Enantioseparation and structural optimisation of β-lactams and stilbene analogues for the treatment of triple negative breast and chemoresistant colorectal cancers

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Eavan C. McLoughlin, BSc. (Pharm), MPharm, MPSI, RSci.

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Under the supervision of
Asst. Prof. Niamh M. O’Boyle, BSc. (Pharm), PhD, MPSI

Co-supervised by Prof. Daniela M. Zisterer
Chapter 7 - Kinetic resolution of β-lactam combretazet racemates using Candida antarctica lipase B
7.1 Rationale for the use of lipase enzyme mediated kinetic resolution of \( \beta \)-lactams racemates

Our family of 3-hydroxyl analogues containing a variety of substitutions and deletions at the B ring \textit{meta} position (Figure 7.1) have dual anti-cancer activity in both TNBC cells and chemoresistant HT-29 colorectal cancer cells.\(^{378, 400}\) Chapters 3 and 4 focused on isolation of the corresponding enantiopure \( \beta \)-lactams through indirect chiral resolution. Chiral diastereomeric resolution has successfully resulted in enantioseparation of (+) and (–) 3-hydroxyl \( \beta \)-lactams as the \( N \)-Boc-L-proline derivatised \( 3S,4S \) and \( 3R,4R \) diastereomers using liquid chromatography (LC).\(^{717}\) Using this method, enantiomers were isolated in very small quantities sufficient only for preliminary \textit{in vitro} biological evaluation. An additional disadvantage noted was co-elution of diastereomers resulting in poorer \( ee \) of 50-80\% for the second eluting \( 3R \),\( 4R \) diastereomer (Table 4.12). LC chiral resolutions were also labour and time intensive, requiring large volumes of organic solvent while yielding only minimal quantities of enantiopure \( \beta \)-lactams. O’Boyle \textit{et al} have previously demonstrated that the aqueous solubility of \( \beta \)-lactam racemates is below the limit of detection measured using HPLC.\(^{380}\) Such solubility issues are potential limiting factors for pre-clinical progression of the eutomers described in Chapter 4 (Figure 7.1). Solubility issues for parent racemates have been overcome by derivatisation toward phosphate or amino acid prodrugs.\(^{380}\) Synthetic optimisation, solubility studies and biological evaluation of prodrug derivatives of lead enantiopure eutomers would require larger yields than chiral resolution has provided.

The biocatalytic approach involving kinetic resolution (KR) for the preparation of enantiomers was investigated, with the aim of increasing the isolated yields of enantiomers listed in Figure 7.1. This chapter explores the use of lipase enzymes, with focus on the use of CAL-B mediated methanolysis of 3-acetoxy \( \beta \)-lactam racemates (Figure 7.1). The use of KR holds potential as an improved strategy for isolation of larger yields of enantiopure 3-hydroxyl \( \beta \)-lactams in a greener and more sustainable manner for the purposes of progression towards the aqueous soluble prodrug derivatives. This chapter aimed to optimise an accessible biocatalytic method using
lipases for isolation of 3-hydroxyl enantiomeric β-lactams in comparable ee, but larger quantities, to chiral diastereomeric resolution. Preliminary phosphate prodrug derivatisation is explored with the aim of synthesising a biocompatible prodrug for future biological evaluation. Long term, it is our vision that a clinically viable prodrug will progress towards pre-clinical and clinical models as a novel agent for treatment of both TNBC and chemoresistant colorectal cancers.

7.2 Enantioselectivity values (E values) for KR reactions

To ensure high enantioselectivity, the rate of reaction for one enantiomer \( k_{fast} \) must be much greater than the rate of conversion for the second enantiomer \( k_{slow} \) (Figure 7.2). If the enantioselectivity is large, the rate of \( k_{slow} \) is negligible and the less reactive enantiomer remains unconverted by the enzyme. KR reactions would therefore cease

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**Figure 7.1:** A: Structural modifications which prevent cis/trans isomerisation of CA-4 (β-lactam ring insertion) and increase anti-proliferative activity in CA-4 resistant HT-29 cells (replacement of the B ring meta hydroxyl moiety with a halogen or methyl group); B: Proposed CAL-B mediated methanolysis of the 3S,4S 3-acetoxy enantiomer to the 3S,4S 3-hydroxyl enantiomer.

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**Chapter 7:** Kinetic resolution of β-lactam combretazet racemates using *Candida antarctica* lipase B
with excellent enantioselectivity at 50% conversion. In practice, this is rare. KR reactions often continue beyond 50% if the lipase enzyme of choice has poor enantioselectivity. Since the substrates are converted at different rates, their ratio in the reaction mixture is not constant as the reaction progresses, varying with degree of conversion. The enantiomeric ratio, E value, or selectivity factor, is a second order rate constant used to describe substrate specificity. The E value is determined by the environment of the system i.e substrate, enzyme, solvent, pH, temperature etc. The E value is also dependent on the ee of substrate (eeₐ), product (eeₚ) and % conversion of reaction. E values are extremely useful constants for determination of KR enantioselectivities. Good E values are considered between 15-30 while above this value infers excellent enantioselectivity.

7.3 Lipase mediated KR of β-lactams and derivatives

There are several examples of lipase-mediated KR of β-lactams reported. For example, lipase B Candida antarctica (CAL-B) has been used for KR of β-lactam racemates for the production of optically pure β-amino acids and β-amino esters via enantioselective ring opening reactions, leaving behind a less reactive β-lactam. Lipases typically have the ability to form amide bonds but cannot cleave them due to their resonance stabilised carbonyl structures. A lack of resonance stabilisation for the β-lactam cyclic amides renders them susceptible to enantioselective ring-opening reactions by lipase enzymes. Ring opening reactions often require structural activation of the NH moiety of the β-lactam ring for e.g N-benzoyl protection to increase ring
reactivity.\textsuperscript{726} NH, N-acetyl and N-chloroacetyl racemates undergo enantioselective ring-opening reactions. In contrast N-\textit{tert}-butyloxycarbonyl and N-aryl substituted racemates have been determined as unsusceptible to methanolysis ring opening mediated by CAL-B.\textsuperscript{724} Steric factors may restrict this enzymatic ring opening reaction for N-aryl substituted \(\beta\)-lactams. Examples of lipase mediated enantioselective ring opening reactions of synthetic interest include the enantioselective ring opening reaction of N-unsubstituted [(3\(R\), 4\(S\))-(\(\pm\))] \textit{cis}-3-acetoxy-4-phenylazetidin-2-one using CAL-B in diisopropyl ether, for the preparation of the optically active taxane side chain component. CAL-B mediated a two-step cascade reaction involving cleavage of the acetoxy ester moiety to the free hydroxyl \(\beta\)-lactam, followed by a highly selective N1-C2 cleavage of the \(\beta\)-lactam ring resulting in \(ee\) of >98\% for the desired \(\beta\) amino acid\textsuperscript{727} (Figure 7.3).

![Figure 7.3: Enantioselective hydrolysis of 3-acetoxy \textit{cis} \(\beta\)-lactam structure in a two-step cascade reaction to the enantioenriched 3-hydroxyl structure in \(<20\%\) \(ee\) followed by enantioselective ring opening to enantiopure taxane side chain precursor in \(>98\%\) \(ee\).]

\textit{CAL-B, iPr2O, H2O, 60 °C}

KR has also been achieved for \(N\)-hydroxymethylated \(\beta\)-lactams \textit{via} lipase catalysed hydroxyl \(O\)-acylation (A, Figure 7.4). \textit{Burkholderia cepacia} (lipase PS) enantioselectively acylated the hydroxyl of the 4\(S\) enantiomer in presence of acyl donor vinyl butanoate in toluene containing 5\% v/v acetone towards the esterified enantiomer, resulting in enantioseparation of racemic \(\beta\)-lactams in 98 and 80\% \(ee\) (A, Figure 7.4). It was noted that cessation of the enzymatic resolution at 30\% conversion was required in order to obtain high \(ee\) values for the preferentially hydrolysed \(S\) enantiomer, while continuation beyond 50\% was necessary in order to obtain higher \(ee\) values for the

\textbf{Chapter 7: Kinetic resolution of \(\beta\)-lactam combretazet racemates using \textit{Candida antarctica} lipase B}
unreacted $R$ enantiomer.\textsuperscript{728} This study also proposed a unique double resolution for the further enantioenrichment of the acylated $S$ enantiomer beyond 80% $ee$. Lipase PS catalysed acylation using vinyl butanoate was quenched at 55% conversion (via filtration to remove the enzyme) followed by LC purification of $R$ and $S$ enantiomers in 98% and 80% $ee$ respectively. The acylated $S$ enantiomer derived from A, Figure 7.4 was subsequently subjected to lipase PS catalysed alcoholysis with 1-butanol, to yield >99% $ee$\textsuperscript{728} (B, Figure 7.4).

Figure 7.4: A: Lipase PS catalysed enantioselective acylation of $\beta$-lactams with vinyl butanoate;\textsuperscript{728} B: Lipase PS catalysed alcoholysis of the enantioenriched acylate (from A) as part of a double resolution strategy to yield the 4$S$ enantiomer in >99% $ee$\textsuperscript{728}

Carr \textit{et al} have reported KR of 3-acetoxy $\beta$-lactam derivatives \textit{via} hydrolysis in phosphate buffers (0.2 M pH 7.2) using lipase PS demonstrating that the enantioselective hydrolysis proceeded in favour of the 3$S$ enantiomer. Conclusions were also made on the effects of the B ring substituent position, size and electronic effects on determining rate and enantioselectivity of reaction. \textit{Para} electron withdrawing substitutions increased rate while electron donating substitutions had lower substrate conversions. \textit{Ortho} electron withdrawing substitutions resulted in lower conversion rates. Conversion also increased with increasing size at the \textit{para} position but decreased with increasing size at either \textit{ortho} or \textit{meta} positions.\textsuperscript{419} These findings are of relevance for KR studies carried out in this chapter since 3-acetoxy $\beta$-lactams are
the substrates of choice. Additionally these 3-acetoxy substrates contain a variety of the substituents at both para and meta positions of the B ring (B, Figure 7.1).

7.4 Proposed mechanism for CAL-B mediated methanolysis of ester bonds

KR strategies reported in this thesis are based on methods reported by Sundell et al employing CAL-B and methanol for methanolysis. The CAL-B active site is a three dimensional structure exhibiting a canonical α/β fold, of parallel β sheets surrounded on both sides by α helices (Scheme 7.1). CAL-B’s active site is tunnel shaped which sterically limits the position of bulky substrates. Its active site is composed of a catalytic triad: a serine (Ser), aspartic acid (Asp) and histidine (His) amino acid residue sequence (S105-H224-D187). The Ser residue acts as the nucleophile in hydrolytic reactions, the His as an acid/base mediating catalytic transfer of protons between the enzyme and its substrate, while the Asp residue stabilises tetrahedral intermediates. The oxyanion hole contains hydrogen bond donors from amides of threonine (T40) and glutamine (Q106) which stabilise the negative charge of tetrahedral intermediate oxygen atoms formed during catalysis.

Lipases differ from typical esterases as they display two conformations: open or closed. In the closed conformation, a lid of amphiphilic α helices precludes the active site from reaching its substrate. Upon exposure of the lid to hydrophobic surfaces, lid displacement occurs, transitioning to the active site open form which in turn leads to interaction of enzyme with its substrate. Most lipases exert their hydrolytic activity on the water-oil interface, a phenomenon known as interfacial activation. Interfacial activation is a two-step mechanism involving enzyme adsorption onto a hydrophobic interface followed by lid opening. Lid opening upon interfacial activation is therefore accompanied by a subsequent augmentation of lipolytic/enzymatic activity. In the absence of an aqueous-lipid interface, the lipase is rendered inactive, since exposure of the active site is energetically unfavourable, stabilising it in closed confirmation. CAL-B however is typically considered as an exception to the requirement for interfacial activation and does not require exposure to a hydrophobic surface to mediate its effects. The lid of CAL-B is composed of two amphiphilic α helices (α5 and α10). However it is now proposed that CAL-B’s immobilisation on hydrophobic surfaces
mediates a form of interfacial activation and increases its activity towards bulkier substrates due to increasing the flexibility of the α5 helix at the lid of the active site which enables entry of a wider variety of substrates. CAL-B has a more open active site when immobilised on a hydrophobic surfaces, thus accommodating substrates that are not typically hydrolysed effectively, e.g. aromatic alcohols.745

The proposed serine hydrolase methanolysis mediated by CAL-B is illustrated as a two-step mechanism in Scheme 7.1. The first step of the catalytic cycle is an acylation reaction. The Ser side chain’s oxygen acts as the nucleophile on the carbonyl of the ester substrate. This occurs concomitantly with proton transfer from the histidine residue to form the first tetrahedral acylated intermediate (INT₁). INT₁ is stabilised by the amino acid side chains of the ‘oxyanion hole’ (backbone amino and side chain hydroxyl of T40 and backbone amino of Q106). In the formation of this acyl intermediate, the 3-hydroxyl β-lactam is liberated (Scheme 7.1). The acyl intermediate is positively charged at the His224 residue, which is stabilised by Asp187.

Secondly, a deacylation step occurs where a second tetrahedral intermediate (INT₂, Scheme 7.1) is formed by nucleophilic attack by the oxygen of an alcohol or water molecule. In the case of hydrolytic reactions, water acts as the nucleophile to form a second tetrahedral intermediate, which proceeds to regenerate the enzyme and release a carboxylic acid.746-748 In this thesis, CAL-B reactions are carried out in organic solvents, where methanol replaces water as the nucleophile.

CAL-B and other lipases preferentially hydrolyse water-insoluble substrates, demonstrating poor activity towards water-soluble substrates.749

This aims of this chapter are as follows.

1) Optimise a KR procedure for isolation of 3S,4S combretazets in similar or higher ee to diastereomeric chiral resolution.

2) Achieve a higher ee for the 3R,4R distomer compared to chiral diastereomeric resolution.

3) Exploration of prodrug synthesis.
4) Application of KR to the B ring meta hydroxyl substituted eutomers derivatised with the N-Boc-L-proline CDR.

![Diagram of serine hydrolase mechanism mediated by CAL-B using methanol as a nucleophile.]

Scheme 7.1: The serine hydrolase mechanism mediated by CAL-B using methanol as a nucleophile. Oxyanion residues illustrated in blue. Acylated intermediate illustrated in red. INT$_1$ = tetrahedral intermediate 1. INT$_2$ = tetrahedral intermediate 2.

Since the combretazets are N-aryl substituted with the 3,4,5-trimethoxyphenyl A ring, this chapter aims to firstly explore the stability of the combretazet β-lactam scaffold to CAL-B and secondly potential reaction pathways of KR mediated by CAL-B.$^{24}$
7.5 Preliminary kinetic resolution of 3-acetoxy racemate 17a using CAL-B

Optimisation of KR approaches were explored using racemate 17a. Intermediate 3-acetoxy 17a was subjected to alcoholysis (methanolysis) by selected lipase enzymes. Lipase B Candida antarctica (CAL-B) immobilised on Immunobead 150, recombinant from yeast 4022 U/g (Lot #BCCF8776, lot result 4022 U/g (Sigma) was selected for initial screening, based on a method reported by Sundell et al.24 Screening was also carried out with Amano Lipase PS, from Burkholderia cepacia (Lipase PS).

An initial quantity of 100 mg (402 U) of CAL-B of was chosen as a starting point with 100 mg (0.25 mmol) of 17a (1mg/ mg of CAL-B/17a) in 10 mL of TBME (Method A, Table 7.2). Preliminary screening of CAL-B aimed to determine if either enantioselective β-lactam ring opening or methanolysis of the 3-acetoxy moiety to an enantioenriched 3-hydroxyl product would proceed. TLC observations determined the formation of a new product and at 110 h the reaction was quenched by gravity filtration to remove CAL-B. Ring opening did not occur, as indicated by 1H NMR analysis, but rather formation of the 3-hydroxyl product occurred in 50% yield (entry 1, Table 7.2). The β-lactam ring hydrogens at δ 5.37 ppm (H₃) and δ 4.85 ppm (H₄) for the 3-acetoxy enantioenriched substrate were accompanied by the presence of additional signals from the 3-hydroxyl H₃ and H₄ hydrogens at δ 4.79 ppm (H₃) and δ 4.74 ppm (H₄). These findings demonstrate the chemical stability of the β-lactam ring of 17a in the presence of CAL-B towards ring opening alcoholysis and infers methanolysis of the 3-acetoxy toward the 3-hydroxyl moiety.

The methanol equivalents remained constant throughout each optimisation reaction while other parameters were selectively varied. A scale up was carried out for 17a (1 mmol, entry 2, Table 7.2) in TBME (10 mL). After 48 hours only 7% conversion was observed. Chiral HPLC determined the ee of isolated substrates and products. Chiral HPLC employed an identical method as described in Chapter 4 using the chiral stationary phase column, Chrompak-IH-3 (150 x 4.6 mm), with a Chiral-IH-3 guard column and mobile phase of 1:1 n-hexane: isopropanol. In order to determine the absolute configuration for the preferentially hydrolysed enantiomer, the isolated 3-hydroxyl product (purified from unreacted 3-acetoxy substrate using LC) was
compared to standard enantiomers isolated in Chapter 3 using LC chiral resolution. In the case of the enantioenriched 3-hydroxyl 17, the first eluting peak was assigned to the 3R,4R 17EN2 by comparison to standard 17EN2 isolated in Chapter 3, while the second eluting and major peak was assigned to 3S,4S 17EN1 (Table 7.1). The major peak for CAL-B methanolysed 3-hydroxyl products and second eluting enantiomer was therefore determined as the 3S, 4S eutomer 17EN1 (as shown previously in Figure 4.2) while the enantioenriched and unreacted substrate was assigned to the 3R, 4R 3-acetoxy distomer 17aEN2 (Table 7.1).

Table 7.1: Chiral chromatograms for enantioenriched 3R,4R 3-acetoxy 17aEN2 and 3S,4S 3-hydroxyl 17EN1 isolated from entry 6, Table 7.1 purified using LC post-reaction.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ER of 3-acetate</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>17aEN2</td>
<td>3S, 4S: 3R, 4R</td>
<td>6:94 (88%)</td>
</tr>
<tr>
<td>17EN1</td>
<td>3S, 4S: 3R, 4R</td>
<td>70:30 (40%)</td>
</tr>
</tbody>
</table>

Entry 2 (Table 7.2) demonstrated poor ee (40%) for the 3-hydroxyl product. Entries 3-5 (Table 7.2) aimed to investigate whether scale or solvent volume determined the rate of reaction for CAL-B mediated methanolysis of 3-acetoxy 17a to 3-hydroxyl 17. Reactions were carried out at 0.25, 0.5 and 1 mmol scales (entry 3-5, Table 7.2)
increasing solvent volume to 10 mL of TBME per 0.25 mmol of 17a (Method A, Table 7.2), to account for reaction scale. Using a lower scale (0.25 mmol), the methanolysis of 3-acetoxo to 3-hydroxyl eutomers proceeded faster with 30% and 47% conversion observed at 24h (1.25%/h conversion) and 48h (0.75%/h) respectively. *Ee* for the 3-hydroxyl product remained modest determined as 32%. Increasing the scale to 0.5 and 1 mmol resulted in a slower rate of methanolysis and higher *ee* values. At 72h only 34% (compared to 39% on 0.5 mmol scale) of the 3-acetoxo 17a had been converted to 3-hydroxyl 17 in 56% and 44% ee. Scale of the reaction influenced both reaction rate and *ee* values with greater conversion observed on the 0.5 mmol scale (47% by 48h) compared to 1 mmol scale (39% by 72h). Greater enantioenrichment was observed using the higher scale when a slower rate of CAL-B mediated was employed [32% versus 56% on 0.5 and 1 mmol scale respectively (entries 3 and 4, Table 7.2)]. Additionally, entries 4 and 5 (Table 7.2) gave rise to preliminary evidence that quenching the enzymatic reaction prior to 50% conversion may yield products in greater *ee*.

Entry 6 aimed to investigate reaction duration. CAL-B was combined with 17a using identical conditions to entry 5 for 111 h. A higher rate of conversion was observed (69% versus 34%) and an excellent *ee* (88%) for the 3-acetoxo (3R,4R) enantioenriched substrate (17a). The *ee* value for the 3-hydroxyl product was however poor (40% versus 44% for entry 6 versus entry 5, Table 7.2). Enantioselectivity using Method A is poor for the desirable 3S,4S 3-hydroxyl eutomer. However the enantioenriched 3-acetoxo β-lactam was isolated in excellent *ee* of >85% when the reaction was allowed to proceed beyond 50% conversion.
Table 7.2: CAL-B optimisation reactions using $17a$ as substrate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme (mg)</th>
<th>Units</th>
<th>Scale mmol (mg)</th>
<th>Method</th>
<th>% conversion $^a$</th>
<th>Time (h)</th>
<th>TBME (mL)</th>
<th>ER of 3-acetoxy 3S, 4S: 3R, 4R (ee)</th>
<th>ER of 3-hydroxy 3S, 4S: 3R, 4R (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>402</td>
<td>0.25 (100)</td>
<td>A</td>
<td>50</td>
<td>110</td>
<td>10</td>
<td>Nd 47:53 (6%)</td>
<td>Nd 70:30 (40%)</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>1608</td>
<td>1 (400)</td>
<td>B</td>
<td>7</td>
<td>48</td>
<td>10</td>
<td>48 33:67 (34%)</td>
<td>48 66:34 (32%)</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>402</td>
<td>0.25 (100)</td>
<td>A</td>
<td>47</td>
<td>48</td>
<td>10</td>
<td>48 30:70 (40%)</td>
<td>48 78:22 (56%)</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>804</td>
<td>0.5 (200)</td>
<td>A</td>
<td>39</td>
<td>72</td>
<td>20</td>
<td>72 39:61 (22%)</td>
<td>72 72:28 (44%)</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>1608</td>
<td>1 (400)</td>
<td>A</td>
<td>34</td>
<td>72</td>
<td>40</td>
<td>72 30:70 (40%)</td>
<td>72 78:22 (56%)</td>
</tr>
<tr>
<td>6</td>
<td>400</td>
<td>1608</td>
<td>1 (400)</td>
<td>A</td>
<td>69</td>
<td>111</td>
<td>40</td>
<td>111 6:94 (88%)</td>
<td>111 70:30 (40%)</td>
</tr>
<tr>
<td>7</td>
<td>1200</td>
<td>5040</td>
<td>1 (400)</td>
<td>C</td>
<td>59</td>
<td>48</td>
<td>40</td>
<td>48 8:93 (85%)</td>
<td>48 70:30 (40%)</td>
</tr>
<tr>
<td>8</td>
<td>300</td>
<td>1206</td>
<td>1 (400)</td>
<td>D</td>
<td>35</td>
<td>144</td>
<td>10</td>
<td>144 37:63 (26%)</td>
<td>144 63:37 (26%)</td>
</tr>
<tr>
<td>9</td>
<td>400</td>
<td>1608</td>
<td>1 (400)</td>
<td>E</td>
<td>51</td>
<td>192</td>
<td>100</td>
<td>192 22:78 (56%)</td>
<td>192 78:22 (56%)</td>
</tr>
</tbody>
</table>

$^a$ = % conversion (alcoholysis from 3-acetoxy racemate to enantioenriched 3-hydroxyl β-lactam) measured using $^1$H NMR in CDCl$_3$ at 400 MHz of reaction following work up

ER = Enantiomeric Ratio, ee = enantiomeric excess (major – minor peak calculated using chiral HPLC)

10 equivalents of methanol used for all reactions

**Method A**: 1 mg CAL B / mg of $17a$. 10 mL of TBME / 0.25 mmol of $17a$, RT.
**Method B**: 1 mg CALB / mg of $17a$. 10 mL of TBME
**Method C**: 30 mg / mL of TBME, 10 mL of TBME / 0.25 mL of $17a$, RT
**Method D**: 30 mg / mL of TBME, solvent volume reduced to 10 mL, RT
**Method E**: 1mg/mg of CAL B/ mg of $17a$, solvent volume increased 100 mL of TBME

Chapter 7: Kinetic resolution of β-lactam combretazet racemates using Candida antarctica lipase B
To investigate the effect of increasing the rate of methanolysis, the enzyme content of CAL-B was increased to 30 mg/mL (Methods C and D, Table 7.2). 59% conversion was observed at 48h. Ee was poor (40%) for the 3-hydroxyl enantiomer but excellent (85%) for the 3-acetoxy enantiomer (entry 7, Table 7.2), due to >50% methanolysis. Reducing the solvent volume to 10 mL resulted in both a slower rate of and poor ee observed for both 3-acetoxy and 3-hydroxyl enantiomers (entry 8, Table 7.2). Since ee for the 3-hydroxyl enantiomer was low using 30 mg/ mL of CAL-B, 1 mg/ mL was trialed with a higher solvent volume (entry 9, Method E, Table 7.2). A combination of reducing the enzyme content and increasing volume yielded a more favouravble ee of 56% for the desirable 3S,4S 3-hydroxyl enantiomer 17EN1. The absolute configuration of the 3S,4S 3-hydroxyl enantiomer was confirmed using XRD analysis for entry 9 (Table 7.2) in 56% ee (Figure 7.5). XRD parameters are in agreement with those obtained for crystals isolated using chiral resolution (Table 4.7).

Figure 7.5: Disordered molecular structure of chiral 3-hydroxyl 17EN1 isolated by CAL-B mediated methanolysis in 56% ee (entry 9, Table 7.2). Atomic displacement shown at 50% probability and heteroatoms labelled only.

7.5.1 Time course analysis of enantiomeric excess for kinetic resolution of 17a
While KR using CAL-B successfully yielded 3-acetoxy 3R,4R 17aEN2 in > 85 % ee (Table 7.1) which was superior to 71% obtained through chiral resolution (Chapter 4), further optimisation was required in order to develop a KR method for isolation of the 3S,4S 3-hydroxyl eutomer in comparable ee values to chiral resolution as discussed in Chapters 3 and 4. A time course analysis of reaction 9 (Figure 7.6) was carried out in order to determine the optimal point of 3-acetoxy to 3-hydroxyl conversion yielding the
greatest ee, using chiral HPLC. However ee values were constant (56-62%) throughout the reaction (Figure 7.6).

![Figure 7.6: % ee of 3S,4S 3-hydroxy 17EN1 and 3R,4R 3-acetoxy 17aEN2 versus % conversion for entry 9, Table 7.1](image)

### 7.5.2 Enantioselectivity of CAL-B using methanol as solvent and 17a as substrate

In order to investigate the effect of the methanol content on the enantioselectivity of CAL-B mediated methanolysis of 3-acetoxy racemate 17a to the 3-hydroxy 3S,4S enantiomer 17, the reaction was carried out in methanol only. Approximately 95% conversion was observed within 3 hours. Ee was determined as 0% for both substrate and product (entry 1, Table 7.3). The reaction was repeated without enzyme in order to investigate solvent effects on conversion of the acetoxy to the free hydroxyl. In the absence of enzyme, conversion was not observed (entry 2, Table 7.3). These findings indicate that the rate of CAL-B mediated hydrolysis is greatly increased in the presence of methanol only and is not enantioselective. The 3-acetoxy is converted to the 3-hydroxyl racemate rather than an enantioenriched substrate.

Table 7.3: CAL-B optimisation reactions varying MeOH content using 17a as substrate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme (mg)</th>
<th>Units</th>
<th>% conversion</th>
<th>Time (h)</th>
<th>ER of 3-acetoxy 3S, 4S: 3R, 4R (ee)</th>
<th>ER of 3-hydroxyl 3S, 4S: 3R, 4R (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>1608</td>
<td>95%</td>
<td>3-4</td>
<td>1:1 (0%)</td>
<td>1:1 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>36</td>
<td>1:1 (0%)</td>
<td>Na</td>
</tr>
</tbody>
</table>

Reactions carried out on 1 mmol scale (400 mg of 17a) in 40 mL of MeOH.
ER = enantiomeric ratio

7.5.3 Investigating the nucleophilic effects of co-solvent and enzyme content on enantioselectivity of CAL-B with 17a as substrate

Next the effects of reducing the methanol content to 2.5 eq rather than 10 eq (Table 7.2) on the ee was investigated. 11% conversion was observed after 3 days (70 h) with 74% of 3S,4S 3-hydroxyl 17 isolated (0.5 mmol scale, entry 1, Table 7.4). In contrast, the ee values were lower (62%) when the reaction scale was increased to 1 mmol (entry 3, Table 7.4). Conversion rates were poor (4% after 79h) using water as the co-solvent or nucleophile (entry 2, Table 7.4). Reducing the enzyme content to 0.5 mg/ mg of CAL-B per 17a with 10 eq of methanol resulted in a larger ee (60%, entry 4, Table 7.4) than previously observed using 1 mg/ mg and 10 eq of methanol (40%, entry 5, Table 7.2). These findings indicate that a combination of the following may enhance ee for the 3S,4S 3-hydroxyl eutomer of 17.

1) Reducing the methanol content to 2.5 eq.
2) Reducing the enzyme content by 50% to 0.5 mg/ mg of substrate.

Using a combination of reduced methanol and enzyme content, ee was increased to 62% for the 3-hydroxyl eutomer 17EN1 with 45% conversion after 78 h (entry 5, Table 7.4).
Table 7.4: Optimisation of CALB KR reactions for 17a varying co-solvent, co-solvent equivalents and enzyme content

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme mg (U)</th>
<th>Scale mmol (mg)</th>
<th>% conversion</th>
<th>Time (h)</th>
<th>TBME (mL)</th>
<th>Co-solvent</th>
<th>Co-solvent volume (eq)</th>
<th>ER of acetate 3S, 4S: 3R, 4R (ee)</th>
<th>ER of 3-hydroxyl 3S, 4S: 3R, 4R (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200 (804)</td>
<td>0.5 (200)</td>
<td>11%</td>
<td>70</td>
<td>20</td>
<td>Methanol</td>
<td>41 µL (2.5)</td>
<td>46:54 (8%)</td>
<td>13:87 (74%)</td>
</tr>
<tr>
<td>2</td>
<td>200 (804)</td>
<td>0.5 (200)</td>
<td>4%</td>
<td>79</td>
<td>20</td>
<td>Water</td>
<td>21 µL (2.5)</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>3</td>
<td>400(1608)</td>
<td>1 (400)</td>
<td>48%</td>
<td>72</td>
<td>40</td>
<td>Methanol</td>
<td>101 µL (2.5)</td>
<td>75:25 (50%)</td>
<td>81:19 (62%)</td>
</tr>
<tr>
<td>4</td>
<td>200 (804)</td>
<td>1 (400)</td>
<td>35%</td>
<td>90</td>
<td>40</td>
<td>Methanol</td>
<td>404 µL (10)</td>
<td>34:66 (32%)</td>
<td>80:20 (60%)</td>
</tr>
<tr>
<td>5</td>
<td>200 (804)</td>
<td>1 (400)</td>
<td>45%</td>
<td>78</td>
<td>40</td>
<td>Methanol</td>
<td>101 µL (2.5)</td>
<td>75:25 (50%)</td>
<td>81:19 (62%)</td>
</tr>
</tbody>
</table>
**7.6 Preliminary kinetic resolution of racemate 17 using amano lipase PS**

KR using amano lipase PS from *Burkholderia cepacia* was trialed for the isolation of enantiomers of 17. A 0.5 mmol scale reaction using 3-acetoxy 17a and 1 mg/mg of lipase PS per 17a at room temperature was carried out. Conversion to the 3-hydroxy β-lactam was not observed after 4 days using Method A adopted from Table 7.2 (entry 1, Table 7.5). The enzyme content was increased to > 5 mg / mg of lipase PS per 17a and at 50 °C for 6 days, again with no observable conversion. Lipase PS was therefore pursued no further in this thesis.

Table 7.5: Preliminary KR reactions of 17a using amano Lipase PS, from *Burkholderia cepacia*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme content (mg)</th>
<th>Enzyme content (U)</th>
<th>Scale mmol (mg)</th>
<th>Temperature</th>
<th>% Conversion</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>6000</td>
<td>0.5 (200)</td>
<td>RT</td>
<td>0</td>
<td>4 days (96)</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>10000</td>
<td>0.1 (40)</td>
<td>50 °C</td>
<td>0</td>
<td>6 days (144)</td>
</tr>
</tbody>
</table>

RT = room temperature
Reactions carried out in 20 mL of TBME with 10 eq of methanol

**7.7 Preliminary kinetic resolution of racemate B ring meta substituted 3-acetoxy 18a using CAL-B**

Preliminary reactions demonstrated a much lower rate of methanolysis for the B ring *meta* fluorine substituted 18a with respect to unsubstituted 17a (entry 1, Table 7.6). After 64h only 7% conversion was observed. However ee was much greater (78%) than prior observations for 17a (Table 7.2). Additionally increasing the volume from 40 mL (Method A, Table 7.6) to 100 mL (Method B, Table 7.6) resulted in a greater relative rate of conversion of 40% at 96 h (0.42%/h) versus 7% at 64h [0.11%/h, (entries 2 and 1, Table 7.6 respectively)] while maintaining a favourable ee of >75%.
Table 7.6: CAL-B optimisation reactions for substrate 18a as substrate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme content mg (U)</th>
<th>Scale mmol (mg)</th>
<th>Method</th>
<th>% conversion a</th>
<th>Time (h)</th>
<th>TBME (mL)</th>
<th>ER of 3-acetoxy 3S, 4S: 3R, 4R (ee)</th>
<th>ER of 3-hydroxyl 3S, 4S: 3R, 4R (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>419 (1685)</td>
<td>1 (419)</td>
<td>A</td>
<td>7%</td>
<td>64 h</td>
<td>40</td>
<td>73:27 (46%)</td>
<td>89:11 (78%)</td>
</tr>
<tr>
<td>2</td>
<td>419 (1685)</td>
<td>1 (419)</td>
<td>A</td>
<td>40%</td>
<td>96h</td>
<td>100</td>
<td>80:20 (60%)</td>
<td>88:12 (76%)</td>
</tr>
<tr>
<td>3</td>
<td>838 (838)</td>
<td>2 (838)</td>
<td>B</td>
<td>27%</td>
<td>168h</td>
<td>80</td>
<td>34:66 (32%)</td>
<td>78:22 (56%)</td>
</tr>
</tbody>
</table>

All reactions carried out with 10 eq of methanol

a = % conversion (alcoholysis from 3-acetoxy to 3-hydroxy) measured using $^1$H NMR in CDCl$_3$ at 400 MHz of reaction following work up

ER = Enantiomeric Ratio, ee = enantiomeric excess (major – minor peak calculated using chiral HPLC)

Method A: 1 mg CAL-B / mg of 17a, 10 mL of TBME / 0.25 mmol of 17a, RT.

Method B: 1 mg CAL-B / mg of 17a, solvent volume increased 100 mL of TBME
7.7.1 Time course analysis of enantiomeric excess for CAL-B mediated KR of 18a

A time course analysis of ee values during formation of the 3-hydroxyl 3S,4S eutomer was carried out in order to determine the time-point of greatest enantioselectivity. For entry 1, Table 7.6, ee values remained constant from 62-82% throughout the reaction illustrated as A, Figure 7.7. However for entry 2 (Table 7.6), ee values were highest (80-90%) at earlier time points of 25-35% methanolation in comparison to 76% ee observed at 96 hours when 40% methanolation had occurred (B, Figure 7.7). This data suggests that as KR approaches 50% methanolation, the rate of non-selective mediated methanolation of the 3R, 4R 3-acetoxy enantiomer increases. Therefore quenching the reaction prior to 40-50% conversion may increase the enantioselectivity of the reaction and yield greater ee for the desired 3S,4S eutomer. XRD analysis of 18a confirmed the absolute configuration for 3-acetoxy 18a and 3-hydroxyl 18 as 3R,4R and 3S,4S respectively. The XRD structure of 18a whose absolute configuration was determined as 3R,4R is illustrated in Figure 7.8.
Figure 7.7: A: % ee for 3S, 4S 18EN1 and 3R,4R 3-acetoxy 18aEN2 (entry 1, Table 7.6) determined by chiral HPLC for B: entry 2, Table 7.6

Figure 7.8: A: XRD structure of enantioenriched 18a with absolute configuration 3R,4R isolated from CAL-B reactions, entry 2, Table 7.6, crystallised from methanol overnight.
A comparative illustration of the rate of CAL-B mediated methanolysis for 17a versus 18a is illustrated in Figure 7.9. In general, a greater rate of 3-acetoxy to 3-hydroxyl methanolysis was observed for B ring meta unsubstituted 17a versus fluorine substituted 18a. Since larger ee values were observed for 18EN1 (56-78%, Table 7.6) versus 17EN1 (30-56%, Table 7.2), a slower rate of methanolysis may be responsible for augmenting the enantioselectivity of KR reactions. The electron withdrawing meta fluorine substitution of 18a’s B ring may favour this slower rate of reaction due to either electronic effects of modifying the conformation of the active site-ligand complex.

Figure 7.9: % conversion of 18a (Table 7.6) and 17a (Table 7.2) using CAL-B (determined by 1H NMR in CDCl₃ at 400 MHz)

7.8 KR optimisation reactions for 20a using CAL-B

Next CAL-B mediated KR was trialled with B ring meta methyl substituted 20a (Table 7.8). Method A which employed 1 mg CAL-B / mg of 20a and 10 mL of TBME / 0.25 mmol of 20a, was trialled on a 0.5 mmol scale (entry 1, Table 7.8). These conditions in combination with 20a as the substrate demonstrated rapid methanolysis by 72h accompanied by poor ee for 3R,4R 3-acetoxy 20a (13%) and 3S,4S 3-hydroxyl 20EN1 (42%). Reducing the enzyme content to 0.5 mg/ mg of 20a did not improve ee (34%, entry 3, Table 7.8). However, reducing both enzyme content and methanol equivalents (from 10 to 3 eq) yielded greater ee of 62% for 3-hydroxyl 20EN1, quenching at 50% conversion (entry 4, Table 7.8). Employing a combination of lower enzyme
content and methanol equivalents led to a much slower rate of reaction. For example, 50% conversion for 20a was achieved by 144 h in contrast to 47% by 60 h for entry 2 (Table 7.8) when using a higher enzyme and methanol content.

Since favouring the slower rate of CAL-B mediated methanolysis appeared to increase the ee for 3S,4S 3-hydroxyl products, further optimisation would involve employing the lower enzyme content (0.5 mg/mg of CAL-B per mg of 3-acetoxy racemate) in combination with 40 mL of TBME and a lower methanol content (3 eq of methanol), in addition to quenching of reactions at approximately 35% conversion. Quenching the reaction prior to 40-50% was anticipated to optimise ee in keeping with findings observed for 18a in B, Figure 7.7.
Table 7.8: CAL B optimisation reactions using 20a as substrate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme (mg)</th>
<th>Units</th>
<th>Scale mmol</th>
<th>Method</th>
<th>% conversion</th>
<th>Time (h)</th>
<th>Methanol µL (Eq)</th>
<th>ER of 3-acetoxy 3S, 4S: 3R, 4R (ee)</th>
<th>ER of 3-hydroxyl 3S, 4S: 3R, 4R (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>207</td>
<td>843</td>
<td>0.5</td>
<td>A</td>
<td>31%</td>
<td>72</td>
<td>202 (10)</td>
<td>43:56 (13%)</td>
<td>29:71 (42%)</td>
</tr>
<tr>
<td>2</td>
<td>207</td>
<td>843</td>
<td>0.5</td>
<td>A</td>
<td>47%</td>
<td>60</td>
<td>202 (10)</td>
<td>68:32 (36%)</td>
<td>65:35 (30%)</td>
</tr>
<tr>
<td>3</td>
<td>103</td>
<td>222</td>
<td>0.5</td>
<td>B</td>
<td>57%</td>
<td>120</td>
<td>202 (10)</td>
<td>66:34 (32%)</td>
<td>67:33 (34%)</td>
</tr>
<tr>
<td>4</td>
<td>103</td>
<td>222</td>
<td>0.5</td>
<td>B</td>
<td>50%</td>
<td>144</td>
<td>62 (3)</td>
<td>72:28 (44%)</td>
<td>81:19 (62%)</td>
</tr>
</tbody>
</table>

Method A: 1 mg CAL B / mg of 20a. 10 mL of TBME / 0.25 mmol of 20a, RT.
Method B: 0.5 mg/mg of CAL B/ mg of 20a, 10 mL of TBME / 0.25 mmol of 20a, RT.
7.9 Investigation of enantioselective CAL-B mediated deacetylation of N-Boc-L-proline derivatised diastereomers

Since a panel of N-Boc-L-proline derivatised diastereomers were already synthesised in Chapter 4 and CAL-B had demonstrated successful methanolysis of the 3-acetoxy ester bond, similar removal of the ester proline moiety from β-lactam racemates was investigated for both 118 and 123 as representative examples of B ring and 3-hydroxyl derivatised diastereomers (Scheme 7.2). After seven days, no methanolysis was observed on either 1H NMR or TLC for either substrate. The steric bulk of the CDR in particular the presence of the t-butoxy moiety may prohibit entry of substrates 118 and 124 within the active site of CAL-B. Future work should focus on Boc removal to determine if decreasing the size of the acyl moiety would favour KR of proline diastereomers to furnish their desirable enantiomers.

It is widely known that CAL-B preferentially hydrolyses fatty materials rather than water soluble substrates. Proline derivatisation may therefore also decrease CAL-B’s preference for methanolysis of diastereomers 118 and 124 due to their increased water solubility with respect to the 3-acetoxy panel. To summarise steric hinderance and aqueous solubility of diastereomers synthetically designed for chiral resolution are factors which potentially prohibit CAL-B mediated KR. Future work could therefore focus on non-polar acyl derivatisation of the B ring meta hydroxyl of optimal size for entry into the CAL-B catalytic triad. Additionally, mechanochemistry is emerging as a strategy for expanding the substrate scope of CAL-B by modifying the shape of the lid α5 and α10 helices. Mechanical force may therefore enhance the enzyme’s activity towards methanolysis of the B ring meta hydroxyl derivatised proline diastereomers.
7.10 Preliminary KR of trans 63a and 23a
Enantiomers of amino-substituted β-lactam 23 were not isolated in Chapter 4. As previously mentioned, the trans racemate of either 23 or 63 are unreported to date in the literature. Since chiral resolution failed to isolate the corresponding 3S,4S eutomer 63EN1 and 23EN1, KR was explored using 3-acetoxy 63a as the substrate (Scheme 7.3). KR was not successful using 3-acetoxy 63a but demonstrated success for the corresponding reduced racemate 23a. The B ring meta amine substituted 23a was successfully obtained by reduction of 63a using zinc and glacial acetic acid over 6 days (a, Scheme 7.3). CAL-B methanolyse 23a to the corresponding enantioenriched 3S,4S 23 in 50% ee. This data suggests that either the highly electron withdrawing effect or steric bulk of the nitro group prevents activity of CAL-B on substrate 63a. In contrast by reducing the nitro to the corresponding amine, biocatalytic activity of CAL-B is observed to afford 3S, 4S 23EN1 in moderate ee (50%).
Scheme 7.3: Pathways for KR of 63a and 23a using CAL-B. a: Reduction of 63a to 23a using zinc and glacial acetic acid for 6 days. b: KR using CAL-B.

7.11 Investigation of lower enzyme and methanol content for KR of B ring meta substituted racemates

The use of 0.5 mg of CAL-B per mg of 3-acetoxy racemate in combination with 3 equivalents of methanol was further investigated to determine if the ee of the product could be ameliorated. Proposed substrates are detailed in B, Figure 7.1 and are specifically designed for dual anti-proliferative activity in both TNBC and HT-29 colorectal cancer cells. Optimisation was trialled for 3-acetoxy racemates 17a-21a and afforded insight on the ee of 3-hydroxyl products obtained via CAL-B mediated methanolysis which depended on the B ring meta substitution. The E or enantioselective factor was calculated for each reaction (using the ENANTIO webtool\(^{751}\)) to comparatively illustrate CAL-B’s enantioselectivity toward the 3S,4S enantiomer amongst the panel of 3-acetoxy β-lactam racemates.

Ee for 3-hydroxyl enantiomers from reactions quenched at <50% (substrates 19a and 21a, Table 7.9) were much greater (70 and 83%) versus reactions which proceeded beyond 50%. For example 17a had undergone 60% conversion at 4 days and yielded only 56% ee for 3-hydroxyl 3S,4S 17EN1. However ee was determined as 91% for the 3-acetoxy 3R,4R enantiomer 17a, when KR was allowed to proceed beyond 50%
conversion. This ee value was superior to the ee values obtained for the corresponding 3R,4R 3-hydroxy 17EN2 via chiral resolution in Chapter 3.

This data suggests two important key findings

1) Quenching KR reactions at 35% conversion improves ee values for the desirable 3-hydroxy 3S,4S eutomer.

2) Allowing the reaction to proceed to 50% enables isolation of the 3R,4R 3-acetoxy distomer in superior ee (i.e. > 91% ee) than has been observed for the 3R,4R 3-hydroxy enantiomer obtained via chiral resolution.

Table 7.9: Optimisation of KR reactions for meta substituted 3-acetoxy β-lactams using CAL-B.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time</th>
<th>% conversion</th>
<th>Yield&lt;sup&gt;1&lt;/sup&gt;</th>
<th>% ee</th>
<th>Yield&lt;sup&gt;1&lt;/sup&gt;</th>
<th>% ee</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>17aEN2</td>
<td>4 days</td>
<td>60</td>
<td>27%</td>
<td>91</td>
<td>61%</td>
<td>56</td>
<td>11</td>
</tr>
<tr>
<td>18aEN2</td>
<td>7 days</td>
<td>57</td>
<td>9%</td>
<td>82</td>
<td>41%</td>
<td>66</td>
<td>12</td>
</tr>
<tr>
<td>19aEN2</td>
<td>6 days</td>
<td>35</td>
<td>18%</td>
<td>30</td>
<td>22%</td>
<td>70</td>
<td>8</td>
</tr>
<tr>
<td>20aEN2</td>
<td>7 days</td>
<td>50</td>
<td>15%</td>
<td>72</td>
<td>16%</td>
<td>56</td>
<td>7</td>
</tr>
<tr>
<td>21aEN2</td>
<td>5 days</td>
<td>30</td>
<td>61%</td>
<td>34</td>
<td>18%</td>
<td>83</td>
<td>15</td>
</tr>
</tbody>
</table>

Reaction conditions: All reactions carried out on 0.5 mmol scale, 0.5 mg of CAL-B per mg of 3-acetoxy β-lactam 17a-21a, 50 mL of TBME, 3 eq of MeOH (62 μL).

<sup>1</sup>isolated yield for 3-acetoxy and 3-hydroxy enantiomers respectively following LC.
The following optimised conditions for CAL-B mediated KR were selected based on these findings:

1) The pre-defined time point for quenching of reaction was ~35% conversion (30-50 considered as acceptable range ensuring to cease all reactions ideally prior to 50% conversion), measured using $^1$H NMR in CDCl$_3$ at 400 MHz.
   
   a. Pre-defined time point for 17a and 22a (meta unsubstituted analogues) was anticipated with a shorter endpoint.

2) Conditions employed were 3 eq of MeOH and 0.5 mg of CAL-B per mg of 3-acetoxy $\beta$-lactam substrate in 50 mL of TBME on the Radley carousel.

3) After the 3-acetoxy and 3-hydroxy were isolated using LC, a second KR was carried out on the unreacted enantioenriched 3$R$,4$R$ 3-acetoxy substrate to an end point of > 50% conversion.

**7.12 Application of optimised KR for the isolation of 3S,4S 3-hydroxyl B ring meta substituted eutomers**

The optimised method was applied to 17a (Table 7.10). $Ee$ values for 17a ranged from 82-86% (average 84%) when the reaction was quenched from 35-41%, typically between 24-36 h. $E$ values were between 16-24 (average 19). Higher $E$ values were observed for reactions carried out on lower scales of 0.5 mmol (24) versus 1 mmol scale [16 (Table 7.10)]. The validity of $E$ values calculated using the ENANTIO online calculator were validated by comparing the measured % conversion via methanolysis versus the ENANTIO predicted % conversion. SEM values were within a range of acceptable experimental error. The $ee$ of the enantioenriched 3-acetoxy 3$R$,4$R$ $\beta$-lactams ranged from 44-60%, isolated in moderate yield of 24-58% for further reaction towards the enantiopure 3$R$,4$R$ 3-acetoxy enantiomers (Table 7.10).

Excellent enantioselectivity was observed on a 0.25 mmol scale for 18a ($E = 28, ee$ of 86% for 3-hydroxyl 18EN1) and 0.5 mmol scale ($E = 14-16, ee$ of 83% for 3-hydroxyl 18EN1). $Ee$ values for the desirable 3$S$,4$S$ 3-hydroxyl eutomer were excellent and constant ranging from 83-86%. The rate of conversion was slower for 18a versus 17a with only 15-36% conversion observed after 4-5 days for 18a versus 30-40% conversion observed for 17a between 24-48 h (Table 7.10). Solubility of 18a in TBME was observed as poor, which may contribute to a slower reaction rate.
Table 7.10: Application of optimised KR procedure to 3-acetoxyl β-lactam substrates 17a - 23a and 65-66a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale (mmol)</th>
<th>Time</th>
<th>conversion&lt;sup&gt;1&lt;/sup&gt;</th>
<th>estimated conversion&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>3&lt;sup&gt;R&lt;/sup&gt;,4&lt;sup&gt;AR&lt;/sup&gt; 3-acetoxy (o) yield</th>
<th>% ee&lt;sub&gt;s&lt;/sub&gt;</th>
<th>3S,4S 3-hydroxyl yield&lt;sup&gt;(p)&lt;/sup&gt;</th>
<th>% ee&lt;sub&gt;p&lt;/sub&gt;</th>
<th>E&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate 17a</td>
<td>1</td>
<td>0.25</td>
<td>36 h</td>
<td>41%</td>
<td>39%</td>
<td>1.41</td>
<td>17aEN2</td>
<td>Nd</td>
<td>52</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>24 h</td>
<td>31%</td>
<td>39%</td>
<td>5.66</td>
<td>20hEN2</td>
<td>54</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>24 h</td>
<td>39%</td>
<td>41%</td>
<td>1.41</td>
<td>21aEN2</td>
<td>60</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.5</td>
<td>24 h</td>
<td>35%</td>
<td>39%</td>
<td>2.83</td>
<td>22aEN2</td>
<td>54</td>
<td>17</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>24 h</td>
<td>29%</td>
<td>35%</td>
<td>4.24</td>
<td>23aEN2</td>
<td>44</td>
<td>36</td>
<td>82</td>
</tr>
<tr>
<td>Substrate 18a</td>
<td>1</td>
<td>0.25</td>
<td>5 d</td>
<td>36%</td>
<td>47%</td>
<td>0.08</td>
<td>18aEN2</td>
<td>76</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>4 d</td>
<td>15%</td>
<td>22%</td>
<td>0.05</td>
<td>19aEN2</td>
<td>24</td>
<td>16</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>8 d</td>
<td>22%</td>
<td>17%</td>
<td>0.04</td>
<td>20aEN2</td>
<td>18</td>
<td>11</td>
<td>86</td>
</tr>
<tr>
<td>Substrate 19a</td>
<td>1</td>
<td>0.5</td>
<td>6 d</td>
<td>35%</td>
<td>36%</td>
<td>0.01</td>
<td>19aEN2</td>
<td>40</td>
<td>22</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>5 d</td>
<td>50%</td>
<td>36%</td>
<td>0.10</td>
<td>20aEN2</td>
<td>44</td>
<td>28</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>5 d</td>
<td>38%</td>
<td>34%</td>
<td>0.03</td>
<td>21aEN2</td>
<td>42</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Substrate 20a</td>
<td>1</td>
<td>0.5</td>
<td>4 d</td>
<td>44%</td>
<td>44%</td>
<td>0</td>
<td>20aEN2</td>
<td>56</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>3 d</td>
<td>38%</td>
<td>39%</td>
<td>0.71</td>
<td>21aEN2</td>
<td>64</td>
<td>17</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>3 d</td>
<td>40%</td>
<td>42%</td>
<td>1.41</td>
<td>22aEN2</td>
<td>52</td>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>4 d</td>
<td>46%</td>
<td>40%</td>
<td>4.24</td>
<td>23aEN2</td>
<td>56</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>3 d</td>
<td>40%</td>
<td>43%</td>
<td>2.12</td>
<td>24aEN2</td>
<td>52</td>
<td>26</td>
<td>70</td>
</tr>
<tr>
<td>Substrate 21a</td>
<td>1</td>
<td>0.5</td>
<td>4 d</td>
<td>16%</td>
<td>18%</td>
<td>1.4</td>
<td>21aEN2</td>
<td>18</td>
<td>25</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>4 d</td>
<td>35%</td>
<td>38%</td>
<td>2.1</td>
<td>22aEN2</td>
<td>42</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>4 d</td>
<td>35%</td>
<td>36%</td>
<td>0.7</td>
<td>23aEN2</td>
<td>40</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Substrate 22a</td>
<td>1</td>
<td>0.5</td>
<td>4 d</td>
<td>32%</td>
<td>34%</td>
<td>1.41</td>
<td>22aEN2</td>
<td>42</td>
<td>42</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>4 d</td>
<td>27%</td>
<td>29%</td>
<td>1.41</td>
<td>23aEN2</td>
<td>32</td>
<td>17</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>4 d</td>
<td>45%</td>
<td>47%</td>
<td>1.41</td>
<td>24aEN2</td>
<td>66</td>
<td>34</td>
<td>76</td>
</tr>
<tr>
<td>Substrate 23a</td>
<td>1</td>
<td>0.1</td>
<td>3 d</td>
<td>41%</td>
<td>39%</td>
<td>1.4</td>
<td>23aEN2</td>
<td>40</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>Substrate 65</td>
<td>1</td>
<td>1</td>
<td>6 d</td>
<td>22%</td>
<td>24%</td>
<td>29%</td>
<td>65aEN2</td>
<td>24</td>
<td>4.3%</td>
<td>62</td>
</tr>
<tr>
<td>Substrate 66a</td>
<td>1</td>
<td>0.5</td>
<td>2 d</td>
<td>34%</td>
<td>44%</td>
<td>7.07</td>
<td>66aEN2</td>
<td>64</td>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>2 d</td>
<td>43%</td>
<td>49%</td>
<td>4.24</td>
<td>66bEN2</td>
<td>66</td>
<td>25</td>
<td>70</td>
</tr>
</tbody>
</table>

<sup>1</sup> measured using <sup>1</sup>H NMR at 400 MHz in CDCl<sub>3</sub>
<sup>2</sup> estimated % conversion (calculated using ENANTIO online tool)<sup>751</sup>
<sup>3</sup> SEM for actual conversions versus estimated conversion using ENANTIO online tool
<sup>4</sup> Total 3-acetoxyl (substrate) and 3-hydroxyl<sup>752</sup> yield
<sup>5</sup> Enantiomeric ratio (calculated using ENANTIO online tool)<sup>751</sup>
<sup>a</sup> substrate<sup>b</sup> product

Chapter 7: Kinetic resolution of β-lactam combretazet racemates using Candida antarctica lipase B

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Excellent ee values of 70-81% were obtained for 19EN1, the 3-hydroxyl 3S,4S meta chlorine substituted enantiomer when reactions were quenched between 35-50% conversion. On a 0.5 mmol scale, at 50% conversion an ee of 77% was determined demonstrating good enantioselectivity for the eutomer 19EN1 despite progression of KR beyond 35% (entry 2, Table 7.10). Ee values were slightly lower for 3S,4S 20EN1 (67-78%) and were typically greater when KR reactions were carried out on a 0.5 versus 1 mmol scale (Table 7.10). E values were quite poor (8-13). Nevertheless, the ee values for 20EN1 were determined as greater than ee values obtained via chiral diastereomeric resolution in Chapter 4 (Table 4.12) indicating the superiority of KR for resolution of 20. Additionally the isolated quantity of 3-hydroxyl 3S, 4S 20EN1 (100 mg) in 70% ee (Table 7.10) was superior to the isolated yield obtained using chiral resolution.

KR for 21a demonstrated moderate ee values of 70-76% with mild E values of 8-9. Nevertheless, KR using CAL-B yielded >100 mg of 3S,4S 21EN1 in 70% ee for future use in prodrug synthetic optimisation (Table 7.10). Excellent ee values and isolated yields were obtained for B ring 3-thiomethyl substituted 3S,4S 3-hydroxyl 22EN1 of 76-80% ee in quantities of 50-150 mg (Table 7.10). XRD analysis also confirmed the absolute configuration as 3S,4S for the 3-hydroxyl product 22EN1 isolated using KR (Figure 7.10)
The poorest ee values recorded using the optimised approach of 62% were for the desired 3S,4S enantiomer of 23EN1 (62%). The rate of methanolysis was fast for the meta amino substituted 23a with 41% conversion observed by 3 days. This suggests that the strong electron donating effect of the amine substituent influenced the rate of CAL-B mediated methanolysis. By increasing the rate, the ee of 62% was lower than for example 18, where only 36% conversion had occurred after 5 days in 86% ee (Table 7.10). The E value for 23 of 6 was the poorest of all E values recorded, indicating that CAL-B reacts with 23a with poor enantioselectivity.

The ee value of 62% for B ring 4-ethoxy substituted 65a was poorer in comparison to the corresponding 4-methoxy substituted analogue 17a [average ee of 84%, (Table 7.10)]. In contrast the ee values for the 4-ethylthio substituted 66a yielded excellent ee values of 70% and 80% and E values of 12 and 17 on 1 and 0.5 mmol scale respectively (Table 7.10). Isolated yields were good with approximately 50-100 mg recovered for prodrug optimisation.

**Figure 7.10:** XRD for 3S,4S 3-hydroxy 22EN1. Disordered molecular structure with two locations for the trimethoxyphenyl ring (58:42%). Atomic displacement shown at 50% probability and heteroatoms labelled only.
7.13 Double KR for the isolation of 3R,4R 3-acetoxy distomers in >90% ee

Enantionenriched 3R,4R 3-acetoxy β-lactams isolated from KR reactions in section 7.12, once purified using LC, were subjected again to a second CAL-B mediated KR otherwise referred to as a double resolution. The enzyme content was increased to 1 mg of CAL-B per mg of 3-acetoxy substrate while 6 eq of MeOH was employed. Despite increasing both the enzyme content and the methanol equivalents, the rate of reactions were slower. For example, only 52% conversion was observed for 17a (commencing at 37% ee) after 4 days, which was determined as 30-39% conversion for the parent racemate (0% ee) at only 24 hours (Table 7.11). This suggests that the activity of CAL-B towards the 3R,4R enantiomer is less favoured and confirmed that while KR reactions are not 100% enantioselective, the 3R,4R enantiomer is not optimally methanolysed by CAL-B. Excellent ee values were obtained for the 3R,4R 3-acetoxy enantiomers of 96-99% ee, by allowing the reaction to approach or progress beyond 50%. As anticipated the ee values for the 3-hydroxyl enantiomers using the double resolution strategy were extremely poor [20-56% ee, (Table 7.11)].
Chapter 7: Kinetic resolution of combretazet racemates using *Candida antarctica* lipase B

Table 7.11: Isolation of 3-acetoxy 3R,4R distomers using CAL-B

<table>
<thead>
<tr>
<th>Compound</th>
<th>% ee prior to reaction</th>
<th>Scale (mmol)</th>
<th>Time</th>
<th>conversion</th>
<th>3-acetoxy (o) yield</th>
<th>% ee</th>
<th>3-hydroxy (p) yield</th>
<th>% ee</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>17aEN2</td>
<td>37</td>
<td>4 d</td>
<td>52%</td>
<td>68%</td>
<td>30%</td>
<td>99</td>
<td>56%</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td>19aEN2</td>
<td>51</td>
<td>5 d</td>
<td>43%</td>
<td>75%</td>
<td>36%</td>
<td>99</td>
<td>20%</td>
<td>33</td>
<td>9</td>
</tr>
<tr>
<td>21aEN2</td>
<td>40</td>
<td>3.5 d</td>
<td>63%</td>
<td>99%</td>
<td>16%</td>
<td>96</td>
<td>10%</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>22aEN2</td>
<td>66</td>
<td>3.5 d</td>
<td>42%</td>
<td>80%</td>
<td>20%</td>
<td>98</td>
<td>20%</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>66aEN2</td>
<td>64</td>
<td>5 d</td>
<td>36%</td>
<td>81%</td>
<td>16%</td>
<td>99</td>
<td>34%</td>
<td>34</td>
<td>9</td>
</tr>
</tbody>
</table>

Reactions carried out at 1 mg/ mg of CAL-B per mg of 3-acetoxy β-lactam and 6 eq of MeOH

### 7.14 Comparison of ee values obtained via chiral diastereomeric resolution versus kinetic enzymatic resolution

Table 7.12 illustrates comparative ee values obtained using two independent methods for enantioseparation of β-lactam racemates; chiral diastereomeric resolution versus kinetic enzymatic resolution. Values for the 3S,4S enantiomers are comparable and in some cases KR was superior for enantioseparation e.g for **18EN1** KR resulted in 86% ee versus 78% ee isolated via chiral diastereomeric resolution. For the 3R,4R enantiomers KR using the two step optimised procedure yielded enantiopure 3-acetoxy enantiomers in greater ee to the corresponding 3R,4R 3-hydroxyl enantiomers isolated via chiral diastereomeric resolution. As discussed previously, due to co-elution of diastereomers during LC chiral resolution, poor ee values were continuously observed for the 3R,4R enantiomer. For example, chiral resolution yielded **17EN2** is 71% ee versus KR which yielded the corresponding 3-acetoxy in 99% ee.
Table 7.12: Comparison of ee values obtained using N-Boc-L-Proline as the CDR in diastereomeric resolution versus kinetic resolution with lipase enzyme CAL-B.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ee (%) obtained via diastereomeric resolution</th>
<th>Compound</th>
<th>Ee (%) obtained via kinetic resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>17EN1 (S,S)</td>
<td>94</td>
<td>17EN1 (S,S)</td>
<td>74</td>
</tr>
<tr>
<td>17EN2 (R,R)</td>
<td>71</td>
<td>17aEN2 (R,R)</td>
<td>99</td>
</tr>
<tr>
<td>18EN1 (S,S)</td>
<td>78</td>
<td>18EN1 (S,S)</td>
<td>86</td>
</tr>
<tr>
<td>18EN2 (R,R)</td>
<td>75</td>
<td>18aEN2 (R,R)</td>
<td>60¹</td>
</tr>
<tr>
<td>19EN1 (S,S)</td>
<td>91</td>
<td>19EN1 (S,S)</td>
<td>81</td>
</tr>
<tr>
<td>19EN2 (R,R)</td>
<td>78</td>
<td>19aEN2 (R,R)</td>
<td>99</td>
</tr>
<tr>
<td>20EN1 (S,S)</td>
<td>66</td>
<td>20EN1 (S,S)</td>
<td>78</td>
</tr>
<tr>
<td>20EN2 (R,R)</td>
<td>50</td>
<td>20aEN2 (R,R)</td>
<td>68</td>
</tr>
<tr>
<td>21EN1 (S,S)</td>
<td>85</td>
<td>21EN1 (S,S)</td>
<td>76</td>
</tr>
<tr>
<td>21EN2 (R,R)</td>
<td>84</td>
<td>21aEN2 (R,R)</td>
<td>96</td>
</tr>
<tr>
<td>22EN1 (S,S)</td>
<td>84</td>
<td>22EN1 (S,S)</td>
<td>81</td>
</tr>
<tr>
<td>22EN2 (R,R)</td>
<td>85</td>
<td>22aEN2 (R,R)</td>
<td>98</td>
</tr>
<tr>
<td>23EN1 (S,S)</td>
<td>Not isolated²</td>
<td>23EN1 (S,S)</td>
<td>61</td>
</tr>
<tr>
<td>23EN2 (R,R)</td>
<td>Not isolated²</td>
<td>23aEN2 (R,R)</td>
<td>Nd</td>
</tr>
<tr>
<td>65EN1 (S,S)</td>
<td>Not isolated²</td>
<td>65EN1 (S,S)</td>
<td>76</td>
</tr>
<tr>
<td>65EN2 (R,R)</td>
<td>Not isolated²</td>
<td>65aEN2 (R,R)</td>
<td>Nd</td>
</tr>
<tr>
<td>66EN1 (S,S)</td>
<td>Not isolated²</td>
<td>66EN1 (S,S)</td>
<td>80</td>
</tr>
<tr>
<td>66EN2 (R,R)</td>
<td>Not isolated²</td>
<td>66aEN2 (R,R)</td>
<td>99</td>
</tr>
</tbody>
</table>

Method with greatest ee highlighted in bold writing.
¹not isolated with optimised CALB kinetic resolution procedure.
²chiral diastereomeric resolution unsuccessful for isolation of enantiomers.
³chiral diastereomeric resolution not carried out.

