Stereoselective Synthesis and Biological Evaluation as Inhibitors of Hepatitis C virus RNA polymerase of GSK3082 Analogues with Structural Diversity at the 5-Position

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Abstract:

GSK3082 – a hepatitis C virus RNA polymerase inhibitor – and a series of analogues with structural diversity at the 5-position were prepared from a 2,2,4,5-tetrasubstituted pyrrolidine obtained with a well-defined stereochemistry from the 1,3-dipolar cycloaddition of the chiral imino ester derived from leucine tert-butyl ester and (R)-2,3-O-isopropylidenglyceraldehyde with methyl acrylate. The chiral 2,2-dimethyl-1,3-dioxolane moiety provided by the glyceraldehyde served as a synthetic equivalent for different substituents and functional groups and these transformations usually required mild reaction conditions and simple work-up procedures. The inhibitory activity of the resulting GSK3082 analogues
was studied *in vitro* in a cell-based assay of the subgenomic HCV RNA replication system. Some of the analogues showed good inhibitory activity with IC50 values in the nanomolar concentration range.

**Key words:** Hepatitis C virus (HCV), N-Acylpyrrolidines, HCV replication inhibitors, Structure-Activity relationships

1. Introduction

Hepatitis C virus (HCV) is a biological pathogenic agent and it is considered to be the most common cause of chronic liver disease worldwide [1]. The progressive destruction and regeneration of the liver parenchyma caused by chronic liver disease is associated with the development of fibrosis, liver cirrhosis, hepatocellular carcinoma and eventually liver failure and death [2]. HCV infection constitutes a major public health problem: a large number of people – about 325 million worldwide in 2015 – are carriers of hepatitis B or C virus infections, each year 1.75 million people newly acquire hepatitis C virus infection and in 2015 alone viral hepatitis caused 1.34 million deaths, most of which were due to chronic liver disease and primary liver cancer [3].

Inhibition of HCV NS5B polymerase, a key enzyme for HCV replication with RNA-dependent RNA polymerase function [4], has proven to be a goal of paramount importance for the development of specifically targeted antiviral therapy for HCV [5]. The potent antiviral effect of polymerase inhibitors has led to the development of nucleos(t)ide and non-nucleoside analogues for the treatment of chronic hepatitis C patients [6].

![Figure 1](attachment:image.png)

**Figure 1.** General structure of N-acylpyrrolidines identified as of HCV NS5B polymerase inhibitors.

In the above context, N-acylpyrrolidines with the general structure A (Figure 1) have been identified as reversible, non-competitive inhibitors of HCV NS5B polymerase [7]. Structural analyses have shown
that compounds from N-acylpyrrolidine series bind in the palm pocket 1 of the active site of the polymerase [6h,8].

Optimization of the structure based on the analysis of structure-activity relationships (Glaxo-SmithKline) led to the identification of GSK3082 [7c], a potent inhibitor of RNA-dependent RNA polymerase (NS5B) in enzymatic assays that also inhibits viral RNA replication in cell-based replicon assays. These SAR studies showed that (i) the presence of a bulky non-polar group in the 2-position increases activity, (ii) aryl groups with lipophilic electron-withdrawing groups in the acyl moiety enhance activity, with the 4-(tert-butyl)-3-methoxybenzoyl group being optimal, and (iii) that the substituent at the 5-position can be widely varied.

1,3-Dipolar cycloaddition has proven to be a flexible and versatile synthetic strategy to gain access to the pyrrolidine core of biologically active compounds [8]. Previously described non-stereoselective [7], diastereoselective [9] and enantioselective [10] syntheses of antiviral compounds with the general structure A have been performed using 1,3-dipolar cycloaddition of iminoglycinates and acrylates as the key step. Substituents \( R_1 \) and \( R_3 \) are provided by the amino acid and the aldehyde from which the iminoglycinate is obtained and substituent \( R_2 \) is generated from the carboxylate moiety in the acrylate (Scheme 1).

Scheme 1. Strategy for synthesis of N-acylpyrrolidines.

One of our research goals is the synthesis of versatile chiral intermediates from imines derived from glyceraldehyde – which is readily accessible from inexpensive D-mannitol – and the development of synthetic routes to obtain bioactive compounds in enantiomerically pure form from these chiral intermediates [11]. With this aim in mind, we studied the behaviour of chiral iminoesters derived from glyceraldehyde acetonide in azomethine ylide cycloadditions and developed a stereoselective and flexible methodology for the synthesis of 2,2,4,5-tetrasubstituted pyrrolidines with a well-defined stereochemistry [12]. We presume that this methodology would be amenable to the synthesis of HCV NS5B polymerase inhibitors and related structural analogues. In the work reported here, we synthesised
a series of novel N-acylpyrrolidines – in which the substituent at 5-position in GSK3082 was varied – starting from a common precursor. The target compounds are potential HCV polymerase inhibitors and their inhibitory activities were evaluated in vitro in a cell-based assay of the subgenomic HCV RNA replication system.

2. Results and discussion

2.1. Chemistry

Based on our previous experience we envisaged that the pyrrolidine core of structural analogues of GSK3082 with different substituents in the 5-position could be obtained by the 1,3-dipolar cycloaddition between an acrylate and an iminoester derived from D-glyceraldehyde acetonide and leucine using silver acetate for metallation. In this way the 2,2,4,5-tetrasubstituted pyrrolidine with the appropriate configuration would be obtained. The chiral 2,2-dimethyl-1,3-dioxolane moiety provided by the glyceraldehyde has a double role: (i) to control the stereochemical course of the reaction and (ii) to serve as a synthetic equivalent for different substituents and functional groups (Scheme 2). The tert-butylimino ester 1 and methyl acrylate were selected as reagents to obtain an orthogonally protected pyrrolidine.

\[
\text{GSK3082 analogues}
\]

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\text{Starting imino ester 1 was prepared by reaction of (S)-leucine tert-butyl ester with (R)-2,3-O-isopropylidenglyceraldehyde in ether at room temperature for 2 h in the presence of anhydrous MgSO}_4\text{ as a dehydrating agent. The crude imino ester was directly submitted to a 1,3-dipolar cycloaddition reaction with methyl acrylate without any purification. Reaction using silver acetate (20}
\]
mol%) as the catalyst and DBU (20 mol%) as the base in toluene at room temperature for 24 h led to an 85/15 mixture of endo adducts 2a/2b in 95% yield, in which compound 2a with the desired (2S,4S,5R) configuration was in excess. The absolute configuration of the major endo adduct was unambiguously established by a single-crystal X-ray diffraction study on compound 4a, which was obtained from 2a as shown below. Acylation of the mixture with 4-(tert-butyl)-3-methoxybenzoyl chloride provided the corresponding N-acyl pyrrolidines 3a/3b as an 85/15 mixture of diastereoisomers in 96% yield (Scheme 3).


The synthesis of GSK3082 was completed as shown in Schemes 4 and 5. Reduction of the methyl ester was achieved with a combination of NaBH4-MeOH (1:2) in the presence of a catalytic amount of NaB(OAc)3H (2.5 mol % relative to NaBH4) [10a]. After stirring 3a/3b in THF for 4 days at room temperature the corresponding 4-hydroxymethylpyrrolidines were obtained as an 85/15 diastereomeric mixture, from which 4a and 4b were isolated as diastereomerically pure compounds in 81% and 12% yield, respectively.

The O-methylation of 4a with an excess of methyl iodide in the presence of potassium tert-butoxide in THF at room temperature was complete after 18 h and provided compound 5 in 88% yield. The 2,2-dimethyl-1,3-dioxolane moiety was converted into the required thiazol-2-yl substituent at the 5-position by first converting the acetonide 5 into the aldehyde 7. Chemoselective deprotection using BiCl3 in dry acetonitrile provided diol 6 in 81% yield [13] and this was subsequently converted into the aldehyde 7 in 94% yield by oxidation with sodium periodate.
Scheme 4. The synthetic route for key intermediate 7.

Further oxidation of aldehyde 7 with potassium permanganate using a mixture of tert-butanol and aqueous NaH$_2$PO$_4$ as the reaction medium [14], followed by treatment with isopropyl chloroformate in the presence of $N$-methylmorpholine in THF at room temperature and subsequent reaction with ammonium hydroxide gave a 79% yield of formamide 8. Direct conversion of formamide 8 into thioamide 10 using Lawesson’s reagent was unsuccessful and we evaluated a two-step procedure using nitrile 9 as the intermediate. Reaction of formamide 8 with POCl$_3$ in pyridine [15] provided a 51% yield of the nitrile intermediate 9, which was subsequently converted into thioamide 10 (95% yield) by the action of 40–44% aqueous ammonium sulfide solution in methanol. At this point we evaluated the direct conversion of aldehyde 7 to nitrile 9. This transformation was successfully achieved in 99% yield using iodine in ammonia water [16]. In an effort to prevent epimerization, the final thiazol-2-yl substituent at the 5-position in GSK3082 was prepared via a modified Hantzsch method [15] that involved the cyclocondensation of thioamide 10 with bromoacetaldehyde in dry dimethoxyethane using NaHCO$_3$ as base followed by dehydratation of the hydroxythiazoline intermediate by treatment with trifluoroacetic anhydride–pyridine. In this way compound 11 was obtained in 87% yield with complete stereocontrol. Finally, hydrolysis of the tert-butyl ester with trifluoroacetic acid in dichloromethane at 0 °C yielded GSK3082 in 95% yield. GSK3082 was obtained from 1 in 10 steps with a good overall yield of 38%.
Scheme 5. The synthetic route for target compound GSK3082.

Cyclocondensation of thioamide 10 with ethyl bromopyruvate using this protocol led to an 88% yield of compound 12, which upon hydrolysis with trifluoroacetic acid in dichloromethane at 0 ºC afforded GSK3082 analogue 13 in 95% yield without detectable epimerization (Scheme 6).

Scheme 6. The synthetic route for GSK3082 analogue 13.

Alternatively, the thiazol-2-yl substituent at the 5-position was installed by reaction of thioamide 10 with α-bromoacetaldehyde diethyl acetal in THF under reflux and in the presence of a catalytic amount of HCl in dioxane [17]. In this way compound 14 – the epimer of 11 at C5 – was obtained in 51% yield, again with complete stereocontrol. tert-Butyl ester 14 was readily hydrolysed to the corresponding acid 15 in 96% yield under the same conditions as above (Scheme 7).
Scheme 7. The synthetic route for GSK3082 analogue 15.

The synthesis of the first set of structural analogues of GSK3082 with substituents with different structural characteristics in the 5-position was readily achieved from intermediates 5, 6 and 8 as detailed below. The chemoselective hydrolysis of the tert-butyl ester compound 5 was carried out under reflux with a methanolic solution of potassium hydroxide and this gave analogue 16 in nearly quantitative yield. Compound 6 was reacted with 1,1'-carbonyldiimidazole in THF at 60 °C to produce carbonate 17 in 90% yield, which upon hydrolysis with trifluoroacetic acid in dichloromethane at 0 °C led to analogue 18 in 94% yield. GSK3082 analogue 19 was obtained in 96% yield by treatment of amide 8 with trifluoroacetic acid in dichloromethane at 0 °C (Scheme 8).

Scheme 8. The synthetic route for GSK3082 analogues 16, 18 and 19.
Other GSK3082 analogues were obtained from versatile chiral intermediate 7 by transformation of the formyl group into methoxymethyl, ethyl and methoxycarbonyl groups, as shown in Scheme 9. Reaction of compound 7 with sodium borohydride in ethanol reduced the C5 formyl group to a hydroxymethyl group, which in turn reacted with methyl iodide to give compound 20 in 85% yield over the two steps. Subsequent hydrolysis of 20 with trifluoroacetic acid in dichloromethane at 0 °C gave GSK3082 analogue 21 in 98% yield. Synthesis of analogue 23 involved oxidation of the formyl group, which was performed with potassium permanganate using a mixture of tert-butanol and aqueous NaH2PO4 as a reaction medium as above. The resulting carboxylic acid was immediately converted into its methyl ester using trimethylsilyldiazomethane. Transformation of aldehyde 7 into methyl ester 22 took place to give 89% yield over the two steps and final hydrolysis with trifluoroacetic acid in dichloromethane at 0 °C afforded a 97% yield of analogue 23. Wittig reaction of 7 and the unstabilised ylide derived from triphenylphosphonium methyl bromide gave olefin 24 in 91% yield. Compound 24 was hydrogenated to 25 (93%) with molecular hydrogen using Pd/C as the catalyst. Hydrolysis of the tert-butyl ester under the usual acidic conditions afforded analogue 26 in 97% yield.


Compounds 13, 15, 16, 18, 19, 21, 23 and 26 were tested as inhibitors by mimicking HCV viral replication in cells using a subgenomic HCV RNA replication system and, on the basis of their in vitro activity (see below), we prepared a second set of structural analogues of GSK3082 taking into account the beneficial effect of the presence of a heteromethyl side chain at C5. Firstly, aldehyde 7 was
efficiently converted into acetate 27, allyl ether 29 or methylcarbonate 31 by reduction 7 with sodium borohydride in ethanol followed by the immediate dimethylaminopyridine-catalysed acetylation with acetic anhydride, reaction with allyl bromide in the presence of sodium hydride or treatment with methyl chloroformate in the presence of a catalytic amount of dimethylaminopyridine. Subsequent hydrolysis of these compounds with trifluoroacetic acid in dichloromethane at 0 °C yielded GSK3082 analogues 28, 30 and 32 in 94–95% yields from starting aldehyde 7 (Scheme 10).

Scheme 10. The synthetic route for GSK3082 analogues 28, 30 and 32.

Compound 7 was also converted into GSK3082 analogues 34, 36 and 38 with a fluoromethyl, azidomethyl or acetylaminomethyl side chain at C5 (Scheme 11). Access to the fluoromethyl side chain was achieved by sequential treatment of aldehyde 7 with sodium borohydride in ethanol and the fluorinating agent diethylaminosulfur trifluoride (DAST). Compound 33, obtained in 52% yield, was hydrolysed with trifluoroacetic acid in dichloromethane at 0 °C to afford analogue 34 in almost quantitative yield. The intermediate hydroxymethyl derivative obtained in the reduction of 7 with sodium borohydride in ethanol was immediately reacted first with methanesulfonyl chloride in the presence of triethylamine and then with sodium azide in the presence of a catalytic amount of sodium iodide to afford 35. Hydrolysis of this compound under the usual conditions gave analogue 36 in 95% from aldehyde 7. Catalytic reduction of azide 35 gave the corresponding amine, which was
immediately acetylated with acetic anhydride-triethylamine to produce acetylamino derivative 37 in 65% yield. Subsequent hydrolysis with trifluoroacetic acid in dichloromethane at 0 ºC yielded analogue 38 in 95% yield.

