

New Peptide-Based Templates

Constrained into a β -Strand by Huisgen

Cycloaddition

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

Ashok D. Pehere

M. Sc. (Org. Chem.)



Department of Chemistry

The University of Adelaide

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Abstract

Chapter One introduces the concept of peptide 'secondary structure' with an emphasis on β -strand geometry in macrocycles. This structural design is crucial for targeting different proteases. The significance of the macrocyclic β -strand 'bioactive' conformation is discussed in detail. In particular the exploitation of the conformationally constrained peptidomimetic macrocyclic backbone, which is constrained by a number of synthetic approaches to lock the 'bioactive' conformation in place.

Chapter Two describes simple and scalable methodology for the preparation of *N*-Cbz protected amino acids by reaction with Cbz-Cl which uses a mixture of aqueous sodium carbonate and sodium bicarbonate to maintain the appropriate pH. This method proceeds without the formation of by-products. The method is extended to large scale preparation of an intermediate zofenopril, an ACE inhibitor.

Chapter Three describes new peptidic templates constrained into a β -strand geometry by linking acetylene and azide containing P₁ and P₃ residues of a tripeptide by Huisgen cycloaddition. The conformations of the macrocycles are defined by NMR studies and those that best define a β -strand are shown to be potent inhibitors of the protease calpain. The β -strand templates presented and defined here are prepared under optimized conditions and should be suitable for targeting a range of proteases and other applications requiring such geometry.

Chapter four describes a new approach to non-covalent peptide-based nanotubular or rod-like structures, whereby the monomeric units are preorganised into a β -strand geometry that templates the formation of an extended and unusual parallel β -sheet rod-like structure. The conformational constraint is introduced by Huisgen cycloaddition to give a triazole-based macrocycle, with the resulting self-assembled structures stabilized by a well-defined series of intermolecular hydrogen bonds.

Chapter Five the 26S proteasome has emerged over the past decade as an attractive therapeutic target in the treatment of cancers. Here, we report new tripeptide aldehydes that are highly specific for the chymotrypsin-like catalytic activity of the proteasome. These new CT-L specific proteasome inhibitors demonstrated high potency and specificity for cancer cells, with therapeutic windows superior to those observed for benchmark proteasome inhibitors, MG132 and Bortezomib. Constraining the peptide backbone into the β -strand geometry was associated with decreased activity *in vitro* and reduced anti-cancer activity, suggesting that the proteasome prefers to bind a conformationally flexible ligand. Using these new proteasome inhibitors, we show that the presence of an intact p53 pathway significantly enhances cytotoxic activity, thus suggesting that this tumor suppressor is a critical downstream mediator of cell death following proteasomal inhibition.

Chapter Six peptide derived protease inhibitors represent an important class of compounds with the potential to treat a wide range of serious medical conditions. Herein we describe the synthesis of a series of triazole containing macrocyclic protease inhibitors preorganised in a β -strand conformation and evaluate their selectivity and potency against a panel of protease inhibitors. A series of acyclic azido-alkyne-based aldehydes is also evaluated for comparison. The macrocyclic peptidomimetics showed considerable activity towards Calpain II, Cathepsin L and S and the 26S proteasome chymotrypsin-like activity. Importantly, the first examples of potent and selective inhibitors of Cathepsin S were identified and shown to adopt a well-defined β -strand geometry by NMR, X-ray and molecular docking studies.

Chapter Seven describes simple and efficient methodology for the selective acylation and alkylation of biotin at its 3'-nitrogen. This methodology is used to prepare of other biotin derivatives.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, contains no material published or written by another person, except where due reference has been made in the text.

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Ashok Pehere

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Date

Publications arising from this thesis:

- 1) “An improved large scale procedure for the preparation of *N*-Cbz amino acids” Pehere, A. D.; Abell, A. D. *Tetrahedron Lett.* **2011**, *52*, 1493-1494.
- 2) “Selective *N*-acylation and *N*-alkylation of biotin” Pehere, A. D.; Abell, A. D. *J. Org. Chem.* **2011**, *76*, 9514-9518.
- 3) “New β -Strand Templates Constrained by Huisgen Cycloaddition” Pehere, A. D.; Abell, A. D. *Org. Lett.* **2012**, *14*, 1330-1333.
- 4) “New Cylindrical Peptide Assemblies Defined by Extended Parallel β -Sheets” Pehere, A. D.; Sumby, C. J.; Abell, A. D. (Manuscript is to be submitted to *J. Am. Chem. Soc.*).
- 5) “New 26S-proteasome inhibitors with high selectivity for chymotrypsin-like activity and p53-dependent cytotoxicity”. Neilsen, P.M.; Pehere, A. D.; Callen, D. F.; Abell, A. D. (Manuscript is to be submitted to *ACS Chem. Biol.*).
- 6) “Synthesis and extended activity of triazole-containing macrocyclic protease inhibitors”. (Manuscript in preparation).

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Abbreviations

aq	aqueous
Boc	<i>tert</i> -butoxycarbonyl
br	broad (spectroscopic)
calcd	calculated
Cbz	benzyloxycarbonyl
conc	concentrated
Cy	cyclohexyl
DCM	dichloromethane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	<i>N,N</i> -diisopropylethylamine
4-DMAP	4-Dimethylaminopyridine
DMF	dimethylformamide
DMP	Dess–Martin periodinane
DMSO	dimethyl sulphoxide
DMTr	4,4'-dimethoxytrityl group
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride
equiv	equivalent
ESI	electrospray ionisation
Et	ethyl
FTIR	Fourier transform infrared
h	hour(s)
HATU	2-(7-aza-1 <i>H</i> -benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HIV	Human Immunodeficiency Virus
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
iPA	isopropylalcohol

IR	infrared
lit.	literature value
Me	methyl
min	minute(s)
mp	melting point
Ms	methylsulphonyl (mesyl)
MS	mass spectrometry
<i>m/z</i>	mass-to-charge ratio
NMR	nuclear magnetic resonance
PDB	Protein Data Bank
Ph	phenyl
PI	protease inhibitor(s)
Ppm	part(s) per million
Pr	propyl
PTSA	<i>p</i> -toulenesulphonic acid
Py	pyridine
quant	quantitative
RCM	ring closing metathesis
rt	room temperature
SAR	structure activity relationship
spec	spectrometry
TBAB	tetrabutylammonium bromide
TBAI	tetrabutylammonium iodide
TCE	1,1,2-trichloroethane
TEA	triethylamine
temp	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Ts	<i>para</i> -toluenesulphonyl (tosyl)
UV	ultraviolet
v/v	volume per unit volume
w/w	weight per unit weight

Statement of Authorship

Paper 1

“An improved large scale procedure for the preparation of *N*-Cbz amino acids” Pehere, A. D.; Abell, A. D. *Tetrahedron Lett.* **2011**, *52*, 1493-1494.

Mr. Ashok Pehere (candidate)

Performed all the experimental work, interpreted data, and prepared manuscript.

I hereby certify that the statement of contribution is accurate.

Signed

date 13/06/2012

Professor Andrew Abell

Supervised development of work, assisted in data interpretation, and revised the manuscript, and is the corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

Signed ..

date 13/6/2012

Statement of Authorship

Paper 2

“New β -Strand Templates Constrained by Huisgen Cycloaddition” Pehere, A. D.; Abell, A. D. *Org. Lett.* **2012**, *14*, 1330-1333.

Mr. Ashok Pehere (candidate)

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Signed ..

date 13/6/2012

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Paper 3

“New Cylindrical Peptide Assemblies Defined by Extended Parallel β -Sheets” Pehere, A. D.; Sumbly, C. J.; Abell, A. D. (Submitted manuscript to *J. Am. Chem. Soc.*).

Mr. Ashok Pehere (candidate)

Performed all the experimental work, interpreted data, and prepared manuscript.

I hereby certify that the statement of contribution is accurate.

Signed .

date...13/06/2012

Dr. Christopher Sumbly

Assisted with analysis by X-ray and interpretation of data, and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

Signed

....

date...19/06/2012

Professor Andrew Abell

Supervised development of work, assisted in data interpretation, and revised the manuscript, and is the corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

Signed

date...13/6/2012

Statement of Authorship

Paper 4

“New 26S-proteasome inhibitors with high selectivity for chymotrypsin-like activity and p53-dependent cytotoxicity.” Neilsen, P.M.[#]; Pehere, A.D.[#]; Callen, D.F.; Abell, A.D. (Submitted manuscript to *ACS Chem. Biol.*).

[#]These authors contributed equally to this work.

Mr. Ashok Pehere (candidate)

Performed all the synthetic experimental work (unless stated otherwise, see below), interpreted data, and prepared manuscript.

I hereby certify that the statement of contribution is accurate.

Signed

date.....13/06/2012

Dr. Paul Neilsen

Performed and interpreted biological assays and other biological work and assisted in the prepared of the manuscript

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

Signed

date.....14/06/12

Professor David Callen

Assisted with data interpretation and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

Signed

date 13/6/2012

Professor Andrew Abell

Supervised development of work, assisted in data interpretation and revised the manuscript, and is the corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

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Statement of Authorship

Paper 6

“Selective N-acylation and N-alkylation of biotin” Pehere, A. D.; Abell, A. D. *J. Org. Chem.* **2011**, *76*, 9514-9518.

Mr. Ashok Pehere (candidate)

Performed all experimental work, interpreted data, and prepared manuscript.

I hereby certify that the statement of contribution is accurate.

Signed

date...13/06/2012

Professor Andrew Abell

Supervised development of work, assisted in data interpretation, and revised the manuscript, and is the corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in this thesis.

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Chapter One

1.1 Protein structure

Proteins display an amazing diversity of structure that allows them to partake in a myriad of functions.¹ This includes catalysis (e.g. enzymes), immune protection (e.g. antibodies), transport and storage (e.g. hemoglobin, myoglobin), nerve impulse transmission (e.g. synaptic receptors), providing mechanical strength (e.g. collagen), coordinated movement (e.g. actin, myosin), cell adhesion (e.g. fibronectin), gene expression (e.g. transcription factors) and overall growth and maintenance of living organisms (e.g. hormones, proteases).^{1,2} Protein structure is not always sufficient for activity, with many enzymes requiring small non amino acid compounds, known as cofactors and coenzymes, in order to function. Good examples of this include NADH, pyridoxal phosphate and biotin. Biotin is a particularly interesting structure in that it is directly associated with the function of biotin protein ligase (BPL), a new identified target for antibiotic discovery.³ Biotin is the topic of chapter seven in this thesis.

The diversity of function of proteins is associated with their ability to form well-defined primary, secondary, tertiary and in some cases quaternary structure. Secondary structure is particularly important in this context and it is a major topic of this thesis. Secondary structure represents the ordered conformation or arrangement of amino acids in localized regions of a polypeptide or protein molecule. Three common secondary structures are found in proteins, α -helices, β -sheets and turns. Each of these is characterised and stabilised by specific hydrogen bonding between the constituent carbonyl and amine functionality.

The three standard secondary structures are characterised by a series of rotational angles designated Φ , Ψ , and Ω . These are shown in the (Figure 1.1) and defined as follows:

Φ = dihedral angle about atoms $C_i, N_{i+1}, C_{ai+1}, C_{i+1}$

Ψ = dihedral angle about atoms $N_i, C_{ai}, C_i, N_{i+1}$

Ω = dihedral angle about atoms $C_{ai}, C_i, N_{i+1}, C_{ai+1}$,

where subscript i represents the i^{th} residue in a sequence and $i+1$ represents the adjacent C-terminal residue.

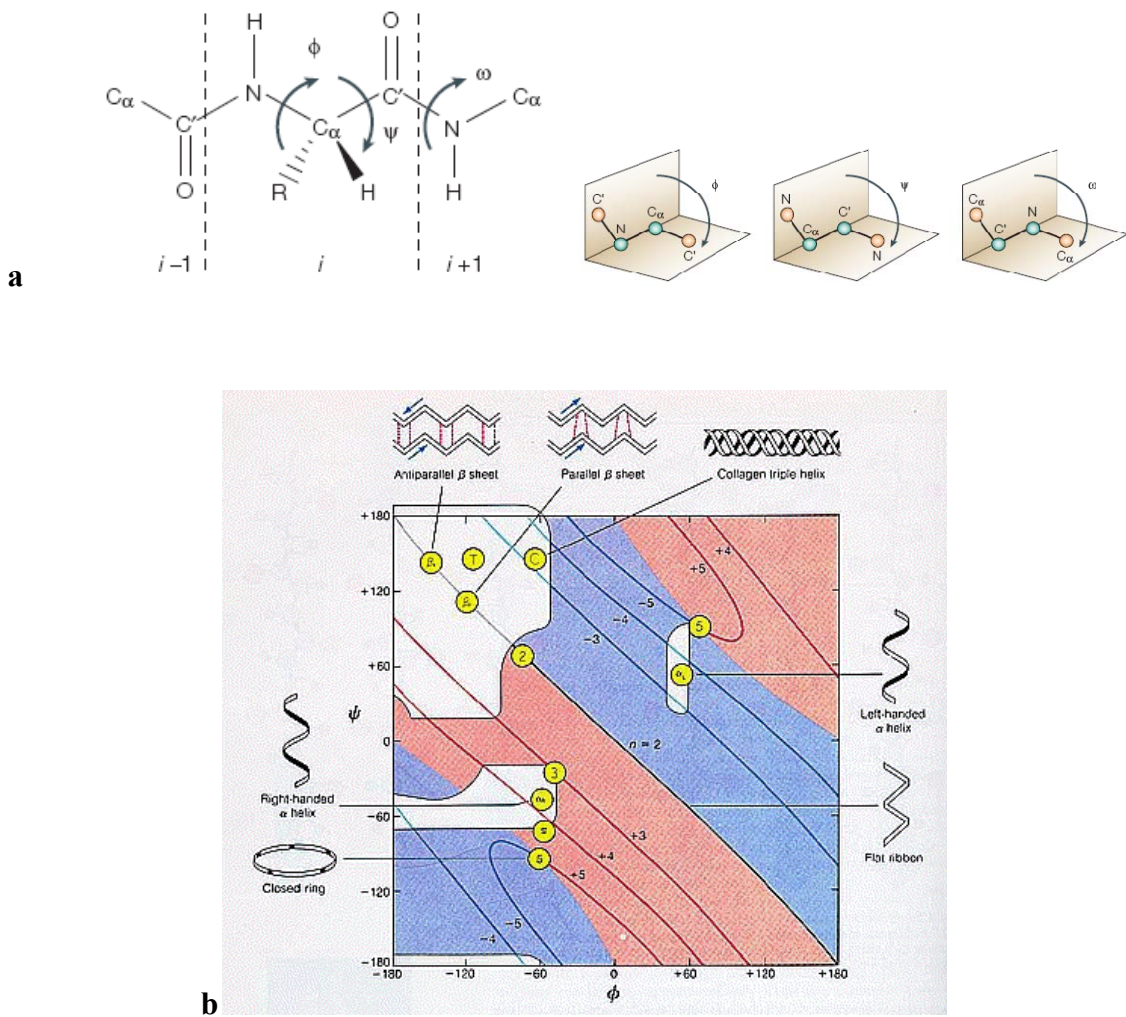


Figure 1.1. a Conformations of peptides definitions of the Φ , Ψ , and Ω torsional angles⁴ **b** Ramachandran (Φ , Ψ) plot, showing the broad, favorable region around the conformation typical for α -helix, β -sheet, α -helix residues.⁵

In peptides and proteins secondary structures conformations for a peptide chain are determined by the Φ and Ψ torsional angles. (Figure 1.1a).⁴ The Φ and Ψ values in polypeptide chains can be plotted on a Ramachandran map⁶ (Figure 1.1b) which provides a useful means of identifying allowable values. The positions of major structures are indicated by yellow circles (i.e. parallel and antiparallel β -sheet, α -helix and 3_{10} -helix) and show allowed Φ and Ψ angles. Blue and pink areas show sterically disallowed conformations. Protein structure all fall within allowed regions (Figure 1.1a).

The α -helix is the most common secondary structure found in proteins, being observed in over 40% of natural polypeptides (Figure 1.2). This structure is stabilised by a specific hydrogen bonding network, through which each amide carbonyl oxygen of residue i is occupied in a hydrogen bond to the amide NH proton of the $i + 4$ residue. Every turn of the helix is composed of about 3.5 amino acids and covers 5.4 Å. A consequence of this structure is that all the amino acid side chains are projected onto the outer face of the helix. Certain amino acids such as Ala, Glu, Leu, Lys and Met are commonly found in α -helices, whereas Gly, Pro, Ser, and Tyr are far less common.

