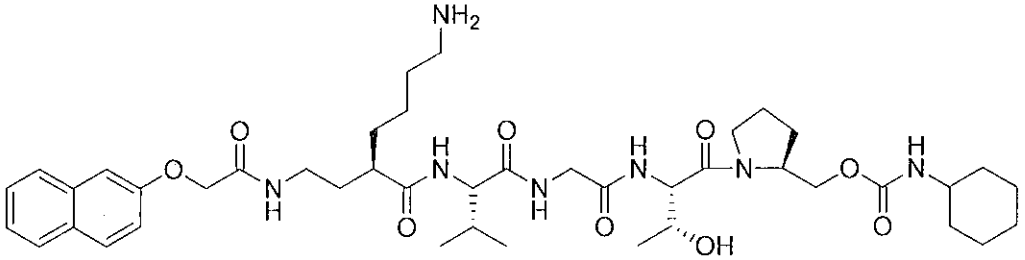
Addendum

Page 2: Compound **1.1** should have the following structure:



Page 3 para 1 line 1: "number of Type II" for "number Type II"

Page 3 para 3 line 4: "looking to convert" for "looking convert"

Page 4 para 1: The first line should read: "Since Arthur described the cycloaddition between alkynes and azides in 1893,[13a] which was further elaborated by Huisgen in 1963,[13b] it has been the most common method of synthesizing 1,2,3-triazoles."

Page 4 scheme 1.1: Change orientation of azide to "linear" from "bent"

Page 5 scheme 1.2: Change orientation of azide to "linear" from "bent"

Page 7 scheme 1.3: Change orientation of azide to "linear" from "bent"

Page 8 scheme 1.4: Change orientation of azide to "linear" from "bent"

Page 10 scheme 1.7: Remove the word "Conceptual"

Page 11 para 2 line 2: "configurations" for "conformations"

Page 12 para 2 line 1: "continued by Ghardiri and coworkers" for "continued by this group"

Page 15 scheme 1.10: Remove the word "Conceptual"

Page 19 para 1 line 2: "integrin inhibitor" for "integrase inhibitor"

Page 20 para 1 line 3: "access to a wide range" for "access to wide range"

Page 24 para 1 line 3 "inhibitor A-74704" for "inhibitor A-74705"

Page 26 para 1 line 4: "of Arora,[53]" for "of Arora, "

Page 29 para 1 line 7: "macrocyclisation" for "macrocyslisation"

Page 33 para 1: add the following sentence to the end of the paragraph: "An iterative synthesis would give access to a range of macrocyclic compounds, with the ability to easily moderate their properties by varying the 'R' groups on the starting materials used."

Page 40 para 1 line 6: "due to the harsh conditions" for "due on the harsh conditions"

Page 47 para 2 line 9: "in 65 % yield" for "in 65 %"

Page 48 para 4 line 6: "(2H, d $^2J_{PH}$ = 70 Hz)" for "(2H, d ^2J_P = 70 Hz)"

Page 55 para 2 line 8: "effected" for "affected"

Page 57 para 1 line 5: "tautomerisation process" for "elimination"

Page 57 para 2 line 1: "would have been a significant setback" for "would have been significant setback"

Page 67 para 3 line 7: "secondary alcohol" for "tertiary alcohol"

Page 69 para 2 line 4: "crude material, no further purification" for "crude material, and no further purification"

Page 71 para 3: final full stop should be after the word "dimethylformamide" instead of on its own line

Page 72 scheme 3.1: Wedge bonds should be small at the sulfur atom and large at the carbon atom

Page 77 para 2 line 4: "72 %" for "71.6 %"

Page 96 para 1 line 3: Remove the words "and corresponding"

Page 96 para 1: Include the following sentence at the end of the paragraph: "Although compound **4.5** was selected as the pre-cyclised linear pentamer, other alkynyl-azides could be used for cyclisation which would give access to an identical cyclised product, if the sequence of residues was kept constant. This particular linear compound was chosen as two of the required monomer units, alkyne **4.9** and alkynyl-azide **(R)-3.25**, were already in hand."

Page 106 para 1 line 8: "-23.1 °" for "-23.1 °C"

Page 110 scheme 4.18: Lysine side-chain protecting group have the "Phth" group connected to nitrogen, not carbon

Page 116 scheme 5.2: Arrows should be regular synthesis arrows, not retrosynthesis arrows

Page 118 para 2 line 3: "molecular ion (*m/z* = 559.2)" for "molecular ion (m/z = 559.2)"

Page 119 para 3 line 8: "2-Chlorotrityl chloride resin" for "Chlorotrityl resin"

Page 120 para 1 line 1: "2-chlorotrityl chloride resin" for "chlorotrityl resin"

Page 121 para 4 line 7: "scheme 5.7 below" for "scheme 5.6 below"

Page 122 para 1 line 7: "seen as a doublet of doublets" for "seen as a triplet"

Page 123 Scheme 5.11: Replace number "**5.30**" under the structure of benzyl azide with "**5.56**"

Page 123 para 1 line 2: "**5.56**" for "**5.30**"

Page 124 scheme 5.12: "propargylamine (**5.30**)" for "propargylamine (**5.31**)"

Page 128 heading 5.4.1: "derivative 5.34" for "derivative 5.33"

Page 128 scheme 5.15: "**(S,S)-4.13**" for "**4.7**" in both diagram and caption

Page 128 scheme 5.15: "derivative" for "deriavive"

Page 128 para 1 line 1: "**(S,S)-4.13**" for "**4.7**"

Page 128 para 1 line 4: "*N*-deprotection" for "Boc-deprotection"

Page 128 para 2 line 1: "*tert*-butyl ester" for "*tert*-buyl ester"

Page 130 para 2 line 9: "correspond to" for "corresponded to"

Page 131 para 1 line 3: "Rink amide resin" for "Rink resin"

Page 132 para 3 line 4: "2-chlorotrityl chloride resin" for "chlorotrityl resin"

Page 134 para 1 line 6: "2-chlorotrityl chloride resin" for "chlorotrityl resin"

Page 136 para 2 line 5: "suitable interactions with this region" for "suitable interactions with at this region"

Page 136 para 3 line 6: "synthesised via solid phase" for "synthesised in via solid phase"

Page 138 para 1 line 8: "synthesis of a phenylalanine" for "synthesis a phenylalanine"

Page 139 para 2 line 5: "ability to synthesise" for "ability to synthesis"

Page 141 para 2 line 1: "analyses were" for "analyses was"

Page 158 para 2 line 8: "amide **3.14**" for "amide 3.14"

Page 223 reference 13: Modified to reference 13a: "Huisgen, R., 1,3-Dipolar Cycloadditions. Past and Future. *Angew. Chem. Int. Ed. Engl.* **1963,** *2* (10), 565-598."

Page 223: Insert reference 13b: "Michael, A., Ueber die Einwirkung von Diazobenzolimid auf Acetylendicarbonsäuremethylester. *J. Prakt. Chem.* **1893,** *48* (1), 94-95."

Page 224 reference 9: "India" for "Inida"

Page 226 reference 47: "Chem. Commun." for "Chem. Commum."

The Synthesis of Triazole Peptidomimetics

Christopher Roderick Opie

B. Med. Chem. (Hons)

A thesis submitted in fulfilment of the requirements for the degree

of Doctor of Philosophy

Department of Medicinal Chemistry

Faculty of Pharmacy and Pharmaceutical Sciences

Monash University

2012

Table of Contents

Statement of originality

To the best of the author's knowledge and belief, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other institution, and contains no material previously published or written, except where due reference is made.

Christopher R Opie

Declaration

In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Candidate's signature: _____ Date: 27/11/2012

Acknowledgements

First and foremost, to Mum and Dad: without your support, this thesis and the work within would not have been possible. Your continued encouragement over the course of my time at Monash was invaluable, and I was very grateful to be able to share the highs and lows of the experience with you. Even if you did have a tendency to ask a lot of questions during our early Saturday morning breakfasts! I really appreciate everything that you have done for me, and I love you both very much. Also a big thank you to my extended family: Pen and Nick, Mark and Rocky, and Charlie and Matilda: you're all amazing.

To my supervisor, Jamie: we got there in the end! I've really enjoyed the time spent working under your supervision, I couldn't ask for a more encouraging, approachable and friendly mentor. Even when you were at your busiest periods during the year, you would still find time to discuss the successes and failures of our troublesome reaction sequences and cyclisations, and for that I am extremely grateful. I hope that, in return, you were happy to have someone who could actually stomach a beer in during our group outings – although as a credit to both of us, we managed to get Brad off the wagon! It only took around 4 years to do it.. Cy [Phe_4] probably got synthesised faster than that!

To the original members of my office: Tim, Will, Jen, Dee, Suze, Brittany and Brad: hanging out with you guys was the highlight of my time at uni. Whether it be discussing the finer points of English grammar, turning our office into a temporary kitchen, or playing snakes and ladders on a giant board with a giant foam die, I'm really thankful for our time together. You've become my closest friends.

To the past and current members of the Simpson group, of which there are too many to name: thank you for all the advice, support and encouragement I've received from all of you over the years. Although our tastes in music have often differed, it was always a very pleasurable environment to work in. I'd also like to thank Phil, Bim, Jase, Simon, David, David and Pete: your time and advice was much appreciated.

To the Med Chem cohort, some of whom I've grown up and matured with since we were little 1^{st} year students: we've really had some amazing times together, generally fuelled by a few drinks at beer club! Our somewhat strangely regimented 12:00 noon lunches (!), along with regular outings down Sydney Rd and Friday night dinners have certainly kept my sanity in check over the years. I wish you all the best, whether it be still in the fields of chemistry and biology, or (gasp!) doing something other than science!

To my other friends and family, people who have given me support and advice over the years, and those that I've forgotten to mention: I've already done the formatting for my thesis, so I'm restricted to keeping this message to one page. But I'm most grateful for having the opportunity to share my life with you guys over the years.

Abbreviations

Boc - *tert*-Butyloxycarbonyl

Cbz – Carboxybenzyl

CD - Circular Dichroism

CD4 - Cluster of Differentiation 4

Cp* - Pentamethylcyclopentadienyl

Cu-AAL - Copper-catalysed Azide-Alkyne Ligation

DBU - 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM - Dichloromethane

DIAD - Diisopropyl azodicarboxylate

DIEA - Diisopropylethylamine

DMF - Dimethylformamide

DNA - Deoxyribonucleic Acid

DOS - Diversity-Oriented Synthesis

DTF - Density Functional Theory

EDC - 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EE - Ethoxyethyl

ESI-MS - Electrospray Ionization Mass Spectrometry

Fmoc - Fluorenylmethyloxycarbonyl

FTIR - Fourier Transform Infrared Spectroscopy

HATU - 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate

HBTU - *O*-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate

HDACs - Histone Deacetylases

HPLC - High Performance Liquid Chromatography

IC_{50} – Half-Maximal Inhibitory Concentration

LCMS - Liquid Chromatography Mass Spectrometry

LDA - Lithium Diisopropylamide

MIC - Minimum Inhibitory Concentration

NMR - Nuclear Magnetic Resonance

NOE - Nuclear Overhauser Effect

PPTS - Pyridinium *p*-toluenesulfonate

PyAOP - (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

PyBOP - (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

ROE - Rotating Frame Overhauser Enhancement

ROESY - Rotating Frame Overhauser Enhancement Spectroscopy

STABASE - 1,1,4,4-Tetramethyldisilyl azacyclopentane

TBAF - Tetrabutylammonium fluoride

TBS - *tert*-Butyldimethylsilyl

TBTA - Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine

TFA - Trifluoroacetic acid

THF - Tetrahydrofuran

TIPS - Triisopropylsilyl

TLC - Thin Layer Chromatography

TMS - Trimethylsilyl

TsDPEN - *N*-Tosyl-1,2-Diphenyl-1,2-ethylenediamine

VO[acac] - Vanadyl acetylacetonate

Abstract

The natural synthesis of peptides and proteins occurs in an efficient iterative manner, and can be considered as a model for highly efficient chemical access to molecular complexity and functional materials. This process is mimicked by modern solid phase peptide synthesis, which can give good, controlled access to molecules with molecular weights above 5,000 Da. The reactions involved in efficient production of these large molecules are required to be high yielding and efficient processes. The Copper-catalysed Azide-Alkyne Ligation reaction (Cu-AAL) described simultaneously by the Sharpless and Meldal groups is also renowned as a high yielding and efficient reaction. Taking inspiration from these processes, we sought to produce triazole peptidomimetics, in which the amide bonds of peptides are replaced by Cu-AAL derived triazoles. While some approaches to this type of molecule have been published, these procedures are not truly general. We sought to devise a synthetic approach to the first cyclic variations of this class of compounds, as they may have interesting conformational properties, and catalytic, coordination, or materials applications.

Peptides can be visualised as a sequence of amino acid monomer units, which have been systematically coupled together. In a similar fashion, triazole peptidomimetics can also be viewed as a series of discrete alkyne and azide-containing units which have been linked through Cu-AAL. In order to facilitate the production of the triazole peptidomimetics, it was of particular importance to explore the synthesis of these monomer units. One method, building on literature precedent, utilised the chiral pool offered by amino acids in a sequence of functional group transformation reactions. While the synthesis was found to be successful for a test case consisting of phenylalanine-derived alkynyl- and azido- monomer units, the approach was not applicable to a wide range of substrates. In addition, the number of functional transformations required resulted in poor yields of the monomer units.

It was also investigated whether these molecules could be constructed from simple starting materials in a stereocontrolled fashion. A particularly promising reaction sequence utilised Ellman's chiral sulfinamide auxiliary, which allowed for the synthesis of analogous phenylalanine monomer units in fewer steps and higher yields. With an acceptable quantity of the phenylalanine-like monomer units in hand, iterative chain extension to linear triazoles was demonstrated in a high-yielding 2-step sequence. The tetramer, pentamer and hexamer peptidomimetics were derived into a form whereby head-to-tail cyclisation could be attempted. Successful cyclisation was seen for both the tetrameric and pentameric compounds, although the cyclic hexameric species was not able to be isolated.

With the knowledge that small cyclic triazole peptidomimetics were accessible via our reaction pathway, we were keen to pursue cyclic compounds that contained diverse chemical functionality. An existing biologically active cyclic pentapeptide was taken, and steps were taken towards the synthesis of its all-triazole analogue. To accomplish this goal, the monomer unit synthesis was adapted for the production of the relevant compounds containing side chains derived from arginine, glycine, aspartic acid and lysine. The 2-step chain extension sequence was performed using the diverse monomer units, giving a linear tetramer. Given the difficulties producing the intended monomer unit analogue of arginine, the synthesis was not completed.

We were curious to determine whether our monomer units could be used in the process of introducing a single triazole, acting as an amide bond surrogate, in established peptide sequences. A serine protease named granzyme B, involved in the apoptosis pathway, was taken as a target enzyme. Inhibitors of the protease were formed by effectively swapping the scissile amide bond of substrates of granzyme B with a triazole linkage, thereby denying the enzyme the means of turning over the molecules. Two compounds were produced via this strategy, which bound to human granzyme B with low millimolar IC_{50}'s in an isolated enzyme assay.

Chapter 1 - Introduction

1.1 Peptidomimetics

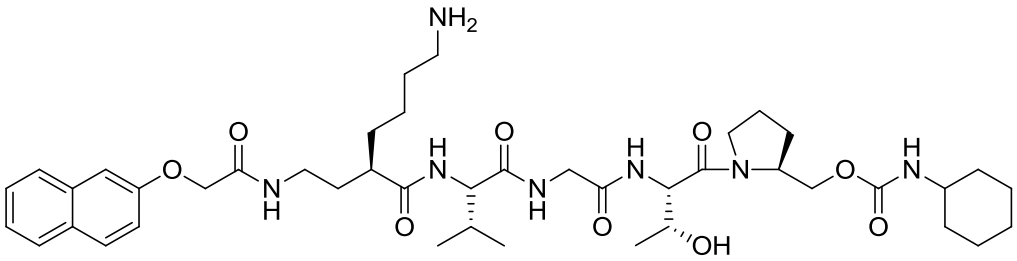
1.1.1 Peptides

The roles of peptides in organisms are very diverse - ranging from signalling molecules to antibiotics, and even analgesics.[1] Biological systems, however, make it difficult for potential peptide drugs to become mainstream. Their use is limited due to: cleavage by intestinal enzymes to di- and tri-peptides, hepatic clearance and poor permeability through cell membranes.[2] In addition, the flexibility of the peptide allows for an enormous number of possible conformations in solution. This decreases selectivity for the protein target, and may reduce the strength of peptide-protein interactions.

Two options are present for medicinal chemists in order to improve the success of peptide derived drugs. The first is traditional drug development, whereby lead peptide drugs are modified chemically in order to improve bioavailability and metabolic stability - while attempting to maintain bioactivity. The second, which this project is an example of, is using the features of a peptide - such as conformation, pharmacophores and electronic properties – and reproducing them on a non-peptidic scaffold. Hypothetically, they may be conformationally stable, resistant to enzyme degradation and better suited to penetrate cell membranes, while still specifically binding to the interaction surfaces of target proteins.[3]

1.1.2 Peptidomimetics

A peptidomimetic is defined as a non-peptidic compound that imitates the structure of a peptide in its receptor-bound conformation.[4] A notable example is the attempt to develop a drug-like molecule from gp120, a ~500 amino acid viral envelope glycoprotein, by Neffe *et al*.[5] The glycoprotein binds to human CD4 – an interaction that is involved in the infection of a human cell with HIV. A range of 85 gp120 peptidomimetics were designed and tested *in silico*; 11 of which were further tested via 2D NMR. The strongest binding affinities came from analogues that had all nonessential amino acids removed, non-peptidic linkages incorporated and hydrophobic residues attached.



1.1

Figure 1.1 A gp120 analogue which had an enzyme affinity of K_D = 35μM, *c.f.* lead peptide (NMWQKVGTPL) with affinity of K_D = 6 mM[6].

Three distinct classes of peptidomimetics have developed over time.[7] The first, sometimes called Type I, mimics the local topography of an amide bond via amide bond isosteres – for example as peptoids or oligocarbamates - or mimics secondary structure; commonly beta turns. Their backbone is closely related to the peptide, while retaining binding affinity with the target site.

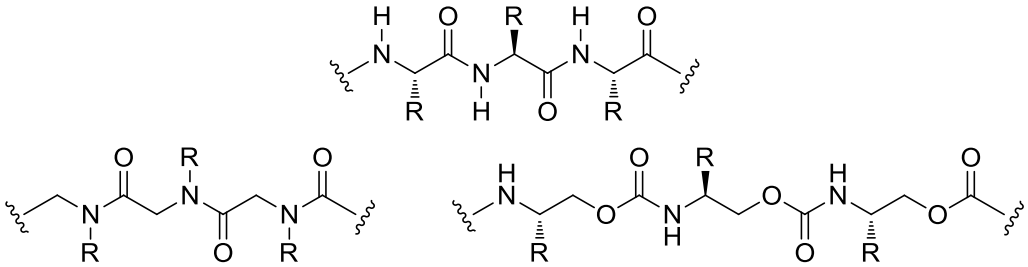


Figure 1.2 Comparison between peptide (top), peptoid (left) and oligocarbamate (right).

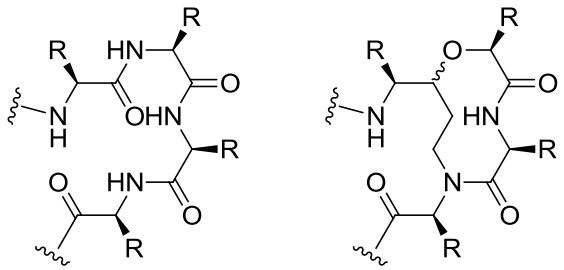
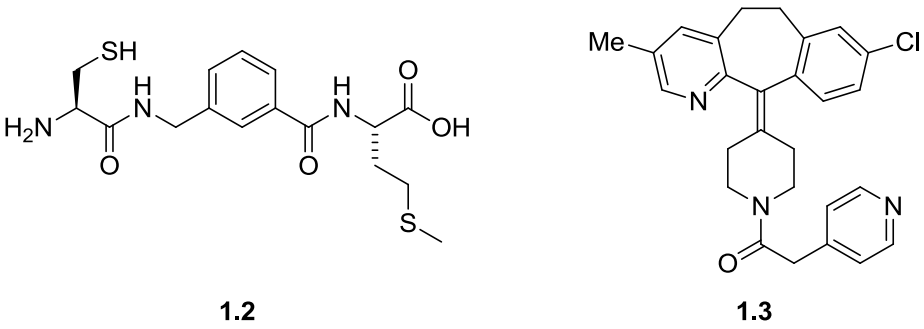


Figure 1.3 Beta turn (left) and conformationally restricted mimic (right).

The second, Type II, are functional mimetics rather than structural ones. They consist of small nonpeptidic molecules that bind to a peptide receptor. It has been shown through a

large number of Type II mimetics that binding does not always occur at the site of the parent peptide – binding to distinct sites is common.

Type III mimetics, ideal for small molecules, have the same spatial arrangement of important chemical groups without the use of a peptide backbone.



1.2 **1.3**

Figure 1.4 Type I (left) vs Type III Ras farnesyltransferase inhibitors.[7]

1.1.3 Amide Bond Isosteres

One branch of type 1 peptidomimetics mimic the local topography of an amide bond via amide bond isosteres. These isosteres were initially used in the stabilization of peptide hormones, and are largely dominated by an effort to improve the pharmacokinetics of potential drug candidates, essentially looking to convert good *in vitro* inhibitors into good *in vivo* inhibitors. This is brought about by replacement of the enzyme targeted amide bond with hydrolysis resistant moieties, or by removing proton donors and acceptors, leading to increased membrane permeability. Existing groups for amide bond replacements in use include thioamides,[8] esters,[9] simple olefins,[10] hydroxyethylenes,[11] and fluorinated ketones.[12]

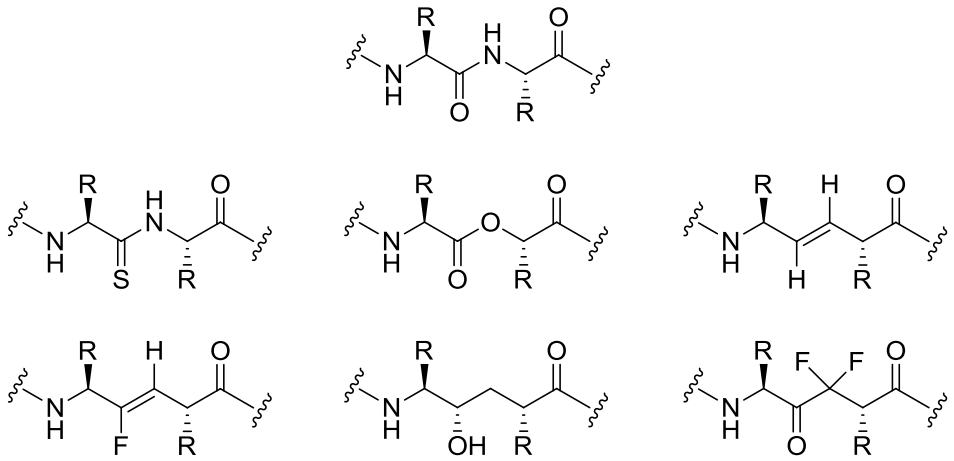


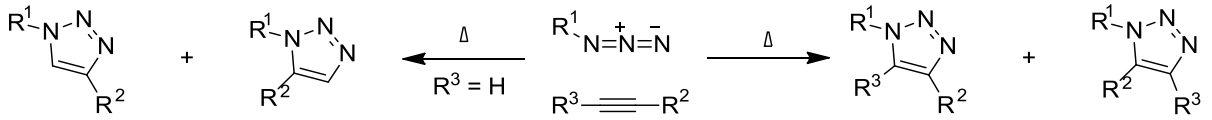
Figure 1.5 Thioamide, ester, alkene (top row) and fluoroalkene, hydroxyethyl and α-difluorokeone (bottom row), as compared to an amide bond (top).

An important consideration in amide bond isosteres is the change in geometry around the bond. Even a small change in a dihedral angle can greatly modify the interaction between peptide and protein.

1.2 Triazoles

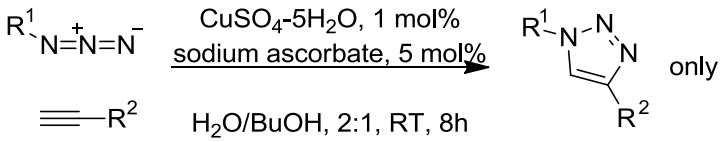
1.2.1 Original Synthesis and Copper modification

The first line should read: “Since Arthur described the cycloaddition between alkynes and azides in 1893,[13a] which was further elaborated by Huisgen in 1963,[13b] it has been the most common method of synthesizing 1,2,3-triazoles. The major drawback of this reaction was the lack of regioselectivity – that is, when terminal alkynes are used, the products are a mixture of both *trans* (1,4-triazole) and *cis* (1,5-triazole) regioisomers. Similarly, when non-terminal alkynes are used for the production of tri-substituted triazoles, a mixture of regioisomers is seen.



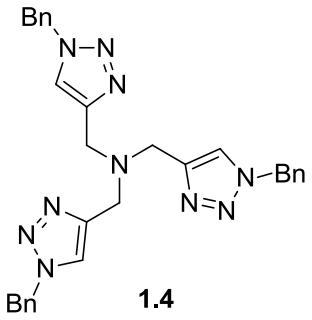
Scheme 1.1 Huisgen cycloaddition between an alkyne and an azide, resulting in a mixture of triazole regioisomers.

Meldal and Sharpless simultaneously reported a copper(I) catalysed version of the reaction in 2002 (copper catalysed azide-alkyne ligation, or Cu-AAL) with terminal alkynes and azides, which led to selective formation of the trans regioisomer.[14, 15] This reaction is able to be conducted in ambient conditions (room temperature), in a variety of solvents including water. It is tolerant to a wide range of functional groups and is generally high yielding.[15]



Scheme 1.2 Copper catalysed azide-alkyne ligation (Cu-AAL).

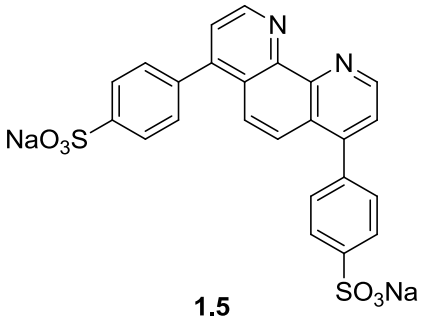
However the general thermodynamic instability of copper(I) - whereby it can be oxidized to copper(II) or disproportionated to copper(0) and copper(II) - may cause variable yields.[16] While the catalytic copper(I) species in the reaction may be introduced via copper(I) salts, it is commonly generated *in situ* via the reduction of a copper(II) species using a reductant such as ascorbate. Chan *et al.*[16] trialled a range of ligands to act as transition metal stabilising agents, and found *tris*-(benzyltriazolylmethyl)amine (TBTA, **1.4**) to improve the rate of the reaction. Inclusion of this ligand in the reaction mixture when performing Cu-AAL is thought to improve yields by enveloping the copper(I) centre, leaving no free binding sites for potential destabilising interactions. Interestingly, **1.4** is formed by a self-catalysed reaction between benzyl azide and tripropargylamine.



1.4

Figure 1.6 *tris*-(Benzyltriazolylmethyl)amide (TBTA).

Further improvements include the use of resin-immobilised copper catalysts[17] or resin-immobilised TBTA,[18] as well as more polar TBTA variants,[19] in addition to the water soluble sulfonated bathophenanthroline (**1.5**) – shown to have superior catalytic ability to TBTA in dilute aqueous solutions.[20]



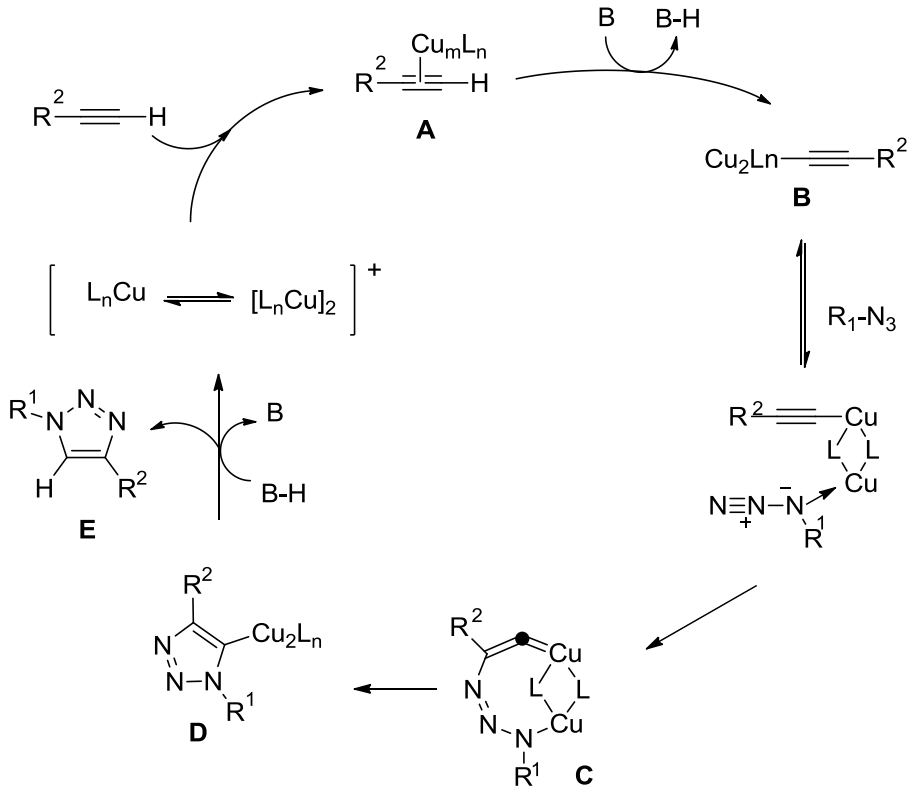
1.5

Figure 1.7 Sulfonated bathophenanthroline.

The ease of Cu-catalysed triazole formation has spurred on a variety of applications in many fields, ranging from material science such as in peptide nanotube formation,[21] to macromolecular chemistry involving the synthesis of dendrimers.[22] The reaction has also been applied to more traditional fields in biology or medicinal chemistry such as β-turn mimics,[23] and peptide chain analogues.[24] Recently, in 2006, Sharpless *et al.* were able to use the reaction as a key diversification point in the synthesis of an HIV protease inhibitor with K_i = 1.7 nM.[25] As a result of the rapid uptake and wide application of this reaction, there have been several reviews documenting the use of the reaction, and the use of triazoles in general, for many different applications.[26, 27, 28]

1.2.2 Mechanism

The mechanism of this copper catalysed variant of the Huisgen triazole synthesis has since attracted much attention.[29, 30] Direct concerted cycloaddition of copper-acetylide π-complexes with appropriate azides (copper catalysed alkyne-azide cycloaddition; Cu-AAC) was not supported by DFT calculations nor kinetic studies.[30] Rather, experiments exploring stepwise processes gave evidence to suggest that they would require lower activation barriers (copper catalysted alkyne-azide ligation; Cu-AAL). A putative mechanism is outlined below.[28]

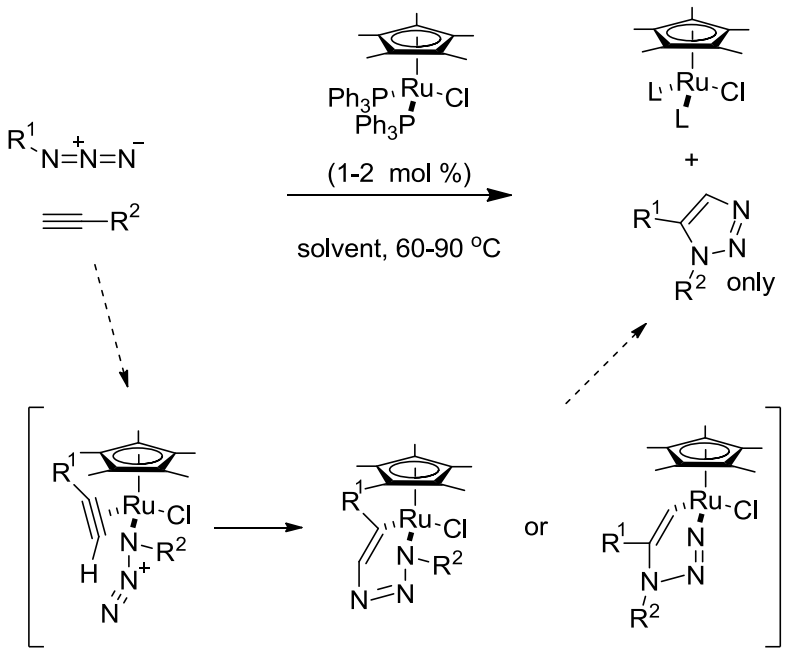


Scheme 1.3 Proposed mechanism of copper-catalysed azide-alkyne ligation.

After activation via formation of a copper-alkyne π complex (**A**), the copper acetylide species **B** is produced. An azide couples to the copper complex, forming the 8-membered ring structure **C**. The ring collapses giving triazole **D**, and in the presence of base (or water) the copper catalyst is recovered, with the concurrent formation of the 1,4-triazole (**E**).

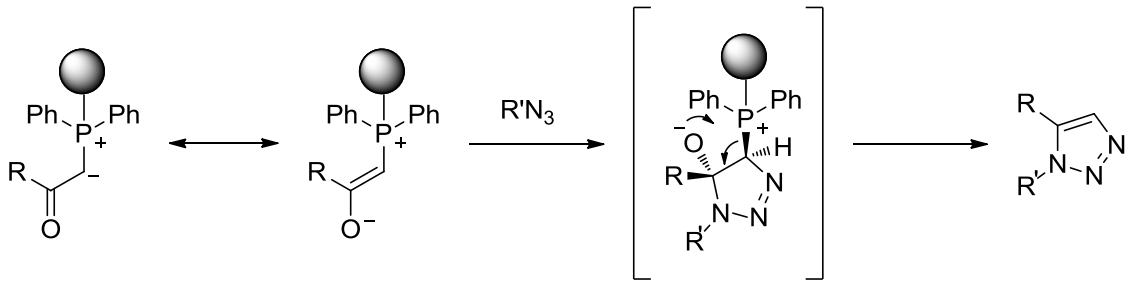
1.2.3 Synthesis of 1,5-Triazoles

Since the discovery of the Cu-catalysed 1,4-triazole formation, there have been similar attempts to selectively access the 1,5-triazole regioisomer. Zhang investigated the use of ruthenium catalysts,[31] due to their prior use in catalytic transformations of alkynes.[32] Several Cp*-based ruthenium-chloride complexes were shown to regioselectively form the 1,5-triazole, albeit at elevated temperatures. Due to a different reaction mechanism to the copper catalysed case, outlined in scheme 1.4 below, the reaction can be applied to internal alkynes as well as terminal alkynes.



Scheme 1.4 Proposed mechanism of ruthenium-catalysed azide-alkyne ligation.

Ahsanullah *et al*.[33] were investigating the possibility of synthesising 1,5-triazoles without the use of the potentially toxic ruthenium-based complexes. Their strategy involved the synthesis of a peptidyl phosphorane, which in the presence of an azide would undergo a cycloaddition, followed by cleavage from the resin. This process thus requires a solid support, and produces only 1,5-triazoles.

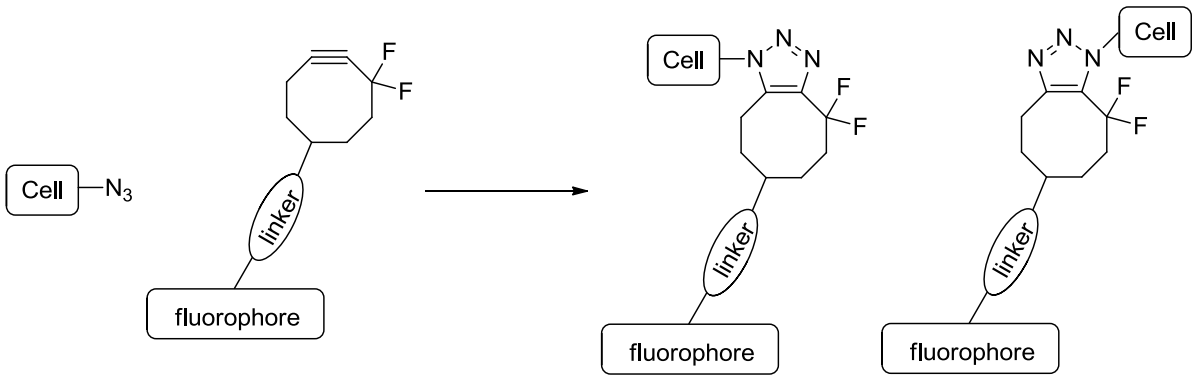


Scheme 1.5 1,5-Triazole formation via the catalytic cleavage of azidopeptidylphosphoranes.

1.2.4 Metal-Free Triazole Synthesis

Although it displays many desirable qualities, the Cu-AAL reaction is limited for use in living systems due to the toxic effects of the copper catalyst. For this reason, Baskin *et al*.[34] developed a copper-free triazole formation reaction, using a strained cyclooctyne system. After labelling a cell with azide tags, a cyclooctyne probe with a fluorophore linker was

introduced which was found to readily couple to the cell, albeit producing a mixture of regioisomers. They later showed that the reaction could be performed in living mice.



Scheme 1.6 Introduction of a fluorophore to an azide-labelled cell using a strained cyclooctyne triazole formation reaction.

1.3 Use of Triazoles as Peptidic Structural Mimics

1.3.1 Triazoles as cis- and trans- Amide Bond Isosteres

Due to similar structural and electronic properties,[26, 35] in addition to that fact that they are proteolytically and metabolically stable, triazoles show significant promise as amide bond isosteres. They are planar, possess a strong dipole, and contain both a hydrogen donor and acceptor. One fundamental difference between the 1,4-triazole and a native *trans*-amide bond is the distance between the side-chains: this distance is elongated by 1.2 A (~25 %), although this discrepancy is less evident in the 1,5-triazole *cis*-amide mimic.

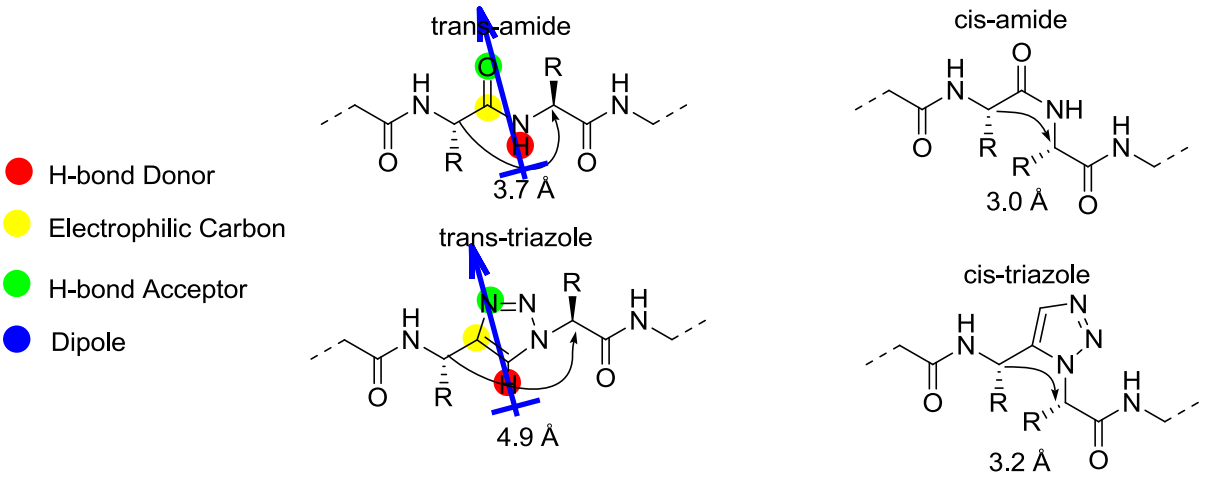
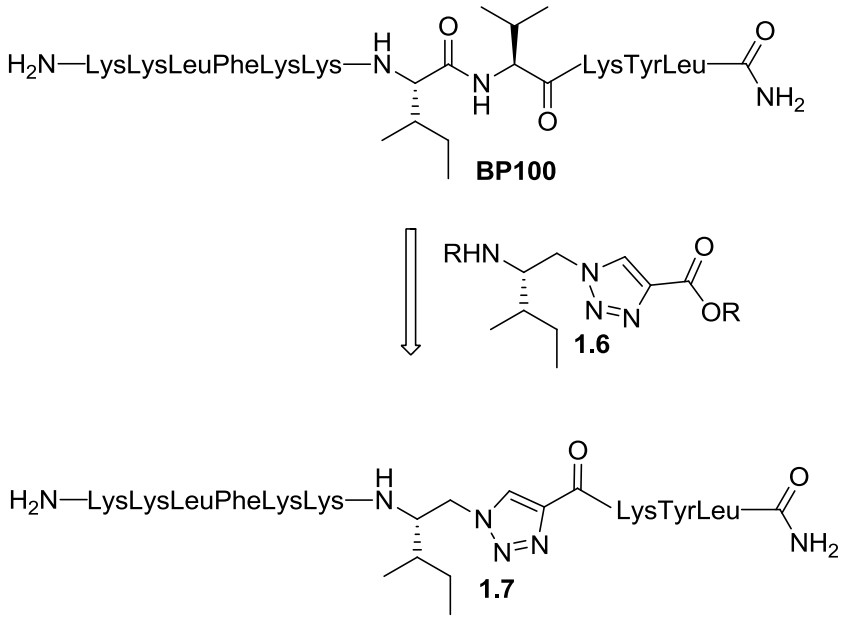


Figure 1.8 Comparison of the physical properties of *trans*- and *cis*- amides and triazoles.

There are many cases in the literature detailing evidence to support the use of the 1,4-triazole as a *trans*-amide mimic. In one example, a known antimicrobial peptide, BP100 (LysLysLeuPheLysLysIleLeuLysTyrLeu-NH_2) was modified by Güell *et al*.,[36] who prepared peptidotriazoles by the replacement of either Ile7-Leu8 or Leu8-Lys9 with the Ile-derived 1,4-triazole unit **1.6**. The reasons for incorporating the triazole into the peptide backbone were not explicitly stated, other than to 'further improve the biological profile of BP100'. It was found that although the compounds in general displayed either similar or lower activity than the parent compound, with MIC values > 6.2μM across a range of pathogenic bacteria and fungi compared to < 6.2 μM for the parent, they were substantially more resistant to enzyme degradation.



Scheme 1.7 Replacement of the Ile-Leu dimer from BP100 with triazole-containing unit 1.6.

In the first investigation of the use of triazoles as *cis*-amide bond isosteres, Tam *et al*.[37] synthesised mimics of RNase A, substituting key resides with 1,4- or 1,5-triazoles. It was proposed that Xaa-1,5-triazole-alanine would be able to act as a Xaa-*cis*-Proline dipeptide analogue, thus Asn_{113}-Pro_{114} of bovine pancreas-derived RNase A, and Ala_{113}-Pro_{114} of *E.coli*-derived RNase A were replaced with the equivalent 1,4- or 1,5- triazole dimer. The synthesis was a two-stage process, with the protected dimers synthesised with copper or ruthenium catalysed azide-alkyne ligation reactions, followed by incorporation into the peptides by solid phase synthesis and protein ligation. As shown by circular dichroism spectroscopy, the secondary structure of the semisynthetic proteins was consistent with the

native proteins, and the structures retained their catalytic activity. While both the 1,4-triazole and 1,5-triazole mimics were catalytically competent, the 1,4-triazole mimics gave lower T_m values compared to the 1,5- and wild type compounds, which suggested that the 1,5-triazole was more stable, and thus better suited for mimicking the *cis*-prolyl bond. Given that the Xaa-Pro *trans*/*cis* ratio is highly dependant on the nature of Xaa, displaying only 10-40 % of the *cis* isomer naturally, the use of a synthetic 1,5-triazole-alanine mimic would eliminate any uncertainty in the geometry of the bond.

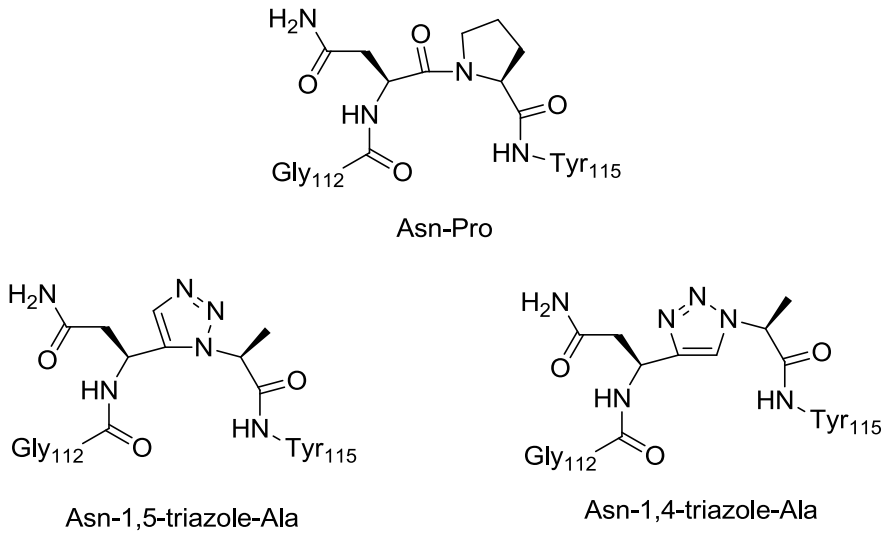
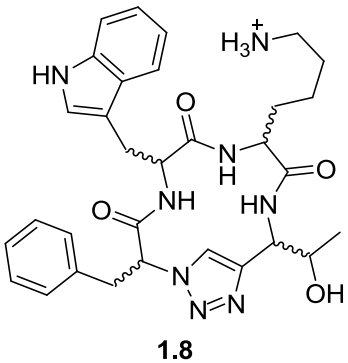


Figure 1.9 Replacement of the Asn-Pro dimer with 1,5- or 1,4- triazole linked alanine residues.

Exploiting the use of 1,4-triazoles as *trans*-amide bond mimics, Beierle *et al*.[38] synthesised and analysed a library of all 16 possible configurations of the somatostatin pharmacophore Cy [Phe-Trp-Lys-Thr], with the 1,4-triazole replacing the Phe-Thr amide bond (**1.8**).

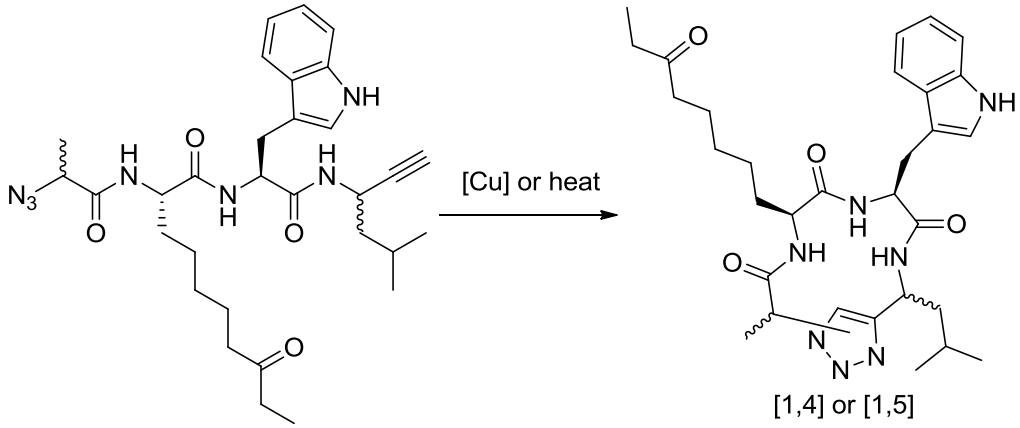


1.8

Figure 1.10 Somatostatin pharmacophore analogue containing a 1,4-triazole replacement of the Phe-Thr amide bond.

With just one amide bond replaced with a triazole, the ^{1}H NMR spectra of the compounds displayed consistently sharp signals, suggesting conformational homogeneity - which is generally not seen with peptides due to the isomerisation of amide bonds. The compounds were assessed by their binding efficiency to a panel of somatostatin receptors ($hSSTR_{1-5}$) and it was found that the constitutional isomers did not bind equally, with conformations showing different selectivities against the receptor subtypes, albeit with poorer activities than the parent cyclic peptides.

The study of triazole-containing cyclic peptide mimics was continued by Ghadiri and coworkers,[39] who incorporated triazoles into Apcidin, a naturally occurring cyclic tetrapeptide inhibitor of histone deacetylases (HDACs), in order to lock the compounds in a defined regiochemistry. The mimics of Apcidin were formed via a head-to-tail triazole-forming cyclisation of tetrapeptide **1.9**, with copper catalysis used for synthesis of the 1,4-triazole mimics, and microwave irradiation at elevated temperatures providing a mixture of 1,5- and 1,4- triazole mimics.



1.9 **1.10**

Scheme 1.8 Head-to-tail cyclisation of tetrapeptide 1.9 via triazole formation.

While natural Apcidin is present as an 80% t-t-t-t (all-*trans*) conformation and 15% c-t-t-t conformation mix, the synthetic compound mimicking c-t-t-t (**1.13**) was found to consistently bind more strongly across the HDAC (Histone deacetylase) enzyme panel than the corresponding t-t-t-t mimic (**1.14**). These studies show the utility of triazoles as either *trans*- and *cis*- amide bonds surrogates.

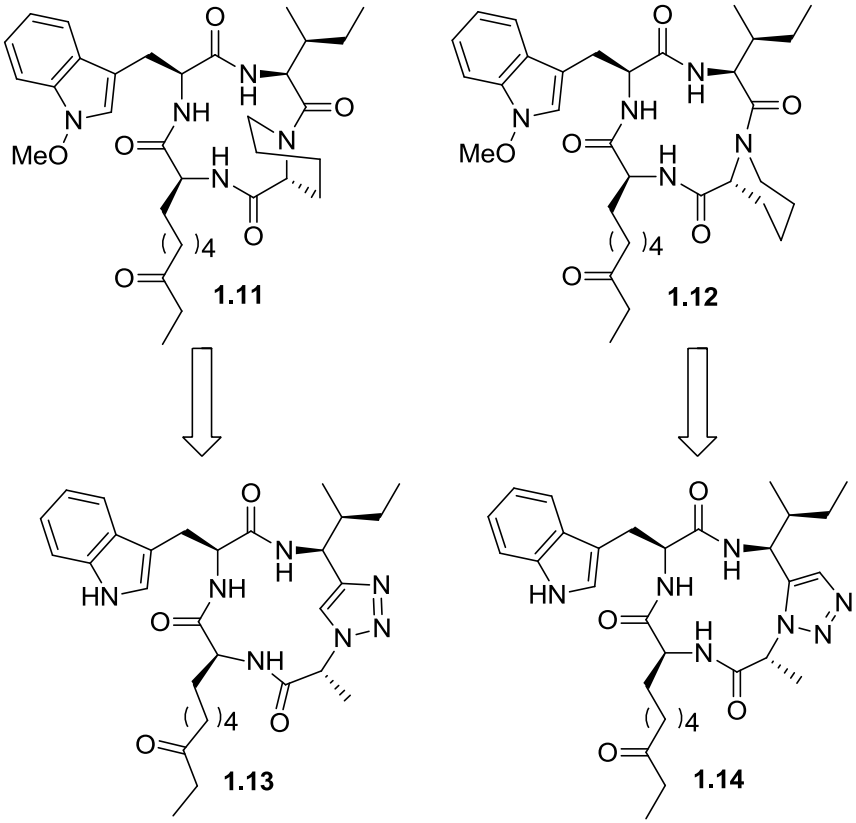


Figure 1.11 Apcidin structures showing *cis-trans-trans-trans* (1.11) conformation and all-*trans* conformation (1.12), with the respective triazole mimics 1.13 and 1.14.

1.3.2 Use of Triazoles in Mimics of Secondary Structures

Horne *et al*.[40] suggested that the addition of a triazole could help change or influence the secondary structure of peptides or proteins. With the inclusion of the triazole as a dipeptide surrogate to form mimics of pLI-GCN4, an α-helical coiled coil, it was shown via X-ray crystallography and CD that the mimics displayed the native α-helical character, with the position of triazole affecting thermodynamic stability. The derived compounds were seen to have either increased or decreased stability compared to the parent compound, depending on the location of the dipeptide surrogate.

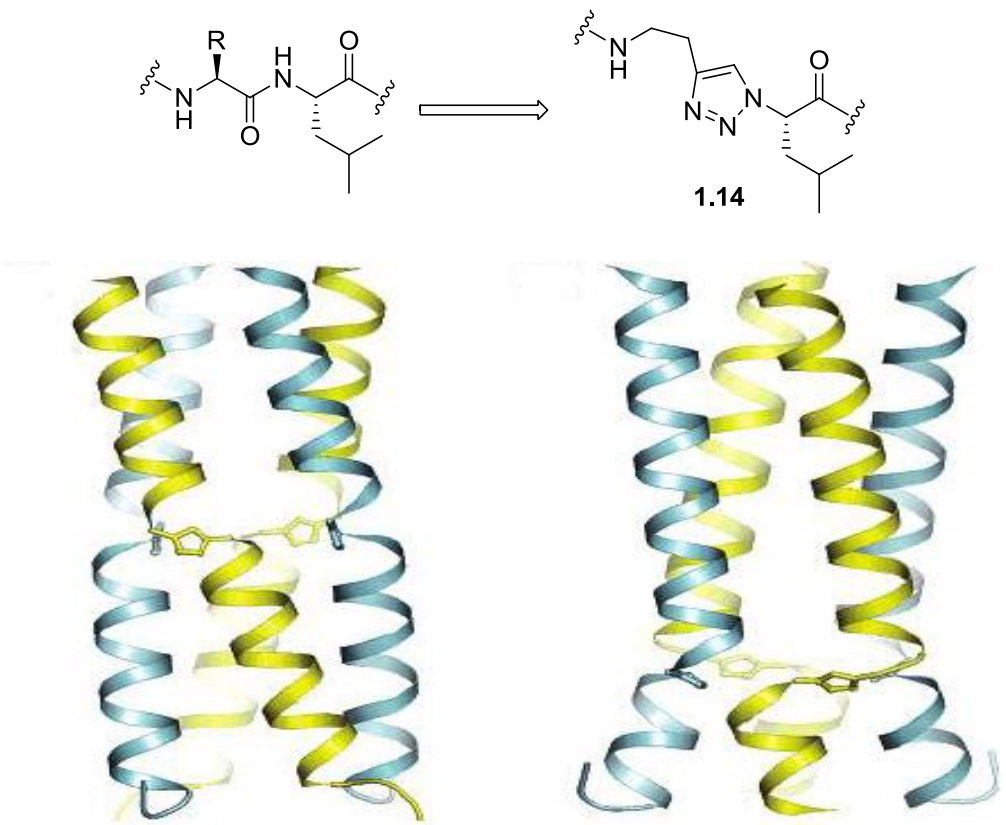
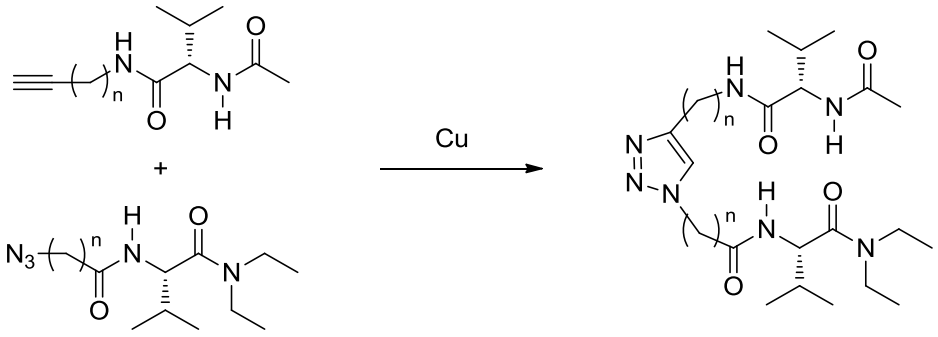


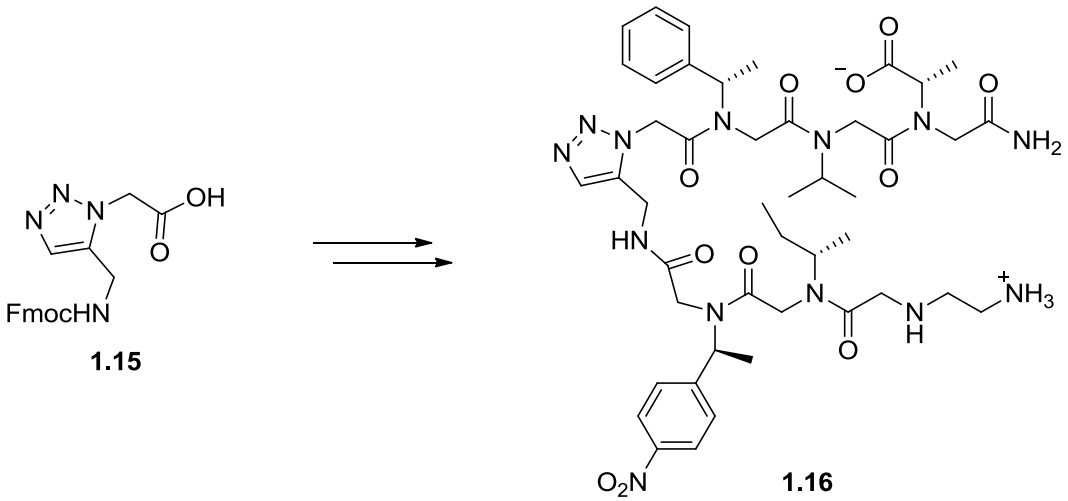
Figure 1.12 Horne's triazole-based dipeptide mimic (1.14, top) and crystal structures of tetrameric coiled coils containing the dipeptide mimics. Reprinted with permission from Horne *et al.*[40]Copyright 2003 American Chemical Society.

Triazole formation was used by Oh *et al*.[41] to give divergent access to β-turn mimics. They coupled short peptide strands by Cu-AAL, and observed the conformations by NMR and FT-IR techniques. By changing the linker lengths between the peptide strands and the 1,4-triazole, they were able to modify the extent of inter-strand hydrogen bonding.



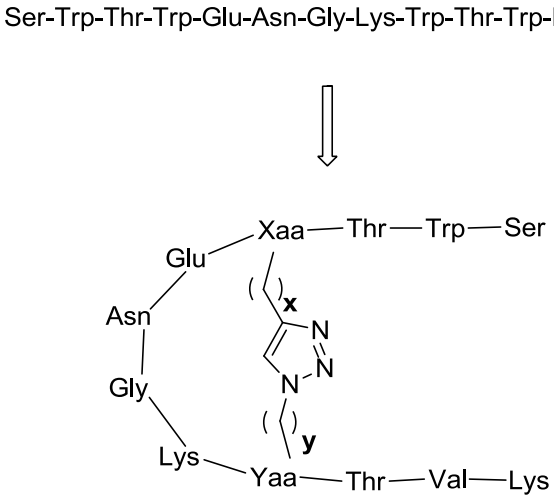
Scheme 1.9 Coupling of azide and alkyne-containing peptide strands leading to a β-turn mimic.

Pokorski *et al*.[42] showed that incorporation of a 1,5-triazole could induce turn formation in peptoid backbones. They synthesised a short peptoid that included triazole **1.15**, and judged the secondary structure to be a hairpin turn by CD and NMR techniques.



Scheme 1.10 Incorporation of 1,5-triazole-containing amino acid 1.15 into a peptoid resulting in a hairpin turn-like structure.

A similar strategy was used by Celentano *et al*.,[43] in this case aiming to stabilize β-hairpin conformations of a known peptide in an effort to increase its *in vivo* stability. Using the trpzip2 peptide (Ser-Trp-Thr-Trp-Glu-Asn-Gly-Lys-Trp-Thr-Trp-Lys-NH_2) as a model system, two of the residues (Trp_4 and Trp_9) were replaced with side-chain containing alkynes or azides of varying length, 6 residues apart. In addition, Trp_{11} was substituted with a valine residue, due to potential stabilisation in the parent peptide with Trp_2. After coupling with standard Cu-AAL conditions the compounds were analysed by 1D and 2D NMR techniques, which involved comparing the level of the amide bond chemical shift dispersion of the key glycine (Gly_7) turn residue with the linear peptide. The triazole-containing bridge was found to stabilise the β-hairpin, with the number and position of methylene groups present on the triazole-containing bridge observed to have an impact on the level of stability seen.



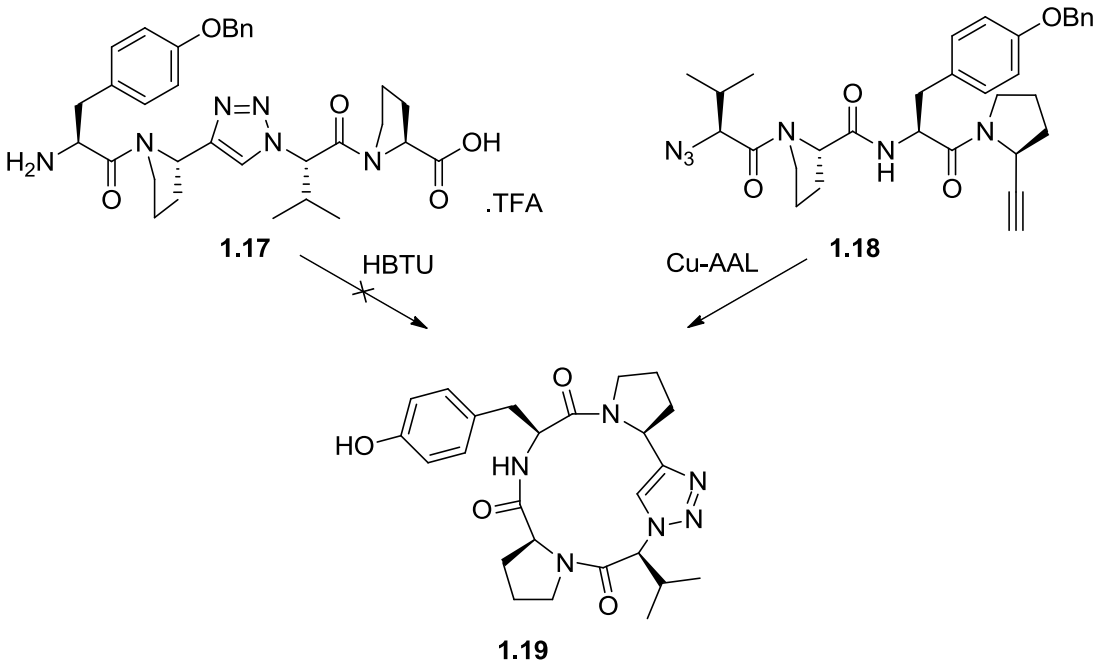
Scheme 1.11 Formation of β-hairpin via the replacement of amino acid residues with alkyne and azide-containing amino acids, and subsequent triazole formation.

Empting *et al*.[44] were interested in determining whether the disulfide bond between residues Cys_3 and Cys_{11} in the sunflower trypsin inhibitor-I (SFTI-1[1,14]) could be replaced with a triazole. To this end, Cys_3 was replaced with either L-β-azidoalanine or L-γ-azidohomoalanine; and Cys_{11} was replaced with propargylglycine. Triazole formation was performed on-resin with either standard Cu-AAL conditions or with a ruthenium catalyst system, to produce the 1,4- and 1,5- triazole regioisomers respectively. It was found that the 1,5- triazole replacement of the disulfide bond had comparable activity to the parent compound (1.6 x relative activity), whereas the 1,4- triazoles showed a large decline in activity. The control non-cyclised peptides did not show any inhibitory activity. The reaction was suggested as a possible replacement for oxidative coupling between cysteine residues, in cases where the reaction may be difficult due to a large number of cysteine residues.

1.3.3 Triazole Formation as Cyclisation Aids

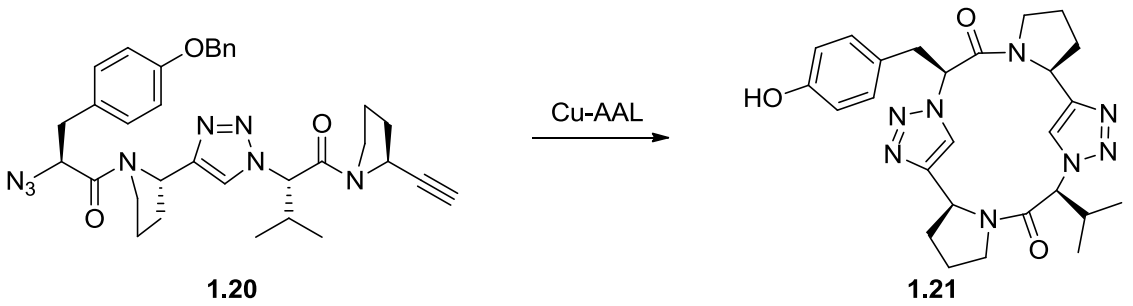
Bock *et al*.[45] investigated the use of the Cu-AAL reaction for the key cyclisation step during the synthesis of a triazole-containing analogue of [*cyclo*-[(L)Pro-(L)Val-(L)Pro-(L)Tyr], where traditional methods of head-to-tail cyclisation had previously failed. They synthesised two linear tetrapeptides: **1.17**, which contained a triazole moiety in place of the central amide bond, which was to be cyclised by traditional peptide bond formation chemistry; and **1.18**, which contained a *C*-terminal alkyne and *N*-terminal azide, with cyclisation to be performed via Cu-AAL. Cyclisation of **1.17** was not achieved, with EDC, HATU and PyAOP reaction

conditions only resulting in dimers and higher oligomers. Various copper-containing catalyst conditions were trialled for the cyclisation of **1.18**, with a mixture of CuBr and DBU in refluxing toluene giving the cyclised product in 70 % yield. No product was seen with the traditional $CuSO_4$/ascorbate catalyst conditions, and they noted significant iodotriazole formation when copper iodide was used.



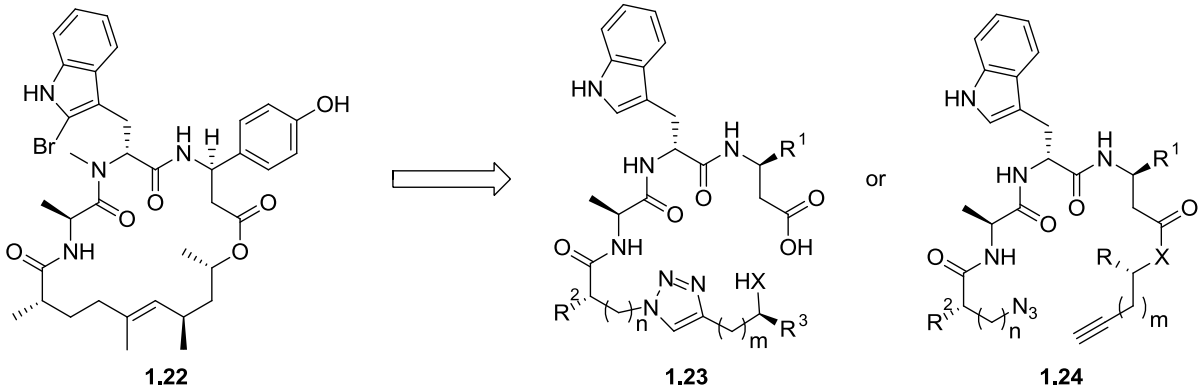
Scheme 1.12 Attempted head-to-tail cyclisation of tetrapeptides 1.17 and 1.18 via either peptide coupling conditions (left) or Cu-AAL (right).

They further investigated the synthesis of these compounds, with the cyclisation of **1.20** (below) resulting in a cyclic mimic of the parent tetrapeptide containing 2 triazoles.[46] They noted that the cyclisation of **1.20** was complicated by the formation of a complex mixture of side-products, and precipitation rather than chromatography was used to purify the desired product. IC_{50} values for the tyrosinase acivity of **1.19** (above) and **1.21** were determined to be 0.6, and 1.6 mM respectively, equal or better than the parent compound (1.5 mM).



Scheme 1.13 Head-to-tail cyclisation of triazole-containing tetrapeptide 1.20 with Cu-AAL.

Cu-AAL was investigated by Hu *et al*.[47] as a substitute for macro-lactonization to provide triazole-containing mimics of Jasplakinolide (**1.22**). A key *E*-substituted alkene was to be replaced by a 1,4-triazole, given the similar structural properties. Thus linear mimics **1.23** and **1.24** were synthesised, and macrocyclisation was performed using either lactonization conditions (for **1.23**) or a CuI-based catalyst system (for **1.24**). In general, the yields for the Cu-AAL-based strategy were higher than those seen for lactonization, with yields between 65-92 % compared with 28-58 %. Biological assay data was not reported for these compounds.



Scheme 1.14 Conceptual replacement of the *E*-alkene of Jasplakinolide (left) with a triazole either by lactonization (middle) or Cu-AAL (right).

By introducing propargylamine and azidoglycine into a linear RGD or RGDX sequence, Liu *et al*.[48] were able to use Cu-AAL for a head-to-tail cyclisation giving cyclic RGD analogues. Copper bromide was used to affect the cyclisation, with a variety of conditions assessed in an attempt to optimise the reaction. A catalytic system consisting of CuBr/DBU (1/3) in DCM at room temperature was found to give the best yields for the reaction. Some of the

derivatives synthesised were found to exhibit cytotoxicity comparable to known cyclopeptide integrin inhibitor Cy [RGDfK].

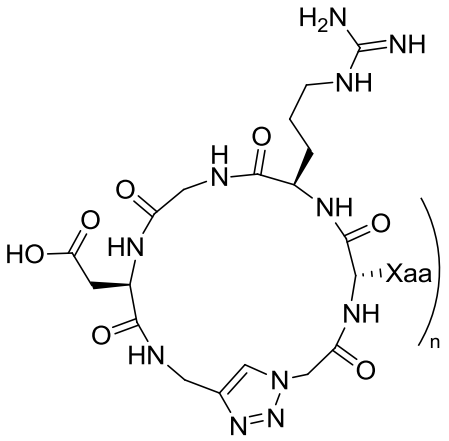
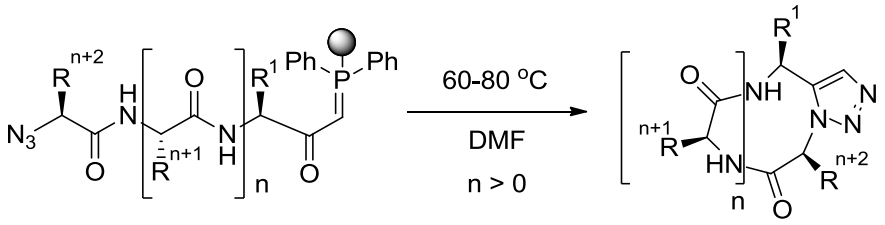


Figure 1.13 General structure of Liu's triazole-containing cyclic RGD analogues.[48]

A cyclic application of the phosphorane cleavage-based catalytic 1,5-triazole formation reaction discussed earlier in section 1.2.3 was used by Ahsanullah *et al*.[49] for the synthesis of *cis*-triazolylcyclopeptides. The catalytic cleavage reaction was investigated due to the failure of ruthenium-based systems to produce cyclic 1,5-triazole containing cyclic peptide mimics of the general structure shown below. Although significant dimerisation was seen during the synthesis of derivatives of tripeptides (n = 1), this effect was less evident with the cyclisation of longer chain oliogomers. The yields for the compounds that were isolated ranged between 32-48 % after purification by HPLC.



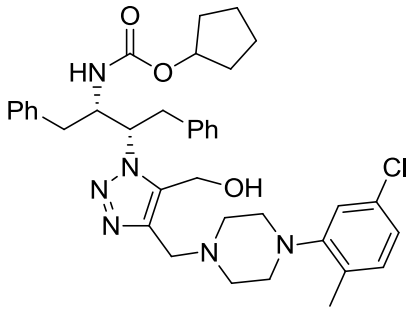
Scheme 1.15 Catalytic azidopeptidylphosphorane cleavage for the formation of 1,5-triazole containing cyclic peptide mimics.

With this literature precedent, it can be seen that the Cu-AAL reaction, as well as other triazole-forming reactions, show great promise for the synthesis of complex cyclic molecules.

1.4 Cu-AAL for Rapid Synthesis of Complex Molecules

1.4.1 Non-Iterative Synthesis of Complex Molecules

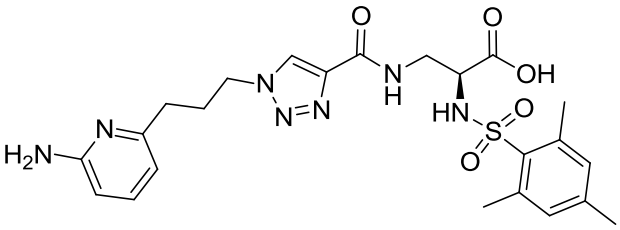
The Cu-AAL reaction was used by Whiting *et al*.[25] for the rapid synthesis of triazole-containing HIV-1 protease inhibitors. The fast coupling and functional group tolerance of the reaction allowed access to a wide range of diverse inhibitors in a short period of time, resulting in compound **1.25** below, with K_i = 9 nM. When the triazole in the compound was replaced with an amide, a drop in activity was seen, suggesting that the triazole was involved in binding and not simply present as a linker.



1.25

Figure 1.14 Triazole-containing protease inhibitor 1.25.

Ni *et al*.[50] used the reaction to synthesise potential inhibitors of integrin αvβ3 with a modified RGD scaffold. By using Cu-AAL as a linkage strategy to bring together two pharmacophores implicated in the binding of compounds to integrin αvβ3, for example a 2-aminopyridine at the *N*-terminus as a guanidine surrogate and an *N*-α-arylsulfonamide-*N*-β-diaminopropionic acid at the *C*-terminus, they were able to produce a number of biologically active compounds, including **1.26** which was found to have an IC_{50} of 13.6 nM against the target receptor.



1.26

Figure 1.15 RGD mimic 1.26 containing a 1,4-triazole.

The Cu-AAL reaction was used by Isidro-Llobet *et al*.[51] as a key macrocylisation step in producing a diversity-oriented synthesis (DOS) library, allowing the synthesis of 14 compounds with the general structures shown in figure 1.16 below. Coupling of amino acid-derived monomer units was effected by EDC-mediated reactions, to give a variety of substrates that underwent either Cu-catalysed or Ru-catalysed triazole macrocyclisation reactions. Subsequent diketopiperazine formation lead to a library of complex structures created from relatively simple amino-acid-derived building blocks. The functional group tolerance of copper or ruthenium-based alkyne-azide macrocyclisations is a key aspect of the synthetic utility of this reaction in producing DOS libraries.

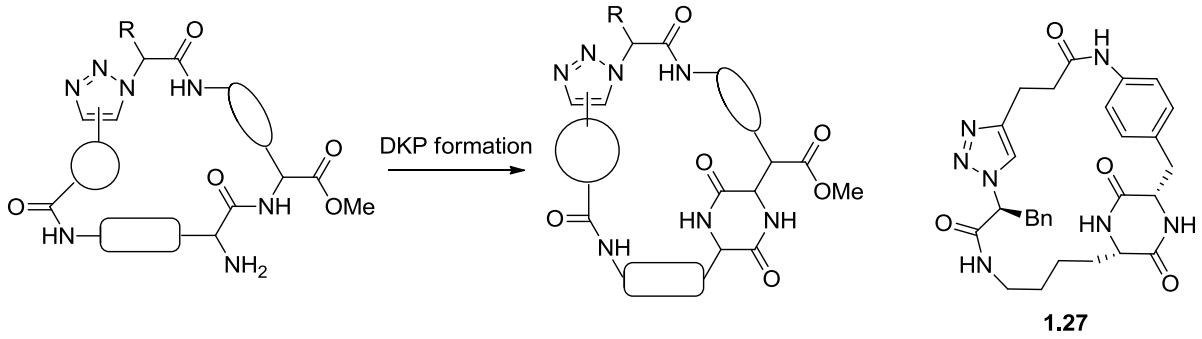
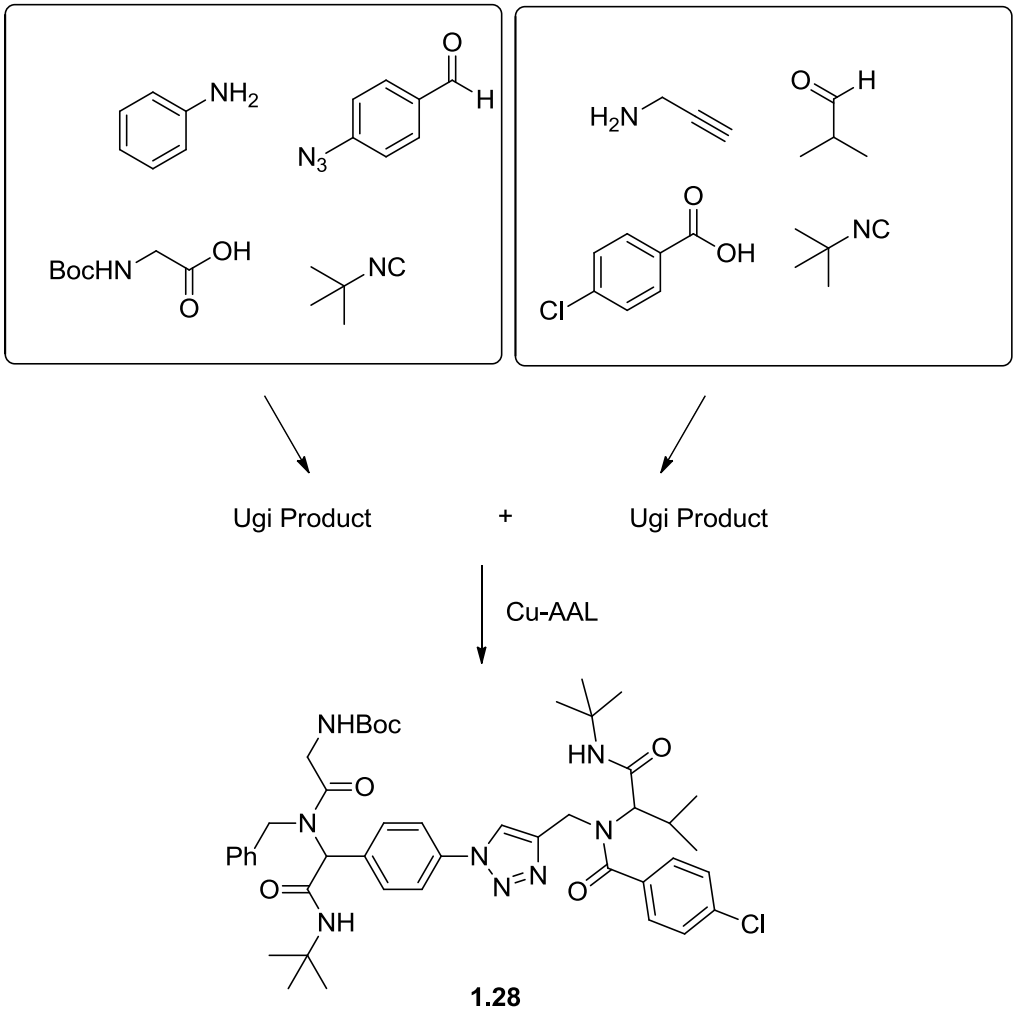


Figure 1.16 General cyclic peptide-like structures synthesised via the key triazole macrocyclisation reaction and diketopiperazine formation, with compound 1.27 shown on the right.

