Supplementary Information

Synthesis and Evaluation of Membrane Permeabilizing Properties of Cationic Amphiphiles Derived from the Disaccharide Trehalose

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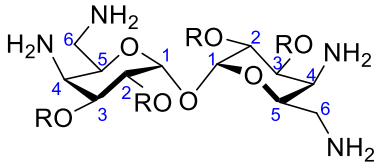
Content:

1. General methods and instrumentation

^{1}H-NMR spectra (including 1D-TOCSY) were recorded on Bruker Avance™ 400 or 500 spectrometers, and chemical shifts (reported in ppm) were calibrated to CD_3OD or $CDCl_3$ (δ = 3.31 or 7.26 respectively). ^{13}C-NMR spectra were recorded on Bruker Avance™ 400 or 500 spectrometers at 100 or 125 MHz. Multiplicities are reported using the following abbreviations: b = broad, s = singlet, d = doublet, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet, app. q = apparent quartet. Coupling constants (J) are given in Hertz. High-resolution electron spray ionization (HR-ESI) mass spectra were measured on a Waters Synapt instrument. Chemical reactions were monitored by TLC (Merck, Silica gel 60 F254). Visualization was achieved using a cerium-molybdate stain [$(NH_4)_2Ce(NO_3)_6$ (5g), $(NH_4)_6Mo_7O_{24}.4H_2O$ (120 g), H_2SO_4 (80 mL), H_2O (720 mL)]. All reactions were carried out under an argon atmosphere with anhydrous solvents, unless otherwise noted. All chemicals, unless otherwise stated, were obtained from commercial sources. Compounds were purified by flash chromatography (SiO_2, Merck, Kieselgel 60).

2. Trehalose numbering system



3. Synthetic procedures

Compound 2: To a solution of compound **1** (3 g, 5.89 mmol) in DCM (50 mL) and pyridine (3.8 mL, 47 mmol) at -40 °C, Tf_2O (8 mL, 47 mmol) was slowly added. The reaction mixture was stirred at 0 °C for 2 h. Reaction progress was monitored by TLC (20% EtOAc, 80% P.E.). Upon completion, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The triflated crude product was dissolved in DMF/HMPA (25/15 mL), and NaN_3 (4.6 gr, 0.07 mol) was added. The reaction was allowed to stir at room temperature overnight; progress was monitored by TLC (35% EtOAc, 65% P.E.). The reaction was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The crude was purified by chromatography (silica gel, P.E./EtOAc) to yield **2** (1.73 g, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36-5.37 (m, 4H, H-2, H-3), 5.24 (d, J=2.8 Hz, H-1), 4.15 (m, H-4), 4.10 (ddd, J_1=1.5, J_2=5.7, J_3=7.3 Hz, H-5), 3.54 (dd, J_1=7.6, J_2=12.7 Hz, H-6), 3.23 (dd, J_1=5.5, J_2=12.7 Hz, H-6), 2.15 (s, 6H, OAc), 2.14 (s, 6H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$) δ

170.1, 169.7, 93.3, 69.8, 68.4, 66.8, 60.7, 51.1, 20.6, 20.4. LRMS (ESI) m/z calculated 633.18 for $C_{20}H_{26}N_{12}O_{11}Na$, found 633.14 $[M+Na]^+$.

Compound 3: A catalytic amount of NaOMe (0.5 M solution in MeOH) was added to a suspension of compound **2** (1.25 g, 2.0 mmol) in MeOH (15 mL). The reaction mixture was stirred at room temperature for 3 h; progress was monitored by TLC (10% MeOH, 90% DCM). The reaction mixture was evaporated to dryness, and the residue was purified by flash chromatography (silica gel, MeOH/DCM) to yield **3** (850 mg, 92%). ^{1}H NMR (500 MHz, CD_3OD) δ 5.07 (d, *J*=3.8 Hz, H-1), 4.30 (ddd, J_1=1.4, J_2=5.2, J_3=7.5 Hz, H-5), 4.19 (dd, J_1=3.8, J_2=10.1 Hz, H-3), 3.91 (dd, J_1=1.5, J_2=3.7 Hz, H-4), 3.84 (dd, J_1=3.8, J_2=10.1 Hz, H-2), 3.54 (dd, J_1=7.8, J_2=12.6 Hz, H-6), 3.30 (dd, J_1=5.2, J_2=12.7 Hz, H-6). ^{13}C NMR (125 MHz CD_3OD) δ 94.3, 69.7, 68.3, 68.2, 64.2, 51.3. LRMS (ESI) m/z calculated 477.14 for $C_{12}H_{18}N_{12}O_7Cl$, found 477.12 $[M+Cl]^-$.

General procedure for etherification of azide-protected trehalose scaffolds (compound 4a): Butyl bromide (137 µL, 1.26 mmol), TBAI (catalytic amount), and NaH (60%, 50 mg, 1.26 mmol) were added to a solution of compound **3** (70 mg, 0.16 mmol) dissolved in dry DMF (2 mL) under argon. The reaction mixture was stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC analysis (P.E./EtOAc 90:10). Upon completion, the reaction mixture was diluted with EtOAc, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, P.E./EtOAc) gave compound **4a** as a colorless oil (72 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (d, *J*=3.7 Hz, H-1), 4.19-4.22 (m, 2H, H-5), 3.96 (dd, J_1=1.5, J_2=3.5 Hz, H-4), 3.80 (dd, J_1=3.6, J_2=9.9 Hz, H-3), 3.59-3.69 (m, 10H, H-2, *n*-butyl (8H)), 3.50 (dd, J_1=7.2, J_2=12.5 Hz, H-6), 3.25 (dd, J_1=6.3, J_2=12.4 Hz, H-6) 1.52-1.62 (m, *n*-butyl (8H)), 1.31-1.45 (m, *n*-butyl (8H)), 0.93 (t, *J*=7.3 Hz, *n*-butyl (6H)), 0.92 (t, *J*=7.3 Hz, *n*-butyl (6H)). ^{13}C NMR (125 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.1, 70.4, 67.8, 60.6, 51.4, 32.2, 19.1, 13.9. LRMS (ESI) m/z calculated 711.39 for $C_{29}H_{51}N_{12}O_9$, found 711.56 $[M+HCO_2]^-$.

Compound **4b** was prepared essentially as described for synthesis of **4a**. Compound **3** (80 mg, 0.18 mmol), 1-bromopentane (181 µL, 1.45 mmol), NaH (60%, 58 mg, 1.45 mmol), TBAI (catalytic amount), and DMF (2 mL) were stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC (P.E./EtOAc, 95:5). Purification by flash column chromatography (silica gel, P.E./EtOAc) gave **4b** (70 mg, 54%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (d, *J*=3.6 Hz, H-1), 4.19-4.23 (m, 2H, H-5), 3.96 (dd, J_1=1.5, J_2=3.5 Hz, H-4), 3.81 (dd, J_1=3.6, J_2=9.9 Hz, H-3), 3.57-3.69 (m, 10H, H-2, *n*-pentyl (8H)),

3.51 (dd, J_1=7.2, J_2=12.5 Hz, H-6), 3.25 (dd, J_1=6.3, J_2=12.4 Hz, H-6) 1.54-1.65 (m, *n*-pentyl (8H)), 1.25-1.38 (m, *n*-pentyl (16H)), 0.90 (t, *J*=6.4 Hz, *n*-pentyl (12H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.4, 70.7, 67.8, 60.6, 51.5, 29.9, 29.8, 28.1, 22.6, 22.5, 14.1, 14.0. LRMS (ESI) m/z calculated 767.46 for $C_{33}H_{59}N_{12}O_9$, found 767.78 $[M+HCO_2]^-$.

Compound **4c** was prepared essentially as described for **4a**. Compound **3 (**70 mg, 0.16 mmol), 1-bromohexane (178 µL, 1.3 mmol), NaH (60%, 50 mg, 1.3 mmol), TBAI (catalytic amount), and DMF (1.5 mL) were stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC (P.E./EtOAc, 95:5). Purification by flash column chromatography (silica gel, P.E./EtOAc) gave **4c** as a colorless oil (88 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (d, *J*=3.6 Hz, H-1), 4.19-4.23 (m, 2H, H-5), 3.96-3.97 (m, 2H, H-4), 3.80 (dd, J_1=3.6, J_2=9.9 Hz, H-3), 3.57-3.69 (m, 10H, H-2, *n*-hexyl (8H)), 3.51 (dd, J_1=7.3, J_2=12.4 Hz, H-6), 3.25 (dd, J_1=6.2, J_2=12.4 Hz, H-6) 1.53-1.64 (m, *n*- hexyl (8H)), 1.25-1.36 (m, *n*- hexyl (24H)), 0.89 (t, *J*=6.5 Hz, *n*- hexyl (12H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.4, 70.8, 67.8, 60.6, 51.5, 31.7, 31.6, 30.1, 25.6, 29.1, 22.6, 22.5, 14.0. LRMS (ESI) m/z calculated 823.52 for $C_{45}H_{83}N_{12}O_9$, found 823.47 $[M+HCO_2]^-$.

Compound **4d** was prepared essentially as described for **4a**. Compound **3** (85 mg, 0.19 mmol), 1-bromoheptane (242 µL, 1.54 mmol), NaH (60%, 62 mg, 1.54 mmol), TBAI (catalytic amount), and DMF (2 mL) were stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC (P.E./EtOAc, 95:5). Purification by flash column chromatography (silica gel, P.E./EtOAc) gave **4d** (90 mg, 56%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (d, *J*=3.6 Hz, H-1), 4.20-4.23 (m, 2H, H-5), 3.94-3.96 (m, 2H, H-4), 3.80 (dd, J_1=3.6, J_2=9.9 Hz, H-3), 3.57-3.69 (m, 10H, H-2, *n*-heptyl (8H)), 3.51 (dd, J_1=7.2, J_2=12.4 Hz, H-6), 3.25 (dd, J_1=6.3, J_2=12.4 Hz, H-6) 1.55-1.65 (m, *n*-heptyl (8H)), 1.28-1.38 (m, *n*-heptyl (32H)), 0.88 (t, *J*=6.6 Hz, *n*-heptyl (12H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.4, 70.8, 67.8, 60.6, 51.5, 31.9, 31.8, 30.1, 29.2, 29.1, 25.9, 22.6, 14.1. LRMS (ESI) m/z calculated 879.58 for $C_{41}H_{75}N_{12}O_9$, found 879.86 $[M+HCO_2]^-$.

Compound **4e** was prepared essentially as described for **4a**. Compound **3** (120 mg, 0.27 mmol), 1-bromooctane (373 µL, 2.16 mmol), NaH (60%, 86 mg, 2.16 mmol), TBAI (catalytic amount), and DMF (2 mL) were stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC (P.E./EtOAc, 95:5). Purification by flash column chromatography (silica gel, P.E./EtOAc) gave **4e** (172 mg, 71%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (d, *J*=3.7 Hz, H-1), 4.20-4.23 (m, 2H, H-5), 3.96 (dd, , J_1=1.5, J_2=3.5 Hz, H-4), 3.80 (dd, J_1=3.6, J_2=9.9 Hz, H-3), 3.58-3.68 (m, 10H, H-2, *n*-octyl (8H)), 3.51 (dd, J_1=7.3, J_2=12.5 Hz, H-6), 3.25 (dd, J_1=6.3, J_2=12.5 Hz, H-6) 1.54-1.64 (m, *n*-octyl

(8H)), 1.27-1.39 (m, *n*-octyl (40H)), 0.88 (t, *J*=6.7 Hz, *n*-octyl (12H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.4, 70.8, 67.8, 60.6, 51.5, 31.8, 30.2, 30.1, 29.5, 29.4, 29.3, 29.2, 26.0, 22.6, 14.1. LRMS (ESI) m/z calculated 935.67 for $C_{45}H_{83}N_{12}O_9$, found 935.77 $[M+HCO_2]^-$.

Compound **4f** was prepared as described for **4a**. Compound **3** (100 mg, 0.23 mmol), 1-bromodecane (375 µL, 1.81 mmol), NaH (60%, 72 mg, 1.81 mmol), TBAI (catalytic amount), and DMF (2 mL) were stirred at ambient temperature overnight. Progress of the reaction was monitored by TLC (P.E./EtOAc, 95:5). Purification by flash column chromatography (silica gel, P.E./EtOAc) gave **4f** (120 mg, 52%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (d, *J*=3.7 Hz, H-1), 4.19-4.22 (m, 2H, H-5), 3.96 (dd, , J_1=1.5, J_2=3.5 Hz, H-4), 3.80 (dd, J_1=3.6, J_2=9.8 Hz, H-3), 3.58-3.68 (m, 10H, H-2, *n*-decyl (8H)), 3.50 (dd, J_1=7.3, J_2=12.5 Hz, H-6), 3.24 (dd, J_1=6.3, J_2=12.4 Hz, H-6) 1.53-1.63 (m, *n*-decyl (8H)), 1.26-1.39 (m, *n*-decyl (56H)), 0.87 (t, *J*=6.8 Hz, *n*-decyl (12H)). ^{13}C NMR (125 MHz, $CDCl_3$) δ 93.6, 77.8, 75.8, 72.4, 70.8, 67.8, 60.6, 51.5, 31.9, 30.2, 30.1, 29.7, 29.6, 29.5, 29.3, 26.0, 22.7, 14.1. LRMS (ESI) m/z calculated 1061.78 for $C_{54}H_{101}N_{12}O_9$, found 1062.10 $[M+ HCO_2]^-$.

General procedure for reduction of azide protecting groups (compound 5a): Compound **4a** (40 mg, 0.06 mmol) was dissolved in THF (2.0 mL) followed by the addition of 0.1 M NaOH (0.5 mL) and 1 M trimethylphosphine in THF (1.2 mL, 1.2 mmol). The reaction mixture was stirred at 50 ºC for 4 hrs. The reaction was monitored by TLC (2.8% NH_4OH solution in MeOH/dichloromethane, 15:85). Upon completion, the reaction mixture was evaporated under reduced pressure, and product was purified by flash column chromatography (silica gel, 2.8% NH_4OH solution in MeOH/dichloromethane). Fractions containing the pure product were concentrated under vacuum, dissolved in H_2O, and freeze-dried to yield compound **5a** as a white foam (35 mg, 78%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.20 (d, *J*=3.8 Hz, H-1), 4.00-4.04 (m, 2H, H-5), 3.60-3.73 (m, 10H, H-2, H-3, *n*-butyl (6H)), 3.53 (dt, J_1=6.4, J_2=9.1 Hz, *n*-butyl (2H)), 3.33 (dd, J_1=1.7, J_2=3.9 Hz, H-4), 2.73-2.83 (m, 4H, H-6, H-6), 1.53-1.63 (m, *n*-butyl (8H)), 1.35-1.49 (m, *n*-butyl (8H)), 0.96 (t, *J*=7.3 Hz, *n*-butyl (6H)), 0.95 (t, *J*=7.3 Hz, *n*-butyl (6H)). ^{13}C NMR (100 MHz, CD_3OD) δ 92.9 (C-1), 77.7 (C-3), 75.3 (C-2), 71.3 (*n*-butyl), 70.4 (C-5), 68.8 (*n*-butyl), 49.5 (C-4), 41.8 (C-6), 32.1 (*n*-butyl), 32.0 (*n*-butyl), 19.1 (*n*-butyl), 18.9 (*n*-butyl), 12.9 (*n*-butyl), 12.8 (*n*-butyl). HR ESI-MS, m/z calcd 563.4384 for $C_{28}H_{59}N_4O_7$, found 563.4382 $[M+H]^+$.

