

APPENDICES

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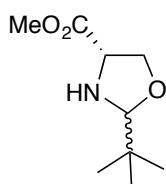
APPENDIX A: SYNTHESIS AND CHARACTERISATION OF REPORTED COMPOUNDS

The synthesis and characterisation of previously reported compounds, prepared using the reported methods, are indicated below.

General procedure P: Synthesis of oxazolidines and thiazolidines¹

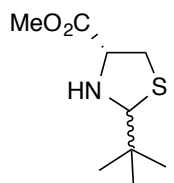
The methyl ester hydrochloride of the L-aminoacid (1.0 eq) was dissolved in petrol (*c* 0.3), and triethylamine (1.5 eq) and trimethylacetaldehyde (1.2 eq) were added. The mixture was heated at reflux with continuous removal of water using a Dean-Stark apparatus for 18 h, then cooled to room temperature, filtered and washed with Et₂O. The combined filtrates were concentrated under reduced pressure to give the oxazolidine or thiazolidine.

(2*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1,3-oxazolidine (**42a**) and (2*S*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1,3-oxazolidine (**42'a**)²

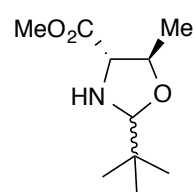


General procedure P (from L-serine methyl ester hydrochloride); yield 97% (5.85 g); yellow oil; 1.4:1 mixture of diastereomers. *R_f* (85% EtOAc in DCM) 0.64; $\nu_{\max}/\text{cm}^{-1}$ 3316 (N-H), 2955 (C-H), 1740 (C=O); δ_{H} (400 MHz, CDCl₃) major diastereomer (**42a**): 0.98 (9H, s, C(CH₃)₃), 2.61 (1H, br s, NH), 3.68-3.73 (1H, m, C(4)*H_AH_B*), 3.76 (3H, s, CO₂CH₃), 3.88-3.97 (2H, m, C(4)*H_AH_B* + C(5)*H*), 4.07 (1H, s, C(2)*H*); minor diastereomer (**42'a**): 0.91 (9H, s, C(CH₃)₃), 2.61 (1H, br s, NH), 3.68-3.73 (1H, m, C(4)*H_AH_B*), 3.74 (3H, s, CO₂CH₃), 3.88-3.97 (1H, m, C(5)*H*), 4.11 (1H, t, *J* 7.6, C(4)*H_AH_B*), 4.32 (1H, s, C(2)*H*); δ_{C} (100 MHz, CDCl₃) major diastereomer (**42a**): 25.2 (C(CH₃)₃), 33.2 (C(CH₃)₃), 52.5 (CO₂CH₃), 59.6 (C(4)), 68.3 (C(5)), 99.9 (C(2)), 172.9 (CO₂CH₃); minor diastereomer (**42'a**): 24.9 (C(CH₃)₃), 34.6 (C(CH₃)₃), 52.4 (CO₂CH₃), 59.4 (C(4)), 68.9 (C(5)), 99.2 (C(2)), 173.1 (CO₂CH₃); *m/z* (ESI⁺) 188.2 (MH⁺, 100%), 210.1 (MNa⁺, 15%); HRMS (ESI⁺) found 188.1285, C₉H₁₈NO₃ (MH⁺) requires 188.1281.

(2*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1,3-thiazolidine (42b) and (2*S*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1,3-thiazolidine (42'b)³


 General procedure P (from L-cysteine methyl ester hydrochloride); yield 98% (2.32 g); yellow oil; 1.5:1 mixture of diastereomers. *R_f* (75% EtOAc in DCM) 0.61; $\nu_{\max}/\text{cm}^{-1}$ 3317 (N-H), 2954 (C-H), 1740 (C=O); δ_{H} (400 MHz, CDCl₃) major diastereomer (**42b**): 1.08 (9H, s, C(CH₃)₃), 2.50 (1H, br s, NH), 2.65-2.72 (1H, m, C(4)*H_AH_B*), 3.23-3.30 (1H, m, C(4)*H_AH_B*), 3.78 (3H, s, CO₂CH₃), 3.79-3.85 (1H, m, C(5)*H*), 4.47 (1H, s, C(2)*H*); minor diastereomer (**42'b**): 0.98 (9H, s, C(CH₃)₃), 2.50 (1H, br s, NH), 3.00-3.06 (1H, m, C(4)*H_AH_B*), 3.09-3.16 (1H, m, C(4)*H_AH_B*), 3.76 (3H, s, CO₂CH₃), 4.13-4.17 (1H, m, C(5)*H*), 4.54 (1H, s, C(2)*H*); δ_{C} (100 MHz, CDCl₃) major diastereomer (**42b**): 27.1 (C(CH₃)₃), 34.0 (C(CH₃)₃), 37.4 (C(4)), 52.4 (CO₂CH₃), 65.4 (C(5)), 81.8 (C(2)), 171.8 (CO₂CH₃); minor diastereomer (**42'b**): 26.6 (C(CH₃)₃), 35.9 (C(CH₃)₃), 37.0 (C(4)), 52.4 (CO₂CH₃), 65.0 (C(5)), 79.8 (C(2)), 172.4 (CO₂CH₃); *m/z* (ESI⁺) 204.1 (MH⁺, 100%); HRMS (ESI⁺) found 226.0880, C₉H₁₇NNaO₂S (MNa⁺) requires 226.0872.

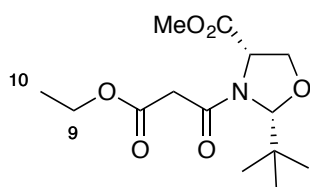
(2*R*,4*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (42c) and (2*S*,4*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (42'c)⁴


 General procedure P (from L-threonine methyl ester hydrochloride); yield 77% (1.84 g); yellow oil; 1:1 mixture of diastereomers. *R_f* (50% EtOAc in DCM) 0.64; $\nu_{\max}/\text{cm}^{-1}$ 3315 (N-H), 2957 (C-H), 1741 (C=O); δ_{H} (400 MHz, CDCl₃) **42c**: 1.00 (9H, s, C(CH₃)₃), 1.36 (3H, d, *J* 6.0, C(4)CH₃), 2.73 (1H, br s, NH), 3.50 (1H, d, *J* 6.8, C(5)*H*), 3.78 (3H, s, CO₂CH₃), 3.85-3.90 (1H, m, C(4)*H*), 4.32 (1H, s, C(2)*H*); **42'c**: 0.93 (9H, s, C(CH₃)₃), 1.40 (3H, d, *J* 6.0, C(4)CH₃), 2.73 (1H, br s, NH), 3.39 (1H, d, *J* 7.2, C(5)*H*), 3.80 (3H, s, CO₂CH₃), 3.81-3.85 (1H, m, C(4)*H*), 4.41 (1H, s, C(2)*H*); δ_{C} (100 MHz, CDCl₃) **42c**: 19.9 (C(4)CH₃), 24.8 (C(CH₃)₃), 34.6 (C(CH₃)₃), 52.4 (CO₂CH₃), 65.7 (C(5)), 76.2 (C(4)), 98.8 (C(2)), 171.9 (CO₂CH₃); **42'c**: 18.9 (C(4)CH₃), 25.2 (C(CH₃)₃), 33.5 (C(CH₃)₃), 52.3 (CO₂CH₃), 67.1 (C(5)), 77.3 (C(4)), 98.2 (C(2)), 172.7 (CO₂CH₃); *m/z* (ESI⁺) 202.1 (MH⁺, 100%); HRMS (ESI⁺) found 224.1266, C₁₀H₁₉NNaO₃ (MNa⁺) requires 224.1257.

General procedure Q: *N*-Acylation of oxazolidines and thiazolidines¹

To a solution of oxazolidine or thiazolidine (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 ethyl hydrogen malonate (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 or thiazolidine.

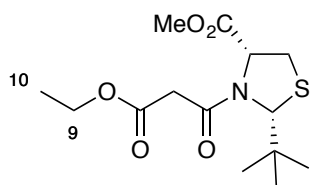
(2*R*,5*S*)-2-(*tert*-Butyl)-1-ethoxycarbonylacetyl-5-methoxycarbonyl-1,3-oxazolidine (43a)¹



General procedure Q (from **42a**); yield 83% (1.68 g); colourless oil.

R_f (10% EtOAc in DCM) 0.52; $[\alpha]_D^{20}$ -52.8 (*c* 1.3, DCM); $\nu_{\max}/\text{cm}^{-1}$ 2960 (C-H), 1739 (C=O), 1672 (C=O); δ_H (400 MHz, CDCl_3) 0.91 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.30 (3H, t, *J* 7.1, $\text{C}(10)\text{H}_3$), 3.52 (1H, d, *J* 15.6, $\text{C}(7)\text{H}_\text{A}\text{H}_\text{B}$), 3.71 (1H, d, *J* 15.6, $\text{C}(7)\text{H}_\text{A}\text{H}_\text{B}$), 3.81 (3H, s, CO_2CH_3), 3.97-4.05 (1H, m, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 4.22 (2H, q, *J* 7.1, $\text{C}(9)\text{H}_2$), 4.54-4.63 (1H, m, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 4.70-4.76 (1H, m, $\text{C}(5)\text{H}$), 5.33 (1H, s, $\text{C}(2)\text{H}$); δ_C (100 MHz, CDCl_3) 14.2 ($\text{C}(10)$), 25.7 ($\text{C}(\text{CH}_3)_3$), 37.4 ($\text{C}(\text{CH}_3)_3$), 43.2 ($\text{C}(7)$), 52.9 (CO_2CH_3), 59.6 ($\text{C}(5)$), 61.8 ($\text{C}(9)$), 67.8 ($\text{C}(4)$), 96.8 ($\text{C}(2)$), 167.4 ($\text{C}(8)$), 167.6 (CO_2CH_3), 170.0 ($\text{C}(6)$); *m/z* (ESI⁺) 302.2 (MH^+ , 33%), 324.1 (MNa^+ , 100%); HRMS (ESI⁺) found 324.1423, $\text{C}_{14}\text{H}_{23}\text{NNaO}_6$ (MNa^+) requires 324.1418.

(2*R*,5*R*)-2-(*tert*-Butyl)-1-ethoxycarbonylacetyl-5-methoxycarbonyl-1,3-thiazolidine (43b)⁵

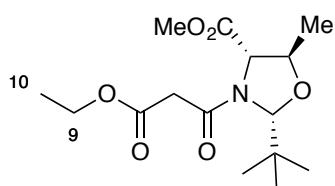


General procedure Q (from **42b**); yield 75% (2.78 g); colourless oil.

R_f (10% EtOAc in DCM) 0.71; $[\alpha]_D^{20}$ -105.7 (*c* 1.0, CHCl_3); $\nu_{\max}/\text{cm}^{-1}$ 2958 (C-H), 1742 (C=O), 1663 (C=O); δ_H (400 MHz, CDCl_3 , 1.5:1 mixture of rotamers, major A and minor B): 1.00 (9H, s, $\text{C}(\text{CH}_3)_3$ A), 1.09 (9H, s, $\text{C}(\text{CH}_3)_3$ B), 1.30 (3H, t, *J* 7.2, $\text{C}(10)\text{H}_3$), 3.25-3.59 (4H, m, $\text{C}(4)\text{H}_2 + \text{C}(7)\text{H}_2$), 3.77 (3H, s, CO_2CH_3 B), 3.83 (3H, s, CO_2CH_3 A), 4.22 (2H, q, *J* 7.2, $\text{C}(9)\text{H}_2$), 4.82 (1H, s, $\text{C}(2)\text{H}$ B), 4.91 (1H, t, *J* 7.8, $\text{C}(5)\text{H}$ A), 5.13 (1H, t, *J* 9.0, $\text{C}(5)\text{H}$ B), 5.56 (1H, s, $\text{C}(2)\text{H}$ A); δ_C (100 MHz, CDCl_3) 14.1 ($\text{C}(10)$), 26.9 ($\text{C}(\text{CH}_3)_3$ A), 27.1

(C(CH₃)₃ B), 32.9 (C(4) B), 34.2 (C(4) A), 39.4 (C(CH₃)₃ A), 40.1 (C(CH₃)₃ B), 42.4 (C(7)), 52.5 (CO₂CH₃ B), 53.0 (CO₂CH₃ A), 61.7 (C(9)), 64.2 (C(5)), 73.3 (C(2) A), 74.7 (C(2) B), 166.8, 166.9, 167.0 (C(8), CO₂CH₃ A, B), 170.6 (C(6) B), 170.8 (C(6) A); *m/z* (ESI⁺) 318.1 (MH⁺, 52%); HRMS (ESI⁺) found 340.1182, C₁₄H₂₃NNaO₅S (MNa⁺) requires 340.1189.

(2*R*,4*R*,5*S*)-2-(*tert*-Butyl)-1-ethoxycarbonylacetyl-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (43c)⁴



General procedure Q (from **42c**); yield 63% (753 mg); yellow oil.

*R*_f (10% EtOAc in DCM) 0.44; [α]_D²⁰ -41.3 (*c* 1.0, CHCl₃);

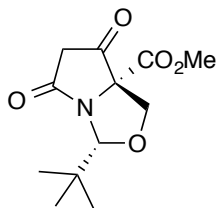
ν_{max} /cm⁻¹ 2979 (C-H), 2956 (C-H), 1740 (C=O), 1670 (C=O); δ_{H} (500

MHz, CDCl₃) 0.92 (9H, s, C(CH₃)₃), 1.30 (3H, t, *J* 7.2, C(10)H₃), 1.38 (3H, d, *J* 6.0, C(4)CH₃), 3.45 (1H, d, *J* 15.5, C(7)H_AH_B), 3.59 (1H, d, *J* 15.5, C(7)H_AH_B), 3.82 (3H, s, CO₂CH₃), 4.22 (2H, q, *J* 7.2, C(9)H₂), 4.29-4.34 (1H, m, C(5)H), 4.74-4.83 (1H, m, C(4)H), 5.45 (1H, s, C(2)H); δ_{C} (100 MHz, CDCl₃) 14.1 (C(10)), 20.1 (C(4)CH₃), 25.8 (C(CH₃)₃), 37.9 (C(CH₃)₃), 42.8 (C(7)), 52.8 (CO₂CH₃), 61.7 (C(9)), 65.5 (C(5)), 76.1 (C(4)), 96.2 (C(2)), 167.3, 167.4 (C(6), C(8)), 170.0 (CO₂CH₃); *m/z* (ESI⁺) 316.2 (MH⁺, 28%), 338.2 (MNa⁺, 100%); HRMS (ESI⁺) found 338.1587, C₁₅H₂₅NNaO₆ (MNa⁺) requires 338.1574.

General procedure R: Dieckmann cyclisation of *N*-acyloxazolidines and thiazolidines¹

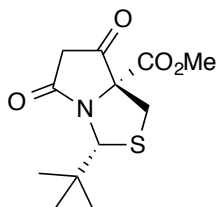
A solution of *N*-acyloxazolidine or thiazolidine (1.0 eq) and potassium *tert*-butoxide (1.05 eq) in anhydrous THF (*c* 0.2) was heated at reflux for 3-8 h. The reaction mixture was then separated between Et₂O and water, the aqueous phase was acidified with 2 M aqueous HCl and extracted with EtOAc. The organic layer was washed with 1 M aqueous NaH₂PO₄ and brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give the bicyclic methyl ester tetramic acid.

(2R,5R)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (44a)¹



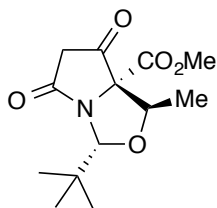
General procedure R (from **43a**, for 3 h); yield 74% (4.38 g); yellow solid; m.p. 131-133 °C (lit.¹ m.p. 115-120 °C). R_f (25% MeOH in EtOAc) 0.20; $[\alpha]_D^{20} +105.5$ (c 1.1, DCM); $\nu_{\max}/\text{cm}^{-1}$ 2958 (C-H), 1744 (C=O); δ_H (500 MHz, CDCl_3) 0.90 (9H, s, $\text{C}(\text{CH}_3)_3$), 3.16 (1H, d, J 21.0, $\text{C}(7)H_AH_B$), 3.62 (1H, d, J 9.0, $\text{C}(4)H_AH_B$), 3.73 (1H, dd, J 21.0, 0.6, $\text{C}(7)H_AH_B$), 3.82 (3H, s, CO_2CH_3), 4.79 (1H, d, J 9.0, $\text{C}(4)H_AH_B$), 5.07 (1H, s, $\text{C}(2)H$); δ_C (125 MHz, CDCl_3) 24.8 ($\text{C}(\text{CH}_3)_3$), 35.7 ($\text{C}(\text{CH}_3)_3$), 44.9 ($\text{C}(7)$), 53.9 (CO_2CH_3), 68.0 ($\text{C}(4)$), 80.7 ($\text{C}(5)$), 98.4 ($\text{C}(2)$), 167.0 (CO_2CH_3), 172.4 ($\text{C}(8)$), 198.4 ($\text{C}(6)$); m/z (ESI^+) 256.1 (MH^+ , 24%), 278.1 (MNa^+ , 67%); HRMS (ESI^+) found 278.0997, $\text{C}_{12}\text{H}_{17}\text{NNaO}_5$ (MNa^+) requires 278.0999; 96% ee (OJ-H column, *n*-hexane/*i*-PrOH (98:2), flow rate 1.0 mL/min, τ_{major} 18.39 min, τ_{minor} 31.77 min).

(2R,5R)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (44b)⁵



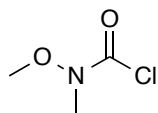
General procedure R (from **43b**, for 3 h); yield 66% (327 mg); yellow solid; m.p. 114-116 °C (lit.⁵ m.p. 109 °C). R_f (20% MeOH in EtOAc) 0.24; $[\alpha]_D^{20} +153.3$ (c 1.0, DCM); $\nu_{\max}/\text{cm}^{-1}$ 2960 (C-H), 1782 (C=O), 1749 (C=O), 1709 (C=O); δ_H (500 MHz, CDCl_3) 0.95 (9H, s, $\text{C}(\text{CH}_3)_3$), 3.01 (1H, d, J 11.5, $\text{C}(4)H_AH_B$), 3.13 (1H, d, J 21.4, $\text{C}(7)H_AH_B$), 3.61 (1H, dd, J 21.4, 0.5, $\text{C}(7)H_AH_B$), 3.78 (1H, d, J 11.5, $\text{C}(4)H_AH_B$), 3.81 (3H, s, CO_2CH_3), 5.32 (1H, s, $\text{C}(2)H$); δ_C (100 MHz, CDCl_3) 26.5 ($\text{C}(\text{CH}_3)_3$), 34.0 ($\text{C}(4)$), 37.3 ($\text{C}(\text{CH}_3)_3$), 43.0 ($\text{C}(7)$), 54.0 (CO_2CH_3), 73.8 ($\text{C}(2)$), 85.0 ($\text{C}(5)$), 167.2 (CO_2CH_3), 170.7 ($\text{C}(8)$), 199.6 ($\text{C}(6)$); m/z (ESI^-) 270.2 (M-H^- , 100%); HRMS (ESI^-) found 270.0807, $\text{C}_{12}\text{H}_{16}\text{NO}_4\text{S}$ (M-H^-) requires 270.0806; 100% ee (OJ-H column, *n*-hexane/*i*-PrOH (98:2), flow rate 1.0 mL/min, τ_{major} 24.03 min, τ_{minor} 39.68 min).

