**Design, Synthesis and in vitro Evaluation of D-Glucose-Based Cationic Glycolipids for Gene Delivery**

Chengxi He*, Shang Wang#, Meiyun Liu*, Chunyan Zhao*, Shuanglin Xiang*, Youlin Zeng*

*National & Local Joint Engineering Laboratory for New Petro-chemical Materials and Fine Utilization of Resources, Hunan Normal University, Changsha, Hunan, 410081, P. R. China.

#Key Laboratory of Protein Chemistry and Developmental Biology of State Education Ministry of China, College of Life Sciences, Hunan Normal University, Changsha, Hunan, 410081, P. R. China.

Contents

- General Experimental Procedures S1
- Synthesis of lipid 2 S1
- Synthesis of lipid 3 S2
- Synthesis of lipid 5 S4
- Synthesis of lipid 6 S5
- NMR spectrums of intermediates and lipids 1 S6
- NMR spectrums of intermediates and lipids 2 S29
- NMR spectrums of intermediates and lipids 3 S41
- NMR spectrums of intermediates and lipids 4 S53
- NMR spectrums of intermediates and lipids 5 S61
- NMR spectrums of intermediates and lipids 6 S69
- Figure S1 S77
- Figure S2 S78

**General experimental procedures.** Chemicals used were reagent grade as supplied except where noted. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates; Compound spots were visualized by UV light (254 nm), or by charring with 30% sulfuric acid-w: alcohol, or by staining with iodine in silica gel. Flash column chromatography was performed on columns (16×240 mm, 18×300 mm, 35×400 mm) of silica gel 60 (200-300 Mesh) with EtOAc-petroleum ether (60-90°C) as the eluent. NMR spectra were referenced using Me$_3$Si (0 ppm), residual CHCl$_3$ (1H-NMR 7.26 ppm, $^{13}$C NMR 77.0 ppm) for CDCl$_3$, or using residual DOH (1H-NMR 4.79 ppm) for D$_2$O, or using residual CD$_2$OD (1H-NMR 4.87 ppm, $^{13}$C NMR 49.0 ppm) for CD$_3$OD. Peak assignments are based on $^1$H NMR, $^1$H-$^1$H gCOSY, $^{13}$C NMR and (or) 1H-$^{13}$C gHSQC and $^1$H-$^{13}$C gHMBC experiments. NMR experiments were conducted at 500, and 125 MHz for $^1$H, $^{13}$C, respectively, using Bruker Avance 500 MHz NMR Spectrometer equipped with a switchable QNP (1H, $^{13}$C) probe enabling back-to-back data acquisition for the different nuclei without the need to remove sample or tune the probe. Electrospray ionization mass spectrometry (ESI-MS) in positive and negative mode was performed on a Finnigan LCQ Advantage (Thermo Finnigan LCQ) equipped with an atmospheric pressure ionization (API) source.

**Synthesis of lipid 2**

$3'$-Azidopropyl 2,3-di-O-tetradecyl-4,6-isopropyldiene-$\beta$-D-glucopyranoside (15b)

NaH (3.3 g, 138.6 mmol) was added slowly to the solution of compound 14 (7.0 g, 23.1 mmol) in dry DMF (100.0 mL) and then myristyl bromide (27.5 mL, 92.4 mmol) was added dropwise. The reaction mixture was stirred until TLC (petroleum ether/ethyl acetate 2:1) showed the starting material was disappeared. The mixture was diluted with DCM (200.0 mL), washed with water for three times. The organic layer was dried by anhydrous Na$_2$SO$_4$ and concentrated to dryness. The residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 16 : 1 as the eluent to give compound 15b (6.5 g, 40.3 %) as a syrup. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) 4.30 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 3.94-3.87 (m, 2 H, OCH$_2$(CH$_2$)$_2$CHN$_3$, H-6a), 3.76-3.70 (m, 3 H, OCH$_2$(CH$_2$)$_2$CH$_2$OH, H-6b), 3.66-3.58 (m, 3 H, OCH$_2$(CH$_2$)$_2$CH$_2$N$_3$, OCH$_2$(CH$_2$)$_2$CH$_3$), 3.53 (dd, 1 H, $J_{1,2}$ = 3.4 Hz, H-2), 3.17-3.08 (m, 2 H, OCH$_2$(CH$_2$)$_2$CH$_3$), 2.74 (dd, 1 H, $J_{1,2}$ = 9.0 Hz, H-3), 3.35 (ddd, 1 H, $J_{2,3}$ = 9.0 Hz, $J_{3,6a}$ = 6.5 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a), 3.16 (dd, 1 H, $J_{1,2}$ = 7.0 Hz, H-3), 3.36 (dd, 1 H, $J_{2,3}$ = 7.5 Hz, $J_{3,6a}$ = 9.0 Hz, H-2), 1.87-1.85 (m, 2 H, OCH$_2$(CH$_2$)$_2$CH$_3$), 1.56-1.46 (m, 4 H, 2 OCH$_2$(CH$_2$)$_2$CH$_2$(CH$_3$)$_2$), 1.46 (s, 3 H, C(CH$_3$)$_2$), 1.39 (s, 3 H, C(CH$_3$)$_2$), 1.38-1.20 (m, 44 H, 2 OCH$_2$(CH$_2$)$_2$(CH$_2$)$_3$(CH$_3$)$_2$), 0.87 (t, 6 H, $J =$ 6.5 Hz, 2

