Total Syntheses of Subereamollines A and B

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General Experimental Procedures

Materials All reactions not involving aqueous solutions or reagents were performed under an atmosphere of argon in oven-dried glassware cooled under vacuum unless otherwise stated. For mixtures of solvents, the ratios given refer to the volumes used. All reagents were obtained from commercial suppliers and used without further purification. Flash column chromatography was performed using Merck Kieselgel (230-400 mesh). All chromatography solvents were distilled before use. NMR spectra were recorded at room temperature on Bruker DPX-400, DRX-500 and DRX-600 spectrometers using the deuterated solvent as internal deuterium lock; 1H spectra at 400, 500 and 600 MHz respectively, and 13C spectra at 100, 125 and 150 MHz. Chemical shifts are reported in parts per million (ppm) and internal references were the residual protic solvent: for d1-chloroform, δH = 7.26 ppm and δC = 77.0 ppm; for d4-methanol, δH = 3.31 ppm and δC = 49.0 ppm; for d6-acetone δH = 2.05 ppm and δC = 29.84 ppm. Coupling constants (J) are reported in Hz and recorded to the nearest 0.1 Hz. In reporting the spectral data, the following abbreviations were used: br = broad, s = singlet, d = doublet, t = triplet, q = quartet. Spectra were assigned with information gained from DEPT, COSY, HMBC and HMQC experiments. Melting points were obtained on a Reichert hot stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum I FTIR spectrometer. Samples were applied neat. Only selected absorbances (νmax) are reported. Peaks are reported in wavenumbers (cm⁻¹). HRMS were recorded on a Waters Micromass LCT Premier spectrometer and were reported in units of m/z. Microanalysis was performed in the microanalytical laboratories at the Department of Chemistry, University of Cambridge. Optical rotations were measured on a Perkin Elmer 343 Polarimeter. Chiral HPLC was performed on HP Agilant 1100.
2-(Benzyl oxy)-3,5-dibromo-4-methoxybenzaldehyde (8)

To a stirred solution of 2-hydroxy-4-methoxybenzaldehyde (7) (8.1 g, 53.3 mmol) in DMF (20 mL) at 0 ºC was added N-bromosuccinimide (19.0 g, 107 mmol) in DMF (40 mL) dropwise over 30 min. After stirring at 0 ºC for a further 20 min Et2O (600 mL) was added and the mixture was washed with H2O (500 mL × 2), 10% Na2S2O3 (400 mL × 3), brine (500 mL), dried over MgSO4, filtered and evaporated to dryness in vacuo to furnish 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (15.3 g, 93%) as a white solid. To a stirred solution of 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (11.2 g, 36.3 mmol), sodium iodide (5.45 g, 36.3 mmol), anhydrous potassium carbonate (12.0 g, 87.0 mmol) in DMF (58 mL) was added benzyl chloride (4.18 mL, 36.3 mmol). The mixture was stirred at room temperature for 13 h before partitioning between distilled water (500 mL) and diethyl ether (500 mL). The aqueous phase was extracted with Et2O (500 mL × 2) and the combined organic layers were washed with 5% LiCl solution (300 mL × 2), H2O (500 mL × 2), brine (500 mL), dried over MgSO4 and evaporated to dryness in vacuo to furnish 8 (14.3 g, 99%) as a white solid.

Rf 0.49 (10% Et2O/Petrol); νmax (thin film)/cm⁻¹1689 s, 1577 m, 1366 s, 1161 s; δH (400 MHz, CDCl₃) 9.94 (1H, s, H-7), 7.99 (1H, s, H-5), 7.40 (5H, m, H-3'/7', H-4'/6' and H-7'), 5.13 (2H, s, H-1'), 3.98 (3H, s, H-8); δC (100 MHz, CDCl₃) 187.1 (C-7), 160.4 (C-3), 159.3 (C-1), 134.9 (C-2'), 131.6 (C-5), 129.2 (C-4'/6'), 128.91 (C-5'), 128.87 (C-3'/7'), 128.4 (C-6), 115.4 (C-4), 114.5 (C-2), 78.1 (C-1'), 61.0 (C-8); m/z (ESI+) found 420.9044, [M+Na]⁺ C₁₅H₁₂Br₂O₃Na requires 420.9045.