Scheme 11. The synthetic route for GSK3082 analogues 34, 36 and 38.

2.2. Biological evaluation

In order to evaluate the potency and cytotoxicity of the 14 synthesised compounds, viral replication was assessed by in vitro HCV replication assays with genotype 1b Con1 HCV subgenomic replicons (Huh 5-2) [18] and the results were compared to those obtained for GSK3082. All of the compounds inhibited HCV repicon replication (measured as the luciferase signal) in a dose-dependent manner, with EC50 values lower than 100 microM (Table 1, Figure 2). The estimated EC50 values for these compounds ranged from nanomolar to micromolar. The anti-HCV activity was not the result of a cytostatic effect [19], since the CC50 values for these compounds were significantly higher than the EC50 values.
Table 1. Evaluation of the potency and cytotoxicity of the obtained compounds in cell assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>EC50(^a) (\text{mM})</th>
<th>CC50(^b) (\text{mM})</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSK3082</td>
<td></td>
<td>0.1</td>
<td>&gt;120</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td>*</td>
<td>15</td>
<td>200</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>60</td>
<td>&gt;120</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>2</td>
<td>&gt;120</td>
</tr>
<tr>
<td>19</td>
<td>CONH(_2)</td>
<td>2</td>
<td>&gt;120</td>
</tr>
<tr>
<td>21</td>
<td>CH(_2)OMe</td>
<td>0.6</td>
<td>&gt;120</td>
</tr>
<tr>
<td>23</td>
<td>CO(_2)Me</td>
<td>7</td>
<td>&gt;120</td>
</tr>
<tr>
<td>26</td>
<td>Et</td>
<td>40</td>
<td>200</td>
</tr>
<tr>
<td>28</td>
<td>CH(_2)OAc</td>
<td>30</td>
<td>&gt;120</td>
</tr>
<tr>
<td>30</td>
<td>CH(_3)OAllyl</td>
<td>7.5</td>
<td>&gt;120</td>
</tr>
<tr>
<td>32</td>
<td>CH(_3)OMoc</td>
<td>30</td>
<td>&gt;120</td>
</tr>
<tr>
<td>34</td>
<td>CH(_2)F</td>
<td>45</td>
<td>&gt;120</td>
</tr>
<tr>
<td>36</td>
<td>CH(_2)N(_3)</td>
<td>100</td>
<td>&gt;120</td>
</tr>
<tr>
<td>38</td>
<td>CH(_2)NHAc</td>
<td>15</td>
<td>&gt;120</td>
</tr>
</tbody>
</table>

\(^a\)EC50, effective concentration 50%; \(^b\)CC50, cytotoxic concentration 50%. Relative error in the parameters is 15%.
2.3. SAR analysis
To uncover relationships between EC50 activities and structural features or physical-chemical properties in the compound series, a simple SAR (Structure-Activity Relationship) analysis was performed. The compound structures were optimized and 36 molecular descriptors were calculated. Then, we sought for linear correlations between effectiveness of compounds against HCV NS5B polymerase (-log(EC50)) and any of the previously calculated molecular descriptors. Although this analysis did not show any high correlation between any of the molecular descriptors selected and the biological activity (not shown) it provided some clues. It was noticed that GSK3082, the most effective compound, presented marked differences with most other compounds in regard to properties such as aromatic ratio (ARR) and reactivity (Re) (Figure 3 panels A and B). According to these plots, only compounds 15 and 13 differentiated from the group in the same direction as GSK3082. This was somewhat expected due to their close structural similarity (15 is a GSK3082’s estereoisomer and 13 presents the same thiazole ring but ramified with an ethyl carboxylate) but did not allow us to explain their lower activity compared to that of GSK3082. On the other hand, compound 13 was that with highest molecular weight (Figure 3 panel C) and displayed one of the lowest activities. Thus, we thought the activity could be qualitatively correlated with structural features of the complexes formed by the compounds and the target enzyme. Based on the availability of a 3D structure for the complex between HCV NS5B polymerase and a ligand structurally close to GSK3082 (PDB code: 2jc1) [7c], we modeled the binding of all the compounds. As shown in SI Figure S1 (panels B and C) both compounds 15 and 13 appear to face steric problems (clashes) to fit in the putative binding site. As for
the remaining compounds, the lower activity of 16, 23, 28, 30, 32 and 38 could also be related to steric issues (SI Figure S2 panel A), while that of the others (e.g. 18, 19, 21, 26, 34 and 36, SI Figure S2 panel B) appears related to loss of polar and/or hydrophobic interactions with the protein binding pocket.

Figure 3. Scatter plots of molecular descriptor values versus the assayed biological activities: (A) aromatic ratio, (B) reactivity and (C) molecular weight values versus the effectiveness of compounds against HCV NS5B polymerase. Substituent structures (R₃) at the 5-position in compounds GSK3082, 13 and 15 are shown.
3. Conclusions

In summary, we have developed a very efficient diastereoselective approach to novel 2,2,4,5-tetrasubstituted N-acyl pyrrolidines with a well-defined stereochemistry starting from a chiral iminoester derived from leucine tert-butyl ester and (R)-2,3-O-isopropylidenglyceraldehyde. The cycloadduct derived from the 1,3-dipolar cycloaddition with methyl acrylate has served as a common precursor for the synthesis of GSK3082 and structurally related compounds with different substituents at C5, with the chiral 2,2-dimethyl-1,3-dioxolane moiety provided by the glyceraldehyde acting as a synthetic equivalent for structural diversity. The inhibitory activity of the obtained GSK3082 analogues has been studied in vitro in a cell-based assay of a subgenomic HCV RNA replication system and some of the analogues have shown a good inhibitory activity with estimated IC50 values ranging from nanomolar to micromolar.

4. Experimental Section

4.1. Chemistry

4.1.1. General Details

Unless otherwise specified, all reagents were obtained from commercial suppliers and were used as received without further purification. For anhydrous conditions, reactions were carried out under Ar in solvents dried using a Solvent Purification System (SPS). TLC was performed on precoated silica gel 60 F254 polyester plates and products were visualised using UV light (254 nm) and potassium permanganate solutions followed by heating. Column chromatography was performed on silica gel (60, 40–63 μm) with air pressure. Melting points were determined in open capillary tubes and are not corrected. FT-IR spectra were recorded as thin films on NaCl plates, \( \nu_{\text{max}} \) values expressed in cm\(^{-1} \) are given for the main absorption bands. Optical rotations were measured on a digital polarimeter at \( \lambda \) 589 nm and 25 °C in cells with 1 or 10 cm path length. \( \alpha \) values are given in 10\(^{\circ}\) deg·cm\(^2\)·g\(^{-1} \) and concentrations are given in g/100 mL. \(^1\)H, \(^19\)F and \(^13\)C NMR spectra were acquired on a 400 MHz spectrometer in the stated deuterated solvent at room temperature unless otherwise stated using a 5-mm probe. \(^{13}\)C NMR spectra were acquired with the \(^1\)H broad-band decoupled mode, chemical shifts (\( \delta \)) are reported in parts per million (ppm) with the solvent resonance as the internal standard [20], and coupling constants (\( \text{J} \)) in hertz (Hz). High-resolution mass spectra were recorded from methanolic solutions on a MICROTOF-Q (quadrupole time-of-flight) micro instrument using the positive electrospray ionization mode (ESI+). The X-ray diffraction data were collected at room temperature on a four-circle diffractometer, using graphite-monochromated Mo-K\( \alpha \) radiation (\( \lambda = 0.71073 \) Å).
4.1.2. Synthesis of crude (E)-tert-butyl N-\{(S)-2,2-dimethyl-1,3-dioxolan-4-yl\}methylidene\-(S)-leucinate (1)

To a solution of (S)-leucine tert-butyl ester hydrochloride (1.79 g, 8.0 mmol) in water (5 mL) at room temperature was added a solution of potassium carbonate (2.21 g, 16.0 mmol) in water (5 mL). The resulting solution was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over anhydrous MgSO\(_4\), filtered and evaporated under reduced pressure to give crude tert-butyl (S)-leucinate. In the meantime to a stirred solution of 1,2:5,6-di-O-isopropylidene-mannitol (1.31 g, 5.0 mmol) in dichloromethane (20 mL) at room temperature was added saturated aqueous solution of sodium hydrogen carbonate (1 mL). Then, sodium metaperiodate (2.14 g, 10.0 mmol) was added with vigorous agitation and the reaction was allowed to proceed for 1 h at 35 °C. The solids were removed by filtration and washed with dichloromethane (30 mL). The combined organic solutions were dried over anhydrous MgSO\(_4\), filtered and evaporated under reduced pressure at less than 30 °C to give crude (R)-2,3-O-isopropylideneglyceraldehyde, which was used immediately in the condensation reaction with tert-butyl (S)-leucinate. To a solution containing crude tert-butyl (S)-leucinate and (R)-2,3-O-isopropylideneglyceraldehyde in dry diethyl ether (20 mL) at room temperature was added anhydrous MgSO\(_4\) (1.0 g) and the resulting suspension was stirred for 2 h. The reaction mixture was then filtered and the solid residue was washed with diethyl ether (20 mL). The combined organic filtrates were evaporated under reduced pressure to afford 2.60 g of crude imine 2 of 65 % purity, as determined by \(^1\)H NMR using 1,3,5-trimethoxybenzene as an internal standard [21]. Crude imine 2 was used without further purification in the next reaction. Oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.85 (d, \(J = 6.6\) Hz, 3H), 0.92 (d, \(J = 6.6\) Hz, 3H), 1.39 (s, 3H), 1.41–1.43 (m, 1H), 1.44 (s, 3H), 1.45 (s, 9H), 1.63–1.99 (m, 2H), 3.78 (dd, \(J = 8.2, 6.2\) Hz, 1H), 3.94 (dd, \(J = 8.5, 5.9\) Hz, 1H), 4.20 (dd, \(J = 8.5, 6.9\) Hz, 1H), 4.65 (ddd, \(J = 6.9, 5.9, 4.7\) Hz, 1H), 7.66 (d, \(J = 4.7\) Hz, 1H).

4.1.3. 2-(tert-Butyl) 4-methyl (2S,4S,5R)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-isobutylpyrrolidine-2,4-dicarboxylate (2a)

To a stirred suspension of crude imine 1 of 65% purity (2.60 g, 5.6 mmol) and silver acetate (183 mg, 1.1 mmol) in dry toluene (30 mL) under argon at room temperature were sequentially added ethyl acrylate (966 mg, 11.2 mmol) and DBU (171 mg, 1.1 mmol), both of them previously solved in dry toluene (1 mL). The reaction mixture was stirred in the dark at room temperature for 24 h. The mixture was quenched with water (5 mL) and filtered through a short Celite\textsuperscript{®} pad. Celite\textsuperscript{®} residues were
thoroughly washed with diethyl ether (50 mL) and the combined organic filtrates were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude cycloadducts were purified by flash chromatography (eluent: diethyl ether/hexane, 1/2) to afford 2 (2.05 g, 95% yield) as a mixture of endo diastereoisomers 2a and 2b (2a/2b = 85/15). Some column chromatography fractions containing exclusively major cycloadduct 2a were used for characterization purposes. Colourless oil; [α]D²⁴ = −39.23 (c = 1.11 in CHCl₃; IR (neat) 3341, 3308, 1734 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.83 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H), 1.29 (s, 3H), 1.39 (s, 3H), 1.41–1.45 (m, 1H), 1.46 (s, 9H), 1.61–1.74 (m, 2H), 1.81 (dd, J = 13.7, 7.9 Hz, 1H), 2.48 (dd, J = 13.7, 5.5 Hz, 1H), 2.92 (ddd, J = 7.9, 6.9, 5.5 Hz, 1H), 3.00 (bs, 1H), 3.26 (dd, J = 6.9, 5.3 Hz, 1H), 3.60 (s, 3H), 3.71 (dd, J = 8.0, 7.1 Hz, 1H), 3.93 (dd, J = 8.0, 6.4 Hz, 1H), 4.12–4.20 (m, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 23.7, 24.4, 25.5, 25.5, 26.6, 28.0, 40.9, 46.2, 49.2, 51.8, 63.2, 67.5, 68.7, 75.0, 81.1, 109.3, 173.5, 175.1; HRMS(ESI⁺) m/z [M+H]+ calcd for C₂₀H₃₆NO₆ 386.2538, found 386.2546.