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (44c**)⁴**



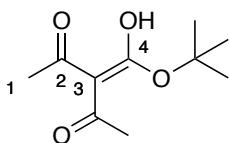
General procedure R (from **43c**, for 8 h); yield 69% (1.26 g); yellow solid; m.p. 132-134 °C (lit.⁶ m.p. 145-147 °C). R_f (50% EtOAc in DCM) 0.23; $[\alpha]_D^{20} +50.0$ (c 1.0, DCM); $\nu_{\max}/\text{cm}^{-1}$ 2960 (C-H), 1749 (C=O), 1725 (C=O); δ_H (400 MHz, CDCl_3) 0.89 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.04 (3H, d, J 6.8, $\text{C}(4)\text{CH}_3$), 3.14 (1H, d, J 21.7, $\text{C}(7)\text{H}_\text{A}\text{H}_\text{B}$), 3.63 (1H, dd, J 21.7, 0.9, $\text{C}(7)\text{H}_\text{A}\text{H}_\text{B}$), 3.79 (3H, s, CO_2CH_3), 5.08 (1H, q, J 6.8, $\text{C}(4)\text{H}$), 5.11 (1H, s, $\text{C}(2)\text{H}$); δ_C (100 MHz, CDCl_3) 15.2 ($\text{C}(4)\text{CH}_3$), 24.8 ($\text{C}(\text{CH}_3)_3$), 35.6 ($\text{C}(\text{CH}_3)_3$), 45.3 ($\text{C}(7)$), 53.7 (CO_2CH_3), 75.1 ($\text{C}(4)$), 83.7 ($\text{C}(5)$), 96.0 ($\text{C}(2)$), 167.3 (CO_2CH_3), 173.0 ($\text{C}(8)$), 199.5 ($\text{C}(6)$); m/z (ESI^+) 270.1 (MH^+ , 100%), 292.1 (MNa^+ , 35%); HRMS (ESI^+) found 292.1158, $\text{C}_{13}\text{H}_{19}\text{NNaO}_5$ (MNa^+) requires 292.1155; 100% ee (OJ-H column, *n*-hexane/*i*-PrOH (99:1), flow rate 0.8 mL/min, τ_{major} 19.46 min, τ_{minor} 25.83 min).

Methoxy(methyl)carbamic chloride (80**)^{7,8}**



Dry pyridine (9.4 mL, 120 mmol) was added dropwise to a suspension of triphosgene (5.75 g, 19.4 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (5.91 g, 60.6 mmol) in anhydrous DCM (58 mL) at -78 °C. The mixture was allowed to cool to room temperature and stirred for 16 h. The reaction mixture was diluted with water and extracted with Et_2O , washed with saturated aqueous NaHCO_3 and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give carbamic chloride **80** (6.36 g, 51.5 mmol, 89%) as a yellow oil along with 1,3-dimethoxy-1,3-dimethylurea **88**. $\nu_{\max}/\text{cm}^{-1}$ 1722 (C=O); δ_H (400 MHz, CDCl_3) 3.35 (3H, s, NCH_3), 3.80 (3H, s, NOCH_3); δ_C (100 MHz, CDCl_3) 36.2 (NCH_3), 61.6 (NOCH_3), 127.2 (CO).

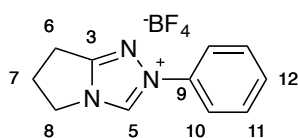
3-(*tert*-butoxy(hydroxy)methylene)pentane-2,4-dione (83'**)⁹**



Pyridine (195 μL , 2.42 mmol) was added dropwise at 0 °C to a solution of magnesium bromide (228 mg, 1.24 mmol) and *tert*-butyl acetoacetate (200 μL , 1.21 mmol) in anhydrous DCM (2 mL), and stirring was continued at 0 °C for 15 min. Acetyl chloride (90 μL , 1.3 mmol) was then added dropwise and the mixture

was stirred at rt for 18 h. 2 M aqueous HCl was then added at 0 °C until the precipitate dissolved. The layers were separated and the aqueous phase was extracted with Et₂O, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (10% EtOAc in petrol) to give **83'** as a light yellow oil (152 mg, 0.759 mmol, 63%). R_f (10% EtOAc in petrol) 0.69; $\nu_{\text{max}}/\text{cm}^{-1}$ 2978 (C-H), 1704 (C=O); δ_{H} (400 MHz, CDCl₃) 1.53 (9H, s, C(CH₃)₃), 2.32 (6H, s, C(1)H₃); δ_{C} (100 MHz, CDCl₃) 25.8 (C(1)), 28.4 (C(CH₃)₃), 81.6 (C(CH₃)₃), 110.6 (C(3)), 166.6 (C(4)), 195.5 (C(2)); m/z (ESI⁺) 223.1 (MNa⁺, 100%); HRMS (ESI⁺) found 223.0950, C₁₀H₁₆NaO₄ (MNa⁺) requires 223.0941.

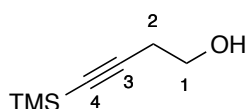
2-Phenyl-6,7-dihydro-5H-pyrrolo[2,1-*c*][1,2,4]triazol-2-ium tetrafluoroborate (**89**)¹⁰



2-Pyrrolidinone (470 μ L, 6.16 mmol) was added to a suspension of trimethyloxonium tetrafluoroborate (1.02 g, 6.96 mmol) in anhydrous DCM (35 mL). After stirring at room temperature for 15 h,

phenylhydrazine (610 μ L, 6.19 mmol) was added and stirred for 2 d, then concentrated *in vacuo*. The residue was dissolved in anhydrous methanol (3.5 mL) and triethyl orthoformate (14 mL), and the mixture was stirred at 100 °C for 21 h. The resultant solid was filtered, washed with MeOH and recrystallised from MeOH to give triazolium salt **89** (504 mg, 1.85 mmol, 30%) as a brown solid; m.p. 170 °C (lit.¹⁰ m.p. 154-156 °C). $\nu_{\text{max}}/\text{cm}^{-1}$ 2981 (C-H), 1601 (C=N), 1589 (C=N); δ_{H} (400 MHz, (CD₃)₂SO) 2.76 (2H, quint, *J* 7.6, C(7)H₂), 3.21 (2H, t, *J* 7.6, C(6)H₂), 4.42 (2H, t, *J* 7.6, C(8)H₂), 7.61-7.65 (1H, m, C(12)H), 7.68-7.72 (2H, m, C(11)H), 7.87-7.90 (2H, m, C(10)H), 10.69 (1H, s, C(5)H); δ_{C} (100 MHz, (CD₃)₂SO) 21.3 (C(6)), 26.6 (C(7)), 46.9 (C(8)), 120.7 (C(10)), 130.3 (C(11)), 130.4 (C(12)), 135.6, 138.4 (C(5), C(9)), 163.0 (C(3)); m/z (ESI⁺) 186.0 (M-BF₄⁺, 100%); HRMS (ESI⁺) found 186.10225, C₁₁H₁₂N₃ (M-BF₄⁺) requires 186.10257.

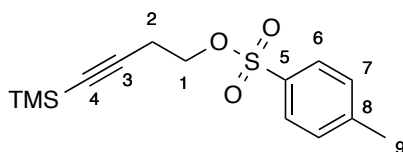
4-(Trimethylsilyl)but-3-yn-1-ol (**149**)¹¹



To a solution of 3-butyn-1-ol (200 μ L, 2.64 mmol) in anhydrous THF (26 mL) was added *n*-BuLi (1.54 M in hexanes, 3.8 mL, 5.9 mmol) dropwise

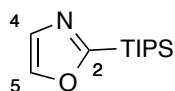
at -78 °C. The reaction mixture was stirred at this temperature for 2 h, and trimethylsilyl chloride (740 μ L, 5.83 mmol) was added dropwise. The solution was then stirred at 0 °C for 1 h, aqueous HCl (2 M, 740 μ L, 1.48 mmol) was added dropwise and stirring was continued for another 45 min. The layers were separated and the organic phase was washed with saturated aqueous NaHCO₃, brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (2% MeOH in DCM) to give protected alkyne **149** (282 mg, 1.98 mmol, 75%) as a colourless oil. R_f (2% MeOH in DCM) 0.44; $\nu_{\max}/\text{cm}^{-1}$ 3339 (O-H), 2959 (C-H), 2177 (C $\equiv$ C); δ_H (400 MHz, CDCl₃) 0.15 (9H, s, Si(CH₃)₃), 1.95 (1H, s, OH), 2.49 (2H, t, J 6.3, C(2)H₂), 3.70 (2H, t, J 6.3, C(1)H₂); δ_C (100 MHz, CDCl₃) 0.2 (Si(CH₃)₃), 24.4 (C(2)), 61.0 (C(1)), 87.1 (C(4)), 103.5 (C(3)); m/z (ESI⁺) 165.1 (MNa⁺, 53%); HRMS (ESI⁺) found 165.07063, C₇H₁₄NaOSi (MNa⁺) requires 165.07061.

4-(Trimethylsilyl)but-3-yn-1yl *p*-toluenesulfonate (**150**)¹²



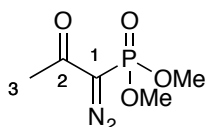
n-Buli (1.54 M in hexanes, 860 μ L, 1.32 mmol) was added dropwise to a solution of alcohol **149** (181 mg, 1.27 mmol) in anhydrous THF (1 mL) at -78 °C. After stirring at -78 °C for 1.5 h, *p*-toluenesulfonyl chloride (271 mg, 1.42 mmol) in anhydrous THF (800 μ L) was added dropwise and the mixture was stirred at room temperature for 6 h. The reaction was then quenched with water and extracted with Et₂O. The combined organic layers were washed with saturated aqueous NaHCO₃ and brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give *p*-toluenesulfonate **150** (358 mg, 1.21 mmol, 95%) as a colourless oil. $\nu_{\max}/\text{cm}^{-1}$ 2960 (C-H), 2181 (C $\equiv$ C), 1598 (C=C), 1362 (S=O); δ_H (400 MHz, CDCl₃) 0.11 (9H, s, Si(CH₃)₃), 2.45 (3H, s, C(9)H₃), 2.59 (2H, t, J 7.2, C(2)H), 4.08 (2H, t, J 7.2, C(1)H), 7.35 (2H, d, J 8.2, C(7)H), 7.80 (2H, d, J 8.2, C(6)H); δ_C (100 MHz, CDCl₃) 0.0 (Si(CH₃)₃), 20.9 (C(2)), 21.8 (C(9)), 67.7 (C(1)), 87.6 (C(4)), 100.4 (C(3)), 128.1 (C(6)), 130.0 (C(7)), 133.0 (C(8)), 145.1 (C(5)); m/z (ESI⁺) 297.0 (MH⁺, 6%), 319.1 (MNa⁺, 100%); HRMS (ESI⁺) found 319.07947, C₁₄H₂₀NaO₃SSi (MNa⁺) requires 319.08001.

2-(Triisopropylsilyl)oxazole (**142**)¹³



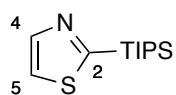
n-Buli (1.6 M in hexanes, 4.0 mL, 6.4 mmol) was added dropwise to a solution of oxazole (400 μ L, 6.08 mmol) in anhydrous THF (20 mL) at -30 °C. TIPS triflate (1.7 mL, 6.3 mL) was then added dropwise at 0 °C and the solution was stirred at room temperature for 30 min. The reaction mixture was quenched with saturated aqueous NH_4Cl , extracted with Et_2O , dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (3% to 5% EtOAc in petrol) to give protected oxazole **142** (1.20 g, 5.33 mmol, 88%) as a colourless oil. R_f (5% EtOAc in petrol) 0.39; $\nu_{\text{max}}/\text{cm}^{-1}$ 2945 (C-H), 2868 (C-H), 1462 (C=C); δ_{H} (400 MHz, CDCl_3) 1.12 (18H, d, J 7.1, $\text{SiCH}(\text{CH}_3)_2$), 1.30-1.48 (3H, m, $\text{SiCH}(\text{CH}_3)_2$), 7.20 (1H, d, J 1.0, C(4)*H*), 7.81 (1H, d, J 1.0, C(5)*H*); δ_{C} (100 MHz, CDCl_3) 11.1 ($\text{SiCH}(\text{CH}_3)_2$), 18.5 ($\text{SiCH}(\text{CH}_3)_2$), 126.7 (C(4)), 140.6 (C(5)), 168.8 (C(2)); m/z (ESI⁺) 226.2 (MH^+ , 100%); HRMS (ESI⁺) found 226.16226, $\text{C}_{12}\text{H}_{24}\text{NOSi}$ (MH^+) requires 226.16217.

Dimethyl (1-diazo-2-oxopropyl)phosphonate, Bestmann reagent (**156**)¹⁴



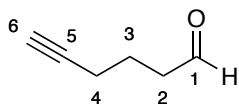
NaH (60% w/w, 334 mg, 8.34 mmol) was added portionwise to a solution of dimethyl (2-oxopropyl)phosphonate (1.0 mL, 7.2 mmol) in anhydrous THF (6.6 mL) and anhydrous toluene (36 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 1 h and 4-acetamidobenzenesulfonyl azide (1.93 g, 8.04 mmol) was added portionwise. The reaction mixture was then stirred at room temperature for 17 h. The suspension was filtered through Celite, washed with toluene and EtOAc, and the filtrate was concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (EtOAc) to give Bestmann reagent **156** (1.11 g, 5.81 mmol, 80%) as a yellow oil. R_f (EtOAc) 0.39; $\nu_{\text{max}}/\text{cm}^{-1}$ 2119 (C=N=N), 1655 (C=O); δ_{H} (400 MHz, CDCl_3) 2.26 (3H, s, C(3)*H*₃), 3.84 (6H, d, J 12.0, OCH_3); δ_{C} (125 MHz, CDCl_3) 27.3 (C(3)), 53.7 (d, J 5.6, OCH_3), 190.0 (d, J 13.1, C(2)); m/z (ESI⁺) 193.1 (MH^+ , 42%), 215.0 (MNa^+ , 41%), 407.0 ($[\text{2M}+\text{Na}]^+$, 100%); HRMS (ESI⁺) found 193.03748, $\text{C}_5\text{H}_{10}\text{N}_2\text{O}_4\text{P}$ (MH^+) requires 193.03727.

2-(Triisopropylsilyl)thiazole (162)¹⁵



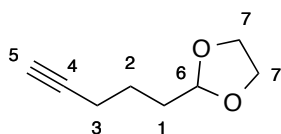
n-Buli (1.6 M in hexanes, 4.8 mL, 7.7 mmol) was added dropwise to a solution of thiazole (500 μ L, 7.05 mmol) in anhydrous THF (11 mL) at -78 °C. After stirring at this temperature for 1 h, TIPS chloride (1.6 mL, 7.5 mL) was added and the solution was stirred at room temperature for 1 h. The reaction mixture was diluted with H₂O, extracted with Et₂O, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (2% EtOAc in petrol) to give protected thiazole **162** (1.61 g, 6.65 mmol, 94%) as an orange oil. *R*_f (2% EtOAc in petrol) 0.19; ν_{max} /cm⁻¹ 2943 (C-H), 2866 (C-H), 1462 (C=C); δ_{H} (400 MHz, CDCl₃) 1.13 (18H, d, *J* 7.5, SiCH(CH₃)₂), 1.46 (3H, sept, *J* 7.5, SiCH(CH₃)₂), 7.54 (1H, d, *J* 2.8, C(5)*H*), 8.16 (1H, d, *J* 2.8, C(4)*H*); δ_{C} (100 MHz, CDCl₃) 11.8 (SiCH(CH₃)₂), 18.6 (SiCH(CH₃)₂), 121.1 (C(5)), 145.5 (C(4)), 169.9 (C(2)); *m/z* (ESI⁺) 242.1 (MH⁺, 100%); HRMS (ESI⁺) found 242.13941, C₁₂H₂₄NSSi (MH⁺) requires 242.13932.

Hex-5-ynal (413)¹⁶



To a suspension of PCC (5.92 g, 27.5 mmol) in anhydrous DCM (90 mL) was added 5-hexyn-1-ol (2.0 mL, 18 mmol) dropwise at 0 °C. After stirring at room temperature for 20 h, Et₂O was added and the reaction mixture was filtered through Celite. The solvent was evaporated *in vacuo* and the residue was distilled under reduced pressure to give hex-5-ynal **413** (856 mg, 8.91 mmol, 49%) as a colourless oil; b.p. 60-65 °C at 55 Torr (lit.¹⁶ 49-50 °C at 15 Torr). δ_{H} (400 MHz, CDCl₃) 1.85 (2H, quint, *J* 7.1, C(3)*H*₂), 1.97 (1H, t, *J* 2.7, C(6)*H*), 2.26 (2H, td, *J* 7.1, 2.7, C(4)*H*₂), 2.60 (2H, td, *J* 7.1, 1.3, C(2)*H*₂), 9.80 (1H, t, *J* 1.3, C(1)*H*); δ_{C} (100 MHz, CDCl₃) 17.9 (C(4)), 20.9 (C(3)), 42.7 (C(2)), 69.5 (C(6)), 83.3 (C(5)), 201.9 (C(1)); HRMS (FI⁺) found 96.0551, C₆H₈O (M⁺) requires 96.0575.

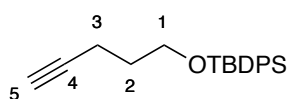
2-(Pent-4-yn-1-yl)-1,3-dioxolane (170)¹⁷



To a solution of aldehyde **413** (856 mg, 8.91 mmol) in anhydrous DCM (30 mL) were added ethylenedioxybis(trimethylsilane) (2.4 mL,

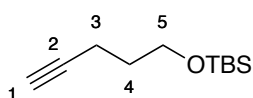
9.8 mmol) and trimethylsilyl triflate (322 μL , 1.78 mmol) at $-78\text{ }^{\circ}\text{C}$. The reaction mixture was stirred at this temperature for 1 h, saturated aqueous NaHCO_3 was added and the mixture was extracted with Et_2O . The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (10% Et_2O in petrol) to give acetal **170** (1.07 mg, 7.66 mmol, 86%) as a colourless oil. R_f (10% Et_2O in petrol) 0.24; $\nu_{\text{max}}/\text{cm}^{-1}$ 3290 (C-H), 2881 (C-H), 2116 ($\text{C}\equiv\text{C}$); δ_{H} (400 MHz, CDCl_3) 1.61-1.69 (2H, m, $\text{C}(2)\text{H}_2$), 1.75-1.80 (2H, m, $\text{C}(1)\text{H}_2$), 1.95 (1H, t, J 2.6, $\text{C}(5)\text{H}$), 2.24 (2H, td, J 7.0, 2.6, $\text{C}(3)\text{H}_2$), 3.80-3.88 (2H, m, $\text{C}(7)\text{H}_2$), 3.91-4.00 (2H, m, $\text{C}(7)\text{H}_2$), 4.87 (1H, t, J 4.6, $\text{C}(6)\text{H}$); δ_{C} (100 MHz, CDCl_3) 18.4 ($\text{C}(3)$), 23.0 ($\text{C}(2)$), 32.8 ($\text{C}(1)$), 65.0 ($\text{C}(7)$), 68.7 ($\text{C}(5)$), 84.2 ($\text{C}(4)$), 104.2 ($\text{C}(6)$); HRMS (CI^+) found 141.0911, $\text{C}_8\text{H}_{13}\text{O}_2$ (MH^+) requires 141.0910.

tert-Butyl(pent-4-yn-1-yloxy)diphenylsilane (**180**)¹⁸



To a solution of pent-4-yn-1-ol (500 μL , 5.37 mmol) in anhydrous DCM (11 mL) were added imidazole (476 mg, 6.99 mmol) and TBDPSCI (1.5 mL, 5.8 mmol) dropwise at $0\text{ }^{\circ}\text{C}$. After stirring at room temperature for 1 h, H_2O was added and the aqueous layer was extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by *flash* column chromatography (2% EtOAc in petrol) to give silyl ether **180** (1.69 g, 5.23 mmol, 97%) as a colourless oil. R_f (2% EtOAc in petrol) 0.36; $\nu_{\text{max}}/\text{cm}^{-1}$ 3307 (C-H), 2931 (C-H), 2858 (C-H), 2119 ($\text{C}\equiv\text{C}$); δ_{H} (200 MHz, CDCl_3) 1.05 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 1.78 (2H, tt, J 7.1, 6.0, $\text{C}(2)\text{H}_2$), 1.92 (1H, t, J 2.6, $\text{C}(5)\text{H}$), 2.35 (2H, td, J 7.1, 2.6, $\text{C}(3)\text{H}_2$), 3.74 (2H, t, J 6.0, $\text{C}(1)\text{H}_2$), 7.32-7.44 (6H, m, Ph), 7.64-7.70 (4H, m, Ph); δ_{C} (100 MHz, CDCl_3) 15.1 ($\text{C}(3)$), 19.4 ($\text{SiC}(\text{CH}_3)_3$), 27.0 ($\text{SiC}(\text{CH}_3)_3$), 31.6 ($\text{C}(2)$), 62.4 ($\text{C}(1)$), 68.4 ($\text{C}(5)$), 84.4 ($\text{C}(4)$), 127.8, 129.7, 133.9, 135.7 (Ph); m/z (ESI^+) 323.1 (MH^+ , 21%); HRMS (ESI^+) found 323.18280, $\text{C}_{21}\text{H}_{27}\text{OSi}$ (MH^+) requires 323.18257.

