Promysalin is a salicylate-containing antimicrobial with a cell-membrane-disrupting mechanism of action on Gram-positive bacteria

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1) Synthesis

1.1 General information

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries by a SMP3 apparatus and are uncorrected. $^1$H spectra were recorded on Bruker AMX 300 MHz and Bruker AV600 spectrometers. TMS was used as an internal standard and the chemical shifts were reported in parts per million ($\delta$). The peak patterns are indicated as follows: $s$, singlet; $d$, doublet; $dd$, doublet of doublet; $t$, triplet; $m$, multiplet; $q$, quartet. The coupling constants, $J$ are reported in Hertz (Hz) and $^{13}$C NMR spectra were recorded on Bruker AMX 300 MHz and Bruker AV600 spectrometers. Optical rotations were measured with a Perkin Elmer 241 polarimeter. The elemental analyses were recorded with a CARLO ERBA EA 1108 instrument. The accurate mass spectra were recorded using Bruker Daltonics model Autoflex III, accurate mass MALDI TOF/TOF MS/MS. Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et$_2$O) were obtained by distillation from sodium-benzophenone ketyl; dry methylene chloride was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow and all glassware were oven dried and/or flame dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230-400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 F$_{254}$, aluminum foil). Compounds on TLC plates were detected under UV light at 254 and 365 nm or were revealed spraying with 10% phosphomolybdic acid (PMA) in ethanol.
1.2 General procedures

Scheme S1: Synthetic routes to compounds 2-8: a) i. aroyl chloride, NEt$_3$, 0 °C to 80 °C, 3h, ii. LiBHe$t_3$, toluene, -78 °C, 1 h, then DIPEA, cat. DMAP, TFAA, -78 °C to rt, 3 h; b) LiOH, EtOH : H$_2$O, 0 °C to rt, 5 h; c) i) 2,4,6-trichlorobenzoyl chloride, NEt$_3$, THF, 0 °C to rt, 2h, ii) alcohol, DMAP, toluene, 0 °C to rt, 12 h; d) TBAF, THF, 0 °C to rt, 1h; e) TiCl$_4$, -20 °C, 15 min.; f) L-proline ethyl ester.HCl, EDCI, HOBT; g) 1N HCl, THF, 0 °C to rt; h) TFA, CH$_2$Cl$_2$, rt, 30 min.
**General procedure A: acylation of ethyl L-pyroglutamate.**

NEt$_3$ (2 eq.), followed by acid chloride (1.2 eq.) were added dropwise to a stirred solution of ethyl L-pyroglutamate (1 eq.) in toluene (0.5 M) at 0 °C under N$_2$ atmosphere. The mixture was stirred at 80 °C for 3 h and cooled to room temperature. Sat. NaHCO$_3$ was added and the organic layer was separated. The aqueous layer was extracted with EtOAc ($\times$ 2). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The product was purified using flash column chromatography in 0-30% EtOAc/hexane.

**General procedure B: reductive elimination.**

To a stirred solution of acylated pyroglutamate (1 eq.) in dry toluene (0.2 M) was added Superhydride® (lithium triethylborohydride) (1.2 eq., 1M in THF) at -78 °C under N$_2$ atmosphere. The mixture was stirred at -78 °C for 1 h, then DMAP (0.1 eq.) and DIPEA (5.7 eq.) were added, followed by very slow addition of TFAA (1.2 eq.). The reaction mixture was gradually warmed to room temperature and stirred for 3 h. Water ($\times$ 10) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate ($\times$ 2); the combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified using flash column chromatography in 0-50 % ethyl acetate: hexane.

**General procedure C: hydrolysis of ethyl ester.**

To a solution of ethyl ester (1 eq.) was added dropwise a solution of LiOH (1.5 eq.) in water (EtOH : H$_2$O 2:1, 0.08 M) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 5 h. EtOH was removed *in vacuo*, the aqueous layer was washed with 40 % ethyl acetate in diethyl ether ($\times$ 2), cooled to 0 °C and acidified using 5% citric acid. The product was extracted using 5% CH$_3$OH : CH$_2$Cl$_2$ ($\times$ 3). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo* to afford carboxylic acid.

**General procedure D: Yamagouchi esterification.**

NEt$_3$ (3 eq.) followed by 2,4,6-trichlorobenzoyl chloride (2 eq.) were added dropwise to a stirred solution of acid (1 eq.) in THF (0.03 M) at 0 °C under N$_2$ atmosphere. The mixture was warmed to room temperature and stirred for 2 h. THF was removed *in vacuo* and the residue was dissolved in toluene (0.03M). DMAP (3 eq.) followed by alcohol (0.8 eq.) in toluene were added at 0 °C under N$_2$ atmosphere. The resulting suspension was stirred overnight at room temperature. EtOAc ($\times$ 15) was added, the organic layer was washed with sat. NH$_4$Cl, brine, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The product was purified by flash column chromatography 0-70 % EtOAc: hexane.

**General procedure E: MEM deprotection.**

To a stirred solution of MEM ether (1 eq.) in CH$_2$Cl$_2$ (0.15 M) was added TiCl$_4$ (2 eq., 1M in CH$_2$Cl$_2$) at -20 °C under N$_2$ atmosphere. The reaction mixture was stirred at -20 °C for 10 min; then aqueous
ammonia (20 times) was added. The aqueous layer was extracted with ethyl acetate ($\times 2$), and the combined organic extracts were washed with brine and dried over anhydrous Na$_2$SO$_4$. The solvent was removed in vacuo. The product was purified using preparative TLC.

General procedure F: TBDPS deprotection.

TBAF (3 eq. 1M in THF) was added dropwise to a stirred solution of silyl ether (1 eq.) in THF (0.2 M) at 0 °C. The reaction mixture was stirred at room temperature for 1h. Sat. NH$_4$Cl was added. The aqueous layer was extracted with ethyl acetate ($\times 2$). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo. The product was purified using preparative TLC.
1.3 Experimental procedures

Promysalin (compound 1) was synthesized as described in the literature.¹

1.3.1 Synthesis of compound 2 (1-Benzoyl-2,3-dihydro-1H-pyrrole-2-carboxylic acid 7-carbamoyl-1-hexyl-7-hydroxy-heptyl ester)

*Ethyl (2S)-1-benzoyl-2,3-dihydro-1H-pyrrole-2-carboxylate (S2)*

Using general procedure A, ethyl L-pyroglutamate (500 mg, 3.28 mmol) yielded ethyl (S)-1-benzoyl-5-oxopyrrolidine-2-carboxylate (S1) as a creamy solid (620 mg, 74 %); Rᵢ (25 % EtOAc: Hexane) = 0.25, [α]²³D = + 23.0 (c 1.00, CHCl₃). mp = 79–80 °C. ¹H NMR (300 MHz, CDCl₃) δ: 7.70–7.63 (2H, m), 7.58–7.50 (1H, m), 7.46–7.37 (2H, m), 4.89 (1H, dd, J = 3.9, 8.9 Hz), 4.26 (2H, q, J = 7.1 Hz), 2.83–2.68 (1H, m), 2.66–2.38 (1H, m), 2.23–2.09 (1H, m), 1.30 (3H, t, J = 7.1 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 173.7, 171.2, 170.6, 134.0, 132.5, 129.3 (×2), 128.1 (×2), 62.1, 59.0, 32.0, 22.1, 14.3. Anal. Calcd. for C₁₄H₁₅NO₄: C, 64.36; H, 5.79; N, 5.36. Found: 64.25; H, 5.78; N, 5.37.

Using general procedure B the ester S1 (478 mg, 1.83 mmol) yielded title compound S2 as a colourless oil (200 mg, 44 %); Rᵢ (30 % EtOAc: Hexane) = 0.4; [α]²³D = - 138.6 (c 1.00, CHCl₃).

