Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Synthesis and bioactivity of fused- and spiro-β-lactone-lactam systems

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General methods and materials

All reagents were obtained from Sigma Aldrich, Alfa Aesar, Apollo Scientific or Acros Organics and used without further purification. All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen unless not using anhydrous solvents. Anhydrous solvents were dried by pre-storing them over activated 3 Å molecular sieves before being passed through an activated alumina column on a solvent tower under N_2 pressure. 'Petrol' refers to light petroleum ether of boiling point 40-60 °C and was used as purchased. Solvents were evaporated at 40 °C under reduced pressure on a Büchi R-114 rotatory evaporator attached to a Vacuubrand CVC2 pump and a pressure control system. Concentrations (*c*) in the general procedures refer to the limiting reagent and are given in mmol/mL.

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium foil backed sheets precoated with 0.2 mm Kielselgel 60 F_{254}. The eluent used is specified in each case. The spots were visualised by UV irradiation (λ 254 nm) and by staining with a $KMnO_4$ solution followed by heating. Retention factors (R_f) are quoted to the nearest 0.01. *Flash* column chromatography was performed on Kielselgel 60 silica gel (230-400 mesh particle size). The eluents are specified in each case.

Optical rotations were recorded at 25 °C on a Perkin-Elmer 241 polarimeter or Unipol L 2000 using the D line of sodium (589 nm) and a path length of 1 dm. Concentrations (*c*) are given in g/100 mL and specific rotations ($[\alpha]_D^{20}$) are quoted in 10^{-1} deg cm^2 g^{-1}.

Melting points were measured using a Stuart Scientific SMP1 melting point instrument and are uncorrected.

Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer equipped with an attached Pike Miracle attenuated total reflectance (ATR) module. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and only selected peaks are reported.

^{1}H NMR spectra were recorded on the following instruments: AVIII HD 400 (400 MHz) and AVII 500 (500 MHz). Chemical shifts (δ_H) are reported in parts per million (ppm) downfield from TMS and are referenced to the residual ^{1}H solvent peak. Coupling constants (*J*) are quoted in Hz. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constant, and assignment. Two-dimensional COSY spectra were recorded on Bruker AVIII HD 400 (400 MHz) and AVII 500 (500 MHz) spectrometers, and NOE experiments were performed on a Bruker AVII 500 (500 MHz).

^{13}C NMR spectra were recorded on a Bruker AVIII HD 400 at 100.6 MHz and AVII 500 at 125.8 MHz with proton decoupling. Chemical shifts (δ_C) are reported in ppm downfield from TMS and are referenced to the residual ^{13}C solvent peak. Assignments of the spectra were made with HSQC and HMBC experiments, which were performed on a Bruker AVII 500 spectrometer.

Low resolution mass spectra (*m/z*) were recorded on a Fison Platform spectrometer using electrospray ionisation (ESI). Selected peaks are reported in Daltons and their intensities given as percentages of the base peak. High resolution mass spectra (HRMS) were recorded on a Bruker microTOF (ESI) or on an Agilent 7200 Q-TOF (CI). LC/MS spectra were recorded on a Bruker 9.4T FT-ICR-MS (ESI).

Crystals for X-ray crystallography were grown from slow vapour diffusion of petrol into a solution of the compound in EtOAc at room temperature.

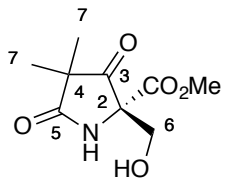
Synthetic procedures

Previously reported compounds **8**,[1] **9**[2] and **14**[2] were prepared using the reported methods.

General procedure A: *N,O*-Acetal deprotection of tetramic acids and pyrrolidinones[3]

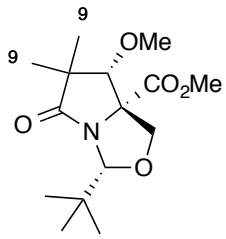
Tetramic acid or pyrrolidinone (1.0 eq) was treated with 1,3-propanedithiol (1.5-5 eq) followed by a freshly prepared solution of 1.5% HCl in 2,2,2-trifluoroethanol (*c* 0.06). The reaction mixture was heated at 50 °C (unless otherwise specified) for 12-28 h and then evaporated under reduced pressure. The crude product was purified by *flash* column chromatography to give the unprotected alcohol.

Methyl (*R*)-2-hydroxymethyl-4,4-dimethyl-3,5-dioxopyrrolidine-2-carboxylate (10)



General procedure A (from **9**, 4.0 eq of 1,3-propanedithiol, for 15 h); yield 78% (249 mg); white solid; m.p. 84 °C. R_f (EtOAc) 0.48; $[\alpha]_D^{20}$ -20.0 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3543, 3338 (N-H, O-H), 2980 (C-H), 1775 (C=O), 1701 (C=O), 1691 (C=O); δ_H (400 MHz, CD_3OD) 1.19 (3H, s, C(7_A)H_3), 1.22 (3H, s, C(7_B)H_3), 3.76 (3H, s, CO_2CH_3), 3.78 (1H, d, *J* 11.6, C(6)H_AH_B), 4.15 (1H, d, *J* 11.6, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.8 (*C*(7_A)), 22.2 (*C*(7_B)), 47.4 (*C*(4)), 53.7 (CO_2CH_3), 63.7 (*C*(6)), 76.0 (*C*(2)), 168.3 (CO_2CH_3), 180.5 (*C*(5)), 209.6 (*C*(3)); *m/z* (ESI^+) 216.1 (MH^+, 27%), 238.1 (MNa^+, 100%); HRMS (ESI^+) found 216.08647, $C_9H_{14}NO_5$ (MH^+) requires 216.08665.

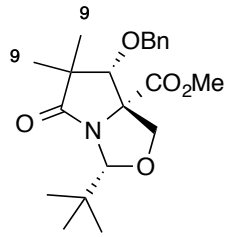
(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (15)



NaH (60% w/w, 20 mg, 0.51 mmol) was added portionwise to a solution of alcohol **14** (97 mg, 0.34 mmol) and methyl iodide (24 µL, 0.38 mmol) in anhydrous THF (3.4 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 4 h. H_2O was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give methyl ether **15** (104 mg, 0.347 mmol, quant.) as a white solid; m.p. 57 °C. R_f (20% EtOAc in petrol) 0.41; $[\alpha]_D^{20}$ +8.9 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2975 (C-H), 2954 (C-H), 1741 (C=O), 1712 (C=O); δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C(CH_3)$_3$), 1.22 (3H, s, C(9_A)H_3), 1.32 (3H, s, C(9_B)H_3), 3.37 (1H, d, *J* 8.4,

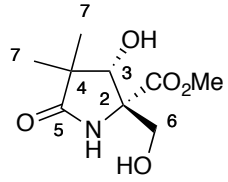
C(4)H_AH_B), 3.39 (3H, s, C(6)OCH_3), 3.55 (1H, s, C(6)*H*), 3.78 (3H, s, CO_2CH_3), 4.82 (1H, s, C(2)*H*), 4.97 (1H, d, *J* 8.4, C(4)H_AH_B); δ_C (100 MHz, $CDCl_3$) 20.1 (*C*(9_B)), 25.1 (C(CH_3)$_3$), 25.5 (*C*(9_A)), 35.7 (*C*(CH_3)$_3$), 49.6 (*C*(7)), 52.5 (CO_2CH_3), 60.3 (C(6)OCH_3), 74.8 (*C*(5)), 75.8 (*C*(4)), 90.5 (*C*(6)), 96.8 (*C*(2)), 170.5 (CO_2CH_3), 182.1 (*C*(8)); *m/z* (ESI^+) 300.2 (MH^+, 66%), 322.2 (MNa^+, 100%); HRMS (CI^+) found 300.1801, $C_{15}H_{26}NO_5$ (MH^+) requires 300.1805.

(2*R*,5*R*,6*S*)-1-Aza-6-benzyloxy-2-(*tert*-butyl)-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (16)



NaH (60% w/w, 20 mg, 0.50 mmol) was added portionwise to a solution of alcohol **14** (88 mg, 0.31 mmol) and benzyl bromide (47 µL, 0.40 mmol) in anhydrous THF (3.5 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 17 h. H_2O was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (petrol to 20% EtOAc in petrol) to give benzyl ether **16** (106 mg, 0.281 mmol, 91%) as a colourless oil. R_f (20% EtOAc in petrol) 0.42; $[\alpha]_D^{20}$ -18.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2958 (C-H), 1714 (C=O); δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.12 (3H, s, C(9_A)H_3), 1.38 (3H, s, C(9_B)H_3), 3.33 (1H, d, *J* 8.4, C(4)H_AH_B), 3.76 (4H, s, C(6)*H* + CO_2CH_3), 4.44 (1H, d, *J* 11.8, OCH_AH_BPh), 4.67 (1H, d, *J* 11.8, OCH_AH_BPh), 4.82 (1H, s, C(2)*H*), 4.96 (1H, d, *J* 8.4, C(4)H_AH_B), 7.26-7.38 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$) 20.5 (*C*(9_B)), 25.1 (C(CH_3)$_3$), 25.1 (*C*(9_A)), 35.8 (*C*(CH_3)$_3$), 49.6 (*C*(7)), 52.6 (CO_2CH_3), 73.6 (OCH_2Ph), 74.8 (*C*(5)), 75.7 (*C*(4)), 87.0 (*C*(6)), 96.8 (*C*(2)), 127.8, 128.3, 128.6, 137.0 (Ph), 170.7 (CO_2CH_3), 182.1 (*C*(8)); *m/z* (ESI^+) 376.2 (MH^+, 35%), 398.2 (MNa^+, 100%); HRMS (ESI^+) found 376.21183, $C_{21}H_{30}NO_5$ (MH^+) requires 376.21185.

