A Thesis Submitted for the Degree of PhD at the University of Warwick

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Novel Oxetane-Containing Spirocycles and Peptidomimetics

by

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

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November 2014

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Acknowledgements

First of all, I would like to thank my supervisor, Mike Shipman, for all of his guidance and support throughout my PhD – it would not have been possible without this help. I would also like to thank Piotr Raubo and Nathaniel Martin for their assistance with the project and, in particular, I thank Piotr for looking after me during my time at Alderley Park. I thank as well the University of Warwick for funding me through the Warwick Postgraduate Research Scholarship and AstraZeneca for the CASE sponsorship.

Thank you to Rebecca Notman for her contribution to this project, and Guy Clarkson for his invaluable help with X-ray crystallography. Thanks to Alison Rodger for all of her help with CD spectroscopy. Thanks also to Lijiang Song, Rebecca Wills, Phil Aston, Edward Tunnah, Robert Perry and Ivan Prokes at the University of Warwick for their analytical expertise. I also thank the analytical staff at AstraZeneca for their assistance during my placement there.

Many thanks to members of the Shipman group, past and present, for all of the fun and laughter: Alpa, Amélie, Ben, Claire, Eduardo, Fran, Greg, Jo, Karen, Mike, Penny, Ricky, Samiullah, Sam and Thuy. A special thank you goes to Alex, for all of his help, knowledge and support over the years - you’ve been a star.

I thank my friends and extended family for all of their support. Finally, I thank my mum, dad and Mel for their endless love and support throughout the years. It would not have been possible without you.

Declaration

Except where clearly indicated, the work reported in this thesis is an account of my own independent research at the University of Warwick and AstraZeneca, Alderley Park, carried out between October 2010 and July 2014.

The research reported in this thesis has not been submitted, either wholly or in part, for a degree at another institution.

At the time of submission, part of this work has appeared in the scientific literature:

Synthesis and Structure of Oxetane Containing Tripeptide Motifs. Powell, N. H.; Clarkson, G. J.; Notman, R.; Raubo, P.; Martin, N. G.; Shipman, M. *Chem. Commun.* **2014**, *50*, 8797-8800.

Abstract

This thesis describes work focused on the use of oxetane rings as isosteres and incorporation of this functional group into pharmaceutically relevant scaffolds.

Chapter 1 describes work directed towards the synthesis of 1,5-dioxaspiro[2.3]hexanes *via* three different strategies, namely ring-closure to form the oxetane ring, Corey epoxidation of the corresponding 3-oxetanones, and epoxidation of the corresponding 3-methylene oxetane. Nucleophilic ring-opening of substituted 1,5-dioxaspiro[2.3]hexanes to give amino acid type isosteres is also investigated.

Chapter 2 details the synthesis of novel oxetane-containing peptidomimetics. A 'one-pot' conjugate addition process from commercially available 3-oxetanone to give nitro dipeptide precursors in good yields was developed. Various methods for the reduction of the nitro group were explored to optimise the synthesis of the corresponding amine. Amide coupling, followed by deprotection, gave peptidomimetics containing the oxetane at the *C*-terminus and mid-chain in good yields over the 3 steps, whilst an *N*-terminus oxetane peptidomimetic was obtained in 45% yield through hydrogenation of the conjugate addition product. X-ray diffraction studies, alongside molecular dynamics simulations, provided structural insights into these new oxetane-containing peptidomimetics.

Detailed experimental procedures for the synthesis of all novel compounds are described in Chapter 3.

Abbreviations

Ac	Acetyl
Anal.	Analysis
aq	Aqueous
Ar	Aromatic ring
atm	Atmosphere
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
br	Broad
Calcd.	Calculated
cat.	Catalytic
Cbz or Z	Carboxybenzyl
δ	Chemical shift
d	Doublet
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicycloundec-7-ene
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
DCE	Dichloroethane
dd	Doublet of doublets
de	Diastereomeric excess
DEPT	Distortionless Enhancement by Polarisation Transfer
DIBAL	Diisobutylaluminium hydride
DIEA	*N*,*N*-Diisopropylethylamine
DKP	Diketopiperazine
DMDO	Dimethyldioxirane

DMF	*N*,*N'*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EDC	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide
EI	Electron Ionisation
ee	Enantiomeric excess
eq or equiv.	Equivalents
ESI	Electrospray Ionisation
Et	Ethyl
EWG	Electron-withdrawing group
FT-IR	Fourier Transform-Infrared
GC-MS	Gas Chromatography-Mass Spectroscopy
h	Hour(s)
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Coherence
HOBt	1-Hydroxybenzotriazole
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectroscopy
IBX	2-Iodoxybenzoic acid
J	Coupling constant
LC-MS	Liquid Chromatography-Mass Spectroscopy
µ	Micro
m.p.	Melting point
m	Multiplet
*m*CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl

MHz	Megahertz
min	Minute(s)
Ms	Methanesulfonyl
MS	Mass Spectrometry
MW	Microwave
NMM	4-Methylmorpholine
Nuc	Nucleophile
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
p-	*para*-
pg	Protecting group
ppm	Parts per million
^{i}Pr	Isopropyl
Py	Pyridine
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	Quartet
R_f	Retention factor
ROESY	Rotating Frame Overhauser Effect Spectroscopy
rt	Room temperature
rtp	Room temperature and pressure
s	Singlet
t	Triplet
TBAF	Tetra-*N*-butylammonium fluoride

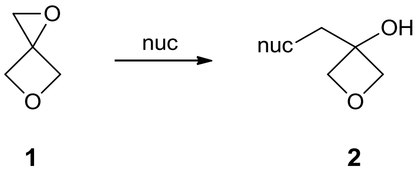
TBD	Triazabicyclodecene
^{t}Bu	*tert*-Butyl
temp.	Temperature
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TBDMS	*tert*-Butyldimethylsilyl
TMS	Trimethylsilyl
Ts	*para*-Toluenesulfonyl
p-TSA	*para*-Toluenesulfonic acid

Chapter 1:

Towards 1,5-Dioxaspiro[2.3]hexanes

1.1 Introduction

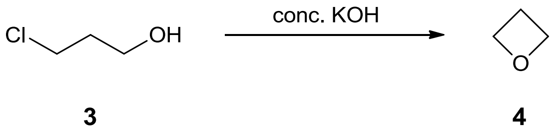
This thesis will describe the efforts made towards the incorporation of oxetane rings into pharmaceutically relevant scaffolds, including novel peptidomimetics. This chapter provides an introduction to oxetane rings, detailing both synthetic routes towards them as well as their use in medicinal chemistry. A number of comprehensive reviews are available on this topic, hence only relevant illustrative examples are included herein. This background is followed by a discussion of the work we have undertaken to develop routes towards spirocyclic scaffolds, such as **1**. Subsequent reaction of these spirocycles with various nucleophiles are also reported (Scheme 1.1).



Scheme 1.1. Proposed nucleophilic ring-opening of spirocycles.

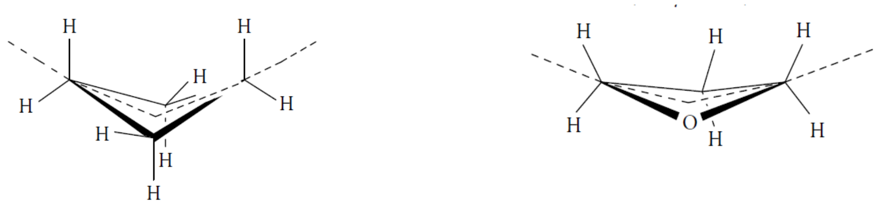
1.1.1 Trimethylene Oxide

The oxetane moiety has been known for many years but it is only over the past few decades that interest in it has grown. Indeed, Reboul was the first to synthesise the simplest oxetane (trimethylene oxide, **4**) in 1878 *via* the route shown in Scheme 1.2.[1-3]



Scheme 1.2. Reboul's synthesis of trimethylene oxide.

Trimethylene oxide was found to have a less puckered ring compared to the analogous cyclobutane (Figure 1.1).[3] The puckering in trimethylene oxide is believed to be lessened due to the replacement of a methylene group by the oxygen, thus reducing unfavourable eclipsing interactions.[2,4-5]



Cyclobutane

Ring puckering ~35°

Trimethylene oxide

Ring puckering ~11°

Figure 1.1. Comparison of ring puckering in cyclobutane and trimethylene oxide, figure reproduced from Process Chemistry in the Pharmaceutical Industry, Volume 2. [3]

1.1.2 Stability of Oxetanes

The inherent ring strain present in oxetanes increases their susceptibility to ring-opening reactions. Indeed, trimethylene oxide readily undergoes acid-catalyzed ring-opening reactions in the presence of sulfuric or perchloric acid in aqueous dioxane, although base-catalyzed ring-opening occurs much more slowly.[3,6] Recently it has been discovered that 3,3-disubstituted oxetanes have ring strain energies (RSE) approximately 14 kJ mol^{-1} below that of the parent oxetane (RSE value of 106.3 kJ mol^{-1}).[7] These 3,3-disubstituted oxetanes undergo no reaction with lithium aluminium hydride at 0 °C whereas monosubstituted oxetanes are opened slowly by the reagent at −78 °C. Despite this observation, 3,3-disubstituted oxetanes are still vulnerable to acid.[8]

1.1.3 Uses of Oxetanes

The oxetane moiety is found in a variety of natural products such as taxol, thromboxane A_2, oxetanocin and oxetin (Figure 1.2).[3]

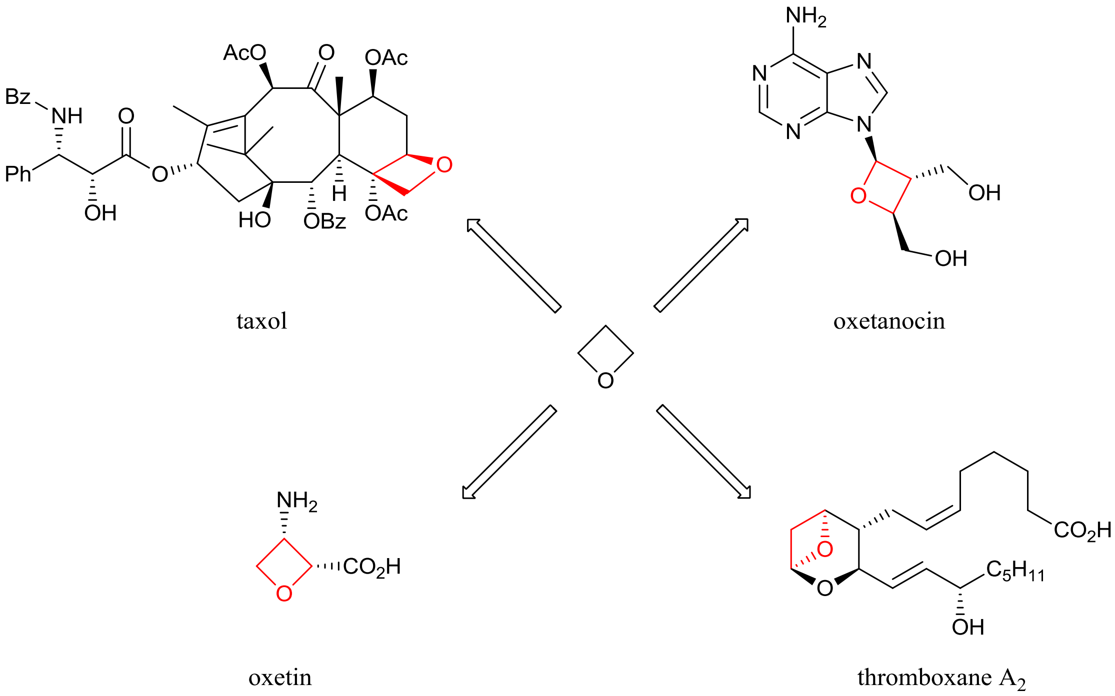


Figure 1.2. The presence of oxetane in natural products.

The contribution of the oxetane ring to the bioactivity of the natural products, however, remains unclear. For example, the importance of the oxetane ring in taxol was investigated through the replacement of the oxetane by the analogous azetidine, thietane and selenetane rings, which all resulted in lower bioactivity for the drug. However, it has not yet been definitively concluded that the oxetane moiety contributes to the drug's activity. Nevertheless, computational studies on taxol have shown that the oxetane ring contributes to the rigidity of the molecule and can act as a hydrogen bond acceptor for a threonine-OH in the supposed binding pocket of the drug.[3]

1.1.4 Oxetanes in Drug Discovery

Oxetanes have traditionally been overlooked in the pharmaceutical industry on account of their perceived instability and difficult syntheses.[3] However, interest in them has grown rapidly over the last few years as new routes and properties have been discovered. Indeed, Carreira and co-workers have hypothesised that the oxetane unit can be used as a *gem*-dimethyl surrogate, a carbonyl bond isostere and for modifying the molecular properties of drug molecules, making the oxetane ring an attractive prospect in drug discovery.[9-11]

1.1.5 Oxetanes as Novel Bioisosteres

Metabolically exposed sites in bioactive drug molecules have conventionally been blocked by the introduction of steric blocking groups such as *gem*-dimethyl units. Unfortunately, introduction of this group increases the lipophilicity of the drug molecule, adversely affecting its metabolic and pharmacokinetic properties.[9] It has been proposed that a better alternative would be the introduction of a more metabolically robust and less lipophilic small molecular unit, which would not have a detrimental effect on the properties of the drug. Such a unit is the oxetane group, which has been found to occupy a similar volume as the *gem*-dimethyl unit, based on calculated Van der Waals volumes.[9] Introduction of the oxetane unit imparts improved solubility and metabolic stability with reduced lipophilicity to the drug candidate. Hence oxetane can be considered analogous to a *gem*-dimethyl unit (Figure 1.3).[8-9,11]

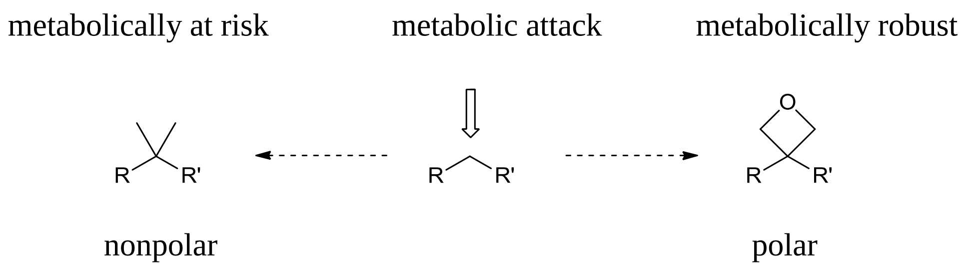


Figure 1.3. Oxetane as a *gem*-dimethyl surrogate.[9]

As mentioned previously, oxetane rings have also been proposed as bioisosteres for carbonyl bonds. The carbonyl bond is a problematic functional group for use in drug discovery due to its susceptibility to enzymatic attack *in vivo*.[8,10-11] Isosteric replacement of the carbonyl bond by an oxetane unit could result in a drug candidate with a similar bioactivity, but improved stability *in vivo*.[8] Additionally, the oxygen lone pairs in a carbonyl bond and a 3,3-disubstituted oxetane have been found to occupy the same spatial arrangement (Figure 1.4). The oxetane ring could potentially also be used to improve the fit of a drug in its receptor pocket through its increased volume and C···O distance compared to a carbonyl bond.[8,10] Furthermore, the hydrogen bonding capability of the oxetane moiety is comparable to aliphatic carbonyls such as ketones, aldehydes and esters.[8,10,12-13] It does, however, remain a much weaker hydrogen bond acceptor than amide carbonyl bonds.[8]

Figure 1.4. Oxetane ring as a carbonyl bond bioisostere.[10]

Another common ploy used in drug development, is to improve the solubility of a potential drug molecule through the introduction of a morpholine unit. However, whilst this improves the solubility of the drug, morpholine is known to be susceptible to oxidative metabolism.[8] It has been proposed that a spirocyclic oxetane unit, such as that shown in Figure 1.5, can be considered structurally analogous to morpholine, improving the solubility of a drug molecule whilst remaining metabolically stable.[8,10]

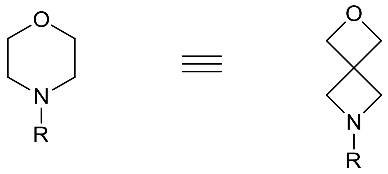
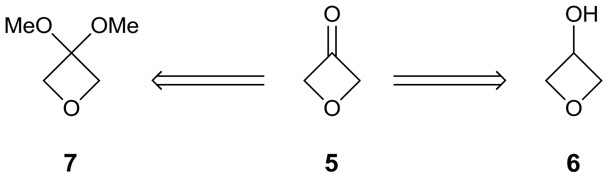


Figure 1.5. Spirocyclic oxetanes as potential morpholine replacements.[10]

One especially desirable property of these spirocyclic analogues is the ability to explore and expand the chemical space around morpholine.[10] With the development of robust synthetic routes to oxetanes that can be readily grafted or incorporated into drug molecules, oxetanes are a promising new scaffold in the pharmaceutical industry.

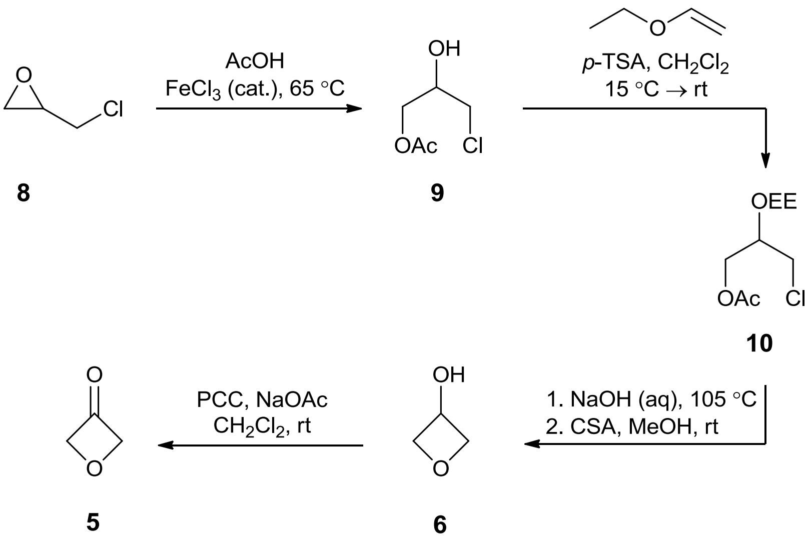
1.1.6 Synthesis of Oxetanes

The most common starting point for the synthesis of oxetane building blocks is oxetan-3-one (**5**), which is commercially available but very expensive. There are two routes primarily used to access oxetan-3-one, one *via* oxetan-3-ol (**6**) and the other *via* 2,2-dimethoxyoxetane (**7**) (Scheme 1.3).



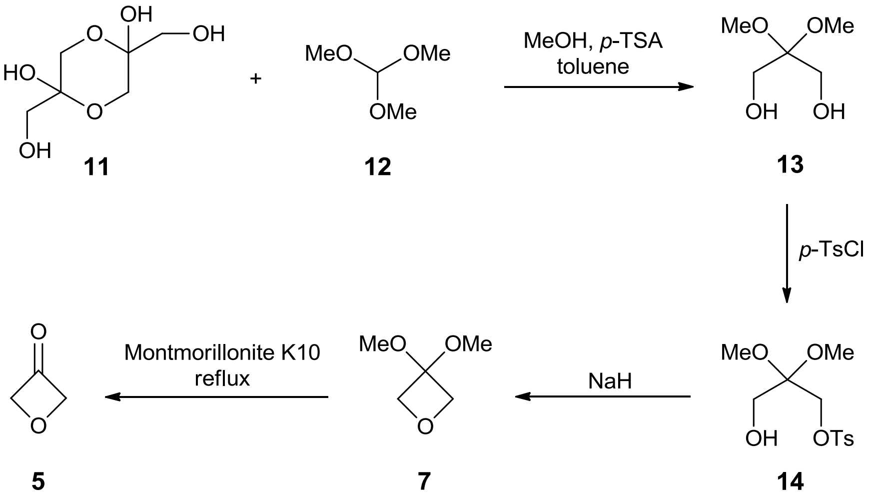
Scheme 1.3. Retrosynthesis of oxetan-3-one.

The synthesis of oxetan-3-one *via* oxetan-3-ol was achieved in 14% overall yield over 5 steps by Kozikowski and Fauq (Scheme 1.4).[14]



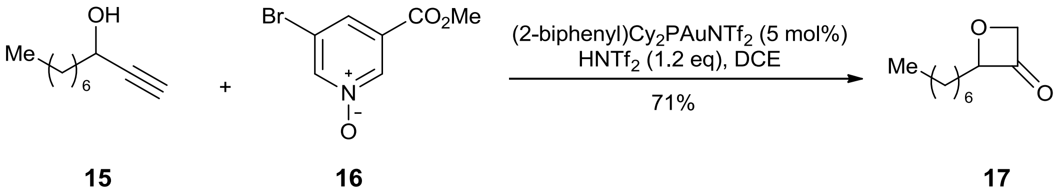
Scheme 1.4. Synthesis of oxetan-3-one *via* oxetan-3-ol.[14]

The synthesis of oxetan-3-one *via* 2,2-dimethoxyoxetane was achieved in 23% overall yield using an improved method developed by Carreira and co-workers (Scheme 1.5).[9] This method was used to synthesise large quantities of oxetan-3-one for use as a building block for incorporation into biologically active molecules.[9] However, the scalability of both routes remains problematic owing to the poor overall yield and multiple steps.



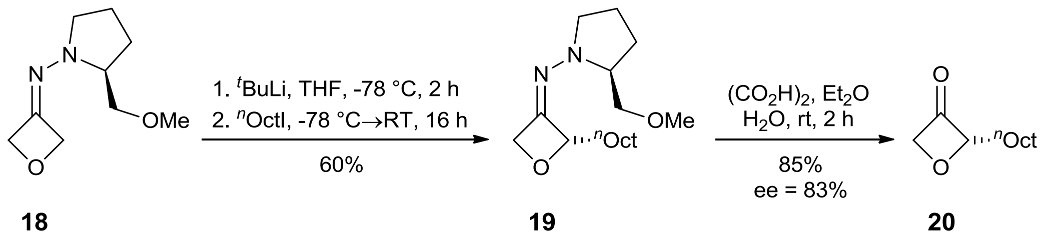
Scheme 1.5. Preparation of oxetan-3-one from 1,3-dihydroxyacetone dimer.[9]

Other methods for the synthesis of substituted oxetan-3-ones have been developed, including recent work published by Zhang and co-workers.[15] These authors utilized a gold catalyst for the cyclisation of propargyl alcohol **15** to oxetan-3-one **17** in a one-pot method (Scheme 1.6).[15] This method allows a wide variety of oxetan-3-ones substituted in the 2- and the 4-positions to be readily synthesised.



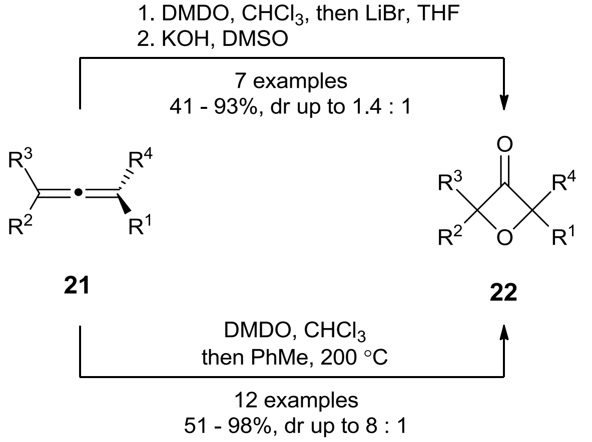
Scheme 1.6. One-pot gold-catalysed cyclisation of **15** to **17**.[15]

An asymmetric route towards 2-substituted oxetan-3-ones has also been developed within our research group.[16] Metalation of the SAMP hydrazone of oxetan-3-one, followed by alkylation with various electrophiles yields substituted oxetan-3-ones in good enantiomeric excesses (Scheme 1.7).



Scheme 1.7. Stereoselective synthesis of 2-substituted oxetan-3-ones.[16]

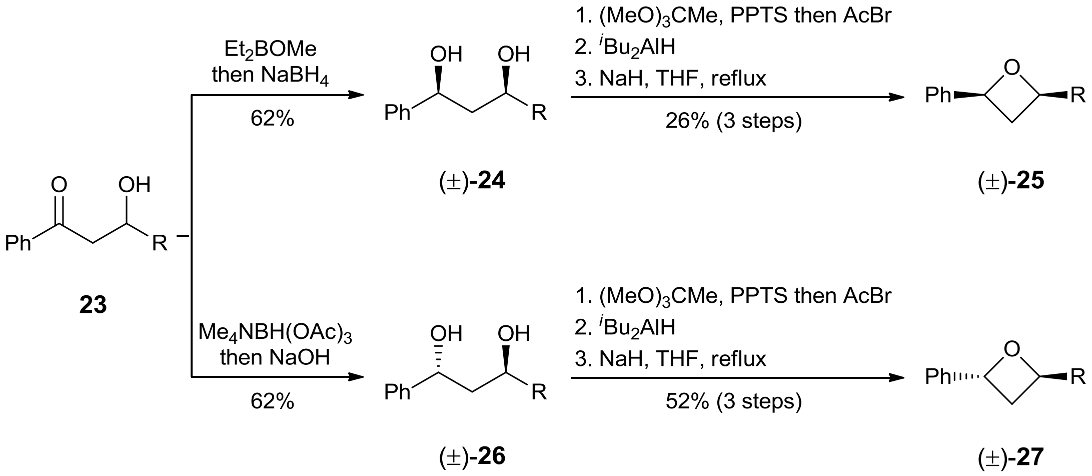
More recently, Sharma and Williams have demonstrated the synthesis of a variety of mono-, di-, tri- and tetra-substituted oxetan-3-ones from allenes, as shown in Scheme 1.8.[17] Initial formation of the spirodiepoxide from allene **21** using DMDO was followed by either halide-induced ring-opening and subsequent base-promoted ring-closure, or thermal rearrangement to oxetan-3-one **22**. When diastereomers were formed, the major diastereomer was dependent on the method employed, thus enabling access to either diastereomer preferentially.



Scheme 1.8. Formation of substituted oxetan-3-ones from allenes.[17]

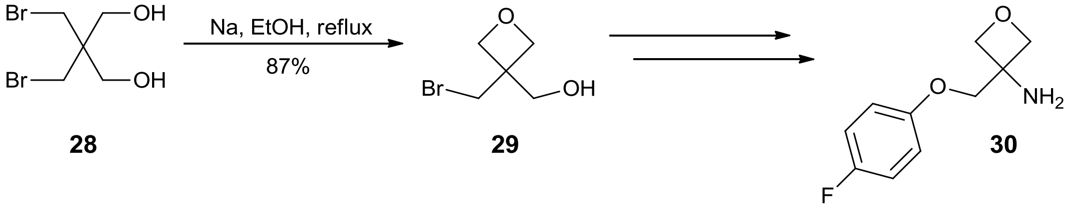
Another synthetic route towards substituted oxetanes is *via* conversion of one hydroxyl group of a 1,3-diol into a better leaving group, followed by ring-closure.

One such method, allowing the stereocontrolled synthesis of a substituted oxetane, has been reported by Aftab *et al* (Scheme 1.9).[18]



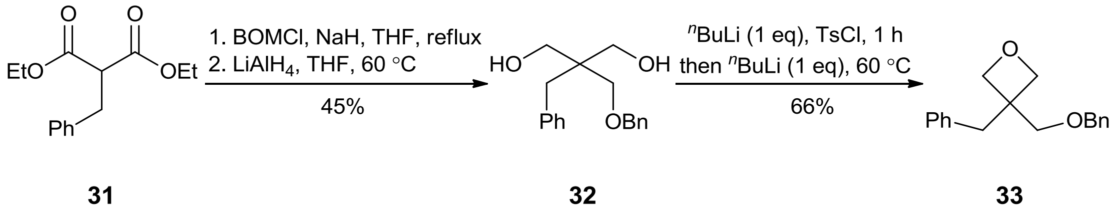
Scheme 1.9. Ring-closure to form an oxetane ring.[18]

Vigo *et al* have recently reported two alternative syntheses for 3,3-disubstituted oxetanes *via* ring-closure.[19] Using dibromide **28**, they formed 3,3-disubstituted oxetane **29** in a good yield, with the opportunity for further functionalities to be introduced through displacement of the second halogen (Scheme 1.10).



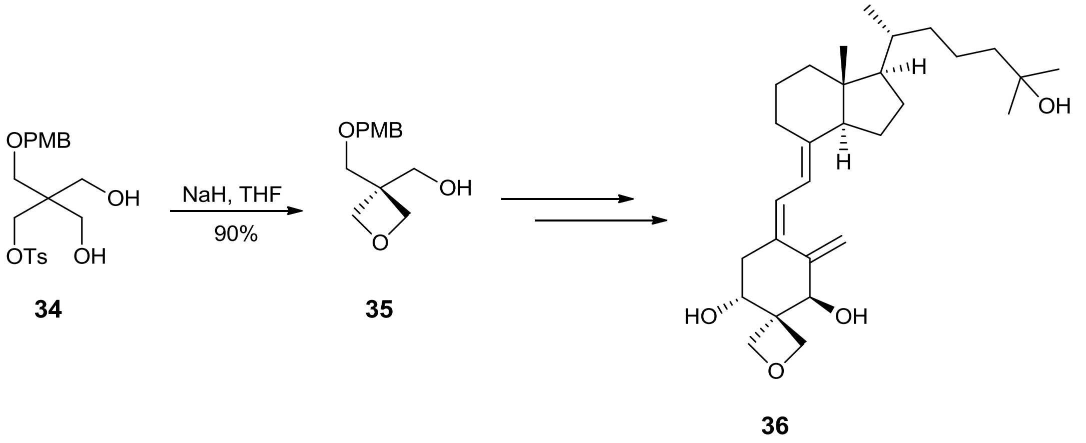
Scheme 1.10. Synthesis of 3,3-disubstituted oxetanes using Br as leaving group.[19]

In the second example, these authors reacted **32** with ^{n}butyllithium and *p*-tosyl chloride to form the monotosylate before adding a further equivalent of ^{n}butyllithium and heating to promote oxetane ring formation to give **33** (Scheme 1.11).



Scheme 1.11. Synthesis of 3,3-disubstituted oxetanes *via* monotosylation.[19]

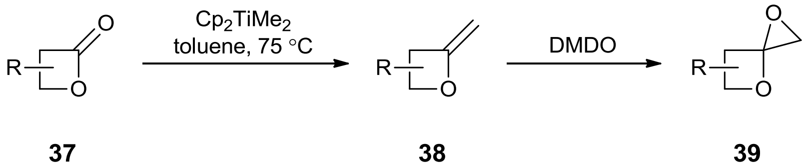
A similar ring-closure strategy was employed by Fujishima *et al*[20] to access oxetane-containing analogues of 1,25-dihydroxyvitamin D_3 (Scheme 1.12). These oxetane analogues were postulated to have improved binding affinity for the vitamin D receptor, based on Carreira and co-workers' suggestion that oxetane enables deeper oxygen placement in receptor pockets.[8,10,20]



Scheme 1.12. Synthesis of oxetane-containing 1,25-dihydroxyvitamin D_3 analogue, **36**.[20]

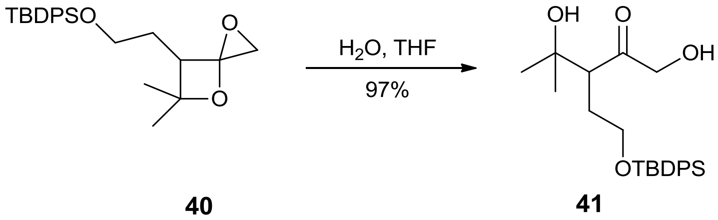
1.1.7 Synthesis and Reactions of Spirocyclic Oxetanes

There has been much interest shown in the synthesis of spirocyclic oxetanes, especially those containing 3- and 4-membered rings, as well as the reactivity of such systems.[21] Indeed, a synthetic route towards 1,5-dioxaspiro[3.2]hexanes (**39**) (Scheme 1.13) has been developed by Ndakala and Howell.[22-23] A general synthesis for the preparation of 2-methyleneoxetanes had previously been established within the Howell group, allowing access to 1,5-dioxaspiro[3.2]hexanes by epoxidation of olefin **38**.[22-23] On account of the potential instability of **39** in acidic or basic conditions, the authors used DMDO as the epoxidation reagent since it can be employed under neutral conditions and generates inert byproducts. Anhydrous “acetone free” DMDO was required for successful reactions.[23] A range of 1,5-dioxaspiro[3.2]hexanes substituted at both positions on the oxetane ring were successfully synthesised and isolated but the parent system **39** (R = H) was only observed by ^{1}H NMR.[23]



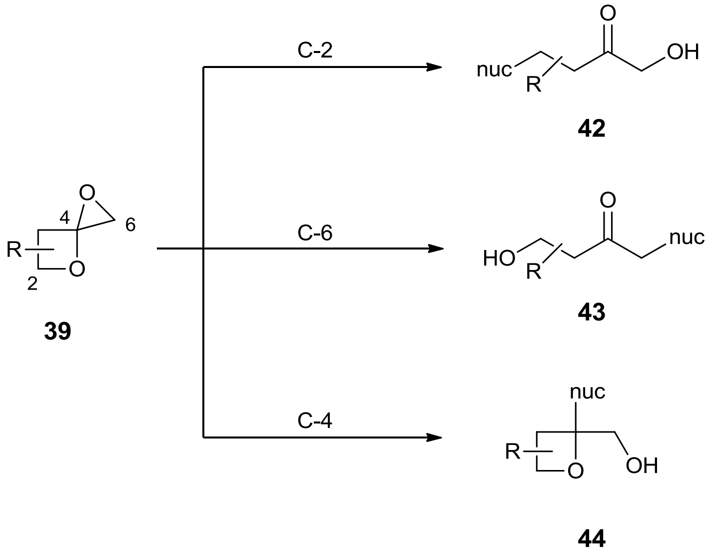
Scheme 1.13. Synthetic route to 1,5-dioxaspiro[3.2]hexanes.

Once a synthetic route to a range of 1,5-dioxaspiro[3.2]hexanes had been established, the reactivity of these systems was investigated. It was discovered that subjection of 1,5-dioxaspiro[3.2]hexane **40** to a water/THF mixture without any acid present, gave the ring-opened product **41** in a 97% yield (Scheme 1.14).[23]



Scheme 1.14. Hydrolysis of 1,5-dioxaspiro[3.2]hexanes.[23]

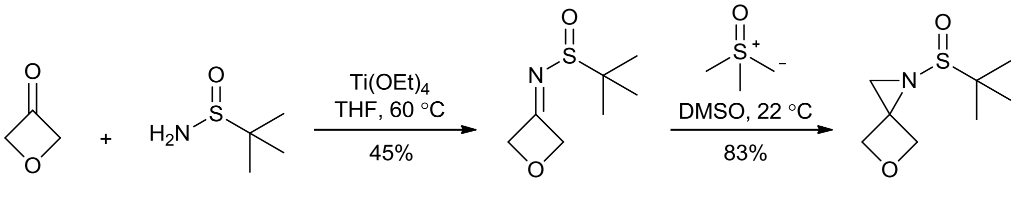
Reactions of 1,5-dioxaspiro[3.2]hexanes with different nucleophiles were then investigated. The ring-opening reactions of 1,5-dioxaspiro[3.2]hexanes can theoretically occur at multiple positions in the molecule (Scheme 1.15).[24]



Scheme 1.15. Potential sites of reaction in 1,5-dioxaspiro[3.2]hexanes.[24]

Further investigation of the ring-opening revealed that the regioselectivity can be controlled through careful selection of both the nucleophile and the reaction conditions used.[24-25] Neutral and anionic oxygen-, nitrogen- and sulfur-based nucleophiles attacked at C-6 to give the expected products, whilst lithium aluminium hydride appeared to react at the C-6 position before reducing the resultant β-hydroxy ketone. However, DIBAL, Me_3Al and $TMSN_3$ were found to react at C-4 to give the unexpected oxetane product **44**.[24] Further investigations were carried out with a variety of nitrogen-containing heteroaromatic nucleophiles and it was concluded that the 2,2-disubstituted oxetane **44** was formed preferentially using acidic nucleophiles, presumably promoted through coordination of the Lewis acid to the epoxide oxygen.[24-25] In contrast, neutral and anionic heteroatom nucleophiles appear to react preferentially at C-6 to give the open chain product **43**.[24-25]

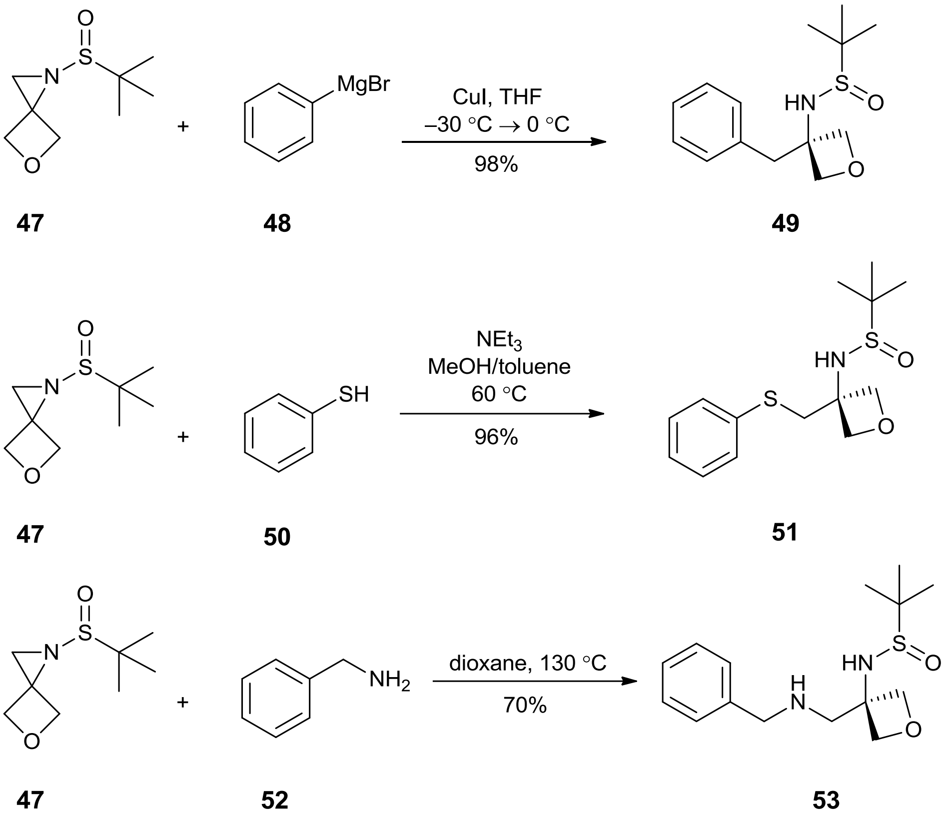
In addition to oxetane/oxirane ring systems, oxetane/aziridine ring systems have also been investigated by Hamzik and Brubaker.[26] Reaction of oxetan-3-one with racemic **45** gave the sulfinimine **46** that was then epoxidised with a sulfur ylide to give the spirocycle **47** (Scheme 1.16). The resultant activity of this spirocycle was then explored.[26]



5 **45** **46** **47**

Scheme 1.16. Synthesis of sulfinyl aziridine oxetane.[26]

It was discovered that the aziridine ring could be regioselectively opened with carbon-, sulfur- and nitrogen-based nucleophiles (Scheme 1.17), which was interesting in terms of offering a useful route to 3-aminooxetanes such as **49**, **51** and **53**.[26] The alternative nucleophilic opening of the oxetane ring was not reported.



Scheme 1.17. The ring-opening reactions of the sulfinyl aziridine oxetane.[26]

1.1.8 Research Hypothesis and Aims

Whilst 1,5-dioxaspiro[3.2]hexanes (**39**) and 5-oxa-1-azaspiro[2.3]hexanes (**47**) are known, 1,5-dioxaspiro[2.3]hexanes such as **1** have not been investigated (Figure 1.6).

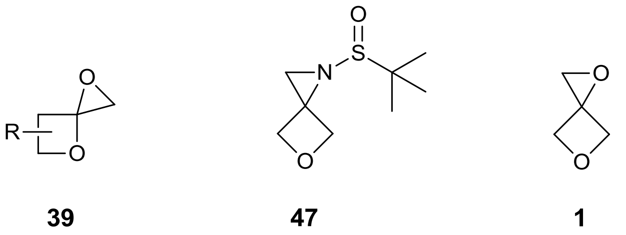
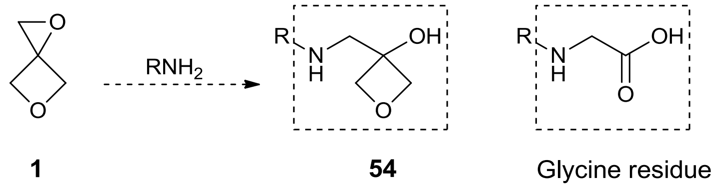


Figure 1.6. Examples of 1,5-dioxaspiro[3.2]hexanes, 5-oxa-1-azaspiro[2.3]hexanes and 1,5-dioxaspiro[2.3]hexanes.

We felt that such systems would be worthy of investigation. The opening of the epoxide ring of a 1,5-dioxaspiro[2.3]hexane (**1**) at C-2 with a nitrogen nucleophile would generate an amino acid isostere containing an oxetane in place of the carbonyl bond (Scheme 1.18).

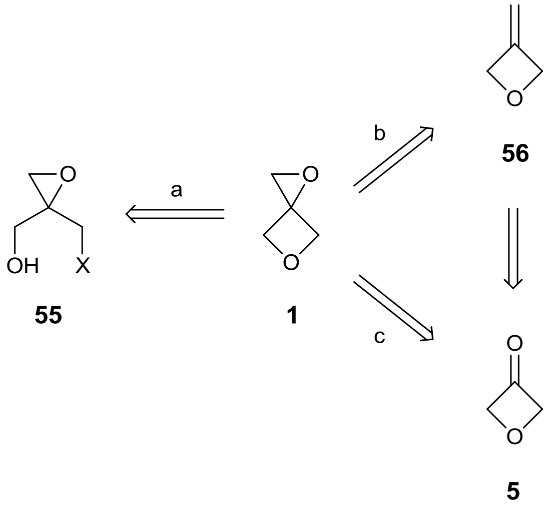


Scheme 1.18. Proposed reaction of spirocycle **1**.

Therefore we decided to investigate routes to spirocycles such as **1**, in order to explore their ring-opening reactions.

1.2 Results and Discussion

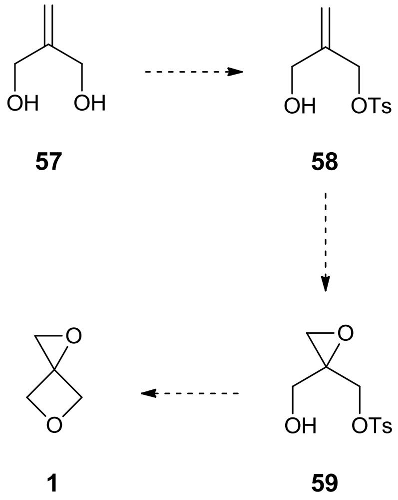
It was proposed that 1,5-dioxaspiro[2.3]hexane (**1**) could be synthesised *via* one of the three routes shown in Scheme 1.19, namely ring-closure of **55** with X as a leaving group (route *a*), epoxidation of alkene **56** (route *b*) or a Corey epoxidation of ketone **5** using a sulfur ylide (route *c*).



Scheme 1.19. Retrosynthesis of **1**.

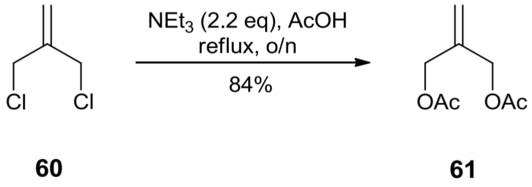
1.2.1 Attempted Synthesis of 1,5-Dioxaspiro[2.3]hexane *via* Ring-Closure

The initial route we investigated for the synthesis of spirocycle **1** involved ring-closure, as detailed in Scheme 1.20.



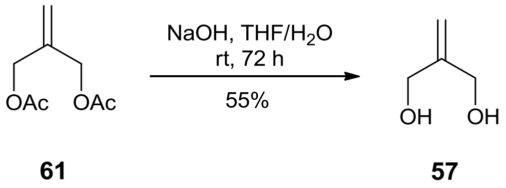
Scheme 1.20. Proposed route to **1** *via* ring-closure.

The first task was to synthesise diol **57**, which is commercially available but expensive, according to literature procedures.[27] Initial replacement of the chlorine groups of commercially available 3-chloro-2-chloromethyl-1-propene (**60**) by acetoxy groups was achieved by heating a solution of the starting material, NEt_3 and glacial acetic acid under reflux overnight (Scheme 1.21). After addition of dichloromethane and an aqueous workup, **61** was obtained in good yields with no need for further purification.



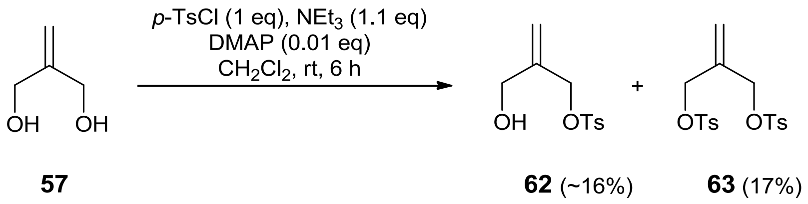
Scheme 1.21. Synthesis of **61**.

The second step involved the hydrolysis of the acetoxy group to give diol **57**. Using K_2CO_3, NaOH or NaOMe in methanol at rt or reflux failed to give any product, forming the monoacetate product preferentially. However, NaOH in THF/water at rt gave diol **57** in ~44% yield (Scheme 1.22). This yield was further improved when a Soxhlet extraction was used rather than an aqueous work-up. Distillation using a Kugelrohr gave **57** in 55% yield. Alternatively **57** could be taken through to the next step without purification.



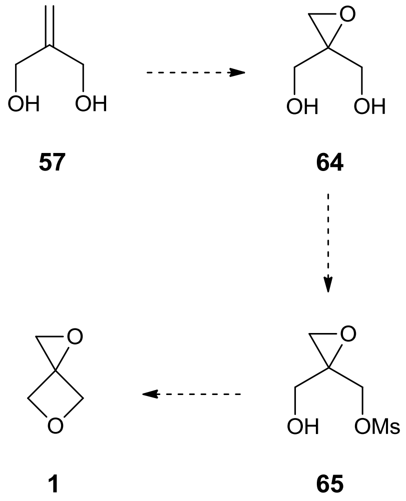
Scheme 1.22. Hydrolysis of **61** to **57**.

Next, we sought to convert one of the hydroxyl groups of **57** into a better leaving group by reacting it with *p*-toluenesulfonyl chloride (Scheme 1.23). The main challenge with this step was preventing monotosylate **62** from reacting further to give ditosylate **63**. Various conditions were investigated, with only *p*-TsCl (1 equiv.), NEt_3 (1.1 equiv.) and DMAP (0.01 equiv.) in anhydrous CH_2Cl_2 giving monotosylate **62** in ~16% yield as well as ditosylate **63** (17% yield). All other conditions explored gave either no product or predominantly **63**. Having obtained monotosylate **62**, albeit impure, epoxidation of the double bond was attempted using *m*CPBA. Unfortunately the desired epoxide could not be isolated.



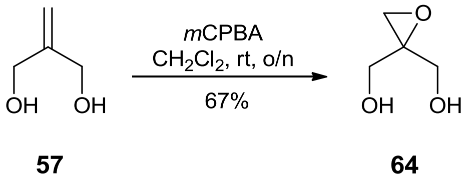
Scheme 1.23. Attempted monotosylation of **57**.

Needing to make more material, we decided to explore an alternative to the difficult tosylation step. We decided to investigate activating one of the hydroxyl groups with methanesulfonyl chloride. Mesylation should proceed faster than tosylation, potentially minimising the chance of overreaction. We also decided to explore epoxidation of diol **57** prior to the protection of the hydroxyl group, as this compound is reported in literature. This modified route is shown in Scheme 1.24.



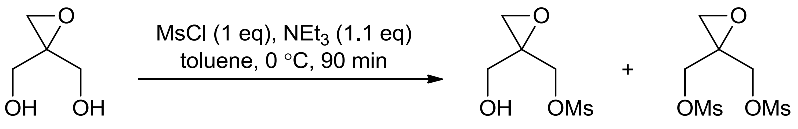
Scheme 1.24. Modified route to **1**.

Epoxide **64** was obtained following a literature procedure, using the conditions shown in Scheme 1.25.[28] Initial yields were poor (23%), probably due to difficulties in purifying epoxide **64**, but were improved to 67% when the reaction was repeated on a larger scale.



Scheme 1.25. Epoxidation to give **64**.

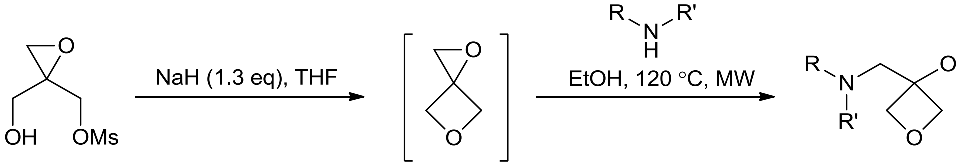
With the epoxide in hand, the mesylation was investigated. Initial attempts using MsCl in toluene at 0 °C gave both monomesylate **65** and dimesylate **66** in poor yields (Scheme 1.26), possibly due to potential product loss when removing the toluene. Changing the reaction solvent from toluene to CH_2Cl_2, and cooling the reaction mixture to −78 °C, enabled us to isolate **65** in an improved yield of 29%, although a large amount of dimesylate **66** (~32%) was also formed.



64 **65** (9%) **66** (5%)

Scheme 1.26. Mesylation of **64**.

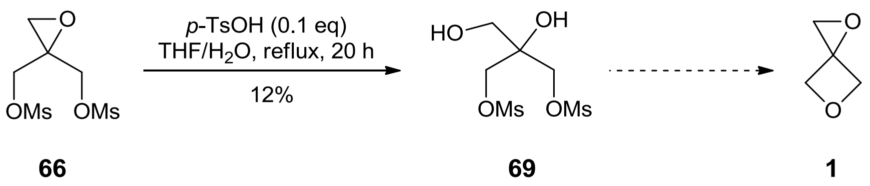
Attempts to form spirocycle **1** from monomesylate **65** using NaH in THF or DMF failed to provide the product, possibly due to loss into the aqueous layer in the work-up or because of the likely volatility of the product itself. For these reasons, direct reaction of the spirocycle with a nucleophile *in situ* was considered (Scheme 1.27). Benzylamine was initially used as the nucleophile but, upon irradiation in the microwave at 120 °C for 10 minutes, only benzylamine was observed by ^{1}H NMR. Dibenzylamine was then used under the same conditions but gave only an insoluble white solid and dibenzylamine.



65 **1** **67** where R = CH_2Ph, R' = H
68 where R, R' = CH_2Ph

Scheme 1.27. Attempted reaction of **1** with amine nucleophiles *in situ*.

As dimesylate **66** was easily obtained, we decided to explore the possibility of opening the epoxide with water to give **69** which could then form spirocycle **1** through a double ring-closure, as shown in Scheme 1.28. However, whilst **69** was obtained, it was only in a low yield and therefore this route was not explored further.

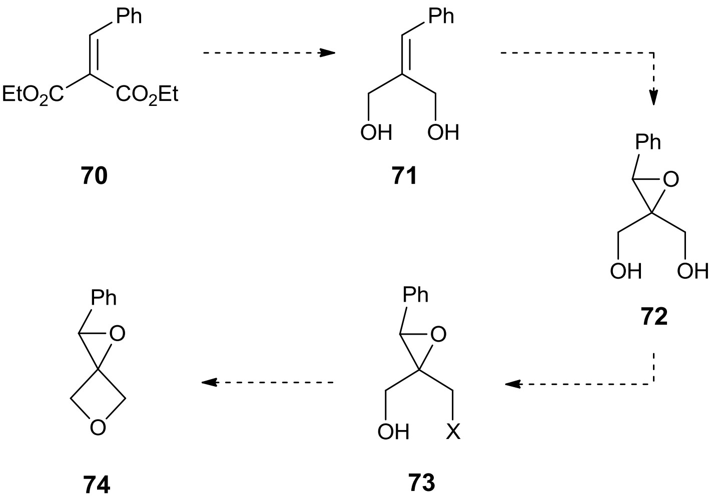


Scheme 1.28. Ring-opening of **66** with water.

Owing to the difficulties in isolating 1,5-dioxaspiro[2.3]hexane **1**, it was decided that it would be better to first establish the synthetic route using a less water-soluble spirocycle of higher molecular weight. Hence, the synthesis of substituted spirocycles was investigated.

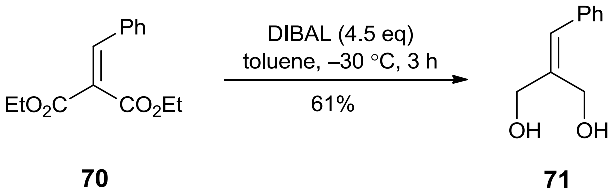
1.2.2 Synthesis of Substituted 1,5-Dioxaspiro[2.3]hexane *via* Ring-Closure

It was reasoned that putting a substituent on the epoxide ring would increase the molecular weight of the spirocyclic compound, making it less volatile and therefore easier to handle. The proposed synthetic route to substituted spirocycle **74** is shown in Scheme 1.29.



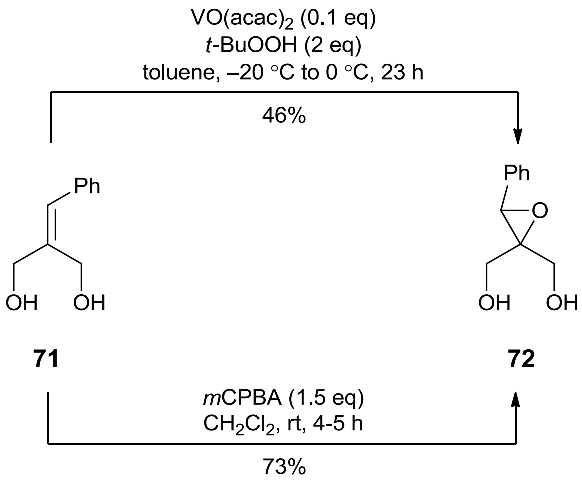
Scheme 1.29. Proposed synthetic route to spirocycle **74**.

The first step in the sequence involved reduction of the ester groups of commercially available diethyl benzylidenemalonate (**70**) to give diol **71**, using a literature method (Scheme 1.30).[29] The solution of **70** in toluene was cooled to –30 °C before DIBAL (1 M in toluene) was added dropwise. Initial attempts at the reaction gave **71** in 28% yield, but purification of the product by simple trituration rather than column chromatography gave **71** in an improved 61% yield. The large volume of expensive DIBAL made this reaction suboptimal for the large scale synthesis of **71**. However, lithium aluminium hydride was ineffective as an alternative reducing agent.



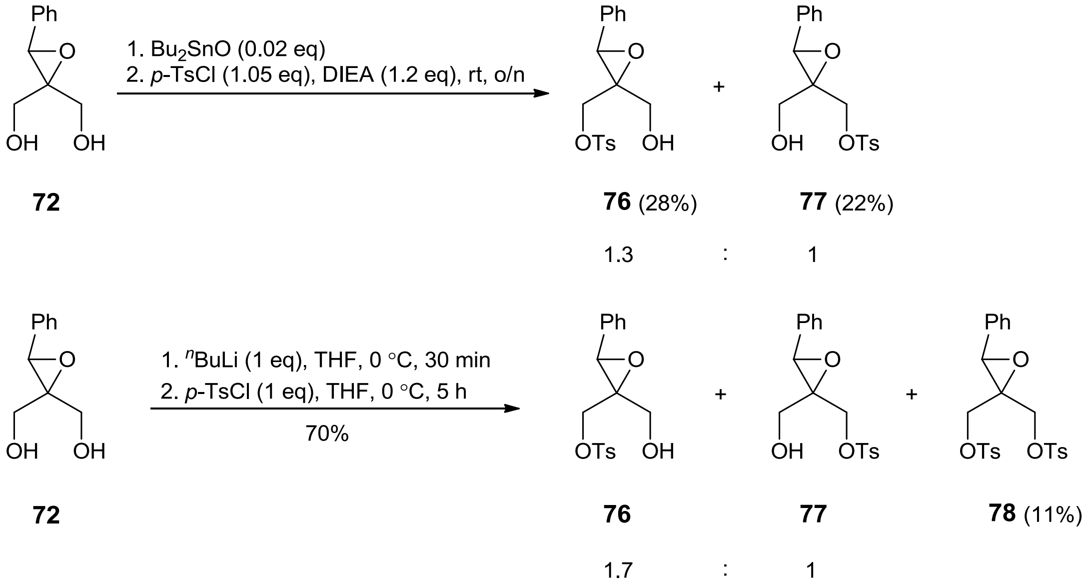
Scheme 1.30. Reduction of the ester groups of **70**.

Having established a route to **71**, the next step was to epoxidise the alkene bond. As it contained allylic alcohols, use of a directed epoxidation was investigated.[30] Vanadyl acetylacetonate and *tert*-butyl hydroperoxide were explored for the reaction (Scheme 1.31).[30-32] Whilst epoxide **72** was obtained, the yield was modest. Subsequent attempts to epoxidise the double bond with *m*CPBA resulted in a much improved yield of 73%.



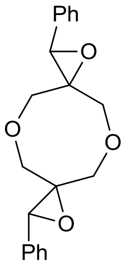
Scheme 1.31. Epoxidation of **71**.

Subjection of epoxide **72** to conventional mesylation conditions failed to isolate monomesylate **75**, so the tosylation of **72** was investigated as an alternative (Scheme 1.32). Dibutyl tin oxide was initially explored for the tosylation, using a modified literature procedure.[33-34] This provided monotosylates **76** and **77** in 28% and 22% yield respectively, separated by column chromatography. Higher yields were obtained using ^{n}BuLi, following a recently published procedure,[35] alongside a small amount of ditosylate **78** (11%), although the regioisomers were not separated. The regioisomers showed different chemical shifts and we were therefore able to assign them using ^{1}H NOE spectroscopy. NOEs of both **76** and **77** were obtained. For example, irradiation of the CH_2 protons adjacent to the OTs group in **76** gave an enhancement of the epoxide CH signal, but no enhancement of the OH.



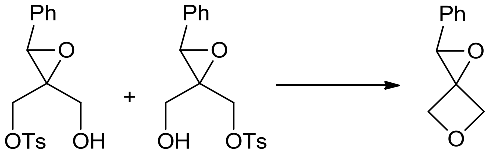
Scheme 1.32. Two methods towards **76** and **77**.

When the reaction was repeated on a large scale, the two regioisomers were not separated as both **76** and **77** can potentially form spirocycle **74**. Next, conditions for the ring-closure were investigated and the results are shown in Table 1.1. Unfortunately, none of the conditions investigated gave **74** in good yield. NaH in DMF at rt gave **74** in 6% yield (entry 1), but attempts to improve upon, or even replicate this reaction were unsuccessful. Using an alternative base such as ^{n}BuLi, Cs_2CO_3, TBD or KH (entries 5-9) failed to yield any product. A mass ion corresponding to $[2M+Na]^+$ was observed (entry 10), which initially led us to believe spirocycle **74** had been formed. However, after we had successfully isolated **74**, it seems more likely that $[2M+Na]^+$ corresponded to a dimer such as **79** (Figure 1.7), formed by combination of two molecules of starting material.



79

Figure 1.7. Dimer resulting from intermolecular reaction of **76**/**77** with **76**/**77**.



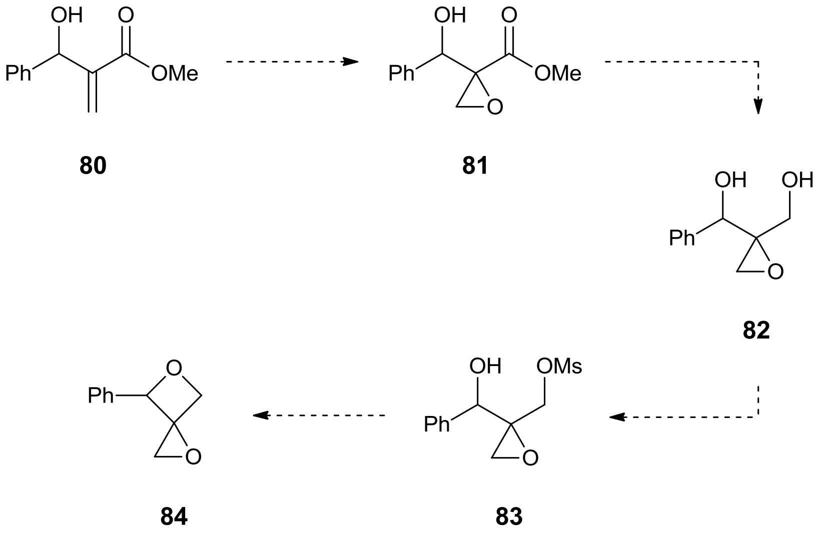
76 **77** **74**

1.7 : 1

Entry	Base	Solvent	Conc. (M)	Temp	Time (h)	Product (s)
1	NaH	DMF	0.02	0 → rt	6	6%
2	NaH	DMF	0.02	0 → rt	24	0%[a]
3	NaH	DMF	0.02	0 → rt	50	Trace[b]
4	NaH	DMF	0.20	0 → rt	24	0%[a]
5	^{n}BuLi	THF	0.02	0 → rt	49	Trace[b]
6	Cs_2CO_3	MeCN	0.02	0 → rt	60	0%[a]
7	Cs_2CO_3	MeCN	0.02	0 → reflux	24	0%
8	TBD	MeCN	0.02	0 → rt	60	0%
9	KH	THF	0.02	0 → rt	24	0%
10	NaH	DMF	0.11	0 → rt	o/n	**79**[c]

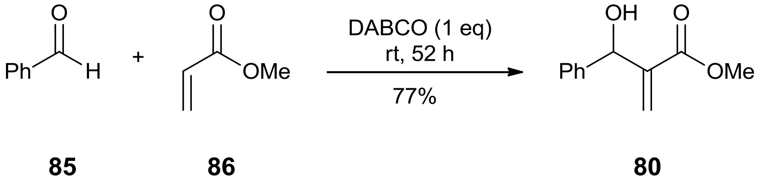
Table 1.1. Investigation into the ring-closure of **76**/**77** to form **74**. [a]Mainly SM observed by ^{1}H NMR. [b]Observed by ^{1}H NMR. [c]Observed by ESI-MS.

As we were unable to isolate **74** in good yield, we decided to investigate the synthesis of spirocycle **84**, with the substituent on the oxetane ring. The proposed route *via* ring-closure is shown in Scheme 1.33.



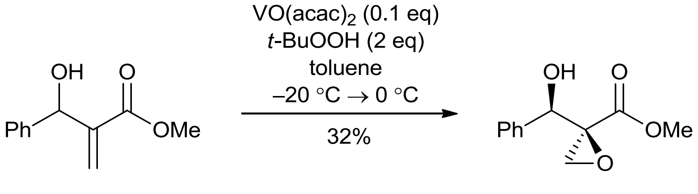
Scheme 1.33. Proposed synthetic route to substituted spirocycle **84**.