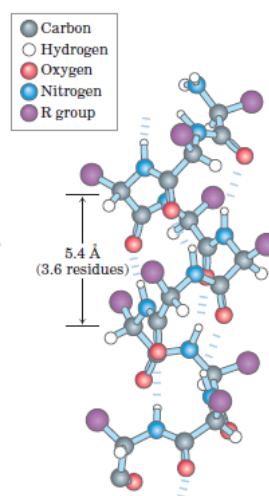


Figure 1.2 Idealized three-dimensional α -Helix.¹

β -Strands are also a particularly common secondary structural element. The backbone of a β -strand is extended with its hydrogen-bonding groups pointing orthogonal to the direction of the chain (Figure 1.3). The side chains of a β -strand alternate between the two faces of the strand; effectively giving it a 2-residue repeat. As a result, amphipathic β -strands contain alternating patterns of hydrophobic and polar amino acids. The positions of the amino acid side chains within an extended β -strand conformation are separated by a maximum distance with the i and $i+4$ residues which are normally 14.5 Å apart. A β -strand peptide (within an antiparallel β -sheet) configuration results in the i and $i + 4$ α -carbons being 13.2 Å apart and amide bond angles of $\Phi = -139^\circ$ and $\Psi = -135^\circ$ (Figure 1.3).⁷

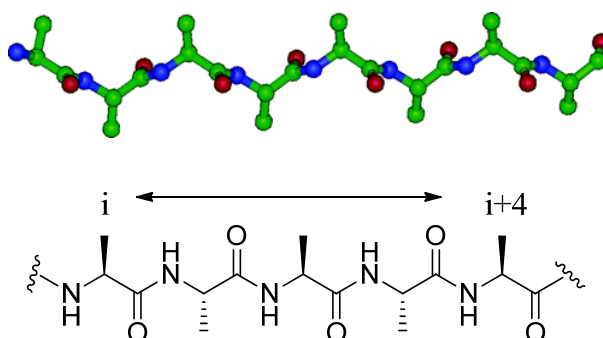


Figure 1.3 Two representations of a β -strand.⁷

β -strands self associate by hydrogen bonding to give β -sheets that adopt a twisted pleated arrangement (Figure 1.4). These structures contribute to the biological functions of proteins and in particular they act as scaffolds to further stabilize protein structure. The amide backbones of each contributing strand project toward each other to allow hydrogen bonding between the respective amide carbonyls and NH protons. Three types of β -sheets are found in proteins and these are classified as either parallel, antiparallel or mixed. The strands of an antiparallel β -sheet run in opposite directions with hydrogen bonding resulting in fourteen-membered rings as shown in (Figure 1.4a). Parallel β -sheets are much less common and consist of strands running in the same direction to give a series of twelve-membered hydrogen-bonded rings, see (Figure 1.4b) and Chapter four for further discussion. Mixed β -sheets contain a mixture of both parallel and antiparallel sheets.

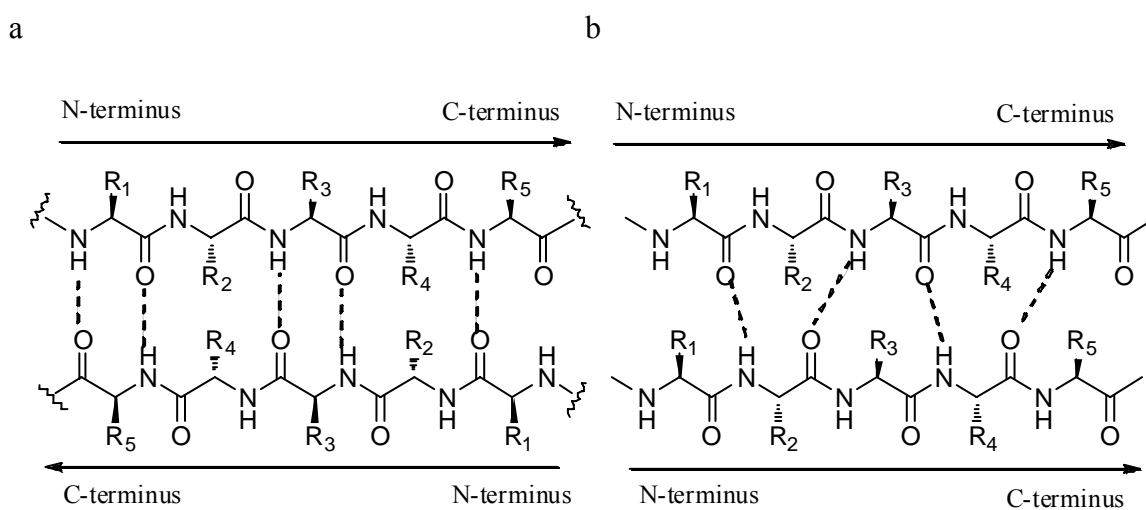


Figure 1.4 (a) Antiparallel β -sheet. (b) Parallel β -sheet.⁷

Peptide chains can form only two consecutive peptide unit bends in the same direction, while the peptide units before and after these bonds maintain the linear (actually zigzag) structure, the protein is said to make a reverse turn (U-Turn) at these two peptide units (Figure 1.5). The peptide chains on either side of the bend run antiparallel. They are further classified on the basis of Φ , Ψ , and Ω bond angles.

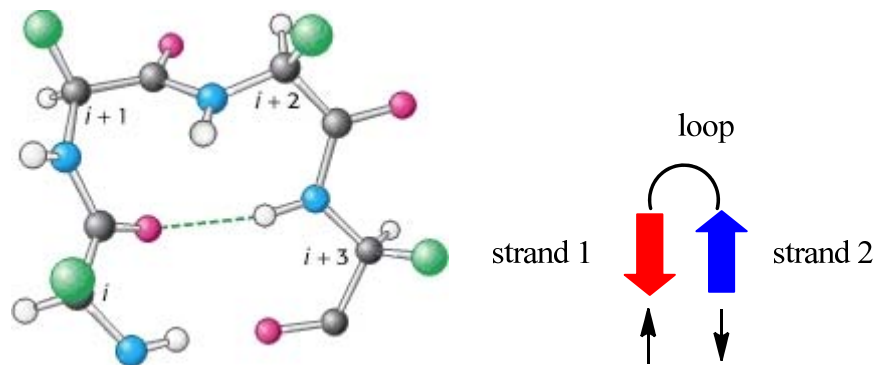


Figure 1.5 Idealized three-dimensional reverse turns.⁸

1.2 Proteases

A protease is an enzyme that hydrolyses peptide (amide) bonds within a protein or peptide substrate.⁹ Each particular protease recognizes and hence cleaves, characteristic sequences of amino acids within its substrate. These recognition sequences are typically incorporated into the design of protease inhibitors to facilitate binding as discussed in Chapter six. Each amino acid R group (designated P) within a substrate or inhibitor, binds with a complementary binding pocket (designated S) in the protease active site (Figure 1.6).¹⁰ These subsites are then numbered depending on their position from the scissile bond as shown.¹¹

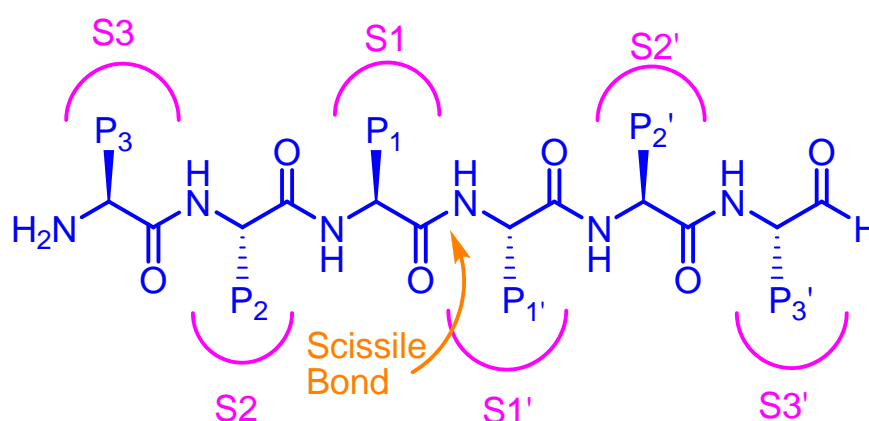


Figure 1.6 Diagram showing the interactions between a peptide substrate (with residues P_x) and a protease (binding sites S_x) using the nomenclature of Schechter and Berger.¹¹

Proteases are categorised by the catalytic residue that effects enzymatic hydrolysis. Six classes of proteases are currently recognised¹² serine, threonine, cysteine, aspartic, glutamic, metallo-proteases.¹³ These enzymes play major roles in the regulation of key biological processes and as such their inhibition provides a basis of therapeutics for treating a wide range of diseases. Examples include, but are not limited to, cancer,^{14,15} cataract,^{16,17} HIV^{18,19} and neurological diseases such as Alzheimer's.²⁰

A β -strand conformation is central to the binding of protease substrates and subsequent inhibitor design. A study of more than 1500 three dimensional X-ray structures of proteases in the Protein Data Bank concluded that proteases universally bind the ligands in a β -strand conformation.²¹ As outline above, a β -strand motif is defined by the bond angles Φ , Ψ and τ , with optimum angles of -139° , 135° and -177.2° , respectively and $d = 8.0 \text{ \AA}$ (Figure 1.7). This conformation allows the side chain residues to have maximum exposure to the protease's active sites, which in turn facilitates binding³⁰ as shown in (Figure 1.6).

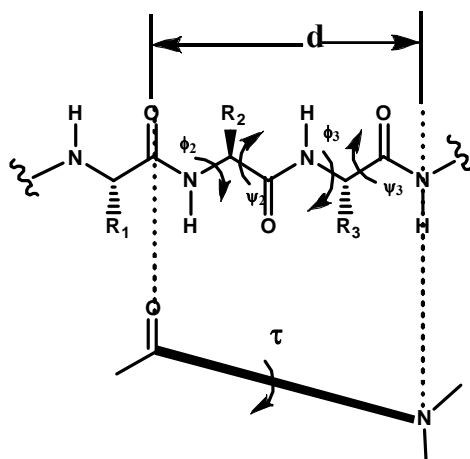


Figure 1.7: Peptide β -strand backbone with torsional angles Φ , Ψ , τ and distance d .²²

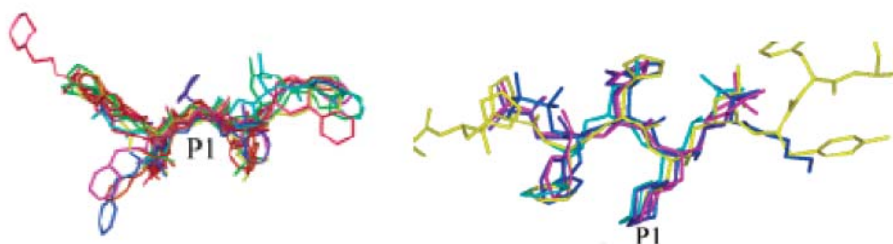


Figure 1.8: Overlay of backbone of known inhibitors when bound to proteases: cathepsin K (cysteine protease) (right) and renin (aspartic protease) (left).²³

An overlay of the backbone of known inhibitors bound to the cysteine protease cathepsin K and aspartic protease renin (Figure 1.8), illustrates that all these inhibitors are bound in a β -strand conformation. The importance of adopting a β -strand conformation³⁰ for ligand binding has significant implications in protease function and inhibitor design. Work in this thesis focuses in Chapters three to six on the design, synthesis and testing of inhibitors of three proteases: the cysteine protease calpain, cathepsins and the proteasomes, using structures pre-organised into a β -strand geometry.

1.2.1 Calpain

Calpain, a member of the cysteine protease family, is an intracellular protease located throughout a multitude of biological systems, which requires calcium ions for activation.²⁴ Over-activation of this protease can result in a number of physiological issues, such as cataract formation,²⁵ Alzheimer's disease,²⁶ stroke²⁷ and diabetes,²⁸ hence its regulation is important.²⁹ Calpain requires calcium for activation, and the two common isoforms, μ -calpain (calpain 1) and m-calpain (calpain 2), require micromolar and millimolar concentrations of calcium respectively for activation *in vitro*.

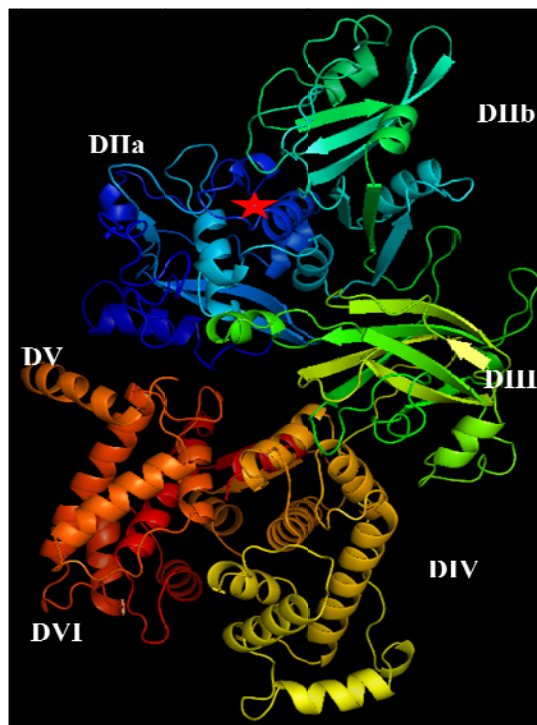


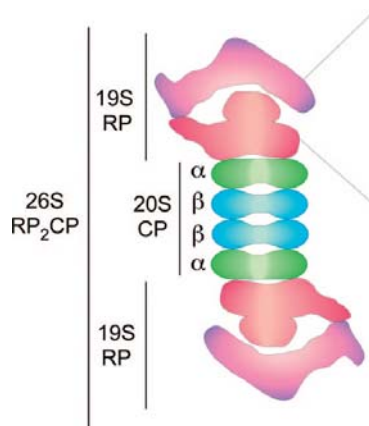
Figure 1.9: Schematic domain representation of m-calpain.³⁰

μ - and m-Calpain are very similar in structure, with both containing a large subunit approximately 80 kD (orange) and a smaller subunit approximately 30 kD (blue) (Figure 1.9). The large subunit consists of domains I to IV and the smaller subunit of domains V and VI. Calcium binding at DIV and DVI is required for activation as this brings the catalytic triad of calpain in the active site (red star) located at DII into the active proteolytic conformation.

1.2.2 26S Proteasome

The 26S proteasome is a multifunctional, 2,500,000 Da proteolytic complex molecular that is classified into the 20S core particle structure and two 19S regulatory caps (Figure 1.10).^{31,32} Proteolysis occurs in the interior of the barrel-shaped 20S structure. The two 19S regulatory subunits overtake the recognition of the ubiquitin-tagged proteins, cleavage of the polyubiquitin chains, unfolding and translocation into the 20S proteasome.^{33,34} The proteasome (20S) has a cylindrical structure that consists of four rings stacked on top of each other that are arranged in a $\alpha_7\beta_7\beta_7\alpha_7$ manner. The two inner rings are comprised of β -subunits, where the proteolytic activities reside. The two outer rings contain α -subunits that do not have enzymatic activity. The β subunits possess three major proteolytic activities: β_1 , β_2 , β_5 .

A



B

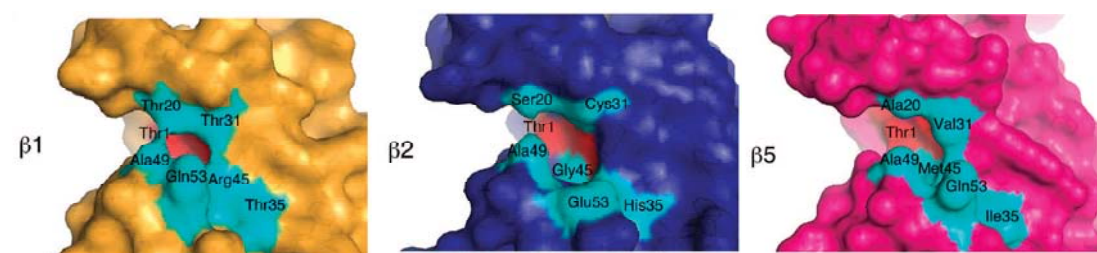


Figure 1.10. A) Schematic representation of the 26S proteasome.³¹ B) Shown are the S1 pockets of the three active site subunits.³¹

The 20S proteasome has three separate proteolytic activities. The β 1-subunit possesses caspase-like activity resulting in preferential cleavage on the C-terminal side of acidic residues (aspartic acid, glutamic acid) within its substrate. The β 2-subunit has trypsin-like activity with associated cleavage of its substrate on the C-terminal side of basic residues (arginine and lysine). The β 5-subunit shows chymotrypsin-like activity to cleave peptide bonds on the C-terminal side of hydrophobic residues (e.g. phenylalanine, tryptophan, and tyrosine).

The proteasome is typically hyperactive in malignancies,³⁵ with the dipeptidylboronic acid PS-341 (bortezomib), recently been approved by the USFDA for the treatment of multiple myeloma³⁶ and is commercially available under its trade name Velcade®.³⁶ See Chapter five for further discussion.

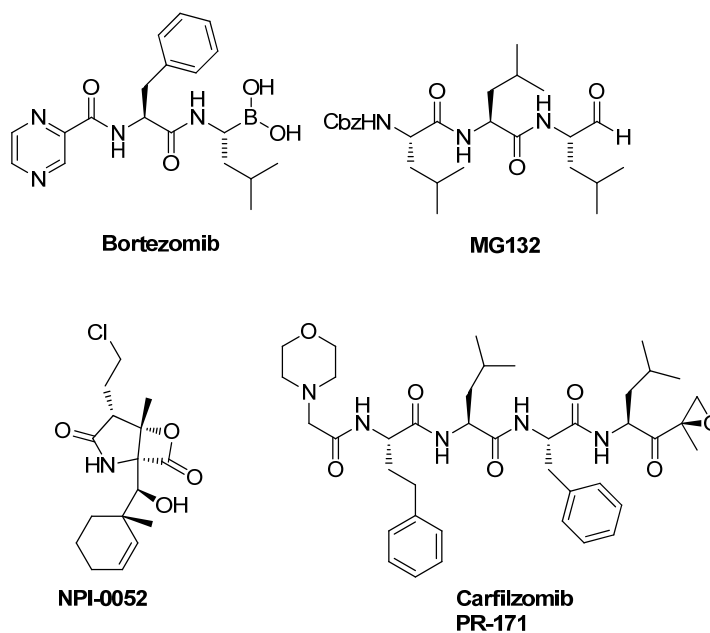


Figure 1.11. Chemical structure of the marketed proteasome inhibitor Bortezomib and of the proteasome inhibitors currently being evaluated in clinical trials.

