Remote asymmetric induction in the organocatalytic aza-Darzens synthesis of peptide aziridines: towards the assembly of new glycopeptide antibiotic analogues

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Thesis submitted in part fulfillment of the requirements for the degree of Doctor of Philosophy
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Declaration

The research presented in this thesis is, to the best of my knowledge, original except where due reference is made.

Polly-Anna Ashford
Abstract

The widespread development of bacterial resistance to antibiotics is an ongoing process which represents a growing problem for modern medicine. Vancomycin is used as a drug of last resort against methicillin-resistant *Staphylococcus aureus* (MRSA), but the increasing prevalence of vancomycin-resistant bacteria has intensified the search for new antibiotics. The introductory chapter of this thesis presents an overview of publications reporting the synthesis of glycopeptide analogues and derivatives in the last decade, highlighting the successful approaches which have brought new pharmaceutical drugs to the market.

The results and discussion section of this work focusses first on the synthesis of a triaryl biether structure, which is a common feature of the glycopeptide antibiotic family and represents the beginning of a versatile synthetic approach towards natural products like orienticin C and vancomycin. The second discussion chapter describes the development of an asymmetric organocatalytic aza-Darzens aziridination protocol using diazopeptides and amino acid imines as substrates. A structurally diverse range of peptide aziridines are reported in yields and diastereoselectivity of up to 99%.

It is anticipated that the remote asymmetric induction methodology described in this thesis will be applicable not only to the synthesis of novel glycopeptide antibiotics and other peptide-based pharmaceutical compounds, but also to the more general asymmetric synthesis of natural and unnatural amino acids.
Publications arising from this research

“Recent advances in the synthesis of new glycopeptide antibiotics”

“Synthesis of Structure and Function Diverse α-D-Diazoacetates, α-D-Diazoacetamides, α-D-Diazoketones, and the Antibiotic α-D-Azaserine”
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Last but not least… Thank you Andy, for putting up with me in the final throes of my thesis writing, for providing unlimited tofu and cider, and for always finding ways to make me smile.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AA</td>
<td>amino acid</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ADME</td>
<td>absorption, distribution, metabolism and excretion</td>
</tr>
<tr>
<td>Aib</td>
<td>α-aminoisobutyric acid</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
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<tr>
<td>BINAP</td>
<td>2,2’-bis(diphenylphosphino)-1,1’-binaphthyl</td>
</tr>
<tr>
<td>BINOL</td>
<td>1,1’-bi-2-naphthol</td>
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<tr>
<td>Bn</td>
<td>benzyl</td>
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<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
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<tr>
<td>Bu</td>
<td>butyl</td>
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<tr>
<td>Bz</td>
<td>benzoyl</td>
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<tr>
<td>CAN</td>
<td>cerium(IV) ammonium nitrate</td>
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<tr>
<td>Cbz</td>
<td>carboxybenzyl</td>
</tr>
<tr>
<td>cod</td>
<td>1,5-cyclooctadiene</td>
</tr>
<tr>
<td>CTAB</td>
<td>cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>Cy</td>
<td>cyclohexyl</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N’-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DCU</td>
<td>N,N’-dicyclohexylurea</td>
</tr>
<tr>
<td>de</td>
<td>diastereomeric excess</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylacetamide</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPA</td>
<td>diphenylphosphoryl azide</td>
</tr>
<tr>
<td>dr</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>DtBPF</td>
<td>1,10-bis(di-tert-butylyphosphino)ferrocene</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>median effective concentration</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
</tbody>
</table>
ee enantiomeric excess
Et ethyl
FT-IR Fourier transform infrared spectroscopy
h hour(s)
HATU 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU O-benzotriazole-\(N,N,N',N'\)-tetramethyluronium hexafluorophosphate
HNESP high-resolution nanoelectrospray
HPLC high performance liquid chromatography
LA Lewis acid
MALDI-TOF matrix-assisted laser desorption/ionization – time of flight
MeCN acetonitrile
MIC minimum inhibitory concentration
MRSA methicillin-resistant *Staphylococcus aureus*
MS molecular sieves
NBS \(N\)-bromosuccinimide
NMR nuclear magnetic resonance
Ph phenyl
PG peptidoglycan
PMP \(para\)-methoxyphenyl
pMZ 9-methoxybenzyloxyacarbonyl
ppm parts per million
Pt protecting group
pTSA \(para\)-toluenesulfonic acid
PyrHOTf pyridinium triflate
RAI remote asymmetric induction
rt room temperature
*S. aureus* *Staphylococcus aureus*
SNAr nucleophilic aromatic substitution
SPhos 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TBAF tetra-\(n\)-butylammonium fluoride
TBDPS \(tert\)-butyldiphenylsilyl
TBTU \(O\)-(benzotriazol-1-yl)-\(N,N,N',N'\)-tetramethyluronium
tetrafluoroborate

′Bu  \textit{tert-}butyl

TBDA  \textit{tert-}butyl diazoacetate

TES  triethylsilyl

Tf  triflyl

TFA  trifluoroacetic acid

THF  tetrahydrofuran

TLC  thin layer chromatography

TMG  1,1,3,3-tetramethylguanidine

TMS  trimethylsilyl

Ts  tosyl

VANOL  3,3′-diphenyl-2,2′-bi-1-naphthol

VAPOL  2,2′-diphenyl-(4-biphenanthrol)

VRE  vancomycin-resistant \textit{enterococci}

VRSA  vancomycin-resistant \textit{Staphylococcus aureus}
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CHAPTER ONE

Synthesis of new glycopeptide antibiotics

1.1 History of vancomycin

The discovery of vancomycin in 1956 was the dawn of a new age for antibacterial research, and over the last 50 years has led to the formation of a large class of compounds, natural and synthetic, known as the glycopeptide antibiotics. Scientists at Eli Lilly isolated vancomycin from the fermentation broth of Streptomyces orientalis, grown from a soil sample originating in the jungles of Borneo.\(^1\) It displays potent activity against a range of Gram-positive bacteria, with an \textit{in vitro} minimum inhibitory concentration (MIC) of 0.25–10.0 \(\mu\)g mL\(^{-1}\) against \textit{Staphylococcus aureus}.\(^2\) Vancomycin was approved by the FDA in 1958, but was initially limited in clinical use due to its apparent side effects,\(^3\) with methicillin becoming the antibiotic of choice against \textit{S. aureus}. However, the sharp rise in drug-resistant strains, in particular the deadly methicillin-resistant \textit{Staphylococcus aureus} (MRSA), has brought vancomycin back into the limelight, prompting a new wave of research into the chemistry and biology of this natural product.

![Molecular structure of vancomycin (1)](image)

**Figure 1.1** • Molecular structure of vancomycin (1)

By 1989, a handful of glycopeptide antibiotics had been isolated and characterised. Among them, ristocetin (4) and teicoplanin (5) displayed antibacterial activity comparable to that of vancomycin (Figure 1.2).\(^4\) Avoparcin (8) was widely used as a growth promoter in animal feed until a link was established between its use and the increased prevalence of vancomycin-resistant \textit{enterococci} (VRE) in the food industry.\(^5\) The use of avoparcin was banned in all EU countries by 1997.
Figure 1.2 • The vancomycin family of glycopeptide antibiotics
While vancomycin continues to be a crucial drug of last resort, the rise in geographically isolated human cases of VRE and the appearance of vancomycin-resistant *Staphylococcus aureus* (VRSA)\(^6\) has signalled urgent research into the development of potent glycopeptide-based antibiotics in both an academic setting and in pharmaceutical and medicinal laboratories within industry. In the last ten years there has been increasing global interest in the design and development of semi-synthetic variants of natural product glycopeptides which have been aimed at providing a solution to this problem, either by modification of the existing scaffold, by the introduction of hydrophobic moieties or by dimerisation.\(^7\) From recent reports, patterns of activity have emerged that may influence and direct future studies.

### 1.2 Structure of the glycopeptide antibiotics

Notable early attempts to elucidate the structure of vancomycin included rigorous NMR experiments carried out by D. H. Williams,\(^8\) and X-ray crystallography of a less complex degradation product by G. Sheldrick,\(^9\) but the precise structure was finally confirmed in 1982 by Harris and Harris.\(^10\) Further NMR studies in 1993 showed that the glycopeptide exists mainly as a dimer in solution,\(^11\) and thanks to advances in X-ray crystallography this observation was confirmed by a full crystal structure of vancomycin in 1996, which also gave an accurate picture of the stereochemistry.\(^12\) The molecular structure of vancomycin consists of the C-O-D-O-E triaryl biether backbone forming two 16-membered ring macrocycles with a heptapeptide framework (Figure 1.1). A 12-membered ring comprising the A-B biaryl system forms a third macrocycle. It is interesting to note the conserved triaryl biether moiety in this class of compounds (Figure 1.2).

Chloro substitution of aromatic rings C and E and the strained nature of the macrocyclic systems causes vancomycin to adopt the low energy conformation (atropisomer) shown in Figure 1.1. There is no rotation of the C or D rings about the adjacent C–C and C–O bonds at ambient temperature; a study by Boger showed that D-O-E ring of vancomycin algycon is able to undergo thermal interconversion to the unnatural atropisomer at 110 °C in *o*-dichlorobenzene with a half-life of 94 h.\(^13\)

There is a second region of atropisomerism associated with the A-B biaryl system, giving a total of 8 possible atropisomers and 18 chiral carbon centres. Selecting the natural
product atropisomer is therefore an important consideration when constructing these large ring systems.

A characteristic vancomycin feature is the disaccharide moiety mounted on the D ring, which consists of a β-D-glucose unit attached to a unique amino-sugar, vancosamine. Of the naturally-occurring glycopeptides, orienticin C (2), chloroeremomycin (6) and balhimycin (7) possess an additional carbohydrate unit on amino acid residue 6, β-hydroxytyrosine, (L-vancosamine, L-4-epivancosamine and 4-oxovancosamine respectively). Teicoplanin (5) is one of the most complex members of the vancomycin family, with N-acetyl-β-D-glucosamine and D-mannose at residue 6 and 7 respectively, and a nine-carbon chain appended to the top of the molecule, as well as an additional 14-membered macrocycle (F-O-G). The complestatin sub-group discussed later in this review contains a tryptophan moiety in place of the D-O-E macrocycle.

1.3 Mode of action

Bacterial cell walls contain a semi-rigid polymer called peptidoglycan, or murein, which acts as a structural support. The peptidoglycan monomers consist of peptide and disaccharide units that are joined in long chains by glycosidic bonds via transglycosidation. Cross-linking of the chains, or transpeptidation, affords the polymer a rigid framework. The glycopeptide antibiotics are able to pass through the peptidoglycan layer to the site of polymerisation, whereupon they form non-covalent bonds with the terminal carbohydrates, in a process that ultimately inhibits cross-linking by the transpeptidase enzyme. The weakened cell wall is no longer able to withstand the positive osmotic pressure within the cell, leading to cytolysis and bacteria death.

Previous NMR and X-ray studies indicate that vancomycin binds to the L-Lys-D-Ala-D-Ala terminus of the peptidoglycan precursor via five hydrogen bonds (Figure 1.3a). It is interesting to note that a number of glycopeptides, such as vancomycin and ristocetin, have the ability to dimerise in aqueous solution. Dimerisation is of particular importance because glycopeptides such as eremomycin, with a high dimerisation constant (ca. 106 M\(^{-1}\)) are potent antibiotics even though binding to cell wall analogs is only moderate (K\(b\) ca. 104 M\(^{-1}\)).15
CHAPTER 1 Synthesis of new glycopeptide antibiotics

Figure 1.3 • Vancomycin binding to a) D-Ala-D-Ala terminus of susceptible bacteria and b) D-Ala-D-Lac terminus of resistant strains

X-ray crystallographic investigations by Sheldrick et al. in 1996 revealed the dimerisation activity of vancomycin (Figure 1.4), but recent X-ray crystal structures by Nitanai et al. provide a more detailed picture of the “back-to-back”, “face-to-face” and “side-to-side” arrangements observed in vancomycin binding to analogues of the peptidoglycan terminus (Figure 1.4). Teicoplanin (5) is unable to dimerise in solution, but contains a structural feature that is absent in vancomycin: an N-acylglucosamine moiety containing a 2-methyloctanyl chain (in the dominant component, teicoplanin A2-2). It has been shown that this lipophilic tail anchors the antibiotic to the phospholipid bilayer of the bacterial cell membrane.15

Figure 1.4 • Stereoview of the vancomycin dimer by Sheldrick et al.12 (left) Stereoview by Nitanai et al. showing the vancomycin dimer-to-dimer structure in the L-Lys-D-Ala-D-Ala complex.18 (right)

Changes in the structure of the peptidoglycan termini lead to vancomycin resistance. The most common phenotypes of vancomycin-resistant enterococci (VRE), VanA and VanB,
were found to have a D-lactate terminus in place of D-alanine.\textsuperscript{16} This removes one of the hydrogen bonding interactions and leads to lone pair-lone pair repulsion (Figure 1.3b). A comprehensive review covering the mode of action of the glycopeptides antibiotics and the mechanisms of bacterial resistance has been compiled by Walsh and Kahne \textit{et al.}\textsuperscript{17}

\section*{1.4 Semi-synthetic glycopeptide antibiotics}

\subsection*{1.4.1 Oritavancin, telavancin and dalbavancin}

Early studies by Nagarajan \textit{et al.} into the development of semi-synthetic glycopeptide antibiotics focused on utilizing vancomycin as a structural starting point, but the inadequate activity of vancomycin analogues\textsuperscript{19} led to the selection of alternatives like teicoplanin (5) and chloroeremomycin (6) as platforms for novel glycopeptide elaboration. Structurally similar to teicoplanin (5) is the semi-synthetic compound dalbavancin (9) which is appended with an additional amide group at the C-terminus. Dalbavancin has come close to clinical success, and is currently undergoing phase III clinical trials funded by Durata Therapeutics.\textsuperscript{1} Although its activity is similar to that of teicoplanin, the semi-synthetic compound has a remarkably long half-life of \textit{ca.} 7 days as a result of strong binding to serum proteins.\textsuperscript{20} By comparison, vancomycin has an elimination half-life of 3–9 h.\textsuperscript{21} A drug that remains in the blood plasma for longer requires less frequent dosing, and is therefore clinically and economically advantageous.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{oritavancin.png}
\caption{Molecular structure of oritavancin (10)}
\end{figure}

\textsuperscript{1} Since publication of this review, Dalbavancin has been approved by the FDA for the treatment of bacterial skin infections (May 2014).
Oritavancin (10) (Figure 1.5) is derived from chloroeremomycin (6) (Figure 1.2). The key modification of natural product 6 was the installation of a 4’-chlorobiphenylmethylene group, which affords oritavancin an additional mode of action that is absent from vancomycin; recent investigations have shown that this biaryl group is involved in cell membrane depolarisation and an increase in membrane permeability. As a consequence of this dual-action mechanism, oritavancin has excellent in vitro activity against gram positive bacteria, with MICs in the range of 0.004–1 µg mL\(^{-1}\) against VRE, and is currently in phase III clinical trials funded by The Medicines Company for the treatment of acute bacterial skin and skin structure infections (ABSSSIs).

A derivative of vancomycin, telavancin (3) (Figure 1.2) also follows the pattern of N-alkylation of the vancomycin moiety with a hydrophobic chain, in this case a decylaminoethyl group. In addition, a methylaminophosphonate group was installed on the A-ring of the A-B biaryl region for its hydrophilic properties, which improved the absorption, distribution, metabolism and excretion (ADME) profile of the compound. With excellent activity against both VRE and VRSA, telavancin was approved by the FDA in September 2009 for complicated skin and skin structure infections (cSSSI). The success of these three semi-synthetic glycopeptides as drug candidates and their potent antibacterial activities in comparison to vancomycin, has encouraged further research in the field of glycopeptide modification. The structure–activity relationships of telavancin, dalbavancin and oritavancin have provided valuable information about the modes of action that this class of compounds possess, with the results having a significant influence on future drug design.

1.4.2 Modification of the amino acids within the polypeptide chain

The challenge of where and how to successfully modify existing natural products can be approached in different ways. The glycopeptide antibiotics are large, complex molecules with plenty of potential for N- and O-alkylation as well as changes to the component amino acids of the heptapeptide chain. Even the chloro substituents of the triaryl biether backbone can be envisaged as sites of reactivity and as such are viable sites for chemical transformation.
Figure 1.6 • Amino acid residues 1-7 of vancomycin (parent amino acids of the biosynthetic pathway are labelled in parentheses)

Modifications of amino acid residue 3 (Figure 1.6) were first reported by Boger et al. in 2002. In the process of developing a total synthesis of vancomycin, the Boger group noted that an intermediate containing a nitrile group at residue 3 rather than the carboxamide of asparagine showed increased biological activity against vancomycin-sensitive and resistant bacteria. Various vancomycin and vancomycin aglycon analogues containing this modification were synthesised and evaluated. Compound 11 was identified as having the best overall activity against a range of relevant bacteria, with promising activity against VRE (VanB) compared to vancomycin (Figure 1.7).

Figure 1.7 • Vancomycin aglycon nitrile methyl ether derivative generated by Boger et al.
However, despite the promising antibacterial activity of compound 11, the nitrile derivatives were generally less active than the corresponding carboxamide. Crucially, removal of the four methyl ether modifications of 11 as well as hydrolysis of the methyl ester resulted in significantly lower activity, with an increase in the MIC against *S. aureus* from 0.625 to 10 µg mL⁻¹. The effect of methylation is therefore clearly more pronounced than that of amino acid modification. When subjected to D-Ala-D-Ala peptide binding tests, nitrile derivative 11 and its corresponding carboxamide showed binding constants that were comparable to vancomycin ($K_a = 9.9 \times 10^5$, $2.0 \times 10^5$ and $2.3–3.9 \times 10^5$ respectively). Boger suggested that this result may be due to the increased hydrophobicity of these compounds, mimicking the effect of the hydrophobic chain in teicoplanin.

A subsequent study on the activity of vancomycin-derived methyl ether analogues demonstrated that O-methylation of the phenolic hydroxyl groups can have mixed results with regard to the biological activity.²⁸ O-methylated derivatives of vancomycin, teicoplanin and ristocetin were prepared from the corresponding aglycons using the following general procedure: *N*-Boc protection of the amine terminus followed by O-methylation by trimethylsilyldiazomethane and removal of the Boc group by 4N hydrochloric acid in dioxane. Activity against *S. aureus* by 12 decreased by half compared to vancomycin and its aglycon, but a significant improvement in activity was observed against the VRE VanB phenotype (Figure 1.8). Similarly, compound 13 performed better against VRE (VanB) than the parent compound, ristocetin, but was less active against *S. aureus*. In all cases the improvements in antibacterial activity afforded by methyl ether derivatisation clearly demonstrated the potential of O-alkylation as a protocol for the development of more potent vancomycin analogues.

![Figure 1.8](image-url)  

12: MIC (*E. faecalis* VanB) = 1.6 µg/mL  
Vancomycin aglycon: MIC (*E. faecalis* VanB) = 25.0 µg/mL  
13: MIC (*E. faecalis* VanB) = 3.2 µg/mL  
Ristocetin aglycon: MIC (*E. faecalis* VanB) = 25.0 µg/mL  

**Figure 1.8** • Methyl ether derivatives of a) vancomycin aglycon and b) ristocetin aglycon by Boger *et al.*
While many of the novel vancomycin derivatives discussed later in this review rely on the addition of groups by alkylation etc., a limited amount of work has been undertaken on modifying the peptide backbone of vancomycin itself. Modification of the peptide backbone presents a considerable synthetic challenge, as changes to the internal peptide structure may involve a full synthesis of the compound rather than simple manipulation of naturally sourced material. Using their experience of vancomycin total synthesis, the Boger group developed an analogue that was specifically designed to target vancomycin resistant organisms (Figure 1.9).\textsuperscript{16} Compound 17 contains a modification in amino acid residue 4 (boxed), which is equipped with a methylene group in place of an amide carbonyl. The effect of this small change is to remove the oxygen-oxygen repulsion responsible for the observed decrease in binding affinity of vancomycin for the D-Ala-D-Lac terminus in the peptidoglycan of VRE type A and B.

![Figure 1.9](image)

**Figure 1.9** • Predicted interaction between vancomycin aglycon derivative 17 and the D-Ala-D-Lac peptidoglycan terminus (Boger et al.).

Analogue 17 was prepared by a similar route to the previously reported total synthesis of vancomycin, with changes to the synthesis of the B and D-ring regions (Scheme 1.1). Thus intermediate 16 was synthesised via a reductive amination reaction between (R)-methyl 2-amino-2-(3-bromo-4-methoxyphenyl)acetate 14 and (R)-tert-butyl-(1-(3,5-bis(benzyloxy)-4-methoxyphenyl)-2-oxoethyl)carbamate 15 in place of the standard peptide coupling procedure.
Gratifyingly, the design of analogue 17 improved affinity by removing lone pair repulsion, but relies on the strength of the four remaining hydrogen bonds involved in the interaction. Boger recognised that while affinity for D-Ala-D-Lac could be improved further by engineering a reverse hydrogen bond into the molecule, this may be clinically disadvantageous as it would disrupt H-bonding to D-Ala-D-Ala. The result would therefore be reduced activity against vancomycin-sensitive bacteria as well as resistant strains such as VanA that initially use the D-Ala-D-Ala terminus.

In binding tests, compound 17 proved to exhibit the desired dual-binding properties, with a 40-fold increase in affinity for D-Ala-D-Lac compared to vancomycin and conversely a 35-fold decrease in affinity for D-Ala-D-Ala, giving it the potential to be equally effective against both vancomycin-sensitive and resistant bacteria. Crucially, this analogue has an MIC of 31 µg mL⁻¹ against VRE (VanA), the most challenging of the resistant bacteria to combat by synthetic design. This compares favourably to the values for vancomycin (2000 µg mL⁻¹) and the aglycon (640 µg mL⁻¹) but remains higher than the MIC of <1 µg mL⁻¹ generally required for clinical applications.

Scheme 1.1 • Construction of a modified polypeptide chain for incorporation into vancomycin derivative 17 (Boger et al.)

Scheme 1.2 • Synthesis of protected hydrazine-containing amino acid 22 reported by Boger and Crane
The Boger group turned next to the \( N \)-methyl-L-leucine residue of vancomycin (residue 1). The aim of this investigation was to introduce additional hydrogen bonding to the peptidoglycan terminus away from the binding pocket of the molecule. Taking this idea further, they predicted that nucleophilic substituents might form a covalent attachment to D-Ala-D-Ala or D-Ala-D-Lac. With this in mind, a series of hydrazine-containing derivatives of vancomycin were synthesised and tested for their antibacterial activity.\(^{29}\)

Preparation of the modified amino acid is shown in Scheme 1.2. Reduction of \((R)\)-Boc-N-methyl-3-cyanophenylalanine methyl ester 18 by RANEY® Ni, hydrogen and 28% aqueous ammonium hydroxide in methanol afforded the corresponding methylamine 19. Amination with oxaziridine 20 afforded \( N \)-Boc protected hydrazine 21 in a 58% yield. Finally, lithium hydroxide hydrolysis of the methyl ester (and a second \( N \)-Boc protection) afforded 22, the free acid substrate for peptide coupling.

![Figure 1.10 - Vancomycin benzylhydrazine derivative generated by Boger et al.](image)

Compound 23 (Figure 1.10) had the same activity against \( S. \) aureus as vancomycin (MIC = 1.23 \( \mu \)g mL\(^{-1} \)), and activity against VRE remained poor. There was no evidence of a general trend in the activity of the hydrazine-containing derivatives or of an improvement in activity arising from exchange of the natural product \( N \)-terminal amino acid for those containing H-bond donors or reactive nucleophiles. Attempts to use the hydrazine group to form a covalent adduct between the vancomycin derivatives and the \( C \)-terminal ester of D-Ala-D-Lac were unsuccessful, even though this reaction could be carried out between the peptidoglycan chain and phenyl hydrazine.

Several members of the glycopeptide antibiotic family are appended with chlorine substituents on the aryl ring of \( \beta \)-hydroxytyrosine amino acid residues 2 and 6 (Figure 1.6), and the significance of these halogen atoms has been examined through structure–
activity relationship (SAR) studies. The presence of chlorine atoms has been shown to strongly enhance the potency of the compounds, probably as a result of their stabilising role in dimerisation. In order to investigate the importance of the chlorine atoms further, Süssmuth et al. synthesised a series of balhimycin variants containing chlorine, bromine and fluoride substituents (Figure 1.11).

Fluorobalhimycin (24) was produced via an enzymatic procedure to incorporate 3-fluoro-β-hydroxytyrosine into the glycopeptide in place of the chlorinated amino acid. In a subsequent report, calcium chloride and magnesium chloride (1:1 ratio) were substituted by the corresponding bromide salts in the culture media of the balhimycin-producing Amycolatopsis balhimicina, resulting in the synthesis of bromobalhimycin 25. Attempts to incorporate fluoride and iodide atoms by this method failed due to the toxicity of the salts. When equimolar amounts of bromide and chloride salts were added, a product with one bromine and one chlorine atom was observed in addition to the balhimycin and bromobalhimycin products. The hetero-halogen species was shown by mass spectrometry to be present as positional isomers 26 and 27 (1:1 ratio). Bromobalhimycin 25 showed similar in vitro antibacterial activity to balhimycin, with the exception of an 8-fold increase in MIC against *S. aureus*. Balhimycin 24 and bromobalhimycin 25 both performed up to 16 times better than dechlorobalhimycin against Gram-positive bacteria, confirming the important role that halogen substituents play within the glycopeptide family.

Following earlier work on the synthesis of chloroorienticin B derivatives, Yasukata et al. turned to extending the heptapeptide chain as a possible method of improving antibiotic activity. Chloroorienticin B derivatives bearing a tripeptide chain at the C-
terminus were prepared by solid-phase parallel synthesis, allowing fast access to a library of over 80 compounds.\textsuperscript{33} These compounds were tested for antibacterial activity against MRSA and VRE; a selection of the data is presented in Table 1.1. Compounds that incorporated tryptophan and tyrosine residues, such as 28 and 29, were found to exhibit activity against VRE as well as matching the activity of the parent compound against MRSA. As a general rule, the tryptophan residue in particular was crucial for activity against VRE. The introduction of glutamic acid totally removed anti-VRE properties of the compound. Yasukata \textit{et al.} concluded that amino acid residues with basic and hydrophobic character provided the most potent derivatives.

\textbf{Table 1.1 • MICs (µg mL\textsuperscript{-1}) of chloroorienticin B tripeptide derivatives by Yasukata \textit{et al.}}

<table>
<thead>
<tr>
<th>A\textsubscript{1}-A\textsubscript{2}-A\textsubscript{3}</th>
<th>\textit{Staph. aureus} SR3637 (MRSA)</th>
<th>\textit{E. faecalis} SR7914\textsuperscript{a}</th>
<th>\textit{E. faecium} SR7917\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>28: Leu-Trp-Tyr</td>
<td>0.5</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>29: Tyr-Trp-His</td>
<td>0.5</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>30: Trp-Lys-His</td>
<td>&lt;0.125</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>31: Glu-Trp-Tyr</td>
<td>8</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Chloroorienticin B</td>
<td>0.5</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Vancomycin-resistant \textit{enterococci} (VanA)
1.4.3 Introduction of hydrophobic side chains to the peripheral glycopeptide structure

Many of the early modifications of vancomycin and the glycopeptide family involved the addition of hydrophobic chains to the molecule, frequently at the vancosamine nitrogen atom. These derivatives mimicked the natural hydrophobic character of teicoplanin (5) (Figure 1.2), but alkylation of the polar hydroxyl, acid and amino groups can compromise the naturally high aqueous solubility of the parent compound.

**Scheme 1.3 • Double modification of chloroorienticin B by Yasukata et al.**

Yasukata *et al.* proposed a solution to this problem with a double modification of chloroorienticin B, inspired by earlier work on the double modification of teicoplanin by Preobrazhenskaya *et al.* In the first step, protection of the amino peptide terminal with a para-methoxybenzyloxycarbonyl (pMZ) group was followed by *N*-alkylation of the amino sugar with \((E)-4-(4\text{-chlorostyryl})\text{benzaldehyde} 33\) (Scheme 1.3). Subsequent pMZ removal afforded 34. This compound has a higher antibacterial activity than chloroorienticin B or vancomycin but the hydrochloride salt has <1% solubility in water, compared to that of vancomycin, which is >99% soluble in water. The second step
employed a Mannich reaction previously reported by the Preobrazhenskaya group\cite{37} to introduce a hydrophilic aminoalkyl group into the A-B biaryl region of the molecule. Thus the reaction of 34 with 5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine-6-methylamine 35 under Mannich conditions afforded doubly-modified derivative 36. Compound 36 exhibited MICs of 0.78 µg mL\(^{-1}\) against MRSA (compared to 1.56 µg mL\(^{-1}\) for vancomycin) and 3.13–6.25 µg mL\(^{-1}\) against VRE (VanA). Crucially, the aqueous solubility of the hydrochloride salt was increased to 10% by the second modification.

\textbf{Scheme 1.4} • Vancomycin glycorandomisation process by Thorson \textit{et al.}

Vancomycin derivatives that contain hydrophobic side chains are disadvantaged by their poor solubility. Yasukata \textit{et al.} have demonstrated that secondary modifications on these glycopeptides can successfully increase solubility, hence making them more suitable for clinical use. One of the most versatile and currently widely employed methods of ligation is the Huisgen or Click reaction; a copper catalysed 1,3-dipolar cycloaddition reaction between an azide and an alkyne. The application of Click chemistry for glycopeptides modification was first reported in 2005 by Thorson \textit{et al.}, who used a chemoenzymatic approach to install 6-azido glucose onto the vancomycin aglycon for subsequent

\begin{center}
\includegraphics[width=\textwidth]{scheme14.png}
\end{center}
glycorandomization (Scheme 1.4). Batta and Herczegh employed the Click reaction for the synthesis of ristocetin and teicoplanin aglycon derivatives bearing a hydrophobic side chain. The N-termini of the glycopeptide aglycons were converted to an azide functionality via a diazo transfer reaction of the amine with triflic azide and catalytic amounts of copper(II) sulfate in pyridine. The alkyne substrates were prepared by propargylation of the corresponding alcohols (Scheme 1.5).

Reaction between the azide and alkyne with 10% CuI and triethylamine at room temperature afforded the 1,4-substituted 1,2,3-triazoles in 44–71% yield. The teicoplanin derivatives showed much higher activity than the parent compound, with 42 and 43 being potent against both VanA and VanB phenotypes of resistant enterococci (MICs of 0.5 µg mL⁻¹ in both cases). Batta and Herczegh postulated that the amphiphilic nature of these derivatives (the presence of the carboxylate group and a lipophilic side chain) favours the formation of a multivalent aggregate in aqueous medium, contributing to increased antibacterial activity. Compound 44, a product of esterification at the C-terminus, showed decreased activity overall, and removal of the N-acetyl glucosamyl group at R₁ (compound 45) drastically reduced potency against vancomycin-resistant and susceptible
strains. The presence of this sugar is key to the biological activity of these compounds. The ristocetin derivatives lost all antibacterial activity, although the ristocetin azide intermediate matched the activity of vancomycin against MRSA (MIC = 2 µg mL⁻¹). Further work by Herczegh et al. employed the Click reaction to add a saccharide moiety to the N-terminus of ristocetin aglycon (Figure 1.12). This modification was shown to improve both antibacterial and anti-influenza activity of the glycopeptide.

![Figure 1.12](image-url)

**Figure 1.12** - Ristocetin triazole saccharide derivatives generated by Herczegh et al.

The anti-influenza virus properties of ristocetin aglycon derivatives were reported by Sztaricskai and Naesens in 2009, with the synthesis of a series of compounds bearing hydrophobic chains via a cyclobutenedione linker (Table 1.2). Coupling of the ristocetin aglycon with dimethyl squarate and subsequent reaction with various primary amines afforded the corresponding asymmetric squaric diamides. The mild reaction conditions are a notable advantage of this ligation method. Furthermore, the lead compound in the series, 4-phenylbenzyl derivative 53, showed excellent anti-viral activity against influenza A and B strains, with an average EC₅₀ of 0.4 µM, which compares favourably with antiinfluenza drugs currently on the market, including ribavirin (8.4 µM) and oseltamivir (2.8 µM).
Interestingly, the corresponding aglycovancomycin derivatives showed no antiviral activity, suggesting that the core aglycoristocetin structure is essential for antiviral activity. There are a number of differences between the two compounds; ristocetin contains additional aromatic rings F and G, connected via a diphenylether linkage, and features a methyl ester at the C-terminus instead of the free carboxylic acid, but lacks the chloro substituents of vancomycin. It is unclear which of these properties contributes to the antiviral characteristics of aglycoristocetin derivatives.

Table 1.2 • Squaric diamide derivatives of ristocetin aglycon by Naesens and Sztaricskai et al.

<table>
<thead>
<tr>
<th>R</th>
<th>Yield (%)</th>
<th>EC₅₀ (µM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>72</td>
<td>1.0</td>
</tr>
<tr>
<td>49</td>
<td>38</td>
<td>&gt;100</td>
</tr>
<tr>
<td>50</td>
<td>62</td>
<td>4.6</td>
</tr>
<tr>
<td>51</td>
<td>87</td>
<td>2.3</td>
</tr>
<tr>
<td>52</td>
<td>73</td>
<td>&gt;100</td>
</tr>
<tr>
<td>53</td>
<td>68</td>
<td>0.36</td>
</tr>
<tr>
<td>54</td>
<td>54</td>
<td>0.50</td>
</tr>
<tr>
<td>55</td>
<td>42</td>
<td>&gt;100</td>
</tr>
<tr>
<td>56</td>
<td>62</td>
<td>&gt;100</td>
</tr>
<tr>
<td>57</td>
<td>75</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* Influenza A/H3N2
Following work on the modification of eremomycin by the incorporation of hydrophobic sugar substituents, Preobrazhenskaya et al. turned their attention to glycopeptides derivatives that were active against Bacillus anthracis. With this in mind they generated a series of eremomycin and vancomycin derivatives appended with an adamantyl amide moiety (Figure 1.13). Anthrax has attracted much attention in the media as a biological weapon, and the mortality rate of patients who are infected by inhalation remains high. The appearance of drug-resistant strains of Bacillus anthracis indicates a particular need for the development of new compounds that are effective against these bacteria. The most potent compound in the series, adamantyl-2-amide eremomycin derivative 59 (Figure 1.13) showed wide-ranging potency against vancomycin-susceptible, intermediate and resistant strains of S. aureus, E. faecalis and E. faecium (MIC = 0.25–8 µg mL⁻¹).

Figure 1.14 • Structure of anti-anthrax drug ciprofloxacin (63)
Compound 59 also proved to be very effective against *Bacillus anthracis* in *in vivo* testing, while it showed excellent *in vitro* activity against strains that are resistant to ciprofloxacin (63), the current drug of choice for treatment of anthrax poisoning (Figure 1.14).

**Scheme 1.6** • Application of the Suzuki-Miyaura cross-coupling on vancomycin by Arimoto *et al.*

Given the importance of the aryl halide derived glycopeptides and the multitude of transition metal-based protocols available to the chemist, it is interesting to note that it was not until 2010 that investigations probing their chemical transformation were undertaken. Focusing on amino acid residues 2 and 6, Arimoto *et al.* reported the application of a Suzuki-Miyaura coupling procedure using trans-2-(4-biphenyl)vinylboronic acid as the key coupling agent.44
Vancomycin has poor solubility in most organic solvents but high solubility in water. In a procedure that did not require any protecting group strategies Arimoto et al. reported the first example of a Suzuki coupling conducted in aqueous solution on a complex natural product. The protocol utilises a water-soluble catalyst combination using ligand 64, originally developed for the Suzuki-Miyaura coupling reaction by Buchwald et al.\(^{45}\) The synthesis of derivatives 65–70 was readily performed in the presence of the corresponding boronic acid, 20 mol% of palladium(II) acetate, sodium acetate (10 equiv.) and water soluble phosphine ligand sodium 2-dicyclohexylphosphino-20,60-dimethoxybiphenyl-30-sulfonate 64 (0.4 equiv.) (Scheme 1.6).

Scheme 1.7 • Suzuki-Miyaura coupling of vancomycin with octenylboronic acid by Arimoto et al.
Although the aryl boronic acids used to generate derivatives 65–69 were found to have low reactivity towards vancomycin, those that did react did so with good selectivity. 2D $^1$H-NMR studies showed that C–C bond formation occurred only at residue 2, where the chloro substituent is on the less-hindered side of the molecule. Alkenylboronic acids were found to be more reactive; the reaction between vancomycin and octenylboronic acid afforded mono-substituted product 70 in a 42% yield, and di-substituted product 71 in a 30% yield (Scheme 1.7).

Novel vancomycin derivatives 65–71 were tested for their biological activity against a range of vancomycin resistant and susceptible bacteria. Simple aryl substitution of vancomycin at residue 2 (compounds 65 and 66) gave a minor decrease in activity against vancomycin-susceptible bacteria and these derivatives remained inactive against VRE and VRSA. Styril derivative 67 showed activity against VRSA (16 µg mL$^{-1}$) but this property was lost as the size of the substituent increased; 68 and 69 were inactive against VRSA but did exhibit activity against VRE (VanB). The overall antibacterial activity of compound 69 was particularly promising, with better activity against MRSA than vancomycin, and excellent activity against the VanB phenotype of vancomycin resistant *E. faecium* (MIC 0.5 µg mL$^{-1}$). Disubstituted products showed no activity against vancomycin-resistant bacteria, and importantly, also lost activity against vancomycin-susceptible strains. Arimoto *et al.* concluded that this behaviour is due to unfavourable steric interactions between the large substituent on residue 6 and the D-Ala-D-Ala peptide terminus, citing the reduced binding affinity of 71 for the D-Ala-D-Ala dipeptide compared to mono-substituted product 70 ($K_D = 11.6$ µM and $3.77$ µM respectively).
1.5 Glycopeptide dimerisation studies

Glycopeptide antibiotics are known to dimerise spontaneously in aqueous solution. Furthermore, dimerisation contributes to their potency. A 1999 study by Williams and Cooper used Surface Plasmon Resonance analysis to show that vancomycin, which dimerises weakly, has a low binding affinity for –Lys-D-Ala-D-Lac peptides found in VRE, whereas strongly dimerising antibiotics like chloroeremomycin had a higher binding affinity for the VRE mimic. In the search for improved biological activity, a number of investigations have aimed to harness or exploit this characteristic by synthesizing covalently-linked glycopeptide dimers. In the design of effective dimers there are a number of options to consider: the site of dimerisation, the length and nature of the bridge, and the reaction employed for ligation.

Following a successful total synthesis of vancomycin, the Nicolaou group began to investigate the efficient synthesis of covalently-linked vancomycin dimers using target-accelerated combinatorial synthesis (TACS). TACS allows the simultaneous evaluation of the effect of both the length of the linker and modifications in the binding pocket on the activity of the novel compounds produced. Initiating this study, the nitrogen atom of the vancomycin sugar was chosen as the linking site of dimerisation, and two ligation methods were tested; disulfide formation and olefin metathesis (Scheme 1.8). Saponification of thioacetate 72 in aqueous sodium hydroxide at 23 °C gave the corresponding disulfide 73, one of the most biologically active in a series consisting of tether lengths $n = 3$–9. Finding suitable conditions for an aqueous olefin metathesis proved more challenging, but the reaction proceeded smoothly in a mixed solvent system (H$_2$O/DCM 95:5) with a phase transfer catalyst, trimethyldecylammonium bromide, and Grubbs 1st generation catalyst, at room temperature. In each case the dimer was isolated as a 1:1 mix of cis and trans isomers in the olefin bridge in a 30–80% yield. The novel dimers were tested for activity against a range of vancomycin-susceptible and resistant strains of bacteria, and a number of general structure–activity trends were observed.
Synthesis of new glycopeptide antibiotics

Scheme 1.8 • Vancomycin dimers by disulfide formation $n = 3$ (top) and olefin metathesis $n = 2$ (bottom) by Nicolaou et al.
The antibacterial activity of both classes of dimer was found to be strongly dependent on the length of the tether employed, especially for the olefin-bridged dimers, where the optimum length was sixteen atoms between the vancosamine nitrogens (compound 75). Dimer 75 had MICs of 1–2 µg mL⁻¹ against VRE (VanA) and vancomycin-intermediate \textit{S. Aureus}, but antibacterial activity was lost on removal of the residue 1 D-leucine from the binding pocket. Of the disulfide dimers, compound 73 was particularly effective against VRE (VanA) with MICs of 0.06–0.5 µg mL⁻¹ compared to the vancomycin MIC of 50–100 µg/mL. In this case, removal of amino acid residue 1 did not remove all antibacterial activity of the dimer, which retained MICs of 2 µg mL⁻¹ against vancomycin susceptible \textit{enterococci} and 2–16 µg mL⁻¹ against VRE (VanA).

With promising biological results using the traditional synthetic approach described above, the Nicolaou group turned to applying a combinatorial methodology that allows an array of building blocks to assemble and react in the presence of a biological target. The biologically optimal combination of building blocks is then generated as the most prevalent species. For this study, three vancomycin analogues with tethers of different lengths were mixed, and under olefin metathesis conditions the reaction was monitored by mass spectrometry. In the presence of the peptide terminus analogue (Ac₂-L-Lys-D-Ala-D-Ala), dimers with shorter tethers were formed preferentially, a result which matched the outcome of the antibacterial activity study. In conducting this study the Nicolaou group has therefore demonstrated that target induced rate acceleration is a powerful method for the rapid generation of biologically active dimers in the vancomycin family. The application of this approach to other biologically active systems would seem to hold promise for the development of more potent bioactive compounds.

The precise mode of action of vancomycin dimers has been called into question by Ellman \textit{et al.}, who compared the antibacterial activities of vancomycin and a series of tail-to-tail dimers with the corresponding desleucyl compounds, which were prepared by double Edman degradation (Scheme 1.9). Desleucyl vancomycin is unable to bind to L-Lys-D-Ala-D-Ala, a fact that is attributed to the lack of the leucine amino acid in the binding pocket, and it is therefore inactive against vancomycin-susceptible or resistant bacteria. However, the desleucyl dimers performed well against VRE (VanA) strains, and showed only a 3.5–8-fold increase in MIC over the vancomycin dimers (Table 1.3).
Scheme 1.9 • Vancomycin tail-to-tail dimers, 76,77 and the corresponding desleucyl dimers 78,79 generated by Ellman et al.
Table 1.3 In vitro antibacterial activity and peptide model binding affinity (Ellman et al.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/mL)\textsuperscript{a}</th>
<th>K\textsubscript{d} (µM)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>1200</td>
<td>1.3</td>
</tr>
<tr>
<td>Desleucylvancomycin</td>
<td>&gt;1200</td>
<td>&gt;60</td>
</tr>
<tr>
<td>76</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>77</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>78</td>
<td>12</td>
<td>&gt;60</td>
</tr>
<tr>
<td>79</td>
<td>5.8</td>
<td>&gt;60</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Against \textit{E. Faecium} (VanA). \textsuperscript{b} Binding to dansyl-Lys(Ac)-D-Ala-D-Ala.

Crucially, a binding affinity study showed that 79 did not bind to the peptide model dansyl-Lys(Ac)-D-Ala-D-Ala or to surface-immobilised L-Lys-D-Ala-D-Lac. This indicates that the \textit{in vitro} behaviour of vancomycin dimers is due to an alternative mechanism.

![Molecular structure of actinomycin D](image)

**Figure 1.15** • Molecular structure of actinomycin D (80)

Many of the previously reported dimers have been connected by long, flexible tethers, but a review of polyvalent interactions by Whitesides \textit{et al.} highlighted the entropic disadvantages of such flexible linkers.\textsuperscript{49} Changing tack, Arimoto \textit{et al.} investigated the effect that incorporating a rigid natural product scaffold from actinomycin 80 (Figure 1.15) would have on the biological activity of vancomycin.\textsuperscript{50} Reductive alkylation of vancomycin with 2-benzyloxy-3-nitrobenzaldehyde 81 was followed by a one-pot catalytic hydrogenation that removed the benzyl protecting group and reduced the nitro group to an amine (Scheme 1.10).
Scheme 1.10 • Synthesis of actinomycin-inspired vancomycin dimers by Arimoto et al. 

The unstable product was subjected to dimerisation using quinine in methanol, and afforded dimer 83 in a good yield (68%). This procedure benefits from mild reaction conditions, good selectivity and high yields. Actinocin-based dimers 83–86 were prepared with variations in linker length and character (Figure 1.16), all of which showed good stability in vivo.

Dimers 83–86 were tested for biological activity against a range of vancomycin-susceptible and resistant bacteria (Table 1.4). As predicted, the short, rigid linker gave the best activity; compound 83 showed particularly promising activity against VRE but somewhat lower potency against MRSA than vancomycin.
The low potency problem was overcome by changing the trifluoroacetate salt for the hydrochloride salt. This simple “anion switch” increased activity up to 32-fold. Furthermore, an in vivo study indicated that dimer 83 rivals the activity of the currently employed anti-pneumonia drug, linezolid, against \textit{S. pneumoniae}.

### Table 1.4 Antibacterial activity of novel vancomycin dimers by Arimoto \textit{et al.}

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. aureus} (MRSA)</td>
<td>1</td>
<td>16 (8)</td>
<td>32</td>
<td>16 (2)</td>
<td>32</td>
</tr>
<tr>
<td>\textit{E. faecium}</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>\textit{E. faecium} (VanA)</td>
<td>&gt;64</td>
<td>32</td>
<td>2</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>\textit{E. faecium} (VanB)</td>
<td>&gt;64</td>
<td>1 (0.25)</td>
<td>1</td>
<td>2 (0.5)</td>
<td>1</td>
</tr>
<tr>
<td>\textit{S. aureus} VRSA-2</td>
<td>&gt;64</td>
<td>8 (4)</td>
<td>-</td>
<td>32 (1)</td>
<td>-</td>
</tr>
<tr>
<td>\textit{S. pneumonia} Type 1</td>
<td>0.5</td>
<td>0.125 (≤0.063)</td>
<td>-</td>
<td>1 (0.25)</td>
<td>-</td>
</tr>
</tbody>
</table>

*a MICs of the HCl salt shown in parentheses.

Acknowledging that the synthesis of covalently-linked dimers can be time-consuming and problematic, the Xu group have focussed on developing a different strategy based on self-assembly\textsuperscript{51} and coordination chemistry. In 2003 Xu \textit{et al.} reported on the exploitation of the unique structural geometry and stability that metal complexes can afford in the formation of vancomycin dimers.\textsuperscript{52} Vancomycin reacted with the corresponding amine to give carboxamide derivatives 87\textit{a–d} in high yields (>65%) (Figure 1.17).

\begin{center}
\textbf{Figure 1.17} • Vancomycin carboxamide derivatives generated by Xu \textit{et al.}
\end{center}
Subsequent co-ordination with cis-platin derivative $[\text{Pt(en)}(\text{H}_2\text{O})_2]^{2+}$ in DMSO (20 h, rt, no light) afforded the dimeric complexes $88a$–$d$ (Figure 1.18). Platinum complex-based vancomycin dimers $88a$–$d$ exhibited higher biological activity against VRE than vancomycin itself, while retaining activity against vancomycin-susceptible enterococci (Table 1.5). Compounds $88a$ and $88c$, with short chains, performed particularly well against a range of VRE, with MICs of 0.03–3.3 µg mL$^{-1}$. Conversely, the more flexible dimers $88b$ and $88d$ lost activity against VRE (VanA) but remained relatively active against the VanB and VanC phenotypes, with MICs in the range of 0.05–4.8 µg mL$^{-1}$. Crucially, platinum complex precursor $90$ was completely inactive against gram positive bacteria, and vancomycin carboxamide derivatives $87a$–$d$ only showed activity against vancomycin-susceptible enterococci and VRE (VanC).
Table 1.5 • Antibacterial activity of Pt-based vancomycin dimers by Xu et al.

<table>
<thead>
<tr>
<th></th>
<th>1: Vancomycin</th>
<th>88a</th>
<th>88b</th>
<th>88c</th>
<th>88d</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em> TCC29212</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>E. faecalis</em> (VanA)</td>
<td>576</td>
<td>0.8</td>
<td>14</td>
<td>3.3</td>
<td>38</td>
</tr>
<tr>
<td><em>E. faecium</em> (VanB)</td>
<td>102</td>
<td>0.28</td>
<td>2</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td><em>E. gallinarum</em> (VanC)</td>
<td>8</td>
<td>0.03</td>
<td>0.05</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

In this preliminary study, Xu et al. have shown that the rigid platinum complexes work well as linkers for the construction of multivalent inhibitors, but the cytotoxicity of such metal complexes is a factor that would need further investigation if the compounds were to find use in a clinical setting.

Figure 1.19 • Mono- and divalent vancomycin-porphyrin conjugates generated by Xu et al.

In a more recent study, Xu et al. demonstrated the potential for application of a divalent vancomycin-porphyrin conjugate within photodynamic antimicrobial chemotherapy (PACT).\textsuperscript{53} Porphyrin acts as a photosensitizer that generates a reactive oxygen species (ROS) on exposure to a suitable wavelength of light. The ROS causes cell death by attacking cell walls and membranes. The synthesis of a vancomycin-porphyrin conjugate
allowed PACT to accurately target areas of bacterial infection. Thus vancomycin was coupled to porphyrin using HBTU affording divalent conjugate 92 in a 54% yield (Figure 1.19). The use of excess quantities of porphyrin afforded monovalent conjugate 91 in a 53% yield. Both conjugates performed poorly against VRE (VanA and VanB) in the in vitro testing, with MICs in excess of 32 µg mL⁻¹. However, divalent vancomycin-porphyrin conjugate 92 proved to be a successful affinity ligand in PACT evaluation, efficiently directing the porphyrin moiety to the surface of vancomycin-resistant bacteria.

The majority of studies investigating glycopeptide dimerisation use vancomycin as the parent compound because of its proven activity against Gram-positive bacteria, and because dimers of other members of the glycopeptide family are rarely seen in the literature. The exact structural requirements for good dimer activity remain elusive, though attempts to clarify patterns and trends have been made.