*Corresponding author. Tel.:+86 139758805056; fax: +86 73188872531
E-mail address: youlinzengcn@gmail.com (Youlin Zeng).
OCH₂CH₂(CH₂)₃CH₂OH; ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 104.0 (1 C, C-1), 99.2 (1 C, C(CH₂)₃), 82.2 (1 C, C-2), 81.7 (1 C, C-3), 73.9 (1 C, C-4), 73.6 (1 C, OCH₂CH₂CH₂CH₂), 73.2 (1 C, OCH₂CH₂CH₂OCH₃), 66.9 (1 C, C-5), 66.8 (1 C, OCH₂CH₂CH₂N), 62.2 (1 C, C-6), 48.2 (1 C, OCH₂CH₂CH₂N), 31.9, 30.3, 30.2, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 26.1, 26.0, 22.7, 19.0 (25 C some signals were overlapped, 2 OCH₂CH₂CH₂OCH₃, OCH₂CH₂CH₂N), 22.7 (1 C, C(CH₂)₃), 19.0 (1 C, C(CH₂)₃), 14.1, 14.1 (2 C, 2 OCH₂CH₂CH₂CH₂).

3'-Azidopropyl 2,3-di-O-tetradecyl-β-D-glucopyranoside (16b)

The mixture of compound 15b (6.5 g, 9.3 mmol) and dry methanol (100.0 ml) was cooled to 0°C under stirring, and then the acetyl chloride (2.0 mL, 28.2 mmol) was added dropwise. The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material was disappeared, during which time the temperature was gradually raised to ambient temperature. The mixture was evaporated to dryness and the residue was purified by silica gel column chromatography with petroleum ether: EtOAc = 2 : 1 as the eluent to give compound 16b (5.0 g, 82.0 %) as a white oil. ¹H NMR (500 MHz, CDCl₃): δ (ppm) 4.30 (d, 1 H, J₃₂ = 7.5 Hz, H-1), 3.97-3.93 (m, 1 H, OCH₂CH₂CH₂N), 3.91-3.87 (m, 2 H, OCH₃(CH₂)₁₂CH₂H₆a), 3.81-3.74 (m, 2 H, OCH₃H (CH₂)₁₂CH₃, H-6b), 3.64-3.56 (m, 3 H, OCH₂CH₂CH₂CH₂N), 3.48-3.41 (m, 3 H, OCH₂CH₂CH₂N, H-4), 3.33 (dd, 1 H, J₅₆ = 9.0 Hz, J₅₆a = 4.5 Hz, J₅₆b = 4.0 Hz, H-5), 3.19 (dd, 1 H, J₃₂ = 9.0 Hz, H-3), 3.06 (dd, 1 H, J₂₃ = 9.5 Hz, J₂₃ = 9.0 Hz, H-2), 1.90-1.85 (m, 2 H, OCH₂CH₂CH₂N), 1.61-1.52 (m, 4 H, 2 OCH₂CH₂(CH₂)₁₂CH₃), 1.37-1.20 (m, 44 H, 2 OCH₂CH₂(CH₂)₁₂H, 0.87 (t, 6 H, J = 3.0 Hz, 2 OCH₂CH₂(CH₂)₁₂CH₃); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 103.7 (1 C, C-1), 84.2 (1 C, C-3), 82.1 (1 C, C-2), 74.8 (1 C, C-5), 73.6 (1 C, OCH₂CH₂CH₂CH₂), 73.0 (1 C, OCH₂CH₂CH₂CH₃), 70.4 (1 C, C-4), 66.5 (1 C, OCH₂CH₂CH₂N), 62.8 (1 C, C-6), 48.2 (1 C, OCH₂CH₂CH₂N), 31.9, 30.4, 30.3, 29.7, 29.6, 29.5, 29.3, 29.2, 26.2, 26.1, 22.7 (25 C some signals were overlapped, 2 OCH₂CH₂CH₂CH₂N, 14.1, 14.1 (2 C, 2 OCH₂CH₂CH₂CH₂).