While Bortezomib is high efficiency in monotherapy it does have undesirable side-effects and resistances profiles. As such it is frequently used in combination with other therapeutics.³⁷ A further three proteasome inhibitors are currently being evaluated in clinical trials, i.e. carfilzomib, NPI-0052 and MG132 (Figure 1.11).^{38,39}

1.2.3 Cathepsin family

Cathepsins are lysosomal peptidases that belong to cysteine, serine, or aspartic protease classes. There are eleven human cathepsins proteases that have been identified (B, C, F, H, K, L, O, S, U, W and X).⁴⁰⁻⁴⁵ This thesis focused on thiol-dependent cathepsins that represent a class of mammalian cysteine proteases mainly located in lysosomes. Cathepsins C, L, K and S are important in several pathological conditions, such as bone remodeling,⁴⁶ keratinocyte differentiation,^{47,48} heart functions⁴⁹ and reproduction.⁵⁰

The catalytic site of papain-like cysteine proteases is highly conserved and is defined by three residues: Cys25, His159, and Asn175 (papain numbering). Cys25 and His159 form an ion pair that is stabilized by hydrogen bonding to Asn175. This triad has some similarities to the active site present in serine proteases (Ser, His, Asp). However, in contrast to serine proteases, the nucleophilic cysteine residue is already ionized prior to substrate binding and thus cysteine proteases can be regarded as a prior activated enzymes.⁵¹ During peptide hydrolysis, the nucleophilic thiolate cysteine attacks the carbonyl carbon of the bound substrate and forms a tetrahedral intermediate which is stabilized by the oxyanion hole. The tetrahedral intermediate transforms into an acyl enzyme with the simultaneous release of the C-terminal portion of the substrate (acylation), followed by the hydrolysis of the acyl enzyme, forming a second tetrahedral intermediate. Finally deacylation splits into the free N-terminal portion and enzyme portion of the substrate. The sequence of reactions is shown in (Figure 1.12).⁴⁴

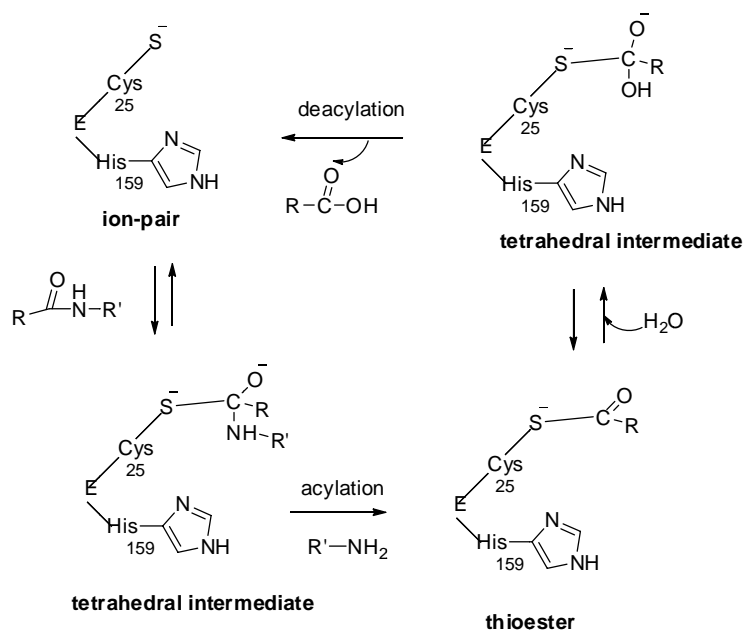


Figure 1.12. Catalytic mechanism of cysteine proteases.⁴⁴

Cathepsin S forms a monomeric structure⁴⁵ consisting of two spherical left (L) and right (R) domains. In the middle of the pocket residues Cys-25 and His-159 form the catalytic site of the enzyme. The L-domain contains three α -helices and a hydrophobic core, whereas the R-domain consists of β -barrel containing a short α -helices motif (Figure 1.13).

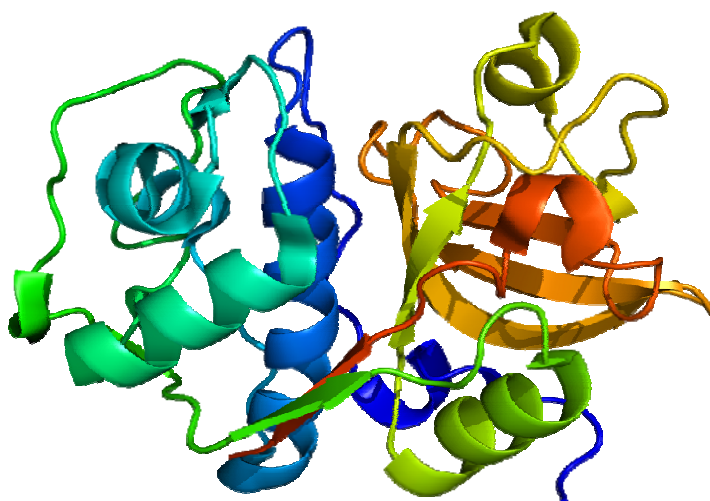


Figure 1.13 Secondary-structure ribbon model of cathepsin S (PDB 1glo).⁴⁵

1.3 Secondary structure mimics of a β -strand

As already discussed a β -strand is an important structural element that is universally recognized by MHC (histocompatibility complex) and proteases proteins.^{7,21}

Peptide-based substrates of proteases do not form specific conformations in solution and thus their arrangement into a β -strand is required for binding. Conformationally pre-organizing or fixing the shape, as recognized by the protease, will impart a higher affinity due to reduced entropy for adopting the receptor binding shape.⁷

The peptidomimetics described here are constrained into a β -strand geometry to give improved drug-like properties relative to peptides. There are a number of approaches for introducing a conformational constrain, including the following three:

1. β -Sheet nucleating templates
2. Backbone-modified β -strand mimetics
3. Macrocyclic β -strand mimetics

1.3.1 β -Sheet nucleating templates

β -Sheet nucleating templates are generated by a two-residue nucleus in a β -turn conformation that is stabilized by a 4 – 1 hydrogen bond. This then provides two reactive functional handles to which can be attached both N- and C- terminal polypeptide chain segments. Figure 1.14 shows a representative set of such a template.

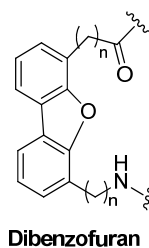


Figure 1.14 β -sheet nucleating template.

The $n=2$ dibenzofuran scaffold (Figure 1.15) is more efficient at stabilizing a β -sheet structure within an attached peptide sequences than is the shorter $n=1$ analogue. The scaffold must then be flanked by hydrophobic amino acids to give the necessary hydrophobic cluster⁵² and has found use within peptides that have antiangiogenic activity.⁵³

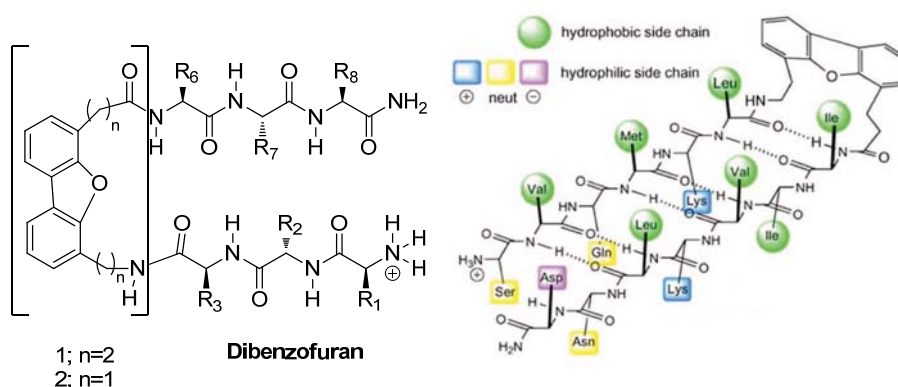
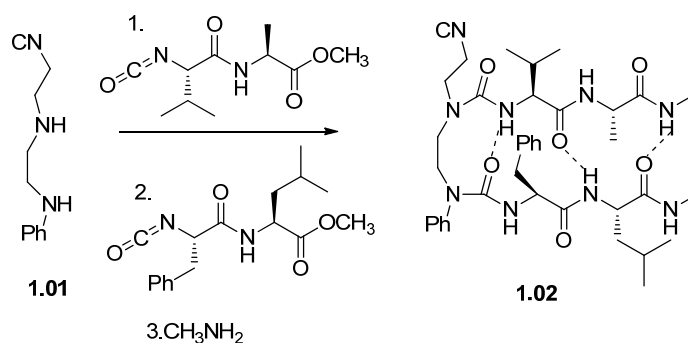


Figure 1.15 Polar residues on the hydrophilic side of the amphipathic β -sheet of 6DBF7 are highlighted with squares, whereas non polar residues on the hydrophobic side of the amphipathic β -sheet of 6DBF7 are highlighted with circles.⁵³

Nowick and coworkers developed nucleating templates in which an intramolecular hydrogen-bonded oligourea molecular scaffold templates a parallel β -sheet structure for example see **1.02** in Scheme 1.1. Structure **1.02** provides a template for parallel β -sheet formation. Artificial parallel β -sheet **1.02** was synthesized by sequential treatment of diamine **1.01** with peptide isonitriles **1** and **2**, followed by aminolysis with methylamine.

^1H NMR spectroscopic studies showed that the molecule adopts a hydrogen-bonded β -sheet structure in chloroform solution and that the phenyl group on the diurea scaffold helps control the relative orientation of the two dipeptide strands. The urea-based turn structure of **1.02** forms a parallel β -sheet.⁵⁴



Scheme 1.1 Parallel β -Sheet.

1.3.2 Backbone-modified β -strand mimetics

Numerous approaches to mimicking a β -strand by replacing all or part of a peptide backbone with a small heterocyclic group designed to restrict conformation have been reported; several examples are shown in (Figure 1.17).

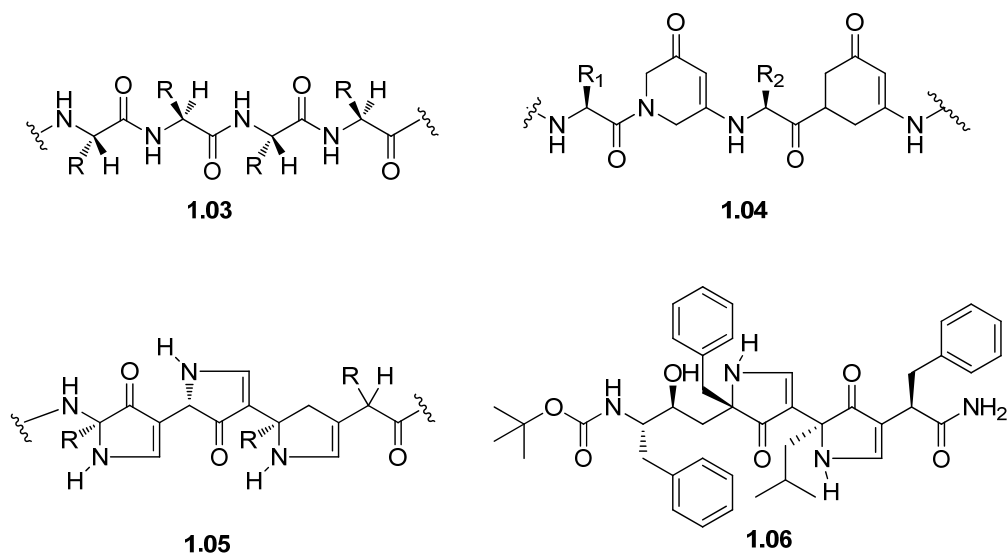


Figure 1.17 Modified backbone β -strand mimics.

For example, Hirschmann and Smith reported pyrrolinone-based non-peptide scaffolds for β -strand mimics **1.06** to target HIV-1 protease in an attempt to improve bioavailability and proteolytic stability.^{55,56}

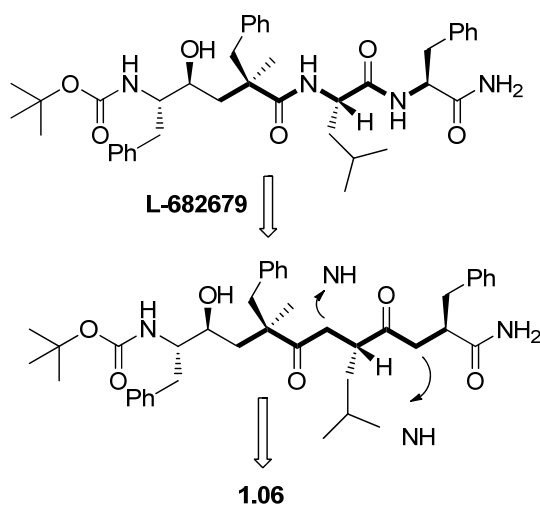


Figure 1.18 Pyrrolinone-based β -strand mimetics. Pyrrolinone motif is generated by NH displacement from peptide backbone.

A pyrrolinone based motif was generated where the P₁'-P₃' sequence in the known HIV-1 protease inhibitor **L-682,679** was replaced by a bis(pyrrolinone) (Figure 1.18). The resulting bis(pyrrolinones) retained potency of inhibition with IC₅₀ of 10 nM, with improved cell transport capacity. In cellular antiviral assay, **L-682,679** and **1.06**^{55,57} showed CIC₉₅ values of 6.0 and 1.5 μM, respectively.

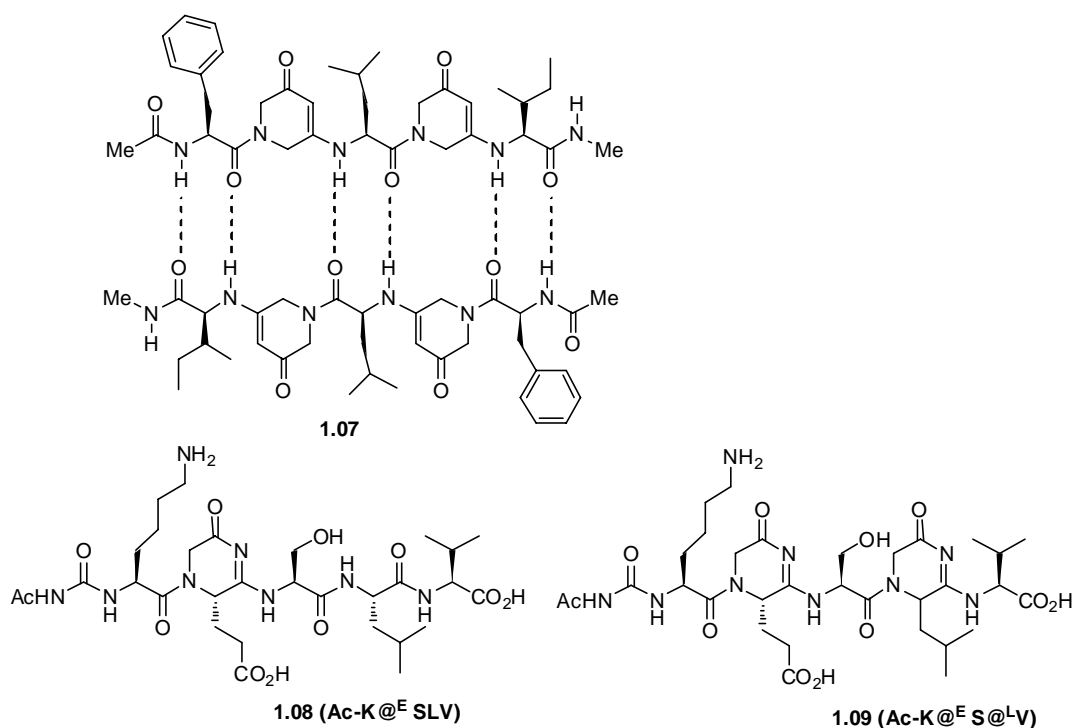


Figure 1.19

Azacyclohexenone units based repeating rigid scaffold have also been reported as a basis of β-strand mimic to target PDZ protein, (see structure 1.07).⁵⁸ These structures were named @-tides, (azacyclohexenone unit is a one letter code “@”). The component cyclic amino acid results in conformational restriction, while also limiting the backbone hydrogen bonding to a single edge of the strand. NMR studies on **1.07** showed that penta-@-tide **1.07** forms a stable β-strand dimer in an antiparallel conformation, (Figure 1.19). Replacing amide groups with @-tides gave **1.08** and **1.09**, resulting in high resistance to protease-mediated degradation,⁵⁹ and were shown to be potent ligands to a PDZ protein-interaction domain.

