 4.69 (1H, t, *J* = 5.8 Hz, NH), 3.86 (2H, dd, *J* = 5.8, 2.6 Hz, CH_2-C3), 2.47 (3H, s, CH_3-C8), 2.14 (1H, t, *J* = 2.6 Hz, CH-C1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8 (C-Arom), 135.5 (C-Arom), 128.7 (CH-C6), 126.4 (CH-C5), 76.9 (C-C2), 72.0 (CH-C1), 31.8 (CH_2-C3), 20.5 (CH_3-C8); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{10}H_{12}NO_2S$ 210.0589, found 210.0589, [Δ +0.3 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 211.1 (12%), 210.1 (100%); Anal. calcd for $C_{10}H_{11}NO_2S$: C, 57.42%; H, 5.26%; N, 6.70%. Found: C, 57.45%; H, 5.31%; 6.69%.

tert-Butyl 2-(4-methyl-*N*-(prop-2-ynyl)phenylsulfonamido)acetate 166

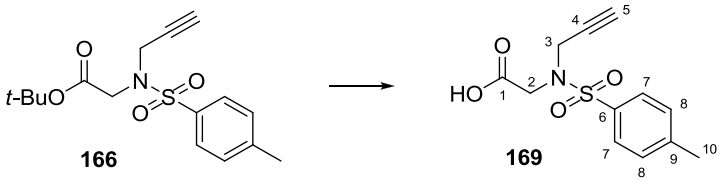


To a solution of sulfonamide **165** (800 mg, 3.83 mmol) in acetone (10 mL) was added anhydrous K_2CO_3 (1.60 g, 11.6 mmol) followed by vigorous stirring at room temperature for 18 hours. The suspension was filtered and washed with acetone and the filtrate was

concentrated *in vacuo*. Ester **166** was isolated after flash column chromatography using silica gel (petroleum ether-ethyl acetate, 9:1) as a white solid (1.16 g, 94%).

R_f = 0.26 (petroleum ether-ethyl acetate 9.1); m.p. = 47–49 °C; ν_{max} 3280, 2983, 1735, 700, 685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (2H, d, *J* = 8.2 Hz, CH-C7), 7.22 (2H, d, *J* = 8.2 Hz, CH-C8), 4.19 (2H, d, *J* = 2.4 Hz, CH_2-C3), 3.94 (2H, s, CH_2-C2), 2.34 (3H, s, CH_3-C10), 2.08 (1H, t, *J* = 2.4 Hz, CH-C5), 1.35 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4 (C-C1), 143.7 (C-Arom), 136.3 (C-Arom), 129.6 (CH-C8), 127.5 (CH-C7), 82.3 (C-*t*Bu), 76.6 (CH-C5), 74.1 (C-C4), 47.4 (CH_2-C2), 37.3 (CH_2-C3), 28.0 (CH_3-*t*Bu), 21.5 (CH_3-C10); HRMS (FAB) $[M+H]^+$ calcd for $C_{16}H_{22}NO_4S$ 324.1270, found 324.1269, [Δ –0.3 ppm]; LRMS (FAB) *m/z* (intensity) 325.1 (20%), 324.1 (100%); Anal calcd for $C_{16}H_{21}NO_4S$: C, 59.44%; H, 6.50%; N, 4.33%. Found: C, 59.65%; H, 6.59%; N, 4.46%.

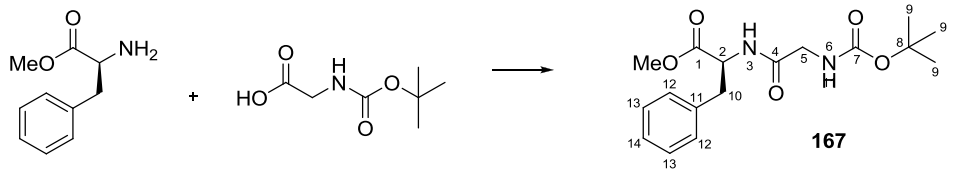
2-(4-Methyl-*N*-(prop-2-ynyl)phenylsulfonamido)acetic acid 169



A solution of the *tert*-butyl ester **166** (594 mg, 1.84 mmol) in CH_2Cl_2 (5 mL) was prepared, to which was added TFA (5 mL) at room temperature. The reaction mixture was stirred for 2 hours followed by concentration *in vacuo*. A small volume of toluene was added to the residue before concentrating *in vacuo* once again. This was repeated a further two times to ensure complete removal of trace amounts of TFA. After further drying under high vacuum, carboxylic acid **169** was isolated as an off-white solid (490 mg, 100%).

R_f = 0.61 (dichloromethane-methanol, 9:1); m.p. = 100–101 °C; ν_{max} 3262, 3050, 2969, 2554, 2120, 1732, 1707, 1599, 816, 766 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.90 (1H, br s, OH), 7.65 (2H, d, *J* = 8.2 Hz, CH-C7), 7.24 (2H, d, *J* = 8.2 Hz, CH-C8), 4.18 (2H, d, *J* = 2.2 Hz, CH_2-C3), 4.07 (2H, s, CH_2-C2), 2.36 (3H, s, CH_3-C10), 2.09 (1H, t, *J* = 2.2 Hz, CH-C5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3 (C-C1), 144.2 (C-Arom), 135.7 (C-Arom), 129.7 (CH-C8), 127.5 (CH-C7), 76.1 (CH-C5), 74.7 (C-C4), 46.6 (CH_2-C2), 37.5 (CH_2-C3), 21.6 (CH_3-C10); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{12}H_{14}NO_4S$ 268.0644, found 268.0650 [Δ +2.4 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 285.1 (17%), 284.1 (100%), 168.1 (47%); Anal calcd for $C_{12}H_{13}NO_4S$: C, 53.92%; H, 4.90%; N, 5.24%. Found: C, 54.05%; H, 5.09%; N, 5.06%.

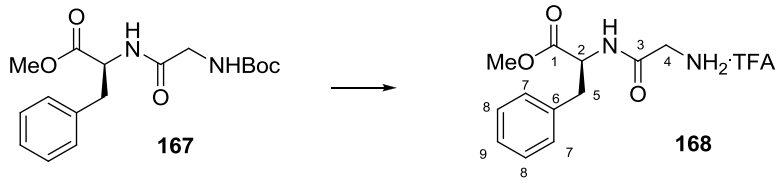
(S)-Methyl 2-(2-(tert-butoxycarbonylamino)acetamido)-3-phenylpropanoate 167



A solution of Boc-glycine (960 mg, 5.49 mmol) and HOBt (675 mg, 5.00 mmol) was prepared in CH_2Cl_2 (10 mL) and DMF (2.5 mL) and cooled to 0 °C. DCC (1.13 g, 5.49 mmol) was added and the reaction stirred for 30 minutes. A solution of L-phenylalanine methyl ester hydrochloride (1.10 g, 5.09 mmol) and TEA (700 μL, 5.03 mmol) in CH_2Cl_2 was added to the reaction mixture followed by a further 30 minutes of stirring at 0 °C and 18 hours at room temperature. The mixture was concentrated and the white residue was suspended in EtOAc (15 mL) and filtered. The filtrate was washed with 5% aqueous citric acid (15 mL), 5% aqueous $NaHCO_3$ (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Dipeptide **167** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 1:1) as a colourless oil (1.4 g, 92%).

R_f = 0.28 (petroleum ether-ethyl acetate, 1:1); $[\alpha]^{27}_D$ +47.8 (*c* = 1.05, $CHCl_3$); ν_{max} 3403, 3311, 2978, 1743, 1717, 1666, 1506, 743, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.24–7.17 (3H, m, CH-Arom), 7.03–7.02 (2H, m, CH-Arom), 6.46 (1H, d, *J* = 7.1 Hz, NH-N3), 5.02 (1H, br s, NH-N6), 4.82 (1H, ddd, *J* = 7.1, 5.9, 5.8 Hz, CH-C2), 3.79-3.61 (2H, m, CH_2-C5), 3.65 (3H, s, CH_3-Me), 3.08 (1H, dd, *J* = 13.9, 5.8 Hz, CH_2-C10a), 3.03 (1H, dd, *J* = 13.9, 5.9 Hz, CH_2-C10b), 1.37 (9H, s, CH_3-C9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C-C1), 169.3 (C-C4), 156.0 (C-C6), 135.7 (C-C11), 129.3 (CH-Arom), 128.6 (CH-Arom), 127.2 (CH-Arom), 80.1 (C-C8), 53.1 (CH-C2), 52.4 (CH_3-Me), 44.1 (CH_2-C5), 37.9 (CH_2-C10), 28.3 (CH_3-C9); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{17}H_{25}N_2O_5$ 337.1763, found 337.1760 [Δ –1.1 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 338.2 (20%), 337.2 (100%); Anal calcd for $C_{17}H_{24}N_2O_5$: C, 60.70%; H, 7.19%; N, 8.33%. Found: C, 60.70%; H, 7.15%; N, 8.33%.

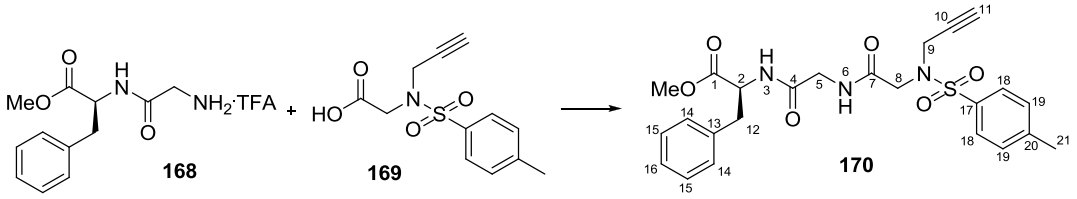
(*S*)-Methyl 2-(2-aminoacetamido)-3-phenylpropanoate TFA salt 168



A solution of carbamate **167** (233 mg, 0.693 mmol) was prepared in CH_2Cl_2 (2 mL) before addition of TFA (2 mL) at room temperature followed by stirring for 2 hours. After this time, the mixture was concentrated *in vacuo*. A small volume of toluene was added to the residue and it was concentrated *in vacuo* once again. This was repeated a further two times to remove trace amounts of TFA. Finally with drying under high vacuum, the target amine **168** was isolated as its TFA salt as a fluffy white solid (242 mg, 100%).

M.p. = 61–63 °C; $[\alpha]^{27}_D$ +9.6 (*c* = 1.13, $CHCl_3$); ν_{max} 3032, 2955, 2855, 2654, 1736, 1667, 1551, 833, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.74 (3H, br s, NH_3^+), 7.32 (1H, d, *J* = 7.9 Hz, NH), 7.15–7.07 (3H, m, CH-Arom), 6.97–6.95 (2H, m, CH-Arom), 4.67 (1H, ddd, *J* = 7.9, 7.3, 5.6 Hz, CH-C2), 3.70 (1H, d, *J* = 15.2 Hz, CH_2-C4a), 3.55 (1H, d, *J* = 15.2 Hz, CH_2-C4b), 3.54 (3H, s, CH_3-Me), 2.96 (1H, dd, *J* = 14.0, 5.6 Hz, CH_2-C5a), 2.85 (1H, dd, *J* = 14.0, 7.3 Hz, CH_2-C5b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1 (C-C1), 166.1 (C-C3), 161.3 (q, $^2J_{F\text{-}C}$ = 30.4, C-TFA), 135.3 (C-C6), 129.0 (CH-Arom), 128.7 (CH-Arom), 127.3 (CH-Arom), 115.6 (q, $^1J_{F\text{-}C}$ = 229.8 Hz, CF_3-TFA), 54.0 (CH-C2), 52.6 (CH_3-Me), 40.9 (CH_2-C4), 37.4 (CH_2-C5); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{12}H_{17}N_2O_3$ 237.1239, found 237.1240, [Δ +0.4 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 238.1 (16%), 237.1 (100%).

Methyl 2-(2-(2-(4-methyl-*N*-(prop-2-ynyl)phenylsulfonamido)acetamido)acetamido)-3-phenylpropanoate 170

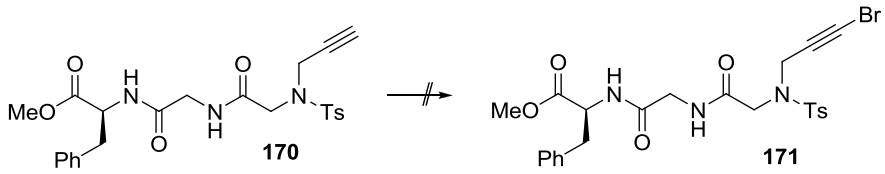


A solution of carboxylic acid **169** (100 mg, 0.374 mmol) and HOBt (51.0 mg, 0.377 mmol), in CH_2Cl_2 (3 mL) and DMF (1 mL) was prepared and cooled to 0 °C. DCC (83.0 mg, 0.402 mmol) was added to the solution and it was stirred at 0 °C for 30 minutes. A solution of TFA salt **168** (132 mg, 0.377 mmol) in CH_2Cl_2 (2 mL) with TEA (52.3 μL, 0.376 mmol) was prepared separately and added to the reaction mixture. The resulting mixture was stirred for

1 hour at 0 °C and a further 16 hours at room temperature. The reaction was concentrated and the resulting white residue was suspended in ethyl acetate (20 mL). The suspension was filtered and the filtrate was washed with 5% aqueous citric acid solution (10 mL), 5% aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Tripeptide **170** was isolated after flash column chromatography on silica gel (dichloromethane-methanol, 19:1) as a white solid (159 mg, 88%).

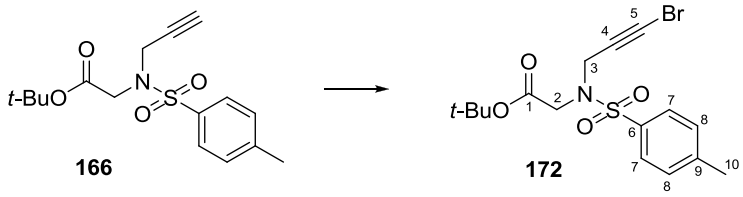
R_f = 0.43 (dichloromethane-methanol, 19:1); m.p. = 47–48 °C; $[\alpha]^{26}_D$ +30.7 (*c* = 1.00, $CHCl_3$); ν_{max} 3364, 3295, 1744, 1659, 1520, 910, 810, 733, 656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (2H, d, *J* = 8.2 Hz, CH-C18), 7.26 (2H, d, *J* = 8.2 Hz, CH-C19), 7.23 – 7.13 (4H, m, CH-Arom and NH-N6), 7.07–7.06 (2H, m, CH-Arom), 6.74 (1H, d, *J* = 7.8 Hz, NH-N3), 4.78 (1H, ddd, *J* = 7.8, 6.7, 5.8 Hz, CH-C2), 4.06 (2H, d, *J* = 2.5 Hz, CH_2-C9), 3.94 (1H, dd, *J* = 16.9, 6.0 Hz, CH_2-C5a), 3.85 (1H, dd, *J* = 16.9, 5.8 Hz, CH_2-C5b), 3.74 (2H, d, *J* = 2.4 Hz, CH_2-C8), 3.62 (3H, s, CH_3-Me), 3.07 (1H, dd, *J* = 13.9, 5.8 Hz, CH_2-C12a), 2.98 (1H, dd, *J* = 13.9, 6.7 Hz, CH_2-C12b), 2.37 (3H, s, CH_3-C21), 2.01 (1H, t, *J* = 2.5 Hz, CH-C11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7 (C-C1), 168.3 (C-C4), 168.2 (C-C7), 144.8 (C-Arom), 135.8 (C-Arom), 133.7 (C-C13), 129.9 (CH-C19), 129.3 (CH-Arom), 128.6 (CH-Arom), 128.0 (CH-C18), 127.1 (CH-Arom), 75.7 (CH-C11), 75.2 (C-C10), 53.4 (CH-C2), 52.4 (CH_3-Me), 50.2 (CH_2-C8), 42.8 (CH_2-C5), 39.3 (CH_2-C9), 37.8 (CH_2-C12), 21.6 (CH_3-C21); HRMS (FAB) $[M+H]^+$ calcd for $C_{24}H_{28}N_3O_6S$ 486.1699, found 486.1707 [Δ +1.6 ppm]; LRMS (FAB) *m/z* (intensity) 488.2 (12%), 487.1 (30.7%), 486.2 (100%).

Bromination of Tripeptidic Alkyne



To a solution of tripeptide **170** (100 mg, 0.206 mmol) in dry acetone (3 mL) at room temperature, was added $AgNO_3$ (3.50 mg, 0.0206 mmol) and the mixture was stirred for 15 minutes. NBS (34.9 mg, 0.196 mmol) was then added and the mixture was stirred at room temperature for a period of 4.5 hours. The mixture was diluted with acetone and filtered, before concentration *in vacuo* to give a yellow oil. Attempts to isolate possible products identified by TLC analysis, using flash column chromatography on silica gel (dichloromethane-methanol 37:3), provided polybrominated products. This approach was abandoned in favour of an alternate route to the alkynyl bromide.

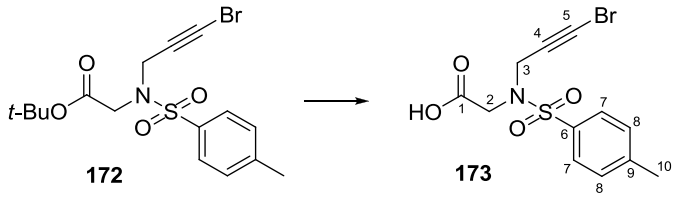
tert-Butyl 2-(*N*-(3-bromoprop-2-ynyl)-4-methylphenylsulfonamido)acetate 172



To a solution of alkyne **166** (1.60 g, 4.95 mmol) in acetone (5 mL) was added silver nitrate (168 mg, 0.988 mmol) and the reaction vessel was covered with aluminium foil. After stirring for 30 minutes, NBS (979 mg, 5.50 mmol) was added. The reaction mixture was stirred for a subsequent 18 hours. The suspension was filtered and washed with EtOAc and the filtrate was concentrated *in vacuo*. Bromide **172** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 4:1), giving a white solid (1.7 g, 88%).

R_f = 0.48 (petroleum ether-ethyl acetate, 3:1); m.p. = 93–94 °C; ν_{max} 2980, 2214, 1922, 1744, 1599, 813, 623, 602 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (2H, d, *J* = 8.2 Hz, CH-C7), 7.24 (2H, d, *J* = 8.2 Hz, CH-C8), 4.20 (2H, s, CH_2-C3), 3.89 (2H, s, CH_2-C2), 2.35 (3H, s, CH_3-C10), 1.36 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3 (C-C1), 143.9 (C-Arom), 135.9 (C-Arom), 129.6 (CH-C8), 127.6 (CH-C7), 82.5 (C-*t*Bu), 73.0 (C-C4), 47.7 (CH_2-C2), 45.5 (C-C5), 38.5 (CH_2-C3), 28.0 (CH_3-*t*Bu), 21.6 (CH_3-C10); HRMS (FAB) $[M+H]^+$ calcd for $C_{16}H_{21}BrNO_4S$ 402.0375, found 402.0372 [Δ –0.7 ppm]; LRMS (FAB) *m/z* (intensity) 404.0 (10%), 402.0 (12%), 345.9 (100%); Anal calcd for $C_{16}H_{20}BrNO_4S$: C, 47.77%; H, 5.01%; N, 3.48%. Found: C, 47.62; H, 5.00%; N, 3.53%.

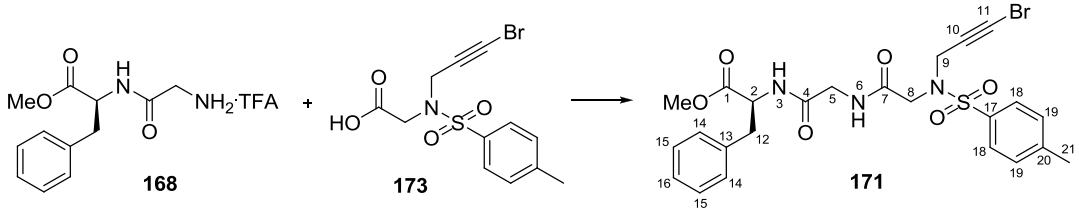
2-(*N*-(3-Bromoprop-2-ynyl)-4-methylphenylsulfonamido)acetic acid 173



A solution of *tert*-butyl ester **172** (50.0 mg, 0.124 mmol) in CH_2Cl_2 (2 mL) was prepared. TFA (2 mL) was added to the solution and it was stirred for 3 hours at room temperature. After this time the reaction was concentrated *in vacuo*. A small volume of toluene was added to the residue, which was then concentrated *in vacuo* once again. This procedure was repeated a further two times to remove traces of TFA, followed by a period of drying under high vacuum. The carboxylic acid **173** was isolated as an off white solid without further purification (43 mg, 100%).

R_f = 0.23 (petroleum ether-ethyl acetate, 3:1); m.p. = 125–127 °C; ν_{max} 2969, 2920, 1729, 1710, 1599, 812, 691, 664, 636 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.82 (1H, br s, OH), 7.65 (2H, d, *J* = 8.2 Hz, CH-C7), 7.26 (2H, d, *J* = 8.2 Hz, CH-C8), 4.19 (2H, s, CH_2-C3), 4.04 (2H, s, CH_2-C2), 2.37 (3H, s, CH_3-C10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C-C1), 143.3 (C-Arom), 134.3 (C-Arom), 128.7 (CH-C8), 126.5 (CH-C7), 71.4 (C-C4), 45.8 (CH_2-C2), 45.3 (C-C5), 37.6 (CH_2-C3), 20.6 (CH_3-C10); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{12}H_{13}BrNO_4S$ 345.9749; found 345.9750 [Δ +0.3 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 348.0 (100%), 347.0 (15%), 346.0 (93%); Anal calcd for $C_{12}H_{12}BrNO_4S$: C, 41.63%; H, 3.49%; N, 4.05%. Found: C, 42.10%; H, 3.56%; N, 4.13%.

(*S*)-Methyl 2-(2-(2-(*N*-(3-bromoprop-2-ynyl)-4-methylphenylsulfonamido)acetamido)acetamido)-3-phenylpropanoate 171

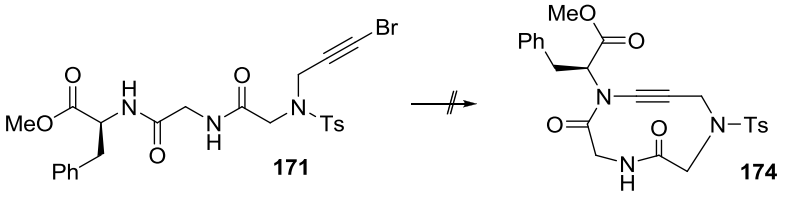


A solution of carboxylic acid **173** (169 mg, 0.488 mmol) was prepared in CH_2Cl_2 (6 mL). HOBt (53.6 mg, 0.397 mmol) and DMF (1 mL) were added to the solution, which was then cooled to 0 °C. After stirring for 30 minutes, a solution of TFA salt **168** (139 mg, 0.397 mmol) and TEA (110 μL, 0.794 mmol) in CH_2Cl_2 (2 mL) was added. The reaction was stirred at 0 °C for 1 hour and a further 16 hours at room temperature. The mixture was concentrated and the residue was suspended in EtOAc (20 mL). This was then filtered and the filtrate was washed with 5% aqueous citric acid (20 mL), 5% aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Tripeptide **171** was isolated by flash column chromatography using silica gel (dichloromethane-methanol, 19:1) to give a white solid (160 mg, 58%).

