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*-isopropylideneglyceraldehyde 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 relationshiphs

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 HVC 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].

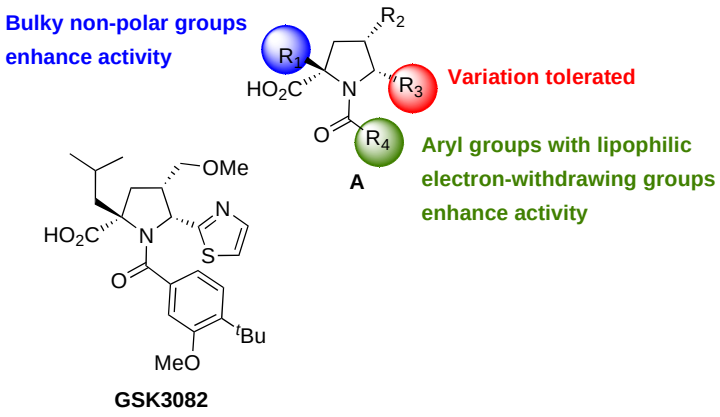


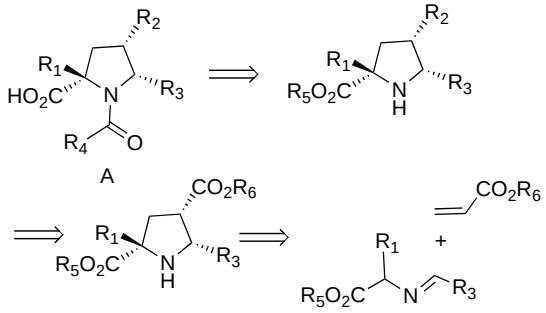
Figure 1. General structure of *N*-acylpyrrolidines identified as of HCV NS5B polymerase inhibitors.

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 the *N*-acylpyrrolidine series bind in the palm pocket I of 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.

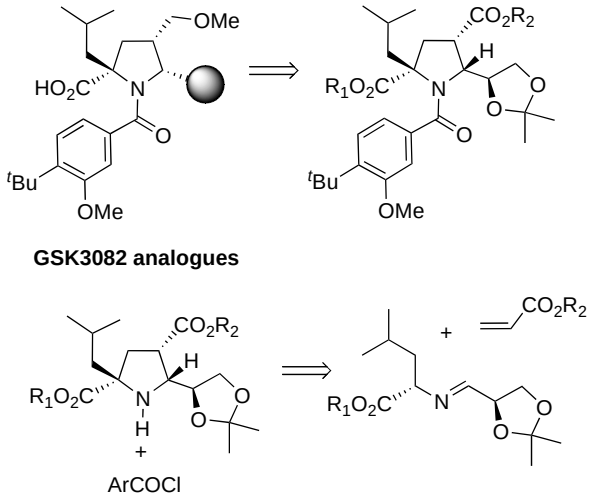
One of our research goals is the synthesis of versatile chiral intermediates from imines derived from glyceraldehyde – which is readily accessible from inexpensive D-mannitol – and the development of synthetic routes to obtain bioactive compounds in enantiomerically pure form from these chiral intermediates [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

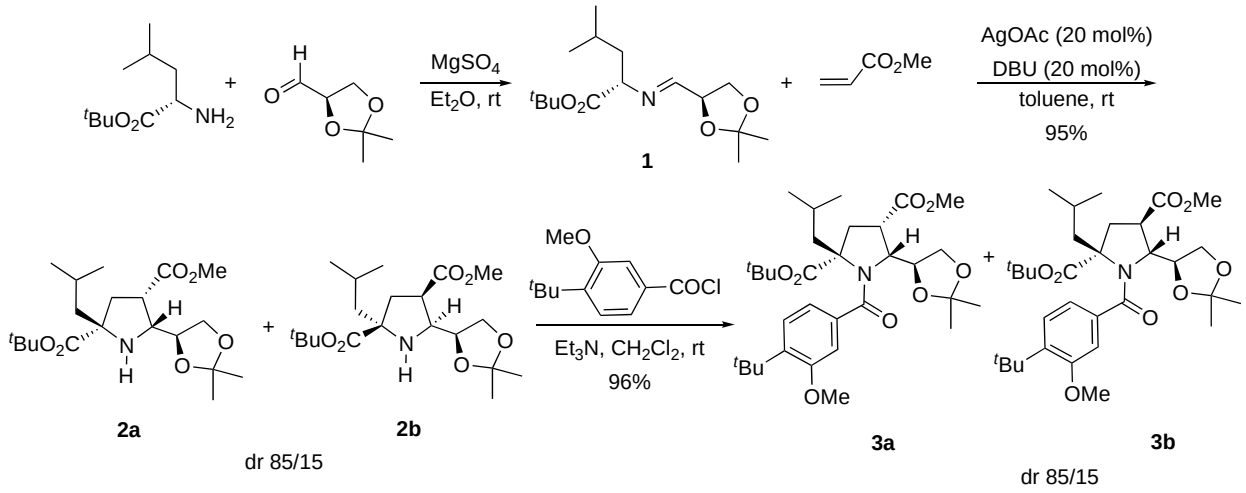
Based on our previous experience we envisaged that the pyrrolidine core of structural analogues of GSK3082 with different substituents in the 5-position could be obtained by the 1,3-dipolar cycloaddition between an acrylate and an iminoester derived from D-glyceraldehyde acetonide and leucine using silver 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.



Scheme 2. Strategy for synthesis GSK3082 analogues.