1.4 Macrocylic β -strand mimetics

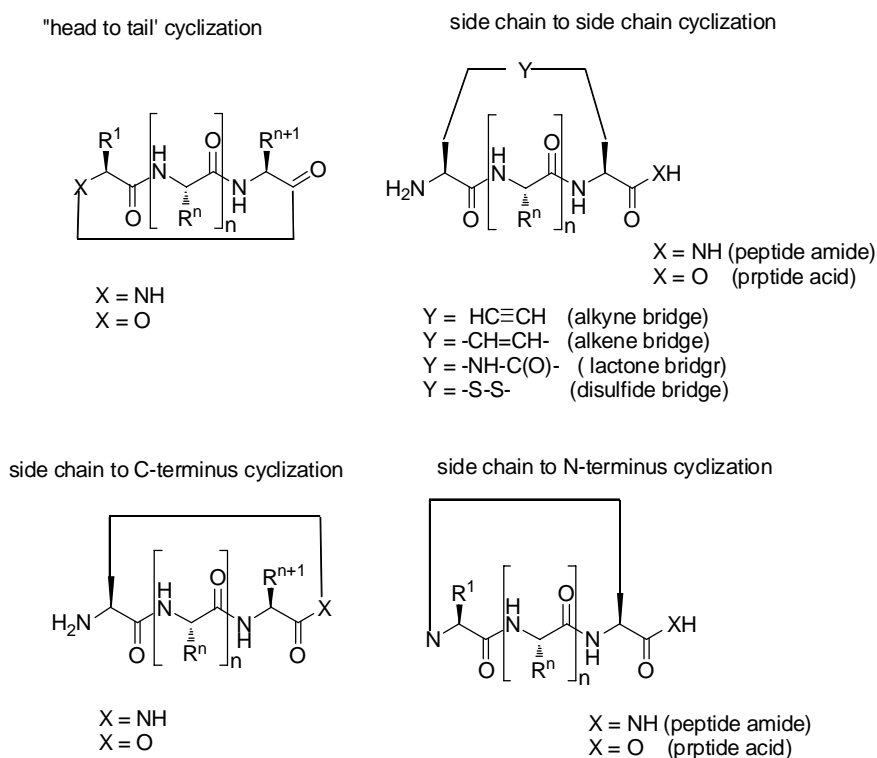


Figure 1.20 Different approaches of peptide cyclization.⁶⁰

As already discussed, a peptide backbone can be preorganized into a β -strand conformation to facilitate protease binding by introducing side chain to side chain or main chain linkages as shown in (Figure 1.20).^{60,61} The requisite macrocycle can be introduced by cyclisation of an appropriate acyclic precursor by alkylation, lactamisation or ring closing metathesis (RCM) (Figure 1.20). There are also naturally occurring examples of these kinds of structures. These structures offer advantages of; i) increased proteolytic stability, ii) improved receptor selectivity, iii) enhanced bioavailability and iv) increased potency.

1.4.1 Naturally occurring macrocylic protease inhibitors

Macrocycles **1.10** (QF4949-IV) and **1.11** (K-13) are potent inhibitors of the aminopeptidase B and ACE^{62,63} respectively. The component cyclic 17-membered biphenyl ether tripeptides contain a biaryl ether diamino acid and an isodityrosine as their basic structural subunit (Figure 1.21). Compound **1.11** was shown to be a potent, non competitive ACE inhibitor ($K_i = 0.349 \mu\text{M}$) and a weak inhibitor of aminopeptidase B.⁶²

These agents showed aminopeptidase B inhibitory activity ($K_i = 61 \mu\text{M}$).^{63,64} Molecular modelling, NMR and X-ray analysis showed that the 17-membered cyclic tripeptide **1.10** and **1.11** is constrained into a β -strand conformation.^{65,66} These cyclic biaryl ether tripeptides inspired the design of several types of β -strand mimics incorporated in macrocyclic compounds including the HIV protease inhibitor **1.12**.⁶⁶

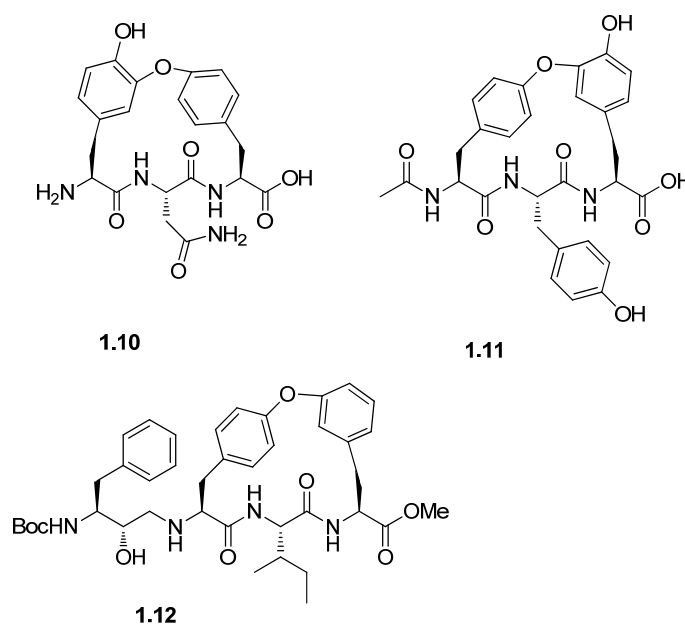
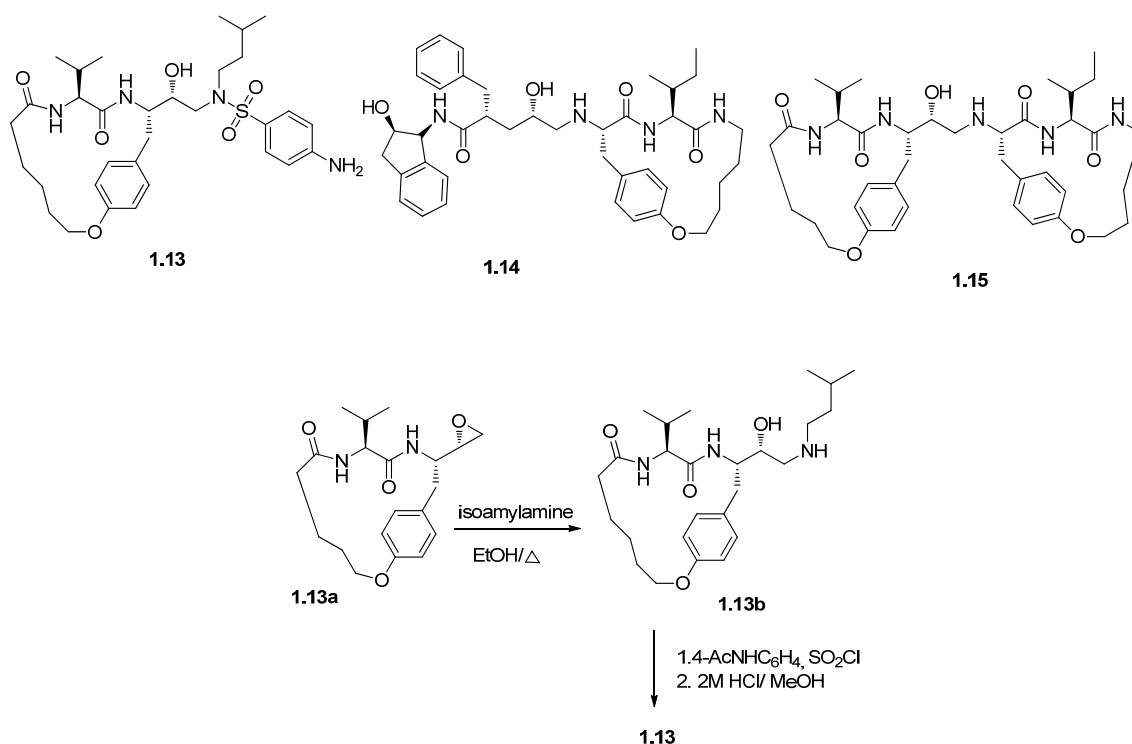


Figure 1.21 Naturally occurring Macrocycles.

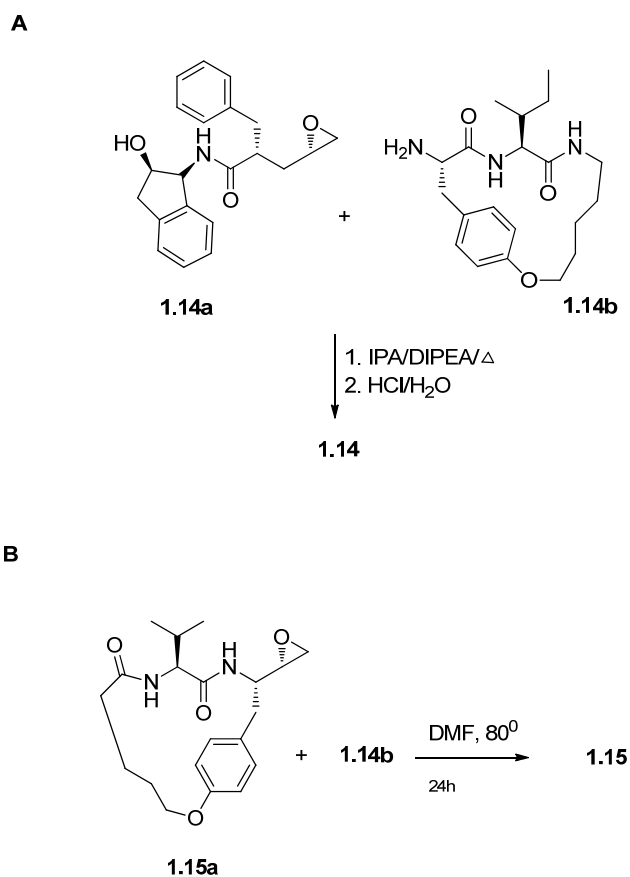
1.4.2 Macrocyclic protease inhibitors prepared by alkylation

Numerous macrocyclic inhibitors of HIV proteases have been prepared by alkylation of para substituted aromatic rings, to form 15 to 17-membered rings.⁶⁷ The component β -strand constraint prevents intramolecular hydrogen bonding and preorganizes the macrocycle into an extended β -strand conformation required for protease binding.⁶⁸ The conformational rigidity enforced by the macrocycles also decreases affinity for other biological receptors to increase selectivity while reducing potential for undesirable side effects. The macrocycles **1.13**, **1.14** and **1.15** are potent and selective inhibitors of HIV proteases, with IC_{50} values of 90, 60 and 5 nM respectively.



Scheme 1.2 Synthesis of macrocycle **1.13**.

The macrocycles **1.13** and **1.14** were made by solution phase coupling of the epoxide. Coupling of epoxide **1.13a** with isoamylamine followed by aminosulfonate gave **1.13**. Also coupling of epoxide **1.14a** with amine **1.14b** gave **1.14**. The synthesis of **1.15** involved coupling the epoxide **1.15a** with the amine **1.14b**. The β -strand mimic **1.13** retains all the amide bonds and the associated hydrogen-bonding interactions evident in the X-ray structure of peptide **1.13** bound to receptor HIV-1(PDB 1mt7) as shown in (Figure 1.22).⁷



Scheme 1.3 A) Synthesis of macrocycle **1.14**. B) Synthesis of macrocycle **1.15**.

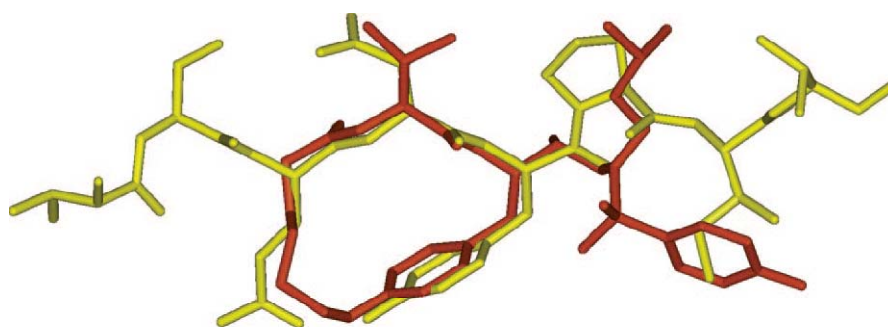


Figure 1.22 Comparison of the HIV-1 protease active site binding conformation of macrocyclic inhibitor **1.13** (red) and the linear peptidic substrate (yellow).³⁴

1.4.3 Macrocyclic protease inhibitors prepared by ring closing metathesis (RCM)

The olefin metathesis reaction has a wide range of applications in the area of macrocyclization.⁶⁹ Ring-closing metathesis (RCM) has subsequently found applicability in our group for the preparation of protease inhibitors with the introduction of both small and larger sized macrocyclic rings.⁷⁰ Others have including natural product-based scaffolds using this methodology.^{71,72} The replacement of natural constraints, such as S–S or CO–NH bridges, with metabolically more stable synthetic bridges is an alternative approach. A C–C covalent bond has the advantage of being less susceptible to proteolytic degradation.

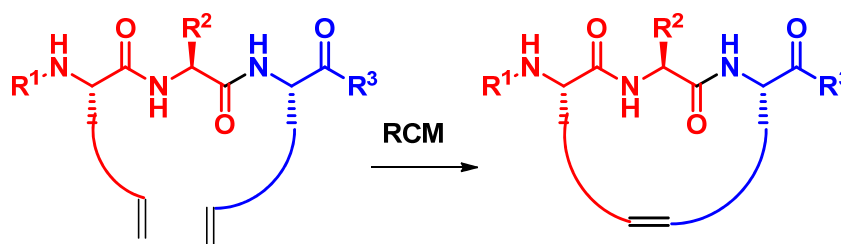
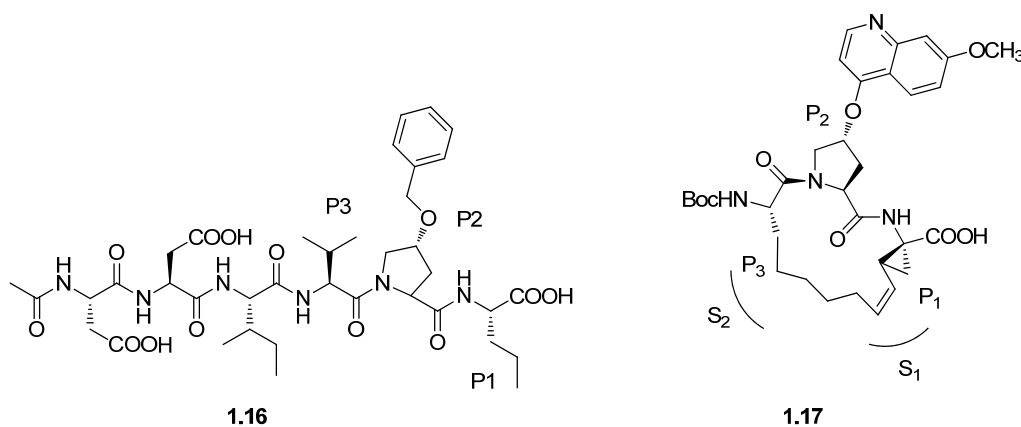


Figure 1.23 Schematic representation of ring closing metathesis (RCM) in macrocyclic peptide.

All these approaches link the P₁ and P₃ amino acid side chain groups in order to constrain the cyclic structure into a β -strand conformation, (see Figure 1.23).

Acyclic compound **1.16** has been shown by NMR and molecular modelling to bind with HCV NS3 serine protease in an extended β -strand conformation ($IC_{50} = 400$ nM). Modification of **1.16** by ring closure between the P₁ and P₃ side chains to a 15-membered macrocycle **1.17** with a *trans* P₂ amide bond and *Z* double bond results in a more potent HCV NS3 serine protease inhibitor (IC_{50} value of 24 nM).⁷³



Macrocycle **1.18** is a potent aspartic protease BACE inhibitor ($IC_{50} = 156$ nM) formed by linking the P₁ and P₃ residues in the acyclic BACE inhibitor.⁷⁴ An overlay of the X-ray crystal structures of the macrocyclic inhibitor **1.18** and the linear peptide **1.19** is shown in (Figure 1.24). The backbones of both adopt an extended β -strand conformation in the active site of the aspartic protease BACE. The crucial hydrogen-bonding interactions observed in the complex of the acyclic inhibitor **1.19** with active site of aspartic protease BACE (including interactions with Gly34, Pro70, Thr72, Gln73, Gly230, and Thr232) are also observed with macrocycle **1.18**. The introduction of the macrocycle enhances the propensity of the bioactive β -strand conformation, known to favour active-site binding and thereby accounting for improved affinity for the enzyme.

