Clustering of $P^k$-trisacharide on Amphiphilic Cyclodextrin Reveals Unprecedented Affinity for the Shiga-like Toxin, Stx2

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1. Synthetic Scheme of Compounds 5 and 6
2. Synthetic Scheme of Compounds 7 and 8

![Synthetic Scheme Diagram]

3. Experimental Section

All commercial reagents were used as supplied unless otherwise stated. Thin-layer chromatography was performed on silica gel 60-F254 with detection by fluorescence, charring with 5% aqueous H₂SO₄. Column chromatography was performed on silica gel 60, and solvent gradients given refer to stepped gradients and concentrations are reported as % v/v. Organic solutions were concentrated and / or evaporated to dryness under vacuum at < 80 °C (bath). Optical rotations were determined in a 5 cm cell at 25 ± 2 °C; [α]D²⁵ values are given in units of 10⁻¹ deg cm² g⁻¹. NMR spectra were recorded at either 400 or 600 MHz (as indicated), and the first-order proton chemical shifts δ_H and δ_C are reported in ppm and referenced to residual solvent (For CDCl₃: CHCl₃ δ_H 7.26 and δ_C 77.00; For CD₃OD: CHD₂OD δ_H 3.31 and δ_C 49.15;) or residual HDO (δ_H 4.80 and external acetone: δ_C 2.05, D₂O).¹H and ¹³C NMR spectra were assigned with the assistance of 2D GCOSY and 2D GHSQC. High-resolution mass spectra were recorded.
on an accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer coupled to and liquid chromatography (LC/MS) system using electrospray ionization (ESI).

3.1 **13,6,9,12-tetraoxapentadec-14-yn-1-ol (14)**

Compound 14 was prepared by following a literature procedure\(^1\). To a solution of tetraethylene glycol (5 g, 0.026 mol) in anhydrous THF (30 mL) cooled to 0° C, was added NaH (60% mineral oil, 1.3 g, 0.03 mol) by small portions, and the mixture was stirred for 10 min. Propargyl bromide (80 wt.% in toluene, 2.8 mL, 0.026 mol, 1.0 eq) was added slowly to the mixture and the reaction was stirred at room temperature for 16 h. Water (0.5 mL) was added to quench the reaction. The reaction mixture was evaporated under reduced pressure, diluted with CH\(_2\)Cl\(_2\) (200 mL), and washed with H\(_2\)O (20 mL). The organic solution was dried over anhydrous Na\(_2\)SO\(_4\) and evaporated. The residue was purified by column chromatography on silica gel using 60% EtOAc – hexane as eluent to afford compound 14 (2.7 g, 45% yield) as pale yellow oil. R\(_f\) 0.23 (acetone : hexane, 25 : 75). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 4.02 (d, 2H, \(J = 2.0\) Hz, OCH\(_2\)C≡CH), 3.56 - 3.45 (m, 14H, 7 \times OCH\(_2\)), 3.43 - 3.38 (m, 2H, CH\(_2\)OH), 3.11 (t, 1H, OH), 2.36 (t, 1H, \(J = 2.4\) Hz, CH\(_2\)C≡CH). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\)C 79.20 (CH\(_2\)C≡CH), 74.35 (CH\(_2\)C≡CH), 72.10 (HOCH\(_2\)CH\(_2\)), 70.07, 70.02, 69.98, 69.83, 69.80, 68.55 (OCH\(_2\)), 61.01 (HOCH\(_2\)), 57.82 (OCH\(_2\)C≡CH).
3.2 3,6,9,12-Tetraoxapentadec-14-yn-1-yl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (15)

A mixture of perbenzoylated lactosyl bromide (13², 4.50 g, 3.97 mmol), alcohol (14, 1.38 g, 5.96 mmol) and crashed molecular sieves 4 Å (5.0 g) in anhydrous toluene (60 mL) was stirred at −78°C for 1 h. Silver triflate (1.53 g, 5.96 mmol) was added, and the temperature was allowed to warm up to ~10°C during one hour. The insoluble material was filtered off and washed with more toluene (3 × 20 mL). The combined filtrate was extracted with a solution of 10% aqueous NH₃·H₂O (2 × 50 mL), and concentrated to afford a mixture, which purified by chromatography on silica gel using EtOAc – hexane (40 → 50%) as eluent to afford the desired compound 15 (4.31 g, 84% yield). Rₛ 0.16 (EtOAc : Hexane, 50 : 50). [α]²⁵°D +31.9° (c 0.37, CHCl₃). ¹H NMR (400 MHz, CDCl₃):

δH 8.07 – 7.95 (m, 10H, Bz), 7.91 (dd, J = 8.3, 1.2 Hz, 2H, Bz), 7.74 (dd, J = 8.4, 1.2 Hz, 2H, Bz), 7.67 – 7.46 (m, 7H, Bz), 7.45 – 7.29 (m, 10H, Bz), 7.22 dd, J = 7.9, 7.9 Hz, 2H, Bz), 7.15 (dd, J = 7.8, 7.8 Hz, 2H, Bz), 5.81 (dd, J = 9.2, 9.7 Hz, 1H, H-3_βGlu), 5.78 – 5.68 (m, 2H, H-2_βGal + H-4_βGal), 5.47 (dd, J = 9.9, 7.9 Hz, 1H, H-2_βGlu), 5.38 (dd, J = 10.3, 3.4 Hz, 1H, H-3_βGal), 4.89 (d, J = 7.9 Hz, 1H, H-1_βGal), 4.83 (d, J = 7.9 Hz, 1H, H-1_βGal), 4.1 (dd, J = 1.8, 12.2 Hz, 1H, H-6a_βGlu), 4.50 (dd, J = 12.2, 4.3 Hz, 1H, H-6b_βGlu), 4.26 (dd, J = 9.5, 9.5 Hz, 1H, H-4_βGlu), 4.19 (d, J = 2.4 Hz, 2H, OCH₂C=CH), 3.88 (m, 3H, H-5_βGlu + H-5_βGal + OCH₂Hb), 3.80 – 3.33 (m, 17H, H-6a_βGal + H-6b_βGal + OCH₂Hb + OCH₂HbCHcHd(OCH₂CH₂)₃), 2.43 (t, J = 2.4 Hz, 1H, OCH₂C=CH). ¹³C NMR (101 MHz, CDCl₃): δC 165.86, 165.57, 165.40 (× 2), 165.22, 165.18, 164.78 (CO), 133.64, 133.53, 133.40, 133.37, 133.25, 133.15, 130.16, 129.99, 129.79, 129.75, 129.74, 129.67, 129.65, 129.59, 129.52, 129.41, 128.95, 128.85,
128.68, 128.63, 128.56, 128.51, 128.46, 128.35, 128.23 (Bz), 101.23 (C-1_βGlu), 100.99 (C-1_βGal), 79.14 (OCH₂C≡CH), 76.05 (C-4_βGlu), 74.52 (OCH₂C≡CH), 72.95 (C-5_βGlu), 72.90 (C-3_βGlu), 71.79 (C-3_βGal), 71.77 (C-2_βGlu), 71.37 (C-5_βGal), 70.59, 70.49, 70.41 (× 2), 70.34, 70.25 ((OCH₂CH₂)₃), 69.88 (C-2_βGal), 69.09 (OCH₂CH₂CH₃), 67.52 (C-4_βGal), 62.42 (C-6_βGlu), 61.05 (C-6_βGal), 58.37 (OCH₃CH₂CH₂CH₂), 58.37 (OCH₃CH₂CH₂CH₂), 67.52 (C-4_βGal), 62.42 (C-6_βGlu), 61.05 (C-6_βGal), 58.37 (OCH₂C≡CH). ESI HRMS m/z calc’d for [C₇₂H₆₈O₂₂ + Na]⁺: 1307.4094; found: 1307.4083.

3.3 3,6,9,12-Tetraoxapentadec-14-yn-1-yl O-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (12)

Compound 15 (4.00 g, 3.11 mmol) was dissolved in anhydrous MeOH (70 mL) containing a catalytic amount of NaOMe, and the mixture was stirred at room temperature overnight, and refluxed for 1 h. After cooling, the mixture was neutralized with Amberlite IR-120 (H⁺) and the solution was concentrated under reduced pressure. The residue was stirred with Et₂O and the precipitate was filtered off to afford the desired heptol 12 (1.59 g, 92% yield). Rf 0.16 (MeOH : CH₂Cl₂, 20 : 80). [α]_25^D -9.1° (c 0.46, MeOH). ¹H NMR (400 MHz, D₂O): δH 4.43 (d, J = 8.0 Hz, 1H, H-1_βGlu), 4.36 (d, J = 7.8 Hz, 1H, H-1_βGal), 4.16 (d, J = 2.4 Hz, 2H, OCH₂C≡CH), 4.01 – 3.94 (ddd, J = 4.1, 4.1, 11.4 Hz, 1H, OCH₃Hb), 3.89 (dd, J = 12.3, 2.0 Hz, 1H, H-6a_βGlu), 3.84 (dd, J = 3.3, <1 Hz, 1H, H-4_βGal), 3.80 – 3.48 (m, 23H, H-3_βGlu + H-4_βGlu + H-5_βGlu + H-6b_βGlu + H-3_βGal + H-5_βGal + H-6a_βGal + H-6b_βGal + OCH₃HbCH₃Hd + (OCH₂CH₂)₃), 3.45 (dd, J = 9.9, 7.8 Hz, 1H, H-2_βGal), 3.26 (high order dd, J = 9.5, 7.9 Hz, 1H, H-1_βGlu), 2.82 (t, J = 2.4 Hz, 1H, OCH₂C≡CH). ¹³C NMR (101 MHz, D₂O): δC
102.91 (C-1_βGal), 102.07 (C-1_βGlu), 78.36 (C-4_βGlu), 78.26 (OCH₂C≡CH), 75.99 (OCH₂C≡CH), 75.31, 74.73, 74.25 (C-3_βGlu + C-5_βGlu + C-3_βGal), 72.77 (C-2_βGlu), 72.49 (C-5_βGal), 69.65, 69.55, 69.51, 69.38, 68.70, 68.62 (OCHₐHₐbCHₐcHₐd + (OCH₂CH₂)₃), 68.51 (C-4_βGal), 60.98 (C-6_βGlu), 60.05 (C-6_βGlu), 57.86 (OCH₂C≡CH). ESI HRMS m/z calc’d for [C₂₃H₄₀O₁₅ + Na]⁺: 579.2259; found: 579.2282.