Griffin et al. took a systematic approach to dimer design, identifying four sites of ligation; the C-terminus (C), N-terminus (N), vancosamine residue (V) and resorcinol ring (R) (Figure 1.20). Vancomycin synthons 93–96 were prepared using appropriate procedures; reductive alkylation was employed for preparation of the N and V synthons, amide bond formation for the C synthon and Mannich aminomethylation for the R synthon. The four vancomycin synthons 93–96 were connected in 10 unique combinations by linker synthons A-E to build an array of 40 dimers (Figure 1.21).
The V-V series was the most effective against vancomycin susceptible bacteria and the VRE *E. faecalis* VanB phenotype, whereas C-C, C-V and V-R combinations afforded broad spectrum activity, with MICs as low as 9.4 µg mL\(^{-1}\) against the VanA resistant strain. The shortest linker, A, which placed a total of 11 atoms between the vancomycin molecules, was preferred for activity against VRE (VanA and VanB), whereas activity against vancomycin-susceptible *enterococci* was generally unaffected by linker length. Ultimately, this comprehensive study did not reveal many easily comprehensible structure–activity relationships for the vancomycin dimers; potency remained highly dependent on the strain of bacteria.

A key problem with the design of effective dimers is the ambiguity over the nature of the interactions between the dimers and the bacterial cells at a molecular level. The precise mode of action of the dimers has been called into question by several groups over the last decade, but there has been little investigation into the subject until earlier this year. The Arimoto group have recently reported the first biochemical study of the mechanism for vancomycin dimer activity.\(^{56}\) Previously reported actinocin-based dimers 83 and 84 (Figure 1.16) were evaluated for their inhibitory action against bacterial macromolecules such as proteins, RNA, DNA, peptidoglycan and lipid biosynthesis. Vancomycin dimers...
joined by the C-terminus of each monomer by the same actinocin linkers were also tested. The incorporation of radio-isotope-labelled precursors was used as a measure of activity for each process; protein synthesis ($^{14}$C-leucine), DNA synthesis ($^{14}$C-thymidine), RNA synthesis ($^3$H-uracil) and fatty acid synthesis ($^{14}$C-acetic acid) were unaffected by the presence of the vancomycin dimers, which exhibited selective inhibition of peptidoglycan synthesis ($^3$H-N-acetyl-D-glucosamine). This represents the first experimental evidence that vancomycin and the dimers that are formed act as VRE cell wall synthesis suppressors.

**Table 1.6** Inhibition of lipid intermediate (LI) and peptidoglycan (PG) synthesis by vancomycin dimers (Arimoto *et al.*)

<table>
<thead>
<tr>
<th></th>
<th>Susceptible Model$^a$</th>
<th>Resistant Model$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LI</td>
<td>PG</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>180</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>83</strong></td>
<td>98</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>84</strong></td>
<td>74</td>
<td>11</td>
</tr>
<tr>
<td>Van-M-02</td>
<td>32</td>
<td>4.2</td>
</tr>
</tbody>
</table>

$^a$ IC50 ($\mu$g/mL)

---

**Figure 1.22** • Structure of novel vancomycin derivative Van-M-02 generated by Arimoto *et al.*

A sophisticated *in vitro* cell wall synthesis assay was then used to establish the effect of these compounds on the two stages in this process: lipid intermediate (LI) synthesis and peptidoglycan (PG) synthesis (Figure 1.23).
Figure 1.23 • In vitro inhibition assay of peptidoglycan (PG) synthesis in the bacterial membrane of S. aureus (Arimoto et al.). [Reproduced from Ref. 56.]

Measurement of the radioactivity derived from $^{14}$C-labelled glycine was used to quantify the LI and the cross-linked peptidoglycan product. For vancomycin-resistant and susceptible models, the dimmers inhibit both steps in this process; LI and PG synthesis. This contrasts with vancomycin, which mainly inhibits peptidoglycan synthesis (Table 1.6). Dimers 83 and 84 were able to inhibit both LI and PG synthesis in the resistant model, with greater inhibition of PG synthesis. A monomeric vancomycin derivative, with a modification at the vancosamine nitrogen, Van-M-02 (Figure 1.22), showed a different inhibition pattern to dimers also linked at the sugar moieties.

The mode of action of dimers 83–84 and Van-M-02 can therefore be assumed to have significant differences. In the light of this recent study, the importance of the traditional “binding pocket” of vancomycin to the activity of the dimers will inevitably re-emerge as a topic of debate. There is an urgent need for further biochemical studies that elucidate the details of dimer antibacterial activity and can subsequently inform the design of new and more effective vancomycin dimers.

1.6 Synthesis of complestatin

Complestatin (chloropeptin II) 98 (Figure 1.24) was first reported in the literature in 1980, as an inhibitor of the alternate pathway of human complement, although details of its original isolation from Streptomyces lavendulae and structural connectivity were not published until 1989. Both complestatin and the structurally related compound
chloropeptin I (97) were isolated from *Streptomyces* sp. WK3419 in 1997, and the full stereochemistry of these compounds were elucidated from NMR data.\(^{59}\)

![Figure 1.24 Structure of the chloropeptins: chloropeptin I, complestatin (or chloropeptin II) and isocomplestatin](image)

The structure of the chloropeptins is reminiscent of the glycopeptides antibiotics, with two macrocycles formed by coupling of the amino acid groups in the linear peptide chain. While the left hand side of the molecule shares the characteristic biaryl ether linkage of the vancomycin family, the second macrocycle is formed by a tryptophan indole ring. Restricted axial rotation about the indole means that there are two possible atropisomers of each compound, and while the natural products 97 and 98 consist only of the \((R)\)-atropisomer, an \((S)\)-atropisomer, isocomplestatin (99), has also been synthesised.\(^{60}\)

Compounds 97 and 98 differ only in the position of the junction between phenyl ring D and the indole ring E. Complestatin (98) can be easily converted to the less strained isomer, chloropeptin I (97), by an acid-catalysed rearrangement.\(^{61}\) Also of note is the \(\alpha\)-
keto amide moiety connecting aryl ring G to the polypeptide chain, a structural feature which is not present in the vancomycin family.

Unlike the glycopeptide antibiotics, the chloropeptins show no activity against gram-positive bacteria, but they have attracted considerable interest for their significant activity against HIV. Studies have shown that this class of compounds inhibits binding of the HIV gp-120 glycoprotein to the CD4 receptor of T-lymphocytes, thus preventing HIV infection of the cells. Isocomplestatin has also been shown to have activity against HIV integrase, the enzyme responsible for integration of viral DNA into the DNA of the infected cell.

Scheme 1.11 • Two macrocyclisation procedures for construction of the D-F ring of complestatin by Rich and Elder.

While complestatin was isolated and characterised in the 1980s, synthetic studies have been slow to emerge. The challenges presented by this compound are centred around the atropisomerism of the D-F macrocycle and, as with vancomycin, finding an effective solution to the problematic biaryl ether formation/cyclisation step. Until 2000, only a handful of complestatin fragments had been synthesised. In the first of these, Gurjar et al. employed a Suzuki-Miyaura cross-coupling method to construct the aryl-indole C–C bond prior to cyclisation, but discovered that subsequent peptide coupling protocols could
not be used to complete this macrocycle.65 The problem was overcome by Rich and Elder two years later (Scheme 1.11).66

The first stereoselective total synthesis of chloropeptin I was published in 2003 by Snapper and Hoveyda.67 The synthetic design involved a copper-mediated biaryl ether formation to close the B-O-D macrocycle followed by palladium-mediated cross-coupling to generate the D-F macrocycle (Scheme 1.12).

**Scheme 1.12 • D-F macrocyclisation step in the synthesis of Chloropeptin I reported by Snapper and Hoveyda**

For this key step, stannyl tryptophane 101 was incorporated via peptide coupling, affording intermediate 102. The subsequent cyclisation step was achieved with one equivalent of bis(tri-tertbutylphosphine) palladium(0), 10 equivalents of collidine and cesium fluoride (5 equiv.). The macrocyclisation product 103 was obtained in a 40% yield from 100. The addition of collidine (10 equiv.) to the standard conditions previously reported68 for this Stille-type C–C bond formation proved crucial to the efficiency of the reaction; in the absence of this additional base the reaction ceased after 30 minutes and
the solution turned black. Snapper and Hoveyda speculated that collidine has a role in the stabilization of the active palladium complex.

![Scheme 1.13](image)

**Scheme 1.13 • Intramolecular Suzuki-Miyaura coupling and SNAr macrocyclisation steps by Zhu et al.**

After chloropeptin I, complestatin (chloropeptide II) became the next target for total synthesis. The Zhu group first chose to apply the well-used nucleophilic aromatic substitution reaction promoted by a nitro substituent on the aromatic ring to close the B-O-D macrocycle (Scheme 1.13). The right hand side of the molecule was then constructed via an intramolecular Suzuki-Miyaura coupling reaction that completed assembly of the D-F macrocycle. It was during this study that Zhu et al. discovered that this approach resulted in the synthesis of the unnatural (S)-atropisomer, isocomplestatin. Reversing the order of cyclisation favoured the correct (R)-atropisomer, a conclusion also reached by the Boger group in their publication of the same year.

Preparation of linear peptide 104 afforded the substrate for intramolecular Suzuki–Miyaura coupling with 1,10-bis(diphenylphosphino)-ferrocene-palladium(II)dichloride, which afforded 105 in a 66% yield. NOE studies confirmed the (R) axial chirality of 105. The intramolecular SNAr reaction of 106 for the formation of the B-O-D biaryl ether linkage in 107 was achieved in a pleasing 62% yield under mild conditions.
CHAPTER 1  Synthesis of new glycopeptide antibiotics

Scheme 1.14 • Larock macrocyclisation by Boger et al.

In a unique approach to the challenge of the D-F ring system of complestatin, Boger et al. investigated the use of an intramolecular Larock indole synthesis\textsuperscript{71} that would provide simultaneous construction of both the right-hand macrocycle and the five-membered indole ring (Scheme 1.14).\textsuperscript{70} Studies by Larock et al. indicated that the steric influence of a large terminal alkyne substituent, such as a triethylsilyl group, would induce the correct regioselectivity in the indole cyclisation process.\textsuperscript{72} The reaction was found to benefit from the incorporation of an electron-rich bidentate phosphine ligand, 1,10-bis(di-tertbutylphosphino) ferrocene (DtBPF). Yields of up to 89\% were obtained with aniline acetamide 108a, with 4:1 (R:S) atropodiastereoselectivity. However, incorporating the larger benzamide group, intermediate 108b, afforded >95\% of the (R)-atropisomer in a 70\% yield.

Scheme 1.15 • Intramolecular S\textsubscript{N}Ar macrocyclisation for formation of the B-O-D biaryl ether linkage of complestatin (Boger et al.)

These promising results were applied to a total synthesis of complestatin with great success. Taking a reverse approach to the order of macrocyclisation chosen by the Zhu group, the Boger group found that construction of the B-O-D ring system before indole
macrocyclisation produced better results. Thus intramolecular nucleophilic aromatic substitution of 110 with potassium carbonate at 60 °C for 12 h in the presence of 4 Å molecular sieves and 18-crown-6 afforded 111 in an excellent 95% yield (Scheme 1.15). The nitro substituent used as a promoter for this step was subsequently removed by a previously reported same two-step procedure. The alkyne required for Larock cyclisation, with its triethylsilyl substituent, was introduced as amino acid component 113 (Scheme 1.16). Fmoc deprotection followed by peptide coupling afforded 114 in a quantitative yield ready for subsequent indole synthesis. Unlike analogue 108 on which the reaction conditions were developed, the substrate in the total synthesis contained a number of potentially problematic functional groups: notably the four unprotected phenols and “four labile aryl chlorides” (circled in Scheme 1.16). However, the intramolecular Larock cyclisation afforded 115 as a single (R)-atropisomer, in an unoptimised yield of 56%. The remaining triethylsilyl substituent was easily removed from the indole ring using dilute hydrochloric acid.

Scheme 1.16 • Intramolecular Larock indole synthesis applied to the total synthesis of complestatin by Boger et al.
1.7 Latest developments in the synthesis of new glycopeptide antibiotic analogues

Employing their expertise in the area of functionalisation of complex molecules, the Miller group investigated the site-selective bromination of the A-B ring of vancomycin (1) (Scheme 1.17), with the expectation that incorporating bromine into the binding pocket region of 1 would have an impact on its biological activity. To this end, peptide catalyst 116 was designed around the D-Ala-D-Ala dipeptide moiety known to have a good binding affinity for vancomycin (see Section 1.3). Catalyst 116 also features the N,N'-dimethylamido functionality which was anticipated to accelerate the rate of bromination in these reactions based on the observed efficiency and selectivity of the NBS-DMF reagent reported by Mitchell et al.

Scheme 1.17 • Site-selective monobromination of vancomycin reported by Miller and Pathak

While the uncatalysed bromination of vancomycin with N-bromophthalimide 117 afforded a 1:1 ratio of monobromination at the two available positions on ring A, the addition of catalyst 116 afforded compound 118 in a 41% yield on a preparative 100 mg scale. Dibromination of ring A was observed to be the major byproduct by HPLC analysis of the reaction mixture. Antibacterial studies carried out on these compounds showed that bromination of ring A failed to improve the biological activity of vancomycin against gram-positive bacteria.

Section 1.7 presents an overview of research reported in this area since publication of the following review: P. Ashford and S. P. Bew, Chem. Soc. Rev., 2012, 41, 957.
A similar approach to the “chemical tailoring” of teicoplanin (5) afforded a series of brominated derivatives with potential as substrates for metal-catalysed cross-coupling reactions. With this in mind, the palladium(II) acetate catalysed Suzuki coupling of compound 119 with a series of aryl and alkyl boronic acids was carried out (Scheme 1.18). The Suzuki reaction was initially unsuccessful, and [substrate+Pd] molecular ions were observed by LC-MS, indicating that the palladium species in the reaction was able to bind to teicoplanin, inhibiting catalysis. An unusually high catalyst loading of 50 mol% was therefore required for these reactions to take place. Thus compound 119 was coupled with 3-furanylboronic acid in the presence of water-soluble S-PHOS ligand 120 (1 equiv.) at 35 °C to afford product 121 in a 28% yield. Employing a higher temperature of 50 °C brought about a second Suzuki coupling reaction at the C-Cl bond on ring E to afford compound 122.

Scheme 1.18 • Suzuki cross-coupling reaction of bromo-teicoplanin 119 reported by Miller and Pathak

3-Furanyl substituted derivatives 121 and 122 showed slightly improved activity against MRSA compared to teicoplanin (MIC = 0.25 μg mL\(^{-1}\) compared to 0.5 μg mL\(^{-1}\) for teicoplanin A\(_2\)-2) but were equally inactive against the VanE strain of vancomycin-resistant enterococci.
Following their earlier work on the application of the Click reaction to the synthesis of novel teicoplanin derivatives (Section 1.4.3), Herczegh et al. set out to synthesise a series of fluorescent teicoplanin and ristocetin derivatives which could be used to elucidate their mode of action at the bacterial cell wall. Inspired by the early studies of Nagarajan et al. on the N-alkylation of glycopeptides described earlier in this review (Section 1.4.1), Herczegh et al. employed a three-component isoindole formation to tag the N-terminus of the peptide chain with a fluorescent moiety (Scheme 1.19).

**Scheme 1.20 • Synthesis of isoindole and benzoisoindole derivatives of teicoplanin aglycon reported by Herczegh et al.**

Thus teicoplanin pseudoaglycon 123 reacted with o-phthalaldehyde and an aryl or alkyl thiol at the N-terminus, affording a series of isoindole and benzoisoindole derivatives 124-127 in moderate to good yields of 31–76%. The new compounds were tested against
a range of resistant and non-resistant bacteria, and almost all exhibited better MICs than the parent natural product, teicoplanin. Compound 125 in particular shows excellent broad activity against both VanA and VanB strains of resistant bacteria, with MICs of 0.0313 µg mL⁻¹ and 0.0625 µg mL⁻¹ respectively, compared to values of >256 µg mL⁻¹ and 4 µg mL⁻¹ for teicoplanin A2-2.

Bacteria are known to be slow in developing resistance to cell membrane disrupting agents such as antimicrobial peptides. In order to counter bacterial resistance to vancomycin as well as increase its potency, Haldar et al. designed a series of derivatives bearing a permanent cationic quaternary ammonium moiety at the C-terminus (Figure 1.25). These new glycopeptides were synthesised by coupling the carboxylic acid group of vancomycin with the desired amine using HBTU. This peptide coupling step was achieved in yields of 70-80% to afford compounds 128-134 bearing alkyl chains between 2 and 18 carbons in length.

![Figure 1.25](image)

**Figure 1.25** • Alkyl quaternary ammonium trifluoroacetate derivatives of vancomycin reported by Haldar et al.

Haldar et al. propose that the lipophilicity of the added alkyl chain disrupts the integrity of the bacterial cell membrane, while the permanent positive charge may strengthen the interaction between the antibiotic and the negatively charged bacterial membrane, “anchoring” the drug in place. By adding a secondary mode of action to the inherent antibacterial activity of vancomycin, Haldar et al. have synthesised a highly potent broad-spectrum antibiotic (compound 133) which shows 25-fold improvement in activity against VRE compared to the second generation glycopeptide dalbavancin 9.
Furthermore, the repeated exposure of MRSA to compounds 131 and 133 resulted in no change in MIC after 52 days, compared to a 20-fold increase in MIC for vancomycin, indicating that they are slow to induce bacterial resistance in this strain.

1.8 Conclusions

It is clear that the race to complete a total synthesis of vancomycin in the late 1990s was not the end of the glycopeptide story; the 21st century demand for effective novel antibiotics is reflected in the plethora of articles published in this field. This review has brought together the many and varied approaches to vancomycin modification; in particular the alkylation of oxygen and nitrogen-containing groups has been shown to significantly increase antibacterial activity. Other modifications take their cues from nature, with the construction of covalent dimers mimicking the dimerisation of vancomycin in aqueous solution, and the addition of long tethers similar to the hydrophobic ‘‘anchor’’ of teicoplanin.

The importance of a tandem approach to drug design and biochemical investigation is particularly highlighted by recent research into the mode of action of glycopeptide dimers, and stronger links forged between antibiotic design and the nature of the intended target will undoubtedly lead to further reports of biologically active molecules. Alongside these ongoing investigations, the total synthesis of the closely-related complestatin family has been completed by a number of research groups in the last decade. Following the success of vancomycin modification, it may well be that future publications will report the design and discovery of highly biologically active analogs and derivatives of complestatin, a natural product whose full potential has yet to be explored.
CHAPTER TWO

Remote asymmetric induction (>1,5-stereocontrol)
2.1 Introduction

Remote stereocontrol, or remote asymmetric induction, occurs when the existing stereochemical centre or axis of the substrate molecule in a chemical reaction influences the formation of a new stereochemical feature in the product through more than five covalent bonds, equivalent to a through-space distance of $>0.7$ nm.\textsuperscript{111}

Figure 2.1 Comparison of cross-membrane communication by the $\beta$-adrenergic G-protein-coupled receptor\textsuperscript{4} (top) with state of the art remote asymmetric induction by a helical peptide foldamer, reported by Clayden et al., 2014 (bottom)

Long-range communication of stereochemical information is common in biological systems. A particularly well understood example is the structure and mechanism of the $\beta$-2 adrenergic G-protein-coupled receptor (GPCR) which takes part in cell signaling by spanning the width of the phospholipid bilayer (Figure 2.1).\textsuperscript{81} When a ligand such as ($R$)-

\textsuperscript{4}Figure 2.1 image top right adapted and reproduced by permission of MacMillan Publishers Ltd: Nature,\textsuperscript{81} copyright 2008.
epinephrine (adrenaline) binds to the β-2 adrenergic receptor outside the cell, a conformational change takes place in the peptide structure which activates G-proteins within the cell, leading to changes in the levels of intracellular messengers such as Ca^{2+} ions. By the long-range communication of stereochemical information, the β-2 adrenergic receptor therefore effects a change in cell processes across an impressive distance of 5 - 7 nm from the ligand binding site.

Remote stereocontrol in synthetic chemistry has generally been limited to a distance of 5 to 8 covalent bonds by the need for rigidity in the reaction transition states. However, research by Clayden and co-workers over the last five years has pushed the boundaries of remote asymmetric induction to through-space distances of up to 4 nm. This advance makes synthetic remote stereocontrol comparable to biological systems. The recent high-impact publications by Clayden et al. have led to a renewed interest in the development of the long-range communication of stereochemical information associated with remote asymmetric induction for potential new applications in the design of artificial peptide-based cell structures such as synthetic receptors.

2.2 Overview of remote stereocontrol

The stereoselective synthesis of a new chiral centre in an organic compound can be achieved either by reagent control (i.e. using a chiral catalyst or additive) or by substrate control, in which existing chiral information is relayed through the covalent bonds of the compound to the site of reactivity. In organocatalysis, C₂-symmetric chiral catalysts based on rigid frameworks such as BINOL and BINAP have become increasingly popular in the synthesis of aziridines, epoxides, and for asymmetric hydrogenation. They are efficient with regard to the amount of chiral material used, and the stereochemistry (R/S, or M/P in the case of helical compounds) of the catalyst can be selected to obtain the desired enantiomer of the reaction product. However, such catalysts can be expensive to purchase and time-consuming to make. As a typical example, (S)-VAPOL 141, employed as a catalyst in asymmetric aziridination, can be purchased at a cost of £104 per mg. The first reported synthesis of (S)-VAPOL by Wulff et al. was achieved in a 37% yield over 7 steps (Scheme 2.1). An (S)-VAPOL-borate complex (10 mol%) catalysed the asymmetric aza-Darzens reaction of N-benzylidene-1,1-diphenylmethanamine with ethyl diazoacetate 144 to afford aziridine (R,R)-145 in an 83% yield and with 90% ee.
Scheme 2.1 • Synthesis of (R)- and (S)-VAPOL reported by Wulff et al. and the Brønsted acid complex formed between the (S)-VAPOL-borate active catalyst and N-benzylidene-1,1-diphenylmethanamine (143) in the asymmetric synthesis of cis-aziridine (R,R)-145

The transmission of stereochemical information through distances of three or more bonds has been a particularly useful synthetic tool since the development of chiral auxiliaries by Corey and Evans in the 1980s.87 Amongst these, the oxazolidinone (147, 148), oxazoline (149),89 and camphorsultam (150)90 “chiral auxiliaries” have received the most attention (Figure 2.2). The chiral auxiliaries listed above share key structural features: they are conformationally constrained, cyclic systems bearing a sterically bulky chiral group, such as the benzyl substituent in example 146.

Figure 2.2 • Oxazolidinone (Evans-type and SuperQuat91), oxazoline and camphorsultam chiral auxiliaries
The Evans oxazolidinone chiral auxiliary is commercially available and has been employed in a number of landmark total syntheses, including the synthesis of talaromycins A and B reported by Smith et al. in 1984, in which (S,R)-152 was obtained as a single diastereomer in a 76% yield (Scheme 2.2). The ability to easily remove the chiral auxiliary from the product makes this method particularly versatile and applicable to a wide range of substrates. 1,4-Stereocontrol of the type shown in Scheme 2.2 is frequently found in the literature, and a number of comprehensive reviews have been published on the subject.

Scheme 2.2 • Use of the Evans chiral auxiliary in the synthesis of talaromycins A and B reported by Smith et al.

Acyclic substrates such as allylstannane 154 (Scheme 2.3) lack the rigid conformation of a 5- or 6-membered ring, and tend to rely on self-assembly of the reagents by chelation with a metal reagent via oxygen or nitrogen lone pairs, forming a highly ordered, conformationally constrained complex. In transition state 156, co-ordination of both the alcohol and aldehyde oxygen atoms to the metal centre (via their lone pairs) bring the existing chiral centre and the newly forming chiral centre into close proximity, bringing about a 1,5-steric induction. Hydrolysis of intermediate 157 releases diol 158, with a 1,7-cis relationship. Despite the restricted conformation of the transition state, Lewis acid-mediated remote asymmetric induction reactions of acyclic substrates generally proceed with moderate yield and diastereomeric excess. As Scheme 2.3 shows, diol 158 (the major syn product of the reaction) was obtained in a low 36% yield, and with a syn:lanti ratio of 90:10.
Metal-free acyclic systems lack the conformational constraint of a cyclic co-ordination complex in the transition state and are therefore extremely rare. Indeed a comprehensive review of “substrate-directable chemical reactions” by Evans et al. published in 1993 contained no examples of organocatalytic substrate-controlled chiral induction reactions, and a search of the literature from 1993-2015 revealed no subsequent examples. A review of stereochemical communication “over nanometer distances” published by Clayden in 2009 found that such transformations were limited to transition metal-catalysed, Lewis acid-mediated, and Grignard reactions.

This introductory chapter will present an overview of the major advances made in remote asymmetric induction (RAI) over the last 30 years, with a particular focus on examples where the stereocentre of the chiral substrate is more than 3 covalent bonds from the newly formed chiral centre in the product. Examples of 1,5- to 1,8-asymmetric induction and beyond, including those comparable to enzyme-catalysed reactions, are rare and particularly difficult to achieve with good diastereoselectivity. Exploring the structural features and mechanisms of these reactions will provide insight and inspiration for the design of future RAI transformations. Achieving absolute communication of stereochemical information on a nanometer scale within a biologically relevant architecture is a challenge that has not yet been met by synthetic chemistry.
2.3 Cyclic substrates in remote asymmetric induction

Substrates with a 5- or 6-membered ring cyclic structure are generally conformationally restricted and inflexible. As a result, the angle of approach of a second molecule to the site of reactivity is limited, causing the preferential formation of a single diastereomer in the product. This is the classical approach to remote asymmetric induction led by the use of the chiral auxiliaries illustrated in Figure 2.2 (Section 2.2). Examining the mechanism of stereocontrol in these examples, and the success of various approaches to improving the diastereomeric ratio of the product, will inform substrate design and optimization of stereoselectivity in future remote asymmetric induction reactions.

2.3.1 Steric control in cyclisation reactions

The Nazarov cyclisation involves the Lewis acid activation of a divinyl ketone followed by the 4π electrocyclic ring closure of a pentadienyl cation. Very few diastereoselective examples of this cyclisation exist. However, in 1999 Pridgen et al. reported a remarkable 1,5-asymmetric induction methodology in the Nazarov cyclisation for the synthesis of non-racemic indanes, which were found to be effective as endothelin receptor antagonists. Substrate 160, bearing the Evans chiral auxiliary, underwent Lewis acid-mediated cyclisation to afford two products, diastereomers trans-161 and cis-162 (Table 2.1).

Table 2.1 • Diastereoselective Nazarov cyclisation reported by Pridgen et al.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Yield (%)</th>
<th>Isomer ratio 161:162</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis acid</td>
<td>SnCl4</td>
<td>85</td>
</tr>
<tr>
<td>Lewis acid</td>
<td>TiCl4</td>
<td>60</td>
</tr>
<tr>
<td>Brønsted acid</td>
<td>CH₃SO₃H</td>
<td>88</td>
</tr>
</tbody>
</table>

When the cyclisation was carried out with Lewis acids tin(IV) tetrachloride and titanium(IV) chloride, indane 161 was obtained as the major diastereomer, with ratios
of 88:12 and 70:30 respectively. This moderate diastereoselectivity was thought to result from the formation of reaction intermediate 163 (Figure 2.3), in which Lewis acid complexation restricts the conformation of the substrate with a temporary 6-membered ring. However, one of the highest diastereomeric ratios (85:15, trans-161:cis-162) was obtained using methanesulfonic acid rather than a Lewis acid, which surprisingly suggests that metal-carbonyl complexation is not crucial for a stereoselective cyclisation in this case.

Figure 2.3 • Mechanistic comparison of the Lewis acid and Brønsted acid mediated Nazarov cyclisation reported by Pridgen et al.
In fact, computational calculations suggested that the lowest energy conformation of the starting material 166 without Lewis acid complexation has a trans-coplanar geometry of the imide carbonyls, as represented in Figure 2.3. In this conformation, steric demands alone bring the alkene into sufficiently close proximity to the aromatic ring for π-π interaction and the formation of a new covalent bond. Thus both the Lewis and Brønsted acid mediated reactions proceed with diastereoselectivity in favour of trans product 161.

An Evans chiral auxiliary was also used by Hsung et al. for a 1,6-asymmetric induction in the stereoselective 6π electrocyclic ring closure of 2-halo-amidotrienes (Scheme 2.5). Substrates 171a-c were synthesised by the electrophilic isomerization of allenamide 169 with an X+ source of the halogen (pyridinium bromide, NCS or NIS) in the presence of two equivalents of DABCO and 4Å molecular sieves (Scheme 2.4). After 16 hours at room temperature, the 1,3,5-hexatrienes 171a-c were obtained, via deprotonation of the acyliminium ion intermediate 170 (X = Cl, Br, I).

Scheme 2.4 • Synthesis of 2-halo-amidotrienes 171a-c reported by Hsung et al.

The 6π-electron electrocyclisation of 171a-c proceeded with good yields of up to 95%, and diastereomeric ratios of up to 90:10 (where X = bromine or chlorine, major product 172a and 172c) (Scheme 2.5). The choice of halogen did not appear to have a significant effect on the diastereomeric ratio, although the yield of the cyclisation was lower where X was chlorine or iodine, affording 172b+173b and 172c+173c in 61% and 73% overall yields respectively. The newly installed vinyl halide was shown to play an important role in relaying the chirality of the Evans auxiliary in the 1,6-asymmetric induction as well as being a site of possible functionalization in subsequent reactions.
Scheme 2.5 • Diastereoselective cyclisation of 1,3,5-hexatrienes 171a-c reported by Hsung *et al.*

The mechanism of the thermally allowed disrotatory ring-closure provides an explanation for the selective formation of 172a-c over 173a-c (Figure 2.4). Clockwise rotation of the \(\text{sp}^2\) orbital of the carbon bearing the phenyl substituent (right) would bring this group into proximity of the benzyl group of the Evans auxiliary, whereas anticlockwise rotation (left) has no such unfavourable steric interactions.

Figure 2.4 • Mechanistic explanation for the diastereoselective synthesis of 1,3,5-hexatrienes 172a-c

2.3.2 Remote stereocontrol by the \(\pi\)-allyltricarbonyliron lactone chiral auxiliary

Ley *et al.* have investigated the effect of a \(\pi\)-allyltricarbonyliron lactone complex as a chiral auxiliary, for example in the addition of trimethylaluminium to ketone 174 (Scheme 2.6).\(^{99}\) The synthesis of 174 was achieved in 4 steps from the commercially available starting material \((E,E)\)-hexa-2,4-dienoic acid, (employing the toxic, highly flammable solid iron source diiron nonacarbonyl) with an overall yield of just 23%. In the reaction between ketone 174 and two equivalents of trimethylaluminium, tertiary alcohol 175 was obtained in an excellent 92% yield, and with >95% diastereomeric excess. The high diastereoselectivity of this reaction is thought to be due to a combination of the stability of the lactone tether, and the steric bulk of the irontricarbonyl group, blocking one face of the ketone against nucleophilic attack (Figure 2.5).
Scheme 2.6 • A π-allyltricarbonyliron lactone complex as a chiral auxiliary, reported by Evans et al.

Scheme 2.7 • Asymmetric fluorination (above) and Mukaiyama aldol reaction (below) reported by Evans et al.
Ley *et al.* have shown that the use of a π-allyltricarbonyliron lactone auxiliary is applicable to a range of reactions, with the benefit of releasing the free ($E,E$)-diene on decomplexation.\(^{101}\) As a chiral auxiliary, the π-allyltricarbonyliron lactone is therefore highly reliable and cheap, although synthesis of the lactone itself is low yielding and employs reagents which may be difficult to handle.

### 2.3.3 Asymmetric keto boronate reduction

Reactions which progress *via* a highly ordered cyclic transition state have excellent potential for remote chiral induction. In a particularly successful example, Molander *et al.* achieved a 1,7-induction in the reduction of keto boronates (180) under optimized conditions of borane-dimethylsulfide (2 equivalents) in dimethylsulfide at 0 °C (Scheme 2.8).\(^{102}\) The diol products **183-187** were obtained in high yields of 81-95% and with excellent enantioselectivity (85-98% ee).

![Scheme 2.8](image)

Scheme 2.8 • Asymmetric keto boronate reduction reported by Molander *et al.*

Molander *et al.* proposed that the steric interactions between the approaching nucleophile and the isopropyl group of boronate 180 result in a preferred face of attack, in what is thought to be a 6-membered cyclic transition state (represented by 181 and 182). The basic solvent, dimethyl sulfide, is also likely to form a sterically bulky complex with the borane reducing agent, further limiting the possible trajectory of the nucleophilic attack. This method provides a straightforward way to synthesise chiral non-racemic secondary alcohols bearing sterically and electronically similar alkyl substituents. In terms of remote asymmetric induction, the synthesis of non-racemic diols **183-187** was achieved by a combination of a cyclic chiral substrate and a conformationally restricted cyclic transition
state – an approach which is also common to the transformations described in Section 2.3.4.

2.3.4  Chiral sulfoxides in remote asymmetric induction

The sulfoxide group is generally represented in Lewis structures as being analogous to a carbonyl moiety. However, when the R substituents are not equivalent, the sulfur atom is a stereogenic centre (Figure 2.6). The energy barrier to interconversion of the two enantiomers is around 40 kcal/mol, meaning that they are conformationally stable at room temperature and will only racemise under harsh conditions of high temperature or irradiation.  

![Figure 2.6](image)

Optically active sulfoxides have been employed as chiral auxiliaries in a diverse range of diastereoselective transformations, particularly those involving 1,3- and 1,4-stereocontrol. Examples of dihydroxylation, reduction, halogenation and ring cleavage using substrates bearing chiral sulfoxides can be readily located in the literature. The popularity of the sulfur-based chiral auxiliaries can be explained by three key points: sulfur lone pairs are able to coordinate to Lewis acids, promoting highly ordered transition states, the large steric difference between the substituents (e.g., lone pair, phenyl group, oxygen) results in good differentiation of the faces of the sulfoxide, and the chiral sulfoxides are straightforward to prepare in very high enantiomeric excess. The stereoselective Mukaiyama aldol reaction of (S)-2-(2-(p-tolylsulfinyl)phenyl)acetaldehyde 188 and O-silylated ketenethioacetal 189 was reported by Ruano and Maestro et al. in 2006 (Scheme 2.9).  

![Scheme 2.9](image)

Scheme 2.9 • 1,5-Asymmetric induction in the Mukaiyama aldol reaction and subsequent hydrolysis of thioester 190 to β-hydroxy carboxylic acid 192
Aldehyde 188 was prepared in 4 steps from 2-bromobenzaldehyde and (S)-(−)-methyl p-toluenesulfinic acid with an overall yield of 55%. The chiral sulfoxide group of 188 exerted a clear 1,5-stereocontrol in the Mukaiyama aldol reaction, which was found to be influenced by the choice of Lewis acid (Table 2.2). Ytterbium(III) triflate (1.2 equiv) gave the highest diastereoselectivity of the series, affording 190 and 191 in a ratio of 88:12, which increased to 96:4 when the reaction was carried out in acetonitrile at -40 °C instead of dichloromethane at -78 °C. Utilising the optimised conditions, this asymmetric Mukaiyama aldol reaction, followed by desulfinylation and further transformations, proved to be an effective route to 1,3-diols and beta-hydroxy acids with >98% ee.

Table 2.2 • Asymmetric Mukaiyama aldol reaction reported by Ruano et al.

<table>
<thead>
<tr>
<th>Lewis acid</th>
<th>Conversion (%)</th>
<th>Ratio 190:191</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiCl₄</td>
<td>87</td>
<td>61:39</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>34</td>
<td>66:34</td>
</tr>
<tr>
<td>ZnI₂</td>
<td>72</td>
<td>72:28</td>
</tr>
<tr>
<td>Yb(OTf)₃</td>
<td>60</td>
<td>88:12</td>
</tr>
</tbody>
</table>

A mechanistic explanation for the diastereoselective synthesis of β-hydroxythioester 190 is presented in Figure 2.7. Bidentate co-ordination of the sulfoxide and aldehyde oxygen atoms of substrate 188 to the metal centre of the Lewis acid creates cyclic complex 193. Steric interactions therefore limit attack of the aldehyde carbonyl to the exposed Si face. However, the degree of stereocontrol was only moderate, with a maximum 76% de in the synthesis of 190. This may be due to the inherent size and flexibility of the 8-membered ring in intermediate 193.

Figure 2.7 • Reaction intermediates in the Mukaiyama aldol reaction

Arai et al. then turned their attention to the Diels-Alder reaction, employing α,β-unsaturated amide 195 as a chiral dienophile in the reaction with cyclopentadiene 196 (Table 2.3). Where the Mukaiyama aldol addition had an 8-membered ring intermediate, the Diels-Alder reaction proceeded via the more conformationally restricted
7-membered ring complex 201 (Figure 2.8). This Lewis acid-promoted [4+2] cycloaddition proceeded smoothly at room temperature in overall yields of up to 99% and favoured the endo product in each case. The endo/exo ratio depended on the Lewis acid used: zinc chloride and the lanthanum triflates gave moderate endo selectivity, while aluminium trichloride gave a 95:5 ratio of endo/exo products and an excellent endo de of 98%.

Table 2.3 • Diels-Alder reaction of chiral sulfinyl dienophile 195 reported by Arai et al.

<table>
<thead>
<tr>
<th>Lewis Acid</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>Ratio endo/exo</th>
<th>endo de (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnCl₂</td>
<td>29</td>
<td>60</td>
<td>77/23</td>
<td>38</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>13</td>
<td>99</td>
<td>95/5</td>
<td>98</td>
</tr>
<tr>
<td>Yb(OTf)₃</td>
<td>45</td>
<td>61</td>
<td>69/31</td>
<td>89</td>
</tr>
</tbody>
</table>

Figure 2.8 • Reaction transition states in the Diels-Alder addition using 1 equivalent of Lewis acid (left) and two equivalents of Lewis acid (right), reported by Arai et al.
When the synthesis of 197 was attempted with two equivalents of Lewis acid (for example aluminium trichloride), the endo/exo selectivity dropped dramatically from 95:5 to 59:41, with a similar decrease in diastereomeric excess of the endo product from 98% to 11%. With more than one equivalent of Lewis acid, the reaction proceeds via transition state 202, which lacks the conformational restraint of the 7-membered ring found in 201 (Figure 2.8).

Following its use in asymmetric reactions, the chiral sulfoxide group is usually cleaved by the reaction with activated Raney nickel, releasing the unstable sulfenic acid, which prevents recycling of the chiral material. Arai et al. demonstrated the recycling of the chiral sulfoxide following the Diels-Alder reaction by treatment of 197 with two equivalents of lithium methanolate to afford methyl ester 203 in a 99% yield (Scheme 2.10). (S)-2-(p-Tolylsulfinyl)-1H-pyrrole 204 was recovered in a 99% yield, and with >99% optical purity, making it economically viable as a reusable chiral auxiliary.

![Scheme 2.10 • Recycling of the (S)-2-(p-Tolylsulfinyl)-1H-pyrrole chiral auxiliary 204 in the asymmetric Diels-Alder reaction, reported by Arai et al.](image)

2.4 Acyclic substrates in remote asymmetric induction

In their 2001 review of acyclic remote stereoccontrol, Mikami and Maryanoff identified common structural features which have been used to improve enantioselectivity in substrate-controlled chiral induction: i) sp² centres, such C=C bonds and amide groups, which restrict rotation of the molecule and provide planarity, ii) biasing substituents, i.e.
sterically bulky nitrogen or oxygen protecting groups, which restrict the conformation of the molecule, and iii) alcohol or amino functional groups capable of coordinating metals via oxygen or nitrogen lone pairs. These characteristics are also desirable for the control of regio- and stereoselectivity in the field of supramolecular chemistry, and are found in architectures such as metal-organic frameworks (MOFs),\textsuperscript{112} calixarenes\textsuperscript{113} and dendrimers.\textsuperscript{114} In the following reports of remote asymmetric induction, the acyclic reaction substrates all bear at least one of the structural features described by Mikami and Maryanoff. In many cases the remarkable through-bond distance over which stereochemical information appears to be communicated can be explained by the formation of highly ordered, cyclic reaction transition states in which the through-bond and through-space distance between the existing and forming chiral centres is in fact shorter than it first appears.

2.4.1 Remote asymmetric induction in the aldol addition reaction

The aldol addition of an enolate to an aldehyde or ketone is one of the most powerful reactions in organic synthesis, and the “primary engine” of C-C bond formation in nature.\textsuperscript{115} The β-hydroxy carbonyl functionality resulting from the aldol addition is a precursor of the 1,3-diol relationship commonly found in natural products, particularly the polyketides.\textsuperscript{116} The low natural abundance of marine-derived polyketides has placed great emphasis on the ability of total synthesis to provide enantiopure samples of these compounds in sufficient quantity for structure elucidation, biological evaluation and possible clinical development.\textsuperscript{117} Stereoselective 1,5-\textit{anti} aldol reactions are a particularly well investigated class of remote asymmetric induction, following boron-mediated aldol methodology developed by Evans and Paterson in the 1990s,\textsuperscript{118} which has since been employed in a number of natural product total syntheses.\textsuperscript{119} Following their previous work on 1,3-induction in the boron-mediated asymmetric aldol reaction,\textsuperscript{120} Evans and co-workers investigated the reaction of an achiral aldehyde with a ketone enolate substrate bearing a chiral β-alkoxy substituent (Scheme 2.11).\textsuperscript{121}

![Scheme 2.11](image-url) • 1,5-Stereocontrol in the boron-mediated aldol reaction, by Evans \textit{et al.}
Ketone 206 first reacted with 1.05 equivalents of dibutylboron triflate in the presence of Hunig’s base at -78 °C to form a boron enolate intermediate. The subsequent addition of dihydrocinnamaldehyde 207 (1.1 equivalents) afforded 1,5-anti diastereomer 208 as the major product, with an antilsyn ratio of 95:5 and a good overall yield of 89%. The high stereoselectivity of the boron-mediated aldol reaction is thought to be the result of the short boron-oxygen bond in the enolate, giving rise to a tight cyclic transition state (Figure 2.9).

**Figure 2.9** • Cyclic transition states for the boron-mediated asymmetric aldol reaction: boat conformations (left) and chair conformations (right), by Goodman *et al.*

By contrast, Evans *et al.* found that a Lewis-acid catalyzed enol silane addition protocol removed any 1,5-stereocontrol by ketone substrate 210 (Scheme 2.12), affording the anti and syn products 211 and 212 in a 1:1 ratio. Effectively “switching off” the 1,5-influence, this route would therefore allow a dominant 1,3-stereocontrol from a chiral aldehyde if desired.

**Scheme 2.12** • Lewis acid-catalysed enol silane aldol addition reported by Evans *et al.*
A comprehensive investigation into the full scope and limitations of these reactions was published by Evans et al. in 2003. In their total synthesis of the anti-cancer polyketide (+)-discodermolide 213 (Figure 2.10), Paterson et al. were able to extend the stereocontrol of the boron-mediated aldol reaction to a 1,6-induction. Model studies demonstrated that the boron enolate of (Z)-enone 215 reacted with isobutyraldehyde 216 to afford 1,6-anti aldol product 217 as the major diastereomer, with an anti/syn ratio of 81:19 (Scheme 2.13).

Figure 2.10 • Molecular structure of (+)-discodermolide 213 (left) and (-)-ericanone 214 (right) showing the aldol disconnection.

Scheme 2.13 • 1,6-Stereocontrol in the boron-mediated aldol reaction, reported by Paterson et al.

This test reaction was successfully exploited in the synthesis of (+)-discodermolide 213, in which the key aldol addition step proceeded with a total yield of 64% and an excellent diastereomeric ratio of >95:5 in favour of the desired natural product isomer. The same boron reagent, chlorodicyclohexylborane, was also used by Dias et al. in their 2013 total synthesis of the natural product (-)-ericanone (214, Figure 2.10), in which the key aldol addition step afforded the desired 1,5-anti diol relationship in the product with a diastereomeric ratio of 94:6 (anti/syn). The absence of 1,5-stereocontrol encountered by Evans et al. in the enol silane aldol addition reaction (Scheme 2.12) indicated that the boron-mediated aldol methodology was unsuitable for such substrates. Following investigations into this limitation, Yamamoto et
al. reported the use of “super silyl” protecting groups\textsuperscript{126} in the aldol reaction of β-siloxy methyl ketones with pivalaldehyde (Table 2.4).\textsuperscript{127}

**Table 2.4** - Asymmetric aldol reaction reported by Yamamoto *et al.*

<table>
<thead>
<tr>
<th>R =</th>
<th>Enolisation reagent</th>
<th>$T$ (°C)</th>
<th>Solvent</th>
<th>Yield (%)</th>
<th>dr (syn/anti)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si(TMS)\textsubscript{3}</td>
<td>LiHMDS</td>
<td>-60</td>
<td>DCM</td>
<td>50</td>
<td>60:40</td>
</tr>
<tr>
<td>Si(TMS)\textsubscript{3}</td>
<td>LiHMDS</td>
<td>-60</td>
<td>Toluene</td>
<td>70</td>
<td>60:40</td>
</tr>
<tr>
<td>Si(TMS)\textsubscript{3}</td>
<td>LiHMDS</td>
<td>-60</td>
<td>DMF</td>
<td>94</td>
<td>87:13</td>
</tr>
<tr>
<td>Si(TES)\textsubscript{3}</td>
<td>LiHMDS</td>
<td>-60</td>
<td>DMF</td>
<td>82</td>
<td>96:4</td>
</tr>
<tr>
<td>Si(TES)\textsubscript{3}</td>
<td>TMSOTf/Et\textsubscript{3}N</td>
<td>-78</td>
<td>Toluene</td>
<td>79</td>
<td>2:98</td>
</tr>
</tbody>
</table>

The extraordinary steric bulk of the super silyl group was found to bring about high selectivity in this reaction, favouring a 1,5-syn product \textbf{220} when lithium hexamethyldisilazide was used as the enolisation reagent. The stereoselectivity of the reaction was found to be highly solvent dependent, with non-coordinating solvents such as dichloromethane and toluene leading to a moderate diastereomeric ratio of 60:40 (\textbf{220}:\textbf{221}) compared with a ratio of 87:13 when the reaction was carried out in $N,N'$-dimethylformamide. This can be explained by the formation of a cyclic transition state in which co-ordination of two molecules of $N,N'$-dimethylformamide to the lithium centre stabilizes the conformation of the 6-membered ring (Figure 2.11).

Changing the protecting group from a TMS-type super silyl to the bulkier TES-type brought about the highest diastereomeric ratio of 96:4 (\textbf{220}:\textbf{221}). Yamamoto *et al.* found that the favoured diastereomer of the product could be switched from syn to anti by employing a silyl enol ether in place of the lithium enolate, with a resulting dr of 2:98 (syn/anti \textbf{220}:\textbf{221}) (Table 2.4, entry 5). Without the formation of a conformationally restricted cyclic transition state around a metal centre, the direction of approach of pivalaldehyde to the silyl enol ether in transition states \textbf{226} and \textbf{227} is influenced by steric interactions only, leading to a preferential formation of the 1,5-anti product \textbf{221} (Figure 2.12).
Figure 2.11 • Transition states of the lithium mediated aldol reaction reported by 
Yamamoto et al.

Figure 2.12 • Transition states in the Mukaiyama aldol synthesis: 1,5-anti 
diastereoselectivity, reported by Yamamoto et al.
In each case (syn or anti favoured) the reaction was found to tolerate a wide range of aldehydes (RCHO where R = cyclohexyl, isopropyl, 4-methoxyphenyl, 4-nitrophenyl) with excellent diastereomeric ratios of between 85:15 and 98:2.

2.4.2 Allylstannanes in remote asymmetric induction

The addition of allylstannanes to aldehydes, promoted by the addition of a tin(IV) halide reagent, has been found to afford homoallylic alcohols with high enantioselectivity. Thomas et al. found that the addition of 4-, 5-, and 6-substituted allylstannanes to benzaldehyde 228 favoured the syn product in each case (Scheme 2.14). The addition of 229 to benzaldehyde proceeded via a transmetallation between stannane 229 and tin(IV) chloride. After five minutes, the addition of benzaldehyde 228 afforded the 1,5-syn-(Z) isomer of allylic alcohol 156 as the major product. The excellent diastereomeric ratio of 98:2 (syn/anti) in the 1,5-asymmetric induction dropped slightly with extension of the substrate carbon chain. Thus, the reaction between stannane 231 and benzaldehyde 228 afforded 1,6-syn product 232 with a diastereomeric ratio of 92:8, which was matched by the corresponding 1,7-induction (addition product 234).

\[ \text{Bu}_3\text{Sn} \underset{\text{i) SnCl}_4 (1.2 \text{ equiv.})}{\xrightarrow{-78 \degree \text{C}, 5 \text{ min}}} \text{HO} \underset{\text{ii) PhCHO 228, -78 \degree \text{C}, 5 \text{ min}}}{\text{Ph}} \underset{\text{BnO}}{\text{Bn}} \text{ Total yield = 90\% syn/anti ratio = 98:2 1,5} \]

\[ \text{Bu}_3\text{Sn} \underset{\text{i) SnBr}_4 (1.2 \text{ equiv.})}{\xrightarrow{-78 \degree \text{C}, 5 \text{ min}}} \text{HO} \underset{\text{ii) PhCHO 228, -78 \degree \text{C}, 5 \text{ min}}}{\text{Ph}} \underset{\text{BnO}}{\text{Bn}} \text{ Total yield = 72\% syn/anti ratio = 92:8 1,6} \]

\[ \text{Bu}_3\text{Sn} \underset{\text{i) SnBr}_4 (1.2 \text{ equiv.})}{\xrightarrow{-78 \degree \text{C}, 5 \text{ min}}} \text{HO} \underset{\text{ii) PhCHO 228, -78 \degree \text{C}, 5 \text{ min}}}{\text{Ph}} \underset{\text{BnO}}{\text{Bn}} \text{ Total yield = 72\% syn/anti ratio = 92:8 1,7} \]

**Scheme 2.14** • 1,5- to 1,7-stereocontrol in the addition of benzaldehyde 228 to allylstannanes 229, 231 and 233, reported by Thomas et al.
The mechanism of the reaction between allylstannane 233 and benzaldehyde 228 reveals the source of the high degree of stereocontrol (Figure 2.13). Following the transmetallation step, the alcohol group is able to co-ordinate to the tin centre via an oxygen lone pair. As a result, 6-membered ring intermediate 236 is formed, in which the vinyl and methyl substituents have an anti-relationship across the ring to achieve the lowest energy conformation. The approach of benzaldehyde is further limited by coordination of the carbonyl oxygen atom to the metal centre (237). Consequently, the addition reaction that follows actually occurs via a 1,5-asymmetric induction through the co-ordinated tin atom, despite the 1,7-relationship in the free diol (234).