Chapter 7: Kinetic resolution of combretazet racemates using *Candida antarctica* lipase B
7.15 Preliminary phosphate prodrug synthesis

Phosphate prodrug synthesis was investigated for 3S,4S 3-hydroxy enantiomer 20EN1 which has been isolated in 70% ee using KR (Table 7.10). The chemistry for phosphate ester derivatisation involved use of carbon tetrachloride (CCl₄), DMAP, dibenzyl phosphite and DIPEA to form the dibenzyl phosphate ester of eutomer 20EN1 and has been previously described by our group for phosphate ester derivatisation of racemate 24 (Scheme 7.4).³⁸⁰

Scheme 7.4: Synthesis of 140 as the dibenzyl phosphate ester of 20EN1 via Atherton-Todd reaction.³⁵³ a: CCl₄ (1.4 eq), ice at 0 °C for 15 minutes in an inert atmosphere, MeCN. Then DMAP, DIPEA and dibenzylphosphite at 0 °C. Then RT overnight.
$^1$H NMR at 600 MHz in CDCl$_3$ confirmed successful coupling of dibenzyl phosphite to 20EN1 to yield 140 in 50% yield. The purified $^1$H NMR demonstrates a multiplet at $\delta$ 5.10 ppm which corresponds to four benzyl protons. The phosphorous is observed on the $^{31}$P NMR spectrum at $\delta$ -2.84 ppm (B, Figure 7.11). The phosphorous of dibenzyl phosphate couples to H$_3$ of the $\beta$-lactam ring to form a double doublet with a $^3$J P-H coupling constant of 7.5 Hz, while a $^3$J coupling constant to H$_4$ is measured as 1.8 Hz (A, Figure 7.11). PYSCHE (pure shift yielded by chirp excitation) NMR experiments are broadband $^1$H decoupled $^1$H NMR spectra and remove proton $J$ coupling enabling visualisation of the $^{31}$P coupling for H$_3$ to the dibenzyl phosphate moiety. A doublet with a $J$ value of 8.5 Hz is observed on the PYSCHE spectrum at $\delta$ 5.06 ppm while H$_4$ resonates as a singlet at 4.94 ppm due to proton decoupling (blue, C, Figure 7.11) rather than the doublet with a $^3$J value of 1.8 Hz observed on the $^1$H NMR spectrum (red, C, Figure 7.11). This data supports the hypothesis that synthesis of phosphate ester prodrugs of the 3-hydroxy panel of combretazet 3S,4S eutomers is synthetically accessible. Future work will involve application and further optimisation of this chemistry to yield a panel of phosphate prodrugs for further physiochemical and biochemical analysis.
Figure 7.11: NMR spectra for dibenzyl phosphate derivative of $3\delta 4\delta$ 140 (70% ee) A: $^1$H NMR in CDCl$_3$ at 600 MHz. B: $^{31}$P NMR spectra at 162 MHz. C: Blue: PYSCHE for 140 (dibenzyl phosphate ether of 20EN1) at 600 MHz in CDCl$_3$ in H$_3$ and H$_4$ region illustrating the $^3$$^J$P-H coupling at $\delta$ 5.05 ppm with of 8.5 Hz while H$_4$ resonates as a singlet. Red: $^1$H NMR spectrum for H$_3$ and H$_4$ at 600 MHz in CDCl$_3$. 

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7.16 Conclusions and future outlook

In summary, in this chapter enantioseparation of a panel of β-lactam enantiomers has been achieved using a biocatalytic approach with the lipase enzyme CAL-B. Preliminary data illustrated that CAL-B did not mediate ring opening reactions for 3-acetoxy β-lactams but instead renders the 3-acetoxy structures susceptible to CAL-B mediated methanolysis towards the 3-hydroxyl enantioenriched eutomers. Initial results demonstrated poor enantioselectivity and moderate enantioenrichment only. Reducing the enzyme content to 0.5 mg/mg with 3 equivalents of methanol in TMBE greatly enhanced the ee (70-80%). XRD analysis confirmed the 3-hydroxyl product as the desirable 3S,4S eutomer. Additionally the 3R,4R distomer was isolated in >95% ee. This provides strong rationale to support KR as a promising strategy for resolution of a panel of racemic 3-hydroxyl β-lactams in high ee comparable to chiral diastereomeric resolution. KR is a rapid, accessible and sustainable strategy for enantioseparation, removing the need for expertise in LC purification and use of large volumes of organic solvents. KR is advantageous for enabling rapid access to large yields of β-lactam enantiomers for synthetic exploration of the most biocompatible prodrug, from a range of phosphate and amino acids, for progression towards pre-clinical and clinical models as a novel agent for treatment of both TNBC and chemoresistant colorectal cancers.
Chapter 8 - Physiochemical analysis, prodrug synthesis and structural optimisation of Pyrazinib
8.1 **Pyrazinib (P3) as a promising radiosensitiser**

Pyrazinib [(E)-2-(2-(pyrazin-2-yl)vinyl)phenol or P3 has demonstrated promising anti-angiogenic and radiosensitising activity both *in-vitro* and *in-vivo* as discussed in the introduction to this thesis.\textsuperscript{448, 449, 451} Its physiochemical properties have not been evaluated to date and are the focus of this chapter. Preliminary observations by our collaborators suggest that P3 has poor aqueous solubility. While P3 was found to have greatest reduction in oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in an *in vitro* isogenic model of OAC radioresistance, its hydrochloride salt, P4, demonstrated superior reduction in OCR and ECAR versus P3 *in vivo*, in zebrafish models, indicating that poor water solubility could be a limiting factor for translation of *in vitro* towards *in vivo* biochemical activity.\textsuperscript{451} Modification of P3’s structure is necessary to achieve the required aqueous solubility for pre-clinical *in vivo* models.

8.2 **Solubility issues with P3 during in vivo pre-clinical animal models**

Poor solubility of P3 in a variety of vehicles trialed during animal xenograft models carried out by Dr. Susan Kennedy at Xenopat, Barcelona (January 2019) has been noted. (Figure 8.1) P3 dissolved in 99% corn oil: 1% DMSO. While corn based vehicles are widely used for delivery of lipophilic agents during drug development, use of an aqueous based vehicle is more desirable for progression toward clinical trials.\textsuperscript{757, 758}

![Figure 8.1](image.png)

*Figure 8.1: (Left) P3 suspended in vehicle A (10% DMSO, 30% EtOH, 60% PBS) and vehicle B (10% DMSO, 30% EtOH, 60% H₂O) at 1 mg/mL. (Right) Post vortex.*

8.3 **Synthesis of P3 using conventional reflux**

P3 was synthesised using the Perkin condensation reaction with 2-methylpyrazine and salicaldehyde in acetic anhydride. Scheme 8.1 details the synthesis of P3 using the Perkin
condensation reaction while Scheme 8.2 illustrates the proposed Perkin condensation reaction mechanism via a 2-methylene reactive species. P3 was isolated in poor yield of < 25% which is explained by limited tautomerisation potential for 2-methylpyrazine (A, Scheme 8.2). The pKₐ of pyrazine is 0.7. Tautomerisation illustrated in A, Scheme 8.2 is therefore not favoured.

Scheme 8.1: Perkin condensation for synthesis of P3. a: Acetic anhydride, 180 °C, 4 days or microwave on standard power, 160 °C, 5 h. b: 1:1 EtOAc/ EtOH with 1M NaOH

The ¹H NMR spectra of P3 confirms the trans configuration with presence of the E alkene doublets resonating at δ 8.03 and 7.37 ppm with ³J values of 16 Hz which are characteristic of trans alkenes, corresponding to H₁ and H₂ respectively. The presence of a broad singlet at δ 10.03 ppm confirmed successful hydrolysis of the acetate group to afford the free phenolic moiety of P3 (Figure 8.2).
Scheme 8.2: Proposed mechanism for the Perkin condensation reaction via A: tautomerisation of 2-methylpyrazine to form a methylene reactive species and B: reaction of 2-methylene reactive species with salicaldehyde to afford P3 acetate (blue box).
8.4 Synthesis of P3 via MAOS

Conventional reflux for the Perkin condensation route to P3 required long reaction times of 5-7 days and in poor yield (20-25%). MAOS was therefore employed with aim of decreasing the reaction time and increasing crude % yield. MAOS was carried out on a 10 mmol scale for 5 hours at 160 °C with a reduced volume of acetic anhydride (5 mL). P3 acetate (Scheme 8.2) was isolated in 15% yield with minimal evidence of the hydrolysed phenolic P3 on crude NMR spectra. This compared to observations using conventional reflux which yielded a mixture of both P3 acetate and P3. The shorter reaction time employed using MAOS, despite the lower yield, enabled rapid reaction repetition for scale up of P3 synthesis when compared to conventional reflux.
8.5 XRD analysis of P3

P3 was recrystallised as an orthorhombic system from a saturated solution of 1:1 \textit{n}-hexane/ethyl acetate over 24 hours. XRD analysis confirmed the \textit{trans} alkene bond of 1.33 Å (Figure 8.3). The XRD structure suggests a potential reason for the poor observed aqueous solubility. Intermolecular hydrogen bonding is observed between P3’s phenolic hydroxyl and the pyrazine N14 nitrogen (Figure 8.3). This may prohibit hydrogen bonding with solvent and therefore limit solvation (B, Figure 8.3).

\[ \text{Figure 8.3: A: Molecular structure of P3 with atomic displacement shown at 50\% probability. B: Detail of the intermolecular hydrogen bonding between molecules of P3 (dotted lines) with atomic displacement shown at 50\% probability.} \]

8.5.1 Phosphate prodrug synthesis for P3

The phosphate prodrug of P3 was selected as a strategy to overcome poor dissolution and underperformance \textit{in vivo} when compared to P3’s \textit{in vitro} activity.\textsuperscript{451} Two alternative synthetic strategies were explored for synthesis of P3 phosphate (P3P) (Scheme 8.3 and 8.4).
8.5.2 Synthesis of P3P using phosphorous oxychloride

A one pot synthesis for the isolation of P3P involving the use of phosphorous oxychloride (POCl₃) was trialled (Scheme 8.3). The reaction of phenolic P3 proceeded with POCl₃ through nucleophilic attack of the phenolic P3’s oxygen with the phosphorous atom of POCl₃ to yield a phosphorochloridate intermediate via a bi-molecular SN₂P mechanism. Typically a base is typically employed for activation of POCl₃. TEA has demonstrated success for activation of POCl₃. The positively charged intermediate is a more reactive phosphorylating reagent than native POCl₃. Additionally, TEA fixes liberated HCl in the form of a corresponding chlorhydrate (TEA.HCl). An excess of TEA is typically employed to scavenge HCl to prevent the formation of byproduct chloroalkanes. The precipitation of this chlorhydrate in appropriate solvent promotes formation of phosphoric acid. Water is employed to hydrolyse the phosphorochloridate intermediate to the free phosphoric acid as the last step (Scheme 8.3). Successful formation of the P3P was determined by HRMS, ¹H NMR and ³¹P NMR, however as a TEA complex in ratio 98:2 TEA: P3P, determined using qNMR. Due to complexities associated with de-salting of small yields, the use of dibenzyl phosphite as a phosphorylating reagent was explored.

Scheme 8.3: Reaction of P3 with POCl₃. a: TEA activates POCl₃ to form the reactive TEA-POCl₃ complex in anhydrous THF b: P3 reacts with TEA-POCl₃ complex yielding the phosphoro chloridate intermediate and TEA. c: Water hydrolyses the phosphorochloridate to the free phosphoric acid.

Chapter 8: Physiochemical analysis, prodrug synthesis and structural optimisation of pyrazinib
8.5.2.1 Synthesis of $P3P$ using dibenzylphosphite

Reaction of $P3$ with dibenzylphosphite via the Atherton-Todd reaction afforded a dibenzyl ether phosphate of $P3$ as $P3DBP$ in good yield (60%) as a yellow oil (Scheme 8.4). Confirmation of dibenzyl phosphite phosphorylation was observed on $^1$H NMR with benzyl CH$_2$ protons integrating as 4H with two singlets at $\delta$ 4.65 and 4.63 ppm. 10 additional aromatic protons were observed as a complex multiplet at $\delta$ 6.83 ppm of $P3$ dibenzyl phosphate’s benzyl rings (A, Figure 8.4). The $^{31}$P NMR spectrum demonstrate the presence of a single phosphate only at $\delta$ -6.6 ppm, (B, Figure 8.4) confirming the purity of desired target.

Scheme 8.4: Synthesis of $P3$ dibenzyl phosphate ($P3DBP$). CCl$_4$, 0 °C, N$_2$, MeCN, 15 minutes with $P3$. Then DMAP, DIPEA, dibenzyl phosphite at 0 °C. Then RT for 24 hours.

Figure 8.4: A: $^1$H NMR spectrum for $P3DBP$ in DMSO-$d_6$ at 400 MHz. B: $^{31}$P for $P3DBP$ in DMSO-$d_6$ at 162 MHz
8.5.2.2 Synthesis of P3 dimethyl phosphate (P3DMP) using dimethyl phosphite

Dimethyl phosphite was also explored as a phosphorylating reagent also via the Atherton-Todd reaction as illustrated in Scheme 8.4. The P3 dimethyl phosphate product (P3DMP) was isolated in 41-50% yield as a brown oil. The dimethyl protons of the phosphate moiety were observed as singlets at δ 3.84 and 3.80 ppm (A, Figure 8.5). The presence of one major phosphorylated product is confirmed by the $^{31}$P NMR spectrum with one resonance signal at δ -2.44 ppm (B, Figure 8.5). However, after storage at 2-8 °C for ~6 months, P3DMP (brown oil) had transformed into a powder. NMR analysis demonstrated transformation of the original NMR spectra. A new and major $^{31}$P resonance was observed at δ -3.94 ppm accompanied by minor presence of the original δ -2.94 ppm resonance (B, Figure 8.5). LC-MS demonstrated presence of two compounds with molecular weight of the major degradation product identified as 293.00 ([M+H$^+$]). Spontaneous atmospheric hydrolysis of a single methyl group occurred during storage to yield the monomethyl phosphoric acid of P3 (C, Figure 8.5). P3DMP was therefore not carried forward as a suitable phosphate prodrug of P3 due to these stability issues. Instead focus was placed on debenzylation of P3DBP (Scheme 8.4 and Scheme 8.5) to yield the free phosphoric acid of P3 (P3P). In the future optimal storage should be investigated followed by optimisation of dimethyl group demethylation.

8.5.2.3 Removal of dibenzyl groups from P3DBP using the McKenna reaction

A simple catalytic hydrogenation in the presence of palladium rapidly typically removes dibenzyl groups to afford free phosphoric acids. This has been achieved for the β-lactam derivatives of CA-4.380 However, the presence of P3’s trans alkene bond prohibits hydrogenation as a method for removal of dibenzyl groups which would otherwise hydrogenate the alkene to an alkane bond. In the synthesis of CA-4 phosphate, which also contains a cis double bond, the McKenna reaction was successfully employed to produce the phosphoric acid in excellent yields.320 The McKenna reaction is a widely known tool for formation of organophosphorus acids from esters.760 However it is water sensitive and accompanied by a plethora of side reactions and decomposition products. P3P was not isolated using the McKenna reaction (b, Scheme 8.5).
Chapter 8: Physiochemical analysis, prodrug synthesis and structural optimisation of pyrazinib
8.5.2.4 Removal of dibenzyl groups using boron tribromide

Boron tribromide (BBr₃) is an inexpensive and effective alternative to trimethylsilane derivatives for dealkylation of dialkyl phosphonates that contain functional groups. A modified version of a reported reaction was explored for debenzylation of P₃DBP (c, Scheme 8.5) and purified using size exclusion chromatography with Sephadex® G-10 medium for isolation of P₃P in 40% yield.

![Scheme 8.5: a: Synthesis of P₃DBP as per Scheme 8.4 b: McKenna reaction for debenzylation. NaI, Me₃SiCl, 0 °C, N₂; c: Debenzylation to P₃P using BBr₃ (1M in hexane), anhydrous toluene, -10 °C, then 80 °C for 2h, N₂. Then MeOH, 1 h. (Yield 40%)](image)

8.6 Aqueous solubility of P₃ and P₃P

The aqueous solubility of P₃ and P₃P was measured using the shake-flask method, a procedure comprising of sample preparation, equilibration in aqueous buffer, separation of solid precipitates from solution containing the dissolved drug and analysis of the dissolved content in the filtered solution using HPLC. Shake flask experiments were carried out in line with WHO guidance. All measurements are made in triplicate and reported at mg/mL. All shake flask experiments were placed on a mechanical (orbital) shaker (Mini gyro-rocker, SSM3, Stuart) with a stirring speed of 70 rpm for 24 hours.
After 24 hours the suspensions were filtered through a 0.22 micron filter to remove undissolved drug and the filtrate was subsequently analysed by HPLC. HPLC studies were carried out at the λ of max absorption of 348 nm and 334 nm for P3 and P3P respectively. A series of standard serial solutions for P3 and P3P were prepared from a stock solution (0.5 mg/ mL) in 70:30 MeCN: H2O. Calibration curves for P3 and P3P were constructed within linear range of absorbance (A and B, Figure 8.6). The solubility of P3P was measured as 0.31 mg/ mL compared to 0.00005 mg/ mL for P3. Derivatisation of P3 to its phosphate prodrug P3P resulted in a 6200-fold increase in aqueous solubility.

![Figure 8.6: Calibration curves used for solubility measurements for A: P3 B: P3P](image)

8.7 Measurement of logP for P3 and P3P

LogP measurements were carried out comparing P3 to P3P using the procedure described in Chapter 4. These values were compared to values obtained using the Swiss ADME webtool and the estimated cLogP values obtained from ChemDraw. Both methods for estimating logP values were comparable to experimentally derived values of 1.55 and -2.35 for P3 and P3P respectively (Table 8.1). The increase in hydrophilicity and solubility imparted by addition of the phosphate for P3P is reflected by the decrease in logP by > 3 units from 1.55 for P3 to -2.35 for P3P (Table 8.1).
Table 8.1: Experimentally measured logP values for P3 and P3P compared to values obtained using Swiss ADME webtool and clogP.

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<th>Swiss ADME derived logP</th>
<th>Experimentally determined logP</th>
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<td>0.31</td>
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8.8 Chemical stability of P3 versus P3P

The stability of P3 and P3P was analysed in phosphate buffers at room temperature at three pH values (acidic, neutral and basic pH with buffer compositions described in Chapter 9) over a period of 1-7 days. Both compounds were extremely stable at all three pH values. >90% of the compound remains for all time points and all pH ranges at 7 days (Figure 8.7).

![Figure 8.7: Stability of P3 and P3P at 24 hr and 7 days in phosphate buffers BP at pH values of 4, 7.4 and 9. Two exceptions noted (P3 pH 7.4 measured at 5 days and P3P at pH 9 measured at 23 days due to Covid-restricted lab access).](image)

8.9 Alkaline phosphatase mediated hydrolysis of P3P

Alkaline phosphatases (AP) are ubiquitous enzymes and are typically anchored to the outside surface of cellular plasma membranes and catalyse the hydrolysis of phosphate groups from a variety of substrates when in alkaline environments. This experiment aimed to demonstrate the rapid dephosphorylation of P3P to P3 in the presence of AP.
A stock solution of **P3P** (2.78 mg/1 mL in borate buffer adjusted to pH 7.4) was prepared and diluted with borate buffer to make a 0.3 mM stock solution. 10 mL of AP solution (Merck P7640-500MG) was prepared as 0.5 mg/mL in distilled water. AP stop solution (Merck A5852-100ML) employed to stop AP enzyme activity, was prepared by reconstitution of the dry powder in 100 mL of distilled water. The solutions were pre-heated separately for 15 min at 37 °C to facilitate optimal AP activity. HPLC vials were prepared containing reconstituted AP stop solution (50 µL). The experiment was performed in triplicate. Replicate 1 involved addition of 5 mL of 0.5 mg/mL AP solution to 10 mL of **P3P** (1:2 AP: **P3P** stock solution) while replicates 2 and 3 added 5 mL of 0.5 mg/mL AP solution to 5 mL of **P3P** stock solution (1:1 AP: **P3P** stock solution). The reaction solution (200 µL) was removed at indicated time points and added to each of the prepared HPLC vials containing the stop solution. Relative peak areas from HPLC analysis for **P3** and **P3P** were used to determine % conversion of **P3P** by AP to parent **P3**.

At higher enzyme concentrations (1:1 volume of AP: **P3P**), 100% dephosphorylation to **P3** was observed by 5 seconds (A, Figure 8.8). At lower enzyme concentration (1:2 volume AP: **P3P**) conversion was marginally slower. 100% conversion was observed between 30 seconds to 1 minute (B and C, Figure 8.8). The phosphate prodrug approach may serve as an effective strategy of ensuring aqueous dissolution of **P3** while maintaining **P3**’s properties due to its rapid conversion to parent in by enzymes anticipated to be present in vivo.
Figure 8.8: Conversion of P3P to P3 by alkaline phosphatase at 37 °C A: 5 mL of AP solution (25 DEA units) was added to 5 mL of P3P of 0.3 mM stock solution B: 5 mL of AP solution (25 DEA units) was added to 10 mL of P3P of 0.3 mM stock solution C: Enlarged area of B from 0-5 minutes
8.10 Comparison of antiproliferative activity for P3 versus P3P in breast cancer cells

The antiproliferative activity of P3 and P3P were compared using in-house cell viability assays (AlamarBlue™) at three concentrations in two breast cancer cell lines; MCF-7 (A, Figure 8.9) and MDA-MB-231 cells (B, Figure 8.9). In MCF-7 cells, P3 demonstrated minimal antiproliferative activity at 1 and 10 µM. Significant antiproliferative activity was however demonstrated at 100 µM for P3 with a p value of <0.001 (A, Figure 8.9). Significant antiproliferative activity was not observed for P3 at any concentration in MDA-MB-231 cells (B, Figure 8.9). A significant difference is observed between % of viable cells treated with P3 (10 µM) versus P3P (10 µM) in both MCF-7 cells (p <0.001, A, Figure 8.9) and MDA-MB-231 cells (p < 0.05, B, Figure 8.9). P3P demonstrated superior antiproliferative activity compared to P3. In vitro IC<sub>50</sub> values were measured as 2.56 µM in MCF-7 cells and 24.7 µM in MDA-MB-231 cells for P3P. P3P is therefore approximately 10-fold more potent in MCF-7 cells compared to MDA-MB-231 cells based on its aforementioned IC<sub>50</sub> values. Future work must determine whether P3P exerts its biological activity through the ER, present in MCF-7 but not MDA-MB-231 cells, which may cause significant variation in antiproliferative activity between the two breast cancer cell lines.

Figure 8.9: Antiproliferative activity of prodrug P3P (pink) versus P3 (green) in A: MCF-7 cells. B: MDA-MB-231 cells. Values represent average of three independent replicates performed in triplicate on three independent occasions. Statistical analysis involved using multiple t-test comparisons corrected for multiple comparisons using the Holm-Sidak method to establish statistical significance with respect to control. * p-value <0.05. *** p-value <0.001.
8.11 **Cell cycle studies for P3 and P3P in MDA-MB-231 cells**

To rationalise the increase in antiproliferative activity for P3P versus P3, cell cycle studies were carried out in MDA-MB-231 cells. Due to limited availability of P3P the data presented in Figure 8.10, Table 8.2 and Table 8.3 represent preliminary data from one replicate (n=1). P3P appeared to induce G2/M phase arrest in MDA-MB-231 cells (Table 8.3) in contrast to P3 which has no effect at any concentration compared to vehicle control and does not cause G2/M phase arrest (Table 8.2). At 8 hours following treatment with P3P (20 µM) 47% of MDA-MB-231 cells had arrested in G2/M phase which increased to 69% at 24 hours. (Table 8.3) Additionally, at 48 hours a rise in the % of sub G1/G0 cells was observed compared to vehicle control. For example 12% of cells treated with P3P (25 and 100 µM) had become apoptotic by 48 hours (Table 8.3). Future work will involve re-synthesis of P3P and confirmation of cell cycle findings *i.e.* the effect of P3P on G2/M cell population in a time and dose dependent manner.
Table 8.2: % of MDA-MB-231 cells in each stage of cell cycle when treated with P3 (25, 32.5, 50 or 100 µM) for 8, 24 or 48 h. Vehicle = DMSO 0.5% v/v.

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Table 8.3: % of MDA-MB-231 cells in each stage of cell cycle when treated with P3P (10, 20, 25, 32.5, 50 or 100 µM) for 8, 24 or 48 h. Vehicle [0.6% v/v ethanol/dH2O].

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Chapter 8: Physiochemical analysis, prodrug synthesis and structural optimisation of pyrazinib
Chapter 8: Physiochemical analysis, prodrug synthesis and structural optimisation of pyrazinib

Figure 8.10: Effects of P3 and P3P on the cell cycle in MDA-MB-231 cells. For P3 cell cycle experiments, cells were treated with vehicle [DMSO 0.5% (v/v)] or P3 at concentrations of 25, 32.5, 50 or 100 µM. For P3P cell cycle experiments, cells were either treated with vehicle (0.6% v/v ethanol) or P3P at 10, 20, 25, 32.5, 50 or 100 µM. Cells were treated for 8, 24 or 48 h. Cells were then fixed, stained with PI and analysed by flow cytometry. Cell cycle analysis was performed on histograms of gated counts per DNA area (FL2-A). The number of cells with <2N (sub-G₁), 2N (G₀/G₁), and 4N (G₂/M). DNA content was determined using the BD accuri C6 flow cytometer. Values represent the mean for one experiment. (n=1)
8.12 Molecular modelling for cis and trans P3

P3 has been synthesised in this thesis as the trans form. The pyrazine A and phenolic B rings of trans P3 have similar trans orientation to the A and B rings of CA-4’s inactive trans configuration (Figure 8.11). Cis CA-4 is active as a CBSI while trans CA-4 is not. A computational study was therefore carried out, modelling the cis isomer of P3 in order to ascertain potential microtubule depolymerising potential with CBS affinity of cis P3 comparable to cis CA-4 (Figure 8.12). This computational study was carried out in MOE using the docking protocol optimised for the docking of β-lactam enantiomers described in Chapter 5 (Figure 5.7).

Figure 8.11: Structures of cis and trans CA-4 and P3

Cis and trans P3 were modelled at the CBS of 1SAO. Computational studies carried out for cis and trans P3 indicate that trans P3 docks unfavourably at the CBS and is therefore unlikely to have in vitro microtubule depolymerising activity (cyan, A and B, Figure 8.12), further explaining its minimal anti-proliferative activity observed in breast cancer cells (Figure 8.9). In comparison, cis P3 (pink, A and B, Figure 8.12) closely aligns with DAMA-colchicine (green, A and B, Figure 8.12). Cis P3 maintains many major interactions at CBS of 1SAO as described for CA-4 in Chapter 5. The pyrazine A ring of cis P3 (Figure 8.11) hydrogen bonds with the SH of Cysβ241 within the β subunit of the CBS (blue dotted line, A, Figure 8.12). This interaction is known to be crucial for stabilising cis-CA-4 at the CBS and is imperative for microtubule destabilising activity as discussed previously in Chapter 5. The pyrazine ring of trans P3...
predominantly interacts with the α subunit of the tubulin heterodimer, in unfavourable orientation and this important interaction with Cysβ241 is not observed (cyan, A and B, Figure 8.12). The cis alkene configuration of P3 is important for orienting P3’s pyrazine A ring at the β subunit of the CBS and in the same orientation as CA-4’s trimethoxyphenyl A ring. It is plausible to suggest that the cis isomer of P3 could have in vitro and in vivo microtubule depolymerisation activity due to this potential CBS affinity imparted by P3’s pyrazine A ring.

Figure 8.12: Molecular docking studies for cis and trans P3 at the CBS of 1SAO A: Cis P3 (pink) and trans P3 (cyan). DAMA-Colchicine is shown in green. CBS binding pocket illustrated in grey. Hydrogen bonding shown as blue dotted lines. B: Overlay of cis P3, trans P3 and DAMA-colchicine with receptor removed for clarity. C:2D Ligand-receptor interaction diagram for cis P3 and D: trans P3 ligand interactions. Atom colours: Red: oxygen, Blue: Nitrogen, Yellow: Sulfur, Grey: Hydrogen.

Most recently it has been demonstrated that a series of CA-4 analogues modified in structure as anilines substituted at the 7-position of a 2-substituted-[1,2,4]triazolo[1,5-
a]pyrimidine nucleus had CBS activity as microtubule destabilisers. This demonstrated that the 3,4,5-trimethoxyaniline ring is not an essential requirement for CBS affinity. The importance of the 3,4,5-trimethoxyaniline ring is subject to debate in the literature. This thesis aimed to further explore the anti-proliferative activity mediated by the pyrazine nucleus of P3 in place of CA-4’s 3,4,5-trimethoxyphenyl ring via cis restriction of P3’s A and B rings using the 2-azetidinone scaffold. This chemistry would yield a panel of P3 β-lactams utilising the cis restriction approach applied successfully applied to β-lactams racemates of CA-4 to yield the combretazets panel, discussed in this thesis from Chapters 2-7.

8.13 Chemistry of P3 β-lactam synthesis – ‘The Pyrazets’

‘The Pyrazets’ (Pyraz- and 2-azet-) were coined as a theoretical panel of cis restricted P3 β-lactams, incorporating P3’s pyrazine A ring and modifications of its B ring, in addition to the 2-azetidione scaffold. Analagues illustrated in Figure 8.13 were considered as potential P3 β-lactam leads. Analogue 145 was designed as a hybrid analogue to mimic the CA-4’s B ring 4-methoxy while replacing the 3,4,5-trimethoxyphenyl A ring of CA-4 with P3’s pyrazine A ring. Analagues 141, 142 and 143 were the most desirable for synthetic isolation, containing the B ring 2-hydroxyl substitution analogous to parent trans P3 (Figure 8.13). This moiety would require OTBDMS protection of aldehyde precursors in preparation for for reaction in Reformatsky or Staudinger reactions.
Two synthetic approaches were considered for TBDMS protection of the B ring hydroxyl moiety, (a and b, Scheme 8.6) Partial TBDMS protection of precursor salicaldehyde was observed on $^1$H NMR (a, Scheme 8.6). However progressive degradation occurred over time, observed as two aldehyde protons at $\delta$ 10.4 ppm for TBDMS protected salicaldehyde and $\delta$ 9.9 ppm for parent salicaldehyde on $^1$H NMR. Direct protection of imine 146 [synthesised in high yields using conventional reflux, (c, Scheme 8.6)] to yield the required imine (147) was also unsuccessful and resulted in imine degradation towards starting material (b, Scheme 8.6).

Figure 8.13: Hypothetical lead analogues of cis restricted P3 $\beta$-lactam racemates
Scheme 8.6: Synthetic routes for synthesis of OTBDMS protected imine 147. **a:** TBDMSCl, DBU, anhydrous DCM, N₂, RT **b:** breakdown of 146 to 2-aminopyrazine and salicaldehyde **c:** Condensation of 2-aminopyrazine and salicaldehyde in ethanol 90 °C

Scheme 8.7: Synthetic routes explored to obtain imines 148-150 and subsequent synthesis of β-lactam 151 **a:** Dean Stark apparatus in anhydrous toluene. **b:** Dean Stark apparatus with anhydrous toluene (30 mL) with ethanol as co-solvent. **c:** TiCl₄, n-butylamine, toluene, 0 °C, then RT overnight. **d:** aminocatalysis: Pyrrolidine (20 mol %), EtOH, 7h, reflux **e:** microwave irradiation: 2-aminopyrazine, substituted aldehyde, 60 minutes. Standard powder (CEM microwave synthesiser) **f:** TEA, toluene, reflux, 5h

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Subsequently attempts at condensing the commercially available benzyl ether protected aldehyde to yield imine 148 was explored using a plethora of trial reactions, including reflux in anhydrous toluene using Dean Stark apparatus (a, Scheme 8.7), reflux in anhydrous toluene with ethanol added as a co-solvent to solubilise 2-aminopyrazine also using the Dean Stark apparatus (b, Scheme 8.7), the use of TiCl\(_4\) in presence of \(n\)-butylamine as base (c, Scheme 8.7), an amino catalytic approach with \(n\)-pyrrolidine as the catalyst (d, Scheme 8.7) and finally the use of the CEM microwave reactor with hot ethanol (e, Scheme 8.7). 148 was not observed or isolated demonstrating that benzyl ether protection of salicaldehyde’s 2-hydroxyl electron donating moiety, prevents the aldehyde condensing with 2-aminopyrazine. Attempts to initiate the reaction using acid catalysis also failed, instead protonating the 2-amino pyrazine’s primary amine to the unreactive salt.

Synthesis of imines using aminocatalytic strategies was also explored. (d, Scheme 8.7) Compounds containing NH\(_2\) moieties (154, Scheme 8.8) may behave as nitrogenated nucleophiles permitting formation of C=N bonds (157, Scheme 8.8) via an iminium organocatalyst intermediate (155, Scheme 8.8).\(^{768}\) The electrophilicity of the iminium intermediate (155, Scheme 8.8) is more than 10 times greater than the carbonyl group in the precursor aldehyde (153, Scheme 8.8).\(^{769}\) This pathway is suggested to proceed in presence of weakly nucleophilic primary amines which ordinarily are inert in presence of aldehydes. Pyrrolidine has successfully demonstrated \(N\)-sulfinyl imine formation and was therefore considered as a synthetic strategy for potential access to P3 imines (148-150, Scheme 8.7) using 2-aminopyrazine and the relevant aldehyde.\(^{768}\) A proposed mechanism is outlined in B, Scheme 8.8. The relevant aldehyde and 2-aminopyrazine were added to ethanol containing 20 mol % of pyrrolidine as the nucleophilic catalyst followed by reflux conditions for 7 hours (d, Scheme 8.7). The amino catalytic method was employed for synthesis of precursor imines 149 and 150 but was unsuccessful for the synthesis of benzyl ether protected 148.
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Scheme 8.8: A: Amino catalytic organocatalysed functionalisation of aldehydes using iminium activation with nitrogen based nucleophiles to form imines. B: Mechanism of pyrrolidine mediated amino catalytic imine formation through the iminium intermediate.768

Titanium tetrachloride (TiCl₄) has also demonstrated efficacy for imine synthesis by employing excesses of both amine and TiCl₄. Scheme 8.9 details the mechanism of TiCl₄ imine synthesis involving coordination of the carbonyl oxygen of the aldehyde (159, Scheme 8.9) with the titanium atom of TiCl₄ which activates the carbonyl for reaction to form intermediate 161 (Scheme 8.9). The intermediate (161) reacts with the primary amine (160), followed by transfer of the carbonyl oxygen to the titanium atom, yielding the desired imine (162) with titanium dioxide (TiO₂) as a by-product.770 2-Nitrobenzaldehyde was successfully reacted with 2-aminopyrazine, albeit in poor yield, using the TiCl₄ approach for imine synthesis (Scheme 8.9). 149 was confirmed by HRMS with target mass of 229.0719 [APCI (M+H⁺)]. P3 imine reactions did not reach completion and due to issues with purification, imines were employed in Staudinger or Reformatsky reactions without further purification.
The synthesis of cis restricted P3 β-lactam analogues was explored using 149 and 150 in Reformatsky and Staudinger reactions. Reformatsky reactions did not yield β-lactam products using either 149 or 150. 151 (Figure 8.14 and Figure 8.15) was the only β-lactam racemate isolated as a cis restricted P3 Pyrazet, synthesised using acetoxyacetyl chloride and the Staudinger reaction. Isolated yields were extremely poor (<26%), with the lowest yield obtained using the TiCl₄ approach for synthesis of 149 followed by reaction with acetoxyacetyl chloride [1.21%, (c, Scheme 8.7)]. Synthesising the required imine using the Dean Stark apparatus with ethanol as co-solvent resulted in a final yield of 25% (b, Scheme 8.7) while the amino catalytic approach resulted in an isolated yield of 26% yield (d, Scheme 8.7) for 151 in the Staudinger reaction. The ¹H NMR spectrum of the Staudinger product 151 demonstrated predominantly cis isomer (at positions 3 and 4 of the β-lactam ring) in 82% yield (Figure 8.14). XRD analysis confirmed the cis configuration for 151 with chiralities confirmed as 3S,4R and 3R,4S for enantiomers of racemate 151 (Figure 8.15). Further reaction to reduce the B ring’s 2-NO₂ substitution of 151 to the 2-NH₂ moiety did not yield the desired product. Similarly hydrazinolysis of the 3-acetoxy using hydrazine dihydrochloride did not afford the final 3-hydroxy product. The desired final Pyrazet, 144 (Figure 8.13) was therefore not isolated in this thesis.
Figure 8.14: $^1$H NMR of 151 in CDCl$_3$ at 600 MHz illustrating majority presence of cis isomer (doublets with $^3J = 5$ Hz at $\delta$ 6.46 and 6.29 ppm) and presence of trans isomer (doublets at $\delta$ 5.96 and 5.76 ppm with $^3J$ values of 2 Hz).

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8.14 Anti-proliferative evaluation of 151 by National Cancer Institute

The National Cancer Institute (NCI) operates a developmental therapeutics program including the NCI-60 tumour cell line screen for over 20 years. It uses 60 tumour cell lines to identify novel compounds as anti-cancer agents and can distinguish between both growth inhibition and cell death in tumour cells. The 60 cell lines encompass 9 different cancer subtypes representing leukaemia, melanoma, lung, colon, brain, ovarian, breast, prostate and kidney cancers. The NCI screen uses a method of protein concentration detection to determine % viabilities in treated cells using a dye called sulforhodamine B (SRB) solution which differs from AlamarBlue™ previously described in this thesis.

Compounds submitted to the NCI undergo a preliminary screen in a one dose assay at a concentration of 10 µM. If the pre-determined threshold for inhibition is met, compounds progress to a 5-dose assay. The NCI-60 screen is a five dose screen that reports three parameters across 60 cell lines (Table 8.4).

1) GI\textsubscript{50} detailing drug concentration resulting in 50% total cell growth inhibition, less commonly referred to as the absolute IC\textsubscript{50}. This compares to relative IC\textsubscript{50} values described in Chapter 6 which report the concentration of a drug required to reduce population of cells growth response to 50% with respect to vehicle control.

2) TGI considered as the concentration required for total growth inhibition

3) LC\textsubscript{50} considered as the concentration required to induce 50% lethality (measurement of cytotoxic rather than antiproliferative effect in cancer cells).
In the one-dose screen, the mean growth % of all 60 cell lines in initial one dose screening for 151 was 17%. Parent P3 was also screened at 10 μM in the NCI-60 panel and was not active as an antiproliferative agent with a mean % growth value of 99%. This data demonstrates that insertion of the β-lactam ring to cis restrict P3’s A and B rings in addition to substitution of the B ring 2-hydroxyl with a 2-nitro moiety dramatically augments the antiproliferative effects of P3. P3P has been further selected for 5 dose screening which has not yet progressed.

GI_{50} values from the five-dose screen are presented for 151 in Table 8.4. For most cell lines, GI_{50} values were determined below 1 μM. In the breast cancer cell panel of interest, 151 demonstrated moderate to good biological activity. GI_{50} values were determined as 0.32 μM, 0.41 μM, 0.74 μM and 0.32 μM in MCF-7, MDA-MB-231, Hs578T and BT-549 cells respectively (Table 8.4 and A, Figure 8.16). This antiproliferative activity did not extend to HT-29 cells which had a much higher GI_{50} value of 2.84 μM (Table 8.4 and B, Figure 8.16). 151 was determined as most active in the non-small-cell-lung cancer cell line NCI-H226 (GI_{50} of 0.257 μM), CNS cancer cell lines SF539 (GI_{50} of 0.244 μM) and SNB-75 (GI_{50} of 0.21 μM) in addition to two melanoma cell lines; LOX-IMV1 (GI_{50} of 0.241 μM) and MDA-MB-435 [GI_{50} of 0.196 μM, (Table 8.4)].

Figure 8.16: Dose-response curves generated by NCI-60 screening for 151 for A: breast cancer cell lines B: colon cancer cell lines.

Table 8.4: GI_{50} values for 151 in NCI 5-dose screen
<table>
<thead>
<tr>
<th>Cell Line</th>
<th>GI50 (μM)</th>
<th>Cell Line</th>
<th>GI50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leukemia</strong></td>
<td></td>
<td><strong>Melanoma</strong></td>
<td></td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>0.452</td>
<td>LOX IMV1</td>
<td>0.241</td>
</tr>
<tr>
<td>HL-60</td>
<td>0.336</td>
<td>MALME-3M</td>
<td>0.755</td>
</tr>
<tr>
<td>K-562</td>
<td>0.337</td>
<td>M14</td>
<td>0.315</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>0.475</td>
<td>MDA-MB-435</td>
<td>0.196</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>0.371</td>
<td>SK-MEL-2</td>
<td>1.96</td>
</tr>
<tr>
<td>SR</td>
<td>0.344</td>
<td>SK-MEL-28</td>
<td>1.12</td>
</tr>
<tr>
<td><strong>Non-Small Cell Lung Cancer</strong></td>
<td></td>
<td><strong>Ovarian Cancer</strong></td>
<td></td>
</tr>
<tr>
<td>A549/ATCC</td>
<td>0.409</td>
<td>UACC-257</td>
<td>0.705</td>
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<tr>
<td>HOP-62</td>
<td>0.594</td>
<td>UACC-62</td>
<td>0.357</td>
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<tr>
<td>HOP-92</td>
<td>0.692</td>
<td><strong>Ovarian Cancer</strong></td>
<td></td>
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<tr>
<td>NCI-H226</td>
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<td>IGROV1</td>
<td>0.531</td>
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<tr>
<td>NCI-H23</td>
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<td>OVCAR-3</td>
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<td>NCI-H332M</td>
<td>6.63</td>
<td>OVCAR-4</td>
<td>0.863</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>3.51</td>
<td>OVCAR-5</td>
<td>0.746</td>
</tr>
<tr>
<td>NCI-H552</td>
<td>1.98</td>
<td>OVCAR-8</td>
<td>0.321</td>
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<tr>
<td>NCI/ADR-RES</td>
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<tr>
<td><strong>Colon Cancer</strong></td>
<td></td>
<td><strong>Renal Cancer</strong></td>
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<tr>
<td>COLO 205</td>
<td>2.58</td>
<td>786-0</td>
<td>0.684</td>
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<tr>
<td>HCT-2998</td>
<td>2.29</td>
<td>A498</td>
<td>&gt;10e-4</td>
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<td>HCT-116</td>
<td>0.326</td>
<td>ACHN</td>
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<tr>
<td>HCT-15</td>
<td>4.54</td>
<td>CAKI-1</td>
<td>0.37</td>
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<td>HT29</td>
<td>2.84</td>
<td>RXF-393</td>
<td>0.271</td>
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<tr>
<td>KM12</td>
<td>3.76</td>
<td>SN12C</td>
<td>0.466</td>
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<tr>
<td>SW-620</td>
<td>3.89</td>
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<td></td>
</tr>
<tr>
<td><strong>CNS Cancer</strong></td>
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<td><strong>Breast Cancer</strong></td>
<td></td>
</tr>
<tr>
<td>SF-268</td>
<td>0.548</td>
<td>UO-31</td>
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<td>SF295</td>
<td>1.24</td>
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<tr>
<td>SF539</td>
<td>0.244</td>
<td>MCF-7</td>
<td>0.32</td>
</tr>
<tr>
<td>SNB-19</td>
<td>2.18</td>
<td>MDA-MB-231/ATCC</td>
<td>0.407</td>
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<tr>
<td>SNB-75</td>
<td>0.21</td>
<td>HS 578T</td>
<td>0.737</td>
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<tr>
<td>U251</td>
<td>0.372</td>
<td>BT-549</td>
<td>0.323</td>
</tr>
<tr>
<td><strong>Prostate cancer</strong></td>
<td></td>
<td><strong>Breast Cancer</strong></td>
<td></td>
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<tr>
<td>PC-3</td>
<td>0.603</td>
<td>MDA-MB-468</td>
<td>0.422</td>
</tr>
<tr>
<td>DU-145</td>
<td>0.512</td>
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</tbody>
</table>

Red = good activity, yellow = moderate activity and green = poor activity
Table 8.5 presents the TGI and LC$_{50}$ values in breast cancer cells for 151. Both values are greater than the GI$_{50}$ values for 50% cell inhibition indicating that the dose required for total inhibition and lethality of cancer cells is much higher than the concentration required for 50% inhibition, inferring potential for semi selective toxicity towards cells at treatment doses.

Table 8.5: GI$_{50}$, TDI$_{50}$ and LC$_{50}$ values for P3BL1 in breast cancer cell lines from NCI-60 5 dose screen

<table>
<thead>
<tr>
<th>Breast cancer cell line</th>
<th>GI$_{50}$ ($\mu$M)</th>
<th>TGI ($\mu$M)</th>
<th>LC$_{50}$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>0.32</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MDA-MB-231/ATCC</td>
<td>0.407</td>
<td>11.9</td>
<td>&gt;100</td>
</tr>
<tr>
<td>HS 578T</td>
<td>0.737</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>BT-549</td>
<td>0.323</td>
<td>1.16</td>
<td>25.8</td>
</tr>
<tr>
<td>T-47D</td>
<td>18.6</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>0.422</td>
<td>17.5</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

These findings demonstrate the potential of the pyrazine $\beta$-lactam scaffold as a potential antiproliferative agent in contrast to the trans P3 which did not demonstrate any antiproliferative activity (Figure 8.9). The $\beta$-lactam pyrazets have potential to supersede P3 as an AA and radiosensitising agent with additional antiproliferative properties. Future work must involve determination of whether insertion of the $\beta$-lactam ring structure maintains the radiosensitising effects of P3 in addition to augmenting its antiproliferative effects. Microtubule depolymerisation potential for 151 remains to be elucidated. A further panel of $\beta$-lactam pyrazets should also be explored i.e reduction of the NO$_2$ substitution of 151 to NH$_2$, hydrazinolysis or methanolysis of 151’s OCOCH$_3$ to the 3-hydroxyl and OTBDMS or other protection of the B ring 2-hydroxyl. Additionally, 3-hydroxyl substituted pyrazet racemates are candidates for further progression toward their enantiomers as described in resolution approaches in Chapters 3,4 and 7.

8.15 Chapter summary and outlook

P3 is highly insoluble and only isolated to date as the trans isomer. Solubility issues have been overcome for P3 using the phosphoric acid derivative P3P. P3P has a better antiproliferative profile than parent P3 in breast cancer cell lines with preliminary cell cycle data (n=1) demonstrating potential anti-mitotic activity (G2/M phase arrest) and

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apoptosis. Additionally, it is rapidly hydrolysed to P3 by AP enzymes demonstrating its viability for use as the phosphate prodrug, rapidly converted into parent P3 in vivo. Computational studies for cis and trans P3 at the CBS have that demonstrated that cis P3 may have microtubule depolymerising activity as a CBSI while trans P3 may not. A pyrazine β-lactam pyrazet analogue 151, was synthesised and its antiproliferative activity evaluated in the NCI-60 5 dose cell line screen. NCI data demonstrates superior antiproliferative activity in the panel of cancer cells lines for 151 compared to parent P3. Future work must involve optimisation of the pyrazine β-lactam scaffold for maximum biological activity. Synthetic or photochemical strategies for isolation of cis P3 should also be explored. The microtubule depolymerising effects of cis P3 and the panel of pyrazets should be evaluated. Additionally, further optimisation for 3-substituted pyrazine β-lactams should involve synthesis of their trans derivatives and isolation of their enantiopure forms for maximum anti-proliferative activity.
Chapter 9 - Concluding remarks and future work
Despite advances in treatment approaches for both TNBC and metastatic colorectal cancer, limitations associated with managing recurrent and advanced disease remain. At present, chemotherapy such as taxanes and anthracyclines are the predominant treatments for patients with TNBC. Adjuvant therapy is often ineffective for metastatic TNBC with median survival estimated at only 13 months. Receptor tyrosine kinases inhibitors such as EGFR inhibitors are now in development as novel agents for the treatment of resistant cancers. Lapatinib is the only anti-EGFR agent approved by the FDA for clinical use, and it is recommended to use in combination with chemotherapy or hormone therapy. Since over 50% of patients with TNBC demonstrate overexpression of EGFR, anti-EGFR was anticipated as a promising target for TNBCs. However clinical trials have consistently demonstrated poor effectiveness of anti-EGFR antibodies. Cyclin dependent kinases (CDKs) 4/6 which participate in cell cycle progression from G1 to DNA synthesis (S phase) mediate tumorigenesis. Three CDK inhibitors have now been approved for ER+ and HER2+ breast cancers e.g palbociclib, robociclib and abemaciclib. However no CDK4/6 inhibitor is currently approved for treating TNBC. Trilaciclib is in trials for patients receiving first or second line treatment for mTNBC. In summary, strategies for the treatment of mTNBC demand novel approaches for combination regimens when other agents fail. Notwithstanding limitations associated with treatment of TNBC, mCRCs are commonly resistant to first line and commonly used clinical agents. Additionally CA-4 has no effect as an anti-proliferative, microtubule depolymerising or anti-mitotic agent in colorectal cancer cells.

Racemates of the combretzet panel discussed in this thesis have previously been reported with excellent in vitro activity in both breast and colorectal cancer cell lines. This thesis has reported methods for enantioseparation of combretazet racemates ranging from 50% to >90% ee. Comparative in vitro biochemical analysis has demonstrated the advantages of eutomers of enantiomeric pairs. Eutomers demonstrated dramatically augmented anti-proliferative, microtubule depolymerising and anti-mitotic activity with respect to both the corresponding parent racemates and distomers. Preliminary pre-clinical in vitro analysis discussed in this thesis demonstrates the potential for treatment of advanced mTNBC and/or mCRC with B ring meta hydroxyl substituted combretazet eutomers.
This project has demonstrated the limitations associated with use of chiral diastereomeric resolution as a method for enantiopure isolation of the combretazet eutomers. Co-elution of diastereomers during LC consistently reduced the yield of the desired eutomers in addition to reducing ee of the pre-distomer diastereomer fractions. While isolated yields in this thesis were sufficient for progression toward preliminary in vitro analysis, larger yields will be required in future for progression towards in vivo studies. The water solubility of parent eutomers remains to be elucidated. Based on prior reports for the parent racemates\textsuperscript{380} it is anticipated that progression toward pre-clinical in vivo models will mandate derivatisation toward the phosphate or phosphate-like prodrugs, such as the novel anti-viral remdesivir as a protide phosphoroamidate prodrug used for the treatment of COVID-19.\textsuperscript{317, 788} Our group have previously demonstrated that β-lactam racemate derivatives of CA-4 are below the detectable limit of solubility measured using HPLC. These solubility issues which would otherwise limit the pre-clinical progression of combretazet eutomers have previously been overcome for the racemic forms by derivatisation toward phosphate or amino acid prodrugs.\textsuperscript{380} Our panel of 3-hydroxyl analogues containing a variety of substitutions and deletions at the B ring meta hydroxyl have dual anti-cancer activity in both TNBC cells and in chemo resistant HT-29 colorectal cancer cells.\textsuperscript{378, 400} They have synthetic potential for development as the phosphate prodrugs due to the availability of the hydroxyl functional group for phosphate ester derivatisation. However, the chemistry required for prodrug synthesis including, synthetic optimisation, solubility studies and subsequent biological evaluation requires larger enantiopure isolated yields than chiral resolution has provided in this thesis. For this reason, KR was explored as a second and alternative method for enantiopure β-lactam isolation in Chapter 7. KR demonstrated improved isolated yields in a more accessible, sustainable and facile approach for enantioseparation. Future adoption of KR may enable synthesis of a panel of prodrug derivatives of the 3S, 4S eutomers for further pre-clinical evaluation. $ee$ values for eutomers isolated in this thesis using KR remain sub-optimal ranging only from 60-80% $ee$.

Initial results using KR demonstrated poor enantioselectivity using 1 mg/mg of CAL-B per 3-acetoxy β-lactam in combination with 10 equivalents of methanol. 3-Hydroxyl 3S,4S 17EN1 was isolated in 56% $ee$. The optimised method demonstrated that by reducing the rate of CAL-B mediated methanolysis using 0.5 mg/mg of CAL-B per mg
of 3-acetoxy β-lactam with 3 equivalents of methanol (in TMBE), ee increased from 40-50% to 70-80% could be obtained. There is currently scope to further augment ee values using a more optimal lipase enzyme or an alternative synthetic route e.g employing lipase enzymes to acylate the 3-hydroxyl moiety following initial methanolysis as a component of a double resolution approach outlined in Scheme 9.1. Double resolution techniques for gram scale preparation of β-lactam enantiomers are previously reported, involving acylation with an acyl donor to yield 88% ee followed by alcoholysis to an ee value of >99% using Pseudomonas cepasia (lipase PS). Specifically, long terms aims are to apply the most optimal approach using lipase enzymes as biocatalysts to obtain enantioenriched, but ideally enantiopure 3S,4S eutomers, followed by synthesis of the most clinically viable amino acid and/or phosphate prodrug. Future visions for the combretazets include progression of a clinically viable B ring meta substituted prodrug e.g the phosphate prodrug of 18EN1 towards pre-clinical and clinical in vivo models, as novel agents with dual activity for the treatment of both TNBC and chemo resistant colorectal cancers.

Recent years have catalysed interest in more environmentally friendly strategies for synthetic chemistry, particularly in the area of green technology development. The use of mechanochemical activation of immobilised lipase enzymes in a field known as mechanochemistry or mechanoenzymology has seen application of KR for asymmetric induction to yield chiral compounds and has led to the synthesis of a wide variety of chiral APIs, e.g precursors of one of the most frequently used β-blockers propranolol. CAL-B specifically, has demonstrated excellent KR of several APIs and precursors by means of mechanoenzymology in enantioselective acylation and/or

Scheme 9.1: Proposed biocatalytic pathway for achieving > 99% ee for 3S,4S eutomers of 3-hydroxyl β-lactam racemates. Pathway carried out and ee values determined to date highlighted in red. TBC = to be confirmed.
hydrolytic procedures. Good results have been achieved for such propranolol precursors via enantioselective esterification in stainless steel, agate or Teflon milling jars accompanied by vibrational milling at 25 Hz in 90% ee with an E value of 21. Hydrolytic KR was also explored using water instead of an acylating reagent producing 96% ee and selective 50% conversion with impressive E values of 259, using agate milling jars. Mechanoenzymatic reactions are typically facilitated by liquid assisted grinding (LAG), crucial for driving the reaction forward by ensuring homogenous distribution of immobilized catalyst and substrate. Best results were obtained using dioxane as the LAG additive, agate milling and CAL-B in a ratio of 1:1 with substrate, with ee of >99%, E value of 300 and conversion ceasing at 50%.

While CAL-B typically is considered as an enzyme which does not demonstrate interfacial activation, it is now suggested that bulkier substrates gain better access to active site due to conformational change of the α5 or α10 helix at the enzyme’s lid. Mechanical force has been demonstrated as altering CAL-B activity most likely due to alteration of α10 helix conformation. This provides strong rationale for further investigation of enantiopure combretazet isolation using mechanoenzymology in future studies.

Additionally, in this thesis, expansion of the 3,4,5-trimethoxyphenyl structure for anti-proliferative activity has been explored by incorporating the pyrazine A ring for P3 β-lactam analogues as a method of cis restriction for AA agent P3 (Chapter 8). This work now requires full elucidation of the mechanisms of anti-proliferative activity including tubulin polymerisation analysis and cell cycle studies. This thesis has demonstrated that the presence of the B ring para-methoxy substituent is essential for anti-proliferative activity due to the dramatic reduction in antiproliferative activity observed for B ring para fluorine and nitro substituted analogues with respect to e.g. 17 (Chapter 6). Formation of CA-4/P3 hybrids as illustrated in Scheme 9.2 with the pyrazine A ring, para methoxy substituted B ring and 3-hydroxyl, would enable chiral resolution and isolation of the respective enantiomeric forms (Scheme 9.2). Enantioseparation in turn has the potential for augmentation of bioactivity due to presence of the desirable substitution pattern.
Synthesis of \textit{cis} P3 should also be explored to determine its microtubule depolymerising activity and CBS affinity. A proposed method is detailed below, involving carboxylation of 2-methylpyrazine towards its corresponding acetic acid for Perkin condensation with salicaldehyde towards the carboxylic acid derivative, which may then be decarboxylated to the \textit{cis} stilbene structure (Scheme 9.3). This method has previously been reported for the synthesis of \textit{cis} CA-4 and its derivatives.\footnote{343}

\begin{scheme}
\textbf{Scheme 9.3: Proposed Perkin condensation synthesis for \textit{cis} P3:} \textit{a:} \textit{n}-butyl lithium, DIPEA, THF, -78 °C, quench with solid CO\textsubscript{2} \textbf{b:} acetic anhydride, TEA, 120 °C, CEM microwave synthesiser, 30 minutes. \textbf{c:} Decarboxylation with copper powder, quinoline, 200 °C, 3h
\end{scheme}
An alternative to the synthetic isolation of cis P3 is the potential for a photoisomerisation reaction which promotes a switch from isolated trans or (E) configuration to the corresponding cis (Z) isomer. Photoisomerisation in opposite trans to cis configuration is a counter thermodynamic process and not commonly found in the literature. However examples are described for sterically hindered cis isomers using solar irradiation, a green way to carry out organic synthesis and is therefore worthy of further investigation.

Both cis P3 and a potential panel of cis restricted P3 β-lactam racemates and/or enantiomers have scope as potential CBSIs, VDAs and AA agents with more potent anti-proliferative and radiosensitising activity with respect to parent trans P3, each of which remain to be investigated.

The underlying mechanism for anti-mitotic activity observed for P3P also requires confirmation and further investigation. The mechanisms for the large improvement in anti-proliferative activity for the phosphate prodrug versus parent trans stilbene structure requires extensive elaboration. Potential explanations include increased cell uptake either by active or passive diffusion.

To summarise, both the combretazets and P3 analogues have demonstrated excellent potential for the treatment of TNBC and colorectal cancers as CBSIs, VDAs, AAs and radiosensitising agents. Synthetic optimisation is required for both series prior to progression toward future pre-clinical in vivo stages. Nevertheless, these agents offer potential strategies for future curative and effective management of resistant, advanced and/or metastatic breast and colorectal cancers, and are therefore worthy of further development.
Chapter 10 - Experimental
Materials and Methods

All reagents were commercially available and were used without any further purification unless otherwise indicated. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Bruker Avance III 400 or Avance II 600 (400.13 MHz/ 600.13 MHz, 1H; 100.61 MHz, 13C/150.61 MHz, 13C)) in either CDCl₃ or DMSO-d₆. For CDCl₃, the internal standard is tetramethylsilane (TMS) with 1H- NMR spectra assigned relative to this TMS peak at δ 0.00 ppm. 13C-NMR spectra were assigned relative to the middle peak of CDCl₃ triplet at δ 77.00 ppm. For 1H NMR assignments, chemical shifts are reported: shift value (multiplicity, integral value, coupling constant(s)). For 1H-NMR assignments, chemical shifts are reported: shift value (multiplicity, the integral value representing number of protons, coupling constant(s), relevant proton or carbon assignment, isomer, diastereomer and or rotamer composition). Quantitative (q) 1H experiments utilised a 90° excitation pulse and a 60 second recycle delay between scans to allow the spins to return to equilibrium ensuring 5 times the T₁ (spin lattice relaxation) was exceeded. High resolution mass spectrometry (HRMS) was carried out by Dr. Gary Hessman of the School of Chemistry, Trinity College Dublin. Experiments were carried out predominantly using atmospheric pressure chemical ionisation (APCI) with occasional use of electrospray ionisation (ESI-MS) in both positive and negative modes. ESI mass spectra were acquired using a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC in positive and negative modes as required. Masses were recorded over the range 100-1400 m/z. APCI experiments were carried out on a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC or direct insertion probe. The instrument was operated in positive or negative mode as required. Masses were recorded over a range of 100-1600 m/z. Low resolution mass spectrometry (LRMS) was performed by Mr Brian Talbot of the School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin using APCI on the LTQ Orbitrap Discovery Mass Spectrometer (ThermoFisher Scientific), operating in positive/negative ion polarity switching mode at mass range of 50 to 2000 m/z. TLC was carried out on silica gel on Merck silica gel on TLC aluminium foils with fluorescent indicator F-254 nm. Rₛ values are quoted for each compound. Flash column chromatography was carried out on Merck silica gel technical grade, pore size 60 Å, 40-63 µM particle size, 230-400 mesh. Microwave experiments were carried out using a Discover CEM microwave synthesizer on standard power setting unless otherwise stated. On standard power setting, the maximum power supplied is 300 W. X-ray crystallography data was collected by Dr. Brendan Twamley of the School of Chemistry, Trinity College Dublin.

Solubility & Analytical HPLC Studies
HPLC instrumentation consisted of a Waters 1525 Binary HPLC pump in-line vacuum degasser, a 717 plus auto sampler and a Waters 2487 dual wavelength detector. The column used was a BDS Hypersil C18, reverse phase, 250 x 4.6mm (ThermoFisher Scientific). 70:30 HPLC grade MeCN:H₂O as mobile phase, injection volume of 10 μL and flow rate of 1 mL/min. A calibration of standard solutions versus concentration was plotted. All standard solutions were made from a 0.5 mg/ mL stock solution in mobile phase. Calibration curves were constructed in triplicate from three independent series of stock solutions made up on three independent occasions. A linear regression equation and correlation coefficient (R² value) were calculated in both Microsoft excel and GraphPad. An R² value of 0.98-0.99 was accepted. The shake flask method for solubility measurements was designed in line with WHO guidance. A suspension of relevant compound was weighed out and added with 1 mL HPLC grade water in a microcentrifuge tube or Eppendorf vial. This suspension was placed onto a mechanical shaker (Mini gyro- rocker, SSM3, Stuart) with a stirring speed of 70 rpm for 24 hours. After 24 hours the solution was filtered through a 0.22 µM filter to remove undissolved compound. The filtrate was transferred to a HPLC vial and diluted to the linear range of relevant calibration curve with mobile phase prior to analysis by HPLC. Solubility studies were carried out in triplicate. Analytical stability studies were carried out at three pH values using the phosphate buffer recipes detailed in the British Pharmacopoeia as follows:

**Phosphate buffer solution in pH 4.0:** 1.7 g of potassium dihydrogen orthophosphate (KH₂PO₄) added to 175 mL of dH₂O and adjusted to pH 4 using 10 % v/v orthophosphoric acid. The solution is then made up to volume of 250 mL with dH₂O.