Starting imino ester **1** was prepared by reaction of (*S*)-leucine *tert*-butyl ester with (*R*)-2,3-*O*-isopropylideneglyceraldehyde in ether at room temperature for 2 h in the presence of anhydrous $MgSO_4$ 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

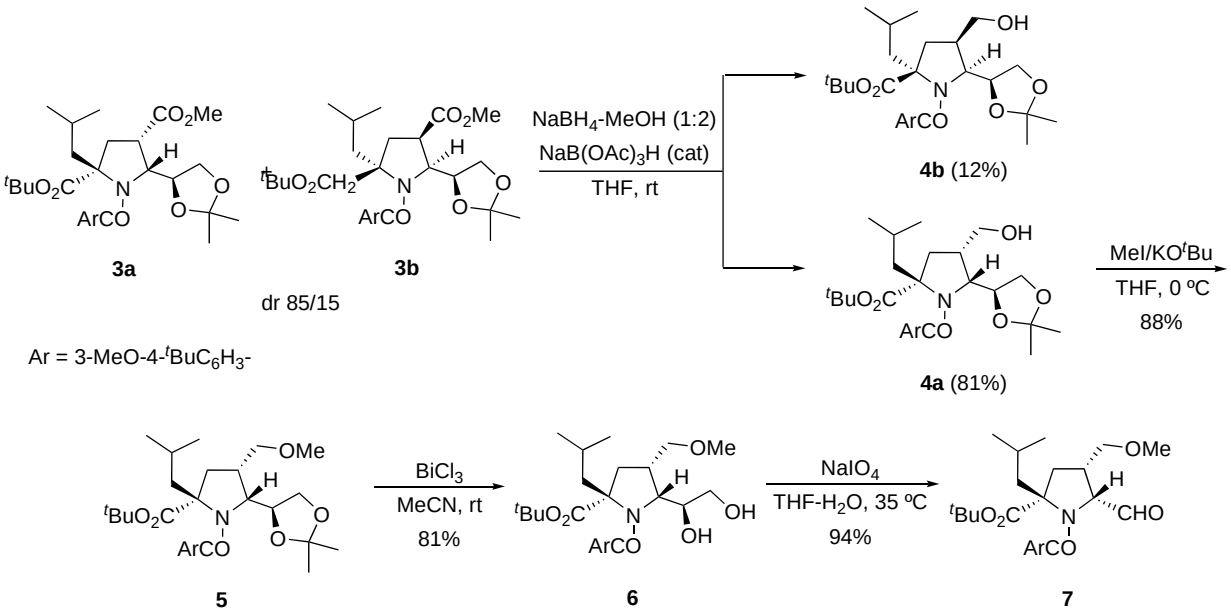
mol%) as the catalyst and DBU (20 mol%) as the base in toluene at room temperature for 24 h led to an 85/15 mixture of *endo* adducts **2a**/**2b** in 95% yield, in which compound **2a** with the desired (2*S*,4*S*,5*R*) configuration was in excess. The absolute configuration of the major *endo* adduct 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).



Scheme 3. Synthesis of *N*-acylpyrrolidine **3a**.

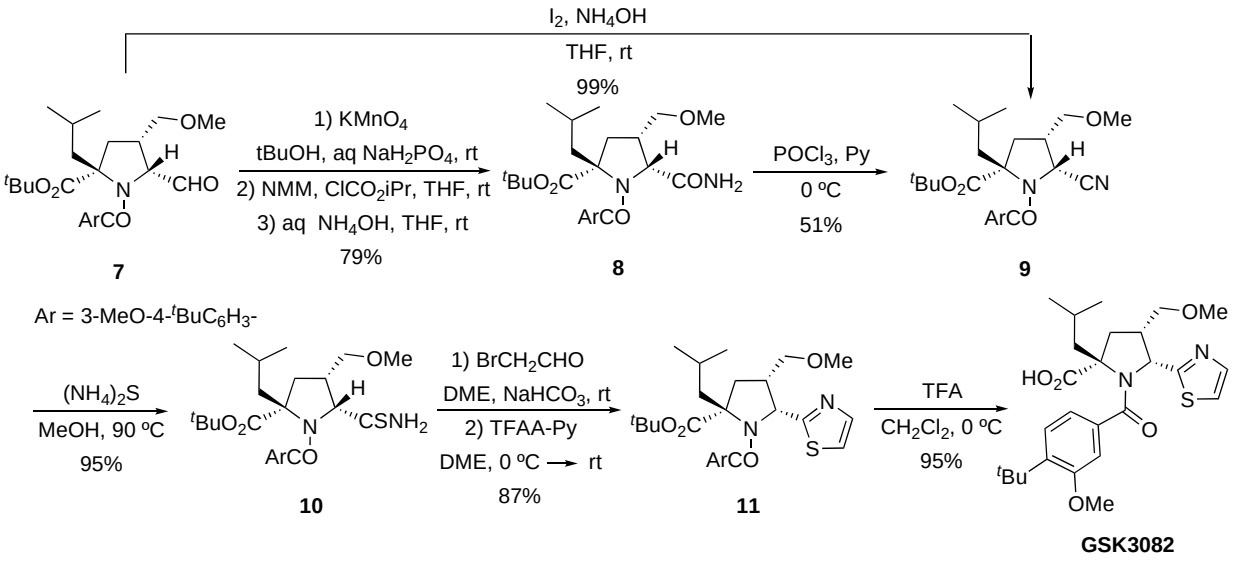
The synthesis of GSK3082 was completed as shown in Schemes 4 and 5. Reduction of the methyl ester was achieved with a combination of $NaBH_4$-MeOH (1:2) in the presence of a catalytic amount of $NaB(OAc)_3H$ (2.5 mol % relative to $NaBH_4$) [10a]. After stirring **3a**/**3b** in THF for 4 days at room 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 $BiCl_3$ 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.