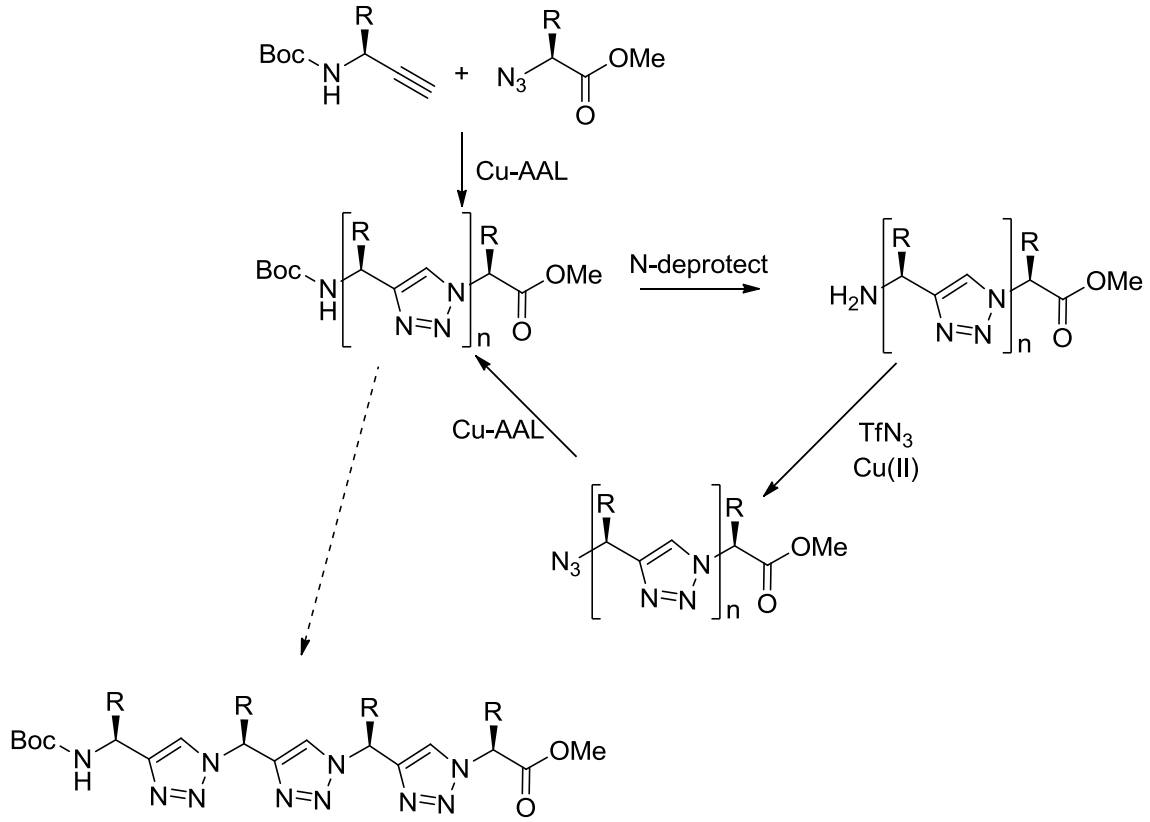
For similar reasons, Niu *et al*.[52] explored pairing together the multicomponent Ugi reaction and Cu-AAL reaction. By combining these reactions, quite complicated and highly functionalised peptidomimetic-like chemical structures could be developed using very basic starting materials. By performing 2 separate Ugi reactions, one containing alkyne functionality and the other containing an azide, and combining the two products with the Cu-AAL reaction, compound **1.28** was produced in a convergent 3-step synthesis without purification of the intermediates.



Scheme 1.16 Synthesis of peptidomimetic 1.28 via Cu-AAL coupling of alkyne and azide-containing Ugi products.

1.4.2 Iterative Synthesis for Complex Molecules

With the knowledge that the triazole moiety was a possible substitute for the amide bond between amino acids, Angelo and Arora used the reaction as a means of linking amino acids to form "triazolomers" – triazole oligomers.[53] They derivatised phenylalanine and lysine into Boc-protected amino alkynes, and used Cu-AAL to connect the alkyne to an azido phenylalanine or lysine methyl ester. A 3-step chain extension process, consisting of Boc-deprotection with TFA and diazotransfer with a sulfonyl azide, gave rise to the triazole oligomers, as shown in scheme 1.17. Extension was thus analogous to peptide synthesis in the *C* to *N*-direction.



Scheme 1.17 Angelo & Arora's synthetic pathway for triazolamers, including a 3-step chain extension sequence.[53]

Through molecular dynamics simulations and *ab initio* calculations, they proposed that the dipoles of neighbouring triazole moieties would prefer to be in opposite directions to one another, thus forming anti-conformations over syn-conformations. As the anti-conformations could lead to either turn or zig-zag secondary structures, 2D ROESY NMR experiments were performed to distinguish between the two. The spectra obtained displayed alternating strong and weak ROE crosspeaks between the triazole NH protons and backbone α-protons, as shown in figure 1.17, thus it was rationalized that these oligomers preferred to adopt a 'zigzag' conformation. They described this phenomenon as being similar to peptide β-sheets, as the distance between the β-carbons on one face of the molecule was calculated to be 7.9 Å, compared to the 7.2 Å seen in β-sheets, while the distance between alternating β-carbons was calculated to be 6.8 Å, longer than the 5.5 Å seen in β-sheets.

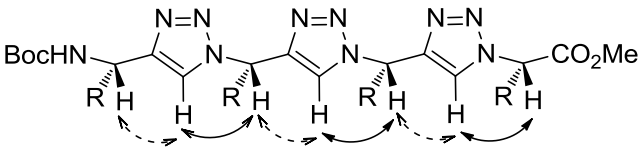
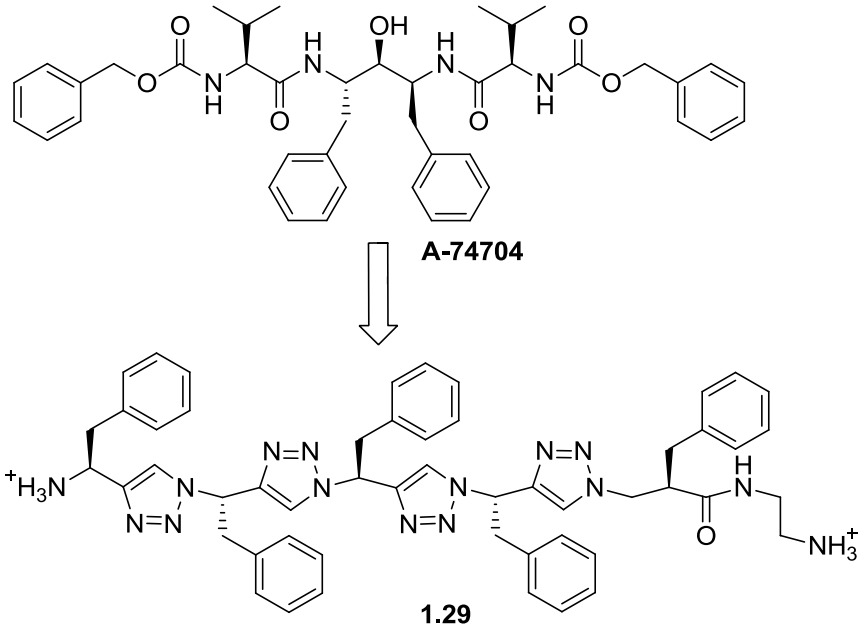


Figure 1.17 Tetrameric triazole oligomer with alternating weak (dashed arrows) and strong (full arrows) NOE crosspeaks indicative of a zig-zag secondary structure.

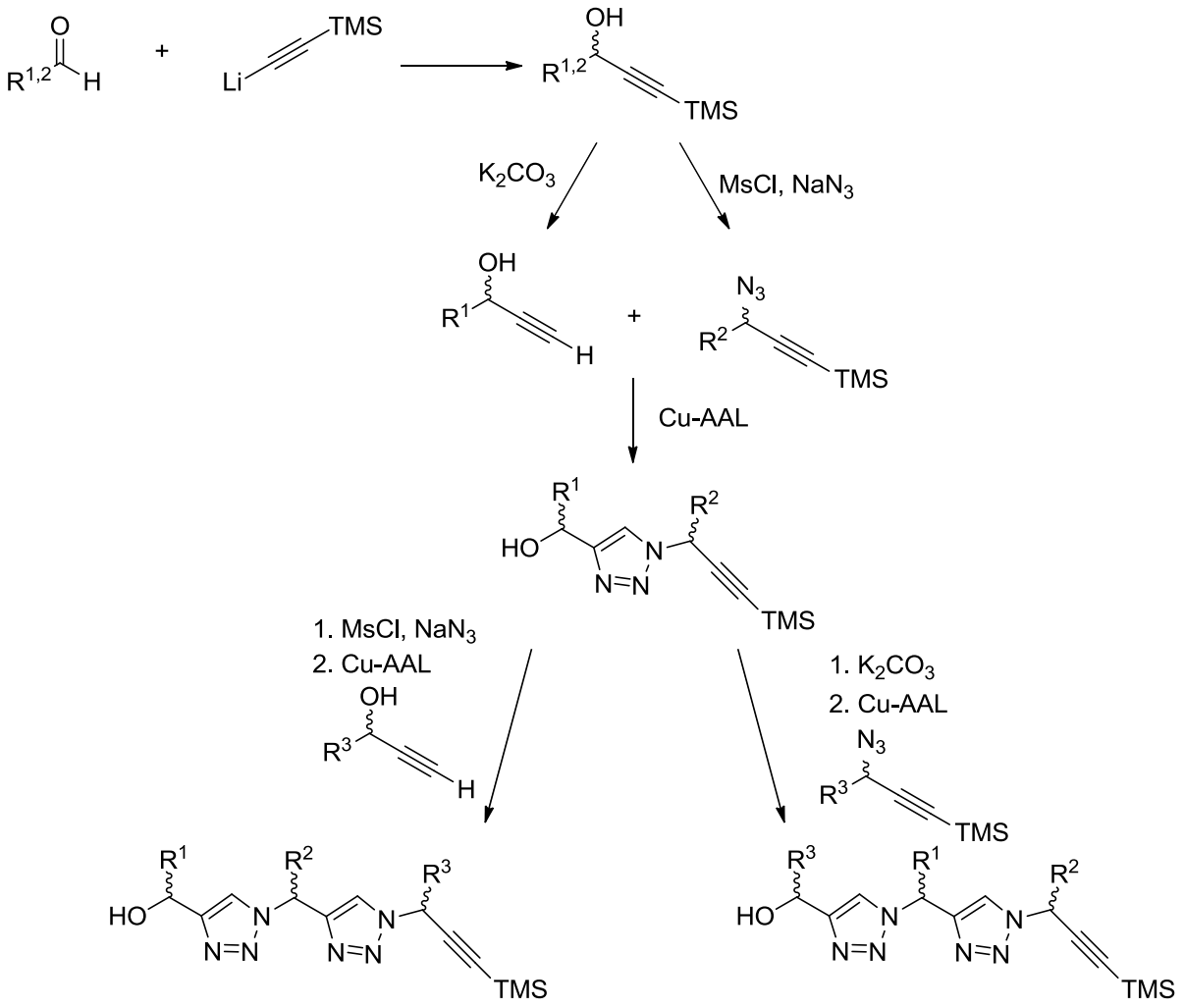
They later elaborated on this method, performing the sequence of reactions on the solid phase, and used their method to synthesize linear HIV-protease 1 inhibitors based on known low-nanomolar inhibitor A-74704 below (IC_{50} = 3.0 nM),[54] with an IC_{50} of 25 μM obtained for **1.29**.[55]



Scheme 1.18 Structure of inhibitor 1.29 in comparison to the lead structure (A-74704).

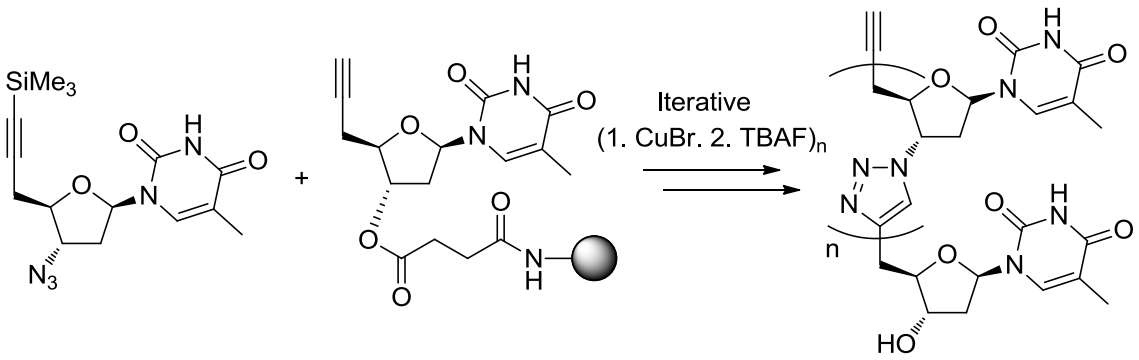
Hughes *et al*.[56] adopted a different approach, exploring the use of alkynyl-azide fragments for the synthesis of triazole oligomers. Racemic trialkylsilyl-protected propargyl alcohols were formed from the addition of lithiotrimethylsilylacetylide to aldehydes, and the hydroxyl moiety was converted into an azide giving bifunctional protected alkyne-azide units. Iterative chain extension from the trialkylsilyl-protected propargyl alcohols could be performed in one of two ways corresponding to extension in the *C*-direction or the *N*-direction respectively: i) silyl deprotection followed by addition of an azido-silyl protected alkyne, or ii) converting the alcohol to an azide, and performing Cu-AAL with the addition of a propargyl alcohol. In either

case, mixtures of diastereomers were formed due to the racemic nature of the starting monomers.



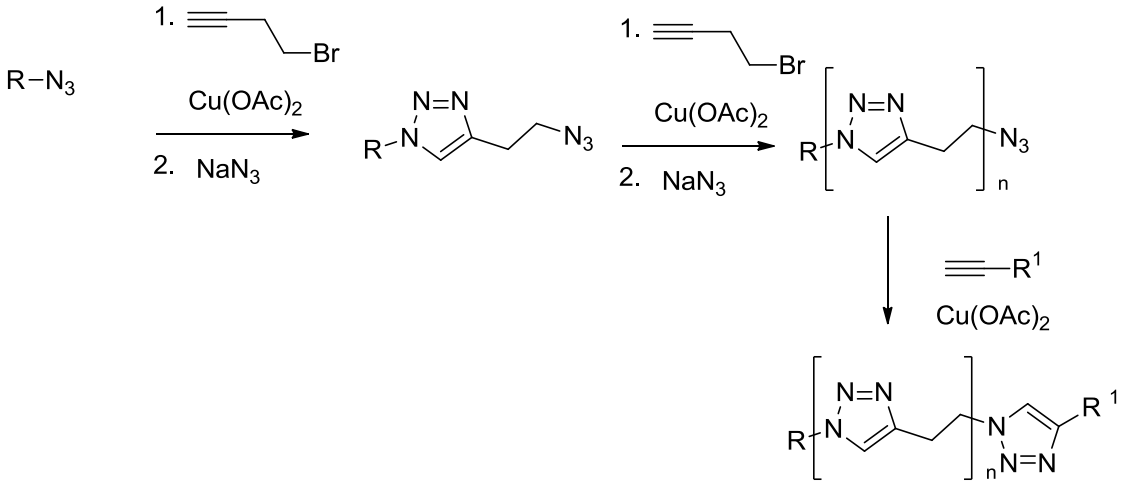
Scheme 1.19 Hughes' alkynyl-azide-based triazole oligomer extension pathway.

By using thymidine-derived monomer units similar to Hughes' silyl protected alkynyl-azide, Isobe *et al*.[57] were able to produce DNA mimics via iterative triazole formation. They reasoned that the triazole units produced were able to serve as a surrogate for the DNA phosphate backbone linkage. The iterative sequence was performed on solid phase leading to a 10-mer, and the DNA mimic was able to form a stable double strand with natural DNA, as determined by UV-visible spectroscopy. The triazoles were therefore seen as suitable replacements for the phosphate backbone.



Scheme 1.20 Solid phase 2-step iterative synthesis of DNA mimics via triazole formation and silyl deprotection.

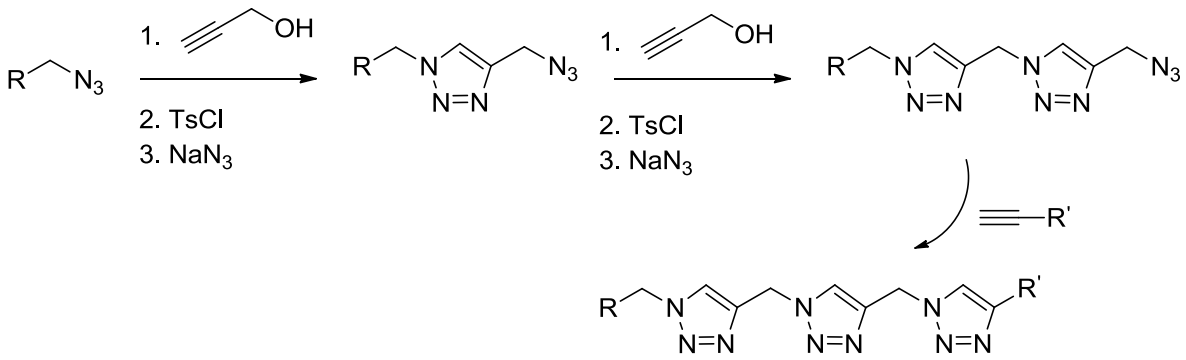
Starting with azido compounds, Fiandenese *et al*.[58] used sequential Cu-AAL with 4-bromo 1-butyne and nucleophilic substitution with sodium azide, as an iterative method of providing a variety of N-C linked 1,2,3-triazole oligomers. The synthesis proceeded in the C-direction in an analogous fashion to the synthesis of Arora,[53] but where Arora used a protected amine as a latent azide, and an amino alkyne as their key extending monomer unit, Fiandenese and co-workers used an alkyne with an attached bromide, which may easily be converted to an azide.



Scheme 1.21 Iterative triazole formation and azide displacement for the synthesis of triazole oligomers.

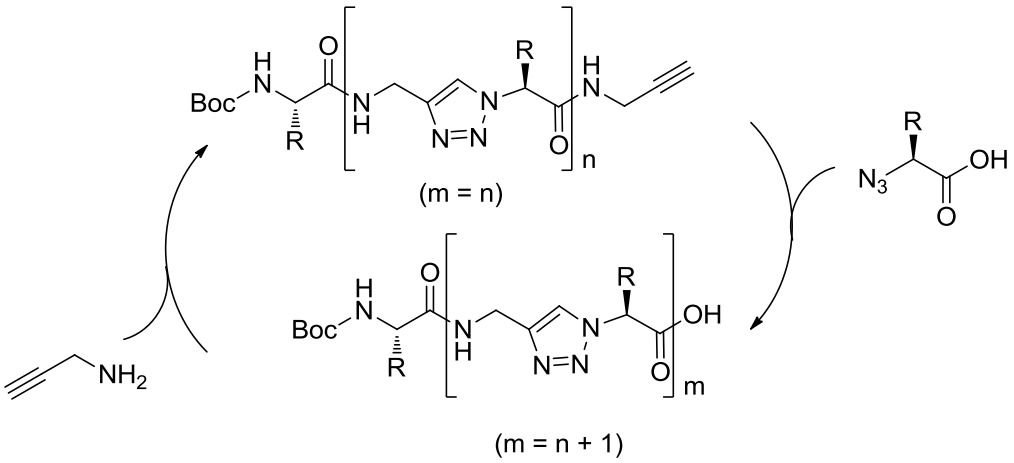
A similar iterative procedure was demonstrated by Stefani *et al*.,[59] whereby azides were coupled to propargyl alcohol, followed by transformation of the alcohol to an azide via a tosylate intermediate. Further extension led to compounds with 3 triazole units present, with the yields for the final coupling between 45 and 50 %. It was suggested that deactivation of

the catalyst via chelation to the bis-triazole could occur, leading to the moderate yields observed.



Scheme 1.22 Iterative triazole formation, tosylate formation and azide displacement for the formation of triazole oligomers.

An iterative synthesis by Ke *et al*.[60] gave rise to oligomers with alternating triazole and amide bonds. The extension process was a two step Cu-AAL with an amino alkyne, followed by a peptide coupling reaction with propargylamine. The yields seen were between 70-85 % for each iteration.



Scheme 1.23 Iterative Cu-AAL / peptide bond formation for the synthesis of oligomers.

They noted concentration dependant amide NH ^{1}H NMR shifts for their oligomers, indicative of intermolecular bonding. The H-bonding event was seen to a greater extent with longer oligomers, suggesting that this was due to a greater number of amide and triazole units. Additionally, 2D NOESY NMR experiments suggested that the *N*-terminal Boc group was in close proximity to the *C*-terminal alkyne of another molecule, showing head-to-tail

dimerisation. They noted that the head-to-tail dimers resemble the antiparallel β-strand motif found in many peptides.

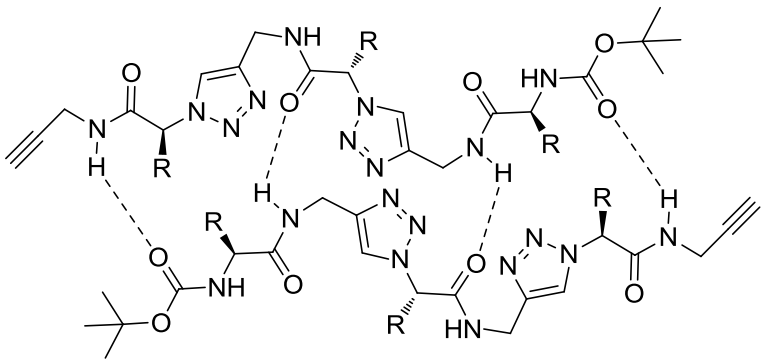
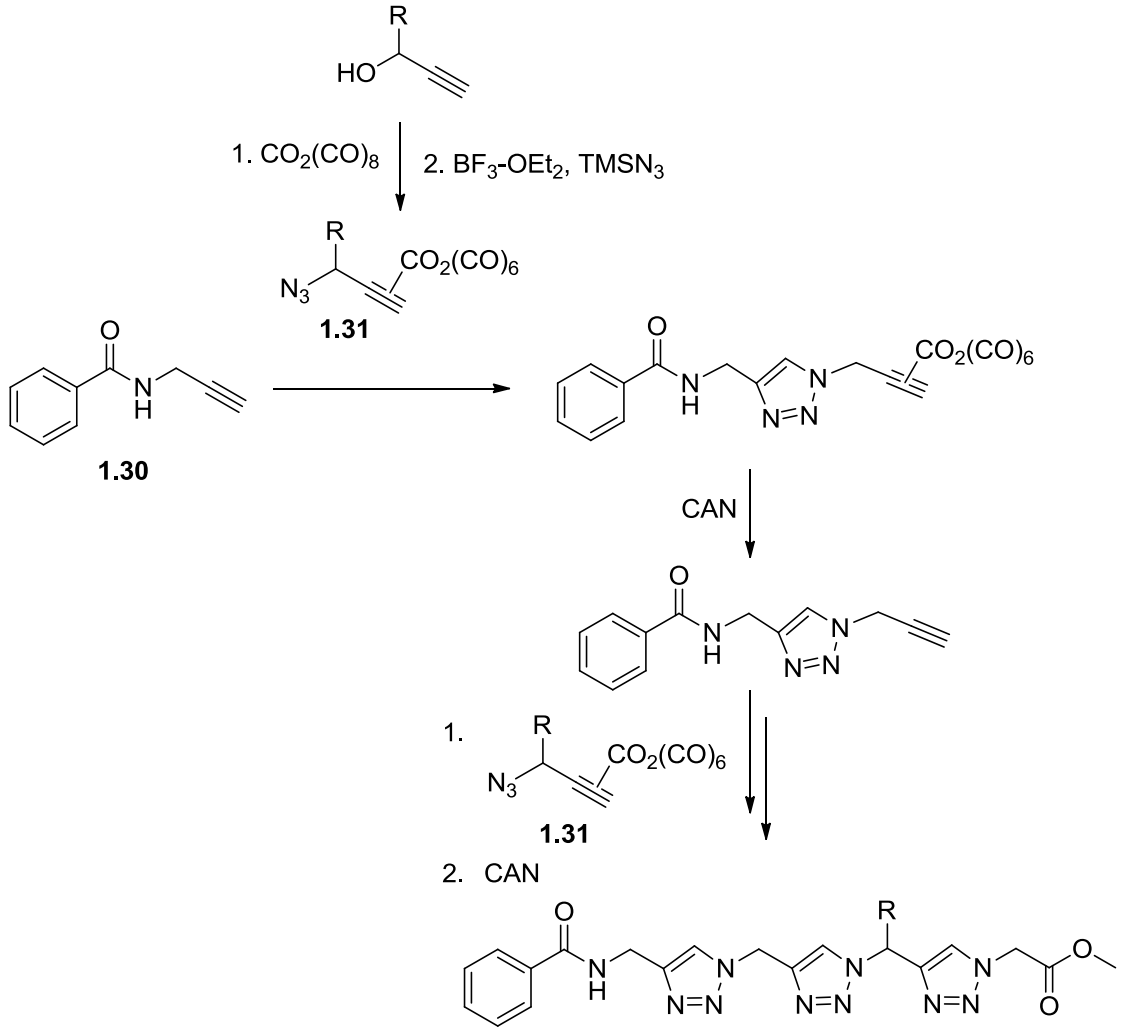


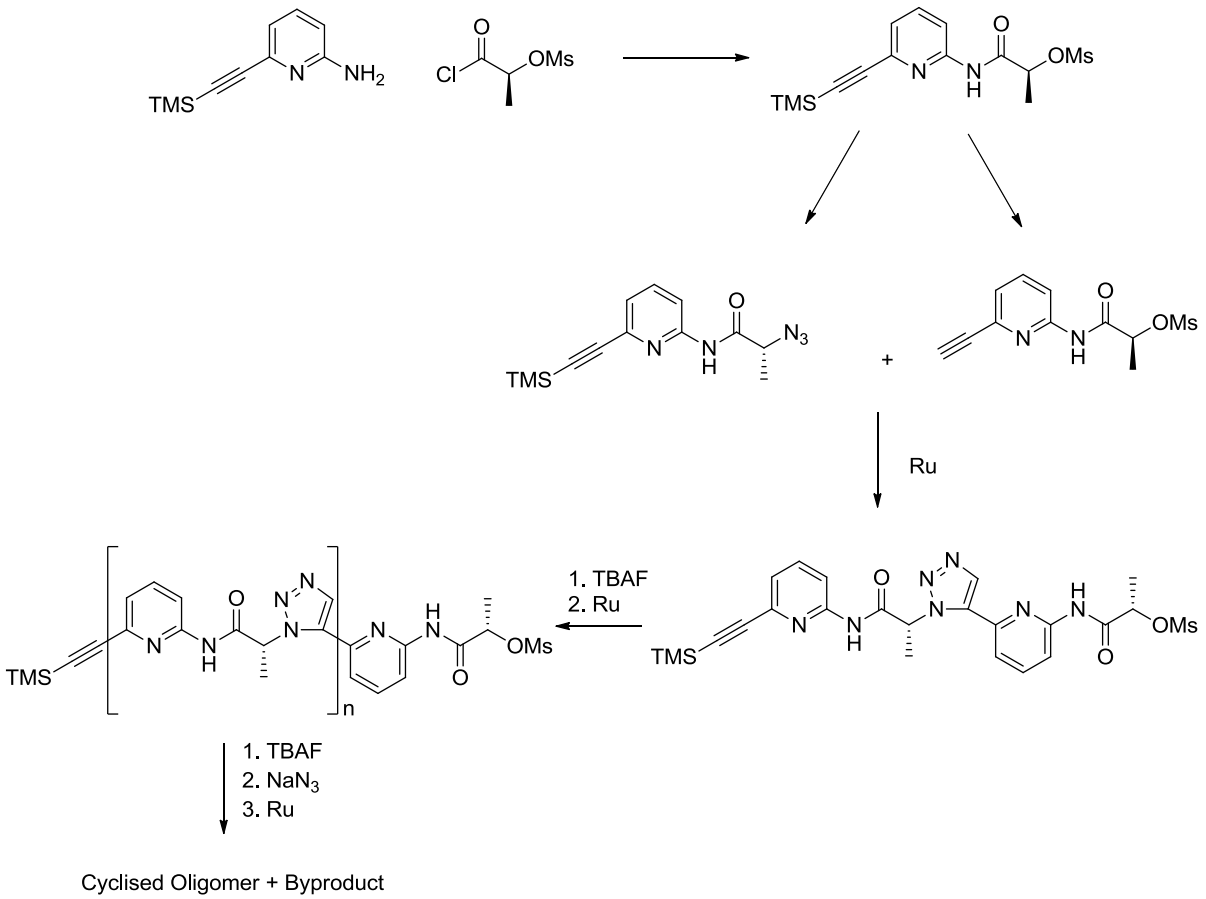
Figure 1.18 Dotted lines showing the intermolecular forces between Ke's oligomers,[60] leading to head-to-tail dimerization.

With an iterative extension strategy similar to Hughes' *N*-direction, Tskuada *et al*.[61] reacted *N*-benzoylpropargylamine (**1.30**) with a cobalt protected alkynyl-azide (**1.31**) derived from a propargyl alcohol. As oxidative deprotection of the cobalt complex with ceric ammonium nitrate exposes a free alkyne, the cobalt-complexed alkynyl-azide monomer unit was performing the same role as the silyl protected alkynyl-azide used by Hughes. The 2-step extension process was continued for multiple cycles, with the coupling – oxidative deprotection sequence giving yields > 70 % over the 2 steps.



Scheme 1.24 Formation of triazole oligomers via two-step Cu-AAL and oxidative deprotection.

Shown in scheme 1.25 below, Krause *et al*.[62] also performed a two-step iterative sequence similar to Hughes' method, with ruthenium coupling of a TMS-protected alkynyl azide with an alkyne-containing molecule with a terminal mesyl group. Deprotection of the silyl alkyne provided a free alkyne, which underwent further Ru-coupling. With the desired linear alkyne length, desilylation of the terminal alkyne, conversion of the terminal mesyl group to an azide, and ruthenium coupling with microwave irradiation gave head-to-tail macrocycles. The Ru-based macrocyclisation gave 2 main products for the reaction.



Scheme 1.25 Synthesis of 1,5-triazole containing cyclic peptidomimetics via iterative Ru-AAL and desilylation.

The ^{1}H NMR spectrum obtained for the major product, (**1.34**, below), was indicative of a highly symmetrical molecule, whereas the minor product, found to have the same molecular weight, had a notably more complex spectrum. It was suggested that the minor product had formed a 1,4-triazole under the thermal conditions used for cyclisation, shown as **1.35**. The major product was isolated in 10 % yield after recrystallisation. Isothermal titration calorimetry was used to study the binding of tetramethylammonium anion salts against either **1.34** or the parent amide bond-containing macrocycle, which indicated a 1:1 salt : triazole macrocycle **1.34** ratio, and 2:1 salt : *cis*-amide macrocycle **1.32**. It was found that the anion binding affinity was intrinsically higher for the triazole-containing mimic, and was suggested that the compound could be used in the development of a synthetic anion receptor.

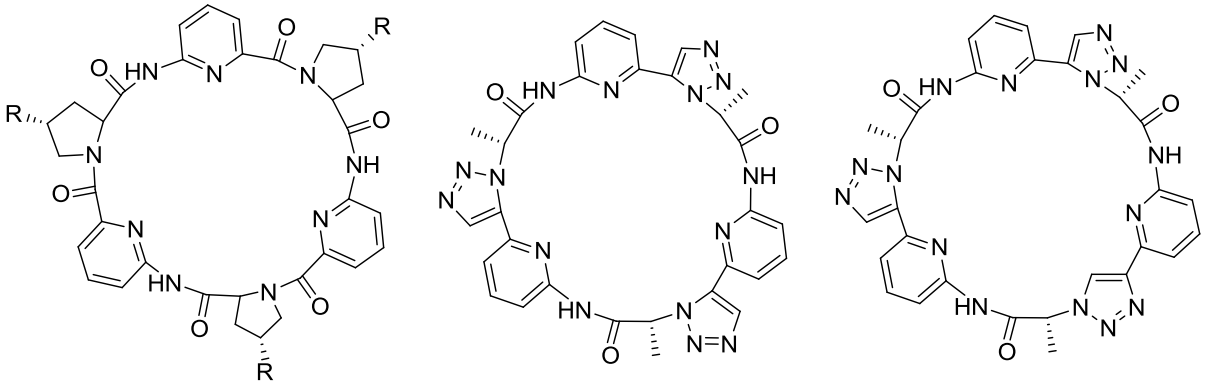
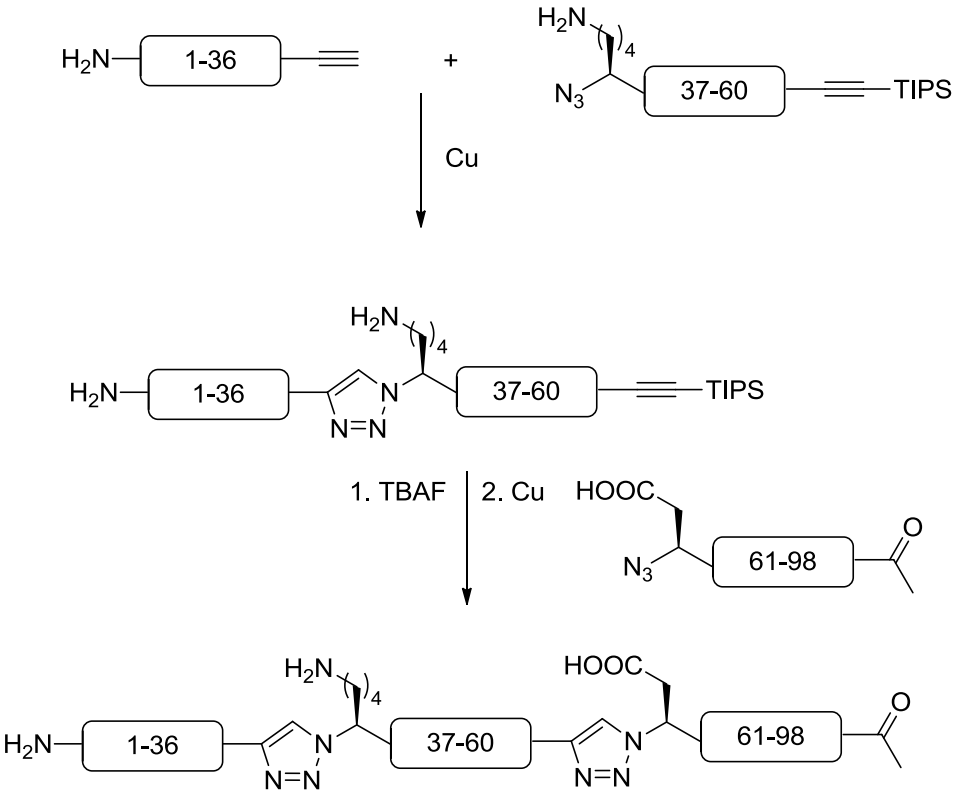


Figure 1.19 Comparison of proline-containing macrocycles 1.32 and 1.33 with macrocycle 1.34, which features 1,5-triazole replacements, and byproduct 1.35.

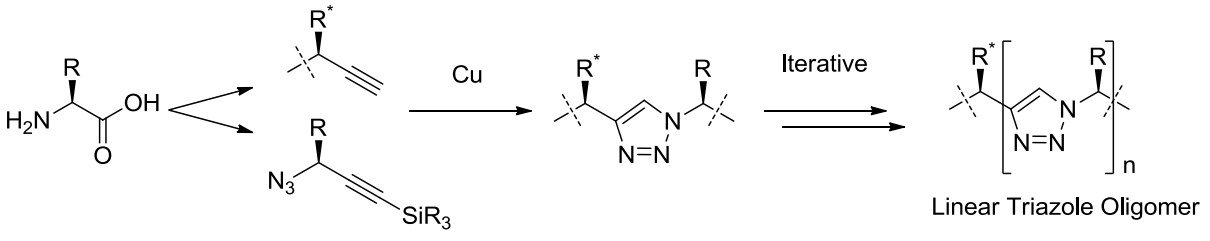
Valverde used a Hughes-like iterative sequence for the coupling of large peptide sequences.[63] They retrosynthetically disconnected a 97 amino acid sequence of cystatin A at two Gly-Xaa amide bonds, and replaced the glycine residues with propargylamine. Thus the first fragment, a *C*-terminal alkyne-containing 36-mer, was coupled using copper catalysis with a 24-mer which contained an *N*-terminal azide and *C*-terminal TIPS-protected alkyne. TBAF cleavage exposed a *C*-terminal alkyne, and the unit was reacted with the final peptide strand, which contained an *N*-terminal azide and an unprotected *C*-terminus, giving an 98-mer in 25.8 % yield over 3 steps after purification. The compound was found to have a similar binding profile to cathespin B, H and L as the parent compound, cystatin A. Circular dichroism spectroscopy was performed, which suggested that the compound showed consistent folds to cystatin A, with a predominant β-sheet structure.



Scheme 1.26 Use of Cu-AAL for the stepwise linkage of peptides.

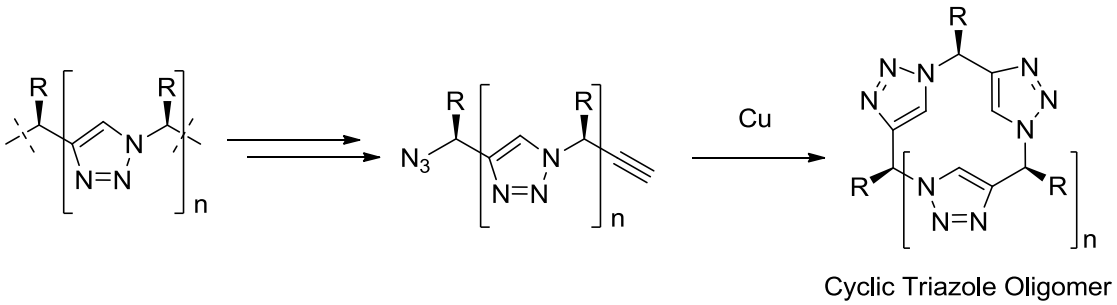
1.5 Thesis Outline

As can be seen from the examples shown, Cu-AAL and related reactions are becoming a popular method to quickly produce triazole-containing compounds with well-defined structures, and have been utilised in many peptidomimetic-based applications. Our aim is to explore the use of Arora and Hughes' methods of iterative triazole peptidomimetic synthesis to produce oligomers with amino acid-derived side-chains, as outlined in scheme 1.27 below.



Scheme 1.27 Conceptual synthesis of linear triazole oligomers from amino acids.

To facilitate the synthesis of the triazole peptidomimetics, a key focus of the thesis is the formation of alkynyl- and azido- monomer units, with side-chain functionality corresponding to those seen in amino acids. Both adaptations of existing literature syntheses (chapter 2) and novel syntheses (chapters 3 and 4) are explored for the production of these units. With the monomer units in hand, potential chain extension sequences are examined and optimised for the synthesis of complex triazole-containing oligomers. Although linear all-triazole peptidomimetics are present in the literature, there are no reports of the all-triazole cyclic peptide mimics. It is hypothesised that these types of molecules can be accessed via head-to-tail cyclisation of the linear oligomers, when the appropriate functionality is present at the *N*- and *C*-termini. An iterative synthesis would give access to a range of macrocyclic compounds, with the ability to easily moderate their properties by varying the 'R' groups on the starting materials used.



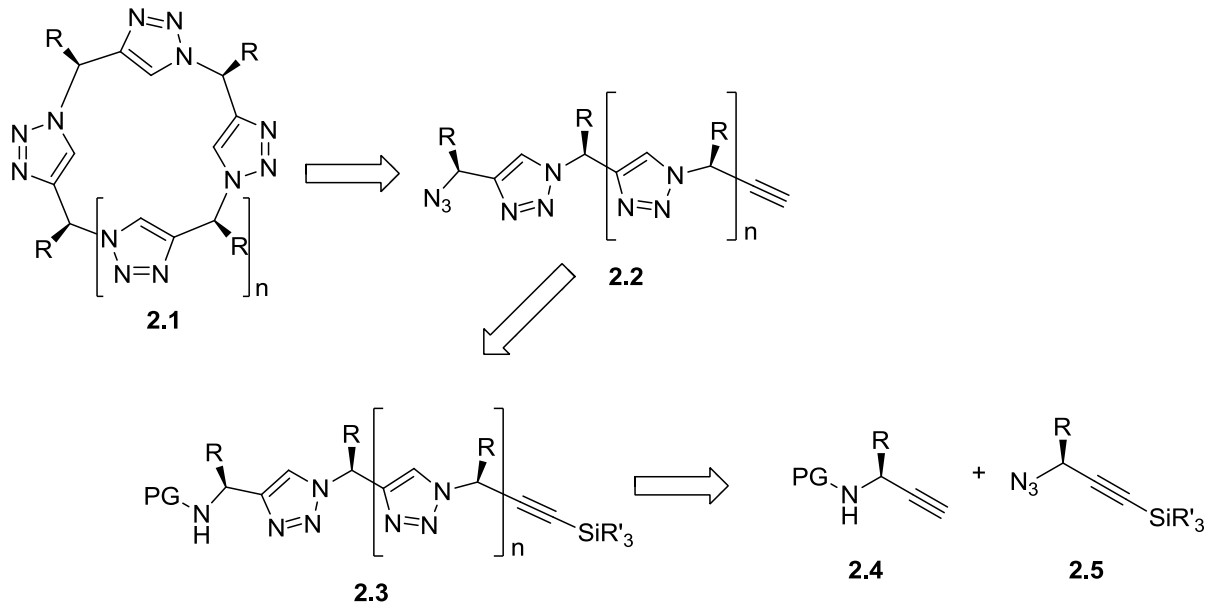
Scheme 1.28 Conceptual synthesis of cyclic triazole oligomers from their linear counterparts.

Finally, granzyme B, a serine protease involved in the cell apoptosis pathway, is introduced in chapter 5. It is investigated whether the scissile amide bonds of known biologically active peptide substrates of granzyme B can readily replaced with triazole functionality. These compounds would be accessed via a combination of standard peptide synthesis and Cu-AAL, incorporating the monomer units synthesised in the earlier chapters. It is expected that replacement of scissile bonds of existing substrates with non-hydrolysable triazoles would transform the substrates into inhibitors.

Chapter 2 - Triazole Peptidomimetics

2.1 Introduction

2.1.1 Retrosynthesis



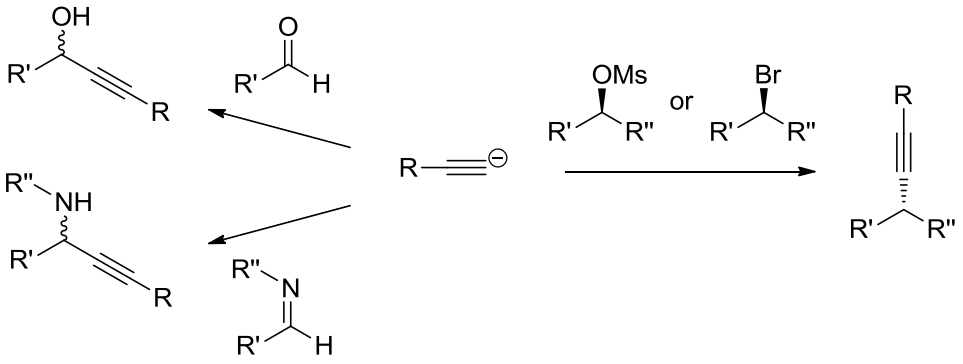
Scheme 2.1 Retrosynthesis of the target cyclic triazole peptidomimetic 2.1.

Scheme 2.1 details a retrosynthesis from the general target cyclic triazole compound **2.1**. It is anticipated that this compound could be synthesised via a head-to-tail Cu-AAL mediated cyclisation of linear alkynyl-azide compound **2.2**. The terminal azide and alkyne functionality of oligomer **2.2** could be obtained via diazotransfer and desilylation reactions on *N*-terminal and *C*-terminal protected linear triazole oligomers, such as **2.3**. These polytriazole oligomers could be formed as a result of Cu-AAL-based iterative synthesis between alkyne and azide-containing monomer units with the general structure of **2.4** and **2.5**, as discussed in chapter 1.4.2. Thus an investigation into the synthesis of these key alkyne and azide-containing monomer units was undertaken.

2.1.2 Synthesis of Alkynes

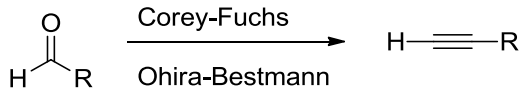
A commonly used method for the introduction of alkynes into molecules involves the nucleophilic addition of a metal acetylide to carbonyl compounds. The main problem with this

reaction is that racemic alkynes would be formed in the event of formation of a new chiral centre. Alternatively, metal acetylides can be utilised in nucleophilic substitution reactions with substrates such as mesylates or alkyl halides, and potentially proceed with an inversion of stereochemistry if present.



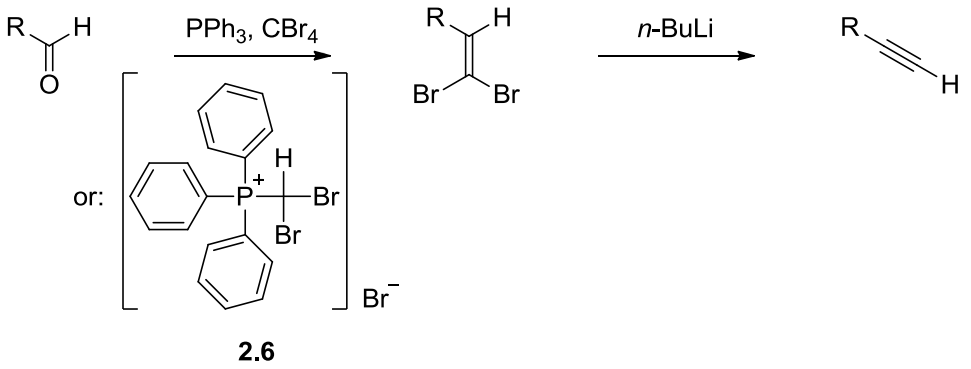
Scheme 2.2 Example nucleophilic addition (left) or substitution (right) reactions with the acetylide ion.

A different approach is the functional group transformation of aldehydes to alkynes, which may be effected via one of two pathways; Corey-Fuchs homologation[64] or the Ohira-Bestmann procedure.[65-66]



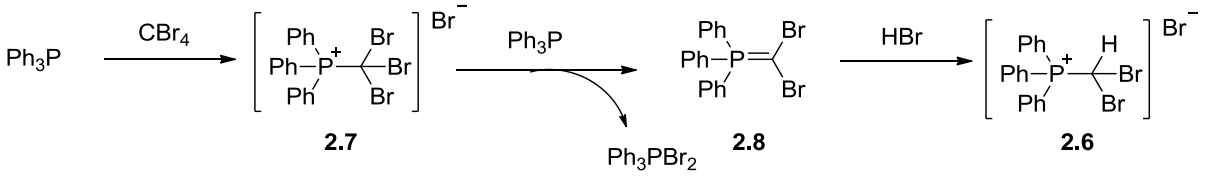
Scheme 2.3 One-step conversion of aldehydes into alkynes via Corey-Fuchs Homologation or the Ohira-Bestmann reaction.[65, 66]

The Corey-Fuchs homologation reaction is based on a special case of the Wittig reaction whereby a phosphorous ylide is formed *in situ* from a reaction between zinc, triphenylphosphine and carbon tetrabromide. When aldehydes are allowed to react with this ylide, a dibromo-olefin intermediate is produced which may be isolated. Reacting the dibromo-olefin with butyllithium results in an alkyne.



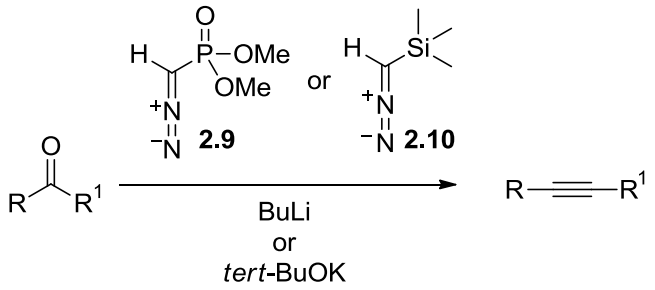
Scheme 2.4 General Corey-Fuchs Homologation scheme showing the formation of a dibromoolefin from an aldehyde, followed by the addition of butyllithium to give an alkyne.[64]

Michel *et al*.[67] documented a procedure for this conversion which did not require isolation of the dibromo-olefin. It uses a preformed ylide: dibromomethylphosphonium bromide (**2.6**), a stable solid which is synthesized in a reaction between triphenylphosphine and carbon tetrabromide. As shown in scheme 2.5 below, Wolkoff synthesized this ylide in a two step process,[68] firstly forming the tribromomethyltriphenylphosphonium bromide (**2.7**) from triphenylphosphine and carbon tetrabromide, followed by reacting with another equivalent of triphenylphosphine to give intermediate **2.8**, which then reacted with hydrogen bromide generated *in situ* following the addition of water, yielding the product.



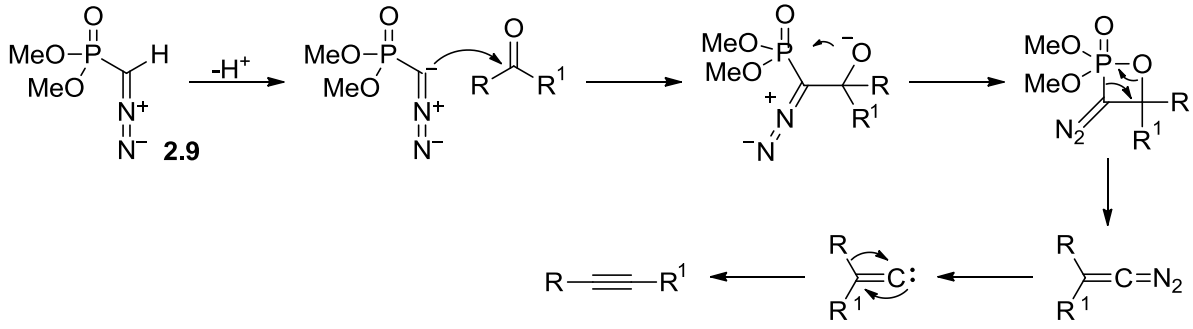
Scheme 2.5 Wolkoff's synthesis of key ylide 2.6 via triphenylphosphine and carbon tetrabromide.[68]

An alternative procedure to the Corey-Fuchs homologation was discovered by Colvin *et al*.,[69] who noticed that after the base-induced deprotonation of Seyferth's compounds dimethyl diazomethylphosphonate (**2.9**)[70] and trimethylsilyldiazomethane (**2.10**),[71] a reaction occurred with carbonyl compounds resulting in the corresponding alkyne derivatives. This method was further explored by Gilbert,[72] which led to phosphonate **2.9** being recognized as the Seyferth-Gilbert reagent.



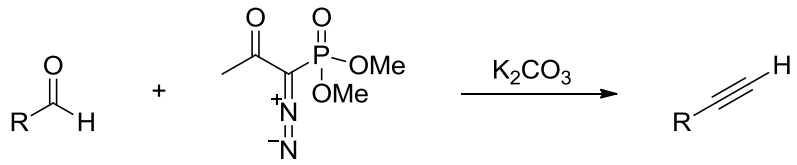
Scheme 2.6 Synthesis of alkynes from azides using dimethyl diazomethylphosphonate (2.9) or trimethylsilyldiazomethane (2.10).

The reaction is initially mechanistically similar to a Horner-Wadsworth-Emmons reaction, followed by the loss of nitrogen and rearrangement of the resulting carbene species to an alkyne, as shown in scheme 2.7 below.



Scheme 2.7 Reaction mechanism for the conversion of an alkyne from an aldehyde or ketone via a reaction with phosphodimethoxydiazomethane (2.9).[69]

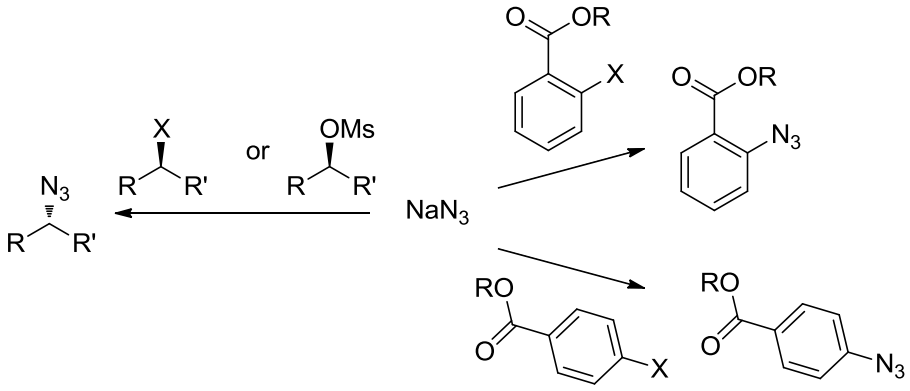
A noticeable improvement of this reaction was initially discovered by Ohira and elaborated by Bestmann, in which the key dimethyl diazomethylphosphonate anion was formed via the addition of the phosphonate **2.11** (the Ohira-Bestmann reagent) to potassium carbonate in methanol.[65, 66] As opposed to the Corey-Fuchs homologation, the reaction does not require anhydrous conditions, low temperatures or a nucleophilic strong base, which allows for a much wider reaction scope.



Scheme 2.8 Conversion of an aldehyde to a terminal alkyne via the Ohira-Bestmann reagent.[65, 66]

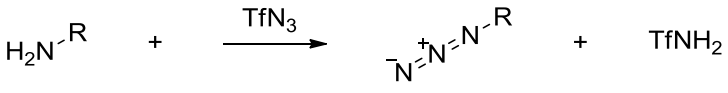
2.1.3 Synthesis of Azides

Two common approaches exist for synthesizing azides, the other component in Cu-AAL. The more common approach is nucleophilic aliphatic substitution with inorganic azides, commonly sodium azide.[73] Nucleophilic aromatic substitution may also be performed, although generally an electron-withdrawing group is required either in the *ortho* or *para* position relative to the leaving group.[74]



Scheme 2.9 Nucleophilic aliphatic (left) or aromatic substitution reactions of sodium azide.

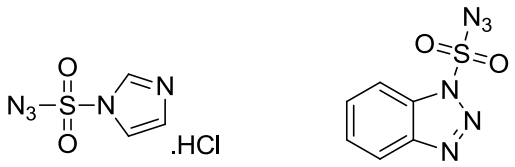
Alternatively, a diazotransfer reaction, involving the transfer of a diazo group from a reactive organic azide to a nucleophile, may be used. When a primary amine serves as the nucleophile, azides are formed (scheme 2.10).



Scheme 2.10 Synthesis of azides via triflic azide-mediated diazotransfer to a primary amine.

The reactive and potentially explosive triflyl azide can be produced by nucleophilic substitution of triflic anhydride with sodium azide.[75] It has been reported that in the biphasic system of CH_2Cl_2 and water, explosive azido-chloromethane and/or diazidomethane can be formed. As a result of this, non-chlorinated solvent systems such as water/toluene/methanol and toluene/THF are now commonly used.[76]

Goddard-Borger *et al*.[77] demonstrated that imidazole based sulfonyl azide **2.12** could also act as a diazo donor, including in reactions involving amino acids. Although it was originally proposed that the hydrochloride salt of the reagent was shelf-stable, it was shown to slowly react with water to produce hydrazoic acid,[78] a shock sensitive and explosive substance. It has since been shown that alternative salts, such as hydrogen sulfate, mesylate and tetrafluoroborate compounds are insensitive to contact.[79] Similarly, benzotriazole-derived azide **2.13** was developed by Katritzky more recently as an alternative shelf-stable diazo-transfer reagent, and was found to have similar reactivity to azide **2.12**.[80] However again, this compound was recently reported to be an explosion risk when the literature work-up procedure was modified.[81]

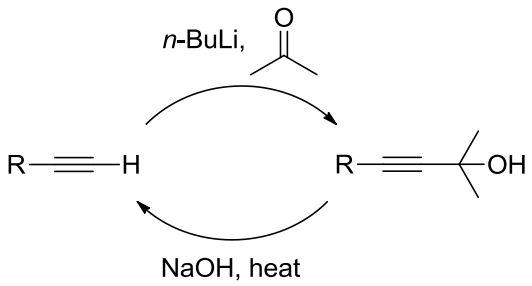


2.12 **2.13**

Figure 2.1 The Goddard-Borger azide (2.12),[77] and Katritzky's azide (2.13).[80]

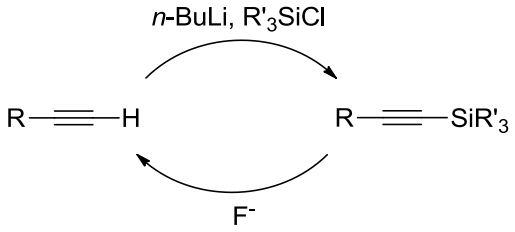
2.1.4 Terminal Alkyne Protecting Groups

Another set of key reactions in our sequence is the protection or deprotection of terminal alkynes. A terminal alkyne may be protected as an isopropyl alcohol via nucleophilic addition of the acetylide to acetone. The terminal alkyne is regenerated with exposure to sodium hydroxide at elevated temperatures.[82]



Scheme 2.11 Protection and deprotection of a terminal alkyne via a tertiary alcohol protecting group.

Alternatively, terminal alkynes may be protected with a variety of trialkylsilyl groups, which differ in their stability to base. For example, the trimethylsilyl protecting group may be cleaved in the presence of methanolic potassium carbonate,[83] whereas the more bulky trisopropylsilyl or *tert*-butyldimethylsilyl groups are unaffected.[84] All trialkylsilyl groups are easily removed via the addition of fluoride ions.[85] They may be formed by the nucleophilic substitution of the acetylide with trialkylsilyl chlorides,[84] although due to the harsh conditions required milder procedures for this protection have been investigated. Such procedures include reacting terminal alkynes with: Zn at high temperature,[86] $Zn(OTf)_2$ at mild temperature,[87] or forming the copper (I) acetylide species with ammonia and copper (I) chloride – all of which form strongly nucleophilic metal acetylides without the use of butyl lithium.

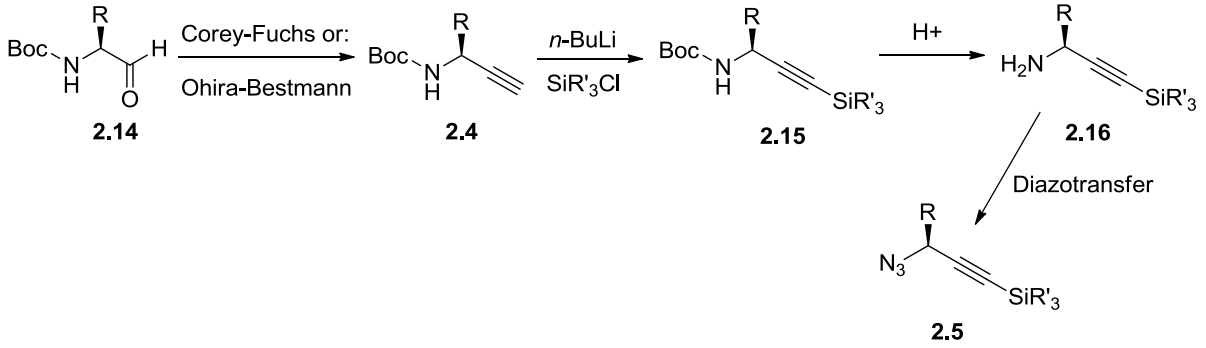


Scheme 2.12 Protection of a terminal alkyne as a trialkylsilane (top), and fluoride-mediated deprotection (bottom).

2.1.5 Initial Plans

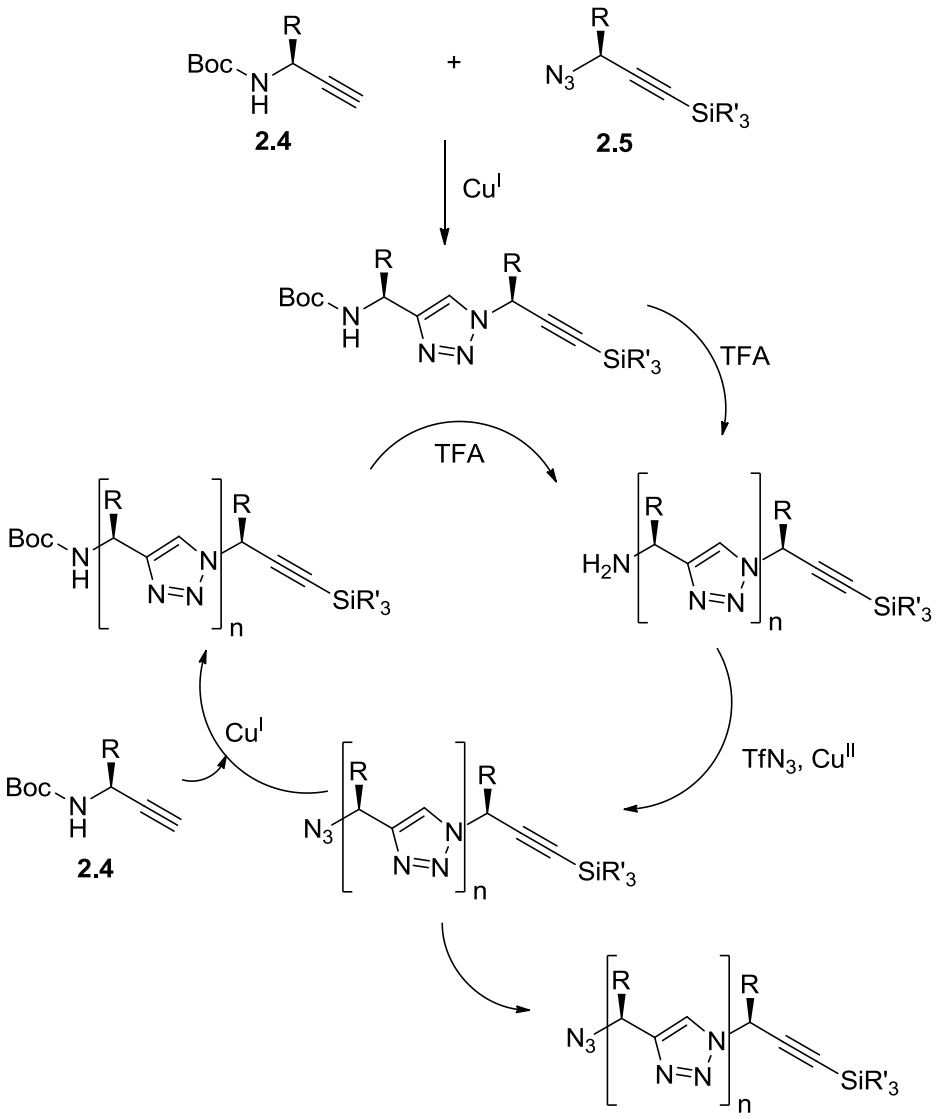
As was detailed in the retrosynthesis in section 2.1.1, an efficient synthesis to monomer units **2.4** and **2.5** was required. It has been shown in the literature that key monomer unit **2.4** may be synthesised from a variety of chiral amino aldehydes (**2.14**) via Corey-Fuchs homologation without racemisation,[88] although potentially the Ohira-Bestmann reaction could be used for the same transformation. Following the synthesis of alkyne **2.4**, protected alkyne

2.15 could be formed via silyl protection of some of the alkyne material with *n*-butyllithium and a trialkylsilyl chloride. Boc-deprotection to amine **2.16** and diazotransfer with either triflic azide or the Goddard-Borger reagent (**2.12**) would give the silyl-protected alkynyl azide monomer unit (**2.5**).

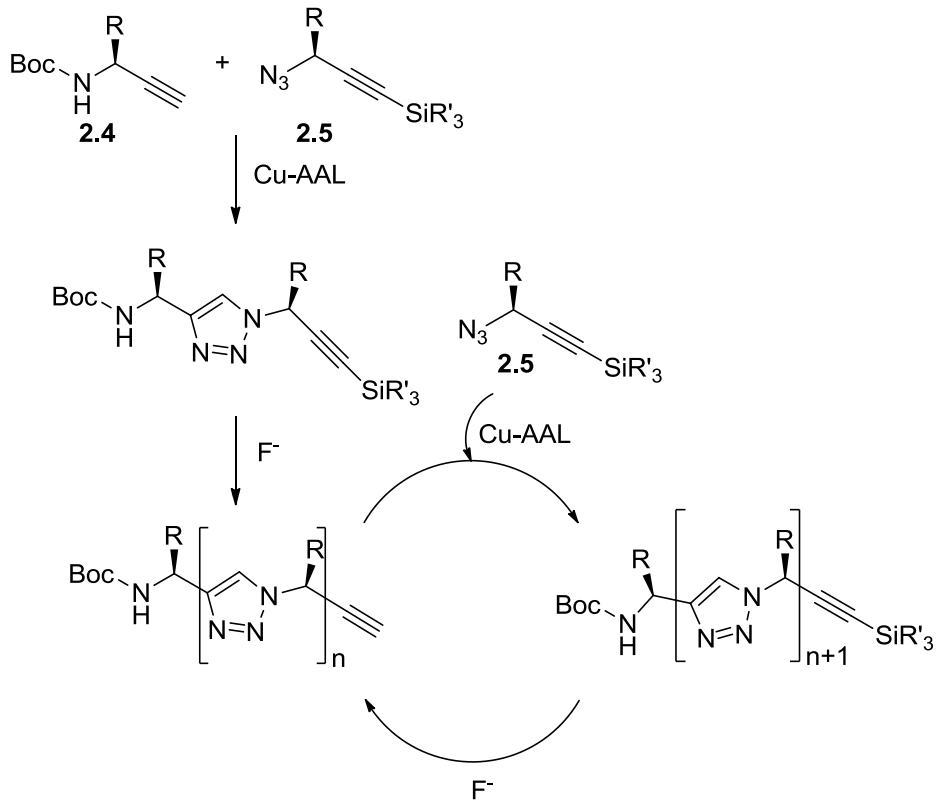


Scheme 2.13 Planned synthesis of key azide monomer unit 2.5 via silyl protection of the terminal alkyne 2.4, followed by *N*-deprotection and diazotransfer.

With both monomer units in hand, either the Cu-AAL *N*-terminal extension strategy (scheme 2.14), or *C*-terminal extension strategy (scheme 2.15) would be used for the formation of triazole oligomers.

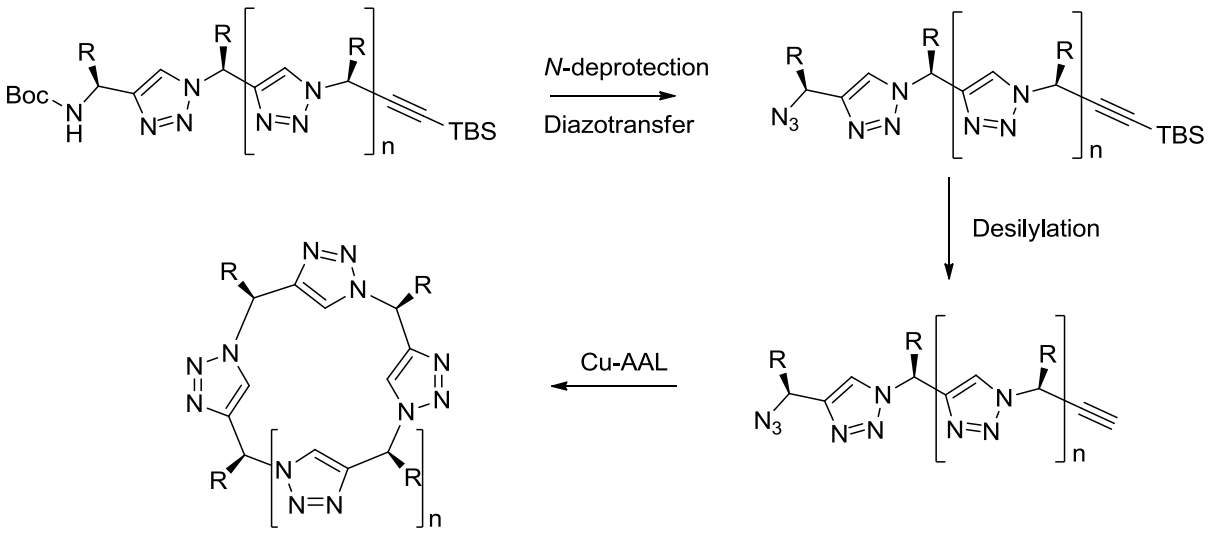


Scheme 2.14 *N*-terminal Cu-AAL based chain extension sequence. Coupling between key monomer units 2.4 and 2.5 is followed by *N*-deprotection and diazotransfer, giving an azide handle for further Cu-AAL reactions with alkyne 2.4.



Scheme 2.15 *C*-terminal Cu-AAL based chain extension sequence. Coupling between key monomer units 2.4 and 2.5 is followed by desilylation, giving an alkyne handle for further Cu-AAL reactions with azide 2.5.

In order to cyclise the linear molecules produced via either extension method, diazotransfer would be performed on the *N*-terminus yielding an azide, and silyl-deprotection woud expose the alkyne on the *C*-terminus as shown in scheme 2.16. It is proposed that, due to the presence of copper ions in the diazotransfer step, silyl-deprotection of the alkyne should take place after diazotransfer to minimise potential Cu-AAL mediated polymerization. Cyclisation would ideally be performed at various oligomer lengths using Cu-AAL to see if steric restrictions are present in terms of ring sizes.

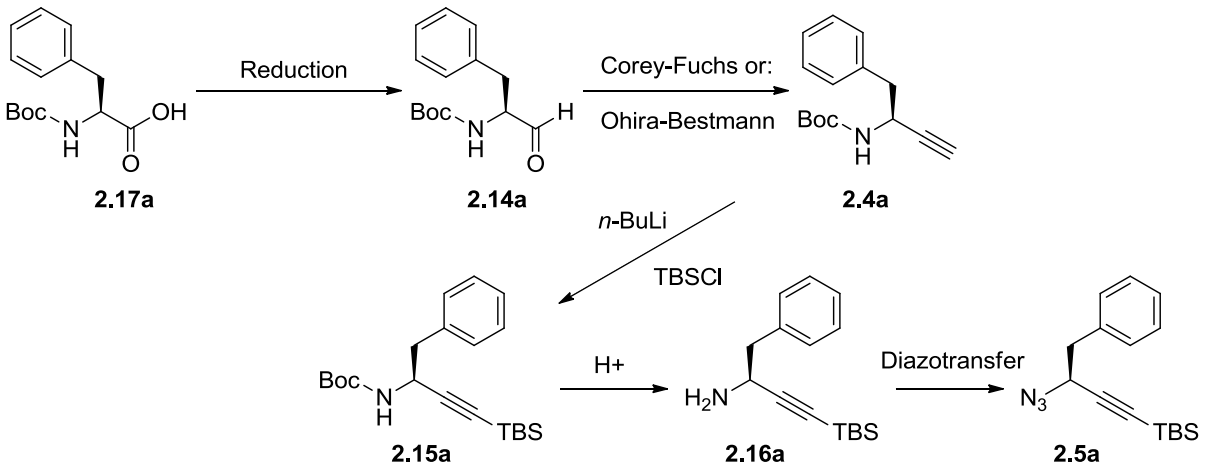


Scheme 2.16 Derivatistion of linear triazole peptidomimetics to the corresponding cyclic forms in a 4-step sequence.

Phenylalanine was selected as the first amino acid to be derivatised, due to its side chain being both non ionisable and UV-active. Once methods for the synthesis of a cyclic phenylalanine triazole mimic had been demonstrated as a test case, biologically active molecules would be investigated and mimicked.

2.2 Monomer Unit Synthesis

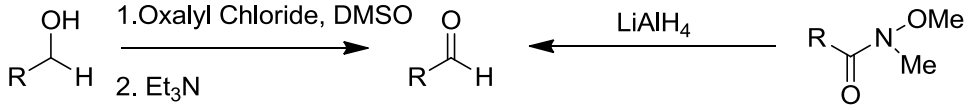
This section focusses on the synthesis of both the starting units: *N*-protected amino alkynes, and silyl-protected amino azides, for use in Cu-AAL-based triazole peptidomimetic extensions (Schemes 2.14 & 2.15).



Scheme 2.17 Synthesis of key alkyne 2.4a from Boc-phenylalanine (2.17a) via an aldehyde intermediate (2.14a), followed by derivatisation to key azide monomer unit 2.5a.

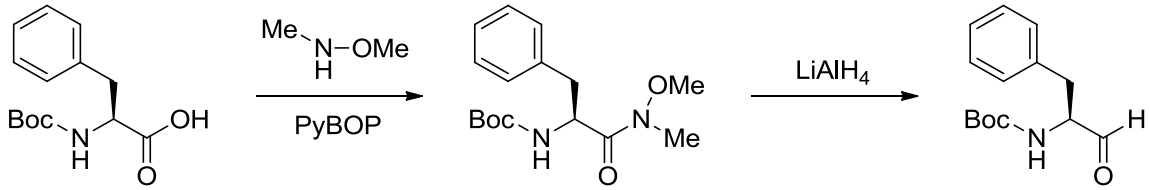
2.2.1 Amino Aldehyde Synthesis

A common pathway to aldehydes is via Swern oxidation,[89] whereby alcohols are oxidized to aldehydes or ketones using oxalyl chloride, dimethyl sulfoxide and a base. Reduction of esters and amides may also produce aldehydes, however a big concern is over-reduction to primary alcohols. This problem is rectified in the reduction of *N*-methoxy-*N*-methylamides (Weinreb amides) by lithium aluminium hydride, in that no alcohol-containing products are observed.[90]



Scheme 2.18 Swern oxidation for the synthesis of aldehydes from alcohols (left),[89] and reduction of Weinreb amides to aldehydes with lithium aluminium hydride (right).[90]

Formation of the amide was initially performed by Weinreb *et al*.[90] via nucleophilic substitution of *N*,*O*-dimethylhydroxylamine with an acid chloride, however it has since been shown that a variety of milder peptide coupling conditions may be used to couple *N*,*O*-dimethylhydroxylamine directly onto carboxylic acids.[91]



2.17a **2.18a** **2.14a**

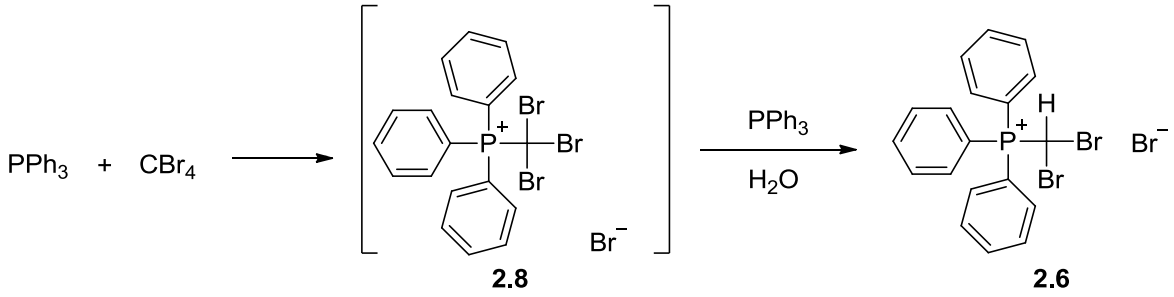
Scheme 2.19 Peptide coupling of Boc phenylalanine to *N,O*-dimethylhydroxylamine giving Weinreb amide 2.18a, followed by reduction to aldehyde 2.14a with lithium aluminium hydride.

Thus Boc-phenylalanine was converted to the Weinreb amide via PyBOP-mediated coupling to *N,O*-dimethylhydroxylamine in DCM. Characteristic methyl signals at δ 3.65 (*O*-methyl) and δ 3.15 (*N*-methyl) observed in the ^{1}H NMR spectrum, and a peak corresponding to the protonated molecular ion (*m/*z = 309), provided evidence for the formation of Weinreb amide **2.18a**, which was consistent with the literature.[91] The reaction was scaled up to 5 g of acid starting material without any appreciable drop in yields (~70 %).

Reduction to Boc-phenylalaninal (**2.14a**) was performed via the addition of 1.25 equivalents of lithium aluminium hydride at 0 ^{o}C. The reaction was performed multiple times with yields of up to 86 %, and quick confirmation of the product by ^{1}H NMR was available via the presence of a characteristic δ 9.65 singlet corresponding to the aldehyde, which was consistent with the literature.[91] The aldehyde produced was found to readily degrade, thus was either freshly prepared or stored in the refrigerator for short periods of time. According to Castro *et al.,*[91] performing column chromatography on amino aldehydes can cause epimerization, and since the desired aldehyde was of good purity no further purification was attempted.

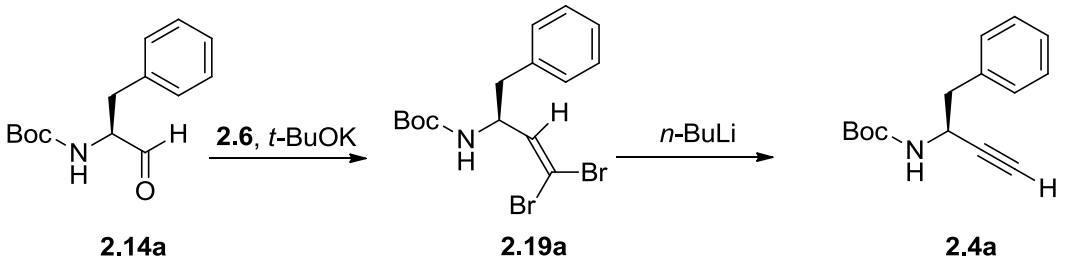
2.2.2 Corey Fuchs Homologation

Corey-Fuchs homologation was then used to transform the *N*-protected amino aldehydes into *N*-protected amino alkynes.



Scheme 2.20 Synthesis of ylide-like reagent 2.6 via a reaction between triphenylphosphine and carbon tetrabromide.

As demonstrated by Wolkoff,[68] reagent **2.6** was synthesized by the addition of water to a stirring solution of carbon tetrabromide (1 eq) and triphenylphosphine (2 eq) in DCM at 0 ^{o}C, giving the product in 84 % yield after isolation. A signal at δ 10.1 in the ^{1}H NMR spectrum appeared to correspond to the CH proton, based on a 1:15 integration pattern compared to the phenyl protons (δ 8.21-7.69, 15H, m), and a signal corresponding to the protonated molecular ion (*m/z* = 354) was observed by mass spectroscopy. The reaction was scaled up to 20 g (product) in order to provide sufficient material for subsequent reactions.



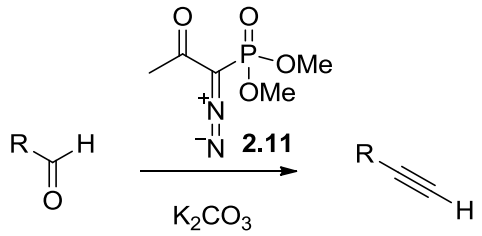
Scheme 2.21 2-step synthesis of key alkyne 2.4a via a Corey-Fuchs procedure from aldehyde 2.14a.[64]

After activation of reagent **2.6** by the addition of potassium *tert*-butoxide (2.4 eq) in anhydrous THF, Boc-phenylalaninal (**2.14a**) was added at 0 ^{o}C. After one hour the reaction was cooled to -78 ^{o}C and *n*-butyllithium (3 eq) was added, and the reaction was worked up after stirring for a further 2 hours at -78 ^{o}C. The ^{1}H NMR spectrum of material purified by column chromatography showed a signal corresponding to the alkyne hydrogen at δ 2.28 (1H, d) which was consistent with the literature,[92] and a signal corresponding to the protonated molecular ion (*m*/z = 246) was observed by mass spectroscopy. The reaction, however, was low-yielding (18 %). Quenching the reaction prior to the addition of *n*-butyllithium allowed isolation of the dibromo-olefin intermediate (**2.19a**) in 65 % yield after

column chromatography. The subsequent *n*-butyllithium-promoted elimination gave the alkyne in 82 % yield, thus giving 53 % over two steps. The stereochemical integrity of the resulting alkyne was investigated by comparison of the optical rotation to the literature value. The experimental value seen, $[\alpha]^{21}_{D}$ = -9.7^{o} (c = 1.07, $CHCl_3$), was indeed consistent with the literature:[92] $[\alpha]^{21}_{D}$ = -10.6^{o} (c = 1.01, $CHCl_3$), showing little evidence of epimerisation.

2.2.3 The Ohira-Bestmann Reaction

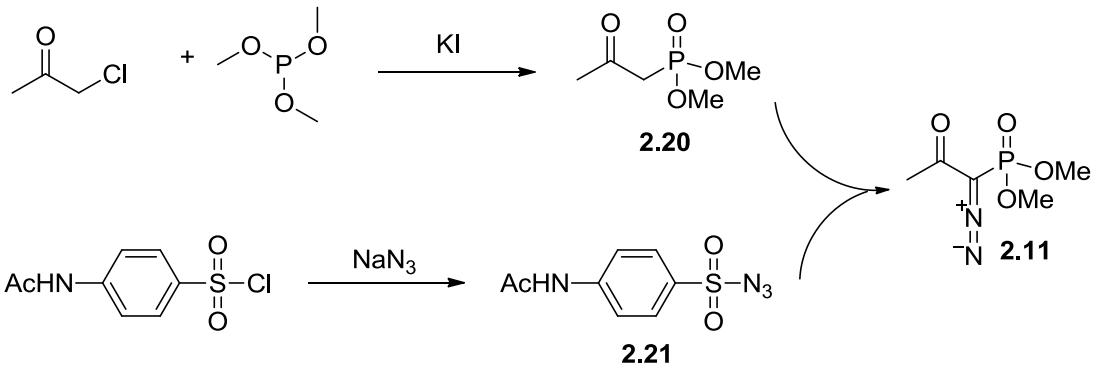
Based on the varying yields and relatively harsh conditions of the Corey-Fuchs homologation reaction, the Ohira-Bestmann reaction was explored as an alternative method for the conversion of *N*-protected amino aldehydes to *N*-protected amino alkynes.



Scheme 2.22 General synthesis of alkynes from aldehydes via the Ohira-Bestman reaction.[65, 66]

The Ohira-Bestmann reagent (**2.11**) is not commercially available, however it may be prepared from phosphonate **2.20**, which was commercially available from Sigma-Aldrich. The scale required suggested that the synthesis of the phosphonate would be more cost effective.