**Phosphate buffer solution in pH 7.4:** 4.08 g KH₂PO₄ added to 150 mL of dH₂O to make a 0.2M solution. Add 125 mL of above solution to 198 mL of 0.1 M NaOH solution.

**Phosphate buffer solution in pH 9.0:** 5.22g of KH₂PO₄ added to 240 mL dH₂O and pH adjusted to 9.0 by dropwise addition of 1M potassium hydroxide solution (5.6 g/ 100 mL of dH₂O). The final volume was diluted to 300 mL using dH₂O.

The test compound was dissolved in mobile phase (70:30 MeCN: H₂O) prior to dilution in phosphate buffer.
Chiral HPLC

Chiral HPLC was carried out for racemates and enantiomers using Chrompak-IH-3 (150 x 4.6 mm) supplied by Chiral Technologies Europe with a Chiral- IH-3 guard column, to protect the analytical column from impurities. The column utilises an immobilised chiral polysaccharide stationary phase, tris(S)-α -methylbenzylcarbamate. The following conditions were used for all chiral HPLC studies: injection volume 5 μL, flow rate of 1 mg/mL and run time of 10-60 minutes, using n-hexane:propan-2-ol, 1:1 at 254 nm. All samples were dissolved in propan-2-ol or ethyl acetate at a concentration of 1-5 mg/ mL for optimal chromatograph resolution. $Ee$ was calculated using the following formula: (% Major peak - % minor peak) X 100 = % ee. Racemates were analysed using a non-chiral reverse phase Zorbax SB-C18 column, 5 μm particle size, 25 cm x 4.6 mm, with an injection volume of 10 μL of sample, flow rate of 1mg/mL, 10 minute run time and mobile phase of 60:40% MeCN/H2O.

Characterisation for of all novel analogues are detailed below while intermediates and previously reported analogues are reported in Appendix 3.

General Method I: Phenol protection using $t$-butyldimethylsilyl chloride (TBDMSCl)

Route A: Direct protection of phenolic imines

To a stirring solution of phenolic imine 46 (1 eq, 6 mmol, 1.9g) in anhydrous DCM (60 mL), $t$-butyldimethylsilyl chloride (1.2 eq, 7.2 mmol, 1.08g) was added under a nitrogenous atmosphere at room temperature. DBU (1.6 eq, 9.6 mmol, 1.44 mL) was added dropwise to the reaction vessel and subsequently stirred for three hours until the starting material had disappeared, indicated by TLC. On completion the reaction mixture was diluted with dichloromethane (60 mL), washed with dH2O (100 mL), 0.1 M HCl (50 mL) and a saturated solution of NaHCO3 (50 mL), retaining the organic layer each time. The organic layer was dried with anhydrous Na2SO4, filtered and the solvent removed under reduced pressure. The resulting product was a brown oil as a mixture of parent aldehyde and amine with TBDMS protected imine.

Route B: Protection of precursor 3-hydroxy-4-methoxybenzaldehyde

To a solution of 3-hydroxy-4-methoxybenzaldehyde (1 eq, 10 mmol, 1.52g) in anhydrous DCM (30 mL) under nitrogen atmosphere at room temperature, $t$-butyldimethylsilyl chloride (1.2 eq, 12 mmol, 1.81g) was added followed by the dropwise addition of DBU (1.8 eq, 18 mmol, 2.68 mL). The reaction was stirred at room temperature until completion as indicated by TLC (1-2 hours). On completion the reaction was diluted with 60-100 mL of DCM which was subsequently washed with dH2O (100 mL), 0.1M HCl (50 mL) and NaHCO3 (50 mL) retaining the organic layer each time. The organic layer was dried with anhydrous Na2SO4,
filtered and the solvent removed under reduced pressure yielding 42 (Appendix 3) as a yellow oil for use without further purification in agreement with literature characterisation.

**General Method II: Protection of amino phenylacetic acid with the CBZ protecting group**

Aminophenylacetic acid (1eq, 5mmol, 0.75g) and sodium bicarbonate (Na$_2$CO$_3$) (2.7 eq, 13.5 mmol, 1.13g) were dissolved in dH$_2$O (50 mL). The reaction vessel was placed on ice and cooled to 0 °C. Benzyl chloroformate (1.1 eq, 5.5 mmol, 0.78 mL) was dissolved in 4 mL of dioxane and injected via septum to the stirring solution. The reaction was stirred for 30 minutes at 0 °C before warming to room temperature and stirring for a further 24 hours. After 24 hours the reaction volume was diluted with 50 mL of ether. The aqueous and ether layers were separated. The aqueous layer was extracted with 2 × 50 mL of ethyl acetate. The organic layers were then combined and dried with anhydrous Na$_2$SO$_4$. The solvent was removed in vacuo to afford (4-benzyloxy carbonylamino phenyl)acetic acid (Appendix 3) as a yellow/white powder. $^1$H NMR data was in line with literature values. The desired product for use without further purification.

**General Method III: Synthesis of imines**

*Method A for imine synthesis*

Imines were synthesised via condensation of the appropriately substituted benzaldehyde (1 eq, 10 mmol) and 3,4,5-trimethoxyaniline (1 eq, 10 mmol, 1.83 g) in ethanol (40 mL) at 90 °C for 4 hours. A catalytic quantity of 0.1 M HCl was added dropwise at the beginning of the reaction. The reaction volume was then reduced to 10-20 mL in vacuo. The imine precipitated overnight and was purified by recrystallization in the minimum volume of hot ethanol. The final product was isolated by gravity filtration and dried under high vacuum.

*Method B for imine synthesis*

The appropriately substituted benzaldehyde (1 eq, 10 mmol) and 3,4,5-trimethoxyaniline (1 eq, 10 mmol, 1.83g) were added to minimum volume of cold ethanol at room temperature followed by addition of one drop of HCl (10% v/v). The reaction was stirred at room temperature until completion, indicated by TLC (1-2 hours). Imine products precipitated in cold ethanol when the reaction approached completion which were isolated via gravity filtration and washing with cold ethanol. Imines were dried overnight under high vacuum. Characterisation for the panel of imines (44-56) synthesised in this thesis is detailed in Appendix 3.

**General Method IV: Synthesis of 3-acetoxy-β-lactams**

*Optimised Staudinger synthesis*
To a stirring solution of acetoxyacetyl chloride (1.2 eq, 7 mmol, 0.78 mL) in an inert atmosphere in dry toluene (50 mL), the relevant imine (1 eq, 5 mmol) was added in stepwise portions. The reaction was heated to 100 °C and stirred for 20 minutes. A bright orange colour was observed on addition of imine to acetoxyacetyl chloride which slowly changed to bright yellow and subsequently pale yellow during the 20 minute reflux period. Anhydrous TEA (1.8 eq, 9 mmol, 1.25 mL) was then added dropwise over 2-5 minutes under nitrogenous conditions. The reaction was stirred at 100 °C for a further 5 hours prior to stirring overnight at room temperature (16-24 hours). The reaction mixture was washed with dH₂O (2 × 30 mL) and the organic layer dried with anhydrous sodium sulphate (Na₂SO₄) before the solvent was removed under reduced pressure. The crude product was purified using flash column chromatography over silica gel using 1:1 n-hexane:ethyl acetate. Characterisation for the panel of 3-acetoxy-β-lactam racemates (16a-22a, 64a-66a, 67a and 67b, 89-91) is detailed in Appendix 3.

2-(4-Methoxy-3-nitrophenyl)-4-oxo-1(3,4,5-trimethoxyphenyl)azetidine-3-yl acetate (63a) was synthesised from 54 using General Method IV and isolated as a novel mixture of predominantly trans isomer in a ratio of 82:18 trans:cis

Yield: 1.33 g (2.98 mmol) 60%
Rf: 0.2 (1:1 n-hexane:ethyl acetate)
Appearance: White solid crystalline powder
Melting Point: 171-172 °C

¹H NMR (400 MHz, CDCl₃): δ (mixture of cis:trans isomers 18:82) 7.89 (d, 1H, J = 2.4 Hz, H₆'), 7.83 (dd, 0.15H, (cis), J = 9.1 Hz, J = 1.9 Hz, H₂'), 7.54 (dd, 1H, J = 9.1 Hz, J = 1.9 Hz, H₂''), 7.51 (d, 0.15H, (cis), J = 2.4 Hz, H₆''), 7.14 (d, 1H, J = 8.6 Hz, H₃''), 7.10 (d, 0.15H, (cis), J = 8.6 Hz, H₃''), 6.54 (s, 0.3H, (cis), H₁&₃'), 6.50 (s, 2H, H₁&₃'), 5.93 (d, 0.11H, (cis), J = 4.8 Hz, H₃), 5.34 (d, 0.11H, (cis), J = 4.78 Hz, H₄), 5.30 (d, 1H, (trans), J = 2.4 Hz, H₄), 4.91 (d, 1H, (trans), J = 2.4 Hz, H₃), 3.98 (s, 3H, H₇'), 3.86 (s, 0.5H, (cis), H₇'') 3.78 (s, 3H, H₈), 3.75 (s, 1H, (cis), H₃₈₀) 3.73 (s, 6H, H₃₈₀), 2.22 (s, 0.4H, (cis), H₁₀), 2.21 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 160.9 (C₂), 153.8 (C₃₈₀), 153.4, 123.6, 132.6, 131.7, 127.7, 124.0, 114.5, 95.3 (C₁&₃'), 82.3 (C₃), 62.7 (C₄), 61.0, 56.7 (C₃₈₀), 56.3, 20.4 (Cₐ₁₀)

HRMS: APCI Calculated for C₂₃H₂₃N₂O₉ [M +H⁺] 447.139807, found 447.139619, error + 0.4 ppm

Chapter 10: Experimental 344
General Method V: Optimised synthesis for 3-substituted β-lactam racemates

To a stirring solution of the appropriate acid chloride (2 eq, 10 mmol), under a nitrogen atmosphere in anhydrous toluene (50 mL), relevant imine (5 mmol) was added. The reaction was heated to reflux at 100 °C and stirred at reflux for 10-15 minutes. Anhydrous TEA (2 eq, 10 mmol, 1.4 mL) was then added dropwise over 10 minutes under a nitrogen atmosphere. The reaction was stirred for 5 hours prior to cooling to room temperature and stirring overnight (16-24 hours). A characteristic darkening of the reaction was observed during reaction progression.

The residue was diluted with dH$_2$O and the organic layer retained. The organic layer was then washed with a saturated solution of brine (2 × 30 mL) and dried with anhydrous Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure to afford a crude yellow/brown sticky oil. The crude product was purified using gradient flash column chromatography over silica gel 5:1 to 1:1 n-hexane:ethyl acetate. The product was washed with a few drops of diethyl ether and placed on high vacuum to give an amorphous white/yellow powder of respective β-lactam.

Characterisation for the panel of 3-substituted racemates 31, 34 and 70-75 are reported in Appendix 3.

3-phenyl-4-(ρ-tolyl)-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (91)

91 was synthesised from 52 and phenylacetyl chloride using General Method Vb.

Yield: 710 mg (1.64 mmol) 48%

Appearance: White crystalline powder

Rf: 0.35 (1:1 n-hexane: ethyl acetate)

Melting Point: 146-148 °C

$^1$H NMR (400 MHz, CDCl$_3$): δ 8.32 (d, 2H, J = 8.4 Hz, H$_{3''}$&$5''$), 7.61 (d, 2H, , J = 8 Hz, H$_{2''}$&$6''$), 7.42 (d, 2H, J = 7.6 Hz), 6.57 (s, 2H, H$_1$&$3$), 5.06 (d, 1H, J = 2.2 Hz, H$_4$), 4.23 (d, 1H, J$_1$= 2.2 Hz, H$_4$), 3.81 (3H, H$_8'$), 3.76 (s, 6H, H$_7'$&$9'$)

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 164.6 (C$_2$), 153.8 (C$_{4&6'}$), 148.2, 144.9, 135.1 (C$_2$), 133.8 (C$_5$), 133.1, 129.3, 128.4, 127.4, 126.8, 124.7, 94.9 (C$_{1'&3'}$), 65.2 (C$_3$), 63.1, 61.0 (C$_8$), 56.2 (C$_7$&$9'$)

IR ν$_{max}$ (ATR): 1745 cm$^{-1}$ (β-lactam C=O), 1506 cm$^{-1}$ (N-O)

HRMS: APCI calculated for C$_{24}$H$_{23}$N$_2$O$_6$ [M+H$^+$] 435.155063; found 435.155063, s -2.1 ppm

General Method VI: Synthesis of 3-phenolic β-lactams using triphosgene-mediated acid activation of substituted acetic acids

Chapter 10: Experimental
A mixture of the relevant substituted acetic acid (1.5 eq, 15 mmol) and triphosgene [bis(trichloromethyl)carbonate] (0.5 eq, 5 mmol, 1.49 g, 0.834 mL) was added to 50 mL of anhydrous toluene and heated to 100 °C. The substituted acetic acid was activated to the respective acid chloride by stirring under reflux for 30 minutes with triphosgene. Next a solution of the appropriately substituted imine (1 eq, 10 mmol) dissolved in anhydrous toluene was injected dropwise to the refluxing solution. This was followed by the addition of anhydrous triethylamine (3 eq, 30 mmol, 5 mL). The reaction was refluxed for a further 5 hours before cooling to room temperature and stirring overnight. The mixture was washed with dH₂O (2 × 50 mL) and NaHCO₃ (sat) (2 × 50 mL). The organic layer was retained and dried using anhydrous Na₂SO₄. The crude product was then purified using LC with isocratic (80:20 n-hexane: ethyl acetate) to afford protected 3-phenolic products. Characterisation for benzylxy protected phenolic racemates 79-81 are presented in Appendix 3.

**General Method VII: Synthesis of β-lactam derivatives via Reformatsky reaction**

**Method A: Reflux**

Zinc dust (900 mg, 2 eq, 13.8 mmol) was suspended in a stirring solution of anhydrous benzene (20 mL) under a nitrogenous atmosphere. The zinc catalyst was activated using trimethylchlorosilane (0.91 mL, 1 eq, 7.1 mmol) by stirring at 40 °C for 15 minutes, followed by heating to reflux (~ 85-88 °C) and stirring vigorously for 5 minutes. The reaction vessel was then cooled on ice. Imine 47 (1 eq, 7 mmol, 3.0 g) was added on ice at 0 °C followed by the respective substituted ethyl bromoacetate (1.8 eq, 12.6 mmol). A vigorous effervescence was observed. The reaction was then heated to reflux temperatures for 8 hours before cooling to room temperature and allowing to stir overnight. The zinc catalyst was removed from solvent by filtration through Celite. The reaction was diluted with dichloromethane (3 × 25 mL) followed by filtration through Celite. The filtrate was retained and washed with NH₄Cl (sat) (40 mL), 0.1M HCl (40 mL) and brine (40 mL). The organic layer was dried with anhydrous Na₂SO₄, filtered and solvent subsequently reduced in vacuo to yield a brown/black gum. The pure product was isolated using flash chromatography over silica gel with isocratic conditions of 4:1 n-hexane: ethyl acetate.

**Method B: Microwave technology**

Zinc powder (4.5 eq, 9 mmol, 0.588 g) was activated using trimethylchlorosilane (3.5 eq, 7 mmol, 0.76 g, 0.65 mL) in anhydrous benzene (4 mL) by heating for 15 min at 40 °C and subsequently for 2 min at 100 °C with microwave irradiation. After cooling the reaction vessel on ice, the imine 03ii (1 eq, 2 mmol, 0.86 g) was added. Next the appropriate substituted ethyl bromoacetate (2.5 eq, 5 mmol) was added to the reaction vessel and the mixture was placed in
the microwave reactor for 30 min at 100 °C. The reaction mixture was filtered through Celite to remove the zinc catalyst and the residue washed with DCM (30 mL). The filtrate was further diluted with DCM (30 mL). This solution was washed with saturated ammonium chloride solution (20 mL), 25 % ammonium hydroxide (20 mL), 0.1 HCl (40 mL), followed by dH₂O (40 mL). The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation in vacuo. A black gum was obtained as the crude product. The purified product was isolated from the crude gum by flash column chromatography over silica gel (eluent: hexane: ethyl acetate gradient). Characterisation for silyl ether protected Reformatsky products are detailed in Appendix 3.

**General Method VIII: Synthesis of 3-hydroxy-β-lactam racemates via hydrazinolysis to the corresponding acetate ester**

Hydrazine dihydrochloride (2 eq) was added to a stirred solution of trans 3-acetoxy-β-lactam intermediates (1 eq) in anhydrous methanol (30 mL) under a nitrogenous atmosphere and on ice at 0 °C. Anhydrous TEA (2.2 eq) was then added dropwise. The mixture was then allowed to reach room temperature before heating to reflux and stirring at 85 °C for 4-6 hours. The solvent was then removed in vacuo and the residue diluted with KHSO₄(aq). The product was extracted with ethyl acetate (2 X 50 mL). The combined organic layers were dried with anhydrous Na₂SO₄ and subsequently filtered and solvent removed under reduced pressure. The crude residue was purified by flash chromatography over silica gel (eluent n-hexane: ethyl acetate; 1:2) to afford the pure product. Previously reported racemates are detailed in Appendix 3 while characterisation for novel racemates is detailed below.

<table>
<thead>
<tr>
<th>Racemate code</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>NO₂</td>
<td>OCH₃</td>
</tr>
<tr>
<td>89</td>
<td>H</td>
<td>F</td>
</tr>
<tr>
<td>90</td>
<td>H</td>
<td>NO₂</td>
</tr>
</tbody>
</table>

The remainder of 3-hydroxy racemates are listed in Appendix 3.

**4-(4-Fluorophenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (89)**

89 was obtained from 89a by General Method VIII.

**Overall yield for Staudinger**: 350 mg (1.01 mmol) 11%
Rₖ: 0.31 (1:1; n-hexane:ethyl acetate)
3-Hydroxy-4-((4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90) trans

90 was synthesised using 90a using General Method VIII and isomers purified using LC over silica gel with isotropic conditions of 1:1 n-hexane:ethyl acetate.

Yield: 113 mg (0.3 mmol) 6% as trans isomer, 100 mg (0.28 mmol) 11% as mixed isomers

Melting Point: 162-167 °C

HRMS: APCI calculated for C_{13}H_{15}FNO_3 [M+H^+] 348.124177; found 348.12425, error +0.2 ppm

3-Hydroxy-4-((4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90) cis

90 cis was synthesised from 90a using General Method VIII and using LC with isocratic conditions (1:1 n-hexane:ethyl acetate).

Yield: 30 mg (0.08 mmol) 1.6% as cis isomer

Melting Point: 165 °C

HRMS: APCI calculated for C_{13}H_{15}FNO_3 [M+H^+] 348.124177; found 348.12425, error +0.2 ppm

3-Hydroxy-4-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (63)

63 was synthesised from 63a using General Method VIII

Yield: 330 mg (0.82 mmol), 27.3% for hydrazinolysis

Overall yield from Staudinger: 16.4%
1H NMR (600 MHz, CDCl3): δ 7.86 (d, 1H, J = 1.9 Hz, H6′′), 7.68 (bs, 1H, OH), 7.48 (dd, 1H, J = 9.3 Hz, J = 2.6 Hz, H2′′), 7.11 (d, 1H, J = 8.9 Hz, H3′′), 6.43 (s, 2H, H1&3′′), 4.83 (d, 1H, J = 1.9 Hz, H3), 4.72 (d, 1H, J = 1.9 Hz, H4), 3.97 (s, 3H, H10′), 3.77 (s, 3H, H8′), 3.72 (s, 6H, H7&9′)

13C NMR (100 MHz, CDCl3): δ 169.9, 161.0, 153.8, 153.4, 139.9, 135.4, 132.3, 131.8, 131.7, 127.6, 123.9, 114.4, 95.5, 82.4, 62.5, 60.9, 56.7, 56.3

15N NMR (60.8 MHz; CDCl3): δ 367.2 (NO2), 155.7 (β-lactam ring)

HRMS: APCI Calculated for C19H21N2O8 [M +H+] 405.12942, found 405.129640 error -1.0 ppm

General Method IX: Debenzylation of 80-81 to afford free 3-phenolic β-lactams 68-89

The respective benzyl ether protected β-lactam was dissolved in a 1:1 mixture of ethanol/ethyl acetate and was hydrogenated over palladium on carbon (10%) at atmospheric pressure until completion as monitored by TLC (2.5 hours). The catalyst was then filtered away using Celite and washed with ethyl acetate before the residual solvent was removed under vacuum. No further purification of the resulting product was required. Characterisation for previously reported intermediates are detailed in Appendix 3 while novel racemates are detailed below.

4-(3-Fluoro-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (68)

68 was synthesised from 80 using General Method IX.

Yield: 550mg (1.21 mmol) 78% for debenzylation, 18.4% overall for Staudinger
Appearance: white/grey fluffy solid.
Rf: 0.42 (1:1 n-hexane: ethyl acetate)
Melting Point: 95ºC

1H NMR (600 MHz, DMSO-d6): δ 9.55 (bs, 1H, OH) 7.40 (dd, 1H, J = 1.8 Hz, J = 9 Hz, H2′′), 7.30 (d, 1H, J = 8.7 Hz, H3′′), 7.19 (apparent triplet, 1H, J = 9.1Hz), 7.13 (d, 2H, J = 9 Hz), 6.78 (d, 2H, J = 9 Hz), 6.59 (s, 2H, H1&3′′), 5.14 (d, 1H, J = 1.9 Hz, H3), 4.34 (d, 1H, J = 1.9 Hz, H3), 3.83 (s, 3H, OCH3, H10′), 3.65 (s, 6H, H7&9′), 3.58 (s, 3H, OCH3, H8′)

19F NMR (376 MHz, DMSO-d6): δ -135.2
IR νmax(ATR): 3298 cm⁻¹(OH), 1726 cm⁻¹(β-lactam C=O)
HRMS: (APCI) Calculated for C_{25}H_{23}FNO_6 [M- H^-] 452.151489, found 452.152126, error +1.4 ppm, Calculated for C_{25}H_{23}FNO_6 [M+ H^+] 454.1666042, found 454.165455, error -1.3 ppm, Calculated for C_{25}H_{23}FNNaO_6 [M+ Na^+] 476.147986, found 476.148814, error -1.7 ppm

(3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (69)

69 was synthesised from 81 using General Method IX.

Yield: 330mg (0.65 mmol), 13% yield from Staudinger

Rf: 0.29 (1:1 n-hexane: ethyl acetate)

Appearance: grey powder

1H NMR (600 MHz, CDCl_3): δ 7.35 (d, 2H, J = 7.8 Hz, H_2''&6''), 7.19 (d, 2H, J = 7.8 Hz), 6.96 (d, 2H, J = 7.8 Hz), 6.83 (d, 2H, J = 7.8 Hz), 6.62 (s, 2H, H_1&3'), 4.82 (d, 1H, J = 2.2 Hz, H_4), 4.22 (d, 1H, J = 2.2 Hz, H_4'), 3.85 (s, 3H, H_10'), 3.79 (s, 3H, H_8'), 3.75 (s, 7H, H_7&9')

13C NMR (100 MHz, CDCl_3): δ 166.3 (C_2), 159.9 (C_4'&6'), 155.6, 153.5 (C_4&6'), 134.5 (C_2'), 133.7 (C_6'), 130.5, 129.3, 128.7, 127.3, 126.6, 115.9, 115.9, 113.8, 94.9 (C_1&3'), 64.5 (C_4), 64.3 (C_3), 61.0 (C_8'), 56.1 (C_7&9'), 56.0 (C_10')

HRMS: (APCI) Calculated for C_{25}H_{23}FNO_6 [M- H^-] 452.151489, found 452.152126, error +1.4 ppm, Calculated for C_{25}H_{23}FNO_6 [M+ H^+] 454.1666042, found 454.165455, error -1.3 ppm, Calculated for C_{25}H_{23}FNNaO_6 [M+ Na^+] 476.147986, found 476.148814, error -1.7 ppm

General Method X: Desilylation of β-lactams

To a stirring solution of TBDMS protected phenol (1 eq) in anhydrous THF (30 mL), TBAF, 1M in THF (1.5 eq) was added dropwise at 0 °C under a nitrogenous atmosphere. The reaction mixture was allowed to stir until the reaction had reached completion as monitored and indicated via TLC (2-3 hours). The solution was diluted with ethyl acetate (50 mL) which was then washed with 0.1M HCl (100 mL). The aqueous layer was then extracted using ethyl acetate (2 X 25 mL). The combined organic layers were then washed with brine (100 mL), water (100 mL) and dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure. The crude product was subjected to column chromatography under gradient elution (1:1 to 1:2 hexane:ethyl acetate) to afford the pure phenolic product. Characterisation for previously reported B ring meta hydroxy substituted racemates are reported in Appendix 3.

Novel characterisation is reported below.

General Method XI: Reduction of -NO_2 to -NH_2 group
The appropriate β-lactam derivative (1 eq) was suspended in glacial acetic acid (5-10 mL) under a nitrogenous atmosphere. Metallic zinc dust (10 eq) was added to the stirring suspension and stirred for 6 days. On completion, the reaction was diluted with DCM (20-50 mL) and filtered through celite to remove the zinc dust. The dichloromethane layer was washed with dH₂O (2 × 20 mL) to remove the glacial acetic acid. The amine derivative was then isolated using flash chromatography over silica gel using a gradient of n-hexane: ethyl acetate (3:1-1:2). Previously reported B ring meta NH₂ substituted racemates are reported in Appendix 3.

4-(3-Amino-4-methoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (64a)
64a was synthesised from 63a using General Method XI.

![Chemical structure of 64a](image)

Yield: <15%
Rf: 0.25 (1:4 n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl₃): δ 8.55 (d, 1H, J = 1.8 Hz, H₆''), 7.81 (bs, 1H, NH₂), 7.02 (dd, 1H, J = 1.8 Hz, J = 8.6 Hz, H₂''), 6.89 (d, 1H, J = 8.6 Hz, H₃''), 6.59 (s, 2H), 5.49 (d, 1H, J = 1.1 Hz, H₃), 4.90 (d, 1H, J = 1.1 Hz, H₄), 3.92 (s, 3H), 3.78 (s, 3H), 3.72 (s, 6H), 2.20 (s, 3H, OCOCH₃).

\(^13\)C NMR (100 MHz, CDCl₃): δ not determined

General Method XII: Diastereomer derivatisation using N-(Boc)-L-proline
To a stirring solution of trans β-lactam racemate (1 eq) in anhydrous MeCN (30 mL) under a nitrogenous atmosphere, HBTU (2.2 eq) was added. N-(tert-Butoxycarbonyl)-L-Proline (2 eq) was then added followed by addition of DIPEA (30 eq). The solution was stirred for 24 hours at room temperature prior to removal of the solvent in vacuo. The residue was diluted with dH₂O and extracted with DCM (3 × 30 mL). The combined organic layers were washed with potassium bisulfate (50 mL), sodium hydrogen carbonate (50 mL) and brine (50 mL). The organic layer was dried with anhydrous Na₂SO₄ before filtration and removal of organic solvent under reduced pressure. The crude residue was then purified by flash chromatography over silica gel using gradient elution; 4:1 to 3:2 n-hexane:ethyl acetate affording a pure mixture of diastereomers in high yield (75-80%). The purified diastereomers were analysed using TLC with various ratios of MTBE: n-hexane. In most cases separation of diastereomers was not observed and thus no further purification was carried out. Diastereomer derivatives are therefore characterised and reported as the diastereomer mixture. A range of proline...
diastereomers were successfully resolved using LC with gradient elution of 4:1 – 1:4 n-hexane: MTBE and are characterized below.

1-(Terz-butyl) 2-(2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (R)-pyrrolidine-1,2-dicarboxylate (107)

107 was synthesised from 17 and N-Boc-D-proline using General Method XII.

Yield: 200 mg (0.36 mmol) 52 %

Appearance: white amorphous powder/ gel

Rf: 0.53 (1:1 n-hexane: ethyl acetate)

^1H NMR (600 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.33-7.29 (m, 2H, H₂-α,β), 6.96-6.9 (m, 2H, H₂-γ,δ), (6.56, 6.55, 6.55, 6.55 (s, 2H), H₂-β,γ), 5.45 (apparent s, 0.2H, H₂), 5.41 (apparent s, 0.2H, H₂), 5.4 (d, 0.16H, J = 1.8 Hz, H₂), 5.4 (d, 0.3H, J = 1.8 Hz, H₂), 5.34 (d, 0.2H, J = 1.8 Hz, H₂), 5.0 (apparent s, 0.3H, H₂), 4.94 (apparent s, 0.3H, H₂), 4.89 (d, 0.2H, J = 1.8 Hz, H₂), 4.86 (apparent s, 0.35H, H₂), 4.42 – 4.37 (m, 1H, H₇-α), (3.85, 3.85, 3.84, 3.83, 3.82, 3.82 (s, 3H, H₆), (3.79, 3.78, (s, 3H, H₇), (3.72, 3.72 (s, 6H), H₈-γδ), 3.66-3.40 (m, 2H, proline ring), 2.36-2.25 (m, 1.5H, proline ring), 2.1 – 2.9 (m, 3H, proline ring), 1.52 (s, 3.5H, t-butyl), 1.47 (s, 2H, t-butyl), 1.45 (s, 3.5H, t-butyl).

^13C NMR (100 MHz, CDCl₃): δ 172.4, 172.3, 172.1, 172.0, 169.7, 161.7, 161.5, 161.4, 161.2, 160.3, 160.2, 160.1, 160.0, 157.7, 154.5, 153.6, 153.5, 153.1, 153.0, 134.9, 134.9, 133.1, 133.1, 133.0, 132.9, 128.0, 127.9, 127.8, 127.7, 127.1, 127.0, 126.8, 114.7, 114.6, 114.6, 114.5, 95.5, 95.4, 95.3, 82.9, 82.8, 82.7, 82.5, 80.4, 80.2, 80.1, 65.9, 63.7, 63.6, 63.5, 63.4, 61.2, 61.0, 59.4, 59.1, 58.8, 58.7, 58.6, 56.3, 56.1, 55.4, 47.3, 46.7, 46.7, 46.4, 43.4, 31.1, 31.0, 29.0, 29.9, 28.5, 28.4, 28.4

^1H NMR (600 MHz, DMSO-d₆): δ 7.44 (m, 1H), 7.41 (m, 1H), 6.97 (m, 2H), 6.56 (s, 0.35H), 6.54 (s, 0.5H), 6.53 (s, 1H), 5.57 (d, 0.2H, J = 1.8 Hz), 5.56 (d, 0.2H, J = 1.8 Hz), 5.51 (d, 0.2H, J = 1.8 Hz), 5.50 (d, 0.2H, J = 1.8 Hz), 5.45 (d, 0.2H, J = 1.8 Hz), 5.52 (apparent s, 0.4H), 5.17 (d, 0.4H, J = 1.8 Hz), 5.13 (apparent s, 0.3H), 4.32-2.29 (m, 1H), 3.75 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 2.32-2.23 (s, 2H), 2.03-1.78 (m, 4H), (1.42, 1.38, 1.35, 1.34 (s, 9H).

^13C NMR (100 DMSO-d₆): δ 174.9, 17.4, 172.4, 172.3, 172.2, 172.0, 169.9, 162.0, 161.6, 161.5, 160.2, 160.1, 160.1, 154.1, 154.0, 154.0, 153.7, 153.2, 134.8, 132.8, 132.8, 132.7, 129.1, 129.0, 129.0, 129.0, 128.9, 127.9, 127.7, 127.6, 127.6, 12.6, 114.8, 96.1, 96.0, 96.0, 92.1, 92.1, 82.3, 82.2, 82.2, 82.1, 79.7, 79.7, 79.1, 65.4, 62.7, 62.6, 62.5, 62.5, 62.4, 61.0, 59.1, 58.8, 58.8, 58.8, 58.7, 58.7, 55.6, 46.9, 46.7, 30.9, 30.8, 29.9, 28.59, 28.5, 28.5, 28.4, 28.4, 24.5, 23.7

HRMS: APCI Calculated for C₂₉H₂₆N₂O₃ [M+ Na⁺] 579.231301, found 579.230838, error + 1.5 ppm

Purity RP-HPLC: 89 -90%
1-(Tert-Butyl) 2-((2S,3S)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (R)-pyrrolidine-1,2-dicarboxylate (107a)

107a was resolved from 107a using LC with a gradient of 4:1 to 1:4 n-hexane: MTBE.

Yield: 30 mg (0.083 mmol) 23.2%

Appearance: white fluffy powder

Rf: 0.33 (4:6 n-hexane: MTBE), plate developed twice

1H NMR (400 MHz, CDCl3): δ (rotamers present) 7.31 (m, 2H), 6.93 (m, 2H), (6.56, 6.56, 6.55, (s, 2H)), 5.41 (d, 0.08H, J = 1.8 Hz), 5.4 (apparent s, 0.25H), 5.4 (d, 0.2H, J = 1.8 Hz), 5.4 (d, 0.3H, J = 1.8 Hz), 5.34 (d, 0.1H, J = 1.8 Hz), 5.0 (apparent s, 0.35H), 4.94 (apparent s, 0.1H), 4.89 (d, 0.2H, J = 1.8 Hz), 4.86 (apparent s, 0.3H), 4.39 (m, 1H), 3.83 (s, 1.7H), 3.81 (s, 1.3H), 3.79 (s, 2.2H), 3.78 (s, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.62-3.4 (m, 2H), 2.35-2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.52 (s, 5H), 1.48 (s, 3H), 1.43 (s, 1H)

13C NMR (100 MHz, CDCl3): δ 172.1, 171.9, 161.5, 161.2, 160.3, 160.0, 154.5, 153.6, 153.5, 153.6, 153.5, 134.9, 133.1, 133.0, 127.1, 126.8, 114.7, 114.6, 95.5, 95.4, 82.8, 82.7, 80.4, 65.9, 63.7, 63.5, 63.4, 63.4, 61.0, 59.4, 59.2, 58.8, 58.5, 56.9, 56.1, 55.4, 46.7, 46.4, 31.1, 31.0, 29.9, 29.7, 28.5, 28.4, 24.6, 23.7

1H NMR (600 MHz, DMSO-d6): δ 7.44 (d, 1.4H, J = 8.9 Hz), 7.42 (d, 0.6H, J = 8.9 Hz), 6.98 (m, 2H), 6.55 (s, 0.7H), 6.52 (s, 1.3H), 5.57 (s, 0.5H, J = 1.8 Hz), 5.51 (s, 0.08H, J = 1.8 Hz), 5.50 (d, 0.2H, J = 1.8 Hz), 5.5 (d, 0.1H, J = 1.8 Hz), 5.22 (d, 0.2H, J = 1.8 Hz), 5.17 (d, 0.7H, J = 1.8 Hz), 4.29 (m, 1H), 3.79 (s, 3H), 3.36 (s, 6H), 3.6 (s, 3H), 2.36-2.25 (m, 2H), 1.98 -1.78 (m, 4H), 1.42 (s, 2.5H), 1.42 (s, 2H), 1.40 (s, 1H), 1.38 (s, 1H), 1.34 (s, 4H), 1.34 (s, 2H)

13C NMR (100, DMSO-d6): δ 172.3, 161.5, 160.1, 153.4, 153.0, 147.6, 146.2, 136.4, 135.0, 132.8, 129.0, 114.71, 102.5, 95.96, 82.3, 79.71, 62.6, 60.48, 59.3, 58.8, 56.31, 55.7, 46.8, 30.6, 28.2, 28.33, 23.68 HRMS: APCI Calculated for C26H23N2O6 [M + H+] 555.23404, found 555.232029, error -5.0 ppm

IR νmax(ATR): 1756 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-D-proline C=O)
Purity RP-HPLC: 85%

1-(Tert-butyl) 2-((2R,3R)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (R)-pyrrolidine-1,2-dicarboxylate (107b)

107b was resolved from 107 using LC with a gradient of 4:1 to 1:4 n-hexane: MTBE.

Yield: not calculated

Appearance: white fluffy powder

Rf: 0.30 (4:6 n-hexane: MTBE), plate developed twice

1H NMR (400 MHz, CDCl3): δ (rotamers present) 7.30 (m, 2H), 6.89 (apparent triplet, 2H, J = 9.6 Hz), 6.56 (s, 2H), 5.4 (apparent s, 0.5H), 5.35 (apparent s, 0.5H), 5.1 (apparent s, 0.1H), 4.93 (apparent s, 0.3H), 4.86 (apparent s, 0.4H), 4.39 (m, 1H), 3.84 (s, 2H), 3.82 (s, 1H), 3.78 (s, 3H), 3.72 (s, 6H), 3.62-3.4 (m, 2H), 2.39 -1.89 (m, 4H), 1.53 (s, 3H), (1.48, 1.47 (s, 3H), 1.43 (s, 5H)

1H NMR (600 MHz, DMSO-d6): δ 7.45 (d, 1H, J = 8.6 Hz), 7.41 (1H, J = 8.9 Hz), 6.97 (d, 2H, J = 8.1 Hz), 6.54 (s, 0.3H), 654 (s, 0.9H), 6.53 (s, 0.8H), 6.55 (d, 0.5H, J = 1.8 Hz), 5.51 (d, 0.6H, J = 1.8 Hz), 5.21 (d, 0.5H, J = 1.8 Hz), 5.17 (d, 0.3H, J = 1.8 Hz), 5.14 (d, J = 0.3H), 4.32 (m, 1H), 3.73 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 2.33-2.16 (m, 3H), 2.04-1.7 (m, 4H), 1.39 (s, 2H), 1.38 (s, 2H), 1.34 (s, 3H), 1.32 (s, 3H).

13C NMR (100 DMSO-d6): δ 174.9, 174.4, 172.3, 172.0, 171.0, 161.7, 154.0 153.7, 153.6, 153.2, 134.9, 132.8, 132.7, 129.6, 129.1, 128.9, 127.6, 127.5, 114.7, 114.6, 96.1, 96.0,
94.0, 92.2, 92.1, 82.2, 82.2, 79.7, 79.7, 78.9, 78.5, 65.4, 63.6, 62.8, 62.4, 60.6, 60.5, 59.1, 58.7, 57.3, 57.1, 56.2, 56.0, 46.8, 46.7, 46.7, 46.6, 37.2, 35.3, 28.5, 28.4, 28.4, 24.9

**IR ν<sub>max</sub>(ATR)**: 1756 (β-lactam C=O) cm<sup>-1</sup>, 1694 cm<sup>-1</sup> (N-Boc-D-proline C=O)

**HRMS**: APCI calculated for C<sub>29</sub>H<sub>33</sub>N<sub>2</sub>O<sub>7</sub>[M - H<sup>+</sup>], 555.234804; found 555.231776, error +0.5 ppm

**ESI** calculated for C<sub>29</sub>H<sub>33</sub>N<sub>2</sub>O<sub>7</sub> [M + Na<sup>+</sup>], 579.231301; found 579.231808, error -0.9 ppm

**Purity RP-HPLC**: 95%

1-(Tert-butyl) 2-(2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (108)

A mixture of diastereomers 108 was synthesised from 17 and N-Boc-L-proline using General Method XII.

**Yield**: 1.08g (1.7 mmol) 70-77%

**Appearance**: white amorphous powder

**R<sub>t</sub>**: 0.53 (1:1 n-hexane: ethyl acetate)

**Melting Point**: 65-69 °C

**¹H NMR (400 MHz, CDCl<sub>3</sub>):** δ 7.32 (doublet, 2H, J = 8.9 Hz, H<sub>2</sub>-κφ'), 6.95 (d, 1.2H, J = 8.9 Hz, H<sub>2</sub>-κϕ'), 6.91 (d, 0.8H, J = 8.9 Hz, H<sub>2</sub>-κφ'), 6.57 (s, 0.7H, H<sub>1</sub>-κɔ'), 6.56 (s, 1.3H, H<sub>1</sub>-κϕ'), 5.45 (d, 0.3H, J = 1.9 Hz, H<sub>4</sub>), 5.41 (apparent s, 0.16H, H<sub>3</sub>), 5.38 (d, 0.3H, J = 1.9 Hz, H<sub>2</sub>), 5.34 (d, 0.2H, J = 1.9 Hz, H<sub>1</sub>), 5.00 (apparent s, 0.25H, H<sub>5</sub>), 4.94 (apparent s, 0.35H, H<sub>4</sub>), 4.86 (apparent s, 0.5H, H<sub>6</sub>), 4.40 (m, 1H, H<sub>3</sub>-ω), 3.87 (s, 1.4H, H<sub>10</sub>), 3.83 (s, 0.1H, H<sub>10</sub>), 3.82 (s, 0.6H, H<sub>9</sub>), 3.79 (s, 1.2H, H<sub>8</sub>), 3.79 (s, 1.7H, H<sub>8</sub>), 3.72 (s, 6H, H<sub>7</sub>-κϕ'), 3.6-3.4 (m, 2H), 2.36-1.9 (m, 4H), 1.53 (s, 3H, t-butyl), 1.47 (s, 2.5H, t-butyl), 1.45 (s, 4.6H, t-butyl)

**¹³C NMR (100 MHz, CDCl<sub>3</sub>):** 172.4, 172.2, 172.0, 171.9, 161.5, 161.2, 160.3, 160.0, 154.5, 153.7, 153.6, 153.5, 135.0, 134.9, 133.1, 133.0, 127.8, 127.0, 126.8, 116.2, 116.4, 114.8, 114.6, 114.4, 112.0, 95.5, 95.3, 82.9, 82.8, 80.4, 80.3, 80.2, 63.7, 63.5, 63.4, 61.0, 59.1, 58.8, 58.6, 56.1, 55.4, 46.7, 46.4, 31.1, 30.1, 29.8, 24.6, 24.5, 23.7

**H NMR (400 MHz, DMSO-d<sub>6</sub>):** δ 7.45 (d, 1.2H, J = 7.8 Hz), 7.40 (d, 0.8H, J = 7.8 Hz), 6.97 (d, 2H, J = 7.8 Hz), 6.54 (s, 0.1H), 6.53 (s, 1H), 5.58 (d, 0.1H, J = 1.7 Hz), 5.56 (d, 0.4H, J = 1.7 Hz), 5.52 (d, 0.3H, J = 1.7 Hz), 5.50 (d, 0.1H, J = 1.7Hz), 5.21 (d, 0.3H, J = 1.7Hz), 5.17 (d, 0.2H, J = 1.7Hz), 5.13 (d, 0.4H, J = 1.7Hz), 4.31 (m, 1H), 3.75 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 3.45 (m, 2H), 2.28 (m, 1H), 2.00 (m, 1H), 1.87 (m, 2H), 1.42 (s, 1H), 1.38 (s, 3H), 1.35 (s, 2H), 1.34 (s, 4H), 1.28 (s, 3H), 1.25 (s, 2H), 1.20 (s, 3H), 1.18 (s, 3H), 1.15 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 1.07 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.81 (s, 3H), 0.79 (s, 3H), 0.77 (s, 3H), 0.75 (s, 3H), 0.73 (s, 3H), 0.71 (s, 3H), 0.69 (s, 3H), 0.67 (s, 3H), 0.65 (s, 3H), 0.63 (s, 3H), 0.61 (s, 3H), 0.59 (s, 3H), 0.57 (s, 3H), 0.55 (s, 3H), 0.53 (s, 3H), 0.51 (s, 3H), 0.49 (s, 3H), 0.47 (s, 3H), 0.45 (s, 3H), 0.43 (s, 3H), 0.41 (s, 3H), 0.39 (s, 3H), 0.37 (s, 3H), 0.35 (s, 3H), 0.33 (s, 3H), 0.31 (s, 3H), 0.29 (s, 3H), 0.27 (s, 3H), 0.25 (s, 3H), 0.23 (s, 3H), 0.21 (s, 3H), 0.19 (s, 3H), 0.17 (s, 3H), 0.15 (s, 3H), 0.13 (s, 3H), 0.11 (s, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H), 0.03 (s, 3H), 0.01 (s, 3H)

**IR ν<sub>max</sub>(ATR):** 1756 cm<sup>-1</sup> (β-lactam C=O), 1694 cm<sup>-1</sup> (N-Boc-D-proline C=O)
1-(Tert-Butyl) 2-((2S,3S)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (108a)

108a was resolved from 108 using LC with a gradient of n-hexane: MTBE

**Yield:** 300mg (0.54 mmol) 30%

**Appearance:** white amorphous powder

**Melting Point:** 66-72°C

**Purity:** >99%

**HRMS:** APCI calculated for C_{29}H_{35}N_{2}O_{9}[M + H]^+; 557.248291; found 557.24937, error -1.9 ppm. ESI calculated for C_{29}H_{35}N_{2}NaO_{9} [M + Na^+]; 579.23239; found 579.2313, error + 1.9 ppm

**1H NMR (400 MHz, CDCl₃):** δ (rotamers present) 7.23 (d, 2H, J = 8 Hz), 6.94 (d, 1H, J = 8.3 Hz), 6.90 (d, 1H, J = 8.3 Hz), 6.55 (s, 1H), 5.4 (apparent s, 0.4H), 5.36 (d, 0.5H, J = 1.3 Hz), 5.0 (apparent s, 0.5), 4.85 (apparent s, 0.5H), 4.38 (m, 1H), 3.83 (s, 1.2H), 3.81 (s, 1.7H), 3.76 (s, 3H), 3.71 (s, 6H), 3.6-3.38 (m, 2H), 2.3-1.9 (m, 4H), 1.51 (s, 6H), 1.46 (s, 6H).

**13C NMR (100 MHz, CDCl₃):** δ 172.4, 172.2, 161.4, 161.2, 160.3, 159.9, 154.5, 153.5, 153.5, 153.4, 134.9, 134.8, 132.9, 130.9, 128.8, 127.8, 127.7, 127.6, 127.0, 127.0, 114.6, 114.4, 95.4, 95.1, 82.8, 82.7, 80.2, 80.2, 63.6, 62.0, 58.6, 58.5, 56.0, 55.4, 55.3, 46.7, 46.4, 31.0, 29.9, 28.4, 24.5, 23.7, 23.6

**IR ν_{max} (ATR):** 1756 cm⁻¹ (β-lactam C=O), 1697 cm⁻¹ (N-Boc-L-proline C=O)

1-(Tert-Butyl) 2-((2R,3R)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (108b)

108b was resolved from 108 using LC with a gradient of n-hexane: MTBE

**Yield:** 350mg (0.63 mmol) 37%

**Appearance:** white amorphous powder

**Melting Point:** 62-65 °C

**Purity:** 78%

**HRMS:** APCI calculated for C_{30}H_{37}N_{2}O_{10}[M + H]^+; 557.248291; found 557.24937, error -1.9 ppm. ESI calculated for C_{30}H_{37}N_{2}NaO_{10} [M + Na^+]; 579.23239; found 579.2313, error + 1.9 ppm
2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (tert-butoxycarbonyl)-L-phenylalaninate (109)

109 was synthesised from 109 and N-Boc-L-phenylalanine using General Method XII.

Yield: 363 mg (0.6 mmol) 60%

Appearance: white fluffy powder

Rf: 0.5 (60:40; n-hexane: ethyl acetate)

Melting point: 72 °C.

1H NMR (400 MHz, CDCl3): δ 7.34-7.2 (m, 7H), 6.94 (d, 2H, J = 8.3 Hz), 6.6 (s, 0.2H), 6.56 (s, 0.8H), 6.53 (s, 1H), 5.4 (apparent s, 0.5H), 5.33 (apparent s, 0.5H), 5 (m, 1H), 4.87 (apparent s, 0.2H), 4.7 (m, 1.6H), 3.83 (s, 3H), 3.8 (s, 3H), 3.73 (s, 6H), 3.18 (s, 2H), 1.34 (s, 4.5H), 1.33 (s, 3H).

13C NMR (100 MHz, CDCl3): δ 171.1, 160.5, 153.6, 153.1, 129.6, 128.7, 127.5, 127.3, 127.0, 126.7, 126.7, 114.6, 95.5, 83.0, 82.8, 80.3, 66.2, 63.5, 63.3, 61.0, 56.1, 55.4, 28.2

Melting point:

Appearance:

Yield:

110 was synthesised from 17 and fmoc-L-phenylalanine using General Method XII.

Yield: 226 mg (0.31 mmol) 31%

Appearance: white fluffy powder

Rf: 0.53 (60:40; n-hexane: ethyl acetate)

Melting point: 77 °C.

1H NMR (400 MHz, CDCl3): δ 7.7 (t, 2H, J = 6.6 Hz), 7.6 (t, 2H, J = 6.6 Hz), 7.41 (m, 2H), 7.32 (m, 5H), 7.2 (m, 2H), 6.93 (apparent d, 2H, J = 8.8 Hz), 6.54 (s, 0.7H), 6.49 (s, 1.3H), 5.44 (apparent s, 0.4H), 5.34 (apparent s, 0.4H), 5.3 (apparent m, 0.4H), 5.23 (apparent m, 0.4H), 4.87 (apparent s, 0.6H), 4.81 (s, 1H), 4.66 (apparent s, 0.5H), 4.47-4.35 (m, 2H), 4.23-4.35 (m, 1H), 3.83 (s, 2H), 3.82 (s, 1H), 3.8 (s, 3H), 3.72 (s, 3.5H), 3.71 (s, 2.5H), 3.23 (m, 2H)

13C NMR (100 MHz, CDCl3): δ 171.8, 168.5, 167.8, 167.0, 164.0, 160.3, 153.5, 141.2, 139.4, 132.9, 129.5, 128.9, 128.0, 127.0, 125.3, 120.0, 114.6, 112.4, 95.4, 66.0, 61.1, 56.0, 55.2, 47.1

1H NMR (400 MHz, DMSO-d6): δ 8.06 (m, 1H, NH), 7.86 (d, 2H, J = 8 Hz), 7.63 (d, 2H, J = 8 Hz), 7.39 (m, 4H), 7.29 (m, 5H), 7.23 (m, 1H), 6.95 (d, 2H, J = 8 Hz), 6.49 (s, 1H), 6.49 (s, 1H), 5.53 (apparent s, 0.6H), 5.3 (apparent s, 0.3H), 5.0 (apparent s, 0.5H), 4.9 (apparent s, 0.5H), 4.4 (m, 1H), 4.31-4.17 (m, 3H), 3.75 (s, 3H), 3.61 (s, 3H), 3.60 (s, 2H), 3.57 (s, 3H), 3.56 (s, 1H), 3.14 (apparent dd, 1H, J = 5 Hz, J = 14 Hz), 3.00 (m, 1H)
1^H NMR (100 MHz, DMSO-d$_6$): δ 171.5, 171.16, 170.8, 161.5, 160.0, 156.4, 153.6, 144.1, 141.2, 137.9, 137.7, 134.8, 132.8, 129.8, 129.6, 128.8, 128.0, 127.5, 127.1, 127.0, 125.6, 121.8, 120.6, 114.7, 95.9, 82.4, 82.3, 66.2, 62.5, 60.5, 60.2, 56.2, 55.7, 55.6, 47.0, 31.2, 31.0

1^C NMR (100 MHz, DMSO-d$_6$): δ 21.2, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8, 16.7, 16.6, 16.5, 16.4, 16.3, 16.2, 16.1, 16.0, 15.9, 15.8, 15.7, 15.6, 15.5, 15.4, 15.3, 15.2, 15.1, 15.0, 14.9, 14.8, 14.7, 14.6, 14.5, 14.4, 14.3, 14.2, 14.1, 14.0, 13.9, 13.8, 13.7, 13.6, 13.5, 13.4, 13.3, 13.2, 13.1, 13.0, 12.9, 12.8, 12.7, 12.6, 12.5, 12.4, 12.3, 12.2, 12.1, 12.0, 11.9, 11.8, 11.7, 11.6, 11.5, 11.4, 11.3, 11.2, 11.1, 11.0, 10.9, 10.8, 10.7, 10.6, 10.5, 10.4, 10.3, 10.2, 10.1, 10.0, 9.9, 9.8, 9.7, 9.6, 9.5, 9.4, 9.3, 9.2, 9.1, 9.0, 8.9, 8.8, 8.7, 8.6, 8.5, 8.4, 8.3, 8.2, 8.1, 8.0, 7.9, 7.8, 7.7, 7.6, 7.5, 7.4, 7.3, 7.2, 7.1, 7.0, 6.9, 6.8, 6.7, 6.6, 6.5, 6.4, 6.3, 6.2, 6.1, 6.0, 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.0

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (tert-butoxycarbonyl)-L-tryptophanate (111)

111 was synthesised from 17 and N-Boc-L-tryptophan using General Method XII.

Yield: 250 mg (0.38 mmol) 59%
Appearance: yellow powder
Rf: 0.35 (1:1 n-hexane: ethyl acetate).

Melting Point: 87-92 °C

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (tert-butoxycarbonyl)-L-tryptophanate (111a)

111a was characterised in CDCl$_3$ at 600 MHz from 111 but was not resolved.
\(^1\)H NMR (600 MHz, CDCl\(_3\)): δ 8.20 (bs, 1H), 7.64 (d, 1H, \(J = 7.8\) Hz), 7.36 (d, 1H, \(J = 8\) Hz), 7.24 (d, 2H, \(J = 8\) Hz), 7.2 (apparent m, 1H), 7.19 (apparent m, 1H), 7.15 apparent m, 1H), 6.91 (d, 2H, \(J = 8\) Hz), 6.54 (s, 2H), 5.41 (apparent s, 1H), 5.1 (d, 1H, \(J = 7.7\) Hz, \(\alpha\)-NH), 4.8 (apparent s, 1H), 4.77 (m, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.72 (s, 6H), 3.39-3.36 (2H), 1.46 (s, 9H).
\(^13\)C NMR (100 MHz, CDCl\(_3\)): δ 171.1, 161.3, 160.2, 155.3, 153.4, 136.1, 135.1, 127.7, 126.8, 123.3, 122.2, 119.8, 118.8, 111.3, 95.3, 82.6, 80.1, 63.3, 60.95, 56.04, 55.34, 54.5, 28.32, 27.69
\(^14\)N NMR (60.8 MHz, CDCl\(_3\)): δ 123.5 (Tryptophan ring NH), 86.3 (\(\alpha\)-NH)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (tert-butoxycarbonyl)-L-tryptophanate (111b)

111b was characterised in CDCl\(_3\) at 600 MHz from 111 but was not resolved.

\(^1\)H NMR (600 MHz, CDCl\(_3\)): δ 8.22 (s, 1H, NH Tryptophan ring), 7.62 (d, 1H, \(J = 7.8\) Hz), 7.36 (d, 1H, \(J = 8.5\) Hz), 7.15 (m, 1H), 7.14 (m, 1H), 7.06 (d, 8 Hz), 7.03 (t, 1H, \(J = 7.3\) Hz), 6.87 (d, 2H, \(J = 8\) Hz), 6.53 (s, 1H), 6.5 (s, 1H), 5.22 (apparent s, 1H), 5.18 (d, 1H, \(J = 7.3\) Hz, \(\alpha\)-NH), 4.79 (m, 1H), 4.37 (apparent s, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.71 (s, 6H), 3.41-3.34 (m, 2H), 1.44 (s, 9H)

\(^13\)C NMR (100 MHz, CDCl\(_3\)): δ 171.2, 161.1, 160.1, 155.2, 153.5, 136.0, 135.0, 127.7, 127.6, 126.9, 123.1, 119.8, 118.7, 114.5, 111.3, 109.7, 95.4, 83.1, 80.1, 63.0, 61.0, 56.0, 55.3, 54.3, 28.3, 27.6

\(^14\)N NMR (60.8 MHz, CDCl\(_3\)): δ 123.6 (Tryptophan ring NH), 86.8 (\(\alpha\)-NH)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (tert-butoxycarbonyl)-L-valinate (112)

112 was synthesised from 17 and N-Boc-L-valine using General Method XII.

**Yield:** 329 mg (0.58 mmol) 58%

**Appearance:** white solid.

**Rt:** 0.38 (20:80; n-hexane: MTBE)

**Melting point:** 78-83 °C

\(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.32 (d, 1H, \(J = 8.3\) Hz), 7.29 (d, 1H, \(J = 8.3\) Hz), 6.95 (d, 1H, \(J = 8.6\) Hz), 6.94 (d, 1H, \(J = 8.6\) Hz), 6.56 (s, 1H), 6.55 (s, 1H), 5.45 (d, 0.6 H, \(J = 1.8\) Hz), 5.35 (apparent s, 0.3H), 5.03 (apparent m, 1, NH), 4.95 (apparent s, 0.6H), 4.88 (apparent s, 0.5H), 4.34 (m, 1H), 3.84 (s, 1.5H), 3.83 (s, 1.5H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.73 (s, 3H), 3.72 (s, 3H), 2.23 (m, 1H), 1.48 (s, 6H), 1.46 (s, 3H), 1.04 (d, 1.5H, \(J = 6.6\) Hz), 1.03 (s, 1.5H, \(J = 6.6\) Hz), 0.98 (d, 1.5H, \(J = 6.6\) Hz), 0.96 (d, 1.5 H, \(J = 6.6\) Hz)

\(^13\)C NMR (100 MHz, CDCl\(_3\)): δ 171.4, 161.2, 160.2, 155.8, 134.8, 131.0, 128.8, 127.8, 126.7, 114.5, 95.3, 83.0, 82.5, 80.1, 65.7, 63.3, 61.06, 58.8, 58.5, 56.1, 55.3, 30.9, 28.2, 19.1, 17.9, 17.4, 15.3

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): δ 7.42 (m, 1H), 7.38 (m, 1H), 6.55 (s, 1.3H), 6.54 (s, 0.7H), 5.55 (apparent m, 1H), 5.89 (apparent s, 0.6H), 5.12 (apparent s, 0.4H), 3.95 (m, 1H), 3.73 (s, 3H), 3.63 (s, 6H), 3.57 (s, 6H), 2.1 (m, 1H), 1.40 (s, 4.5H), 1.38 (s, 4.5H), 0.94 (m, 6H).

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)): δ 1718, 171.6, 162.0, 153.7, 134.8, 132.9, 129.0, 127.7, 114.7, 96.0, 79.0, 62.5, 60.6, 59.8, 56.3, 55.6, 38.7, 30.1, 29.9, 28.7, 28.6, 19.5, 19.4, 19.1, 18.9

**IR** \(\nu_{max}(ATR)\): 1757 (C=O) cm\(^{-1}\)

**HRMS:** ESI calculated for C\(_{29}\)H\(_{38}\)N\(_4\)O\(_8\)[M + Na\(^+\)], 581.246952; found 581.247950, error -1.7 ppm

**Purity RP-HPLC:** 97%
2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (2-(9H-fluoren-9-yl)acetyl)-L-valinate (113)

113 was synthesised from 17 and fmoc-L-valine using General Method XII.

Yield: 370 mg, (0.54 mmol) 54%

Appearance: white solid.

Rf: 0.38 (20:80; n-hexane: MTBE)

Melting point: 78-83 °C

1H NMR (400 MHz, CDCl3): \( \delta \) 7.78 (t, 2H, \( J = 7.6 \) Hz), 7.642 (m, 2H, \( J = 7.6 \) Hz), 6.93 (apparent triplet, 2H, \( J = 8 \) Hz), 6.55 (s, 0.91H), 6.52 (s, 1.1), 5.47 (apparent s, 0.4H), 5.38 (d, 0.4H, \( J = 1.8 \) Hz), 5.30 (apparent s, 0.3H), 5.29 (apparent s, 0.4H), 4.94 (apparent s, 0.5H), 4.88 (apparent s, 0.5H), 4.49-4.36 (m, 3H), 4.25 (m, 1H), 3.83 (s, 1.5H), 3.82 (s, 1.5H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.72 (s, 3H), 3.69 (s, 3H), 2.3 (m, 1H), 1.0-0.98 (m, 6H)

13C NMR (100 MHz, CDCl3): \( \delta \) 176.9, 175.9, 161.5, 153.4, 143.9, 141.4, 133.0, 129.9, 127.1, 120.0, 114.7, 95.5, 95.2, 67.1, 61.1, 60.4, 56.1, 55.4, 47.0, 30.9, 21.8, 21.3

IR \( \nu_{max} \) (ATR): 1730 cm\(^{-1}\) (valine C=O), 1757 cm\(^{-1}\) (\( \beta \)-lactam C=O)

HRMS: ESI calculated for C\textsubscript{39}H\textsubscript{40}NO\textsubscript{9}[M + Na\(^{+}\)], 703.262602; found 703.264394, error +2.5 ppm

Purity RP-HPLC: >90%

Numbering nomenclature for 3- hydroxy \( N \)-Boc-L-proline derivatised diastereomers.

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Chapter 10: Experimental
1-(Tert-butyl) 2-((2S,3S)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (114)

114 was synthesised from 18 and N-(Boc)-L-proline using General Method XII.

Yield: 1.04g (1.81 mmol) 80%

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (diastereomers and rotamers present) 7.11 (m, 2H), 7.00 (m, 1H), 6.55 (s, 0.7H), 6.54 (s, 0.7H), 6.53 (s, 0.6H), 5.40 (d, 0.2H, $J = 1.8$ Hz), 5.36 (d, 0.2H, $J = 1.8$ Hz), 5.34 (d, 0.3H, $J = 1.8$ Hz), 5.31 (d, 0.3H, $J = 1.8$ Hz), 4.99 (apparent s, 0.4H), 4.92 (apparent s, 0.3H), 4.83 (apparent s, 0.3H), 4.83 (apparent s, 0.3H), 4.39 (m, 1H), 3.94 (s, 1H), 3.91 (s, 1H), 3.90 (s, 1H), 3.81 (s, 1H), 3.80 (s, 2H), 3.75 (s, 5H), 3.74 (s, 2H), 3.58- 3.41 (m, 2H), 2.33 – 2.18 (m, 2H), 2.10- 1.89 (m, 2H), 1.53 (s, 2H), 1.47 (m, 2H), 1.43 (s, 6H)

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -132.9, -133.3, -133.4, -133.5

IR $\nu_{\text{max}}$(ATR): 1757 (β-lactam C=O) cm$^{-1}$, 1691 cm$^{-1}$ (N-Boc-L-proline C=O)

1-(Tert-butyl) 2-((2S,3S)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (114a)

114a was resolved from 114 using LC as described in General Method XII.