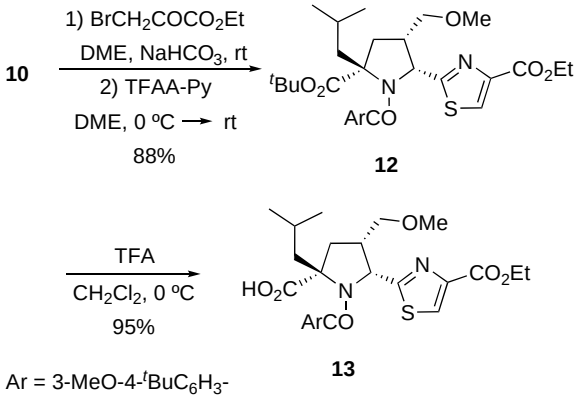
Scheme 4. The synthetic route for key intermediate **7**.

Further oxidation of aldehyde **7** with potassium permanganate using a mixture of *tert*-butanol and aqueous NaH_2PO_4 as the reaction medium [14], followed by treatment with of 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 ºC yielded GSK3082 in 95% yield. GSK3082 was obtained from **1** in 10 steps with a good overall yield of 38%.



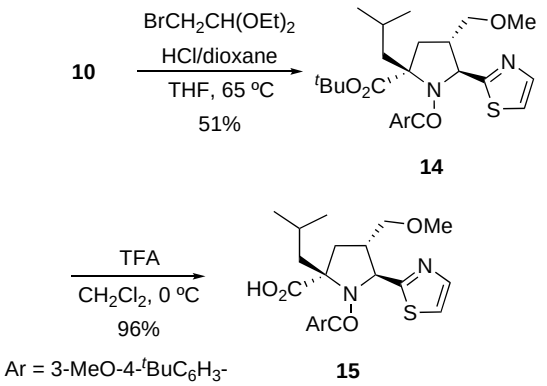
Scheme 5. The synthetic route for target compound **GSK3082**.

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).



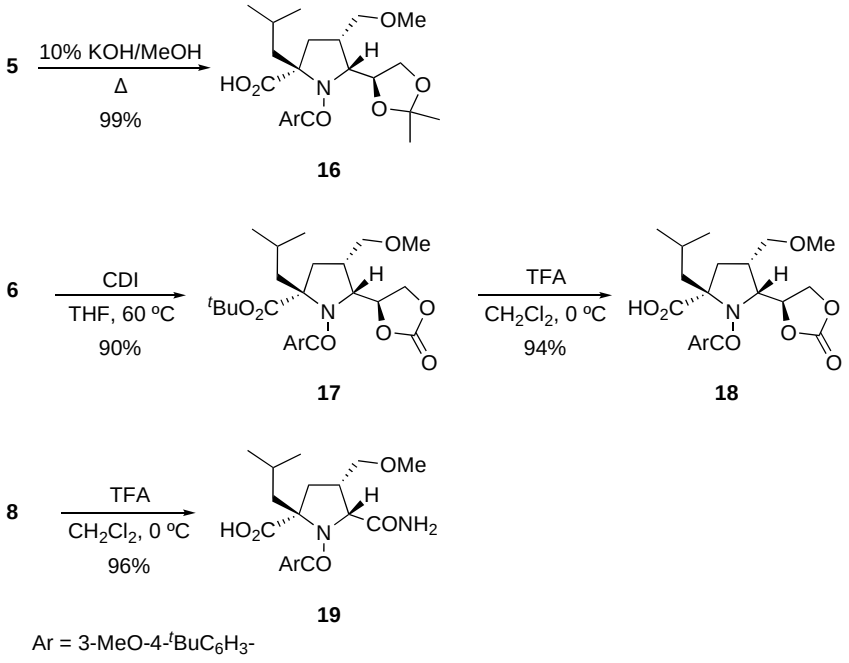
Scheme 6. The synthetic route for GSK3082 analogue **13**.

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).



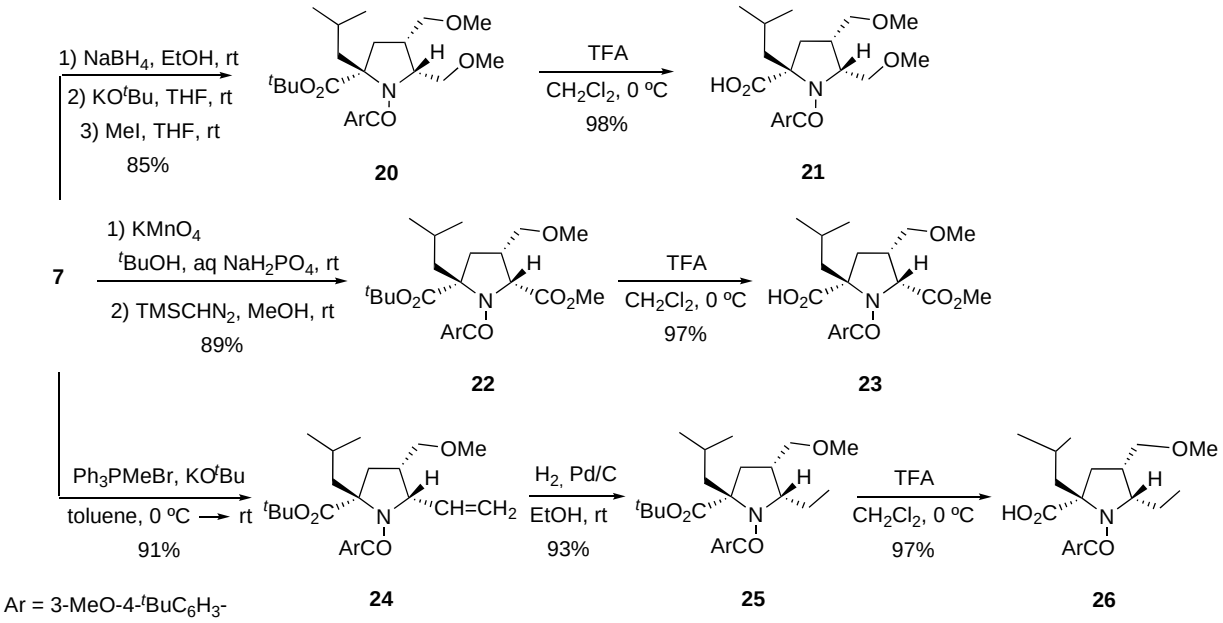
Scheme 7. The synthetic route for GSK3082 analogue **15**.