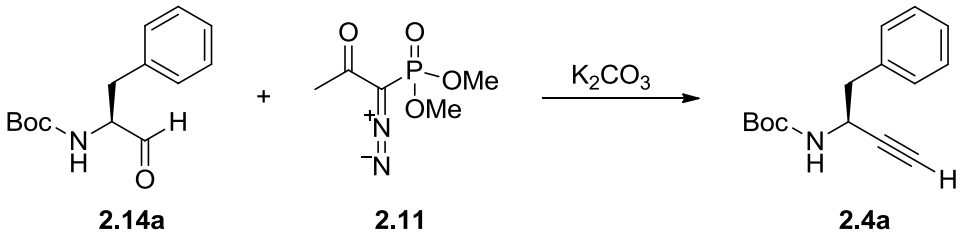
Formation of the starting material for the Ohira-Bestmann reagent, dimethyl-2-oxopropylphosphonate (**2.20**) was performed by a Michaelis-Arbuzov reaction between trimethyl phosphate and iodoacetone - formed *in situ* via the addition of potassium iodide (1 eq) to chloroacetone (1 eq). Vacuum distillation (2.7 mbar, 105 ^{o}C) gave phosphonate **2.20** in 39 % yield. The peaks found in the ^{1}H spectrum, including the key methylene signal at δ 3.11, (2H, d $^{2}J_{PH}$ = 70 Hz), were consistent with the literature.[93]



Scheme 2.23 Synthesis of key phosphonate 2.20 from a reaction between chloroacetone and triphenylphosphite.

Diazotransfer to the phosphonate results in production of the Ohira-Bestmann reagent (**2.11**). As shown by Vanderwall,[94] sulfonyl azide **2.21**, as a shelf-stable substitute for the more commonly used triflyl azide, is a suitable reagent for this reaction. The azide was prepared via the nucleophilic substitution of a sulfonyl chloride (1 eq) with sodium azide (1.6 eq), giving 72 % yield after isolation. FTIR indicated the presence of an azide with a sharp peak appearing at ~2120 cm^{-1}, consistent with the literature value.[93]

A diazo-transfer reaction between azide **2.21** (1.2 eq) and phosphonate **2.20** (1 eq) was then performed in toluene at 0 ^{o}C, mediated by the addition of sodium hydride (1.5 eq) to the phosphonate (1 eq), giving the Ohira-Bestman reagent (**2.11**) in 40 % yield. FTIR indicated the presence of the diazo group at 2128 cm^{-1},[93] and a peak corresponding to the protonated molecular ion (*m/z* = 193) was observed by ESI-MS.



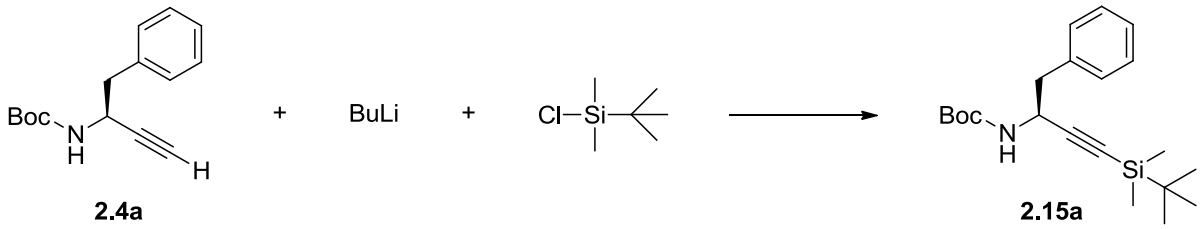
Scheme 2.24 Synthesis of key alkyne 2.4a via an Ohira-Bestmann reaction with aldehyde 2.14a.

Boc-phenylalaninal (**2.14a**) was converted to the corresponding alkyne (**2.4a**) in 67 % yield, via the addition of the Ohira-Bestmann reagent (**2.11**, 1.2 eq) to a solution of the aldehyde (**2.14a**, 1 eq) and potassium carbonate in methanol. The ^{1}H NMR spectrum of the crude product suggested it was of high purity, and identical to that of previously prepared alkyne

2.4a. However the optical rotation was found to be -6.67° (c = 1.0, $CHCl_3$), rather than the expected rotation of -10.6° (c = 1.01, $CHCl_3$),[92] showing that racemisation had occurred. Although a lower temperature was expected to reduce the extent of racemisation, the reaction did not proceed at -78 °C, and between -20 °C to 0 °C racemisation was still apparent. Replacing the reaction solvent with ethanol instead of methanol showed a slight improvement in enantiopurity; presumably due to the weakening of the base formed *in situ* with potassium carbonate.

Thus although the Ohira-Bestmann procedure[65, 66] had increased yields, easier purification and a significant atom-economy advantage over the ylide-based conversion, due to the inability to completely negate racemisation, the phenylalanine-derived alkyne used in further reactions was synthesized using the Corey-Fuchs procedure outlined previously (2.2.2).

2.2.4 Silyl Protection of Amino Alkynes



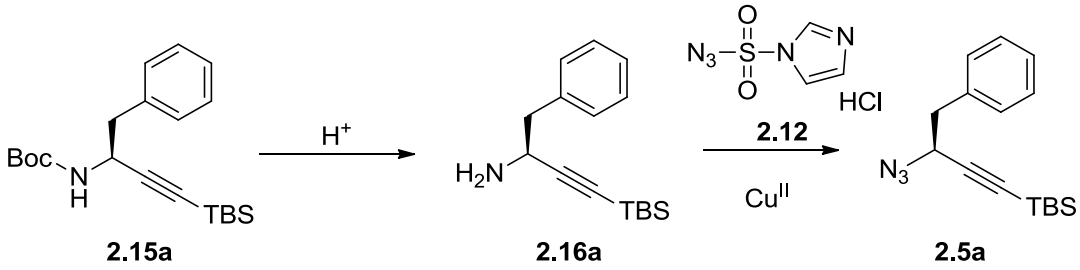
Scheme 2.25 TBS protection of alkyne 2.4a via formation of the acetylide and addition of *tert*-butyldimethylsilyl chloride.

Protection of the alkyne with *tert*-butyl dimethylsilyl chloride was investigated as a means of retaining alkyne functionality on the *C*-terminus of a growing peptidomimetic chain (see scheme 2.14) or as a key feature of the 2-step chain extension sequence (scheme 2.15). *tert*-Butyl dimethylsilyl chloride was used because the protecting group formed is more stable than the trimethylsilyl group to the acidic conditions required for Boc-deprotection at a later stage.

After deprotonation of **2.4a** with *n*-butyl lithium (2.2 eq) in THF at -78 °C, addition of *tert*-butyldimethylsilyl chloride (1 eq) resulted in the TBS-protected alkyne (**2.15a**). Characteristic signals corresponding to the silyl group were found in the ^{1}H NMR spectrum: δ 0.89 (s, 9H) and 0.10 (s, 6H), and a peak corresponding to the protonated molecular ion (*m/z* = 361) was observed in the ESI-MS, confirming that the silyl group was attached to the alkyne. As the

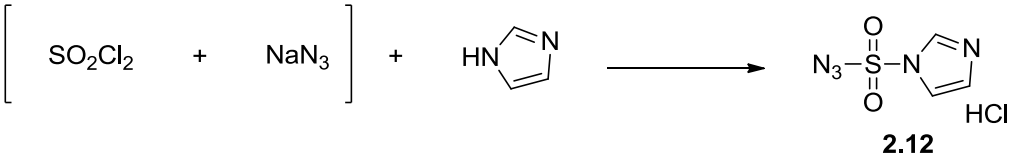
NMR spectrum indicated that the material was of high purity, no further purification was undertaken.

2.2.5 N-Deprotection and Diazotransfer to form Azido Alkynes



Scheme 2.26 2-step *N*-deprotection and diazotransfer of alkyne intermediate 2.15a to give key azide monomer unit 2.5a.

Derivatisation of the TBS-protected alkyne (**2.15a**) to the corresponding azide was required to produce key monomer unit **2.5a**. This was to be accomplished in a 2-step process: *N*-deprotection followed by diazotransfer. Although triflic azide is commonly used for diazotransfer reactions of primary amines to azides due to its high reactivity, it is not shelf stable, and is required to be prepared freshly prior to diazotransfer reactions. Goddard-Borger *et al.*[77] showed that azide **2.12** could perform in diazo-transfer reactions, and was considered to be shelf stable at the time.



Scheme 2.27 Synthesis of the Goddard-Borger diazotransfer reagent (2.12) from sulfuryl chloride, sodium azide and imidazole.[77]

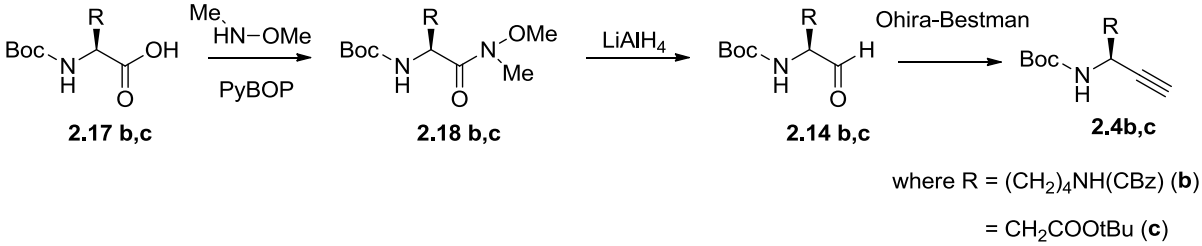
Dropwise addition of sulfuryl chloride (1 eq) to a cooled solution of sodium azide (1 eq) in acetonitrile, followed by treatment with imidazole (1.9 eq), gave azide **2.12**. Formation of the hydrochloride salt of the azide was mediated by the addition of an ethanolic solution of HCl, formed *in situ* via a reaction between acetyl chloride and ethanol. The three signals observed in the ^{1}H NMR spectrum were somewhat different to those reported in the

literature, with the peaks δ 8.57 (1H, s), 7.83 (1H, s) and 7.39 (1H, s) present, as opposed to δ 9.53 (1 H, dd, *J* = 1.3, 1.6 Hz), 8.09 (1 H, dd, *J* = 1.6, 2.2 Hz) and 7.68 (1H, dd, *J* = 1.3, 2.2 Hz) in the literature.[77] A peak corresponding to the protonated molecular ion (*m/z* = 174) was observed in the ESI-MS.

TFA-mediated *N*-deprotection of Boc-amine **2.15a** was performed, with the ^{1}H NMR spectrum and ESI-MS confirming both removal of the Boc protecting group, and that the silyl group remained attached to the alkyne (*m/z* = 260 [MH^+]) after the acidic conditions. Free amine **2.16a** (1 eq) was subsequently converted to the azide (**2.5a**) via a diazo-transfer reaction with reagent **2.12** (1.2 eq), catalysed by copper sulfate (0.02 eq). The TBS-protected alkynyl azide (**2.5a**) was isolated in 29 % yield in the 3-step conversion from alkyne **2.4a** with purification only after the final reaction. Although the protonated molecular ion was not observed by ESI-MS, a peak corresponding to the fragmentation product (*m/z* = 258 [$(M\text{-}N_2)H^+$]) was present.

2.2.6 Monomer Synthesis of other Amino Acid Side-Chains

To demonstrate that the monomer unit synthesis was a general procedure that could be applied to a wide variety of amino acids, the synthesis of derivatives of both lysine and aspartic acid were attempted. These amino acids were chosen as they contain functionality that could potentially interfere with the monomer unit synthesis protocol in ways that the other non-polar amino acids would not. The side chain of lysine was orthogonally protected as the Cbz carbamate, and aspartic acid as the *tert*-butyl ester. Both of these side-chain protected amino acids are commercially available due to their use in Boc-based peptide synthesis.



Scheme 2.28 Derivatisation of Boc-(Cbz)-lysine and Boc-(O*t*Bu)-aspartic acid to the corresponding Weinreb amides (2.18b,c) via peptide coupling to *N*,*O*-dimethylhydroxylamine, followed by reduction to the aldehydes (2.14b,c) and Ohira-Bestmann homologation to the alkynes (2.4b,c).

The Weinreb amide derivatives of Boc-(Cbz)-lysine (**2.17b**) and Boc-(O*t*Bu)-aspartic acid (**2.17c**) were prepared via standard peptide coupling to *N*,*O*-dimethylhydroxylamine, giving **2.18b** and **2.18c** in 82 % and 90 % yield respectively. For lysine derivative **2.18b**, the ^{1}H NMR spectrum displayed one of the Weinreb amide methyl signals at δ 3.68 (3H, s), while the other methyl signal was located at the same region as the Cbz-methylene peak: δ 3.12 (5H, m). These peaks were distinctly seen in the ^{1}H NMR spectrum of the aspartic acid derivative **2.18c** at δ 3.80 (3H, s) and 3.23 (3H, s).

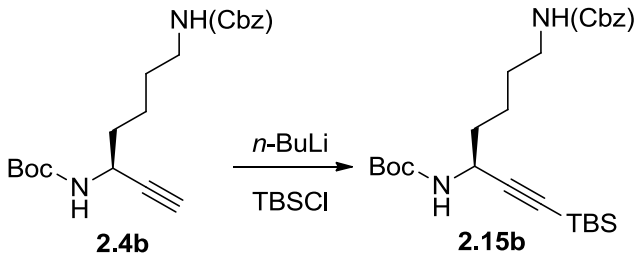
Lithium aluminium hydride was used to reduce Weinreb amides **2.18b** and **2.18c** to the corresponding aldehydes. The Boc-(Cbz)-lysine derived Weinreb amide (**2.18b**) was found to be less reactive than the corresponding phenylalanine derivative (**2.18a**), and up to 4 equivalents of lithium aluminium hydride was required for complete conversion of the starting material. The key aldehyde proton signal was found in the ^{1}H NMR spectrum at δ 9.51 (1H, s). As with the analogous Boc-phenylalanal case, column chromatography was not performed on this compound due to the potential for racemisation.

Unidentified byproducts were found when the Boc-(O*t*Bu)-aspartic acid-derived Weinreb amide (**2.18c**) was reacted at both 0 ^{o}C and at room temperature, suggesting that the side-chain ester was not compatible with the conditions used. Reducing the reaction temperature to – 78 ^{o}C appeared to give cleaner conversion to the aldehyde. The key aldehyde proton signal was found in the ^{1}H NMR spectrum at δ 9.66 (1H, s).

Homologation of the aldehydes was performed via the milder Ohira-Bestmann protocol, as the use of *n*-butyllithium as part of the Corey-Fuchs procedure was anticipated to be incompatible with the side-chain protecting groups of amino aldehydes **2.14b** and **2.14c**. Similar yields were seen for the lysine derivative (**2.4b**) to phenylalanine derivative **2.4a**, and the key alkyne ^{1}H NMR signal was seen in the spectrum at δ 2.27 (1H, d). Although the compound was optically active, the magnitude of optical rotation was less than the literature value[88] ($[\alpha]^{21}_{D}$ = -9.3 compared with $[\alpha]^{21}_{D}$ = -23.1) suggesting that some racemisation may have occurred.

Much lower yields were seen for the aspartic acid derivative, with yields between 10-30 % of isolated product (**2.4c**) consistently seen, in part due unexpected transesterification of the side-chain *tert*-butyl ester with methanol to the corresponding methyl ester. The alkyne signal of the product was again located in the ^{1}H NMR spectrum at δ 2.27 (1H, d). The optical rotation of the compound was determined to be $[\alpha]^{21}_{D}$ = -11.1, but without literature precedent it was not evident whether partial racemisation had occurred at this stage. Given that the reactions leading to alkyne **2.4c** were poorly yielding, synthesis of the aspartic acid-

alkyne derivative was put on hold, and this issue of stereochemistry was not addressed until aspartic acid derivatives were re-explored at a later stage (chapter 4.3.1).



Scheme 2.29 Silylation of lysine-derived alkyne 2.10b via formation of the acetylide with *n*-butyllithium and addition of *tert*-butyl dimithylsilylchloride.

Initially, synthesis of TBS-protected alkyne **2.15b** was attempted with the same conditions as the phenylalanine derivative (**2.15a**), with the acetylide formation with *n*-butyllithium (3 eq) and TBS-chloride addition procedure attempted. However the presence of a carbamate on the side-chain appeared to interfere with the formation of the acetylide, with variable amounts of unreacted alkyne detected in the ^{1}H NMR spectrum. Modifications to the procedure including changing the amount of *n*-butyllithium and *tert*-butyldimethylsilyl chloride, and performing the reaction between -78 ^{o}C and 0 ^{o}C did not improve the procedure, with the ^{1}H NMR spectrum showing up to 95 % of an unprotected terminal alkyne signal. As large quantities of *n*-butyllithium (~4 eq) resulted in cleavage of the Cbz-side chain protecting group, it was proposed that the use of a less nucleophilic base would negate this side-reaction.

LDA (lithiumdiisopropylamide) was used as a substitute for *n*-butyllithium as a strong base, as its steric bulk results in a reduction of its nucleophilicity. LDA was formed *in situ* by the addition of *n*-butyllithium to diisopropylamine in THF at -78 ^{o}C. After titration, it was added to lysine-derived alkyne **2.4b** in THF at -78 ^{o}C, followed by the addition of *tert*-butyldimethylsilyl chloride. However again, incomplete conversion of up to 50 % was observed as shown by the presence of the starting material alkyne peak in the crude ^{1}H NMR spectrum at δ 2.27 (1H, d, ^{4}J = 8Hz). When larger amounts of LDA were used, as with *n*-butyllithium, Cbz-deprotection was evident.

As a response to requiring the stoichiometric use of either organolithium or Grignard reagents to effect the silyl-protection of terminal alkynes, which may result in issues when performing the reactions on large scale, Sugita *et al*.[86] reported the direct silylation of terminal alkynes with trialkylchlorosilanes mediated by with zinc powder at elevated

temperatures (120 ^{o}C). It was proposed that this reaction proceeded via an *in situ* generated reactive alkynylzinc species.

Similarly, it has been suggested that the formation of a π-complex between transition metals and alkynes results in the terminal C-H bond becoming labile to the point that where deprotection may occur with a relatively weak base.[95] This was exploited by Carreira *et al*.[96] who reported the catalytic formation of alkynylzinc molecules from terminal alkynes at room temperature, utilising zinc (II) triflate in the presence of a mild amine base. Silyl protection using the zinc triflate-derived alkynylzinc species and trialkylchlorosilanes or trialkylsilyltriflates was shown by Zhu *et al*.[87] and Rahaim Jr. *et al*.[97] respectively, who effected the transformation at room temperature without the requirement of a strong base.

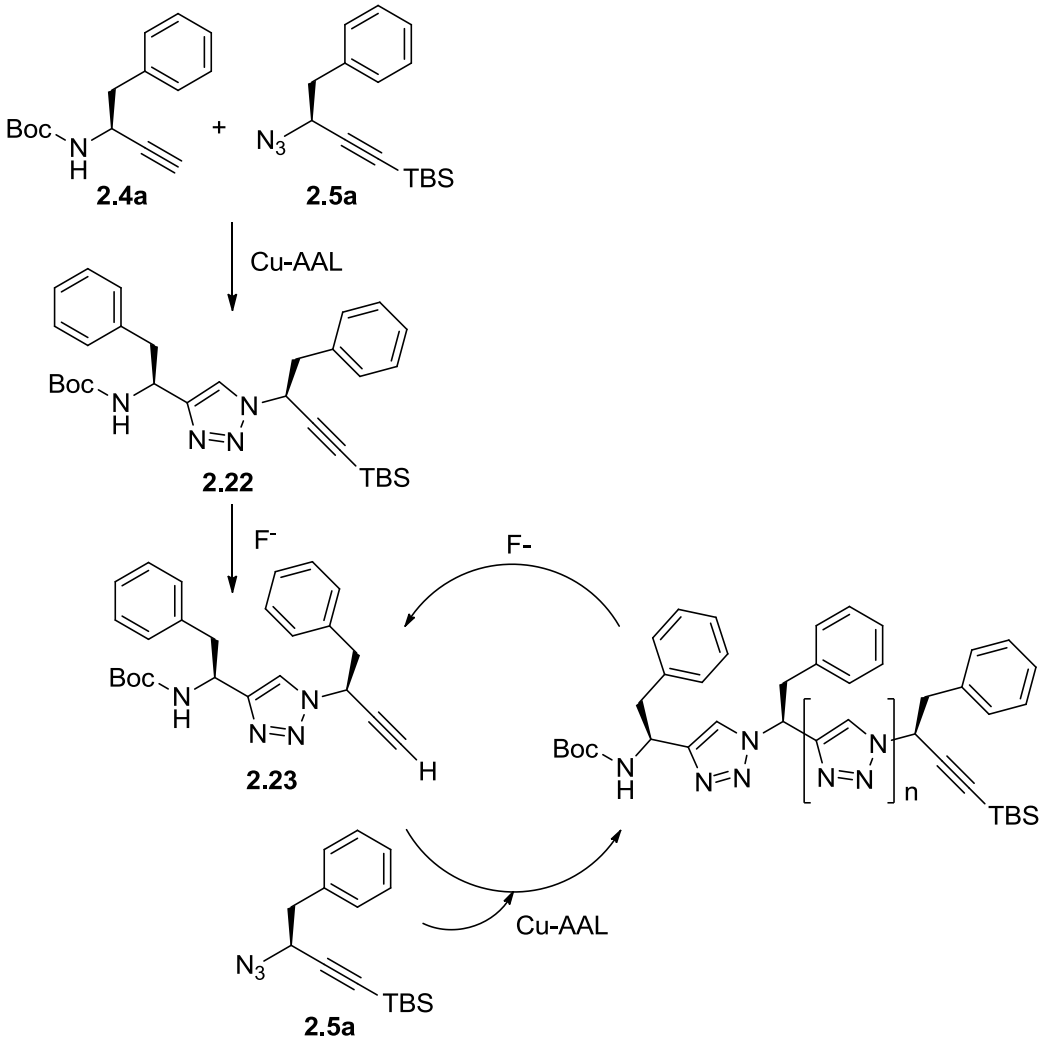
Following the conditions of Sugita,[86] activated zinc dust (4 eq) was added to lysine-derived alkyne **2.4b** (1 eq) with TBS chloride (2 eq) in acetonitrile. After heating to 120 ^{o}C in a sealed tube for 5 hours, the reaction was left to cool to room temperature and the zinc dust was filtered. Analysis of the crude ^{1}H NMR spectrum was performed, showing that again complete protection had not occurred, as shown by the presence in part of the alkyne signal at δ 2.35. In addition, the Boc-protecting group had been cleaved, presumably due to the high temperature used to mediate the reaction.

Due to the incompatibility of the Boc group to the high temperatures, the conditions of Zhu were attempted,[87] which involved the addition of alkyne **2.4b** (1 eq) to a solution of zinc triflate (1.2 eq) and triethylamine at 30 ^{o}C. After an hour TBS chloride was added and the reaction was allowed to stir overnight. The crude ^{1}H NMR spectrum showed that 66 % of the alkyne still remained unprotected. In light of the promising results discussed in chapter 4 for the synthesis of a silyl-protected lysine derivative compound similar to alkyne **2.15b**, no further optimisation of the reaction was performed.

2.3 Chain Extension

2.3.1 Click Coupling C-Extension

Although the synthesis of the alkynyl-azide derivative of phenylalanine (**2.5a**) had been successful, the corresponding derivatives of lysine and aspartic acid (**2.5b,c**) had not been synthesised. Regardless, the click coupling chain extension cycle was attempted using only the phenylalanine-derived monomer units **2.4a** and **2.5a** as a test case for the 2-step *C*-terminal chain extension cycle.

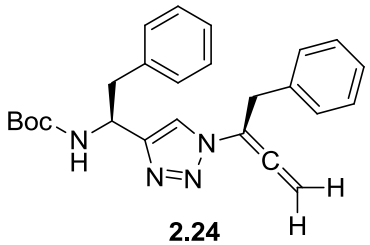


Scheme 2.30 *C*-terminal triazole peptidomimetic iterative chain extension with phenylalanine-derived alkyne and azide monomer units 2.4a and 2.5a.

Formation of the first triazole link between the phenylalanine-derived monomer units was performed by coupling alkyne **2.4a** (1 eq) and azide **2.5a** (1 eq) in a solution of *tert*-butanol : water (2 : 1), with copper sulfate pentahydrate (0.1 eq), ascorbic acid (1 eq), and TBTA (**1.4**, 0.1 eq), giving dimer **2.22** in 60 % yield. The ^{1}H NMR spectrum showed the 2 key α-proton signals at δ 5.64 (1H, t) and 5.08 (1H, q), but the triazole peak was obscured by the side-chain aromatic signals. A peak corresponding to the protonated molecular ion (*m/z* = 532) was observed in the ESI-MS.

TBS-protected dimer **2.22** was treated with TBAF for the production of alkyne **2.23**, but no alkyne or silyl peaks were observed by the ^{1}H NMR spectrum of the purified material. This was surprising given that TLC had showed that complete consumption of the starting material, and mass spectrometry showed a signal corresponding to the anticipated

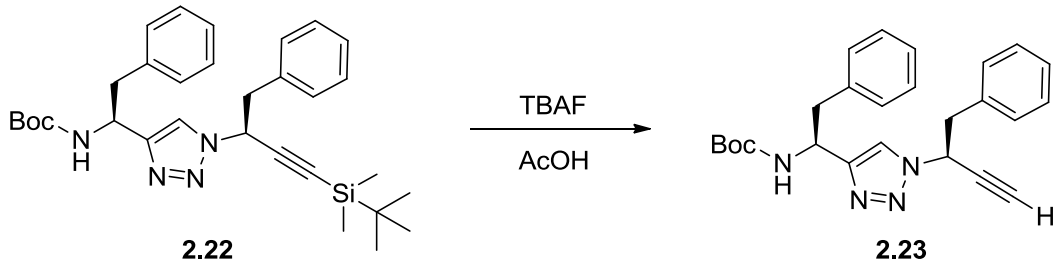
molecular weight (*m/z* = 417 [MH^+]). Interestingly, two sets of apparent triplet signals were observed in the ^{1}H spectrum: δ 5.42 (2H, t, ^5J = 2.7 Hz), δ 4.10 (2H, t, ^5J = 2.5 Hz), and only one signal corresponding to an α-proton was present, at δ 5.02 (1H, t, *J* = 6.3 Hz). Only one set of peaks characteristic of the β-protons seen in dimer **2.22** were observed in the region between δ 3.5-3.0 (2H, m). It was speculated that elimination, presumably base catalysed by the fluoride, resulted in the formation of allene **2.24** below. This was supported by the presence of a characteristic peak in the ^{13}C NMR spectrum at δ 201.9, corresponding to the central allene carbon.



2.24

Figure 2.2 Allene biproduct 2.24.

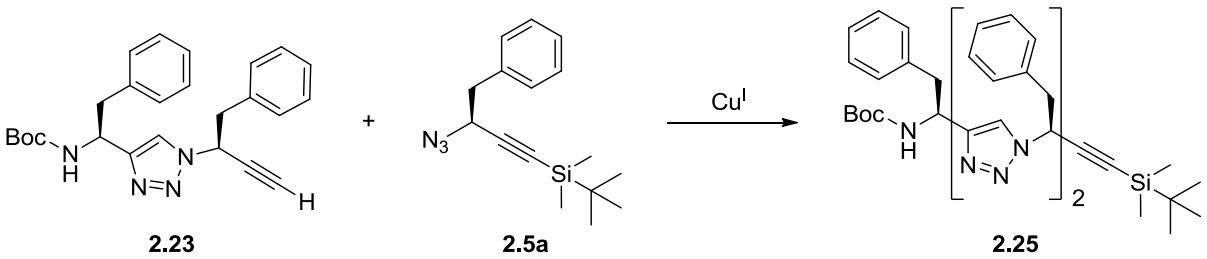
This rearrangement would have been a significant setback for our silyl-alkyne protecting group approach. A literature search showed that on occasion TBAF-mediated silyl deprotection was buffered with acid in an attempt to circumvent the basicity of the fluoride ion.[97]



2.22 **2.23**

Scheme 2.31 TBAF-mediated desilylation of dimer 2.17 buffered with acetic acid.

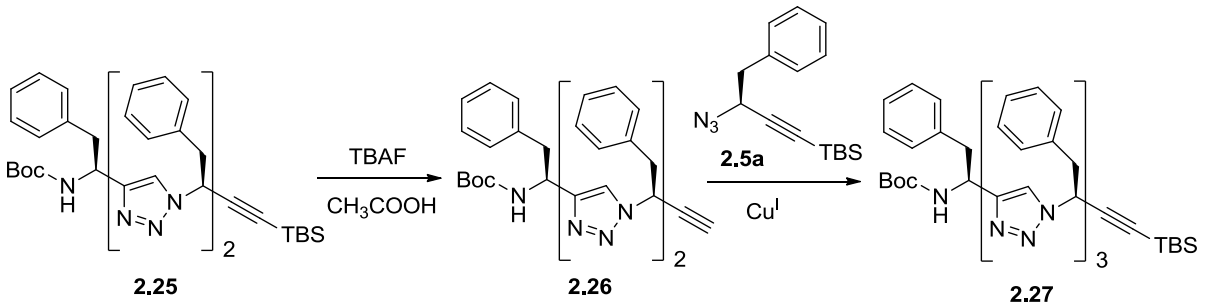
Repeating the reaction by the addition of a solution of TBAF (4 eq) and acetic acid (4 eq) in THF gave complete consumption of starting material after 1 hour by TLC, and no spot was observed with an R_f corresponding to allene **2.24**. After purification, the desired dimer (**2.23**) was isolated in 79 % yield, with the terminal alkyne signal observed at δ 2.58 (1H, d) in the ^{1}H NMR spectrum.



2.23 **2.5a** **2.25**

Scheme 2.32 Cu-AAL reaction between alkyne dimer 2.23 and azide 2.5a to give trimer 2.25.

The terminal alkyne-containing dimer **2.23** was then coupled to azido monomer unit **2.5a** under similar Cu-AAL conditions as previously shown. Trimer **2.25** was isolated in 68 % yield, with an additional α-proton peak seen in the ^{1}H NMR spectrum at δ 5.91 (1H, t), and a triazole peak at δ 7.37 (1H, s).



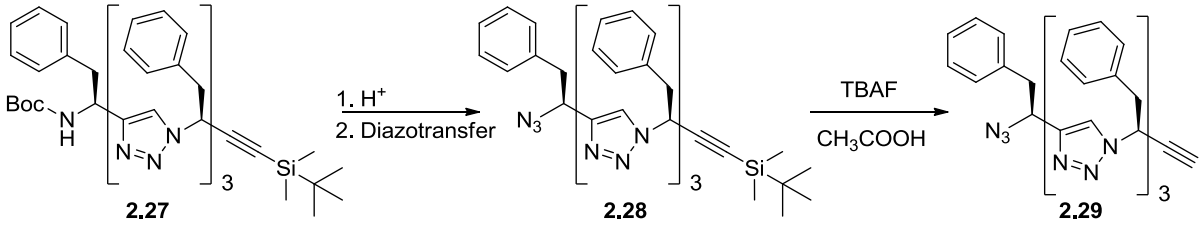
2.25 **2.26** **2.27**

Scheme 2.33 TBAF-mediated desilylation of trimer 2.25 buffered with acetic acid, followed by a Cu-AAL reaction with azide 2.5a to give tetramer 2.27.

As before, the chain was then extended in two steps. Silyl deprotection of trimer **2.25** was accomplished by the addition of TBAF (4 eq) and acetic acid (4 eq) in THF, followed by column chromatography to give trimer **2.26** in 84 % yield. A peak corresponding to the protonated molecular ion (*m/z* = 588.8) was observed in the ESI-MS, and the alkyne signal was present at δ 2.64 (1H, d) in the ^{1}H NMR spectrum.

Cu-AAL coupling of alkynyl-trimer **2.26** with azide **2.5a** gave tetramer **2.27** in 63 % yield. The ^{1}H spectrum displayed three α-proton signals, with signal in the δ 5.91 region integrating for two protons, suggesting superimposition of the non-terminal α-proton signals. The other α-proton signals were located at δ 5.66 (1H, m) and 5.27 (1H, m). A peak corresponding to the protonated molecular ion (*m/z* = 874.4) was observed in the ESI-MS.

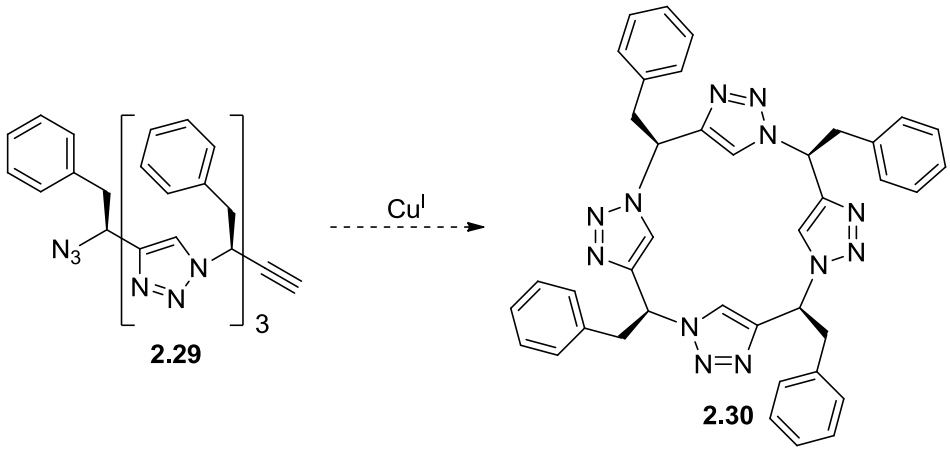
2.3.2 Cyclisation Attempts



Scheme 2.34 N-deprotection and diazotransfer of tetramer 2.27 to give azide 2.28, followed by TBAF-mediated desilylation with acetic acid to give the precyclisation unit azido-alkyne 2.29.

For cyclisation to occur, a linear chain containing an *N*-terminal azide and *C*-terminal alkyne was required. Starting from the phenylalanine-derived tetramer **2.27**, *N*-deprotection with TFA and diazotransfer using the Goddard-Borger reagent (**2.12**) was performed, giving azide **2.28** in 47 % yield after isolation.

Silyl deprotection of azido-tetramer **2.28** was accomplished by the addition of a solution of TBAF (4 eq) and acetic acid (4 eq) in THF, with the key alkyne signal appearing in the ^{1}H NMR spectrum at δ 2.66 (1H, d). After the extension sequence and subsequent diazotransfer and silyl-deprotection reactions, only 5.3 mg of crude azido-alkyne **2.29** was obtained. It was noted by Arora that long-chain azides may decompose during column chromatography,[53] thus the small amount of compound produced was reacted further without purification.



Scheme 2.35 Attempted head-to-tail Cu-AAL based cyclisation of tetramer 2.29 for the synthesis of cyclic tetramer 2.30.

The same Cu-AAL catalyst mixture and solvent system as used in the linear extension sequence was used for attempting the head-to-tail cyclisation of azido-alkyne **2.29**. In an attempt to minimise the possible polymerisation side-reaction, a concentration of 1.5 mM was used, roughly 100 times more dilute than during the chain extension Cu-AAL reactions, and comparable to the 1.0 mM concentration used by Bock for Cu-AAL based cyclisation reactions.[45] After allowing to stir for 3 days, the solvent was concentrated and a ^{1}H NMR spectrum of the crude material was taken to determine whether any cyclised product had formed. It was expected that unreacted starting material would polymerise during the work-up process.

The cyclised product was anticipated to be completely symmetrical, hence one set of signals was expected to be observed for the product by ^{1}H NMR. No distinct signals were seen in the crude spectrum however, thus column chromatography was performed in order to separate any products or side-products. When the fractions were analysed by ^{1}H NMR there was no indication of the presence of the desired product, nor any residual starting alkyne material. It was inferred that either polymerisation had been the favoured reaction pathway, or that the rate of the reaction was too slow to allow for the formation of an appreciable amount of product over 3 days.

2.4 Conclusions

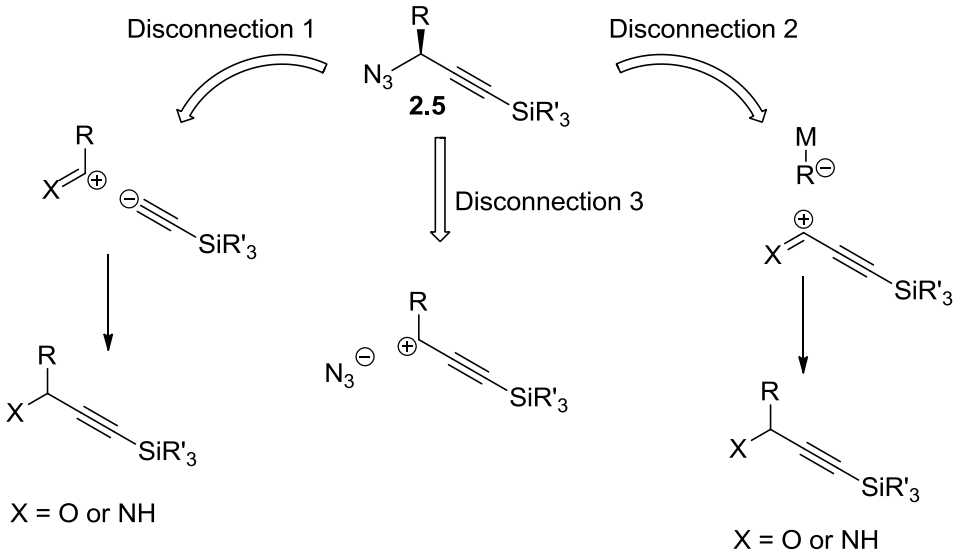
With a limited amount of the tetrameric cyclisation precursor **2.29** at our disposal, phenylalanine-based cyclic tetramer **2.30** was unable to be isolated. It was hypothesised that the polymerisation pathway was causing consumption of a large proportion of the starting material, suggesting that a larger dilution factor or a different catalyst set may be required to favour the head-to-tail cyclisation reaction pathway. To perform such test reactions, a larger quantity of linear precursor **2.29** was required. The solution phase chain extension route performed adequately, consistently giving yields greater than 60 % for both the Cu-AAL and TBAF-mediated silyl-deprotection. Thus it was thought that the biggest limitation in this synthesis was the access to the monomer units themselves. Synthesis of the key phenylalanine-derived azido-alkyne monomer **2.4a** was a 7-step synthesis with an overall yield of 10 %, with the conversion from the aldehyde to the alkyne (sections 2.2.2 and 2.2.3) being the transformation which caused the most difficulties, giving variable and generally very poor yields. A bigger issue was seen with the derivatisation of other amino

acids, with various reaction incompatibilities seen with different amino acid side-chains. These issues are addressed in chapters 3 and 4.

Chapter 3 - A New Approach to Triazole Peptidomimetics

3.1 Retrosynthesis of the Key Silyl-protected Azido-alkyne

It became apparent that it was not feasible to use the synthetic pathway described in chapter 2 for the synthesis of appropriate amounts of the 'extending unit' monomers (**2.5**). The reactions employed, specifically formation of the dibromoolefin (section 2.2.2) and the diazotransfer reaction to the azide (section 2.2.5) did not consistently give high yields of products, which made it difficult to establish a large enough amount of material for use in the chain extension cycles. Furthermore, when mimics of amino acids, containing more elaborate side-chain functionality than phenylalanine were attempted, additional problems were encountered. Thus new routes for generating silyl-protected azido-alkynes were devised.



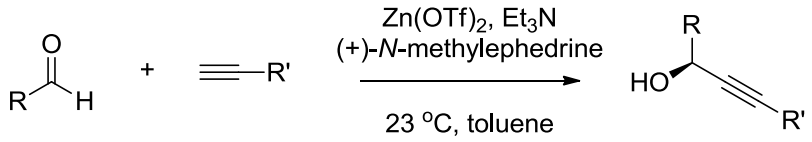
Scheme 3.1 Retrosynthetic analysis for key monomer unit 2.5.

Retrosynthetically, 3 possible disconnections are shown in scheme 3.1 for the formation of the key monomer unit **2.5**. Disconnection 1 can be thought of as an attack of a silyl-protected acetylide ion on an appropriate electrophile, such as an aldehyde or an imine. The aldehyde or imine would contain functionality analogous to the amino acid side-chain to be mimicked. In a similar light, disconnection 2 could be viewed as an attack of an organometallic reagent onto an appropriate alkyne substituted electrophile. In this case however, the organometallic reagent would be required to contain the amino acid side chain-

like functionality. In either case, transformation of either the alcohol or amine produced from nucleophillic attack would be required to provide the azide. The electrophile in disconnection 3 would be considerably more difficult to synthesise than those in disconnections 1 and 2, particularly in a manner consistent with straightforward variation of the side-chain R, and was not examined in detail. For all 3 approaches, special consideration would need to be taken to ensure that the products produced were enantiomerically pure.

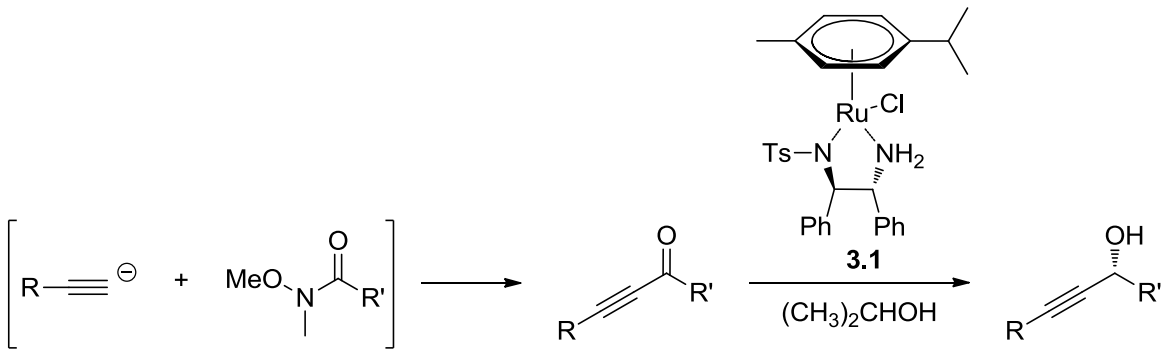
3.1.1 Disconnection 1: The acetylide anion approach

It has been shown that achiral secondary propargylic alcohols are formed by the reaction of an aldehyde and an acetylide ion.[98] As detailed in scheme 3.2, Carreira *et al.*[99] produced an asymmetric version of this reaction, starting with aldehydes and terminal alkynes. They noted that a catalytic system consisting of zinc triflate and *N*-methylephedrine was able to perform this reaction on a range of substrates, with up to 99% ee under mild conditions. They suggest that an *in situ* generated zinc alkynylide is able to attack an aldehyde in a nucleophilic manner, unlike the similar copper (I) acetylides, which are not prone to undergo nucleophilic addition to aldehydes.[99] The mechanism by which *N*-methylephedrine is used to impart stereoselectivity on the reaction was not discussed, other than that it was a putative ligand for Zn(II). It is worth noting that, for our purposes, this reaction pathway would require access to aldehydes which correspond to the amino acid side chain of the amino acid to be mimicked.



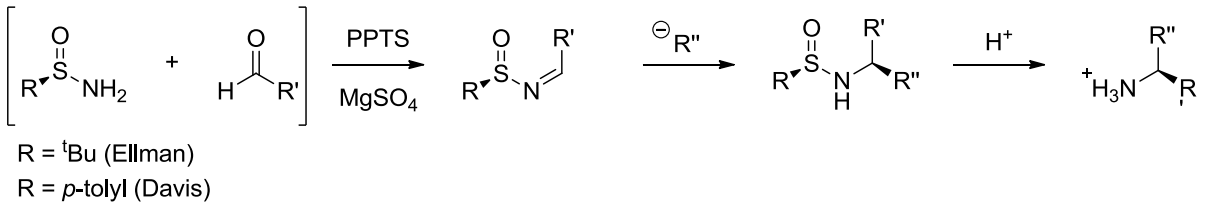
Scheme 3.2 Carreira's catalytic system for formation of propargylic alcohols.[99]

Another approach for the production of chiral propargylic alcohols was demonstrated by Noyori *et al*.,[100] who used chiral Ru(II) catalysts, such as **3.1** below, to enantioselectively reduce acetylenic ketones. With the use of 2-propanol as the hydrogen donor, mild reduction of the ketone could be performed with no concurrent reduction of the alkyne. Acetylenic ketones themselves would be able to be accessed via addition of a lithium acetylide to a Weinreb amide.[101] Therefore the amino acid side chain to be mimicked in this pathway would originate from Weinreb amides derived from carboxylic acids.



Scheme 3.3 Acetylenic ketone formation (left), followed by Noyori asymmetric ketone reduction.[100]

Metal acetylides may also react with imines, in this case resulting in the formation of achiral propargyl amines. As an extension of this reaction, both Davis and Ellman have demonstrated a general stereoselective addition of organometallic reagents to imines via the use of chiral sulfinylimines.[102, 103] The two auxiliaries developed by the groups differ in the R group attached to the sulfinylimine, while the mechanism imparting stereoselectivity is the same: chirality present on the sulfur atom causes nucleophilic attack of an organometallic reagent to occur in a particular orientation on the adjacent imine, resulting in a sulfinylamide diastereomer. Diastereoselective excesses are said to be >95 % by both groups for a wide range of substrates. The auxiliaries may be removed via mild acid conditions to provide chiral amines in high enantiomeric excesses. A side-reaction, consisting of the addition of small organometallic reagents to the sulfur atom of either the *p*-tolyl or *tert*-butyl directing groups has been shown to occur, leading to sulfoxides,[104, 105] although this effect may be reduced by the co-addition of a Lewis acid catalyst.[105] Recent advances in the facile large scale chiral synthesis of the *tert*-butyl sulfinamine resulted in our investigation of this chiral auxiliary over the *p*-tolyl variant.[106]



Scheme 3.4 Synthesis and nucleophilic attack of organometallic reagents with sulfinylimines.

Upon addition of organometallic reagents to *tert*-butyl sulfinylimines, it has been suggested that a 6-membered cyclic transition state may arise with the metal coordinated with the sulfinyl oxygen as shown in figure 3.1.[103, 107] In this case, with the bulky *tert*-buyl group in the

less hindered equatorial position, the smaller substituent of the imine would preferentially sit in the axial position, *i.e.* as the *E*-regioisomer, with the larger substituent in the equatorial position, leading to nucleophilic attack of the organometallic reagent from the Si-face of the imine. Bulky substituents on the imine would therefore increase the ratio of *E*-imine to *Z*-imine in the transition state, leading to higher stereoselectivities.[108] In addition, non-coordinating solvents such as DCM are proposed to give the highest stereoselectivities, with coordinating solvents such as THF and diethyl ether disrupting the proposed transition state.[103]

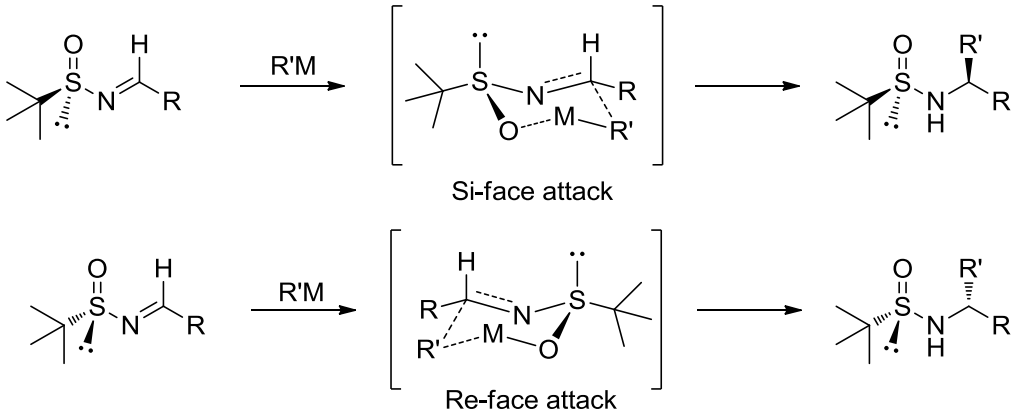


Figure 3.1 Six-membered transition states of chiral sulfinylimines.

Interestingly, in the synthesis of 1,2-amino alcohols from *tert*-butyl *N*-sulfinylimino ethers, Barrow noticed a reversal of selectivity.[109] They proposed that coordination of the incoming metal with the ether could stabilise the *Z*-imine, as depicted in figure 3.2 below.

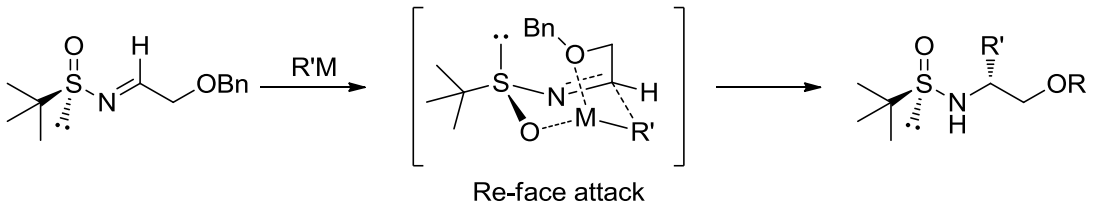


Figure 3.2 Cross-coordination of a metal ion with both the sulfinylimine and ether functional groups.

Alternatively, Davis proposed that if the reaction proceeds in a non chelation-controlled manner the Cram product would be favoured,[105] as shown in figure 3.3 below. Addition to the R-sulfinylimine would occur via the Re-face, and conversely addition to the S-sulfinylimine occurring via the Si-face. The addition of a Lewis acid catalyst, such as trimethylaluminum or boron trifluoride etherate, increased the stereoselectivity slightly -

presumably due to chelation to the sulfinyl oxygen to further shield the unfavourable face of attack.[105]

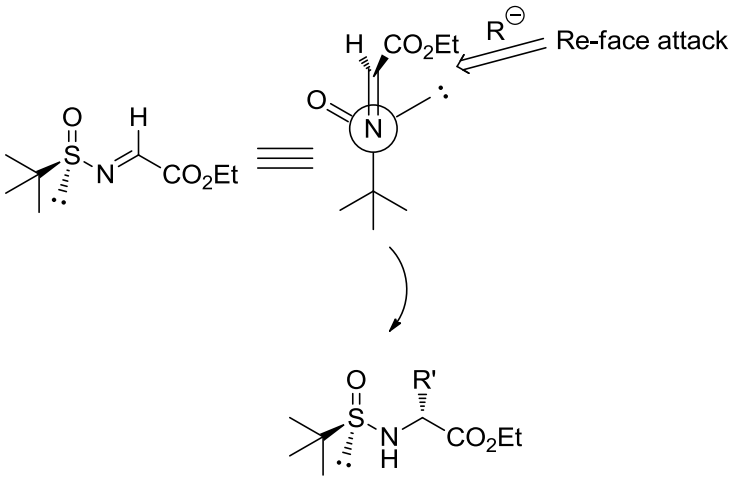
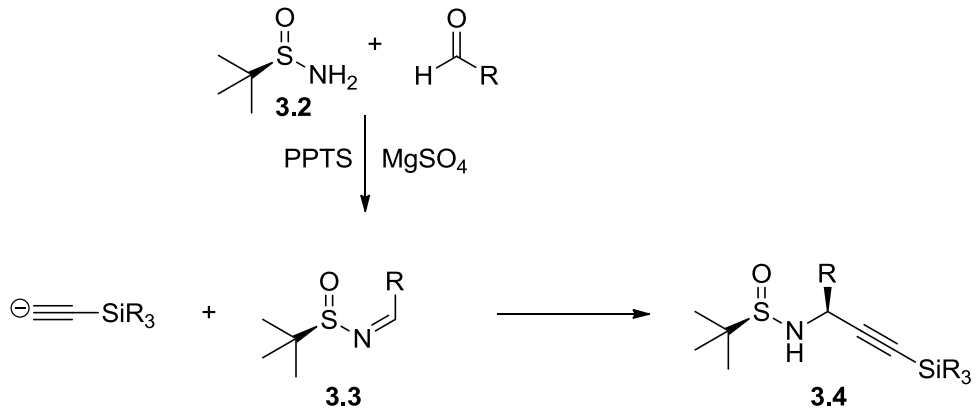


Figure 3.3 Proposed transition state for the non-chelation-controlled organometallic addition to sulfinylimines resulting in Cram products.

Scheme 3.5 below shows this chiral auxiliary chemistry as applied to our desired chiral alkynyl amines, utilising disconnection 1. As shown, formation of the key *tert*-butyl sulfinylimine proceeds via the condensation of a sulfinamide with an aldehyde, with the side-chain of the amino acid mimic derived from an aldehyde, as would also be required for the Carreira propargylic alcohol approach. Potentially this is drawback for these methods, as the preparation of side chain-specific aldehydes may require several steps.



Scheme 3.5 Synthesis of protected alkyne 3.4 via trialkylacetylide addition to an Ellman sulfinylimine derived from an aldehyde and sulfinamide 3.2.

3.1.2 Disconnection 2: The nucleophilic side-chain approach

If the sulfinylimine strategy was approached in a different fashion, whereby the protected alkyne group was already present in the key sulfinylimine, organometallic reagents acting as source of the amino acid side-chain could be used for the addition reactions. Therefore only one aldehyde; and thus one sulfinylimine, would need to be produced in order to synthesise many different mimics, resulting in a late stage divergent synthesis.

The synthesis of 3-(trialkylsilyl)propiolaldehydes (**3.5**) which may be used in production of sulfinylimine **3.6** have already been reported in the literature,[110] and following the addition of the organometallic reagent, key sulfinylamide **3.4** above may be formed. Many commercially available Grignard reagents would be able to react with the sulfinylimine intermediate forming mimics of the hydrophobic side chain-containing amino acids - such as valine, isoleucine and phenylalanine. More complex side-chains may not be as accessible, and would potentially require further derivatisations after the initial organometallic addition.

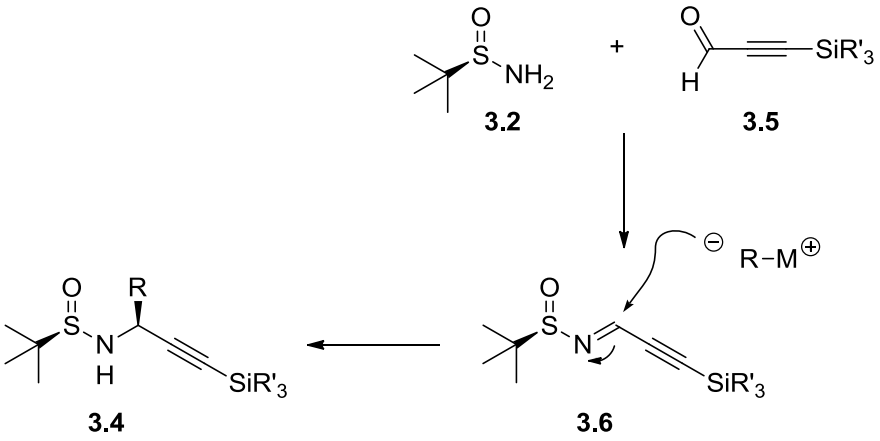
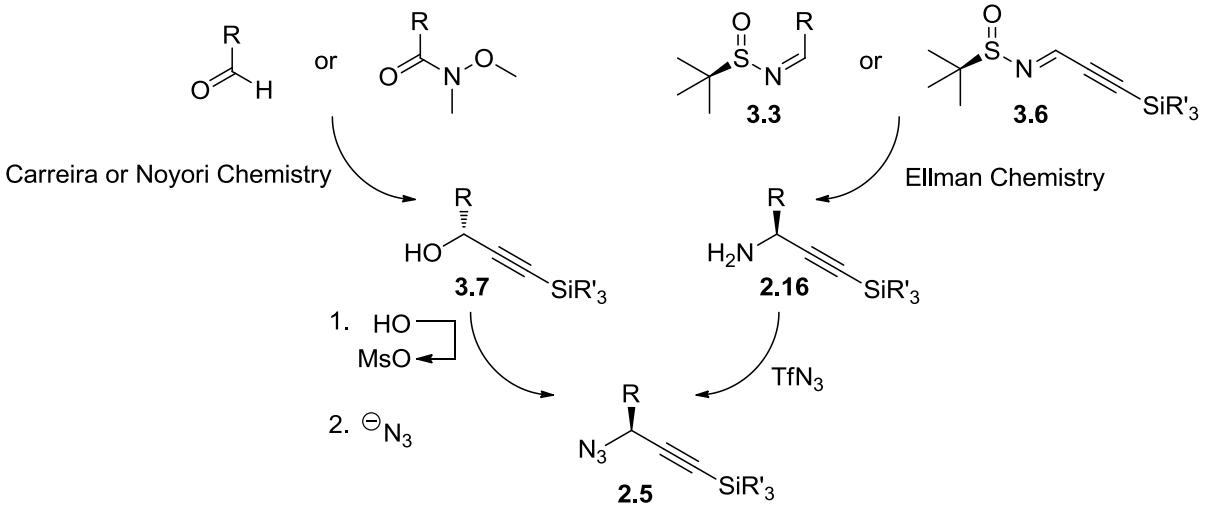


Figure 3.4 Organometallic reagents acting as the amino acid side-chain source during nucleophilic addition to sulfinylimines.

3.1.3 Azide Formation

With the side-chain functionality set in the molecules, whether it be via Carriera, Noyori or Ellman chemistry, conversion of the alcohol or amine present on the molecules to an azide would be required to complete the synthesis of the alkynyl-azide monomer units (**2.5**). For the alcohol-containing intermediates, activation with an appropriate reagent (such as conversion to a mesylate or via the Mitsunobu protocol) would allow for nucleophilic displacement by an appropriate source of azide anion. It is important to note that these reactions would result in complete inversion of the secondary alcohol, which would need to

be considered when selecting the chiral catalysts. For the amine-containing products formed via the use of the Ellman chemistry, diazotransfer as seen in section 2.2.5 would be used. The *tert*-butyl sulfinamide auxiliary may be cleaved under similar acidic conditions to the Boc protecting group used previously.[111]

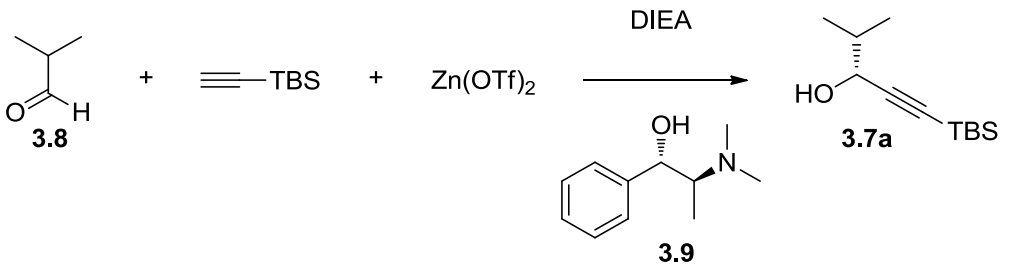


Scheme 3.6 Synthesis of monomer unit 2.5 via azide-forming reactions of intermediates formed via Carreira, Noyori or Ellman chemistry.

3.2 Monomer Unit Synthesis

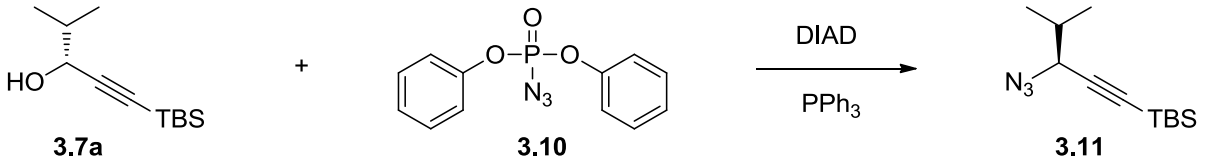
3.2.1 Monomer unit synthesis via Carreira chemistry

Carreira's method of secondary propargylic alcohol generation was attempted for synthesis of a valine mimic, as the required starting material, isobutyraldehyde (**3.8**) was readily available. This would allow for quick assessment as to whether the reaction was worth pursing further.



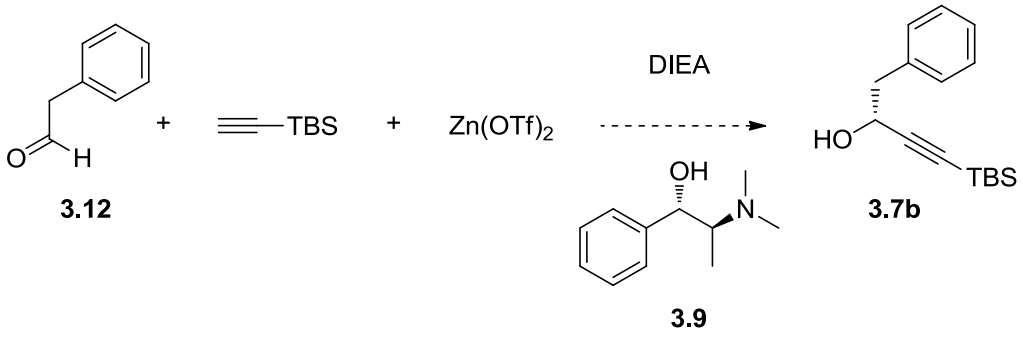
Scheme 3.7 First step in the synthesis of the valine mimic, using Carreira's catalytic conditions.[99]

After forming the activated acetylene by adding TBS-acetylene to a solution of zinc triflate (1.1 eq), *N*-methylephedrine (**3.9**, 1.2 eq) and triethylamine, isobutyraldehyde (**3.8**, 1 eq) was added and the reaction was stirred overnight at 60 ^{o}C. After purification, alcohol **3.7a** was isolated in 33 % yield. The ^{1}H NMR spectrum in deuterochloroform showed a signal at δ 1.78 (1H, d) which was attributed to the alcohol proton, and a signal at δ 4.14 (1H, dd) which was indicative of the proton adjacent to the alcohol. In order to confirm the presence of the alcohol signal, the NMR experiment was repeated in deuteromethanol. The proton signal assigned to the alcohol in $CDCl_3$ was not present, suggesting that exchange had occurred with deuterium. In addition the proton adjacent to the alcohol was now a doublet.



Scheme 3.8 Mitsunobu reaction with alkynyl alcohol 3.7a and diphenylphosphoryl azide (3.10) giving monomer unit 3.11.

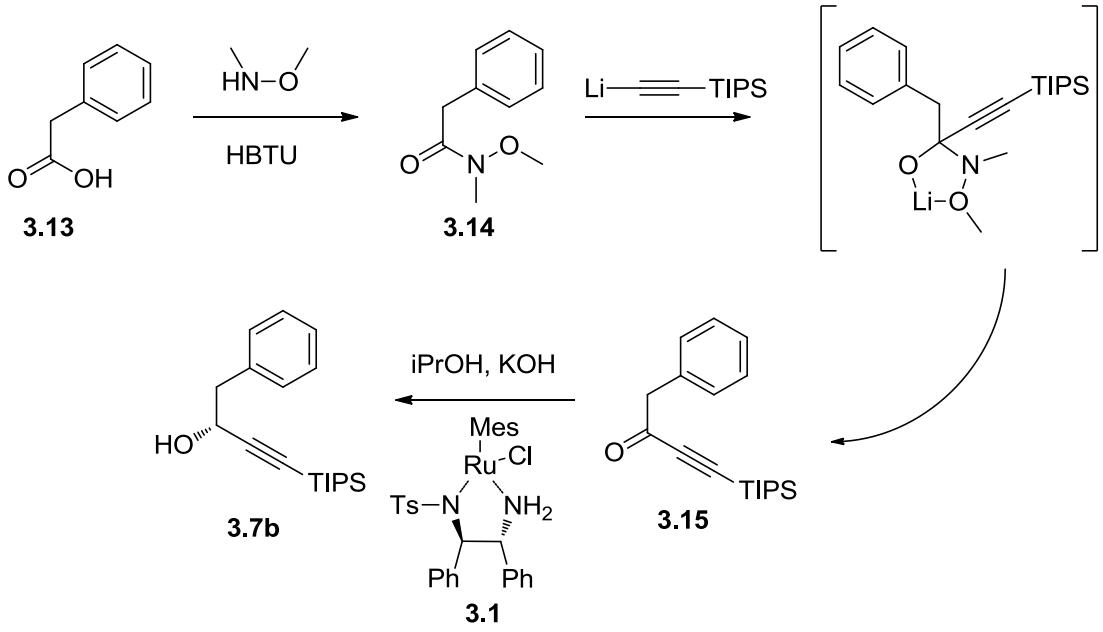
Conversion of the alcohol to an azide was then attempted using the Mitsunobu protocol in THF, with diphenylphosphoryl azide (**3.10**, 1 eq) acting as the source of the azide anion.[112] Although evidence of the product was shown by the ^{1}H NMR spectrum and ESI-MS of the crude material, no further purification nor characterisation were performed, as we were more interested in the synthesis of a phenylalanine mimic. Thus the reactions were repeated with freshly distilled phenyl acetaldehyde (**3.12**), in the same manner as before, as shown in scheme 3.9 below.



Scheme 3.9 Conversion of phenyl acetaldehyde to alkynyl alcohol 3.7b using Carreira's conditions.[99]

However when the zinc-mediated addition of TBS-acetylene to phenyl acetaldehyde was attempted, no signals corresponding to the expected product were apparent in the crude ^{1}H NMR spectrum. The failure of this reaction was attributed to the acidity of the benzylic protons of phenyl acetaldehyde, which may lead to aldol-type products in the presence of a base. This was supported by the complete consumption of the aldehyde starting material. A different route to the desired phenylbutynol compound (**3.7b**) was required.

3.2.2 Monomer Unit Synthesis via Noyori Chemistry

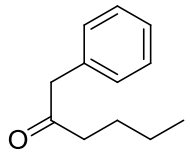


Scheme 3.10 Three step synthesis of alkynyl alcohol 3.7b from phenylacetic acid (3.13) via Weinreb amide formation, TIPS-acetylide addition and Noyori asymmetric transfer hydrogenation.[100]

Based on Noyori's work with chiral ruthenium catalysts,[100] a two-step α, β-acetylenic ketone formation and subsequent asymmetric transfer hydrogenation was attempted for the synthesis of phenylbutynol **3.7b**. This required firstly formation of the phenylacetic acid-derived Weinreb amide (**3.14**), which was isolated in 65 % after HBTU-mediated coupling of phenylacetic acid (**3.13**) to *N,O*-dimethylhydroxyl amine. Characteristic methyl peaks in the ^{1}H NMR spectrum corresponding to the Weinreb amide signals were observed at δ 3.61 (3H, s) and δ 3.20 (3H, s).

Subsequent attack of a TIPS-protected lithium acetylide species at -78 ^{o}C occurred with retention of the carbonyl functionality, due to the stabilisation of the metal-chelated

intermediate. However, the major product from the reaction appeared to be 1-phenylhexan-2-one (**3.16**), in a 5 : 1 molar ratio, presumably derived from attack on the Weinreb amide intermediate by *n*-butyl anion remaining from the acetylide formation step. The minor product (9.3 %) was found to be the desired alkynyl ketone, supported by the ^{1}H NMR spectrum which displayed the benzyl protons at δ 3.83 (2H, s) and TIPS protecting group at δ 1.03 (21H, m).



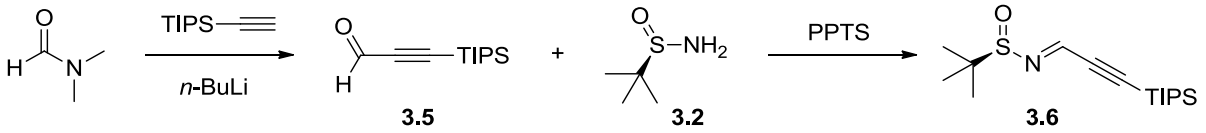
3.16

Figure 3.5 Biproduct 1-phenylhexan-2-one.

It was anticipated that optimisation of this reaction could eliminate or reduce the by-product, however sufficient alkynylketone was produced to test Noyori's transfer hydrogenation procedure.[100] The procedure involved the addition of ketone **3.15** (1 eq) in isopropanol to a solution of RuCl(*p*-cymene)[(R,R)-TsDPEN] (**3.1**) (0.05 eq) and potassium hydroxide (0.06 eq) in isopropanol. The reaction was worked up after 12 hours and a crude ^{1}H NMR spectrum was recorded, which displayed the expected α-H and β-H signals for alcohol **3.7b** at δ 4.64 (1H, t) and 3.03 (2H, d) respectively, but suggested only ~15 % conversion in comparison with a peak representing the benzyl protons from the starting ketone material. Although the transfer hydrogenation reaction pathway showed that the intended product could be accessed, the reactions had been performed simultaneously with experiments exploring Ellman's chiral sulfinamide auxiliary, and the latter pathway showed more promising results. For this reason, no further investigations of this pathway were attempted.

3.2.3 Monomer Unit Synthesis via Ellman Chemistry

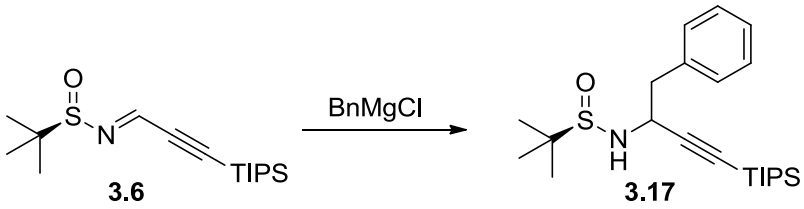
To incorporate a silyl-protected alkyne to an Ellman-like sulfinylimine, an appropriate propiolaldehyde must initially be synthesised. Conveniently, these molecules may be accessed via nucleophilic substitution of a silyl-protected acetylide on dimethylformamide.



Scheme 3.11 Formation of key sulfinylimine 3.6 via a condensation reaction between sulfinamide 3.2 and silyl propiolaldehyde 3.5.

After the formation of the TIPS-acetylide with *n*-butyllithium at 0 ^{o}C, the solution was cannulated into a flask containing DMF, which resulted in the formation of 3-(triisopropylsilyl)propiolaldehyde (**3.5**). The aldehyde peak was detected in the ^{1}H NMR spectrum at δ 9.22 (s), consistent with the literature.[110]

The crude TIPS-propiolaldehyde material (~ 1 eq) was then condensed with sulfinamide **(R)-3.2** (1 eq) in the presence of magnesium sulfate as a water scavenger, with pyridinium *p*-toluenesulfonate (0.05 eq) acting as a mild acid catalyst. The key sulfinylimine intermediate (**3.6**) was isolated in 59 % yield, with the ^{1}H NMR spectrum showing the imine proton signal at δ 7.84 (1H, s), in addition to the *tert*-butyl and TIPS signals at δ 1.25 (9H, s) and 1.12 (21H, m) respectively.

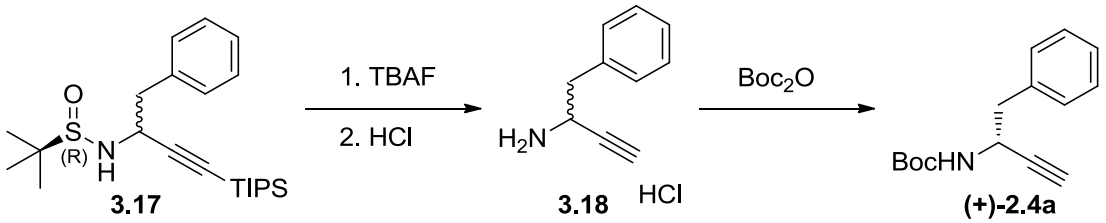


Scheme 3.12 Synthesis of phenylalanine-derived sulfinylamide 3.17 via the addition of benzylmagnesium chloride to sulfinylimine 3.6.

With key sulfinylimine **3.6** in hand, the addition of organometallic reagents acting as amino acid side-chains would provide sulfinylamides such as **3.17** above, which is analogous to the monomer unit intermediate **2.15a** synthesised in chapter 2. For synthesis of **3.17**, benzylmagnesium chloride was added to sulfinylimine **3.6** in DCM at 0 ^{o}C, and an aliquot of the crude mixture was analysed by ^{1}H NMR after 30 minutes. Almost complete consumption of starting material was seen based on the imine peak at δ 7.84 (s), and two peaks corresponding to α-protons were observed at δ 4.41 (ddd) and 4.32 (ddd), with a 1.25 : 1.00 integration ratio respectively, suggesting an d.e. of only 11 %. When the reaction was repeated with the temperature was further reduced to -78 ^{o}C, again a mixture of

diastereomers were seen, but remarkably a reversal of the major diastereomer was observed, with an integration ratio of 0.22 : 1.00, giving an overall d.e. of 64 %. A further improvement to 95 % d.e.,[113] isolated in 45 % yield, was obtained when the imine was pre-activated with 2 equivalents of boron trifluoride diethyletherate, followed by the addition of benzylmagnesium chloride at -78 ^{o}C, as suggested by Davis.[105]

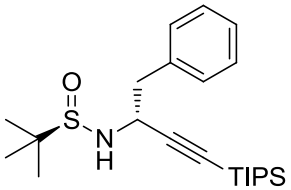
Although it was anticipated that the (R_S)-(S)-isomer had been synthesised, derivatisation to known alkyne **2.4a** was performed for a comparison of the optical rotation, and therefore to unambiguously assign the stereochemistry.



3.17 **3.18** **(+)-2.4a**

Scheme 3.13 Derivatisation of sulfinylamide 3.17 to Boc-protected alkyne 2.4a.

After TBAF-mediated desilylation, the crude compound was dissolved in diethyl ether and a solution of hydrogen chloride in diethyl ether added, resulting in the precipitation of an off-white solid. Presuming the solid to be amine **3.18**, Boc-protection via Boc anhydride and triethylamine was performed, resulting in alkyne **2.4a** in 7.7 % isolated yield over the 3 reactions. Both the ^{1}H NMR and ESI-MS gave identical spectra to the compound produced in chapter 2.2. To our surprise, the optical rotation of the alkyne was determined to be [α] = + 8, which was the opposite stereochemistry to that which was desired, based on the literature values reported by Reginato.[92] This indicated that the parent sulfinylamide compound (**3.17**) was in fact the (R_S-R)-diastereomer, as shown below in figure 3.6.



(R,R)-3.17

Figure 3.6 (R,R)-sulfinylamide 3.17.

A possible explanation for the reversed diastereoselectivity demonstrated in this reaction is depicted in figure 3.7, based on the transition states proposed in the literature.[103, 107] It is

known that more bulky groups preferentially occupy the equatorial positions of such 6-membered ring transition states. Comparatively small groups, such as the fluoride, cyano and alkynyl groups display a greatly reduced preference for the equatorial position, thus exhibit a more equal equatorial to axial ratio. Therefore in this reaction, the presence of the alkyne could lead to of a mixture of the proposed transition states. However a steric clash between the TIPS-protecting group and the incoming Grignard reagent may result in the *Z*-imine being the more favourable transition state. This transition state leads to Re-addition to the sufinylimine giving **(R,R)-3.17** as the major stereoisomer, and this stereoselectivity would become more noticeable at reduced temperatures, as less energy is available to the system to convert the more stable intermediate into the less stable one.

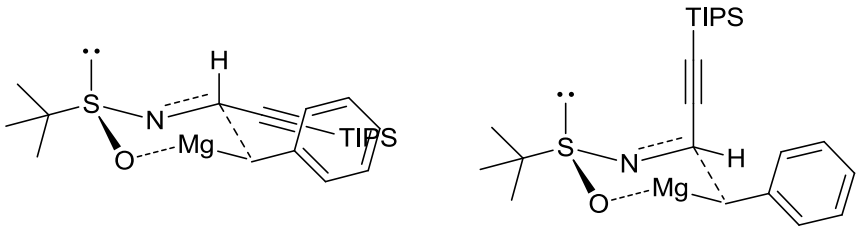
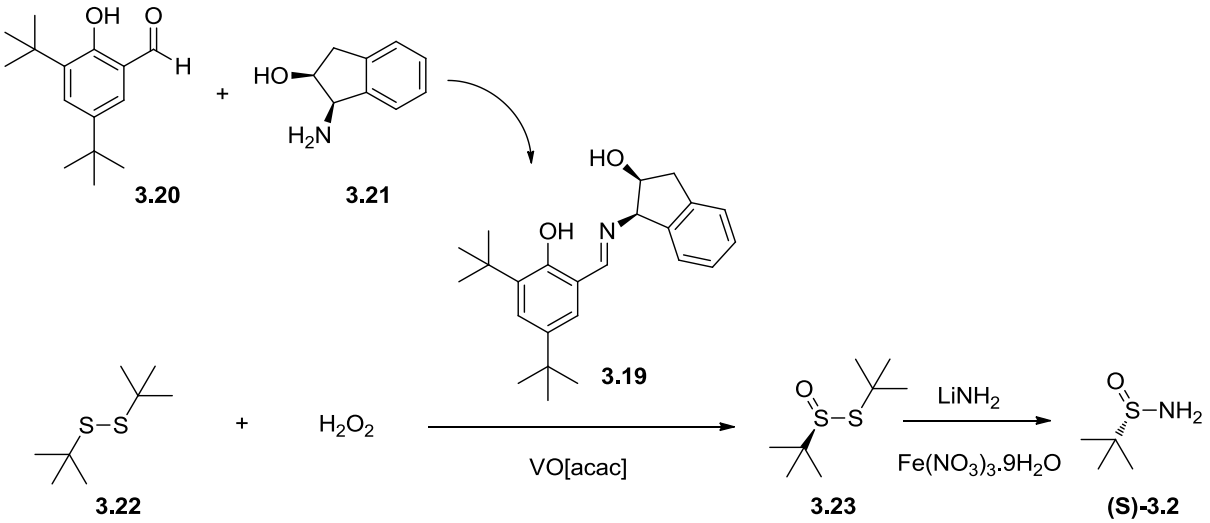


Figure 3.7 Proposed transition states of nucleophilic attack on sulfinylimine 3.6 showing unfavoured 'E-imine' conformation (left) and favoured 'Z-imine' conformation (right).

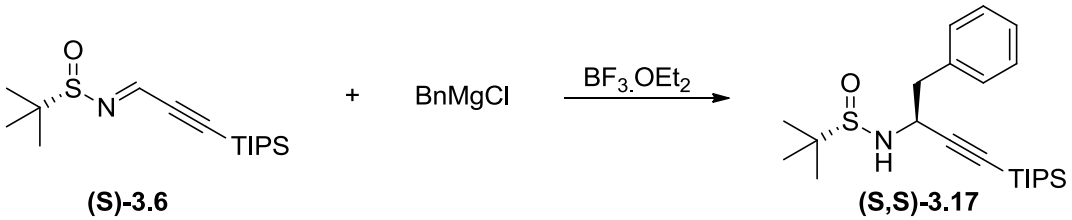
Given the promising results shown by the sulfinylimine route using the commercially available (R)-(+)-*tert*-butyl sulfinamide (**(R)-3.2**), we were interested in investigating the epimer (**(S)-3.2**) to give compounds with the reversed stereochemistry. However the cost of the enantiomerically pure sulfinamide was prohibitive. To acquire enough material for future chain extension reactions, a large quantity was required. Fortunately, a synthesis that allows for large-scale preparation of this amine was published by Ellman *et al*.[106] Firstly a chiral catalyst was required, which may be readily synthesised in one step. As detailed in scheme 3.14 below, the (1*R*, 2*S*)-(+)-*cis*-1-amino-2-indanol derived catalyst (**3.19**) leads to the production of the (S) enantiomer of *tert*-butyl sulfinamide **3.2**.



Scheme 3.14 Synthesis of (S)-*tert*-butyl sulfinamide from commercially available starting materials.

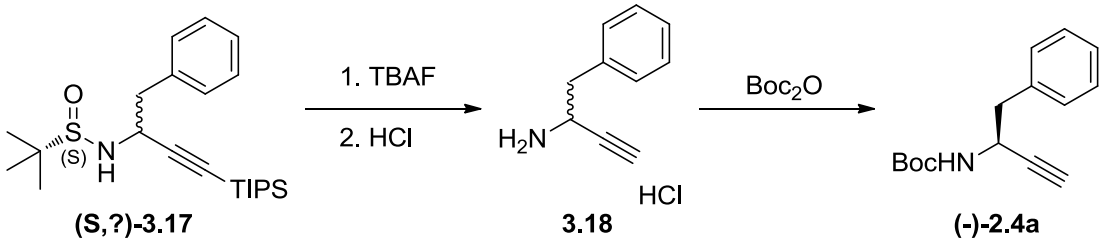
The desired chiral catalyst (**3.19**) was synthesised following the procedure of Ruck *et al*.,[114] which involved the condensation between (1R, 2S)-(+)-*cis*-1-amino-2-indanol (**3.21**) (1.05 eq) and 3,5-di-*tert*-butylsalicylaldehyde (**3.20**) (0.64 g, 1 eq) in ethanol, giving the compound in quantitative yield. Subsequently, asymmetric oxidation of di-*tert*-butyl *di*-sulfide (**3.22**) to sulfinothioate **3.23** was accomplished via the addition of hydrogen peroxide over 20 hours to a solution of catalyst **3.19** (0.005 eq) and VO[acac] (0.00506 eq) in acetone at 0 ^{o}C. The ^{1}H NMR spectrum of the crude material showed complete conversion of the starting material, as shown by the presence of only 2 peaks in the spectrum, corresponding to the two *tert*-butyl groups, at δ 1.74 (9H, s) and 1.38 (9H, s). No further purification was performed on the crude material.