Yield resolved from diastereomer mixture: 472mg (0.82 mmol) 45%

Appearance: white amorphous powder

Melting Point: 78 °C

$R_f$: 0.36 - 0.39 (2:1 MTBE: n-hexane): plate developed twice
1H NMR (400 MHz, CDCl3): δ (rotamers present) 7.25 (d, 1H, J=7.5 Hz, H2,-80˚), 7.18 (d, 1H, J=7.5 Hz, H3,-80˚), 7.09 (t 1H, J=8 Hz, H5, rotamer 1), 6.95 (t, 1H, J= 8 Hz, H5, rotamer 2), 6.54 (s, 2H, H12,13), 5.36 (s, 0.5 H), 5.34 (s, 0.5H, rotamer 2), 4.99 (s, 0.5H, rotamer 2), 4.83 (s, 0.5H, rotamer 2), 4.38 (m, 1H, H1), 3.91 (s, 1.5H, H5, rotamer 1), 3.89 (s, 1.5H, H5, rotamer 2), 3.79 (s, 3H, H2, 3, 5), 3.73 (s,6H, H7,8,9), 3.50 (m, 2H, proline CH2), 2.27 (m,2H, proline CH2), 1.99 (m, 2H, proline CH2), 1.50 (s, 4.5H, , t-butyl, H8-es10-), 1.47 (s, 9H, rotamer 2, H5-es10-)

13C NMR (100 MHz, CDCl3): δ 161.5, 154.1,154.6, 153.2, 152.7, 152.7, 151.1, 148.0 (m), 134.9, 132.6, 128.4 (m), 124.4 (d, J= 25 Hz), 115.3 (d, J= 25 Hz), 114.5 (d, J= 7 Hz), 96.1 (d, J= 15 Hz), 82.1 (d, J = 13 Hz), 79.7 (d, 5.3 Hz), 62.1, 61.9, 60.6, 58.8 (d, J = 9 Hz), 56.5, 56.3, 46.9, 46.7, 38.7, 30.8, 29.8, 28.5, 28.4, 24.5, 23.7

19F NMR (376 MHz, CDCl3): δ -132.9, -133.2, -133.5

IR νmax (ATR): 1756 (β-lactam C=O) cm⁻¹1690 cm⁻¹ (N-Boc-L-proline C=O).

HRMS: APCI calculated for C20H15FN2NaO [M +H⁺], 575.238645; found 575.239935, error + 2.2 ppm ESI calculated for C20H15FN2NaO [M+Na⁺], 597.221775; found 597.221880, error + 0.2 ppm

Purity (RP-HPLC): 86 %

1-(Tert-butyl) 2-((2R,3R)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (114b)

114b was resolved from 114 using LC described in General Method XII.

Yield resolved from diastereomer mixture: 250mg (0.44 mmol) 24%

Appearance: white powder

Melting Point: 71°C

Rf: 0.31-0.34 (2:1; MTBE: n-hexane), plate developed twice

1H NMR (400 MHz, CDCl3): δ 7.12 (t, 2H, J=7 Hz), 7.18 (m, 1H, J=7.9 Hz), 6.53 (s, 1H, rotamer 1), 6.52 (s, 2H, rotamer 2), 5.38 (apparent s, 0.5H, H5, rotamer 1), 5.31 (s, 0.5H, H5, rotamer 2), 4.91 (s, 0.5H, H4, rotamer 1), 4.82 (s, 0.5H, H4,rotamer 2), 4.39 (m, 1H, H1, 7.5 Hz), 3.92 (s, 1.5H, rotamer 1), 3.9 (s, 1.5H, rotamer 2), 3.79 (s,3H), 3.74 (s,3H), 3.7 (s,3H, rotamer 2), 3.50 (m, 2H, proline CH2), 2.20 (m,2H, proline CH2), 2.05 (m, 2H, proline CH2), 1.40 (s, 9H, H5-es10-)

13C NMR (100 MHz, CDCl3): δ 172.5, 172.3, 161.2, 160.2, 154.6, 153.8, 53.3, 153.7, 153.6, 151.4, 151.3, 148.2, 148.0, 135.3, 135.2, 132.7, 129.9, 128.3, 128.0, 127.7, 127.6, 126.5, 126.2, 125.3, 122.4, 22.4, 117.7, 114.4, 114.3, 113.9, 113.7, 95.5, 95.3, 82.8, 82.6, 82.5, 80.4, 80.2, 64.1, 63.1, 60.9, 58.7, 58.6, 56.3, 56.2, 46.8, 46.4, 46.4, 31.0,30.8, 29.9, 28.5, 28.3, 24.5, 23.6

19F NMR (376 MHz, CDCl3): δ -132.9, -133.11, -133.4, -133.5

IR νmax (ATR): 1757 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-proline C=O).

HRMS: APCI calculated for C20H15FN2NaO [M+H⁺], 575.238613; found 575.239935, error -2.3 ppm.

ESI calculated for C20H15FN2NaO [M+Na⁺], 597.222267; found 597.22188, error -0.6 ppm

Purity (HPLC): 82%

1-(Tert-butyl) 2-((2R,3R)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (115)

115 was synthesised from 67a and N-Boc-L-proline using General Method XII.

Chapter 10: Experimental
Yield: 58%
Re: 0.49 (2:1 MTBE:n-hexane), plate developed once

\( ^1H \) NMR (400 MHz, CDCl3): \( \delta \) (diastereomers and rotamers present) 6.78 (complex m, 3H), 6.52 (s, 1H), 6.51 (s, 1H), 5.39 (d, \( J=1.3 \) Hz, 0.2H), 5.36 (apparent s, 0.18H), 5.34 (d, 0.18H, \( J=1.3 \) Hz), 5.32 (d, 0.24H, \( J=1.3 \) Hz), 5.3 (apparent s, 0.19H), 4.9 (apparent s, 0.2H), 4.8 (apparent s, 0.19H), 4.79 (d, \( J=1.3 \) Hz, 0.18H), 4.77 (apparent s, 0.4H) 4.35 (complex m, 1H, \( H_{1''} \)), 3.79, 3.78, 3.77 (3 X s, 3H), 3.74 & 3.72 (2 X s, 3H), 3.68 (s, 6H), 3.42 (m, 2H), 2.27 (m, 2H), 1.90 (m, 2H), 1.42 & 1.41 (2 X s, 3H, t-butyl (C(CH3)3)), 0.92 & 0.89 (2 X s, 3H, (C(CH3)3)), 0.06 & 0.05 (2 X s, 1.5H, CH3 of TBDMS), 0.04 & 0.03 (2 X S, 1.5H, CH3 of TBDMS)

\( ^13C \) NMR (100 MHz, CDCl3): \( \delta \) 172.1, 172.0, 171.9, 171.8, 170.6, 169.5, 167.7, 161.5, 161.4, 161.2, 161.1, 154.5, 153.6, 153.5, 153.4, 152.4, 151.7, 151.6, 154.1, 151.4, 151.4, 147.5, 145.6, 145.5, 145.4, 144.6, 138.2, 135.0, 134.9, 134.8, 133.0, 132.9, 132.9, 130.9, 130.4, 130.4, 130.3, 127.4, 127.3, 127.1, 120.19, 120.1, 120.0, 119.9, 119.4, 118.9, 118.8, 118.8, 112.4, 112.3, 111.3, 107.6, 95.5, 95.4, 95.3, 83.2, 82.6, 82.5, 82.3, 80.3, 80.2, 80.1, 80.0, 79.9, 63.8, 63.8, 63.5, 63.5, 63.3, 63.1, 61.6, 60.9, 60.8, 59.0, 58.9, 58.8, 58.6, 58.4, 56.0, 56.0, 55.9, 55.8, 55.7, 55.5, 55.4, 46.60, 46.3, 46.3, 31.9, 31.0, 30.8, 29.8, 29.6, 28.4, 28.5, 28.3, 28.2, 25.6, 25.6, 24.4, 23.6, 23.5, 23.5, 20.5, 20.4, 20.4, 18.4, 18.3, 15.1, -4.8, -4.9

1-(Tert-butyl) 2-((2S,3S)-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (116)

115 was deprotected using General Method X to yield a mixture of diastereomers 116.
Yield for desilylation and purification: 82%
Total yield for diastereomer coupling: 42%
Appearance: Yellow/white gel
Re: 0.75 (1:1 ethyl acetate: n-hexane), 0.49 (2:1 MTBE: n-hexane)

\( ^1H \) NMR (400 MHz, CDCl3): \( \delta \) 6.94-6.89 (m, 3H), 6.57 (s, 0.5H), 6.56 (s, 1H), 6.56 (s, 0.7H), 5.45 (s, 0.3H), 0.42 (s, 0.2H), 5.38 (s, 0.25H), 5.36 (s, 0.3H), 4.94 (s, 0.2H), 4.88 (s, 0.4H), 4.81 (s, 0.4H), 4.39 (m, 1H), 3.92 (s, 1.4H), 3.91 (s, 1H), 3.9 (s, 1H), 3.79 (s, 1H), 3.78 (s, 2H), 3.74 (s, 6H), 3.61-3.63 (m, 2H), 2.35-2.24 (m, 2H), 2.10-1.91 (m, 2H), 1.61 (s, 3H), 1.45 (s, 6H)

\( ^13C \) NMR (100 MHz, CDCl3): \( \delta \) 172.1, 172.0, 171.9, 171.8, 170.6, 169.5, 167.7, 161.5, 161.4, 161.2, 161.1, 154.5, 153.6, 153.5, 153.4, 152.4, 151.7, 151.6, 154.1, 151.4, 151.5, 151.4, 150.8, 145.7, 145.6, 145.5, 145.4, 144.6, 138.2, 135.0, 134.9, 134.8, 133.0, 132.9, 132.9, 130.9, 130.4, 130.4, 130.3, 127.4, 127.3, 127.1, 120.19, 120.1, 120.0, 119.9, 119.4, 118.9, 118.8, 118.8, 112.4, 112.3, 111.3, 107.6, 95.5, 95.4, 95.3, 83.2, 82.6, 82.5, 82.3, 80.3, 80.2, 80.1, 80.0, 79.9, 63.8, 63.8, 63.5, 63.5, 63.3, 63.1, 61.6, 60.9, 60.8, 59.0, 58.9, 58.8, 58.6, 58.4, 56.0, 56.0, 55.9, 55.8, 55.7, 55.5, 55.4, 46.60, 46.3, 46.3, 31.9, 31.0, 30.8, 29.8, 29.6, 28.4, 28.5, 28.3, 28.2, 25.6, 25.6, 24.4, 23.6, 23.5, 23.5, 20.5

1-(Tert-butyl) 2-((2S,3S)-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (116a)
116a was resolved from 116 using LC as described in General Method XII.
Yield resolved from diastereomer mixture: 112 mg (0.2 mmol) 20%
Appearance: white powder
Re: 0.26 (plate development 1), 0.56 (plate development 2), (3:1 MTBE : n-hexane), 0.42 (1:1 n-hexane: ethyl acetate)

Chapter 10: Experimental
\[ ^1 \text{H NMR (400 MHz, CDCl}_3): \delta (\text{rotamers present}) 6.93-6.84 (m, 3H), 6.57 (s, 2H), 5.42 (s, 0.45H), 5.38 (s, 0.48H), 4.95 (s, 0.6H), 4.81 (s, 0.4H), 4.38 (s, 1H), 3.92 (s, 2H), 3.90 (s, 1H), 3.78 (s, 3H), 3.74 (s, 6H), 3.61-3.40 (m, 3H), 2.30-2.21 (m, 1H), 2.1-1.9 (m, 3H), 1.52 (s, 6H), 1.47 (s, 3H) \]

\[ ^1 \text{H NMR (600 MHz, DMSO-\text{d}_6):} \delta (\text{rotamers present}) 9.12 (bs, 1H, OH), 9.09 (bs, 1H, OH), 6.94 (apparent t, 1H, J = 7.6 Hz, H_{\text{y}}), 6.89 (d, 1H, J = 8.6 Hz, H_{\text{x}}), 6.84 (d, 1H, J = 9 Hz, H_{\text{z}}), 6.57 (s, 1H, H_{1'\&3'}), 6.55 (s, 1H, H_{1'\&3'}), 5.51, 5.47 (apparent s, 1H, H_2), 5.13, 5.10 (apparent s, 1H, H_3), 4.30 4.30 (m, 1H, H_{10''}), 3.76 (s, 3H, H_{10''}) 3.762 (s, 6H, H_{7'\&9'}), 3.58 (s, 3H, H_2), 2.30 (m, 2H), 1.95 (m, 2H), 1.86 (m, 2H, H_{1''\&2''\&3''}), 1.42, 1.37, 1.30 (s, 9H, H_{6''\&7''\&8''}) \]

\[ ^{13} \text{C NMR (100 MHz, DMSO-\text{d}_6):} \delta 172.3 (C_{5''}), 172.1 (C_{6''}'), 161.6 (C_2), 154.1, 153.6, 153.2 (C_{5'\&6'}'), 148.7, 148.6 (C_{5'}'), 147.7 (C_3'), 134.9 (C_3), 132.58 (C_2), 128.1, 128.01 (C_{1'\&2'}), 118.7, 118.6 (C_{6'''}), 114.23 (C_{5''}), 112.7 (C_{2'}), 96.1, 95.9 (C_{1'\&2'}), 82.3 (C_3), 72.3 (C_{7'\&9'}), 62.7 (C_{1''}), 60.53 (C_6), 56.2 (C_{7'\&9'}), 56.0 (C_{10''}), 46.8, 46.7, 28.3, 24.3, 23.6 \]

IR \( \nu_{\text{max}} \) (ATR): 3356 cm\(^{-1}\) (OH), 1754 cm\(^{-1}\) (\( \beta \)-lactam C=O), 1690 cm\(^{-1}\) (N-Boc-L-proline C=O)

HRMS: APCI calculated for C\(_{35}\)H\(_{43}\)N\(_2\)O\(_{10}\) [M+H\(^{+}\)], 573.24453; found 573.24427, error -0.3 ppm

Purity (RP-HPLC): 88%

1-(Tert-butyl) 2-((2R,3R-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (116b)

116b was resolved from 116 using LC as described in General Method XII.

Yield: 100 mg (0.17 mmol) 17%

Appearance: white powder

R\(_2\): 0.26 (plate development 1), 0.54 [plate development 2 (3:1 MTBE : n-hexane)]

\[ ^1 \text{H NMR (400 MHz, CDCl}_3): \delta (\text{rotamers present}) 9.11 (bs, 1H, OH, H_2), 6.94 (d, 1H, J=8.1, H_{\text{y}}), 6.89 (d, 1H, J=8.1 Hz, H_{\text{x}}), 6.85 (d, 1H, J= 2 Hz, H_{\text{z}}), 6.55 (s, 1H, H_{1'\&3'}), 6.54 (s, 1H, H_{1'\&3'}), 5.51, 5.47 (apparent s, 1H, H_3), 5.13, 5.08 5.04 (apparent s, 1H, H_2), 4.31 (m, 1H, H_{10''}), 3.76 (s, 3H, H_{10''}), 3.64 (s, 6H, H_{7'\&9'}), 3.59 (s, 3H, H_2), 2.29 (m, 2H), 1.99 (m, 2H), 1.84 (m, 2H), 1.39 (s, 3H, H_{1''\&2''\&3''}), 1.34 (s, 6H, H_{8''\&7''\&6''}) \]

\[ ^{13} \text{C NMR (100 MHz, CDCl}_3): \delta 172.3 (C_{5''}), 172.3 (C_{6''}'), 172.192 (C_{6''}), 161.47 (C_2), 154.07, 153.9, 153.3 (rotamers) (C_{5'\&6'}'), 48.6 (C_{6'''}), 147.2 (C_{5''}), 134.9 (C_3), 132.91, 132.83 (C_2), 128.2, 128.1 (C_{1'\&2'}), 118.71, 118.57 (C_{2'}), 114.26, 114.34, 114.28 (C_{1''}), 112.7 (C_{1''}), 96.0 (C_{1'\&2'}), 95.9 (C_{1'\&2'}), 82.2(C_3), 82.2 (C_3), 79.7, 79.7, 62.9, 62.6 (C_4), 62.5 (C_4), 60.5 (C_{10''}), 58.8, 56.3 (C_{7'\&9'}), 56.1 (C_{7'\&9'}), 28.5 (C_{2''}), 28.4 (C_{8''\&10''}), 27.3, 24.5, 23.6 (C_{2''\&3''\&4''}) \]

IR \( \nu_{\text{max}} \) (ATR): 3356 cm\(^{-1}\) (OH), 1754 cm\(^{-1}\) (\( \beta \)-lactam C=O), 1687 cm\(^{-1}\) (N-Boc-L-proline C=O)

HRMS: APCI calculated for C\(_{35}\)H\(_{43}\)N\(_2\)O\(_{10}\) [M+H\(^{+}\)], 573.242595; found 573.244272, error -2.9 ppm

Purity (RP-HPLC): 95%

1-(Tert-butyl) 2-((2R,3R-2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (117)

117 was synthesised from 19 and N-Boc-L-proline using General Method XII.

Yield: 912 mg (1.54 mmol) 61%

Appearance: white amorphous solid

Melting Point: 77-82°C
**1H NMR (400 MHz, CDCl\textsubscript{3}):** \(\delta\) (rotamers and diastereomers present) 7.39 (dd, 1H, \(J = 2.3\) Hz, \(J = 8.6\) Hz, H\(_2\)), 7.27-7.21 (m, 1H, H\(_6\)), 7.96 (apparent triplet, 1H, \(J = 8.0\) Hz, H\(_7\)). 6.54 (s, 0.4H, H\(_{10b}\)), 6.53 (s, 0.6H, H\(_{10a}\)), 6.52 (s, 0.8H, H\(_{1\&3}\)), 5.4 (apparent s, 0.3H, H\(_3\)), 5.38 (apparent s, 0.1H, H\(_6\)), 5.34 (apparent s, 0.2H, H\(_5\)), 5.3 (apparent s, 0.4H, H\(_4\)), 4.97 (apparent s, 0.2H, H\(_4\)), 4.91 (apparent s, 0.4H, H\(_3\)), 4.81 (apparent s, 0.4H, H\(_2\)), 4.39 (m, H\(_{18\&19}\)), 3.92 (s, 1.5H, OCH\(_3\) H\(_6\)), 3.91 (s, 1H, H\(_1\)), 3.90 (s, 0.5H, H\(_8\)), 3.78 (s, 0.6H, OCH\(_3\)), 3.77 (s, 2.4H, H\(_{1\&2}\)), 3.74 (s, 3H, H\(_{16\&17}\)), 3.73 (s, 3H, H\(_{16\&17}\)), 3.6-3.41 (m, 2H, proline CH\(_2\)), 2.35 – 1.89 (m, 4H, proline CH\(_2\)), 1.52 (s, 1.6H, t- butyl), 1.46 (s, 1.2H, t- butyl), 1.44 (s, 3H, t- butyl), 1.43 (s, 4H, t- butyl)

**13C NMR (100 MHz, CDCl\textsubscript{3}):** 172.5, 172.1, 172.0, 161.2, 160.9, 155.7, 155.5, 154.6, 153.6, 153.5, 153.1, 132.8, 128.4, 128.2, 127.9, 126.0, 123.4, 112.4, 112.6, 112.4, 95.5, 95.4, 95.3, 82.8, 82.6, 80.4, 80.2, 63.0, 62.8, 62.7, 61.0, 59.1, 58.8, 58.6, 56.2, 46.6, 46.3, 31.0, 29.8, 28.5, 28.4, 28.4, 27.0, 24.7, 24.6, 23.8, 23.7

**IR \(\nu_{max}\) (ATR):** 1759 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1693 cm\(^{-1}\) (N-Boc-L-proline C=O)

**HRMS:** ESI calculated for C\(_{29}\)H\(_{53}\)Cl\(_5\)N\(_2\)Na\(_2\) [M+Na\(^+\)], 613.192329; found 613.192975, error -1.1 ppm

1-(tert-butyl) 2-(((2S,3S)-2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (117a)

117a was resolved from 117 using LC as described in General Method XII.

**Yield resolved from diastereomer mixture:** 339 mg (0.57 mmol) 37%

**Appearance:** white amorphous solid

**Melting Point:** 75-82 °C

**Rf:** 0.26 (plate development 1), 0.46 (plate development 2), (2:1 MTBE : i-hexane)

**1H NMR (400 MHz, CDCl\textsubscript{3}):** \(\delta\) (rotamers present) 7.39 (s, 1H), 7.32 (dd, 1H, \(J = 8.7\) Hz, \(J = 1.8\) Hz), 6.98 (d, 0.4H, \(J = 8.7\) Hz), 6.94 (d, 0.4H, \(J = 8.7\) Hz), 5.39 (s, 0.4H), 5.35 (s, 0.6H), 4.98 (s, 0.7H), 4.82 (s, 0.4H), 4.39 (m, 1H), 3.93 (s, 1H), 3.91 (s, 2H), 3.83 (s, 2H), 3.81 (s, 3H), 3.74 (s, 6H), 3.63-3.41 (m, 2H), 2.36-2.19 (m, 1H), 2.11 – 1.91 (m, 3H), 1.53 (s, 6H), 1.48 (s, 3H)

**1H NMR (600 MHz, DMSO-d\(_6\)):** \(\delta\) 7.63 (d, 1H, \(J = 2.9\) Hz, H\(_6\)), 7.45 (dd, 1H, \(J = 8.8\) Hz, \(J = 2.9\) Hz, H\(_7\)), 7.17 (t, 1H, \(J = 8.8\) Hz, H\(_8\)), 6.57 (s, 1H, H\(_{10b}\)), 6.49 (s, 1H, H\(_{10a}\)), 5.62, 5.55 (apparent ss, H\(_3\)), 5.21, 5.19 (apparent ss, 1H, H\(_4\)), 4.30 (m, 1H, H\(_{18\&19}\)), 3.86 (s, 3H, H\(_{1\&2}\)), 3.65 (s, 6H, H\(_{16\&17}\)), 3.59 (s, 3H, H\(_5\)), 3.36 (m, 2H), 2.27 (m, 1H), 1.98 (m, H\(_{18\&19}\)), 1.86 (m, 2H, H\(_{1\&2}\)), 1.39 (s, 3H, H\(_{16\&17}\)), 1.36 (s, 6H, H\(_{1\&2}\))

**13C NMR (100 MHz, DMSO-d\(_6\)):** \(\delta\) 172.33 (C\(_{18\&19}\)), 172.21 (C\(_{18\&19}\)), 161.53 (C\(_2\)), 155.31, 155.24 (C\(_{1\&2}\)), 154.1 (C\(_3\)), 153.6 (C\(_{1\&3}\)), 153.1 (C\(_{1\&3}\)), 134.9 (C\(_4\)), 132.6 (C\(_2\)), 129.4, 128.9, 128.8 (C\(_2\)), 127.8, 127.6 (C\(_2\)), 121.7, 121.6 (C\(_7\)), 113.5, 113.4 (C\(_{10b}\)), 96.1 (C\(_{10a}\)), 96.1 (C\(_{1\&2}\)), 82.1, 82.1 (C\(_3\)), 79.8 79.7 (C\(_7\)), 61.9, 61.8 (C\(_6\)), 60.6 (C\(_8\)), 58.9, 58.8 (C\(_{10}\)), 56.6, 56.3 (C\(_{16\&17}\)), 46.9, 46.7 (C\(_{1\&2}\)), 30.9 (C\(_1\)), 28.8, 28.6, 28.4 (C\(_{18\&19}\)), 27.3 (C\(_{1\&2}\)), 24.5, 23.7 (C\(_9\))

**IR \(\nu_{max}\) (ATR):** 1760 cm\(^{-1}\) (β-lactam C=O), 1692 cm\(^{-1}\) (N-Boc-L-proline C=O)

**HRMS:** ESI calculated for C\(_{29}\)H\(_{53}\)Cl\(_5\)N\(_2\)Na\(_2\) [M+Na\(^+\)], 613.192329; found 613.192975, error -1.1 ppm

1-Tert-butyl 2-(((2R,3R)-2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (117b)

117b was resolved from 117 using LC as described in General Method XII.

**Yield resolved from diastereomer mixture:** 112mg (0.19 mmol) 12.2%
**Appearance:** White amorphous powder  
**Melting Point:** 74-77 °C

Rf: 0.24 (plate development 1), 0.37 (plate development 2), (2:1 MTBE : n-hexane)

**1H NMR (600 MHz, CDCl₃):** 8 7.41 (dd, 1H, J = 8 Hz, J₁ = 1.8 Hz), 7.25 (m, 1H), 7.97 (apparent t, 1H, J = 8 Hz), 6.54 (s, 0.6H), 6.53 (s, 1H), 5.41 (d, 0.3H, J₁ = 1.8 Hz), 5.32 (d, 0.4H, J = 1.8 Hz), 4.92 (apparent s, 0.5H), 4.82 (apparent s, 0.4H), 4.40 (m, 1H), 3.96 (s, 1H), 3.92 (s, 2H), 3.80 (s, 3H), 3.74 (s, 2H), 3.73 (s, 4H), 3.62-3.41 (m, 2H), 2.38 - 2.25 (m, 1H), 2.12 - 1.89 (m, 3H), 1.45 (s, 3H), 1.44 (s, 6H)

**IR:** 2857 cm⁻¹

**HRMS:** APCI calculated for C₂₉H₅₃ClN₂O₉ [M+H⁺], 535.145950; found 535.147758.

**ESI** calculated for C₂₉H₅₃ClN₂O₉ [M+Na⁺], 613.193189; found 613.192329, error + 1.4 ppm

1-(*Tert-butyl*-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (118)

118 was synthesised from 20 and N-Boc-L-proline by General Method XII.

**Yield:** 1.16 g (2.04 mmol) 58%

**Appearance:** white amorphous solid

**Melting Point:** 65-67 °C

Rf: 0.35 (2:1 MTBE: n-hexane)

**1H NMR (400 MHz, CDCl₃):** 8 (diastereomers and rotamers present) 7.14 (apparent m, 2H, H), 6.83 (t, 1H, J = 8.1 Hz), 6.57 (s, 0.5H), 6.56 (s, 0.4H), 6.56 (s, 0.4H), 5.48 (d, 0.3H, J = 1.7 Hz), 5.43 (d, 0.3H, J = 1.5 Hz), 5.39 (d, 0.3H, J = 1.5 Hz), 5.36 (d, 0.3H, J = 1.7 Hz), 4.92 (apparent s, 0.4H), 4.89 (apparent s, 0.3H), 4.82 (apparent s, 0.4H), 4.39 (m, 1H, H₁–H), 3.84 (s, 1.2H), 3.83 (s, 0.9H), 3.82 (s, 0.7H), 3.78 (s, 0.9H), 3.78 (s, 2H), 3.72 (s, 3.5H), 3.72 (s, 2.5H), 3.6-3.4 (m, 2H), 2.3-2.0 (m, 4H), 1.46 (s, 1H, t-butyl), 1.43 (s, 8H, t-butyl, H₁ & H₃)

**IR νmax (ATR):** 1757 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (N-Boc-L-proline C=O)

**1-(*Tert-butyl*-2-(2S,3S)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (118a)

118a was resolve from 118 using LC as described in General Method XII.

**Yield resolved from diastereomer mixture:** 430 mg (0.75 mmol) 36.8%

**Appearance:** white fluffy dust

**Melting Point:** 63 °C

Rf: 0.28 (plate development 1), 0.44 (plate development 2), 0.64 (plate development 3), (2:1 MTBE: n-hexane). **Appearance:** white fluffy amorphous solid

**1H NMR (400 MHz, CDCl₃):** 8 (rotamers present) 7.15 (d, 1H, J = 8.6 Hz), 7.12 (d, 1H, J = 8.6 Hz), 6.83 (0.5H, J = 8.6 Hz), 6.79 (d, 0.5H, J = 8.6 Hz), 6.57 (s, 2H, H₁ & H₃), 5.43 (apparent s, 0.4H), 4.95 (apparent s, 0.3H), 4.82 (apparent s, 0.4H), 4.39 (m, 1H, H₁–H), 3.84 (s, 1.2H), 3.83 (s, 0.9H), 3.82 (s, 0.7H), 3.78 (s, 0.9H), 3.78 (s, 2H), 3.72 (s, 3.5H), 3.72 (s, 2.5H), 3.6-3.4 (m, 2H), 2.3-2.0 (m, 4H), 1.46 (s, 1H, t-butyl), 1.43 (s, 8H, t-butyl, H₁ & H₃)

**IR νmax (ATR):** 1757 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (N-(Boc)-L-proline C=O)
0.4H, H3), 5.39 (apparent s, 0.5H, H3), 4.95 (apparent s, 0.5H, H3), 4.82 (apparent s, 0.4H, H3),
4.38 (m, 1H, H7'), 3.84 (s, 1.4H, H6'), 3.82 (s, 1.7H, H6'), 3.78 (s, 3H, H9'), 3.72 (s, 6H, H7, H8, H9,)
3.58-3.38 (m, 2H), 2.36–1.9 (m, 4H), 1.51 (s, 6H, t-butyl, H8' & H9'), 1.47 (s, 3H, H7')
\[^{13}C\text{ NMR (100 MHz, CDCl}_3\]: \(\delta 172.4, 171.9, 161.6, 158.8, 158.2, 153.5, 153.4, 135.0, 133.1,
128.6, 128.4, 126.6, 125.1, 125.0, 110.2, 110.1, 95.5, 95.4, 82.9, 82.8, 80.2, 80.1, 63.8, 60.9, 58.7,
58.5, 56.1, 55.4, 45.6, 46.5, 46.4, 29.9, 28.44, 24.5, 23.5
IR \(\nu_{max}(\text{ATR})\): 1756 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1694 cm\(^{-1}\) (N-(Boc-L-proline C=O)
HRMS: APCI calculated for [M+H\(^+\)], 571.265007 found, 571.265100 error + 0.2 ppm

1-(Tert-butyl) 2-((2R,3R)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-
trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (118b)
118b was resolved from 118 using LC as described in General Method XII.
Yield resolved from diastereomer mixture: 76 mg (0.13 mmol) 6%
Appearance: white fluffy dust
Melting Point: 63 °C
Rf: 0.28 (plate development 1), 0.39 (plate development 2), 0.56 (plate development 3), (2:1
MTBE: n-hexane)
Appearance: white amorphous powder
\[^{1}H\text{ NMR (400 MHz, CDCl}_3\]: \(\delta 7.16\) (apparent m, 2H), 6.83 (apparent triplet, \(J = 8.3\) Hz), 6.57
(s, 1H), 6.56 (s, 1H), 5.48 (d, \(J = 1.7\) Hz, 0.45H), 5.36 (d, \(J = 1.7\) Hz, 0.5H), 4.89 (d, \(J = 1.5\) Hz,
0.47H), 4.83 (d, \(J = 1.5\) Hz, 0.47H), 3.85 (s, 1.7H), 3.84 (s, 1H), 3.79 (s, 3H), 3.73 (s, 3H), 3.73
(s, 3H), 3.73 (s, 3H), 3.65-3.4 (m, 2H), 3.17–3.1 (m, 2H), 2.37-2.26 (m, 2H), 2.19 (s, 9H, t-butyl,
H8''', 8'''&9'''
\[^{13}C\text{ NMR (100 MHz, CDCl}_3\]: \(\delta 171.7, 160.2, 153.4, 146.2, 138.3, 135.8, 134.4, 133.7, 129.4,
127.6, 125.8, 96.4, 92.5, 62.0, 56.5, 55.9, 46.9, 28.9
IR \(\nu_{max}(\text{ATR})\): 1756 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1694 cm\(^{-1}\) (N-(Boc-L-proline C=O)
HRMS: APCI calculated for [M+H\(^+\)], 571.265007 found, 571.264529 error -0.8 ppm

1-(Tert-butyl) 2-((2S,3S)-2-(4-(methylthio)phenyl)-4-oxo-1-(3,4,5-
trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (119)
119 was synthesised from 22 and N-(Boc)-L-proline using General Method XII.
Yield: 290 mg (0.51 mmol) 32%
Appearance: white fluffy powder
Rf: 0.37 (2:1 MTBE: n-hexane)
\[^{1}H\text{ NMR (400 MHz, CDCl}_3\]: \(\delta\) (diastereomers and rotamers present) 7.33 (m, 1H), 7.27 (m,
2H), 6.53 (m, 2H), 5.41 (d, 0.2H), 5.38 (d, 0.2H), 5.36 (d, 0.1H), 5.34 (d, 0.3H), 5.33 (d, 0.2H),
5.0 (apparent s, 0.3H), 4.93 (apparent s, 0.3H), 4.86 (apparent s, 0.4H), 4.38 (m, 1H), 3.78 (s,
1H, OCH_3), 3.77 (s, 3H, OCH_3), 3.72 (bs, 9H, OCH_3), 3.61-3.43 (m, 2H), 2.33 – 1.85 (m, 4H),
1.42 (apparent m, 9H, H8''''-10''''
\[^{13}C\text{ NMR (100 MHz, CDCl}_3\]: \(\delta 171.7, 160.2, 153.4, 146.2, 138.3, 135.8, 134.4, 133.7, 129.4,
127.6, 125.8, 96.4, 92.5, 62.0, 56.5, 55.9, 46.9, 28.9
IR \(\nu_{max}(\text{ATR})\): 1756 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1694 cm\(^{-1}\) (N-(Boc-L-proline C=O)
HRMS: APCI calculated for [M+H\(^+\)], 571.265007 found, 571.264529 error -0.8 ppm

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\[ \text{HRMS: APCI calculated for C}_{29}\text{H}_{30}\text{N}_2\text{NaO}_8\text{S} \ [\text{M+Na}^+], 595.208459 \text{ found}, 595.208878, \text{ error - 0.7 ppm} \]

APCI calculated for C\(_{29}\)H\(_{30}\)KN\(_2\)O\(_8\)S \ [\text{M+K}^+], 611.182395, found 611.183278, error - 1.4 ppm

1-(Tert-butyl) 2-((2R,3R)-2-(4-methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (119b)

119b was resolved from 119 using LC as described in General Method XII.

Yield resolved from diastereomer mixture: 38 mg (0.06 mmol) 12%

Appearance: white fluffy dust

R\(_c\): 0.37 (plate development 1), 0.38 (plate development 2), 0.57 (plate development 3), (2:1 MTBE:n-hexane)

\[ \text{HRMS: APCI calculated for C}_{29}\text{H}_{30}\text{N}_2\text{NaO}_8\text{S} \ [\text{M+Na}^+], 595.208459 \text{ found}, 595.208878, \text{ error +0.3 ppm, APCI calculated for C}_{29}\text{H}_{30}\text{KN}_2\text{O}_8\text{S} \ [\text{M+K}^+], 611.182395, found 611.183392, error +1.6 ppm} \]

2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(tert-butyl) (S)-pyrrolidine-1,2-dicarboxylate (120)

120 was synthesised from 21 and N-Boc-L-proline using General Method XII.

Yield: 810 mg (1.28 mmol) 61%

Appearance: white fluffy dust

Melting Point: 71-74 °C

R\(_c\): 0.54 (three developments, 2:1 MTBE: n-hexane)
**13C NMR (100 MHz, CDCl₃)**: δ 172.4, 172.1, 161.2, 161.4, 160.9, 156.6, 156.3, 154.8, 153.6, 153.5, 132.7, 131.5, 131.3, 128.6, 128.7, 128.3, 126.8, 126.6, 112.5, 112.4, 112.3, 112.2, 112.1, 95.5, 95.3, 95.3, 82.8, 82.7, 80.4, 80.2, 80.1, 65.8, 65.3, 62.9, 62.7, 62.5, 60.9, 59.1, 58.7, 58.55, 56.3, 56.1, 53.7, 46.7, 46.4, 42.0, 41.9, 31.0, 30.9, 30.1, 29.8, 29.1, 28.6, 28.5, 28.4, 28.3, 24.7, 24.6, 23.7, 23.6, 23.4, 23.1, 18.6, 17.3, 15.3

**IR ν max (ATR):** 1756 cm⁻¹ (B-lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

**HRMS:** APCI calculated for C₂₅H₃₆BrN₂O₇ [M+ H⁺], 635.159869 found 635.158822, error + 1.6 ppm

2-((2S,3S)-2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(tert-butyl) (S)-pyrrolidine-1,2-dicarboxylate (120a)

120a was resolved from the mixture of 120 using LC as described in **General Method XII**.

**Yield resolved from diastereomer mixture:** 210 mg (0.33 mmol) 26%

**Appearance:** white fluffy dust

**Melting Point:** 72-74 °C

Rf: 0.17 (plate development 1), 0.24 (plate development 2), 0.54 (plate development 3), (2:1 TBME:n-hexane)

**1H NMR (400 MHz, CDCl₃):** δ (rotamers present) 7.59 (d, 0.1H, J = 1.7 Hz, H₂), (7.57 (d, 0.9H, J = 1.7 Hz, H₂), 7.27 (dd, 1H, J = 9.1Hz, J = 2.1 Hz, H₅), 6.94 (d, 0.2H, J = 9.1Hz, J = 2.1 Hz, H₅), 6.93 (d, 0.2H, J = 9.1Hz, H₅), 6.89 (d, 0.6H, J = 9.1Hz, H₅), 6.54 (s, 2H, H₁ₓy), 5.39 (apparent s, 0.3H, H₁), 5.37 (d, 0.12H, J = 1.9 Hz, H₁), 5.35 (d, 0.5H, J = 1.9 Hz), 4.97 (apparent s, 0.6H, H₁), 4.84 (d, 0.1H, J = 1.9 Hz, H₁), 4.82 (apparent s, 0.3H), 4.39 (m, 1H, H₁), 3.92 (s, 1.5H, H₁), 3.91 (s, 2H, H₁), 3.79 (s, 3H, H₁₀), 3.74 (s, 6H, H₇ₓ₈₉), 3.49 (m, 2H), 1.92 (m, 2H), 1.95 (m, 2H), 1.53 (s, 6H, t-butyl, H₆⁻ₓ₁₀⁻), 1.47 (s, 3H, t-butyl, H₆⁻ₓ₁₀⁻)

**1C NMR (100MHz, CDCl₃):** δ 172.4 (C₆⁻), 172.2 (C₅⁻), 161.1 (C₂, rotamer 1), 160.9 (C₂, rotamer 2), 156.3 (C₄⁻), 154.8 (C₄ₓy), 153.7, 153.6, 135.2 (C₃), 132.7 (C₂), 131.5 (C₂⁻), 131.3, 128.7, 128.4, 126.6 (C₁⁻), 126.5 (C₀⁻), 112.3 (C₃⁻), 112.14, 95.5 (C₁ₓy), 95.3, 82.7, 82.6, 82.4 (C₁), 80.2, 65.3, 62.9 (C₄), 60.95 (C₅), 58.6, 56.3 (C₁₀⁻), 56.2 (C₇ₓ₈₉), 46.7, 46.4, 41.9, 29.8, 29.1, 28.5, 26.9, 24.6

**IR ν max (ATR):** 1756 cm⁻¹ (B-lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

**HRMS:** APCI calculated for C₂₅H₃₆BrN₂O₇ [M+ H⁺], 635.159869 found 635.158775, error - 3.1 ppm

2-((2R,3R)-2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(tert-butyl) (S)-pyrrolidine-1,2-dicarboxylate (120b)

120b was resolved from 120 using LC as described in **General Method XII**.

**Yield recovered from diastereomer mixture:** 100 mg (0.158 mmol) 12%

**Appearance:** white fluffy dust

**Melting Point:** 73-74 °C

Rf: 0.17 (plate development 1), 0.21 (plate development 2), 0.44 (plate development 3), (2:1 TBME: n-hexane)

**Appearance:** white amorphous solid

**1H NMR (400 MHz, CDCl₃):** δ 7.59 (d, 0.5H, J = 1.9Hz, H₂), 7.57 (d, 0.5H, J = 1.9Hz, H₂), 7.32 (dd, J = 8.9 Hz, J = 1.9Hz, H₁), 6.93 (apparent triplet, 1H, J = 8.9 Hz, H₁), 6.54 (s, 0.65H, H₁ₓy), 6.53 (s, 0.9H, H₁ₓy), 5.42 (d, J = 1.8 Hz, 0.43H, H₁), 5.31 (d, J = 1.8 Hz, 0.6H, H₁), 4.92 (apparent s, 0.6H, H₁), 4.82 (apparent s, 0.5H, H₁), 4.39 (m, 1H, H₁₀), 3.93 (s, 1H, H₁), 3.91 (s, 2H, H₁), 3.79 (s, 3H, H₁₀), 3.74 (s, 6H, H₇ₓ₈₉), 3.49 (m, 2H), 1.92 (m, 2H), 1.95 (m, 2H), 1.53 (s, 6H, t-butyl, H₆⁻ₓ₁₀⁻), 1.47 (s, 3H, t-butyl, H₆⁻ₓ₁₀⁻)
3.91 (s, 2H, H₆), 3.8 (s, 3H, H₉), 3.75 (s, 3H, H₇&₈), 3.74 (s, 3H, H₇&₈), 3.58-3.41 (m, 2H), 2.37-2.19 (m, CH₂), 2.1-1.9 (m, 2H, CH₂), 1.45 (s, 3H, H₈), 1.44 (s, 6H, H₆9,10-)

1³C NMR (100 MHz, CDCl₃): δ 172.1, 161.4, 157.0, 154.4 (C₆&₇), 135.5, 133.6, 131.4, 127.1, 113.1 (C₃), 95.5 (C₁&₂), 82.9 (C₃), 80.1, 62.7 (C₄), 61.5 (C₅), 58.6 (C₁₀), 56.7 (C₇&₈), 47.2, 39.9, 30.6, 28.8 (C₅ & C₆), 28.1 (C₉), 24.0, 22.9

IR νₑ₅ₑᵣₑ (ATR): 1756 cm⁻¹ (lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₁₀H₁₀BrN₂O₉ [M+ H⁺], 635.159869 found 635.157875, error - 1.0 ppm

1-(tert-butyl) 2-(2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121)

121 was synthesised from 89 and N-Boc-L-proline using General Method XII.

Yield: 240 mg (0.44 mmol) 44%

Appearance: pale yellow powder

Rₛ: 0.16 (2:1 TBME:n-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.38 (apparent m, 2H, H₁&₂), 7.11 (m, 2H, H₃&₄), 6.52 (s, 0.7H, H₁&₂), 6.51 (s, 0.65H, H₁&₂), 6.51 (s, 0.5H, H₁&₂), 5.39 (d, 0.2H, J = 1.5 Hz, H₃), 5.36 (d, 0.1H, J = 1.5 Hz, H₃), 5.35 (d, 0.06H, J = 1.5 Hz, H₃), 5.34 (d, 0.1H, J = 1.5 Hz, H₃), 5.32 (d, 0.2H, J = 1.5 Hz, H₃), 5.05 (d, 0.2H, J = 0.2Hz, H₄), 4.93 (0.3H, J = 1.4 Hz, H₄), 4.92 (d, 0.06H, J = 1.4 Hz, H₄), 4.89 (apparent s, 0.3H, H₄), 4.39 (m, 1H, H₈), 3.79 (s, 0.8H, H₈), 3.78 (s, 2.2H, H₈), 3.72 (s, 3H, H₉&₁₀), 3.71 (s, 3H, H₉&₁₀), 3.6- 3.3 (m, 2H), 2.43 – 1.87 (4H), 1.43 (apparent m, 9H, t-buty1, H₆9,10-)

¹F NMR (376 MHz): δ -111.6, -111.9, -112.4, -112.5, -113.6, -113.6, -113.7

1-(tert-butyl) 2-((2S,3S)-2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121a)

121a was resolved from 121 using LC as described in General Method XII.

Yield recovered from diastereomer mixture: 120 mg (0.22 mmol) 50%

Appearance: white fluffy amorphous dust

Rₛ: 0.16 (plate development 1), 0.24 (plate development 2), 0.38 (plate development 3), 0.48 (plate development 4), (2:1 MTBE:n-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.36 (m, 2H), 7.13 (t, 0.8H, J = 9.8 Hz), 7.06 (t, 1.3H, J = 9.8 Hz), 6.52 (s, 1.3H, H₁&₂), 6.51 (s, 1H, H₁&₂), 5.37 (apparent s, 0.3H, H₃), 5.35 (d, 0.2H, J = 1.8 Hz, H₃), 5.34 (d, 0.6H, J = 1.8 Hz, H₃), 5.01 (apparent s, 0.7H, H₄), 4.89 (s, 0.4H, H₄), 4.28 (m, 1H H₁&₂), 3.79 (s, 3H, H₈), 3.72 (bs, 6H, H₉&₁₀), 3.6-3.3 (m, 2H), 2.36–1.86 (m, 4H), 1.49 (m, 9H, H₆9,10-)

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 164.3, 161.8, 154.6, 154.0, 134.0, 132.7, 130.8, 128.2, 116.2, 108.7, 107.6, 95.4, 82.6, 81.0, 63.3, 61.0, 58.6, 56.0, 46.8, 46.3, 30.9, 23.9

¹⁹F NMR (376 MHz, CDCl₃): δ -111.6, -111.9, -112.5, -113.6

HRMS: APCI calculated for C₁₀H₁₀BrN₂O₉ [M+ H⁺], 545.229371, found545.229371, error - 1.9 ppm

1-(tert-butyl) 2-((2R,3R)-2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121b)

121b was resolved from 121 using LC as described in General Method XII.

Yield recovered from diastereomer mixture: 80 mg (0.15mmol) 34%

Appearance: white fluffy amorphous dust
Rf: 0.16 (plate development 1), 0.24 (plate development 2), 0.33 (plate development 3), 0.43 (plate development 4), (2:1 TBME: n-hexane)

\( ^1H \) NMR (400 MHz, CDCl\(_3\))\(): \delta 7.40 (m, 2H), 7.09 (m, 2H), 6.52 (s, 0.3H, H\( _{1'&3'} \)), 6.52 (s, 0.3H, H\( _{1'&3'} \)), 6.51 6.52 (s, 1.5H, H\( _{1'&3'} \)), 5.34 (apparent s, 0.4H, H\( _3 \)), 5.31 (d, 0.6H, J = 1.3 Hz, H\( _3 \)), 4.98 (apparent s, 0.4H, H\( _3 \)), 4.89 (apparent s, 0.4H, H\( _3 \)), 4.39 (m, 1H, H\( _{1'&3'} \)), 3.79 (s, 3H, H\( _3 \)), 3.71 (bs, 6H, H\( _{1'&3'} \)), 3.6-3.4 (m, 2H), 2.39-1.88 (m, 4H), 1.5 (s, 3H, \textit{t}-butyl, H\( _{17} \)), 1.43 (s, 6H, H\( _{18,19} \))

\( ^1^C \) NMR (100 MHz, CDCl\(_3\))\(): \delta 172.1, 161.2, 154.7, 153.6, 135.2, 132.7, 128.5, 128.2, 126.4, 117.1, 116.5, 116.2, 114.9, 111.3, 107.6, 95.3, 82.7, 80.3, 63.1, 61.0, 58.9, 56.0, 56.0, 46.5, 30.9, 29.9, 28.3, 23.7, 20.7

\( ^1^F \) NMR (376 MHz): \(-111.6, -112.4, -113.4, -113.5\)

HRMS: APCI calculated for C\(_{28}H\(_{36}\)F\(_2\)N\(_3\)O\(_8\) [M+H\(^+\)], 545.229371 found 545.230533, error - 2.1 ppm

1-(\textit{tert}-butyl)-2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (122)

122 was synthesised from 63 using General Method XII.

Yield: 360mg (0.6 mmol) 60%

Appearance: white fluffy amorphous solid

Rf: 0.15 (2:1 TBME:n-hexane), white fluffy amorphous solid

\( ^1H \) NMR (400 MHz, CDCl\(_3\))\(): \delta \text{(mixed diastereomers and rotamers present) 7.92 (d, 0.1H, } J = 2.9 \text{ Hz, } H\( _0 \text{-rotamer 1} \)), 7.90 (d, 0.7H, } J = 2.9 \text{ Hz, } H\( _0 \text{-rotamer 2} \)), 7.88 (d, 0.2H, } J = 2.9 \text{ Hz, } H\( _0 \)), 7.61 (dd, 0.4H, } J = 7.9 \text{ Hz, } 2.1 \text{ Hz, } H\( _0 \)), 7.55 (dt, 0.6H, } J = 7.9 \text{ Hz, } 2.1 \text{ Hz, } H\( _0 \)), 7.18 – 7.13 (apparent m, 0.7H, H\( _3 \)), 7.11 (d, 0.3H, } J = 8.9 \text{ Hz, } H\( _3 \)), 6.54 (s, 0.4H, H\( _{1'&3'} \)), 6.53(s,0.25H, H\( _{1'&3'} \)), 6.52 (s, 0.3H, H\( _{1'&3'} \)), 6.51 (s, 1H, H\( _{1'&3'} \)), 5.36 (apparent bs, 0.2H, H\( _3 \)), 5.35 (apparent bs, 0.1H, H\( _3 \)), 5.32 (apparent m, 0.3H, H\( _3 \)), 5.29 (d, 0.35H, } J = 1.7 \text{ Hz, } H\( _3 \)), 5.07 (d, 0.2H, H\( _3 \)), 5.01 (d, 0.3H, H\( _3 \)), 4.92 (d, 0.1H, H\( _3 \)), 4.88 (apparent bs, 0.3H, H\( _3 \)), 4.39 (m, 1H, H\( _3 \)), 4.01 (s, 0.8H, OCH\(_3\), H\( _8 \)), 4.00 (s, 0.8H, OCH\(_3\), H\( _8 \)), 3.99 (s, 0.9H, OCH\(_3\), H\( _8 \)), 3.98 (s, 0.6H, OCH\(_3\), H\( _8 \)), 3.81 (s, 1H, OCH\(_3\), H\( _{10} \)), 3.80 (s, 2H, OCH\(_3\), H\( _{10} \)), 3.76 (s, 2H, H\( _{1'&3'} \)), 3.75 (s, 2H, H\( _{1'&3'} \)), 3.75 (s, 2H, H\( _{1'&3'} \)), 3.62-3.40 (m, 2H), 2.37-1.91 (m, 4H), 1.53-1.44 (m, 9H, H\( _{18,19} \))

\( ^1^C \) NMR (100 MHz, CDCl\(_3\))\(): \delta 172.2, 160.8, 154.6, 153.8, 153.8, 153.7, 153.2, 139.9, 132.45, 132.5, 132.4, 131.8, 127.7, 127.3, 124.1, 123.9, 114.6, 114.4, 114.2, 95.6, 95.4, 82.7, 82.5, 80.5, 80.4, 77.3, 72.8, 62.7, 62.1, 59.2, 58.7, 58.6, 56.2, 56.2, 49.5, 49.7, 46.4, 38.7, 31.1, 29.9, 28.4, 28.3, 27.0, 24.8, 24.7

HRMS: APCI calculated for C\(_{28}H\(_{36}\)N\(_3\)O\(_8\) [M+H \(^+\)\)], 600.219882, found 600.219239, error -1.1 ppm

1-(\textit{tert}-butyl)-2-((2S,3S)-2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (122a)

122a was resolved from 122 using LC as described in General Method XII.

Yield resolved from diastereomer mixture: 160 mg (0.27 mmol) 27%

Rf: 0.15 (plate development 1), 0.33 (plate development 2), 0.6 (plate development 3), (2:1 TBME:n-hexane), white fluffy amorphous solid

\( ^1H \) NMR (400 MHz, CDCl\(_3\))\(): \delta \text{(rotamers present) 7.88 (d, 1H, } J = 2.9 \text{ Hz, } H\( _0 \)), 7.54 (d, 1H, } J = 9.9 \text{ Hz, } 2.2 \text{ Hz, } H\( _0 \)), 7.17 (d, 0.3H, } J = 10.8 \text{ Hz, } H\( _0 \)), 7.12 (d, 0.7H, } J = 8.1 \text{ Hz, } H\( _0 \)), 6.54 (s, 1.3H, H\( _{1'&3'} \)), 6.52 (s, 0.8H, H\( _{1'&3'} \)), 5.36 (apparent s, 0.3H, H\( _3 \)), 5.3 (s, 0.8H, H\( _3 \)), 5.07 (s, 0.7H, H\( _3 \)), 4.89 (s, 0.3H, H\( _3 \)), 4.38 (m, 1H, H\( _{1'&3'} \)), 4.0 (s, 1H, H\( _{10} \)), 3.98 (s, 2H, H\( _{10} \)), 3.81 (s, 3H, H\( _8 \)),

Chapter 10: Experimental
3.76 (s, 6H, $H_{7,8''}$), 3.59-3.42 (m, 2H), 2.4-1.89 (m, 4H), 1.52 (s, 6H, $t$-butyl, $H_{8'\sim 10''}$), 1.48 (s, 3H, $H_{9''}$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.8, 172.3, 160.7, 160.4, 154.6, 153.8, 153.7, 153.4, 153.2, 139.8, 132.3, 131.9, 131.7, 127.7, 127.3, 124.1, 123.8, 114.5, 114.1, 95.6, 95.2, 82.7, 82.6, 80.3, 65.8, 62.6, 61.0, 58.6, 56.8, 56.7, 56.4, 46.7, 46.4, 38.7, 31.0, 29.9, 24.6, 23.5, 18.7, 17.6, 15.3

HRMS: APCI calculated for C$_{29}$H$_{34}$N$_{2}$O$_{11}$ [M-H$^-$], 600.219820, found 600.219239, error -1.1 ppm

1-(tert-butyl) 2-((2R,3R)-2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)(S)-pyrrolidine-1,2-dicarboxylate hydrate (122b)

122b was resolved from 122 using LC as described in using General Method XII.

Yield: 100mg (0.17 mmol) 17% TBME:n-hexane, white fluffy amorphous solid

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (rotamers present) 7.92 (d, 0.1H, $J = 1.9$ Hz, $H_{9''}$), 7.90 (d, 0.8H, $J = 1.9$ Hz, $H_{9''}$), 7.6 (dd, 0.6H, $J = 9.9$ Hz, 2.2 Hz, $H_{2''}$), 7.5 (dd, 0.6H, $J = 9.9$ Hz, 2.2 Hz, $H_{2''}$), 7.2 (apparent m, 1H, $H_{7''}$), 6.52 (s, 0.3H, $H_{8''}$), 6.52 (s, 0.6H, $H_{9''}$), 6.51 (s, 0.9H, $H_{1''}$) 5.37 (d, 0.3H $J = 1.5$ Hz, $H_{3''}$), 5.32 (d, 0.15H, $J = 2.2$ Hz, $H_{3''}$), 5.29 (d, 0.4H, $J = 2.2$ Hz, $H_{2''}$), 5.01 (d, $0.5H, J = 2.2$ Hz, $H_{3''}$), 4.92 (d, 0.1H, $J = 2.2$ Hz, $H_{3''}$), 4.88 (apparent s, 0.3H, $H_{4''}$), 4.39 (m, 1Hm $H_{4''}$), 4.05 (s, 0.5H, $H_{10''}$), 4.01 (s, 0.5H, $H_{10''}$), 3.99 (s, 1.5H, $H_{10''}$), 3.80 (s, 3H, $H_{6''}$), 3.76 (s, 3H, $H_{7''}$), 3.75 (s, 3H, $H_{7''}$), 3.67-3.42 (m, 2H), 2.38-1.91 (m, 4H), 1.44 (s, 3H, $t$-butyl, $H_{9''}$), 1.48 (s, 6H, $H_{8'\sim 10''}$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.4, 172.2, 169.9, 160.9, 160.9, 154.7, 153.8, 153.4, 153.5, 153.2, 139.8, 135.6, 135.4, 132.5, 132.3, 132.1, 131.8, 131.7, 127.7, 127.5, 127.3, 124.1, 123.9, 123.8, 114.6, 114.4, 114.3, 95.4, 95.3, 82.7, 82.6, 80.5, 80.3, 62.7, 62.5, 62.1, 61.09, 59.2, 58.7, 56.8, 56.7, 56.2, 56.1, 46.7, 46.4, 31.1, 29.8, 28.4, 28.3, 27.0, 24.8, 23.8, 20.4

Numbering nomenclature for 3-phenolic diastereomers

![Diagram](https://via.placeholder.com/150)

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1-(tert-butyl) 2-((2R,3R)-2-(4-Methoxy-3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)(S)-pyrrolidine-1,2-dicarboxylate hydrate (134)

134 was synthesised from 68 and N-Boc-L-Proline using General Method XII.

Yield: 80%

$R$: 0.25 (2:1 n-hexane: ethyl acetate), 0.83 (2:1 ethyl acetate: n-hexane)
\textbf{\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6):} \delta 7.45 - 7.32 (apparent m, 3H), 7.17 (d, 2H, \textit{J} = 8 Hz), 6.95 (d, 1H, \textit{J} = 7.9 Hz), 6.62 (s, 2H, H\textsubscript{1'&3'}), 5.29 (d, 0.6 H, \textit{J} = 1.7 Hz), 5.27 (d, 0.4H, \textit{J} = 1.7 Hz), 4.55 (d, 1H, \textit{J} = 1.7 Hz), 4.14 (m, 1H), 3.84 (s, 3H), 3.67 (s, 6H, H\textsubscript{7'&9'}), 3.59 (s, 3H), 3.4 - 3.2 (m, 2H), 2.15 - 1.83 (m, 4H), 1.43 (s, 3H), 1.39 (s, 6H, t-butyl)

\textbf{\textsuperscript{19}F NMR (376 MHz, DMSO-\textit{d}_6):} -134.33

1-(tert-butyl) 2-(4-(2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl) (2S)-pyrrolidine-1,2-dicarboxylate hydrate (135)

135 was synthesised from 69 and \textit{N}-Boc-L-proline using General Method XII.

\textbf{Yield:} 71 %

\textbf{Rf:} 0.56 (1:1 \textit{n}-hexane: ethyl acetate)

\textbf{\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6):} \delta 7.49 (d, 2H, \textit{J} = 8.3 Hz), 7.45 (d, 1H, \textit{J} = 7.8 Hz), 7.16 (apparent doublet, 1H, \textit{J} = 8.3 Hz), 7.16 (d, 1H, \textit{J} = 1.8 Hz, H\textsubscript{6''}), 6.99 (d, 2H, \textit{J} = 8.3 Hz), 6.6 (s, 2H, H\textsubscript{7'&9'}), 5.27 (d, 0.6H, \textit{J} = 1.7 Hz), 5.25 (apparent s, 0.4H), 4.5 (apparent s, 1H), 4.42 (m, 1H), 3.76 (s, 3H), 3.65 (s, 6H), 3.58 (s, 3H), 2.41 - 2.2 (m, 2H), 2.16 - 1.86 (m, 4H), 1.42 (s, 3H, t-butyl), 1.34 (s, 6H, t-butyl)

\textbf{\textsuperscript{19}F NMR (376 MHz, DMSO-\textit{d}_6):} -134.33

4-(2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl(tert-butoxycarbonyl)-L-phenylalaninate (136)

136 was synthesised from 68 and \textit{N}-Boc-L-phenylalanine using General Method XII.

\textbf{Yield:} 250 mg (0.37 mmols) 81.6 %

\textbf{Appearance:} yellow oil

\textbf{Rf:} 0.66 - 0.71 (1:1 \textit{n}-hexane: ethyl acetate)

\textbf{\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6):} \delta 7.58 - 7.13 (complex m, 11H, Ar-H), 7.03 (dd, 1H, \textit{J}= 2.2 Hz, 8.6 Hz, H\textsubscript{2'}), 6.6 (s, 2H, H\textsubscript{1'&3'}), 5.28 (apparent s,1H, H\textsubscript{3}), 4.53 (apparent s, 1H, H\textsubscript{4}), 4.38 (m, phenylalanine CH), 3.84 (s, 3H, H\textsubscript{10'}), 3.67 (s, 6H, H\textsubscript{7'&9'}), 3.59 (s, 3H, H\textsubscript{8'}), 3.07 (apparent m), 1.37 (s, 9H, t-butyl)
Numbering nomenclature for B ring *meta* hydroxyl proline esterified diastereomers

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2-(5-(3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (2S)-pyrrolidine-1,2-dicarboxylate (123)

123 was synthesised from 67b and N-Boc-L-proline using General Method XII.

Yield: 380mg (0.62 mmol) 65%

Appearance: white fluffy dust

Rf: 0.2 (2:1 TBME: n-hexane)

1H NMR (400 MHz, DMSO-d6): δ (diastereomers and rotamers present) 7.41 (m, 1H), 7.28 (d, J 6-2 = 2.2 Hz, 0.15H, H6'), 7.24 (d, J 6-2 = 2.2 Hz, 0.2H, H6'), 7.19 (dd, 1H, J2-6 = 2.2 Hz, J2-3 = 7.6 Hz, H2'), 7.16 (2Xd, 1H, J3-2 = 7.6 Hz, H3'), 6.53 (s, 1H, H1', 8''), 6.52 (s, 1H, H1'), 5.51 (d, J3-4 = 2 Hz, 0.05H, H3'), 5.48 (d, J5-4 = 2 Hz, 0.2H, H5'), 5.45 (m, J5-4 = 0.5H, H5'), 5.29 (d, J3-2 = 2 Hz, 0.3H, H3'), 5.26 (d, J5-3 = 2 Hz 0.35H, H5'), 5.24 (m, 0.35H, H1'), 4.43 (m, 1H, H3'), 3.77 (s, 2H, H2'), 3.76 (s, 1H, H1'), 3.64 (s, 2H, H7', 8), 3.62 (s, 2H, H7', 8), 3.61 (s, 2H, H7', 8), 3.58 (s, 3H, H5), 4.49 – 3.8 (m, 2H), 2.38 – 1.7 (m, 4H), 1.4 (s, 1.5H), 1.39 (s, 1.5H), 1.34 (s, 6H, t-butyl), 1.29 (s, 3H, t-butyl)

13C NMR (100 MHz, DMSO-d6): δ 171.1, 170.9, 165.3 (C2), 161.9, 161.8, 154.0, 153.9, 153.3, 151.6, 151.5, 139.6, 139.6, 139.5, 134.8, 132.2, 131.9, 130.1, 128.9, 128.5, 128.4, 126.5, 126.3, 126.2, 125.5, 122.4, 122.2, 121.8, 121.5, 120.3, 115.9, 115.6, 113.8, 113.7, 95.9, 95.89, 95.84, 82.2, 81.2, 41.9, 81.8, 79.6, 79.5, 66.1, 66.1, 62.0, 60.5, 58.9, 58.8, 56.6, 56.4, 56.2, 46.9, 46.7, 38.7, 31.0, 28.5, 28.3, 28.2, 24.4, 23.6, 20.7

HRMS: APCI calculated for C31H30N2O11 [M+ H+], 613.240284; found 613.23886, error + 2.3 ppm

2-(5-((2S,3S)-3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (2S)-pyrrolidine-1,2-dicarboxylate (123a)

123a was resolved from 123 using LC as described in General Method XII.

Yield resolved from diastereomer mixture: 100mg (0.16 mmol) 26%

Appearance: white fluffy dust

Rf: 0.2 (plate development 1), 0.45 (plate development 2) (2:1 TBME: n-hexane)

1H NMR (600 MHz, DMSO-d6): δ 7.41 (apparent triplet, J = 2.3 Hz, 0.5H, H6'), 7.39 (apparent triplet, J = 2.3 Hz, 0.5H, H6'), 7.39 (d, 0.3H, J = 2.8 Hz, H6'), 7.25 (apparent triplet, 0.5H, J = 2.3 Hz), 7.19 (m, 1H)), 7.16 (d, 0.7H, J = 8.1 Hz, H6'), 6.53 (s, 1.4H, H1', 8''), 6.52 (s, 0.6H, H1', 8''), 5.51 (d, J = 1.6 Hz, 0.3H, H5), 5.48 (apparent m, 0.2H), 5.45 (d, J = 1.6 Hz, 0.5H, H5), 5.29 (d, J = 1.6 Hz, 0.4H, H5), 5.27 (d, J = 1.6 Hz, 0.4H, H5), 3.77 (s, 2H, H2'), 3.76 (s, 1H, H1'), 3.63 (s, 6H, H7', 8), 3.56 (s, 3H, H3), 2.17 (s, OOCCH)

13C NMR (100 MHz, DMSO-d6): δ 171.1 (C5''), 170.9 (C5''), 169.9, 161.9 (C2), 154.6 (C4'', 5''), 151.6 (C2''), 139.5 (C3''), 134.8 (C7''), 132.8 (C2''), 128.4 (C1'' 126.3 (C2''), 121.8 (C6''), 113.8 (C3''), 95.8 (C1'', 8), 82.0 (C), 81.8 (C), 79.6, 79.3, 62.0 (C3), 60.6 (C2), 58.9, 56.7 (C7'', 8), 56.4 (C2), 56.2 (C13), 46.9, 46.6, 30.9, 28.6, 28.2

HRMS: APCI calculated for C31H30N2O11 [M+ H+], 613.240284; found 613.23886, error + 2.3 ppm

2-(5-((2R,3R)-3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (2S)-pyrrolidine-1,2-dicarboxylate (123b)

123b was resolved from 123 using LC as described in General Method XII.