The remote stereocontrol shown by the tin(IV) halide mediated addition of substituted allylstannanes to simple aldehydes (Scheme 2.14) has led to the development of new strategies in the synthesis of various natural products. For example, the total synthesis of the antibacterial macroide (±)-patulolide C 163 by Thomas et al. (Scheme 2.15) featured a 1,5-remote asymmetric induction in the reaction between tributylstannane 239 and acrolein 240 to afford alcohol 241 with a diastereomeric ratio of 96:4 in favour of the syn product, and a good overall yield of 77%. An Ireland-Claisen rearrangement was then used to relay the stereochemical information down the chain, resulting in the 1,6-syn relationship found in the natural product (242).
Scheme 2.15 • 1,5-Stereocontrol in the synthesis of (±)-patulolide C, reported by Thomas et al.

While the organotin chemistry described above provided a reliable method for acyclic remote stereocontrol, the reactions require very low temperatures (-78 °C) and anhydrous conditions. Organotin compounds are also notoriously toxic, and cause well-documented damage to the environment. Finally, the removal of tin residues such as tributyltin chloride from the final product of the reaction can be very challenging. In order to overcome these limitations, Thomas et al. turned to allylgermane substrates in place of the allylstannanes (Table 2.5).133

Table 2.5 • 1,6-Stereocontrol in the addition of aldehydes to allylgermane 243

(E)-243 was found to react with tin(IV) tetrabromide and then benzaldehyde 263 with promising 1,6-stereocontrol to afford diol 244 as the major product, with a diastereomeric ratio of 89:11 (244:245). This diastereomeric ratio, as well as the overall yield, was found to improve when the (Z)-isomer of 243 was used, increasing the dr to 94:6 in favour of the syn product 244. The reaction shown in Table 2.5 was also able to tolerate a range of
aldehyde substrates including those bearing aliphatic chains (entry 3) and substituted benzaldehydes (entries 4-6, Table 2.5).

### 2.4.3 Remote asymmetric induction in the Grignard reaction

Tamai et al. employed (R)-BINOL as the chiral auxiliary in the Grignard reaction of ketoester 246 (Table 2.6). By extending the carbon chain of the ketoacid starting material, they were able to achieve up to 1,12-asymmetric induction with significant enantiomeric excess. However, the ee decreased with increasing length of the carbon chain, with an optimum of n = 1 (1,8-stereocontrol), which afforded the (R)-isomer 247a as the major product with 97% ee and an overall yield of 82%.

#### Table 2.6 • 1,8- to 1,10-stereocontrol in the Grignard reaction, reported by Tamai et al.

<table>
<thead>
<tr>
<th>Product</th>
<th>n</th>
<th>R</th>
<th>1,X-induction</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-247a</td>
<td>1</td>
<td>-C₉H₁₉</td>
<td>1,8</td>
<td>82</td>
<td>97</td>
</tr>
<tr>
<td>(S)-247b</td>
<td>2</td>
<td>-CH₃</td>
<td>1,9</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>(S)-247c</td>
<td>3</td>
<td>-CH₃</td>
<td>1,10</td>
<td>89</td>
<td>4</td>
</tr>
</tbody>
</table>

The reaction was thought to proceed via a pseudo-macrocyclic chelate such as 248, where the oligoether substituent functions as a podand around the Mg²⁺ centre. The stability of this complex fixes the orientation of the ketone carbonyl group, allowing nucleophilic attack only from the outside of the macrocycle. A short chain ketoester, where n = 1, appears to hinder the formation of complex 248, and no enantioselectivity was observed in the product. As the chain extends beyond n = 3, the complex becomes too flexible to limit the direction of nucleophilic attack. The stability of the pseudo-macrocyclic intermediate therefore governs the stereoselectivity of this reaction. The in situ lactonisation means that the (R)-BINOL chiral framework is released during the reaction, and could therefore be recovered and re-used if desired.
Table 2.7 • 1,6-Stereocontrol in the nucleophilic addition to ketones, reported by Takahashi et al.

Following their earlier work on the 1,5-asymmetric induction in the diastereoselective reaction of carbonyl compounds with organotitanium reagents, Takahashi et al. reported a comparison of the 1,6 remote asymmetric induction achieved using three different organometallic reagents: the Grignard reagent methylmagnesium bromide, methyllithium, and methyltitanium triisopropoxide (Table 2.7). Valinol-derived (S)-4-amino-1-phenylbutanones 249a and 249b reacted with methyltitanium triisopropoxide in tetrahydrofuran at room temperature to afford diastereomeric mixtures of products 250a and 250b. When the same reaction was carried out with methyllithium or methylmagnesium bromide as the organometallic reagent, little diastereoselectivity was observed.

Scheme 2.16 • Cyclic reaction intermediate complex 251 in the methylation of ketone (S)-249a, reported by Takahashi et al.

With methyltitanium triisopropoxide, moderate diastereoselectivity was achieved in the synthesis of 250a, favouring the R,S diastereomer in a ratio of 89:11 (R,S: S,S). However, there was no stereocontrol observed in the synthesis of 250b (where R = -CH₃), suggesting that the free hydroxyl group is necessary for a cyclic chiral reaction intermediate. To explain the reagent- and substrate-dependent diastereoselectivity of the
reaction, Takahashi et al. proposed the formation of transition state 251 (Scheme 2.16), in which co-ordination of the carbonyl and hydroxyl oxygen atoms to the titanium centre brought the existing and forming chiral centres into close proximity - a conformation further stabilized by the possible co-ordination of the tertiary amine via the nitrogen lone pair. The steric bulk of the isopropoxy ligands compared to the methyl ligand would give rise to preferential addition of the methyl group to the same face as the existing chiral centre. Methylmagnesium bromide and methyl lithium are stronger nucleophiles than methyltitanium triisoproxide, as reflected in the yield of the reactions (63% and 53% yield respectively, compared to 27% with MeTi(OiPr)₃). When these reagents are used, methylation occurs before the formation of a highly ordered metal complex can take place.

2.4.4 Organolithium reagents in remote asymmetric induction

Phosphorylated amino acids are found in a range of antibiotics, antivirals, and enzyme inhibitors as well as natural products. In their 2001 synthesis of β-amidophosphonates, Quirion et al. compared a number of synthetic routes, each involving a 1,5-asymmetric induction. In the first approach, β-amidobenzene 252 was deprotonated at the benzylic carbon by s-butyllithium, before the addition of diethyl chlorophosphite as an electrophile (Scheme 2.17). While compound 253 was obtained in a 55% yield, no diastereoselectivity was observed for this reaction due to the flexibility of the substrate molecule.

![Scheme 2.17](image)

Scheme 2.17 • Synthesis of β-amidophosphonates 253 reported by Quirion et al.

However, in a more successful approach, diethylphosphite reacted with a series of α,β-unsaturated chiral amide 254 via a Michael addition (Table 2.8). The diastereoselectivity of this reaction was found to be particularly dependant on the nitrogen protecting group, with an N-benzyl group affording the highest de of 92%, and the more hindered N-isopropyl group resulted in a significant loss of selectivity, with a de of just 22%. A mechanistic rationale for this effect is presented in Figure 2.14. In A, where there is no N-substituent, the molecule is largely flat due to the planarity of the alkene and amide bonds.
Table 2.8 • Asymmetric synthesis of β-amidophosphonates, reported by Quirion et al.

<table>
<thead>
<tr>
<th>Major Product</th>
<th>R</th>
<th>Yield (%)</th>
<th>de (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,R)-255</td>
<td>H</td>
<td>55</td>
<td>82</td>
</tr>
<tr>
<td>(R,R)-256</td>
<td>Me</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>(R,R)-257</td>
<td>Pr</td>
<td>45</td>
<td>22</td>
</tr>
<tr>
<td>(R,R)-258</td>
<td>Bn</td>
<td>50</td>
<td>92</td>
</tr>
</tbody>
</table>

Diethyl phosphite therefore preferentially approaches the upper face of the alkene to avoid interactions with the phenyl ring. Similarly in B, the lower face is blocked by the phenyl group, and further steric interactions with the N’-benzyl substituent also limit the trajectory of the nucleophile, leading to a slight increase in diastereomeric excess from 82% to 92%. Finally, the N’-isopropyl substituent in C lies perpendicular to the plane of the amide, cause steric hindrance on both the upper and lower face of the alkene substrate which is reflected the lower de of 22%.

Figure 2.14 • Mechanistic explanation for the diastereoselectivity in the Michael addition of diethylphosphite to α,β-unsaturated chiral amides, reported by Quirion et al.

Examples of remote chiral induction beyond a distance of 8 covalent bonds are extremely rare – only a handful exist, and these tend to rely on supramolecular complexation of an acyclic substrate (bearing a chiral auxiliary) around a metal centre, such as the work by Magnus et al. described below.
In 1997, following earlier investigations into 1,9-stereocontrol for the same methodology, Magnus and Magnus reported a 1,13- and 1,14-asymmetric induction in the synthesis of linear chiral sulfones (Scheme 2.18). Substrate 259, bearing the (R)-α-methylbenzylamine chiral auxiliary, was deprotonated by n-butyllithium at -70 °C before the addition of benzaldehyde 228 at -100 °C. There are four possible diastereomeric products of this reaction: two syn-isomers (R,R,R/S,S,R) and two anti-isomers (R,S,R/S,R,R). However, only two diastereomers were observed in the 1H-NMR of the impure reaction mixture: one syn, and one anti.

Scheme 2.18 • 1,13 and 1,14-asymmetric induction in the synthesis of chiral sulfones, reported by Magnus and Magnus.

Magnus and Magnus proposed that the unexpected long-range stereoselectivity of this reaction may be due to the formation of bicyclic chelate intermediate 262, in which the chiral centre of the starting material is only three atoms from the C-Li bond. The stereocontrol therefore operates over a much shorter distance than it would appear from the long-chain structure of starting material 259, being a 1,4- rather than a 1,14- remote asymmetric induction. The ratio of 260:261 deteriorated as the chain length increased from n = 2 to n = 4, as the corresponding change in ring size from 7 to 9 atoms increased the flexibility of the transition state.

Taking a highly flexible substrate such as 259, with no C=C bonds, alcohol groups or cyclic features, and achieving an apparent 1,14-stereocontrol is undoubtedly a significant achievement. However, the examples of RAI described so far in this review lack truly impressive through-space distance in the chiral induction.
2.5 Atropisomeric and helical chiral substrates for RAI

In order to achieve the long-range communication of stereochemical information over nanometer distances, it is necessary to employ a substrate which is structurally linear and has a global conformational constraint. Clayden and co-workers initially investigated the use of atropisomeric amide compounds for this purpose, and in 1997 they reported a rare case of remote chiral induction by a rotationally restricted amide (Scheme 2.19).\textsuperscript{141} Naphthamide 263 was deprotonated by s-butyl lithium before the addition of benzaldehyde 228, to afford secondary alcohol diastereomers 264 and 265 in an overall yield of 51%. Unfortunately, no significant diastereoselectivity was observed in this reaction. However, when an N-benzylidenemethylamine 266 was employed as the electrophile, secondary amine 267 was obtained as a single diastereomer in a 74% yield.

\begin{center}
\textbf{Scheme 2.19} • 1,5-Stereocontrol by atropisomerism, reported by Clayden \textit{et al.}
\end{center}

The conformation of amides can be controlled by nearby stereogenic centres, and can further influence the conformation of neighbouring amide groups.\textsuperscript{142} This approach mirrors the “chiral relay” methodology introduced by Davies \textit{et al.} in the synthesis of enantiomerically pure \(\alpha\)-amino acids (Scheme 2.20).\textsuperscript{143} Sterically bulky groups such as the N-benzyl substituents of cyclic amide 269 can increase the influence of the existing chiral centre on the remote site of reactivity. Thus diastereomer 270 was obtained by 1,4-stereocontrol with 93% de and an overall yield of 72%.
Scheme 2.20 • Chiral relay auxiliary for the synthesis of optically active $\alpha$-amino acids $(R)$-alanine 271 and $(S)$-valine 272, reported by Davies et al.

Viewing substrate 263 as a promising building block, Clayden et al. constructed a highly rigid polyxanthene structure (273) bearing rotationally restricted amides whose atropisomeric stereochemistry was fixed by a $(1S,2R)$-ephedrine moiety (Scheme 2.21). At the far end of the molecule, an aldehyde group provided a target for asymmetric nucleophilic attack by a suitable Grignard reagent.

Scheme 2.21 • 1,23-Stereocontrol in a polyxanthene structure, reported by Clayden et al.
The conformationally constrained nature of compound 273 meant that the chiral centre was both spatially remote from the aldehyde carbonyl group as well as separated by the chain of covalent bonds (coloured purple, Scheme 2.21). The energy barrier to epimerization of this class of compounds, such as amide 275 (85 kJ mol⁻¹) is sufficiently high to prevent changes in conformation through the structure, and each amide substituent is angled anti to its neighbours. Remarkably, Clayden et al. found that the distance of the stereocontrol was limited by difficulties in synthesis of suitable substrates rather than the reliability of the chiral relay, as they were unable to synthesise a tetraxanthene starting material.

In a change of strategy, Clayden et al. instead built substrate 276: a peptide chain consisting of multiple aminoisobutyric acid (Aib) subunits, which adopts a helical conformation. The direction of the helix was established by either one or two (S)-α-methyl valine residues at one end (276a and 276b respectively). Under hydrogenation conditions of Crabtree’s catalyst (10% w/w) and a hydrogen atmosphere, peptides 277a and 277b were obtained as the major diastereomers, featuring (S)-phenylalanine residues highlighted in red.

Scheme 2.22 • Asymmetric hydrogenation for the synthesis of an (S)-phenylalanine residue within a right-handed helical peptide foldamer: 1,16-asymmetric induction, reported by Clayden et al.
Table 2.9 • Asymmetric hydrogenation in a peptide foldamer reported by Clayden et al.

<table>
<thead>
<tr>
<th>Product</th>
<th>n</th>
<th>Solvent</th>
<th>dr (S)-Phe-277 : (R)-Phe-277</th>
</tr>
</thead>
<tbody>
<tr>
<td>277a</td>
<td>1</td>
<td>EtOH</td>
<td>79:21</td>
</tr>
<tr>
<td>277a</td>
<td>1</td>
<td>CH₂Cl₂</td>
<td>88:12</td>
</tr>
<tr>
<td>277b</td>
<td>2</td>
<td>EtOH</td>
<td>89:11</td>
</tr>
<tr>
<td>277b</td>
<td>2</td>
<td>CH₂Cl₂</td>
<td>&gt;95:5</td>
</tr>
</tbody>
</table>

The diastereoselectivity of the hydrogenation reaction was improved by using dichloromethane as the solvent rather than ethanol, with an increase in dr from 79:21 to 88:12 in the synthesis of 277a. This can be explained by the ability of polar protic solvents such as ethanol and methanol to disrupt hydrogen bonding, which would compromise the conformational integrity of right-handed helical foldamer 276a. The highest dr (>95:5) was achieved with two units of (S)-α-methyl valine in the substrate (n = 2, compound 276b).

Aiming to improve on the 1,16-asymmetric induction achieved for the hydrogenation reaction (Scheme 2.22), Clayden et al. increased the length of the peptide chain, maintaining the ordered helix by the incorporation of cyclohexane (Ac6c) residues (compound 278, Scheme 2.23). The resulting peptide formed a helical foldamer at low
temperatures (down to -50 °C) held in a stable conformation by multiple hydrogen bonds. The chiral centres of the (S)-α-methyl valine dimer again influenced a preference for a right-handed screw-sense of the helix. Thus chiral information could be relayed through up to 60 covalent bonds to the site of reactivity. In the Friedel-Crafts reaction between N-alkenes 278a-c and 1,3,5-trimethoxybenzene 279 in the presence of trifluoromethanesulfonic acid in THF at -50 °C, a mixture of two diastereomers were formed, where (S,S,R)-280 was the major product. In the 1,31-induction (n = 1), a diastereomeric ratio of 92:8 was achieved in favour of 280a, which dropped only slightly to 90:10 for the 1,46-induction (280b) and 88:12 for the 1,61-induction (280c). The yields of the reactions also remained high, with an overall range of 78-91%.

The 1,61-asymmetric induction described in Scheme 2.23 represents the communication of stereochemical information over a distance of around 4 nm,\textsuperscript{146} which is far greater than any remote stereocontrol reaction previously reported. The mode of chiral induction showcased by this peptide foldamer is comparable to the stereoselectivity shown by enzymes, and may therefore find potential use in artificial cell structures for biological applications.

2.6 Conclusions

Remote asymmetric induction is a concept that has been used by organic chemists for half a century. Chiral auxiliaries have been widely employed in the synthesis of chiral non-racemic small compounds and in large natural products total syntheses. They are efficient, commercially available, and in many cases recyclable. However, the future of remote asymmetric induction lies beyond the synthesis of isolated small molecules. Mastering the transfer of information \textit{via} remotely-triggered conformational changes in large molecules, particularly peptides, opens up the possibility of designing and manipulating artificial trans-membrane receptors in human cells.
2.7 Project Aims

In the first chapter of this thesis, an introductory review of previously published work on the synthesis and biological activity of glycopeptide antibiotic analogues showed that this is an important class of antibiotics to pursue, and that an efficient, low cost route to the triaryl biether backbone (C-O-D-O-E) would be highly desirable for future syntheses of compounds featuring this structural motif. To this end, the early aim of this project (presented in Chapter 3) was to investigate the feasibility of a double nucleophilic aromatic substitution between methyl gallate and 4-fluorobenzaldehyde, and to then employ previously reported transformations for the synthesis of the ring D α-amino acid moiety of orienticin C (Scheme 2.24).

Scheme 2.24 • Plan for the construction of the C-O-D-O-E triaryl biether backbone of the glycopeptide antibiotic family

In a parallel study, the aldehyde functional group of the C-O-D-O-E backbone would be subject to imine condensation and aza-Darzens aziridination, with the intention of ring-opening a suitable aziridine to afford the functional group protected β-hydroxy α-amino acid (Scheme 2.25), which is a key component of the D-O-E macrocycle of orienticin C.

Scheme 2.25 • Proposed aza-Darzens aziridination for the synthesis of the β-hydroxy α-amino acid moiety of orienticin C

In order to increase the efficiency of this approach in the synthesis of glycopeptide antibiotic analogues, the direct synthesis of peptide from amino acid-based precursors was also investigated. As the literature review of remote asymmetric induction in Chapter 2 shows, existing chiral centres in reaction substrates can influence the stereochemical outcome of a reaction, even when the substrates are acyclic. It was anticipated that amino
acid derived imines or diazoacetamides might exert a 1,3- or 1,5-induction in the Brønsted acid catalyzed aza-Darzens aziridination reaction (Scheme 2.26).

Scheme 2.26 · Brønsted acid catalyzed aza-Darzens synthesis of a peptide aziridine precursor to the β-hydroxy α-amino acid moiety of orienticin C

As well as being investigated for the synthesis of glycopeptide antibiotic analogues, the remote chiral induction strategy described in Chapter 4 may prove more generally useful in the synthesis of chiral non-racemic peptide aziridines as precursors for unnatural dipeptides and α-amino acids.
CHAPTER THREE

Construction of the orienticin C backbone
3.1 Introduction

In order to explore the application of an organocatalytic asymmetric aza-Darzens reaction to the synthesis of vancomycin-type compounds, an aldehyde-functionalised core starting point for the C-O-D-O-E backbone was required (Figure 3.1). Ideally this would feature the central \((R)-3,4,5\text{-tri}-\text{hydroxyphenylglycine derivative (ring D)}\) already in place. Protecting group Pt\(^2\) at the amino acid on ring D will be necessary to prevent an unwanted imine condensation at this position during subsequent aziridination steps, and protecting group Pt\(^3\) will be required for ease of purification. Phenol derivatives are poor nucleophiles, but still under specific conditions are able to ring-open substituted aziridines.\(^{147}\) This potential side reaction will be prevented by incorporating a phenol protecting group on ring D (Pt\(^1\)).

**Figure 3.1** • Summary of project aims for construction of the triaryl vancomycin backbone

The initial starting material for the synthesis of the C-O-D-O-E backbone, methyl gallate 281, is commercially available and can be easily obtained by esterification of gallic acid,\(^ {148}\) an inexpensive natural product found in a range of plant sources including acacia bark and grape seeds.\(^ {149}\) It was anticipated that the ester functionality of compound 281 would influence the chemoselectivity of the S\(_{N}\)Ar reaction with 4-fluorobenzaldehyde 282, leading to coupling only at the 3- and 5-hydroxy positions of 281 (Scheme 3.1). Protection of the dialdehyde functionality on 283 in the form of a cyclic or acyclic acetal is necessary during the reduction of the methyl ester to primary alcohol 284 and the subsequent oxidation that leads to the formation of the Wittig reaction precursor, aldehyde 285.
**Scheme 3.1** • Retrosynthetic analysis for the construction of the triaryl biether core 287 of orienticin C (R = -CH₃, -Bn; R' = -Cbz, -Boc)

With the styrene moiety of 286 in place, a Sharpless asymmetric aminohydroxylation reaction should afford an amino alcohol which could be oxidised to 287, completing the synthesis of the (R)-alpha-amino acid of ring D. Acid-catalysed hydrolysis of the two acetal protecting groups on rings C and E would restore the aldehyde functionality in preparation for imine condensation and subsequent asymmetric aza-Darzens aziridination.

### 3.2 Diaryl ether coupling

Since Fritz Ullmann reported the copper(0)-catalysed reaction between phenol and bromobenzene in 1905, a myriad of methods have been developed for diaryl ether synthesis. Traditionally, the Ullmann ether synthesis uses high temperatures (100–300 °C), long reaction times (48–72 hours) and stoichiometric metal reagents or high catalyst loading. Modern variations include the use of 2 mol% of copper(II) trans-bis-(glycinato) catalyst 288 by Jain *et al.*, which enables lower reaction temperatures, shorter reaction times and lower catalyst loading than previous protocols (Scheme 3.2).\(^{152}\)
Scheme 3.2 • Copper(II)-catalysed diaryl ether coupling reported by Jain et al., 2012.

The copper(II)-mediated Chan-Lam coupling of a hydroxyl group with 4-methoxyphenylboronic acid 290 was employed by Evans et al. in their 1998 formal synthesis of L-thyroxine 292 from (S)-ethyl 2-acetamido-3-(4-hydroxy-3,5-diiodophenyl)propanoate 289 (Scheme 3.3).\textsuperscript{153} A range of palladium(II)-catalysed protocols have also been reported, exploiting the recent interest in using platinum-group metals in coupling methodologies, such as that of Buchwald et al. which employed the bulky and sterically hindered phosphorus diaryl ligand, ‘BuXPhos 297 (Scheme 3.3).\textsuperscript{154}

Scheme 3.3 • Copper(II)-catalysed arylboronic acid coupling reported by Evans et al., 1998; palladium(II)-catalysed coupling reported by Buchwald et al., 2006.

Previous syntheses of vancomycin and its analogues have employed as key strategies either copper(I)-catalysed or transition metal-free coupling methods. In their 1997 synthesis of monomacrocyclic vancomycin analogues, the Boger group performed a nucleophilic aromatic substitution to generate the C-O-D macrocycles 299 and 300 (atropisomers which were separable by flash column chromatography) (Scheme 3.4).\textsuperscript{155}
The presence of an electron withdrawing nitro group ortho to the fluoro substituent (compound 298, ring C) has previously been shown to activate the halogen towards nucleophilic attack.\textsuperscript{156} The macrocyclisation of 298 proceeded in a moderate 50\% yield overall, although there was no selectivity for the natural (M)-diastereomer 300 over the unnatural (P)-diastereomer 299 in this case. The nitro group of 300 was converted in three steps to the chloro substituent found in vancomycin (1, p. 1) later in the synthesis.

Scheme 3.4 • Synthesis of vancomycin C-O-D ring analogues reported by Boger et al.

Taking a novel approach to this problem for their 1998 total synthesis of vancomycin, the Nicolaou group developed a triazene-based macrocyclisation reaction which employed an excess of a copper(I) bromide dimethyl sulfide complex (Scheme 3.5).\textsuperscript{157} It was proposed that the reaction proceeded via intermediate 303, in which the attacking nucleophile was brought into the proximity of the aryl bromide by coordination of the metal counterion to the triazene functionality of ring D, which itself became an “electron sink” for the nucleophilic substitution. Following the synthesis of model C-O-D ring 302 in a 77\% yield, this methodology also proved to be moderately efficient in the synthesis of vancomycin, closing the D-O-E macrocycle in a 60\% yield.\textsuperscript{158}
3.2.1 S\textsubscript{N}Ar synthesis of triaryl biether and diaryl ether aldehydes

An aldehyde-functionalised triaryl biether was required as the starting point for our synthesis of the C-O-D-O-E backbone of orienticin C. With this in mind, a double nucleophilic aromatic substitution reaction between methyl gallate 281 and 4-fluorobenzaldehyde 282 (Scheme 3.6) was investigated, exploring a range of reaction conditions (Table 3.1 and 3.2).

![Scheme 3.6 • General scheme for the S\textsubscript{N}Ar reaction between methyl gallate 281 and 4-fluorobenzaldehyde 282](image)

At room temperature, no reaction was observed between methyl gallate 281 and 4-fluorobenzaldehyde 282 in the presence of 2.5 equivalents of potassium carbonate (Table 3.1). Increasing the temperature to 70 °C afforded 304 in a poor 20% yield after prolonged heating for 48 hours.

<table>
<thead>
<tr>
<th>Base</th>
<th>Solvent</th>
<th>4-Fluoro- benzaldehyde (equiv)</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield 304 (%)</th>
<th>Yield 305 (%)</th>
<th>Total Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>DMF</td>
<td>1.1</td>
<td>25</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>DMA</td>
<td>2</td>
<td>70</td>
<td>48</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>DMF</td>
<td>2</td>
<td>160</td>
<td>4</td>
<td>&lt;5</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

In an attempt to reduce the reaction time, the flask was heated to reflux (~160 °C) in DMF. After four hours, a white crystalline precipitate was observed, whose physicochemical properties matched those of 1-(4-((dimethylamino)phenyl)ethanone 306. Formation of 306 was undoubtedly due to the in situ decomposition of DMF into dimethylamine and carbon monoxide. Presumably the dimethylamine underwent a nucleophilic aromatic substitution with 4-fluorobenzaldehyde (Scheme 3.7). Indeed this
construction of the orienticin C backbone

reaction has recently been exploited by Ulven et al. in a flow chemistry protocol using a range of aryl halides. DMF was therefore deemed an unsuitable solvent for this reaction at the temperature indicated. Instead, using DMA or DMSO as the reaction solvent for the synthesis of 304 and 305 prevented the formation of byproduct 306.

Scheme 3.7 • Mechanism for the S_NAr reaction between dimethylamine and 4-fluorobenzaldehyde following the in situ decomposition of DMF

Compared to the low yield, high temperature and long reaction time of using a conventional oil bath set-up, the application of microwave irradiation offered several significant advantages for this reaction. A comparable 21% yield of 304 was obtained in just 30 minutes by changing the heating method to microwave irradiation at the same temperature (160 °C), in DMA (Table 3.2). Changing the base to cesium carbonate and the solvent to DMSO increased the yield of 304 to 32%, and triaryl biether 305 was observed for the first time in a 5% yield. By adding 3.2 equivalents of 4-fluorobenzaldehyde, dialdehyde 305 could be obtained in a 56% yield, with a total reaction yield of compounds 304 and 305 of 77%.

Table 3.2 Base and solvent study for the synthesis of 304 and 305 (microwave irradiation)

<table>
<thead>
<tr>
<th>Base</th>
<th>Solvent</th>
<th>4-Fluoro-benzaldehyde (equiv)</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield 304 (%)</th>
<th>Yield 305 (%)</th>
<th>Total Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_2CO_3</td>
<td>DMA</td>
<td>1</td>
<td>160</td>
<td>0.5</td>
<td>21</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>K_2CO_3</td>
<td>DMSO</td>
<td>1</td>
<td>160</td>
<td>2</td>
<td>&lt;5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Cs_2CO_3</td>
<td>DMSO</td>
<td>1</td>
<td>160</td>
<td>2</td>
<td>32</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td>Cs_2CO_3</td>
<td>DMSO</td>
<td>1.2</td>
<td>160</td>
<td>1</td>
<td>42</td>
<td>&lt;5</td>
<td>42</td>
</tr>
<tr>
<td>Cs_2CO_3</td>
<td>DMSO</td>
<td>3.2</td>
<td>160</td>
<td>2</td>
<td>21</td>
<td>56</td>
<td>77</td>
</tr>
</tbody>
</table>

Increasing the reaction time, temperature or number of equivalents beyond those reported in Table 3.2 did not increase the yield of either 304 or 305. Instead only decomposition of
the starting materials and products was observed, presumably by base-mediated hydrolysis of the methyl ester. The marked increase in yield of 304 on switching from potassium carbonate to cesium carbonate in DMSO (Table 3.2, entries 2 and 3) may be a result of the “cesium effect”, a term which refers to the large electronic polarizability and cationic radius (1.67 Å vs. 1.33 Å for K⁺) of Cs⁺ compared to other alkali metal ions. Cesium carbonate therefore releases the “naked” carbonate anion more readily. In addition, cesium carbonate has a much higher solubility in DMSO, DMF, and DMA than potassium carbonate (Table 3.3).

Table 3.3 Solubility of alkali carbonates determined by Cella et al. (g/10 mL)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Cs₂CO₃</th>
<th>K₂CO₃</th>
<th>Li₂CO₃</th>
<th>Na₂CO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>1.195</td>
<td>0.075</td>
<td>0.003</td>
<td>0.038</td>
</tr>
<tr>
<td>DMSO</td>
<td>3.625</td>
<td>0.470</td>
<td>0.014</td>
<td>0.143</td>
</tr>
<tr>
<td>DMA</td>
<td>0.490</td>
<td>0.047</td>
<td>0.004</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Scheme 3.8 • Synthesis of dialdehyde 305 on a 10 g scale by oil bath heating

Though the microwave reactor provided a relatively fast and efficient route to 305, the key aldehyde starting material of the orienticin C backbone, the scale was limited to a ‘batch synthesis’ using 500 mg of methyl gallate. Fortunately, the improved reaction conditions could be applied to a 10 g scale oil bath protocol with a minimal reduction in yield (Scheme 3.8). In this case, the impure reaction product could be brought to 98% purity (estimated by ¹H-NMR) without column chromatography by washing the orange solid with cold diethyl ether.

3.2.2 Mechanistic rationale and spectroscopic analysis of diaryl ether 304 and triaryl diether 305

The nucleophilic aromatic substitution described above (Scheme 3.7) shows chemoselectivity for the 3- and 5-hydroxy positions of methyl gallate 281. The most
acids proton is at the 4-hydroxy position, with a calculated pKa of 8 compared to a pKa of 12 for the 3- and 5-hydroxy positions.\cite{162} Deprotonation at the 4-hydroxy position of 281 results in anion 307 which is stabilised by conjugation with the ester group \textit{para} to the phenoxide (Figure 3.2). By contrast, the anion formed by deprotonation at the 3-hydroxy position (309) is not stabilised by the ester, making intermediate 309 the more reactive nucleophile.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.2.png}
\caption{Resonance stabilisation of the reaction intermediates formed by deprotonation of methyl gallate 281}
\end{figure}

This analysis indicates that two equivalents of base are required for the first S\textsubscript{N}Ar. Deprotonation is followed by an addition-elimination mechanism (Scheme 3.9) in which the electron-withdrawing aldehyde \textit{para} to the halide provides stabilisation of anion 312.

\begin{scheme}[h]
\centering
\includegraphics[width=\textwidth]{scheme3.9.png}
\caption{Postulated mechanism of the S\textsubscript{N}Ar reaction between deprotonated methyl gallate 281 and 4-fluorobenzaldehyde 282}
\end{scheme}
The carbon-fluorine bond has been referred to as “the strongest single bond in organic chemistry”. The short C-F bond length (1.4 Å compared to 1.9 Å for C-Br) and high bond dissociation energy (averaging around 500 kJ mol$^{-1}$ compared to 290 kJ mol$^{-1}$ for C-Br) are due to the large difference in electronegativity between carbon and fluorine (2.5 vs. 4.0). Consequently the C-F bond is highly polar, and the electron-poor carbon makes an excellent electrophile in the rate-determining addition step of the S$_{N}$Ar reaction. Re-aromatisation of the benzene ring in 312 is sufficiently exothermic to permit the energetically disfavourable breaking of the strong C-F bond.

![Figure 3.3](image)

**Figure 3.3** • X-ray crystal structure of dialdehyde 305: front view (left) and side view (right)

![Figure 3.4](image)

**Figure 3.4** • Intra- and intermolecular hydrogen bonding in the X-ray crystal structure of dialdehyde 305

Pale brown crystals of dialdehyde 305 were obtained by crystallisation from diethyl ether and subjected to X-ray crystallographic analysis. This confirmed the symmetry of the
double nucleophilic aromatic substitution product (Figure 3.3). In the monoclinic crystal, compound 305 has a non-planar conformation where aryl rings C and E are tilted at an angle of approximately 70° to the central D-ring, compared to 80° in the crystal structure of vancomycin 1. There is evidence of intermolecular hydrogen bonding between the hydroxyl group on ring D and the aldehyde carbonyl of ring E, with a bond length of 2.65 Å and an angle of 118° (O—H---O, Figure 3.4). A possible intramolecular hydrogen bond was also identified between the hydroxyl on ring D and the oxygen of the neighbouring diaryl ether, with a bond length of 2.41 Å compared to 0.84 Å for the O-H covalent bond.

Figure 3.5 • Partial ¹H-NMR spectra of 305 (300 MHz, CDCl₃) and 304 (400 MHz, CD₃OD)

The ¹H-NMR spectrum supported our hypothesis that aldehyde 304 is substituted only at the 3-hydroxy position (Figure 3.5). The non-equivalent aromatic protons of the D ring appear as doublets at 7.30 and 7.12 ppm. The coupling constant ⁴J = 2 Hz is consistent with meta proton-proton coupling across an aromatic ring. By contrast, the magnetically equivalent D-ring protons in dialdehyde 305 appear as one peak: a singlet at 7.62 ppm. In each case the aldehyde protons appear at 9.95 ppm (di-305, CDCl₃) and 9.77 ppm (mono-304, CD₃OD) and the methyl ester signals appear in the appropriate region at
3.85 ppm (305) and 3.73 ppm (304). The FT-IR spectrum confirmed the presence of the aldehyde and ester functional groups, with carbonyl stretch bands at 1692 and 1669 cm\(^{-1}\) for dialdehyde 305 and an overlapping band at 1686 cm\(^{-1}\) for mono-aldehyde 304. The C=O signals appear at a lower frequency than the expected range for carbonyl stretch bands due to the conjugation in these molecules.\(^{169}\)

### 3.2.3 Diaryl ether coupling under continuous flow conditions

Continuous processes in the synthesis of fine chemicals and pharmaceuticals have grown increasingly sophisticated over the last three decades, and versatile modular flow systems are now commercially available and within the reach of both academic and industrial laboratories. Chemical reactions conducted in flow micro-reactors benefit from rapid heat transfer, increased safety and the ability to handle unstable reaction species.\(^{170}\) On an industrial scale, continuous flow processes have attractive economic and environmental benefits due to their high energy efficiency and reduced consumption of resources compared with batch processes.\(^{171}\) Flow system software offers precise control over reaction conditions and continuous measurement of temperature and pressure inside the reactor. Flow systems are therefore also highly compatible with continuous in-line and on-line reaction monitoring by analytical techniques such as ReactIR infrared spectroscopy and flow NMR spectroscopy.\(^{172,173}\) The differences in mass and heat transfer, surface area, catalyst exposure and reaction concentration between flow systems and corresponding batch processes can have profound effects on the efficiency and selectivity of a chemical reaction.\(^{174}\) This so-called “flash chemistry” concept coined by Jun-ichi Yoshida\(^{175}\) was applied to the synthesis of diaryl ethers by Wiles and Watts in 2011 (Scheme 3.10).\(^{176}\)

![Scheme 3.10](image_url)

**Scheme 3.10** • Continuous flow synthesis of 1-chloro-4-nitro-2-(4-nitrophenoxy)benzene 316 reported by Wiles *et al.*

Using an organic base, DBU, to ensure the reaction was homogenous, 1,2-dichloro-4-nitrobenzene 314 was coupled with 4-hydroxynitrobenzene 315 in acetonitrile at 195 °C to afford diaryl ether 316. Employing this flow protocol reduced the reaction time from...
ten minutes to two minutes compared to the microwave-assisted reaction reported by Moseley et al. in 2010, with an accompanying increase in yield from 70% to >99%.\textsuperscript{177} Subsequent investigation into the use of a biphasic aqueous potassium carbonate / acetonitrile mixture within the micro-reactor indicated that inorganic bases could be used in place of DBU, which significantly reduced the costs of the reaction.\textsuperscript{i}

Preliminary investigations into the continuous flow synthesis of dialdehyde 305 were carried out using a Uniqsis FlowSyn reactor. Employing the most favourable base/solvent conditions (outlined in Section 3.2.1, Table 3.2) of cesium carbonate in DMSO, a system was devised in which separate solutions of methyl gallate 281 and 4-fluorobenzaldehyde 282 in DMSO were simultaneously pumped at a flow rate of 1 mL/min \textit{via} a static mixer through a preheated glass column packed with cesium carbonate (Figure 3.6).

\begin{quote}
\textbf{Figure 3.6} Continuous flow synthesis of triaryl biether 305 and diaryl ether 304: 1) using a heated column only and 2) with an additional 20 mL heated coil
\end{quote}

\textsuperscript{i} Potassium carbonate ~£20 per kg compared to DBU ~£400 per kg based on current prices from Sigma-Aldrich Co.
The concentrations of the organic starting materials were such that 2.5 equivalents of 4-fluorobenzaldehyde 282 were present to one equivalent of methyl gallate 281. It was anticipated that the presence of the inorganic base cesium carbonate in a large excess relative to the amount of methyl gallate flowing through the column would increase the efficiency of the diaryl ether coupling. The amount of base inside the column decreased slightly over the course of the reaction, partly due to the slight solubility of cesium carbonate in DMSO (Section 3.2.1, Table 3.3) but mainly due to reaction with methyl gallate to form the soluble cesium salt 311 (Scheme 3.9, Section 3.2.2). However, the high temperature and high polarity of the solvent ensured that no precipitation of the intermediates or byproducts occurred inside the PTFE tubing of the reactor.

In an attempt to improve the yield of dialdehyde 305, a 20 mL PTFE coil reactor heated to 150 °C was added to the FlowSyn set-up between the glass column and the collection flask (Figure 3.6). This increased the reaction time by 10 minutes and changed the ratio of the mono- and di-substitution products 304 and 305 to ~1:1 by 1H-NMR spectroscopy of the impure reaction mixture (Figure 3.7).

Figure 3.7 • Partial 1H-NMR spectra of the impure reaction mixture of flow synthesis reaction 2 (Figure 3.6) (CDCl3, 500 MHz)
Measuring the integration of the methyl ester –OCH₃ protons shows a total conversion of 81% from the starting material methyl gallate 281 to products 304 and 305. However, the isolated yields of 304 and 305 (29% and 33% respectively) add up to an overall reaction yield of 62%, indicating some loss of material due to base hydrolysis of the methyl ester moiety. The yield of the diaryl ether coupling step using a flow reactor is comparable to (but no better than) the yield of the corresponding microwave-assisted reaction discussed in Section 3.2.1, but these initial studies demonstrate the potential of continuous flow systems for the fast automated synthesis of the triaryl biether core of orienticin C.

In the microwave irradiation, flow synthesis and conventional oil bath procedures discussed above, the yield of the desired dialdehyde 305 remained frustratingly low. It is likely that employing an ortho nitro electron-withdrawing group on the electrophile (as demonstrated by both Evans and Boger, Section 3.2) would increase the yield of the reaction. There is also the possibility of switching to a transition metal-catalysed reaction such as that developed by Nicolaou et al. (Scheme 3.4, Section 3.2). However, the commercial availability and low cost of methyl gallate (and its precursor gallic acid) make our nucleophilic aromatic substitution route more appealing in terms of minimising the time and number of synthetic steps required. The byproduct of the S_NAr discussed above, diaryl ether 304, is extremely useful as the starting material for the single-ring glycopeptide analogues which could be employed as test systems for new macrocyclisation chemistry in this field. Alternatively, diaryl ether 304 could be resubmitted to the S_NAr reaction conditions for triaryl biether formation.

3.3 Towards the synthesis of the ring D α-amino acid

Aldehydes 304 and 305 described in Section 3.2 contain phenolic hydroxyl groups which required functional group protection at this stage to prevent possible ring-opening of the aziridines later in the synthesis. The phenol functional group can be routinely protected by a methyl, benzyl or allyl ether. The synthesis of a cyclic para-methoxybenzylidene acetal of methyl gallate, compound 281, was reported simultaneously by the groups of S. Tsuboi and T. H. Chan in 2005.

Following a similar procedure to Chan et al., the transacetalisation reaction of methyl gallate 281 with para-methoxybenzaldehyde dimethyl acetal 317 was carried out in toluene under reflux conditions, with a catalytic amount (0.5 mol%) of para-
toluenesulfonic acid (Scheme 3.11). The round-bottom flask was fitted with a Soxhlet apparatus containing flame-dried 4Å molecular sieves to remove methanol from the reaction, and cyclic acetal 318 was obtained in a 41% yield. Methyl gallate 281 was recovered in a 47% yield after purification by flash column chromatography.

Scheme 3.11 • Protection of methyl gallate 281 as PMB acetal 318, and subsequent S_{N}Ar diaryl ether coupling with 4-fluorobenzaldehyde

Coupling of acetal 318 with 4-fluorobenzaldehyde 282 under the conditions described in Section 3.2.1 was initially unsuccessful (Scheme 3.11); microwave irradiation at 80-120 °C for several hours produced no change by thin layer chromatography. After further heating at 160 °C for one hour the starting material was consumed and the reaction was quenched with distilled water. However, the $^1$H-NMR spectrum of the impure reaction mixture contained a mixture of 4-fluorobenzaldehyde 282, methyl gallate 281 and para-methoxybenzaldehyde, indicating that hydrolysis of the acetal occurred during the workup. The same result was obtained using a copper(II) oxide coupling protocol reported by Kodama et al.

Scheme 3.12 • Protection of compound 304 as para-methoxybenzylidene acetal 319
Compound \textit{319} was finally obtained in a poor 20\% yield by oil bath heating at 75 °C over a period of three days, employing potassium carbonate as the base and acetonitrile as the solvent (Scheme 3.11). Compound \textit{319} could also be obtained by transacetalisation of diaryl ether \textit{304} with \textit{para}-methoxybenzaldehyde dimethyl acetal \textit{317} (Scheme 3.12). However the overall yield of this two-step synthesis from methyl gallate \textit{281} was only 11\%, and with an 8\% overall yield for the reactions described in Scheme 3.10, neither route is suitable for this synthesis.

Protecting the catechol functional group as the \textit{O}-benzyl ether was more successful (Scheme 3.13). The reaction of diaryl ether \textit{304} with benzyl bromide and potassium carbonate in DMF at 120 °C for 7 hours afforded compound \textit{320} in a 73\% yield.\textsuperscript{184} The mono-protected product was not observed in a quantity large enough to isolate.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{304}};
\node (b) at (2,0) {\textbf{320: 73\% yield}};
\node (c) at (0.5,0) {\text{BnBr}};
\node (d) at (1,0) {K$_2$CO$_3$};
\node (e) at (1.5,0) {DMF};
\node (f) at (2,0) {120 °C, 7 h};
\draw[->] (a) -- (b);
\draw[->] (a) -- (c);
\draw[->] (a) -- (d);
\draw[->] (a) -- (e);
\draw[->] (a) -- (f);
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.13 • \textit{O}-Benzyl protection of the 1,2-diol functionality of diaryl ether \textit{304}}

Cleavage of a benzyl ether is generally carried out \textit{via} hydrogenation in the presence of a catalytic amount of palladium on carbon.\textsuperscript{185} In the case of diaryl ether \textit{320} it is unlikely that any chemoselectivity would be shown, and both benzyl groups would be removed simultaneously under these conditions. To achieve a chemoselective deprotection, two protecting groups of differing reactivity were required. The 4-hydroxyl group (\textit{para} to the methyl ester) of compound \textit{304} is present in the aglycon of orienticin C (Chapter 1, Figure 1.2) and may remain protected until the end of the synthesis. In their 1999 synthesis of vancomycin, Boger \textit{et al.} chose to install a methyl ether at the 4-hydroxyl position; a highly robust group which was removed only by a global demethylation in the final stages of the synthesis using aluminium tribromide and ethanethiol.\textsuperscript{13} Following a procedure by Nishiyama \textit{et al.},\textsuperscript{186} the reaction of compound \textit{304} with one equivalent of lithium carbonate and one equivalent of iodomethane in DMF chemoselectively protected the 4-hydroxy group, affording compound \textit{321} in a 71\% yield (Scheme 3.14).
Scheme 3.14 • Phenolic hydroxyl protection of 304 as i) methyl ether 321 and ii) TBDPS ether 322

The chemoselectivity of this reaction is likely to be due to the influence of the electron withdrawing methyl ester on the acidity of the hydroxyl groups of compound 304. Deprotonation by lithium carbonate at the more acidic 4-hydroxy position on 304 results in anion 324 which is stabilised by conjugation with the ester group para to the phenoxide (Scheme 3.15). By contrast, deprotonation at the 3-hydroxy position of 323 is not stabilised by the ester, resulting in a higher pKa. As only one equivalent of base was been added, anion 324 will be the major deprotonated species and will further react with iodomethane via an S_N2 mechanism to generate compound 321.

Scheme 3.15 • Mechanism of the S_N2 nucleophilic substitution of iodomethane with diaryl ether 304

To confirm that the methyl ether was located para to the methyl ester, a second reaction was carried out, in which methyl 3,5-dihydroxy-4-methoxybenzoate 326 was coupled
with one equivalent of 4-fluorobenzaldehyde 282 under the S\textsubscript{N}Ar reaction conditions described in Section 3.2.1 (Scheme 3.16). The \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra of the resulting product matched those of compound 321 obtained by the alternative route illustrated by Scheme 3.14.

\begin{center}
\textbf{Scheme 3.16} • S\textsubscript{N}Ar reaction of 4-fluorobenzaldehyde 282 with methyl 3,5-dihydroxy-4-methoxybenzoate 326
\end{center}

A tert-butylidiphenylsilyl (TBDPS) group was chosen for protection of the remaining phenolic hydroxyl of compound 321. More robust than the trimethyl silyl (TMS) ether group, aryl TBDPS ethers are stable towards weak acid, such as 1 M hydrochloric acid at room temperature, and can be selectively deprotected using TBAF\textsuperscript{187} or DBU\textsuperscript{188}. Following a procedure by Coelho \textit{et al.}, compound 321 was added to a mixture of tert-butylidiphenylsilyl chloride (1.1 equiv.) and imidazole (2.2 equiv.) in DMF at room temperature (Scheme 3.14).\textsuperscript{189} After 18 hours, TBDPS ether 322 was obtained in a moderate 57% yield after flash column chromatography. The yield of 322 is comparable to those reported by Coelho \textit{et al.}, however it has since been found that the addition of 3 equivalents of iodine dramatically accelerates the reaction between aryl alcohols and TBDPS-Cl by the formation of a polyhalide I\textsubscript{2}Cl\textsuperscript{-} anion, so there is potential to reduce the reaction time of the protection described in Scheme 3.14.\textsuperscript{190}

The methyl ether and benzyl ether protections described in Section 3.3.1 above were also applied to triaryl biether 305 in excellent yields of 92% and 87% respectively (Schemes 3.17 and 3.18).

\begin{center}
\textbf{Scheme 3.17} • Reaction of triaryl diether 305 with iodomethane to form methyl ether 327
\end{center}
The benzyl ether was ultimately chosen as a convenient group to both synthesise and cleave later in the synthesis, as benzyl ethers can be efficiently removed by hydrogenation with palladium on carbon but are stable towards standard reduction and oxidation reactions as well as the mildly acidic conditions of the aza-Darzens aziridination reaction.\textsuperscript{37}

As it was decided to focus initially on conversion of the methyl ester of compound 328 to an $\alpha$-amino acid (analogous to the core ring D of orienticin C), it was necessary to protect the aldehyde functionality of 328, which would be incompatible with subsequent reduction and oxidation steps. The acetal moiety is a widely used protecting group of the aldehyde carbonyl, and can be synthesised by the acid-catalysed reaction of an aldehyde with an alcohol, as discussed previously in this section.

Dimethyl acetals can be readily synthesised by the reaction of aldehydes with trimethyl orthoformate in methanol with a catalytic amount of $p$-toluenesulfonic acid.\textsuperscript{191} However, they are prone to hydrolysis during aqueous reaction work-ups, and can be challenging to purify using flash column chromatography due to the slightly acidic nature of silica gel.\textsuperscript{192} Cyclic acetals tend to be more stable towards hydrolysis than acyclic acetals, for entropic reasons (two compounds are obtained by hydrolysis of a cyclic acetal compared with three compounds for an acyclic acetal), and steric hindrance in the case of substituted cyclic acetals, which increases resistance to nucleophilic attack.\textsuperscript{193}
Following a similar procedure originally reported by Ovaa et al.,\textsuperscript{194} compound 328 reacted with 2.3 equivalents of pinacol in refluxing toluene in the presence of 0.5 mol% \textit{p}-toluenesulfonic acid to afford the \textit{bis}-acetal 329 in an 86% yield (Scheme 3.19). A Dean-Stark apparatus was used to collect the water produced in the reaction. The formation of a cyclic acetal is accelerated by the kinetically favourable intramolecular ring-closing step (intermediate 333, Scheme 3.20). In the case of pinacol, the \textit{gem}-dimethyl groups may aid the cyclisation step due to the Thorpe-Ingold effect, by which the size of the methyl substituents compresses the angle of the neighbouring substituents, bringing the nucleophilic alcohol group into closer proximity to the electrophilic carbon reaction site.\textsuperscript{195}

\begin{center}
\includegraphics[width=\textwidth]{Scheme3_20.png}
\end{center}

\textbf{Scheme 3.20 • Mechanism of acetal condensation between dialdehyde 328 and pinacol}

With the acetal protecting groups in place, methyl ester 329 was reduced to primary alcohol 336 using 2.5 equivalents of lithium aluminium hydride in an excellent 97% yield following a similar procedure reported by Percec \textit{et al.}\textsuperscript{196} Oxidation of the alcohol 336 proceeded smoothly using 20 equivalents of activated manganese(II) dioxide to afford aldehyde 337 in a moderate yield of 69% after 18 hours at room temperature. The reduction of a methyl ester to an aldehyde has previously been achieved in one step using diisobutylaluminium hydride (DIBAL-H), which would be an advantage in this synthesis.\textsuperscript{197} However, significant over-reduction of the ester to alcohol 336 was observed
by thin layer chromatography during the reaction between 329 and one equivalent of DIBAL-H at -78 °C. This route was therefore disregarded in favour of the less efficient but more reliable two-step process shown in Scheme 3.21.