Following the procedure of Ellman,[115] careful addition of lithium metal (2.5 eq) to iron (III) nitrate (0.0025 eq) in liquid ammonia at -45 ^{o}C resulted in *in situ* lithium amide formation, which then was allowed to add to (S_S)-sulfinothioate **3.23** (1 eq) at -78 ^{o}C, displacing *tert*-butyl thiol. Sulfinamide **3.2** was isolated in 50.5 % yield, and the optical rotation was observed to be $[\alpha]_D$ = -5.5^{o}, consistent with the literature value for the *S* enantiomer.[116]



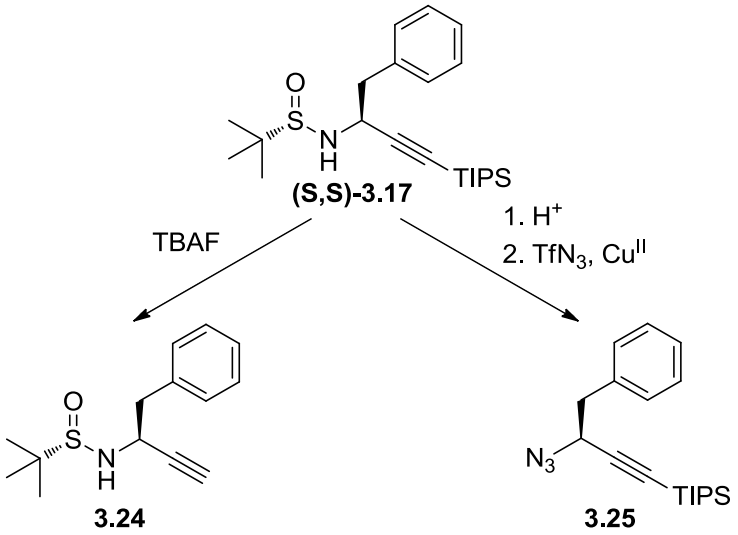
Scheme 3.15 Synthesis of phenylalanine-derived sulfinylamide 3.17 via the addition of benzyl magnesiumchloride to sulfinylimine (S)-3.6.

Using a similar procedure as detailed previously, the key sulfinylimine (**(S)-3.6**) was prepared via a condensation reaction between the newly prepared *tert*-butylsulfinamide (**(S)-3.2**) and TIPS-protected propiolaldehyde (**3.5**). With the exception of the optical rotation, which as expected had the same magnitude but the opposite sign ($[\alpha]^{20}_{D}$ = +169.1), the compounds had identical spectral properties. Again synthesis of the phenylalanine mimic was attempted, by firstly pre-activating sulfinylimine **(S)-3.6** (1 eq) with boron trifluoride diethyletherate (2 eq), followed by the addition of benzylmagnesium chloride (2.1 eq) at -78 ^{o}C. A d.e. of 42.2 % was suggested by the crude ^{1}H NMR spectrum, with 2 α-proton signals present at δ 4.39 (ddd, 0.27H) and 4.31 (ddd, 1.0H). The poorer diastereoselectivity seen with this sulfinylimine was attributed the reaction being performed on a larger scale, which may result in a localised increase in temperature upon the addition of the Grignard reagent over a short period of time. The major isomer was isolated in 73.5 % yield, with a peak corresponding to the protonated molecular ion (*m/z* = 406.2) in the ESI-MS.



Scheme 3.16 Derivatisation of sulfinylamide 3.17 to Boc-protected alkyne 2.4a.

Performing a similar set of reactions as outlined previously, a small amount of sulfinylamide **3.17** was derivatised to known alkyne **2.4a** to determine the absolute stereochemistry. After isolation of **2.4a**, the optical rotation was determined to be $[\alpha]^{20}_{D}$ = -10.7 showing that indeed the (S)-stereoisomer had been synthesised.[92]



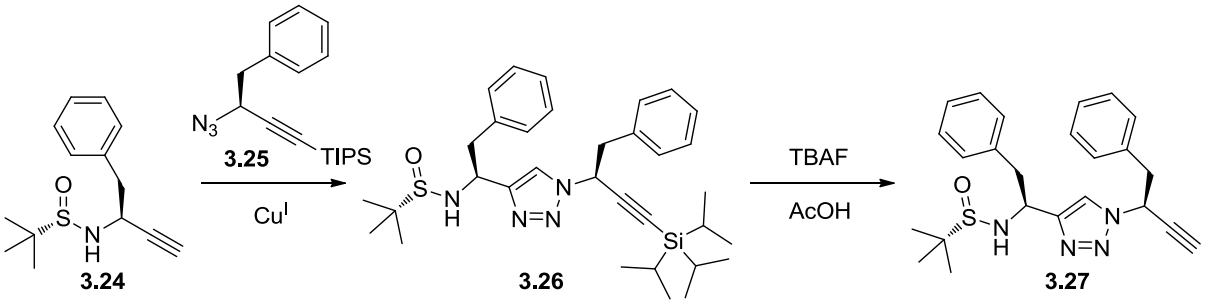
Scheme 3.17 Derivatisation of sulfinylimine intermediate (S,S)-3.17 to the key monomer units 3.24 and 3.25.

With a route now established to provide orthogonally protected phenylalanine mimic **(S,S)-3.17**, our attention turned to the conversion of these molecules into a form whereby a chain extension sequence could be applied. For extension in the *C*-direction, it was shown in chapter 2.3.1 that a Boc-protected amino-alkyne could act as the 'anchor' in the growing chain. As the *tert*-butyl sulfinamide functional group displays a very similar reactivity profile to the Boc-protecting group, it would be able to remain as the *N*-terminal protecting group. Thus TBAF-mediated desilylation was applied to sulfinylamide **(S,S)-3.17**, exposing the terminal alkyne for a subsequent Cu-AAL chain extension reaction. Alkyne **3.24** was isolated in 78 % yield, with the key alkyne signal observed in the ^{1}H NMR spectrum at δ 2.41 (1H, d).

Formation of the 'extending unit' may be performed similarly to the method used for Boc-protected derivative **2.15a**. Thus **(S,S)-3.17** was transformed into its azido derivative in a two-step process: *N*-deprotection with a solution of hydrogen chloride in ether, and subsequent diazotransfer on the crude amine with triflic azide, giving azide **3.25** in 72 % yield over two steps. The α-proton signal in the ^{1}H NMR had shifted from δ 4.33 (ddd, J = 7.3, 6.4, 5.7 Hz, 1H) in the sulfinylamide starting material, to 4.30 (t, J = 6.9 Hz, 1H) in the azide. Although the protonated molecular ion was not observed by ESI-MS, a peak corresponding to the fragmentation product (m/z = 300.2 [(M-N_2)H^+]) was seen.

3.3 Chain Extension

With a divergent, high-yielding, and stereoselective synthesis of 'anchor unit' molecules (**3.24**) and 'extending unit' molecules (**3.25**) in hand, linear chain extension was investigated. As has been mentioned earlier, the *tert*-butyl sulfinamide functional group displays similar reactivity to the Boc amine protecting group, thus the extension performs in an almost identical fashion to that discussed in chapter 2.3.



Scheme 3.18 Copper-mediated coupling of the monomer units 3.24 and 3.25, followed by desilylation to give terminal alkyne 3.27.

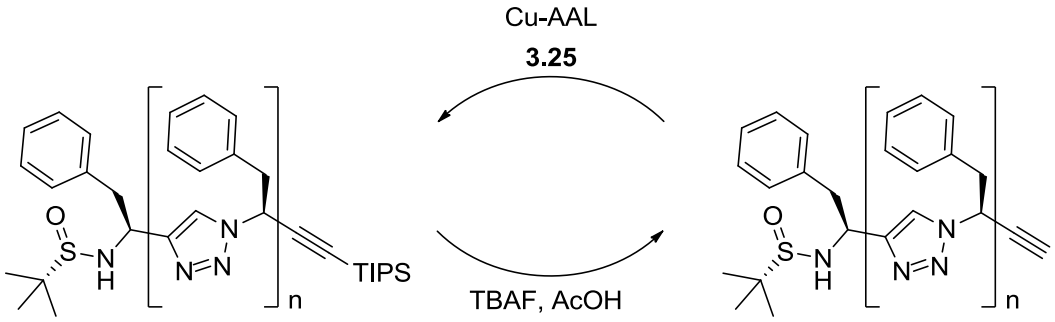
Direct coupling of alkyne **3.24** with the alkynyl-azide **3.25** was performed under Cu-AAL conditions, with a catalyst cocktail consisting of copper sulfate pentahydrate (0.1 eq), ascorbic acid (1 eq) and TBTA (**1.4**, 0.1 eq). The reaction was performed in a DCM : methanol solvent mixture, as opposed to the more classical *tert*-butanol in water mixtures, to ensure complete dissolution of the starting materials. Test reactions suggested that both DCM : methanol mixtures and DMF perform equally in these reactions, although the former allowed for a more straightforward work-up procedure. Dimer **3.26** was isolated in 95 % yield after purification by column chromatography, with the two key α-proton signals seen in the ^{1}H NMR spectrum at δ 5.66 (dd, *J* = 7.9, 5.3 Hz, 1H) and 4.77 (q, *J* = 7.0 Hz, 1H), and the newly formed triazole signal present at δ 7.40 (s, 1H). A peak corresponding to the protonated molecular ion (*m/z* = 577.3) was observed in the ESI-MS.

Desilylation of the *C*-terminal TIPS-protecting group was effected by treatment with TBAF (4 eq) buffered with acetic acid (2 eq) in THF, giving alkyne **3.27** in 84.4 % yield. The exposed alkyne signal was present in the ^{1}H NMR spectrum at δ 2.62 (d, *J* = 2.4 Hz, 1 H). Coupling between this signal to the α-proton signal at δ 5.61 (ddd, *J* = 8.0, 5.8, 2.4 Hz, 1H) was observed, providing evidence to suggest that this particular signal arose from the *C*-terminal α-proton. Thus the signal at δ 4.76 (td, *J* = 8.0, 6.3 Hz, 1H) was assigned to the *N*-terminal

α-proton. A peak corresponding to the protonated molecular ion (*m/z* = 421.2) was observed in the ESI-MS.

Iterative chain extension was performed using the described Cu-AAL extension and TBAF-mediated desilylation procedures, leading to the tetrameric, pentameric and hexameric triazole oligomers. The yields and basic spectral data are summarised in table 3.1 below.

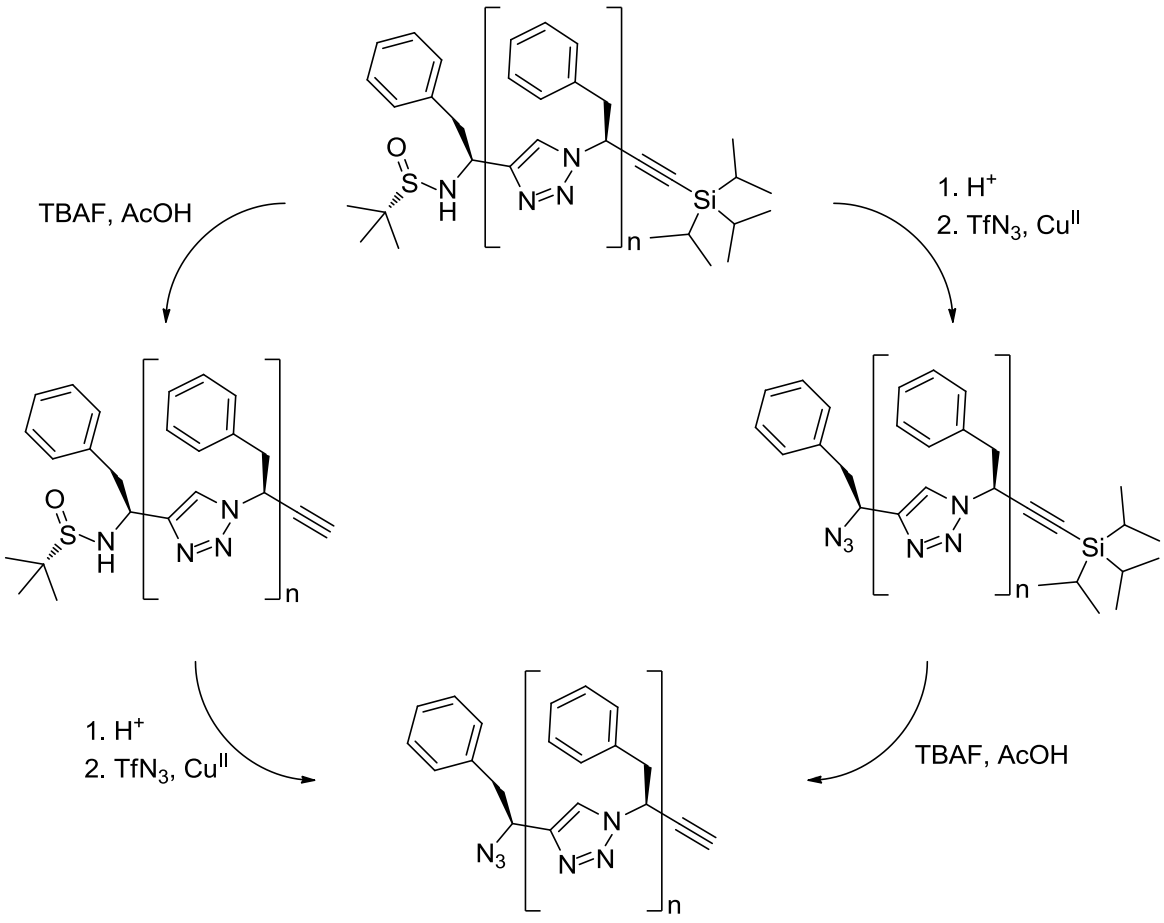
Table 3.1 The yields, diagnostic ^{1}H NMR α-proton signals and ESI-MS peaks of TIPS-protected-alkyne triazole oligomers (left) and terminal alkyne oligomers (right) synthesised during the iterative 2-step chain extension sequence.



n =	Yield (%)	Key ^{1}H NMR α-proton signals (δ)	ESI-MS (*m/z*) [M + H^{+}]		n =	Yield (%)	Key ^{1}H NMR α-proton signals (δ)	ESI-MS (*m/z*) [M + H^{+}]
1 (3.26)	95	5.66 (1H) 4.77 (1H)	577.3		1 (3.27)	84	5.61 (1H) 4.76 (1H)	421.2
2 (3.28)	65	5.95 (1H) 5.68 (1H) 4.71 (1H)	748.2		2 (3.29)	87	5.92 (1H) 5.63 (1H) 4.72 (1H)	592.3
3 (3.30)	80	5.92 (2H) 5.70 (1H) 4.71 (1H)	919.5		3 (3.31)	93	5.93 (2H) 5.64 (1H) 4.71 (1H)	763.4
4 (3.32)	73	5.96 (3H) 5.71 (1H) 4.71 (1H)	1090.6		4 (3.33)	87	5.92 (3H) 5.64 (1H) 4.70 (1H)	934.4
5 (3.34)	71	5.95 (4H) 5.72 (1H) 4.72 (1H)	1261.6					

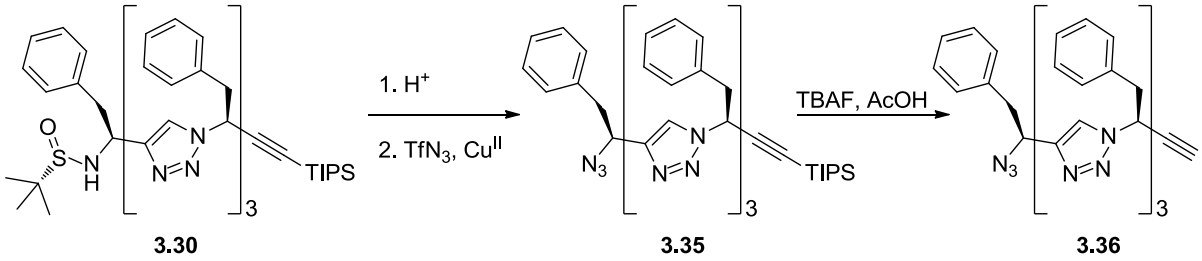
3.4 Cyclisation Reactions

With linear phenylalanine-based triazole peptidomimetic chains containing 4-6 residues in hand, progression to the cyclic forms of the molecules was planned. For head-to-tail cyclisation to occur, an *N*-terminal azide and *C*-terminal alkyne were required. Of the 2 possible reaction sequences to these molecules shown in scheme 3.19 below, diazotransfer followed by desilylation was selected as more appropriate than the alternative order. The requirement of copper as a catalyst during the diazotransfer reaction was particularly concerning. It was identified that spontaneous polymerisation alkynyl-azide products could occur during the copper(II)-catalysed diazotransfer step of a terminal alkyne-containing molecule. By having TIPS-deprotection as the final step in the sequence, this problem would potentially be avoided.



Scheme 3.19 The two possible routes to the linear azido-alkynes: i) desilylation followed by *N*-deprotection and diazotransfer (left) and ii) *N*-deprotection and diazotransfer followed by desilylation.

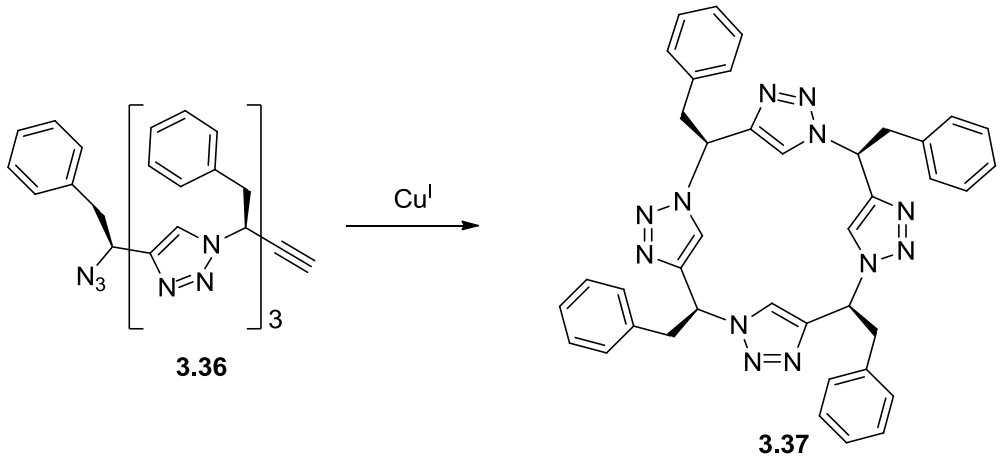
3.4.1 Cyclisation of Tetramer



Scheme 3.20 Derivatisation of tetramer 3.30 to linear alkynyl-azide compound 3.36.

N-Deprotection of tetramer **3.30** (1 eq) with a solution of hydrogen chloride in diethyl ether (2 eq), followed by diazotransfer with triflic azide (1.5 eq), gave azido-tetramer **3.35** in 80 % yield for the 2 reactions. A peak corresponding to the protonated molecular ion (*m/z* = 841.5) was observed in the ESI-MS.

The terminal alkyne of azido-tetramer **3.35** (1 eq) was exposed via the addition of TBAF (4 eq) buffered with acetic acid (2 eq). After completion of the reaction as shown by TLC, the product (**3.36**) was precipitated by the addition of water, filtered, and washed, and dried under high-vacuum to give alkynyl-azide tetramer **3.36** in 75 % yield. The ^{1}H NMR spectrum produced showed that the product was of high purity, and the newly exposed terminal alkyne signal was seen at δ 2.65 (d, *J* = 2.5 Hz, 1H). The compound was stored at 0-4 ^{o}C as a highly dilute solution in DCM (0.5 mM), to minimise the potential for polymerisation.



Scheme 3.21 Copper-mediated head-to-tail cyclisation of tetramer 3.36.

Examination of compound **3.36** suggests that, with the addition of copper, various competing reaction pathways could be realised. Where the desired pathway was head-to-tail cyclisation, dimerisation or polymerisation to higher oligomers could result. In an attempt to force the starting material to react via the head-to-tail cyclisation pathway, a large dilution factor was seen to be important. In the analogous peptide head-to-tail cyclisation reactions, concentrations of 1 mM are commonly seen,[117] and thus similar or more dilute solutions were used. By using highly dilute solutions, however, a problem of effectively being able to monitor the completeness of the reaction arises. Due to the fast rate at which Cu-AAL commonly occurs, if an aliquot of a solution undergoing cyclisation was taken and concentrated, potentially any unreacted starting material would quickly undergo polymerisation, which would thus not give a realistic impression of the progress of the reaction. As the cyclised and linear products share the same molecular weight, mass spectrometry was not considered a useful analytical approach for monitoring this reaction, although LC-MS or HPLC could potentially be used. Performing the reaction in deuterated solvents could be used as a viable method for monitoring the reaction directly via ^{1}H NMR, although not without its limitations: the selection of solvents would be quite limited, the products may precipitate out of solution and not be observed, and the polymerised side-products may look very similar to the linear starting material.

The initial attempts at this cyclisation used the same coupling conditions as the linear extension: alkynyl-azide tetramer **3.36** (1 eq) was mixed with copper sulfate pentahydrate (0.1 eq), ascorbic acid (1 eq) and TBTA (**1.4**) (0.1 eq) in a solution of methanol and DCM. A solute concentration of 0.5 mM to 10 mM was used, as opposed to 0.1 M which was regularly used for the linear extension. No peaks corresponding to the product were observed in the crude ^{1}H NMR spectra, with polymerisation proposed as the main reaction pathway due to insoluble particulate matter being the only observable product. A different set of conditions as used for macrocyclisation were trialled,[45] which included the addition of copper bromide (0.2 eq) to a refluxing solution of alkynyl-azide **3.36** (1 eq) and DBU (3 eq) in toluene (1 mM). Again, the expected product could not be isolated, with polymerisation proposed as the expected reaction pathway.

A slightly modified version of Meldal's original Cu-AAL conditions were trialled, consisting of the alkynyl-azide (**3.36**) (1 eq, 0.5 mM), copper iodide (2 eq) and DIEA (50 eq) in THF in a sealed microwave tube. The reaction was heated up to 150 ^{o}C and stirred for 45 minutes. When the reaction had cooled back to room temperature an HPLC trace was run, which suggested that all starting material had been consumed, and a new peak was observed. After the solution was concentrated, acetonitrile (2 mL) and water (5 mL) were added, which caused dissolution of some, but not all, of the material present. The acetonitrile / water

solution was injected through a preparative HPLC column, with a very small peak at 15.3 minutes collected. After lyophilisation only a white solid of negligible mass remained, however the product was soluble in $CDCl_3$ and the ^1H NMR spectrum produced gave quite an interesting result. The intended product was a symmetrical tetramer, with 4 identical monomer units arranged in cycle. Thus the ^1H NMR spectrum would be expected to show just one set of these signals, and this is indeed what was seen. Only 1 distinct signal triazole signal was observed: δ 6.87 (s, 4H), 1 distinct α-proton signal: δ 5.93 (t, 4H) and 1 distinct set of β-proton signals: δ 3.84 (dd + dd, 8H). The ^1H NMR spectrum did not contain enough information to unambiguously assign the compound as the cyclised tetramer compound however, as higher oligomers would presumably display similar information, and the integration of the peaks was thus only tentatively assigned. The ^1H NMR spectra of both the stating material and cyclic product are shown in figures 3.8 and 3.9 below.

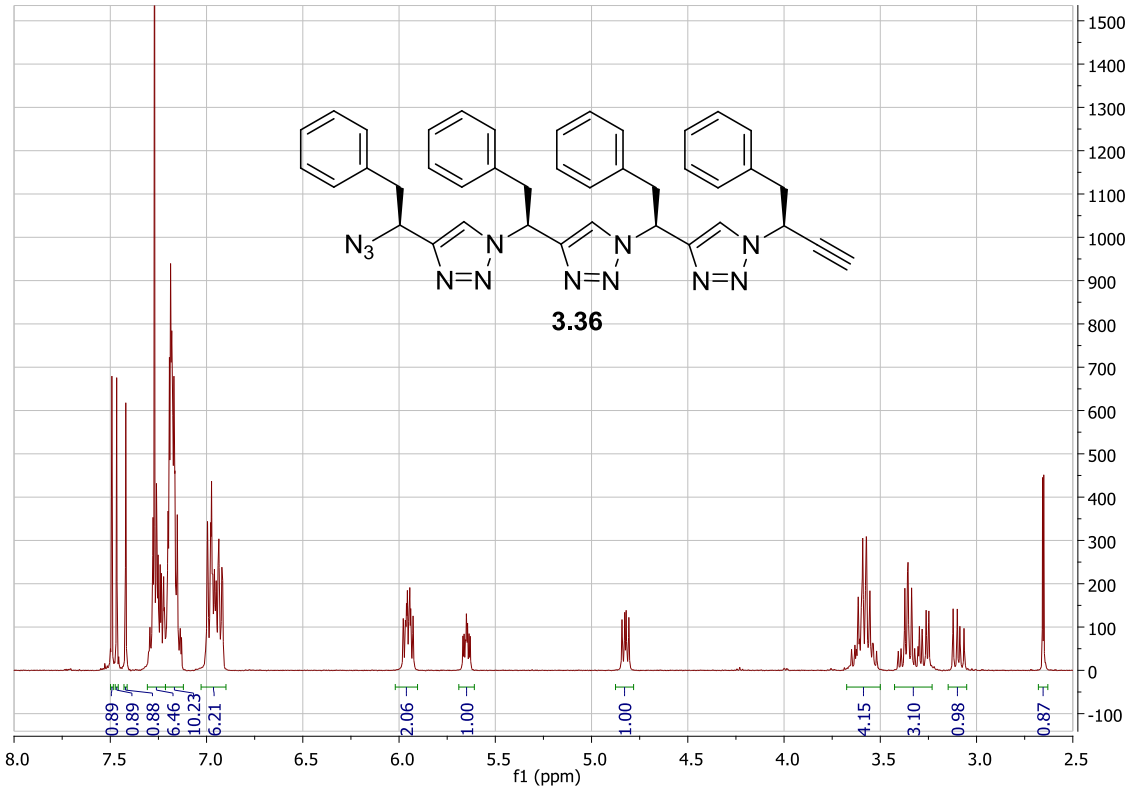


Figure 3.8 ^{1}H NMR spectrum of linear alkynyl-azide tetramer 3.36.

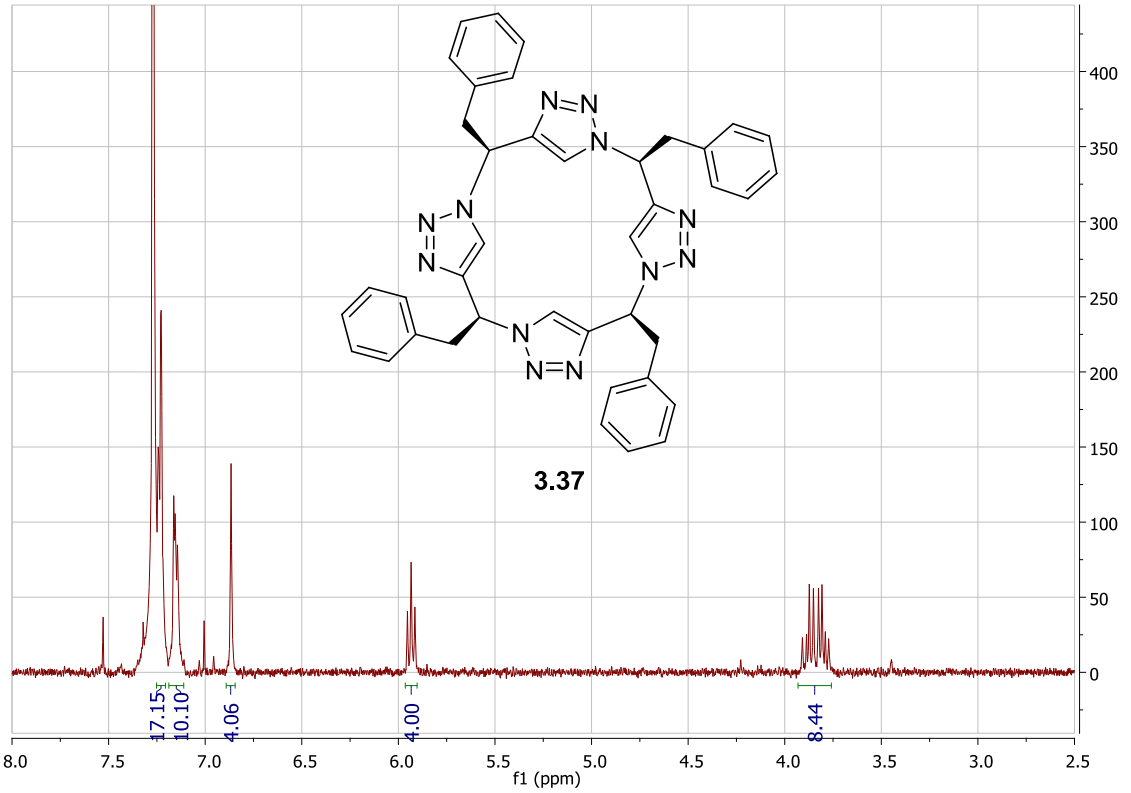


Figure 3.9 ^{1}H NMR spectrum of cyclic tetramer 3.37.

Further experiments were undertaken in order to improve the efficiency of the reaction. A series of reactions at 0.5 mM were performed in THF for 45 minutes at 150 ^{o}C under microwave irradiation, using a variety of copper sources (No copper, CuI, CuBr·Me_2S, copper powder) although in all cases after evaporation of the reaction solvent and addition of $CDCl_3$, appreciable amounts of insoluble material, presumed to be polymerised starting material, were present.

A recent article by Chouhan,[118] investigating macrocyclisation of an ephedrine-derived alkynyl-azide, suggested that head-to-tail cyclisation by Cu-AAL was adversely affected by halide-counterions of copper, due to coordination effects. It was proposed that non-coordinating counterions would thus improve the yield of the reaction. Thus tetrakis(acetonitrile)copper(I) hexafluorophosphate and tetrafluoroborate were obtained and the cyclisation reaction repeated.

An initial test reaction with similar reaction conditions to Chouhan was performed. To a solution of **3.36** (1 eq, 0.5 mM) in DCM in a pressure vial was added TBTA (**1.4**, 0.05 eq) as a solution in DCM and tetrakis(acetonitrile)copper(I) hexafluorophosphate (0.05 eq) as a solution in acetonitrile. The reaction was heated to 55-60 ^{o}C and left to stir overnight. After cooling to room temperature the combined organic phases were concentrated under reduced pressure. The ^{1}H NMR spectrum obtained from the crude material was consistent with the cyclic compound produced earlier, although again only product of a negligible weight was obtained by preparative HPLC.

In order to get a reasonable estimation of the conversion of the starting material to product via ^{1}H NMR, an internal standard was included in the reaction mixture. 4-Chloro-2-nitroaniline was selected as an appropriate internal standard, due to the presence of a signal at δ 8.13 in the ^{1}H NMR, which is further downfield than any of the signals in the starting materials or products from the reaction, and was not expected to participate or interfere with the reaction. The other signals from the internal standard were present in the aromatic region, therefore would not interfere with the integration of either the α-protons or β-protons. The reaction was repeated using tetrakis(acetonitrile)copper(I) tetrafluoroborate with the addition of 1 equivalent of the internal standard. After stirring overnight at 55-60 ^{o}C the reaction solvents were evaporated. It was seen via ^{1}H NMR analysis of the crude reaction mixture that 2.7 molecules of 4-chloro-2-nitroaniline were present for every molecule of product, corresponding to a 29 % conversion. As no starting material was present, the remainder was thought to have reacted via the polymerisation pathway (either during the course of the reaction or during concentration of the reaction mixture).

The reaction was repeated without the internal standard over 5.5 days at 55-60 ^{o}C, giving cyclic tetramer **3.37** as a white solid after chromatography. Given the previous conversion, and the scale of the reaction (1.7 mg), it was anticipated that less than 0.5 mg would result prior to purification. The material was carefully weighed in a small vial, employing triplicate weighings of empty and sample containing vial on a five-figure balance (+/- 0.01 mg), giving an average value of 0.1 ± 0.03 mg (6 %). Reassuringly, this value is qualitatively consistent with the observed signal-to-noise ratio of the ^{1}H NMR spectrum obtained, however, given the recently published discussion on the accuracy of such low mass measurements, we acknowledge the likely errors in this value.[113] The peaks in the $CDCl_3$ ^{1}H NMR spectrum were consistent with the product produced in the earlier trials. Interestingly, 1 new peak was present when d_6-DMSO was used as the ^{1}H NMR solvent - at δ 4.03 (s, 4H). With the addition of a few drops of deuterium oxide to the DMSO sample, this peak disappeared - suggesting that the proton seen was exchangeable. Potentially, this means that coordination to the cyclic tetramer by some unknown species, or protonation of the triazole moieties, had occurred.

Analysis of the isotope pattern of the HRMS spectrum gave further evidence that the major component of the sample was the anticipated tetrameric material. As shown in the figure below, mass differences of 1 amu were seen different isotope peaks for the molecular ion. This rules out the signals being derived from a multiply charged higher oligomer, such as a doubly charged octamer, as mass differences of 1 / n amu (where n is the number of units present in the oligomer) would necessarily be seen.

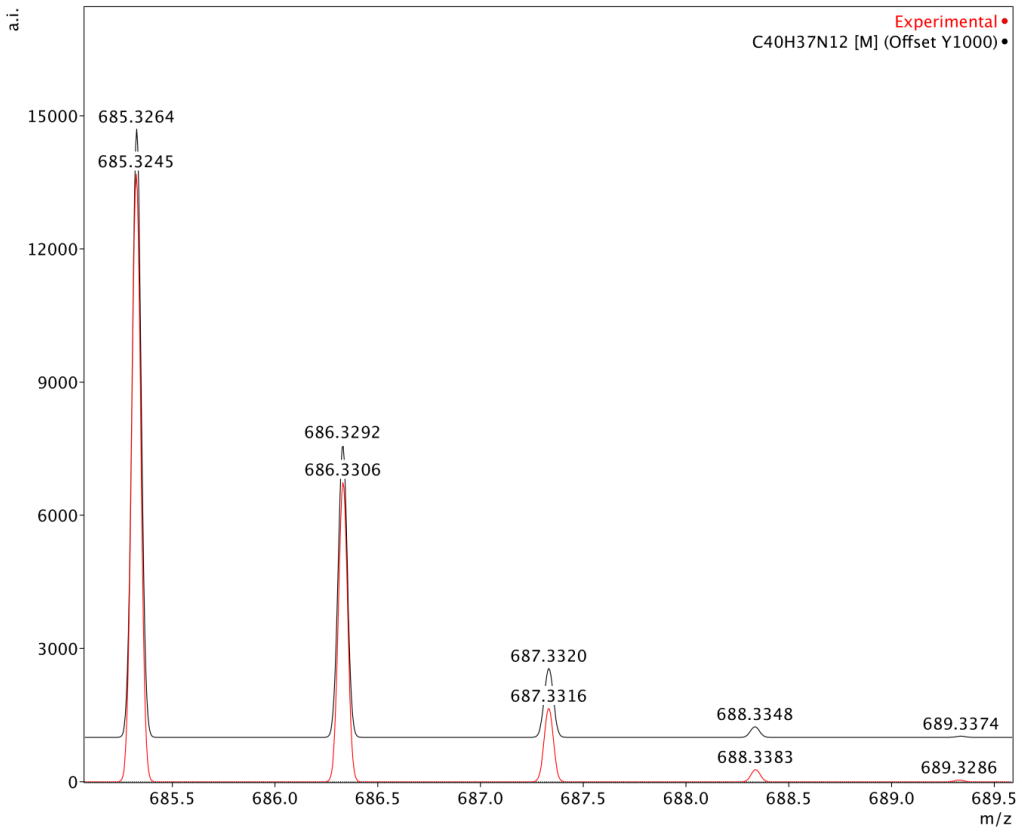
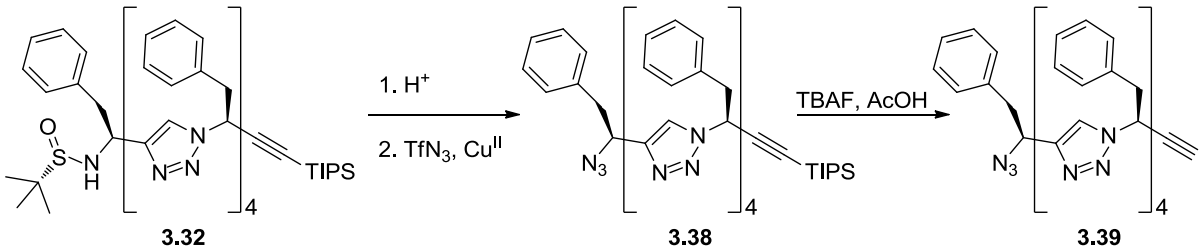


Figure 3.10 HRMS spectrum overlay of synthesised cyclic tetramer (red) overlaid with predicted isotope pattern (black) using mMass v3.12.1[119].

Although the reaction was performed on larger scales (up to 8.0 mg of alkynyl-azide **3.36**), the resulting isolated material was consistently less than 1.0 mg per reaction.

3.4.2 Cyclisation of Pentamer

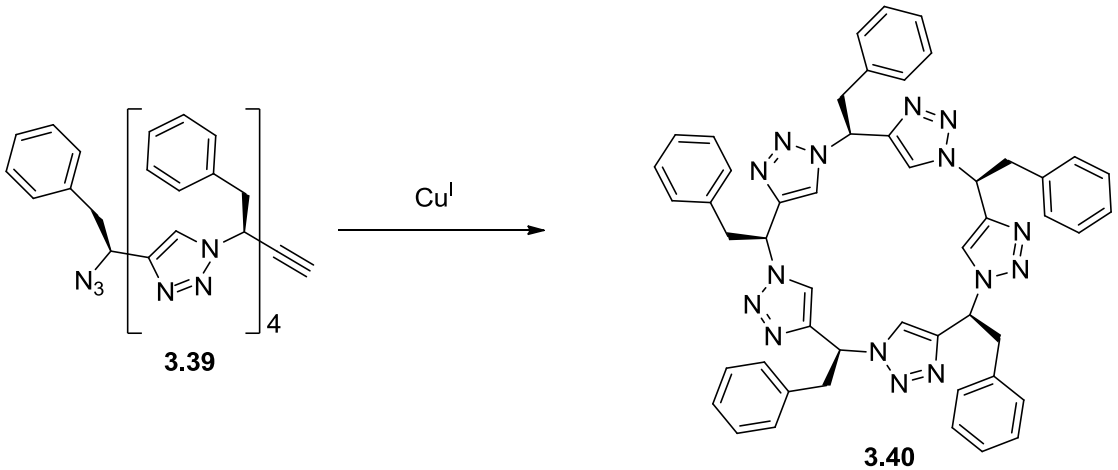
With conditions found for the cyclisation of the linear tetramer **3.36**, it remained to be seen whether the same conditions could be used for the cyclisation of longer triazole oligomers. Thus the linear pentameric triazole oligomer (**3.32**) was derived in a similar fashion to give a compound with an *N*-terminal azide and *C*-terminal alkyne, as shown in scheme 3.22 below.



Scheme 3.22 Derivatisation of pentamer 3.32 to pre-cyclic alkynyl-azide pentamer 3.39.

N-Deprotection of pentamer **3.32** with a solution of hydrogen chloride in diethyl ether (2 eq), followed by diazotransfer with triflic azide (7 eq) gave azido-pentamer **3.38** in 46 % yield for the 2 reactions. A peak corresponding to the protonated molecular ion (*m/z* = 1012.5) was observed in the ESI-MS.

The terminal alkyne of azido-pentamer **3.38** was exposed via the addition of TBAF (4 eq) buffered with acetic acid (2 eq). After completion of the reaction as shown by TLC, the product was precipitated by the addition of water. The solid was filtered and washed, and dried under high-vacuum to give **3.39** in 67 % yield. The ^{1}H NMR spectrum produced showed that the product was of high purity, and the newly exposed terminal alkyne signal was observed at δ 2.67 (d, *J* = 2.4 Hz, 1H). The compound was stored at 0-4 ^{o}C as a highly dilute solution in DCM (0.5 mM), to minimise the potential for polymerisation.



Scheme 3.23 Copper-mediated head-to-tail cyclisation of pentamer 3.39.

The optimised conditions found during cyclisation of the phenylalanine-based alkynyl-azide tetramer were used for cyclisation of the pentamer, and after reacting for 4 days, ^{1}H NMR analysis of an aliquot of the solution showed complete consumption of starting material. After

chromatography, cyclic pentamer **3.40** was obtained as a white solid. As before, due to the scale of the reaction (2.1 mg starting material), the isolated product was carefully weighed in triplicate on a 5-figure balance (+/- 0.01 mg), however unfortunately the mass was not able to be reliably determined (average of six weighings = 0.07 ± 0.06 mg (3 %)). Similar peaks were obtained in the ^{1}H NMR spectrum as observed for the tetramer, with one set of peaks present for each type of proton. Interestingly, the β-protons (δ 3.65 (d, 10H)) were clearly observed as a doublet in the cyclic pentamer, as opposed to 2 doublet of doublets in the tetramer. It was speculated that this may have been a result of the molecule existing in a different conformation to the tetramer, although no further evidence was obtained to test this hypothesis.

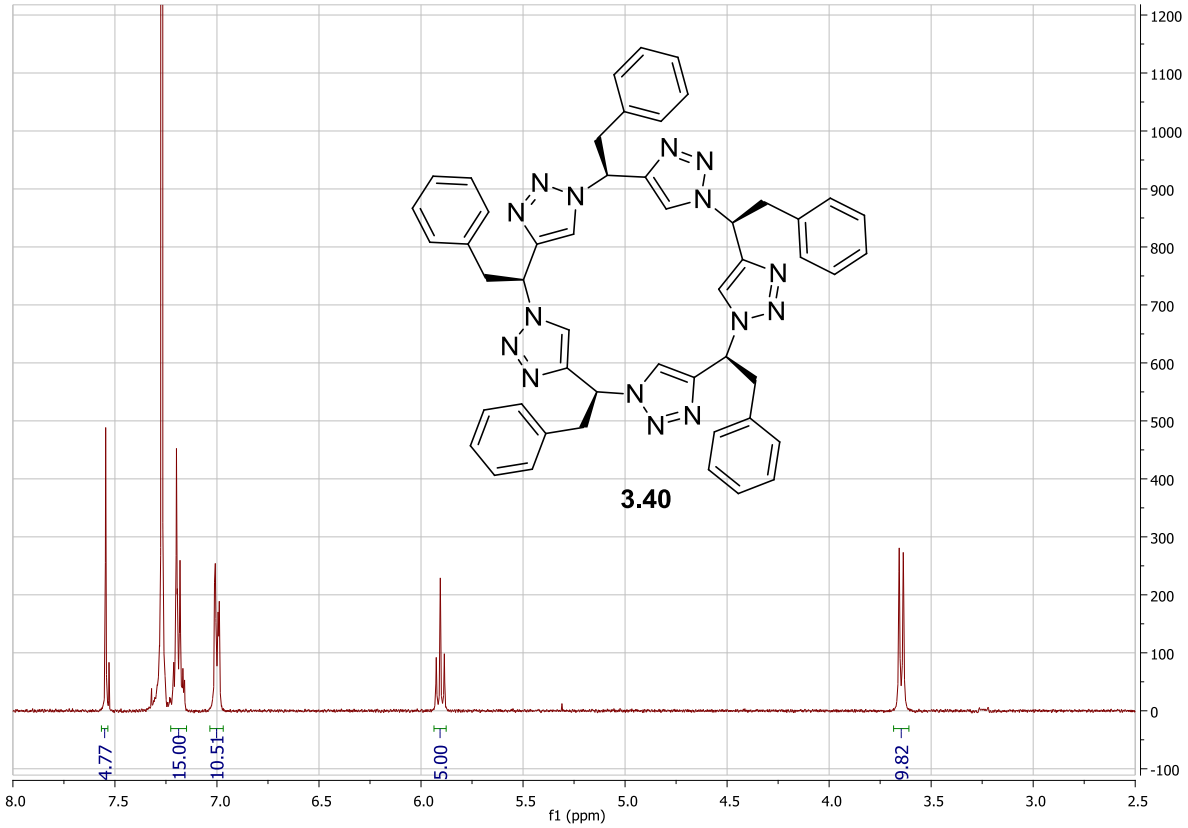
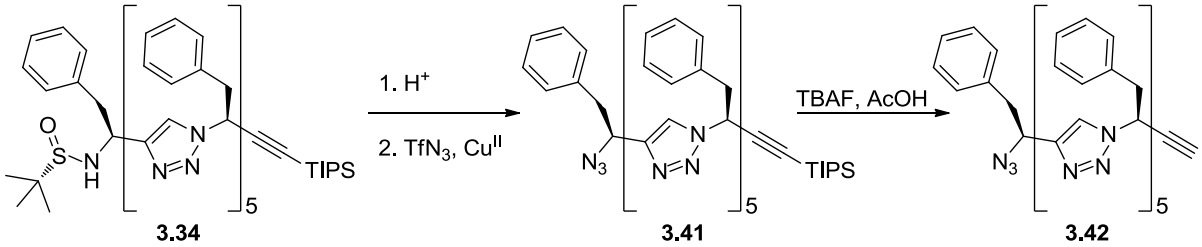


Figure 3.11 ^{1}H NMR spectrum of cyclic pentamer 3.40.

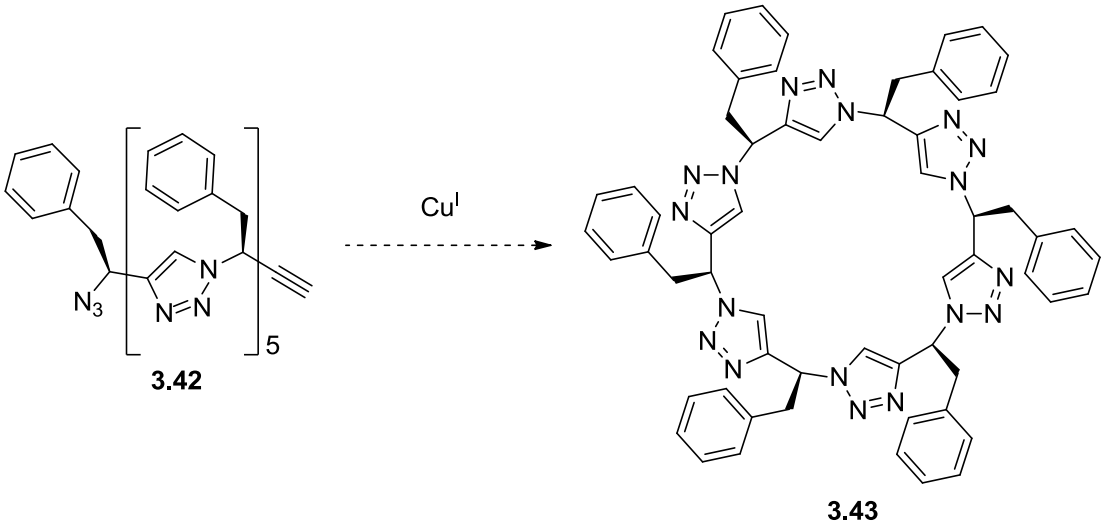
3.4.3 Cyclisation of Hexamer



Scheme 3.24 Derivatisation of hexamer 3.34 to linear azido-alkyne hexamer 3.42.

N-Deprotection of hexamer **3.34** with a solution of hydrogen chloride in diethyl ether (2 eq), followed by diazotransfer with triflic azide (7 eq), gave azido-hexamer **3.41** in 71.6 % yield for the 2 reactions. A peak corresponding to the protonated molecular ion (*m/z* = 1183.6) was observed in the ESI-MS.

The terminal alkyne of azido-hexamer **3.41** was exposed via the addition of TBAF (4 eq) buffered with acetic acid (2 eq). After completion of the reaction as shown by TLC, the product was precipitated by the addition of water. The solid was filtered and washed, and drying under high vacuum gave **3.42** in 66 % yield. A peak corresponding to the protonated molecular ion (m/z = 1027.5) was observed in the ESI-MS, and signals corresponding to the *C*-terminal α-proton, *N*-terminal α-proton and terminal alkyne were observed at δ 5.88 (td, *J* = 7.4, 2.4 Hz, 13H), 4.96 (dd, *J* = 8.4, 6.8 Hz, 11H), 3.74 (d, *J* = 2.4 Hz, 11H) respectively in the d_6-DMSO ^{1}H NMR spectrum. However, the signals corresponding to the internal α-protons integrated for 5 protons, potentially indicative of a small amount of polymerised material. The compound was stored at 0-4 ^{o}C as a highly dilute solution in DCM (0.5 mM), to minimise the potential for further polymerisation.



Scheme 3.25 Attempted copper-mediated head-to-tail cyclisation of hexamer 3.42.

The optimised conditions found during cyclisation of the phenylalanine-based alkyne-azide tetramer and pentamer were used for cyclisation of the hexamer, however the expected product was not isolated from the reaction. A steady decrease in solubility was observed for the growing chain lengths, and it is possible that the cyclised product was simply not soluble in the reaction solvents or NMR solvents. Alternatively, the product itself may not be a favoured ring size for cyclisation. If the seven atoms of the triazole and adjacent carbons are considered as a discrete, largely inflexible entity, the cyclisation series for triazole peptidomimetics could be viewed as mirroring that of the cycloalkanes series. In that series, overall strain, resulting from angle, conformational and transannular strain, is at a global maximum for small ring sizes (3,4) and minimum for common ring sizes (5,6). Interestingly, a local maximum strain is observed for medium ring sizes (7-11), which is followed by decreased strain for large ring sizes (≥ 12).[120] It is possible that the cyclic hexamer **3.43** is in an analogous region of the series correlating to a local maximum strain, which correlates with problematic cyclisation. The inference is that select pre-cyclised linear compounds in this series may display a reduced amount of time spent in a conformation that allows for cyclisation. To provide evidence in support of this theory, attempts to make larger members of the series would be required, however this is beyond the aims of this project.

3.5 Conclusions

A major focus of this project was the efficient synthesis of monomer units for use in an iterative chain extension sequence. The linear pathway detailed in chapter 2 gave the key alkynyl-azide extension monomer unit in 10 % yield over 7 steps. Via the Ellman sufinamide auxiliary, a novel, a late-stage divergent synthesis of chiral phenylalanine-derived monomer units was established. The key alkynyl-azide extension monomer unit was able to be synthesised in 44 % over 3 steps from an easily accessible sulfinylimine intermediate. The pathway to these units was designed in such a way that it would allow the synthesis of a range of amino acid mimics, which are explored in chapter 4.

The 2-step *C*-terminal linear extension pathway was repeated and optimised with the new monomer units, allowing the synthesis of up to the hexameric linear triazole oligomers, and the extension reactions consistently gave yields > 70 %. Facile derivatisation of the terminal units of the oligomers gave access to the starting materials for head-to-tail cyclisation. Conditions for the cyclisation were investigated, identifying the use of non coordinating counter-ions as being critical. Successful cyclisation of the tetra and pentameric series were conducted, which gave the corresponding cyclised compounds, albeit in low yields. Given that such reactions are often not trivial, the results were encouraging.

With the ability to produce a cyclic pentamer, our attention moved to the use of this chemistry for the synthesis of a biologically active peptide mimic, based on an existing cyclic pentapeptide.

Chapter 4 - Synthesis Towards an RGD-based Triazole Peptidomimetic

4.1 Introduction to Integrins and the RGD motif

Having demonstrated a successful synthetic approach to a cyclic all-triazole peptidomimetic, our attention turned to the synthesis of a biologically active cyclic peptide mimic. Many pentameric and hexameric RGD-containing cyclic peptides have been synthesised for the production of potent and selective integrin inhibitors.[121] For the $\alpha_v\beta_3$ integrin, implicated in human tumour metastasis and angiogenesis, the cyclic peptide RGDfV (**4.1**) was very potent with an IC_{50} value of 4.9 nM, inhibiting the binding of the RGD-containing vitronectin protein to integrins.[121] As shown in figure 4.1 below, the lower-case 'f' in RGDfV denotes the use of the D-phenylalanine stereoisomer. Other derivatives found to be strong binders were Cy [R G D f K] (**4.2**, 4.2 nM), Cy [R G D f G] (**4.3**, 1.6 nM) and Cy [R G D f L] (**4.4**, 0.95 nM).[122]

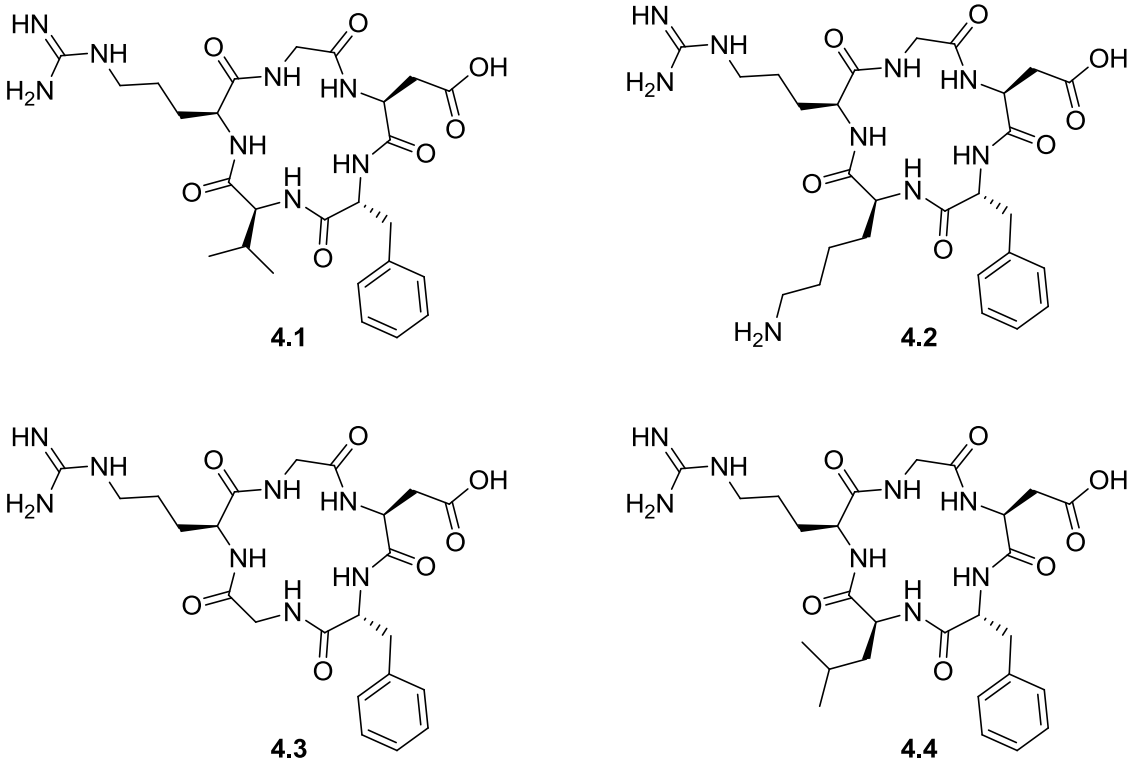


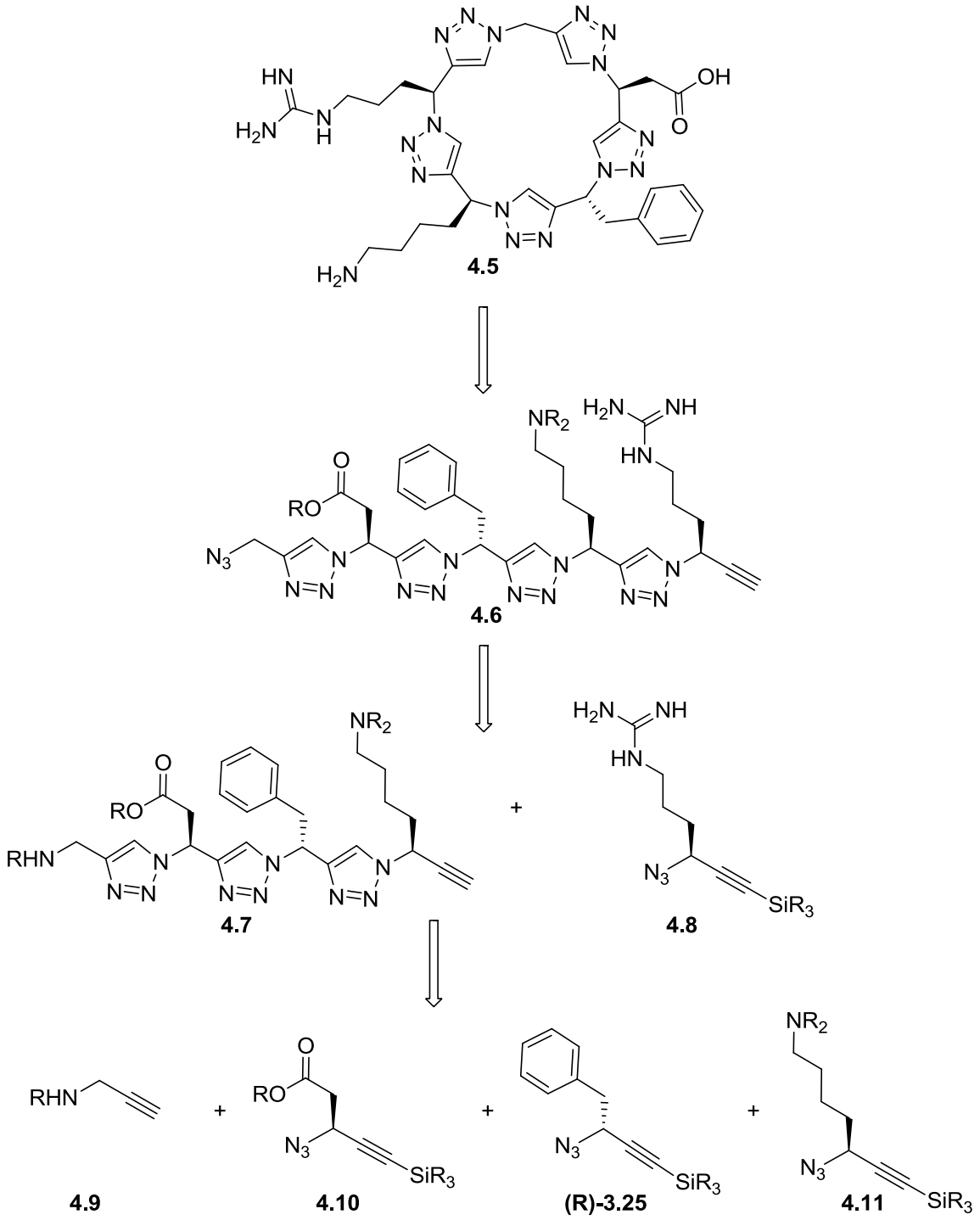
Figure 4.1 $\alpha_v\beta_3$ integrin cyclic pentapeptide inhibitors with a common RGDfX motif, with X = V, K, G and L for compounds 4.1, 4.2, 4.3 and 4.4 respectively.

Endothelial cells, which line blood vessels, bind via surface-based $\alpha_v\beta_3$ integrins to extracellular matrix components. These cells provide an interface between the luminal components in the circulatory system and the ECM, which allows for signal transduction – both inside-out and outside-in. In tumour tissues $\alpha_v\beta_3$ is upregulated,[123] which promotes angiogenesis and tumour metastasis. The interaction between the ECM and the endothelial cells can be disrupted by blocking the $\alpha_v\beta_3$ integrins, which in turn causes apoptosis of the angiogenic vascular cells in tumour tissue.[124]

Integrins are made up of 2 distinct domains: α and β, of which 15 α and 8 β subunits are known, which associate into many different α-β heterodimers. Many of these heterodimers contain an Arg-Gly-Asp (RGD) binding region, to which ECM glycoproteins that contain the key RGD motif, such as fibronectin and collagen, may bind.[122] Hautanen *et al*.[125] showed that short RGD-containing peptides may mimic these cell-adhesion proteins, although the affinity compared to the parent protein was relatively low - with the fibronectin-derived GRGDSP peptide showing 1000 times less affinity in a cell attachment assay. The RGD motif was found to be critical for binding, with the replacement of the aspartic acid residue with glutamic acid, or the replacement of the glycine residue with alanine, each causing a 100-fold decrease in affinity.

We sought to develop the requisite monomer units to enable production of the cyclic all-triazole version of the inhibitor Cy [R G D f K]. This particular sequence was chosen to explore the versatility of the monomer unit synthesis shown in chapter 3, by attempting to demonstrate the synthesis of a diverse range of alkynyl-azide based amino acid mimics.

4.2 Plan for the Synthesis of a Triazole-based RGD Peptidomimetic

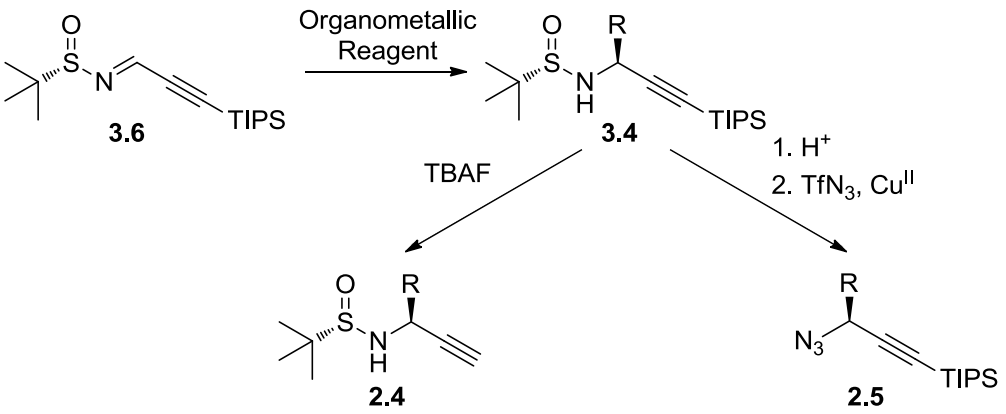


Scheme 4.1 Retrosynthesis of the all-triazole Cy [RGDfK] peptidomimetic (4.5) leading to amino acid-derived alkynyl-azide monomer units.

It is shown in scheme 4.1 above that the target all-triazole cyclic pentamer (**4.5**) could be synthesised via a head-to-tail cyclisation of alkynyl-azide **4.6**. The linear compound could be

prepared via a Cu-AAL reaction between tetrameric alkyne **4.7** and the silyl-protected alkynyl-azide arginine analogue **4.8**, followed by derivatisation of the *N*-terminus and *C*-terminus to an azide and alkyne respectively. In turn, the tetrameric alkyne **4.7** and corresponding could be synthesised in a similar iterative fashion as demonstrated in chapter 3, using monomer units derived from glycine (**4.9**), aspartic acid (**4.10**), phenylalanine (**(R)-3.25**) and lysine (**4.11**). Although compound **4.5** was selected as the pre-cyclised linear pentamer, other alkynyl-azides could be used for cyclisation which would give access to an identical cyclised product, if the sequence of residues was kept constant. This particular linear compound was chosen as two of the required monomer units, alkyne **4.9** and alkynyl-azide **(R)-3.25**, were already in hand.

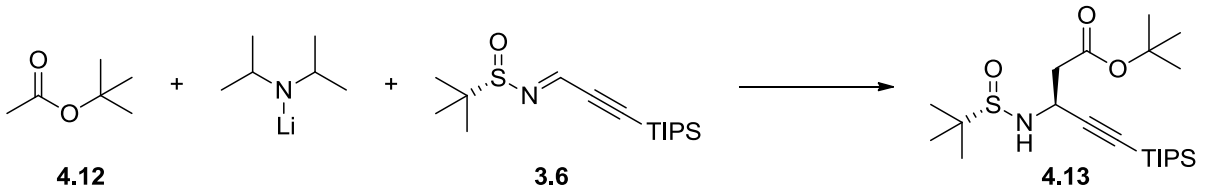
4.3 Monomer Unit Synthesis



Scheme 4.2 General synthesis of monomer units from sulfinylimine 3.6.

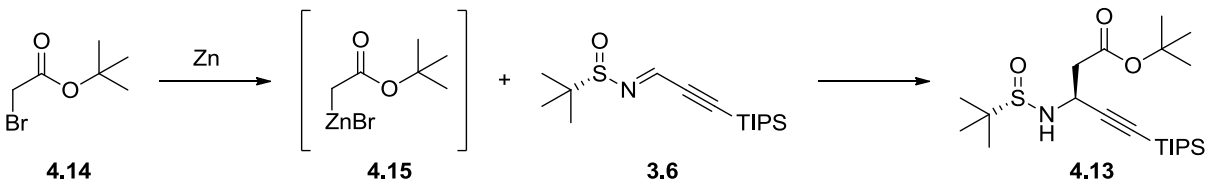
As shown in chapter 3.2.3, synthesis of the amino acid side chain-containing alkynyl-azide variants (**2.5**) would involve the addition of an organometallic group containing the amino acid side-chain functionality onto sulfinylimine **3.6**, followed by *N*-deprotection and diazotransfer. The *N*-protected alkyne monomer unit (**2.4**) would be synthesised via TBAF-mediated desilylation of the intermediate sulfinylamide **3.4**.

4.3.1 Synthesis of the aspartic acid mimic



Scheme 4.3 Synthesis of aspartic acid alkyne mimic 4.13 via a reaction between *tert*-butyl acetate (4.12), LDA and sulfinylimine 3.6.

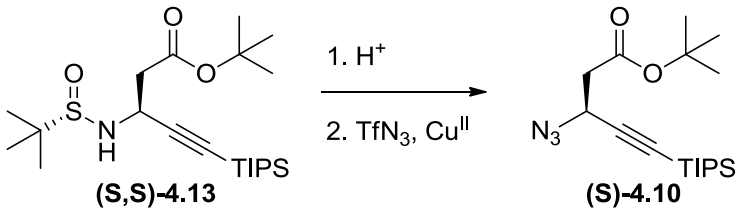
For the synthesis of an aspartic acid mimic, an organometallic reagent derived from a protected acetate ester was required. An enolate was formed by the addition of LDA (1.1 eq) to *tert*-butyl acetate (**4.12**, 1 eq) in THF at -78 ^{o}C,[126] to which was added, sulfinylimine **3.6** (1 eq) in THF. TLC analysis of the reaction mixture showed a pair of spots which potentially indicated the formation of a pair of sulfinylamide diastereomers. The crude ^{1}H NMR spectrum showed signals consistent with the α-protons (δ 4.45, 1H, m) and β-protons (δ 2.74, 2H, m), however estimation of the diastereomeric excess was not possible due to overlapping peaks. After separation by column chromatography, the compounds were isolated in 21 % and 8.6 % yield. The α-proton for both compounds was observed at δ 4.47 (1H, q) in the ^{1}H NMR spectrum, and the diastereomeric excess was found to be 42 % by mass. The modest diastereoselectivity displayed by the enolate was attributed to its highly reactive nature.



Scheme 4.4 Synthesis of aspartic acid alkyne mimic 4.13 via a reaction between the *in situ* generated Reformatsky reagent (4.15) and sulfinylimine 3.6.

With the failure of the *tert*-butyl acetate-derived enolate to give the aspartic acid sulfinylamide mimic **4.13** in good yields, a procedure which involved the use of a less

reactive enolate was expected to give higher yields. Brinner *et al.*[127] demonstrated that the organozinc Reformatsky reagent (**4.15**) could react with a *tert*-butyl sulfinylimine, thus their procedure was replicated using sulfinylimine **3.6**. Firstly the Reformatsky reagent was formed *in situ* by the addition of *tert*-butyl bromoacetate (**4.14,** 2 eq) to a suspension of activated zinc (4 eq) in THF. After cooling the solution to 0 ^{o}C, sulfinylimine **3.6** (1 eq) in THF was added. Similar signals to those noted above were observed in the crude ^{1}H NMR spectrum at δ 4.56-4.46 for the α-protons, and δ 2.89-2.66 for the β-protons. After separation by column chromatography, the major diastereomer was isolated in 57 % yield, and the minor diastereomer in 16 % yield, giving an overall d.e. of 56 %. Interestingly, the major diastereomer from this reaction was in fact the minor diastereomer from the enolate addition shown above, which may suggest that the products are being formed by different mechanisms.

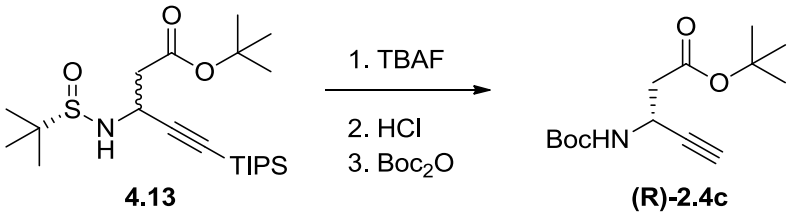


(S,S)-4.13 **(S)-4.10**

Scheme 4.5 Conversion of aspartic acid derivative 4.13 to the protected-alkynyl azide form (4.10).

Transformation of the major diastereomer from the Reformatsky addition (assumed to be **(S,S)-4.13**) to the azide monomer unit was achieved via *N*-deprotection and diazotransfer with triflic azide, giving azide **4.10** in 26 % yield for the two steps. The ^{1}H NMR spectrum showed the α-proton peak at δ 4.53 (1H, t) and β-proton peaks at δ 2.63 (2H, m), and a signal corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 352).

In order to confirm the absolute stereochemistry of the diastereomers described earlier, the major product from the Reformatsky addition (**4.13**) was derivatised to the Boc-protected desilylated product **2.4c**, of which the 'S' enantiomer had previously been synthesised in chapter 2.2.6 and was found to have an optical rotation of $[\alpha]^{21}_{D}$ = -11.1^{o}. Therefore TBAF-mediated desilylation, *N*-deprotection with a solution of hydrogen chloride in diethyl ether, and re-protection of the amine as a *tert*-butyl carbamate using Boc anhydride were performed in succession, which gave alkyne **2.4c** in 35 % yield over the 3 steps.



Scheme 4.6 3-step conversion of aspartic acid alkyne mimic 4.13 to Boc-protected derivative 2.4c.

The $[\alpha]^{21}_{D}$ was determined to be +21.4, which was interesting for 2 reasons. Firstly, as the absolute value of the optical rotation was higher than the expected 11.1, it suggested (as was seen with the analogous phenylalanine case) that alkyne **(S)-2.4c** synthesised through the Ohira-Bestmann route was not optically pure. Secondly, as the opposite sign was seen it suggested that the major product of the Reformatsky addition reaction was in fact the (R)-stereoisomer. In turn, the minor product from the Reformatsky addition was also derivatised to the Boc-protected desilylated form and was found to have an optical rotation of $[\alpha]^{21}_{D}$ = -20.8^{o}. Therefore it would appear that addition of the Reformatsky reagent occurred preferentially from the *Si*-face, whereas the opposite was true for the lithium enolate addition. As a consequence of this discovery, it became apparent that azide **(R)-4.10** below, rather than azide **(S)-4.10**, had been synthesised earlier. Although the desired stereochemistry was not obtained, it was decided to proceed with this stereochemistry in a model reaction series for chain extension (section 4.4).

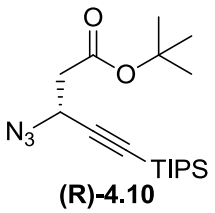
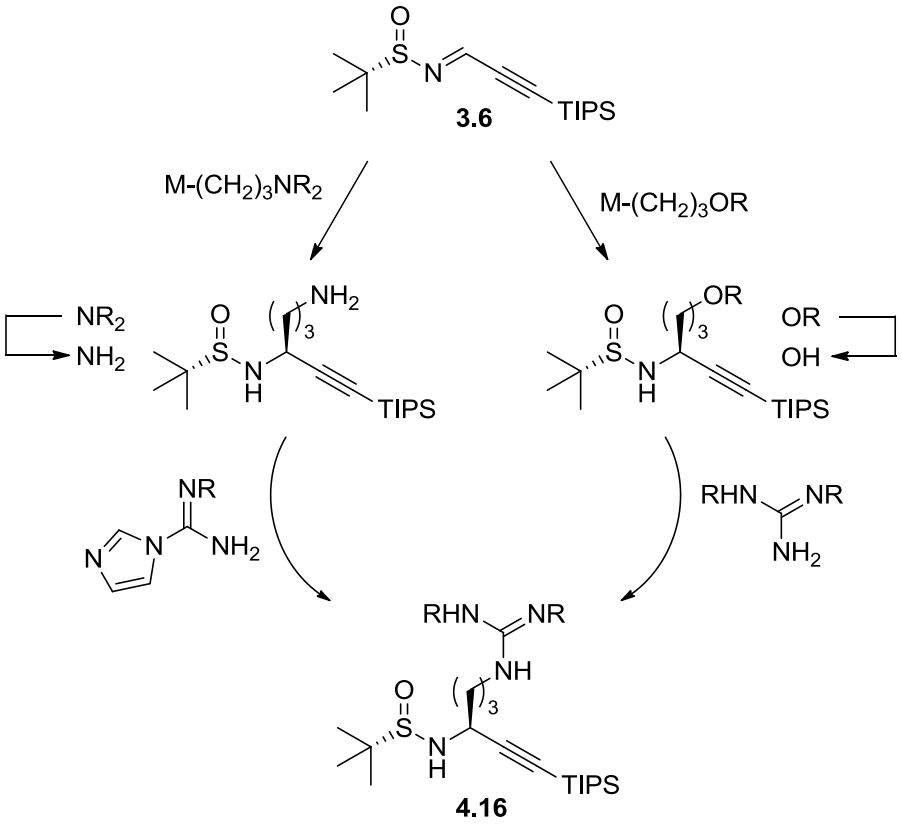


Figure 4.2 Protected alkynyl-azide aspartic acid mimic (R)-4.10.

4.3.2 Synthesis of the arginine mimic

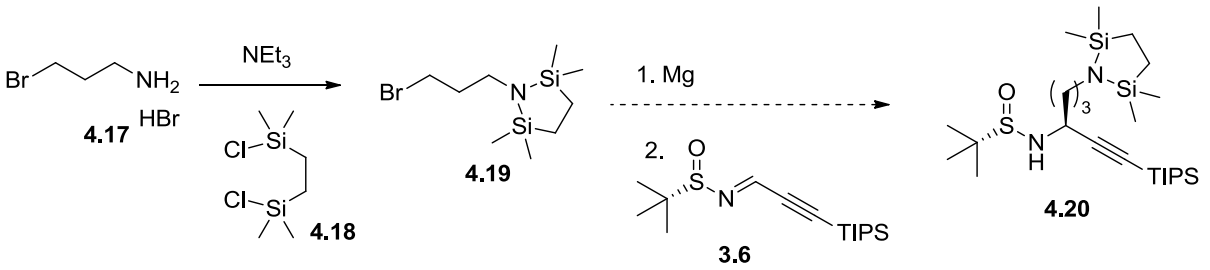
When considering the synthesis of the arginine mimic, we predicted that formation of a guanidine-containing Grignard reagent for addition to the sulfinylimine would be problematic. As such, we proposed that the guanidine moiety could be incorporated after the addition of the carbon atoms of the side-chain to sulfinylimine **3.6**. There are a number of methods available for the installation of a guanidine, and these will dictate the functional groups

chosen. As outlined in scheme 4.7 below, a guanidine-containing group could be introduced via nucleophilic substitution of an amino side-chain, or in a Mitsunobu reaction with an alcohol side-chain. These reactions could be modified for the production another of our target monomer units: the lysine analogue.



Scheme 4.7 Synthesis of arginine alkyne mimic 4.16 via either addition of a protected amine-containing Grignard (left) or a protected alcohol-containing Grignard (right) to key sulfinylimine 3.6, followed by substitution to a guanidine moiety.

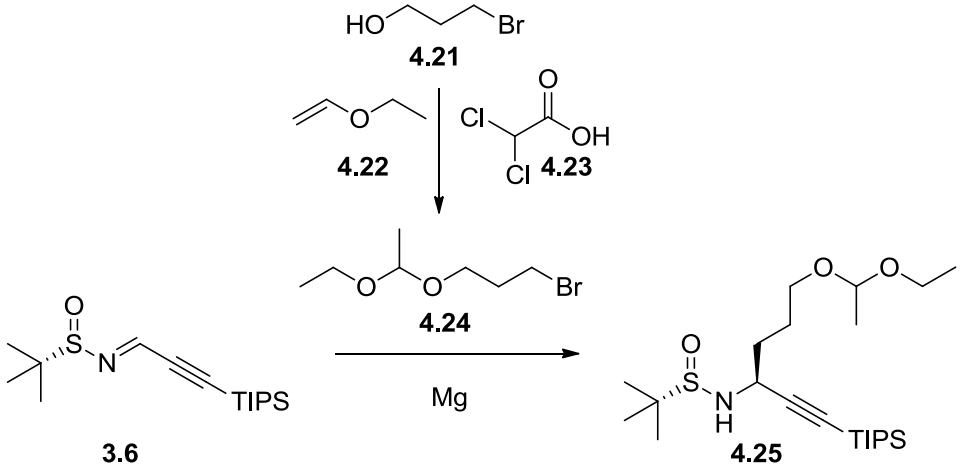
For the addition of the propylamine moiety onto sulfinylimine **3.6**, bis-protection of the halo-alkylamine was necessary as secondary amines may interfere with the formation of Grignard reagents. It was shown by Xu *et al*.[128] that the STABASE bis-protecting group was inert to Grignard conditions, and thus was investigated.



Scheme 4.8 Proposed protection of 3-bromopropylamine (4.17) with the STABASE protecting group, followed by addition to sulfinylimine 3.6 giving sulfinylamide 4.20.

The STABASE group was installed by the addition of 1,2-bis(chlorodimethylsilyl)ethane (**4.18**, 1 eq) to a solution of 3-bromopropylamine hydrobromide (**4.17**, 1 eq) and triethylamine, which gave protected amine **4.19** in 17.5 % yield. The ^{1}H NMR spectrum showed 3 sets of aliphatic protons at δ 3.41 (1H, t), δ2.95 (1H, t) and δ 1.96 (1H, p), as well as the silyl attached signals at δ 0.71 (4H, s) and δ 0.07 (12H, s). The product was found to be very sensitive, and was found to degrade quickly, even when kept neat at 4 ^{o}C. Due to the apparent instability shown with these compounds, the alternative, alcohol functionalised route was investigated.

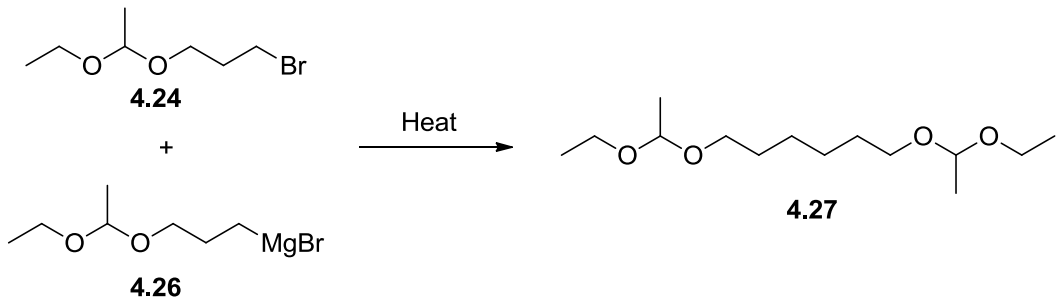
It was hypothesised that propanol functionality could be installed onto sulfinylimine **3.6** via the addition of an appropriately protected alcohol-containing Grignard reagent. It was shown by Song *et al*.,[129] who were also performing reactions with Ellman sulfinylimines, that ethoxyethyl ether-protected Grignard reagents derived from 3-bromopropanol could be used.



Scheme 4.9 Protection of 3-bromopropanol (4.21) as an ethoxyethyl ether, followed by Grignard formation and addition to sulfinylimine 3.6 giving sulfinylamide 4.25.

Following the procedure of Eaton,[130] 3-bromopropanol (**4.21**) was protected as the ethoxyethyl ether via dichloroacetic acid-catalysed addition of the alcohol to ethyl vinyl ether (**4.22**), giving crude bromide **4.24** after the excess ethyl vinyl ether was removed by rotary evaporation. The key acetal proton from the protecting group was observed in the ^{1}H NMR spectrum at δ 4.70 (1H, q). Heating the crude material during distillation caused the material to degrade, therefore the following reaction was performed with a crude mixture of the product and residual 3-bromopropanol (5 : 1 ratio by NMR).

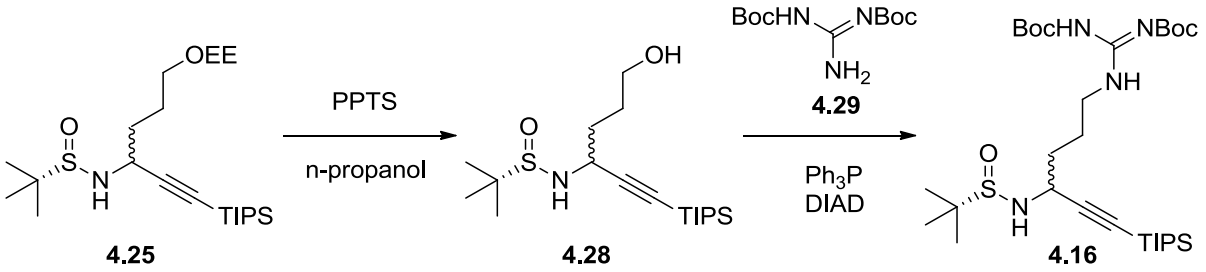
After formation of the Grignard via addition of freshly prepared bromide **4.24** (4 eq) over 20 minutes to magnesium turnings (4 eq), the Grignard solution was added dropwise to a solution of sulfinylimine **3.6** (1 eq) in DCM at -78 ^{o}C. Only 1 diastereomer was formed during the addition of the Grignard to the sulfinylimine as judged by the ^{1}H NMR spectrum and TLC, as the crude ^{1}H NMR spectrum only showed one signal that could be attributed to product α-proton at δ 4.15 (1H, q). Interestingly, when a shorter addition time of the alkylbromide to magnesium was performed (10 minutes), this signal did not appear in the ^{1}H NMR spectrum, with magnesium-catalysed homocoupling of the bromide becoming the dominant reaction pathway, as shown in scheme 4.10 below. This is presumably due to the rate of formation of the Grignard reagent being slower than the subsequent homo-coupling reaction, as well as localised heating due to the exothermic magnesium insertion reaction, which may react with excess alkylbromide in the solution.