Yield resolved from diastereomer mixture: 80mg (0.13 mmol) 21%

Appearance: white fluffy dust

Rf: 0.2 (plate development 1), 0.39 (plate development 2) (2:1 TBME: n-hexane)
1H NMR (600 MHz, DMSO-d$_6$): δ (rotamers present) 7.41 (d, 0.25H, $J=2.4$ Hz, H$_e$), 7.40 (d, 0.3H, $J=2.4$ Hz, H$_a$), 7.38 (d, 0.15H, $J=2.4$ Hz, H$_c$), 7.28 (d, 0.1H, $J=2.1$ Hz, H$_b$), 7.25 (d, 0.25H, $J=2.1$ Hz, H$_b$), 7.19 (d, $J=8$ Hz, 1H, H$_e$), 7.16 (dd, 1H, $J=7.3$ Hz, $J=2.1$ Hz, H$_c$), 6.53 (s, 0.8H, H$_{1\&3}$), 6.52 (s, 1.2H, H$_{1\&3}$), 5.51 (d, 0.1H, $J=1.8$ Hz, H$_b$), 5.48 (d, 0.3H, $J=1.8$ Hz, H$_a$), 5.49 (d, 0.6H, $J=1.8$ Hz, H$_c$), 5.29 (d, 0.2H, $J=1.8$ Hz, H$_d$), 5.26 (d, 0.5H, $J=1.8$ Hz, H$_e$), 5.25 (d, 0.3H, $J=1.8$ Hz, H$_f$), 4.42 (m, 1H, H$_{10}$), 3.78 (s, 2H, H$_{30}$), 3.77 (s, 1H, H$_{40}$), 3.64 (s, 2H, H$_{40}$), 3.63 (s, 3H, H$_s$), 3.62 - 3.33 (m, 2H), 2.39 - 2.26 (m, 2H), 1.94 - 1.85 (m, 2H), 1.39 (s, 3H, H$_{10''}$), 1.29 (s, 6H, $\delta^4_{-8}$)

13C NMR (100 MHz, DMSO-d$_6$): δ 177.0, 170.9, 169.9, 165.1, 161.9, 154.0, 153.6, 153.3, 151.6, 161.5, 139.6, 139.5, 133.4, 130.4, 130.3, 129.9, 128.3, 128.2, 122.3, 121.5, 113.8, 95.9, 95.8, 82.1, 81.9 79.57, 62.1, 62.0, 59.0, 56.5, 56.2, 55.9, 46.9, 46.7, 40.5, 40.4, 39.9, 28.5, 28.3, 28.2, 20.7

HRMS: APCI calculated for C$_{31}$H$_{37}$N$_2$O$_{11}$ [M+H$^+$/], 613.240284; found 613.239433 error + 1.4 ppm

1-(Tert-buty1)-2-(2-methoxy-5-(4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (124)

124 was synthesised from 24 and N-Boc-L-proline using General Method XII.

Yield: 363 mg (0.57 mmol) 57%

Melting Point: 77-82 °C

1H NMR (400 MHz, CDCl$_3$): δ (diastereomers and rotamers present) 7.44 - 7.33 (m, 5H), 7.30 - 7.25 (m, 2H), 7.23 (d, 0.3H, $J=2.1$ Hz, H$_c$), 7.19 (d, 0.3H, $J=2.1$ Hz, H$_b$), 7.08 (apparent triplet, 0.6H), 7.01 (apparent triplet, 1H), 6.61 (s, 2H, H$_{1\&3}$), 4.86 (m, 1H, H$_e$), 4.59 (m, 0.4H, H$_{10}$), 4.34 (m, 0.6H, H$_{10}$), 4.31 (d, 0.2H, $J=1.9$ Hz, H$_d$), 4.30 (d, 0.2H, $J=1.9$ Hz, H$_a$), 4.29 (apparent s, 0.6H, H$_a$), 3.86 (s, 1.6H, H$_b$), 3.85 (s, 1.1H, H$_e$), 3.84 (s, 0.5H, H$_c$), 3.79 (s, 3H, H$_s$), 3.73 (s, 6H, H$_{30}$), 3.66 - 3.4 (m, 2H), 2.4 - 1.9 (m, 4H), 1.5 - 1.39 (5X s, 9H, H$_{10''-11''}$)

13C NMR (100 MHz, CDCl$_3$): δ 170.9, 170.8, 169.1, 165.5, 165.4, 153.7, 153.6, 153.5, 151.4, 140.2, 134.6, 133.6, 130.1, 129.5, 128.0, 127.9, 127.4, 124.4, 124.1, 121.5, 121.3, 120.4, 120.2, 113.1, 113.0, 97.6, 94.8, 80.1, 79.9, 72.8, 65.1, 64.9, 63.44, 61.0, 59.0, 58.99, 56.0, 55.9, 49.5, 46.7, 46.4, 31.1, 30.9, 30.0, 28.4, 28.3, 27.0, 24.4, 23.6

IR $\nu_{max}$(ATR): 1751 cm$^{-1}$ (β-lactam C=O), 1693 cm$^{-1}$ (N-Boc-L-proline C=O)

1-(Tert-buty1)-2-(2-methoxy-5-((2R,3S)-4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (124a)

124a was resolved from 124 using LC as described in General Method XII.

Yield: 93 mg (0.15 mmol) 26%

Appearance: white fluffy dust

Melting Point: 73-77 °C

1H NMR (600 MHz, DMSO-d$_6$): δ 7.38 (m, 6H), 7.21 (d, 2H, $J=12$ Hz), 6.61 (s, 2H, H$_{1\&3}$), 5.30, 4.95, (1H, apparent s, H$_e$), 4.52, 4.45 (1H, apparent s, H$_c$), 4.41 (m, H$_{10}$), 3.78, 3.77 (s, 3H, H$_b$), 3.64 (s, 6H, H$_{30}$), 3.59 (s, 3H, H$_{10}$), 1.40 (s, 3H, H$_{10''}$), 1.34 (s, 6H, H$_{10''-11''}$)

13C NMR (100 MHz, DMSO-d$_6$): δ 171.2, 170.8, 165.8, 165.7, 153.9, 153.6, 153.3, 151.4, 148.5, 147.4, 139.7, 139.7, 139.6, 135.2, 134.4, 133.4, 130.4, 130.3, 129.9, 128.3, 128.2, 128.0, 125.9, 122.0, 121.3, 114.1, 114.0, 95.5, 93.9, 79.6, 79.5, 64.1, 63.9, 61.8, 61.7, 60.5, 59.0, 58.9, 56.5, 56.4, 56.1, 46.9, 46.7, 31.3, 30.0, 28.5, 28.4, 28.3, 27.3, 24.4, 23.6

HRMS: APCI calculated for C$_{30}$H$_{38}$N$_2$O$_{10}$ [M+Na$^+$], 655.26202; found 655.262713, error -0.2 ppm

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IR ν_max(ATR): 1750 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-proline C=O)

1-(Tert-butyl) 2-(2-methoxy-5-(253R)-4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (124b)

124b was resolved from 124 using LC as described in General Method XII.

Yield: 74 mg (0.12 mmol) 21 %

Appearance: white fluffy dust

Melting Point: 66-71 °C

¹H NMR (600 MHz, DMSO-d₆): δ 7.46-7.40 (m, 3H), 7.37-7.32 (m, 4H), 7.21 (m, 2H), 7.20 (m, 2H), 6.60 (2 X s, 2H, H₃), 5.30 (broad apparent s, 1H, H₁), 4.48 (apparent s, 0.3H, H₄), 4.46 (apparent s, 0.6H, H₅), 4.42 (m, H₁′−), 3.78 (s, 1.5H, H₆), 3.77 (s, 1.5H, H₇), 3.65 (s, 6H, H₈), 3.64 (s, 3H, H₉), 3.58 (s, 3H, H₁₀), 1.42 (2H), 1.38 (3H), 1.33 (1H), 1.29 (3H)

¹³C NMR (100 MHz, DMSO-d₆): δ 171.2, 170.9, 169.4, 165.7, 165.6, 154.0, 153.6, 153.3, 153.2, 151.4, 139.8, 139.7, 136.4, 135.8, 134.4, 133.4, 130.4, 129.6, 129.4, 128.8, 128.0, 127.0, 126.1, 125.9, 121.7, 121.3, 121.0, 114.1, 113.9, 97.2, 95.5, 95.5, 79.6, 79.5, 64.1, 63.9, 61.8, 61.7, 60.6, 60.5, 59.0, 58.9, 56.5, 56.2, 56.1, 49.2, 46.9, 43.9, 31.4, 30.9, 29.9, 28.5, 28.3, 27.3, 24.4, 23.6, 23.6, 22.5

IR ν_max(ATR): 1752 cm⁻¹ (β-lactam C=O), 1692 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₅₃H₅₈N₂O₅ [M + Na⁺], 655.26202; found 655.262619, error - 0.0 ppm

1-(Tert-butyl) 2-(2-methoxy-5-(4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (2S)-pyrrolidine-1,2-dicarboxylate (125)

125 was synthesised from 27 and N-Boc-L-proline using General Method XII.

Yield: 724 mg (1.27 mmol) 53 %

Appearance: white/yellow fluffy dust

Melting Point: 75 °C

Rf: 0.26 (3:1 TBME:n-hexane)

¹H NMR (600 MHz, CDCl₃): δ 7.26 (dd, 0.7H, J₂-₃= 2.5 Hz, J₁₂-₁₃= 9.7 Hz, H₁₂), 7.22 (dd, 0.35H, J₂-₃ = 2.5 Hz, J₁₂-₁₃= 9.7 Hz, H₁₂), 7.20 (d, 0.25H, J₁₂-₁₃= 2.5 Hz, H₁₂), 7.18 (d, 0.25H, J₁₂-₁₃ = 2.5 Hz, H₁₂), 7.05 (d, 0.5H J₁₂-₁₃= 2.5 Hz, H₁₂), 6.99 (d, 0.5H, J₁₂-₁₃ = 2.5 Hz, H₁₂), 6.97 (d, 1H, J₁₂-₁₃ = 9.7 Hz, H₁₂), 6.54 (d, 2H, H₁₃), 4.93 (m, 0.5H, H₃), 4.90 (m, 0.5H, H₃), 4.58 (m, 0.5H, H₃), 4.48 (m, 0.5H, H₃), 3.88 (s, 2H), 3.84 (s, 1H), 3.78 (s, 3H), 3.74 (s, 1H), 3.74 (s, 5H), 3.52 (m, 4H, proline CH₂ & H₂), 3.0 (dd, 0.5H, J₁₃₁₄= 2.9 Hz, J₁₃₁₅ = 14 Hz, H₁₃) 2.9 (dd, 0.5H, 1H, J₁₃₁₄= 2.9 Hz, J₁₃₁₅ = 14 Hz, H₁₃)

Chapter 10: Experimental
Chapter 10: Experimental

1-(Tert-butyl) 2-(2-methoxy-5-((S)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)
(S)-pyrrolidine-1,2-dicarboxylate (125a)

d125a was resolved from 125 LC described in General Method XII.

Yield resolved from diastereomer mixture: 106 mg (0.19 mmol) 15%

Appearance: white fluffy amorphous dust

Melting Point: 75 ºC

Rf: 0.26 (plate development 1), 0.44 (plate development 2), 0.56 (plate development 3), (4:1 TBME:n-hexane)

1H NMR (600 MHz, DMSO-d6): δ (rotamers present) 7.40 (dd, 0.4H, J2''-6'' = 2.5 Hz, J2''-3'' = 9.7 Hz, H2''), 7.38 (dd, 0.6H, J2''-6'' = 2.5 Hz, J2''-3'' = 9.7 Hz, H2''), 7.20 (d, 0.3H, J6''-2'' = 2.5 Hz, H6''), 7.21 (d, 0.1H, J6''-2'' = 2.5 Hz, H6''), 7.18 (d, 0.3H, J6''-2'' = 9.7 Hz, H6''), 7.16 (d, 0.2H, J2''-6'' = 9.7 Hz, H2''), 7.14 (d, 0.3H J3'-2' = 9.7 Hz, H3''), 7.13 (d, 0.2H, J1''-6'' = 9.7 Hz, H1''), 6.53 (s, 0.45H, H1''&3''), 6.52 (s, 1.1H, H1''&3''), 5.19 (m, 0.4H, H1''&3''), 4.42 (dd, 1H, J2''-6'' = 9 Hz, J3''-4'' = 4 Hz, H3''), 3.77 (s, 2H), 3.75, (s, 1H), 3.63 (s, 3H), 3.57 (s, 3H), 3.40, (m, 1H, H3''), 2.97 (dd, 0.4H, J2''-6'' = 2.9 Hz, J3''-4'' = 14 Hz, H2''), 2.91 (dd, 0.6H, J3''-4'' = 2.9 Hz, J3''-4'' = 14 Hz, H3''), 2.3-2.27 (m, 2H), 1.9-1.85 (m, 2H), 1.42 (s, 2H, t-butyl), 1.38 (s, 1H t-butyl), 1.34 (s, 4H, t-butyl), 1.29 (s, 2H, t-butyl)

13C NMR (100 MHz, DMSO-d6): δ 171.2, 164.8, 153.6, 153.3, 151.2, 151.1, 139.7, 134.2, 134.0, 131.4, 125.9, 125.5, 122.2, 122.0, 121.6, 114.0, 113.9, 79.6, 79.4, 60.5, 59.0, 58.9, 56.4, 56.2, 52.9, 46.9, 46.7, 46.2, 38.7, 31.0, 29.9, 28.6, 28.3, 28.0, 24.4, 23.5

IR νmax (ATR): 1749 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-Proline C=O).

HRMS: APCI calculated for C20H36N2NaO6 [M + Na⁺], 579.2321303 found 579.231643, error + 0.6 ppm

1-(Tert-butyl) 2-(2-methoxy-5-((S)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)
(S)-pyrrolidine-1,2-dicarboxylate (125b)

125b was resolved from 125 using LC described in General Method XII.

Yield: 100 mg (0.17 mmol) 14%

Appearance: white fluffy dust

Melting Point: 75 ºC

Rf: 0.26 (plate development 1), 0.41 (plate development 2), 0.50 (plate development 3) (4:1 TBME:n-hexane); plate developed three times

1H NMR (600 MHz, DMSO-d6): δ (rotamers present) 7.40 (dd, 0.6H, J = 2.7 Hz, J = 8.4 Hz, H2''), 7.37 (dd, 0.4H, J = 2.7 Hz, J = 8.4 Hz, H2''), 7.26 (d, 0.2H, J = 1.7 Hz, H6''), 7.20 (d, 0.2H J = 1.8 Hz, H6''), 7.19 (d, 0.65H, J = 9 Hz, H6''), 7.16 (d, 0.4H, J = 9 Hz, H6''), 7.14 (d, 0.2H, J = 9 Hz, H6''), 7.13 (d, 0.45H, J = 9 Hz, H6''), 6.52 (s, 1H, H1''&3''), 6.51 (s, 1H, H1''&3''), 5.17 (m, 1H, H1''), 4.41 (dd, 1H, J3''-4'' = 10.3 Hz, J3''-4'' = 4 Hz, H3''), 3.77 (s, 2H), 3.76 (s, 1H), 3.64 (s,1.5H), 3.63 (s, 1.5H), 3.63 (s, 3H), 3.60 (s, 1.2H), 3.56 (s, 1.8H), 3.35, (m, 1H, H3''), 2.97 (dd, 0.5H, J3''-4'' = 2.9 Hz, J3''-4'' = 14 Hz, H3''), 2.91 (dd, 0.5H, J3''-4'' = 2.9 Hz, J3''-4'' = 14 Hz, H3''), 2.3-2.27 (m, 2H),
1.9-1.85 (m, 4H), 1.42 (s, 1H, t-butyl), 1.39 (s, 2H t-butyl), 1.34 (s, 1H, t-butyl), 1.3 (s, 4H, t-butyl)

\[^{13}\text{C} \text{NMR} (100 \text{ MHz, DMSO-d}_6): \delta 171.3 164.8, 153.6, 153.7, 151.1, 139.6, 134.1, 131.4, 125.9, 126.0, 122.8, 113.8, 79.6, 60.5, 59.0, 56.5, 56.1, 53.1, 46.7, 46.7, 46.5, 38.6, 36.1, 31.0, 28.6, 28.2, 24.4, 23.2

IR \( \nu_{\text{max}}(\text{ATR}) \): 1749 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1695 cm\(^{-1}\) (N-Boc-L-proline C=O)

HRMS: APCI calculated for C\(_{29}H\(_{36}\)N\(_2\)NaO\(_6\) [M+Na\(^+\)], 579.2321301 found 579.231780, error -0.8 ppm

1-(\textit{t}-butyl) 2-(2-methoxy-5-(4-oxo-3-(thiophen-2-yl)-1-(3,4,5-
trimethoxyphenyl)azetidin-2-yl)phenyl) (2S)-pyrrolidine-1,2-dicarboxylate (127)

127 was synthesised from 30 and N-Boc-L-proline using General Method XII.

Yield: 770 mg (1.23 mmol) 77%

Appearance: white fluffy dust

Melting Point: 87-90 °C

R\(_f\): 0.06 (2:1 TBME:n-hexane)

\(^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3): \delta (\text{rotamers}) 7.31 (m, 2H), 7.24 (dd, 0.5H, \text{J} = 1.7 Hz, \text{J} = 8.6 Hz, H\(_{29}\)), 7.2 (d, 0.2H, \text{J}= 2.5 Hz, H\(_{29}\)), 7.09 (d, 2H \text{J}= 5 Hz), 7.05-6.99 (m, 2H), 6.59 (s, 2H, H\(_{1-\text{ax}}\)), 4.9 (\text{apparent m, 1H, H\(_3\))}, 4.60-4.46 (m, 2H, H\(_{1-\text{ax}}\)), 3.86 (s, 2H), 3.85 (s, 1H), 3.79 (s, 3H), 3.75 (s, 6H, H\(_{29}\)), 3.66-3.41 (m, 2H), 2.41-2.26 (m, 2H), 1.21-1.92 (m, 2H), 1.49 (s, 2H), 1.48 (s, 3H), 1.43 (s, 2H), 1.39 (s, 3H)

\[^{13}\text{C} \text{NMR} (100 \text{ MHz, CDCl}_3): \delta \text{ not determined}

IR \( \nu_{\text{max}}(\text{ATR}) \): 1753 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1695 cm\(^{-1}\) (N-Boc-L-proline C=O)

HRMS: APCI calculated for C\(_{29}H\(_{36}\)N\(_2\)NaO\(_6\) [M+Na\(^+\)], 579.2321301 found 579.231780, error -0.8 ppm

1-(\textit{t}-butyl) 2-(2-methoxy-5-((2S,3R)-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-
trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (127a)

127a was resolved from 127 using LC as described in General Method XII.

Yield recovered from diastereomer mixture: 105 mg (0.17 mmol) 14%

Appearance: yellow/white fluffy powder

Melting Point: 85 °C

R\(_f\): 0.06 (plate development 1), 0.19 (plate development 1), 0.39 (plate development 1), 0.46 (plate development 1), (2:1 TBME:n-hexane)

\(^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3): \delta 7.31 (m, 2H), 7.25 (d, 0.4H, \text{J} = 2.2 Hz, H\(_{29}\)), 7.24 (d, 0.4H, \text{J} = 2.2 Hz, H\(_{29}\)), 7.10-6.99 (m, 4H), 6.59 (s, 2H, H\(_{1-\text{ax}}\)), 4.90 (d, \text{J}= 2.3 Hz, 0.6H, H\(_3\)), 4.89 (d, \text{J}= 2.3 Hz, 0.4H, H\(_3\)), 4.6-4.47 (m, 2H, H\(_{1-\text{ax}}\)), 3.86 (s, 2H, H\(_{10}\)), 3.85 (s, 1H, H\(_{18}\)), 3.79 (s, 3H, H\(_8\)), 3.75 (s, 6H, H\(_{29}\)), 2.41-2.25 (m, 2H), 2.09-1.88 (m, 2H), 1.49 (s, 3H, H\(_{10\text{ax}}\)), 1.43 (6H, t-butyl, H\(_{10\text{ax}}\))

\[^{13}\text{C} \text{NMR} (100 \text{ MHz, CDCl}_3): \delta 170.9, 170.8, 153.6, 153.1, 135.9, 133.6, 129.5, 127.4, 125.8, 125.4, 121.4, 120.3, 112.3, 123.7, 94.9, 80.1, 79.9, 64.2, 60.9, 60.3, 60.1, 59.9, 58.7, 56.1, 46.7, 46.4, 30.9, 29.9, 28.5, 24.4, 23.6

IR \( \nu_{\text{max}}(\text{ATR}) \): 1753 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1694 cm\(^{-1}\) (N-Boc-L-proline C=O)

HRMS: APCI calculated for C\(_{33}H\(_{37}\)N\(_2\)O\(_6\)S [M-H\(^-\)]; m/z 637.222525; found 637.222326, error -0.3 ppm
1-(Tert-butyl) 2-(2-methoxy-5-((2R,3S)-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (127b)

127b was resolved from 127 using LC as described in General Method XII.

Yield recovered from diastereomer mixture: 200 mg (0.32 mmol) 20 %

Appearance: yellow/white fluffy powder

Melting Point: 83 °C

Rf: 0.06 (plate development 1), 0.19 (plate development 1), 0.29 (plate development 1), 0.41 (plate development 1), (2:1 TBME:n-hexane)

1H NMR (400 MHz, CDCl3): δ 7.31 (m, 1H), 7.24 (dd, 0.3H, J = 2.2 Hz, J = 7.9 Hz, H2-), 7.21 (d, 0.3H, J = 2.2 Hz, H2-), 7.09 (m, 2H), 7.05 (m, 2H), 6.56 (s, 2H, H7(κα)), 4.91 (d, 0.6H, J = 2.7 Hz, H3), 4.9 (d, 0.4H, J = 2.2 Hz, H3), 4.61-4.5 (m, 3H, H10 & H11), 3.86 (s, 2H, H9'), 3.85 (s, 1H, H9'), 3.79 (s, 3H, H9), 3.75 (s, 6H, H7(κδ)), 3.66-3.42 (m, 2H), 2.42 – 2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.47 (s, 3H, H10-), 1.40 (s, 6H, H9- & H11-)

13C NMR (100 MHz, CDCl3): δ 170.9, 170.81, 164.3, 164.1, 154.5, 153.9, 153.6, 151.6, 140.4, 135.9, 134.8, 134.6, 133.5, 129.6, 129.5, 127.5, 127.3, 125.9, 125.4, 124.5, 124.1, 121.3, 120.4, 120.2, 113.2, 95.0, 80.1, 80.0, 64.2, 60.9, 60.3, 59.1, 56.1, 46.2, 46.4, 31.1, 30.1, 28.4, 28.3, 27.0, 24.4, 23.5

IR (ATR): νmax (cm⁻¹) (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C33H37N2O5S [M-H⁺]; m/z 637.222525; found 637.22556, error -0.0 ppm

1-(Tert-butyl) 2-(2-methoxy-5-((4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (2S)-pyrrolidine-1,2-dicarboxylate (128)

128 was synthesised from 33 and N-Boc-L-proline using General Method XII.

Yield: 310 mg (0.515 mmol) 43%

Rf: 0.1 (2:1 TBME: n-hexane)

1H NMR (400 MHz, CDCl3): δ 7.24 (dd, 0.2H, J2-6 = 2.5 Hz, J2-3 = 9.1 Hz, H2-), 7.23 (d, 0.4H, J6-2 = 2.5 Hz, H6-), 7.18 (dd, 0.8H, J2-6 = 2.5 Hz, J2-3 = 9.1 Hz, H2-), 7.03 (d, 0.6H, J6-2 = 2.5 Hz, H6-), 7.00 (d, 0.6H, J3-6 = 9.1 Hz, H3-), 6.97 (d, 0.4H, J3-6 = 9.1 Hz, H3-), 6.54 (s, 2H, H1(κδ), 6.01 (apparent m, 1H, H8), 5.40 (apparent doublet, 1H, J1,5 = 16 Hz, H1), 5.35 (apparent doublet, 1H, J1,5 = 10 Hz, H8), 4.73 (d, 0.6H, J4,3 = 2.2 Hz, H4), 4.70 (d, 0.4H, J4,3 = 2.2 Hz, H4), 4.57 (m, 0.4H, H8-), 4.46 (m, 0.6H, H8-), 3.84 (s, 2H), 3.83 (s, 1H), 3.77 (s, 3H), 3.73 (apparent s, 7H, H7(κδ) & H10), 3.66-3.42 (m, 2H), 2.43-2.25 (m, 3H), 2.1 – 1.9 (m, 2H), (1.49 (s, 1.5H), 1.45 (s, 2.5H), 1.44 (s, 2H), 1.39 (s, 3H)

Chapter 10: Experimental
1\textsuperscript{C} NMR (100 MHz, CDCl\textsubscript{3}); \(\delta\) 170.9, 165.1, 153.7, 151.3, 140.3, 134.5, 133.7, 130.3, 129.9, 124.5, 123.9, 120.1, 113.1, 94.7, 80.1, 63.9, 61.0, 59.1, 56.1, 46.8, 46.4, 38.9, 31.1, 30.2, 28.5, 24.4, 23.7

HRMS: APCI calculated for C\textsubscript{31}H\textsubscript{37}N\textsubscript{2}O\textsubscript{6} [M-H\textsuperscript{+}]; m/z 581.250454; found 581.250273, error + 0.3 ppm

1-(\textit{Terti}l-butyl) 2-(2-methoxy-5-((2S,3R)-4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (128a)

128a was resolved from 128 using LC as described in General Method XII.

\[
\text{Yield resolved from diastereomer mixture: } 120\text{mg (0.21 mmol) 40\%}
\]

R\textsubscript{f}: 0.1 (plate development 1), 0.18 (plate development 1), 0.25 (plate development 1), 0.35 (plate development 1), 0.45 (plate development 1), (2: 1 TBME: \(n\)-hexane).

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}); \(\delta\) 7.31 (dd, 0.3H, \(J_{\text{F,H}} = 2.8\) Hz, \(J_{\text{F,F}} = 8.6\) Hz, \(H_2\)), 7.24 (dd, 0.3H, \(J_{\text{F,F}} = 2.8\) Hz, \(J_{\text{F,F}} = 8.6\) Hz, \(H_2\)), 7.21 (d, 0.2H, \(J_{\text{F,F}} = 2.5\) Hz, \(H_6\)), 7.19 (d, 0.3H, \(J_{\text{F,F}} = 2.5\) Hz, \(H_6\)), 7.03 (d, 0.4H, \(J_{\text{F,F}} = 2.5\) Hz, \(H_6\)), 7.01 (d, 0.5H, \(J_{\text{F,F}} = 8.5\) Hz, \(H_3\)), 6.98 (d, 0.5H, \(J_{\text{F,F}} = 8.5\) Hz, \(H_3\)), 6.59 (s, 0.35H, \(H_4\)), 6.55 (s, 1.6H, \(H_4\)), 6.03 (apparent m, 1H, \(H_3\)), 5.40 (apparent doublet, 1H, \(J_{\text{F,F}} = 17.1\) Hz, \(H_2\)), 5.34 (apparent doublet, 1H, \(J_{\text{F,F}} = 10.2\) Hz, \(H_6\)), 4.73 (d, 0.5H, \(J_{\text{F,F}} = 2.5\) Hz, \(H_6\)), 4.7 (d, 0.4H \(J_{\text{F,F}} = 2.5\) Hz, \(H_6\)), 4.59 (m, 0.5H, \(H_4\)), 4.48 (m, 0.5H, \(H_4\)), 3.86 (s, 1H), 3.84 (s, 1H), 3.83 (s, 1H), 3.77 (s, 3H), 3.75 (s, 1H, \(J_{\text{F,F}} = 3\)), 3.73 (s, 5H, \(H_4\)), 3.66-3.42 (m, 3H, \(H_3\)), 2.4-2.25 (m, 2H, \(H_3\)), 2.1-2.0 (m, 1H, \(H_3\)), 1.99 (dd, 1H, \(J_{\text{F,F}} = 2.5\) Hz, \(J_{\text{F,F}} = 7.2\) Hz, \(H_3\)), 1.4 (s, 6H, \(t\)-butyl), 1.49 (s, 3H, \(t\)-butyl)

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}); \(\delta\) 380, 170.8, 170.7, 165.23, 164.0, 163.8, 153.6, 151.4, 147.3, 142.3, 140.1, 133.7, 130.3, 129.8, 129.6, 128.3, 127.2, 125.2, 124.4, 124.1, 123.9, 122.2, 121.5, 120.4, 120.1, 112.0, 113.1, 112.9, 112.2, 94.7, 94.5, 80.2, 80.0, 63.9, 63.7, 62.7, 61.6, 60.97, 59.1, 58.8, 56.1, 56.0, 46.7, 46.4, 31.0, 30.0, 28.5, 28.3, 27.0, 24.3, 23.5, 18.3

HRMS: APCI calculated for C\textsubscript{31}H\textsubscript{37}N\textsubscript{2}O\textsubscript{6} [M-H\textsuperscript{+}]; m/z 581.250454; found 581.250145, error + 0.3 ppm

1-(\textit{Terti}l-butyl) 2-(2-methoxy-5-((2S,3R)-4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (128b)

128b was resolved from 128 using LC as described in General Method XII.

Yield: 100 mg (0.17 mmol) 33 %

R\textsubscript{f}: 0.1 (plate development 1), 0.18 (plate development 1), 0.25 (plate development 1), 0.30 (plate development 1), 0.402 (plate development 1), (2: 1 TBME: \(n\)-hexane)
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1H NMR (400 MHz, CDCl3): δ 7.24 (dd, 0.4H, J2-6,6′ = 1.6 Hz, J2-3,3′ = 8.5 Hz, H2′-), 7.21 (dd, 0.6H, J2-6,6′ = 1.6 Hz, J2-3,3′ = 8.5 Hz, H2′-), 7.16 (d, 0.4H, J6′-2′ = 2 Hz, H6′-), 7.03 (d, 0.6H, J6′-2′ = 2 Hz, H6′-), 7.0 (d, 0.5H, J y-2′ = 8 Hz, H y′), 6.97 (d, 0.5H, J y-2′ = 8 Hz, H y′), 6.58 (s, 0.2H, H y1& y3), 6.55 (s, 1.8H, H y1& y3), 6.02 (apparent m, 1H, H3′), 5.39 (d, J y1,5 = 17.4 Hz), 5.34 (d, 1H, J6,5 = 10.1 Hz), 4.73 (d, 0.5H, J3,3′ = 2.5 Hz, H3′), 4.71 (d, 0.4H, J3,3′ = 2.5 Hz, H3′), 4.58 (m, 0.4H, H1′-), 4.48 (m, 0.6H, H1′-), 3.84 (s, 2H, H2′), 3.83 (s, 1H, H3′), 3.78 (s, 3H, H10′), 3.74 (s, 6H, H13&15′), 3.66-3.42 (m, 2H), 2.42-2.24 (m, 2H), 2.14-1.19 (m, 2H), 1.48 (s, 3H, H10′), 1.4 (s, 6H, H13&15′).

13C NMR (100 MHz, CDCl3): δ 170.9, 170.7, 165.6, 153.6, 151.4, 140.2, 133.7, 130.4, 129.9, 129.8, 124.4, 124.0, 120.2, 119.9, 113.0, 94.7, 80.0, 79.93, 64.0, 63.7, 60.9, 59.0, 56.1, 55.9, 46.6, 46.3, 38.7, 31.0, 30.0, 28.4, 28.3, 25.7, 24.4, 23.6

HRMS: APCI calculated for C31H43N3O8 [M-H]−: m/z 581.250454; found 581.250273, error + 0.3 ppm

1-(Tert-butyl) 2-(2-methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (129)

129 was synthesised using General Method XII from 38 and N-Boc-L-proline.
Yield: 70 mg (0.11 mmol) 11%

Appearance: white fluffy dust

Melting Point: 64-66 °C
Rf: 0.22 (1:1 n-hexane: ethyl acetate). 0.15 (plate development 1), 0.3 (plate development 2), 0.5 (plate development 3), (1:1 TBME:n-hexane)

1H NMR (400 MHz, CDCl3): δ 7.24 (m, 2H), 7.08-8.66 (m, 6H), 6.57 (s, 1H, H1&y3), 6.54 (s, 1H H1&y3), 5.1 (apparent s, 1H, H3′), 4.89 (m, 1H, H2′), 4.59-4.85 (m, 1H, H1′-), 3.93 (s, 1H), 3.85 (s, 2H), 3.75 (s, 3H), 3.71 (s, 3H, H13&15′), 3.70 (s, H10′), 3.66-3.99 (m, 2H), 2.41-1.90 (m, 2H, CH2), 1.48-1.36 (multiple s, 9H, t-butoxy, H13&15′).

13C NMR (400 MHz, CDCl3): δ 170.9, 162.6, 157.1, 153.4, 151.9, 130.1, 129.7, 122.3, 121.5, 118.5, 115.6, 112.4, 95.6, 95.2, 87.7, 63.5, 56.3, 61.0, 59.0, 56.5, 46.7, 46.4, 31.0, 30.0, 28.6, 28.2

IR νmax (ATR): 1760 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C30H33N3O8·[M + H]+, 549.223142, found 549.222837, error - 0.6 ppm, (mass and formula for 21DSMix without t-buty1 group)

1-(Tert-butyl) 2-(5-(3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (2S)-pyrrolidine-1,2-dicarboxylate (130)

130 was synthesised from 39 and N-Boc-L-Proline using General Method XII.
Yield: 470mg (0.72 mmol) 37%
Appearance: white/yellow fluffy dust
Melting Point: 79-83 °C  
Rr: 0.23 (2:1 TBME:n-hexane)

1H NMR (400 MHz, CDCl3): δ 7.32 (apparent dd, 2H, J= 4 Hz, J= 3.8 Hz, H5\&(6)\&(7)), 7.25 (dd, 0.5H, J= 6.7 Hz, J= 1.5Hz, H1\&(2)), 7.20 (dd, 0.5H, J= 12.5 Hz, J= 1.8 Hz, H3\&(4)), 7.11 – 6.9 (m, 4H, H6\&(7)\&(8), H1\&(2)\&(3)), 6.60 (s, 2H, H1\&(2)), 4.82 (apparent m, 1H, H4), 4.57 (m, 0.5H, H5\&(6)), 4.48 (m, 0.5H, H7\&(8)), 4.32 (d, 0.15H, J= 2.4 Hz, H3), 4.29 (d, 0.4H, J= 2.4 Hz, H2), 4.27 (apparent s, 1H, 0.5H, H4), 3.86 (s, 2H), 3.85 (s, 2H), 3.79 (s, 3H), 3.75 (s, 6H), 3.67 (m, 2H), 2.34-2.19 (m, 2H), 2.11- 1.96 (m, 2H), (1.49 (s, 1.5H), 1.47 (s, 3H), 1.43 (s, 1.5H), 1.39 (s, 3H)

13C NMR (100 MHz, CDCl3): not obtained due to low sample concentration

19F NMR (376 MHz, CDCl3): δ -113.82 (major peak), -113.95 (minor peak) -114.07 (minor peak), -114.05 (main peak), -114.4 (minor peak)

IR νmax (ATR): 1749 cm⁻¹ (β-lactam C=O), 1639 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C29H36N2NaO9 [M+Na⁺], 579.2321301 found 575.231780, error - 0.8 ppm

1-(Tert-butyl) 2-(5-((2S,3R)-3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (S)-pyrrolidine-1,2-dicarboxylate (130a)

130a was resolved from 130 using LC conditions described in General Method XII.

Yield resolved from diastereomer mixture: 50mg (0.08 mmol) 11%

Appearance: white fluffy dust

Rr: 0.13 (plate development 1), 0.2 (plate development 2), 0.31 (plate development 3), (2:1 TBME:n-hexane)

1H NMR (600 MHz, CDCl3): δ 7.32 (dd, 2H, J= 8 Hz, J= 3.6 Hz, H5\&(6)\&(7)), 7.22 (d, 0.6H, J= 1.8 Hz, H4\&(5)), 7.09 (apparent triplet, 2H, J= 8 Hz, H6\&(7)\&(8)), 7.06 (d, 0.6H, J= 1.8 Hz, H3\&(4)), 7.03 (d, 0.5H, J= 8.8 Hz, H5\&(6)), 7.00 (d, 0.6H, J= 8 Hz), 6.61 (s, 0.4H, H1\&(2)), 6.58 (s, 1.6H, H1\&(2)), 5.43 (apparent s, 0.4H, H3), 5.34 (apparent s, 0.5H, H2), 4.94 (apparent s, 0.5H, H3), 4.81 (apparent s, 0.45H, H4), 4.31 (m, 1H, H5), 3.83 (s, 1.5H, H6\&(7)), 3.82 (s, 1.5H, H6\&(7)), 3.78 (s, 3H, H8\&(9)), 3.72 (s, 3H, H7\&(8)), 3.58 – 3.78 (m, 2H), 2.36-2.14 (m, 2H), 2.12 – 1.86 (m, 2H), 1.51 (s, 6H, H9\&(10)), 1.46 (s, 3H, H10\&(11))

13C NMR (100 MHz, CDCl3): δ 172.4, 171.9, 161.6, 158.8, 158.2, 153.5, 153.4, 133.1, 128.6, 128.4, 126.6, 125.1, 110.23, 110.1, 95.6, 95.4, 82.8, 80.2, 80.1, 63.8, 61.0, 59.3, 58.6, 56.1, 55.4, 46.7, 46.5, 46.4, 29.9, 28.4, 27.0, 24.5, 23.6, 16.3

19F NMR (376 MHz, CDCl3): δ -113.8 (major peak), -113.9 (minor peak), -114.0 (major peak).

HRMS: APCI calculated for C29H36N2NaO9 [M+Na⁺], 579.2321301 found 575.231780, error - 0.8 ppm
1-(tert-butyl) 2-(5-((2R,3S)-3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (S)-pyrrolidine-1,2-dicarboxylate (130b)

130b was resolved from 130 using LC described in General Method XIII.

Yield resolved from diastereomer mixture: 70 mg (0.11 mmol) 15%

Appearance: white fluffy dust

R_6: 0.13 (plate development 1), 0.13 (plate development 1), 0.28 (plate development 1), (2:1 TBME:n-hexane);

^1H NMR (400 MHz, CDCl₃): δ 7.32 (dd, 2H, J = 7.1 Hz, J = 3.7 Hz), 7.24 (m, 1H), 7.09 (m, 3H), 7.02 (apparent triplet, J = 8.6 Hz), 6.59 (s, 2H, H₃), 4.82 (apparent m, 1H, H₃), 4.57 (m, 0.5H, H₃), 4.49 (m, 0.5H, H₃), 4.32 (apparent s, 0.2H, H₂), 4.29 (apparent s, 0.3H, H₂), 4.28 (apparent s, 0.6H, H₂), 3.86 (s, 2H, H₂), 3.85 (s, 1H, H₂), 3.79 (s, 3H, H₃), 3.75 (s, 6H, H₃), 3.68 – 3.42 (m, 2H), 2.42– 1.98 (m, 4H), 1.49– 1.35 (apparent m, 9H, H₃)

^13C NMR (100 MHz, CDCl₃): not obtained due to low sample concentration

^19F NMR (376 MHz, CDCl₃): δ -113.82 (major peak), -114.03 (minor peak), -114.09 (major peak), -114.4 (minor peak)

HRMS: APCI calculated for C₉₀H₆₀Na₂O₉ [M+Na⁺], 579.2321301 found 575.231780, error -0.8 ppm

1-(tert-butyl) 2-(2-methoxy-5-(3-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (2S)-pyrrolidine-1,2-dicarboxylate (131)

131 was synthesised from 41 and N-Boc-L-proline using General Method XII.

Yield: 500 mg (0.76 mmol) 35%

Appearance: white fluffy dust

Melting Point: 98-103 ºC

R_6: 0.2 (2:1 TBME:n-hexane)

^1H NMR (400 MHz, CDCl₃): δ (rotamers) 7.26 (d, 3H, J = 8.1), 7.21 (d, 0.14H, J = 2 Hz, 0.14H, H₂), 7.18 (d, 0.3H, J = 2 Hz, H₂), 7.06 (d, 0.6H, J = 2 Hz, H₂), 7.01 (d, 1H, J = 8.4 Hz, H₂), 6.92 (apparent m, 2H), 6.61 (s, 2H, H₃), 4.8 (m, 1H, H₂), 4.58 (m, 0.4H, H₂), 4.49 (m, 0.6H, H₂), 4.29 (d, 0.15H, J = 2.3 Hz, H₃, rotamer 1), 4.26 (d, 0.3H, J = 2.3 Hz, H₃, rotamer 2), 4.23 (apparent s, 0.6H, H₃, rotamer 3), 3.85 (s, 1H, H₂, rotamer 1), 3.84 (s, 2H, H₂, rotamer 2), 3.83 (s, 1H, H₃), 3.79 (s, 2H, H₃), 3.75 (s, 6H, H₃), 3.68–3.42 (m, 2H), 2.41 – 1.92 (m, 4H), 1.49 (s, 2H), 1.47 (s, 2H), 1.43 (s, 2H), 1.39 (s, 3H)

^13C NMR (100 MHz, CDCl₃): δ 170.9, 165.9, 165.8, 159.4, 159.3, 153.6, 151.4, 140.3, 140.2, 133.7, 130.1, 126.7, 126.6, 124.4, 121.5, 121.3, 120.4, 113.2, 113.1, 80.1, 80.0, 79.9, 64.5, 64.4, 63.8, 63.8, 63.8, 60.9, 59.1, 58.8, 56.1, 55.9, 55.3, 56.6, 46.4, 38.7, 37.2, 31.1, 30.1, 30.0, 28.4, 28.3, 28.3, 27.0, 24.4, 24.3, 23.6

IR ν max (ATR): 1749 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (N-Boc-L-proline C=O)

1-(tert-butyl) 2-(2-methoxy-5-((2S,3R)-3-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (131a)

131a was resolved from the mixture of 131 using the column chromatography conditions described in General Method XII.

Yield: 90 mg (0.14 mmol) 18%

Appearance: white fluffy dust

Melting Point: 101-103 ºC

R_6 0.4 (4:1 TBME:n-hexane) plate developed twice

Chapter 10: Experimental
Chapter 10: Experimental

1H NMR (400 MHz, CDCl3): δ (rotamers present) 7.26 (d, J = 8.1 Hz, 3H), 7.19 (d, J = 2 Hz, H\textsubscript{10}), 7.10 (d, 0.14H, J = 2 Hz, H\textsubscript{19}), 7.06 (d, 1H, J = 2 Hz, H\textsubscript{1}), 7.01 (apparent triplet, 1H, J = 8.4 Hz), 6.93 (d, 2H, J = 9 Hz), 6.61 (s, 2H, H\textsubscript{18}), 4.8 (d, J = 2.3 Hz, 1H, H\textsubscript{1}), 4.59 (m, 0.5H, H\textsubscript{1a}), 4.48 (m, 0.5H, H\textsubscript{1b}), 4.29 (d, 0.3H, J = 2.3 Hz, H\textsubscript{2}), 4.24 (d, 0.7H, J = 2.3 Hz, H\textsubscript{3}), 3.85 (s, 2H, H\textsubscript{6}), 3.84 (s, 1H, H\textsubscript{9}), 3.83 (s, 3H), 3.79 (s, 3H), 3.75 (s, 6H, H\textsubscript{2,4,6}), 3.67-3.43 (m, 2H), 2.41 – 1.9 (m, 4H), 1.49 (s, 3H, H\textsubscript{10}), 1.43 (s, 6H, H\textsubscript{9}, H\textsubscript{14}, 1H).

13C NMR (100 MHz, CDCl3): δ 170.8, 170.0, 165.9 (C\textsubscript{2}), 165.8 (C\textsubscript{3}), 159.4, 153.8 (C\textsubscript{13,16}), 153.4 (C\textsubscript{10}), 151.4, 151.3, 140.2, 133.7, 133.1, 128.7, 126.5, 124.4, 124.1, 121.5, 120.4, 114.5, 113.2, 113.1, 94.8 (C\textsubscript{2}), 80.1, 80.0, 79.9, 64.6, 64.4, 63.9, 60.9, 59.0, 58.8, 56.1, 55.9, 55.4, 46.61, 46.4, 31.0, 30.1, 28.5, 28.4, 24.3, 23.6

HRMS: APCI calculated for C\textsubscript{36}H\textsubscript{41}N\textsubscript{2}O\textsubscript{10} [M-H\textsuperscript{-}]; 661.27669; found 661.277348, error + 1.1 ppm

1-(tert-butyl) 2-(2-methoxy-5-((2R,3S)-3-(4-methoxynyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (S)-pyrrolidine-1,2-dicarboxylate (131b)

131b was resolved from the mixture of 131 using the LC as described in General Method XII.

Yield: 110 mg (0.17 mmol) 23% Appearance: white fluffy dust

Melting Point: 100-103 °C

Rt: 0.38 (2:1 TBME:n-hexane), plate developed twice

1H NMR (400 MHz, CDCl3): δ 7.26 (d, 3H, J = 8.1 Hz), 7.19 (d, 0.3H, J = 2 Hz), 7.07 (d, 0.6H, J = 2 Hz), 7.02 (d, 1H, J = 8.4 Hz), 6.92 (apparent m, 2H), 6.6 (s, 2H, H\textsubscript{18}), 4.8 (m, 0.85, H\textsubscript{2}), 4.7 (d, 0.15H, J = 2.3 Hz, H\textsubscript{3}), 4.57 (m, 0.45H, H\textsubscript{1}), 4.49 (m, 0.6H, H\textsubscript{1}), 4.28 (d, 0.15H, J = 2.3 Hz, H\textsubscript{4}), 4.26 (d, 0.3H, J = 2.3 Hz, H\textsubscript{5}), 4.24 (apparent s, 0.6H, H\textsubscript{6}), 3.85 (s, 2H, H\textsubscript{7}), 3.84 (s, 2H, H\textsubscript{8}), 3.83 (s, 1H, 3-Ph-OCH\textsubscript{3}), 3.79 (s, 3H, H\textsubscript{9}), 3.74 (s, 6H, H\textsubscript{2,4,6}), 3.67-3.43 (m, 2H), 2.42 – 1.93 (m, 4H), 1.49 (s, 1H), 1.47 (s, 2H), 1.43 (s, 1H), 1.39 (s, 3H).

13C NMR (100 MHz, CDCl3): not obtained due to low sample concentration

HRMS: APCI calculated for C\textsubscript{36}H\textsubscript{41}N\textsubscript{2}O\textsubscript{10} [M-H\textsuperscript{-}]; 661.27669; found 661.276838, error + 1.1 ppm

General Method XIII: Coupling of N-Boc-L-proline to NH\textsubscript{2} to form β-lactam diastereomers using HOBT and DCC

To a stirring solution of amino substituted β-lactam (1 eq, 0.23 mmol) in 10 mL of DMF under a nitrogenous atmosphere, HOBT (1.6 eq, 0.36 mmol, 50mg) and DCC (1.2 eq, 0.28 mmol, 57 mg) were added. N-(Boc)-L-Proline (1.2 eq, 0.28 mmol, 60mg) was then added to the reaction vessel. The reaction was stirred for 24 hours and subsequently diluted with ethyl acetate (40 mL) and filtered to remove the DCC prior to work up. The organic filtrate was washed with dH\textsubscript{2}O (3 X 30 mL) to remove residual DMF, followed by 30 mL of NaHCO\textsubscript{3} solution to remove excess proline. The organic layer was dried with anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and solvent removed in vacuo to afford a crude yellow gel. The crude mixture was purified using flash chromatography over silica gel using isocratic conditions of 1: 1 n-hexane: ethyl acetate. TLC using various ratios of TBME: n-hexane failed to achieve separation of amide diastereomer derivatives. No further diastereomer purification was carried out.
### Numbering nomenclature for B ring meta derivatised amide diastereomers

<table>
<thead>
<tr>
<th>Diastereomer Code</th>
<th>R</th>
</tr>
</thead>
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<tr>
<td>137</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>138</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

*Tert*-butyl-(S)-2-((2-methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (137)

137 was synthesised from 37 *trans* and N-Boc-L-proline using General Method XIII.  
**Yield:** not calculated  
**Appearance:** brown oil  
**R:** 0.18 (1:1 n-hexane: ethyl acetate)  
**1H NMR (400 MHz, CDCl₃):** δ 7.25 (m, 2H), 7.01 (t, 1H, J = 7.6 Hz), 6.93 (m, 4H), 6.63 (s, 1.5H, H₇,₈), 6.62 (s, 0.5H, H₉), 5.18 (apparent s, 1H, H₄), 4.97 (apparent s, 1H, H₃), 4.37 (m, 1H, H₁'''), 3.91 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H, H₇,₈), 3.67-3.44 (m, 2H, proline CH₂), 2.42-1.89 (m, 4H, proline CH₂), 1.51 (s, 6H, H₉,₁₀), 1.45 (s, 3H, H₁₀'')

**13C NMR (100 MHz, CDCl₃):** δ 173.9, 162.7, 157.1, 153.4, 134.8, 133.2, 129.7, 129.3, 126.6, 122.4, 121.5, 118.6, 117.5, 114.8, 114.4, 111.2, 110.8, 95.7, 87.2, 81.9, 80.8, 64.2, 61.1, 59.4, 59.0, 56.2, 55.9, 47.3, 46.6, 46.3, 30.9, 28.2, 24.2, 23.7

**HRMS:** APCI calculated for C₃₅H₄₀N₃O₉ [M-H⁺]; 646.277003; found 646.278552, error -2.4 ppm

*Tert*-butyl-(R)-2-((2-methoxy-5-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (138)

138 was synthesised from 32 *trans* and N-Boc-L-proline using both General Method XII and General Method XIII with good agreement of spectroscopic data using both methods.  
**Yield General Method XII:** 30 mg (0.048 mmol) 20%.  
**Yield General Method XIII:** 50 mg (0.08 mmol) 35 %  
**R:** 0.39 (1:1 n-hexane: ethyl acetate), 0.11 (2:1 n-hexane: TBME).

**Melting Point:** 65-68 °C  
**1H NMR (600 MHz, CDCl₃):** δ 9.29 (bs, NH, H₇−), 8.58 (bs, 1H), 7.29 (d, 1H, J = 4.5 Hz), 7.1 (bs, 1H), 7.08 (apparent s, 1H), 7.02 (t, 1H, J = 5.9 Hz), 6.91 (bs, 1H), 6.62 (2H, H₃,₄), 4.93

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1H NMR (500 MHz, CDCl₃): δ 7.53 (d, 1H, H₃), 6.70 (d, 1H, H₄), 5.60 (s, 2H, proline CH₂), 3.90 (s, 3H, H₁'), 3.78 (s, 3H, H₈'), 3.75 (s, 6H, H₇'₉'), 3.66 - 3.40 (broad m, 2H, proline CH₂), 2.05 - 1.70 (broad m, 4H, proline CH₂), 1.55 - 1.32 (apparent m, 9H, H₉' - 11').

13C NMR (125 MHz, CDCl₃): δ 172.5, 164.5, 153.4, 148.3, 136.1, 134.5, 133.7, 127.2, 125.9, 125.2, 118.3, 110.7, 110.4, 97.7, 95.2, 95.0, 80.6, 72.7, 65.8, 64.8, 60.9, 60.1, 56.1, 55.9, 49.3, 47.2, 33.8, 28.3, 25.5, 24.9.

15N NMR (125 MHz, CDCl₃): δ 117.8 (NH, H₇').

IR νmax (ATR): 1752 cm⁻¹ (β-lactam C=O), 1690 cm⁻¹ (N-Boc-L-proline C=O).

HRMS: APCI calculated for C₃₂H₃₈N₃O₈S [M - H]⁺: 636.238510; found 636.238493, error -0.0 ppm.

General Method XIV: Hydrazinolysis of N-Boc-L-proline from β-lactam diastereomers to afford optically pure enantiomers

Hydrazine dihydrochloride (5 eq) was added to a stirring solution of the respective diastereomer (1 eq) in anhydrous methanol (30 mL) at 0 °C (on ice) under a nitrogenous atmosphere. Anhydrous TEA (9 eq) was added dropwise to the vessel. The solution was allowed reach room temperature before heating to reflux for 6 hours. The temperature was maintained at 80 °C. The solvent was removed under reduced pressure and residue treated with a saturated solution of potassium hydrogen sulfate (KHSO₄). Extraction was performed using ethyl acetate (2 × 30 mL). The organic layers were washed with sodium bicarbonate (3 × 20 mL) to ensure removal of proline. The organic phase was dried with anhydrous Na₂SO₄, filtered and solvent removed under reduced pressure. The crude was then purified using flash chromatography over silica gel (eluent: n-hexane:ethyl acetate; 1:2) to afford the desired product. Spectroscopic data is in line with racemates for enantiomers listed below as detailed in Appendix 3.

(3S,4S)-3-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN1)

16EN1 was synthesised from 116a and 123a using General Method XIV.

Yield: (116a to 16EN1): 22.5 mg (0.06 mmol) 30%
Yield: (123a to 16EN1): 30mg (0.08 mmol) 49%

Melting Point: 64-68 °C
Rf: 0.2 (1:1; n-hexane:ethyl acetate)
HRMS: APCI calculated for [M + H']⁺, 376.139869; found 376.139078, error + 2.1 ppm
IR νmax (ATR): 3221 cm⁻¹ (OH), 1727 cm⁻¹ (β-lactam C=O)
Purity (RP-HPLC): 97%
Ee: 75%

(3R,4R)-3-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN2)

16EN2 was synthesised from 116b and 123b using General Method XIV.

Yield: (116b to 16EN1): 19 mg (0.05 mmol), 31%
Yield: (123b to 16EN1): 30 mg (0.08 mmol) 61%
Melting Point: 69-73 °C
Rf: 0.2 (1:1; n-hexane:ethyl acetate)
IR $\nu_{\text{max}}$(ATR): 3225 cm$^{-1}$ (OH) 1743 cm$^{-1}$ ($\beta$-lactam C=O)
HRMS: APCI calculated for [M+H$^+$], 376.139204; found 376.139078, error -0.3 ppm
Purity (RP-HPLC): 90%
$Ee$: 41%

Dextrorotatory - (3S, 4S)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN1)
17EN1 was synthesised from 108a using General Method XIV.
Yield: 58mg (0.16 mmol), 30%
Appearance: white powder
Melting Point: 120-122°C
Rf: 0.36 (1:1; n-hexane:ethyl acetate) 
$[\alpha]_{D}^{20}$: +25.14 ($c$ 17.9mg/5mL in chloroform)
IR $\nu_{\text{max}}$(ATR): 3284 cm$^{-1}$ (OH), 1726 cm$^{-1}$ (b-lactam C=O)
APCI calculated for [M+H$^+$], 360.144560; found 360.144164, error -2.1 ppm and [M+ Na$^+$], 382.126696; found 382.126108, error -2.1 ppm
Purity (RP-HPLC): 97%
$Ee$: 94%

Levorotatory- (3R,4R)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN2)
17EN2 was synthesised from 108b using General Method XIV.
Yield: 150mg (0.42mmol) 66%
Appearance: White powder
Melting Point: 122-127 °C
Rf: 0.36 (1:1; n-hexane: ethyl acetate) 
$[\alpha]_{D}^{20}$: -25.38 ($c$ 19.7mg/5mL in chloroform)
IR $\nu_{\text{max}}$(ATR): 3295.35 cm$^{-1}$ (OH), 1723.34 cm$^{-1}$ ($\beta$-lactam C=O)
HRMS: APCI calculated for [M +H$^+$], 360.144560; found 360.144164, error +1.5 ppm and [M+ Na$^+$], 382.126696; found 382.126108, error +1.5 ppm
Purity (RP-HPLC): 91%
$Ee$: 74%

Dextrorotatory- (3S,4S)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN1)
18EN1 was synthesised from 114a using General Method IX.
Yield: 90mg, (0.24mmol) 46%
Appearance: white powder
Melting Point: 143-146 °C
Rf: 0.25 (1:1 n-hexane:ethyl acetate) 
$[\alpha]_{D}^{20}$: +22.15 ($c$ 15.8mg/5mL in chloroform)
IR $\nu_{\text{max}}$(ATR): 3288 cm$^{-1}$ (OH), 1726 cm$^{-1}$ ($\beta$-lactam C=O)
HRMS: APCI calculated for [M +H$^+$], 378.134910; found 378.134742, error +0.4 ppm
Purity (HPLC): 95%  
Ee: 79%

Levorotatory-\((3R,4R)-4-(3\text{-fluoro}-4\text{-methoxyphenyl})-3\text{-hydroxy}-1-(3,4,5\text{-trimethoxyphenyl})\) azetidin-2-one (18EN2)

18EN2 was synthesised from 114b using General Method XIV.  
Yield: 100mg (0.27mmol) 67%  
Appearance: white powder  
Melting Point: 144-146 °C

Rf: 0.24 (1:1 n-hexane:ethyl acetate)  
\(\alpha\): \(-17.6^\circ\) (c 15.1mg/5mL in chloroform) ee (%): 77%

IR \(\nu_{\text{max}}\) (ATR): 3288 cm\(^{-1}\) (OH), 1726 cm\(^{-1}\) (\(\beta\)-lactam C=O)

HRMS: APCI calculated for \([\text{M}^+\text{H}]\), 378.135181; found 378.134742, error -1.2 ppm

Purity (HPLC): 91%  
Ee: 75%

\((3S,4S)-4-(3\text{-chloro-4\text{-methoxyphenyl}})-3\text{-hydroxy}-1-(3,4,5\text{-trimethoxyphenyl})\) azetidin-2-one (19EN1)

19EN1 was synthesised from 117a using General Method XIV.  
Yield: 157 mg (0.4 mmol) 71%  
Melting Point: 135.9 °C

Rf: 0.34 (1:1; n-hexane:ethyl acetate)  
IR \(\nu_{\text{max}}\) (ATR): 3321 cm\(^{-1}\) (OH), 1727 cm\(^{-1}\) (\(\beta\)-lactam C=O)

HRMS: APCI calculated for \([\text{M}^+\text{H}]\), 394.106003; found 394.105191, \([\text{M}^+\text{Na}]\), error -2.1 ppm

Purity (RP-HPLC): 99%  
Ee: 91%

\((3R,4R)-4-(3\text{-chloro-4\text{-methoxyphenyl}})-3\text{-hydroxy}-1-(3,4,5\text{-trimethoxyphenyl})\) azetidin-2-one (19EN2)

19EN2 was synthesised from 117b using General Method XIV.  
Yield: 53 mg (0.133 mmol) 70%  
Melting Point: 135.9 °C

Rf: 0.3 (1:1; n-hexane: ethyl acetate)  
HRMS: APCI calculated for \([\text{M}^+\text{H}]\), 394.105635; found 394.105191, error -1.1 ppm

IR \(\nu_{\text{max}}\) (ATR): 3279 cm\(^{-1}\) (OH), 1720 cm\(^{-1}\) (\(\beta\)-lactam C=O)

Purity (RP-HPLC): 99%  
Ee: 78%

\((3S,4S)-3\text{-hydroxy-4-(4\text{-methoxy-3-methylphenyl}})-1-(3,4,5\text{-trimethoxyphenyl})\) azetidin-2-one (20EN1)

20EN1 was obtained from 118a using General Method XIV.  
Yield: 99 mg (0.27 mmol) 35%  
Melting Point: 128-130 °C

Rf: 0.2 (2:1 n- hexane: ethyl acetate), 0.33 (1:1 n- hexane: ethyl acetate)  
IR \(\nu_{\text{max}}\) (ATR): 3298 cm\(^{-1}\) (OH), 1725 cm\(^{-1}\) (\(\beta\)-lactam C=O)
HRMS: APCI calculated for C$_{20}$H$_{24}$NO$_{6}$ [M+H$^+$], 374.159814; found 374.159814, error +0.3 ppm
Purity (RP-HPLC): 99%
Ee: 66%

(3R,4R)-3-Hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN2)
20EN2 was obtained from 118b using General Method XIV.
Yield: 20 mg (0.054 mmol) 41.2 %
Melting Point: 122-128 ºC
Rf: 0.2 (2:1 n- hexane: ethyl acetate), 0.33 (1:1 n- hexane: ethyl acetate),
IR $\nu_{\text{max}}$(ATR): 3298 cm$^{-1}$ (OH), 1724 cm$^{-1}$ (β-lactam C=O)
HRMS: APCI calculated for C$_{20}$H$_{24}$NO$_{6}$ [M+H$^+$], 374.159814; found 374.159575, error -0.6 ppm
Ee: 50%

(3R,4S)-4-(3-Bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN1)
21EN1 was synthesised from 120a using General Method XIV.
Yield: 80mg (0.25 mmol) 76%
Melting Point: 63 ºC
Rf: 0.25 (1:1; n-hexane:ethyl acetate)
IR $\nu_{\text{max}}$(ATR): 1747.53 cm$^{-1}$ (β-lactam C=O)
HRMS: APCI calculated for C$_{19}$H$_{21}$BrNO$_6$ [M+H$^+$]; 438.054676; found 438.055315; error +1.5 ppm Ee: 50%
Ee: 76%

(3R,4R)-4-(3-Bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN2)
21EN2 was synthesised from 120b using General Method XIV.
Yield: 45 mg (0.249 mmol) 78 %
Melting Point: 60-63 ºC
Rf: 0.25 (1:1; n-hexane:ethyl acetate)
IR $\nu_{\text{max}}$(ATR): 1750 cm$^{-1}$ (β-lactam C=O)
HRMS: APCI calculated for C$_{19}$H$_{23}$NO$_5$S [M+H$^+$] 376.121320; found 376.121995, error -1.8 ppm
Ee: 96%

(3S,4S)-3-Hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN1)
22EN1 was obtained from 119a using General Method XIV.
Rf: 0.25 (1:1; n-hexane:ethyl acetate)
IR $\nu_{\text{max}}$(ATR): 3300 cm$^{-1}$ (OH), 1721 cm$^{-1}$ (β-lactam C=O)
HRMS: APCI calculated for C$_{19}$H$_{23}$NO$_5$S [M+ H$^+$] 376.121320; found 376.121995, error -1.8 ppm
Ee: 84%

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(3R,4R)-3-Hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN2)

22EN2 was obtained from 119b using General Method XIV.

Yield: 6 mg (0.02 mmol) 27%

R<sub>f</sub>: 0.25 (1:1 n-hexane: ethyl acetate)

IR ν<sub>max</sub>(ATR): 3300 cm<sup>-1</sup> (OH), 1721 cm<sup>-1</sup> (β-lactam C=O).

HRMS: APCI calculated for C<sub>19</sub>H<sub>21</sub>NClO<sub>5</sub>S [M+ Cl]<sup>-</sup> 410.083445; found 410.084225, error -1.9 ppm, APCI calculated for C<sub>19</sub>H<sub>22</sub>NO<sub>5</sub>S [M+ H]<sup>+</sup> 376.121320; found 376.121269, error +0.1 ppm, APCI calculated for C<sub>10</sub>H<sub>21</sub>NaO<sub>5</sub>S [M+ Na]<sup>+</sup> 398.103264; found 398.10254 Ee: 85%

3S, 4R)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN1)

24EN1 was synthesised from 124a using General Method XIV.