4.1.4. 2-(tert-Butyl) 4-methyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-isobutylpyrrolidine-2,4-dicarboxylate (3a)
A mixture of 4-tert-butyl-3-methoxybenzoic acid (1.56 g, 7.5 mmol) and thionyl chloride (12 mL) was refluxed for 4 hours. Thionyl chloride in excess was removed by evaporation under reduced pressure and the residue was solved in toluene (5 mL). The organic solvent was evaporated under reduced pressure to remove thionyl chloride traces and the crude 4-tert-butyl-3-methoxybenzoyl chloride was solved in anhydrous dichloromethane (10 mL). To this stirred solution at 0 ºC was added a solution of compound 2 as an 85/15 mixture of diastereoisomers 2a and 2b (1.93 g, 5.0 mmol) in dichloromethane (10 mL) followed by triethylamine (2.8 mL, 20.1 mmol) and this mixture was stirred at room temperature for 36 hours. After that, the reaction mixture was washed with saturated aqueous sodium bicarbonate solution (2 x 20 mL). The organic layer was dried over MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (1ˢᵗ eluent: diethyl ether/hexane, 1/3; 2ⁿᵈ eluent: diethyl ether/hexane 1/1) to afford 3 (2.76 g, 96% yield) as a mixture of endo diastereoisomers 3a and 3b (3a/3b = 85/15). Following the same experimental procedure starting from major diastereoisomer 2a, diastereomerically pure compound 3a was obtained and fully characterised. Colourless oil; [α]D²⁴ = 0.65 (c = 1.94 in CHCl₃; IR (neat) 1734, 1636, 1567 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.52 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 1.12 (s, 3H), 1.32 (s, 9H), 1.52 (s, 9H), 1.72–1.83 (m, 1H), 2.00 (dd, J = 15.1, 4.4 Hz, 1H), 2.25 (dd, J = 13.4, 6.9 Hz, 1H), 2.24–2.32 (m, 1H), 2.64 (dd, J = 13.4, 13.4 Hz, 1H), 3.28 (dd, J = 7.7, 7.7 Hz, 1H), 3.47 (ddd,
$J = 13.4, 7.3, 6.9 \text{ Hz, 1H}$; $3.67 - 3.73 (m, 1H)$; $3.71 (s, 3H)$; $3.84 (s, 3H)$; $4.32 - 4.42 (m, 1H)$; $4.73 (dd, J = 9.3, 7.3 \text{ Hz, 1H})$; $6.92 (dd, J = 7.9, 1.6 \text{ Hz, 1H})$; $7.04 (d, J = 1.6 \text{ Hz, 1H})$; $7.18 (d, J = 7.9 \text{ Hz, 1H})$; $13\text{C-APT}^{(1\text{H})} \text{ NMR (100 MHz, CDCl}_3 \delta 24.4, 24.7, 24.9, 25.3, 25.7, 28.2, 29.6, 35.0, 36.1, 41.6, 44.6, 52.3, 55.1, 65.3, 66.4, 69.3, 75.2, 81.5, 108.6, 112.6, 118.5, 125.7, 137.0, 140.4, 158.8, 169.7, 170.8, 172.8; \text{HRMS(ESI$^+$) m/z [M+H$^+$] calcd for C}_{32}\text{H}_{50}\text{NO}_8 576.3531, found 576.3518.$

4.1.5. **tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-(hydroxymethyl)-2-isobutylpyrrolidine-2-carboxylate (4a)**

To a stirred solution of compound 3 as an 85/15 mixture of diastereoisomers 3a and 3b (2.30 g, 4.0 mmol) in dry THF (40 mL) were sequentially added NaBH$_4$ (606 mg, 16.0 mmol) and NaB(OAc)$_3$H$_2$ (172 mg, 0.8 mmol). To the white slurry under argon at 0 ºC dry methanol (1.3 mL, 32.1 mmol) was added and the mixture was stirred at room temperature for 4 days until complete disappearance of the starting material was observed by TLC. The resulting reaction mixture was quenched with saturated aqueous NH$_4$Cl solution (20 mL) and the organic solvents were evaporated under reduced pressure. The aqueous layer was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (1$^{st}$ eluent: diethyl ether/hexane, 1/1; 2$^{nd}$ eluent: diethyl ether) to provide diastereomerically pure 4a (1.78 g, 81% yield) as a white solid. M.p. = 204 ºC; $[\alpha]_{D}^{24} = -3.07 (c = 0.99 \text{ in CHCl}_3$); IR (nujol) 3379, 1730, 1593, 1563 cm$^{-1}$; $^1\text{H NMR (400 MHz, CDCl}_3 \delta 0.73 (s, 3H), 0.97 (d, J = 6.6 \text{ Hz, 3H}), 1.00 (d, J = 6.6 \text{ Hz, 3H}), 1.11 (s, 3H), 1.32 (s, 9H) 1.50 (s, 9H), 1.77–1.89 (m, 1H), 1.93 (dd, J = 12.9, 12.9 Hz, 1H), 2.01 (dd, J = 14.9, 4.4 Hz, 1H), 2.14 (dd, J = 12.9, 6.8 Hz, 1H), 2.25 (dd, J = 14.9, 6.0 Hz, 1H), 2.68 (bs, 1H), 2.80–2.94 (m, 1H), 3.48–3.63 (m, 2H), 3.67 (dd, J = 8.8, 8.8 Hz, 1H), 3.83 (s, 3H), 3.98 (dd, J = 8.8, 6.5 Hz, 1H), 4.14–4.23 (m, 1H), 4.61 (dd, J = 7.5, 7.5 Hz, 1H), 6.93 (dd, J = 7.9, 1.7 Hz, 1H), 7.01 (d, J = 1.7 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H); $^{13}\text{C-APT}^{(1\text{H})} \text{ NMR (100 MHz, CDCl}_3 \delta 24.5, 24.7, 24.9, 25.0, 25.7, 28.2, 29.7, 35.0, 37.1, 41.9, 43.4, 55.2, 61.1, 63.9, 66.9, 69.7, 75.5, 81.2, 108.0, 112.0, 118.5, 125.9, 137.0, 140.3, 158.7, 170.0, 173.2; \text{HRMS(ESI$^+$) m/z [M+H$^+$] calcd for C}_{32}\text{H}_{50}\text{NO}_7 548.3582, found 548.3598.$

4.1.6. **tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (5)**

To a stirred solution of compound 4a (1.64 g, 3.0 mmol) in dry THF (30 mL) under argon at 0 ºC was added KO'Bu (673.3 mg, 6.0 mmol) and the mixture was stirred at room temperature for 15 min. Then,
iodomethane (0.94 mL, 15.1 mmol) was added and the slurry was stirred for 18 hours at room temperature. The resulting reaction mixture was quenched with saturated aqueous NH₄Cl solution (20 mL) and the organic solvent was evaporated under reduced pressure. The aqueous layer was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane: 1/1), to provide compound 5 (1.78 g, 88% yield) as a white solid. M.p. = 109 ºC; [α]D²⁴ = -13.72 (c = 1.45 in CHCl₃); IR (nujol) 1729, 1612 cm –¹; ¹H NMR (400 MHz, CDCl₃) δ 0.54 (s, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 1.15 (s, 3H), 1.32 (s, 9H), 1.50 (s, 9H), 1.81-1.99 (m, 1H), 1.93-2.05 (m, 2H), 2.13 (dd, J = 12.8, 6.8 Hz, 1H), 2.28 (dd, J = 14.8, 6.5 Hz, 1H), 2.83-2.96 (m, 1H), 3.17 (dd, J = 9.7, 9.2 Hz, 1H), 3.30 (s, 3H), 3.34 (dd, J = 9.7, 5.1 Hz, 1H), 3.58 (dd, J = 8.6, 7.4 Hz, 1H), 3.83 (s, 3H), 3.87 (dd, J = 8.6, 6.4 Hz, 1H), 4.26 (ddd, J = 9.3, 7.4, 6.4 Hz, 1H), 4.55 (dd, J = 9.3, 6.9 Hz, 1H), 6.92 (dd, J = 8.0, 1.7 Hz, 1H), 7.06 (d, J = 1.7 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.4, 24.7, 25.0, 25.4, 25.7, 28.2, 29.7, 34.9, 36.8, 40.9, 41.8, 55.1, 58.8, 65.2, 67.0, 69.2, 71.2, 75.3, 81.0, 108.1, 112.7, 118.8, 125.6, 137.5, 140.1, 158.7, 170.1, 173.4; HRMS(ESI) m/z [M+H]+ calcd for C₃₂H₅₂NO₇ 562.3739, found 562.3751.

4.1.7. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-((S)-1,2-dihydroxyethyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (6)

Compound 5 (1.40 g, 2.5 mmol) and bismuth(III) chloride (79 mg, 0.25 mmol) were mixed in wet acetonitrile (50 mL) and the mixture was stirred at room temperature for 4 hours. The resulting reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (20 mL) and the organic solvent was evaporated under reduced pressure. The aqueous layer was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (1st eluent: diethyl ether/hexane, 1/1; 2nd eluent: diethyl ether) to provide compound 6 (1.06 g, 81% yield) as colourless oil. [α]D²⁴ = -17.34 (c = 0.88 in CHCl₃); IR (nujol) 3579, 3411, 1737, 1699, 1639, 1566 cm –¹; ¹H NMR (400 MHz, CDCl₃) δ 0.99 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.34 (s, 9H), 1.53 (s, 9H), 1.78-1.90 (m, 1H), 1.95 (dd, J = 14.8, 5.0 Hz, 1H), 2.00 (dd, J = 13.0, 13.0 Hz, 1H), 2.20 (dd, J = 13.0, 6.9 Hz, 1H), 2.37 (dd, J = 14.8, 6.0 Hz, 1H), 2.83-2.98 (m, 1H), 3.02-3.14 (m, 1H), 3.20-3.29 (m, 1H), 3.30 (s, 3H), 3.33-3.42 (m, 3H), 3.57-3.66 (m, 1H), 3.83 (s, 3H), 4.36 (dd, J = 6.7, 6.7 Hz, 1H), 5.26 (d, J = 5.4 Hz, 1H), 6.93 (dd, J = 7.9, 1.7 Hz,
1H), 7.04 (d, J = 1.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H); 13C-APT{1H} NMR (100 MHz, CDCl$_3$) δ 24.5, 25.1, 25.7, 28.1, 29.7, 35.0, 37.8, 41.6, 41.7, 55.3, 59.0, 62.0, 64.9, 70.0, 70.4, 70.8, 83.3, 112.1, 119.1, 126.0, 136.2, 140.4, 158.6, 171.2, 176.6; HRMS(ESI +) m/z [M+Na$^+$] calcd for C$_{29}$H$_{47}$NNaO$_7$ 544.3245, found 544.3256.

4.1.8. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-formyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (7)

To a stirred solution of compound 6 (1.04 g, 2.0 mmol) in THF (30 mL) at room temperature was added water (6 mL) followed by sodium metaperiodate (1.71 g, 8.0 mmol) and the mixture was vigorously stirred at 35 ºC for 16 hours. After that, the solvents were evaporated under reduced pressure and the residue was partitioned between a mixture of dichloromethane (30 mL) and water (10 mL). The aqueous layer was extracted with dichloromethane (20 mL) and the combined organic layers were dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 7 (921 mg, 94% yield) as a white solid. M.p. = 175 ºC; $\alpha$D$_{24}$ = −3.55 (c = 1.01 in CHCl$_3$); IR (nujol) 2727, 1728, 1630, 1563 cm$^{-1}$; 1H NMR (400 MHz, CDCl$_3$) δ 1.03 (d, J = 6.5 Hz, 6H), 1.32 (s, 9H), 1.53 (s, 9H), 1.77−1.91 (m, 1H), 2.07 (dd, J = 14.9, 3.7 Hz, 1H), 2.17 (dd, J = 13.1, 7.6 Hz, 1H), 2.26 (dd, J = 13.1, 13.1 Hz, 1H), 2.35 (dd, J = 14.9, 8.2 Hz, 1H), 2.88−2.99 (m, 1H), 3.19 (s, 3H), 3.24 (dd, J = 9.8, 4.8 Hz, 1H), 3.43 (dd, J = 9.8, 5.2 Hz, 1H), 3.80 (s, 3H), 4.38 (dd, J = 9.1, 3.7 Hz, 1H), 6.75 (d, J = 1.7 Hz, 1H), 6.97 (d, J = 3.7, 1H); 13C-APT{1H} NMR (100 MHz, CDCl$_3$) δ 24.0, 24.7, 25.8, 28.1, 29.6, 35.1, 38.0, 41.8, 43.2, 55.1, 58.7, 69.6, 70.2, 70.9, 81.7, 109.6, 117.9, 127.0, 135.0, 140.6, 158.8, 169.5, 172.7, 200.1; HRMS(ESI +) m/z [M+H$^+$] calcd for C$_{28}$H$_{44}$NO$_6$ 490.3149, found 490.3144.

4.1.9. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-carbamoyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (8)

A solution of compound 7 (146.9 mg, 0.3 mmol) in tert-butanol (12 mL) was diluted with an aqueous 1.25M sodium phosphate buffer (4 mL) and the resulting mixture was treated with an aqueous 1M potassium permanganate solution (1.8 mL, 1.8 mmol) at room temperature and vigorously stirred for 2 hours. Then, saturated aqueous sodium sulfite solution (4 mL) was added and the pH of the mixture was adjusted to 3 with 1M hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts were dried over MgSO$_4$ and evaporated under reduced
pressure to afford a residue containing the crude acid. To a stirred solution of the resulting crude acid and N-methylmorpholine (30.5 mg, 0.3 mmol) in anhydrous THF (10 mL) at room temperature was added a 1M solution of isopropyl chloroformate in toluene (0.45 mL, 0.45 mmol) and the mixture stirred for 30 minutes. Then, 28% ammonium hydroxide solution (1 mL) was added and the reaction mixture was stirred for 2 hours. The organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (elucent: diethyl ether/hexane, 1/1) to afford compound 8 (119.5 mg, 79% yield) as colourless oil. [α]D²⁴ = 7.83 (c = 1.01 in CHCl₃); IR (neat) 3324, 3189, 1697, 1650, 1563 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.7 Hz, 3H), 1.32 (s, 9H), 1.55 (s, 9H), 1.76–1.88 (m, 1H), 2.02 (dd, J = 13.1, 13.1 Hz, 1H), 2.08–2.19 (m, 2H), 2.41 (dd, J = 13.1, 6.4 Hz, 1H), 2.82–2.99 (m, 1H), 3.14 (dd, J = 9.6, 6.4 Hz, 1H), 3.29 (s, 3H), 4.44 (d, J = 9.6 Hz, 1H), 5.58 (bs, 1H), 6.86 (d, J = 1.7 Hz, 1H), 6.89 (dd, J = 7.9, 1.7 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 9.25 (bs, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.3, 25.0, 25.7, 28.1, 29.6, 35.0, 39.0, 41.3, 41.7, 55.1, 59.2, 65.9, 71.4, 72.3, 83.6, 109.8, 118.1, 126.8, 134.7, 140.4, 158.5, 169.8, 173.0, 175.8; HRMS(ESI⁺) m/z [M+H⁺] calcd for C₂₈H₄₅N₂O₆ 505.3273, found 505.3290.

4.1.10. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-cyano-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (9)

**Method A:** Phosphorus oxychloride (192 mg, 1.25 mmol) was added to a stirred solution of compound 8 (252 mg, 0.5 mmol) in pyridine (3 mL) at 0 ºC and the solution was stirred for 6 hours at the same temperature. The resulting solution was poured into ice, diluted with water (3 mL) and extracted with diethyl ether (3 x 10 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (elucent: diethyl ether/hexane, 1/2) to yield compound 9 (124 mg, 51% yield) as colourless oil.