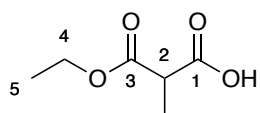
tert-Butyldimethyl(pent-4-yn-1-yloxy)silane (**180'**)¹⁹



To a solution of pent-4-yn-1-ol (1.4 mL, 15 mmol) in anhydrous DCM (33 mL) were added imidazole (1.34 g, 19.7 mmol) and TBSCl (2.51 g,

16.7 mmol) dropwise at 0 °C. After stirring at room temperature for 1.5 h, H₂O was added and the aqueous layer was extracted with DCM, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by *flash* column chromatography (2% Et₂O in petrol) to give silyl ether **180'** (2.95 g, 14.9 mmol, 99%) as a colourless oil. R_f (2% Et₂O in petrol) 0.50; $\nu_{\max}/\text{cm}^{-1}$ 3314 (C-H), 2954 (C-H), 2930 (C-H), 2121 (C $\equiv$ C); δ_{H} (400 MHz, CDCl₃) 0.05 (6H, s, Si(CH₃)₂), 0.89 (9H, s, OSi(CH₃)₃), 1.72 (2H, tt, *J* 7.0, 6.0, C(4)H₂), 1.93 (1H, t, *J* 2.7, C(1)H), 2.27 (2H, td, *J* 7.0, 2.7, C(3)H₂), 3.69 (2H, t, *J* 6.0, C(5)H₂); δ_{C} (100 MHz, CDCl₃) -5.2 (OSi(CH₃)₂), 15.0 (C(3)), 18.5 (OSi(CH₃)₃), 26.1 (OSi(CH₃)₃), 31.7 (C(4)), 61.6 (C(5)), 68.4 (C(1)), 84.4 (C(2)); HRMS (CI⁺) found 199.1516, C₁₁H₂₃OSi (MH⁺) requires 199.1513.

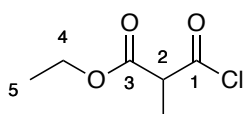
Ethyl α -methylmalonic acid (**294**)²⁰



Diethylmethylmalonate (9.0 mL, 53 mmol) was added to a solution of KOH (2.99 g, 53.3 mmol) in EtOH (55 mL) at 0 °C. The mixture was stirred at

room temperature for 2 h and refluxed for 30 min. The solution was cooled to room temperature, filtered and concentrated under reduced pressure to give a white solid. Water was then added, washed with petrol and acidified with HCl at 0 °C. The suspension was then extracted with Et₂O, washed with brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give acid **294** (5.63 g, 38.6 mmol, 73%) as a colourless oil. $\nu_{\max}/\text{cm}^{-1}$ 3700-2400 (O-H), 2990 (C-H), 2945 (C-H), 1712 (C=O); δ_{H} (400 MHz, CDCl₃) 1.28 (3H, t, *J* 7.1, C(5)H₃), 1.45 (3H, d, *J* 7.3, C(2)CH₃), 3.47 (1H, q, *J* 7.3, C(2)H₃), 4.21 (2H, q, *J* 7.1, C(4)H₂), 10.47 (1H, br s, CO₂H); δ_{C} (100 MHz, CDCl₃) 13.7 (C(2)CH₃), 14.1 (C(5)), 46.1 (C(2)), 61.9 (C(4)), 170.0 (C(3)), 176.0 (C(1)); *m/z* (ESI⁺) 147.1 (MH⁺, 42%), 169.1 (MNa⁺, 100%); HRMS (ESI⁺) found 147.06506, C₆H₁₁O₄ (MH⁺) requires 147.06519.

Ethyl α -methylmalonyl chloride (**295**)²⁰

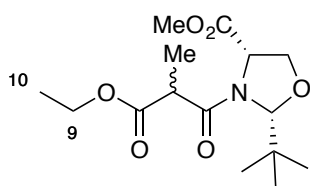


Thionyl chloride (5.6 mL, 76 mmol) was added dropwise to acid **294** (5.58 g, 38.2 mmol) at 0 °C and the mixture was stirred at 40 °C for 24 h.

Excess thionyl chloride was removed *in vacuo* to give malonyl chloride **295** (6.10 g, 37.0 mmol, 97%) as an orange oil, which was used in the following step without further purification.

δ_{H} (400 MHz, CDCl_3) 1.30 (3H, t, J 7.0, $\text{C}(5)\text{H}_3$), 1.52 (3H, d, J 7.2, $\text{C}(2)\text{CH}_3$), 3.84 (1H, q, J 7.2, $\text{C}(2)\text{H}$), 4.21-4.28 (2H, m, $\text{C}(4)\text{H}_2$); δ_{C} (100 MHz, CDCl_3) 13.9 ($\text{C}(5)$), 14.0 ($\text{C}(2)\text{CH}_3$), 57.0 ($\text{C}(2)$), 62.3 ($\text{C}(4)$), 167.4 ($\text{C}(3)$), 170.7 ($\text{C}(1)$).

(2*R*,5*S*)-2-(*tert*-Butyl)-1-((*R*)-1-ethoxycarbonyl-1-methylacetyl)-5-methoxycarbonyl-1,3-oxazolidine (296**) and (2*R*,5*S*)-2-(*tert*-Butyl)-1-((*S*)-1-ethoxycarbonyl-1-methylacetyl)-5-methoxycarbonyl-1,3-oxazolidine (**296'**)¹**

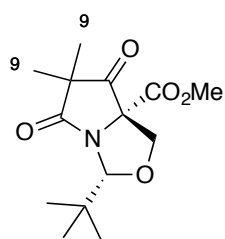


A solution of malonyl chloride **295** (6.64 g, 40.3 mmol) in anhydrous DCM (8 mL) was added to a solution of oxazolidine **42a** (3.78 g, 20.2 mmol) and dry pyridine (3.4 mL, 42 mmol) in anhydrous DCM (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 6 h, it was then washed with saturated aqueous NH_4Cl and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (20% to 40% EtOAc in petrol) to afford *N*-acyloxazolidine **296** as a 1:1.5 mixture of diastereomers *7R* and *7S* (6.37 g, 20.2 mmol, quant.), which were only separated for characterisation purposes and were assigned by comparison to reported characterisation by M. D. Andrews *et al.*¹

Major diastereomer (**(S)-296'**): Orange oil. R_{f} (40% EtOAc in petrol) 0.62; $[\alpha]_{\text{D}}^{20}$ -70.0 (c 1.0 in DCM); $\nu_{\text{max}}/\text{cm}^{-1}$ 2979 (C-H), 2959 (C-H), 1745 (C=O), 1672 (C=O); δ_{H} (400 MHz, CDCl_3) 0.87 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.23 (3H, t, J 7.0, $\text{C}(10)\text{H}_3$), 1.45 (3H, d, J 6.9, $\text{C}(7)\text{CH}_3$), 3.77 (1H, q, J 6.9, $\text{C}(7)\text{H}$), 3.79 (3H, s, CO_2CH_3), 3.95 (1H, t, J 7.8, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 4.15 (2H, q, J 7.0, $\text{C}(9)\text{H}_2$), 4.56 (1H, dd, J 7.8, 0.8, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 4.90 (1H, dd, J 7.8, 0.8, $\text{C}(5)\text{H}$), 5.33 (1H, s, $\text{C}(2)\text{H}$); δ_{C} (100 MHz, CDCl_3) 14.2 ($\text{C}(10)$), 14.5 ($\text{C}(7)\text{CH}_3$), 25.7 ($\text{C}(\text{CH}_3)_3$), 37.3 ($\text{C}(\text{CH}_3)_3$), 45.8 ($\text{C}(7)$), 53.0 (CO_2CH_3), 59.5 ($\text{C}(5)$), 61.8 ($\text{C}(9)$), 67.7 ($\text{C}(4)$), 96.6 ($\text{C}(2)$), 170.2, 170.3, 171.9 ($\text{C}(6)$, $\text{C}(8)$, CO_2CH_3); m/z (ESI⁺) 316.1 (MH^+ , 16%), 338.2 (MNa^+ , 100%); HRMS (ESI⁺) found 338.15659, $\text{C}_{15}\text{H}_{25}\text{NNaO}_6$ (MNa^+) requires 338.15741.

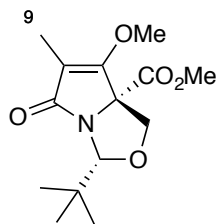
Minor diastereomer (**(R)**-**296**): Orange oil. R_f (40% EtOAc in petrol) 0.49; $[\alpha]_D^{20}$ -13.3 (c 1.0 in DCM); $\nu_{\max}/\text{cm}^{-1}$ 2980 (C-H), 2958 (C-H), 1738 (C=O), 1667 (C=O); δ_H (500 MHz, CDCl_3) 0.91 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.24 (3H, t, J 7.2, $\text{C}(10)\text{H}_3$), 1.37-1.42 (3H, m, $\text{C}(7)\text{CH}_3$), 3.63-3.70 (1H, m, $\text{C}(7)\text{H}$), 3.75 (3H, s, CO_2CH_3), 3.93-4.00 (1H, m, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 4.13-4.20 (2H, m, $\text{C}(9)\text{H}_2$), 4.47-4.53 (2H, m, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$ + $\text{C}(5)\text{H}$), 5.33 (1H, s, $\text{C}(2)\text{H}$); δ_C (125 MHz, CDCl_3) 13.8 ($\text{C}(7)\text{CH}_3$), 14.2 ($\text{C}(10)$), 25.7 ($\text{C}(\text{CH}_3)_3$), 37.7 ($\text{C}(\text{CH}_3)_3$), 46.0 ($\text{C}(7)$), 52.8 (CO_2CH_3), 59.5 ($\text{C}(5)$), 61.5 ($\text{C}(9)$), 68.2 ($\text{C}(4)$), 96.8 ($\text{C}(2)$), 169.7, 170.4, 171.6 ($\text{C}(6)$, $\text{C}(8)$, CO_2CH_3); m/z (ESI^+) 316.1 (MH^+ , 8%), 338.2 (MNa^+ , 100%); HRMS (ESI^+) found 338.15664, $\text{C}_{15}\text{H}_{25}\text{NNaO}_6$ (MNa^+) requires 338.15741.

(2R,5R)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7,7-dimethyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (52)²¹



Potassium *tert*-butoxide (2.21 g, 19.7 mmol) was added to a solution of *N*-acyloxazolidine **296** (5.61 g, 17.8 mmol) in anhydrous THF (90 mL). The reaction mixture was heated at reflux for 3 h, methyl iodide (4.4 mL, 71 mmol) was added dropwise and refluxed for another 1 h. The mixture was cooled to room temperature, quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by *flash* column chromatography (DCM) to give the *gem*-dimethyl tetramate **52** (2.74 g, 79.7 mmol, 55%) as a colourless oil. R_f (DCM) 0.50; $[\alpha]_D^{20}$ +100.4 (c 1.0 in DCM); $\nu_{\max}/\text{cm}^{-1}$ 2961 (C-H), 1779 (C=O), 1748 (C=O), 1720 (C=O); δ_H (400 MHz, CDCl_3) 0.93 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.26 (3H, s, $\text{C}(9_\text{A})\text{H}_3$), 1.51 (3H, s, $\text{C}(9_\text{B})\text{H}_3$), 3.44 (1H, d, J 8.8, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 3.83 (3H, s, CO_2CH_3), 4.85 (1H, d, J 8.8, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 5.05 (1H, s, $\text{C}(2)\text{H}$); δ_C (100 MHz, CDCl_3) 19.7 ($\text{C}(9_\text{A})$), 23.9 ($\text{C}(9_\text{B})$), 24.7 ($\text{C}(\text{CH}_3)_3$), 35.3 ($\text{C}(\text{CH}_3)_3$), 51.1 ($\text{C}(7)$), 53.6 (CO_2CH_3), 68.9 ($\text{C}(4)$), 78.5 ($\text{C}(5)$), 99.0 ($\text{C}(2)$), 167.2 (CO_2CH_3), 180.7 ($\text{C}(8)$), 205.7 ($\text{C}(6)$); m/z (ESI^+) 284.1 (MH^+ , 72%), 306.1 (MNa^+ , 100%); HRMS (ESI^+) found 306.13127, $\text{C}_{14}\text{H}_{21}\text{NNaO}_5$ (MNa^+) requires 306.13119.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-oct-6-ene (297)²¹



Side-product (15%, 750 mg) from the Dieckmann cyclisation of oxazolidine

296; white solid; m.p. 135 °C. R_f (DCM) 0.27; $[\alpha]_D^{20} +175.9$ (c 0.9, CHCl_3);

$\nu_{\text{max}}/\text{cm}^{-1}$ 2959 (C-H), 2919 (C-H), 1738 (C=O), 1711 (C=O), 1662 (C=C);

δ_H (500 MHz, CDCl_3) 0.90 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.94 (3H, s, $\text{C}(9)\text{H}_3$), 3.37 (1H, d,

J 8.4, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 3.77 (3H, s, CO_2CH_3), 3.99 (3H, s, $\text{C}(6)\text{OCH}_3$), 4.67 (1H, s, $\text{C}(2)\text{H}$), 4.79 (1H, d, J 8.4,

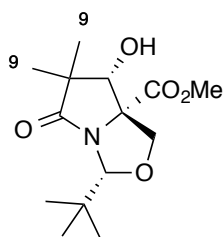
$\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$); δ_C (125 MHz, CDCl_3) 8.5 ($\text{C}(9)$), 24.8 ($\text{C}(\text{CH}_3)_3$), 35.2 ($\text{C}(\text{CH}_3)_3$), 53.2 (CO_2CH_3), 59.3

($\text{C}(6)\text{OCH}_3$), 70.1 ($\text{C}(4)$), 74.0 ($\text{C}(5)$), 96.9 ($\text{C}(2)$), 104.9 ($\text{C}(7)$), 167.7 ($\text{C}(6)$), 169.4 (CO_2CH_3), 179.8

($\text{C}(8)$); m/z (ESI⁺) 284.1 (MH^+ , 14%), 306.0 (MNa^+ , 100%); HRMS (ESI⁺) found 284.14931,

$\text{C}_{14}\text{H}_{22}\text{NO}_5$ (MH^+) requires 284.14925.

(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (303)²¹



NaBH_4 (331 mg, 8.74 mmol) was added portionwise to a solution of tetramate

52 (2.28 g, 8.06 mmol) in EtOH (81 mL) at 0 °C. After stirring at this

temperature for 1 h, saturated aqueous NH_4Cl was added and the organic

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 (20% to 30% EtOAc in petrol) to give alcohol **303** (1.15 g, 4.04 mmol, 47%) as a

white crystalline solid; m.p. 162-164 °C (lit.²¹ m.p. 148 °C). R_f (40% EtOAc in petrol) 0.30;

$[\alpha]_D^{20} +34.5$ (c 1.0, DCM); $\nu_{\text{max}}/\text{cm}^{-1}$ 3434 (O-H), 2960 (C-H), 1744 (C=O), 1693 (C=O); δ_H (500 MHz,

CDCl_3) 0.88 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.21 (3H, s, $\text{C}(9_\text{A})\text{H}_3$), 1.34 (3H, s, $\text{C}(9_\text{B})\text{H}_3$), 2.88 (1H, d, J 6.8, OH), 3.35

(1H, d, J 9.0, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$), 3.79 (3H, s, CO_2CH_3), 4.10 (1H, d, J 6.8, $\text{C}(6)\text{H}$), 4.84 (1H, s, $\text{C}(2)\text{H}$), 4.91

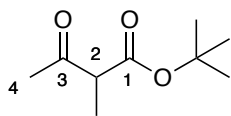
(1H, d, J 9.0, $\text{C}(4)\text{H}_\text{A}\text{H}_\text{B}$); δ_C (100 MHz, CDCl_3) 19.8 ($\text{C}(9_\text{B})$), 24.7 ($\text{C}(9_\text{A})$), 25.0 ($\text{C}(\text{CH}_3)_3$), 35.7

($\text{C}(\text{CH}_3)_3$), 49.6 ($\text{C}(7)$), 52.7 (CO_2CH_3), 75.5 ($\text{C}(4)$), 75.6 ($\text{C}(5)$), 80.0 ($\text{C}(6)$), 97.0 ($\text{C}(2)$), 171.1

(CO_2CH_3), 182.8 ($\text{C}(8)$); m/z (ESI⁺) 286.1 (MH^+ , 49%), 308.1 (MNa^+ , 100%); HRMS (ESI⁺) found

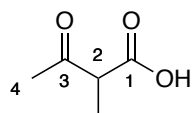
286.16462, $\text{C}_{14}\text{H}_{24}\text{NO}_5$ (MH^+) requires 286.16490.

***tert*-Butyl α -methylacetoacetate (**416**)⁶**



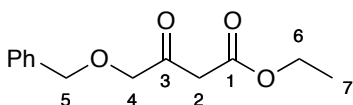
To *tert*-butyl acetoacetate (3.0 mL, 18 mmol) in anhydrous THF (18 mL) was added potassium *tert*-butoxide (2.10 g, 18.7 mmol) portionwise at 0 °C. After stirring at room temperature for 40 min, the yellow solution was cooled again to 0 °C and methyl iodide (1.2 mL, 6.6 mmol) was added dropwise. After stirring at room temperature for 5 h, water was added and extracted with Et₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give *tert*-butyl α -methylacetoacetate **416** (2.97 g, 17.2 mmol, 95%) as a yellow oil. $\nu_{\text{max}}/\text{cm}^{-1}$ 2981 (C-H), 2940 (C-H), 1735 (C=O), 1714 (C=O); δ_{H} (400 MHz, CDCl₃) 1.27 (3H, d, J 7.1, C(2)CH₃), 1.45 (3H, s, C(4)H₃), 2.21 (9H, s, C(CH₃)₃), 3.89 (1H, q, J 7.1, C(2)H); δ_{C} (100 MHz, CDCl₃) 12.8 (C(2)CH₃), 28.0 (C(CH₃)₃), 28.5 (C(4)), 54.8 (C(2)), 81.9 (C(CH₃)₃), 169.9 (C(1)), 204.2 (C(3)); m/z (ESI⁺) 195.1 (MNa⁺, 100%); HRMS (ESI⁺) found 195.09982, C₉H₁₆NaO₃ (MNa⁺) requires 195.09917.