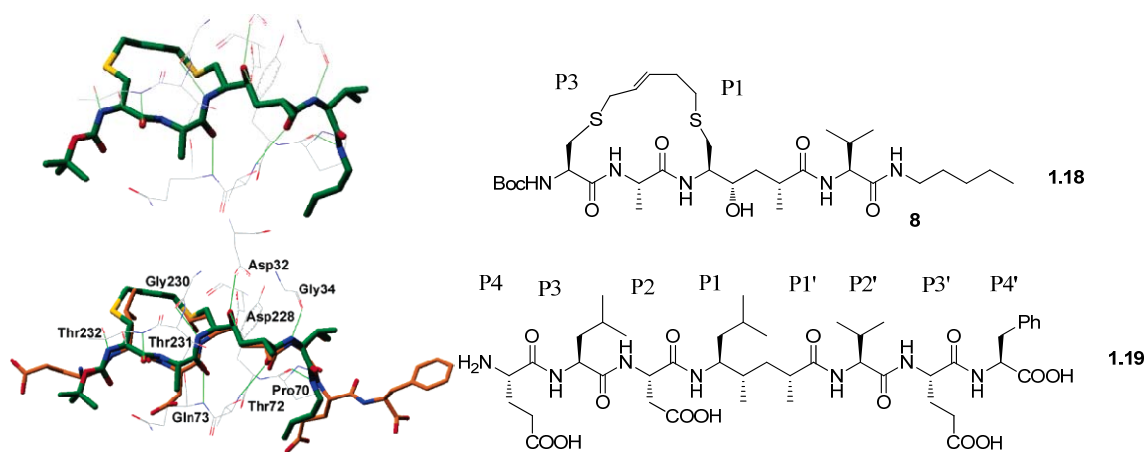
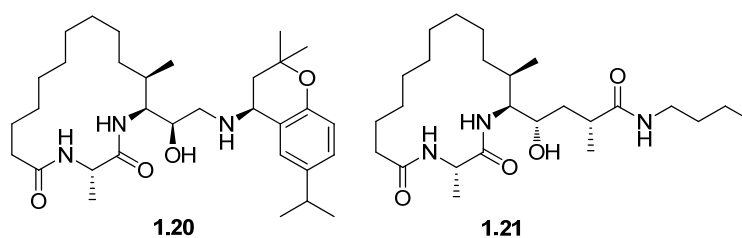
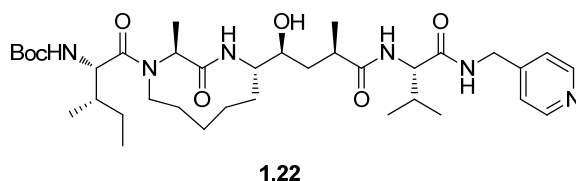


Figure 1.24. X-ray structure of macrocycle **1.18** (dark green)-BACE complex (top) and overlay of the X-ray structures of BACE complexes with macrocycle **1.19** (dark green) and acyclic **1.19** (orange) (bottom).

Compounds **1.20** and **1.21** are macrocyclic BACE-1 inhibitors that have been shown to inhibit cellular release of A β -40 **1.20** and block A β -42 production in mice **1.21**.^{75,76}



Macrocycle **1.22** is a β -strand mimetic-based inhibitor of secretase (BACE) ($IC_{50} = 650$ nM), one of the enzymes involved in producing β -amyloid peptide associated with Alzheimer's disease which have been cocrystallized with BACE.



The 17-membered tyrosine based macrocycle **1.23** (**CAT811**) has been reported by our research group as a potent inhibitor of calpain II with an $IC_{50} = 30$ nM.⁷⁰

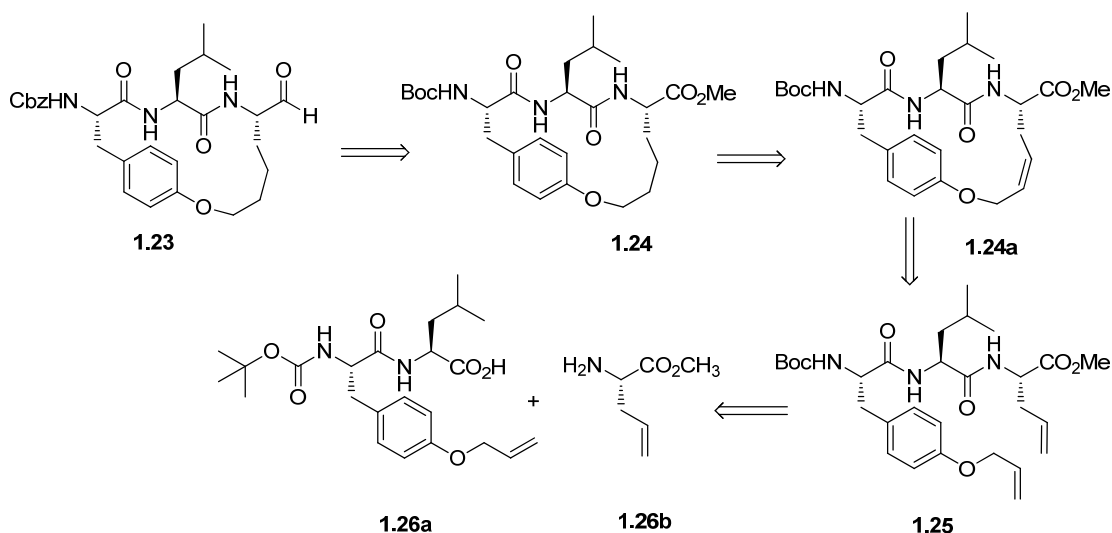


Figure 1.25 Tyrosine based macrocyclic calpain inhibitor using RCM.

Macrocycle **1.23** contains a C-terminal aldehyde to interact with the active site cysteine to give reversible inhibition. This macrocycle was prepared as shown in (Figure 1.25) by reaction of *N*-Boc-allylamino acid **1.26a** and allylamino acid methyl ester **1.26b** to give the diene **1.25**, which underwent RCM on treatment with Grubbs 2nd generation catalyst to give **1.24a**. The alkene of the macrocycle **1.24a** was hydrogenated and Boc group removed. The resulting amine was protected with a Cbz group and the ester reduced to the aldehyde **1.23**.

A conformational search on XCluster^{70,78} shows that these tyrosine based macrocycles exhibit a high percentage of β -strand conformers. The macrocycle **1.23** was also docked into calpain II. As shown in (Figure 1.26) the computational studies revealed the following key points:

1. Macrocyclic **1.23** exhibit three essential hydrogen bonds with Gly271 and Gly208 of the active site of calpain that stabilise a β -strand conformation of the peptide chain.
2. The warhead distance for aldehyde **1.23**, as defined by the distance between the warhead carbonyl carbon and the active cysteine sulfur in Å, is less than 4.5 Å. A distance of less than 4.5 Å is required for nucleophilic attack by the sulfur of cysteine for a reversible covalent inhibitor. Φ , Ψ
3. The macrocycle **1.23** adopts a high percentage of β -strand conformers. The Boltzmann weighted percentage of β -strand for macrocycles was based on the Ψ (Psi) and Φ (Phi) angles of the P₂ leucine amino acid. Ramachandran plots of Ψ , Φ angles for β -strand or β -sheet regions of protein X-ray crystal structures show typical Ψ angles to be between 90° and 160° and that of Φ angles to be between -90° and -160° (see Figure 1.26).⁷⁸

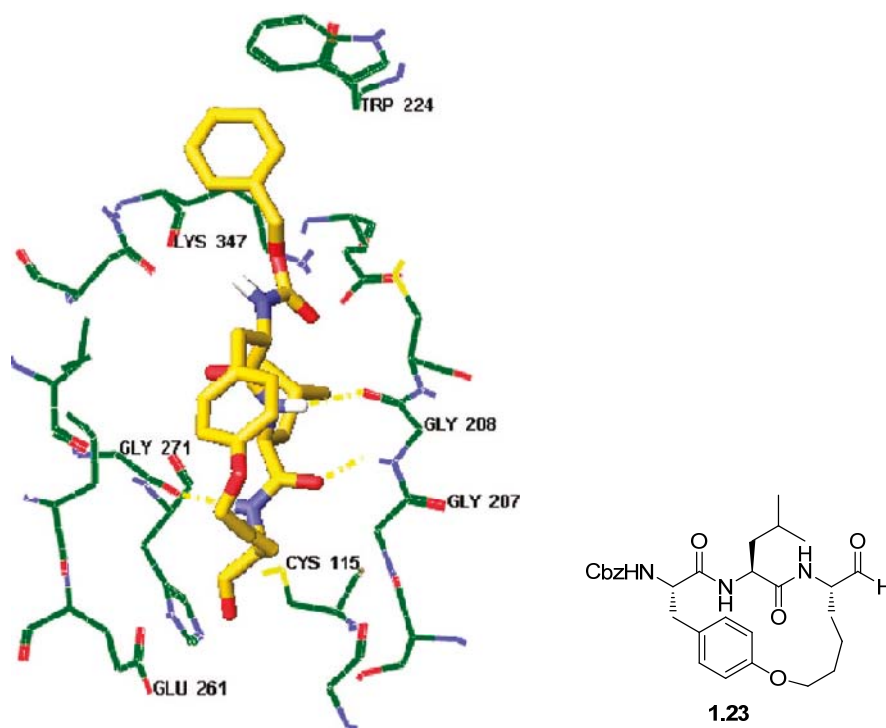
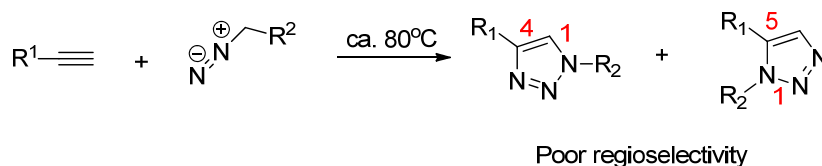


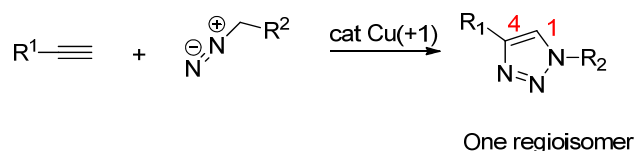
Figure 1.26 Macrocycle **1.23** and its best docked pose.⁷⁸

1.4. 4 A new method for protease inhibitors prepared by azide-alkyne cycloadditions

The Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC) has recently emerged as a powerful new tool in organic synthesis with widespread use in the preparation of modified peptide and peptidomimetics.⁷⁹ The broad chemical orthogonality and versatility of CuAAC gives it broad applicability to a range of applications ranging from drug discovery, pharmacology, materials science and nanotechnology.⁸⁰ The original thermal conditions reported for this general reaction give rise to two regioisomers (1,4 and 1,5).



(reaction 1)



(reaction 2)

Scheme 1.2 Cu-Catalyzed azide alkyne cycloaddition

The groups of Meldal⁸¹ then Sharpless,⁸² independently, found that copper(I) catalysis results in high regioselectivity, with formation of the 1,4-isomer (see reaction 2). This transformation also has the advantage of high chemoselectivity since few functional groups react with azides or alkynes in the absence of other reagents.

This example of a 1,3-dipolar cycloaddition (Huisgen Cycloaddition) has several attributes that are highly attractive for peptidomimetic synthesis:

1. A 1,2,3-triazole is compatible with the side chains of all the amino acids, at least in protected form.
2. The reaction is high yielding with few by products.
3. Triazoles bear a strong physicochemical resemblance to amide bonds due to their relative planarity and strong dipole moment.

Mechanism of copper-catalyzed azide-alkyne cycloaddition reaction

The mechanism of the copper-catalysed azide-alkyne Huisgen cycloaddition reaction is proposed as shown in Figure 1.27.^{83,84} Step one involves the alkyne coordinating to the Cu(I) species resulting in the displacement of a ligand (L_n) and the terminal hydrogen. The azide then replaces another ligand as it binds to the copper atom forming intermediate **c**. The terminal nitrogen then interacts with the C-2 carbon of the acetylide forming a six membered copper (III) metallocycle **d**, this structure has a low energy barrier for ring contraction hence the triazolyl-copper derivative quickly forms **e**. In the final step proteolysis occurs releasing the free 1,4-disubstituted triazole **f** and the copper catalyst [L_nCu]⁺.

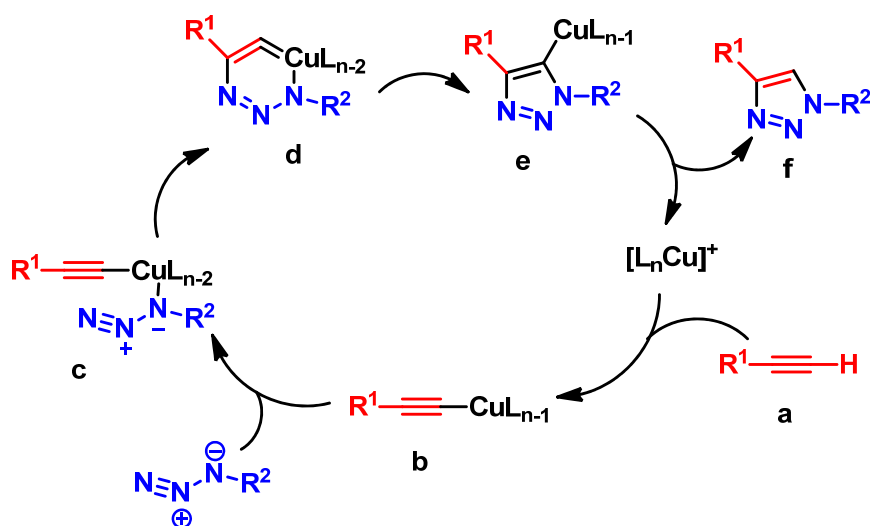


Figure 1.27 Putative mechanism of the copper-mediated azide-alkyne Huisgen cycloaddition.^{83,84}

To date there have not been any reports on using CuAAC to define a specific β -strand geometry. In Chapter three of this thesis this reaction is used to construct new β -strand templates of the type shown in (Figure 1.28). Unlike other methods discussed above this approach is fully compatible with nature's aqueous environment. The high functional group tolerance of the CuAAC reaction and the relatively high resistance to metabolic degradation of the 1,2,3-triazole moiety provides access to a range of β -strand peptides containing a side chain-to-side chain triazole bridge. This provides access to a range of related structures using a variety of 'clickable' building blocks with an opportunity to target key proteases associated with important medical conditions and nano structure.

1.5 Research described in this thesis

A biocompatible click-based[#] synthetic methodology for cyclising tripeptides in order to stabilize their known biological active conformation is developed. This leads to improved biostability and hence therapeutic potential. Chapters three, four, five and six describe these synthetic methodologies, conformational studies and biological inhibition of key metabolic enzymes, namely the proteases calpain, cathepsin and 20S proteasome.

The work in Chapter Two describes a simple and scalable methodology for protection of *N*^α-amino acids with Cbz-Cl that is a crucial part of additional recognition moieties for macrocyclic compounds described in chapters three to six. Chapter Three describes the methodology to synthesize β -strand templates by using ring closing Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC). This methodology is used for synthesising different target compounds in chapters four to six. Chapter Four describes a new approach to peptide-based nanotubular structure, whereby macrocyclic units are constrained into a β -strand conformation, linked together by parallel hydrogen bonding to form cylindrical structures. Chapter Five describes the design and synthesis of a series of acyclic 20S proteasome inhibitors and investigates their anticancer activity and p53-dependent cytotoxicity. Chapter Six describes testing of a β -strand macrocyclic library of inhibitors against a series of calpain 2, cathepsin L, cathepsin S and 20S proteasome protease enzymes.

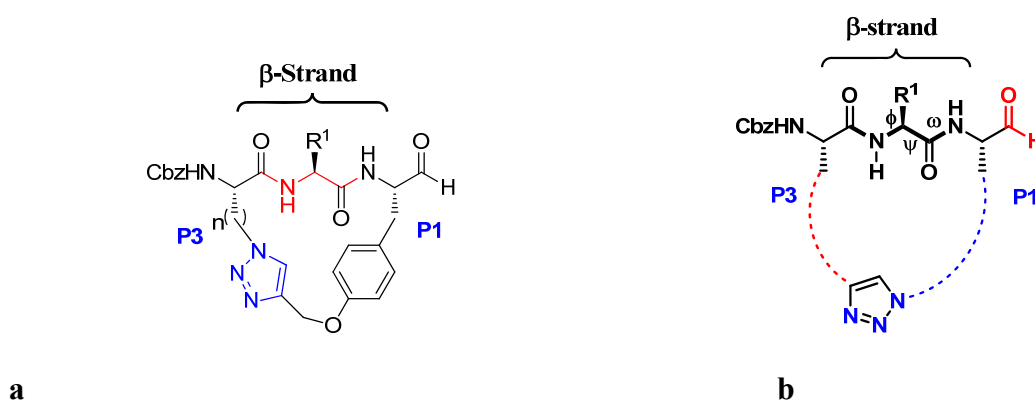


Figure 1.28 Macrocyclisation template.⁸⁵

[#]Click refers to a Huisgen 1,3-dipolar-cycloaddition reaction of an azide with an acetylene to give a triazole.

Short sequences of L-amino acids, specifically Tyr, Leu and Phe, were used in the design of the macrocycles in order to favour the formation of a β -strand in the sequence.^{7,70} The P₁ and P₃ residues of the backbone are linked by CuAAC using a number of different acetylenes and azides building blocks as shown in (Figure 1.28). In particular, three series of triazole containing macrocycles were targeted.

i) Macrocycles containing an aryl group at P₃ and triazole at P₁ **1.29** (see Figure 1.29), this feature is favored for calpain and cathepsins.

ii) Macrocycles containing an aryl group at P₁ and the triazole at P₃ **1.30** (see Figure 1.29), to target the chymotrypsin-like activity of the proteasome .

iii) Third series lacking the aryl group **1.27** and **1.28** (see Figure 1.29), with the structures remaining constrained into a β -strand geometry for self assembly studies.