Consistent with literature data.
(Z)-4-(2-(Benzyloxy)-3,5-dibromo-4-methoxybenzylidene)-2-methyloxazol-5(4H)-one (9)

A slurry of 8 (6.4 g, 16.0 mmol), sodium acetate (1.31 g, 16.0 mmol) and N-acetylglycine (1.87 g, 16.0 mmol) in acetic anhydride (30 mL) was heated to 120 ºC for 6 h. The reaction mixture was allowed to cool to room temperature and the resulting precipitate isolated by filtration. The filtrate was dissolved in CH$_2$Cl$_2$ (200 mL), washed with distilled water (200 mL), dried over MgSO$_4$ and evaporated to dryness *in vacuo* to afford 9 (6.93 g, 90%) as an orange solid which was used without further purification:

For characterisation purposes, a sample was purified by flash column chromatography (50% CH$_2$Cl$_2$/Petrol) to furnish 9 as a bright yellow solid.

R$_f$ 0.65 (20% EtOAc/Petrol); ν$_{max}$ (thin film)/cm$^{-1}$ 2944 w, 1802 s, 1655 s, 1599 s, 1192 s; δ$_H$ (400 MHz, CDCl$_3$) 8.87 (1H, s, H-5), 7.44-7.36 (5H, m, H-3'/7', H-4'/6' and H-7'), 7.23 (1H, s, H-7), 4.99 (2H, s, H-1'), 3.95 (3H, s, H-12), 2.39 (3H, s, H-11); δ$_C$ (100 MHz, CDCl$_3$) 167.0 (C-10), 166.8 (C-9), 157.1 (C-3), 156.4 (C-1), 135.2 (C-2'), 134.7 (C-5), 133.3 (C-8), 128.9 (C-4'/6'), 128.71 (C-5'), 128.69 (C-3'/7'), 126.3 (C-6), 122.7 (C-7), 114.7 (C-4), 113.8 (C-2), 77.3 (C-1'), 60.8 (C-12), 15.6 (C-11); m/z (ESI+) found 479.9464, [M+H]$^+$ C$_{19}$H$_{16}$Br$_2$NO$_4$ requires 479.9446; *elem. anal.* C$_{19}$H$_{15}$Br$_2$NO$_4$ requires C 47.43%, H 3.14 %, N 2.91 % found C 47.06 %, H 3.10 %, N 2.80 %.

Consistent with literature data.ii
(E)-3-(2-(Benzyloxy)-3,5-dibromo-4-methoxyphenyl)-2-((benzyloxy)imino)propanoic acid (10)

A solution of 9 (4 g, 8.32 mmol) and Ba(OH)₂•8H₂O (18.3 g, 58.2 mmol) in 1,4-dioxane (60 mL) and H₂O (60 mL) was heated to 60 ºC. After 1 h O-benzylhydroxylamine (3.07 g, 25.0 mmol) was added and the mixture was stirred for a further 16 h. The reaction mixture was cooled to 0 ºC, acidified to pH 0 with 3N HCl and extracted with CH₂Cl₂ (250 mL × 3). The combined organic layers were dried over MgSO₄, evaporated to dryness in vacuo and the resulting residue triturated with petroleum ether to afford 10 (2.31 g, 4.10 mmol, 49%) as an off white solid. The remaining liquor was evaporated to dryness in vacuo and the resulting residue was purified by flash column chromatography (10% Et₂O/Petrol) to furnish 11 (924 mg, 22%) as a white solid.

10: Rₜ 0.59 (10% MeOH/ CH₂Cl₂); ν max (thin film)/cm⁻¹ 2991 br, 1701 s, 1455 s, 1427 s, 1216 s, 993 s; δH (400 MHz, CDCl₃) 7.50-7.48 (2H, m, H-10'/14'), 7.39-7.33 (6H, m, Ph-H), 7.21-7.19 (2H, m, Ph-H), 7.16 (1H, s, H-5), 5.22 (2H, s, H-1'), 5.01 (2H, s, H-8'), 3.89 (2H, s, H-7), 3.88 (3H, s, H-10); δC (100 MHz, CDCl₃) 163.4 (C-9), 154.3 (C-1), 154.1 (C-3), 149.3 (C-8), 136.4 (C-9'), 135.6 (C-2'), 131.9 (C-5), 128.7 (C-6), 128.6 (Ph-C), 128.4 (Ph-C), 128.3 (C-5' or C-12'), 128.2 (Ph-C), 128.03 (C-5' or C-12'), 127.96 (Ph-C), 114.6 (C-4), 112.8 (C-2), 78.4 (C-1'), 74.7 (C-8'), 60.6 (C-10), 25.3 (C-7); HRMS (ESI+): m/z: 583.9688 (calcd for C₂₄H₂₁Br₂NO₅Na, 583.9679).