α -Methylacetoacetic acid (330**)⁶**



To *tert*-butyl α -methylacetoacetate **416** (960 mg, 5.58 mmol) was added TFA (2.6 mL) at 0 °C and the solution was stirred at room temperature for 24 h. Concentration of the mixture *in vacuo* gave α -methylacetoacetic acid **330** (534 mg, 4.60 mmol, 82%) as a brown oil, which was used in the following step without further purification. $\nu_{\text{max}}/\text{cm}^{-1}$ 2993 (C-H), 2947 (C-H), 1790 (C=O), 1702 (C=O); δ_{H} (400 MHz, CDCl₃) 1.39 (3H, d, J 7.2, C(2)CH₃), 2.31 (3H, s, C(4)H₃), 3.59 (1H, q, J 7.2, C(2)H), 10.5 (CO₂H); δ_{C} (100 MHz, CDCl₃) 12.9 (C(2)CH₃), 28.8 (C(4)), 53.2 (C(2)), 176.3 (C(1)), 204.5 (C(3)); m/z (ESI⁺) 139.1 (MNa⁺, 100%); HRMS (ESI⁺) found 139.03665, C₅H₈NaO₃ (MNa⁺) requires 139.03657.

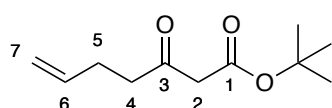
Ethyl 4-benzyloxy-3-oxobutanoate (417**)²²**



To a suspension of NaH (60% w/w, 4.45 g, 111 mmol) in anhydrous THF (37 mL) was added benzyl alcohol (4.0 mL, 39 mmol) dropwise over 1 h at 0 °C. After stirring at room temperature for 45 min, ethyl

4-chloroacetate (5.0 mL, 37 mmol) was added dropwise over 1.5 h at 0 °C and the mixture was stirred for 20 h at room temperature. The solution was acidified with 2 M aqueous HCl to pH 6, extracted with EtOAc, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (15% EtOAc in petrol) to give benzyloxy **417** (8.05 g, 34.1 mmol, 92%) as a yellow oil. *R*_f (15% EtOAc in petrol) 0.27; ν_{max} /cm⁻¹ 2983 (C-H), 1721 (C=O); δ_{H} (400 MHz, CDCl₃) 1.24 (3H, t, *J* 7.2, C(7)H₃), 3.53 (2H, s, C(2)H₂), 4.14 (2H, s, C(4)H₂), 4.16 (2H, q, *J* 7.2, C(6)H₂), 4.58 (2H, s, C(5)H₂), 7.28-7.39 (5H, m, Ph); δ_{C} (100 MHz, CDCl₃) 14.1 (C(7)), 46.2 (C(2)), 61.5 (C(6)), 73.6 (C(5)), 74.9 (C(4)), 127.9, 128.2, 128.6, 137.0 (Ph), 167.1 (C(1)), 201.8 (C(3)); *m/z* (ESI⁺) 259.1 (MNa⁺, 100%); HRMS (ESI⁺) found 259.09424, C₁₃H₁₆NaO₄ (MNa⁺) requires 259.09518.

***tert*-Butyl 3-oxohept-6-enoate (**422**)**^{23,24}

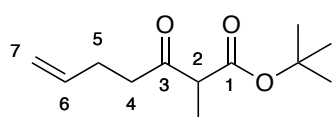


To a solution of 4-pentenoic acid (4.0 mL, 39 mmol) in anhydrous DCM (23 mL) was added oxalyl chloride (3.3 mL, 39 mmol) dropwise at 0 °C. The mixture was heated at 40 °C for 2 h and concentrated under reduced pressure to give pent-4-enoyl chloride as a yellow oil.

Dry pyridine (6.3 mL, 78 mmol) was added dropwise to a solution of Meldrum's acid (5.10 g, 35.4 mmol) in anhydrous DCM (80 mL) at -10 °C. After 20 min at this temperature, pent-4-enoyl chloride prepared as described above in anhydrous DCM (9 mL) was added dropwise. The reaction mixture was stirred at -10 °C for 30 min and at room temperature for 2 h, and quenched with 1 M aqueous HCl. The aqueous layer was extracted with DCM and the combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give 2,2-dimethyl-5-(pent-4-enoyl)-1,3-dioxane-4,6-dione, which was dissolved in toluene (51 mL) and *tert*-butanol (51 mL), heated at reflux for 2 h and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (5% Et₂O in petrol) to give β -ketoester **422** (3.50 g, 17.6 mmol, 50%) as a light yellow oil.

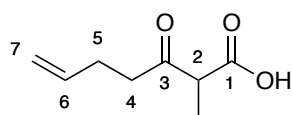
R_f (5% Et₂O in petrol) 0.11; $\nu_{\max}/\text{cm}^{-1}$ 3081 (C-H), 2980 (C-H), 1716 (C=O), 1643 (C=C); δ_H (400 MHz, CDCl₃) 1.45 (9H, s, C(CH₃)₃), 2.30-2.36 (2H, m, C(5)H₂), 2.62 (2H, t, J 7.2, C(4)H₂), 3.33 (2H, s, C(2)H₂), 4.97 (1H, dq, J 10.4, 1.4, C(7)H_AH_B), 4.97 (1H, dq, J 17.0, 1.4, C(7)H_AH_B), 5.79 (1H, ddt, J 17.0, 10.4, 6.4, C(6)H); δ_C (100 MHz, CDCl₃) 27.5 (C(5)), 28.1 (C(CH₃)₃), 42.0 (C(4)), 50.8 (C(2)), 82.1 (C(CH₃)₃), 115.6 (C(7)), 136.8 (C(6)), 166.5 (C(1)), 202.6 (C(3)); m/z (ESI⁺) 221.2 (MNa⁺, 100%); HRMS (ESI⁺) found 221.11494, C₁₁H₁₈NaO₃ (MNa⁺) requires 221.11482.

***tert*-Butyl 2-methyl-3-oxohept-6-enoate (423)²⁴**



To β -ketoester **422** (3.50 g, 18.6 mmol) in anhydrous THF (37 mL) was added potassium *tert*-butoxide (2.20 g, 19.6 mmol) portionwise at 0 °C. After stirring at room temperature for 45 min, the yellow solution was cooled again to 0 °C and methyl iodide (1.3 mL, 21 mmol) was added dropwise. After stirring at room temperature for another 6 h, water was added and extracted with Et₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give methyl β -ketoester **423** (3.39 g, 16.0 mmol, 86%) as a light yellow oil. R_f (5% Et₂O in petrol) 0.17; $\nu_{\max}/\text{cm}^{-1}$ 3080 (C-H), 2980 (C-H), 1714 (C=O), 1642 (C=C); δ_H (400 MHz, CDCl₃) 1.27 (3H, d, J 7.1, C(2)CH₃), 1.44 (9H, s, C(CH₃)₃), 2.30-2.36 (2H, m, C(5)H₂), 2.52-2.72 (2H, m, C(4)H₂), 3.41 (1H, q, J 7.1, C(2)H), 4.95-4.99 (1H, m, C(7)H_AH_B), 4.99-5.06 (1H, m, C(7)H_AH_B), 5.79 (1H, ddt, J 16.8, 10.4, 6.4, C(6)H); δ_C (100 MHz, CDCl₃) 12.8 (C(2)CH₃), 27.7 (C(5)), 28.0 (C(CH₃)₃), 40.6 (C(4)), 54.0 (C(2)), 81.9 (C(CH₃)₃), 115.5 (C(7)), 137.0 (C(6)), 169.8 (C(1)), 205.5 (C(3)); HRMS (CI⁺) found 213.1481, C₁₂H₂₀O₃ (MH⁺) requires 213.1485.

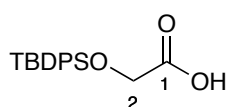
2-Methyl-3-oxohept-6-enoic acid (424)²⁴



TFA (13 mL, 170 mmol) was added to a solution of β -ketoester **423** (2.82 g, 13.3 mmol) in anhydrous DCM (13 mL) at 0 °C. The solution was stirred at room temperature for 1.5 h and concentrated under reduced pressure to give acid **424** (2.10 g, 13.3 mmol, quant.) as an orange oil, which was used in the following step without further

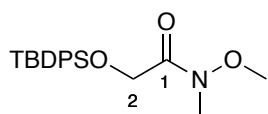
purification. $\nu_{\max}/\text{cm}^{-1}$ 3083 (C-H), 2984 (C-H), 1789 (C=O), 1703 (C=O), 1643 (C=C); δ_{H} (400 MHz, CDCl_3) 1.38 (3H, d, J 7.2, C(2) CH_3), 2.32-2.39 (2H, m, C(5) H_2), 2.63-2.80 (2H, m, C(4) H_2), 3.61 (1H, q, J 7.2, C(2) H), 4.97-5.07 (2H, m, C(7) H_2), 5.79 (1H, ddt, J 16.8, 10.4, 6.4, C(6) H), 11.05 (1H, s, CO_2H); δ_{C} (100 MHz, CDCl_3) 13.0 (C(2) CH_3), 27.6 (C(5)), 41.0 (C(4)), 52.5 (C(2)), 115.9 (C(7)), 136.5 (C(6)), 176.5 (C(1)), 205.7 (C(3)); m/z (ESI $^{+}$) 157.0 (MH $^{+}$, 43%), 179.0 (MNa $^{+}$, 100%); HRMS (ESI $^{+}$) found 157.08620, $\text{C}_8\text{H}_{13}\text{O}_3$ (MH $^{+}$) requires 157.08592.

2-((*tert*-Butyldiphenylsilyl)oxy)acetic acid (**374**)²⁵



To a solution of glycolic acid (497 mg, 6.54 mmol) in anhydrous THF (26 mL) were added DMAP (18 mg, 0.15 mmol) and Et $_3$ N (930 μL , 6.65 mmol) at 0 $^{\circ}\text{C}$. After stirring at this temperature for 5 min, TMSCl (880 μL , 6.93 mmol) was added dropwise over 30 min and stirring was continued at 0 $^{\circ}\text{C}$ for 1 h and at room temperature for 1 h. Et $_3$ N (930 μL , 6.65 mmol) was then added, followed by TBDPSCl (1.8 mL, 6.9 mmol) dropwise. The reaction mixture was stirred at room temperature for 8 h and then treated with acetic acid (5.9 mL, 100 mmol) and H $_2$ O (18 mL). After stirring at room temperature for 1 h, the mixture was extracted with Et $_2$ O, dried over anhydrous Na $_2$ SO $_4$ and concentrated under reduced pressure. The residue was purified by *flash* column chromatography (20% to 50% Et $_2$ O in petrol) to give acid **374** (1.55 mmol, 4.92 mmol, 75%) as a thick colourless oil. R_f (50% Et $_2$ O in petrol) 0.22; $\nu_{\max}/\text{cm}^{-1}$ 3072 (C-H), 2932 (C-H), 2858 (C-H), 1727 (C=O); δ_{H} (400 MHz, CDCl_3) 1.11 (9H, s, SiC(CH $_3$) $_3$), 4.23 (2H, s, C(2) H_2), 7.40-7.48 (6H, m, Ph), 7.64-7.66 (4H, m, Ph); δ_{C} (100 MHz, CDCl_3) 19.3 (SiC(CH $_3$) $_3$), 26.8 (SiC(CH $_3$) $_3$), 62.1 (C(2)), 128.2, 130.5, 131.7, 135.5 (Ph), 174.9 (C(1)); m/z (ESI $^{+}$) 337.2 (MNa $^{+}$, 100%); HRMS (ESI $^{+}$) found 337.12298, $\text{C}_{18}\text{H}_{22}\text{NaO}_3\text{Si}$ (MNa $^{+}$) requires 337.12304.

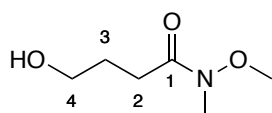
2-((*tert*-Butyldiphenylsilyl)oxy)-*N*-methoxy-*N*-methylacetamide (**375**)²⁶



DCC (2.30 g, 11.1 mmol) was added to a solution of acid **374** (1.18 g, 3.76 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (1.10 mg, 11.3 mmol) in anhydrous DCM (7.5 mL) at 0 $^{\circ}\text{C}$. The reaction mixture was heated at reflux for 3 h

and quenched with water. The aqueous phase was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (DCM to 2% EtOAc in DCM) to give Weinreb amide **375** (995 mg, 2.78 mmol, 74%) as a white solid; m.p. 55 °C (lit.²⁶ m.p. 53.4-54.8 °C). R_f (2% EtOAc in DCM) 0.28; $\nu_{\max}/\text{cm}^{-1}$ 3071 (C-H), 2931 (C-H), 2855 (C-H), 1669 (C=O); δ_H (400 MHz, CDCl_3) 1.10 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 3.14 (3H, s, NCH_3), 3.44 (3H, s, NOCH_3), 4.42 (2H, s, $\text{C}(2)\text{H}_2$), 7.37-7.43 (6H, m, Ph), 7.72-7.74 (4H, m, Ph); δ_C (125 MHz, CDCl_3) 19.5 ($\text{SiC}(\text{CH}_3)_3$), 26.8 ($\text{SiC}(\text{CH}_3)_3$), 32.5 (NCH_3), 61.3 (NOCH_3), 62.1 ($\text{C}(2)$), 127.8, 129.9, 133.3, 135.8 (Ph), 171.9 ($\text{C}(1)$); m/z (ESI^+) 380.2 (MNa^+ , 100%); HRMS (ESI^+) found 380.16554, $\text{C}_{20}\text{H}_{27}\text{NNaO}_3\text{Si}$ (MNa^+) requires 380.16524.

4-Hydroxy-*N*-methoxy-*N*-methylbutanamide (**381**)²⁷



Trimethylaluminium (2 M in toluene, 7.5 mL, 15 mmol) was added over 30 min to a solution of *N,O*-dimethylhydroxylamine hydrochloride (1.54 g, 15.8 mmol) in anhydrous DCM (6.5 mL) at -78 °C. After stirring at room temperature for 4 h, the solution was cooled to -5 °C and γ -butyrolactone (500 μL , 6.50 mmol) was added dropwise. The solution was stirred at room temperature for 1.5 h and then quenched at 0 °C with a saturated aqueous solution of Rochelle's salt. The mixture was stirred at room temperature overnight, filtered through Celite, washed with DCM, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give Weinreb amide **381** (783 mg, 5.32 mmol, 82%) as a light yellow oil. $\nu_{\max}/\text{cm}^{-1}$ 3421 (O-H), 2925 (C-H), 1638 (C=O); δ_H (400 MHz, CDCl_3) 1.80-1.88 (2H, m, $\text{C}(3)\text{H}_2$), 2.54 (2H, t, J 6.1, $\text{C}(2)\text{H}_2$), 3.14 (3H, s, NCH_3), 3.61-3.66 (5H, m, $\text{C}(4)\text{H}_2 + \text{NOCH}_3$); δ_C (100 MHz, CDCl_3) 27.3 ($\text{C}(3)$), 29.1 ($\text{C}(2)$), 32.2 (NCH_3), 61.3 (NOCH_3), 62.4 ($\text{C}(4)$), 174.9 ($\text{C}(1)$); m/z (ESI^+) 148.0 (MH^+ , 33%), 170.0 (MNa^+ , 100%); HRMS (ESI^+) found 148.09682, $\text{C}_6\text{H}_{14}\text{NO}_3$ (MH^+) requires 148.09682.

References

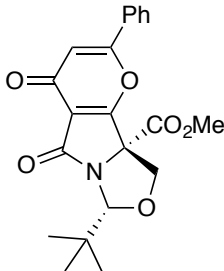
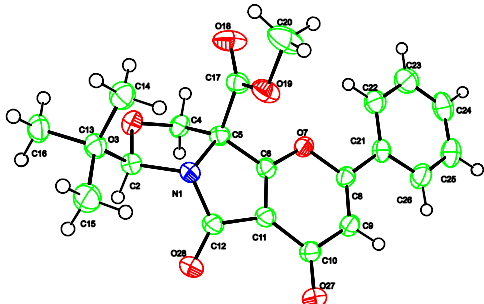
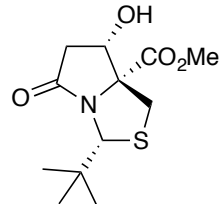
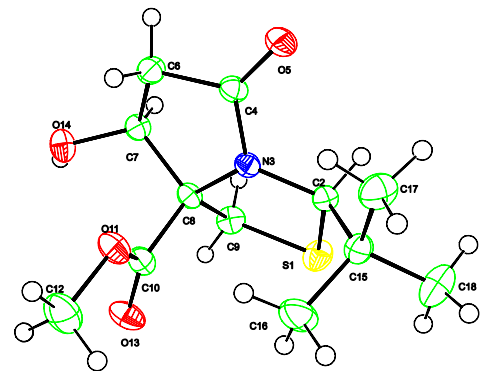
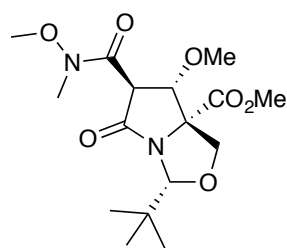
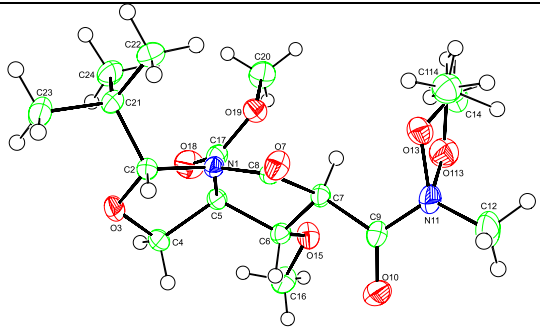
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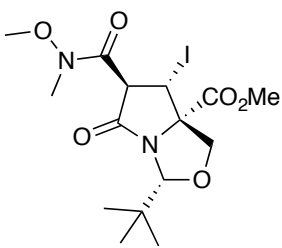
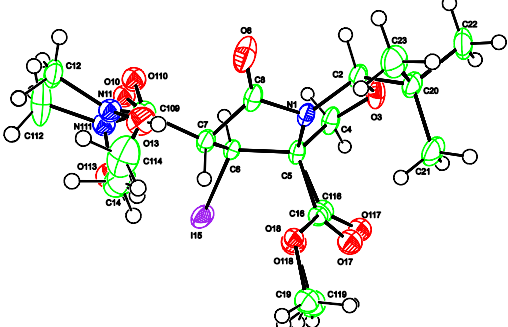
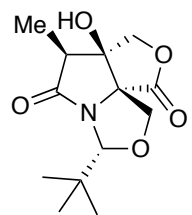
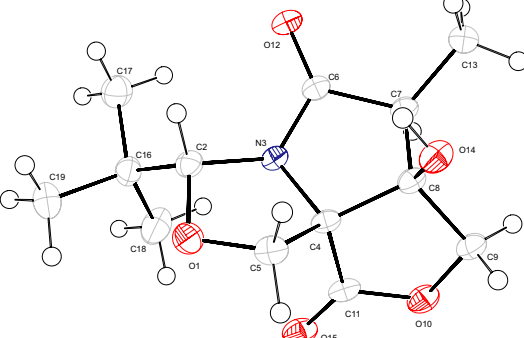
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APPENDIX B: X-RAY CRYSTALLOGRAPHY DATA

Crystal structures of the compounds indicated in Table B.1 were determined by the Oxford Chemistry Crystallography Service, and the data are provided separately on the CD.