The first step involved synthesis of **80** *via* a Baylis-Hillman reaction (Scheme 1.34). This was performed according to a literature procedure[36] and occurred in good yield, despite a long reaction time.



Scheme 1.34. Baylis-Hillman reaction to give **80**.

Epoxidation of alkene **80** was performed using vanadyl acetylacetonate, as shown in Scheme 1.35, which gave (±)-**81** in a relatively low yield of 32%. The stereochemistry of (±)-**81** was tentatively assigned through comparison with data reported in the literature.[37]



80

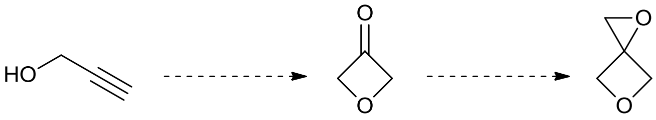
(±)-81

Scheme 1.35. Epoxidation of **80**.

Reduction of the ester group of **81** was attempted using DIBAL in a 2.5-fold excess, but this gave mainly starting material. As this route had low yields and there were difficulties reducing the methyl ester, an alternative route was sought.

1.2.3 Synthesis of 1,5-Dioxaspiro[2.3]hexane *via* Corey Epoxidation

A recent paper published on the synthesis of substituted oxetan-3-ones using a gold-catalysed cyclisation provided an interesting alternative route to 1,5-dioxaspiro[2.3]hexanes.[15] The cyclisation of propargyl alcohol (**87**) to oxetan-3-one (**5**) could be followed by a Corey epoxidation to give **1** (Scheme 1.36).



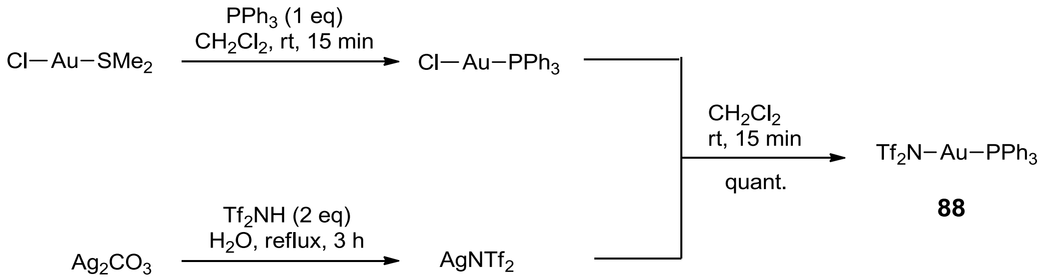
87

5

1

Scheme 1.36. Gold-catalysed cyclisation followed by Corey epoxidation to **1**.

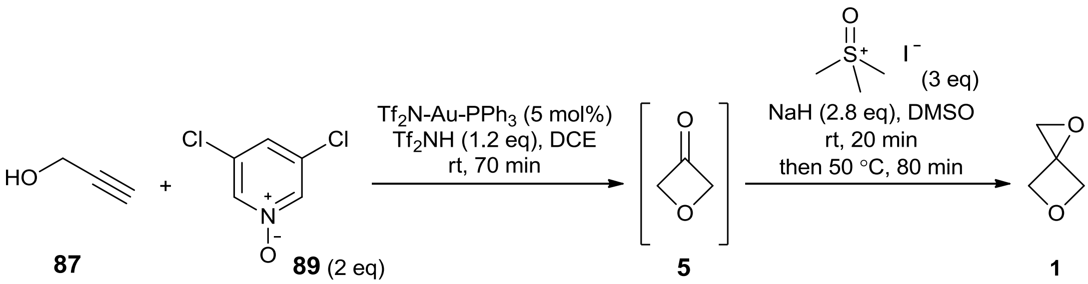
Ye *et al* investigated a range of gold catalysts, oxidants and acids in order to find the optimal conditions for the cyclisation.[15] We elected to use catalyst **88**, which was accessed using literature methods,[38] as shown in Scheme 1.37, as well as *N*-oxide **89** and bis(trifluoromethane)sulfonimide as the acid source. These were not the best reagents/catalysts reported by Ye *et al*, but they were shown by these authors to be effective and were more readily accessible.



88

Scheme 1.37. Synthesis of gold catalyst **88**.

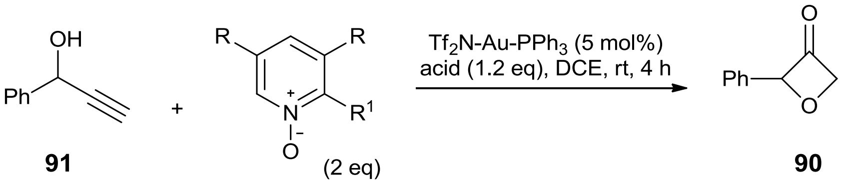
Having synthesised the catalyst, the cyclisation and subsequent epoxidation *in situ* using a sulfur ylide, according to a literature procedure, was attempted (Scheme 1.38).[39] The reaction mixture was worked-up with diethyl ether, which was then removed by distillation. However, only dichloroethane was observed in the distillate by ^{1}H NMR.



87 **89** (2 eq) **5** **1**

Scheme 1.38. Attempted synthesis of **1**.

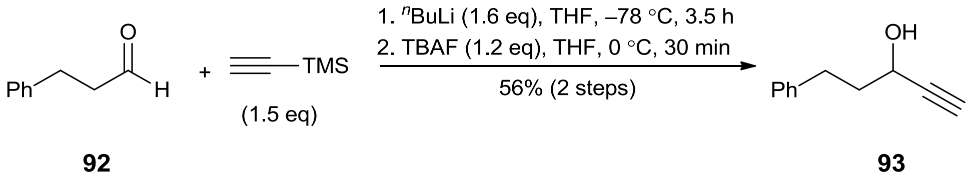
As attempts to obtain spirocycle **1** using this method were unsuccessful, likely due to the volatility and reactivity of the product, we decided to synthesise an oxetan-3-one with a substituent in the 2-position. We initially tried to synthesise oxetan-3-one **90** from the commercially available alkyne **91**, as reported by Ye *et al*.[15] Unfortunately, despite exploring different conditions, we could not synthesise **90** in a good yield using our catalyst and *N*-oxide combination (Table 1.2). In contrast, Ye *et al* synthesised **90** in 57% yield using 2-bromopyridine *N*-oxide and (2-biphenyl)$Cy_2PAuNTf_2$.[15]



Entry	R	R^1	Acid	Yield (%)
1	H	Br	MsOH	0[a]
2	Cl	H	MsOH	~16
3	Cl	H	$HNTf_2$	~11

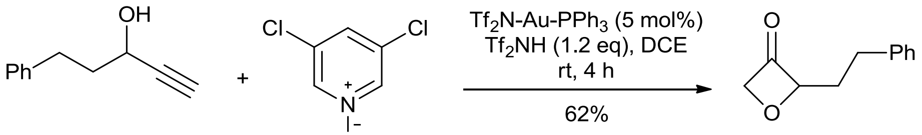
Table 1.2. Reaction conditions explored for the formation of **90**. [a]Mainly SM recovered.

An alternative substituted oxetan-3-one was selected and the starting alkyne **93** synthesised in 56% overall yield according to a literature method (Scheme 1.39).[15]



Scheme 1.39. Synthesis of substituted alkyne **93**.

Cyclisation to **94** was achieved in reasonable yields under gold catalysis (Scheme 1.40).

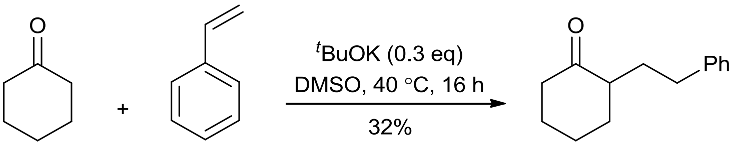


93 **89** (2 eq) **94**

Scheme 1.40. Synthesis of **94** *via* gold-catalysed cyclisation.

Before we explored the epoxidation of **94** using the Corey method, we decided to optimise the conditions using ketone **95**, as quantities of **94** were limited.

95 was synthesised following a literature method (Scheme 1.41).[40] Cyclohexanone was added to styrene in the presence of a catalytic amount of potassium *tert*-butoxide. The reaction was performed at 40 °C in order to minimise the formation of the disubstituted product.[40] However, the low yield indicates that these conditions are not optimal.

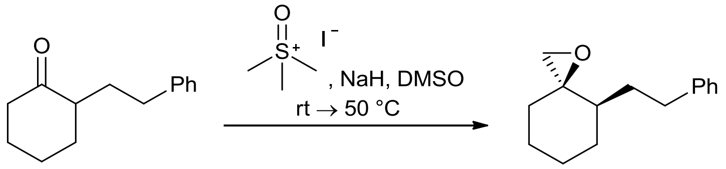


96 **97** **95**

Scheme 1.41. Synthesis of **95**.

Nevertheless, sufficient quantities of **95** were produced to explore its epoxidation and the results are shown in Table 1.3.

Dimethylsulfoxonium methylide, generated from trimethylsulfoxonium iodide, was chosen as the ylide source rather than dimethylsulfonium methylide as the latter is prone to spontaneous thermal decomposition.[39] To begin with, we used a slight excess of base, but found this gave the epoxide in a fairly poor yield (Table 1.3, entry 1). As we were concerned that any excess base may react with the epoxide, we instead explored the reaction using the sulfur ylide in excess. This gave a marked improvement in yield (entry 2), which was further improved when the equivalents of the sulfur ylide were increased (entries 3-4). Additionally, it was discovered that fresh reagents were required for best results. Only one diastereomer of (±)-**98** was isolated by column chromatography although it was difficult to determine from the crude ^{1}H NMR if small amounts of the other diastereomer had been formed. The stereochemistry is tentatively assigned as (3*R**,4*R**) based on work done on the subsequent oxetane system (p.45).



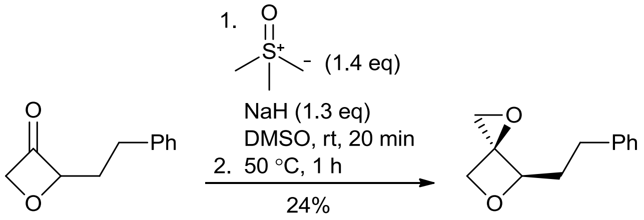
95

(±)-**98**

Entry[a]	Ylide (equiv.)	NaH (equiv.)	Yield (%)[b]
1	1.2	1.3	27
2	1.2	1.1	52
3	1.6	1.5	61
4	1.4	1.3	~77

Table 1.3. Exploration of the Corey epoxidation of **95**. [a]0.5 M concentration. [b]Isolated single diastereomer.

With these optimised conditions in hand, the next step was to explore the epoxidation of **94** (Scheme 1.42).



94 **(±)-99a**

Scheme 1.42. The Corey epoxidation of **94**.

Dimethylsulfoxonium methylide was generated in the first step, which was then reacted with **94**. An aqueous work-up and purification *via* column chromatography gave (±)-**99a** as predominantly one diastereomer in 24% yield, although a small amount of the other diastereomer (~5%) was also isolated. The stereochemistry of (±)-**99a** was tentatively assigned as (3*R**,4*R**) on the basis that ylide attack will likely arise from the opposite face to the alkyl substituent (Figure 1.8).

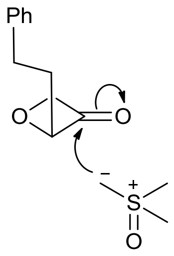
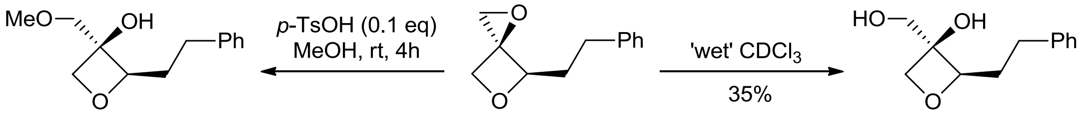


Figure 1.8. Nucleophilic attack of the sulfur ylide on **94**.

This epoxide slowly opened in the presence of 'wet' deuterated chloroform in the freezer over a period of 2 months to give (±)-**99a** and (±)-**100** in a 1.9 : 1 ratio (Scheme 1.43). Epoxide (±)-**99a** was then stirred in MeOH in the presence of an acid catalyst at room temperature for 4 hours. The crude ^{1}H NMR suggested the epoxide ring was opened preferentially, although the reaction did not go to completion and (±)-**101** was not isolated due to the small quantities involved. The ring strain energies for oxiranes and oxetanes are very similar (26.3 and 24.7 kcal mol^{-1} respectively[41]), so it was interesting to observe selective opening of the epoxide ring.



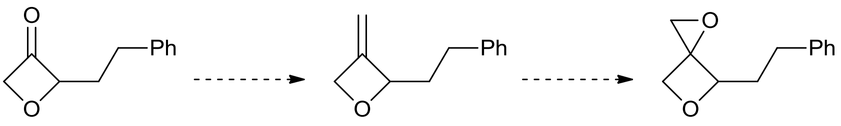
(±)-**101** (±)-**99a** (±)-**100**

Scheme 1.43. Ring-opening of (±)-**99a**.

The formation of (±)-**99a** proved capricious and even using fresh reagents we were unable to improve on the 24% yield (Scheme 1.42). As the epoxidation of ketone **94** with a sulfur ylide was unreliable, giving inconsistent results, we decided to explore a different route to these spirocycles.

1.2.4 Synthesis of Substituted 1,5-Dioxaspiro[2.3]hexane *via* Epoxidation

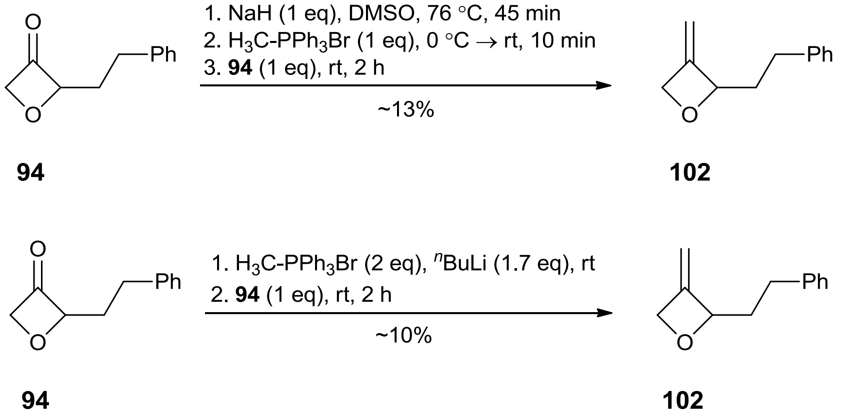
An alternative route to spirocycle **99** *via* alkene **102** was proposed, as shown in Scheme 1.44.



94 **102** **99**

Scheme 1.44. Alternative route to **99**.

Initial attempts at Wittig reactions using standard conditions provided **102** in poor yields (Scheme 1.45).[42-43]



Scheme 1.45. Wittig reaction on **94**.

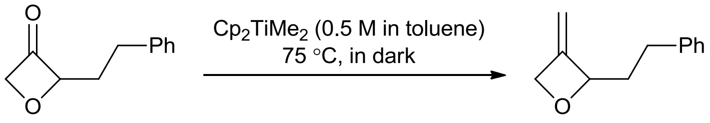
On account of work done by Dollinger and Howell in which 2-methyleneoxetanes were successfully synthesised from *β*-lactones,[22] it was decided to try an alternative methylenation approach. We decided to explore the Petasis reagent as it is milder and less acidic than the Tebbe reagent.[22]

The Petasis reagent (**104**) was synthesised successfully from commercially available titanocene dichloride (**103**) and methyllithium in a good yield (Scheme 1.46).[44] A 0.5 M solution in toluene was prepared and stored in the dark under nitrogen as **104** is known to be unstable in the presence of light, especially in its solid form.[45]

$$\underset{\mathbf{103}}{\mathrm{Cp_2TiCl_2}} \xrightarrow[98\%]{\mathrm{MeLi,\ Et_2O}} \underset{\mathbf{104}}{\mathrm{Cp_2TiMe_2}}$$

Scheme 1.46. Synthesis of the Petasis reagent, **104**.

Toluene was used rather than THF as it was reported to be a superior solvent for use in the methylenation.[22] Optimisation studies for this reaction are shown in Table 1.4. This investigation revealed that the best results were obtained using 1.5 equivalents of the Petasis reagent and performing the reaction at 75 ˚C (entry 1).[22,45-46] Reducing the reaction time led to a decrease in yield (entry 2) whilst reducing the equivalents led to a slightly lower yield (entries 3 and 4). Unfortunately, when the reaction was repeated on a larger scale (1.3 mmol vs 0.6 mmol) using the optimal conditions (entry 1), the yield of the reaction decreased to 17 – 25%. It is known that the Petasis intermediate can react with the product alkene once all of the ketone has been consumed, thus decreasing the yield.[46] Therefore the yield could potentially be improved by adding a sacrificial carbonyl compound, although this was not explored in this case.[46]

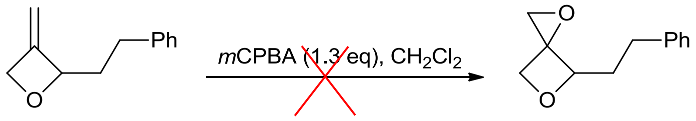


94 **102**

Entry[a]	Cp_2TiMe_2 (equiv.)	Time (h)	Yield (%)
1	1.5	16	47
2	1.5	7	30
3	1.2	14.5	45
4	1.05	14	45 (+ rec SM)
5[b]	1.2	15	4

Table 1.4. Investigation into optimal conditions for the methylenation of **94**. [a]Reactions performed on a 0.6 mmol scale. [b]Reaction run at 0.1 M rather than 0.5 M.

Having synthesised **102**, the epoxidation of the double bond was investigated. Initially *m*CPBA was used as the oxidant (Scheme 1.47), despite some concern that the ring-opening of the spirocycle could occur *in situ*, due to the generation of 3-chlorobenzoic acid during the reaction. However, epoxidation of **102** using *m*CPBA was very slow, giving both diastereomers, as observed by ^{1}H NMR, and the reaction did not go to completion.

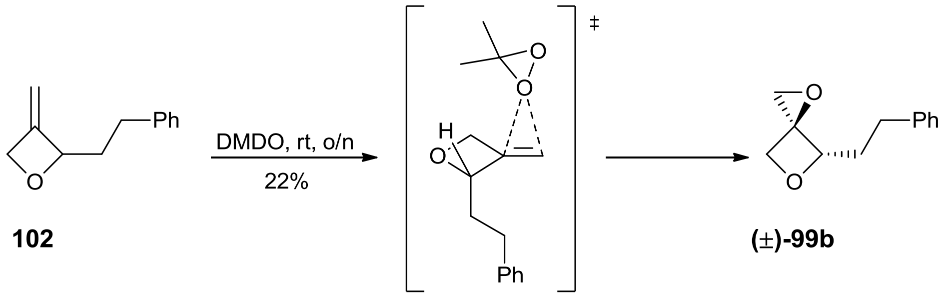


102

99

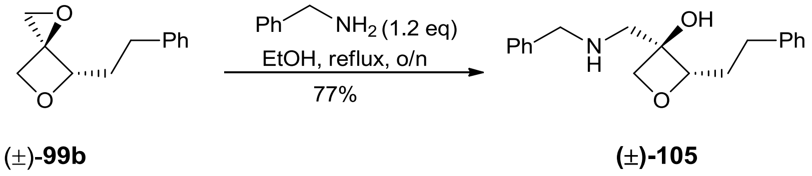
Scheme 1.47. Epoxidation of **102** using *m*CPBA.

When DMDO[47] was used (Scheme 1.48), one diastereomer ((±)-**99b**) was observed preferentially, which was the opposite one to that formed *via* the Corey epoxidation. (±)-**99a** was observed in the crude ^{1}H NMR (ratio (±)-**99a** : (±)-**99b**, 0.5 : 1), but was not isolated. We tentatively assign (±)-**99b** as the (3*S**,4*R**)-diastereomer, arising from concerted epoxidation[48] from the face opposite the -CH_2CH_2Ph group. Unfortunately, the yield of the epoxidation was low, possibly due to loss of product during purification or further reaction of the product *in situ*. Another issue with the DMDO epoxidation is that the generation of DMDO usually occurs in poor yields, making scale-up of the reaction challenging.



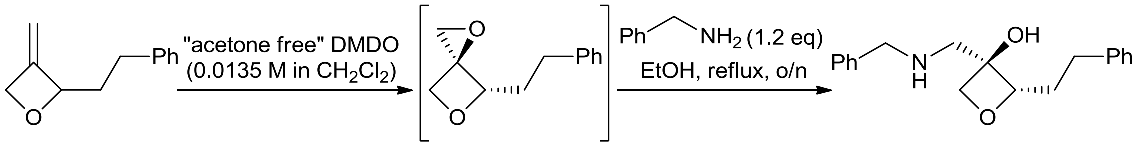
Scheme 1.48. Epoxidation of **102** using DMDO.

(±)-**99b**, obtained from the DMDO epoxidation, was immediately reacted with benzylamine (Scheme 1.49) due to concerns about its stability. Initial attempts at room temperature failed to give any product but, after heating under reflux overnight and purification *via* column chromatography, (±)-**105** was obtained in a good yield.



Scheme 1.49. Nucleophilic ring-opening of (±)-**99b** by benzylamine.

On account of the poor isolated yields of (±)-**99b** from the DMDO epoxidation, it was decided to try the epoxidation using “acetone free” DMDO[23,49] followed by an *in situ* ring-opening with benzylamine, without isolating the intermediate epoxide (Scheme 1.50). DMDO was generated as before, but the distillate obtained was diluted with water and extracted with CH_2Cl_2, before the combined organics were washed multiple times with 0.1 M phosphate buffer. Unfortunately, this one-pot method failed to yield (±)-**105**.

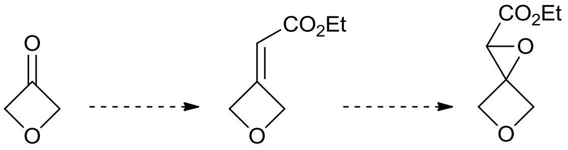


102 (±)-**99b** (±)-**105**

Scheme 1.50. DMDO epoxidation of **102** followed by *in situ* ring-opening with benzylamine.

1.2.5 Synthesis of 1,5-Dioxaspiro[2.3]hexane-2-carboxylic Acid Ethyl Ester

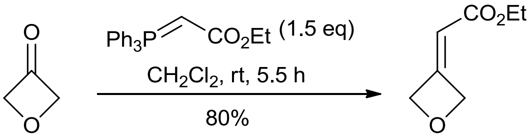
Next, the synthesis of spirocycle **107**, with an ester substituent on the epoxide, *via* the proposed synthetic route shown in Scheme 1.51, was explored, primarily because **106** is readily accessible.[9,15] Specifically, we wanted to ascertain if the presence of an electron withdrawing group on the epoxide would influence the regioselectivity or reactivity of the resultant spirocycle.



5 **106** **107**

Scheme 1.51. Proposed synthetic route to **107**.

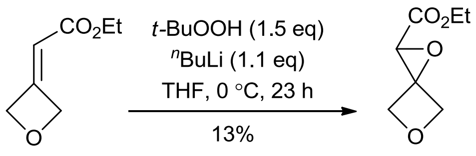
Initially, a Wittig reaction between carbethoxymethylene triphenylphosphorane and oxetan-3-one was carried out according to a known method,[15] giving **106** in 80% yield (Scheme 1.52).



5 **106**

Scheme 1.52. Wittig reaction using oxetan-3-one to give **106**.

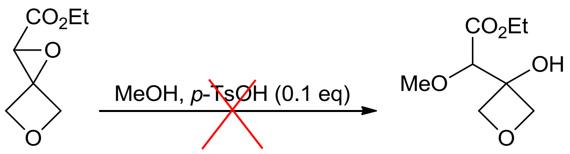
Subsequent epoxidation of the double bond, however, proved to be quite difficult, owing to its electron deficient nature. Attempts to epoxidise the double bond with DMDO were unsuccessful and returned starting material. Nucleophilic epoxidation procedures using peroxides were then investigated.[50-52] Reaction of lithium *tert*-butylhydroperoxide with **106** gave spirocycle **107** in 13% yield (Scheme 1.53). However, the reaction did not go to completion, even using extended reaction times.



106 **107**

Scheme 1.53. Epoxidation of **106** *via* lithium *tert*-butyl hydroperoxide.

Difficulties were also encountered with the ring-opening of **107**. Conditions that had been used successfully with spirocycle **99a** returned starting material at room temperature and, upon heating, transesterification appeared to take place and **108** could not be isolated (Scheme 1.54).



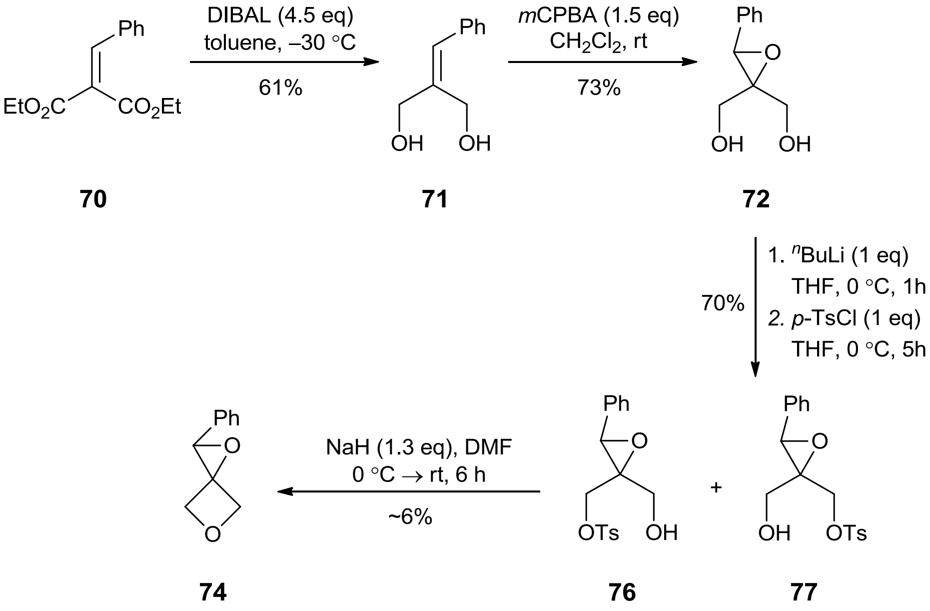
107 **108**

Scheme 1.54. Attempted ring-opening of **107** with MeOH.

1.3 Conclusions

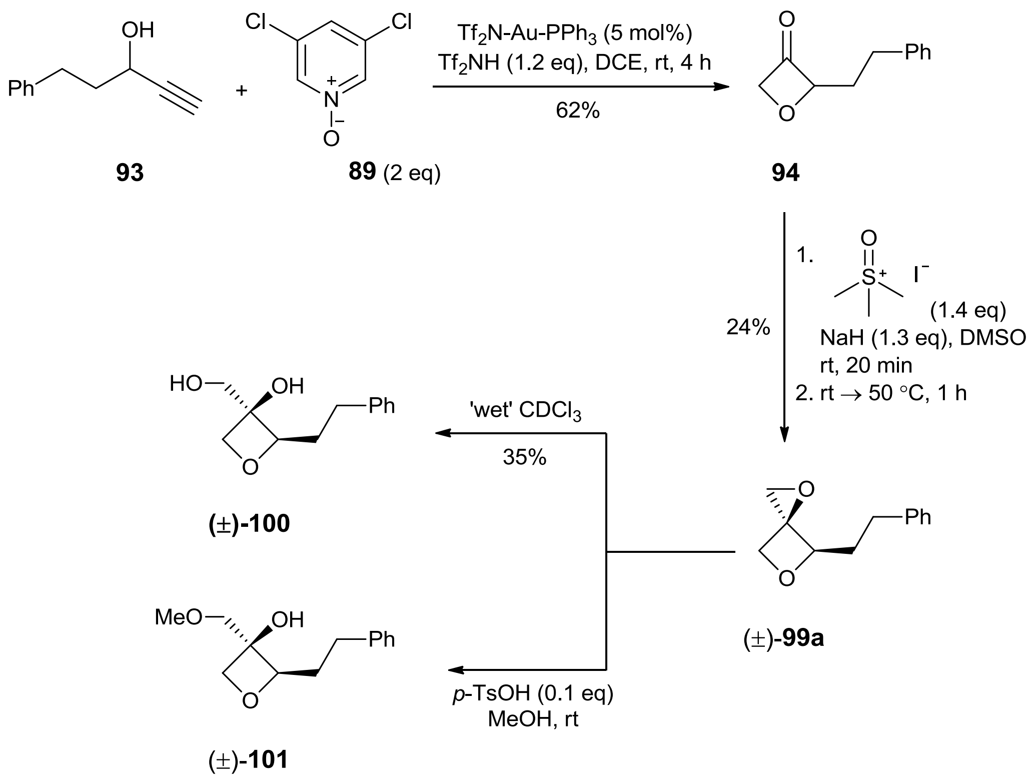
Initial attempts to synthesise 1,5-dioxaspiro[2.3]hexane **1** were unsuccessful, probably due to issues with its volatility, stability and/or water solubility. However, some success was noted with the preparation of substituted derivatives **74**, **99a/b** and **107**.

Spirocycle **74** was obtained in 4 steps from **70** using a ring-closure method, although there were significant problems with low yields and reproducibility of the final ring closure.



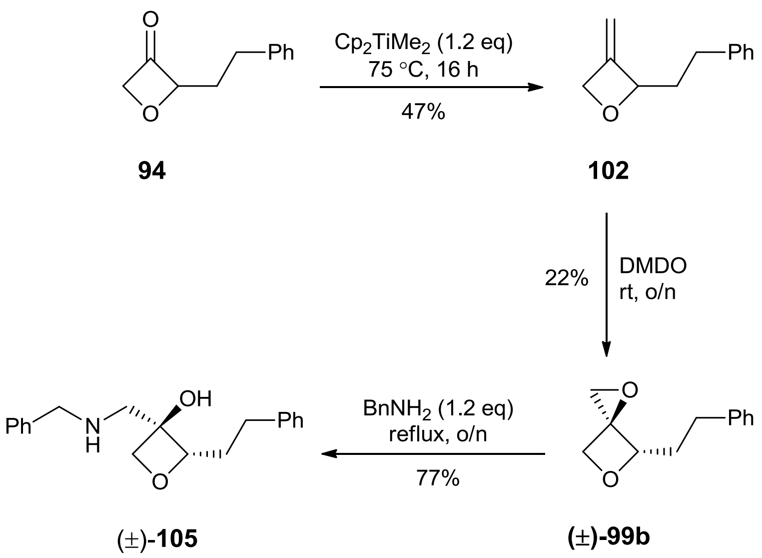
Scheme 1.55. Synthetic route to **74**.

The synthesis of spirocycle (±)-**99a** was achieved in two steps through the gold-catalysed cyclisation of alkyne **93**, followed by epoxidation using a sulfur ylide (Scheme 1.56). This spirocycle was shown to react readily with nucleophiles, as demonstrated by the ring-opening reaction occurring during storage in wet $CDCl_3$.



Scheme 1.56. Summary of route to spirocycle (±)-**99a**.

An alternative route to spirocycle (±)-**99b** *via* the alkene was also developed that was complementary with respect to stereoselectivity (Scheme 1.57). Conversion of ketone **94** to the corresponding alkene was realised using the Petasis reagent. Epoxidation of alkene **102** was then achieved with DMDO and the resultant spirocycle reacted successfully with benzylamine to give amine (±)-**105**.



Scheme 1.57. Alternative route to (±)-**99b** and subsequent ring-opening.

Spirocycle **107**, containing an electron withdrawing group on the epoxide was also obtained, albeit in a poor yield (Scheme 1.53).

Of the routes explored, the methodology described in Scheme 1.56 is the most attractive, although low yields deterred us from developing this chemistry further. Since this study was completed, an asymmetric route to 2-substituted oxetanones has been devised in the group (Scheme 1.7).[16] Thus it might be feasible to produce spirocycles such as **99** as single enantiomers, and this could form the basis for future work.

Chapter 2:

Synthesis of Oxetane-Containing Tripeptide Motifs

2.1 Introduction

As discussed in Chapter 1, oxetanes have become of increasing interest in medicinal chemistry because of their ability to act as isosteres for *gem*-dimethyl groups and carbonyl bonds. Also of interest to medicinal chemists, are peptidomimetics. Whilst there are examples of biologically active peptides being used as drugs, peptides have traditionally been considered to be undesirable drug candidates due to their poor bioavailability, susceptibility to proteolysis and rapid excretion from the body.[53-57] Peptidomimetics, however, can be designed to retain the biological activity of a peptide whilst having an improved bioavailability and stability, thus making them better drug candidates.[53-59]

We sought to develop routes to short peptide sequences wherein one of the carbonyl bonds had been replaced with an oxetane ring, as illustrated in Figure 2.1. This is because the amide bond in peptides is vulnerable to proteolysis and replacement of the C=O bond by the isosteric oxetane unit should result in novel peptidomimetics with increased stability. Once a route had been established, the properties of these novel peptidomimetics could then be compared and contrasted with the properties of a peptide chain such as their preferred conformations, ability to act as receptor ligands, and zwitterionic character.

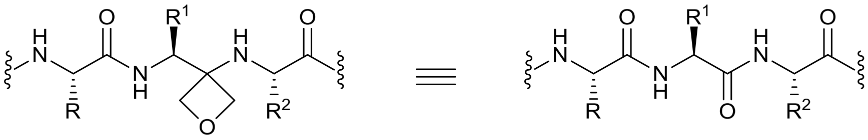


Figure 2.1. Peptide chain containing an aminooxetane unit as an amide bond isostere.

This chapter begins by introducing the concept of peptidomimetics and then details our work directed towards the development of routes to peptide chains containing an oxetane as a carbonyl bond replacement. The structural implications of this replacement are also investigated using X-ray diffraction, CD and NMR spectroscopy, and molecular modelling.

2.1.1 Introduction to Bioisosteres

An important part of the drug discovery process is the optimisation of a lead molecule that has been identified from a high-throughput screening assay. Changes to the molecule are usually made in order to investigate the properties of the drug against its target and to identify any deleterious effects. These changes often involve structural modifications, of which the use of bioisosteres is an important tool. Functional groups present in a drug molecule can impart undesirable properties *in vivo* such as toxicity or high rates of metabolism and so the introduction of bioisosteres that retain the desirable properties of the original functional group, whilst improving the pharmacological profile of the drug can be important.[60-61] Bioisosteres can also be used to help identify the pharmacophore in a drug molecule, thus enabling the medicinal chemists to concentrate the optimisation of the lead compound on a specific set of structural features.[62]

In 1919, Langmuir defined isosteres as compounds having the same number of atoms as well as the same total number of electrons that can arrange themselves in the same manner.[63] Subsequent revisions of the definition occurred, including the introduction of Grimm’s Hydride Displacement Law in 1925 that states “atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with one to four hydrogen atoms, in such a manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups one to four places respectively, to their right."[62] This was further built on by Erlenmeyer, who classified isosteres as atoms, ions and molecules whose outermost layers of electrons are the same.[62]

In 1951, Friedman expanded on the definition of an isostere and introduced the term 'bioisostere', stating that compounds were considered to be bioisosteric if they "fit the broadest definition for isosteres and have the same type of biological activity."[64] Building on this, Thornber later gave a more flexible definition which stated that "bioisosteres are groups or molecules which have chemical and physical similarities producing broadly similar biological properties."[65]

Today, medicinal chemists have a broad range of bioisosteres at their disposal when optimising a lead compound, and these can be divided into two main types, as defined by Burger: classical and nonclassical bioisosteres.[62,66]

2.1.2 Classical and Nonclassical Bioisosteres

Classical bioisosteres are generally considered to be those that satisfy the definitions initially proposed by Langmuir and Grimm[67] and are usually subdivided into five main categories: 1) monovalent atoms or groups; 2) divalent atoms or groups; 3) trivalent atoms or groups; 4) tetrasubstituted atoms; and 5) ring equivalents.[60,62,68-69]

There have been many successful applications of classical bioisosteres in medicinal chemistry, but the more general definition for bioisosteres, as developed by Friedman and Thornber, allowed the introduction of nonclassical bioisosteres.

Nonclassical bioisosteres do not necessarily have the same number of atoms, or the same steric or electronic configurations as the original drug molecule and can be broadly divided into two main categories: 1) cyclic vs noncyclic isosteres; and 2) exchangeable groups.[62,68]

2.1.3 Carbonyl Bond Bioisosteres

Functional groups containing carbonyl bonds are not very common in drug discovery, due to the inherent reactivity of the carbonyl bond, especially when found in aldehydes and ketones.[8] Examples of carbonyl bond bioisosteres are shown in Figure 2.2. More recently, work done by Carreira and co-workers has identified the oxetane moiety as a new, nonclassical bioisostere for the carbonyl bond,[10] as discussed in Chapter 1.

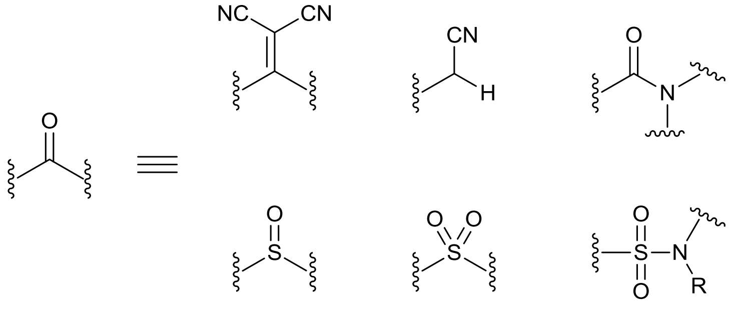


Figure 2.2. Common bioisosteres of carbonyl bonds.[60]

2.1.4 Amide Bioisosteres

Isosteres for the amide functionality are often needed in order to improve the bioavailability of a drug molecule and to enhance its stability *in vivo*.[60,62] Whilst the amide bond is usually stable to acidic or basic conditions, it is susceptible to cleavage under mild conditions by proteases and peptidases.[56] Examples of amide isosteres are shown in Figure 2.3 and include esters, although these come with their own issues as drug candidates, as well as heterocyclic compounds such as 1,2,4-oxadiazoles and triazoles.

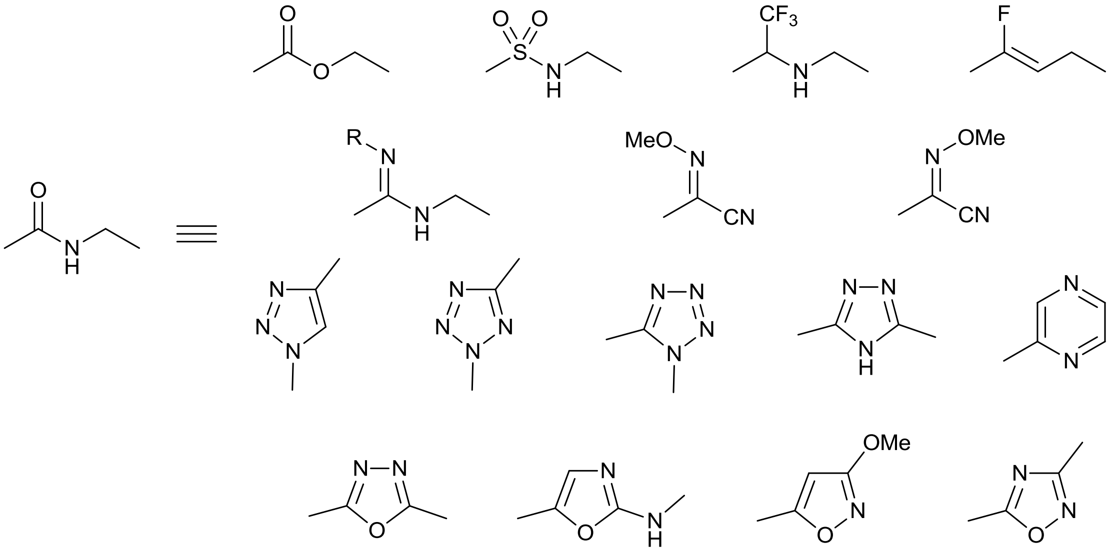


Figure 2.3. Amide isosteres.[60]

The choice of bioisostere for the amide bond depends on the role that the amide plays in the biological activity of the drug molecule. If, for example, the hydrogen bond donor and acceptor abilities of the amide are required, then the bioisostere needs to be able to mimic these properties in order to elicit the same biological response. If, however, the amide bond merely acts as a spacer between functional groups rather than being essential for the biological activity then a different isostere may be appropriate.[62]

Amide bonds are omnipresent in peptides and applying bioisosteric replacements for these bonds led to the development of the field of peptidomimetics.

2.1.5 Peptidomimetics

There are numerous possible peptides and proteins arising from different combinations of the 20 natural amino acids. However, whilst many show biological activity, generally speaking peptides are not suitable for application as drugs. This is primarily because of their susceptibility to proteases *in vivo*, poor oral bioavailability, rapid excretion and undesirable side effects caused by the conformationally flexible peptides interacting with receptors other than the target.[57] These issues have led to the development of peptide isosteres, known as peptidomimetics. Peptidomimetics are designed to have improved metabolic stability and bioavailability whilst retaining or improving the desired peptide-receptor interactions and reducing unwanted side effects.[54,57] They can be divided into three distinct categories:

- Type I (structural) mimetics – these mimic the local topography around the amide bond and may utilise amide bond isosteres. The functionalities responsible for the activity remain intact.[55,70]

- Type II (functional) mimetics – these peptidomimetics retain the ability to bind to the desired peptide receptor, but do not necessarily resemble the structure of the natural peptide.[55,70]

- Type III (functional-structural) mimetics – these examples contain novel scaffolds unrelated to the parent peptide but containing the necessary functional groups arranged in the spatial orientation needed in order to elicit the same biological response.[55,70]

2.1.6 Examples of Peptidomimetics

There are many reviews covering the vast area of peptidomimetics[53-57,71] and this section simply serves to highlight some relevant examples.

One method for creating peptidomimetics is to modify the amino acid residues contained within the chain, either through alterations to the side chain or by altering the peptide backbone. The potential modifications to the amino acid residue are numerous and can involve the substitution of the natural amino acid with the unnatural D-amino acid, use of β-, γ-, δ-amino acids, or alkylation of the α-C or the α-N.[57] Some examples of peptide bond isosteres are shown in Figure 2.4.

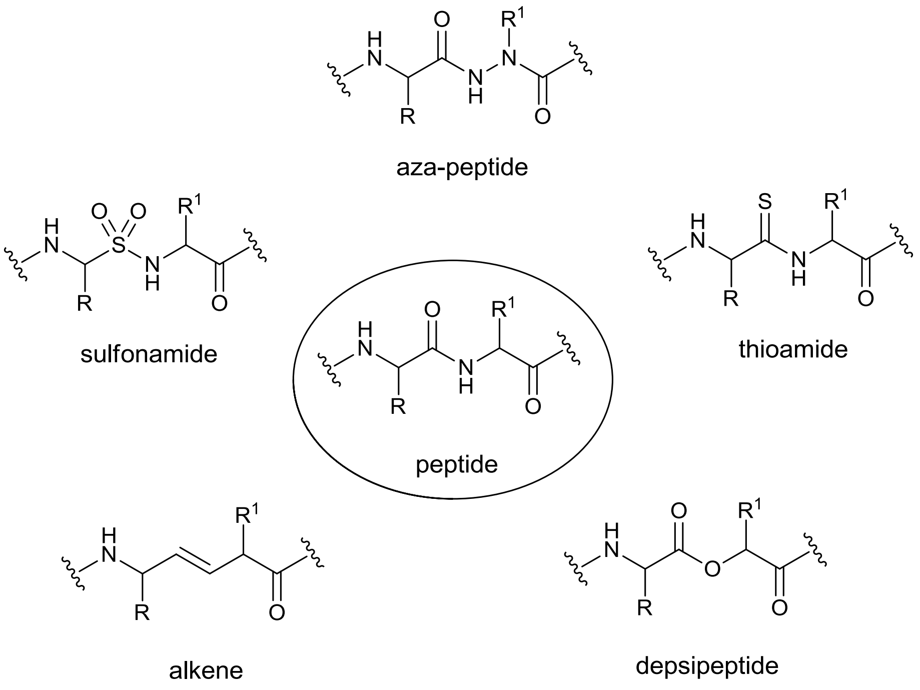


Figure 2.4. Examples of peptide bond isosteres.

Aza-peptides are an attractive class of peptidomimetics due to the ease with which they can be accessed, for example through the acylation of hydrazines.[53] Sulfonamide peptidomimetics are examples of those known as "transition-state" isosteres,[72] whereby the isostere mimics the intermediate formed during the hydrolysis of the amide bond.[56] Other examples of these transition-state isosteres are phosphinates[73] and hydroxyethylamines.[74]

Simon and co-workers developed a class of peptidomimetics called peptoids[75] in which the side chain of the amino acid has been moved onto the nitrogen of the amide bond (Figure 2.5).[53] These peptoids can be synthesised relatively easily using solid-phase methods and have increased stability to proteases as well as a greater conformational flexibility compared to peptides. Some peptoids also retain, or even exceed, the activity shown by the natural peptides.[53,75]

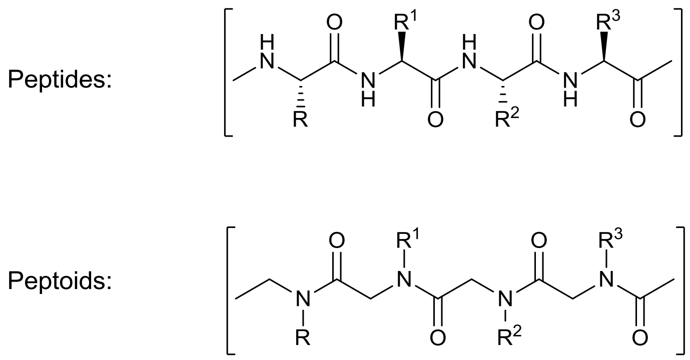


Figure 2.5. Peptoids vs peptides.[75]

Cai *et al* have recently reported the synthesis of 'AApeptides', so termed because they consist of *N*-acylated-*N*-aminoethyl amino acids.[76] In these peptidomimetics, the nitrogen has been acylated whilst alternate carbonyl bonds have been removed from the peptide backbone (Figure 2.6).

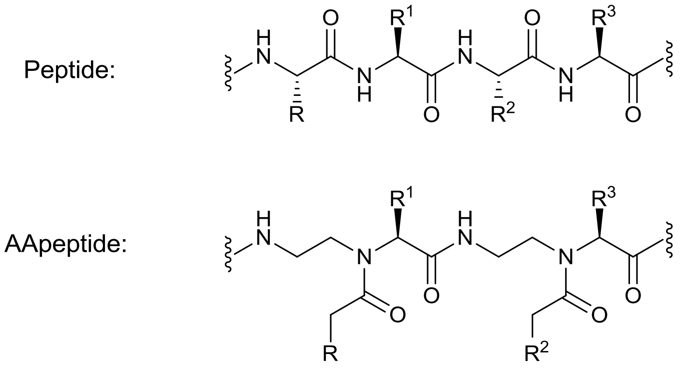
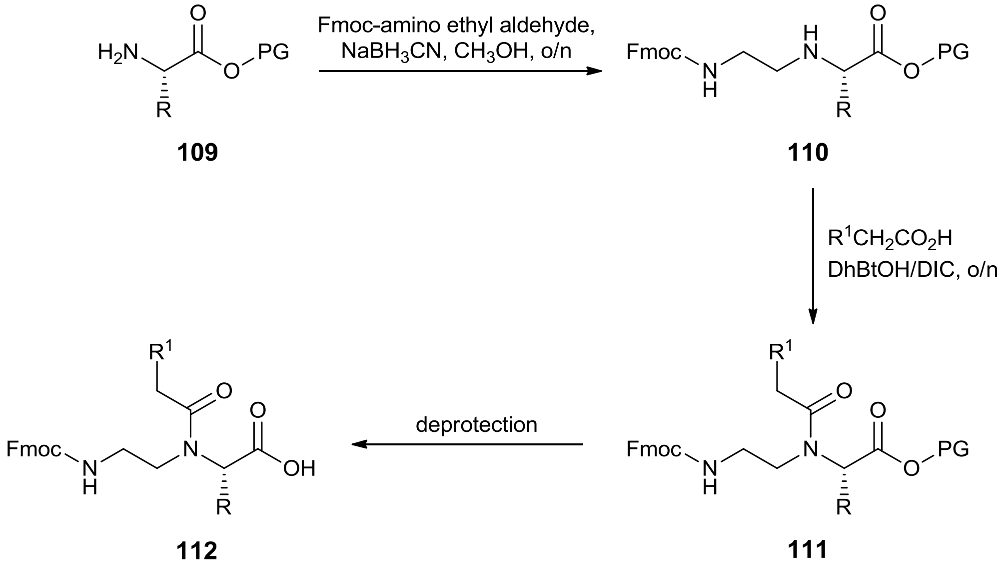


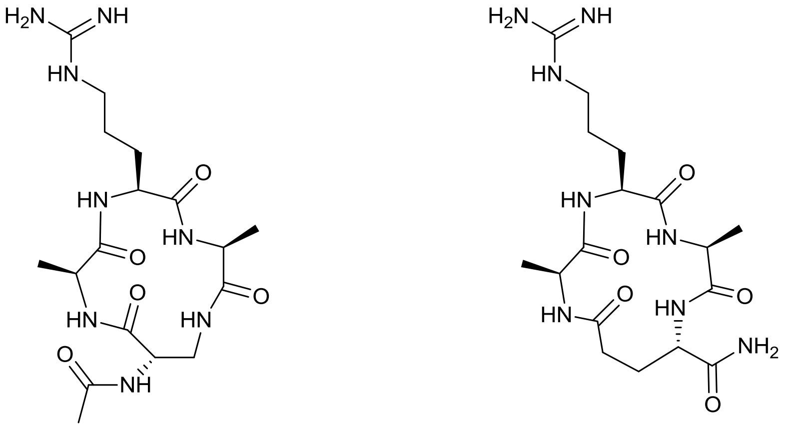
Figure 2.6. Peptide vs AApeptide.[76]

The synthesis of these AApeptides involves reaction of the amino ester with Fmoc-amino ethyl aldehyde, followed by acylation with a carboxylic acid and deprotection to give the building block **112** (Scheme 2.1). Initial investigations into the stability of these peptidomimetics indicate an improved stability to proteolysis and good bioactivity.[76]



Scheme 2.1. Illustrative synthesis of an AApeptide building block.[76]

Alterations can also be made to the peptide chain to create peptidomimetics that can adopt conformations mimicking the structural elements found in natural peptides. Hoang *et al* have reported the ability of cyclic tetrapeptides **113** and **114**, shown in Figure 2.7, to mimic two of the potential nine distinct α-turn types present in protein structures.[77]

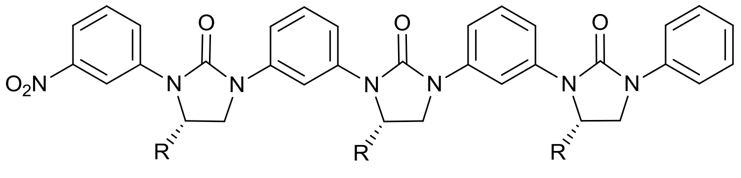


113 **114**

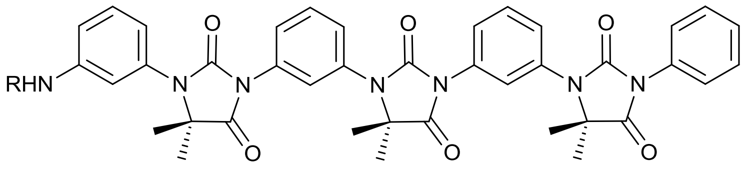
Figure 2.7. Cyclic peptides mimicking α-turn types.[77]

Vicinal disulfide constrained cyclic peptidomimetics have also been reported by Brust *et al*, synthesised by grafting an active pharmacophore onto the constrained cyclised scaffold.[78]

Secondary structures of peptides, such as turns, can also be recreated by oligomers that do not represent traditional peptide scaffolds. For example, Hamilton and co-workers have developed oligomers containing aryl linkers that mimic a peptide β-strand (Figure 2.8).[79-80]



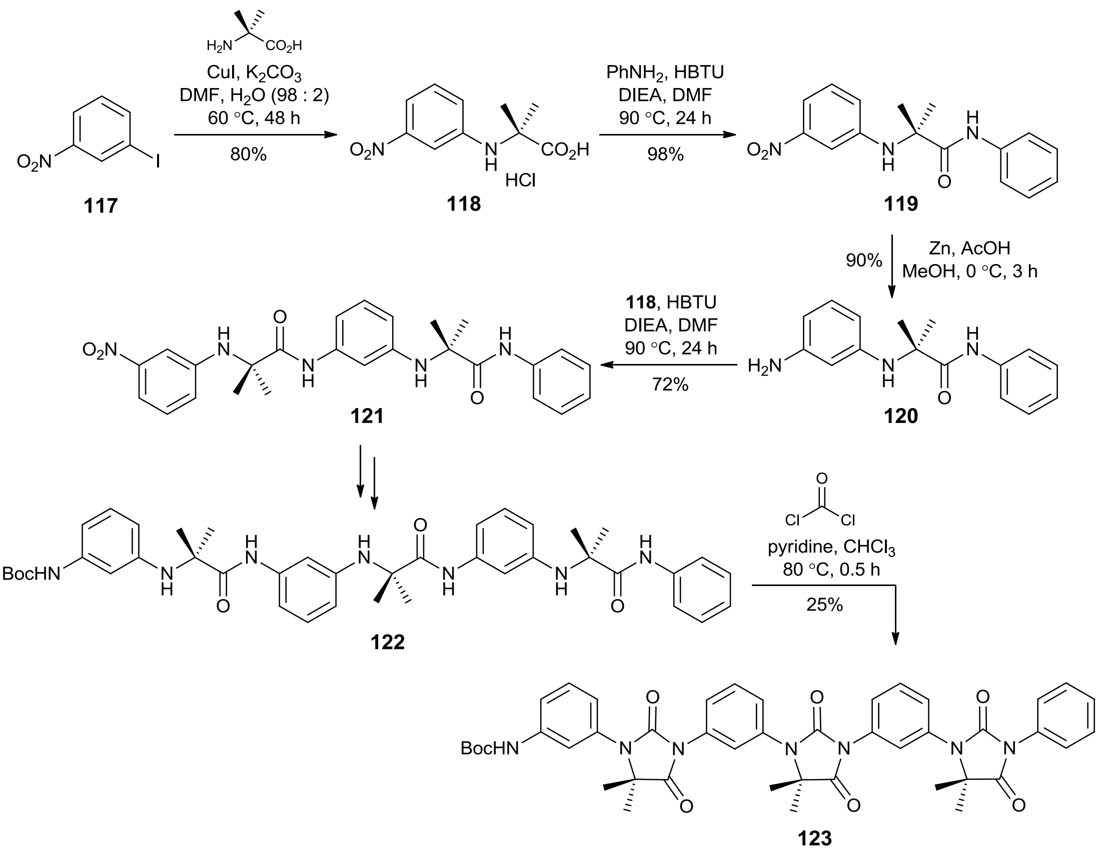
115



116

Figure 2.8. β-strand mimics.[79-80]

The monomer **118** was initially synthesised before repeat nitro reduction and amide coupling using HBTU as the coupling reagent, followed by heating in basic phosgene solution gave the mimetic **123** (Scheme 2.2).[79]



Scheme 2.2. Synthetic route towards the β-strand mimetic **123**.[79]

2.1.7 Oxetane-Containing Peptidomimetics

In 1984, Matsumoto and co-workers[81] identified and isolated a new antibiotic from *Streptomyces* sp. OM-2317 that showed activity against *Bacillus subtilis* and *Pyricularia oryzae* in minimal media, as well as demonstrating a herbicidal effect. This antibiotic was oxetin (Figure 2.9) and was the first reported natural product containing an oxetane ring that is also a β-amino acid.[81-82]

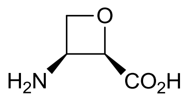
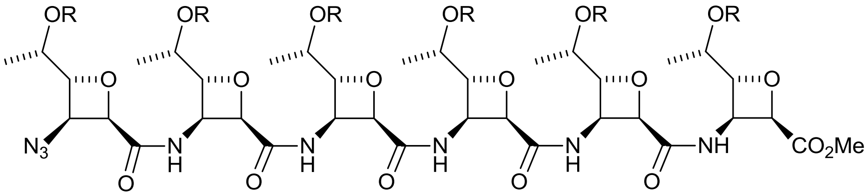

124

Figure 2.9. The antibiotic oxetin.

Like α-amino acids, β-amino acids can form chains (known as β-peptides) that contain amide bonds along the backbone, potentially allowing them to form stable secondary structures and thereby classifying them as foldamers.[83] These β-peptides are consequently examples of peptidomimetics and, as such, have potential for application in medicinal chemistry. Indeed, Porter *et al* demonstrated that a β-peptide analogue of a known antibiotic had comparable antimicrobial activity to that of the natural antibiotic itself.[84] Fleet and co-workers have developed a route to oxetane β-amino acids as scaffolds for the synthesis of new foldamers that form the hexamers **125**, **126** and **127** (Figure 2.10).[82]


R = Bn (**125**)
R = TBDMS (**126**)

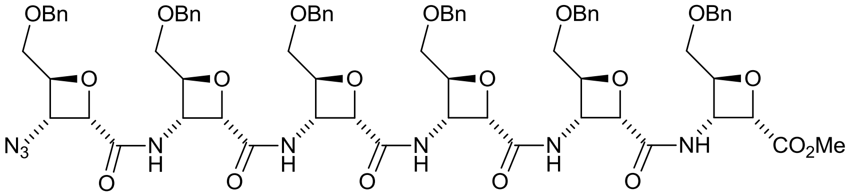
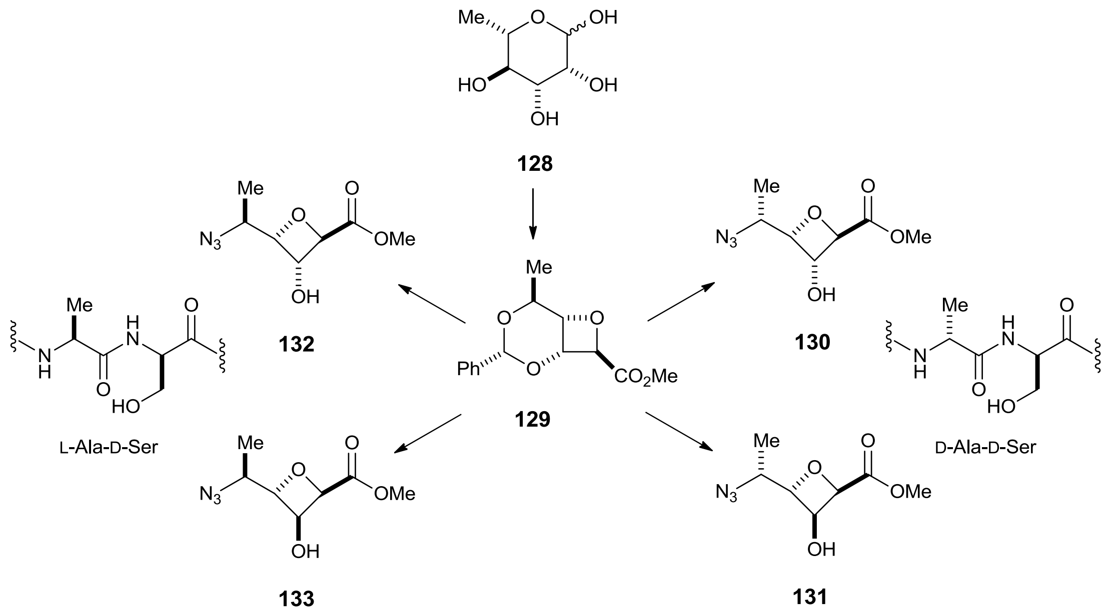

127

Figure 2.10. Hexamers from *cis*-β-azidoesters.[82]

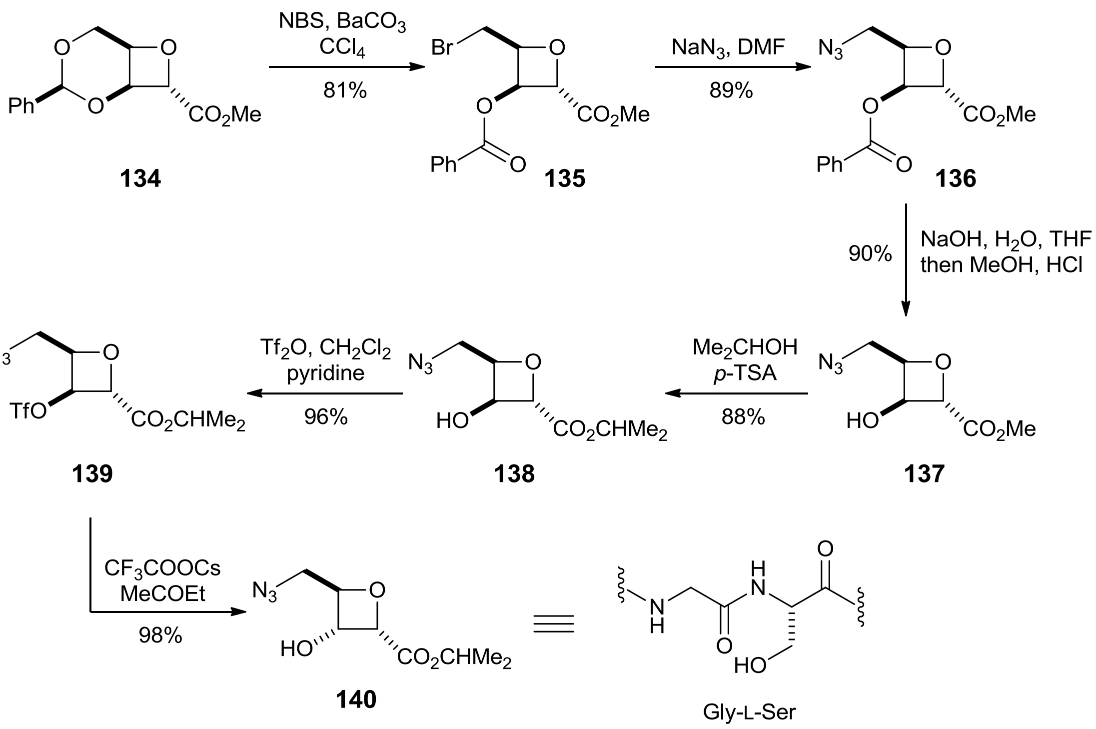
NMR studies showed that hexamers **125** and **126**, which have the same absolute configuration at C-2 and C-3 as oxetin, form a left-handed helix stabilised by inter-residue hydrogen bonds between the amide and carbonyl groups, to form ten-membered rings.[82,85] In contrast, hexamer **127** has the opposite absolute configuration at C-2 and C-3 compared to oxetin and forms a right-handed helix.[82,85]

Johnson *et al* utilised similar methodology to synthesise dipeptide isosteres of D/L-alanine-D-serine and glycine-L-serine based on oxetane δ-amino acid scaffolds,[86] as shown in Schemes 2.3 and 2.4. L-Rhamnose (**128**) was converted into **129** through sequential reaction with bromine water then benzaldehyde to form the lactone, which was then esterified with triflic anhydride and reacted with potassium carbonate in methanol to provide **129**.[82,87] Once **129** had been obtained, various conditions were employed to introduce the azide functionality, either with retention of configuration at C-5 to give **132** and **133** as conformationally restricted mimetics of L-Ala-D-Ser, or with inversion of configuration to give **130** and **131** as mimetics of D-Ala-D-Ser (Scheme 2.3).[86]



Scheme 2.3. Synthetic strategy for D/L-alanine-D-serine dipeptide isosteres derived from L-rhamnose.[86]

A similar strategy was employed to access **134** from D-xylose (Scheme 2.4). Bromination of **134** allows the introduction of the azide functionality to give **136**. Hydrolysis of the benzoate ester of **136** with NaOH also removed the methyl ester, which was readily reinstated using methanol and hydrochloric acid. Transesterification of **137** to the isopropyl ester was required to prevent *in situ* oligomerisation after reduction of the azide to the amine. Formation of triflate **139**, followed by S_N2 displacement gave **140**, which can be considered to be a conformationally restricted mimetic of Gly-L-Ser.