3.4 3,6,9,12-Tetraoxapentadec-14-yn-1-yl O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (11)

Compound 12 (221 mg, 0.397 mmol) was dissolved in water (4 mL) and pH of the solution was adjusted to ~ 8 by adding NaHCO₃, then a solution of HEPES buffer (pH 8.0, 1 M, 2.4 mL) was added followed by aq. DTT solution (0.4 M, 0.38 mL), alkaline phosphatase (38 μL) and UDP-glucose (16, 363 mg, 0.595 mmol). The mixture was gently shaken to dissolve it and crude fusion enzyme GalT/UDP-4’-epimerase (716 μL) was added. The reaction mixture was incubated at 37 °C and gently shaken for 18 h. TLC (CH₂Cl₂ : MeOH : H₂O : AcOH, 12 : 6 : 1 : 0.1) and ¹H NMR of a small sample of the reaction confirmed that the reaction was complete. The mixture was treated with Dowex resin (H⁺) till pH ~ 4, filtered and freeze-dried. The crude product (17), a white powder was suspended in pyridine (7 mL) and sonicated to help to dissolve it, then acetic anhydride (7 mL) was added and the mixture was sonicated for a while, then left stirring for 3 days. The reaction mixture was concentrated, co-evaporated with toluene, and the obtained off-white solid was suspended in water and extracted with CH₂Cl₂ (3 times).
The combine organic layers were concentrated and the residue was purified by chromatography on silica gel using 40% acetone-hexane as the eluent to provide the desired Pk-trisaccharide decaacetate 11 (289 mg, 66% yield) as white foam. Rf 0.48 (MeOH : CH2Cl2, 5 : 95). Rf 0.38 (hexane : acetone, 1 : 1). [α]25°D +31.2° (c 0.26, CHCl3).

1H NMR (500 MHz, CDCl3): δH 5.60 (dd, J = <1, 2.7 Hz, 1H, H-4_αGal), 5.41 (dd, J=11.1, 3.4 Hz, 1H, H-3_αGal), 5.18 - 5.24 (m, 2H, H-3_βGlu + H-2_αGal), 5.12 (dd, J=10.9, 7.8 Hz, 1H, H-2_βGal), 5.00 (d, J=3.7 Hz, 1H, H-1_αGal), 4.91 (dd, J=9.3, 8.1 Hz, 1H, H-1_βGlu), 4.75 (dd, J=10.9, 2.7 Hz, 1H, H-3_βGal), 4.58 (d, J=7.9 Hz, 1H, H-1_βGlu), 4.42 - 4.55 (m, 4H, H-6a_βGlu + H-1_βGal + H-6a_βGal + H-5_αGal), 4.22 (d, J=2.4 Hz, 2H, OCH2Propargyl), 4.09 - 4.21 (m, 4H, H-6b_βGlu + H-6b_αGal + H-6a_αGal + H-6b_αGal), 4.03 (dd, J=1.2 Hz, 1H, H-4_βGal), 3.93 (ddd, J=11.1, 4.4, 4.4 Hz, 1H, OCH3_H6(CH2O), 3.80 - 3.83 (m, 1H, H-4_βGlu), 3.60 - 3.80 (m, 17H, H-5_βGlu + H-5_βGal + OCH3_H6 + 7 × CH2_PEG), 2.45 (t, 1H, CH_propargyl), 2.15 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc). 13C NMR (125 MHz, CDCl3): δC 170.70 (C=O), 170.51 (C=O), 170.48 (C=O), 170.47 (C=O), 170.45 (C=O), 170.10 (C=O) 169.77 (C=O), 169.66 (C=O), 169.53 (C=O), 168.88 (C=O), 101.15, 100.65, 99.66 (C-1_βGlu, C-1_βGal, C-1_αGal), 74.54 (OCH2C=CH), 76.98, 76.51, 73.15, 72.85, 72.58, 71. 85, 71.78, 69.01, 68.89, 67.93, 67.17, 67.11 (OCH2C=CH + C-2_βGlu + C-2_βGal + C-2_αGal + C-3_βGlu + C-3_αGal + C-4_βGlu + C-4_βGal + C-4_αGlu + C-5_βGlu + C-5_βGal + C-5_αGal), 70.73, 70.64, 70.64, 70.43, 70.26, 69.15 (CH2_PEG), 62.2, 61.3, 60.3 (C-6_βGlu + C-6_βGal + C-6_αGal), 58.42 (OCH2C=CH), 20.96 (OAc), 20.89 (OAc),
20.76 (OAc), 20.75 (OAc), 20.74 (OAc), 20.73 (OAc), 20.70 (OAc), 20.66 (OAc), 20.62 (OAc), 20.54 (OAc). ESI HRMS m/z calc’d for [C_{49}H_{70}NaO_{30} + Na]^+: 1161.3844; found: 1161.3829.

3.5 Compound (18)

To a solution of compound 11 (60 mg, 52.7 µmol) and per-6-azide 9 (15 mg, 6 µmol) in acetone (5 mL), was added N,N-diisopropylethylamine (20 µL, 114.8 µmol) and CuI (10 mg, 52.5 µmol), and the reaction mixture was heated to reflux overnight. The mixture was diluted with ethyl acetate (40 mL), and the solution was washed with a saturated solution of EDTA (1 × 40 ml), water (1 × 40 mL), brine (1 × 40 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel using 4% MeOH – CH₂Cl₂ as the eluent to afford compound 18 as a white foam (42.0 mg, yield: 67%). Rf 0.37 (MeOH : CH₂Cl₂, 5 : 95). [α]_{D}^{25} +49.4° (c 0.26, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δH 7.66 (s, 7H, 7 × triazole), 5.56 (dd, J = <1 Hz, 3.0 Hz, 7H, 7 × H-4_αGal), 5.47 (br, 7H, 7 × H-1_αGlu_CD), 5.36 (dd, J = 11.0, 3.3 Hz, 7H, 7 × H-3_αGal), 5.24 – 5.12 (m, 14H, 7 × H-3_βGlu + 7 × H-2_αGal), 5.08 (dd, J = 10.8, 7.8 Hz, 7H, 7 × H-2_βGal), 4.96 (d, J = 3.6 Hz, 7H, 7 × H-1_αGal), 4.87 (dd, J = 9.3, 8.1 Hz, 7H, 7 × H-2_βGlu), 4.71 (dd, J = 10.8, 2.5 Hz, 7H, 7 × H-3_βGal), 4.69 – 3.29 (m, 273H, 7 × H-1_βGal + 7 × H-1_βGlu + 7 × H-4_βGlu + 7 × H-4_βGal + 7 × H-5_βGlu + 7 × H-5_βGal + 7 × H-5_αGal + 7 × H-6a_βGlu + 7 × H-6b_βGlu + 7 × H-6a_βGal + 7 × H-6b_βGal + 7 × H-6a_αGal + 7 × H-6b_αGal + 7 × H-6a_αGlu_CD + 7 × H-6b_αGlu_CD + 7 × H-5_αGlu_CD + 7 × H-
3_αGlu_CD + 7 × OCHAhbO + 7 × OCHAhbO + 49 × OCH₂ + 7 × OCH₂-triazole + 14 × OCHAhb_hexyl + 14 × OCHAhb_hexyl), 3.21 (br, 7H, 7 × H-4_αGlu_CD), 3.05 (br, 7H, 7 × H-2_αGlu_CD), 2.11 (s, 21H, 7 × Ac), 2.09 (s, 21H, 7 × Ac), 2.08 – 1.99 (m, 147H, 49 × Ac), 1.96 (s, 21H, 7 × Ac), 1.69-1.44 (br, 28H, 14 × CH₂_hexyl), 1.40-1.14 (br, 84H, 42 × CH₂_hexyl), 0.95-0.77 (br, 42H, CH₃_hexyl). Selected ¹³C NMR (101 MHz, CDCl₃): δC 170.61, 170.41, 170.39, 170.36, 170.02, 169.65, 169.59, 169.45, 168.81 (C=O), 101.06, 100.61 (βGlu, C-1_βGal), 99.94 (C-1_αGlu_CD), 99.58 (C-1_αGal), 79.82 (C-4_αGal), 76.89 (C-4_βGlu), 76.42, 73.06, 72.79, 72.53, 71.79, 71.67, 70.56, 70.45, 70.43, 70.40, 70.29, 70.04, 69.84, 69.09, 68.95, 68.80, 67.88, 67.10, 67.03, 62.18, 61.31, 60.25, 31.99, 31.74, 30.42, 30.14, 29.63, 25.81, 25.63, 22.68, 22.61 (CH₂_hexyl), 20.88, 20.82, 20.67, 20.63, 20.59, 20.54, 20.46 (OAc), 14.00, 13.96 (CH₃_hexyl). ESI HRMS m/z calc’d for [¹²C₄₆₄¹³C₅₇₂₁N₂₁O₂₃₈ + 4Na]⁺⁺: 2638.1174; found: 2638.1067.