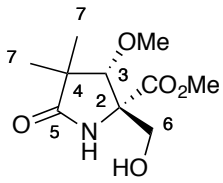
Methyl (2*R*,3*S*)-3-hydroxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (17)



General procedure A (from **14**, 4.0 eq of 1,3-propanedithiol, for 15 h); yield quant. (219 mg); white solid; m.p. 139-141 °C. R_f (10% MeOH in EtOAc) 0.42;

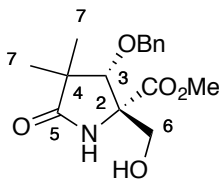
$[\alpha]_D^{20}$ -16.6 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3347, 3217 (N-H, O-H), 2971 (C-H), 1716 (C=O), 1677 (C=O); δ_H (400 MHz, CD_3OD) 1.07 (3H, s, C(7_A)H_3), 1.15 (3H, s, C(7_B)H_3), 3.67 (1H, d, *J* 11.2, C(6)H_AH_B), 3.75 (3H, s, CO_2CH_3), 4.01 (1H, s, C(3)*H*), 4.06 (1H, d, *J* 11.2, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.6 (*C*(7_A)), 25.3 (*C*(7_B)), 45.5 (*C*(4)), 52.7 (CO_2CH_3), 66.0 (*C*(6)), 71.9 (*C*(2)), 78.5 (*C*(3)), 172.9 (CO_2CH_3), 183.7 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 100%), 240.1 (MNa^+, 80%); HRMS (ESI^+) found 218.10245, $C_9H_{16}NO_5$ (MH^+) requires 218.10230.

Methyl (2*R*,3*S*)-2-hydroxymethyl-3-methoxy-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (18)



General procedure A (from **15**, 4.0 eq of 1,3-propanedithiol, for 13 h); yield 95% (89 mg); white solid; m.p. 132 °C. R_f (10% MeOH in EtOAc) 0.48; $[\alpha]_D^{20}$ -36.4 (*c* 1.1, MeOH); ν_{max}/cm^{-1} 3339 (N-H, O-H), 2953 (C-H), 1738 (C=O), 1684 (C=O); δ_H (400 MHz, CD_3OD) 1.09 (3H, s, C(7_A)H_3), 1.21 (3H, s, C(7_B)H_3), 3.47 (3H, s, C(3)OCH_3), 3.74 (1H, d, *J* 11.4, C(6)H_AH_B), 3.74 (4H, s, CO_2CH_3 + C(3)*H*), 4.05 (1H, d, *J* 11.4, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.7 (*C*(7_A)), 26.0 (*C*(7_B)), 45.5 (*C*(4)), 52.7 (CO_2CH_3), 61.0 (C(3)OCH_3), 66.2 (*C*(6)), 71.3 (*C*(2)), 88.5 (*C*(3)), 172.5 (CO_2CH_3), 183.1 (*C*(5)); *m/z* (ESI^+) 232.0 (MH^+, 37%), 254.0 (MNa^+, 100%); HRMS (ESI^+) found 232.11828, $C_{10}H_{18}NO_5$ (MH^+) requires 232.11795.

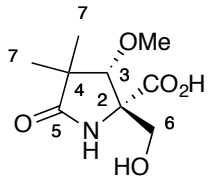
Methyl (2*R*,3*S*)-3-benzyloxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (19)



General procedure A (from **16**, 4.0 eq of 1,3-propanedithiol, for 12 h); yield quant. (81 mg); colourless oil. R_f (EtOAc) 0.24; $[\alpha]_D^{20}$ -39.1 (*c* 0.5, DCM); ν_{max}/cm^{-1} 3338 (N-H, O-H), 2951 (C-H), 1743 (C=O), 1698 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7_A)H_3), 1.14 (3H, s, C(7_B)H_3), 3.70 (3H, s, CO_2CH_3), 3.75 (1H, d, *J* 11.6, C(6)H_AH_B), 3.99 (1H, s, C(3)*H*), 4.07 (1H, d, *J* 11.6, C(6)H_AH_B), 4.59 (1H, d, *J* 11.6, OCH_AH_BPh), 4.72 (1H, d, *J* 11.6, OCH_AH_BPh), 7.27-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 20.3 (*C*(7_B)), 25.7 (*C*(7_A)), 45.6 (*C*(4)), 52.8 (CO_2CH_3), 66.1 (*C*(6)), 71.4 (*C*(2)), 75.3 (OCH_2Ph), 85.7 (*C*(3)), 128.9, 129.4, 129.4, 139.2 (Ph), 172.6

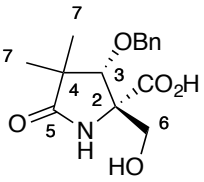
(CO_2CH_3), 183.2 (C(5)); *m/z* (ESI$^+$) 308.1 (MH^+, 14%), 330.1 (MNa^+, 100%); HRMS (ESI$^+$) found 308.14931, $C_{16}H_{22}NO_5$ (MH^+) requires 308.14925.

(2*R*,3*S*)-2-Hydroxymethyl-3-methoxy-4,4-dimethyl-5-oxopyrrolidine-2-carboxylic acid (21)



NaOH (20 mg, 0.49 mmol) was added to a solution of ester **18** (82 mg, 0.35 mmol) in 1:1:1 MeOH/THF/H_2O (3.6 mL). The reaction mixture was stirred at room temperature for 24 h, diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **21** (65 mg, 0.30 mmol, 85%) as a colourless oil. $[\alpha]_D^{20}$ -32.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3304 (N-H, O-H), 2937 (C-H), 1669 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7$_A$)H_3), 1.21 (3H, s, C(7$_B$)H_3), 3.50 (3H, s, C(3)OCH_3), 3.76 (1H, d, *J* 11.5, C(6)H_AH_B), 3.76 (1H, s, C(3)*H*), 4.04 (1H, d, *J* 11.5, C(6)H$_A$$H_B$); δ_C (100 MHz, CD_3OD) 19.8 (C(7$_A$)), 25.9 (C(7$_B$)), 45.6 (C(4)), 61.0 (C(3)OCH$_3$), 66.2 (C(6)), 70.9 (C(2)), 88.3 (C(3)), 173.7 (CO_2H), 183.2 (C(5)); *m/z* (ESI$^+$) 218.1 (MH^+, 51%), 240.1 (MNa^+, 100%); *m/z* (ESI$^-$) 216.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 216.08728, $C_9H_{14}NO_5$ (M-H$^-$) requires 216.08775.

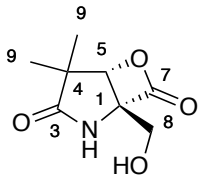
(2*R*,3*S*)-3-Benzyloxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylic acid (22)



Sodium hydroxide (6 mg, 0.1 mmol) was added to a solution of ester **19** (26 mg, 0.084 mmol) in 1:1:1 MeOH/THF/H_2O (840 µL). The mixture was stirred at room temperature for 24 h, diluted with H_2O and washed with DCM. The aqueous layer was acidified with 1 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **22** (24 mg, 0.080 mmol, 95%) as white solid; m.p. 66 °C. $[\alpha]_D^{20}$ -31.1 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3293 (O-H), 2928 (C-H), 1665 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7$_A$)H_3), 1.17 (3H, s, C(7$_B$)H_3), 3.77 (1H, d, *J* 11.6, C(6)H_AH_B), 4.00 (1H, s, C(3)*H*), 4.06 (1H, d, *J* 11.6, C(6)H$_A$$H_B$), 4.59 (1H, d, *J* 11.6, OCH_AH_BPh), 4.79 (1H, d, *J* 11.6, OCH$_A$$H_B$Ph), 7.28-

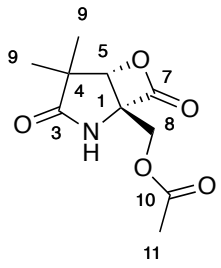
7.38 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 19.0 (*C*(7_B)), 24.3 (*C*(7_A)), 44.3 (*C*(4)), 64.8 (*C*(6)), 69.7 (*C*(2)), 73.9 (O*C*H_2Ph), 84.2 (*C*(3)), 127.4, 127.6, 127.9, 137.9 (Ph), 172.4 (*C*O_2H), 181.9 (*C*(5)); *m/z* (ESI^+) 294.1 (MH^+, 30%), 316.1 (MNa^+, 100%), *m/z* (ESI^-) 292.1 ($M\text{-}H^-$, 100%); HRMS (ESI^-) found 292.11899, $C_{15}H_{18}NO_5$ ($M\text{-}H^-$) requires 292.11905.