¹H NMR (600 MHz, acetone-d₆) δ: 7.60–7.31 (5H, m); 6.57 (1H, s); 5.17 (1H, s); 4.95 (1H, dd, J = 5.1, 11.7 Hz); 4.28–4.12 (2H, m); 3.20–3.12 (1H, m); 2.70–2.63 (1H, m); 1.35–1.19 (3H, m). ¹³C NMR (150 MHz, acetone-d₆) δ: 170.5, 166.0, 135.6, 130.6, 130.4, 128.4 (× 2), 127.6 (× 2), 108.4, 60.6, 58.4, 33.4, 13.5. Anal. Calcd. for C₁₄H₁₅NO₃: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.64; H, 6.15; N, 5.72.
Using general procedure C the ester S2 (170 mg, 0.69 mmol) was hydrolyzed to give (S)-1-benzoyl-2,3-dihydro-1H-pyrrole-2-carboxylic acid (S3) as a pale yellow solid (130 mg). R_f (5 % MeOH : CH_2Cl_2) = 0.2. [α]_D = -85.3 (c 1.20, CHCl_3). ^1H NMR (300 MHz, CD_2OD) δ: 7.62-7.55 (2H, m); 7.52-7.42 (3H, m); 6.51-6.47 (1H, m); 5.26-5.21 (1H, m); 4.84 (1H, dd, J = 4.5, 11.2 Hz); 3.18-3.05 (1H, m); 2.78-2.67 (1H, m); ^13C NMR (150 MHz, CD_2OD) δ: 177.3, 168.0, 135.4, 130.9, 128.4, 127.66 (× 2), 111.2, 60.6, 34.2, 26.5. Compound S3 was used for the next step without further purification.

NEt_3 (0.09 mL, 0.69 mmol) followed by 2,4,6-trichlorobenzoyl chloride (0.07 mL, 0.46 mmol) were added dropwise to a stirred solution of the above acid S3 (50 mg, 0.23 mmol) in THF (4.1 mL, 0.03 M) at 0 °C under N_2 atmosphere. The mixture was warmed to room temperature and stirred for 2h. THF was removed in vacuo and the residue was dissolved in toluene (3 mL). DMAP (84 mg, 0.69 mmol) followed by (2R,8R)-2-((tert-butyldiphenylsilyl)oxy)-8-hydroxytetradecanamide (S4) \(^1\) (57 mg, 0.115 mmol) in toluene were added at 0 °C under N_2 atmosphere. The resulting suspension was stirred overnight at room temperature. EtOAc (8 mL) was added, the organic layer was washed with sat. NH_4Cl (5 mL), brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude silyl ether (54 mg, 0.076 mmol) was dissolved in THF (1 mL) and cooled to 0 °C; TBAF (0.22 mL, 0.23 mmol, 1M in THF) was added dropwise under N_2 atmosphere. The reaction mixture was stirred at room temperature for 1 h. Sat. NH_4Cl (7 mL) was added and the aqueous layer was extracted with ethyl acetate (2 × 7 mL) The combined organic extracts were washed with brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified preparative TLC in 6% MeOH : CH_2Cl_2, to afford the title compound 2 as a colourless oil (28 mg, 52 % over two steps). R_f (5 % MeOH : CH_2Cl_2) = 0.4. [α]_D = -42.0 (c 0.45, CHCl_3). ^1H NMR (300 MHz, CDCl_3) δ: 7.57-7.36 (5H, m); 6.85-6.72 (1H, m); 6.53-6.46 (1H, m); 5.20-5.14 (1H, m); 5.13-5.01 (1H, m); 4.97 (1H, dd, J = 4.1, 12.1 Hz); 4.27 (1H, m); 4.11-4.02 (1H, m); 3.50 (1H, brs); 3.15-3.07 (1H, m); 2.77-2.64 (1H, m); 1.90-1.17 (20H, m); 0.88 (3H, t, J = 6.9 Hz); ^13C NMR (75 MHz, CDCl_3) δ: 177.6, 170.7, 167.9, 134.8, 131.1, 130.9, 128.8 (× 2), 127.8 (× 2), 110.0, 75.3, 70.7, 58.7, 34.9, 34.2, 34.1, 33.7, 31.9, 29.3, 27.6, 25.7, 24.8, 24.3, 22.8, 14.3. Anal.
Calcd. for C$_{26}$H$_{38}$N$_2$O$_5$: C, 68.10; H, 8.35; N, 6.11. Found: C, 68.01; H, 8.33; N, 6.12. HRMS: (ES$^+$) calculated for C$_{26}$H$_{38}$N$_2$O$_5$Na (M + Na)$^+$ 481.26729, Found: 481.26861.
1.3.2. Synthesis of compound 3. (R)-14-amino-14-oxotetradecan-7-y1 (S)-1-(2-hydroxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate.

Using general procedure D, (S)-1-(2-((2-methoxyethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylic acid S5 (50 mg, 0.15 mmol) was reacted with (R)-8-hydroxytetradecanamide (11, see below) (32 mg, 0.139 mmol) to give (R)-14-amino-14-oxotetradecan-7-yl (S)-1-(2-((2-methoxyethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate S6 (40 mg, 54%) as a colourless oil. Rf (4 % MeOH:CH2Cl2) = 0.3. [α]D23 = -54.5 (c 0.75, CHCl3). 1H NMR (300 MHz, CDCl3) δ: 7.41-7.31 (2H, m); 7.22 (1H, dd, J = 8.2, 1.0 Hz); 6.30 (1H, brs); 6.21-6.14 (1H, m); 5.28 (2H, s); 5.09-4.91 (3H, m); 3.84-3.78 (2H, m); 3.57-3.50 (2H, m); 3.36 (3H, s); 3.20-3.07 (1H, m); 2.73-2.63 (1H, m); 2.42-2.34 (1H, m); 2.19 (2H, t, J = 7.5 Hz); 1.72-1.18 (20H, m); 0.87 (3H, t, J = 6.7 Hz); 13C NMR (75 MHz, CDCl3) δ = 176.3, 170.8, 165.3, 153.6, 131.6, 131.0, 128.9, 125.9, 115.6, 109.0, 94.0, 75.5, 71.7, 68.2, 59.2, 58.2, 36.1, 34.7, 34.4, 31.9, 29.4, 28.9, 28.6, 25.6, 25.2, 24.8, 22.8, 14.3. Anal. Calcd. for C30H46N2O7: C, 65.91; H, 8.48; N, 5.12. Found: C, 65.83; H, 8.50; N, 5.10.

Using general procedure E, compound S6 (85 mg, 0.159 mmol) was deprotected to afford the title compound 3 as a colourless oil (38 mg, 52%). Rf (2 % MeOH:CH2Cl2) = 0.4. [α]D23 = -50.1 (c 1.5, CHCl3). 1H NMR (300 MHz, CDCl3) δ: 7.45-7.33 (2H, m); 6.99 (1H, dd, J = 8.2, 1.0 Hz); 6.89 (1H, ddd, J = 8.2, 8.2, 1.0 Hz); 6.79 (1H, brs); 5.67 (1H, brs); 5.32-5.16 (2H, m); 5.09-4.91 (2H, m); 3.23-3.05 (1H, m); 2.76-2.62 (1H, m); 2.20 (2H, t, J = 7.6 Hz); 1.74-1.11 (20H, m); 0.86 (3H, t, J = 6.9 Hz). 13C NMR (75 MHz, CDCl3) δ = 176.1, 170.1, 167.5, 158.8, 133.5, 131.1, 128.5, 119.2, 118.1, 117.5, 110.8, 76.1, 59.6, 36.1, 34.4, 34.2, 31.9, 29.3, 29.1, 29.0, 25.5 × 3, 25.0, 22.7, 14.3. Anal. Calcd. for C26H38N2O5: C, 68.10; H, 8.35; N, 6.11. Found: C, 68.02; H, 8.37; N, 6.11. HRMS: (ES+) calculated for C26H38N2O5Na (M + Na)+ 481.26729, Found: 481.26829.
1.3.3. Synthesis of compound 4. (7R,13R)-14-amino-13-hydroxy-14-oxotetradecan-7-yl (S)-1-(2-methoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate

*Ethyl (S)-1-(2-methoxybenzoyl)-5-oxopyrrolidine-2-carboxylate (S7)*

Using general procedure A, ethyl L-pyroglutamate (500 mg, 3.28 mmol) afforded compound S7 as a white solid (723 mg, 77 %); R_f (30 % EtOAc: Hexane) = 0.4. mp = 93-94 °C. [α]_D^23 = + 65.5 (c 1.00, CHCl₃). ¹H NMR (600 MHz, CDCl₃) δ: 7.44 (1H, ddd, J = 8.4, 7.7, 1.7 Hz); 7.33 (1H, dd, J = 7.7, 1.7 Hz); 7.01 (1H, dd, J = 7.7, 7.7 Hz); 6.93 (1H, d, J = 8.4 Hz); 4.92 (1H, dd, J = 9.5, 2.8 Hz); 4.29 (2H, q, J = 6.9 Hz); 3.83 (3H, s); 2.73-2.66 (1H, m); 2.57-2.52 (1H, m); 2.47-2.40 (1H, m); 2.19-2.13 (1H, m); 1.34 (3H, t, J = 7.1 Hz).