The Wittig reaction of aldehyde 337 to form styrene 338 was carried out using standard conditions, in which 3 equivalents of sodium hydride reacted with an equimolar amount of methyltriphenylphosphonium bromide (initially at a reaction temperature of -25 °C) to form a phosphorus ylide which then reacted with aldehyde 337 to afford styrene 338 in a 51% yield.

![Scheme 3.21 • Functional group interconversions from ester 329 to aldehyde 337 and subsequent Wittig olefination](image)

The initially disappointing yield and slow rate of this reaction was thought to be a result of the low temperature employed. Indeed, maintaining the reaction temperature at 0 °C afforded compound 338 in a much improved 76% yield. Cyclic acetals such as those on 338 can be challenging to remove, often requiring strongly acidic conditions (10% aqueous hydrochloric acid / THF or 50% aqueous trifluoroacetic acid). In order to be sure that the pinacol acetals of 338 could be hydrolysed when required, a test deprotection was carried out (Scheme 3.22). The deprotection of a pinacol acetal in the presence of a 1,2-disubstituted alkene with similar success has been reported on multiple occasions in the literature, with no reports of alkene polymerisation.

105
Scheme 3.22 • Test hydrolysis of pinacol acetal 338 using 1 M aqueous hydrochloric acid in THF

Following a procedure adapted from Eichen et al.,201 acetal 338 was dissolved in a 1:1 homogeneous mixture of tetrahydrofuran and 1 M aqueous hydrochloric acid. The reaction was monitored regularly by thin layer chromatography. After stirring at room temperature for 2 hours no change was observed. The reaction temperature was increased to 50 °C, and after 3 hours the starting material was completely consumed. $^1$H-NMR analysis of the impure reaction mixture indicated that the pinacol deprotection was complete. A small amount of the corresponding mono-aldehyde was observed by $^1$H-NMR (400 MHz) but was not isolated in this case. Dialdehyde 339 was obtained in an excellent 90% yield after purification by flash column chromatography on silica gel.
CHAPTER FOUR

Synthesis of peptide aziridines via the aza-Darzens reaction
4.1 Racemic aza-Darzens aziridination of biaryl and triaryl ether aldehydes

Initial attempts to synthesise N-aryl aziridines from the triaryl and biaryl ether aldehydes described in chapter 2 were based on racemic organocatalytic methodology previously established within the Bew group (Scheme 4.1).\textsuperscript{202} The reaction between imine 345 and tert-butyl diazoacetate (TBDA) 346 was catalysed by 10 mol% of pyridinium triflate 347 to afford racemic cis-aziridine 348 in a 71% yield. This reaction was also reported as a one-pot procedure from benzaldehyde, with the addition of 4Å molecular sieves to facilitate the initial imine condensation.

\begin{center}
\textbf{Scheme 4.1} • Pyridinium triflate-catalysed aza-Darzens synthesis of racemic cis-aziridine 348 reported by Bew et al., 2009
\end{center}

Applying this methodology to the triaryl biether system, a three-component one-pot reaction between aldehyde 328, 2-tert-butoxyaniline 349 and tert-butyl diazoacetate 346 was catalysed by pyridinium triflate 347 (10 mol%) at -60 °C, affording bis-aziridine cis-350 in a 59% yield. Byproduct racemic, cis-351 was isolated in a 23% yield (Scheme 4.2).

\begin{center}
\textbf{Scheme 4.2} • One-pot imine condensation / racemic aza-Darzens aziridination reaction of aldehyde 328 catalysed by pyridinium triflate 347
\end{center}
The one-pot protocol shown in Scheme 4.2 is a reaction in two parts; first the imine condensation between aldehyde 328 and 2-tert-butoxyaniline 349, which took place at room temperature. Only 1.95 equivalents of 349 were added, to ensure complete consumption of the amine, which would otherwise be protonated by the pyridinium triflate catalyst and slow down the reaction. For the second part, the aza-Darzens reaction of the activated imine with a diazo compound, catalyst pyridinium triflate (347) was added at room temperature, and the sealed vial was cooled to -60 °C before the addition of tert-butyl diazoacetate 346.

C2,3-disubstituted aziridines can be identified as their cis or trans diastereomer by their 1H-NMR doublet coupling constant.203 The Karplus equation provides a relationship between the three-bond coupling constant ($^3J$) and the H-C-C-H dihedral angle (Figure 4.1).204 In the cis-aziridine, the protons are eclipsed, where the dihedral angle $\phi = 0 \, ^{\circ}$, therefore coupling constant $^3J = \sim7$-11 Hz. In the trans-aziridine, the dihedral angle $\phi = 120 \, ^{\circ}$, giving a coupling constant of $^3J = \sim2$-4 Hz.

![Figure 4.1](image)

**Figure 4.1** The Karplus relationship applied to cis and trans C2,3-disubstituted aziridines

Compounds 350 and 351 isolated from the reaction in Scheme 4.2 have aziridine C-H doublets with coupling constants of 6.7 Hz, which is consistent with a cis configuration. An overall diastereoselectivity of >99:1 (cis:trans) was observed by 1H-NMR analysis of the impure reaction mixture (Figure 4.2). When the same reaction was conducted at room temperature rather than -60 °C, the overall cis/trans ratio dropped to 90:10, although the ratio of mono:bi aziridines (351:350) remained approximately the same.
Figure 4.2 • Partial $^1$H-NMR spectra of the impure reaction mixtures in the formation of aziridines 350 and 351

The reaction conditions outlined in Scheme 4.2 were applied to biaryl ether aldehyde 320 with similar success (Scheme 4.3). It was found that the one-pot imine condensation / racemic aza-Darzens aziridination reaction between 320, 2-tert-butoxyaniline 349 and TBDA 346 could be carried out at 0 °C rather than -60 °C, affording racemic cis-aziridine 352 in a moderate 57% yield. The trans aziridine diastereomer was not observed in this case.

Scheme 4.3 • One-pot imine condensation / racemic aza-Darzens reaction of aldehyde 320 with 2-tert-butoxyaniline 349 and tert-butyl diazoacetate 346

Attempting to carry out a selective mono-aziridination of aldehyde 339 using one equivalent of tert-butyl diazoacetate 346 proved more problematic (Scheme 4.4). Cis-
aziridine 353 was isolated in a poor 19% yield after purification by flash column chromatography, which is unlikely to reflect the true outcome of the reaction, as compound 353 was very difficult to separate from 2-tert-butoxyaniline 349 which was present in the impure reaction mixture. 2-tert-Butoxyaniline 349 was likely to have been produced by hydrolysis of the unreacted imine intermediate upon removal of the 4Å molecular sieves by filtration, or during the flash column chromatography itself.

Scheme 4.4 • One-pot imine condensation / racemic aza-Darzens reaction of aldehyde 339 with 2-tert-butoxyaniline 349 and tert-butyl diazoacetate 346

The cis-selectivity of the racemic pyridinium triflate-catalysed aza-Darzens reactions described above are consistent with previous findings by the Bew group.202 Wulff et al. have put forth a number of mechanistic explanations for this selectivity in relation to their VANOL/VAPOL-boroxinate catalyst.244 With regard to the pyridinium triflate-catalysed reactions described above, a possible mechanism is proposed in Scheme 4.5. Aldehyde 354 first reacts with 2-tert-butoxyaniline 349 in a condensation reaction to form imine 359 in situ. On the addition of pyridinium triflate 347, imine 359 is in equilibrium with protonated species 360, which now is activated towards attack at the electrophilic sp2-hybridised carbon. Due to the difference in pKa of the imine (pKa around 7-8)205 and pyridinium triflate 347 (pKa = 5.2),206 the equilibrium lies towards the protonated imine species 358. Diazoacetate 346 is nucleophilic at the –CHN2 carbon due to resonance of the diazocarbonyl group,207 and can attack either the top or bottom face of the imine on 360.
The orientation of the diazoacetate relative to the imine as it approaches defines the stereochemistry of the chiral centre in intermediate 361 produced by C-C bond formation. The approach of diazoacetate 346 to imine 360 is drawn such that the diazo group is in close proximity to the imine bond, which is consistent with tentative evidence that the reaction may alternatively proceed via a concerted cycloaddition mechanism. In intermediate 361 the bulky tert-butyl group occupies the least sterically hindered position relative to the phenyl group of the imine. The ring-closing step requires a rotation of the bond indicated in 361 so that the nitrogen lone pair overlaps with the empty $\sigma^*$ orbital of
the electrophilic carbon in a “backside attack” mechanism. The –N$_2^+$ group leaves irreversibly as nitrogen gas, observed as bubbles produced during the reaction.

Overall, the results of the racemic aza-Darzens aziridination reactions described in this section were encouraging; the triaryl biether aldehydes 328 and 339 and biaryl ether 320 all reacted successfully in a one-pot procedure. These reactions all produced the cis-aziridine and little or no trans-aziridine. The yields of the aziridination reaction were poor to moderate, however, mainly due to problems with purification. 2-tert-Butoxyaniline 349 and its decomposition products proved difficult to remove from the cis-aziridine by column chromatography. The 2-tert-butoxyphenyl group has been found to enhance the enantioselectivity of the non-racemic version of the organocatalytic aza-Darzens reaction catalysed by BINOL-derived triflamides. However, there have so far been no reports of methods to cleave the 2-tert-butoxyphenyl group from an amine, making it an undesirable protecting group for this multi-step synthesis. Following these initial investigations it was clear that a higher yielding, more flexible, and crucially non-racemic aza-Darzens aziridination route was required.

4.2 Remote asymmetric induction in the organocatalytic synthesis of peptide aziridines

The initial approach to the synthesis of orienticin C described in this work involved the construction of (S)-phenylalanine derivative ring E as a protected α-amino acid which would be subsequently deprotected by hydrolysis of the ester ready for peptide coupling with the asparagine residue of the peptide chain (Route 1, Figure 4.3).

![Figure 4.3](image_url)

**Figure 4.3** Retrosynthetic analysis outlining the construction of the E-ring of orienticin C via a peptide aziridine
However, an approach involving the synthesis of a peptide aziridine (Route 2) would remove the need for a deprotection and peptide coupling step, making this route more efficient. The synthesis of $N$-aryl C$_{2,3}$-disubstituted $\alpha$-amino acid derived aziridines by aza-Darzens reaction of an imine with a diazopeptide has not yet been reported in the literature. Diazo substrates have so far been restricted to diazoacetates and simple phenyl diazoacetamides. It was anticipated that the reaction illustrated by Scheme 4.7 would be catalysed by achiral Brønsted acid pyridinium triflate 347 as in Section 4.1.

![Scheme 4.7](image)

**Scheme 4.7** • General scheme for the pyridinium triflate-catalysed aza-Darzens reaction of an imine with a diazopeptide to afford a peptide-derived cis-aziridine

However, the influence of an $\alpha$-amino acid chiral centre in the diazoacetamide starting material may result in a diastereoselective reaction, due to a 1,5-asymmetric induction (Scheme 4.7). In order to investigate this hypothesis, a series of chiral non-racemic diazopeptides were required. Their synthesis and characterisation are described in Section 4.2.1.

4.2.1 Synthesis of diazopeptides using $N,N'$-ditosylhydrazine

$\alpha$-Diazocarbonyl compounds have found a wide range of applications since the work of Theodor Curtius on their synthesis in the late 19$^{th}$ century (Scheme 4.8).$^{210}$ Diazopeptides exhibit biological activity in a number of areas, and notably they have been shown to display antibacterial$^{211}$ and anti-cancer properties.$^{212}$ Ethyl diazoacetate 144 was first synthesised by Curtius in 1883 by diazotisation of glycine methyl ester 366 in aqueous acid.$^{210}$ $N$-(Diazooacetamido)glycine ethyl ester 369 was subsequently synthesised from $N$-glycylglycine ethyl ester 368 employing the same conditions.$^{213}$ Despite the early synthesis of 369, only a small number of diazopeptides have been reported, of which the majority are benzyl, benzoyl and methyl ester variants of $N$-(diazooacetamido)-glycine and phenylalanine.$^{214}$
Scheme 4.8 • Synthesis of ethyl diazoacetate 144 and N-(diazoacetamido)glycine ethyl ester 369 reported by Curtius et al.

In 2007, Fukuyama et al. reported the synthesis of diazoacetates from the corresponding alcohol in two steps using $N,N'$-ditosylhydrazine as the diazoacetylating reagent (Scheme 4.9).$^{215}$ This method represented a significant improvement on the conventional diazotisation route, affording a diverse range of phenyl, alkyl, allyl and benzyl diazoacetates in yields of 52-90%. $N,N'$-Ditosylhydrazine reacts with organic bases such as DBU and TMG according to the mechanism in Scheme 4.9 put forth by Ragnarsson et al. to afford nitrogen gas and two equivalents of a tosylate salt.$^{216}$

Scheme 4.9 • Synthesis of diazoacetates reported by Fukuyama et al.

Scheme 4.10 • Mechanism of reaction between $N,N'$-ditosylhydrazine 376 and a base (B), where B = DBU, TMG postulated by Ragnarsson and Grehn.
However, in the presence of an alkyl bromide, the deprotonated species acts as a nucleophile, and a diazo compound is formed as the major product. In order to synthesise \( N\)-(diazoacetamido)-L-phenylalanine methyl ester by the method reported by Fukuyama et al., the corresponding bromoacetamide was first made according to a procedure by Calorini et al (Scheme 4.11).

![Scheme 4.11 • Synthesis of α-amino acid bromoacetamides 381-385](image)

L-Phenylalanine methyl ester hydrochloride reacted with one equivalent of triethylamine at room temperature to release the free amine in situ before the flask was cooled to -10 °C for the addition of one equivalent of bromoacetyl bromide and a further one equivalent of triethylamine to react with the HBr produced in the reaction. Gratifyingly, \( N\)-(bromoacetamido)-L-phenylalanine methyl ester was obtained in an excellent 94% yield and could be used in the next step without further purification. These reaction conditions were applied to a further five \( \alpha\)-amino acids, affording compounds in yields of 76-91%. \( \alpha\)-Amino acid bromoacetamides, and their physicochemical properties (\( ^1\)H-NMR, FT-IR, and MS) were consistent with those reported previously.

The conversion of bromoacetamide to \( N\)-(diazoacetamido)-L-phenylalanine methyl ester proceeded smoothly at 0 °C in a 60% yield (Scheme 4.12). The product was isolated as a bright yellow solid, and the physicochemical data was consistent with that published for \( rac\)-386 reported previously by Lowe et al. Diazopeptides were also synthesised by this method in moderate yields of 57-79%.
The striking yellow colour is common to compounds containing the diazo functional group, which also has a characteristic peak of strong intensity known as the “diazoband” at around 2106 cm\(^{-1}\) in the FT-IR spectrum (Figure 4.3). This has been attributed to the effect of the \(-\text{C}=\text{N}=\text{N}\) cumulative double bond, as the stretching frequency of diazomethane in the gas phase is 3712 cm\(^{-1}\). Figure 4.3 also shows the ester and amide carbonyl stretch of diazopeptide 390 at 1738.6 and 1621.2 cm\(^{-1}\) respectively.

**Figure 4.3 • FT-IR (thin film) spectrum of compound 390**
In the case of $N$-(diazoacetamido)-$(S)$-phenylalanine methyl ester 386, which was reported previously by Lowe et al., the specific rotation is comparable to the literature value: $[\alpha]_D^{21} +175.0$ (c 1.0, CHCl$_3$) cf. $[\alpha]_D^{20} +186.0$ (c 1.0, CHCl$_3$).\textsuperscript{221}

Unfortunately, the Fukuyama diazoacetate synthesis described above could not be applied successfully to the synthesis of diazopeptide with side-chains bearing more reactive functional groups such as $N$-(diazoacetamido)-$(S)$-methionine methyl ester 393 (Scheme 4.13). A $^1$H-NMR of the impure bromoacetamide 392 was obtained which appeared to show a successful reaction between (S)-methionine methyl ester hydrochloride 391 and bromoacetyl bromide to afford 392 as the major product.\textsuperscript{1} However, this compound proved to be very unstable and could not be fully characterised. Consequently, an impure sample of 392 was submitted to the Fukuyama reaction conditions ($N,N'$-ditosylhydrazine / DBU) immediately after workup. The impure $^1$H-NMR of diazopeptide 393 contained no peaks that could be identified as corresponding either to the starting material 392 or the product 393. In order to obtain a diazopeptide with a methionine side-chain such as 393, an alternative method of synthesis was investigated, which is described in section 4.2.2.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {391};
\node (b) at (2,0) {392 (not isolated)};
\node (c) at (4,0) {393};
\draw[->] (a) -- (b) node[midway,above] {ET$_3$N Br$_2$CO Br$_2$};
\draw[->] (b) -- (c) node[midway,above] {Ta(NH)NHTs DBU} node[midway,below] {THF 0 $^\circ$C, 30 min} node[midway,above] {45 min DCM -10 $^\circ$C - r.t.};
\end{tikzpicture}
\end{center}

**Scheme 4.13 • Attempted synthesis of $N$-(diazoacetamido)-$(S)$-methionine methyl ester 393**

### 4.2.2 Synthesis of $\alpha$-amino acid diazopeptides using succinimidyl diazooacetate

Succinimidyl diazoacetate 396 was first synthesised in 1993 by Badet et al. as a convenient and versatile reagent for diazoacylation.\textsuperscript{222} The Steiglich esterification of $N$-hydrosuccinimide 394 with glyoxyllic acid tosylhydrazine 395 using DCC in 1,4-dioxane afforded succinimidyl diazoacetate 396 in a reported yield of 65%. Compound 396 reacted with a range of aliphatic and aromatic amine nucleophiles, displacing $N$-hydroxysuccinimide to afford the corresponding $N$-diazopeptides in yields of 30-98%. A

\textsuperscript{1} Attempted synthesis of 393 (Scheme 4.13) carried out by Miss Haseena Seylani, BSc Chemistry final year dissertation (University of East Anglia)
number of novel $N$-diazopeptides were reported, including $N$-diazoacetamido-(S)-phenylalanine benzyol ester 397.

![Chemical structures and reactions](image)

**Scheme 4.14** • Synthesis of succinimidyl diazoacetate 396 and $N$-(diazoacetamido)-(S)-phenylalanine benzyol ester 397 by Badet *et al.*

The yield of succinimidyl diazoacetate 396 reported by Badet proved challenging to reproduce on a large (5-10 g) scale, partly due to the difficulty of removing unreacted DCC and the byproduct DCU from the product by column chromatography on silica gel. A 47% yield of 396 was the highest achieved. The reaction of compound 396 with (S)-methionine methyl ester hydrochloride 391 was carried out in THF at room temperature with three equivalents of Hünig’s base (ethyl diisopropylamine or DIPEA), to afford 393 in 50% yield (Scheme 4.15).

![Chemical structures and reactions](image)

**Scheme 4.15** • Synthesis of amino acid diazoacetamides 393, 386, 387 and 398-402 using succinimidyl diazoacetate 396
This method was used to synthesise novel \( N \)-(diazoacetamido)-\( \alpha \)-amino acids 238-240 in moderate to good yields of 65-75%. The \( \beta \)-amino acid diazoacetamide 237 was obtained in a 61% yield from the commercially available free amine \textit{tert}-butyl (3S)-3-amino-3-phenylpropanoate.

The poor yield of \( N \)-(diazoacetamido)-(\( R,S \))-phenylalanine benzyl ester 402 can be explained by problems with purification. Overall the reaction was consistently reliable, with \( \alpha \)-amino acid diazopeptides 386 and 387 obtained in similar yields to those observed using the \( N,N' \)-ditosylhydrazine method described in Section 3.2.2. The disadvantages of the succinimidyldiazooacetate (396) route are the long reaction times (<24 hours) and the low yield of the 396 itself, which invariably required more than one purification by column chromatography following the DCC-mediated esterification (Scheme 4.14).

4.2.3 Asymmetric organocatalytic aza-Darzens aziridination of non-chiral \( N \)-aryl imines with \( \alpha \)-amino acid diazopeptides

\( N \)-Benzylidene-4-methoxyaniline 405 was chosen as the achiral imine substrate for our initial investigations into the asymmetric synthesis of peptide aziridines (Scheme 4.17). Compound 405 has previously been found by the Bew group to react successfully with ethyl diazoacetate 144 to afford a \textit{cis}-aziridine.\textsuperscript{223} Mayer \textit{et al.} have established that the \( p \)-methoxyphenyl nitrogen protecting group can be removed with relative ease using cerium(IV) ammonium nitrate (CAN) (Scheme 4.16).\textsuperscript{224}

\[ \text{Scheme 4.16 • CAN-mediated cleavage of the } N-p \text{-methoxyphenyl group from aziridine 242 reported by Mayer et al.} \]

Employing similar conditions to the aza-Darzens reactions described in Section 3.1, imine 405 was cooled to 0 C in an ice bath before the addition of 10 mol% pyridinium triflate 347 and 1.2 equivalents of \( N \)-(diazoacetamido)-(\( S \))-phenylalanine methyl ester 386 (Scheme 4.17). The sealed reaction vial was allowed to warm slowly to room temperature over 16 hours.
Scheme 4.17 • Pyridinium triflate-catalysed aza-Darzens synthesis of cis-aziridine 406a

The $^1$H-NMR of the impure reaction mixture shows poor conversion of imine 405 to aziridine 406a (Figure 4.4). There is also a singlet peak at 10 ppm consistent with the –CHO proton of benzaldehyde, indicating partial hydrolysis of the imine during the reaction. Overall the reaction mixture contains benzaldehyde, imine 405 and aziridine 406a in a ratio of approximately 1.5:2.5:1, which is consistent with the 23% isolated yield of 406a.

Figure 4.4 • Partial $^1$H-NMR of cis-aziridine 406a (blue) overlaid with the impure reaction mixture (red)

Absolute stereochemistry of 406a inferred from x-ray crystal structure, Figure 4.5
The $^1$H-NMR of compound 406a has aziridine C-H doublet peaks at 3.57 ppm and 3.05 ppm with coupling constants of 7.0 Hz, which is characteristic of a cis-aziridine. N-(Diazoacetamido)-(S)-phenylalanine methyl ester 386 is absent from the $^1$H-NMR of the impure reaction, which would prevent further conversion of imine 405 to product 406a. It is likely that compound 386 decomposed during the course of the reaction; diazo compounds are known to be both light and acid sensitive. In the synthesis of 406a it may be that the rate of decomposition of 386 is faster than the rate of aziridination.

A small sample of aziridine 406a crystallised in the eluent of the column chromatography during purification (petroleum ether / diethyl ether 1:1). X-ray crystallographic analysis revealed that compound 406a crystallised in the centrosymmetric spacegroup triclinic P-1, indicating a racemic mixture of the cis-enantiomers (R,R,S) and (S,S,R) (Figure 4.5).

![Figure 4.5 • X-ray crystal structure of racemic cis-aziridine 406a](image)

The diazopeptide substrate 386 was synthesised with the S configuration (as supported by the specific rotation), but the ee was not confirmed by chiral HPLC, so either a small amount of the R configuration was present in the sample, or some racemisation of 386 occurred during the reaction. The two or three crystals formed during column chromatography may not be representative of the whole sample, as opposite enantiomers can have a greater affinity for each other than the same enantiomer, resulting in a small
amount of the “true racemate” crystals. Taking this into account, from the crystal structure of 406a we can still infer that for the 1,5-induction there is a mismatch in the stereochemical outcome of the aziridination reaction: an $S$ configuration in the diazopeptide substrate leads to an $R,R$ configuration in the aziridine ring.

A second reaction was attempted under the same conditions using $N$-((diazoacetamido)-(S)-methionine methyl ester 393 (Scheme 4.18). Again only a single cis-aziridine product was obtained, according to the $^1$H-NMR of the isolated product, 407a or 407b, which showed an aziridine C-H doublet coupling constant of 7.0 Hz. However, the yield was poor at 27% and the $^1$H-NMR of the impure reaction mixture did not conclusively confirm the diastereoselectivity of this reaction. No crystals of the aziridine product were obtained in this case. The cis-selectivity of the aziridination reactions in Scheme 4.17 and 4.18 is surprising in the context of similar research reported in the literature. It has been generally accepted that acid catalysed aza-Darzens reactions with a diazoacetate substrate afford a cis-aziridine as the major product, while diazoacetamides generally afford a trans aziridine.

In 2008 Maruoka et al. reported the use of BINOL-based dicarboxylic acid catalyst ($R$)-410 for the trans-selective asymmetric aziridination of phenyl diazoacetamide 409 and N-Boc imine 408 (Scheme 4.19). Aziridine ($R,S$)-411 was isolated in a 61% yield, with an excellent 97% ee determined by chiral HPLC and a trans:cis ratio of >20:1. Similarly, Zhong et al. used BINOL-phosphoric acid catalyst ($R$)-414 for the synthesis of $p$-methoxyphenyl aziridine ($R,S$)-415 with a trans:cis ratio of >50:1.
Scheme 4.19 • BINOL-derived Bronsted acid-catalysed aza-Darzens aziridinations reported by Maruoka et al. (top) and Zhong et al. (bottom)

In order to make a comparison to the work by Maruoka and Zhong, N-Boc imine 408iii was reacted with N-(diazoacetamido)-(S)-phenylalanine methyl ester 386 by the addition of 10 mol% of pyridinium triflate 347 (Scheme 4.20). Contrary to the outcome of the reactions using p-anisidine imine 405, trans-aziridine 416 (a or b) was obtained in an isolated yield of 72% as the only product observed by 1H-NMR of the impure reaction mixture (Scheme 4.20).

Scheme 4.20 • Pyridinium triflate-catalysed trans-selective aza-Darzens aziridination between N-Boc imine 408 and diazopeptide 386

iii Kindly provided by postgraduate research student Mr Victor Zdorichenko, University of East Anglia.
Thus replacing the \( p \)-methoxyphenyl group on the imine with a Boc group resulted in a switch from \( cis \) selectivity to \( trans \) selectivity, showing that the nitrogen protecting group significantly influences the reaction. An explanation for the formation of a \( trans \) aziridine is presented in Figure 4.6. For the \( trans \) isomer (right), the approach of the diazoacetamide is influenced by hydrogen bonding between the amide \( –NH– \) and the carbonyl of the Boc group on the imine, affording intermediate 418 which is free from steric repulsion. Conversely reaction transition state 417 for the \( cis \) isomer (left) is made unfavourable by steric repulsion between the amide and the phenyl group. A single set of peaks corresponding to a \( trans \) aziridine was observed in the crude 1H-NMR of 416, indicating that there is excellent 1,5-stereocontrol in this reaction, although the absolute stereochemistry of 416 (either \( a \) or \( b \)) cannot be assigned without further investigation.

**Figure 4.6** Possible explanation for the \( trans \) selectivity of the reaction between \( N \)-Boc imine 408 and \( N \)-(diazoacetamido)-(S)-phenylalanine 386.
4.2.4 One-pot asymmetric organocatalytic aza-Darzens reaction of peptide imines with diazopeptides

Peptide aziridines have attracted particular interest over the last 15 years as protease enzyme inhibitors. The natural product miraziridine A, for example, is a cysteine protease inhibitor isolated from a marine sponge found in the Japanese archipelago (Figure 4.7).

![Figure 4.7 • Molecular structure of miraziridine A](image)

Recent work by the Yudin group has highlighted the use of aziridines in peptide ligation and macrocyclisation. The chemical synthesis of peptide aziridines reported in the literature largely relies on peptide coupling reagents to bring together simple carboxylate aziridines and amino acids or peptide chains. For example, T. Schirmeister et al. reported the synthesis of cis-aziridine 424 as a potential inhibitor of the secreted aspartic protease 2 (SAP2) of Candida albicans (Scheme 4.21).

![Scheme 4.21 • Synthesis of peptide aziridine cis-424 reported by Schirmeister et al.](image)
Racemic aziridine 421 was synthesised from *trans*-420 as a mixture of two enantiomers (*R,R*) and (*S,S*). Hydrolysis of the ethyl ester of 421 afforded carboxylic acid 422 ready for DPPA coupling with dipeptide 423. The resulting mixture of two diastereomers of *cis*-424 could then be separated by a combination of column chromatography and preparative HPLC.

Following the initial work on the synthesis of α-amino acid derived *cis*-aziridines by an aza-Darzens route presented in section 3.3.4, it was decided that to be practical and useful this route needed improved flexibility and higher yields. To this end, the *N*-aryl group would be replaced by an α-amino acid, and functional groups introduced on the aldehyde substrate (Scheme 4.22). This approach was designed to install three directions of functionality, with the aziridine ring as the “connector” scaffold.

**Scheme 4.22** • Summary of the one-pot asymmetric aza-Darzens synthesis of peptide aziridines

The route illustrated in Scheme 4.22 offers considerable advantages over the conventional stepwise approach outlined in Scheme 4.21; there are significantly fewer steps required, and crucially the chirality of the α-amino acid substrates were anticipated to influence the stereochemistry of the aziridine formation by both 1,3- and possibly 1,5-asymmetric induction.

**Scheme 4.23** • Synthesis of α-amino acid imine 425 reported by Spring *et al.*
The imine condensation reaction between benzaldehyde 228 and (S)-phenylalanine methyl ester hydrochloride 380 was reported previously by Spring et al. (Scheme 4.23).\textsuperscript{233} This reaction was successfully reproduced, and imine 425 was obtained in a 90% yield. The optical rotation of 425, [\(\alpha\)]\textsubscript{D}\textsuperscript{25} -198.5 (c 0.92, CHCl\textsubscript{3}), was close to the literature value of [\(\alpha\)]\textsubscript{D}\textsuperscript{25} -206.7 (c 0.92, CHCl\textsubscript{3}). However, despite being stored at low temperature, the rotation was found to have dropped to [\(\alpha\)]\textsubscript{D}\textsuperscript{25} -11.2 (c 1.0, CHCl\textsubscript{3}) after 24 hours, possibly due to isomerism of the imine. It was important to avoid racemisation of 425 prior to the aziridination step, so the synthesis of 425 was carried out in situ from benzaldehyde 228 and (S)-phenylalanine methyl ester 426 (Scheme 4.24). After one hour, 10 mol% of pyridinium triflate 347 and 1.2 equivalents of \(N\)-(diazoacetamido)-(S)-phenylalanine methyl ester 386 were added to the vial at 0 °C.

![Scheme 4.24](image)

**Scheme 4.24** Synthesis of peptide aziridine cis-427\textsuperscript{iv} via a one-pot pyridinium triflate-catalysed aza-Darzens reaction

Gratifyingly, the \(^1\)H-NMR of the impure reaction mixture after 16 hours showed complete consumption of benzaldehyde 228 and imine intermediate 425, with the exclusive formation of a single cis-aziridine product 427 (where the aziridine doublet coupling constant \(J = 7.2\) Hz) which was isolated in an excellent 91% yield. The same reaction conditions were applied to the synthesis of a further three peptide aziridines 428-430 which bear \(\alpha\)-amino acid side-chains including \(N\)-Cbz-(S)-tryptophan, (S)-methionine, (S)-leucine and O-allyl-(S)-tyrosine (Figure 4.8). In each case, a single cis aziridine diastereomer was observed in the \(^1\)H-NMR of the crude reaction mixture, where the absolute stereochemistry has not yet been determined.

\textsuperscript{iv} Absolute stereochemistry established in Section 4.2.7
Aziridine 429, featuring allyl ester and allyl amide functional groups, was designed as a potential precursor for transition metal-catalysed ring-closing metathesis to form a peptide macrocycle.

To achieve the “three directional” functionality described in Scheme 4.22, benzaldehyde 228 was replaced by commercial available aldehydes 4-trifluoromethylbenzaldehyde 431 and fluorene-2-carboxaldehyde 434 (Scheme 4.25). The incorporation of trifluoromethyl groups into peptides as a labelling technique in $^{19}$F-NMR is a key tool in structural biology. Fluorene derivatives have been employed as fluorescent probes in two photon fluorescence microscopy.

Scheme 4.25 • Synthesis of CF$_3$-bearing peptide aziridine cis-433a/b via a pyridinium triflate-catalysed aza-Darzens methodology
Scheme 4.26 • Synthesis of fluorene-based peptide aziridine cis-436a/b via a pyridinium triflate-catalysed aza-Darzens methodology

CF₃-bearing cis-aziridine 433 was obtained in a disappointing 49% yield, indicating that this methodology may be less able to tolerate strongly electron withdrawing groups on the aldehyde substrate. The one-pot imine condensation / aziridination of fluorene-2-carboxaldehyde 434, by contrast, afforded cis-aziridine 436 in a 79% yield after purification.

Ferrocenecarboxaldehyde 438 (Scheme 4.26) was identified as a potentially useful substrate for the aza-Darzens reaction due to the redox activity of ferrocene in biochemical processes.²³⁶ For example, ferrocene-peptide conjugates have been used in the electrochemical probing of HIV enzymes.²³⁷ Aldehyde 276 has been previously reported to react successfully with amines to form stable Schiff base ligands for transition metal complexes.²³⁸ Indeed ¹H-NMR analysis of intermediate 277 contained a singlet peak at 8.16 ppm consistent with the imine –CH=N- proton. However, on the addition of catalyst 347 and N-(diazooacetamido)-(S)-leucine methyl ester 387 there was no further reaction observed. This may be due to stabilisation of the partial positive charge of the α-carbon attached to the Cp ring by the iron(II) centre – a recognised property of substituted ferrocene compounds.²³⁹ In order to obtain a ferrocene-based peptide aziridine, therefore, a spacer was required between the Cp and the aziridine ring.
Imrie et al. synthesised 4-(ferrocenyl)benzaldehyde \(443\) by a Suzuki coupling reaction between iodoferrocene \(441\) and 4-formylphenylboronic acid \(442\) (Scheme 4.27).\(^{240}\) However, the yield of this reaction was just 13\%, and iodoferrocene \(441\) is not commercially available; it had to be synthesised in 2 steps from ferrocene in an overall 31\% yield.

**Scheme 4.27 • Synthesis of 4-(ferrocenyl)benzaldehyde 443 reported by Imrie et al.**

In order to obtain 4-(ferrocenyl)benzaldehyde \(443\) in a reasonable quantity, an alternative synthesis was designed in which methyl-4-aminobenzoate first reacted with sodium nitrite in a solution of aqueous hydrochloric acid to afford diazonium salt \(444\) *in situ* (Scheme 4.28). The addition of ferrocene \(445\) afforded methyl 4-(ferrocenyl)benzoate \(446\) as a deep red solid in a 37\% yield. Subsequent DIBAL-H reduction of the ester group of \(446\) to alcohol \(447\) proceeded in an excellent 90\% yield, and a manganese(IV) oxide oxidation of \(447\) afforded the desired aldehyde \(443\) in an 86\% yield.
Unlike ferrocenecarboxaldehyde 438, 4-(ferroceny1)benzaldehyde 443 formed an imine with (S)-phenylalanine methyl ester 426 and then reacted successfully with \(N\)-(diazoacetamido)-(S)-leucine methyl ester 387 in the presence of 10 mol% of pyridinium triflate 347 to afford cis-aziridine 440 in a 73% yield.

**Scheme 4.28** • Four step synthesis of ferrocene-based peptide aziridine 440 from ferrocene 445 and diazoester 444

### 4.2.5 Asymmetric synthesis of \(N\)-\(\alpha\)-methylbenzyl peptide aziridine 455

The \(\alpha\)-methylbenzyl group is a highly useful nitrogen protecting group; it has found numerous applications in remote chiral induction, \(^{241}\) for example in the synthesis of isoquinoline alkaloids by Czarnocki et al. \(^{242}\) After use as a chiral auxiliary the group can be subsequently cleaved under Pd/C hydrogenation conditions. \(^{243}\) The asymmetric synthesis of aziridines bearing an (R)- or (S)-\(N\)-\(\alpha\)-methylbenzyl group has been reported by the groups of Wulff and Lee (Scheme 4.29). Lee et al. investigated the Lewis acid-catalysed aza-Darzens reaction of ethyl diazoacetate 144 with a series of (S)-\(N\)-\(\alpha\)-methylbenzyl imines using a combination of 20 mol% cobalt chloride and 40 mol% silver tetrafluoroborate. \(^{244}\)
Cis diastereomers 449 and 450 were obtained in yields of up to 65%, however the diastereomeric ratio was generally poor (≤ 27:73 in favour of 450). Wulff et al. employed a double stereodifferentiation approach, using the chiral (R)-VANOL boroxinate catalyst for the reaction between (R)-N-α-methylbenzyl imine 451 and phenyl diazoacetamide 409. They reported a trans/cis ratio of 96:4 by this method, and ratio of the two trans diastereomers 452 and 453 of 95:5 (diastereomeric ratios obtained from the \(^1\)H-NMR spectrum of the impure reaction mixture).

The pyridinium triflate-catalysed aza-Darzens reaction described in section 4.3.5 was applied to the synthesis of N-α-methylbenzyl aziridine 451 as a comparison to the work by Wulff et al. In the one-pot protocol (Scheme 4.30), benzaldehyde 228 reacted with (R)-α-methylbenzyl amine 454 to form imine intermediate 451 by a condensation reaction. Following the addition of 10 mol% of achiral catalyst 347 and 1.2 equivalents of N-(diazoacetamido)-(S)-leucine methyl ester 387, \(^1\)H-NMR analysis of the impure reaction mixture after 16 hours (Figure 4.9) showed the formation of a single major cis-aziridine product 455, which was isolated in a 90% yield.

**Scheme 4.29** • Synthesis of aziridines bearing the N-α-methylbenzyl protecting group reported by Lee et al., 2005, and Wulff et al., 2012.
Scheme 4.30 • One-pot pyridinium triflate-catalysed synthesis of N-α-methylbenzyl aziridine 455 (a or b)

Figure 4.9 • Partial $^1$H-NMR of the impure reaction mixture (Scheme 4.30)

Figure 4.9 shows the presence of two further doublets in the aziridine ring C-H region, with similar coupling constants to that of 455 ($J = 7.1$ Hz). It is presumed that the doublet at 2.44 ppm (far right) represents a diastereomer of 455, giving a $dr$ of 95:5. The starting material for this reaction, (R)-α-methylbenzyl amine 454, was purchased at reagent grade, with an ee of 96% by GLC (see Experimental section for details). This may account for the presence of a third diastereomer 456 indicated by the doublet at 2.46 ppm, and the peak integration of 0.04 (4% of the reaction products) supports this theory.

Employing the remote chiral induction protocol in this way removes the need for expensive catalysts and introduces more functionality into the molecule in a single step than the reactions shown in Scheme 4.29. Isolated as a single isomer, 455 is potentially a direct precursor to a range of natural and unnatural, nitrogen-protected, non-racemic dipeptides accessible via nucleophilic ring opening of the cis-aziridine moiety.
4.2.6 Investigating the effect of temperature and chiral distance on stereoselectivity

The peptide aziridines described in Section 4.2.5 contain two α-amino acid chiral centres – the first directly connected to the nitrogen of the aziridine ring, and the second separated from the aziridine ring by a single amide bond. In order to establish the degree of influence of each chiral centre on the stereochemistry of the aziridine ring being formed, a comparison was made between aziridines 457 and 459, which feature the achiral α-aminoisobutyric acid (Aib) amino acid (Scheme 4.31).

Scheme 4.31 • Pyridinium triflate-catalysed aza-Darzens synthesis of aziridines 457 and 459

The synthesis of cis-457 (where the aziridine doublet coupling constant $J = 7.2$ Hz) was carried out at 0 °C using the protocol described previously (Section 4.3.5). The solvent was changed from chloroform to chloroform-$d_3$ to make monitoring the reaction by $^1$H-NMR more convenient. The reaction was complete after 10 hours, and a single aziridine product 457 was observed in the $^1$H-NMR of the impure reaction mixture, which was isolated in an 80% yield.

In the synthesis of cis-459, the formation of a major aziridine product was observed, with a set of doublets at 2.78 ppm ($J = 6.9$ Hz) and 3.27 ppm ($J = 6.9$ Hz) corresponding to the aziridine C-H protons. However, a second minor set of doublets was observed with an identical coupling constant of 6.9 Hz. It is presumed that these peaks correspond to the
second diastereomer of 459. A temperature study showed that the ratio of the two diastereomers was influenced by the reaction temperature (Figure 4.10).

When the reaction was carried out at room temperature (25 °C), the ratio of major:minor diastereomers (measured by integration of the C-H doublets in the 1H-NMR) was 47.6:1, increasing to 60.1:1 when a 0 °C chiller bath was used. The optimum temperature for the synthesis of 459 was -30 °C, achieving a diastereomeric ratio of 94.4:1. Below this temperature, precipitation of N-(diazoacetamido)-(S)-phenylalanine methyl ester 386 was observed and the reaction failed.

The findings from the synthesis of 457 and 459 are consistent with the expectation in remote chiral induction reactions that the closer the chiral centre of the starting material to the chiral centre formed during the reaction, the stronger the influence, and the higher the stereoselectivity.

Figure 4.10 • Partial 1H-NMR of the impure reaction mixture in the synthesis of cis-aziridine 459 carried out at 25 °C, 0 °C, and -30 °C

Figure 4.11 • Comparison of the distance between the two key chiral centres of aziridines 457, 459 and 460
Thus in aziridine 457, the (S)-phenylalanine chiral centre is two bonds from the first aziridine chiral centre formed during the aza-Darzens reaction (Figure 4.11), and a single diastereomer product is observed. In the synthesis of compound 459, a 1,5-chiral induction takes place through four bonds, and selectivity is slightly lower, with a maximum \(dr\) of 94:1. It was hoped that inserting an Aib unit between the phenylalanine chiral centre and the aziridine ring would still result in a degree of stereoselectivity. This would be a 1,8 chiral induction (compound 460, Figure 4.11). To this end, diazodipeptide 464 was synthesised from Boc-Aib-(S)-phenylalanine methyl ester 461 in three steps with an overall yield of 36% (Scheme 4.32).

Scheme 4.32 • Synthesis of diazoacetamide dipeptide 464

Compound 464 was found to be poorly soluble in chloroform at low temperature, so for the one-pot aziridination step (Scheme 4.33) the reaction was cooled to -30 °C for the addition of 464 using a dry ice bath, which was allowed to warm slowly to room temperature overnight. Two cis-aziridine diastereomers were identified in the \(^1\)H-NMR of the impure reaction mixture and were subsequently separated by column chromatography (Figure 4.12). The \(dr\) based on \(^1\)H-NMR integration of the aziridine C-H doublets and the isolated yields were in close agreement at 2:1.
It is clear that despite the conformationally restricted nature of the Aib unit, the 1,8 chiral induction was much less successful in terms of stereoselectivity than the 1,5- and 1,3-chiral induction reactions. Further work to increase the 2:1 ratio obtained in Scheme 4.33 may include changing the Aib unit for a cyclohexyl group or a dehydro amino acid, as
sections of fixed planarity and restricted rotation are known to relay chiral information over longer distances.²⁴⁶

### 4.2.7 Assigning the absolute stereochemistry of cis-aziridine 427a

The peptide aziridines described in Section 4.3.5 were isolated and characterised as single cis diastereoisomers. However, it was not possible to assign the absolute stereochemistry of the aziridine ring in these compounds without further investigation. An alternative method of synthesis was required in which these unknown chiral centres would be derived from known enantiomerically pure starting materials. To simplify this process, the cis-aziridine would first be submitted to palladium(0)-catalysed hydrogenation, affording a pseudopeptide with just three chiral centres; a more convenient target for synthesis from commercially available amino acid starting materials (Scheme 4.34). Thus palladium on carbon (10 mol%) was added to a solution of aziridine 427a in ethanol and the reaction pressurised to 50 psi with hydrogen in a Biotage Endeavour system to afford compound 466 in a 96% yield.

Scheme 4.34 • Hydrogenation of the aziridine ring of 427 catalysed by palladium on carbon

A retrosynthetic summary of the alternative route to \((S,R,S)-466\) is presented in Scheme 4.35. Peptide coupling of O-acetyl-(S)-phenyllactic acid 467 with (S)-phenylalanine methyl ester 426 affords compound 468, which can be deprotected under basic conditions to afford alcohol 469. The corresponding triflate 470 will then be displaced by (S)-phenylalanine methyl ester 426 via an S_N2 mechanism to afford compound 466.
Scheme 4.35 • Retrosynthetic analysis of the synthesis of pseudopeptide \((S,R,S)\)-466

O-Acetyl-(S)-phenyllactic acid 467 was prepared from (S)-phenylalanine 471 according to a procedure by Plenkiewicz \textit{et al.}\textsuperscript{247} a reaction in which the (S) stereochemistry is retained in the product (Scheme 4.36). Subsequent coupling of 467 with (S)-phenylalanine methyl ester hydrochloride was achieved using TBTU, affording compound 468 in a 38% yield.

Scheme 4.36 • Synthesis of (S)-methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-3-phenylpropanoate 468 from (S)-phenylalanine 471

Total yield 468 + 473 = 75%

Scheme 4.37 • Synthesis of 468 and 473 as a mixture of two diastereomers: (S,S) and (R,S)
These reactions were repeated using rac-phenylalanine as the initial starting material to obtain diastereomers 468 and 473 (Scheme 4.37) from O-acetyl-(R,S)-phenyllactic acid 472. Compounds 468 and 473 could not be separated by column chromatography and were used in the next step of the synthesis as a 1:1 mixture of the two diastereomers.

The TBTU-mediated coupling steps in Scheme 4.36 and 4.37 were repeated with the hydrochloride salts of (S)-methionine methyl ester, (S)-leucine methyl ester and α-aminoisobutyric acid methyl ester to afford compounds 474-477 (Figure 4.13), with the intention of assigning the stereochemistry of a further range of peptide aziridines. In the synthesis of 476, a minor diastereomer 477 was also formed, in a ratio of 8:1 (476:477). This indicates that the O-Acetyl-(S)-phenyllactic acid 467 was not enantiomerically pure, and diastereomers 476 and 477 proved impossible to separate by flash column chromatography.

Figure 4.13 • Compounds 474-477 synthesised by TBTU-mediated coupling illustrated by Scheme 4.37

Hydrolysis of the acetyl protecting group of compound 468 was achieved under basic conditions using 0.25 equivalents of potassium carbonate in aqueous methanol, following a procedure by Yang et al.\textsuperscript{248} Complete consumption of the starting material was observed by thin layer chromatography after one hour, however the moderate 57% yield of the reaction indicates that some material may be lost by hydrolysis of the methyl ester.

Scheme 4.38 • Base hydrolysis of acetate 468 to afford alcohol 469
The acetate hydrolysis reaction was carried out on compounds 474-476, affording peptide alcohols 478-480 as a single (S,S) diastereomer (Figure 4.14). Racemic mixtures of (R,S) and (S,S) acetate diastereomers were hydrolysed by the method shown in Scheme 4.36 to afford the corresponding alcohols, which could be separated easily by column chromatography on silica gel (Scheme 4.37).

**Figure 4.14** • Alcohols 478-480 synthesised by base hydrolysis of acetates 474-476

**Scheme 4.39** • Hydrolysis of racemic acetates to afford separable (R,S) and (S,S) diastereomers 469, 479 and 480–483
Scheme 4.40 • Synthesis of methyl iminodiacetic derivative 486 reported by Langlois et al.

In their 2008 publication on the synthesis of iminodiacetic acid derivatives as potential inhibitors of HIV-1 aspartyl protease, Langlois et al. reported the reaction between triflate 485 and (S)-phenylalanine methyl ester 426 to afford compound 486 in a 42% yield (Scheme 4.40). This route was applied to the synthesis of peptides 305 and 328 (Scheme 4.41). It was found that triflates 488 and 490 were highly unstable and had to be used immediately in the second step without purification. Despite careful handling, and the low reaction temperature of -78 °C, compounds 489 and 466 were obtained in low yields of 19% and 24% respectively. Fortunately, for the purposes of assigning absolute stereochemistry only pure samples of 489 and 466 were required for characterisation, and the yield was of less importance.

Scheme 4.41 • Synthesis of pseudopeptide diastereomers 489 and 466 from phenyllactic acid derivatives 487 and 469
Figure 4.15 • Partial 1H-NMR of the crude reaction mixture of hydrogenation of aziridine 427a (top), pure diastereomer 466 (middle) and pure diastereomer 489 (bottom)
The impure reaction mixture of the aza-Darzens reaction for the synthesis of aziridine 427a was submitted for hydrogenation by palladium on carbon in a vial pressurised with hydrogen (50 psi). Purification of 427a by column chromatography was avoided to prevent enrichment of the diastereomeric ratio. The $^1$H-NMR spectrum of the impure hydrogenation reaction mixture was compared with the spectra of pure diastereomers 466 and 489 (Figure 4.15), and there is a clear match to (S,R,S) diastereomer 466. The (S,S,S) diastereomer 489 is not visible in the $^1$H-NMR of the impure reaction mixture, indicating that the diastereoselectivity of the aziridination reaction is >99%. Cis-aziridine 427a therefore has (R,R) stereochemistry at the chiral centres of the three-membered ring. A proposed mechanistic rationale for the formation of 427a as the only product is presented in Figure 4.16.

Figure 4.16 • Mechanistic rationale for the selective synthesis of cis-(S,R,R,S) diastereomer 427a over cis-(S,S,S,S) 427b

In Figure 4.16a (above), the N-diazopeptide is shown approaching the bottom face of the imine, in a suitable orientation for the addition of the diazo group to the protonated imine.
(as discussed in Section 4.1). In this proposed orientation of the two substrates (ii), the phenyl rings of the amino acid side chains are directed away from each other, minimising unfavourable steric interactions. Conversely, the approach of the diazopeptide to the top face of the imine in representations (iii) and (iv) brings the benzyl side chains into close proximity, leading to unfavourable steric interactions which prevent the formation of diastereomer 427b.