(1*R*,5*S*)-1-Hydroxymethyl-4,4-dimethyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (23)



Sodium hydroxide (43 mg, 1.1 mmol) was added to a solution of ester **17** (187 mg, 0.862 mmol) in 1:1:1 MeOH/THF/H_2O (8.4 mL). The mixture was stirred at room temperature for 1 h, acidified with 1 M aqueous HCl and concentrated *in vacuo* to give a white solid. Triethylamine (240 µL, 1.72 mmol) and BOP chloride (440 mg, 1.73 mmol) were added to a suspension of the crude acid in anhydrous DCM (17 mL). After stirring at room temperature for 19 h, brine was added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (EtOAc) to give fused-β-lactone **23** (71 mg, 0.38 mmol, 45%) as a white solid; m.p. 163 °C. R_f (EtOAc) 0.36; $[\alpha]_D^{20}$ -82.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3306, 3217 (N-H, O-H), 2930 (C-H), 1833 (C=O), 1686 (C=O); δ_H (400 MHz, CD_3OD) 1.20 (3H, s, C(9_A)H_3), 1.23 (3H, s, C(9_B)H_3), 3.74 (1H, d, *J* 12.2, C(8)H_AH_B), 4.00 (1H, d, *J* 12.2, C(8)H_AH_B), 4.81 (1H, s, C(5)*H*); δ_C (100 MHz, CD_3OD) 17.0 (*C*(9_B)), 24.3 (*C*(9_A)), 44.2 (*C*(4)), 58.7 (*C*(8)), 77.8 (*C*(1)), 81.8 (*C*(5)), 170.4 (*C*(7)), 182.5 (*C*(3)); *m/z* (ESI^-) 184.1 ($M\text{-}H^-$, 100%); HRMS (ESI^-) found 184.0610, $C_8H_{10}NO_4$ ($M\text{-}H^-$) requires 184.06153.

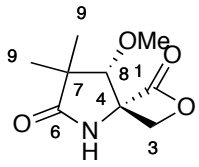
((1*R*,5*S*)-4,4-Dimethyl-3,7-dioxo-6-oxa-2-azabicyclo[3.2.0]heptan-1-yl)methyl acetate (24)



Pyridine (4 µL, 0.05 mmol) and acetic anhydride (5 µL, 0.05 mmol) were added to a suspension of β-lactone **23** (6 mg, 0.03 mmol) in anhydrous DCM (300 µL). After stirring at room temperature for 13 h, the product was concentrated

under reduced pressure to give pure acetylated β-lactone **24** (7 mg, 0.03 mmol, quant.) as a white solid; m.p. 153 °C. R_f (50% EtOAc in DCM) 0.48; $[\alpha]_D^{20}$ -40.8 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 3171 (N-H), 2971 (C-H), 1843 (C=O), 1746 (C=O), 1706 (C=O); δ_H (500 MHz, $CDCl_3$) 1.23 (3H, s, C(9_A)H_3), 1.31 (3H, s, C(9_B)H_3), 2.13 (3H, s, C(11)H_3), 4.46 (1H, d, *J* 12.5, C(8)H_AH_B), 4.50 (1H, d, *J* 12.5, C(8)H_AH_B), 4.67 (1H, s, C(5)*H*), 6.63 (1H, br s, N*H*); δ_C (125 MHz, $CDCl_3$) 16.8 (*C*(9_B)), 20.7 (*C*(11)), 24.2 (*C*(9_A)), 42.9 (*C*(4)), 59.4 (*C*(8)), 73.4 (*C*(1)), 81.3 (*C*(5)), 166.8 (*C*(7)), 170.3 (*C*(10)), 179.6 (*C*(3)); *m/z* (ESI^+) 228.1 (MH^+, 10%), 250.1 (MNa^+, 100%); HRMS (ESI^+) found 228.08668, $C_{10}H_{14}NO_5$ (MH^+) requires 228.08665.

(4*R*,8*S*)-8-Methoxy-7,7-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (26)



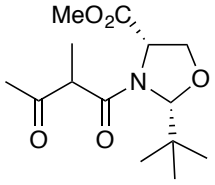
HATU (228 mg, 0.600 mmol) and DIPEA (100 μL, 0.574 mmol) were added to a solution of acid **21** (65 mg, 0.30 mmol) in anhydrous THF (10 mL) at 0 °C. The mixture was stirred at room temperature for 36 h and quenched with brine. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (40% to 50% EtOAc in petrol) to give spiro-β-lactone **26** (16 mg, 0.081 mmol, 27%) as a 2.5:1 inseparable mixture with tetramethylurea. R_f (EtOAc) 0.51; ν_{max}/cm^{-1} 3228 (N-H), 2973 (C-H), 1827 (C=O), 1714 (C=O); δ_H (500 MHz, CD_3OD) 1.25 (3H, s, C(9_A)H_3), 1.28 (3H, s, C(9_B)H_3), 3.58 (3H, s, C(8)OCH_3), 4.10 (1H, s, C(8)*H*), 4.43 (1H, d, *J* 6.0, C(3)H_AH_B), 4.51 (1H, d, *J* 6.0, C(3)H_AH_B); δ_C (125 MHz, CD_3OD) 20.2 (*C*(9_A)), 24.2 (*C*(9_B)), 45.4 (*C*(7)), 60.4 (C(8)OCH_3), 70.6 (*C*(3)), 77.2 (*C*(4)), 88.8 (*C*(8)), 171.3 (*C*(1)), 182.0 (*C*(6)); *m/z* (ESI^+) 200.2 (MH^+, 51%), 222.0 (MNa^+, 100%); HRMS (ESI^+) found 200.09190, $C_9H_{14}NO_4$ (MH^+) requires 200.09173.

General procedure B: *N*-Acylation of oxazolidines[1]

To a solution of oxazolidine (1.0 eq) in anhydrous DCM (*c* 0.5) were added DMAP (0.05 eq) and DCC (1.05 eq). The mixture was cooled to 0 °C and the required β-carbonyl carboxylic acid (1.05 eq) was added. After stirring 30 min at 0 °C and 5 h at room temperature, the reaction mixture was filtered

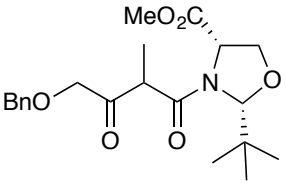
and washed with DCM. The combined filtrates were concentrated *in vacuo* and purified by *flash* column chromatography to give the *N*-acyloxazolidine.

(2*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1-(2-methyl-3-oxobutanoyl)-1,3-oxazolidine (28)



General procedure B (from (2*RS*,5*S*)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine,[4] with α-methylacetoacetic acid[5]); yield 43% (506 mg); yellow solid; m.p. 93 °C. R_f (5% EtOAc in DCM) 0.22; $[\alpha]_D^{20}$ -16.6 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1744 (C=O), 1730 (C=O), 1656 (C=O); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 1.31 (3H, d, *J* 6.8, C(7)CH_3), 2.28 (3H, s, C(6)CH_3), 3.62 (1H, q, *J* 6.8, C(7)*H*), 3.81 (3H, s, CO_2CH_3), 4.06 (1H, t, *J* 8.8, C(4)H_AH_B), 4.52-4.58 (2H, m, C(4)H_AH_B + C(5)*H*), 5.35 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 12.9 (C(7)*C*H_3), 25.9 (C(*C*H_3)$_3$), 27.7 (C(6)*C*H_3), 37.9 (*C*(CH_3)$_3$), 53.0 (CO_2CH_3), 53.7 (*C*(7)), 59.6 (*C*(5)), 68.2 (*C*(4)), 97.0 (*C*(2)), 170.0 (CO_2CH_3), 172.5 (*C*(8)), 203.0 (*C*(6)); *m/z* (ESI^+) 286.1 (MH^+, 19%), 308.1 (MNa^+, 100%); HRMS (ESI^+) found 286.16519, $C_{14}H_{24}NO_5$ (MH^+) requires 286.16490.