¹³C NMR (150 MHz, CDCl₃) δ: 173.1, 171.4, 168.3, 157.2, 132.3, 129.0, 125.3, 120.8, 111.3, 62.1, 58.6, 56.1, 53.8, 31.9, 22.1, 14.5. Anal. Calcd. for C₁₅H₁₇NO₅: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.95; H, 5.89; N, 4.80.

Using general procedure B, ethyl (S)-1-(2-methoxybenzoyl)-5-oxopyrrolidine-2-carboxylate S7 (723 mg, 2.48 mmol) afforded compound S8 as a clear oil (405 mg, 60 %). R_f (30 % EtOAc: Hexane) = 0.5. [α]_D^23 = -117.8 (c 1.25, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.43-7.33 (2H, m); 7.00 (1H, ddd, J = 8.2, 8.2, 1.1 Hz); 6.94 (1H, d, J = 8.2 Hz); 6.18-6.12 (1H, m); 5.08-5.03 (1H, m); 5.00 (1H, dd, J = 5.02, 11.7 Hz); 4.34-4.19 (2H, m); 3.84 (3H, s); 3.20-3.05 (1H, m); 2.76-2.65 (1H, m); 1.32 (3H, t, J = 7.1 Hz);¹³C NMR (75 MHz, CDCl₃) δ: 171.2, 165.3, 156.1, 131.5, 131.0, 129.2, 125.1, 121.0, 111.6, 108.7, 61.6, 58.2, 56.0, 34.3, 14.3. Anal. Calcd. for C₁₅H₁₇NO₄: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.54; H, 6.21; N, 5.08.

*Using general procedure A, ethyl L-pyroglutamate (500 mg, 3.28 mmol) afforded compound S7 as a white solid (723 mg, 77 %); R_f (30 % EtOAc: Hexane) = 0.4. mp = 93-94 °C. [α]_D^23 = + 65.5 (c 1.00, CHCl₃). ¹H NMR (600 MHz, CDCl₃) δ: 7.44 (1H, ddd, J = 8.4, 7.7, 1.7 Hz); 7.33 (1H, dd, J = 7.7, 1.7 Hz); 7.01 (1H, dd, J = 7.7, 7.7 Hz); 6.93 (1H, d, J = 8.4 Hz); 4.92 (1H, dd, J = 9.5, 2.8 Hz); 4.29 (2H, q, J = 6.9 Hz); 3.83 (3H, s); 2.73-2.66 (1H, m); 2.57-2.52 (1H, m); 2.47-2.40 (1H, m); 2.19-2.13 (1H, m); 1.34 (3H, t, J = 7.1 Hz). ¹³C NMR (150 MHz, CDCl₃) δ: 173.1, 171.4, 168.3, 157.2, 132.3, 129.0, 125.3, 120.8, 111.3, 62.1, 58.6, 56.1, 53.8, 31.9, 22.1, 14.5. Anal. Calcd. for C₁₅H₁₇NO₅: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.95; H, 5.89; N, 4.80.*
Using general procedure C, compound S8 (250 mg, 0.908 mmol) was hydrolyzed to yield S9 ((S)-1-(2-methoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylic acid) as a yellow solid (185 mg, 82 %). Rf (4 % MeOH : CH₂Cl₂) = 0.4. mp = 145-146 °C. [α]D²³ = -82.8 (c 0.5, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.47-7.35 (2H, m); 7.03 (1H, ddd, J = 8.2, 8.2, 1.0 Hz); 6.96 (1H, d, J = 8.2 Hz); 6.07-6.02 (1H, m); 5.26-5.20 (1H, m); 5.13 (1H, dd, J = 4.3, 10.8 Hz); 3.84 (3H, s); 3.28-3.16 (1H, m); 3.11-2.96 (1H, m). Rf (4 % MeOH : CH₂Cl₂) = 0.4. mp = 145-146 °C. [α]D = -82.8 (c 0.5, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.47-7.35 (2H, m); 7.03 (1H, ddd, J = 8.2, 8.2, 1.0 Hz); 6.96 (1H, d, J = 8.2 Hz); 6.07-6.02 (1H, m); 5.26-5.20 (1H, m); 5.13 (1H, dd, J = 4.3, 10.8 Hz); 3.84 (3H, s); 3.28-3.16 (1H, m); 3.11-2.96 (1H, m). ¹³C NMR (75 MHz, CDCl₃) δ: 173.1, 167.3, 156.0, 132.2, 130.0, 129.3, 124.0, 121.1, 111.6, 111.3, 59.1, 56.0, 33.2. Anal. Calcd. for C₁₃H₁₃NO₄: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.23; H, 5.29; N, 5.66.

Using general procedure D, the above acid S9 (35 mg, 0.14 mmol) was reacted with S4 ((2R,8R)-2-((tert-butyldiphenylsilyl)oxy)-8-hydroxytetradecanamide) (56 mg, 0.112 mmol) to give (7R,13R)-14-amino-13-((tert-butyldiphenylsilyl)oxy)-14-oxotetradecan-7-yl (S)-1-(2-methoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate (S10) as a pale yellow oil (42 mg, 50 %). Rf (50 % EtOAc: hexane) = 0.3. [α]D²³ = -39.0 (c 0.4, CHCl₃). ¹H NMR (600 MHz, CDCl₃) δ: 7.67 (2H, d, J = 7.0 Hz); 7.62 (2H, d, J = 7.0 Hz); 7.50-7.34 (8H, m); 7.00 (1H, dd, J = 7.0, 7.0 Hz); 6.95 (1H, d, J = 8.3 Hz); 6.74 (1H, d, J = 4.5 Hz); 6.18-6.14 (1H, m); 5.58-5.54 (1H, m); 5.06-4.92 (3H, m); 4.27 (1H, dd, J = 3.9, 5.2 Hz); 3.85 (3H, s); 3.17-3.10 (1H, m); 2.71-2.66 (1H, m); 1.70-1.12 (29H, m); 0.98 (3H, t, J = 7.1 Hz). ¹³C NMR (150 MHz, CDCl₃) δ: 176.2, 170.8, 164.9, 155.9, 135.7 (× 2), 135.6 (× 2), 133.0, 132.6, 131.2, 130.9, 130.2, 130.1, 127.9 (× 2), 127.8 (× 2), 125.1, 120.8, 111.3, 108.3, 75.4, 74.2, 58.1, 55.7, 34.3, 34.2, 33.9, 33.8, 31.7, 29.7, 29.3, 29.2, 27.0 (× 2), 25.2, 24.8, 23.3, 22.6, 19.3, 14.0.

Compound S10 (30 mg, 0.041 mmol) was deprotected using general procedure F to give the title compound 4 as a colourless oil (19 mg, 99 %). Rf (4 % MeOH : CH₂Cl₂) = 0.3, [α]D²³ = -32.8 (c 0.5, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.44-7.37 (1H, m); 7.33 (1H, dd, J = 1.8, 7.6 Hz); 7.03-6.97 (1H, m); 6.95 (1H, d, J = 8.2 Hz); 6.91 (1H, brs); 6.18-6.12 (1H, m); 5.14-4.99 (3H, m); 4.96 (1H, dd, J = 4.5, 11.6 Hz); 4.36 (1H, d, J = 5.4 Hz); 4.11-4.01 (1H, m); 3.83 (3H, s); 3.21-3.07 (1H, m); 2.73-2.62 (1H, m); 1.91-1.17 (20H, m); 0.88 (3H, t, J = 6.7 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 177.9, 170.7, 166.0, 156.0, 131.9, 130.8, 129.0, 124.3, 121.1, 111.6, 109.8, 75.1, 70.5, 58.2, 56.0, 35.0, 34.3, 33.7, 31.9, 29.9, 29.3, 27.5, 25.7, 24.8, 24.4, 22.8, 14.3. Anal. Calcd. for C₂₇H₄₀N₂O₆: C, 66.37; H, 8.25; N, 5.73. Found: C, 66.21; H, 8.26; N, 5.72. HRMS: (ES⁺) calculated for C₂₇H₄₀N₂O₆Na (M + Na)⁺ 511.27786, Found: 511.28881.
1.3.4. Synthesis of compound 5. Heptyl (S)-1-(2-hydroxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate

Using general procedure F, compound S5 (S)-1-((2-methoxyethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylic acid 1 (50 mg, 0.15 mmol) was reacted with heptanol (0.026 mL, 0.19 mmol) to give heptyl (S)-1-((2-methoxyethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate S11 as a colourless oil (50 mg, 58 %). Rf (30 % EtOAc: hexane) = 0.2. [α]D = -85.7 (c 1.25, CHCl3).