R_f = 0.32 (dichloromethane-methanol, 19:1); m.p. = 49–50 °C; $[\alpha]^{26}_D$ +47.8 (*c* = 0.99, $CHCl_3$); ν_{max} 3358, 3066, 2950, 2215, 1741, 1659, 1518, 803, 747, 701, 662, 625 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (2H, d, *J* = 8.2 Hz, CH-C18), 7.29 (2H, d, *J* = 8.2 Hz, CH-C19), 7.25–7.16 (3H, m, CH-Arom), 7.06–7.04 (2H, m, CH-Arom), 6.99 (1H, dd, *J* = 6.0, 5.8 Hz, NH-N6), 6.40 (1H, d, *J* = 7.8 Hz, NH-N3), 4.88 (1H, ddd, *J* = 7.8, 6.4, 5.8 Hz, CH-C2), 4.11 (1H, d, *J* = 18.3 Hz, CH_2-C9a), 4.06 (1H, d, *J* = 18.3 Hz, CH_2-C9b), 3.94 (1H, dd, *J* =16.7, 6.0 Hz, CH_2-C5a), 3.87 (1H, dd, *J* = 16.7, 5.8 Hz, CH_2-C5b), 3.72 (2H, s, CH_2-C8), 3.66 (3H, s, CH_3-

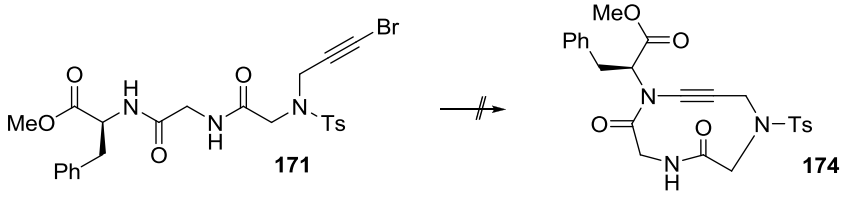
Me), 3.09 (1H, dd, *J* = 13.9, 5.8 Hz, CH_2-C12a), 3.01 (1H, dd, *J* = 13.9, 6.4 Hz, CH_2-C12b), 2.39 (3H, s, CH_3-C21); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6 (C-C1), 168.1 (C-C4), 168.0 (C-C7), 144.8 (C-Arom), 135.7 (C-Arom), 133.7 (C-Arom), 129.9 (CH-C19), 129.3 (CH-Arom), 128.6 (CH-Arom), 128.1 (CH-C18), 127.2 (CH-Arom), 77.2 (C-C10), 72.1 (C-C11), 53.3 (CH-C2), 52.4 (CH_3-Me), 50.4 (CH_2-C8), 42.8 (CH_2-C5), 40.2 (CH_2-C9), 37.8 (CH_2-C12), 21.6 (CH_3-C21); HRMS (FAB) $[M+H]^+$ calcd for $C_{24}H_{27}BrN_3O_6S$ 564.0804, found 564.0801 [Δ –0.6 ppm]; LRMS (FAB) *m/z* (intensity) 567.1 (30%), 566.1 (100%), 565.1 (29%), 564.1 (97%).

Hsung 2nd Generation Coupling Using Potassium Carbonate



To a solution of alkynyl bromide **171** (15.0 mg, 0.0266 mmol) in toluene (2 mL) was added, sequentially, $CuSO_4 \cdot 5H_2O$ (0.700 mg, 0.00280 mmol), 1,10-phenanthroline (1.00 mg, 0.00555 mmol) and K_2CO_3 (7.30 mg, 0.528 mmol). The reaction mixture was heated to 70 °C and stirred for 41 hours. The mixture was diluted with EtOAc and filtered through a pad of Celite®, before concentration *in vacuo*. Analysis of the residue indicated that no reaction had taken place; the starting material was recovered using flash column chromatography on silica gel (petroleum ether-ethyl acetate 4:1).

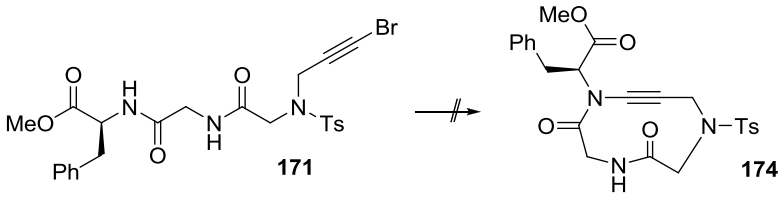
Hsung 2nd Generation Coupling Using Potassium Phosphate



A solution of tripeptide **171** (21.0 mg, 0.0372 mmol) in toluene (10 mL) was prepared, before the sequential addition of $CuSO_4 \cdot 5H_2O$ (1.90 mg, 0.00761 mmol), 1,10-phenanthroline (2.70 mg, 0.0150 mmol) and K_3PO_4 (15.0 mg, 0.0707 mmol). The reaction mixture was heated to reflux and stirred continuously for 48 hours. The reaction was allowed to cool to room

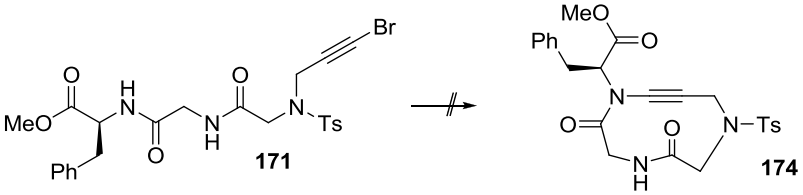
temperature before concentration *in vacuo*. TLC analysis indicated traces of new products and so the mixture was separated using flash column chromatography on silica gel (dichloromethane-methanol, 19:1). Analysis of the new products using NMR methods revealed them to be trace decomposition products rather than the desired macrocycle.

Zhang Iron Catalysed Coupling



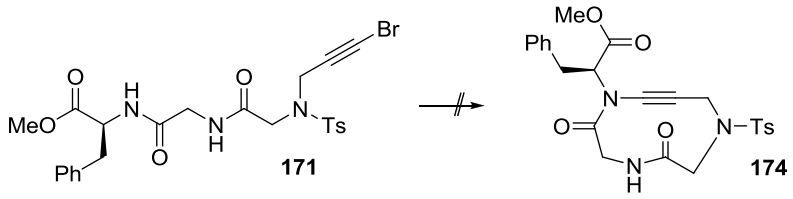
A solution of tripeptide **171** (20.0 mg, 0.0354 mmol) was prepared in toluene (5 mL), before the sequential addition of $FeCl_3 \cdot 6H_2O$ (0.960 mg, 0.00355 mmol), DMEDA (0.630 mg, 0.00715 mmol) and K_2CO_3 (9.70 mg, 0.0702 mmol). The mixture was heated to 90 °C and stirred for a period of 22 hours. TLC analysis indicated no progression of reaction or consumption of starting material. The reaction mixture was filtered and concentrated *in vacuo*. The starting material was successfully recovered from the reaction mixture using flash column chromatography on silica gel (dichloromethane-methanol, 19:1).

Danheiser Coupling Protocol



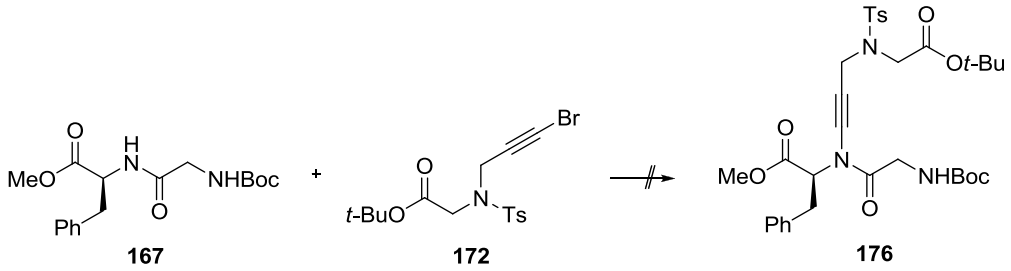
A solution of tripeptide **171** (20.0 mg, 0.0354 mmol) and pyridine (0.1 mL) in THF (10 mL) was prepared and cooled to 0 °C. KHMDS (70.0 μL of a 0.5 M solution in toluene, 0.0350 mmol) was added dropwise and the resulting mixture was stirred for 15 minutes. CuI (6.70 mg, 0.0352 mmol) was added and the reaction mixture was allowed to warm to room temperature. After stirring for 20 hours, the reaction was diluted with Et_2O (10 mL) and washed with a 2:1 solution of brine and saturated aqueous NH_4Cl (3 × 20 mL). The aqueous washings were then extracted with Et_2O (3 × 20 mL) and the combined extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo*. TLC analysis of the residue indicated no consumption of starting material and no progression of reaction.

Hsung 1st Generation Protocol



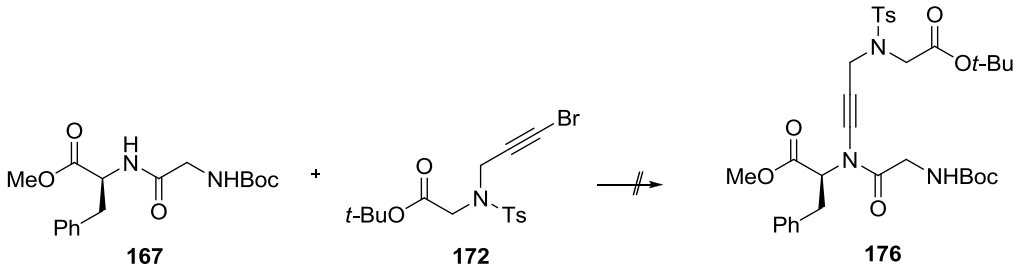
A solution of tripeptide **171** (20.0 mg, 0.0354 mmol) in toluene (0.5 mL) was prepared before the addition of K_3PO_4 (15.0 mg, 0.0707 mmol) and CuCN (0.160 mg, 0.00179 mmol). Finally, DMEDA (0.380 μL, 0.00353 mmol) was added to the mixture and the reaction was heated to 90 °C. After stirring at this temperature for 48 hours, the reaction mixture was allowed to cool to room temperature. The mixture was filtered through a pad of silica and concentrated *in vacuo*. Analysis of the residue indicated that the desired product had not formed and that there was no consumption of the starting material.

Intramolecular Approach Using Hsung 2nd Generation Protocol



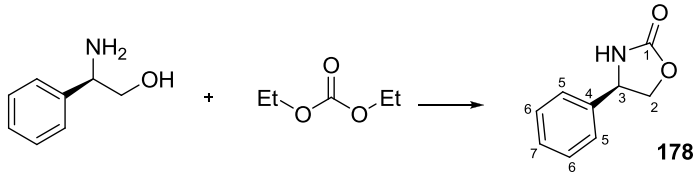
To a solution of protected dipeptide **167** (50.0 mg, 0.149 mmol) in toluene (3 mL) was added $CuSO_4 \cdot 5H_2O$ (3.70 mg, 0.0148 mmol), 1,10-phenanthroline (5.40 mg, 0.0300 mmol) and K_3PO_4 (63.0 mg, 0.297 mmol). Finally, alkynyl bromide **172** (66.0 mg, 0.164 mmol) was added to the reaction mixture, before heating it to 75 °C. The reaction was stirred for 24 hours, after which time TLC analysis revealed that reaction had not taken place. The reaction mixture was concentrated *in vacuo* and the starting materials were recovered by flash column chromatography on silica gel (petroleum ether-ethyl acetate 4:1).

Intramolecular Approach Using Danheiser Stoichiometric Protocol



A solution of dipeptide **167** (17.0 mg, 0.0505 mmol) in pyridine (0.13 mL) was prepared and cooled to 0 °C. KHMDS (0.100 mL of a 0.5 M solution in toluene, 0.0500 mmol) was added dropwise and the resulting mixture was stirred for 15 minutes. A solution of CuI (9.50 mg, 0.0499 mmol) in pyridine (0.12 mL) was added and the reaction mixture was allowed to warm to room temperature, followed by another 2 hours of stirring. Finally, a solution of alkynyl bromide **172** (40.0 mg, 0.0994 mmol) in THF (0.25 mL) was added slowly to the mixture, before stirring for 20 hours. The mixture was diluted with Et_2O (15 mL) and washed with a 2:1 mixture of brine and saturated aqueous NH_4Cl (3 × 15 mL). The combined aqueous washings were extracted with Et_2O (3 × 15 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. TLC analysis did not indicate product formation, and so the starting materials were recovered from the residue using flash column chromatography on silica gel (petroleum ether-ethyl acetate, 4:1).

(*R*)-4-Phenyloxazolidin-2-one 178

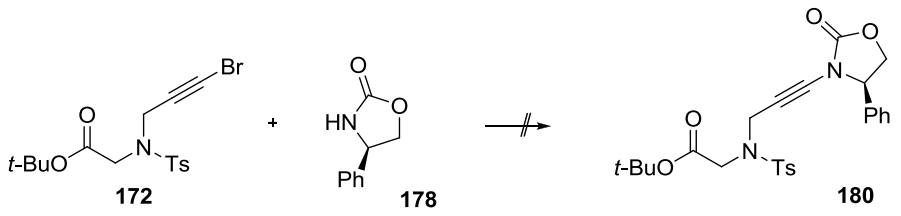


A 2 neck round-bottomed flask with attached distillation condenser was flame dried under vacuum. After cooling, the flask was charged with (*R*)-(–)-2-phenylglycinol (2.00 g, 14.6 mmol), dried K_2CO_3 (201 mg, 1.46 mmol) and diethyl carbonate (3.50 mL, 28.9 mmol). The flask was lowered into a pre-heated oil bath at 135 °C. The reaction was stirred at this temperature, allowing ethanol by-product to be distilled into a separate flask. The reaction was complete upon cessation of ethanol formation, at which point the reaction was cooled and diluted with CH_2Cl_2 (1 L). The solution was washed with water (500 mL) and the organic layer was dried ($MgSO_4$), filtered and concentrated yielding a yellow solid. The product was washed with Et_2O and filtered to give a clean white solid. Further product crystallised out of

the Et_2O washings upon cooling. This was also filtered and collected, giving oxazolidinone **178** as a white solid (887 mg, 37%).

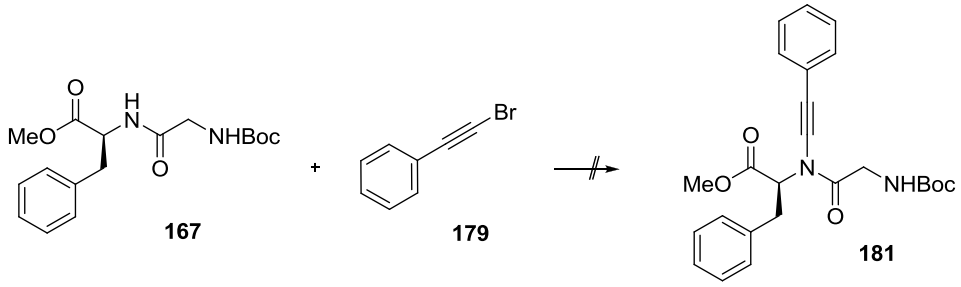
R_f = 0.23 (petroleum ether-ethyl acetate, 1:1); m.p. = 127–129 °C {Lit. m.p. = 129–131 °C}[189]; $[\alpha]^{26}_D$ –55.3 (*c* = 1.05, $CHCl_3$) {Lit. $[\alpha]^{20}_D$ –54.0 (*c* = 1.00, $CHCl_3$)}[190]; ν_{max} 3237, 3140, 1736, 1701, 748, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.26 (5H, m, CH-Arom), 5.71 (1H, s, NH), 4.89 (1H, dd, *J* = 8.6, 7.0 Hz, CH-C3), 4.67 (1H, dd, *J* = 8.6, 8.6 Hz, CH_2-C2a), 4.12 (1H, dd, *J* = 8.6, 7.0 Hz, CH_2-C2b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7 (C-C1), 139.5 (C-C4), 129.2 (CH-Arom), 128.9 (CH-Arom), 126.1 (CH-Arom), 72.5 (CH_2-C2), 59.4 (CH-C3); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_9H_{10}NO_2$ 164.0712, found 164.0709 [Δ –1.3 ppm]; LRMS *m/z* (intensity) 165.1 (10%), 164.1 (100%); Anal. calcd for $C_9H_9NO_2$: C, 66.25%; H, 5.56%; N, 8.58%. Found C, 66.27%; H, 5.51%; N, 8.55%.

Alkynyl Bromide Fragment Test Reaction



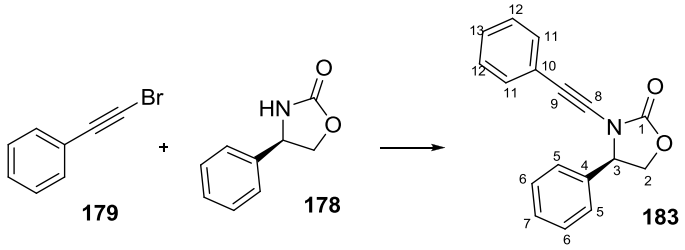
To a solution of oxazolidinone **178** (100 mg, 0.613 mmol) in toluene (10 mL) was added, sequentially, $CuSO_4 \cdot 5H_2O$ (15.3 mg, 0.0613 mmol), 1,10-phenanthroline (22.1 mg, 0.123 mmol) and K_3PO_4 (260 mg, 1.22 mmol). Finally, alkynyl bromide **172** (271 mg, 0.674 mmol) was added and the reaction mixture was heated to 75 °C for 48 hours. The reaction mixture was then concentrated *in vacuo*. TLC analysis indicated that reaction had not occurred and so the starting materials were recovered by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:1).

Amide Fragment Test Reaction



To a solution of dipeptide **167** (169 mg, 0.502 mmol) in toluene (10 mL) was added $CuSO_4 \cdot 5H_2O$ (12.5 mg, 0.0501 mmol), 1,10-phenanthroline (18.1 mg, 0.100 mmol) and K_3PO_4 (213 mg, 1.00 mmol). Finally, phenyl acetylene bromide **179** (100 mg, 0.552 mmol) was added and the mixture was heated to 75 °C. After stirring for 20 hours, analysis of the reaction mixture by TLC indicated the formation of a new product. The reaction mixture was allowed to cool to room temperature before it was filtered, washing through with excess dichloromethane and methanol. The filtrate was then concentrated *in vacuo*. The reaction mixture was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 2:3) and the new product was analysed using NMR spectroscopy. This new material was not the desired ynamide and there was strong evidence that it was, in fact, the alkynyl dimer product.

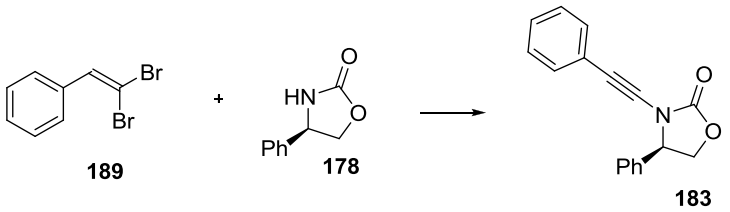
(*R*)-4-Phenyl-3-(phenylethynyl)oxazolidin-2-one 183



A vial was charged with (*R*)-4-phenyloxazolidin-2-one, **178** (25.0 mg, 0.153 mmol), $CuSO_4 \cdot 5H_2O$ (3.80 mg, 0.0152 mmol), 1,10-phenanthroline (5.50 mg, 0.0306 mmol) and dried potassium phosphate (65.0 mg, 0.307 mmol). A solution of phenylacetylene bromide **179** (30.0 mg, 0.166 mmol) in dry toluene (0.2 mL) was added to the vial, which was then heated to 75 °C. The reaction was stirred for 3 days before cooling to room temperature The mixture was filtered and washed with EtOAc and the filtrate was concentrated *in vacuo*. The target ynamide **183** was isolated by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 2:1) giving a white solid (17.3 mg, 43%).

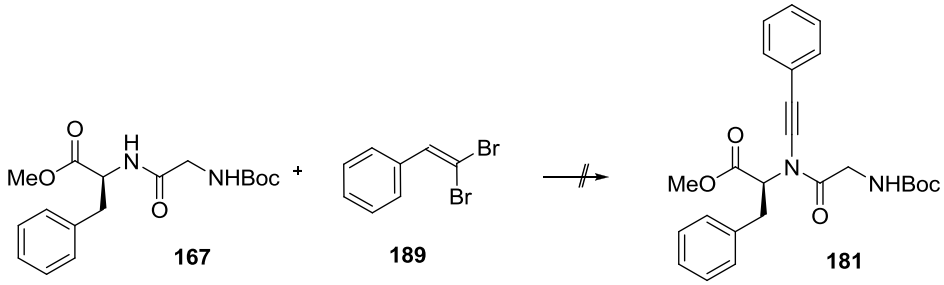
R_f = 0.63 (petroleum ether-ethyl acetate, 1:1); m.p. = 140–143 °C {Lit. m.p. 139–141 °C}[122]; $[\alpha]^{26}_D$ –180.3 (*c* = 1.00, $CHCl_3$) {Lit. $[\alpha]^{20}_D$ –208.5 (*c* = 1.00, CH_2Cl_2)}[122]; ν_{max} 2979, 2252, 1746, 702, 692, 642 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.30 (5H, m, CH-Arom), 7.18–7.12 (5H, m, CH-Arom), 5.05 (1H, dd, *J* = 8.6, 7.2 Hz, CH-C3), 4.69 (1H, dd, *J* = 9.0, 8.6 Hz, CH-C2a), 4.21 (1H, dd, *J* = 9.0, 7.2 Hz, CH_2-C2b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6 (C-C1), 136.1 (C-Arom), 131.5 (CH-Arom), 129.5 (CH-Arom), 129.3 (CH-Arom), 128.2 (CH-Arom), 128.1 (CH-Arom), 126.9 (CH-Arom), 122.1 (C-Arom), 78.1 (C-C8), 72.8 (C-C9), 70.8 (CH_2-C2), 62.2 (CH-C3); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{17}H_{14}NO_2$ 264.1025, found 264.1024 [Δ –0.1 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 265.1 (20%), 264.1 (100%).

(*R*)-4-Phenyl-3-(phenylethynyl)oxazolidin-2-one (183) Using Evano Approach



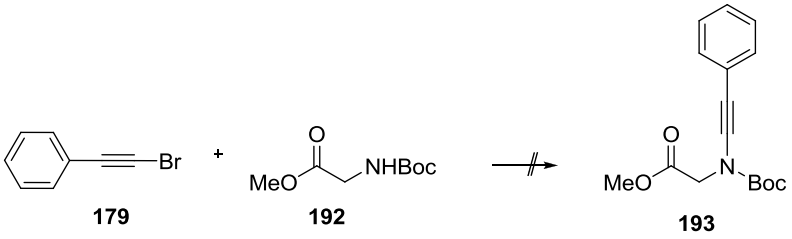
A vial was charged with oxazolidinone **178** (16.0 mg, 0.0981 mmol), 1,1-dibromoalkene **189** (39.0 mg, 0.149 mmol), Cs_2CO_3 (130 mg, 0.399 mmol) and CuI (2.30 mg, 0.0121 mmol). Dry dioxane (0.3 mL) and DMEDA (1.60 mg, 0.0182 mmol) were added to the vessel and the mixture was heated to 60 °C with stirring. The reaction mixture was stirred for 18 hours and then cooled to room temperature and filtered through a pad of Celite®. The filtrate was then concentrated *in vacuo*. The target ynamide **183** was isolated by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:2) to give a white solid (10 mg, 39%).

Evano Test Reaction Using Dipeptide Fragment



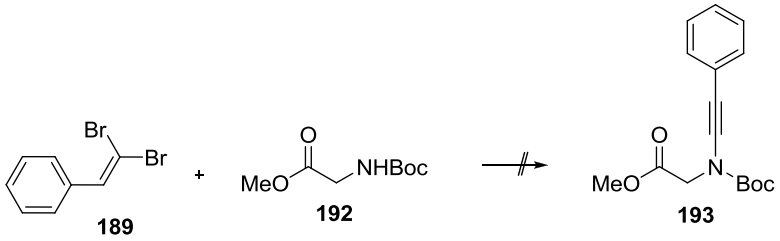
To a solution of dipeptide **167** (33.0 mg, 0.981 mmol) in dioxane (0.5 mL) was added 1,1-dibromoalkene **189** (39.0 mg, 0.149 mmol), CuI (2.30 mg, 0.0121 mmol) and Cs_2CO_3 (130 mg, 0.399 mmol). Finally, DMEDA (1.60 mg, 0.0182 mmol) was added and the reaction was heated to 60 °C with stirring. After 24 hours, there appeared to have been no reaction and so further portions of CuI (2.30 mg, 0.0121 mmol) and DMEDA (1.60 mg, 0.0182 mmol) were added to the mixture and the temperature was raised to 75 °C. The reaction was then stirred for a further 24 hours, during which time no reaction was observed. The mixture was allowed to cool to room temperature, before filtering through a pad of Celite® and concentrating *in vacuo*. The starting materials were recovered by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 4:1).