Compound **5b** was prepared as described for **5a**. Compound **4b** (65 mg, 0.09 mmol), THF (3.0 mL), NaOH (0.1 M, 0.5 mL), and 1 M trimethylphosphine in THF (1.8 mL, 1.8 mmol) were stirred at 50 ºC for 4 hrs. Purification by flash column chromatography (silica gel, 2.8%

NH_4OH solution in MeOH/dichloromethane) gave **5b** (25 mg, 45%) as a white foam. ^1H NMR (400 MHz, CD_3OD) δ 5.20 (d, *J*=3.8 Hz, H-1), 4.02 (ddd, J_1=1.5, J_2=5.9, J_3=7.7 Hz, H-5), 3.60-3.73 (m, 10H, H-2, H-3, *n*-pentyl (6H)), 3.51 (dt, J_1=6.4, J_2=9.1 Hz, *n*-pentyl (2H)), 3.32 (dd, J_1=1.6, J_2=3.8 Hz, H-4), 2.74-2.84 (m, 4H, H-6, H-6), 1.55-1.63 (m, *n*-pentyl (8H)), 1.31-1.42 (m, *n*-pentyl (16H)), 0.93 (t, *J*=7.0 Hz, *n*-pentyl (6H)), 0.92 (t, *J*=7.0 Hz, *n*-pentyl (6H)). ^{13}C NMR (100 MHz, CD_3OD) δ 92.8 (C-1), 77.6 (C-3), 75.2 (C-2), 71.5 (*n*-pentyl), 70.4 (C-5), 69.1 (*n*-pentyl), 49.5 (C-4), 41.8 (C-6), 29.7 (*n*-pentyl), 29.5 (*n*-pentyl), 28.2 (*n*-pentyl), 28.1 (*n*-pentyl), 22.3 (*n*-pentyl), 22.2 (*n*-pentyl), 13.1 (*n*-pentyl), 13.0 (*n*-pentyl). HR ESI-MS, m/z calcd 619.5010 for $C_{32}H_{67}N_4O_7$, found 619.5011 $[M+H]^+$.

Compound **5c** was prepared as described for **5a**. Compound **4c** (40 mg, 0.05 mmol), THF (2.0 mL), NaOH (0.1 M, 0.5 mL), and 1 M trimethylphosphine in THF (1 mL, 1.1 mmol) were stirred at 50 ºC for 4 hrs. Purification by flash column chromatography (silica gel, 2.8% NH_4OH solution in MeOH/dichloromethane) gave **5c** (28 mg, 75%) as a white foam. ^1H NMR (400 MHz, CD_3OD) δ 5.20 (d, *J*=3.8 Hz, H-1), 4.02 (ddd, J_1=1.5, J_2=5.9, J_3=7.7 Hz, H-5), 3.60-3.73 (m, 10H, H-2, H-3, *n*-hexyl (6H)), 3.51 (dt, J_1=6.4, J_2=9.1 Hz, *n*-hexyl (2H)), 3.32 (m, 2H, H-4), 2.72-2.84 (m, 4H, H-6, H-6), 1.54-1.65 (m, *n*-hexyl (8H)), 1.33-1.46 (m, *n*-hexyl (24H)), 0.91 (t, *J*=6.4 Hz, *n*-hexyl (12H)). ^{13}C NMR (100 MHz, CD_3OD) δ 92.7 (C-1), 77.7 (C-3), 75.3 (C-2), 71.5 (*n*-hexyl), 70.6 (C-5), 69.1 (*n*-hexyl), 49.5 (C-4), 41.9 (C-6), 31.5 (*n*-hexyl), 31.4 (*n*-hexyl), 29.9 (*n*-hexyl), 29.8 (*n*-hexyl), 25.7 (*n*-hexyl), 25.6 (*n*-hexyl), 22.4 (*n*-hexyl), 22.3 (*n*-hexyl), 13.1 (*n*-hexyl), 13.0 (*n*-hexyl). HR ESI-MS, m/z calcd 675.5636 for $C_{36}H_{75}N_4O_7$, found 675.5637 $[M+H]^+$.

Compound **5d** was prepared as described for **5a**. Compound **4d** (70 mg, 0.08 mmol), THF (3.0 mL), NaOH (0.1 M, 0.5 mL), and 1 M trimethylphosphine in THF (1.6 mL, 1.6 mmol) were stirred at 50 ºC for 4 hrs. Purification by flash column chromatography (silica gel, 2.8% NH_4OH solution in MeOH/dichloromethane) gave **5d** (27 mg, 46%) as a white foam. ^1H NMR (400 MHz, CD_3OD) δ 5.21 (d, *J*=3.8 Hz, H-1), 4.02 (ddd, J_1=1.4, J_2=5.8, J_3=7.6 Hz, H-5), 3.60-3.75 (m, 10H, H-2, H-3, *n*-heptyl (6H)), 3.51 (dt, J_1=6.5, J_2=9.1 Hz, *n*-heptyl (2H)), 3.32 (m, 2H, H-4), 2.72-2.84 (m, 4H, H-6, H-6), 1.54-1.65 (m, *n*-heptyl (8H)), 1.32-1.43 (m, *n*-heptyl (32H)), 0.91 (t, *J*=6.6 Hz, *n*-heptyl (12H)). ^{13}C NMR (100 MHz, CD_3OD) δ 92.7 (C-1), 77.7 (C-3), 75.2 (C-2), 71.5 (*n*-heptyl), 70.6 (C-5), 69.1 (*n*-heptyl), 49.5 (C-4), 41.9 (C-6), 31.7 (*n*-heptyl), 31.6 (*n*-heptyl), 30.0 (*n*-heptyl), 29.9 (*n*-heptyl), 29.0 (*n*-heptyl), 28.9 (*n*-heptyl), 26.0 (*n*-heptyl), 25.9 (*n*-heptyl), 22.3 (*n*-heptyl), 13.0 (*n*-heptyl). HR ESI-MS, m/z calcd 731.6262 for $C_{40}H_{83}N_4O_7$, found 731.6258 $[M+H]^+$.

Compound **5e** was prepared as described for **5a**. Compound **4e** (85 mg, 0.09 mmol), THF (3.0 mL), NaOH (0.1 M, 0.5 mL) and 1 M trimethylphosphine in THF (1.9 mL, 1.9 mmol) were stirred at 50 ºC for 4 hrs. Purification by flash column chromatography (silica gel, 2.8% NH_4OH solution in MeOH/dichloromethane) gave **5e** (37 mg, 49%) as a white foam. ^{1}H NMR (400 MHz, CD_3OD) δ 5.21 (d, *J*=3.8 Hz, H-1), 4.02 (ddd, J_1=1.4, J_2=5.8, J_3=7.6 Hz, H-5), 3.60-3.73 (m, 10H, H-2, H-3, *n*-octyl (6H)), 3.51 (dt, J_1=6.5, J_2=9.1 Hz, *n*-octyl (2H)), 3.32 (m, 2H, H-4), 2.72-2.84 (m, 4H, H-6, H-6), 1.54-1.65 (m, *n*-octyl (8H)), 1.31-1.43 (m, *n*-octyl (40H)), 0.90 (t, *J*=6.6 Hz, *n*-octyl 12H). ^{13}C NMR (100 MHz, CD_3OD) δ 92.6 (C-1), 77.7 (C-3), 75.2 (C-2), 71.5 (*n*-octyl), 70.6 (C-5), 69.1 (*n*-octyl), 49.5 (C-4), 41.9 (C-6), 31.6 (*n*-octyl), 30.0 (*n*-octyl), 29.9 (*n*-octyl), 29.4 (*n*-octyl), 29.3 (*n*-octyl), 29.2 (*n*-octyl), 29.1 (*n*-octyl), 26.1 (*n*-octyl), 26.0 (*n*-octyl), 22.3 (*n*-octyl), 13.0 (*n*-octyl). HR ESI-MS, m/z calcd 787.6888 for $C_{44}H_{91}N_4O_7$, found 787.6891 $[M+H]^+$.

Compound **5f** was prepared as described for **5a**. Compound **4f** (40 mg, 0.04 mmol), THF (2.0 mL), NaOH (0.1 M, 0.5 mL) and 1 M trimethylphosphine in THF (0.8 mL, 0.8 mmol) were stirred at 50 ºC for 4 hrs. Purification by flash column chromatography (silica gel, 2.8% NH_4OH solution in MeOH/dichloromethane) gave **5f** (11 mg, 30%) as a white foam. ^{1}H NMR (400 MHz, CD_3OD) δ 5.21 (d, *J*=3.7 Hz, H-1), 4.02 (ddd, J_1=1.4, J_2=5.6, J_3=7.5 Hz, H-5), 3.60-3.74 (m, 10H, H-2, H-3, *n*-decyl (6H)), 3.51 (dt, J_1=6.5, J_2=9.1 Hz, *n*-decyl (2H)), 3.32 (m, 2H, H-4), 2.72-2.84 (m, 4H, H-6, H-6), 1.54-1.63 (m, *n*-decyl (8H)), 1.30-1.42 (m, *n*-decyl (56H)), 0.90 (t, *J*=6.7 Hz, *n*-decyl (12H)). ^{13}C NMR (100 MHz, CD_3OD) δ 92.6 (C-1), 77.7 (C-3), 75.2 (C-2), 71.4 (*n*-decyl), 70.6 (C-5), 69.1(*n*-decyl), 49.6 (C-4), 41.9 (C-6), 31.7 (*n*-decyl), 30.0 (*n*-decyl), 29.9 (*n*-decyl), 29.5 (*n*-decyl), 29.4 (*n*-decyl), 29.3 (*n*-decyl), 29.1(*n*-decyl), 26.1 (*n*-decyl), 26.0 (*n*-decyl), 22.3 (*n*-decyl), 13.0 (*n*-decyl). HR ESI-MS, m/z calcd 899.8140 for $C_{52}H_{107}N_4O_7$, found 899.8146 $[M+H]^+$.

4. Biological evaluation protocols.

A. Minimal inhibitory concentration test (anti-bacterial activity): All strains were tested using the double dilution method in 96 well plates (Corning).[1] Starter cultures were incubated for 24 h (37 °C, 5% CO_2, aerobic conditions) and then diluted in fresh medium to obtain an optical density of OD_{600}=0.004. All compounds were dissolved in ethanol (5 mg/mL) and then added to the LB media to form the mother liquor (32 μL in 1218 μL of LB) at the starting concentration of 64 μg/mL. Next, 100 μL of serial double dilutions of compounds in LB (64, 32, 16, 8, 4, 2 and 1 μg/mL) were prepared in a flat-bottomed 96 well microplates (Corning). Control wells with no compounds and wells without bacteria containing each tested concentration of the compounds (blanks) were also prepared. An equal volume (100 μL) of bacterial suspensions in LB was added to

each well to reach a final volume of 200 μL (the final concentration of ethanol in each well ranged between 0.01 - 1.3%). After incubation of 24 h at 37°C in 5% CO_2, MTT (50 μL of a 1 mg/mL solution in H_2O) was added to each well followed by additional incubation at 37 °C for 2 h. All of the bacterial strains in this study were grown in Lysogeny Broth (LB). The medium was prepared by dissolving 20 g of LB in 1 L purified water; medium was autoclaved at 121 °C for 15 minutes. Each concentration of compound was tested in triplicate, and the results were confirmed in two independent experiments. MIC values (μg/mL) were the lowest concentration at which no bacterial growth was observed.

B. Minimal inhibitory concentration test (anti-fungal activity): All of the yeast strains in this study were grown in RPMI 1640. Starter cultures were incubated for 24 h (37 °C, 5% CO_2, aerobic conditions) and then diluted in fresh medium 1:100. All strains were tested using the double-dilution method starting at 64 μg/mL in 96-well plates (Corning) as described for the antibacterial activity test. After 24 h of incubation, MTT (50 μL of a 1 mg/mL solution in H_2O) was added to each well followed by additional incubation at 37 °C for 2 h. MIC values (μg/mL) were the lowest concentration at which no *Candida* growth was observed. Results were confirmed in two independent experiments, and each concentration was tested in triplicate.

C. Erythrocyte hemolysis assay: The hemolysis assay was performed following a previously reported protocol[1]. In each experiment, samples were analyzed in triplicate, and two independent experiments were performed. The results are an average of experiments on blood samples taken from two laboratory rats.

Table 1S: Hemolysis of rat erythrocytes (%) in the presence of indicated concentrations of evaluated compounds.[a]

	Concentration [μg/mL]						
Compound #	**256**	**128**	**64**	**32**	**16**	**8**	**4**
Colistin	0	0	0	0	0	0	0
Gramicidin D	97±3	97±2	97±2	95±4	92±2	69±4	48±2
5a	90±9	84±10	79±4	7±1	0.6±0.5	0	0
5b	100	100	100	100	78±5	43±3	7±6
5c	100	100	100	100	100	60±3	9±0
5d	100	100	100	100	100	77±10	28±6
5e	100	100	100	100	100	99±0.5	55±4
5f	100	100	90±10	51±11	25±5	14±4	5±1
C1	100	95±4	75±3	23±5	3±1	2±2	1±1
C2	95±2	95±4	95±4	92±7	88±2	6±2	0
C3	89±2	28±3	4±0	0	0	0	0

[a] Rat erythrocytes were incubated with the tested compounds for 1 hour at 37 ºC. All experiments were performed in triplicate, and the results are the average of two independent experiments using blood samples from two different laboratory rats.

D. Microscopy data acquisition: *C. albicans* that express ENO1-GFP were cultured on YPD agar plates overnight at 37 °C. A single colony was picked and diluted in Dulbecco's phosphate buffered saline (DPBS 1X) to an OD_{600} of 2.5. A 50 µL sample containing cells sample and compound **5b** at a concentration of 10 µg/mL was incubated at 37 °C for 30 min. Aliquots (2 µL) were placed on glass slides and covered with a cover slip. The cells were visualized on a MORE imaging system (TILL photonics GmbH) with an Olympus UPlanApo 100X 1.3 NA oil immersion objective. A 150 W Xenon lamp with galvanometer driven filter switching was used as an excitation source. The bandpass filter sets used to image GFP stained were 485/20ex and 523/21em.

(1) Berkov-Zrihen, Y.; Herzog, I. M.; Feldman, M.; Sonn-Segev, A.; Roichman, Y.; Fridman, M. *Bioorg. Med. Chem.* 2013, **21**, 3624–3631.

5. ^{1}H and ^{13}C NMR spectra

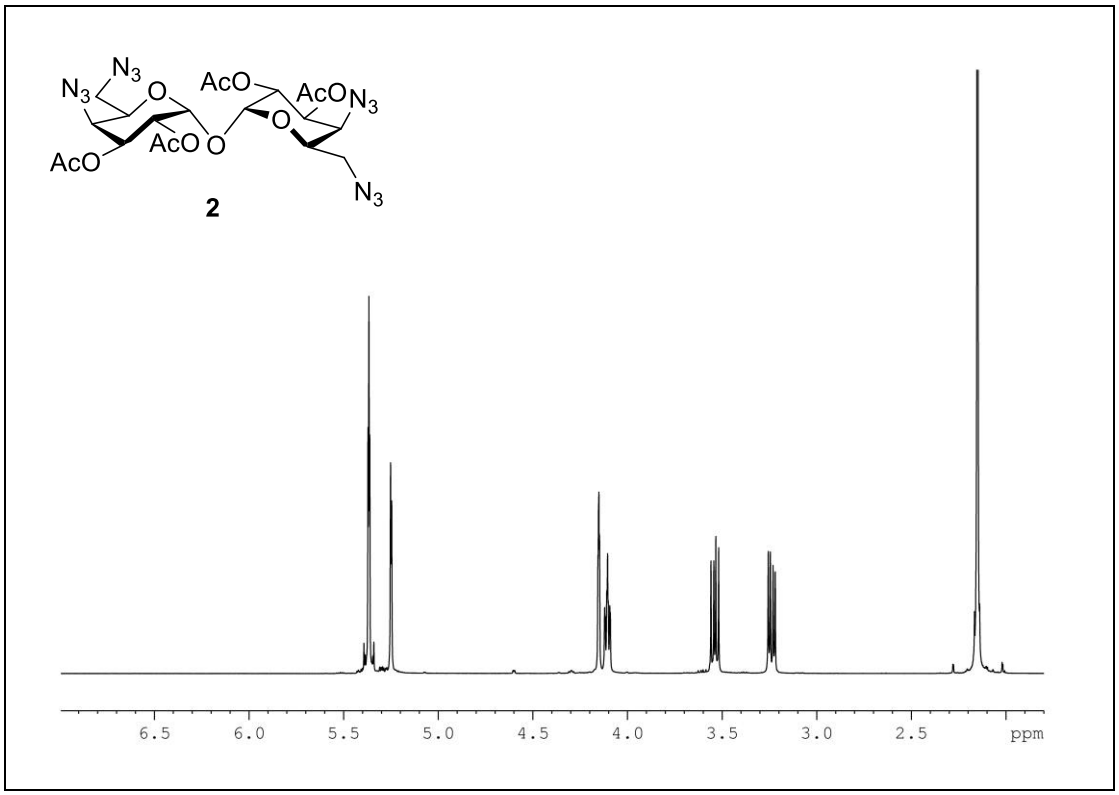


Fig. S1. 500 MHz ^{1}H-NMR in $CDCl_3$ for compound **2**.