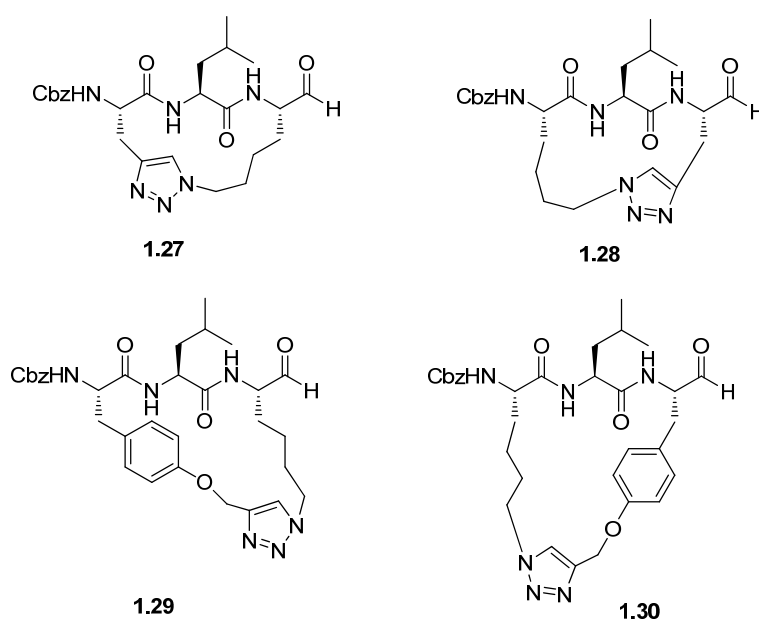
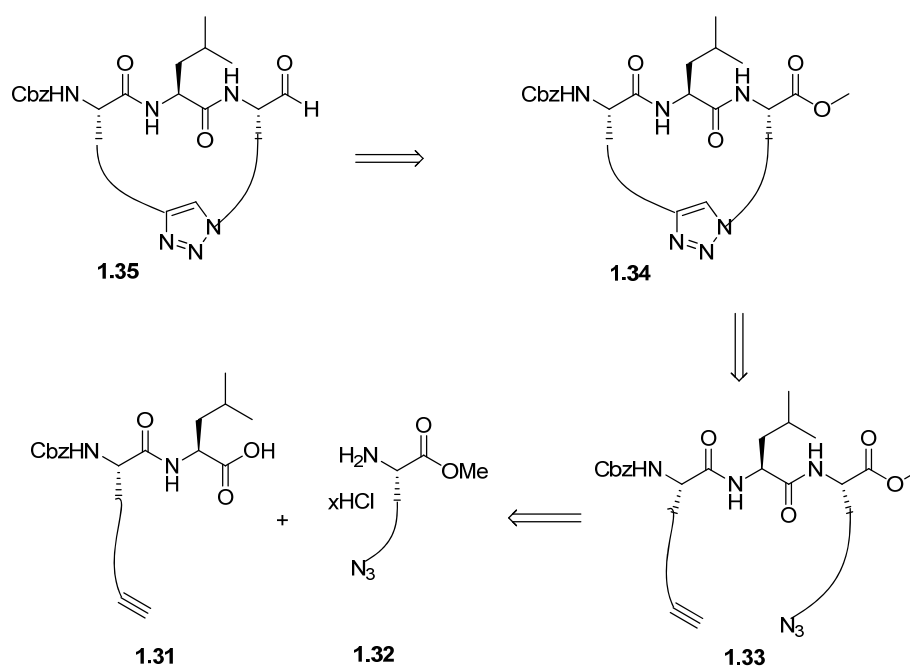


Figure 1.29 Targets macrocycles

Conformation search will be carried out on target macrocycles (see Figure 1.29) in order to define their ability to adopt β -strand conformation as is required for protease binding.



Scheme 1.5 Retrosynthetic analysis.

A general synthetic method is developed to prepare a library of macrocyclic β-strand compounds as shown in scheme 1.5 and discussed in detail in chapters three, four and six. This synthesis involves a series of peptide coupling with copper catalysed Huisgen 1,3 dipolar cycloaddition to give the triazole.

Chapter seven targets enzymes that utilise Biotin. Biotin is a water-soluble B vitamin that functions as a prosthetic group for the biotin-dependent carboxylases, key metabolic enzymes found in all organisms. Biotin protein ligase (BPL) is a crucial enzyme, its primary function is to catalyze the post-translational attachment of the co-factor biotin onto specific lysine residue on the biotin carboxyl carrier protein (BCCP). Due to its biological relevance, biotin and its derivatives attract considerable interest. The N1'-substituted derivative is an important inhibitor of biotin CoA carboxylase, and the N3' substituted derivative is a substrate for biotin protein ligase. The ureido nitrogens of biotin involve hydrogen-bonding interaction with the protein. Hence, selective methods for functionalizing the two ureido nitrogens of biotin is significant. Chapter seven exclusively describes the selective functionalization of biotin at its N1' and N3' derivatives.

1.5.1 Linkage between publications

Listed below are the six papers that comprise Chapters 2, 3, 4, 5, 6 and 7. All were completed during candidature, and Chapters 2, 3, and 7 have been published in peer-reviewed, international journals. Chapter 4 and 5 manuscript to be submitted for publication. Chapter 6 comprises in preparation manuscript.

- Chapter 2. “An improved large scale procedure for the preparation of *N*-Cbz amino acids” Pehere, A. D.; Abell, A. D. *Tetrahedron Lett.* **2011**, *52*, 1493-1494.
- Chapter 3. “New β -Strand Templates Constrained by Huisgen Cycloaddition” Pehere, A. D.; Abell, A. D. *Org. Lett.* **2012**, *14*, 1330-1333.
- Chapter 4. “New Cylindrical Peptide Assemblies Defined by Extended Parallel β -Sheets” Pehere, A. D.; Sumby, C. J.; Abell, A. D. (Manuscript is to be submitted to *J. Am. Chem. Soc.*).
- Chapter 5. “New 26S-proteasome inhibitors with high selectivity for chymotrypsin-like activity and p53-dependent cytotoxicity”. Neilsen , P.M.; Pehere, A. D.; Callen, D. F.; Abell, A. D. (Manuscript is to be submitted to *ACS Chem. Biol.*).
- Chapter 6. “Synthesis and extended activity of triazole-containing macrocyclic protease inhibitors”. (In preparation manuscript).
- Chapter 7. “Selective *N*-acylation and *N*-alkylation of biotin” Pehere, A. D.; Abell, A. D. *J. Org. Chem.* **2011**, *76*, 9514-9518.

1.6 References

- (1) Lehninger's Principles of Biochemistry (4th ed.). New York, New York: W. H. Freeman and Company.
- (2) Gutteridge, A.; Thornton, J. M. *Trends Biochem. Sci.* **2005**, *30*, 622.
- (3) Hooper, N. M., Proteases: a primer. *Essays in Biochemistry* 2002, *38*, (Proteases in Biology and Medicine), 1-8.
- (4) Hruby, V. J. *Nat. Rev. Drug Discov.* **2002**, *1*, 847.
- (5) Mathews, C. K.; van Holde, K. E. *Biochemistry* (2th ed.). The Benjamin/Cummings Publishing Company Menlo Park, CA.
- (6) Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 283.
- (7) Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Hill, T. A.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, Pr32.
- (8) Berg, J.M.; Tymoczko, J. L.; Stryer L.; Freeman, W.H. *Biochemistry*. 5th edition, New York: W H Freeman; 2002.
- (9) Turk, B. *Nat. Rev. Drug Discov.* **2006**, *5*, 785.
- (10) Cuerrier, D.; Moldoveanu, T.; Davies, P. L. *J. Biol. Chem.* **2005**, *280*, 40632.
- (11) Schechte, I; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
- (12) Hilt, W. *Cell. Mol. Life Sci.* **2004**, *61*, 1615.
- (13) Hooper, N. M. *Essays Biochem.* **2002**, *38*, 1.
- (14) Johnson, L. L.; Dyer, R.; Hupe, D. J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 466.
- (15) Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Discov. Today* **1996**, *1*, 16.
- (16) Biswas, S.; Harris, F.; Dennison, S.; Singh, J.; Phoenix, D. A. *Trends Mol. Med.* **2004**, *10*, 78.
- (17) Nixon, R. A. *Ageing Res. Rev.* **2003**, *2*, 407.
- (18) West, M. L.; Fairlie, D. P. *Trends Pharmacol. Sci.* **1995**, *16*, 67.
- (19) Tyndall, J. D. A.; Reid, R. C.; Tyssen, D. P.; Jardine, D. K.; Todd, B.; Passmore, M.; March, D. R.; Pattenden, L. K.; Bergman, D. A.; Alewood, D.; Hu, S. H.; Alewood, P. F.; Birch, C. J.; Martin, J. L.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 3495.
- (20) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, L.;

- Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
- (21) Madala, P. K.; Tyndall, J. D. A.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, Pr1.
- (22) Glenn, M. P.; Fairlie, D. P. *Mini-Rev. Med. Chem.* **2002**, *2*, 433.
- (23) Tyndall, J. D.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 973.
- (24) Pietsch, M.; Chua, K. C.; Abell, A. D. *Curr. Top. Med. Chem.* **2010**, *10*, 270.
- (25) Stradner, A.; Foffi, G.; Dorsaz, N.; Thurston, G.; Schurtenberger, P. *Phys. Rev. Lett.* **2007**, *99*, 198103.
- (26) Tsuji, T.; Shimohama, S.; Kimura, J.; Shimizu, K. *Neurosci. Lett.* **1998**, *248*, 109.
- (27) Tsuji, T. *American Physiological Society* **2001**, *281*, H1286.
- (28) Horikawa, Y, O. N., Cox, N, Li, Xiangquan, Orho-Melander, M, Hara, M, Hinokio, Y, Linder, T. H, Mashima, H, Schwarz, P., del Bosque-Plata, L, Horikawa, Y, Oda, Y, Yoshiuchi, I, Colilla, S, Polonsky, K, Wei, S, Concannon, P, Iwasaki, N, Schulze. *Nat. Genet.* **2000**, *26*, 163.
- (29) Abell, A. D.; Jones, M. A.; Neffe, A. T.; Aitken, S. G.; Cain, T. P.; Payne, R. J.; McNabb, S. B.; Coxon, J. M.; Stuart, B. G.; Pearson, D.; Lee, H. Y.-Y.; Morton, J. D. *J. Med. Chem.* **2007**, *50*, 2916.
- (30) PyMOL software was used to render the image, PDB 3DF0.
- (31) Marques, A. J.; Palanimurugan, R.; Matias, A. C.; Ramos, P. C.; Dohmen, R. J. *Chem. Rev.* **2009**, *109*, 1509.
- (32) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.
- (33) Eytan, E.; Ganoth, D.; Armon, T.; Hershko, A. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7751.
- (34) Hough, R.; Pratt, G.; Rechsteiner, M. *J. Biol. Chem.* **1987**, *262*, 8303.
- (35) Kisselev, A. F.; Goldberg, A. L. *Chem. Bio.* **2001**, *8*, 739.
- (36) Delforge, M. *Expert Opin. Pharmacol.* **2011**, *12*, 2553.
- (37) Naujokat, C.; Fuchs, D.; Berges, C. *Bba-Mol. Cell Res.* **2007**, *1773*, 1389.
- (38) Sterz, J.; von Metzler, I.; Hahne, J. C.; Lamottke, B.; Rademacher, J.; Heider, U.; Terpos, E.; Sezer, O. *Expert Opin. Investig. Drugs* **2008**, *17*, 879.
- (39) Moore, B. S.; Eustaquio, A. S.; McGlinchey, R. P. *Curr. Opin. Chem. Biol.* **2008**, *12*, 434.
- (40) Buhling, F.; Gerber, A.; Hackel, C.; Kruger, S.; Kohnlein, T.; Bromme, D.; Reinhold, D.; Ansorge, S.; Welte, T. *Am. J. Respir. Cell Mol. Biol.* **1999**, *20*, 612.

-
- (41) Buhling, F.; Peitz, U.; Kruger, S.; Kuster, D.; Vieth, M.; Gebert, I.; Roessner, A.; Weber, E.; Malfertheiner, P.; Wex, T. *Biol. Chem.* **2004**, *385*, 439.
- (42) Haeckel, C.; Krueger, S.; Buehling, F.; Broemme, D.; Franke, K.; Schuetze, A.; Roese, I.; Roessner, A. *Dev. Dyn.* **1999**, *216*, 89.
- (43) Krueger, S.; Kalinski, T.; Hundertmark, T.; Wex, T.; Kuster, D.; Peitz, U.; Ebert, M.; Nagler, D. K.; Kellner, U.; Malfertheiner, P.; Naumann, M.; Rocken, C.; Roessner, A. *J. Pathol.* **2005**, *207*, 32.
- (44) Lecaille, F.; Kaleta, J.; Bromme, D. *Chem. Rev.* **2002**, *102*, 4459.
- (45) Turkenburg, J. P.; Lamers, M. B.; Brzozowski, A. M.; Wright, L. M.; Hubbard, R. E.; Sturt, S. L.; Williams, D. H. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2002**, *58*, 451.
- (46) Saftig, P.; Hunziker, E.; Wehmeyer, O.; Jones, S.; Boyde, A.; Rommerskirch, W.; Moritz, J. D.; Schu, P.; von Figura, K. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13453.
- (47) Shi, G. P.; Villadangos, J. A.; Dranoff, G.; Small, C.; Gu, L.; Haley, K. J.; Riese, R.; Ploegh, H. L.; Chapman, H. A. *Immunity* **1999**, *10*, 197.
- (48) Roth, W.; Deussing, J.; Botchkarev, V. A.; Pauly-Evers, M.; Saftig, P.; Hafner, A.; Schmidt, P.; Schmahl, W.; Scherer, J.; Anton-Lamprecht, I.; Von Figura, K.; Paus, R.; Peters, C. *FASEB J.* **2000**, *14*, 2075.
- (49) Stypmann, J.; Glaser, K.; Roth, W.; Tobin, D. J.; Petermann, I.; Matthias, R.; Monnig, G.; Haverkamp, W.; Breithardt, G.; Schmahl, W.; Peters, C.; Reinheckel, T. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6234.
- (50) Robker, R. L.; Russell, D. L.; Espey, L. L.; Lydon, J. P.; O'Malley, B. W.; Richards, J. S. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 4689.
- (51) Polgar, L.; Halasz, P. *Biochem J.* **1982**, *207*, 1.
- (52) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988.
- (53) Mayo, K. H.; Dings, R. P. M.; Flader, C.; Nesmelova, I.; Hargittai, B.; van der Schaft, D. W. J.; van Eijk, L. I.; Walek, D.; Haseman, J.; Hoye, T. R.; Griffioen, A. W. *J. Biol. Chem.* **2003**, *278*, 45746.
- (54) Nowick, J. S.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1995**, *60*, 7386.
- (55) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. *J. Med. Chem.* **1994**, *37*, 215.

-
- (56) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokoyama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc.* **1995**, *117*, 11113.
- (57) Smith, A. B.; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947.
- (58) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 58.
- (59) Hammond, M. C.; Harris, B. Z.; Lim, W. A.; Bartlett, P. A. *Chem. Biol.* **2006**, *13*, 1247.
- (60) Liskamp, R. M. J.; Rijkers, D. T. S.; Bakker, S. E. In *Modern supramolecular chemistry: strategies for macrocyclic synthesis*; Diederich, F., Stang, P. J., Tykwinski, R. R., Eds.; Wiley: Weinheim, 2008, p 1-27.
- (61) Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945.
- (62) Kase, H.; Kaneko, M.; Yamada, K. *J. Antibiot.* **1987**, *40*, 450.
- (63) Sano, S.; Ikai, K.; Katayama, K.; Takesako, K.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1685.
- (64) Sano, S.; Ikai, K.; Kuroda, H.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1674.
- (65) Janetka, J. W.; Satyshur, K. A.; Rich, D. H. *Acta Crystallogr. C* **1996**, *52*, 3112.
- (66) Janetka, J. W.; Raman, P.; Satyshur, K.; Flentke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1997**, *119*, 441.
- (67) Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Hill, T. A.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, Pr32.
- (68) Martin, J. L.; Begun, J.; Schindeler, A.; Wickramasinghe, W. A.; Alewood, D.; Alewood, P. F.; Bergman, D. A.; Brinkworth, R. I.; Abbenante, G.; March, D. R.; Reid, R. C.; Fairlie, D. P. *Biochemistry* **1999**, *38*, 7978.
- (69) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606.
- (70) Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaff, R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1455.
- (71) Gradillas, A.; Perez-Castells, J. *Angew. Chem. Int. Ed.* **2006**, *45*, 6086.
- (72) Colombo, M.; Peretto, I. *Drug Discov. Today* **2008**, *13*, 677.