Consistent with literature data.ii

2-(Benzyloxy)-3,5-dibromo-4-methoxybenzaldehyde O-benzyl oxime (11) Rₜ 0.84 (30% Et₂O/Petrol); ν max (thin film)/cm⁻¹ 2945 w, 1599 w, 1580 w, 1457 m, 1365 s, 1155 s, 943 s; δH (400 MHz, CDCl₃) 8.40 (1H, s, H-7), 8.20 (1H, s, H-5), 7.59-7.43 (10H, s, Ph-H), 5.36 (2H, s, H-1'), 5.05 (2H, s, H-1 H-1''), 4.04 (3H, s, H-8); δC (100
MHz, CDCl₃) 156.0 (C-3), 154.4 (C-1), 142.8 (C-7), 137.0 (C-2’), 135.4 (C-2”), 128.9 (Ph-C), 128.28 (Ph-C), 128.25 (Ph-C), 128.14 (Ph-C), 128.06 (Ph-C), 128.02 (Ph-C), 127.7 (Ph-C), 124.7 (C-6), 114.6 (C-4), 113.6 (C-2), 76.4, 76.2, 60.4 (C-8); m.pt. 69-71 °C; elem. anal. C₂₂H₁₉Br₂NO₃ requires C 52.3 %, H 3.79 %, N 2.77 %, found C 51.85 %, H 3.79 %, N 2.76 %.

***(E)-Methyl-3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)-2-(hydroxyimino)propanoate (13)***

To a stirred solution of 10 (307 mg, 0.545 mmol) in toluene/MeOH (3:1, 8 mL) at 0 °C was added TMS diazomethane (2.0 M in hexane, 409 uL, 0.812 mmol) dropwise. The mixture was stirred for 1 h at room temperature and quenched by the addition of 3 N HCl (100 uL). The solvent was removed *in vacuo* and the residue partitioned between H₂O (30 mL) and EtOAc (30 mL). The aqueous phase was extracted with EtOAc (30 mL × 2) and the combined organic layers were dried (anhyd. MgSO₄) and evaporated to dryness *in vacuo* to furnish (E)-methyl-3-(2-(benzyloxy)-3,5-dibromo-4-methoxyphenyl)-2-((benzyloxy)imino)propanoate (300 mg, 95%) as an off white solid. A mixture of (E)-methyl-3-(2-(benzyloxy)-3,5-dibromo-4-methoxyphenyl)-2-((benzyloxy)imino)propanoate (100 mg, 0.173 mmol) and palladium black (18 mg, 0.17 mmol) in dioxane/AcOH (1:1, 2.46 mL) was stirred at room temperature under an H₂ atmosphere for 3 h. The mixture was filtered through a pad of celite and the solvent removed *in vacuo*. The residue was taken up in EtOAc (15 mL), washed with water (15 mL), dried (anhyd. MgSO₄). The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (25% EtOAc/Hexane) to furnish 13 (63 mg, 92%) as a white solid.
Rf 0.37 (30% EtOAc/Hexanes); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1} \) 3267 br, 1730 s, 1593 w, 1015 s; \( \delta_{\text{H}} \) (400 MHz, CDCl\(_3\)) 7.40 (1H, s, H-5), 3.92 (2H, s, H-7), 3.90 (3H, s, H-11), 3.85 (3H, s, C-10); \( \delta_{\text{C}} \) (100 MHz, CDCl\(_3\)) 164.4 (C-9), 153.8 (C-3), 151.5 (C-1), 149.9 (C-8), 133.4 (C-5), 119.4 (C-4), 107.6 (C-2), 60.5 (C-11), 53.4 (C-10), 25.5 (C-7); \( m/z \) (ESI+) found 395.9100, [M+H]+ C\(_{11}\)H\(_{12}\)Br\(_2\)NO\(_5\) requires 395.9082.