Yield: 28 mg (0.06 mmol) 43%

R<sub>f</sub>: 0.4-0.5 (2:1 n-hexane:ethyl acetate)

IR ν<sub>max</sub>(ATR): 3282 cm<sup>-1</sup> (OH), 1746 cm<sup>-1</sup> (β-lactam C=O).

HRMS: APCI calculated for C<sub>25</sub>H<sub>25</sub>NaO<sub>6</sub> [M+ Na]<sup>+</sup> 458.157408; found 458.157727; error -0.7 ppm

Ee: 96%

(3R, 4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN2)

24EN2 was synthesised from 124b using General Method XIV.

Yield: 20 mg (0.05 mmol) 38%

R<sub>f</sub>: 0.4-0.5 (2:1 n-hexane: ethyl acetate)

IR ν<sub>max</sub>(ATR): 3282 cm<sup>-1</sup> (OH), 1746 cm<sup>-1</sup> (β-lactam C=O)

HRMS: APCI calculated for C<sub>25</sub>H<sub>24</sub>NaO<sub>6</sub> [M-H]<sup>-</sup> 434.160911; found 434.160898; error -0.0 ppm

Ee: 59%

4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN1)

(absolute configuration not determined)

27EN1 was synthesised from 125a using General Method XIV.

Yield: 40 mg (0.11 mmols) 58 %

Appearance: yellow powder

R<sub>f</sub>: 0.25 (1:1 n-hexane: ethyl acetate)

IR ν<sub>max</sub>(ATR) 1731 cm<sup>-1</sup> (β-lactam C=O)

HRMS: APCI calculated for C<sub>19</sub>H<sub>22</sub>NO<sub>6</sub> [M+H]<sup>+</sup> 360.144164 found 360.144342, error +0.2 ppm

Ee: 61%

4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN2)

(absolute configuration not determined)

27EN2 was synthesised from 125b using General Method XIV.

Yield: 50 mg (0.134 mmols) 80 %

Appearance: yellow powder
\( R_f: 0.25 \) (1:1 \( n \)-hexane: ethyl acetate)

IR \( \nu_{\text{max}} (\text{ATR}) \): 1731 cm\(^{-1}\) (\( \beta \)-lactam C=O)

HRMS: APCI calculated for C\(_{19}\)H\(_{22}\)NO\(_6\) [M+H\(^+\)]: 360.144164 found 360.144282, error + 0.3 ppm

\( Ee: 45\% \)

(35, 4R)-4-(3-Hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one

(30EN1)

30EN1 was synthesised from 127a using General Method XIV.

Yield: 47 mg (0.11 mmol) 63%

Appearance: yellow powder

Melting Point: 94-96 °C

IR \( \nu_{\text{max}} (\text{ATR}) \): 1720.29 cm\(^{-1}\) (\( \beta \)-lactam C=O)

HRMS: APCI calculated for C\(_{23}\)H\(_{24}\)NO\(_6\)S [M+H\(^+\)]: 442.131885; found 442.1312129, error -0.6 ppm

IR \( \nu_{\text{max}} (\text{ATR}) \): 1720.29 cm\(^{-1}\) (\( \beta \)-lactam C=O)

\( Ee: 100\% \)

(3R, 4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN2)

30EN2 was synthesised from 127b using General Method XIV.

Yield: 110 mg (0.25 mmol) 78%

Appearance: pale yellow powder

Melting Point: 94-96 °C

IR \( \nu_{\text{max}} (\text{ATR}) \): 1720.29 cm\(^{-1}\) (\( \beta \)-lactam C=O)

HRMS: APCI calculated for C\(_{23}\)H\(_{22}\)NO\(_6\)S [M-H\(^-\)]: 442.131885; found 442, error -0.9 ppm

\( Ee: 56\% \)

(35, 4R)-4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33EN1)

33EN1 was synthesised from 128a using General Method XIV.

Yield: 30 mg (0.07 mmol) 38%

Appearance: white powder

IR \( \nu_{\text{max}} (\text{ATR}) \): 1741 cm\(^{-1}\) (\( \beta \)-lactam C=O)

HRMS (APCI): Calculated for C\(_{21}\)H\(_{22}\)NO\(_6\) [M-H\(^-\)]: 384.145261, found 384.145315. Error -0.1 ppm

\( Ee: 85\% \)

(35, 4R)-3-(4-Fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN1)

39EN1 was synthesised from 130a using General Method XIV.

Yield: 30 mg (0.07 mmol) 86%

IR \( \nu_{\text{max}} (\text{ATR}) \): 1741 cm\(^{-1}\) (\( \beta \)-lactam C=O)
HRMS: APCI calculated for C$_{25}$H$_{25}$FNO$_6$ [M+H$^+$], 454.166042; found 454.166042; error + 0.6 ppm

(3R, 4S)-3-(4-Fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN2)

39EN2 was synthesised from 139b using General Method XIV.

Yield: 40 mg (0.09 mmol) 81%

R$_f$: 0.35 (1:1 n-hexane: ethyl acetate)

IR $\nu_{max}$(ATR): 1740 cm$^{-1}$ (b-lactam C=O)

HRMS: APCI calculated for C$_{25}$H$_{25}$FNO$_6$ [M + H$^+$], 454.166042; found 454.165688; error + 0.8 ppm

(3S,4R)-4-(3-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN1)

41EN1 was synthesised from 131a using General Method XIV.

Yield: 54 mg (0.12 mmol) 85%

Appearance: yellow powder

R$_f$: 0.35 (1:1 n-hexane: ethyl acetate)

IR $\nu_{max}$(ATR): 2937 cm$^{-1}$ (OH), 1736 cm$^{-1}$ (C=O)

HRMS: APCI calculated for C$_{26}$H$_{28}$NO$_7$ [M+H$^+$] 466.186029; found 466.186117, error + 0.2 ppm

(3R,4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN2)

41EN2 was synthesised from 131b using General Method XIV.

Yield: 47 mg (0.10 mmol) 61%

Appearance: yellow powder

R$_f$: 0.35 (1:1 n-hexane: ethyl acetate)

IR $\nu_{max}$(ATR): 2937 cm$^{-1}$ (OH), 1736 cm$^{-1}$ (C=O)

HRMS: APCI calculated for C$_{26}$H$_{28}$NO$_7$ [M+H$^+$] 466.186029; found 466.186243, error + 0.2 ppm

**General Method XV: Optimised biocatalytic methanolysis of racemic 3-acetoxy β-lactams using Candida antarctica lipase B (CAL-B) for isolation of (3S,4S) 3-hydroxy and (3R,4R) 3-acetoxy β-lactams**

3-Acetoxy β-lactam (1 eq) was added to TBME (40 mL) in glass tubes of the Radley 12 plus carousel reaction station. 0.5 mg of CAL-B per mg of 3-acetoxy β-lactam was added to the reaction vessel followed by methanol (3 eq). The reaction was stirred and monitored by $^1$H NMR until 35-45% conversion to the 3-hydroxy enantioenriched 3S,4R β-lactam (2-6 days). The reaction was quenched via filtration of the enzyme and washing the residue with ethyl acetate. The solvent was removed *in vacuo* followed by separation of 3R,4R 3-acetoxy β-lactam from the enantioenriched (3S,4R) 3-hydroxy β-lactam using flash column chromatography with isocratic elution of 1:1 n-hexane: ethyl acetate. Following calculation of enantiomeric excess
(ee) for both analogues using chiral HPLC [with Chrompak-IH-3 (150 × 4.6 mm) column and Chiral-IH-3 guard column, mobile phase 1:1 n-hexane: isopropanol], the 3-acetoxy β-lactam was reacted further with 1 mg/mg of CALB, methanol (6 eq) and TBME (40 mL). The reaction was again monitored by 1H NMR and quenched at 50-60% conversion. Work up and purification was repeated to isolate enantiopure 3R,4R 3-acetoxy β-lactam.

**General Method XVI: Addition of dibenzylphosphite to afford dibenzylphosphate esters**
The phenol containing compound (1 eq) was added to a stirring solution of anhydrous MeCN under a nitrogen atmosphere (20 mL) and sonicated for 10 minutes until a homogenous suspension was observed. CCl4 (3 eq) was added via syringe and septum on an ice/sodium chloride mixture at -20°C and stirred for 15 minutes while maintaining the nitrogenous atmosphere. DMAP (0.2 mmol) and DIPEA (4 mmol) were dissolved in MeCN (2 mL) and injected. Dibenzylphosphite (1.4 eq) was added dropwise to the reaction vessel. The reaction was then warmed to RT and stirred overnight. The reaction was quenched with KHSO4(sat) (10 mL). The MeCN/water layer was then extracted with ethyl acetate (4 × 15 mL). The organic layers were combined and dried with anhydrous Na2SO4. The solvent was removed in vacuo and crude product purified using LC and isocratic elution (1:1 n-hexane/ethyl acetate).

Dibenzy1 ((2S,3R)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) phosphate (140)
20EN1 was isolated using General Method XV and 140 was synthesised from using General Method XVI.

![Dibenzy1 phosphate](image)

1H NMR (600 MHz, CDCl3): δ 7.31 (m, 10H, benzyl), 7.10 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 7.05 (apparent s, 1H), 6.78 (d, 1H, J = 8.8 Hz), 6.54 (s, 2H), 5.10-5.06 (m, 5H, H3 and benzyl CH2X2), 4.94 (apparent s, 1H, H4), 3.85 (s, 3H), 3.79 (s, 3H), 3.72 (s, 6H)
13C NMR (100 MHz, CDCl3): δ 161.1 (d, J2,P = 9 Hz), 158.4, 153.5, 135.3, 135.1, 132.9, 128.7, 128.6, 128.1, 127.8, 126.1, 125.3, 110.2, 95.5, 95.4, 84.3 (d, J3,P = 9 Hz), 70.0, 63.9, 60.9, 56.1, 55.4
31P NMR (162 MHz, DMSO-d6): δ -2.84
Ee: 71%

**General Method XVII: Perkin condensation for synthesis of P3 acetate using reflux**
A stirring solution of salicaldehyde (40 mmol, 1 eq, 4.84 g, 4.18 mL) and 2-methylpyrazine (40 mmol, 1 eq, 3.76g, 3.65 mL) in acetic anhydride (211 mmol, 5.29 eq, 20 mL) was refluxed at

**Chapter 10: Experimental**
180 °C for 4-5 days and monitored by TLC until visualisation of required product at 365 nm under UV light. A characteristic yellow colouring was seen on day 1 followed by darkening of the reaction vessel thereafter to a brown/black colour. After 4-5 days, the acetic anhydride was reduced in vacuo, dried under N₂ gas to afford a brown oil. A mixture of the free phenol and acetate intermediate were observed on TLC and on ¹H NMR. The acetate intermediate was hydrolysed to the free phenol using 1M NaOH (aq.) (1-2 drops) prior to further purification.

**General Method XVIII: Perkin condensation for synthesis of P3 acetate using microwave technology**

Salicaldehyde (1 eq, 10 mmol, 1.05 mL) and 2-methylpyrazine (1 eq, 10 mmol, 0.92 mL) were added to 5 mL of acetic anhydride in a microwave tube. The reaction vessel was placed in microwave reactor for 5 hours at 160° C on standard power (300 W). After 5 hours the vessel contents were diluted with dH₂O (30 mL) and extracted with ethyl acetate (2×40 mL). The extracted organic layer was dried with anhydrous MgSO₄, filtered and solvent reduced in vacuo. The crude brown oil was purified using flash column chromatography with a gradient of 1:1 – 2:1, ethyl acetate: n-hexane to afford P3 acetate as a fine yellow solid.

((E)-2-(2-(Pyrazin-2-yl)vinyl)phenyl acetate) (P3 acetate)
P3 acetate was synthesised using General Method XVII and XVIII.

**Yield:** Not calculated for Perkin condensation using reflux. 17% using microwave technology  
**Appearance:** fine yellow powder.  
**Melting Point:** 131-132 °C  
**Rf:** 0.55 (1:1 n-hexane: ethyl acetate)  

¹H NMR (600 MHz, DMSO-d₆): δ 8.96 (d, 1H, J = 1.1 Hz, H₂''), 8.66 (apparent triplet, 1H, J = 1.5 Hz, H₃''), 8.54 (d, 1H, J = 2.4 Hz, H₄''), 7.93 (dd, 1H, J = 7.22, J = 1.5 Hz, H₂'), 7.75 (d, 1H, J = 16 Hz, CH=CH, H₁''), 7.43 (d, 1H, J = 16 Hz, CH=CH, H₁'''), 7.42 (dd, 1H, J = 1 Hz, J = 9 Hz, H₃'), 7.36 (t, 1H, J = 7.6 Hz,H₄'), 7.2 (dd, 1H, J = 8.4 Hz, J = 1.3 Hz, H₃), 2.39 (s, 3H, COOCH₃, H₅''')

¹³C NMR (100 MHz, DMSO-d₆): δ 169.1 (C₆''), 150.8 (C₁''), 149.9 (C₅''), 145.1 (C₃''), 144.6 (C₂''), 144.0(C₄''), 130.4 (C₅), 129.0 (C₂), 128.0 (C₃), 127.4 (C₂),127.2 (C₁), 126.9 (C₄), 123.8 (C₃'), 23.9 (C₅''')

**HRMS:** APCI calculated for C₁₄H₁₃N₂O₂[M+H⁺]: 241.097154, found 241.06916, error + 0.2 ppm

Chapter 10: Experimental
General Method XIX: Hydrolysis of Perkin condensation product P3 acetate to free phenolic P3.

**P3 acetate** was added to a stirring solution of 1:1 ethyl acetate/ethanol (40 mL). 1M NaOH (1-2 mL) was added to this suspension which was left to stir vigorously until completion as indicated by TLC (10-20 minutes). The solvent was then removed *in vacuo*. The crude product was purified using dry loading in methanol over silica gel using gradient elution to remove residual salicaldehyde (9:1 to 1:2 hexane:ethyl acetate).

**(E)-2-(2-(Pyrazin-2-yl)vinyl)phenol (P3)**

P3 was synthesised from **P3 acetate** using General Method XIX.

![Chemical structure of P3](image)

**Yield:** 1.5-2g (< 25%)

**Melting Point:** 206-209 °C

**Appearance:** Bright yellow crystalline solid

**Rf:** 0.45 (1:1; n-hexane: ethyl acetate)

**1H NMR 600 MHz, DMSO-**
\[\delta 9.97 \text{ (bs, 1H, H}_7\text{'')}, 8.70 \text{ (s, 1H, H}_2\text{''}), 8.51 \text{ (apparent s, 1H, H}_4\text{''}), 8.44 \text{ (d, 1H, J = 2.4 Hz, H}_2\text{')}, 8.09 \text{ (d, 1H, J = 16.2 Hz, H}_3\text{')}, 7.62 \text{ (1H, J = 8 Hz, H}_5\text{')}, 7.39 \text{ (d, 1H, J = 16.2 Hz, H}_2\text{')}, 7.15 \text{ (t, 1H, J = 7.2 Hz, H}_3\text{')}, 6.89 \text{ (d, 1H, J = 6.5 Hz, H}_2\text{')}, 6.83 \text{ (1H, J = 6.5 Hz, H}_3\text{')}

**13C NMR (100 MHz, DMSO-**
\[\delta 156.4 \text{ (C}_6\text{'')}, 151.7 \text{ (C}_1\text{''}), 144.9 \text{ (C}_2\text{''}), 144.2 \text{ (C}_2\text{‘’), 143.17 \text{ (C}_3\text{‘’), 130.5 \text{ (C}_3\text{), 130.4 \text{ (C}_1\text{), 128.0 \text{ (C}_3\text{), 124.0 \text{ (C}_2\text{, 123.2 \text{ (C}_1\text{‘’), 119.9 \text{ (C}_3\text{), 116.5 \text{ (C}_2\text{)})}}

**HRMS:** APCI Calculated for C$_{12}$H$_9$N$_2$O[S-H$^+$]: 197.072405, found 197.0236, error + 1.9 ppm

**((E)-Dibenzyl (3-(2-(pyrazin-2-yl)vinyl)phenyl) phosphate) (P3DBP)**

P3DBP was synthesised from P3 and dibenzyl phosphite using General Method XVI.

**Rf:** 0.45 (1:1; n-hexane: ethyl acetate)

**Appearance:** Yellow oil

**Yield:** 60%

**1H NMR (600 MHz, DMSO-**
\[\delta 8.70 \text{ (s, 1H)}, 8.62 \text{ (s, 1H)}, 8.53 \text{ (d, 1H, J = 2.2 Hz)}, 8.04 \text{ (d, 1H, J = 16 Hz)}, 7.91 \text{ (d, 1H, J = 6.7 Hz)}, 7.43 \text{ (d, 1H, J = 16 Hz)}, 7.33 \text{ (complex m, 11H)}, 5.20 \text{ (s, 2H)}, 5.19 \text{ (s, 2H)}

**13C NMR (100 MHz, DMSO-**
\[\delta 150.2, 148.1, 148.0, 144.6, 143.5, 135.5, 135.5, 130.1, 128.5, 128.4, 127.9, 127.7, 127.6, 127.1, 127.0, 126.4, 125.7, 129.7, 69.5, 69.4

**3P NMR (162 MHz, DMSO-**
\[\delta -6.1 ppm

**HRMS:** APCI calculated for C$_{26}$H$_{24}$N$_2$O$_4$P [M+H$^+$], 459.146881; found 459.146820, error + 0.1 ppm

**ESI** calculated for C$_{26}$H$_{22}$N$_2$O$_4$PNa [M +Na$^+$], 481.128765; found 481.128652, error -0.2 ppm

General Method XX: Debenzylation of P3 dibenzyl phosphate to P3P using boron tribromide

Chapter 10: Experimental
P3DBP (200 mg, 0.5 mmol, 1 eq) was weighed and added to a stirring solution of anhydrous toluene (20 mL), under N₂ and on ice water/methanol trough at -10 °C. Boron tribromide 1M in anhydrous toluene (0.5 mmol, 1 eq, 0.5 mL) was added dropwise to the stirring solution while maintaining a temperature of -10 °C. The reaction was warmed to room temperature before bringing the temperature to 80 °C. On completion of the debenzylation after two hours reflux, a yellow precipitate was observed. The reaction vessel was cooled once again to room temperature. An excess of methanol (10mL) was then added. The solvent was then removed in vacuo and left under vacuum conditions at 60 °C to allow for evaporation of the excess boron tribromide. The crude solids were purified using Sephadex gel filtration (Sephadex® G-10 Medium) to afford the pure phosphoric acid derivative as a solid yellow powder.

(E)-2-(2-(Pyrazin-2-yl)vinyl)phenyl dihydrogen phosphate (P3P)

P3 phosphoric acid (P3P) was synthesised from P3DBP using General Method XX.

Yield: 55mg (0.198 mmol) 40%

Appearance: bright yellow powder.

\[ \text{Rf: } 0.05 \text{ (4:1 ethyl acetate: methanol)} \]

\[ \text{1H NMR (600 MHz, DMSO-}d_6\text{): } \delta 8.75 \text{ (apparent singlet, 1H, } H_\text{4''}, \text{ 8.64 (apparent singlet, 1H, } H_\text{3''}, \text{ 8.50 (d, 1H, } J_{2''-3''} = 2.2 \text{ Hz, } H_2'', \text{ 8.04 (d, 1H, } J_{2-1} = 16.4 \text{ Hz, } H_2, \text{ 7.83 (d, 1H, } J_{2-3} = 8.2 \text{ Hz, } H_2, \text{ 7.4 (d, 1H, } J_{1-2} = 15.98 \text{ Hz, } H_1, \text{ 7.44 (d, 1H, } J_{5-4} = 4.6 \text{ Hz, } H_5, \text{ 7.31 (t, 1H, } J_{3''-5''} = 7.3 \text{ Hz, } H_3, \text{ 7.17 (t, 1H, } J_{3-4} = 7.3 \text{ Hz, } H_3, \text{)} \]

\[ \text{13C NMR (100 MHz, DMSO-}d_6\text{): } \delta 151.3 \text{ (C_1''}, \text{ 150.7 (d, } J_{C-C} = 8.1 \text{ Hz), 145.0 (C_3''}, \text{ 144.4 (C_3}, \text{ 143.6 (C_2''}, \text{ 130.7 (C_2}, \text{ 129.2 (C_3}, \text{ 127.7 (d, } J_{C-C} = 7.6 \text{ Hz, C_1}, \text{ 127.1 (C_2}, \text{ 125.6 (C_1}, \text{ 124.2 (C_3}, \text{ 121.2 (C_3}) \]

\[ \text{31P NMR (162 MHz, DMSO-}d_6\text{): } \delta -6.1 \]

\[ \text{15N NMR (41 MHz, DMSO-}d_6\text{): } \delta 341.4 \text{ (N_1}, \text{ 321.0 (N_6}) \]

**HRMS:** APCI Calculated for C_{12}H_{12}N_2O_4P [M+H^+]: 279.05290, found 279.052943, error + 0.1 ppm

**General Method XXI:** Aminocatalytic synthesis of P3 imines using pyrrolidine

2-Nitrobenzaldehyde (1 mmol, 1 eq, 151 mg) and aminopyrazine (1 mmol, 1 eq, 95 mg) was added to 10 mL of ethanol under a nitrogen atmosphere containing 20 mol % pyrrolidine catalyst (0.2 eq, 0.2 mmol, 1µL). The reaction was refluxed at 90 °C for 7 hours. Completion was not achieved. The solvent was reduced in vacuo and imine used prior to further purification

**General Method XXII:** Synthesis of P3 Imines using TiCl₄
TiCl₄ (2.5 mmol, 0.5 eq, 2.5 mL) was added slowly to anhydrous toluene (40 mL) under nitrogenous conditions at 0 °C prior to addition of 2-nitrobenzaldehyde (5 mmol, 1 eq, 755 mg). After stirring for 10 minutes, 2-aminopyrazine (5 mmol, 1 eq, 475 mg) was added. Tri-­‐n-­‐butylamine (15 mmol, 3 eq, 3.57 mL) was added dropwise while ensuring to keep the temperature at 0 °C. The reaction was allowed to stir for 30 minutes at 0 °C prior to warming to room temperature and stirring overnight. The reaction was quenched with sodium bicarbonate (5 mL). The organic layer was diluted with ethyl acetate (20 mL), separated from aqueous layer and dried with anhydrous Na₂SO₄ prior to removal of the solvent in vacuo. The crude imine was used without further purification.

**General Method XXIII: Imine Synthesis using Dean Stark apparatus, ethanol and toluene**

Aminopyrazine (5 mmol, 1 eq, 475 mg) was dissolved in ethanol (5 mL) prior to addition to anhydrous toluene (30 mL) under reflux conditions using the Dean Stark apparatus at 100 °C. The amino pyrazine was allowed to dissolve in the reaction vessel prior to addition of the aldehyde for 10-15 minutes. 2-Nitrobenzaldehyde (5 mmol, 1 eq, 755 mg) was subsequently added and the mixture was refluxed for 48-72 hours until formation of imine was evident on TLC. The solvent was reduced in vacuo and imine used without further purification.

(E)-1-(2-Nitrophenyl)-N-(pyrazin-2-yl)methanimine (149)

149 was synthesised using General Method XXI, XXII and XXIII.

**Rₚ:** 0.64 (1:1 ethyl acetate: n-hexane)

**Yield:** Not calculated. *Imine used without further purification.*

**HRMS:** (APCI): Calculated for C₁₁H₉N₄O₂, [M+H⁺] 229.070202, found 229.071942, error + 0.3 ppm.

2-(2-Nitrophenyl)-4-oxo-1-(pyrazin-2-yl)azetidin-3-yl acetate (151 cis)

141 was synthesised from 149 using General Method IV in 9:1 *cis:trans* ratio

**Rₚ:** 0.47 (3:2 ethyl acetate: n- hexane)

**Appearance:** yellow powder

**Yield for 151:** (Imine 149 synthesised using Dean Stark, toluene and ethanol as co-solvent): 210 mg (0.64 mmol) 24%

**Yield for 151:** (Imine 149 synthesised using aminocatalysis): 160 mg (0.5 mmol) 25%

**Yield for 151:** (Imine 149 synthesised using TiCl₄): 20 mg (0.06 mmol) 1.21%

**¹H NMR (600 MHz, CDCl₃):** δ 8.42 (d, 1H, J = 2.9 Hz), 8.26 (dd, 1H, J = 7.2 Hz, 2.2 Hz), 8.2 (apparent multiplet, 1H), 7.65 (td, 1H, J = 2.2 Hz, J = 6.7 Hz), 7.58 (td, 1H, J = 2.2 Hz, J = 6.7 Hz)
Hz), 7.4 (d, 1H, J = 7.4 Hz), 6.46 d, 1H, J = 6.2 Hz), 6.28 (d, 1H, J = 6.2 Hz), 1.89 (s,3H, OCOCH₃)

13C NMR (100 MHz, CDCl₃): δ 162.4 (β-lactam C=O), 147.6, 145.7, 142.4, 141.2, 137.0, 134.0, 129.3, 128.8, 125.5, 76.1, 58.0, 31.1, 19.8

HRMS: APCI calculated for C₁₅H₁₃N₄O₅ [M+H⁺] 329.088046; found 329.087494, error + 1.7 ppm
Appendices
Appendix 1: Additional material related to Chapter 2: Synthesis and chemistry of trans β-lactam racemates

1H NMR of β-lactam H₃ and H₄ region (δ 4.1 - 6.0 ppm) for crude 3-acetoxy-β-lactam 17a in CDCl₃ at 400 MHz.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H₃ &amp; H₄ region</th>
<th>Cis: trans ratio</th>
<th>Entry</th>
<th>H₃ &amp; H₄ region</th>
<th>Cis: trans ratio</th>
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1 Ratios calculated via integration of H₃ and H₄ and normalization to 100% for cis and trans H₃ signals. J = 1.8-2 Hz for trans isomer J = 4-5 Hz for cis isomer. Trans H₃ & H₄ signals appear at δ 4.86 and 5.37 ppm respectively. Cis H₃ & H₄ signals appear at δ 5.29 and 5.90 respectively.
$^1$H NMR of $\beta$-lactam H$_3$ and H$_4$ region (δ 4.1-6.0 ppm) for $\beta$-lactam 18a in CDCl$_3$ at 400 MHz.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H$_3$ &amp; H$_4$ region</th>
<th>Cis: trans ratio$^1$</th>
<th>Entry</th>
<th>H$_3$ &amp; H$_4$ region</th>
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$^1$ Ratios calculated via integration of H$_3$ and H$_4$ and normalisation to 100% for cis and trans H$_3$ signals. J = 1.8-2 Hz for trans isomer J = 4-5 Hz for cis isomer. Trans H$_3$ & H$_4$ signals appear at δ 4.83 and 5.32 ppm respectively. Cis H$_3$ & H$_4$ signals appear at δ 5.26 and 5.90 respectively.
Stereochemical outcomes for Staudinger reactions 1-3 using optimised Staudinger conditions for isolation of 3-acetoxy-63a

<table>
<thead>
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1Calculated via integration of H at δ 5.32 (trans) and δ 5.37 ppm (cis) followed by normalization to 100%. J values for trans isomer = 1.8 Hz and cis isomer = 3 Hz.

Reflux versus microwave (MAOS) cis:trans yields for 77 in crude Reformatsky products calculated using 1H NMR spectrum in CDCl3 at 400 MHz.

<table>
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<tr>
<th>Reflux</th>
<th>MAOS</th>
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<tr>
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<td>Cis: trans ratio1</td>
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<tr>
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<td>76:23</td>
</tr>
</tbody>
</table>

1 Ratios are calculated by integration of H4 doublets followed by normalisation to 100%. 2 Coupling constants are J = 5.5 Hz for cis isomer and J = 2.2 Hz for trans isomer.
Appendix 2: Additional material related to Chapter 8: Physiochemical analysis, prodrug synthesis and structure optimisation of pyrazinib

NMR spectroscopy data of P3P isolated using POCl₃/TEA: A: ¹H NMR in DMSO-d₆ at 600 MHz integrating TEA: P3P and normalising to 100% to afford a ratio of 98:2. B: Enlargement of P3P aromatic region from δ 9.5 -7 ppm at 600 MHz in DMSO-d₆. C: ³¹P for P3P at 162 MHz in DMSO-d₆.
Spontaneous hydrolysis of P3DMP (1) mediated by atmospheric water to the monomethyl phosphate derivative. Ionisation of 2 yields phosphate anion 3a in equilibrium with resonance structure 3b (The electrophilic phosphate of the dimethyl derivative (1) undergoes nucleophilic attack by water to yield a methanol by-product and a monomethylated phosphate ester. The monomethylated ester is acidic in nature and has potential for ionisation to 3a, the monomethylated phosphate anion in equilibrium with 3b the resonance delocalised phosphate anion. Resonance delocalisation of the O- negative charge reduces the electrophilicity of the phosphate atom, thus preventing a second demethylation mediated by water as a weak nucleophile to the free phosphoric acid.

Solubility data for P3 and phosphate prodrug P3P for three independent studies with average solubility reported as μg/mL ± SEM

<table>
<thead>
<tr>
<th>Solubility data for P3</th>
<th>Solubility data for P3P</th>
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<tbody>
<tr>
<td>Replicate 1</td>
<td>0.046 μg/mL</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>0.067 μg/mL</td>
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<tr>
<td>Replicate 3</td>
<td>0.040 μg/mL</td>
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<tr>
<td><strong>Average solubility ± SEM</strong></td>
<td><strong>0.051 ± 0.014 μg/mL</strong></td>
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</table>
Appendix 3: Characterisation of previously reported intermediates and racemates

3-((Tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (42)\(^{286}\)

42 was synthesised from 3-hydroxy-4-methoxybenzaldehyde using General Method I, Route B.

![Chemical Structure]

**Yield:** 2.6g (8.8 mmol) 88%

**Appearance:** Yellow oil

**R\(_f\):** 0.78 (1:1; n-hexane: ethyl acetate)

**\(^1\)H NMR (400 MHz, CDCl\(_3\)):** \(\delta\) 9.77 (s, 1H, H\(_1\)), 7.43 (dd, \(J = 8.5\) Hz, \(J = 2\) Hz, 1H,H\(_2\)), 7.33 (d, 1H, J = 2.1 Hz, H\(_1\)), 6.92 (d, 1H, J = 8.31 Hz, H\(_3\)), 3.86 (s, 3H, OCH\(_3\), H\(_6\)), 0.973 (s, 9H, C(CH\(_3\))\(_3\), H\(_{9,10,11}\)), 0.86 (s, 6H, SiCH\(_3\), H\(_{7,8}\))

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\)):** \(\delta\) 190.7 (C\(_1\)), 156.6 (C\(_4\)), 145.6 (C\(_5\)), 130.1, 126.5, 120.0, 111.2, 55.5, 25.6, 25.5, 18.4, 18.0

(4-Benzoxycarbonylaminophenyl)acetic acid\(^{405}\)

(4-Benzoxycarbonylaminophenyl)acetic acid was synthesised using General Method II.

![Chemical Structure]

**Yield:** 1.03g (3.7 mmol) 75%

**Appearance:** off white/yellow amorphous powder

**R\(_f\):** 0.42 (1:1 n-hexane: ethyl acetate)

**\(^1\)H NMR (400 MHz, DMSO-\(d_6\)):** \(\delta\) 9.74 (bs, 1H, H\(_9\)), 7.43 (m, 5H, H\(_{1,5,10,14}\)), 7.32 (d, 2H, H\(_{16}\)), 7.15 (d, 2H, H\(_{11,13}\)), 5.14 (s, 2H, H\(_3\)), 3.46 (s, 2H, H\(_6\))

**\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)):** \(\delta\) 173.6, 153.9, 143.0, 138.0, 137.1, 130.2, 129.5, 128.9, 128.6, 128.5, 127.1, 126.9, 118.6, 63.4, 66.2
Numbering nomenclature for imines

$$\begin{align*}
\text{Imine code} & \quad R_1 & \quad R_2 \\
44 & \quad H & \quad \text{OCH}_3 \\
45 & \quad \text{F} & \quad \text{OCH}_3 \\
46 & \quad \text{OH} & \quad \text{OCH}_3 \\
47 & \quad \text{OTBDMS} & \quad \text{OCH}_3 \\
48 & \quad \text{Cl} & \quad \text{OCH}_3 \\
49 & \quad \text{CH}_3 & \quad \text{OCH}_3 \\
50 & \quad H & \quad \text{SCH}_3 \\
51 & \quad H & \quad \text{F} \\
52 & \quad H & \quad \text{NO}_2 \\
53 & \quad \text{Br} & \quad \text{OCH}_3 \\
54 & \quad \text{NO}_2 & \quad \text{OCH}_3 \\
55 & \quad H & \quad \text{OCH}_2\text{CH}_3 \\
56 & \quad H & \quad \text{SCH}_2\text{CH}_3 \\
\end{align*}$$

\((E)-1-(4\text{-Methoxyphenyl})-N-(3,4,5\text{-trimethoxyphenyl})\text{methanimine (44)}\)\(^{378}\)

44 was synthesised from 4-methoxybenzaldehyde using General Method III, Method A.

Yield: 88%

Appearance: bright yellow crystalline solid

Melting Point: 119.1-119.5 °C

R\(_f\): 0.6. (1:1; \(n\)-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, \(\text{CDCl}_3\)): \(\delta\) 8.4 (s, 1H, \(H_7\)), 7.8 (d, 2H, \(J=8.7\) Hz, \(H_{3&4}\)), 6.9 (d, 2H, \(J=8.7\) Hz, \(H_{5&6}\)), 6.45 (s, 2H, \(H_1&H_2\)), 3.83 (s,6H, \(H_{8&10}\)), 3.81 (s, 3H, \(H_9\)), 3.71 (s, 3H, \(H_9\))

\(^1\)C NMR (100 MHz, \(\text{CDCl}_3\)): \(\delta\) 159.3, 153.6, 139.6, 114.1, 97.7, 60.9, 56.1, 55.3

\((E)-1-(3\text{-Fluoro-4-methoxyphenyl})-N-(3,4,5\text{-trimethoxyphenyl})\text{methanimine (45)}\)\(^{400}\)

45 was synthesised from 3-fluoro-4-methoxybenzaldehyde using General Method III, Method A.

Yield: 90%

Appearance: bright yellow crystalline solid

R\(_f\): 0.40 (1:1; \(n\)-hexane: ethyl acetate)

Melting Point: 107.1-108.6 °C

\(^1\)H NMR (400 MHz, \(\text{CDCl}_3\)): \(\delta\) 8.35 (1H, s, \(H_7\)), 7.98 (d, 1H, \(J=2\) Hz, \(H_3\)), 7.75 (1H, apparent doublet, \(J=8.2\) Hz, \(H_2\)), 6.99 (d, 1H, \(J=9\) Hz, \(H_3\)), 6.48 (s, 2H, \(H_1&H_2\)), 3.96 (s,\(H_9\)), 3.88 (s,6H, \(H_{8&10}\)), 3.85 (s, 3H, \(H_9\))

\(^1\)C NMR (100MHz, \(\text{CDCl}_3\)): \(\delta\) 159.3, 153.6, 139.6, 114.1, 97.7, 60.9, 56.1, 56.

\(^19\)F NMR (376 MHz, \(\text{CDCl}_3\)): \(\delta\) -134.3

\((E)-2\text{-Methoxy-5-(((3,4,5\text{-trimethoxyphenyl})imino)methyl)phenol (46)}\)\(^{378}\)

Appendices
was synthesised from 3-hydroxy-4-methoxybenzaldehyde using General Method III, Method A.

Appearance: Bright yellow crystalline solid

Melting Point: 165 °C

Rf: 0.5 (1:1; n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.33 (s,1H, H$_7$), 8.00 (bs, OH, H$_{10}$), 7.50 (d,1H, $J=1.8$ Hz, H$_3$), 7.34 (dd, 1H, $J=8.5$ Hz, $J=1.8$ Hz, H$_4$), 6.92 (d, 1H, $J=8.5$ Hz, H$_5$), 6.45 (s,2H, H$_{1&2}$), 3.95 (s,2H, H$_{11}$), 3.86 (s,3H, H$_9$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 159.2, 153.5, 149.3, 148.1, 147.4, 146.0, 129.0, 122.3, 113.8, 110.3, 98.2, 61.0, 56.1

(E)-1-(3-((Tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-N-(3,4,5 trimethoxyphenyl)methanimine (47)

47 was synthesised from 42 using General Method III, Method A.

Appearance: yellow/brown powder

Rf: 0.7 (1:1; n-hexane: ethyl acetate)

Melting Point: 79-82 °C

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.34 (s, 1H, H$_7$), 7.43 (s, 1H, H$_4$), 7.36 (d, 1H, $J=8$ Hz, H$_3$), 6.9 (d, 1H, $J=8.6$ Hz, H$_5$), 6.45 (s, 2H, H$_{1&2}$), 3.89 (s, 6H, H$_{8&10}$), 3.87 (s, 3H, H$_9$), 3.85 (s, 3H, H$_{11}$), 1.01 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 6H, C(CH$_2$)$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 159.3, 154.0, 153.5, 153.2, 148.3, 145.3, 129.4, 123.8, 120.7, 111.4, 98.1, 61.0, 56.1, 55.5, 25.7, 18.4

(E)-1-(3-Chloro-4-methoxyphenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (48)

48 was synthesised from 3-chloro-4-methoxybenzaldehyde using General Method III, Method B.

Appearance: Bright yellow crystalline solid

Melting Point: 99.3-100.7 °C

Rf: 0.66 (1:1; n-hexane: ethyl acetate); 0.51 (2:1; n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.66 (1H, s, H$_7$), 7.31 (dd, 1H, J=12 Hz, J=1.8 Hz, H$_3$), 7.54 (d, 1H, J=8.6 Hz, H$_5$), 7.03 (apparent triplet, 1H, J=8.4 Hz, H$_4$), 6.47 (s, 2H, H$_{1&2}$), 3.95 (s, 3H, H$_{11}$), 3.89 (s, 6H, H$_{8&10}$), 3.86 (s, 3H, H$_9$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 157.5, 157.4, 153.5, 153.2, 148.3, 145.3, 129.4, 123.8, 120.7, 111.4, 98.1, 61.0, 56.3, 56.1

(E)-1-(4-Methoxy-3-methylphenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (49)

49 was synthesised from 3-methyl-4-methoxybenzaldehyde and 3,4,5-trimethoxyaniline by General Method III Method B.

Appearance: Yellow crystals

Melting Point: 140-141 °C

Rf: 0.76 (1:1; n-hexane: ethyl acetate)
\( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.40 (s, 1H, H\(_7\)), 7.68 (d, 1H, \( J=8 \) Hz, H\(_3\)), 6.91 (d, 1H, \( J=8 \) Hz, H\(_3\)), 6.51 (s, 2H, H\(_1&2\)), 5.95 (s, 1H), 3.91 (s, 6H, H\(_8&10\)), 3.88 (s, 3H, H\(_9\)), 3.82 (s, 3H, H\(_9\)), 2.30 (s, 3H, CH\(_3\))

\( ^13C \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 160.6, 159.6, 142.9, 135.6, 130.43, 129.0, 127.4, 125.2, 109.7, 98.2, 92.71, 61.1, 61.2, 56.1, 56.0, 55.6, 24.0

(E)-1-(4-(Methylthio)phenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (50) was synthesised from (4-(methylmercapto)benzaldehyde) using General Method III, Method B.

**Yield:** 95%

**Appearance:** Pale yellow solid.

**Melting Point:** 119-121 °C

R\(_f\): 0.55 (1:1; n-hexane: ethyl acetate)

\( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.43 (s, 1H, H\(_7\)), 7.82 (d, 2H, \( J=9 \) Hz, H\(_5&6\)), 7.3 (d, 2H, \( J=7.7 \) Hz, H\(_5&6\)), 6.51 (s, 2H, H\(_1&2\)), 3.92 (s, 6H, H\(_8&10\)), 3.87 (s, 3H), 2.55 (s, 3H, S(CH\(_3\)))

\( ^13C \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 158.9, 153.6, 147.9, 143.4, 142.9, 136.4, 132.62, 129.94, 129.0, 125.7, 98.3, 61.1, 56.2, 56.1, 45.0

IR \( \nu_{\text{max}}(\text{ATR}) \): 1583 cm\(^{-1}\) (C=N)

(E)-1-(4-Fluorophenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (51) was synthesised from 4-fluorobenzaldehyde using General Method III, Method B.

**Yield:** 95%

**Appearance:** Pale yellow powdery solid

R\(_f\): 0.7 (1:1 n-hexane: ethyl acetate)

\( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.45 (s, 1H, H\(_7\)), 7.92 (apparent multiplet, 2H, H\(_5&6\)), 7.18 (apparent triplet, 2H, H\(_3&4\)), 6.50 (s, 2H, H\(_1&2\)), 3.93 (s, 6H, H\(_8&10\)), 3.89 (s, 3H)

\( ^13C \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 158.2, 153.5, 147.7, 143.4, 142.9, 136.4, 132.62, 129.94, 129.0, 125.7, 98.3, 61.1, 56.2, 56.1, 45.0

\( ^19F \) NMR (376 MHz, CDCl\(_3\)): \( \delta \) -107.9

(E)-1-(4-Nitrophenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (52) was synthesised from 4-nitrobenzaldehyde and 3,4,5-trimethoxyaniline by General Method III, Method B.

**Yield:** > 90%

**Appearance:** pale yellow solid

R\(_f\): 0.51 (1:1 n-hexane: ethyl acetate)

\( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.59 (s, 1H, H\(_7\)), 8.36 (d, 2H, \( J=9.1 \) Hz, H\(_5&6\)), 8.1 (d, 2H, \( J=9.1 \) Hz, H\(_5&6\)), 6.58 (s, 2H, H\(_1&2\)), 3.95 (s, 6H, H\(_8&10\)), 3.91 (s, 3H, H\(_6\))

\( ^13C \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 156.5, 149.0, 146.7, 141.4, 129.3, 124.1, 98.4, 61.1, 56.2

\( ^{19}F \) NMR (376 MHz, CDCl\(_3\)): \( \delta \) -107.9

(E)-1-(3-Bromo-4-methoxyphenyl)-N-(3,4,5-trimethoxyphenyl)methanimine (53) was synthesised from 3-bromo-4-methoxybenzaldehyde using General Method III, Method B.

**Yield:** > 90%

**Appearance:** Bright yellow solid

R\(_f\): 0.65 (1:1 n-hexane: ethyl acetate)
\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.36 (s, 1H, H\(_7\)), 8.30 (s, 1H, H\(_4\)), 8.04 (d, 2H, \(J = 10.8\) Hz, H\(_5\)), 7.12 (d, 1H \(J = 9\) Hz, H\(_3\)), 6.44 (s, 2H, H\(_1\&2\)), 3.97 (s, 3H, H\(_9\)), 3.84 (s, 6H, H\(_8\&10\)), 3.80 (s, 3H, H\(_6\))

\(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 151.4, 150.1, 148.9, 142.2, 135.1, 132.1, 128.9, 124.2, 108.9, 93.5, 56.2, 53.7, 52.1, 51.4

\((E)-1-(4-Methoxy-3-nitrophenyl)-N-(3,4,5-trimethoxyphenyl) methanimine (54)\)

54 was synthesised from 4-methoxy-3-nitrobenzaldehyde using General Method III, Method B.

Yield: > 90%

Appearance: Yellow solid

Melting Point: 145-149 °C

Rf: 0.55 (1:1; n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.36 (s, 1H, H\(_7\)), 8.30 (s, 1H, H\(_4\)), 8.04 (d, 2H, \(J = 10.8\) Hz, H\(_5\)), 7.12 (d, 1H \(J = 9\) Hz, H\(_3\)), 6.44 (s, 2H, H\(_1\&2\)), 3.97 (s, 3H, H\(_9\)), 3.84 (s, 6H, H\(_8\&10\)), 3.80 (s, 3H, H\(_6\))

\(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 151.4, 150.1, 148.9, 142.2, 135.1, 132.1, 128.9, 124.2, 108.9, 93.5, 56.2, 53.7, 52.1, 51.4

\((E)-1-(4-Ethylphenyl)-N-(3,4,5-trimethoxyphenyl) methanimine (55)\)

55 was synthesised from 4-methoxy-3-nitrobenzaldehyde using General Method III, Method A.

Yield: > 90%

Appearance: white solid

Rf: 0.8 (1:1; n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.41 (s, 1H, H\(_7\)), 7.94 (d, 2H, \(J = 6.6\) Hz), 6.97 (d, 2H, \(J = 10\) Hz), 6.59 (s, 2H, H\(_1\&2\)), 3.90 (s, 6H, H\(_8\&10\)), 3.85 (s, 3H, H\(_9\)), 3.71 (q, 2H, \(J = 8\) Hz, CH\(_2\)), 1.44 (t, 3H, \(J = 8.1\) Hz).

\(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 159.7, 154.1, 131.7, 114.9, 98.2, 63.9, 61.0, 58.4, 56.2, 56.0, 18.4, 14.6

\((E)-1-(4-(ethylthio)phenyl)-N-(3,4,5-trimethoxyphenyl) methanimine (56)\)

56 was synthesised from 4-methoxy-3-nitrobenzaldehyde using General Method III, Method A.

Yield: > 90%

Appearance: bright orange solid

Rf: 0.67 (1:1; n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.42 (s, 1H, H\(_7\)), 7.84 (d, 2H, \(J = 9\) Hz, H\(_3\&4\)), 7.34 (d, 2H, \(J = 9\) Hz, H\(_5\&6\)), 6.54 (s, 2H, H\(_1\&2\)), 3.89 (s, 6H, H\(_8\&10\)), 3.89 (s, 3H, H\(_9\)), 3.02 (q, 2H, \(J = 9\) Hz, SCH\(_2\)), 1.37 (t, 3H, \(J = 8.2\) Hz, CH\(_3\))

\(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 158.9, 153.6, 130.1, 129.4, 127.2, 126.4, 98.3, 61.0, 56.2, 26.5, 25.9, 14.1, 13.8

Numbering nomenclature for 3-acetoxy β-lactams
2-Azetidinone

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*one enantiomer shown

2-(3-((Tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (16a) 378

16a was synthesised from 47 using General Method IV.

Yield: 1.46g (0.3 mmol) 60%

Melting Point: 133.4-138.9 °C

Appearance: yellow powder

R₁: 0.63 (2:1; n-hexane: ethyl acetate; 0.6 (1:1; n-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.91 (dd, 1H, J=8.7 Hz, J= 2.5 Hz, H₂''), 6.85 (d, 1H, J= 8.2 Hz, H₃'''), 6.78 (d,1H, J=2.5 Hz, H₄'''), 6.52 (s, 2H, H₁₃&₁₄), 5.34 (1H, d, J=1.5 Hz, H₅'), 4.80 (d, 1H, J=1.5 Hz, H₆'), 3.79 (s, 3H, H₇), 3.74 (s, 3H, H₇'''), 3.68 (s, 6H, H₈&₉), 2.18 (s,3H, H₁₀), 0.91 (s,9H, t- butyl C(CH₃)₃), 0.06 (s, 3H, Si-CH₃), 0.04 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): 169.6 (C₁₁), 161.6 (C₁), 153.4 (C₈&₉), 151.6, 145.8, 142.5, 135.0, 133.0, 127.4, 119.9, 119.0, 112.5, 95.4 (C₁₃&₁₄), 82.3 (C₃), 63.6 (C₆), 62.0, 56.0, 55.4, 20.5 (C₁₀), 18.5 (C(CH₃)₃), 0.9 (Si-CH₃), -4.7 (Si-CH₃)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (17a) 378

17a was synthesised from 44 using General Method IV.

Yield (preliminary): 926 mg (2.3 mmol) 46%, isolated as a 1:1 trans:cis isomeric mixture.

Yield (optimised) 1.16 g (2.9 mmol) 58%, 95:5 trans:cis

Appearance: white crystalline solid

R₁: 0.41 (1:1; n-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ (isomeric mixture): 7.29 (d, 2H, J = 8.5 Hz, H₂₃&₂₄: cis), 7.24 (d, 2H, J = 8.5 Hz, H₃₃&₃₄: trans), 6.92 (d, 2H, J = 8.5 Hz, H₃₃&₃₄: cis), 6.88 (d, 2H, J = 8.2 Hz, H₃₃&₃₄: trans ), 6.57 (s, 2H, H₁₃&₁₄: cis ), 6.53 (s, 2H H₁₃&₁₄: trans), 5.89 (d, 1H, J= 4.5 Hz, H₅, Appendices 410
cis), 5.37 (d, 1H, J = 1.8 Hz, H₂, trans), 5.29 (d, 1H, J = 4.5 Hz, H₄, cis), 4.86 (d, 1H, J = 1.8 Hz, H₅, trans), 3.77 (s, 3H), 3.76 (s, 3H), 3.76, (s, 3H), 3.72 (s, 6H), 3.69, (s, 6H, H₃&₈), 2.21 (s, 3H, H₁₀)

¹H NMR (400 MHz, CDCl₃): δ (trans) 7.28 (d, 2H, J = 8.6 Hz, H₂ & H₂'), 6.90 (d, 2H, J = 8.6 Hz, H₃ & H₃'), 6.52 (s, 2H, H₁ & H₁'), 5.36 (d, 1H, J = 1.8 Hz, H₂), 4.85 (d, 1H, J = 1.8 Hz, H₃), 3.80 (s, 3H, H₅), 3.79 (s, 3H, H₆), 3.75 (s, 3H, H₇), 2.03 (s, 3H, H₁₀)

¹³C (100 MHz, CDCl₃): δ (trans) 161.7 (C₁₁), 160.2 (C₂), 153.5 (C₃ & H₅'), 135.0, 133.0, 129.4, 128.0, 127.7, 127.0, 114.6, 95.4 (C₇ & H₇'), 82.5 (C₃), 63.7 (C₁₁), 61.0 (C₆), 56.0 (C₇₈₉), 55.4 (C₇'), 20.5 (C₁₀)

IR ν<sub>max</sub> (ATR): 1746 cm⁻¹ (β-lactam C=O)

2-(3-Fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (18a)

18a was synthesised from 45 using General Method IV.

Yield: 1.22 g (2.9 mmol) 58%, 96:4 cis:trans

R<sub>ç</sub>: 0.4 (1:1; n-hexane: ethyl acetate)

Appearance: white powder

¹H NMR (400 MHz, CDCl₃): δ 7.08 (apparent d, 2H, J = 9 Hz, H₀ & H₂'), 6.96 (t, 1H, J = 8.3 Hz, H₂'), 6.51 (s, 2H, H₁ & H₁'), 5.31 (d, 1H, J = 1.3 Hz, H₃), 4.81 (d, 1H, J = 1.3 Hz, H₄), 3.88 (s, 3H, H₅), 3.76 (s, 3H, H₆), 3.69 (s, 3H, H₇), 2.17 (s, 3H, H₁₀)

¹³C (100 MHz, CDCl₃): δ 169.7 (C₁₁), 161.4 (C₂), 153.4 (C₃ & H₅'), 132.7, 127.8, 122.2, 114.1, 113.8, 95.3 (C₇ & H₇'), 82.5 (C₃), 63.0 (C₁₁), 61.0, 56.3 (C₇₈₉), 55.9, 20.3 (C₁₀)

¹⁹F NMR (376 MHz, CDCl₃): δ -133.13

2-(3-Chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidin-3-yl acetate (19a)

19a was synthesised from 48 using General Method IV.

Yield: 1.63g (3.6 mmol) 72%

R<sub>ç</sub>: 0.13 (2:1; n-hexane:ethyl acetate)

Appearance: white solid powder

Melting Point: 115 °C

¹H NMR (400 MHz, CDCl₃): δ 7.37 (apparent s, 1H, H₀), 7.20 (apparent d, 1H, J=8.0 Hz, H₂'), 6.91 (d, 1H, J=8.0 Hz H₂'), 6.49 (s,2H, H₃ & H₅'), 5.31 (apparent s, 1H, H₄), 4.80 (apparent s, 1H, H₄), 3.87 (s, 3H, H₅), 3.74 (s, 3H, H₆), 3.69 (s, 3H, H₇), 2.17 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 169.6 (C₁₁), 161.5 (C₂), 155.7, 153.5 (C₃ & H₅'), 135.2, 132.7, 128.3, 128.1, 125.8, 123.4, 120.0, 118.8, 112.5, 95.3 (C₇ & H₇'), 82.4 (C₃), 63.1 (C₁₁), 60.9, 56.2, 56.1, 20.0 (C₁₀)

IR ν<sub>max</sub> (ATR): 1746 cm⁻¹ (β-lactam C=O)

2-(4-Methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (20a)

20a was synthesised from 49 using General Method IV.

Yield: 2.03 g (4.89 mmol) 49 %

Melting Point: 59-61 °C

R<sub>ç</sub>: 0.38 (2:1; n-hexane:ethyl acetate)

Appearance: yellow solid powder

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2-(4-(Methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (22a)\textsuperscript{378}

22a was synthesised from 50 using General Method IV.

**Yield:** 700 mg (1.79 mmol) 36%
**Appearance:** white solid powder
**Melting Point:** 121 – 122 °C.

**H NMR (400 MHz, CDCl\textsubscript{3}):** δ 7.29 (d, J = 6 Hz, H\textsubscript{2'}&6'), 6.25 (d, 2H, J = 8 Hz, H\textsubscript{3'}&5'), 5.35 (d, 1H, J = 1.9 Hz, H\textsubscript{3}), 4.92 (d, 1H, J = 2.0 Hz, H\textsubscript{4}), 3.72 (s, 6H, H\textsubscript{2}$_{\text{acetoxy}}$), 2.49 (s, 3H, H\textsubscript{3}), 2.20 (s, 3H, H\textsubscript{10})

**C NMR (100 MHz, CDCl\textsubscript{3}):** δ 169.8 (C\textsubscript{4}), 161.7, 153.5 (C\textsubscript{1'}&3'), 140.1, 132.9, 131.67, 130.02, 129.1, 128.2, 126.89, 125.3, 125.2, 95.4 (C\textsubscript{1'}&3'), 83.5 (C\textsubscript{5}'), 63.7 (C\textsubscript{4}), 60.1, 56.1, 20.52, 15.5

**IR ν\textsubscript{max} (ATR):** 1720 cm\textsuperscript{-1} (β-lactam C=O)

2-(4-(5-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (89a)

89a was synthesised from 51 using General Method IV.

**Yield:** 1.03g (2.65 mmol) 30%
**Appearance:** yellow powder
**Melting Point:** 52-56 °C

**H NMR (400 MHz, CDCl\textsubscript{3}):** δ 8.6 Hz, 2H, H\textsubscript{2'}&6'), 6.52 (s, 2H, H\textsubscript{1'}&3'), 5.35 (d, 1H, J = 1.9 Hz, H\textsubscript{3}), 4.92 (d, 1H, J = 2.0 Hz, H\textsubscript{4}), 3.72 (s, 6H, H\textsubscript{2}$_{\text{acetoxy}}$), 2.22 (s, 3H, H\textsubscript{10})

**C NMR (100 MHz, CDCl\textsubscript{3}):** δ 169.5 (C\textsubscript{4}), 164.2 (C\textsubscript{2}), 161.7, 153.5 (C\textsubscript{1'}&3'), 134.8 (C\textsubscript{5}'), 133.2 (C\textsubscript{6}), 128.0, 116.4 (C\textsubscript{2'}&6'), 116.2 (C\textsubscript{3'}&5'), 95.5 (C\textsubscript{1'}&3'), 83.9 (C\textsubscript{3}), 65.1 (C\textsubscript{4}), 61.0 (C\textsubscript{5}), 56.1 (C\textsubscript{3})

**F NMR (376 MHz, CDCl\textsubscript{3}):** δ -111.7

**IR ν\textsubscript{max} (ATR):** 1750 cm\textsuperscript{-1} (β-lactam C=O)

2-(4-(Nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (90a)

90a was synthesised from 52 using General Method IV.

**Yield:** 610 mg (1.48 mmol) 37%
**Appearance:** white solid powder
**Melting Point:** 66-68 °C

**H NMR (400 MHz, CDCl\textsubscript{3}):** (mixture of cis:trans isomers; 44:66) δ 8.32 (d, 2H, J = 8.1 Hz, (trans), H\textsubscript{2'}&6'), 8.27 (d, 1.4H, J = 8.6 Hz, (cis) H\textsubscript{2'}&6'), 7.59 (d, 2H, J = 8.1 Hz, (trans) H\textsubscript{2'}&6'), 7.54 (d, 1.4H, J = 8.6 Hz, (cis) H\textsubscript{2'}&6'), 6.54 (s, 1.4H, (cis) H\textsubscript{1'}&3'), 6.49 (s, 2H, (trans) H\textsubscript{1'}&3'), 6.03 (d, 0.44H, J = 4.7 Hz, (cis), H\textsubscript{5}), 5.49 (d, 0.44H, J = 4.7 Hz, (cis), H\textsubscript{5}), 5.29 (d, 0.66H, J = 1.7 Hz,
(trans), H₂), 5.04 (d, 0.66H, J = 1.7 Hz, (trans) H₂), 3.80 (s, 1.6H, (cis), H₈), 3.79 (s, 3H, (trans), H₈), 3.73 (s, 6H, (trans), H₃₇₆), 2.25 (s, 3H, H₁₀) ¹³C NMR (100 MHz, CDCl₃): δ 164.0 (C₀), 161.9 (C₂), 153.8 (C₇₈₉), 142.3, 132.3, 128.9, 127.4, 124.5, 123.9, 116.2, 100.2, 95.4 (C₉₆₈), trans), 95.1 (C₇₈₉, cis), 85.9 (C₃, trans), 82.3 (C₃, cis), 63.1 (C₁), 61.0, 60.9, 56.2, 56.1, 20.5 (C₁₀, trans), 19.9 (C₁₀, cis)

IR νₓᵧ₉ (ATR): 1751 cm⁻¹ (β-lactam C=O), 1506.2 cm⁻¹ (N–O)

2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (21a)
21a was synthesised from 53 using General Method IV.
Yield: 2.39g (4.79 mmol) 63%

Appearance: off white solid

Melting Point: 98-101 °C

IR: 0.45 (1:1; n-hexane:ethyl acetate)

Appearance: white solid powder ¹H NMR (400 MHz, CDCl₃): δ 7.57 (apparent s, 1H, H₂⁻⁻), 7.27 (d, 1H, J = 8 Hz, H₃⁻⁻), 6.91 (d, 1H, J = 8 Hz, H₃⁻⁻), 6.52 (s, 1H, H₁⁻), 5.34 (apparent singlet, 1H, H₂), 4.81 (apparent singlet, 1H, H₃), 3.91 (s, 3H, H₄), 3.75 (s, 3H, H₅), 3.71 (s, 6H, H₆₇₈), 2.16 (s, 3H, H₁₀) ¹³C NMR (100 MHz, CDCl₃): δ 169.7 (C₁₀), 161.3 (C₂), 156.6 (H₂⁻⁻), 153.3, 135.9, 132.7, 131.4, 128.4, 126.8, 112.5, 95.7 (C₇₈₉), 82.6 (C₃), 65.8 (C₄), 62.8, 60.8, 56.5 (C₉₆₈), 20.9 (C₁₀)

IR νₓᵧ₉ (ATR): 1760 cm⁻¹ (β-lactam C=O)

4-(4-Ethoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (65a)
65a was synthesised from 55 using General Method IV.
Yield: 0.91g (2.25 mmol) 45%

Appearance: off white solid ¹H NMR (400 MHz, CDCl₃): δ 7.27 (d, 2H, J = 8.8 Hz, H₂⁻⁻), 6.90 (d, 2H, J = 8.8 Hz, H₂⁻⁻), 6.53 (s, 2H, H₁⁻), 5.37 (apparent singlet, 1H, H₂), 4.85 (apparent singlet, 1H, H₃), 4.01 (q, 2H, J = 6.3 Hz, OCH₂), 3.76 (s, 3H, H₄), 3.69 (s, 6H, H₆₇₈), 2.12 (s, 3H, H₁₀), 1.40 (t, 3H, J = 6.3 Hz CH₃ of OCH₂CH₃)
¹³C NMR (100 MHz, CDCl₃): δ 169.8, 161.8, 159.6, 153.5, 133.1, 129.1, 127.7, 126.9, 114.9, 95.2, 82.4, 63.8, 63.5, 61.0, 56.0, 20.4, 14.8

4-(4-Ethylthio)phenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (66a)
66a was synthesised from 56 using General Method IV.
Yield: 0.86 g (2.05 mmol) 40%

Appearance: yellow solid ¹H NMR (400 MHz, CDCl₃): δ 7.32 (d, 2H, J = 8.5 Hz, H₂⁻⁻), 7.3 (d, 2H, J = 8.5 Hz, H₂⁻⁻), 6.54 (s, 2H, H₁⁻), 5.39 (d, 1H, J = 1.8 Hz, H₂), 4.89 (d, 1H, J = 1.8 Hz, H₃), 3.78 (s, 3H, H₄), 3.72 (s, 6H, H₆₇₈), 2.97 (q, 2H, J = 7.4 Hz, S–CH₂), 2.21 (s, 3H, H₁₀), 1.34 (t, 3H, J = 7.8 Hz, CH₃ of S–CH₂CH₃)
¹³C NMR (100 MHz, CDCl₃): δ 169.7, 161.4, 153.5, 140.6, 138.4, 135.1, 132.8, 132.2, 129.0, 126.9, 95.4, 82.4, 63.8, 61.0, 56.1, 27.1, 20.6, 14.3

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Numbering nomenclature for 3-substituted β-lactams

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*one enantiomer shown

4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl) 2-azetidine-2-one (70)$^{78}$

70 was synthesised with 47 and phenylacetyl chloride using General Method V.

Yield: 878 mg (1.6 mmol) 32%

Rf: 0.2 (3:1 n-hexane: ethyl acetate); 0.55 (2:1 n-hexane: ethyl acetate)

Appearance: yellow powder
Melting Point: 297 °C

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.32 (m, 5H, H$_{2''}$-$H_{6''}$), 6.95 (dd, 1H, J = 8.5 Hz, J = 2.1 Hz, H$_{7''}$), 6.85 (m, 2H, H$_{5''}$ & H$_{6''}$), 6.58 (s, 2H, H$_{5''}$&$H_{6''}$), 4.78 (d, 1H, J = 2 Hz, H$_3$), 4.24 (d, 1H, J = 2 Hz, H$_4$), 3.79 (s,3H, H$_3$), 3.75 (s, 3H, H$_5$), 3.70 (s, 6H, H$_{7''}$&$H_{8''}$), 0.91 (s,9H, t - butyl, H$_{9''}$,10''&11''), 0.07 (s, 3H, SiCH$_3$), 0.05 (s, 3H, Si-CH$_3$)

$^1$C NMR (100 MHz, CDCl$_3$): δ 165.4 (C$_2$), 153.4 (C$_{5''}$&$C_{6''}$), 151.3, 145.7, 134.8, 133.6, 129.7, 129.4, 128.3, 128.7, 127.8, 119.5, 118.4, 112.4, 112.9, 94.9 (C$_{1''}$&$C_{2''}$), 64.9 (C$_5$), 63.7 (C$_6$), 55.9, 55.5, 25.6, -4.7 (Si-CH$_3$), -4.8 (Si-CH$_3$)

IR $\nu_{\text{max}}$ (ATR): 1748 cm$^{-1}$ (β-lactam C=O)

4-((tert-butyldimethylsilyl)(oxy)-3-hydroxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (71)

71 was synthesised from 2-thiopheneacetylchloride and 47 using General Method V.

