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

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Table of Contents

Abstract v

Declaration vii

Acknowledgement ix

Abbreviations x

Statements of Authorship xiii

Chapter One

1.1 Protein structure 1

1.2 Proteases 5

1.2.1 Calpain 8

1.2.2 26S Proteasome 9

1.2.3 Cathepsin family 11

1.3 Secondary structure mimics (β-strand) 13

1.3.1 β-Sheet nucleating template 13

1.3.2 Backbone-modified β-strand mimetics 15

1.4 Macrocyclic β-strand mimetics 17

1.4.1 Naturally occurring macrocyclic protease inhibitors 17

1.4.2 Macrocyclic protease inhibitors prepared by alkylation 19

1.4.3 Macrocyclic protease inhibitors prepared by ring closing metathesis (RCM) 21

1.4.4 A new method for protease inhibitors prepared by azide-alkyne cycloadditions 25

1.5 Research described in this thesis 27

1.6 References 31

Chapter Two

2.1 Abstract 37

2.2 Introduction 38

2.3 Acknowledgment 42

2.4 References 42

2.5 Experimental 44

Chapter Three

3.1 Abstract 52

3.2 Introduction 53

3.3 Synthesis 54

3.4 Conformation of the macrocycles 57

3.5 Biological Data 58

3.6 Acknowledgment 59

3.7 References 60

3.8 Experimental 62

3.8.1 Enzyme assays 62

3.8.2 Preparation of the compounds 62

3.8.3 Synthesis and characterization 66

Chapter Four

4.1 Abstract 90

4.2 Introduction 91

4.3 Synthesis 93

4.4 X-ray crystal structure analysis 94

4.5 NMR analysis 96

4.6 IR analysis 97

4.7 ESI mass spectrum 98

4.8 Scanning electron microscopy (SEM) 98

4.9 Acknowledgment 100

4.10 References 101

4.11 Experimental 104

4.11.1 Preparation of the compounds 104

4.11.2 Synthesis of **2c** and **3c** 108

4.11.3 Synthesis and characterization 110

4.11.4 Enzyme assays 119

4.11.5 X-ray Crystallographic data for **1a** 120

4.11.6 SEM Images, Face Indexing, Infrared spectra and (ESI-MS) spectra 122

Chapter Five

5.1 Abstract 128

5.2 Introduction 129

5.3 Results and discussion 131

5.4 Synthesis of tripeptide MG132 analogues 132

5.5 Inhibition of the proteasome 135

5.6 Tripeptide MG132 analogues specifically kill cancer cells 136

5.7 Tripeptide MG132 analogues mediate cell death in part through the p53 pathway 138

5.8 Materials and Methods 140

5.9 Acknowledgment 143

5.10 References 143

5.11 Experimental 147

5.11.1 Preparation of the compounds 147

5.11.2 Synthesis and characterization 149

Chapter Six

6.1 Abstract 162

6.2 Introduction 163

6.3 Synthesis 165

6.4 Conformational analysis 169

6.5 Biological data 171

6.6 References 174

6.7 Experimental 176

Chapter Seven

7.1 Abstract 190

7.2 Introduction 191

7.3 Synthesis 192

7.4 Experimental 196

7.5 Acknowledgment 205

7.6 References 206

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 macrocylic β-strand 'bioactive' conformation is discussed in detail. In particular the exploitation of the conformationally constrained peptidomimetic macrocylic 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 P_1 and P_3 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 macrocylic 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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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	*tert*-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	*N*,*N*-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*H*-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
m/*z*	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	*p*-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	*para*-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.

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Paper 2

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Paper 3

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

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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.*).

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Professor David Callen

Assisted with data interpretation and revision of manuscript.

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Paper 6

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

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Professor Andrew Abell

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

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