1H NMR (300 MHz, CDCl3) δ: 7.39-7.32 (2H, m); 7.21 (1H, dd, J = 1.2, 8.2 Hz); 7.04 (1H, ddd, J = 1.2, 8.2, 8.2 Hz); 5.28 (2H, s); 5.05-5.01 (1H, m); 4.99 (1H, dd, J = 5.0, 11.8 Hz); 4.23-4.12 (2H, m); 3.83-3.76 (2H, m); 3.56-3.48 (2H, m); 3.35 (3H, s); 3.19-3.03 (1H, m); 2.74-2.63 (1H, m); 1.71-1.60 (2H, m); 1.42-1.18 (8H, m); 0.86 (3H, t, J = 6.7 Hz).

13C NMR (75 MHz, CDCl3) δ: 171.2, 165.1, 153.7, 131.4, 131.0, 129.1, 126.1, 122.3, 115.5, 108.7, 93.9, 71.7, 68.1, 65.8, 59.2, 58.1, 34.4, 31.9, 29.1, 28.8, 26.0, 22.8, 14.3. Anal. Calcd. for C23H33NO6 C, 65.85; H, 7.93; N, 3.34. Found: C, 65.73; H, 7.91; N, 3.33.

Using general procedure E the above MEM ether S11 (25 mg, 0.04 mmol) was deprotected to give the title compound 5 as a colourless oil (14 mg, 99 %). Rf (20 % EtOAc: hexane) = 0.4. [α]D = -112.7 (c 0.65, CHCl3).

1H NMR (300 MHz, CDCl3) δ: 9.78 (1H, s); 7.45-7.33 (2H, m); 7.01 (1H, dd, J = 1.2, 8.3 Hz); 6.88 (1H, ddd, J = 1.2, 8.3, 8.3 Hz); 6.84-6.80 (1H, m); 5.31-5.23 (1H, m); 5.03 (1H, dd, J = 5.3, 11.4 Hz); 4.27-4.09 (2H, m); 3.20-3.04 (1H, m); 2.78-2.67 (1H, m); 1.72-1.61 (2H, m); 1.40-1.16 (8H, m); 0.87 (3H, t, J = 7.1 Hz).

13C NMR (75 MHz, CDCl3) δ: 170.9, 167.6, 159.2, 134.1, 130.9, 128.3, 118.8, 118.0, 116.8, 110.6, 65.8, 59.3, 33.5, 31.7, 28.8, 28.5, 25.7, 22.5, 14.0. Anal. Calcd. for C19H25NO4: C, 68.86; H, 7.60; N, 4.23. Found: C, 68.68; H, 7.58; N, 4.24. HRMS: (ES+) calculated for C19H25NO4Na (M + Na)+ 354.16758, Found: 354.16798.
1.3.5. Synthesis of compound 6. Ethyl (S)-1-(2-hydroxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate

Using general procedure E ethyl (S)-1-(2-((2-methoxyethoxy)methoxy)benzoyl)-5-oxopyrrolidine-2-carboxylate\(^1\) (S12) (35 mg, 0.10 mmol) was deprotected to give the title compound 6 as a colourless oil (20 mg, 76 %), \(R_f\) (20 % EtOAc : hexane) = 0.3; \([\alpha]_D^{23} = -132.3\) (c 1.00, CHCl\(_3\)). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 9.76 (1H, s); 7.46-7.34 (2H, m); 7.00 (1H, dd, \(J = 1.2, 8.3\) Hz); 6.88 (1H, ddd, \(J = 8.3, 8.3, 1.2\) Hz); 6.84-6.78 (1H, m); 5.30-5.24 (1H, m); 5.02 (1H, dd, \(J = 5.2, 11.4\) Hz); 4.30-4.19 (2H, m); 3.18-3.05 (1H, m); 2.78-2.66 (1H, m); 1.29 (3H, t, \(J = 7.1\) Hz). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\): 171.1, 167.8, 159.3, 133.7, 131.1, 128.5, 119.1, 118.2, 117.0, 110.9, 61.9, 59.5, 53.6, 14.3. Anal. Calcd. for C\(_{14}\)H\(_{15}\)NO\(_4\): C, 64.36; H, 5.79, N, 5.36. Found: C, 64.52; H, 5.78; N, 5.34. HRMS: (ES+) calculated for C\(_{14}\)H\(_{15}\)NO\(_4\)Na (M + Na)\(^+\) 284.08933, Found: 284.08971.
1.3.6. Synthesis of compound S. (1-(2-hydroxybenzoyl)-pyrrolidine-2-carboxylic acid 7-carbamoyl-1-hexyl-7-hydroxyheptyl ester)

_Ethyl 2-(((2-methoxyethoxy)methoxy)benzoyl)-L-prolinate (S13)._ \[
\begin{array}{c}
\text{OH} \\
\text{OMEM} \\
\text{L-proline ethyl ester HCl, EDCI, HOBT} \\
\text{DIPEA, DMF, 0 °C to rt.} \\
\text{16 h, 79%} \\
\text{S13} \\
\text{HOt (125 mg, 0.93 mmol) and EDC·HCl (168 mg, 0.93 mmol) were added sequentially to a stirred solution of 2-((2-methoxyethoxy)methoxy)benzoic acid (150 mg, 0.66 mmol) and L-proline ethyl ester·HCl (143 mg, 0.80 mmol) in DMF (2 mL, 0.3 M) at 0 °C under N\textsubscript{2} atmosphere. DIPEA (0.54 mL, 3.31 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was poured into ice cold water (15 mL) and the aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were washed with cold brine (2 × 5 mL), dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated in vacuo. Purification using flash column chromatography in 2 % MeOH : CH\textsubscript{2}Cl\textsubscript{2} afforded the compound S13 as a colourless oil (185 mg, 79 %). \text{R}_{\text{f}} (2 \% \text{EtOAc: hexane}) = 0.15. [\alpha]_{D}^{23} = - 69.7 (c 1.2, CHCl\textsubscript{3}). \text{H NMR (300 MHz, CDCl\textsubscript{3}) mixture of rotamers (major) \delta: 7.34-7.12 (3H, m); 7.07-7.00 (1H, m); 5.29 (2H, s); 4.65 (1H, dd, J = 4.3, 8.6 Hz); 4.23 (2H, q, J = 6.9 Hz); 3.86-3.76 (2H, m); 3.57-3.51 (2H, m); 3.36 (3H, s); 3.38-3.25 (2H, m); 2.37-1.78 (4H, m); 1.31 (3H, t, J = 6.9 Hz). \text{C NMR (75 MHz, CDCl\textsubscript{3}) \delta: (major) 172.2, 168.0, 153.1, 130.2, 128.0, 127.7, 122.2, 115.4, 94.0, 71.5, 67.9, 61.0, 59.0, 58.6, 48.2, 29.6, 24.7, 14.2. Anal. Calcd. for C\textsubscript{18}H\textsubscript{25}NO\textsubscript{6}: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.39; H, 7.16; N, 4.00.}
\end{array}
\]
Using general procedure C ester S13 (165 mg, 0.47 mmol) was hydrolyzed to (2-((2-methoxyethoxy)methoxy)benzoyl)-L-proline S14 as a colourless gummy mass (140 mg, 92 %); Rf (10 % MeOH: CH2Cl2) = 0.3. [α]D23 = -79.3 (c 1.7, CHCl3). 1H NMR (300 MHz, CDCl3) mixture of rotamers (major) δ: 7.41-7.17 (3H, m); 7.06 (1H, dd, J = 7.8, 7.8 Hz); 5.29 (2H, s); 4.76 (1H, dd, J = 4.2, 8.2 Hz); 3.83-3.77 (2H, m); 3.57-3.52 (2H, m); 3.37 (3H, s); 3.40-3.32 (2H, m); 2.55-2.36 (1H, m); 2.26-1.80 (3H, m). 13C NMR (75 MHz, CDCl3) δ = major: 170.2, 168.5, 153.2, 131.4, 128.0, 126.6, 122.5, 115.5, 114.8, 94.1, 71.7, 68.2, 59.7, 59.2, 49.0, 28.6, 24.8. Anal. Calcd. for C16H21NO6: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.55; H, 6.54; N, 4.34.