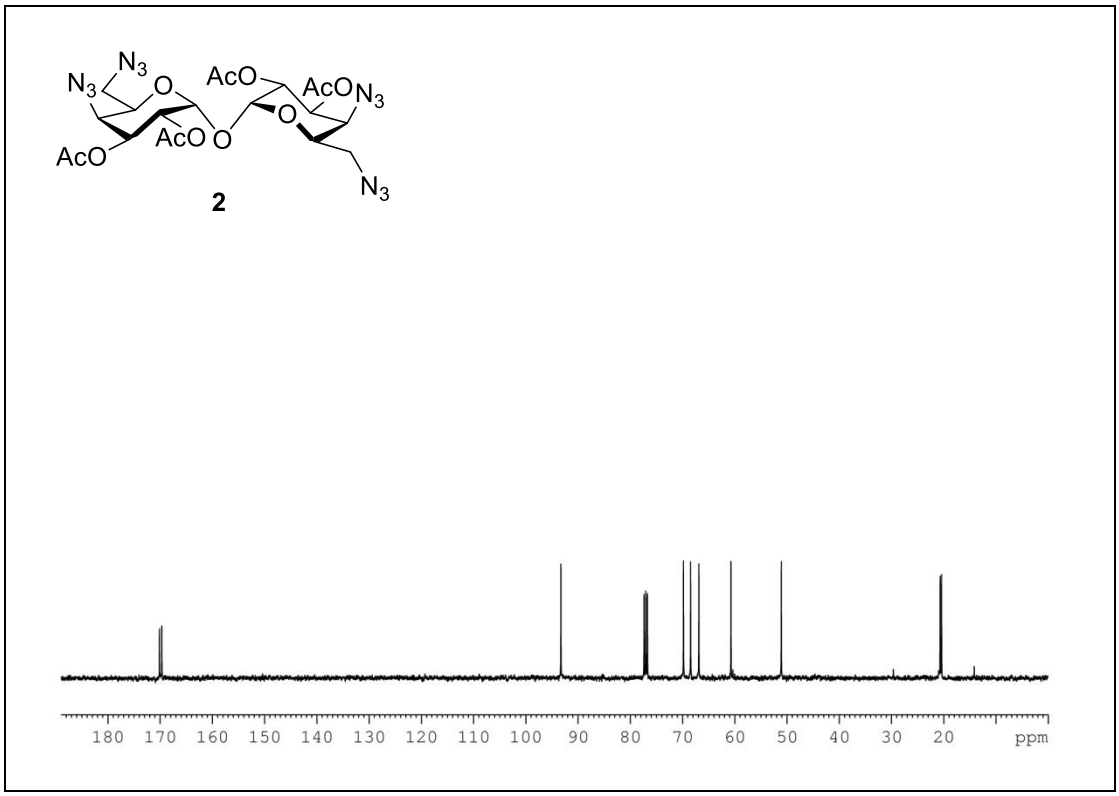


Fig. S2. 100 MHz ^{13}C-NMR in $CDCl_3$ for compound **2**.

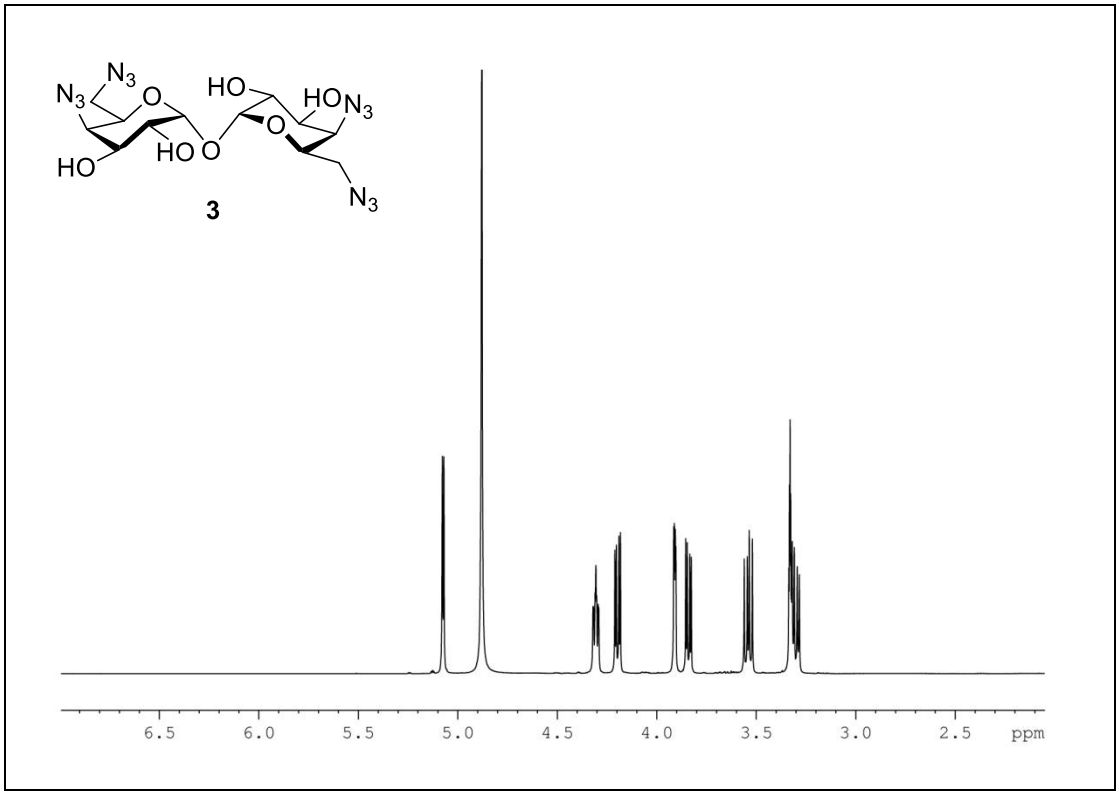


Fig. S3. 500 MHz ^{1}H-NMR in CD_3OD for compound **3**.

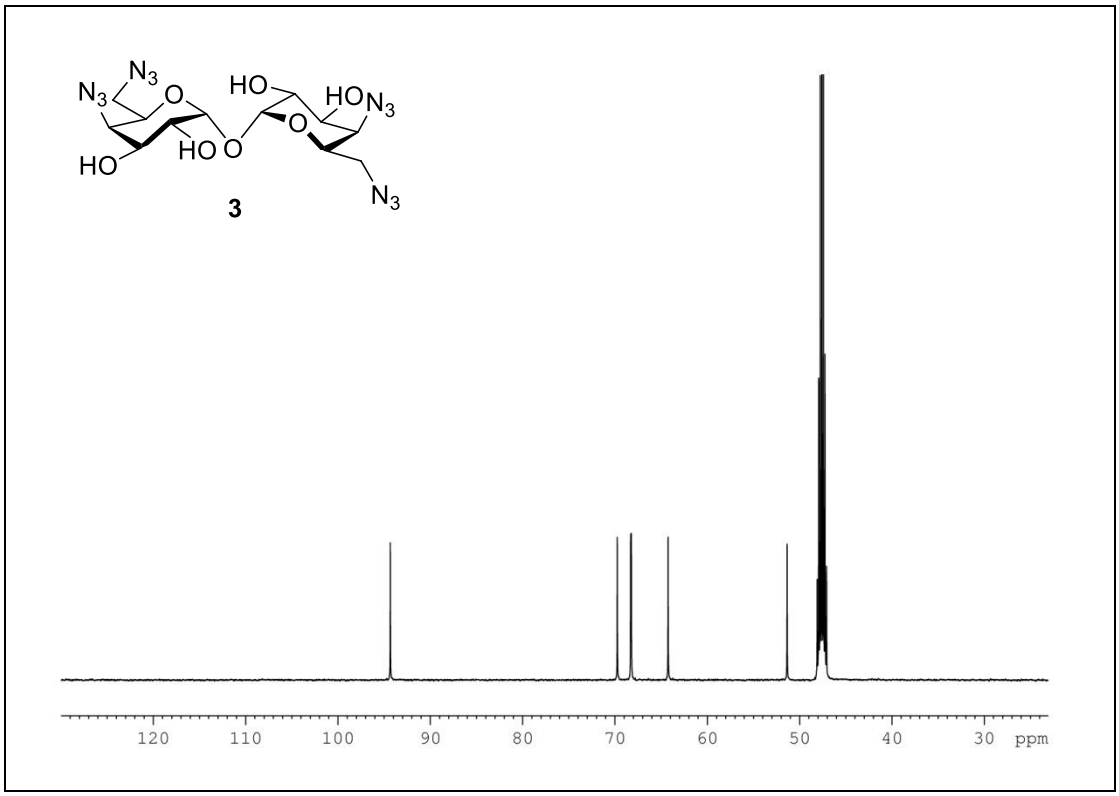


Fig. S4. 125 MHz ^{13}C-NMR in CD_3OD for compound **3**.

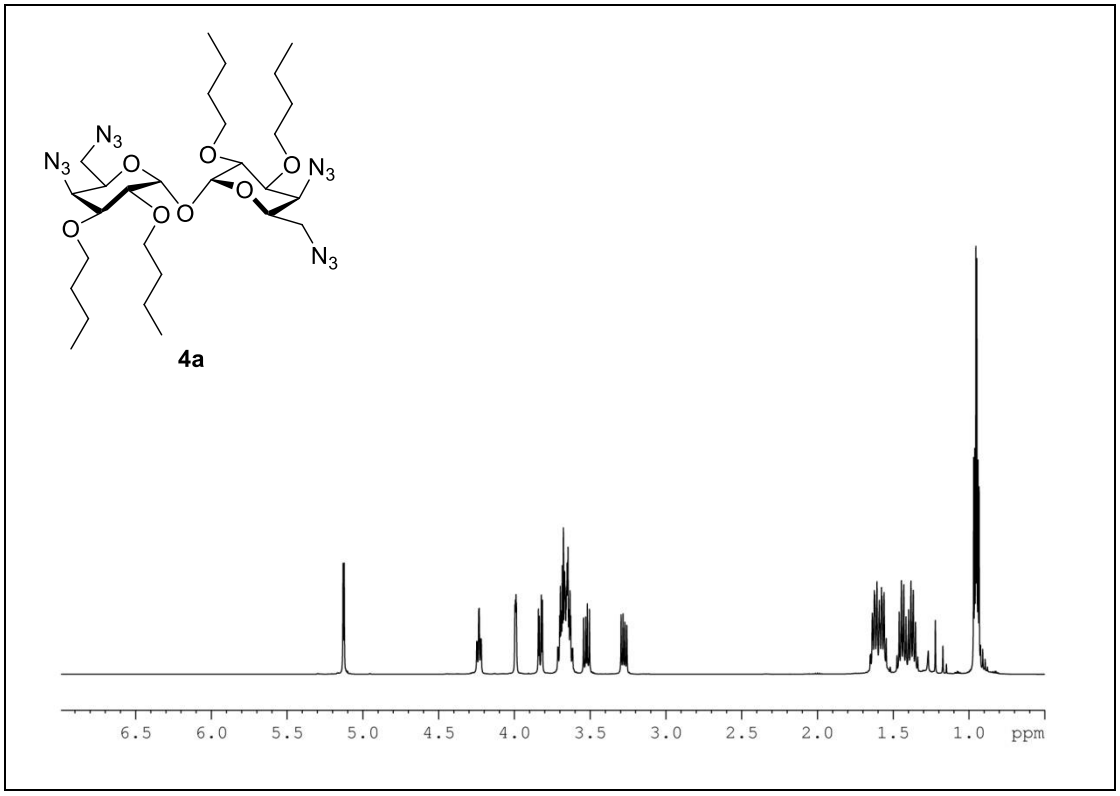


Fig. S5. 500 MHz ^{1}H-NMR in $CDCl_3$ for compound **4a**.

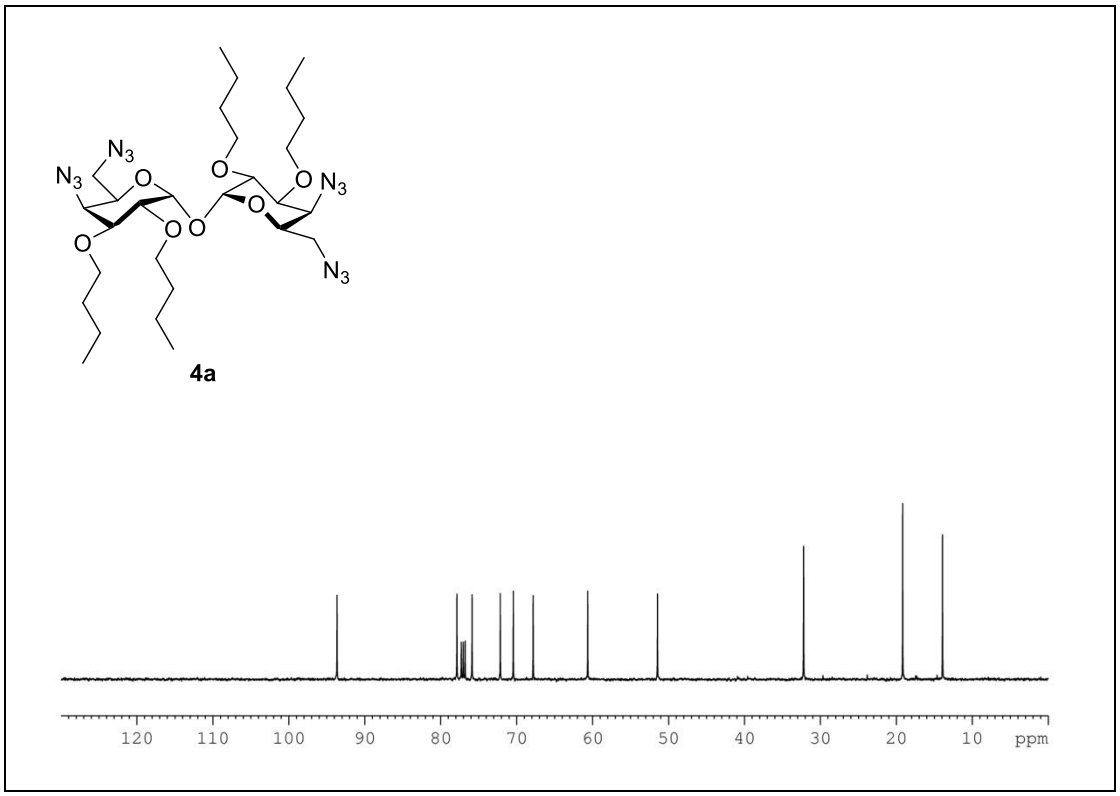


Fig. S6. 125 MHz ^{13}C-NMR in $CDCl_3$ for compound **4a**.