Scheme 4.10 Homocoupling of ethoxyethyl ether-protected 3-bromopropanol.

The crude material was purified with column chromatography to give sulfinylamide **4.25**, however a small amount of the homodimerised byproduct **4.27** remained in the sample, due to the R_f being identical that of the product. Both the acetal proton from the protecting group and the equivalent signals from the byproduct were superimposed in the ^{1}H NMR spectrum,

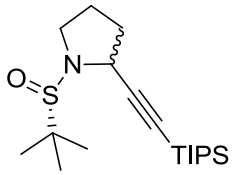
at δ 4.73-4.66 (1.4H, m). It was anticipated that the byproduct could be separated after deprotection of the ethoxyethyl protecting group.



Scheme 4.11 Derivatisation of sulfinylamide 4.25 to arginine minic 4.16.

Functional group transformation of the protected alcohol to the guanidine group was effected in two steps. Firstly the alcohol was exposed via PPTS-catalysed transetherification of the ethoxyethyl ether group to *n*-propanol, giving deprotected alcohol **4.28**. The side-products were able to be separated from the product at this stage of the synthesis, and the alcohol was isolated in 61 % yield over the 2 steps from sulfinylimine **3.6**. The α-proton signal and γ-proton signals were observed in the ^{1}H NMR spectrum at δ 4.17 (1H, q), and δ 3.68 (2H, t) respectively, and no signals were observed from the byproduct (**4.27**) or derivatives thereof. Unfortunately, no compounds existed in the literature to which alcohol **4.28** could be derivatised for comparison of the optical rotation, thus the overall stereochemistry of the compound was not determined. Potentially a Mosher's amide analysis after *N*-deprotection would be able to differentiate between the 2 possible epimers.[131] In the meantime, functional group transformation to the intended guanidine derivative (**4.16**) was attempted.

A Mitsunobu reaction between alcohol **4.28** and 1,3-bis(*tert*-butoxycarbonyl)guanidine (**4.29**) (2 eq) with triphenylphosphine and DIAD was performed. After column chromatography, a compound was isolated which had a mass that corresponded to the starting material minus water, as observed by ESI-MS (*m/z* = 356). The compound was proposed to be byproduct **4.30** shown in figure 4.3 below, which could have formed via intramolecular cyclisation of the activated alcohol intermediate. This was supported by the ^{1}H NMR spectrum, which contained plausible signals for an α-proton at δ 4.59 (1H, m), δ-protons at 3.50 (1H, m) and 3.35 (1H, m), as well as the β- and γ-protons at 2.05-1.86 (4H, m). The *tert*-butyl sulfinamide and TIPS signals remained, but no other signals were present.



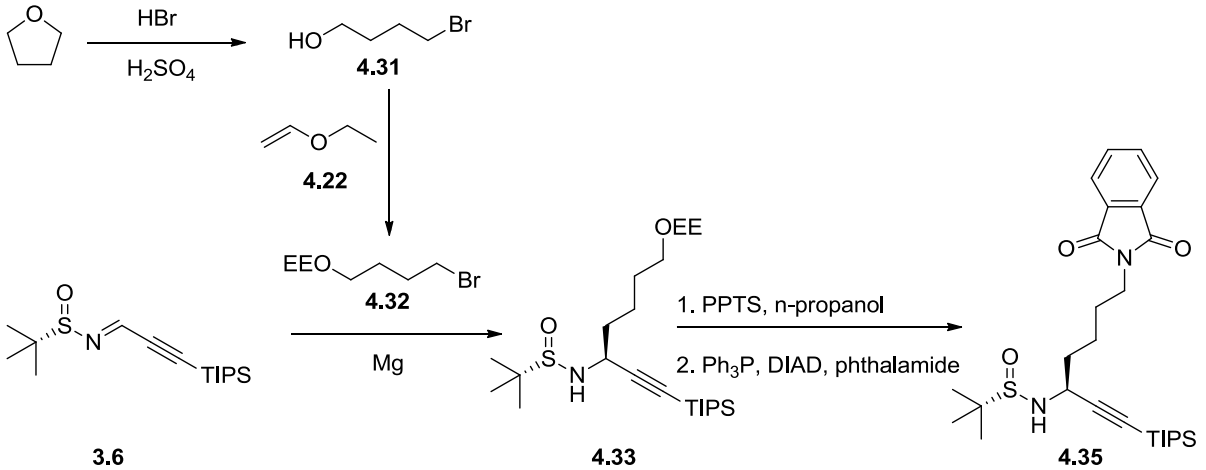
4.30

Figure 4.3 Pyrollidine byproduct 4.30.

The reaction was repeated using 10 equivalents of the guanidine in an attempt to circumvent formation of pyrrolidine **4.30**. The procedure was found to be successful, as the crude ^{1}H NMR spectrum showed no signals corresponding to the byproduct. Signals resulting from the α-proton were observed at δ 4.18 (1H, q), γ-protons at δ 3.71 (2H, t), and the side-chain Boc signals were observed at δ 1.28 (9H, s) and δ 1.26 (9H, s). The product was not able to be isolated, however, and it was hypothesised that the Boc-protecting groups were not stable to the mildly acidic column chromatography employed. Interestingly the starting alcohol material was isolated, in 34 %. Given that the ^{1}H NMR spectrum of the crude material did not show any evidence of the residual alcohol starting material, it was proposed that reversion via an unknown mechanism on the silica column had taken place. The use of an acid-stable protecting group such as the *bis*-Cbz guanidine, in place of the *bis*-Boc guanidine, would be required to stop this side-reaction from occurring.

4.3.3 Synthesis of the lysine mimic

Similar reactions to those used for the production of the arginine mimic were used for the synthesis of the corresponding lysine analogue, as detailed below in scheme 4.13.



Scheme 4.13 Synthesis and protection of 4-bromobutanol (4.31) as an ethoxyethyl ether, Grignard formation and addition to sulfinylimine 3.6, followed by deprotection of sulfinylamide intermediate 4.33 and a Mitsunobu reaction installing phthalimide-protected amine functionality.

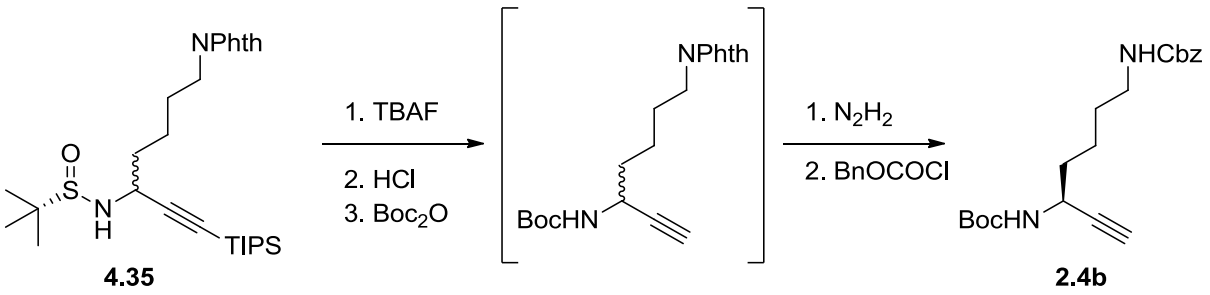
A two-step reaction was performed for the synthesis of the ethoxyethyl-protected 4-bromo-butanol (**4.32**). Firstly, the addition of concentrated sulfuric acid and hydrobromic acid to THF gave crude 4-bromobutanol (**4.31**), in ~ 38 % yield. In the crude ^{1}H NMR spectrum the methylene protons α to the alcohol were observed at δ 3.67 (2H, t) and the protons α to the bromine were seen at δ 3.45 (2H, t). Similar reaction conditions as detailed in 4.3.2 were then used for the protection of the alcohol as an ethoxyethyl ether, giving crude bromide **4.32**. The ^{1}H NMR spectrum showed the key acetal signal from the protecting group at δ 4.6 (1H, q), as well as methylene signals at δ 1.97 (2H, m) and δ 1.72 (2H, m). The methylene signals α to the bromine and protected alcohol were superimposed with signals from the protecting group, at δ 3.65-3.40. As with the analogous protected 3-bromobutanol (**4.24**), no further purification was performed.

After formation of the Grignard reagent via addition of freshly prepared bromide **4.32** (3 eq) over 20 minutes to magnesium turnings (3 eq), the Grignard solution was added dropwise to a solution of sulfinylimine **3.6** (1 eq) in DCM at -78 ^{o}C. The ^{1}H NMR spectrum of the crude material suggested that a mixture of diastereomers were present in a 1 : 2 ratio, by comparison of the α-proton signals at δ 4.17 (1H, q) and δ 4.11 (2H, q). Some material resulting from homodimerisation of the starting bromide also appeared to be present. Again, column chromatography could not completely remove the homodimerised byproduct, although the diastereomers could be separated.

Functional group transformation of the protected alcohol to a phthalimide-protected amine was effected in two steps. Firstly the alcohol was exposed via PPTS-catalysed transetherification of the ethoxyethyl ether group to n-propanol, which gave the primary alcohol (**4.34**), isolated in 13 % yield over the 2 steps from sulfinylimine **3.6**. The α-proton and ε-protons were observed in the ^{1}H NMR spectrum at δ 4.11 (1H, m) and δ 3.66 (2H, t) respectively, and no signals were found corresponding to the homodimerised byproduct or its derivatives.

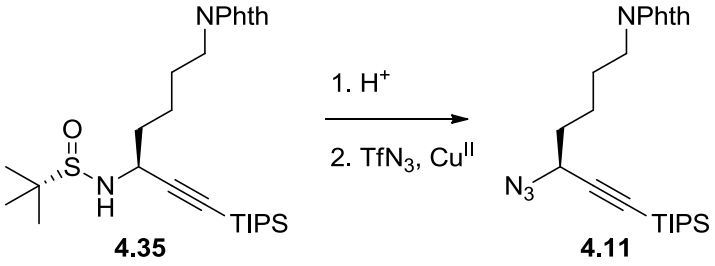
Next, a Mitsunobu reaction using standard conditions (triphenylphosphine, DIAD) with phthalimide (2 eq) was used to effect the transformation of the alcohol (1 eq), to protected amine **4.35**, which was isolated in in 84 % yield. The phthalimide peaks were observed in

the ^{1}H NMR spectrum at δ 7.84 (2H, m) and δ 7.71 (2H, m), as was the α-proton at δ 4.09 (1H, m). The ε-protons had moved slightly downfield to δ 3.70 (2H, t).



Scheme 4.14 Functional group transformation of sulfinylamide 4.36 to literature compound alkyne 2.4b.

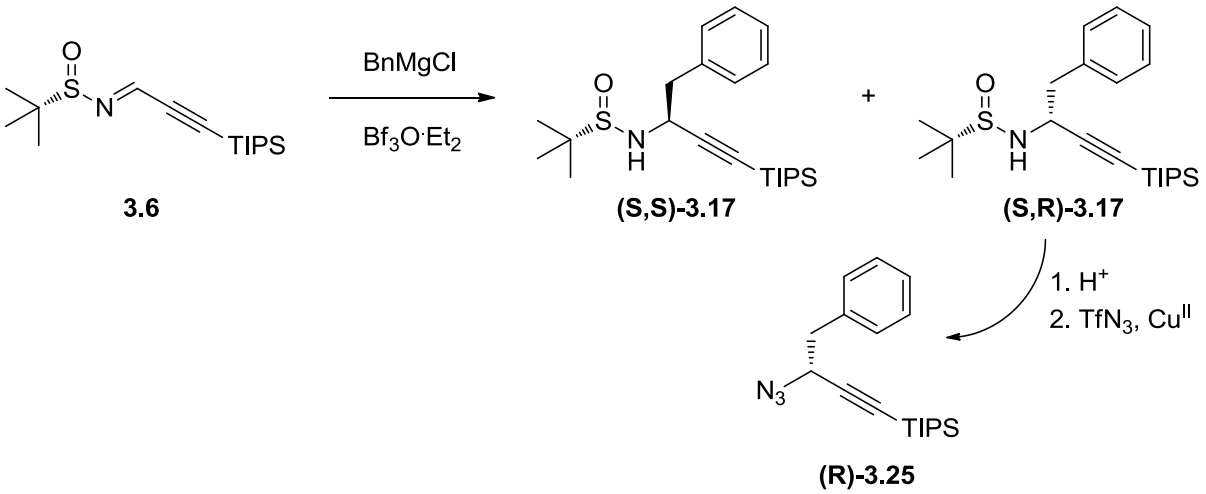
To determine the stereochemistry of the product, derivatisation to known alkyne **2.4b** was performed using the major isolated sulfinylamide diastereomer (**4.35**). Desilylation with TBAF, *N*-deprotection with hydrogen chloride and Boc-protection with Boc anhydride gave the Boc-protected alkyne derivative in 53 % yield for the 3 steps. As suggested by Maryanoff *et al.*,[132] the side-chain phthalimide group was then cleaved with hydrazine in methanol with 1-heptene present as scavenger of diimide. The scavenger was present to prevent diimide, an oxidation product of hydrazine, further oxidising to nitrogen concurrently reducing the alkyne. The primary amine was protected as a Cbz-carbamate via nucleophilic addition to benzyl choroformate, giving **2.4b** in 17 % yield for the final 2 steps. The optical rotation was determined to be $[\alpha]^{21}_{D}$ = -14.3, lower than the literature value of -23.1 ^{o}. As the sign of the optical rotation was the same as the literature compound, the compound being analysed was the (S)-enantiomer. The difference in the magnitude of the optical rotation seen may be a result of the solvent used to run the experiment, as the literature did not describe either the concentration or solvent used. It was not believed to be due to a result of racemisation of the product.



Scheme 4.15 *N*-deprotection and diazotransfer leading to azide 4.11.

For use in a Cu-AAL chain extension sequence, conversion of sulfinylamide **4.35** to azido-TIPS protected alkyne **4.11** was required. The side-chain amino group was not deprotected at this stage, as it would interfere with the diazotransfer reaction. Firstly removal of the sulfinyl group was effected by a solution of hydrogen chloride in diethyl ether, and the amine peaks were seen in the ^{1}H NMR spectrum (d_6-DMSO) at δ 8.34 (2H, 2 x s), and the adjacent α-proton was observed at δ 4.14 (1H, m). Triflic azide-mediated diazotransfer was then performed, which gave azide **4.11** in 80 % yield. The ^{1}H NMR spectrum showed the α-proton as a clear triplet (δ 4.11, 1H), giving evidence to suggest that conversion from the primary amine had occurred.

4.3.4 Synthesis of the (D)-phenylalanine Mimic

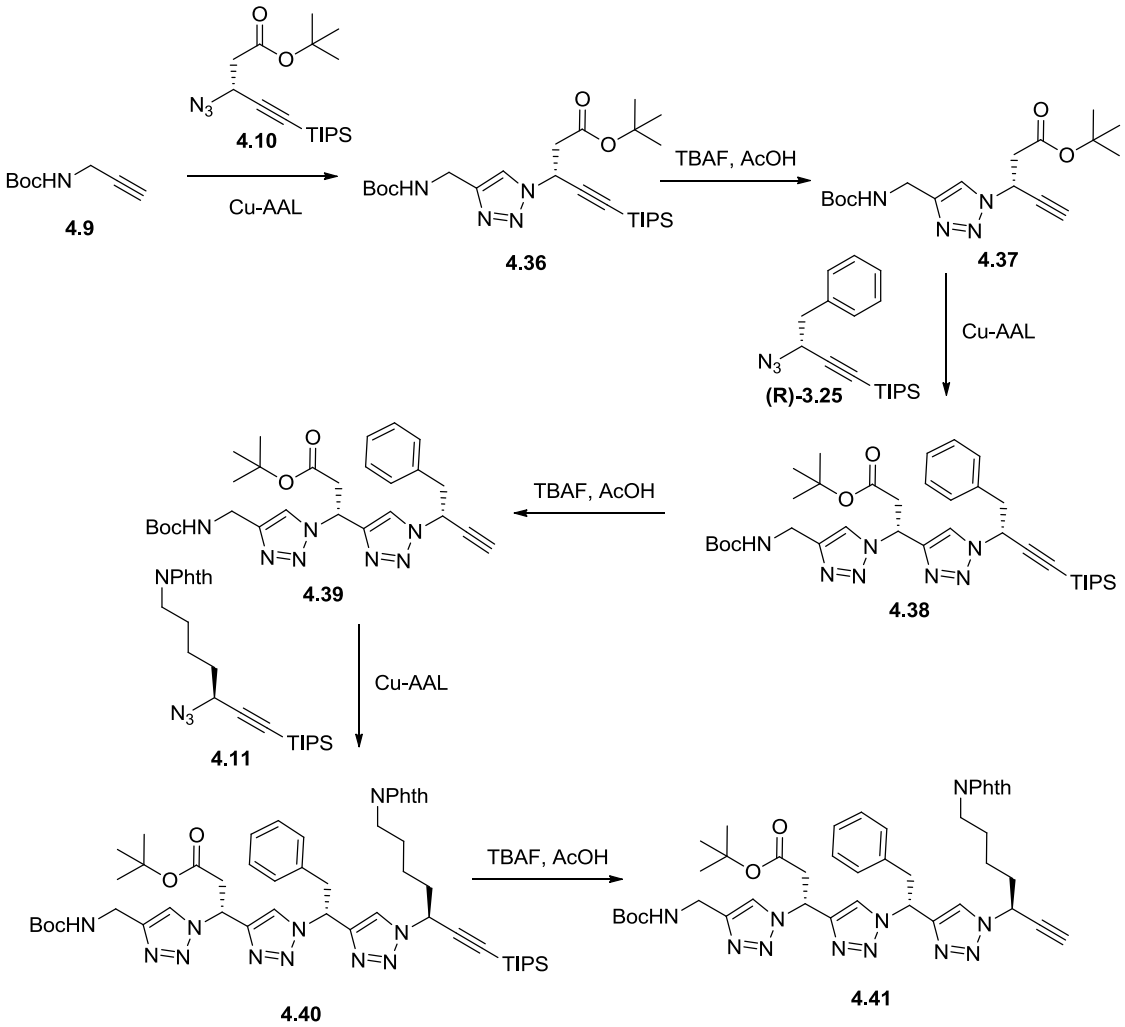


Scheme 4.16 Synthesis of azide (R)-3.25 from the addition of benzylmagnesium chloride to sulfinylamide 3.6, separation of the diastereomers, *N*-deprotection and diazotransfer.

The synthesis of the phenylalanine mimic was demonstrated in chapter 3.2.3, via the addition of benzylmagnesium chloride to sulfinylimine **3.6** at -78 ^{o}C, which gave **3.17** as a 4 : 1 mixture of diastereomers. The desired stereochemistry for the RGD cyclic mimic was the epimer of the compound used in chapter 3. As the diastereomers **(S,S)-3.17** and **(S,R)-3.17** were able to be separated by column chromatography, sulfinylamide **(S,R)-3.17** was isolated in 18 % yield in a reaction from sulfinylimine **3.6**. Key signals observed in the ^{1}H NMR spectrum were the α-proton signal at δ 4.40 (1H, q), as well as the β-proton signals at δ 3.05 (2H, 2 x dd). If a greater amount of this compound were required, the epimer of sulfinylimine **3.6** could have been used.

Sulfinylamide **(S,R)-3.17** was then derivatised for use in the linear chain extension pathway. Firstly, *N*-deprotection with a solution of ethereal hydrogen chloride cleaved the *N*-terminal sulfinylamide, and the free amine was transformed into an azide via triflic azide-mediated diazotransfer. Azide **(R)-3.25** was isolated in 46 % yield over the 2 steps. The ^{1}H NMR spectrum now displayed the α-proton peak at δ 4.30 (1H, t) and β-proton peak at δ 2.97 (2H, d). A signal corresponding to the protonated molecular ion (*m/z* = 328) was not present in the ESI-MS spectrum, however a mass corresponding to the loss of nitrogen from the protonated molecular ion was observed (*m/z* = 300).

4.4 Linear Extension Cycle



Scheme 4.17 Iterative Cu-AAL and desilylation for the synthesis of the tetramer Boc-GdfK using amino acid-like alkynyl azides.

With Boc-protected propargylamine acting as the glycine analogue, and the alkynyl-azide analogues of aspartic acid, phenylalanine and lysine, we investigated the coupling of these residues using the chain extension sequence outlined in the previous chapter, to produce a linear triazole peptidomimetic.

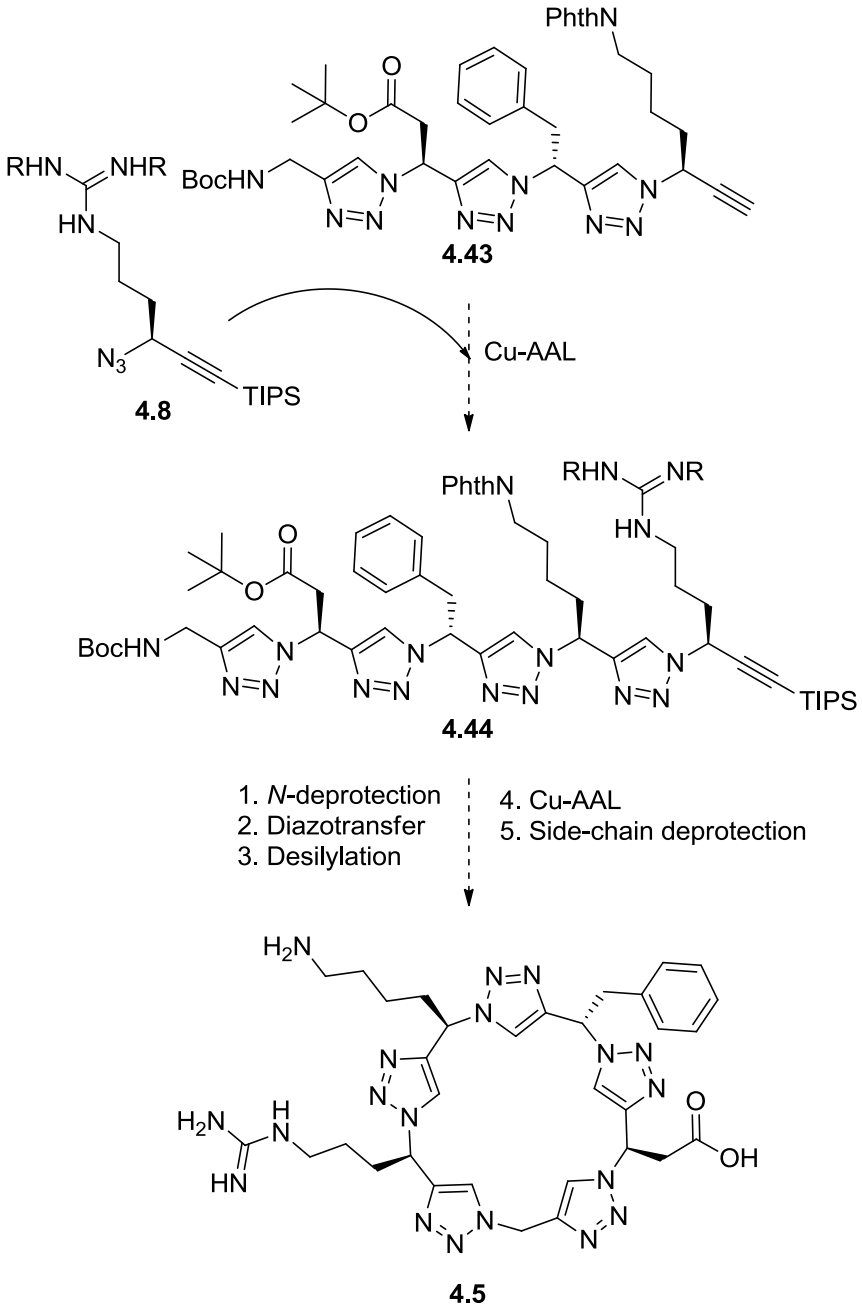
In the first step, Boc-propargylamine was coupled with the aspartic acid mimic **(R)-4.10** under Cu-AAL conditions, leading to dimer **4.36** in 93 % yield. The α-proton from the aspartic acid mimic and methylene protons from the glycine mimic were observed in the ^{1}H NMR spectrum at δ 5.77 (1H, t) and δ 4.39 (2H, d) respectively. A peak corresponding to the sodium adduct of the molecular ion was seen by ESI-MS (*m/z* = 529). TBAF-mediated desilylation buffered with acetic acid was then performed to give alkyne **4.37**, which was isolated in 80 % yield. The terminal alkyne signal was observed at δ 2.63 (1H, d) in the ^{1}H NMR spectrum, with the aspartic acid-mimic residue α-proton moving upfield to δ 5.73 (1H, t). The methylene protons from the glycine mimic remained at δ 4.38 (2H, d). A peak corresponding to the protonated molecular ion was seen by ESI-MS (m/z = 351).

Coupling to the phenylalanine mimic **(R)-3.25** was then performed, which gave trimer **4.38** in 96 % yield. The α-proton signals for the mimics of aspartic acid, phenylalanine and glycine were observed in the ^{1}H NMR spectrum at δ 6.23 (1H, dd); 5.71 (1H, dd) and 4.38 (2H, d) respectively. A peak corresponding to the protonated molecular ion was seen by ESI-MS (*m/z* = 678). Again, TBAF-mediated desilylation buffered with acetic acid was then performed to give alkyne **4.39**, which was isolated in 68 % yield. The terminal alkyne signal was observed in the ^{1}H NMR spectrum at δ 2.64 (1H, d), and the α-proton from the phenylalanine-mimic residue had moved upfield to δ 5.63 (1H, ddd). A peak corresponding to the protonated molecular ion was seen by ESI-MS (m/z = 522).

Coupling to the phthalimide-protected lysine mimic (**4.11**) gave tetramer **4.40** in 63 % yield. The α-proton signals for the mimics of aspartic acid, phenylalanine, lysine and glycine were observed in the ^{1}H NMR spectrum at δ 6.21 (1H, dd); 5.99 (1H, t); 5.42 (1H, dd) and 4.39 (2H, d) respectively. A peak corresponding to the protonated molecular ion was seen by ESI-MS (*m/z* = 960). TBAF-mediated desilylation buffered with acetic acid was then performed to give alkyne **4.41**, which was isolated in 74 % yield. The terminal alkyne signal was observed in the ^{1}H NMR spectrum at δ 2.62 (1H, d), and the α-proton from the lysine-mimic residue had moved upfield to δ 5.40 (1H, dt). A peak corresponding to the protonated molecular ion was seen by ESI-MS (m/z = 748). The overall yield for the iterative chain extension to alkynyl tetramer **4.40** was 23 % over 6 steps.

4.5 Conclusions

The synthesis of the monomer units corresponding to aspartic acid, D-phenylalanine and lysine was successfully completed, however further work was required for the synthesis of the arginine analogue (**4.8**). Even though the key alcohol intermediate (**4.28**) had been synthesised, there were issues relating to substitution to the guanidine moity. It was shown that a *bis*-Boc guanidine unit was successfully used to replace the alcohol functionality, however the compound produced was unable to be isolated via chromatography. It was believed the formation of side-products during column chromatography was a direct result of the acid-sensitivity of the Boc-groups on the guanidine. To prevent these side-reactions, the guanidine moiety should be protected with an acid-stable group such as Cbz. Nevertheless, linear triazole tetramer **4.41** was synthesised using the 2-step Cu-AAL / desilylation sequence, demonstrating the synthesis and chain extension sequence for other amino acid mimics. For completion of the linear sequence, synthesis of the arginine alkynyl-azide mimic and linear extension using the S-aspartic acid monomer unit (**(S)-4.10**), rather than **(R)-4.10**, would be required.



Scheme 4.18 Proposed completion of the synthesis of an all-triazole RGDfK integrin inhibitor.

Following the synthesis of linear triazole pentamer **4.44**, derivatisation to an *N*-terminal azide and *C*-terminal alkyne, head-to-tail cyclisation under Cu-AAL conditions and global deprotection would give rise to the desired RGD mimic (**4.5**). Even though this target compound was not synthesised, in this chapter it has been shown that sulfinylimine **3.6** can be used as a powerful intermediate for the synthesis of mimics of a variety of amino acid residues, leading to compounds that contain both azide and alkyne functionality. It was demonstrated that these units could be coupled together in an iterative fashion, forming linear peptidomimetics with diverse side-chain functionality.

Chapter 5 - Synthesis of Triazole-containing Granzyme B Inhibitors

5.1 Introduction to Granzyme B

5.1.1 Proteases

Proteases are enzymes that cleave the amide bonds between amino acid residues present in peptides or proteins. The physiological function of proteases is defined by which native substrates they cleave, as well as the cleavage location. They can be separated into 6 families: serine, threonine, cysteine, aspartate, glutamate and metalloproteases, with the names derived from the key residue found in the respective protease active sites.[133, 134] Substrate specificity is generated by the preference of proteases for particular amino acids located either side of the cleaved, or scissile, bond. The nomenclature of Scheter and Berger states that amino acid residues in the direction of the *N*-terminus from the scissile bond are designated P1, P2 … Pn, and amino residues in the direction of the *C*-terminus from the scissile bond are designated P1', P2' … Pn'.[135] Binding pockets of the proteases are similarly named, as shown below.

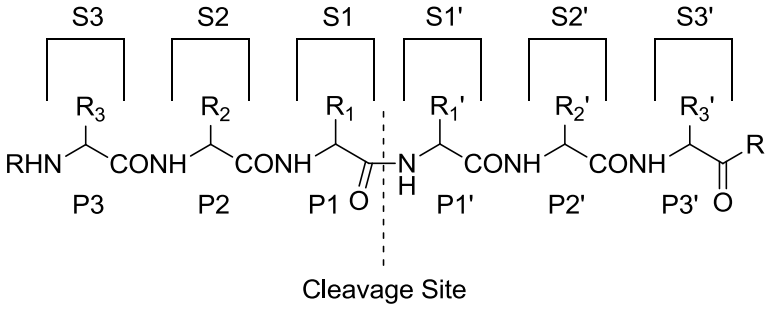
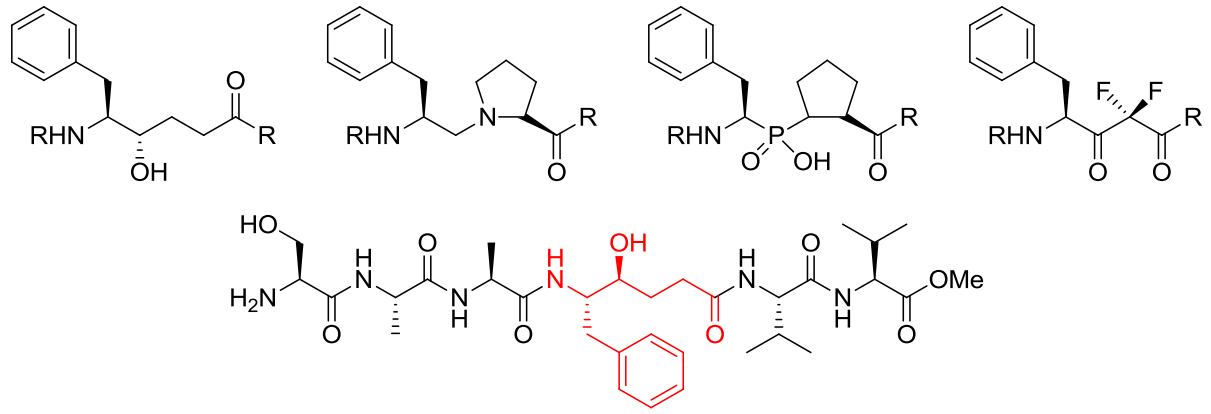


Figure 5.1 Nomenclature of a protease-substrate interaction.

By taking known native sequences for proteases and modifying the scissile bond with appropriate amide isosteres, substrates may be potentially transformed into specific inhibitors. This approach has been applied to HIV-1 protease extensively, with the seminal paper published in 1989.[136] Analogues of a heptapeptide substrate of HIV-1 protease, derived from the cleavage site of the native substrate ($Pr55^{gag}$) were synthesised. Derivatisations of the native substrate involved replacing the Phe-Pro scissile bond with various non-hydrolysable isosteres, which were proposed to mimic the transition state. Such

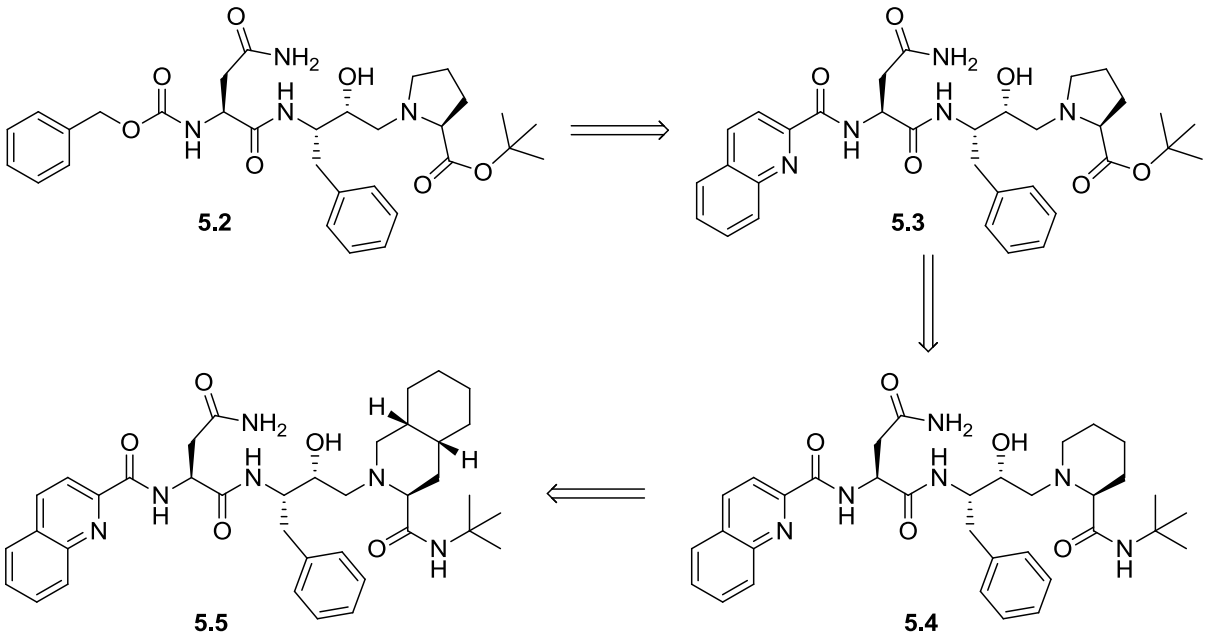
replacements included: hydroxyethylene isosteres, reduced amides, phosphinates and α-fluoroketones, with the hydroxyethylene moiety providing the tightest binding inhibitors. The K_i of the strongest binding inhibitor (**5.1**, shown below) was 62 nM.



5.1

Figure 5.2 Inhibitor 5.1 and amide bond isosteres applied to HIV-protease by Dreyer *et al.*[136]

Roberts *et al.*[137] continued this approach with compounds based on the *gag-pol* fragment Leu^{165}-Ile^{169}, again replacing a Phe-Pro scissile bond with a hydroxyethylamine moiety. Initial studies of hydroxyethylamine-containing fragments led to compound **5.2** below, which had an IC_{50} of 140 nM against HIV-1 and demonstrated a preference for the *R*-hydroxyethylamine. Replacement of the *N*-terminal Cbz group with either a β-napthoyl or quinolone-2-carbonyl structure (**5.3**) gave improved affinity (IC_{50} = 52 and 23 nM respectively). The *C*-terminal proline residue was then replaced with piperidine-2-*S*-*tert*-butyl carbamide, giving compound **5.4** with an IC_{50} of 2 nM. Finally, elaboration of the piperidine structure to decahydroisoquinoline, gave Saquinavir (**5.5**), with an IC_{50} <0.4 nM, which is currently being used in HIV antiretroviral therapy.



Scheme 5.1 Functionalisation of lead compound 5.2 to Saquinavir (5.5), through modification of the *N*-terminus (5.3) and *C*-terminus (5.4).

5.1.2 Granzymes

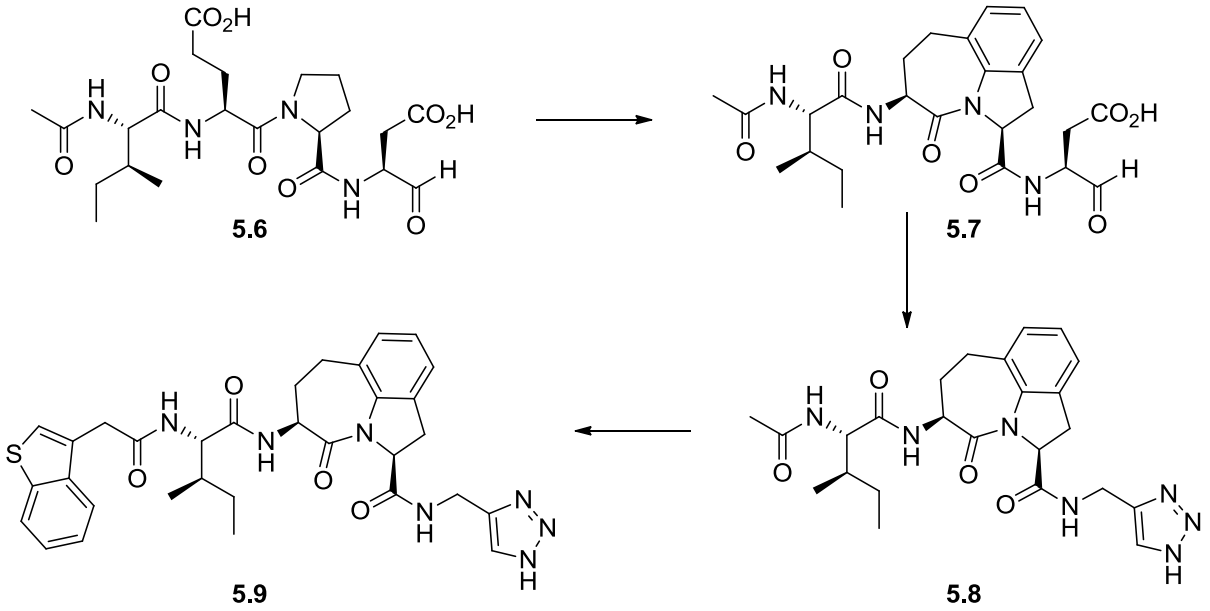
Granzymes are serine proteases classified within the chymotrypsin family,[133] due to the presence of the catalytic triad His-Asp-Ser. They are located within lymphocyte granules in cytotoxic T-lymphocytes and natural killer cells. Exocytosis of the granules from these cells triggers apoptosis of abnormal cells, such as tumour cells and pathogen-infected cells, via the products generated by granzyme-mediated cleavage of various proteins.[138] Out of the 5 distinct human granzyme isoforms (A, B, H, K and M), granzyme B is the most potent activator of cell death.[139] Also contained within these granules is perforin – a pore-forming protein, the role of which is a subject of debate in the literature. It was thought that the mechanism by which apoptosis occurs involved perforin inserting into target cell membranes resulting in a pore by which granzymes may transverse into the cytoplasm.[140] However, it has been shown that perforin-independent granzyme-mediated apoptosis may occur.[141] Uptake into target cells may be regulated by the cation-independent mannose 6-phosphate receptor.[142] Given the role of granzyme B as an anti-viral and anti-tumour agent,[140] it can be seen that down-regulation could result in the proliferation of harmful tissue, whereas up-regulation could lead to autoimmune disorders. Higher levels of granzyme B are seen in atherosclerotic legions and in the synovial fluid and tissue of patients with rheumatoid arthritis.[143] Granzyme B therefore can both serve as a biomarker for detecting the onset of

such disease states, and also potentially become an attractive drug target for their prevention.

Compared to the 5 granzyme isoforms found in humans, mice have 10 isoforms. Knock-out mice, as well as the use of purified granzymes, have been used to investigate their role in the apoptosis pathway.[144] Interestingly, mice lacking in either granzyme A or B do not show a greatly altered apoptotic response, although the loss of both isoforms results in immunological defects.[145] Perforin-deficient mice however do not undergo granule-mediated apoptosis.[146] When comparing granzyme B from the two species, it has been shown that the cytotoxicity, substrate preferences and inhibitor interactions are markedly different. As a result, binding data from *in vivo* mouse studies cannot be extrapolated to humans, which severely limits the ability of potential granzyme B inhibitors to progress as drug candidates.[145]

5.1.3 The Merck Granzyme B Inhibitor

A group from Merck sought to produce a selective inhibitor of granzyme B,[147] as a compound that bound selectively to granzyme B and not to the closely related caspases was essential in being able to more closely study its biological role. By screening a positional scanning combinatorial library, the tetrapeptide IEPD was elucidated as the preferred P4-P1 tetramer motif for Granzyme B. As indicated in scheme 5.2 below, the corresponding *C*-terminal aldehyde **5.6** was produced, and found to have a K_i of 80 nM. Modification of the P2-P3 residues resulted in compound **5.7**, which was shown to have a 10-fold improvement of the parent, with a K_i of 8 nM. The compound was still active against several of the caspases, thus more compounds were produced in order to explore selectivity. By modifying the P1 position, inhibitors were found which completely abolished the ability of the compounds to bind to the caspases, attributed to the removal of the electrophilic serine trap present in compounds **5.6** and **5.7**. Monotriazole-containing compound **5.8** was the best inhibitor produced in this series, with a K_i of 38 nM. Finally, the P4 residue was optimised, which resulted in compound **5.9** shown below, which was found to have a K_i of 7 nM against human granzyme B, and showed no caspase inhibition.



Scheme 5.2 Representation of the derivatisation of tetrapeptide IEPD to potent inhibitor 5.9 by Thornberry *et al.*[147]

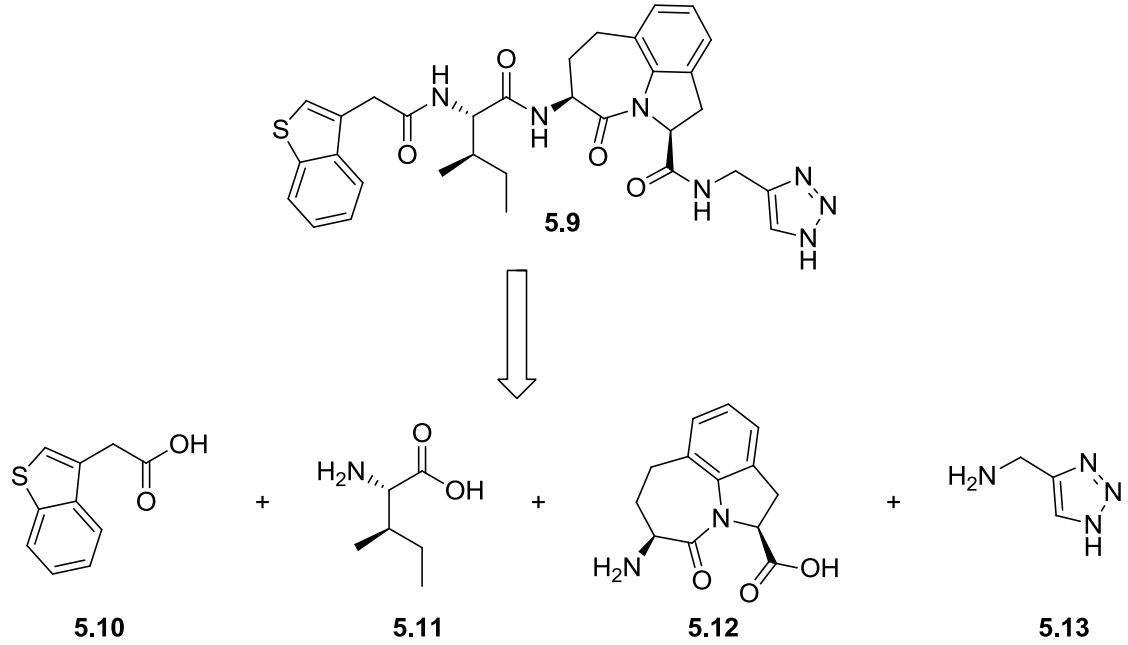
5.1.4 Initial Plans

Given that the Merck compound contained a terminal mono-triazole, we were interested in determining whether Cu-AAL chemistry could be used to synthesise analogues which extended from the triazole moiety, resulting in more interactions with the target enzyme. The Merck compound itself would also be synthesised, and used as a positive control in the granzyme assay.

Additionally, if the consensus sequences of both human and mouse granzyme B substrates were taken, the scissile bonds (P1-P1') could be replaced with a triazole acting as an amide bond isostere which would potentially result in the formation of inhibitors. Using the substrate specificity of the consensus sequences, selective inhibitors of both human and mouse granzyme B could be envisioned, which may act as useful tools or as lead compounds. The different consensus sequences were determined by our collaborators, the Bird group, using substrate phage display.[145] For the human granzyme-selective compound the sequence IGADVLT was chosen, and for the mouse compound the sequence to be mimicked was LVFDWGG, with the triazole moiety to be inserted between the D-V and D-W residues respectively.

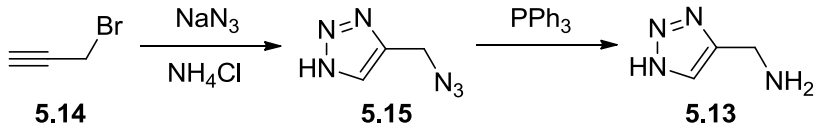
5.2 Synthesis of the Merck Compound

5.2.1 Solution phase synthesis



Scheme 5.3 Splitting of the Willoughby inhibitor into monomer units.

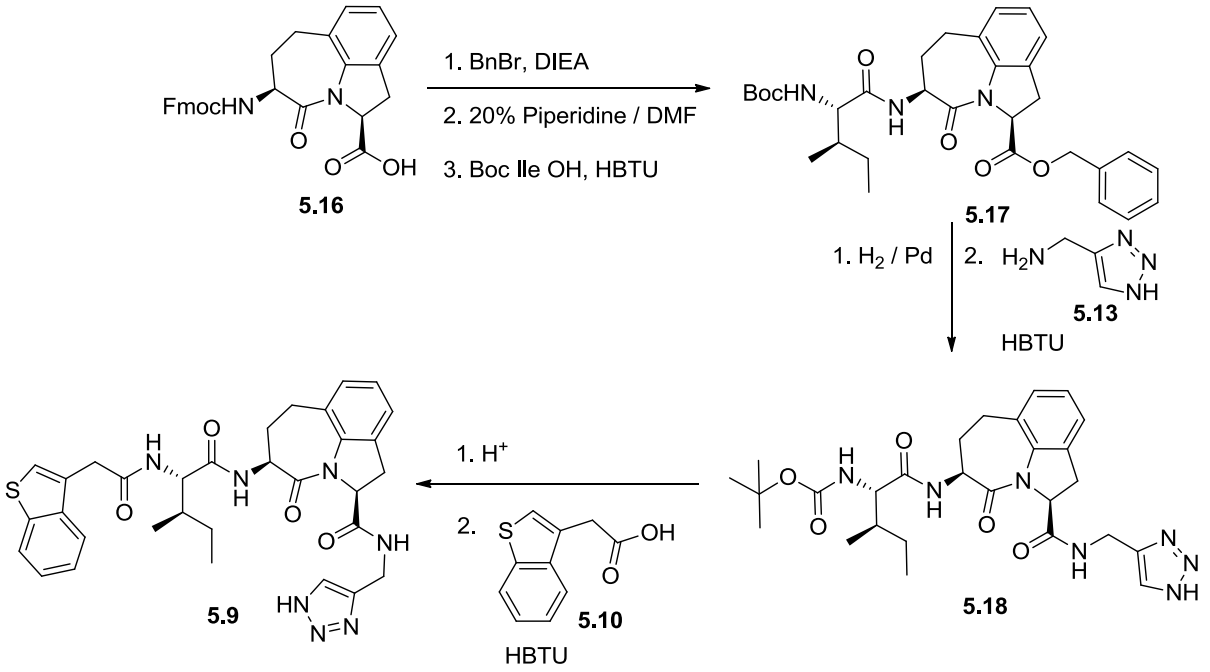
Initially synthesis of the Merck compound (**5.9**) was performed in the solution phase, in a similar reaction scheme to Willoughby *et al*.[147] Benzothiophene **5.10** was purchased as the free acid, isoleucine (**5.11**) was available as either the Boc or Fmoc-protected amino acid, and **5.12** was purchased as the Fmoc *N*-protected carboxylic acid. However, aminomethyl triazole **5.13** was synthesised as per scheme 5.3 below.



Scheme 5.4 Synthesis of aminomethyl triazole 5.13.

As shown by Banert *et al*.,[148] azide **5.15** may be synthesised in a cascade reaction from propargyl halides or the tosylate. Thus, propargyl bromide (**5.14**) in a solution of dioxane and water was heated in the presence of sodium azide, which resulted in azide **5.15**. A characteristic azide signal was observed in the IR spectrum of the crude material at 2102 cm^{-1}, and the C-H triazole peak was seen in the ^{1}H NMR spectrum at δ 7.75 (1H, s). A Staudinger reduction of the crude azide to the amine was effected with triphenylphosphine in refluxing methanol, which gave amine **5.13** in 63 % yield over the two steps. The ^{1}H NMR

peaks seen for the product were different to the literature values in D_2O, with δ 8.01 (1H, s) and 4.35 (2H, s) seen compared to δ 7.57 (1H, s) and 4.05 (2H, s).[148] A peak corresponding to the protonated molecular ion *m/z* = 99.1 was observed by ESI-MS. Following the synthesis of this compound, chain extension to tetrapeptide **5.9** was performed following the procedure shown in scheme 5.5 below.



Scheme 5.5 Solution phase synthesis of tetramer 5.5.

Benzylation of acid **5.16** was performed with a solution of DIEA and benzyl bromide, giving the benzyl ester in quantitative yield after isolation. A peak corresponding to the protonated molecular ion (*m/z* = 559.2) was observed by ESI-MS.

After *N*-deprotection via a solution of 20% piperidine in DMF, the free amine was coupled to Boc-isoleucine via HBTU-mediated peptide coupling conditions, giving dipeptide **5.17** in 54 % yield after isolation. An new α-proton signal corresponding to the isoleucine residue was observed in the ^{1}H NMR spectrum at δ 4.22 (1H, m), and other key signals present were the α-proton signal from the dihydroindole moiety at δ 4.54 (1H, dd) and the Boc signal at δ 1.57 (9H, s).

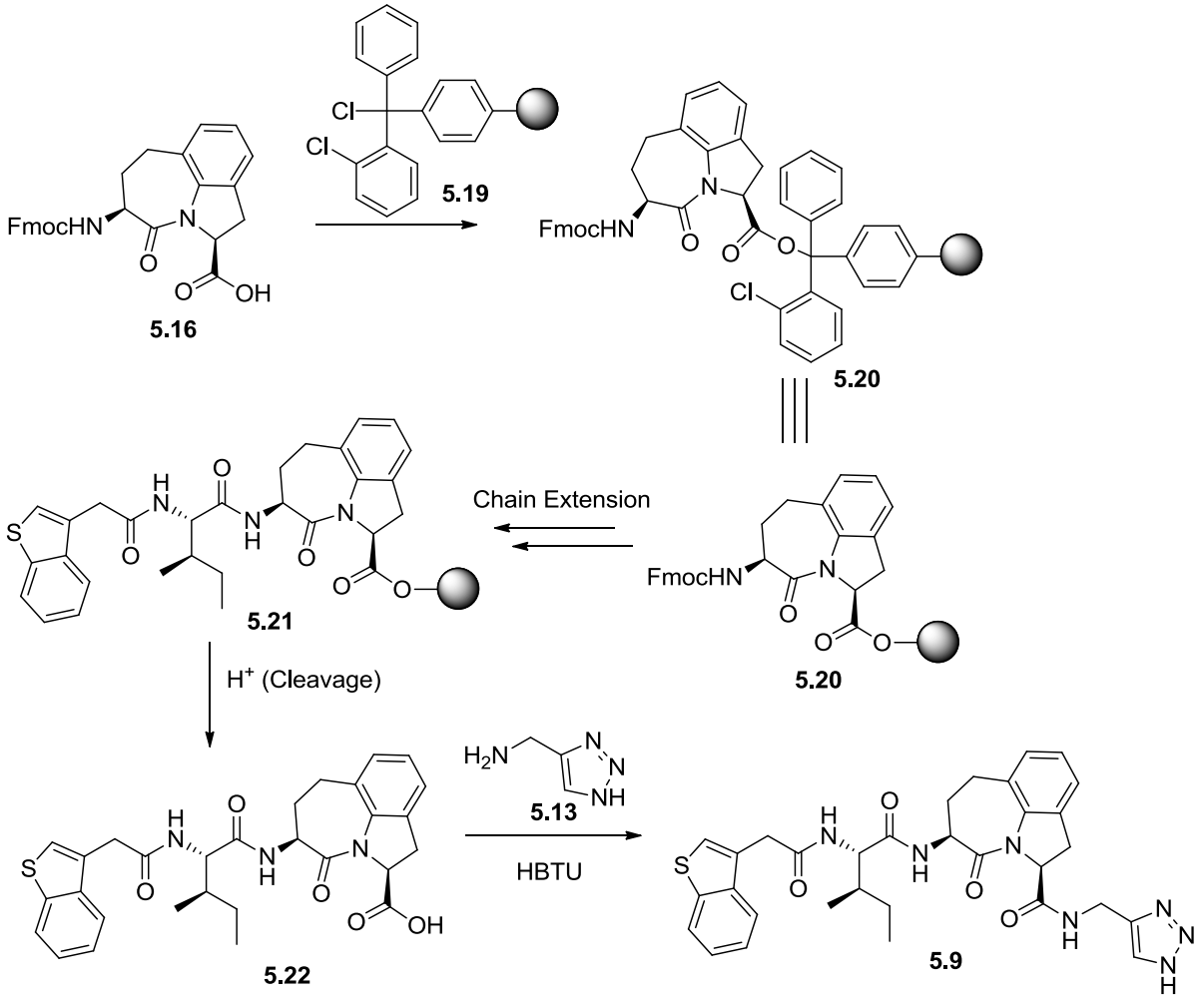
After benzyl deprotection of dipeptide **5.17** via hydrogenation with 10% Pd/C at 60 ^{o}C in a solution of 1 : 1 ethanol : ethyl acetate, HBTU-mediated peptide coupling to aminomethyl triazole **5.13** was performed, which after isolation gave tripeptide **5.18** in 35 % yield. The new triazole C-H signal was observed in the ^{1}H NMR spectrum at δ 7.69 (1H, s), while the dihydroindole α-proton signal had shifted downfield to δ 5.14 (1H, dd).

Boc-deprotection with TFA, followed by HBTU-mediated peptide coupling to benzothiophene **5.10** was performed which gave tetrapeptide **5.9** in 55.6% yield. The proton in the 2-position of the benzothiophene was observed in the ^{1}H NMR spectrum at δ 7.23 (1H, s), and the dihydroindole α-proton signal remained at δ 5.14 (1H, dd).

Overall, the yield of tetrapeptide **5.9** from the tricyclic acid **5.16** was 10.5 % for the 7-step synthesis.

5.2.2 Solid phase synthesis

Although the solution phase synthesis allowed for production of the target tetrapeptide **5.9**, it required tedious functional group manipulation, as well as several column chromatography steps, which lowered the overall yield of the product. The prohibitive cost of acid **5.16** necessitated an attempt to optimise the reaction conditions, which led to an investigation of solid phase synthesis. By attaching tricyclic unit **5.16** onto a solid support via the *C*-terminal carboxylic acid, essentially using the resin as a protecting group, chain extension to the tripeptide could be performed, using excess reagents to maximise the yields, and several washing steps instead of chromatography. 2-Chlorotrityl chloride resin was selected as this resin reforms the carboxylic acid on cleavage, which allows for coupling of the terminal amine-containing residue in the solution phase.



Scheme 5.6 Solid phase synthesis to tetrapeptide 5.9 using the chlorotrityl resin (5.19).

Free acid **5.16** was attached to 2-chlorotrityl chloride resin (**5.19**) (1.12 mmol g^{-1}) via stirring the two components in DMF with the addition of DIEA. After 4 hours the suspension was drained, and the beads were washed with additional DMF.

A 2-step iterative chain extension was sequence was performed, which consisted of *N*-deprotection with a solution of piperidine in DMF, followed by HBTU-mediated coupling to Fmoc-isoleucine then benzothiophene **5.10**. Reaction completeness was measured by use of the chloranil test,[149] in which the solid phase beads become a green colour in the presence of free primary amines, and remain yellow otherwise. This method of assessing reaction completeness was used over the more commonly used ninhydrin test,[150] because of the simpler procedure.

Cleavage from the resin was effected by a solution consisting of DCM/TFA/triisopropylsilane (95/2.5/2.5), which gave tripeptide **5.22** in 40 % yield. Key ^{1}H NMR (d_6-DMSO) signals seen in the spectrum were the benzothiophene 2-position signal at δ 7.52 (1H, s), and the dihydroindole α-H at δ 5.05 (1H, dd). The ^{1}H NMR spectrum showed that the compound was

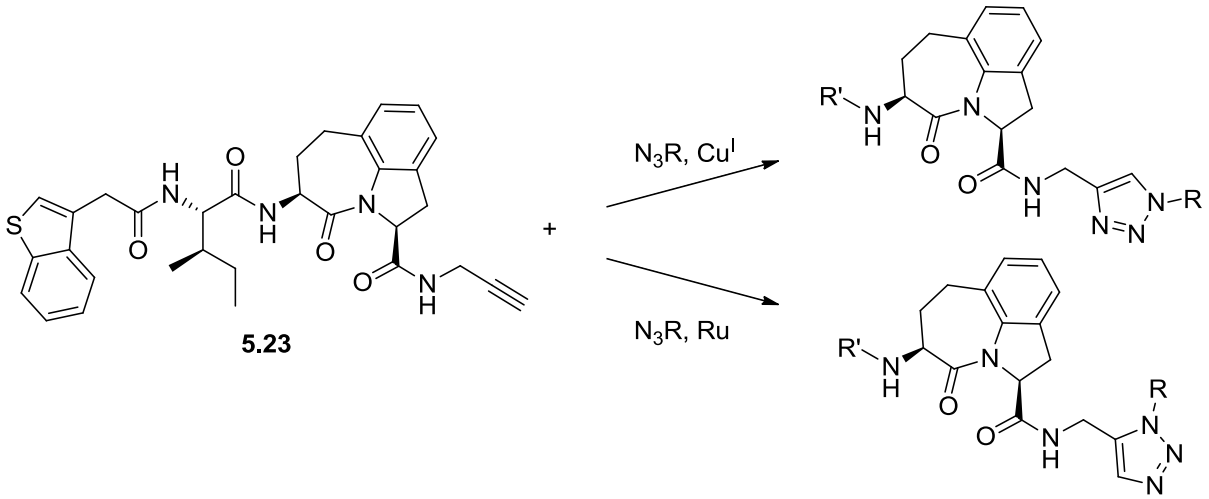
of high purity, and no further purification was found to be necessary after cleavage from the resin. It was seen that the solid phase extension allowed for 5 successive steps to be performed with very high conversion rates.

Tripeptide **5.22** was then coupled to aminomethyl triazole **5.13** via HBTU-mediated peptide coupling conditions, giving the target compound in 49 % yield. The compound was found to be spectroscopically identical to the material produced via the solution phase route (5.2.1).

The overall yield for the solution phase synthesis was 10.5 % which consisted of 4 separate purifications by column chromatography. On the other hand, synthesis utilising the chlorotrityl resin gave an overall yield of 19.6 %, giving product of good purity with just one purification step performed.

5.3 Derivatives of the Merck Compound: C-terminal Extension

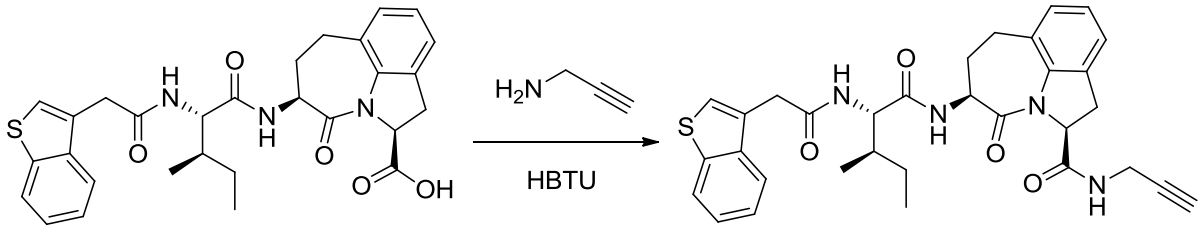
Given that granzyme B substrates extend further than the P1 position, it was thought that there might be room in the enzyme to fit larger compounds which may bind more tightly than tetrapeptide **5.9**, although at the time of this work, the bound position of **5.9** was not explicitly known. If the analogous *C*-terminal alkyne tetrapeptide was synthesised (**5.23**), coupling of various azides via Huisgen 1,3-dipolar cycloaddition at elevated temperatures would produce a mixture of *cis*- and *trans*- triazoles. Chromatography could then be used to isolate both regioisomers. As shown in scheme 5.6 below, rather than deal with a potentially tedious separation, either copper-based or ruthenium-based catalysts could be used in separate reactions, which would selectively give the *trans*-triazoles and the *cis*-triazoles respectively.



Scheme 5.7 Derivatisation of alkyne 5.23 via Cu-AAL to 1,4-triazoles or Ru-AAL to 1,5-triazoles.

The azides chosen for the analogue synthesis were derived from amino acids located at the P1' position in the consensus sequence. It was not known whether 1,4-*trans*- or 1,5-*cis*-triazoles would be the preferred regiochemistry at the C-terminus, thus both regioisomers were considered. For this series of compounds, the P4-P2 tripeptide mimic (**5.22**) would be conserved, and the triazole formed was expected to act as a mimic of the P1 aspartic acid residue.

5.3.1 Synthesis of the alkyne analogue of the Merck compound



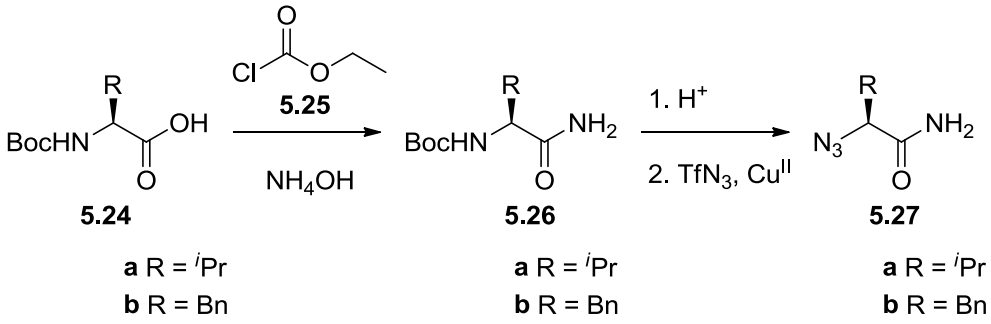
5.22 **5.23**

Scheme 5.8 Peptide coupling of key acid 5.22 with propargylamine.

Tripeptide **5.22**, synthesised via solid phase chemistry (section 5.2.2), was coupled via HBTU-mediated peptide coupling conditions with propargylamine. Tetrapeptide **5.23** was seen to be very poorly soluble in both aqueous and organic solvents, thus was isolated in 84 % yield after precipitation from the solution by the addition of water. Key ^{1}H NMR (d_6-DMSO) signals found in the spectrum were the alkyne at δ 3.11 (1H, t) and the signal corresponding to the 2-position on the benzothiophene at δ 7.51 (1H, s). If the reaction was allowed to proceed to completion, no starting materials were present in the ^{1}H NMR spectrum after precipitation, and was of acceptable purity for further reactions.

5.3.2 Synthesis of amino acid-derived azido-amides

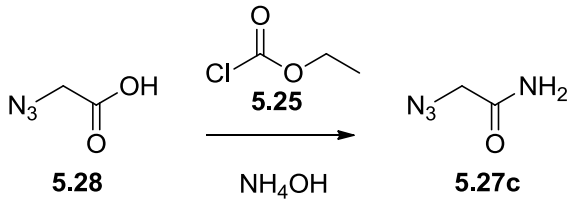
As shown in scheme 5.9 below, amino acids were converted to azido-amides for use in the coupling reactions to alkynyl tetrapeptide **5.22**.



Scheme 5.9 Synthesis of azido-amide derivatives from amino acids.

After activation of Boc-valine (**5.24a**) by formation of the mixed anhydride with the addition of ethyl chloroformate (**5.25**), aqueous ammonia was added which gave amide **5.26a** in 75 % yield after isolation. The new amide protons were seen in the ^{1}H NMR spectrum at δ 5.92 (0.7 H, br) and 5.43 (0.7 H, br), and the α-proton was seen at δ 3.96 (1H, t). After *N*-deprotection with a solution of hydrogen chloride in diethyl ether, diazotransfer with triflic azide gave azide **5.27a** in 47 % yield. The α-H was seen in the ^{1}H NMR spectrum at δ 3.86 (1H, d). This signal was seen as a doublet of doublets in Boc-amide **5.26a** due to the close proximity of the carbamate NH, and as expected this splitting pattern was no longer seen in azide **5.27a**.

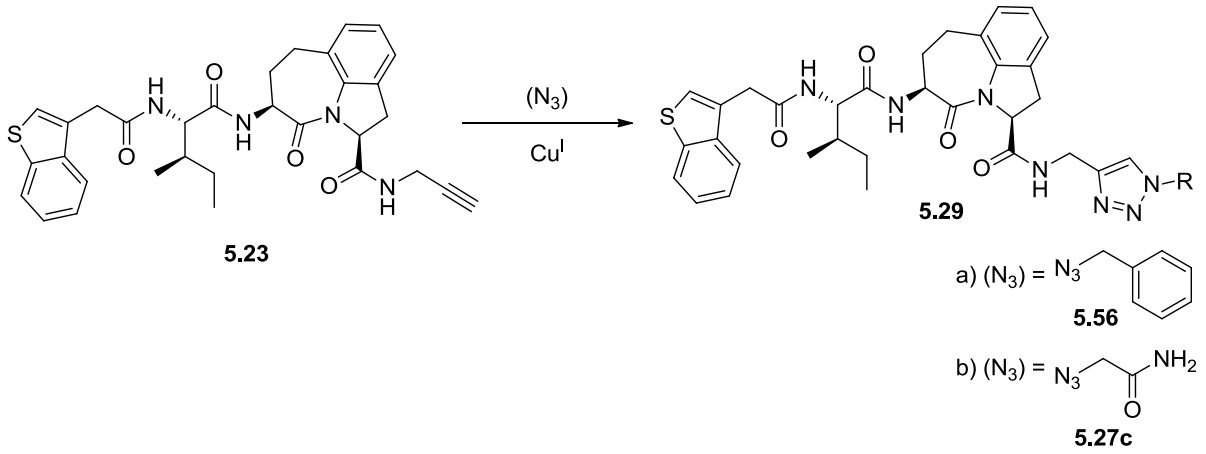
The same *C*-amidation procedure was performed using Boc-phenylalanine (**5.24b**), giving **5.26b** in quantitative yield. The new amide protons were seen in the ^{1}H NMR spectrum at δ 5.78 (0.87 H, br) and δ 5.38 (0.85 H, br), and the phenylalanine α-proton was observed at δ 4.37 (1H, m). An similar procedure was performed as previously shown for *N*-deprotection and diazotransfer, giving **5.27b** in 48 % yield over the 2 steps. The α-H in the ^{1}H NMR spectrum was observed as a doublet of doublets (δ 4.21, 1H), giving evidence to suggest that conversion to the azide had taken place, as no carbamate protons were present to cause a different splitting pattern.



Scheme 5.10 Conversion of azidoglycine to the amide derivative.

C-amidation was performed on azidoglycine (**5.28**) in a similar procedure to above, giving **5.27c** in 10 % yield. The ^{1}H NMR obtained was consistent with the literature,[151] with the methylene signal observed at δ 4.00 (2H, s).

5.3.3 Cu-catalysed coupling



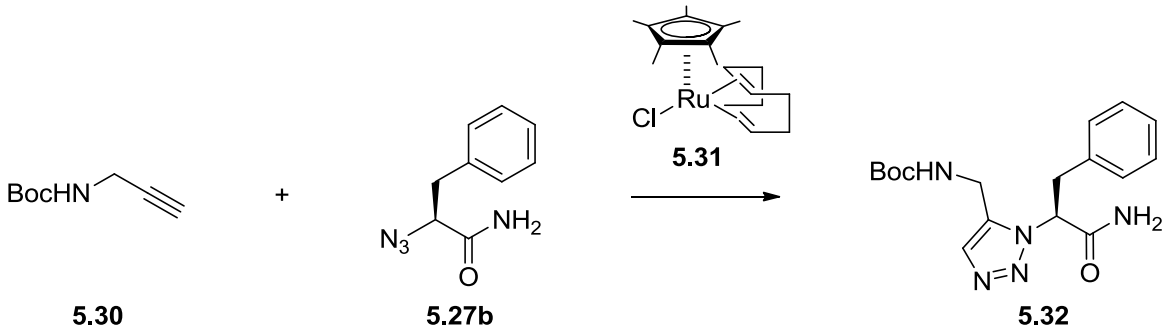
Scheme 5.11 Cu-AAL of key alkyne 5.23 to benzylazide.

As a test Cu-AAL reaction, alkyne **5.23** was suspended in THF and coupled to benzyl azide (**5.56**) over 16 hours using a copper bromide-dimethyl sulfide complex (0.25 eq) and DIEA. The product (**5.29a**) was purified using preparative HPLC, however only a small amount of material (< 1 mg) was isolated. A peak corresponding to the protonated molecular ion was observed in the ESI-MS (m/z = 704).

The reaction was repeated with glycine-derived azide **5.27c** in DMF, to ensure complete dissolution of the alkyne starting material. After stirring overnight the crude material was analysed by ESI-MS, and a peak corresponding to the expected protonated molecular ion was observed (*m/z* = 671). The crude product appeared to be only sparingly soluble in hot acetonitrile for preparative HPLC purposes, and tetramer **5.29b** could not be separated from the alkyne starting material on the column.

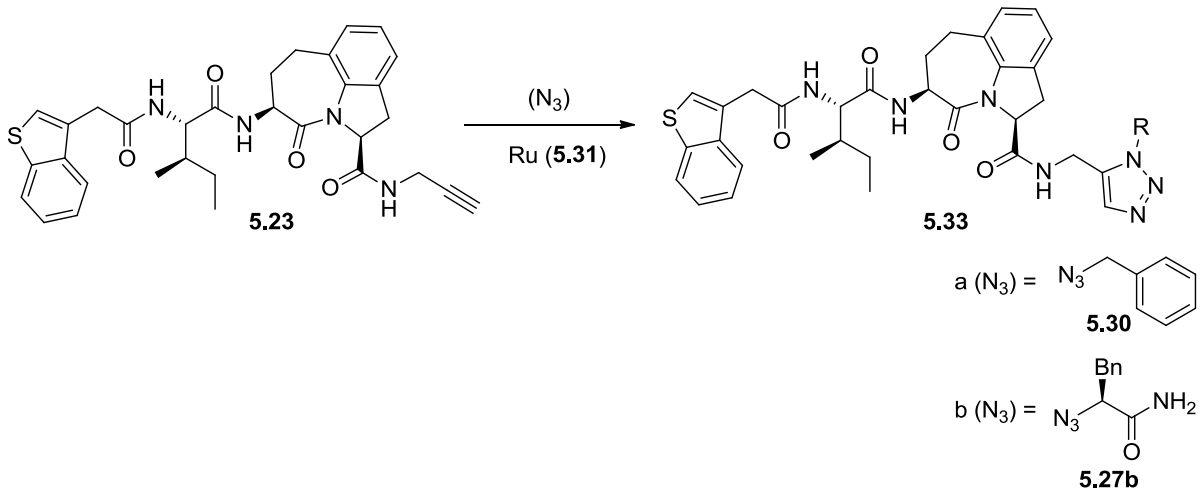
It did not appear that the reactions were working particularly well for the synthesis of the extended 1,4-triazole derivatives of tetramer **5.29**, primarily due to the poor solubility of both the starting material (alkyne **5.23**) and the products. Synthesis of the 1,5-triazole compounds was performed simultaneously and is described in the next section.

5.3.4 Ru-catalysed coupling



Scheme 5.12 Test Ru-AAL reaction between Boc propargylamine (5.30) and phenylalanine-derived azido-amide 5.27b using ruthenium catalyst 5.32.

Following the catalytic conditions of Zhang,[31] a test ruthenium-catalysed coupling reaction between phenylalanine-derived azide **5.27b** and Boc-propargylamine (**5.30**) was performed, which involved the addition of ruthenium complex **5.31** (0.02 eq) to a solution of the alkyne and azide in THF. A ^{1}H NMR spectrum was taken of an aliquot of the reaction mixture after 20 hours, and only one sharp triazole-like peak (δ 7.51, s) was observed which indicated the synthesis of a single triazole regioisomer. The reaction had gone to 60 % completion as determined by comparison of the newly formed triazole signal with the α-proton signal from the azide starting material at δ 4.21 (1H, dd). After stirring for another 28 hours, analysis of an aliquot by ^{1}H NMR suggested that the reaction had gone to 67 % completion. After stirring for a further 4 days, the reaction had appeared to have gone to 75 % completion. As the reaction was performed simply to test the reaction conditions, the product was not isolated.



Scheme 5.13 Ru-AAL reaction between key alkyne tetrapeptide 5.23 and azides 5.30 and 5.27b.

Alkynyl tetramer **5.23** was suspended with benzyl azide (**5.30**) and ruthenium complex **5.31** in THF, with the reaction being left to stir at reflux temperature for 4 days. ESI-MS of the crude material showed a peak corresponding to the protonated molecular ion (*m/z* = 704). The desired product was not able to be isolated however, with poor organic and aqueous solubility of the product restricting the use of both normal and reverse-phase column chromatography.

The same conditions were used with alkyne **5.23** and phenylalanine-derived azide **5.27b**. After stirring for 5 days, the solution was concentrated and the crude material was dissolved in acetonitrile, with non-soluble components filtered off. ESI-MS on the filtrate showed a peak corresponding to the protonated molecular ion (*m/z* = 761). The crude material was purified by preparative HPLC, giving pentamer **5.33b** (<1 mg).

5.3.5 Conclusions for triazole-containing analogues of the Merck compound

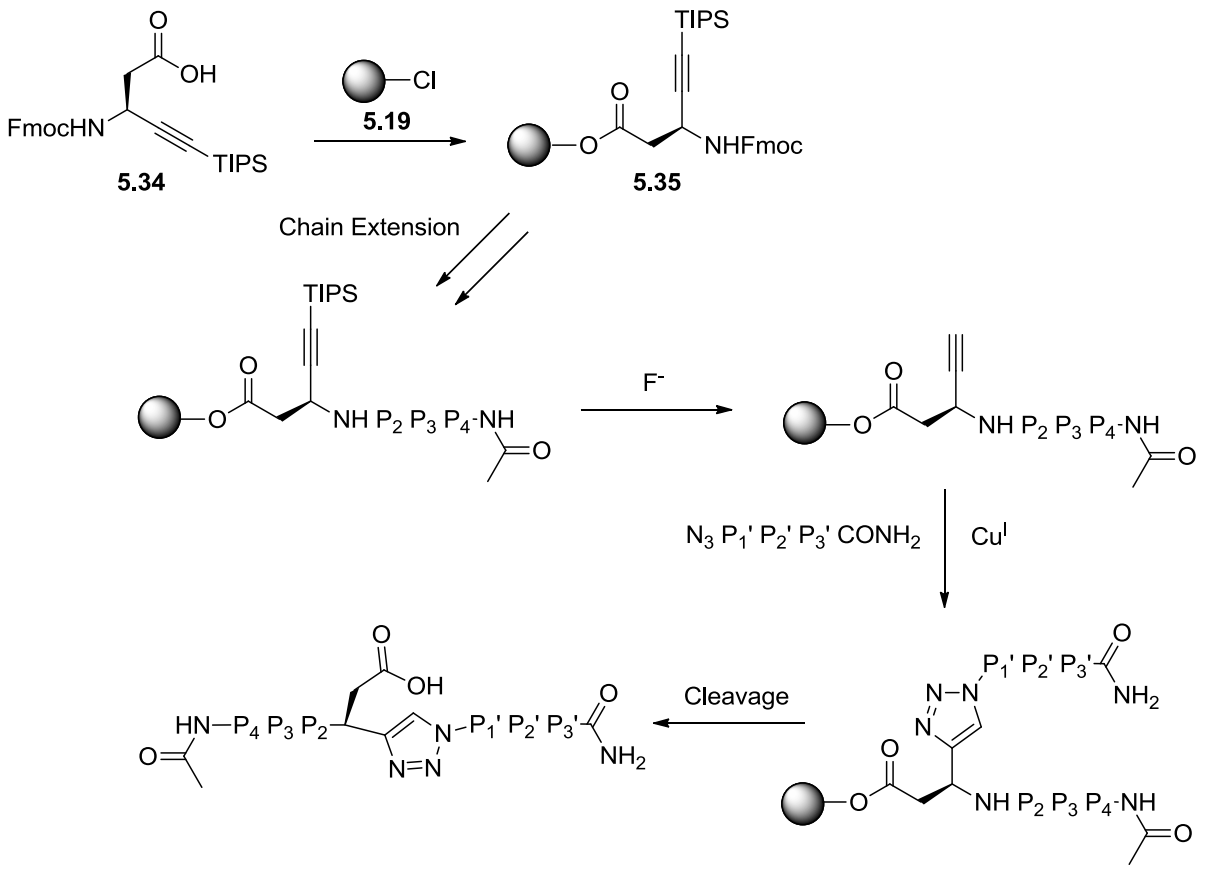
Although both the copper-catalysed and ruthenium-catalysed coupling reactions appeared to give the triazole-containing analogues, the poor quality ^{1}H NMR data obtained was not able to be used to unambiguously differentiate between the triazole regioisomers. This was due to the obtained products being very poorly soluble in most solvents other than DMF and DMSO, which made purification quite difficult. As a result of these solubility related challenges, insufficient quantities of the analogues were isolated for biological testing purposes.

5.4 Human and Mouse-specific Granzyme B Inhibitors

Concurrently, isosteric modification of the granzyme substrate consensus sequences was being looked into. The initial plan was to take the consensus sequences of both human and mouse granzyme B and replace the scissile bonds with triazoles, turning the substrates into inhibitors. As discussed in section 5.1.4, the sequences IGA-(triazole)-DVLT and LVF-(triazole)-DWGG were chosen to mimic the human and mouse granzyme B enzymes respectively.

It can be seen that the scissile bond replacement could be prepared by the coupling of an amino alkyne mimic of aspartic acid and an amino azide mimic of either alanine (human) or phenylalanine (mouse). Rather than synthesise the peptide-like compounds in a linear

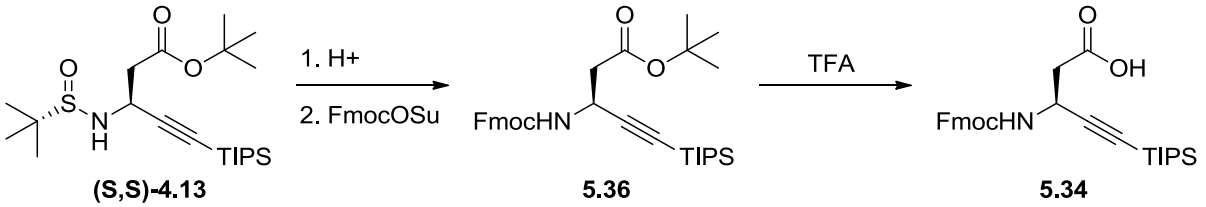
fashion from the *P3*' residue, a more convergent approach was considered as outlined in scheme 5.14 below.



Scheme 5.14 General solid phase synthesis of triazole-containing human and mouse granzyme B inhibitors.

The presence of the aspartic acid residue at P1 in the sequence was exploited by connecting the side-chain carboxylic acid to a solid support. Chain extension to include the P2-P4 residues could be performed using standard peptide synthesis techniques directly on the resin. Separately the P1'-P3' azido-tripeptide could be synthesised, which would be coupled to the solid supported alkyne. Following the coupling reaction, cleavage from the resin would furnish the heptapeptide mimics. Again, the chlorotrityl resin (**5.19**) was selected for the ability to easily attach carboxylic acids, which are regenerated by mild acid-mediated cleavage.