3'- (N,N-Dimethylamino)propyl 2,3-di-O-tetradecyl-β-D-glucopyranoside (17b)

The mixture of compound 16b (1.0 g, 1.5 mmol) and formaldehyde (36 %, 1.0 mL, 12.0 mmol), Pd/C (5 %, 300 mg), methanol (60.0 mL) was stirred at the room temperature under H₂ atmosphere. The reaction mixture was stirring until TLC (ethanol : methanol = 5 : 2) showed the starting material was disappeared. The mixture was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography with EtOAc : methanol = 5 : 1 as the eluent to give compound 17b (0.5 g, 40.6 %) as a white oil.

3'- (N,N,N-Trimethylamininium iodine)propyl 2,3-di-O-tetradecyl-β-D-glucopyranoside (18b, lipid 2)

The mixture of compound 17b (300.0 mg, 0.46 mmol) and iodomethane (112.0 µL, 1.8 mmol), THF (3.0 ml) was stirring at the room temperature until TLC (ethyl acetate : methanol = 2 : 1) showed the starting material was disappeared. The reaction mixture was cooled with ice bath and a solid was precipitated. The mixture was filtered, and the filter cake was washed with acetone (5.0 mL × 3) and dried by vacuum to give white solid 18b (220.0 mg, 59.5 %). ¹H NMR (500 MHz, CDCl₃): δ (ppm) 4.31 (d, 1 H, J₁₂ = 7.5 Hz, H-1), 3.91-3.78 (m, 5 H, OCH₂CH₂CH₂H(N(CH₃)₂), OCH₂CH₂CH₂N(CH₃)₂, OCH₂CH₂CH₂N(CH₃)₂, H-6a), 3.77-3.65 (m, 4 H, OCH₂CH₂(CH₂)₁₂CH₃, OCH₂CH₂CH₂H(N(CH₃)₂), H-6b), 3.58-3.54 (m, 1 H, OCH₂CH₂CH₂(CH₃)₂), 3.46-3.32 (m, 11 H, OCH₂CH₂CH₂N(CH₃)₂, H-4, H-5), 3.19 (dd, 1 H, J₁₂ = 9.0 Hz, H-3), 3.10 (s, 1 H, OCH₂CH₂CH₂H(N(CH₃)₂), 3.01 (dd, 1 H, J₁₂ = 7.5 Hz, J₂₃ = 9.0 Hz, H-2), 2.22-2.20 (m, 1 H, OCH₂CH₂CH₂H(N(CH₃)₂), 2.06-2.00 (m, 1 H, OCH₂CH₂CH₂H(N(CH₃)₂), 1.60-1.50 (m, 4 H, 2 OCH₂CH₂(CH₂)₁₂H), 1.37-1.20 (m, 44 H, 2 OCH₂CH₂(CH₂)₁₂CH₃), 0.87 (t, 6 H, J = 7.0 Hz, 2 OCH₂CH₂(CH₂)₁₂CH₃); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 103.9 (1 C, C-1), 84.2 (1 C, C-3), 81.9 (1 C, C-2), 75.6 (1 C, C-5), 73.6 (1 C, OCH₂CH₂CH₂CH₂), 73.0 (1 C, OCH₂CH₂CH₂CH₃), 70.0 (1 C, C-4), 66.5 (1 C, OCH₂CH₂CH₂N(CH₃)₂), 64.8 (1 C, OCH₂CH₂CH₂N(CH₃)₂), 61.6 (1 C, C-6), 53.9, 53.9, 53.9 (3 C, OCH₂CH₂CH₂N(CH₃)₂), 31.8, 30.4, 29.7, 29.6, 29.3, 26.2, 26.1, 24.4, 22.7 (25 C some signals were overlapped, 2 OCH₂CH₂(CH₂)₁₂CH₃, OCH₂CH₂CH₂N(CH₃)₂), 14.0, 14.0 (2 C, 2 OCH₂CH₂(CH₂)₁₂CH₃). ESI-MS: m/z = 673.0, in agreement with the calculated mass for [M]⁺ = C₄₀H₆₂N₂O₆⁺.
Synthesis of lipid 3