The \(^{13}\text{C}\) chemical shift for C-6 is not visible, however, this is consistent with literature data. \(^{\text{iii}}\)

**Methyl 7,9-dibromo-8-methoxy-10-oxo-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxylate (14)**

To a stirred solution of 13 (381 mg, 1 mmol) in MeCN (45 mL) at 0 °C was added iodobenzene diacetate (354 mg, 1.1 mmol). After 2 h, H\(_2\)O (50 mL) was added and the mixture stirred for a further 10 min at room temperature. The mixture was extracted with CH\(_2\)Cl\(_2\) (200 mL × 2) and the combined organic extracts were dried (anhyd. MgSO\(_4\)) and evaporated to dryness in vacuo to furnish (±)-14 a dark orange oil which was used without further purification.

For characterisation purposes, a sample was purified by flash column chromatography (10-40% EtOAc/Hexane) to furnish (±)-14 as a white solid.

Rf 0.62 (40% EtOAc/Hexanes); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1} \) 1725 s, 1679 m, 1239 s, 732 s; \( \delta_{\text{H}} \) (400 MHz, CDCl\(_3\)) 6.77 (1H, s, H-5), 4.18 (3H, s, H-10), 3.91 (3H, s, H-11), 3.62 (1H, d, \( J = 17.8 \) Hz, H-7a), 3.29 (1H, d, \( J = 17.8 \) Hz, H-7b); \( \delta_{\text{C}} \) (100 MHz, CDCl\(_3\)) 188.6 (C-1), 163.2 (C-9), 159.6 (C-3), 149.9 (C-8), 135.9 (C-5), 120.9 (C-4), 106.7 (C-2), 86.8 (C-6), 62.1 (C-11), 53.1 (C-10), 44.4 (C-7).

Consistent with literature data. \(^{\text{iv}}\)
Methyl 7,9-dibromo-10-hydroxy-8-methoxy-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxylate (6)

To a stirred solution of (±)-14 (1 mmol) in CH₂Cl₂ (5 mL) at room temperature was added a freshly prepared solution of Zn(BH₄)₂ (0.21 M in Et₂O, 1.73 mL). After 10 min, H₂O (0.4 mL) was added and the mixture stirred for a further 20 min. Following the addition of anhyd. MgSO₄, the mixture was filtered and concentrated in vacuo. The residue was purified by careful flash column chromatography (10-20% EtOAc/Petrol) to furnish (±)-6a (122 mg, 32% over 2 steps) and (±)-6b (60 mg, 16% over 2 steps from 13) as pale yellow oils. Both compounds could be crystallized by slow evaporation from Et₂O.

(5S*,10R*)-Methyl 7,9-dibromo-10-hydroxy-8-methoxy-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxylate (6a) Rᶠ 0.53 (40% EtOAc/Petrol); νmax (thin film)/cm⁻¹ 3370 br, 2958 w, 1732 s, 1578 m, 1431 m, 1268 s; δH (400 MHz, d6-acetone) 6.51 (1H, s, H-5), 5.38 (1H, d, J 8.1 Hz, OH), 4.22 (1H, d, J 8.1 Hz, H-1), 3.84 (1H, d, J 18.2 Hz, C-7a), 3.83 (3H, s, H-11), 3.73 (3H, s, H-10), 3.21 (1H, d, J 18.2); δC (100 MHz, d₆-acetone) 161.1 (C-9), 152.4 (C-8), 148.7 (C-3), 132.0 (C-5), 122.1 (C-4), 113.7 (C-2), 92.4 (C-6), 75.1 (C-1), 60.2 (C-11), 52.8 (C-10), 39.9 (C-7); m.pt. 134-136 ºC; m/z (ESI+) found 395.9099, [M+H]⁺ C₁₁H₁₂Br₂NO₅ requires 395.9082.

Consistent with literature data.¹

The enantiomers were separated using preparative chiral HPLC (CHIRALPAK AD-H column and 4% iPr₂OH in hexane at a flow rate of 5 mL/min). Retention times: (+)-6a = 30.9 min; (–)-6a = 32.9 min
(+)-6a \( [\alpha]_D^{27.9} +165.5 \) \((c 0.325, \text{C}_6\text{H}_6)\) \(-\)\(-\)
\[\\alpha\]_D^{27.9} –150.9 \((c 0.43, \text{C}_6\text{H}_6)\)