**Method B:** Iodine (1.27 g, 5.0 mmol) was added to a stirred solution of compound 7 (245 mg, 0.5 mmol) in aqueous 28% ammonium hydroxide solution (20 mL) and THF (5 mL) at room temperature and the reaction mixture was stirred for 16 hours. After that, the resulting dark mixture was treated with saturated aqueous sodium sulfite solution (5 mL), followed by extraction with dichloromethane (2 x 20 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (elucent: diethyl
ether/hexane, 1/2) to afford compound 9 (240.9 mg, 99% yield) as colourless oil. [α]D$_{24}^{24}$ = 32.95 (c = 1.01 in CHCl$_3$); IR (neat) 2250, 1734, 1657, 1565 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.98 (d, $J$ = 6.6 Hz, 3H), 1.01 (d, $J$ = 6.6 Hz, 3H), 1.36 (s, 9H), 1.55 (s, 9H), 1.70–1.82 (m, 1H), 2.00 (dd, $J$ = 13.1, 13.1 Hz, 1H); 2.05 (dd, $J$ = 15.0, 3.4 Hz, 1H), 2.20 (dd, $J$ = 13.1, 6.5 Hz, 1H), 2.39 (dd, $J$ = 15.0, 8.8 Hz, 1H), 2.83–2.96 (m, 1H), 3.33 (s, 3H), 3.47 (dd, $J$ = 9.6, 9.6 Hz, 1H), 3.58 (dd, $J$ = 9.6, 5.0 Hz, 1H), 3.86 (s, 3H), 4.76 (d, $J$ = 7.2 Hz, 1H), 6.92 (dd, $J$ = 7.9, 1.7 Hz, 1H), 6.97 (d, $J$ = 1.7 Hz, 1H), 7.30 (d, $J$ = 7.9 Hz, 1H); 13C-APT(1H) NMR (100 MHz, CDCl$_3$) δ 23.5, 24.4, 25.7, 28.1, 29.6, 35.1, 37.4, 41.2, 41.7, 55.1, 55.1, 59.4, 70.2, 72.0, 82.4, 109.3, 116.1, 117.3, 127.1, 134.7, 140.5, 158.7, 169.4, 171.9; HRMS(ESI$^+$) m/z [M+H]$^+$ calcd for C$_{28}$H$_{43}$N$_2$O$_5$ 487.3167, found 487.3171.

4.1.11. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-carbamothioyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (10)

Compound 9 (195.7 mg, 0.4 mmol) was solved in methanol (6 mL) and aqueous 40-44% ammonium sulfide solution (3 mL) was added. The reaction mixture was stirred at 90 ºC for 4 days in an autoclave. The resulting mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (20 mL) and water (20 mL). The aqueous layer was further extracted with dichloromethane (2 x 20 mL) and the combined organic extracts were dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/2) to yield compound 10 (197.9 mg, 95% yield) as a white solid. M.p. = 158 ºC; [α]D$_{24}^{24}$ = −27.95 (c = 1.25 in CHCl$_3$); IR (nujol) 3302, 3138, 1743, 1700, 1653, 1565, 1500 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 1.00 (d, $J$ = 6.6 Hz, 3H), 1.01 (d, $J$ = 6.6 Hz, 3H), 1.32 (s, 9H), 1.55 (s, 9H), 1.75–1.90 (m, 1H), 1.99 (dd, $J$ = 13.2, 13.2 Hz, 1H), 2.11 (d, $J$ = 5.7 Hz, 2H), 2.42 (dd, $J$ = 13.2, 6.4 Hz, 1H), 2.85–2.98 (m, 1H), 3.12 (dd, $J$ = 9.5, 9.2 Hz, 1H), 3.28 (s, 3H), 3.74 (dd, $J$ = 9.5, 4.6 Hz, 1H), 3.81 (s, 3H), 5.06 (d, $J$ = 9.1 Hz, 1H), 6.79 (d, $J$ = 1.7 Hz, 1H), 6.85 (dd, $J$ = 7.9, 1.7 Hz, 1H), 7.21 (d, $J$ = 8.0 Hz, 1H), 7.62 (bs, 1H), 10.66 (bs, 1H); 13C-APT(1H) NMR (100 MHz, CDCl$_3$) δ 24.3, 25.0, 25.7, 28.0, 29.6, 35.0, 39.0, 41.2, 41.6, 55.2, 59.1, 70.9, 72.3, 72.8, 83.9, 109.9, 118.2, 126.7, 134.2, 140.4, 158.5, 170.0, 176.6, 205.2; HRMS(ESI$^+$) m/z [M+H]$^+$ calcd for C$_{28}$H$_{45}$N$_2$O$_5$S 521.3044, found 521.3058.

4.1.12. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-(thiazol-2-yl)pyrrolidine-2-carboxylate (11)

To a solution of thioamide 10 (52.1 mg, 0.1 mmol) and crude bromoacetaldehyde with a 21.5 % purity
(228.7 mg, 0.4 mmol) in dry dimethoxyethane (2 mL) was added sodium hydrogen carbonate (67.2 mg, 0.8 mmol) and the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (10 mL) and water (10 mL). The aqueous layer was further extracted with dichloromethane (2 x 10 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was solved in dry dimethoxiethane (1 mL) and cooled to 0 ºC. A solution of trifluorooacetic anhydride (84.1 mg, 0.4 mmol) in dry dimethoxyetnane (0.5 mL) was added followed by a solution of pyridine (63.3 mg, 0.8 mmol) in dry dimethoxyetane (0.5 mL). The reaction mixture was stirred at room temperature for 24 hours. After that, the resulting mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (20 mL) and water (10 mL). The aqueous layer was further extracted with dichloromethane (2 x 10 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 11 (47.4 mg, 87% yield) as a brown solid. M.p. = 144 ºC; [α]D²⁴ = 66.42 (c = 0.55 in CHCl₃); IR (neat) 3113, 1713, 1608, 1567, 1502 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.06 (d, J = 6.6 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 1.27 (s, 9H), 1.58 (s, 9H), 1.91−2.02 (m, 1H), 2.10 (dd, J = 14.6, 7.0 Hz, 1H), 2.25−2.35 (m, 3H), 2.81 (dd, J = 9.4, 7.1 Hz, 1H), 2.95 (dd, J = 9.4, 6.6 Hz, 1H), 3.02−3.14 (m, 1H), 3.06 (s, 3H), 3.55 (s, 3H), 5.46 (d, J = 8.5 Hz, 1H), 6.31 (d, J = 1.7 Hz, 1H), 6.62 (dd, J = 7.9, 1.7 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 7.19 (d, J = 3.2 Hz, 1H), 7.48 (d, J = 3.2 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.6, 25.3, 25.6, 28.3, 29.6, 34.9, 37.3, 42.7, 42.8, 54.8, 59.0, 63.9, 70.9, 72.0, 81.9, 109.4, 117.1, 119.8, 126.4, 135.9, 139.2, 141.7, 158.3, 169.5, 169.8, 172.2; HRMS(ESI⁺) m/z [M+H]⁺ calcd for C₃₀H₄₅N₂O₆S 545.3044, found 545.3061.

4.1.13. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-(thiazol-2-yl)pyrrolidine -2-carboxylic acid (GSK3082)

To a solution of compound 11 (27.2 mg, 0.05 mmol) in dichloromethane (0.6 mL) at 0 ºC was added trifluoroacetic acid (0.2 mL) and the resulting solution was stirred at 0 ºC for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 10 mL). The combined organic extracts washed with water (10 mL), dried over anhydrous MgSO₄ filtered and
evaporated under reduced pressure to give compound 1 (23.1 mg, 95% yield) as a brown solid. All physical and spectroscopic data are in agreement with those previously described in the literature.\textsuperscript{7c}

4.1.14. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-(4-(ethoxycarbonyl)thiazol-2-yl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (12)
To a solution of thioamide 10 (52.1 mg, 0.1 mmol) and ethyl bromopyruvate 85% (91.8 mg, 0.4 mmol) in dry dimethoxyethane (2 mL) was added sodium hydrogen carbonate (67.2 mg, 0.8 mmol) and the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (10 mL) and water (10 mL). The aqueous layer was further extracted with dichlorometane (2 x 10 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was solved in dry dimethoxyethane (1 mL) and cooled to 0 °C. A solution of trifluoroacetic anhydride (84.1 mg, 0.4 mmol) in dry dimethoxyetnane (0.5 mL) was added followed by a solution of pyridine (63.3 mg, 0.8 mmol) in dry dimethoxyetane (0.5 mL). The reaction mixture was stirred at room temperature for 24 hours. After that, the resulting mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (20 mL) and water (10 mL). The aqueous layer was further extracted with dichlorometane (2 x 10 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 12 (54.2 mg, 88% yield) as a brown solid. M.p. = 114 °C; [α]\textsubscript{D}\textsuperscript{24} = 53.75 (c = 1.05 in CHCl₃); IR (nujol) 3092, 1730, 1653, 1591 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, CDCl₃) δ 1.07 (d, J = 6.5, 6H), 1.25 (s, 9H), 1.34 (t, J = 7.1 Hz, 3H), 1.59 (s, 9H), 1.89–2.00 (m, 1H), 2.07 (dd, J = 14.8, 7.3 Hz, 1H), 2.28 (dd, J = 13.0, 13.0 Hz, 1H), 2.28 (dd, J = 14.8, 3.9 Hz, 1H), 2.37 (dd, J = 13.0, 6.3 Hz, 1H), 2.78 (dd, J = 9.2, 7.5 Hz, 1H), 3.02–3.12 (m, 1H), 3.06 (s, 3H), 3.16 (dd, J = 9.2, 5.4 Hz, 1H), 3.58 (s, 3H), 4.26–4.41 (m, 2H), 5.59 (d, J = 8.7 Hz, 1H), 6.37 (d, J = 1.7 Hz, 1H), 6.64 (dd, J = 7.9, 1.7 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 0.7 Hz, 1H); \textsuperscript{13}C-APT\textsuperscript{1}H NMR (100 MHz, CDCl₃) δ 14.5, 24.6, 25.3, 25.6, 28.3, 29.5, 34.9, 37.6, 42.5, 42.7, 54.9, 59.0, 61.5, 64.0, 71.1, 71.8, 82.2, 109.3, 117.3, 126.5, 128.4, 135.4, 139.6, 146.0, 158.2, 161.2, 169.6, 171.0, 172.3; HRMS(ESI+) m/z [M+H]+ calcd for C\textsubscript{33}H\textsubscript{49}N\textsubscript{2}O\textsubscript{7}S 617.3255, found 617.3272.

4.1.15. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-5-(4-(ethoxycarbonyl)thiazol-2-yl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (13)
To a solution of compound 12 (30.8 mg, 0.05 mmol) in dichloromethane (0.6 mL) at 0 °C was added trifluoroacetic acid (0.2 mL) and the resulting solution was stirred at 0 °C for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 10 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 13 (26.5 mg, 95% yield) as a brown solid. M.p. = 55 ºC; [α]D²⁴ = 61.89 (c = 0.64 in CHCl₃); IR (nujol) 3500–2400, 1741, 1653, 1607, 1565 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.09 (d, J = 6.6 Hz, 3H), 1.12 (d, J = 6.6 Hz, 3H); 1.28 (s, 9H), 1.45 (t, J = 7.1 Hz, 1H), 1.90–2.00 (m, 1H), 2.17–2.23 (m, 2H), 2.30 (dd, J = 15.0, 4.0 Hz, 1H), 2.40 (dd, J = 15.0, 8.1 Hz, 1H), 3.11 (s, 3H), 3.17–3.30 (m, 1H), 3.33 (dd, J = 9.4, 4.5 Hz, 1H), 3.56 (s, 3H), 4.44 (q, J = 7.1 Hz, 2H), 5.50 (d, J = 8.1 Hz, 1H), 6.31 (d, J = 1.7 Hz, 1H), 6.61 (dd, J = 7.9, 1.7 Hz, 1H), 7.12 (d, J = 7.9 Hz, 1H), 8.06 (s, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 14.4, 24.2, 24.9, 25.7, 29.6, 35.0, 35.6, 42.6, 43.5, 55.0, 58.9, 62.4, 64.0, 70.8, 71.3, 109.1, 116.7, 126.6, 128.3, 135.0, 140.2, 145.9, 158.6, 160.4, 169.9, 172.0, 173.6; HRMS(ESI⁺) m/z [M+Na⁺] calcd for C₂₉H₄₀N₂NaO₇S 583.2449, found 583.2441.

4.1.16. tert-Butyl (2S,4S,5S)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-(thiazol-2-yl)pyrrolidine-2-carboxylate (14)

To a solution of thioamide 10 (156.2 mg, 0.3 mmol) and bromoacetaldehyde diethyl acetal (295.6 mg, 1.5 mmol) in dry THF (12 mL) was added HCl/dioxane (4N, 0.1 mL) and the mixture was stirred at 65 ºC for 24 hours. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 14 (83.3 mg, 51% yield) as an oil. [α]D²⁴ = −28.67 (c = 0.99 in CHCl₃); IR (neat) 3082, 1734, 1652, 1610, 1566 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.05 (d, J = 6.3 Hz, 3H), 1.07 (d, J = 6.4 Hz, 3H); 1.28 (s, 9H), 1.51 (s, 9H), 2.02–2.11 (m, 2H), 2.20 (dd, J = 12.9, 10.7 Hz, 1H), 2.39–2.49 (m, 2H), 2.75–2.86 (m, 1H), 3.28 (s, 3H), 3.34 (dd, J = 9.6, 6.4 Hz, 1H), 3.47 (dd, J = 9.6, 5.2 Hz, 1H), 3.63 (s, 3H), 5.16 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 1.7 Hz, 1H), 6.76 (dd, J = 7.9, 1.7 Hz, 1H), 7.06 (d, J = 3.2 Hz, 1H), 7.10 (d, J = 7.9 Hz, 1H), 7.42 (d, J = 3.2 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 25.4, 25.4, 25.5, 28.1, 29.6, 34.9, 38.2, 44.1, 47.4, 54.9, 59.1, 64.8, 71.6, 72.3, 81.5, 109.6, 117.6, 119.2, 126.4, 135.9, 139.3, 142.1, 158.4, 170.4, 172.0, 172.3; HRMS(ESI⁺) m/z [M+H⁺] calcd for C₃₀H₄₅N₂O₇S 545.3044, found 545.3056.
4.1.17. (2S,4S,5S)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-(thiazol-2-yl)pyrrolidine-2-carboxylic acid (15)

To a solution of compound 14 (27.2 mg, 0.05 mmol) in dichloromethane (0.6 mL) at 0 ºC was added trifluoroacetic acid (0.2 mL) and the resulting solution was stirred at 0 ºC for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 10 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO$_4$ filtered and evaporated under reduced pressure to give compound 15 (23.5 mg, 96% yield) as a brown oil. [α]$^\text{D}_{24} = -52.63$ (c = 0.90 in CHCl$_3$); IR (neat) 3500−2400, 1728, 1650, 1607, 1566, 1501 cm$^{-1}$; 1H NMR (400 MHz, CDCl$_3$) δ 1.03 (d, J = 6.6 Hz, 3H), 1.04 (d, J = 6.6 Hz, 3H), 1.29 (s, 9H), 1.79−1.91 (m, 1H), 2.20 (dd, J = 14.0, 6.2 Hz, 1H), 2.42 (dd, J = 13.2, 7.2 Hz, 1H), 2.48−2.58 (m, 1H), 2.83 (dd, J = 13.2, 3.5 Hz, 1H), 2.87 (dd, J = 14.0, 5.8 Hz, 1H); 3.33 (s, 3H), 3.34 (dd, J = 9.8, 8.3 Hz, 1H), 3.42 (dd, J = 9.8, 5.1 Hz, 1H), 3.61 (s, 3H), 5.26 (d, J = 3.3 Hz, 1H), 6.38 (d, J = 1.6 Hz, 1H), 6.63 (dd, J = 7.9, 1.6 Hz, 1H), 7.13 (d, J = 7.9 Hz, 1H), 7.15 (d, J = 3.3 Hz, 1H), 7.60 (d, J = 3.3 Hz, 1H), 8.51 (bs, 1H); 13CAPT{1H} NMR (100 MHz, CDCl$_3$) δ 24.0, 24.8, 25.2, 29.5, 35.0, 36.6, 43.8, 46.0, 55.0, 59.1, 65.6, 72.8, 74.5, 108.8, 117.3, 119.6, 126.7, 135.0, 140.1, 142.3, 158.3, 170.9, 173.9, 174.6; HRMS(ESI$^+$) m/z [M+Na]$^+$ C$_{26}$H$_{36}$N$_2$NaO$_5$S 511.2238, found 511.2251.