4.3 Conclusions

The peptide aziridines described in this chapter were prepared by a previously unreported Bronsted acid-catalysed remote asymmetric induction methodology, achieving a 2:1 diastereomeric ratio in the 1,8-asymmetric induction which increased to 94:1 in the 1,5-induction, and >99:1 in the 1,3-induction, where only a single stereoisomer of the product was observed. The absolute stereochemistry of cis-aziridine 427 was established by hydrogenation of the three-membered ring to afford pseudopeptide 466. It was revealed that the natural (S)-stereochemistry of the phenylalanine imine and diazoacetamide starting materials influenced the formation of the (R,R) stereochemistry in the aziridine product. This methodology therefore provides access not only to the synthesis of simple (R,S)-dipeptides, but also to the incorporation of unnatural, functionalised (R)-amino acid residues into an existing peptide chain, with potential applications in synthesis of the artificial cell structures discussed in Chapter 2.
4.4 Future work

Taking the conclusions from the work described in chapters 3 and 4, the next step would be to apply the aza-Darzens remote asymmetric induction methodology to the synthesis of a glycopeptide antibiotic analogue. A proposed strategy is presented in Scheme 4.42, in which the (S)-α-methylbenzylamine group is employed as both a chiral auxiliary for 1,3-stereocontrol in the aza-Darzens reaction, and an easily cleaved nitrogen protecting group.

Scheme 4.42 • Application of aza-Darzens remote asymmetric induction methodology to the synthesis of an orienticin C analogue

In order to further investigate the mechanism of the asymmetric aza-Darzens aziridination, it would be useful to establish the effect of different stereochemical configurations in the reaction substrates on the diastereoselectivity of the reaction. In the examples presented in Chapter 4, the (S)-amino acids were used exclusively to prepare both the imine and diazopeptide starting materials. This led to a mis-matched stereochemistry in the product, with cis-\( (R,R) \) configuration of the aziridine ring. Changing the configuration of the stereocentre in one or both of the substrates may produce the opposite diastereomer, or have a detrimental effect on the diastereoselectivity of the reaction, which may provide useful information about the competing or synergetic effect of combining 1,3- and 1,5- remote asymmetric induction in this context.
EXPERIMENTAL SECTION
General Information

Air and water-sensitive reactions were carried out under a nitrogen atmosphere and anhydrous conditions with magnetic stirring unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.2 mm), with visualization by UV light and/or potassium permanganate stain followed by heating. Flash column chromatography was performed using Davisil silica gel 60 (40-63 µm). ¹H- and ¹³C-NMR spectra were recorded on a Bruker Avance spectrometer 500 (500 MHz), an Oxford Gemini 400 spectrometer (400 MHz) or an Oxford Gemini 300 spectrometer (300 MHz). Chemical shifts are reported in δ (ppm) and referenced to residual solvent signals (¹H-NMR: CDCl₃ at 7.26 ppm, CD₃CN at 1.94 ppm; ¹³C-NMR: CDCl₃ at 77.16 ppm, CD₃CN at 1.32, 118.26 ppm). Signal multiplicities are described as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad. FT-IR spectra were recorded on a Perkin Elmer Spectrum 100 spectrometer as thin films on sodium chloride plates and are reported in wavenumbers (cm⁻¹). Optical rotations were measured using a Perkin Elmer 241 polarimeter with a 1 mL cell (path length 1 dm) and are reported as follows: [α]D⁻¹⁰°C concentration (c = g / 100 mL, solvent). MALDI-TOF mass spectra were recorded on a Shimadzu Axima-CFR spectrometer. High resolution mass spectrometry (HRMS) was carried out by the EPSRC National Mass Spectrometry Facility (Swansea, UK). Melting points of crystalline solids were recorded using a Stuart Scientific SMP1 apparatus (solvent of crystallization in brackets) and are uncorrected. Percentage yield refers to the isolated yield of analytically pure material unless otherwise stated. Dichloromethane was distilled from calcium hydride before use. Chloroform and d-chloroform were filtered through basic alumina and stored over 4Å molecular sieves. Tetrahydrofuran was distilled from sodium/benzophenone prior to use. All other solvents and reagents were purchased from commercial sources (Sigma-Aldrich, Alfa Aesar, Fluorochem and Fisher Scientific) and used as received.
Experimental Section

Methyl 3,5-bis(4-formylphenoxy)-4-hydroxybenzoate (305)

Synthesis A: Microwave irradiation

To a 15 mL microwave vial under a nitrogen atmosphere was added methyl 3,4,5-trihydroxybenzoate 281 (500 mg, 2.72 mmol, 1 equiv.), cesium carbonate (2.41 g, 7.40 mmol, 2.7 equiv.) and a magnetic stirrer bar. Dimethyl sulfoxide (7 mL) was added, and the resulting mixture stirred vigorously for 10 minutes at room temperature before the addition of 4-fluorobenzaldehyde (0.93 mL, 8.69 mmol, 3.2 equiv.) via a 2.5 mL syringe. The vial was sealed with a PTFE-lined aluminium crimp cap and the dark red suspension heated at 160 °C in a Biotage microwave reactor for three hours, after which the reaction mixture was allowed to cool to room temperature and poured into a 250 mL separating funnel containing 1 M aqueous hydrochloric acid (50 mL). The aqueous mixture was extracted with ethyl acetate (2 x 50 mL) and the combined organic extracts were washed with distilled water (3 x 50 mL) and then brine (50 mL). The solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo to afford a dark brown residue. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 9:1; Rf = 0.30) afforded a pale yellow solid (600 mg, 1.53 mmol, 56% yield) whose physicochemical characteristics were consistent with those of the title compound (305).

M.p. 161–162 °C; ^1H-NMR (300 MHz, CDCl3) δ = 9.93 (s, 2H, -CHO), 7.88 (d, J = 8.7 Hz, 4H, Ar-H), 7.62 (s, 2H, Ar-H), 7.13 (d, 4H, J = 8.8 Hz, Ar-H), 6.44 (s, 1H, -OH), 3.84 (s, 3H, -CO2CH3); ^13C-NMR (125 MHz, CDCl3) δ = 190.8 (-CHO), 165.5 (-CO2CH3), 161.8, 144.8, 143.1, 132.3(1), 132.2(7), 122.9, 119.1, 117.6, 52.6 (-CO2CH3); FT-IR (ATR): ν (cm^-1) = 3079 (O-H), 2846 (C-H), 1692 (ester C=O), 1669 (aldehyde C=O), 1578, 1502, 1423, 1341, 1194; MS (MALDI-TOF): m/z 414.82 [M+Na]^+; HRMS (APCI) exact mass calculated for C_{22}H_{17}O_{7} requires m/z 393.0969, found m/z 393.0965 [M+H]^+.
A byproduct was isolated during the column chromatography ($R_f = 0.60$; dichloromethane / methanol 2%); this was identified as methyl 3-(4-formylphenoxy)-4,5-dihydroxybenzoate 304 (165 mg, 0.57 mmol, 21% yield).

M.p. 191–193 °C (chloroform); $^1$H-NMR (300 MHz, CD$_3$OD) $\delta = 9.87$ (s, 1H, -CHO), 7.88 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.40 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.22 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.05 (d, $J = 8.8$ Hz, 2H, Ar-H), 3.83 (s, 3H, -CO$_2$CH$_3$); $^{13}$C-NMR (125 MHz, CD$_3$OD) $\delta = 192.8$ (-CHO), 168.0 (-CO$_2$CH$_3$), 164.7, 148.2, 144.3, 143.2, 133.0, 132.7, 122.2, 117.7, 116.0, 114.6, 52.5 (-CO$_2$CH$_3$); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3217 (O-H), 1686 (C=O), 1593, 1504, 1439, 1319, 1224, 1156; MS (MALDI-TOF) $m/z$ 311.12 [M+Na]$^+$; HRMS (NESI) exact mass calculated for C$_{15}$H$_{11}$O$_6$ requires $m/z$ 287.0561, found $m/z$ 287.0566 [M-H]$^-$. 

**Methyl 3,5-bis(4-formylphenoxy)-4-hydroxybenzoate (305)**

**Synthesis B: Conventional heating**

![Chemical structure](image)

To a 500 mL two-necked round-bottom flask was added methyl 3,4,5-trihydroxybenzoate 281 (9.93 g, 53.9 mmol, 1 equiv.) and a magnetic stirrer bar. The white solid was dissolved in dimethyl sulfoxide (160 mL) under a nitrogen atmosphere. Cesium carbonate (52.7 g, 162 mmol, 3 equiv.) was added in one portion and the resulting mixture stirred at room temperature for 15 minutes before the addition of 4-fluorobenzaldehyde (14.4 ml, 135 mmol, 2.5 equiv.) via a 25 mL syringe. The flask was fitted with a reflux condenser and the reaction mixture heated at 120 °C for 48 hours using an aluminium heating block mounted on a hotplate magnetic stirrer. When no further conversion from 281 to 305 was observed by thin layer chromatography (eluent: dichloromethane / ethyl acetate 9:1), the reaction mixture was cooled to 0 °C, quenched with 1 M aqueous hydrochloric acid (200 mL) and poured into a 2 L separating funnel. The aqueous mixture was extracted with ethyl acetate (2 x 200 mL) and the combined organic extracts were washed with distilled water (3 x 300 mL) and then brine (250 mL). The solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo to afford a dark brown solid.
Trituration with diethyl ether afforded a cream solid (8.89 g, 22.7 mmol, 42% yield) whose physicochemical properties were consistent with those of the title compound \(305\) described previously.

**Methyl 3,5-bis(4-formylphenoxy)-4-hydroxybenzoate (305)**

**Synthesis C: Flow reactor**

A Uniqsis Flow Syn reactor was fitted with a 6.6 x 100 mm glass column packed with cesium carbonate (5 g). The column was pre-heated to 150 °C. A solution of methyl 3,4,5-trihydroxybenzoate \(281\) (200 mg, 1.09 mmol, 1 equiv.) in dimethyl sulfoxide (2 mL), solution A, and a solution of 4-fluorobenzaldehyde \(282\) (0.4 mL, 2.78 mmol, 2.5 equiv.) in dimethyl sulfoxide (2 mL), solution B, were pumped simultaneously through the glass column, each with a flow rate of 1 mL / min. The dark red solution was collected in a 250 mL separating funnel containing 1 M hydrochloric acid (50 mL) at room temperature. The aqueous mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with distilled water (3 x 50 mL) and then brine (50 mL). The organic solution was dried over magnesium sulfate, filtered, and the solvent removed *in vacuo*. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 9:1) afforded \(305\) as a pale yellow solid (141 mg, 0.36 mmol, 33% yield) and \(304\) as a light brown solid (91 mg, 0.32 mmol, 29% yield). The physicochemical properties of these compounds matched the data obtained from the microwave-promoted synthesis outlined above.
Methyl 3-(4-formylphenoxy)-4,5-dihydroxybenzoate (304)

To a 15 mL microwave vial was added methyl 3,4,5-trihydroxybenzoate 281 (1 g, 5.43 mmol, 1 equiv.), cesium carbonate (3.89 g, 11.9 mmol, 2.2 equiv.) and a magnetic stirrer bar. The vial was purged with nitrogen, sealed with a PTFE-lined aluminium crimp cap and dimethyl sulfoxide (10 mL) was added via a 20 mL syringe. The resulting reaction mixture was stirred vigorously for 10 minutes at room temperature before the addition of 4-fluorobenzaldehyde (0.7 mL, 6.53 mmol, 1.2 equiv.) via a 2 mL syringe. The dark red suspension was heated to 120 °C in a Biotage microwave reactor for 90 minutes. The reaction mixture was allowed to cool to room temperature and poured into a 250 mL separating funnel containing 1 M aqueous hydrochloric acid (50 mL). The aqueous mixture was extracted with ethyl acetate (2 x 50 mL) and the combined organic extracts were washed with distilled water (2 x 100 mL) and then brine (100 mL). The resulting solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo to afford a dark brown residue. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 9:1) afforded a yellow solid (603 mg, 2.09 mmol, 39% yield) whose physicochemical properties matched those of the title compound (304). Byproduct 305 was isolated in a 7% yield (149 mg, 0.38 mmol); the physicochemical properties were as outlined previously.
Methyl 3,4-\textit{bis}(benzylxy)-5-(4-formylphenoxy)benzoate (320)

To a 250 mL round-bottom flask containing methyl 3-(4-formylphenoxy)-4,5-dihydroxybenzoate 304 (333 mg, 1.16 mmol, 1 equiv.) under a nitrogen atmosphere was added \textit{N},\textit{N}-dimethylformamide (35 mL) and a magnetic stirrer bar. Potassium carbonate (481 mg, 3.48 mmol, 3 equiv.) was added to the stirred orange solution in one portion, following by the addition of benzyl bromide (0.41 mL, 3.48 mmol, 3 equiv.) via a 1 mL syringe. The resulting suspension was heated to 120 °C for 7 hours. After cooling to room temperature, distilled water (30 mL) was added, followed by 1 M aqueous hydrochloric acid (40 mL). The mixture was extracted with ethyl acetate (2 x 50 mL) and the combined organic layers were washed with distilled water (2 x 100 mL) and then brine (100 mL) before being dried over magnesium sulfate, filtered, and the solvent removed \textit{in vacuo}. Purification \textit{via} flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 7:3; \textit{R} \text{f} = 0.20) afforded a cream solid whose physicochemical properties were consistent with those of the title compound 320 (398 mg, 0.85 mmol, 73% yield).

M.p. 88–90 °C (hexane / dichloromethane); \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 9.91 (s, 1H, -CHO), 7.81 (d, \(J = 8.6\) Hz, 2H, Ar-H), 7.62 (s, 1H, Ar-H), 7.49 – 7.33 (m, 6H, Ar-H), 7.24 – 7.14 (m, 5H, Ar-H), 6.96 (d, \(J = 8.6\) Hz, 2H, Ar-H), 5.20 (s, 2H, -CH\textsubscript{2}Ph), 5.07 (s, 2H, -CH\textsubscript{2}Ph), 3.89 (s, 3H, -CO\textsubscript{2}C\textsubscript{H}\textsubscript{3}); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 190.9 (-CHO), 166.1 (-CO\textsubscript{2}C\textsubscript{H}\textsubscript{3}), 163.0, 153.3, 147.9, 144.8, 136.7, 136.2, 132.1, 131.5, 128.8, 128.5, 128.4, 128.3, 127.8, 126.0, 116.8, 116.8, 112.2, 75.4 (-CH\textsubscript{2}Ph), 71.5 (-CH\textsubscript{2}Ph), 52.5 (-CO\textsubscript{2}C\textsubscript{H}\textsubscript{3}); FT-IR (thin film): \(\nu\) (cm\textsuperscript{-1}) = 3064 (O-H), 3033, 2952, 2736 (C-H), 1724 (C=O), 1697, 1584, 1503, 1427, 1341, 1220, 1157, 1076; MS (MALDI-TOF) \textit{m/z} 491.05 [M+Na]\textsuperscript{+}, 507.00 [M+K]\textsuperscript{+}; HRMS (HNESP) exact mass calculated for C\textsubscript{29}H\textsubscript{25}O\textsubscript{6} requires \textit{m/z} 469.1646, found \textit{m/z} 469.1642 [M+H]\textsuperscript{+}. 
Methyl 3-(4-formylphenoxy)-5-hydroxy-4-methoxybenzoate (321)

To a 15 mL microwave vial containing methyl 3-(4-formylphenoxy)-4,5-dihydroxybenzoate 304 (603 mg, 2.09 mmol, 1 equiv.) and a magnetic stirrer bar under a nitrogen atmosphere was added N,N-dimethylformamide (10 mL). To the stirred orange solution was added lithium carbonate (155 mg, 2.09 mmol, 1 equiv.) in one portion, followed by the addition of iodomethane (0.13 mL, 2.09 mmol, 1 equiv.) dropwise via a 1 mL syringe. The vial was sealed with a PTFE-lined aluminium crimp cap and heated to 55 °C for 18 hours using a sand bath. After cooling to room temperature the mixture was poured into a 250 mL separating funnel containing distilled water (20 mL), to which 1 M aqueous hydrochloric acid (50 mL) was added. The mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with distilled water (3 x 100 mL) and subsequently brine (100 mL). The organic solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: dichloromethane / diethyl ether 5%; Rf = 0.31) afforded an orange oil whose physicochemical properties were consistent with those of the title compound 321 (449 mg, 1.49 mmol, 71% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) δ 9.94 (s, 1H, -CHO), 7.87 (d, J = 8.8 Hz, 2H, Ar-H), 7.54 (d, J = 2.0 Hz, 1H, Ar-H), 7.32 (d, J = 2.0 Hz, 1H, Ar-H), 7.05 (d, J = 8.8 Hz, 2H, Ar-H), 5.94 (s, 1H, -OH), 3.95 (s, 3H, -OCH$_3$), 3.87 (s, 3H, -OCH$_3$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 190.8 (-CHO), 166.0 (-CO$_2$CH$_3$), 162.4, 149.8, 145.9, 142.8, 132.2, 131.9, 126.3, 117.0, 115.7, 113.7, 61.6 (Ar-OCH$_3$), 52.5 (-CO$_2$CH$_3$); FT-IR (thin film): ν (cm$^{-1}$) = 3384 (O-H), 2955, 2843 (C-H), 1709 (C=O), 1582, 1503, 1436, 1354, 1227, 1156; MS (MALDI-TOF) $m/z$ 325.03 [M+Na]$^+$; HRMS (NESI) exact mass calculated for C$_{16}$H$_{15}$O$_6$ [M+H]$^+$ requires $m/z$ 303.0863, found $m/z$ 303.0866.
Methyl 3-((tert-butyldiphenylsilyl)oxy)-5-(4-formylphenoxy)-4-methoxybenzoate (322)

To a 5 mL round-bottom flask containing methyl 3-(4-formylphenoxy)-5-hydroxy-4-methoxybenzoate 321 (100 mg, 0.33 mmol, 1 equiv.) and a magnetic stirrer bar under a nitrogen atmosphere was added N,N-dimethylformamide (2 mL). To the stirred orange solution was added imidazole (50 mg, 0.73 mmol, 2.2 equiv.) in one portion, followed by the addition of tert-butyldiphenylchlorosilane (95 μL, 0.36 mmol, 1.1 equiv.) via a 250 μL syringe. The pale yellow solution was stirred at room temperature for 18 hours before being poured into a 50 mL separating funnel containing 5% aqueous sodium bicarbonate solution (10 mL) and the organics extracted with hexane (3 x 10 mL). The combined organic layers were washed with brine (30 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 1:1; R$_f$ = 0.56) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound 322 (102 mg, 0.19 mmol, 57% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) δ 9.91 (s, 1H, -CHO), 7.80 (d, $J$ = 8.6 Hz, 2H, Ar-H), 7.73 (d, $J$ = 7.8 Hz, 4H, Ar-H), 7.46 (t, $J$ = 7.4 Hz, 2H, Ar-H), 7.39 (t, $J$ = 7.4 Hz, 4H, Ar-H), 7.36 – 7.31 (m, 2H, Ar-H), 6.83 (d, $J$ = 8.6 Hz, 2H, Ar-H), 3.75 (s, 3H, -CO$_2$C$_{13}$H$_3$), 3.65 (s, 3H, -OC$_3$H$_3$), 1.14 (s, 9H, -C(CH$_3$)$_3$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 190.8 (-CHO), 165.8 (-CO$_2$CH$_3$), 162.9, 149.9, 147.7, 147.3, 135.6, 132.4, 132.0, 131.3, 130.2, 127.9, 125.5, 119.6, 117.3, 116.4, 60.9 (Ar-OC$_3$H$_3$), 52.3 (-CO$_2$CH$_3$), 26.7 (-C(CH$_3$)$_3$), 19.8 (-C(CH$_3$)$_3$); FT-IR (thin film): ν (cm$^{-1}$) = 3074 (C-H), 2956 (C-H), 2859 (C-H), 1726 (C=O), 1700 (C=O), 1603, 1582, 1506, 1430, 1345, 1227, 1155, 1109, 1071, 999; MS (MALDI-TOF) m/z 579.28 [M+K]$^+$; HRMS (NESI) exact mass calculated for C$_{32}$H$_{33}$O$_6$Si requires m/z 541.2041, found m/z 541.2038 [M+H]$^+$. 
Methyl 7-hydroxy-2-(4-methoxyphenyl)benzo[d][1,3]dioxole-5-carboxylate (318)\textsuperscript{181}

\[
\begin{align*}
\text{OCH}_3 & \quad \quad \text{OCH}_3 \\
\text{O} & \quad \quad \text{O} \\
317 & \quad \quad 281 \\
\end{align*}
\]

A flame-dried 250 mL two-necked round-bottom flask fitted with a Soxhlet apparatus containing 4Å molecular sieves was charged with a solution of \( p \)-anisaldehyde dimethyl acetal 317 (2.18 g, 12.0 mmol, 1.25 equiv.) in anhydrous toluene (100 mL) and a magnetic stirrer bar. Methyl gallate 281 (1.77 g, 9.60 mmol, 1 equiv.) and para-toluenesulfonic acid (8 mg, 0.046 mmol, 0.5 mol%) were added, and the solution heated at reflux for 3 hours. After cooling to room temperature the majority of the solvent was removed in vacuo. The residue was redissolved in dichloromethane (50 mL) and washed with saturated aqueous sodium carbonate solution (50 mL). The addition of hexane (50 mL) resulted in the precipitation of a pale yellow solid, which was filtered and recrystallised from dichloromethane / hexane (1:1) to afford a cream solid whose physicochemical properties matched those previously reported for this compound (1.20 g, 3.97 mmol, 41% yield).

M.p. 130–132 °C (dichloromethane / hexane); \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.50 (d, \( J = 8.8 \) Hz, 2H, Ar-H), 7.34 (d, \( J = 1.5 \) Hz, 1H, Ar-H), 7.18 (d, \( J = 1.5 \) Hz, 1H, Ar-H), 7.00 (s, 1H, -CH\(_3\)), 6.96 (d, \( J = 8.8 \) Hz, 2H, Ar-H), 5.06 (s, 1H, -OH), 3.88 (s, 3H, -OCH\(_3\)), 3.84 (s, 3H, -OCH\(_3\)); FT-IR (thin film): \( \nu \) (cm\(^{-1}\)) = 3347 (O-H), 2920 (C-H), 2850 (C-H), 1696 (C=O), 1637, 1617, 1516, 1449, 1375, 1257, 1081; MS (MALDI-TOF) \( m/z \) 325.08 [M+Na]\(^+\).
Methyl 7-(4-formylphenoxy)-2-(4-methoxyphenyl)benzo[d][1,3]dioxole-5-carboxylate (319)

A 15 mL microwave vial containing 4Å molecular sieves was flame-dried and allowed to cool to room temperature under a flow of nitrogen. Methyl 3-(4-formylphenoxy)-4,5-dihydroxybenzoate 304 (100 mg, 0.347 mmol, 1 equiv.), anhydrous toluene (7 mL), para-anisaldehyde dimethyl acetal 317 (66 mg, 0.364 mmol, 1.05 equiv.), and para-toluenesulfonic acid (0.7 mg, 0.0035 mmol, 1 mol%) were added. The vial was sealed with a PTFE-lined aluminium crimp cap and the stirred suspension was heated at 110 °C for 6 hours. After cooling to room temperature, sodium carbonate (0.4 mg, 0.0038 mmol, 1.09 mol%) was added and the mixture stirred at room temperature for one hour before being filtered to remove the molecular sieves. The solvent was removed in vacuo, and purification via flash column chromatography on silica gel (eluent: dichloromethane 100%; Rf = 0.30) afforded a pale yellow solid whose physicochemical properties were consistent with those of the title compound 319 (20 mg, 0.049 mmol, 28% yield).

M.p. 76–78 °C; ¹H-NMR (400 MHz, CDCl₃) δ 9.93 (s, 1H, -CHO), 7.85 (d, J = 8.7 Hz, 2H, Ar-H), 7.48 (d, J = 1.5 Hz, 1H, Ar-H), 7.46 – 7.40 (m, 3H, Ar-H), 7.09 (d, J = 8.7 Hz, 2H, Ar-H), 7.05 (s, 1H, Ar-H), 6.93 (d, J = 8.7 Hz, 2H, Ar-H), 3.88 (s, 3H, -CO₂CH₃), 3.83 (s, 3H, -OCH₃); ¹³C-NMR (126 MHz, CDCl₃) δ 190.8 (-CHO), 165.9 (-CO₂CH₃), 162.0, 161.6, 150.1, 142.8, 136.5, 132.1, 131.8, 128.1, 127.0, 125.0, 118.4, 117.0, 114.2, 112.8, 106.9, 55.5 (-OCH₃), 52.5 (-CO₂CH₃); FT-IR (thin film): ν (cm⁻¹) = 3076 (C-H), 3011 (C-H), 2949 (C-H), 2843 (C-H), 2733 (C-H), 1717 (ester C=O), 1696 (aldehyde C=O), 1614, 1600, 1518, 1500, 1442, 1367, 1301, 1253, 1222, 1178, 1157, 1020, 1006; MS (MALDI-TOF) m/z 405.25 [M-H]⁻; HRMS (HNESP) exact mass calculated for C₂₃H₁₉O₇ requires m/z 407.1125, found m/z 407.1128 [M+H]⁺.
Methyl 4-(benzyloxy)-3,5-bis(4-formylphenoxy)benzoate (328)

A 250 mL round-bottom flask was charged with a solution of methyl 3,5-bis(4-formylphenoxy)-4-hydroxybenzoate 305 (3.01 g, 7.65 mmol, 1 equiv.) in N,N-dimethylformamide (50 mL) under a nitrogen atmosphere. Potassium carbonate (712 mg, 11.5 mmol, 1.5 equiv.) was added in one portion, followed by the addition of benzyl bromide (0.91 mL, 7.65 mmol, 1 equiv.) via a 2 mL syringe. The mixture was stirred at room temperature for 18 hours before being transferred to a 250 mL separating funnel containing 1 M aqueous hydrochloric acid (100 mL). The organics were extracted with ethyl acetate (3 x 75 mL). The combined organic layers were washed with water (3 x 100 mL) and subsequently brine (100 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / ethyl acetate 30%; Rf 0.38) afforded a pale yellow solid whose physicochemical characteristics were consistent with those of the title compound (328) (3.21 g, 6.64 mmol, 87% yield).

M.p. 80–82 °C; ^1H-NMR (400 MHz, CDCl₃) δ 9.94 (s, 2H, -CHO), 7.86 (d, J = 8.8 Hz, 4H, Ar-H), 7.66 (s, 2H, Ar-H), 7.23 – 7.13 (m, 3H, Ar-H), 7.06 – 6.99 (m, 6H, Ar-H), 5.05 (s, 2H, -CH₂Ph), 3.87 (s, 3H, -OCH₃); ^13C NMR (100 MHz, CDCl₃) δ 190.8 (-CHO), 165.3 (-CO₂CH₃), 162.3, 148.7, 147.2, 135.9, 132.0, 128.6, 128.5, 128.3, 126.6, 120.5, 117.1, 75.7 (-CH₂Ph), 52.7 (-CO₂CH₃); FT-IR (thin film): ν (cm⁻¹) = 3069 (C-H), 2956 (C-H), 2835 (C-H), 2745 (C-H), 1724 (d) (ester and aldehyde C=O), 1602, 1503, 1422, 1337, 1229, 1157; MS (MALDI-TOF) m/z 505.0 [M+Na]^+, 520.96 [M+K]^+; HRMS (NESP) exact mass calculated for C₂₉H₂₂O₇Na requires m/z 505.1258, found m/z 505.1250 [M+Na]^+. 
**Methyl 3,5-\textit{bis}(4-formylphenoxy)-4-methoxybenzoate (327)**

To a 15 mL microwave vial was added methyl 3,5-\textit{bis}(4-formylphenoxy)-4-hydroxybenzoate 305 (500 mg, 1.27 mmol, 1 equiv.), potassium carbonate (352 mg, 2.55 mmol, 2 equiv.) and a magnetic stirrer bar. N,N’-Dimethylformamide (6 mL) was added and the vial sealed with a PTFE-lined aluminium crimp cap. The suspension was stirred at room temperature for 15 minutes. Iodomethane (111 µL, 1.78 mmol, 1.4 equiv.) was added \textit{via} a 250 µL syringe, and the mixture heated at 80 °C for 24 hours. After cooling to room temperature, the suspension was poured into a 250 mL separating funnel containing ethyl acetate (50 mL) and 1 M aqueous hydrochloric acid (50 mL). The aqueous layer was re-extracted with ethyl acetate (50 mL) and the combined organic layers were washed with distilled water (2 x 100 mL) and subsequently brine (100 mL) before being dried over magnesium sulfate and filtered. The solvent was removed \textit{in vacuo} to afford a yellow oil whose physicochemical characteristics were consistent with those of the title compound (327) (478 mg, 1.18 mmol, 92%). 327 was used without further purification.

$^1$H-NMR (500 MHz, CDCl$_3$) δ 9.94 (s, 2H, -CHO), 7.88 (d, $J = 8.7$ Hz, 4H, Ar-H), 7.68 (s, 2H, Ar-H), 7.07 (d, $J = 8.7$ Hz, 4H, Ar-H), 3.88 (s, 3H, -OCH$_3$), 3.78 (s, 3H, -OCH$_3$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 190.7 (-CHO), 165.3 (-CO$_2$CH$_3$), 162.5, 148.7, 148.1, 132.2, 131.9, 126.2, 120.8, 117.0, 61.5 (-OCH$_3$), 52.7 (-CO$_2$CH$_3$); FT-IR (thin film): υ (cm$^{-1}$) = 2955 (C-H), 2836 (C-H), 2738 (C-H), 1727 (ester C=O), 1697 (aldehyde C=O), 1602, 1579, 1503, 1421, 1335, 1223, 1156; MS (MALDI-TOF) $m/z$ 429.2 [M+Na]$^+$, 445.21 [M+K]$^+$; HRMS (NESP) exact mass calculated for C$_{23}$H$_{19}$O$_7$ requires $m/z$ 407.1125, found $m/z$ 407.1125 [M+H]$^+$. 
Methyl 4-(benzylxy)-3,5-bis(4-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)phenoxy)benzoate (329)

A 100 mL two-neck round-bottom flask was charged with a solution of methyl 4-(benzylxy)-3,5-bis(4-formylphenoxy)benzoate 328 (1.0 g, 2.55 mmol, 1 equiv.) in dry toluene (40 mL). The flask was fitted with a Dean-Stark apparatus and reflux condenser under a nitrogen atmosphere. Pinacol (692 mg, 5.86 mmol, 2.3 equiv.) and para-toluenesulfonic acid (20 mg, 0.11 mmol, 5 mol%) were added, and the solution was heated at reflux for 6 hours. After cooling to room temperature, triethylamine (0.1 mL, 0.72 mmol, 0.3 equiv.) was added via a 1 mL syringe and the solution was stirred for a further 30 minutes before the solvent was removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 1:1; Rf = 0.58) afforded a white solid whose physicochemical properties were consistent with those of the title compound (329) (1.50 g, 2.19 mmol, 86% yield).

M.p. 132–134 °C; ^1^H-NMR (500 MHz, CDCl3) δ 7.47 (d, J = 8.7 Hz, 4H, Ar-H), 7.41 (s, 2H, Ar-H), 7.24 – 7.16 (m, 5H, Ar-H), 6.96 (d, J = 8.7 Hz, 4H, Ar-H), 5.98 (s, 2H, -CH2Ph), 5.12 (s, 2H, -CH2Ph), 3.80 (s, 3H, -CO2C3H3), 1.33 (s, 12H, -C(CH3)2-), 1.30 (s, 12H, -C(CH3)2-); ^13^C-NMR (126 MHz, CDCl3) δ 165.8 (-CO2CH3), 157.4, 150.3, 146.0, 136.6, 134.9, 128.6, 128.3, 128.2, 125.8, 117.8, 117.4, 99.7 (-CH2Ph), 82.8 (-C(CH3)2-), 75.3 (-CH2Ph), 52.3 (-CO2CH3), 24.6 (-C(CH3)2-), 22.3 (-C(CH3)2-); FT-IR (thin film): ν (cm⁻¹) = 2980 (C-H), 2932 (C-H), 2877 (C-H), 1727 (C=O), 1614, 1583, 1504, 1453, 1425, 1391, 1380, 1373, 1332, 1222, 1157, 1075, 1040, 1006; MS (MALDI-TOF) m/z 681.7; HRMS (HNESP) exact mass calculated for C41H50O9N requires m/z 700.3480, found m/z 700.3496 [M+NH4]^+.
(4-(Benzyloxy)-3,5-bis(4-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)phenoxy)phenyl) methanol (336)

To a flame-dried 250 mL round-bottom flask was added lithium aluminium hydride (118 mg, 3.11 mmol, 2.5 equiv.) and dry tetrahydrofuran (40 mL) under a nitrogen atmosphere. The mixture was cooled to -15 °C using an acetone / dry ice bath before a solution of methyl 4-(benzyloxy)-3,5-bis(4-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)phenoxy)benzoate 329 (2.62 g, 4.56 mmol, 1 equiv.) in tetrahydrofuran (15 mL) was added dropwise via a 20 mL syringe. The mixture was allowed to warm to 0 °C and stirred for 2 hours. On complete disappearance of the starting material by thin layer chromatography (petroleum ether / diethyl ether 1:1), ethyl acetate (50 mL) and 1 M aqueous sodium hydroxide (50 mL) were added simultaneously in small portions while maintaining the temperature at 0 °C. The reaction mixture was transferred to a 500 mL separating funnel and the aqueous phase extracted with ethyl acetate (2 x 75 mL). The combined organic phases were washed with brine (150 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo to afford a colourless oil whose physicochemical properties were consistent with those of the title compound (336) (2.42 g, 4.42 mmol, 97% yield); no further purification was required.

R<sub>f</sub> = 0.21 (eluent: petroleum ether / diethyl ether 1:1); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 7.46 (d, J = 8.6 Hz, 4H, Ar-H), 7.22 – 7.15 (m, 5H, Ar-H), 6.97 (d, J = 8.6 Hz, 4H, Ar-H), 6.73 (s, 2H, Ar-H), 5.97 (s, 2H, -CH-pin.), 5.01 (s, 2H, -CH<sub>2</sub>Ph), 4.48 (s, 2H, -CH<sub>2</sub>OH), 1.33 (s, 12H, -C(CH<sub>3</sub>)<sub>2</sub>), 1.29 (s, 12H, -C(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C-NMR (126 MHz, CDCl<sub>3</sub>) δ 157.7, 150.8, 141.1, 137.2, 137.0, 134.5, 128.6, 128.3, 128.1, 128.1, 117.9, 114.4, 99.8 (-CH<sub>pin</sub>), 82.8 (-C(CH<sub>3</sub>)<sub>2</sub>), 75.4 (-CH<sub>2</sub>Ph), 64.5 (-CH<sub>2</sub>OH), 42.6 (-C(CH<sub>3</sub>)<sub>2</sub>), 22.3 (-C(CH<sub>3</sub>)<sub>2</sub>); FT-IR (thin film): ν (cm<sup>-1</sup>) = 3458 (O-H), 2984 (C-H), 2932 (C-H), 2877 (C-H), 1610, 1586, 1507, 1435, 1370, 1322, 1219, 1154, 1075, 1034, 989; MS (MALDI-TOF) m/z
693.12 [M+K]^+; HRMS (HNESP) exact mass calculated for C_{40}H_{50}O_{8}N requires \textit{m/z} 672.3530, found \textit{m/z} 672.3531 [M+NH_4]^+.

4-(Benzyloxy)-3,5-bis(4-(dimethoxymethyl)phenoxy)benzaldehyde (337)

A 250 mL round-bottom flask was charged with a solution of 4-(benzyloxy)-3,5-bis(4-(dimethoxymethyl)phenoxy)benzyl alcohol 336 (447 mg, 0.68 mmol, 1 equiv.) in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere. Activated manganese dioxide (1.19 g, 13.7 mmol, 20 equiv.) was added in small portions and the mixture stirred at room temperature for 18 hours. On complete disappearance of starting material 336 by thin layer chromatography (eluent: petroleum ether / ethyl acetate 20%), the mixture was filtered through celite and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / ethyl acetate 20%; R_f = 0.38) afforded a white solid whose physicochemical characteristics were consistent with those of the title compound 337 (307 mg, 0.47 mmol, 69% yield).

M.p. 118–120 °C; \textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}) \delta 9.70 (s, 1H, -CHO), 7.49 (d, J = 8.6 Hz, 4H, Ar-H), 7.25 – 7.22 (m, 5H, Ar-H), 7.20 (s, 2H, Ar-H), 6.98 (d, J = 8.6 Hz, 4H, Ar-H), 5.98 (s, 2H, -CH- pin.), 5.19 (s, 2H, -CH_2Ph), 1.34 (s, 12H, -C(CH_3)_2), 1.29 (s, 12H, -C(CH_3)_2); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}) \delta 189.7 (C=O), 156.7, 151.1, 146.6, 136.3, 135.2, 131.8, 128.4, 128.2, 128.1(1), 128.0(5), 117.9, 116.3, 99.3 (-CH- pin.), 82.6 (-C(CH_3)_2), 75.2 (-CH_2Ph), 24.3 (-C(CH_3)_2), 22.1 (-C(CH_3)_2); FT-IR (thin film): \nu (cm\textsuperscript{-1}) = 2977 (C-H), 2936 (C-H), 2864 (C-H), 1696 (C=O), 1614, 1579, 1507, 1435, 1377, 1325, 1222, 1150, 1119, 1075, 1044; MS (MALDI-TOF) \textit{m/z} 691.44 [M+K]^+; HRMS (HNESP) exact mass calculated for C_{40}H_{48}O_{8}N requires \textit{m/z} 670.3374, found \textit{m/z} 670.3390 [M+NH_4]^+. 
A 250 mL round-bottom flask under a nitrogen atmosphere was charged with methyl triphenylphosphonium bromide (1.09 g, 3.06 mmol, 4 equiv.) and tetrahydrofuran (40 mL). The suspension was stirred while being cooled to 0 °C in an ice bath before the addition of sodium hydride (60 % in mineral oil, 122 mg, 3.06 mmol, 4 equiv.). After 1 hour, a solution of 4-(benzyloxy)-3,5-bis(4-(dimethoxymethyl)phenoxy)benzaldehyde 337 (500 mg, 0.77 mmol, 1 equiv.) in tetrahydrofuran (10 mL) was added dropwise to the flask via a 20 mL syringe, and the resulting mixture stirred at 0 °C for a further 3 hours or until complete disappearance of the starting material by thin layer chromatography (eluent: petroleum ether / ethyl acetate 20%). Diethyl ether (75 mL) was added, and the mixture transferred to a 500 mL separating funnel containing cold distilled water (100 mL). The aqueous phase was extracted with diethyl ether (2 x 100 mL) and the combined organic layers washed with brine (150 mL) before being dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / ethyl acetate 20%; Rf = 0.53) afforded a white solid whose physicochemical properties were consistent with those of the title compound 338 (379 mg, 0.58 mmol, 76% yield).

M.p. 116–118 °C; 1H-NMR (500 MHz, CDCl3) δ 7.46 (d, J = 8.6 Hz, 4H, Ar-H), 7.23 – 7.11 (m, 5H, Ar-H), 6.97 (d, J = 8.6 Hz, 4H, Ar-H), 6.81 (s, 2H, Ar-H), 6.47 (dd, J = 17.5, 11.0 Hz, 1H, -CH=CH2), 5.97 (s, 2H, -CH- pin.), 5.51 (dd, J = 17.5, 0.4 Hz, 1H, -CH=CH2), 5.16 (d, J = 11.0 Hz, 1H, -CH=CH2), 5.00 (s, 2H, -CH2Ph), 1.33 (s, 12H, -CH(CH3)2-), 1.29 (s, 12H, -CH(CH3)2-); 13C-NMR (126 MHz, CDCl3) δ 157.9, 150.5, 141.8, 137.0, 135.4, 134.4, 133.9, 128.6, 128.3, 128.1, 128.0, 117.6, 114.5, 114.3, 99.8 (-CH- pin.), 82.8 (-C(CH3)2-), 75.4 (-CH2Ph), 24.6 (-CH(CH3)2-), 22.3 (-CH(CH3)2-); FT-
Experimental Section

IR (thin film): $\nu$ (cm$^{-1}$) = 2983 (C-H), 2928 (C-H), 2855 (C-H), 1610, 1569, 1503, 1428, 1410, 1390, 1321, 1228, 1155, 1076, 1031, 990; MS (MALDI-TOF) $m/z$ 689.69 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{41}$H$_{50}$O$_7$N requires $m/z$ 668.3582, found $m/z$ 668.3581 [M+NH$_4$]$^+$.

4,4’-(2-(Benzylxy)-5-vinyl-1,3-phenylene)bis(oxy)dibenzaldehyde (339)

A 5 mL microwave vial was charged with a colourless solution of 4,4’-((2-(benzylxy)-5-vinyl-1,3-phenylene)bis(oxy))bis((dimethoxymethyl)benzene) 338 (50 mg, 0.08 mmol, 1 equiv.) in tetrahydrofuran (1 mL). 1 M aqueous hydrochloric acid (1 mL) was added, and the solution was heated at 50 ºC with stirring for 3 hours. On complete disappearance of the starting material by thin layer chromatography (eluent: petroleum ether / ethyl acetate 20%), the organic solvent was removed in vacuo and the residue extracted with dichloromethane (3 x 5 mL). The combined organic layers were washed with brine (20 mL) before being dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 50%; $R_f$ = 0.31) afforded a white solid whose physicochemical characteristics were consistent with those of the title compound (31 mg, 0.069 mmol, 90% yield).

M.p. 101–103 ºC; $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 9.92 (s, 2H, -CHO), 7.84 (d, $J$ = 8.6 Hz, 4H, Ar-H), 7.22 – 7.10 (m, 3H, Ar-H), 7.06 (s, 2H, Ar-H), 7.03 (d, $J$ = 8.6 Hz, 4H, Ar-H), 7.00 – 6.96 (m, 2H, Ar-H), 6.59 (dd, $J$ = 17.5, 11.0 Hz, 1H, -CH=CH$_2$), 5.65 (d, $J$ = 17.5 Hz, 1H, -CH=CH$_2$), 5.29 (d, $J$ = 11.0 Hz, 1H, -CH=CH$_2$), 4.92 (s, 2H, -CH$_2$Ph); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 190.9 (-CHO), 162.9, 148.9, 142.8, 136.3, 134.9, 134.9, 132.2, 131.6, 128.4(1), 128.3(7), 117.2, 116.9, 115.6, 75.7 (-CH$_2$Ph); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3070, 3033, 2834, 2741, 1696 (C=O), 1600, 1585, 1569, 1501, 1454, 1430, 1408, 1321, 1225, 1154, 1104, 1057, 1026; MS (MALDI-TOF) $m/z$ 473.32 [M+Na]$^+$; HRMS
Experimental Section

(HNESPI) exact mass calculated for C_{29}H_{26}O_{5}N requires \textit{m/z} 468.1805, found \textit{m/z} 468.1802 [M+NH\textsubscript{4}]\textsuperscript{+}.

\textit{tert}-Butyl 3-(4-(2,3-bis(benzyloxy)-5-(methoxycarbonyl)phenoxy)phenyl)-1-(2-(\textit{tert}-butoxy)phenyl)aziridine-2-carboxylate (\textit{rac},\textit{cis}-352)

To a flame-dried 5 mL microwave vial containing crushed 4Å molecular sieves under a nitrogen atmosphere was added a solution of methyl 3,4-bis(benzyloxy)-5-(4-formylphenoxy)benzoate 320 (100 mg, 0.21 mmol, 1 equiv.) and 2-\textit{tert}-butoxy aniline\textsuperscript{209} 349 (33 mg, 0.20 mmol, 0.95 equiv.) in anhydrous dichloromethane (2 mL). The mixture was stirred slowly at room temperature for 18 hours. After the addition of pyridinium triflate 347 (5 mg, 0.02 mmol, 10 mol%), the vial was sealed with a PTFE-lined aluminium crimp cap and cooled to 0 °C. \textit{Ter}-butyl diazoacetate 346 (30 µL, 0.21 mmol, 1 equiv.) was added dropwise via a 100 µL syringe. The solution was stirred at 0 °C for 18 hours before being filtered through a short plug of silica and eluted with diethyl ether (3 x 5 mL). The organic solvent was removed in vacuo to afford a dark yellow residue. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether / dichloromethane 8:1:1; R\textsubscript{f} = 0.20), afforded a white solid whose physicochemical properties were consistent with those of the title compound 352 (89 mg, 0.12 mmol, 57% yield).

M.p. 39–41 °C; \textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}) δ 7.52 – 7.43 (m, 5H, Ar-H), 7.42 – 7.31 (m, 5H, Ar-H), 7.28 – 7.21 (m, 4H, Ar-H), 7.05 – 6.99 (m, 1H, Ar-H), 6.98 – 6.92 (m, 3H, Ar-H), 6.90 (d, J = 8.7 Hz, 2H, Ar-H), 5.17 (s, 2H, -CH\textsubscript{2}Ph), 5.13 (s, 2H, -CH\textsubscript{2}Ph), 3.84 (s, 3H, -OCH\textsubscript{3}), 3.46 (d, J = 6.7 Hz, 1H, aziridine C-H), 3.04 (d, J = 6.7 Hz, 1H, aziridine C-H), 1.38 (s, 9H, -C(CH\textsubscript{3})\textsubscript{3}), 1.23 (s, 9H, -C(CH\textsubscript{3})\textsubscript{3}); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}) δ 167.3 (-CO\textsubscript{2}Bu), 166.3 (-CO\textsubscript{2}CH\textsubscript{3}), 156.8, 153.1, 150.3, 148.1, 146.7, 144.2,
A 5 mL microwave vial containing 4Å molecular sieves was flame-dried and cooled under a flow of nitrogen. A solution of methyl 4-(benzyloxy)-3,5-bis(4-formylphenoxy)benzoate 328 (100 mg, 0.21 mmol, 1 equiv.) and 2-tert-butoxyaniline 349 (67 mg, 0.40 mmol, 1.95 equiv.) in dry chloroform (1.5 mL) was added, along with a magnetic stirrer bar. The vial was sealed with a PTFE-lined aluminium crimp cap and the reaction mixture was stirred slowly at room temperature for 18 hours. Pyridinium triflate 347 (5 mg, 0.021 mmol, 10 mol%) was added, and the vial was resealed before being cooled to -30 °C in a chiller bath. Tert-butyl diazoacetate 346 (32 µL, 0.23 mmol, 2.2 equiv.) was added via a 100 µL syringe and the mixture was stirred at -30 °C for 16 hours. After warming to room temperature, the solution was filtered through a short plug of silica, eluting with diethyl ether (3 x 5 mL). The solvent was removed in vacuo, and
purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether / dichloromethane 7:2:1; R_f = 0.26) afforded a white solid whose physicochemical properties were consistent with those of the title compound 350 (224 mg, 0.22 mmol, 59% yield).

cis-350: M.p. 78–80 °C; ^1H-NMR (300 MHz, CDCl_3) δ 7.52 (d, J = 8.6 Hz, 4H, Ar-H), 7.34 (s, 2H, Ar-H), 7.33 – 7.24 (m, 4H, Ar-H), 7.05 – 6.92 (m, 13H, Ar-H), 5.17 (s, 2H, -CH_2Ph), 3.78 (s, 3H, -CO_2CH_3), 3.47 (d, J = 6.7 Hz, 2H, aziridine C-H), 3.05 (d, J = 6.7 Hz, 2H, aziridine C-H), 1.38 (s, 18H, tert-butyl), 1.24 (s, 9H, -CH(CH_3)_2), 1.17 (s, 9H, -CH(CH_3)_2, -CO_2CH_3), 80.5 (-C(CH_3)_3), 75.4 (-C(CH_3)_2), 52.3 (-CO_2CH_3), 47.5 (aziridine C-H), 47.1 (aziridine C-H), 28.8 (-C(CH_3)_3), 28.0 (-C(CH_3)_3); FT-IR (thin film): ν (cm^-1) = 2979 (C-H), 1721 (C=O), 1699, 1580, 1515, 1486, 1369, 1334, 1265, 1161, 1043, 978, 846; MS (MALDI-TOF) m/z 1005.3 [M]^+; HRMS (NESP) exact mass calculated for C_{61}H_{72}N_3O_{14} requires m/z 1022.5169 [M+NH_4]^+.

A byproduct was isolated during the flash column chromatography (R_f = 0.23); this was identified as rac, cis-tert-butyl 3-(4-(2-(benzoxyl)-3-(4-formylphenoxy)-5-(methoxycarbonyl)phenoxy)phenyl)-1-(2-(tert-butoxy)phenyl)aziridine-2-carboxylate 351 (64 mg, 0.087 mmol, 23% yield; R_f = 0.23).

rac,cis-351: ^1H-NMR (500 MHz, CDCl_3) δ 9.93 (s, 1H, -CHO), 7.84 (d, J = 8.8 Hz, 2H, Ar-H), 7.56 – 7.53 (m, 3H, Ar-H), 7.46 (d, J = 2.0 Hz, 1H, Ar-H), 7.24 – 7.13 (m, 5H, Ar-H), 7.04 – 6.92 (m, 8H, Ar-H), 5.11 (s, 2H, -CH_2Ph), 3.83 (s, 3H, -CO_2CH_3), 3.48 (d, J = 6.7 Hz, 1H, aziridine C-H), 3.06 (d, J = 6.7 Hz, 1H, aziridine C-H), 1.38 (s, 9H, -C(CH_3)_3), 1.24 (s, 9H, -C(CH_3)_3); ^13C-NMR (126 MHz, CDCl_3) δ 190.9 (-CHO), 167.1 (-CO_2Bu), 165.5 (-CO_2CH_3), 162.7, 156.0, 151.1, 148.3, 148.1, 146.5, 146.3, 145.3, 146.3, 132.1, 131.6, 131.1, 129.8, 128.4(0), 128.3(8), 126.1, 123.2(4), 123.1(7), 121.0, 118.8, 118.1, 117.8, 116.9, 81.5 (-C(CH_3)_3), 80.5 (-C(CH_3)_3), 75.5 (-CH_2Ph), 52.5 (-CO_2CH_3), 47.5 (aziridine C-H), 47.0 (aziridine C-H), 28.8 (-C(CH_3)_3), 28.0 (-C(CH_3)_3); FT-IR (thin film): ν (cm^-1) = 3063 (C-H), 2978 (C-H), 1724 (ester C=O), 1699 (aldehyde C=O), 1580, 1504, 1491, 1452, 1422, 1392, 1368, 1333, 1306, 1223, 1157, 1040, 1005; MS (MALDI-
TOF) \( m/z \) 782.1 [M+K]⁺; HRMS (HNESP) exact mass calculated for \( C_{45}H_{46}NO_9 \) requires \( m/z \) 744.3167, found \( m/z \) 744.3165 [M+H]⁺.

