β-Strand Mimicry as the Basis for a Universal Approach to Protease Inhibition

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By

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Abstract

This thesis describes the design, preparation, and testing of a range of protease inhibitors. **Chapter One** introduces the concept of peptidomimetics, and discusses how proteases almost universally bind their ligands in a *β*-strand conformation. The idea of constraining a compound into a biologically active conformation by the introduction of a ring or bridge is discussed. The technique of ring closing metathesis as a strategy for macrocyclisation is introduced. The chapter also discusses calpain and HIV proteases and their structures and implications in human disease.

Chapter Two surveys the acyclic calpain inhibitors reported in the literature. A series of *N*-heterocyclic peptidic calpain inhibitors were docked *in silico* into an ovine m-calpain homology model using Glide, which revealed that compounds **2.60** – **2.67** all adopted a *β*-strand conformation upon binding. The modelling revealed low energy conformations of **2.60**, **2.61** and **2.66** not in a *β*-strand geometry. The synthesis and testing of these inhibitors is described, with **2.63** displaying an IC_{50} of 40 nM against m-calpain in an *in vitro* assay.

Chapter Three describes the design and synthesis of the *β*-strand mimic macrocycle **3.8**, which was prepared using ring closing metathesis. The chapter also describes the design of a number of calpain and HIV protease inhibitors that incorporate **3.8**. Each inhibitor is designed to bind and inhibit a specific protease target.

Chapter Four describes the synthesis and testing of a series of macrocyclic calpain and proteasome 20S inhibitors. The preparation of the aldehydes **3.9** and **3.10** by elaboration of the macrocycle **3.8** is described. As well, the preparation of **3.10** from the *N*-capped 4-fluorosulphonyl diene **4.4** is described. The most potent macrocycle in the series was **3.10**, which displays an IC_{50} against m-calpain of 2000 nM, and an IC_{50} against the chymotrypsin like activity of proteasome 20S of 2 nM.

Chapter Five describes the synthesis of a series of building blocks, and their use in the attempted preparation of the potential HIV protease inhibitor **3.12a**, as well as the successful preparation of the potential HIV protease inhibitors **3.11** and **3.12b**. Preliminary studies testing the biological activity of compounds **3.11**, **3.12b** and **5.21** found that they displayed a percentage inhibition of HIV-1 subtype B protease of 86, 63, and 26%, respectively. The K_i of **3.11** against HIV-1 subtype B protease was also determined to be 62 nM. The activity of **3.11** against HIV-1 protease establishes that the common macrocyclic core **3.8** can be incorporated into inhibitors of both calpain, and HIV-1 protease.

Chapter Six describes the preparation of a key macrocycle by cross-metathesis. The preparation of **6.4** by cross-metathesis of the olefins **6.5** and **6.24** is described, as well as the elaboration of **6.4** to give the macrocycle **6.1**. A systematic study of the cross-metathesis of the olefins **6.5**, **6.6**, **6.23** and **6.24** is described. Their percentage conversion to **6.4** was calculated using high performance liquid chromatography analysis. The highest conversion to **6.4** was found to be 60%, from the cross metathesis of an equimolar mixture of **6.6** and **6.23**.

Chapter Seven describes a multi-gram synthesis of the potent macrocyclic calpain inhibitor **CAT0811**. The key step in the synthesis is the base induced macrocyclisation of the iodo-peptide **7.10** to give **7.6**. The macrocycle **7.6** was also prepared by macrolactamisation of the pseudopeptide **7.9**. The synthesis was found to be scalable, affordable and efficient, and removes the need for Grubbs' 2nd generation catalyst (**II**).

Declaration and Published Works

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

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Work in this thesis has appeared in the following publications:

"*N*-Heterocyclic Dipeptide Aldehyde Calpain Inhibitors", Jones, S. A.; Jones, M. A.; McNabb, S. B.; Aitken, S. G.; Coxon, J. M.; Abell, A. D. *Protein Pept. Lett.* **2009**, *16*, 1466-1472.

"Efficient Large-Scale Synthesis of CAT811, a Potent Calpain Inhibitor of Interest in the Treatment of Cataracts", Jones, M. A.; Coxon, J. M.; McNabb, S. B.; Mehrtens, J. M.; Alexander, N. A.; Jones, S.; Chen, H.; Buisan, C.; Abell, A. D. *Aust. J. Chem.* **2009**, *62*, 671-675.

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Abbreviations

18-crown-6	1,4,7,10,13,16-hexaoxacyclooctadecane
aq	aqueous
AIDS	Acquired Immunodeficiency Syndrome
Boc	*tert*-butoxycarbonyl
br	broad (spectroscopic)
calcd	calculated
Cbz	benzyloxycarbonyl
CM	cross-metathesis
conc	concentrated
Cy	cyclohexyl
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	N,N-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride
equiv	equivalent

ESI	electrospray ionisation
Et	ethyl
FTIR	Fourier transform infrared
h	hour(s)
HAART	highly active antiretroviral therapy
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
NHC	*N*-heterocyclic carbene
NMR	nuclear magnetic resonance
PB	4-phenylbutyryl-

PDB	Protein Data Bank
Ph	phenyl
PI	protease inhibitor(s)
ppm	part(s) per million
Pr	propyl
PTC	phase transfer catalyst
PTSA	*p*-toulenesulphonic acid
Py	pyridine
quant	quantitative
RCM	ring closing metathesis
ROM	ring-opening metathesis
ROMP	ring-opening metathesis polymerisation
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