Using general procedure F, the above acid was reacted with (2R,8R)-2-((tert-butyldiphenylsilyl)oxy)-8-hydroxytetradecanamide1 S4 (61 mg, 0.12 mmol) to give (7R,13R)-14-amino-13-((tert-butyldiphenylsilyl)oxy)-14-oxotetradecan-7-yl(2-((2-methoxyethoxy) methoxy)benzoyl)-L-proline S15 as a yellow sticky solid (58 mg, 59 %); Rf (3 % MeOH: CH2Cl2) = 0.4. [α]D23 = -25.1 (c 0.35, CHCl3). 1H NMR (300 MHz, CDCl3) mixture of rotamers (major) δ: 7.69-6.98 (14H, m); 6.72 (1H, brs; 5.52 (1H, brs; 5.29 (2H, s); 4.70-4.58 (1H, m); 4.29-4.20 (1H, m); 3.87-3.72 (3H, m); 3.58-3.47 (2H, m); 3.35 (3H, s); 3.45-3.24 (2H, m); 2.40-1.77 (4H, m); 1.74-1.04 (29H, m); 0.87 (3H, t, J = 6.7 Hz). 13C NMR (75 MHz, CDCl3) δ (major) 176.4, 172.1, 167.8, 153.3, 136.0 (× 2), 135.8 (× 4), 133.2, 132.8, 130.7, 130.4, 128.2, 128.1 (× 4), 122.4, 115.2, 94.1, 75.3, 74.4, 71.8, 68.1, 59.2, 48.4, 34.5, 34.2, 32.0, 31.9, 31.5, 30.0, 29.9, 29.5, 29.4, 29.3, 27.3, 25.4, 25.0, 23.0, 22.8, 22.78, 19.5, 14.3. Anal. Calcd. for C46H66N2O8Si: C, 68.79; H, 8.28; N, 3.49. Found: C, 68.67; H, 8.26; N, 3.48.
2N HCl (0.5 mL) was added dropwise to a stirred solution of the S15 (50 mg, 0.06 mmol) in THF (0.5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. THF was removed *in vacuo*; the aqueous layer was extracted with ethyl acetate (2 × 7 mL). The combined organic extracts were washed with brine (5 mL), dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The product was purified using preparative TLC in 10% MeOH : CH$_2$Cl$_2$ to afford the title compound 7 as a pale yellow oil (25 mg, 75 %). $R_f$ (10 % MeOH : CH$_2$Cl$_2$) = 0.4. $[\alpha]_D^{23}$ = -13.0 (c 1.0, CHCl$_3$).$^1$H NMR (300 MHz, CDCl$_3$) δ: 10.66 (1H, s); 7.55-7.44 (1H, m); 7.40-7.30 (1H, m); 6.96 (1H, dd, $J$ = 1.0, 8.2 Hz); 6.87 (1H, dd, $J$ = 8.2, 8.2 Hz); 6.59 (1H, brs); 5.29 (1H, brs); 4.98 (1H, brs); 4.65 (1H, dd, $J$ = 5.5, 8.6 Hz); 4.15-4.06 (1H, m); 3.95-3.74 (2H, m); 3.40-3.31 (1H, m); 2.43-2.21 (1H, m); 2.17-1.89 (3H, m); 1.83-1.07 (20H, m); 0.87 (3H, t, $J$ = 6.7 Hz). $^{13}$C NMR (75 MHz, CDCl$_3$) δ: 177.3, 172.4, 170.4, 159.1, 133.3, 128.2, 118.9, 117.95, 117.87, 75.5, 71.7, 60.7, 50.8, 34.6, 34.5, 34.4, 31.9, 29.4, 29.3, 28.6, 25.9, 25.6, 25.0, 24.7, 22.8, 14.3. Anal. Calcd. for C$_{26}$H$_{40}$N$_2$O$_6$: C, 65.52; H, 8.46; N, 5.88. Found: C, 65.63; H, 8.48; N, 5.89. HRMS: (ES+) calculated for C$_{26}$H$_{40}$N$_2$O$_6$Na (M + Na)$^+$ 499.27786, Found: 499.27697.
1.3.7. Synthesis of compound 8. (7R,13R)-14-amino-13-hydroxy-14-oxotetradecan-7-yl (1S)-9-oxo-1,2,3,3a-tetrahydro-9H-benzo[e]pyrrolo[2,1-b][1,3]oxazine-1-carboxylate.

Compound 8 was synthesized as described in the literature.\(^2\)

More polar diastereomer: \(R_f\) (50 % EtOAc: hexane) = 0.32. \([\alpha]_D^{23} = -54.0\) (c 0.75, CHCl\(_3\)). \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\): 7.83 (1H, d, \(J = 7.6\) Hz); 7.44 (1H, dd, \(J = 7.6, 7.6\) Hz); 7.09 (1H, dd, \(J = 7.6, 7.6\) Hz); 6.99 (1H, d, \(J = 7.6\) Hz); 6.87 (1H, s); 5.56 (1H, dd, \(J = 6.2, 7.3\) Hz); 5.48 (1H, s); 4.99-4.93 (1H, m); 4.60 (1H, d, \(J = 8.9\) Hz); 4.29-4.26 (1H, m); 4.13-4.08 (1H, m); 2.50-2.44 (1H, m); 2.40-2.23 (2H, m); 2.20-2.15 (1H, m); 1.84-1.04 (20H, m); 0.85 (3H, t, \(J = 7.3\) Hz);\(^1\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\): 177.3, 170.6, 161.4, 157.8, 134.4, 127.8, 122.8, 118.9, 116.9, 88.5, 75.5, 71.0, 57.1, 34.4, 33.8, 33.7, 31.7, 30.2, 29.0, 27.7, 26.0, 25.3, 24.5, 24.0, 22.5, 14.0. Anal. Calcd. for \(C_{26}H_{38}N_2O_6\) C, 65.80; H, 8.07; N, 5.90. Found: C, 65.87; H, 8.09; N, 5.91. HRMS: (ES+) calculated for \(C_{26}H_{38}N_2O_6Na\) (M + Na)\(^+\) 497.26221, Found: 497.26346.
1.3.8. Synthesis of compound 9. (2R,8R)-2,8-dihydroxytetradecanamide

Using general procedure F, silyl ether S4 (38 mg, 0.076 mmol) yielded the title compound 9 as an off white solid (12 mg, 63%). Rf (2 % MeOH:CH2Cl2) = 0.3. mp. 100-102 °C. [α]D23 = +15.0 (c 0.5, CHCl3). 1H NMR (300 MHz, CH3OH - d4) δ: 3.97 (1H, dd, J = 3.9, 7.7 Hz); 3.50 (1H, brs); 1.83-1.67 (1H, m); 1.66-1.52 (1H, m); 1.50-1.22 (20H, m); 0.90 (3H, t, J = 7.0 Hz). 13C NMR (75 MHz, CH3OH - d4) δ: 179.5, 71.5, 71.2, 37.3, 37.2, 34.4, 31.9, 29.4, 29.4, 25.6, 25.5, 24.9, 22.5, 13.2. Anal. Calcd. for C14H29NO3: C, 64.83; H, 11.27; N, 5.40. Found: C, 64.75; H, 11.29; N, 5.41. HRMS: (ES+) calculated for C14H29NO3Na (M + Na)+ 282.20396, Found: 282.20448.
1.3.9. Synthesis of compound 10. (2R, 8R)-2,8 dihydroxytetradecanol.