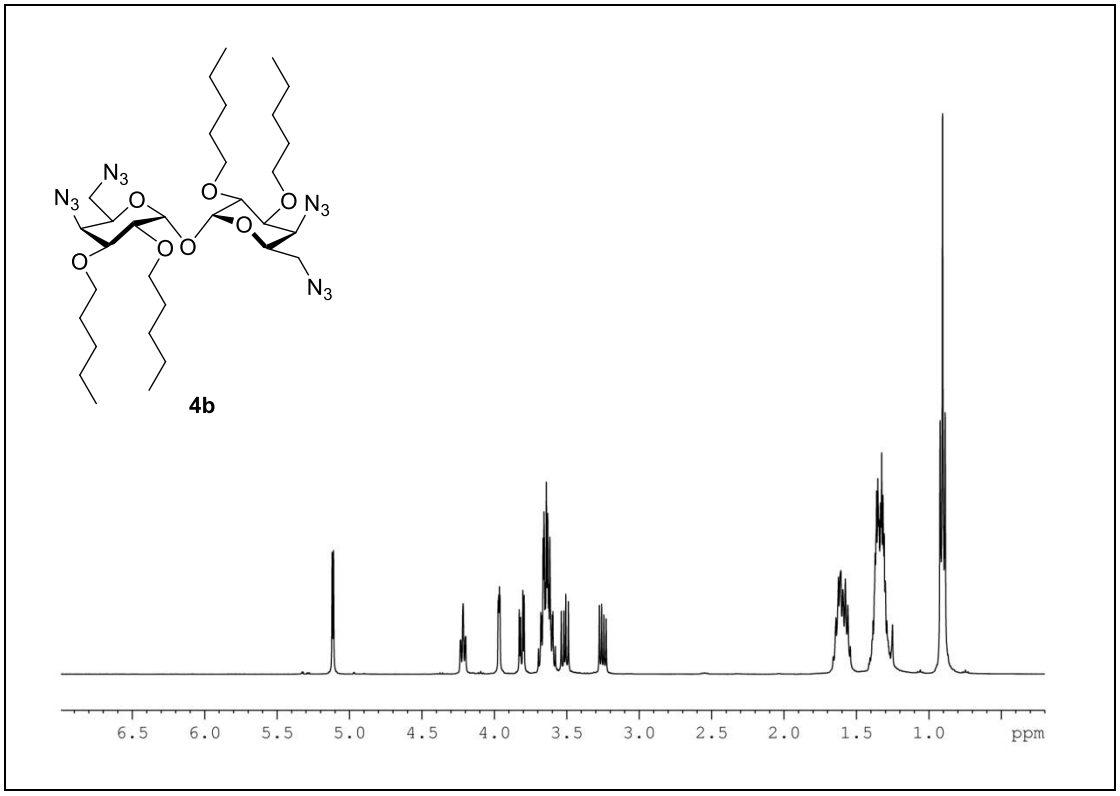


Fig. S7. 400 MHz ^{1}H-NMR in $CDCl_3$ for compound **4b**.

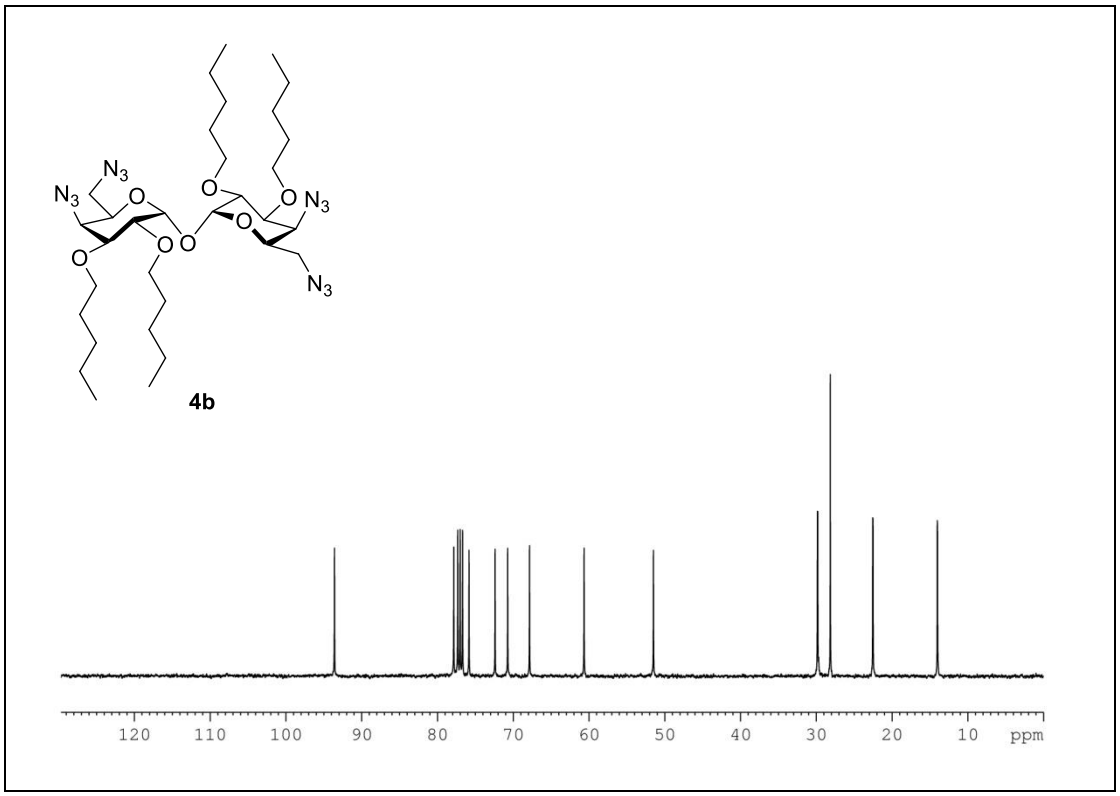


Fig. S8. 400 MHz ^{13}C-NMR in $CDCl_3$ for compound **4b**.

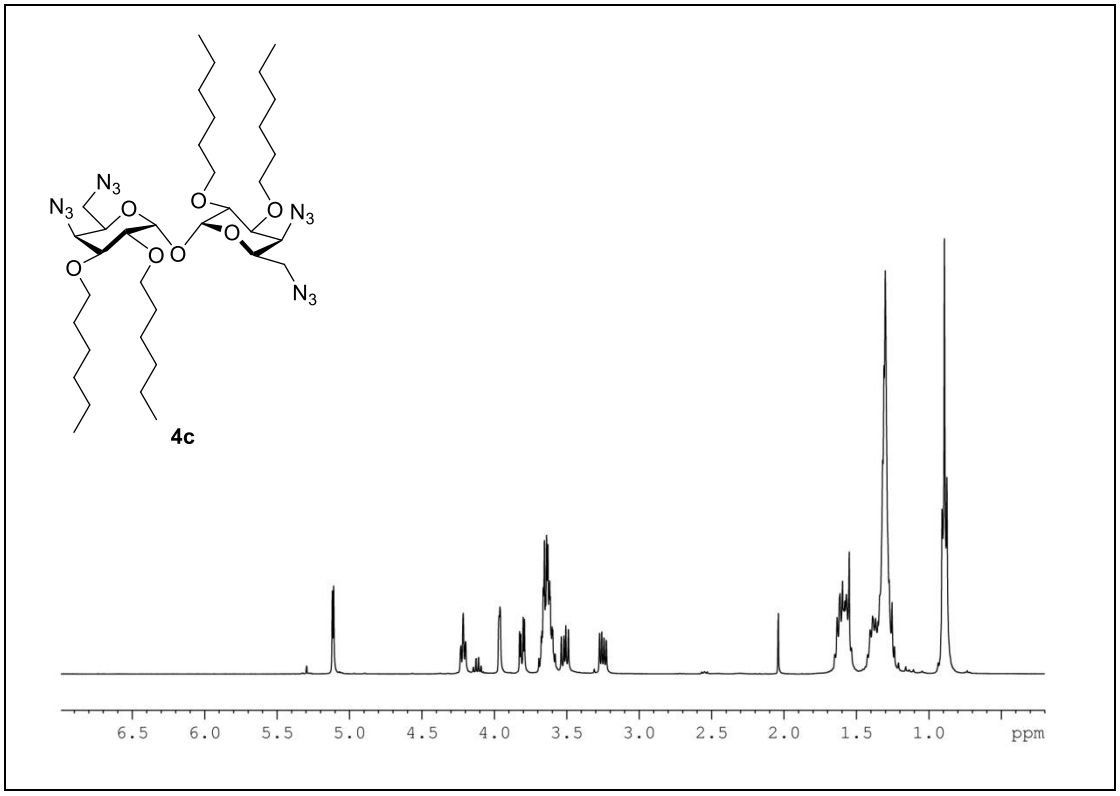


Fig. S9. 400 MHz ^{1}H-NMR in $CDCl_3$ for compound **4c**.

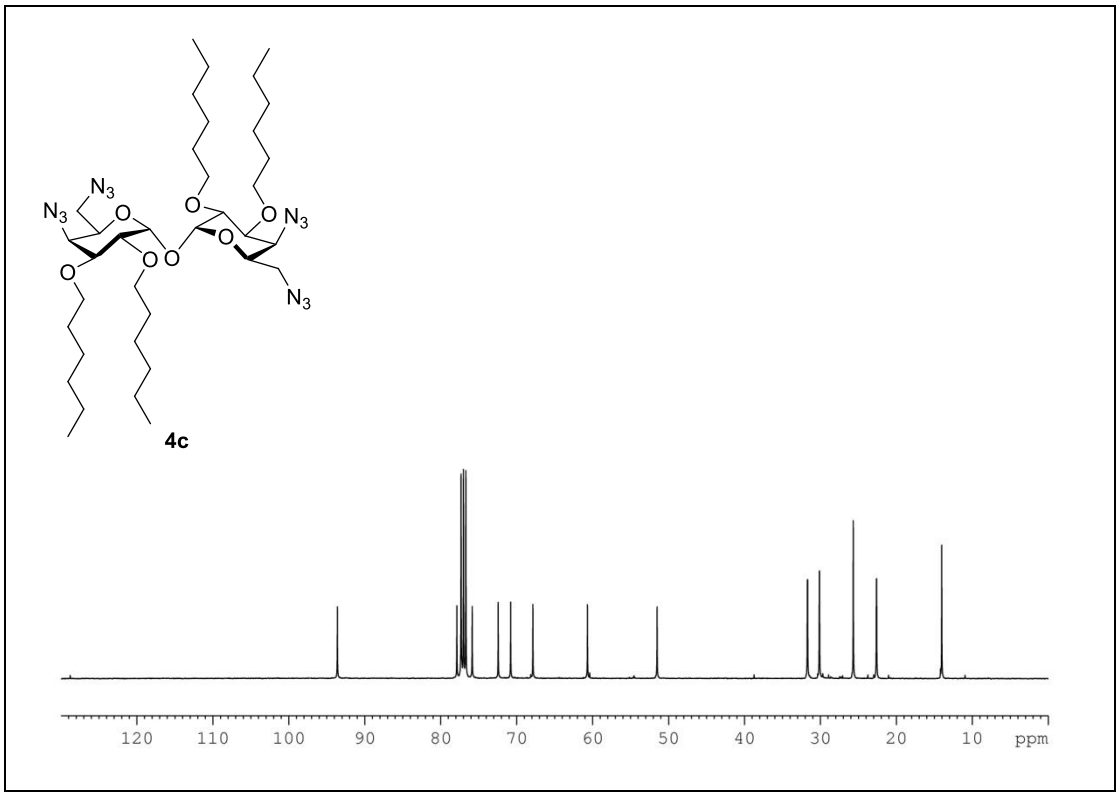


Fig. S10. 100 MHz ^{13}C-NMR in $CDCl_3$ for compound **4c**.

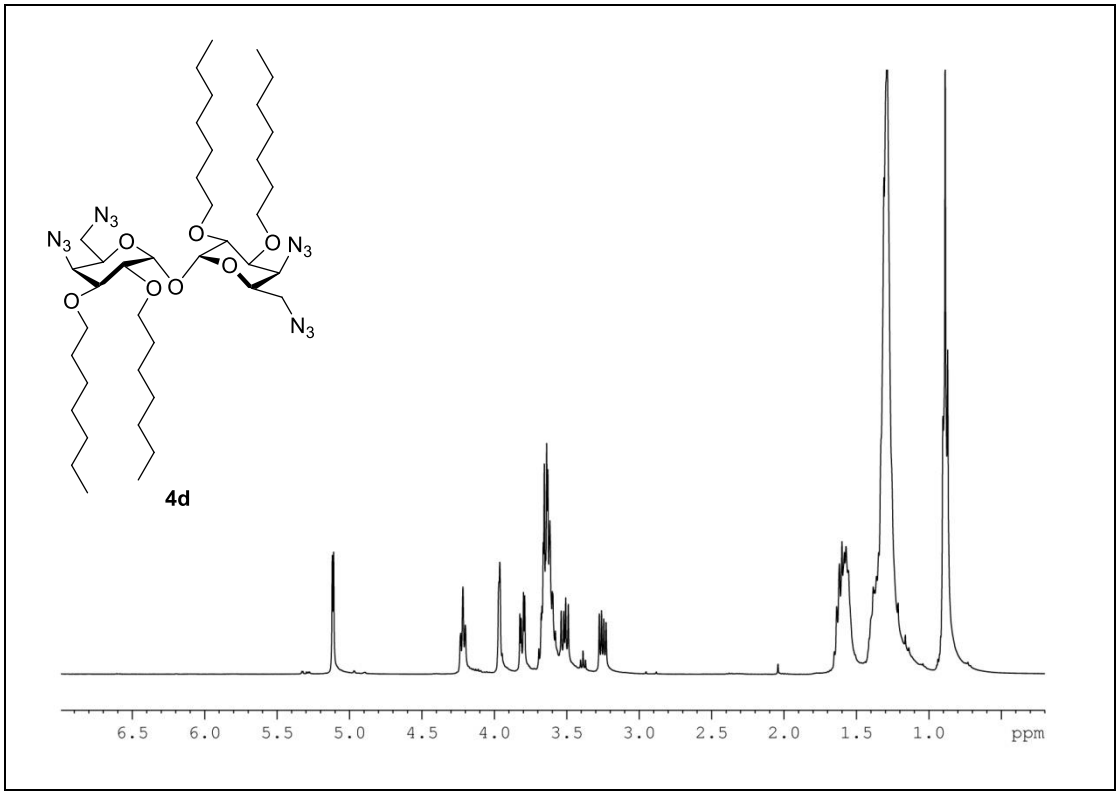


Fig. S11. 400 MHz ^{1}H NMR in $CDCl_3$ for compound **4d**.

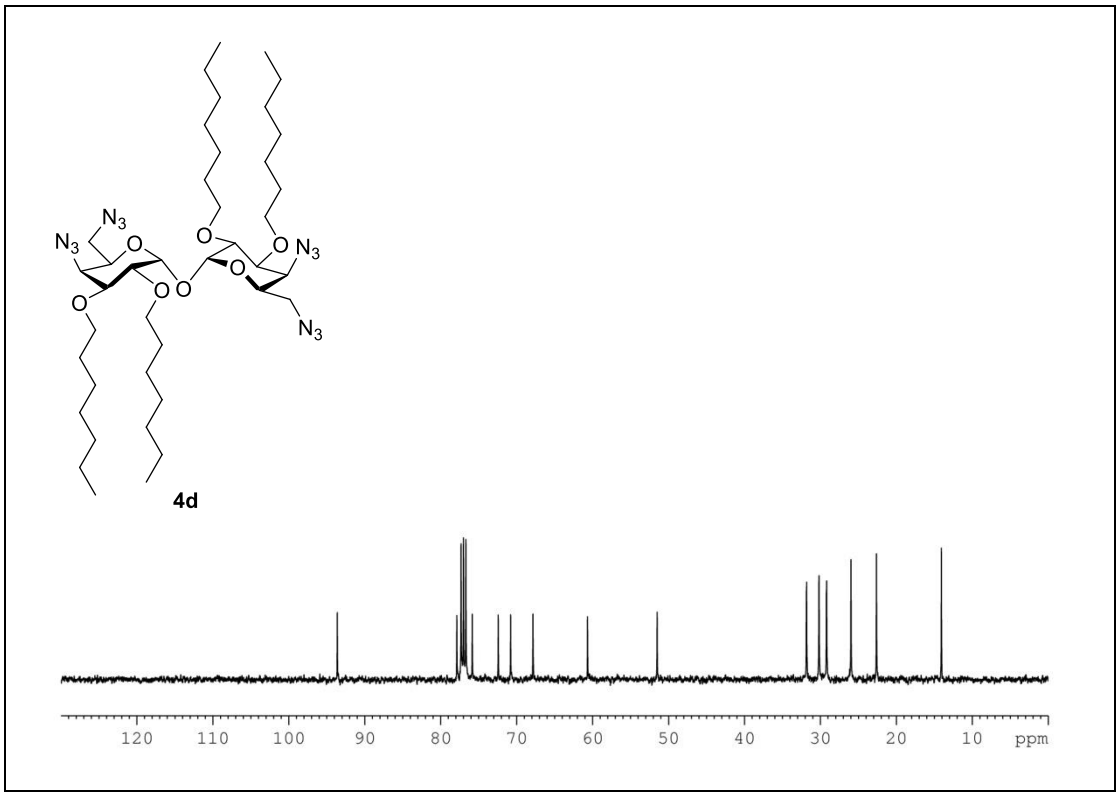


Fig. S12. 100 MHz ^{13}C-NMR in $CDCl_3$ for compound **4d**.