Coupling of Methyl Ester Analogue of Glycine Using Hsung 2nd Generation Protocol



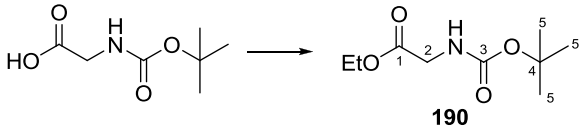
To a solution of carbamate **192** (19.0 mg, 0.100 mmol) in toluene (1 mL) was added alkynyl bromide **179** (20.0 mg, 0.110 mmol), $CuSO_4 \cdot 5H_2O$ (5.00 mg, 0.0200 mmol), 1,10-phenanthroline (7.20 mg, 0.0400 mmol) and K_3PO_4 (42.0 mg, 0.198 mmol). The mixture was heated to 80 °C and stirred for a period of 48 hours before being cooled to room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. TLC analysis indicated that the reaction had not taken place and so purification was abandoned.

Coupling of Methyl Ester Analogue of Glycine Using Evano Protocol



A vial was charged with carbamate **192** (19.0 mg, 0.100 mmol), 1,1-dibromoalkene **189** (39.0 mg, 0.149 mmol), Cs_2CO_3 (130 mg, 0.399 mmol) and CuI (2.30 mg, 0.0121 mmol). Dioxane (0.5 mL) was added to the vessel, followed by DMEDA (1.60 mg, 0.0182 mmol) and the mixture was heated to 65 °C with stirring. The mixture was stirred for 24 hours and then filtered through a pad of Celite® and concentrated *in vacuo*. TLC analysis showed that reaction had not occurred and so further purification and characterisation of the mixture was abandoned.

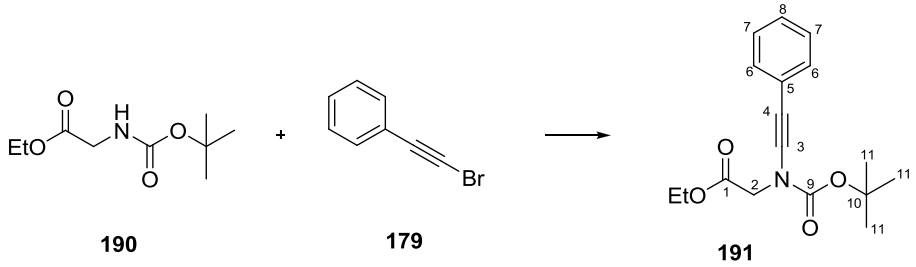
Ethyl 2-(*tert*-butoxycarbonylamino)acetate 190



To a solution of glycine ethyl ester hydrochloride (2.00 g, 14.3 mmol) in acetonitrile (68 mL) was added $NaHCO_3$ (3.60 g, 42.9 mmol). The mixture was cooled to 0 °C followed by addition of di-*tert*-butyl dicarbonate (3.10 g, 14.2 mmol). The reaction mixture was warmed to room temperature and stirred continuously for 18 hours. Upon completion, it was concentrated *in vacuo* and the residue was suspended in a mixture of CH_2Cl_2 (70 mL) and H_2O (70 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 70 mL). The combined organic layers were washed with H_2O (70 mL) followed by drying ($MgSO_4$), filtration and concentration *in vacuo* to yield the ester **190** as a colourless oil (2.7 g, 94%).

R_f = 0.46 (petroleum ether-ethyl acetate, 17:3); ν_{max} 3372, 2978, 1697, 1512 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.02 (1H, br s, NH), 4.23 (2H, q, *J* = 7.2 Hz, CH_2-Et), 3.92 (2H, d, *J* = 5.4 Hz, CH_2-C2), 1.48 (9H, s, CH_3-C5), 1.30 (3H, t, *J* = 7.2 Hz, CH_3-Et); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C-C1), 155.7 (C-C3), 80.0 (C-C4), 61.3 (CH_2-Et), 42.5 (CH_2-C2), 28.3 (CH_3-C5), 14.2 (CH_3-Et); HRMS, (CI, Me_3CH) $[M+H]^+$ calcd for $C_9H_{18}NO_4$ 204.1236, found 204.1234 [Δ –1.1 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 205.1 (13%), 204.1 (100%).

Ethyl 2-(*tert*-butoxycarbonyl(phenylethynyl)amino)acetate 191

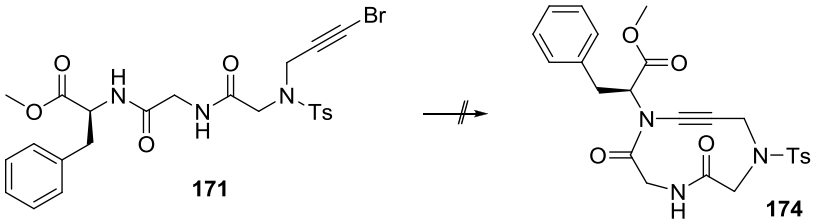


190 179 191

A sealed vial was charged with carbamate **190** (37.0 mg, 0.182 mmol) and phenylacetylene bromide **179** (27.0 mg, 0.149 mmol) in toluene (1 mL), that had been degassed with argon. Freshly ground K_3PO_4 (95.0 mg, 0.448 mmol) was added to the reaction mixture, followed by $CuSO_4 \cdot 5H_2O$ (7.50 mg, 0.0300 mmol), 1,10-phenanthroline (10.8 mg, 0.0600 mmol) and $Ni(acac)_2$ (4.00 mg, 0.0156 mmol). The reaction was heated to 80 °C and stirred for 48 hours. The crude mixture was cooled to room temperature and filtered through a pad of Celite®, washing it through with Et_2O. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 17:3). Ynamide **191** was isolated as a white solid (33 mg, 73%).

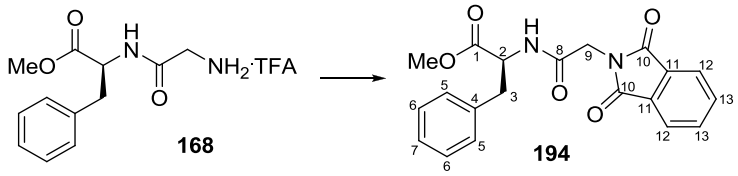
R_f = 0.53 (petroleum ether-ethyl acetate, 17:3); m.p. = 76–77 °C; ν_{max} 2978, 2253, 1751, 1713, 694, 633 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.28 (2H, m, CH-Arom), 7.22–7.16 (3H, m, CH-Arom), 4.19 (2H, q, *J* = 7.0 Hz, CH_2-Et), 4.16 (2H, s, CH_2-C2), 1.48 (9H, s, CH_3-C11), 1.23 (3H, t, *J* = 7.0 Hz, CH_3-Et); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9 (C-C1), 153.8 (C-C9), 130.8 (CH-Arom), 128.2 (CH-Arom), 127.3 (CH-Arom), 123.4 (C-C5), 83.6 (C-C3), 83.2 (C-C10), 69.8 (C-C4), 61.5 (CH_2-Et), 51.0 (CH_2-C2), 27.9 (CH_3-C11), 14.2 (CH_3-Et); HRMS (EI+) $[M]^+$ calcd for $C_{17}H_{21}NO_4$ 303.1471, found 303.1469 [Δ –0.7 ppm]; LRMS (EI+) *m/z* (intensity) 305.0 (28%), 303.1 (17%), 247.0 (100%); Anal. calcd for $C_{17}H_{21}NO_4$: C, 67.31%; H, 6.98%; N, 4.62%. Found: C, 67.28%; H, 7.02%; N, 4.70%.

Application of Optimised Hsung Conditions to Intramolecular Coupling



To a solution of tripeptide **171** (56.0 mg, 0.0992 mmol) in dry, degassed toluene (10 mL) was added $CuSO_4 \cdot 5H_2O$ (5.00 mg, 0.0200 mmol), 1,10-phenanthroline (7.10 mg, 0.0394 mmol) and freshly ground K_3PO_4 (42.0 mg, 0.198 mmol). Finally, $Ni(acac)_2$ (2.55 mg, 0.00993 mmol) was added, and the mixture was heated to 80 °C with stirring. There was no evidence of reaction after 20 hours and so further amounts of $CuSO_4$ (5.00 mg, 0.0200 mmol) and 1,10-phenanthroline (7.10 mg, 0.0394 mmol) were added with an extended period of stirring. After the reaction had progressed for a total of 48 hours, TLC analysis showed that formation of new products and consumption of the tripeptide starting material had not occurred. The reaction was allowed to cool to room temperature, filtered and concentrated *in vacuo*. The residue was purified using flash column chromatography on silica gel (dichloromethane-methanol, 19:1) and pure starting material was recovered.

(*S*)-Methyl 2-(2-(1,3-dioxoisoindolin-2-yl)acetamido)-3-phenylpropanoate 194

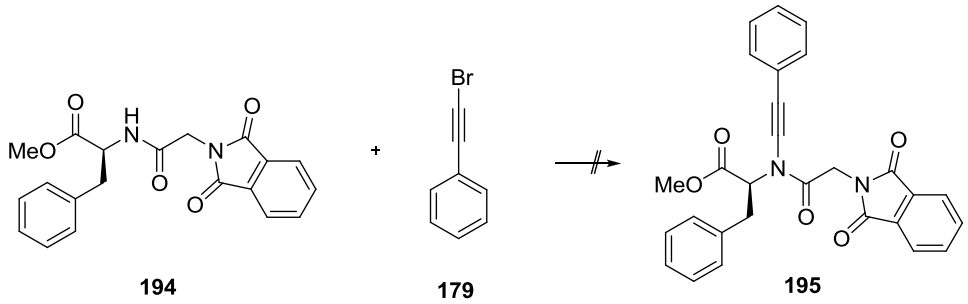


A vessel was flame dried with attached Dean-Stark condenser. The apparatus was charged with TFA salt **168** (70.0 mg, 0.200 mmol) and toluene (2.4 mL). Phthalic anhydride (30.0 mg, 0.203 mmol) and TEA (30.0 μL, 0.216 mmol) were added to the reaction mixture and it was heated to reflux for 4 hours forming water (~4 μL) as byproduct, which was isolated as the reaction progressed. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Phthalimide **194** was isolated by flash column chromatography using silica gel (dichloromethane-methanol, 99:1) as a colourless solid (28 mg, 38%).

R_f = 0.57 (dichloromethane-methanol, 19:1); m.p. = 169–170 °C {Lit. m.p. 174–175 °C}[191]; $[\alpha]^{25}_D$ +88.6 (*c* = 1.01, $CHCl_3$) {Lit. $[\alpha]^{21}_D$ +91.6 (*c* = 1.00, EtOAc)}[189]; ν_{max} 3310, 2947, 1720, 1659, 1543, 702, 648 cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$) δ 7.82 (2H, dd, *J* = 5.5, 3.1

Hz, CH-Phthalimide), 7.69 (2H, dd, *J* = 5.5, 3.1 Hz, CH-Phthalimide), 7.18–7.13 (3H, m, CH-Arom), 7.02–7.00 (2H, m, CH-Arom), 6.14 (1H, d, *J* = 7.5 Hz, NH), 4.81 (1H, ddd, *J* = 7.5, 5.8, 5.1 Hz, CH-C2), 4.32 (1H, d, *J* = 16.1 Hz, CH_2-C9a), 4.24 (1H, d, *J* = 16.1 Hz, CH_2-C9b), 3.67 (3H, s, CH_3-Me), 3.11 (1H, dd, *J* = 13.9, 5.8 Hz, CH_2-C3a), 3.05 (1H, dd, *J* = 13.9, 5.1 Hz, CH_2-C3b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7 (C-C1), 167.9 (C-C8), 165.8 (C-C10), 135.5 (C-Arom), 134.2 (CH-Phthalimide), 132.0 (C-C11), 129.4 (CH-Arom), 128.6 (CH-Arom), 127.1 (CH-Arom), 123.6 (CH-Phthalimide), 53.4 (CH-C2), 52.5 (CH_3-Me), 40.6 (CH_2-C9), 37.6 (CH_2-C3); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{20}H_{19}N_2O_5$ 367.1294, found 367.1296 [Δ +0.6 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 368.1 (23%), 367.1 (100%).

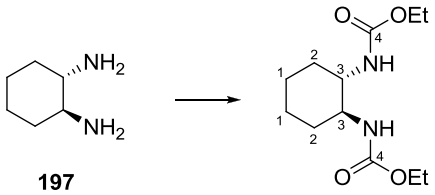
Optimised Conditions Test Reaction



194 **179** **195**

A solution of protected dipeptide **194** (35.0 mg, 0.0993 mmol) and freshly ground K_3PO_4 (51.0 mg, 0.240 mmol) was prepared in toluene (0.5 mL) that was first purged with argon. Alkynyl bromide **179** (14.0 mg, 0.0773 mmol), $CuSO_4 \cdot 5H_2O$ (4.00 mg, 0.0160 mmol), 1,10-phenanthroline (6.00 mg, 0.0333 mmol) and $Ni(acac)_2$ (2.00 mg, 0.00778 mmol) were added and the mixture was heated to 80 °C with stirring. After 24 hours, the mixture was allowed to cool to room temperature and then filtered through Celite® before being concentrated *in vacuo*. TLC analysis found that no new compounds had been formed and starting material had not been consumed. Consequently, the reaction was abandoned.

Diethyl (1*S*,2*S*)-cyclohexane-1,2-diyldicarbamate 198

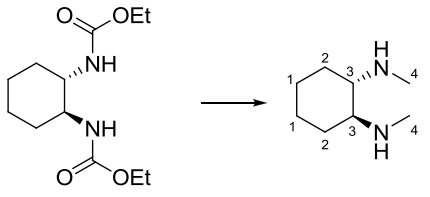


197 198

A solution of NaOH (3.80 g, 95.0 mmol) in water (18 mL) was prepared, followed by addition of (±)-trans-1,2-diaminocyclohexane **197** (2.40 mL, 20.0 mmol). The reaction mixture was cooled to 0 °C and stirred for 10 minutes. Ethyl chloroformate (4.00 mL, 42.2 mmol) was added as a solution in toluene (18 mL) by addition funnel over a period of 30 minutes. The reaction mixture was warmed to room temperature and stirred for 2.5 hours. It was then filtered and concentrated in vacuo. The target carbamate **198** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 2:1) as a white solid (3.1 g, 60%).

R_f = 0.61 (petroleum ether-ethyl acetate, 2:1); m.p. = 144–147 °C; ν_{max} 3314, 2930, 1678, 1533 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.94 (2H, br s, NH), 4.19–4.06 (4H, m, CH_2-Et), 3.39–3.37 (2H, m, CH-C3), 2.11–2.07 (2H, m, CH_2-C2a), 1.78–1.75 (2H, m, CH_2-C1a), 1.36–1.30 (2H, m, CH_2-C1b), 1.27–1.22 (2H, m, CH_2-C2b), 1.25 (6H, t, *J* = 7.0 Hz, CH_3-Et); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1 (C-C4), 60.8 (CH_2-Et), 55.4 (CH-C3), 32.9 (CH_2-C2), 24.8 (CH_2-C1), 14.6 (CH_3-Et); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_4$ 259.1658, found 159.1659 [Δ +0.5 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 260.2 (14%), 259.2 (100%).

(1*S*,2*S*)-*N*1,*N*2-Dimethylcyclohexane-1,2-diamine, 196



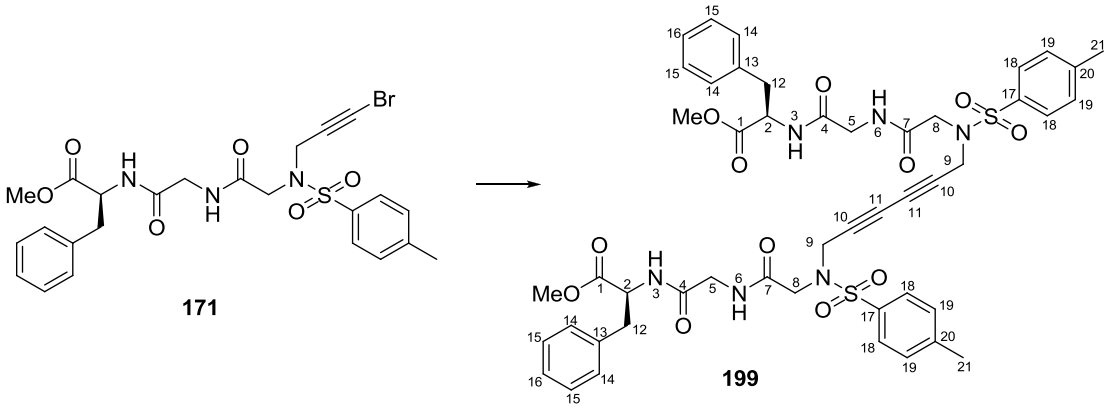
198 196

A suspension of $LiAlH_4$ (2.80 g, 73.7 mmol) in dry THF (100 mL) was prepared in a three-necked flask fitted with a condenser. The suspension was cooled to 0 °C before addition of dicarbamate **198** (3.10 g, 12.0 mmol) portionwise. The reaction was stirred at room temperature for 1 hour and then at reflux for 18 hours. The mixture was cooled to 0 °C followed by careful sequential addition of water (3 mL), 15% aqueous NaOH (3 mL) and a further quantity of water (11 mL). The suspension was filtered, washing through with THF

(2 × 50 mL) and the filtrate was concentrated *in vacuo*. The residue was acidified with 1M HCl until pH 2 and then extracted with CH_2Cl_2 (3 × 40 mL). The aqueous layer was adjusted to pH 13 with saturated aqueous NaOH solution and extracted with CH_2Cl_2 (3 × 80 mL). These extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield the pure diamine **196** as a yellow liquid (1.5 g, 88%).

R_f = 0.47 (dichloromethane-methanol, 9:1); ν_{max} 3243, 2933, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (6H, s, CH_3-C4), 2.06–2.02 (2H, m, CH_2-C2a), 1.98–1.94 (2H, m, CH-C3), 1.68–1.64 (2H, m, CH_2-C1a), 1.54 (2H, s, NH), 1.19–1.13 (2H, m, CH_2-C1b), 0.94–0.84 (2H, m, CH_2-C2b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.3 (CH-C3), 33.7 (CH_3-C4), 30.8 (CH_2-C2), 25.0 (CH_2-C1); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_8H_{19}N_2$ 143.1549, found 143.1544 [Δ –3.1 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 143.2 (100%).

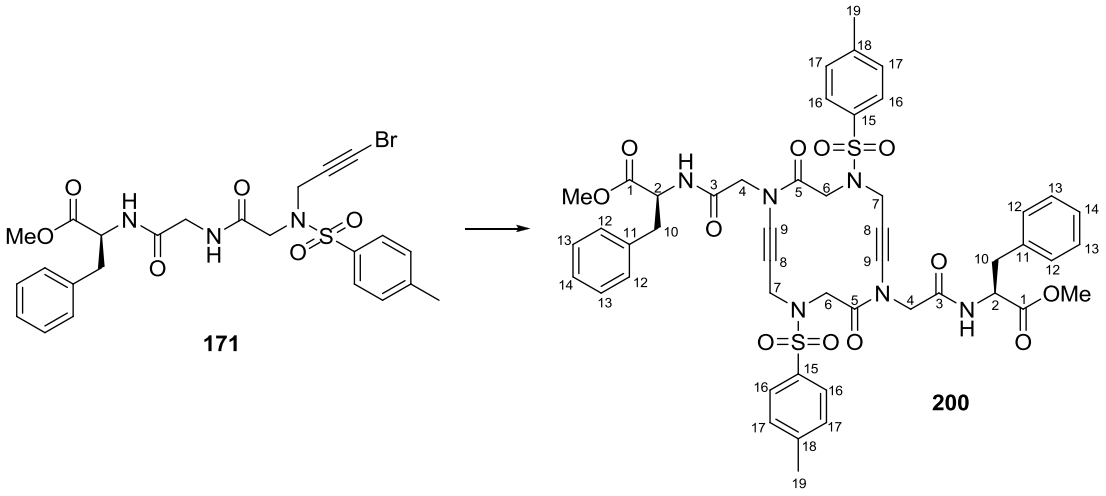
Linear Alkynyl Dimer 199



A flask was charged with alkynyl bromide **171** (100 mg, 0.177 mmol), CuTC (2.40mg, 0.0126 mmol) and freshly ground K_3PO_4 (75.0 mg, 0.354 mmol). The flask was evacuated and flushed with argon three times. The mixture was dissolved in toluene (0.75 mL) that had been degassed with argon. Finally, diamine **196** (3.80 mg, 0.0268 mmol) was added and the reaction was heated to 85 °C. The vessel was covered with aluminium foil for the duration of the reaction. The reaction mixture was stirred for 24 hours, at which point it was cooled to room temperature. Water (10 mL) was added and the solution was extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (dichloromethane-methanol, 19:1). The dialkyne **199** was isolated as a slightly yellow oil (41 mg, 24%).

R_f = 0.40 (dichloromethane-methanol, 40:3); $[\alpha]^{23}_D$ +30.1 (*c* = 1.00, $CHCl_3$); ν_{max} 3343, 3302, 3063, 2926, 2359, 1744, 1663, 1526, 816, 752, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.61 (4H, d, *J* = 8.2 Hz, CH-C18), 7.26 (4H, d, *J* = 8.2 Hz, CH-C19), 7.23–7.15 (6H, m, CH-Arom), 7.12 (2H, dd, *J* = 5.7, 5.7 Hz, NH-N6), 7.06–7.04 (4H, m, CH-Arom), 6.61 (2H, d, *J* = 7.7 Hz, NH-N3), 4.78 (2H, ddd, *J* = 7.7, 6.6, 5.7 Hz, CH-C2), 4.12 (2H, d, *J* = 18.5 Hz, CH_2-C9a), 4.07 (2H, d, *J* = 18.5 Hz, CH_2-C9b), 3.90 (2H, dd, *J* = 16.7, 5.7 Hz, CH_2-C5a), 3.83 (2H, dd, *J* = 16.7, 5.7 Hz, CH_2-C5b), 3.72 (2H, d, *J* = 17.1 Hz CH_2-C8a), 3.64 (2H, d, *J* = 17.1 Hz, CH_2-C8b), 3.64 (6H, s, CH_3-Me), 3.08 (2H, dd, *J* = 13.9, 5.7 Hz, CH_2-C12a), 2.99 (2H, dd, *J* = 13.9, 6.6 Hz, CH_2-C12b), 2.37 (6H, s, CH_3-C21); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C-C1), 168.1 (C-C4), 167.9 (C-C7), 145.0 (C-Arom), 135.7 (C-Arom), 133.7 (C-Arom), 130.0 (CH-C19), 129.3 (CH-Arom), 128.6 (CH-Arom), 127.9 (CH-C18), 127.2 (CH-Arom), 71.6 (C-C10), 70.0 (C-C11), 53.4 (CH-C2), 52.5 (CH_3-Me), 50.4 (CH_2-C8), 42.7 (CH_2-C5), 39.5 (CH_2-C9), 37.7 (CH_2-C12), 21.7(CH_3-C21); HRMS (FAB) $[M+H]^+$ calcd for $C_{48}H_{53}N_6O_{12}S_2$ 969.3163, found 969.3171 [Δ +0.8 ppm]; LRMS (FAB) *m/z* (intensity) 969.3 (100%).

Cyclic dimer 200

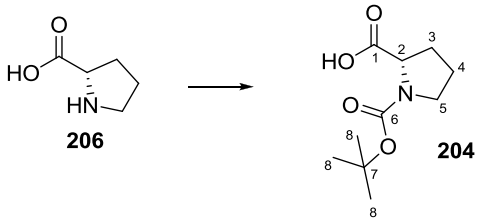


To a solution of alkynyl bromide **171** (100 mg, 0.177 mmol) in toluene that had been degassed with argon (40 mL) was added CuTC (2.40 mg, 0.0126 mmol) and freshly ground K_3PO_4 (75.0 mg, 0.354 mmol). Finally diamine **196** (3.80 mg, 0.0268 mmol) was added and the reaction mixture was heated to 85 °C. The flask was wrapped in aluminium foil and the reaction was stirred for 40 hours before cooling to room temperature and diluting with water (40 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL) and the combined organic extracts were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

The residue was separated by flash column chromatography using silica gel (dichloromethane-methanol, 49:1). Cyclic ynamide **200** was isolated as a colourless oil (21 mg, 10%).