![Chemical structure of 71]

Yield: 1.58 g (2.92 mmol) 58 %

Appearance: pink/red powder

Rf: 0.54 (1:1 n-hexane: ethyl acetate)

Melting Point: 51-55 °C

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.31 (dd, 1H, J = 5.63 Hz, J = 1.3 Hz, H$_9$), 7.10 (d, 1H, J = 3.1 Hz, H$_6$), 7.09 (dd, 7.09, 1H, J = 8 Hz, 1.9Hz, H$_5$), 6.99 (dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H$_7$), 6.90 (d, 1H, J = 8.9 Hz, H$_3$), 6.89 (d, 1H, J = 1.8 Hz, H$_{1''}$), 6.61 (s, 2H, H$_{1''}$&$H_{2''}$), 4.86 (d, 1H, J = 1.8 Hz, H$_4$), 4.45 (d, 1H, J = 1.8 Hz, H$_5$), 3.84 (s, 3H, H$_7$), 3.79 (s, 3H, H$_8$), 3.73 (s, 6H, H$_{3''}$&$H_{4''}$), 0.96 (s,9H, t - butyl, H$_{9''}$,10''&11''), 0.12 (s, 3H, Si-CH$_3$), 0.09 (s, 3H, Si-CH$_3$)

$^1$C NMR (100 MHz, CDCl$_3$): δ 164.3 (C$_2$), 153.5 (C$_{5''}$&$C_{6''}$), 151.4 (C$_{1''}$), 145.8 (C$_{3''}$), 136.2 (C$_8$), 134.6 (C$_2$), 133.6 (C$_1$), 129.2 (C$_{1''}$) 127.2 (C$_6$), 125.7 (C$_2$), 125.3 (C$_7$), 119.4 (C$_{2''}$), 118.4 (C$_{6''}$), 112.5 (C$_{3''}$), 95.04 (C$_{1''}$&$C_{2''}$), 64.5 (C$_4$), 60.9 (C$_5$), 60.1 (C$_6$), 56.0 (C$_{3''}$&$C_{4''}$), 25.6 (C$_{3''}$,10''&11''), -4.7, -4.7 (Si-CH$_3$)

IR $\nu_{\text{max}}$ (ATR): 1751 cm$^{-1}$ (β-lactam C=O)

LRMS: APCI calculated for [M+H$^+$] 556 m/z; found 556.11

4-((tert-butyldimethylsilyl)(oxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (72)

72 was synthesised from crotonyl chloride and 47 using General Method V.
Yield: not calculated. (De-silylated prior to purification)

Appearance: orange gel

Rf: 0.7 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl$_3$): δ 6.96 (dd, 1H, $^1$J = 2.2 Hz, $^1$J = 8 Hz, H$_{12''}$), 6.87 (d, 1H, $^1$J = 8 Hz, H$_{13''}$), 6.83 (d, 1H, $^1$J = 2.1 Hz, H$_{15''}$), 6.57 (s, 2H, H$_{7''}$), 6.04 (td, 1H, $^1$J$_{13''}$, $^1$J = 17.1 Hz, $^1$J$_{13''}$, $^1$J$_{14''}$ = 9.9 Hz, $^1$J$_{13''}$, $^1$J$_{14''}$ = 2.2 Hz, H$_{14''}$), 5.40 (dd, 1H, $^1$J$_{15''}$, $^1$J$_{14''}$ = 16 Hz, $^1$J$_{15''}$, $^1$J$_{14''}$ = 2.2 Hz, H$_{15''}$), 5.34 (dd, 1H, $^1$J$_{14''}$, $^1$J$_{13''}$ = 9.9 Hz, $^1$J$_{14''}$, $^1$J$_{13''}$ = 2.2 Hz, H$_{14''}$), 4.69 (d, 1H, $^1$J = 2.2 Hz, H$_3$), 3.83 (s, 3H, H$_8$), 3.80 (s, 3H, H$_{10''}$), 3.75 (apparent multiplet, 1H, H$_3$), 3.73 (s, 6H, H$_{7''}$), 2.69 (s, 3H, H$_{9''}$), 0.96 (s, 3H, t-butyl, H$_{9''}$, $^1$J$_{9''}$, $^1$J$_{10''}$), 0.1 (s, 3H, H$_{11''}$), 0.09 (s, 3H, H$_{10''}$)

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 165.3 (C$_1$), 153.5 (C$_{3''}$, C$_{4''}$), 151.3 (C$_{4''}$), 145.7 (C$_{5''}$), 134.4 (C$_5$), 133.8 (C$_5$), 130.64 (C$_6$), 129.64 (C$_{15''}$), 119.8 (C$_{13''}$), 119.8 (C$_{13''}$), 112.4 (C$_{13''}$), 94.8 (C$_{2''}$), 65.9 (C$_4$), 65.3 (C$_3$), 61.3 (C$_{1''}$), 60.9, 60.4 (C$_5$), 56.0 (C$_{7''}$), 55.5 (C$_{10''}$), 42.0, 30.1, 29.1, 25.6, 23.4, 23.1, 18.5, 15.3, -4.7 (Si-CH$_3$)

4-(4-methoxy-3-nitrophenyl)-3-phenoxy-1(3,4,5-trimethoxyphenyl)azetidine-2-one (36) was synthesised from 54 and benzzyloxyacetyl chloride using General Method V as an isomeric mixture. 36 cis and trans isomers were purified using LC over silica gel with an n-hexane:ethyl acetate gradient.

Yield (isomeric mixture): 1.2g (2.5 mmol) 36 %

Yield (isolated cis isomer): 460 mg (0.95 mmol) 19%

Rf: 0.13 (cis), 0.25 (trans) (1:1 n-hexane: ethyl acetate)

Yield: 620 mg (1.29 mmol), 26% (cis)

Melting Point: 150 °C solid, 71-75 °C amorphous powder (cis)

$^1$H NMR (400 MHz, CDCl$_3$): δ (cis) 7.94 (d, 1H, J = 2.5 Hz, H$_{6''}$), 7.62 (dd, 1H, J = 9.6 Hz, 2.5 Hz, H$_{1''}$), 7.22 (t, 2H, J = 7.8 Hz), 7.01 (d, 1H, J = 9.6 Hz, H$_8$), 6.97 (t, 1H, J = 7.0 Hz, H$_{10''}$), 6.83 (d, 2H, J = 7.4 Hz), 6.62 (s, 2H, H$_{15''}$), 5.6 (d, 1H, J = 5.2 Hz, H$_5$), 5.41 (d, 1H, J = 5.2 Hz, H$_9$), 3.96 (s, 3H, H$_7$), 3.81 (s, 3H, H$_{7''}$), 3.78 (s, 6H, H$_{7''}$)

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\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) (trans) 7.95 (d, 1H, \(J = 3.5\) Hz, H\(_{6}^-\)), 7.60 (dd, 1H, \(J = 9.6\) Hz, 2.6 Hz, H\(_2\)), 7.3 (apparent multiplet, 2H, \(J = 7.8\) Hz, H\(_{4}^-\&6^-\)), 7.21 (d, 1H, \(J = 9.6\) Hz, H\(_3^-\)), 6.98 (t, 1H, \(J = 7.0\) Hz, H\(_{5}^-\)), 6.84 (d, 2H, \(J = 7.4\) Hz, H\(_{2}^-\&3^-\)), 6.55 (s, 2H, H\(_{1}^-\&3^-\)), 5.14 (d, 1H, \(J = 1.7\) Hz, H\(_3\)), 5.04 (d, 1H, \(J = 1.7\) Hz, H\(_4\)), 4.03 (s, 3H, H\(_6\)), 3.81 (s, 3H, H\(_7^-\&9^-\)), 3.76 (s, 6H, H\(_{7}^-\&9^-\))

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) (cis) 162.4, 156.6, 153.8, 139.2, 135.4, 133.6, 132.6, 129.7, 125.8, 125.2, 122.6, 115.5, 113.8, 95.3, 80.9, 61.0, 60.9, 56.7, 56.2

IR \(\nu_{\max}\) (ATR): 1749 cm\(^{-1}\) (\(\beta\)-lactam C=O), 1622.52 cm\(^{-1}\) (N-O).

4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (73)

73 was synthesised from 47 and benzyloxyacetyl chloride using General Method V in predominantly trans yield (90:10 trans:cis).

![Compound 73](image)

**Yield:** 1.05g (1.9 mmol) 37%

**Appearance:** white fluffy powder (90:10% trans: cis)

R\(_f\): 0.7 (1:1 n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.0-6.48 (m, 8H, H\(_{2}^-\&3^-\&\&\&\&\&4^-\&5^-\&\&\&\&6^-\&7^-\&\&\&\&\&8^-\&9^-\)), 6.56 (s, 2H, H\(_{1}^-\&3^-\)), 5.07 (apparent singlet, 1H, H\(_3\)), 4.87 (apparent singlet, 1H, H\(_4\)), 4.38 (s, 3H, H\(_6\)), 3.76 (s, 3H, H\(_8\)), 3.69 (s, 6H, H\(_{7}^-\&9^-\)), 0.93 (s, 9H, t-butyl), 0.09 (s, 3H, Si-CH\(_3\)), 0.06 (s, 3H, Si-CH\(_3\))

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 162.5, 157.0, 153.5, 151.8, 145.8, 135.0, 133.1, 130.2, 129.7, 127.8, 126.3, 122.3, 115.4, 112.6, 111.2, 95.5, 87.1, 65.9, 64.0, 61.0, 56.0, 55.6, 25.8, -4.8 (Si-CH\(_3\)), -4.7 (Si-CH\(_3\))

4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-fluorophenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (74)

74 was synthesised using General Method V from 47 and 4-fluorophenylacetyl chloride.

![Compound 74](image)

**Yield:** 1.2g (2.11 mmol) 42%

R\(_f\): 0.65 (1:1 n-hexane: ethyl acetate), 0.45 (3:1 n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.73 (m, 2H, H\(_{4}^-\&6^-\)), 7.07 (m, 2H, H\(_{2}^-\&3^-\)), 6.96 (d, 1H, \(J = 8.6\) Hz, H\(_5\)), 6.87 (d, 1H, \(J = 7.7\) Hz, H\(_7\)), 6.88 (d, 1H, \(J = 2.6\) Hz, H\(_6\)), 6.59 (s, 2H, H\(_{1}^-\&3^-\)), 4.75 (d, \(J = 2.1\) Hz, H\(_3\)), 4.28 (s, \(J = 2.1\) Hz, H\(_4\)), 3.81 (s, 3H, H\(_6\)), 3.77 (s, 3H, H\(_8\)), 3.71 (s, 6H, H\(_{7}^-\&9^-\)), 0.94 (s, 9H, t-butyl), 0.09 (s, 3H, Si-CH\(_3\)), 0.07 (s, 3H, Si-CH\(_3\))

\(^19\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) -114.1

Appendices
4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (75)

75 was synthesised using General Method V from 47 and 4-methoxyphenylacetyl chloride.

Yield: 2.34 g (3.66 mmol) 57%
Appearance: yellow/white powder
Melting Point: 121-123 °C
Rf: 0.48 (2:1 n-hexane: ethyl acetate), 0.58 (1:1 n-hexane: ethyl acetate).
Melting Point: 121-124 °C

^1H NMR (400 MHz, CDCl₃): δ 7.27 (d, 2H, J = 9 Hz, H₂₋₋₋₋), 6.97 (dd, 1H, J = 1.9 Hz, H₃₋₋₋₋), 6.93 (d, 2H, J = 7.3 Hz, H₄₋₋₋₋), 6.89 (d, 1H, J= 8 Hz, H₅₋₋₋₋), 6.87 (d, 1H, J= 2.19 Hz, H₆₋₋₋₋), 6.62 (s, 2H, H₇₋₋₋₋), 4.76 (d, 1H, J = 2.2 Hz, H₈₋₋₋₋), 4.22 (d, 1H, J = 2.2 Hz, H₉₋₋₋₋), 3.84 (s, 3H, H₁₀₋₋₋₋), 3.79 (s, 3H, H₁₁₋₋₋₋), 3.74 (s, 3H, H₁₂₋₋₋₋), 0.96 (s, 9H, t-buty), 0.1 (s, 3H, Si-CH₃), 0.1 (s, 3H Si-CH₂).

^13C NMR (100 MHz, CDCl₃): δ 166.1(C₂₋₋₋₋), 159.4 (C₃₋₋₋₋), 153.5 (C₄₋₋₋₋), 151.4 (C₅₋₋₋₋), 145.8 (C₆₋₋₋₋), 134.4 (C₇₋₋₋₋), 133.8 (C₈₋₋₋₋), 129.9 (C₉₋₋₋₋), 128.6 (C₁₀₋₋₋₋), 126.9 (C₁₁₋₋₋₋), 119.5 (C₁₂₋₋₋₋), 118.7 (C₁₃₋₋₋₋), 114.5 (C₋₋₋₋₋), 112.6 (C₋₋₋₋₋), 95.1 (C₋₋₋₋₋), 65.9, 64.5 (C₋₋₋₋₋), 64.2 (C₋₋₋₋₋), 60.9 (C₋₋₋₋₋), 56.0 (C₋₋₋₋₋), 55.7 (C₋₋₋₋₋), 55.4 (C₋₋₋₋₋), 25.6 (t-buty), -4.7 (Si-CH₃)

IR νₒ_max (ATR): 1739 cm⁻¹ (β-lactam C=O)

4-(4-methoxy-3-nitrophenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (31)

31 was synthesised from 53 and 2-thiophenyl acetyl chloride using General Method V and as an isomeric mixture 9:1 cis:trans.

Yield: 1.51g (3.19 mmol) 40 %
Appearance yellow/white powder
Rf: 0.33 (1:1 n-hexane: ethyl acetate)

^1H NMR (400 MHz, CDCl₃): δ 8.30 (d, 0.09 H, cis, J = 1.9 Hz, H₃₋₋₋₋), 8.09 (dd, 0.09H, cis, J = 2.4, Hz, J = 8.8 Hz, H₂₋₋₋₋), 7.94 (d, 1H, J = 1.9 Hz, H₃₋₋₋₋), 7.72 (apparent s, 0.09H, cis, H₄₋₋₋₋), 7.62 (dd, 1H, J= 2.4 Hz, J = 8.8 Hz, H₅₋₋₋₋), 7.34 (d, 1H, J = 5.5 Hz, H₆₋₋₋₋), 7.18 (d, 1H, J = 9.5 Hz, H₇₋₋₋₋), 7.10 (d, 1H, J = 3 Hz, H₈₋₋₋₋), 7.05 (t, 1H, J = 4 Hz, H₉₋₋₋₋), 6.57 (s, 2H, H₋₋₋₋₋), 5.39 (d, 0.09H, cis, J =
3.7 Hz, H₂), 5.18 (d, 0.09H, cis, J = 3.7 Hz, H₂), 4.98 (d, 1H, J = 2.5 Hz, H₁), 4.49 (d, 1H, J = 2.5 Hz), 4.09 (s, 3H, H₁0'), 3.80 (s, 3H, H₈), 3.77 (s, 6H, H₇,₈,₉,₁₀')

$^1$H NMR (100 MHz, CDCl₃): δ 163.8 (C₂), 153.8 (C₄,₆'), 153.3 (C₃'), 140.1 (C₅'), 135.2 (C₆), 135.1 (C₇), 133.1 (C₇'), 131.2 (C₂'), 129.3 (C₁'), 127.5 (C₆), 126.1 (C₇), 125.7 (C₈), 123.6 (C₉'), 114.7 (C₃'), 95.0 (C₁₅,₁₆'), 63.4 (C₃), 61.0 (C₅), 60.2 (C₃), 56.8 (C₁₀), 56.2 (C₇,₈,₉,₁₀')

4-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (34)$^{389}$

34 was synthesised from 47 and crotonyl chloride using General Method V.

Yield: 480 mg (1.15 mmol) 11%
Rₛ: 0.25 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl₃): δ 7.91 (d, 1H, J = 2.9 Hz, H₁'), 7.56 (dd, 1H, J = 1.9, 1.9 Hz, H₃'), 7.27 (d, 1H, J = 8 Hz, H₅'), 6.54 (s, 2H, H₁₁',₁₃'), 6.05 (td, 1H, J = 17 Hz, J = 2.6 Hz, 1.9 Hz, H₁₂'), 5.41 (apparent multiplet, 2H, H₃₀₉,₁₃'), 4.81 (d, 1H, J = 2.1 Hz, H₁₀'), 4.01 (s, 3H, H₈'), 3.88 (apparent multiplet, 1H, H₅'), 3.81 (s, 3H, H₈), 3.77 (s, 6H, H₇',₈',₉',₁₀')

Numbering nomenclature for 3-phenolic β-lactams

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3-(4-(Benzyloxy)phenyl)-4-(3-(tert-butyldimethylsilyloxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidin-2-one (79a)

79a was synthesised from 4-(benzylxyloxyphenyl)acetic acid and 47 using General Method VI.

Yield: 1.9g (2.9 mmol) 29%

Melting Point: 98-103 ºC
Rₛ: 0.31 (2:1 n-hexane: ethyl acetate), 0.75 (1:1 n-hexane: ethyl acetate) Appearance: white solid.

$^1$H NMR (400, MHz CDCl₃): δ 7.37 (m, 5H), 7.23 (d, 2H, J=9 Hz), 6.95 (apparent triplet, 3H, J= 8.8 Hz), 6.84 (d, 2H, J= 6.8 Hz), 6.58 (s, 2H, H₁₅,₁₆'), 5.05 (s, 2H, CH₂ (benzyl)), 4.72 (d, 1H, J= 1.9 Hz, H₅'), 4.17 (d, 1H, J= 1.9 Hz, H₃'), 3.84 (s, 3H, H₁₀'), 3.75 (s, 3H, H₈'), 3.7 (s, 6H, H₇',₈',₉',₁₀') 0.92 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H)
13C NMR (100, MHz CDCl3): δ 176.15, 166.0, 158.4, 158.0, 153.5, 151.3, 145.7, 136.9, 136.8, 134.4, 133.7, 130.4, 129.8, 128.6, 128.6, 127.9, 127.4, 127.1, 125.6, 119.5, 118.5, 115.4, 115.0, 112.5, 94.9, 70.0, 70.0, 64.4, 64.1, 60.9, 60.9, 56.0, 55.5, 39.9, 25.6, 18.4, -4.7, -4.8

IR ν max( ATR): 1740 cm⁻¹ (β-lactam C=O)

3-(4-(benzyloxy)phenyl)-4-(3-fluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (80)

80 was synthesised from (4-benzyloxyphenyl)acetic acid and 45 using General Method VI

Yield: 820mg (1.55 mmol) 24%

Appearance: Grey amorphous powder

Melting Point: 60-63°C

Rf: 0.54 (1:1 n-hexane: ethyl acetate), 0.75 (1:1 n-hexane: ethyl acetate)

1H NMR (600 MHz, DMSO-d6): δ 7.42 (m, 5H), 7.33 (d, 1H, J= 8.3 Hz), 7.26 (d, 2H, J= 8.3 Hz), 7.20 (t, 1H, J= 8.3 Hz), 7.05 (d, 2H, J= 8.3 Hz), 6.60 (d, 2H, H1J=2), 5.19 (d, 1H, J= 2 Hz, H3), 5.12 (s, 2H, benzyl CH2), 4.22 (d, 1H, J= 2 Hz, H2), 3.83 (s, 3H, H10), 3.65 (s, 6H, H7&9), 3.58 (s, 3H, H6)

13C NMR (100 MHz, DMSO-d6): δ 166.1 (C2), 158.4 (C4&5), 153.6 (Cα&β), 152.7, 151.3, 147.5, 137.5, 134.5, 133.7, 133.9, 130.9, 130.7, 129.25, 128.9, 128.27, 128.1, 127.5, 123.9, 115.7, 115.1, 114.8, 114.7, 95.6 (Cα&β), 69.6, 63.4 (Cα), 62.15 (C5), 60.6 (C8), 60.2, 56.5 (C7&9), 56.2 (C10)

19F NMR: (376 MHz, DMSO-d6): δ -134.8

IR ν max( ATR): 1746 cm⁻¹ (C=O)

3-(4-(benzyloxy)phenyl)-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (81)

81 was synthesised from (4-benzyloxyphenyl)acetic acid and 44 using General Method VI

Yield: not calculated

Rf: 0.54 (2:1 n-hexane: ethyl acetate), 0.75 (1:1 n-hexane: ethyl acetate)

Appearance: White solid.

1H NMR (600 MHz, CDCl3): δ 7.45 (d, 2H, J=6.8Hz), 7.41 (t, 2H, J= 7.3 Hz), 7.34 (d, 2H, 8.5 Hz, H3&4), 7.27 (d, 2H, J= 8.4 Hz), 6.99 (d, 2H, J= 8.4 Hz), 6.95 (d, 2H, J= 7.8 Hz, H2&5), 6.61 (s, 2H, H1&3), 5.09 (s, 2H, benzyl CH2), 4.82 (d, 1H, J= 3 Hz), 4.23 (d, 1H, J= 3 Hz), 3.84 (s, 3H, H10), 3.79 (s,3H, H2), 3.74 (s, 6H, H8&9)

13C NMR (100 MHz, CDCl3): δ 166.4 (C2), 160.2 (C4&5), 158.7 (Cα&β), 153.6 (Cα&β), 137.2, 134.7 (Cα), 134.0, 130.8, 129.7, 128.9, 128.0, 125.8, 115.8, 114.9, 95.4 (Cα&β), 70.2 (Benzyl CH2), 64.2, 61.0 (Cα), 56.3 (C7&9), 56.8 (C10)

Benzyl (4-(2-(3-((tert-butylidimethylsilyl)oxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl) azetidin-3-yl)phenyl)carbamate (43)

43 was synthesised from (4-benzyloxycarbonylaninophenyl) acetic acid and 47 using General Method VI.

Yield: 470 mg (0.67 mmol) 37% yield

Rf: 0.63 (1:1 n-hexane: ethyl acetate)

1H NMR (600 MHz, CDCl3): δ 7.43-7.34 (m, 7H, CBZ Ar-H, H1J=3&4), 7.29 (d, 2H, J= 8.7 Hz, H4&5), 6.97 (dd, 1H, J= 1.9, J= 8.6 Hz, H5), 6.89 (d, 1H, J= 8.3 Hz, H6), 6.87 (d, 1H, J= 1.8 Hz, H7), 6.74 (bs, 1H, NH), 6.61 (s, 2H, H1J=2), 5.23 (s, 2H, CH2 of CBZ group), 4.76 (d,
1H, J = 2.6 Hz, H₃), 4.23 (d, 1H, J= 2.6 Hz, H₃), 3.84 (s, 3H, H₁₀), 3.8 (s, 3H, H₈), 3.7 (s, 6H, H₇&₉), 0.9 (s, 9H, t-butyl), 0.1 (s, 3H, Si-CH₃), 0.1 (s, 3H, Si-CH₃)

Numbering nomenclature for Reformatsky products

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(S)-4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (76)³⁸⁴

76 was synthesised from 47 and ethyl bromoacetate using General Method VII A and General Method VII B.

Yield using reflux: 1.05 g (2.8 mmol) 40%
Yield using microwave: 403 mg (0.85 mmol) 22%

Appearance: Yellow brown oil
Rf: 0.78 (1:1 n-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.95 (d, 1H, J₆-₂= 2.7 Hz, H₆⁻), 6.89 (dd, 1H, J₂⁻₋₆⁻= 9.3 Hz, J₂⁻₋₆⁰⁻= 2.7 Hz, H₂⁻), 6.86 (d, 1H, J₃⁻₋₂⁻= 8.3 Hz, H₃⁻), 6.56 (s, 2H, H₁₁&₁₃⁻), 4.88 (dd, 1H, J₄⁻₋₅⁻= 5.5 Hz, J₄⁻₋₃⁻= 15 Hz, H₄⁻), 3.82 (s, 3H, H₁₀⁻), 3.77 (s, 3H, H₈), 3.72 (s, 6H, H₇&₉), 3.52 (dd, 1H, J₃⁻₋₅⁻= 14.9 Hz, J₄⁻₋₃⁻= 5.5 Hz, H₃⁻), 2.93 (dd, 1H, J₅⁻₋₃⁻= 15 Hz, J₄⁻₋₃⁻= 2.1 Hz, H₅⁻), 0.96 (s, 9H, t-butyl), 0.1 (s, 3H, Si-CH₃), 0.08 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 164.6 (C₂), 153.5 (C₄&₆⁻), 151.2 (C₅⁻), 145.7 (C₇⁻), 134.3 (C₂⁻), 130.5 (C₇⁻), 119.4, 118.5 (C₂⁻), 112.2(C₀⁻), 110.9 (C₇⁻), 95.6 (C₁₁&₁₃⁻), 60.9 (C₈⁻), 56.0 (C₇&₉⁻), 55.3 (C₁₀⁻), 54.1 (C₄), 46.8 (C₆⁻), 25.6 (t-butyl), -4.7 (Si-CH₃)

(4-((3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (77)³⁸⁴

The isomeric mixture of 77 was synthesised from 47 and ethyl bromopropionate using General Method VII A and General Method VII B.

Appendices
Yield using microwave: 1.7g (3.49 mmol) 39%
Yield using reflux: 1.34g (2.75 mmol) 63%
Rt: 0.9 (1:1 n-hexane: ethyl acetate), 0.7 (2:1 n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 6.95 (dd, 1H, \(J_{2',3'} = 2.8 \text{ Hz}, J_{2',5'} = 8.3 \text{ Hz}, H_{2}\), 6.86 (d, 1H, \(J_{3',2'} = 8.3 \text{ Hz}, H_{3'}\), 6.83 (d, 1H, \(J_{6',2'} = 1.9 \text{ Hz}, H_{6'}\), 6.71 (d, 0.6H, \(J_{6',2'} = 1.9 \text{ Hz}, H_{6'}\)), 6.58 (s, 1.4H, \(H_{1',\&3'}\), 6.56 (s, 0.55H, \(H_{2',\&3'}\), 5.07 (d, 0.7H, \(J_{3'} = 4.8 \text{ Hz}, cis, H_{3}\)), 4.45 (d, 0.25H, \(J_{3} = 1.9 \text{ Hz}, trans, H_{3}\)), 3.8 (s, 3H, \(H_{10}\)), 3.76 (s, 2H, \(H_{5}\)), 3.75 (s, 1H, \(H_{6}\)), 3.71 (s, 4H, \(H_{7,\&8}\)), 3.7 (2H, \(H_{7,\&8}\)), 3.61 (apparent multiplet, 1H, \(H_{5}\)), 3.09 (dq, 0.3H, \(J_{1}, CH_3 = 7.32 \text{ Hz}, J_{3,4} = 1.9 \text{ Hz}, H_{3}\)). 1.49 (d, 3H, \(J = 8.1 \text{ Hz}, H_{3}\)), 0.94 (s, 2H, TBDMS, \(t\)-butyl), 0.92 (s, 7H, TBDMS, \(t\)-butyl), 0.07 (s, 3H, Si-CH\(_3\)), 0.03 (s, 2H, Si-CH\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 168.4, 153.4, 151.2, 145.6, 134.1, 133.9, 130.2, 120.5, 119.8, 118.5, 112.3, 112.0, 94.9, 94.6, 62.7, 61.0, 58.3, 56.0, 55.9, 55.5, 55.4, 55.1, 25.6, 18.5, 13.1, -4.8

\(\text{(4-}((\text{tert-butyldimethylsilyloxy})-4\text{-methoxyphenyl})-3\text{-methyl-}(3,4,5\text{-trimethoxyphenyl})azetidine-2\text{-one (77 trans)}\)

77 trans was isolated from the mixture of 77 isomers using gravity LC and isocratic conditions of 2:1 n-hexane: ethyl acetate.

Yield of trans isomer isolated from mixture: 87mg (0.18 mmol) 5%.

Rt: 0.69 (2:1 n-hexane: ethyl acetate)

\(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 6.95 (dd, 1H, \(J_{2',3'} = 2.2 \text{ Hz}, J_{2',5'} = 8.34 \text{ Hz}, H_{2}\)), 6.86 (d, 1H, \(J_{3',2'} = 8.34 \text{ Hz}, H_{3'}\), 6.83 (d, 1H, \(J_{6',2'} = 1.9 \text{ Hz}, H_{6'}\)), 6.56 (s, 2H, \(H_{1',\&3'}\), 4.46 (d, \(J_{3} = 1.9 \text{ Hz}, H_{3}\)), 3.83 (s, 3H, \(H_{10}\)), 3.79 (s, 3H, \(H_{5}\)), 3.73 (s, 6H, \(H_{7,\&8}\)), 3.12 (dq, \(J_{1}, CH_{3} = 7.3 \text{ Hz}, J_{3,4} = 1.9 \text{ Hz}, H_{3}\)), 1.49 (d, 3H, \(J_{CH_{3,\&3}} = 8.1 \text{ Hz}, H_{3}\)), 0.96 (s, 9H, \(t\)-butyl), 0.12 (s, 3H, Si-CH\(_3\)), 0.11 (s, 3H, Si-CH\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 168.39 (C\(_2\)), 153.5 (C\(_{\&8}\)), 151.2 (C\(_{\&7}\)), 145.7 (C\(_{\&5}\)), 134.3 (C\(_{1}\)), 134.0 (C\(_{\&1}\)), 130.3, 127.1 (C\(_{\&1}\)), 119.7 (C\(_{\&1}\)), 118.5 (C\(_{\&8}\)), 111.0 (C\(_{\&1}\)), 94.9 (C\(_{\&1}\)), 118.5 (C\(_{\&8}\)), 62.7 (C\(_{4}\)), 61.0 (C\(_{5}\)), 58.3 (C\(_{10}\)), 56.1 (C\(_{3}\)), 55.1 (C\(_{\&1}\)), 13.1 (C\(_{3}\)), -4.8 (CH\(_3\)-Si)

\(\text{4-}((\text{tert-butyldimethylsilyloxy})-4\text{-methoxyphenyl})-3\text{-methyl-}(3,4,5\text{-trimethoxyphenyl})azetidine-2\text{-one (77 cis)}\)

Yield of cis isomer isolated from mixture: 110mg (0.23 mmol) 7%

Rt: 0.66 (2:1 n-hexane: ethyl acetate)

\(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 6.86 (d, \(J_{3',2'} = 8.3 \text{ Hz}, H_{3}\)), 6.81 (d, \(J_{2',5'} = 2.1 \text{ Hz}, J_{2',3'} = 8.4 \text{ Hz}, H_{2}\)), 6.71 (d, 1H, \(J_{6',2'} = 2.1 \text{ Hz}, H_{6'}\)), 6.58 (s, 2H, \(H_{1',\&3'}\), 5.08 (d, 1H, \(J_{3} = 5.9 \text{ Hz}, H_{3}\)), 3.82 (s, 3H, \(H_{10}\)), 3.78 (s, 3.79, \(H_{5}\)), 3.73 (s, 6H, \(H_{7,\&8}\)), 3.64 (dq, \(J_{3,5} = 12 \text{ Hz}, J_{3,4} = 2.6 \text{ Hz}, H_{1}, H_{3}\)), 1.56 (apparent bs, 3H, \(H_{3}\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 168.7 (C\(_2\)), 153.3 (C\(_{\&8}\)), 151.4, 134.3, 133.9, 127.2, 120.6, 119.8, 112.1, 95.0, 94.8 (C\(_{\&1}\)), 65.9, 62.7, 61.0, 58.3, 56.1, 55.4, 49.4, 25.7, 18.4, 15.3, -4.6, -4.9
4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-fluoro-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (78)

78 was synthesised with ethyl bromofluoroacetate and 47 using General Method VII B.

Yield using microwave: 756 mg (1.54 mmol) 52%

Rf: 0.56 (2:1 n-hexane: ethyl acetate), 0.73 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.95 (dd, 1H, $J_{2''-6''} = 2.7$ Hz, $J_{2''-3''} = 7.8$ Hz, H$_{2''}$), 6.86 (d, 1H, $J_{6''-2''} = 1.7$ Hz, H$_{6''}$), 6.84 (d, 1H, $J_{3''-2''} = 7.8$ Hz, H$_{3''}$), 5.83 (s, 2H, H$_{1'&3'}$), 5.14 (apparent dd, 1H, $J_{F-H3} = 47.4$ Hz, $J_{3-4} = 2.1$ Hz, H$_3$), 4.85 (apparent dd, 1H, $J_{F-H4} = 25.3$ Hz, $J_{4-3} = 2.1$ Hz, H$_4$), 3.81 (s, 3H, H$_{10'}$), 3.73 (s, 6H, H$_{7'&9'}$), 3.73 (s, 3H, H$_{8'}$), 0.97 (s, 9H, t-butyldimethylsilyl), 0.10 (s, 3H, Si-CH$_3$), 0.09 (s, 3H, Si-CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 167.9 (d, $J_{C-F} = 25.6$ Hz, C$_2$), 153.8 (C$_{4'&6'}$), 150.8, 145.3, 130.7, 130.1, 120.3 (C$_2$), 119.7 (C$_6''$), 112.2 (C$_3''$), 95.0 (C$_{1'&3'}$), 91.7 (C$_4$, $J_{F-C} = 95$ Hz), 69.0, 62.0, 61.5, 61.1 (C$_7$), 58.9 (C$_3$, $J_{F-C} = 21$ Hz), 55.9 (C$_{7'&9'}$), 55.6 (C$_{10'}$)

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -203.7

HRMS: APCI calculated for C$_{25}$H$_{35}$FNO$_6$Si; 492.221219, found 492.221424, error + 0.4ppm

Numbering nomenclature for 3-hydroxyl racemates

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3-Hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17) trans

17 trans was synthesised from 17a using General Method VIII.

Overall yield for Staudinger: 900 mg (2.5 mmol) 50%

Appearance: white powder

Rf: 0.31 (2:1; n-hexane: ethyl acetate)

Melting Point: 122-127 °C

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.26 Hz (d, 2H, $J=8.4$ Hz, H$_{2''&6''}$), 6.90 (d, 2H, $J=8.4$ Hz, H$_{3''&5''}$), 6.5 (s, 2H, H$_{1'&3'}$), 5.29 (s, 1H, OH), 4.79 (d, 1H, $J=1.9$ Hz, H$_3$), 4.74 (d, 1H, $J=1.89$ Hz, H$_3$), 3.80 (s, 3H, H$_{10'}$), 3.75 (s, 3H, H$_8'$), 3.68 (s, 6H, H$_{7'&9'}$)

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Melting Point:

Overall Yield for Staudinger: 67a
3-trimethoxyphenyl

Purity (RP-LRMS): 144.5, 114.5, 95.3, 76.7, 65.3, 65.3, 62.3, 60.9, 56.0, 55.3

IR ν_{max}(ATR): 3390 cm⁻¹ (OH) · 1752 cm⁻¹ (β-lactam C=O).

LRMS: APCI calculated for [M+H+] 360 m/z; found 360.07
Purity (RP-HPLC): 99 %

3-Hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17) Cis

17 cis was synthesized from 17a using General Method VIII and purified from the isomeric mixture by LC.

Overall Yield for Staudinger: 1.5%
Appearance: white powder
Rt: 0.25 (1:1 n-hexane:ethyl acetate)
Melting Point: 146-148 °C

1H NMR (400 MHz, CDCl₃): δ 7.27 Hz (d,2H, J=8.4 Hz, H_2-κ₆), 6.94 (d, 2H, J=8.4 Hz, H_κ-κ'), 6.58 (s, 2H, H_1', 5.29 (bs,1H, OH), 5.22 (d, 1H, J=5.5 Hz, H_3), 5.15 (d, 1H, J=5.5 Hz, H_2), 3.80 (s, 3H, H_1'), 3.76 (s, 3H, H_2), 3.71 (s, 6H, H_κ-κ')

13C NMR (100 MHz, CDCl₃): δ 166.6 (C_2), 160.0, 153.5, 134.9, 133.1, 128.9, 127.9, 124.7, 114.5, 95.3, 76.7, 65.3, 65.3, 62.3, 60.9, 56.0, 55.3

IR ν_{max}(ATR): 1712 cm⁻¹ (β-lactam C=O)
LRMS: APCI calculated for [M+H+] 360 m/z; found 360.03
Purity (RP-HPLC): 99%}

4-(3-Fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18)

18 was obtained from 18a using General Method VIII.

Overall Yield for Staudinger: 38%
Appearance: white powder
Rt: 0.32 (1:1 n-hexane: ethyl acetate)
Melting Point: 143-146 °C

1H NMR (400 MHz, CDCl₃): δ 7.05 Hz (2H, apparent multiplet, H_2-κ₆'), 6.95 (t, 1H, J=8.5 Hz, H_3'), 6.47 (s, 2H, H_κ-κ'), 4.75 (s, 1H, OH), 4.71 (d, 1H, J=1.3 Hz, H_2), 4.69 (d, 1H, J=1.3 Hz, H_3), 3.88 (s, 3H, H_1'), 3.75 (s, 3H, H_2), 3.69 (s, 6H, H_κ-κ')

13C NMR (100 MHz CDCl₃): δ 166.7 (C_2), 153.8 (C_κ-κ'), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8, 95.3 (C_1',κ'), 83.6 (C_4), 65.1 (C_3), 60.9 (C_κ'), 56.3 (C_κ-κ'), 56.0 (C_κ')

19F NMR (376 MHz, CDCl₃): δ -133.2

IR ν_{max}(ATR): 3288 cm⁻¹ (OH), 1726 cm⁻¹ (β-lactam C=O)
LRMS: APCI calculated for [M+H'] 379 m/z; found 378.04
Purity (RP-HPLC): 95%

3-((Tert-butyl(dimethyl)silyl)oxy)-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (67a)

67a was obtained from 16a using General Method VIII

Overall Yield for Staudinger: 24%
Melting Point: 144.5-146.9 °C

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**4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19)**

19 was obtained from 19a using General Method VIII.

**Overall Yield for Staudinger:** 0.931g (2.37 mmol) 57%

**Melting Point:** 140-144 ºC

**Rf:** 0.34 (1:1; n-hexane:ethyl acetate)

**1H NMR (400 MHz, CDCl₃):** δ 6.89 (dd, 1H, J=8 Hz, J=2 Hz, H₃''), 6.82 (d, 1H, J=8.1 Hz, H₅''), 6.75 (d, 1H, J=2.2 Hz, H₃''), 6.49 (s, 2H, H₁'), 4.71 (d,1H, J= 1.8 Hz, H₆), 4.69 (d, 1H, J= 1.8 Hz, H₃), 3.78 (s,3H, H₉''), 3.73 (s, 3H, H₆'), 3.66 (s, 6H, H₆''&₉'') 0.9 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H)

**IR νₘₐₓ (ATR):** 3279 cm⁻¹ (OH), 1720 cm⁻¹ (β-lactam C=O)

**LRMS:** APCI calculated for [M+H⁺] 391 m/z; found 391.12

**Purity (RP-HPLC):** 92.6%

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**3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20)**

20 was obtained from 20a using General Method VIII.

**Overall yield for Staudinger:** 1.72 g (4.6 mmol) 46%

**Melting Point:** 118-122 ºC

**Rf:** 0.20 (2:1 n-hexane: ethyl acetate), 0.33 (1:1 n-hexane: ethyl acetate)

**1H NMR (400 MHz, DMSO-d₆):** δ 7.14 (dd, 1H, J = 8.1 Hz, 2 Hz, H₂''), 7.11 (d, 1H, J = 1.75 Hz, H₀''), 6.81 (d, 1H, J= 7.8 Hz, H₃''), 6.54 (s, 2H, H₁''&₃''), 4.77 (d, 1H, J= 1.8 Hz, H₆), 4.75(d, 1H, J = 1.8 Hz, H₃), 3.83 (s, 3H,H₁''&₃''), 3.77 (s, 3H, H₆''), 3.71 (s, 6H, H₆''&₉''), 2.187 (s, 3H, CH₃, H₃'')

**13C NMR (100 MHz, DMSO-d₆):** δ 166.7 (C₂), 158.1 (C₆''), 153.4 (C₃''&₅''), 134.6 (C₂''), 133.4 (C₃), 128.4 (C₅''), 127.6 (C₅''), 127.4 (C₂''), 124.9 (C₆''), 110.1 (C₆''), 95.3 (C₄''&₅''), 83.61 (C₃), 65.7 (C₆''), 60.9 (C₉''), 55.9 (C₇''&₉''), 55.4 (C₁₀''), 31.0 (CH₃)

**IR νₘₐₓ (ATR):** 3287 cm⁻¹ (OH), 1727 cm⁻¹ (β-lactam C=O)

**LRMS:** APCI calculated for [M+H⁺] 374 m/z; found 374.02

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**3-hydroxy-4-(4-methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22)**

22 was obtained from 22a by General Method VIII.

**Overall yield from Staudinger:** 600mg (1.6 mmol) 32%

**Melting Point:** 160-161 ºC

**Rf:** 0.25 (1:1; n-hexane: ethyl acetate)

**1H NMR (400 MHz, DMSO-d₆):** δ 7.38 (d,1H (H₅''&₉''), 7.28 (d, 2H, J=8.6 Hz, H₆''&₅''), 6.72 (d,1H, J= 8.6 Hz, 3-OH), 6.52 (s,2H, H₁''&₃''), 4.89 (d,1H, J=1.9 Hz, H₆), 4.75 (dd, 1H, J= 7.6 Hz, J= 1.9 Hz, H₃), 3.63 (s, 6H, H₆''&₉''), 3.57 (s, 3H, H₆''), 2.46, (s, 3H, H₁'')
**C NMR (100 MHz, DMSO-d$_6$):** δ 166.9, 153.5, 138.9, 134.3, 133.8, 133.4, 127.9, 126.6, 83.8, 65.1, 60.5, 56.3, 14.9

**HRMS: ESI** calculated for C$_{19}$H$_{21}$NNaO$_5$S for [M+Na$^+$] 398.103264; found 398.103717, error -1.1 ppm

**IR $\nu_{max}$(ATR):** 3300 cm$^{-1}$ (OH), 1721 cm$^{-1}$ ($\beta$-lactam C=O)

4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21)

21 was obtained from 21a using **General Method VIII**.

**Overall yield for Staudinger:** 1.48 g (3.38 mmol) 44%

**Melting Point:** 70 ºC

**R$_f$:** 0.25 (1:1; n-hexane:ethyl acetate)

**H NMR (400 MHz, CDCl$_3$):** δ 7.57 (d, 1H, J= 1.6 Hz, H$_{6''}$), 7.25 (dd, 1H, J= 8.3 Hz, J= 1.8 Hz, H$_{2''}$), 6.91 (d,1H, J= 8.3 Hz, H$_{3''}$), 6.51 (s, 1H, H$_{1'&3'}$), 4.78 (d, 1H, J= 1.5 Hz, H$_3$), 4.74 (d, 1H, J= 1.5 Hz, H$_4$), 3.92 (s, 3H, H$_{10'}$), 3.78 (s, 3H, H$_{8'}$), 3.73 (s, 6H, H$_{7'&9'}$)

**C NMR (100 MHz, CDCl$_3$):** δ 165.9 (C$_2$), 157.2 (C$_{4''}$), 153.4 (C$_{5'&6'}$), 134.9 (C$_5$), 132.9 (C$_2$), 131.2 (C$_{7'}$), 129.5 (C$_{1'}$), 126.3 (C$_{2'}$), 112.5 (C$_{4'}$), 112.3 (C$_{5'}$), 95.37 (C$_{1'&3'}$), 86.6 (C$_3$), 64.4 (C$_4$), 60.9 (C$_{8'}$), 56.4 (C$_{7'&9'}$), 56.1 (C$_{10'}$

**IR $\nu_{max}$(ATR):** 3287 cm$^{-1}$ (OH), 1726 cm$^{-1}$ ($\beta$-lactam C=O)

**LRMS:** APCI calculated for [M+H$^+$] 437.98 m/z; found 437.98

4-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (79)

79 was synthesised from 79a using **General Method IX**.

**Yield:** 880mg (1.56 mmol) 55%

**Appearance:** white oil

**R$_f$:** 0.65 (1:1; n-hexane:ethyl acetate)

**H NMR (400 MHz, CDCl$_3$):** δ 7.14 (d, 2H, J= 8 Hz), 6.99 (d,1H, J= 1.5 Hz, H$_{6'}$), 6.80 (d, 1H, J= 8 Hz, H$_{3'}$), 6.77 (d, 2H, J= 8 Hz), 6.64 (s, 2H, H$_{1'&3'}$), 4.72 (d, 1H, J= 1.9 Hz, H$_3$), 4.16 (d, 1H, J= 1.9 Hz, H$_4$), 3.79 (3H, s, H$_{10'}$), 3.75 (s, 3H, H$_{8'}$), 3.65 (s, 6H, H$_{7'&9'}$), 0.91 (s, 9H, C(CH$_3$)$_2$), 0.07 (s, 3H, Si-CH$_3$), 0.04 (s, 3H, Si-CH$_3$)

**C NMR (100 MHz, CDCl$_3$):** δ 166.3, 153.5, 133.6, 130.5, 129.7, 128.7, 119.5, 118.4, 115.9, 115.5, 112.5, 94.9, 64.3, 64.2, 61.13, 60.9, 55.9, 55.5

**IR $\nu_{max}$(ATR):** 3299.01 cm$^{-1}$ (OH), 1737 cm$^{-1}$ ($\beta$-lactam C=O)
Numbering nomenclature for B ring *meta* hydroxyl substituted racemates

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3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16)

16 was synthesised from both 67a using **General Method X**.

**Overall yield for Staudinger from 67a:** 1.2g (2.6 mmol) 43%

**Overall Yield for Staudinger from 67b:** 56 mg (0.15 mmol) 67%

R<sub>f</sub>: 0.2 (1:1; n-hexane:ethyl acetate)

**Melting Point:** 62–64 °C

**<sup>1</sup>H NMR (400 MHz, CDCl₃):** δ 7.29 (singlet, 1H, OH), 6.65 (apparent singlet, 1H, H<sub>4</sub>), 6.85 (apparent singlet, 2H, H<sub>2''</sub>,H<sub>3''</sub>), 6.53 (s, 2H, H<sub>1''</sub>,H<sub>3</sub>), 4.75 (1H, apparent singlet, H<sub>3</sub>), 4.73 (1H, apparent singlet, H<sub>4</sub>), 3.90 (s, 3H, H<sub>10</sub>'), 3.77 (s, 3H, H<sub>8</sub>'), 3.71 (s, 6H, H<sub>7''</sub>,H<sub>9''</sub>), 3.63 (s, 3H, H<sub>8''</sub>), 3.56 (s, 3H, H<sub>9''</sub>), 3.42 (s, 3H, H<sub>8''</sub>), 3.32 (s, 3H, H<sub>9''</sub>)

**<sup>13</sup>C NMR (100 MHz, CDCl₃):** δ 167.0, 153.8 (C<sub>1'</sub>,C<sub>3'</sub>), 147.1 (C<sub>4'</sub>), 147.0 (C<sub>5'</sub>), 135.0 (C<sub>5</sub>), 133.3 (C<sub>2</sub>), 130.3 (C<sub>3</sub>), 118.2 (C<sub>2''</sub>), 112.2 (C<sub>3''</sub>), 111.0 (C<sub>5''</sub>), 95.5 (C<sub>1''</sub>,C<sub>3</sub>), 83.8 (C<sub>7''</sub>), 65.7 (C<sub>3</sub>), 61.0 (C<sub>8</sub>), 56.0 (C<sub>7''</sub>,C<sub>9''</sub>), 56.0 (C<sub>10</sub>), 54.0 (C<sub>8</sub>)

**IR ν<sub>max</sub> (ATR):** 3357 cm⁻¹ (OH), 1747 cm⁻¹ (β-lactam C=O)

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2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate

(67b)

67b was synthesised from 16a using General Method X.

Overall Yield for Staudinger: 680 mg (1.63 mmol) 32%

Rf: 0.4 (1:1 n-hexane:ethyl acetate)

1H NMR (400 MHz, CDCl3): δ 6.02 (d, 1H, J = 1.6 Hz, Hα), 6.88 (m, 2H, H2′,H3′), 6.54 (s, 2H, H1′,H5′), 5.38 (d, J = 1.5 Hz, 1H, Hβ), 4.80 (d, J = 1.5 Hz, 1H, Hγ), 3.89 (s, 3H, H10′), 3.76 (s, 3H, H8), 3.70 (s, 6H, H7′,H9′), 2.17 (s, 3H, OOCH3)

13C NMR (100 MHz, CDCl3): δ 169.6 (C=O, OOCH3), 161.6 (C2), 153.5 (C2′,C6′), 147.3 (C4′), 146.3 (C3′), 134.2 (C2′), 133.1 (C5′), 128.5 (C3′), 118.5 (C2′), 112.7 (C6′), 111.1 (C3′), 95.5 (C1′,C5′), 82.3 (C2), 63.5 (C1), 60.9 (C8), 56.2 (C10′), 55.9 (C7′,C9′), 20.7 (CH3, OOCH3)

4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24)

24 was synthesised from 70 using General Method X.

Overall yield for Staudinger: 766 mg (1.76 mmol) 17.6%

Rf: 0.4-0.5 (2:1 n-hexane: ethyl acetate)

Melting Point: 81-85 °C

1H NMR (400 MHz, CDCl3): δ 7.35 (m, 5H, 3-phenyl), 6.99 (d, 1H, J = 2 Hz, Hα), 6.91 (dd, 1H, H β, J = 8 Hz,J = 2 Hz, Hγ), 6.86 (d, 1H, J = 8 Hz, H3′), 6.64 (s, 2H, H1′,H5′), 5.79 (s, OH), 4.80 (d, 1H, J = 2.5 Hz, Hδ), 4.29 (d, 1H, J = 2.5 Hz, H2′), 3.90 (s, 3H, H10′), 3.76 (s, 3H, H8), 3.72 (s, 6H, H7′,H9′)

13C NMR (100 MHz, CDCl3): δ 165.5 (C2), 153.4 (C2′,C6′), 146.8 (C4′), 146.3 (C3′), 133.7 (C3′), 129.0 (C1′), 127.8, 127.4, 117.7 (C2′), 112.0 (C4′), 110.9 (C5′), 94.9 (C1′,C5′), 64.9 (C3′), 63.7 (C1), 61.0 (C8), 56.0 (C7′,C9′), 55.9 (C10′)

IR νmax(ATR): 3183 cm−1 (OH), 1775 cm−1 (β-lactam C=O)

LRMS: APCI calculated for [M+H+] 436 m/z; found 436.13

3-(4-(benzylxoy)phenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (83)

83 was synthesised from 79a using General Method X.

Yield: not calculated

Appearance: yellow oil/gum

Rf: 0.67 (1:1 n-hexane:ethyl acetate)

1H NMR (400 MHz, CDCl3): δ 7.45 – 7.33 (m, 5H, Bn Ar-H), 7.25 (d, 2H, J = 8.8 Hz, H2′,H6′), 7.22 (dd, 1H, J = 7.0 Hz, J = 2.2 Hz, Hγ), 7.16 (d, 1H, J = 2.2 Hz, Hα), 6.99 (d, 3H, J = 8.8 Hz, H3′,H5′), 6.63 (2H, H1′,H5′), 5.09 (s, 2H, benzyl CH2), 4.80 (d, 1H, J = 2.6 Hz, Hδ), 2.47 (d, 1H, J = 2.6 Hz, H2′), 3.87 (s, 3H, H10′), 3.79 (s, 3H, H8), 3.76 (s, 6H, H7′,H9′)

13C NMR (100 MHz, CDCl3): δ 166.2, 158.5, 153.3, 153.2, 146.8, 146.2, 136.7, 133.9, 130.7, 128.6, 127.6, 127.1, 117.8, 115.6, 112.1, 111.1, 97.5, 94.7, 70.1, 64.5, 64.1, 60.9, 56.2

4-(3-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (26)

26 was synthesised from 79b using General Method X.

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Overall yield for Staudinger: 880 mg (1.56 mmol) 11%
Appearance: white powder
Rf: 0.24 (1:1 n-hexane: ethyl acetate)
Melting Point: 73-75 °C

{H NMR (400 MHz, CDCl₃): δ 7.13 (d, 2H, J=8 Hz), 6.98 (d, 1H, J=1.5 Hz, H6-), 6.91 (dd, 1H, J=8 Hz, J=1.5 Hz, H5-), 6.87 (d, 1H, J=8 Hz, H3-), 6.78 (d, 2H, J=8 Hz), 6.64 (s, 2H, H1&2), 4.78 (d, 1H, J=1.9 Hz, H4), 4.19 (d, 1H, J=1.9 Hz, H3), 3.92 (3H, s, H2O), 3.80 (s, 3H, H5), 3.75 (s, 6H, H7&8)

IR νmax(ATR): 1725.88 cm⁻¹ (β-lactam C=O)
LRMS: APCI calculated for [M+H]+ 452 m/z; found 451.10

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27)

Yield (microwave): 110 mg (0.31 mmol) 5%
Yield (reflux): 900 mg (2.5 mmol) 36%
Rf: 0.25 (1:1 n-hexane: ethyl acetate)
Melting Point: 103-104 °C

{H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, J=8 Hz, H6-), 6.91 (dd, 1H, J=8 Hz, J=1.3 Hz, H5-), 6.86 (d, 1H, J=8 Hz, H3-), 6.58 (s, 2H, H1&2), 5.7 (bs, 1H, H3OH), 4.89 (dd, 1H, J=J=5.5 Hz, J=2.7 Hz, H4), 3.92 (s, 3H, H2O), 3.78 (s, 3H, H5), 3.75 (s, 6H, H7&8), 3.53 (dd, 1H, J=13.7 Hz, J=5.1 Hz, H3), 2.95 (dd, 1H, J=14.6 Hz, J=2.7 Hz, H3)

{C NMR (100 MHz, CDCl₃): δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

IR νmax(ATR): 1732.58 cm⁻¹ (β-lactam C=O)
LRMS: APCI calculated for [M+H]+ 360 m/z; found 360.11

(4-(3-hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (28 cis)

28 cis was synthesised from 77 cis using General Method X.

Yield for Reformatsky: 50 mg (0.13 mmol) 2.6%
Appearance: yellow powder
Rf: 0.3 (1:1 n-hexane: ethyl acetate)
Melting Point: 144-145 °C

{H NMR (400 MHz, CDCl₃): δ 6.83 (d, 1H, J=8.2 Hz, H3-), 6.81 (d, 1H, J=8.2 Hz, H3-), 6.72 (dd, 1H, J=8.2 Hz, J=1.7 Hz, H2-), 6.57 (s, 2H, H1&2), 5.71 (bs, 1H, OH),
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5.06 (d, 1H, J₅,₆ = 6.1 Hz, H₅), 3.84 (s, 3H, H₁₀), 3.77 (s, 3H, H₈), 3.72 (s, 6H, H₇&₉), 3.62 (dq, 1H, J₃,₄ = 6.1 Hz, J₃,₅ = 6.7 Hz, H₃), 0.91 (d, 3H, J₃,₃ = 6.92 Hz, H₃)

¹³C NMR (100 MHz, CDCl₃): δ 168.6, 153.5, 145.6, 145.8, 134.3, 134.0, 128.1, 119.3, 118.6, 113.8, 113.3, 111.3, 110.8, 95.5, 95.2, 94.6, 57.9, 56.2, 49.4, 49.2

IR νmax(ATR): 3196.7 cm⁻¹ (OH, intermolecular bonded), 2930.4 cm⁻¹ (OH, intramolecular bonded) 1708 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₃₅H₅₂NO₆ [M + H⁺] 734.159814; 734.159684, error + 0.3 ppm

(4-(3-hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (28 trans)

28 trans was synthesised from 77) trans using General Method X.

Yield (reflux) for desilylation and purification: 40 mg (0.11 mmol) 61%. Yield for

Reformatsky: 140 mg (0.38 mmol) 7.6%

Appearance: pale yellow powder

Rᵣ: 0.35 (1:1 n-hexane: ethyl acetate), 0.5 (1:2 n-hexane: ethyl acetate)

Melting Point: 140-142 °C

¹H NMR (600 MHz, DMSO-d₆): δ 6.92 (d, 1H, J₅,₆ = 8.5 Hz, H₅'), 6.81 (dd, 1H, J₂,₃ = 8.9 Hz, J₂,₆ = 1.7 Hz, H₂'), 6.82 (d, 1H, J₆,₇ = 1.7 Hz, H₆'), 6.55 (s, OH), 6.53 (s, 2H, H₁₃&₁₄'), 4.69 (d, 1H, J₃,₄ = 2.5 Hz, H₄'), 3.71 (s, 3H, H₁₀'), 3.63 (s, 6H, H₇&₉'), 3.59 (s, 3H, H₈'), 3.10 (dq, 1H, J₃,₃ = 2.5 Hz, J₆,₆ = 8 Hz, H₆'), 1.34 (d, 3H, J₃,₃ = 6.92 Hz, H₃)

¹H NMR (400 MHz, CDCl₃): δ 6.96 (d, 1H, J = 1.8 Hz, H₆), 6.87 (m, 3H, H₂',₃',₄'), 6.58 (s, 2H, H₁₃&₁₄'), 5.70 (s, 1H, OH), 4.46 (d, 1H, J = 2 Hz, H₉), 3.92 (s, 3H, H₁₀), 3.78 (s, 3H, H₈), 3.75 (s, H₇&₉), 3.13 (d, 1H, J = 8.9 Hz, J = 2 Hz, H₉), 1.47 (d, 3H, J = 6.4 Hz)

¹³C NMR (100 MHz, DMSO-d₆): δ 167.8 (C₂'), 153.0 (C₄&₆'), 147.7 (C₇'), 146.9 (C₅'), 133.6 (C₇'), 130.3 (C₁'), 117.6 (C₂'), 113.1 (C₅'), 112.2 (C₃'), 94.6 (C₄&₅'), 61.2 (C₆'), 56.1 (C₇&₉'), 55.6 (C₃'), 54.8 (C₅), 53.9 (C₆), 12.8 (CH₃)

IR νmax(ATR): 2930 cm⁻¹ (OH), 1732 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₃₅H₅₂NO₆ [M+H⁺] 734.159814; 734.159938, error + 0.3 ppm

3-fluoro-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (29)

29 was synthesised from 78 using General Method X.

Yield for Reformatsky: 640 mg (1.7 mmol) 56%

Appearance: Yellow powder

Rᵣ: 0.25 (1:1 n-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, J = 1.9 Hz, H₆'), 6.88 (dd, 1H, J = 8.1 Hz, J = 1.9 Hz, H₅'), 6.83 (d, 1H, J = 8.9 Hz, H₇'), 5.83 (s, 2H, H₁₃&₁₄'), 5.11 (apparent dd, 1H, J₃,₃ = 48.3 Hz, J₆,₆ = 2.6 Hz, H₆'), 4.86 (apparent dd, 1H, J₃,₃ = 26.1 Hz, J₆,₆ = 2.6 Hz, H₆'), 3.87 (s, 3H, H₁₀'), 3.74 (s, 6H, H₇&₉'), 3.72 (s, 3H, H₈'),

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\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 167.91 (\(J_{\text{F-C}} = 31\) Hz, C\(_2\)), 153.8 (C\(_{\text{ax}}\&\text{eq}\)), 146.2 (\(J_{\text{F-C}} = 62\) Hz, C\(_2^\prime\)), 142.8, 131.4 (C\(_5\)), 130.4 (C\(_3\)), 118.6 (C\(_{\text{ax}}\)), 113.1 (C\(_{\text{eq}}\)), 110.8 (C\(_{\text{ax}}\)), 95.2 (\(J_{\text{F-C}} = 75\) Hz, C\(_3\)), 92.3 (C\(_{\text{ax}}\)), 62.1 (C\(_8\)), 56.0 (C\(_8^\prime\)), 55.9 (C\(_7^\prime&\text{eq}\))

\(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) -203.74

HRMS: APCI calculated for C\(_{19}\)H\(_{20}\)FNO\(_6\) [M + H\(^+\)] 378.1353; found 378.1347.

4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30)

30 was synthesised from 71 using General Method X.

Overall yield for Staudinger: 870 mg (1.61 mmol) 32%

Appearance: yellow powder

Melting point: 118-120 °C

\(R_f\): 0.29 (1:1 n-hexane: ethyl acetate)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.31 (dd, 1H, \(J= 7\) Hz, \(J= 1.7\) Hz), 7.10 (d, 1H, \(J= 1.8\) Hz), 7.05 (dd, 1H, \(J= 3.5\) Hz, H\(_6\)), 7.01 (d, 1H, \(J= 2.1\) Hz), 6.94 (dd, 1H, \(J= 8.14\) Hz, \(J= 1.9\) Hz), 6.89 (d, 1H, \(J= 8.9\) Hz, H\(_3\)), 6.61 (s, 2H, H\(_{\text{ax}}\&\text{eq}\)), 5.70 (bs,1H, OH), 4.87 (d, 1H, \(J= 1.8\) Hz, H\(_4\)), 4.48 (d, 1H, \(J= 1.8\) Hz, H\(_5\)), 3.93 (s, 3H, H\(_{10}\)), 3.80 (s, 3H, H\(_8\)), 3.76 (s, 6H, H\(_{\text{ax}}\&\text{eq}\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 164.4 (C\(_2\)), 153.6 (C\(_{\text{ax}}\&\text{eq}\)), 146.9 (C\(_{\text{eq}}\)), 146.4 (C\(_{\text{ax}}\)), 136.1, 134.7, 133.6, 129.9, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.1

IR \(\nu_{\text{max}}\) (ATR): 3528 cm\(^{-1}\) (free OH), 3187 cm\(^{-1}\) (intramolecular bonded OH), 1719 cm\(^{-1}\) (\(\beta\)-lactam C=O)

LRMS: APCI calculated for [M+H\(^+\)] 442 m/z; found 442.09

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33)

33 was synthesised from 72 using General Method X.

Yield: 530 mg (1.38 mmol) 28%

Appearance: white powder

\(R_f\): 0.35 (1:1 n-hexane: ethyl acetate)

Melting Point: 58-60 °C
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1H NMR (400 MHz, CDCl₃): δ 6.97 (d, 1H, H₆, J₆–₂′ = 2.1 Hz), 6.88 (dd, 1H, H₇, J₇–₆ = 2.1 Hz, H₂), 6.87 (d, 1H, J₁’, 3’ = 8 Hz, J₂’–₃’ = 8 Hz, H₄), 6.58 (s, 2H, H₁’₃’), 6.03 (td, 1H, J₁’₁₂ = 17.1 Hz, J₁’₁₂ = 9.9 Hz, J₁’₁₂ = 2.2 Hz, H₁’), 5.71 (bs, OH), 5.40 (dd, 1H, J₁₂–₁₂ = 17 Hz, J₁₂–₁₂ (geminal) = 2.2 Hz, H₁₂), 5.33 (dd, 1H, J₁₀–₁₁ = 9.9 Hz, J₁₀–₁₁ (geminal) = 2.2 Hz, H₁₀), 4.69 (d, 1H, J₄₃ = 2.2 Hz, H₃), 3.92 (s, 3H, H₆), 3.78 (s, 3H, H₁₀), 3.75 (s, 6H, H₇₈₉), 3.74 (dt, 1H, J₃–₂’ = 8.5 Hz, J₃–₃’ = 2.2 Hz, H₃)

13C NMR (100 MHz, CDCl₃): δ 165.3 (C₂), 153.5 (C₁’₃’), 146.9 (C₄’), 146.3 (C₅’), 135.2 (C₆’), 133.9 (C₇’), 130.6 (C₈’), 130.5 (C₉’), 119.8 (C₉), 117.8 (C₂’), 112.0 (C₆’), 111.9 (C₅’), 94.8 (C₁’₉’), 63.9 (C₃’), 61.4 (C₄’), 60.9 (C₅’), 56.1 (C₇₈₉), 56.0 (C₁₀’)

IR νmax (ATR): 1738 cm⁻¹ (β-lactam C=O)

4-(3-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (38)

was synthesised from 73 using General Method X.