Scheme 2.4. Synthetic strategy for glycine-L-serine dipeptide isosteres derived from D-xylose.[86]

This methodology allowed the synthesis of *cis*- and *trans*-oxetane δ-amino acids of the type shown in Figure 2.11 to be realised. Interestingly, ^{1}H NMR and IR experiments showed that oligomers (dimers, tetramers and hexamers) of δ-2,4-*trans*-oxetane amino acids such as **143**, **144** and **145** displayed no regular secondary structure in solution whilst the oligomers of **141** and **142** showed ordered conformations, primarily due to the steric bulk of the TBDMS group.[88] Tetramers and hexamers of δ-2,4-*cis*-oxetane amino acid **146**, on the other hand, had defined secondary structures in solution due to the formation of 10-membered rings through intramolecular hydrogen bonds.[89]

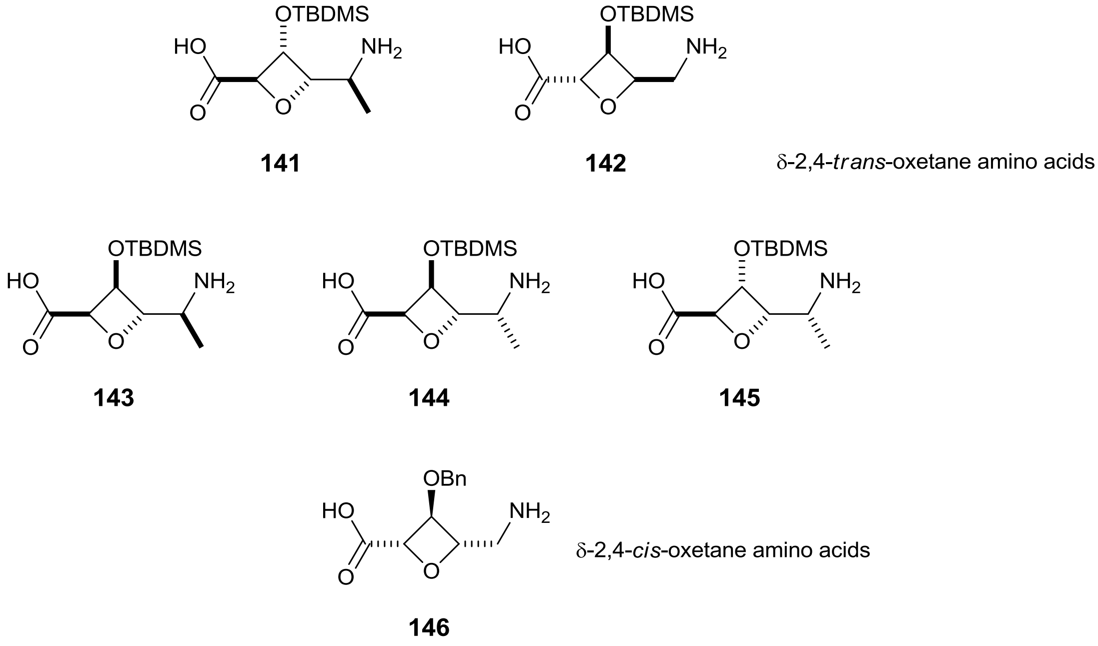
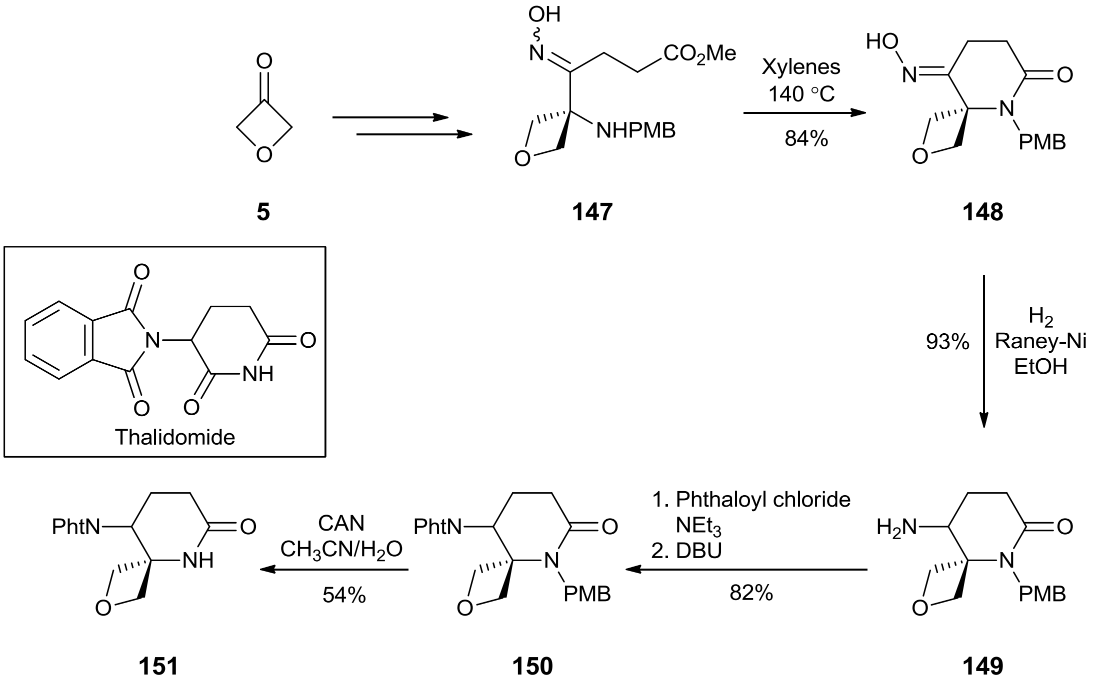


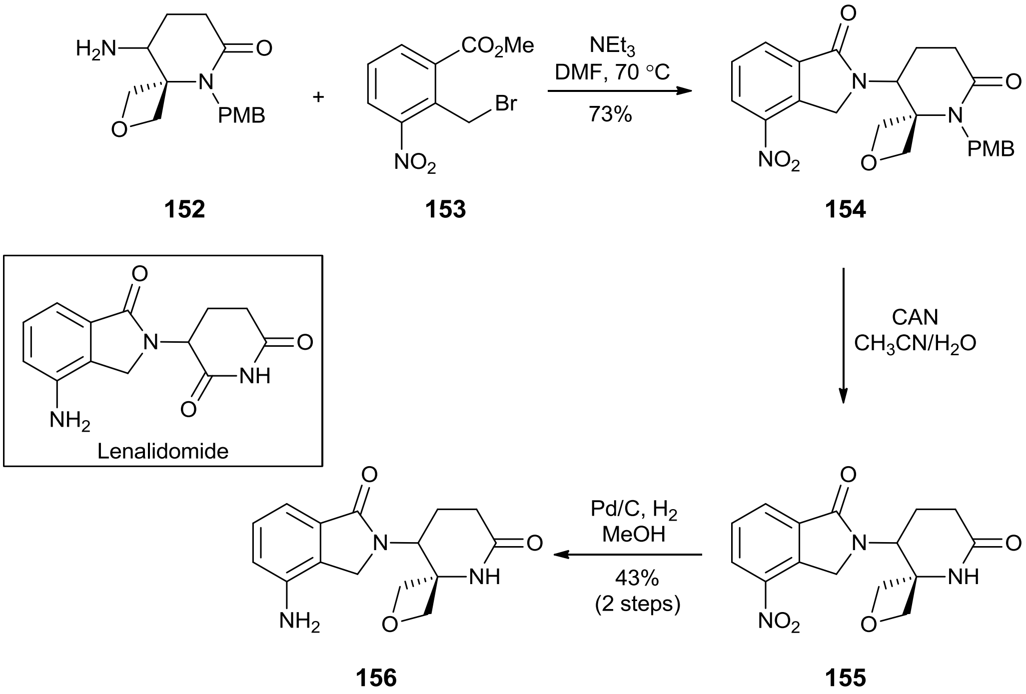
Figure 2.11. δ-*cis*- and δ-*trans*-oxetane amino acids.[89]

2.1.8 Oxetane Replacement of Carbonyl Bonds in Drug Molecules

The effect that the presence of the oxetane ring has on the activity of known drug molecules has been investigated. Burkhard *et al* synthesised an oxetane-containing analogue of the known drug thalidomide, as well as an analogue of the closely related lenalidomide, which is being investigated for the treatment of various cancers (Schemes 2.5 and 2.6).[90]



Scheme 2.5. Synthesis of oxetane-containing thalidomide analogue, **151**.[90]



Scheme 2.6. Synthesis of oxetane-containing lenalidomide analogue, **156**.[90]

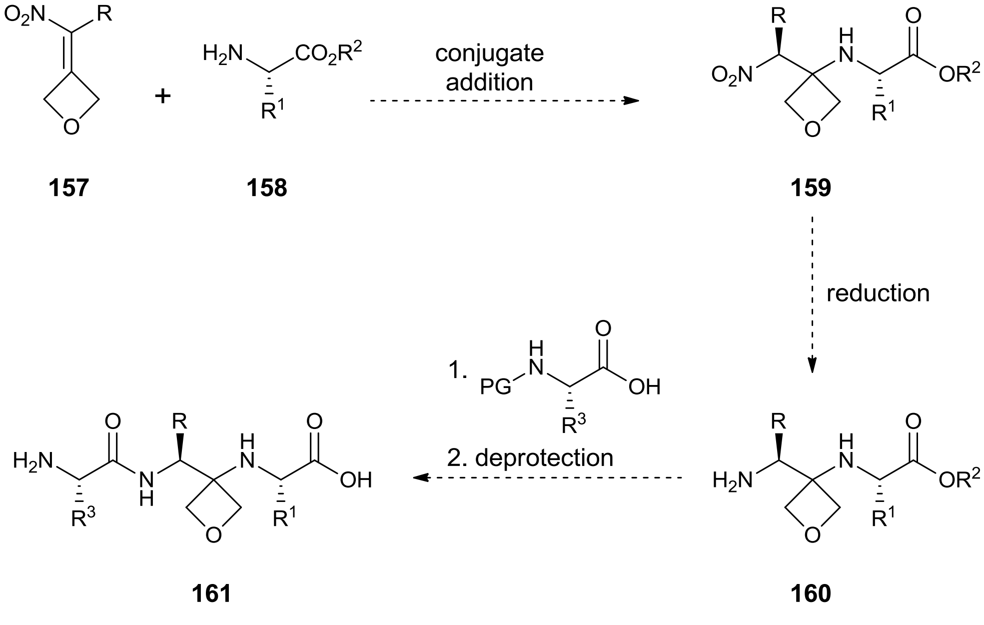
The authors discovered that the introduction of the oxetane as a replacement for one of the carbonyl bonds resulted in little change in the physicochemical and *in vitro* metabolic profiles of the compounds. However, oxetane thalidomide is much more stable than thalidomide itself in human plasma.[90]

2.1.9 Research Hypothesis and Aims

These results lend credence to the idea that an oxetane-containing peptidomimetic could potentially form defined secondary structures in solution, retain the bioactivity of the analogous parent peptide, and be more stable.

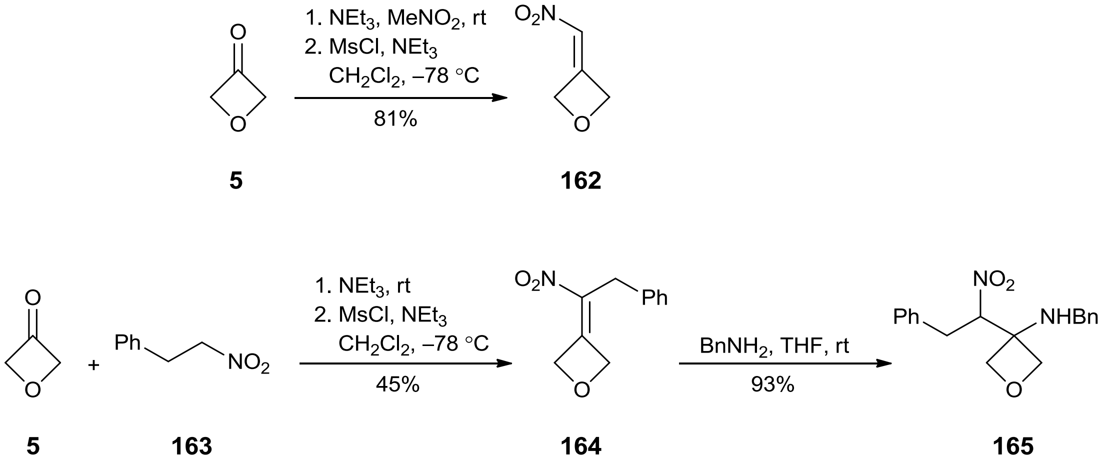
2.2 Results and Discussion

A route towards the synthesis of a peptidomimetic containing an oxetane ring as a replacement for the carbonyl bond in the middle of a tripeptide chain was initially proposed (Scheme 2.7).



Scheme 2.7. Proposed synthetic route towards oxetane-containing peptidomimetics.

This was inspired by work by Carreira and co-workers, who demonstrated that oxetane-containing Michael acceptors, **162** and **164**, are readily accessible from commercially available 3-oxetanone.[9,91] Moreover, Burkhard *et al* demonstrated the ease with which **164** reacts with benzylamine (Scheme 2.8),[91] suggesting that conjugate addition to form **159** should be facile.



Scheme 2.8. The synthesis and reactions of **162** and **164**.[9,91]

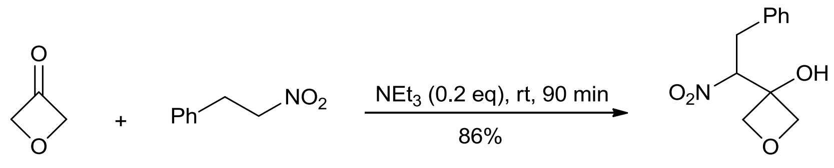
Whilst this synthetic route appeared promising, several questions would need to be addressed in order to develop a general route to oxetane-containing peptidomimetics. What substituents and protecting groups can be tolerated in the sequence? Can the conjugate addition to **157** be achieved with stereocontrol when $R \neq H$? Can the reduction of the nitro group be achieved with control? Can further coupling (**160** → **161**) be achieved without protection of the secondary amine? What protecting groups are optimal in this sequence?

Initially, we elected to focus on the synthesis of peptidomimetics containing oxetane mimetics of glycine and phenylalanine, as **162** and **164** were known and readily accessible.

2.2.1 Conjugate Additions to 'Phe' *via* a Two-Step Method

As we perceived **164** to be easier to handle than **162**, we decided to explore the synthesis of mimetics containing an oxetane 'Phe' residue first since this would also allow us to investigate the potential for a stereoselective conjugate addition to **164**.

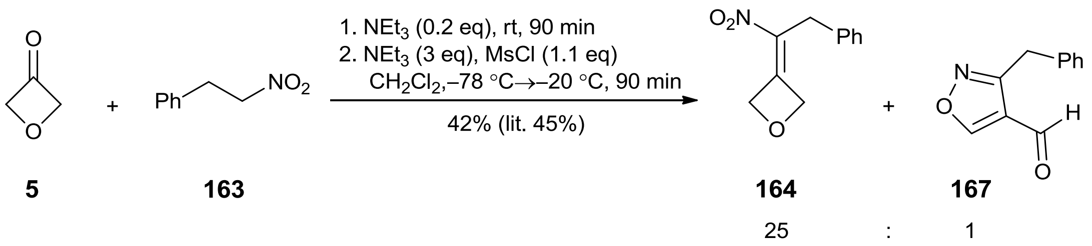
Whilst our initial target was to develop a route towards *pseudo*-tripeptides containing an oxetane instead of a carbonyl bond in the mid-chain position, the potential for incorporation of the oxetane at the *N*- or *C*-terminus was also of interest. During the synthesis of **164**, **166** is formed as an intermediate, which could be used as a precursor for the synthesis of a peptidomimetic containing an oxetane at the *C*-terminus. Therefore, nitroalkane **163** was reacted with 3-oxetanone in the presence of catalytic NEt_3 according to a literature procedure,[91] to give **166** in excellent yield after purification by column chromatography (Scheme 2.9). To our knowledge, this intermediate has not been isolated previously.



5 **163** **166**

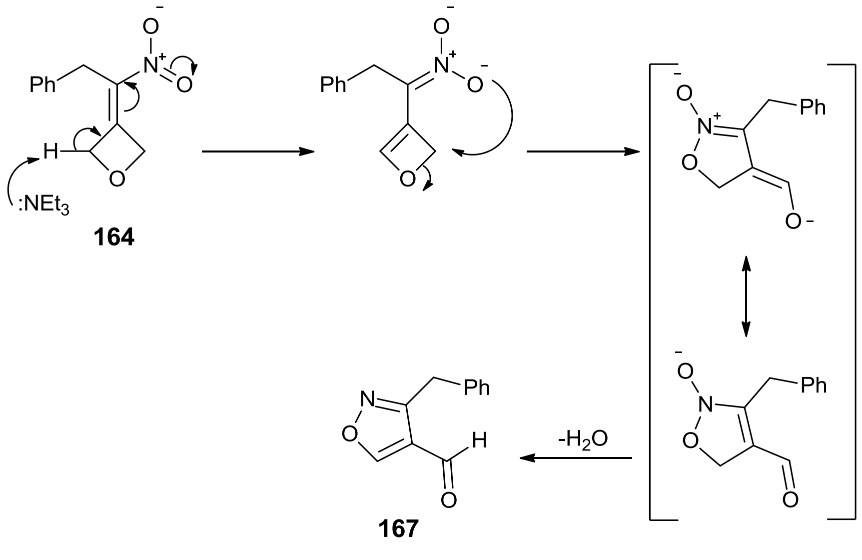
Scheme 2.9. Henry addition of **163** to 3-oxetanone to give **166**.

To obtain nitroalkene **164**, the Henry addition was repeated using 3-oxetanone and nitroalkane **163**, followed by mesylation and elimination of the resulting mesylate, according to the method reported by Burkhard *et al* (Scheme 2.10).[91] Nitroalkene **164** was obtained in a similar yield to that reported and the rearranged product, **167**, was also observed.[91]



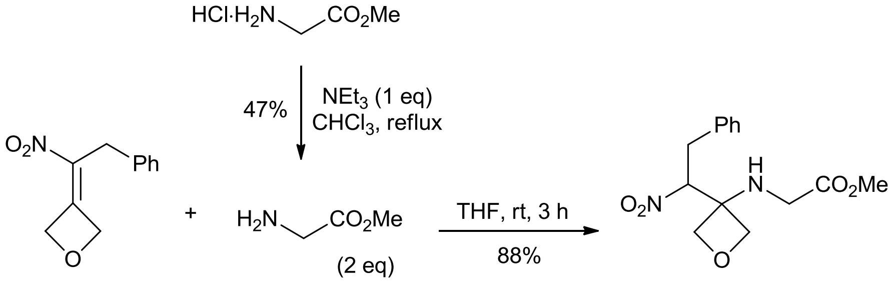
Scheme 2.10. Synthesis of **164**.

Isoxazole **167** is formed through deprotonation of the oxetane ring of **164**, leading to the rearrangement shown in Scheme 2.11, which is promoted by secondary or tertiary amines.[91] Unfortunately **167** cannot be completely removed from **164** using column chromatography.



Scheme 2.11. Rearrangement of **164** to form isoxazole **167**.[91]

Having obtained **164** in reasonable yield, the next step was to explore the conjugate addition with commercially available amino esters, which are generally sold as their hydrochloride or *p*-toluenesulfonate salts. Glycine methyl ester hydrochloride was investigated initially, due to the lack of steric hindrance. On account of the propensity of **168** to polymerise, it was used in the conjugate addition immediately after it had been free based according to literature methods (Scheme 2.12).[92-93] **168** was used in a 2-fold excess to compensate for loss due to polymerisation *in situ* and reacted smoothly with **164** at rt in THF to give **169** in 88% yield. Formation of **169** was indicated by the appearance of a proton at 5.12 ppm in the ^{1}H NMR, which was found to be adjacent to the benzyl group based on 2-D NMR experiments.

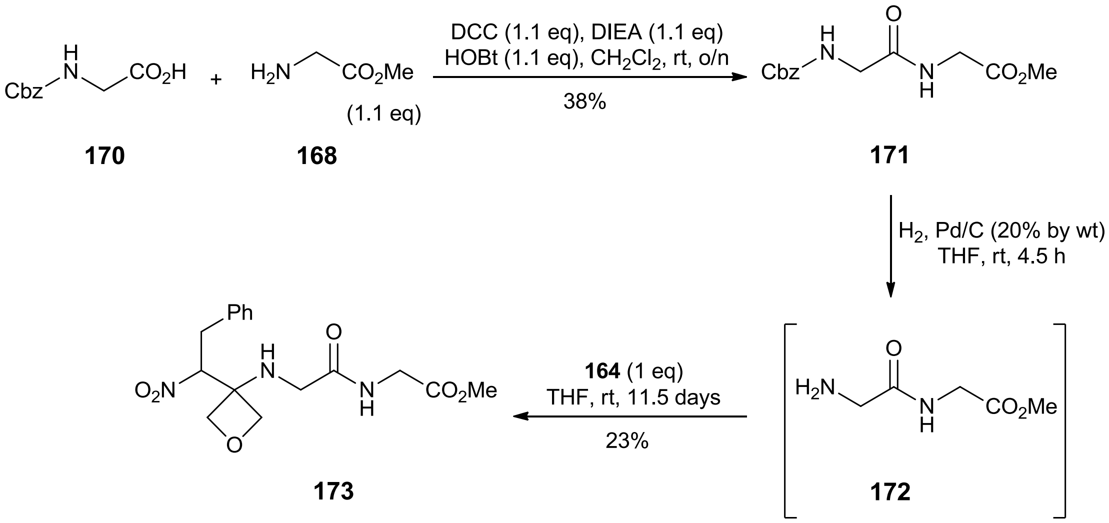


164 **168** **169**

Scheme 2.12. Conjugate addition of **168** to **164**.

We were also interested in exploring the conjugate addition of a dipeptide ester such as **172** to give a tripeptide precursor containing an oxetane at the *N*-terminus (Scheme 2.13). Reaction of *N*-Z-glycine (**170**) with **168** using standard amide coupling conditions gave protected dipeptide ester **171** in which the *N*-protecting group could be selectively removed by hydrogenation. The moderate yield for the synthesis of **171** could be attributed to polymerisation of **168** in the reaction mixture.

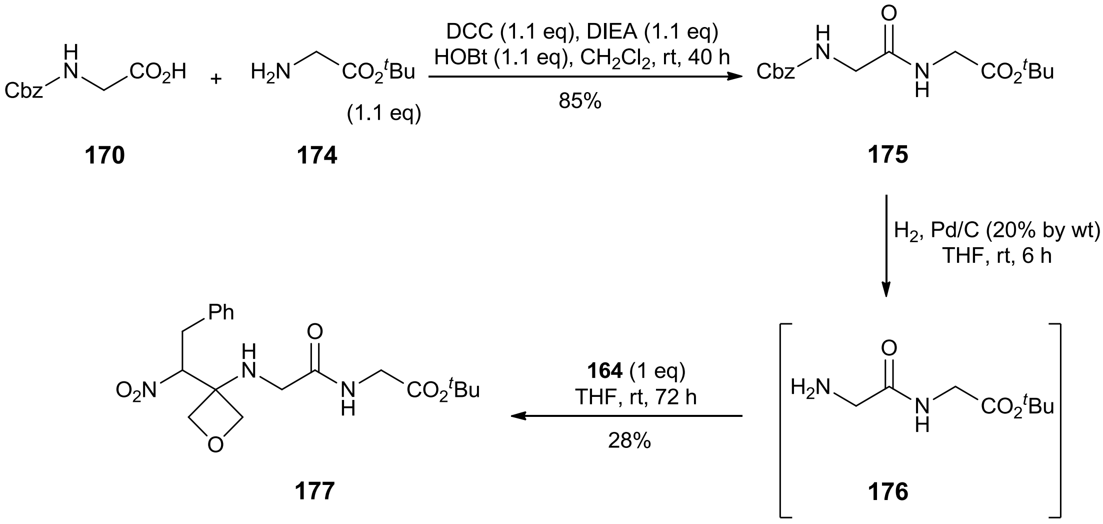
Dipeptide ester **171** was hydrogenated in THF, then filtered through Celite® before the filtrate was added immediately to a solution of **164** in THF. The reaction was stirred at rt for 11.5 days and followed by TLC. However, after work-up, the crude ^{1}H NMR showed **173** and unreacted **164** in a 1 : 2 ratio despite the dipeptide ester being used in a 2.5-fold excess. Purification by column chromatography gave **173** in 23% yield. The presence of unreacted starting material suggested that **172** had potentially cyclised *in situ* before it could react with **164**, a known issue with dipeptide esters, particularly those containing glycine residues.[94]



Scheme 2.13. Conjugate addition of dipeptide ester **172** to **164**.

In order to prevent the possible cyclisation of **172**, we decided to use a bulkier ester protecting group, in this case the *tert*-butyl ester as they are known to be more stable to cyclisation due to the steric bulk inhibiting nucleophilic attack.[95]

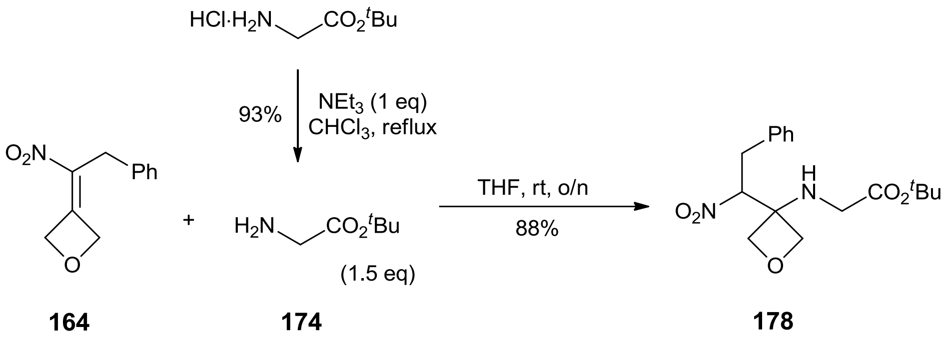
Dipeptide ester **175** was synthesised using the same amide coupling conditions in a much higher yield (Scheme 2.14). Deprotection, followed by conjugate addition afforded **177** in 28% yield. However, a large proportion of the starting material was recovered, possibly due to cyclisation of the dipeptide ester still occurring.



Scheme 2.14. Conjugate addition of dipeptide ester **176** to **164**.

Whilst using a *tert*-butyl ester rather than a methyl ester did not significantly improve the yields for the conjugate addition, we hypothesised that a *tert*-butyl ester may prove beneficial when reducing the nitro group of peptidomimetic precursors such as **159**, on account of the propensity of dipeptides to undergo cyclisation.[96-97]

Therefore we investigated the conjugate addition of glycine *tert*-butyl ester to **164** and found the reaction proceeded smoothly to give **178** in 88% yield (Scheme 2.15).

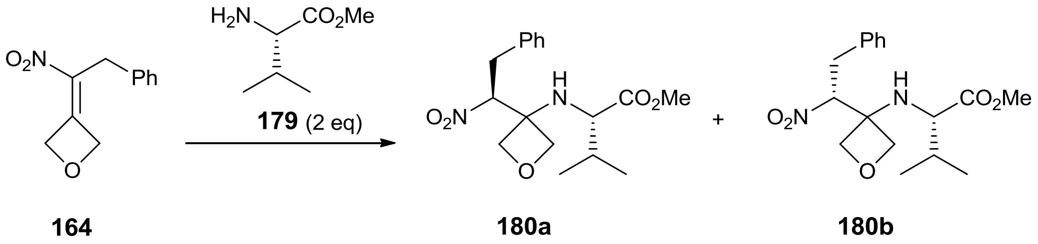


Scheme 2.15. Conjugate addition of **174** to **164**.

Having demonstrated that the conjugate addition occurred readily, albeit in varying yields, with unsubstituted amino and dipeptide esters, we wanted to explore the reaction using an amino ester containing a substituent on the α-carbon. We elected to investigate L-valine methyl ester, since it is a branched-chain amino ester and would provide a good indication of whether steric hindrance might slow the conjugate addition. Additionally, it would also allow us to probe if a chiral amino ester might influence the diastereoselectivity of the conjugate addition. Immediately before the conjugate addition, L-valine methyl ester hydrochloride was free based[92] and reacted with **164**.

The reaction of L-Val-OMe and **164** in THF at rt was followed by TLC and, after no reaction was observed, the reaction mixture was heated under reflux (Table 2.1, entry 1). The crude ^{1}H NMR showed both diastereomers **180a**/**180b**, as well as starting material **164** and isoxazole **167**. Observation of **167** suggested that the rearrangement of **164** can occur with primary amines under more forcing conditions such as heating. Alternative solvents were then investigated. Using methanol, a mixture of products were formed, including isoxazole **167** as well as the product resulting from conjugate addition of methanol to **164**, as indicated by ESI-MS (entry

2). When DMSO was used as the solvent, and the reaction was heated at 85 °C, only isoxazole **167** was observed (entry 3). Finally it was discovered that the reaction proceeded well in THF at rt, but required a longer reaction time than for glycine methyl ester (entry 4). Unfortunately the two diastereomers **180a** and **180b** were inseparable by column chromatography and were isolated in near equal quantities (55 : 45).



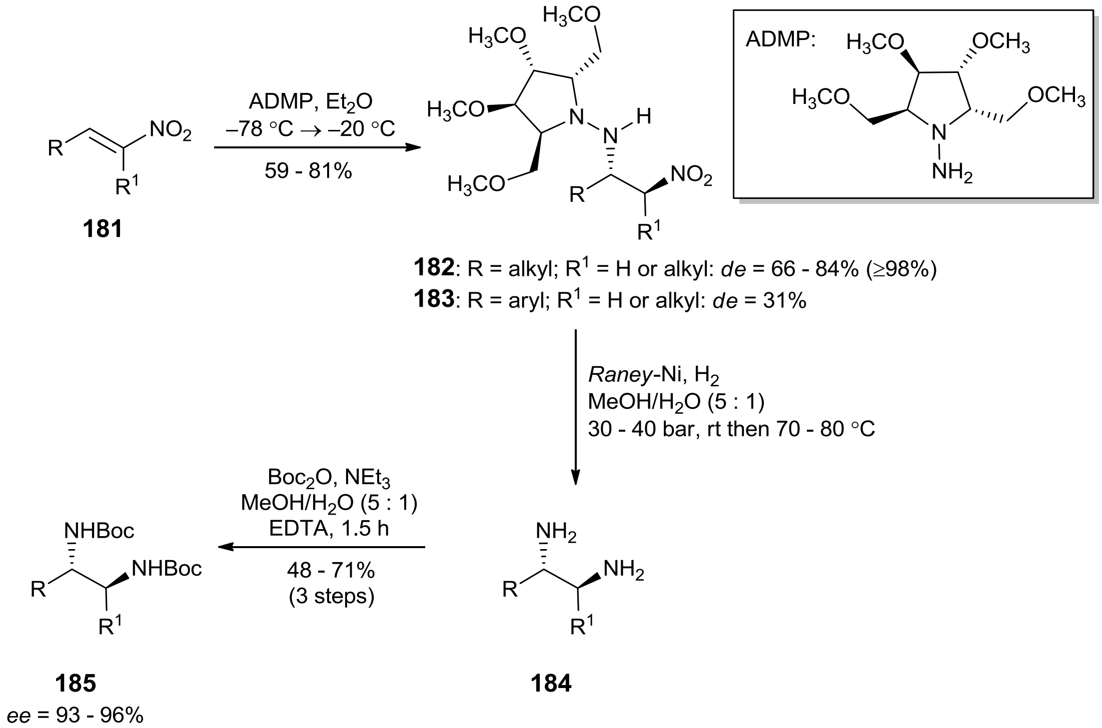
Entry	Reaction Conditions	Product(s)
1	THF, rt → reflux, 72 h	**164**, **167**, **180a**/**180b**[a]
2	MeOH, rt → reflux, 51 h	**167** and unknown products[a]
3	DMSO, rt → 85 °C, 120 h	**167**[a]
4	THF, rt, 64 h	**180a**/**180b** (80%)[b]

Table 2.1. Optimisation of the conjugate addition of L-valine methyl ester to **164**. [a]Observed in crude ^{1}H NMR. [b]Isolated as a 55 : 45 mixture, as determined by ^{1}H NMR.

Based on these results, we had established that the conjugate addition occurred readily with substituents on the nitroalkene and/or the amino ester α-carbon. However, poor diastereoselectivity was observed in the addition to **164**. Next, we were interested in investigating if stereocontrol could be achieved in the conjugate addition using a chiral catalyst.

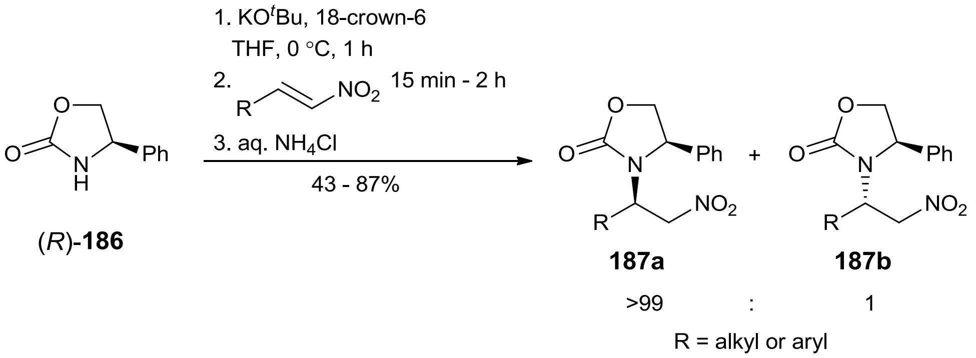
2.2.1.1 Control of Stereoselectivity in Conjugate Addition

It was hypothesised that the diastereoselectivity of the conjugate addition could be improved by addition of a chiral catalyst during the reaction. There are few reported examples of asymmetric aza-Michael additions to nitroalkenes[98-99] and of these, the majority involve the use of an enantiopure nucleophile to control the selectivity of the reaction, rather than a chiral catalyst. For example, Enders and Wiedemann developed a route towards the diastereo- and enantioselective synthesis of protected vicinal diamines, by addition of ADMP, as a chiral equivalent of ammonia, to various nitroalkenes (Scheme 2.16).[100]



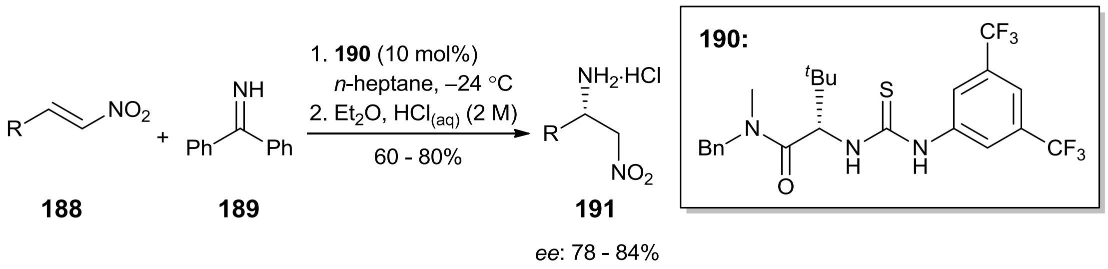
Scheme 2.16. Diastereo- and enantioselective route towards protected vicinal diamines.[100]

Similarly, Lucet *et al* added (*R*)- or (*S*)-4-phenyl-2-oxazolidinone (**186**) to monosubstituted nitroalkenes in the presence of 18-crown-6 and potassium *t*-butoxide to give a single diastereomer of the conjugate product that could then be reacted further as required (Scheme 2.17).[101] Indeed, Mioskowski and co-workers expanded on this chemistry to develop routes towards the synthesis of various α,α-disubstituted amines.[102]



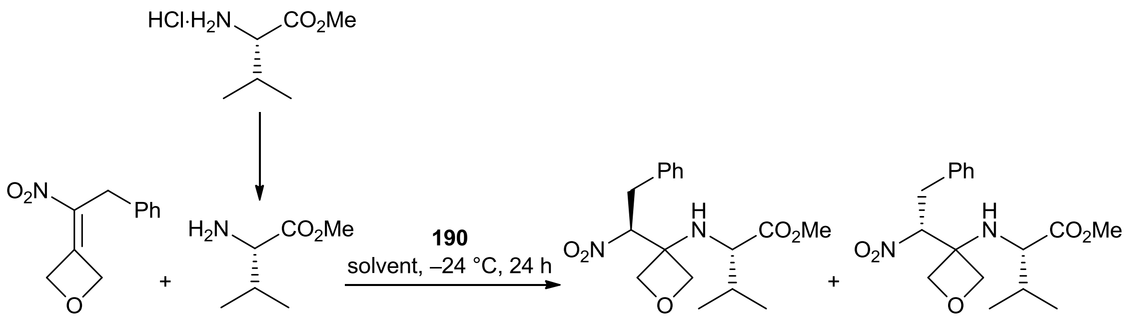
Scheme 2.17. Diastereoselective aza-Michael reaction of Lucet *et al*.[101]

However, examples of asymmetric organocatalytic aza-Michael additions involving nitroalkenes had not been established until Wang *et al*'s work in 2006, which demonstrated the reaction using *N*-heterocycles as nucleophiles.[103] Whilst examples of asymmetric organocatalytic aza-Michael additions are still relatively rare, a method published by Lykke *et al*[99] interested us because of its potential applicability to this project. Using benzophenone imine (**189**) as an ammonia equivalent, the authors added it to a range of nitroalkenes in the presence of various chiral catalysts. They found that thiourea-based catalysts, used to activate nitroalkenes towards nucleophilic attack, gave the most promising results.[99,104] Optimal results were obtained when 10 mol% of **190** was used in *n*-heptane at −24 °C.[99] Application of these conditions enabled the isolation of β-amino nitro compounds as their hydrochloride salts in good yields and enantioselectivities (Scheme 2.18).[99]



Scheme 2.18. Organocatalysed aza-Michael addition to nitroalkenes.[99]

Based on their investigations, we decided to explore their optimised conditions for the conjugate addition of L-valine methyl ester to **164**, as changes in diastereoselectivity would be readily observed by ^{1}H NMR. Entry 1 in Table 2.2 was performed according to the optimal conditions developed by Lykke *et al*[99] and showed a preference for the opposite diastereomer to that observed under the standard conjugate addition conditions. As the conjugate additions were traditionally performed with an excess of L-valine methyl ester rather than an excess of nitroalkene **164**, we decided to explore the catalysed reaction using a 1 : 2 ratio of **164** : **179**. The control reaction (entry 7), performed in the absence of any catalyst, showed that the solvent choice, and/or the temperature, has some effect on the ratio of the diastereomers, increasing the selectivity for **180a** over **180b** compared to the reaction in THF at rt. In the presence of catalyst **190**, the ratios of the diastereomers did change, decreasing the selectivity for **180a** compared to **180b**. However, the results were rather inconsistent (entries 2 vs 3, entries 4 vs 5), which could be in part due to issues of solubility in *n*-heptane. When the reaction was performed in toluene (entry 6), there were no solubility issues and the amount of the minor diastereomer increased, although there was still very little diastereoselectivity.



164 **179** **180a** **180b**

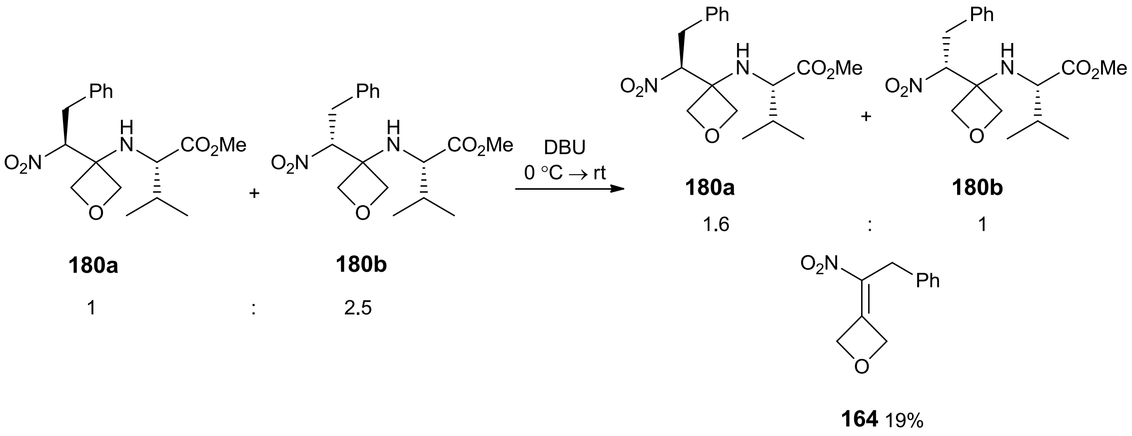
stereochemistry arbitrarily assigned

Entry	Ratio of 164 : 179	Catalyst Loading	Solvent	Ratio of diastereomers	Conversion
1	2 : 1	10 mol%	Heptane	0.68 : 1	~99%
2[a]	1 : 2	10 mol%	Heptane	0.76 : 1	>99%
3[a]	1 : 2	10 mol%	Heptane	1.15 : 1	>99%
4[b]	1 : 2	20 mol%	Heptane	1.08 : 1	86%
5[b]	1 : 2	20 mol%	Heptane	0.98 : 1	~94%
6	1 : 2	10 mol%	Toluene	1.06 : 1	>99%
7	1 : 2	N/A	Heptane	1.43 : 1	98%

Table 2.2. Investigation into the diastereoselectivity of the addition of **179** to **164**. [a]Reaction repeated under the same conditions. [b]Reaction repeated under the same conditions.

These results suggest that an organocatalyst can influence the diastereoselectivity of the conjugate addition, however, there is much to be done to optimise this process.

Due to the acidic nature of the proton on the carbon adjacent to the nitro group, we decided to explore whether the ratio of the diastereomers is changed by equilibration *via* deprotonation/protonation (Scheme 2.19). Reaction of **180a/b** (**180a** : **180b**, 1 : 2.5) with NaOMe led to the consumption of the starting material and the formation of unknown products alongside **164**. However, using a milder base such as DBU, the diastereoselectivity did change (1 : 2.5 → 1.6 : 1), although elimination of L-valine methyl ester from **180a/b** to give **164** (19%) was also observed.



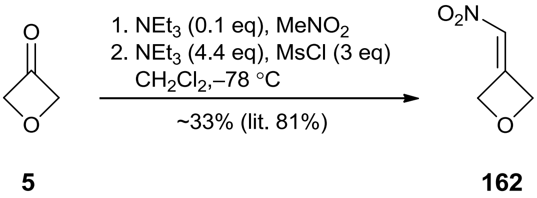
Scheme 2.19. Deprotonation of **180a/b** to alter the diastereomeric ratio.

Based on these early investigations, it is possible to affect the stereoselectivity of the conjugate addition to nitroalkene **164**, but the optimal conditions have not yet been found. Similarly, the diastereomeric ratio can be altered by deprotonation with a weak base, but there is a competing elimination reaction. Lack of stereocontrol in this conjugate addition led us to turn our attention to the reactions of unsubstituted nitroalkene **162**.

2.2.2 Conjugate Additions to ‘Gly’ *via* a Two-Step Method

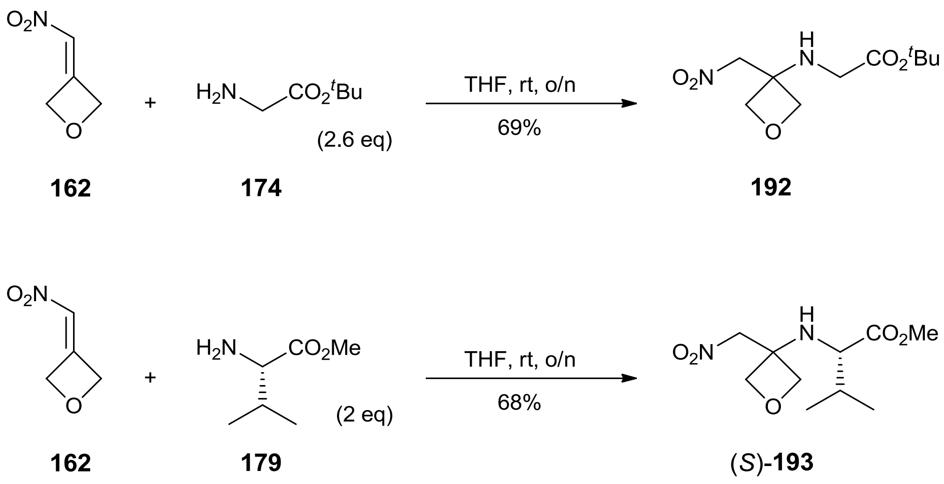
We were interested in developing a route towards peptidomimetics containing an oxetane ‘Gly’ residue as this would allow us to establish the methodology without the need to address the issue of stereocontrol in the conjugate addition.

Nitroalkene **162** was prepared according to the literature procedure[9] (Scheme 2.20). However, **162** was isolated in a much lower yield than reported, probably due to its volatility and difficulties identified in purifying it.



Scheme 2.20. Synthesis of **162**.

162 was taken through and reacted with both L-valine methyl ester and glycine *tert*-butyl ester. The amino esters were free based from their hydrochloride salts as described previously then immediately reacted with **162** to give **192** and **193** in good yields (Scheme 2.21).

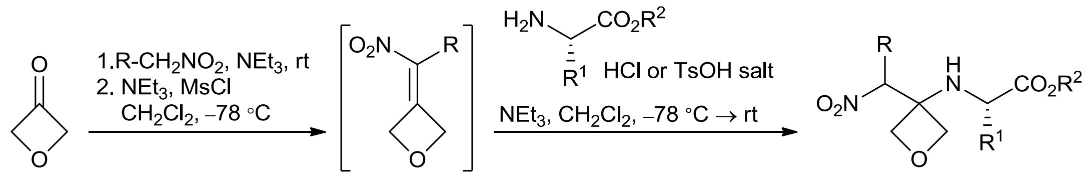


Scheme 2.21. Conjugate addition of **174** and **179** to **162**.

Whilst the conjugate additions to **162** occurred in good yields, the difficulties encountered in obtaining **162** cleanly led to a poor yield over the 2 steps. Therefore an alternative method of obtaining the addition products was sought.

2.2.3 Conjugate Additions *via* a One-Pot Method

The limiting step in the synthesis of the nitro amino esters, was the formation of Michael acceptors **162** and **164**. On the basis that these compounds were formed in low yields because of their volatility and/or susceptibility to rearrangement, we decided to investigate the potential for generating them *in situ*. Our initial reaction conditions involved using the nitroalkene as the limiting reagent and the results are shown in Table 2.3. Encouragingly, (*S*)-**193** was formed in a higher yield of 47% compared to the 22% overall yield achieved with the two step method (entry 1). High yields were also obtained with L-valine benzyl ester (entry 2, 83%) and L-phenylalanine benzyl ester (entry 3, 73%), whilst moderate to good yields were obtained with other amino esters. Good yields were also obtained for **199** and **200**, which suggests that reacting **164** *in situ* may help prevent the rearrangement to isoxazole **167**.



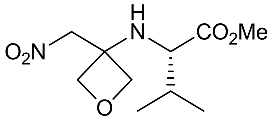
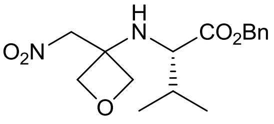
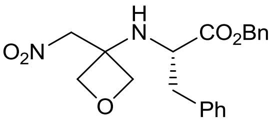
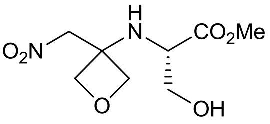
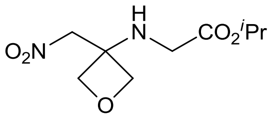
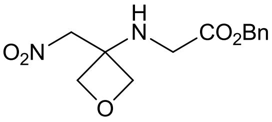
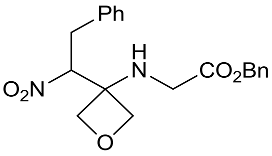
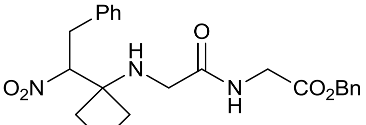
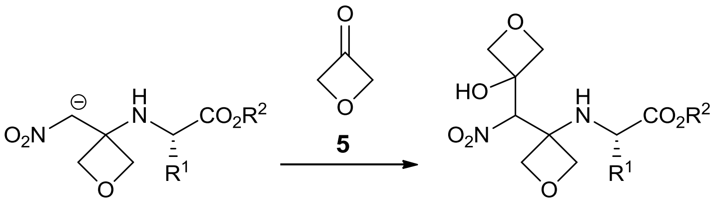
Entry[a,b]	R	Amino ester salt	Product	Yield (%)
1	H	H-L-Val-OMe·HCl	 (*S*)-**193**	47
2	H	H-L-Val-OBn·TsOH	 **194**	83
3	H	H-L-Phe-OBn·HCl	 **195**	73
4	H	H-L-Ser-OMe·HCl	 **196**	48
5	H	H-Gly-O^{i}Pr·HCl	 **197**	61
6	H	H-Gly-OBn·TsOH	 **198**	64
7	Bn	H-Gly-OBn·TsOH	 **199**	58
8	Bn	H-Gly-Gly-OBn·TsOH	 **200**	64

Table 2.3. One-pot reactions to form nitro amino esters. [a]Method for entries 1-6: CH_3NO_2 (1 eq), 3-oxetanone (1.3 eq), NEt_3 (0.2 eq), 30 min; NEt_3 (2 eq), MsCl (1 eq) 30 – 40 min; amino ester salt (2 eq), NEt_3 (2 eq), 16 – 25 h. [b]Method for entries 7-8: $BnCH_2NO_2$ (1 eq), 3-oxetanone (1.3 eq), NEt_3 (0.2 eq), 90 min; NEt_3 (2 eq), MsCl (1 eq), 90 min; amino ester salt (2.2 eq), NEt_3 (2 eq), o/n.

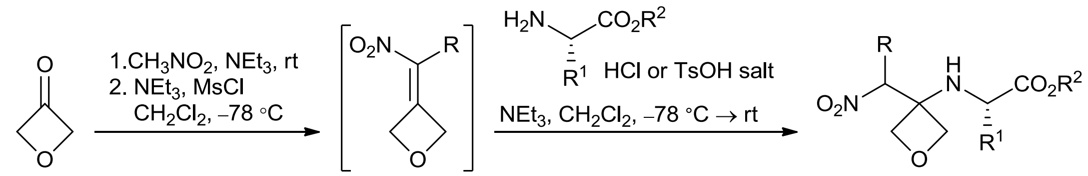
Whilst the yields were generally good, close-running impurities were often formed that were difficult to remove by column chromatography. It was postulated that one of these impurities could have been **202**, resulting from the reaction of the amino ester anion **201** with excess 3-oxetanone (Scheme 2.22), due to the presence of extra peaks in the oxetane region of the ^{1}H NMR.



201 **202**

Scheme 2.22. Potential over-reaction of **201**.

In order to minimise this possible side reaction, we investigated the one-pot reaction using 3-oxetanone as the limiting reagent. The results are shown in Table 2.4. With 3-oxetanone as the limiting reagent, the yield for (*S*)-**193** increased from 47% to 65% (entry 1) and the reaction conditions gave the products from a range of amino and dipeptide esters in generally good yields. The synthesis of (*S*)-**193** using reduced equivalents of the amino ester (entry 2), gave (*S*)-**193** in a lower yield of 40%. Therefore the amino ester was used in a 2-fold excess in all subsequent additions.



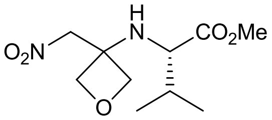
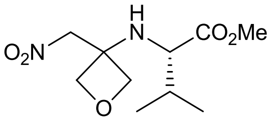
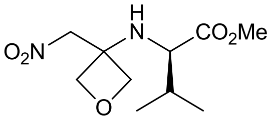
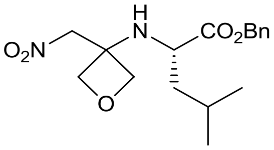
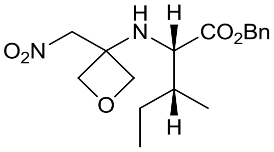
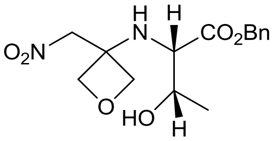
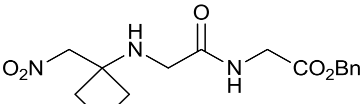
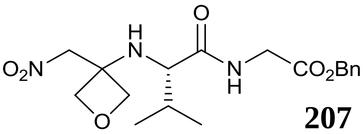
Entry[a]	Amino ester salt	Product	Yield (%)
1	H-L-Val-OMe·HCl	 (*S*)-**193**	65
2	H-L-Val-OMe·HCl	 (*S*)-**193**	40[b]
3	H-D-Val-OMe·HCl	 (*R*)-**193**	68
4	H-L-Leu-OBn·TsOH	 **203**	59
5	H-L-Ile-OBn·TsOH	 **204**	52
6	H-L-Thr-OBn·HCl	 **205**	56
7	H-Gly-Gly-OBn·TsOH	 **206**	57
8	H-L-Val-Gly-OBn·HCl	 **207**	62

Table 2.4. One-pot reactions to form nitro amino esters using 3-oxetanone as the limiting reagent. [a]Method: CH_3NO_2 (1.4 eq), 3-oxetanone (1 eq), NEt_3 (0.2 eq), 30 min; NEt_3 (2 eq), MsCl (1 eq) 40 – 120 min; amino ester salt (2 eq), NEt_3 (2 eq), 20 h. [b]Method as above with 1.2 eq of L-Val-OMe.

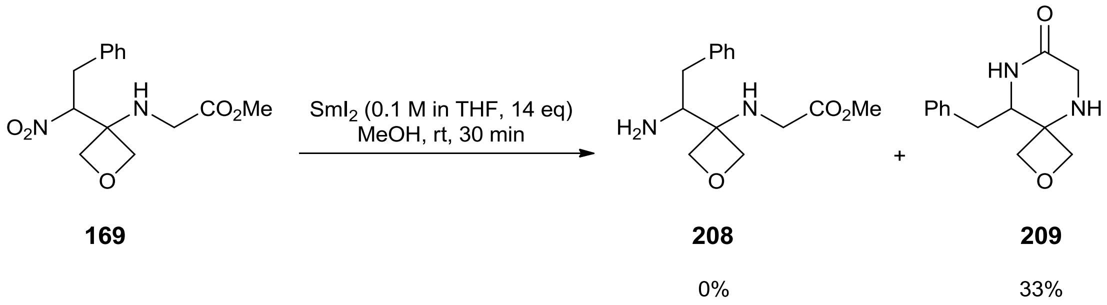
A variety of amino esters, including polar and hydrophobic examples, have been successfully used in these one-pot reactions, as well as dipeptide esters, which provide easy access to longer chain oxetane-containing peptidomimetics. Importantly, chiral HPLC analysis of (*R*)-**193** and (*S*)-**193** (synthesised from D- and L-valine methyl ester respectively), indicated that no detectable racemisation occurred during the conjugate additions (Appendix I). Thus this methodology appears promising for the synthesis of enantiopure peptidomimetics.

Having developed a reliable method for the synthesis of nitro peptide precursors in good yields, with the reaction conditions tolerated by a range of substituents and protecting groups, we next focused our attention on progressing the synthesis of the target peptidomimetics by reduction of the nitro group.

2.2.4 Reduction of the Nitro Group

Whilst there is much literature precedent for the reduction of aromatic nitro groups to the corresponding amine, the reduction of nitroalkanes is generally more challenging.

We initially chose to explore the reduction of **169** to discover if the methyl ester would be susceptible to cyclisation, or if the presence of the oxetane and the benzyl group would inhibit this potential side reaction. Initial attempts to reduce the nitro group using hydrogenation or transfer hydrogenation failed to yield amine **208**.[9,105] Reduction of the nitro group with samarium(II) iodide (Scheme 2.23) gave only the cyclised oxetane-containing diketopiperazine (**209**), whose structure was confirmed by X-ray crystallography (Figure 2.12).



Scheme 2.23. Reduction of the nitro group of **169**.

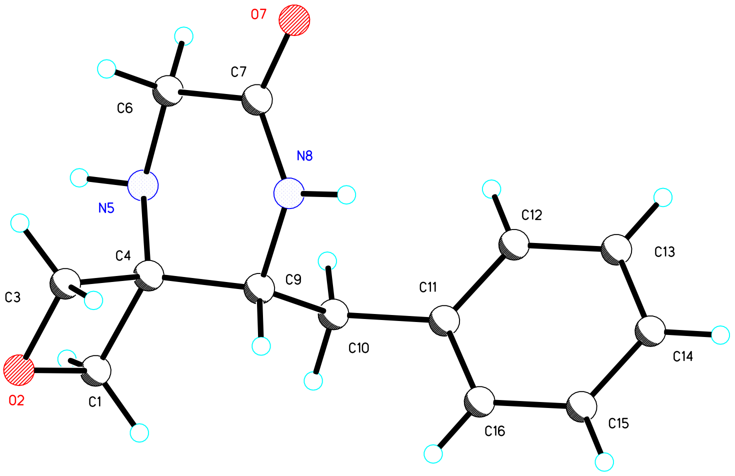
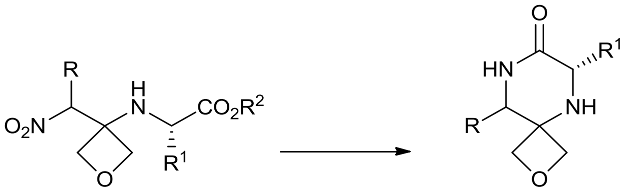


Figure 2.12. Crystal structure of **209**.

As feared, these results indicated that the methyl ester is susceptible to the cyclisation.[97] Oxetane-containing diketopiperazine **209**, however, was itself of interest as diketopiperazines are often regarded as peptidomimetics and are also present in various natural products.[96,106] Therefore, we decided to try to optimise this cyclisation.

Generally, acidic conditions promoted the cyclisation, even in the presence of bulkier protecting groups such as benzyl esters. Indeed, diketopiperazine synthesis often involves heating in acidic medium.[96,106] Even in the absence of acid, the cyclisation still occurs to give **210** in a reasonable yield under standard hydrogenation conditions (Table 2.5, entry 1). Interestingly, the reduction and *in situ* cyclisation of **194** (entry 2) did not go to completion despite longer reaction times being investigated and the amine was observed in the crude ^{1}H NMR, along with **210** (2 : 1 amine : **210**). In this example, the isopropyl substituent did not prevent nucleophilic attack onto the methyl ester. It is believed that the unsubstituted example, **198**, (entry 5) most likely cyclised but the resultant product was not isolated on account of its potential water solubility.



Entry	Substrate	R	R^1	R^2	Method^a	Product(s)
1	(*S*)-**193**	H	^{i}Pr	Me	B, 17 h	**210** (45%)
2	**194**	H	^{i}Pr	Bn	A, 67 h	**210** (22%)
3	**195**	H	Bn	Bn	A, 67 h	**211** (68%)
4	**196**	H	CH_2OH	Me	A, 72 h	0%^{b}
5	**198**	H	H	Bn	A, 67 h	0%^{b}
6	**199**	Bn	H	Bn	A, 24 h	**209** (35%)

Table 2.5. Optimisation of the cyclisation. ^{a}Method A: Zn (35 eq), AcOH, THF, rt;[107] Method B: Pd/C (50% by wt), H_2, MeOH, rt. ^{b1}H NMR of crude product was inconclusive.

Our results indicate that both the choice of ester protecting group and the amino ester itself can affect the efficiency of the cyclisation. Heating the reaction in acid may encourage complete conversion to the cyclised product.

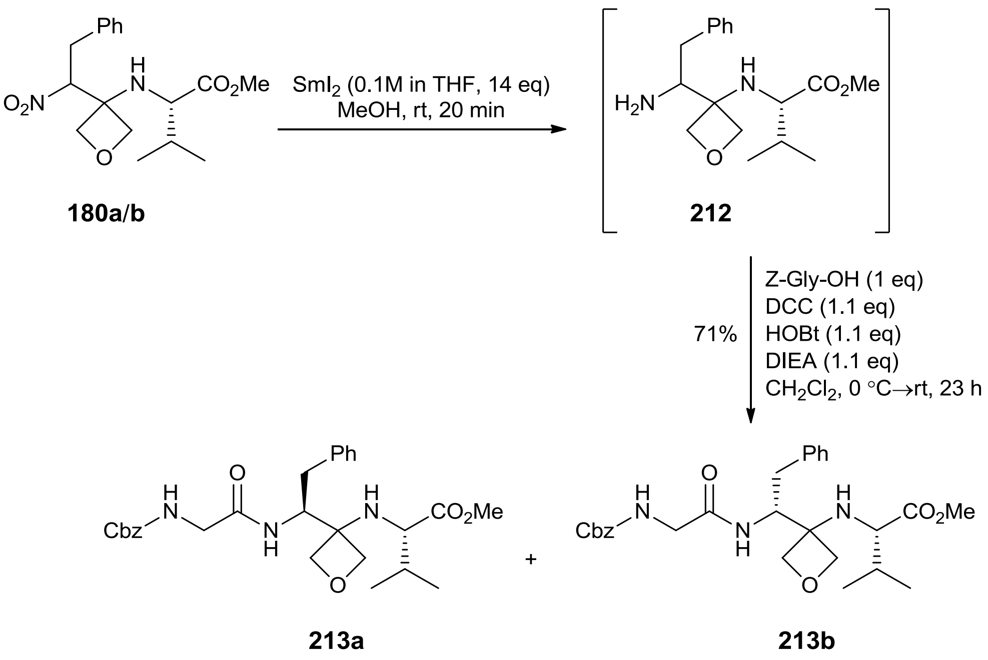
Investigations into the reduction of the nitro group indicated that whilst the corresponding amines can be obtained, there is a strong propensity for cyclisation to the diketopiperazine-like structure. The extent of this cyclisation is dependent on the nature of the ester group, α-carbon substituents and reaction conditions. Zn in AcOH generally promoted cyclisation with various α-carbon substituents, even when a benzyl ester was used, and heating the reaction may result in full conversion to the cyclised product. Hydrogenation when the ester was a methyl group also gave the cyclised product despite the isopropyl substituent on the α-carbon. To minimise loss of the amine through cyclisation, we decided to investigate the reduction of the nitro group, followed by immediate coupling to the next amino acid (Scheme 2.7, p.75).

2.2.5 Nitro Reduction and Amide Couplings

Efforts focused on the reduction and subsequent coupling of compounds with either a substituent on the α-carbon of the amino ester, a bulky ester group, or both.