3'-Azidopropyl 2,3-di-O-hexadecyl-4,6-O-isoproplidene-β-D-glucopyranoside (15c)

NaH (4.0 g, 99.6 mmol) was added slowly to the solution of compound 14 (5.0 g, 16.6 mmol) in dry DMF (200.0 mL) and then 1-bromohexadecane (203.0 mL, 66.4 mmol) was added dropwise. The reaction mixture was stirring at 50°C until TLC (petroleum ether/ethyl acetate 2:1) showed the starting material was disappeared. The mixture was diluted with DCM (200.0 mL), washed with water for three times. The organic layer was dried by anhydrous Na₂SO₄ and concentrated to dryness. The residue was purified by silica gel column chromatography with petroleum ether:EtOAc = 16 : 1 as the eluent to give compound 15c (10.0 g, 80.1%) as a white oil.

3'-Azidopropyl 2,3-di-O-hexadecyl-β-D-glucopyranoside (16c)

The mixture of compound 15c (10.0 g, 13.3 mmol) and dry methanol (100.0 mL) was cooled to 0°C under stirring, and then the acetyl chloride (4.2 mL, 53.2 mmol) was added dropwise. The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material was disappeared, during which time the temperature was gradually raised to ambient temperature. The mixture was evaporated to dryness and the residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 2 : 1 as the eluent to give compound 16c (9.0 g, 95.0%) as a white oil. ¹H NMR (500 MHz, CDCl₃): δ (ppm) 4.31 (d, 1 H, J₁₂ = 7.5 Hz, H-1), 3.97-3.93 (m, 2 H, OCH₂CH₂CH₂H₂N₃), 3.90-3.88 (m, 2 H, OCH₂CH₂CH₂H₂N₃), 3.81-3.73 (m, 14 H, C(CH₃)₂), 3.48-3.40 (m, 6 H, OCH₂CH₂CH₂N₃), 3.39-3.37 (m, 19 H, C(CH₃)₂), 3.22-3.20 (m, 1 H, J₁₂ = 7.5 Hz, H-1), 1.38-1.30 (m, 2 H, OCH₂CH₂CH₂CH₃), 0.87 (t, 6 H, J = 6.5 Hz, 2 OCH₃CH₂CH₂CH₃); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 104.0 (1 C, C-1), 99.2 (1 C, C(CH₃)₂), 82.2 (1 C, C-2), 81.7 (1 C, C-3), 73.9 (1 C, C-4), 73.6 (1 C, OCH₂CH₂CH₂CH₃), 73.1 (1 C, OCH₂CH₂CH₂CH₃), 67.0 (1 C, C-5), 66.8 (1 C, OCH₂CH₂CH₂CH₃), 62.3 (1 C, C-6), 48.2 (1 C, OCH₂CH₂CH₂CH₃), 31.9, 30.3, 30.2, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 26.1, 22.7, 19.0 (29 C some signals were overlapped, 2 OCH₂CH₂CH₂CH₃, OCH₂CH₂CH₂CH₃), 26.0 (1 C, C(CH₃)₂), 19.1 (1 C, C(CH₃)₂), 14.1, 14.1 (2 C, 2 OCH₂CH₂CH₃)

3'- (N,N-Dimethylamino)propyl 2,3-di-O-hexadecyl-β-D-glucopyranoside (17c)

The mixture of compound 16c (1.5 g, 2.1 mmol) and formaldehyde (36%, 1.3 mL, 16.8 mmol), Pd/C (5%, 450 mg), methanol (60.0 mL) was stirred at the room temperature under H₂ atmosphere. The reaction mixture was stirring until TLC (ethyl acetate : methanol = 5 : 2) showed the starting material was disappeared. The mixture was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography with EtOAc : methanol = 5 : 1 as the eluent to give compound 17c (0.6 g, 40.0%) as a white oil.