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 ºC to produce carbonate **17** in 90% yield, which upon hydrolysis with trifluoroacetic acid in dichloromethane at 0 ºC 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 ºC (Scheme 8).



Scheme 8. The synthetic route for GSK3082 analogues **16**, **18** and **19**.

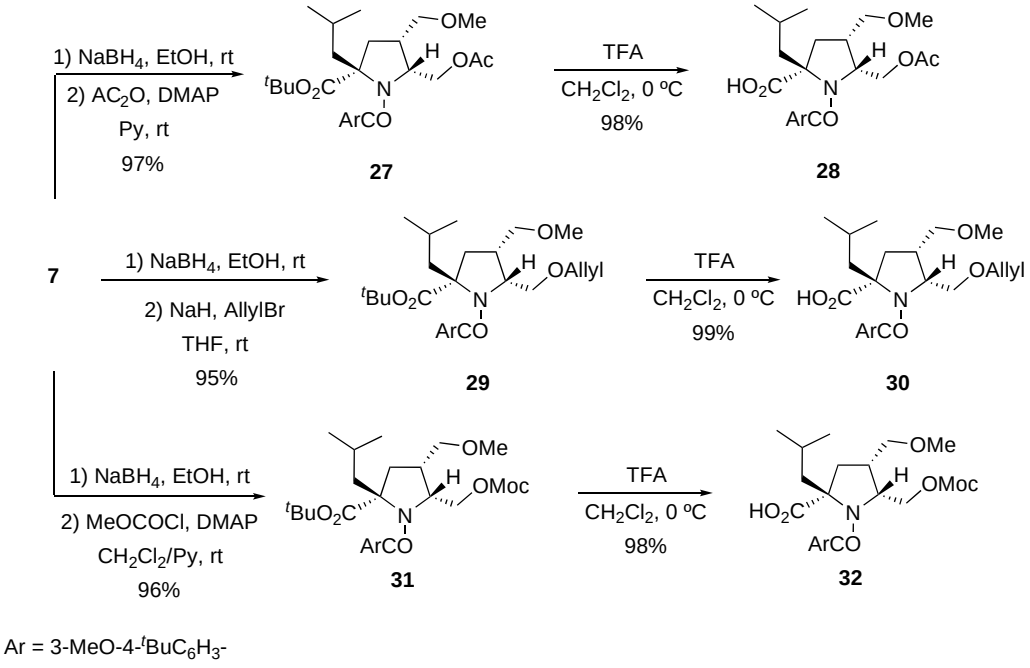
Other GSK3082 analogues were obtained from versatile chiral intermediate **7** by transformation of the formyl group into methoxymethyl, ethyl and methoxycarbonyl groups, as shown in Scheme 9. Reaction of compound **7** with sodium borohydride in ethanol reduced the C5 formyl group to a hydroxymethyl 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 ºC 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 NaH_2PO_4 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 ºC 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.



Scheme 9. The synthetic route for GSK3082 analogues **21**, **23** and **26**.

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

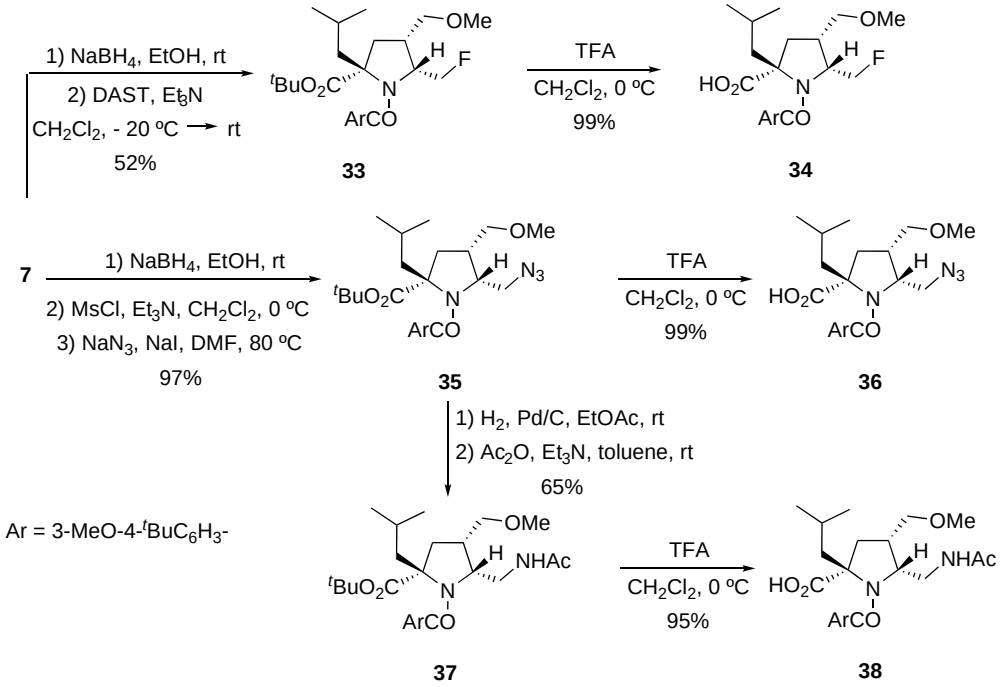
efficiently converted into acetate **27**, allyl ether **29** or methylcarbonate **31** by reduction **7** with sodium borohydride in ethanol followed by the immediate dimethylaminopyridine-catalysed acetylation with acetic anhydride, reaction with allyl bromide in the presence of sodium hydride or treatment with methyl chloroformate in the presence of a catalytic amount of dimethylaminopyridine. Subsequent hydrolysis of these compounds with trifluoroacetic acid in dichloromethane at 0 ºC 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 acetylaminometyl 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 ºC 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.