R_f = 0.47 (dichloromethane-methanol, 24:1); $[\alpha]^{23}_D$ +0.77 (*c* = 0.39, $CHCl_3$); ν_{max} 2961, 2922, 2854, 2359, 2342, 1684, 1559, 756, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (4H, d, *J* = 8.2 Hz, CH-C16), 7.28 (4H, d, *J* = 8.2 Hz, CH-C17), 7.21–7.17 (6H, m, CH-Arom), 7.01–6.99 (4H, m, CH-Arom), 5.94 (2H, d, *J* = 7.5 Hz, NH), 4.73 (2H, ddd, *J* = 7.5, 5.5, 5.3 Hz, CH-C2), 4.64 (2H, d, *J* = 16.5 Hz, CH_2-C4a), 4.58 (2H, d, *J* = 16.5 Hz, CH_2-C4b), 3.94 (4H, s, CH_2-C7), 3.91 (2H, d, *J* = 13.0 Hz, CH_2-C6a), 3.86 (2H, d, *J* = 13.0 Hz, CH_2-C6b), 3.65 (6H, s, CH_3-Me), 3.05 (2H, dd, *J* = 13.7, 5.5 Hz, CH_2-C10a), 3.00 (2H, dd, *J* = 13.7, 5.3 Hz, CH_2-C10b), 2.37 (6H, s, CH_3-C19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4 (C-C1), 166.4 (C-C3), 165.4 (C-C5), 144.8 (C-Arom), 135.4 (C-Arom), 135.0 (C-Arom), 130.1 (CH-C17), 129.3 (CH-Arom), 128.7 (CH-Arom), 127.8 (CH-C16), 127.3 (CH-Arom), 90.7 (C-C9), 77.2 (C-C8), 53.3 (CH-C2), 52.5 (CH_3-Me), 50.4 (CH_2-C6), 49.0 (CH_2-C4), 47.4 (CH_2-C7), 37.7 (CH_2-C10), 21.6 (CH_3-C19).

1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carboxylic acid 204

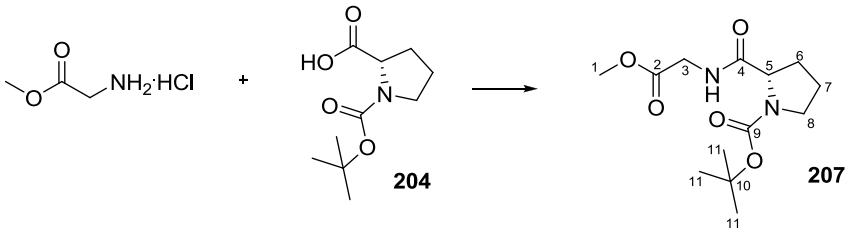


A solution of L-proline **206** (1.15 g, 10.0 mmol) was prepared in a 1:1 mixture of dioxane and water (10 mL). Saturated aqueous $NaHCO_3$ (20 mL) was added to the solution, which was then cooled to 0 °C. Di-*tert*-butyl dicarbonate (4.30 g, 19.7 mmol) was added portionwise and the mixture was stirred vigorously for 18 hours. The pH was adjusted to pH 3 by addition of 1M aqueous HCl solution and the mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give a colourless oil. Boc-protected proline **204** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 1:1) as a white solid (1.7 g, 79%). This compound was isolated as a mixture of rotamers.

R_f = 0.25 (petroleum ether-ethyl acetate, 1:1); m.p. = 132–133 °C {Lit. m.p. 133 °C}[192]; $[\alpha]^{27}_D$ –98.0 (*c* = 1.02, $CHCl_3$) {Lit. $[\alpha]^{27}_D$ –57.0 (*c* = 1.00, $CHCl_3$)}[193]; ν_{max} 2970, 2893, 2716, 1736, 1636, 725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.45 (2H, br s, OH), 4.29 (1H, dd, *J* = 8.2, 2.3 Hz, CH-C2a), 4.17 (1H, dd, *J* = 8.2, 4.2 Hz, CH-C2b), 3.52–3.28 (4H, m, CH_2-C5), 2.25–1.96

(4H, m, CH_2-C3), 1.93–1.78 (4H, m, CH_2-C4), 1.41 (9H, s, CH_3-C8a), 1.35 (9H, s, CH_3-C8b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.9 (C-C1a), 176.1 (C-C1b), 155.9 (C-C6a), 153.9 (C-C6b), 81.0 (C-C7a), 80.4 (C-C7b), 59.0 (CH-C2), 46.9 (CH_2-C5a), 46.3 (CH_2-C5b), 30.8 (CH_2-C3a), 29.0 (CH_2-C3b), 28.4 (CH_3-C8a), 28.3 (CH_3-C8b), 24.3 (CH_2-C4a), 23.6 (CH_2-C4b); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{10}H_{18}NO_4$ 216.1236, found 216.1234 [Δ –1.0 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 217.1 (18%), 216,1 (100%); Anal. calcd for $C_{10}H_{17}NO_4$: C, 55.80%; H, 7.96%; N, 6.51%. Found: C, 55.71%; H, 8.03%; N, 6.53%.

(*S*)-*tert*-Butyl 2-(2-methoxy-2-oxoethylcarbamoyl)pyrrolidine-1-carboxylate 207

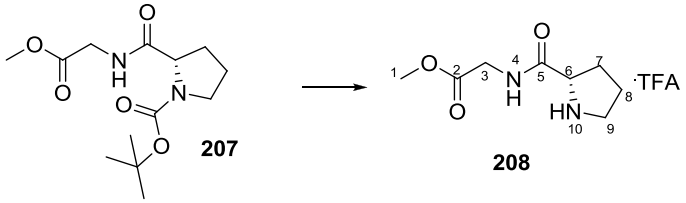


A solution of Boc-proline **204** (344 mg, 1.60 mmol), and glycine methyl ester hydrochloride (200 mg, 1.59 mmol) was prepared in CH_2Cl_2 (16 mL). BOP (707 mg, 1.60 mmol) and DIPEA (835 μL, 4.80 mmol) were added to the solution, and the mixture was stirred for 18 hours at room temperature. The mixture was then concentrated *in vacuo* and the residue was dissolved in EtOAc (25 mL). This solution was washed with 1M aqueous $KHSO_4$ (3 × 25 mL), 10% aqueous Na_2CO_3 (3 × 25 mL) and brine (3 × 25 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The target dipeptide **207** was isolated after flash column chromatography using silica gel (dichloromethane-methanol, 19:1) as a colourless solid (323 mg, 71%). This compound was isolated as a mixture of rotamers.

R_f = 0.54 (dichloromethane-methanol, 9:1); m.p. = 67–68 °C {Lit. m.p. 70–71 °C}[194]; $[\alpha]^{27}_D$ –4.3 (*c* = 1.03, $CHCl_3$) {Lit. $[\alpha]^{27}_D$ –16.0 (*c* = 1.00, $CHCl_3$)}[195]; ν_{max} 3306, 2976, 1755, 1666, 1530, 731 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (1H, br s, NHa), 6.49 (1H, br s, NHb), 4.28–4.20 (2H, m, CH-C5), 4.00 (2H, dd, *J* = 18.3, 5.6 Hz, CH_2-C3a), 3.99–3.96 (2H, m, CH_2-C3b), 3.68 (6H, s, CH_3-C1), 3.40–3.28 (4H, m, CH_2-C8), 2.27–2.09 (4H, m, CH_2-C6), 1.84–1.80 (4H, m, CH_2-C7), 1.40 (18H, s, CH_3-C11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (C-C2a), 172.4 (C-C2b), 170.2 (C-C4), 155.8 (C-C9a), 154.6 (C-C9b), 80.5 (C-C10), 61.1 (CH-C5a), 59.9 (CH-C5b), 52.3 (CH_3-C1), 47.1 (CH_2-C8), 41.1 (CH_2-C3), 31.0 (CH_2-C6), 28.3 (CH_3-C11), 24.5 (CH_2-C7a), 23.7 (CH_2-C7b); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{13}H_{23}N_2O_5$ 287.1607, found 287.1608 [Δ +0.5 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity)

288.2 (15%), 287.2 (100%); Anal. calcd for $C_{13}H_{22}N_2O_5$: C, 54.53%; H, 7.74%; N, 9.78%. Found: C, 54.36%; H, 7.76%; N, 9.61%.

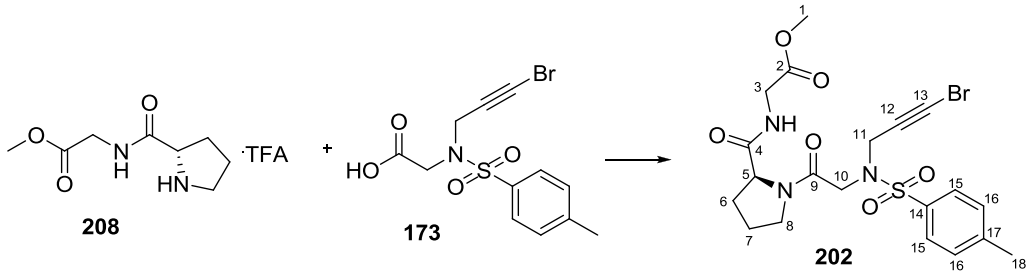
(*S*)-Methyl 2-(pyrrolidine-2-carboxamido)acetate TFA Salt 208



A solution of Boc-protected dipeptide **207** (1.00 g, 3.50 mmol) in dry CH_2Cl_2 (3 mL) was prepared. TFA (3 mL) was added to the solution followed by continuous stirring at room temperature for 4 hours. The mixture was then concentrated *in vacuo*. A small volume of toluene was added to the residue and it was concentrated *in vacuo*. This was repeated another two times followed by a period of drying under high vacuum to remove any traces of excess TFA from the product. The TFA salt of the target dipeptide **208** was isolated as a pale yellow oil (1.05 g, 100%). This compound was isolated as a mixture of rotamers.

$[\alpha]^{23}_D$ –20.5 (*c* = 0.98, $CHCl_3$); ν_{max} 3287, 3094, 2963, 1744, 1667, 1559, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.54 (1H, s, NH-N10a), 10.44 (1H, s, NH-N10b), 7.91 (2H, dd, *J* = 6.0, 5.4 Hz, NH-N4), 4.80 (2H, br s, CH-C6), 4.14 (1H, dd, *J* = 17.9, 6.0 Hz, CH_2-C3a), 4.04 (1H, dd, *J* = 17.9, 5.4 Hz, CH_2-C3b), 3.78 (3H, s, CH_3-C1), 3.51 (2H, br s, CH_2-C9), 2.57–2.47 (1H, m, CH_2-C7a), 2.21–2.13 (3H, m, CH_2-C7b and CH_2-C8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (C-C2), 169.5 (C-C5), 59.7 (CH-C6), 52.4 (CH_3-C1), 46.8 (CH_2-C9), 41.4 (CH_2-C3), 30.1 (CH_2-C7), 24.3 (CH_2-C8); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_8H_{15}N_2O_3$ 187.1083, found 187.1086 [Δ +1.6 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 187.1 (100%).

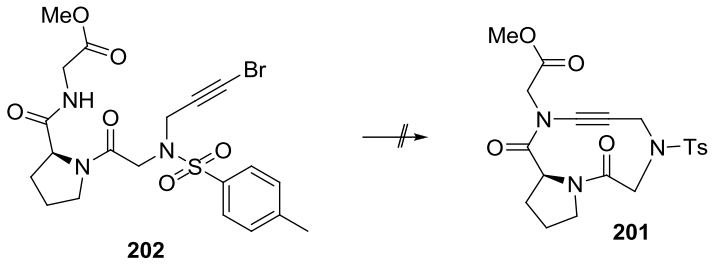
Methyl 2-(1-(2-(*N*-(3-bromoprop-2-ynyl)-4-methylphenylsulfonamido)acetyl)pyrrolidine-2-carboxamido)acetate 202



A solution of TFA salt **208** (694 mg, 2.31 mmol) and carboxylic acid **173** (800 mg, 2.31 mmol) in CH_2Cl_2 (23 mL) was prepared at room temperature. Coupling reagent BOP (1.02 g, 2.31 mmol) and DIPEA (1.20 mL, 6.90 mmol) were added and the solution was stirred for 24 hours. The reaction was concentrated *in vacuo* upon completion and the residue was redissolved in ethyl acetate (100 mL). The solution was washed with 1M aqueous $KHSO_4$ (3 × 50 mL), 10% aqueous Na_2CO_3 (3 × 50 mL) and brine (50 mL). The washed solution was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Tripeptide **202** was isolated by flash column chromatography using silica gel (dichloromethane-methanol, 19:1) as a white solid (728 mg, 61%). This compound was isolated as a mixture of rotamers.

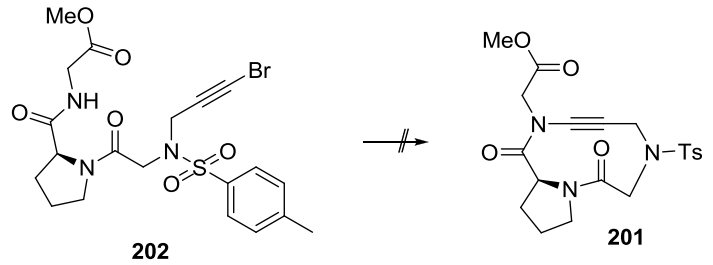
R_f = 0.40 (dichloromethane-methanol, 19:1); m.p. = 60–62 °C; $[\alpha]^{26}_D$ –62.4 (*c* = 0.985, $CHCl_3$); ν_{max} 3310, 2955, 2214, 1751, 1651, 1528, 810, 664, 602 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.67 (2H, d, *J* = 8.2 Hz, CH-C15), 7.26 (2H, d, *J* = 8.2 Hz, CH-C16), 7.20 (1H, dd, *J* = 5.9, 5.4 Hz, NH), 4.55 (1H, dd, *J* = 8.2, 2.3 Hz, CH-C5), 4.24 (1H, d, *J* = 18.3 Hz, CH_2-C11a), 4.18 (1H, d, *J* = 18.3 Hz, CH_2-C11b), 4.00 (1H, d, *J* = 15.8 Hz, CH_2-C10a), 3.96 (1H, dd, *J* = 18.1, 5.9 Hz, CH_2-C3a), 3.94 (1H, d, *J* = 15.8 Hz, CH_2-C10b), 3.88 (1H, dd, *J* = 18.1, 5.4 Hz, CH_2-C3b), 3.69–3.62 (1H, m, CH_2-C8a), 3.66 (3H, s, CH_3-C1), 3.50 (1H, td, *J* = 9.3, 7.2 Hz, CH_2-C8b), 2.37 (3H, s, CH_3-C18), 2.32–2.29 (1H, m, CH_2-C6a), 2.10–2.04 (1H, m, CH_2-C7a), 1.99–1.93 (1H, m, CH_2-C7b), 1.91–1.83 (1H, m, CH_2-C6b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (C-C2), 170.1 (C-C4), 167.2 (C-C9), 144.1 (C-Arom), 135.1 (C-Arom), 129.7 (CH-C16), 127.8 (CH-C15), 72.9 (C-C12), 60.2 (CH-C5), 52.3 (CH_3-C1), 48.6 (CH_2-C10), 47.2 (CH_2-C8), 45.6 (C-C13), 41.2 (CH_2-C3), 38.9 (CH_2-C11), 27.5 (CH_2-C6), 25.1 (CH_2-C7), 21.6 (CH_3-C18); HRMS (FAB) $[M+H]^+$ calcd for $C_{20}H_{25}BrN_3O_6S$ 514.0647, found 514.0650 [Δ –0.5 ppm]; LRMS (FAB) *m/z* (intensity) 517.1 (23%), 516.1 (100%), 515.1 (16%), 514.1 (96%); Anal. calcd for $C_{20}H_{24}BrN_3O_6S$: C, 46.70%; H, 4.70%; N, 8.17%. Found: C, 46.61; H, 4.68%; N, 8.04%.

Cyclisation Using Hsung 2nd Generation Protocol



The tripeptide **202** (50.0 mg, 0.0972 mmol) and freshly ground K_3PO_4 (41.0 mg, 0.193 mmol) were dissolved in dry toluene (20 mL) that had been bubbled through with argon. To this solution was added $CuSO_4 \cdot 5H_2O$ (4.90 mg, 0.0196 mmol) and 1,10-phenanthroline (7.00 mg, 0.0388 mmol), and the mixture was heated to 75 °C with stirring. After 20 hours, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* and a new compound was detected by TLC analysis of the residue. The new compound was isolated by flash column chromatography using silica gel (dichloromethane-methanol 24:1) and found to be a decomposition product following NMR analysis.

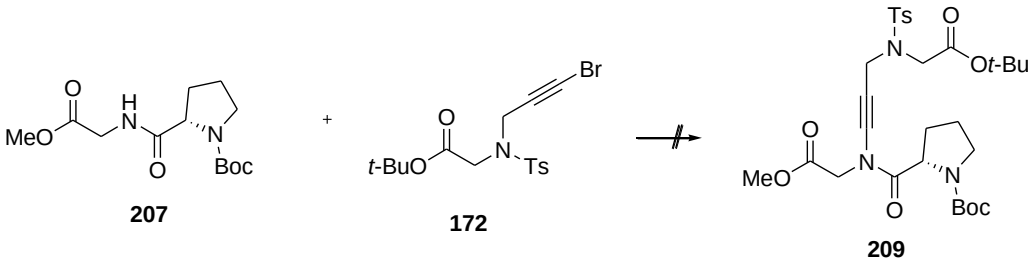
Cyclisation using CuTC conditions



A dried flask was charged with tripeptide **202** (50.0 mg, 0.0972 mmol), CuTC (1.30 mg, 0.00682 mmol) and freshly ground K_3PO_4 (41.0 mg, 0.193 mmol). The flask was evacuated and charged with argon several times before addition of dry toluene (25 mL) that was first saturated with argon. Finally, (*S*,*S*)-(+)-*N*,*N*'-dimethyl-1,2-cyclohexanediamine ligand **196** (2.10 mg, 0.0148 mmol) was added to the mixture and the reaction vessel was then wrapped in aluminium foil. The mixture was then heated to 85 °C and stirred at this temperature for 24 hours. After this time, the mixture was allowed to cool to room temperature and water (20 mL) was added. The mixture was then extracted with EtOAc (3 × 15 mL) and the combined extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (dichloromethane-

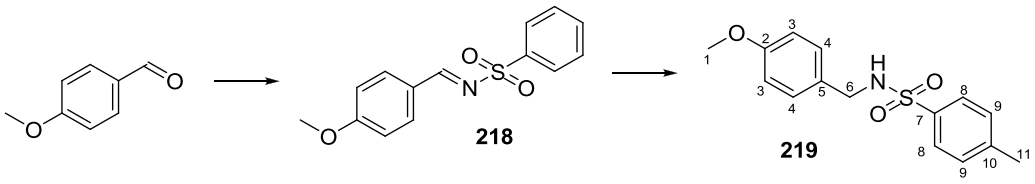
methanol, 39:1) to afford a single product. Analysis of this material suggested that it was the dimer formed by coupling of two alkyne units.

Intermolecular Reaction With Proline Dipeptide



A dried vessel was charged with dipeptide **207** (50.0 mg, 0.175 mmol), CuTC (2.30 mg, 0.0121 mmol) and freshly ground K_3PO_4 (74.0 mg, 0.349 mmol). The vessel was then evacuated and charged with argon several times. Dry toluene (1 mL), that had been deoxygenated with argon, was added followed by (*S*,*S*)-(+)-*N*,*N*'-dimethyl-1,2-cyclohexanediamine ligand **196** (3.70 mg, 0.0260 mmol). Finally, alkynyl bromide **172** (119 mg, 0.296 mmol) was added to the reaction mixture and the reaction vessel was wrapped in aluminium foil. The mixture was then heated to 85 °C and stirred at this temperature for 24 hours. TLC analysis showed the development of traces of a new material and so the mixture was stirred for another 24 hours to allow the reaction to progress. The mixture was cooled to room temperature, diluted with water (10 mL) and then extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 4:1) to give an unwanted decomposition product.

N-(4-Methoxybenzyl)-4-methylbenzenesulfonamide 219

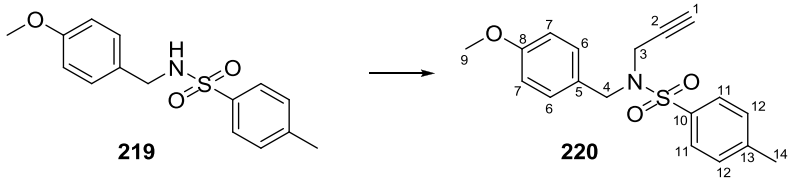


A solution of *p*-anisaldehyde (2.40 mL, 19.7 mmol) was prepared in CH_2Cl_2 (60 mL). *p*-Toluenesulfonamide (4.40 g, 25.7 mmol) and TEA (8.30 mL, 59.7 mmol) were added sequentially and the reaction was cooled to 0 °C before dropwise addition of distilled $TiCl_4$ (2.90 mL, 26.3 mmol) as a solution in CH_2Cl_2 (15 mL). The reaction progressed over 5 hours at which point it was quenched by the addition of saturated aqueous $NaHCO_3$ (30 mL). The

aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine (50 mL) before being dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude imine product **218** was used without further purification by dissolving in MeOH (60 mL), followed by portionwise addition of $NaBH_4$ (760 mg, 20.0 mmol) at 0 °C. The reaction was allowed to warm to room temperature and it was stirred for 16 hours. The reaction was quenched with water (60 mL) and extracted with Et_2O (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Sulfonamide **219** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:1) as a white solid (3.3 g, 57%).

R_f = 0.53 (petroleum ether-ethyl acetate, 3:2); m.p. = 120–121 °C {Lit. m.p. 121–123 °C}; ν_{max} 3248, 3024, 2924, 1929, 1613, 1512, 810 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* = 8.2 Hz, CH-C8), 7.33 (2H, d, *J* = 8.2 Hz, CH-C9), 7.13 (2H, d, *J* = 8.6 Hz, CH-Arom), 6.82 (2H, d, *J* = 8.6 Hz, CH-Arom), 4.61 (1H, t, *J* = 6.0 Hz, NH), 4.07 (2H, d, *J* = 6.0 Hz, CH_2-C6), 3.80 (3H, s, CH_3-C1), 2.46 (3H, s, CH_3-C11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-Arom), 143.5 (C-Arom), 136.9 (C-Arom), 129.7 (CH-C9), 129.3 (CH-Arom), 128.3 (C-Arom), 127.2 (CH-C8), 114.1 (CH-Arom), 55.3 (CH_3-C1), 46.8 (CH_2-C6), 21.6 (CH_3-C11); HRMS (EI+) $[M]^+$ calcd for $C_{15}H_{17}NO_3S$ 291.0929, found 291.0925 [Δ –1.3 ppm]; LRMS (EI+) *m/z* (intensity) 292.1 (20%), 291.1 (100%); Anal. calcd for $C_{15}H_{17}NO_3S$: C, 61.83%; H, 5.88%; N, 4.81%. Found: C, 61.41%; H, 5.90%; N, 4.83%.

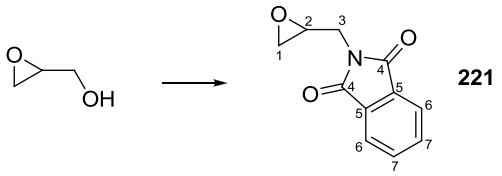
N-(4-Methoxybenzyl)-4-methyl-*N*-(prop-2-ynyl)benzenesulfonamide 220



A solution of sulfonamide **219** (200 mg, 0.687 mmol) was prepared in acetone (4 mL), to which was added anhydrous K_2CO_3 (190 mg, 1.38 mmol) and 80% propargyl bromide in toluene (92.0 μL, 0.825 mmol). The reaction was stirred vigorously at 60 °C for 16 hours. Water (20 mL) was added to the reaction mixture and it was then extracted with Et_2O (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated. Flash column chromatography with silica gel (petroleum ether-ethyl acetate, 3:1) isolated the alkyne **220** as a white solid (192 mg, 85%).