4.1.18. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (16)

A solution of compound 5 (112.4 mg, 0.2 mmol) in 10% KOH/methanol (5 mL) was refluxed for 5 days. The resulting mixture was cooled and the solvent evaporated under reduced pressure. The residue was diluted in water (10 mL) and washed with dichloromethane (5 mL). The aqueous layer was then acidified with 1M hydrochloric acid and extracted with dichloromethane (2 x 20 mL). The combined organic extracts were dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure to provide compound 16 (100.1 mg, 99% yield) as a white solid. M.p. = 155 °C; [α]$^\text{D}_{24} = -99.59$ (c = 1.11 in CHCl$_3$); IR (nujol) 3400–2200, 1739, 1605, 1558 cm$^{-1}$; 1H NMR (400 MHz, CDCl$_3$) δ 0.96 (d, J = 6.4 Hz, 3H), 0.98 (d, J = 6.3 Hz, 3H), 1.15 (s, 3H), 1.22 (s, 3H), 1.34 (s, 9H), 1.73−1.85 (m, 2H), 2.18 (dd, J = 12.6, 6.5 Hz, 1H), 2.36 (dd, J = 15.4, 7.7 Hz, 1H), 2.70−2.82 (m, 1H), 2.82 (dd, J = 12.6, 12.6 Hz, 1H), 3.24 (dd, J = 9.6, 9.6 Hz, 1H), 3.29 (s, 3H), 3.38 (dd, J = 8.0, 8.0 Hz,
1H), 3.46 (dd, J = 9.6, 4.4 Hz, 1H), 3.67 (dd, J = 8.0, 6.0 Hz, 1H), 3.35 (s, 3H), 3.88–3.95 (m, 1H), 4.49 (dd, J = 6.2, 6.2 Hz, 1H), 6.91 (dd, J = 7.9, 1.7 Hz, 1H), 7.04 (bs, 1H), 7.26 (d, J = 7.9 Hz, 1H);

$^{13}$C-APT$^1$H) NMR (100 MHz, CDCl$_3$) δ 23.9, 24.7, 24.9, 25.1, 25.9, 25.9, 29.5, 35.3, 35.8, 39.2, 42.7, 55.2, 59.0, 65.1, 66.3, 71.2, 73.0, 73.7, 109.1, 112.1, 119.3, 126.7, 134.1, 142.5, 150.0, 174.0, 175.4;

HRMS(ESI$^+$) m/z [M+Na]$^+$ calcd for C$_{28}$H$_{43}$NNaO$_7$ 528.2932, found 528.2919.

4.1.19. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-((S)-2-oxo-1,3-dioxolan-4-yl)pyrrolidine-2-carboxylate (17)

To a solution of compound 6 (104.3 mg, 0.2 mmol) in THF (5 mL) was added 1,1’-carbonyldiimidazole (97.3 mg, 0.6 mmol) and the mixture was stirred at 60 ºC for 24 hours. After that, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (eluent: diethyl ether/hexane, 3/1) to afford compound 17 (98.5 mg, 90% yield) as a white solid. M.p. = 90 ºC; $[\alpha]_D^{24} = -37.76$ (c = 1.01 in CHCl$_3$); IR (nujol) 1815, 1729, 1639, 1565 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.93−1.00 (m, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.34 (s, 9H), 1.52 (s, 9H), 1.76−1.90 (m, 1H), 1.91−2.05 (m, 1H), 2.10−2.22 (m, 2H), 2.28−2.42 (m, 1H), 2.86−3.02 (m, 1H), 3.15−3.26 (m, 1H), 3.29 (s, 3H), 3.46 (dd, J = 10.2, 4.0 Hz, 1H), 3.88 (s, 3H); 4.03−4.15 (m, 1H), 4.20−4.31 (m, 1H), 4.55−4.68 (m, 1H), 4.84−4.95 (m, 1H), 6.84 (bd, J = 7.9 Hz, 1H), 7.08 (d, J = 1.7 Hz, 1H), 7.17 (d, J = 7.9 Hz, 1H); $^{13}$C$^1$H) NMR (100 MHz, CDCl$_3$) δ 24.4, 25.2, 25.6, 28.1, 29.6, 35.0, 36.5, 40.8, 41.7, 55.1, 59.1, 63.6, 67.3, 69.3, 70.3, 75.7, 77.4, 82.1, 112.4, 117.8, 125.8, 135.8, 140.4, 153.4, 159.1, 170.5, 173.1; HRMS(ESI$^+$) m/z [M+H]$^+$ calcd for C$_{30}$H$_{46}$NO$_8$ 548.3218, found 548.3232.

4.1.20. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-((S)-2-oxo-1,3-dioxolan-4-yl)pyrrolidine-2-carboxylic acid (18)

To a solution of compound 17 (54.7 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 15 mL). The combined organic extracts washed with water (10 mL), dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure to give compound 18 (46.1 mg, 94% yield) as a brown solid. M.p. = 194 ºC; $[\alpha]_D^{24} = -146.28$ (c = 0.92 in CHCl$_3$); IR (nujol) 3700−2500, 1818, 1691, 1653, 1608, 1572
cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.7 Hz, 3H), 1.02 (d, J = 6.7 Hz, 3H), 1.36 (s, 9H), 1.76–1.85 (m, 2H), 2.25 (dd, J = 13.9, 7.8 Hz, 1H), 2.41–2.52 (m, 1H), 2.72–2.84 (m, 1H), 2.98 (dd, J = 13.9, 13.9 Hz, 1H), 3.18 (dd, J = 10.5, 7.8 Hz, 1H), 3.28 (s, 3H), 3.52 (dd, J = 10.5, 3.5 Hz, 1H), 3.89 (s, 3H), 3.95 (dd, J = 9.2, 5.8 Hz, 1H), 4.32 (dd, J = 9.2, 8.0 Hz, 1H), 4.45 (dd, J = 9.9, 6.2 Hz, 1H), 4.58 (dd, J = 9.9, 8.0, 5.8 Hz, 1H), 6.79 (dd, J = 7.9, 1.7 Hz, 1H), 7.10 (d, J = 1.7 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 23.8, 24.7, 25.1, 29.5, 34.7, 35.5, 39.1, 42.6, 55.3, 59.1, 66.3, 67.0, 69.7, 71.9, 74.1, 112.0, 117.6, 126.6, 133.7, 142.3, 153.1, 159.5, 173.8, 176.1; HRMS(ESI⁺) m/z [M+Na]+ calcd for C₂₆H₃₇NNaO₈ 514.2412, found 514.2425.

4.1.21. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-5-carbamoyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (19)

To a solution of compound 8 (50.4 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 8 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 19 (43.1 mg, 96% yield) as a brownish solid. M.p. = 253 ºC; [α]D²⁴ = 7.12 (c = 1.01 in CHCl₃); IR (nujol) 3600–2200, 3433, 3337, 3209, 1720, 1650, 1608, 1570 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.01 (d, J = 6.7 Hz, 3H), 1.31 (s, 9H), 1.75–1.89 (m, 1H), 2.01 (dd, J = 13.4, 13.4 Hz, 1H), 2.16 (dd, J = 15.1, 3.6 Hz, 1H), 2.20 (dd, J = 13.4, 6.7 Hz, 1H), 2.34 (dd, J = 15.1, 8.3 Hz, 1H), 2.94–3.14 (m, 1H), 3.28 (s, 3H), 3.31–3.40 (m, 2H), 3.77 (s, 3H), 4.64 (d, J = 8.5 Hz, 1H), 6.45 (bs, 1H), 6.80–6.84 (m, 2H), 7.05 (bs, 1H), 7.22 (d, J = 8.0 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 23.9, 24.6, 25.7, 29.6, 35.1, 37.0, 41.5, 41.9, 55.2, 59.1, 65.4, 70.6, 71.5, 109.8, 117.6, 126.8, 134.3, 140.6, 158.7, 170.4, 175.4, 175.4; HRMS(ESI⁺) m/z [M+Na]+ calcd for C₂₆H₃₇NNaO₈ 471.2466, found 471.2481.

4.1.22. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4,5-bis(methoxymethyl)pyrrolidine-2-carboxylate (20)

A solution of compound 7 (97.9 mg, 0.2 mmol) in ethanol (2 mL) was reacted with sodium borohydride (75.7 mg, 2 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (2 mL) and concentrated under reduced pressure. The
residue was partitioned between dichloromethane (20 mL) and water (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol in dry THF (5 mL) under argon at 0 ºC KOtBu (44.9 mg, 0.4 mmol) was added and the mixture was stirred at room temperature for 15 min. Then, iodomethane (172 mg, 1.0 mmol) was added and the slurry was stirred for 18 hours at room temperature. The resulting reaction mixture was quenched with saturated aqueous NH₄Cl solution (10 mL) and the organic solvent was evaporated under reduced pressure. The aqueous layer was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to provide compound 20 (85.9 mg, 85% yield) as a white solid. M.p. = 157 ºC; [α]D²⁴ = 29.15 (c = 1.04 in CHCl₃); IR (nujol) 1727, 1620, 1566 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.33 (s, 9H), 1.50 (s, 9H), 1.78−1.89 (m, 1H), 1.91 (dd, J = 13.0, 13.0 Hz, 1H), 1.99 (dd, J = 14.8, 3.6 Hz, 1H), 2.22 (dd, J = 13.0, 6.8 Hz, 1H), 2.30 (dd, J = 14.8, 8.2 Hz, 1H), 2.78−2.91 (m, 1H), 2.85 (s, 3H), 3.09 (dd, J = 10.8, 2.8 Hz, 1H), 3.25 (dd, J = 9.5, 7.7 Hz, 1H), 3.31 (s, 1H), 3.52 (dd, J = 9.8, 2.8 Hz, 1H), 6.93 (d, J = 1.7 Hz, 1H), 6.96 (dd, J = 7.9, 1.7 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.1, 24.5, 25.8, 28.1, 29.7, 35.0, 38.0, 41.4, 41.7, 55.2, 57.9, 59.0, 60.2, 69.4, 72.0, 72.1, 81.1, 110.4, 118.4, 126.5, 136.0, 140.3, 158.6, 169.2, 173.3; HRMS(ESI +) m/z [M+H]+ calcd for C₂₉H₄₈NO₆ 506.3477, found 506.3488.

4.1.23. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-4,5-bis(methoxymethyl)pyrrolidine-2-carboxylic acid (21)

To a solution of compound 20 (50.5 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 21 (44.0 mg, 98% yield) as a brown oil. [α]D²⁴ = −53.18 (c = 1.00 in CHCl₃); IR (neat) 3400−2400, 1734, 1636, 1608, 1558 cm⁻¹; ¹H NMR (400 MHz,
CDCl₃ δ 1.01 (d, J = 6.7 Hz, 3H), 1.02 (d, J = 6.6 Hz, 3H), 1.36 (s, 9H), 1.75–1.89 (m, 1H), 2.00 (dd, J = 14.2, 6.1 Hz, 1H), 2.19 (dd, J = 13.4, 8.0 Hz, 1H), 2.29 (dd, J = 14.2, 5.5 Hz, 1H), 2.59 (dd, J = 13.4, 13.4 Hz, 1H), 2.72–2.85 (m, 1H), 3.18 (dd, J = 10.2, 4.5 Hz, 1H), 3.21 (s, 3H), 3.25 (dd, J = 10.2, 4.5 Hz, 1H), 3.31 (s, 3H), 3.31 (dd, J = 9.5, 9.5 Hz 1H), 3.42 (dd, J = 9.5, 5.5 Hz, 1H), 3.85 (s, 3H), 4.42 (ddd, J = 7.6, 4.5, 4.5 Hz, 1H), 6.91 (dd, J = 8.0, 1.7 Hz, 1H), 6.96 (d, J = 1.7 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.5, 24.6, 24.9, 29.6, 35.2, 36.7, 39.2, 42.5, 55.4, 59.1, 59.2, 63.1, 71.4, 71.9, 72.6, 110.9, 118.5, 126.8, 134.6, 141.6, 159.0, 173.8, 174.2; HRMS(ESI⁺) m/z [M+Na⁺] calcd for C₂₅H₃₉NNaO₆ 472.2670, found 472.2687.