\{lit. \[\alpha\]_D^{26} +217.1 \((c 1.0, \text{C}_6\text{H}_6)\}\}

\{lit. \[\alpha\]_D^{26} –210.3 \((c 1.0, \text{C}_6\text{H}_6)\}\}

(5S*,10S*)-Methyl 7,9-dibromo-10-hydroxy-8-methoxy-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxylate (6b) \( R_f 0.47 \) \((40\% \text{EtOAc/Petrol})\); \( \nu_{\text{max}} \) \((\text{thin film})/\text{cm}^{-1}\) 3433 br, 2938 w, 1725 s, 1597 m, 1575 m, 1441 s, 1248 s; \( \delta_H \) \((400 \text{MHz, d}_6\text{-acetone})\) 6.61 \((1\text{H, s, H-5})\), 5.00 \((1\text{H, d, J 8.5 Hz, OH})\), 4.56 \((1\text{H, d, J 8.4 Hz, H-1})\), 3.82 \((3\text{H, s, H-11})\), 3.71 \((3\text{H, s, H-10})\), 3.48 \((1\text{H, d, J 17.9 Hz, H-7a})\), 3.40 \((1\text{H, d, J 17.9 Hz, H-7b})\); \( \delta_C \) \((100 \text{MHz, d}_6\text{-acetone})\) 161.2 \((\text{C-9})\), 152.3 \((\text{C-8})\), 148.4 \((\text{C-3})\), 132.8 \((\text{C-5})\), 121.1 \((\text{C-4})\), 115.5 \((\text{C-2})\), 91.4 \((\text{C-6})\), 75.2 \((\text{C-1})\), 60.1 \((\text{C-11})\), 52.8 \((\text{C-10})\), 43.3 \((\text{C-7})\); m.pt. 125-127 \(^{\circ}\)\(\text{C}\); \( m/z \) \((\text{ESI}+)\) found 395.9073, \([\text{M+H}]^+\) \(\text{C}_{11}\text{H}_{12}\text{Br}_2\text{NO}_5\) requires 395.9082.

Consistent with literature data\(^{\text{v}}\)

(5S*,10R*)-7,9-Dibromo-10-hydroxy-8-methoxy-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxylic acid (3)

To a solution of trans \((\pm)-6\) \((100 \text{mg, 0.252 mmol})\) in \(\text{H}_2\text{O}/\text{MeOH}\) \((1:3, 15 \text{ml})\) was added \(\text{LiOH}\) monohydrate \((32 \text{mg, 0.723 mmol})\). After 70 min \(3\text{ N HCl}\) \((1.5 \text{mL})\) was added followed by \(\text{H}_2\text{O}\) \((25 \text{mL})\). The mixture was extracted with \(\text{EtOAc}\) \((25 \text{mL} \times 2)\)
and the combined organic extracts were dried (anhyd. MgSO₄) and evaporated to dryness in vacuo to furnish (±)-3 (96 mg, 0.250 mmol, 99%) as a light brown foam.

ν<sub>max</sub> (thin film)/cm<sup>-1</sup> 3402 br, 2978 w, 2935 w, 1709 s, 1598 m, 1431 m, 1255 s; δ<sub>1H</sub> (600 MHz, CD₃OD) 6.43 (1H, s, H-5), 4.11 (1H, s) 3.75 (1H, d, J 18.3 Hz, C-7a), 3.73 (3H, s, H-10) 3.10 (1H, d, J 18.3 Hz, H-7b); δ<sub>C</sub> (150 MHz, CD₃OD) 162.6 (C-9), 153.8 (C-3), 149.3 (C-8), 132.2 (C-5), 122.8 (C-4), 114.1 (C-2), 93.1 (C-6), 75.5 (C-1), 60.4 (C-10), 43.3 (C-7).

Consistent with literature data.<sup>vi</sup>

**Ethyl (4-aminobutyl)carbamate (4)**

To a stirred solution of butane-1,4-diamine (15) (943 mg, 10.6 mmol) in absolute EtOH (13 mL) at room temperature was added a solution of phenyl ethyl carbonate (1.77 g, 10.6 mmol) in absolute EtOH (2 mL) in a dropwise fashion. After 12 h, the solvent was evaporated in vacuo and the residue was taken up in H₂O (25 mL) and acidified to pH 2-3 by the dropwise addition of 3 N HCl. The aqueous phase was washed with CH₂Cl₂ (50 mL × 3), basified by the addition of 10% aqueous sodium hydroxide solution and extracted with CH₂Cl₂ (50 mL × 3). The combined organic layers were dried over MgSO₄ and evaporated to dryness in vacuo to furnish 4 (1.36 g, 80%) as a pale yellow oil.