**tert-Butyl 3-(4-(2-benzzyloxy)-3-(4-formylphenoxy)-5-vinylphenoxy)phenyl)-1-(2-tert-butoxyphenyl)aziridine-2-carboxylate (rac, cis-353)**

A 5 mL microwave vial containing crushed 4Å molecular sieves was flame-dried and cooled under nitrogen. The vial was charged with 4,4'-(2-(benzyloxy)-5-vinyl-1,3-phenylene)bis(oxy))dibenzaldehyde 339 (100 mg, 0.22 mmol, 1 equiv.) and a solution of 2-tert-butoxyaniline 349 (36 mg, 0.22 mmol, 0.98 equiv.) in chloroform (1 mL). The mixture was stirred at room temperature for 16 hours. Pyridinium triflate 347 (5 mg, 0.02 mmol, 10 mol%) was added, and the vial sealed with a PTFE-lined aluminium crimp cap. Tert-butyl diazoacetate 346 (31 µL, 0.22 mmol, 1 equiv.) was added dropwise via syringe. The mixture was stirred at room temperature for a further 16 hours and filtered through a short plug of silica gel, eluting with diethyl ether (3 x 5 mL). The solvent was removed in vacuo to afford a brown oil. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether / dichloromethane (8:1:1); Rf 0.20) afforded a pale red liquid whose physicochemical properties were consistent with those of the title compound 353 (18 mg, 0.025 mmol, 19%).

\(^1\)H-NMR (500 MHz, CDCl₃) δ 9.91 (s, 1H -CHO), 7.83 (d, \( J = 8.7 \) Hz, 2H, Ar-H), 7.53 (d, \( J = 8.7 \) Hz, 2H, Ar-H), 7.10 – 7.24 (m, 5H, Ar-H), 6.90 – 7.05 (m, 9H, Ar-H), 6.83 (d, \( J = 2.0 \) Hz, 1H, Ar-H), 6.50 (dd, \( J = 10.9, 17.5 \) Hz, 1H, -CH=CH₂), 5.55 (d, \( J = 17.5 \) Hz, 1H, -CH=CH₂), 5.21 (d, \( J = 10.9 \) Hz, 1H, -CH=CH₂), 5.00 (s, 2H, -CH₂Ph), 3.48 (d, \( J = 6.7 \) Hz, 1H, aziridine -CH_), 3.05 (d, \( J = 6.7 \) Hz, 1H, aziridine -CH_), 1.38 (s, 9H, -C(CH₃)₃), 1.23 (s, 9H, -C(CH₃)₃); \(^{13}\)C-NMR (100 MHz, CDCl₃) δ 190.9 (-CHO), 167.1 (-CO₂Bu), 163.1, 156.4, 151.3, 148.5, 148.0, 146.5, 141.9, 136.7, 135.2, 134.3, 132.1, 131.3, 130.6, 129.6, 128.4, 128.3, 128.2, 123.2(4), 123.2(2), 123.1, 121.0, 117.7, 116.6,
115.2, 114.9, 114.8, 81.4 (-C(CH$_3$)$_3$), 80.5 (-C(CH$_3$)$_3$), 75.6 (-CH$_2$Ph), 47.5 (aziridine -CH$_2$-), 47.1 (aziridine -CH$_2$-), 28.8 (-C(CH$_3$)$_3$), 28.0 (-C(CH$_3$)$_3$); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3065, 3033, 2976, 2930, 2733, 2250, 1744 (ester C=O), 1696 (aldehyde C=O), 1596, 1584, 1568, 1490, 1452, 1428, 1392, 1367, 1319, 1225, 1155, 1111, 1028; [$\alpha$]$_D^{21}$ +6.1 (c 1.0, CHCl$_3$); HRMS (HNESP) exact mass calculated for C$_{45}$H$_{46}$NO$_7$ requires m/z 712.3269, found m/z 712.3265 [M+H]$^+$.

**General procedure for the synthesis of amino acid bromoacetamides:**

(S)-Methyl 2-(2-bromoacetamido)-3-phenylpropanoate (381)$^{218}$

A 250 mL round-bottom flask containing a magnetic stirrer bar was flame-dried and allowed to cool to room temperature under a nitrogen atmosphere. To the flask was added (S)-phenylalanine methyl ester hydrochloride 380 (2 g, 9.27 mmol, 1 equiv.) and dry dichloromethane (70 mL). Triethylamine (1.3 mL, 9.27 mmol, 1 equiv.) was added dropwise via a 2.5 mL syringe and the suspension stirred at room temperature for 20 minutes. The reaction was cooled to -10 °C using an acetone / dry ice bath, and bromoacetyl bromide (0.8 mL, 9.27 mmol, 1 equiv.) was added dropwise via a 2 mL syringe followed by a further 1 equivalent of triethylamine (1.3 mL, 9.27 mmol, 1 equiv.). The solution continued to be stirred at -10 °C for 15 minutes before the cooling bath was removed and the reaction mixture was allowed to warm to room temperature. The solution was stirred for a further 30 minutes before the addition of distilled water (50 mL). The mixture was then poured into a 250 mL separating funnel and the aqueous layer extracted with dichloromethane (2 x 50 mL). The combined organic layers were washed with 1 M aqueous hydrochloric acid (100 mL), then half-saturated aqueous sodium bicarbonate solution (100 mL) and finally brine (100 mL), before being dried over magnesium sulfate and filtered. Removal of the solvent *in vacuo* afforded a pale brown solid (used without further purification) whose physicochemical properties matched those previously reported for the title compound (2.62 g, 8.71 mmol, 94% yield).
\[ ^1 \text{H-NMR (500 MHz, CDCl}_3 \] \( \delta = 7.33-7.24 \ (m, \ 3\text{H, Ar-H}), \ 7.14-7.10 \ (m, \ 2\text{H, Ar-H}), \] 6.83 (br d, \( J = 6.0 \) Hz, 1H, N-H), 4.85 (dt, \( J = 7.9, \ 5.8 \) Hz, 1H, -NHCH-), 3.86 (d, \( J = 13.7 \) Hz, 1H, AB -CH\textsubscript{2}Br), 3.83 (d, \( J = 13.7 \) Hz, 1H, AB -CH\textsubscript{2}Br), 3.74 (s, 3H, -OCH\textsubscript{3}), 3.18 (dd, \( J = 13.9, \ 5.8 \) Hz, 1H, -CH\textsubscript{2}Ph), 3.13 (dd, \( J = 13.9, \ 5.8 \) Hz, 1H, -CH\textsubscript{2}Ph); \[ ^{13} \text{C-NMR (125 MHz, CDCl}_3 \] \( \delta = 171.4 \ (-\text{CONH-}), \ 165.2 \ (-\text{CO}_2\text{CH}_3), \ 135.5, \ 129.4, \ 128.9, \ 127.5, \] 53.9 (-NHCH-), 52.6 (-OCH\textsubscript{3}), 37.9 (-CH\textsubscript{2}Ph), 28.8 (-CH\textsubscript{2}Br); FT-IR (thin film, cm\textsuperscript{-1}) \( \nu = 3295 \) (N-H), 3033, 2959, 2854, 1746 (ester C=O), 1664 (amide C=O), 1533, 1440, 1365, 1216. \]

(S)-Methyl 2-(2-bromoacetamido)-4-methylpentanoate (382\textsuperscript{250})

Compound 382 was prepared according to the general procedure described above to afford a white solid (1.33 g, 5.01 mmol, 91% yield) which was used without further purification. The physicochemical properties matched those previously reported for the title compound.

\[ ^1 \text{H-NMR (500 MHz, CDCl}_3 \] \( \delta = 6.79 \ (d, \ J = 6.8 \) Hz, 1H, N-H), 4.62 (dt, \( J = 8.5, \ 5.1 \) Hz, 1H, -NHCH-), 3.91 (d, \( J = 13.7 \) Hz, 1H, AB -CH\textsubscript{2}Br), 3.87 (d, \( J = 13.7 \) Hz, 1H, AB -CH\textsubscript{2}Br), 3.75 (s, 3H, -OCH\textsubscript{3}), 1.74-1.55 (m, 3H, -CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 0.95 (d, \( J = 6.3 \) Hz, 6H, -C(CH\textsubscript{3})\textsubscript{2}); \[ ^{13} \text{C-NMR (125 MHz, CDCl}_3 \] \( \delta = 172.9 \ (-\text{CONH-}), \ 165.4 \ (-\text{CO}_2\text{CH}_3), \ 52.6 \ (-\text{OCH}_3), \] 51.5 (-NHCH-), 41.5 (-CHCH\textsubscript{2}), 28.9 (-CH\textsubscript{2}Br), 25.0 (-CH(CH\textsubscript{3})\textsubscript{2}), 22.9 (-CH(CH\textsubscript{3})\textsubscript{2}), 22.1 (-CH(CH\textsubscript{3})\textsubscript{2}); FT-IR (thin film, cm\textsuperscript{-1}) \( \nu = 3295 \) (N-H), 3074, 2959, 2873, 1746 (ester C=O), 1660 (amide C=O), 1544, 1440, 1208, 1156; \([\alpha]_D^{21}\ +5.6 \) (c 1.0, CHCl\textsubscript{3}).
(S)-Allyl 2-(2-bromoacetamido)-3-phenylpropanoate (384)

Compound 384 was prepared according to the general procedure described above to afford a pale brown oil (1.26 g, 3.86 mmol, 76% yield) that was used without further purification.

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.33 – 7.27 (m, 3H, Ar-H), 7.17 – 7.10 (m, 2H, Ar-H), 6.84 (br d, $J = 7.6$ Hz, 1H, N-H), 5.88 (ddt, $J = 17.2$, 10.4, 5.9 Hz, 1H, -CH=CH$_2$), 5.32 (dd, $J = 17.2$, 1.4 Hz, 1H, -CH=CH$_2$), 5.27 (dd, $J = 10.4$, 1.4 Hz, 1H, -CH=CH$_2$), 4.87 (dt, $J = 7.9$, 5.9 Hz, 1H, -NHCH$-$), 4.63 (dt, $J = 5.9$, 1.4 Hz, 2H, -OCH$_2$), 3.87 (d, $J = 13.7$ Hz, 1H, AB -CH$_2$Br), 3.83 (d, $J = 13.7$ Hz, 1H, AB -CH$_2$Br), 3.19 (dd, $J = 13.9$, 5.9 Hz, 1H, -CH$_2$Ph), 3.01 (dd, $J = 13.9$, 5.9 Hz, 1H, -CH$_2$Ph); $^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$ 170.7 (amide C=O), 165.3 (ester C=O), 135.4, 131.4 (-CH=CH$_2$), 129.5, 128.8, 127.5, 119.4 (-CH=CH$_2$), 66.4 (-OCH$_2$), 53.9 (-NHCH$-$), 37.9 (-CH$_2$Ph), 28.8 (-CH$_2$Br); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3307 (N-H), 3032 (C-H), 2935 (C-H), 2863 (C-H), 1743 (ester C=O), 1662 (amide C=O), 1536, 1498, 1455, 1379, 1197, 1138, 1049; [$\alpha$]$_D$ $^{21}$ +22.3 (c 1.0, CHCl$_3$); MS (MALDI-TOF) $m/z$ 364.08, 366.07 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{14}$H$_{17}$BrNO$_3$ requires $m/z$ 326.0386, 328.0366, found $m/z$ 326.0390, 328.0367 [M+H]$^+$.

(S)-Methyl 2-(2-bromoacetamido)-2-phenylacetate (385)

Compound 385 was prepared according to the general procedure described above to afford an orange solid (1.18 g, 4.12 mmol, 83% yield) which was used without further
The physicochemical properties matched those previously reported for the title compound.

M.p. 74–76 °C; \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.45 (br d, \(J = 6.1\) Hz, 1H, N-H), 7.41 – 7.31 (m, 5H, Ar-H), 5.54 (d, \(J = 7.1\) Hz, 1H, -CH\(_2\)Br), 3.86 (d, \(J = 13.7\) Hz, 1H, AB -CH\(_2\)Br), 3.75 (s, 3H, -OCH\(_3\)); FT-IR (thin film): \(\nu\) (cm\(^{-1}\)) = 3298 (N-H), 3041 (C-H), 2955 (C-H), 1746 (ester C=O), 1660 (amide C=O), 1529, 1339, 1216; [\(\alpha\)]\(_D\)\(^{19}\) +84.4 (c 1.0, CHCl\(_3\)).

**Methyl 2-(2-bromoacetamido)-2-methylpropanoate (383)**

![Chemical Reaction Diagram]

Compound 383 was prepared according to the general procedure described above to afford a white solid (1.27 g, 5.33 mmol, 78% yield) that was used without further purification.

M.p. 83–85 °C; \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.06 (s, 1H, -NH-), 3.83 (s, 2H, -CH\(_2\)Br), 3.77 (s, 3H, -OCH\(_3\)), 1.59 (s, 6H, -C(CH\(_3\))\(_2\)); \(^{13}\)C-NMR (126 MHz, CDCl\(_3\)) \(\delta\) 174.7 (ester –C=O), 164.9 (amide –C=O), 57.3 (-C(CH\(_3\))\(_2\)), 53.0 (-OCH\(_3\)), 29.2 (-CH\(_2\)Br), 24.4 (-C(CH\(_3\))\(_2\)); FT-IR (thin film): \(\nu\) (cm\(^{-1}\)) = 3273 (N-H), 3074 (C-H), 2922 (C-H), 2859 (C-H), 1734 (ester C=O), 1650 (amide C=O), 1548, 1493, 1472, 1282, 1197, 1157, 1049; MS (MALDI-TOF) \(m/z\) 261.92, 263.90 [M+Na]+; HRMS (HNESP) exact mass calculated for C\(_7\)H\(_{13}\)BrNO\(_3\) requires \(m/z\) 238.0073, 240.0053, found \(m/z\) 238.0076, 240.0051 [M+H]+.
**General procedure for the synthesis of amino acid diazoacetamides (Method A: N,N'-Ditosylhydrazine):**

(S)-Methyl 2-(2-diazoacetamido)-3-phenylpropanoate (386)

![Chemical structure]

A 250 mL round-bottom flask containing (S)-methyl 2-(2-bromoacetamido)-3-phenylpropanoate 381 (1.23 g, 4.10 mmol, 1 equiv.) and a stirrer bar under a nitrogen atmosphere was cooled to 0 °C. Dry tetrahydrofuran (40 mL) was added via a 50 mL syringe, followed by the sequential addition of N,N'-ditosylhydrazine (2.79 g, 8.20 mmol, 2 equiv.) in one portion, and 1,8-diazabicycloundec[7]ene (3.1 mL, 20.5 mmol, 5 equiv.) via a 5 mL syringe. The resulting deep yellow solution was stirred at 0 °C for 30 minutes. The reaction was quenched by the addition of saturated sodium bicarbonate solution (20 mL) and poured into a 250 mL separating funnel containing distilled water (50 mL) and diethyl ether (50 mL). The mixture was extracted with diethyl ether (3 x 50 mL), and the combined organic layers were washed with brine (100 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo. The yellow residue was redisssolved in chloroform (50 mL) and the mixture filtered to remove the unreacted white solid N,N'-ditosylhydrazine. The yellow filtrate was concentrated in vacuo. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1, Rf = 0.28) afforded a yellow solid whose physicochemical properties were consistent with those of the title compound (598 mg, 0.24 mmol, 60% yield).

M.p. 126–128 °C (dichloromethane / diethyl ether / petroleum ether); $^1$H-NMR (300 MHz, CD$_3$CN) $\delta$ = 7.39 – 7.15 (m, 5H, Ar-H), 6.33 (br s, 1H, -NH-), 5.03 (s, 1H, -CHN$_2$), 4.68 (dt, $J$ = 8.2, 5.6 Hz, 1H, -NHCH-), 3.65 (s, 3H, -OCH$_3$), 3.11 (dd, $J$ = 13.9, 5.6 Hz, 1H, -CH$_2$Ph), 2.94 (dd, $J$ = 13.9, 8.2 Hz, 1H, -CH$_2$Ph); $^{13}$C-NMR (100 MHz, CD$_3$CN) $\delta$ = 173.1 (-C=O ester), 166.3 (-C=O amide), 137.8, 130.2, 129.4, 127.8, 55.0 (-NHCH-), 52.7 (-OCH$_3$), 47.5 (-CHN$_2$), 38.4 (-CH$_2$Ph); FT-IR (thin film, cm$^{-1}$) $\nu$ = 3106 (N-H), 3027, 2115 (diazo C=N$_2$), 1748 (ester C=O), 1605 (amide C=O), 1527, 1385, 1208; [$\alpha$]$_D$


Experimental Section

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+175.0 (c 1.0, CHCl₃); MS (MALDI-TOF): \( m/z = 270.0 \ [M+Na]^+ \); HRMS (ESI) exact mass calculated for \( C_{12}H_{13}N_3NaO_3 \) \( m/z \) 270.0849, found \( m/z \) 270.0850 [M+Na]^+.

(S)-Methyl 2-(2-diazoacetamido)-4-methylpentanoate (387)

\[
\text{Br} \quad \text{TsNHNTs} \quad \text{DBU} \quad \text{THF} \quad 0 \ ^\circ \text{C}, 30 \text{ min} \quad \text{382} \quad \text{387: 65\% yield}
\]

Compound 387 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; \( R_f = 0.25 \)) afforded a yellow oil whose physicochemical properties were consistent with those of the title compound (1.28 mg, 6.03 mmol, 65\% yield).

\(^1\)H-NMR (300 MHz, CD₃CN) \( \delta = 6.41 \) (br s, 1H, -NH-), 5.06 (s, 1H, -CHN₂), 4.68 (m, 1H, -NHCH-), 3.65 (s, 3H, -OCH₃), 1.69 – 1.49 (m, 3H, -CH₂CH(CH₃)₂), 0.92 (d, \( J = 6.9 \) Hz, 3H, -CH(CH₃)₂), 0.89 (d, \( J = 6.9 \) Hz, 3H, -CH(CH₃)₂); \(^13\)C-NMR (100 MHz, CD₃CN) \( \delta = 174.3 \) (-C=O ester), 166.5 (-C=O amide), 52.7 (-NHCH-), 52.0 (-OCH₃), 47.5 (-CHN₂), 41.4 (-CH₂), 25.5 (-CH(CH₃)₂), 23.1 (-CH(CH₃)₂), 21.8 (-CH(CH₃)₂); FT-IR (ATR, cm\(^{-1}\)) \( \nu = 3105 \) (N-H), 2959 (C-H), 2877 (C-H), 2100 (diazo C=N₂), 1741 (C=O ester), 1616 (C=O amide), 1536, 1380, 1204, 1148 cm\(^{-1}\); \([\alpha]_D^{19} = -6.3 \) (c 1.0, CHCl₃); MS (MALDI-TOF): \( m/z = 252.0 \ [M+K]^+ \); HRMS (ESI) exact mass calculated for \( C_{9}H_{16}N_{3}O_{3} \) requires \( m/z \) 214.1186, found \( m/z \) 214.1187 [M+H]^+.

(S)-Methyl 2-(2-diazoacetamido)-2-phenylacetate (389)

\[
\text{Br} \quad \text{TsNHNTs} \quad \text{DBU} \quad \text{THF} \quad 0 \ ^\circ \text{C}, 30 \text{ min} \quad \text{385} \quad \text{389: 68\% yield}
\]

Compound 389 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; \( R_f = 0.31 \)) afforded a yellow solid whose physicochemical properties were consistent with those of the title compound (1.00 g, 4.29 mmol, 68\% yield).
M.p. 87–89 °C; \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.42 – 7.28 (m, 5H, Ar-\(\text{H}\)), 6.26 (br s, 1H, -NH-), 5.63 (d, \(J = 7.2\) Hz, 1H, -CHN\(_2\)), 4.83 (s, 1H, -NHCH-), 3.73 (s, 3H, -OCH\(_3\)); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.6 (-C=O ester), 164.8 (-C=O amide), 136.6, 129.0, 128.6, 127.2, 56.7 (-NHCH-), 52.9 (-OCH\(_3\)), 47.6 (-CHN\(_2\)); FT-IR (thin film): \(\nu\) (cm\(^{-1}\)) = 3307 (N-H), 3100 (C-H), 2965 (C-H), 2857 (C-H), 2111 (-HC=N\(_2\)), 1746 (ester C=O), 1625 (amide C=O), 1535, 1386, 1265, 1211, 1174, 1103; \([\alpha]\)\(_D\)^{20} +12.6 (c 1.0, CHCl\(_3\)); MS (MALDI-TOF) \(m/z\) 271.9 \([\text{M+K}]^+\); HRMS (HNESP) exact mass calculated for C\(_{11}\)H\(_{12}\)N\(_3\)O\(_3\) requires \(m/z\) 234.0873, found \(m/z\) 234.0876 \([\text{M+H}]^+\).

**Methyl 2-(2-diazoacetamido)-2-methylpropanoate (390)**

![Chemical Structure](image)

Compound 390 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether 100%; \(R_f = 0.55\)) afforded a yellow oil whose physicochemical properties were consistent with those of the title compound (829 mg, 4.48 mmol, 57% yield).

\(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.65 (s, 1H, -NH-), 4.73 (s, 1H, -CHN\(_2\)), 3.76 (s, 3H, -OCH\(_3\)), 1.58 (s, 6H, -C(CH\(_3\))\(_2\)); \(^{13}\)C-NMR (126 MHz, CDCl\(_3\)) \(\delta\) 175.4 (C=O ester), 164.9 (C=O amide), 57.2 (-C(CH\(_3\))\(_2\)), 53.0 (-OCH\(_3\)), 47.8 (-CHN\(_2\)), 25.3 (-C(CH\(_3\))\(_2\)); FT-IR (thin film): \(\nu\) (cm\(^{-1}\)) = 3319 (N-H), 3096, 2991, 2951, 2106 (diazo C=N\(_2\)), 1739 (ester C=O), 1621 (amide C=O), 1539, 1383, 1289, 1246, 1152; MS (MALDI-TOF) \(m/z\) 224.0 \([\text{M+K}]^+\); HRMS (HNESP) exact mass calculated for C\(_7\)H\(_{12}\)N\(_3\)O\(_3\) requires \(m/z\) 186.0873, found \(m/z\) 186.0873 \([\text{M+H}]^+\).
(S)-Allyl 2-(2-diazoacetamido)-3-phenylpropanoate (388)

Compound 388 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 2:1; $R_f = 0.37$) afforded a yellow solid whose physicochemical properties were consistent with those of the title compound (836 mg, 3.06 mmol, 79% yield).

M.p. 66–68 °C; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.32 – 7.22 (m, 3H, Ar-H), 7.15 – 7.08 (m, 2H, Ar-H), 5.88 (ddt, $J = 17.2$, 10.4, 5.9 Hz, 1H, -CH=CH$_2$), 5.46 (d, $J = 7.5$ Hz, 1H, -NH-), 5.32 (d, $J = 17.2$ Hz, 1H, -CH=CH$_2$), 5.27 (d, $J = 10.4$ Hz, 1H, -CH=CH$_2$), 4.98 (br s, 1H, -NHCH$_2$-), 4.73 (s, 1H, -CHN$_2$), 4.62 (d, $J = 5.9$ Hz, 2H, -OCH$_2$-), 3.18 (dd, $J = 13.9$, 5.7 Hz, 1H, -CH$_2$Ph), 3.13 (dd, $J = 13.9$, 5.7 Hz, 1H, -CH$_2$Ph); $^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$ 171.9 (C=O ester), 165.5 (C=O amide), 135.9, 131.4, 129.4, 128.6, 127.2, 119.2 (-CH=CH$_2$), 66.2 (-OCH$_2$-), 53.6 (-NHCH$_2$-), 47.4 (-CHN$_2$), 38.3 (-CH$_2$Ph); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3306 (N-H), 3092, 2953, 2108 ($\text{diazo C}=\text{N}_2$), 1742 (ester C=O), 1625 (amide C=O), 1534, 1458, 1385, 1196; [$\alpha$]$^D_{20}$ = -47.4 (c 1.0, CHCl$_3$); MS (MALDI-TOF) m/z 312.05 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{14}$H$_{16}$N$_3$O$_3$ requires m/z 274.1186, found m/z 274.1188 [M+H]$^+$.

**General procedure for the synthesis of amino acid diazoacetamides (Method B):**

(S)-Methyl 2-(2-diazoacetamido)-4-(methylthio)butanoate (393)

To a flame-dried 25 mL round-bottom flask containing (S)-methionine methyl ester hydrochloride 391 (218 mg, 1.09 mmol, 2 equiv.) and a magnetic stirrer bar was added dry tetrahydrofuran (5 mL) via syringe under a nitrogen atmosphere. The resulting
suspension was cooled to 0 °C using an ice bath and N,N-diisopropylethylamine (0.95 mL, 5.46 mmol, 3 equiv.) was added dropwise via a 2 mL syringe. The solution was stirred for 10 minutes while maintaining the temperature at 0 °C. A solution of succinimidyldiazoacetate (100 mg, 0.55 mmol, 1 equiv.) in tetrahydrofuran (10 mL) was added dropwise to the flask, and the solution was allowed to warm to room temperature. After stirring at room temperature for 24 hours, the solvent was removed in vacuo and the yellow residue extracted with dichloromethane (2 x 20 mL). The combined organic layers were washed with distilled water (3 x 30 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether; Rf = 0.24) afforded a yellow solid (63 mg, 0.27 mmol, 50% yield) whose physicochemical properties were consistent with those of the title compound.

M.p. = 40–42 °C; 1H-NMR (500 MHz, CD3CN) δ = 6.39 (br s, 1H, -NH), 5.06 (s, 1H, -C=H2N2), 4.54 (dt, J = 8.9, 4.9 Hz, 1H, -NHCH2-), 3.68 (s, 3H, -OCH3), 2.57–2.45 (m, 2H, -CH2S-), 2.06 (s, 3H, -SCH3), 2.05–2.00 (m, 1H, -CH2CH2S-), 1.92–1.85 (m, 1H, -CH2CH3S-); 13C-NMR (125 MHz, CD3CN) δ = 173.5 (C=O ester), 166.6 (C=O amide), 52.8 (-NHCH-), 52.6 (-OCH3), 47.6 (-CHN2), 32.2 (-CH2CH2S-), 30.6 (-CH2S-), 15.3 (-SCH3); FT-IR (thin film, cm⁻¹) ν = 3095 (N-H), 2925, 2111 (diazo C=N2), 1746 (ester C=O), 1620 (amide C=O), 1544, 1386, 1215; [α]D²⁰ +5.31 (c 1.0, CHCl3); MS (MALDI-TOF) m/z 269.9 [M+K]+; HRMS (HNESP) exact mass calculated for C8H14N3O3S requires m/z 232.0750, found m/z 232.0754 [M+H]+.

(S)-tert-Butyl 3-(2-diazoacetamido)-3-phenylpropanoate (398)

Compound 398 was prepared according to method B. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; Rf = 0.31) afforded a yellow oil whose physicochemical properties were consistent with those of the title compound (33 mg, 0.114 mmol, 61% yield).
$^1$H-NMR (300 MHz, CD$_3$CN) $\delta$ 7.49 – 7.20 (m, 5H, Ar-H), 6.66 (d, $J = 6.7$ Hz, 1H, -NH-), 5.28 (m, 1H, -NHCH-), 5.02 (s, 1H, -CH$_2$N$_2$), 2.71 (dd, $J = 14.5$, 7.0 Hz, 1H, -CH$_2$CO$_2$Bu), 2.64 (dd, $J = 14.5$, 7.0 Hz, 1H, -CH$_2$CO$_2$Bu), 1.34 (s, 9H, -C(CH$_3$)$_3$); $^{13}$C-NMR (100 MHz, CD$_3$CN) $\delta$ 170.6 (ester $-$C=O), 165.7 (amide –C=O), 142.9, 129.4, 128.3, 127.5, 81.4 (-C(CH$_3$)$_3$), 51.6 (-NHCH-), 47.5 (-CH$_2$N$_2$), 42.9 (-CH$_2$CO$_2$Bu), 28.1 (-C(CH$_3$)$_3$); FT-IR (ATR): $\nu$ (cm$^{-1}$) = 3277 (N-H), 3095 (C-H), 2983 (C-H), 2938 (C-H), 2099 (diazo C=N$_2$), 1727 (ester C=O), 1619 (amide C=O), 1561, 1375, 1251, 1146, 701; $[\alpha]_D^{19}$ -54.7 (c 1.5, CHCl$_3$); MS (MALDI-TOF) m/z 327.9 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{15}$H$_{20}$N$_3$O$_3$ requires m/z 290.1499, found m/z 290.1503 [M+H]$^+$. 

(S)-Methyl 1-(2-diazoacetyl)pyrroldine-2-carboxylate (401)

$\begin{array}{c}
\text{Cl}^+ \text{H}_3\text{CO} \text{N} \text{H} \text{C} \text{H}_2 \text{N}^+ \\
\text{N}_2 \text{O} \text{O} \text{N} \text{H} \text{C} \text{H}_2 \text{N} \\
\text{H}_3\text{CO} \text{N} \text{H} \text{C} \text{H}_2 \text{N} \\
\text{N}_2 \text{O} \text{O} \text{N} \text{H} \text{C} \text{H}_2 \text{N} \\
\text{H}_3\text{CO} \text{N} \text{H} \text{C} \text{H}_2 \text{N} \\
\text{N}_2 \text{O} \text{O} \text{N} \text{H} \text{C} \text{H}_2 \text{N} \\
\text{H}_3\text{CO} \text{N} \text{H} \text{C} \text{H}_2 \text{N}
\end{array}$

Compound 401 was prepared according to method B. Purification via flash column chromatography on silica (diethyl ether 100%; $R_f = 0.19$) afforded a yellow oil whose physicochemical properties were consistent with those of the title compound (70 mg, 0.35 mmol, 65% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 4.88 (s, 1H, -CH$_2$N$_2$), 4.53 (br s, 1H, -NCH-), 3.72 (s, 3H, -OCH$_3$), 3.43 (br s, 1H, -NCH$_2$-), 3.28 (br s, 1H, -NCH$_2$-), 2.25–1.90 (m, 4H, -CH$_2$-); $^{13}$C-NMR (101 MHz, CD$_3$CN) $\delta$ 174.0 (-C=O ester), 165.1 (-C=O amide), 59.8 (-NHCH-), 52.6 (-OCH$_3$), 47.2 (-NCH$_2$- + -CH$_2$N$_2$), 30.0 (-CH$_2$-), 25.2 (-CH$_2$-); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3075 (C-H), 2861 (C-H), 2927 (C-H), 2855 (C-H), 2107 (diazo C=N$_2$), 1736 (ester C=O), 1607 (amide C=O), 1413, 1163, 730; $[\alpha]_D^{22}$ -11.4 (c 0.8, CHCl$_3$); MS (MALDI-TOF) m/z 220.9 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_8$H$_{12}$N$_3$O$_3$ requires m/z 198.0873, found m/z 198.0873 [M+H]$^+$. 

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(S)-Methyl 2-(2-diazoacetamido)-3-(4-iodophenyl)propanoate (400)

Compound 400 was prepared according to method B. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; R_f = 0.37) afforded a yellow solid whose physicochemical properties were consistent with those of the title compound (89 mg, 0.24 mmol, 67% yield).

M.p. 73–75 °C; 1H-NMR (500 MHz, CDCl_3) δ 7.61 (d, J = 8.2 Hz, 2H, Ar-H), 6.84 (d, J = 8.2 Hz, 2H, Ar-H), 5.50 (d, J = 6.0 Hz, 1H, -NHCH-), 4.93 (m, 1H, -NHCH-), 4.75 (s, 1H, -CHNH), 3.13 (dd, J = 13.9, 6.0 Hz, 1H, -CH_2Ph), 3.05 (dd, J = 13.9, 6.0 Hz, 1H, -CH_2Ph); 13C-NMR (126 MHz, CDCl_3) δ 172.3 (-C=O ester), 165.4 (-C=O amide), 137.7, 135.6, 131.4, 92.7 (C-I), 53.3 (-NHCH-), 52.6 (-OCH_3), 47.5 (-CHNH), 37.7 (-CH_2Ar); FT-IR (thin film): ν (cm⁻¹) = 3361 (N-H), 3091 (C-H), 3011 (C-H), 2922 (C-H), 2854 (C-H), 2106 (diazo C=N), 1743 (ester C=O), 1540, 1493, 1447, 1383, 1206, 1054; [α]_D^{20} +151.8 (c 1.0, CHCl_3); MS (MALDI-TOF) m/z 412.02 [M+K]^⁺; HRMS (HNESP) exact mass calculated for C_{12}H_{13}IN_3O_2 requires m/z 373.9996, found m/z 373.997 [M+H]^⁺.

rac-Benzyl 2-(2-diazoacetamido)-3-phenylpropanoate (402)

Compound 402 was prepared according to method B. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; R_f = 0.47) afforded a yellow solid whose physicochemical properties were consistent with those of the title compound (2.01 g, 6.22 mmol, 37% yield).
M.p. 78–80 °C; \(^1\)H-NMR (500 MHz, CDCl\(_3\)) δ 7.41 – 7.34 (m, 3H, Ar-H), 7.34 – 7.29 (m, 2H, Ar-H), 7.24 – 7.20 (m, 3H, Ar-H), 7.02 – 6.98 (m, 2H, Ar-H), 5.46 (d, \(J = 7.2\) Hz, 1H, -NH-), 5.18 (d, \(J = 12.1\) Hz, 1H, -OCH\(_2\)Ph), 5.13 (d, \(J = 12.1\) Hz, 1H, -OCH\(_2\)Ph), 4.99 (br s, 1H, -NHCH-), 4.72 (s, 1H, -CHN\(_2\)), 3.15 (dd, \(J = 13.9, 5.9\) Hz, 1H, -CH\(_2\)Ph), 3.12 (dd, \(J = 13.9, 5.9\) Hz, 1H, -CH\(_2\)Ph); \(^{13}\)C-NMR (126 MHz, CDCl\(_3\)) δ 171.8 (-C=O ester), 165.3 (-C=O amide), 135.8, 135.1, 129.5, 128.8, 128.7, 127.2, 67.5 (-CO\(_2\)C\(_6\)H\(_5\)Ph), 53.6 (-NHCH-), 47.5 (-CHN\(_2\)), 38.3 (-CH\(_2\)Ph); FT-IR (thin film): \(\nu\) (cm\(^{-1}\)) = 3289 (NH), 3092, 3070, 3035, 2946, 2107 (-diazo C=N\(_2\)), 1741 (-ester C=O), 1621 (-amide C=O), 1538, 1456, 1387, 1195, 1109, 1078, 1027, 992; MS (MALDI-TOF) \(m/z\) 361.80 [M+K]+; HRMS (HNESP) exact mass calculated for C\(_{18}\)H\(_{18}\)N\(_3\)O\(_3\) requires \(m/z\) 324.1343, found \(m/z\) 324.1344 [M+H]+.

(S)-Benzyl 3-(2-(2-diazoacetamido)-3-methoxy-3-oxopropyl)-1H-indole-1-carboxylate (399)

Compound 399 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether / dichloromethane / petroleum ether 7:1:2; \(R_f = 0.38\)) afforded a pale yellow solid whose physicochemical properties were consistent with those of the title compound (876 mg, 2.08 mmol, 75% yield).

M.p. 120–122 °C; \(^1\)H-NMR (500 MHz, CDCl\(_3\)) δ 8.07 (br s, 1H, -CH\(_{ind}\)), 7.45 – 7.39 (m, 3H, Ar-H), 7.38 – 7.28 (m, 4H, Ar-H), 7.25 (t, \(J = 7.3\) Hz, 1H, Ar-H), 7.21 – 7.16 (m, 1H, Ar-H), 5.52 (d, \(J = 7.6\) Hz, 1H, -NH-), 5.37 (s, 2H, -CH\(_2\)Ph), 4.93 (m, 1H, -NHCH-), 4.62 (s, 1H, -CHN\(_2\)), 3.60 (s, 3H, -OCH\(_3\)), 3.28 (dd, \(J = 14.7, 5.6\) Hz, 1H, -CH\(_2\)-indole), 3.22 (dd, \(J = 14.7, 5.6\) Hz, 1H, -CH\(_2\)-indole); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)) δ 172.3 (-C=O ester), 165.2 (-C=O amide), 150.8 (-C=O carbamate), 135.2, 130.7, 128.9, 125.1, 123.9, 123.2, 119.1, 116.0, 115.5, 68.9 (-CH\(_2\)Ph), 52.9 (-NHCH-), 52.7 (-OCH\(_3\)), 47.7 (-CHN\(_2\)), 27.9 (-CH\(_2\)-indole); FT-IR (thin film, cm\(^{-1}\)) \(\nu\) = 3335 (N-H), 3092, 2920, 2106, 1739
(ester C=O), 1617 (amide C=O), 1539, 1457, 1394, 1250; [α]D25 +23.9 (c 1.0, acetone);
MS (MALDI-TOF): m/z = 443.12 [M+Na]+; HRMS (NSI) exact mass calculated for
C22H21O3N4 requires m/z 421.1506, found m/z 421.1505 [M+H]+.

**trans-**Tert-butyl 2-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3-
phenylaziridine-1-carboxylate (416a/b)

A 5 mL microwave vial containing crushed 4Å molecular sieves was flame-dried and
cooled under a nitrogen atmosphere. The vial was charged with a solution of tert-butyl
benzylidenecarbamate 408 (100 mg, 0.49 mmol, 1 equiv.) in chloroform-d3 (1 ml) via
syringe and a magnetic stirrer bar before being cooled to 0 °C using an ice bath.
Pyridinium triflate (11 mg, 0.05 mmol, 10 mol%) was added, and the vial was sealed with
a PTFE-lined aluminium crimp cap. A solution of (S)-methyl 2-(2-diazoacetamido)-3-
phenylpropanoate 386 (145 mg, 0.59 mmol, 1.2 equiv.) in chloroform-d3 (1 mL) was
added dropwise via syringe, and the reaction was allowed to warm slowly to room
temperature over 16 hours. On complete disappearance of imine 408 by 1H-NMR, the
mixture was filtered through a short plug of silica, eluting with diethyl ether, and the
solvent removed *in vacuo*. Purification *via* flash column chromatography on silica gel
(eluent: petroleum ether / diethyl ether 1:1; Rf = 0.34) afforded a white solid whose
physicochemical properties were consistent with those of a single diastereomer of the title
compound (149 mg, 0.35 mmol, 72% yield).

M.p. 125–127 °C; 1H-NMR (500 MHz, CDCl3) δ 7.39 – 7.22 (m, 8H, Ar-H), 7.11 (m, 2H,
Ar-H), 6.56 (d, J = 8.0 Hz, 1H, -NH-), 4.93 (dt, J = 8.0, 5.8 Hz, 1H, -NHCH3-), 3.73 (m,
4H, -CO2CH3 + aziridine C-H), 3.19 (d, J = 2.2 Hz, 1H, aziridine C-H), 3.18 (m, 1H, -
cis-(2S)-Methyl 2-((1-(4-methoxyphenyl)-3-phenylaziridine-2-carboxamido)-3-phenylpropanoate (406a)

Compound 406a was prepared according to the procedure for compound 416 above. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 1:1; Rf = 0.13) afforded a white solid whose physicochemical properties were consistent with those of the title compound (47 mg, 0.11 mmol, 23% yield).

M.p. 140–142 °C (petroleum ether / diethyl ether); 1H-NMR (500 MHz, CDCl3) δ 7.42 – 7.36 (m, 2H, Ar-H), 7.32 – 7.21 (m, 6H, Ar-H), 7.14 – 7.06 (m, 2H, Ar-H), 6.92 (d, J = 9.0 Hz, 2H, Ar-H), 6.88 – 6.81 (m, 3H, Ar-H + N-H), 4.60 (dt, J = 8.3, 6.0 Hz, 1H, -NHCH-), 3.80 (s, 3H, Ar-OCH3), 3.57 (d, J = 7.0 Hz, 1H, aziridine -CH-), 3.47 (s, 3H, -CO2CH3), 3.10 (dd, J = 13.7, 6.0 Hz, 1H, -CH2Ph), 3.05 (d, J = 7.0 Hz, 1H, aziridine -CH-), 3.00 (dd, J = 13.7, 6.0 Hz, 1H, -CH2Ph); 13C-NMR (126 MHz, CDCl3) δ 171.0 (-C=O ester), 166.7 (-C=O amide), 156.2 (-COCH3), 145.2, 136.0, 134.5, 129.5, 128.6, 128.4, 127.9, 127.7, 127.2, 120.9, 114.7, 55.8 (-NHCH-), 52.7 (Ar-OCH3), 52.2 (-CH-aziridine), 48.0 (-CO2CH3), 47.1 (-CH- aziridine), 38.3 (-CH2Ph); FT-IR (thin film): ν (cm⁻¹) = 3393 (N-H), 3033, 2961, 1745 (ester C=O), 1673 (amide C=O), 1506, 1242, 1152, 1086; [α]D23 +17.5 (c 1.0, CHCl3); MS (MALDI-TOF) m/z 463.27 [M+K]+; HRMS (HNESP) exact mass calculated for C24H28N2O5Na requires m/z 447.1890, found m/z 447.1887 [M+Na]+.
1033; [α]$_D^{21}$ -21.7 (c 1.0, CHCl$_3$); MS (MALDI-TOF) m/z 453.08 [M+Na]$^+$; HRMS (HNESP) exact mass calculated for C$_{26}$H$_{27}$N$_2$O$_4$ requires m/z 431.1965, found m/z 431.1973 [M+H]$^+$.

cis-(2S)-Methyl 2-(1-(4-methoxyphenyl)-3-phenylaziridine-2-carboxamido)-4-(methylthio)butanoate (407a/b)

Compound 407a/b was prepared according to the procedure for compound 416 above. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 1:1; R$_f$ = 0.10) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (18 mg, 0.04 mmol, 27% yield).

$^1$H-NMR (400 MHz, CDCl$_3$) δ 7.46 (d, J = 7.1 Hz, 2H, Ar-H), 7.36 – 7.24 (m, 3H, Ar-H), 7.05 – 6.96 (m, 3H, Ar-H + -NH-), 6.85 (d, J = 8.9 Hz, 2H, Ar-H), 4.35 (dt, J = 6.1, 5.6 Hz, 1H, -NHCH-), 3.79 (s, 3H, -OCH$_3$), 3.59 (d, J = 7.0 Hz, 1H, aziridine -CH-), 3.54 (s, 3H, -CO$_2$CH$_3$), 3.13 (d, J = 7.0 Hz, 1H, aziridine -CH-), 2.38 (t, J = 7.8 Hz, 2H, -SCH$_2$-), 2.12 – 2.00 (m, 1H, -SCH$_2$CH$_2$-), 2.06 (s, 3H, -SCH$_3$), 1.98 – 1.84 (m, 1H, -SCH$_2$CH$_2$-);

$^{13}$C-NMR (126 MHz, CDCl$_3$) δ 171.3 (-CO$_2$CH$_3$), 167.2 (-CONH-), 156.3 (C-OCH$_3$), 145.1, 134.5, 128.5, 127.9, 127.8, 121.0, 114.8, 55.8 (Ar-OCH$_3$), 52.4 (-NHCH-), 51.1 (aziridine -CH-), 48.1 (-CO$_2$CH$_3$), 46.8 (aziridine -CH-), 32.0 (-SCH$_2$CH$_2$-), 29.9 (-SCH$_2$-).
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), 15.6 \textcolor{red}{{(SCH}_3\text{)}}; FT-IR (thin film): \upsilon (cm\textsuperscript{-1}) = 3312 (N-H), 2962 (C-H), 2923 (C-H), 1740 (ester C=O), 1653 (amide C=O), 1508, 1245, 1036; \{\alpha\}_D\textsuperscript{21} = \textcolor{red}{{-23.8}} (c 1.0, CHCl\textsubscript{3}); MS (MALDI-TOF) \textcolor{red}{m/z} 452.8 [M+K]\textsuperscript{+}; HRMS (ASAP, solid) exact mass calculated for C\textsubscript{22}H\textsubscript{27}N\textsubscript{2}O\textsubscript{4}S requires \textcolor{red}{{m/z} 415.1686}, found \textcolor{red}{{m/z} 415.1687} [M+H]\textsuperscript{+}.

\textbf{General procedure for the one-pot synthesis of peptide aziridines:}

\textcolor{red}{(S)-Methyl 2-((2R,3R)-1-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)-3-phenylaziridine-2-carboxamido)-3-phenylpropanoate (427)}

\begin{align*}
\text{A 5 mL microwave vial containing crushed 4Å molecular sieves was flame-dried and cooled under a nitrogen atmosphere, followed by the addition of a magnetic stirrer bar. Benzaldehyde 228 (56 mg, 0.528 mmol, 1.1 equiv.) and (S)-phenylalanine methyl ester 426 (91 mg, 0.508 mmol, 1 equiv.) were weighed into a sample vial, dissolved in chloroform (1 mL) and transferred to the microwave vial via a 2 mL syringe. The solution was stirred at room temperature for 1 hour before being cooled to 0 °C using an ice-water bath. Pyridinium triflate 347 (12 mg, 0.0524 mmol, 10 mol%) was added and the vial was sealed with a PTFE-lined aluminium crimp cap. (S)-Methyl 2-(2-diazoacetamido)-3-phenylpropanoate 386 (150 mg, 0.607 mmol, 1.2 equiv.) was dissolved in chloroform (1 mL) and added dropwise to the reaction vial via a 2 mL syringe. Effervescence indicated the evolution of nitrogen gas during the reaction. The reaction was allowed to warm slowly to room temperature. After 16 hours the mixture was filtered through a short plug of silica, eluting with diethyl ether, and the solvent removed \textit{in vacuo}. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; R\textsubscript{f} = 0.17) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (224 mg, 0.460 mmol, 91% yield).}
\end{align*}
1H-NMR (500 MHz, CDCl$_3$) $\delta = 7.32$-$7.10$ (m, 15H, Ar-$H$), 4.57 (dt, $J = 8.2$, 6.2 Hz, 1H, -NHCH$\text{-}$), 3.64 (s, 3H, -OCH$_3$), 3.47 (s, 3H, -OCH$_3$), 3.29 (dd, $J = 13.8$, 6.2 Hz, 1H, -CH$_2$Ph), 3.12 – 3.05 (m, 2H, -CH$_2$Ph), 3.02 (dd, $J = 13.8$, 6.2 Hz, 1H, -CH$_2$Ph), 2.90 (t, $J = 6.6$ Hz, 1H, -NCH(Bn)CO$_2$CH$_3$), 2.78 (d, $J = 7.2$ Hz, 1H, aziridine -CH$\text{-}$), 2.60 (d, $J = 7.2$ Hz, 1H, aziridine -CH$\text{-}$); $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta = 171.7$ (-C=O ester), 171.3 (-C=O amide), 136.7, 136.1, 134.4, 129.5, 129.4, 128.6, 128.5, 128.0, 127.6, 127.5, 127.1, 126.9, 72.5 (-NCH), 52.7 (-NHCH$\text{-}$), 52.2 (-OCH$_3$), 52.1 (-OCH$_3$), 47.4 (-CH- aziridine), 46.8 (-CH- aziridine), 39.2 (-CH$_2$Ph), 38.3 (-CH$_2$Ph); FT-IR (thin film, cm$^{-1}$) $\nu = 3391$ (N-H), 3035, 2955, 2935, 2867, 1745 (ester C=O), 1681 (amide C=O), 1521, 1497, 1217, 1181, 1125, 1053, 828; $\left[\alpha\right]_{D}^{20}$ $-48.3$ (c 1.0, CHCl$_3$); MS (MALDI-TOF): $m/z = 509.4$ [M+Na]$^+$; HRMS (NSI) exact mass calculated for C$_{29}$H$_{31}$O$_5$N$_2$ requires $m/z$ 487.2227, found $m/z$ 487.2221 [M+H]$^+$.

cis-Benzyl 3-((2S)-3-methoxy-2-(2-((S)-1-methoxy-4-(methylthio)-1-oxobutan-2-ylcarbamoyl)-3-phenylaziridin-1-yl)-3-oxopropyl)-1H-indole-1-carboxylate (428a/b)

![Chemical Structure]

Compound 428a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 1:1; $R_f = 0.13$) afforded a colourless oil whose physicochemical
properties were consistent with those of a single diastereomer of the title compound (188 mg, 0.292 mmol, 83% yield).