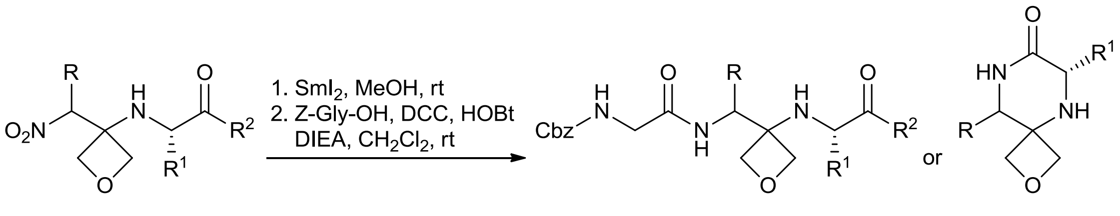
2.2.5.1 Synthesis of Protected Mid-Chain Peptidomimetics

Reduction of **180a**/**b**, as a 55 : 45 mixture of diastereomers, with samarium(II) iodide in MeOH gave the corresponding amine (**212**), which was coupled with *N*-Z-glycine, using DCC as the coupling reagent, to give a mixture of **213a** and **213b** in 71% yield. These were subsequently separated by chiral HPLC to give one diastereomer in 26% isolated yield and the other diastereomer in 19% isolated yield, although the relative stereochemistry of each diastereomer was not determined.



Scheme 2.24. Reduction and coupling of **180a**/**b**.

As the samarium(II) iodide reduction and *in situ* DCC coupling had been successful with **180a**/**b**, we decided to examine these conditions with a range of nitro compounds, the results of which are shown in Table 2.6. When a *tert*-butyl ester was used, amide **214** was isolated in 50% yield (entry 1). This was promising as, traditionally, the presence of glycine residues facilitates diketopiperazine formation due to the lack of an obstructive side chain.[97] Attempts to improve this yield using EDC as the coupling reagent instead of DCC gave **214** in 35% yield (entry 2). Unsurprisingly, the reduction of **197**, containing two glycine residues, yielded no amide product, despite the presence of the isopropyl ester group (entry 3). Interestingly, the reduction of **195** gave only diketopiperazine **211** (entry 4). Benzyl esters are generally believed to be less reactive to cyclisation than other esters, based on a study using H-Gly-Gly-OR.[97] The reduction of (*S*)-**193** on the other hand, gave **215** in ~17% yield containing the cyclised product **210** as well (entry 5). This indicates that a benzyl ester, in conjunction with a branched substituent on the α-carbon of the amino ester, enables some of the intermediate amine to react before it cyclises. Similarly, the reduction of **194** allowed the isolation of **216** containing trace cyclised product (entry 6). These results implied that the choice of ester group may not be as important as the α-carbon substituent with respect to suppressing the cyclisation. Reduction and coupling of **200** demonstrated the ability to access longer chain peptidomimetics (entry 7). Under the conditions employed, however, transesterification of the ester group occurred, giving the final amide product as the methyl ester rather than the expected benzyl ester. As samarium(II) iodide is highly oxophilic and can be used as a Lewis acid, the transesterification likely occurred in the reduction step.[108-109] Indeed, samarium(II) iodide has been used to catalyse the transesterification of dimethylcarbonate and phenol.[110]



214-217 **211**

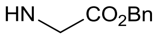
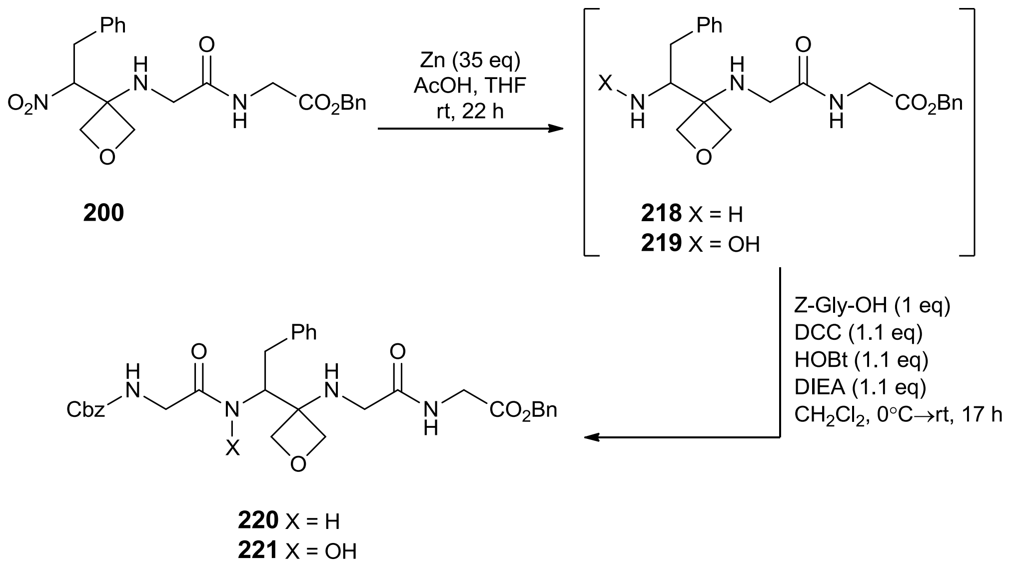
Entry	Substrate	R	R^1	R^2	Product(s)
1	**178**	Bn	H	O^{t}Bu	**214** (50%)[a]
2[b]	**178**	Bn	H	O^{t}Bu	**214** (35%)
3	**197**	H	H	O^{i}Pr	0
4	**195**	H	Bn	OBn	**211** (~88%)
5	(*S*)-**193**	H	^{i}Pr	OMe	**215** (~17%)[c]
6	**194**	H	^{i}Pr	OBn	**216** (~41%)
7	**200**	Bn	H		**217** (45%)[d]

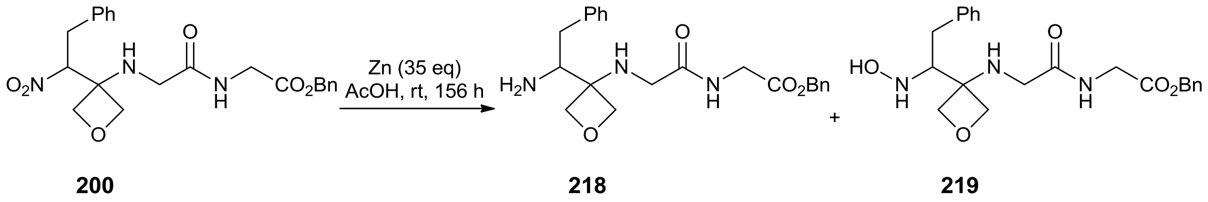
Table 2.6. Investigation of sequential nitro reduction and amide coupling. [a]Yield calculated for amide coupling step due to crude amine used in excess. [b]Coupling conditions: EDC·HCl, HOBt, NMM, EtOH, rt. [c]As a 2.5 : 1 mixture of **215** : **210**. [d]Methyl ester amide isolated due to transesterification.

We decided to investigate the reduction of **200** with Zn in AcOH/THF as an alternative to samarium(II) iodide and applied these conditions to the synthesis of **220** (Scheme 2.25). We discovered that these reduction conditions did not reduce the nitro group completely and the intermediate hydroxylamine also underwent amide coupling to Z-Gly-OH. Unfortunately this product was inseparable from **220**.



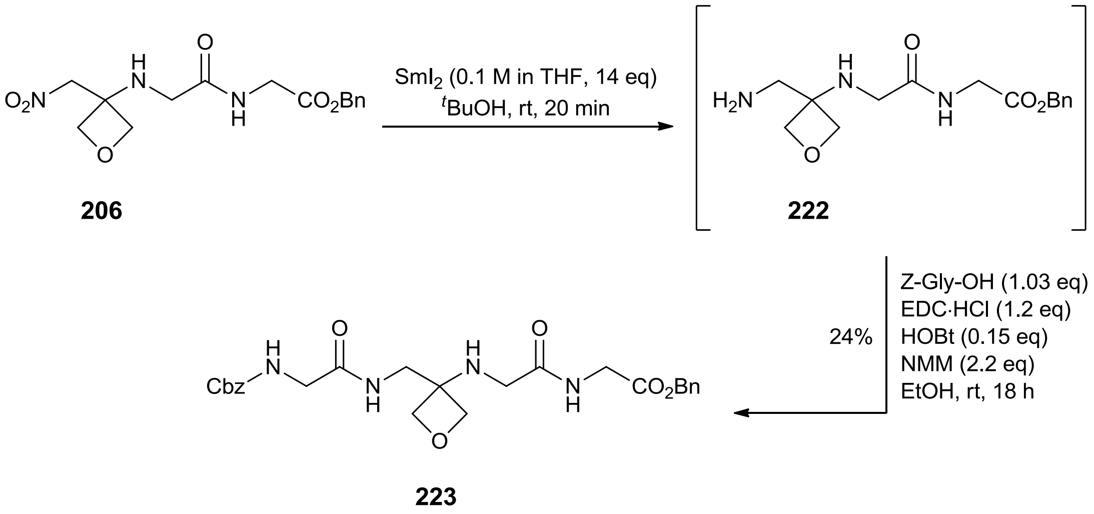
Scheme 2.25. Reduction and coupling of **200** using Zn in AcOH.

We then decided to try the reduction in pure acetic acid, without a co-solvent, and left it for a much longer reaction time (Scheme 2.26). **200** was initially subjected to the reaction conditions for 84 h, after which ^{1}H NMR indicated the presence of both amine **218** and hydroxylamine **219** in an approximately 1 : 1 ratio. The crude material was resubjected to the reaction conditions for a further 72 h. Unfortunately, whilst there was a slight increase in conversion to amine **218**, the reaction did not go to completion, implying the need for more forcing conditions. The low yield suggests **218** was being lost, either through cyclisation or on work-up.



Scheme 2.26. Reduction of **200** with Zn in AcOH.

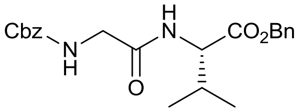
As samarium(II) iodide reduction had been successful for **200**, despite transesterification occurring, we decided to attempt the reduction of **206** using ^{t}BuOH as the proton source rather than MeOH (Scheme 2.27). When the reduction was performed in ^{t}BuOH no transesterification was observed but **223** was only isolated in a 24% yield over the 2 steps. The low yield may be attributed to the presence of the glycine residues encouraging DKP formation. Attempts to reduce **206** with indium, followed by an EDC coupling failed to give any product.



Scheme 2.27. Reduction and coupling of **206** using samarium(II) iodide and EDC.

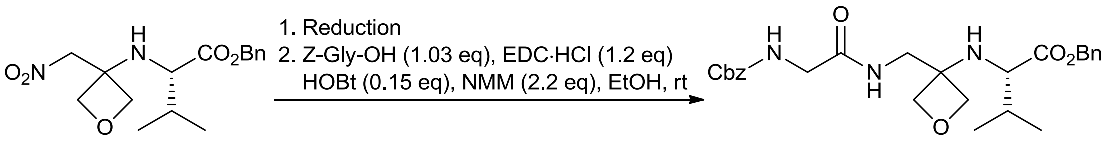
Based on our earlier results, we decided to focus on optimising the reduction of **194**. This substrate was chosen on the theory that the combination of benzyl ester and the isopropyl group of L-valine would inhibit the cyclisation enough to enable the amide coupling to proceed. Additionally, the use of the benzyl ester group would allow the concomitant deprotection of the *N*- and *C*-termini in the final step of the sequence. We chose EDC as the coupling reagent rather than DCC due to the comparative ease of removing the urea by-product.

A screen of various conditions for the reduction of **194**, followed by an amide coupling with Z-Gly-OH was performed and the results are shown in Table 2.7. The reduction of **194** with nickel boride, generated *in situ*, and sodium borohydride returned mainly starting material (entry 1). Reduction of **194** with tin chloride in methanol (entry 3) showed no reaction at rt, as judged by TLC, whilst heating the mixture under reflux for 2 hours showed consumption of starting material. After the subsequent amide coupling, the main product isolated was amide **224**, shown in Figure 2.13. Clearly, heating the reaction mixture had resulted in the elimination of the amino ester from **194** to yield L-valine benzyl ester and nitroalkene **162**. Reduction using samarium(II) iodide in ^{t}BuOH gave **216** in 35% yield whereas reduction with indium in aqueous HCl improved the yield to 49% (entries 2 and 4). Changing the acid from HCl to HCO_2NH_4 or AcOH had a detrimental effect on the yield of **216** (entries 5 and 6). Shorter reaction times had no effect (entry 7).



224

Figure 2.13. Amide from reaction of L-Val-OBn with Z-Gly-OH.

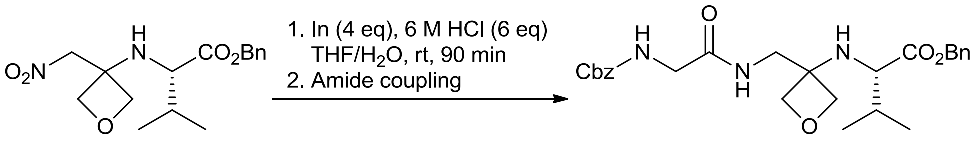


194 **216**

Entry	Reduction Conditions	Yield (%)
1	$NiCl_2 \cdot 6H_2O$ (0.5 eq), $NaBH_4$ (5 eq), MeOH, rt, 24 h[111]	0
2	SmI_2 (0.1 M in THF, 14 eq), ^{t}BuOH, rt, 25 min	35
3	$SnCl_2 \cdot 2H_2O$ (5 eq), MeOH, rt, 24 h then reflux, 2 h[112]	0
4	In (4 eq), 6 M HCl (6 eq), THF/H_2O, rt, 3 h[112]	49
5	In (4 eq), HCO_2NH_4 (6 eq), THF/H_2O, rt, 24 h then 80 °C, 4 h[113]	0
6	In (4 eq), AcOH (6 eq), THF/H_2O, rt, 3 h[113]	37
7	In (4 eq), 6 M HCl (6 eq), THF/H_2O, rt, 90 min	49

Table 2.7. Investigation of sequential nitro reduction and EDC coupling of **194**.

With good reduction conditions in hand, the amide coupling conditions were varied to determine if the yield of **216** could be improved (Table 2.8). This involved varying the solvent used (entry 1), the equivalents of Z-Gly-OH (entry 2), the coupling reagent employed (entry 3) and using Et_2O rather than EtOAc in the work-up (entry 4). However, no further improvements were realised and the optimal conditions were reduction with indium in aqueous HCl, followed by an amide coupling using EDC, as for entry 7 in Table 2.7. The similar yields obtained from investigating the amide coupling conditions indicate the nitro reduction is the limiting step.

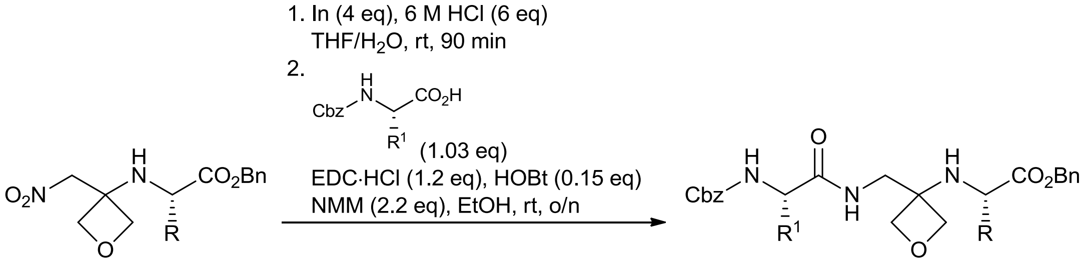


194 **216**

Entry	Coupling Conditions	Yield (%)
1	Z-Gly-OH (1.03 eq), HOBt (0.15 eq), NMM (2.2 eq), EDC·HCl (1.2 eq), CH_2Cl_2, rt, 18 h	45
2	Z-Gly-OH (1.2 eq), HOBt (0.15 eq), NMM (2.6 eq), EDC·HCl (1.4 eq), EtOH, rt, 18 h	47
3	Z-Gly-OH (1.03 eq), HOBt (0.15 eq), DIEA (2.2 eq), PyBOP (1.2 eq), CH_2Cl_2, rt, 18 h[114]	47
4	Z-Gly-OH (1.03 eq), HOBt (0.15 eq), NMM (2.2 eq), EDC·HCl (1.2 eq), EtOH, rt, 18 h[a]	42

Table 2.8. Optimisation of indium reduction and amide coupling of **194**. [a]Worked-up with Et_2O rather than EtOAc.

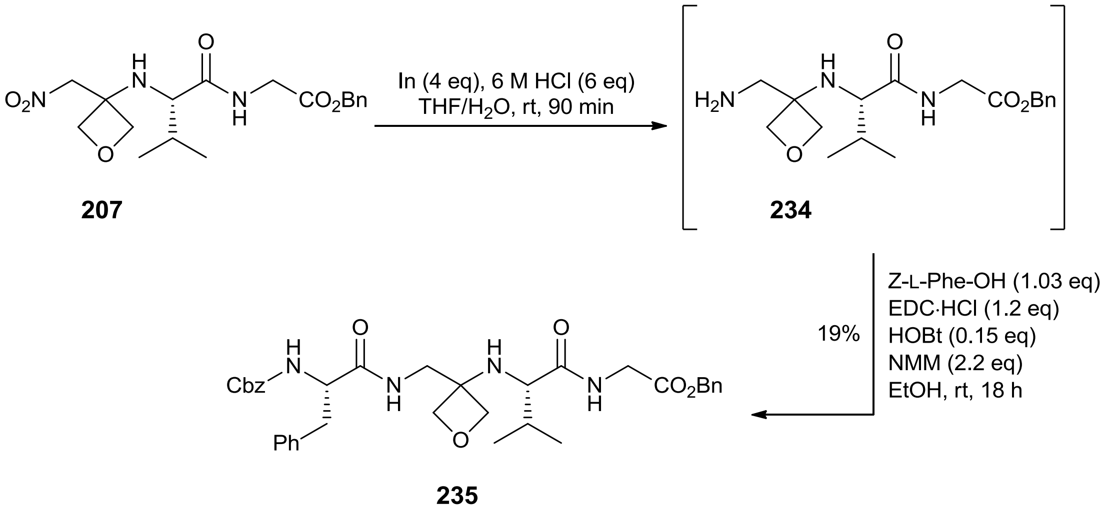
The next step was to apply these reduction and coupling conditions to a range of nitro compounds. The reduction of amino esters **194**, **203**, **204** and **205**, followed by coupling with a range of *N*-carboxybenzyl protected amino acids afforded protected oxetane-containing peptidomimetics (Table 2.9). Interestingly, the highest yields were obtained when Z-L-Phe-OH was coupled with both the L-valine analogue **194** (entry 1) and the L-isoleucine analogue **204** (entry 2), but no product was observed with the L-leucine or L-threonine analogues **203** and **205** (entries 8 and 9). The lack of product from the L-threonine example (entry 9) may be due to the presence of the free hydroxyl group, which could make it more difficult to extract from the aqueous reaction mixture or compete in the coupling. Attempts to reduce **205** with samarium(II) iodide in ^{t}BuOH, followed by coupling with Z-L-Phe-OH, were also unsuccessful. One solution to this would be to protect the free hydroxyl with a benzyl group, which would still allow all protecting groups to be removed concomitantly (*vide infra*).



Entry	Oxetane	R	R^1	Product	Yield (%)
1	**194**	^{i}Pr	Bn	**225**	60
2	**204**	CH(Me)Et	Bn	**226**	58
3	**194**	^{i}Pr	Me	**227**	44
4	**194**	^{i}Pr	^{i}Pr	**228**	47
5	**204**	CH(Me)Et	$CH_2{}^i$Pr	**229**	54
6	**194**	^{i}Pr	CH_2(indole)	**230**	20
7	**194**	^{i}Pr	$CH_2CH_2SCH_3$	**231**	37
8	**203**	$CH_2{}^i$Pr	Bn	**232**	0
9	**205**	CH(OH)Me	Bn	**233**	0

Table 2.9. Synthesis of **225**-**233**.

Having established a route towards the synthesis of protected peptidomimetics containing the oxetane mid-chain, we applied the reaction conditions to the reduction of **207** in order to access the protected tetrapeptide **235** (Scheme 2.28). **235** was isolated in a low yield of 19% despite the presence of the valine residue in **207** to help minimise cyclisation. As cyclisation onto an amide bond should be much slower (compared to the ester), this suggests there are other issues with this chemistry. Whilst this example demonstrates feasibility, further work is clearly required to optimise this sequence.



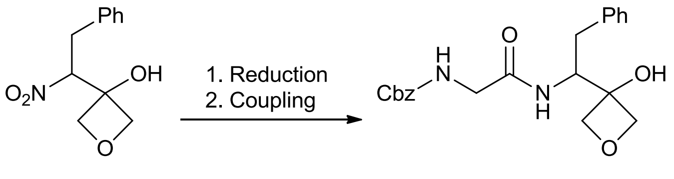
Scheme 2.28. Reduction and coupling of **207** to make protected tetrapeptide **235**.

The reduction and amide coupling conditions, whilst not optimal, do allow the nitro group of the conjugate addition products to be reduced and coupled with another *N*-protected amino acid. The best overall yields were achieved using indium or samarium(II) iodide for the reduction step.

2.2.5.2 Synthesis of Protected *C*-Terminus Peptidomimetics

The synthesis of *C*-terminus peptidomimetics involves the reduction of the nitro group in **166**, followed by amide coupling and deprotection, as for the mid-chain examples. Various conditions for the reduction and immediate amide coupling of

166 were investigated (Table 2.10). Reduction using Zn in AcOH and coupling with DCC gave **236** in ~46% yield (entry 1), but this was contaminated with the hydroxylamine derived by-product. Samarium(II) iodide reduction, combined with DCC coupling, gave impure product in a low yield (entry 2). Hydrogenation of **166** in AcOH, followed by amide coupling with either EDC or DCC gave **236** in similar yields (entries 3 and 4). Finally, changing the hydrogenation solvent from AcOH to methanol gave **236** in the highest yield (entry 5).

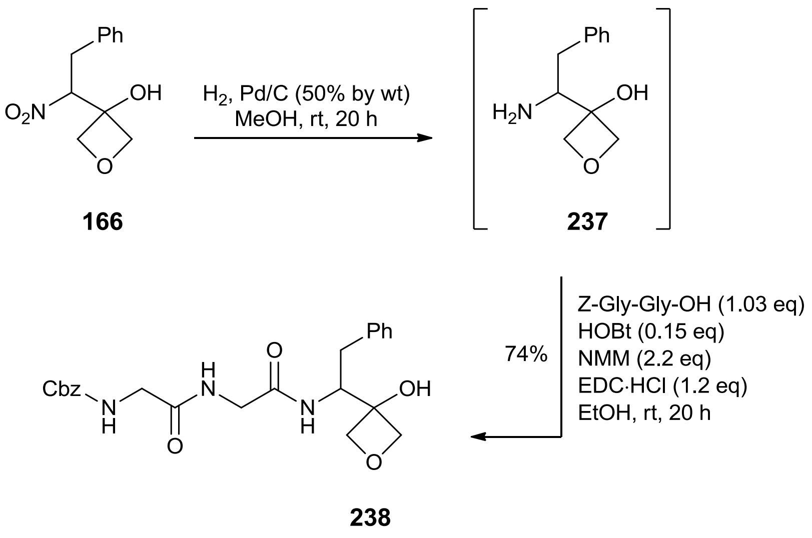


166 **236**

Entry	Reduction Conditions	Coupling Method[a]	Yield (%)
1	Zn (35 eq), AcOH, rt, 96 h	A, 18 h	~46[b]
2	SmI_2 (0.1 M, 14 eq), MeOH, rt, 20 min	A, 21 h	~15
3	Pd/C (50% by wt), H_2, AcOH, rt, 20 h[115]	A, 18 h	44
4	Pd/C (50% by wt), H_2, AcOH, rt, 20 h[115]	B, 5.5 h	42
5	Pd/C (50% by wt), H_2, MeOH, rt, 22 h	B, 17 h	65

Table 2.10. Reduction and coupling of **166**. [a]Method A: Z-Gly-OH (1 eq), DCC (1.1 eq), HOBt (1.1 eq), DIEA (1.1 eq), CH_2Cl_2, 0 ˚C, 1 h then rt; Method B: Z-Gly-OH (1.03 eq), EDC·HCl (1.2 eq), HOBt (0.15 eq), NMM (2.2 eq), EtOH, rt. [b]Hydroxylamine product co-eluted.

These optimised reaction conditions were also used to provide *C*-terminus oxetane tripeptide mimetic **238** in 74% yield (Scheme 2.29).



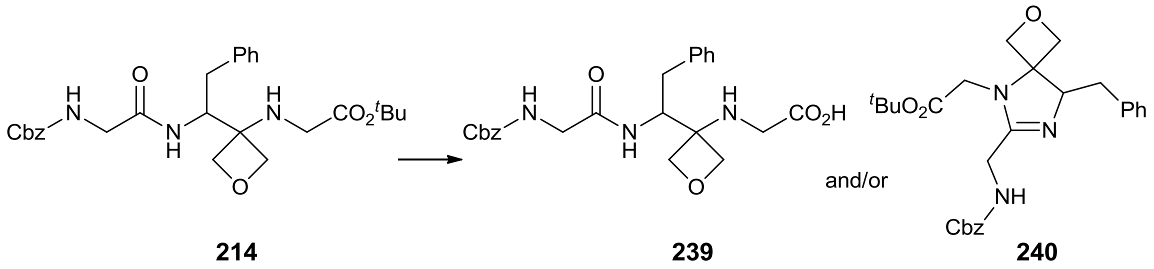
Scheme 2.29. Reduction and coupling of **166** to give tripeptide mimic **238**.

2.2.6 Deprotection to Free Peptidomimetics

With routes developed for the synthesis of peptidomimetics containing the oxetane ring at different positions along the peptide chain, the next step was to investigate the removal of the terminal protecting groups.

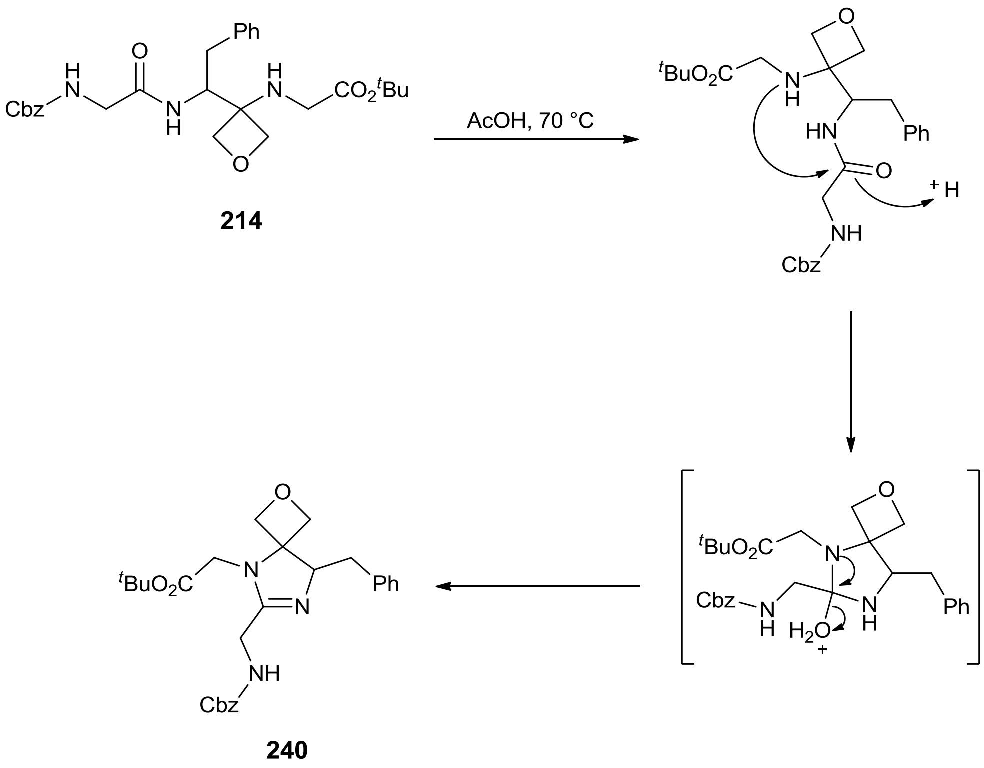
2.2.6.1 Attempted Removal of the *tert*-Butyl Ester

We focused our initial investigations into the hydrolysis of the *tert*-butyl ester group of **214**. From the outset, we were concerned about the potential susceptibility of the oxetane ring to the acidic conditions that might be needed to cleave this ester group.[11,116] Traditionally *tert*-butyl esters are removed using acids such as TFA[117] and, as 3,3-disubstituted oxetanes are thought to be relatively stable to TFA at lower temperatures,[116] we decided to initially explore these conditions (Table 2.11, entries 1 and 2). When the reaction was performed at 0 °C, the starting material was not consumed (according to TLC), but at rt a mixture of unknown products was formed. During the cleavage of the *tert*-butyl ester, a *tert*-butyl cation is formed that can potentially alkylate any nucleophilic sites within the molecule.[118] As the peptidomimetic contained a secondary amine we decided to perform the deprotection with TFA in the presence of triethylsilane (TES) as a carbocation scavenger.[119] Unfortunately these conditions failed to give **239** either at 0 °C or rt (entries 3 and 4). As carboxybenzyl groups are reported to be removed under acidic conditions, we were concerned that the TFA could be opening the oxetane ring and/or removing the *N*-protecting group. Therefore, the next step was to try the deprotection using milder conditions. Using formic acid, only **214** and unknown products were observed (entry 5). Work done in the group has demonstrated that the oxetane ring is stable to heating in AcOH[120] and so we decided to explore the use of this acid (entry 6).[121] Deprotection in acetic acid at rt gave no reaction, as determined by TLC, but upon heating the starting material was fully consumed. However, it was determined through mass spectrometry (m/z = 480 $[M+H]^+$) and NOE experiments that the product observed was not **239**, but **240**, resulting from cyclisation of the secondary amine onto the carbonyl group of the amide bond (Scheme 2.30). Heating the reaction obviously promotes this cyclisation as **240** was not formed at rt. Attempts to remove the *tert*-butyl group with silica gel in refluxing toluene[122] (entry 7) gave **214** as well as **240** (2.6 : 1). Finally, we investigated the use of phosphoric acid to deprotect the *tert*-butyl ester at rt (entry 8).[123] Unfortunately, **214** had poor solubility in toluene so a mixture of CH_2Cl_2 and MeOH had to be added to the reaction mixture. ESI-MS indicated possible product formation (m/z = 464 $[M+Na]^+$), but the crude ^{1}H NMR was messy and inconclusive.



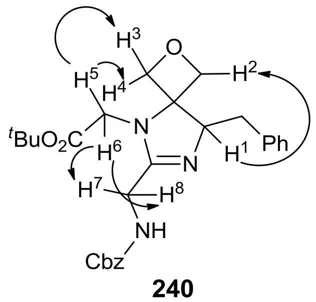
Entry	Reaction Conditions	Product(s)
1	TFA/CH_2Cl_2 (1 : 1), rt, 5 h[124]	0%[a]
2	TFA/CH_2Cl_2 (1 : 4), 0 °C → rt, 5 h[125]	0%[a]
3	TES (10 eq), TFA/CH_2Cl_2 (1 : 2.5), 0 °C → rt, 5 h[119]	0%[a]
4	TES (11 eq), TFA/CH_2Cl_2 (1 : 1), 0 °C, 48 h[119]	0%[a]
5	HCO_2H, rt, o/n[126]	**214** and unknown
6	AcOH, 70 °C, 5.5 h[121]	**240** (~68%)[b]
7	Silica gel (1 eq), toluene, reflux, 90 min[122]	**214** and **240**[c]
8	H_3PO_4 (5 eq), toluene, CH_2Cl_2/MeOH (5 : 1), rt, 17 h[123]	**239** and unknown[d]

Table 2.11. Investigation into the hydrolysis of the *tert*-butyl ester group of **214**. [a]Unknown products formed. [b]Observed in crude ^{1}H NMR. [c]**214** : **240** in 2.6 : 1.0 ratio according to ^{1}H NMR. [d]Based on ESI-MS.



Scheme 2.30. Proposed mechanism for the formation of **240**.

Evidence for **240** was primarily based on NOE experiments that showed irradiation of H-1 caused an enhancement of the oxetane H-2, whilst irradiation of the CH_2 protons (H-5/H-6) gave an enhancement of the oxetane protons (H-3 and H-4) as well as H-7 and H-8 (Figure 2.14). The enhancement of H-7 and H-8, caused by irradiation of H-5 and H-6 is particularly indicative of the formation of **240**, as this would not be expected if the compound was **214** or **239**.



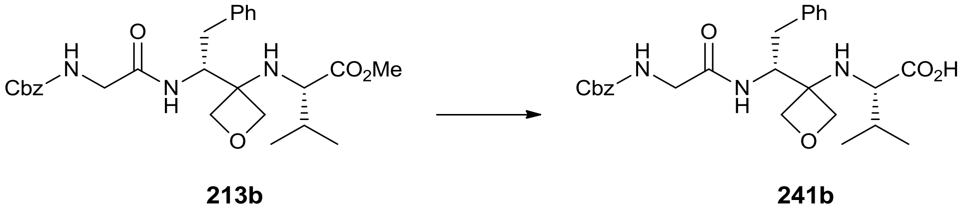
240

Figure 2.14. NOE enhancements in **240**.

These results showed that the *tert*-butyl ester was not a suitable protecting group, despite an improved resistance towards cyclisation, as it was difficult to remove in the presence of the oxetane ring and the secondary amine. Therefore we decided to focus our attentions on the deprotection of the methyl ester of **213a** and **213b** instead.

2.2.6.2 Attempted Removal of the Methyl Ester

Basic conditions for the hydrolysis of the methyl ester of **213b** were investigated and the results are shown in Table 2.12. The hydrolysis of the methyl ester using different bases failed to give **241b**. Even conditions that had been successfully used previously to remove a methyl ester from an *N*-carboxybenzyl protected Gly-Gly dipeptide failed to give **241b** (entry 2). One possible explanation for this is the steric bulk of the isopropyl group blocking nucleophilic attack.

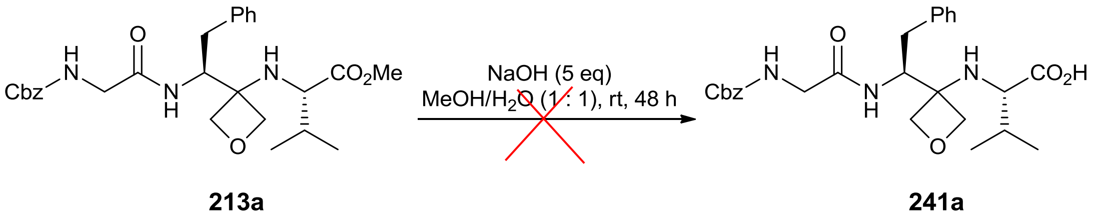


stereochemistry arbitrarily assigned

Entry	Reaction Conditions	Product[a]
1	LiOH·H_2O (6 eq), THF/MeOH/H_2O (3 : 1 : 1), 0 °C → rt, 89 h[127]	Unknown
2	LiOH (2 eq), H_2O_2 (5 eq), THF/H_2O (3 : 1), 0 °C → rt, 17 h[128-129]	**213b**
3	K_2CO_3 (1.5 eq), MeOH/H_2O (1 : 1), rt, 20 h[130]	Unknown
4	LiOH·H_2O (5 eq), THF/H_2O (1 : 1), reflux, 15 h	Unknown

Table 2.12. Investigation into the hydrolysis of the methyl ester of **213b**. [a]According to crude ^{1}H NMR.

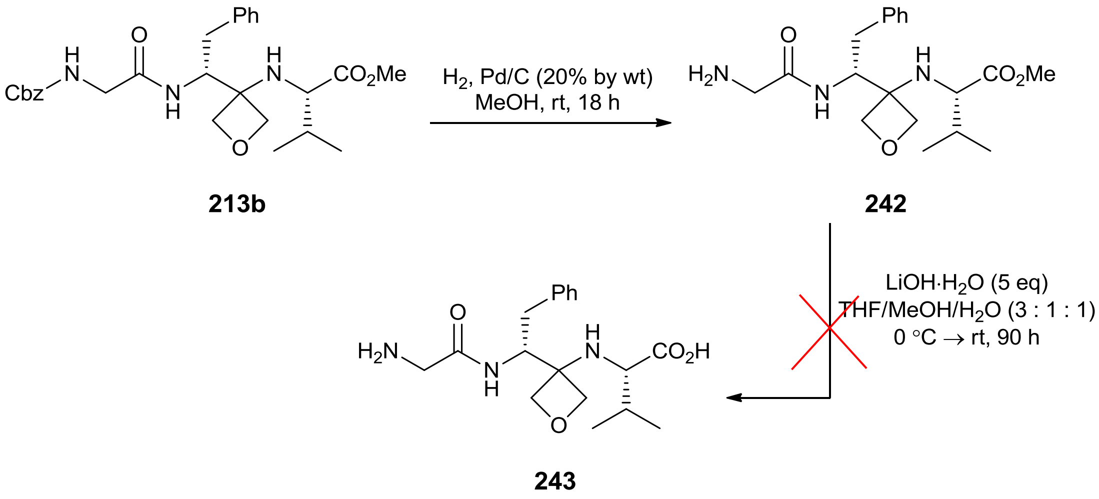
Attempts to hydrolyse the other diastereomer, **213a**, with NaOH also failed (Scheme 2.31).



stereochemistry arbitrarily assigned

Scheme 2.31. Attempted hydrolysis of the methyl ester group in **213a**.

Removing the *N*-protecting group before attempted hydrolysis of the methyl ester failed to generate **243**, despite ESI-MS evidence to suggest the amine **242** (*m*/*z* = 364 $[M+H]^+$, 386 $[M+Na]^+$) had been successfully formed (Scheme 2.32).



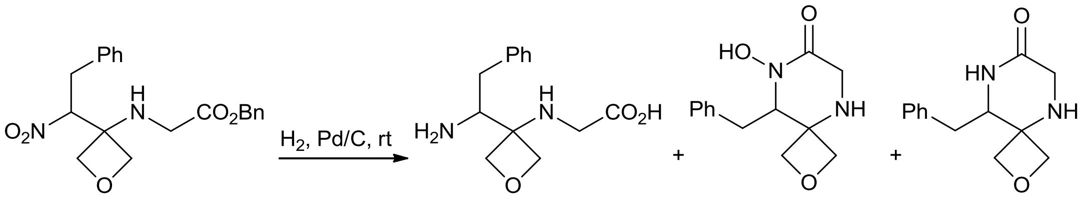
Scheme 2.32. Attempted hydrogenation and hydrolysis of **213b**.

The difficulties encountered removing the methyl ester protecting group, as well as the tendency of the methyl ester analogues to undergo cyclisation, led us to ultimately discount the use of this ester.

2.2.6.3 Hydrogenation of Nitro and Benzyl Ester Groups

Next, the hydrogenation of oxetane **199** was explored with a view to providing dipeptide derivatives. The results are presented in Table 2.13. It was demonstrated that hydrogenation could be used to reduce the nitro group and remove the benzyl ester concurrently. However, the cyclised side-products, **245** and **209**, were often observed in the crude ^{1}H NMR, sometimes along with **244**. This suggested that the nitro group was being reduced faster than the benzyl ester was cleaved. Diketopiperazine **209** could arise from further reduction of **245**, or alternatively, arise from cyclisation of amine **244**. Resubjecting **245** to the reaction conditions led to **209**, suggesting that the former pathway may be in operation.

The hydrogenations were initially performed in the presence of acid, in order to prevent the amine poisoning the palladium catalyst.[131] However, ring-closure was observed when acid was present (Table 2.13, entries 1-4). When methanol was used as the solvent, a mixture of products was obtained, including **244** (entries 5-6). When no acid was present, a larger proportion of Pd/C was required to compensate for inhibition by the amine. Attempts to purify **244** using reverse-phase HPLC gave a mixture of **244** and increased amounts of **209**, likely due to the use of formic acid in the eluent. Unfortunately, on account of these difficulties, the dipeptide derivatives were not isolated.

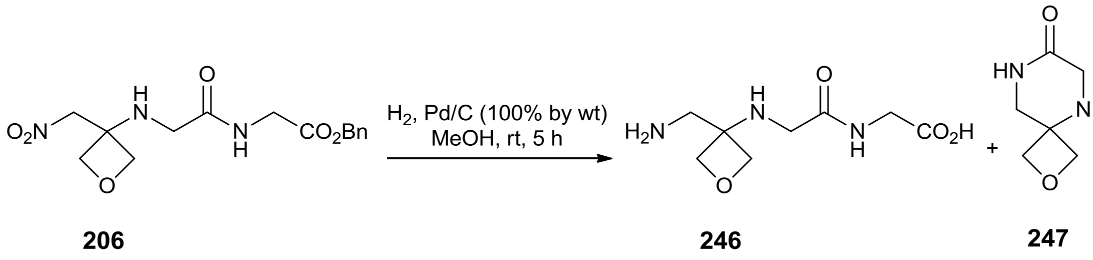


199 **244** **245** **209**

Entry	Reaction Conditions	Product(s)[a]
1	Method A, Pd/C (50% by wt), 2 M, 20 h	**245** and **209** (3.2 : 1)
2	Method A, Pd/C (75% by wt), 0.2 M, 21 h	**245** and **209** (3.3 : 1)
3	Method B, Pd/C (30% by wt), 0.01 M, 17 h	**245** and **209** (2.7 : 1)
4	Method B, Pd/C (50% by wt), 0.1 M, 20 h	**245** and **209** (1.1 : 1)
5	Method C, Pd/C (100% by wt), 0.05 M, 22 h	**244** and **209** (1.4 : 1)
6	Method C, Pd/C (50% by wt), 0.1 M, 21 h	**244**, **245** and **209** (4.5 : 1 : 1.2)

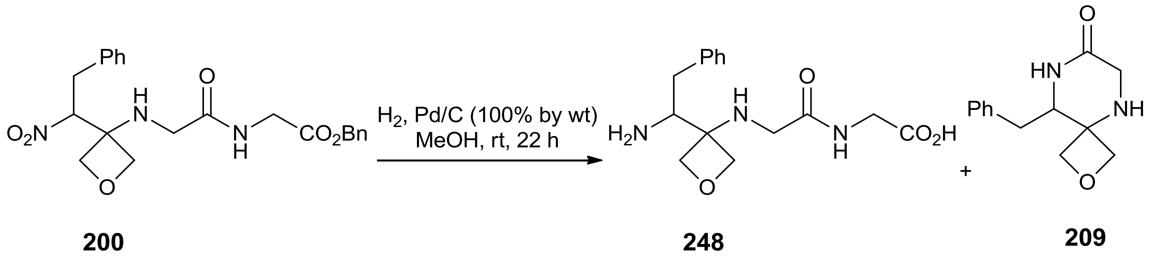
Table 2.13. Hydrogenation conditions to access dipeptide isosteres. [a]As observed by ^1H NMR. Method A: H_2, Pd/C, AcOH, rt. [115] Method B: H_2, Pd/C, AcOH/EtOAc/MeOH (1 : 2 : 1), rt.[132] Method C: H_2, Pd/C, MeOH, rt.[133]

Next, we explored the hydrogenation of longer chain nitro compounds to access *N*-terminus peptidomimetics. Initial attempts to hydrogenate **206**, a Gly-Gly-Gly analogue, resulted in a poor mass recovery and a mixture of 2 compounds, believed to be **246** and **247**, as judged by the ^1H NMR (Scheme 2.33).



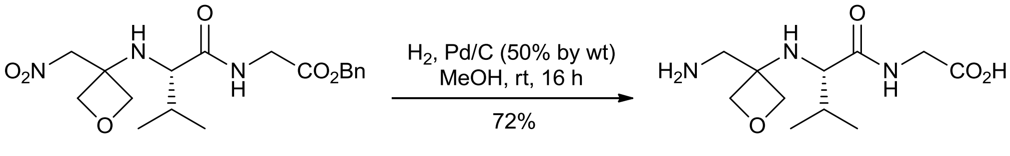
Scheme 2.33. Hydrogenation of **206**.

As the glycine residues in **206** could promote the cyclisation, we decided to explore the hydrogenation of analogues containing substituents at different positions on the chain. Hydrogenation of **200** under standard conditions gave primarily **248**, as observed by crude ^{1}H NMR, as well as some impurities (Scheme 2.34). Purification by reverse-phase HPLC, however, resulted in conversion of **248** to **209**, catalysed by the presence of formic acid in the eluent. This implied the amine had cyclised onto the amide bond, eliminating glycine. Based on our previous work optimising the reduction of the nitro group, we decided to introduce an L-valine residue into the tripeptide in order to minimise this cyclisation.



Scheme 2.34. Hydrogenation of **200**.

Pleasingly, hydrogenation of **207** gave peptidomimetic **249** in 72% yield (Scheme 2.35). The presence of the isopropyl group presumably providing sufficient steric hindrance to minimise cyclisation. This chemistry could therefore provide easy access to a range of *N*-terminus peptidomimetics, of varying chain length, provided the amide bond is protected from nucleophilic attack by adjacent substituents.



207

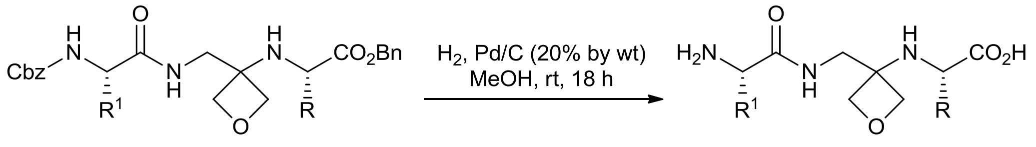
249

Scheme 2.35. Hydrogenation of **207**.

This work demonstrated that the benzyl ester is readily cleaved without harsh conditions that could affect the oxetane ring. Once we had established a route to tripeptides containing an oxetane at the *N*-terminus, we focused our attention on the concomitant removal of the *N*-carboxybenzyl and benzyl ester groups to furnish mid-chain oxetane-containing peptidomimetics.

2.2.6.4 Concomitant Removal of Cbz and Benzyl Ester Groups

Deprotection of compounds **216**, **225**–**229** proceeded smoothly using standard hydrogenolysis conditions[134] to give the oxetane peptidomimetics in excellent yields (Table 2.14).



216, **225-229** **250-255**

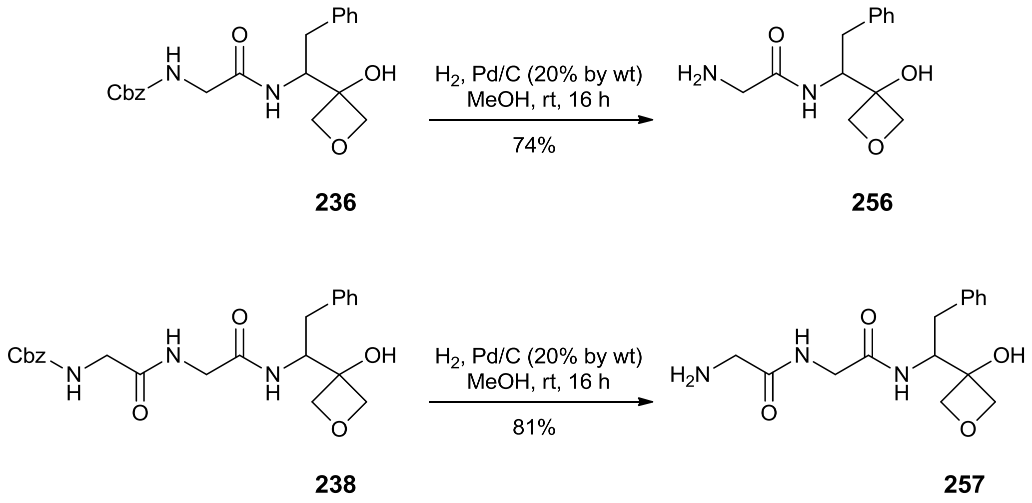
Entry	Substrate	R^1	R	Product	Yield (%)
1	**216**	H	^{i}Pr	**250**	84
2	**225**	Bn	^{i}Pr	**251**	96
3	**226**	Bn	CH(Me)Et	**252**	96
4	**227**	Me	^{i}Pr	**253**	100
5	**228**	^{i}Pr	^{i}Pr	**254**	94
6	**229**	$CH_2{}^i$Pr	CH(Me)Et	**255**	99

Table 2.14. Deprotection to give peptidomimetics **250-255**.

The high yields obtained from the removal of the *N*-carboxybenzyl and benzyl ester protecting groups led us to consider these as the optimal protecting groups for this chemistry, especially when sterically bulky amino esters were used.

2.2.6.5 Removal of Cbz Group to afford *C*-Terminus Peptidomimetics

The final step in the synthesis of the *C*-terminus oxetane-containing peptidomimetics was the removal of the *N*-carboxybenzyl protecting group. This was achieved under standard hydrogenolysis conditions to give dipeptide **256** and tripeptide **257** in high yields (Scheme 2.36).



Scheme 2.36. Deprotection of **236** and **238**.

Therefore an easy route to peptidomimetics containing an oxetane at the *C*-terminus has been developed. Longer chain peptide isosteres should be readily accessible *via* standard homologation of the *N*-terminus using conventional peptide chemistries.

2.2.7 Structural Investigations

In collaboration with Dr Rebecca Notman of the University of Warwick, molecular dynamics simulations of **255** were run, along with simulations of the parent peptide **258** (Figure 2.15). This model system was chosen as **258** has shown activity as an angiotensin I-converting enzyme (ACE) inhibitor and it would be interesting to compare the effect of the oxetane on its properties.[135]

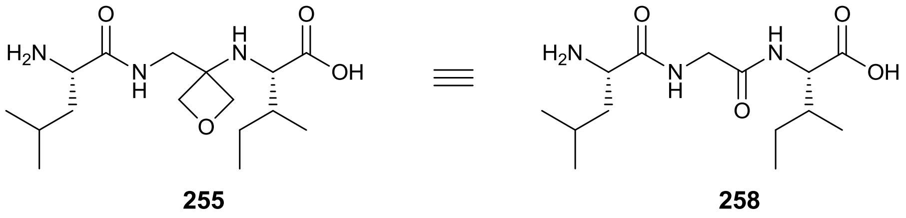
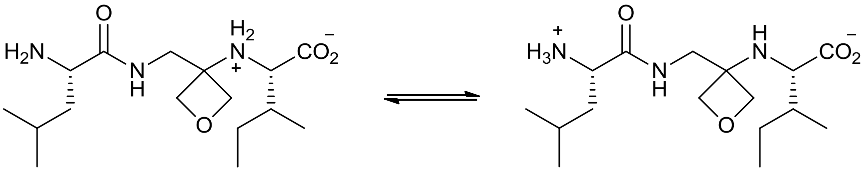


Figure 2.15. Peptidomimetic **255** and its parent peptide **258**.

We were interested in exploring how the replacement of an sp^2 hybridised carbon centre in the natural peptide with an sp^3 hybridised 3-aminooxetane unit would affect the conformation of the peptidomimetic. We were also concerned as to whether the presence of the secondary amine within the peptide backbone might influence the behaviour of the molecule, for example by competitive protonation in the zwitterionic form (Scheme 2.37).



Scheme 2.37. Competitive protonation in **255**.

CHARMM-compatible forcefield parameters for the 3-aminooxetane residue were first derived from quantum mechanical calculations. Each peptide was then simulated for 100 ns in water at 500 K and from this trajectory 10 distinct conformations of each peptide were selected for an additional 100 ns of simulation at 300 K, to yield a total simulation time of 1 μs per peptide. Cluster analysis was performed using 100000 structures extracted from the trajectories at 10 ps intervals.

The simulations showed that there were eleven distinct structures for the peptidomimetic **255** compared to seven for the peptide **258**. These results suggested that the oxetane-containing peptidomimetic has greater conformational flexibility than the traditional peptide, as might be expected on account of the substitution of a rigid amide bond with a 3-aminooxetane unit.

Snapshots of the two most populated clusters for **255** and **258** are shown in Figure 2.16.

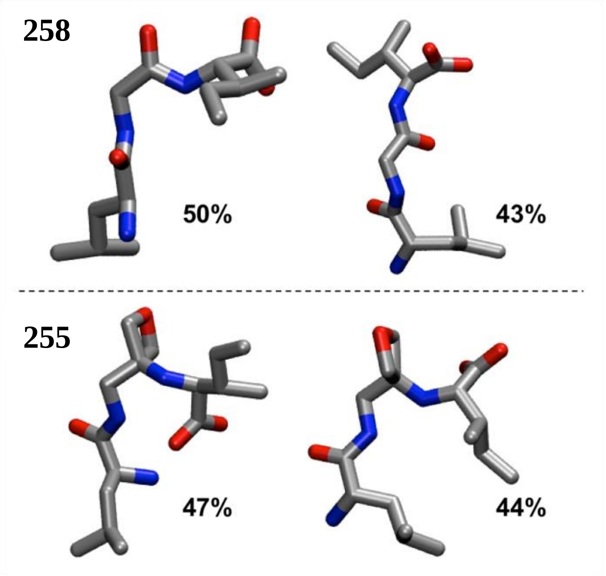


Figure 2.16. Snapshots of the two most populated clusters of **258** (top) and **255** (bottom) showing the percentage of total structures accounted for by the cluster. Hydrogen atoms have been omitted for clarity. Snapshots were generated using the Visual Molecular Dynamics software.[136]

The natural tripeptide **258** adopts conformations in which the C and N termini are separated by > 7 Å. In contrast, the oxetane peptidomimetic spends more time in a folded conformation with a C-N separation distance of 3-4 Å, presumably driven by the electrostatic interaction of the terminal $–CO_2^-$ and $–NH_3^+$ ions in this arrangement (Figure 2.17). The flexibility of the peptidomimetic, arising from the change in hybridisation, allows formation of these folded conformations, which could in turn give rise to turn-like behaviour in longer chain peptidomimetics.

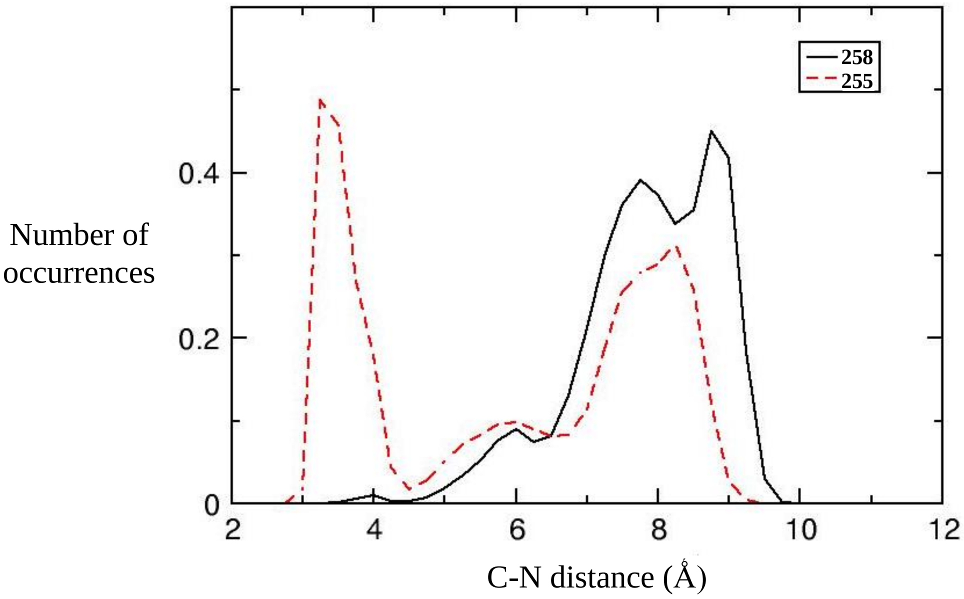


Figure 2.17. Normalised distribution of the distance between the C-terminal C atom and the N-terminal N atom obtained from MD simulations in solution.

Having synthesised these oxetane peptidomimetics, we also wanted to determine their conformations in solution, to compare them to the results of the molecular dynamics simulations. Consequently, NOESY and ROESY experiments of **253**, **254** and **255** were run. The NOESY and ROESY spectra for **255** are presented in Figures 2.18 and 2.19, whilst those for **253** and **254** are in Appendix II. However, there was no discernible interaction between the two side chains present in the molecules. This means that the molecules are likely not, in solution, spending appreciable amounts of time in folded conformations where these side chains would be in close contact.

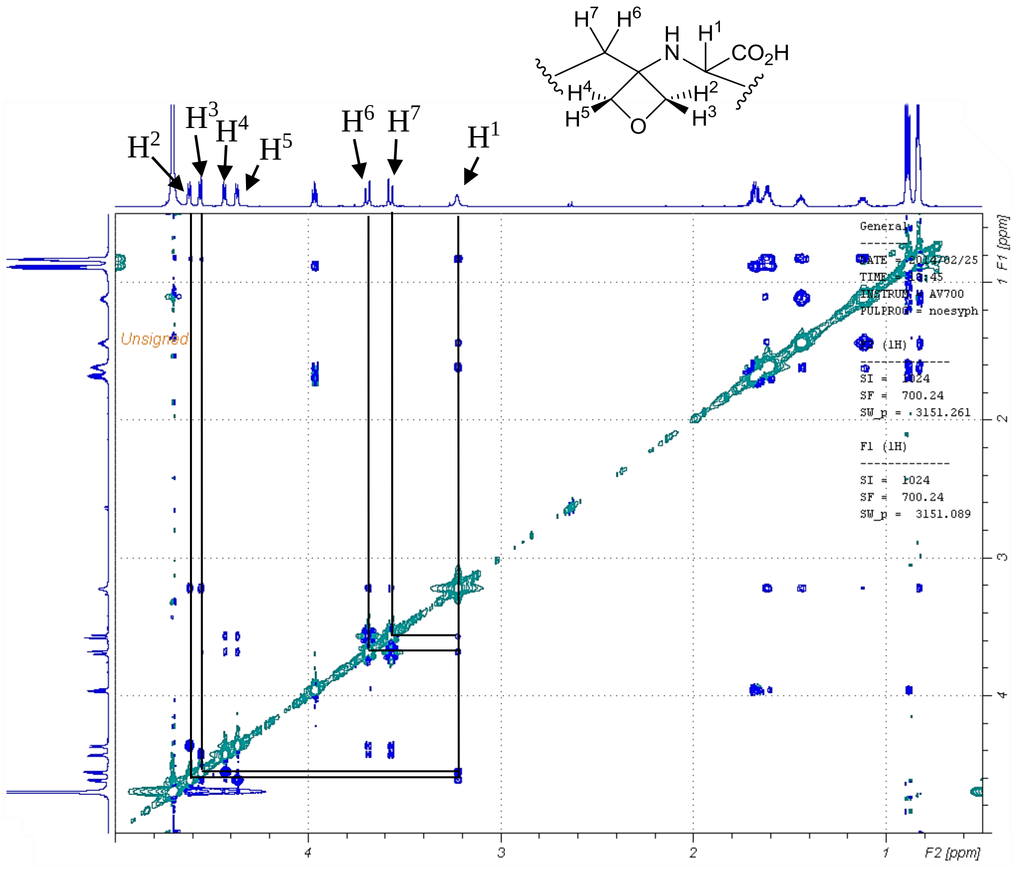


Figure 2.18. NOESY spectrum of **255**.

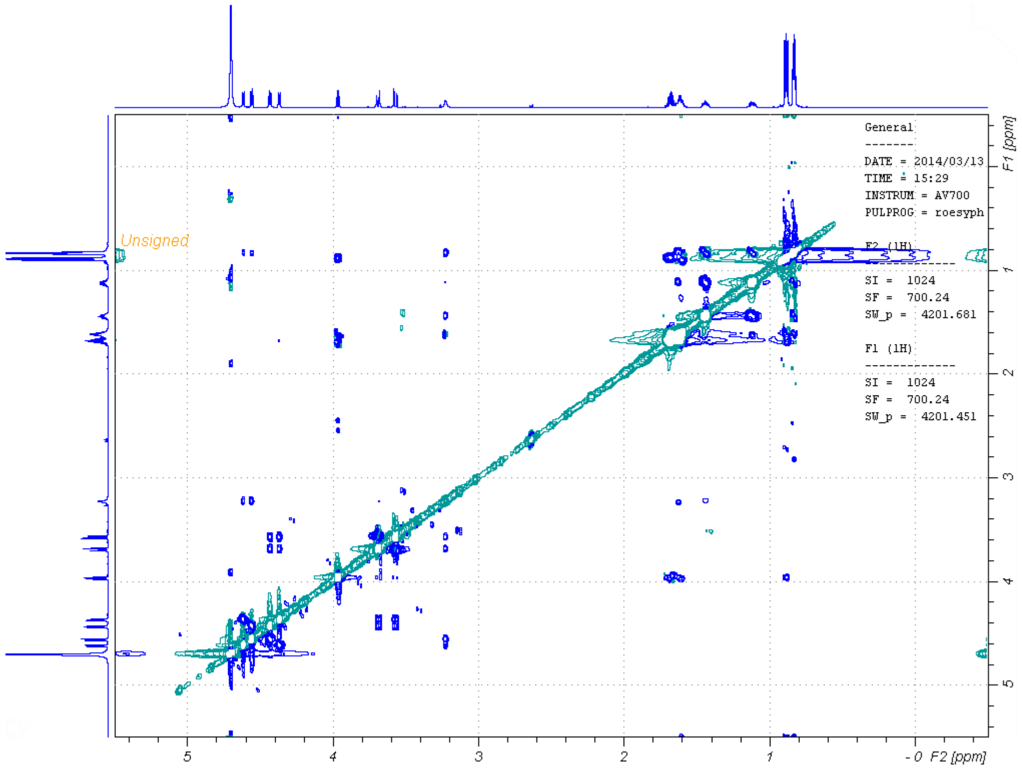


Figure 2.19. ROESY spectrum of **255**.

Fortunately, we were able to grow crystals of **252** and **255** from methanol and determine the solid state structure of these peptidomimetics using X-ray crystallography (Figures 2.20 and 2.21).

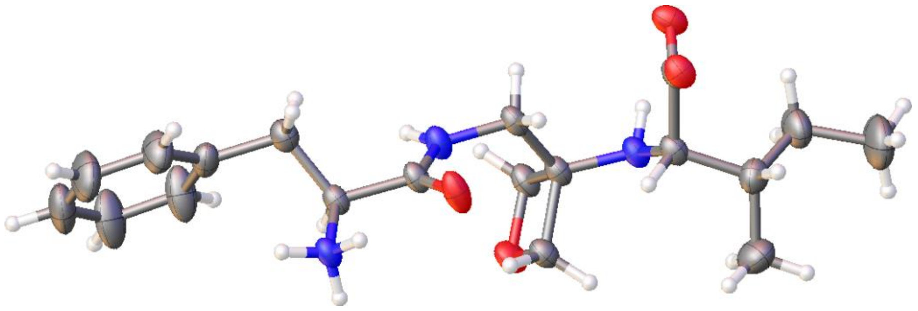


Figure 2.20. Crystal structure of **252**.