ν<sub>max</sub> (thin film)/cm<sup>-1</sup> 3328 w, 2954 w, 1683 s, 1531 s; δ<sub>1H</sub> (400 MHz, CDCl₃) 5.28 (1H, br s, NH), 3.96 (2H, q, J 6.8 Hz, H-2), 3.03 (2H, br s, H-4), 2.58 (2H, t, J 6.7 Hz, H-7), 1.45-1.30 (4H, m, H-5 and H-6), 1.10 (3H, t, J 7.0 Hz, H-1), 1.03 (2H, br s, NH₂); δ<sub>C</sub> (125 MHz, CDCl₃) 156.6 (C-3), 60.4 (C-2), 41.7 (C-7), 40.6 (C-4), 30.7 (C-6), 27.3 (C-5), 14.5 (C-1); m/z (ESI+) found 161.1287, [M+H]<sup>+</sup> C₇H₁₇N₂O₂ requires 161.1285.
Ethyl (5-aminopentyl)carbamate (5)

To a stirred solution of pentane-1,5-diamine (16) (2.19 mL, 18.7 mmol) in absolute EtOH (70 mL) at room temperature was added phenyl ethyl carbonate (3.10 g, 18.7 mmol) in absolute EtOH (5 mL) in a dropwise fashion. After 12 h the solvent was evaporated in vacuo and the residue was taken up in H2O (50 mL) and acidified to pH 2-3 by the dropwise addition of 3 N HCl. The aqueous phase was washed with CH2Cl2 (100 mL × 3), basified by the addition of 10% aqueous sodium hydroxide solution and extracted with CH2Cl2 (100 mL × 3). The combined organic layers were dried over MgSO4 and evaporated to dryness in vacuo to furnish 5 (2.67 g, 15.3 mmol, 82%) as a pale yellow oil:

Rf 0.11 (12% MeOH/CH2Cl2); νmax (thin film)/cm−1 3316 w, 2932 w, 1690 s, 1540 s, 1255 s; δH (400 MHz, CDCl3) δ 4.99 (1H, br s, NH), 4.01 (2H, q, J 7.0 Hz, H-2), 3.07 (2H, q, J 6.5 Hz, H-4), 2.60 (2H, t, J 6.9 Hz, H-8), 1.46-1.34 (4H, m, H-5 and H-7), 1.27-1.24 (2H, m, H-6), 1.22 (2H, br s, NH2), 1.14 (3H, t, J 7.0 Hz, H-1); 13C NMR (100 MHz, CDCl3) δ 156.6 (C-3), 60.3 (C-2), 41.8 (C-8), 40.6 (C-4), 33.1 (C-7), 29.7 (C-5), 23.8 (C-6), 14.5 (C-1); m/z (ESI+) found 175.1447, [M+H]+ C8H19N2O2, requires 175.144.

Subereamolline A. Ethyl (4-((5S,10R)-7,9-dibromo-10-hydroxy-8-methoxy-1-oxa-2-azaspiro[4.5]deca-2,6,8-triene-3-carboxamido)butyl)carbamate (1)

Procedure 1. To a stirred solution of (±)-3 (25 mg, 0.0653 mmol) and HOBt (11.5 mg, 0.0849 mmol) in CH2Cl2 (2 mL) at 0 °C was added a solution of DCC (17.5 mg, 0.0849 mmol) in CH2Cl2 (1 mL). After 10 min, a solution of amine 4 (13.6 mg, 0.0849 mmol) in CH2Cl2 (2 mL) was added in one portion and the mixture was
stirred at room temperature. After 18 h H₂O (20 mL) and CH₂Cl₂ (15 mL) were added and the organic phase removed. The aqueous layer was extracted with CH₂Cl₂ (20 mL × 2) and the combined organic layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (20-60% EtOAc/Petrol) to furnish (±)-subereamolline A (1) (31.3 mg, 91%) as a colourless oil.