(2*R*,5*S*)-1-(4-(Benzyloxy)-2-methyl-3-oxobutanoyl)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine (29)[6]



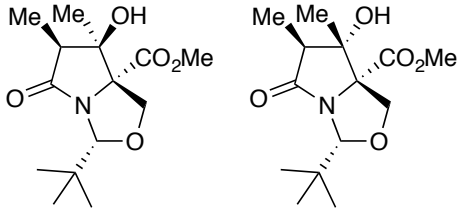
General procedure B (from (2*RS*,5*S*)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine,[4] with 4-benzyloxy-2-methyl-3-oxobutanoic acid[6]); yield 38% (2.26 g); colourless oil. R_f (20% EtOAc in petrol) 0.32; $[\alpha]_D^{20}$ -79.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 1731 (C=O), 1657 (C=O); δ_H (400 MHz, $CDCl_3$) 0.85 (9H, s, C(CH_3)$_3$), 1.43 (3H, d, *J* 6.8, C(7)CH_3), 3.25 (1H, t, *J* 7.8, C(4)H_AH_B), 3.76 (3H, s, CO_2CH_3), 3.78 (1H, q, *J* 6.8, C(7)*H*), 4.03 (1H, d, *J* 16.4, C(6)CH_AH_B), 4.13-4.17 (1H, m, C(4)H_AH_B), 4.15 (1H, d, *J* 16.4, C(6)CH_AH_B), 4.49 (2H, s, OCH_2Ph), 4.76-4.78 (1H, m, C(5)*H*), 5.32 (1H, s, C(2)*H*), 7.28-7.31 (2H, m, Ph), 7.33-7.38 (3H, m, Ph); δ_C (100 MHz, $CDCl_3$) 13.6 (C(7)*C*H_3), 25.7 (C(*C*H_3)$_3$), 37.6 (*C*(CH_3)$_3$), 50.7 (*C*(7)), 52.7 (CO_2CH_3), 60.2 (*C*(5)), 67.9 (*C*(4)), 74.2 (O*C*H_2Ph), 74.7 (C(6)*C*H_2), 96.2 (*C*(2)), 128.4, 128.7, 128.9, 136.5 (Ph), 170.5

(CO_2CH_3), 174.0 (*C*(8)), 204.5 (*C*(6)); *m/z* (ESI^+) 392.2 (MH^+, 74%), 414.2 (MNa^+, 100%); HRMS (ESI^+) found 392.20552, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

General procedure C: Aldol cyclisation of *N*-acyloxazolidines[7]

Sodium methoxide (1.05 eq) was added to a solution of *N*-acyloxazolidine (1.0 eq) in anhydrous MeOH (*c* 0.2), and stirring was continued at room temperature for 18 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography to give the bicyclic pyrrolidinone.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-6,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (30a) and (2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-6,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (30b)

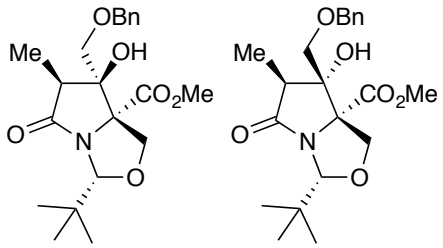


General procedure C (from **28**).

Major diastereomer (**(*R*)-30a**): Yield 55% (355 mg); white solid; m.p. 162 °C. R_f (40% EtOAc in petrol) 0.27; $[\alpha]_D^{20}$ +18.8 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3405 (O-H), 2955 (C-H), 1733 (C=O), 1692 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 7.2, C(7)CH_3), 1.26 (3H, s, C(6)CH_3), 2.24 (1H, br s, O*H*), 3.12 (1H, q, *J* 7.2, C(7)*H*), 3.78 (3H, s, CO_2CH_3), 4.29 (1H, d, *J* 8.9, C(4)H_AH_B), 4.52 (1H, d, *J* 8.9, C(4)H_AH_B), 4.92 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 6.6 (C(7)CH_3), 22.2 (C(6)CH_3), 25.1 (C(CH_3)$_3$), 36.7 ($C(CH_3)_3$), 48.9 (*C*(7)), 52.8 (CO_2CH_3), 68.3 (*C*(4)), 79.4 (*C*(5)), 80.2 (*C*(6)), 96.7 (*C*(2)), 172.0 (CO_2CH_3), 179.0 (*C*(8)); *m/z* (ESI^+) 286.2 (MH^+, 44%), 308.1 (MNa^+, 22%); HRMS (ESI^+) found 286.16494, $C_{14}H_{24}NO_5$ (MH^+) requires 286.16490.

Minor diastereomer (**(*S*)-30b**): Yield 23% (150 mg); white solid; m.p. 179 °C. R_f (40% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ +15.9 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3356 (O-H), 2959 (C-H), 1733 (C=O), 1686 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.08 (3H, d, *J* 7.5, C(7)CH_3), 1.23 (3H, s, C(6)CH_3), 2.31 (1H, br s, O*H*), 3.44 (1H, q, *J* 7.5, C(7)*H*), 3.81 (3H, s, CO_2CH_3), 3.96 (1H, d, *J* 9.5, C(4)H_AH_B), 4.62 (1H, d, *J* 9.5, C(4)H_AH_B), 4.90 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 7.9 (C(7)*C*H_3), 20.2 (C(6)*C*H_3), 25.1 (C(*C*H_3)$_3$), 36.7 (*C*(CH_3)$_3$), 49.1 (*C*(7)), 52.8 (CO_2CH_3), 69.1 (*C*(4)), 78.8 (*C*(5)), 80.7 (*C*(6)), 96.2 (*C*(2)), 171.9 (CO_2CH_3), 177.0 (*C*(8)); *m/z* (ESI^+) 286.2 (MH^+, 100%), 308.1 (MNa^+, 22%); HRMS (ESI^+) found 286.16497, $C_{14}H_{24}NO_5$ (MH^+) requires 286.16490.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (31a) and (2*R*,5*R*,6*S*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (31b)[6]



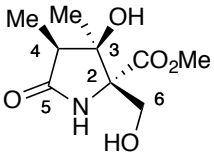
General procedure C (from **29**).

Major diastereomer (**(*R*)-31a**): Yield 59% (1.20 g); white solid; m.p. 116 °C (lit.[6] m.p. 100-102 °C). R_f (30% EtOAc in petrol) 0.36; $[\alpha]_D^{20}$ +27.8 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 3421 (O-H), 2961 (C-H), 1746 (C=O), 1704 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 7.0, C(7)CH_3), 2.91 (1H, d, *J* 1.0, O*H*), 3.20 (1H, q, *J* 7.0, C(7)*H*), 3.41 (1H, d, *J* 9.7, C(6)CH_AH_B), 3.46 (1H, d, *J* 9.7, C(6)CH_AH_B), 3.66 (3H, s, CO_2CH_3), 4.15 (1H, d, *J* 9.0, C(4)H_AH_B), 4.45 (1H, d, *J* 11.5, OCH_AH_BPh), 4.54 (1H, d, *J* 11.5, OCH_AH_BPh), 4.60 (1H, d, *J* 9.0, C(4)H_AH_B), 4.89 (1H, s, C(2)*H*), 7.29-7.39 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 7.4 (C(7)*C*H_3), 25.1 (C(*C*H_3)$_3$), 36.5 (*C*(CH_3)$_3$), 46.0 (*C*(7)), 52.7 (CO_2CH_3), 68.4 (*C*(4)), 71.4 (C(6)*C*H_2), 74.0 (O*C*H_2Ph), 78.2 (*C*(5)), 81.4 (*C*(6)), 96.3 (*C*(2)), 128.1, 128.4, 128.7, 136.9 (Ph), 171.7

(CO_2CH_3), 178.0 (*C*(8)); *m/z* (ESI^+) 392.2 (MH^+, 57%), 414.2 (MNa^+, 100%); HRMS (ESI^+) found 392.20681, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

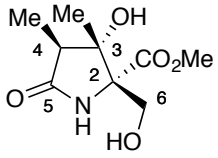
Minor diastereomer (**(*S*)-31b**): Yield 29% (592 mg); white solid; m.p. 52 °C. R_f (30% EtOAc in petrol) 0.26; $[\alpha]_D^{20}$ +22.3 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3392 (O-H), 2957 (C-H), 1744 (C=O), 1693 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.04 (3H, d, *J* 7.5, C(7)CH_3), 3.16 (1H, s, O*H*), 3.43 (2H, s, C(6)CH_2), 3.51 (1H, q, *J* 7.5, C(7)*H*), 3.67 (1H, d, *J* 9.5, C(4)H_AH_B), 3.79 (3H, s, CO_2CH_3), 4.50 (1H, d, *J* 11.5, OCH_AH_BPh), 4.58 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.74 (1H, d, *J* 9.5, C(4)H$_A$$H_B$), 4.78 (1H, s, C(2)*H*), 7.29-7.39 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 7.8 (C(7)CH_3), 25.1 (C(CH_3)$_3$), 36.3 (*C*(CH$_3$)$_3$), 47.4 (*C*(7)), 52.6 (CO_2CH_3), 69.3 (C(6)CH_2), 70.5 (*C*(4)), 74.1 (OCH_2Ph), 78.5 (*C*(5)), 80.5 (*C*(6)), 95.5 (*C*(2)), 128.2, 128.6, 128.9, 136.4 (Ph), 171.2 (CO_2CH_3), 175.7 (*C*(8)); *m/z* (ESI^+) 392.2 (MH^+, 63%), 414.2 (MNa^+, 100%); HRMS (ESI^+) found 392.20660, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