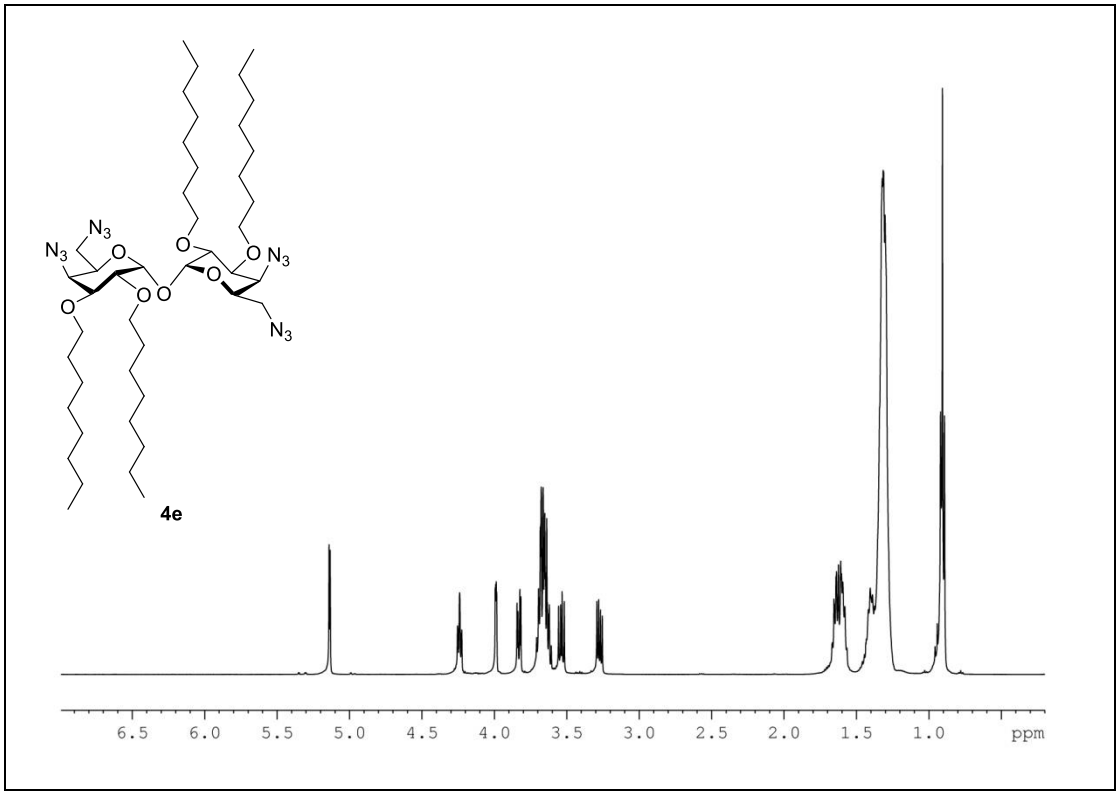


Fig. S13. 400 MHz ^{1}H-NMR in $CDCl_3$ for compound **4e**.

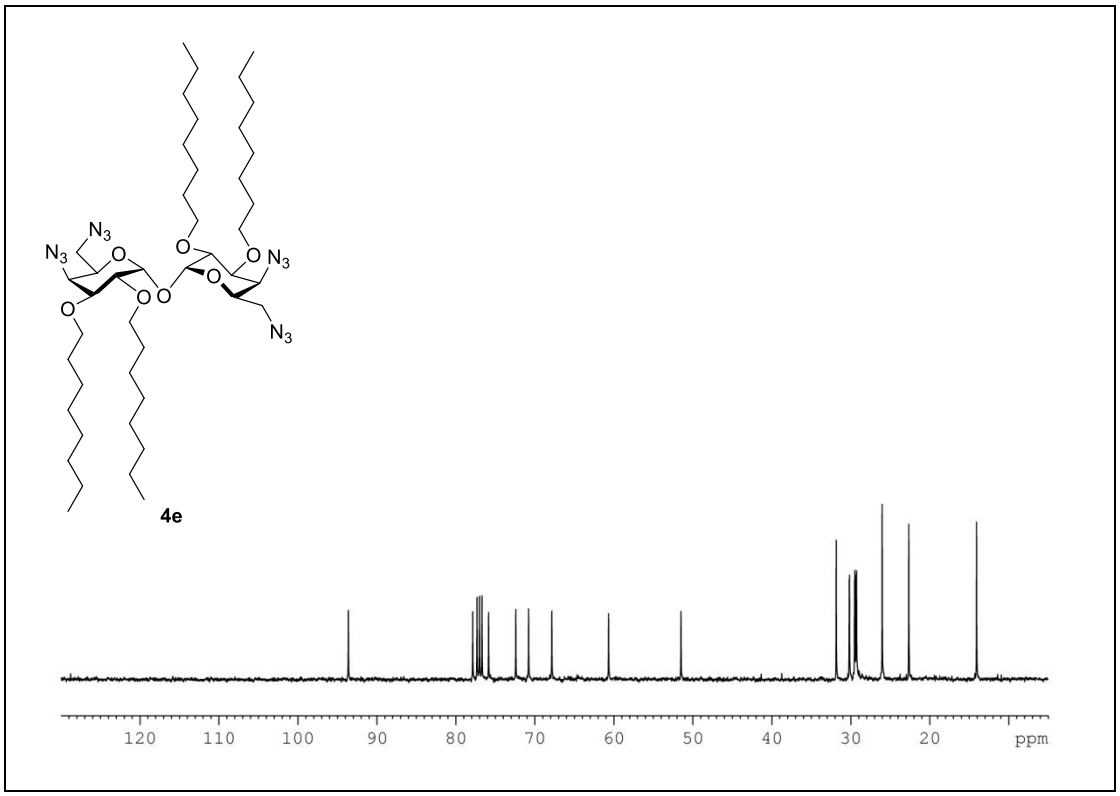


Fig. S14. 100 MHz ^{13}C-NMR in $CDCl_3$ for compound **4e**.

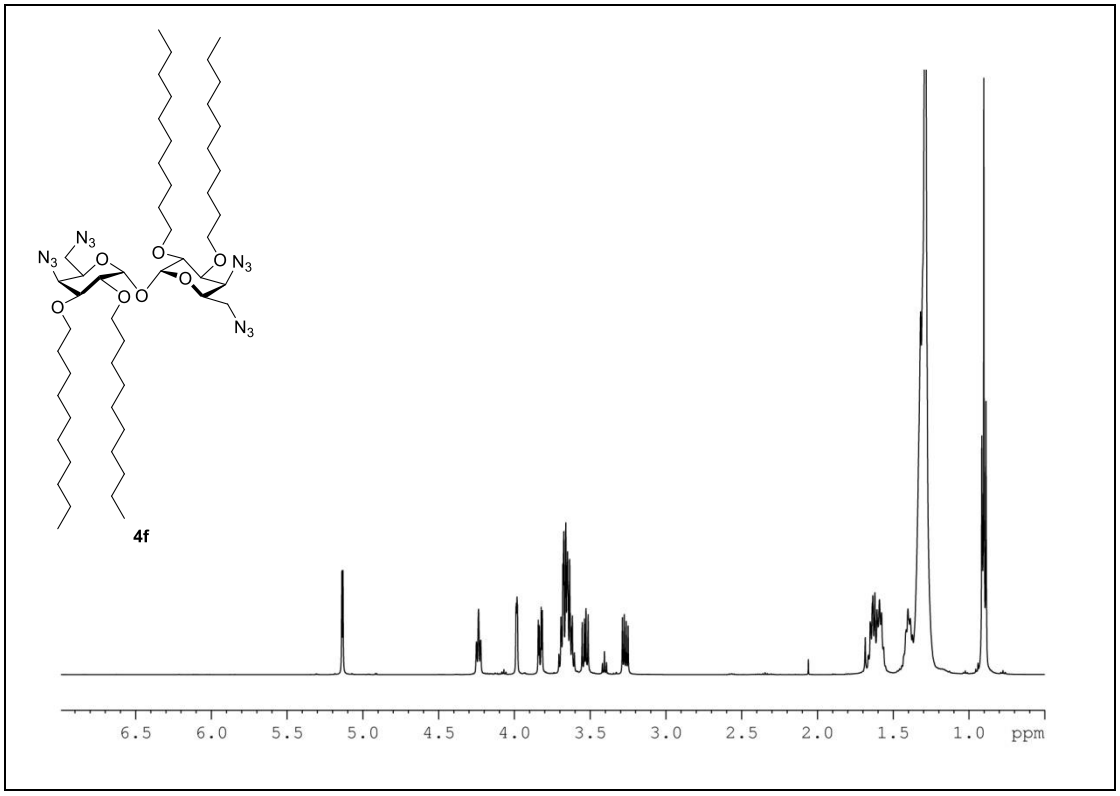


Fig. S15. 500 MHz ^{1}H-NMR in $CDCl_3$ for compound **4f**.

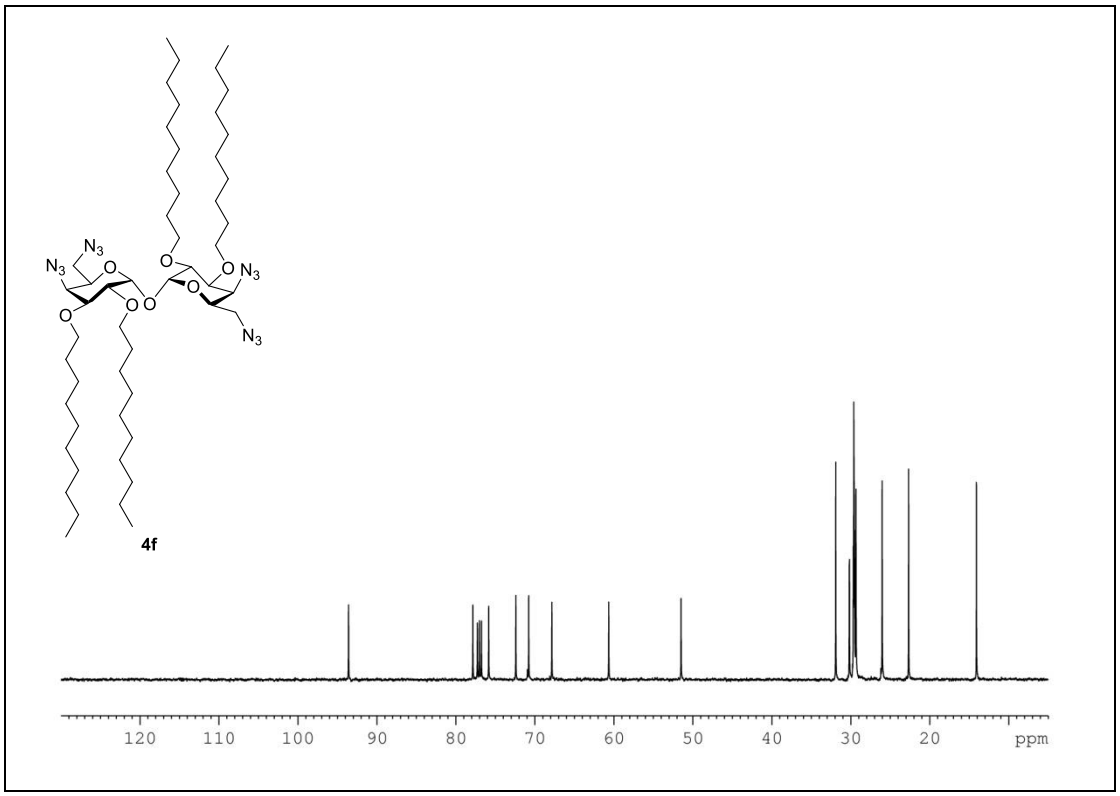


Fig. S16. 125 MHz ^{13}C-NMR in $CDCl_3$ for compound **4f**.

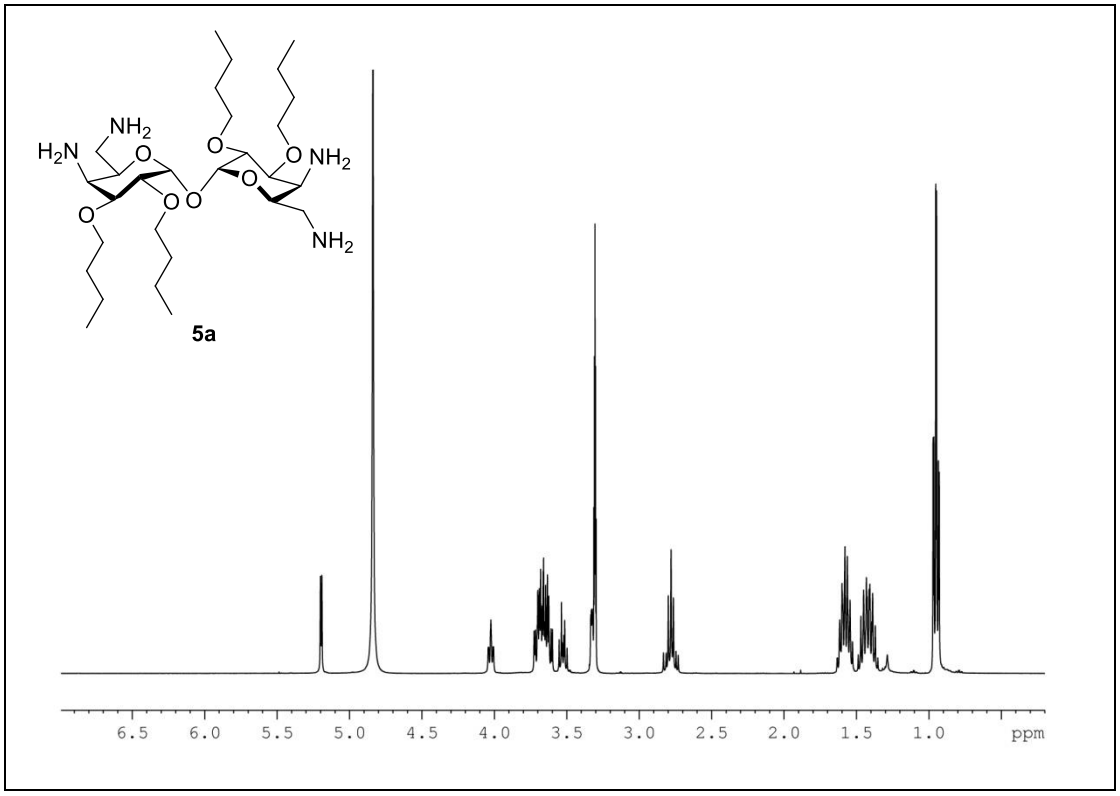


Fig. S17. 400 MHz ^{1}H-NMR in CD_3OD for compound **5a**.