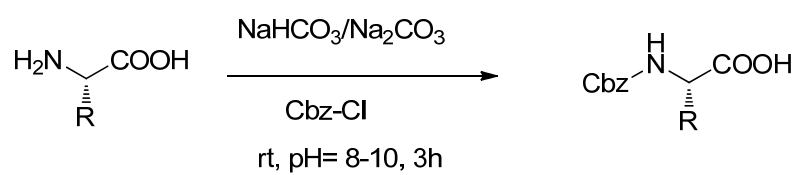
-
- (73) Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukulj, G.; LaPlante, S. R.; Llinas-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem. Int. Ed.* **2003**, *42*, 1355.
- (74) Hanessian, S.; Yang, G. Q.; Rondeau, J. M.; Neumann, U.; Betschart, C.; Tintelnot-Blomley, M. *J. Med. Chem.* **2006**, *49*, 4544.
- (75) Machauer, R.; Laumen, K.; Veenstra, S.; Rondeau, J. M.; Tintelnot-Blomley, M.; Betschart, C.; Jatou, A. L.; Desrayaud, S.; Staufenbiel, M.; Rabe, S.; Paganetti, P.; Neumann, U. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1366.
- (76) Machauer, R.; Veenstra, S.; Rondeau, J. M.; Tintelnot-Blomley, M.; Betschart, C.; Neumann, U.; Paganetti, P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1361.
- (77) Griffiths-Jones, S. R.; Sharman, G. J.; Maynard, A. J.; Searle, M. S. *J. Mol. Biol.* **1998**, *284*, 1597.
- (78) Stuart, B. G.; Coxon, J. M.; Morton, J. D.; Abell, A. D.; McDonald, D. Q.; Aitken, S. G.; Jones, M. A.; Bickerstaffe, R. *J. Med. Chem.* **2011**, *54*, 7503.
- (79) Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, 2399.
- (80) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249.
- (81) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
- (82) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
- (83) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.
- (84) Angell, Y. L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674.
- (85) Pehere, A. D.; Abell, A. D. *Org. Lett.* **2012**, *14*, 1330.

Chapter Two

An improved large scale procedure for preparation of *N*-Cbz amino acids[†]

Ashok D. Pehere and Andrew D. Abell *

School of Chemistry and Physics, The University of Adelaide, North Terrace, Adelaide SA 5005, Australia.



[†]Pehere, A.D.; Abell, A.D. *Tetrahedron Lett.* **2011**, 52, 1493–1494.

2.1 Abstract

A simple and scalable method for the preparation of *N*-Cbz protected amino acids is presented which uses a mixture of aqueous sodium carbonate and sodium bicarbonate to maintain the appropriate pH during the addition of benzyl chloroformate. The method has been extended to other *N*-protections and is amenable to large scale preparation of an intermediate toward Zofenopril, an ACE inhibitor.

2.2 Introduction

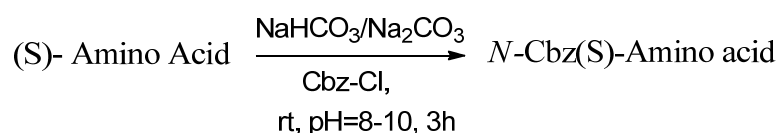
The benzyloxycarbonyl (Cbz) amine protecting group has found wide application in organic synthesis, particularly in solution phase peptide synthesis.¹ The required Cbz-protected amino acid building blocks are traditionally prepared^{1b} by the portion wise addition of benzyl chloroformate (Cbz-Cl) to an alkaline solution of the amino acid, with careful maintenance of pH (8–10) by the simultaneous addition of 2 N aqueous NaOH. While this methodology generally works well, it does have some drawbacks. In particular, control of the pH during the addition of Cbz-Cl can be problematic, especially for larger multi-gram scale reactions, and especially industrial applications. A drop in pH below 8 can lead to decomposition of Cbz-Cl, while too high a pH can give rise to racemisation of the amino acid.

Here we present a simple method for the introduction of a Cbz protecting group by addition of Cbz-Cl to a mixture of the amino acid in an aqueous mixture of 2 mol equiv of sodium carbonate and 1 mol equiv of sodium bicarbonate to maintain the required pH, see Table 1. The methodology is particularly amenable to larger scale reactions since it does not require concomitant addition of base and also to substrates containing labile functionality. We have investigated its applicability for the benzyloxycarbonylation of natural and non-natural α -amino acids (Table 1, entries 1–9), the introduction of other *N*-terminal protecting groups (Table 2), and finally in the synthesis of **4**, a key intermediate in the industrial synthesis of Zofenopril² [an angiotensin converting enzyme (ACE) inhibitor].

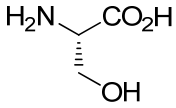
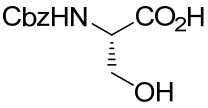
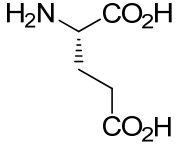
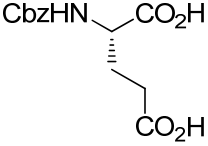
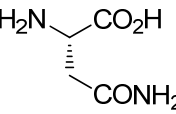
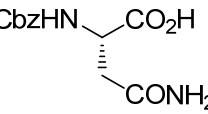
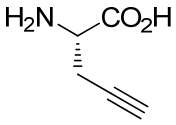
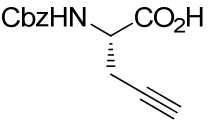
The Cbz-protected amino acids were prepared in high yield (75–97%)³ as summarised in Table 1. The methodology involves adding 1.25 equiv of Cbz-Cl dropwise to a pH 8–10 mixture of the (*S*)-amino acid in water (30 volumes) and acetone (4 volumes) containing sodium carbonate (2 equiv) and sodium bicarbonate (1 equiv). The reactions were complete (as monitored by TLC) after 3 h stirring at rt, and the products were isolated by simply washing the crude mixture with ether, followed by precipitation of the residue with the slow addition of aqueous hydrochloric acid. In all cases the product *N*-Cbz protected amino acids were obtained in high optical purity, with optical rotations in agreement with literature. Interestingly the equivalent reactions of amino acids with Cbz-Cl in either aqueous sodium carbonate or sodium hydroxide alone gave low yields of the Cbz-protected amino acid, even after extended reaction times.

The reaction conditions were also suitable for introducing other *N*-protecting groups, such as dihydrocinamoyl⁴ (Table 2, entry 1) and sulfonyl⁵ (Table 2, entry 2). In these cases the product was isolated by flash chromatography on silica to remove excess reagent and/or its hydrolysed equivalent. Finally, we used this simple methodology to prepare the *N*-protected proline derivative **4** (Scheme 1), a key intermediate in the industrial synthesis of the ACE inhibitor Zofenopril.² In this case, addition of the acid chloride **2** to a mixture of the hydrochloride **3**, sodium carbonate (4 equiv) and sodium bicarbonate (2 equiv) in aqueous acetone, gave **4** in a yield of 78%, after purification, as its dicyclohexylamine salt.

Table 1. Preparation of *N*-Cbz amino acids.



Entry	Substrate	Product ^a	Yield (%)
1			85
2			92
3			95
4			97
5			90

6			89
7			75
8			75
9			95

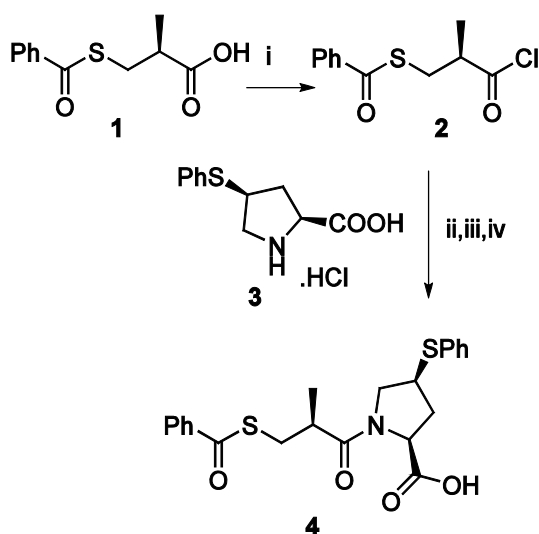
^aAll products were characterised by IR, ¹HNMR spectroscopy and optical rotations.

Acylation of **3** under these conditions gave similarly high yields of **4** on both 100 g and 10 kg scales. It should be noted that the literature method² for the preparation of **4**, that is, separate and simultaneous addition of **2** and aqueous sodium bicarbonate to a solution of **3** in 25% sodium carbonate, works poorly on large scale due to difficulties in maintaining the appropriate pH (8-10). This method leads to a diminished yield, optical purity and reproducibility, particularly on a larger industrial scale. An attempted preparation of **4** by the simultaneous addition of **2** and 2% aqueous sodium hydroxide to a solution of **3** in 2% aqueous sodium hydroxide, gave similar problems. The addition of **2** to a solution of **3** in a mixture of boric acid, potassium chloride and sodium hydroxide (pH 9.5) buffer⁶ also gave a good yield (84%) of **4**, however very large volumes of water (85 volumes) were required in this case due to the poor solubility of boric acid. Our new method reported here gives **4** in high yield and optical purity using manageable volumes of water (30 volumes) and it is as such amenable to both small and large scale reactions. This methodology has recently been used in an industrial scale preparation of Zofenopril.⁷

Table 2

(S)- Amino Acid $\xrightarrow[\text{RCl, rt, pH=8-10, 3h}]{\text{NaHCO}_3/\text{Na}_2\text{CO}_3}$ <i>N</i> -R-(S)-Amino acid			
Entry	Substrate	Product	Yield ^a
1			75
2			80

RCl= Ph(CH₂)₂COCl and 4-FC₆H₄SO₂Cl, ^aYield after column chromatography.



Scheme 1. Reagents: (i) SOCl₂, toluene; (ii) NaHCO₃, Na₂CO₃; (iii) Dicyclohexylamine, EtOH, CH₂Cl₂; (iv) KHSO₄.

In summary, we report a simple and convenient method for the preparation of Cbz-protected amino acids in high yield and optical purity. The methodology is amenable to both small and large scale and it can also be used for the introduction of other amine protecting groups.

2.3 Acknowledgments

We thank Dr. Vinayak Gore (Mylan India Private Limited) for carrying out the large scale reactions. The authors acknowledge the financial support of an ARC DP grant (A.D.A) and the University of Adelaide for an AFSI scholarship to A.D.P.

2.4 References

1. (a) Isidro-Llobet, A.; Alvarez, M.; Fernando Albericio, F. *Chem. Rev.* **2009**, *109*, 2455-2504; (b) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1994, p11; (c) Hernandez, J. N.; Martin, V. S. *J. Org. Chem.* **2004**, *69*, 3590–3592; (d) Pavan Kumar, V.; Somi Reddy, M.; Narender, M.; Surendra, K.; Nageswar, Y. V. D.; Rama Rao, K. *Tetrahedron Lett.* **2006**, *47*, 6393-6396; (e) Berkowitz, D. B.; Pedersen, M. L. *J. Org. Chem.* **1994**, *59*, 5476–5478; (f) Maligres, P. E.; Houpis, I.; Rossen, K.; Molina, A.; Sager, J.; Upadhyay, V.; Wells, K. M.; Reamer, R. A.; Lynch, J. E.; Askin, D.; Volante, R. P.; Reider, P. J. *Tetrahedron* **1997**, *32*, 10983–10992; (g) Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y.-Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1455–1458.
2. Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnvak, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwa, K. S.; Petrillo, E. W. *J. Med. Chem.* **1988**, *31*, 1148–1160.
3. The (S)-amino acid (10.0 gm) was dissolved in H₂O (300 ml) and Na₂CO₃ (2.0 equiv) and NaHCO₃ (1.0 equiv) were added at rt, with stirring, to give a clear solution. Acetone (4.0 vol, with respect to the amino acid) was added and the slightly turbid solution was cooled in an ice water bath to 15-20 °C. Cbz-Cl (1.25 equiv) was added slowly, with stirring, and the reaction mixture allowed to warm to rt. After stirring for an additional 3 h at rt the mixture was extracted with Et₂O. To the aqueous phase was slowly added aqueous HCl to give a pH of 2. The resulting oil was extracted into EtOAc (150 ml) and this was washed with H₂O (100 ml) and

then concentrated in *vacuo* to give the N-Cbz amino acid as a white solid, see table 1.

4. Kiviranta, P. H.; Salo, H. S.; Leppanen, J.; Rinne, V. M.; Kyrlylenko, S.; Kuusisto, E.; Suuronen, T.; Salminen, A.; Poso, A.; Lahtela-Kakkonen, M.; Wallen, E. A. *Bioorg. Med. Chem.* **2008**, *16*, 8054.
5. Shirasaki, Y.; Nakamura, M.; Yamaguchi, M.; Miyashita, H.; Sakai, O.; Inoue, J. J. *Med. Chem.* **2006**, *49*, 3926–3932.
6. European Pharmacopoeia, 5th ed., 2005, p. 434.
7. Work performed by our collaborators Dr. Vinayak Gore of Mylan India (formally known as Merck development Center) unpublished results.

2.5 Experimental

1. Preparation of the compounds

General Methods.

All chemicals commercially obtained were utilised as received. Thin-layer chromatography (TLC) was carried out using Merck aluminium sheets with silica gel 60 F₂₅₄. The compounds were visualised with an Oliphant (6W – 254 nm tube) UV lamp and/or potassium permanganate stain (1.5 g KMnO₄, 10 g K₂CO₃, 1.25 mL 10% NaOH_(aq) and 200 mL water). All yields reported were isolated yields judged to be homogenous by TLC and NMR spectroscopy. ¹H NMR spectra were recorded on a 300 spectrometer, ¹³C NMR spectra (151 MHz) on a Varian Inova spectrometer. All spectra were reported relative to TMS ($\delta_{\text{H}} = 0.00$ ppm), CDCl₃ ($\delta_{\text{H}} = 7.26$ ppm, $\delta_{\text{C}} = 77.0$ ppm), (CD₃)₂SO ($\delta_{\text{H}} = 2.50$ ppm, $\delta_{\text{C}} = 39.5$ ppm). Chemical shifts (δ) are reported in ppm and all coupling constants were calculated to one decimal place. Spin multiplicities are represented by the following signals: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Melting points were measured on a microscope hot stage melting point apparatus and are uncorrected. Specific rotation values were determined using an Atago Automatic Polarimeter AP-100, and a 100 mm observation tube (5 mL capacity). Oven dried glassware was used in all reactions carried out under an inert atmosphere (either dry nitrogen or argon). All starting materials and reagents were obtained commercially unless otherwise stated. Removal of solvents “under reduced pressure” refers to the process of bulk solvent removal by rotary evaporation (low vacuum pump) followed by application of high vacuum pump (oil pump) for a minimum of 30 min.

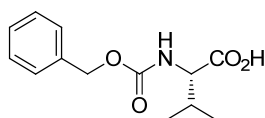
2. General Procedure:

The (*S*)-amino acid (10.0 g) was dissolved in H₂O (300 ml) and Na₂CO₃ (2.0 equiv) and NaHCO₃ (1.0 equiv) were added at rt, with stirring, to give a clear solution. Acetone (4.0 vol, with respect to the amino acid) was added and the slightly turbid solution was cooled in an ice water bath to 15–20°C. Cbz-Cl (1.25 equiv) was added slowly, with stirring, and the reaction mixture allowed to warm to rt. After stirring for an additional 3 h at rt the mixture was extracted with Et₂O (50 ml). To the aqueous phase was slowly added aqueous

HCl to give a pH of 2. The resulting oil was extracted into EtOAc (150 ml) and this was washed with H₂O (100 ml) and then concentrated in vacuo to give the *N*-Cbz amino acid, (see individual experiments for details).

3. Synthesis and characterization

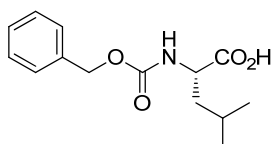
(*S*)-2-(((Benzyloxy)carbonyl)amino)-3-methylbutanoic acid¹



L-Val-OH (2.50 g, 32.3 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Val-OH (4.5 g, 85%) as a transparent oil.

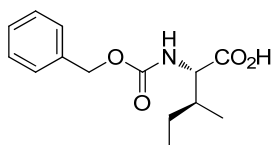
$[\alpha]^{24.0}_{\text{D}} = -5.6$ (c 2.0, AcOH); ¹H NMR (300 MHz, CDCl₃) δ 7.50 – 7.29 (m, 5H), 5.23 (d, *J* = 8.2 Hz, 1H), 5.21 (s, 2H), 4.54 – 4.31 (m, 1H), 2.35 – 2.15 (m, 1H), 1.25 – 0.91 (m, 6H).

(*S*)-2-(((Benzyloxy)carbonyl)amino)-4-methylpentanoic acid^{2,3}



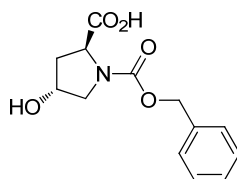
L-Leu-OH (5.00 g, 38.1 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Leu-OH (9.3 g, 92%) as a transparent oil.

$[\alpha]^{24.0}_{\text{D}} = -13.8$ (c 2.0 MeOH)²; ¹H NMR (300 MHz, CDCl₃) δ 7.36 – 7.29 (s, 5H), 5.28 (d, *J* = 8.4 Hz, 1H), 5.13 (s, 2H), 4.51. – 4.37 (m, 1H), 1.78 – 1.53 (m, 3H), 1.06 – 0.83 (m, 6H).

(2S,3S)-2-(((Benzyloxy)carbonyl)amino)-3-methylpentanoic acid⁴

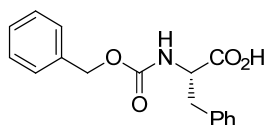
L-Ile-OH (2.5 g, 19.0 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Ile-OH (4.8 g, 95%) as a transparent oil.