Table B.1. Crystal structures and file names.

Compound	Structure	Crystal structure	File name
98a			6517
197b			6515
239			6603

264			6604
350			6570

APPENDIX C: NOE CORRELATIONS

NOE experiments were performed on a Bruker AVII 500 (500 MHz). Figure C.1, Figure C.2 and Figure C.3 show the key correlations which were used to assign the stereochemistry of the compounds.

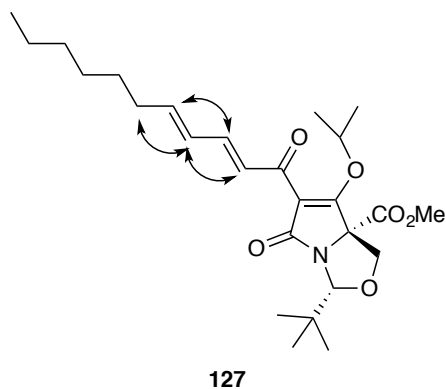


Figure C.1. NOE correlations of compounds from Chapter 2.

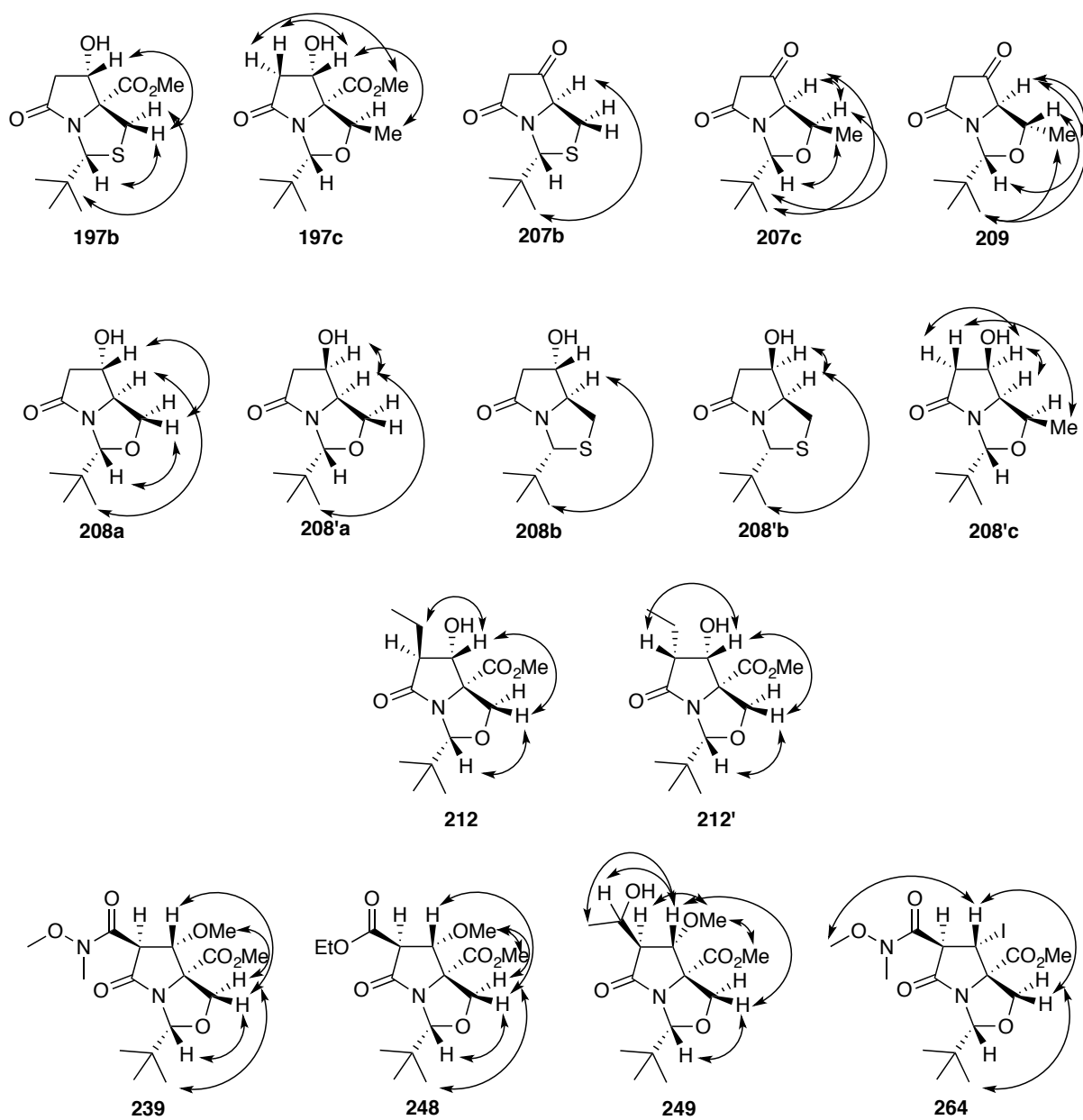


Figure C.2. NOE correlations of compounds from Chapter 3.

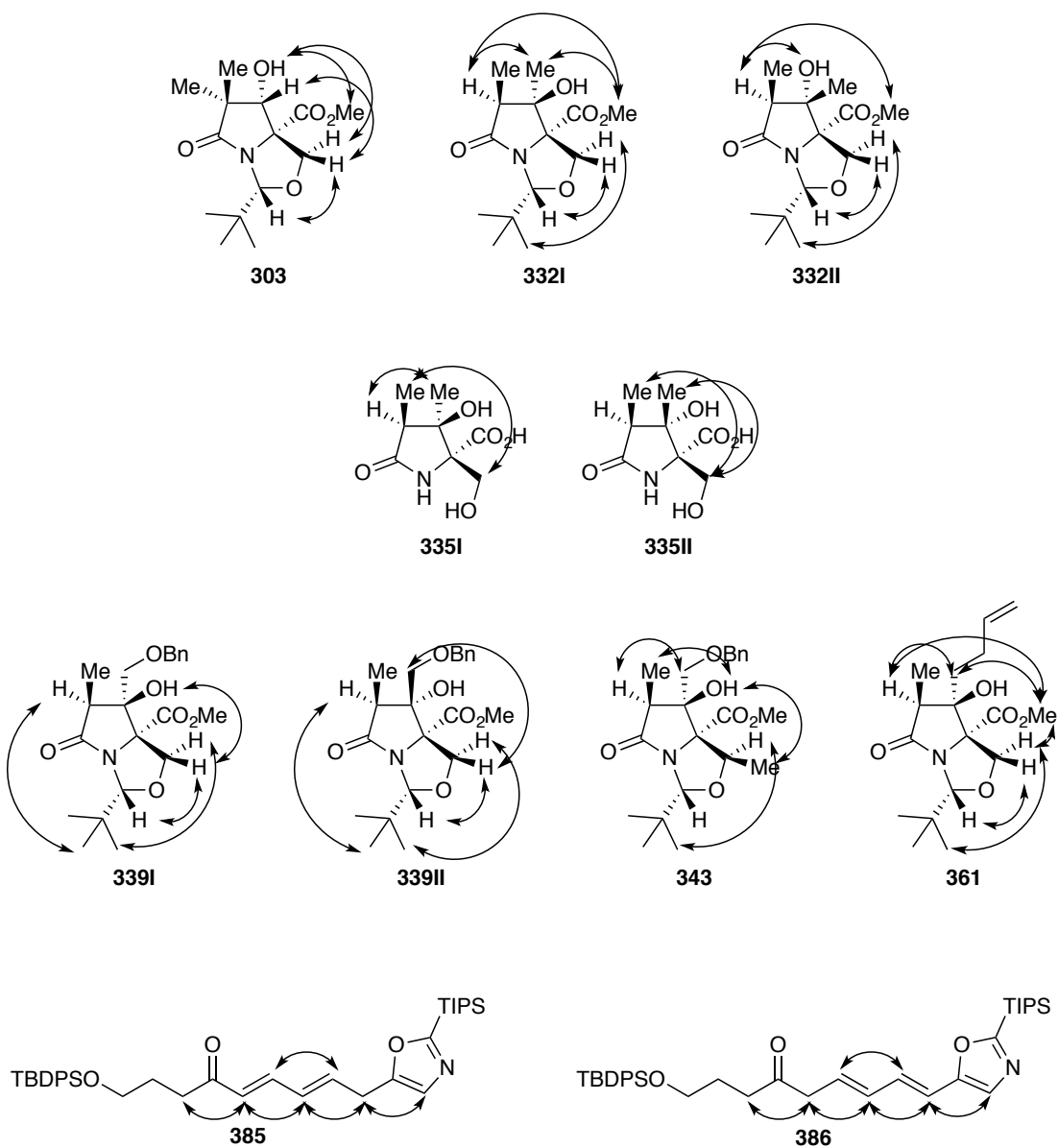


Figure C.3. NOE correlations of compounds from Chapter 4.

APPENDIX D: LC/MS CHROMATOGRAMS

The diastereomeric excess of the reduction of tetramic acids **44a-c** (Figure D.1, Figure D.2 and Figure D.3) and **207a-c** (Figure D.4, Figure D.5 and Figure D.6) was determined by integration of the peaks from LC/MS analysis of the crude products obtained.

All analyses presented were performed using a Thermo Exactive mass spectrometer equipped with an Agilent 1100 chromatography system. Instrument control and data processing were performed using Thermo Xcalibur Software. The system was calibrated on the day of the analysis and its mass accuracy with external calibration is better than 5 ppm for 24 hours following calibration. The mass spectrometer was operated using the heated electrospray (HESI-II) probe. Analysis was performed with positive ion mode. Electrospray source conditions were adjusted to maximise sensitivity.

LC eluents consisted of deionised water from a Millipore milli-Q dispenser (resistivity 18.2 M Ω , 0.2 μ m filtered) with 0.1% formic acid (v/v) (solvent A) and HPLC grade acetonitrile (solvent B). The HPLC column used was Chromolith Fastgradient RP18e 50 x 2 mm from Merck. The column temperature was maintained at 40 °C and UV absorption data was collected from 190-400 nm using a diode array detector. Elution gradients were optimised for each sample. A flow rate of 0.4 mL/min was used in all cases.

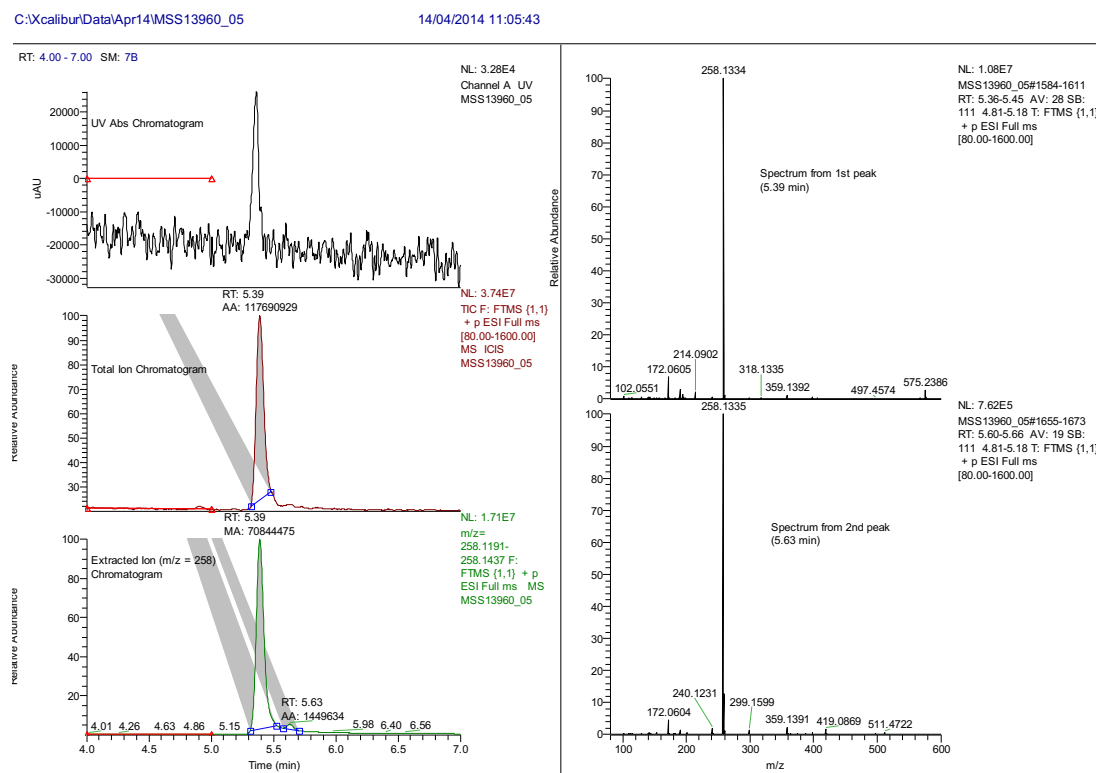
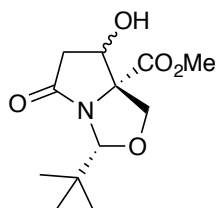


Figure D.1. LC/MS chromatogram of the crude product from the reduction of **44a**.

(2R,5R,6S)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-8-oxo-3-thiabicyclo[3.3.0]-octane (197b)

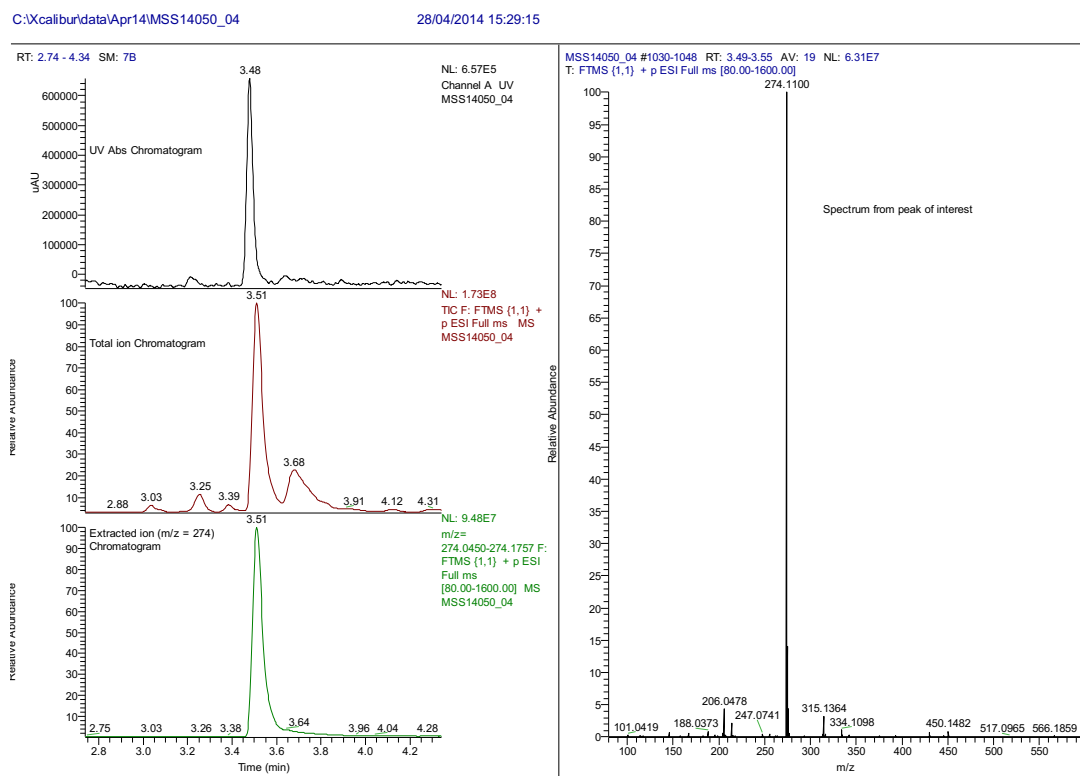
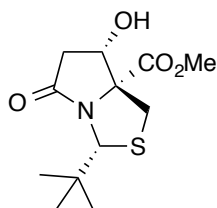


Figure D.2. LC/MS chromatogram of the crude product from the reduction of **44b**.

(2*R*,4*R*,5*R*,6*RS*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (197*c*, 197'*c*)

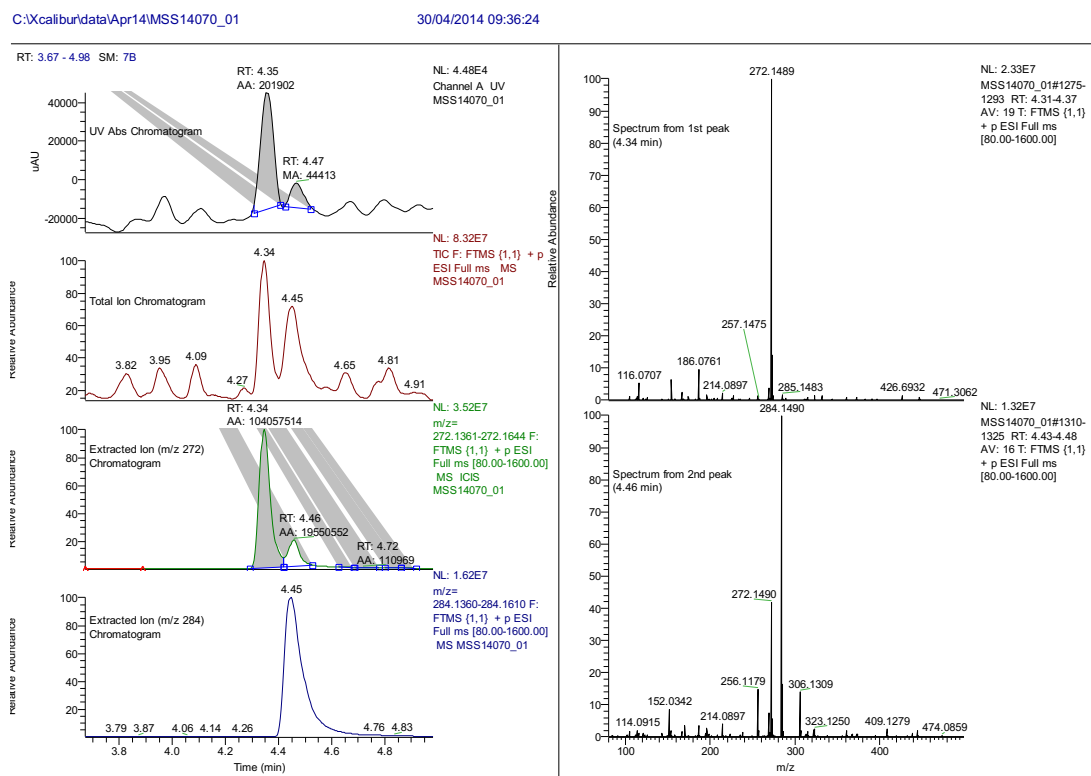
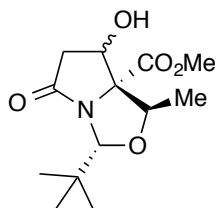


Figure D.3. LC/MS chromatogram of the crude product from the reduction of **44c**.