### 3.6 Compound (5)

Compound 18 (40 mg, 3.8 μmol) was dissolved in anhydrous MeOH (2.5 mL), and a solution of NaOMe in MeOH (1.5 M, 80 uL) was added. The mixture was stirred at room temperature for 2 days. Dry ice was added to neutralize the reaction to pH ~ 9, and the mixture was evaporated under reduced pressure. The crude residue was purified by size exclusion column on Sephadex LH-20 using MeOH as the eluent to afford the desired compound 5 which was freeze-dried as a colorless fluffy solid (26.1 mg, yield: 91%). [α]²⁵_D +40° (c 0.11, H₂O). Selected ¹H NMR (600 MHz, D₂O) δH 7.92 (br s, 7H, 7 × triazole), 5.37 (br, 7H, 7 × H-1_αGlu_CD), 4.85 (d, J = 3.5 Hz, 7H, 7 × αGal), 4.42 (d, J = 7.8 Hz, 7H, 7 × H-1_βGal), 4.42 (d, J = 7.8 Hz, 7H, 7 × H-1_βGlu), 4.27 (m, 7H, H-
5_αGal), 3.26 (br, 7H, 7 × H-2_βGlu), 3.09 – 2.94 (m, 7H, 7 × H-2_αGlu_CD), 1.73-0.96 (m, 112H, CH₂-n-hexyl), 0.75 (s, 42H, CH₃-n-hexyl). Selected ¹³C NMR (150 MHz, D₂O, from HSQC): δC 103.24, 102.15, 100.25, 78.57, 77.27, 75.31, 74.70, 74.26, 72.81, 72.04, 70.80, 70.72, 69.59, 69.53, 69.50, 68.85, 68.77, 68.56, 68.42, 60.40, 60.16, 60.12, 59.95, 29.23, 22.33, 22.27, 13.51. ESI HRMS m/z calc’d for [¹²C₃₂⁵¹³C₄H₈₈₁N₂₁O₁₆₈ + 4Na]⁺⁺: 1902.6817; found: 1902.6701.

### 3.7 Compound (19)

To a solution of compound 11 (60 mg, 52.67 µmol) and per-6-azide 10 (20.3 mg, 5.54 µmol) in acetone (5 mL), was added N,N-diisopropylethylamine (20 µL, 114.8 µmol) and CuI (10 mg, 52.5 µmol), and the reaction mixture was heated to reflux overnight. The mixture was diluted with ethyl acetate (40 mL), and the solution was washed with a saturated solution of EDTA (1 × 40 ml), water (1 × 40 mL), brine (1 × 40 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel using 4% MeOH – CH₂Cl₂ as the eluent to provide 19 as a white foam (50.1 mg, yield: 77.2 %). Rf 0.42 (MeOH : CH₂Cl₂, 5 : 95). [α]₂⁵D +35.4° (c 0.28, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δH 7.65 (br, 7H, 7 × triazole), 5.57 (dd, J = <1 Hz, 3.0 Hz, 7H, 7 × H-4_αGal), 5.46 (br, 7H, 7 × H-1_αGlu_CD), 5.37 (dd, J = 11.1, 3.3 Hz, 7H, 7 × H-3_αGal), 5.22 – 5.14 (m, 14H, 7 × H-3_βGlu + 7 × H-2_αGal), 5.08 (dd, J = 10.7, 7.8 Hz, 7H, 7 × H-2_βGal), 4.97 (d, J = 3.6 Hz, 7H, 7 × H-1_αGal), 4.87 (dd, J = 9.3, 8.1 Hz, 7H, 7 × H-2_βGlu), 4.72 (dd, J = 10.8, 2.3 Hz, 7H, 7 × H-3_βGal), 4.69 – 3.30 (m, 273H, 7 × H-1_βGal + 7 × H-1_βGlu +
7 × H-4_βGlu + 7 × H-4_βGal + 7 × H-5_βGlu + 7 × H-5_βGal + 7 × H-5_αGal + 7 × H-6a_βGlu + 7 × H-6b_βGlu + 7 × H-6a_βGal + 7 × H-6b_βGal + 7 × H-6a_αGal + 7 × H-6b_αGal + 7 × H-6a_αGlu_CD + 7 × H-6b_αGlu_CD + 7 × H-5_αGlu_CD + 7 × H-3_αGlu_CD + 7 × OCHaHb + 7 × OCHaHb + 49 × OCH2 + 7 × OCH2-triazole + 14 × OCHaHb_dodecyl + 14 × OCHaHb_dodecyl), 3.21 (br, 7H, 7 × H-4_αGlu_CD), 3.03 (br, 7H, 7 × H-2_αGlu_CD), 2.11 (s, 21H, 7 × Ac), 2.09 (s, 21H, 7 × Ac), 2.08 – 1.99 (m, 147H, 49 × Ac), 1.97 (s, 21H, 7 × Ac), 1.69-1.44 (br, 28H, 14 × CH2_hexyl), 1.40-1.14 (br, 252H, 126 × CH2_dodecyl), 0.90-0.81 (br, 42H, CH3_dodecyl). Selected 13C NMR (101 MHz, CDCl3): δC 170.62, 170.42, 170.39, 170.36, 170.02, 169.65, 169.59, 169.45, 168.81 (CO), 101.07, 100.62 (βGlu, C-1_βGal), 99.98 (C-1_αGlu_CD), 99.58 (C-1_αGal), 76.90 (C-4_αGal), 76.44 (C-4_βGlu), 73.06, 72.79, 72.53, 71.79, 71.67, 70.59, 70.57, 70.46, 70.44, 70.40, 70.37, 70.29, 70.04, 69.83, 69.09, 68.95, 68.80, 67.88, 67.10, 67.04, 62.19, 61.31, 60.25, 31.92, 29.99, 29.90, 29.78, 29.73, 29.68, 29.43, 29.39, 26.32, 26.06, 22.66, 20.89, 20.82, 20.67, 20.63, 20.59, 20.54, 20.47 (OAc), 14.06, 14.05 (CH3_dodecyl). ESI HRMS m/z calc’d for [12C54713C6H889N21O238 + 4Na]4+: 2932.6969; found: 2932.6811.

3.8 Compound (6)

Compound 19 (47 mg, 4.04 µmol) was dissolved in anhydrous MeOH (3 mL), and a solution of NaOMe in MeOH (1.5 M, 100 µL) was added. After stirring at room temperature for 2 days, the reaction mixture was neutralized with dry ice to pH ~9. The solvent was evaporated under reduced pressure and the obtained crude product was
purified by dialysis using a membrane tube (MW 3500). The collected solution was freeze-dried to afford compound 6 as a white solid (31 mg, yield: 88.3%). Selected \(^1\)H NMR (600 MHz, D\(_2\)O) \(\delta_H 8.47\) (br, triazole), \(5.49\) (br, H-1\(\_\alpha\)Gal), \(2.26\)-1.54 (br, CH\(_2\)_dodecyl), \(1.38\) (br, CH\(_3\)_dodecyl). ESI HRMS m/z calc’d for \([^{12}\text{C}_{409}^{13}\text{C}_4\text{H}_{749}\text{N}_2\text{O}_{168} + 4\text{Na}]^{4+}\): 2197.0104; found: 2197.0053.

3.9  
**Methyl 4,6-O-benzylidene-2,3-di-O-hexyl-\(\alpha\)-D-glucopyranoside (21)**

To a solution of compound 20 (5.0 g, 17.7 mmol) in anhydrous DMF (120 mL), was added NaH (60% in mineral oil, 2.8 g, 70.8 mmol). The mixture was stirred for 5 minutes, and 1-bromohexane (9.9 ml, 70.8 mmol) was added dropwise at 0 °C. After stirring at ambient temperature overnight, the excess amount of NaH was destroyed by the addition of MeOH. The mixture was diluted with ethyl acetate (200 ml), and the organic solution was washed with 10% NaCl solution (2 \times 300 mL), dried over anhydrous Na\(_2\)SO\(_4\), and concentrated under reduced pressure. The obtained syrop was purified by column chromatography on silica gel using a mixture of hexane – ethyl acetate (7 : 93) as the eluent to afford the desired compound 21 as an oil (7.39 g, yield: 92.6%). \(R_f\) 0.39 (AcOEt : hexane 1 : 9). [\(\alpha\)]\(^{25}\)_D +42° (c 0.6, CHCl\(_3\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)):
- \(\delta_H 7.55 – 7.45\) (m, 2H, Ph), \(7.43 – 7.30\) (m, 3H, Ph), \(5.54\) (s, 1H, PhCH), \(4.80\) (d, \(J=3.6\) Hz, 1H, H-1), \(4.29\) (dd, \(J=4.5, 9.8\) Hz, 1H, H-6a), \(3.86 – 3.58\) (m, 7H, H-3 + H-5 + H-6b + 2 \times OCH\(_a\)H\(_b\) + 2 \times OCH\(_a\)H\(_b\)), \(3.51\) (dd, \(J=9.3, 9.3\) Hz, 1H, H-4), \(3.44\) (s, 3H, OCH\(_3\)), \(3.36\) (dd, \(J=9.3, 3.7\) Hz 1H, H-2), \(1.67-1.52\) (m, 4H, 2 \times OCH\(_a\)H\(_b\)CHcH\(_d\) + 2 \times OCH\(_a\)H\(_b\)CHcH\(_d\)), \(1.45 – 1.18\) (m, 12H, 2 \times (CH\(_2\))\(_3\)CH\(_3\)), \(0.90\) (t, \(J=7.0\) Hz, 3H, (CH\(_2\))\(_3\)CH\(_3\)), \(0.86\) (t, \(J=7.0\) Hz, 3H, t, \(J=7.0\) Hz, 3H, (CH\(_2\))\(_3\)CH\(_3\)). \(^{13}\)C NMR (100 MHz,
CDCl₃): δₗ 137.51 (Ph), 128.78 (Ph), 128.11 (× 2, Ph), 126.00 (× 2, Ph), 101.20 (PhCH),
99.11 (C-1), 82.01 (C-4), 80.44 (C-2), 78.25 (C-3), 73.43 (OCH₃b), 72.24 (OCH₃b),
69.09 (C-6), 62.40 (C-5), 55.23 (OCH₃), 31.68 (CH₂), 31.64 (CH₂), 30.25 (CH₂), 30.00
(CH₂), 25.75 (CH₂), 25.61 (CH₂), 22.61 (× 2, CH₂), 14.02 (× 2, CH₃). ESI HRMS m/z