5.4.1 Synthesis of aspartic acid derivative 5.34



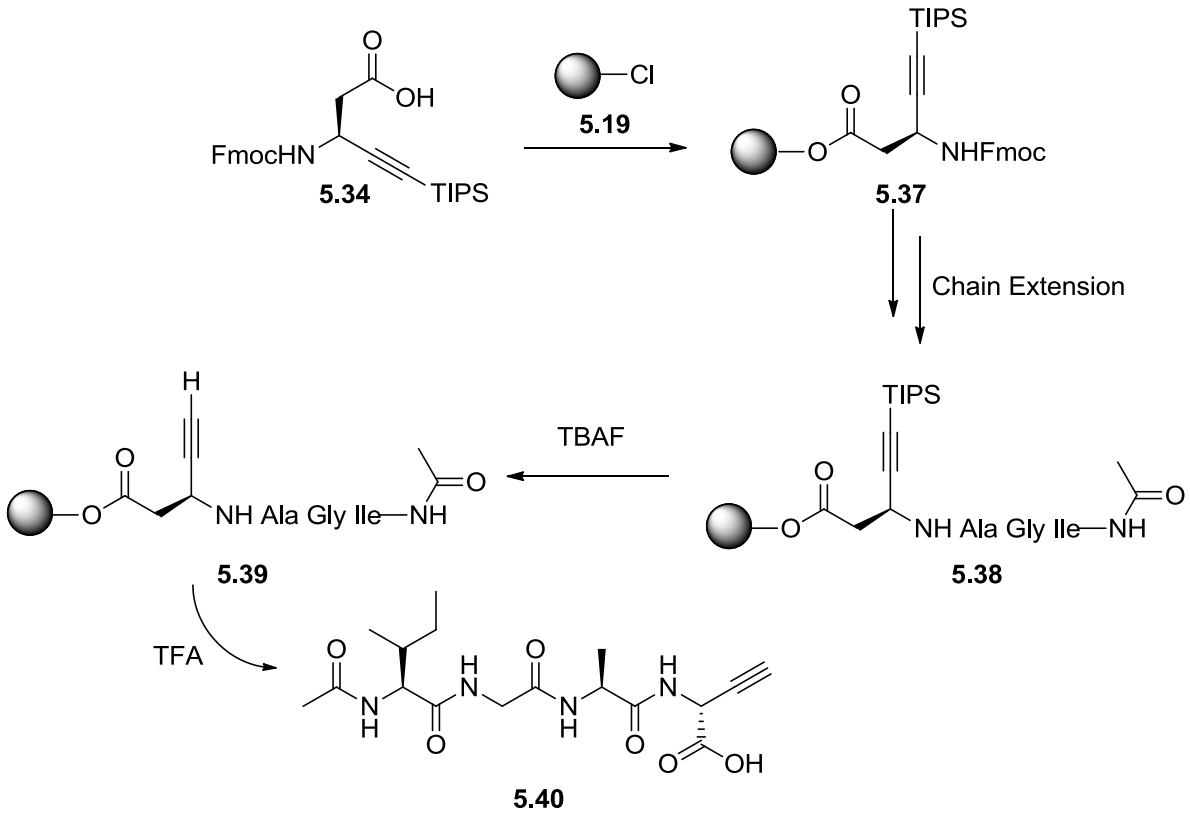
(S,S)-4.13 **5.36** **5.34**

Scheme 5.15 Conversion of aspartic acid-derivative (S,S)-4.13 to Fmoc-protected derivative 5.34.

Synthesis of the aspartic acid mimic **(S,S)-4.13** shown above was performed in section 4.3.1. As the proposed synthesis required the unit to be attached to an acid-labile resin, the *N*-terminal protecting group was converted into the acid-stable Fmoc group. This was accomplished by *N*-deprotection with a solution of hydrogen chloride in diethyl ether, followed by stirring the crude amine in a solution of Fmoc-succinimide and sodium carbonate, giving derivative **5.36** in 33 % yield for the 2 steps. The aliphatic Fmoc signals were observed in the ^{1}H NMR spectrum at δ 4.26 (1H, t) and δ 4.40 (2 H, m), while the α-proton was present at δ 4.87 (1H, m).

The side-chain *tert*-butyl ester was then deprotected by stirring alkyne **5.36** in TFA over 2 hours, giving acid **5.34** in quantitative yield. A signal corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 492.3).

5.4.2 Synthesis of the P4-P1 portion of the human inhibitor



Scheme 5.16 Attachment of aspartic acid derivative 5.34 to chlorotrityl resin (5.19) via the side-chain carboxylic acid, followed by chain extension to tetrapeptide 5.40.

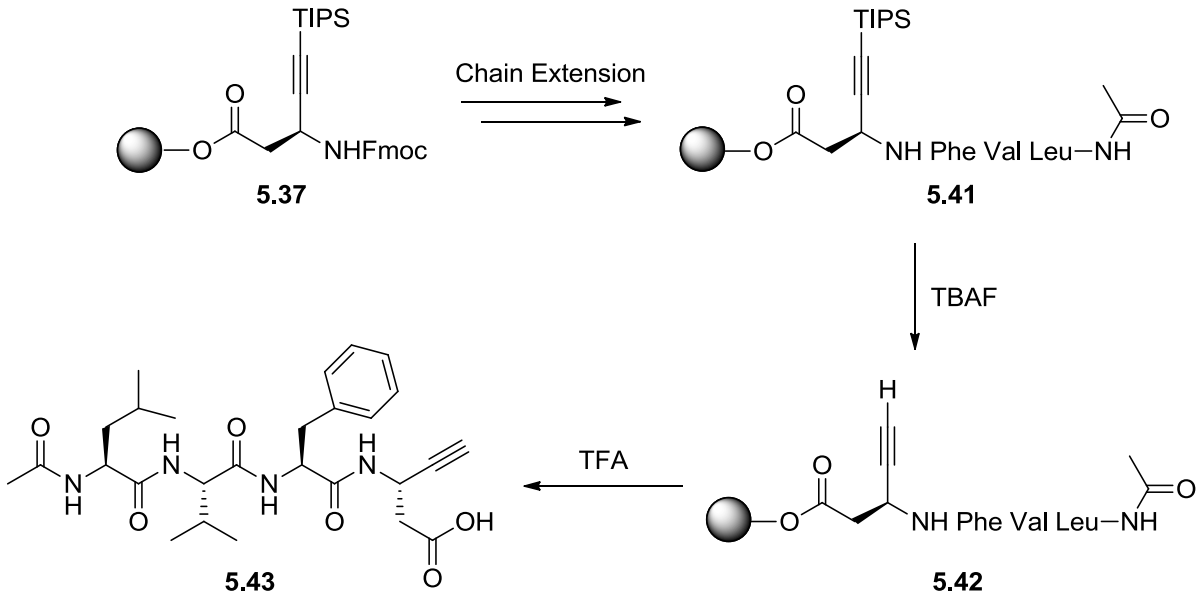
With acid **5.34** in hand, the unit (1 eq) was attached to chlorotrityl resin (**5.19**) (1 eq, 1.22 mmol g^{-1}) by stirring the two in a solution of DIEA (3 eq) in DMF. While the TIPS-protecting group on the alkyne was not required to be present for either attachment to the resin or chain extension, it could potentially be cleaved at any stage while attached onto the solid support.

Iterative peptide synthesis involving *N*-deprotection with a solution of 20 % piperidine in DMF followed by HBTU-mediated coupling to Fmoc-amino acids, followed by acetylation, resulted in tetramer **5.38** attached to the resin. Again, reaction completeness was monitored by use of the chloranil test,[149] with complete coupling to the HBTU-activated amino acids occurring within 30 minutes.

Desilylation of the TIPS protecting group was then performed, by adding resin **5.38** to a solution of TBAF in THF. The P4-P1 alkyne-containing tetrapeptide analogue (**5.40**) was isolated by TFA-mediated cleavage of a proportion of resin **5.39**, followed by preparative HPLC, which gave the tetrapeptide in 6.3 % yield. A peak corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 397). The key alkyne signal was seen in the

^{1}H NMR at δ 2.68 (1H, d), and α-proton signals were observed at δ 4.97 (ddd, *J* = 10.6, 6.9, 3.2 Hz, 1H), 4.33 (q, *J* = 7.2 Hz, 1H) and 4.13 (t, *J* = 6.1 Hz, 1H). The methylene signal from the glycine residue was observed as doublet of doublets at δ 3.88 (2H).

5.4.3 Synthesis of the P4-P1 portion of the mouse inhibitor

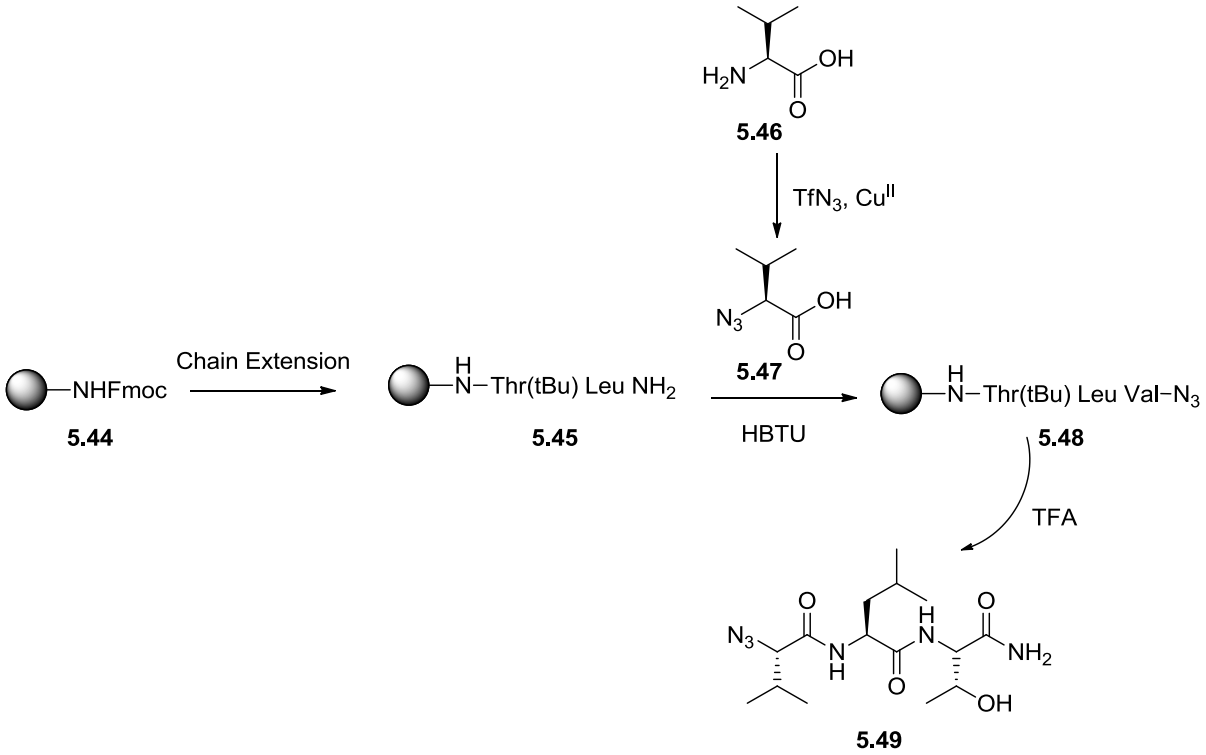


Scheme 5.17 Iterative peptide coupling, desilylation and cleavage from the chlorotrityl resin.

An identical synthetic approach was used for the synthesis of the P4-P1 portion of the mouse granzyme B-targeting molecule, starting from resin **5.37**. Again for the synthesis of a P4-P1 test compound, some of the TIPS-deprotected resin **5.42** was taken and cleaved with a mild TFA solution, giving alkyne **5.43** in 6.1 % yield after purification by preparative HPLC. A peak corresponding to the protonated molecular ion was detected by ESI-MS (*m/z* = 515). The key alkyne signal was seen in the ^{1}H NMR spectrum at δ 2.63 (d, *J* = 2.3 Hz, 1H), and α-proton signals were observed at δ 4.97 – 4.91 (m, 1H), 4.57 (dd, *J* = 8.3, 6.1 Hz, 1H), 4.38 (dd, *J* = 9.7, 5.4 Hz, 1H), 4.11 (d, *J* = 7.1 Hz, 1H). Interestingly, signals were observed which appeared to correspond to tetrabutylammonium ions at δ 1.03 (3H, t) and in the region between δ 1.70-1.35 (overall 7 protons, overlapping with protons from the valine and leucine side-chains). The relative ratios of the integration between the tetrapeptide and the tetrabutylammonium ion signals suggested 4 : 1 product : counterion.

5.4.4 Synthesis of full human inhibitor

For synthesis of the P1'-P3' portion of the compounds, it was decided that the C-terminus should be a primary amide. For this reason, chain extension to the tripeptide was performed using the Rink resin, which results in *C*-terminal amides upon cleavage.

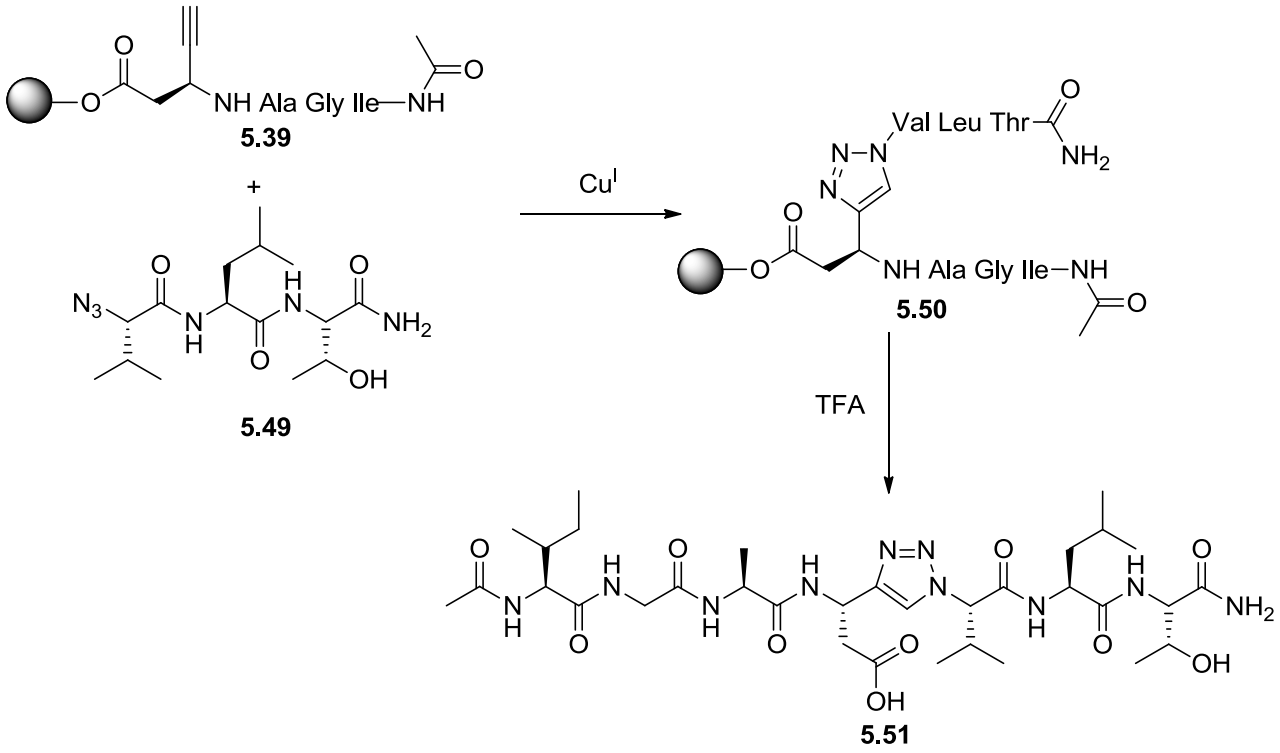


Scheme 5.18 Synthesis of azido-valine (5.47) via diazotransfer with triflic azide, and solid phase chain extension to azido-tripeptide 5.49 using the Rink resin (5.44).

As the P1' valine residue was to be coupled to the *C*-terminal alkyne present in tetrapeptide **5.40**, synthesis of the azido derivative of valine (**5.46**) was performed via a triflic azide-mediated diazotransfer reaction, which gave azide **5.47** in 81.8 % yield. The α-proton signal was seen in the ^{1}H NMR spectrum at δ 3.79 (1H, d); β-proton at δ 2.26 (1H, dq); one set of γ-protons at δ 1.08 (3H, d) and the other set at δ 1.03 (3H, d), with the carboxylic acid signal seen as a very broad peak (δ 10.23-9.10, 1 H). These signals matched the literature.[152]

Iterative peptide synthesis using the Rink resin (**5.44**) involving *N*-deprotection with a solution of 20 % piperidine in DMF followed by HBTU-mediated coupling to Fmoc-amino acids and freshly prepared azide **5.47** resulted in tripeptide **5.48** attached to the resin. Again, reaction completeness was monitored by use of the chloranil test,[149] with complete coupling to the HBTU-activated amino acids occurring within 30 minutes.

Azido tripeptide **5.49** was isolated after cleavage from the resin via a solution of TFA (97.5 %) and TIPS (2.5 %), giving a white precipitate in 15 % yield after purification by chromatography. A peak corresponding to the sodium adduct of the molecular ion was observed by ESI-MS (*m/z* = 379.2). The α-proton signals were observed in the ^{1}H NMR spectrum at δ 4.51 (1H, dd), 4.31 (1H, d) and δ 3.62 (1H, d) representing leucine, threonine and valine respectively. The threonine β-proton was located in the same region as the α-proton signals, at δ 4.18 (qd, 1H).



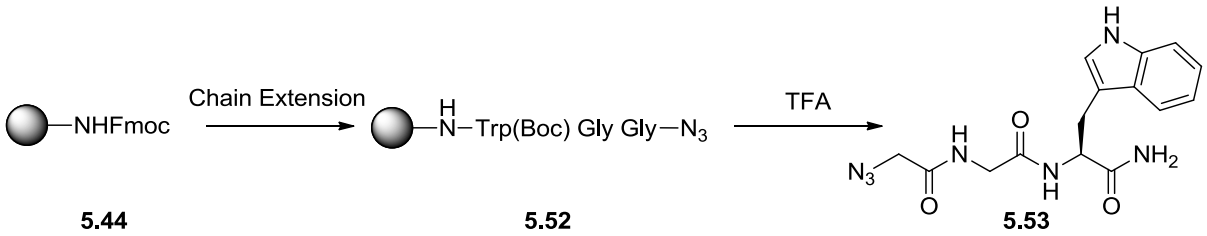
Scheme 5.19 Cu-AAL between azide 5.49 and resin-bound alkyne 5.39, followed by cleavage to give heptamer 5.51.

Cu-AAL was used for the formation of the heptamers on solid phase, using Meldal's original solid phase Cu-AAL conditions.[153] Thus azido tripeptide **5.49** and resin-bound alkynyl tetrapeptide **5.39** were mixed in THF in the presence of DIEA and copper iodide (2 eq), with coupling allowed to take place over 20 hours.

The heptamer (**5.51**) was cleaved from the resin with TFA, and analytically pure compound was achieved via preparative HPLC. The yield was tentatively assigned as 2.6 %, which was calculated using an assumption that resin **5.39** had an identical loading to the parent 2-chlorotriyl chloride resin (1.12 mmol per gram). In reality, the loading of the derivatised resin was likely to be lower than this figure. A peak corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 753), and the key triazole signal was observed in the ^{1}H

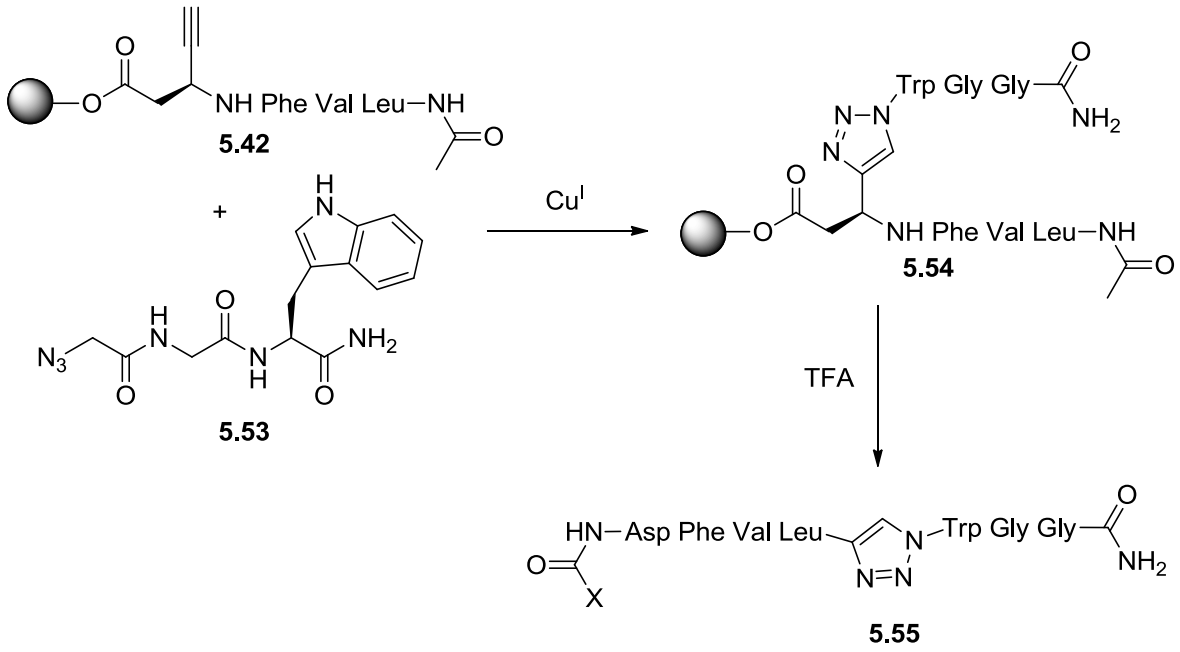
NMR spectrum at δ 8.04 (1H, s), with the α-proton signals present at δ 5.48 (t, *J* = 6.8 Hz, 1H), 4.94 (d, *J* = 7.1 Hz, 1H), 4.43 – 4.37 (m, 1H), 4.34 (q, *J* = 7.2 Hz, 1H), 4.30 (d, *J* = 3.7 Hz, 1H), 4.17 (dd, *J* = 6.4, 3.7 Hz, 1H) and 4.13 (d, *J* = 7.4 Hz, 1H). The glycine methylene protons were observed as a doublet of doublets at δ 3.85 (2H).

5.4.5 Synthesis of full mouse inhibitor



Scheme 5.20 Iterative peptide coupling and cleavage from the Rink resin for azido-trimer 5.53.

A similar procedure was used for the synthesis of azido tripeptide **5.53**, which was isolated in 14 % yield, and corresponded to the P3'-P1' portion of the mouse inhibitor. The ^{1}H NMR spectrum showed a clear signal for the 2-position of the indole at δ 7.53 (1H, s), with the glycine methylene signals present between δ 3.96-3.74 (m, 4H). A signal corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 344.1).



Scheme 5.21 Cu-AAL between azide 5.53 and resin-bound alkyne 5.42, followed by cleavage to give heptamer 5.55.

Thus azido tripeptide **5.53** and resin-bound alkynyl tetrapeptide **5.42** were mixed in THF in the presence of DIEA and copper iodide (2 eq), with coupling allowed to take place over 20 hours. The heptamer (**5.55**) was cleaved from the resin with TFA, and analytically pure compound was achieved via preparative HPLC. The yield was tentatively assigned as 1.7 %, again calculated using an assumption that resin **5.42** had an identical loading to the parent 2-chlorotrityl chloride resin (1.12 mmol per gram). A peak corresponding to the protonated molecular ion was observed by ESI-MS (*m/z* = 858), and the key triazole proton was observed in the ^{1}H NMR spectrum at δ 7.37 (1H, s), with the α-proton signals present at δ 5.44 (t, *J* = 6.8 Hz, 1H), 4.68 (dd, *J* = 8.2, 5.3 Hz, 1H), 4.62 (dd, *J* = 8.3, 6.4 Hz, 1H), 4.39 (dd, *J* = 9.9, 5.3 Hz, 1H) and 4.11 (d, *J* = 7.1 Hz, 1H). The signals corresponding to the glycine methylene protons were observed as doublet of doublets at δ 5.00 (2H) and 3.83 (2H).

5.4.6 Assay Results

Four compounds were tested against human granzyme B in a competitive fluorescent quenching assay to determine the binding efficiency and selectivity of the compounds.[154] Two compounds were expected to bind: the human granzyme B-targeted heptapeptidomimetic **5.51** and its shortened variant **5.40**, and two compounds were not expected to bind: the mouse granzyme B-targeted heptapeptidomimetic **5.55** and its shortened variant **5.43**. The compounds (2.5 mM to 3.9 µM) were pre-incubated with human granzyme B (47 nM), and the substrate acetyl-IETD-7-amino 4-trifluoromethyl coumarin (50 µM) was added. The effect of test compounds on the rate of substrate cleavage, measured by fluorescence of the cleaved coumarin, was evaluated across a range of test concentrations. The assays were performed in duplicate, with the results shown in figure 5.3 below.

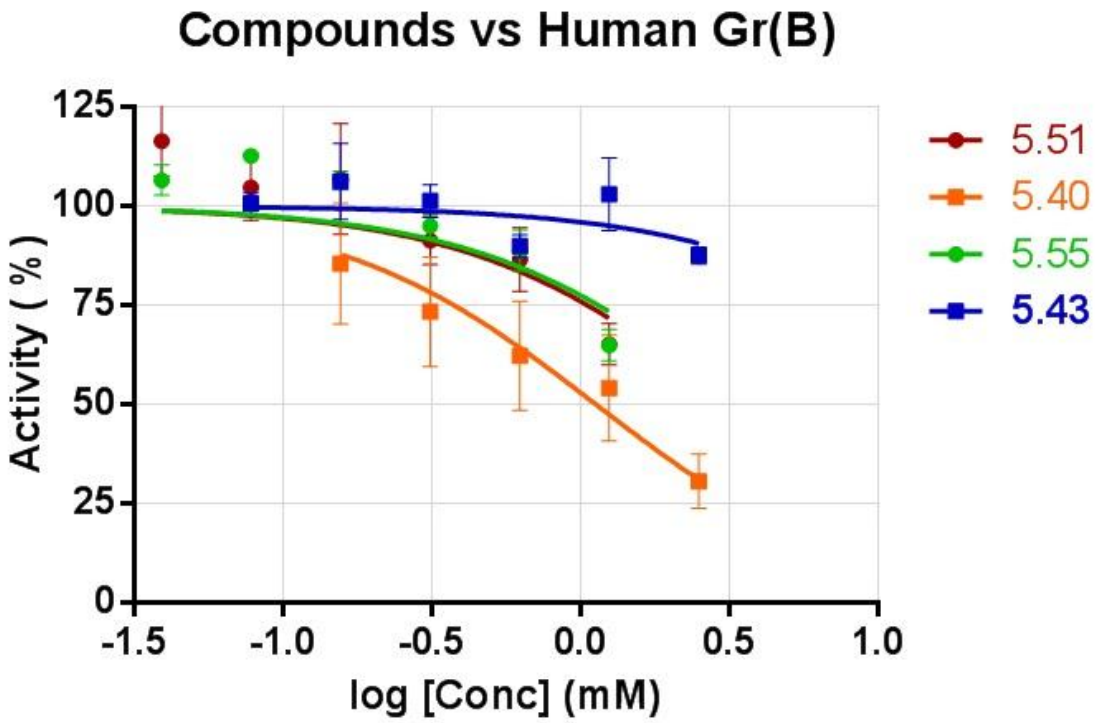


Figure 5.3 Binding data displayed in graph form showing the log concentration of substrate compound (mM) against the residual activity of human granzyme B (%).

Due to the high concentrations of compound required, and the limited material available, the IC_{50}'s of the heptameric compounds **5.51** and **5.55** were extrapolated by fitting the data to a nonlinear regression (curve fit) using GraphPad Prism 5.

Compound	***IC50 (mM)***
5.51	*3.17*
5.40	*1.13*
5.55	*3.45*
5.43	*> 10*

Table 5.1 IC_{50} data of the substrate compounds as determined from figure 5.4.

It can be seen that the three out of the four compounds do inhibit the binding of the substrate to human granzyme B, albeit in the low millimolar range, with alkyne tetramer **5.40** binding the strongest to granzyme B out of four compounds with an IC_{50} of 1.13 mM. No significant substrate selectivity was seen for the human-directed heptapeptide (**5.51**) compared with the mouse-directed heptapeptide (**5.55**), a surprising result given the compounds were derived from the different consensus sequence specificities. Alkyne tetramer **5.43** was found to effectively not bind at all.

During the course of the production of these compounds, a crystal structure of human granzyme B with the Merck inhibitor (**5.9**, synthesised in chapter 5.2) bound in the active site was obtained by our collaborators, shown in figure 5.4 below.

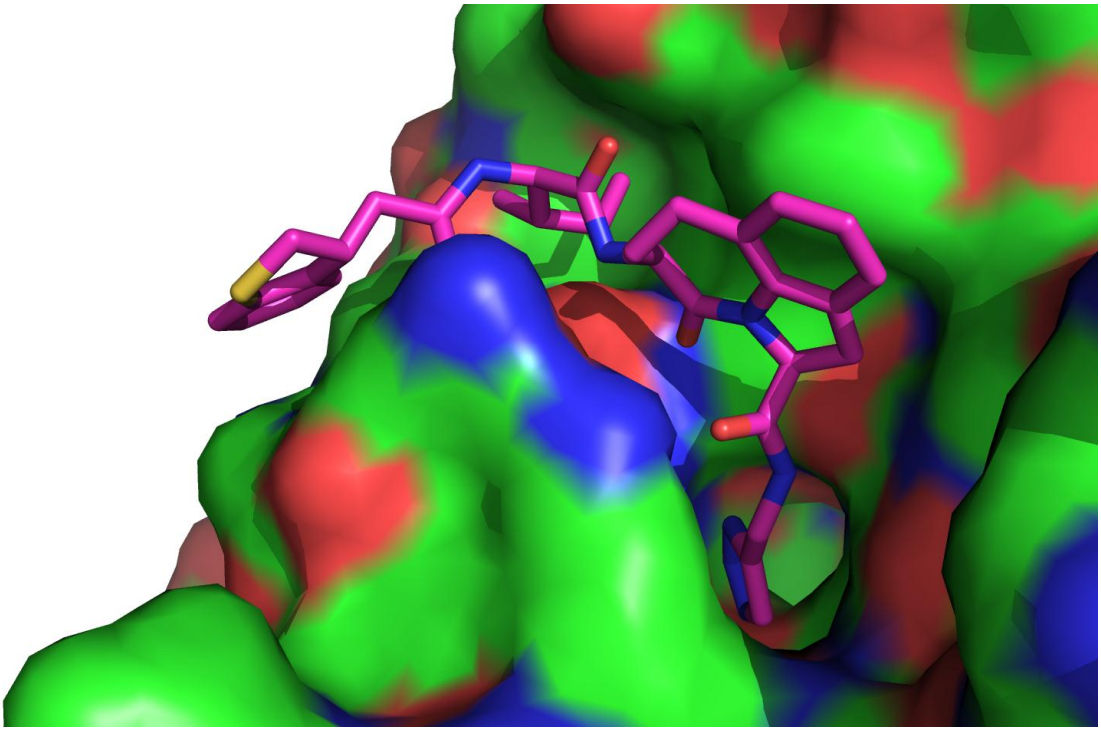


Figure 5.4 Crystal structure of inhibitor 5.9 bound to human granzyme B.

It can be seen from the portion of the crystal structure shown that the *C*-terminal triazole of the inhibitor is concealed in a pocket of the enzyme. It is clear that the triazole present in our heptamer mimics cannot bind in the same orientation, as there is no space available for extension from the 1-position of the triazole in the pose shown. The compounds appear to be unable to pick up suitable interactions with this region of the enzyme, leading to the weak binding seen.

5.5 Conclusions

A new synthesis of the Merck human granzyme B inhibitor (**5.9**) was described, and attempts were made to produce analogues of this compound extending from the *C*-terminus. However the compounds were found to have issues with solubility, which restricted both their synthesis and purification, and prevented any assessment of inhibition. At a later stage a crystal structure was obtained by our collaborators showing the binding of the Merck inhibitor (**5.9**) synthesised via solid phase synthesis in chapter 5.2.2 to granzyme B, which

suggested that the proposed compounds were unlikely to bind, or at least take up a different binding mode.

An attempt to take the consensus sequences of both human and mouse granzyme B and derive inhibitors via a triazole-for-amide replacement showed limited success, as the synthesised compounds (heptamers **5.51** and **5.55**) were shown to bind to human granzyme B in the low millimolar range, yet selectivity was not seen. This suggests that the triazole-for-amide strategy could be used to provide lead compounds for the inhibition of proteases – although as seen with Saquinvair (**5.4**) and the Merck inhibitor (**5.9**), extensive derivatisation may be required to produce tightly binding compounds.

Chapter 6 - Conclusions and Future Directions

A major focus of this project has been the synthesis of appropriate alkynyl-azide monomer units which, when systematically coupled together, form triazole peptidomimetics. An existing literature approach was replicated, involving a number of functional group transformations, including key Corey-Fuchs homologation and diazotransfer steps, from *N*-protected amino acids. The variety of conditions required, in addition to racemisation issues, meant that this particular pathway could only be used for a small subset of amino acids, namely those with simple hydrophobic side-chains. It was successfully used for the synthesis of a phenylalanine derivative, giving the key alkynyl-azide monomer unit in 10 % yield over 7 steps.

In order to broaden the scope and improve the yields of the monomer units, we sought to develop a new synthetic approach. Key requirements were stereochemical control, and flexibility in the installation of a wide range of side-chain groups. Our strategy employs Ellman-type sulfinylimine chiral auxiliaries to facilitate organometallic addition of the amino acid side-chain to the monomer unit backbone, present as a TIPS-propiolaldehyde-derived *tert*-butyl sulfinylimine. The late-stage divergent synthesis allowed for the production of an analogous phenylalanine-derived alkynyl azide monomer unit in 44 % yield in just 3 steps. This approach was also used in the synthesis of analogues of aspartic acid and lysine. Access to an arginine analogue was restricted by difficulties associated with the guanidine moiety, although alternative syntheses to this molecule have previously been discussed. Ideally, the synthesis of analogues of all common amino acids, with the exception of proline, would be achieved through the use of the key sulfinylimine. Many amino acid analogues, such as the mimics of valine, leucine and isoleucine, are anticipated to be easily accessed via the use of commercial Grignard reagents. Other side-chains, such as those of tryptophan, histidine and cysteine, may present chemoselectivity challenges, however judicious protecting group selection should allow suitable access. Additionally, the method used to confirm the stereochemistry of the monomer units during this project, conversion to other known compounds, is not a general procedure. For other derivatives, determination of the stereochemistry by *de novo* methods such as Mosher's amide analysis of the intermediate amino alkynes would be preferable.

The second key aim of this project was the use of these monomer units for the synthesis of linear and cyclic triazole peptidomimetics. A 2-step chain extension cycle was demonstrated, which gave yields upwards of 70 % yield per cycle, and could be performed in the presence

of a variety of side-chain functionality. Three all-triazole oligomers, consisting of 4, 5 and 6 phenylalanine-like units, were derivatised in a sequence of reactions to the terminal alkynyl-azide functionality to allow for head-to-tail cyclisation. A set of conditions was found which gave access to the cyclic tetramer and pentamer, validating the reaction sequence for the synthesis of these novel structures. As the synthesis of small cyclic peptides is often problematic, these compounds may become useful alternatives. Further work would be required for this to be achieved, with optimisation of the cyclisation yields and an investigation into the allowable ring sizes critical.

It was shown that the tetramer and pentamer compounds were able to form in low yields at a concentration of 0.5 mM, with the competing polymerisation reaction consuming the majority of the starting material. A higher dilution factor would presumably give higher yields, but would concurrently reduce the rate of the reaction. This may be off-set by higher catalyst loadings, an increase in the temperature of the reaction, or patience. The ability to synthesise a larger amount of these compounds would allow an investigation of their structural properties, in particular the orientation of the side-chains in space, with a direct comparison to the analogous peptidic structures. Either NOE experiments using NMR, or X-ray crystallography, could be used to gain an insight into the structural properties. It is proposed that, unlike cyclic peptides, cyclic triazole peptidomimetics would be less flexible, and thus would form conserved, well-defined structures. As a result of this they may prove to be useful in the synthesis of mimics of peptide secondary structures. It remains to be seen whether all-triazole analogues of biologically active cyclic peptides retain biological activity.

Finally, the aspartic acid-derived alkyne monomer unit was an integral part in the synthesis of triazole-containing heptamer sequences, as potential granzyme B inhibitors, based on existing substrates. With the P4-P1 and P1'-P3' portion of the molecules synthesised using solid phase peptide synthesis, Cu-AAL was used to link both components, concurrently forming a triazole in lieu of a scissile bond. Although the compounds synthesised were intended to selectively act on the human and mouse isoforms of granzyme B, no such selectivity was observed, and only relatively weak binding (IC_{50} = low millimolar) by both inhibitors was observed against the human isoform. Recent crystallographic data of the complex of granzyme B and a known tight-binding inhibitor suggests that there may not be space available in the active site to incorporate many residues in the prime position while containing a triazole moiety, thus, in hindsight, this strategy may not have been ideal for producing inhibitors of granzyme B. Given the apparent ease in inserting a triazole moiety into a peptide sequence as compared to other isosteres, the synthetic strategy demonstrated could be readily applied to a variety of other protease targets.

Chapter 7 - Experimental

7.1 General Experimental

All materials were reagent grade and purchased commercially from Sigma-Aldrich/Fluka, Alfa-Aesar, Merck, Auspep, Acros, NeoMPS, Chem-Impex and GL Biochem. HPLC-grade THF, DCM and DMF were dried using an Mbraun solvent purification system (SPS-800) according to the manufacturer's instructions. All other solvents were reagent grade and used as required.

Thin layer chromatography (TLC) was performed using Merck Silica Gel 60 F254 pre-coated plates (0.25 mm) and visualised by ultra violet light as well as staining with solutions of vanillin or phosphomolybdic acid. Flash column chromatography used Merck Silica Gel 60, 230-400 mesh ASTM or DAVSIL 60A 30-70 micron silica, following the method described by Still *et al*.[155] Compounds were either pre-adsorbed onto silica prior to column chromatography or dissolved in the appropriate solvent.

^{1}H NMR spectra were routinely recorded at 300.13 MHz using a Bruker Avance DPX-300 spectrometer or at 400.13 MHz using a Bruker Ultrashield-Avance III NMR spectrometer, both at 298 K unless otherwise stated. Data acquisition and processing was managed using XWINNMR (Bruker) software package v3.5 and plotting was managed using XWINPLOT or MestReNova v6.0.2. Chemical shifts (δ) for all ^{1}H NMR spectra were reported in parts per million (ppm) referenced to an internal standard of residual proteo-solvent: δ 2.50 ppm for d_6-dimethylsulfoxide (DMSO), δ 3.31 ppm for d_4-methanol (CD_3OD), δ 4.79 ppm for d_2-deuterium oxide (D_2O) and δ 7.27 ppm for d_1-chloroform ($CDCl_3$).[156] The ^{1}H NMR spectra were reported as follows: chemical shift (δ), multiplicity, coupling constant (*J*) in Hertz (quoted to one decimal place) and peak integration. In reporting the spectral data, the following abbreviations have been used: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, app = apparent.

^{13}C NMR spectra were routinely recorded at 75.5 MHz using a Bruker Avance DPX-300 spectrometer or at 100.62 MHz using a Bruker Ultrashield-Avance III NMR spectrometer, both at 298 K unless otherwise stated. Data acquisition and processing were managed using XWINNMR (Bruker) software package v3.5 and plotting was managed using XWINPLOT or MestReNova v6.0.2. Chemical shifts

(δ) for all ^{13}C NMR were reported in parts per million (ppm) referenced to an internal standard of residual proteo-solvent: δ 39.52 ppm for d_6-dimethylsulfoxide (DMSO), δ 49.00 ppm for d_4-methanol (CD_3OD) and δ 77.16 ppm for d_1-chloroform ($CDCl_3$).[156]

High resolution mass spectrometry analyses were performed on a Waters Micromass LCT Premier XE time-of-flight mass spectrometer fitted with an electrospray (ESI) ion source controlled by MassLynx v4.5 software. Low resolution mass spectrometry analyses were performed using a Micromass Platform II single quadrupole mass spectrometer equipped with an atmospheric pressure (ESI/APCI) ion source. Sample management was facilitated by an Agilent 1100 series HPLC system and the instrument was controlled using MassLynx v3.5 software.

Microwave reactions were conducted in a Biotage Initiator TM, in 2.0-10.0 mL vials according to manufacturer's instructions.

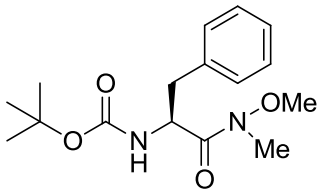
Analytical reverse-phase high performance liquid chromatography (RP-HPLC) was conducted on a Waters Millenium 2690 system fitted with a Phenomenex® Luna C8, 100 Å, 5 µm (50 x 4.60 mm I.D.) column. A binary solvent system was used (solvent A: 0.1% TFA, 99.9% H_2O; solvent B: 0.1% TFA, 19.9% H_2O, 80% acetonitrile), with UV detection at 214 or 254 nm. The method used gradient elution beginning with 100% solvent A going to 20% solvent A, 80% solvent B, over 20 min at a flow rate of 1 mL/min. EmpowerPro software managed the running and processing of samples.

Optical rotations were measured on a Jasco P-2000 series digital polarimeter (Jasco Corp., Tokyo, Japan) operating at the sodium D line (589 nm) and at ambient temperature (approximately 23 °C). Samples were measured in a 100 mm path length Jasco cell with a digital integration time (DIT) interval of 5 sec, 10 cycles, and a cycle interval of 1 sec. Compound samples were prepared by dissolving in chloroform (2-7 mg/ml). The optical rotation of each enantiomer of a compound was measured three separate times in the polarimeter. Specific rotations of compounds [α] were calculated according to the equation $[\alpha]^T_D = \alpha / (l \times c)$ where T = temperature (^oC), α = experimental optical rotation (^{o}), l = path length (dm) and c = concentration (g per 100 mL).

7.2 Synthesis of the Compounds Described in Chapter 2

7.2.1 Corey-Fuchs approach to amino alkyne

(S)-tert-Butyl (1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate



2.18a

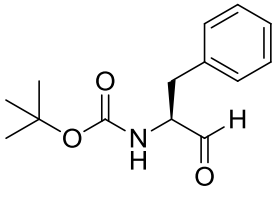
PyBOP (1.2 eq, 7.1 g) was added to a solution of Boc-phenylalanine (1 eq, 4.51 g) and triethylamine (1.1 eq, 2.61 mL) in DCM (30 mL). After allowing 30 minutes stirring for activation of the acid, *N,O*-dimethylhydroxylamine hydrochloride (1 eq, 1.66 g) and triethylamine (1.1 eq, 2.61 mL) were added and the reaction allowed to stir for 16 hours. After concentrating the solution, the crude mixture was redissolved in ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (3 x 30 mL), saturated sodium bicarbonate solution (3 x 30 mL) and brine (30 mL). Column chromatography (25 to 33 % ethyl acetate in petroleum spirits) furnished Weinreb amide **2.18a** as a colourless oil (3.47 g, 66 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.12 (m, 5H), 5.14 (s, 1H), 4.94 (s, 1H), 3.65 (s, 3H), 3.15 (s, 3H), 3.05 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.87 (dd, *J* = 13.0, 7.0 Hz, 1H), 1.38 (s, 9H).

ESI-MS: *m/z* = 309 [MH^+].

The spectral data are consistent with those reported in the literature.[91]

(S)-tert-Butyl (1-oxo-3-phenylpropan-2-yl)carbamate



2.14a

Lithium aluminium hydride (1.25 eq, 0.46 g) was added to a solution of Weinreb amide **2.18a** (1 eq, 3.0 g) in THF (50 mL) at 0 ^oC. After stirring for one hour, the reaction was quenched via the addition of an aqueous potassium hydrogen sulfate solution (0.85 M, 20 mL). The solution was diluted with ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (2 x 20 mL), saturated sodium bicarbonate

solution (2 x 20 mL) and brine (10 mL), before drying over magnesium sulfate and concentrating, giving crude aldehyde **2.14a** as a colourless oil (2.09 g, ~ 86 % yield).

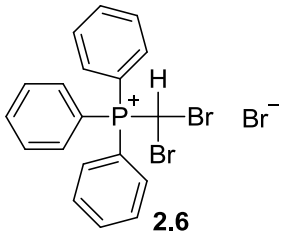
^{1}H NMR (300 MHz, $CDCl_3$): δ 9.65 (s, 1H), 7.37 – 7.15 (m, 5H), 5.06 (s, 1H), 4.45 (app q, *J* = 6.6 Hz, 1H), 3.14 (d, *J* = 6.5 Hz, 2H), 1.45 (s, 9H).

ESI-MS: *m/z* = 250 [MH^+].

$[\alpha]^{21}_D$ = -53^o (c = 1.00, MeOH).

The spectral data are consistent with those reported in the literature.[91]

(Dibromomethyl)triphenylphosphonium bromide



2.6

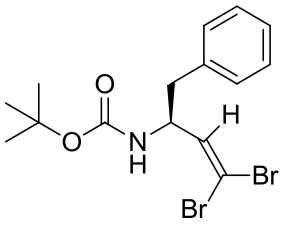
The title compound was synthesised following the procedure of Wolkoff:[68] carbon tetrabromide (1 eq, 26 g) was added to a solution of triphenylphosphine (2 eq, 40.1 g) in DCM (200 mL) at 0 ^{o}C. After stirring for 5 minutes, the reaction was allowed to warm to room temperature and stir for a further 20 minutes. After cooling back to 0 ^{o}C, water (0.17 eq, 12.6 mL) was added and the reaction was left to stir for 30 minutes. The aqueous layer was separated, and the organic layer was concentrated. Acetonitrile was added to the syrup produced, causing precipitation of the crude product. Recrystallisation from hot acetonitrile gave phosphonium bromide **2.6** as a white powder (33.5 g, 84 %).

^{1}H NMR: δ 10.1 (s, 1H), 8.21-7.69 (m, 15 H).

ESI-MS: *m/z* = 354 [$(M - Br)H^+$].

The spectral data are consistent with those reported in the literature.[157]

(S)-tert-Butyl (4,4-dibromo-1-phenylbut-3-en-2-yl)carbamate



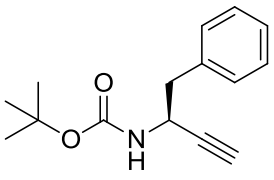
2.19a

Potassium *tert*-butoxide (2.4 eq, 2.28 g) was added to a solution of **2.6** (2 eq, 8.71 g) in THF (55 mL) and cooled to 0 ^{o}C. After stirring for 10 minutes, the solution was allowed to return to room temperature and stir for a further 10 minutes. After the addition of aldehyde **2.14a** (1 eq, 2.11 g), the reaction was allowed to stir for 30 minutes. Brine (50 mL) was added to quench the reaction, and the organic layer was extracted with ethyl acetate (3 x 50 mL). The combined organic fractions were washed with brine (20 mL), dried with magnesium sulfate and concentrated. Column chromatography (25 to 50 % ethyl acetate in petroleum spirits) furnished dibromo-olefin **2.19a** as an off-white solid (2.22 g, 65%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.16 (m, 5H), 6.39 (d, *J* = 8.3 Hz, 1H), 4.56 (br s, 1H), 4.50 (m, 1H), 2.90 (d, *J* = 5.4 Hz, 2H), 1.42 (s, 10H).

The spectral data are consistent with those reported in the literature.[92]

(S)-tert-Butyl (1-phenylbut-3-yn-2-yl)carbamate



2.4a

n-Butyl lithium (3 eq, 2.17 M, 12.6 mL) was added to a solution of **2.19a** (1 eq, 3.68 g) in THF (10 mL) at -78 ^{o}C, and the solution was stirred for 2 hours, before warming to 0 ^{o}C and stirring for a further 30 minutes. Saturated sodium bicarbonate solution (20 mL) was added to quench the reaction, brine (30 mL) was added and the organic phase was extracted with ethyl acetate (2 x 20 mL). The combined organic fractions were washed with brine (3 x 25 mL). After drying with magnesium sulfate, the

solution was concentrated. Column chromatography (5% ethyl acetate in petroleum spirits) furnished alkyne **2.4a** as a white solid (1.82 g, 82 %).

^{1}H NMR (300 MHz, CDCl3): δ 7.34-7.27 (m, 5H), 4.69 (br s, 2H), 2.99-2.95 (m, 2H), 2.29 (d, *J* = 2.1 Hz, 1H), 1.44 (s, 9H).

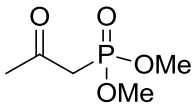
ESI-MS: m/z = 246 [MH^+].

$[\alpha]^{21}_D$ = -9.7^o (c = 1.07, $CHCl_3$).

The spectral data are consistent with those reported in the literature.[92]

7.2.2 Ohira-Bestmann approach to amino alkyne

Dimethyl (2-oxopropyl)phosphonate



2.20

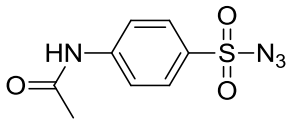
Chloroacetone (1 eq, 10 mL) was added to a solution of potassium iodide (1 eq, 20.9 g) in acetonitrile (30 mL) and acetone (24 mL). After stirring for one hour, trimethyl phosphite (1 eq, 14.7 mL) was added, and the reaction mixture left to stir overnight. The solution was then filtered through celite with acetone. The slightly yellow filtrate was concentrated, and vacuum distillation (2.7 mbar, 105 ^{o}C) gave phosphonate **2.20** as a colourless yellow liquid (6.85 g, 39%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (d, ^3J_P = 11.2 Hz, 6H), 3.11 (d, ^2J_P = 22.8 Hz, 2H), 2.33 (s, 3H).

ESI-MS: *m/z* = 167 [MH^+].

The spectral data are consistent with those reported in the literature.[93]

4-Acetamidobenzenesulfonyl azide



2.21

Sulfonyl chloride (1 eq, 19.5 g) and tetrabutylammonium bromide (0.0025 eq, 70 mg) were dissolved in toluene (120 mL), before sodium azide (1.6 eq, 8.7 g) in water (40 mL) was added. After allowing to stir for 16 hours, the solution was diluted with ethyl acetate (100 mL) and the organic layer was washed with water (3 x 50 mL) and brine (50 mL), before drying over magnesium sulfate and concentrating, giving azide **2.21** as a pink powder (14.4 g, 72%).

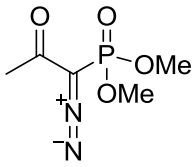
^{1}H NMR: δ 7.91 (app d, *J* = 9 Hz, 2H), 7.78 (app d, *J* = 9 Hz, 2H), 7.65 (br s, 1H), 2.36 (s, 3H).

ESI-MS: *m/z* = 241 [MH^+].

IR: 2120 cm^{-1} (N_3).

The spectral data are consistent with those reported in the literature.[93]

Dimethyl (1-diazo-2-oxopropyl)phosphonate



2.11

Phosphonate **2.20** (1 eq, 2.05 mL) was dissolved in toluene (30 mL) and cooled to 0 ^oC. Sodium hydride (1.2 eq; 60 % in paraffin, 1 g) was added slowly. After the evolution of gas ceased, azide **2.21** (1.2 eq, 5.6 g) in THF (10 mL) was added and the solution was left to stir overnight at room temperature. The crude mixture was diluted with petroleum spirits (50 mL) and washed through celite with diethyl ether, and the filtrate was concentrated. Column chromatography (66 to 75 % ethyl acetate in petroleum spirits) furnished the title compound as a clear liquid (1.16 g, 40 %).

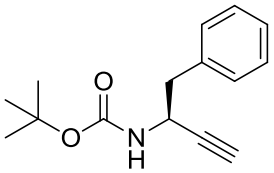
^{1}H NMR ($CDCl_3$): δ 3.86 (d, ^3J_P = 11.9 Hz, 6H), 2.28 (s, 3H).

ESI-MS: *m/z* = 193 [MH^+].

IR (Neat): 2128 cm^{-1} (N_2).

The spectral data are consistent with those reported in the literature.[93]

(S)-tert-Butyl (1-phenylbut-3-yn-2-yl)carbamate



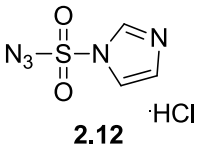
2.4a

Aldehyde **2.14a** (1eq, 0.19 g) was dissolved in methanol (4 mL) with potassium carbonate (2 eq, 0.23 g). The Ohira-Bestmann reagent (**2.11**; 1.2 eq, 0.19 g) in methanol (4 mL) was added to the reaction mixture, which was left at room temperature to stir overnight. After the addition of saturated sodium bicarbonate solution (10 mL), the reaction mixture was diluted with ethyl acetate (20 mL) and washed with brine (3 x 10 mL). Column chromatography (6 % ethyl acetate in petroleum spirits) furnished alkyne **2.4a** as a white solid (0.12 g, 59 %).

The spectral data are identical to those from the compound produced via the Corey-Fuchs approach, with the exception of the optical rotation: $[\alpha]^{21}_{D} = -6.67^{o}$ (c = 1.0, $CHCl_3$).

7.2.3 Diazo-transfer reactions

1H-Imidazole-1-sulfonyl azide hydrochloride



2.12

The diazotransfer reagent was synthesised following the procedure of Goddard-Borger:[77] sodium azide (1 eq, 3.75 g) was cooled in an ice bath to 0 ^{o}C in acetonitrile (60 mL). Sulfuryl chloride (1 eq, 4.62 mL) was added dropwise, at which point the ice bath was removed. After stirring for 16 hours, the suspension was again cooled to 0 ^{o}C in an ice bath, and imidazole (1.9 eq, 7.46 g) was added. After stirring at room temperature for 3 hours, the reaction was diluted with ethyl acetate (50 mL) and washed with water (2 x 50 mL) and saturated sodium bicarbonate solution (2 x 50 mL).

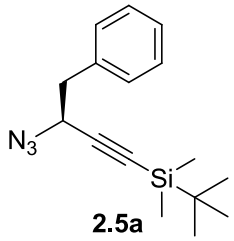
Acetyl chloride (1.5 eq, 6.15 mL) was added dropwise to 100 % ethanol (30 mL) at 0 ^{o}C. This solution was added dropwise to the filtrate produced prior, resulting in the precipitation of **2.12** as a white solid (5.46 g, 45 %).

^{1}H NMR (300 MHz, D_2O): δ 8.57 (s, 1H), 7.83 (s, 1H), 7.39 (s, 1H).

Lit:[77] ^{1}H NMR (600 MHz, D_2O): δ 9.53 (dd, *J* = 1.6, 1.3 Hz, 1H), 8.09 (dd, *J* = 2.2, 1.6 Hz, 1H), 7.68 (dd, *J* = 2.2, 1.3 Hz, 1H).

ESI-MS: *m/z* = 174 [MH^{+}].

(S)-(3-azido-4-phenylbut-1-yn-1-yl)(tert-butyl)dimethylsilane



2.5a

Amino-alkyne **2.4a** (1 eq, 1.5 g) was cooled to -78 ^{o}C in THF (20 mL). *n*-Butyl lithium (2.2 eq, 1.52 M, 8.9 mL) was added dropwise, and the solution was stirred for 30 minutes before *tert*-butyl dimethylsilyl chloride (1 eq, 0.92 g) was added. The solution was stirred at -78 ^{o}C for 30 minutes before being allowed to warm to 0 ^{o}C, and after stirring continued for an hour, before the reaction was quenched with saturated sodium bicarbonate solution (10 mL). The solution was diluted with ethyl acetate (30 mL) and washed with brine (3 x 20 mL), before being dried with magnesium sulfate and concentrated. The crude material (1 eq, 2.0 g) was then stirred in a solution of TFA in DCM (30 %, 10 mL) for one hour, before being evaporated, giving amine **2.16a** as a TFA salt. The crude free amine (1 eq) was dissolved in methanol (30 mL), to which was added potassium carbonate (2.5 eq, 2.12 g), copper sulfate pentahydrate in water (0.02 eq, 1 M, 0.12 mL). After 10 minutes of stirring, diazotransfer reagent **2.12** (1.5 eq, 1.92 g) was added and stirring was allowed to continue overnight at room temperature. After evaporation of the solvents, the residue was redissolved in ethyl acetate (30 mL) and washed with hydrochloric acid (2 x 20 mL), saturated sodium bicarbonate solution (2 x 20 mL) and brine (2 x 20 mL). Column chromatography (1 % ethyl acetate in petroleum spirit) furnished azide **2.5a** as a colourless oil (29 %).

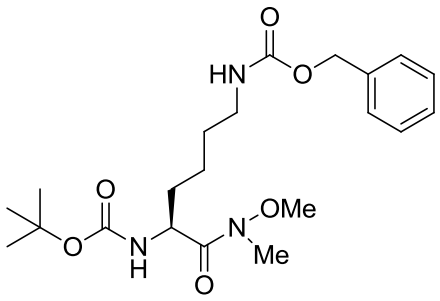
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 – 7.23 (m, 5H), 4.28 (t, *J* = 6.8 Hz, 1H), 2.98 (d, *J* = 6.8 Hz, 2H), 0.94 (s, 9H), 0.13 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 136.2, 129.7, 128.5, 127.2, 100.7, 91.7, 54.8, 41.7, 26.1, 16.4, 4.6.

ESI-MS: *m/z* = 258 [$(M-N_2)H^+$].

7.2.4 Other amino acid mimics

(S)-Benzyl tert-butyl (6-(methoxy(methyl)amino)-6-oxohexane-1,5-diyl)dicarbamate



2.18b

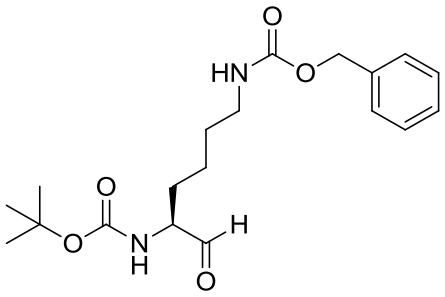
PyBOP (1.2 eq, 4.5 g) was added to a solution of Boc-(Cbz)-lysine (1 eq, 2.63 g) and triethylamine (1.1 eq, 1.64 mL) in DCM (50 mL). After allowing 30 minutes stirring for activation of the acid, *N,O*-dimethylhydroxylamine hydrochloride (1.2 eq, 1.25 g) and triethylamine (1.1 eq, 1.64 mL) were added and the reaction allowed to stir for 16 hours. After concentrating the solution, the crude mixture was redissolved in ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (3 x 30 mL), saturated sodium bicarbonate solution (3 x 30 mL) and brine (30 mL). Column chromatography (33 to 66 % ethyl acetate in petroleum spirits) furnished Weinreb amide **2.18b** as a colourless oil (2.54 g, 82 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 – 7.17 (m, 5H), 5.12 (d, *J* = 8.0 Hz, 1H), 5.01 (s, 2H), 4.77 (br s, 1H), 4.58 (br s, 1H), 3.68 (s, 3H), 3.16 – 3.07 (m, 2H), 3.12 (s, 3H), 1.69 – 1.56 (m, 1H), 1.48 – 1.39 (m, 3H), 1.34 (s, 9H).

ESI-MS: *m/z* = 446.3 [MNa^+].

The spectral data are consistent with those reported in the literature.[91]

(S)-Benzyl tert-butyl (6-oxohexane-1,5-diyl)dicarbamate



2.14b

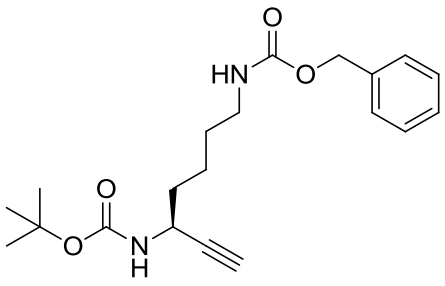
Lithium aluminium hydride (2 eq, 1.26 g) was added to a solution of Weinreb amide **2.18b** (1 eq, 7.0 g) in THF (50 mL) at 0 ^{o}C. After stirring for one hour, the reaction was quenched via the addition of an aqueous potassium hydrogen sulfate solution (0.83 M, 50 mL). The solution was diluted with ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (2 x 20 mL), saturated sodium bicarbonate solution (2 x 20 mL) and brine (10 mL), before drying over magnesium sulfate and concentrating, giving crude aldehyde **2.14b** as a colourless oil (4.34 g, ~ 72 % yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.51 (s, 1H), 7.35 – 7.19 (m, 5H), 5.13 (br s, 1H), 5.04 (s, 2H), 4.78 (br s, 1H), 4.15 (app q, *J* = 7.2 Hz, 1H), 3.20 – 3.10 (m, 2H), 1.85 – 1.76 (m, 2H), 1.50 (m, 4H), 1.40 (s, 9H).

ESI-MS: *m/z* = 387.3 [MNa^{+}].

The spectral data are consistent with those reported in the literature.[91]

(S)-Benzyl tert-butyl hept-6-yne-1,5-diyldicarbamate



2.4b

Aldehyde **2.14b** (1eq, 1.9 g) was dissolved in methanol (20 mL) with potassium carbonate (2 eq, 1.43 g). The Ohira-Bestmann reagent (**2.11**; 1.2 eq, 1.19 g) in methanol (10 mL) was added to the reaction mixture, which was left at room temperature to stir overnight. The solution was concentrated and redissolved in ethyl acetate (20 mL), which was washed with brine (2 x 10 mL). Column

chromatography (25 % ethyl acetate in petroleum spirits) furnished alkyne **2.4b** as a colourless oil (1.02 g, 55 %).

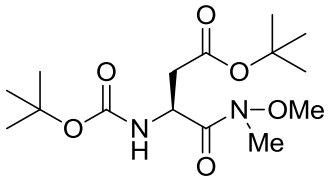
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39-7.36 (m, 5H), 5.10 (s, 2H), 4.40 (m, 2H), 3.21 (app q, *J* = 6.4 Hz, 1H), 2.27 (d, *J* = 2.2 Hz, 1H), 1.74-1.62 (m, 2H), 1.59-1.47 (m, 4H), 1.45 (s, 9H).

ESI-MS: *m/z* = 383.3 [MNa^+].

$[\alpha]^{21}_D$ = -9.3^o (c = 1.00, $CHCl_3$).

The spectral data are consistent with those reported in the literature,[53] with the exception of the optical rotation,[88] $[\alpha]^T_D$ = -23.1^o (no solvent given).

(S)-tert-Butyl 3-((tert-butoxycarbonyl)amino)-4-(methoxy(methyl)amino)-4-oxobutanoate



2.18c

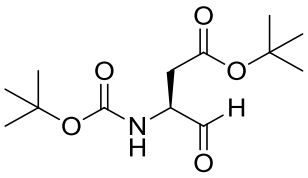
HOBt (1.1 eq, 1.28 g) and HBTU (1 eq, 3.28 g) were added to a solution of Boc-(O*t*Bu)-aspartic acid (1 eq, 2.5 g) and diisopropylethylamine (1.2 eq, 1.85 mL) in DCM (20 mL). After allowing 1 hour of stirring for activation of the acid, *N,O*-dimethylhydroxylamine hydrochloride (1.2 eq, 1.01 g) and diisopropylethylamine (1.2 eq, 1.85 mL) were added and the reaction allowed to stir for 1.5 hours. After concentrating the solution, the crude mixture was redissolved in ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (3 x 30 mL), saturated sodium bicarbonate solution (3 x 30 mL) and brine (30 mL). Column chromatography (25 % ethyl acetate in petroleum spirits) furnished Weinreb amide **2.18c** as a colourless oil (2.59 g, 90 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (d, *J* = 8.6 Hz, 1H), 4.98 (br s, 1H), 3.79 (s, 3H), 3.22 (s, 3H), 2.69 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.51 (dd, *J* = 14.9, 7.2 Hz, 1H), 1.44 (s, 9H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 169.5, 155.2, 81.3, 79.8, 61.8, 48.1, 38.7, 32.4, 28.4, 28.1.

ESI-HRMS-TOF calcd for $C_{15}H_{28}N_2O_6$ $[M+H]^+$: 333.2020 found: 333.2030.

(S)-tert-butyl 3-((tert-butoxycarbonyl)amino)-4-oxobutanoate



2.14c

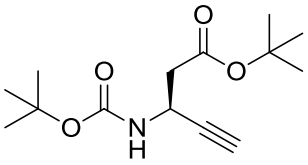
Lithium aluminium hydride (5 eq, 0.16 g) was added to a solution of Weinreb amide **2.18c** (1 eq, 0.22 g) in THF (6.6 mL) at 0 ^{o}C. After stirring for 2 hours, the reaction was quenched via the addition of an aqueous potassium hydrogen carbonate solution (0.82 M, 10 mL). The solution was diluted with ethyl acetate (30 mL) and washed with 1 M hydrochloric acid (3 x 10 mL), saturated sodium bicarbonate solution (3 x 10 mL) and brine (10 mL) before drying over magnesium sulfate and concentrating, giving crude aldehyde **2.14c** as a colourless oil (87 mg, ~ 48 % yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 5.61 (d, *J* = 7.5 Hz, 1H), 4.37 – 4.29 (m, 1H), 2.92 (dd, *J* = 17.0, 4.5 Hz, 1H), 2.74 (dd, *J* = 17.0, 5.0 Hz, 1H), 1.47 (s, 9H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 199.5, 170.4, 155.7, 82.0, 80.4, 56.3, 35.8, 28.3, 28.1.

ESI-MS: *m/z* = 274.5 [MH^+].

(S)-tert-Butyl 3-((tert-butoxycarbonyl)amino)pent-4-ynoate



2.4c

Aldehyde **2.14c** (1eq, 88 mg) was dissolved in methanol (5 mL) with potassium carbonate (2 eq, 89 mg). The Ohira-Bestmann reagent (**2.11**; 1.2 eq, 74 mg) was added to the reaction mixture, which was left at room temperature to stir overnight. A solution of saturated sodium hydrogen carbonate (5 mL) was added, before the methanol was evaporated by rotary evaporation. Ethyl acetate (30 mL) was added, and the organic phase was washed with brine (2 x 10 mL). Column chromatography (10 % ethyl acetate in petroleum spirits) furnished alkyne **2.4c** as a colourless oil (25 mg, 30 %).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.34 (m, 1H), 4.75 (m, 1H), 2.69-2.53 (m, 2H), 2.27 (d, *J* = 2.4 Hz, 1H), 1.46 (s, 9H), 1.44 (s, 9H).

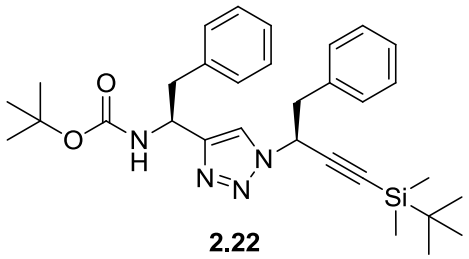
^{13}C NMR (101 MHz, CDCl3) δ 169.6, 154.7, 82.3, 81.7, 77.4, 71.2, 41.4, 39.7, 28.5, 28.2.

ESI-HRMS-TOF calcd for $C_{14}H_{23}NO_4$ $[M+H]^+$: 270.1705 found: 270.1683.

$[\alpha]^{21}_D$ = -11.1^o (c = 0.235, $CHCl_3$).

7.2.5 Chain extension sequence

tert-Butyl ((S)-1-(1-((S)-4-(tert-butyldimethylsilyl)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)carbamate



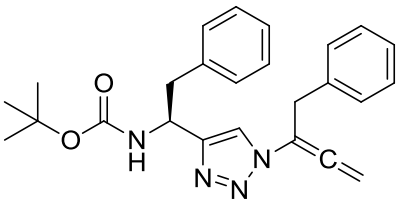
2.22

Alkyne **2.4a** (1 eq, 0.34 g) was added to azide **2.5a** (1eq, 0.38 g) in a solution of *tert*-butanol : water (2 : 1, 10 mL). Copper sulfate pentahydrate in water (0.1 eq, 1 M, 0.14 mL), ascorbate (1 eq, 27 mg) and TBTA (0.1 eq, 73 mg) were added, and the suspension was allowed to stir overnight. The suspension was diluted with water (20 mL), extracted with ethyl acetate (3 x 20 mL) and the combined organic phases were washed with brine (3 x 20 mL), before drying over magnesium sulfate and concentrating. Column chromatography (10 % ethyl acetate in petroleum spirits) was used to isolate dimer **2.22** as a colourless oil (0.44 g, 60 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.31 – 7.00 (m, 10H), 5.66 (t, *J* = 5.9 Hz, 1H), 5.42 (d, *J* = 7.8 Hz, 1H), 5.09 (dd, *J* = 14.6, 7.2 Hz, 1H), 3.42 – 3.11 (m, 4H), 1.48 (s, 9H), 0.93 (s, 9H), 0.14 (d, *J* = 1.0 Hz, 6H).

ESI-MS: *m/z* = 532 $[MH^+]$.

(S)-tert-Butyl (2-phenyl-1-(1-(1-phenylbuta-2,3-dien-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)carbamate



2.24

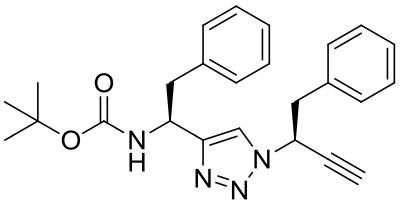
To dimer **3.26** (1 eq, 50 mg) in THF (0.87 mL) was added a solution of TBAF in THF (4 eq, 1 M, 0.35 mL). After stirring for one hour water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (2 x 10 mL). The combined organic phases were dried using magnesium sulfate and concentrated. Column chromatography gave allene **2.24** as a colourless oil (23.7 mg, 65 %).

^{1}H NMR (400 MHz, CDCl3) δ 7.31 – 7.15 (m, 9H), 7.05 (m, 2H), 5.42 (t, *J* = 2.7 Hz, 2H), 5.02 (t, *J* = 6.3 Hz, 1H), 4.10 (d, *J* = 2.4 Hz, 2H), 3.43 – 3.04 (m, 2H), 1.40 (s, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 201.9, 155.3, 137.4, 136.8, 129.7, 129.2, 128.6, 128.4, 127.1, 126.6, 120.5, 111.1, 87.1, 79.8, 48.7, 41.8, 36.2, 28.5, 17.9.

ESI-MS: *m/z* = 417.3 [MH^+].

tert-Butyl ((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)carbamate



2.23

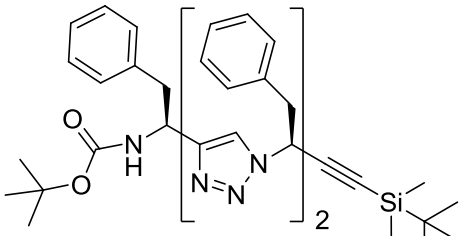
To dimer **2.22** (1 eq, 0.18 g) in THF (7 mL) was added a solution of TBAF in THF (4 eq, 1 M, 1.37 mL) and acetic acid (4 eq, 79 µL) in THF (7 mL). After stirring for one hour the solution was diluted with ethyl acetate (20 mL) and water (20 mL), and the aqueous phase was extracted with further ethyl acetate (20 mL). The combined organic phases were washed with brine (2 x 20 mL), dried using magnesium sulfate and concentrated. Column chromatography (20 % ethyl acetate in petroleum spirits) gave alkyne **2.23** as a white solid (0.11 g, 79 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.01 (m, 11H), 5.63 (m, 1H), 5.37 (br s, 1H), 5.08 (dd, *J* = 15.2, 7.3 Hz, 1H), 3.44 – 3.09 (m, 4H), 2.63 (d, *J* = 2.4 Hz, 1H), 1.45 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 147.4, 137.4, 134.6, 129.7, 129.6, 128.6, 128.4, 127.6, 126.6, 120.7, 78.4, 76.6, 53.8, 48.8, 43.0, 41.7, 28.4.

ESI-MS: *m/z* = 439.3 [MNa^+].

tert-Butyl ((S)-1-(1-((S)-1-(1-((S)-4-(tert-butyldimethylsilyl)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)carbamate



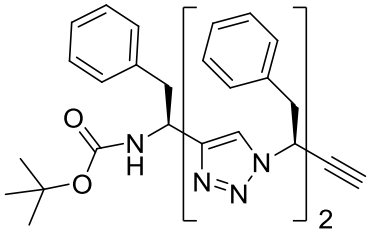
2.25

Alkyne **2.23** (1 eq, 0.10 g) was added to azide **2.5a** (1 eq, 70 mg) in a solution of *tert*-butanol : water (2 : 1, 3 mL). Copper sulfate pentahydrate in water (0.1 eq, 1 M, 25 µL), ascorbate (1 eq, 48 mg) and TBTA (0.1 eq, 13 mg) were added, and the suspension was allowed to stir overnight. The solution was diluted with ethyl acetate (20 mL) and brine (20 mL), and the aqueous phase was extracted with ethyl acetate (20 mL). Magnesium sulfate was added to the organic phase, which was subsequently concentrated and purified with column chromatography (25 % ethyl acetate in petroleum spirits), giving trimer **2.25** as a colourless oil (0.12 g, 68 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.26 – 6.85 (m, 16H), 5.94 (m, 1H), 5.66 (m, 1H), 5.32 (d, *J* = 8.3 Hz, 1H), 5.03 (app q, *J* = 6.5 Hz, 1H), 3.60 (d, *J* = 7.6 Hz, 2H), 3.40 – 2.99 (m, 4H), 1.40 (s, 9H), 0.89 (s, 9H), 0.11 (s, 6H).

ESI-MS: *m/z* = 703 [MH^+].

tert-Butyl ((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)carbamate



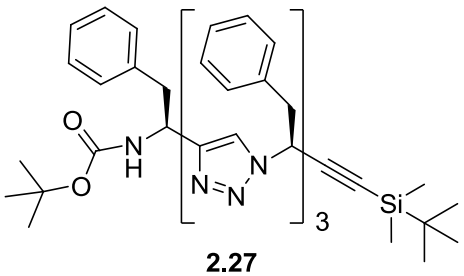
2.26

To trimer **2.25** (1 eq, 0.10 g) in THF (10 mL) was added a solution of TBAF in THF (4 eq, 1 M, 0.58 mL) and acetic acid (4 eq, 33 µL) in THF (1 mL). After stirring for one hour the solution was diluted with ethyl acetate (20 mL) and water (20 mL), and the aqueous phase was extracted with further ethyl acetate (20 mL). The combined organic phases were washed with brine (2 x 20 mL), dried using magnesium sulfate and concentrated. Column chromatography (33 % ethyl acetate in petroleum spirits) gave alkyne **2.26** as a white solid (59 mg, 84 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.24 – 6.87 (m, 16H), 5.87 (t, *J* = 7.7 Hz, 1H), 5.61 (ddd, *J* = 6.9, 5.9, 2.5 Hz, 1H), 5.28 (br s, 1H), 4.99 (app q, *J* = 6.6 Hz, 1H), 3.55 (d, *J* = 7.8 Hz, 2H), 3.36 – 2.98 (m, 4H), 2.64 (d, *J* = 2.4 Hz, 1H), 1.38 (s, 9H).

ESI-MS: *m/z* = 588.8 [MH^+].

tert-Butyl ((S)-1-(1-((S)-1-(1-((S)-1-(1-((S)-4-(tert-butyldimethylsilyl)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)carbamate



2.27

Alkyne **2.26** (1 eq, 56 mg) was added to azide **2.5a** (3.5 eq, 94 mg) in a solution of *tert*-butanol : water (2 : 1, 1 mL).. Copper sulfate pentahydrate in water (0.1 eq, 1 M, 10 µL), ascorbate (1 eq, 19 mg) and TBTA (0.1 eq, 5 mg) were added, and the suspension was allowed to stir overnight. The solution was diluted with ethyl acetate (20 mL) and water (1 mL), and the aqueous phase was extracted with ethyl acetate (20 mL), before being washed with brine (2 x 20 mL). Magnesium sulfate was added to the

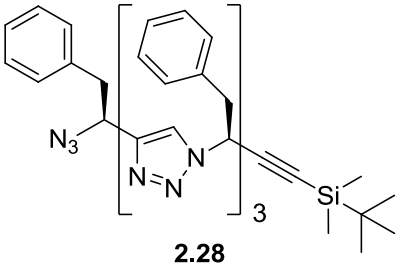
organic phase, which was subsequently concentrated and purified with column chromatography (33 % ethyl acetate in petroleum spirits), giving tetramer **2.27** as a colourless oil (52 mg, 63 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.44 (s, 1H), 7.25 – 6.87 (m, 21H), 5.99 – 5.82 (m, 2H), 5.71 – 5.61 (m, 1H), 5.27 (br s, 1H), 4.98 (app q, *J* = 6.0 Hz, 1H), 3.57 (d, *J* = 6.4 Hz, 4H), 3.42 – 3.13 (m, 3H), 3.12 – 2.96 (m, 1H), 1.40 (s, 9H), 0.89 (s, 9H), 0.11 (s, 6H).

ESI-MS: *m/z* = 874.4 [MH^+].

7.2.6 Steps toward head-to-tail cyclisation of Phe-derived tetramer

4-((S)-1-Azido-2-phenylethyl)-1-((S)-1-(1-((S)-1-(1-((S)-4-(tert-butyldimethylsilyl)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazole

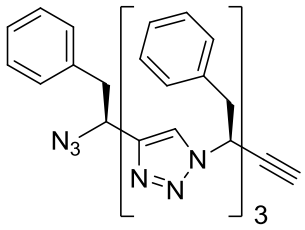


2.28

Tetramer **2.27** (1 eq, 16 mg) was stirred in a solution of TFA in DCM (30 %, 3 mL) solution for one hour, after which the solution was evaporated. The residue was dissolved in methanol (1 mL) and potassium carbonate (6.3, eq, 6.4 mg), as well as copper sulfate pentahydrate in water (0.1 eq, 1 M, 5 µL), were added. After allowing to stir for 5 minutes, azide **2.12** (2 eq, 7.6 mg) was added, and the reaction was allowed to stir overnight. The solution was then diluted with ethyl acetate (20 mL), washed with 1 M hydrochloric acid (3 x 10 mL), saturated sodium bicarbonate solution (3 x 10 mL) and brine (10 mL). The organic phase was dried with magnesium sulfate and concentrated, after which column chromatography (33 % ethyl acetate in petroleum spirits) was used to isolate azide **2.28** as a colourless oil (6.6 mg, 47 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.43 (s, 1H), 7.31 – 7.10 (m, 16H), 7.01 – 6.90 (m, 5H), 6.02 – 5.92 (m, 2H), 5.67 (dd, *J* = 7.3, 5.0 Hz, 1H), 4.83 (dd, *J* = 8.6, 5.6 Hz, 1H), 3.62 – 3.53 (m, 4H), 3.40 – 3.18 (m, 3H), 3.09 (dd, *J* = 13.9, 8.7 Hz, 1H), 0.89 (s, *J* = 7.2 Hz, 9H), 0.11 (s, *J* = 2.3 Hz, 6H).

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



2.29

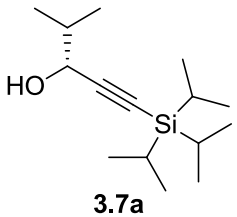
To tetramer **2.28** (1 eq, 6.6 mg) in THF (0.8 mL) was added a solution of TBAF in THF (4 eq, 1 M, 33 µL) and acetic acid (4 eq, 2 µL) in THF (0.8 mL). After stirring for one hour the solution was diluted with ethyl acetate (10 mL) and water (10 mL), and the aqueous phase was extracted with further ethyl acetate (10 mL). The combined organic phases washed with brine (1 x 20 mL), dried using magnesium sulfate and concentrated, giving alkyne **2.29** as a crude white solid (5.3 mg, ~ 94 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.48 (s, 1H), 7.42 (s, 1H), 7.30 – 6.88 (m, 20H), 5.99 – 5.89 (m, 2H), 5.68 – 5.60 (m, 1H), 4.82 (dd, *J* = 8.4, 5.8 Hz, 1H), 3.67 – 3.47 (m, 4H), 3.41 – 3.18 (m, 3H), 3.08 (dd, *J* = 13.9, 8.7 Hz, 1H), 2.65 (d, *J* = 2.4 Hz, 1H).