**Procedure 2.** To a stirred solution of (±)-3 (44 mg, 0.115 mmol), 4 (18.4 mg, 0.115 mmol) and iPr₂EtN (50 µL, 0.288 mmol) in CH₂Cl₂ (1.2 mL) at 0 ºC was added propylphosphonic anhydride (®T3P) (82 µL of a 50% wt.% in EtOAc, 0.138 mmol) dropwise. After 2 h H₂O (10 mL) and CH₂Cl₂ (10 mL) were added and the organic phase removed. The aqueous layer was extracted with CH₂Cl₂ (10 mL × 2) and the combined organic layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (40-60% EtOAc/Petrol) to furnish (±)-subereamolline A (1) (58 mg, 96%) as a colourless oil.

Rᵥ 0.42 (60% EtOAc/Petrol); νₘₐₓ (thin film)/cm⁻¹ 3330 br, 2936 w, 1658 s, 1536 m, 1438 m, 1260 m; δₜ (500 MHz, CD₃OD) 6.42 (1H, s, H-5), 4.08 (1H, s, H-1), 4.05 (2H, q, J 7.1 Hz, H-15), 3.77 (1H, d, J 18.3 Hz, H-7a), 3.73 (3H, s, H-17), 3.29 (2H, hidden t, J 6.9 Hz, H-10), 3.11 (2H, t, J 6.7 Hz, H-13), 3.10 (1H, d, J 18.1 Hz, H-7b), 1.60-1.49 (4H, m, H-11 and H-12), 1.22 (3H, t, J 7.1 Hz, H-16); δₙ (125 MHz, CD₃OD) 161.5 (C-9), 159.2 (C-14), 155.3 (C-8), 149.3 (C-3), 132.3 (C-5), 122.7 (C-4), 114.1 (C-2), 92.3 (C-6), 75.5 (C-1), 61.6 (C-15), 60.4 (C-17), 41.2 (C-13), 40.2 (C-7), 40.1 (C-10), 28.3 (C-12), 27.6 (C-11), 15.0 (C-16); m/z (ESI+) found 545.9872, [M+Na⁺] C₁₇H₂₃Br₂N₃O₆Na requires 545.9851.

The enantiomers were separated using preparative chiral HPLC (CHIRALPAK AD-H column and 10% iPr₂OH in hexane at a flow rate of 5 mL/min). Retention times: (+)-1 = 40.3 min; (−)-1 = 43.6 min.
Subereamolline B. Ethyl (5-((5S,10R)-7,9-dibromo-10-hydroxy-8-methoxy-1-oxa-
2-azaspiro[4.5]deca-2,6,8-triene-3-carboxamido)pentyl)carbamate (2)

**Procedure 1.** To a stirred solution of (+)-3 (17 mg, 0.0444 mmol) and HOBt (7.2
mg, 0.0533 mmol) in CH$_2$Cl$_2$ (2 mL) at 0 ºC was added a solution of DCC (11 mg,
0.0533 mmol) in CH$_2$Cl$_2$ (1 mL). After 10 min, a solution of amine 5 (9.3 mg,
0.0533 mmol) in CH$_2$Cl$_2$ (1 mL) was added in one portion and the mixture was
stirred at room temperature. After 18 h H$_2$O (20 mL) and CH$_2$Cl$_2$ (15 mL) were
added and the organic phase removed. The aqueous layer was extracted with
CH$_2$Cl$_2$ (20 mL × 2) and the combined organic layers were dried over MgSO$_4$ and
concentrated in vacuo. The residue was purified by flash column chromatography
(20-60% EtOAc/Petrol) to furnish (+)-subereamolline B (2) (18 mg, 0.0334, 75%)
as a colourless oil.

**Procedure 2.** To a stirred solution of (+)-3 (51.5 mg, 0.134 mmol), 4 (23.4 mg,
0.134 mmol) and iPr$_2$EtN (58.5 µL, 0.336 mmol) in CH$_2$Cl$_2$ (1.3 mL) at 0 ºC was
added propylphosphonic anhydride (iT$_3$P) (96 µL of a 50% wt.% in EtOAc, 0.161
mmol) dropwise. After 2 h H$_2$O (10 mL) and CH$_2$Cl$_2$ (10 mL) were added and the
organic phase removed. The aqueous layer was extracted with CH$_2$Cl$_2$ (10 mL × 2)
and the combined organic layers were dried over MgSO$_4$ and concentrated in vacuo.
The residue was purified by flash column chromatography (40-60%
EtOAc/Petrol) to furnish (±)-suberemolline B (2) (70 mg, 97%) as a colourless oil.