R_f = 0.42 (petroleum ether-ethyl acetate, 3:1); m.p. = 91–92 °C; ν_{max} 3248, 2924, 2839, 2122, 1605, 1512, 810, 656 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, *J* = 8.1 Hz, CH-C11), 7.25 (2H, d, *J* = 8.1 Hz, CH-C12), 7.20 (2H, d, *J* = 8.7 Hz, CH-Arom), 6.79 (2H, d, *J* = 8.7 Hz, CH-Arom), 4.22 (2H, s, CH_2-C4), 3.86 (2H, d, *J* = 2.5 Hz, CH_2-C3), 3.73 (3H, s, CH_3-C9), 2.37 (3H, s, CH_3-C14), 1.93 (1H, t, *J* = 2.5 Hz, CH-C1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5 (C-Arom), 143.6 (C-Arom), 136.0 (C-Arom), 130.2 (CH-Arom), 129.5 (CH-C12), 127.9 (CH-C11), 126.7 (C-Arom), 114.1 (CH-Arom), 76.4 (C-C2), 74.0 (CH-C1), 55.3 (CH_3-C9), 49.2 (CH_2-C4), 35.3 (CH_2-C3), 21.6 (CH_3-C14); HRMS (EI+) $[M]^+$ calcd for $C_{18}H_{19}NO_3S$ 329.1086, found 329.1089 [Δ +0.9 ppm]; LRMS (EI+) *m/z* (intensity) 330.1 (21%), 329.1 (100%).

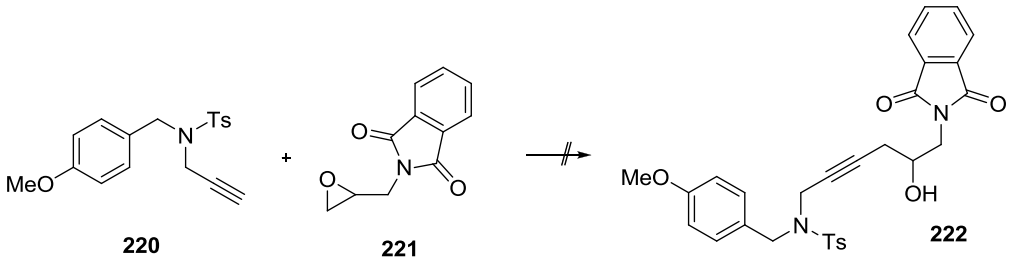
2-(Oxiran-2-ylmethyl)isoindoline-1,3-dione 221



A solution of phthalic anhydride (294 mg, 2.00 mmol) in dry THF (5 mL) was prepared, followed by addition of PPh_3 (524 mg, 2.00 mmol) and glycidol (150 μL, 2.26 mmol). Finally DEAD (0.310 mL, 1.97 mmol) was added and the solution was stirred for 18 hours. The resulting mixture was concentrated *in vacuo* and diluted with Et_2O (15 mL) before stirring for a further 2 hours. The mixture was filtered and concentrated *in vacuo*. Phthalimide **221** was isolated by flash column chromatography (petroleum ether-ethyl acetate, 4:1) as a colourless solid (222 mg, 48%).

R_f = 0.41 (petroleum ether-ethyl acetate, 3:2); m.p. = 96–98 °C {Lit. m.p. 99 °C}[196]; ν_{max} 3215, 3085, 2940, 1771, 1710, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (2H, dd, *J* = 5.4, 3.0 Hz, CH-Arom), 7.76 (2H, dd, *J* = 5.4, 3.0 Hz, CH-Arom), 3.98 (1H, dd, *J* = 14.4, 5.0 Hz, CH_2-C3a), 3.83 (1H, dd, *J* = 14.4, 5.0 Hz, CH_2-C3b), 3.26 (1H, dddd, *J* = 5.0, 5.0, 4.3, 2.6 Hz, CH-C2), 2.83 (1H, dd, *J* =4.9, 4.3 Hz, CH_2-C1a), 2.71 (1H, dd, *J* = 4.9, 2.6 Hz, CH_2-C1b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0 (C-C4), 134.2 (CH-Arom), 132.0 (C-C5), 123.5 (CH-Arom), 49.1 (CH-C2), 46.1 (CH_2-C1), 39.7 (CH_2-C3); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{11}H_{10}NO_3$ 204.0661, found 204.0656 [Δ –2.1 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 205.1 (12%), 204.1 (100%); Anal. calcd for $C_{11}H_9NO_3$: C, 65.02%; H, 4.46%; N, 6.89%. Found: C, 64.66%; H, 4.44%; N, 6.95%.

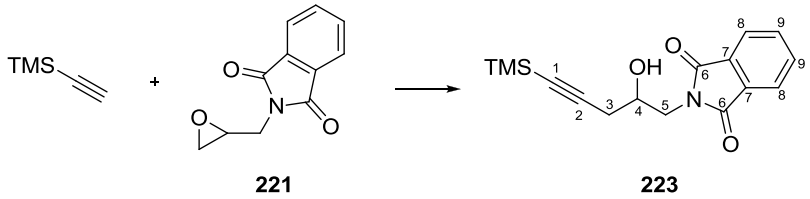
Attempted Epoxide Opening Using PMB Protected Sulfonamide



220 **221** **222**

A solution of alkyne **220** (132 mg, 0.401 mmol) in toluene (1 mL) was prepared and cooled to 0 °C. *n*-BuLi (150 μL of a 2.5 M solution in hexanes, 0.375 mmol) was added to the solution, dropwise, and the mixture was stirred for 15 minutes. Et_2AlCl (390 μL of a 1M solution in hexanes, 0.390 mmol) was added dropwise, and the mixture was stirred for a further 1 hour. Finally, a solution of epoxide **221** (41.0 mg, 0.202 mmol) in toluene (1.5 mL) was added to the mixture, which was stirred for another hour, maintaining the temperature at 0 °C throughout. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) and the mixture was allowed to warm to room temperature. The biphasic mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the combined extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 7:3) to afford a mixture of unreacted starting material and traces of decomposition products.

2-(2-Hydroxy-5-(trimethylsilyl)pent-4-ynyl)isoindoline-1,3-dione 223



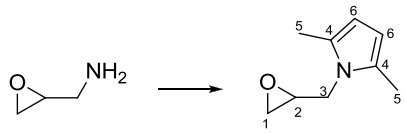
221 **223**

A solution of TMS-acetylene (73.0 μL, 0.518 mmol) in THF (3.6 mL) was prepared and cooled to –78 °C. *n*-BuLi (160 μL of a 2.5 M solution in hexanes, 0.400 mmol) was added dropwise with continuous stirring. After 10 minutes $BF_3{\cdot}OEt_2$ (76.0 μL, 0.599 mmol) was added. The mixture was stirred for a further 10 minutes before addition of a solution of epoxide **221** (81.0 mg, 0.399 mmol) in THF (0.4 mL). The reaction continued for 2.5 hours, maintaining the temperature at –78 °C. Excess *n*-BuLi was quenched by addition of saturated aqueous NH_4Cl (5 mL), and the mixture was then extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (20 mL) before drying ($MgSO_4$), filtering

and concentrating *in vacuo*. Flash column chromatography using silica gel (petroleum ether-ethyl acetate, 2:1) delivered the target alcohol **223** as a clear, colourless oil (31 mg, 26%).

R_f = 0.53 (dichloromethane-methanol, 49:1); ν_{max} 3472, 2963, 2901, 2176, 1775, 1712, 1613, 718, 640 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, dd, *J* = 5.5, 3.1 Hz, CH-Arom), 7.59 (2H, dd, *J* = 5.5, 3.1 Hz, CH-Arom), 3.96–3.91 (1H, m, CH-C4), 3.77 (1H, dd, *J* = 14.2, 7.2 Hz, CH_2-C5a), 3.74 (1H, dd, *J* = 14.2, 4.3 Hz, CH_2-C5b), 2.56 (1H, d, *J* = 6.8 Hz, OH), 2.43 (1H, dd, *J* = 17.0, 5.9 Hz, CH_2-C3a), 2.37 (1H, dd, *J* = 17.0, 5.5 Hz, CH_2-C3b), 0.00 (9H, s, CH_3-TMS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8 (C-C6), 134.2 (CH-Arom), 132.0 (C-C7), 123.5 (CH-Arom), 101.5 (C-C1), 88.7 (C-C2), 68.3 (CH-C4), 43.3 (CH_2-C5), 26.7 (CH_2-C3), 0.0 (CH_3-TMS); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{16}H_{20}NO_3Si$ 302.1212, found 302.1215 [Δ +0.3 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 303.1 (21%), 302.1 (89%), 284.1 (100%).

2,5-Dimethyl-1-(oxiran-2-ylmethyl)-1*H*-pyrrole 224



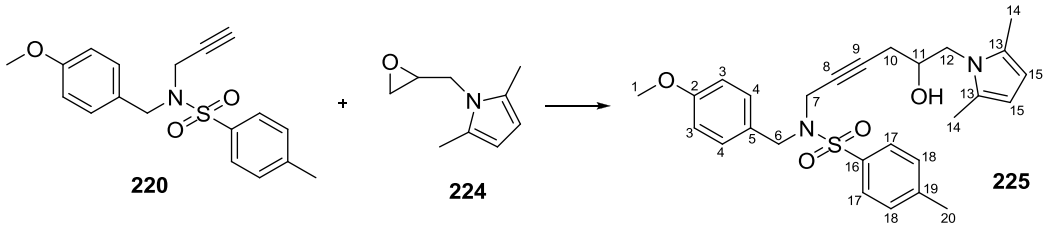
224

A suspension of 60% NaH dispersed on mineral oil (600 mg, 15.0 mmol) in anhydrous DMF (15 mL) was prepared followed by addition of 2,5-dimethylpyrrole (1.00 mL, 9.84 mmol) in anhydrous DMF (15 mL). This mixture was stirred for 18 hours before cooling to 0 °C. Epibromohydrin (940 μL, 11.0 mmol) was added dropwise and the mixture was stirred for a further 7 hours at 0 °C. The reaction was quenched by careful addition of H_2O (20 mL) before extraction with Et_2O (4 × 30 mL). The combined extracts were washed with brine (30 mL), dried ($MgSO_4$) filtered and concentrated *in vacuo*. Flash column chromatography using silica gel (petroleum ether-ethyl acetate, 9:1) afforded the pyrrole **224** as a cloudy oil (864 mg, 58%).

R_f = 0.40 (petroleum ether-ethyl acetate, 17:3); ν_{max} 3050, 2934, 1521, 747, 723, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (2H, s, CH-C6), 4.09 (1H, dd, *J* = 15.7, 3.2 Hz, CH_2-C3a), 3.95 (1H, dd, *J* = 15.7, 4.4 Hz, CH_2-C3b), 3.16 (1H, dddd, *J* = 4.4, 4.0, 3.2, 2.5 Hz, CH-C2), 2.79 (1H, dd, *J* = 4.9, 4.0 Hz, CH_2-C1a), 2.42 (1H, dd, *J* = 4.9, 2.5 Hz, CH_2-C1b), 2.25 (6H, s, CH_3-C5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.1 (C-C4), 105.5 (CH-C6), 51.2 (CH-C2), 45.2 (CH_2-C1), 44.4 (CH_2-C3), 12.6 (CH_3-C5); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_9H_{14}NO$

152.1075, found 152.1074 [Δ –0.8 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 153.1 (12%), 152.1 (100%).

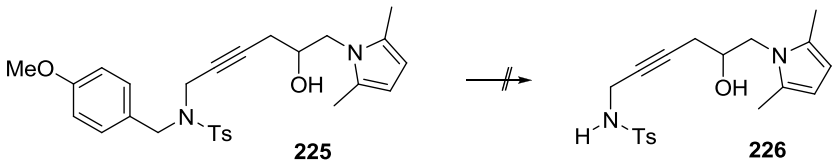
N-(6-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-hydroxyhex-2-ynyl)-*N*-(4-methoxybenzyl)-4-methylbenzenesulfonamide 225



A solution of alkyne **220** (165 mg, 0.502 mmol) in THF (3.5 mL) was prepared and cooled to –78 °C. *n*-BuLi (260 μL of a 2.5 M solution in hexanes, 0.650 mmol) was added dropwise and the resulting mixture was stirred for 30 minutes. Subsequently, BF_3·THF (72.0 μL, 0.652 mmol) was added followed by a further 15 minutes of stirring. Finally, epoxide **224** (91.0 mg, 0.602 mmol) was added as a solution in THF (0.5 mL). The mixture was stirred for 2 hours, maintaining the temperature at –78 °C, and the reaction was quenched by the addition of saturated aqueous NH_4Cl (4 mL). The resulting biphasic mixture was extracted with Et_2O (3 × 10 mL) and the combined extractions were washed with brine (10 mL). The organic solution was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 2:1) to give alcohol **225** as a colourless oil (83 mg, 34%).

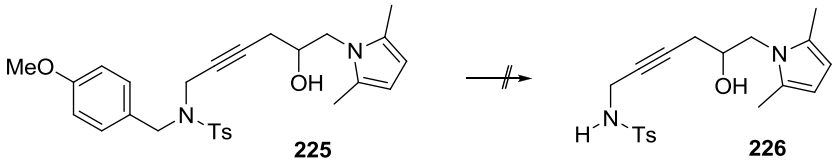
R_f = 0.29 (petroleum ether-ethyl acetate, 7:3); ν_{max} 3470, 2974, 2913, 1611, 835, 658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, *J* = 8.2 Hz, CH-C17), 7.24 (2H, d, *J* = 8.2 Hz, CH-C18), 7.17 (2H, d, *J* = 8.6 Hz, CH-Arom), 6.78 (2H, d, *J* = 8.6 Hz, CH-Arom), 5.72 (2H, s, CH-C15), 4.21 (2H, s, CH_2-C6), 3.85 (2H, s, CH_2-C7), 3.72 (3H, s, CH_3-C1), 3.70–3.64 (1H, m, CH-C11), 3.61 (1H, dd, J = 14.5, 4.4 Hz, CH_2-C12a), 3.60 (1H, dd, *J* = 14.5, 8.0 Hz, CH_2-C12b), 2.32 (3H, s, CH_3-C20), 2.12 (6H, s, CH_3-C14), 2.13–2.11 (2H, m, CH_2-C10), 1.90 (1H, d, *J* = 3.9 Hz, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5 (C-Arom), 143.7 (C-Arom), 136.2 (C-Arom), 130.0 (CH-Arom), 129.5 (CH-C18), 128.0 (C-C13), 127.9 (CH-C17), 126.9 (C-Arom), 114.1 (CH-Arom), 105.9 (CH-C15), 81.5 (C-C9), 75.8 (C-C8), 69.5 (CH-C11), 55.3 (CH_3-C1), 49.4 (CH_2-C6), 48.5 (CH_2-C12), 35.7 (CH_2-C7), 24.9 (CH_2-C10), 21.5 (CH_3-C20), 12.8 (CH_3-C14); HRMS (EI) $[M+]^+$ calcd for $C_{27}H_{32}N_2O_4S$ 480.2083, found 480.2084 [Δ +0.3 ppm]; LRMS (EI) *m/z* (intensity) 481.2 (35%), 480.2 (100%).

Attempted PMB Deprotection Using Pd/C



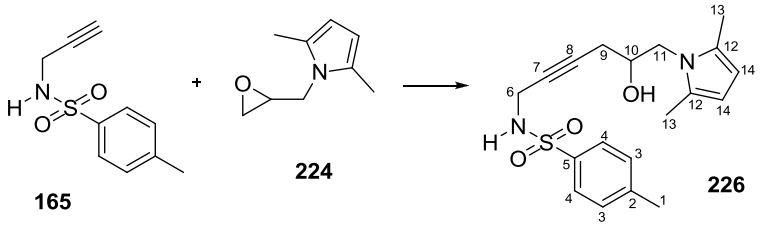
To a solution of alkyne **225** (80.0 mg, 0.166 mmol) in methanol (15 mL) was added 10% Pd/C (2.80 mg, 0.00263 mmol) and ammonium formate (105 mg, 1.67 mmol). The mixture was heated to reflux and stirred at this temperature for 2.5 hours. The mixture was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with water (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo*. TLC analysis revealed that no reaction had taken place.

Attempted PMB Deprotection Using *α*-Chloroethyl Chloroformate



A solution of alkyne **225** (48.0 mg, 0.0999 mmol) in dichloromethane (1 mL) was prepared and cooled to 0 °C. *α*-Chloroethyl chloroformate (14.0 *μ*L, 0.130 mmol) was added slowly to the solution of the alkyne and the mixture was stirred at 0 °C for 1 hour. The mixture was allowed to reach room temperature before being concentrated *in vacuo*. Residual material was redissolved in methanol (2 mL) and the solution was heated at reflux for 1 hour. The reaction mixture was concentrated *in vacuo* but TLC analysis of the residue indicated that the reaction had not taken place.

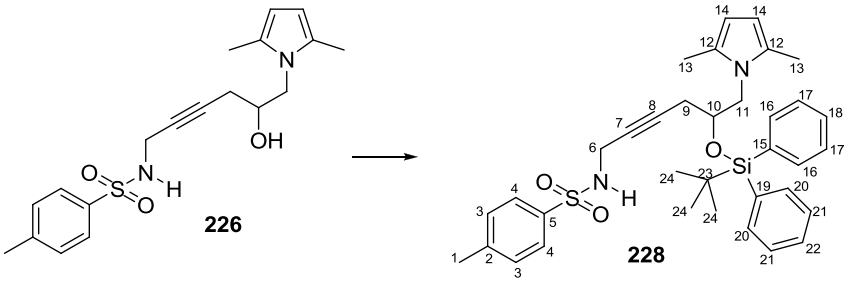
N-(6-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-hydroxyhex-2-ynyl)-4-methylbenzenesulfonamide 226



A solution of alkyne **165** (2.10 g, 10.0 mmol) in THF (120 mL) was prepared before cooling to –78 °C. *n*-BuLi (9.20 mL of a 2.5 M solution in hexanes, 23.0 mmol) was added dropwise followed by 30 minutes of stirring. Subsequently, BF_3·THF (1.40 mL, 12.6 mmol) was added followed by a further 15 minutes of stirring. Finally, a solution of epoxide **224** (1.80 g, 11.9 mmol) in THF (2 mL) was added and the reaction was stirred for 5 hours, maintaining the temperature at –78 °C. Excess *n*-BuLi was quenched by the addition of saturated aqueous NH_4Cl (120 mL). The resulting biphasic solution was extracted with Et_2O (3 × 60 mL) and the combined extracts were washed with brine (60 mL). The Et_2O solution was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Alcohol **226** was isolated by column chromatography using silica gel (petroleum ether-ethyl acetate, 3:2) as a colourless oil (2.1 g, 58%).

R_f = 0.18 (petroleum ether-ethyl acetate, 3:2); ν_{max} 3387, 3113, 2901, 1732, 1597, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.79 (2H, d, *J* = 8.3 Hz, CH-C4), 7.33 (2H, d, *J* = 8.3 Hz, CH-C3), 5.80 (2H, s, CH-C14), 4.71 (1H, t, *J* = 5.9 Hz, NH), 3.88–3.86 (1H, m, CH-C10), 3.82 (2H, dt, *J* = 5.9, 2.2 Hz, CH_2-C6), 3.76 (1H, dd, *J* = 14.6, 4.4 Hz, CH_2-C11a), 3.73 (1H, dd, *J* = 14.6, 7.8 Hz, CH_2-C11b), 2.43 (3H, s, CH_3-C1), 2.27 (2H, dd, *J* = 5.6, 2.2 Hz, CH_2-C9), 2.25 (1H, br s, OH), 2.22 (6H, s, CH_3-C13); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8 (C-Arom), 136.7 (C-Arom), 129.7 (CH-C3), 128.1 (C-C12), 127.4 (CH-C4), 105.8 (CH-C14), 80.6 (C-C8), 77.4 (C-C7), 69.5 (CH-C10), 48.5 (CH_2-C11), 33.2 (CH_2-C6), 24.9 (CH_2-C9), 21.5 (CH_3-C1), 12.8 (CH_3-C13); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_3S$ 361.1586, found 361.1583 [Δ –0.8 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 362.2 (23%), 361.2 (100%).

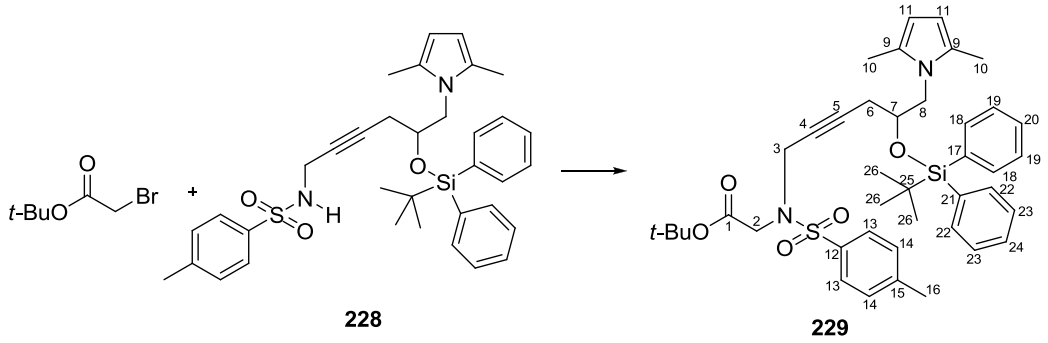
N-(5-(*tert*-Butyldiphenylsilyloxy)-6-(2,5-dimethyl-1*H*-pyrrol-1-yl)hex-2-ynyl)-4-methylbenzenesulfonamide 228



To a solution of alcohol **226** (72.0 mg, 0.200 mmol) in dry CH_2Cl_2 (0.2 mL) was added 2,6-lutidine (33.0 μL, 0.296 mmol) followed by dropwise addition of TBDPSOTf (78.0 μL, 0.231 mmol). The mixture was stirred for 18 hours and then diluted with EtOAc (10 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The EtOAc solution was then dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 9:1) to give silyl ether **228** as a colourless oil (56 mg, 47%).

R_f = 0.46 (petroleum ether-ethyl acetate, 4:1); ν_{max} 3264, 3048, 2932, 2855, 2245, 1713, 1597, 810, 702, 617 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.62 (2H, d, *J* = 8.2 Hz, CH-C4), 7.60–7.58 (2H, m, CH-Arom), 7.39–7.24 (8H, m, CH-Arom), 7.17 (2H, d, *J* = 8.2 Hz, CH-C3), 5.65 (2H, s, CH-C14), 4.31 (1H, t, *J* = 5.8 Hz, NH), 3.86–3.81 (1H, m, CH-C10), 3.65 (1H, dd, *J* = 14.7, 6.6 Hz, CH_2-C11a), 3.62 (1H, dd, *J* = 14.7, 7.1 Hz, CH_2-C11b), 3.54 (2H, dt, *J* = 5.8, 2.3 Hz, CH_2-C6), 2.30 (3H, s, CH_3-C1), 1.92 (6H, s, CH_3-C13), 1.92–1.90 (2H, m, CH_2-C9), 0.93 (9H, s, CH_3-C24); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.6 (C-Arom), 136.6 (C-Arom), 135.9 (CH-Arom), 135.9 (CH-Arom), 133.8 (C-Arom), 132.7 (C-Arom), 130.0 (CH-Arom), 130.0 (CH-Arom), 129.6 (CH-C3), 128.1 (C-Arom), 127.8 (CH-Arom), 127.8 (CH-Arom), 127.3 (CH-C4), 105.6 (CH-C14), 81.1 (C-C8), 76.4 (C-C7), 70.4 (CH-C10), 48.8 (CH_2-C11), 33.2 (CH_2-C6), 26.8 (CH_3-C24), 25.1 (CH_2-C9), 21.5 (CH_3-C1), 19.1 (C-C23), 12.7 (CH_3-C13); HRMS (EI) $[M+]^+$ calcd for $C_{35}H_{42}N_2O_3SSi$ 598.2685, found 598.2679 [Δ –1.0 ppm]; LRMS (EI) *m/z* (intensity) 600.3 (20%), 599.3 (47%), 598.3 (100%).