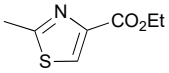
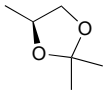


Scheme 11. The synthetic route for GSK3082 analogues **34**, **36** and **38**.

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 replicon 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

Compound	R	EC50[a] (∝M)	CC50[b] (∝M)
GSK3082		0.1	>120
13		60	200
15	 *	15	200
16		60	>120
18		2	>120
19	$CONH_2$	2	>120
21	CH_2OMe	0.6	>120
23	CO_2Me	7	>120
26	Et	40	200
28	CH_2OAc	30	>120
30	$CH_2OAllyl$	7.5	>120
32	CH_2OMoc	30	>120
34	CH_2F	45	>120
36	CH_2N_3	100	>120
38	CH_2NHAc	15	>120

[a]EC50, effective concentration 50%; [b]CC50, cytotoxic concentration 50%. Relative error in the parameters is 15%.

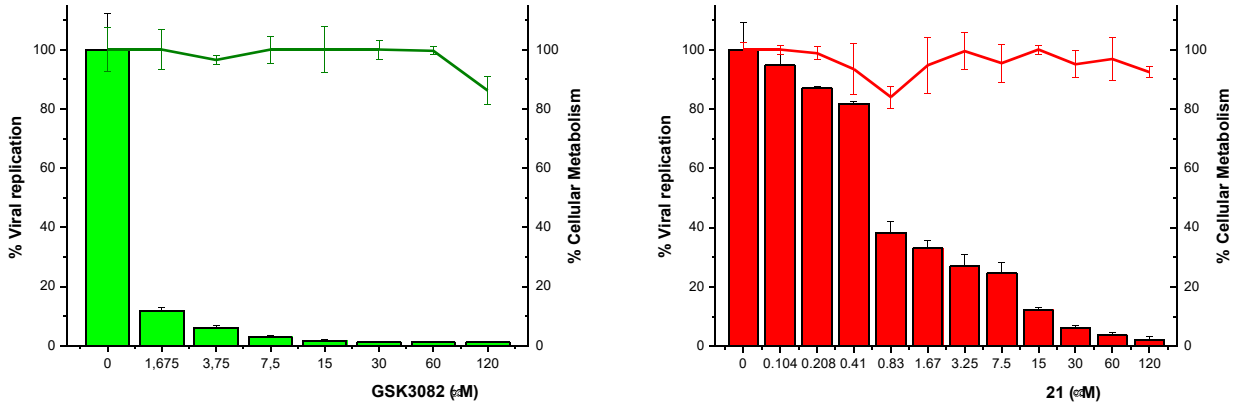


Figure 2. Evaluation of viral load reduction (bars) of the HCV replicon system in human hepatoma cells (Huh5-2) with cell growth (lines) upon increasing the concentration of compound GSK3082 (green) and **21** (red).*UTC: untreated controls.

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.