R_f 0.45 (60% EtOAc/Petrol); ν_{max} (thin film)/cm^{-1} 3349 br, 2934 w, 1663 s, 1439 m, 1260 m; δ_{H} (500 MHz, CD_{3}OD) 6.42 (1H, s, H-5), 4.08 (1H, s, H-1), 4.05 (2H, q, J 7.1 Hz, H-16), 3.77 (1H, d, J 18.3 Hz, H-7a), 3.73 (3H, s, H-18), 3.28 (2H, t, J 7.1 Hz, H-10), 3.10-3.07 (2H, m, H-14), 3.09 (1H, hidden d, J 18.5 Hz, H-7b), 1.60-1.54 (2H, m, H-11), 1.54-1.48 (2H, m, H-13), 1.39-1.33 (2H, m, H-12), 1.22 (3H, t, J 7.1 Hz, H-17); δ_{C} (125 MHz, CD_{3}OD) 161.5 (C-9), 159.2 (C-15), 155.3 (C-8), 149.3 (C-3), 132.3 (C-5), 122.7 (C-4), 114.1 (C-2), 92.3 (C-6), 75.5 (C-1), 61.6 (C-16), 60.4 (C-18), 41.5 (C-14), 40.3 (C-7), 40.2 (C-10), 30.6 (C-13), 30.0 (C-11), 25.1 (C-12), 15.0 (C-17); m/z (ESI+) found 560.0029, [M+Na]^+ C_{18}H_{25}Br_{2}N_{3}O_{6}Na requires 560.0008.

The enantiomers were separated using preparative chiral HPLC (CHIRALPAK AD-H column and 10% iPr_{2}OH in hexane at a flow rate of 5 mL/min). Retention times: (+)-2 = 48.3 min; (−)-2 = 52.9 min.

(+)-2 [α]_{b}^{27.7} +55.4 (c 0.33, CH_{2}Cl_{2})

(−)-2 [α]_{b}^{27.6} −50.0 (c 0.29, CH_{2}Cl_{2})

{lit. [α]_{b}^{28} +22.9 (c 6.25, CH_{2}Cl_{2})}
$^1$H NMR spectrum of synthetic Subereamolline A (CD$_3$OD 300 MHz)

$^1$H NMR spectrum of synthetic Subereamolline A (CD$_3$OD 500 MHz)

$^{13}$C NMR spectrum of natural Subereamolline A (CD$_3$OD 75 MHz)
$^{13}$C NMR spectrum of synthetic Subereamolline A (CD$_3$OD 125 MHz)

$^1$H NMR spectrum of natural Subereamolline B (CD$_3$OD 300 MHz)
$^1$H NMR spectrum of synthetic Subereamolline B (CD$_3$OD 500 MHz)

$^{13}$C NMR spectrum of natural Subereamolline B (CD$_3$OD 75 MHz)
$^{13}$C NMR spectrum of synthetic Subereamolline B (CD$_3$OD 125 MHz)

Analytical Chiral HPLC
The following HPLC analyses were performed on an analytical CHIRALPAK AD-H column. Conditions: 10% iPr₂OH in hexane at a flow rate of 1 mL/min.

Trace for (±)-1

Trace for (+)-1 synthesised from (+)-6a.

Trace for (±)-2
Trace for (−)-2 synthesised from (+)-6a.

Biological Assays

Cell culture and MTS assay SK-OV-3 cells were obtained from Cell Services (CRUK LRI) grown in T75 flasks (NUNC™), and maintained in RPMI-1640 medium (Gibco) supplemented with 10% (v/v) FBS (Invitrogen™) at 37 °C in humidified air containing 5% CO₂. Cell viability was assessed using the Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay. Single cell suspensions were prepared and seeded into 96 well plates (96F Nunclon Delta microwell) with 3 × 10^4 cells/mL (90 µL/well). Cells were cultured for 24 h before the addition of agent in culture medium (10 µL) at the desired concentration. Drug samples were dissolved in DMSO (to form a 0.1 M stock solution) and diluted with the culture medium to the
required concentrations. The final DMSO concentration did not exceed 0.1% to the total incubation solution (v/v) and a vehicle control was conducted with 0.1% DMSO concentration in each assay. Cells were incubated at 37 ºC for 48 h. MTS reagent was aliquoted to each well (20 µL) and the plates were incubated at 37 ºC for 2 h. Absorbance at 490 nm was determined for each well using a TECAN infinite M200. Each experimental point was performed in triplicate.

References