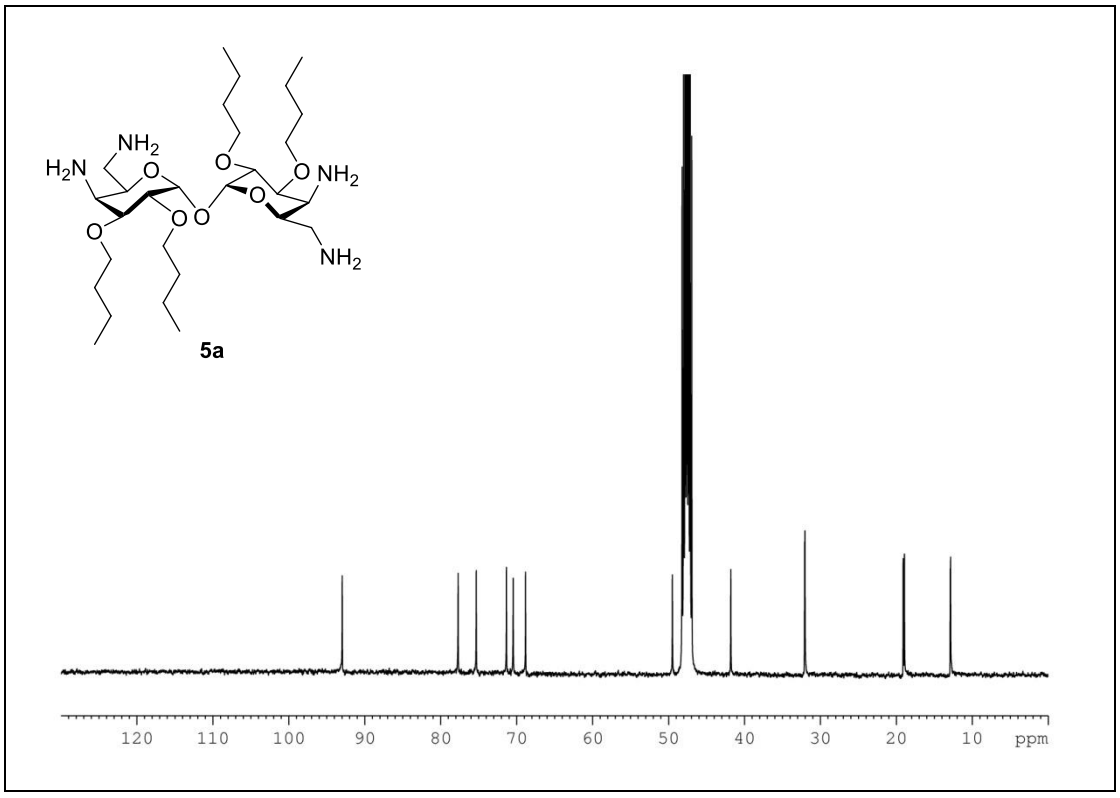


Fig. S18. 100 MHz ^{13}C-NMR in CD_3OD for compound **5a**.

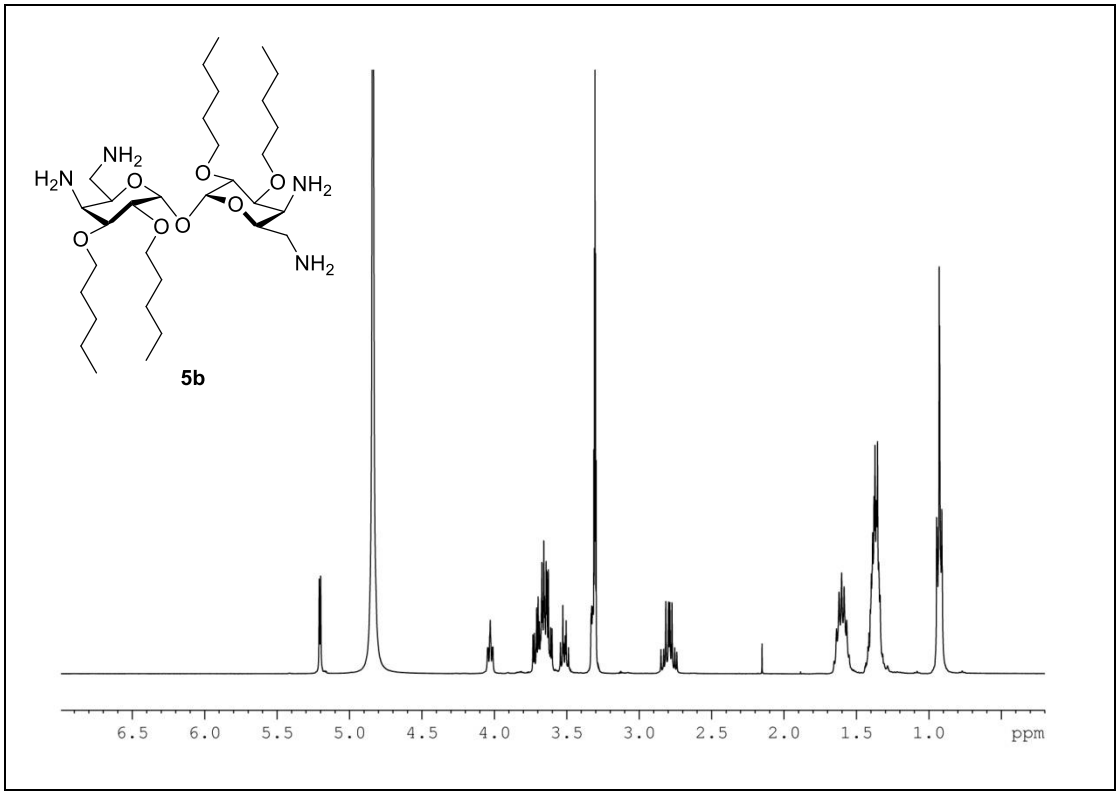


Fig. S19. 400 MHz ^{1}H-NMR in CD_3OD for compound **5b**.

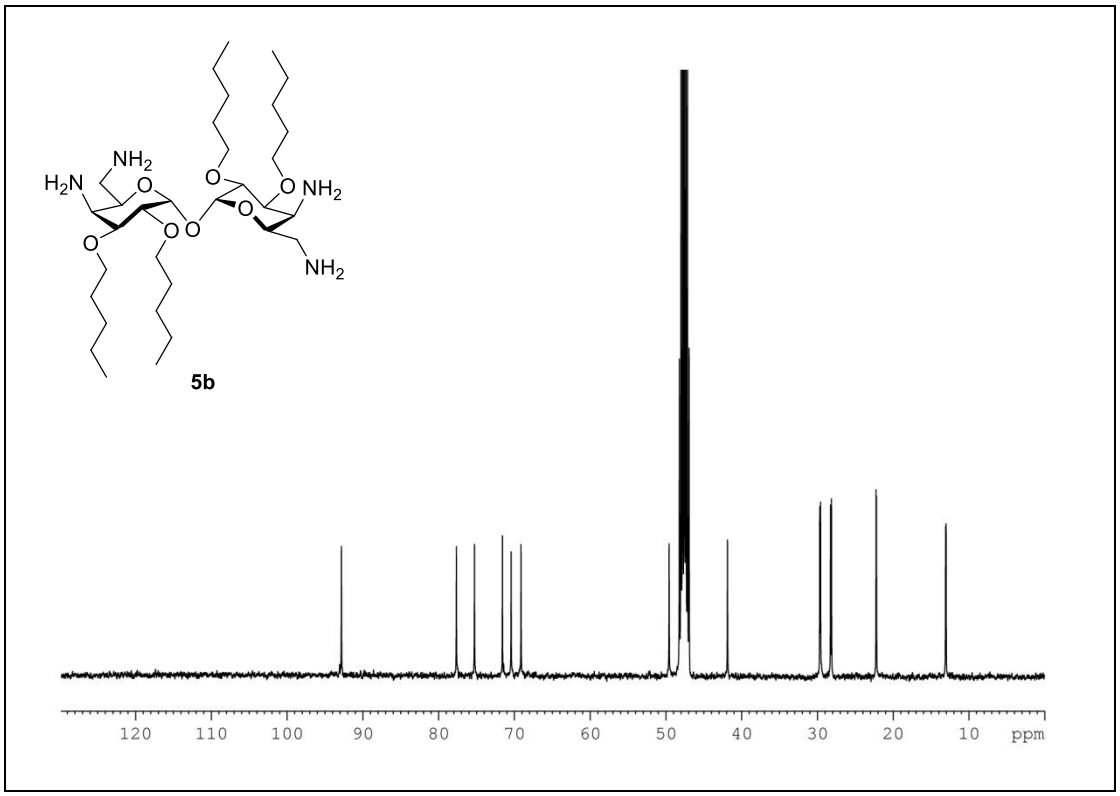


Fig. S20. 100 MHz ^{13}C-NMR in CD_3OD for compound **5b**.

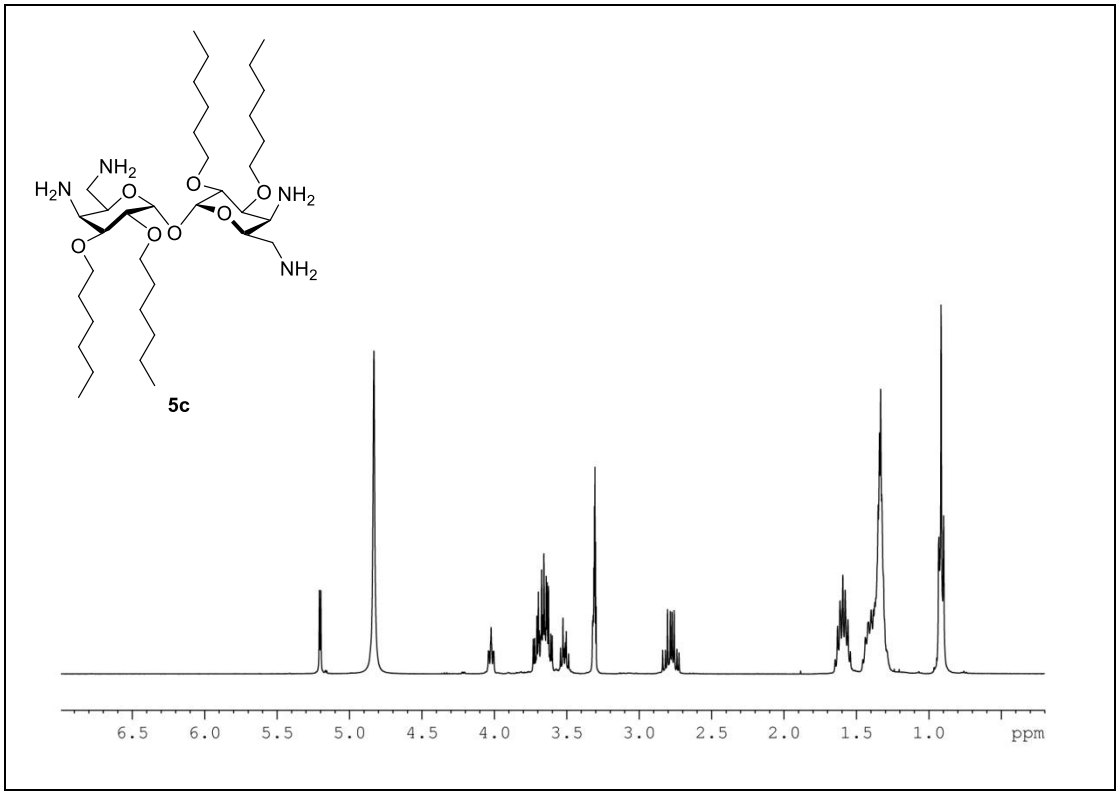


Fig. S21. 400 MHz ^{1}H-NMR in CD_3OD for compound **5c**.

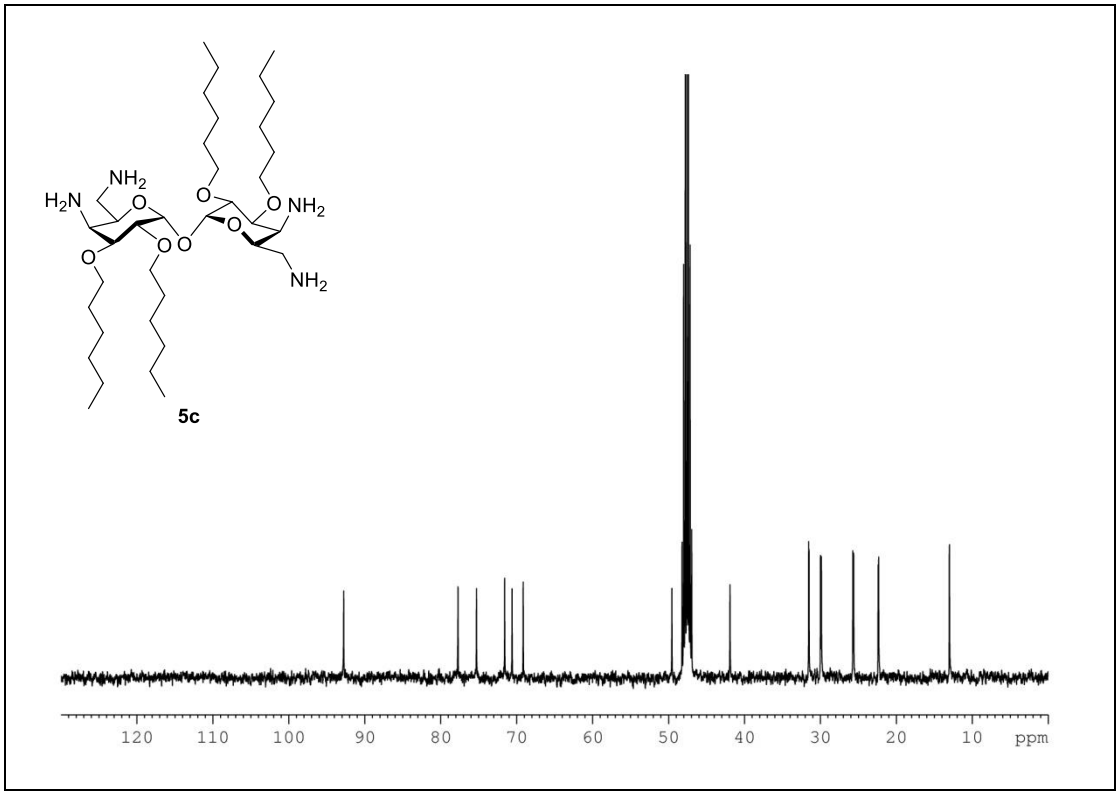


Fig. S22. 100 MHz ^{13}C-NMR in CD_3OD for compound **5c**.