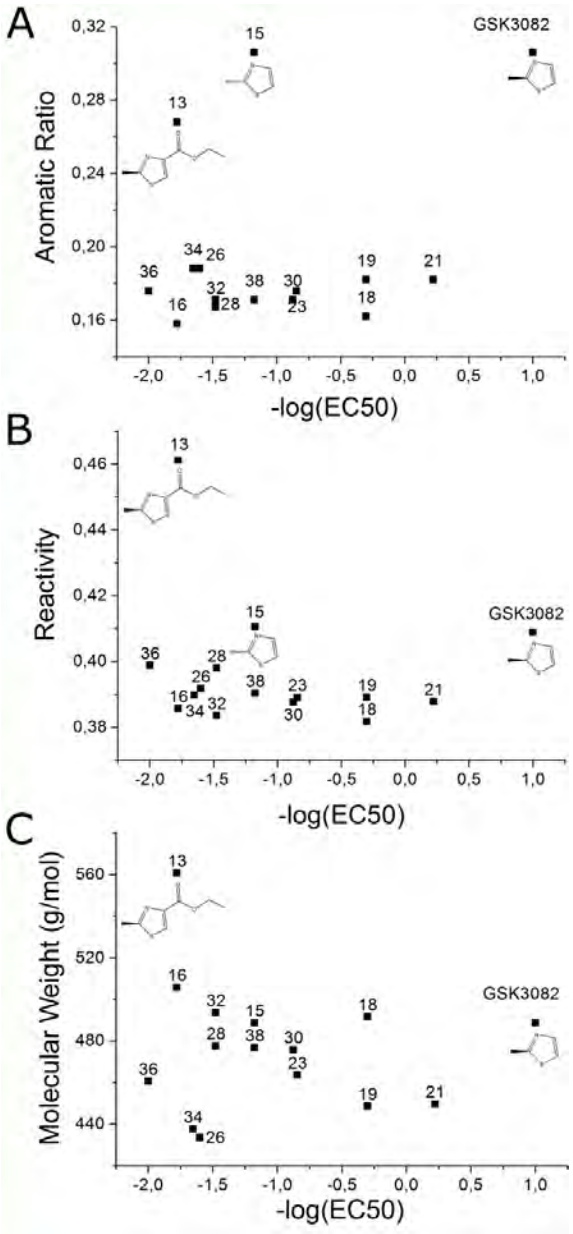


Figure 3. Scatter plots of molecular descriptor values versus the assayed biological activities: (A) aromatic ratio, (B) reactivity and (C) molecular weight values versus the effectiveness of compounds against HCV NS5B polymerase. Substituent structures (R_3) at the 5-position in compounds **GSK3082**, **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*-isopropylideneglyceraldehyde. 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 F_{254} 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, ν_{max} values expressed in cm^{-1} are given for the main absorption bands. Optical rotations were measured on a digital polarimeter at λ 589 nm and 25 ºC in cells with 1 or 10 cm path length; $[\alpha]_D$ values are given in 10^{-1} $deg \cdot cm^2 \cdot g^{-1}$ and concentrations are given in g/100 mL. ^1H, ^{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 ^1H broad-band decoupled mode, chemical shifts (δ) are reported in parts per million (ppm) with the solvent resonance as the internal standard [20], and coupling constants (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-*Ka* radiation (l = 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-D-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 ºC. 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 ºC 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, $CDCl_3$) δ 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® pad. Celite® residues were

thoroughly washed with diethyl ether (50 mL) and the combined organic filtrates were dried over anhydrous $MgSO_4$, 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* diasteroisomers **2a** and **2b** (**2a**/**2b** = 85/15). Some column chromatography fractions containing exclusively major cycloadduct **2a** were used for characterization purposes. Colourless oil; $[\alpha]_D^{24}$ = −39.23 (*c* = 1.11 in $CHCl_3$); IR (neat) 3341, 3308, 1734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{20}H_{36}NO_6$ 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 choride 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 $MgSO_4$, 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; $[\alpha]_D^{24}$ = 0.65 (*c* = 1.94 in $CHCl_3$); IR (neat) 1734, 1636, 1567 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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 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 Hz, 1H), 6.92 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.04 (d, *J* = 1.6 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 1H); ^{13}C-APT{^{1}H} NMR (100 MHz, $CDCl_3$) δ 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; HRMS(ESI$^+$) *m/z* $[M+H]^+$ calcd for $C_{32}H_{50}NO_8$ 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 $NaB(OAc)_3H$ (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_4Cl 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 in $CHCl_3$); IR (nujol) 3379, 1730, 1593, 1563 cm $^{-1}$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (s, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.6 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}C-APT{^{1}H} NMR (100 MHz, $CDCl_3$) δ 24.5, 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; HRMS(ESI$^+$) *m/z* $[M+H]^+$ calcd for $C_{31}H_{50}NO_7$ 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^{t}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 NH_4Cl 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 $MgSO_4$, 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; $[\alpha]_D^{24}$ = −13.72 (*c* = 1.45 in $CHCl_3$); IR (nujol) 1729, 1612 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{32}H_{52}NO_7$ 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 $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography chromatography (1st eluent: diethyl ether/hexane, 1/1; 2nd eluent: diethyl ether) to provide compound **6** (1.06 g, 81% yield) as colourless oil; $[\alpha]_D^{24}$ = −17.34 (*c* = 0.88 in $CHCl_3$); IR (nujol) 3579, 3411, 1737, 1699, 1639, 1566 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^{1}H} 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}; ^{1}H 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), 6.77 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 9.67 (d, *J* = 3.7, 1H); ^{13}C-APT{^{1}H} 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 $C_{28}H_{44}NO_6$ 490.3164, found 490.3149.

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 $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound **8** (119.5 mg, 79% yield) as colourless oil. $[\alpha]_D^{24}$ = 7.83 (*c* = 1.01 in $CHCl_3$); IR (neat) 3324, 3189, 1697, 1650, 1563 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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, 8.4 Hz, 1H), 3.29 (s, 3H), 3.59 (dd, *J* = 9.6, 4.9 Hz, 1H), 3.80 (s, 3H), 4.44 (d, *J* = 9.0 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{28}H_{45}N_2O_6$ 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 $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: 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 $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: diethyl

ether/hexane, 1/2) to afford compound **9** (240.9 mg, 99% yield) as colourless oil. $[\alpha]_D^{24}$ = 32.95 (*c* = 1.01 in $CHCl_3$); IR (neat) 2250, 1734, 1657, 1565 cm^{-1}; ^1H 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); ^{13}C-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 $C_{28}H_{43}N_2O_5$ 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; $[\alpha]_D^{24}$ = −27.95 (*c* = 1.25 in $CHCl_3$); IR (nujol) 3302, 3138, 1743, 1700, 1653, 1565, 1500 cm^{-1}; ^1H 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); ^{13}C-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 $C_{28}H_{45}N_2O_5S$ 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