4.1.24. 2-(tert-Butyl) 5-methyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2,5-dicarboxylate (22)

A solution of compound 7 (97.9 mg, 0.2 mmol) in tert-butanol (8 mL) was diluted with an aqueous 1.25 M sodium phosphate buffer (2 mL) and the resulting mixture at room temperature was treated with an aqueous 1M potassium permanganate solution (1.2 mL, 1.2 mmol) and vigorously stirred for 2 hours. Then, saturated aqueous sodium sulfite solution (4 mL) was added and the pH of the mixture was adjusted to 3 with 1M hydrochloric acid. The reaction mixture was diluted with diethyl ether (20 mL) and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 x 20 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to afford a residue containing the crude acid. A stirred solution of the resulting crude acid in methanol (5 mL) was treated with a 2M solution of (trimethylsilyl)diazomethane in diethyl ether at room temperature until yellow color persisted. The reaction mixture was stirred for 1 hour and then quenched with a few drops of acetic acid until yellow color disappeared. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/2) to afford compound 22 (92.5 mg, 89% yield) as a white solid. M.p. = 154 °C; [α]_D²⁴ = 45.14 (c = 1.01 in CHCl₃); IR (nujol) 1753, 1737, 1720, 1631, 1609, 1567 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.3 Hz, 3H); 1.02 (d, J = 6.3 Hz, 3H), 1.33 (s, 9H), 1.54 (s, 9H), 1.82–1.93 (m, 1H), 2.07–2.27 (m, 4H), 2.84–2.96 (m, 1H), 3.23 (s, 3H), 3.23–3.29 (m, 2H), 3.37 (s, 3H), 3.80 (s, 3H), 4.48 (d, J = 8.7 Hz, 1H), 6.73 (d, J = 1.7 Hz, 1H), 6.75 (dd, J = 7.8, 1.7 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.4, 24.9, 25.6, 28.1, 29.7, 35.0, 37.6, 41.5, 42.7, 51.5, 55.1, 59.1, 64.4, 70.6, 71.8, 81.0, 109.5, 117.5, 126.5, 136.0, 139.6, 158.4, 169.2, 170.3, 171.8; HRMS(ESI⁺) m/z [M+H⁺] calcd for C₂₉H₄₆NO₇ 520.3269, found 520.3281.
4.1.25.  \((2S,4S,5R)-1-(4-(\text{tert-Butyl})-3-methoxybenzoyl)-2\text{-isobutyl}-5-(\text{methoxycarbonyl})-4-(\text{methoxymethyl})\text{pyrrolidine-2-carboxylic acid (23)}\)

To a solution of compound 22 (52.0 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 °C was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 °C for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 23 (44.0 mg, 97% yield) as a brown solid. M.p. = 107 °C; [\(\alpha\)_D] 24 = −13.35 (c = 1.06 in CHCl₃); IR (nujol) 3500–2400, 1753, 1717, 1628, 1564 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.02 (d, J = 6.6 Hz, 6H), 1.34 (s, 9H), 1.75–1.88 (m, 1H), 2.08 (dd, J = 14.5, 7.2 Hz, 1H), 2.20 (dd, J = 13.6, 7.0 Hz, 1H), 2.36 (dd, J = 14.5, 4.8 Hz, 1H), 2.57 (dd, J = 13.6, 11.4 Hz, 1H), 2.77–2.91 (m, 1H), 3.23 (s, 3H), 3.27 (dd, J = 9.9, 6.8 Hz, 1H), 3.33 (dd, J = 9.9, 4.6 Hz, 1H), 3.54 (s, 3H), 3.81 (s, 3H), 4.64 (d, J = 8.1 Hz, 1H), 6.74–6.8 (m, 2H), 7.25 (d, J = 7.9 Hz, 1H); ¹³C-APT(¹H) NMR (100 MHz, CDCl₃) δ 24.2, 24.8, 24.8, 29.5, 35.2, 36.3, 41.4, 42.3, 52.9, 55.3, 59.1, 65.5, 70.4, 72.7, 109.8, 117.7, 126.9, 134.0, 141.3, 158.8, 172.5, 173.0, 173.0; HRMS(ESI⁺) m/z [M+Na]+ calcd for C₂₅H₃₇NNaO₇ 486.2463, found 486.2448.

4.1.26.  \((2S,4S,5S)-1-(4-(\text{tert-Butyl})-3-methoxybenzoyl)-2\text{-isobutyl}-4-(\text{methoxymethyl})-5-\text{vinylpyrrolidine-2-carboxylate (24)}\)

To a suspension of methyl triphenylphosphonium bromide (428.7 mg, 1.2 mmol) in dry toluene (9 mL) at 0 °C and under argon atmosphere was added potassium tert-butoxide (112.2 mg, 1.0 mmol) and the mixture was stirred at room temperature for 2 hours. Then, a solution of aldehyde 7 (98.0 mg, 0.2 mmol) in dry toluene (3 mL) was added dropwise and the reaction was allowed to proceed for 24 hours. The reaction was quenched with saturated aqueous ammonium chloride solution (10 mL) and extracted with ethyl ether (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 24 (88.6 mg, 91% yield) as a white solid. M.p. = 162 °C; [\(\alpha\)_D] 24 = −27.86 (c = 0.96 in CHCl₃); IR (nujol) 3076, 1728, 1653, 1610, 1567 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.01 (d, J = 6.6 Hz, 3H), 1.05 (d, J = 6.5 Hz, 3H), 1.33 (s, 9H), 1.52 (s, 9H), 1.82–1.92 (m, 1H), 1.90 (dd, J = 13.1, 13.1 Hz, 1H), 2.04 (dd, J = 14.8, 3.7 Hz, 1H), 2.15 (dd, J = 13.1, 6.7 Hz, 1H), 2.29 (dd, J = 14.8, 8.2 Hz, 1H), 2.75–2.87 (m, 1H), 3.20 (d, J = 7.1 Hz,
2H), 3.24 (s, 3H), 3.79 (s, 3H), 4.35 (dd, J = 17.2, 1.6 Hz, 1H), 4.52 (dd, J = 9.4, 8.0 Hz, 1H), 4.79 (dd, J = 10.2, 1.6 Hz, 1H), 5.87 (ddd, J = 17.2, 10.2, 9.4 Hz, 1H), 6.77 (d, J = 1.7 Hz, 1H), 6.83 (dd, J = 7.9, 1.7 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 9.6 Hz, 1H); 6.77 (d, J=1.6 Hz, 1H); 6.83 (dd, J=7.9, 1.6 Hz, 1H); 7.18 (d, J=7.9 Hz, 1H); 13C-APT{1H} NMR (100 MHz, CDCl 3 ) δ 24.1, 24.6, 25.8, 28.1, 29.7, 35.0, 37.9, 41.8, 41.9, 55.2, 59.1, 66.1, 69.8, 72.5, 81.2, 110.5, 117.6, 118.2, 126.0, 134.0, 136.5, 139.7, 158.2, 169.9, 173.3; HRMS(ESI+) m/z [M+H]+ calcd for C 29 H 46 NO 5 488.3371, found 488.3370.

4.1.27. tert-Butyl (2S,4S,5S)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-ethyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (25)

A solution of compound 24 (48.7 mg, 0.1 mmol) in ethanol (5 mL) was hydrogenated with Pd/C 10% (20 mg) as catalyst at room temperature for 12 hours. The catalyst was removed by filtration through a short Celite® pad and the filtrate evaporated to dryness to afford compound 25 (45.5 mg, 93% yield) as a white solid. M.p. = 152 ºC; [α] D 24 = –8.40 (c = 1.04 in CHCl 3 ); IR (nujol) 1728, 1621, 1567 cm–1; 1H NMR (400 MHz, CDCh 3 ) δ 0.46 (t, J = 7.5 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.34 (s, 9H), 1.44–1.58 (m, 2H), 1.49 (s, 9H), 1.78–1.92 (m, 1H), 1.87 (dd, J = 12.7, 12.7 Hz, 2H), 1.99 (dd, J = 15.1, 3.9 Hz, 1H), 2.09 (dd, J = 12.7, 6.5 Hz, 1H), 2.31 (dd, J = 15.1, 7.7 Hz, 1H), 2.78–2.91 (m, 1H), 3.29 (s, 3H), 3.26–3.39 (m, 2H), 3.82 (s, 3H), 4.06–4.16 (m, 1H), 6.90–6.95 (m, 2H), 7.22 (d, J = 8.3 Hz, 1H); 13C-APT{1H} NMR (100 MHz, CDCl 3 ) δ 11.9, 24.3, 24.5, 25.6, 25.8, 28.1, 29.7, 35.0, 37.3, 41.6, 42.1, 55.2, 58.9, 63.9, 69.1, 71.8, 80.9, 110.8, 118.6, 126.2, 136.6, 139.9, 158.5, 169.5, 173.5; HRMS(ESI+) m/z [M+H]+ calcd for C 29 H 48 NO 5 490.3527, found 488.3370.

4.1.28. (2S,4S,5S)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-5-ethyl-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (26)

To a solution of compound 25 (24.5 mg, 0.05 mmol) in dichloromethane (0.6 mL) at 0 ºC was added trifluoroacetic acid (0.2 mL) and the resulting solution was stirred at 0 ºC for 14 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 10 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO 4 , filtered and evaporated under reduced pressure to give compound 26 (21.0 mg, 97% yield) as a brown oil. [α] D 24 = –106.76 (c = 1.00 in CHCl 3 ); IR (neat) 3500–2300, 1740, 1635, 1607, 1542 cm–1; 1H NMR (400 MHz, CDCl 3 ) δ 0.66 (t, J = 7.4 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H); 1.00 (d, J = 6.6 Hz, 3H), 1.37 (s, 9H),
1.30–1.40 (m, 2H), 1.74–1.84 (m, 1H), 2.16–2.26 (m, 1H), 2.40–2.47 (m, 1H), 2.64–2.76 (m, 2H), 3.31 (s, 3H), 3.31 (dd, J = 9.6, 9.6 Hz, 1H), 3.41 (dd, J = 9.6, 4.8 Hz, 1H), 3.86 (s, 3H), 4.35 (dd, J = 10.0, 4.8, 4.8 Hz, 1H), 6.94 (dd, J = 7.9, 1.7 Hz, 1H), 6.98 (d, J = 1.7 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H);

$^{13}$C-APT{¹H} NMR (100 MHz, CDCl$_3$) $\delta$ 10.8, 22.8, 23.9, 24.7, 25.0, 29.6, 35.3, 35.6, 40.1, 42.4, 55.4, 59.2, 66.0, 71.7, 72.2, 111.6, 119.5, 126.9, 134.2, 142.3, 159.0, 174.5, 175.0; HRMS(ESI $^+$) m/z [M+Na]$^+$ calcd for C$_{25}$H$_{39}$NNaO$_5$ 456.2721, found 456.2741.

4.1.29. tert-Butyl (2S,4S,5R)-5-(acetoxymethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (27)

A solution of compound 7 (97.9 mg, 0.2 mmol) in ethanol (2 mL) was reacted with sodium borohydride (75.7 mg, 2 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (2 mL) and concentrated under reduced pressure. The residue was partitioned between dichloromethane (20 mL) and water (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol in pyridine (5 mL) was successively added DMAP (12.2 mg, 0.1 mmol) and acetic anhydride (2.04, 20.0 mmol) and the resulting mixture was stirred at room temperature for 18 hours. Then, the reaction mixture was evaporated under reduced pressure and the residue was partitioned between dichloromethane (20 mL) and water (10 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 27 (103.5 mg, 97% yield) as a white solid. M.p. = 79 ºC; $[\alpha]_D^{24}$ = 14,16 (c = 1.25 in CHCl$_3$); IR (nujol) 1736, 1622, 1566 cm $^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.99 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 1.33 (s, 9H), 1.48 (s, 9H), 1.75 (s, 3H), 1.77–1.88 (m, 1H), 1.90 (dd, J = 13.2, 13.2 Hz, 1H), 1.99 (dd, J = 14.9, 3.7 Hz, 1H), 2.17 (dd, J = 13.2, 6.9 Hz, 1H), 2.31 (dd, J = 14.9, 8.1 Hz, 1H), 2.83–2.95 (m, 1H), 3.29 (s, 3H), 3.31 (dd, J = 9.5, 6.8 Hz, 1H), 3.38 (dd, J = 9.5, 7.5 Hz, 1H), 3.82 (s, 3H), 3.89 (dd, J = 11.6, 5.2 Hz, 1H), 4.23 (dd, J = 11.6, 7.3 Hz, 1H), 6.91–6.96 (m, 2H), 7.22 (d, J = 7.8 Hz, 1H); $^{13}$C-APT{¹H} NMR (100 MHz, CDCl$_3$) $\delta$ 20.7, 24.2, 24.4, 25.8, 28.0, 29.6, 35.0, 37.3, 41.3, 41.3, 55.1, 59.1, 60.3, 63.5, 69.4, 71.4, 81.3, 110.7, 118.4, 126.4, 135.8, 140.2, 158.6, 169.4, 170.3, 173.0; HRMS(ESI $^+$) m/z [M+H]$^+$ calcd for C$_{30}$H$_{48}$NO$_7$ 534.3426, found 534.3447.
4.1.30. \((2S,4S,5R)-5-(\text{Acetoxymethyl})-1-(4-(\text{tert-butyl})-3-\text{methoxybenzoyl})-2\text{-isobutyl}-4-(\text{methoxymethyl})\text{pyrrolidine-2-carboxylic acid} \) (28)

To a solution of compound 27 (53.4 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 °C was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 °C for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO\(_4\), filtered and evaporated under reduced pressure to give compound 28 (46.7 mg, 98% yield) as a brownish oil. \([\alpha]_{D}^{24} = -72.69 \ (c = 1.06 \text{ in CHCl}_3); \ IR \ (\text{neat}) \ 3700-2200, 1739, 1638, 1609, 1564 \text{ cm}^{-1}; \ ^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3) \ \delta 1.00, 1.00 \ (d, J = 6.6 \text{ Hz, } 3\text{H}), 1.36 \ (s, 9\text{H}), 1.75-1.86 \ (m, 1\text{H}), 2.02 \ (s, 3\text{H}), 2.22 \ (d, J = 11.6, 5.9 \text{ Hz, } 1\text{H}), 2.36 \ (d, J = 13.8, 5.7 \text{ Hz, } 1\text{H}), 2.67-2.84 \ (m, 2\text{H}), 3.29 \ (s, 3\text{H}), 3.29 \ (d, J = 9.6, 9.6 \text{ Hz, } 1\text{H}), 3.45 \ (d, J = 9.6, 3.8 \text{ Hz, } 1\text{H}), 4.53 \ (d, J = 9.8, 3.8 \text{ Hz, } 1\text{H}), 6.90 \ (d, J = 7.9, 1.7 \text{ Hz, } 1\text{H}), 6.96 \ (d, J = 1.7 \text{ Hz, } 1\text{H}), 7.29 \ (d, J = 7.9 \text{ Hz, } 1\text{H}); \ ^{13}\text{C NMR} \ (100 \text{ MHz, CDCl}_3) \ \delta 21.0, 24.0, 24.6, 25.0, 29.5, 35.2, 36.6, 38.4, 42.7, 55.3, 59.3, 62.4, 62.8, 71.6, 74.0, 110.6, 118.2, 127.0, 133.8, 141.9, 159.0, 170.8, 173.9, 174.7; \ \text{HRMS(ESI +)} \ m/z [\text{M+Na}]^+ \ C_{26}H_{39}NNaO_{7} 500.2619, \text{ found 500.2603.}