7.3 Synthesis of the Compounds Described in Chapter 3

7.3.1 Investigation of other methods for alkynyl-azide synthesis

(R)-4-Methyl-1-(triisopropylsilyl)pent-1-yn-3-ol



3.7a

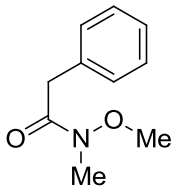
To zinc triflate (1.1 eq, 0.28 g) was added *N*-methylephedrine (1.2 eq, 0.15 g), toluene (0.7 mL) and triethylamine (1.2 eq, 0.12 mL). The solution was stirred for two hours when TBS-acetylene (1.2 eq, 0.16 mL) was added, and isopropaldehyde (1 eq, 0.063 mL) was added after 15 minutes, and the solution was warmed to 60 ^{o}C. After stirring for a further 16 hours, saturated ammonium chloride (10 mL) and diethyl ether (20 mL) were added, and the aqueous phase was further extracted with diethyl ether (2 x 10 mL). The combined organic phases were dried over magnesium chloride and

concentrated. Column chromatography (2.5 % ethyl acetate in petroleum spirits) gave alcohol **3.7a** as a colourless oil (104 mg, 33 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.14 (app t, *J* = 5.7 Hz, 1H), 1.85 (m, 1H), 1.78 (d, *J* = 6.0 Hz, 1H), 1.01 (t, *J* = 6.6 Hz, 6H), 0.96 (s, 9H), 0.12 (s, 1H).

ESI-MS: m/z = 255.2 [MH^+].

N-Methoxy-N-methyl-2-phenylacetamide



3.14

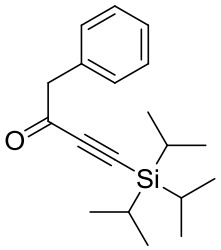
To phenylacetic acid (1 eq, 1 g) in DCM (8 mL) was added DIEA (1.2 eq, 1.54 mL), HOBt (1.1 eq, 1.09 g) and HBTU (1 eq, 2.78 g) and the solution was left to stir for 60 minutes, before N,O-dimethylhydroxylamine hydrochloride (1.2 eq, 0.86 g) was added, and the solution was stirred for a further 2 hours. After diluting with ethyl acetate (30 mL), the combined organic phases were washed with a 1 M hydrochloric acid solution (2 x 10 mL), a saturated sodium hydrogen carbonate solution (2 x 10 mL) and brine (10 mL). After drying over magnesium sulfate the organic phases were concentrated. The crude product was purified using column chromatography (25 % ethyl acetate in petroleum spirits) giving amide **3.14** as a colourless liquid (0.77 g, 65 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.23 (m, 5H), 3.79 (s, 2H), 3.61 (s, 3H), 3.20 (s, 3H).

ESI-MS: *m/z* = 179.3 [MH^+].

The spectral data are consistent with those reported in the literature.[158]

1-Phenyl-4-(triisopropylsilyl)but-3-yn-2-one



3.15

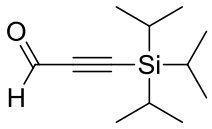
To TIPS acetylene (1 eq, 0.96 mL) in THF (4.3 mL) at -78 ^{o}C was added *n*-butyllithium (1 eq, 1.6 M, 6.9 mL) dropwise. After stirring for 30 minutes the solution was transferred via cannula to Weinreb amide **3.14** (1 eq, 0.77 g) in THF (4.3 mL) at -78 ^{o}C. After stirring for one hour the solution was warmed to room temperature, diluted with diethyl ether (30 mL) and the combined organic phases were washed with 1 M hydrochloric acid (2 x 10 mL) and brine (10 mL). Column chromatography (1.5 % ethyl acetate in petroleum spirits) was used to give ketone **3.15** as a colourless oil (0.12 g, 9.3 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.52 (m, 5H), 3.83 (s, 2H), 1.03 (m, 21H).

ESI-MS: *m/z* = 300.2 [MH^+].

7.3.2 Test Ellman sulfinamide reactions

3-(Triisopropylsilyl)propiolaldehyde



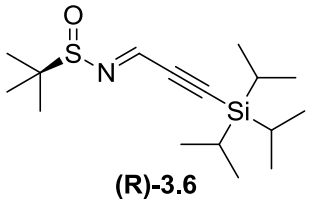
3.5

To TIPS-acetylene (2.30 mL, 1 eq) in diethyl ether (9.2 mL) at 0 ^{o}C was added *n*-butyllithium (1.41 M, 8.17 mL, 1.11 eq) dropwise. After stirring for 1 hour at 0 ^{o}C the solution was cannulated into a solution of DMF (2.4 mL, 3 eq) and diethyl ether (9.2 mL) at -78 ^{o}C over 30 minutes. After stirring at -78 ^{o}C for one hour the reaction was warmed to 0 ^{o}C and stirred for 1 hour. The reaction was quenched by pouring into an aqueous 5 % sulfuric acid solution (23 mL) at 0 ^{o}C. After stirring for 1 hour the aqueous layer was extracted with diethyl ether (3 x 20 mL) and dried with magnesium sulfate. The solution was concentrated by rotary evaporation at 10 ^{o}C, resulting in an amber liquid (1.2 g, ~ 97 %). No further purification was performed on the crude material.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H), 1.12 (m, 21H).

The spectral data are consistent with those reported in the literature.[110]

(R)-2-Methyl-N-(3-(triisopropylsilyl)prop-2-ynylidene)propane-2-sulfinamide



(R)-3.6

To (R)-(+)-2-methyl-2-propanesulfinamide (1 eq, 0.3 g) in DCM (4.1 mL) was added PPTS (0.05 eq, 31 mg), magnesium sulfate (5 eq, 1.5 g) and freshly prepared crude aldehyde **3.5** (1.5 eq, 0.78 g) in DCM (4.1 mL). After stirring for 16 hours the solution was filtered through celite with DCM, and concentrated. Column chromatography (1.25 – 2.5 % ethyl acetate in petroleum spirits) gave sulfinylimine **(R)-3.6** as a yellow oil (0.46 g, 59 %).

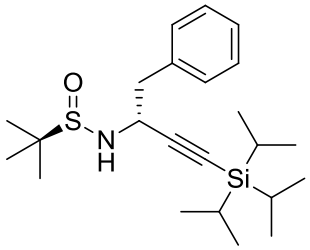
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 1.25 (s, 9H), 1.12 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.9, 105.6, 101.9, 58.5, 22.7, 18.7, 11.2.

ESI-MS: *m/z* = 314.3 [MH^+].

$[\alpha]^{20}_{D}$ = -166.5^{o} (c = 1.0, CHCl3).

(R)-2-Methyl-N-((R)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)propane-2-sulfinamide



(R,R)-3.17

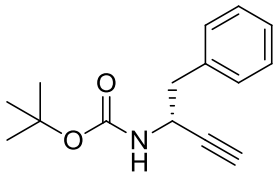
To **(R)-3.6** (0.10 g, 1 eq) in DCM (4.6 mL) at -78 ^{o}C was added $BF_3.OEt_2$ (2 eq, 81 µL). After stirring for 5 minutes, benzylmagnesium chloride (2 eq, 2 M, 0.33 mL) was added in one portion. After further stirring for 10 minutes, saturated ammonium chloride (2.3 mL) was added, and the solution was warmed to room temperature. The solution was diluted with DCM (10 mL) and water (2.3 mL), and the

aqueous phase was extracted with DCM (3 x 10 mL). The combined organic extracts were dried over magnesium sulfate and concentrated. Purification of the crude material was performed with column chromatography (20 % ethyl acetate in petroleum spirits) giving **(R,R)-3.17** as a colourless oil (58.0 mg, 45 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.17 (m, 5H), 4.33 (app q, *J* = 6.8 Hz, 1H), 3.35 (d, *J* = 5.6 Hz, 1H), 3.11 (dd, *J* = 13.6, 6.4 Hz, 1H), 3.01 (dd, *J* = 13.2, 7.2 Hz, 1H), 1.20 – 1.16 (s, 9H), 1.06 – 1.03 (m, 21H).

ESI-MS: *m/z* = 406.3 [MH^+].

(R)-tert-Butyl (1-phenylbut-3-yn-2-yl)carbamate



(R)-2.4a

To **(R,R)-3.17** (1 eq, 49.2 mg) in THF (1.2 mL) was added a solution of TBAF in THF (2 eq, 1 M, 0.24 mL). After stirring for 1.5 hours, water (2 mL) was added, and the solution was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were dried over magnesium sulfate and concentrated. The crude alkyne (~ 1 eq) was dissolved in diethyl ether (4.8 mL) and a solution of hydrogen chloride in diethyl ether (4 eq, 2 M, 0.24 mL) was added. After stirring for 30 minutes a white solid that had precipitated was filtered and rinsed with diethyl ether. The solid (1 eq, 9 mg) was suspended in THF (1 mL), and after the addition of Boc carbamate (1.1 eq, 13 µL) the suspension was cooled to 0 ^oC, before triethylamine (2.1 eq, 14 µL) was added, and the solution was stirred for 16 hours at room temperature. Water (2 mL) was added, and the solution was extracted with diethyl ether (3 x 10 mL). After drying over magnesium sulfate, the combined organic phases were concentrated. Column chromatography (2.5 to 5 % ethyl acetate in petroleum spirits) gave **(R)-2.4a** as a white solid (2.3 mg, 7.7 % over 3 steps).

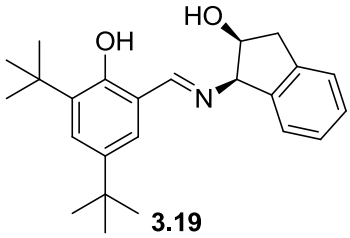
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 5H), 4.71 (m, 2H), 3.05 – 2.90 (m, 2H), 2.29 (s, 1H), 1.43 (s, 9H).

ESI-MS: *m/z* = 246.3 [MH^+].

$[\alpha]^{21}_{D}$ = +8.0^{o} (c = 0.10, $CHCl_3$).

7.3.3 Ellman sulfinamide approach to amino alkynes

(2R)-1-((E)-3,5-di-tert-Butyl-2-hydroxybenzylideneamino)-2,3-dihydro-1H-inden-2-ol



3.19

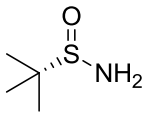
Compound 3.19 was synthesised following the procedure of Ellman:[115] (1R, 2S)-(+)-*cis*-1-amino-2-indanol (430 mg, 1.05 eq) was added in one portion to 3,5-di-*tert*-butylsalicylaldehyde (640 mg, 1 eq) in ethanol (0.16 M, 17 mL) and stirred for two hours. The solvent was evaporated, and DCM (10 mL) was added and evaporated three times. The yellow residue was dried under vacuum, giving **3.19** in quantitative yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.35 – 7.20 (m, 4H), 7.18 (d, *J* = 2.4 Hz, 1H), 4.82 (d, *J* = 5.3 Hz, 1H), 4.70 (app q, *J* = 5.3 Hz, 1H), 3.27 (dd, *J* = 15.9, 5.8 Hz, 1H), 3.18 – 3.10 (m, 1H), 1.43 (s, 9H), 1.33 (s, 9H).

ESI-MS: *m/z* = 366.3 [MH^+].

The spectral data are consistent with those reported in the literature.[114]

(S)-2-Methylpropane-2-sulfinamide



(S)-3.2

Ligand **3.19** (0.005 eq, 0.48 g) and vanadyl acetylacetonate (0.005 eq, 0.34 g) were stirred in acetone (65 mL) for 30 minutes before di-*tert*-butyl disulfide (1 eq, 49.7 mL) was added. The solution was cooled to 0 ^{o}C, and aqueous hydrogen peroxide (1.1 eq, 30 %, 32 mL) was added over 20 hours at 0 ^{o}C via syringe pump. The reaction was quenched via the addition of a saturated aqueous solution of sodium thiosulfate (15 mL) dropwise over 30 minutes. The aqueous solution was extracted with

hexanes (3 x 75 mL) and the combined organic phases were washed with brine (15 mL), dried over magnesium sulfate and concentrated, giving crude disulfide **3.23** as a brown oil (47 g, ~ 94 %).

Lithium pieces (4.19 g, 2.5 eq) were added over 45 minutes to a suspension of iron(III) nitrate nonahydrate (242 mg, 0.00248 eq) in liquid ammonia (~ 700 mL) at -78 ^{o}C under argon. The reaction flask was periodically raised out of the dry ice/acetone bath to allow lithium to react, which occurred at ~ -45 ^{o}C as shown by the initially blue colour going grey over time. After the final addition the reaction was left to stir for 30 minutes, before cooling back down to -78 ^{o}C. Freshly prepared disulfide **3.23** (47 g, 1 eq) was added in THF (17 mL) via an addition funnel. The argon gas was replaced with nitrogen, and the reaction was allowed to warm to room temperature overnight. After cooling to 0 ^{o}C, ice (130 g, 30 eq) was added and the mixture stirred until mostly homogeneous. Chloroacetic acid (25.1 g, 1.1 eq) was added in 5 portions over 20 minutes. The solution was allowed to stir for 16 hours. The solution was extracted with DCM (6 x 200 mL), dried with sodium sulfate, filtered through celite with DCM and concentrated. Hexanes (50 mL) was added and evaporated, followed by more hexanes (15 mL) and evaporated. The crude mixture was trituated with hexanes (70 mL) for 30 minutes with stirring. The crude solid material was filtered and recrystallised with hot hexanes, followed by cooling to RT with fast stirring. The white crystals which formed were washed with cold hexanes, giving **(S)-3.2** as white crystals (14.8 g, 50.5 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 2H), 1.19 (s, 9H).

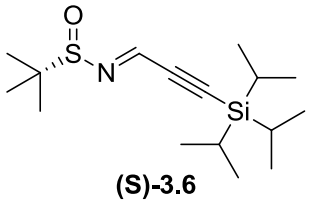
^{13}C NMR (101 MHz, $CDCl_3$) δ 55.4, 22.2.

ESI-MS: *m/z* = 122.1 [MH^+].

$[\alpha]^{20}_{D}$ = -5.5^{o} (c = 1.0, $CHCl_3$).

Note: Literature for this compound gives the optical rotation for the (S)-sulfinamide as +5.5^{o},[106] however the earlier literature gives the optical roation for the (R)-sulfinamide as +5.5.[116] As **(R)-3.2** purchased from Sigma-Adrich used in section 6.3.2 was found to have an optical rotation of +5.5, we believe that the earlier literature reflects the true value of the optical rotation. The other spectral data are consistent with those reported in the literature.[106]

(S)-2-Methyl-N-(3-(triisopropylsilyl)prop-2-ynylidene)propane-2-sulfinamide



(S)-3.6

Sulfinamide **(S)-3.2** (6.5 g, 1 eq), PPTS (0.05 eq, 0.67 g) and magnesium sulfate (5 eq, 32.3 g) were suspended in DCM (90 mL). Crude aldehyde **3.5** (~1 eq, 11.3 g) in DCM (90 mL) was added and the reaction was left to stir overnight. The suspension was then filtered through celite with DCM and concentrated. Purification of the crude material was performed by column chromatography (1.25 % to 5 % ethyl acetate in petroleum spirits), giving sulfinylimine **(S)-3.6** as a yellow oil (12.66 g, 75.3 %).

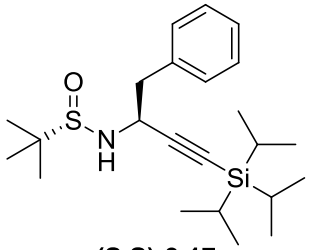
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 1.24 (s, 9H), 1.11 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.9, 105.5, 101.9, 58.4, 22.7, 18.6, 11.2.

ESI-HRMS-TOF calcd for $C_{16}H_{31}NOSSi$ $[M+H]^+$: 314.1968 found: 314.1966.

$[\alpha]^{20}_D$ = +169.1^o (c = 1.0, $CHCl_3$).

(S)-2-Methyl-N-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)propane-2-sulfinamide



(S,S)-3.17

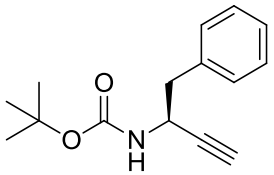
To sulfinylimine **(S)-3.6** (3.19 g, 1 eq) in DCM (143 mL) at -78 ^{o}C was added $BF_3.OEt_2$ (2 eq, 2.5 mL). After stirring for 10 minutes, benzylmagnesium chloride (1 M, 21.3 mL, 2.1 eq) was added via addition funnel. After stirring for 30 minutes, saturated ammonium chloride (25 mL) was added, followed by water (50 mL) and the aqueous phase was extracted with DCM (3 x 150 mL). The combined organic extracts were dried over magnesium sulfate and concentrated. Purification of the crude material was performed with column chromatography (ethyl acetate : petroleum spirits 1 : 4) giving 3.3 g of the major diastereomer **(S,S)-3.17** as a yellow oil (73 %) as well as 0.76 g of the minor diasteromer **(S,R)-3.17** as a yellow oil (18 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.20 (m, 5H), 4.33 (ddd, *J* = 7.3, 6.4, 5.7 Hz, 1H), 3.34 (d, *J* = 5.7 Hz, 1H), 3.10 (dd, *J* = 13.4, 6.4 Hz, 1H), 3.01 (dd, *J* = 13.4, 7.3 Hz, 1H), 1.18 (s, 9H), 1.06 – 1.03 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 136.8, 130.0, 128.4, 127.0, 106.5, 87.1, 77.5, 77.2, 76.8, 56.4, 49.6, 43.7, 22.6, 18.69, 18.68, 11.3.

ESI-HRMS-TOF calcd for $C_{23}H_{39}NOSSi$ $[M+H]^+$: 406.2494 found: 406.2596.

(S)-tert-Butyl (1-phenylbut-3-yn-2-yl)carbamate

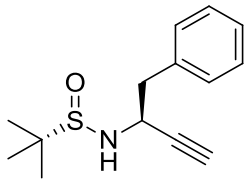


(S)-2.4a

To **(S,S)-3.17** (1 eq, 0.76 g) in methanol (0.93 mL) was added a solution of hydrogen chloride in methanol (2 eq, 4 M, 0.93 mL), and the solution was left to stir for 45 minutes, after which the off-white solid which had precipitated from the reaction was filtered with diethyl ether. To a proportion of the solid (1 eq, 0.1 g) in methanol (2.96 mL) was added diisopropylethylamine (2 eq, 0.1 mL) and Boc carbonate (1.1 eq, 0.075 mL). After stirring for 1 hour at 40 ^{o}C the solution was concentrated, ethyl acetate (20 mL) added and the organic phase was washed with a solution of 1M hydrochloric acid (2 x 10 mL), sodium hydrogen carbonate (2 x 10 mL) and brine (10 mL). After drying over magnesium sulfate the solution was concentrated. The crude material (~1 eq) was dissolved in THF (2.96 mL), and a solution of TBAF in THF (4 eq, 1 M, 1.2 mL). After stirring for 30 minutes water (5 mL) was added, and the solution was extracted with ethyl acetate (3 x 20 mL), before drying over magnesium sulfate and concentrated. Column chromatography (10 % ethyl acetate in petroleum spirits) gave **(S)-2.4a** as a white solid (68.1 mg, 70 % over 3 steps).

The spectral data are consistent with those reported for the identical compound synthesised in section 6.2.1.

(S)-2-Methyl-N-((S)-1-phenylbut-3-yn-2-yl)propane-2-sulfinamide



3.24

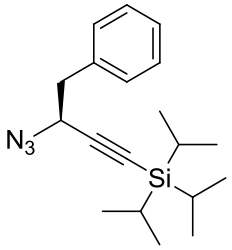
To sulfinylimine **(S,S)-3.17** (0.436 g, 1 eq) in THF (1 M, 1 mL) was added TBAF (4.3 mL, 1 M, 4 eq) and the solution stirred for 35 minutes. Water (5 mL) was added and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried over magnesium sulfate and concentrated. Column chromatography was used to purify the crude material using 33 % ethyl acetate in petroleum spirits, giving alkyne **3.24** as a colourless oil (0.25 g, 94.3 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.22 (m, 5H), 4.27 (app qd, *J* = 6.8, 2.3 Hz, 1H), 3.39 (d, *J* = 7.1 Hz, 1H), 3.07 – 3.02 (m, 2H), 2.46 (d, *J* = 2.3 Hz, 1H), 1.15 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 136.4, 130.0, 128.5, 127.1, 83.2, 74.2, 56.4, 49.0, 43.2, 22.6.

ESI-HRMS-TOF calcd for $C_{14}H_{19}NOS$ $[2M + H]^+$: 499.2447 found: 499.2466.

(S)-(3-Azido-4-phenylbut-1-ynyl)triisopropylsilane



3.25

To sulfinylimine **(S,S)-3.17** (5.5 g, 1 eq) in methanol (1.0 M, 13.6 mL) was added a solution of hydrogen chloride in diethyl ether (2 M, 13.6 mL, 2 eq), and the reaction was allowed to stir for one hour at room temperature. The solution was evaporated to dryness, and the remaining solid was filtered using cold petroleum spirits giving the free amine as an orange-white solid (3.87 g, 84.3 %).

A solution of triflic azide was concurrently prepared as follows: to sodium azide (2.23 g, 3 eq) in water (7 mL) was added toluene (7 mL) and cooled to 0 ^{o}C, prior to the dropwise addition of triflic anhydride (2.89 mL, 1.5 eq). After stirring at 0 ^{o}C for 30 minutes, the reaction was allowed to warm to room temperature and react for a further 2 hours. An aqueous saturated sodium hydrogen chloride solution

(1 mL) was added and the aqueous phase was further extracted with toluene (2 x 7 mL) giving a solution of triflic azide in toluene.

The amine prepared earlier (3.87 g, 1 eq) was dissolved in a solution of water (12.0 mL) and methanol (82.0 mL), and sodium hydrogen carbonate (3.85 g, 4 eq), copper sulfate pentahydrate (0.14 g, 0.05 eq) and the freshly prepared solution of triflic azide in toluene (~1.5 eq, ~1.4 M, 21 mL) were added. After stirring for 17 hours, the solvents were evaporated by rotary evaporation, water (10 mL) was added, and the aqueous solution was extracted with ethyl acetate (2 x 30 mL). After drying over magnesium sulfate the solution was concentrated. The crude material was purified via column chromatography (2.5% ethyl acetate in petroleum spirits) giving a colourless oil (2.65 g, 60.0 % over 2 steps).

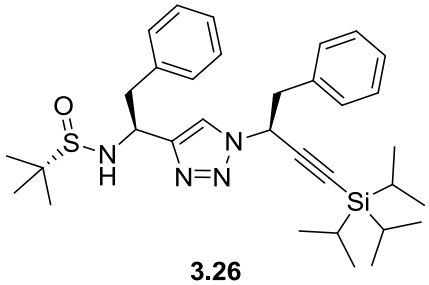
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.21 (m, 5H), 4.30 (t, *J* = 6.9 Hz, 1H), 2.97 (d, *J* = 6.9 Hz, 2H), 1.05 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 129.8, 128.5, 127.2, 101.7, 89.7, 55.0, 41.9, 18.6, 11.2.

ESI-HRMS-TOF calcd for $C_{19}H_{29}N_3Si$ $[(M - N_2) + H]^+$: 300.2142 found: 300.2134.

7.3.4 Chain extension sequence

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.26

To alkyne **3.24** (0.59 g, 1 eq) in DCM (16 mL) was added azide **3.25** (0.86 g, 1.1 eq) in methanol (8 mL), followed by copper sulfate pentahydrate (60 mg, 0.1 eq), ascorbic acid (0.47 g, 1 eq) and TBTA (63 mg, 0.1 eq). After stirring for 1 hour, no starting material was seen by TLC (33% ethyl acetate in petroleum spirits). The solvents were evaporated, water (20 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 40 mL). After drying over magnesium sulfate the solution was concentrated. The crude material was purified via column chromatography with a

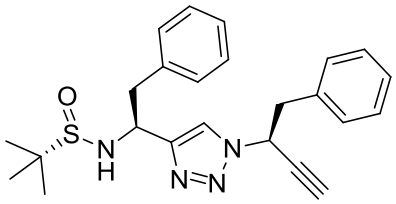
gradient of 25% ethyl acetate in petroleum spirits to 33 % ethyl acetate in petroleum spirits, giving dimer **3.26** as a colourless oil (1.30 g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.21 (m, 6H), 7.13 – 7.04 (m, 4H), 5.66 (dd, *J* = 7.9, 5.3 Hz, 1H), 4.77 (app q, *J* = 7.0 Hz, 1H), 3.89 (d, *J* = 6.8 Hz, 1H), 3.38 (dd, *J* = 13.4, 5.3 Hz, 1H), 3.30 – 3.23 (m, 3H), 1.13 (s, 9H), 1.01 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.2, 137.5, 134.9, 129.81, 129.79, 128.5, 128.4, 127.5, 126.7, 121.3, 101.0, 90.7, 56.3, 54.9, 54.0, 43.5, 42.8, 22.6, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{33}H_{48}N_4OSSi$ $[M+H]^+$: 577.3391 found: 577.3412.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.27

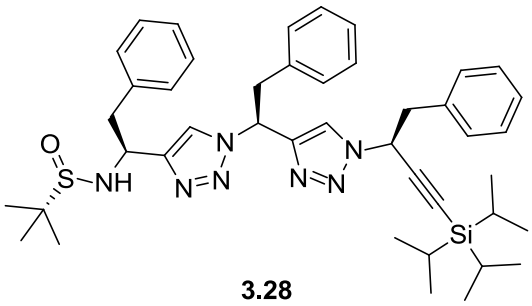
To dimer **3.26** (1 eq, 1.21 g) in THF (0.2 M, 10 mL) was added TBAF (8.5 mL, 1 M, 4 eq) buffered with acetic acid (0.24 mL, 2 eq) in THF (0.2 M, 10 mL). The reaction was stirred for 40 minutes, after which TLC showed complete consumption of starting material (50% ethyl acetate in petroleum spirits). Water (20 mL) was added and the aqueous phase was extracted with ethyl acetate (3 x 30 mL). The combined organic phases were dried over magnesium sulfate and concentrated. Column chromatography was used to purify the crude material using 50% ethyl acetate in petroleum spirits, giving alkyne **3.27** as an amorphous solid (0.76 g, 84.4 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.30 – 7.24 (m, 5H), 7.24 – 7.18 (m, 1H), 7.17 – 7.13 (m, 2H), 7.11 – 7.06 (m, 2H), 5.61 (ddd, *J* = 8.0, 5.8, 2.4 Hz, 1H), 4.76 (app dt, *J* = 8.0, 6.3 Hz, 1H), 3.93 (d, *J* = 8.1 Hz, 1H), 3.43 – 3.19 (m, 4H), 2.62 (d, *J* = 2.4 Hz, 1H), 1.09 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.9, 137.5, 134.7, 129.8, 129.7, 128.6, 128.5, 127.6, 126.8, 121.9 (2 x C's), 78.3, 76.8, 56.6, 54.9, 54.0, 43.0, 42.5, 22.5.

ESI-HRMS-TOF calcd for $C_{24}H_{28}N_4OS$ $[M+H]^+$: 421.2057 found: 421.2072.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.28

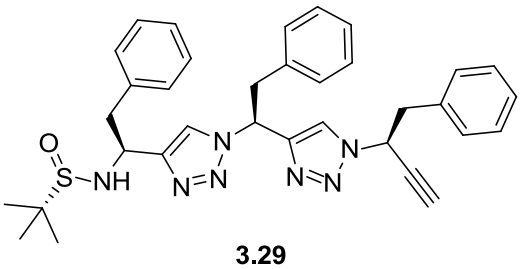
To alkyne **3.27** (0.75 g, 1 eq) in DCM (12 mL) was added azide **3.25** (0.64 g, 1.1 eq) in methanol (6 mL), followed by copper sulfate pentahydrate (45 mg, 0.1 eq), ascorbic acid (0.35 g, 1 eq) and TBTA (95 mg, 0.1 eq). After stirring for 1 hour, no starting material was seen by TLC (33 % ethyl acetate in petroleum spirits). The solvents were evaporated, water (20 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 40 mL). After drying over magnesium sulfate the solution was concentrated. The crude material was purified via column chromatography (33 % to 60 % ethyl acetate in petroleum spirits), giving trimer **3.28** as a colourless oil (0.87 g, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.47 (s, 1H), 7.25 – 7.15 (m, 9H), 7.07 (m, 2H), 7.04 – 6.98 (m, 4H), 5.95 (dd, *J* = 8.3, 7.2 Hz, 1H), 5.68 (dd, *J* = 7.7, 5.0 Hz, 1H), 4.74 (dd, *J* = 14.2, 7.1 Hz, 1H), 3.77 (d, *J* = 7.2 Hz, 1H), 3.68 – 3.53 (m, 2H), 3.38 (dd, *J* = 13.4, 5.0 Hz, 1H), 3.32 – 3.16 (m, 3H), 1.09 (s, 9H), 1.02 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 144.7, 137.5, 136.1, 134.6, 129.8, 129.7, 129.2, 128.7, 128.50, 128.45, 127.6, 127.2, 126.7, 122.1 (2 x C's), 100.7, 91.3, 59.2, 56.3, 55.0, 54.3, 43.3, 42.7, 41.9, 22.6, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{43}H_{57}N_7OSSi$ $[M+H]^+$: 748.4187 found: 748.4207.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.29

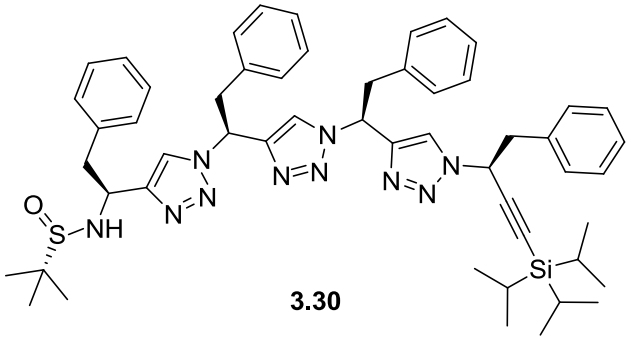
To trimer **3.28** (0.80 g, 1 eq) in THF (0.1 M, 11 mL) was added TBAF (4.3 mL, 1 M, 4 eq) buffered with acetic acid (0.12 mL, 2 eq) in THF (0.1 M, 11 mL). The reaction was stirred at room temperature for 30 minutes, after which TLC showed complete consumption of starting material (50% ethyl acetate in petroleum spirits). Water (10 mL) was added and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried over magnesium sulfate and concentrated. Column chromatography was used to purify the crude material using 50 % ethyl acetate in petroleum spirits, giving alkyne **3.29** as a white solid (0.55 g, 87 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.36 (s, 1H), 7.26 – 7.17 (m, 9H), 7.09 – 6.98 (m, 6H), 5.92 (dd, *J* = 8.8, 6.6 Hz, 1H), 5.63 (ddd, *J* = 7.5, 5.7, 2.4 Hz, 1H), 4.72 (app q, *J* = 7.1 Hz, 1H), 3.78 (d, *J* = 7.2 Hz, 1H), 3.66 – 3.53 (m, 2H), 3.42 – 3.29 (m, 2H), 3.29 – 3.16 (m, 2H), 2.63 (d, *J* = 2.4 Hz, 1H), 1.10 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6, 145.3, 137.5, 136.1, 134.4, 129.8, 129.7, 129.2, 128.8, 128.7, 128.5, 127.8, 127.3, 126.8, 122.4, 122.1, 78.1, 77.2, 59.6, 56.3, 54.3, 54.2, 43.0, 42.7, 42.0, 22.6.

ESI-HRMS-TOF calcd for $C_{34}H_{37}N_7OS$ $[M+H]^+$: 592.2853 found: 592.2877.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.30

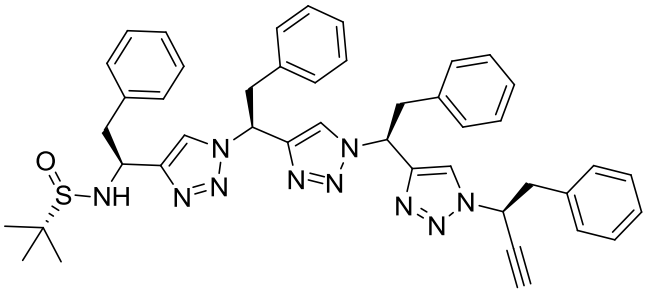
To alkyne **3.29** (0.55 g, 1 eq) in DCM (9.4 mL) was added azide **3.25** (0.38 g, 1.25 eq) in methanol (3.2 mL), followed by copper sulfate pentahydrate (23 mg, 0.1 eq), ascorbic acid (0.19 g, 1 eq) and TBTA (50 mg, 0.1 eq). After stirring for 1 hour, no starting material was seen by TLC (50 % ethyl acetate in petroleum spirits). The solvents were evaporated, water (10 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 25 mL). After drying over magnesium sulfate the solution was concentrated. The crude material was purified via column chromatography (40 % to 50 % ethyl acetate in petroleum spirits), giving tetramer **3.30** as a white foam (0.70 g, 80.1%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.46 (s, 1H), 7.38 (s, 1H), 7.24 – 7.10 (m, 12H), 7.06 (m, 2H), 7.03 – 6.92 (m, 6H), 5.99 – 5.90 (m, 2H), 5.70 (dd, *J* = 7.6, 4.9 Hz, 1H), 4.71 (app q, *J* = 7.1 Hz, 1H), 3.75 (d, *J* = 7.3 Hz, 1H), 3.69 – 3.50 (m, 4H), 3.37 (dd, *J* = 13.4, 4.9 Hz, 1H), 3.32 – 3.14 (m, 3H), 1.08 (s, 9H), 1.04 – 1.01 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6, 145.3, 144.1, 137.6, 136.2, 135.9, 134.5, 129.8, 129.7, 129.2, 129.1, 128.74, 128.73, 128.50, 128.46, 127.7, 127.3, 127.2, 126.7, 122.3 (2 x C's), 122.2, 100.6, 91.5, 59.5, 59.4, 56.3, 55.1, 54.4, 43.4, 42.7, 42.0, 41.8, 22.6, 18.7, 11.2.

ESI-HRMS-TOF calcd for $C_{53}H_{66}N_{10}OSSi$ $[M+H]^+$: 919.4984 found: 919.4990.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.31

To tetramer **3.30** (0.54 g, 1 eq) in THF (0.1 M, 5.9 mL) was added TBAF (2.4 mL, 1 M, 4 eq) buffered with acetic acid (0.07 mL, 2 eq) in THF (0.1 M, 5.9 mL). The reaction was stirred at RT for 30 minutes, after which TLC showed complete consumption of starting material (50 % ethyl acetate in petroleum spirits). Water (10 mL) was added and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried over magnesium sulfate and concentrated. Column

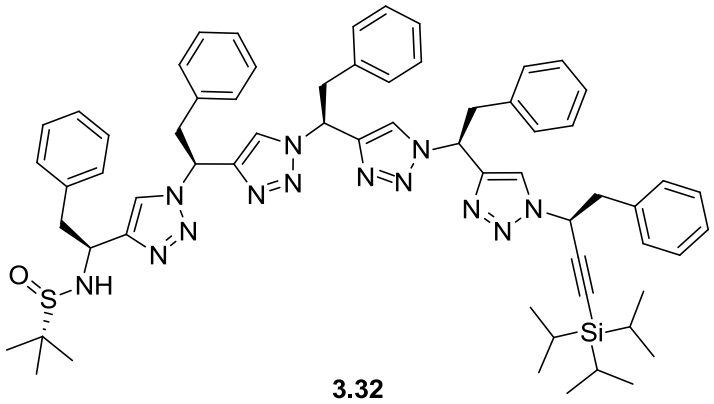
chromatography (60 % ethyl acetate in petroleum spirits) gave alkyne **3.31** as a white solid (0.42 g, 93 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.44 (s, 1H), 7.37 (s, 1H), 7.26 – 7.11 (m, 12H), 7.08 – 6.91 (m, 8H), 5.93 (app dt, *J* = 14.1, 7.0 Hz, 2H), 5.64 (ddd, *J* = 8.0, 5.7, 2.4 Hz, 1H), 4.71 (app q, *J* = 7.2 Hz, 1H), 3.79 (d, *J* = 7.4 Hz, 1H), 3.68 – 3.50 (m, 4H), 3.35 (m, 2H), 3.28 – 3.14 (m, 2H), 2.66 (d, *J* = 2.4 Hz, 1H), 1.09 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 145.2, 144.6, 137.5, 136.1, 135.9, 134.3, 129.8, 129.6, 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 127.8, 127.4, 127.2, 126.8, 122.5, 122.4, 122.1, 78.1, 77.4, 59.6, 59.4, 56.3, 54.4, 54.2, 43.0, 42.7, 42.0, 41.7, 22.6.

ESI-HRMS-TOF calcd for $C_{44}H_{46}N_{10}OS$ $[M+H]^+$: 763.3650 found: 763.3666.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.32

To alkyne **3.31** (0.37 g, 1 eq) in DCM (3.2 mL) was added azide **3.25** (0.20 g, 1.25 eq) in methanol (1.6 mL), followed by copper sulfate pentahydrate (12 mg, 0.1 eq), ascorbic acid (96 mg, 1 eq) and TBTA (26 mg, 0.1 eq). After stirring for 1 hour, no starting material was seen by TLC (50 % ethyl acetate in petroleum spirits). The solvents were evaporated, water (10 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 20 mL). After drying over magnesium sulfate the solution was concentrated. The crude material was purified via column chromatography (50% ethyl acetate in petroleum spirits), giving pentamer **3.32** as a white solid (0.38 g, 73 %).

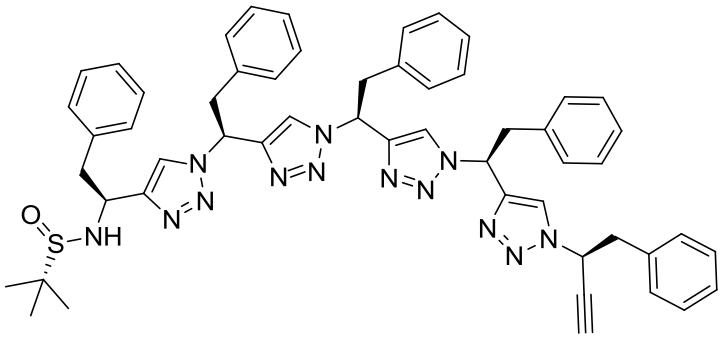
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 7.38 (s, 1H), 7.21 – 7.09 (m, 15H), 7.06 (m, 2H), 7.00 – 6.89 (m, 8H), 5.99 – 5.88 (m, 3H), 5.71 (dd, *J* = 7.5, 4.9 Hz, 1H), 4.71 (app

q, *J* = 7.2 Hz, 1H), 3.78 (d, *J* = 7.4 Hz, 1H), 3.67 – 3.49 (m, 6H), 3.37 (dd, *J* = 13.4, 4.9 Hz, 1H), 3.31 – 3.14 (m, 3H), 1.09 (s, 9H), 1.04 – 1.00 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 145.2, 144.7, 144.0, 137.6, 136.2, 135.9, 135.8, 134.4, 129.8, 129.7, 129.2, 129.08, 129.05, 128.8, 128.7, 128.7, 128.48, 128.45, 127.7, 127.4, 127.3, 127.2, 126.7, 122.5, 122.3, 122.1, 100.6, 91.6, 77.5, 77.2, 76.8, 59.5, 59.4, 59.3, 56.3, 55.1, 54.4, 43.4, 42.7, 42.1, 41.8, 41.7, 22.6, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{63}H_{75}N_{13}OSSi$ $[M+H]^+$: 1090.5780 found: 1090.5808.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.33

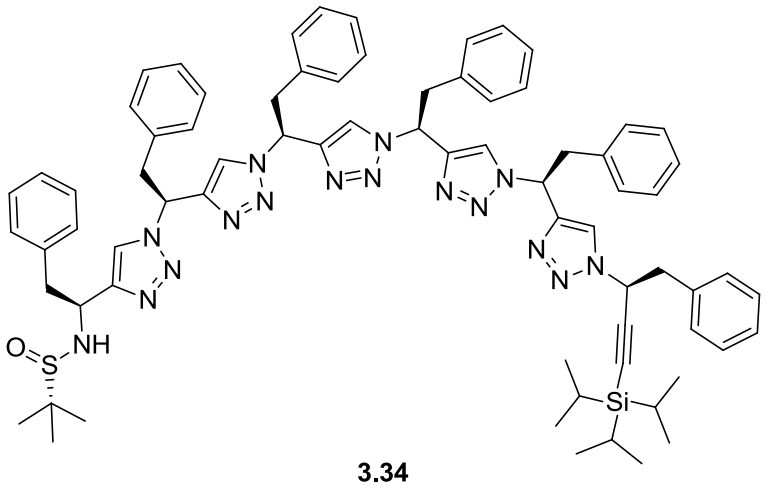
To pentamer **3.32** (0.28 g, 1 eq) in THF (0.1 M, 2.6 mL) was added TBAF (1.0 mL, 1 M, 4 eq) buffered with acetic acid (0.03 mL, 2 eq) in THF (0.1 M, 2.6 mL). The reaction was stirred at RT for 30 minutes, after which TLC showed complete consumption of starting material (50% ethyl acetate in petroleum spirits). Water (10 mL) was added, which caused the product to crash out of the solution. After filtration of the solid, recrystallisation from ethanol gave alkyne **3.33** as a while solid (0.21 g, 87 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.49 (s, 1H), 7.45 (s, 1H), 7.38 (s, 1H), 7.22 – 7.09 (m, 15H), 7.06 (m, 2H), 7.01 – 6.88 (m, 8H), 5.92 (m, 3H), 5.64 (ddd, *J* = 7.2, 5.7, 2.4 Hz, 1H), 4.70 (app q, *J* = 7.3 Hz, 1H), 3.80 (d, *J* = 7.4 Hz, 1H), 3.64 – 3.49 (m, 6H), 3.41 – 3.29 (m, 2H), 3.21 (m, 2H), 2.69 (d, *J* = 2.4 Hz, 1H), 1.08 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 145.3, 144.6, 144.5, 137.5, 136.1, 135.9, 135.8, 134.3, 129.8, 129.6, 129.2, 129.12, 129.07, 128.78, 128.76, 128.7, 128.6, 128.5, 127.8, 127.4, 127.3, 127.2, 126.8, 122.8, 122.4, 122.3, 122.1, 78.0, 77.5, 59.6, 59.4, 59.4, 56.3, 54.5, 54.2, 43.0, 42.7, 42.2, 41.75, 41.72, 22.6.

ESI-HRMS-TOF calcd for $C_{54}H_{55}N_{13}OS$ $[M+H]^+$: 934.4446 found: 934.4401.

(S)-2-Methyl-N-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-2-sulfinamide



3.34

To pentamer **3.33** (0.16 g, 1 eq) in DCM (2.1 mL) was added azide **3.25** (0.07 g, 1.25 eq) in methanol (1.1 mL), followed by copper sulfate pentahydrate (4.2 mg, 0.1 eq), ascorbic acid (33 mg, 1 eq) and TBTA (8.9 mg, 0.1 eq). After stirring for 2 hours, water (10 mL) was added, and the organic solvents were removed via rotary evaporation. The residual precipitate was filtered and recrystallised from ethanol, giving hexamer **3.34** as a green-white solid (0.15 g, 71 %).

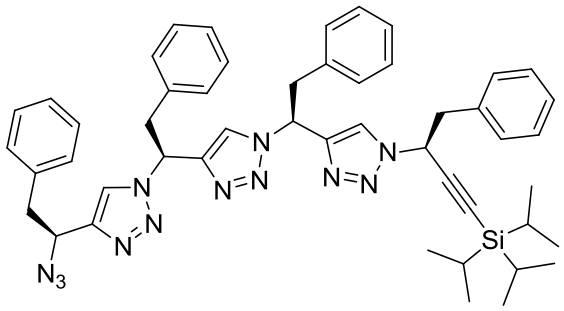
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.48 (s, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.39 (s, 1H), 7.22 – 7.04 (m, 20H), 6.96 (m, 4H), 6.91 (m, 6H), 5.95 (m, 4H), 5.72 (dd, *J* = 7.5, 4.9 Hz, 1H), 4.72 (app q, *J* = 7.2 Hz, 1H), 3.85 (d, *J* = 7.4 Hz, 1H), 3.64 – 3.45 (m, 8H), 3.37 (dd, *J* = 13.4, 4.8 Hz, 1H), 3.25 (m, 3H), 1.08 (s, 9H), 1.05 – 1.01 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6, 145.1, 144.53, 144.51, 143.8, 137.5, 136.1, 135.9, 135.8, 135.7, 134.4, 129.8, 129.7, 129.2, 129.13, 129.06, 129.0, 128.70, 128.66, 128.44, 128.42, 128.3, 127.6, 127.33, 127.30, 127.25, 127.2, 126.7, 125.4, 122.6, 122.5, 122.3, 122.3, 122.0, 100.5, 91.5, 59.41, 59.38, 59.4, 59.3, 59.2, 56.3, 55.0, 54.4, 43.3, 42.6, 42.0, 41.9, 41.7, 41.6, 22.6, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{73}H_{84}N_{16}OSSi$ $[M+H]^+$: 1261.6577 found: 1261.6515.

7.3.5 Cyclisation reactions

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



3.35

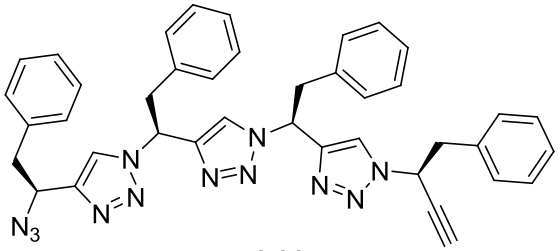
To tetramer **3.30** (0.117 g, 1 eq) in methanol (0.5 M, 0.26 mL) was added a solution of hydrogen chloride in diethyl ether (2 eq, 2 M, 0.13 mL). The reaction was left to stir for 45 minutes, and a solid precipitated. Diethyl ether (5 mL) was added and the solid was filtered, washing with further diethyl ether, giving a white solid. The solid (91 mg, 1 eq) was suspended in a solution of in water (1.14 mL) and methanol (7.62 mL), sodium hydrogen carbonate (36.0 mg, 4 eq), a solution of freshly prepared triflic azide in toluene (0.08 M, 1.93 mL, 1.5 eq) and copper sulfate pentahydrate (1.3 mg, 0.05 eq) were added. After stirring for 16 hours the organic solvents were evaporated via the rotary evaporator, water (5 mL) was added and the combined aqueous phases were extracted with ethyl acetate (3 x 20 mL). After drying with magnesium sulfate the solution was concentrated, and the crude material was purified by column chromatography (25 % ethyl acetate in petroleum spirits) to give azide **3.35** as a white solid (71.6 g, 79.6 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.48 (s, 1H), 7.44 (s, 1H), 7.31 – 7.10 (m, 14H), 7.00 – 6.91 (m, 6H), 6.02 – 5.94 (m, 2H), 5.72 (dd, *J* = 7.5, 4.9 Hz, 1H), 4.83 (dd, *J* = 8.6, 5.6 Hz, 1H), 3.66 – 3.50 (m, 4H), 3.37 (dd, *J* = 13.4, 4.9 Hz, 1H), 3.27 (m, 2H), 3.09 (dd, *J* = 14.0, 8.7 Hz, 1H), 1.05 – 1.02 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.2, 144.7, 143.9, 137.0, 135.9, 135.7, 134.4, 129.7, 129.5, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 127.7, 127.4, 127.3, 127.1, 122.6, 122.3, 121.4, 100.5, 91.6, 59.3, 59.2, 55.1, 43.3, 42.1, 42.0, 40.8, 21.6, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{49}H_{56}N_{12}Si$ $[M+H]^+$: 841.4593 found: 841.4597.

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



3.36

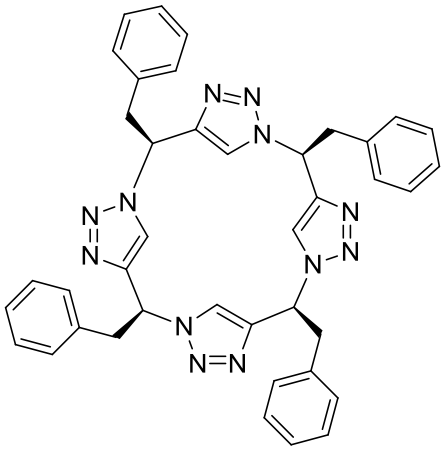
To tetramer **3.35** (1 eq, 48 mg) in THF (0.07 M, 0.8 mL) was added a solution of TBAF (0.23 mL, 4 eq) buffered with acetic acid (6.5 uL, 2 eq) in THF (0.07 M, 0.8 mL). After stirring for 2 hours water (7 mL) was added, which caused a white solid to precipitate out of the solution. The solid was filtered and washed with water, and dried under high-vacuum, giving azido-alkyne **3.36** as a white solid (29.3 mg, 75 %). The compound was stored at 0-4 ^{o}C as a dilute solution in DCM (0.5 mM).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.47 (s, 1H), 7.42 (s, 1H), 7.31 – 7.21 (m, 4H), 7.21 – 7.12 (m, 10H), 7.03 – 6.90 (m, 6H), 5.95 (m, 2H), 5.65 (ddd, *J* = 8.0, 5.6, 2.4 Hz, 1H), 4.82 (dd, *J* = 8.6, 5.6 Hz, 1H), 3.67 – 3.50 (m, 4H), 3.43 – 3.23 (m, 3H), 3.09 (dd, *J* = 14.0, 8.7 Hz, 1H), 2.65 (d, *J* = 2.5 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.3, 144.7, 144.4, 137.1, 136.0, 135.8, 134.3, 129.7, 129.5, 129.13, 129.08, 128.8, 128.7, 128.6, 127.8 (2 x C's), 127.44, 127.36, 127.1, 122.7, 122.3, 121.3, 78.0, 77.4, 59.7, 59.3, 59.2, 54.2, 43.0, 42.1, 42.0, 40.9.

ESI-HRMS-TOF calcd for $C_{40}H_{36}N_{12}$ $[M+H]^+$: 685.3259 found: 685.3287.

tetra-((S)-2-Phenyl-1H-1,2,3-triazol-4-ylethane)



3.37

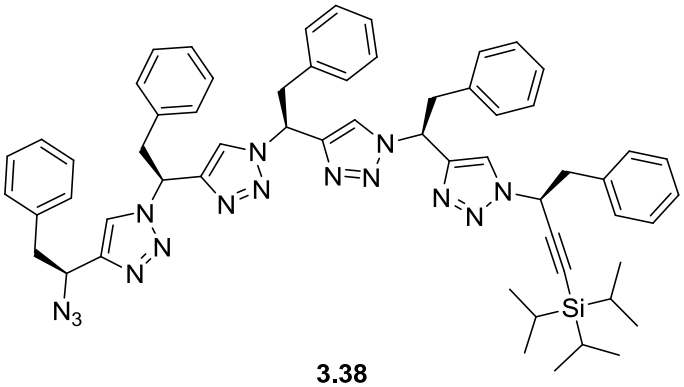
To a solution of azido-alkyne **3.36** in DCM (5 mL, 0.5 mM, 1.7 mg, 1 eq) in a pressure vial was added TBTA as a solution in DCM (33.3 µL, 3.75 mM, 0.05 eq) and tetrakis(acetonitrile)copper(I) tetrafluoroborate as a solution in acetonitrile (33.3 µL, 3.75 mM, 0.05 eq). The reaction was heated to 55-60 ^{o}C and left to stir for 5.5 days. The reaction was cooled to RT, diluted with DCM (5 mL) then filtered through cotton wool with additional DCM. After concentration of the solution under reduced pressure, the crude material was purified using column chromatography, with a series of solvents (20 mL x 100 % DCM, 20 mL x 0.625 % MeOH in DCM, 2 x 10 mL x 1.25 % MeOH in DCM, 2 x 10 mL x 2.5 % MeOH in DCM), giving a white solid (0.1 ± 0.03 mg, 6 %).

^{1}H NMR (400 MHz, DMSO) δ 8.08 (s, 4H), 7.32 – 7.13 (m, 20H), 6.14 (app t, *J* = 8.1 Hz, 4H), 4.03 (s, 4H), 3.74 (dd, *J* = 13.9, 9.0 Hz, 1H), 3.66 (dd, *J* = 13.9, 7.3 Hz, 1H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (m, 12H), 7.19 – 7.11 (m, 8H), 6.87 (s, 4H), 5.93 (app t, *J* = 7.7 Hz, 4H), 3.88 (dd, *J* = 14.1, 8.6 Hz, 4H), 3.80 (dd, *J* = 14.1, 7.0 Hz, 4H).

^{13}C NMR (400 MHz, DMSO) δ 147.6, 136.4, 129.4, 128.9, 126.8, 121.6, 58.5, 38.9.

ESI-HRMS-TOF calcd for $C_{40}H_{36}N_{12}$ $[M+H]^{+}$: 685.3259 found: 685.3254.

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



3.38

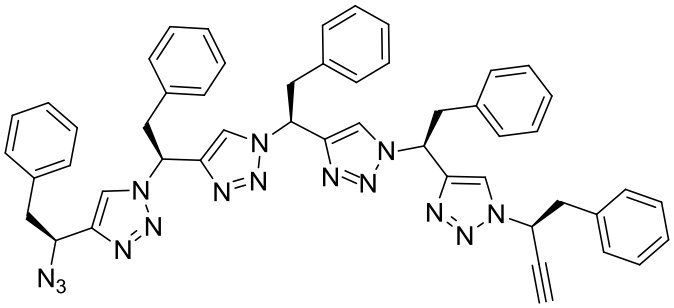
To a suspension of pentamer **3.32** (50 mg, 1 eq) in diethyl ether (0.01 M, 4.6 mL) was added a solution of hydrogen chloride in diethyl ether (0.046 mL, 2 M, 2 eq) and methanol (1 mL). After stirring overnight, the appearance of the suspension had changed, with a fine white precipitate apparent. The powder was filtered and washed with diethyl ether. The solid (38.6 mg) was suspended in a solution of water (0.4 mL) and methanol (2.69 mL), and sodium hydrogen carbonate (12.7 mg, 4 eq), a solution of freshly prepared triflic azide in toluene (0.39 M, 0.68 mL, 7 eq) and copper sulfate pentahydrate (0.47 mg, 0.05 eq) were added. After 22 hours the organic solvents were evaporated via the rotary evaporator. Ethyl acetate (25 mL) was added and the organic phase was washed with water (3 x 10 mL). The organic phase was dried with magnesium sulfate the solution was concentrated, giving a white solid (37 mg, 46 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.46 (s, 2H), 7.43 (s, 1H), 7.32 – 7.21 (m, 3H), 7.21 – 7.09 (m, 14H), 6.99 – 6.89 (m, 8H), 5.99 – 5.91 (m, 3H), 5.71 (dd, *J* = 7.5, 4.8 Hz, 1H), 4.81 (dd, *J* = 8.7, 5.6 Hz, 1H), 3.68 – 3.50 (m, 6H), 3.37 (dd, *J* = 13.4, 4.8 Hz, 1H), 3.31 – 3.22 (m, 2H), 3.09 (dd, *J* = 14.0, 8.7 Hz, 1H), 1.05 – 1.00 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.2, 144.7, 144.5, 143.9, 137.1, 136.0, 135.8, 135.7, 134.4, 129.7, 129.5, 129.12, 129.06 (2 x C's), 128.80 (2 x C's), 128.76, 128.7, 128.5, 127.7, 127.4 (2 x C's), 127.3, 127.1, 122.52, 122.46, 122.4, 121.3, 100.6, 91.6, 59.5, 59.3 (2 x C's), 59.2, 55.1, 43.4, 42.1, 42.01, 41.96, 40.8, 18.7, 11.2.

ESI-HRMS-TOF calcd for $C_{59}H_{65}N_{15}Si$ $[M+H]^+$: 1012.5389 found: 1012.5370.

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



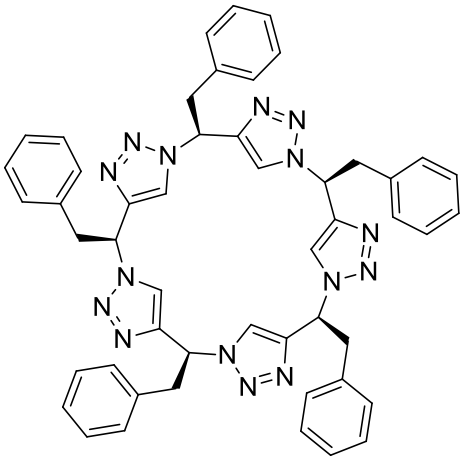
3.39

To azide **3.38** (22.6 mg, 1 eq) in THF (0.07 M, 0.32 mL) was added a solution of TBAF (0.09 mL, 4 eq) buffered with acetic acid (2 eq, 2.6 µL), in THF (0.07 M, 0.32 mL). After stirring for 2 hours water (2 mL) was added, which caused a white solid to precipitate out of the solution. The solid was filtered and washed with water, and dried under high-vacuum, giving azido-alkyne **3.39** as a white solid (13.1 mg, 69 %). The compound was stored at 0-4 ^{o}C as a dilute solution in DCM (0.5 mM).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.48 (s, 2H), 7.43 (s, 1H), 7.26 – 7.10 (m, 15H), 6.95 (m, 8H), 5.94 (m, 3H), 5.70 – 5.60 (m, 1H), 4.81 (dd, *J* = 8.7, 5.6 Hz, 1H), 3.64 – 3.49 (m, 6H), 3.31 (m, 5H), 3.09 (dd, *J* = 13.9, 8.7 Hz, 1H), 2.67 (d, *J* = 2.4 Hz, 1H).

ESI-HRMS-TOF calcd for $C_{50}H_{45}N_{15}$ $[M+H]^{+}$: 856.4055 found: 856.4089.

penta-((S)-2-Phenyl-1H-1,2,3-triazol-4-ylethane)



3.40

To a solution of azido-alkyne **3.39** in DCM (5 mL, 0.5 mM, 2.1 mg, 1 eq) in a pressure vial was added TBTA as a solution in DCM (33.3 µL, 3.75 mM, 0.05 eq) and tetrakis(acetonitrile)copper(I)

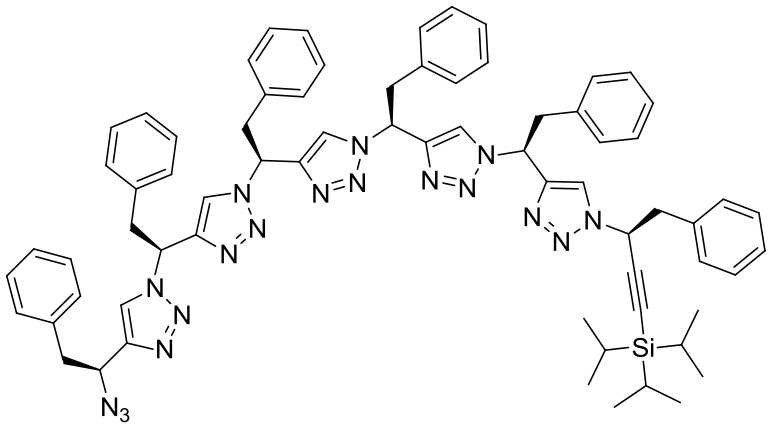
tetrafluoroborate as a solution in acetonitrile (33.3 µL, 3.75 mM, 0.05 eq). The reaction was heated to 55-60 ^{o}C and left to stir for 4 days. The reaction was cooled to RT, diluted with DCM (7 mL) then filtered through cotton wool with additional DCM. After concentration of the solution under reduced pressure, the crude material was purified using chromatography with a series of solvents: (20 mL x 100 % DCM, 20 mL x 0.625 % MeOH in DCM, 2 x 10 mL x 1.25 % MeOH in DCM, 2 x 10 mL x 2.5 % MeOH in DCM), giving a white solid (0.07 ± 0.06 mg, 3 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 5H), 7.23 – 7.15 (m, 15H), 7.03 – 6.97 (m, 10H), 5.91 (t, *J* = 7.9 Hz, 5H), 3.65 (d, *J* = 7.9 Hz, 10H).

^{13}C NMR (400 MHz, $CDCl_3$) δ 144.1, 135.2, 128.9, 128.7, 127.4, 121.8, 59.3, 41.8.

ESI-HRMS-TOF calcd for $C_{50}H_{45}N_{15}$ $[M+H]^+$: 856.4055 found: 856.4083.

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



3.41

To a suspension of hexamer **3.34** (37.3 mg, 1 eq) in diethyl ether (0.01 M, 3.0 mL) was added a solution of hydrogen chloride in diethyl ether (0.030 mL, 2 M, 2 eq) and methanol (0.5 mL). After stirring overnight, the appearance of the suspension had changed, with a fine white precipitate apparent. The powder was filtered and washed with diethyl ether. A portion of the solid (22.4 mg) was suspended in a solution of water (0.19 mL) and methanol (1.27 mL), was added sodium hydrogen carbonate (6.0 mg, 4 eq), a solution of freshly prepared triflic azide in toluene (0.39 M, 0.32 mL, 7 eq) and copper sulfate pentahydrate (0.22 mg, 0.05 eq) was added. After 22 hours the organic solvents

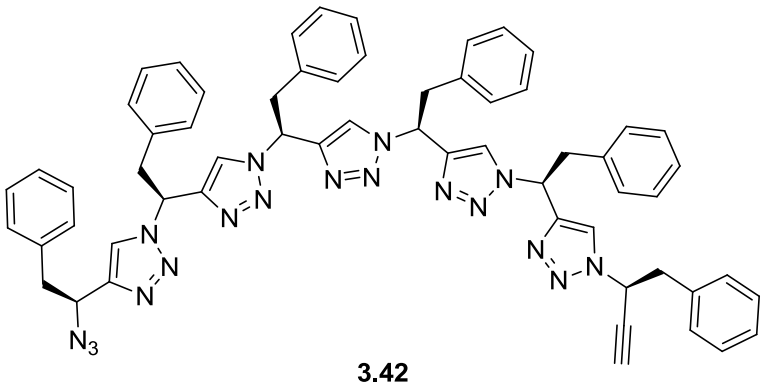
were evaporated via the rotary evaporator. Ethyl acetate (25 mL) was added and the organic phase was washed with water (3 x 10 mL). The organic phase was dried with magnesium sulfate and concentrated, giving a white solid (20 mg, 71.6 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.47 (s, 2H), 7.46 (s, 1H), 7.44 (s, 1H), 7.32 – 7.07 (m, 20H), 7.00 – 6.86 (m, 10H), 6.02 – 5.89 (m, 4H), 5.72 (dd, *J* = 7.4, 4.9 Hz, 1H), 4.83 (dd, *J* = 8.6, 5.7 Hz, 1H), 3.70 – 3.47 (m, 8H), 3.37 (dd, *J* = 13.4, 4.8 Hz, 1H), 3.27 (m, 2H), 3.10 (dd, *J* = 14.0, 8.7 Hz, 1H), 1.05 – 1.01 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.2, 144.7, 144.5, 144.4, 143.8, 137.1, 136.0, 135.76, 135.75, 135.7, 134.4, 129.7 (2 x C's), 129.5 (2 x C's), 129.1 (2 x C's), 129.0 (3 x C's), 128.8 (3 x C's), 128.7 (2 x C's), 128.6 (2 x C's), 128.5 (2 x C's), 127.7, 127.4 (3 x C's), 127.3, 127.1, 122.51, 122.50, 122.46, 122.3, 121.3, 100.5, 91.6, 59.5, 59.4, 59.27, 59.25, 59.2, 55.1, 43.3, 42.0, 41.9 (3 x C's), 40.8, 18.6, 11.1.

ESI-HRMS-TOF calcd for $C_{69}H_{74}N_{18}Si$ $[M+H]^+$: 1183.6186 found: 1183.6188.

4-((S)-1-Azido-2-phenylethyl)-1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-2-phenyl-1-(1-((S)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-1,2,3-triazole



3.42

To azide **3.41** (13.4 mg, 1 eq) in THF (0.04 M, 0.28 mL) was added a solution of TBAF (0.045 mL, 4 eq) buffered with acetic acid (1.3 uL, 2 eq), in THF (0.04 M, 0.28 mL). The suspension was stirred for 2 hours until water (2 mL) was added, and the solid was filtered and washed with water, and dried under high-vacuum, giving azido-alkyne **3.42** as crude white solid (7.6 mg, ~ 65.5 %). The 1H NMR suggested that the solid was a mixture of the azido-alkyne **3.42** and polymerised products, however no further purification was performed. The compound was stored at 0-4 ^{o}C as a dilute solution in DCM (0.5 mM).

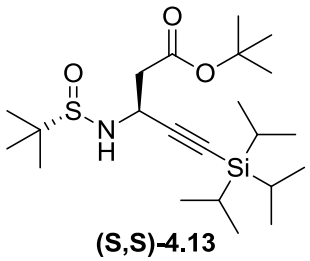
^{1}H NMR (400 MHz, DMSO) δ 8.30 (s, 2H), 8.27 (s, 1H), 8.23 (s, 1H), 8.20 (s, 1H), 7.28 – 6.98 (m, 30H), 6.26 – 6.12 (m, 4H), 5.89 (dd, *J* = 7.3, 2.3 Hz, 1H), 4.96 (app t, *J* = 7.6 Hz, 1H), 3.74 (d, *J* = 2.3 Hz, 1H), 3.54 (m, 10H), 3.16 (dd, *J* = 13.9, 6.7 Hz, 1H), 3.08 (dd, *J* = 13.7, 8.4 Hz, 1H).

ESI-HRMS-TOF calcd for $C_{60}H_{54}N_{18}$ $[M+H]^+$: 1027.4852 found: 1027.4849.

7.4 Synthesis of the Compounds Described in Chapter 4

7.4.1 Aspartic acid monomer unit synthesis

(S)-tert-Butyl 3-((S)-1,1-dimethylethylsulfinamido)-5-(triisopropylsilyl)pent-4-ynoate



(S,S)-4.13

To disopropylamine (1.1 eq, 0.025 mL) in THF (1.1 M, 0.15 mL) at -78 ^{o}C was added *n*-butyllithium (1.1 eq, 0.125 mL). After stirring for 1 hour tert-butyl acetate (1 eq, 43 µL) was added, and after stirring for a further 1 hour a solution of sulfinylimine **(S)-3.6** (1 eq, 50 mg) in THF (40 µL) was added, and the combined solutions were stirred for 30 minutes at -78 ^{o}C. The solution was then poured into a 1 M hydrochloric acid solution (2 mL) at 0 ^{o}C, extracted with diethyl ether (3 x 3 mL), and the combined organic fractions were washed with a saturated sodium hydrogen carbonate solution (2 x 3 mL) and brine (3 mL) before drying over magnesium sulfate and concentrated. Column chromatography (15 % ethyl acetate in petroleum spirits) gave **(S,S)-4.13** as a colourless oil (13.8 mg, 21 %) and **(S,R)-4.13** as a colourless oil (5.6 mg, 8.6 %).

(S,S)-4.13: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.48 – 4.42 (m, 1H), 3.91 (d, *J* = 6.2 Hz, 1H), 2.84 (dd, *J* = 16.2, 8.2 Hz, 1H), 2.70 (dd, *J* = 16.2, 6.8 Hz, 1H), 1.46 (s, 9H), 1.21 (s, 9H), 1.07 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 136.8, 130.0, 128.4, 127.0, 106.5, 87.1, 56.4, 49.6, 43.7, 22.6, 18.7, 11.3.

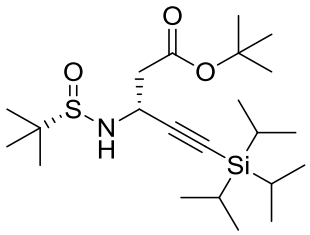
ESI-MS: *m/z* = 452.4 $[MNa^+]$.

(S,R)-4.13: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 – 4.45 (m, 1H), 4.37 (d, *J* = 6.1 Hz, 1H), 2.81 (d, *J* = 16.2, 4.8 Hz, 1H), 2.70 (dd, *J* = 16.2, 7.1 Hz, 1H), 1.46 (s, 9H), 1.23 (s, 9H), 1.07 – 1.04 (m, 21H).

^{13}C NMR (101 MHz, $CDCl^3$) δ 169.7, 105.4, 86.2, 81.8, 55.9, 45.4, 42.9, 28.2, 22.6, 18.6, 11.2.

ESI-MS: *m/z* = 452.2 [MNa^+].

(R)-tert-Butyl 3-((S)-1,1-dimethylethylsulfinamido)-5-(triisopropylsilyl)pent-4-ynoate

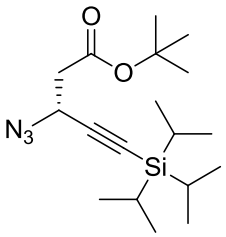


(S,R)-4.13

To zinc power (4 eq, 0.83 g) in THF (0.1 M, 32 mL) was added trimethylsilyl chloride (0.2 eq, 81 µL). After 30 minutes, *tert*-butyl bromoacetate (2 eq, 0.94 mL) was added dropwise, the solution was heated to 40 ^{o}C and stirred for 30 minutes. The reaction was then cooled to 0 ^{o}C, and sulfinylimine **(S)-3.6** (1 eq, 1.0 g) in THF (0.1 M, 32 mL) was added dropwise via cannula. The reaction was stirred at 0 ^{o}C for 30 minutes, and then at room temperature for 2 hours. A solution of ammonium chloride (20 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 40 mL). After drying over magnesium sulfate, the combined organic phases were concentrated. Column chromatography ethyl acetate in petroleum spirits (10 to 50 % ethyl acetate in petroleum spirits) giving **(S,R)-4.13** as a colourless oil (0.78 g, 57 %) and **(S,S)-4.13** as a colourless oil (0.22 g, 16 %).

The spectral data of both diastereomers are identical to those seen above.

(R)-tert-Butyl 3-azido-5-(triisopropylsilyl)pent-4-ynoate



(R)-4.10

To **(S,R)-4.13** (1 eq, 0.21 g) in diethyl ether (0.1 M, 4.9 mL) was added a solution of hydrogen chloride in diethyl ether (3 eq, 2 M, 0.73 mL) and the solution was stirred for two hours, before being

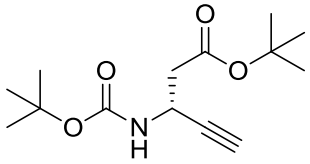
concentrated and left under vacuum overnight. The crude material was redissolved in water (1 mL) and methanol (6.8 mL), and to this solution was added sodium hydrogen carbonate (4 eq, 0.16 g), a solution of triflic azide in toluene (2.5 eq, 0.7 M, 1.75 mL) and copper sulfate pentahydrate (0.05 eq, 6.0 mg). The mixture was stirred for 16 hours, before being concentrated. Ethyl acetate (20 mL) and water (10 mL) were added, and the aqueous phase was extracted with ethyl acetate (2 x 20 mL). The combined organic fractions were dried over magnesium sulfate and concentrated. Column chromatography (1.25 % ethyl acetate in petroleum spirits) was used to isolate azide **(R)-4.10** as a colourless oil (43.6 mg, 26 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (app t, *J* = 7.2 Hz, 1H), 2.66 (dd, *J* = 13.4, 4.2 Hz, 1H), 2.61 (dd, *J* = 13.4, 5.3 Hz, 1H), 1.46 (s, 9H), 1.08 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.4, 101.2, 89.0, 81.7, 50.2, 42.0, 28.2, 18.6, 11.1.

ESI-MS: *m/z* = 352 [MH^+].

(R)-tert-Butyl 3-((tert-butoxycarbonyl)amino)pent-4-ynoate



(R)-2.4c

To **(S,R)-4.13** (1 eq, 0.34 g) in THF (8 mL) was added a solution of TBAF in THF (2 eq, 1 M, 1.6 mL). After stirring for 30 minutes, water (10 mL) was added, and the solution was extracted with ethyl acetate (3 x 20 mL). Column chromatography (20 % ethyl acetate in petroleum spirits) gave the free alkyne as a yellow oil (0.174 g).

A portion of the alkyne (1 eq, 40 mg) was dissolved in diethyl ether (0.05 M, 2.9 mL), and a solution of hydrogen chloride in diethyl ether (2 eq, 1M, 0.29 mL) was added. After stirring for one hour, the white solid which had precipitated was filtered with diethyl ether. The solid (18 mg) was redissolved in THF (0.05 M, 1.75 mL) and Boc anhydride (1.1 eq, 22 µL) was added. After cooling the solution to 0 ^{o}C, triethylamine (2.1 eq, 26 µL) was added, and the solution was stirred for 16 hours at room temperature. Water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic fractions were dried over magnesium sulfate and concentrated. The

crude material was purified by column chromatography (5 % ethyl acetate in petroleum spirits) giving **(R)-2.4c** as a white solid (13.8 mg, 28 % over 3 steps).

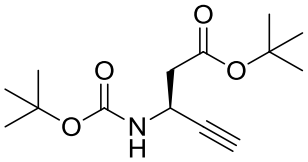
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (br s, 1H), 4.74 (m, 1H), 2.65 (dd, *J* = 15.6, 5.6 Hz, 1H), 2.59 (dd, *J* = 15.6, 6.0 Hz, 1H), 2.28 (d, *J* = 2.4 Hz, 1H), 1.47 (s, 9H), 1.45 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 154.8, 82.3, 81.7, 80.2, 71.2, 41.4, 39.6, 28.5, 28.2.

ESI-MS: *m/z* = 270 [MH^+].

$[\alpha]^{21}_D$ = +21.4^o (c = 0.5, $CHCl_3$).

(S)-tert-Butyl 3-((tert-butoxycarbonyl)amino)pent-4-ynoate



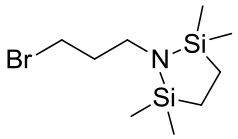
(S)-2.4c

A solution of TBAF in THF (2 eq, 1 M, 0.49 mL) was added to **(S,S)-4.7** (1 eq, 0.1 g), and after stirring for 1 hour water (2 mL) was added. The aqueous phase was extracted with ethyl acetate (3 x 10 mL), and the combined organic phases were dried over magnesium sulfate and concentrated. The crude material was redissolved in diethyl ether (2.5 mL), and a solution of hydrogen chloride in diethyl ether (2 eq, 2 M, 0.25 mL) was added. After stirring for 1 hour the solvents were evaporated, the crude material was redissolved in THF (2.5 mL) and cooled to 0 ^{o}C. Boc anhydride (1.1 eq, 61 µL) and triethylamine (2.1 eq, 71 µL) were added, and the solution was stirred at room temperature for 16 hours. Water (5 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were dried over magnesium sulfate and concentrated. Column chromatography (5 % ethyl acetate in petroleum spirits) gave alkyne **(S)-2.4c** as a white solid (7.5 mg, 11 %).

The spectral data are identical to that which was seen for the compound produced in chapter 6.2.4, with the exception of the optical rotation: $[\alpha]^{21}_D$ = -20.8^o (c = 0.25, $CHCl_3$).

7.4.2 Arginine monomer unit synthesis

1-(3-Bromopropyl)-2,2,5,5-tetramethyl-1,2,5-azadisilolidine



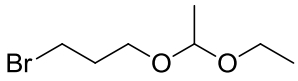
4.19

To 3-bromopropylamine hydrobromide (1 eq, 5 g) and triethylamine (3 eq, 9.5 mL) in dry DCM (0.95 M, 24 mL) was added 1,2-bis(chlorodimethylsilyl)ethane (1 eq, 4.9 g) in DCM (1.3 M, 17 mL) via cannula. After stirring for 2 hours at room temperature the reaction mixture was filtered and washed with DCM (3 x 5 mL). After drying over magnesium sulfate the solution was concentrated under reduced pressure. Petroleum spirits were added (25 mL) and the solution was refiltered and concentrated. Vacuum distillation (118 ^{o}C, 5 mbar), give **4.19** as a colourless liquid (4.4 g, 17.5 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (t, *J* = 6.6 Hz, 2H), 2.94 (t, *J* = 6.6 Hz, 2H), 1.95 (p, *J* = 6.6 Hz, 2H), 0.71 (s, 4H), 0.11 – 0.03 (m, 12H).

The spectral data are consistent with those reported in the literature.[128]

1-bromo-3-(1-ethoxyethoxy)propane



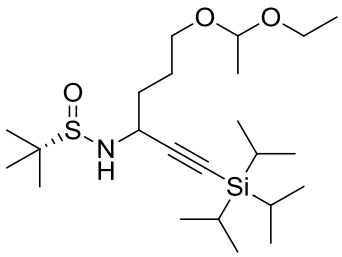
4.24

The title compound was synthesised following the procedure of Eaton:[130] after 3-bromo propanol (1 eq, 3.73 g) was neutralised with sodium carbonate, it was added to ethyl vinyl ether (1.6 eq, 3.73 mL), and dichloroacetic acid (0.017 eq, 34 µL) was added. After stirring for 1 hour, additional dichloroacetic acid was added (0.006 eq, 12 µL) and the reaction was left to stir overnight. Sodium carbonate (1.29 eq, 1.29 g) was then added, and the reaction was left to stir for a further 2.5 hours, after which diethyl ether (10 mL) was added and the sodium carbonate was filtered off. Concentration of the reaction mixture gave bromide **4.24** as a crude colourless liquid (4.80 g, ~ 93 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.69 (q, *J* = 5.3 Hz, 1H), 3.74 – 3.61 (m, 2H), 3.59 – 3.44 (m, 4H), 2.10 (app p, *J* = 6.2 Hz, 2H), 1.31 (d, *J* = 5.3 Hz, 3H), 1.21 (t, *J* = 7.1 Hz, 3H).

The spectral data are consistent with those reported in the literature.[130]

(S)-N-(6-(1-ethoxyethoxy)-1-(triisopropylsilyl)hex-1-yn-3-yl)-2-methylpropane-2-sulfinamide



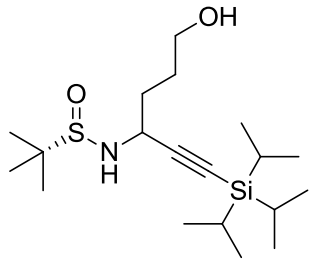
4.25

A portion of bromide **4.24** (0.67 g, ~4 eq) was added dropwise to magnesium turnings (0.078 g, 4 eq) in THF (0.8 mL) over 20 minutes. THF (4 mL) was then added, and the solution was left to react over 1 hour, after which the majority of the magnesium had been visibly consumed. The Grignard solution was then added dropwise to a solution of sulfinylimine **(S)-3.6** (0.25 g, 1 eq) in DCM (9.5 mL, 0.084 M) at -78 ^{o}C. After an hour of stirring, a solution of ammonium chloride (3 mL) was added, and the solution was allowed to return to room temperature. Water (3 mL) was added, and extraction with DCM (3 x 10 mL) was performed. After drying over magnesium sulfate the solution was concentrated under reduced pressure. The crude material was purified with column chromatography (20 % ethyl acetate in petroleum spirits) to give sulfinylamide **4.25** as a crude colourless oil (0.26 g, ~ 73 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (q, *J* = 5.3 Hz, 1H), 4.13 (app q, *J* = 5.4 Hz, 1H), 3.67 – 3.55 (m, 2H), 3.50 – 3.42 (m, 2H), 1.91 – 1.69 (m, 4H), 1.29 (d, *J* = 5.4 Hz, 3H), 1.20 (s, 9H), 1.18 (t, *J* = 7.0 Hz, 3H), 1.06 – 1.04 (m, 21H).

ESI-MS: *m/z* = 468 [MNa^{+}].

(S)-N-(6-Hydroxy-1-(triisopropylsilyl)hex-1-yn-3-yl)-2-methylpropane-2-sulfinamide



4.28

A portion of the oil (0.23 g, 1 eq) was dissolved in *n*-propanol (9.3 mL, 0.054 eq), to which PPTS (31.7 mg, 0.25 eq) was added and the reaction was allowed to stir for 16 hours. The solution was concentrated under reduced pressure, and column chromatography (100% ethyl acetate) was used to give sufinylamide **4.28** as a colourless oil (0.16 g, 63 % over 2 steps from sulfinylimine **3.6**).

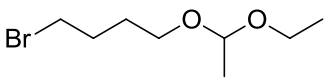
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (app q, *J* = 6.0 Hz, 1H), 3.68 (t, *J* = 5.9 Hz, 2H), 3.53 (d, *J* = 5.2 Hz, 1H), 1.98 – 1.68 (m, 4H), 1.22 (s, 9H), 1.09 – 1.04 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 107.1, 86.0, 62.3, 56.3, 47.7, 33.8, 28.9, 22.7, 18.7, 11.3.

ESI-MS: *m/z* = 374.3 [MH^+].

7.4.3 Lysine monomer unit synthesis

1-bromo-4-(1-ethoxyethoxy)butane



4.32

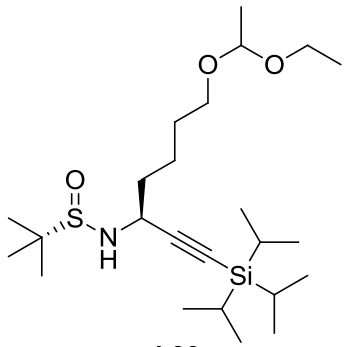
Concentrated sulfuric acid (0.5 mL, 0.14 eq) and hydrobromic acid (11.9mL, 1 eq) were added dropwise to THF (1 eq, 5.6 mL) at 0 ^{o}C. The solution was heated to reflux for 90 minutes, before being cooled back to 0 ^{o}C. After neutralizing with sodium hydrogen carbonate, water (25 mL) was added, and the aqueous solution was extracted with ethyl ether (3 x 30 mL). The combined organic fractions were washed with brine (25 mL), dried over magnesium sulfate and the solvents were evaporated, giving a slightly yellow liquid (4.75 g).