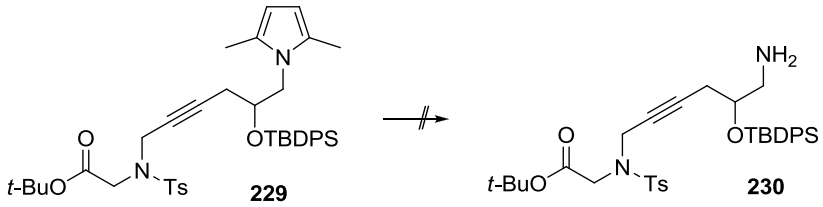
tert-Butyl 2-(_N_-(5-(_tert_-butyldiphenylsilyloxy)-6-(2,5-dimethyl-1_H_-pyrrol-1-yl)hex-2-ynyl)-4-methylphenylsulfonamido)acetate 229



To a solution of sulphonamide **228** (88.0 mg, 0.147 mmol) in acetone (2 mL) was added K_2CO_3 (61.0 mg, 0.442 mmol) and *tert*-butyl bromoacetate (33.0 μL, 0.224 mmol). The reaction mixture was stirred at 65 °C for 20 hours, after which it was allowed to cool to room temperature. The reaction mixture was filtered, washing through with excess acetone, and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 4:1) to give *tert*-butyl ester **229** as a colourless oil (72 mg, 69%).

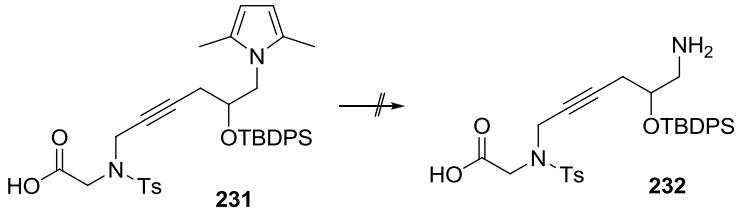
R_f = 0.54 (petroleum ether-ethyl acetate, 2:1); ν_{max} 3071, 2931, 1746, 822, 743, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.62–7.59 (2H, m, CH-Arom), 7.61 (2H, d, *J* = 8.1 Hz, CH-C13), 7.38–7.22 (8H, m, CH-Arom), 7.14 (2H, d, *J* = 8.1 Hz, CH-C14), 5.67 (2H, s, CH-C11), 4.11 (2H, t, *J* = 2.2 Hz, CH_2-C3), 3.89 (2H, d, *J* = 1.4 Hz, CH_2-C2), 3.83 (1H, tdd, *J* = 6.6, 6.3 4.3 Hz, CH-C7), 3.65 (2H, d, *J* = 6.6 Hz, CH_2-C8), 2.27 (3H, s, CH_3-C16), 1.95 (6H, s, CH_3-C10), 1.89 (1H, ddt, *J* = 16.8, 6.3, 2.2 Hz, CH_2-C6a), 1.84 (1H, ddt, *J* = 16.8, 4.3, 2.2 Hz, CH_2-C6b), 1.33 (9H, s, CH_3-*t*Bu), 0.92 (9H, s, CH_3-C26); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5 (C-C1), 143.5 (C-Arom), 136.6 (C-Arom), 135.9 (CH-Arom), 135.8 (CH-Arom), 133.8 (C-Arom), 132.6 (C-Arom), 130.0 (CH-Arom), 130.0 (CH-Arom), 129.5 (CH-C14), 127.9 (C-Arom), 127.8 (CH-Arom), 127.8 (CH-Arom), 127.5 (CH-C13), 105.7 (CH-C11), 82.2 (C-*t*Bu), 82.2 (C-C5), 75.5 (C-C4), 70.4 (CH-C7), 48.4 (CH_2-C8), 47.2 (CH_2-C2), 37.7 (CH_2-C3), 28.0 (CH_3-*t*Bu), 26.8 (CH_3-C26), 24.7 (CH_2-C6), 21.5 (CH_3-C16), 19.0 (C-25), 12.8 (CH_3-C10); HRMS (FAB) $[M+H]^+$ calcd for $C_{41}H_{53}N_2O_5SSi$ 713.3444, found 713.3441, [Δ –0.3 ppm]; LRMS (FAB) *m/z* (intensity) 714.3 (53%), 713.3 (100%).

Attempted Removal of 2,5-Dimethylpyrrole



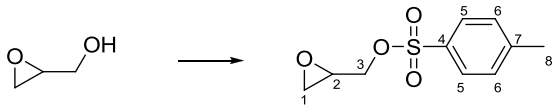
To a solution of protected amine **229** (68.0 mg, 0.0954 mmol) in isopropanol (4 mL) and water (1 mL) was added hydroxylamine hydrochloride (134 mg, 1.93 mmol) followed by TEA (130 μL, 0.932 mmol). The reaction mixture was heated to reflux and stirred for a period of 4 hours. The mixture was then cooled to room temperature and NaOH (38.0 mg, 0.950 mmol) was added. The mixture was stirred for a period of 20 hours. It was apparent that reaction had not taken place and so the mixture was heated to reflux for a further two hours with stirring. Upon cooling, the reaction mixture was filtered through Celite® and concentrated *in vacuo*. Reaction had not taken place and the starting material was recovered by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:1).

Alternative Approach to 2,5-Dimethylpyrrole Removal



To a solution of alkyne **231** (13.0 mg, 0.0198 mmol) in isopropanol (4 mL) and water (1 mL) was added hydroxylamine hydrochloride (28.0 mg, 0.441 mmol) followed by TEA (28 μL, 0.201 mmol). The reaction mixture was heated to reflux and stirred at this temperature for 18 hours. After this time the mixture was cooled to room temperature and NaOH (8.00 mg, 0.200 mmol) was added. The mixture was stirred for a further 20 hours, then filtered through a pad of Celite® and concentrated *in vacuo*. TLC analysis of the residual material revealed that reaction had not taken place. The starting material could not be recovered by column chromatography due to polarity issues relateing to the carboxylic acid.

Oxiran-2-ylmethyl 4-methylbenzenesulfonate 233

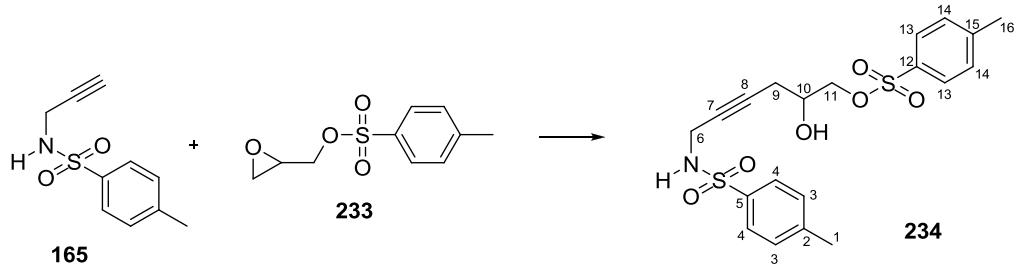


233

A solution of glycidol (5.00 mL, 75.5 mmol) in CH_2Cl_2 (60 mL) was prepared and cooled to 0 °C. TEA (14.0 mL, 101 mmol) was added, followed by *p*-toluene sulfonyl chloride (17.3 g, 89.0 mmol) and the reaction mixture was warmed to room temperature with stirring. After 18 hours, the reaction mixture was washed with brine (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting oil was purified by flash column chromatography (petroleum ether-ethyl acetate, 3:1) to produce sulfonamide **233** as a colourless oil (15.8 g, 92%).

R_f = 0.38 (petroleum ether-ethyl acetate, 3:2); ν_{max} 2959, 1597, 957, 808 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (2H, d, *J* = 8.2 Hz, CH-C5), 7.37 (2H, d, *J* = 8.2 Hz, CH-C6), 4.27 (1H, dd, *J* = 11.4, 3.4 Hz, CH_2-C3a), 3.96 (1H, dd, *J* = 11.4, 6.1 Hz, CH_2-C3b), 3.20 (1H, dddd, *J* = 6.1, 4.4, 3.4, 2.5 Hz, CH-C2), 2.82 (1H, dd, *J* = 4.8, 4.4 Hz, CH_2-C1a), 2.60 (1H, dd, *J* = 4.8, 2.5 Hz, CH_2-C1b), 2.47 (3H, s, CH_3-C8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.2 (C-Arom), 132.7 (C-Arom), 129.9 (CH-C6), 128.0 (CH-C5), 70.5 (CH_2-C3), 48.8 (CH-C2), 44.6 (CH_2-C1), 21.7 (CH_3-C8); HRMS (EI+) $[M]^+$ calcd for $C_{10}H_{12}O_4S$ 228.0456 found, 228.0448 [Δ –3.5 ppm]; LRMS (EI+) *m/z* (intensity) 229.0 (11%), 228.0 (59%), 83.9 (100%).

2-Hydroxy-6-(phenylsulfonamido)hex-4-ynyl 4-methylbenzenesulfonate, 234



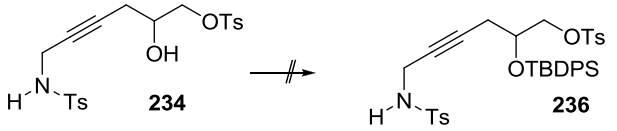
165 **233** **234**

A solution of alkyne **165** (2.10 g, 10.0 mmol) in dry THF (120 mL) was prepared and cooled to –78 °C. *n*-BuLi (9.20 mL of a 2.5 M solution in hexanes, 23.0 mmol) was added dropwise and the mixture was stirred for 30 minutes. BF_3·THF (1.40 mL, 12.7 mmol) was added and the mixture stirred for another 15 minutes. Finally, epoxide **233** (1.80 g, 11.9 mmol) was added as a solution in THF (2 mL). The reaction was stirred for 5 hours, maintaining the temperature at –78 °C. The reaction was then quenched by careful addition of saturated aqueous NH_4Cl (120 mL). The mixture was allowed to warm to room temperature and then extracted with

Et_2O (3 × 60 mL). The combined organic extracts were washed with brine (60 mL) before drying ($MgSO_4$), filtration and concentration *in vacuo*. Alcohol **234** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:2) to give a colourless oil (2.1 g, 58%).

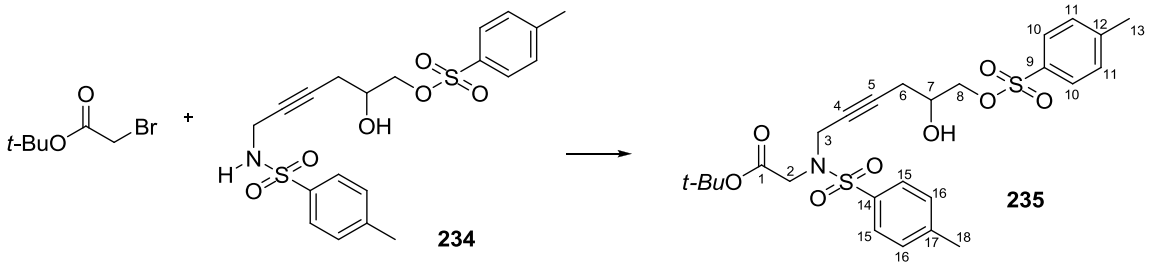
R_f = 0.29 (petroleum ether-ethyl acetate, 1:1); ν_{max} 3448, 3387, 2970, 2360, 1598, 814, 660 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (2H, d, *J* = 8.2 Hz, CH-C13), 7.77 (2H, d, *J* = 8.2 Hz, CH-C4), 7.40 (2H, d, *J* = 8.2 Hz, CH-C14), 7.33 (2H, d, *J* = 8.2 Hz, CH-C3), 4.70 (1H. t, *J* = 6.0 Hz, NH), 4.01 (1H, dd, *J* = 10.3, 4.5 Hz, CH_2-C11a), 3.95 (1H, dd, *J* = 10.3, 5.6 Hz, CH_2-C11b), 3.87–3.80 (1H, m, CH-C10), 3.77 (2H, dt, *J* = 6.0, 2.3 Hz, CH_2-C6), 2.49 (3H, s, CH_3-C16), 2.45 (3H, s, CH_3-C1), 2.33 (1H, d, *J* = 6.0 Hz, OH), 2.30 (2H, dt, *J* = 6.1, 2.3 Hz, CH_2-C9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.3 (C-Arom), 143.9 (C-Arom), 136.6 (C-Arom), 132.4 (C-Arom), 130.0 (CH-C14), 129.7 (CH-C3), 128.0 (CH-C13), 127.4 (CH-C4), 79.7 (C-C8), 77.7 (C-C7), 71.8 (CH_2-C11), 67.6 (CH-C10), 33.2 (CH_2-C6), 23.5 (CH_2-C9), 21.7 (CH_3-C16), 21.5 (CH_3-C1); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{20}H_{24}NO_6S_2$ 438.1045, found 438.1041 [Δ –1.0 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 439.1 (27%), 438.1 (100%).

Attempted Protection of Secondary Alcohol Prior to *N*-Alkylation



To a solution of secondary alcohol **234** (40.0 mg, 0.0914 mmol) in dichloromethane (1 mL) was added sequentially DMAP (22.0 mg, 0.180 mmol), TBDPSCl (48.0 μL, 0.185 mmol) and TEA (14.0 μL, 0.100 mmol). The mixture was stirred for 18 hours at room temperature before the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL). The aqueous phase was separated and extracted with Et_2O (3 × 10 mL) and the combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. TLC analysis indicated that no reaction had taken place and so the starting material was recovered by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:2)..

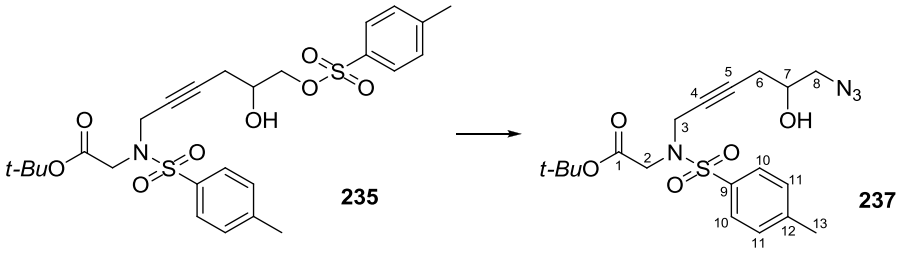
tert-Butyl 2-(*N*-(5-hydroxy-6-(tosyloxy)hex-2-ynyl)-4-methylphenylsulfonamido)acetate 235



A solution of alcohol **234** (874 mg, 2.00 mmol) was prepared in acetone (7 mL). K_2CO_3 (828 mg, 5.99 mmol) was added, followed by *tert*-butyl bromoacetate (310 μL, 2.10 mmol). The reaction was stirred for 18 hours at which point it was filtered and concentrated *in vacuo*. The target ester **235** was isolated by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:1) as a colourless oil (512 mg, 46%).

R_f = 0.20 (petroleum ether-ethyl acetate, 7:3); ν_{max} 3525, 3476, 2981, 1741, 1598, 813, 660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, *J* = 8.2 Hz, CH-C10), 7.62 (2H, d, *J* = 8.4 Hz, CH-C15), 7.29 (2H, d, *J* = 8.2 Hz, CH-C11), 7.21 (2H, d, *J* = 8.4 Hz, CH-C16), 4.07 (2H, s, CH_2-C3), 3.89 (1H, dd, *J* = 10.3, 4.8 Hz, CH_2-C8a), 3.87 (2H, s, CH_2-C2), 3.84 (1H, dd, *J* = 10.3, 5.8 Hz, CH_2-C8b), 3.77–3.71 (1H, m, CH-C7), 2.48 (1H, d, *J* = 5.7 Hz, OH), 2.38 (3H, s, CH_3-C13), 2.33 (3H, s, CH_3-C18), 2.21–2.20 (2H, m, CH_2-C6), 1.34 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6 (C-C1), 145.3 (C-Arom), 143.8 (C-Arom), 136.2 (C-Arom), 132.4 (C-Arom), 130.0 (CH-C11), 129.6 (CH-C16), 128.0 (CH-C10), 127.5 (CH-C15), 82.4 (C-*t*Bu), 80.9 (C-C5), 76.0 (C-C4), 71.8 (CH_2-C8), 67.6 (CH-C7), 47.8 (CH_2-C2), 37.8 (CH_2-C3), 28.0 (CH_3-*t*Bu), 23.5 (CH_2-C6), 21.7 (CH_3-C13), 21.5 (CH_3-C18); HRMS (FAB) $[M+H]^+$ calcd for $C_{26}H_{34}NO_8S_2$ 552.1726, found 552.1730, [Δ +0.4 ppm]; LRMS (FAB) *m/z* (intensity) 553.2 (30%), 552.2 (100%); Anal. calcd for $C_{26}H_{33}NO_8S_2$: C, 56.61%; H, 6.03%; N, 2.54%. Found: C, 56.54%; H, 6.22%; N, 2.59%.

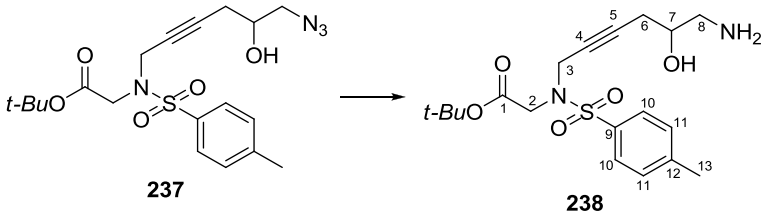
tert-Butyl 2-(*N*-(6-azido-5-hydroxyhex-2-ynyl)-4-methylphenylsulfonamido)acetate 237



A solution of tosylate **235** (200 mg, 0.363 mmol) in DMF (2.5 mL) was added to a flask charged with NaN_3 (71.0 mg, 1.09 mmol). The mixture was heated to 80 °C and stirred for 5 hours. Upon completion of the reaction, the mixture was cooled to room temperature before H_2O (15 mL) was added. The biphasic solution was extracted with Et_2O (3 × 10 mL) and the combined organic phases were washed with brine (20 mL). The solution was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:2) to give azide **237** as a colourless solid (139 mg, 91%).

R_f = 0.41 (petroleum ether-ethyl acetate, 3:2); m.p. = 53–54 °C; ν_{max} 3570, 3480, 2981, 2102, 1744, 1598, 813, 658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.66 (2H, d, *J* = 8.1 Hz, CH-C10), 7.24 (2H, d, *J* = 8.1 Hz, CH-C11), 4.14 (2H, t, *J* = 2.0 Hz, CH_2-C3), 3.92 (2H, s, CH_2-C2), 3.71–3.65 (1H, m, CH-C7), 3.22 (1H, dd, *J* = 12.5, 4.5 Hz, CH_2-C8a), 3.18 (1H, dd, *J* = 12.5, 6.3 Hz, CH_2-C8b), 2.37 (3H, s, CH_3-C13), 2.24 (2H, dt, *J* = 6.1, 2.0 Hz, CH_2-C6), 2.17 (1H, d, *J* = 5.4 Hz, OH), 1.36 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6 (C-C1), 143.8 (C-Arom), 136.3 (C-Arom), 129.6 (CH-C11), 127.6 (CH-C10), 82.5 (C-*t*Bu), 81.3 (C-C5), 76.2 (C-C4), 69.0 (CH-C7), 55.2 (CH_2-C8), 47.9 (CH_2-C2), 37.8 (CH_2-C3), 28.0 (CH_3-*t*Bu), 24.8 (CH_2-C6), 21.5 (CH_3-C13); HRMS (FAB) $[M+H]^+$ calcd for $C_{19}H_{27}N_4O_5S$ 423.1702, found 423.1704, [Δ +0.2 ppm]; LRMS (FAB) *m/z* (intensity) 423.2 (16%), 366.8 (100%).

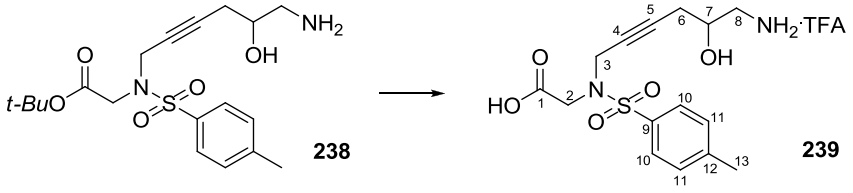
tert-Butyl 2-(*N*-(6-amino-5-hydroxyhex-2-ynyl)-4-methylphenylsulfonamido)acetate 238



To a solution of azide **237** (860 mg, 2.04 mmol) in THF (9 mL) was added PPh_3 (561 mg, 2.14 mmol). The mixture was stirred at room temperature for 3 days. The reaction mixture was poured into H_2O (22 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (methanol-dichloromethane, 9:1) to yield the primary amine **238** as a colourless oil (713 mg, 88%).

R_f = 0.13 (dichloromethane-methanol, 9:1); ν_{max} 3534, 3372, 2978, 2870, 1744, 1597, 656 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.66 (2H, d, *J* = 8.2 Hz, CH-C10), 7.23 (2H, d, *J* = 8.2 Hz, CH-C11), 4.13 (2H, t, *J* = 2.0 Hz, CH_2-C3), 3.92 (2H, s, CH_2-C2), 3.52–3.47 (1H, m, CH-C7), 2.75 (1H, dd, *J* = 12.8, 3.6 Hz, CH_2-C8a), 2.52 (1H, dd, *J* = 12.8, 7.7 Hz, CH_2-C8b), 2.36 (3H, s, CH_3-C13), 2.20 (1H, ddt, *J* = 16.7, 6.2, 2.0 Hz, CH_2-C6a), 2.15 (1H, ddt, *J* = 16.7, 6.1, 2.0 Hz, CH_2-C6b), 2.09 (3H, br s, OH and NH_2), 1.35 (9H, s, CH_3-*t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6 (C-C1), 143.7 (C-Arom), 136.3 (C-Arom), 129.5 (CH-C11), 127.6 (CH-C10), 82.5 (C-*t*Bu), 82.4 (C-C5), 75.2 (C-C4), 69.9 (CH-C7), 47.7 (CH_2-C2), 46.1 (CH_2-C8), 37.9 (CH_2-C3), 28.0 (CH_3-*t*Bu), 24.8 (CH_2-C6), 21.5 (CH_3-C13); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{19}H_{29}N_2O_5S$ 397.1797, found 397.1787 [Δ –2.5 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 397.2 (64%), 230.2 (100%).

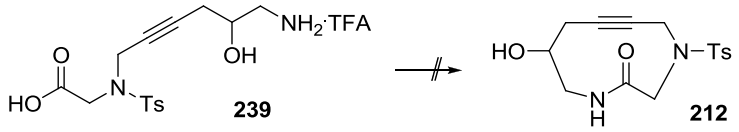
2-(*N*-(6-Amino-5-hydroxyhex-2-ynyl)-4-methylphenylsulfonamido)acetic acid TFA salt 239



A solution of *tert*-butyl ester **238** (98.0 mg, 0.247 mmol) in CH_2Cl_2 (1.8 mL) was prepared and TFA (1.8 mL) was added. The mixture was stirred for 16 hours before concentrating *in vacuo*. The residue was mixed with a small volume of toluene and concentrated *in vacuo* once again. This last procedure was repeated two more times to ensure complete removal of traces of TFA, followed by further drying under high vacuum. The TFA salt **239** was isolated as a white solid (112 mg, 100%).