To a solution of diol S16\(^1\) (100 mg, 0.274 mmol) in ethanol (7 mL) was added 10\% Pd/C (10 mg). The suspension was evacuated under vacuum and flushed with H\(_2\) gas (4 times). The reaction mixture was stirred under H\(_2\) atmosphere at room temperature for 12 h. The reaction mixture was filtered through a plug of celite and the residue was washed with ethyl acetate (2 × 5 mL). The filtrate was concentrated in vacuo, the concentrate was triturated with diethyl ether (5 mL) to obtain alcohol 10 (50 mg, 74 \%) as white solid. \(R_f\) (70 \% ethyl acetate: hexane) = 0.2. mp 103-104 °C. \([\alpha]_D^{23} = +8.0\) (c 0.5, MeOH). \(^1\)H NMR (300 MHz, 300 MHz, CH\(_3\)OH - d\(_4\)) \(\delta\): 3.62-3.34 (4H, m); 1.60-1.22 (20H, m), 0.90 (3H, t, \(J = 6.9\) Hz). \(^1^3\)C NMR (75 MHz, CH\(_3\)OH - d\(_4\)) \(\delta\): 72.1, 71.2, 66.2, 37.3, 37.2, 33.2, 31.9, 29.7, 29.3, 25.6 (× 2), 25.5, 22.5, 13.2. Anal. Calcd. for C\(_{14}\)H\(_{30}\)O\(_3\): C, 68.25; H, 12.27. Found: C, 68.33; H, 12.29. HRMS: (ES+) calculated for C\(_{14}\)H\(_{30}\)O\(_3\)Na (M + Na)\(^+\) 269.20872, Found: 269.20931.
1.3.10. Synthesis of compound 11. (R)-8-hydroxytetradecanamide

A suspension of alcohol S17 (700 mg, 2.02 mmol), NaHCO₃ (509 mg, 6.06 mmol) in CH₃CN : H₂O 1 : 1 (25 mL) was cooled to 0 °C and stirred for 10 min. TEMPO (63 mg, 0.40 mmol) and bis(acetoxy)iodobenzene (1.62 gm, 5.05 mmol), were added sequentially in one portion and the solution was stirred at 0 °C for 4 h in the dark. Saturated aq. NaHCO₃ (20 mL) was added at 0 °C and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude oil was purified using flash column chromatography with 0-30% ethyl acetate: petroleum ether) to furnish carboxylic acid S18 (475 mg, 65 %) as white translucent oil. Rf (30 % ethyl acetate: hexane) = 0.25. [α]D²³ = +28.5 (c 1.00, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.24 (2H, d, J = 9.1 Hz); 6.86 (2H, d, J = 9.1 Hz); 5.58 (1H, dt, J = 11.2, 7.4 Hz); 5.32 (1H, dd, J = 11.2, 9.5 Hz); 4.50 (1H, d, J = 12.3 Hz); 4.26 (1H, d, J = 12.3 Hz); 4.11-4.02 (1H, m); 3.80 (3H, s); 2.35 (2H, t, J = 7.3 Hz); 2.16-1.93 (2H, m); 1.74-1.18 (14H, m); 0.87 (3H, t, J = 7.3 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 179.7, 159.2, 132.6, 131.9, 131.3, 129.5 (× 2), 113.9 (× 2), 74.0, 69.6, 55.5, 35.9, 34.1, 32.1, 29.5, 29.4, 27.7, 25.6, 24.6, 22.8, 14.3. Anal. Calcd. for C₂₂H₃₄O₄: C, 72.89; H, 9.45. Found: C, 72.77; H, 9.44.

A solution of carboxylic acid S18 (475 mg, 1.31 mmol), NH₄Cl (282.3 mg, 5.27 mmol) in dry DMF (18 mL, 0.07 M) was cooled to 0 °C. HOBT (534 mg, 3.93 mmol) and HBTU (1.5 gm, 3.93 mmol) were added, followed by DIPEA (1.87 mL, 5.27 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h. Ice pieces were added, and then the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with cold brine (3 × 15 mL) and dried over anhydrous Na₂SO₄. After removal of the solvent in vacuo, the residue was purified using flash column chromatography with 0-50% ethyl acetate: petroleum ether to furnish S19 (389 mg, 82 %) as a colourless oil. Rf (50 % ethyl acetate: hexane) = 0.33. [α]D²³ = +17.4 (c 0.5, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ: 7.24 (2H, d, J = 8.9 Hz); 6.86 (2H, d, J = 8.9 Hz); 5.58 (1H, dt, J = 11.2, 7.4 Hz); 5.45 (2H, brs); 5.31 (1H, dd, J = 11.2, 9.3 Hz); 4.49 (1H, d, J = 11.7 Hz); 4.26 (1H, d, J = 11.7 Hz); 4.12-4.00 (1H, m); 3.80 (3H, s); 2.20 (2H, t, J = 7.6 Hz); 2.15-1.93 (2H, m); 1.74-1.17 (14H, m); 0.87 (3H, t, J = 7.7 Hz). ¹³C NMR (75 MHz, CDCl₃) δ = 175.4, 159.2, 132.7, 131.8, 131.3, 129.5 (× 2), 113.9 (× 2), 74.0, 69.6, 55.5, 35.9, 34.1, 32.1, 29.5, 29.4, 27.7, 25.6, 24.6, 22.8, 14.3.
To a solution of S19 (389 mg, 1.083 mmol) in ethanol (30 mL) was added 10% Pd/C (200 mg). The suspension was evacuated under vacuum and flushed with H₂ gas (× 4). The reaction mixture was stirred under H₂ at room temperature for 12 h, then it was filtered through a plug of celite and the residue was washed with ethyl acetate (2 × 20 mL). The filtrate was concentrated in vacuo; the concentrate was triturated with diethyl ether (10 mL) to obtain (R)-8-hydroxytetradecanamide 11 (150 mg, 71 %) as white solid. Rᵣ (50 % ethyl acetate: hexane) = 0.15. mp :97-99 °C.[α]D²³ = +9.0 (c 1.0, CHCl₃). ¹H NMR (300 MHz, CD₃OD) δ = 3.49 (1H, brs), 2.49-2.40 (1H, m); 2.22-2.15 (3H, s); 1.66-1.22 (20H, m); 0.90 (3h, t, J = 6.7 Hz); ¹³C NMR (75 MHz, CD₃OD) δ = 178.1, 71.2, 37.3, 35.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 25.7, 25.6, 22.5, 13.3. Anal. Calcd. for C₁₄H₂₉NO₂: C, 69.09; H, 12.01; N, 5.75. Found: C, 69.15; H, 12.03; N, 5.74. HRMS: (ES+) calculated for C₁₄H₂₉NO₂Na (M + Na)⁺ 266.20905, Found: 266.20960.
1.4. References

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2) A. D. Steele, C. E. Keohane, K. W. Knouse, S. E. Rossiter, S. J. Williams, W. M. Wuest Diverted total synthesis of promysalin analogs demonstrates that an iron-binding motif is responsible for its narrow-spectrum antibacterial activity *J. Am. Chem. Soc.* 2016, 138, 5833–5836.
1.4 Spectral data
Compound S2
Compound S3

RK-471P_13C
RK-471P

RK-471P_1H
RK-471P
Compound 2
Compound S6
Compound S7
Compound S8
Compound S9
Compound S10
Compound 4
Compound S11
Compound 5
Compound 6
Compound S14

[Chemical structure and NMR spectrum image]

S 41
Compound 7
Compound 8
Compound 9
Compound 10
Compound S18
Compound 11
Mass Spectra

Compound 1.

Compound 2.
Compound 3.

Compound 4.
Compound 5.

ESI positive, *m/z* 354 (MeOH). BB mode, 17 Nov 2016
Resolution (*m/z* 354) = 63 000, Flow Rate = 120 μL/h

Compound 6.

ESI positive, *m/z* 284 (MeOH). BB mode, 17 Nov 2016
Resolution (*m/z* 284) = 81 000, Flow Rate = 120 μL/h
Compound 7.

Compound 8.
Compound 9.

Compound 10.
Compound 11.

Compound S5.
2) Materials and methods related to microbiological assays

2.1 Microbiological media and culture condition

M17 broth (Difco, Laboratories, Detroit, MI) for *Streptococcus* and *Lactococcus* species, and in MRS broth (Difco) for all *Lactobacillus*, *Pediococcus*, and *Enterococcus* species. *Staphylococcus* species were cultivated in Brain Heart Infusion broth (Difco). *Streptococcus pneumoniae* was routinely maintained in Trypticase Soy Broth (TSB) (Difco, Laboratories, Detroit, MI) with 3% (v/v) defibrinated horse blood in a 5% CO2 incubator. *Escherchia coli*, *Pseudomonas* species and *Bacillus subtilis* were cultivated in TSB (Difco, Laboratories, Detroit, MI). Cultures were incubated at 30 °C and 37 °C for mesophilic and thermophilic species, respectively.