A proportion of the crude material (4.33 g, 1 eq) was added to ethyl vinyl ether (4.44 mL, 1.6 eq) which had been cooled to 0 ^{o}C. Dichloroacetic acid (0.017 eq, 40 µL) was added, the reaction was stirred for one hour, further dichloroacetic acid (0.006 eq, 14 µL) was added and the reaction was left

to stir overnight at room temperature. Sodium carbonate (0.5 eq, 1.5 g) was added and the dispersion was left to stir for 2 hours, before filtering through cotton wool and the solution was concentrated under reduced pressure, giving bromide **4.32** as a crude colourless oil (5.76 g, ~ 90 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (q, *J* = 5.3 Hz, 1H), 3.69 – 3.57 (m, 2H), 3.52 – 3.41 (m, 4H), 1.97 (app p, *J* = 7.6 Hz, 2H), 1.72 (app p, *J* = 6.7 Hz, 2H), 1.30 (d, *J* = 5.3 Hz, 3H), 1.21 (t, *J* = 7.1 Hz, 3H).

(S)-N-((3S)-7-(1-ethoxyethoxy)-1-(triisopropylsilyl)hept-1-yn-3-yl)-2-methylpropane-2-sulfinamide



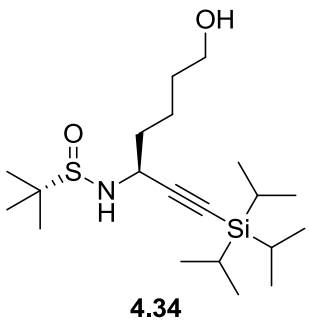
4.33

To magnesium (3 eq, 70 mg) in THF (0.24 mL) was added a portion of crude bromide **4.32** (~3 eq, 0.65 g) dropwise over 20 minutes. After complete addition, THF (1.2 mL) was added and the solution was left to stir at room temperature for 45 minutes. The Grignard solution was transferred to a solution of sulfinylimine **(S)-3.6** (1 eq, 0.3 g) in DCM (0.084 M, 11.5 mL) at -78 ^{o}C, and the combined solutions were stirred for one hour, before the reaction was quenched with a solution of ammonium chloride (5 mL). Water (5 mL) was added, and the solution was warmed to room temperature before the aqueous phase was extracted with ethyl acetate (2 x 20 mL). The combined organic phases were dried with magnesium sulfate and concentrated under reduced pressure. Column chromatography (33 % ethyl acetate in petroleum spirits) gave **4.33** as a crude colourless oil (0.12 g, ~ 28 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (q, 5.4 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.68 – 3.37 (m, 4H), 1.83 – 1.71 (m, 2H), 1.66 – 1.53 (m, 6H), 1.30 (d, *J* = 5.4 Hz, 3H), 1.22 – 1.21 (m, 3H), 1.22 (s, 9H), 1.08 – 1.06 (m, 21H).

ESI-MS: *m/z* = 482 [MNa^+].

(S)-N-((S)-7-Hydroxy-1-(triisopropylsilyl)hept-1-yn-3-yl)-2-methylpropane-2-sulfinamide



4.34

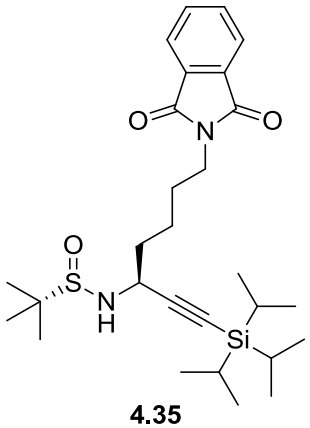
The crude mixture (~ 1 eq, 0.12 g) was dissolved in n-propanol (0.054 M, 5 mL), and PPTS (0.25 eq, 17 mg) was added. After stirring for 16 hours the solution was concentrated, and column chromatography (90 % ethyl acetate in petroleum spirits) was used to give alcohol **4.34** as a colourless oil (46 mg, 13 % over 2 steps from sulfinylimine **3.6**).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (app q, *J* = 6.8 Hz, 1H), 3.66 (t, *J* = 6.0 Hz, 2H), 3.37 (app t, *J* = 5.2 Hz, 1H), 1.90 – 1.72 (m, 2H), 1.67 – 1.55 (m, 4H), 1.23 (s, 9H), 1.10 – 1.04 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 107.2, 85.8, 62.3, 56.2, 47.9, 36.8, 32.1, 22.6, 21.8, 18.7, 11.2.

ESI-MS: *m/z* = 388.4 [MH^+].

(S)-N-((S)-7-(1,3-Dioxoisoindolin-2-yl)-1-(triisopropylsilyl)hept-1-yn-3-yl)-2-methylpropane-2-sulfinamide



4.35

To alcohol **4.34** (1 eq, 97 mg), phthalimide (2 eq, 74 mg) and triphenylphosphine (1.5 eq, 98 mg) in toluene (2.5 mL) at 0 ^{o}C was added DIAD (1.5 eq, 74 µL) dropwise over 15 minutes, after which the reaction was left to stir for 16 hours. Water (5 drops) was added, and the mixture was concentrated

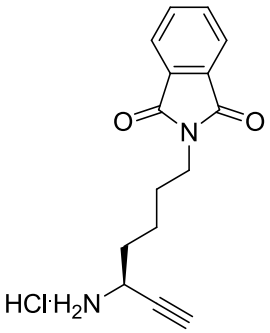
under reduced pressure. Column chromatography (33 to 50 % ethyl acetate in petroleum spirits) gave **4.35** as colourless oil (109 mg, 84 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 2H), 7.76 – 7.68 (m, 2H), 4.15 – 4.07 (app dt, *J* = 7.6, 6.8 Hz, 1H), 3.70 (t, *J* = 6.8 Hz, 2H), 3.30 (d, *J* = 5.2 Hz, 1H), 1.87 – 1.67 (m, 4H), 1.60 – 1.48 (m, 2H), 1.21 (m, 9H), 1.05 – 1.01 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 134.0, 132.2, 123.3, 107.0, 86.0, 56.2, 47.7, 37.9, 36.6, 28.2, 23.0, 22.6, 18.6, 11.2.

ESI-MS: *m/z* = 517.3 [MH^+].

(S)-2-(5-Aminohept-6-yn-1-yl)isoindoline-1,3-dione



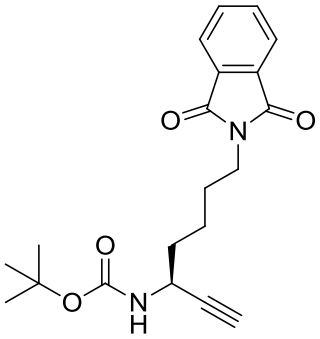
4.45

To **(S,S)-4.30** (1 eq, 50 mg) in THF (0.1 M, 1 mL) was added a solution of TBAF (4 eq) and acetic acid (2 eq, 11 µL) in THF (1.4 mL). After stirring for one hour the water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (2 x 10 mL). The combined organic fractions were dried over magnesium sulfate and concentrated. The crude material was dissolved in diethyl ether (2 mL) and a solution of hydrogen chloride in diethyl ether (2 eq, 2 M, 0.1 mL) was added, which caused precipitation of a white solid. After stirring for one hour the solid was filtered, giving alkyne **4.45** (20 mg, 72 %).

^{1}H NMR (400 MHz, MeOD) δ 7.89 – 7.84 (m, 2H), 7.84 – 7.79 (m, 2H), 4.58 (s, 1H), 4.05 (m, 1H), 3.71 (t, *J* = 7.0 Hz, 2H), 3.17 (d, *J* = 2.3 Hz, 1H), 1.93 – 1.71 (m, 4H), 1.71 – 1.47 (m, 2H).

ESI-MS: *m/z* = 257 [MH^+].

(S)-tert-Butyl (7-(1,3-dioxoisoindolin-2-yl)hept-1-yn-3-yl)carbamate



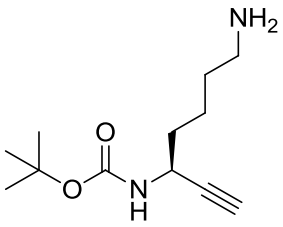
4.46

To **4.45** (20 mg) in THF (0.1 M, 0.68 mL) was added Boc anhydride (1.1 eq, 18 mg), and the solution was cooled to 0 ^{o}C before triethylamine (2.1 eq, 20 µL) was added dropwise. After the solution was stirred for 16 hours at room temperature, ethyl acetate (3 mL) and water (1 mL) were added, and the aqueous phase was extracted with a further 2 x 3 mL ethyl acetate. The combined organic phases were dried over magnesium sulfate and concentrated. Column chromatography (20 % ethyl acetate in petroleum spirits) gave **4.46** as a colourless oil (18.3 mg, 74 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.80 (m, 2H), 7.74 – 7.67 (m, 2H), 4.74 (m, 1H), 4.39 (m, 1H), 3.70 (t, *J* = 7.2 Hz, 2H), 2.25 (d, *J* = 2.3 Hz, 1H), 1.78 – 1.64 (m, 4H), 1.54 – 1.45 (m, 2H), 1.45 (s, 9H).

ESI-MS: *m/z* = 379 [MNa^{+}].

(S)-tert-Butyl (7-aminohept-1-yn-3-yl)carbamate



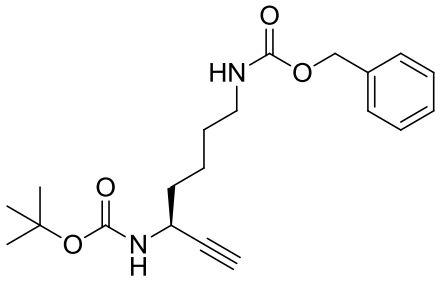
4.47

To **4.46** (1 eq, 18.3 mg) in methanol (0.02 M, 2.6 mL) was added 1-heptene (15 eq, 0.11 mL) and hydrazine monohydrate (10 eq, 25 µL) and the solution was stirred for 16 hours, before being concentrated. The solid crude material was filtered with diethyl ether and purified with column chromatography (1 % triethylamine, 20 % methanol in ethyl acetate), giving alkyne **4.47** as an orange oil (7.5 mg, 64 %).

^{1}H NMR (400 MHz, MeOD) δ 4.31 – 4.22 (m, 1H), 2.68 (m, 2H), 2.62 (d, *J* = 2.3 Hz, 1H), 1.66 (app q, *J* = 7.2 Hz, 2H), 1.55 – 1.47 (m, 4H), 1.44 (m, 9H).

ESI-MS: *m/z* = 227.2 [MH^+].

(S)-Benzyl tert-butyl hept-6-yne-1,5-diyldicarbamate

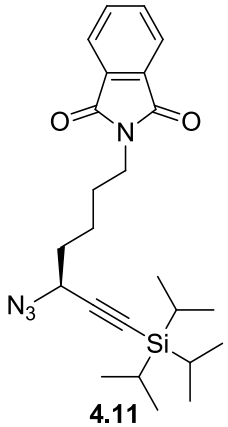


(S)-2.4b

To **4.47** (1 eq, 7.4 mg) in THF (0.1 M, 0.35 mL) was added triethylamine (2.2 eq, 10 µL) and benzyl chloroformate (1.1 eq, 5.1 µL). After stirring for 30 minutes, water (1 mL) was added and the aqueous solution was extracted with ethyl acetate (3 x 3 mL). The combined organic fractions were dried over magnesium sulfate and concentrated. The crude material was purified by column chromatography (20 % ethyl acetate in petroleum spirits) to give alkyne **(S)-2.4b** as a white solid (3.1 mg, 26 %).

The spectral data are identical to that which was seen for the compound produced in chapter 6.2.4, with the exception of the optical rotation: $[\alpha]^{21}_D = -14.3^o$ (c = 0.1, $CHCl_3$).

(S)-2-(5-Azido-7-(triisopropylsilyl)hept-6-yn-1-yl)isoindoline-1,3-dione



4.11

To sulfinylamide **4.35** (1 eq, 47.2 mg) in diethyl ether (0.9 mL) was added a solution of hydrogen chloride in diethyl ether (2 eq, 2 M, 0.09 mL) and the reaction was stirred for 1 hour, before the

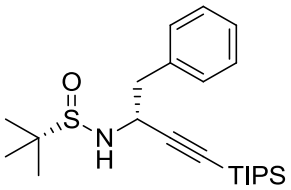
volatile components were removed under reduced pressure giving a colourless oil. Water (1.2 mL), methanol (8.3 mL), sodium hydrogen carbonate (4 eq, 30.7 mg), a solution of triflic azide in toluene (3 eq, 0.13 M, 2.1 mL) and copper sulfate pentahydrate (0.05 eq, 1.1 mg) were added in succession. The reaction was left to stir at room temperature for 18 hours. After evaporation of the organic solvents under reduced pressure, water (10 mL) was added, and the aqueous phases were extracted with ethyl acetate (3 x 40 mL). The combined organic phases were dried under magnesium sulfate, and concentrated. Column chromatography (2 to 5 % ethyl acetate in petroleum spirits gave azide **4.11** as a colourless oil (32 mg, 79.8 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.82 (m, 2H), 7.75 – 7.69 (m, 2H), 4.11 (t, *J* = 6.7 Hz, 1H), 3.70 (t, *J* = 7.1 Hz, 2H), 1.80 – 1.69 (m, 4H), 1.52 (m, 2H), 1.07 – 1.03 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 134.0, 132.3, 123.4, 102.2, 88.7, 53.6, 37.8, 35.2, 28.2, 23.1, 18.6, 11.1.

ESI-MS: *m/z* = 439.3 [MH^+].

(S)-2-Methyl-N-((R)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)propane-2-sulfinamide



(S,R)-3.17

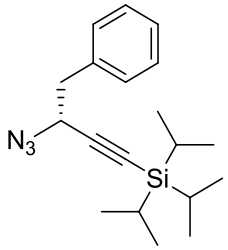
Produced as a byproduct during the synthesis of **(S,S)-3.17**; chapter 6.3.3. Isolated as a yellow oil (18 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.19 (m, 5H), 4.44 – 4.35 (app q, *J* = 5.6 Hz, 1H), 3.33 (d, *J* = 5.2 Hz, 1H), 3.08 (dd, *J* = 13.2, 6.8 Hz, 1H), 3.01 (dd, *J* = 13.3, 6.4 Hz, 1H), 1.15 (s, 9H), 1.04 – 0.99 (m, 21H).

ESI-MS: *m/z* = 406.3 [MH^+].

7.4.4 Phenylalanine monomer unit synthesis

(R)-(3-Azido-4-phenylbut-1-yn-1-yl)triisopropylsilane



(R)-3.25

To **(S,R)-3.17** (1 eq, 0.48 g) in diethyl ether (0.1 M, 12 mL) was added a solution of hydrogen chloride in diethyl ether (2 eq, 2 M, 1.2 mL) and the solution was stirred for one hour, and the white solid white precipitated out of the solution was filtered with cold diethyl ether, giving a solid (0.26 g). To a proportion of the solid (1 eq, 0.2 g) was added water (1.3 mL), methanol (8.7 mL), sodium hydrogen carbonate (4 eq, 0.2 g), a solution of triflic azide in toluene (2.5 eq, 0.7 M, 2.3 mL) and copper sulfate pentahydrate (0.05 eq, 7.4 mg). The mixture was stirred for 16 hours, before being concentrated. Ethyl acetate (20 mL) and water (10 mL) were added, and the separated aqueous phase was extracted with ethyl acetate (2 x 20 mL). The combined organic layers were dried over magnesium sulfate and concentrated. Column chromatography (0.625 % ethyl acetate in petroleum spirits) was used to isolate azide **(R)-3.25** as a colourless oil (0.13 g, 46 % over 2 steps).

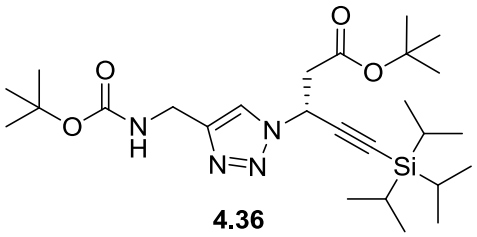
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.22 (m, 5H), 4.30 (t, *J* = 6.8 Hz, 1H), 2.97 (d, *J* = 7.2 Hz, 2H), 1.07 – 1.04 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 129.8, 128.5, 127.2, 101.7, 89.6, 55.0, 41.9, 18.6, 11.2.

ESI-MS: *m/z* = 300 [$(M-N_2)H^+$].

7.4.5 Chain extension sequence

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-5-(triisopropylsilyl)pent-4-ynoate



4.36

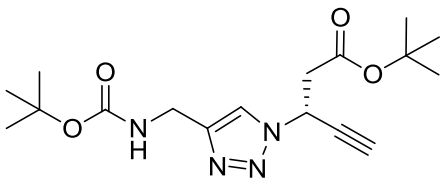
To Boc-propargylamine (1.5 eq, 75 mg) in DCM (2.1 mL) was added a solution of azide **(R)-4.10** (1 eq, 0.11 g) in methanol (1.1 mL), followed by copper sulfate pentahydrate (0.1 eq, 8 mg), ascorbate (1 eq, 63 mg) and TBTA (0.1 eq, 17 mg). After stirring for 2 hours the solution was diluted with ethyl acetate (20 mL), and water (5 mL) was added. The aqueous phase was extracted with ethyl acetate (2 x 20 mL), and the combined organic fractions were washed with brine (10 mL), dried over magnesium sulfate and concentrated. Column chromatography (10 to 20 % ethyl acetate in petroleum spirits) gave dimer **4.36** as a colourless oil (0.15 g, 93 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 5.77 (app t, *J* = 7.0 Hz, 1H), 5.14 (s, 1H), 4.39 (d, *J* = 5.9 Hz, 2H), 3.15 (dd, *J* = 15.9, 6.4 Hz, 1H), 2.96 (dd, *J* = 15.9, 7.4 Hz, 1H), 1.42 (s, 9H), 1.41 (s, 9H), 1.07 – 1.04 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 155.9, 145.3, 121.2, 100.9, 89.8, 82.1, 79.7, 50.0, 43.1, 36.2, 28.5, 28.0, 18.6, 11.1.

ESI-MS: *m/z* = 529 [MNa^+].

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)pent-4-ynoate



4.37

To dimer **4.36** (1 eq, 0.14 g) in THF (0.1 M, 2.8 mL) was added a solution of TBAF (4 eq) and acetic acid (2 eq, 33 µL) in THF (2.9 mL). After stirring for 90 minutes water (10 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were

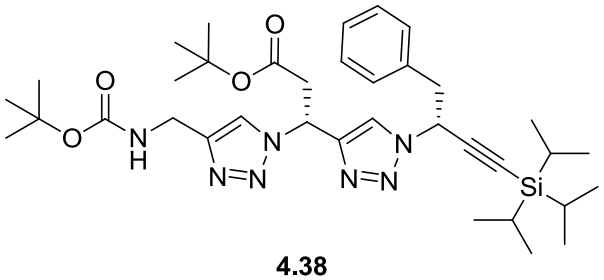
dried over magnesium sulfate and concentrated. Column chromatography (25 to 33 % ethyl acetate in petroleum spirits) gave alkyne **4.37** as a colourless oil (79 mg, 80 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 5.73 (td, *J* = 7.1, 2.4 Hz, 1H), 5.17 (br s, 1H), 4.37 (d, *J* = 6.0 Hz, 2H), 3.15 (dd, *J* = 16.0, 7.1 Hz, 1H), 2.99 (dd, *J* = 16.0, 7.1 Hz, 1H), 2.63 (d, *J* = 2.4 Hz, 1H), 1.42 (s, 9H), 1.40 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 155.9, 145.5, 121.5, 82.3, 79.8, 78.1, 75.8, 49.1, 42.3, 36.2, 28.5, 28.0.

ESI-MS: *m/z* = 351 [MH^+].

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-3-(1-((R)-1-phenyl-4-(triisopropylsilyl)but-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)propanoate



4.38

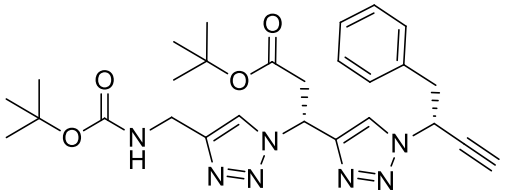
To alkyne **4.37** (1 eq, 74 mg) in DCM (1.4 mL) was added a solution of azide **(R)-3.25** (1.25 eq, 86 mg) in methanol (0.7 mL), followed by copper sulfate pentahydrate (0.1 eq, 5.2 mg), ascorbate (1 eq, 42 mg) and TBTA (0.1 eq, 11 mg). After stirring for 2 hours, water (10 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL) . The combined organic fractions were washed with brine (10 mL), dried over magnesium sulfate and concentrated. Column chromatography (30 % ethyl acetate in petroleum spirits) gave trimer **4.38** as a colourless oil (0.14 g, 96 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.45 (s, 1H), 7.25 – 7.15 (m, 3H), 6.98 (m, 2H), 6.23 (app q, *J* = 7.4 Hz, 1H), 5.71 (dd, *J* = 7.6, 4.8 Hz, 1H), 5.05 (br s, 1H), 4.38 (d, *J* = 5.7 Hz, 2H), 3.41 – 3.23 (m, 4H), 1.43 (s, 9H), 1.34 (s, 9H), 1.04 – 0.99 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3, 155.8, 145.4, 144.3, 134.4, 129.7, 128.5, 127.7, 121.9, 121.4, 100.5, 91.6, 81.9, 79.8, 55.0, 54.0, 43.3, 40.8, 36.3, 28.5, 28.0, 18.6, 11.1.

ESI-MS: *m/z* = 678 [MH^+].

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-3-(1-((R)-1-phenylbut-3-yn-2-yl)-1H-1,2,3-triazol-4-yl)propanoate



4.39

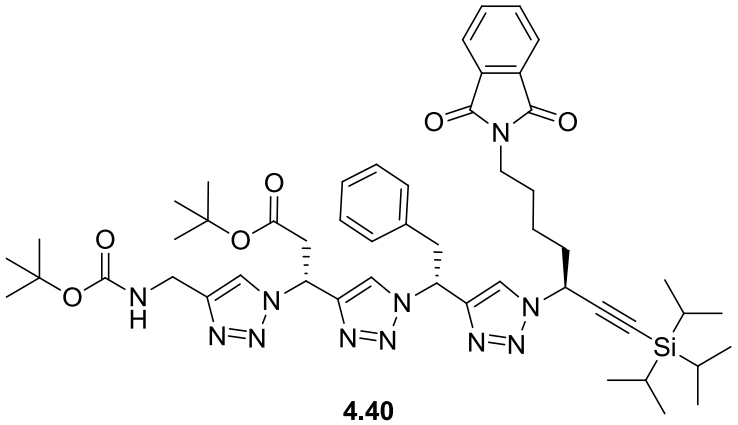
To trimer **4.38** (1 eq, 0.14 g) in THF (0.1 M, 2 mL) was added a solution of TBAF (4 eq) and acetic acid (2 eq, 23 µL) in THF (2.8 mL). After stirring for 2 hours water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were dried over magnesium sulfate and concentrated. Column chromatography (30 % ethyl acetate in petroleum spirits) gave alkyne **4.39** as a white solid (68 mg, 68 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.51 (s, 1H), 7.26 – 7.15 (m, 3H), 7.00 – 6.94 (m, 2H), 6.22 (dd, *J* = 8.1, 6.9 Hz, 1H), 5.63 (ddd, *J* = 7.9, 5.6, 2.4 Hz, 1H), 5.22 (br s, 1H), 4.41 (d, *J* = 6.0 Hz, 2H), 3.39 – 3.24 (m, 4H), 2.64 (d, *J* = 2.4 Hz, 1H), 1.41 (s, 9H), 1.33 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3, 155.8, 145.4, 144.5, 134.1, 129.5, 128.6, 127.7, 122.1, 121.7, 82.0, 79.8, 77.9, 77.4, 54.1, 53.9, 42.9, 40.7, 36.1, 28.4, 27.9.

ESI-MS: *m/z* = 522.3 [MH^+].

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-3-(1-((R)-1-(1-((S)-7-(1,3-dioxoisoindolin-2-yl)-1-(triisopropylsilyl)hept-1-yn-3-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)propanoate



4.40

To alkyne **4.39** (1 eq, 29 mg) in DCM (0.75 mL) was added a solution of azide **4.11** (1 eq, 24 mg) in methanol (0.35 mL), followed by copper sulfate pentahydrate (0.1 eq, 1.4 mg), ascorbate (1 eq, 11

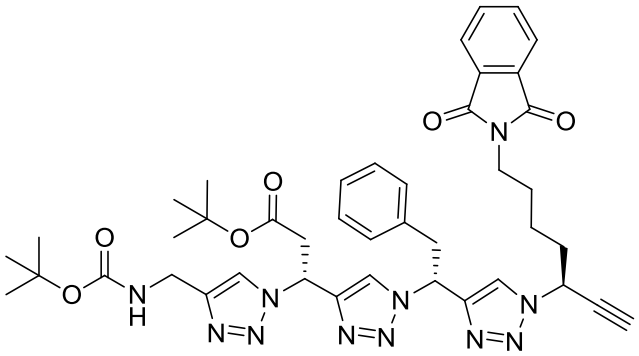
mg) and TBTA (0.1 eq, 2.9 mg). After stirring for 2 hours, water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were washed with brine (10 mL), dried over magnesium sulfate and concentrated. Column chromatography (30 to 50 % ethyl acetate in petroleum spirits) gave tetramer **4.40** as a white foam (33.4 mg, 63 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.81 (m, 2H), 7.75 (s, 1H), 7.71 (m, 2H), 7.69 (m, 1H), 7.58 (s, 1H), 7.20 – 7.15 (m, 3H), 6.95 (m, 2H), 6.21 (dd, *J* = 8.7, 6.3 Hz, 1H), 5.99 (t, *J* = 7.9 Hz, 1H), 5.42 (dd, *J* = 7.5, 6.2 Hz, 1H), 5.08 (br s, 1H), 4.39 (d, *J* = 5.6 Hz, 2H), 3.65 (app t, *J* = 6.8 Hz, 2H), 3.57 (d, *J* = 8.0 Hz, 2H), 3.36 (dd, *J* = 16.4, 8.4 Hz, 1H), 3.26 (dd, *J* = 16.2, 6.0 Hz, 1H), 2.14 – 2.02 (m, 2H), 1.70 (p, *J* = 7.6 Hz, 2H), 1.44 (s, 9H), 1.40 (m, 2H), 1.32 (s, 9H), 1.04 – 0.98 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 168.2, 168.0, 145.3, 144.7, 144.2, 135.8, 134.1, 132.2, 129.1, 128.8, 127.4, 123.4, 122.4, 121.6, 121.6, 101.2, 90.4, 81.9, 79.7, 59.4, 54.0, 53.8, 42.5, 40.7, 37.6, 36.9, 36.3, 28.5, 28.0, 27.9, 22.8, 18.6, 11.1.

ESI-MS: *m/z* = 960 [MH^+].

(R)-tert-Butyl 3-(4-(((tert-butoxycarbonyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-3-(1-((R)-1-(1-((S)-7-(1,3-dioxoisoindolin-2-yl)hept-1-yn-3-yl)-1H-1,2,3-triazol-4-yl)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)propanoate



4.41

To tetramer **4.40** (1 eq, 33 mg) in THF (0.1 M, 0.35 mL) was added a solution of TBAF (4 eq) and acetic acid (2 eq, 23 µL) in THF (0.49 mL). After stirring for 3.5 hours water (5 mL) was added, the solid which precipitated from the solution was filtered, giving alkyne **4.41** as a white solid (19 mg, 74 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.81 (m, 2H), 7.75 (m, 1H), 7.74 – 7.70 (m, 2H), 7.64 (m, 1H), 7.58 (s, 1H), 7.22 – 7.16 (m, 3H), 6.96 (dt, *J* = 7.4, 3.8 Hz, 2H), 6.21 (dd, *J* = 8.5, 6.5 Hz, 1H), 5.98 (t, *J* = 7.9 Hz, 1H), 5.40 (td, *J* = 6.9, 2.4 Hz, 1H), 5.07 (s, 1H), 4.38 (d, *J* = 5.7 Hz, 2H), 3.65 (t, *J* = 7.3

Hz, 2H), 3.60 (t, *J* = 7.8 Hz, 2H), 3.36 (dd, *J* = 16.4, 8.7 Hz, 1H), 3.27 (dd, *J* = 16.4, 6.4 Hz, 1H), 2.62 (d, *J* = 2.4 Hz, 1H), 2.08 (dd, *J* = 15.7, 7.2 Hz, 2H), 1.69 (p, *J* = 7.6 Hz, 2H), 1.43 (s, 9H), 1.41 – 1.34 (m, 2H), 1.34 (m, 9H).

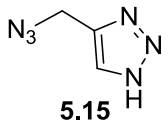
^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 168. 3, 144.8, 144.5, 135.8, 134.1, 132.2, 129.1, 128.8, 127.5, 123.4, 122.4, 121.8, 121.6, 82.0, 78.4, 76.5, 59.6, 54.0, 52.9, 42.4, 40.7, 37.5, 36.4, 28.5, 28.0, 27.8, 22.6, 17.9, 17.8, 12.4.

ESI-MS: *m/z* = 748 [MH^+].

7.5 Synthesis of the Compounds Described in Chapter 5

7.5.1 Solution phase synthesis of Merck inhibitor 5.9

4-(Azidomethyl)-1H-1,2,3-triazole



5.15

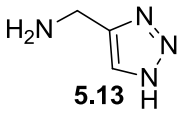
Sodium azide (3.28 g, 4 eq) and ammonium chloride (1.35 g, 2 eq) were added to propargyl bromide (1.40 mL; 80% in toluene, 1 eq) in 3 : 1 dioxane : water (64 mL). After stirring at 75 ^{o}C overnight, the solution was allowed to return to room temperature before being diluted with ethyl acetate (90 mL) and washed with water (40 mL). The aqueous layer was extracted ethyl acetate (2 x 20mL) and the combined organic extracts were dried with magnesium sulfate before being concentrated, giving azide **5.15** with trace amounts of dioxane (1.72 g, 110 %) as a yellow liquid, which was not purified further.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (s, 1H), 4.53 (s, 2H).

ESI-MS: *m/z* = 125.3 [MH^+].

IR: 2102 cm^{-1} (m, N_3).

(1H-1,2,3-Triazol-4-yl)methanamine



5.13

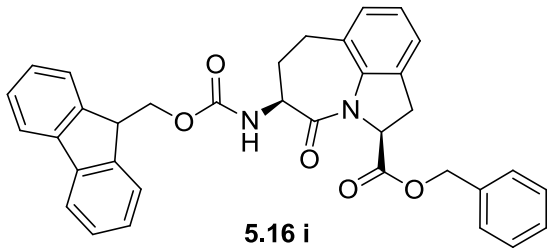
Triphenylphosphine (5.46 g, 1.5 eq) was added to azide **5.15** (1.72 g, 1 eq) in methanol (140 mL), and refluxed for 1 hour. The solution was allowed to cool to room temperature before being concentrated. The residue was redissolved in toluene (40 mL), which was extracted with 2 M hydrochloric acid (2 x 30 mL). The aqueous fractions were concentrated, and the off-white solid produced was recrystallised from methanol and chloroform giving amine **5.13** as an off-white solid (0.86 g, 63 %).

^{1}H NMR (D_2O): δ 8.01 (s, 1H); 4.35 (s, 2H).

ESI-MS: *m/z* = 99.1 [MH^+].

The spectral data are similar to the literature.[148]

(2S,5S)-Benzyl 5-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxylate



5.16 i

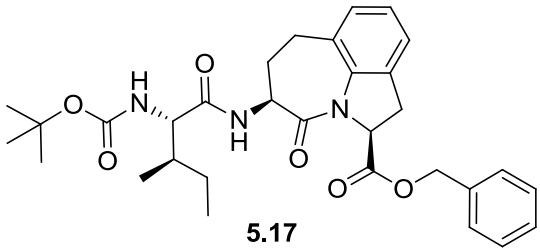
Diisopropylethylamine (100 uL, 1.3 eq) and benzyl bromide (60 uL, 1.2 eq) were added to commercially available (2S,5S)-5-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxylic acid (0.2 g, 1 eq) in DMF (600 uL), and stirring continued overnight. The solution was then diluted with ethyl acetate (30 mL) and washed with water (3 x 10 mL) and brine (3 x 10 mL). The organic phase was concentrated after drying with magnesium sulfate. The residue was purified using column chromatography with petroleum spirits and ethyl acetate (3 : 1) giving **5.16 i** as a colourless oil (0.24 g, 101%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (m, 2H), 7.71(m, 2H), 7.53-7.23 (m, 9H), 7.19-6.98 (m, 3H) 6.35 (s, 1H), 5.43 (m, 1H), 5.38-5.12 (m, 2H), 4.52-4.26 (m, 4H), 3.63-3.29 (m, 2H), 3.28-3.10 (m, 2H), 2.51-2.38 (m, 1H), 2.29-2.12 (m, 1H).

ESI-MS: *m/z* = 559.2 [MH^+].

The spectral data are consistent with those reported in the literature.[147]

(2S,5S)-Benzyl 5-((2S,3R)-2-(tert-butoxycarbonylamino)-3-methylpentanamido)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxylate



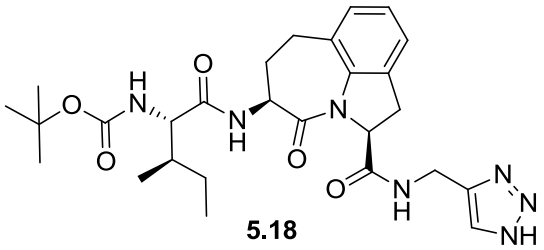
5.17

Peptide **5.16 i** (0.2415 g, 1 eq) was dissolved in a solution of 20% piperidine in DMF (2 mL) and stirred for 1 hour. After rotary evaporation of the DMF and piperidine, the residue was dissolved in DCM (2 mL) and added to a mixture of Boc-isoleucine (0.125 g, 1.2 eq), diisopropyl ethylamine (140 uL, 1.5 eq) HOB*t* (0.14 g, 2.4 eq) and HBTU (0.295 g, 1.8 eq) in DCM (4 mL). After stirring overnight, the mixture was concentrated, diluted in ethyl acetate (30 mL) and washed with 1 M hydrochloric acid (2 x 10 mL), saturated sodium bicarbonate solution (2 x 10 mL) and brine (2 x 10 mL). After drying with magnesium sulfate, the organic phase was concentrated. The residue was purified using column chromatography with petroleum spirits and ethyl acetate (3 : 1) giving peptide **5.17** as a clear oil (150 mg, 54%).

^{1}H NMR (300 MHz, MeOH) δ 7.39 – 7.23 (m, 5H), 7.08 – 6.95 (m, 3H), 5.34 (dd, *J* = 10.9, 2.6 Hz, 1H), 5.22 – 5.09 (m, 3H), 4.43 (dd, *J* = 9.2, 5.4 Hz, 1H), 3.48 (dd, *J* = 16.8, 10.9 Hz, 1H), 3.41 – 3.27 (m, 2H), 3.17 – 3.01 (m, 1H), 2.30 (dt, *J* = 13.2, 3.7 Hz, 1H), 2.13 – 1.99 (m, 1H), 1.99 – 1.83 (m, 1H), 1.56 – 1.48 (m, 1H), 1.46 (s, 9H), 0.96 – 0.91 (m, 6H).

ESI-MS: *m/z* = 550.4 [MH^+].

tert-Butyl (2S,3R)-1-((2S,5S)-2-((1H-1,2,3-triazol-4-yl)methylcarbamoyl)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indol-5-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate



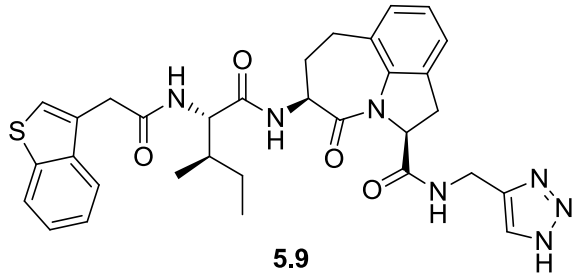
5.18

Benzyl deprotection of peptide **5.17** (1 eq, 80 mg) was performed using the H-Cube using a 10 % Pd/C with cartridge and following settings: (0 pressure, 60 ^{o}C, Full H_2 mode) in a 50mL solution of 1 : 1 ethanol : ethyl acetate, with TLC confirming complete consumption of starting material. To the free acid (59 mg, 1 eq) was added diisopropyl ethylamine (27 uL, 1.25 eq) HOB*t* (33 mg, 2 eq) and HBTU (70 mg, 1.5 eq) in DCM (1 mL), followed by amine **5.13** (24 mg, 2 eq). After stirring overnight, the mixture was concentrated, diluted with ethyl acetate (30 mL) and washed with 1 M hydrochloric acid (2 x 10mL), saturated sodium bicarbonate solution (2 x 10 mL) and brine (2 x 10mL). After drying with magnesium sulfate, the organic phase was concentrated. The residue was purified using column chromatography with petroleum spirits and ethyl acetate (4 : 1), furnishing peptide **5.18** as a white solid (24 mg, 35 %).

^{1}H NMR (300 MHz, MeOH) δ 8.58 (t, *J* = 5.7 Hz, 1H), 8.33 (d, *J* = 6.7 Hz, 1H), 7.68 (s, 1H), 7.12 – 7.03 (m, 2H), 6.99 (dd, *J* = 9.0, 5.9 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 5.14 (dd, *J* = 10.8, 3.2 Hz, 1H), 4.48 (s, 2H), 4.47 – 4.40 (m, 1H), 4.09 – 3.95 (m, 2H), 3.49 (dd, *J* = 16.6, 10.8 Hz, 1H), 3.24 – 3.01 (m, 3H), 2.29 – 2.13 (m, 2H), 1.84 (m, 1H), 1.60 – 1.47 (m, 1H), 1.45 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.8 Hz, 3H).

ESI-MS: *m/z* = 540.2 [MH^+].

(2S,5S)-N-((1H-1,2,3-Triazol-4-yl)methyl)-5-((2S,3R)-2-(2-(benzo[b]thiophen-3-yl)acetamido)-3-methylpentanamido)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxamide



5.9

Peptide **5.18** (24 mg, 1 eq) was Boc-deprotected by stirring in a 30% TFA in DCM solution (1 mL) with triethylsilane (8 uL, 1.1 eq) for one hour, before being concentrated. A solution of commercially available 2-(benzo[b]thiophen-3-yl)acetic acid (8 mg, 1.2 eq), diisopropyl ethylamine (20 uL, 3 eq) HOBT (12 mg, 2.4 eq) and HBTU (25 mg, 1.8 eq) in DCM (2 mL), was added and the reaction was allowed to stir overnight. The mixture was then concentrated, diluted with ethyl acetate (25 mL) and washed with 1 M hydrochloric acid, (2 x 10 mL), saturated sodium bicarbonate solution (2 x 10 mL) and brine (2 x 10 mL). After drying with magnesium sulfate, the organic phase was concentrated and purified via column chromatography with petroleum spirits and ethyl acetate (1 : 9) to give **5.9** as a white solid (12 mg, 55.6 %). Further purification via preparative HPLC gave a white solid (0.8 mg, 3.7 %).

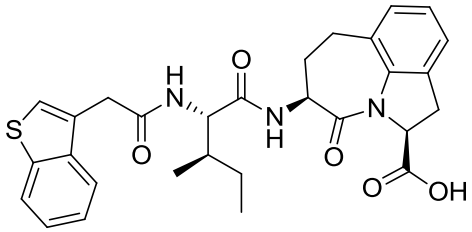
^{1}H NMR (400 MHz, MeOD) δ 8.49 (t, *J* = 5.6 Hz, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 8.26 (d, *J* = 8.9 Hz, 1H), 8.00 – 7.92 (m, 1H), 7.87 (dd, *J* = 5.8, 3.1 Hz, 1H), 7.61 (s, 1H), 7.51 (s, 1H), 7.43 – 7.32 (m, 2H), 7.08 (m, 2H), 6.96 (dd, *J* = 14.3, 7.0 Hz, 1H), 5.04 (dd, *J* = 10.9, 2.8 Hz, 1H), 4.41 – 4.35 (m, 1H), 4.32 (app t, *J* = 5.3 Hz, 2H), 4.28 (dd, *J* = 10.2, 5.8 Hz, 1H), 3.79 (AB q, *J* = 35.3, 14.9 Hz, 2H), 3.45 (dd, *J* = 16.7, 10.9 Hz, 1H), 3.08 (m, 2H), 2.92 (dd, *J* = 16.9, 2.3 Hz, 1H), 2.16 – 1.96 (m, 2H), 1.79 (m, 1H), 1.46 (m, 1H), 1.20 – 1.06 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H).

ESI-MS: *m/z* = 614.8 [MH^{+}].

The spectral data are consistent with those reported in the literature.[147]

7.5.2 Solid phase synthesis of Merck inhibitor 5.9

(2S,5S)-5-((2S,3R)-2-(2-(Benzo[b]thiophen-3-yl)acetamido)-3-methylpentanamido)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxylic acid



5.22

After chlorotrityl resin (0.5 g, 1.12 mmol g^{-1}) was washed with DMF (3 x 5 mL), a solution of acid **5.16** (1.1 eq, 0.29 g) and DIEA (3 eq, 0.29 mL) in DMF (5 mL) was added. The suspension was stirred sporadically over 4 hours, before being drained and washed with an acetic acid: DIEA solution (1 : 2, 6 mL), followed by washing with DMF (3 x 7.5 mL).

To the resin was added 20 % piperidine in DMF (2 x 5 mL), and the resin was stirred for 10 minutes for each piperidine aliquot, and finally washed with DMF (3 x 7.5 mL). In a separate flask, Fmoc-isoleucine (3 eq, 0.59g), DIEA (3 eq, 0.29 mL) and HBTU (3 eq, 0.64 g) were mixed in DMF (7.5 mL), which was stirred for 15 minutes. The coupling solution was added to the resin, and the suspension was stirred for 30 minutes.

The resin was then washed with DMF (3 x 75 mL), after which 20 % piperidine in DMF (2 x 5 mL) was added, and the resin was stirred for 10 minutes for each piperidine aliquot. In a separate flask, acid **5.10** (1.5 eq, 0.16 g), DIEA (1.5 eq, 0.15 mL) and HBTU (1.5 eq, 0.32 g) were mixed in DMF (7.5 mL), which was stirred for 15 minutes. After the resin was washed with DMF (3 x 7.5 mL) the coupling solution was added, and the suspension was stirred for 30 minutes.

After washing with DMF (3 x 7.5 mL), a solution of acetic anhydride (1.25 mL) and DIEA (2.5 mL) in DMF (3.75 mL) was added, and the suspension was stirred for 15 minutes, before being drained and washed with DMF (3 x 7.5 mL). The resin was dried under vacuum for 15 minutes.

A cleavage solution consisting of 95 % DCM 2.5 % TFA 2.5 % triisopropylsilane (7.5 mL) was added to the resin, and after 2 minutes the solution was drained into cold diethyl ether (60 mL). The procedure was repeated twice more, after which the diethyl ether solution was left in the fridge

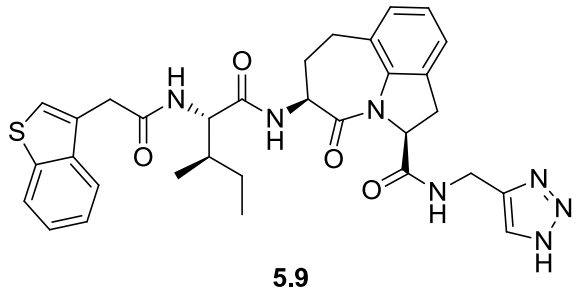
overnight, resulting in a suspension of while solid in the organic solution. The white solid was filtered, giving product **5.22** (0.12 g, 40 %).

^{1}H NMR (400 MHz, DMSO) δ 8.30 (m, 2H), 7.99 – 7.93 (m, 1H), 7.91 – 7.84 (m, 1H), 7.52 (s, 1H), 7.41 – 7.33 (m, 2H), 7.09 (m, 2H), 6.98 (t, *J* = 7.4 Hz, 1H), 5.03 (dd, *J* = 11.0, 2.4 Hz, 1H), 4.36 (dd, *J* = 13.0, 5.8 Hz, 1H), 4.29 (d, *J* = 7.4 Hz, 1H), 3.80 (dd, *J* = 35.0, 14.5 Hz, 2H), 3.54 – 3.43 (m, 2H), 3.11 – 2.95 (m, 2H), 2.10 – 1.94 (m, 2H), 1.80 (m, 1H), 1.46 (m, 1H), 1.19 – 1.10 (m, 1H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 172.5, 170.4, 169.2, 168.5, 139.4, 138.8, 138.2, 132.1, 130.8, 129.6, 125.1, 124.2, 124.2, 123.9, 123.6, 123.0, 122.7, 122.2, 59.7, 56.9, 52.6, 36.8, 35.2, 31.4, 31.0, 28.1, 24.2, 15.4, 11.1.

ESI-HRMS-TOF calcd for $C_{29}H_{31}N_3O_5S$ $[M+H]^+$: 534.2057 found: 534.2076.

(2S,5S)-N-((1H-1,2,3-triazol-4-yl)methyl)-5-((2S,3R)-2-(2-(benzo[b]thiophen-3-yl)acetamido)-3-methylpentanamido)-4-oxo-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxamide



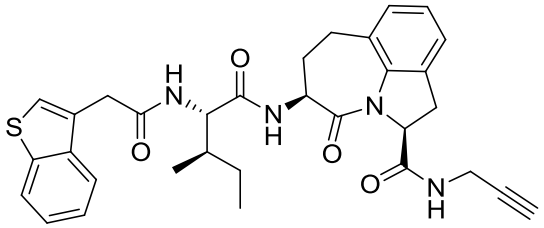
5.9

To acid **5.22** (1 eq, 99 mg) in DMF (0.07 M, 2.6 mL) was added DIEA (2 eq, 64 µL) and HBTU (1 eq, 70 mg), and the solution was left to stir for 30 minutes. Over time, some material precipitated out of solution. Amine **5.13** (2 eq, 36 mg) was added, and the suspension was left for stir for 17 hours, over which time the solid material redissolved in DMF. Water (10 mL) was added, and the solution was filtered with further water followed by diethyl ether. Hot acetonitrile (25 mL) was added, and any insoluble material was discarded. Upon cooling the filtrate to 0 ^{o}C a fine white solid precipitated out of the solution, which was filtered with cold acetonitrile, giving product **5.9** (55.3 mg, 49 %).

The spectral data are identical to those for material that was synthesised using the method described in 6.5.1.

7.5.3 Progression towards analogues of Merck inhibitor 5.9

(2S,5S)-5-((2S,3R)-2-(2-(Benzo[b]thiophen-3-yl)acetamido)-3-methylpentanamido)-4-oxo-N-(prop-2-yn-1-yl)-1,2,4,5,6,7-hexahydroazepino[3,2,1-hi]indole-2-carboxamide



5.23

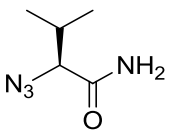
To acid **5.22** (1 eq, 83 mg) in DMF (0.07 M, 2.2 mL) was added DIEA (2 eq, 54 µL) and HBTU (1 eq, 58.4 mg). After stirring for 30 minutes, propargylamine hydrochloride (2 eq, 28.2 mg) and DIEA (1.5 eq, 41 µL) was added, the the solution was left to stir for 17 hours. The solvent was evaporated under vacuum, 1 M hydrochloric acid (10 mL) and ethyl acetate (10 mL) were added, and the resultant precipitate was filtered with water, followed by diethyl ether, giving **5.23** as a fine off-white powder (74.1 mg, 89 %).

^{1}H NMR (400 MHz, DMSO) δ 8.44 (t, *J* = 5.5 Hz, 1H), 8.33 (d, *J* = 7.1 Hz, 1H), 8.27 (d, *J* = 8.9 Hz, 1H), 7.99 – 7.93 (m, 1H), 7.86 (m, 1H), 7.51 (s, 1H), 7.41 – 7.33 (m, 2H), 7.08 (m, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 5.01 (dd, *J* = 10.8, 2.8 Hz, 1H), 4.40 – 4.33 (m, 1H), 4.27 (dd, *J* = 8.8, 7.2 Hz, 1H), 3.81 (m, 4H), 3.50 – 3.43 (m, 2H), 3.11 (dd, *J* = 4.6, 2.1 Hz, 1H), 3.06 (d, *J* = 6.0 Hz, 2H), 2.11 – 1.99 (m, 2H), 1.79 (m, 1H), 1.46 m, 1H), 1.14 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 170.4, 170.4, 169.1, 168.9, 139.4, 138.8, 138.8, 132.3, 130.8, 129.3, 125.4, 124.2, 124.2, 123.9, 123.6, 122.9, 122.7, 122.2, 80.9, 73.1, 60.5, 56.9, 52.5, 36.7, 35.2, 31.9, 30.9, 28.5, 28.0, 24.3, 15.4, 11.1.

ESI-HRMS-TOF calcd for $C_{32}H_{34}N_4O_4S$ $[M+H]^+$: 571.2374 found: 571.2346.

(S)-2-Azido-3-methylbutanamide



5.27a

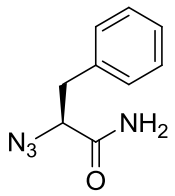
To Boc-valine (1 eq, 0.2 g) and triethylamine (1 eq, 0.13 mL) in anhydrous THF (0.1 M, 9.2 mL) at -10 ^{o}C was added ethyl chloroformate (1 eq, 88 µL) dropwise. After stirring for 30 minutes, aqueous

ammonia (30%, 2 eq, 0.24 mL) was added and the solution was stirred for 45 minutes. Ethyl acetate (20 mL) and water (20 mL) were added, the aqueous phase was separated and extracted with ethyl acetate (20 mL). The combined organic phases were washed with sodium hydrogen carbonate solution (20 mL) and brine (20 mL). After drying over magnesium sulfate the solvents were evaporated under reduced pressure, giving a white solid. The solid (~1 eq, 0.15 g) was redissolved in methanol (0.1 M, 6.9 mL), a solution of hydrogen chloride in diethyl ether (2 M, 2 eq, 0.7 mL) was added and the solution was left to stir for 1 hour. After evaporation of the solvents under reduced pressure, the crude material was filtered with cold diethyl ether, giving a white solid (80 mg). The filtered material was transferred to a round bottomed flask, and water (1.05 mL), methanol (7.1 mL), sodium hydrogen carbonate (4 eq, 0.23 g), a solution of triflic azide in toluene (0.58 M, 1.8 mL) and copper sulfate pentahydrate (0.05 eq, 8.7 mg) were added. The suspension was left to stir for 20 hours. After evaporation of the organic solvents, water (10 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were dried with magnesium sulfate and concentrated under reduced pressure. Column chromatography with a ethyl acetate : petroleum spirits gradient (25 % to 50 % ethyl acetate in petroleum spirits) to give **5.27a** as a white solid (47 mg, 47 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (d, *J* = 4.1 Hz, 1H), 2.37 (dq, *J* = 6.8, 4.1 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H).

ESI-MS: *m/z* = 143.2 [MH^+].

(S)-2-Azido-3-phenylpropanamide



5.27b

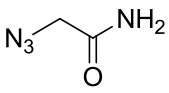
To Boc-phenylalanine (1 eq, 0.25 g) and triethylamine (1 eq, 0.13 mL) in anhydrous THF (0.1 M, 9.4 mL) at -10 ^{o}C was added ethyl chloroformate (1 eq, 90 µL) dropwise. After stirring for 30 minutes, aqueous ammonia (30%, 2 eq, 0.25 mL) was added and the solution was stirred for 45 minutes. Ethyl acetate (20 mL) and water (20 mL) were added, the aqueous phase was separated and extracted

with ethyl acetate (20 mL). The combined organic phases were washed with sodium hydrogen carbonate solution (20 mL) and brine (20 mL). After drying over magnesium sulfate the solvents were evaporated under reduced pressure, giving a white solid. The solid (~1 eq, 0.25 g) was redissolved in methanol (0.5 M, 2 mL), and a solution of hydrogen chloride in diethyl ether (2M, 2 eq, 1.0 mL) was added and the solution was left to stir for 1 hour. After evaporation of the solvents under reduced pressure, the remaining solid material was filtered with cold diethyl ether, giving a white solid (0.16 g). The material was transferred to a round bottomed flask and water (1.5 mL), methanol (9.6 mL), sodium hydrogen carbonate (4 eq, 0.32 g), a solution of triflic azide in toluene (0.75 M, 2.5 mL) and copper sulfate pentahydrate (0.05 eq, 12.0 mg) were added. The suspension was left to stir for 18 hours. After evaporation of the organic solvents, water (10 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were dried with magnesium sulfate and concentrated under reduced pressure. Column chromatography (50 % ethyl acetate in petroleum spirits) gave **5.27b** as a white solid (86 mg, 60 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H), 6.18 (br s, 0.7H), 5.38 (br s, 0.7H), 4.22 (dd, *J* = 8.3, 4.3 Hz, 1H), 3.37 (dd, *J* = 14.1, 4.3 Hz, 1H), 3.05 (dd, *J* = 14.1, 8.3 Hz, 1H).

ESI-MS: *m/z* = 191.1 [MH^+].

2-Azidoacetamide



5.27c

Sodium azide (1.05 eq, 0.98 g) and ammonium chloride (2 eq, 1.55 g) were added to bromoacetic acid (2 eq, 2 g) in water (9.6 mL). After stirring for 48 hours, the solution was acidified to ph = 2 via the addition of concentrated hydrogen chloride solution (~ 0.5 mL). After extracting with diethyl ether (2 x 20 mL) the combined organic fractions were dried over magnesium sulfate and concentrated, giving azidoglycine as a colourless oil (0.95 g, 62 %). No further purification was performed on the compound.

To azidoglycine (1 eq, 0.22 g) and triethylamine (1 eq, 0.28 mL) in anhydrous THF (0.5 M, 10 mL) at -10 ^{o}C was added ethyl chloroformate (1 eq, 0.19 mL) dropwise. After stirring for 30 minutes, aqueous

ammonia (30%, 2 eq, 0.52 mL) was added and the solution was stirred for 45 minutes. Ethyl acetate (20 mL) and water (10 mL) were added, the aqueous phase was separated and extracted with ethyl acetate (20 mL). The combined organic phases were washed with sodium hydrogen carbonate solution (10 mL) and brine (10 mL). After drying over magnesium sulfate the solvents were evaporated under reduced pressure. Column chromatography (2.5 % methanol in DCM) was used to purify the compound, giving **5.27c** as an off-white solid (20 mg, 10 %).

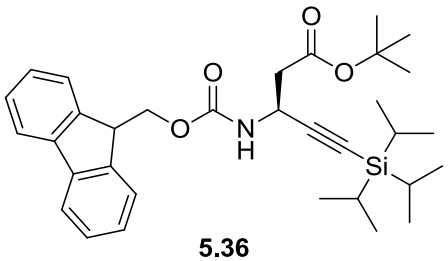
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (s, 2H).

ESI-MS: *m/z* = 198.9 [2M-H$^-$].

The spectral data are consistent with those reported in the literature.[151]

7.5.4 Synthesis of the human granzyme B-directed inhibitors

(S)-tert-Butyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(triisopropylsilyl)pent-4-ynoate



5.36

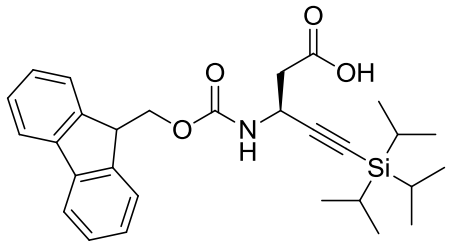
To alkyne **(S,S)-4.7** (1 eq, 330 mg) in methanol (1 M, 0.77 mL) was added hydrochloric acid in diethyl ether (2 M, 2 eq 0.77 mL) and the solution was left to stir for 1 hour, at which point the solvents were evaporated, giving a colourless oil. The oil (~1 eq, 0.26 g) was redissolved in water (0.1 mL, 7.1 mL), and sodium carbonate (2 eq, 0.15 g), followed by Fmoc succinimide (1 eq, 0.24 g) in dioxane (5.7 mL) was added. The solution was left to stir for 3.5 hours, at which point water (15 mL) was added, and the aqueous solution was extracted with ethyl acetate (3 x 30 mL). After drying over magnesium sulfate, the combined organic fractions were concentrated under reduced pressure. Column chromatography with an ethyl acetate in petroleum spirits gradient (5% to 20 %) gave **5.36** as a colourless oil (0.17 g, 33 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.4 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (app tt, *J* = 7.4, 1.1 Hz, 2H), 5.71 (d, *J* = 7.1 Hz, 1H), 4.91 – 4.82 (m, 1H), 4.45 – 4.34 (m, 2H), 4.25 (t, *J* = 7.1 Hz, 1H), 2.76 – 2.57 (m, 2H), 1.48 (s, 9H), 1.08 – 1.05 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 155.4, 144.0, 144.0, 141.4, 127.8, 127.2, 125.3, 120.1, 105.5, 81.6, 67.3, 47.3, 41.6, 40.9, 28.2, 18.7, 11.2.

ESI-MS: *m/z* = 548 [MH^+].

(S)-3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(triisopropylsilyl)pent-4-ynoic acid



5.34

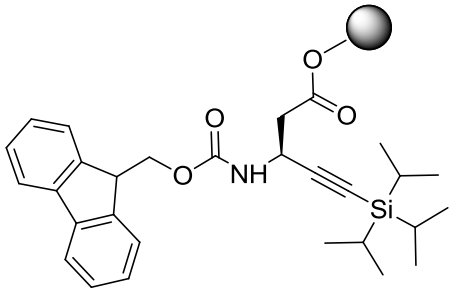
Alkyne **5.36** (1 eq, 30.9 mg) was stirred in TFA (0.1 M, 0.6 mL) for 2 hours, after which TFA was removed under reduced pressure, giving **5.34** as a colourless oil in quantitative yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.44 – 7.38 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 5.58 (d, *J* = 6.9 Hz, 1H), 4.93 (m, 1H), 4.49 – 4.40 (m, 2H), 4.25 (t, *J* = 6.9 Hz, 1H), 2.83 (d, *J* = 14.2 Hz, 2H), 1.06 (m, 21H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.7, 155.5, 143.9, 141.4, 127.9, 127.2, 125.2, 120.2, 104.5, 85.4, 67.3, 47.3, 40.7, 40.5, 18.6, 11.2.

ESI-MS: *m/z* = 492.3 [MH^+].

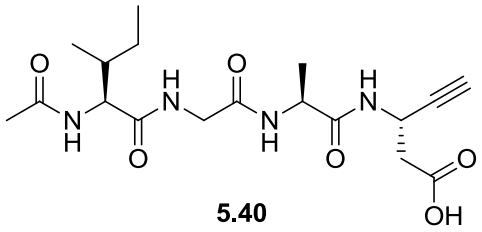
(S)-3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(triisopropylsilyl)pent-4-yn-chlorotrityl resin



5.37

After washing chlorotrityl resin (1 eq, 0.145 g, 1.12 mmol g^{-1}) with DMF (3 x 2 mL) a solution of **5.34** (1 eq, 80 mg) and DIEA (3 eq, 84 µL) in DMF (2 mL) was added, and the resin was stirred sporadically over 4 hours. After draining the DMF solution, the resin was washed with a solution of acetic anhydride and DIEA (1 :2 v/v, 2 mL), DMF (3 x 2mL) and methanol (2 x 2 mL), at which point the resin was dried under vacuum. Acid-mediated cleavage of a small amount of the resin using a solution of TFA (2.5 %) in $CDCl_3$ gave a compound with identical spectral properties as acid **5.34**.

(4S,10S,13S)-4-((R)-sec-Butyl)-13-ethynyl-10-methyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazapentadecan-15-oic acid



5.40

Resin **5.37** (1 eq, 0.21 g, 1.12 mmol g^{-1}) was washed with DMF (2 x 2 mL DMF), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (2 x 2 mL), methanol (2 x 2 mL) and again with DMF (2 x 2 mL).

To Fmoc alanine (2.25 eq, 0.15 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (3 eq, 0.18 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL) and methanol (3 x 2 mL).

The resin was washed with DMF (2 mL), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

To Fmoc glycine (2.25 eq, 0.14 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (2.25 eq, 0.082 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL) and methanol (3 x 2 mL).

The resin was then washed with DMF (2 mL), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

To Fmoc isoleucine (2.25 eq, 0.17 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (2.25 eq, 0.18 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL), methanol (3 x 2 mL) and DMF (3 x 2 mL).

A 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

A solution of acetic anhydride (25 eq, 0.38 mL) and DIEA (50 eq, 0.7 mL) in DMF (1 mL) was added to the resin, which was swirled for 30 minutes before being drained. The resin was then washed with DMF (3 x 2 mL), methanol (3 x 2 mL), DMF (3 x 2 mL) and THF (3 x 2 mL).

A solution of TBAF in THF (10 eq, 1 M, 2.5 mL) was added to the resin, and the suspension was swirled for 2 hours, before being drained and washed with THF (3 x 2 mL), DMF (3 x 2 mL) and methanol (3 x 2 mL), giving resin **5.39**.

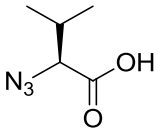
A portion of the resin (50 mg, ~ 1.12 mmol g^{-1}) was taken and cleaved with 5 x 1 mL aliquots of a cleavage solution consisting of DCM (95 %), TFA (2.5 %) and TIPS (2.5 %). The cleavage solution

was concentrated and the crude material purified by preparative HPLC, giving peptide **5.40** as a white solid (1.4 mg, 6.3 %).

^{1}H NMR (400 MHz, MeOD) δ 4.98 (app td, *J* = 6.4, 2.4 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 1H), 4.13 (d, *J* = 6.1 Hz, 1H), 3.88 (dd, *J* = 41.8, 16.6 Hz, 2H), 2.76 (dd, *J* = 15.9, 6.5 Hz, 1H), 2.69 (dd, J = 15.9, 7.5 Hz, 1H), 2.68 (d, *J* = 2.4 Hz, 1H), 2.00 (s, 3H), 1.92 – 1.80 (m, 1H), 1.58 (m, 1H), 1.36 (d, 7.1 Hz, 3H), 1.29 – 1.17 (m, 1H), 0.97 – 0.91 (m, 6H).

ESI-HRMS-TOF calcd for $C_{18}H_{28}N_4O_6$ $[M+H]^+$: 397.2082 found: 397.2163.

(S)-2-Azido-3-methylbutanoic acid



5.47

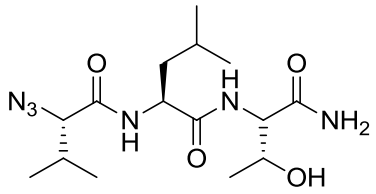
To valine (1 eq, 0.5 g) in water (4.6 mL) and methanol (30.6 mL) was added potassium carbonate (4 eq, 2.36 g), copper sulfate pentahydrate (0.05 eq, 53 mg) and a solution of triflic azide in toluene (0.82 M, 7.8 mL). After the suspension was left to stir for 16.5 hours, the organic solvents were removed under reduced pressure. water (50 mL) was added, and concentrated hydrochloric acid was added to achieve a pH of 6. The solution was diluted with 0.25 M pH 6.2 phosphate buffer (50 mL), and the aqueous solution was washed with ethyl acetate (4 x 20 mL). Concentrated hydrochloric acid was then used to acidify the aqueous layer to pH 2. The aqueous layer was extracted with ethyl acetate (3 x 25 mL) and the combined organic phases were dried over magnesium sulfate and concentrated under reduced pressure, giving azide **5.47** as an off-white oil (0.50 g, 81.8 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (br s, 1H), 3.79 (d, *J* = 5.7 Hz, 1H), 2.34 – 2.19 (m, 1H), 1.08 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H).

ESI-MS: *m/z* = 129 $[(M-N)^-]$.

The spectral data are consistent with those reported in the literature.[152]

(S)-N-((2R,3R)-1-Amino-3-hydroxy-1-oxobutan-2-yl)-2-((S)-2-azido-3-methylbutanamido)-4-methylpentanamide



5.49

Rink resin (1 eq, 1 g, 0.75 mmol g^{-1}) was washed with DMF (10 mL DMF), before a 20 % piperidine in DMF solution (10 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 10 mL), methanol (3 x 10 mL) and again with DMF (3 x 10 mL).

To Fmoc threonine with the side-chain protected as the *tert*-butyl ester (3 eq, 0.85 g) in DMF (0.07 M, 10.7 mL) was added DIEA (3 eq, 0.39 mL) and HBTU (3 eq, 0.85 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 10 mL) and methanol (3 x 10 mL).

The resin was then was washed with DMF (10 mL DMF), before a 20 % piperidine in DMF solution (10 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 10 mL), methanol (3 x 10 mL) and again with DMF (3 x 10 mL).

To Fmoc leucine (3 eq, 0.98 g) in DMF (0.07 M, 10.7 mL) was added DIEA (3 eq, 0.39 mL) and HBTU (3 eq, 0.85 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 10 mL) and methanol (3 x 10 mL).

A portion of the resin (1 eq, 0.47 g, ~0.75 mmol g^{-1}) was then washed with DMF (5 mL DMF), before a 20 % piperidine in DMF solution (5 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 5 mL), methanol (3 x 5 mL) and again with DMF (3 x 5 mL).

To azide **5.47** (5 eq, 0.25 g) in DMF (5 mL) was added DIEA (5 eq, 0.30 mL) and HBTU (5 eq, 0.66 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension

was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 5 mL) and methanol (3 x 5 mL).

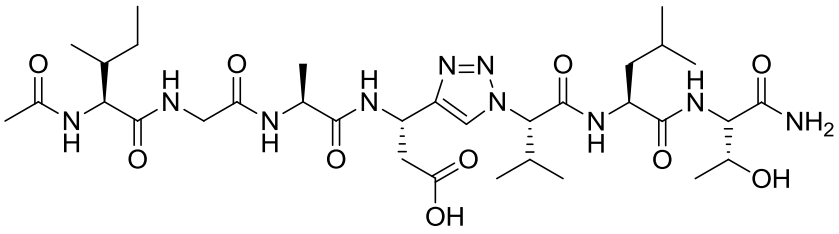
A cleavage solution (5 mL) consisting of TFA (97.5 %) and TIPS (2.5 %) was added, and the suspension was swirled sporadically over 2.5 hours, before being drained into cold diethyl ether (40 mL), with a precipitate forming after the solution was left at reduced temperatures overnight. The precipitate was filtered and purified by column chromatography (50 to 100 % ethyl acetate in petroleum spirits) giving azide **5.49** as a white solid (19.4 mg, 15 %).

^{1}H NMR (400 MHz, MeOD) δ 4.56 – 4.49 (m, 1H), 4.32 (m, 1H), 4.18 (m, 1H), 3.62 (d, *J* = 7.2 Hz, 1H), 2.17 (m, 1H), 1.78 – 1.58 (m, 3H), 1.17 (d, *J* = 6.4 Hz, 3H), 1.03 – 0.91 (m, 12H).

^{13}C NMR (101 MHz, MeOD) δ 174.8, 174.5, 172.2, 70.5, 68.3, 59.6, 53.5, 41.4, 32.1, 25.9, 23.5, 21.8, 20.0, 19.7, 18.5.

ESI-HRMS-TOF calcd for $C_{15}H_{28}N_6O_4$ $[M+H]^+$: 357.2250 found: 357.2269.

(4S,10S,13S)-13-(1-((S)-1-(((S)-1-(((2R,3R)-1-Amino-3-hydroxy-1-oxobutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1H-1,2,3-triazol-4-yl)-4-((R)-sec-butyl)-10-methyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazapentadecan-15-oic acid



5.51

Resin **5.39** (1 eq, 0.22 g, 1.12 mmol g^{-1}) was washed successively with DMF (3 x 2 mL) and THF (3 x 2 mL) before a solution of TBAF in THF (10 eq, 1 M, 2.1 mL) was added, and the suspension was shaken sporadically over 2 hours. After drainage of the TBAF solution, the resin was washed with THF (3 x 2 mL), DMF (3 x 2 mL) and methanol (3 x 2 mL) and dried under vacuum.

To a proportion of the deprotected resin (1 eq, 45 mg, 1.12 mmol g^{-1}) in THF (1 mL) was added azide **5.49** (1.1 eq, 19 mg) in THF (1 mL), DIEA (25 eq, 0.22 mL) and copper iodide (0.5 eq, 4.8 mg). The suspension gently mixed continuously for 17 hours. After drainage of the solution, the resin was washed with THF (3 x 2 mL), water (3 x 2 mL) and THF (3 x 2 mL).

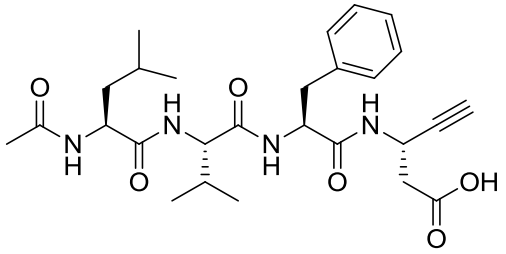
The resin was cleaved with 5 x 1 mL repetitions of a cleavage solution consisting of TFA (2.5 %) and TIPS (2.5 %) in DCM, and the combined organic extracts were evaporated under reduced pressure, giving a crude white-green solid. Preparative HPLC was used to purify the compound, giving **5.51** as a white solid (1.0 mg, 2.6 %).

^{1}H NMR (400 MHz, MeOD) δ 8.04 (s, 1H), 5.48 (t, *J* = 6.8 Hz, 1H), 4.94 (m, 1H), 4.40 (dd, *J* = 8.2, 6.8 Hz, 1H), 4.34 (q, *J* = 7.2 Hz, 1H), 4.30 (d, *J* = 3.7 Hz, 1H), 4.17 (dd, *J* = 6.4, 3.7 Hz, 1H), 4.13 (d, *J* = 7.4 Hz, 1H), 3.86 (dd, *J* = 47.8, 16.8 Hz, 2H), 3.06 – 2.92 (m, 2H), 2.58 – 2.46 (m, 1H), 2.00 (s, 3H), 1.91 – 1.80 (m, 1H), 1.65 – 1.57 (m, 3H), 1.58 – 1.53 (m, 1H), 1.35 (d, *J* = 7.2 Hz, 3H), 1.22 (m, 1H), 1.18 (d, *J* = 6.4 Hz, 3H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.97 – 0.92 (m, 6H), 0.92 – 0.90 (m, 3H), 0.83 (d, *J* = 6.3 Hz, 3H), 0.73 (d, *J* = 6.6 Hz, 3H).

ESI-HRMS-TOF calcd for $C_{33}H_{56}N_{10}O_{10}$ $[M+H]^+$: 753.4254 found: 753.4023.

7.5.5 Synthesis of the mouse granzyme B-directed inhibitors

(4S,7S,10S,13S)-10-Benzyl-13-ethynyl-4-isobutyl-7-isopropyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazapentadecan-15-oic acid



5.43

Resin **5.37** (1 eq, 0.21 g, 1.12 mmol g^{-1}) was washed with DMF (2 x 2 mL DMF), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (2 x 2 mL), methanol (2 x 2 mL) and again with DMF (2 x 2 mL).

To Fmoc phenylalanine (2.25 eq, 0.18 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (3 eq, 0.18 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL) and methanol (3 x 2 mL).

The resin was then was washed with DMF (2 mL DMF), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

To Fmoc valine (2.25 eq, 0.16 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (2.25 eq, 0.082 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL) and methanol (3 x 2 mL).

The resin was then washed with DMF (2 mL DMF), before a 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

To Fmoc leucine (2.25 eq, 0.17 g) in DMF (2 mL) was added DIEA (2.25 eq, 0.082 mL) and HBTU (2.25 eq, 0.18 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 2 mL) methanol (3 x 2 mL) and DMF (3 x 2 mL).

A 20 % piperidine in DMF solution (2 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 2 mL), methanol (3 x 2 mL) and again with DMF (3 x 2 mL).

A solution of acetic anhydride (25 eq, 0.38 mL) and DIEA (50 eq, 0.7 mL) in DMF (1 mL) was added to the resin, which was swirled for 30 minutes before being drained. The resin was then washed with DMF (3 x 2 mL), methanol (3 x 2 mL), DMF (3 x 2 mL) and THF (3 x 2 mL).

A solution of TBAF in THF (10 eq, 1 M, 2.5 mL) was added to the resin, and the suspension was swirled for 2 hours, before being drained and washed with THF (3 x 2 mL), DMF (3 x 2 mL) and methanol (3 x 2 mL), giving resin **5.42**.

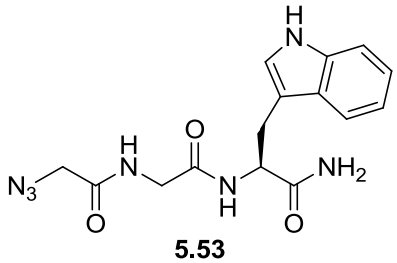
A portion of the resin (50 mg, ~ 1.12 mmol g^{-1}) was taken and cleaved with 5 x 1 mL aliquots of a cleavage solution consisting of DCM (95 %), TFA (2.5 %) and TIPS (2.5 %). The cleavage solution

was concentrated and the crude material purified by preparatve HPLC, giving alkyne **5.43** as a white solid (2.6 mg, 6.1 %).

^{1}H NMR (400 MHz, MeOD) δ 7.29 – 7.14 (m, 5H), 4.94 (app td, *J* = 6.9, 2.3 Hz, 1H), 4.57 (dd, *J* = 8.3, 6.1 Hz, 1H), 4.38 (dd, *J* = 9.7, 5.4 Hz, 1H), 4.11 (d, *J* = 7.1 Hz, 1H), 3.15 – 3.08 (m, 1H), 2.92 (dd, *J* = 13.8, 8.3 Hz, 1H), 2.70 – 2.56 (m, 2H), 2.63 (d, *J* = 2.3 Hz, 1H), 2.05 – 1.96 (m, 1H), 1.98 (s, 3H), 1.72 – 1.60 (m, 3H), 1.60 – 1.48 (m, 2H), 1.41 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 6H).

ESI-HRMS-TOF calcd for $C_{27}H_{38}N_4O_6$ $[M+H]^+$: 515.2864 found: 515.2888.

(S)-2-(2-(2-Azidoacetamido)acetamido)-3-(1H-indol-3-yl)propanamide



5.53

Rink resin (1 eq, 1 g, 0.75 mmol g^{-1}) was washed with DMF (10 mL DMF), before a 20 % piperidine in DMF solution (10 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 10 mL), methanol (3 x 10 mL) and again with DMF (3 x 10 mL).

To Fmoc tryptophan with the side-chain protected as the *tert*-butyl carbamate (3 eq, 1.18 g) in DMF (0.07 M, 10.7 mL) was added DIEA (3 eq, 0.39 mL) and HBTU (3 eq, 0.85 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 10 mL) and methanol (3 x 10 mL).

The resin was then was washed with DMF (10 mL DMF), before a 20 % piperidine in DMF solution (10 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 10 mL), methanol (3 x 10 mL) and again with DMF (3 x 10 mL).

To Fmoc glycine (3 eq, 0.67 g) in DMF (0.07 M, 10.7 mL) was added DIEA (3 eq, 0.39 mL) and HBTU (3 eq, 0.85 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 10 mL) and methanol (3 x 10 mL).

A fraction of the resin (1 eq, 0.68 g, ~0.75 mmol g^{-1}) was then washed with DMF (5 mL DMF), before a 20 % piperidine in DMF solution (5 mL) was added, and the suspension was swirled for 10 minutes, before being drained. After repeating the deprotection step one further time, the resin was washed successively with DMF (3 x 5 mL), methanol (3 x 5 mL) and again with DMF (3 x 5 mL).

To azidoglycine (5 eq, 0.26 g) in DMF (5 mL) was added DIEA (5 eq, 0.44 mL) and HBTU (5 eq, 0.97 g). After stirring for 15 minutes, the solution was added to the deprotected resin, and the suspension was swirled sporadically over 30 minutes, before being drained and washed with DMF (3 x 5 mL) and methanol (3 x 5 mL).

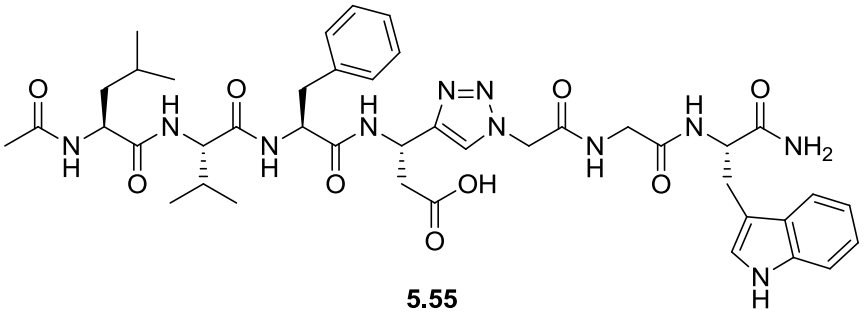
A cleavage solution (5 mL) consisting of TFA (97.5 %) and TIPS (2.5 %) was added, and the suspension was swirled sporadically over 2.5 hours, before being drained into cold diethyl ether (40 mL). The precipitate was filtered with cold diethyl ether and purified with column chromatography (2.5 % methanol in ethyl acetate) giving azide **5.53** as a white solid (24.5 mg, 14 %).

^{1}H NMR (400 MHz, MeOD) δ 7.62 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.10 (m, 2H), 7.03 (t, *J* = 7.4 Hz, 1H), 4.71 (ddd, *J* = 7.9, 6.7, 4.0 Hz, 1H), 3.96 – 3.74 (m, 4H), 3.38 – 3.28 (m, 1H), 3.22 – 3.10 (m, 1H).

^{13}C NMR (101 MHz, MeOD) δ 176.6, 171.0, 170.8, 138.0, 128.8, 124.6, 122.4, 119.9, 119.3, 112.3, 110.7, 55.2, 52.7, 43.5, 28.7.

ESI-HRMS-TOF calcd for $C_{15}H_{17}N_7O_3$ $[M+H]^+$: 344.1466 found: 344.1478.

(4S,7S,10S,13S)-13-(1-(2-((2-(((S)-1-Amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)-10-benzyl-4-isobutyl-7-isopropyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazapentadecan-15-oic acid



5.55

Resin **5.42** (1 eq, 0.19 g, 1.12 mmol g^{-1}) was washed successively with DMF (3 x 1 mL) and THF (3 x 1 mL) before a solution of TBAF in THF (10 eq, 1 M, 2.1 mL) was added, and the suspension was shaken sporadically over 2 hours. After drainage of the TBAF solution, the resin was washed with THF (3 x 1 mL), DMF (3 x 1 mL) and methanol (3 x 1 mL) and dried under vacuum.

To a proportion of the deprotected resin (1 eq, 50 mg, 1.12 mmol g^{-1}) was added azide **5.53** (2 eq, 16.7 mg) in THF (1 mL), DIEA (25 eq, 0.25 mL) and copper iodide (0.5 eq, 5.3 mg). The suspension was gently mixed continuously for 17 hours at atmospheric pressure. After drainage of the solution, the resin was washed with THF (3 x 1 mL), water (3 x 1 mL) and THF (3 x 1 mL).

The resin was cleaved with 5 x 1 mL repetitions of a cleavage solution consisting of TFA (2.5 %) and TIPS (2.5 %) in DCM, and the combined organic extracts were evaporated under reduced pressure, giving a crude brown solid. Preparative HPLC was used to further purify the compound, giving **5.55** as a white solid (0.8 mg, 1.7 %).

^{1}H NMR (400 MHz, MeOD) δ 7.61 (d, *J* = 7.7 Hz, 1H), 7.37 (s, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.25 – 7.13 (m, 5H), 7.11 (s, 1H), 7.10 – 6.99 (m, 2H), 5.44 (t, *J* = 6.8 Hz, 1H), 5.00 (AB q, 2H), 4.68 (dd, *J* = 8.2, 5.3 Hz, 1H), 4.62 (dd, *J* = 8.3, 6.4 Hz, 1H), 4.57 (s, 2H), 4.39 (dd, *J* = 9.9, 5.3 Hz, 1H), 4.11 (d, *J* = 7.1 Hz, 1H), 3.83 (q, *J* = 16.6 Hz, 2H), 2.92 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.85 (dd, *J* = 6.5, 4.2 Hz, 1H), 2.05 – 1.95 (m, 4H), 1.71 – 1.44 (m, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.85 (dd, *J* = 6.7, 4.5 Hz, 6H).

ESI-HRMS-TOF calcd for $C_{42}H_{55}N_{11}O_9$ $[M+H]^+$: 858.4257 found: 858.4265.

7.6 Peptide Assay

Assay buffer: 20 mM Tris pH 7.4, 150 mM NaCl.

Substrate: Carboxybenzoyl-IEPD-7-amido-4-trifluoromethylcoumarin, K_m = 145 µM.

Procedure: The assay was performed following the procedure of de Poot:[159] A serial two-fold dilution of inhibitor (2.5 mM to 0.078 mM or 1.25 mM to 0.039 mM, in assay buffer) for 6 data points was incubated with 94 nM human granzyme B in 100 µL of assay buffer at 37 ^{o}C for 10-15 min. The substrate (100 µM diluted in assay buffer) was then added and fluorescence followed for 50 min on a FLUOstar Galaxy plate reader (BMG Labtech; excitation filter 380 +/- 10 nm, emission filter 510 +/- 10 nm). The linear portion of the progress curves were used to determine residual human granzyme B activity, which was plotted against inhibitor concentration to estimate the IC_{50}.

Chapter 8 - References

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