3.10 Methyl 4,6-O-benzylidene-2,3-di-O-dodecyl-α-D-glucopyranoside (22)

To a solution of compound 20 (5.464 g, 20 mmol) in dry DMF (80 mL) was slowly
added NaH (3.2 g, 80 mmol). Then 1-bromododecane 2 (192 mL, 19.938 g, 80 mmol)
was added to the mixture, and the reaction mixture was stirred for 3 hours at room
temperature. Water was added to quench the reaction, extracted with CH₂Cl₂ and washed
with brine. The organic solution was dried over anhydrous MgSO₄ and evaporated. The
residue was purified by column chromatography on silica gel using CH₂Cl₂ : hexane (2 : 1)
as eluent to afford the product 22 (11.486 g, 93 % yield) as a colorless solid. Rᵣ 0.28
(CH₂Cl₂ : Hexane 3 : 1). [α]D₂₅: +29.4° (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 600 MHz): δₗ
7.54 - 7.44 (m, 2H, Ph), 7.41 - 7.29 (m, 3H, Ph), 5.54 (s, 1H, PhCH), 4.79 (d, J = 3.6 Hz,
1H, H-1), 4.28 (dd, J = 10.0, 4.4 Hz, 1H, H-6a), 3.83 - 3.60 (m, 7H, H-3 + H-5 + H-6b +
2 × OCH₃bH₂ + 2 × OCH₃bH₂), 3.50 (dd, J = 9.2, 9.2 Hz, 1H, H-4), 3.43 (s, 3H, OCH₃),
3.35 (dd, J = 9.2, 3.6 Hz, 1H, H-2), 1.64 - 1.53 (m, 4H, 2 × OCH₃bCH₃ + 2 ×
OCH₃bCH₃H₂), 1.37 - 1.22 (m, 36H, 2 × (CH₂)₉CH₃), 0.88 (t, J = 6.8 Hz, 6H, 2 ×
CH₃). ¹³C NMR (CDCl₃, 150 MHz): δ 137.64, 128.92, 128.27, 126.14 (Ph), 101.34
(PhCH), 99.26 (C-1), 82.17 (C-4), 80.58 (C-2), 78.40 (C-3), 73.60 (OCH₃b), 72.41
(OCH₃b), 69.25 (C-6), 62.54 (C-5), 55.39 (OCH₃), 32.06 (2 × CH₂), 30.47 (CH₂), 30.20
(CH₂), 29.84 (CH₂), 29.82 (3 × CH₂), 29.79 (4 × CH₂), 29.68 (CH₂), 29.62 (CH₂), 29.50
(2 × CH₂), 26.27 (CH₂), 26.12 (CH₂), 22.83 (2 × CH₂), 14.25 (2 × CH₃). ESI HRMS m/z calc’d for [C₃₈H₆₇O₆ + H]⁺: 619.4932; found: 619.4935.

3.11 Methyl 4-O-benzoyl-6-bromo-6-deoxy-2,3-di-O-hexyl-α-D-glucopyranoside (23)

To a solution of compound 21 (4.0 g, 8.8 mmol) and N-bromosuccinimide (2.37 g, 13.3 mmol) in 1,2-dichloroethene (40 mL), was added barium carbonate (3.5 g 17.7 mmol), and the mixture was heated to reflux for 2 hours. The insoluble solid was filtered off, and the organic solution was evaporated. The obtained residue was purified by column chromatography on silica gel using a mixture of hexane – ethyl acetate (1 : 99) as the eluent to afford the desired compound 23 (4.4 g, yield: 93.6%). Rₐ 0.39 (AcOEt : Hexane 1 : 9). [α]25°D +12.5° (c 2.8, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δH 8.06 (m, 2H, Bz), 7.59 (m, 1H, Bz), 7.46 (m, 2H, Bz), 5.07 (dd, J=9.9, 9.3 Hz, 1H, H-4), 4.88 (d, J=3.6 Hz, 1H, H-1), 3.77 (ddd, J=2.3, 8.0, 9.9 Hz, 1H, H-5), 3.82 – 3.73 (m, 2H, OCHaHb), 3.70 – 3.58 (m, 2H, OCHaHb + OCHaHb), 3.52 (s, 3H, OCH₃), 3.52 – 3.41 (m, 3H, OCHaHb + H-2 + H-6a), 3.39 (dd, J=11.2, 8.0 Hz, 1H, H-6b), 1.65-1.55 (m, 2H, OCHaHbCHcHd + OCHaHbCHcHd), 1.44 – 1.24 (m, 8H, OCHaHbCHcHd + OCHaHbCHcHd + (CH₂)₃CH₃), 1.20 – 0.95 (m, 6H, (CH₂)₃CH₃), 0.89 (t, J=6.9 Hz, 3H, (CH₂)₃CH₃), 0.71 (t, J=6.8 Hz, 3H, (CH₂)₃CH₃). ¹³C NMR (100 MHz, CDCl₃): δC 165.33 (C=O), 133.35 (Ph), 129.78 (× 2, Ph), 129.43 (Ph), 128.45 (× 2, Ph), 98.11 (C-1), 80.27 (C-2), 78.93 (C-3), 73.72 (OCHaHb), 73.17 (C-4), 71.89 (OCHaHb), 69.35 (C-5), 55.52 (OCH₃), 31.94 (C-6), 31.57 (CH₂), 31.49 (CH₂), 30.17 (CH₂), 29.93 (CH₂), 25.58 (CH₂), 22.58 (× 2, CH₂), 22.36 (CH₂), 13.98 (CH₃), 13.87 (CH₃). ESI HRMS m/z calc’d for [C₂₆H₄₁O₆Br + H]⁺: 529.2159; found 529.21589.
3.12  *Methyl 4-O-benzoyl-6-bromo-6-deoxy-2,3-di-O-dodecyl-α-D-glucopyranoside (24)*

To a solution of compound 22 (6.189 g, 10 mmol) in DCE (60 mL) were added NBS (2.670 g, 15 mmol) and BaCO₃ (3.947 g, 20 mmol). The reaction mixture was stirred for 2 hours at reflux temperature. The white precipitates were filtered off, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel using CH₂Cl₂ : hexane (1 : 1) as eluent to afford the product 24 (6.098 g, 87 % yield) as a colorless liquid. Rf 0.34 (CH₂Cl₂ : Hexane 2 : 1). [α]D₂⁵: +22.8° (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 600 MHz): δ 8.06 (m, 2H, Ph), 7.59 (m, 1H, Ph), 7.46 (m, 2H, Ph), 5.07 (dd, J = 9.6, 9.6 Hz, 1H, H-4), 4.88 (d, J = 3.2 Hz, 1H, H-1), 4.01 (ddd, J=10.2, 8.0, 2.4 Hz, 1H, H-5), 3.82 - 3.70 (m, 2H, H-3 + OCH₃Hb), 3.68 - 3.58 (m, 2H, OCH₃Hb + OCHAHb), 3.52 (s, 3H, OCH₃), 3.50 - 3.31 (m, 4H, OCHAHb + H-2 + H-6a + H-6b), 1.63 - 1.55 (m, 2H, OCHAHbCHcHd + OCHAHbCHcHd), 1.39 - 1.00 (m, 38H, OCHAHbCHcHd + OCHAHbCHcHd + 2 × (CH₂)₉CH₃), 0.89 (t, J = 6.8 Hz, 3H, CH₃), 0.88 (t, J = 6.8 Hz, 3H, CH₃). ¹³C NMR (CDCl₃, 150 MHz): δ 165.53 (C=O), 133.54 (Ph), 129.98 (× 2, Ph), 129.64 (Ph), 128.65 (× 2, Ph), 98.32 (C-1), 80.47 (C-2), 79.12 (C-3), 73.94 (OCHAHb), 73.38 (C-4), 72.10 (OCHAHb), 69.56 (C-5), 55.73 (OCHAHb), 32.14 (C-6), 32.08 (2 × CH₂), 30.43 (CH₂), 30.18 (CH₂), 29.84 (CH₂), 29.79 (5 × CH₂), 29.70 (CH₂), 29.62 (CH₂), 29.60 (CH₂), 29.54 (CH₂), 29.51 (2 × CH₂), 26.15 (CH₂), 26.12 (CH₂), 22.85 (2 × CH₂), 14.27 (2 × CH₃). ESI HRMS m/z calc’d for [C₃₈H₆₆BrO₆ + H]⁺: 697.40373; found: 697.40269.
3.13  **Methyl 6-azido-4-O-benzoyl-6-deoxy-2,3-di-O-hexyl-α-D-glucopyranoside (25)**