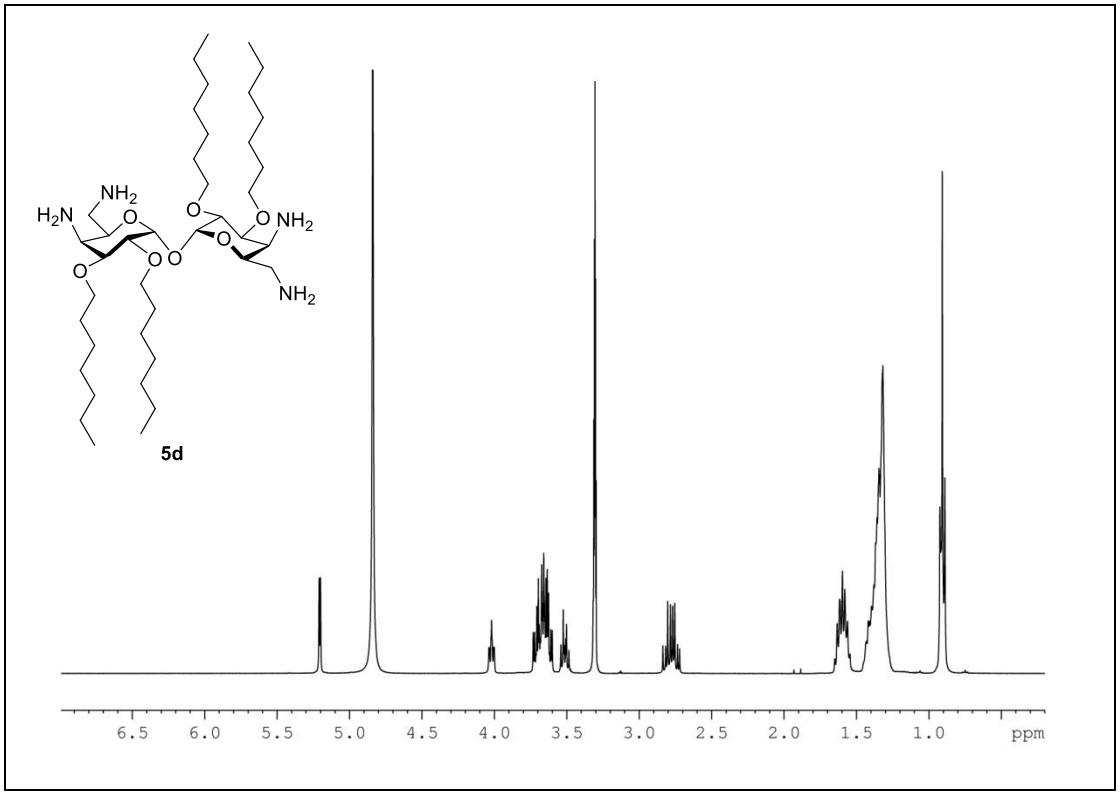


Fig. S23. 400 MHz ^{1}H-NMR in CD_3OD for compound **5d**.

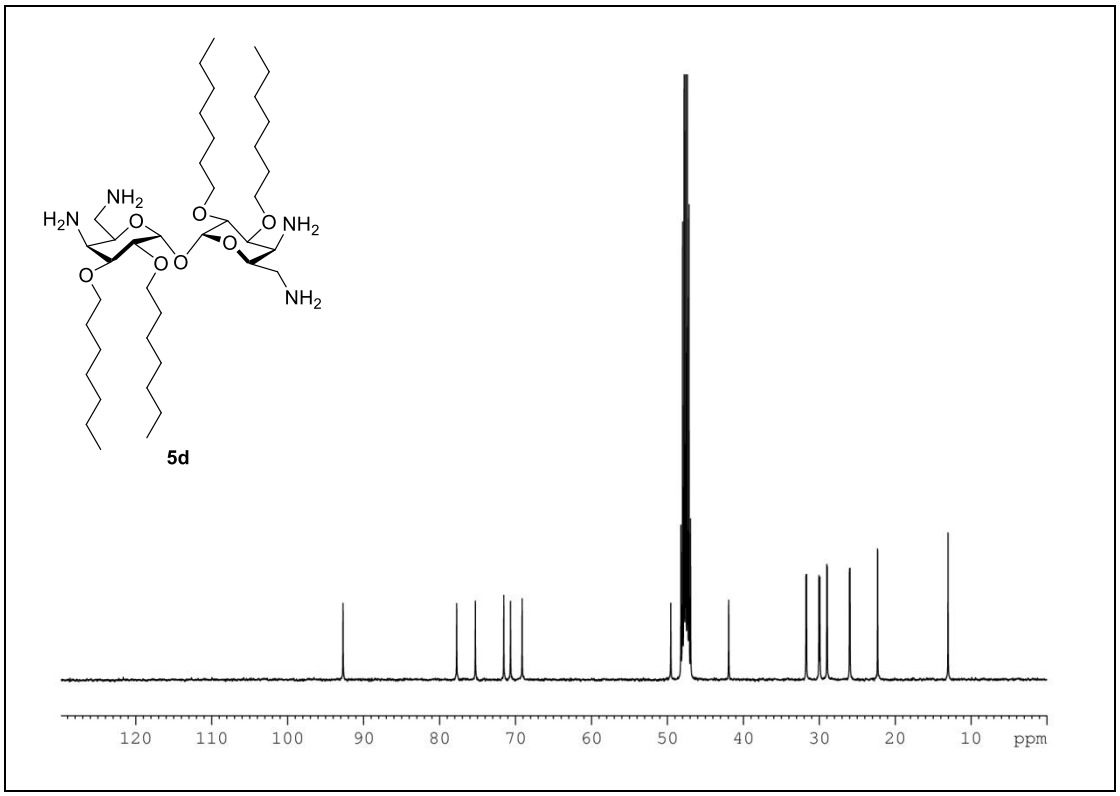


Fig. S24. 100 MHz ^{13}C-NMR in CD_3OD for compound **5d**.

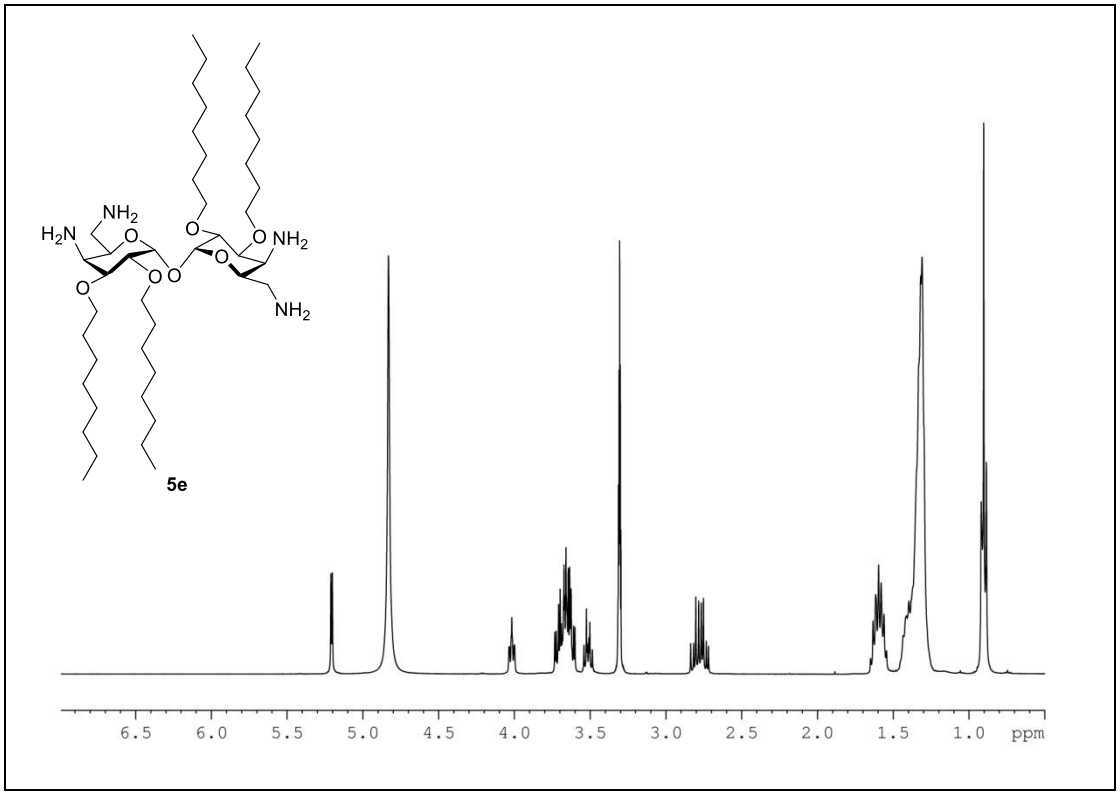


Fig. S25. 400 MHz ^{1}H NMR in CD_3OD for compound **5e**.

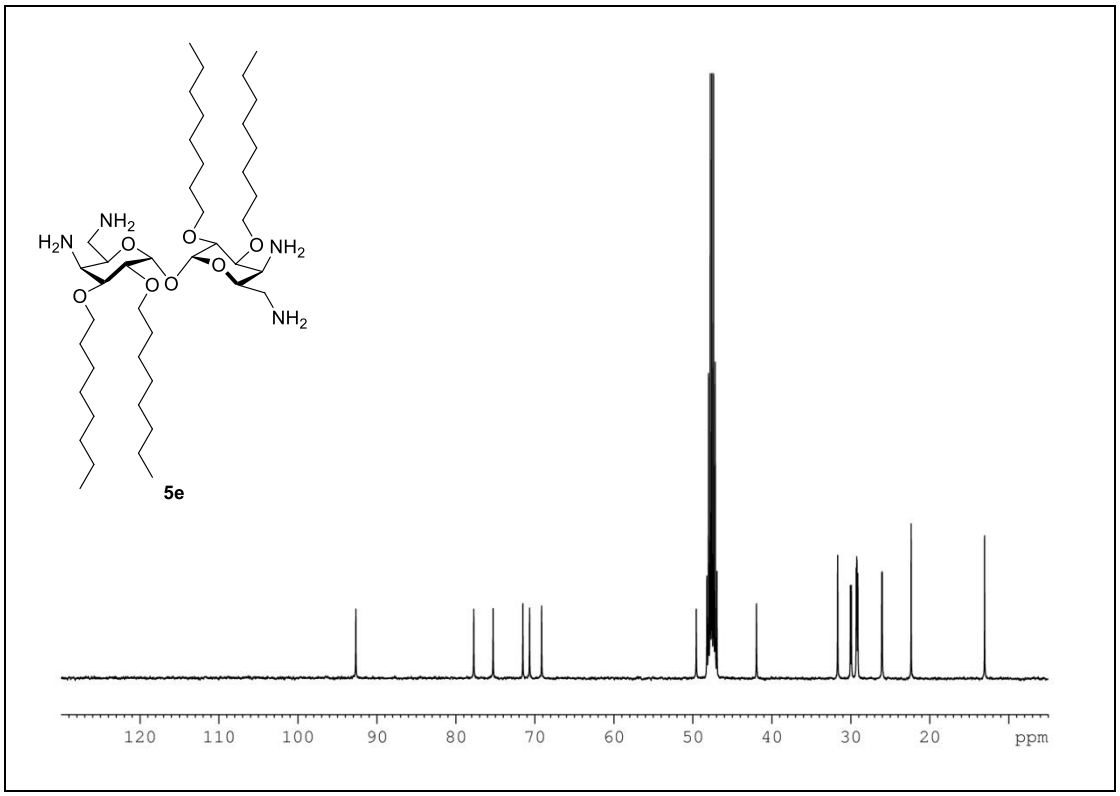


Fig. S26. 100 MHz ^{13}C NMR in CD_3OD for compound **5e**.

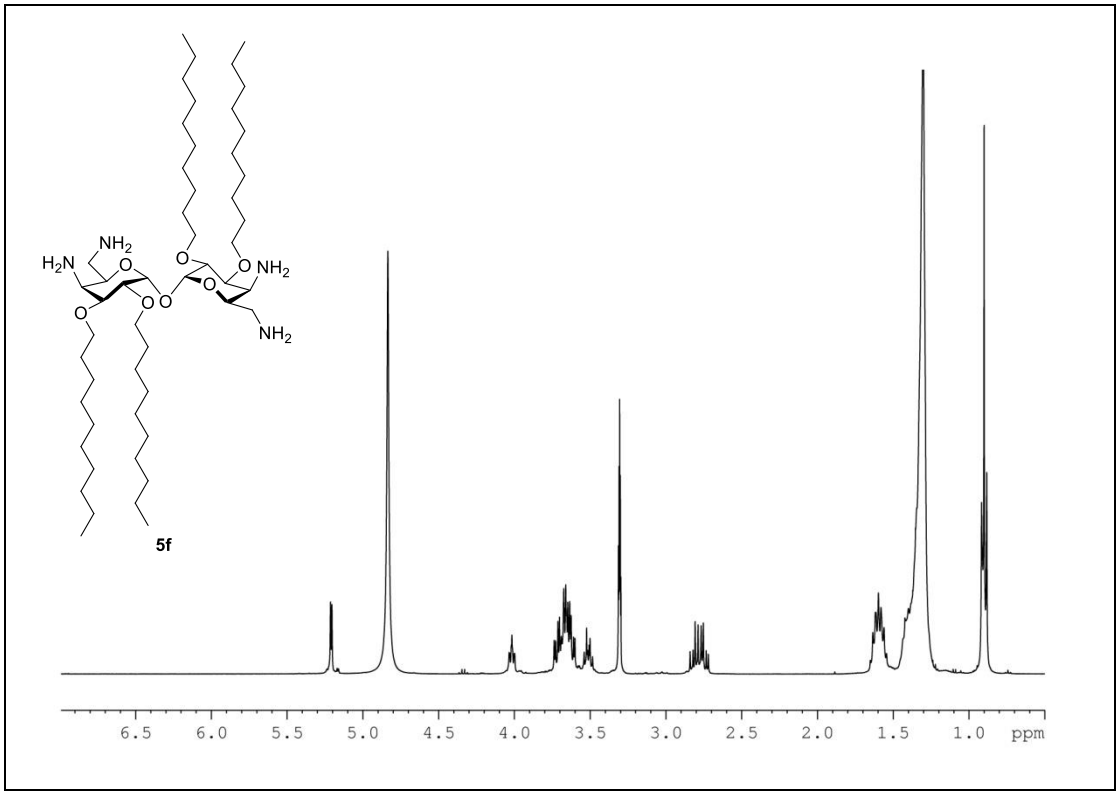


Fig. S27. 400 MHz ^{1}H-NMR in CD_3OD for compound **5f**.

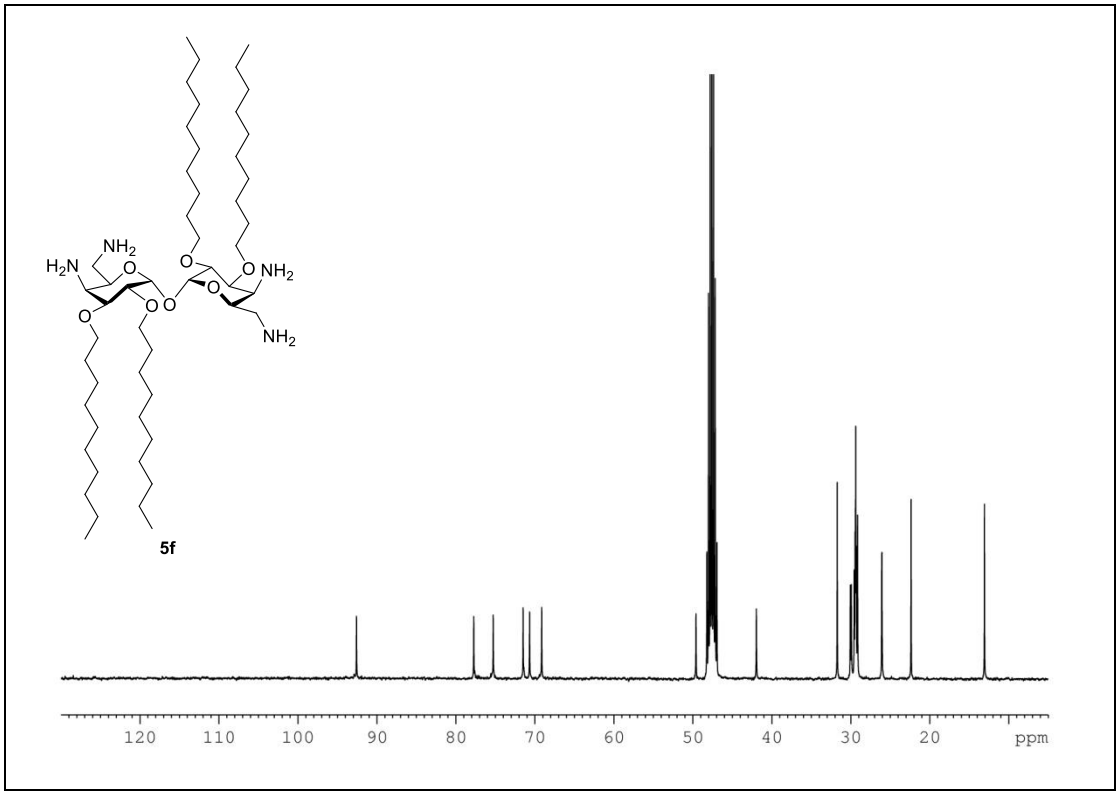


Fig. S28. 100 MHz ^{13}C-NMR in CD_3OD for compound **5f**.