M.p. = 38–39 °C; ν_{max} 3337, 3156, 2928, 2643, 1736, 1670, 799, 671 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.59 (2H, d, *J* = 8.2 Hz, CH-C10), 7.25 (2H, d, *J* = 8.2 Hz, CH-C11), 4.05 (2H, t, *J* = 2.2 Hz, CH_2-C3), 3.90 (2H, s, CH_2-C2), 3.69–3.64 (1H, m, CH-C7), 2.90 (1H, dd, *J* = 12.8, 3.0 Hz, CH_2-C8a), 2.65 (1H, dd, *J* = 12.8, 9.3 Hz, CH_2-C8b), 2.29 (3H, s, CH_3-C13), 2.18 (1H, ddt, *J* = 16.9, 5.4, 2.2 Hz, CH_2-C6a), 2.17 (1H, s, OH), 2.11 (1H, ddt, *J* = 16.9, 7.1, 2.2 Hz, CH_2-C6b); ^{13}C NMR (100 MHz, CD_3OD) δ 171.9 (C-C1), 145.4 (C-Arom), 137.6 (C-Arom), 130.8 (CH-C11), 128.8 (CH-C10), 82.3 (C-C5), 76.8 (C-C4), 67.2 (CH-C7), 48.1 (CH_2-C2), 44.9 (CH_2-C8), 38.8 (CH_2-C3), 25.9 (CH_2-C6), 21.5 (CH_3-C13); HRMS (FAB) [M^+] calcd for $C_{15}H_{21}N_2O_5S$ 341.1171, found 341.1168 [Δ –1.0 ppm]; LRMS (FAB) *m/z* (intensity) 341.1 (17%), 154.1 (100%).

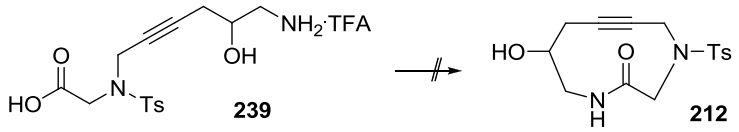
Lactamisation Reaction Using BOP



To a solution of carboxylic acid **239** (30.0 mg, 0.0660 mmol) in dry CH_2Cl_2 (248 mL) and DMF (2.5 mL) was added HOBt (89.0 mg, 0.659 mmol) and DIPEA (230 μL, 1.32 mmol). The mixture was cooled to –15 °C and BOP (292 mg, 0.660 mmol) was added. The mixture was stirred for 24 hours and during this time it was allowed to warm to room temperature. The mixture was concentrated *in vacuo* to 30 mL and washed sequentially with 2% aqueous citric acid solution (2 × 50 mL), 2% saturated aqueous Na_2CO_3 (2 × 50 mL) and brine (2 × 50 mL).

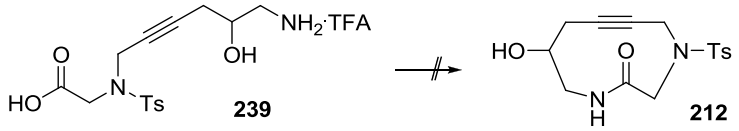
The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo*. No new products were visible upon TLC analysis of the residue.

Lactamisation Reaction Using EDCI



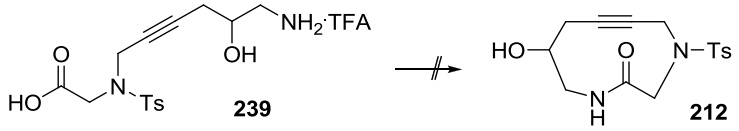
A solution of carboxylic acid **239** (20.0 mg, 0.0440 mmol) in dichloromethane (396 mL) and DMF (4 mL) was prepared and cooled to –15 °C. EDCI (211 mg, 1.10 mmol) and DMAP (54.0 mg, 0.442 mmol) were added to the mixture, followed by stirring for 20 hours as it reached room temperature. The mixture was concentrated *in vacuo* to 30 mL and then washed sequentially with 5% aqueous citric acid solution (2 × 50 mL), 5% aqueous Na_2CO_3 solution (2 × 50 mL) and brine (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo*. TLC analysis of the crude mixture showed that the desired product had not been obtained.

Lactamisation Reaction Using FDPP



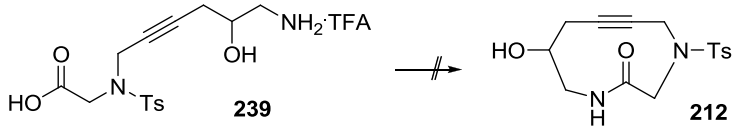
To a solution of alkyne **239** (15.0 mg, 0.0330 mmol) in DMF (3 mL) was added FDPP (38.0 mg, 0.0989 mmol) and DIPEA (29.0 μL, 0.166 mmol). The mixture was stirred at room temperature for 4 days and then acidified to pH 5 by the addition of 1M HCl solution. The mixture was partitioned between water (20 mL) and a 3:1 mixture of chloroform/isopropanol (20 mL). The biphasic solution was extracted with a 3:1 mixture of chloroform/isopropanol (3 × 20 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 1:1), but the new products were found to be decomposition products.

Lactamisation Reaction Using DMTMM



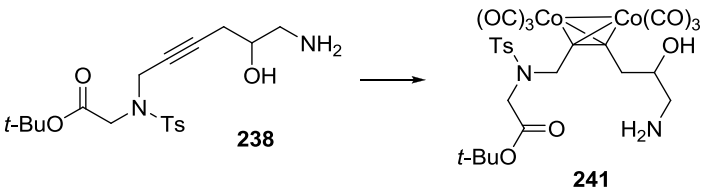
To a solution of alkyne **239** (15.0 mg, 0.0330 mmol) in DMF (3 mL) was added DMTMM (27.0 mg, 0.0976 mmol) and DIPEA (29.0 μL, 0.166 mmol). The mixture was stirred at room temperature for 4 days and was then acidified to pH 5 using 1M HCl solution. The mixture was partitioned between water (20 mL) and a 3:1 mixture of chloroform/isopropanol (20 mL). The resulting biphasic solution was extracted with a 3:1 mixture of chloroform/isopropanol (3 × 20 mL) and the organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:2) to afford trace amounts of decomposition products and none of the desired lactam.

Lactamisation Reaction Using PyBOP



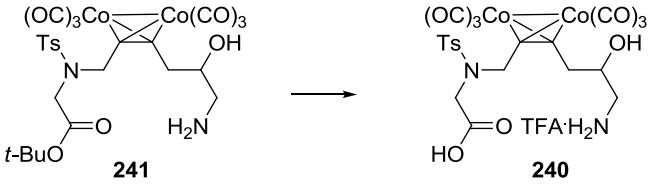
To a solution of alkyne **239** (15.0 mg, 0.0330 mmol) in dry DMF (1 mL) was added PyBOP (52.0 mg, 0.0999 mmol) and DIPEA (29.0 μL, 0.166 mmol), and the mixture was then stirred at room temperature for 3 days. The solution was acidifed to pH 5 using 1M HCl solution and the resulting mixture was partitioned between water (20 mL) and a 3:1 solution of chloroform/isopropanol (20 mL). The resulting biphasic mixture was extracted with 3:1 chloroform/isopropanol (3 × 20 mL) and the combined extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude reaction mixture was purified by flash column chromatography on silica gel (dichloromethane-methanol, 19:1), to deliver trace amounts of decomposition products.

Synthesis of the Cobalt Complex 241



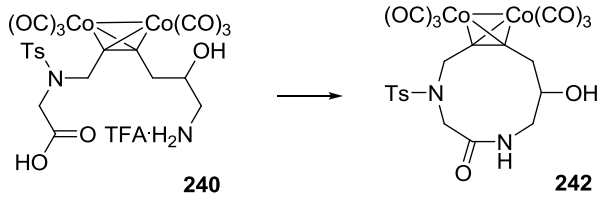
To a solution of alkyne **238** (700 mg, 1.77 mmol) in dichloromethane (20 mL) was added cobalt octacarbonyl (635 mg, 1.86 mmol) at room temperature. The mixture was stirred for 5 hours and then concentrated *in vacuo*. Flash column chromatography on silica gel (dichloromethane-methanol, 9:1) delivered the cobalt complex **241** (624 mg, 52%) as a brown solid. The purified product was dissolved in a small volume of CH_2Cl_2 and passed through a pad of Celite® to remove any traces of free cobalt in preparation for NMR analysis. This was unsuccessful and no useful NMR data could be obtained. Similarly, mass spectrometric data was unrevealing.

Deprotection of the Cobalt Complex



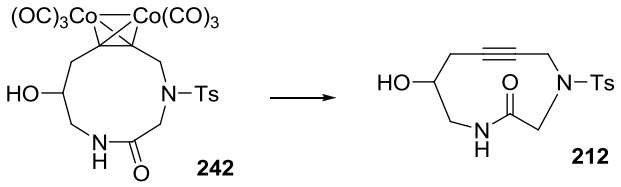
To a solution of cobalt complex **241** (624 mg, 0.914 mmol) in dichloromethane (3 mL) was added TFA (3 mL). The mixture was stirred at room temperature for 5 hours and then concentrated *in vacuo*. The residue was dissolved in toluene and concentrated once again *in vacuo*. This procedure was performed twice more, followed by drying under high vacuum, to ensure complete removal of traces of free TFA. The deprotected product **234** was isolated as a brown oil (677 mg, 100%) and used without further purification. Attempts to remove traces of cobalt for NMR analysis were unsuccessful and so useful NMR data could not be obtained.

Formation of the Cobalt Complex Lactam



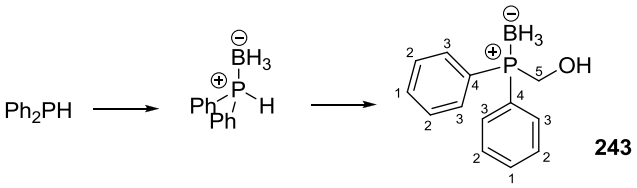
To a solution of cobalt complex **240** (20.0 mg, 0.0270 mmol) in dichloromethane (2.7 mL) was added DIPEA (9.40 μL, 0.0540 mmol) and BOP (14.0 mg, 0.0317 mmol) The reaction mixture was stirred for 2 days at room temperature, after which time it was acidified to pH 5 using 1M HCl solution. The mixture was partitioned between water (10 mL) and dichloromethane (10 mL). The aqueous layer was separated and extracted with dichloromethane (3 × 10 mL) and the combined extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. A possible product was identified by TLC analysis and this compound was isolated by flash column chromatography on silica gel (dichloromethane/methanol, 9:1). Attempts to obtain useful NMR data from this compound were unsuccessful and so the next step involving removal of the cobalt group was carried out as a means of validating this structure.

Attempted Removal of Cobalt Complex



To a solution of the isolated product **242** (5.70 mg, 0.00937 mmol) in dichloromethane (1 mL) was added NMO (20.0 mg, 0.171 mmol). The mixture was stirred for 2 hours, before partitioning it between Et_2O (10 mL) and saturated aqueous NH_4Cl (10 mL). The organic layer was washed with saturated aqueous NH_4Cl (10 mL) and brine (10 mL), then dried (Na_2SO_4), filtered and concentrated *in vacuo*. The reaction did not show a visible progression by TLC analysis, suggesting that the product that was isolated from the previous reaction was not the desired lactam.

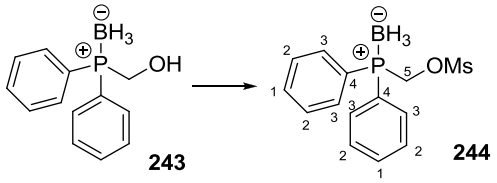
Diphenylphosphino(borane)methyl alcohol 243



Borane-dimethylsulfide (19.0 mL of a 2M solution in THF, 38.0 mmol) was diluted by addition of THF (20 mL) before the addition of diphenylphosphine (3.50 mL, 20.1 mmol) and the mixture was stirred for 2.5 hours. The reaction was quenched slowly with ice (~20 mL) and then diluted with brine (20 mL). The mixture was extracted with EtOAc (3 × 20 mL) and the combined extracts were washed with brine (20 mL). The organic solution was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give the diphenylphosphane borane. This product was used without further purification by dissolving in a 37% aqueous solution of formaldehyde (15 mL) and THF (10 mL). KOH (2.40 g, 42.9 mmol) was added and the reaction was stirred for 2 hours. The mixture was concentrated *in vacuo* and the remaining aqueous solution was extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:1) to yield alcohol **243** as a white solid (4.6 g, 100%).

R_f = 0.46 (petroleum ether-ethyl acetate, 3:1); m.p. = 38–40 °C; ν_{max} 3442, 3056, 2972, 2370, 1587, 734, 703, 690 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.75–7.73 (4H, m, CH-Arom), 7.56–7.54 (2H, m, CH-Arom), 7.51–7.47 (4H, m, CH-Arom), 4.46 (2H, d, J = 6.0 Hz, CH_2-C5), 2.23 (1H, br s, OH), 1.29–0.90 (3H, m, BH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.8 (d, $^3J_{P\text{-}C}$ = 9.0 Hz, CH-C2), 131.8 (d, $^4J_{P\text{-}C}$ = 2.6 Hz, CH-C1), 129.0 (d, $^2J_{P\text{-}C}$ = 9.8 Hz, CH-C3), 126.7 (d, $^1J_{P\text{-}C}$ = 55.0 Hz, C-C4), 60.4 (d, $^1J_{P\text{-}C}$ = 41.4 Hz, CH_2-C5); HRMS (EI+) $[M]^+$ calcd for $C_{13}H_{13}OP$ 216.0704, found 216.0708 [Δ +1.8 ppm]; LRMS (EI+) *m/z* (intensity) 217.1 (13%), 216.1 (100%).

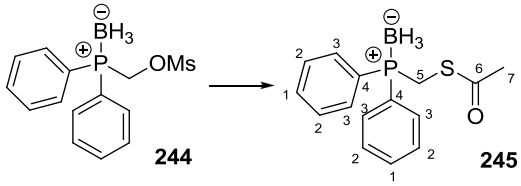
Diphenylphosphino(borane)methyl methanesulfonate 244



The alcohol **243** (1.10 g, 4.78 mmol) was dissolved in CH_2Cl_2 (10 mL) and TEA (1.40 mL, 10.1 mmol) was added. The solution was cooled to 0 °C before dropwise addition of MsCl (580 μL, 7.46 mmol). The reaction was stirred continuously for 18 hours. Upon completion of the reaction, the resulting solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petroleum ether-diethyl ether, 1:1) to give mesylate **244** as a white solid (1.4 g, 95%).

R_f = 0.15 (petroleum ether-ethyl acetate, 1:1); m.p. = 63–65 °C; ν_{max} 3055, 2932, 2330, 1588, 704, 689 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.80–7.75 (4H, m, CH-Arom), 7.64–7.59 (2H, m, CH-Arom), 7.57–7.52 (4H, m, CH-Arom), 4.94 (2H, d, J = 2.2 Hz, CH_2-C5), 2.92 (3H, s, CH_3-Ms), 1.60–0.67 (3H, m, BH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.9 (d, $^3J_{P\text{-}C}$ = 9.9 Hz, CH-C2), 132.3 (d, $^4J_{P\text{-}C}$ = 2.5 Hz, CH-C1), 129.2 (d, $^2J_{P\text{-}C}$ = 10.7 Hz, CH-C3), 125.1 (d, $^1J_{P\text{-}C}$ = 56.9 Hz, C-C4), 64.5 (d, $^1J_{P\text{-}C}$ = 38.5 Hz, CH_2-C5), 37.5 (CH_3-Ms).

Diphenylphosphino(borane)methanethiol acetate 245

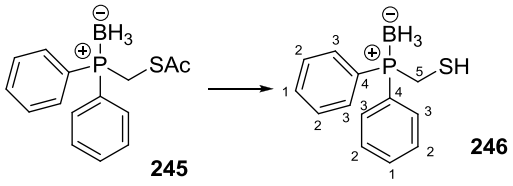


To a solution of mesylate **244** (150 mg, 0.487 mmol) in dry DMF (1 mL) was added thioacetic acid (52.0 μL, 0.731 mmol). The mixture was cooled to –20 °C before addition of Cs_2CO_3 (317 mg, 0.975 mmol). The reaction was stirred for 3 days, allowing it to reach room temperature. The reaction mixture was then diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 4:1) to give thioacetate **245** as a white solid (94 mg, 67%).

R_f = 0.38 (petroleum ether-ethyl acetate, 4:1); m.p. = 52–54 °C; IR ν_{max} 3061, 2960, 2388, 1694, 1587, 702, 691 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.64–7.60 (4H, m, CH-Arom), 7.46–7.43 (2H, m, CH-Arom), 7.40–7.36 (4H, m, CH-Arom), 3.64 (2H, d, J = 6.9 Hz, CH_2-

C5), 2.18 (3H, s, CH_3-C7), 1.18–0.66 (3H, m, BH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.3 (d, $^3J_{P\text{-}C}$ = 2.8 Hz, C-C6), 132.5 (d, $^3J_{P\text{-}C}$ = 9.3 Hz, CH-C2), 131.8 (d, $^4J_{P\text{-}C}$ = 2.5 Hz, CH-C1), 128.9 (d, $^2J_{P\text{-}C}$ = 10.1 Hz, CH-C3), 127.6 (d, $^1J_{P\text{-}C}$ = 55.3 Hz, C-C4), 30.1 (CH_3-C7), 23.8 (d, $^1J_{P\text{-}C}$ = 35.7 Hz, CH_2-C5).

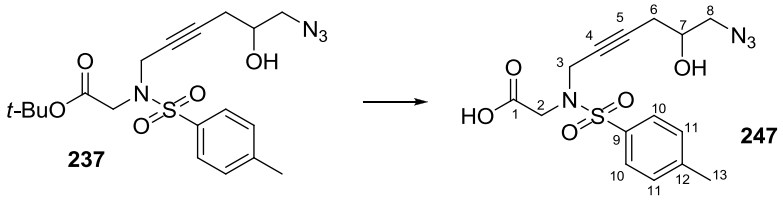
Diphenylphosphino(borane)methanethiol 246



A solution of thioacetate **245** (200 mg, 0.694 mmol) in methanol (1.4 mL) was prepared and a solution of NaOH (56.0 mg, 1.40 mmol) dissolved in the minimum volume of methanol was added. The reaction was stirred for 3 hours before concentration *in vacuo*. The residue was redissolved in EtOAc (20 mL) and washed sequentially with saturated aqueous NH_4Cl (20 mL), water (20 mL) and brine (20 mL). The solution was then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 4:1) to give thiol **246** as a cloudy oil (119 mg, 70%).

R_f = 0.39 (petroleum ether-ethyl acetate, 4:1); ν_{max} 3055, 2918, 2379, 2244, 1588, 689, 648 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65–7.61 (4H, m, CH-Arom), 7.48–7.45 (2H, m, CH-Arom), 7.43–7.39 (4H, m, CH-Arom), 3.12 (2H, dd, *J* = 8.1, 6.1 Hz, CH_2-C5), 1.82 (1H, td, *J* = 8.1, 6.8 Hz, SH), 1.22–0.67 (3H, m, BH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.5 (d, $^3J_{P\text{-}C}$ = 7.1 Hz, CH-C2), 131.7 (d, $^4J_{P\text{-}C}$ = 1.5 Hz, CH-C1), 129.0 (d, $^2J_{P\text{-}C}$ = 7.9 Hz, CH-C3), 127.8 (d, $^1J_{P\text{-}C}$ = 44.0 Hz, C-C4), 19.7 (d, $^1J_{P\text{-}C}$ = 25.7 Hz, CH_2-C5).

2-(*N*-(6-Azido-5-hydroxyhex-2-ynyl)-4-methylphenylsulfonamido)acetic acid 247

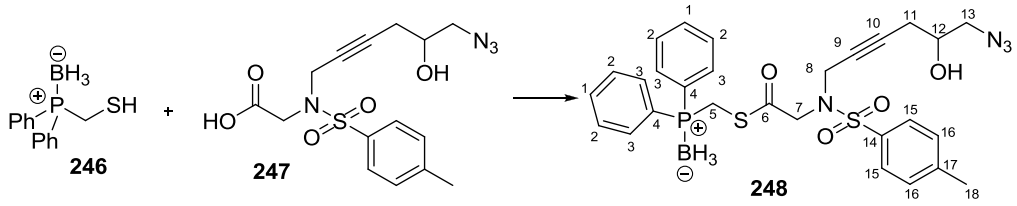


To a solution of *tert*-butyl ester **237** (20.0 mg, 0.0474 mmol) in CH_2Cl_2 (1 mL) was added TFA (1 mL). The reaction was stirred for 18 hours after which it was concentrated *in vacuo*. The residue was mixed with a small volume of toluene and concentrated again *in vacuo*. This last procedure was repeated twice more to remove traces of excess TFA. The sample was

further dried under high vacuum. This yielded carboxylic acid **247** as a clear oil (17 mg, 100%).

R_f = 0.10 (petroleum ether-ethyl acetate, 3:2); ν_{max} 3063, 2924, 2102, 1728, 1597, 810, 656 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.66 (2H, d, *J* = 8.2 Hz, CH-C10), 7.26 (2H, d, *J* = 8.2 Hz, CH-C11), 4.09 (2H, s, CH_2-C3), 4.03 (2H, s, CH_2-C2), 3.70 (1H, dtd *J* = 6.2, 5.7, 4.6 Hz, CH-C7), 3.20 (1H, dd, *J* = 13.0, 4.6 Hz, CH_2-C8a), 3.16 (1H, dd, *J* = 13.0, 6.2 Hz, CH_2-C8b), 2.37 (3H, s, CH_3-C13), 2.34 (1H, s, OH), 2.23 (2H, d, *J* = 5.7 Hz, CH_2-C6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (C-C1), 144.3 (C-Arom), 135.6 (C-Arom), 129.8 (CH-C11), 127.6 (CH-C10), 82.1 (C-C5), 75.5 (C-C4), 69.0 (CH-C7), 55.1 (CH_2-C8), 47.5 (CH_2-C2), 38.3 (CH_2-C3), 24.5 (CH_2-C6), 21.5 (CH_3-C13); HRMS (CI, Me_3CH) $[M+H]^+$ calcd for $C_{15}H_{19}N_4O_5S$ 367.1076, found 367.1074 [Δ –0.6 ppm]; LRMS (CI, Me_3CH) *m/z* (intensity) 368.1 (22%), 367.1 (100%).

13-Azido-12-hydroxy-5-oxo-2,2-diphenyl-7-tosyl-4-thia-7-aza-2-phosphonia-1-boranuidatridec-9-yne, 243

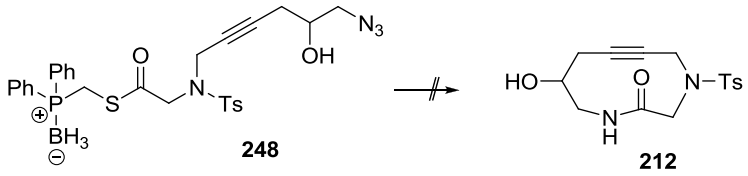


To a solution of azide **247** (8.00 mg, 0.0219 mmol) in CH_2Cl_2 (1 mL) was added thiol **246** (5.00 mg, 0.0203 mmol). The solution was cooled to 0 °C and DCC (5.00 mg, 0.0243 mmol) was added. The reaction mixture was stirred for 18 hours, allowing it to reach room temperature, before concentrating *in vacuo*. The residue was redissolved in a mixture of ethyl acetate and petroleum ether (1:4, 10 mL). The resulting solution was filtered and concentrated *in vacuo* once again. The residue was purified by flash column chromatography using silica gel (petroleum ether-ethyl acetate, 3:2) to give thioether **248** as a clear oil (11 mg, 85%).

R_f = 0.28 (petroleum ether-ethyl acetate, 3:2); ν_{max} 3522, 3059, 2924, 2388, 2102, 1697, 814, 748, 702, 664 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.62 (2H, d, *J* = 7.6 Hz, CH-C15), 7.62–7.59 (4H, m, CH-Arom), 7.48–7.45 (2H, m, CH-Arom), 7.41–7.38 (4H, m, CH-Arom), 7.24 (2H, d, *J* = 7.6 Hz, CH-C16), 3.99 (2H, s, CH_2-C7), 3.94 (2H, t, *J* = 2.2 Hz, CH_2-C8), 3.65–3.60 (1H, m, CH-C12), 3.63 (2H, d, *J* = 6.9 Hz, CH_2-C5), 3.17 (1H, dd, *J* = 12.5, 4.3 Hz, CH_2-C13a), 3.12 (1H, dd, *J* = 12.5, 6.4 Hz, CH_2-C13b), 2.37 (3H, s, CH_3-C18), 2.15 (2H, dt, *J* = 6.1, 2.2 Hz, CH_2-C11), 1.98–1.30 (3H, m, BH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8 (C-C6),

144.5 (C-Arom), 135.1 (C-Arom), 132.5 (d, $^{3}J_{P\text{-}C}$ = 9.4 Hz, CH-C2), 131.9 (d, $^{4}J_{P\text{-}C}$ = 2.4 Hz, CH-C1), 129.8 (CH-C16), 129.0 (d, $^{2}J_{P\text{-}C}$ = 10.1 Hz, CH-C3), 127.9 (CH-C15), 127.5 (d, $^{1}J_{P\text{-}C}$ = 54.8, C-C4), 82.5 (C-C10), 75.1 (C-C9), 68.9 (CH-C12), 55.2 (CH_2-C13), 54.9 (CH_2-C7), 38.6 (CH_2-C8), 24.6 (CH_2-C11), 23.3 (d, $^{1}J_{P\text{-}C}$ = 34.4 Hz, CH_2-C5), 21.6 (CH_3-C18).