The above obtained 6-bromoide 23 (4.3 g, 8.1 mmol) was dissolved in dry DMF (30 mL), and sodium azide (1.5 g , 2.4 mmol) was added; the reaction mixture was heated at 70 °C for 17 h. Water (40 mL) was added, and the mixture was extracted with ethyl acetate (80 mL). The organic layer was washed with brine (2 × 40 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using a mixture of hexane – ethyl acetate (1 : 99) as the eluent to afford the desired 6-azide 25 (3.8 g, yield: 95%). \( \alpha \) \( ^{25} \) \( \text{D} \) +29.2° (c 0.37, CHCl₃). \( ^1 \)H NMR (400 MHz, CDCl₃): δ 8.06 (m, 2H, Bz), 7.59 (m, 1H, Bz), 7.46 (m, 2H, Bz), 5.10 (dd, \( J=10.1, 9.3 \) Hz, 1H, H-4), 4.88 (d, \( J=3.6 \) Hz, 1H, H-1), 3.99 (ddd, \( J=10.0, 7.4, 2.5 \) Hz, 1H, H-5), 3.79 (dd, \( J=9.4, 9.4 \) Hz, 1H, H-3), 3.77 (ddd, \( J=9.4, 6.3, 6.3 \) Hz, 1H, OCHaHb), 3.71 – 3.60 (m, 2H, OCHaHb + OCHaHb), 3.52 (s, 3H, OCH₃), 3.49 (ddd, \( J=9.3, 6.7, 6.7 \) Hz, 1H, OCHaHb), 3.45 (dd, \( J=9.7, 3.6 \) Hz, 1H, H-2), 3.39 (dd, \( J=13.3, 7.4 \) Hz, 1H, H-6a), 3.27 (dd, \( J=13.2, 2.5 \) Hz, 1H, H-6b), 1.61 (m, 2H, OCHaHbCHcHd + OCHaHbCHcHd), 1.44 – 1.24 (m, 8H, OCHaHbCHcHd + OCHaHbCHcHd + (CH₂)₃CH₃), 0.89 (t, \( J=6.9 \) Hz, 3H, (CH₂)₃CH₃), 0.72 (t, \( J=6.9 \) Hz, 3H, (CH₂)₃CH₃). \( ^{13} \)C NMR (100 MHz, CDCl₃): δc 165.40 (C=O), 133.36 (Ph), 129.79 (× 2, Ph), 129.47 (Ph), 128.48 (× 2, Ph), 98.16 (C-1), 80.28 (C-2), 78.90 (C-3), 73.73 (OCHaHb), 71.92 (OCHaHb), 71.89 (C-4), 69.25 (C-5), 55.56 (OCH₃), 51.49 (C-6), 31.61 (CH₂), 31.52 (CH₂), 30.21 (CH₂), 29.98 (CH₂), 25.62 (× 2, CH₂), 22.61 (CH₂), 22.40 (CH₂), 14.01 (CH₃), 13.89 (CH₃). ESI HRMS m/z calc’d for [C₂₆H₄₁O₈N₃ + Na]⁺: 514.2888; found: 514.2902.
To a solution of compound 24 (4.187 g, 6 mmol) in dry DMF (35 mL) was added NaN₃ (0.780 g, 12 mmol). The reaction mixture was stirred at 65 °C overnight. The mixture was concentrated to few milliliters, then water was added to the residue. The solution was extracted with AcOEt, washed with brine. The organic solution was dried over anhydrous MgSO₄ and evaporated. The residue was purified by column chromatography on silica gel using AcOEt : hexane (1 : 20) as eluent to afford the product 26 (3.077 g, 78 % yield) as a colorless solid. Rf 0.19 (AcOEt : Hexane 1 : 15). [α]D 25°: +19.4° (c 1.0, CHCl₃).

1H NMR (CDCl₃, 600 MHz): δ 8.04 (m, 2H, Ph), 7.57 (m, 1H, Ph), 7.44 (m, 2H, Ph), 5.08 (dd, J = 9.6, 9.6 Hz, 1H, H-4), 4.86 (d, J = 3.6 Hz, 1H, H-1), 3.97 (ddd, J =10.0, 7.4, 2.4 Hz, 1H, H-5), 3.81 - 3.71 (m, 2H, H-3 + OC₉H₆), 3.68 - 3.59 (m, 2H, OC₉H₆ + OCH₃), 3.51 (s, 3H, OCH₃), 3.48 - 3.42 (m, 2H, OCH₉H₆ + H-2), 3.37 (dd, J =7.5, 13.3 Hz, H-6a), 3.25 (dd, J = 13.2, 2.4 Hz, 1H, H-6b), 1.65 - 1.54 (m, 2H, OCH₉H₆CHcHd + OCH₉H₆CHcHd), 1.41 - 1.00 (m, 38H, OCH₉H₆CHcHd + OCH₉H₆CHcHd + 2 × (CH₂)₉CH₃), 0.88 (t, J = 6.8 Hz, 3H, CH₃), 0.87 (t, J = 6.8 Hz, 3H, CH₃).

13C NMR (CDCl₃, 150 MHz): δ 165.51 (C=O), 133.47 (Ph), 129.91 (× 2, Ph), 129.61 (Ph), 128.59 (× 2, Ph), 98.28 (C-1), 80.41 (C-2), 79.02 (C-3), 73.87 (OCH₉H₆), 72.05 (OCH₉H₆), 72.03 (C-4), 69.39 (C-5), 55.68 (OCH₃), 51.64 (C-6), 32.05 (× 2, CH₂), 30.40 (CH₂), 30.15 (CH₂), 29.80 (CH₂), 29.76 (× 5, CH₂), 29.67 (CH₂), 29.59 (CH₂), 29.56 (CH₂), 29.50 (CH₂), 29.48 (× 2, CH₂), 26.12 (CH₂), 26.10 (CH₂), 22.81 (× 2, CH₂), 14.23 (× 2, CH₃). ESI HRMS m/z calc’d for [C₃₈H₆₆N₃O₆ + H]+: 660.49461; found: 660.49398.
3.15  *Methyl 6-azido-6-deoxy-2,3-di-O-hexyl-α-D-glucopyranoside (27)*

Compound 25 (3.7 g, 7.5 mmol) was dissolved in anhydrous MeOH (30 mL), and a solution of NaOMe in MeOH (1.5 M, 1.5 mL) was added. The mixture was stirred at room temperature for 2 hrs, and the solution was neutralized with Amberlite IR-120 (H+). The mixture was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel using a mixture of hexane – ethyl acetate (5 : 90) as eluent to afford the desired compound 27 (2.8 g, yield: 96%). $\text{R}_f 0.14$ (AcOEt : Hexane 1 : 9). $[\alpha]_{25}^{20} +60.4^\circ$ (c 0.5, CHCl$_3$). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 4.80 (d, $J=3.5$ Hz, 1H, H-1), 3.91 (ddd, $J=9.4, 6.9, 6.9$ Hz, 1H, OCHaHb), 3.75 (ddd, $J=9.7, 6.2, 2.5$ Hz, 1H, H-5), 3.64 – 3.55 (m, 2H, OCHaHb + OCHaHb), 3.55 – 3.47 (m, 3H, OCHaHb + H-6a + H-3), 3.46 – 3.34 (m, 5H, OCH$_3$ + H-4 + H-6b), 3.29 (dd, $J=9.5, 3.5$ Hz, 1H, H-2), 2.53 (d, $J=2.4$ Hz, 1H, OH-4), 1.63 – 1.50 (m, 4H, 2 × OCHaHbCHcHd + 2 × OCHaHbCHcHd), 1.40 – 1.21 (m, 12H, 2 × (CH$_2$)$_3$CH$_3$), 0.88 (t, $J=6.8$ Hz, 3H, (CH$_2$)$_3$CH$_3$), 0.88 (t, $J=6.8$ Hz, 3H, (CH$_2$)$_3$CH$_3$), 0.87 (t, $J=6.8$ Hz, 3H, (CH$_2$)$_3$CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$C 98.03 (C-1), 80.79 (C-3), 80.54 (C-2), 73.56 (OCHaHb), 71.19 (OCHaHb), 70.70 (C-4), 70.30 (C-5), 55.32 (OCH$_3$), 51.55 (C-6), 31.64 (CH$_2$), 31.57 (CH$_2$), 30.29 (CH$_2$), 29.94 (CH$_2$), 25.70 (CH$_2$), 25.63 (CH$_2$), 22.56 (× 2, CH$_2$), 13.96 (× 2, CH$_3$). ESI HRMS m/z calc’d for [C$_{19}$H$_{37}$O$_3$N$_3$ + NH$_4$]$^+$: 405.3072; found: 405.3066.

3.16  *Methyl 6-azido-6-deoxy-2,3-di-O-dodecyl-α-D-glucopyranoside (28)*

To a solution of compound 26 (2.970 g, 4.5 mmol) in dry MeOH (30 mL) was added a solution of NaOMe in MeOH (1.5 M, 1.8 mL). The reaction mixture was stirred for 1 day at room temperature. AcOH was added to quench the reaction. The reaction mixture was
concentrated. The residue was purified by column chromatography on silica gel using AcOEt : hexane (1 : 15) as eluent to afford the product 28 (2.264 g, 91 % yield) as a colorless solid. Rf 0.17 (AcOEt : Hexane 1 : 10). \([\alpha]_{D}^{25}: +44^\circ (c 1.0, \text{CHCl}_3)\). \(^1\)H NMR (CDCl\(_3\), 600 MHz): \(\delta\) 4.81 (d, \(J = 3.2\) Hz, 1H, H-1), 3.95 - 3.89 (ddd, \(J = 6.8, 6.8, 9.4\) Hz, 1H, OCH\(_a\)Hb), 3.75 (ddd, \(J = 9.7, 6.0, 2.4\) Hz, 1H, H-5), 3.63 - 3.56 (m, 2H, OCH\(_a\)Hb + OCH\(_a\)Hb), 3.55 - 3.48 (m, 3H, OCH\(_a\)Hb + H-6a + H-3), 3.44 (s, 3H, OCH\(_3\)), 3.43 - 3.36 (m, 2H, H-4 + H-6b), 3.29 (dd, \(J = 9.2, 3.6\) Hz, 1H, H-2), 2.43 (d, \(J = 2.3\) Hz, 1H, OH-4), 1.62 - 1.51 (m, 4H, 2 \times OCH\(_a\)HbCHcHd + 2 \times OCH\(_a\)HbCHcHd), 1.34 - 1.22 (m, 36H, 2 \times (CH\(_2\))\(_9\)CH\(_3\)), 0.87 (t, \(J = 6.8\) Hz, 6H, 2 \times CH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 600 MHz): \(\delta\) 98.21 (C-1), 80.96 (C-3), 80.72 (C-2), 73.75 (OCH\(_a\)Hb), 71.36 (OCH\(_a\)Hb), 70.91 (C-4), 70.47 (C-5), 55.50 (OCH\(_3\)), 51.74 (C-6), 32.05 (\(\times 2\), CH\(_2\)), 30.54 (CH\(_2\)), 30.17 (CH\(_2\)), 29.81 (\(\times 2\), CH\(_2\)), 29.77 (\(\times 6\), CH\(_2\)), 29.66 (CH\(_2\)), 29.58 (CH\(_2\)), 29.49 (\(\times 2\), CH\(_2\)), 26.25 (CH\(_2\)), 26.16 (CH\(_2\)), 22.82 (\(\times 2\), CH\(_2\)), 14.23 (\(\times 2\), CH\(_3\)). ESI HRMS m/z calc’d for [C\(_{31}\)H\(_{65}\)N\(_4\)O\(_5\) + NH\(_4\)]\(^+\): 573.4950; found: 573.4950.