$^1$H-NMR (500 MHz, CD$_3$CN) $\delta$ = 8.06 (d, $J$ = 8.0 Hz, 1H, -NH-), 7.55 (d, $J$ = 7.7 Hz, 1H, Ar-H), 7.52 – 7.37 (m, 6H, Ar-H), 7.35 – 7.29 (m, 2H, Ar-H), 7.25 (t, $J$ = 7.5, 1H, Ar-H), 7.16 – 7.04 (m, 5H, Ar-H), 5.34 (d, $J$ = 12.2 Hz, 1H, -CH$_2$Ph), 5.26 (d, $J$ = 12.2 Hz, 1H, -CH$_2$Ph), 4.34 (dt, $J$ = 8.3, 5.0 Hz, 1H, -CONHCH$_2$), 3.70 (s, 3H, -CO$_2$C$_6$H$_3$), 3.59 (s, 3H, -CO$_2$C$_6$H$_3$), 3.32 (dd, $J$ = 14.4, 4.9 Hz, 1H, -CH$_2$-indole), 3.25 – 3.14 (m, 1H, -NCH-), 2.68 (d, $J$ = 7.2 Hz, 1H, -CH- aziridine), 2.61 (d, $J$ = 7.2 Hz, 1H, -CH- aziridine), 2.04 – 1.99 (m, 2H, -CH$_2$SCH$_3$), 2.07-1.98 (m, 1H, -CH$_2$CH$_2$SCH$_3$), 2.03 (s, 3H, -SCH$_3$), 1.87 (ddt, $J$ = 14.0, 8.2, 6.2 Hz, 1H, -CH$_2$SCH$_3$); $^{13}$C-NMR (126 MHz, CD$_3$CN) $\delta$ = 173.4 (-C=O ester), 172.7 (-C=O ester), 167.3 (-C=O amide), 151.2 (-C=O carbamate), 136.6, 136.4, 136.3, 131.0, 129.7, 129.5, 129.3, 128.8, 128.2, 128.1, 125.7, 125.6, 123.7, 120.2, 117.1, 115.9, 70.0 (-NCH-), 69.3 (-CH$_2$PH), 52.8(6) (-NHCH-), 52.8(5) (-OCH$_3$), 51.5 (-OCH$_3$), 48.0 (-CH- aziridine), 47.7 (-CH- aziridine), 32.6 (-NHCHCH$_2$), 30.1 (-CH$_2$-), 29.0 (-CH$_2$-indole), 15.3 (-SCH$_3$); FT-IR (thin film, cm$^{-1}$) $\nu$ = 3385 (N-H), 2951, 2920, 2848, 1737 (ester C=O), 1677 (amide C=O), 1519, 1455, 1396, 1248, 1215, 1083; [\(\alpha\)]$_D$$_{23}$ = –41.4 (c 1.0, CHCl$_3$); MS (MALDI-TOF): $m/z$ = 666.5 [M+Na]$^+$; HRMS (NSI) exact mass calculated for C$_{35}$H$_{38}$O$_7$N$_3$S requires $m/z$ 644.2425, found $m/z$ 644.2440 [M+H]$^+$. 

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cis-Benzyl 3-((2S)-3-methoxy-2-(1-((S)-1-methoxy-4-methyl-1-oxopentan-2-yl)-3-(4-(trifluoromethyl)phenyl)aziridine-2-carboxamido)-3-oxopropyl)-1H-indole-1-carboxylate (433a/b)

Compound 433a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether / dichloromethane 3:2:1; R_f = 0.16) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (122 mg, 0.18 mmol, 51% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) δ 8.16 (br s, 1H, -NH-), 7.52 (d, J = 7.8 Hz, 2H, Ar-H), 7.50 – 7.46 (m, 2H, Ar-H), 7.43 – 7.36 (m, 5H, Ar-H), 7.34 – 7.29 (m, 4H, Ar-H), 7.28 – 7.23 (m, 1H, Ar-H), 5.47 (d, J = 12.1, 1H, -CH$_2$Ph), 5.43 (d, J = 12.1, 1H, -CH$_2$Ph), 4.59 (dt, J = 8.0, 5.9 Hz, 1H, -CONHCH-), 3.57 (s, 3H, -CO$_2$C$_2$H$_3$), 3.38 (s, 3H, -CO$_2$C$_2$H$_3$), 3.19 (dd, J = 14.8, 5.8 Hz, 1H, -CH$_2$-indole), 3.13 (dd, J = 14.8, 5.8 Hz, 1H, -CH$_2$-indole), 3.03 (d, J = 7.1 Hz, 1H, -CH- aziridine), 2.67 (t, J = 6.4 Hz, 1H, -NCH-), 2.61 (d, J = 7.1 Hz, 1H, -CH- aziridine), 1.85 – 1.78 (m, 1H, -CH(CH$_3$)$_2$), 1.75 – 1.62 (m, 2H, -CH$_2$CH(CH$_3$)$_2$), 0.89 (d, J = 6.2 Hz, 3H, -CH(CH$_3$)$_2$), 0.88 (d, J = 6.2 Hz, 3H, -CH(CH$_3$)$_2$), $^{19}$F-NMR (471 MHz, CDCl$_3$) δ -62.6; $^{13}$C-NMR (125 MHz, CDCl$_3$) δ 172.6 (-C=O ester), 171.4 (-C=O ester), 166.3 (-C=O amide), 150.9 (-C=O carbamate), 138.7, 135.2, 130.6, 129.8 (q, J =
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32 Hz), 128.9, 128.8, 128.5, 128.0, 125.6, 125.2 (q, \( J = 3.7 \) Hz), 125.0, 124.2 (q, \( J = 270 \) Hz, \(-\text{CF}_3\)), 124.1, 123.2, 119.1, 116.0, 115.4, 68.8 (\(-\text{NHCH}-\)), 68.5 (\(-\text{CH}_2\text{Ph}\)), 52.1 (\(-\text{NHCH}-\)), 51.7 (\(-\text{OCH}_3\)), 46.7 (\(-\text{OCH}_3\)), 46.2 (\(-\text{CH-aziridine}\)), 42.0 (\(-\text{CH-aziridine}\)), 30.4 (\(-\text{CH}_2\text{CH}(\text{CH}_3)_2\)), 27.8 (\(-\text{CH}_2\text{-indole}\)), 24.8 (\(-\text{CH}(\text{CH}_3)_2\)), 22.8 (\(-\text{CH}(\text{CH}_3)_2\)), 22.7 (\(-\text{CH}(\text{CH}_3)_2\)); FT-IR (thin film, cm\(^{-1}\)) \( \nu = \) 3385 (N-H), 2956, 2872, 1747 (ester C=O), 1685 (amide C=O), 1622, 1520, 1399, 1358, 1245, 1164, 1131, 1095, 1065, 1018; \([\alpha]_D^{20} -48.3\) (c 1.0, CHCl\(_3\)); MS (MALDI-TOF): \( m/z = 732.55 \) [M+K]\(^+\); HRMS (NSI) exact mass calculated for C\(_{37}\)H\(_{38}\)F\(_3\)O\(_7\)N\(_3\) requires \( m/z = 694.2735 \), found \( m/z = 694.2730 \) [M+H]\(^+\).

cis-Benzyl 3-((2S)-2-(2-(9H-fluoren-2-yl)-3-((S)-1-methoxymethylthio)-1-oxobutan-2-ylcarbamoyl)aziridin-1-yl)-3-methoxy-3-oxopropyl)-1H-indole-1-carboxylate (436a/b)

Compound 436a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether / dichloromethane 3:2:1; \( R_f = 0.16 \)) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (204 mg, 0.279 mmol, 79% yield).
$^1$H-NMR (500 MHz, CD$_3$CN) δ 8.02 (br s, 1H, -NH-), 7.78 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.60 – 7.55 (m, 4H, Ar-H), 7.39 – 7.25 (m, 8H, Ar-H), 7.22 – 7.20 (m, 2H, Ar-H), 7.17 (s, 1H, Ar-H), 6.98 (d, $J = 12.2$ Hz, 1H, -CH$_2$Ph), 5.00 (d, $J = 12.2$ Hz, 1H, -CH$_2$Ph), 4.38 (dt, $J = 8.4$, 4.9 Hz, 1H, -NHCH-), 3.80 (d, $J = 21.0$ Hz, 1H, AB -CH$_2$- fluorene), 3.75 (s, 3H -OCH$_3$), 3.40 – 3.32 (m, 1H, -CH$_2$-indole), 3.27 – 3.18 (m, 2H, -CH$_2$-indole + -NCH-), 2.62 (d, $J = 7.2$ Hz, 1H, -CH- aziridine), 2.56 (d, $J = 7.2$ Hz, 1H, -CH- aziridine), 2.52 – 2.42 (m, 2H, -CH$_2$S-), 2.07-2.00 (m, 1H, -CH$_2$CH$_2$SCH$_3$), 2.03 (s, 3H, -SCH$_3$), 1.92 – 1.86 (m, 1H, -CH$_2$CH$_2$SCH$_3$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 172.3 (-C=O ester), 172.0 (-C=O ester), 166.5 (-C=O amide), 150.1 (-C=O carbamate), 143.6, 143.2, 141.5, 141.1, 135.7, 135.1, 133.2, 129.7, 128.6, 128.5, 128.0, 126.9, 126.8, 126.1, 125.2, 125.0, 124.0, 123.1, 119.9, 119.3, 118.7, 115.7, 115.6, 70.0 (-NCH-), 68.3 (-CH$_2$Ph), 52.5 (-NHCH-), 52.3 (-OCH$_3$), 50.9 (-OCH$_3$), 48.2 (-CH- aziridine), 47.3 (-CH- aziridine), 36.8 (-CH$_2$- fluorene), 32.2 (-NHCHCH$_2$-), 29.7 (-CH$_2$S-), 29.1 (-CH$_2$-indole), 15.5 (-SCH$_3$); FT-IR (ATR, cm$^{-1}$) ν = 3385 (N-H), 2925, 1737 (ester C=O), 1675 (amide C=O), 1519, 1455, 1398, 1253, 1212, 1088; [$\alpha$]$_D^{23}$ –106.2 (c 1.0, CHCl$_3$); MS (MALDI-TOF): m/z = 770.4 [M+K]$^+$; HRMS (NSI) exact mass calculated for C$_{42}$H$_{41}$O$_3$N$_3$SO$_2$H requires m/z 764.2636, found m/z 764.2654 [M+O$_2$+H]$^+$. *

*Oxidation of methionine and/or tryptophan residues during MS analysis are commonly observed.
cis-Ferroceny1-(S)-methyl 2-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)-3-phenylaziridine-2-carboxamido)-4-methylpentanoate (447a/b)

Compound 447a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 1:1; R_f = 0.19) afforded a brown oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (160 mg, 0.252 mmol, 73% yield).

1^H-NMR (500 MHz, CDCl_3) δ 7.33 (d, J = 8.3 Hz, 2H, Ar-H), 7.18 – 7.13 (m, 8H, Ar-H), 4.59 (t, J = 1.5 Hz, 2H, Cp-H), 4.43 (dt, J = 8.7, 5.0 Hz, 1H, -NHCH-), 4.29 (t, J = 1.9 Hz, 2H, Cp-H), 4.03 (s, 5H, Cp-H), 3.71 (s, 3H, -CO_2CH_3), 3.53 (s, 3H, -CO_2CH_3), 3.33 (dd, J = 13.8, 6.1 Hz, 1H, -CH_2Ph), 3.13 (dd, J = 13.8, 7.2 Hz, 1H, -CH_2Ph), 2.91 (dd, J = 7.2, 6.1 Hz, 1H, -NCH-), 2.76 (d, J = 7.2 Hz, 1H, -CH- aziridine), 2.60 (d, J = 7.2 Hz, 1H, -CH- aziridine), 1.65 – 1.51 (m, 3H, -CH_2CH(CH_3)_2), 0.92 (d, J = 4.6 Hz, 3H, -CH(CH_3)_2), 0.91 (d, J = 4.6 Hz, 3H, -CH(CH_3)_2); 1^3C-NMR (126 MHz, CDCl_3) δ 172.5 (-C=O ester), 171.8 (-C=O ester), 166.3 (-C=O amide), 138.4, 136.6, 131.9, 129.3, 128.4, 127.6, 126.9, 125.7, 85.2 (Cp), 72.6 (-NCH-), 69.6 (Cp), 68.8(9) (Cp), 68.8(7) (Cp), 66.6 (Cp), 66.3 (Cp), 52.1 (-OCH_3), 52.0 (-OCH_3), 50.0 (-NHCH-), 47.5 (-CH- aziridine), 46.9 (-CH- aziridine), 41.8 (-CH_2CH(CH_3)_2), 39.1 (-CH_2Ph), 24.6 (-CH(CH_3)_2), 22.8 (-CH(CH_3)_2), 22.1 (-CH(CH_3)_2); FT-IR (thin film, cm\(^{-1}\)) ν = 3385 (N-H), 2951, 1742 (ester...
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C=O), 1677 (amide C=O), 1522, 1437, 1204, 1104, 1026, 819; [α]D23 –93.1 (c 1.0, CHCl3); MS (MALDI-TOF): m/z = 659.6 [M+Na]+; HRMS (NSI): exact mass calculated for C36H41O5N2Fe requires m/z 635.2406, found m/z 635.2406 [M+H]+.

cis-(S)-Methyl 2-((S)-3-((S)-3-(4-(allyloxy)phenyl)-1-methoxy-1-oxopropan-2-yl)-3-phenylaziridine-2-carboxamido)-4-methylpentanoate (429a/b)

Compound 429a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 1:1; Rf = 0.20) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (320 mg, 0.63 mmol, 73% yield).

1H-NMR (500 MHz, CDCl3) δ 7.25 – 7.18 (m, 5H, Ar-H), 7.15 (d, J = 8.9 Hz, 1H, -NH-), 7.03 (d, J = 8.7 Hz, 2H, Ar-H), 6.68 (d, J = 8.7 Hz, 2H, Ar-H), 6.01 (ddt, J = 17.3, 10.5, 5.3 Hz, 1H, -CH=CH2), 5.37 (dd, J = 17.3, 1.6 Hz, 1H, -CH=CH2), 5.26 (dd, J = 10.5, 1.4 Hz, 1H, -CH=CH2), 4.47 – 4.37 (m, 3H, -NHCH- + -OCH2), 3.71 (s, 3H, -OCH3), 3.53 (s, 3H, -OCH3), 3.24 (dd, J = 13.9, 6.0 Hz, 1H, -CH2Ph), 3.05 (dd, J = 13.9, 7.3 Hz, 1H, -CH2Ph), 2.85 (dd, J = 7.2, 6.0 Hz, 1H, -NCH-), 2.77 (d, J = 7.2 Hz, 1H, -CH- aziridine),
2.57 (d, J = 7.2 Hz, 1H, -CH- aziridine), 1.62 – 1.50 (m, 3H, -CH(CH₃)₂), 0.91 (d, J = 6.4 Hz, 3H, -CH(CH₃)₂), 0.90 (d, J = 6.4 Hz, 3H, -CH(CH₃)₂); ¹³C-NMR (126 MHz, CDCl₃) δ 172.6 (-C=O ester), 172.0 (-C=O ester), 166.4 (-C=O amide), 157.6 (C-OAllyl), 134.6, 133.4, 130.4, 128.9, 128.0, 127.7, 127.6, 117.7, 114.8, 72.8 (-NCH-), 68.9 (-OCH₂-), 52.2 (-OCH₃), 52.1 (-OCH₃), 50.1 (-NHCH-), 47.6 (-CH- aziridine), 47.0 (-CH- aziridine), 41.9 (-CH₂CH(CH₃)₂), 38.4 (-CH₂Ph), 24.8 (-CH(CH₃)₂), 22.9 (-CH(CH₃)₂), 22.2 (-CH(CH₃)₂); FT-IR (thin film): ν (cm⁻¹) = 3388, 2955, 1742, 1679, 1512, 1436, 1244, 1204, 1027; [α]D ²⁴ = -25.4 (c 1.0, CHCl₃); MS (MALDI-TOF) m/z 531.5 [M+Na]+; HRMS (HNESP) exact mass calculated for C₂₉H₄₇N₂O₆ requires m/z 509.2646, found m/z 509.2637 [M+H]+.

 cis-(2S)-Allyl 2-(1-((S)-1-(allylamino)-4-methyl-1-oxopentan-2-yl)-3-phenylaziridine-2-carboxamido)-3-phenylpropanoate (430a/b)

Compound 430a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; Rf = 0.12) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (78 mg, 0.16 mmol, 53% yield).

¹H-NMR (500 MHz, CDCl₃) δ 7.38 – 7.24 (m, 6H, Ar-H), 7.20 – 7.12 (m, 3H, Ar-H + -NH-), 6.69 – 6.64 (m, 2H, Ar-H), 6.16 (d, J = 7.5 Hz, 1H, -NH-), 5.87 – 5.70 (m, 2H,
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CH=CH₂, 5.25 – 5.17 (m, 3H, -CH=CH₂), 5.13 (dd, J = 10.3, 1.4 Hz, 1H, -CH=CH₂), 4.59 (dt, J = 7.5, 6.1 Hz, 1H, -NHCH-), 4.44 (dd, J = 5.9, 1.2 Hz, 2H, -OCH₂-), 3.98 – 3.90 (m, 1H, -NCH₂-), 3.84 – 3.77 (m, 1H, -NCH₂-), 3.09 (d, J = 6.9 Hz, 1H, -CH-aziridine), 2.91 (dd, J = 13.8, 5.1 Hz, 1H, -CH₂Ph), 2.72 (dd, J = 13.8, 6.2 Hz, 1H, -CH₂Ph), 2.61 (d, J = 6.9 Hz, 1H, -CH-aziridine), 2.55 (t, J = 6.5 Hz, 1H, -NC₂H₂), 1.86 – 1.66 (m, 3H, -CH₂CH(CH₃)₂), 0.90 (d, J = 3.4 Hz, 3H, -CH(CH₃)₂), 0.89 (d, J = 3.5 Hz, 3H, -CH(CH₃)₂); ¹³C-NMR (126 MHz, CDCl₃) δ 172.3 (-C=O ester), 170.6 (-C=O ester), 166.3 (-C=O amide), 135.5, 134.9, 134.3, 131.4, 129.4, 128.6, 128.2, 127.5, 127.2, 119.2 (-CH=CH₂), 116.5 (-CH=CH₂), 71.0 (-NCH-), 66.1 (-OCH₂-), 53.1 (-NHCH-), 47.3 (-CH-aziridine), 46.9 (-CH-aziridine), 43.2 (-NCH₂-), 41.8 (-CH₂CH(CH₃)₂), 38.2 (-CH₂Ph), 24.7 (-CH(CH₃)₂), 23.1 (-CH(CH₃)₂), 23.0 (-CH(CH₃)₂); FT-IR (ATR, cm⁻¹) v = 3310 (N-H), 3066, 3032, 2956, 2936, 2860, 1744 (ester C=O), 1662 (amide C=O), 1531, 1452, 1422, 1384, 1367, 1342, 1322, 1260, 1191, 1092, 1027, 930; [α]D²³ +31.1 (c 1.0, CHCl₃); MS (MALDI-TOF): m/z = 526.3 [M+Na]+; HRMS (NSI) exact mass calculated for C₃0H₃₈O₄N₃ requires m/z 504.2857, found m/z 504.2852 [M+H]+.

Methyl 4-(ferrocenyl)benzoate (446)²⁵⁴

![Methyl 4-(ferrocenyl)benzoate (446)²⁵⁴](image)

To a 250 mL round-bottom flask was added methyl-4-aminobenzoate (5 g, 33.1 mmol, 1 equiv.) and distilled water (16 mL). Concentrated hydrochloric acid (8 mL) was added, and the reaction mixture was cooled to 0 °C using an ice bath. A solution of sodium nitrite (2.28 g, 33.1 mmol, 1 equiv.) in distilled water (13 mL) was added dropwise via pipette. A colour change from pale brown to yellow indicated the formation of diazonium salt 444. The solution was stirred for a further 30 minutes and the temperature maintained at 0 °C until use. To a 250 mL round-bottom flask containing a solution of ferrocene 445 (6.16 g, 33.1 mmol, 1 equiv.) in diethyl ether (50 mL) was added cetyltrimethylammonium bromide (330 mg, 0.906 mmol, 2.7 mol%). The mixture was cooled to 0 °C and the aqueous solution of diazonium salt 444 was added dropwise via pipette. The biphasic mixture was stirred vigorously for a further two hours before being
diluted with diethyl ether (50 mL). Saturated aqueous sodium bicarbonate solution (50 mL) was added, and the aqueous layer extracted with diethyl ether (2 x 100 mL). The combined organic layers were washed with distilled water (200 mL) and brine (150 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 5%) afforded dark red crystals (3.92 g, 12.2 mmol, 37% yield); the physicochemical properties matched those previously reported.

M.p. 119–121 °C (petroleum ether / diethyl ether); $^1$H-NMR (500 MHz, CDCl$_3$) δ 7.94 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.48 (d, $J = 7.6$ Hz, 2H, Ar-H), 4.77 (br s, 2H, Cp-H), 4.45 (br s, 2H, Cp-H), 4.09 (s, 5H, Cp-H), 3.92 (s, 3H, -OCH$_3$); FT-IR (thin film, cm$^{-1}$) $\nu = 2951$ (C-H), 1715 (C=O), 1609, 1430, 1281, 1183, 1105.

4-(Ferrocenyl)benzyl alcohol (447)$^{240}$

A flame-dried 250 mL round-bottom flask was cooled under nitrogen and charged with methyl 4-(ferrocenyl)benzoate 446 (290 mg, 0.91 mmol, 1 equiv.) and anhydrous toluene (10 mL). The resulting solution was cooled to -78 °C using a dry ice/acetone bath. Diisobutylaluminium hydride (1.5 mL, 1.82 mmol, 2 equiv., 1.2 M in toluene) was added dropwise via a 2.5 mL syringe. The reaction was allowed to warm up slowly to room temperature over 18 hours. Ethyl acetate (20 mL) and distilled water (20 mL) were added to the reaction mixture, which was stirred vigorously for a further 30 minutes. The aqueous layer was extracted with ethyl acetate (30 mL) and the combined organic layers washed with distilled water (50 mL) and subsequently brine (50 mL). The orange solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo to afford an orange solid whose physicochemical properties matched those previously reported (238 mg, 0.82 mmol, 90% yield).

$R_f = 0.10$ (9:1 petroleum ether / ethyl acetate); m.p. 104–106 °C (hexane); $^1$H-NMR (500 MHz, CDCl$_3$) δ 7.48 (d, $J = 8.3$ Hz, 2H, Ar-H), 7.29 (d, $J = 8.3$ Hz, 2H, Ar-H), 4.67 (d,
4-(Ferrocenyl)benzaldehyde (443)\textsuperscript{254}

To a 100 mL round-bottom flask containing 4-(ferrocenyl)benzyl alcohol 447 (237 mg, 0.81 mmol, 1 equiv.) dissolved in anhydrous dichloromethane (30 mL) under an atmosphere of nitrogen was added activated manganese dioxide (1.76 g, 20.3 mmol, 25 equiv.). The heterogeneous mixture was stirred at room temperature for 18 hours. The black solid manganese dioxide was removed by filtration and the solvent removed \textit{in vacuo} to afford a red solid that was used without further purification (202 mg, 0.70 mmol, 86% yield); the physicochemical properties matched those previously reported for the title compound 443.

M.p. 121–123 °C (hexane / ethyl acetate); $^1$H-NMR (500 MHz, CDCl$_3$) $\delta = 9.97$ (s, 1H, -CHO), 7.79 (d, $J = 8.3$ Hz, 2H, Ar-H), 7.60 (d, $J = 8.3$ Hz, 2H, Ar-H), 4.74 (t, $J = 1.9$ Hz, 2H, Cp-H), 4.44 (t, $J = 1.9$ Hz, 2H, Cp-H), 4.05 (s, 5H, Cp-H); MS (MALDI-TOF): $m/z = 290.05$ [M]$^+$. 

$J =$ 5.6 Hz, 2H, CH$_2$OH), 4.64 (t, $J =$ 1.9 Hz, 2H, Cp-H), 4.32 (t, $J =$ 1.9 Hz, 2H, Cp-H), 4.04 (s, 5H, Cp-H), 1.60 (t, $J =$ 5.6 Hz, 1H, -OH); MS (MALDI-TOF): $m/z = 292.1$ [M]$^+$. 

4-\textit{(Ferrocenyl)benzaldehyde (443)}\textsuperscript{254}
**cis-(2S)-Methyl 4-methyl-2-(3-phenyl-1-((R)-1-phenylethyl)aziridine-2-carboxamido)pentanoate (455a/b)**

Compound 455a/b was prepared according to the procedure for compound 427. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; R<sub>f</sub> = 0.50) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (154 mg, 0.39 mmol, 91% yield).

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 7.39 (d, J = 8.0 Hz, 2H, Ar-H), 7.28 (t, J = 7.5 Hz, 2H, Ar-H), 7.23 – 7.20 (m, 2H, Ar-H), 7.12 – 7.03 (m, 4H, Ar-H), 6.77 (br d, J = 8.6 Hz, 1H, -NH), 4.32 – 4.26 (m, 1H, -NHC<sub>H</sub>-), 3.40 (s, 3H, -OC<sub>3</sub>H<sub>3</sub>), 3.01 (d, J = 7.1 Hz, 1H, -C<sub>H</sub>-aziridine), 2.85 (q, J = 6.5 Hz, 1H, -NCH-), 2.55 (d, J = 7.1 Hz, 1H, -CH-aziridine), 1.55 – 1.39 (m, 6H, -CH<sub>3</sub> + -CH<sub>2</sub>CH<sub>-</sub>-), 0.85 (d, J = 3.5 Hz, 3H, -CH(CH<sub>3</sub>)<sub>2</sub>), 0.84 (d, J = 3.5 Hz, 3H, -C(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C-NMR (126 MHz, CDCl<sub>3</sub>) δ 172.4 (-C=O ester), 167.5 (-C=O amide), 143.2, 134.9, 128.7, 128.1, 127.8, 127.7, 127.3, 127.0, 69.1 (-NCH-), 52.1 (-NHCH-), 50.2 (-OCH<sub>3</sub>), 47.7 (-CH-aziridine), 46.7 (-CH-aziridine), 42.0 (-CH<sub>2</sub>-), 25.0 (-CH(CH<sub>3</sub>)<sub>2</sub>), 22.9 (-CH(CH<sub>3</sub>)<sub>2</sub>), 22.8 (-CH(CH<sub>3</sub>)<sub>2</sub>), 22.4 (-CH<sub>3</sub>); FT-IR (thin film): ν (cm<sup>-1</sup>) = 3392 (N-H), 3036 (C-H), 2960 (C-H), 2872 (C-H), 1744 (ester C=O), 1680 (amide C=O), 1516, 1452, 1372, 1328, 1260, 1204, 1156, 1100, 1028; [α]<sub>D</sub> <sup>18</sup> = −167.0 (c 0.5, CHCl<sub>3</sub>); MS (MALDI-TOF) m/z 433.4 [M+K]<sup>+</sup>; HRMS (HNESP) exact mass calculated for C<sub>24</sub>H<sub>31</sub>N<sub>2</sub>O<sub>3</sub> requires m/z 395.2329, found m/z 395.2320 [M+H]<sup>+</sup>.
cis-Methyl 2-(2-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3-phenylaziridin-1-yl)-2-methylpropanoate (459a/b)

A 5 mL microwave vial containing crushed 4Å molecular sieves was flame-dried and cooled under a nitrogen atmosphere, followed by the addition of a magnetic stirrer bar. Benzaldehyde 228 (15 mg, 0.14 mmol, 1.1 equiv.) and methyl 2-amino-2-methylpropanoate (15 mg, 0.13 mmol, 1 equiv.) were weighed into a sample vial and dissolved in chloroform-d$_3$ (1 mL) before being transferred to the microwave vial via a 2 mL syringe. The solution was stirred at room temperature under nitrogen for 1 hour before pyridinium triflate (3 mg, 0.013 mmol, 10 mol%) was added and the vial was sealed with a PTFE-lined aluminium crimp cap. The reaction was cooled to -30 °C using a chiller bath before the addition of a solution of (S)-methyl 2-(2-diazoacetamido)-3-phenylpropanoate 386 (38 mg, 0.15 mmol, 1.2 equiv.) in chloroform-d$_3$ (1 mL) was added dropwise to the reaction vial via a 2 mL syringe. The progress of the reaction was monitored by $^1$H-NMR until complete disappearance of the imine intermediate (~24 h). After being allowed to warm to room temperature, the mixture was filtered through a short plug of silica eluting with diethyl ether and the solvent was removed in vacuo. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; R$_f$ = 0.19) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (51 mg, 0.12 mmol, 93% yield).
1H-NMR (500 MHz, CDCl$_3$) δ 7.34 – 7.16 (m, 8H, Ar-H), 7.12 – 7.06 (m, 2H, Ar-H), 6.94 (d, $J = 7.9$ Hz, 1H, -NH-), 6.51 (d, $J = 8.0$, 5.9 Hz, 1H, -NHCH-), 3.68 (s, 3H, -OCH$_3$), 3.44 (s, 3H, -OCH$_3$), 3.35 (d, $J = 6.9$ Hz, 1H, -CH- aziridine), 3.07 (dd, $J = 13.8$, 6.1 Hz, 1H, -CH$_2$Ph), 2.99 (dd, $J = 13.8$, 5.7 Hz, 1H, -CH$_2$Ph), 2.86 (d, $J = 6.9$ Hz, 1H, -CH- aziridine), 1.36 (s, 3H, -CH$_3$), 1.34 (s, 3H, -CH$_3$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 173.8 (-C=O ester), 171.1 (-C=O ester), 167.3 (-C=O amide), 136.0, 134.9, 129.4, 128.6, 128.1, 127.8, 1274, 127.1, 61.4 (-NC-), 52.5 (-NHCH-), 52.2 (-OCH$_3$), 52.1 (-OCH$_3$), 42.1 (-CH- aziridine), 41.6 (-CH- aziridine), 38.1 (-CH$_2$Ph), 23.7 (-CH$_3$), 22.9 (-CH$_3$); FT-IR (thin film): γ (cm$^{-1}$) = 3384 (N-H), 3062, 3033, 2990, 2953, 1738 (ester C=O), 1676 (amide C=O), 1512, 1450, 1366, 1279, 1195, 1147, 1078, 1031; [$\alpha$]$_{D}^{23}$ –31.7 (c 1.0, CHCl$_3$); MS (MALDI-TOF) m/z 462.9 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{24}$H$_{29}$N$_2$O$_5$ requires m/z 425.2071, found m/z 425.2069 [M+H]$^+$.  

**cis-Methyl 2-(1-((R)-1-methoxy-1-oxo-3-phenylpropan-2-yl)-3-phenylaziridine-2-carboxamido)-2-methylpropanoate (457a/b)**

Compound 457a/b was prepared according to the procedure for compound 459 above. Purification via flash column chromatography on silica gel (eluent: diethyl ether / petroleum ether 3:1; $R_f$ = 0.14) afforded a colourless oil whose physicochemical properties were consistent with those of a single diastereomer of the title compound (76 mg, 0.18 mmol, 80% yield).
(S)-Methyl 2-(2-(tert-butoxycarbonylamino)-2-methylpropanamido)-3-phenylpropanoate (461)

A 250 mL round-bottom flask was charged with 2-((tert-butoxycarbonylamino)-2-methylpropanoic acid (1 g, 4.92 mmol, 1 equiv.) and the solid dissolved in a mixture of dichloromethane and N,N-dimethylformamide (5:1, 30 mL) under a nitrogen atmosphere. (S)-Phenylalanine methyl ester hydrochloride (1.27 g, 5.90 mmol, 1.2 equiv.) was added in one portion, followed by the addition of N,N-diisopropylethylamine (3.9 mL, 22.1 mmol, 4.5 equiv.) via a 10 mL syringe. The solution was stirred at room temperature for 18 hours before the addition of distilled water (40 mL). The reaction mixture was extracted with dichloromethane (2 x 40 mL) and the combined organic layers washed with brine (50 mL), dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 9:1; Rf = 0.33) afforded a white solid whose physicochemical properties matched those previously reported (1.29 g, 3.54 mmol, 72% yield).
Experimental Section

M.p. 139–140 °C; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.30 – 7.20 (m, 3H, Ar-H), 7.14 – 7.09 (m, 2H, Ar-H), 6.85 (br s, 1H, -NH-), 4.87 (br s, 1H, -NH-), 4.85 (dt, $J$ = 7.6, 5.9 Hz, 1H, -NHCH-), 3.69 (s, 3H, -OCH$_3$), 3.17 – 3.07 (m, 2H, Ar-H), 3.11 (s, 3H, -OC$_3$H$_3$), 1.45 (s, 3H, -CC(CH$_3$)$_2$), 1.42 (s, 12H, -CC(CH$_3$)$_2$ + -CC(CH$_3$)$_3$); FT-IR (thin film, cm$^{-1}$) $\nu$ = 3319 (N-H), 2979 (C-H), 2932 (C-H), 1750 (C=O ester), 1688 (C=O carbamate), 1656 (C=O amide), 1519, 1285, 1167, 1085.

(S)-Methyl 2-(2-amino-2-methylpropanamido)-3-phenylpropanoate (462)$^{252}$

A 250 mL round-bottom flask was charged with a solution of (S)-methyl 2-(2-(tert-butoxycarbonylamino)-2-methylpropanamido)-3-phenylpropanoate 461 (1.29 g, 3.54 mmol, 1 equiv.) in dichloromethane (30 mL) under a nitrogen atmosphere. Trifluoroacetic acid (2.5 mL, 28.3 mmol, 8 equiv.) was added dropwise via a 5 mL syringe and the solution stirred at room temperature for 8 hours or until complete disappearance of the starting material was observed by thin layer chromatography (eluent: dichloromethane / ethyl acetate 9:1). The reaction was cooled to 0 °C using an ice bath, and triethylamine (3.9 mL, 28.3 mmol, 8 equiv.) added via a 5 mL syringe. After stirring at room temperature for 10 minutes, the solution was diluted with distilled water (40 mL) and the organics extracted with dichloromethane (2 x 30 mL). The combined organic layers were washed with distilled water (2 x 50 mL) and brine (50 mL), and filtered. The solvent was removed in vacuo to afford a yellow oil which was used without further purification (725 mg, 2.74 mmol, 77% yield); the physicochemical properties were consistent with those previously reported for the title compound.

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.98 (br d, $J$ = 7.6 Hz, 1H, -NH-), 7.31 – 7.21 (m, 3H, Ar-H), 7.15 – 7.11 (m, 2H, Ar-H), 4.83 – 4.78 (m, 1H, -NHCH-), 3.72 (s, 3H, -OCH$_3$), 3.17 (dd, $J$ = 13.9, 5.8 Hz, 1H, -CH$_2$-), 3.06 (dd, $J$ = 13.9, 6.9 Hz, 1H, -CH$_2$-), 1.47 (s, 2H, -NH$_2$), 1.32 (s, 3H, -CC(CH$_3$)$_2$), 1.25 (s, 3H, -CC(CH$_3$)$_3$); FT-IR (thin film, cm$^{-1}$) $\nu$ = 3358 (N-H), 3028 (C-H), 2960 (C-H), 2931 (C-H), 2867 (C-H), 1738 (ester C=O), 1667 (amide C=O), 1493, 1451, 1358, 1180, 1079; [$\alpha$]$_D^{21}$ +50.1 (c 1.0, CHCl$_3$).
**Experimental Section**

(S)-Methyl 2-(2-(2-bromoacetamido)-2-methylpropanamido)-3-phenylpropanoate (463)

![Chemical structure of 463]

Compound 463 was prepared according to the general procedure described above. Purification via flash column chromatography on silica gel (eluent: diethyl ether 100%; \( R_f = 0.23 \)) afforded a white solid (514 mg, 1.34 mmol, 68% yield) whose physicochemical properties were consistent with those of the title compound.

M.p. 128–130 °C (diethyl ether); \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.34 – 7.22 (m, 3H, Ar-\( H \)), 7.17 – 7.07 (m, 3H, Ar-\( H \) + -NH-), 6.50 (br d, \( J = 7.5 \) Hz, 1H, -NH-), 4.85 (dt, \( J = 7.6, 5.8 \) Hz, 1H, -NHCH-), 3.79 (s, 2H, -CH\(_2\)Br), 3.75 (s, 3H, -OCH\(_3\)), 3.19 (dd, \( J = 13.9, 5.7 \) Hz, 1H, -CH\(_2\)Ph), 3.11 (dd, \( J = 13.9, 6.0 \) Hz, 1H, -CH\(_2\)Ph), 1.55 (s, 3H, -C(CH\(_3\))\(_2\)), 1.53 (s, 3H, -C(CH\(_3\))\(_2\)) \(^{13}\)C-NMR (126 MHz, CDCl\(_3\)) \( \delta \) 173.5 (C=O amide), 171.9 (C=O amide), 165.2 (C=O ester), 135.8, 129.4, 128.7, 127.4, 57.7 (-C(CH\(_3\))\(_2\)), 53.5 (-NHCH-), 52.6 (-OCH\(_3\)), 37.8 (-CH\(_2\)Ph), 29.4 (-CH\(_2\)Br), 24.7 (-C(CH\(_3\))\(_2\)), 24.6 (-C(CH\(_3\))\(_2\)); FT-IR (thin film): \( \nu \) (cm\(^{-1}\)) = 3303 (N-H), 2931 (C-H), 2863 (C-H), 1734 (C=O), 1658 (C=O), 1544, 1493, 1455, 1219, 1117, 1058; \([\alpha]_D^{19}\) +42.2 (c 1.0, CHCl\(_3\)); MS (MALDI-TOF) \( m/z \) 407.2, 409.2 [M+Na]\(^+\); HRMS (HNESI) exact mass calculated for \( C_{16}H_{22}BrN_2O_4 \) requires \( m/z \) 385.0757, 387.0737 found \( m/z \) 385.0762, 387.0740 [M+H]\(^+\).

(S)-Methyl 2-(2-(2-diazoacetamido)-2-methylpropanamido)-3-phenylpropanoate (464)

![Chemical structure of 464]

Compound 464 was prepared according to method A. Purification via flash column chromatography on silica gel (eluent: diethyl ether 100%; \( R_f = 0.17 \)) afforded a pale
yellow solid whose physicochemical properties were consistent with those of the title compound (548 mg, 1.62 mmol, 68% yield).

M.p. 136–138 °C; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.33 – 7.21 (m, 3H, Ar-H), 7.14 – 7.10 (m, 2H, Ar-H), 6.79 (d, $J = 7.5$ Hz, 1H, -NH-), 5.61 (s, 1H, -NH-), 4.84 (dt, $J = 7.6$, 5.9 Hz, 1H, -NHCH-), 4.69 (s, 1H, -CHN$_2$), 3.74 (s, 3H, -OCH$_3$), 3.17 (dd, $J = 13.9$, 5.6 Hz, 1H, -CH$_2$Ph), 3.11 (dd, $J = 13.9$, 6.2 Hz, 1H, -CH$_2$Ph), 1.53 (s, 3H, -C(CH$_3$)$_2$), 1.51 (s, 3H, -C(CH$_3$)$_2$); $^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$ 174.2 (C=O ester), 172.1 (C=O amide), 165.3 (C=O amide), 135.9, 129.5, 128.6, 127.3, 57.9 (-C(CH$_3$)$_2$), 53.5 (-NHCH-), 52.5 (-OCH$_3$), 48.1 (-CHN$_2$), 37.9 (-CH$_2$Ph), 25.9 (-C(CH$_3$)$_2$), 25.4 (-C(CH$_3$)$_2$); FT-IR (thin film): $\nu$ (cm$^{-1}$) = 3389 (N-H), 3252 (N-H), 3059, 2973, 2929, 2112 (diazo C=N$_2$), 1734 (ester C=O), 1648 (amide C=O), 1631 (amide C=O), 1542, 1470, 1435, 1380, 1356, 1343, 1246, 1216, 1198, 1164, 1016, 972; [\(\alpha\)]$_D^{20}$ +51.7 (c 1.0, CHCl$_3$); MS (MALDI-TOF) $m/z$ 370.8 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{16}$H$_{21}$N$_4$O$_4$ requires $m/z$ 333.1557, found $m/z$ 333.1557 [M+H]$^+$.

cis-Methyl 2-(2-((1-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamoyl)-3-phenylaziridin-1-yl)-2-methylpropanoate (460a and 460b)
Compound 460a and 460b were prepared according to the procedure for compound 459 above. Purification via flash column chromatography on silica gel (eluent: diethyl ether 100%) afforded two separable diastereomers, (S,S,S)-460a and (R,R,S)-460b as colourless oils whose physicochemical properties were consistent with those of the title compound.

R<sub>f</sub> = 0.60 (diethyl ether 100%); 261 mg, 0.51 mmol, 60% yield; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 7.39 – 7.36 (m, 2H, Ar-H), 7.31 – 7.18 (m, 6H, Ar-H), 7.12 – 7.08 (m, 2H, Ar-H), 6.84 (d, J = 7.9 Hz, 1H, -NH-), 4.71 – 4.63 (m, 1H, -NHCH-), 3.68 (s, 3H, -OCH<sub>3</sub>), 3.67 (s, 3H, -OCH<sub>3</sub>), 3.37 (d, J = 6.9 Hz, 1H, -CH- aziridine), 3.06 (dd, J = 13.9, 5.8 Hz, 1H, -CH$_2$Ph), 2.98 (dd, J = 13.8, 6.8 Hz, 1H, -CH$_2$Ph), 2.77 (d, J = 6.9 Hz, 1H, -CH-aziridine), 1.43 (s, 3H, -CH$_3$), 1.30 (s, 3H, -CH$_3$), 1.05 (s, 3H, -CH$_3$), 0.92 (s, 3H, -CH$_3$); <sup>13</sup>C-NMR (126 MHz, CDCl<sub>3</sub>) δ 174.3 (C=O ester), 173.8 (C=O ester), 172.0 (C=O amide), 167.7 (C=O amide), 136.3, 135.2, 129.5, 128.5, 128.3, 128.0, 127.8, 127.0, 61.3 (-NC-), 56.7 (-NHC-), 53.4 (-NHCH-), 52.4 (-OCH$_3$), 52.2 (-OCH$_3$), 41.4 (-CH-aziridine), 41.3 (-CH- aziridine), 37.9 (-CH$_2$Ph), 25.9 (-CH$_3$), 24.5 (-CH$_3$), 23.3 (-CH$_3$), 21.4 (-CH$_3$); FT-IR (thin film): ν (cm$^{-1}$) = 3341 (N-H), 3066, 3033, 2985, 2952, 1743 (ester C=O), 1670 (amide C=O), 1512, 1457, 1388, 1366, 1278, 1212, 1150, 1029; [α]$_D^{21}$ +61.1 (c 1.0, CHCl<sub>3</sub>); MS (MALDI-TOF) m/z 547.76 [M+K]$^+$; HRMS (HNESP) exact mass calculated for C$_{28}$H$_{36}$N$_3$O$_6$ requires m/z 510.2599, found m/z 510.2589 [M+H]$^+$. 

R<sub>f</sub> = 0.36 (diethyl ether 100%); 126 mg, 0.25 mmol, 29% yield; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 7.39 – 7.36 (m, 2H, Ar-H), 7.30 – 7.18 (m, 6H, Ar-H), 7.13 – 7.05 (m, 3H, Ar-H + -NH-), 4.68 (dt, J = 7.2, 6.2 Hz, 1H, -NHCH-), 3.68 (s, 3H, -OCH$_3$), 3.68 (s, 3H, -OCH$_3$), 3.36 (d, J = 6.9 Hz, 1H, -CH- aziridine), 3.10 (dd, J = 13.8, 5.7 Hz, 1H, -CH$_2$Ph), 2.96 (dd, J = 13.8, 6.8 Hz, 1H, -CH$_2$Ph), 2.75 (d, J = 6.9 Hz, 1H, -CH- aziridine), 1.42 (s, 3H, -CH$_3$), 1.31 (s, 3H, -CH$_3$), 1.06 (s, 3H, -CH$_3$), 0.97 (s, 3H, -CH$_3$); <sup>13</sup>C-NMR (126 MHz, CDCl<sub>3</sub>) δ 174.3 (C=O ester), 174.0 (C=O ester), 172.0 (C=O amide), 167.8 (C=O amide), 136.5, 135.2, 129.5, 128.5, 128.3, 127.9, 127.8, 127.0, 61.3 (-NC-), 56.9 (-NHC-), 53.7 (-NHCH-), 52.4 (-OCH$_3$), 52.3 (-OCH$_3$), 41.4 (-CH- aziridine), 41.3 (-CH- aziridine), 37.9 (-CH$_2$Ph), 25.9 (-CH$_3$), 24.4 (-CH$_3$), 23.4 (-CH$_3$), 21.6 (-CH$_3$); FT-IR (thin film): ν (cm$^{-1}$) = 3341 (N-H), 3033, 2989, 2952, 2864, 1739 (ester C=O), 1666 (amide C=O), 1512, 1450, 1388, 1366, 1278, 1208, 1150, 1029; [α]$_D^{21}$ -5.1 (c 1.0,
CHCl₃; MS (MALDI-TOF) m/z 547.81 [M+K]⁺; HRMS (HNESP) exact mass calculated for C₂₈H₃₆N₃O₆ requires m/z 510.2599, found m/z 510.2588 [M+H]⁺.

(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-3-phenylpropanoate (468)

A 150 mL round-bottom flask containing (S)-2-acetoxy-3-phenylpropanoic acid 467 (500 mg, 2.40 mmol, 1 equiv.) under a nitrogen atmosphere was charged with dichloromethane (25 mL), N,N-dimethylformamide (5 mL) and a magnetic stirrer bar. (S)-Phenylalanine methyl ester hydrochloride (621 mg, 2.88 mmol, 1.2 equiv.) and N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate (1.08 g, 3.36 mmol, 1.4 equiv.) were added in one portion, followed by the addition of N,N-diisopropylethylamine (0.4 mL, 10.8 mmol, 4.5 equiv.) dropwise via a 1 mL syringe. The solution was stirred at room temperature for 18 hours before the addition of distilled water (30 mL). The mixture was extracted with dichloromethane (3 x 30 mL), washed with water (60 mL) and brine (60 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rᵣ = 0.60) afforded a pale yellow oil whose physicochemical properties were consistent with those of the title compound (340 mg, 0.92 mmol, 38% yield).

¹H-NMR (500 MHz, CDCl₃) δ 7.33 – 7.27 (m, 2H, Ar-H), 7.25 – 7.20 (m, 4H, Ar-H), 7.20 – 7.17 (m, 2H, Ar-H), 6.87 – 6.82 (m, 2H, Ar-H), 6.38 (d, J = 8.2 Hz, 1H, -NH-), 5.37 (dd, J = 6.6, 4.8 Hz, 1H, AcOCH-), 4.85 (dt, J = 8.1, 5.5 Hz, 1H, -NHCH-), 3.68 (s, 3H, -CO₂CH₃), 3.19 (dd, J = 14.3, 4.7 Hz, 1H, -CH₂Ph), 3.12 (dd, J = 14.3, 6.7 Hz, 1H, -CH₂Ph), 3.07 (dd, J = 13.8, 5.2 Hz, 1H, -CH₂Ph), 2.93 (dd, J = 13.8, 5.8 Hz, 1H, -CH₂Ph), 2.03 (s, 3H, CH₃CO₂-); ¹³C-NMR (126 MHz, CDCl₃) δ 171.5 (-C=O ester), 169.4 (-C=O ester), 168.5 (-C=O amide), 135.9, 135.5, 129.5, 129.4, 128.64, 128.56, 127.3, 127.2, 74.2 (AcOCH-), 52.6, 52.5, 38.1 (-CH₂Ph), 37.6 (-CH₂Ph), 21.0 (CH₃CO₂-); FT-IR (thin film, cm⁻¹) ν = 3323 (N-H), 3030 (C-H), 2955 (C-H), 1746 (ester C=O), 1684
Experimental Section

(amide C=O), 1531, 1457, 1371, 1226; [α]_D^{22} +52.1 (c 1.0, CHCl_3); MS (MALDI-TOF): m/z = 392.2 [M + Na]^+; HRMS (HNESP): exact mass calculated for C_{21}H_{24}O_5N requires m/z 370.1649, found m/z 370.1653 [M+H]^+.

(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-3-phenylpropanoate (468) and (S)-methyl 2-((R)-2-acetoxy-3-phenylpropanamido)-3-phenylpropanoate (473)

Compounds 468 and 473 were prepared according to the procedure for compound 468 above. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate (10 %) R_f = 0.61) afforded an inseparable mixture of two diastereomers 468 and 473 as a white solid whose physicochemical properties were consistent with those of the title compounds (318 mg, 0.86 mmol, 75% yield).

^1H-NMR (500 MHz, CDCl_3) δ 7.33 – 7.15 (m, 16H, Ar-H), 7.00 – 6.96 (m, 2H, Ar-H), 6.87 – 6.82 (m, 2H, Ar-H), 6.39 (d, J = 7.9 Hz, 1H, -NH-), 6.34 (d, J = 7.6 Hz, 1H, -NH-), 5.40 – 5.35 (m, 2H, AcOCH-), 4.90 – 4.83 (m, 2H, -NHCH-), 3.72 (s, 3H, -CO_2CH_3), 3.68 (s, 3H, -CO_2CH_3), 3.24 – 2.99 (m, 7H, -CH_2Ph), 2.93 (dd, J = 13.8, 5.9 Hz, 1H, -CH_2Ph), 2.02 (s, 3H, CH_3CO_2-), 1.96 (s, 3H, CH_3CO_2-); ^13C-NMR (126 MHz, CDCl_3) δ 171.5 (-C=O), 171.5 (-C=O), 169.4 (-C=O), 169.4 (-C=O), 168.9 (-C=O), 168.5 (-C=O), 136.0, 135.9, 135.5(1), 135.4(7), 129.9, 129.6, 129.4, 128.7, 128.6, 128.5(3), 128.5(2), 127.4, 127.3, 127.2, 127.1, 74.3 (AcOCH-), 74.2 (AcOCH-), 52.7, 52.6, 52.5(2), 52.4(5), 38.0 (-CH_2Ph), 37.9 (-CH_2Ph), 37.8 (-CH_2Ph), 37.6 (-CH_2Ph), 20.9 (CH_3CO_2-), 20.8 (CH_3CO_2-); FT-IR (thin film): ν (cm^{-1}) = 3327 (N-H), 3066 (C-H), 3032 (C-H), 2949 (C-H), 2850 (C-H), 1748 (ester C=O), 1679 (amide C=O), 1528, 1497, 1452, 1439, 1370, 1219, 1119, 1061; MS (MALDI-TOF) m/z 392.2 [M+Na]^+.
(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-4-methylpentanoate (476)

![Chemical Structure](image)

Compound 476 was prepared according to the procedure for compound 468. Purification via flash column chromatography on silica gel (elucent: dichloromethane / ethyl acetate (5%) Rf = 0.31) afforded a colourless oil whose physicochemical properties were consistent with a mixture of 476 and 477 in a diastereomeric ratio (S,S,R,S) of 8:1 by 1H-NMR (491 mg, 1.46 mmol, 61% yield).