2.2 Agar and well diffusion assay

An agar diffusion assay was carried out using *P. putida* RW10S1 (promysalin producers), and *P. stutzeri* LMG 2333 (promysalin sensitive) as reference strains. Strains RW10S1 and LMG 2333 were spotted on the surface of agar Trypticase Soy Broth (TSB) (Difco, Laboratories, Detroit, MI) and incubated for 10-18 h at 30 °C. After growth, the surface of the Petri plate was exposed to a saturated atmosphere of chloroform for 10 min, and an overlay containing a suspension of $10^7$ CFU/mL of the target strain in the appropriate soft agar medium was poured onto the surface. The soft agar overlay, containing agar 7.5 g/L, was prepared in TSB or M17 medium (Difco, Laboratories, Detroit, MI) for *Streptococcus thermophilus* DSM 20617T, and in TSB or MRS medium (Difco, Laboratories, Detroit, MI) for *Pediococcus acidilactici* DSM20284T. After solidification, the plates were incubated at the appropriate temperature for 18 h, and the presence or the absence of an inhibition halo around the *P. putida* RW10S1 was verified. For agar well diffusion assay, $10^7$ CFU/ml were inoculated in melted M17 or MRS or TSB agar media (15 g/l). After the medium solidification, a well of 1 cm of diameter was created using a sterile tip and loaded with a 50 µl of DMSO of promysalin at different concentration. The plates were then incubated at the appropriate temperature for 18 h, and the presence or the absence of an inhibition halo around the wells were verified.
**Figure S1.** Agar diffusion assay carried out using *P. putida* RW10S1 (promysalin producers), and *P. stutzeri* LMG 2333 (promysalin sensitive) as reference strains spotted in TSB agar. Promysalin production and activity was tested against the M17 soft agar overlay containing *Streptococcus thermophilus* DSM 20617T (A), and the MRS soft agar overlay containing *Pediococcus acidilactici* PAC1.0 (B) and the TSB soft agar overlay containing *Pseudomonas stutzeri* LMG 2333 (C). P, and S in the figure represent the growth of the promysalin-producer *P. putida* RW10S1, and the promysalin-sensitive strain respectively in TSB agar.

**Figure S2.** Agar diffusion assay carried out using *P. putida* RW10S1 (promysalin producers) as reference strains spotted in TSB agar. Promysalin production and activity was tested against the TSB soft agar overlay containing *Streptococcus thermophilus* DSM 20617T (A), and *Pediococcus acidilactici* PAC1.0 (B). P in the figure represent the growth of the promysalin-producer *Pseudomonas putida* RW10S1 in TSB agar.

**Figure S3.** Agar well diffusion assay carried out using promysalin at different concentrations. Promysalin production and activity was tested against M17 or MRS or TSB agar inoculated with 10^7 CFU/ml of *Streptococcus thermophilus* DSM 20617T (A), *Pediococcus acidilactici* PAC1.0 (B), *Pseudomonas stutzeri* LMG 2333 (C), and the promysalin-producer (promysalin-resistant) *Pseudomonas putida* RW10S1 (D). DMSO (indicated in figure) was used in a volume equal to that used for promysalin solutions. The total amount of promysalin (µg) loaded in each well is indicated in the figure.
Evaluation of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of promysalin and its derivative analogues against Gram-positive and Gram-negative bacteria

Minimum inhibitory concentration (MIC) was determined using the broth microdilution method: after overnight growth on Mueller-Hinton broth-II (MHB-II) (Sigma-Aldrich, Milan, Italy) plates, strains were suspended in MHB-II to a standardized OD$_{590nm}$ of 0.5. Three 10-fold dilutions were performed, and each cell suspension was inoculated in the presence of promysalin or its analogues at the indicated concentrations. Chlorhexidine digluconate (20 % w/v aqueous solution, code C9394), benzalkonium chloride (code 12060) and surfactin (code S3523) were purchased by Sigma-Aldrich. Determination of the minimum bactericidal concentration (MBC) was performed by subculturing 10 µL from each well without visible microbial growth. After 48 hours of incubation, the promysalin or analogue dilutions yielding three colonies or less were scored as the MBC for starting inocula of $10^5$ CFU/ml. The experiments were performed in triplicate. MIC and MBC were performed according to CLSI (Clinical and Laboratory Standards Institute) methods for dilution antimicrobial susceptibility tests for aerobic bacteria (approved standard, Wayne, PA, USA: CLSI; 2009).
Table S1. Minimal inhibitory concentration (MIC, µg/ml) and minimal bactericidal concentration (MBC, µg/ml) values of chlorhexidine and benzalconium chloride against Gram-negative and Gram-positive bacteria

| Compound                | Pseudomonas aeruginosa ATCC 10145 | Pseudomonas stutzeri LMG 2333 | Pseudomonas putida RW 10S1 | Streptococcus thermophilus DSM20617<sup>T</sup> |
|-------------------------|-----------------------------------|-------------------------------|----------------------------|-----------------------------------------------|
|                         | MIC | MBC  | MIC | MBC  | MIC | MBC  | MIC | MBC  |
| Chlohexidine            | 32  | 32   | 8   | 16   | 8   | 16   | 4   | 32   |
| Benzalconium chloride   | 64  | 128  | 16  | 32   | 32  | 64   | 2   | 16   |
2.4 Flow cytometry evaluation of cell membrane damage and measurement of cFSE fluorescence cell leakage

To evaluate whether membrane damage was linked to cell leakage of intracellular components, microbial cells grown for 18 h in the appropriate medium in Petri dishes were collected and diluted in sterile filtered (0.2 µm) phosphate-buffered saline (PBS) (NaCl 8 g/L; KCl 0.2 g/L; Na₂HPO₄ 1.44 g/L; KH₂PO₄ 0.24 g/L; pH 7.4) to a final concentration of 10⁸ events per mL. The cell suspension was diluted to 10⁶ events/mL and then exposed to promysalin (100 µg/mL) or its derivative analogues (100 µg/mL), chlorhexidine (100 µg/mL) (Sigma-Aldrich) or benzalkonium chloride (100 µg/mL) (Sigma-Aldrich) at 37 °C. The cell suspension was also exposed to a DMSO control. At the time requested, a sample was collected and subjected to SYBR Green I/PI double staining and analysis by flow cytometry and, when necessary, to a standard plate count in the appropriate medium. In flow cytometry, particles/cells that pass through the beam will scatter light, which is detected as forward scatter (FSC) and side scatter (SSC). FSC correlates with cell size, cell shape and cell aggregates, whereas SSC depends on the density of the particles/cells (i.e., the number of cytoplasmic granules and membrane size). In this manner, cell populations can often be distinguished based on differences in their size and density. Cell suspensions were subjected to dual nucleic acid staining with cell permeant SYBR Green I (1X) and cell impermeant propidium iodide (PI) (5 µg/mL) (Sigma-Aldrich, Milan, Italy). SYBR Green I permeates the membrane of total cells and stains nucleic acids with green fluorescence. After incubation at room temperature for 15 min, the labeled cell suspensions were diluted to approximately 10⁶ events per mL, and analyzed by flow cytometry. Cell suspensions that were prepared as described above were analyzed using a flow cytometer with the following threshold settings: FSC 5,000, SSC 4,000, and 20,000 total events collected. All parameters were collected as logarithmic signals, and a 488-nm laser was used to measure the FSC values. The rate of events in the flow was generally lower than 2,000 events/s. The obtained data were analyzed using BD AccuriTM C6 software 1.0 (BD Biosciences, Milan, Italy). Cell-membrane damage was carried out by applying double staining with SYBR Green I and PI. The SYBR Green I fluorescence intensity of stained cells was recovered in the FL1 channel (excitation 488 nm, emission filter 530/30 nm). PI fluorescence was recovered in the FL3 channel (excitation 488 nm, emission filter 670 nm long pass). PI penetrates only bacteria with damaged membranes, causing a reduction in SYBR Green I fluorescence when both dyes are present. Thus, live bacteria with intact cell membranes fluoresce bright green (defined as active fluorescent cells), bacteria with slightly damaged membranes exhibit both green and red fluorescence (defined as slightly membrane damaged cells) and cells with broken membranes fluoresce red (defined as non-active fluorescent cells) (ISO 19344:2015; IDF 232:2015). Active fluorescent cells, damaged cells and non-active fluorescent cells were electronically gated in density plots of green fluorescence (FL1) versus red (FL3) fluorescence. Green and red fluorescence allowed for optimal distinction between stained
microbial cells and instrument noise or sample background. Active fluorescent cells were gated in G1, cells with a slightly damaged membrane were gated in G2, and cells with broken membranes fluoresced were gated in G3.