(2*R*,5*R*,6*RS*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-3-oxa-8-oxobicyclo[3.3.0]-octane (208a, 208'a)

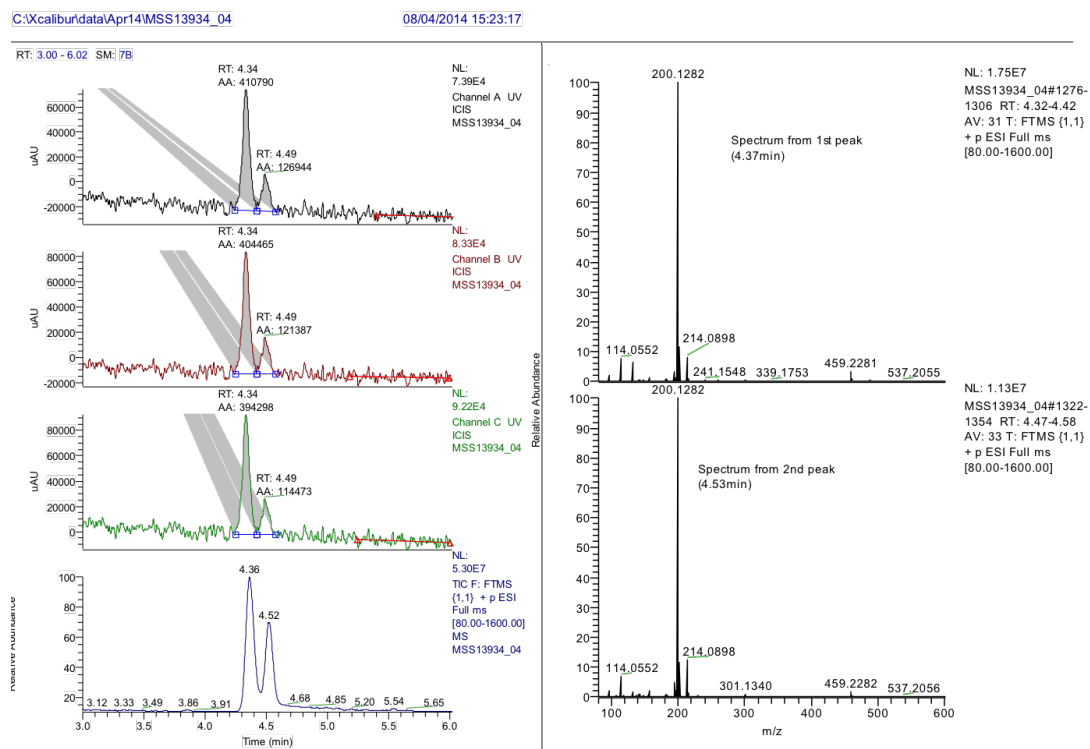
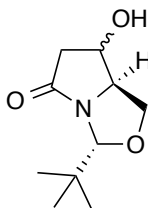


Figure D.4. LC/MS chromatogram of the crude product from the reduction of **207a**.

(2*R*,5*S*,6*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]-octane (208b, 208'b)

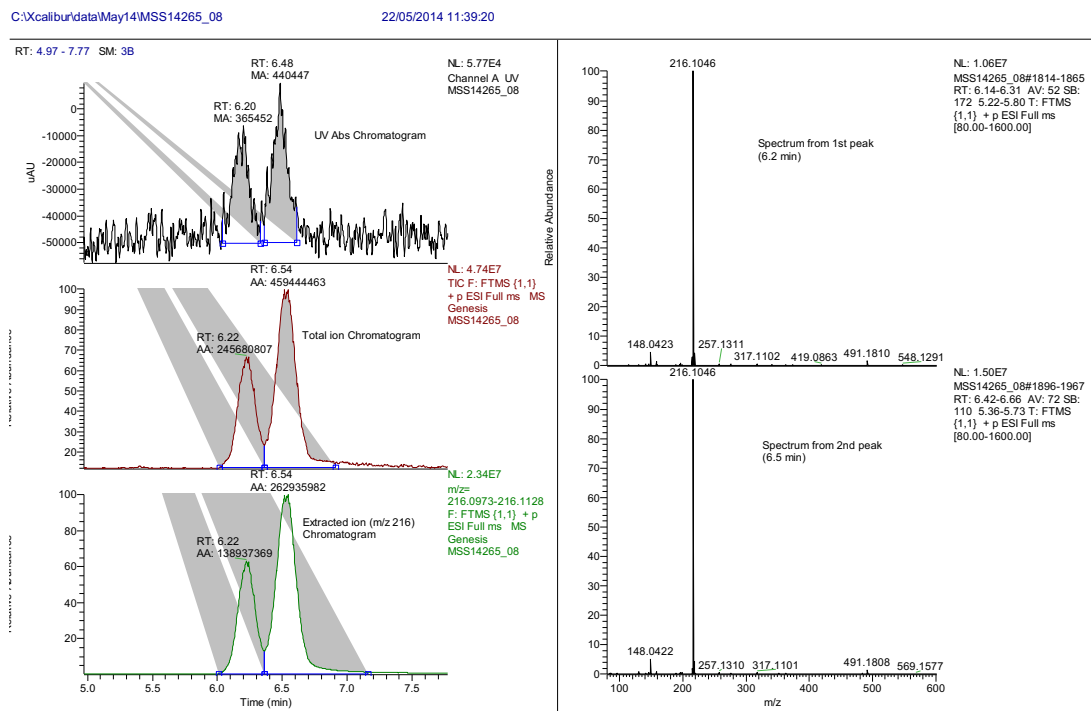
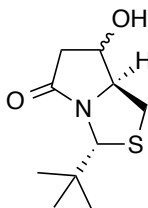


Figure D.5. LC/MS chromatogram of the crude product from the reduction of **207b**.

**(2R,4R,5S,6RS)-1-Aza-2-(*tert*-butyl)-6-hydroxy-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (208c, 208'c) and
(2R,4S,5S,6RS)-1-Aza-2-(*tert*-butyl)-6-hydroxy-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane**

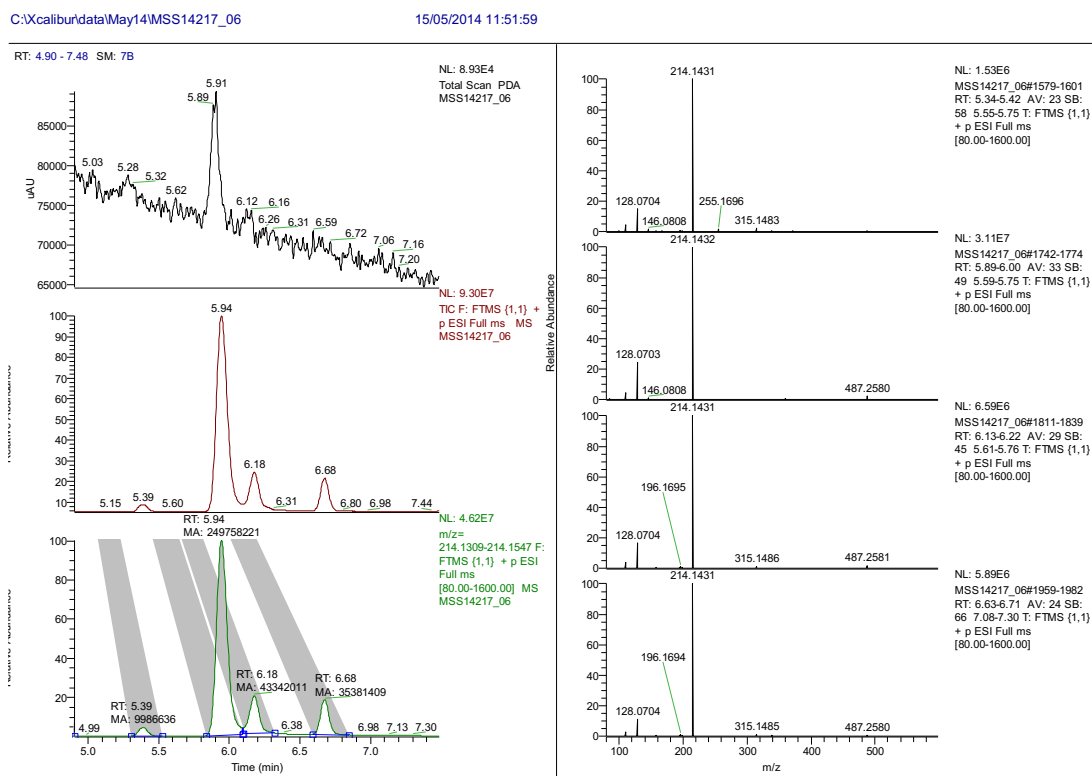
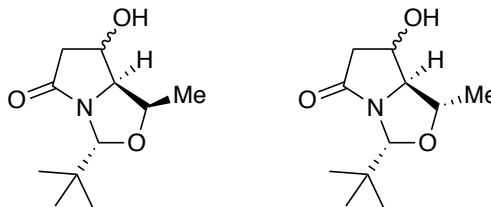
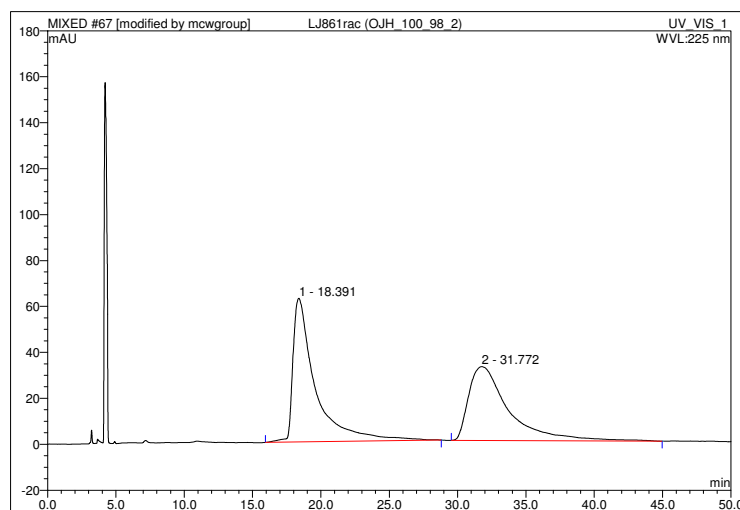
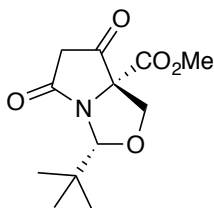


Figure D.6. LC/MS chromatogram of the crude product from the reduction of **207c**.

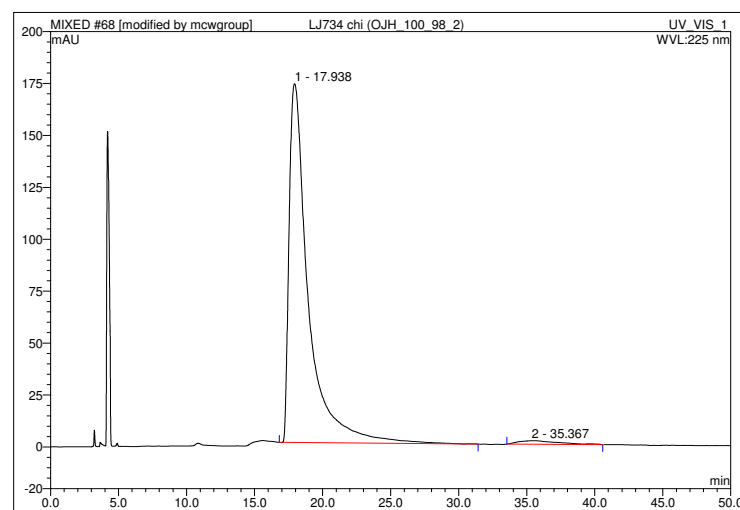
APPENDIX E: CHIRAL HPLC CHROMATOGRAMS

Chiral HPLC chromatograms were obtained on a chiral stationary phase HPLC in a Dionex P680 chromatography with a Dionex UVD170U detector, and with Daicel Chiralpak OJ-H or AD-H columns. The first chromatogram corresponds to the racemic sample and the second one to the enantiopure compound. Racemic samples were prepared analogously to the enantiopure compounds, but starting from the DL-aminoacids.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (44a)



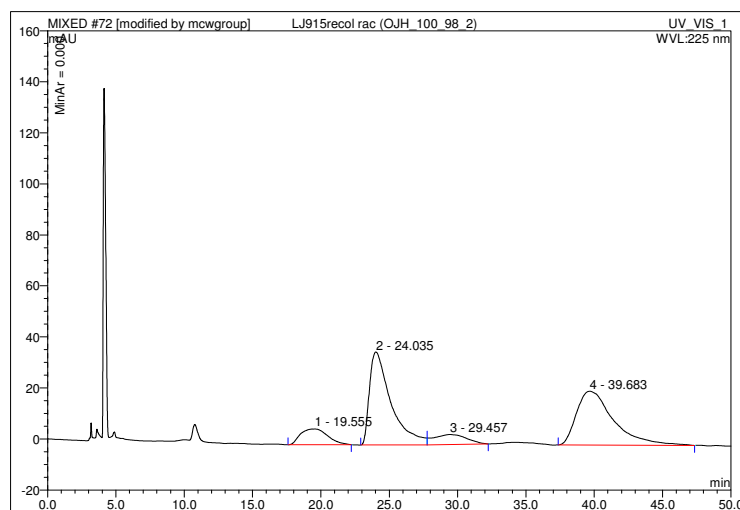
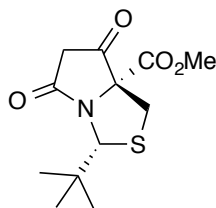
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	18.39	n.a.	62.495	119.188	52.25	n.a.	BMB*
2	31.77	n.a.	32.104	108.907	47.75	n.a.	BMB*
Total:			94.599	228.095	100.00	0.000	



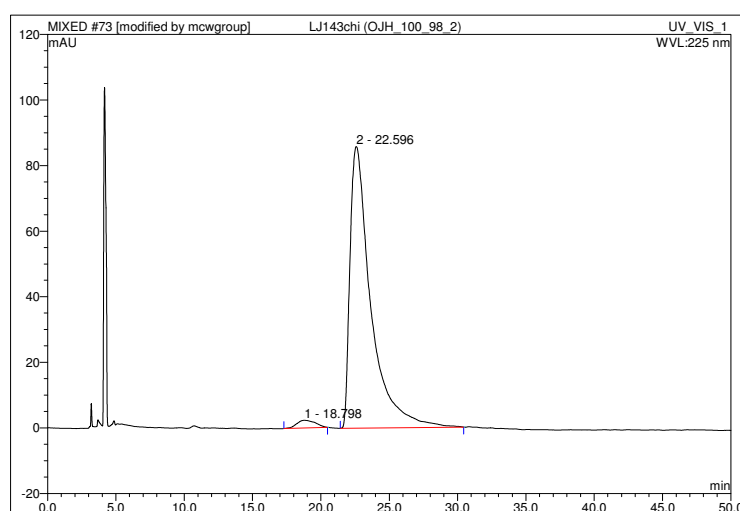
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	17.94	n.a.	172.706	282.903	98.02	n.a.	BMB*
2	35.37	n.a.	1.644	5.705	1.98	n.a.	BMB*
Total:			174.349	288.607	100.00	0.000	

Figure E.1. Chiral HPLC chromatogram of racemic (top) and enantiopure (bottom) **44a**.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (44b)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.55	n.a.	6.136	14.149	8.97	n.a.	BMB*
2	24.03	n.a.	36.419	66.673	42.26	n.a.	BM *
3	29.46	n.a.	3.920	10.891	6.90	n.a.	MB*
4	39.68	n.a.	21.064	66.048	41.87	n.a.	BMB*
Total:			67.539	157.761	100.00	0.000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	18.80	n.a.	2.334	3.654	2.35	n.a.	BMB*
2	22.60	n.a.	85.850	151.646	97.65	n.a.	BMB*
Total:			88.185	155.300	100.00	0.000	

Figure E.2. Chiral HPLC chromatogram of racemic (top) and enantiopure (bottom) **44b**.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]octane (44c**)**

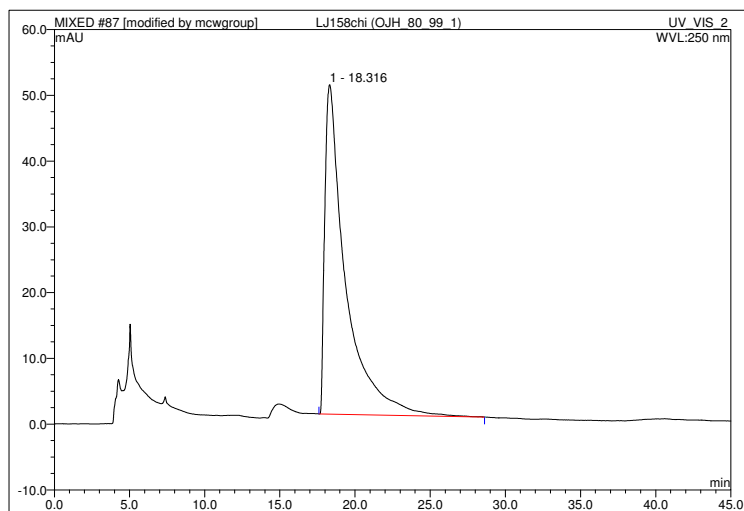
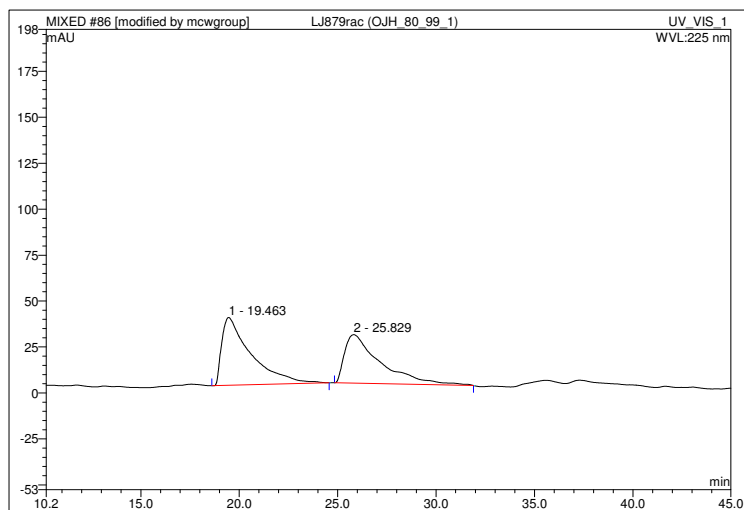
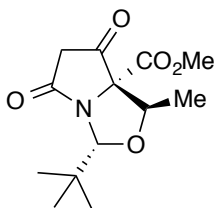


Figure E.3. Chiral HPLC chromatogram of racemic (top) and enantiopure (bottom) **44c**.

APPENDIX F: ANTIBACTERIAL ACTIVITY

F.1 Hole-plate antibacterial assay

Antibacterial assays were performed using the hole-plate method with agar plates inoculated with Gram-positive *Staphylococcus aureus* DS267 and Gram-negative *Escherichia coli* X580. The agar plates were prepared by Wendy Sobey at the Department of Chemistry, University of Oxford. Samples were prepared as 4 mg/mL solutions in 1:1 MeOH/DMSO, and diluted to lower concentrations where necessary. A 100 μ L aliquot of each solution was loaded into wells of 10 mm of diameter in agar plates and incubated at 37 °C for 16-20 h. The diameters of the resultant inhibition zones were then measured along two perpendicular axes and averaged to obtain the zone of inhibition. The assays were repeated in duplicate, and a negative control was run with solvent alone.