Methyl (2*R*,3*R*,4*S*)-3-hydroxy-2-hydroxymethyl-3,4-dimethyl-5-oxopyrrolidine-2-carboxylate (32a)



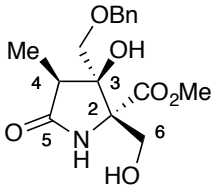
General procedure A (from **30a**, 1.5 eq of 1,3-propanedithiol, for 12 h); yield quant. (357 mg); colourless oil. R_f (10% MeOH in EtOAc) 0.37; $[\alpha]_D^{20}$ -41.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3342 (O-H), 2981 (C-H), 2945 (C-H), 1729 (C=O), 1684 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.2, C(4)CH_3), 1.26 (3H, s, C(3)CH_3), 2.41 (1H, q, *J* 7.2, C(4)*H*), 3.77 (3H, s, CO_2CH_3), 3.83 (1H, d, *J* 11.3, C(6)H_AH_B), 4.18 (1H, d, *J* 11.3, C(6)H$_A$$H_B$); δ_C (100 MHz, CD_3OD) 7.3 (C(4)CH_3), 22.9 (C(3)CH_3), 47.9 (*C*(4)), 53.0 (CO_2CH_3), 64.1 (*C*(6)), 75.5 (*C*(2)), 79.4 (*C*(3)), 173.7 (CO_2CH_3), 181.0 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 23%), 240.1 (MNa^+, 54%); HRMS (ESI^+) found 240.08436, $C_9H_{15}NNaO_5$ (MNa^+) requires 240.08534.

Methyl (2*R*,3*S*,4*S*)-3-hydroxy-2-hydroxymethyl-3,4-dimethyl-5-oxopyrrolidine-2-carboxylate (32b)



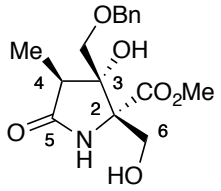
General procedure A (from **31b**, 1.5 eq of 1,3-propanedithiol, for 16 h); yield 92% (70 mg); white solid; m.p. 166 °C. R_f (10% MeOH in EtOAc) 0.30; $[\alpha]_D^{20}$ -31.6 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3373 (O-H or N-H), 3334 (O-H or N-H), 2960 (C-H), 1743 (C=O), 1661 (C=O); δ_H (400 MHz, CD_3OD) 1.12 (3H, d, *J* 7.6, C(4)CH_3), 1.28 (3H, s, C(3)CH_3), 2.47 (1H, q, *J* 7.6, C(4)*H*), 3.61 (1H, d, *J* 10.8, C(6)H_AH_B), 3.76 (3H, s, CO_2CH_3), 4.06 (1H, d, *J* 10.8, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 11.7 (C(4)CH_3), 19.0 (C(3)CH_3), 50.3 (*C*(4)), 52.7 (CO_2CH_3), 65.1 (*C*(6)), 76.5 (*C*(2)), 79.4 (*C*(3)), 172.4 (CO_2CH_3), 180.8 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 11%), 240.1 (MNa^+, 29%); HRMS (ESI^+) found 218.10251, $C_9H_{16}NO_5$ (MH^+) requires 218.10230.

Methyl (2*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylate (33a)[6]



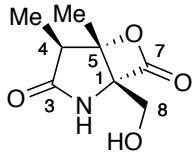
General procedure A (from **31a**, 1.5 eq of 1,3-propanedithiol, for 24 h, at rt); yield quant. (269 mg); white solid, m.p. 55 °C. R_f (10% MeOH in EtOAc) 0.46; $[\alpha]_D^{20}$ -51.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3324 (O-H, N-H), 2948 (C-H), 1731 (C=O), 1690 (C=O); δ_H (400 MHz, CD_3OD) 1.02 (3H, d, *J* 7.2, C(4)CH_3), 2.84 (1H, q, *J* 7.2, C(4)*H*), 3.35 (1H, d, *J* 9.6, C(3)CH_AH_B), 3.56 (3H, s, CO_2CH_3), 3.59 (1H, d, *J* 9.6, C(3)CH_AH_B), 3.83 (1H, d, *J* 11.2, C(6)H_AH_B), 4.21 (1H, d, *J* 11.2, C(6)H_AH_B), 4.41 (1H, d, *J* 11.9, OCH_AH_BPh), 4.50 (1H, d, *J* 11.9, OCH_AH_BPh), 7.26-7.35 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.4 (C(4)CH_3), 43.2 (*C*(4)), 53.0 (CO_2CH_3), 64.7 (*C*(6)), 70.9 (C(3)CH_2), 73.6 *C*(2), 74.2 (OCH_2Ph), 81.6 (*C*(3)), 128.7, 128.7, 129.4, 139.1 (Ph), 173.1 (CO_2CH_3), 180.8 (*C*(5)); *m/z* (ESI^+) 324.1 (MH^+, 30%), 346.1 (MNa^+, 100%); HRMS (ESI^+) found 324.14436, $C_{16}H_{22}NO_6$ (MH^+) requires 324.14416.

Methyl (2*R*,3*S*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylate (33b)



General procedure A (from **31b**, 5 eq of 1,3-propanedithiol, for 24 h, at rt); yield 81% (167 mg); white solid; m.p. 151 °C. R_f (10% MeOH in EtOAc) 0.45; $[\alpha]_D^{20}$ -34.5 (*c* 0.8, MeOH); ν_{max}/cm^{-1} 3361 (O-H, N-H), 2951 (C-H), 1688 (C=O); δ_H (400 MHz, CD_3OD) 1.18 (3H, d, *J* 7.6, C(4)CH_3), 2.65 (1H, q, *J* 7.6, C(4)*H*), 3.58 (1H, d, *J* 9.2, C(3)CH_AH_B), 3.64 (1H, d, *J* 9.2, C(3)CH_AH_B), 3.74 (1H, d, *J* 10.8, C(6)H_AH_B), 3.75 (3H, s, CO_2CH_3), 4.11 (1H, d, *J* 10.8, C(6)H_AH_B), 4.49 (1H, d, *J* 11.8, OCH_AH_BPh), 4.55 (1H, d, *J* 11.8, OCH_AH_BPh), 7.27-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 10.6 (C(4)*C*H_3), 48.2 (*C*(4)), 52.8 (CO_2CH_3), 63.9 (*C*(6)), 70.7 (C(3)*C*H_2), 74.4 (O*C*H_2Ph), 75.4 *C*(2), 80.6 (*C*(3)), 128.8, 128.9, 129.3, 139.0 (Ph), 172.3 (CO_2CH_3), 180.4 (*C*(5)); *m/z* (ESI^+) 324.1 (MH^+, 56%), 346.1 (MNa^+, 83%); HRMS (ESI^+) found 324.14444, $C_{16}H_{22}NO_6$ (MH^+) requires 324.14416.

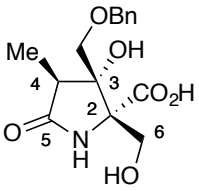
(1*R*,4*S*,5*S*)-1-Hydroxymethyl-4,5-dimethyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (34)



Sodium hydroxide (16 mg, 0.39 mmol) was added to a solution of ester **32b** (64 mg, 0.29 mmol) in 1:1:1 MeOH/THF/H_2O (3 mL). The mixture was stirred at room temperature for 2 h, acidified with 2 M aqueous HCl and concentrated *in vacuo*. The residue was dissolved in anhydrous DCM (5.9 mL), and triethylamine (82 µL, 0.59 mmol) and BOP chloride (152 mg, 0.597 mmol) were added. After stirring at room temperature for 15 h, brine was added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (EtOAc) to give fused-β-lactone **34** (8 mg, 0.04 mmol, 21%) as a white solid; m.p. 133 °C. R_f (EtOAc) 0.34; $[\alpha]_D^{20}$ -56.3 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3391 (O-H or N-H), 3229 (O-H or N-H), 2929 (C-H), 1825 (C=O), 1666 (C=O); δ_H (500 MHz, CD_3OD) 1.23 (3H, d, *J* 8.0, C(4)CH_3), 1.68 (3H, s, C(5)CH_3), 2.77 (1H, q, *J* 8.0, C(4)*H*), 3.81 (1H, d, *J* 12.6, C(8)H_AH_B), 3.95 (1H, d, *J* 12.6, C(8)H_AH_B); δ_C (125 MHz, CD_3OD) 13.2 (C(4)*C*H_3), 15.7 (C(5)*C*H_3), 46.5 (*C*(4)), 57.0 (*C*(8)), 79.1 (*C*(1)), 87.5 (*C*(5)),

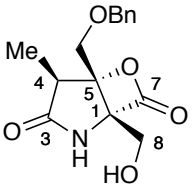
170.4 (*C*(7)), 180.4 (*C*(3)); *m/z* (ESI$^-$) 184.1 (M-H$^-$, 39%); HRMS (ESI$^+$) found 186.07628, $C_8H_{12}NO_4$ (MH$^+$) requires 186.07608.