1H-NMR (500 MHz, CDCl3) δ 7.29 – 7.20 (m, 3H, Ar-H), 7.20 – 7.14 (m, 2H, Ar-H), 6.31 (d, J = 8.5 Hz, 1H, -NHCH3), 5.40 (dd, J = 6.3, 4.8 Hz, 1H, -AcOCH-), 4.62 – 4.56 (m, 1H, -NHC=H-), 3.70 (s, 3H, -CO2CH3), 3.20 (dd, J = 14.3, 4.8 Hz, 1H, -CH2Ph), 3.15 (dd, J = 14.3, 6.3 Hz, 1H, -CH2Ph), 2.12 (s, 3H, -CO2CH3), 1.57 – 1.49 (m, 1H, -CH2CH(CH3)2), 1.45 – 1.32 (m, 2H, -CH2CH(CH3)2 + -CH(CH3)2), 0.87 (d, J = 6.5 Hz, 3H, -CH(CH3)2), 0.85 (d, J = 6.5 Hz, 3H, -CH(CH3)2); 13C-NMR (126 MHz, CDCl3) δ 173.2 (-C=O), 169.4 (-C=O), 168.6 (-C=O), 135.8, 129.8, 128.5, 127.1, 74.3 (AcOCH-), 52.5, 50.4, 41.7, 37.6, 24.6 (-CH(CH3)2), 22.8 (-CH(CH3)2), 22.0 (-CH(CH3)2), 21.1 (CH3CO2-); FT-IR (thin film): ν (cm⁻¹) = 3319 (N-H), 2959 (C-H), 1750 (ester C=O), 1668 (amide C=O), 1535, 1371, 1226, 1066; [α]D²⁴ = 3.2 (c 0.5, CHCl3); MS (MALDI-TOF) m/z 374.39 [M+K]+; HRMS (HNESP) exact mass calculated for C18H26NO5 requires m/z 336.1807, found m/z 336.1805 [M+H]+.

(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-4-methylpentanoate (476) and (S)-methyl 2-((R)-2-acetoxy-3-phenylpropanamido)-4-methylpentanoate (477)

![Chemical Structure](image)
Compounds 476 and 477 were prepared according to the procedure for compound 468. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 5%; R_f = 0.31) afforded an inseparable mixture of two diastereomers 476 and 477 as a colourless oil whose physicochemical properties were consistent with those of the title compound (540 mg, 1.61 mmol, 67% yield).

\[^1\text{H}-\text{NMR}\ (500\ \text{MHz, CDCl}_3)\ \delta\ 7.24 - 7.08\ (m, 10H, Ar-\text{H}),\ 6.27 - 6.20\ (m, 2H, -NH-),\ 5.37 - 5.29\ (m, 2H, AcOCH-),\ 4.56 - 4.50\ (m, 2H, -NHCH-),\ 3.65\ (s, 3H, -CO_2CH_3),\ 3.64\ (s, 3H, -CO_2CH_3),\ 3.17\ (dd, J = 14.2, 5.0\ Hz, 1H, -CH_2Ph),\ 3.14 - 3.06\ (m, 2H, -CH_2Ph),\ 3.02\ (dd, J = 14.2, 7.7\ Hz, 1H, -CH_2Ph),\ 2.06\ (s, 3H, CH_3CO_2-),\ 2.00\ (s, 3H, CH_3CO_2-),\ 1.59 - 1.25\ (m, 6H, -CH_2CH(CH_3)_2),\ 0.83\ (d, J = 4.4\ Hz, 3H, -CH(CH_3)_2),\ 0.82\ (d, J = 4.4\ Hz, 3H, -CH(CH_3)_2),\ 0.81\ (d, J = 6.5\ Hz, 3H, -CH(CH_3)_2),\ 0.79\ (d, J = 6.5\ Hz, 3H, -CH(CH_3)_2);\ ^{13}\text{C}-\text{NMR}\ (126\ MHz, CDCl_3)\ \delta\ 173.2\ (-C=O),\ 173.0\ (-C=O),\ 169.6\ (-C=O),\ 169.4\ (-C=O),\ 169.0\ (-C=O),\ 168.6\ (-C=O),\ 136.0,\ 135.8,\ 129.8,\ 129.6,\ 128.5(2),\ 128.4(6),\ 127.1,\ 127.0,\ 74.4\ (AcOCH-),\ 74.3\ (AcOCH-),\ 52.5,\ 50.6,\ 50.4,\ 41.7(3),\ 41.6(7),\ 37.8,\ 37.6,\ 24.9\ (-CH(CH_3)_2),\ 24.6\ (-CH(CH_3)_2),\ 22.8\ (-CH(CH_3)_2),\ 22.1\ (-CH(CH_3)_2),\ 22.0\ (-CH(CH_3)_2),\ 21.1\ (CH_3CO_2-),\ 20.9\ (CH_3CO_2-);\ MS\ (MALDI-TOF)\ m/z\ 358.6\ [M+Na]^+.

\((S)\)-Methyl 2-(2-acetoxy-3-phenylpropanamido)-2-methylpropanoate (474)

\[
\begin{align*}
\text{Ph} & \quad \text{O} & \quad \text{O} & \quad \text{N} & \quad \text{O} & \quad \text{O} \\
\text{Ac} & \quad \text{H} & \quad \text{N} & \quad \text{O} & \quad \text{CH}_3 \\
\text{467} & & & & & \\
\text{NH}_3^+ & & & & & \\
\text{Cl}^- & & & & & \\
\text{DCM / DMF} & & & & & \\
\text{r.t., 16 h} & & & & & \\
\text{TBTU} & & & & & \\
\text{DIPEA} & & & & & \\
\text{474: 51% yield} & & & & & 
\end{align*}
\]

Compound 474 was prepared according to the procedure for compound 468. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; R_f = 0.56) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (374 mg, 1.22 mmol, 51% yield).

\[^1\text{H}-\text{NMR}\ (500\ MHz, \text{CDCl}_3)\ \delta\ 7.30 - 7.21\ (m, 3H, Ar-\text{H}),\ 7.20 - 7.16\ (m, 2H, Ar-\text{H}),\ 6.62\ (s, 1H, -NH-),\ 5.32\ (dd, J = 6.7, 5.0\ Hz, 1H, AcOCH-),\ 3.73\ (s, 3H, -CO_2CH_3),\ 3.20\ (dd, J = 14.3, 5.0\ Hz, 1H, -CH_2Ph),\ 3.13\ (dd, J = 14.3, 6.7\ Hz, 1H, -CH_2Ph),\ 2.10\ (s, 3H, -CH_3CO_2-),\ 1.52\ (s, 3H, -C(CH_3)_2),\ 1.48\ (s, 3H, -C(CH_3)_2);\ ^{13}\text{C}-\text{NMR}\ (126\ MHz, \text{CDCl}_3)
δ 175.1 (-C=O), 169.5 (-C=O), 168.2 (-C=O), 136.0, 129.8, 128.5, 127.0, 74.4 (AcOCH-), 56.8, 52.9, 37.6, 24.5 (-C(CH3)2), 24.3 (-C(CH3)2), 21.1 (CH3CO2-); FT-IR (thin film): ν (cm⁻¹) = 3335 (N-H), 3034 (C-H), 2991 (C-H), 2955 (C-H), 1746 (ester C=O), 1676 (amide C=O), 1531, 1453, 1371, 1230, 1152, 1070; [α]D²⁰ = 10.7 (c 1.0, CHCl₃); MS (MALDI-TOF) m/z 346.1 [M+K]+; HRMS (HNESP) exact mass calculated for C₁₆H₂₁NO₅ requires m/z 308.1492, found m/z 308.1496 [M+H]+.

(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-4-(methylthio)butanoate (475)

Compound 475 was prepared according to the procedure for compound 468. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rf = 0.44) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (302 mg, 0.85 mmol, 36% yield).

¹H-NMR (500 MHz, CDCl₃) δ 7.31 – 7.22 (m, 3H, Ar-H), 7.19 – 7.15 (m, 2H, Ar-H), 6.65 (d, J = 7.7 Hz, 1H, -NH-), 5.41 (t, J = 5.4 Hz, 1H, AcOCH-), 4.68 – 4.63 (m, 1H, -NHCH-), 3.73 (s, 3H, -CO₂CH₃), 3.19 (d, J = 5.9 Hz, 2H, -CH₂Ph), 2.31 – 2.16 (m, 2H, -CH₂S-), 2.14 (s, 3H, CH₃CO₂-), 2.07 – 2.00 (m, 1H, -CH₂CH₂S-), 2.03 (s, 3H, -SCH₃), 1.93 – 1.84 (m, 1H, -CH₂CH₂S-); ¹³C-NMR (126 MHz, CDCl₃) δ 172.1 (-C=O), 169.4 (-C=O), 168.8 (-C=O), 135.7, 129.8, 128.5, 127.2, 74.3 (AcOCH-), 52.8, 51.3, 37.6 (-CH₂Ph), 31.4 (-CH₂CH₂S-), 29.6 (-CH₂S-), 21.1 (CH₃CO₂-), 15.4 (-SCH₃); FT-IR (thin film): ν (cm⁻¹) = 3324 (N-H), 3030 (C-H), 2955 (C-H), 2921 (C-H), 2856 (C-H), 1746 (ester C=O), 1674 (amide C=O), 1531, 1439, 1374, 1227, 1063; [α]D²⁰ = 20.5 (c 1.0, CHCl₃); MS (MALDI-TOF) m/z 376.3 [M+Na]+, 392.3 [M+K]+; HRMS (HNESP) exact mass calculated for C₁₇H₂₄NO₅S requires m/z 354.1370, found m/z 354.1372 [M+H]+.
(S)-Methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-4-(methylthio)butanoate (475) and (S)-methyl 2-((R)-2-acetoxy-3-phenylpropanamido)-4-(methylthio)butanoate (491)

Compounds 475 and 491 were prepared according to the procedure for compound 468. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rf = 0.44) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (441 mg, 1.25 mmol, 52% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) δ 7.26 (s, 6H, Ar-H), 7.21 – 7.16 (m, 4H, Ar-H), 6.65 (d, J = 7.3 Hz, 2H, -NH-), 5.41 (t, J = 5.4 Hz, 1H, AcOCH-), 5.39 – 5.36 (m, 1H, AcOCH-), 4.69 – 4.63 (m, 2H, -NHCH-), 3.74 (s, 3H, CO$_2$C$_3$H$_3$), 3.73 (s, 3H, CO$_2$C$_3$H$_3$), 3.24 (dd, J = 14.2, 4.9 Hz, 1H, -CH$_2$Ph), 3.19 (d, J = 5.8 Hz, 2H, -CH$_2$Ph), 3.09 (dd, J = 14.1, 7.7 Hz, 1H, -CH$_2$Ph), 2.39 (m, 1H, -CH$_2$S-), 2.30 – 2.16 (m, 2H, -CH$_2$S-), 2.14 (s, 3H, CH$_3$CO$_2$-), 2.13 – 2.09 (m, 1H, -CH$_2$S-), 2.09 (s, 3H, CH$_3$CO$_2$-), 2.06 (s, 3H, -SCH$_3$), 2.05 – 2.00 (m, 4H, -SCH$_3$ + -CH$_2$CH$_2$S-), 1.96 (m, 2H, -CH$_2$CH$_2$S-), 1.88 (m, 1H, -CH$_2$CH$_2$S-); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 172.1 (-C=O), 172.0 (-C=O), 169.7 (-C=O), 169.4 (-C=O), 169.1 (-C=O), 168.8 (-C=O), 136.0, 135.7, 129.9, 129.6, 128.6, 128.6, 127.2, 127.1, 74.5 (AcOCH-), 74.3 (AcOCH-), 52.8, 51.5, 51.3, 37.8 (-CH$_2$Ph), 37.6 (-CH$_2$Ph), 31.4(4) (-CH$_2$CH$_2$S-), 31.3(5) (-CH$_2$CH$_2$S-), 30.0 (-CH$_2$S-), 29.6 (-CH$_2$S-), 21.1 (CH$_3$CO$_2$-), 21.0 (CH$_3$CO$_2$-), 15.6 (-SCH$_3$), 15.4 (-SCH$_3$); FT-IR (thin film): ν (cm$^{-1}$) = 3326 (N-H), 2950 (C-H), 2923 (C-H), 2852 (C-H), 1745 (ester C=O), 1677 (amide C=O), 1528, 1437, 1376, 1227, 1061; MS (MALDI-TOF) m/z 376.33 [M+Na]$^+$, 392.34 [M+K]$^+$. 

![Experimental Section](image.png)
(S)-Methyl 2-((S)-2-hydroxy-3-phenylpropanamido)-3-phenylpropanoate (469)

A 150 mL round-bottom flask containing (S)-methyl 2-((S)-2-acetoxy-3-phenylpropanamido)-3-phenylpropanoate 468 (320 mg, 0.87 mmol, 1 equiv.) was charged with a mixture of methanol and distilled water (30 mL, 9:1), and a magnetic stirrer bar. Potassium carbonate (30 mg, 0.22 mmol, 0.25 equiv.) was added in one portion. The colourless solution was stirred at room temperature for 1 hour, and then concentrated by rotary evaporation. The aqueous residue was extracted with dichloromethane (3 x 25 mL) and the combined organic layers were washed with 1 M aqueous hydrochloric acid (50 mL) and subsequently brine (50 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rf = 0.22) afforded a white solid whose physicochemical properties were consistent with those of the title compound (162 mg, 0.49 mmol, 57% yield).

M.p. 104–106 °C; $^1$H-NMR (500 MHz, CDCl$_3$) δ 7.36 – 7.30 (m, 2H, Ar-H), 7.29 – 7.20 (m, 6H, Ar-H), 6.96 (d, J = 7.9 Hz, 2H, Ar-H), 6.91 (d, J = 8.4 Hz, 1H, -NH-), 4.89 (dt, J = 8.2, 6.0 Hz, 1H, -NHCH-), 4.30 (m, 1H, -CHOH), 3.71 (s, 3H, -OCH$_3$), 3.15 (dd, J = 14.0, 4.0 Hz, 1H, -CH$_2$Ph), 3.05 (d, J = 6.0 Hz, 2H, -CH$_2$CHOH), 2.82 (dd, J = 13.9, 8.1 Hz, 1H, -CH$_2$Ph), 2.42 (d, J = 5.0 Hz, 1H, -OH); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 172.2 (-C=O), 171.9 (-C=O), 136.7, 135.8, 129.8, 129.4, 128.9, 128.7, 127.2(8), 127.2(6), 72.9 (-CHOH), 52.8 (-NHCH-), 52.5 (-OCH$_3$), 40.8 (-CH$_2$Ph), 38.2 (-CH$_2$Ph); FT-IR (thin film, cm$^{-1}$) ν = 3398 (N-H), 2967, 2854, 1739 (ester C=O), 1653 (amide C=O), 1539, 1441, 1093, 1030; [α]$^D_{19}$ +5.0 (c 1.0, CHCl$_3$); MS (MALDI-TOF): m/z = 350.2 [M+Na]$^+$; HRMS (HNESP) exact mass calculated for C$_{19}$H$_{22}$NO$_4$ requires m/z 328.1543, found m/z 328.1537 [M+H]$^+$. 


(S)-Methyl 2-((S)-2-hydroxy-3-phenylpropanamido)-3-phenylpropanoate (469) and (S)-methyl 2-((R)-2-hydroxy-3-phenylpropanamido)-3-phenylpropanoate (481)

Following the procedure for compound 469 above, a mixture of two diastereoisomers 469 and 481 were synthesised and separated via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate (10%). Compound 469 was isolated as a colourless oil (278 mg, 0.85 mmol, 36% yield) whose physicochemical properties matched those reported above. Compound 481 was isolated as a colourless oil (332 mg, 1.01 mmol, 43% yield).

316: Rf = 0.43; 1H-NMR (500 MHz, CDCl3) δ 7.35 – 7.19 (m, 8H, Ar-H), 7.12 – 7.07 (m, 2H, Ar-H), 6.89 (d, J = 7.7 Hz, 1H, -NH-), 4.88 (dt, J = 8.2, 6.1 Hz, 1H, -NHCH-), 4.27 (dd, J = 9.0, 4.0 Hz, 1H, -CHOH), 3.71 (s, 3H, -OCH3), 3.21 (dd, J = 14.0, 4.0 Hz, 1H, -CH2Ph), 3.12 (qd, J = 13.9, 6.1 Hz, 2H, -CH2Ph), 2.84 (dd, J = 14.0, 9.0 Hz, 1H, -CH2Ph); 13C-NMR (126 MHz, CDCl3) δ 172.3 (-C=O), 171.8 (-C=O), 136.8, 135.9, 129.6, 129.4, 129.0, 128.8, 127.3, 127.3, 73.0 (-CHOH), 53.0 (-NHCH-), 52.5 (-OCH3), 40.9 (-CH2Ph), 38.1 (-CH2Ph); FT-IR (thin film): ν (cm⁻¹) = 3390 (N-H), 3066, 3028, 2952, 2924, 1741 (ester C=O), 1655 (amide C=O), 1521, 1497, 1455, 1438, 1362, 1217, 1086; [α]D⁰ +86.4 (c 1.0, CHCl3); MS (MALDI-TOF) m/z 350.2 [M+Na]+; HRMS (HNESP) exact mass calculated for C₁₉H₂₂NO₄ requires m/z 328.1543, found m/z 328.1543 [M+H]+.

(S)-Methyl 2-((R)-2-hydroxy-3-phenylpropanamido)-4-methylpentanoate (480)
Compound 480 was prepared according to the procedure for compound 469. Purification via flash column chromatography on silica (dichloromethane / ethyl acetate 10%; R_f = 0.42) afforded a white solid whose physicochemical properties were consistent with those of the title compound (354 mg, 1.21 mmol, 81% yield).

M.p. 45–47 °C; ^1H-NMR (500 MHz, CDCl_3) δ 7.37 – 7.31 (m, 2H, Ar-H), 7.30 – 7.23 (m, 3H, Ar-H), 6.77 (d, J = 8.3 Hz, 1H, -NHCH-), 4.36 – 4.30 (m, 1H, -CHOH), 3.73 (s, 3H, -OCH_3), 3.25 (s, 2H, -CH(CH_3)_2), 2.91 (dd, J = 14.0, 4.2 Hz, 1H-CH_2Ph), 2.39 (s, 1H, -OH), 1.50 – 1.49 (m, 3H, -CH(CH_3)_2), 0.91 (d, J = 2.7 Hz, 3H, -CH(CH_2CH(CH_3)_2)), 0.90 (d, J = 2.8 Hz, 3H, -CH(CH(CH_3)_2)_2); ^13C-NMR (126 MHz, CDCl_3) δ 173.1 (-C=O), 172.9 (-C=O), 137.0, 129.6, 128.7, 126.9, 72.9 (-CHOH), 52.4 (-NHCH-), 50.4 (-OCH_3), 41.5 (-CH_2CH(CH_3)_2), 40.8 (-CH_2Ph), 24.8 (-CH(CH_3)_2), 22.8 (-CH(CH_3)_2), 21.9 (-CH(CH_3)_2); FT-IR (thin film): v (cm⁻¹) = 3389 (N-H), 3032, 2956, 2874, 1744 (ester C=O), 1658 (amide C=O), 1531, 1456, 1439, 1370, 1277, 1209, 1154, 1088; [α]_D¹⁹ +48.6 (c 1.0, CHCl_3); MS (MALDI-TOF) m/z 332.4 [M+K]⁺; HRMS (HNESP) exact mass calculated for C₁₆H₂₄N⁴O₄ requires m/z 294.1700, found m/z 294.1702 [M+H]⁺.

(S)-Methyl 2-((S)-2-hydroxy-3-phenylpropanamido)-4-methylpentanoate (480) and (S)-methyl 2-((R)-2-hydroxy-3-phenylpropanamido)-4-methylpentanoate (482)

Following the procedure for compound 469, a mixture of two diastereoisomers 480 and 482 were synthesised and separated via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10 %). Compound 480 was isolated as a white solid (111 mg, 0.38 mmol, 41% yield) whose physicochemical properties matched those reported above. Compound 482 was isolated as a white solid (103 mg, 0.35 mmol, 38% yield).

482: R_f = 0.20; M.p. 83–85 °C; ^1H-NMR (500 MHz, CDCl_3) δ 7.31 – 7.25 (m, 2H, Ar-H), 7.25 – 7.19 (m, 3H, Ar-H), 6.93 (d, J = 8.5 Hz, 1H, -NH-), 4.55 (dt, J = 8.9, 5.1 Hz, 1H, -
NHCH-), 4.33 (dt, J = 8.3, 4.4 Hz, 1H, -CHOH), 3.68 (s, 3H, -OCH3), 3.43 (brs, 1H, -OH), 3.16 (dd, J = 14.0, 3.9 Hz, 1H, -CH2Ph), 2.91 (dd, J = 14.0, 7.6 Hz, 1H, -CH2Ph), 1.60 – 1.52 (m, 1H, -CH(CH3)2), 1.50 – 1.34 (m, 2H, -CH2CH(CH3)2), 0.87 (d, J = 2.8 Hz, 3H, -OCH(CH3)2), 0.86 (d, J = 2.9 Hz, 3H, -OCH(CH3)2); 13C-NMR (126 MHz, CDCl3) δ 173.6 (-C=O), 173.1 (-C=O), 137.0, 129.8, 128.5, 126.9, 72.8 (-CHOH), 52.4 (-NHCH-), 50.3 (-OCH3), 41.3 (-CH2CH(CH3)2), 40.6 (-CH3Ph), 24.7 (-CH(CH3)2), 22.9 (-CH(CH3)2), 21.8 (-CH(CH3)2); FT-IR (thin film): ν (cm⁻¹) = 3372 (NH), 3186, 2955, 2924, 2866, 1752 (ester C=O), 1662 (amide C=O), 1541, 1448, 1421, 1366, 1269, 1235, 1190, 1152, 1093; [α]D²⁰ = 60.2 (c 1.0, CHCl3); MS (MALDI-TOF) m/z 332.37 [M+K]+; HRMS (HNESP) exact mass calculated for C16H24N04 requires m/z 294.1700, found m/z 294.1702 [M+H]+.

(S)-Methyl 2-(2-hydroxy-3-phenylpropanamido)-2-methylpropanoate (478)

Compound 478 was prepared according to the procedure for compound 469. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rf = 0.13) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (154 mg, 0.58 mmol, 84% yield).

1H-NMR (500 MHz, CDCl3) δ 7.36 – 7.29 (m, 2H, Ar-H), 7.29 – 7.23 (m, 3H, Ar-H), 6.89 (s, 1H, -NH-), 4.26 (dd, J = 8.1, 4.5 Hz, 1H, -NHCH-), 3.74 (s, 3H, -OCH3), 3.17 (dd, J = 14.0, 4.4 Hz, 1H, -CH2Ph), 2.93 (dd, J = 13.9, 8.1 Hz, 1H, -CH2Ph), 2.55 (s, 1H, -OH), 1.52 (s, 3H, -CH3), 1.51 (s, 3H, -CH3); 13C-NMR (126 MHz, CDCl3) δ 174.9 (-C=O), 172.4 (-C=O), 137.0, 129.8, 128.6, 127.0, 72.8 (-CHOH), 56.2 (-NHCH-), 52.7 (-OCH3), 40.7 (-CH2Ph), 25.0 (-CH3), 24.7 (-CH3); FT-IR (thin film): ν (cm⁻¹) = 3382 (N-H), 3033, 2991, 2953, 1742 (ester C=O), 1654 (amide C=O), 1528, 1456, 1385, 1296, 1162, 1090; [α]D²⁰ = 49.1 (c 1.0, CHCl3); MS (MALDI-TOF) m/z 304.3 [M+K]+; HRMS (HNESP) exact mass calculated for C14H20N04 requires m/z 266.1387, found m/z 266.1390 [M+H]+.
(S)-Methyl 2-((R)-2-hydroxy-3-phenylpropanamido)-4-(methylthio)butanoate (479)

Compound 479 was prepared according to the procedure for compound 469. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; R_f = 0.10) afforded a white solid whose physicochemical properties were consistent with those of the title compound (166 mg, 0.53 mmol, 88% yield).

M.p. 60–62 °C; 1H-NMR (500 MHz, CDCl_3) δ 7.36 – 7.23 (m, 5H, Ar-H), 7.14 (d, J = 8.0 Hz, 1H, -NH-), 4.69 (dt, J = 7.9, 5.2 Hz, 1H, -NHCH-), 4.39 (dd, J = 7.6, 3.9 Hz, 1H, -CHOH), 3.74 (s, 3H, -OCH_3), 3.20 (dd, J = 13.9, 4.0 Hz, 1H, -CH_2Ph), 2.97 (dd, J = 13.9, 7.6 Hz, 2H, -CH_2Ph), 2.38 – 2.26 (m, 2H, -CH_2S-), 2.13 – 2.07 (m, 1H, -CH_2CH_3S-), 2.06 (s, 3H, -SCH_3), 1.96 – 1.86 (m, 1H, -CH_2CH_3S-); 13C-NMR (126 MHz, CDCl_3) δ 173.0 (-C=O), 172.3 (-C=O), 136.6, 129.8, 128.7, 127.1, 72.7 (-CHOH), 52.7 (-NHCH-), 51.1 (-OCH_3), 40.7 (-CH_2Ph), 31.6 (-CH_2CH_2S-), 29.8 (-CH_2S-), 15.5 (-SCH_3); FT-IR (thin film): ν (cm⁻¹) = 3377 (N-H), 3255, 2957, 2913, 1752 (ester C=O), 1660 (amide C=O), 1542, 1447, 1352, 1274, 1223, 1196, 1189, 1169, 1125, 1088; [α]_D^20 – 29.5 (c 1.0, CHCl_3); MS (MALDI-TOF) m/z 334.3 [M+Na]^+, 350.3 [M+K]^+; HRMS (HNESP) exact mass calculated for C_{15}H_{21}NO_4S requires m/z 312.1264, found m/z 312.1267 [M+H]^+.

(S)-Methyl 2-((S)-2-hydroxy-3-phenylpropanamido)-4-(methylthio)butanoate (479) and (S)-Methyl 2-((R)-2-hydroxy-3-phenylpropanamido)-4-(methylthio)butanoate (483)
Using the procedure for compound 469, a 1:1 mixture of diastereoisomers 479 and 483 were made and separated via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 20%). Compound 479 was isolated as a white solid (87 mg, 0.28 mmol, 33% yield) whose physicochemical properties matched those reported above. Compound 483 was isolated as a white solid (74 mg, 0.24 mmol, 28% yield).

319: R<sub>f</sub> = 0.28; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ 7.30 – 7.24 (m, 2H, Ar-H), 7.23 – 7.16 (m, 3H, Ar-H), 6.98 (d, J = 8.1 Hz, 1H, -NH-), 4.65 (dt, J = 7.8, 5.3 Hz, 1H, -NHCH-), 4.27 (dd, J = 8.8, 4.1 Hz, 1H, -CHOH), 3.68 (s, 3H, -OCH<sub>3</sub>), 3.18 (dd, J = 14.0, 4.1 Hz, 1H, -C<sub>6</sub>H<sub>2</sub>Ph), 2.83 (dd, J = 14.0, 8.8 Hz, 1H, -CH<sub>2</sub>Ph), 2.40 (t, J = 7.4 Hz, 2H, -CH<sub>2</sub>S-), 2.14 – 2.04 (m, 1H, -CH<sub>2</sub>CH<sub>2</sub>S-), 2.02 (s, 3H, -SC<sub>H</sub>3), 1.97 – 1.86 (m, 1H, -C<sub>6</sub>H<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>S-); <sup>13</sup>C-NMR (126 MHz, CDCl<sub>3</sub>) δ 172.8 (-C=O), 172.1 (-C=O), 136.9, 129.6, 128.9, 127.1, 73.0 (-CHOH), 52.7 (-NHCH-), 51.3 (-OCH<sub>3</sub>), 40.9 (-CH<sub>2</sub>Ph), 31.7 (-CH<sub>2</sub>CH<sub>2</sub>S-), 30.0 (-CH<sub>2</sub>S-), 15.5 (-SCH<sub>3</sub>); FT-IR (thin film): ν (cm<sup>-1</sup>) = 3382 (N-H), 3059, 3028, 2953, 2919, 2850, 1741 (ester C=O), 1658 (amide C=O), 1524, 1435, 1360, 1277, 1226, 1171, 1088, 1030; [α]<sub>D</sub><sup>22</sup> +33.1 (c 1.0, CHCl<sub>3</sub>); MS (MALDI-TOF) m/z 349.7 [M+K]<sup>+</sup>; HRMS (HNESP) exact mass calculated for C<sub>15</sub>H<sub>22</sub>NO<sub>4</sub>S requires m/z 312.1264, found m/z 312.1265 [M+H]<sup>+</sup>.

(S)-Methyl 2-((R)-2-((S)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-3-phenylpropanamido)-3-phenylpropanoate (466)

A Biotage Endeavor glass hydrogenation vial was charged with a solution of (S)-methyl 2-(((2R,3R)-1-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)-3-phenylaziridine-2-carboxamido)-3-phenylpropanoate 427 (100 mg, 0.21 mmol, 1 equiv.) in ethanol (4 mL). Palladium on carbon (10 wt%) (22 mg, 0.021 mmol, 10 mol%) was added, and the mixture stirred under 50 psi of hydrogen at room temperature for 12 hours. The mixture
was filtered through a short plug of celite and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; Rf = 0.44) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (96 mg, 0.20 mmol, 96% yield).

$^1$H-NMR (500 MHz, CDCl$_3$) δ 7.71 (s, 1H, -NH-), 7.25 – 7.14 (m, 9H, Ar-H), 7.10 (d, J = 6.6 Hz, 2H, Ar-H), 7.04 – 6.99 (m, 2H, Ar-H), 6.81 (d, J = 5.9 Hz, 2H, Ar-H), 4.85 (dt, J = 8.3, 6.5 Hz, 1H, -NHCH-), 3.70 (s, 3H, -OCH$_3$), 3.56 (s, 3H, -OCH$_3$), 3.34 (dd, J = 9.1, 4.3 Hz, 1H, -NHCH-), 3.29 (t, J = 6.5 Hz, 1H, -NHCH-), 3.18 – 3.03 (m, 3H, -CH$_2$Ph), 2.76 – 2.63 (m, 3H, -CH$_2$Ph); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 173.3 (-C=O), 173.2 (-C=O), 172.0 (-C=O), 137.2, 136.4, 136.3, 129.4, 129.3, 129.2, 129.0, 128.8, 128.7, 127.1, 127.0(2), 126.9(6), 62.6 (-NHCH-), 61.6 (-NHCH-), 53.2 (-NHCH-), 52.3 (-OCH$_3$), 52.0 (-OCH$_3$), 39.1 (-CH$_2$Ph), 38.1 (-CH$_2$Ph), 38.0 (-CH$_2$Ph); FT-IR (thin film): ν (cm$^{-1}$) = 3339, 2920, 2858, 1746, 1672, 1500, 1457, 1203, 1027; [α]$_D$ +11.9 (c 1.0, CHCl$_3$); MS (MALDI-TOF) m/z 489.6 [M+Na]$^+$; HRMS (HNESP) exact mass calculated for C$_{29}$H$_{33}$N$_2$O$_5$ requires m/z 489.2384, found m/z 489.2378 [M+H]$^+$.

(S)-Methyl 2-((R)-2-((S)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-3-phenylpropanamido)-3-phenylpropanoate (466)

A flame-dried 5 mL microwave vial containing a magnetic stirrer bar was charged with a solution of trifluoromethanesulfonic anhydride (51 µL, 0.31 mmol, 1 equiv.) in dry dichloromethane (1 mL) and cooled to 0 °C under a nitrogen atmosphere using an ice bath. A solution of (S)-methyl 2-((S)-2-hydroxy-3-phenylpropanamido)-3-phenylpropanoate 469 (100 mg, 0.31 mmol, 1 equiv.) and pyridine (25 µL, 0.31 mmol, 1 equiv.) in dry dichloromethane (2 mL) was added dropwise via syringe over 1 hour. The reaction was allowed to warm slowly to room temperature, and the solvent removed in vacuo. Pentane (5 mL) was added and the resulting white precipitate was removed by
filtration. The filtrate was concentrated *in vacuo* before being redissolved in dry dichloromethane (2 mL) and added dropwise to a solution of L-phenylalanine methyl ester 426 (109 mg, 0.61 mmol, 2 equiv.) in dichloromethane (2 mL) at -70 °C in a 5 mL microwave vial under a nitrogen atmosphere. The reaction was stirred at -70 °C for one hour before being allowed to warm to room temperature and stirred for a further 16 hours. The white solid precipitate was removed by filtration and the filtrate concentrated *in vacuo*. Purification via flash column chromatography on silica gel (eluent: dichloromethane / ethyl acetate 10%; \( R_f = 0.44 \)) afforded a colourless oil (28 mg, 0.058 mmol, 19% yield) whose physicochemical properties were consistent with those of the title compound and matched those obtained by hydrogenation of the corresponding aziridine 427 described above.

**(S)-Methyl 2-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-3-phenylpropanamido)-3-phenylpropanoate (489)**

![Chemical structure of compound 489](image)

Compound 489 was prepared according to the procedure for compound 466. Purification via flash column chromatography on silica (dichloromethane / ethyl acetate 10%; \( R_f = 0.32 \)) afforded a colourless oil whose physicochemical properties were consistent with those of the title compound (36 mg, 0.073 mmol, 24% yield).

\(^1^H\)-NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.32 – 7.20 (m, 10H, Ar-H), 7.11 – 7.05 (m, 4H, Ar-H), 6.96 (d, \( J = 7.2 \) Hz, 2H, Ar-H), 4.71 (td, \( J = 8.3, 5.3 \) Hz, 1H, -NHCH-), 3.73 (s, 3H, -OCH\(_3\)), 3.48 (s, 3H, -OCH\(_3\) + -NHCH-), 3.25 (dd, \( J = 8.8, 3.9 \) Hz, 1H, -NHCH-), 2.99 (dd, \( J = 13.9, 5.3 \) Hz, 1H, -CH\(_2\)Ph), 2.94 (dd, \( J = 13.9, 4.1 \) Hz, 1H, -CH\(_2\)Ph), 2.83 – 2.63 (m, 3H, -CH\(_2\)Ph), 2.46 – 2.39 (m, 1H, -CH\(_2\)Ph); \(^{13}^C\)-NMR (126 MHz, CDCl\(_3\)) \( \delta \) 173.3 (-C=O), 172.9 (-C=O), 171.8 (-C=O), 137.0, 136.7, 129.6, 129.7, 129.5, 129.3, 128.7(1), 128.6(7), 128.5, 127.2, 127.1, 127.0, 62.2 (-NHCH-), 61.1 (-NHCH-), 52.4 (-NHCH- + -OCH\(_3\)), 51.9 (-OCH\(_3\)), 40.0 (-CH\(_2\)Ph), 39.6 (-CH\(_2\)Ph), 37.4 (-CH\(_2\)Ph); FT-IR (thin film): \( \nu \)
(cm⁻¹) = 3351 (N-H), 2924, 2854, 1743 (ester C=O), 1680 (amide C=O), 1504, 1453, 1203; [α]D²⁰ -7.2 (c 0.6, CHCl₃); MS (MALDI-TOF) m/z 511.37 [M+Na]+; HRMS (HNESP) exact mass calculated for C₂₉H₳N₂O₅ requires m/z 489.2384, found m/z 489.2375 [M+H]+.

(S)-tert-Butyl 1-(allylamino)-4-methyl-1-oxopentan-2-ylcarbamate (494)²⁵³

A 150 mL round-bottom flask was charged with a solution of (S)-2-((tert-butoxycarbonyl)amino)-4-methylpentanoic acid 493 (1.0 g, 4.32 mmol, 1 equiv.) in dry tetrahydrofuran (20 mL) and cooled to -25 °C under a nitrogen atmosphere. 4-Methylmorpholine (0.48 mL, 4.32 mmol, 1 equiv.), ethyl chloroformate (0.41 mL, 4.32 mmol, 1 equiv.) and allylamine (0.36 mL, 4.75 mmol, 1.1 equiv.) were added sequentially via syringe (3 x 1 mL) and with vigorous stirring of the reaction mixture. The resulting suspension was allowed to warm to room temperature and stirred for 18 hours. The solvent was removed in vacuo and the residue diluted with distilled water (15 mL) and adjusted to pH 1 with 1 M aqueous potassium hydrogen sulfate. The resulting mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers washed with distilled water (100 mL), then saturated aqueous sodium bicarbonate (100 mL) and finally brine (100 mL). The separated organic solution was dried over magnesium sulfate, filtered, and the solvent removed in vacuo. Purification via flash column chromatography on silica gel (eluent: petroleum ether / diethyl ether 1:1; Rf = 0.24) afforded a white solid whose physicochemical properties matched those previously reported (527 mg, 1.95 mmol, 45% yield).

M.p. 78–80 °C; ¹H-NMR (500 MHz, CDCl₃) δ = 6.19 (br s, 1H, -NH-), 5.87 – 5.78 (m, 1H, -CH=CH₂), 5.19 (dd, J = 17.2, 1.4 Hz, 1H, -CH=CH₂), 5.13 (dd, J = 10.3, 1.3 Hz, 1H, -CH=CH₂), 4.85 (br s, 1H, BocNH-), 4.08 (br s, 1H, BocNH₂-), 3.88 (br s, 2H, -NHCH₂-), 1.73 – 1.63 (m, 2H, -CH₂CH(CH₃)₂), 1.52 – 1.40 (m, 1H, -CH(CH₃)₂), 1.44 (s, 9H, -C(CH₃)₃), 0.95 (d, J = 4.8 Hz, 3H, -CH(CH₃)₂), 0.93 (d, J = 4.8 Hz, 3H, -CH(CH₃)₂);
$^{13}$C-NMR (126 MHz, CDCl$_3$) δ 172.7 (amide –C=O), 156.0 (carbamate -C=O), 134.1 (-CH=CH$_2$), 116.3 (-CH=CH$_2$), 80.2 (-C(CH$_3$)$_3$), 53.2 (-NHCH$_2$-), 41.8, 41.4, 28.4 (-C(CH$_3$)$_3$), 24.9 (-CH(CH$_3$)$_2$), 23.1 (-CH(CH$_3$)$_2$), 22.1 (-CH(CH$_3$)$_2$); MS (MALDI-TOF): m/z 309.2 [M+K]$^+$. 

(S)-$N$-allyl-2-amino-4-methylpentanamide (495)

![Chemical structure](image)

To a 150 mL round-bottom flask containing a solution of (S)-$tert$-butyl 1-(allylamino)-4-methyl-1-oxopentan-2-ylcarbamate 494 (400 mg, 1.48 mmol, 1 equiv.) in dry dichloromethane under a nitrogen atmosphere was added trifluoroacetic acid (0.68 mL, 8.88 mmol, 8 equiv.) dropwise via a 2 mL syringe. The resulting solution was stirred at room temperature for 6 hours. When the starting material was no longer observed by thin layer chromatography (eluent: petroleum ether / diethyl ether 1:1), the reaction mixture was cooled to 0 °C and quenched by the dropwise addition of triethylamine (1.5 mL, 11.1 mmol, 10 equiv.) via a 2.5 mL syringe. After stirring for a further one hour at room temperature, the solvents were removed in vacuo to afford a colourless oil that was used without further purification (146 mg, 0.86 mmol, 58% yield); the physicochemical properties were consistent with those of the title compound.

$^1$H-NMR (500 MHz, CDCl$_3$) δ = 7.38 (br s, 1H, -NH-), 5.84 (ddt, J = 17.2, 10.2, 5.6 Hz, 1H, -CH=CH$_2$), 5.17 (dd, J = 17.2, 1.5 Hz, 1H, -CH=CH$_2$), 5.12 (dd, J = 10.2, 1.5 Hz, 1H, -CH=CH$_2$), 3.88 (t, J = 5.6 Hz, 2H, -NH$_2$CH-), 3.40 (dd, J = 9.8, 3.3 Hz, 1H, -NHCH$_2$-), 1.76 – 1.68 (m, 2H, -CH$_2$CH(CH$_3$)$_2$), 1.48 (br s, 2H, -NH$_2$), 1.37 – 1.32 (m, 1H, -CH(CH$_3$)$_2$), 0.96 (d, J = 6.1 Hz, 3H, -CH(CH$_3$)$_2$), 0.93 (d, J = 6.1 Hz, 3H, -CH(CH$_3$)$_2$); $^{13}$C-NMR (126 MHz, CDCl$_3$) δ 175.5 (-C=O), 134.6 (-CH=CH$_2$), 116.1 (-CH=CH$_2$), 53.7 (-CHNH$_2$), 44.3 (-NHCH$_2$-), 41.5 (-CH$_2$CH(CH$_3$)$_2$), 25.0 (-CH$_2$CH(CH$_3$)$_2$), 23.6 (-CH(CH$_3$)$_2$), 21.4 (-CH(CH$_3$)$_2$); FT-IR (thin film, cm$^{-1}$) ν = 3290 (N-H), 3074 (C-H), 2952 (C-H), 2935 (C-H), 2876 (C-H), 1654 (C=O), 1540, 1472, 1371, 1261, 1147, 990, 918;
[α]D 218 -20.6 (c 1.0, CHCl₃); MS (MALDI-TOF): m/z = 209.1 [M+K]+; HRMS (HNESP) exact mass calculated for C₉H₁₉N₂O requires m/z 171.1492, found m/z 171.1491 [M+H]+.

(5)-3-((Benzyloxy)carbonyl)-1H-indol-3-yl)-1-methoxy-1-oxopropan-2-aminium trifluoroacetate (497)

To a 500 mL two-necked round-bottom flask under a nitrogen atmosphere was added (S)-benzyl 3-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indole-1-carboxylate 329 (9.33 g, 20.6 mmol, 1 equiv.) and a magnetic stirrer bar. The white solid was dissolved in dichloromethane (60 mL) and trifluoroacetic acid (12.6 mL, 165 mmol, 8 equiv.) was added via a 20 mL syringe. The solution was stirred at room temperature for 16 hours, by which time complete disappearance of the starting material was observed by thin layer chromatography (eluent: petroleum ether / diethyl ether 1:1). The solvent was removed in vacuo to afford a colourless oil, and a white solid precipitate was formed on the addition of diethyl ether. The solid was filtered, washed with diethyl ether and dried in vacuo. The physicochemical properties were consistent with those of the title compound (6.71 g, 20.2 mmol, 98% yield).

M.p. 138–140 °C; 1H-NMR (500 MHz, MeOD) δ 8.18 (d, J = 8.1 Hz, 1H, Ar-H), 7.66 (s, 1H, ind. C-2 H), 7.60 – 7.56 (m, 1H, Ar-H), 7.55 – 7.51 (m, 2H, Ar-H), 7.46 – 7.33 (m, 4H, Ar-H), 7.30 (t, J = 7.5 Hz, 1H, Ar-H), 5.48 (s, 2H, -CH₂Ph), 4.39 (br s, 1H, -CHNH₂), 3.78 (s, 3H, -OCH₃), 3.42 (dd, J = 15.2, 5.1 Hz, 1H, -CH₂-ind), 3.34 – 3.25 (m, 1H, -CH₂-ind); 13C-NMR (126 MHz, MeOD) δ 170.5 (-C=O), 162.2 (-C=O), 151.8 (-C=O), 137.0, 136.6, 130.9, 129.8, 129.7, 129.6, 126.1, 126.1, 124.2, 119.7, 116.3, 114.9, 69.9 (-CH₂Ph), 53.7 (-OCH₃), 27.0 (-CH₂-ind); FT-IR (thin film): v (cm⁻¹) = 3441 (N-H), 3038 (C-H), 2959 (C-H), 2921 (C-H), 2855 (C-H), 1741 (ester C=O), 1679 (carbamate C=O), 1610, 1521, 1462, 1403, 1362, 1345, 1303, 1255, 1203, 1135, 1093, 1024, 945; [α]D 352 +4.8 (c 1.0, MeOH); MS (MALDI-TOF) m/z 352.5 [M]+ (free amine); HRMS (HNESP) exact mass calculated for C₂₀H₂₁N₂O₄ requires m/z 353.1496, found m/z 353.1494 [M+H]+ (free amine).
BIBLIOGRAPHY
Bibliography


162. Theoretical pKa values were calculated in Marvinsketch 15.1.19 using the protonation plugin.

204. Graph based on the equation $^{3}J_{HH} = A + B \cos \phi + C \cos 2 \phi$ where $A = 4.22$, $B = -0.5$ and $C = 4.5$ Hz from M. Karplus, J. Am. Chem. Soc., 1963, 85, 2870.


APPENDIX

X-ray crystallographic data
Appendix 1: X-ray crystal structure and crystallographic data for methyl 3,5-bis(4-formylphenoxy)-4-hydroxybenzoate 305

Figure 1 • An Ortep representation of the crystal structure of 305. Thermal ellipsoids are drawn at 50% probability level.

Crystal data and structure refinement details:

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\(^{1}\) All x-ray crystallographic data collection and structure analysis carried out by Dr Mateusz B. Pitak, UK National Crystallography Service, Southampton.
APPENDIX  X-ray crystallographic data

\( \theta \) range for data collection  
2.94 – 24.99°

Index ranges  
\(-13 \leq h \leq 13, \ -10 \leq k \leq 10, \ -21 \leq l \leq 21\)

Reflections collected  
13357

Independent reflections  
3079 \([R_{int} = 0.0355]\)

Completeness to \( \theta = 24.99° \)  
98.7 %

Absorption correction  
Semi–empirical from equivalents

Max. and min. transmission  
0.9923 and 0.9782

Refinement method  
Full-matrix least-squares on \( F^2 \)

Data / restraints / parameters  
3079 / 0 / 264

Goodness-of-fit on \( F^2 \)  
1.101

Final \( R \) indices \([F^2 > 2\sigma(F^2)]\)  
\( R1 = 0.0456, \ wR2 = 0.1014 \)

\( R \) indices (all data)  
\( R1 = 0.0574, \ wR2 = 0.1108 \)

Largest diff. peak and hole  
0.351 and \(-0.276 \, \text{e Å}^{-3}\)

**Table 1** • Atomic coordinates \([\times 10^4]\), equivalent isotropic displacement parameters \([\text{Å}^2 \times 10^3]\) and site occupancy factors. \( U_{eq} \) is defined as one third of the trace of the orthogonalized \( U^{ij} \) tensor.

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### Table 3 • Anisotropic displacement parameters \([\text{Å}^2 \times 10^3]\). The anisotropic displacement factor exponent takes the form: 
\[-2\pi^2 [h^2a^*2U_{11} + \cdots + 2hka^*b^*U_{12}]\].

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## APPENDIX  X-ray crystallographic data

### Table 4 • Hydrogen coordinates \([\times 10^4]\) and isotropic displacement parameters \([\text{Å}^2 \times 10^3]\).

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<th>(z)</th>
<th>(U_{eq})</th>
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### Table 5 • Hydrogen bonds \([\text{Å} \text{ and } ^\circ]\).

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<th>(d(H\cdots A))</th>
<th>(d(D\cdots A))</th>
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Possible intramolecular H-bond

| O5–H5…O2        | 0.84        | 2.41             | 2.834(2)          | 112.0           |

Symmetry transformations used to generate equivalent atoms:

(i) \(-x+3/2, y-1/2, -z+3/2\)

**Diffractometer**: Nonius KappaCCD area detector (\(\phi\) scans and \(\omega\) scans to fill asymmetric unit sphere).


**Data collection**: Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998).


Appendix 2: X-ray crystal structure and crystallographic data for cis-(S)-methyl 2-(1-(4-methoxyphenyl)-3-phenylaziridine-2-carboxamido)-3-phenylpropanoate 406a

**Figure 1** Molecular structure of rac,cis-406a. Displacement ellipsoids – 50% probability.

Crystal data and structure refinement details:

- **Identification code**: 2012ncs0332 (PA531)
- **Empirical formula**: C_{26}H_{36}N_{2}O_{4}
- **Formula weight**: 430.49
- **Temperature**: 120(2) K
- **Wavelength**: 0.71075 Å
- **Crystal system**: Triclinic
- **Space group**: P–1
- **Unit cell dimensions**:
  - $a = 9.6355(2)$ Å
  - $b = 9.9156(2)$ Å
  - $c = 12.9883(9)$ Å
  - $\alpha = 98.751(7)^\circ$
  - $\beta = 97.164(7)^\circ$
  - $\gamma = 113.516(8)^\circ$
- **Volume**: $1100.83(8)$ Å$^3$
- **Z**: 2
- **Density (calculated)**: 1.299 Mg / m$^3$
**Absorption coefficient**: 0.088 mm\(^{-1}\)

\(F(000)\) 456

**Crystal** Prism; colourless

**Crystal size** \(0.60 \times 0.37 \times 0.17 \text{ mm}^3\)

\(\theta\) range for data collection 2.99 – 27.45°

**Index ranges** 
- \(-12 \leq h \leq 12, -12 \leq k \leq 12, -16 \leq l \leq 14\)

**Reflections collected** 19839

**Independent reflections** 5019 \([R_{int} = 0.0140]\)

**Completeness to \(\theta = 27.45^\circ\)** 99.7 %

**Absorption correction** Semi–empirical from equivalents

**Max. and min. transmission** 0.9852 and 0.9491

**Refinement method** Full-matrix least-squares on \(F^2\)

**Data / restraints / parameters** 5019 / 0 / 295

**Goodness-of-fit on \(F^2\)** 1.045

**Final \(R\) indices \([F^2 > 2\sigma(F^2)]\)** \(R1 = 0.0348, wR2 = 0.0926\)

**\(R\) indices (all data)** \(R1 = 0.0377, wR2 = 0.0948\)

**Largest diff. peak and hole** 0.325 and –0.198 e \(\text{Å}^{-3}\)

---

**Special details:**

The crystal structure of 2012ncs0332 (PA531) crystallises in centrosymmetric spacegroup triclinic P-1. This means, that the crystal structure is a 50/50 racemic mixture of two enantiomers.
Table 1 • Atomic coordinates \([ \times 10^4]\), equivalent isotropic displacement parameters \([\text{Å}^2 \times 10^3]\) and site occupancy factors. \(U_{eq}\) is defined as one third of the trace of the orthogonalized \(U^{ij}\) tensor.

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<th>(y)</th>
<th>(z)</th>
<th>(U_{eq})</th>
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Table 2 • Bond lengths [Å] and angles [°].

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C13–N2–C12  62.25(6)
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C24–O4–C27  117.50(8)
Table 3 • Anisotropic displacement parameters [Å$^2 \times 10^3$]. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^*2U_{11} + ... + 2hk a^* b^* U_{12}]$.

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Table 4 • Hydrogen coordinates \([\times 10^4]\) and isotropic displacement parameters \([\AA^2 \times 10^3]\).

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Diffractometer: Rigaku R-Axis Spider including curved Fujifilm image plate and a graphite monochromated sealed tube Mo generator.


Data reduction, cell refinement and absorption correction: CrystalClear-SM Expert 2.0 r13 (Rigaku, 2011).