3.17 Methyl 6-azido-6-deoxy-2,3-di-O-hexyl-4-O-methyl-\(\alpha\)-D-glucopyranoside (29)

To a solution of compound 27 (2.66 g, 6.86 mmol) in anhydrous DMF (30 mL), was added NaH (60% in mineral oil, 0.55 g, 13.72 mmol), and the reaction mixture was stirred at room temperature for 5 minutes. After cooling to 0° C, methyl iodide (850 \(\mu\)L, 13.65 mmol) was added dropwise, and the mixture was allowed to warm-up to room temperature. After stirring for 2 hrs, MeOH (1 mL) was added to quench the reaction, and the mixture was concentrated under reduced pressure. The residue was dissolved in
EtOAc (150 mL), and the solution was washed with H₂O (1 × 100 mL), 2N HCl (1 × 100 mL) and 10% NaHCO₃ (1 × 100 mL), dried over anhydrous Na₂SO₄, and evaporated. The crude mixture was purified by column chromatography on silica gel using 3% AcOEt – hexane as eluent to afford compound 29 as a colorless foam (2.6 g, yield 94.2%). Rₚ 0.33 (AcOEt : Hexane 1 : 9). [α]₂₅²⁰ +90.4° (c 0.9, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ_H 4.77 (d, J =3.6 Hz, 1H, H-1), 3.83 (ddd, J =9.1, 6.8, 6.8 Hz, 1H, OCHaHb), 3.73 – 3.52 (m, 8H, 2 × OCHaHb + OCHaHb + OCH₃ + H-3 + H-5), 3.50 (dd, J =13.0, 2.4 Hz, 1H, H-6a), 3.42 (s, 3H, OCH₃), 3.39 (dd, J =13.0, 5.5, 1H, H-6b), 3.27 (dd, J =9.7, 3.6 Hz, 1H, H-2), 3.07 (dd, J =9.8, 8.9 Hz, 1H, H-4), 1.66 – 1.53 (m, 4H, 2 × OCHaHbCHcHd + 2 × OCHaHbCHcHd), 1.43 – 1.22 (m, 12H, 2 × (CH₂)₃CH), 0.89 (t, J =6.8 Hz, 3H, (CH₂)₃CH₃), 0.88 (t, J =6.8 Hz, 3H, (CH₂)₃CH₃). ¹³C NMR (100 MHz, CDCl₃): δ_C 97.99 (C-1), 81.36 (C-3), 80.60 (C-2), 80.37 (C-4), 73.58 (OCHaHb), 71.74 (OCHaHb), 70.04 (C-5), 60.76 (OCH₃), 55.28 (OCH₃), 51.49 (C-6), 31.73 (CH₂), 31.63 (CH₂), 30.46 (CH₂), 30.01 (CH₂), 25.82 (CH₂), 25.66 (CH₂), 22.62 (CH₂), 22.61 (CH₂), 14.01 (CH₃), 14.00 (CH₃). ESI HRMS m/z calc’d for [C₂₀H₃₉O₅N₃ + Na]⁺: 424.2782; found: 424.2785.

3.18 Methyl 6-azido-6-deoxy-2,3-di-O-dodecyl-4-O-methyl-α-D-glucopyranoside (30)

To a solution of compound 28 (2.223 g, 4 mmol) in dry DMF (10 mL) was slowly added NaH (0.32 g, 8 mmol). The reaction mixture was stirred for 10 min at room temperature. Then iodomethane (0.5 mL, 1.136 g, 8 mmol) was added to the mixture, and the reaction mixture was stirred for 0.5 hour at room temperature. Water was added to quench the reaction, extracted with AcOEt and washed with brine. The organic solution was dried over anhydrous MgSO₄ and evaporated. The residue was purified by column
chromatography on silica gel using AcOEt : hexane (1 : 20) as eluent to afford the product 30 (2.225 g, 98 % yield) as a colorless solid. Rf 0.21 (AcOEt : Hexane 1 : 20). 

[$\alpha$]$^D_{25}$: +63.6° (c 1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ 4.76 (d, $J = 3.6$ Hz, 1H, H-1), 3.84 - 3.79 (ddd, $J = 9.1$, 6.8, 6.8 Hz, 1H, OCH$_a$Hb), 3.69 - 3.52 (m, 8H, 2 × OCH$_a$Hb + OCH$_a$Hb + OCH$_3$ + H-3 + H-5), 3.49 (dd, $J = 13.2$, 5.6 Hz, 1H, H-6a), 3.41 (s, 3H, OCH$_3$), 3.38 (dd, $J = 13.2$, 5.6 Hz, 1H, H-6b), 3.26 (dd, $J = 9.6$, 3.6 Hz, 1H, H-2), 3.06 (dd, $J = 9.6$, 9.6 Hz, 1H, H-4), 1.62 - 1.54 (m, 4H, 2 × OCH$_a$HbCHcHd + 2 × OCH$_a$HbCHcHd), 1.35 - 1.22 (m, 36H, 2 × (CH$_2$)$_9$CH$_3$), 0.87 (t, $J = 6.4$ Hz, 6H, 2 × CH$_3$). $^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ 98.13 (C-1), 81.50 (C-3), 80.74 (C-2), 80.52 (C-4), 73.73 (OCH$_a$Hb), 71.89 (OCH$_a$Hb), 70.18 (C-5), 60.91 (OCH$_3$), 55.43 (OCH$_3$), 51.64 (C-6), 32.06 (× 2, CH$_2$), 30.67 (CH$_2$), 30.21 (CH$_2$), 29.82 (× 2, CH$_2$), 29.79 (× 6, CH$_2$), 29.71 (CH$_2$), 29.61 (CH$_2$), 29.50 (× 2, CH$_2$), 26.34 (CH$_2$), 26.16 (CH$_2$), 22.82 (× 2, CH$_2$), 14.24 (× 2, CH$_3$). ESI HRMS m/z calc’d for [C$_{32}$H$_{67}$N$_4$O$_5$ + H]$^+$: 588.5184; found: 588.5142.

3.19 Compound (31)

To a solution of compound 11 (27 mg, 23.7 µmol) and 6-azide 29 (20 mg, 50 µmol) in acetone (3 mL), was added N,N-diisopropylethylamine (10 µL, 57.5 µmol) and CuI (10 mg, 52.5 µmol). The reaction was stirred overnight at room temperature. The mixture was dissolved in ethyl acetate (40 mL), and the organic solution was washed with a saturated solution of EDTA (1 × 40 ml), water (1 × 40 mL), brine (1 × 40 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 25% acetone – toluene as eluent to afford the desired compound 31 (36 mg, yield 98.6%). R$_f$ 0.14 (Acetone : Toluene, 3 : 7). [$\alpha$]$^{25}_{D}$
+41.1° (c 0.6, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δH 7.67 (s, 1H, Triazole), 5.57 (dd, J=3.4, 1.1 Hz, 1H, H-4_αGal_p⁰), 5.37 (dd, J=11.0, 3.3 Hz, 1H, H-3_αGal_p⁰), 5.18 (dd, J=9.1, 9.1 Hz, 1H, H-3_βGlu_p⁰), 5.17 (dd, J=3.6, 9.6 Hz, 1H, H-2_αGal_p⁰), 5.09 (dd, J=10.8, 7.7 Hz, 1H, H-2_βGal_p⁰), 4.97 (d, J=9.1 Hz, 1H, H-1_βGlu_p⁰), 4.88 (dd, J=9.4, 8.0 Hz, 1H, H-2_βGlu_p⁰), 4.76 – 4.65 (m, 4H, H-3_βGlu_p⁰ + H-1_αGlu + triazole-CH₃HbO + triazole-CH₃HbO), 4.62 (dd, J=5.7, 14.2 Hz, 1H, H-6a_αGlu), 4.56 (dd, J=3.1, 14.2 Hz, 1H, H-6b_αGlu), 4.54 (d, J=7.9 Hz, 1H, H-1_βGlu_p⁰), 4.51 (d, J=7.8 Hz, 1H, H-1_βGlu_p⁰), 4.50 – 4.05 (m, 7H, H-5_βGlu_p⁰ + H-6a_αGlu + H-6b_αGlu + H-6a_βGlu_p⁰ + H-6b_βGlu_p⁰ + H-6a_βGlu_p⁰ + H-6b_βGlu_p⁰ + H-6b_βGlu_p⁰), 4.00 (dd, J=2.2, <1.0 Hz, 1H, H-4_βGlu_p⁰), 3.90 (ddd, J=4.3, 4.3, 11.0 Hz, 1H, OCH₃ + H-3_βGlu_p⁰ + H-5_αGlu + H-4_βGlu_p⁰ + H-5_βGlu_p⁰ + H-5_αGlu + OCH₃HbCH₃Hd(OCH₂CH₂)₃O), 3.23 (s, 3H, OCH₃), 3.13 (dd, J=9.7, 3.5 Hz, 1H, H-2_αGlu), 2.74 (dd, J=9.9, 8.9 Hz, 1H, H-4_αGlu), 2.11 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.55 (m, 4H, 2 × OCH₃HbCH₃Hd + 2 × OCH₃HbCH₃Hd), 1.43 – 1.16 (m, 12H, 2 × (CH₂)₂CH₃), 0.87 (t, J=6.8 Hz, 3H, (CH₂)₂CH₃), 0.86 (t, J=6.8 Hz, 3H, (CH₂)₂CH₃). ¹³C NMR (100 MHz, CDCl₃): δC 170.61 (C=O), 170.40 (× 4, C=O), 170.03 (C=O), 169.68 (C=O), 169.59 (C=O), 169.46 (C=O), 168.81 (C=O), 145.05 (triazone), 124.01 (triazone), 101.07 (C-1_βGal_p⁰), 100.59 (C-1_βGlu_p⁰), 99.59 (C-1_αGal_p⁰), 97.99 (C-1_αGlu), 81.36 (C-4_αGal_p⁰), 80.40 (C-3_αGlu), 79.86 (C-2_αGlu), 76.90 (C-4_αGlu), 76.43 (C-4_βGal_p⁰), 73.59, 73.08, 72.79, 72.53, 71.79, 71.75, 71.71, 70.64,
70.53, 70.47, 70.16, 69.07, 68.96, 68.81, 67.87, 67.11, 67.04, 64.63 (triazole-CHaHbO), 62.18, 61.29, 60.72 (OCH₃), 60.24, 55.27 (OCH₃), 50.65 (C-6_αGlu), 31.67 (CH₂), 31.58 (CH₂), 30.40 (CH₂), 29.96 (CH₂), 25.77 (CH₂), 25.60 (CH₂), 22.56 (× 2, CH₂), 20.88 (CH₂), 20.82 (CH₂), 20.67 (× 4, Ac), 20.58 (Ac), 20.54 (Ac), 20.46 (Ac), 13.98 (× 2, CH₃). ESI HRMS m/z calc’d for [C₆₉H₁₀₉O₃₅N₃ + H]⁺: 1540.6914; found: 1540.6894.