(2*R*,3*S*,4*S*)-3-((Benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylic acid (47b)



NaOH (16 mg, 0.40 mmol) was added to a solution of methyl ester **33b** (101 mg, 0.312 mmol) in 1:1:1 MeOH/THF/H_2O (3.1 mL). The reaction mixture was stirred at room temperature for 2 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **47b** (97 mg, 0.31 mmol, quant.) as a white solid; m.p. 146 °C. $[\alpha]_D^{20}$ -32.9 (*c* 0.7, MeOH); ν_{max}/cm^{-1} 3306 (O-H, N-H), 2924 (C-H), 1684 (C=O); δ_H (500 MHz, CD_3OD) 1.19 (3H, d, *J* 7.6, C(4)CH_3), 2.67 (1H, q, *J* 7.6, C(4)*H*), 3.61 (1H, d, *J* 9.7, C(3)CH_AH$_B$), 3.68 (1H, d, *J* 9.7, C(3)CH$_A$$H_B$), 3.74 (1H, d, *J* 11.0, C(6)H_AH$_B$), 4.07 (1H, d, *J* 11.0, C(6)H$_A$$H_B$), 4.51 (1H, d, *J* 11.7, OCH_AH$_B$Ph), 4.56 (1H, d, *J* 11.7, OCH$_A$$H_B$Ph), 7.26-7.29 (1H, m, Ph), 7.31-7.37 (4H, m, Ph); δ_C (125 MHz, CD_3OD) 10.8 (C(4)CH_3), 48.6 (*C*(4)), 64.1 (*C*(6)), 70.9 (C(3)CH_2), 74.5 (OCH_2Ph), 75.1 *C*(2), 80.4 (*C*(3)), 128.8, 128.9, 129.4, 139.1 (Ph), 173.3 (CO_2H), 180.6 (*C*(5)); *m/z* (ESI$^-$) 308.1 (M-H$^-$, 100%); HRMS (ESI$^+$) found 310.12862, $C_{15}H_{20}NO_6$ (MH$^+$) requires 310.12851.

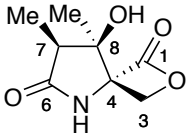
(1*R*,4*S*,5*S*)-5-((Benzyloxy)methyl)-1-hydroxymethyl-4-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (35)



Triethylamine (25 μL, 0.18 mmol) and BOPCl (49 mg, 0.19 mmol) were added to a solution of acid 4**7b** (27 mg, 0.088 mmol) in anhydrous DCM (1.8 mL). After stirring at room temperature for 14 h, the solution was diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography (70% EtOAc in petrol) to give fused-β-lactone **35** (16 mg, 0.056 mmol, 64%) as a white solid; m.p. 96 °C. R_f (70% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ -4.2 (*c* 0.4, MeOH); δ_H (500 MHz, CD_3OD) 1.26 (3H, d, *J* 8.0, C(4)CH_3), 2.82 (1H, q, *J* 8.0, C(4)*H*),

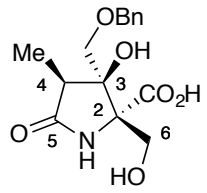
3.82 (1H, d, *J* 12.5, C(8)H_AH_B), 4.06 (1H, d, *J* 11.7, C(5)CH_AH_B), 4.07 (1H, d, *J* 12.5, C(8)H_AH_B), 4.09 (1H, d, *J* 11.7, C(5)H_AH_B), 4.60 (1H, d, *J* 11.7, OCH_AH_BPh), 4.65 (1H, d, *J* 11.7, OCH_AH_BPh), 7.27-7.36 (5H, m, Ph); δ_C (125 MHz, CD_3OD) 12.4 (C(4)CH_3), 45.1 (*C*(4)), 57.2 (*C*(8)), 68.3 (C(5)CH_2), 74.9 (OCH_2Ph), 79.1 (*C*(1)), 87.6 *C*(5), 128.9, 128.9, 129.5, 139.0 (Ph), 170.0 (*C*(7)), 180.0 (*C*(3)); *m/z* (ESI^+) 292.1 (MH^+, 33%), 314.1 (MNa^+, 100%); HRMS (ESI^+) found 292.11812, $C_{15}H_{18}NO_5$ (MH^+) requires 292.11795.

(4*R*,7*S*,8*R*)-8-Hydroxy-7,8-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (36)



Sodium hydroxide (21 mg, 0.53 mmol) was added to a solution of ester **32a** (90 mg, 0.41 mmol) in 1:1:1 MeOH/THF/H_2O (3.9 mL). The mixture was stirred at room temperature for 2 h, acidified with 2 M aqueous HCl and concentrated *in vacuo*. To a solution of the residue in anhydrous THF (14 mL) at 0 °C were added HATU (329 mg, 0.865 mmol) and DIPEA (288 µL, 1.65 mmol), and the mixture was stirred at room temperature for 32 h. Brine was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (30% to 80% EtOAc in petrol) to give spiro-β-lactone **36** (27 mg, 0.037 mmol, 9%) as a yellow oil, as a 1:4 inseparable mixture with tetramethylurea. R_f (EtOAc) 0.41; ν_{max}/cm^{-1} 2944 (C-H), 2885 (C-H), 1832 (C=O), 1720 (C=O); δ_H (500 MHz, CD_3OD) 1.10 (3H, d, *J* 7.2, C(7)CH_3), 1.42 (3H, s, C(8)CH_3), 2.48 (1H, q, *J* 7.2, C(7)*H*), 4.39 (1H, d, *J* 6.3, C(3)H_AH_B), 5.67 (1H, d, *J* 6.3, C(3)H_AH_B); δ_C (125 MHz, CD_3OD) 7.3 (C(7)CH_3), 20.9 (C(8)CH_3), 47.9 (*C*(7)), 67.7 (*C*(3)), 77.5 (*C*(8)), 81.8 (*C*(4)), 172.6 (*C*(1)), 180.1 (*C*(6)); HRMS (Cl^+) found 186.0761, $C_8H_{12}NO_4$ (MH^+) requires 186.0761.

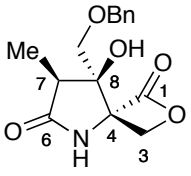
(2*R*,3*R*,4*S*)-3-((Benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylic acid (47a)[6]



NaOH (14 mg, 0.34 mmol) was added to a solution of methyl ester **33a** (95 mg, 0.29 mmol) in 1:1:1 MeOH/THF/H_2O (3 mL). The reaction mixture was stirred at room temperature for 24 h, then diluted with brine and washed with EtOAc. The

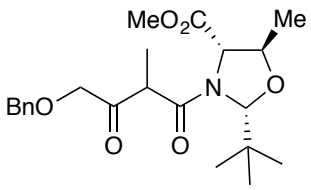
aqueous layer was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **47a** (83 mg, 0.27 mmol, 93%) as a white solid; m.p. 94 °C (lit.[6] m.p. 90-100 °C). $[\alpha]_D^{20}$ -44.2 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3321 (O-H, N-H), 2919 (C-H), 2955 (C-H), 1691 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.4, C(4)CH_3), 2.82 (1H, q, *J* 7.4, C(4)*H*), 3.46 (1H, d, *J* 10.0, C(3)CH_AH_B), 3.64 (1H, d, *J* 10.0, C(3)CH_AH_B), 3.88 (1H, d, *J* 11.2, C(6)H_AH_B), 4.22 (1H, d, *J* 11.2, C(6)H_AH_B), 4.43 (1H, d, *J* 11.9, OCH_AH_BPh), 4.55 (1H, d, *J* 11.9, OCH_AH_BPh), 7.23-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.8 (C(4)*C*H_3), 43.9 (*C*(4)), 64.8 (*C*(6)), 72.0 (C(3)*C*H_2), 73.4 (*C*(2)), 74.2 (O*C*H_2Ph), 81.4 (*C*(3)), 128.6, 128.7, 129.3, 139.3 (Ph), 174.3 (*C*O_2H), 180.9 (*C*(5)); *m/z* (ESI^-) 308.1 ($M\text{-}H^-$, 100%); HRMS (ESI^-) found 308.11430, $C_{15}H_{18}NO_6$ ($M\text{-}H^-$) requires 308.11396.

