

# Defining Peptide Structure with Metathesis

A thesis submitted for the  
degree of Doctor of Philosophy

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# ABSTRACT

Understanding protein structure and function is central for the development of therapeutics for the treatment of diseases and also novel biocompatible materials. Herein describes studies on the control of peptide structure and function through synthetic modifications, for the synthesis of novel enzyme inhibitors and biomaterials, primarily using olefin metathesis chemistry. Metathesis is chosen for the manipulation of peptide structure in order to induce conformational constraint in novel macrocyclic peptidomimetic inhibitors and to develop novel hydrogel matrices, which are of importance in the advancement of the pharmaceutical and medical industries.

The realization that enzymes bind their substrates in an extended  $\beta$ -stranded conformation has led to the development of inhibitors that mimic this bioactive conformation. The controlled organization of secondary structures in peptides by conformational constraint has been utilized to design two novel series of macrocyclic inhibitors, which are constrained by the P<sub>1</sub> and P<sub>3</sub> residues or the P<sub>2</sub> and P<sub>4</sub> residues using ring closing metathesis (RCM). These inhibitors contain a pyrrole group in the peptide backbone, thereby decreasing the peptidic nature of these inhibitors minimising susceptibility to proteolysis, while maintaining the appropriate geometry for inhibitor binding. The corresponding P<sub>1</sub>-P<sub>3</sub> and P<sub>1</sub>-P<sub>4</sub> acyclic inhibitors are designed and synthesized to provide an insight into the importance of cyclisation on the potency of inhibition against serine and cysteine protease.

The macrocyclic and acyclic inhibitors synthesized are assayed against a series of cysteine (calpain and cathepsin) and serine proteases ( $\alpha$ -chymotrypsin, human leukocyte elastase and trypsin). These enzyme assays analyse the efficacy of the inhibitors against the enzymes tested. The potency of the inhibitors against the aforementioned proteases provides an insight into the effect of cyclisation, ring size and introduction of aryl groups into the ring system, as well as trends in selectivity between proteases of the same family (calpain vs. cathepsin and  $\alpha$ -chymotrypsin vs. HLE and trypsin) and between the cysteine and serine protease families.

The ability to mimic the natural environment of structural proteins in wound healing, has led to the development of biocompatible materials, such as hydrogels, through the manipulation of natural peptide structure. The controlled organization of the tertiary structure of naturally occurring peptides is investigated by aqueous metathesis in the synthesis of biocompatible hydrogels derived from gelatin. Novel gelatin-gels are obtained by reacting methacrylate-functionalized gelatin and norbornene dicarboxylic acid in the presence of a catalyst in aqueous media. Optimisation of the hydrogel formation is investigated by; i) varying catalyst utilised and ii) varying ratios of starting gelatin and norbornene dicarboxylic acid. These polymer gels exhibited physical and chemical properties that might be useful in regenerative medicine. Mechanistic studies using MALDI is also performed to provide an insight into the mode of hydrogel formation.

# DECLARATION

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# ABBREVIATIONS

$\delta$	chemical shift (in NMR)
Å	angstrom
anh.	anhydrous
aq.	aqueous
bCT	bovine $\alpha$ -chymotrypsin
Boc	<i>tert</i> -butoxycarbonyl
BODIBY	4,4-difluoro-5,7-dimethyl-4-bora-3a,4-diaza- <i>s</i> -indacene-3-propionic acid (in assay)
CatL	cathepsin L
CatS	cathepsin S
COSY	H-H correlation spectroscopy
CM	cross metathesis
CM-ROMP	cross metathesis-ring opening metathesis polymerization
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DIEA	<i>N,N</i> -diisopropylethylamine
4-DMAP	4- <i>N,N</i> -dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
ECM	extracellular matrix
EDCI	1-[3-(dimethylamino)propyl]-3-carbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid (in assay)
EGTA	ethylene glycol tetraacetic acid (in assay)
EI	electron impact ionization (in mass spectrometry)
equiv	equivalent(s)
ESI	electrospray ionization (in mass spectrometry)
EtOAc	ethyl acetate
Et <sub>2</sub> O	diethyl ether

gel-GMA	methacrylate-functionalized gelatin
GMA	glycidyl methacrylate
Grubbs 1 <sup>st</sup> Generation Catalyst (GI)	benzylidene-bis(tricyclohexylphosphine)dichlororuthenium
Grubbs 2 <sup>nd</sup> Generation Catalyst (GII)	benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium
h	hour(s)
HATU	<i>N,N,N',N'</i> -tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate
HLE	human leukocyte elastase
HOBt	1-hydroxybenzotriazole
Hoveyda-Grubbs 1 <sup>st</sup> Generation Catalyst	dichloro( <i>o</i> -isopropoxyphenylmethylene)(tricyclohexylphosphine)ruthenium(II)
Hoveyda-Grubbs 2 <sup>nd</sup> Generation Catalyst	(1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro( <i>o</i> -isopropoxyphenylmethylene)ruthenium
rp-HPLC	reversed phased high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz (in NMR)
IC <sub>50</sub>	half maximal inhibitory constant
IR	infrared
<i>J</i>	coupling constant (in NMR)
<i>K<sub>i</sub></i>	inhibitor disassociation constant
kDa	kilodalton
LRMS	low resolution mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
MeOH	methanol
min	minute(s)
MOPS	3-( <i>N</i> -morpholino)propanesulfonic acid (in assay)
m.p.	melting point
NaAsc	sodium ascorbate
NBE-OH	norbornene dicarboxylic acid
NMR	nuclear magnetic resonance
o-CAPN1	ovine calpain 1 (μ-calpain)

o-CAPN2	ovine calpain 2 (m-calpain)
PEGMA	polyethylene glycol methacrylate
Pet. ether	petroleum ether (50-70 °C)
Pd/C	palladium on carbon catalyst
ppm	parts per million
rCAPN1	rat calpain 1 ( $\mu$ -calpain)
rCAPN2	rat calpain 2 (m-calpain)
RCM	ring closing metathesis
ROCM	ring opening cross metathesis
ROMP	ring opening metathesis polymerization
rt	room temperature
SOCl <sub>2</sub>	thionyl chloride
SO <sub>3</sub> Py	sulfur trioxide-pyridine complex
T <sub>c</sub>	helix-to-coil transition temperature
T <sub>m</sub>	crystalline melting temperature
<i>t</i> -BuOH	<i>tert</i> -butanol
TBAI	tetrabutylammonium iodide
TFA	trifluoroacetic acid
TGA	thermal gravimetric analysis
THF	tetrahydrofuran
TLC	thin layer chromatography
TMDSC	temperature modulated differential scanning calorimetry
TMS	trimethylsilyl
TNBS	trinitrobenzene sulfonate (colorimetric assay)
TRIS	tris(hydroxymethyl)aminomethane
Yb(OTf) <sub>3</sub>	ytterbium triflate