4.1.31. tert-Butyl \((2S,4S,5R)-5-(\text{allyloxy)methyl})-1-(4-(\text{tert-butyl})-3-\text{methoxybenzoyl})-2\text{-isobutyl}-4-(\text{methoxymethyl})\text{pyrrolidine-2-carboxylate} \) (29)

A solution of compound 7 (97.9 mg, 0.2 mmol) in ethanol (2 mL) was reacted with sodium borohydride (75.7 mg, 2 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (2 mL) and concentrated under reduced pressure. The residue was partitioned between dichloromethane (20 mL) and water (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO\(_4\), filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol in dry THF (5 mL) under argon at 0 °C was added sodium hydride (16.0 mg of a 60% dispersion in mineral oil, 0.4 mmol) and the mixture was stirred at room temperature for 15 min. Then, allylbromide (173 ml, 242 mg, 2.0 mmol) was added and the slurry was stirred for 18 hours at room temperature. The resulting reaction
mixture was quenched with saturated aqueous NH₄Cl solution (10 mL) and the organic solvent was evaporated under reduced pressure. The aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to provide compound 29 (101.0 mg, 95% yield) as a white solid. M.p. = 97 ºC; [α]D²⁴ = 20.90 (c = 1.15 in CHCl₃); IR (nujol) 1725, 1624, 1566 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.99 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), 1.33 (s, 9H), 1.48 (s, 9H), 1.78–1.88 (m, 1H), 1.89 (dd, J = 13.1, 13.1 Hz, 1H), 1.98 (dd, J = 14.8, 3.5 Hz, 1H), 2.22 (dd, J = 13.1, 6.8 Hz, 1H), 2.29 (dd, J = 14.8, 8.4 Hz, 1H), 2.79–2.91 (m, 1H), 3.17 (dd, J = 10.9, 2.7 Hz, 1H), 3.27 (dd, J = 9.6, 7.8 Hz, 1H), 3.30 (s, 3H), 3.35–3.42 (m, 1H), 3.43–3.50 (m, 1H), 3.56 (dd, J = 9.6, 6.3 Hz, 1H), 3.57 (dd, J = 10.9, 10.9 Hz, 1H), 3.83 (s, 3H), 4.31 (dd, J = 9.8, 7.0, 2.7 Hz, 1H), 4.90 (dd, J = 17.2, 3.3, 1.5 Hz, 1H), 4.97 (dq, J = 10.4, 2.9, 1.3 Hz, 1H), 5.49 (dd, J = 17.2, 10.7, 5.4 Hz, 1H), 6.90 (d, J = 1.7 Hz, 1H), 6.95 (dd, J = 7.9, 1.7 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.0, 24.4, 25.8, 28.1, 29.6, 35.0, 38.1, 41.3, 41.7, 55.1, 59.0, 60.7, 69.2, 69.4, 71.1, 72.1, 81.0, 110.4, 116.6, 118.3, 126.5, 134.5, 135.9, 140.1, 158.6, 169.2, 173.2; HRMS(ESI⁻) m/z [M+H⁻] calcd for C₃₁H₅₀NO₆ 532.3633, found 532.3646.

4.1.32. (2S,4S,5R)-5-((Allyloxy)methyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (30)

To a solution of compound 29 (53.2 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 30 (47.1 mg, 99% yield) as a brownish oil. [α]D²⁴ = −52.62 (c = 1.05 in CHCl₃); IR (neat) 3700–2100, 1739, 1641, 1608, 1550 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.7 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.35 (s, 9H), 1.75–1.88 (m, 1H), 1.97 (dd, J = 14.2, 6.0 Hz, 1H), 2.20 (dd, J = 13.3, 7.8 Hz, 1H), 2.30 (dd, J = 14.2, 5.6 Hz, 1H), 2.62 (dd, J = 13.3, 13.3 Hz, 1H), 2.71–2.84 (m, 1H), 3.20–3.28 (m, 2H), 3.30 (s, 3H), 3.32 (dd, J = 9.5, 9.5 Hz, 1H), 3.42 (dd, J = 9.5, 5.6 Hz, 1H), 3.79–3.83 (m, 2H), 3.84 (s, 3H), 4.45 (dd, J = 7.4, 4.8, 4.8 Hz, 1H), 5.12–5.16 (m, 1H), 5.16–5.19 (m, 1H), 5.79 (dd, J = 17.8, 9.8, 5.9, 5.9 Hz, 1H), 6.90 (dd, J = 7.9,
1.7 Hz, 1H), 6.95 (d, J = 1.7 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H); $^{13}$C-APT{$^1$H} NMR (100 MHz, CDCl$_3$) δ 24.4, 24.6, 24.9, 29.6, 35.2, 36.6, 39.0, 42.5, 55.3, 59.2, 63.2, 68.4, 71.8, 72.5, 72.7, 111.0, 118.3, 118.4, 126.7, 133.9, 134.6, 141.5, 158.9, 174.0, 174.3; HRMS(ESI') m/z [M+Na]$^+$ calcd for C$_{27}$H$_{41}$NNaO$_6$ 498.2827, found 498.2841.

4.1.33. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-5-(((methoxycarbonyl)oxy)methyl)-4-(methoxymethyl)pyrrolidine-2-carboxylate (31)

A solution of compound 7 (97.9 mg, 0.2 mmol) in ethanol (2 mL) was reacted with sodium borohydride (75.7 mg, 2 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (2 mL) and concentrated under reduced pressure. The residue was partitioned between dichloromethane (20 mL) and water (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol and DMAP (12.2 mg, 0.1 mmol) in a mixture of dry dichloromethane (2 mL) and dry pyridine (2 mL) under argon at 0°C was added dropwise methyl chloroformate (2.32 mL, 30.0 mmol) and the mixture was stirred at room temperature for 2 hours. Then, the reaction mixture was diluted with dichloromethane (30 mL) and washed with water (2 x 10 mL). The organic layer was dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to give compound 31 (105.5 mg, 96% yield) as a white solid. M.p. = 78 ºC; [α]$_{D}$$^{24}$ = 18.01 (c = 1.10 in CHCl$_3$); IR (nujol) 1754, 1727, 1614, 1566 cm–1; $^1$H NMR (400 MHz, CDCl$_3$) δ 1.00 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.33 (s, 9H), 1.50 (s, 9H), 1.77–1.90 (m, 1H), 1.96 (dd, J = 13.1, 13.1 Hz, 1H); 1.99 (dd, J = 15.1, 4.0 Hz, 1H), 2.18 (dd, J = 13.1, 6.9 Hz, 1H), 2.32 (dd, J = 15.1, 7.8 Hz, 1H), 2.82–2.95 (m, 1H), 3.28 (s, 3H), 3.32 (dd, J = 9.6, 6.3 Hz, 1H), 3.40 (dd, J = 9.6, 7.3 Hz, 1H), 3.60 (s, 3H), 3.83 (s, 3H), 3.94 (dd, J = 11.2, 4.6 Hz, 1H), 4.30 (dd, J = 11.2, 8.1 Hz, 1H), 4.52 (dd, J = 7.6, 7.6, 4.7 Hz, 1H), 6.90 (dd, J = 7.9, 1.7 Hz, 1H), 6.93 (dd, J = 1.7 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H); $^{13}$C-APT{$^1$H} NMR (100 MHz, CDCl$_3$) δ 24.3, 24.4, 25.8, 28.0, 29.6, 35.0, 37.2, 41.3, 41.3, 54.7, 55.0, 59.0, 60.1, 66.5, 69.3, 71.2, 81.4, 110.7, 118.1, 126.4, 135.6, 140.2, 155.1, 158.6, 169.5, 173.0; HRMS(ESI') m/z [M+H]$^+$ calcd for C$_{40}$H$_{48}$NO$_8$ 550.3375, found 550.3393.

4.1.34. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-5-(((methoxycarbonyl)oxy)methyl)-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (32)
To a solution of compound 31 (55.0 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts washed with water (10 mL), dried over anhydrous MgSO4, filtered and evaporated under reduced pressure to give compound 32 (48.4 mg, 98% yield) as a brownish solid. M.p. = 81 ºC; [α]D 24 = −72.29 (c = 1.08 in CHCl3); IR (nujol) 3700–2300, 1757, 1739, 1638, 1562 cm −1; 1H NMR (400 MHz, CDCl3) δ 0.99 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 1.35 (s, 9H), 1.73–1.84 (m, 1H), 1.86 (dd, J = 13.8, 5.6 Hz, 1H), 2.24 (dd, J = 12.7, 6.7 Hz, 1H), 2.36 (dd, J = 13.8, 5.6 Hz, 1H), 2.69 (dd, J = 12.7, 12.7 Hz, 1H), 2.70–2.83 (m, 1H), 3.29 (s, 3H), 3.30 (dd, J = 9.6, 8.6 Hz, 1H), 3.46 (dd, J = 9.6, 5.0 Hz, 1H), 3.66 (s, 3H), 3.80 (dd, J = 11.7, 6.9 Hz, 1H), 3.86 (s, 3H), 4.16 (dd, J = 11.7, 4.0 Hz, 1H), 4.55 (ddd, J = 6.9, 6.9, 4.0 Hz, 1H), 6.86 (dd, J = 7.9, 1.7 Hz, 1H), 7.02 (d, J = 17 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H); 13C-APT{1H} NMR (100 MHz, CDCl3) δ 24.0, 24.6, 24.9, 29.5, 35.2, 36.1, 38.5, 42.5, 55.0, 55.2, 59.2, 62.4, 65.8, 71.1, 73.0, 111.3, 118.0, 126.7, 134.0, 141.6, 155.2, 158.9, 173.9, 174.9; HRMS(ESI +) m/z [M+Na]+ calcd for C26H39NNaO8 516.2568, found 516.2580.

4.1.35. tert-Butyl (2S,4S,5R)-1-(4-(tert-butyl)-3-methoxybenzoyl)-5-(fluoromethyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (33)

A solution of compound 7 (97.9 mg, 0.2 mmol) in ethanol (2 mL) was reacted with sodium borohydride (75.7 mg, 2 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (2 mL) and concentrated under reduced pressure. The residue was partitioned between dichloromethane (20 mL) and water (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO4, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol and triethylamine (40.5 mg, 0.4 mmol) in dry dichloromethane (2 mL) under argon at −20 ºC was added dropwise DAST (53 mg, 0.4 mmol). After the addition, the reaction mixture was warmed to room temperature and stirred for 14 hours. Then, the resulting mixture was quenched by adding saturated aqueous NaHCO3 solution (10 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic layers were dried over anhydrous MgSO4, filtered and concentrated under reduced pressure. The crude product was purified
by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 33 (51.3 mg, 52% yield) as a white solid. M.p. = 136 ºC; [α]D24 = 7.11 (c = 1.09 in CHCl₃); IR (nujol) 1736, 1622, 1567 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.01 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.35 (s, 9H), 1.50 (s, 9H), 1.80–1.90 (m, 1H), 1.95 (dd, J = 13.1, 13.1 Hz, 1H), 2.00 (dd, J = 14.9, 3.8 Hz, 1H), 2.22 (dd, J = 13.1, 6.9 Hz, 1H), 2.32 (dd, J = 14.9, 8.1 Hz, 1H), 2.87–2.99 (m, 1H), 3.32 (s, 3H), 3.37 (ddd, J = 9.3, 6.9, 1.8 Hz, 1H), 3.50 (ddd, J = 9.3, 1.8 Hz, 1H), 3.83 (s, 3H), 4.22 (ddd, J = 45.9, 8.9, 2.9 Hz, 1H), 4.47 (dd, J = 42.5, 8.3, 8.3 Hz, 1H), 4.43–4.51 (m, 1H), 6.91 (d, J = 1.4 Hz, 1H), 6.94 (dd, J = 7.9, 1.4 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.2, 24.4, 25.8, 28.1, 29.7, 35.1, 37.6, 41.2, 41.4, 55.1, 59.2, 60.7 (d, J = 23.7 Hz), 69.5, 71.6 (d, J = 5.1 Hz), 81.3, 81.6 (d, J = 174.1 Hz), 110.2, 118.1, 126.7, 135.6, 140.4, 158.7, 169.3, 173.1; HRMS(ESI⁺) m/z [M+H]⁺ calcd for C₂₈H₄₅FNO₅ 494.3277, found 494.3296.