### 3.20 Compound (32)

To a solution of compound 11 (28 mg, 24.6 μmol) and 6-azide 30 (30 mg, 52.6 μmol) in acetone (5 mL), was added N,N-diisopropylethylamine (10 μL, 57.5 μmol) and CuI (10 mg, 52.5 μmol), and the reaction mixture was stirred at room temperature overnight. Ethyl acetate (40 mL) was added and the organic solution was washed with a saturated solution of EDTA (1 × 40 ml), water (1 × 40 mL), brine (1 × 40 mL), dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 25% acetone – toluene as the eluent to provide the desired product 32 as a white foam (41.0 mg, yield 97.6%). Rf 0.33 (Methanol : CH₂Cl₂, 5 : 95). [α]²⁵⁺D +40.5° (c 0.41, CHCl₃). ^1H NMR (400 MHz, CDCl₃): δH 7.67 (s, 1H, Triazole), 5.57 (dd, J=3.0, ~1 Hz, 1H, H-4_αGal_p[^b]), 5.36 (dd, J=11.0, 3.3 Hz, 1H, H-3_αGal_p[^b]), 5.18 (dd, J=9.1, 9.1 Hz, 1H, H-3_βGlu_p[^b]), 5.17 (dd, J=3.6, 9.6 Hz, 1H, H-2_αGal_p[^b]), 5.08 (dd, J=7.8, 10.7 Hz, 1H, H-2_βGlu_p[^b]), 4.97 (d, J=3.5 Hz, 1H, H-1_αGal_p[^b]), 4.88 (dd, J=9.4, 8.0 Hz, 1H, H-2_βGlu_p[^b]), 4.76 – 4.66 (m, 4H, H-3_βGal_p[^b] + H-1_αGlu + triazole-CHaHbO + triazole-CHaHbO), 4.60 (dd, J=5.9, 14.4 Hz, 1H, H-6a_αGlu), 4.56 (dd, J=2.8, 14.2 Hz, 1H, H-6b_αGlu), 4.55 (d, J=7.9 Hz,
1H, H-1_βGlu_p\(^k\)), 4.51 (d, \(J=8.0\) Hz, 1H, H-1_βGlu_p\(^k\)), 4.53 – 4.05 (m, 7H, H-5_βGal_p\(^k\) + H-6a_αGal_p\(^k\) + H-6a_βGal_p\(^k\) + H-6b_αGal_p\(^k\) + H-6b_βGal_p\(^k\) + H-6b_βGlu_p\(^k\) + H-6b_βGlu_p\(^k\)), 4.00 (dd, \(J=2.2, <1.0\) Hz, 1H, H-4_βGal_p\(^k\)), 3.90 (dd, \(J=4.9, 4.9, 10.7\) Hz, 1H, OCHA HB), 3.84 – 3.47 (m, 27H, 2 × OCH\(_2\)CH\(_2\)O), 3.23 (s, 3H, OCH\(_3\)), 3.14 (dd, \(J=3.5, 9.7\) Hz, 1H, H-2_αGlu), 2.74 (dd, \(J=9.2, 9.2\) Hz, 1H, H-4_αGlu), 2.11 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 6H, 2 × Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.60 – 1.51 (m, 4H, 2 × OCH\(_2\)CH\(_2\)CH\(_2\)O + 2 × OCH\(_2\)CH\(_2\)CH\(_2\)O), 1.37 – 1.17 (m, 36H, 2 × (CH\(_2\)\(_9\)CH\(_3\)). 13C NMR (100 MHz, CDCl\(_3\)): δ\(_C\) 170.62 (C=O), 170.40 (× 4, C=O), 170.03 (C=O), 169.68 (C=O), 169.59 (C=O), 169.46 (C=O), 168.81 (C=O), 145.04 (triazole), 124.01 (triazole), 101.07 (C-1_βGal_p\(^k\)), 100.59 (C-1_βGlu_p\(^k\)), 99.59 (C-1_αGal_p\(^k\)), 97.97 (C-1_αGlu), 81.35 (C-4_αGal_p\(^k\)), 80.40 (C-3_αGlu), 79.87 (C-2_αGlu), 76.90 (C-4_αGlu), 76.43 (C-4_βGal_p\(^k\)), 73.60, 73.08, 72.79, 72.53, 71.79, 71.71, 70.64, 70.53, 70.47, 70.17, 69.59, 69.19, 69.07, 68.96, 68.82, 67.87, 67.11, 67.04, 64.63, 62.18, 61.29, 60.72 (OCH\(_3\)), 60.24, 55.27 (OCH\(_3\)), 50.66 (C-6_αGlu), 31.87 – 22.63 (CH\(_2\)_dodecyl), 20.88 – 20.46 (Ac), 14.06 (× 2, CH\(_3\)). ESI HRMS m/z calc’d for [C\(_{81}H_{133}O_{35}N_3 + H]^+: 1708.8792; found: 1708.8754.

### 3.21 Compound (7)

Compound 31 (26 mg, 16.88 μmol) was dissolved in anhydrous MeOH (3 mL), and a solution of NaOMe in MeOH (1.5 M, 50 μL) was added. After stirring at room
temperature overnight, the reaction mixture was neutralized with Amberlite IR-120 (H\(^+\)). After filtration, the mixture was evaporated under reduced pressure, and the crude product was purified by reverse phase C18 Sep-Pak column using a gradient of methanol-H\(_2\)O as the eluent (0-100%) to afford the desired compound 7 which was freeze-dried (17.4 mg, yield: 92.5%). R\(_f\) 0.12 (Methanol : CH\(_2\)Cl\(_2\) 4 : 6). \([\alpha]\)\(^{25}\)\(_D\) +44.5° (c 0.2, MeOH). \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\)H 8.00 (s, 1H, triazole), 4.96 (d, \(J\)=3.6 Hz, 1H, H-1_\(\alpha\)Gal\(_p\)), 4.77 (d, \(J\)=3.6 Hz, 1H, H-1_\(\alpha\)Glu), 4.71 (dd, \(J\)=14.3, 2.8 Hz, 1H, H-6a_\(\alpha\)Glu), 4.66 (s, 2H, triazole-CH\(_2\)HbO + triazole-CH\(_a\)HbO), 4.63 (dd, \(J\)=6.9, 14.3 Hz, 1H, H-6b_\(\alpha\)Glu), 4.43 (high order d, \(J\)=7.4 Hz, 1H, H-1_\(\beta\)Glu\(_p\)), 4.35 (d, \(J\)=7.8 Hz, 1H, H-1_\(\beta\)Gal\(_p\)), 4.26 (ddd, \(J\)=~1, 5.3, 6.3 Hz, 1H, H-5_\(\alpha\)Gal\(_p\)), 4.14 (d, \(J\)=7.8 Hz, 1H, H-5_\(\beta\)Gal\(_p\)), 4.04 – 3.96 (m, 2H, H-4_\(\alpha\)Glu), 3.93 (dd, \(J\)=0.9, 2.9 Hz, 1H, H-4_\(\alpha\)Gal\(_p\)), 3.92 – 3.47 (m, 37H, H-2_\(\alpha\)Gal\(_p\) + H-3_\(\alpha\)Gal\(_p\) + H-5_\(\alpha\)Gal\(_p\) + H-6a_\(\alpha\)Gal\(_p\) + H-6b_\(\alpha\)Gal\(_p\) + H-3_\(\beta\)Gal\(_p\) + H-6a_\(\beta\)Gal\(_p\) + H-6b_\(\beta\)Gal\(_p\) + H-2_\(\beta\)Glu\(_p\) + H-3_\(\beta\)Glu\(_p\) + H-4_\(\beta\)Glu\(_p\) + H-6a_\(\beta\)Glu\(_p\) + H-6b_\(\beta\)Glu\(_p\) + H-2_\(\alpha\)Glu + H-3_\(\alpha\)Glu + H-5_\(\alpha\)Glu + OCH\(_3\) + OCH\(_3\)HbCHcHd(OCH\(_2\)CH\(_2\))\(_3\)O + OCH\(_3\)Hb + 2 × OCH\(_3\)Hb), 3.42 (m, 1H, H-5_\(\beta\)Glu\(_p\)), 3.27 (dd, \(J\)=8.4, 8.7 Hz, 1H, H-2_\(\beta\)Gal\(_p\)), 3.22 (dd, dd, \(J\)=3.6, 9.8 Hz, 1H, H-2_\(\alpha\)Glu), 3.18 (s, 3H, OCH\(_3\)), 2.85 (dd, \(J\)=9.9, 8.8 Hz, 1H, H-4_\(\alpha\)Glu), 1.64 – 1.50 (m, 4H, 2 × OCH\(_3\)HbCHcHd + 2 × OCH\(_3\)HbCHcHd), 1.44 – 1.25 (m, 12H, 2 × (CH\(_2\))\(_3\)CH\(_3\)), 0.91 (t, \(J\)=6.8 Hz, 2 × CH\(_3\)). \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \(\delta\)C 125.23 (triazole), 103.93 (C-1_\(\beta\)Glu\(_p\)), 102.85 (C-1_\(\beta\)Gal\(_p\)), 101.26 (C-1_\(\alpha\)Gal\(_p\)), 97.58 (C-1_\(\alpha\)Glu), 81.38, 80.26, 80.18, 79.59, 78.34, 75.09, 75.03, 74.86, 73.40, 73.24, 73.05, 71.42, 71.22, 70.81, 70.09, 70.05, 69.84, 69.60, 69.30, 69.17, 69.13, 68.31, 63.51 (triazole-CH\(_3\)HbO), 61.24, 60.50, 60.01, 59.78 (OCH\(_3\)), 54.14 (OCH\(_3\)), 50.56 (C-6_\(\alpha\)Glu), 31.49, 31.39, 30.15,