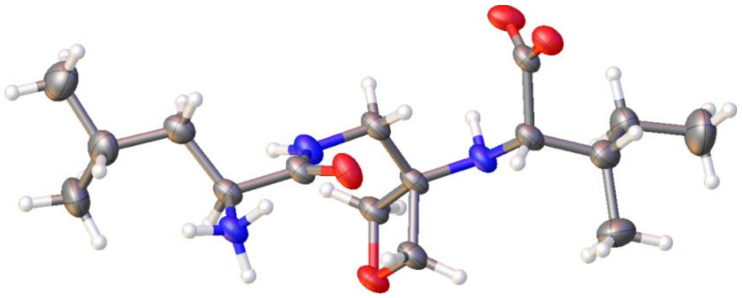


Figure 2.21. Crystal structure of **255**.

The crystal structures confirmed that both **252** and **255** were zwitterionic and the secondary amine was not protonated in the solid state. Both peptidomimetics also showed an antiparallel sheet-like arrangement, which was interesting as molecular dynamics simulations had shown the peptidomimetic adopting a folded conformation, to a significant extent, in water (Figures 2.22 and 2.23). The antiparallel sheet arises from hydrogen bonds between the amides, carboxylates, ammonium ions and secondary amines in the antiparallel oxetane tripeptide mimics.

The oxetane oxygen, however, is approximately orthogonal to this hydrogen-bonded sheet and is involved in hydrogen bonding outside of the plane of the sheet.

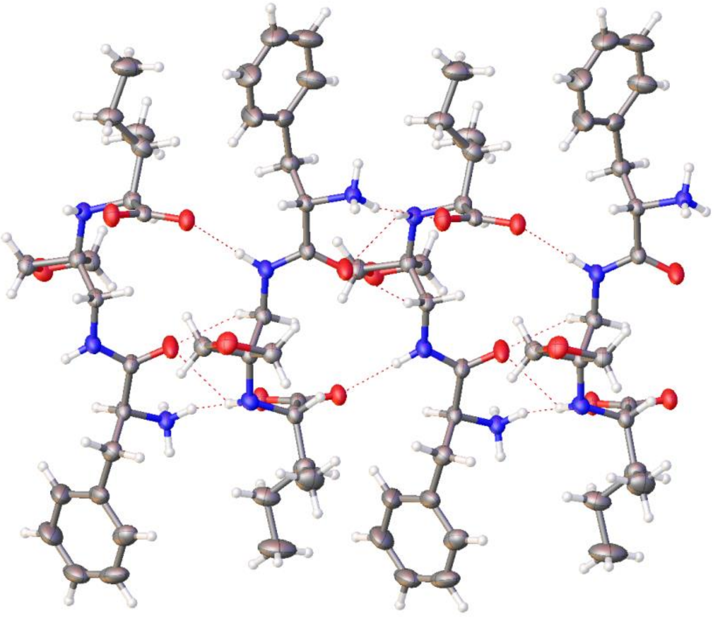


Figure 2.22. Antiparallel sheet arrangement of **252** in the solid state.

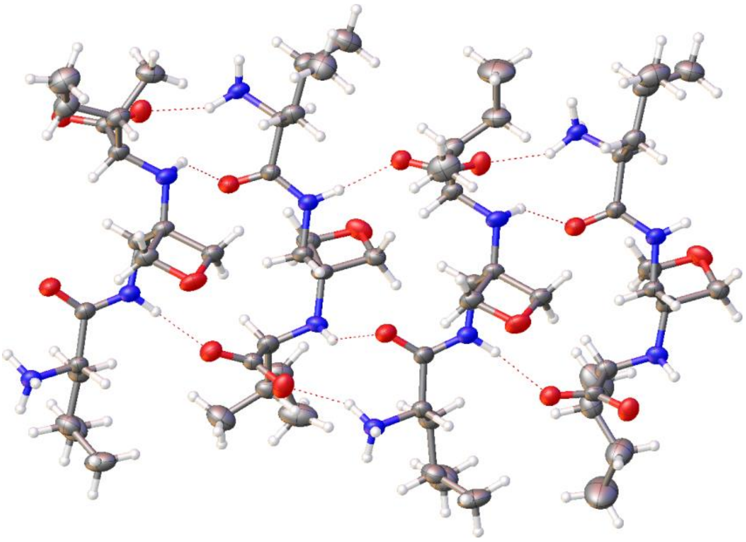


Figure 2.23. Antiparallel sheet arrangement of **255** in the solid state.

The presence of the oxetane ring also had a notable effect on the C-N-C_{ox}-C torsional angle in the *pseudo*-tripeptides due to the pyramidal nature of the nitrogen atom (Figure 2.24). For **252**, $\bar{\omega}$ = 55.7° and for **255**, the C-N-C_{ox}-C torsional angle was 60.2° (values derived from the average of all molecules in the unit cell). This is in comparison to a traditional peptide that has a trigonal nitrogen and ω = ~180°.

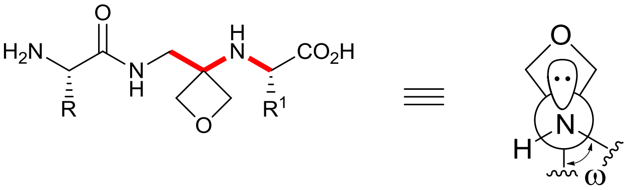


Figure 2.24. Torsional angle (ω) in oxetane-containing peptidomimetics.

CD spectroscopy on the oxetane-containing peptidomimetics in water (Figure 2.25) showed that when the oxetane was in the mid-chain position (**250**–**255**), there was no evidence of conventional peptide secondary structure elements, such as α-helices, β-sheets or turns.[56] In fact, secondary structures such as α-helices are not seen in tripeptides as they are stabilised by a hydrogen bond between the carbonyl oxygen of one residue (n) and the nitrogen of the amide four residues along the chain (n+4).[137] However, when the oxetane was at the *N*-terminus, as in **249**, the peptidomimetic showed indications of β-sheet formation. This is because the negative peak that occurs near 217 nm and the positive peak at around 195 nm are characteristic of β-sheets spectra.[138-139] As there are two sequential amino acid residues present in **249**, it could be that these impart rigidity to the peptide chain, decreasing its flexibility compared to the peptidomimetics containing the oxetane in the mid-chain position and therefore enabling it to adopt a sheet-like structure normally seen in a traditional peptide. If any of the peptidomimetics formed α-helices in solution, two negative peaks of similar intensity would be observed at ~222 nm and ~208 nm, as well as a positive peak at ~192 nm of higher intensity.[138-139]

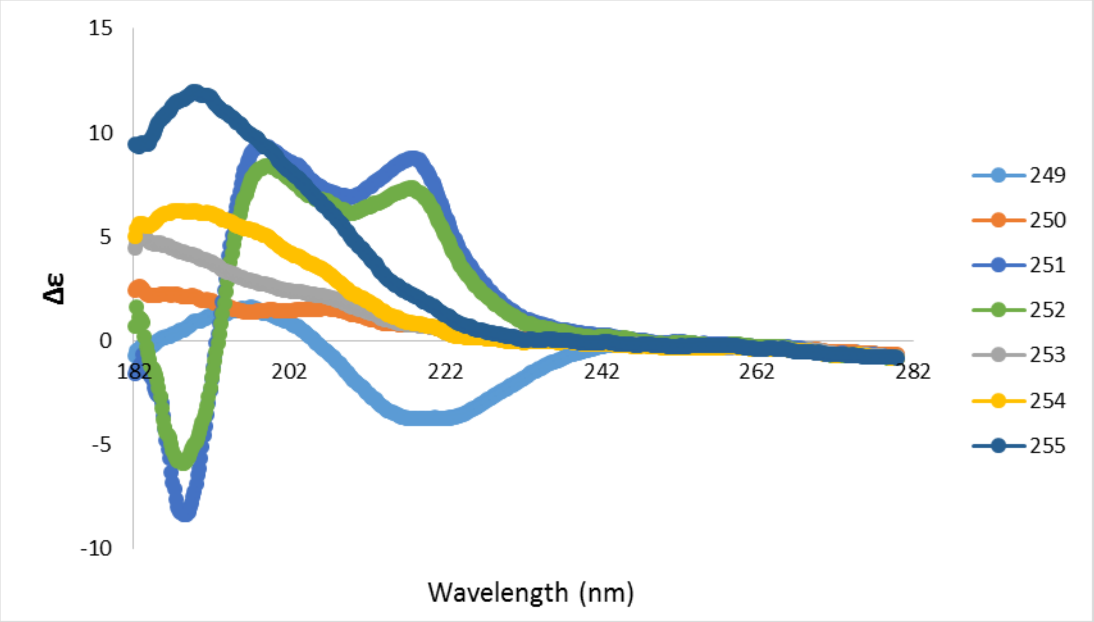


Figure 2.25. CD spectroscopy of oxetane peptidomimetics **249**–**255**.

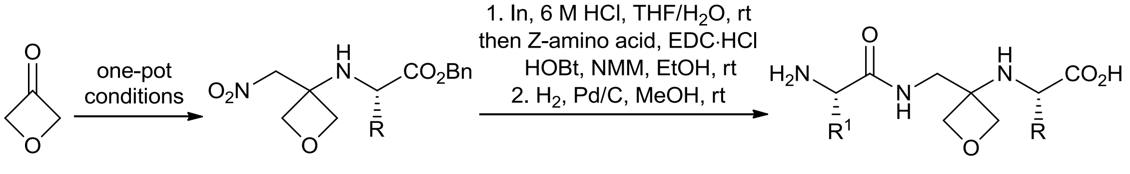
Our initial structural investigations indicate that replacement of a carbonyl bond with an oxetane ring in a tripeptide does have a significant effect on the properties. The increased flexibility resulting from the change in hybridisation of the carbon from sp^2 to sp^3 allows the oxetane peptidomimetic to adopt a wider range of conformations in water, as evidenced by molecular dynamics simulations. However, the peptidomimetic remains zwitterionic like a natural tripeptide and can produce antiparallel sheet-like structures in the solid state, along similar lines to conventional peptides.

2.3 Conclusions

In summary, we have developed routes towards the synthesis of peptidomimetics containing an oxetane as a carbonyl bond isostere at the *N*-terminus, *C*-terminus and mid-chain from commercially available 3-oxetanone.[140]

A range of peptidomimetics containing different amino acid residues have been synthesised. The initial conjugate addition between a nitroalkene (**162** or **164**) and an amino ester has been successfully demonstrated, as well as more efficient, higher yielding one-pot reactions to give the conjugate addition product directly from 3-oxetanone. This one-pot reaction has been performed with a range of amino and dipeptide esters, including polar and hydrophobic amino esters, in moderate to good yields (Schemes 2.38 and 2.39).

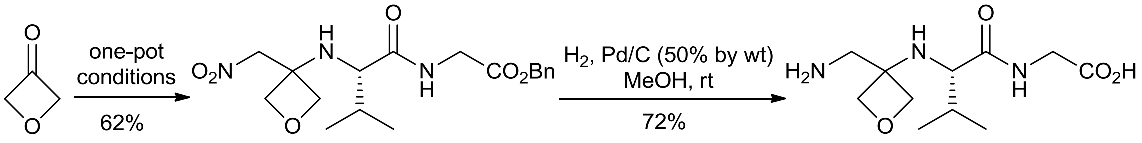
The optimal ester protecting group for the synthesis of mid-chain oxetane-containing peptidomimetics was found to be the benzyl ester. The reduction of the nitro group proceeded best when a branched substituent was present on the α-carbon of the amino ester in order to minimise competitive cyclisation to the DKP derivatives. Nitro reduction using indium in aqueous HCl, followed immediately by an amide coupling gave the best results and, using this method, a range of protected peptidomimetics were synthesised in good yields (Table 2.9, p.106). Hydrogenation to remove the protecting groups gave the final peptidomimetics in excellent yields (Table 2.14, p.119).



194: R = ^{i}Pr 83%
195: R = Bn 73%
198: R = H 44%
203: R = $CH_2{}^i$Pr 59%
204: R = CH(Me)Et 52%
205: R = CH(OH)Me 56%

250: R = ^{i}Pr, R^1 = H 41%
251: R = ^{i}Pr, R^1 = Bn 58%
252: R = CH(Me)Et, R^1 = Bn 56%
253: R = ^{i}Pr, R^1 = Me 44%
254: R = ^{i}Pr, R^1 = ^{i}Pr 44%
255: R = CH(Me)Et, R^1 = $CH_2{}^i$Pr 53%

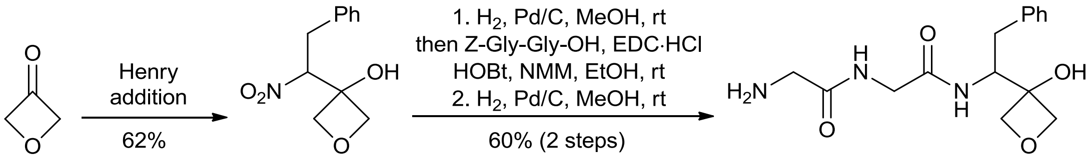
Scheme 2.38. Routes to mid-chain oxetane peptidomimetics using the benzyl ester protecting group.



5 **207** **249**

Scheme 2.39. Route to *N*-terminus oxetane peptidomimetic **249**.

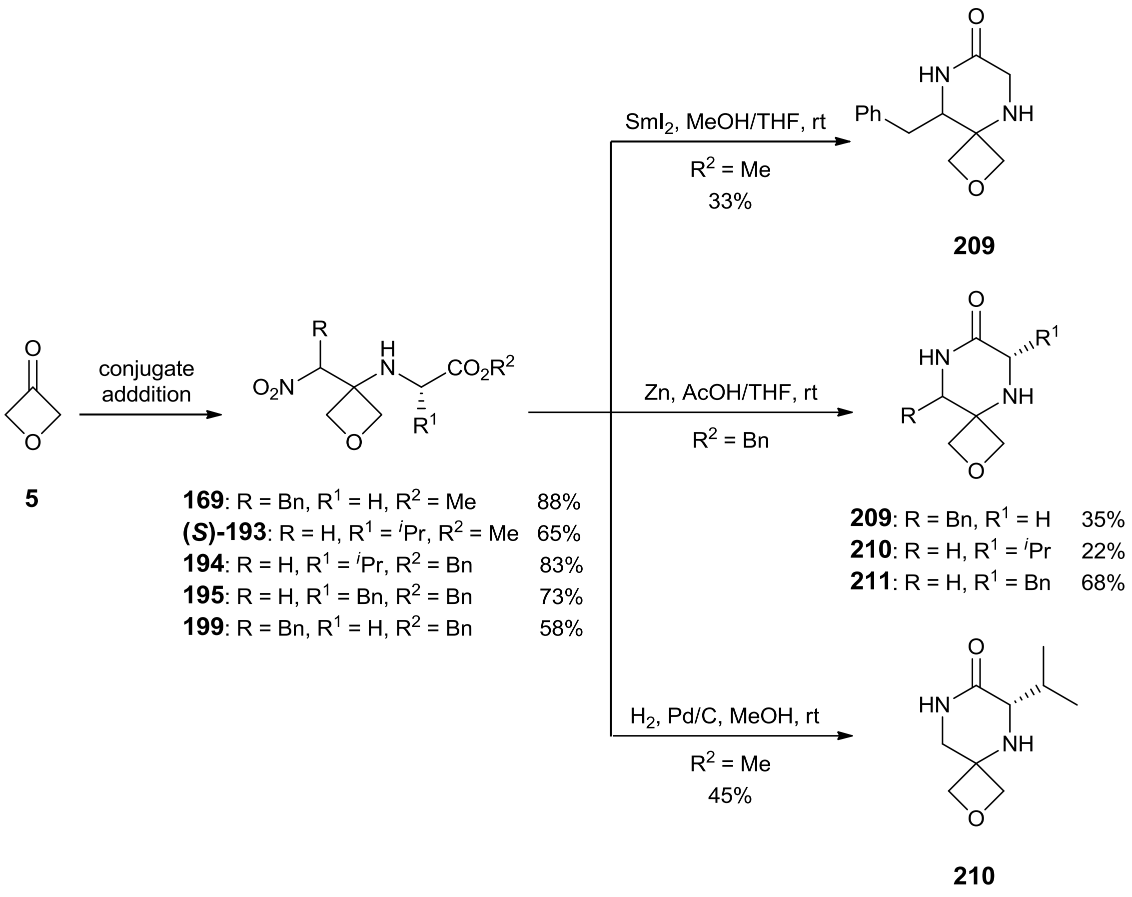
Where there was no potential for cyclisation, reduction of the nitro group occurred readily under standard hydrogenation conditions and amide coupling to give the protected *C*-terminus peptidomimetic **257** in good yield (Scheme 2.40).



5 **166** **257**

Scheme 2.40. Route to *C*-terminus oxetane peptidomimetic **257**.

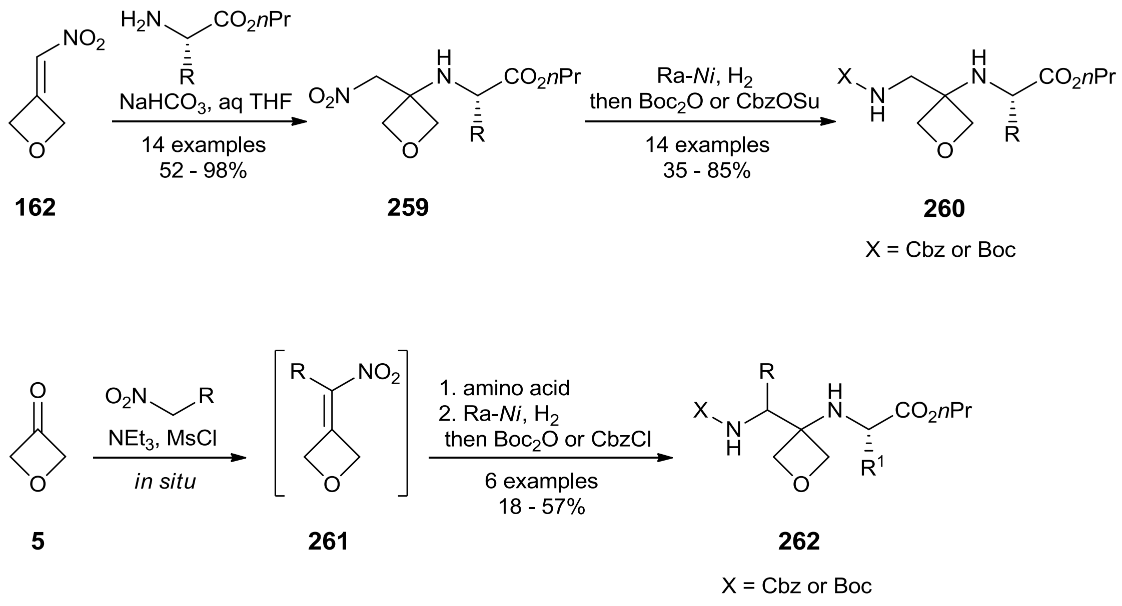
Cyclisation to the oxetane-containing diketopiperazine was promoted in acidic conditions and examples of these pharmaceutically interesting molecules have been synthesised (Scheme 2.41).



Scheme 2.41. Routes developed to oxetane-containing diketopiperazines.

Exploration of the structures of these oxetane peptidomimetics using X-ray crystallography, CD spectroscopy and molecular dynamics simulations provided interesting insights. When the oxetane is in the middle of a tripeptide chain, CD spectroscopy indicated that there is no dominant structure in solution, although in the solid state they appear to form antiparallel sheet-like arrangements, as evidenced by the crystal structures of **252** and **255**. The crystal structures also showed the formation of conventional zwitterions rather than by protonation of the secondary amine. Molecular dynamics simulations indicate that these oxetane peptidomimetics have a greater flexibility than their analogous tripeptides and favour folded conformations, presumably driven by electrostatic interactions and the change in hybridisation at the central “peptide” bond. Interestingly, when the oxetane is at the *N*-terminus, CD spectroscopy provided some evidence of β-sheet formation.

The development of oxetane-containing peptidomimetics is a rapidly evolving area of research. Recently, appearing in print shortly after our initial findings had been published,[140] Carreira and co-workers have demonstrated a strategy to accessing protected oxetane-containing peptidomimetics closely related to that described in this thesis.[141] These authors established a similar synthetic route to oxetane-containing *pseudo*-dipeptides such as **260** through conjugate addition of the amino ester in DMSO to **162**, followed by nitro reduction with Raney Ni and immediate protection of the resultant amine. As well as polar and lipophilic side chains, these authors also had success using protected amino esters, including derivatives of L-tyrosine, L-serine, L-glutamic acid, and L-asparagine. Interestingly, they reported that the use of the *n*-propyl esters gave higher yields than the corresponding methyl or benzyl esters. The reaction also worked with unprotected amino acids, proceeding in similar yields. Similar strategies have been developed to access oxetane peptidomimetics such as **262**, although the ability to separate the diastereomers was dependent on the substrate. Subsequent cleavage of either the *N*-protecting group or the ester, followed by a standard amide coupling to elongate the peptide chain was further established. Carreira did not report any structural insights into the folding of the resulting peptidomimetics.[141]



Scheme 2.42. Synthetic routes to oxetane-containing peptidomimetics developed by Carreira *et al.*[141]

This complementary work can only help to expand our knowledge of, and access to, oxetane-containing peptidomimetics.

2.4 Future Work

Due to time constraints, we were unable to investigate fully the potential for a diastereoselective conjugate addition of amino esters to the substituted nitroalkene **164**. Optimisation of the reaction in the presence of a chiral catalyst to preferentially form one diastereomer is of the utmost interest, as it would allow access to many different oxetane-containing peptidomimetics, and not just ‘Gly’ or racemic ‘Phe’ examples produced herein. Alternative routes such as chiral protonation strategies also merit investigation.

Having established routes towards these oxetane peptidomimetics, it would be of interest to expand the chemistry further to longer peptide chains, and to remove the need for the presence of branched-chain substituents at the residue downstream of the oxetane. It would be of great interest to see the impact of the oxetane unit on the flexibility and secondary structure of longer peptidomimetics (4 amino acids or longer).

Finally, the impact of the oxetane unit on a peptide's biological activity and stability needs to be investigated. In particular, enantiopure peptidomimetics containing the oxetane 'Phe' residue could be exposed to chymotrypsin assays in order to compare their stability and reactivity to the natural peptides. Additionally, the peptide Leu-Gly-Ile has been shown to have activity as an angiotensin I-converting enzyme (ACE) inhibitor.[135] As our structural studies have shown that the oxetane analogue **255** has more rotational freedom than the parent peptide, it would be interesting to explore the activity of the peptidomimetic in such an assay. Another tripeptide of interest, is the neuropeptide, Pro-Leu-Gly-NH_2 (PLG), a modulator of the dopamine D_2 receptor.[142] Studies have shown that PLG forms a type II β-turn and it would be interesting to explore the impact of the introduction of the oxetane on both the secondary structure of the peptide chain and its bioactivity.[143]

Chapter 3:

Experimental

3.1 General Information

All reactions were performed under an atmosphere of dry nitrogen in flame- or oven-dried glassware unless otherwise stated. Anhydrous solvents were purchased from Sigma-Aldrich or Fisher Scientific in Sure-Seal™ bottles for use as reaction solvents. All other solvents were reagent grade and used as received or purified by standard protocols where stated. Petrol refers to the petroleum ether fraction which boils in the range 40 – 60 ^{o}C for column chromatography.

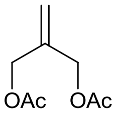
Commercially available starting materials were used without further purification unless otherwise stated. Thin layer chromatography was performed on pre-coated aluminium-backed plates (Merck Silicagel 60 F254), visualised by UV 254 nm then stained with potassium permanganate or ceric ammonium molybdate solution. Flash chromatography was performed using Fluorochem LC60A 40-63 micron silica. Chiral HPLC was performed using an Agilent 1100 series or an Agilent 1260 Infinity system. Melting points were recorded on a Gallenkamp MPD350 apparatus or a Büchi B-545 Melting Point Apparatus and are reported as observed. Single crystal X-ray diffraction data were obtained using a Siemens SMART XRD system or an Oxford Diffraction Gemini XRD system. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer, Thermo Nicolet Avatar 370 FT-IR or a Bruker Alpha Platinum ATR spectrometer with internal calibration and are given in cm^{-1}.

Low resolution mass spectra were recorded on an Esquire 2000 platform with electrospray ionisation or on a Waters Alliance Micromass ZQ / CapLC. High resolution mass spectra were obtained using a Bruker MicroTOF spectrometer or a

Thermo Accela CTC – LTQ FT. Optical rotations were measured using an AA-1000 Polarimeter from Optical Activity Ltd. Warwick Analytical Service carried out all elemental analysis.

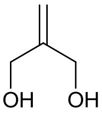
Nuclear magnetic resonance (NMR) spectra were recorded on Bruker DPX (300, 400, 500 or 600 MHz) or AV (400 or 700 MHz) spectrometers. Chemical shifts are reported in parts per million relative to the standard tetramethylsilane. The peak multiplicities were specified as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint). Coupling constants (J) are reported in Hertz.

2-Methylenepropane-1,3-diyl diacetate (61)[144]



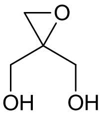
This known compound was prepared according to a literature procedure.[27] To a solution of 3-chloro-2-chloromethyl-1-propene (5.57 g, 44.6 mmol) in glacial acetic acid (40 mL) at 0 °C was added NEt_3 (12.5 mL, 89.7 mmol) and the reaction mixture was heated under reflux overnight. On cooling, the solution was diluted with CH_2Cl_2 (50 mL) and washed with water (3 x 50 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **61** (6.48 g, 84%) as an orange liquid. Data in agreement with literature values.[144] IR (film) 1735, 1370, 1214, 1026, 917 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.18 (2H, t, *J* = 1.0, =CH_2), 4.50 (4H, t, *J* = 1.0, CH_2), 1.99 (6H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 170.4 (C=O), 138.7 (C), 116.6 (=*C*H_2), 64.4 (CH_2), 20.7 (CH_3); MS (ES^+) *m/z* 195 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_8H_{12}NaO_4$ $[M+Na]^+$: 195.0628; found: 195.0626.

2-Methylenepropane-1,3-diol (57)[145]



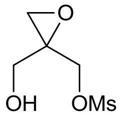
This known compound was prepared as follows. To a solution of **61** (11.66 g, 67.7 mmol) in THF (36 mL) and H_2O (36 mL) at 0 °C was added NaOH (6.01 g, 150.3 mmol) and the reaction mixture was stirred at rt for 72 h. The mixture was concentrated *in vacuo* and the resultant solid extracted with diethyl ether by Soxhlet extraction. The ether solution was concentrated *in vacuo* and the resulting oil distilled (110 °C, 0-1 mbar) to give **57** (3.30 g, 55%) as a colourless liquid. Data in agreement with literature values.[145] IR (film) 3286, 2919, 1662, 908 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.14 (2H, t, *J* = 0.9, =C*H*$_2$), 4.24 (4H, s, CH_2), 1.94 (2H, br s, O*H*); δ_C (75 MHz, $CDCl_3$) 147.6 (C), 112.6 (=*C*H_2), 64.8 (CH_2).

(2-Hydroxymethyloxiranyl)-methanol (64)[28]



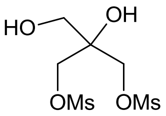
This known compound was prepared according to a literature procedure.[28] To a stirred solution of **57** (3.6 g, 40.9 mmol) in anhydrous CH_2Cl_2 (100 mL) was added *m*CPBA (77% in water, 11.1 g, 49.5 mmol). The reaction mixture was stirred at rt for 5 h then concentrated *in vacuo*. Purification by column chromatography (100% EtOAc) provided **64** (2.86 g, 67%) as a colourless liquid. Data in agreement with literature values.[28] R_f = 0.25 (100% EtOAc); δ_H (300 MHz, $CDCl_3$) 3.90 (2H, dd, *J* = 12.4, 5.6, C*H*H), 3.72 (2H, dd, *J* = 12.4, 7.2, CH*H*), 2.89 (2H, s, epoxide CH_2), 2.64 (2H, dd, *J* = 7.2, 5.6, O*H*); δ_C (100 MHz, $CDCl_3$) 62.5 (CH_2), 60.6 (C), 49.0 (CH_2).

[2-(Hydroxymethyl)oxiran-2-yl]methyl methanesulfonate (65)



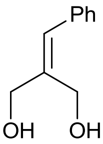
This novel compound was prepared using a modified literature procedure.[146] To a solution of **64** (126 mg, 1.21 mmol) and NEt_3 (0.19 mL, 1.36 mmol) in anhydrous toluene (3 mL) at 0 °C was added a solution of MsCl (0.1 mL, 1.29 mmol) in anhydrous toluene (2 mL) dropwise. The reaction mixture was stirred at 0 °C for 90 min then filtered and concentrated *in vacuo*. Purification by column chromatography (67 → 80% EtOAc in petroleum ether) provided dimesylate **66** (15 mg, 5%), followed by the more polar **65** (20 mg, 9%) as a colourless oil. R_f = 0.2 (75% EtOAc in petroleum ether); δ_H (400 MHz, $CDCl_3$) 4.43 (1H, d, *J* = 11.6, C*H*HOMs), 4.35 (1H, d, *J* = 11.6, CH*H*OMs), 3.88 (1H, dd, *J* = 12.5, 5.9, C*H*HOH), 3.80 (1H, dd, *J* = 12.5, 7.2, CH*H*OH), 3.08 (3H, s, CH_3), 2.96 (1H, d, *J* = 4.4, epoxide C*H*H), 2.89 (1H, d, *J* = 4.4, epoxide CH*H*), 1.91 (1H, dd, *J* = 7.2, 5.9, O*H*); δ_C (100 MHz, $CDCl_3$) 69.3 (CH_2), 61.5 (CH_2), 57.5 (C), 49.5 (CH_2), 37.9 (CH_3). Dimesylate **66**: δ_H (400 MHz, $CDCl_3$) 4.42 (2H, d, *J* = 11.8, C*H*HOMs), 4.34 (2H, d, *J* = 11.8, CH*H*OMs), 3.10 (6H, s, CH_3), 2.98 (2H, s, CH_2); δ_C (100 MHz, $CDCl_3$) 68.3 (CH_2), 55.1 (C), 50.2 (CH_2), 38.0 (CH_3).

2-Hydroxy-2-(hydroxymethyl)propane-1,3-diyl dimethanesulfonate (69)



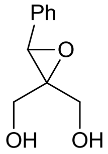
This novel compound was prepared as follows. To **66** (180 mg, 0.69 mmol) and *p*-toluenesulfonic acid monohydrate (18 mg, 0.09 mmol) was added THF (5 mL) and water (5 mL). The reaction mixture was stirred at rt overnight then heated under reflux for 20 h. On cooling, the reaction mixture was concentrated *in vacuo*. The residue was extracted with EtOAc (3 x 30 mL) and the combined organic extracts washed with water (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (83% EtOAc in petroleum ether) provided **69** (24 mg, 12%) as a white solid. R_f = 0.27 (100% EtOAc); M.p. 80 – 81 °C; IR (film) 3489, 3424, 1411, 1332, 1166, 1122, 1062 cm^{-1}; δ_H (300 MHz, MeOD) 4.25 (4H, s, C*H*$_2$OMs), 3.58 (2H, s, C*H*$_2$OH), 3.13 (6H, s, C*H*$_3$), OHs not observed; δ_C (75 MHz, MeOD) 73.7 (C), 70.9 (CH_2), 63.2 (CH_2), 37.1 (CH_3); MS (ES$^+$) *m/z* 301 $[M+Na]^+$; HRMS (ES$^+$) calcd. for $C_6H_{14}NaO_8S_2$ $[M+Na]^+$: 301.0022; found: 301.0024.

2-Benzylidenepropane-1,3-diol (71)



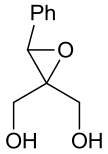
This known compound was prepared according to a literature procedure.[29] To a solution of diethyl benzylidenemalonate (2.21 g, 8.90 mmol) in anhydrous toluene (4 mL) at -30 °C was added DIBAL (1 M in toluene, 40.0 mL, 40.0 mmol) dropwise. The reaction mixture was stirred at -30 °C for 3 h then quenched with methanol (10 mL). Potassium sodium tartrate (36 g) in water (100 mL) was added to the solution, which was stirred at rt for 1 h before it was extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Resultant solid was washed with petroleum ether, filtered and dried to give **71** (890 mg, 61%) as a white solid. R_f = 0.23 (75% EtOAc in petroleum ether); δ_H (400 MHz, DMSO-*d*$_6$) 7.37 – 7.30 (4H, m, Ar*H*), 7.26 – 7.21 (1H, m, Ar*H*), 6.56 (1H, br s, =C*H*), 4.89 (1H, t, *J* = 5.6, O*H*), 4.81 (1H, t, *J* = 5.2, O*H*), 4.15 (2H, dd, *J* = 5.6, 1.4, C*H*$_2$), 4.07 (2H, d, *J* = 5.2, C*H*$_2$); δ_C (100 MHz, DMSO-*d*$_6$) 142.6 (C), 136.9 (C, Ar), 128.6 (CH, Ar), 128.1 (CH, Ar), 126.5 (CH, Ar), 124.7 (CH), 63.0 (CH_2), 57.3 (CH_2); MS (ES$^+$) *m/z* 187 $[M+Na]^+$; HRMS (ES$^+$) calcd. for $C_{10}H_{12}NaO_2$ $[M+Na]^+$: 187.0730; found: 187.0736.

(3-Phenyloxirane-2,2-diyl)dimethanol (**72**)



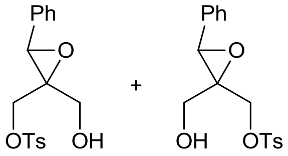
This novel compound was prepared using a modified literature procedure.[31-32] To a solution of **71** (160 mg, 0.97 mmol) and vanadyl acetylacetonate (29 mg, 0.11 mmol) in anhydrous toluene (15 mL) at -25 °C was added anhydrous *t*-butyl hydroperoxide (5-6 M in decane, 0.4 mL, 1.95 mmol). The reaction mixture was stirred at 0 °C for 23 h before water (50 mL) was added and the solution extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with water (2 x 50 mL) then brine (50 mL) before being dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (75% EtOAc in petroleum ether) provided **72** (80 mg, 46%) as a white solid. R_f = 0.27 (75% EtOAc in petroleum ether); M.p. 99 - 100 °C; IR (film) 3242, 2925, 1602, 1498, 1450, 1244, 953, 862 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.40 – 7.28 (5H, m, Ar*H*), 4.27 (1H, s, C*H*), 4.11 (1H, d, *J* = 12.4, C*H*H), 3.95 (1H, d, *J* = 12.4, CH*H*), 3.59 (2H, s, C*H*$_2$), 2.15 (1H, br s, O*H*), 1.72 (1H, br s, O*H*); δ_C (75 MHz, acetone-*d*$_6$) 136.8 (C, Ar), 128.9 (CH, Ar), 128.2 (CH, Ar), 127.2 (CH, Ar), 66.5 (C), 63.0 (CH_2), 60.9 (CH), 60.1 (CH_2); MS (ES^+) *m/z* 203 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{10}H_{12}NaO_3$ $[M+Na]^+$: 203.0679; found: 203.0684.

(3-Phenyloxirane-2,2-diyl)dimethanol (**72**)



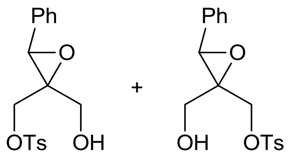
To a solution of **71** (888 mg, 5.41 mmol) in anhydrous CH_2Cl_2 (35 mL) was added *m*CPBA (77% in water, 1.86 g, 8.30 mmol) and the reaction mixture stirred at rt for 4.5 h. EtOAc (100 mL) was added to the solution, which was washed with saturated aqueous $Na_2S_2O_3$ solution (100 mL) then saturated aqueous $NaHCO_3$ solution (100 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (75% EtOAc in petroleum ether) provided **72** (715 mg, 73%) as a white solid. Data as previously reported.

[2-(Hydroxymethyl)-3-phenyloxiran-2-yl]methyl 4-methylbenzenesulfonate (**76** and **77**)



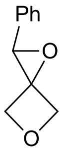
These novel compounds were prepared using a modified literature procedure.[33-34] A solution of **72** (103 mg, 0.57 mmol) and dibutyltin(IV) oxide (4 mg, 0.02 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred at rt for 1 h. Diisopropyl ethylamine (0.12 mL, 0.69 mmol) was added and the reaction mixture stirred for 5 min, before *p*-toluenesulfonyl chloride (115 mg, 0.60 mmol) was added. The reaction mixture was stirred at rt for 21 h before 1 M HCl (20 mL) was added. The solution was extracted with EtOAc (3 x 30 mL) before the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (11 → 33% EtOAc in petroleum ether) provided the less polar **76** (54 mg, 28%) as a colourless oil. R_f = 0.24 (33% EtOAc in petroleum ether); IR (film) 3568, 1359, 1265, 1175, 968, 815, 733, 700, 666 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.84 (2H, d, *J* = 8.3, Ar*H*), 7.40 – 7.26 (7H, m, Ar*H*), 4.39 (1H, d, *J* = 11.0, C*H*HOTs), 4.30 (1H, d, *J* = 11.0, CH*H*OTs), 4.15 (1H, s, C*H*), 3.51 (2H, s, CH_2OH), 2.46 (3H, s, CH_3), 1.62 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 145.4 (C, Ar), 133.6 (C, Ar), 132.7 (C, Ar), 130.2 (CH, Ar), 128.5 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 126.4 (CH, Ar), 69.8 (*C*H_2OTs), 62.9 (C), 61.7 (CH), 59.4 (CH_2OH), 21.8 (CH_3); MS (ES^+) *m/z* 357 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{17}H_{18}NaO_5S$ $[M+Na]^+$: 357.0767; found: 357.0766. Followed by the more polar **77** (42 mg, 22%) as a colourless oil. R_f = 0.27 (40% EtOAc in petroleum ether); IR (film) 3546, 1362, 1270, 1176, 973, 814, 735, 701, 667 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.58 (2H, d, *J* = 8.3, Ar*H*), 7.29 – 7.24 (5H, m, Ar*H*), 7.19 – 7.15 (2H, m, Ar*H*), 4.25 (1H, s, C*H*), 3.99 – 3.88 (2H, m, C*H*$_2$OH), 3.98 (1H, d, *J* = 10.9, C*H*HOTs), 3.86 (1H, d, *J* = 10.9, CH*H*OTs), 2.44 (3H, s, C*H*$_3$), 1.92 (1H, t, *J* = 6.0, O*H*); δ_C (100 MHz, $CDCl_3$) 145.2 (C, Ar), 133.5 (C, Ar), 132.2 (C, Ar), 130.0 (CH, Ar), 128.5 (CH, Ar), 128.3 (CH, Ar), 128.1 (CH, Ar), 126.3 (CH, Ar), 67.2 (*C*H$_2$OTs), 62.8 (C), 61.7 (CH_2OH), 60.4 (CH), 21.8 (CH_3); MS (ES$^+$) *m/z* 357 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{17}H_{18}NaO_5S$ [M+Na]$^+$: 357.0767; found: 357.0767. The regioisomers were identified using NOE experiments.

[2-(Hydroxymethyl)-3-phenyloxiran-2-yl]methyl 4-methylbenzenesulfonate (**76** and **77**)



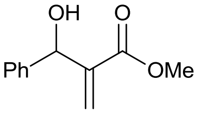
These novel compounds were prepared using a modified literature procedure.[35] To a solution of **72** (505 mg, 2.80 mmol) in anhydrous THF (24 mL) at 0 °C was added ^{n}BuLi (1.6 M in hexanes, 1.8 mL, 2.88 mmol) dropwise and the reaction mixture stirred at 0 °C for 30 min. A solution of *p*-toluenesulfonyl chloride (538 mg, 2.82 mmol) in anhydrous THF (11 mL) was added dropwise to the reaction mixture, which was stirred at 0 °C for 5 h before it was quenched with saturated aqueous NH_4Cl solution (20 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) before the combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (22% → 29% EtOAc in hexane) provided a mixture of **76** and **77** (654 mg, 70%) as a pale, viscous liquid. Ratio of **76** : **77** was 1.7 : 1. Data as previously reported.

2-Phenyl-1,5-dioxaspiro[2.3]hexane (74)



This novel compound was prepared as follows. To a stirred suspension of NaH (60% in mineral oil, 20 mg, 0.50 mmol) in anhydrous DMF (7 mL) at 0 °C, was added a solution of **76** and **77** (103 mg, 0.31 mmol) in anhydrous DMF (8.4 mL). The reaction mixture was stirred at 0 °C for 1 h then warmed to rt and stirred for a further 6 h. Saturated aqueous NH_4Cl solution (15 mL) was added to the solution, which was extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (17 → 25% EtOAc in petroleum ether) provided **74** (3 mg, 6%) as a colourless oil. R_f = 0.24 (25% EtOAc in petroleum ether); δ_H (300 MHz, $CDCl_3$) 7.52 – 7.46 (2H, m, Ar*H*), 7.41 – 7.35 (3H, m, Ar*H*), 5.41 (1H, s, C*H*), 4.71 (1H, d, *J* = 7.0, OC*H*H), 4.65 (1H, d, *J* = 7.0, OCH*H*), 4.54 (1H, d, *J* = 7.4, OC*H*H), 4.48 (1H, d, *J* = 7.4, OCH*H*).

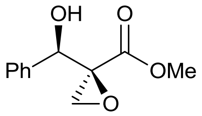
Methyl 2-[hydroxy(phenyl)methyl]acrylate (80)[147]



This known compound was prepared according to a literature procedure.[36] A solution of methyl acrylate (5.1 mL, 56.6 mmol), benzaldehyde (4.8 mL, 47.3 mmol) and 1,4-diazobicyclo[2.2.2]octane (5.4 g, 48.1 mmol) were added together at 0 °C then stirred at rt for 52 h. Diethyl ether (80 mL) was added to the solution, which was washed with 1 M HCl (2 x 50 mL), saturated aqueous $NaHCO_3$ solution (2 x 50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10 → 20% EtOAc in petroleum ether) provided **80** (7.02 g, 77%) as a white solid. Data in agreement with literature values.[147] R_f = 0.2 (20% EtOAc in petroleum ether); δ_H (400 MHz, $CDCl_3$) 7.32 –

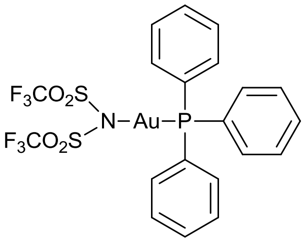
7.24 (4H, m, Ar*H*), 7.23 – 7.17 (1H, m, Ar*H*), 6.26 (1H, s, =C*H*H), 5.76 – 5.74 (1H, m, =CH*H*), 5.51 – 5.47 (1H, m, C*H*), 3.64 (3H, s, C*H*$_3$), 2.93 (1H, d, *J* = 4.9, O*H*); δ_C (100 MHz, CDCl$_3$) 166.8 (C=O), 142.1 (C), 141.4 (C, Ar), 128.5 (CH, Ar), 127.9 (CH, Ar), 126.7 (CH, Ar), 126.1 (CH$_2$), 73.2 (CH), 52.0 (CH$_3$); MS (ES$^+$) *m/z* 215 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{11}H_{12}NaO_3$ [M+Na]$^+$: 215.0679; found: 215.0680.

Methyl (*S**)-2-((*R**)-hydroxy(phenyl)methyl)oxirane-2-carboxylate ((±)-81)[37]



This known compound was prepared using a modified literature procedure.[32] To a solution of **80** (507 mg, 2.64 mmol) and vanadyl acetylacetonate (69 mg, 0.26 mmol) in anhydrous toluene (25 mL) at -20 °C, was added anhydrous *t*-butyl hydroperoxide (5-6 M in decane, 0.95 mL, 5.20 mmol). The reaction mixture was stirred at 0 °C for 25 h before water (25 mL) was added and the solution was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (2 x 25 mL) then dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (17 → 50% EtOAc in petroleum ether) provided (±)-**81** (176 mg, 32%) as a colourless oil. Data in agreement with literature values.[37] R$_f$ = 0.27 (40% EtOAc in petroleum ether); δ_H (400 MHz, CDCl$_3$) 7.41 (2H, d, *J* = 7.5, Ar*H*), 7.37 – 7.27 (3H, m, Ar*H*), 5.18 (1H, s, C*H*), 3.71 (3H, s, C*H*$_3$), 3.14 (1H, br s, O*H*), 3.10 (1H, d, *J* = 5.8, C*H*H), 2.83 (1H, d, *J* = 5.8, CH*H*); δ_C (100 MHz, CDCl$_3$) 169.9 (C=O), 138.4 (C, Ar), 128.4 (CH, Ar), 127.1 (CH, Ar), 71.8 (CH), 59.0 (C), 52.8 (CH$_3$), 49.8 (CH$_2$); MS (ES$^+$) *m/z* 231 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{11}H_{12}NaO_4$ [M+Na]$^+$: 231.0628; found: 231.0625.

[Bis(trifluoromethanesulfonyl)imidate](triphenylphosphine)gold(I) (88)[38]

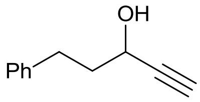


This known compound was prepared according to literature procedures.[38] Silver carbonate (470 mg, 1.70 mmol) and bis(trifluoromethane)sulfonimide (980 mg, 3.49 mmol) were dissolved in water (17 mL) and the reaction mixture was heated under reflux for 3 h. On cooling, the reaction mixture was concentrated *in vacuo* to give silver bis(trifluoromethanesulfonyl)imide as a white solid, which was immediately taken through to the next step.

Meanwhile, chloro(dimethylsulfide)gold(I) (498 mg, 1.69 mmol) and triphenylphosphine (445 mg, 1.70 mmol) were dissolved in anhydrous CH_2Cl_2 (42 mL) and the reaction mixture was stirred at rt for 15 min. The reaction mixture was concentrated *in vacuo* and the resultant solid washed with hexane, filtered and dried to give chloro(triphenylphosphine)gold(I) as a white solid, which was immediately taken through to the next step.

A solution of chloro(triphenylphosphine)gold(I) in anhydrous CH_2Cl_2 (9 mL) was added to a solution of silver bis(trifluoromethanesulfonyl)imide in anhydrous CH_2Cl_2 (10 mL) and the reaction mixture was stirred at rt for 15 min. The solution was then filtered through Celite®, washed with CH_2Cl_2 and concentrated *in vacuo* to afford **88** (1.25 g, 100%) as a white solid. Data in agreement with literature values.[38] δ_H (300 MHz, $CDCl_3$) 7.63 – 7.44 (15H, m, Ar*H*); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ_P 29.8.

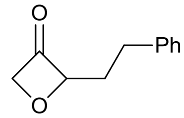
5-Phenylpent-1-yn-3-ol (93)[15]



This known compound was prepared according to a literature procedure.[15] To a solution of trimethylsilylacetylene (3.2 mL, 22.6 mmol) in anhydrous THF (56 mL) at -78 °C, was added ^{n}BuLi (1.6 M in hexanes, 14.9 mL, 23.8 mmol) and the reaction mixture was stirred for 15 min. A solution of 3-phenylpropionaldehyde (2.0 mL, 15.2 mmol) in anhydrous THF (56 mL) was added dropwise and the reaction mixture was stirred at -78 °C for 3.5 h before saturated aqueous NH_4Cl solution (20 mL) was added. The aqueous layer was extracted with diethyl ether (4 x 50 mL) then the combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (13% EtOAc in petroleum ether; 11 → 13% EtOAc in petroleum ether) provided the TMS-protected alkyne. To a stirred solution of this alkyne (2.46 g, 10.6 mmol) in anhydrous THF (93 mL) at 0 °C, was added a solution of tetrabutylammonium fluoride (1 M in THF, 12.7 mL, 12.7 mmol) and the reaction mixture was stirred for 30 min. Saturated aqueous NH_4Cl solution (30 mL) was added to the reaction mixture and the aqueous layer was extracted with diethyl ether (3 x 75 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (13 → 17% EtOAc in petroleum ether) provided **93** (1.29 g, 76%) as a pale yellow liquid. Data in agreement with literature values.[15] R_f = 0.23 (17% EtOAc in petroleum ether); IR (film) 3485, 3283, 2931, 1604, 1496, 1042, 746, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.33 – 7.27 (2H, m, Ar*H*), 7.25 – 7.18 (3H, m, Ar*H*), 4.37 (1H, t, *J* = 5.8, C*H*OH), 2.81 (2H, t, *J* = 7.8, CH_2), 2.50 (1H, d, *J* = 2.0, C*H*), 2.32 (1H, br s, O*H*), 2.12 – 1.97 (2H, m, CH_2); δ_C (100 MHz, $CDCl_3$) 141.2 (C, Ar), 128.6 (CH, Ar), 128.6 (CH, Ar), 126.1 (CH, Ar), 84.8

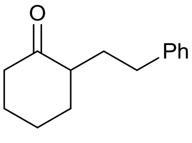
(CH), 73.5 (C), 61.6 (CHOH), 39.1 (Bn*C*H_2), 31.3 (Ph*C*H_2); MS (ES$^+$) *m/z* 178 [M+NH_4]$^+$.[148]

2-Phenethyloxetan-3-one (94)[15]



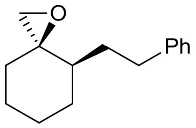
This known compound was prepared according to a literature procedure.[15] To a solution of **93** (707 mg, 4.41 mmol) in anhydrous DCE (62 mL) was added 3,5-dichloropyridine *N*-oxide (1.48 g, 9.02 mmol), bis(trifluoromethane)sulfonimide (1.52 g, 5.41 mmol) and **88** (169 mg, 0.23 mmol). The reaction mixture was stirred at rt for 4 h in the absence of light before saturated aqueous $NaHCO_3$ solution (50 mL) was added and the solution extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0 → 15% EtOAc in petroleum ether) provided **94** (485 mg, 62%) as a colourless oil. Data in agreement with literature values.[15] R_f = 0.22 (17% EtOAc in petroleum ether); δ_H (400 MHz, $CDCl_3$) 7.32 – 7.27 (2H, m, Ar*H*), 7.23 – 7.17 (3H, m, Ar*H*), 5.48 – 5.42 (1H, m, C*H*), 5.29 (1H, dd, *J* = 15.1, 1.3, OC*H*H), 5.24 (1H, dd, *J* = 15.1, 4.0, OCH*H*), 2.85 – 2.73 (2H, m, C*H*$_2$), 2.23 - 2.06 (2H, m, C*H*$_2$); δ_C (100 MHz, $CDCl_3$) 203.2 (C=O), 140.4 (C, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 126.4 (CH, Ar), 102.9 (CH), 89.0 (OCH_2), 32.9 (CH_2), 30.3 (CH_2); MS (ES$^+$) *m/z* 199 [M+Na]$^+$.

2-Phenethylcyclohexan-1-one (95)[40]



This known compound was prepared according to a literature procedure.[40] To a stirred suspension of potassium *t*-butoxide (558 mg, 4.97 mmol) in anhydrous DMSO (52 mL) was added cyclohexanone (5.13 mL, 49.5 mmol) and styrene (1.9 mL, 16.5 mmol). The reaction mixture was heated at 40 °C for 16 h then cooled to 0 °C and diluted with water (50 mL). The solution was extracted with CH_2Cl_2 (3 x 100 mL) and the combined organic extracts washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0 → 10% EtOAc in petroleum ether) provided **95** (1.06 g, 32%) as a pale yellow liquid. Data in agreement with literature values.[40] R_f = 0.29 (10% EtOAc in petroleum ether); δ_H (400 MHz, $CDCl_3$) 7.24 – 7.18 (2H, m, Ar*H*), 7.15 – 7.09 (3H, m, Ar*H*), 2.63 – 2.49 (2H, m, C*H₂*), 2.40 – 2.30 (1H, m, C*H*), 2.27 – 2.17 (2H, m, C*H₂*), 2.13 – 2.03 (2H, m, C*H₂*), 2.02 – 1.91 (1H, m, C*H*), 1.85 – 1.74 (1H, m, C*H*), 1.68 – 1.52 (2H, m, C*H₂*), 1.49 – 1.31 (2H, m, C*H₂*); δ_C (100 MHz, $CDCl_3$) 213.2 (C=O), 142.3 (C, Ar), 128.5 (CH, Ar), 128.4 (CH, Ar), 125.9 (CH, Ar), 50.0 (CH), 42.2 (CH_2), 34.1 (CH_2), 33.3 (CH_2), 31.3 (CH_2), 28.2 (CH_2), 25.0 (CH_2); MS (ES^+) *m/z* 225 $[M+Na]^+$.

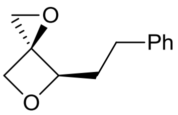
(3*R**,4*R**)-4-Phenethyl-1-oxaspiro[2.5]octane ((±)-98)



This novel compound was prepared using a modified literature procedure.[39] Anhydrous DMSO (1.2 mL) was added to NaH (60% in mineral oil, 76 mg, 1.90 mmol) and trimethylsulfoxonium iodide (395 mg, 1.79 mmol). After 15 – 20 min, a solution of **95** (300 mg, 1.49 mmol) in anhydrous DMSO (1.8 mL) was added and the reaction mixture was stirred at rt for 20 min before being heated at 50 °C for 3 h. After cooling, water (10 mL) was added to the solution, which was extracted with diethyl ether (4 x 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (6 → 10% EtOAc in petroleum ether) provided (±)-**98** (88 mg, 27%) as a colourless oil. R_f = 0.26 (2% EtOAc in petroleum ether); IR (film)

2931, 2859, 1604, 1496, 1240, 922, 845, 734, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.31 – 7.25 (2H, m, Ar*H*), 7.21 – 7.15 (3H, m, Ar*H*), 2.74 – 2.64 (1H, m, C*H*H), 2.70 (1H, dd, *J* = 4.8, 0.9, OC*H*H), 2.61 – 2.49 (1H, m, CH*H*), 2.52 (1H, d, *J* = 4.8, OCH*H*), 1.85 – 1.34 (11H, m, C*H*$_2$, C*H*); δ_C (100 MHz, $CDCl_3$) 142.7 (C, Ar), 128.5 (CH, Ar), 128.4 (CH, Ar), 125.8 (CH, Ar), 61.5 (C), 53.7 (OCH_2), 40.2 (CH), 33.9 (CH_2), 32.0 (CH_2), 30.6 (CH_2), 29.5 (CH_2), 25.2 (CH_2), 22.4 (CH_2); MS (ES$^+$) *m/z* 239 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{15}H_{20}NaO$ [M+Na]$^+$: 239.1406; found: 239.1408.

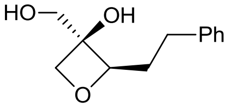
(3*R**,4*R**)-4-Phenethyl-1,5-dioxaspiro[2.3]hexane ((±)-99a)



This novel compound was prepared using a modified literature procedure.[39] Anhydrous DMSO (1.45 mL) was added to NaH (60% in mineral oil, 53 mg, 1.33 mmol) and trimethylsulfoxonium iodide (319 mg, 1.45 mmol) and the reaction mixture was stirred at rt for 20 min. A solution of **94** (182 mg, 1.03 mmol) in anhydrous DMSO (0.7 mL) was then added and the reaction mixture was stirred at rt for 20 min before being heated at 50 °C for 2.5 h. After cooling, water (20 mL) was added to the solution, which was extracted with diethyl ether (4 x 20 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (13 $\rightarrow$ 22% EtOAc in petroleum ether) provided one diastereomer, (±)-**99a** (47 mg, 24%) as a colourless oil. R_f = 0.25 (20% EtOAc in petroleum ether); IR (film) 2944, 1604, 1496, 1454, 962, 864 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.27 – 7.21 (2H, m, Ar*H*), 7.17 – 7.12 (3H, m, Ar*H*), 5.01 (1H, ddd, *J* = 7.3, 5.9, 1.1, C*H*), 4.89 (1H, d, *J* = 7.9, OC*H*H), 4.78 (1H, d, *J* = 7.9, OCH*H*), 2.80 (1H, d, *J* = 4.3, epoxide C*H*H), 2.75 – 2.70 (1H, m, C*H*H), 2.68 (1H, d, *J* = 4.3, epoxide CH*H*), 2.66 – 2.57 (1H, m, CH*H*), 2.19 – 2.09 (1H, m C*H*H), 2.02 – 1.93 (1H, m, CH*H*); δ_C

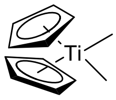
(100 MHz, $CDCl_3$) 141.4 (C, Ar), 128.6 (CH, Ar), 128.5 (CH, Ar), 126.1 (CH, Ar), 87.1 (CH), 76.0 (OCH_2), 61.4 (C), 48.8 (epoxide CH_2), 33.4 (CH_2), 30.3 (CH_2); MS (ES^+) *m/z* 213 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{12}H_{14}NaO_2$ $[M+Na]^+$: 213.0886; found: 213.0887. A small amount of the other diastereomer (**99b**) was also obtained impure (9 mg, ~ 5%).

3-(Hydroxymethyl)-2-phenethyloxetan-3-ol ((±)-100)



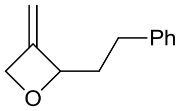
This novel compound was prepared as follows. (±)-**99a** was left in the freezer with residual $CDCl_3$ over a period of 2 months. Purification by column chromatography (25 → 70% EtOAc in petroleum ether) provided (±)-**100** (18 mg, 35%) as a colourless oil. $R_f = 0.39$ (70% EtOAc in petroleum ether); IR (film) 3380, 2881, 1601, 1496, 1454, 1067, 954, 751, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.24 – 7.18 (2H, m, Ar*H*), 7.15 – 7.09 (3H, m, Ar*H*), 4.43 (1H, dd, *J* = 8.2, 5.9, C*H*), 4.41 – 4.37 (2H, m, OC*H*$_2$), 3.79 – 3.71 (2H, m, OHC*H*$_2$), 2.72 – 2.64 (2H, m, O*H*, C*H*H), 2.59 – 2.51 (1H, m, CH*H*), 2.16 – 1.95 (3H, m, C*H*$_2$, O*H*); δ_C (100 MHz, $CDCl_3$) 141.6 (C, Ar), 128.6 (CH, Ar), 126.1 (CH, Ar), 88.4 (CH), 78.5 (OCH_2), 74.6 (C), 67.5 (CH_2OH), 32.5 (CH_2), 30.8 (CH_2); MS (ES^+) *m/z* 231 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{12}H_{16}NaO_3$ $[M+Na]^+$: 231.0992; found: 231.0993.

Dimethyl titanocene (Petasis reagent) (104)[44]



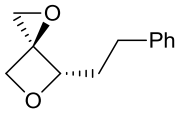
This known compound was prepared according to a literature procedure.[44] To a stirred solution of bis(cyclopentadienyl)titanium (IV) dichloride (2.02 g, 8.11 mmol) in anhydrous Et_2O (20 mL) at 0 °C in the dark, was added methyl lithium (1.6 M in diethyl ether, 11.0 ml, 17.6 mmol) dropwise. The reaction mixture was stirred at rt for 1 h then cooled to 0 °C and quenched with water (30 mL). The solution was extracted with Et_2O (3 x 50 mL) and the combined organic extracts washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **104** (1.66 g, 98%) as a bright orange solid. A 0.5 M solution was made up using anhydrous toluene. Data in agreement with literature values.[44] δ_H (400 MHz, $CDCl_3$) 6.06 (10H, s, Cp), -0.15 (6H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 113.2 (CH, Cp), 45.6 (CH_3).

3-Methylene-2-phenethyloxetane (102)



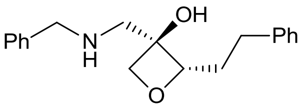
This novel compound was prepared using a modified literature procedure.[22] A stirred solution of **94** (101 mg, 0.57 mmol) and **104** (0.5 M solution in toluene, 1.72 mL, 0.86 mmol) was heated at 75 °C for 16 h then cooled to rt. Petroleum ether (2 mL) was added and the cloudy solution formed was filtered through Celite® repeatedly until the filtrate was clear. The filtrate was concentrated *in vacuo* and subsequent purification by column chromatography (5 → 20% Et_2O in petroleum ether) provided **102** (47 mg, 47%) as a colourless liquid. R_f = 0.36 (20% EtOAc in petroleum ether); IR (film) 3026, 2930, 2860, 1699, 1602, 1495, 1454, 1073, 956, 885, 746, 697 cm^{-1}; δ_H (400 MHz, acetone-d_6) 7.34 – 7.28 (2H, m, Ar*H*), 7.25 (2H, d, *J* = 7.8, Ar*H*), 7.20 (1H, t, *J* = 7.3, Ar*H*), 5.26 – 5.20 (1H, m, C*H*), 5.10 – 5.04 (1H, m, OC*H*H), 5.04 – 4.98 (1H, m, OCH*H*), 4.92 (1H, d, *J* = 1.1, =C*H*H), 4.86 (1H, d, *J* = 1.1, =CH*H*), 2.85 – 2.69 (2H, m, CH_2), 2.13 – 1.98 (2H, m, CH_2); δ_C (100 MHz, acetone-d_6) 149.8 (*C*=), 142.7 (C, Ar), 129.2 (CH, Ar), 129.2 (CH, Ar), 126.6 (CH, Ar), 103.6 (=CH_2), 89.4 (OCH), 76.3 (OCH_2), 38.5 (CH_2), 30.9 (CH_2); MS (ES^+) *m/z* 197 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{12}H_{14}NaO$ $[M+Na]^+$: 197.0937; found: 197.0937.

(3*S**,4*R**)-4-Phenethyl-1,5-dioxaspiro[2.3]hexane ((±)-99b)



DMDO was generated according to a literature procedure.[47] A solution of $NaHCO_3$ (12 g), water (20 mL) and acetone (13 mL) was stirred at 0 °C and equipped with distillation apparatus cooled with dry ice. Oxone (25 g) was added and the reaction mixture was stirred at rt under a slight vacuum for 1 h. The DMDO solution collected was added to a solution of (±)-**102** (46 mg, 0.26 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture was warmed to rt and stirred overnight before it was concentrated *in vacuo*. Purification by column chromatography (0 → 10% Et_2O in petroleum ether + 0.5% NEt_3) provided **99b** (11 mg, 22%) as a colourless oil. δ_H (400 MHz, acetone-d_6) 7.30 – 7.25 (2H, m, Ar*H*), 7.24 – 7.15 (3H, m, Ar*H*), 4.90 (1H, dd, *J* = 8.0, 4.3, C*H*), 4.75 (1H, dd, *J* = 7.8, 1.3, OC*H*H), 4.67 (1H, d, *J* = 7.8, OCH*H*), 2.92 (1H, d, *J* = 4.4, epoxide C*H*H), 2.80 – 2.72 (1H, m, C*H*H), 2.77 (1H, d, *J* = 4.4, epoxide CH*H*), 2.69 – 2.60 (1H, m, CH*H*), 2.10 – 2.00 (1H, m, C*H*H), 1.94 – 1.85 (1H, m, CH*H*). The compound was taken through to the next step immediately due to its instability.