$[\alpha]^{24.0}_{\text{D}} = +5.3$ (c 6.0 EtOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.55 – 7.29 (m, 5H), 5.28 (d, $J = 10.4$ Hz, 1H), 5.11 (s, 2H), 4.51 – 4.33 (m, 1H), 1.63 – 1.35 (m, 1H), 1.35 – 1.10 (m, 1H), 1.04 – 0.77 (m, 7H).

(2S,4R)-1-(((Benzyloxy)carbonyl)-4-hydroxypyrrolidine-2-carboxylic acid⁵

L-Pro-OH (2.0 g, 15.3 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Pro-OH (3.9 g, 95%) as a transparent oil.

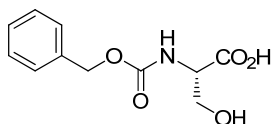
$[\alpha]^{24.0}_{\text{D}} = -39.3$ (c 2.0 EtOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.57 – 7.47 (m, 5H), 5.27 – 5.04 (m, 2H), 4.45 (m, 2H), 3.63 (m, 2H), 2.36 (m, 1H), 2.13 (m, 1H).

(S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoic acid⁶

L-Tyr-OH (1.0 g, 5.5 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Tyr-OH (1.5 g, 90%) as a white solid.

mp 55-58 °C; $[\alpha]_{\text{D}}^{24.0} = +6.3$ (c 5.0 AcOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.45 – 7.23 (m, 7H), 7.20 – 7.10 (m, 2H), 5.17 (d, $J = 8.0$ Hz, 1H), 5.10 (s, 2H), 4.80 – 4.64 (m, 1H), 3.29 – 3.05 (m, 2H).

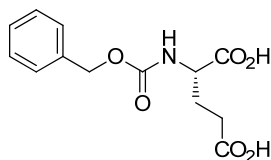
(S)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxypropanoic acid^{7,8}



L-Ser-OH (10.0 g, 95.0 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Ser-OH (20.5 g, 89%) as a white solid.

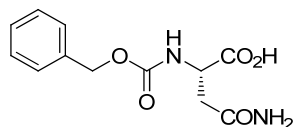
m.p. 113-116 °C; $[\alpha]_{\text{D}}^{24.0} = +4.4$ (c 3.0 AcOH); $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 7.50 – 7.25 (m, 6H), 5.04 (s, 2H), 4.11 – 3.99 (m, 1H), 3.72 – 3.57 (m, 2H).

(S)-2-(((Benzyloxy)carbonyl)amino)pentanedioic acid⁹



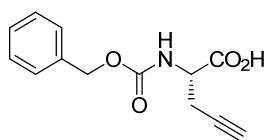
L-Glu-OH (1.0 g, 6.8 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Glu-OH (1.45 g, 75%) as a white solid.

m.p. 110-112 °C; $[\alpha]_{\text{D}}^{24.0} = -8.6$ (c 10.0 AcOH); $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 7.59 (d, $J = 8.3$ Hz, 1H), 7.37 – 7.29 (m, 5H), 5.03 (s, 2H), 4.10 – 3.96 (m, 1H), 2.32 – 2.26 (m, 2H), 1.95 – 1.78 (m, 2H).

(S)-4-Amino-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoic acid¹⁰

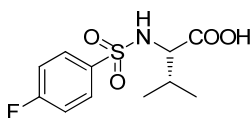
L-Asn-OH (1.0 g, 5.5 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-Asn-OH (1.5 g, 75%) white solid. mp 159-162.

$[\alpha]^{24.0}_{\text{D}} = +8.1$ (c 2.0 AcOH); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 7.46 (d, $J = 8.4$ Hz, 1H), 7.42 – 7.26 (m, 5H), 6.93 (s, 2H), 5.02 (s, 2H), 4.42 – 4.26 (m, 1H), 2.61 – 2.36 (m, 2H).

(S)-2-(((Benzyloxy)carbonyl)amino)pent-4-ynoic acid

L-propargylglycine (1.0 g, 19.0 mmol) was *N*-protected with Cbz-Cl according to General Procedure. The product was to give compound *N*-Cbz-L-propargylglycine (2.08 g, 95%) as a transparent oil.

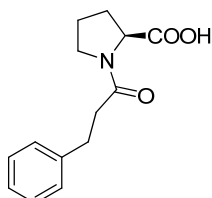
$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.51 – 7.29 (m, 5H), 5.60 (d, $J = 7.6$ Hz, 1H), 5.15 (s, 2H), 4.68 – 4.52 (m, 1H), 2.97 – 2.68 (m, 2H), 2.07 (t, $J = 3.6$ Hz, 1H).

***N*-(4-Fluorophenylsulfonyl)-L-valine**¹¹

L-Val-OH (0.5 g, 4.20 mmol) was *N*-protected with 4-fluorophenylsulfonyl chloride according to General Procedure. The product was to give compound *N*-(4-fluorophenylsulfonyl)-L-Val-OH (0.9 g, 80%) as a semisolid.

^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 8.07 (d, $J = 9.1$ Hz, 1H), 7.85 – 7.77 (m, 2H), 7.41 – 7.23 (m, 2H), 3.48 – 3.52 (m, 1H), 1.98 – 1.85 (m, 1H), 0.80 – 0.77 (m, 6H).

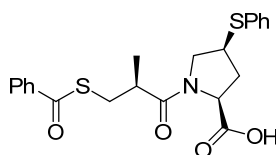
1-Hydrocinnamoyl- L- proline^{12,13}



L-Pro-OH (1.5 g, 13.0 mmol) was reacted with hydrocinnamoyl chloride according to General Procedure. The product was to give compound 1-Hydrocinnamoyl-L-Pro-OH (2.4 g, 75%) as a transparent oil.

$[\alpha]^{24.0}_{\text{D}} = -10.1$ (c 1.0 MeOH)¹³; ^1H NMR (300 MHz, CDCl_3) δ 7.30 – 7.24 (s, 5H), 4.46 – 4.46 (m, 1H), 3.32 – 3.29 (m, 2H), 2.76 – 2.72 (m, 4H), 1.98 – 1.89 (m, 4H).

(2S,4S)-1-((S)-3-(Benzoylthio)-2-methylpropanoyl)-4-(phenylthio)pyrrolidine-2-carboxylic acid 4¹⁴



Compound **3** (132.0 g) was coupled according to General Procedure to give **4** as a colorless oil (183 g 79%).

^1H NMR (CDCl_3): δ 7.93–7.22 (m, 10H), 4.56 (t, $J=8.0, 7.0$ Hz, 1H), 4.11 (dd, $J=10.1, 8.8$ Hz, 1H); 3.71 (m, 1H); 3.41 (dd, $J=10.1, 8.8$ Hz, 1H); 3.27(dd, $J=13.4, 7.9$ Hz, 1H); 3.14 (dd, $J=13.6, 6.2$ Hz, 1H); 2.89 (m, 1H); 2.65 (m, 1H); 2.06 (m, 1H); 1.27 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 192.6, 176.0, 174.5, 137.1, 134.0, 133.9, 132.2, 129.6, 129.1, 128.0, 127.6, 59.0, 53.6, 44.7, 39.0, 35.8, 32.2, 17.1. HRMS (ES) 430.1147 (M + H)⁺; $\text{C}_{22}\text{H}_{23}\text{NO}_4\text{S}_2$ requires 430.1141.

4 References

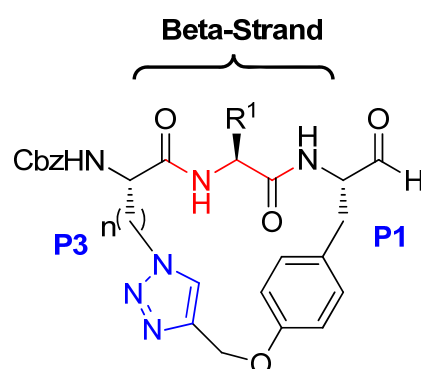
- (1) Aldrich catalog, Beil. 6 ,IV, 2344.
- (2) Frelek, J.; Fryszkowska, A.; Kwit, M.; Ostaszewski, R. *Tetrahedron: Asymmetry* **2006**, *17*, 2469.
- (3) Aldrich catalog, Beil. 6, III, 1503.
- (4) Aldrich catalog, Beil. 6, IV, 2370.
- (5) Aldrich catalog, Beil. 22, V,1, 84.
- (6) Aldrich catalog, Beil. 14, IV, 1596.
- (7) Lall, M. S.; Karvellas, C.; Vederas, J. C. *Org. Lett.* **1999**, *1*, 803-806.
- (8) Aldrich catalog, Beil. 6, IV, 2374.
- (9) Aldrich catalog, Beil. 6, IV, 2402.
- (10) Aldrich catalog, Beil. 6, IV, 2398.
- (11) Inoue, J.; Nakamura, M.; Cui, Y.-S.; Sakai, Y.; Sakai, O.; Hill, J. R.; Wang, K. K. W.; Yuen, P.-W. *J. Med. Chem.* **2003**, *46*, 868-871.
- (12) Kiviranta, P. H.; Salo, H. S.; Leppänen, J.; Rinne, V. M.; Kyrylenko, S.; Kuusisto, E.; Suuronen, T.; Salminen, A.; Poso, A.; Lahtela-Kakkonen, M.; Wallén, E. A. A. *Bioorg. Med. Chem.* **2008**, *16*, 8054-8062.
- (13) Abraham, D. J.; Gazze, D. M.; Kennedy, P. E.; Mokotoff, M. *J. Med. Chem.* **1984**, *27*, 1549-1559.
- (14) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G. *J. Med. Chem.* **1988**, *31*, 1148-1160.

Chapter Three

New β -Strand Templates Constrained by Huisgen Cycloaddition[†]

Ashok D. Pehere and Andrew D. Abell*

*School of Chemistry and Physics, The University of Adelaide, North Terrace, Adelaide SA
5005, Australia.*



[†]Pehere, A.D.; Abell, A.D. *Org. Lett.* **2012**, *14*, 1330-1333.

Pehere, A.D. & Abell, A.D. (2012) New Beta-strand templates constrained by Huisgen Cycloaddition.
Organic Letters, v. 14(5), pp. 1330-1333

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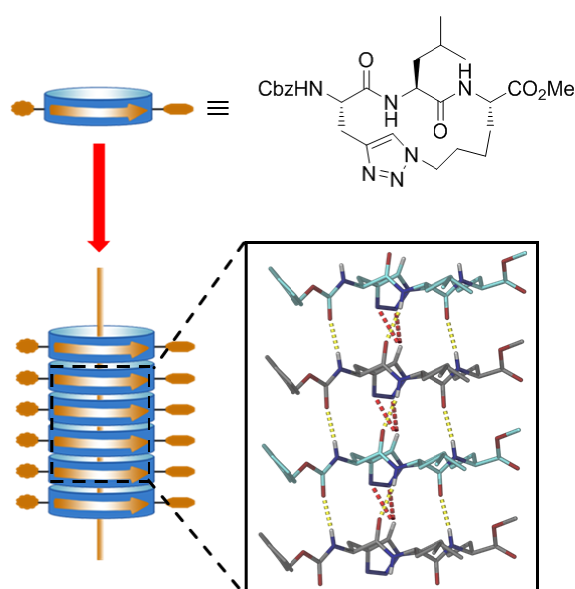
<http://doi.org/10.1021/o13002199>

Chapter Four

New Cylindrical Peptide Assemblies Defined by Extended Parallel β -Sheets[†]

Ashok D. Pehere, Christopher J. Sumby and Andrew D. Abell*

School of Chemistry and Physics, The University of Adelaide, North Terrace, Adelaide SA 5005, Australia.



[†]Pehere, A.D.; Sumby, C.J. and Abell, A.D. *J. Am. Chem. Soc.* to be submitted manuscript.

Pehere, A.D., Sumby, C.J. & Abell, A.D. (2013) New cylindrical peptide assemblies defined by extended parallel beta-sheets.

Organic & Biomolecular Chemistry, v. 11(3), pp. 425-429

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Chapter Five

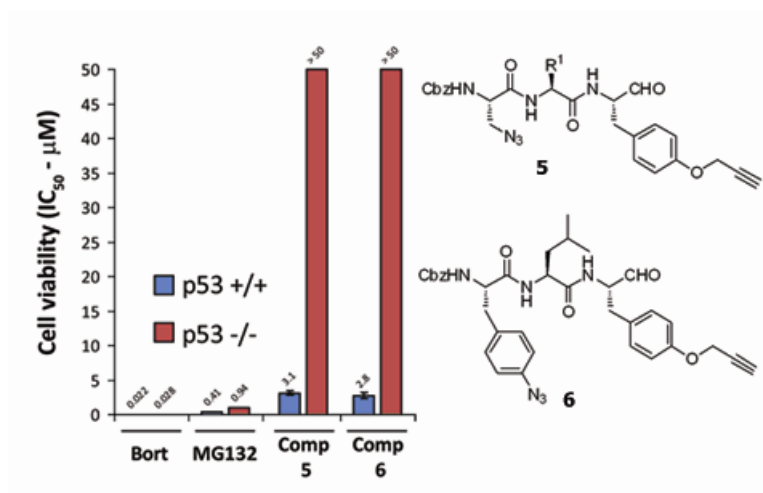
New 26S-proteasome inhibitors with high selectivity for chymotrypsin-like activity and p53-dependent cytotoxicity[†]

Paul M. Nielsen,^{1#} Ashok D. Pehere,^{1,2#} David F. Callen,¹ and Andrew D. Abell^{1,2*}

¹ Centre for Personalised Cancer Medicine, The University of Adelaide, North Terrace, Adelaide SA 5005, Australia.

² School of Chemistry & Physics, The University of Adelaide, North Terrace, Adelaide SA 5005, Australia.

These authors contributed equally



[†]Nielsen, P.M.; Pehere, A.D.; Callen, D.F.; Abell, A.D. *ACS Chem. Biol.* to be submitted manuscript.

Neilsen, P.M., Pehere, A.D., Pishas, K.I., Callen, D.F. & Abell, A.D. (2013) New 26S-proteasome inhibitors with high selectivity for chymotrypsin-like activity and p53-dependent cytotoxicity. *ACS Chemical Biology*, v. 8(2), pp. 353-359

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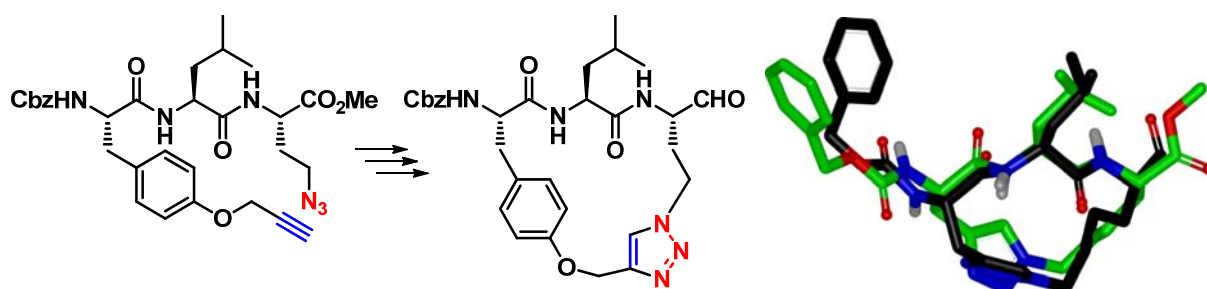
It is also available online to authorised users at:

<http://doi.org/10.1021/cb300549d>

Chapter Six

Synthesis and Extended Activity of Triazole-Containing Macrocyclic Protease Inhibitors[†]

*School of Chemistry and Physics, The University of Adelaide, North Terrace, Adelaide SA
5005, Australia.*



[†]Pehere *et al.* (In preparation manuscript).

Pehere, A.D., Pietsch, M., Gutschow, M., Neilsen, P.M., Pederson, D.S., Nguyen, S., Zvarec, O., Sykes, M.J., Callen, D.F. & Abell, A.D. (2013) Synthesis and extended activity of triazole-containing macrocyclic protease inhibitors.

Chemistry a European Journal, v. 19(24), pp. 7975-7981

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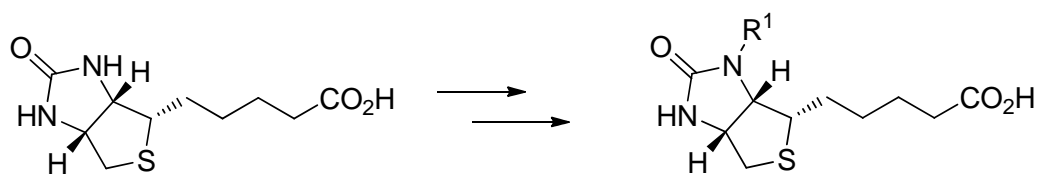
<http://dx.doi.org/10.1002/chem.201204260>

Chapter Seven

Selective *N*-Acylation and *N*-Alkylation of Biotin[†]

Ashok D. Pehere and Andrew D. Abell*

School of Chemistry and Physics, The University of Adelaide, North Terrace, Adelaide SA 5005, Australia.



[†]Pehere, A.D.; Abell, A.D. *J. Org. Chem.* **2011**, 76, 9514–9518.

Pehere, A.D. & Abell, A.D. (2011) Selective N-acylation and N-alkylation of biotin.
Journal of Organic Chemistry, v. 76(22), pp. 9514-9518

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<http://doi.org/10.1021/jo201615j>