(4*R*,7*S*,8*R*)-8-((Benzyloxy)methyl)-8-hydroxy-7-methyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (37)



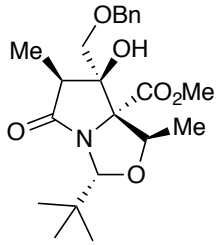
HATU (70 mg, 0.18 mmol) and DIPEA (64 μL, 0.37 mmol) were added to a solution of acid **47a** (28 mg, 0.091 mmol) in anhydrous THF (3 mL) at 0 °C. The mixture was stirred at room temperature for 33 h and quenched with brine. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (50% EtOAc in petrol) to give spiro-β-lactone **37** (23 mg, 0.080 mmol, 88%) as a white solid; m.p. 198 °C (deg). R_f (EtOAc) 0.56; $[\alpha]_D^{20}$ -27.1 (*c* 0.3, MeOH); ν_{max}/cm^{-1} 3448 (O-H or N-H), 3242 (O-H or N-H), 2902 (C-H), 2864 (C-H), 1813 (C=O), 1733 (C=O), 1675 (C=C); δ_H (500 MHz, CD_3OD) 1.09 (3H, d, *J* 7.3, C(7)CH_3), 2.54 (1H, q, *J* 7.3, C(7)*H*), 3.70 (1H, d, *J* 10.0, C(8)CH_AH_B), 3.73 (1H, d, *J* 10.0, C(8)CH_AH_B), 4.28 (1H, d, *J* 6.0, C(3)H_AH_B), 4.51 (1H, d, *J* 11.5, OCH_AH_BPh), 4.55 (1H, d, *J* 11.5, OCH_AH_BPh), 4.86 (1H, d, *J* 6.0, C(3)H_AH_B), 7.27 (1H, tt, *J* 7.0, 1.5, Ph), 7.31-7.38 (4H, m, Ph); δ_C (125 MHz, CD_3OD) 8.2 (C(7)*C*H_3), 44.8 (*C*(7)), 69.4 (*C*(3)), 74.2 (C(8)*C*H_2), 74.9 (O*C*H_2Ph), 79.6 (*C*(8)), 80.7 *C*(4), 128.8, 128.9, 129.3, 139.0 (Ph), 172.6 (*C*(1)), 179.5 (*C*(6)); *m/z* (ESI^+) 314.1 (MNa^+, 49%); HRMS (ESI^+) found 314.09986, $C_{15}H_{17}NNaO_5$ (MNa^+) requires 314.09989.

(2*R*,4*R*,5*S*)-1-(4-(Benzyloxy)-2-methyl-3-oxobutanoyl)-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (38)



General procedure B (from (2*RS*,4*R*,5*S*)-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-1,3-oxazolidine,[8] with 4-benzyloxy-2-methyl-3-oxobutanoic acid[6]); yield 28% (1.71 g); white solid; m.p. 124 °C. R_f (30% EtOAc in petrol) 0.18; $[\alpha]_D^{20}$ -13.0 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2975 (C-H), 2962 (C-H), 1748 (C=O), 1650 (C=O); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.33-1.36 (6H, m, C(4)CH_3 + C(7)CH_3), 3.62 (1H, q, *J* 6.9, C(7)*H*), 3.72 (3H, s, CO_2CH_3), 4.09 (1H, d, *J* 4.3, C(5)*H*), 4.28 (2H, s, OCH_2Ph), 4.58 (1H, d, *J* 11.9, C(6)CH_AH$_B$), 4.63 (1H, d, *J* 11.9, C(6)CH$_A$$H_B$), 4.73-4.78 (1H, m, C(4)*H*), 5.41 (1H, s, C(2)*H*), 7.29-7.36 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 13.2 (C(7)*C*H$_3$), 20.4 (C(4)*C*H$_3$), 26.1 (C(*C*H$_3$)$_3$), 38.3 (*C*(CH$_3$)$_3$), 49.6 (*C*(7)), 53.0 (CO_2CH_3), 65.9 (*C*(5)), 72.9 (O*C*H$_2$Ph), 73.5 (C(6)*C*H$_2$), 76.2 (*C*(4)), 96.5 (*C*(2)), 128.1, 128.3, 128.6, 137.3 (Ph), 169.6 (CO_2CH_3), 171.9 (*C*(8)), 202.5 (*C*(6)); *m/z* (ESI^+) 428.2 (MNa^+, 100%); HRMS (ESI^+) found 428.20411, $C_{22}H_{31}NNaO_6$ (MNa^+) requires 428.20436.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (39)



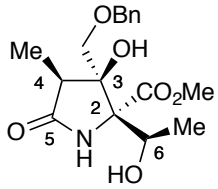
(2*R*,5*S*)-1-(4-(Benzyloxy)-2-methyl-3-oxobutanoyl)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine (29)[6]

General procedure B (from (2*RS*,5*S*)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine,[4] with 4-benzyloxy-2-methyl-3-oxobutanoic acid[6]); yield 38% (2.26 g); colourless oil. R_f (20% EtOAc in petrol) 0.32; $[\alpha]_D^{20}$ -79.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 1731 (C=O), 1657 (C=O); δ_H (400 MHz, $CDCl_3$) 0.85 (9H, s, C(CH_3)$_3$), 1.43 (3H, d, *J* 6.8, C(7)CH_3), 3.25 (1H, t, *J* 7.8, C(4)H_AH$_B$), 3.76 (3H, s, CO_2CH_3), 3.78 (1H, q, *J* 6.8, C(7)*H*), 4.03 (1H, d, *J* 16.4, C(6)CH_AH$_B$), 4.13-4.17 (1H, m, C(4)H$_A$$H_B$), 4.15 (1H, d, *J* 16.4, C(6)CH$_A$$H_B$), 4.49 (2H, s, OC$H_2$Ph), 4.76-4.78 (1H, m, C(5)*H*), 5.32 (1H, s, C(2)*H*), 7.28-7.31 (2H, m, Ph), 7.33-7.38 (3H, m, Ph); δ_C (100 MHz, $CDCl_3$) 13.6 (C(7)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.6 (*C*(CH$_3$)$_3$), 50.7 (*C*(7)), 52.7 (CO_2CH_3), 60.2 (*C*(5)), 67.9 (*C*(4)), 74.2 (O*C*H$_2$Ph), 74.7 (C(6)*C*H$_2$), 96.2 (*C*(2)), 128.4, 128.7, 128.9, 136.5 (Ph), 170.5 (CO_2CH_3), 174.0 (*C*(8)),

204.5 (*C*(6)); *m/z* (ESI^+) 392.2 (MH^+, 74%), 414.2 (MNa^+, 100%); HRMS (ESI^+) found 392.20552, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

General procedure C: (from **38**); yield 82% (1.29 g); white solid; m.p. 146 °C. R_f (30% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ -13.7 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3353 (O-H), 2959 (C-H), 1745 (C=O), 1690 (C=O); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.05 (3H, d, *J* 7.0, C(7)CH_3), 1.66 (3H, d, *J* 6.5, C(4)CH_3), 2.60 (1H, s, O*H*), 3.24 (1H, q, *J* 7.0, C(7)*H*), 3.47 (1H, d, *J* 10.0, C(6)CH_AH$_B$), 3.56 (1H, d, *J* 10.0, C(6)CH$_A$$H_B$), 3.60 (3H, s, CO_2CH_3), 4.44 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.72 (1H, q, *J* 6.5, C(4)*H*), 5.03 (1H, s, C(2)*H*), 7.27-7.32 (3H, m, Ph), 7.33-7.37 (2H, m, Ph); δ_C (125 MHz, $CDCl_3$) 6.7 (C(7)*C*H$_3$), 15.7 (C(4)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 37.5 (*C*(CH$_3$)$_3$), 45.6 (*C*(7)), 52.7 (CO_2CH_3), 70.1 (C(6)*C*H$_2$), 73.7 (O*C*H$_2$Ph), 77.6 (*C*(5)), 79.0 (*C*(4)), 85.5 (*C*(6)), 96.6 (*C*(2)), 127.8, 128.1, 128.6, 137.1 (Ph), 171.6 (*C*O$_2$CH$_3$), 180.5 (*C*(8)); *m/z* (ESI^+) 428.2 (MNa^+, 100%); HRMS (ESI^+) found 428.20403, $C_{22}H_{31}NNaO_6$ (MNa^+) requires 428.20546.

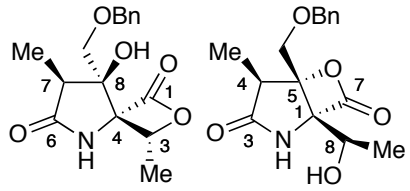
Methyl (2*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-((*R*)-1-hydroxyethyl)-4-methyl-5-oxopyrrolidine-2-carboxylate (40)



General procedure A (from **39**, 5 eq of 1,3-propanedithiol, for 28 h, at rt); yield quant. (314 mg); colourless oil. R_f (EtOAc) 0.30; $[\alpha]_D^{20}$ -51.1 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3337 (O-H, N-H), 2942 (C-H), 1690 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.4, C(4)CH_3), 1.25 (3H, d, *J* 6.5, C(6)CH_3), 2.85 (1H, q, *J* 7.4, C(4)*H*), 3.44 (1H, d, *J* 9.4, C(3)CH_AH$_B$), 3.55 (3H, s, CO_2CH_3), 3.80 (1H, d, *J* 9.4, C(3)CH$_A$$H_B$), 4.18 (1H, q, *J* 6.5, C(6)*H*), 4.43 (1H, d, *J* 11.8, OCH_AH$_B$Ph), 4.50 (1H, d, *J* 11.8, OCH$_A$$H_B$Ph), 7.26-7.36 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.9 (C(4)*C*H$_3$), 19.1 (C(6)*C*H$_3$), 43.4 (*C*(4)), 52.6 (CO_2CH_3), 70.8 (*C*(6)), 72.4 (C(3)*C*H$_2$), 74.1 (O*C*H$_2$Ph), 74.2 *C*(2), 81.7 (*C*(3)), 128.7, 128.7, 129.4, 139.1 (Ph), 173.2 (*C*O$_2$CH$_3$), 181.2 (*C*(5)); *m/z* (ESI^+) 338.2 (MH^+, 14%), 360.2 (MNa^+, 100%); HRMS (ESI^+) found 360.14172, $C_{17}H_{23}NNaO_6$ (MNa^+) requires 360.14286.