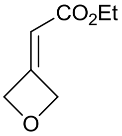
3-[(Benzylamino)methyl]-2-phenethyloxetan-3-ol ((±)-105)



This novel compound was prepared as follows. To a stirred solution of (±)-**99b** (10 mg, 52.6 µmol) in anhydrous ethanol (2 mL), was added benzylamine (6.9 µL, 63.1 µmol). The reaction mixture was heated under reflux for 16 h then cooled to rt and concentrated *in vacuo*. Purification by column chromatography (80% EtOAc in petroleum ether → 10% MeOH in EtOAc) provided (±)-**105** (12 mg, 77%) as a colourless oil. R_f = 0.41 (10% MeOH in EtOAc); IR (film) 3337, 3027, 2944, 2871, 1603, 1496, 1454, 1329, 964, 746, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.25 (7H, m, Ar*H*), 7.22 –

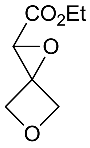
7.15 (3H, m, Ar*H*), 4.74 (1H, dd, *J* = 9.5, 4.3, C*H*), 4.49 (1H, d, *J* = 6.3, OC*H*H), 4.23 (1H, d, *J* = 6.3, OCH*H*), 3.81 (2H, s, PhCH_2NH), 3.01 – 2.94 (2H, m, BnNHCH_2), 2.80 – 2.50 (4H, m, CH_2, O*H*, N*H*), 1.98 – 1.79 (2H, m, CH_2); δ_C (125 MHz, $CDCl_3$) 141.4 (C, Ar), 138.6 (C, Ar), 128.8 (CH, Ar), 128.6 (CH, Ar), 128.5 (CH, Ar), 128.4 (CH, Ar), 127.8 (CH, Ar), 126.2 (CH, Ar), 90.9 (CH), 79.4 (OCH_2), 73.9 (C), 54.1 (Ph*C*H_2NH), 51.2 (BnNH*C*H_2), 33.3 (CH_2), 31.4 (CH_2); MS (ES^+) *m/z* 298 $[M+H]^+$, 320 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{19}H_{24}NO_2$ $[M+H]^+$: 298.1802; found: 298.1802.

Ethyl 2-(oxetan-3-ylidene)acetate (106)[15]



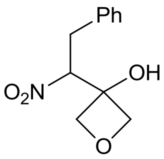
This known compound was prepared according to a literature procedure.[15] To a stirred solution of 3-oxetanone (390 mg, 5.41 mmol) in anhydrous CH_2Cl_2 (90 mL) at rt was added carbethoxymethylene triphenylphosphorane (2.84 g, 8.15 mmol). The reaction mixture was stirred at rt for 5.5 h and then concentrated *in vacuo*. Purification by column chromatography (10 → 20% EtOAc in petroleum ether) provided **106** (615 mg, 80%) as a colourless liquid. Data in agreement with literature values.[15] R_f = 0.33 (30% EtOAc in petroleum ether); IR (film) 2913, 2860, 1727, 1703, 1471, 1370, 1343, 1296, 1252, 1200, 1096, 958, 862, 831 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.61 – 5.58 (1H, m, C*H*), 5.48 – 5.44 (2H, m, OCH_2), 5.28 – 5.24 (2H, m, OCH_2), 4.12 (2H, q, *J* = 7.2, CH_2), 1.23 (3H, t, *J* = 7.2, CH_3); δ_C (100 MHz, $CDCl_3$) 165.3 (C=O), 159.3 (C), 111.2 (CH), 81.1 (OCH_2), 78.5 (OCH_2), 60.4 (CH_2), 14.3 (CH_3); GC-MS (EI) *m/z* 142 $[M]^+$, 84, 68, 55.

Ethyl 1,5-dioxaspiro[2.3]hexane-2-carboxylate (107)



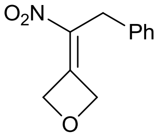
This novel compound was prepared using a modified literature procedure.[52] To a stirred solution of anhydrous THF (4 mL) at -78 °C was added *t*-BuOOH (~ 5.5 M in decane, 0.19 mL, 1.05 mmol), followed by ^{n}BuLi (2.5 M in hexane, 0.31 mL, 0.78 mmol). The reaction mixture was stirred at -78 °C for 5 min before a solution of **106** (97 mg, 0.68 mmol) in anhydrous THF (2 mL) was added. The reaction mixture was stirred at -78 °C for 5 min then warmed to 0 °C and maintained at this temperature for 23 h before sodium sulfite (0.5 g) was added. The reaction mixture was warmed to rt and stirred for 20 min then diluted with Et_2O (15 mL), filtered through Celite®, washed with Et_2O and concentrated *in vacuo*. Purification by column chromatography (0 → 50% EtOAc in petroleum ether) provided **107** (14 mg, 13%) as a colourless oil. $R_f = 0.27$ (30% EtOAc in petroleum ether); IR (film) 2953, 2878, 1738, 1452, 1339, 1291, 1198, 1026 cm^{-1}; δ_H (400 MHz, acetone-d_6) 4.96 – 4.93 (1H, m, OC*H*H), 4.87 – 4.84 (1H, m, OC*H*H), 4.79 (2H, d, *J* = 8.5, OCH*H*), 4.27 – 4.14 (2H, m, C*H*$_2$CH$_3$), 3.63 (1H, s, epoxide C*H*), 1.26 (3H, t, *J* = 7.2, C*H*$_3$); δ_C (100 MHz, acetone-d_6) 168.0 (C=O), 78.0 (OCH_2), 78.0 (OCH_2), 64.3 (C), 62.2 (CH_2), 54.5 (epoxide CH), 14.5 (CH_3); GC-MS (CI) *m/z* 159 $[M+H]^+$; HRMS (ES$^+$) calcd. for $C_7H_{11}O_4$ $[M+H]^+$: 159.0652; found: 159.0653.

3-(1-Nitro-2-phenylethyl)oxetan-3-ol (166)



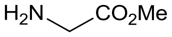
This novel compound was prepared using a modified literature procedure.[91] (2-Nitroethyl)benzene (194 mg, 1.28 mmol), 3-oxetanone (107 µL, 1.67 mmol) and NEt_3 (36 µL, 0.26 mmol) were stirred at rt for 90 min, giving a pale yellow solid. Purification by column chromatography (40 → 50% EtOAc in hexane) provided **166** (246 mg, 86%) as a white solid. M.p. 96 – 97 °C; R_f = 0.32 (50% EtOAc in hexane); IR (film) 3354, 2955, 2884, 1602, 1548, 1497, 1455, 1369, 1274, 1079, 971, 744, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.23 (5H, m, Ar*H*), 5.20 (1H, t, *J* = 7.5, C*H*), 4.63 (1H, d, *J* = 7.4, OC*H*H), 4.59 (1H, d, *J* = 7.4, OCH*H*), 4.39 (1H, d, *J* = 7.7, OC*H*H), 4.03 (1H, d, *J* = 7.7, OCH*H*), 3.50 (1H, s, O*H*), 3.43 (1H, dd, *J* = 14.1, 8.0, C*H*HPh), 3.27 (1H, dd, *J* = 14.1, 7.0, CH*H*Ph); δ_C (100 MHz, $CDCl_3$) 134.5 (C, Ar), 129.2 (CH, Ar), 129.1 (CH, Ar), 128.0 (CH, Ar), 92.4 (CH), 81.6 (OCH_2), 79.7 (OCH_2), 74.3 (C), 35.0 (*C*H_2Ph); MS (ES^+) *m/z* 246 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{11}H_{13}NNaO_4$ $[M+Na]^+$: 246.0737; found: 246.0738; CHN calcd. for $C_{11}H_{13}NO_4$: C, 59.19 H, 5.87 N, 6.27%; found: C, 59.08 H, 5.86 N, 6.23%.

3-(1-Nitro-2-phenylethylidene)oxetane (164)[91]



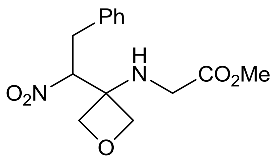
This known compound was prepared according to a literature procedure.[91] (2-Nitroethyl)benzene (630 mg, 4.17 mmol), 3-oxetanone (0.35 mL, 5.46 mmol) and NEt_3 (0.12 mL, 0.86 mmol) were stirred at rt for 90 min then diluted with anhydrous CH_2Cl_2 (24.5 mL) and cooled to -78 °C. NEt_3 (1.74 mL, 12.5 mmol) was added, followed by MsCl (0.35 mL, 4.52 mmol). The reaction mixture was stirred at -78 °C for 30 min then gradually warmed to -20 °C over 1 h and quenched with saturated aqueous NH_4Cl solution (20 mL). The solution was diluted with water (10 mL) and CH_2Cl_2 (30 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10 → 30% Et_2O in petroleum ether) provided **164** (359 mg, 42%) in an inseparable mixture with its rearrangement product **167** (~25 : 1) as a white solid. Data in agreement with literature values but full data had not been reported.[91] R_f = 0.18 (20% EtOAc in petroleum ether); M.p. 51 – 52 °C; IR (film) 2915, 2850, 1602, 1513, 1496, 1454, 1337, 1305, 960, 770, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.28 (3H, m, Ar*H*), 7.24 – 7.20 (2H, m, Ar*H*), 5.60 – 5.57 (2H, m, OCH_2), 4.92 – 4.88 (2H, m, OCH_2), 3.82 (2H, s, CH_2); δ_C (100 MHz, $CDCl_3$) 151.3 (C), 140.0 (C), 134.8 (C, Ar), 129.2 (CH, Ar), 129.0 (CH, Ar), 127.7 (CH, Ar), 79.9 (OCH_2), 75.5 (OCH_2), 34.0 (CH_2); MS (ES$^+$) *m/z* 228 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{11}H_{11}NNaO_3$ [M+Na]$^+$: 228.0631; found: 228.0634.

Methyl glycinate (168)



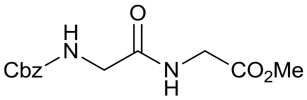
This known compound was prepared according to a literature procedure.[92] To a stirred solution of glycine methyl ester hydrochloride (1.06 g, 8.44 mmol) in anhydrous chloroform (6 mL) was added a solution of NEt_3 (1.18 mL, 8.47 mmol) in anhydrous chloroform (6 mL). The reaction mixture was stirred at rt for 5 h then at 70 °C for 1 h before it was cooled to rt and concentrated *in vacuo*. The white solid formed was diluted with Et_2O and filtered under vacuum. The filtrate was concentrated *in vacuo* to give **168** (352 mg, 47%) as a pale yellow liquid, which was used without further purification. Data in agreement with literature values.[149] δ_H (400 MHz, $CDCl_3$) 3.72 (3H, s, CH_3), 3.43 (2H, s, CH_2), 1.42 (2H, br s, NH_2); MS (ES$^+$) *m/z* 112 [M+Na]$^+$.[150]

Methyl [3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycinate (169)



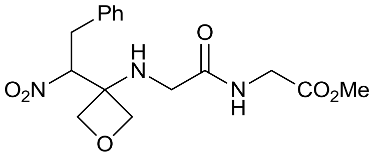
This novel compound was prepared using a modified literature procedure.[91] To a stirred solution of **164** (357 mg, 1.74 mmol) in anhydrous THF (16 mL) was added a solution of **168** (311 mg, 3.49 mmol) in anhydrous THF (10.5 mL). The reaction mixture was stirred at rt for 4 h then concentrated *in vacuo*. Resultant solid was recrystallised (EtOH) to give **169** (450 mg, 88%) as white crystals. M.p. 102 - 104 °C; IR (film) 3339, 2955, 2885, 1738, 1606, 1550, 1497, 1456, 1437 1368, 1213, 1181, 981, 747, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.35 – 7.21 (5H, m, Ar*H*), 5.12 (1H, dd, *J* = 9.8, 4.5, C*H*), 4.65 (1H, d, *J* = 7.5, OC*H*H), 4.62 (1H, d, *J* = 7.5, OCH*H*), 4.51 (1H, d, *J* = 8.0, OC*H*H), 4.48 (1H, d, *J* = 8.0, OCH*H*), 3.78 (3H, s, C*H₃*), 3.72 (1H, dd, *J* = 17.3, 6.0, C*H*HCO_2Me), 3.61 (1H, dd, *J* = 17.3, 6.0, CH*H*CO_2Me), 3.52 (1H, dd, *J* = 14.6, 9.8, C*H*HPh), 3.29 (1H, dd, *J* = 14.6, 4.5, CH*H*Ph), 2.47 (1H, br t, *J* = 5.9, N*H*); δ_C (100 MHz, acetone-d_6) 173.1 (C=O), 137.2 (C, Ar), 129.9 (CH, Ar), 129.5 (CH, Ar), 128.0 (CH, Ar), 92.7 (CH), 77.6 (OCH_2), 77.2 (OCH_2), 63.1 (C), 52.2 (CH_3), 45.0 (*C*H_2CO_2Me), 35.2 (*C*H_2Ph); MS (ES$^+$) *m/z* 295 [M+H]$^+$, 317 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{14}H_{18}N_2NaO_5$ [M+Na]$^+$: 317.1108; found: 317.1108.

Methyl [(benzyloxy)carbonyl]glycylglycinate (171)



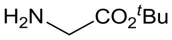
This known compound was prepared using a modified literature procedure.[151] To a stirred solution of Z-Gly-OH (73 mg, 0.35 mmol), DCC (79 mg, 0.38 mmol), HOBt (59 mg, 0.38 mmol) and DIEA (66.4 µL, 0.38 mmol) in anhydrous CH_2Cl_2 (2 mL) was added a solution of **168** (34 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (3 mL). The reaction mixture was stirred at rt overnight then filtered through Celite®, washed with CH_2Cl_2 and concentrated *in vacuo*. Purification by column chromatography (70 → 75% EtOAc in petroleum ether + 0.5% NEt_3) provided **171** (37 mg, 38%) as a white solid. Data was not fully reported in the literature. M.p. 64 – 66 °C; R_f = 0.23 (70% EtOAc in petroleum ether); IR (film) 3317, 3037, 2955, 2851, 1733, 1688, 1655, 1533, 1495, 1235, 1209, 997, 733, 694 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.35 – 7.27 (5H, m, Ar*H*), 6.87 – 6.81 (1H, m, N*H*), 5.76 – 5.70 (1H, m, N*H*), 5.11 (2H, s, PhC*H₂*O), 4.01 (2H, d, *J* = 5.0, C*H₂*), 3.90 (2H, d, *J* = 4.5, C*H₂*), 3.72 (3H, s, C*H₃*); δ_C (100 MHz, $CDCl_3$) 170.3 (C=O), 169.6 (C=O), 156.8 (C=O), 136.2 (C, Ar), 128.6 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 67.3 (Ph*C*H₂O), 52.5 (CH_3), 44.4 (CH_2), 41.2 (CH_2); MS (ES$^+$) *m/z* 281 [M+H]$^+$, 303 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{13}H_{16}N_2NaO_5$ [M+Na]$^+$: 303.0951; found: 303.0952.

Methyl [[3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycyl]glycinate (173)



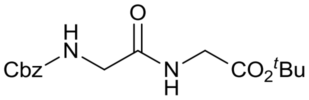
This novel compound was prepared using modified literature procedures.[91,134] A suspension of **171** (307 mg, 1.10 mmol) and 10% Pd/C (66 mg) in anhydrous THF (9 mL) was stirred under an atmosphere of hydrogen at rt for 4.5 h, then filtered through Celite® and washed with anhydrous THF (10 mL). This solution was added to a stirred solution of **164** (92 mg, 0.45 mmol) in anhydrous THF (1 mL). The reaction mixture was stirred at rt for 11.5 days then concentrated *in vacuo*. Purification by column chromatography (85 → 100% EtOAc in petroleum ether) provided **173** (37 mg, 23%) as a pale yellow oil. R_f = 0.38 (100% EtOAc); IR (film) 3368, 2955, 1746, 1669, 1595, 1550, 1545, 1498, 1456, 1371, 1209, 1183, 733, 699 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.40 – 7.20 (6H, m, Ar*H*, N*H*), 5.18 (1H, dd, *J* = 10.4, 3.8, C*H*), 4.71 – 4.61 (3H, m, OC*H*H, OCH*H*), 4.56 (1H, d, *J* = 7.9, OCH*H*), 4.16 (1H, dd, *J* = 18.3, 5.8, C*H*HCO₂Me), 4.01 (1H, dd, *J* = 18.3, 5.3, CH*H*CO₂Me), 3.76 (3H, s, C*H₃*), 3.72 – 3.51 (3H, m, C*H₂*, C*H*HPh), 3.30 (1H, dd, *J* = 14.7, 3.8, CH*H*Ph), 2.40 (1H, br s, N*H*); δ_C (75 MHz, $CDCl_3$) 171.3 (C=O), 170.3 (C=O), 135.1 (C, Ar), 129.2 (CH, Ar), 128.8 (CH, Ar), 127.9 (CH, Ar), 93.2 (CH), 76.9 (OCH_2), 76.5 (OCH_2), 62.4 (C), 52.6 (CH_3), 46.0 (CH_2), 40.9 ($\mathit{C}H_2CO_2Me$), 34.7 ($\mathit{C}H_2Ph$); MS (ES^+) *m/z* 352 $[M+H]^+$, 374 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{16}H_{21}N_3NaO_6$ $[M+Na]^+$: 374.1323; found: 374.1326.

***tert*-Butyl glycinate (174)**[152]



This known compound was prepared according to a literature procedure.[92] To a stirred solution of glycine *tert*-butyl ester hydrochloride (878 mg, 5.24 mmol) in anhydrous chloroform (6.1 mL) was added a solution of NEt_3 (0.73 mL, 5.24 mmol) in anhydrous chloroform (6.1 mL). The reaction mixture was stirred at rt for 3 h then at 70 °C for 1 h before it was cooled to rt and concentrated *in vacuo*. The white solid formed was diluted with Et_2O and filtered under vacuum. The filtrate was concentrated *in vacuo* to give **174** (642 mg, 93%) as a pale yellow liquid, which was used without further purification. Data in agreement with literature values.[152] δ_H (400 MHz, $CDCl_3$) 3.27 (2H, s, CH_2), 1.88 (2H, br s, NH_2), 1.41 (9H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 173.3 (C=O), 81.2 (C), 44.5 (CH_2), 28.1 (CH_3); MS (ES^+) *m/z* 132 $[M+H]^+$, 154 $[M+Na]^+$.

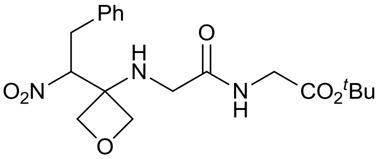
***tert*-Butyl [(benzyloxy)carbonyl]glycylglycinate (175)**[153]



This known compound was prepared using a modified literature procedure.[154] To a stirred solution of Z-Gly-OH (512 mg, 2.45 mmol), DCC (550 mg, 2.67 mmol), HOBt (410 mg, 2.67 mmol) and DIEA (0.46 mL, 2.64 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C was added a solution of **174** (351 mg, 2.68 mmol) in anhydrous CH_2Cl_2 (10 mL). The reaction mixture was stirred at rt for 40 h, then filtered through Celite®, washed with CH_2Cl_2

and concentrated *in vacuo*. Purification by column chromatography (45 → 60% EtOAc in petroleum ether + 0.5% NEt_3) provided **175** (674 mg, 85%) as a pale yellow oil. Data in agreement with literature values.[153] R_f = 0.29 (60% EtOAc in petroleum ether); IR (film) 3323, 2979, 1725, 1667, 1527, 1368, 1224, 1153, 737, 697 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.28 (5H, m, Ar*H*), 6.61 – 6.53 (1H, m, N*H*), 5.60 – 5.52 (1H, m, N*H*), 5.12 (2H, s, $PhCH_2O$), 3.95 – 3.89 (4H, m, CH_2), 1.46 (9H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 169.2 (C=O), 168.9 (C=O), 156.7 (C=O), 136.2 (C, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 82.6 (C), 67.4 ($PhCH_2O$), 44.5 (CH_2), 42.0 (CH_2), 28.1 (CH_3); MS (ES^+) *m/z* 345 $[M+Na]^+$.

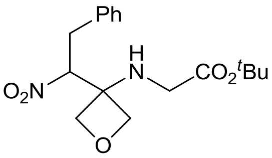
tert-Butyl [[3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycyl]glycinate (177)



This novel compound was prepared using modified literature procedures.[91,134] A suspension of **175** (250 mg, 0.78 mmol) and 10% Pd/C (51 mg) in anhydrous THF (4 mL) was stirred under an atmosphere of hydrogen at rt for 6 h before it was filtered through Celite® and washed with anhydrous THF (4 mL). This crude solution was added to a stirred solution of **164** (96 mg, 0.47 mmol) in anhydrous THF (0.5 mL). The reaction mixture was stirred at rt for 72 h then concentrated *in vacuo*. Purification by column chromatography (75 → 90% EtOAc in petroleum ether) provided **177** (52 mg, 28%) as a colourless oil as well as recovered **164** (68 mg). R_f = 0.27 (90% EtOAc in petroleum ether); IR (film) 3323, 2981, 1738, 1671, 1596, 1553, 1537, 1457, 1369, 1230, 1157, 985, 701 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.20 (6H, m, Ar*H*, CON*H*), 5.21 – 5.15 (1H, m, C*H*), 4.69 – 4.65 (1H, m, OC*H*H), 4.63 – 4.57 (2H, m, OCH*H*, OC*H*H), 4.56 – 4.52 (1H, m, OCH*H*), 4.08 – 3.99 (1H, m, $CHHCO_2{}^tBu$), 3.95 – 3.86 (1H, m, $CHHCO_2{}^tBu$), 3.68

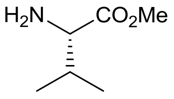
– 3.52 (3H, m, NHC*H*$_2$CO, C*H*HPh), 3.34 – 3.26 (1H, m, CH*H*Ph), 2.52 (1H, br s, N*H*), 1.47 (9H, s, C*H*$_3$); δ$_C$ (100 MHz, CDCl$_3$) 171.0 (C=O), 168.9 (C=O), 135.2 (C, Ar), 129.2 (CH, Ar), 128.8 (CH, Ar), 127.9 (CH, Ar), 93.3 (CH), 82.6 (C, ^{t}Bu), 77.0 (OCH$_2$), 76.7 (OCH$_2$), 62.4 (C, oxetane), 46.1 (NH*C*H$_2$CO), 41.8 (*C*H$_2$CO$_2$^{t}Bu), 34.8 (*C*H$_2$Ph), 28.2 (CH$_3$); MS (ES$^+$) *m/z* 394 [M+H]$^+$, 416 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{19}H_{27}N_3NaO_6$ [M+Na]$^+$: 416.1792; found: 416.1796.

tert-Butyl [3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycinate (178)



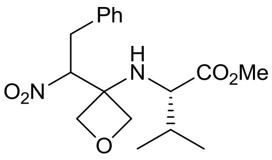
This novel compound was prepared using a modified literature procedure.[91] To a stirred solution of **164** (27 mg, 0.13 mmol) in anhydrous THF (2 mL) was added a solution of **174** (26 mg, 0.20 mmol) in anhydrous THF (0.5 mL). The reaction mixture was stirred at rt overnight then concentrated *in vacuo*. Purification by column chromatography (30% EtOAc in petroleum ether) provided **178** (39 mg, 88%) as a white solid. M.p. 124 – 125 °C; R$_f$ = 0.35 (30% EtOAc in petroleum ether); IR (film) 3334, 2977, 1729, 1605, 1552, 1497, 1455, 1367, 1237, 1153, 983, 749, 699 cm^{-1}; δ$_H$ (400 MHz, CDCl$_3$) 7.34 – 7.20 (5H, m, Ar*H*), 5.12 (1H, dd, *J* = 10.0, 4.3, C*H*), 4.64 (1H, d, *J* = 7.3, OC*H*H), 4.61 (1H, d, *J* = 7.3, OCH*H*), 4.52 – 4.48 (2H, m, OC*H*$_2$), 3.60 (1H, d, *J* = 17.4, NHC*H*H), 3.53 (1H, dd, *J* = 14.7, 10.0, C*H*HPh), 3.49 (1H, d, *J* = 17.4, NHCH*H*), 3.27 (1H, dd, *J* = 14.7, 4.3, CH*H*Ph), 2.46 (1H, br s, N*H*), 1.49 (9H, s, C*H*$_3$); δ$_C$ (100 MHz, CDCl$_3$) 171.3 (C=O), 135.5 (C, Ar), 129.1 (CH, Ar), 128.9 (CH, Ar), 127.7 (CH, Ar), 93.1 (CH), 82.2 (C, ^{t}Bu), 77.3 (OCH$_2$), 77.3 (OCH$_2$), 62.4 (C, oxetane), 45.5 (NHCH$_2$), 34.5 (*C*H$_2$Ph), 28.2 (CH$_3$); MS (ES$^+$) *m/z* 337 [M+H]$^+$, 359 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{17}H_{24}N_2NaO_5$ [M+Na]$^+$: 359.1577; found: 359.1582.

Methyl *L*-valinate (179)[155]



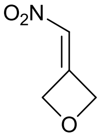
This known compound was prepared according to a literature procedure.[92] To a stirred solution of L-valine methyl ester hydrochloride (151 mg, 0.90 mmol) in anhydrous chloroform (1 mL) was added a solution of NEt_3 (0.12 mL, 0.86 mmol) in anhydrous chloroform (1.5 mL). The reaction mixture was stirred at rt for 2 h then at 70 °C for 1 h before it was cooled to rt and concentrated *in vacuo*. The white solid formed was diluted with Et_2O and filtered under vacuum. The filtrate was concentrated *in vacuo* to give **179** (88 mg, 74%) as a pale yellow liquid, which was used without further purification. Data in agreement with literature values.[155] δ_H (400 MHz, $CDCl_3$) 3.72 (3H, s, CO_2CH_3), 3.32 (1H, d, J = 5.0, CH^iPr), 2.08 – 1.98 (1H, m, $CHMe_2$), 1.83 (2H, br s, NH_2), 0.97 (3H, d, J = 6.8, CH_3), 0.91 (3H, d, J = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 176.1 (C=O), 60.0 (CH^iPr), 51.8 (CH_3), 32.2 ($CHMe_2$), 19.3 (CH_3), 17.3 (CH_3); MS (ES^+) *m/z* 132 $[M+H]^+$.

Methyl [3-(1-nitro-2-phenylethyl)oxetan-3-yl]-*L*-valinate (180a/b)



This novel compound was prepared using a modified literature procedure.[91] To a stirred solution of **164** (105 mg, 0.51 mmol) in anhydrous THF (4 mL) was added a solution of **179** (130 mg, 0.99 mmol) in anhydrous THF (4 mL). The reaction mixture was stirred at rt for 64 h then concentrated *in vacuo*. Purification by repeat column chromatography (10 → 25% EtOAc in petroleum ether; 50 → 80% CH_2Cl_2 in petroleum ether) provided **180a/b** (137 mg, 80%) as a 55 : 45 mixture of diastereoisomers as an oily white solid. R_f = 0.28, 0.36 (30% EtOAc in petroleum ether); IR (film) 3336, 2962, 2882, 1729, 1552, 1496, 1455, 1366, 1200, 1153, 985, 744, 699 cm^{-1}; δ_H (400 MHz, CDCl$_3$) 7.35 – 7.20 (9.3H, m, Ar*H* D$_1$ and D$_2$), 5.17 (1H, dd, *J* = 10.0, 4.3, C*H*Bn D$_1$), 5.10 (0.86H, dd, *J* = 10.3, 3.8, C*H*Bn D$_2$), 4.72 (0.86H, d, *J* = 7.7, OC*H*H D$_2$), 4.59 (0.86H, d, *J* = 7.7, OC*H*H D$_2$), 4.56 (0.86H, d, *J* = 7.7, OCH*H* D$_2$), 4.54 – 4.51 (2H, m, OC*H*H D$_1$), 4.45 (1H, d, *J* = 7.7, OCH*H* D$_1$), 4.41 (1.86H, d, *J* = 7.7, OCH*H* D$_1$ and D$_2$), 3.75 (2.58H, s, CO$_2$C*H*$_3$ D$_2$), 3.74 (3H, s, CO$_2$C*H*$_3$ D$_1$), 3.62 – 3.43 (3.72H, m, C*H*HPh D$_1$ and D$_2$, C*H*^{i}Pr D$_1$ and D$_2$), 3.28 – 3.20 (1.86H, m, CH*H*Ph D$_1$ and D$_2$), 2.63 (1H, d, *J* = 10.5, N*H* D$_1$), 2.24 (0.86H, d, *J* = 10.3, N*H* D$_2$), 2.10 – 2.01 (1H, m, C*H*Me$_2$ D$_1$), 2.01 – 1.90 (0.86H, m, C*H*Me$_2$ D$_2$), 1.00 (3H, d, *J* = 6.5, C*H*$_3$ D$_1$), 0.92 (3H, d, *J* = 6.5, C*H*$_3$ D$_1$), 0.91 (2.58H, d, *J* = 6.8, C*H*$_3$ D$_2$), 0.89 (2.58H, d, *J* = 6.8, C*H*$_3$ D$_2$); δ_C (100 MHz, CDCl$_3$) 176.2 (C=O, D$_2$), 176.0 (C=O, D$_1$), 135.6 (C, Ar, D$_2$), 135.5 (C, Ar, D$_1$), 129.1 (CH, Ar, D$_1$ and D$_2$), 128.9 (CH, Ar, D$_1$ and D$_2$), 127.7 (CH, Ar, D$_1$), 127.7 (CH, Ar, D$_2$), 94.3 (CH, D$_1$), 92.1 (CH, D$_2$), 78.2 (OCH$_2$, D$_2$), 78.0 (OCH$_2$, D$_2$), 77.6 (OCH$_2$, D$_1$), 77.3 (OCH$_2$, D$_1$), 62.4 (C, D$_2$), 62.3 (C, D$_1$), 60.8 (*C*H^{i}Pr, D$_2$), 60.6 (*C*H^{i}Pr, D$_1$), 52.3 (CO$_2$*C*H$_3$, D$_1$), 52.2 (CO$_2$*C*H$_3$, D$_2$), 34.7 (*C*H$_2$Ph, D$_1$), 34.1 (*C*H$_2$Ph, D$_2$), 32.5 (*C*HMe$_2$, D$_1$), 32.3 (*C*HMe$_2$, D$_2$), 19.6 (CH$_3$, D$_1$), 19.3 (CH$_3$, D$_2$), 18.1 (CH$_3$, D$_2$), 17.9 (CH$_3$, D$_1$); MS (ES$^+$) *m/z* 337 [M+H]$^+$, 359 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{17}H_{24}N_2NaO_5$ [M+Na]$^+$: 359.1577; found: 359.1579.

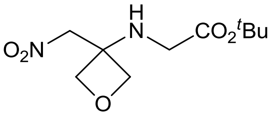
3-(Nitromethylene)oxetane (162)[9]



This known compound was prepared according to a literature procedure.[9] To a stirred solution of 3-oxetanone (178 µL, 2.77 mmol) in nitromethane (3.2 mL) was added NEt$_3$ (50 µL, 0.36 mmol). The reaction mixture was stirred at rt for 20 min then concentrated *in vacuo*. The residue was diluted with anhydrous CH$_2$Cl$_2$ (10 mL) then cooled to -78 °C. To this solution was added NEt$_3$

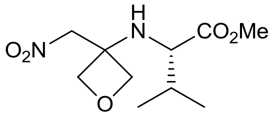
(1.7 mL, 12.2 mmol), followed by MsCl (640 µL, 8.27 mmol) dropwise over 10 min. The reaction mixture was stirred at -78 °C for 20 min then loaded directly onto a silica column. Purification by column chromatography (50% Et_2O in petroleum ether) provided **162** (104 mg, ~33%) as a pale yellow solid containing some impurities. Product was taken through without further purification.

tert-Butyl [3-(nitromethyl)oxetan-3-yl]glycinate (192)



This novel compound was prepared using a modified literature procedure.[91] A solution of **174** (144 mg, 1.10 mmol) in anhydrous THF (2 mL) was added to a stirred solution of **162** (49 mg, 0.43 mmol) in anhydrous THF (2 mL). The reaction mixture was stirred at rt overnight then concentrated *in vacuo*. Purification by column chromatography (75% Et_2O in petroleum ether) provided **192** (72 mg, 69%) as white crystals. M.p. 67 – 68 °C; R_f = 0.30 (80% Et_2O in petroleum ether); IR (film) 3330, 2977, 1731, 1554, 1455, 1436, 1369, 1238, 1155 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.81 (2H, s, NO_2CH_2), 4.62 (2H, d, *J* = 7.3, OC*H*H), 4.56 (2H, d, *J* = 7.3, OCH*H*), 3.42 (2H, d, *J* = 5.0, $CH_2CO_2{}^tBu$), 2.35 (1H, br s, N*H*), 1.46 (9H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 171.0 (C=O), 82.3 (C, ^{t}Bu), 78.8 (CH_2), 78.4 (2 x CH_2), 59.6 (C, oxetane), 45.6 ($CH_2CO_2{}^tBu$), 28.1 (CH_3); MS (ES$^+$) *m/z* 247 [M+H]$^+$, 269 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{10}H_{18}N_2NaO_5$ [M+Na]$^+$: 269.1108; found: 269.1108.

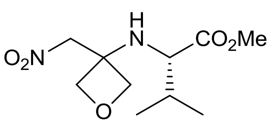
Methyl [3-(nitromethyl)oxetan-3-yl]-*L*-valinate ((*S*)-193)



This novel compound was prepared using a modified literature procedure.[91] A solution of **179** (100 mg, 0.76 mmol) in anhydrous THF (2 mL) was added to a stirred solution of **162** (44 mg, 0.38

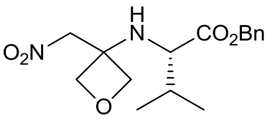
mmol) in anhydrous THF (2 mL). The reaction mixture was stirred at rt overnight then concentrated *in vacuo*. Purification by column chromatography (75% Et_2O in petroleum ether) provided (*S*)-**193** (64 mg, 68%) as a yellow oil. $R_f = 0.19$ (100% CH_2Cl_2); $[\alpha]_D^{33}$ –19.4 (*c* 0.24, $CHCl_3$); IR (film) 3333, 2962, 2878, 1730, 1555, 1467, 1435, 1379, 1276, 1202 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.85 (1H, d, *J* = 13.1, NO_2CHH), 4.77 (1H, d, *J* = 13.1, NO_2CH*H*), 4.60 (2H, d, *J* = 7.0, OC*H*H), 4.50 (1H, d, *J* = 7.0, OCH*H*), 4.46 (1H, d, *J* = 7.0, OCH*H*), 3.72 (3H, s, CO_2CH_3), 3.22 (1H, dd, *J* = 10.8, 6.0, CH^iPr), 2.27 (1H, d, *J* = 10.8, N*H*), 1.98 – 1.85 (1H, m, C*H*Me_2), 0.92 (3H, d, *J* = 6.8, CH_3), 0.89 (3H, d, *J* = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 175.9 (C=O), 79.0 (CH_2), 78.7 (CH_2), 78.6 (CH_2), 61.4 (CH^iPr), 59.5 (C), 52.3 (CO_2CH_3), 32.2 (*C*HMe_2), 19.4 (CH_3), 18.1 (CH_3); MS (ES^+) *m/z* 269 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{10}H_{18}N_2NaO_5$ $[M+Na]^+$: 269.1108; found: 269.1105.

Methyl [3-(nitromethyl)oxetan-3-yl]-*L*-valinate ((*S*)-193)



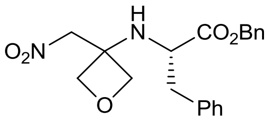
This novel compound was prepared as follows. Nitromethane (70 µL, 1.29 mmol), 3-oxetanone (108 µL, 1.68 mmol) and NEt_3 (36 µL, 0.26 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (6.4 mL) and cooled to -78 °C. To this solution was added NEt_3 (360 µL, 2.58 mmol), followed by MsCl (100 µL, 1.29 mmol) dropwise over 10 min. The reaction mixture was stirred at -78 °C for 40 min. Meanwhile, to a stirred solution of L-valine methyl ester hydrochloride (434 mg, 2.59 mmol) in anhydrous CH_2Cl_2 (8 mL) was added NEt_3 (360 µL, 2.58 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at -78 °C. The reaction mixture was allowed to warm to rt, stirred for 23 h, then quenched with saturated aqueous NH_4Cl solution (10 mL). The mixture was extracted with CH_2Cl_2 (5 x 30 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (20 → 25% EtOAc in hexane; 0 → 2% MeOH in CH_2Cl_2) provided (*S*)-**193** (149 mg, 47%) as a pale yellow oil. Data as previously reported.

Benzyl [3-(nitromethyl)oxetan-3-yl]-*L*-valinate (194)



This novel compound was prepared as follows. Nitromethane (177 µL, 3.27 mmol), 3-oxetanone (273 µL, 4.26 mmol) and NEt_3 (91 µL, 0.65 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (16 mL) and cooled to –78 °C. To this solution was added NEt_3 (0.91 mL, 6.53 mmol), followed by MsCl (255 µL, 3.29 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of L-valine benzyl ester *p*-toluenesulfonate salt (2.49 g, 6.56 mmol) in anhydrous CH_2Cl_2 (19 mL) was added NEt_3 (0.91 mL, 6.53 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 21 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (2 x 40 mL) and EtOAc (2 x 40 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 40% EtOAc in heptane) provided **194** (876 mg, 83%) as a pale pink oil. R_f = 0.33 (30% EtOAc in heptane); $[\alpha]_D^{17}$ –4.5 (*c* 0.20, $CHCl_3$); IR (KBr) 3338, 2963, 2877, 1732, 1560, 1498, 1457, 1379, 1268, 1184, 983, 738 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.40 – 7.31 (5H, m, Ar*H*), 5.17 (1H, d, *J* = 12.0, OC*H*HPh), 5.11 (1H, d, *J* = 12.0, OCH*H*Ph), 4.80 (1H, dd, *J* = 13.1, 0.9, NO_2C*H*H), 4.72 (1H, dd, *J* = 13.1, 0.9, NO_2CH*H*), 4.55 (1H, d, *J* = 7.1, OC*H*H), 4.53 (1H, d, *J* = 7.1, OC*H*H), 4.45 (1H, d, *J* = 7.1, OCH*H*), 4.35 (1H, d, *J* = 7.1, OCH*H*), 3.25 (1H, dd, *J* = 10.9, 5.9, C*H*^{i}Pr), 2.29 (1H, d, *J* = 10.9, N*H*), 2.00 – 1.88 (1H, m, C*H*Me$_2$), 0.91 (3H, d, *J* = 6.8, C*H*$_3$), 0.86 (3H, d, *J* = 6.8, C*H*$_3$); δ_C (175 MHz, CDCl$_3$) 175.3 (C=O), 135.4 (C, Ar), 128.9 (CH, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 78.9 (CH$_2$), 78.7 (CH$_2$), 78.7 (CH$_2$), 67.3 (O*C*H$_2$Ph), 61.5 (*C*H^{i}Pr), 59.6 (C), 32.2 (*C*HMe$_2$), 19.4 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) *m/z* 323 [M+H]$^+$; HRMS (ES$^+$) calcd. for $C_{16}H_{23}N_2O_5$ [M+H]$^+$: 323.1602; found: 323.1606.

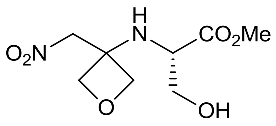
Benzyl [3-(nitromethyl)oxetan-3-yl]-*L*-phenylalaninate (195)



This novel compound was prepared as follows. Nitromethane (220 µL, 4.06 mmol), 3-oxetanone (339 µL, 5.28 mmol) and NEt$_3$ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH$_2$Cl$_2$ (20 mL) and cooled to –78 °C. To this solution was added NEt$_3$ (1.13 mL, 8.11 mmol), followed by MsCl (317 µL, 4.09 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 35 min. Meanwhile, to a stirred solution of L-phenylalanine benzyl ester hydrochloride (2.38 g, 8.16 mmol) in anhydrous CH$_2$Cl$_2$ (25 mL) was added NEt$_3$ (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 22 h, then quenched with saturated aqueous NH$_4$Cl solution (30 mL). The mixture was extracted with CH$_2$Cl$_2$ (1 x 30 mL) and EtOAc (3 x 40 mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 40% EtOAc in heptane) gave a yellow solid. Recrystallisation (MeOH) provided **195** (1.10 g, 73%) as pale yellow crystals. M.p. 92 – 93 °C (MeOH); R$_f$ = 0.25 (30% EtOAc in heptane); $[\alpha]_D^{17}$ –9.6 (*c* 0.20, CHCl$_3$);

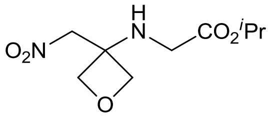
IR (KBr) 3335, 2957, 2880, 1732, 1603, 1556, 1497, 1455, 1379, 1275, 1175, 982, 749 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.37 – 7.33 (3H, m, Ar*H*), 7.28 – 7.21 (5H, m, Ar*H*), 7.13 – 7.09 (2H, m, Ar*H*), 5.11 (1H, d, *J* = 12.0, OC*H*HPh), 5.05 (1H, d, *J* = 12.0, OCH*H*Ph), 4.75 (1H, dd, *J* = 13.1, 0.8, NO_2C*H*H), 4.69 (1H, d, *J* = 13.1, NO_2CH*H*), 4.47 (1H, d, *J* = 7.2, OC*H*H), 4.35 (1H, d, *J* = 7.3, OC*H*H), 4.33 (1H, d, *J* = 7.2, OCH*H*), 4.25 (1H, d, *J* = 7.3, OCH*H*), 3.77 – 3.70 (1H, m, C*H*), 2.97 (1H, dd, *J* = 13.4, 6.3, C*H*HPh), 2.87 (1H, dd, *J* = 13.4, 7.1, CH*H*Ph), 2.37 (1H, d, *J* = 9.6, N*H*); δ_C (175 MHz, $CDCl_3$) 174.5 (C=O), 136.6 (C, Ar), 135.2 (C, Ar), 129.6 (CH, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.6 (CH, Ar), 127.2 (CH, Ar), 78.8 (CH_2), 78.7 (CH_2), 78.6 (CH_2), 67.5 (O*C*H_2Ph), 59.6 (C), 57.7 (CH), 40.8 (*C*H_2Ph); MS (ES$^+$) *m/z* 371 [M+H]$^+$; HRMS (ES$^+$) calcd. for $C_{20}H_{23}N_2O_5$ [M+H]$^+$: 371.1607; found: 371.1607; CHN calcd. for $C_{20}H_{22}N_2O_5$: C, 64.73 H, 5.92 N, 7.53%; found: C, 64.85 H, 5.99 N, 7.56%.

Methyl [3-(nitromethyl)oxetan-3-yl]-*L*-serinate (196)



This novel compound was prepared as follows. Nitromethane (70 µL, 1.29 mmol), 3-oxetanone (107 µL, 1.67 mmol) and NEt_3 (36 µL, 0.26 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (6.3 mL) and cooled to –78 °C. To this solution was added NEt_3 (357 µL, 2.56 mmol), followed by MsCl (99 µL, 1.28 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 30 min. Meanwhile, to a stirred solution of L-serine methyl ester hydrochloride (401 mg, 2.58 mmol) in anhydrous CH_2Cl_2 (10 mL) was added NEt_3 (357 µL, 2.56 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 25 h, then quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with CH_2Cl_2 (2 x 30 mL) and EtOAc (2 x 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (65 → 70% EtOAc in hexane) provided **196** (146 mg, 48%) as a colourless oil. R_f = 0.28 (80% EtOAc in petroleum ether); $[\alpha]_D^{30}$ –23.3 (*c* 0.20, $CHCl_3$); IR (film) 3346, 2956, 2886, 1732, 1553, 1459, 1380, 1207, 1060 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.85 (1H, d, *J* = 13.2, $NO_2C\mathit{H}H$), 4.80 (1H, d, *J* = 13.2, $NO_2CH\mathit{H}$), 4.61 (2H, d, *J* = 7.3, $OC\mathit{H}H$), 4.53 (2H, d, *J* = 7.3, $OCH\mathit{H}$), 3.79 – 3.73 (4H, m, $C\mathit{H_3}$, $C\mathit{H}HOH$), 3.65 – 3.59 (2H, m, $CH\mathit{H}OH$, $C\mathit{H}$), 2.70 (2H, br s, $O\mathit{H}$, $N\mathit{H}$); δ_C (100 MHz, $CDCl_3$) 173.6 (C=O), 78.9 (CH_2), 78.7 (CH_2), 78.5 (CH_2), 63.9 (CH_2OH), 59.3 (C), 57.5 (CH), 52.9 (CH_3); MS (ES^+) *m/z* 257 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_8H_{14}N_2NaO_6$ $[M+Na]^+$: 257.0744; found: 257.0746.

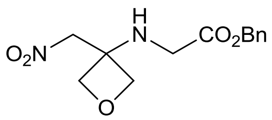
Isopropyl [3-(nitromethyl)oxetan-3-yl]glycinate (197)



This novel compound was prepared as follows. Nitromethane (220 µL, 4.06 mmol), 3-oxetanone (339 µL, 5.28 mmol) and NEt_3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (20 mL) and cooled to -78 °C. To this solution was added NEt_3 (1.13 mL, 8.11 mmol), followed by MsCl (320 µL, 4.13 mmol) dropwise over 10 min. The reaction mixture was stirred at -78 °C for 30 min. Meanwhile, to a stirred solution of glycine isopropyl ester hydrochloride (1.25 g, 8.14 mmol) in anhydrous CH_2Cl_2 (25 mL) was added NEt_3 (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at -78 °C. The reaction mixture was allowed to warm to rt, stirred for 22 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2

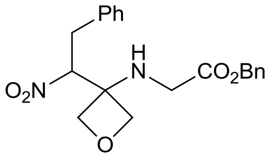
(1 x 30 mL) and EtOAc (4 x 30 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (30 → 50% EtOAc in heptane) provided **197** (580 mg, 61%) as a pale pink oil. R_f = 0.20 (40% EtOAc in heptane); IR (KBr) 3335, 2982, 2881, 1731, 1556, 1469, 1384, 1207, 1108 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.07 (1H, septet, J = 6.2, C*H*), 4.82 (2H, s, NO_2CH_2), 4.64 (2H, d, J = 7.3, OC*H*H), 4.58 (2H, d, J = 7.3, OCH*H*), 3.50 (2H, s, $CH_2CO_2{}^iPr$), 2.38 (1H, br s, N*H*), 1.27 (6H, d, J = 6.2, CH_3); δ_C (175 MHz, $CDCl_3$) 171.4 (C=O), 78.7 (CH_2), 78.4 (2 x CH_2), 69.4 (CH), 59.6 (C), 45.2 ($CH_2CO_2{}^iPr$), 21.9 (CH_3); MS (ES^+) *m/z* 233 $[M+H]^+$; HRMS (ES^+) calcd. for $C_9H_{17}N_2O_5$ $[M+H]^+$: 233.1137; found: 233.1139.

Benzyl [3-(nitromethyl)oxetan-3-yl]glycinate (198)



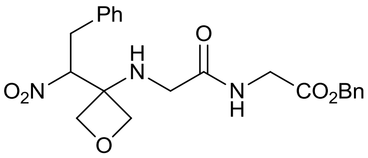
This novel compound was prepared as follows. Nitromethane (220 µL, 4.06 mmol), 3-oxetanone (340 µL, 5.30 mmol) and NEt_3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (20 mL) and cooled to –78 °C. To this solution was added NEt_3 (1.13 mL, 8.11 mmol), followed by MsCl (315 µL, 4.07 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of glycine benzyl ester *p*-toluenesulfonate salt (2.75 g, 8.15 mmol) in anhydrous CH_2Cl_2 (25 mL) was added NEt_3 (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 21 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (4 x 75 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (60 → 80% Et_2O in hexane) gave a yellow solid. Recrystallisation (EtOH/hexane) provided **198** (728 mg, 64%) as off-white crystals. M.p. 77 – 79 °C (EtOH); R_f = 0.30 (50% EtOAc in petroleum ether); IR (film) 3342, 2962, 2885, 1736, 1550, 1498, 1456, 1436, 1380, 1190, 1102, 976, 737, 696 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.41 – 7.32 (5H, m, Ar*H*), 5.18 (2H, s, OC*H*$_2$Ph), 4.82 (2H, s, NO_2CH_2), 4.61 (2H, d, *J* = 7.3, OC*H*H), 4.56 (2H, d, *J* = 7.3, OCH*H*), 3.58 (2H, d, *J* = 5.3, C*H*$_2$$CO_2$Bn), 2.42 – 2.36 (1H, m, N*H*); δ_C (100 MHz, $CDCl_3$) 171.7 (C=O), 135.2 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 78.5 (CH_2), 78.3 (2 x CH_2), 67.3 (O*C*H_2Ph), 59.5 (C), 44.9 (*C*H_2CO_2Bn); MS (ES^+) *m/z* 281 $[M+H]^+$, 303 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{16}N_2NaO_5$ $[M+Na]^+$: 303.0951; found: 303.0957.

Benzyl [3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycinate (199)



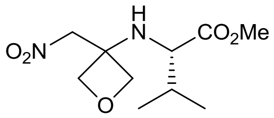
This novel compound was prepared as follows. (2-Nitroethyl)benzene (196 mg, 1.30 mmol), 3-oxetanone (108 µL, 1.68 mmol) and NEt_3 (36 µL, 0.26 mmol) were stirred at rt for 90 min then diluted with anhydrous CH_2Cl_2 (6.4 mL) and cooled to –78 °C. To this solution was added NEt_3 (358 µL, 2.57 mmol), followed by MsCl (100 µL, 1.29 mmol) dropwise. The reaction mixture was stirred at –78 °C for 90 min. Meanwhile, to a stirred solution of glycine benzyl ester *p*-toluenesulfonate salt (965 mg, 2.86 mmol) in anhydrous CH_2Cl_2 (9 mL) was added NEt_3 (360 µL, 2.58 mmol). The mixture was stirred at rt for 10 min before it was added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt overnight, then quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with CH_2Cl_2 (2 x 50 mL) and EtOAc (2 x 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (35 → 100% EtOAc in hexane; 20 → 30% EtOAc in hexane) provided **199** (280 mg, 58%) as a pale yellow solid. M.p. 53 – 54 °C; R_f = 0.34 (40% EtOAc in hexane); IR (film) 3342, 2958, 2883, 1736, 1604, 1551, 1496, 1455, 1367, 1189, 1083, 981, 737 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.19 (10H, m, Ar*H*), 5.20 (2H, s, OCH_2Ph), 5.11 (1H, dd, *J* = 9.9, 4.4, C*H*), 4.61 (1H, d, *J* = 7.5, OC*H*H), 4.59 (1H, d, *J* = 7.5, OCH*H*), 4.47 (2H, s, OCH_2), 3.75 (1H, d, *J* = 17.6, C*H*HCO_2Bn), 3.65 (1H, d, *J* = 17.6, CH*H*CO_2Bn), 3.51 (1H, dd, *J* = 14.7, 9.9, C*H*HPh), 3.27 (1H, dd, *J* = 14.7, 4.4, CH*H*Ph), 2.50 (1H, br s, N*H*); δ_C (100 MHz, acetone-d_6) 172.6 (C=O), 137.2 (C, Ar), 137.1 (C, Ar), 129.9 (CH, Ar), 129.5 (CH, Ar), 129.3 (CH, Ar), 129.1 (CH, Ar), 129.0 (CH, Ar), 128.0 (CH, Ar), 92.8 (CH), 77.5 (OCH_2), 77.1 (OCH_2), 67.1 (OCH_2Ph), 63.1 (C), 45.2 (*C*H_2CO_2Bn), 35.1 (CH_2Ph); MS (ES^+) *m/z* 371 $[M+H]^+$, 393 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{20}H_{23}N_2O_5$ $[M+H]^+$: 371.1601; found: 371.1602.

Benzyl [[3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycyl]glycinate (200)



This novel compound was prepared as follows. (2-Nitroethyl)benzene (303 mg, 2.00 mmol), 3-oxetanone (167 µL, 2.60 mmol) and NEt_3 (56 µL, 0.40 mmol) were stirred at rt for 90 min then diluted with anhydrous CH_2Cl_2 (12.5 mL) and cooled to –78 °C. To this solution was added NEt_3 (559 µL, 4.01 mmol), followed by MsCl (156 µL, 2.01 mmol) dropwise. The reaction mixture was stirred at –78 °C for 90 min. Meanwhile, to a stirred solution of glycylglycine benzyl ester *p*-toluenesulfonate salt (1.74 g, 4.41 mmol) in anhydrous CH_2Cl_2 (10 mL) was added NEt_3 (559 µL, 4.01 mmol). The mixture was stirred at rt for 10 min before being added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt overnight, then quenched with saturated aqueous NH_4Cl solution (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 50 mL) and EtOAc (1 x 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (65 → 100% EtOAc in heptane; 45 → 80% EtOAc in heptane) provided **200** (549 mg, 64%) as a sticky yellow solid. $R_f = 0.23$ (80% EtOAc in heptane); IR (KBr) 3379, 3342, 2956, 2887, 1744, 1668, 1605, 1549, 1521, 1497, 1455, 1189, 1030, 736, 698 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.39 – 7.28 (9H, m, Ar*H*, CON*H*), 7.22 (2H, d, *J* = 7.3, Ar*H*), 5.19 (2H, s, OCH_2Ph), 5.17 (1H, dd, *J* = 10.3, 3.8, C*H*), 4.66 (1H, d, *J* = 7.7, OC*H*H), 4.61 (1H, d, *J* = 7.7, OCH*H*), 4.59 (1H, d, *J* = 8.0, OC*H*H), 4.53 (1H, d, *J* = 8.0, OCH*H*), 4.20 (1H, dd, *J* = 18.4, 6.1, C*H*HCO_2Bn), 4.06 (1H, dd, *J* = 18.4, 6.1, CH*H*CO_2Bn), 3.66 (1H, d, *J* = 17.2, NHC*H*HCO), 3.60 (1H, d, *J* = 17.2, NHCH*H*CO), 3.56 (1H, dd, *J* = 14.8, 10.3, C*H*HPh), 3.28 (1H, dd, *J* = 14.8, 3.8, CH*H*Ph), 2.51 (1H, br s, N*H*); δ_C (175 MHz, $CDCl_3$) 171.3 (C=O), 169.7 (C=O), 135.2 (C, Ar), 135.2 (C, Ar), 129.3 (CH, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 127.9 (CH, Ar), 93.3 (CH), 77.0 (OCH_2), 76.6 (OCH_2), 67.5 (O*C*H_2Ph), 62.4 (C), 46.1 (*C*H_2CO_2Bn), 41.2 (NH*C*H_2CO), 34.8 (*C*H_2Ph); MS (ES^+) *m/z* 428 $[M+H]^+$, 450 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{22}H_{26}N_3O_6$ $[M+H]^+$: 428.1816; found: 428.1812.

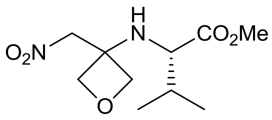
Methyl [3-(nitromethyl)oxetan-3-yl]-*L*-valinate ((*S*)-193)



This novel compound was prepared as follows. 3-Oxetanone (130 µL, 2.03 mmol), nitromethane (154 µL, 2.85 mmol) and NEt_3 (57 µL, 0.41 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (10 mL) and cooled to –78 °C. To this solution was added NEt_3 (565 µL, 4.05 mmol), followed by MsCl (157 µL, 2.03 mmol) dropwise over 10 min.

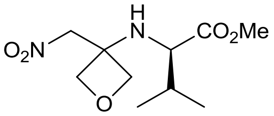
The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of L-valine methyl ester hydrochloride (681 mg, 4.06 mmol) in anhydrous CH_2Cl_2 (12.5 mL) was added NEt_3 (565 µL, 4.05 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with CH_2Cl_2 (5 x 30 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 25% EtOAc in hexane) provided (*S*)-**193** (323 mg, 65%) as a yellow oil. Data as previously reported.

Methyl [3-(nitromethyl)oxetan-3-yl]-*L*-valinate ((*S*)-193)



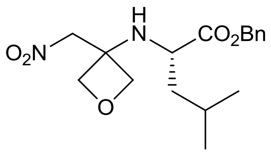
This novel compound was prepared as follows. 3-Oxetanone (130 µL, 2.03 mmol), nitromethane (154 µL, 2.85 mmol) and NEt_3 (57 µL, 0.41 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (10 mL) and cooled to -78 °C. To this solution was added NEt_3 (565 µL, 4.05 mmol), followed by MsCl (157 µL, 2.03 mmol) dropwise over 10 min. The reaction mixture was stirred at -78 °C for 40 min. Meanwhile, to a stirred solution of L-valine methyl ester hydrochloride (409 mg, 2.44 mmol) in anhydrous CH_2Cl_2 (12.5 mL) was added NEt_3 (339 µL, 2.43 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at -78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with CH_2Cl_2 (5 x 30 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 25% EtOAc in hexane) provided (*S*)-**193** (202 mg, 40%) as a yellow oil. Data as previously reported.

Methyl [3-(nitromethyl)oxetan-3-yl]-*D*-valinate ((*R*)-193)



This novel compound was prepared as follows. 3-Oxetanone (130 µL, 2.03 mmol), nitromethane (154 µL, 2.85 mmol), and NEt_3 (57 µL, 0.41 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (10 mL) and cooled to –78 °C. To this solution was added NEt_3 (565 µL, 4.05 mmol), followed by MsCl (157 µL, 2.03 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of D-valine methyl ester hydrochloride (682 mg, 4.07 mmol) in anhydrous CH_2Cl_2 (12.5 mL) was added NEt_3 (565 µL, 4.05 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with CH_2Cl_2 (5 x 30 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 25% EtOAc in hexane) provided (*R*)-**193** (337 mg, 68%) as a yellow oil. R_f = 0.23 (40% EtOAc in hexane); $[\alpha]_D^{25}$ +16.6 (*c* 0.22, $CHCl_3$); IR (film) 3330, 2960, 2877, 1729, 1555, 1466, 1434, 1379, 1275, 1201 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.85 (1H, dd, *J* = 13.1, 0.8, $NO_2CH\!H$), 4.76 (1H, d, *J* = 13.1, NO_2CHH), 4.60 (1H, d, *J* = 7.3, $OCHH$), 4.59 (1H, d, *J* = 7.3, $OCHH$), 4.49 (1H, d, *J* = 7.3, $OCHH$), 4.45 (1H, d, *J* = 7.3, $OCHH$), 3.71 (3H, s, CO_2CH_3), 3.21 (1H, dd, *J* = 8.5, 6.5, CH^iPr), 2.27 (1H, d, *J* = 8.5, NH), 1.97 – 1.85 (1H, m, $CHMe_2$), 0.91 (3H, d, *J* = 6.8, CH_3), 0.88 (3H, d, *J* = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 175.9 (C=O), 79.0 (OCH_2), 78.7 (OCH_2), 78.5 (NO_2CH_2), 61.3 (CH^iPr), 59.5 (C), 52.3 (CO_2CH_3), 32.1 ($CHMe_2$), 19.4 (CH_3), 18.1 (CH_3); MS (ES^+) *m/z* 247 $[M+H]^+$, 269 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{10}H_{18}N_2NaO_5$ $[M+Na]^+$: 269.1108; found: 269.1101.

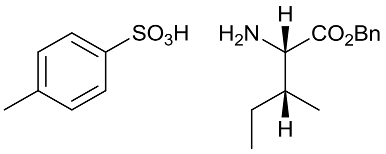
Benzyl [3-(nitromethyl)oxetan-3-yl]-*L*-leucinate (203)



This novel compound was prepared as follows. 3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt$_3$ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH$_2$Cl$_2$ (20 mL) and cooled to –78 °C. To this solution was added NEt$_3$ (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of L-leucine benzyl ester *p*-toluenesulfonate salt (3.19 g, 8.11 mmol) in anhydrous CH$_2$Cl$_2$ (25 mL) was added NEt$_3$ (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH$_4$Cl solution (30 mL). The mixture was extracted with CH$_2$Cl$_2$ (5 x 50 mL) and the combined organic extracts were dried over MgSO$_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15 → 30% EtOAc in hexane) provided **203** (798 mg, 59%) as a pale yellow oil. R$_f$ = 0.31 (30% EtOAc in hexane); $[\alpha]_D^{19}$ +5.0 (*c* 0.16, CHCl$_3$); IR (film) 3338, 2958, 2874, 1730, 1553, 1499, 1457, 1379, 1268, 1169, 976, 737, 697 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 7.42 – 7.31 (5H, m, Ar*H*), 5.17 (1H, d, *J* = 12.1, OC*H*HPh), 5.08 (1H, d, *J* = 12.1, OCH*H*Ph), 4.83 (1H, dd, *J* = 12.8, 0.8, NO$_2$C*H*H), 4.75 (1H, d, *J* = 12.8, NO$_2$CH*H*), 4.58 (1H, d, *J* = 7.2, OC*H*H), 4.53 (1H, d, *J* = 7.0, OC*H*H), 4.45 (1H, d, *J* = 7.2, OCH*H*), 4.26 (1H, d, *J* = 7.0, OCH*H*), 3.51 (1H, s, C*H*CO$_2$Bn), 2.22 (1H, br s, N*H*), 1.74 – 1.57 (1H, m, C*H*Me$_2$), 1.55 – 1.35 (2H, m, C$H_2{}^i$Pr), 0.90 (3H, d, *J* = 6.8, CH_3), 0.88 (3H, d, *J* = 6.8, CH_3); δ_C (75 MHz, CDCl$_3$) 175.9 (C=O), 135.2 (C, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 79.1 (OCH$_2$), 78.9 (OCH$_2$), 78.3 (NO$_2$CH$_2$), 67.3 (O*C*H$_2$Ph), 59.7 (C), 54.5 (*C*HCO$_2$Bn), 43.5 (*C*H$_2{}^i$Pr), 24.7 (*C*HMe$_2$), 22.9 (CH$_3$), 22.3 (CH$_3$); MS (ES$^+$)

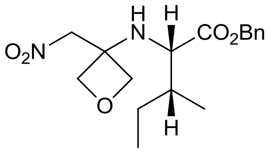
m/z 337 $[M+H]^+$, 359 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{17}H_{24}N_2NaO_5$ $[M+Na]^+$: 359.1577; found: 359.1564.

Benzyl *L*-isoleucinate 4-methylbenzenesulfonate (263)



This known compound was prepared according to a literature procedure.[156] L-isoleucine (3.0 g, 22.9 mmol) and *p*-toluenesulfonic acid monohydrate (4.78 g, 25.1 mmol) were added to a mixture of benzyl alcohol (29 mL) and toluene (43 mL). The reaction mixture was heated under reflux for 16 h with a Dean-Stark trap before being cooled to rt and diluted with Et_2O (30 mL). The solution was cooled in an ice bath for 3 h and the precipitate formed was filtered under vacuum and washed with Et_2O. Recrystallisation (MeOH – Et_2O) gave **263** (7.55 g, 84%) as a white solid. Data has not been fully reported. M.p. 150 – 151 °C (MeOH – Et_2O); $[\alpha]_D^{22}$ +3.89 (*c* 0.22, MeOH); IR (film) 2967, 1738, 1604, 1528, 1497, 1397, 1214, 1175, 1125, 1037, 1013, 699, 678 cm^{-1}; δ_H (300 MHz, MeOD) 7.71 (2H, d, *J* = 8.2, Ar*H*), 7.44 – 7.34 (5H, m, Ar*H*), 7.22 (2H, d, *J* = 8.2, Ar*H*), 5.32 (1H, d, *J* = 12.1, OC*H*HPh), 5.21 (1H, d, *J* = 12.1, OCH*H*Ph), 4.03 (1H, d, *J* = 4.0, C*H*CO_2Bn), 2.36 (3H, s, CH_3, Ts), 2.02 – 1.89 (1H, m, EtC*H*Me), 1.52 – 1.37 (1H, m, C*H*HMe), 1.36 – 1.20 (1H, m, CH*H*Me), 0.96 – 0.86 (6H, m, CH_3), NH_2, SO_3H not observed; δ_C (75 MHz, MeOD) 169.8 (C=O), 143.4 (C, Ar), 141.7 (C, Ar), 136.4 (C, Ar), 130.0 (CH, Ar), 129.8 (CH, Ar), 129.7 (CH, Ar), 126.9 (CH, Ar), 69.0 (O*C*H_2Ph), 58.2 (*C*HCO_2Bn), 37.8 (Et*C*HMe), 26.6 (*C*H_2Me), 21.3 (CH_3, Ts), 14.8 (CH_3), 11.9 (CH_3); MS (ES^+) *m/z* 222 [M free base+H]$^+$, 244 [M free base+Na]$^+$; HRMS (ES^+) calcd. for $C_{13}H_{20}NO_2$ $[M+H]^+$: 222.1489; found: 222.1486.