To evaluate whether membrane damage was linked to cell leakage of intracellular components, microbial cells grown for 18 h in the appropriate medium in Petri dishes were collected and diluted in PBS to a final concentration of $10^8$ per mL. The obtained cell suspension was supplemented with 4 µM cFDASE (Sigma-Aldrich, Milan, Italy), which is a precursor molecule of cFSE. The suspensions were incubated for 30 min at 37 °C. During this incubation, membrane-permeating cFDASE was cleaved by intracellular esterases, and the resulting cFSE molecules were conjugated to the aliphatic amines of intracellular proteins. After centrifugation at 15,000 x g for 1 min and washing with PBS solution, the cells were suspended in an equal volume PBS. To ensure that unconjugated and free probes were eliminated by the cells, we periodically monitored cell fluorescence by flow cytometry as described below. The stability of the cell fluorescence was assessed; stained cells kept on ice in PBS after staining maintained a stable fluorescence, indicating that no free cFSE was inside the cells. Cell suspensions, prepared as described above and diluted to $10^6$ events/mL, were analyzed using a flow cytometer with the previously described threshold settings. The cFSE fluorescence intensity of stained cells was recovered in the FL1 channel (excitation 488 nm, emission filter 530/30, provided by BD Biosciences, Milan, Italy). The cFSE-labeled cell suspension was then exposed to promysalin (100 µg/mL) or its derivative analogues (100 µg/mL), chlorhexidine (100 µg/mL) (Sigma-Aldrich) or benzalkonium chloride (100 µg/mL) (Sigma-Aldrich) at 37 °C. As a control, the cFSE-labeled cell suspension was also exposed to a volume of DMSO solvent equal to that used for promysalin and its derivative analogues.

At the time point, two samples of each cell suspension were collected: i) one sample was labeled with PI as described above, incubated at room temperature for 15 min, and analyzed by flow cytometry. The second sample was used to measure cFSE-fluorescence cell leakage. In flow cytometry, density plots of cFSE-green fluorescence (FL1) and FSC allowed for optimal distinction between cFSE-stained microbial cells and instrument noise or sample background. Active cells showing only cFSE fluorescence were gated in G1, and cells with a slightly or heavily damaged membranes showing cFSE and PI fluorescence were gated in G2 and G3. Electronic gates on the green fluorescence/FSC density plot were used to select the measured bacterial concentration expressed as a % equal to the number of events in the gate divided by the total events counted. The sample for the measurement of cFSE-fluorescence cell leakage was centrifuged (13000 rpm, 2 min), and the cell-free supernatant transferred to a 96-microtiter plate for measurement of cFSE-fluorescence in a Victor 3 fluorometer (PerkinElmer). The fluorescence data were calculated as the average of three independent assays and expressed in arbitrary units of fluorescence ± the standard deviation.
Figure S4. The effect of benzalkonium chloride on *Streptococcus thermophilus* DSM 20617<sup>T</sup> and *Pseudomonas aeruginosa* ATCC 10145 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin or benzalkonium chloride (100 and 200 µg/mL, respectively). A) and C) Cells before exposure to benzalkonium chloride (100 µg/mL). B) and D) Cells after 15 min of exposure to benzalkonium chloride. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S5. The effect of promysalin and gramicidin on *Streptococcus thermophilus* DSM 20617^T^ cell membrane integrity. Flow cytometry density diagrams show the cFSE vs PI fluorescence of cells exposed to promysalin or gramicidin (100 μg/mL and 100 mM, respectively). A) Cells before exposure to antimicrobials. B) Cells after 60 min of exposure to DMSO. C) Cells after 60 min of exposure to promysalin. D) Cells after 60 min of exposure to gramicidin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
**Figure S6.** The effect of DMSO and chlorhexidine on *Streptococcus thermophilus* DSM 20617<sup>T</sup> cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to DMSO (a volume equal to that used for promysalin and its derivative analogs), and chlorhexidine (100 µg/mL). A) Cells before exposure to antimicrobials. B) Cells after 60 min of exposure to DMSO. C) Cells after 60 min of exposure to promysalin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S7. The effect of DMSO and promysalin on *Lactobacillus paracasei* DSM 5622<sup>T</sup> cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to DMSO (a volume equal to that used for promysalin) and promysalin (100 µg/mL). A) Cells incubated 60 min at 37 °C in presence of DMSO. B) Cells after 60 min of exposure to promysalin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S8. The effect of DMSO and promysalin on *Staphylococcus aureus* ATCC 25923 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to DMSO (a volume equal to that used for promysalin) and promysalin (100 µg/mL). A) Cells incubated 60 min at 37 °C in presence of DMSO. B) Cells after 60 min of exposure to promysalin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S9. The effect of DMSO, promysalin and chlorhexidine on *Bacillus subtilis* DSM 347 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to DMSO (a volume equal to that used for promysalin) and promysalin (100 µg/mL). A) Cells incubated 60 min at 37 °C in presence of DMSO. B) Cells after 60 min of exposure to promysalin. C) Cells after 60 min of exposure to chlorhexidine. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S10. The effect of promysalin and chlorhexidine on *Pseudomonas stutzeri* LMG 2333 cell-membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin or chlorhexidine (100 µg/mL) for 1h at 37 °C. A) Cells exposed to DMSO as control; B) Cells exposed to promysalin; C) Cells exposed to chlorhexidine. Viable cells are gated in G1, Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S11. The effect of promysalin and chlorhexidine on *Pseudomonas aeruginosa* ATCC 10145 cell-membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin or chlorhexidine (100 µg/mL) for 1h at 37 °C. A) Cells exposed to DMSO as control; B) Cells exposed to promysalin; C) Cells exposed to chlorhexidine. Viable cells are gated in G1, Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S12. The effect of promysalin and chlorhexidine on *Escherichia coli* ATCC 25922 cell-membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin or chlorhexidine (100 and 200 µg/mL, respectively). A) and B) Cells after 20 min of exposure to antibacterials. C) and D) Cells after 1.5 h-exposure to antimicrobials. Viable cells are gated in G1, and viable cells with slightly damaged cell membranes are gated in G2. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S13. The effect of promysalin and chlorhexidine on *Acetobacter aceti* MIM2000/28 cell-membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin or chlorhexidine (100 µg/mL) for 1 h at 37 °C. A) Cells exposed to DMSO as control; B) Cells exposed to promysalin; D) Cells exposed to chlorhexidine. Viable cells are gated in G1, Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S14. The effect of promysalin and chlorhexidine on *Saccharomyces cerevisiae* BC1 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to promysalin and chlorhexidine (100 µg/mL). A) Cells before exposure to antimicrobials. B) Cells after 60 min of exposure to promysalin. C) Cells after 60 min of exposure to chlorhexidine. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S15. The effect of surfactin on *Streptococcus thermophilus* DSM 20617 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to surfactin (200 µg/mL). A) Cells before exposure to antimicrobials. B) Cells after 60 min of exposure to surfactin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
Figure S16. The effect of surfactin on *Pseudomonas aeruginosa* ATCC 10145 cell membrane integrity. Flow cytometry density diagrams show SYBR Green I vs PI fluorescence of cells exposed to surfactin (200 µg/mL). A) Cells before exposure to antimicrobials. B) Cells after 60 min of exposure to surfactin. Viable cells are gated in G1. Dead cells with damaged membranes are gated in G3. The transition of cell populations from gate G1 to gate G3 is correlated to cell membrane damage.
2.5 Evaluation of growth-kinetic parameters of *Saccharomyces cerevisiae* BC1 in the absence and presence of promysalin

*S. cerevisiae* BC1 growth was monitored in 96-well plates that were filled using an automatic liquid handling system (EpMotion, Eppendorf, Italy) to a final volume of 200 μL in the presence and absence of promysalin at different concentrations (4-128 μg/mL). A set of promysalin solutions at different concentrations in DMSO was prepared to add the same volume to each well, regardless of the final promysalin concentration. The growth of *S. cerevisiae* BC1 in the presence of promysalin was compared to its growth in the presence of a DMSO control added to the medium. Microbial growth was monitored using a spectrophotometer (MicroWave RS2, Biotek, USA) programmed for 145 readings (O.D. 600 nm) every 10 min for 24 h at 37 °C. At the end of the incubation, the growth curve and lag time (h:min) were obtained using Gen5 software (Biotek, USA). The data were calculated as the average of three independent assays ± the standard deviation.

![Figure S17](image-url)

**Figure S17.** Growth of *Saccharomyces cerevisiae* BC1 in the absence and presence of different concentrations of promysalin. The calculated lag time (h:min) for each growth condition is indicated.
Figure S18. Growth of *Saccharomyces cerevisiae* BC1 in the absence and presence of different concentrations of chlorhexidine. Chlorhexidine concentrations (µg/ml) are indicated. The MIC for chlorhexidine was 8 µg/ml. In presence of chlorhexidine 1, 2 and 4 µg/ml, the growth curves of *S. cerevisiae* were not significantly different from the growth in absence of the biocide (control).