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29.70, 25.59, 25.44, 22.29 (× 2) (CH₂), 12.97 (× 2) (CH₃). ESI HRMS m/z calc’d for [C₄₉H₈₉O₂₅N₃ + H]⁺: 1120.5858; found 1120.5848.

3.22 Compound (8)

Compound 32 (24 mg, 14.0 µmol) was dissolved in anhydrous MeOH (2 mL), and a solution of NaOMe in MeOH (1.5 M, 50 µL) was added. After stirring the mixture at room temperature overnight, the reaction was neutralized with Amberlite IR-120 (H⁺). After filtration, the mixture was evaporated under reduced pressure and the crude product was purified by reversed phase column C18 Sep-Pak column using a gradient of methanol-H₂O as the eluent (0-100%) to yield the desired compound 8 which was freeze-dried (16 mg, yield: 88.4%). Rf 0.12 (Methanol : CH₂Cl₂ 4 : 6). [α]²⁵_D +58.3° (c 0.12, MeOH).

¹H NMR (600 MHz, CD₃OD): δH 8.00 (s, 1H, triazole), 4.96 (d, J=3.8 Hz, 1H, H-1_αGal_p^k), 4.78 (d, J=3.5 Hz, 1H, H-1_αGlu), 4.71 (dd, J=14.3, 2.7 Hz, 1H, H-6a_αGlu), 4.66 (s, 2H, triazole-CHaHbO + triazole-CHaHbO), 4.63 (dd, J=14.3, 7.0 Hz, 1H, H-6b_αGlu), 4.43 (d, J=7.2 Hz, 1H, H-1_βGlu_p^k), 4.35 (d, J=7.8 Hz, 1H, H-1_βGal_p^k), 4.26 (ddd, J=0.9, 5.4, 6.5 Hz, 1H, H-5_βGal_p^k), 4.02 – 3.97 (m, 2H, H-4_βGal_p^k + OCHaHb), 3.93 (dd, J=3.1, 1.0 Hz, 1H, H-4_αGal_p^k), 3.92 – 3.48 (m, 37H, H-2_αGal_p^k + H-3_αGal_p^k + H-5_αGal_p^k + H-6a_αGal_p^k + H-6b_αGal_p^k + H-3_βGal_p^k + H-6a_βGal_p^k + H-6b_βGal_p^k + H-2_βGlu_p^k + H-3_βGlu_p^k + H-4_βGlu_p^k + H-6a_βGlu_p^k + H-6b_βGlu_p^k + H-3_αGlu + H-5_αGlu + OCH₃ + OCHaHbCHcHd(OCH₂CH₂)₂O + OCHaHb + 2 × OCHaHb), 3.42 (ddd, J=2.8, 3.9, 9.4 Hz, 1H, H-5_βGlu_p^k), 3.27 (dd, J=9.0, 7.9 Hz, 1H, H-2_βGal_p^k), 3.20 (dd, J=3.6, 9.7 Hz, 1H, H-2_αGlu), 3.17 (s, 3H, OCH₃), 2.85 (dd, J=9.9, 8.8 Hz, 1H, H-4_αGlu), 1.63 –
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1.51 (m, 4H, 2 × OCH₃HbCHd + 2 × OCH₃HbCHcH₄), 1.43 – 1.24 (m, 36H, 2 × (CH₂)₉CH₃), 0.91 (t, J=7.1 Hz, 3H, (CH₂)₉CH₃), 0.90 (t, J=7.1 Hz, 3H, (CH₂)₉CH₃). ¹³C NMR (150 MHz, CD₃OD): δC 144.39 (triazole), 125.21 (triazole), 103.96 (C-1_βGlu_p⁵), 102.73 (C-1_βGal_p⁵), 101.28 (C-1_αGal_p⁵), 97.54 (C-1_αGlu), 81.39, 80.28, 80.18, 79.59, 78.36, 75.10, 75.06, 74.87, 73.39, 73.25, 73.05, 71.43, 71.22, 70.74, 70.04, 70.02, 70.01, 69.99, 69.93, 69.86, 69.62, 69.25, 69.18, 69.13, 68.25, 63.50 (triazole-CH₃HbO), 61.27, 60.48, 60.03, 59.78 (OCH₃), 54.15 (OCH₃), 50.57 (C-6_αGlu), 31.69, 30.24, 29.77, 29.44, 29.43, 29.42, 29.39, 29.31, 29.21, 29.10, 25.96, 25.83, 22.34 (× 2) (CH₂_dodecyl), 13.06 (× 2) (CH₃). ESI HRMS m/z calc’d for [C₆₁H₁₁₃O₂₅N₃ + H]⁺: 1310.7555; found: 1310.7542.

4. Toxin Binding Assays

The Stx toxin assays were performed according to established protocol.briefly, a test compound (10 μg/mL, 100 μL/well) was added to polystyrene microtiter plates, incubated overnight at room temperature then washed with PBST (5x) blocked with BSA (1% in PBS, 100 μL/well) for 1 h at room temperature and washed again with PBST (5x). Serially diluted toxin solutions (in PBS, dilution factor 3.16) was applied in triplicates and incubated for 2 h at room temperature. The plates were washed with PBST (5x) and treated with respective anti-Stx polyclonal antibodies in PBST (ATCC ascites #1794 (Stx1) or #1907 (Stx2), 1 μL/mL; 100 μL/well) for 1 h at room temperature, then washed (5x) with PBST. Horseradish peroxidase-labeled anti-mouse antibody (KPL, 0.2 mg/mL) was applied and the plates were incubated for 30 min at room temperature, then washed (5x) with PBST. A peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB) with
H$_2$O$_2$, was added. After 15 min the reaction was quenched by addition of H$_3$PO$_4$ (1M, 100 μL/well). The plates were read at 450 nm.

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\(^1\)H NMR Spectrum in CDCl\(_3\) (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in D$_2$O (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H NMR Spectrum in D$_2$O (600 MHz)
$^1$H–$^1$H GCOSY NMR Spectrum in D$_2$O (600 MHz)
ESI HRMS Spectrum of Compound 5

Counts vs. Mass-to-Charge (m/z)

+ESI Scan (rt: 1.099 min) Frag=120.0V 130419PZ5072_006.d

Counts vs. Mass-to-Charge (m/z)

+ESI Scan (rt: 1.099 min) Frag=120.0V 130419PZ5072_006.d
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1\text{H NMR Spectrum in D}_2\text{O (600 MHz)}$
ESI HRMS Spectrum of Compound 6

+ESI Scan (rt: 1.250 min) Frags=120.0V 130503PZ5071_007.d

Counts vs. Mass-to-Charge (m/z)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^{1}H^{13}C$ GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1\text{H} - ^1\text{H}$ GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^{1}H-^{1}H$ GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^{1}H-^{13}C$ GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^{1}H^{13}C$ GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
\(^{1}\text{H NMR Spectrum in CDCl}_3\) (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)

[Chemical structure and spectrum image]

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$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)

[Chemical structure diagram]

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$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1\text{H}-^1\text{H}$ GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1\text{H}-^{13}\text{C} \text{ GHSQC NMR Spectrum in CDCl}_3 (400 \text{ MHz})$
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)

![NMR Spectrum Image]

Chemical shifts:
- 98.13
- 81.50 & 80.74 & 80.52
- 73.73
- 71.89
- 70.18
- 60.91
- 55.43
- 51.64

Chemical structure:

![Chemical Structure Image]
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CDCl$_3$ (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1\text{H}^1\text{H}$ GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
\(^1\)H NMR Spectrum in CDCl\(_3\) (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CDCl$_3$ (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H-$^{13}$C GHSQC NMR Spectrum in CDCl$_3$ (400 MHz)
$^1$H NMR Spectrum in CD$_3$OD (400 MHz)
$^{13}$C (DEPTQ) NMR Spectrum in CD$_3$OD (100 MHz)
$^1$H-$^1$H GCOSY NMR Spectrum in CD$_3$OD (400 MHz)
$^1$H NMR Spectrum in D$_2$O (400 MHz)
"$^1$H NMR Spectrum in CD$_3$OD (600 MHz)"
$^{13}$C (DEPTQ) NMR Spectrum in CD$_3$OD (150 MHz)
$^{1}H-^{1}H$ GCOSY NMR Spectrum in CD$_3$OD (600 MHz)
$^{1}\text{H}^{13}\text{C}$ GHSQC NMR Spectrum in CD$_3$OD (600 MHz)
$^1$H NMR Spectrum in D$_2$O (400 MHz)