F.1.1 Calibration data for hole-plate antibacterial assay

Cephalosporin C (CephC) was used as a standard. Calibration curves were performed at 2-100 μ g/mL for *E. coli* and 200-1000 μ g/mL for *S. aureus*, and these were collected for each set of bioassays performed. An example of calibration data is illustrated below (Table F.1, Table F.2), with the respective calibration curves (Figure F.1, Figure F.2).

Table F.1. Example of calibration data for *E. coli*.

Conc. stock solution [µg/mL]	Ceph C stock solution [µL/well]	H ₂ O [µL/well]	Conc. CephC [µg/well]	Conc. CephC [nmol/well]	log(nmol/well) Ceph C	Zone size
10	20	80	0.2	0.48	-0.32	12
	40	60	0.4	0.96	-0.02	15
	60	40	0.6	1.44	0.16	19.5
	80	20	0.8	1.93	0.28	22.5
	100	0	1	2.41	0.38	24
100	20	80	2	4.81	0.68	28
	40	60	4	9.63	0.98	33
	60	40	6	14.44	1.16	36
	80	20	8	19.26	1.28	37
	100	0	10	24.07	1.38	37.5

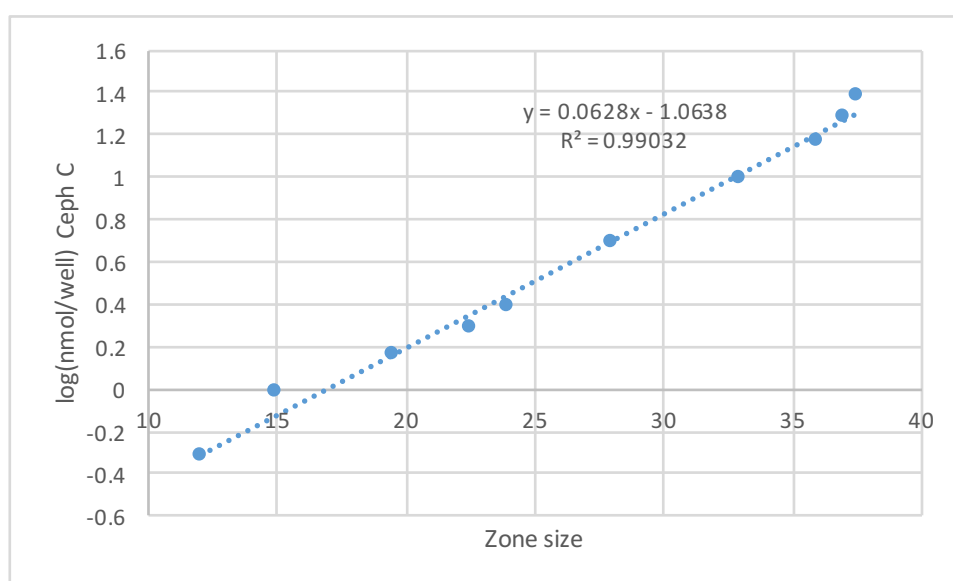
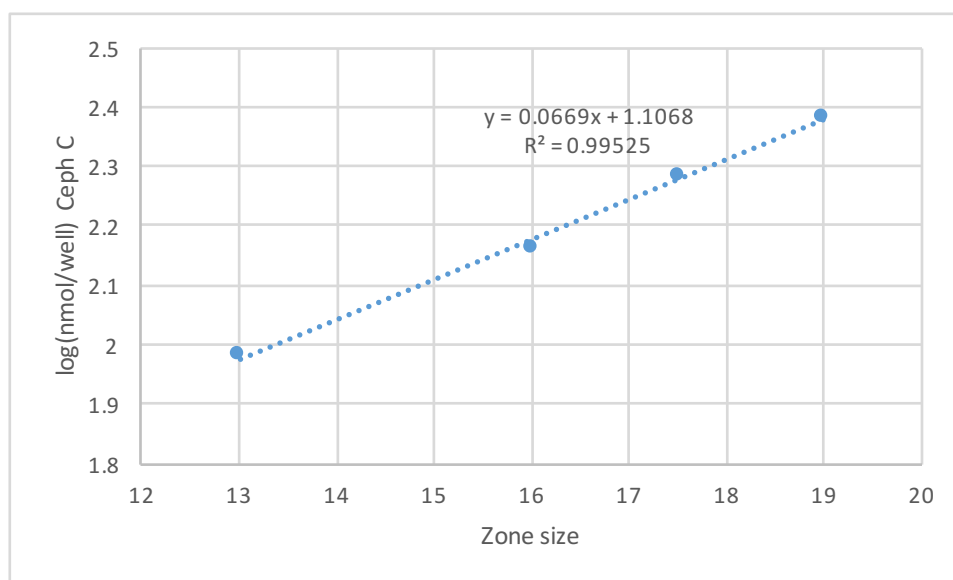

Figure F.1. Calibration curve for *E. coli*.

Table F.2. Example of calibration data for *S. aureus*.

Conc. stock solution [μg/mL]	Ceph C stock solution [μL/well]	H ₂ O [μL/well]	Conc. CephC [μg/well]	Conc. CephC [nmol/well]	log(nmol/well) Ceph C	Zone size
1000	20	80	20	48.15	1.68	
	40	60	40	96.29	1.98	13
	60	40	60	144.44	2.16	16
	80	20	80	192.59	2.28	17.5
	100	0	100	240.73	2.38	19


Figure F.2. Calibration curve for *S. aureus*.

F.1.2 Data from hole-plate antibacterial assay

Table F.3 indicates the zone sizes obtained for the compounds tested at the indicated concentration, which are the average of duplicates. The bactericidal effect of the compounds was compared to that of the standard CephC via the relative potency, which corresponds to the equivalent moles of CephC (that would have given the same zone size as the compound) / moles of the compound. The equivalent number of moles of Ceph C was calculated from the calibration curves.

Table F.3. Antibacterial activity of synthesised analogues against *E. coli* and *S. aureus* using the hole-plate method. n.a. = not active, 'H' = 'halo' zone of inhibition, 'P' = compound precipitated during incubation.

Compound	<i>E. coli</i>			<i>S. aureus</i>		
	Concentration [mg/mL]	Zone size [mm]	Relative potency	Concentration [mg/mL]	Zone size [mm]	Relative potency
Compounds from Chapter 2						
44a	4	n.a.	-	4	n.a.	-
44b	4	n.a.	-	4	n.a.	-
44c	4	n.a.	-	4	n.a.	-
77a	4	n.a.	-	4	n.a.	-
77b	4	n.a.	-	4	n.a.	-
77b	4	n.a.	-	4	n.a.	-
79	4	n.a.	-	4	13.5	0.07
81a	4	n.a.	-	4	n.a.	-
81b	4	n.a.	-	4	n.a.	-
81c	4	n.a.	-	4	n.a.	-
86	4	n.a.	-	4	23	0.28
87	4	n.a.	-	4	n.a.	-
93	4	n.a.	-	4	n.a.	-
94a	4	n.a.	-	4	n.a.	-
94b	4	15 H	$7.09 \cdot 10^{-4}$	4	13.75 H	0.10
94c	4	n.a.	-	4	n.a.	-
95a	4	n.a.	-	4	n.a.	-
95b	4	15.5 H	$8.23 \cdot 10^{-4}$	4	13.5 H	0.10
95c	4	n.a.	-	4	n.a.	-
96a	4	16 H	$8.41 \cdot 10^{-4}$	4	30	1.25
				0.5	18	1.58
96b	4	15.5 H	$8.15 \cdot 10^{-4}$	4	41	7.10
				0.1	17.75	7.91
96c	4	14.5 H	$7.02 \cdot 10^{-4}$	4	35	2.80
				0.5	20	2.22
98a	4	13.25 P	$5.62 \cdot 10^{-4}$	4	14 P	0.11
98b	4	15 P	$7.54 \cdot 10^{-4}$	4	13 P	0.09
98c	4	17 P	$1.00 \cdot 10^{-3}$	4	13.5	0.10
100	4	n.a.	-	4	n.a.	-

105	4	n.a.	-	4	n.a.	-
107	4	n.a.	-	4	n.a.	-
109	4	n.a.	-	4	15.75	0.07
110a	4	n.a.	-	0.5	26.5	4.37
110b	4	n.a.	-	0.5	37	26.91
				0.1	26.5	22.93
110c	4	n.a.	-	0.5	27.75	5.63
114	4	n.a.	-	0.5	20	1.68
115	4	16.25 P	$8.54 \cdot 10^{-4}$	4	n.a. P	-
116I	4	n.a.	-	4	n.a.	-
117	4	n.a.	-	4	n.a.	-
122	4	n.a.	-	0.5	32	14.15
				0.1	27.25	31.78
404	4	n.a.	-	0.5	28	6.21
				0.1	24.25	16.52
123	4	n.a.	-	0.5	20.5	2.24
124	4	n.a.	-	0.5	27.5	6.03
125	4	n.a.	-	0.5	35.5	25.78
				0.1	33	84.57
126	4	n.a.	-	0.5	19.5	1.91
127	4	n.a.	-	4	n.a.	-
129	4	n.a.	-	4	22	0.34
138	4	n.a.	-	4	20.5	0.24
139	4	16 H	$1.19 \cdot 10^{-3}$	4	24	0.46
171	4	n.a.	-	4	15 H	0.10
186	4	n.a.	-	4	n.a.	-

Compounds from Chapter 3

197a	4	n.a.	-	4	n.a.	-
197b	4	n.a.	-	4	n.a.	-
197c	4	n.a.	-	4	n.a.	-
207a	4	n.a.	-	4	20	0.14
207b	4	14.5	$3.75 \cdot 10^{-4}$	4	27.75	0.49
207c	4	n.a.	-	4	15	0.07
208a	4	n.a.	-	4	n.a.	-

208b	4	13 H	$3.05 \cdot 10^{-4}$	4	12.75 H	0.05
208c	4	n.a.	-	4	n.a.	-
210	4	14.5 H	$4.20 \cdot 10^{-4}$	4	n.a.	-
217	4	n.a. P	-	4	n.a. P	-
239	4	n.a.	-	4	n.a.	-
248	1	21	$6.60 \cdot 10^{-3}$	1	22	1.07
255	1	27.5	$1.07 \cdot 10^{-2}$	1	23	1.04
256	1	21.5	$6.95 \cdot 10^{-3}$	1	27.75	2.83
257	1	18.75	$5.67 \cdot 10^{-3}$	1	24.5	1.78
258	1	18.75	$5.61 \cdot 10^{-3}$	1	28.25	3.31
259	1	14.25 H	$3.49 \cdot 10^{-3}$	1	28.5	3.44
260	1	19.5	$5.36 \cdot 10^{-3}$	1	14.25 H	0.28
261	1	21	$5.98 \cdot 10^{-3}$	1	23	1.15
264	1	19.5	$7.46 \cdot 10^{-3}$	1	14.5 H	0.40

Compounds from Chapter 4

52	4	n.a.	-	4	n.a.	-
297	4	n.a.	-	4	n.a.	-
298	4	n.a.	-	4	n.a.	-
299	4	16.25	$6.09 \cdot 10^{-4}$	4	14.5 H	0.06
303	4	n.a.	-	4	n.a.	-
304	4	n.a.	-	4	n.a.	-
305	4	n.a.	-	4	n.a.	-
306	4	n.a.	-	4	n.a.	-
308	4	14.5	$4.45 \cdot 10^{-4}$	4	n.a.	-
319	4	13.5 H	$6.55 \cdot 10^{-4}$	4	14 H	0.07
321	4	n.a.	-	4	n.a.	-
323	4	n.a.	-	4	n.a.	-
332I	4	n.a.	-	4	n.a.	-
332II	4	n.a.	-	4	n.a.	-
334I	4	n.a.	-	4	n.a.	-
334II	4	n.a.	-	4	n.a.	-
336	4	18.5	$6.48 \cdot 10^{-4}$	4	13 H	0.04
339I	4	20.25	$1.61 \cdot 10^{-3}$	4	14.25 H	0.10
339II	4	21.25	$1.77 \cdot 10^{-3}$	4	13.75 H	0.09

352I	4	n.a.	-	4	n.a.	-
352II	4	n.a.	-	4	n.a.	-
420I	4	n.a.	-	4	n.a.	-
420II	4	n.a.	-	4	n.a.	-
340	4	14 H	$6.68 \cdot 10^{-4}$	4	13.25 H	0.06
341	4	n.a.	-	4	n.a.	-
343	4	n.a. P	-	4	n.a. P	-
421	4	n.a.	-	4	n.a.	-
345/346	4	15.5	$8.06 \cdot 10^{-4}$	4	n.a.	-
348I	4	n.a.	-	4	n.a.	-
350	4	n.a.	-	4	14 H	0.07
353	4	n.a.	-	4	n.a.	-
356	4	n.a.	-	4	n.a.	-
358	4	n.a. P	-	4	n.a. P	-
361	4	14.5 P	$7.82 \cdot 10^{-4}$	4	n.a. P	-
425	4	n.a. P	-	4	n.a. P	-
362	4	n.a.	-	4	n.a.	-
363	4	n.a. P	-	4	n.a. P	-
365	4	n.a. P	-	4	n.a. P	-
366	4	n.a.	-	4	n.a.	-
391	4	n.a. P	-	4	n.a. P	-

F.2 Broth dilution assay

Determination of the MIC values of selected compounds with a broth dilution method was performed by SeaLife Pharma.

The extracts were tested in a primary 96 well plate screening assay, according to SOP 0906. The substances were diluted in "MHB" for bacterial screening to a stock solution of 1000 µg/mL or 1% serial diluted and overlaid with a microbe solution in a concentration of 10^4 CFU/ml. The plates were incubated at 35 °C for 24 h.

All the compounds were tested against Gram-negative *Escherichia coli* and Gram-positive MRSA and *Streptococcus pneumoniae*. Some compounds were also tested for antibacterial activity against Gram-negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, Gram-positive *Enterococcus faecalis*, and antifungal activity against *Candida albicans*, *Candida krusei* and *Aspergillus fumigatus*. Toxicity of some compounds was evaluated via determination of their activity against mammalian HaCaT and HUVEC (human umbilical vein endothelial cell) cell lines. The full data is indicated in Table F.4.

Table F.4. Antibacterial activity, antifungal activity and toxicity of selected synthesised analogues determined by SeaLife Pharma (n.a. = not active).

Compound	Antibacterial activity, MIC [$\mu\text{g/mL}$]						Antifungal activity, MIC [$\mu\text{g/mL}$]			Toxicity, EC ₅₀ [$\mu\text{g/mL}$]	
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	MRSA	<i>S. pneumoniae</i>	<i>E. faecalis</i>	<i>C. albicans</i>	<i>C. krusei</i>	<i>A. fumigatus</i>	HaCaT	HUVEC
44a	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	750
44b	n.a.	n.a.	n.a.	>500	n.a.	>500	n.a.	n.a.	n.a.	375	500
44c	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	750
77a	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	1000
77b	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	800
77b	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	1000
81a	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	750
81b	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	375	500
81c	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	400	375
94a	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	600
94b	n.a.	n.a.	n.a.	>250	n.a.	>250	n.a.	n.a.	n.a.	250	250
94c	n.a.	n.a.	n.a.	n.a.	n.a.	>250	n.a.	n.a.	n.a.	400	500
95a	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	500	375
95b	n.a.	n.a.	n.a.	>250	>500	>250	n.a.	n.a.	n.a.	250	375
95c	n.a.	n.a.	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	250	500
96a	n.a.	n.a.	n.a.	>16	>16	>16	n.a.	n.a.	n.a.	62.5	125
96b	n.a.	n.a.	n.a.	>2	>2	>2	n.a.	n.a.	n.a.	75	31.25
96c	n.a.	n.a.	n.a.	>31	>16	>16	n.a.	n.a.	n.a.	62.5	62.5

98a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	125	45
98b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	45	31.25
98c	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	>500	>500	n.a.	100	-
105	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	400	500
107	n.a.	-	-	n.a.	n.a.	-	-	-	-	-	-
109	250	-	-	250	-	-	-	-	-	-	-
110a	n.a.	-	-	7.81	3.91	-	-	-	-	-	-
110b	n.a.	-	-	0.98	0.45	-	-	-	-	-	-
110c	250	-	-	7.81	3.91	-	-	-	-	-	-
114	n.a.	n.a.	n.a.	>31	>31	>31	>250	>250	>250	175	150
122	n.a.	n.a.	n.a.	<1	<1	<1	>250	>250	62	87.5	100
123	n.a.	n.a.	n.a.	<2	<2	<2	>500	>500	n.a.	150	45
124	n.a.	n.a.	>500	<2	>6	<2	>500	>500	>200	25	9
125	n.a.	n.a.	n.a.	<2	<2	<2	>250	>250	>8	45	12
126	n.a.	n.a.	n.a.	<2	<2	<2	>250	>250	>250	24	12
127	n.a.	-	-	n.a.	n.a.	-	-	-	-	-	-
138	250	-	-	125	125	-	-	-	-	-	-
139	250	-	-	62.5	62.5	-	-	-	-	-	-
171	250	-	-	125	125	-	-	-	-	-	-
197a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1000	600
197b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	600	500
197c	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1000	1000

207a	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	n.a.	n.a.	1000	200
207b	n.a.	n.a.	n.a.	>31	>125	>125	n.a.	n.a.	n.a.	600	125
207c	n.a.	n.a.	n.a.	>500	n.a.	n.a.	n.a.	n.a.	n.a.	1000	375
208a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	800	750
208b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	900	375
208c	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	800	700
210	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	500	400	375

APPENDIX G: ANTICANCER ACTIVITY

G.1 High-throughput screen

The anticancer high-throughput screen was performed at the Target Discovery Institute (TDI), in collaboration with Dr Daniel Ebner's research group. The screen was performed with four cancer cell lines: HeLa, H460, MCF7 and PC3 and it was ran twice, with a different set of compounds each time.

G.1.1 General methods and materials

HeLa and H460 cells were purchased from American Type Culture Collection (ATCC). MCF7 cells were provided by Prof Xin Lu from Ludwig Institute for Cancer Research. PC3 cells were provided by Dr Richard Bryant from Ludwig Institute for Cancer Research.

Cell banks of HeLa and H460 cells were prepared at the Department of Oncology, University of Oxford, and MCF7 and PC3 cells were obtained from Miss Alison Howarth and Dr Madalina Raducu respectively.

HeLa, H460 and MCF7 cells were grown in DMEM medium (Sigma) and PC3 cells in RPMI 1640 medium (Lonza). All media were supplemented with 10% FBS. All cultures were maintained in a humidified incubator at 37 °C with 5% CO₂.

Cell passage: cells were grown to confluence, washed with PBS (Oxoid Ltd) and incubated in Trypsin/EDTA (Sigma) for 5 min at 37 °C. Lifted cells were resuspended in 3-5 times the original volume of culture medium by gentle pipetting and divided over fresh culture dishes.

Frozen storage: Subconfluent cultures were detached in Trypsin/EDTA, further resuspended in PBS. Cells were centrifuged for 5 min at 1200 rpm in a bench top centrifuge. Cells were then

resuspended in freezing medium (DMEM with 20% FBS and 10% DMSO) and transferred to cryo-vials (1 mL per vial) for storage at -80 °C or transferred to liquid nitrogen after 1-2 days at -80 °C.

Recovery of frozen cells: Cells were rapidly thawed by addition of pre-warmed culture medium to the cryo-vial before transfer to 10 mL of culture medium. Cells were centrifuged for 5 min at 800 rpm, the supernatant was removed and the cells were resuspended in culture medium and transferred to a culture flask.