6. LC-MS method for the determination of compounds purity

Purities of new compounds were determined by using ULC-ESI-MS. The chromatographic separation was achieved by using a Waters e2695 HPLC Separation Module and a 3.0 x 100 mm column. Sample aliquots of 10 µL were injected onto the column at a flow-rate of 300 µL/min. The HPLC separation conditions and the MS data are listed in the following tables.

Table 2S: LC program.

Time (min)	**Solution A:** 10 mM ammonium formate in 90% water /10% ACN/ 0.125% formic acid	**Solution B:** 10 mM ammonium formate in 90% ACN /10% water/ 0.125% formic acid
0	0	100
1	0	100
12	100	0
12.01	0	100
15	0	100

7. Retention times, purity percentages, and LRMS values

Table 3S: Retention times, purities, and LRMS data.

Compound	**Retention Time (min)**	**Purity (%)**	**LRMS ($M+H^+$)**
5a	8.92	>99.0	563.57
5b	8.63	98.1	619.67
5c	8.46	95.3	675.72
5d	8.42	98.2	731.78
5e	8.34	>99.0	787.86
5f	8.37	96.0	900.13

8. ULC chromatograms and the corresponding ESI-LRMS spectra

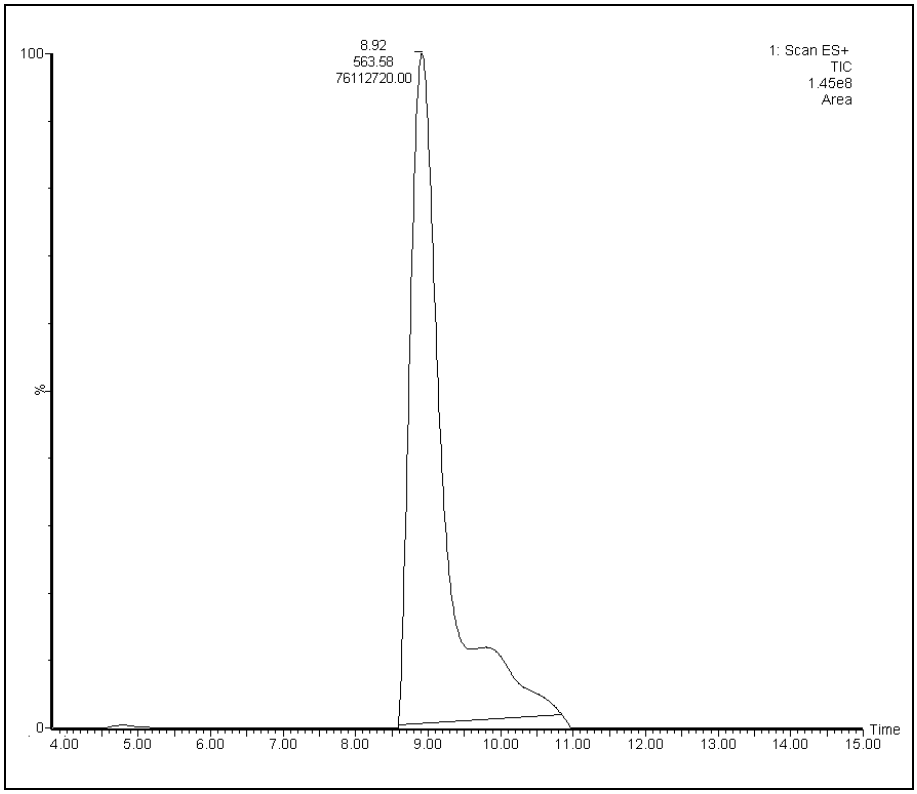


Fig. S29. ULC chromatogram of compound **5a**

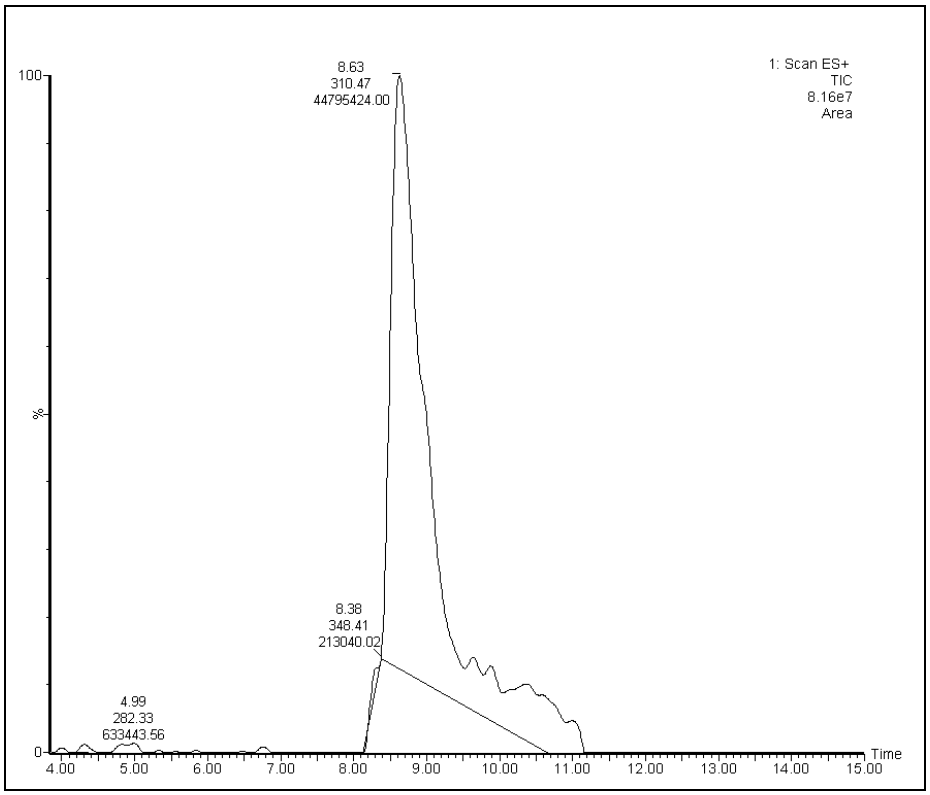


Fig. S30. ULC chromatogram of compound **5b**.

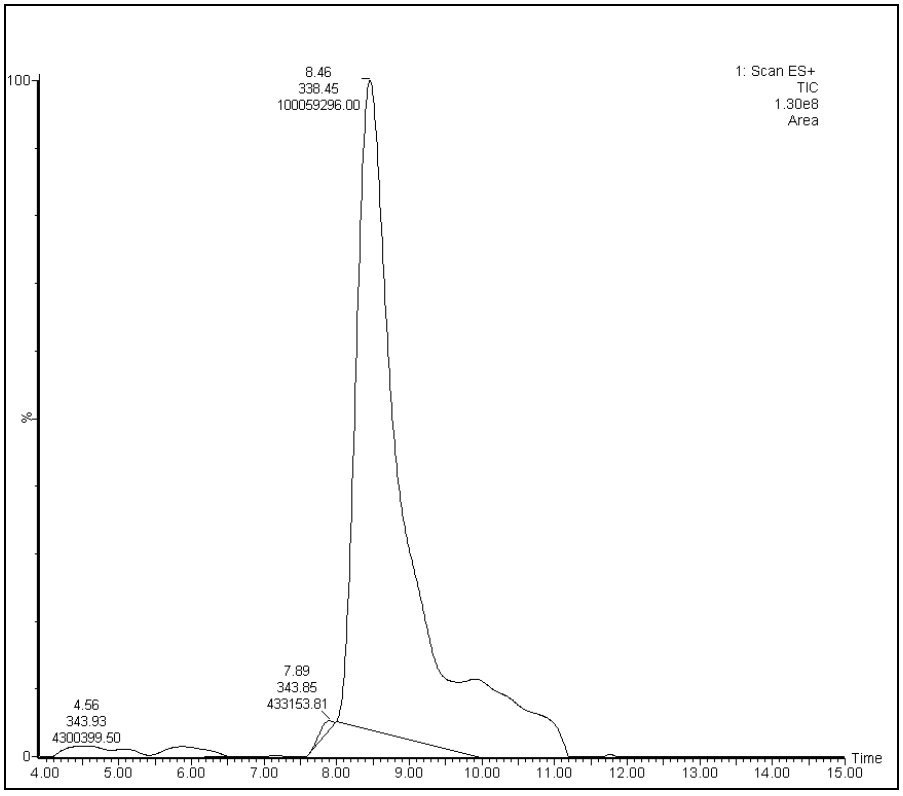


Fig. S31. ULC chromatogram of compound **5c**.

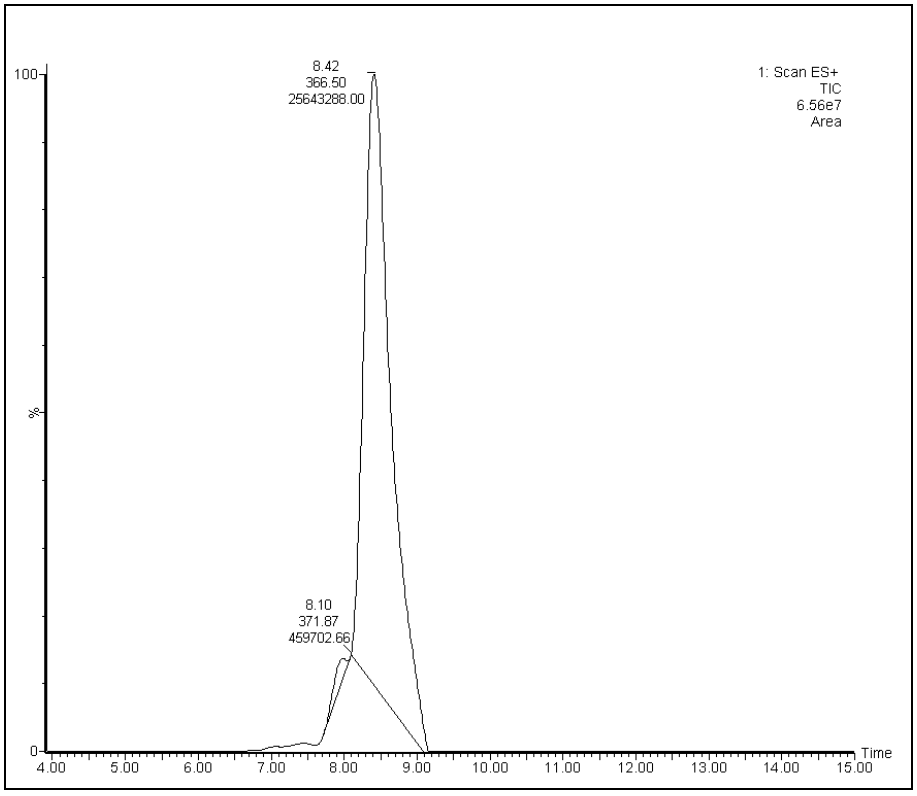


Fig. S32. ULC chromatogram of compound **5d.**

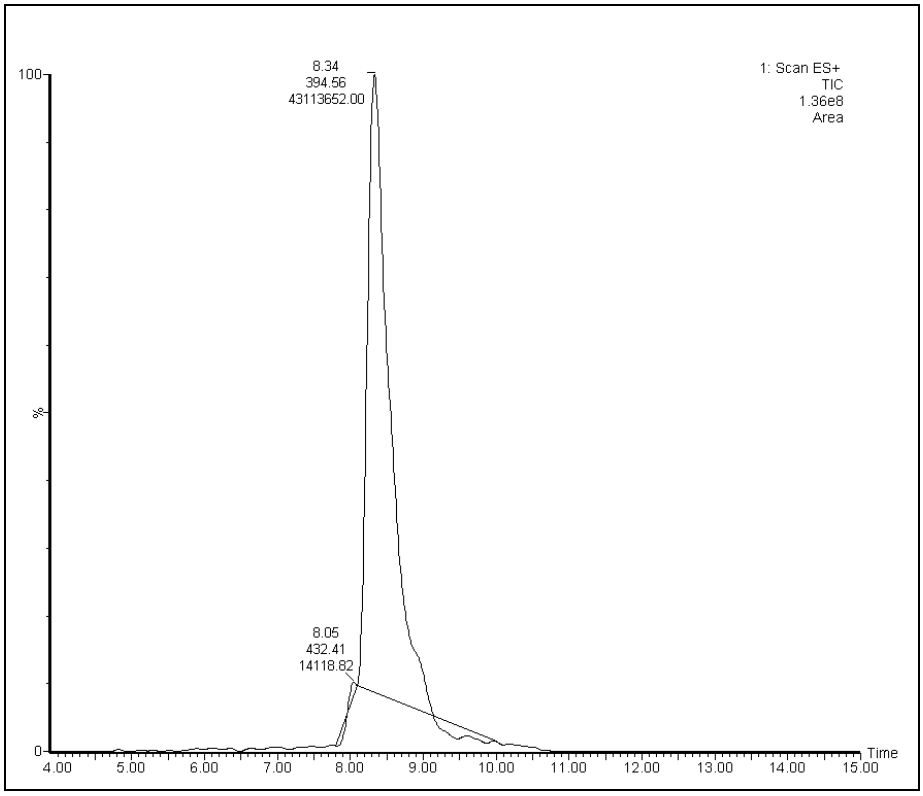


Fig. S33. ULC chromatogram of compound **5e**.

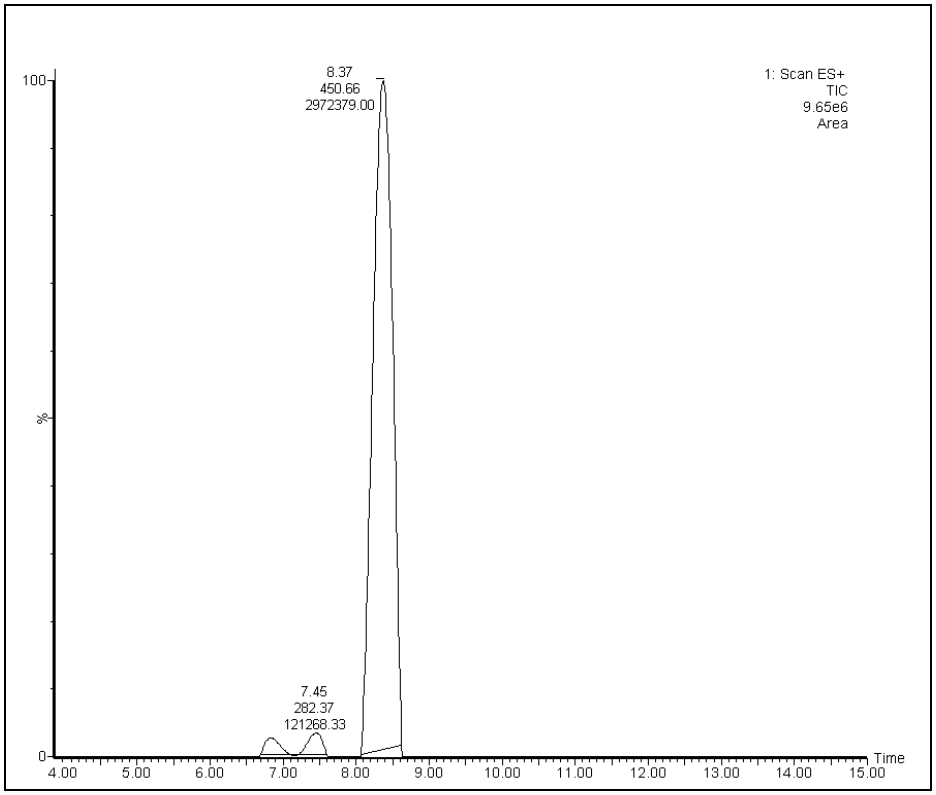


Fig. S34. ULC chromatogram of compound **5f.**