[22] (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 $MgSO_4$, filtered and evaporated under reduced pressure. The residue was solved in dry dimethoxiethane (1mL) and cooled to 0 ºC. 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 dichloromethane (2 x 10 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, 1/1) to afford compound **11** (47.4 mg, 87% yield) as a brown solid. M.p. = 144 ºC; $[\alpha]_D^{24}$ = 66.42 (*c* = 0.55 in $CHCl_3$); IR (neat) 3113, 1713, 1608, 1567, 1502 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{30}H_{45}N_2O_5S$ 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 $MgSO_4$, 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.[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 $MgSO_4$, filtered and evaporated under reduced pressure. The residue was solved in dry dimethoxyethane (1 mL) and cooled to 0 ºC. 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 $MgSO_4$, 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 ºC; $[\alpha]_D^{24}$ = 53.75 (*c* = 1.05 in $CHCl_3$); IR (nujol) 3092, 1730, 1653, 1591 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{33}H_{49}N_2O_7S$ 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 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 **13** (26.5 mg, 95% yield) as a brown solid. M.p. = 55 ºC; $[\alpha]_D^{24}$ = 61.89 (*c* = 0.64 in $CHCl_3$); IR (nujol) 3500−2400, 1741, 1653, 1607, 1565 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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, 3H), 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), 2.54 (dd, *J* = 10.3, 9.4 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{29}H_{40}N_2NaO_7S$ 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. $[\alpha]_D^{24}$ = −28.67 (*c* = 0.99 in $CHCl_3$); IR (neat) 3082, 1734, 1652, 1610, 1566 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{30}H_{45}N_2O_5S$ 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 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. $[\alpha]_D^{24}$ = −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); ^{13}C-APT{^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* $[M+Na]^+$ $C_{26}H_{36}N_2NaO_5S$ 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 ºC; $[\alpha]_D^{24}$ = −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, 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}; ^{1}H NMR (400 MHz, $CDCl_3$) 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 (ddd, *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); $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) 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 calcd for $C_{26}H_{37}NNaO_8$ 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 $MgSO_4$, filtered and evaporated under reduced pressure to give compound **19** (43.1 mg, 96% yield) as a brownish solid. M.p. = 253 ºC; $[\alpha]_D^{24}$ = 7.12 (*c* = 1.01 in $CHCl_3$); IR (nujol) 3600−2200, 3433, 3337, 3209, 1720, 1650, 1608, 1570 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (d, *J* = 6.7 Hz, 3H), 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); ^{13}C-APT$\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) 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 calcd for $C_{24}H_{36}N_2NaO_6$ 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 $MgSO_4$, 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 ºC KO^{t}Bu (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 NH_4Cl 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 $MgSO_4$, 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; $[\alpha]_D^{24}$ = 29.15 (*c* = 1.04 in $CHCl_3$); IR (nujol) 1727, 1620, 1566 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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* = 9.5, 6.2 Hz, 1H), 3.60 (dd, *J* = 10.8, 9.8 Hz, 1H), 3.84 (s, 3H), 4.32 (ddd, *J* = 9.8, 7.0, 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{29}H_{48}NO_6$ 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 (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 **21** (44.0 mg, 98% yield) as a brown oil. $[\alpha]_D^{24}$ = −53.18 (*c* = 1.00 in $CHCl_3$); IR (neat) 3400−2400, 1734, 1636, 1608, 1558 cm^{-1}; ^1H NMR (400 MHz,

$CDCl_3$) 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); ^{13}C-APT{^{1}H} NMR (100 MHz, $CDCl_3$) 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 $C_{25}H_{39}NNaO_6$ 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 m<l) 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 $MgSO_4$ 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 ºC; $[\alpha]_D^{24}$ = 45.14 (*c* = 1.01 in $CHCl_3$); IR (nujol) 1753, 1737, 1720, 1631, 1609, 1567 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^{1}H} NMR (100 MHz, $CDCl_3$) 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 $C_{29}H_{46}NO_7$ 520.3269, found 520.3281.

4.1.25. (2S,4S,5R)-1-(4-(tert-Butyl)-3-methoxybenzoyl)-2-isobutyl-5-(methoxycarbonyl)-4-(methoxymethyl)pyrrolidine-2-carboxylic acid ***(23)***

To a solution of compound **22** (52.0 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 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 $MgSO_4$, filtered and evaporated under reduced pressure to give compound **23** (44.0 mg, 97% yield) as a brown solid. M.p. = 107 ºC; $[\alpha]_D^{24}$ = −13.35 (*c* = 1.06 in $CHCl_3$); IR (nujol) 3500−2400, 1753, 1717, 1628, 1564 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{25}H_{37}NNaO_7$ 486.2463, found 486.2448.

4.1.26. tert-Butyl (2S,4S,5S)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)-5-vinylpyrrolidine-2-carboxylate ***(24)***

To a suspension of methyl triphenylphosphonium bromide (428.7 mg, 1.2 mmol) in dry toluene (9 mL) at 0 ºC 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 dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure and the residue was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound **24** (88.6 mg, 91% yield) as a white solid. M.p. = 162 ºC; $[\alpha]_D^{24}$ = −27.86 (*c* = 0.96 in $CHCl_3$); IR (nujol) 3076, 1728, 1653, 1610, 1567 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^{1}H} 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; $[\alpha]_D^{24}$ = −8.40 (*c* = 1.04 in $CHCl_3$); IR (nujol) 1728, 1621, 1567 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^{1}H} 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 490.3540.

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. $[\alpha]_D^{24}$ = −106.76 (*c* = 1.00 in $CHCl_3$); IR (neat) 3500−2300, 1740, 1635, 1607, 1542 cm^{-1}; ^{1}H 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, 2H), 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 (ddd, *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{^{1}H} NMR (100 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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{^{1}H} NMR (100 MHz, $CDCl_3$) δ 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-(Acetoxymethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid ***(28)***

To a solution of compound **27** (53.4 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 $MgSO_4$, 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 in $CHCl_3$); IR (neat) 3700−2200, 1739, 1638, 1609, 1564 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.36 (s, 9H), 1.75−1.86 (m, 1H), 1.88 (dd, *J* = 13.8, 5.6 Hz, 1H), 2.02 (s, 3H), 2.22 (dd, *J* = 11.6, 5.9 Hz, 1H), 2.36 (dd, *J* = 13.8, 5.7 Hz, 1H), 2.67−2.84 (m, 2H), 3.29 (s, 3H), 3.29 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.48 (dd, *J* = 9.6, 4.7 Hz, 1H), 3.78 (dd, *J* = 12.2, 3.8 Hz, 1H), 3.86 (s, 3H), 4.06 (dd, *J* = 12.2, 3.8 Hz, 1H), 4.53 (ddd, *J* = 7.3, 3.8, 3.8 Hz, 1H), 6.90 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.96 (d, *J* = 1.7 Hz, 1H), 7.29 (d, *J* = 7.9 Hz, 1H); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) δ 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; HRMS(ESI $^+$) *m/z* $[M+Na]^+$ $C_{26}H_{39}NNaO_7$ 500.2619, found 500.2603.