All cell lines were tested for mycoplasma using MycoAlert (Lonza)

G.1.2 Screening protocol

The protocol followed had been previously designed by the Ebner group.

Stock solutions of the compounds tested were prepared at 10 mM in DMSO, and the set of approved oncology drugs (for full list see Section G.1.3) was purchased at the same concentration. Serial dilutions were performed using an Echo 550 acoustic dispenser in 384-well plates (Corning 3656).

Cells (75 µL) were added to 384-well plates (PerkinElmer CellCarrier 6007558) using a PerkinElmer Janus liquid handling workstation. The number of cells added was previously optimised for each cell line and is indicated in Table G.1.

Table G.1 Number of cells seeded for each cell line.

Cell line	Cell number / well
HeLa	800
H460	1000
MCF7	1200
PC3	2750

Cells were incubated at 37 °C, 5% CO₂ for 16-22 h, then 5 µL of compound solutions were added to give final concentrations of 10 µM, 1 µM, 100 nM and 10 nM. Addition of the compounds was performed every 2 hours for each concentration, from lowest to highest. Each concentration was performed in duplicate. Several wells in each plate were also treated with positive control (15 nM Docetaxel) and negative control (0.1% DMSO).

Cells exposed to the compounds were incubated for 72 h before removing the media and adding 20 µL of the previously prepared dye solution. The solution of live cell dyes was prepared in the dark in phenol-free DMEM medium to give the concentrations indicated in Table G.2.

Table G.2. Volume of stock solutions used to prepare 50 mL of live cell dye solution in phenol-free medium, and final concentrations.

Live cell dye	Stock solution	Volume	Final concentration
Hoechst 33342	16 mM, diluted to 160 µM	312.5 µL	1 µM
Annexin V	-	750 µL	15 µL/mL
TMRM	1 mM	50 µL	1 µM
Yo-Pro 3	1 mM	50 µL	1 µM

Plates were incubated for another 1 h and then read on a PerkinElmer Operetta High Content Imager, at three fields per well. The setup parameters are indicated in Table G.3

Table G.3. Setup parameters used for the high-content imaging with Operetta.

Channel / dye	Main emission wavelength [nm]	Main excitation wavelength [nm]	Exposure [ms]
Hoechst 33342	445	380	25
Annexin V	525	475	40
TMRM	595	535	5
Yo-Pro 3	705	630	20
Brightfield	0	740	5

G.1.3 FDA-approved drugs

A set of approved oncology drugs was purchased from the National Cancer Institute, which contained the 114 FDA-approved drugs indicated in Table G.4.

Table G.4. FDA-approved drugs used for the high-throughput screen.

NSC number	CAS number	Drug name (USAN)
32065	127-07-1	Hydroxyurea
1390	315-30-0	Allopurinol
19893	51-21-8	Fluorouracil
752	154-42-7	Thioguanine
755	50-44-2	Mercaptopurine
762	55-86-7	Mechlorethamine hydrochloride
6396	52-24-4	Thiotepa
18509	5451-09-2	Aminolevulinic acid hydrochloride
362856	85622-93-1	Temozolomide
750	55-98-1	Busulfan
13875	645-05-6	Altretamine
27640	50-91-9	Floxuridine
45923	298-81-7	Methoxsalen
79037	13010-47-4	Lomustine
102816	320-67-2	Azacitidine
127716	2353-33-5	Decitabine
409962	154-93-8	Carmustine
26271	6055-19-2	Cyclophosphamide
34462	66-75-1	Uracil mustard
63878	69-74-9	Cytarabine hydrochloride
66847	50-35-1	Thalidomide
77213	366-70-1	Procarbazine hydrochloride
85998	18883-66-4	Streptozocin
105014	4291-63-8	Cladribine
109724	3778-73-2	Ifosfamide
119875	15663-27-1	Cisplatin
122758	302-79-4	Tretinoin
169780	24584-09-6	Dexrazoxane

218321	53910-25-1	Pentostatin
686673	121032-29-9	Nelarabine
701852	149647-78-9	Vorinostat
713563	107868-30-4	Exemestane
719344	120511-73-1	Anastrozole
719345	112809-51-5	Letrozole
747972	191732-72-6	Lenalidomide
775351	19171-19-8	Pomalidomide
3088	305-03-3	Chlorambucil
26980	50-07-7	Mitomycin
38721	53-19-0	Mitotane
606869	123318-82-1	Clofarabine
25154	54-91-1	Pipobroman
71423	595-33-5	Megestrol acetate
138783	3543-75-7	Bendamustine hydrochloride
312887	75607-67-9	Fludarabine phosphate
681239	179324-69-7	Bortezomib
712807	154361-50-9	Capecitabine
719627	169590-42-5	Celecoxib
750690	557795-19-4	Sunitinib
757441	319460-85-0	Axitinib
279836	65271-80-9	Mitoxantrone
698037	137281-23-3	Pemetrexed
715055	184475-35-2	Gefitinib
755986	879085-55-9	Vismodegib
756645	877399-52-5	Crizotinib
740	59-05-2	Methotrexate
609699	119413-54-6	Topotecan hydrochloride
732517	863127-77-9	Dasatinib
737754	635702-64-6	Pazopanib hydrochloride
743414	152459-95-5	Imatinib
747971	284461-73-0	Sorafenib
747974	84449-90-1	Raloxifene
750691	439081-18-2	Afatinib

754230	146464-95-1	Pralatrexate
755605	915087-33-1	Enzalutamide
760766	443913-73-3	Vandetanib
761431	1029872-54-5	Vemurafenib
763932	755037-03-7	Regorafenib
256439	57852-57-0	Idarubicin hydrochloride
747599	641571-10-0	Nilotinib
747973	219989-84-1	Ixabepilone
754143	128517-07-7	Romidepsin
758253	26833-87-4	Omacetaxine mepesuccinate
758487	943319-70-8	Ponatinib
761068	849217-68-1	Cabozantinib
765694	380843-75-4	Bosutinib
82151	23541-50-6	Daunorubicin hydrochloride
123127	25316-40-9	Doxorubicin hydrochloride
141540	33419-42-0	Etoposide
180973	54965-24-1	Tamoxifen citrate
256942	56390-09-1	Epirubicin hydrochloride
702294	52205-73-9	Estramustine phosphate sodium
745750	231277-92-2	Lapatinib
616348	100286-90-6	Irinotecan hydrochloride
719276	129453-61-8	Fulvestrant
758246	871700-17-3	Trametinib
764134	1195768-06-9	Dabrafenib mesylate
122819	29767-20-2	Teniposide
246131	56124-62-0	Valrubicin
758252	868540-17-4	Carfilzomib
628503	114977-28-5	Docetaxel
761432	183133-96-2	Cabazitaxel
125973	33069-62-4	Paclitaxel
49842	143-67-9	Vinblastine sulfate
67574	2068-78-2	Vincristine sulfate
226080	53123-88-9	Sirolimus
733504	159351-69-6	Everolimus

3053	50-76-0	Dactinomycin
24559	18378-89-7	Plicamycin
125066	9041-93-4	Bleomycin sulfate
608210	125317-39-7	Vinorelbine tartrate
683864	162635-04-3	Temsirolimus
613327	122111-03-9	Gemcitabine hydrochloride
369100	99011-02-6	Imiquimod
761388	110078-46-1	Plerixafor
45388	4342-03-4	Dacarbazine
92859	1327-53-3	Arsenic trioxide
9706	51-18-3	Triethylenemelamine
241240	41575-94-4	Carboplatin
718781	183319-69-9	Erlotinib hydrochloride
296961	20537-88-6	Amifostine
721517	118072-93-8	Zoledronic acid
266046	61825-94-3	Oxaliplatin
8806	3223-07-2	Melphalan hydrochloride
749226	154229-19-3	Abiraterone

G.1.4 Image analysis and viability results

The generated data was analysed by Columbus software, which had been previously optimised by Miss Alison Howarth to extract 460 features using the data collected from the high-content imager. The full list of features, with details of the building blocks used and the information extracted from the different channels is provided in a separate file on the CD.

Viability of the compounds was obtained using the information from the Hoechst dye (blue channel), which gave the number of cells per well for each concentration of compound. Cytotoxicity of the compounds was evaluated by comparison with the negative control, and it was calculated as the percentage number of cells in the well treated with the compound over the number of cells in the well treated with negative control. The full data is provided in Excel files on the CD.

G.2 Viability assay

Determination of the IC₅₀ values of selected compounds was performed by Dr Frances Willenbrock and Dr Chris Towers, from the research group of Dr Valentine Macaulay at the CRUK prevalidation facility.

G.2.1 Materials and methods

The SKOV-3 cells were purchased from ATCC and KMS-B12 from DSMZ, H460 cells were provided by Dr Remko Prevo and MCF7 by Dr Guillaume Rieunier. HeLa cells were already owned by the lab. All cell lines were authenticated. Hoechst 33342 was purchased from Sigma, and propidium iodide from Biolegend.

Stock solutions of the compounds were prepared at 10 mM in DMSO, stored at -15 °C and defrosted each time before use.

Cells were plated in 96-well dishes (Corning 3595), with the cell number per well previously optimised, and incubated overnight at 37 °C, 5% CO₂. The medium was then removed, and 150 µL of a serial dilution of the compounds in DMEM was added, with final concentrations ranging from 0.01-50 µM, and 0.1% DMSO except for the highest concentration (50 µM), which had 0.5% DMSO. After incubating for 3 days, Hoechst and propidium iodide solutions were added to give final concentrations of 1.5 µM and 0.75 µM respectively. Plates were incubated for 30 min in the dark and read in a Nexcelom Celigo cytometer.

G.2.2 Results

The concentration of compounds resulting in 50% growth inhibition (IC_{50}) was calculated for each compound and each cell line in GraphPad Prism, and is indicated in Table G.5 for the active compounds. The assays were performed in duplicates.

Table G.5. IC_{50} values of compounds tested at the CRUK prevalidation facility.

Compound	IC_{50} [μ M]				
	HeLa	H460	MCF7	SKOV-3	KMS-12BM
110a	14.5	20.3	16.7	31.5	16.2
	15.3	18	15.7	20.3	28.7
110b	9.3	9.2	9	21.9	7.15
	7.9	9.2	9.8	10.5	11
114	12.3	7.8	15.4	18.3	>50
	60	37.5	16.1	20.8	>50
		40.4			
122	11.9	15.4	11.8	19.2	>50
	36.3	35.9	14.2	30.6	>50
		36			
125	15.3	14.2	17.8	23.3	28.3
	20.6	17.8	16.8	20.2	>50
127	10.5	12.1	8.9	25.6	9.5
	10.4	13.2	10	10.1	13.6
207c	27	>50	>50	>50	>50
	-	-	-	-	31.3
299	>50	>50	>50	20.4	>50
	>50	>50	-	-	-

APPENDIX H: PHYSICOCHEMICAL PROPERTIES

Physicochemical properties of the compounds tested for biological activity were determined using Calculator Plugins in Marvin 16.7.25.0, 2016, ChemAxon (<http://www.chemaxon.com>), and are indicated in Table H.1.

Table H.1. Physicochemical properties of synthesised compounds tested for antibacterial activity. %PSA = (PSA/MSA)*100.

Compound	MW	clogP	clogD _{7.4}	PSA	MSA	%PSA	H-bond donor count	H-bond acceptor count
Compounds from Chapter 2								
44a	255.27	1.29	1.29	72.91	393.14	18.55	0	4
44b	271.33	1.74	1.74	88.98	397.44	22.39	0	3
44c	269.30	1.70	1.70	72.91	423.41	17.22	0	4
77a	342.35	0.15	-3.36	105.61	501.19	21.07	1	6
77b	358.41	0.60	-2.88	121.68	508.76	23.92	1	5
77c	356.38	0.56	-2.94	105.61	532.77	19.82	1	6
79	327.33	1.09	-2.44	102.37	482.98	21.20	1	5
81a	297.31	1.22	-1.19	93.14	435.18	21.40	1	5
81b	313.37	1.67	-0.67	109.21	439.55	24.85	1	4
81c	311.33	1.64	-0.72	93.14	464.45	20.05	1	5
86	297.31	0.68	0.68	82.14	440.69	18.64	0	4
87	342.35	0.94	0.94	94.61	509.53	18.57	0	5
93	298.34	0.56	0.56	68.31	468.82	14.57	0	5
94a	359.38	2.59	1.01	93.14	509.20	18.29	1	5
94b	375.44	3.04	1.51	109.21	514.43	21.23	1	4
94c	373.40	3.00	1.47	93.14	537.92	17.31	1	5
95a	389.40	2.43	0.84	102.37	558.01	18.35	1	6
95b	405.46	2.88	1.33	118.44	563.33	21.02	1	5
95c	403.43	2.85	1.29	102.37	586.87	17.44	1	6
96a	385.42	3.20	0.96	93.14	542.24	17.18	1	5
96b	401.48	3.65	1.46	109.21	547.41	19.95	1	4
96c	399.44	3.61	1.41	93.14	571.84	16.29	1	5

114	377.44	3.40	1.35	93.14	560.91	16.61	1	5
116l	356.38	0.26	0.26	94.61	538.48	17.57	0	6
117	341.36	1.20	1.20	91.37	520.44	17.56	0	5
122	419.52	4.73	2.65	93.14	653.50	14.25	1	5
404	361.48	4.73	2.86	66.84	576.39	11.60	1	4
123	461.60	6.06	3.98	93.14	746.07	12.48	1	5
124	381.47	3.96	1.59	93.14	618.58	15.06	1	5
125	423.55	5.30	2.89	93.14	710.14	13.12	1	5
126	465.63	6.63	4.22	93.14	802.90	11.60	1	5
127	461.60	5.46	5.46	82.14	751.68	10.93	0	5
138	402.40	1.59	-0.97	119.17	538.34	22.14	1	6
139	418.46	2.52	0.06	134.27	546.28	24.58	1	6
171	421.45	2.04	-0.17	111.60	593.46	18.80	1	7
129	435.52	3.95	1.72	105.67	669.70	15.78	1	6
186	365.38	1.23	-0.97	113.37	509.20	22.26	2	6
100	274.23	-2.50	-6.00	125.40	366.61	34.21	3	6
105	229.19	-1.42	-3.99	112.93	298.46	37.84	3	5
107	359.33	0.99	-1.36	119.00	469.23	25.36	2	5
98a	383.40	2.43	2.43	82.14	525.97	15.62	0	5
98b	399.46	2.88	2.88	98.21	530.48	18.51	0	4
98c	397.43	2.85	2.85	82.14	555.10	14.80	0	5
115	377.44	3.19	3.19	82.14	573.77	14.32	0	4
109	239.27	1.22	-0.95	66.84	358.58	18.64	1	4
110a	327.38	3.19	1.17	66.84	525.97	12.71	1	4
110b	343.44	3.64	1.67	82.91	471.10	17.60	1	3
110c	341.41	3.61	1.62	66.84	495.23	13.50	1	4

Compounds from Chapter 3

197a	257.29	0.37	0.37	76.07	403.26	18.86	1	4
197b	273.35	0.82	0.82	92.14	407.59	22.61	1	3
197c	271.31	0.78	0.78	76.07	432.37	17.59	1	4
207a	197.23	1.28	1.28	46.61	316.90	14.71	0	3
207b	213.30	1.73	1.73	62.68	322.83	19.42	0	2
207c	211.26	1.70	1.70	46.61	346.72	13.44	0	3
208a	199.25	0.51	0.51	49.77	326.85	15.23	1	3

208b	215.31	0.97	0.97	65.84	332.56	19.80	1	2
208c	213.28	0.93	0.93	49.77	356.11	13.98	1	3
210	239.27	1.44	1.44	55.84	366.30	15.24	0	3
217	371.55	2.89	2.89	65.07	639.72	10.17	0	4
239	358.39	0.54	0.51	94.61	565.06	16.74	0	6
248	343.38	1.25	1.25	91.37	543.16	16.82	0	5
255	281.31	1.36	1.36	72.91	423.02	17.24	0	4
256	343.38	2.78	2.78	72.91	499.51	14.60	0	4
257	373.41	2.62	2.62	82.14	548.48	14.98	0	5
258	369.42	3.72	3.72	72.91	532.30	13.70	0	4
259	367.40	3.73	3.73	72.91	519.56	14.03	0	4
260	326.35	0.82	0.82	85.38	492.62	17.33	0	5
261	311.33	1.76	1.76	82.14	473.38	17.35	0	4
264	454.26	1.68	1.68	85.38	538.18	15.86	0	5

Compounds from Chapter 4

52	283.32	2.39	2.39	72.91	457.79	15.93	0	4
297	283.32	1.28	1.28	65.07	447.70	14.53	0	4
298	225.29	2.38	2.38	46.61	381.47	12.22	0	3
299	215.20	-0.26	-0.26	92.70	321.29	28.85	2	4
303	285.34	1.47	1.47	76.07	468.48	16.24	1	4
304	255.31	1.16	1.16	66.84	420.50	15.90	1	4
305	257.33	0.85	0.85	70.00	435.20	16.08	2	4
306	217.22	-1.18	-1.18	95.86	330.85	28.97	3	4
308	185.18	-0.66	-0.66	75.63	267.30	28.29	2	3
319	299.37	2.11	2.11	65.07	503.81	12.92	0	4
321	231.25	-0.53	-0.53	84.86	367.98	23.06	2	4
323	217.22	-0.68	-4.02	95.86	332.56	28.82	3	5
332I	285.34	1.19	1.19	76.07	467.09	16.29	1	4
332II	285.34	1.19	1.19	76.07	467.90	16.26	1	4
334I	217.22	-1.45	-1.45	95.86	330.84	28.97	3	4
334II	217.22	-1.45	-1.45	95.86	331.38	28.93	3	4
336	185.18	-0.93	-0.94	75.63	268.04	28.22	2	3
339I	391.46	2.51	2.51	85.30	619.71	13.76	1	5
339II	391.46	2.51	2.51	85.30	619.61	13.77	1	5

352I	323.34	-0.13	-0.13	105.09	483.40	21.74	3	5
352II	323.34	-0.13	-0.13	105.09	483.24	21.75	3	5
420I	309.32	-0.28	-0.28	116.09	448.01	25.91	4	6
420II	309.32	-0.28	-3.63	116.09	446.65	25.99	4	6
340	291.30	0.39	0.39	84.86	418.55	20.27	2	4
341	291.30	0.39	0.39	84.86	420.48	20.18	2	4
343	405.49	2.93	2.93	85.30	648.37	13.16	1	5
421	337.37	0.28	0.28	105.09	510.91	20.57	3	5
345	305.33	0.80	0.80	84.86	447.21	18.98	2	4
346	305.33	0.80	0.80	84.86	448.56	18.92	2	4
348I	301.34	0.14	0.14	96.30	476.29	20.22	2	5
350	269.30	0.66	0.66	76.07	409.21	18.59	1	4
353	365.38	0.31	0.31	111.16	545.21	20.39	2	5
356	499.56	1.19	1.19	120.01	807.42	14.86	1	9
358	491.58	4.35	4.35	117.23	802.20	14.61	1	5
361	325.40	2.30	2.30	76.07	527.65	14.42	1	4
425	307.39	3.11	3.11	55.85	492.69	11.34	0	3
362	343.42	1.17	1.17	96.30	568.89	16.93	2	5
363	457.56	2.49	2.49	111.60	776.46	14.37	1	6
365	411.54	3.27	3.27	85.30	699.27	12.20	1	5
366	393.52	4.08	4.08	65.07	662.96	9.82	0	4
391	406.32	2.71	2.71	76.07	576.35	13.20	1	4