4.1.36. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-5-(fluoromethyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (34)

To a solution of compound 33 (49.4 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound 34 (43.3 mg, 99% yield) as a brownish oil. [α]D24 = −79.74 (c = 1.05 in CHCl₃); IR (neat) 3700–2300, 1739, 1711, 1631, 1607, 1564 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.01 (d, J = 6.5 Hz, 6H), 1.36 (s, 9H), 1.76–1.87 (m, 1H), 1.92 (dd, J = 14.0, 5.8 Hz, 1H), 2.25 (dd, J = 13.4, 7.7 Hz, 1H), 2.37 (dd, J = 14.0, 5.7 Hz, 1H), 2.66 (dd, J = 13.4, 13.4 Hz, 1H), 2.72–2.86 (m, 1H), 3.31 (s, 3H); 3.34 (dd, J = 9.6, 9.6 Hz, 1H), 3.47 (dd, J = 9.6, 5.4 Hz, 1H), 3.85 (s, 3H), 4.27 (dd, J = 47.0, 4.9 Hz, 2H), 4.52 (dddd, J = 21.9, 7.2, 4.9, 4.9 Hz, 1H), 6.89 (dd, J = 7.9, 1.7 Hz, 1H), 6.94 (d, J = 1.7 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.2, 24.6, 24.9, 29.6, 35.2, 36.3, 38.6 (d, J = 1.9 Hz), 42.4, 55.3, 59.2, 63.1 (d, J = 18.5 Hz), 71.3, 73.0, 82.0 (d, J =174.3 Hz), 110.6, 118.2, 127.0, 134.0, 141.8, 159.1, 174.1, 174.5; HRMS(ESI⁺) m/z [M+Na]⁺ calcd for C₂₄H₃₆FNNaO₅ 460.2470, found 460.2485.
4.1.37. tert-Butyl (2S,4S,5R)-5-(azidomethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylate (35)

A solution of compound 7 (195.8 mg, 0.4 mmol) in ethanol (6 mL) was reacted with sodium borohydride (151.4 mg, 4 mmol) for 16 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (6 mL) and concentrated under reduced pressure. The residue was partitioned between dichloromethane (20 mL) and water (10 mL); the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol and triethylamine (121.5 mg, 1.2 mmol) in dry dichloromethane (20 mL) under argon at 0 ºC was added methanesulfonyl chloride (93 ml, 1.2 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Then the resulting mixture was quenched by the addition of a saturated aqueous NaHCO₃ solution (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The crude product was used in the next step without further purification. A mixture of crude mesylate, NaI (60.0 mg, 0.4 mg) and NaN₃ (260.1 mg, 4.0 mmol) in dry DMF (6 mL) was stirred under argon at 80 ºC for 14 hours. The solvent was removed at reduced pressure, the residue partitioned between ethyl ether (20 mL) and water (10 mL), the organic layer separated and the aqueous layer extracted with ethyl ether (2 x 20 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound 35 (200.5 mg, 97% yield) as a white solid. M.p. = 113 ºC; [α]D²⁴ = 5.73 (c = 1.20 in CHCl₃); IR (nujol) 2104, 1731, 1626, 1566 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), 1.34 (s, 9H), 1.49 (s, 9H), 1.78−1.89 (m, 1H), 1.88 (dd, J = 13.1, 13.1 Hz, 1H), 1.98 (dd, J = 14.9, 3.7 Hz, 1H), 2.17 (dd, J = 13.1, 6.8 Hz, 1H), 2.30 (dd, J = 14.9, 8.1 Hz, 1H), 2.83−2.96 (m, 1H), 3.17 (dd, J = 13.0, 9.4 Hz, 1H), 3.25 (dd, J = 13.0, 3.6 Hz, 1H), 3.31 (s, 3H), 3.33 (dd, J = 9.5, 6.4 Hz, 1H), 3.42 (dd, J = 9.5, 7.3 Hz, 1H), 3.84 (s, 3H), 4.36−4.44 (m, 1H), 6.93 (d, J = 1.7 Hz, 1H), 6.96 (dd, J = 7.9, 1.7 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H); ¹³C-APT{¹H} NMR (100 MHz, CDCl₃) δ 24.1, 24.4, 25.8, 28.1, 29.6, 35.1, 37.2, 41.4, 41.6, 51.3, 55.2, 59.0, 60.9, 69.4, 71.3, 81.4, 110.1, 118.2, 126.7, 135.4, 140.8, 158.8, 169.2, 173.1; HRMS(ESI⁺) m/z [M+H]⁺ calcd for C₂₈H₄₅N₄O₅ 517.3385, found 517.3401.
4.1.38. \((2S,4S,5R)-5-(\text{Azidomethyl})-1-(4-(\text{tert-butyl})-3\text{-methoxybenzoyl})-2\text{-isobutyl}-4-(\text{methoxymethyl})\text{pyrrolidine-2-carboxylic acid} \) (36)

To a solution of compound \(35\) (51.7 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 °C was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 °C for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give compound \(36\) (45.6 mg, 99% yield) as a brownish oil. \(\alpha\) \(_{D}^{24}\) = −58.82 \((c = 1.00\ \text{in CHCl}_3);\ IR (\text{neat}) \ 3700\text{−}2400, 2102, 1738, 1712, 1639, 1607, 1564 \text{ cm}^{-1};\ 1H\ \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 1.00 (d, J = 6.7 \text{ Hz, 3H}), 1.00 (d, J = 6.6 \text{ Hz, 3H}), 1.36 (s, 9H), 1.75−1.86 (m, 1H), 1.92 (dd, J = 14.1, 5.9 Hz, 1H), 2.24 (dd, J = 13.6, 7.6 Hz, 1H), 2.34 (dd, J = 14.1, 5.6 Hz, 1H), 2.63 (dd, J = 13.6, 13.6 Hz, 1H), 2.73−2.85 (m, 1H), 3.18 (dd, J = 12.9, 7.3 Hz, 1H), 3.24 (dd, J = 12.9, 5.5 Hz, 1H), 3.28 (dd, J = 9.6, 9.6 Hz, 1H), 3.31 (s, 3H), 3.45 (dd, J = 9.6, 5.3 Hz, 1H), 3.87 (s, 3H), 4.45−4.52 (m, 1H), 6.91 (dd, J = 7.9, 1.7 Hz, 1H), 7.02 (d, J = 1.7 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H); \(^{13}\text{C-APT}{^1\text{H}}\) NMR (100 MHz, CDCl₃) \(\delta\) 24.2, 24.5, 29.5, 35.2, 35.8, 39.2, 42.3, 51.0, 55.4, 59.2, 62.7, 71.1, 111.0, 118.5, 126.8, 134.0, 142.0, 159.2, 174.2, 174.7; \text{HRMS(ESI +)} m/z [M+Na]⁺ calcd for C₂₄H₃₆N₄NaO₅ 483.2577, found 483.2581.

4.1.39. \(\text{tert-Butyl} \ (2S,4S,5R)-5-(\text{acetamidomethyl})-1-(4-(\text{tert-butyl})-3\text{-methoxybenzoyl})-2\text{-isobutyl}-4-(\text{methoxymethyl})\text{pyrrolidine-2-carboxylate} \) (37)

A mixture of compound \(35\) (103.4 mg, 0.2 mmol) and 10% Pd/C (20 mg) in ethyl acetate (5 mL) was stirred at room temperature under hydrogen atmosphere for 2 hours. The reaction mixture was filtered through a Celite® pad and concentrate under reduced pressure to afford a residue containing the crude amine. To a stirred solution of the crude amine in toluene (5 mL) at room temperature was subsequently added triethylamine (101.2 mg, 1.0 mmol) and acetic anhydride (102.1 mg, 1.0 mmol) and the reaction mixture was stirred at 100 °C for 4 hours. The reaction was quenched with water (10 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether) to give compound \(37\) (69.3 mg, 65% yield) as a colourless oil. \(\alpha\) \(_{D}^{24}\) = 43.81 \((c = 1.07 \text{ in CHCl}_3);\ IR (\text{nujol}) 3304, 1727, 1700, 1678, 1639, 1564 \text{ cm}^{-1};\ 1H\ \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 1.01 (d, J = 6.6 \text{ Hz, 6H}), 1.33 (s, 9H), 1.55 (s, 9H), 1.78−1.88 (m, 1H), 1.86 (dd, J
= 12.8, 12.8 Hz, 1H), 1.89 (s, 3H), 2.01 (dd, \( J = 14.9, 4.0 \) Hz, 1H), 2.14 (dd, \( J = 12.8, 7.2 \) Hz, 1H), 2.26 (dd, \( J = 14.9, 7.1 \) Hz, 1H), 2.78 (dd, \( J = 14.8, 3.4 \) Hz, 1H), 2.84–2.98 (m, 1H), 3.30 (dd, \( J = 9.3, 9.3 \) Hz, 1H), 3.32 (s, 3H); 3.34 (dd, \( J = 9.3, 6.1 \) Hz, 1H), 3.54 (ddd, \( J = 14.8, 8.3, 2.6 \) Hz, 1H), 3.83 (s, 3H), 4.36–4.44 (m, 1H), 6.87–6.92 (m, 2H), 7.22 (d, \( J = 8.5 \) Hz, 1H), 7.92 (d, \( J = 8.3 \) Hz, 1H); \(^{13}\)C-APT\(^{1}\)H NMR (100 MHz, CDCl\(_3\)) \( \delta \) 23.2, 24.5, 24.7, 25.7, 28.1, 29.6, 35.0, 38.3, 39.3, 41.1, 42.1, 55.3, 59.2, 60.7, 70.4, 71.5, 82.7, 110.0, 117.9, 126.6, 135.5, 140.5, 158.8, 169.4, 169.9, 175.4; HRMS(ESI\(^{+}\)) \( m/z \) [M+H]\(^{+}\) calcd for C\(_{30}\)H\(_{49}\)N\(_2\)O\(_6\) 533.3585, found 533.3578.

4.1.40. \((2S,4S,5R)-5-(Acetamidomethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid (38)\)

To a solution of compound 37 (53.3 mg, 0.1 mmol) in dichloromethane (1.2 mL) at 0 ºC was added trifluoroacetic acid (0.4 mL) and the resulting solution was stirred at 0 ºC for 6 hours. The reaction mixture was diluted with dichloromethane (15 mL), neutralised with saturated aqueous sodium hydrogen carbonate solution and carefully acidified at pH 1 with 1M hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous MgSO\(_4\), filtered and evaporated under reduced pressure to give compound 38 (45.3 mg, 95% yield) as a brownish solid. M.p. = 85 ºC; \([\alpha]_D^{24} = -28.00 \) (c = 1.02 in CHCl\(_3\)); IR (nujol) 3600–2200, 3279, 1730, 1711, 1627, 1563 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.00 (d, \( J = 6.6 \) Hz, 6H), 1.34 (s, 9H), 1.76–1.88 (m, 1H), 1.83 (s, 3H), 2.06 (dd, \( J = 14.4, 6.5 \) Hz, 1H), 2.21 (dd, \( J = 13.4, 8.0 \) Hz, 1H), 2.24 (dd, \( J = 14.4, 5.0 \) Hz, 1H), 2.43 (dd, \( J = 13.4, 13.4 \) Hz, 1H), 2.74–2.87 (m, 1H), 3.12 (ddd, \( J = 14.4, 8.9, 5.6 \) Hz, 1H), 3.24 (ddd, \( J = 14.4, 4.8, 4.8 \) Hz, 1H), 3.33 (s, 3H), 3.36–3.46 (m, 2H), 3.82 (s, 3H), 4.45–4.52 (m, 1H), 6.82 (bt, \( J = 4.8 \) Hz, 1H), 6.93 (dd, \( J = 7.9, 1.7 \) Hz, 1H), 7.06 (d, \( J = 1.7 \) Hz, 1H), 7.26 (d, \( J = 7.9 \) Hz, 1H);

\(^{13}\)C-APT\(^{1}\)H NMR (100 MHz, CDCl\(_3\)) \( \delta \) 23.2, 24.5, 24.8, 24.8, 29.6, 35.2, 36.8, 39.5, 40.0, 42.1, 55.3, 59.2, 62.2, 71.0, 71.7, 110.9, 118.2, 126.7, 134.5, 141.3, 158.9, 170.9, 172.5, 176.0; HRMS(ESI\(^{+}\)) \( m/z \) [M+Na]\(^{+}\) calcd for C\(_{26}\)H\(_{40}\)N\(_2\)NaO\(_6\) 499.2779, found 499.2797.

4.2. Biological assays

4.2.1. Cells and replicon system.

The highly permissive cell clone Huh /-Lunet, as well as HuH 7 cells containing subgenomic (HCV) replicons I389luc-ubi-neo/NS3-3'/5.1 (Huh 5-2), I377NS3-3'/wt (Huh 9-13), or I389/hygro-ubi-NS3-3'/5.1 (a kind gift from Dr. V. Lohmann and Dr. R. Bartenschaler) has been described [18]. Briefly,
this system allowed the efficient propagation of genetically modified HCV RNAs (replicons) in a human hepatoma cell line (Huh). The amount of RNA that has been transcribed and translated is determined through the quantification of a reporter contained the replicon system (luciferase). The amount of luminescence detected (after adding the substrate specific for this enzyme) is proportional to the virus replication rate. Cells were grown in a DMEM supplemented with 10% heat-inactivated fetal bovine serum 1 x non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin and 250 µg/mL geneticin (G418).

4.2.2. Antiviral and cytostatic assays with Huh 5-2 cells
Antiviral and cytostatic assays were performed according to the protocol already described in Abian et al [19]. The 50% effective concentration (EC50) was defined as the concentration of compound that reduced the luciferase signal by 50 % and the 50% cytotoxic concentration (CC50) was determined employing the dose–response equation (i.e., Hill equation).

4.3.3 Geometry optimization and calculation of molecular descriptors
Structures of the compounds were optimized at the quantum level with the DFT (Density Functional Theory) functional b3lyp/6-31+g(d,p) using GaussView 5.0.8 and Gaussian09 [24]. Charge zero (0) and a multiplicity of one (1) were settled, in the Gaussian input files, for all the compounds. The Homo and Lumo energies (in a.u.) of the optimized structures were extracted from the .log Gaussian output files, and used to calculate the reactivity (Re) of compounds as:

\[
Re = \frac{\eta}{(\eta + 27.2114)}
\]

where, \( \eta \), is the chemical hardness, and 27.2114 is the conversion rate from a.u. to eV units.

Thirty-five additional molecular descriptors, including steric ones (e.g. molecular weight, sum of atomic van der Waals volumes, radius of gyration, molecular eccentricity, spherocity, etc.), constitutional ones (e.g. heteroatom percentages, rotatable bond fraction, etc.), ring ones (e.g. cyclomatic number, total ring size, aromatic ratio, etc.), molecular properties (e.g. hydrophilic factor, molar refractivity, octanol-water partition coefficient, etc.) and a consensus drug-like score were calculated with the DRAGON 6.0 program [25].
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REFERENCES


[12] See, for example:  


**Supplementary data**

Copies of 1H NMR and 13C NMR spectra of all compounds, X-ray crystallographic data (ORTEP) for compound 4a (PDF), copies of antiviral activity and cell viability graphs of compounds 13, 15, 16, 18, 19, 23, 26, 28, 30, 32, 34, 36 and 38, and figures for binding poses of GSK3082 and compounds 13, 15, 16, 18, 19, 21, 23, 26, 28, 30, 32, 34, 36 and 38.

X-ray crystallographic data for compound 4a (CIF).