Yield from Staudinger: 700 mg (1.57 mmol) 32%

Appearance: white powder

Melting Point: 61-64 °C (amorphous powder)

Rf: 0.15 (1:1 n-hexane: ethyl acetate)

1H NMR (400 MHz, CDCl₃): δ 7.28 (d, 2H, J = 8.9 Hz), 7.03 (t, 1H, J = 8.9 Hz), 7.0 (d, 1H, J = 3.2 Hz), 6.96 (dd, 1H, J = 7.9 Hz, J = 3.2 Hz), 6.92 (d, 1H, J = 7.9 Hz), 6.90 (d, 2H, J = 8.9 Hz), 6.61 (s, 2H), 5.74 (s, 1H, OH), 5.13 (d, 1H, J = 1.3 Hz), 4.90 (d, 1H, J = 1.3 Hz), 3.96 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H)

13C NMR (100 MHz, CDCl₃): δ 162.5, 157.1, 153.6, 147.3, 146.6, 134.9, 133.1, 129.7, 122.2, 118.5, 115.9, 115.4, 112.4, 111.1, 95.4, 87.1, 64.0, 60.8, 55.9

IR νmax (ATR): 3386 cm⁻¹ (OH), 1749 cm⁻¹ (β-lactam C=O)

3-(4-fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39)

was synthesised from 73 using General Method X.

Yield: 900 mg (1.98 mmol) 40%

Appearance: yellow fluffy powder

Melting Point: 99-101 °C

Rf: 0.35 (1:1 n-hexane: ethyl acetate)

1H NMR (400 MHz, CDCl₃): δ 7.33 (apparent dd, 2H, J = 8.1 Hz, J = 3.1 Hz, H₉’₆’), 7.09 (apparent triplet, 2H, J = 8.1 Hz, H₉’₆’), 7.00 (d, 1H, J = 2.1 Hz, H₁’), 6.93 (dd, 1H, J = 7.9, J = 1.8 Hz, H₇’), 6.89 (d, 1H, J = 7.9, H₇’), 6.63 (s, 2H, H₁’₃’), 5.72 (s, 1H, OH), 4.78 (d, 1H, J = 1.8 Hz, H₃), 4.27 (d, 1H, J = 1.8 Hz, H₄), 3.94 (s, 3H, H₁₀), 3.80 (s, 3H, H₆), 3.76 (s, 6H, H₁’₉’₈’₉’)

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1H NMR (400 MHz, CDCl₃): δ 7.42-7.32 (m, 7H), 7.27 (d, 2H, J = 8.6 Hz), 6.99 (d, 1H, J = 8 Hz, H₆⁻), 6.9 (dd, 1H, J = 8.6 Hz, H₄⁻), 6.88 (d, 1H, J = 8.6 Hz, H₃⁻) 6.81 (bs, 1H, NH), 6.63 (s, 2H, H₁⁻), 5.22 (s, 2H, CH₂ of CBZ group), 4.77 (d, 1H, J = 2.2 Hz, H₅), 4.24 (d, 1H, J = 8.5 Hz, H₄), 3.92 (s, 3H, H₂O), 3.8 (s, 3H, H₈), 3.75 (s, 6H, H₂O), 1.24 (s, 3H, H₃OCH₃)

13C NMR (100 MHz, CDCl₃): δ 166.0 (C₇), 159.3 (C₄⁻), 153.5 (C₃⁻), 146.6, 146.4, 134.5 (C₅), 133.8 (C₂⁻), 130.7, 128.6 (C₈⁻), 126.9 (C₁⁻), 117.8 (C₆⁻), 114.4, 112.1 (C₉⁻), 111.0, 94.9 (C₁₆), 64.5 (C₁₅), 64.2 (C₄), 56.1 (C₇⁻), 56.0 (C₁₀⁻), 55.3 (C₈⁻)
4-(3-amino-4-methoxyphenyl)-3-phenoxy-1-(3,4,5trimethoxyphenyl)azetidin-2-one (37 trans)

37 trans was synthesised from 36 trans using General Method XI.

Yield for reduction and purification: 160 mg (0.36 mmol) 38%

Overall yield for Staudinger: 7%

Appearance: brown oil/powder

Rf: 0.32 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (400 MHz, CDCl$_3$): 7.28 (m, 2H), 7.01 (t, 2H, J = 7.0 Hz), 6.95 – 6.85 (m, 4H), 6.62 (s, 2H), 5.13 (d, 1H, J = 1.9 Hz), 4.88 (d, 1H, J = 1.9 Hz), 3.91 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H)

$^13$C NMR (100 MHz, CDCl$_3$): not determined as yield was too low.

4-(3-amino-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5trimethoxyphenyl)azetidin-2-one (32 as an isomeric mixture)

32 was synthesised from 31 as a mixture of cis:trans isomers (ratio 32: 68) prior to further purification as cis and trans isomers using General Method XI.

Yield for reduction and purification: 410 mg (0.93 mmol) 33%

Yield for Staudinger: 11%

Appearance: yellow powder

Melting Point: 65-69 °C (amorphous powder)

Rf: 0.31 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (600 MHz, CDCl$_3$): δ 7.29 (dd, 1H, J = 4.5 Hz, J = 1.3 Hz, thiophene), 7.28 (m, 2H, H$_{5&6}$), 7.24 (bs, 1H, NH$_2$), 7.1 – 7.05 (m, 3H, thiophene ring), 7.03 (d, 1H, J = 3.3 Hz, thiophene ring), 6.91 (d, 0.4H, cis isomer, J = 3.3 Hz, H$_{6'}$), 6.83 (m, 1H), 6.64 (s, 2H, H$_{5&6'}$), 6.58 (d, 0.4H, cis isomer, J = 9 Hz, H$_{5'}$), 5.30 (d, 0.4H, cis isomer, J = 4.6 Hz, H$_s$), 5.08 (d, 0.4H, cis isomer, J = 4.6 Hz, H$_a$), 4.83 (d, 1H, J = 3.3 Hz, H$_d$), 4.48 (d, 1H, J = 3.3 Hz, H$_s$), 3.96 (s, 1.8H, cis isomer, H$_{10'}$), 3.89 (s, 3H, H$_{10'}$), 3.82 (s, 3H, H$_{10'}$), 3.79 (s, 2.5H, cis isomer, H$_{7&8'}$), 3.76 (6H, H$_{7&8'}$)

$^13$C NMR (100 MHz, CDCl$_3$): δ 164.5, 153.6, 136.2, 134.7, 133.8, 129.6, 127.8, 126.6, 125.3, 110.8, 95.0 (C$_{1&3}$), 65.0 (C$_5$ trans), 61.13 (C$_8$), 60.8, 60.0, 56.3, 55.9 (C$_{7&8'}$), 55.6 (C$_{10'}$)

$^1$H NMR (400 MHz, DMSO-d$_6$): δ 7.56 (dd, 1H, J = 5.2 Hz, J = 1.2 Hz), 7.15 (d, 1H, J = 3.7 Hz), 7.09 (dt, 1H, J = 5.3, J = 1.5 Hz), 6.83 (d, 1H, J = 8.5 Hz, H$_{6'}$), 6.77 (d, 1H, J = 1.5 Hz, H$_{6'}$), 6.75 (dd, 1H, J = 9.1 Hz, J = 1.5 Hz, H$_{2'}$), 6.63 (s, 2H, H$_{5&6'}$), 5.07 (d, 1H, J = 2.2 Hz, H$_s$), 4.87 (bs, 1H, OH), 4.64 (d, 1H, J = 2.2 Hz, H$_d$), 3.79 (s, 3H, OCH$_3$), 3.68 (s, 6H, H$_{7&8'}$), 3.61 (s, 3H, OCH$_3$)

$^13$C NMR (100 MHz, DMSO-d$_6$): δ 164.8 (C$_2$), 153.6, 147.2, 138.8, 136.9, 134.5, 133.7, 129.5, 127.8, 126.6, 126.4, 115.4, 111.0, 97.3, 95.6, 64.2, 60.6, 56.3, 55.8

$^{15}$N NMR (60.8 MHz, CDCl$_3$): δ 131.2 (NH$_2$)

IR $\nu_{v_{max}}$(ATR): 1745 cm$^{-1}$ (β-lactam C=O)

HRMS: APCI calculated for C$_{25}$H$_{28}$N$_2$O$_5$S [M +H$^+$], 441.147869; found 441.147664, error + 0.5 ppm

4-(3-amino-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5trimethoxyphenyl)azetidin-2-one (32 trans)

32 trans was isolated from a mixture of cis:trans isomers using LC

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Yield: 240 mg (0.55 mmol) 59 % of isomeric composition

**Appearance:** yellow powder

Rf: 0.35 (1:1 n-hexane: ethyl acetate)

**1H NMR (600 MHz, CDCl3):** δ 7.30 (dd, 1H, J = 4.5 Hz, J = 1.3 Hz, H6), 7.2 (bs, 2H, NH2), 7.09 (apparent d, 1H, J = 2.6 Hz, H7), 7.05 (dd, 1H, J = 2.1 Hz, H8), 6.93 (m, 2H, H1''&3''), 6.85 (d, 1H, J = 8.5 Hz, H3''), 6.63 (s, 2H, H1''&3''), 4.85 (d, 1H, J = 1.8 Hz, H4), 4.48 (d, 1H, J = 1.8 Hz, H5), 3.89 (s, 3H, H7'), 3.79 (s, 3H, H6'), 3.76 (s, 3H, H5''&7'').

**13C NMR (100 MHz, CDCl3):** δ 164.4, 153.6, 136.2, 133.7, 129.5, 127.3, 125.8, 125.3, 111.0, 95.1, 64.7, 61.0, 60.2, 56.2, 55.9

**15N NMR (60.8 MHz, CDCl3):** δ 130.8

IR νmax (ATR): 1745 cm⁻¹ (β-lactam C=O)

**HRMS:** APCI calculated for C20H22N2O5S [M+H⁺], 441.147869; found 441.147664, error + 0.5 ppm

4-(3-amino-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (35)

35 was synthesised from 34 using General Method XI

Yield for reduction and purification: 400mg (1.04 mmol) 90 %

Yield for Staudinger: 20%

**Appearance:** off white amorphous powder

Rf 0.41 (1:1 n-hexane: ethyl acetate)

Melting Point: 60-65 °C

**1H NMR (400 MHz, CDCl3):** δ 6.78 (apparent doublet, J = 6.6 Hz, H3, H2''&3''&6''), 6.6 (s, 2H, H1''&3''), 6.01 (td, 1H, J13''-11'' = 17.1 Hz, J13''-12'' = 9.9 Hz, J13''-3'' = 2.2 Hz, H13''), 5.38 (d, 1H, J17-13' = 16 Hz, H17'), 5.32 (d, 1H, J12-13'' = 9.9 Hz, H12'), 5.32 (d, 1H, Jk,3'' = 2.2 Hz, H3''), 3.88 (s, 3H, H10'), 3.78 (s, 3H, H8'), 3.75 (apparent singlet, 7H, H7''&9'&6'')

**13C NMR (100 MHz, CDCl3):** δ 165.7, 153.9, 134.3, 133.9, 129.9, 119.7, 110.7, 94.9, 63.8, 60.9, 56.2, 55.7, 30.9

IR νmax (ATR): 1736 cm⁻¹ (β-lactam C=O)

4-(3-Amino-4-methoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (23a)

23a was synthesised from 63a using General Method XII.

Yield: <15%

Rf: 0.25 (1:4 n-hexane: ethyl acetate)

**1H NMR (400 MHz, CDCl3):** δ 8.55 (d, 1H, J = 1.8 Hz, H6''), 7.81 (bs, 1H, NH2), 7.02 (dd, 1H, J = 1.8 Hz, J = 8.6 Hz, H2''), 6.89 (d, 1H, J = 8.6 Hz, H3''), 6.59 (s, 2H), 5.49 (d, 1H, J = 1.1 Hz, H4), 4.90 (d, 1H, J = 1.1 Hz, H5'), 3.92 (s, 3H), 3.78 (s,3H), 3.72 (s, 6H), 2.20 (s, 3H, OCOCH3).

**13C NMR (100 MHz, CDCl3):** δ not determined

Appendices
Oxazinone 88 was synthesised as a by-product in high yield during the synthesis of 4-(4-methoxy-3-nitropheryl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (25) using General Method V using phenylacetyl chloride and 54.

Rf: 0.38 (1:1 n-hexane: ethyl acetate)

$^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.71 (d, 1H, $J = 1.9$ Hz, H$_{6'''}$), 7.42 (dd, 1H, $J = 2.2$ Hz, $H_{2'''}$), 7.34 (t, 1H, $J = 8.3$ Hz, H$_{2'''}$), 7.19 (d, 1H, $J = 2.1$ Hz, H$_4$), 7.17 (apparent multiplet, 2H, H$_{3&5'''}$), 6.93 (apparent m, 2H, H$_{2&6''}$), 6.92 (d, 1H, $J = 2.8$ Hz, H$_{3'''}$), 6.59 (s, 1H, H$_{11}$), 6.54 (s, 2H, H$_{2'''}&6''$), 3.97 (s, 3H, H$_{10'''}$), 3.81 (s, 3H, H$_{8'''}$), 3.8 (s, 6H, H$_{7'''}&9''$), 3.53 (d, $J = 13.8$ Hz, 1H, H$_7$), 3.41 (d, 1H, $J = 13.8$ Hz, H$_8$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 161.8 (C$_{10}$), 161.1 (C$_9$), 153.4 (C$_{3'''}&5''$), 153.3 (C$_{4'''}$), 139.3 (C$_5$), 136.8 (C$_4$), 135.0 (C$_3$), 134.9 (C$_{1'''}$), 132.6 (C$_{1'}$), 132.3 (C$_{3'''}$), 131.0 (C$_{2&6''}$), 129.1 (C$_{2&6'}$), 128.8 (C$_{1'''}$), 128.4 (C$_{3&5'}$), 128.5 (C$_3$), 128.4 (C$_2$), 128.4 (C$_{3'''}&5'$), 127.9 (C$_{4'}$), 127.1 (C$_4$), 124.6 (C$_{3'''}$), 115.5 (C$_5$), 113.0 (C$_{4'''}$), 103.6 (C$_{2'''}&6'$$'$), 88.2 (C$_{11}$), 60.8 (C$_8$), 56.7 (C$_{10'''}$), 56.3 (C$_{7'''}&9'$), 37.5 (C$_7$).

HRMS: APCI measured for C$_{33}$H$_{31}$N$_2$O$_8$; 583.27492, found 583.207483, error + 0.0 ppm

Amides 92, 93, 95 an 153 were isolated as by-products of General Method VIII. They were subsequently isolated using the method detailed below.

General Method XXIV: Condensation of primary amines and acid chlorides to form novel amide structures via base independent dehydration.

3,4,5-trimethoxyaniline (458 mg, 1 eq, 2.5 mmol) was added to a stirring solution of anhydrous toluene (50 mL) with the respective acid chloride (1 eq, 1.5 mmol). The reaction was allowed to stir at room temperature until a white precipitate was formed and starting materials had disappeared as indicated by TLC. The crude reaction mixture was filtered to remove the toluene and the crude product recrystallised from hot ethanol. Selected amides where indicated were isolated spontaneously from the Staudinger reaction crude mixture but not directly synthesised via base independent condensation.

2-phenyl-N-(3,4,5-trimethoxyphenyl)acetamide (92)
92 was synthesised from phenylacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV.
It was also isolated during the synthesis of 70 from 47 in the Staudinger reaction.
Yield from General Method XII: 590 mg (1.96 mmol) 78%

Rf: 0.47 (1:1 n-hexane: ethyl acetate)

Appearance: white fluffy crystals

Melting Point: 173-176 °C

$^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.43 (t, 2H, $J = 8.6$ Hz, H$_{15&16}$), 7.36 (apparent t, 3H, H$_{17-19}$), (7.07, bs, 1H, H$_{10}$), 6.75 (s, 2H, H$_{1&3}$), 3.82 (s, 6H, H$_7&9$), 3.82 (s, 3H, H$_8$), 3.75 (s, 2H, H$_{12}$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.0 (C$_{13}$), 153.3 (C$_{4&6}$), 134.9 (C$_{20}$), 134.3 (C$_3$), 133.7 (C$_{14}$), 129.7 (C$_{17&19}$), 129.2 (C$_{15&16}$), 127.8 (C$_{21}$), 97.7 (C$_4$), 60.9 (C$_8$), 56.3 (C$_{7&9}$), 43.1 (C$_{11}$)

$^{15}$N NMR (60.8 MHz, CDCl$_3$): $\delta$ 142.8 (N$_{10}$)

HRMS: APCI measured for C$_{17}$H$_{20}$NO$_4$ [M+ H$^+$]; 302.138685, found 302.138685, error + 0.2 ppm

IR $\nu_{\text{max}}$(ATR): 3294 cm$^{-1}$ (NH)

Purity (RP-HPLC): >95%

2-oxo-2-((3,4,5-trimethoxyphenyl)amino)ethyl acetate (93)

93 was synthesised from acetoxyacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV. It was also isolated during the synthesis of 17a and 63a in the Staudinger reaction.

Yield calculated from Staudinger (17a): 20-30%

Yield from General Method XXIV: 430mg (1.67 mmol) 64%

Rf: 0.21 (1:1 n-hexane: ethyl acetate)

Appearance: Grey crystalline powder

Melting Point: 137 °C

$^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.72 (bs, 1H, NH, H$_{10}$), 6.84 (s, 1H, H$_{1&3}$), 4.7 (s, 2H, CH$_2$, H$_{12}$), 3.88 (s, 6H, H$_{7&9}$), 3.84 (s, 3H, H$_8$), 2.26 (s, 3H, H$_{23}$)

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.3 (C$_{21}$), 165.0, (C$_{13}$), 153.4 (C$_{4&6}$), 135.4 (C$_3$), 132.7 (C$_{20}$), 98.0 (C$_{1&3}$), 63.3 (C$_{11}$), 61.8 (C$_8$), 56.2 (C$_{7&9}$), 20.6 (C$_{23}$)

$^{15}$N NMR (60.8 MHz, CDCl$_3$): $\delta$ 123.5 (N$_{10}$)

HRMS: APCI measured for C$_{13}$H$_{16}$NO$_6$ [M- H$^-$]; 282.098311, found 282.097997, error + 1.1 ppm, ESI measured for C$_{13}$H$_{16}$NO$_6$ [M+ H$^+$]; 284.112864, found 284.112864, error + 2.1 ppm.

IR $\nu_{\text{max}}$(ATR): 3313 cm$^{-1}$ (NH)

Purity (RP-HPLC): >95%

2-(4-fluorophenyl)-N-(3,4,5-trimethoxyphenyl)acetamide (94)

94 was not isolated from crude Staudinger reaction but synthesised from 4-fluorophenylacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV.

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Yield: 422 mg (1.32 mmol) 58%

Rf: 0.42 (1:1 n-hexane: ethyl acetate)

Appearance: Grey crystalline powder

Melting Point: 178-179 °C

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.32 (apparent m, 2H), 7.11 (apparent triplet, 2H, \(J = 8\) Hz), 6.77 (s, 2H, \(H_{1\&3}\)), 3.84 (s, 6H, \(H_{7\&9}\)), 3.82 (s, 3H, \(H_8\)), 3.72 (s, 2H, \(H_{11}\)).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 168.9, 163.5, 161.1, 153.4, 135.0, 133.8, 131.1, 130.1, 116.3, 116.0, 97.6, 61.0, 58.5, 56.2, 43.9, 18.5

\(^19\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) -114.3

HRMS: APCI measured for C\(_{13}\)H\(_{16}\)NO\(_6\) [M-H\(^+\)]; 318.114710, found 318.114652, error +0.2 ppm, ESI measured for C\(_{17}\)H\(_{20}\)FO\(_4\) [M-H\(^+\)]; 320.12963, found 320.129138, error -0.4 ppm.

IR \(v_{max}\) (ATR): 3277 cm\(^{-1}\) (NH)

Purity (RP-HPLC): >95%

2-phenoxy-N-(3,4,5-trimethoxyphenyl)acetamide (95)

95 was synthesised from phenoxyacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV. It was also isolated during the synthesis of 73 from 47 in the Staudinger reaction.

Yield: 704 mg (2.3 mmol) 89%

Rf: 0.63 (1:1 n-hexane: ethyl acetate)

Appearance: White/silver crystalline powder

Melting Point: 144-145 °C

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.26 (bs, 1H, NH, \(H_{10}\)), 7.37 (t, 2H, \(J = 7.8\) Hz, \(H_{17\&19}\)), 7.09 (t, 1H, \(J = 5.8\) Hz, \(H_{18}\)), 7.03 (d, 2H, \(J = 7.8\) Hz, \(H_{15\&16}\)) 6.9 (s, 2H, \(H_{1\&3}\)), 4.63 (s, 2H, \(H_{12}\)), 3.88 (s, 6H, \(H_{7\&9}\)), 3.84 (s, 3H, \(H_8\))

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 166.4 (C\(_{13}\)), 157.1, 153.5, 135.0, 132.8, 129.9, 122.2, 114.8, 98.1, 67.6, 61.1, 56.2.

HRMS: APCI measured for C\(_{17}\)H\(_{20}\)NO\(_5\) [M+ H\(^+\)]; 318.133599, found 318.133933, error –1.0 ppm,

IR \(v_{max}\) (ATR): 3287 cm\(^{-1}\) (NH)

Purity (RP-HPLC): >95%

2-(thiophen-2-yl)-N-(3,4,5-trimethoxyphenyl)acetamide (96)

96 was not isolated from the Staudinger reaction. It was synthesised from 2-thiopheneacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV.
Yield: 510 mg (1.66 mmol), 66%
Rf: 0.47 1:1 n-hexane: ethyl acetate
Appearance: pink/grey crystalline powder
Melting Point: 152-153°C

^1H NMR (400 MHz, CDCl₃): δ 7.34 (dd, 1H, J = 5.39 Hz, J = 1.1 Hz), 7.24 (bs, 1H, NH, H₁₀), 7.07 (m, 3H), 6.75 (s, 2H, H₁&₃), 3.96 (s, 2H, H₁₂), 3.85 (s, 6H, H₇&₉), 3.82 (s, 3H, H₈)

^1H NMR (400 MHz, DMSO-d₆): δ 10.15 (s, 1H, NH, H₁₀), 7.39 (t, 1H, J = 2.3 Hz), 6.98 (apparent s, 4H, H₁&₃, 3 X thiophene H), 3.8 (s, 2H, H₁₂), 3.74 (s, 6H, H₇&₉), 3.62 (s, 3H, H₈)

^13C NMR (100 MHz, DMSO-d₆): δ 168.3, 153.2, 137.6, 135.7, 133.8, 127.2, 126.9, 125.6, 97.3, 60.5, 56.1, 38.1

HRMS: APCI measured for C₁₅H₈NO₄ [M+ H⁺]; 308.095105, found 308.095447, error +0.3 ppm,
IR νmax (ATR): 3260 cm⁻¹ (NH)
Purity (RP-HPLC): >95%

2-(4-methoxyphenyl)-N-(3,4,5-trimethoxyphenyl)acetamide (97)
97 was not isolated from the crude Staudinger reaction. It was synthesised from 4-methoxyphenylacetyl chloride and 3,4,5-trimethoxyaniline using General Method XXIV.

Yield: 650 mg (1.95 mmol) 78%
Rf: 0.37 (1:1 n-hexane: ethyl acetate)
Appearance: silver grey crystalline powder
Melting Point: 174-175 °C

^1H NMR (400 MHz, CDCl₃): δ 7.26 (d, 2H, J = 7.5 Hz, H₁₄&₁₅), 7.13 (bs, 1H, NH, H₁₀), 6.96 (d, 2H, J = 8 Hz, H₁₆&₁₇), 6.75 (s, 2H, H₁&₃), 3.85 (s, 3H), 3.82 (s, 6H, H₁₆&₁₇), 3.81 (s, 3H), 3.68 (s, 2H, H₁₂).

^13C NMR (100 MHz, CDCl₃): δ 169.5 (C₁₃), 159.1, 153.5, 134.0, 130.7, 126.1, 115.0, 97.6, 61.0, 56.3, 55.1, 43.4
HRMS: APCI measured for C₁₈H₂₀NO₅ [M- H⁻]; 330.134696, found 330.134547, error –1.0 ppm,
IR νmax (ATR): 3330 cm⁻¹ (NH)
Purity (RP-HPLC): >95%

2-(4-benzylphenyl)-N-(3,4,5-trimethoxyphenyl)acetamide (153)
153 was isolated when synthesising 79a from 47 and (4-benzyloxyphenyl)acetic acid. It was not directly synthesised using General Method XXIV.
\( ^1H \text{ NMR (400 MHz; CDCl}_3 \): } \delta 7.48-7.33 (m, 5H, Benzyl Ar-H), 7.26 (d, 2H, \( J = 8.1 \text{ Hz} \)), 7.06 (bs, 1H, NH, H\(_{10}\)), 7.03 (d, 2H, \( J = 8.1 \text{ Hz} \)), 6.74 (s, 2H, H\(_{1&3}\)), 5.11 (s, 2H, Benzyl CH\(_2\), H\(_{21}\)), 3.83 (s, 6H, H\(_{7&9}\)), 3.81 (s, 3H, H\(_8\)), 3.69 (s, 2H, H\(_{12}\)).

\( ^1C \text{ NMR (100 MHz; CDCl}_3 \): } \delta 169.6 (C\(_{13}\)), 158.5, 153.3, 136.5, 130.6, 128.6, 128.1, 127.5, 126.6, 115.7, 97.6, 70.1, 61.0, 56.8, 56.2, 55.9, 47.2, 44.0, 28.3.

Numbering nomenclature for 3-hydroxy EN1 (3S,4S) and EN2 (3R,4R) enantiomers

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(3S,4S)-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN1)

\( ^1H \text{ NMR (400 MHz, CDCl}_3 \): } \delta 7.29 (s, 1H, OH), 6.65 (apparent s, 1H, H\(_{6}\)), 6.85 (apparent s, 2H, H\(_{2}\) & H\(_{1}\)), 6.53 (s, 2H, H\(_{1&3}\)), 4.75 (1H, apparent s, H\(_{1}\)), 4.73 (1H, apparent s, H\(_{3}\)), 3.90 (s, 3H, H\(_{10}\)), 3.77 (s, 3H, H\(_{8}\)), 3.71 (s,6H, (H\(_{7&9}\)).
1^1^C NMR (100 MHz, CDCl_3): δ 167.0 (C), 153.8 (C_4<sub>4</sub>&<sub>6</sub>), 147.1 (C_7&<sub>-</sub>), 147.0 (C_5&<sub>-</sub>), 135.0 (C_5), 135.3 (C_2&<sub>-</sub>), 130.0 (C_1&<sub>-</sub>), 118.2 (C_2&<sub>-</sub>), 112.2 (C_3&<sub>-</sub>), 111.0 (C_6&<sub>-</sub>), 95.5 (C_1<sub>1</sub>&<sub>3</sub>), 83.8 (C_4), 65.7 (C_3), 61.0 (C_5), 56.0 (C_7&<sub>8</sub>), 56.0 (C_10), 54.0 (C_8)

(3R, 4R)-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN2)

1^H NMR (400 MHz, CDCl_3): δ 7.29 (s, 1H, OH), 6.65 (apparent s, 1H, H_6&<sub>-</sub>), 6.85 (apparent s, 2H, H_2&<sub>-</sub> & H_7&<sub>-</sub>), 6.53 (s, 2H, H_1<sub>1</sub>&<sub>3</sub>), 4.75 (1H, apparent s, H_3), 4.73 (1H, apparent s, H_4), 3.90 (s, 3H, H_3&<sub>5</sub>), 3.77 (s, 3H, H_6), 3.71 (s, 6H, H_7&<sub>8</sub>)

1^3^C NMR (100 MHz, CDCl_3): δ 167.0 (C), 153.8 (C_4<sub>4</sub>&<sub>6</sub>), 147.1 (C_7&<sub>-</sub>), 147.0 (C_5&<sub>-</sub>), 135.0 (C_5), 135.3 (C_2&<sub>-</sub>), 130.0 (C_1&<sub>-</sub>), 118.2 (C_2&<sub>-</sub>), 112.2 (C_3&<sub>-</sub>), 111.0 (C_6&<sub>-</sub>), 95.5 (C_1<sub>1</sub>&<sub>3</sub>), 83.8 (C_4), 65.7 (C_3), 61.0 (C_5), 56.0 (C_7&<sub>8</sub>), 56.0 (C_10), 54.0 (C_8)

Dextrorotatory(+)-(3S, 4S)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN1)

1^H NMR (400 MHz, CDCl_3): δ 7.26 Hz (d, 2H, J=8.4 Hz, H_2&<sub>-</sub>&<sub>6</sub>), 6.90 (d, 2H, J=8.4 Hz, H_3&<sub>5</sub>&<sub>-</sub>), 6.5 (s, 2H, H_1<sub>1</sub>&<sub>3</sub>), 5.29 (s, 1H, OH), 4.79 (d, 1H, J=1.9 Hz, H_3), 4.74 (d, 1H, J=1.89 Hz, H_5), 3.80 (s, 3H, H_3&<sub>5</sub>), 3.75 (s, 3H, H_6), 3.68 (s, 6H, H_7&<sub>8</sub>)

1^3^C NMR (100 MHz, CDCl_3): δ 167.9 (C_2), 160.0 (C_4&<sub>-</sub>), 153.3 (C_4&<sub>4</sub>&<sub>6</sub>), 135.2 (C_5), 134.2 (C_2), 127.8 (C_1&<sub>-</sub>), 127.5 (C_2&<sub>-</sub>&<sub>6</sub>), 114.5 (C_3&<sub>3</sub>&<sub>-</sub>), 95.2 (C_1<sub>3</sub>&<sub>3</sub>), 83.6 (C_3), 66.0 (C_4), 60.93 (C_8), 55.9 (C_7&<sub>8</sub>), 55.32 (C_10)

Levorotatory-(3R,4R)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN2)

17EN2 was synthesised from 01DS2 using General Method IX.

1^H NMR (400 MHz, CDCl_3): δ 7.26 Hz (d, 2H, J=8.4 Hz, H_2&<sub>-</sub>&<sub>6</sub>), 6.90 (d, 2H, J=8.4 Hz, H_3&<sub>5</sub>&<sub>-</sub>), 6.5 (s, 2H, H_1<sub>1</sub>&<sub>3</sub>), 5.29 (s, 1H, OH), 4.79 (d, 1H, J=1.9 Hz, H_3), 4.74 (d, 1H, J=1.89 Hz, H_5), 3.80 (s, 3H, H_3&<sub>5</sub>), 3.75 (s, 3H, H_6), 3.68 (s, 6H, H_7&<sub>8</sub>)

1^3^C NMR (100 MHz, CDCl_3): δ 167.9 (C_2), 160.0 (C_4&<sub>-</sub>), 153.3 (C_4&<sub>4</sub>&<sub>6</sub>), 135.2 (C_5), 134.2 (C_2), 127.8 (C_1&<sub>-</sub>), 127.5 (C_2&<sub>-</sub>&<sub>6</sub>), 114.5 (C_3&<sub>3</sub>&<sub>-</sub>), 95.2 (C_1<sub>3</sub>&<sub>3</sub>), 83.6 (C_3), 66.0 (C_4), 60.93 (C_8), 55.9 (C_7&<sub>8</sub>), 55.32 (C_10)

dextrorotatory-(3S,4S)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN1)

1^H NMR (400 MHz, CDCl_3): δ 7.05 Hz (2H, apparent m, H_2&<sub>-</sub>&<sub>6</sub>), 6.95 (t, 1H, J=8.5 Hz, H_3&<sub>-</sub>), 6.47 (s, 2H, H_7&<sub>8</sub>), 4.75 (s, 1H,OH), 4.71 (d, 1H, J=1.3 Hz, H_3), 4.69 (d, 1H, J=1.3 Hz, H_3), 3.88 (s, 3H, H_10), 3.75 (s, 3H, H_6), 3.69 (s, 6H, H_7&<sub>8</sub>)

1^3^C NMR (100 MHz CDCl_3): δ 166.7 (C_2), 153.8 (C_4&<sub>4</sub>&<sub>6</sub>), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8), 95.3 (C_1<sub>1</sub>&<sub>3</sub>), 83.56 (C_4), 65.1 (C_3), 60.9 (C_8), 56.3 (C_7&<sub>8</sub>), 56.0 (C_10)

1^3^F NMR (376 MHz, CDCl_3): δ -133.2

Levorotatory-(3R,4R)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN2)

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\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.05 Hz (2H, apparent m, H\(_{2-\&6^-}\)), 6.95 (t, 1H, J=8.5 Hz, H\(_7^-\)), 6.47 (s, 2H, H\(_{1-\&5^-}\)), 4.75 (s, 1H,OH), 4.71 (d, 1H, J=1.3 Hz, H\(_4^-\)), 4.69 (d, 1H, J=1.3 Hz, H\(_3^-\)), 3.88 (s, 3H, H\(_6^-\)), 3.75 (s, 3H, H\(_2^-\)), 3.69 (s, 6H, H\(_{2-\&5^-}\))

\(^{13}\)C NMR (100 MHz CDCl\(_3\)): \(\delta\) 166.7 (C\(_2^-\)), 153.8 (C\(_{4-\&6^-}\)), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8, 95.3 (C\(_{1-\&5^-}\)), 83.56 (C\(_4^-\)), 65.1 (C\(_3^-\)), 60.9 (C\(_5^-\)), 56.3 (C\(_{7-\&9^-}\)), 56.0 (C\(_{10^-}\))

\(^19\)F NMR (376 MHz, CDCl\(_3\)): \(\delta -133.2\)

\((3S,4S)-4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN1)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.38 (apparent s,1H, H\(_{6^-}\)), 7.21 (d,1H, J=7.5 Hz, H\(_2^-\)), 6.95 (d,1H, J=7.5 Hz, H\(_1^-\)), 6.5 (s,2H, H\(_{1-\&5^-}\)), 4.77 (apparent s, 1H, H\(_3^-\)), 4.75 (apparent s, 1H, H\(_4^-\)), 3.9 (s, 3H, H\(_6^-\)), 3.9 (s, 3H, H\(_2^-\)), 3.7 (s, 6H, H\(_{2-\&5^-}\)).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 153.6 (C\(_2^-\)), 153.5 (C\(_{4-\&6^-}\)), 135.4 (C\(_{5^-\&7^-}\)), 128.4 (C\(_{3^-}\)), 127.7 (C\(_{2^-}\)), 122.5 (C\(_{1^-}\)), 112.3 (C\(_{3^-}\)), 95.4 (C\(_{1-\&5^-}\)), 83.1 (C\(_3^-\)), 65.0 (C\(_4^-\)), 61.0 (C\(_5^-\)), 56.3 (C\(_{10^-}\)), 56.0 (C\(_{7-\&9^-}\))

\((3R,4R)-4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN2)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.38 (apparent s,1H, H\(_{6^-}\)), 7.21 (d,1H, J=7.5 Hz, H\(_2^-\)), 6.95 (d,1H, J=7.5 Hz, H\(_1^-\)), 6.5 (s,2H, H\(_{1-\&5^-}\)), 4.77 (apparent s, 1H, H\(_3^-\)), 4.75 (apparent s, 1H, H\(_4^-\)), 3.9 (s, 3H, H\(_6^-\)), 3.9 (s, 3H, H\(_2^-\)), 3.7 (s, 6H, H\(_{2-\&5^-}\)).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 153.6 (C\(_2^-\)), 153.5 (C\(_{4-\&6^-}\)), 135.4 (C\(_{5^-\&7^-}\)), 128.4 (C\(_{3^-}\)), 127.7 (C\(_{2^-}\)), 122.5 (C\(_{1^-}\)), 112.3 (C\(_{3^-}\)), 95.4 (C\(_{1-\&5^-}\)), 83.1 (C\(_3^-\)), 65.0 (C\(_4^-\)), 61.0 (C\(_5^-\)), 56.3 (C\(_{10^-}\)), 56.0 (C\(_{7-\&9^-}\))

\((3S,4S)-3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN1)

20EN1 was obtained from 06DS1 using General Method IX.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.12 (dd, 1H, J = 8.6 Hz, J = 1.8 Hz, H\(_2^-\)), 7.10 (apparent s, 1H, H\(_{6^-}\)), 6.79 (d, 1H, J = 8.6 Hz, H\(_3^-\)), 6.52 (s, 2H, H\(_{1-\&5^-}\)), 4.74 (apparent s, 2H, H\(_{1-\&5^-}\)), 3.82 (s, 3H), 3.76 (s, 3H), 3.69 (s, 6H), 2.20 (s, 3H, B ring meta CH\(_3\))

\(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \(\delta\) 7.14 (dd, 1H, J = 8.1 Hz, 2 Hz, H\(_2^-\)), 7.11 (d, 1H, J = 1.75 Hz, H\(_1^-\)), 6.81 (d, 1H, J = 8.1 Hz, H\(_3^-\)), 6.54 (s, 2H, H\(_{1-\&5^-}\)), 4.77 (d, 1H, J = 1.8 Hz, H\(_4^-\)), 4.75(d, 1H, J = 1.8 Hz, H\(_3^-\)), 3.83 (s, 3H,H\(_{10^-}\)), 3.77 (s, 3H, H\(_6^-\)), 3.71 (s, 6H, H\(_{2-\&5^-}\)), 2.187 (s, 3H, CH\(_3\), H\(_{2^-}\))

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)): \(\delta\) 166.7 (C\(_2^-\)), 158.1 (C\(_{4^-}\)), 153.4 (C\(_{4-\&6^-}\)), 134.6 (C\(_2^-\)), 133.4 (C\(_{3^-}\)), 128.4 (C\(_{3^-}\)), 127.6 (C\(_{2^-}\)), 124.9 (C\(_{1^-}\)), 110.1 (C\(_{5^-}\)), 95.3 (C\(_{1-\&5^-}\)), 83.6 (C\(_3^-\)), 65.7 (C\(_4^-\)), 60.9 (C\(_5^-\)), 55.9 (C\(_{7-\&9^-}\)), 55.4 (C\(_{10^-}\)), 30.9 (CH\(_3\))

\((3R,4R)-3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN2)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.12 (dd, 1H, J = 8.6 Hz, J = 1.8 Hz, H\(_2^-\)), 7.10 (apparent s, 1H, H\(_{6^-}\)), 6.79 (d, 1H, J = 8.6 Hz, H\(_3^-\)), 6.52 (s, 2H, H\(_{1-\&5^-}\)), 4.74 (apparent s, 2H, H\(_{1-\&5^-}\)), 3.82 (s, 3H), 3.76 (s, 3H), 3.69 (s, 6H), 2.20 (s, 3H, B ring meta CH\(_3\))

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$^1$H NMR (400 MHz, DMSO-d$_6$): δ 7.14 (dd, 1H, $J = 8.1$ Hz, 2 Hz, H$_{2''}$), 7.11 (d, 1H, $J = 1.75$ Hz, H$_{3''}$), 6.81 (d, 1H, $J = 8.1$ Hz, H$_{2'}$), 6.54 (s, 2H, H$_{1'''}$), 4.77 (dd, 1H, $J = 1.8$ Hz, H$_1$), 4.75 (dd, 1H, $J = 1.8$ Hz, H$_1$), 3.83 (s, 3H, H$_{10'''}$), 3.77 (s, 3H, H$_8$), 3.71 (s, 6H, H$_7$), 2.187 (s, 3H, CH$_3$)

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.23 (apparent s, 3H, H$_{2'}$), 6.5 (s, 2H, H$_{1'''}$), 4.80 (d, 1H, $J = 1.8$ Hz, H$_1$), 4.73 (apparent s, 1H, H$_4$), 3.76 (s, 3H, H$_8$), 3.69 (s, 6H, H$_7$), 2.48 (s, S CH$_3$, H$_{10'''}$)

$^1$H NMR (400 MHz, DMSO-d$_6$): δ 7.38 (d, 1H, H$_{2'''}$), 7.28 (d, 2H, $J = 8.6$ Hz, H$_{3'''}$), 6.72 (d, 1H, $J = 8.6$ Hz, 3-OH), 6.52 (s, 2H, H$_{1'''}$), 4.89 (dd, 1H, $J = 1.9$ Hz, H$_1$), 4.75 (dd, 1H, $J = 1.9$ Hz, H$_1$), 3.63 (s, 6H, H$_7$), 3.57 (s, 3H, H$_8$), 2.46 (s, 3H, H$_{10'''}$)

$^1$C NMR (400 MHz, DMSO-d$_6$): δ 166.9, 153.5, 138.9, 134.3, 133.8, 133.4, 127.9, 129.7, 126.6, 83.8, 65.1, 60.5, 56.3, 14.9

(3R,4R)-3-hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN1)

(3R,4R)-3-hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN2)

(35,45)-3-hydroxy-4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN1)

(3R,4R)-3-hydroxy-4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN2)

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**1³C NMR (100 MHz, CDCl₃):** δ 165.9 (C₂), 157.2 (C₆), 153.4 (C₄,₅,₆), 134.9 (C₇), 132.9 (C₂), 131.2 (C₃), 129.5 (C₄,₅), 126.3 (C₃,₄), 112.5 (C₇), 112.3 (C₃,₄), 95.4 (C₁,₆), 86.6 (C₃), 64.4 (C₄), 60.9 (C₆), 56.4 (C₇,₈,₉), 56.1 (C₁₀)

Numbering nomenclature for 3-substituted enantiomers

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4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN1)
(absolute configuration not determined)

**¹H NMR (400 MHz, CDCl₃):** δ 6.98 (d, 1H, J₆₂= 1.8 Hz, H₆), 6.91 (dd, 1H, J₂₃= 8.4 Hz, J₃₂= 1.3 Hz, H₃), 6.86 (d, 1H, J₃₂= 8.3 Hz, H₃), 6.58 (s, 2H, H₇,₈), 5.7 (bs, 1H, H₉,OH), 4.89 (dd, 1H, J₃₂=5.5 Hz, J₃₂= 2.7 Hz, H₃), 3.92 (s, 3H, H₁₀), 3.78 (s, 3H, H₁₀), 3.75 (s, 6H, H₇,₈,₉), 3.53 (dd, 1H, J₃₂= 13.7 Hz, J₃₄= 5.1 Hz, H₃), 2.95 (dd, 1H, J₃₂= 14.6 Hz, J₃₄= 2.7 Hz, H₃)

**¹C NMR (100 MHz, CDCl₃):** δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN2)
(absolute configuration not determined)

**¹H NMR (400 MHz, CDCl₃):** δ 6.98 (d, 1H, J₆₂= 1.8 Hz, H₆), 6.91 (dd, 1H, J₂₃= 8.4 Hz, J₃₂= 1.3 Hz, H₃), 6.86 (d, 1H, J₃₂= 8.3 Hz, H₃), 6.58 (s, 2H, H₇,₈), 5.7 (bs, 1H, H₉,OH), 4.89 (dd, 1H, J₃₂=5.5 Hz, J₃₂= 2.7 Hz, H₃), 3.92 (s, 3H, H₁₀), 3.78 (s, 3H, H₁₀), 3.75 (s, 6H, H₇,₈,₉), 3.53 (dd, 1H, J₃₂= 13.7 Hz, J₃₄= 5.1 Hz, H₃), 2.95 (dd, 1H, J₃₂= 14.6 Hz, J₃₄= 2.7 Hz, H₃)

**¹C NMR (100 MHz, CDCl₃):** δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

(3S, 4R)-4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN1)
1H NMR (400 MHz, CDCl3): δ 7.31 (dd, 1H, J = 7 Hz, J = 1.7 Hz), 7.10 (d, 1H, J = 1.8 Hz), 7.05 (dd, 1H, J = 8.41 Hz, J = 3.5 Hz, Hc), 7.01 (d, 1H, J = 2.1 Hz), 6.94 (dd, 1H, J = 8.14 Hz, J = 1.9 Hz), 6.89 (d, 1H, J = 8.9 Hz, Hc), 6.61 (s, 2H, H1&3), 5.70 (bs, 1H, OH), 4.87 (d, 1H, J = 18 Hz, Hbz), 4.48 (d, 1H, J = 1.8 Hz, Hb), 3.93 (s, 3H, H10), 3.80 (s, 3H, H6), 3.76 (s, 6H, H7&9)

13C NMR (100 MHz, CDCl3): δ 164.4 (C2), 153.6 (C4&6), 146.9 (C3'), 146.4 (C5'), 136.1, 134.7, 133.6, 129.9, 127.3, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.05

(3R,4S)-4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN2)

1H NMR (400 MHz, CDCl3): δ 7.31 (dd, 1H, J = 7 Hz, J = 1.7 Hz), 7.10 (d, 1H, J = 1.8 Hz), 7.05 (dd, 1H, J = 8.41 Hz, J = 3.5 Hz, Hc), 7.01 (d, 1H, J = 2.1 Hz), 6.94 (dd, 1H, J = 8.14 Hz, J = 1.9 Hz), 6.89 (d, 1H, J = 8.9 Hz, Hc), 6.61 (s, 2H, H1&3), 5.70 (bs, 1H, OH), 4.87 (d, 1H, J = 18 Hz, Hbz), 4.48 (d, 1H, J = 1.8 Hz, Hb), 3.93 (s, 3H, H10), 3.80 (s, 3H, H6), 3.76 (s, 6H, H7&9)

13C NMR (100 MHz, CDCl3): δ 164.4 (C2), 153.6 (C4&6), 146.9 (C3'), 146.4 (C5'), 136.1, 134.7, 133.6, 129.9, 127.3, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.05

(3S,4R)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33EN1)

1H NMR (400 MHz CDCl3): δ 6.97 (d, 1H, H6), 3J6-C2= 2.1 Hz), 6.88 (dd, 1H, 3J2-3= 8Hz, 3J2-6= 2.1 Hz, H2'), 6.87 (d, 1H, 3J3-2= 8Hz, H3'), 6.58 (s, 2H, H1&3), 6.03 (td, 1H, J= 17.1 Hz, J= 9.9 Hz, J= 2.2 Hz, H11'), 5.71 (bs, OH), 5.40 (dd, 1H, J= 17 Hz, J= 2.2 Hz, H6), 5.33 (dd, 1H, J= 9.9 Hz, J= 2.2 Hz, H10), 4.69 (d, 1H, J= 2.2 Hz, H5), 3.92 (s, 3H, H6), 3.78 (s, 3H, H10), 3.75 (s, 6H, H7&9), 3.74 (dt, 1H, J= 8.5 Hz, 3J3-5= 2.2 Hz, H4)

13C NMR (100 MHz CDCl3): δ 165.3 (C2), 153.5 (C4&6), 146.9 (C3'), 146.3 (C5'), 135.2 (C3), 133.9 (C2), 130.6 (C1'), 130.5 (C6'), 119.8 (C4'), 117.8 (C2'), 112.0 (C6'), 111.9 (C3'), 94.8 (C4&6), 63.9 (C3), 61.4 (C6), 60.9 (C6'), 56.1 (C7&9), 56.0 (C10)

Numbering nomenclature for 3-phenyl-substituted 3S,4R EN1 and 3R,4S EN2 enantiomers
(3S, 4R)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN1)

1H NMR (400 MHz, CDCl3): δ 7.35 (m, 5H, 3-phenyl, Hs,α), 6.99 (d, 1H, J=2 Hz, H9), 6.91 (d, 1H, H2, J=8 Hz, J=2 Hz, H2, H2'), 6.86 (d, 1H, J=8 Hz, H2'), 6.64 (s, 2H, H1',H3'), 5.79 (s, OH), 4.80 (d, 1H, J=2.5 Hz, H1), 4.29 (d, 1H, J=2.5 Hz, H1), 3.90 (s, 3H, H10'), 3.76 (s, 3H, H8), 3.72 (s, 6H, H3'), 63.7 (C3), 61.0 (C6), 56.0 (C7,αγ), 55.9 (C10)

13C NMR (100 MHz, CDCl3): δ 165.5 (C2), 153.4 (C4,αγ), 146.8 (C4', αγ), 146.3 (C5', αγ), 134.7 (C5), 133.7 (C2), 129.0 (C1', αγ), 127.87, 127.4, 117.7 (C2', αγ), 112.0 (C3', αγ), 110.9 (C8'), 94.9 (C1', αγ), 64.92 (C3), 63.7 (C4), 61.0 (C6), 56.0 (C7,αγ), 55.9 (C10)

(3R, 4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN2)

1H NMR (400 MHz, CDCl3): δ 7.35 (m, 5H, 3-phenyl, Hs,α), 6.99 (d, 1H, J=2 Hz, H9), 6.91 (d, 1H, H2, J=8 Hz, J=2 Hz, H2, H2'), 6.86 (d, 1H, J=8 Hz, H2'), 6.64 (s, 2H, H1',H3'), 5.79 (s, OH), 4.80 (d, 1H, J=2.5 Hz, H1), 4.29 (d, 1H, J=2.5 Hz, H1), 3.90 (s, 3H, H10'), 3.76 (s, 3H, H8), 3.72 (s, 6H, H3'), 63.7 (C3), 61.0 (C6), 56.0 (C7,αγ), 55.9 (C10)

13C NMR (100 MHz, CDCl3): δ 165.5 (C2), 153.4 (C4,αγ), 146.8 (C4', αγ), 146.3 (C5', αγ), 134.7 (C5), 133.7 (C2), 129.0 (C1', αγ), 127.87, 127.4, 117.7 (C2', αγ), 112.0 (C3', αγ), 110.9 (C8'), 94.9 (C1', αγ), 64.92 (C3), 63.7 (C4), 61.0 (C6), 56.0 (C7,αγ), 55.9 (C10)

(3S, 4R)-3-(4-Fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN1)

1H NMR (400 MHz, CDCl3): δ 7.33 (apparent dd, 2H, J= 8.1 Hz, J = 3.1 Hz, H3,α8), 7.09 (apparent triplet, 2H, J= 8.1 Hz, H3,α8), 7.00 (d, 1H, J= 2.1 Hz, H9), 6.93 (dd, 1H, J = 7.9, J = 1.8 Hz, H2'), 6.89 (d, 1H, J = 7.9, H1',αγ), 6.63 (s, 2H, H1',H3'), 5.72 (s, OH), 4.78 (d, 1H, J= 1.8 Hz, H1), 4.27 (d, 1H, H1, J= 1.8 Hz, H1), 3.94 (s, 3H, H10'), 3.80 (s, 3H, H8), 3.76 (s, 6H, H3',αγ)

13C NMR (100 MHz CDCl3): δ 161.3 (C2), 153.6 (C4,αγ), 147.0, 146.4, 130.3, 129.0, 117.8, 116.2, 115.9, 115.3, 112.0, 111.2, 94.9 (C1', αγ), 85.05 (C3), 64.3 (C3), 64.0, 64.0, 61.1, 56.2 (C7, αγ)

19F NMR (376 MHz, CDCl3): δ -114.1

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(3R,4S)-3-(4-fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN2)

1H NMR (400 MHz, CDCl3): δ 7.33 (apparent dd, 2H, J= 8.1 Hz, J = 3.1 Hz, H3&8), 7.09 (apparent triplet, 2H, J= 8.1 Hz, H5&6), 7.00 (d, 1H, J= 2.1 Hz, H6'), 6.93 (dd, 1H, J = 1.8 Hz, H2''), 6.89 (d, 1H, J = 7.9, H1''), 6.63 (s, 2H, H11&13), 5.72 (s, OH), 4.78 (d, 1H, J = 1.8 Hz, H4), 4.27 (d, 1H, J= 1.8 Hz, H3), 3.94 (s, 3H, H10), 3.80 (s, 3H, H3'), 3.76 (s, 6H, H7&9')

13C NMR (100 MHz CDCl3): δ 161.3 (C2), 153.6 (C3&5), 147.0, 146.4, 130.3, 129.0, 117.8, 116.2, 115.9, 115.3, 112.0, 111.2, 94.9 (C1&3), 85.05 (C3), 64.3 (C4), 64.0, 64.0, 61.1, 56.2 (C7&8)

19F NMR (376 MHz, CDCl3): δ -114.1

(3S,4R)-4-(3-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN1)

1H NMR (400 MHz CDCl3): δ 7.25 (d, 2H, J= 8.2 Hz, H3&8), 7.0 (d, 1H J= 2 Hz, H6'), 6.92 (d, 2H, J = 8.2 Hz, H5&6), 6.92 (dd, J= 8.1 Hz, J= 2 Hz, H2''), 6.9 (d, J=J'= 8.1 Hz, H1''), 6.63 (s, 2H, H11&13), 5.75 (broad s, OH), 4.77 (d, 1H, J= 2.1 Hz, H3), 4.23 (d, 1H, J= 2.1 Hz, H4), 3.92 (s, 3H, H10), 3.83 (s, 3H, H3'), 3.79 (s, 3H, H5), 3.75 (s, 6H, H7&9')

13C NMR (100 MHz CDCl3): δ 166.0 (C2), 159.3 (C4'), 153.5 (C3&5), 146.8, 146.4, 134.5 (C7), 133.8 (C2'), 130.7, 128.6 (C5'), 126.9 (C2'), 117.8 (C6'), 114.4, 112.1 (C3'), 111.0 , 94.9 (C1&3), 64.5 (C4), 64.2 (C3), 56.1 (C7&8), 56.0 (C10), 55.3 (C5')

(3S,4S)-4-(3-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN2)

1H NMR (400 MHz CDCl3): δ 7.25 (d, 2H, J= 8.2 Hz, H3&8), 7.0 (d, 1H J= 2 Hz, H6'), 6.92 (d, 2H, J = 8.2 Hz, H5&6), 6.92 (dd, J= 8.1 Hz, J= 2 Hz, H2''), 6.9 (d, J=J'= 8.1 Hz, H1''), 6.63 (s, 2H, H11&13), 5.75 (broad s, OH), 4.77 (d, 1H, J= 2.1 Hz, H3), 4.23 (d, 1H, J= 2.1 Hz, H4), 3.92 (s, 3H, H10), 3.83 (s, 3H, H3'), 3.79 (s, 3H, H5), 3.75 (s, 6H, H7&9')

13C NMR (100 MHz CDCl3): δ 166.0 (C2), 159.3 (C4'), 153.5 (C3&5), 146.8, 146.4, 134.5 (C7), 133.8 (C2'), 130.7, 128.6 (C5'), 126.9 (C2'), 117.8 (C6'), 114.4, 112.1 (C3'), 111.0 , 94.9 (C1&3), 64.5 (C4), 64.2 (C3), 56.1 (C7&8), 56.0 (C10), 55.3 (C5')

General Method XXV: Addition of dimethylphosphite to P3 to afford P3 dimethylphosphate

P3 (250mg, 1.16 mmol, 1 eq) was added to a stirring solution of anhydrous MeCN (20 mL) under a nitrogen atmosphere and sonicated for 10 minutes. The reaction vessel was then placed on ice followed by dropwise addition of CCl4 (0.4 mL, 3.5 mmol, 3 eq). The solution was stirred vigorously for 20 minutes. DMAP (16mg, 0.125 mmol, 0.1 eq) and DIPEA (0.45 mL, 2.52 mmol, 2 Eq) were dissolved in MeCN (5 mL) and injected The solution was stirred for a further 5 minutes before the addition of dimethylphosphate (0.16 mL, 1.8 mmol, 1.4 eq). The solution was stirred for a further 4 hours and monitored by TLC until a decrease in Rf was observed. The reaction was then quenched with KHSO4 (5mL). The reaction was diluted with ethyl acetate (20 mL). After phase separation the aqueous layer was retained and was then

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extracted with 3 X 20 mL ethyl acetate. The combined organic layers were washed with brine and dried using anhydrous Na₂SO₄. A crude yellow oil was obtained. The purified product was isolated from parent material using flash chromatography over silica gel with a gradient of ethyl acetate: MeOH (100:0 - 80:20).

\((E)\)-dimethyl (2-(2-(pyrazin-2-yl)vinyl)phenyl) phosphate (P3DMP)

P3DMP was synthesised from P3 and dimethyl phosphite using General Method XXV.

Yield: 160mg, 50%
Appearance: dark brown oil
Rf: 2:1 n-hexane: ethyl acetate: 0.16 (plate development 1), 0.22 (plate development 2)

1H NMR (600 MHz, DMSO-d₆): δ 8.76 (d, 1H, J₄''-₃'' = 1.6 Hz, H₄''), 8.67 (t, 1H, J₃''-₂'' = 2.3 Hz, J₃''-₄'' = 1.6 Hz, H₃''), 8.53 (d, 1H, J₂''-₃'' = 2.2 Hz, H₂''), 8.01 (d, J₂-₁ = 16 Hz, H₂), 7.93 (d, 7.47 J₂'-₃' = 7.67 Hz, H₂') (d, J₁₇ = 16 Hz, H₂), 7.43 (dt, Jₚ₄'' = 1.6 Hz, J₄''-₅''-₃'' = 8.1 Hz, H₄''), 7.32 (apparent multiplet, 2H, H₃'₅'), 3.84 (s, 3H, P-O-CH₃), 3.83 (s, 3H, P-O-CH₃)

13C NMR (100 MHz, DMSO-d₆): δ 150.7 (C₁''), 148.6 (C₆'), 145.2 (C₃''), 144.7 (C₄''), 144.1 (C₂''), 130.7 (C₁'), 128.0 (C₂'), 127.7 (C₃), 126.9 (C₁), 126.1 (C₃'), 120.9 (C₅'), 55.5 (C₇₈₉₉)

31P NMR (162 MHz, DMSO-d₆): δ -3.6

HRMS: APCI Calculated for C₁₄H₁₆N₂O₄P [M-H⁻]: 307.084220, found 307.084214, error +0 ppm. APCI calculated for C₁₄H₁₅N₂NaO₄P [M+Na⁺]: 329.06611, found 329.066428, error +0.8 ppm

General Method XXVI: Synthesis of P3 phosphoric acid using phosphorous oxychloride

Pyrazinib (50mg, 0.25mmol, 1 eq) was added to a stirring solution of anhydrous THF (30 mL) and sonicated for 20 minutes under a nitrogen atmosphere, prior to commencing the reaction to aid dissolution of the solids. The reaction vessel was then cooled on an ice/NaCl bath to -15 °C and set up under reflux conditions to allow for condensation of THF following addition of reagents. Phosphorous oxychloride (0.03 mL, 0.3 mmol, 1.2 eq) was added dropwise to the vessel via injection. Following cooling of the vessel, anhydrous TEA was added dropwise (0.04 mL, 1.2 mmol, 1.2 eq). The reaction vessel was allowed to warm to room temperature and stirred for 3 hours. dH₂O (20 mL) was added and stirred overnight. The solvents were reduced in vacuo and the residue dissolved in methanol (30mL) before drying with anhydrous Na₂SO₄. The solvent was once again removed in vacuo to afford the crude phosphoric acid intermediate in complex with TEA.

2-((pyrazin-2-ylimino)methyl)phenol (146)

146 was synthesised from 2-aminopyrazine and salicaldehyde using General Method III.

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Yield: >90% Appearance: yellow crystalline material

$^1$H NMR (600 MHz); CDCl$_3$: δ 13.00 (bs, OH), 9.48 (s, 1H, H$_6$), 8.707 (d, 1H, H$_3$, $J$=1.6Hz), 8.53 (d, 1H, $J$= 1.9Hz, H$_1$), 8.51(dd, 2H, $J$=16Hz, $J$= 2.5Hz, H$_2$), 7.54 (d, 1H, $J$= 8.5Hz, H$_{5'}$), 7.48(t, 1H, $J$= 8Hz, $J$= 2Hz, H$_{5''}$), 7.083 (d, 1H, $J$= 8.2Hz, $J$= 2Hz, H$_{3''}$), 7.01(t, 1H, $J$= 8Hz, $J$= 2Hz, H$_{4''}$)

$^{13}$C NMR (100 MHz); CDCl$_3$: δ 166.9 (C$_6$), 162.1 (C$_1'$), 153.8 (C$_3$), 143.2 (C$_2$), 143.4 (C$_2$), 143.2 (C$_2$), 142.6(C$_3$), 134.8 (C$_5'$), 133.9 (C$_6'$), 117.5 (C$_5$)

HRMS: APCI calculated for C$_{11}$H$_9$N$_3$O [M$^+$+H], 200.081612; found 200.081838

(E)-1-(4-methoxyphenyl)-N-(pyrazin-2-yl) methanimine (150)

150 was synthesised from 2-aminopyrazine and 4-methoxybenzaldehyde (para-anisaldehyde) using General Method XXI and General Method XXII.

HRMS: APCI calculated for C$_{12}$H$_{12}$N$_3$O [M$^+$+H], 214.097488; found 214.097617
References
19. HSE.ie, National Cancer Control Programme: Cancer Incidence, Survival and Mortality Data.
References

34. ACS, Breast Cancer Hormone Receptor Status. https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-hormone-receptor-status.html#:%7E:text=Hormone%20receptor%20positive%20(or%20hormone%20levels%20or%20block%20estrogen%20receptors. (accessed 7-2-22).


130. NCCP, NCCP Chemotherapy Regimen CARBOplatin (AUC 6) and Weekly PACLitaxel 80mg/m2 followed by Dose Dense DOXorubicin Cyclophosphamide Therapy-Triple Negative Breast Cancer Therapy. https://www.hse.ie/eng/services/list/5/cancer/profinfo/chemoprotocols/breast/348.pdf (accessed 22-5-21).


References
321. Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W.; Shnyder, S. D.; Cooper, P. A., Bibby, M. C., Comparative Preclinical


anti-cancer agents inspired by the vascular disrupting agent 2-(3'-hydroxy-4'-methoxyphenyl)-3-(3",4",5"-trimethoxybenzoyl)-6-methoxyindole (OXi8006). Bioorganic & medicinal chemistry 2013, 21 (21), 6831-6843.


References
References


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484. Clader, J. W.; Burnett, D. A.; Caplen, M. A.; Domalski, M. S.; Dugar, S.; Vaccaro, W.; Sher, R.; Browne, M. E.; Zhao, H.; Burrier, R. E.; Salisbury, B., Davis,


References

References


References

588. ucalgary.com, Chapter 3: Conformations of Alkanes and Cycloalkanes What are Conformational Isomers? http://www.chem.ucalgary.ca/courses/350/Casey5th/Ch03/ch3-02.html#:~:text=Conformational%20isomers%20(or%20conformers%20or,rapidly%20interconverting%20at%20room%20temperature.


624. NCI, pharmacokinetics


(accessed 5-9-21).


References
666. X.org Foundation, I. *XQuartz 2.7.11*, 2016.

**References**


690. GlutaMAX media, Keep your cells healthier for longer. gibco, Ed. Gibco.


References


705. Lin, C. M.; Singh Sb Fau - Chu, P. S.; Chu Ps Fau - Dempey, R. O.; Dempey Ro Fau - Schmidt, J. M.; Schmidt Jm Fau - Pettit, G. R.; Petit Gr Fau - Hamel, E.,Hamel, E., Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin: a structure-activity study. (0026-895X (Print)).


References


References


782. H捕捉语义，生成自然文本：


784. H捕捉语义，生成自然文本：


786. H捕捉语义，生成自然文本：

References

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