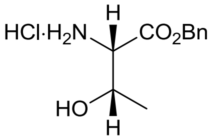
Benzyl [3-(nitromethyl)oxetan-3-yl]-*L*-isoleucinate (204)



This novel compound was prepared as follows. 3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt_3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (20 mL) and cooled to –78 °C. To this solution was added NEt_3 (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of **263** (3.19 g, 8.11 mmol) in anhydrous CH_2Cl_2 (25 mL) was added NEt_3 (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (5 x 50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10 → 25% EtOAc in hexane) provided **204** (704 mg, 52%) as a pale yellow oil. R_f = 0.23 (30% EtOAc in hexane); $[\alpha]_D^{23}$ –2.2 (*c* 0.24, $CHCl_3$); IR (film) 3339, 2965, 2879, 1728, 1555, 1499, 1457, 1379, 1177, 1147, 979, 751, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.41 – 7.32 (5H, m, Ar*H*), 5.16 (1H, d, *J* = 12.1, OC*H*HPh), 5.12 (1H, d, *J* = 12.1, OCH*H*Ph), 4.80 (1H, dd, *J* = 13.1, 0.8, NO_2C*H*H), 4.72 (1H, d, *J* = 13.1, NO_2CH*H*), 4.55 (1H, d, *J* = 7.3, OC*H*H), 4.54 (1H, d, *J* = 7.3, OC*H*H), 4.45 (1H, d, *J* = 7.3, OCH*H*), 4.33 (1H, d, *J* = 7.3, OCH*H*), 3.31 (1H, dd, *J* = 11.0, 6.2, C*H*CO_2Bn), 2.30 (1H, d, *J* = 11.0, N*H*), 1.72 – 1.61 (1H, m, EtC*H*Me), 1.52 – 1.41 (1H, m, C*H*HMe), 1.13 – 1.01 (1H, m, CH*H*Me), 0.88 – 0.81 (6H, m, C*H_3*); δ_C (100 MHz, $CDCl_3$) 175.3 (C=O), 135.3 (C, Ar), 128.9 (CH, Ar), 128.8 (CH, Ar), 78.9 (CH_2), 78.7 (CH_2), 78.5 (CH_2), 67.2 (O*C*H_2Ph), 60.6 (*C*HCO_2Bn), 59.6 (C), 39.0 (Et*C*HMe), 24.9 (*C*H_2Me), 15.7 (CH_3), 11.6 (CH_3); MS (ES^+) *m/z* 337

[M+H]$^+$, 359 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{17}H_{24}N_2NaO_5$ [M+Na]$^+$: 359.1577; found: 359.1576.

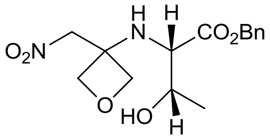
Benzyl *L*-threoninate hydrochloride (264)[157]



This known compound was prepared using a modified literature procedure.[156,158] L-threonine (2.0 g, 16.8 mmol) and *p*-toluenesulfonic acid monohydrate (3.51 g, 18.5 mmol) were added to a mixture of benzyl alcohol (21.3 mL) and toluene (32 mL). The reaction mixture was heated under reflux for 16 h with a Dean-Stark trap before being cooled to rt and diluted with Et_2O (25 mL). Cooling of the solution in an ice bath gave no precipitate, so the toluene was removed *in vacuo* and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic extracts were washed with water (4 x 100 mL) before $NaHCO_3$ (3.5 g) was added to the combined aqueous phases. The aqueous was then extracted with EtOAc (5 x 100 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. To this was added HCl solution (4 M in dioxane, 17 mL) and the reaction mixture was stirred at rt for 3 h before it was concentrated *in vacuo*. Addition of CH_2Cl_2 and Et_2O formed a white precipitate, which was filtered under vacuum to give **264** (2.79 g, 68%) as a white solid. Data in agreement with literature values.[157] M.p. 125 – 126 °C (Et_2O – CH_2Cl_2); $[\alpha]_D^{23}$ –7.12 (*c* 0.28, MeOH); IR (film) 3337, 2975, 2875, 1742, 1596, 1490, 1196, 1050, 913, 753, 700 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.59 (3H, br s, NH_2·HCl), 7.45 – 7.31 (5H, m, ArH), 5.75 (1H, d, J = 5.0, OH), 5.26 – 5.19 (2H, m, OCH_2Ph), 4.21 – 4.13 (1H, m, CHMe), 3.98 (1H, d, J = 3.8, CHCO$_2$Bn), 1.21 (3H, d, J = 6.5, CH_3); δ_C (100 MHz, DMSO-d_6) 168.1 (C=O), 135.3 (C, Ar), 128.5 (CH, Ar), 128.3 (CH, Ar), 128.1 (CH, Ar), 67.0 (OCH$_2$Ph), 65.3 (CHMe), 58.0 (CHCO$_2$Bn), 20.0

(CH_3); MS (ES^+) *m/z* 210 [M free base+H]$^+$, 232 [M free base+Na]$^+$; HRMS (ES^+) calcd. for $C_{11}H_{16}NO_3$ [M+H]$^+$: 210.1125; found: 210.1123.

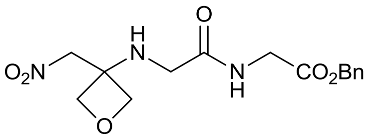
Benzyl [3-(nitromethyl)oxetan-3-yl]-*L*-threoninate (205)



This novel compound was prepared as follows. 3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt_3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (20 mL) and cooled to –78 °C. To this solution was added NEt_3 (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of **264** (1.99 g, 8.10 mmol) in anhydrous CH_2Cl_2 (25 mL) was added NEt_3 (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (5 x 50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (30 → 50% EtOAc in hexane; 0 → 2% MeOH in CH_2Cl_2; 70 → 85% Et_2O in hexane) provided **205** (741 mg, 56%) as a pale yellow oil. R_f = 0.22 (50% EtOAc in hexane); $[\alpha]_D^{23}$ –7.7 (*c* 0.22, $CHCl_3$); IR (film) 3347, 2925, 1729, 1552, 1499, 1457, 1378, 1265, 1173, 974, 736, 697 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.41 – 7.33 (5H, m, Ar*H*), 5.20 (1H, d, *J* = 12.1, OC*H*HPh), 5.15 (1H, d, *J* = 12.1, OCH*H*Ph), 4.82 (1H, d, *J* = 13.3, NO_2C*H*H), 4.73 (1H, d, *J* = 13.3, NO_2CH*H*), 4.57 – 4.52 (2H, m, OC*H*H), 4.45 (1H, d, *J* = 7.3, OCH*H*), 4.43 (1H, d, *J* = 7.3, OCH*H*), 3.84 – 3.76 (1H, m, C*H*Me), 3.27 (1H, dd, *J* = 10.6, 6.2, C*H*CO_2Bn), 2.87 (1H, d, *J* = 3.5, O*H*), 2.70 (1H, d, *J* = 10.6, N*H*), 1.18 (3H, d, *J* = 6.3, C*H*$_3$); δ_C

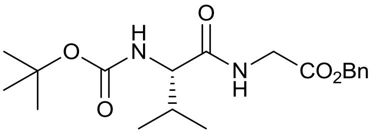
(100 MHz, $CDCl_3$) 173.7 (C=O), 134.8 (C, Ar), 129.0 (CH, Ar), 128.9 (CH, Ar), 78.8 (CH_2), 78.5 (CH_2), 78.3 (CH_2), 68.5 (*C*HMe), 67.8 (O*C*H_2Ph), 62.1 (*C*HCO_2Bn), 59.2 (C), 19.2 (CH_3); MS (ES^+) *m/z* 325 $[M+H]^+$, 347 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{20}N_2NaO_6$ $[M+Na]^+$: 347.1214; found: 347.1213.

Benzyl [[3-(nitromethyl)oxetan-3-yl]glycyl]glycinate (206)



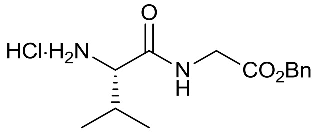
This novel compound was prepared as follows. 3-Oxetanone (303 µL, 4.72 mmol), nitromethane (358 µL, 6.61 mmol) and NEt_3 (132 µL, 0.95 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (23.6 mL) and cooled to -78 °C. To this solution was added NEt_3 (1.32 mL, 9.47 mmol), followed by MsCl (366 µL, 4.73 mmol) dropwise over 10 min. The reaction mixture was stirred at -78 °C for 2 h. Meanwhile, to a stirred solution of glycylglycine benzyl ester *p*-toluenesulfonate salt (3.73 g, 9.46 mmol) in anhydrous CH_2Cl_2 (29 mL) was added NEt_3 (1.32 mL, 9.47 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at -78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (5 x 60 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (70 → 100% EtOAc in hexane) provided **206** (916 mg, 57%) as a pale yellow solid. M.p. 82 – 84 °C; R_f = 0.26 (100% EtOAc); IR (film) 3359, 2969, 2884, 1744, 1668, 1551, 1526, 1499, 1456, 1382, 1266, 1188, 979, 731, 697 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.42 (1H, br t, *J* = 5.6, CON*H*), 7.39 – 7.31 (5H, m, Ar*H*), 5.17 (2H, s, OC*H*$_2$Ph), 4.81 (2H, s, NO_2C*H*$_2$), 4.60 (2H, d, *J* = 7.4, OC*H*H), 4.54 (2H, d, *J* = 7.4, OCH*H*), 4.09 (2H, d, *J* = 5.6, C*H*$_2CO_2$Bn), 3.45 (2H, s, NHC*H*$_2$CO), 2.47 (1H, br s, N*H*); δ$_C$ (100 MHz, CDCl$_3$) 171.1 (C=O), 169.6 (C=O), 135.2 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.5 (CH, Ar), 78.6 (NO$_2$CH$_2$), 77.8 (2 x OCH$_2$), 67.4 (O*C*H$_2$Ph), 59.5 (C), 46.2 (NH*C*H$_2$CO), 41.1 (*C*H$_2$CO$_2$Bn); MS (ES$^+$) *m/z* 360 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{15}H_{19}N_3NaO_6$ [M+Na]$^+$: 360.1166; found: 360.1174.

Benzyl (*tert*-butoxycarbonyl)-*L*-valylglycinate (265)[159]



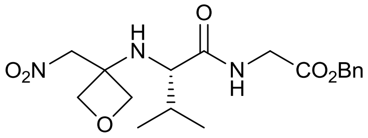
This known compound was prepared according to a literature procedure.[160] A stirred solution of glycine benzyl ester *p*-toluenesulfonate salt (4.61 g, 13.7 mmol), *N*-(*tert*-butoxycarbonyl)-L-valine (3.07 g, 14.1 mmol), HOBt (317 mg, 2.06 mmol) and NMM (4.83 mL, 43.9 mmol) in EtOH (137 mL) was cooled in an ice-water bath before EDC·HCl (3.16 g, 16.5 mmol) was added. The reaction mixture was stirred at rt for 64 h before water (800 mL) was added and the precipitate formed was filtered under vacuum. The resultant white solid was dissolved in EtOAc, dried over MgSO$_4$, filtered and concentrated *in vacuo* to afford **265** (4.29 g, 86%) as a cream solid. Data in agreement with literature values.[159] M.p. 81 – 82 °C; $[\alpha]_D^{23}$ – 23.2 (*c* 0.24, MeOH); δ$_H$ (400 MHz, CDCl$_3$) 7.39 – 7.30 (5H, m, Ar*H*), 6.64 (1H, br t, *J* = 5.2, N*H*), 5.17 (2H, s, OC*H$_2$*Ph), 5.09 (1H, br d, *J* = 8.0, N*H*), 4.12 (1H, dd, *J* = 18.3, 5.2, NHC*H*H), 4.08 – 3.97 (2H, m, NHCH*H*, C*H*^{i}Pr), 2.22 – 2.10 (1H, m, C*H*Me$_2$), 1.43 (9H, s, C*H$_3$*, Boc), 0.97 (3H, d, *J* = 6.8, C*H$_3$*), 0.91 (3H, d, *J* = 6.8, C*H$_3$*); δ$_C$ (100 MHz, CDCl$_3$) 172.1 (C=O), 169.7 (C=O), 156.0 (C=O), 135.2 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 80.1 (C), 67.4 (O*C*H$_2$Ph), 59.9 (*C*H^{i}Pr), 41.4 (NHCH$_2$), 31.0 (*C*HMe$_2$), 28.4 (CH$_3$, Boc), 19.4 (CH$_3$), 17.7 (CH$_3$); MS (ES$^+$) *m/z* 387 [M+Na]$^+$.

Benzyl *L*-valylglycinate hydrochloride (266)



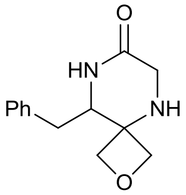
This known compound was prepared using a modified literature procedure.[161] **265** (4.0 g, 11.0 mmol) was stirred in an HCl solution (4 M in dioxane, 22.0 mL, 88.0 mmol) at rt for 2 h before it was concentrated *in vacuo*. The resultant white solid was diluted with Et_2O, filtered under vacuum and washed with Et_2O to give **266** (3.28 g, 99%) as a cream solid. Data has not been fully reported. M.p. 71 – 73 °C; $[\alpha]_D^{23}$ +8.54 (*c* 0.24, MeOH); IR (film) 3207, 3036, 2935, 2880, 2634, 1745, 1671, 1554, 1497, 1186, 1105, 736, 695 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.60 (1H, br s, N*H*), 8.16 (3H, br s, NH_2·*H*Cl), 7.31 – 7.27 (5H, m, Ar*H*), 5.09 (2H, s, OCH_2Ph), 4.30 (1H, d, *J* = 4.5, C*H*^{i}Pr), 4.15 – 3.95 (2H, m, NHCH_2), 2.39 – 2.29 (1H, m, C*H*Me_2), 1.06 (3H, d, *J* = 6.8, CH_3), 1.02 (3H, d, *J* = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 169.8 (C=O), 169.1 (C=O), 135.1 (C, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.6 (CH, Ar), 67.5 (O*C*H_2Ph), 58.6 (*C*H^{i}Pr), 41.6 (NHCH_2), 30.4 (*C*HMe_2), 18.5 (CH_3), 18.2 (CH_3); MS (ES^+) *m/z* 265 [M free base+H]$^+$, 287 [M free base+Na]$^+$; HRMS (ES^+) calcd. for $C_{14}H_{21}N_2O_3$ [M+H]$^+$: 265.1547; found: 265.1546.

Benzyl [[3-(nitromethyl)oxetan-3-yl]-*L*-valyl]glycinate (207)



This novel compound was prepared as follows. 3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt_3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with anhydrous CH_2Cl_2 (20 mL) and cooled to –78 °C. To this solution was added NEt_3 (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at –78 °C for 40 min. Meanwhile, to a stirred solution of **266** (2.44 g, 8.11 mmol) in anhydrous CH_2Cl_2 (25 mL) was added NEt_3 (1.13 mL, 8.11 mmol). The mixture was stirred at rt for 10 min then added to the oxetane solution at –78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (5 x 50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (40 → 50% EtOAc in hexane; 80 → 100% Et_2O in hexane; 0 → 2% MeOH in CH_2Cl_2) provided **207** (949 mg, 62%) as a pale yellow oil. R_f = 0.31 (60% EtOAc in hexane); $[\alpha]_D^{23}$ –17.1 (*c* 0.22, $CHCl_3$); IR (film) 3321, 2962, 2878, 1746, 1652, 1552, 1499, 1457, 1380, 1278, 1187, 977, 738, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.40 – 7.30 (6H, m, Ar*H*, CON*H*), 5.20 – 5.13 (2H, m, OC*H*$_2$Ph), 4.85 (1H, d, *J* = 13.8, NO_2C*H*H), 4.79 (1H, dd, *J* = 13.8, 0.5, NO_2CH*H*), 4.69 (1H, d, *J* = 7.3, OC*H*H), 4.59 (1H, d, *J* = 7.5, OC*H*H), 4.47 (1H, d, *J* = 7.3, OCH*H*), 4.45 (1H, d, *J* = 7.5, OCH*H*), 4.14 (1H, dd, *J* = 18.2, 6.3, NHC*H*H), 4.02 (1H, dd, *J* = 18.2, 5.5, NHCH*H*), 3.36 (1H, t, *J* = 4.0, C*H*^{i}Pr), 2.26 – 2.14 (2H, m, C*H*Me$_2$, N*H*), 1.01 (3H, d, *J* = 6.8, C*H*$_3$), 0.91 (3H, d, *J* = 7.0, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 174.3 (C=O), 169.7 (C=O), 135.2 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 79.5 (NO_2CH_2), 77.9 (OCH_2), 77.5 (OCH_2), 67.4 (O*C*H$_2$Ph), 62.4 (*C*H^{i}Pr), 59.3 (C), 41.0 ($NHCH_2$), 31.7 (*C*HMe$_2$), 19.7 (CH_3), 17.6 (CH_3); MS (ES^+) *m/z* 380 $[M+H]^+$, 402 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{18}H_{25}N_3NaO_6$ $[M+Na]^+$: 402.1636; found: 402.1631.

9-Benzyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one (209)

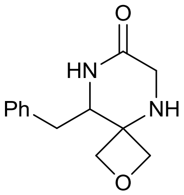


This novel compound was prepared using a modified literature procedure.[113] To a stirred solution of **169** (50 mg, 0.17 mmol) in anhydrous methanol (4.5 mL), samarium(II) iodide solution (0.085 M in THF, 28.0 mL, 2.38 mmol) was added. The reaction mixture was stirred at rt for 30 min then quenched with saturated aqueous $NaHCO_3$ solution (30 mL) and extracted with CH_2Cl_2 (5 x 70 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10 → 20% MeOH in EtOAc) provided **209** (13 mg, 33%) as a cream solid. M.p. 157 – 160 °C; R_f = 0.19 (10% MeOH in EtOAc); IR (film) 3297, 2948, 2874, 1657, 1494, 1454, 1312, 977, 734, 700 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.31 – 7.26 (2H, m, Ar*H*), 7.24 – 7.19 (1H, m, Ar*H*), 7.15 (2H, d, *J* = 7.0, Ar*H*), 5.74 (1H, br s, N*H*), 4.63 (1H, d, *J* = 7.3, OC*H*H), 4.58 (1H, d, *J* = 6.8, OC*H*H), 4.52 (1H, d, *J* = 6.8, OCH*H*), 4.41 (1H, d, *J* = 7.3, OCH*H*), 3.75 (1H, dt, *J* = 10.6, 3.0, C*H*), 3.55 (1H, d, *J* = 17.8, C*H*HNH), 3.49 (1H, d, *J* = 17.8, CH*H*NH), 3.03 (1H, dd, *J* = 13.6, 3.0, C*H*HPh), 2.70 (1H, dd, *J* = 13.6, 10.6, CH*H*Ph), 2.17 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 168.4 (C=O), 136.5 (C, Ar), 129.4 (CH, Ar), 129.3 (CH, Ar), 127.4 (CH, Ar), 80.1 (OCH_2), 77.9 (OCH_2), 58.7 (CH), 58.3 (C), 45.8 (CH_2NH), 38.1 ($\mathit{C}H_2Ph$); MS (ES^+) *m/z* 233 $[M+H]^+$, 255 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{17}N_2O_2$ $[M+H]^+$: 233.1285; found: 233.1284. A crystal for X-ray analysis was grown using the solvent diffusion method (EtOAc/pentane).

Crystal Data for $C_{13}H_{16}N_2O_2$, *M* = 232.28, orthorhombic, *a* = 12.8111 (17) Å, *b* = 8.7861 (9) Å, *c* = 10.4514 (12) Å, *U* = 1176.4 (2) Å^3, *T* = 150 (2) K, space group Pna2 (1), *Z* = 4, μ(MoKα) = 0.725 mm^{-1}, 7966 reflections measured, 2322 unique

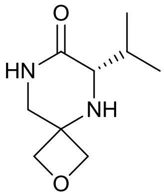
(R_{int} = 0.1297) which were used in all calculations. The final wR_2 was 0.1388 and R_1 was 0.0466 (>2 sigma (I)).

9-Benzyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one (209)



This novel compound was prepared using a modified literature procedure.[107] To a stirred solution of **199** (81 mg, 0.22 mmol) in glacial AcOH (3.6 mL), Zn (503 mg, 7.69 mmol) was added. The reaction mixture was stirred vigorously at rt for 24 h then filtered through Celite®, washed with Et_2O and concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$ solution (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Recrystallisation (EtOH) provided **209** (18 mg, 35%) as a pale orange solid. Data as previously reported.

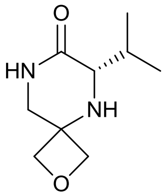
(*S*)-6-Isopropyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one (210)



This novel compound was prepared using a modified literature procedure.[107] To a stirred solution of **194** (126 mg, 0.39 mmol) in glacial AcOH (6.5 mL) was added Zn (901 mg, 13.8 mmol). The reaction mixture was stirred vigorously at rt for 67 h then filtered through Celite® and concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$ solution (15 mL) and extracted with EtOAc (4 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by repeat column chromatography (0 → 15% MeOH in EtOAc + 0.5% NEt_3; 0 → 5% MeOH in EtOAc + 0.5% NEt_3), followed by recrystallisation (EtOAc-hexane) provided **210** (16 mg, 22%) as white flakes. M.p. 153 – 154 °C (EtOAc – hexane);

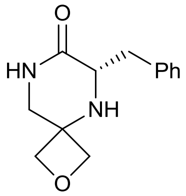
R_f = 0.27 (10% MeOH in EtOAc); $[\alpha]_D^{28}$ –107 (*c* 0.16, $CHCl_3$); IR (film) 3255, 2968, 2871, 2822, 1669, 1491, 1455, 1323 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.75 (1H, br s, N*H*CO), 4.63 – 4.58 (2H, m, OC*H$_2$*), 4.51 (1H, d, *J* = 7.1, OC*H*H), 4.45 (1H, d, *J* = 7.1, OCH*H*), 3.65 (1H, dd, *J* = 11.5, 4.7, C*H*HNH), 3.49 – 3.40 (2H, m, CH*H*NH, C*H*^{i}Pr), 2.50 – 2.34 (1H, m, C*H*Me_2), 1.82 (1H, br s, N*H*), 1.00 (3H, d, *J* = 7.2, C*H$_3$*), 0.91 (3H, d, *J* = 6.8, C*H$_3$*); δ_C (75 MHz, $CDCl_3$) 171.3 (C=O), 82.2 (OCH_2), 80.1 (OCH_2), 60.1 (*C*H^{i}Pr), 55.4 (C), 49.1 (CH_2NH), 30.2 (*C*HMe_2), 19.2 (CH_3), 16.3 (CH_3); MS (ES^+) *m/z* 185 $[M+H]^+$, 207 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_9H_{17}N_2O_2$ $[M+H]^+$: 185.1285; found: 185.1282.

(*S*)-6-Isopropyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one (210)



This novel compound was prepared using a modified literature procedure.[133] A suspension of (*S*)-**193** (107 mg, 0.43 mmol) and 10% Pd/C (53 mg) in MeOH (8 mL) was stirred under an atmosphere of hydrogen at rt for 17 h then filtered through Celite®, washed with MeOH and concentrated *in vacuo*. Purification by column chromatography (0 → 4% MeOH in CH_2Cl_2 + 0.5% NEt_3), followed by recrystallisation (EtOAc-hexane) provided **210** as a cream solid (36 mg, 45%). Data as previously reported.

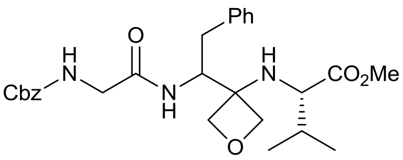
(*S*)-6-Benzyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one (211)



This novel compound was prepared using a modified literature procedure.[107] To a stirred solution of **195** (98 mg, 0.26 mmol) in glacial AcOH (4.5 mL) was added Zn (628 mg, 9.60 mmol). The reaction mixture was stirred vigorously at rt for 67 h then filtered through Celite® and concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$

solution (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0 → 10% MeOH in EtOAc + 0.5% NEt_3) provided **211** (42 mg, 68%) as a white solid. M.p. 156 – 158 °C; $R_f = 0.30$ (10% MeOH in EtOAc); $[\alpha]^{29}_{D}$ –98.9 (*c* 0.18, $CHCl_3$); IR (film) 3286, 3061, 2945, 2869, 1665, 1604, 1495, 1455, 1314, 975, 734, 700 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.37 – 7.32 (2H, m, Ar*H*), 7.31 – 7.25 (3H, m, Ar*H*), 6.30 (1H, br s, N*H*CO), 4.57 (1H, d, *J* = 6.5, OC*H*H), 4.49 (1H, d, *J* = 6.5, OCH*H*), 4.41 (1H, d, *J* = 7.0, OC*H*H), 4.38 (1H, d, *J* = 7.0, OCH*H*), 3.72 (1H, dd, *J* = 10.0, 3.4, C*H*), 3.68 (1H, dd, *J* = 11.5, 4.0, C*H*HNH), 3.52 – 3.44 (2H, m, CH*H*NH, C*H*HPh), 2.80 (1H, dd, *J* = 13.7, 10.0, CH*H*Ph), 1.86 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 170.8 (C=O), 137.8 (C, Ar), 129.4 (CH, Ar), 129.0 (CH, Ar), 127.0 (CH, Ar), 81.8 (OCH_2), 79.9 (OCH_2), 56.3 (CH), 55.2 (C), 49.4 (CH_2NH), 38.5 (CH_2Ph); MS (ES^+) *m/z* 233 $[M+H]^+$, 255 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{16}N_2NaO_2$ $[M+Na]^+$: 255.1104; found: 255.1106.

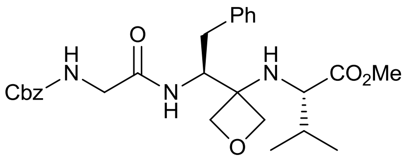
Methyl [3-[1-[2-[[(benzyloxy)carbonyl]amino]acetamido]-2-phenylethyl]oxetan-3-yl]-*L*-valinate (213a/b)

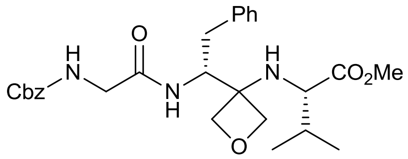


This novel compound was prepared using modified literature procedures.[113,154] To a stirred solution of **180a/b** (137 mg, 0.41 mmol) in anhydrous MeOH (7.3 mL) was added samarium(II) iodide solution (0.1 M in THF, 57.0 mL, 5.70 mmol). The reaction mixture was stirred at rt for 20 min before saturated aqueous $NaHCO_3$ solution (30 mL) was added and the solution extracted with CH_2Cl_2 (5 x 70 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude amine. To this was added anhydrous CH_2Cl_2 (2.5 mL) and the resultant solution was added to a stirred mixture of Z-Gly-OH (79 mg, 0.38 mmol), DCC (85 mg, 0.41 mmol), HOBt (66 mg, 0.43 mmol) and DIEA (70.9 µL, 0.41 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then at rt for 22 h, before it was filtered through Celite®, washed with CH_2Cl_2 and concentrated *in vacuo*. Purification by repeat column chromatography (40 → 70% EtOAc in petroleum ether + 0.5% NEt_3; 50 → 55% EtOAc in petroleum ether + 0.5% NEt_3) provided **213a**/**b** (133 mg, 71%) as a colourless oil. Both diastereomers were observed in a 1 : 1.3 ratio. R_f = 0.29 (70% EtOAc in petroleum ether); IR (film) 3321, 2958, 1724, 1662, 1502, 1497, 1454, 1239, 1151, 1049, 980, 733, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.16 (23H, m, Ar*H*), 6.95 (1.3H, br d, *J* = 8.5, CON*H*), 6.62 (1H, br d, *J* = 8.5, CON*H*), 5.55 – 5.49 (1.3H, m, CbzN*H*), 5.45 – 5.39 (1H, m, CbzN*H*), 5.11 (4.6H, s, PhC*H*₂O), 4.75 – 4.65 (2.3H, m, C*H*Bn), 4.42 – 4.32 (3.3H, m, OC*H*₂, OC*H*H), 4.29 – 4.22 (2.6H, m, OCH*H* and OC*H*H), 4.19 (1.3H, d, *J* = 6.8, OCH*H*), 4.07 (1H, d, *J* = 7.3, OC*H*H), 4.01 (1H, d, *J* = 7.3, OCH*H*), 3.86 (1.3H, dd, *J* = 16.8, 5.5, NHC*H*H), 3.79 – 3.69 (10.2H, m, 2 x CO_2CH_3, NHC*H*₂, NHCH*H*), 3.62 (1H, d, *J* = 4.8, C*H*^{i}Pr), 3.55 (1.3H, d, *J* = 5.0, C*H*^{i}Pr), 2.94 – 2.72 (4.6H, m, C*H*₂Ph), 2.26 – 1.78 (4.6H, m, C*H*Me₂, N*H*), 0.99 (3.9H, d, *J* = 6.7, C*H*₃), 0.96 (3H, d, *J* = 6.7, C*H*₃), 0.90 (3.9H, d, *J* = 6.8, C*H*₃), 0.89 (3H, d, *J* = 6.8, C*H*₃); δ_C (150 MHz, $CDCl_3$) 177.1 (C=O), 176.9 (C=O), 169.3 (C=O), 168.9 (C=O), 156.7 (C=O), 137.8 (C, Ar), 137.3 (C, Ar), 136.3 (C, Ar), 136.2 (C, Ar), 129.3 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.4 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 128.1 (CH, Ar), 126.9 (CH, Ar), 126.9 (CH, Ar), 79.4 (OCH_2), 79.3 (OCH_2), 79.0 (OCH_2), 77.8 (OCH_2), 67.3 (Ph*C*H₂O), 67.2 (Ph*C*H₂O), 62.6 (C), 62.5 (C), 61.1 (*C*H^{i}Pr), 60.1 (*C*H^{i}Pr), 56.4 (*C*HBn), 55.9 (*C*HBn), 52.4 (CO₂*C*H₃), 52.3 (CO₂*C*H₃), 44.9 ($NHCH_2$), 44.7

(NHCH$_2$), 35.9 (*C*H$_2$Ph), 35.5 (*C*H$_2$Ph), 32.6 (*C*HMe$_2$), 32.1 (*C*HMe$_2$), 19.7 (CH$_3$), 19.7 (CH$_3$), 18.1 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) *m/z* 498 [M+H]$^+$, 520 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{27}H_{36}N_3O_6$ [M+H]$^+$: 498.2599; found: 498.2594.

Methyl [3-[(S)-1-[2-[[(benzyloxy)carbonyl]amino]acetamido]-2-phenylethyl]oxetan-3-yl]-*L*-valinate (213a and 213b)

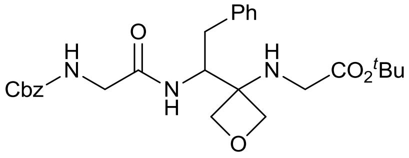




These diastereomers were separated by preparative HPLC (Phenomonex Lux C2 column, 20 µm silica, 50 mm diameter, 250 mm length), 50 : 50 heptane : EtOH at 90 ml / min. to afford the less polar **213a** (36 mg, 19%) as a yellow waxy solid. R$_f$ = 0.39 (70% EtOAc in heptane); $[\alpha]_D^{17}$ +9.35 (*c* 0.26, CHCl$_3$); IR (KBr) 3319, 2959, 2876, 1729, 1670, 1518, 1455, 1245, 1050, 751 cm^{-1}; δ_H (700 MHz, CDCl$_3$) 7.30 – 7.11 (10H, m, Ar*H*), 6.44 (1H, br d, *J* = 7.9, CON*H*), 5.22 (1H, br s, CbzN*H*), 5.04 (2H, s, PhC*H$_2$*O), 4.68 – 4.64 (1H, m, C*H*Bn), 4.31 (2H, s, OC*H$_2$*), 4.00 (1H, d, *J* = 6.9, OC*H*H), 3.94 (1H, d, *J* = 6.9, OCH*H*), 3.71 (2H, d, *J* = 5.7, NHC*H$_2$*), 3.70 (3H, s, CO$_2$C*H$_3$*), 3.55 (1H, d, *J* = 4.8, C*H*^{i}Pr), 2.81 – 2.74 (1H, m, C*H*HPh), 2.71 (1H, dd, *J* = 13.9, 7.5, CH*H*Ph), 1.97 – 1.90 (1H, m, C*H*Me$_2$), 0.90 (3H, d, *J* = 6.8, C*H$_3$*), 0.82 (3H, d, *J* = 6.8, C*H$_3$*), secondary NH not observed; δ_C (175 MHz, CDCl$_3$) 176.9 (C=O), 168.9 (C=O), 156.7 (C=O), 137.3 (C, Ar), 136.2 (C, Ar), 129.3 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.5 (CH, Ar), 128.3 (CH, Ar), 127.0 (CH, Ar), 79.3 (OCH$_2$), 77.9 (OCH$_2$), 67.4 (Ph*C*H$_2$O), 62.6 (C), 60.2 (*C*H^{i}Pr), 56.4 (*C*HBn), 52.3 (CO$_2$*C*H$_3$), 45.0 (NHCH$_2$), 36.1 (*C*H$_2$Ph), 32.7 (*C*HMe$_2$), 19.7 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) *m/z* 498 [M+H]$^+$, 520 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{27}H_{36}N_3O_6$ [M+H]$^+$: 498.2599;

found: 498.2604. And the more polar **213b** (48 mg, 26%) as a yellow waxy solid. R$_f$ = 0.34 (70% EtOAc in heptane); $[\alpha]_D^{17}$ –22.3 (*c* 0.20, $CHCl_3$); IR (KBr) 3323, 2959, 2875, 1730, 1668, 1520, 1454, 1243, 1053, 982, 751 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.30 – 7.11 (10H, m, Ar*H*), 6.74 (1H, br d, *J* = 7.8, CON*H*), 5.35 (1H, br s, CbzN*H*), 5.05 (2H, s, PhC*H$_2$*O), 4.63 (1H, dd, *J* = 14.3, 7.8, C*H*Bn), 4.27 (1H, d, *J* = 7.2, OC*H*H), 4.20 (1H, d, *J* = 6.8, OC*H*H), 4.16 (1H, d, *J* = 7.2, OCH*H*), 4.11 (1H, d, *J* = 6.8, OCH*H*), 3.81 (1H, dd, *J* = 16.6, 4.6, NHC*H*H), 3.70 (1H, dd, *J* = 16.6, 5.3, NHCH*H*), 3.64 (3H, s, CO_2CH_3), 3.49 (1H, d, *J* = 5.1, C*H*^{i}Pr), 2.83 (1H, dd, *J* = 14.3, 5.7, C*H*HPh), 2.76 (1H, dd, *J* = 14.3, 7.8, CH*H*Ph), 2.01 – 1.94 (1H, m, C*H*Me_2), 1.77 (1H, br s, N*H*), 0.93 (3H, d, *J* = 6.8, CH_3), 0.83 (3H, d, *J* = 7.0, CH_3); δ_C (175 MHz, $CDCl_3$) 177.1 (C=O), 169.3 (C=O), 156.7 (C=O), 137.7 (C, Ar), 136.4 (C, Ar), 129.3 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 127.0 (CH, Ar), 79.5 (OCH_2), 79.0 (OCH_2), 67.3 (Ph*C*H_2O), 62.7 (C), 61.2 (*C*H^{i}Pr), 56.1 (*C*HBn), 52.4 (CO_2CH_3), 44.8 ($NHCH_2$), 35.7 (*C*H_2Ph), 32.1 (*C*HMe_2), 19.7 (CH_3), 18.1 (CH_3); MS (ES$^+$) *m/z* 498 [M+H]$^+$, 520 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{27}H_{36}N_3O_6$ [M+H]$^+$: 498.2599; found: 498.2602.

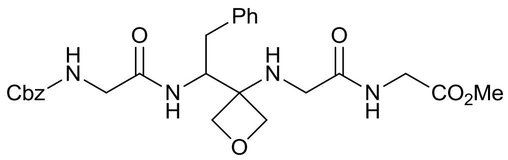
***tert*-Butyl [3-[1-[2-[[(benzyloxy)carbonyl]amino]acetamido]-2-phenylethyl]oxetan-3-yl]glycinate (214)**



This novel compound was prepared using modified literature procedures.[113,154] To a stirred solution of **178** (165 mg, 0.49 mmol) in anhydrous MeOH (10 mL) was added samarium(II) iodide solution (0.1 M in THF, 68.8 mL, 6.88 mmol). The reaction mixture was stirred at rt for 20 min before saturated aqueous $NaHCO_3$ solution (35 mL) was added and the solution extracted with

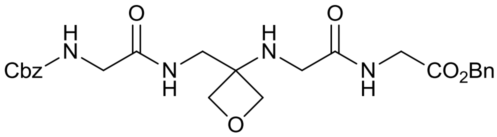
CH_2Cl_2 (5 x 80 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude amine. To this was added anhydrous CH_2Cl_2 (4 mL) and the resultant solution was added to a stirred mixture of Z-Gly-OH (94 mg, 0.45 mmol), DCC (103 mg, 0.50 mmol), HOBt (75 mg, 0.49 mmol) and DIEA (85.4 µL, 0.49 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then at rt for 24 h, before it was filtered through Celite®, washed with CH_2Cl_2 and concentrated *in vacuo*. Purification by column chromatography (45 → 80% EtOAc in petroleum ether + 0.5% NEt_3) provided **214** (121 mg, 54%) as a white solid. M.p. 132 – 133 °C; R_f = 0.32 (70% EtOAc in petroleum ether); IR (film) 3312, 2974, 1725, 1662, 1522, 1455, 1367, 1235, 1154, 979, 737, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.31 – 7.10 (10H, m, Ar*H*), 6.61 (1H, d, *J* = 8.8, CON*H*), 5.39 – 5.33 (1H, m, CbzN*H*), 5.04 (2H, s, PhCH_2O), 4.68 – 4.60 (1H, m, C*H*), 4.35 – 4.27 (2H, m, OCH_2), 4.18 (1H, d, *J* = 7.0, OC*H*H), 4.09 (1H, d, *J* = 7.0, OCH*H*), 3.75 (1H, dd, *J* = 16.2, 5.8, CbzNHC*H*H), 3.66 (1H, dd, *J* = 16.2, 5.3, CbzNHCH*H*), 3.55 (1H, d, *J* = 17.6, C*H*HCO$_2{}^t$Bu), 3.47 (1H, d, *J* = 17.6, CH*H*CO$_2{}^t$Bu), 2.79 – 2.64 (2H, m, CH_2Ph), 1.89 (1H, br s, N*H*), 1.42 (9H, s, CH_3); δ_C (150 MHz, $CDCl_3$) 172.4 (C=O), 169.0 (C=O), 156.6 (C=O), 137.5 (C, Ar), 136.3 (C, Ar), 129.1 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.3 (CH, Ar), 126.9 (CH, Ar), 82.2 (C, ^{t}Bu), 78.5 (OCH_2), 78.0 (OCH_2), 67.3 (Ph*C*H_2O), 62.6 (C, oxetane), 55.6 (CH), 45.6 (*C*H_2CO$_2{}^t$Bu), 44.7 (CbzNH*C*H_2), 35.8 (*C*H_2Ph), 28.2 (CH_3); MS (ES$^+$) *m/z* 498 $[M+H]^+$, 520 $[M+Na]^+$; HRMS (ES$^+$) calcd. for $C_{27}H_{36}N_3O_6$ $[M+H]^+$: 498.2599; found: 498.2601.

Methyl [[3-[1-[2-[[(benzyloxy)carbonyl]amino]acetamido]-2-phenylethyl]oxetan-3-yl]glycyl]glycinate (217)



This novel compound was prepared using modified literature procedures.[113,154] To a stirred solution of **200** (168 mg, 0.39 mmol) in anhydrous MeOH (7.9 mL) was added samarium(II) iodide solution (0.1 M in THF, 55.0 mL, 5.50 mmol). The reaction mixture was stirred at rt for 20 min before saturated aqueous $NaHCO_3$ solution (35 mL) was added and the solution extracted with CH_2Cl_2 (5 x 70 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude amine. To this was added anhydrous CH_2Cl_2 (2 mL) and the resultant solution was added to a stirred mixture of Z-Gly-OH (83 mg, 0.40 mmol), DCC (91 mg, 0.44 mmol), HOBt (67 mg, 0.44 mmol) and DIEA (75.4 µL, 0.43 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min, then at rt for 66 h, before it was filtered through Celite®, washed with CH_2Cl_2 and concentrated *in vacuo*. Purification by repeat column chromatography (0 → 9% MeOH in CH_2Cl_2 + 0.5% NEt_3; 2 → 4% MeOH in EtOAc + 0.5% NEt_3) provided **217** (90 mg, 45%) as a white solid. M.p. 55 – 57 °C (Et_2O); R_f = 0.24 (7% MeOH in EtOAc); IR (film) 3321, 2952, 1721, 1659, 1530, 1454, 1440, 1234, 1182, 737, 700 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.50 (1H, br s, N*H*), 7.40 – 7.14 (10H, m, Ar*H*), 6.69 (1H, br d, *J* = 8.3, N*H*), 5.52 (1H, br t, *J* = 5.8. N*H*), 5.13 – 5.06 (2H, m, PhC*H₂*O), 4.71 – 4.64 (1H, m, C*H*), 4.56 (1H, d, *J* = 7.5, OC*H*H), 4.39 – 4.29 (3H, m, OCH*H*, OC*H₂*), 4.13 (1H, dd, *J* = 18.2, 5.5, C*H*H), 4.03 (1H, dd, *J* = 18.2, 5.3, CH*H*), 3.81 – 3.51 (7H, m, C*H₃*, C*H₂*, C*H₂*), 2.83 (1H, dd, *J* = 14.1, 4.3, C*H*HPh), 2.72 (1H, dd, *J* = 14.1, 9.0, CH*H*Ph), 2.10 (1H, br s, N*H*); δ_C (125 MHz, $CDCl_3$) 172.4 (C=O), 171.1 (C=O), 169.7 (C=O), 157.0 (C=O), 137.4 (C, Ar), 136.3 (C, Ar), 129.0 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 127.0 (CH, Ar), 78.3 (OCH_2), 77.7 (OCH_2), 67.3 (Ph*C*H_2O), 63.4 (C), 55.7 (CH), 52.6 (CH_3), 46.4 (CH_2), 44.8 (CH_2), 40.9 (CH_2), 35.6 (*C*H_2Ph); MS (ES$^+$) *m/z* 535 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{26}H_{33}N_4O_7$ [M+H]$^+$: 513.2344; found: 513.2348.

Benzyl [[3-[[2-[[(benzyloxy)carbonyl]amino]acetamido]methyl]oxetan-3-yl]glycyl]glycinate (223)



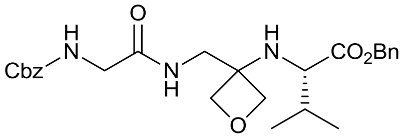
This novel compound was prepared using modified literature procedures.[113,160] To a stirred solution of **206** (153 mg, 0.45 mmol) in anhydrous *ᵗ*BuOH (10.6 mL) was added samarium(II) iodide solution (0.1 M in THF, 63.5 mL, 6.35 mmol). The reaction mixture was stirred at rt for 20 min before saturated aqueous $NaHCO_3$ solution (40 mL) was added and the solution extracted with CH_2Cl_2 (5 x 75 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude amine. To this was added Z-Gly-OH (97 mg, 0.46 mmol), HOBt (12 mg, 0.08 mmol), NMM (108 µL, 0.98 mmol) and EtOH (4.5 mL). The reaction mixture was cooled in an ice-water bath before EDC·HCl (103 mg, 0.54 mmol) was added. The reaction mixture was stirred at rt for 18 h before water (25 mL) was added and the solution extracted with EtOAc (4 x 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (4 → 9% MeOH in CH_2Cl_2) provided **223** (55 mg, 24%) as a colourless oil. R_f = 0.24 (9% MeOH in CH_2Cl_2); IR (film) 3318, 2938, 2879, 1717, 1660, 1530, 1456, 1230, 1190, 974, 740, 699 cm^{-1}; δ_H (400 MHz, MeOD) 7.36 – 7.26 (10H, m, Ar*H*), 5.15 (2H, s,

CO_2CH_2Ph), 5.07 (2H, s, $PhCH_2OCONH$), 4.48 (2H, d, *J* = 6.8, OC*H*H), 4.41 (2H, d, *J* = 6.8, OCH*H*), 4.04 (2H, s, CH_2CO_2Bn), 3.77 (2H, s, $CbzNHCH_2$), 3.59 (2H, s, CH_2), 3.40 (2H, s, CH_2), NHs not observed; δ_C (100 MHz, MeOD) 175.5 (C=O), 173.0 (C=O), 171.3 (C=O), 159.1 (C=O), 138.1 (C, Ar), 137.1 (C, Ar), 129.6 (CH, Ar), 129.5 (CH, Ar), 129.3 (CH, Ar), 129.1 (CH, Ar), 128.9 (CH, Ar), 79.8 (2 x OCH_2), 68.0 (CH_2), 67.8 (CH_2), 61.5 (C), 46.7 (CH_2), 45.1 (CH_2), 44.7 (CH_2), 41.9 (CH_2CO_2Bn); MS (ES^+) *m/z* 499 $[M+H]^+$, 521 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{25}H_{30}N_4NaO_7$ $[M+Na]^+$: 521.2007; found: 521.1997.

General Method A: Nitro reduction[112] and amide coupling[160]

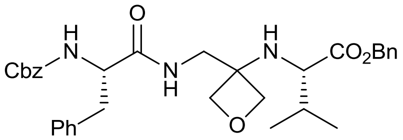
To a stirred suspension of the nitro compound (1.0 molar equiv.) in THF/H_2O (1:3, 1.0 mL) was added In metal (4.0 molar equiv.), followed by 6 M HCl (6.0 molar equiv.). The reaction mixture was stirred at rt for 90 min, then basified with saturated aqueous $NaHCO_3$ solution (8 mL) and diluted with EtOAc (20 mL). The solution was filtered through Celite® and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude amine. To this amine (1.0 molar equiv.) was added the Cbz protected amino acid (1.03 molar equiv.), HOBt (0.15 molar equiv.), NMM (2.2 molar equiv.) in EtOH. The reaction mixture was cooled in an ice-water bath before EDC·HCl (1.2 molar equiv.) was added. The reaction mixture was stirred at rt for 18 h before water (18 mL) was added and the solution extracted with EtOAc (4 x 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography provided the product.

Benzyl [3-[[2-[[(benzyloxy)carbonyl]amino]acetamido]methyl]oxetan-3-yl]-*L*-valinate (216)



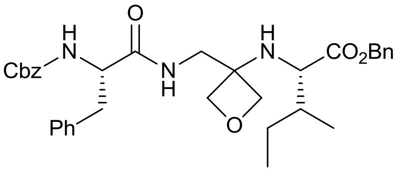
This novel compound was prepared as follows. **194** (97 mg, 0.30 mmol), In metal (142 mg, 1.24 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-Gly-OH (68 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (70 mg, 0.37 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (60 → 80% EtOAc in hexane) provided **216** (71 mg, 49%) as a colourless oil. R_f = 0.31 (80% EtOAc in petroleum ether); $[\alpha]_D^{33}$ +12.3 (*c* 0.22, $CHCl_3$); IR (film) 3316, 2960, 2875, 1722, 1666, 1520, 1456, 1241, 1152, 974, 737, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.29 (10H, m, Ar*H*), 6.50 (1H, br s, CON*H*), 5.41 (1H, br s, CbzN*H*), 5.16 – 5.08 (4H, m, CO_2CH_2Ph and PhC*H*$_2$OCONH), 4.37 (1H, d, *J* = 6.4, OC*H*H), 4.30 (1H, d, *J* = 6.5, OC*H*H), 4.23 (1H, d, *J* = 6.5, OCH*H*), 4.18 (1H, d, *J* = 6.4, OCH*H*), 3.86 (2H, d, *J* = 5.5, CbzNHC*H*$_2$), 3.78 (1H, dd, *J* = 14.0, 5.8, C*H*H), 3.41 (1H, dd, *J* = 14.0, 4.9, CH*H*), 3.12 (1H, d, *J* = 5.3, C*H*^{i}Pr), 2.01 – 1.65 (2H, m, C*H*Me$_2$, N*H*), 0.94 (3H, d, *J* = 6.8, C*H*$_3$), 0.87 (3H, d, *J* = 6.8, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 176.0 (C=O), 169.7 (C=O), 156.7 (C=O), 136.2 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 79.7 (OCH_2), 79.4 (OCH_2), 67.3 (CH_2), 67.2 (CH_2), 61.1 (*C*H^{i}Pr), 59.4 (C), 44.7 (CbzNH*C*H$_2$), 43.6 (CH_2), 32.0 (*C*HMe$_2$), 19.5 (CH_3), 17.9 (CH_3); MS (ES$^+$) *m/z* 484 [M+H]$^+$, 506 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{26}H_{34}N_3O_6$ [M+H]$^+$: 484.2442; found: 484.2444.

Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-3-phenylpropanamido]methyl]oxetan-3-yl]-*L*-valinate (225)



This novel compound was prepared as follows. **194** (101 mg, 0.31 mmol), In metal (145 mg, 1.26 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Phe-OH (97 mg, 0.32 mmol), HOBt (9 mg, 0.06 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (73 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (40 → 50% EtOAc in hexane) provided **225** (107 mg, 60%) as a colourless oil. R_f = 0.26 (50% EtOAc in hexane); $[\alpha]_D^{21}$ +15.5 (*c* 0.20, $CHCl_3$); IR (film) 3320, 2962, 2875, 1724, 1663, 1525, 1498, 1456, 1254, 1179, 1152, 977, 747, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.21 (13H, m, Ar*H*), 7.18 (2H, d, *J* = 7.5, Ar*H*), 6.19 – 6.13 (1H, m, CON*H*), 5.43 – 5.36 (1H, m, CbzN*H*), 5.12 – 5.04 (4H, m, CO_2CH_2Ph and $PhCH_2OCONH$), 4.43 – 4.35 (1H, m, C*H*Bn), 4.28 (1H, d, *J* = 6.5, OC*H*H), 4.14 (1H, d, *J* = 6.5, OC*H*H), 4.06 (1H, d, *J* = 6.5, OCH*H*), 4.02 (1H, d, *J* = 6.5, OCH*H*), 3.64 (1H, dd, *J* = 13.9, 5.4, NHC*H*H), 3.33 (1H, dd, *J* = 13.9, 5.0, NHCH*H*), 3.15 (1H, dd, *J* = 13.8, 6.3, C*H*HPh), 3.04 – 2.97 (2H, m, CH*H*Ph, C*H*^{i}Pr), 1.92 – 1.78 (2H, m, C*H*Me_2, N*H*), 0.86 (3H, d, *J* = 6.8, CH_3), 0.78 (3H, d, *J* = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 176.0 (C=O), 171.3 (C=O), 155.9 (C=O), 136.6 (C, Ar), 136.3 (C, Ar), 135.3 (C, Ar), 129.4 (CH, Ar), 128.9 (CH, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 127.2 (CH, Ar), 79.6 (OCH_2), 79.6 (OCH_2), 67.2 (CH_2), 67.2 (CH_2), 61.0 (*C*H^{i}Pr), 59.0 (C), 56.6 (*C*HBn), 43.7 ($NHCH_2$), 38.8 (*C*H_2Ph), 32.0 (*C*HMe_2), 19.5 (CH_3), 18.0 (CH_3); MS (ES^+) *m/z* 596 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{33}H_{39}N_3NaO_6$ $[M+Na]^+$: 596.2731; found: 596.2722.

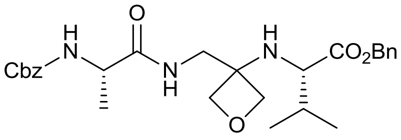
Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-3-phenylpropanamido]methyl]oxetan-3-yl]-*L*-isoleucinate (226)



This novel compound was prepared as follows. **204** (100 mg, 0.30 mmol), In metal (137 mg, 1.19 mmol) and 6 M HCl (298 µL, 1.79 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Phe-OH (95 mg, 0.32 mmol), HOBt (7 mg, 0.05 mmol), NMM (72 µL, 0.65 mmol), EDC·HCl (70 mg, 0.37 mmol) in EtOH (3 mL). Work-up, followed by purification by column chromatography (30 → 50% EtOAc in hexane) provided **226** (102 mg, 58%) as a colourless oil. R_f = 0.31 (50% EtOAc in hexane); $[\alpha]_D^{23}$ +16.3 (*c* 0.20, $CHCl_3$); IR (film) 3313, 3033, 2963, 2876, 1724, 1663, 1523, 1498, 1456, 1255, 1174, 976, 746, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.20 (13H, m, Ar*H*), 7.18 (2H, d, *J* = 7.3, Ar*H*), 6.17 – 6.12 (1H, m, CON*H*), 5.41 (1H, d, *J* = 7.5, CbzN*H*), 5.10 (1H, d, *J* = 12.3, C*H*H), 5.08 (2H, s, C*H*$_2$), 5.07 (1H, d, *J* = 12.3, CH*H*), 4.43 – 4.36 (1H, m, C*H*Bn), 4.28 (1H, d, *J* = 6.5, OC*H*H), 4.14 (1H, d, *J* = 6.5, OC*H*H), 4.06 (1H, d, *J* = 6.5, OCH*H*), 4.01 (1H, d, *J* = 6.5, OCH*H*), 3.64 (1H, dd, *J* = 13.9, 5.4, NHC*H*H), 3.32 (1H, dd, *J* = 13.9, 4.9, NHCH*H*), 3.16 (1H, dd, *J* = 13.7, 5.8, C*H*HPh), 3.05 (1H, d, *J* = 5.8, C*H*CO_2Bn), 2.99 (1H, dd, *J* = 13.7, 7.8, CH*H*Ph), 1.83 (1H, br s, N*H*), 1.64 – 1.54 (1H, m, EtC*H*Me), 1.39 – 1.28 (1H, m, C*H*HMe), 1.04 – 0.90 (1H, m, CH*H*Me), 0.83 – 0.78 (6H, m, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 176.0 (C=O), 171.3 (C=O), 155.9 (C=O), 136.6 (C, Ar), 136.3 (C, Ar), 135.2 (C, Ar), 129.4 (CH, Ar), 128.9 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 127.2 (CH, Ar), 79.7 (OCH_2), 79.6 (OCH_2), 67.2 (2 x O*C*H_2Ph), 60.2 (*C*HCO_2Bn), 59.0 (C), 56.5 (*C*HBn), 43.7 ($NHCH_2$), 38.9 (*C*H_2Ph), 38.8 (Et*C*HMe), 24.9 (*C*H_2Me), 15.9 (CH_3), 11.6 (CH_3);

MS (ES$^+$) *m/z* 588 [M+H]$^+$, 610 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{34}H_{42}N_3O_6$ [M+H]$^+$: 588.3068; found: 588.3050.

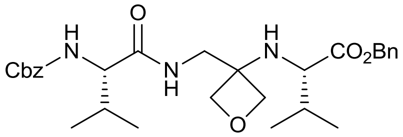
Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]propanamido]methyl]oxetan-3-yl]-*L*-valinate (227)



This novel compound was prepared as follows. **194** (101 mg, 0.31 mmol), In metal (146 mg, 1.27 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Ala-OH (72 mg, 0.32 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (71 mg, 0.37 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (50 → 70% EtOAc in hexane) provided **227** (69 mg, 44%) as a colourless oil. R_f = 0.31 (70% EtOAc in hexane); $[\alpha]_D^{20}$ +9.4 (*c* 0.16, $CHCl_3$); IR (film) 3327, 2962, 2876, 1721, 1663, 1521, 1499, 1456, 1239, 1178, 1153, 974, 737, 697 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.28 (10H, m, Ar*H*), 6.48 (1H, br s, CON*H*), 5.31 (1H, br s, CbzN*H*), 5.14 (1H, d, *J* = 12.1, $CO_2C\mathit{H}HPh$), 5.11 (1H, d, *J* = 12.1, $CO_2CH\mathit{H}Ph$), 5.10 (2H, s, $PhC\mathit{H_2}OCONH$), 4.35 (1H, d, *J* = 6.5, OC*H*H), 4.28 (1H, d, *J* = 6.5, OC*H*H), 4.25 – 4.19 (2H, m, OCH*H*, C*H*Me), 4.17 (1H, d, *J* = 6.5, OCH*H*), 3.75 (1H, dd, *J* = 13.9, 5.8, NHC*H*H), 3.39 (1H, dd, *J* = 13.9, 4.9, NHCH*H*), 3.13 (1H, d, *J* = 5.5, $C\mathit{H}^i$Pr), 2.03 – 1.89 (2H, m, $C\mathit{H}Me_2$, N*H*), 1.38 (3H, d, *J* = 7.0, $C\mathit{H_3}$, Ala), 0.94 (3H, d, *J* = 6.8, $C\mathit{H_3}$, Val), 0.87 (3H, d, *J* = 6.8, $C\mathit{H_3}$, Val); δ_C (100 MHz, $CDCl_3$) 176.1 (C=O), 173.0 (C=O), 156.0 (C=O), 136.3 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 79.7 (OCH_2), 79.4 (OCH_2), 67.2 (CH_2), 67.1 (CH_2), 61.1 ($\mathit{C}H^i$Pr), 59.5 (C), 50.9 (*C*HMe), 43.7 ($NHCH_2$), 32.0 ($\mathit{C}HMe_2$), 19.6

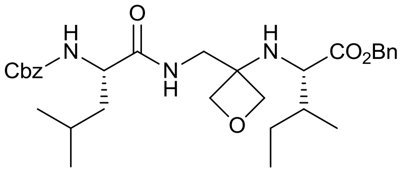
(CH_3, Val), 18.8 (CH_3, Ala), 18.0 (CH_3, Val); MS (ES^+) *m/z* 520 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{27}H_{35}N_3NaO_6$ $[M+Na]^+$: 520.2418; found: 520.2420.

Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-3-methylbutanamido]methyl]oxetan-3-yl]-*L*-valinate (228)



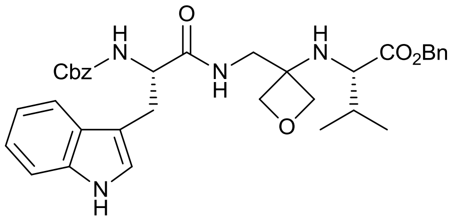
This novel compound was prepared as follows. **194** (102 mg, 0.32 mmol), In metal (144 mg, 1.25 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Val-OH (82 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (73 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (35 → 50% EtOAc in hexane) provided **228** (78 mg, 47%) as a white oily solid. $R_f = 0.24$ (50% EtOAc in hexane); $[\alpha]_D^{20}$ +20.5 (*c* 0.20, $CHCl_3$); IR (film) 3311, 2963, 2876, 1723, 1659, 1521, 1498, 1456, 1233, 1177, 1152, 1027, 976, 737, 696 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.28 (10H, m, Ar*H*), 6.46 – 6.41 (1H, m, CON*H*), 5.39 (1H, d, *J* = 8.3, CbzN*H*), 5.14 (1H, d, *J* = 12.1, OC*H*HPh), 5.11 (1H, d, *J* = 12.1, OCH*H*Ph), 5.10 (2H, s, PhCH_2OCONH), 4.35 (1H, d, *J* = 6.5, OC*H*H), 4.29 (1H, d, *J* = 6.5, OC*H*H), 4.22 (1H, d, *J* = 6.5, OCH*H*), 4.17 (1H, d, *J* = 6.5, OCH*H*), 3.99 (1H, dd, *J* = 8.3, 6.0, C*H*^{i}Pr, Z-Val), 3.74 (1H, dd, *J* = 13.8, 5.5, NHC*H*H), 3.42 (1H, dd, *J* = 13.8, 5.0, NHCH*H*), 3.13 (1H, d, *J* = 5.3, C*H*^{i}Pr, Val-OBn), 2.22 – 2.09 (1H, m, C*H*Me_2, Z-Val), 2.07 – 1.90 (2H, m, C*H*Me_2, Val-OBn, and N*H*), 0.98 – 0.93 (6H, m, CH_3), 0.91 (3H, d, *J* = 7.0, CH_3), 0.87 (3H, d, *J* = 6.8, CH_3); δ_C (100 MHz, $CDCl_3$) 176.1 (C=O), 171.8 (C=O), 156.5 (C=O), 136.4 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 79.7 (OCH_2), 79.5 (OCH_2), 67.2 (CH_2), 67.2 (CH_2), 61.1 (*C*H^{i}Pr, Val-OBn), 60.8 (*C*H^{i}Pr, Z-Val), 59.4 (C), 43.7 ($NHCH_2$), 32.0 (*C*HMe$_2$, Val-OBn), 31.0 (*C*HMe$_2$, Z-Val), 19.6 (CH_3), 19.3 (CH_3), 18.0 (CH_3), 17.9 (CH_3); MS (ES$^+$) *m/z* 526 [M+H]$^+$, 548 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{29}H_{39}N_4NaO_6$ [M+Na]$^+$: 548.2731; found: 548.2724.

Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-4-methylpentanamido]methyl]oxetan-3-yl]-*L*-isoleucinate (229)



This novel compound was prepared as follows. **204** (104 mg, 0.31 mmol), In metal (145 mg, 1.26 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Leu-OH (98 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (73 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by repeat column chromatography (30 → 45% EtOAc in hexane; 2 → 4% MeOH in CH_2Cl_2) provided **229** (92 mg, 54%) as a colourless oil. R_f = 0.37 (50% EtOAc in hexane); $[\alpha]_D^{23}$ +9.1 (*c* 0.20, $CHCl_3$); IR (film) 3314, 3034, 2960, 2875, 1724, 1718, 1660, 1529, 1456, 1237, 1172, 975, 737, 697 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.39 – 7.28 (10H, m, Ar*H*), 6.58 – 6.52 (1H, m, CON*H*), 5.23 (1H, d, *J* = 7.8, CbzN*H*), 5.12 (2H, s, CO_2C*H*$_2$Ph), 5.09 (2H, s, PhC*H*$_2$OCONH), 4.35 (1H, d, *J* = 6.3, OC*H*H), 4.29 (1H, d, *J* = 6.3, OC*H*H), 4.23 – 4.14 (3H, m, OCH*H*, C*H*CH_2), 3.74 (1H, dd, *J* = 14.0, 5.7, NHC*H*H), 3.39 (1H, dd, *J* = 14.0, 5.0, NHCH*H*), 3.20 (1H, d, *J* = 5.5, C*H*CO_2Bn), 2.03 (1H, br s, N*H*), 1.75 – 1.60 (3H, m, C*H*H^{i}Pr, C*H*Me$_2$, EtC*H*Me), 1.55 – 1.37 (2H, m, CH*H*^{i}Pr, C*H*HMe), 1.17 – 1.04 (1H, m, CH*H*Me), 0.97 – 0.81 (12H, m, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 176.1 (C=O), 172.9 (C=O), 156.2 (C=O), 136.3 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 79.8 (OCH_2), 79.4 (OCH_2), 67.2 (2 x O*C*H_2Ph), 60.3 (*C*HCO_2Bn), 59.5 (C), 53.8 (*C*HCH_2), 43.7 ($NHCH_2$), 41.7 (*C*$H_2{}^{i}$Pr), 39.0 (Et*C*HMe), 24.9 (*C*H_2Me), 24.9 (*C*HMe_2), 23.0 (CH_3), 22.1 (CH_3), 16.0 (CH_3), 11.7 (CH_3); MS (ES^+) *m/z* 554 $[M+H]^+$, 576 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{31}H_{44}N_3O_6$ $[M+H]^+$: 554.3225; found: 554.3210.