4.1.31. tert-Butyl (2S,4S,5R)-5-((allyloxy)methyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)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 ºC 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 NH_4Cl 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 $MgSO_4$, 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; $[\alpha]_D^{24}$ = 20.90 (*c* = 1.15 in $CHCl_3$); IR (nujol) 1725, 1624, 1566 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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 (ddd, *J* = 9.8, 7.0, 2.7 Hz, 1H), 4.90 (ddd, *J* = 17.2, 3.3, 1.5 Hz, 1H), 4.97 (dq, *J* = 10.4, 2.9, 1.3 Hz, 1H), 5.49 (dddd, *J* = 17.2, 10.7, 5.4, 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{31}H_{50}NO_6$ 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 $MgSO_4$, filtered and evaporated under reduced pressure to give compound **30** (47.1 mg, 99% yield) as a brownish oil. $[\alpha]_D^{24}$ = −52.62 (*c* = 1.05 in $CHCl_3$); IR (neat) 3700−2100, 1739, 1641, 1608, 1550 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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 (ddd, *J* = 7.4, 4.8, 4.8 Hz, 1H), 5.12−5.16 (m, 1H), 5.16−5.19 (m, 1H), 5.79 (dddd, *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 0ºC 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; $[\alpha]_D^{24}$ = 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 (d, *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 $MgSO_4$, filtered and evaporated under reduced pressure to give compound **32** (48.4 mg, 98% yield) as a brownish solid. M.p. = 81 ºC; $[\alpha]_D^{24} = -72.29$ (*c* = 1.08 in $CHCl_3$); IR (nujol) 3700−2300, 1757, 1739, 1638, 1562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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* = 1.7 Hz, 1H), 7.26 (d, *J* = 7.9 Hz, 1H); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{26}H_{39}NNaO_8$ 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 $MgSO_4$, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol and triethylamine (40.5 mg, 0.4 mmol) in dry dichloromethane (2 mL) under argon at −20 ºC was added dropwise DAST (53 ml, 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 $NaHCO_3$ solution (10 mL) and extracted with dichloromethane (3 x 20 mL). 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 **33** (51.3 mg, 52% yield) as a white solid. M.p. = 136 ºC; $[\alpha]_D^{24}$ = 7.11 (*c* = 1.09 in $CHCl_3$); IR (nujol) 1736, 1622, 1567 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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), 1.93 (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, 7.1, 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{28}H_{45}FNO_5$ 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 $MgSO_4$, filtered and evaporated under reduced pressure to give compound **34** (43.3 mg, 99% yield) as a brownish oil. $[\alpha]_D^{24}$ = −79.74 (*c* = 1.05 in $CHCl_3$); IR (neat) 3700−2300, 1739, 1711, 1631, 1607, 1564 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{24}H_{36}FNNaO_5$ 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 $MgSO_4$, filtered and evaporated under reduced pressure to afford a residue containing the crude alcohol. To a stirred solution of the crude alcohol and 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 $NaHCO_3$ solution (10 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 10 mL). The combined organic exracts were dried over anhydrous $MgSO_4$, filtered and concentrated 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 NaN_3 (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 $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: diethyl ether/hexane, 1/1) to afford compound **35** (200.5 mg, 97% yield) as a white solid. M.p. = 113 ºC; $[\alpha]_D^{24}$ = 5.73 (*c* = 1.20 in $CHCl_3$); IR (nujol) 2104, 1731, 1626, 1566 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C-APT{^1H} NMR (100 MHz, $CDCl_3$) 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 $C_{28}H_{45}N_4O_5$ 517.3385, found 517.3401.

*4.1.38. (2S,4S,5R)-5-(Azidomethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)pyrrolidine-2-carboxylic acid **(36)***

To a solution of compound **35** (51.7 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 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 **36** (45.6 mg, 99% yield) as a brownish oil. $[\alpha]_D^{24}$ = −58.82 (*c* = 1.00 in $CHCl_3$); IR (neat) 3700−2400, 2102, 1738, 1712, 1639, 1607, 1564 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, *J* = 6.7 Hz, 3H), 1.00 (d, *J* = 6.6 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}C-APT{^1H} NMR (100 MHz, $CDCl_3$) δ 24.2, 24.5, 24.9, 29.5, 35.2, 35.8, 39.2, 42.3, 51.0, 55.4, 59.2, 62.7, 71.1, 72.3, 111.0, 118.5, 126.8, 134.0, 142.0, 159.2, 174.2, 174.7; HRMS(ESI $^+$) *m/z* $[M+Na]^+$ calcd for $C_{24}H_{36}N_4NaO_5$ 483.2577, found 483.2581.

*4.1.39. tert-Butyl (2S,4S,5R)-5-(acetamidomethyl)-1-(4-(tert-butyl)-3-methoxybenzoyl)-2-isobutyl-4-(methoxymethyl)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 ºC 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 $MgSO_4$, 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 in $CHCl_3$); IR (nujol) 3304, 1727, 1700, 1678, 1639, 1564 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (d, *J* = 6.6 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, $CDCl_3$) δ 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 $C_{30}H_{49}N_2O_6$ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{26}H_{40}N_2NaO_6$ 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:

$$Re = \frac{!}{!} = \frac{!}{\frac{(!"\#\$!!"\#" * !".!""\#)}{!}} = \frac{!.!"\#\$}{(!"\#\$!\,!"\#"\quad)} \qquad (1)$$

where, η, 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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Supplementary data

Copies of ^{1}H NMR and ^{13}C 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).