Cyclisation of Diphenylphosphine Borane Tethered Intermediate



A solution of azide **248** (20.0 mg, 0.0336 mmol) in THF (3 mL) was prepared and heated to reflux. DABCO (19.0 mg, 0.169 mmol) was added to the solution and the reaction mixture was stirred at reflux for 2 days. The reaction showed no progression after this time and so wet THF (2 mL) was added to the mixture and it was heated for a further period of 2 days at reflux. The reaction mixture was allowed to cool to room temperature and concentrated *in vacuo*. Residual material was purified by flash column chromatography on silica gel (petroleum ether-ethyl acetate, 3:2) which resulted in the recovery of starting material only.

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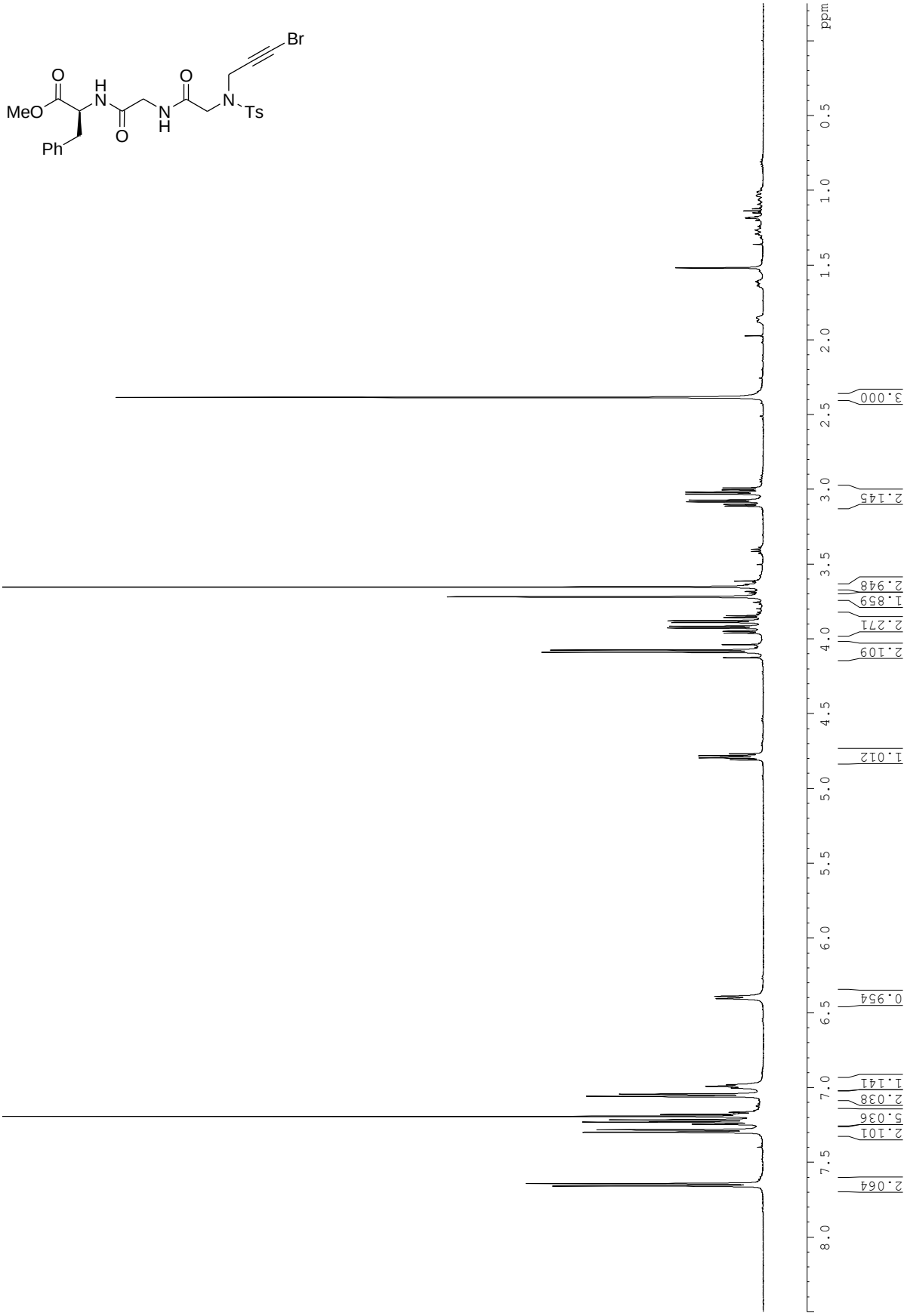
Appendices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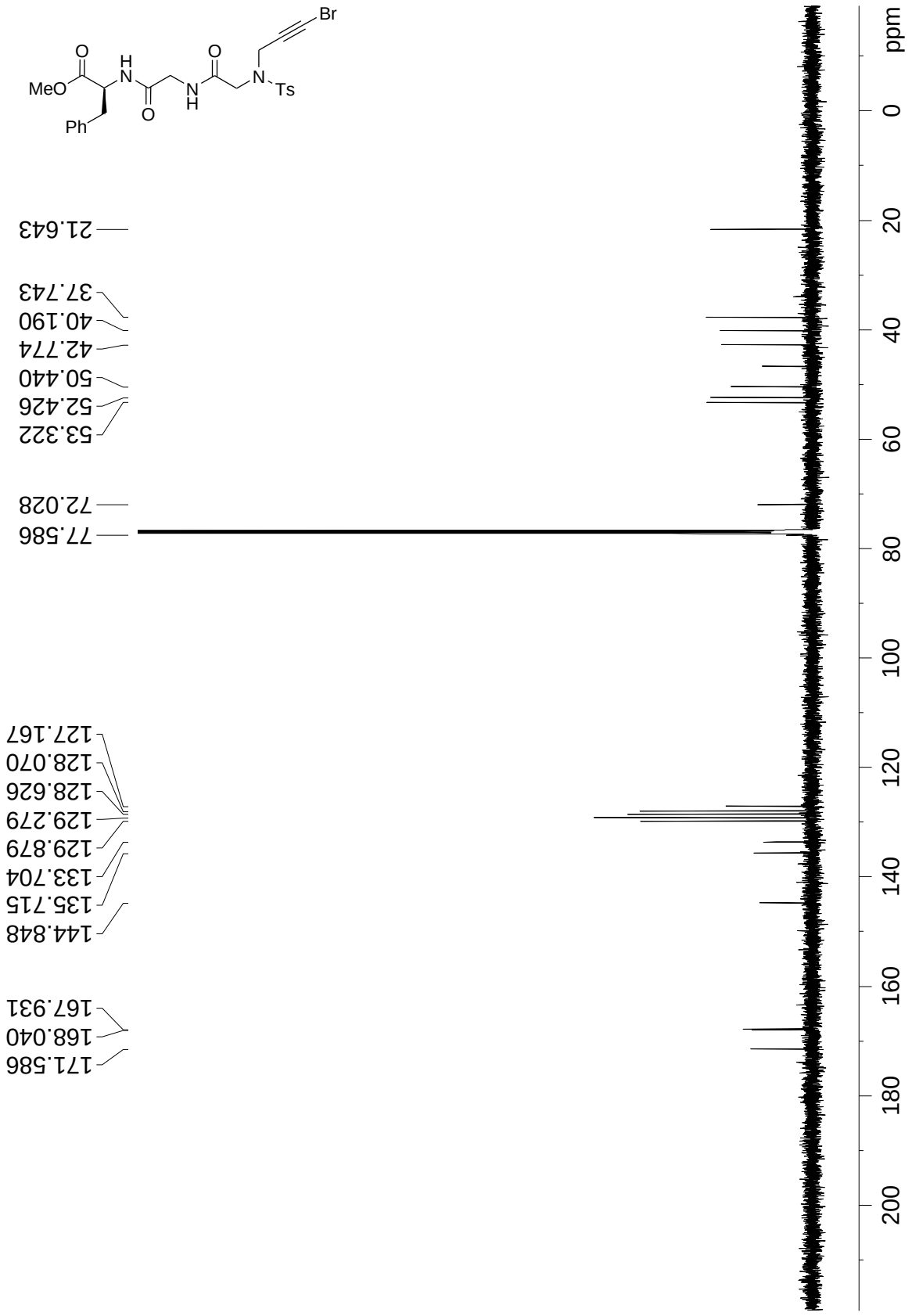
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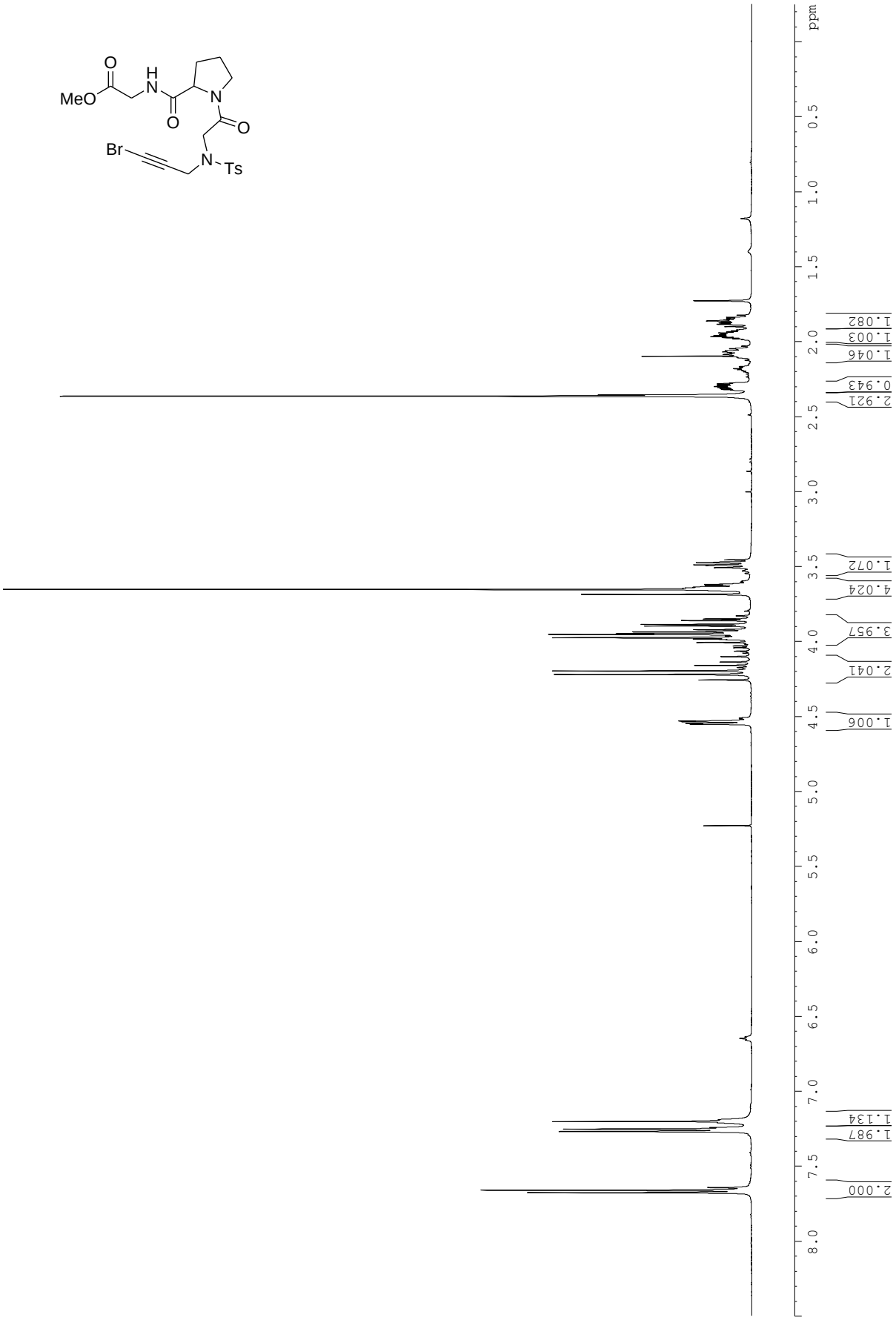
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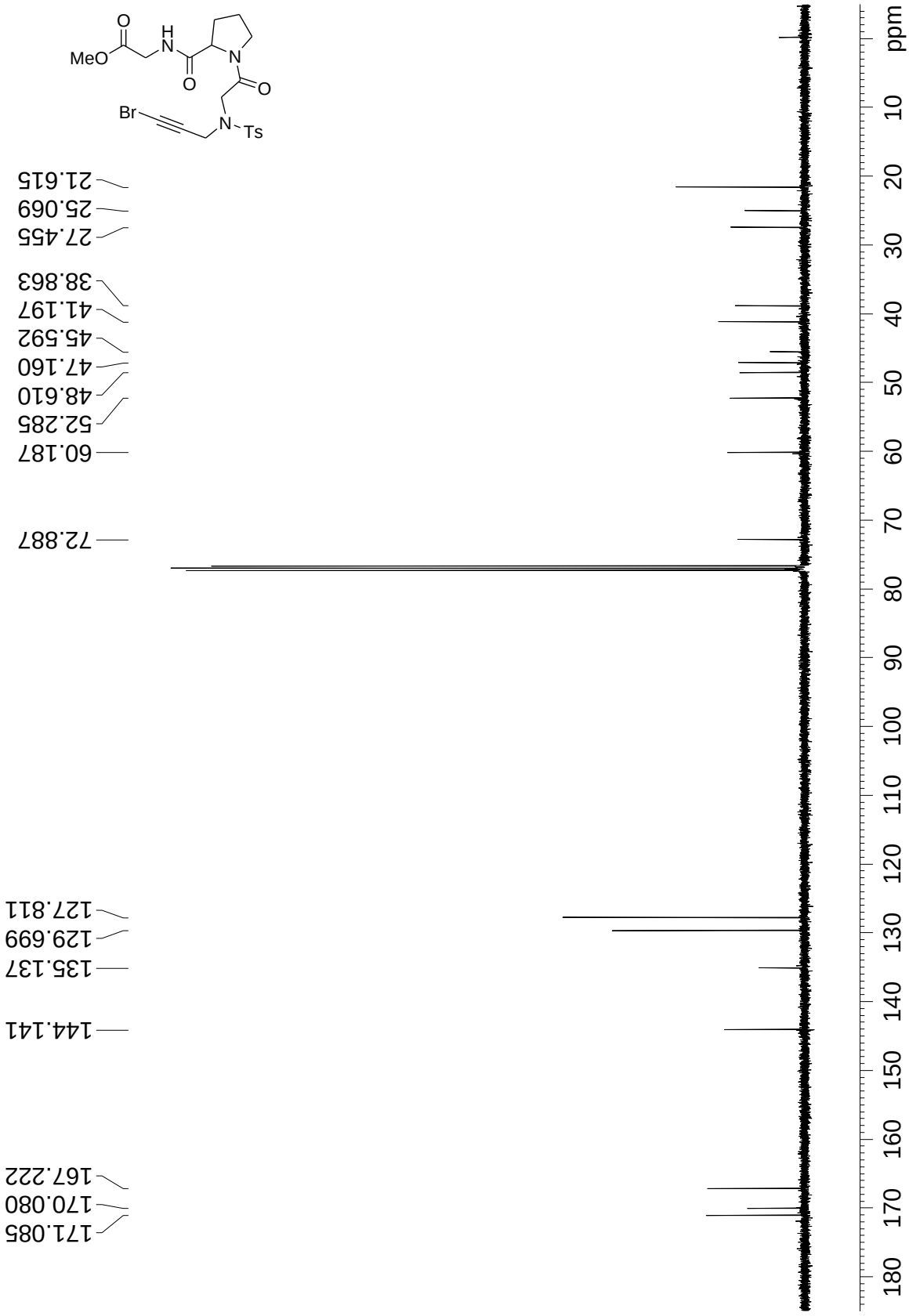
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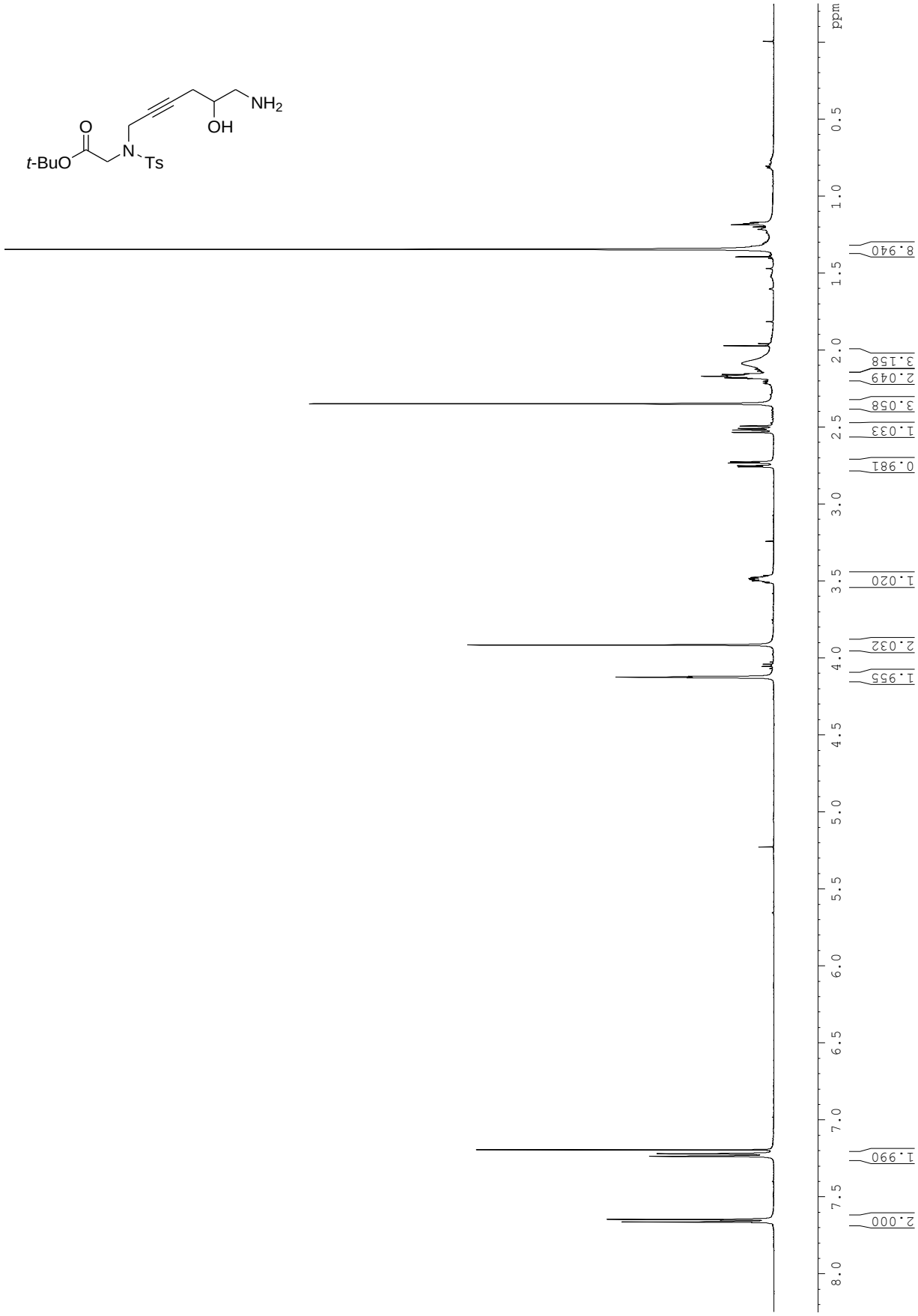
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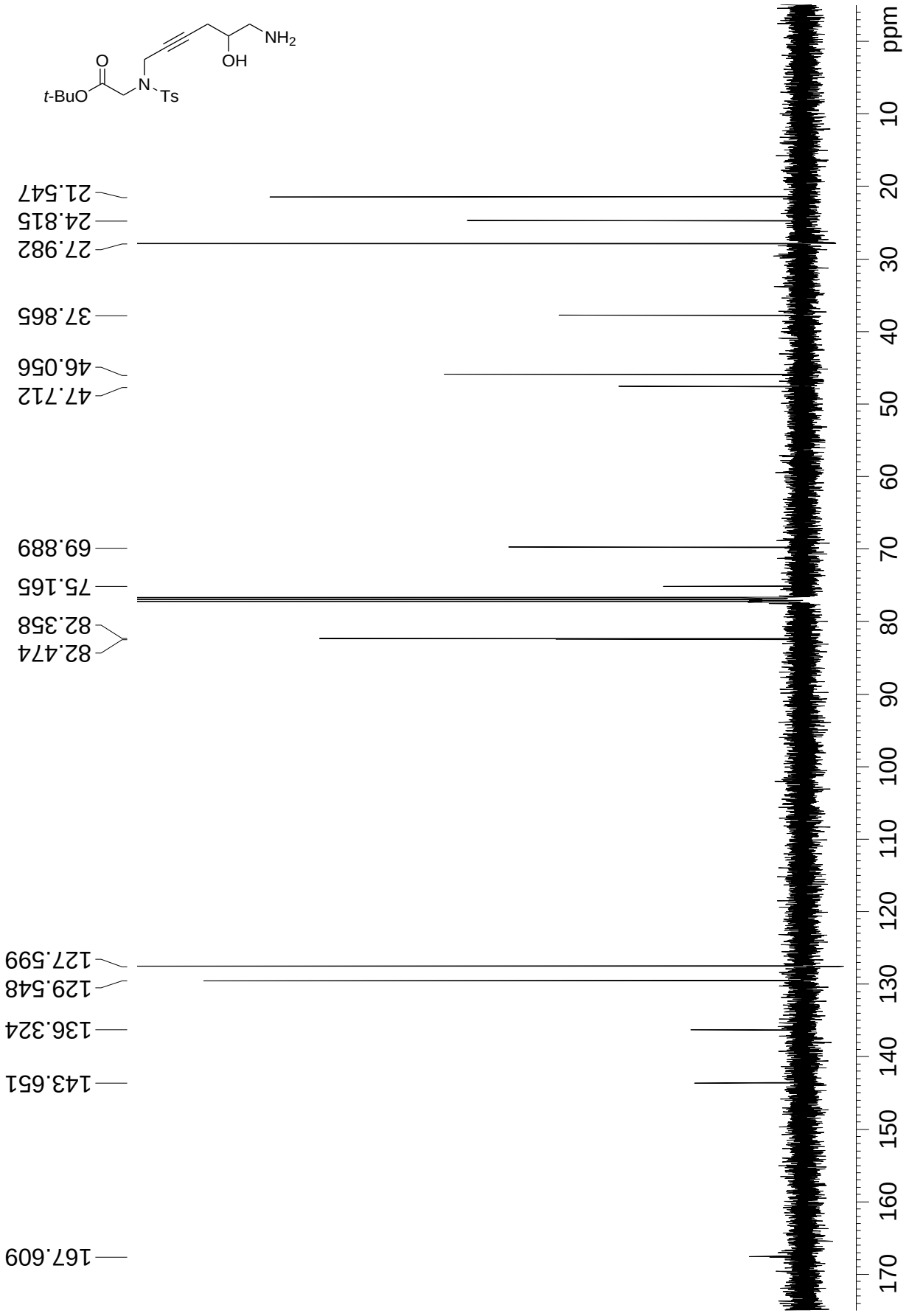
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