(3*R*,4*R*,7*S*,8*R*)-8-((Benzyloxy)methyl)-8-hydroxy-3,7-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (42) or

(1*R*,4*S*,5*S*)-5-((Benzyloxy)methyl)-1-((*R*)-1-hydroxyethyl)-4-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (43)

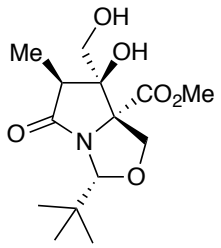


Method 1: NaOH (18 mg, 0.45 mmol) was added to a solution of methyl ester **40** (123 mg, 0.365 mmol) in 1:1:1 MeOH/THF/H_2O (3.6 mL). The reaction mixture was stirred at room temperature for 42 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. HATU (158 mg, 0.416 mmol) and DIPEA (71 µL, 0.41 mmol) were added to a solution of the crude acid in anhydrous THF (6.8 mL) at 0 °C. The mixture was stirred at room temperature for 36 h, diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (40% to 50% EtOAc in petrol) to give β-lactone **42** or **43** (21 mg, 0.069 mmol, 18%).

Method 2: NaOH (3 mg, 0.07 mmol) was added to a solution of methyl ester **40** (21 mg, 0.061 mmol) in 1:1:1 MeOH/THF/H_2O (600 µL). The reaction mixture was stirred at room temperature for 42 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in anhydrous DCM (820 µL), and triethylamine (11 µL, 0.079 mmol) and BOPCl (23 mg, 0.088 mmol) were added. After stirring at room temperature for 15 h, the solution was diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography (40% to 60% EtOAc in petrol) to give β-lactone **42** or **43** (3 mg, 0.01 mmol, 16%).

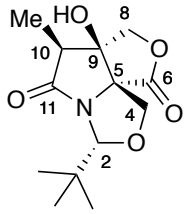
White solid; m.p. >250 °C (deg). R_f (80% EtOAc in petrol) 0.37; $[\alpha]_D^{20}$ -8.7 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3267 (O-H or N-H), 2874 (C-H), 1821 (C=O), 1708 (C=O); δ_H (500 MHz, CD_3OD) 1.09 (3H, d, *J* 7.0, C(7/4)CH_3), 1.47 (3H, d, *J* 6.5, C(3/8)CH_3), 2.53 (1H, q, *J* 7.0, C(7/4)*H*), 3.69 (1H, d, *J* 10.1, C(8/5)CH_AH$_B$), 3.72 (1H, d, *J* 10.1, C(8/5)CH$_A$$H_B$), 4.52 (1H, d, *J* 11.7, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.7, OCH$_A$$H_B$Ph), 4.25 (1H, q, *J* 6.5, C(3/8)*H*), 7.25-7.38 (5H, m, Ph); δ_C (125 MHz, CD_3OD) 8.4 (C(7/4)*C*H$_3$), 15.2 (C(3/8)*C*H$_3$), 44.8 (*C*(7/4)), 74.1 (C(8/5)*C*H$_2$), 74.8 (O*C*H$_2$Ph), 77.5 (*C*(3/8)), 79.5 (*C*(8/5), 81.1 (*C*(4/1)), 128.8, 128.9, 129.4, 139.0 (Ph), 172.8 (*C*(1/7)), 180.2 (*C*(6/3)); *m/z* (ESI$^+$) 306.1 (MH$^+$, 45%); HRMS (ESI$^+$) found 306.13358, $C_{16}H_{20}NO_5$ (MH$^+$) requires 306.13360.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-6-hydroxymethyl-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (45)



A suspension of benzyloxy **31a** (206 mg, 0.525 mmol) and 10% Pd/C (59 mg, 0.056 mmol) in methanol (7 mL) was stirred under H_2 for 14 h. The mixture was filtered through Celite and purified by *flash* column chromatography (40% to 60% EtOAc in petrol) to give alcohol **45** (147 mg, 0.488 mmol, 93%) as a colourless oil. R_f (60% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ +37.1 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 3437 (O-H), 2958 (C-H), 1696 (C=O); δ_H (400 MHz, $CDCl_3$) 0.86 (9H, s, C(CH_3)$_3$), 1.08 (3H, d, *J* 7.2, C(7)CH_3), 2.87 (1H, t, *J* 5.8, C(6)CH$_2$O*H*), 3.07 (1H, q, *J* 7.2, C(7)*H*), 3.56-3.62 (3H, m, C(6)CH_2 + C(6)O*H*), 3.80 (3H, s, CO$_2$CH_3), 4.21 (1H, d, *J* 8.9, C(4)H_AH$_B$), 4.52 (1H, d, *J* 8.9, C(4)H$_A$$H_B$), 4.88 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 7.3 (C(7)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 36.5 (*C*(CH$_3$)$_3$), 45.8 (*C*(7)), 53.1 (CO$_2$*C*H$_3$), 64.6 (C(6)*C*H$_2$), 68.9 (*C*(4)), 78.5 (*C*(5)), 82.7 (*C*(6)), 96.5 (*C*(2)), 172.9 (*C*O$_2$CH$_3$), 178.6 (*C*(8)); *m/z* (ESI$^+$) 302.1 (MH$^+$, 69%), 324.1 (MNa$^+$, 23%); HRMS (ESI$^+$) found 302.15992, $C_{14}H_{24}NO_6$ (MH$^+$) requires 302.15981.

(2*R*,5*R*,9*R*,10*S*)-1-Aza-2-(*tert*-butyl)-9-hydroxy-10-methyl-3,7-dioxa-6,11-dioxotricyclo[6.3.0.0^{5,9}]-undecane (46)



Side-product from the hydrogenation of benzyloxy **31a**; off-white solid; m.p. 227 °C. R_f (60% EtOAc in petrol) 0.47; $[\alpha]_D^{20}$ +27.9 (*c* 0.7, MeOH);

ν_{max}/cm^{-1} 3406 (O-H), 2954 (C-H), 1794 (C=O), 1689 (C=O); δ_H (500 MHz, $CDCl_3$) 1.03 (9H, s, C(CH_3)$_3$), 1.22 (3H, d, *J* 7.0, C(10)CH_3), 3.04 (1H, br s, C(9)O*H*), 3.08 (1H, q, *J* 7.0, C(10)*H*), 4.06 (1H, d, *J* 9.8, C(8)H_AH_B), 4.20 (1H, d, *J* 9.0, C(4)H_AH_B), 4.33 (1H, d, *J* 9.8, C(8)H_AH_B), 4.36 (1H, d, *J* 9.0, C(4)H_AH_B), 4.98 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 6.7 (C(10)CH_3), 25.6 (C(CH_3)$_3$), 36.4 ($C(CH_3)_3$), 47.4 (*C*(10)), 68.0 (*C*(4)), 71.0 (*C*(8)), 72.9 (*C*(5)), 82.2 (*C*(9)), 99.3 (*C*(2)), 173.5 (*C*(6)), 179.7 (*C*(11)); *m/z* (ESI^+) 292.1 (MNa^+, 70%); HRMS (ESI^-) found 268.11908, $C_{13}H_{18}NO_5$ ($M-H^-$) requires 268.11905.

Proteasome inhibition assay

H460 cells were obtained from the ATCC cell bank, and KMS-12-BM from the DSMZ cell bank. The medium used for H460 cells was DMEM (10% FBS + Pen Strep), and RPMI (20% FBS + Pen Strep) for KMS-12-BM cells.

Proteasome activity was measured via the Promega Proteasome-Glo™ Chymotrypsin-like Cell based assay kit (G8660), in two different cell lines (H460 and KMS-12-BM), in a 96-well white walled plate. For H460 cells, 7.5 x 10^4 cells/per well were seeded overnight before performing the assay the next day. For KMS-12-BM cells, 1.5 x 10^5 cells/per well were seeded on the day of the experiment. Compounds at the required concentration were added in triplicate wells for 30 minutes before the assay was started via the addition of Proteasome-Glo reagent. Luminescence readings were taken after 15 minutes using an OMEGA POLARstar plate reader. Readings were normalised as a percentage of a control value obtained in the absence of compound. Values represent the mean of three separate measurements.

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