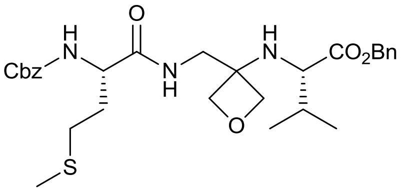
Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)propanamido]methyl]oxetan-3-yl]-*L*-valinate (230)



This novel compound was prepared as follows. **194** (100 mg, 0.31 mmol), In metal (147 mg, 1.28 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Trp-OH (109 mg, 0.32 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (72 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (50 → 70% EtOAc in hexane) provided **230** (38 mg, 20%) as oily white crystals. M.p. 65 – 67 °C; $R_f = 0.35$ (70% EtOAc in hexane); $[\alpha]_D^{26}$ +17.8 (*c* 0.16, $CHCl_3$); IR (film) 3318, 2961, 2875, 1718, 1665, 1508, 1504, 1457, 1233, 1215, 1181, 1153, 974, 740, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.27 (1H, br s, indole N*H*), 7.66 (1H, d, *J* = 7.5, Ar*H*), 7.39 – 7.28 (11H, m, Ar*H*), 7.17 (1H, t, *J* = 7.3, Ar*H*), 7.11 (1H, t, *J* = 7.3, Ar*H*), 7.01 (1H, s, Ar*H*), 6.11 – 6.05 (1H, m, CON*H*), 5.53 (1H, d, *J* = 7.0, CbzN*H*), 5.13 (1H, d, *J* = 12.3, PhC*H*HOCONH), 5.09 (1H, d, *J* = 12.3, PhCH*H*OCONH), 5.04 (2H, s, CO_2C*H_2*Ph), 4.56 – 4.48 (1H, m, C*H*CH_2), 4.20 (1H, d, *J* = 6.5, OC*H*H), 4.00 (1H, d, *J* = 6.5, OC*H*H), 3.94 (1H, d, *J* = 6.5, OCH*H*), 3.86 (1H, d, *J* = 6.5, OCH*H*), 3.53 – 3.45 (1H, m, NHC*H*H), 3.42 – 3.33

(2H, m, NHCH*H*, CHC*H*H), 3.14 (1H, dd, *J* = 14.3, 7.9, CHCH*H*), 2.81 (1H, d, *J* = 4.5, CH^iPr), 1.81 – 1.64 (2H, m, C*H*Me_2, N*H*), 0.74 (3H, d, *J* = 6.5, CH_3), 0.64 (3H, d, *J* = 6.5, CH_3); δ_C (100 MHz, $CDCl_3$) 175.9 (C=O), 171.9 (C=O), 156.0 (C=O), 136.4 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.6 (CH, Ar), 128.3 (CH, Ar), 128.2 (CH, Ar), 127.3 (C, Ar), 123.2 (CH, Ar), 122.5 (CH, Ar), 120.0 (CH, Ar), 119.0 (CH, Ar), 111.4 (CH, Ar), 110.5 (C, Ar), 79.6 (OCH_2), 79.4 (OCH_2), 67.1 (2 x O*C*H_2Ph), 60.8 (*C*H^iPr), 58.6 (C), 55.7 (*C*HCH_2), 43.6 ($NHCH_2$), 31.8 (*C*HMe_2), 28.8 (CH*C*H_2), 19.3 (CH_3), 17.9 (CH_3), quarternary aryl carbon not observed; MS (ES^+) *m/z* 613 $[M+H]^+$, 635 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{35}H_{40}N_4NaO_6$ $[M+Na]^+$: 635.2840; found: 635.2839.

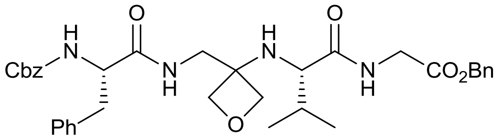
Benzyl [3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-4-(methylthio)butanamido]methyl]oxetan-3-yl]-*L*-valinate (231)



This novel compound was prepared as follows. **194** (101 mg, 0.31 mmol), In metal (145 mg, 1.26 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Met-OH (92 mg, 0.32 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (72 mg, 0.38 mmol) in EtOH (3.10 mL). Work-up, followed by purification by repeat column chromatography (40 → 50% EtOAc in heptane, then 2 → 4% MeOH in CH_2Cl_2) provided **231** (65 mg, 37%) as a colourless oil. R_f = 0.33 (60% EtOAc in hexane); $[\alpha]_D^{26}$ +8.54 (*c* 0.24, $CHCl_3$); IR (film) 3307, 2960, 2875, 1724, 1663, 1526, 1498, 1456, 1238, 1180, 1151, 1052, 975, 741, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.40 – 7.29 (10H, m, Ar*H*), 6.63 – 6.57 (1H, m, CON*H*), 5.53 (1H, br d, *J* = 7.3, CbzN*H*), 5.16 – 5.09 (4H, m, CO_2CH_2Ph and PhCH_2OCONH), 4.38 –

4.31 (2H, m, OC*H*H, C*H*CH$_2$), 4.29 (1H, d, *J* = 6.5, OC*H*H), 4.21 (1H, d, *J* = 6.5, OCH*H*), 4.16 (1H, d, *J* = 6.5, OCH*H*), 3.75 (1H, dd, *J* = 13.9, 5.7, NHC*H*H), 3.43 (1H, dd, *J* = 13.9, 5.0, NHCH*H*), 3.14 (1H, d, *J* = 5.3, C*H*^{i}Pr), 2.62 – 2.47 (2H, m, C*H$_2$*), 2.16 – 1.91 (4H, m, C*H$_2$*, C*H*Me$_2$, N*H*), 2.09 (3H, s, SC*H$_3$*), 0.94 (3H, d, *J* = 6.8, C*H$_3$*), 0.87 (3H, d, *J* = 6.8, C*H$_3$*); δ_C (100 MHz, CDCl$_3$) 176.1 (C=O), 171.8 (C=O), 156.2 (C=O), 136.3 (C, Ar), 135.3 (C, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.2 (CH, Ar), 79.6 (OCH$_2$), 79.4 (OCH$_2$), 67.2 (2 x CH$_2$), 61.1 (*C*H^{i}Pr), 59.5 (C), 54.2 (*C*HCH$_2$), 43.9 (NHCH$_2$), 32.0 (*C*HMe$_2$), 31.7 (CH$_2$), 30.2 (CH$_2$), 19.6 (CH$_3$), 18.0 (CH$_3$), 15.3 (SCH$_3$); MS (ES$^+$) *m/z* 558 [M+H]$^+$, 580 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{29}H_{39}N_3NaO_6S$ [M+Na]$^+$: 580.2452; found: 580.2456.

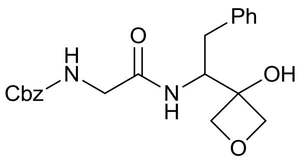
Benzyl [[3-[[(*S*)-2-[[(benzyloxy)carbonyl]amino]-3-phenylpropanamido]methyl]oxetan-3-yl]-*L*-valyl]glycinate (235)



This novel compound was prepared as follows. **207** (102 mg, 0.27 mmol), In metal (123 mg, 1.07 mmol) and 6 M HCl (0.27 mL, 1.62 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Phe-OH (84 mg, 0.28 mmol), HOBt (6 mg, 0.04 mmol), NMM (65 µL, 0.59 mmol), EDC·HCl (62 mg, 0.32 mmol) in EtOH (2.7 mL). Work-up, followed by purification by repeat column chromatography (65 → 80% EtOAc in hexane; 0 → 6% MeOH in CH$_2$Cl$_2$) provided **235** (33 mg, 19%) as a colourless oil. R$_f$ = 0.27 (80% EtOAc in hexane); $[\alpha]_D^{26}$ +19.0 (*c* 0.16, CHCl$_3$); IR (film) 3305, 2959, 2875, 1715, 1654, 1518, 1497, 1455, 1240, 1188, 970, 733, 696 cm^{-1}; δ_H (400 MHz, CDCl$_3$) 7.50 (1H, br s, N*H*), 7.37 – 7.19 (13H, m, Ar*H*), 7.17 –

7.14 (2H, m, Ar*H*), 6.85 (1H, br s, N*H*), 5.48 (1H, d, *J* = 8.3, CbzN*H*), 5.16 – 5.09 (2H, m, CO$_2$C*H*$_2$Ph), 5.06 – 4.99 (2H, m, PhC*H*$_2$OCONH), 4.41 (1H, d, *J* = 6.8, OC*H*H), 4.39 – 4.26 (4H, m, OCH*H*, OC*H*H, C*H*Bn, C*H*H), 4.20 (1H, d, *J* = 6.8, OCH*H*), 3.87 (1H, dd, *J* = 13.9, 7.5, C*H*H), 3.80 (1H, dd, *J* = 18.3, 3.8, CH*H*), 3.30 (1H, dd, *J* = 13.9, 4.3, CH*H*), 3.23 (1H, d, *J* = 3.8, C*H*^{i}Pr), 3.07 (1H, dd, *J* = 13.8, 7.0, C*H*HPh), 3.00 (1H, dd, *J* = 13.8, 7.3, CH*H*Ph), 2.12 – 2.00 (1H, m, C*H*Me$_2$), 1.79 (1H, br s, N*H*), 0.98 (3H, d, *J* = 6.8, C*H*$_3$), 0.89 (3H, d, *J* = 6.8, C*H*$_3$); δ_C (100 MHz, CDCl$_3$) 175.0 (C=O), 172.4 (C=O), 171.3 (C=O), 156.3 (C=O), 136.6 (C, Ar), 136.3 (C, Ar), 135.0 (C, Ar), 129.4 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.4 (CH, Ar), 128.1 (CH, Ar), 127.1 (CH, Ar), 79.2 (OCH$_2$), 78.5 (OCH$_2$), 67.5 (CO$_2$*C*H$_2$Ph), 67.1 (Ph*C*H$_2$OCONH), 62.6 (*C*H^{i}Pr), 60.6 (C), 56.3 (*C*HBn), 45.0 (CH$_2$), 41.0 (CH$_2$), 38.1 (*C*H$_2$Ph), 31.8 (*C*HMe$_2$), 19.7 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) *m/z* 631 [M+H]$^+$, 653 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{35}H_{42}N_4NaO_7$ [M+Na]$^+$: 653.2946; found: 653.2929.

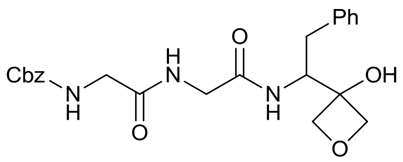
Benzyl [2-[[1-(3-hydroxyoxetan-3-yl)-2-phenylethyl]amino]-2-oxoethyl]carbamate (236)



This novel compound was prepared using modified literature procedures.[133,160] A suspension of **166** (302 mg, 1.35 mmol) and 10% Pd/C (151 mg) in anhydrous MeOH (13.4 mL) was stirred under an atmosphere of hydrogen at rt for 22 h before it was filtered through Celite$^{\circledR}$, washed with MeOH and concentrated *in vacuo* to give the crude amine. To this was added Z-Gly-OH (291 mg, 1.39 mmol), HOBt (31 mg, 0.20 mmol), NMM (325 µL, 2.95 mmol) and EtOH (13.4 mL). The reaction mixture was cooled in an ice-water bath before EDC·HCl (309 mg, 1.61 mmol) was added.

The reaction mixture was stirred at rt for 17 h before water (30 mL) was added and the precipitate formed was filtered under vacuum then washed with water and dried to afford **236** (336 mg, 65%) as a white solid. M.p. 144 – 146 °C; R_f = 0.31 (100% EtOAc); IR (film) 3311, 3264, 2963, 2894, 1686, 1648, 1538, 1495, 1455, 1436, 1258, 1139, 951, 749, 695 cm^{-1}; δ_H (400 MHz, MeOD) 7.38 – 7.13 (10H, m, Ar*H*), 5.09 (2H, s, PhCH_2O), 4.58 – 4.52 (2H, m, OC*H*H, C*H*), 4.47 (1H, d, *J* = 6.8, OCH*H*), 4.37 (1H, d, *J* = 7.0, OC*H*H), 4.30 (1H, d, *J* = 7.0, OCH*H*), 3.72 (1H, d, *J* = 16.8, NHC*H*H), 3.63 (1H, d, *J* = 16.8, NHCH*H*), 2.90 (1H, dd, *J* = 13.8, 5.3, C*H*HPh), 2.70 (1H, dd, *J* = 13.8, 9.5, CH*H*Ph); δ_C (75 MHz, MeOD) 172.3 (C=O), 158.9 (C=O), 139.7 (C, Ar), 138.1 (C, Ar), 130.2 (CH, Ar), 129.5 (CH, Ar), 129.4 (CH, Ar), 129.0 (CH, Ar), 128.9 (CH, Ar), 127.4 (CH, Ar), 83.5 (OCH_2), 82.9 (OCH_2), 76.6 (C), 67.8 (PhCH_2O), 57.1 (CH), 44.8 ($NHCH_2$), 35.8 (CH_2Ph); MS (ES$^+$) *m/z* 407 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{21}H_{24}N_2NaO_5$ [M+Na]$^+$: 407.1577; found: 407.1575.

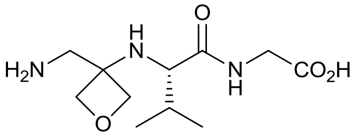
Benzyl [2-[[2-[[1-(3-hydroxyoxetan-3-yl)-2-phenylethyl]amino]-2-oxoethyl]amino]-2-oxoethyl]carbamate (238)



This novel compound was prepared using modified literature procedures.[133,160] A suspension of **166** (97 mg, 0.43 mmol) and 10% Pd/C (50 mg) in anhydrous MeOH (4.35 mL) was stirred under an atmosphere of hydrogen at rt for 20 h before it was filtered through Celite®, washed with MeOH and concentrated *in vacuo* to give the crude amine. To this was added Z-Gly-Gly-OH (119 mg, 0.45 mmol), HOBt (11 mg, 0.07 mmol), NMM (105 µL, 0.95 mmol) and EtOH (11 mL). The reaction mixture was cooled in an ice-water bath before EDC·HCl (102 mg, 0.53

mmol) was added. The reaction mixture was stirred at rt for 20 h before water (20 mL) was added and the solution was extracted with EtOAc (4 x 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0 → 6% MeOH in EtOAc) provided **238** (142 mg, 74%) as a waxy white solid. M.p. 61 – 62 °C; R_f = 0.20 (5% MeOH in EtOAc); IR (film) 3295, 2953, 2877, 1674, 1654, 1532, 1454, 1242, 1050, 970, 737, 699 cm^{-1}; δ_H (300 MHz, MeOD) 7.40 – 7.13 (10H, m, Ar*H*), 5.15 (1H, d, *J* = 12.4, PhC*H*HO), 5.10 (1H, d, *J* = 12.4, PhCH*H*O), 4.60 (1H, d, *J* = 6.9, OC*H*H), 4.54 (1H, dd, *J* = 9.8, 4.9, C*H*), 4.48 (1H, d, *J* = 6.9, OCH*H*), 4.40 (1H, d, *J* = 7.0, OC*H*H), 4.34 (1H, d, *J* = 7.0, OCH*H*), 3.86 (1H, d, *J* = 16.8, CONHC*H*H), 3.78 (2H, s, CbzNHCH_2), 3.70 (1H, d, *J* = 16.8, CONHCH*H*), 2.92 (1H, dd, *J* = 13.8, 4.9, C*H*HPh), 2.74 (1H, dd, *J* = 13.8, 9.8, CH*H*Ph), OH and NHs not observed; δ_C (100 MHz, MeOD) 172.7 (C=O), 171.6 (C=O), 159.2 (C=O), 139.7 (C, Ar), 137.9 (C, Ar), 130.2 (CH, Ar), 129.5 (CH, Ar), 129.4 (CH, Ar), 129.1 (CH, Ar), 128.9 (CH, Ar), 127.4 (CH, Ar), 83.2 (OCH_2), 82.9 (OCH_2), 76.7 (C), 68.0 (Ph*C*H_2O), 57.2 (CH), 45.1 (CbzNH*C*H_2), 43.3 (CONH*C*H_2), 35.6 (*C*H_2Ph); MS (ES^+) *m/z* 464 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{23}H_{27}N_3NaO_6$ $[M+Na]^+$: 464.1792; found: 464.1796.

[[3-(Aminomethyl)oxetan-3-yl]-*L*-valyl]glycine (249)



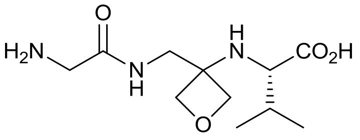
This novel compound was prepared using a modified literature procedure.[133] A suspension of **207** (47 mg, 0.12 mmol) and 10% Pd/C (24 mg, 50% by wt) in MeOH (1.32 mL) was stirred under an atmosphere of hydrogen at rt for 16 h. The mixture was filtered through Celite®, washed with MeOH and concentrated *in vacuo*.

The resultant solid was centrifuged in cold EtOH to give **249** (23 mg, 72%) as a white solid. M.p. 183 – 185 °C (dec); $[\alpha]_D^{25}$ –49.5 (*c* 0.20, MeOH); IR (film) 3314, 2941, 2874, 2600, 1650, 1555, 1545, 1473, 1380 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.10 – 8.05 (1H, m, CON*H*), 4.46 (1H, d, *J* = 6.5, OC*H*H), 4.31 (1H, d, *J* = 6.8, OC*H*H), 4.27 – 4.23 (2H, m, OCH*H*), 3.60 (1H, dd, *J* = 16.7, 6.8, C*H*HCO$_2$H), 3.33 (1H, dd, *J* = 16.7, 4.5, CH*H*CO$_2$H), 3.24 (1H, d, *J* = 13.3, NH$_2$C*H*H), 3.09 (1H, d, *J* = 13.3, NH$_2$CH*H*), 2.88 (1H, d, *J* = 5.5, C*H*^{i}Pr), 1.87 – 1.75 (1H, m, C*H*Me$_2$), 0.94 – 0.87 (6H, m, C*H*$_3$), NH, NH$_2$ and CO$_2$H not observed; δ_C (100 MHz, DMSO-d_6) 174.1 (C=O), 173.1 (C=O), 78.4 (OCH$_2$), 78.1 (OCH$_2$), 62.7 (*C*H^{i}Pr), 58.6 (C), 43.0 (CH$_2$), 42.9 (CH$_2$), 31.3 (*C*HMe$_2$), 19.4 (CH$_3$), 18.6 (CH$_3$); MS (ES$^+$) *m/z* 260 [M+H]$^+$, 282 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{11}H_{22}N_3O_4$ [M+H]$^+$: 260.1605; found: 260.1612.

General Method B: Deprotection[134]

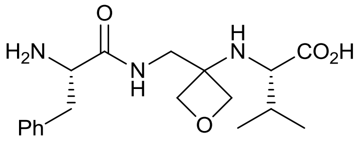
A suspension of the protected oxetane tripeptide and 10% Pd/C (20% by wt) in MeOH was stirred under an atmosphere of hydrogen at rt for 18 h then filtered through Celite®, washed with MeOH and concentrated *in vacuo* to give the product.

[3-[(2-Aminoacetamido)methyl]oxetan-3-yl]-*L*-valine (250)



This novel compound was prepared as follows. **216** (73 mg, 0.15 mmol) and 10% Pd/C (15 mg) in MeOH (3 mL) were reacted according to General Method B to give **250** (33 mg, 84%) as a cream oily solid. $[\alpha]_D^{25}$ +12.3 (*c* 0.20, MeOH); IR (film) 3266, 3077, 2963, 2879, 1682, 1568, 1557, 1470, 1436, 1393 cm^{-1}; δ_H (500 MHz, D$_2$O) 4.68 (1H, d, *J* = 7.3, OC*H*H), 4.62 (1H, d, *J* = 7.3, OC*H*H), 4.50 (1H, d, *J* = 7.3, OCH*H*), 4.43 (1H, d, *J* = 7.3, OCH*H*), 3.86 (2H, s, $\mathrm{NH_2C}\mathit{H}_2$), 3.79 (1H, d, *J* = 14.3, CONHC*H*H), 3.65 (1H, d, *J* = 14.3, CONHCH*H*), 3.13 (1H, d, *J* = 5.2, $\mathrm{C}\mathit{H}^{i}\mathrm{Pr}$), 1.98 – 1.87 (1H, m, $\mathrm{C}\mathit{H}\mathrm{Me_2}$), 0.95 (3H, d, *J* = 7.6, $\mathrm{C}\mathit{H}_3$), 0.94 (3H, d, *J* = 7.6, $\mathrm{C}\mathit{H}_3$), NH_2, NHs and CO_2H not observed; δ_C (125 MHz, D_2O) 181.2 (C=O), 167.9 (C=O), 79.0 (OCH_2), 78.8 (OCH_2), 64.2 ($\mathit{C}\mathrm{H}^{i}\mathrm{Pr}$), 59.9 (C), 43.3 ($\mathrm{CONH}\mathit{C}\mathrm{H_2}$), 40.5 ($NH_2CH_2$), 31.3 ($\mathit{C}\mathrm{HMe_2}$), 18.3 ($CH_3$), 18.2 ($CH_3$); MS ($ES^+$) *m/z* 260 $[M+H]^+$, 282 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{11}H_{22}N_3O_4$ $[M+H]^+$: 260.1605; found: 260.1605.

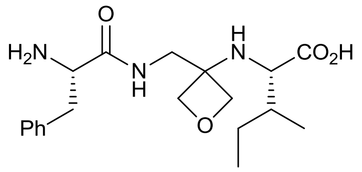
[3-[((*S*)-2-Amino-3-phenylpropanamido)methyl]oxetan-3-yl]-*L*-valine (251)



This novel compound was prepared as follows. **225** (89 mg, 0.16 mmol) and 10% Pd/C (18 mg) in MeOH (3.1 mL) were reacted according to General Method B to give **251** (52 mg, 96%) as a pale yellow solid. M.p. 165 – 167 °C (dec); $[\alpha]^{26}_{D}$ +41.3 (*c* 0.18, MeOH); IR (film) 3045, 2958, 2890, 1666, 1559, 1512, 1455, 1436, 1390, 974, 742, 697 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 8.23 – 8.19 (1H, m, CON*H*), 7.31 – 7.27 (2H, m, Ar*H*), 7.25 – 7.20 (3H, m, Ar*H*), 4.35 (1H, d, *J* = 6.2, OC*H*H), 4.29 (1H, d, *J* = 6.3, OC*H*H), 4.17 (1H, d, *J* = 6.3, OCH*H*), 4.13 (1H, d, *J* = 6.2, OCH*H*), 3.67 – 3.63 (1H, m, C*H*Bn), 3.49 – 3.40 (2H, m, $\mathrm{NHC}\mathit{H}_2$), 3.06 (1H, d, *J* = 5.7, $\mathrm{C}\mathit{H}^{i}\mathrm{Pr}$), 3.02 (1H, dd, *J* = 13.6, 5.2, C*H*HPh), 2.75 (1H, dd, *J* = 13.6, 8.0, CH*H*Ph), 1.87 – 1.79 (1H, m, $\mathrm{C}\mathit{H}\mathrm{Me_2}$), 0.88 (3H, d, *J* = 6.8, $\mathrm{C}\mathit{H}_3$), 0.83 (3H, d, *J* = 6.8, $\mathrm{C}\mathit{H}_3$), NH_2, NH and CO_2H not observed; δ_C (125 MHz, DMSO-d_6) 177.1 (C=O), 172.8 (C=O), 137.4 (C, Ar), 129.4 (CH, Ar), 128.3 (CH, Ar), 126.5 (CH, Ar), 78.4 (OCH_2), 78.2 (OCH_2), 60.7 ($\mathit{C}\mathrm{H}^{i}\mathrm{Pr}$), 59.7 (C), 55.3 (*C*HBn), 43.4 ($NHCH_2$), 38.9

(*C*H$_2$Ph), 31.1 (*C*HMe$_2$), 19.4 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) *m/z* 350 [M+H]$^+$, 372 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{18}H_{28}N_3O_4$ [M+H]$^+$: 350.2074; found: 350.2070.

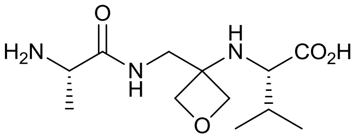
[3-[((*S*)-2-Amino-3-phenylpropanamido)methyl]oxetan-3-yl]-*L*-isoleucine (252)



This novel compound was prepared as follows. **226** (91 mg, 0.15 mmol) and 10% Pd/C (18 mg) in MeOH (3.1 mL) were reacted according to General Method B to give **252** (54 mg, 96%) as a pale orange solid. M.p. 162 – 164 °C (dec); $[\alpha]_D^{26}$ +43.2 (*c* 0.18, MeOH); IR (film) 3313, 3030, 2958, 2874, 1670, 1560, 1555, 1497, 1453, 1437, 1387, 972, 742, 696 cm^{-1}; δ_H (500 MHz, DMSO-*d$_6$*) 8.20 (1H, br t, *J* = 5.4, CON*H*), 7.31 – 7.26 (2H, m, Ar*H*), 7.25 – 7.18 (3H, m, Ar*H*), 4.34 (1H, d, *J* = 6.2, OC*H*H), 4.31 (1H, d, *J* = 6.3, OC*H*H), 4.18 (1H, d, *J* = 6.3, OCH*H*), 4.13 (1H, d, *J* = 6.2, OCH*H*), 3.61 (1H, dd, *J* = 7.8, 5.4, C*H*Bn), 3.48 – 3.40 (2H, m, NHC*H$_2$*), 3.10 (1H, d, *J* = 5.5, C*H*CO$_2$H), 3.00 (1H, dd, *J* = 13.6, 5.4, C*H*HPh), 2.74 (1H, dd, *J* = 13.6, 7.8, CH*H*Ph), 1.59 – 1.44 (2H, m, EtC*H*Me, C*H*HMe), 1.12 – 1.01 (1H, m, CH*H*Me), 0.87 – 0.79 (6H, m, C*H$_3$*), NH$_2$, NH and CO$_2$H not observed; δ_C (125 MHz, DMSO-*d$_6$*) 177.2 (C=O), 173.2 (C=O), 137.6 (C, Ar), 129.3 (CH, Ar), 128.2 (CH, Ar), 126.4 (CH, Ar), 78.5 (OCH$_2$), 78.2 (OCH$_2$), 60.1 (*C*HCO$_2$H), 59.8 (C), 55.4 (*C*HBn), 43.4 (NHCH$_2$), 39.7 (*C*H$_2$Ph), 38.1 (Et*C*HMe), 24.5 (*C*H$_2$Me), 15.7 (CH$_3$), 11.6 (CH$_3$); MS (ES$^+$) *m/z* 364 [M+H]$^+$, 386 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{19}H_{30}N_3O_4$ [M+H]$^+$: 364.2231; found: 364.2228. A crystal for X-ray analysis was grown from methanol.

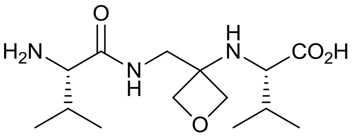
Crystal Data for $C_{19}H_{29}N_3O_4$ (M=366.65): triclinic, space group P1 (no. 1), a = 8.4127(4) Å, b = 9.5002(5) Å, c = 13.3202(8) Å, α = 96.096(5)°, β = 104.312(5)°, γ = 90.121(4)°, V = 1025.27(10) Å^3, Z = 2, T = 150(2) K, μ(CuKα) = 0.683 mm^{-1}, *Dcalc* = 1.188 g/mm^3, 19052 reflections measured (6.89 ≤ 2Θ ≤ 158.46), 8088 unique (R_{int} = 0.0470, R_{sigma} = 0.0526) which were used in all calculations. The final R_1 was 0.0506 (I > 2σ(I)) and wR_2 was 0.1426 (all data).

[3-[((*S*)-2-Aminopropanamido)methyl]oxetan-3-yl]-*L*-valine (253)



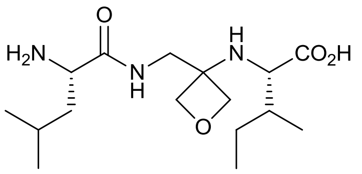
This novel compound was prepared as follows. **227** (53 mg, 0.11 mmol) and 10% Pd/C (11 mg) in MeOH (2.13 mL) were reacted according to General Method B to give **253** (29 mg, 100%) as a cream solid. M.p. 113 – 114 °C; $[\alpha]_D^{25}$ +31.5 (*c* 0.16, MeOH); IR (film) 3245, 2960, 2876, 1672, 1558, 1539, 1468, 1393 cm^{-1}; δ_H (400 MHz, D_2O) 4.65 (1H, d, *J* = 7.0, OC*H*H), 4.60 (1H, d, *J* = 7.3, OC*H*H), 4.48 (1H, d, *J* = 7.3, OCH*H*), 4.40 (1H, d, *J* = 7.0, OCH*H*), 4.03 (1H, q, *J* = 7.0, C*H*Me), 3.75 (1H, d, *J* = 14.3, NHC*H*H), 3.59 (1H, d, *J* = 14.3, NHCH*H*), 3.07 (1H, d, *J* = 5.3, C*H*^{i}Pr), 1.95 – 1.83 (1H, m, C*H*Me$_2$), 1.52 (3H, d, *J* = 7.0, C*H*$_3$, Ala), 0.93 (6H, d, *J* = 6.8, C*H*$_3$, Val), NH$_2$, NHs and CO$_2$H not observed; δ_C (100 MHz, D_2O) 182.3 (C=O), 172.6 (C=O), 79.4 (OCH$_2$), 79.2 (OCH$_2$), 64.1 (*C*H^{i}Pr), 59.7 (C), 49.4 (*C*HMe), 43.6 (NHCH$_2$), 31.5 (*C*HMe$_2$), 18.5 (CH$_3$, Va;), 18.2 (CH$_3$, Val), 17.0 (CH$_3$, Ala); MS (ES$^+$) *m/z* 274 [M+H]$^+$, 296 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{12}H_{24}N_3O_4$ [M+H]$^+$: 274.1761; found: 274.1769.

[3-[((*S*)-2-Amino-3-methylbutanamido)methyl]oxetan-3-yl]-*L*-valine (254)



This novel compound was prepared as follows. **228** (61 mg, 0.12 mmol) and 10% Pd/C (12 mg) in MeOH (2.32 mL) were reacted according to General Method B to give **254** (33 mg, 94%) as a cream solid. M.p. 102 – 103 °C; $[\alpha]_D^{25}$+56.6 (*c* 0.16, MeOH); IR (film) 3244, 2960, 2875, 1680, 1555, 1540, 1467, 1393 cm^{-1}; δ_H (400 MHz, D_2O) 4.66 (1H, d, *J* = 7.0, OC*H*H), 4.61 (1H, d, *J* = 7.3, OC*H*H), 4.48 (1H, d, *J* = 7.3, OCH*H*), 4.42 (1H, d, *J* = 7.0, OCH*H*), 3.77 (1H, d, *J* = 14.3, NHC*H*H), 3.75 (1H, d, *J* = 6.0, C*H*^{i}Pr, H-Val), 3.61 (1H, d, *J* = 14.3, NHCH*H*), 3.09 (1H, d, *J* = 5.3, C*H*^{i}Pr, Val-OH), 2.28 – 2.15 (1H, m, C*H*Me_2, H-Val), 1.95 – 1.83 (1H, m, C*H*Me_2, Val-OH), 1.04 (3H, d, *J* = 7.0, CH_3, H-Val), 1.03 (3H, d, *J* = 7.0, CH_3, H-Val), 0.93 (6H, d, *J* = 6.8, CH_3, Val-OH), NH_2, NHs and CO_2H not observed; δ_C (100 MHz, D_2O) 182.3 (C=O), 171.1 (C=O), 79.3 (OCH_2), 79.1 (OCH_2), 64.1 (*C*H^{i}Pr, Val-OH), 59.7 (C), 59.1 (*C*H^{i}Pr, H-Val), 43.8 ($NHCH_2$), 31.5 (*C*HMe_2, Val-OH), 30.0 (*C*HMe_2, H-Val), 18.5 (CH_3, Val-OH), 18.2 (CH_3, Val-OH), 17.8 (CH_3, H-Val), 16.9 (CH_3, H-Val); MS (ES$^+$) *m/z* 302 [M+H]$^+$, 324 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{14}H_{28}N_3O_4$ [M+H]$^+$: 302.2074; found: 302.2066.

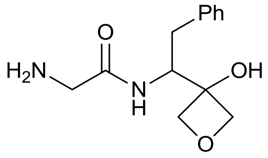
[3-[((*S*)-2-Amino-4-methylpentanamido)methyl]oxetan-3-yl]-*L*-isoleucine (255)



This novel compound was prepared as follows. **229** (90 mg, 0.16 mmol) and 10% Pd/C (18 mg) in MeOH (3.25 mL) were reacted according to General Method B to give **255** (53 mg, 99%) as a pale cream/orange solid. M.p. 130 – 132 °C (dec); $[\alpha]_D^{25}$ +36.6 (*c* 0.18, MeOH); IR (film) 3200, 2960, 2875, 1673, 1557, 1550, 1466, 1391 cm^{-1}; δ_H (500 MHz, D_2O) 4.70 (1H, d, *J* = 7.4, OC*H*H), 4.64 (1H, d, *J* = 7.6, OC*H*H), 4.52 (1H, d, *J* = 7.6, OCH*H*), 4.45 (1H, d, *J* = 7.4, OCH*H*), 4.05 (1H, t, *J* = 7.3, NH_2C*H*), 3.77 (1H, d, *J* = 14.5, NHC*H*H), 3.66 (1H, d, *J* = 14.5, NHCH*H*), 3.32 (1H, d, *J* = 4.6, C*H*CO_2H), 1.81 – 1.65 (4H, m, CH_2^{i}Pr, EtC*H*Me, C*H*Me_2), 1.57 – 1.48 (1H, m, C*H*HMe), 1.25 – 1.15 (1H, m, CH*H*Me), 0.98 (3H, d, *J* = 6.5, CH_3), 0.96 (3H, d, *J* = 6.5, CH_3), 0.93 – 0.89 (6H, m, CH_3), NH_2, NHs and CO_2H not observed; δ$_C$ (125 MHz, D_2O) 179.9 (C=O), 171.5 (C=O), 78.3 (OCH_2), 78.0 (OCH_2), 63.0 (*C*HCO_2H), 60.4 (C), 52.1 (NH_2CH), 43.2 ($NHCH_2$), 39.8 (*C*$H_2{}^i$Pr), 38.2 (Et*C*HMe), 25.5 (*C*H_2Me), 23.9 (*C*HMe_2), 21.7 (CH_3), 20.9 (CH_3), 14.8 (CH_3), 11.2 (CH_3); MS (ES$^+$) *m/z* 330 $[M+H]^+$, 352 $[M+Na]^+$; HRMS (ES$^+$) calcd. for $C_{16}H_{31}N_3NaO_4$ $[M+Na]^+$: 352.2207; found: 352.2208. A crystal for X-ray analysis was grown from methanol.

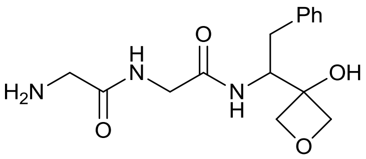
Crystal Data for $C_{16}H_{31}N_3O_4$ (M =329.44): triclinic, space group P1 (no. 1), a = 8.5557(2) Å, b = 14.0932(5) Å, c = 16.3070(4) Å, α = 77.134(3)°, β = 81.935(2)°, γ = 80.958(2)°, V = 1881.70(9) Å^3, Z = 4, T = 150(2) K, μ(CuKα) = 0.679 mm^{-1}, *Dcalc* = 1.163 g/mm^3, 34050 reflections measured (6.488 ≤ 2Θ ≤ 156.87), 14895 unique (R_{int} = 0.0468, R_{sigma} = 0.0504) which were used in all calculations. The final R_1 was 0.0644 (I > 2σ(I)) and wR_2 was 0.1836 (all data).

2-Amino-*N*-[1-(3-hydroxyoxetan-3-yl)-2-phenylethyl]acetamide (256)



This novel compound was prepared as follows. **236** (50 mg, 0.13 mmol) and 10% Pd/C (10 mg) in MeOH (2.6 mL) were reacted according to General Method B. Purification by column chromatography (10 → 15% MeOH in CH_2Cl_2 + 0.5% $NH_{3(aq)}$) provided **256** (24 mg, 74%) as a colourless oil. R$_f$ = 0.25 (20% MeOH in CH_2Cl_2 + 0.5% $NH_{3(aq)}$); IR (film) 3294, 3030, 2915, 2872, 1651, 1528, 1497, 1456, 1368, 1228, 967, 752, 697 cm^{-1}; δ$_H$ (400 MHz, MeOD) 7.28 – 7.23 (4H, m, Ar*H*), 7.21 – 7.15 (1H, m, Ar*H*), 4.60 – 4.55 (2H, m, C*H*, OC*H*H), 4.49 (1H, d, *J* = 6.8, OCH*H*), 4.41 (1H, d, *J* = 7.0, OC*H*H), 4.35 (1H, d, *J* = 7.0, OCH*H*), 3.29 – 3.13 (2H, m, NH_2C*H_2*), 2.94 (1H, dd, *J* = 13.8, 5.0, C*H*HPh), 2.71 (1H, dd, *J* = 13.8, 9.8, CH*H*Ph), NH_2, NH and OH not observed; δ$_C$ (100 MHz, MeOD) 174.2 (C=O), 139.8 (C, Ar), 130.3 (CH, Ar), 129.4 (CH, Ar), 127.5 (CH, Ar), 83.4 (OCH_2), 83.0 (OCH_2), 76.7 (C), 57.0 (CH), 44.3 (NH_2CH_2), 35.8 (CH_2Ph); MS (ES^+) *m/z* 251 $[M+H]^+$, 273 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{19}N_2O_3$ $[M+H]^+$: 251.1390; found: 251.1389.

2-Amino-*N*-[2-[[1-(3-hydroxyoxetan-3-yl)-2-phenylethyl]amino]-2-oxoethyl]acetamide (257)



This novel compound was prepared as follows. **238** (64 mg, 0.14 mmol) and 10% Pd/C (13 mg) in MeOH (2.9 mL) were reacted according to General Method B. Purification by column chromatography (20 → 25% MeOH in CH_2Cl_2 + 0.5% $NH_{3(aq)}$) provided **257** (36 mg, 81%) as a colourless oil. $R_f = 0.33$ (30% MeOH in CH_2Cl_2 + 0.5% $NH_{3(aq)}$); IR (film) 3294, 3063, 2948, 2875, 1652, 1604, 1532, 1496, 1454, 1363, 1219, 971, 753, 700 cm^{-1}; δ_H (400 MHz, MeOD) 7.26 (4H, d, *J* = 4.3, Ar*H*), 7.21 – 7.15 (1H, m, Ar*H*), 4.60 – 4.55 (2H, m, C*H*, OC*H*H), 4.49 (1H, d, *J* = 7.0, OCH*H*), 4.39 (1H, d, *J* = 7.0, OC*H*H), 4.34 (1H, d, *J* = 7.0, OCH*H*), 3.85 (1H, d, *J* = 16.6, CONHC*H*H), 3.74 (1H, d, *J* = 16.6, CONHCH*H*), 3.29 (2H, s, NH_2CH_2), 2.92 (1H, dd, *J* = 13.9, 5.0, C*H*HPh), 2.71 (1H, dd, *J* = 13.9, 9.7, CH*H*Ph), NH_2, NHs and OH not observed; δ_C (100 MHz, MeOD) 175.8 (C=O), 171.6 (C=O), 139.8 (C, Ar), 130.2 (CH, Ar), 129.4 (CH, Ar), 127.4 (CH, Ar), 83.4 (OCH_2), 82.9 (OCH_2), 76.7 (C), 57.1 (CH), 45.0 (NH_2CH_2), 43.1 ($CONHCH_2$), 35.7 (CH_2Ph); MS (ES^+) *m/z* 308 $[M+H]^+$, 330 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{21}N_3NaO_4$ $[M+Na]^+$: 330.1424; found: 330.1433.

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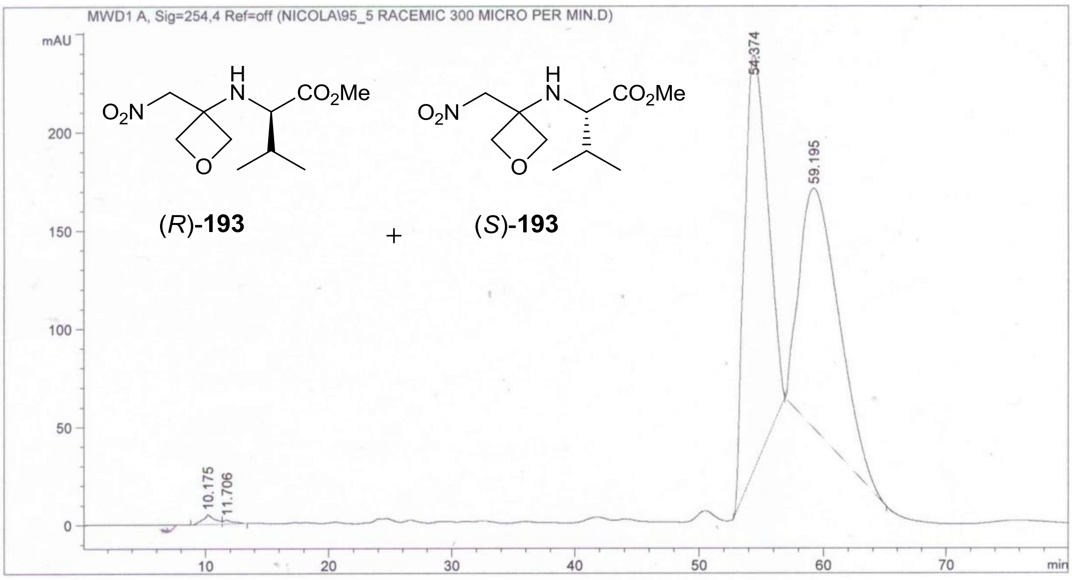
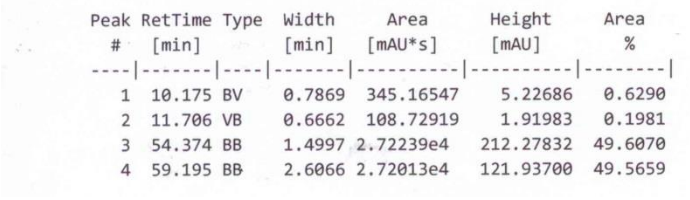
Appendix I – Chiral HPLC analysis of (*R*)-193 and (*S*)-193

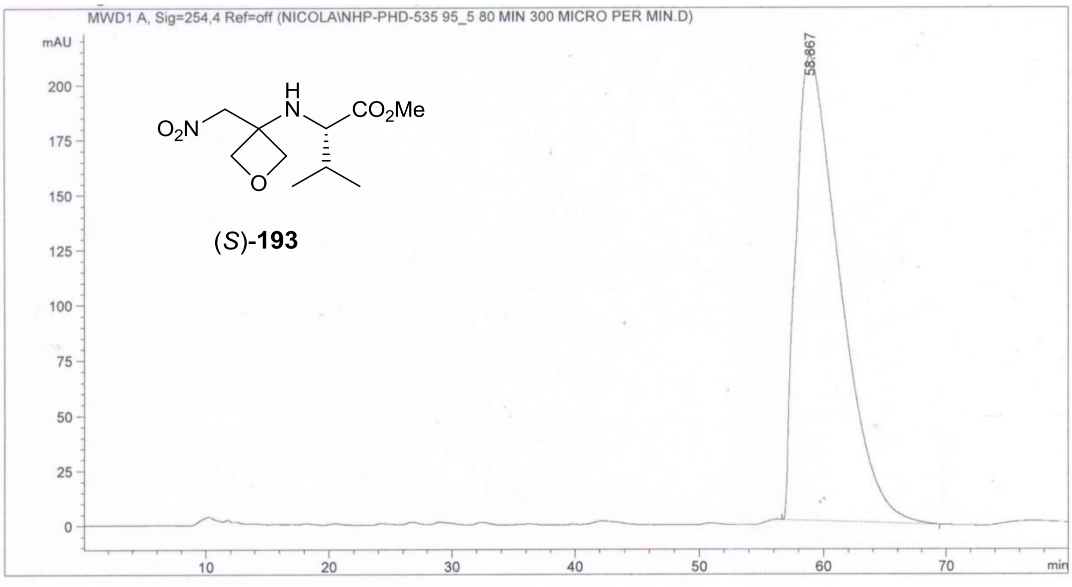
(HPLC OD-H, 95 : 5 hexane : ^{i}PrOH, flow rate = 0.30 μL/min, λ = 254 nm)





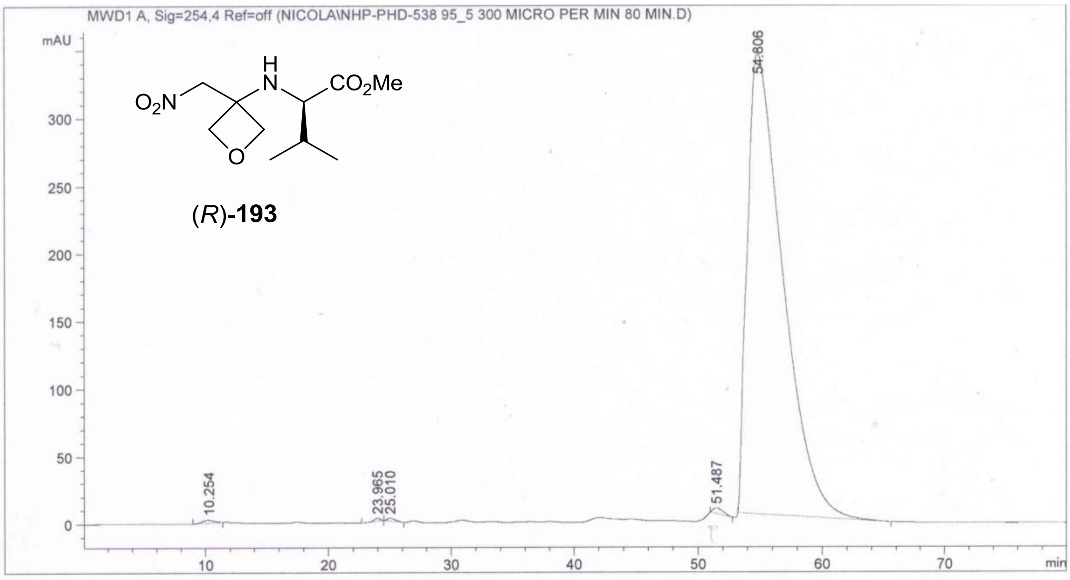
```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]         [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1  10.175 BV    0.7869  345.16547    5.22686   0.6290
  2  11.706 VB    0.6662  108.72919    1.91983   0.1981
  3  54.374 BB    1.4997 2.72239e4   212.27832  49.6070
  4  59.195 BB    2.6066 2.72013e4   121.93700  49.5659
```

Chiral HPLC trace of the racemic mixture of (*R*)- and (*S*)-**193**.



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]         [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1  58.867 BB     2.8600 5.15828e4  210.71313 100.0000
```

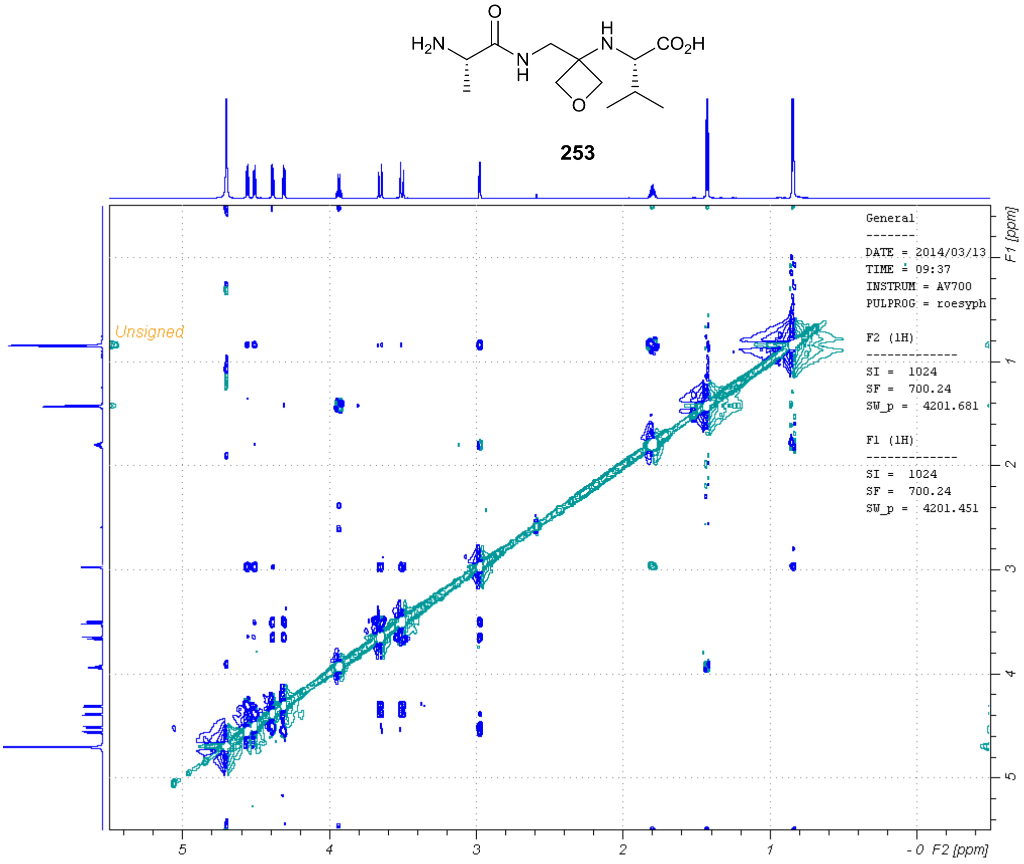
Chiral HPLC trace of enantiomerically pure (*S*)-**193**.



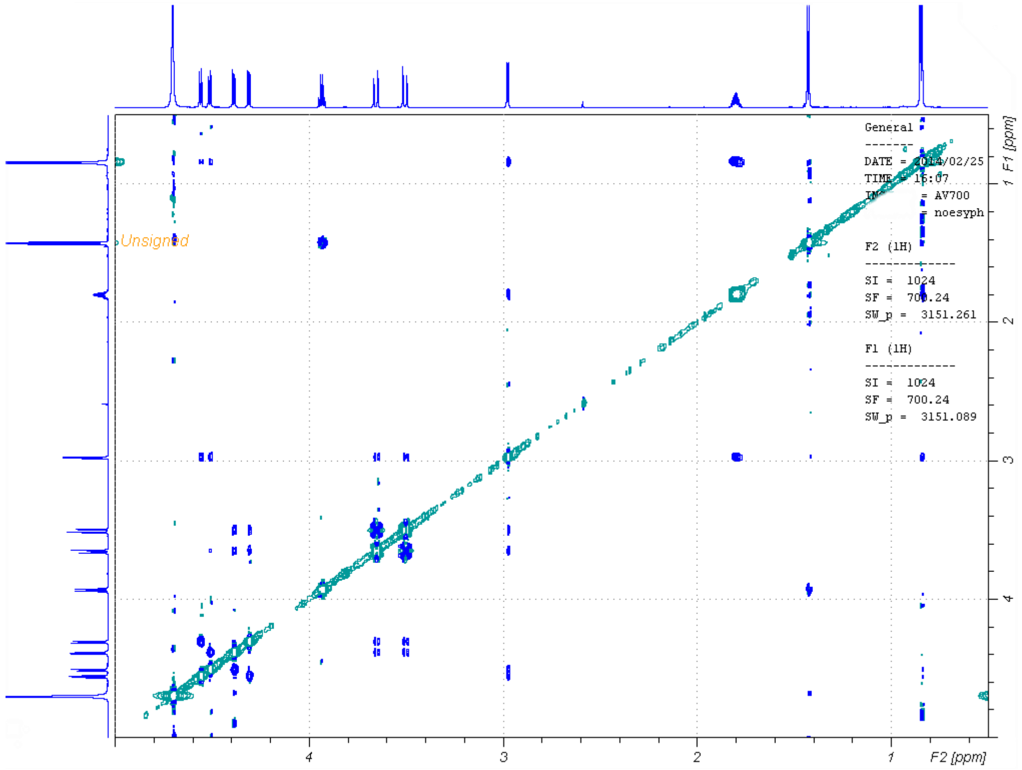
```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]         [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1  10.254 BB     0.7384  152.92067    2.42422   0.2249
  2  23.965 BB     0.4219   76.26684    2.12404   0.1122
  3  25.010 BB     0.5285   88.67345    1.96676   0.1304
  4  51.487 BB     0.6835  232.87225    3.99550   0.3425
  5  54.806 BB     2.3168 6.74385e4  340.03842  99.1900
```

Chiral HPLC trace of enantiomerically pure (*R*)-**193**.

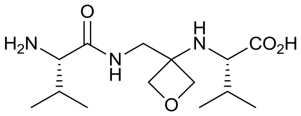
Appendix II – NOESY and ROESY spectra of 253 and 254



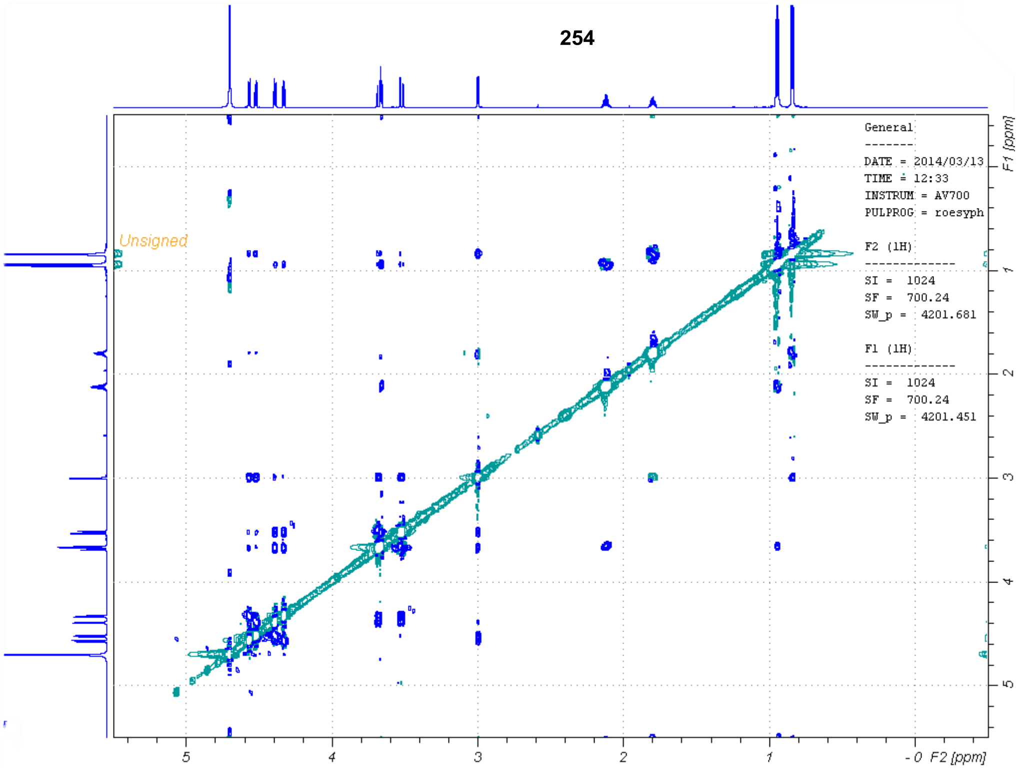
ROESY spectrum of **253**.



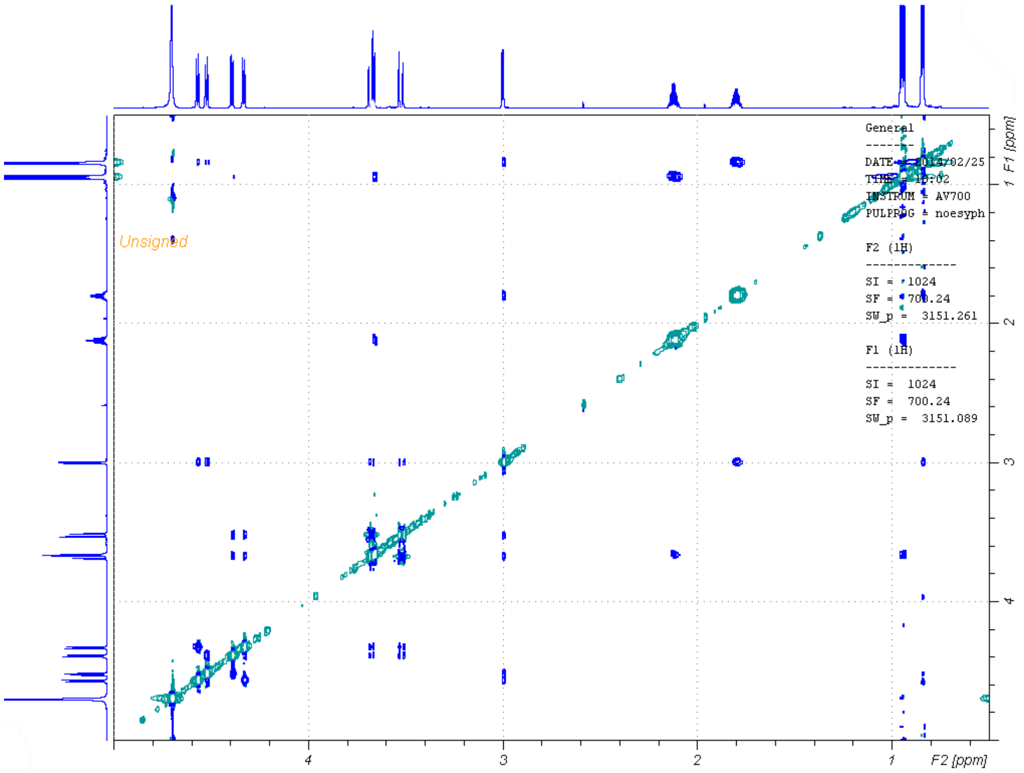
NOESY spectrum of **253**.



254

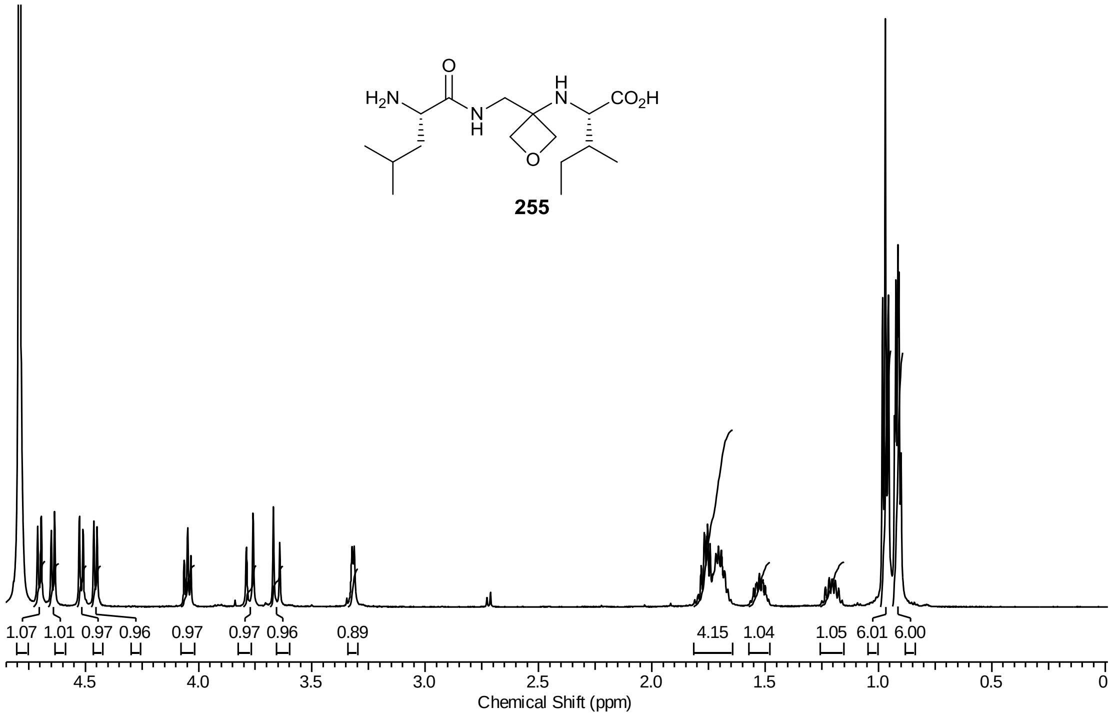


ROESY spectrum of **254**.

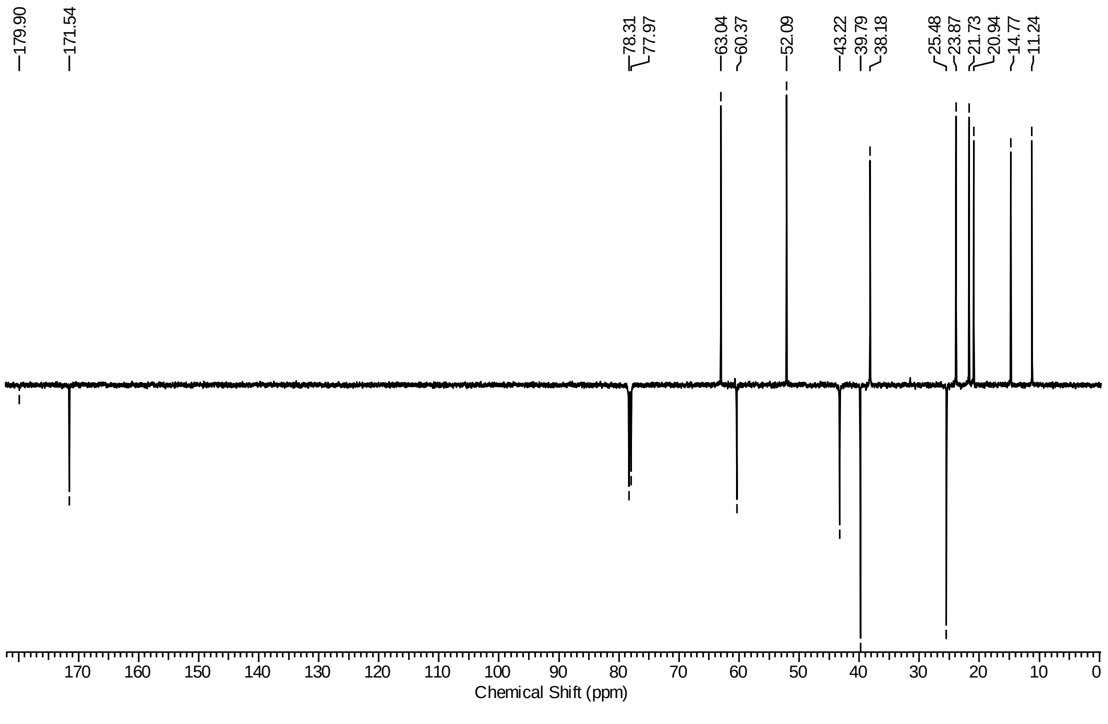


NOESY spectrum of **254**.

Appendix III – ^{1}H and DEPT Spectra of 255



^{1}H NMR of **255**.



DEPT spectrum of **255**.