3'(N,N,N-Trimethylaminonium iodine)propyl 2,3-di-O-hexadecyl-β-D-glucopyranoside (18c, lipid 3)

The mixture of compound 17c (250.0 mg, 0.35 mmol) and iodomethane (86.8 µL, 1.4 mmol), THF (3.0 mL) was stirring at the room temperature until TLC (ethyl acetate : methanol = 2 : 1)
showed the starting material was disappeared. The reaction mixture was cooled with ice bath and a solid was precipitated. The mixture was filtered, and the filter cake was washed with ace tone (5.0 mL \times 3) and dried by vacuum to give white solid 18c (170.0 mg, 56.7%). ^1H NMR (500 MHz, CDCl3): δ (ppm) 4.31 (d, 1 H, J1,2 = 7.5 Hz, H-1), 3.91-3.82 (m, 3 H, OCH2CH2CH2N(CH3)3), H-(6a), 3.80-3.77 (m, 2 H, OCH2CH2CH2N(CH3)3, OCH2CH2CH2N(CH3)3), 3.75-3.66 (m, 4 H, OCH2CH2CH2N(CH3)3, OCH2CH2CH2N(CH3)3, H-6b), 3.58-3.54 (m, 1 H, OCH2CH2CH2N(CH3)3, 3.45-3.32 (m, 11 H, OCH2CH2CH2N(CH3)3, H-4, H-5), 3.19 (dd, 1 H, J1,2 = J3,4 = 9.0 Hz, H-3), 3.03 (dd, 1 H, J2,3 = 7.5 Hz, J2,3 = 9.0 Hz, H-2), 2.28-2.18 (m, 1 H, OCH2CH2CH2N(CH3)3), 2.09-2.00 (m, 1 H, OCH2CH2CH2N(CH3)3), 1.60-1.50 (m, 4 H, 2 OCH2CH2CH2N(CH3)3), 1.37-1.20 (m, 52 H, 2 OCH2CH2CH2N(CH3)3), 0.87 (t, 6 H, J = 7.0 Hz, 2 OCH3CH2CH2N(CH3)3); ^13C NMR (125 MHz, CDCl3): δ (ppm) 104.0 (1 C, C-1), 84.2 (1 C, C-3), 82.0 (1 C, C-2), 75.7 (1 C, C-5), 73.6 (1 C, OCH2CH2CH2N(CH3)3), 73.0 (1 C, OCH2CH2CH2N(CH3)3), 70.1 (1 C, C-4), 66.6 (1 C, OCH2CH2CH2N(CH3)3), 64.9 (1 C, OCH2CH2CH2N(CH3)3), 61.6 (1 C, C-6), 53.9, 53.9, 53.9 (3 C, OCH2CH2CH2N(CH3)3), 31.9, 30.4, 29.7, 29.6, 29.3, 26.2, 26.1, 24.5, 22.7 (29 C some signals were overlapped, 2 OCH2CH2CH2N(CH3)3, OCH2CH2CH2N(CH3)3), 14.1, 14.1 (2 C, 2 OCH2CH2CH2N(CH3)3). ESI-MS: m/z = 729.0, in agreement with the calculated mass for [M]+ = C44H90NO6+. 

**Synthesis of lipid 5**

**3’-[(N,N-di-tetradecy lamino)-propyl-β-D-glucopyranoside (20b)**

The mixture of compound 19 (1.9 g, 8.0 mmol), myristyl bromide (8.9 g, 32.0 mmol), anhydrous K2CO3 (2.2 g, 16.0 mmol), CH3OH (20.0 mL), CH3CH2OH (20.0 mL) was refluxed at 75°C until TLC (methanol) showed the starting material was disappeared. The mixture was diluted with DCM (30.0 mL), washed with water for two times. The organic layer was dried by anhydrous Na2SO4 and concentrated to dryness. The residue was purified by silica gel column chromatography with EtOAc : methanol = 4 : 1 as the eluent to give compound 20b (1.6 g, 32.9 %) as a syrup. ^1H NMR (500 MHz, CDCl3): δ (ppm) 4.30 (d, 1 H, J1,2 = 7.5 Hz, H-1), 3.93-3.87 (m, 1 H, OCH2CH2CH2N(CH3)3), 3.82-3.77 (m, 2 H, H-6b), 3.64-3.50 (m, 3 H, OCH2CH2CH2N(CH3)3), 3.30-3.25 (m, 1 H, H-5), 2.78-2.65 (m, 2 H, OCH2CH2CH2N(CH3)3), 2.57 (t, J = 6.5 Hz, 4 H, N(CH2CH2CH2N(CH3)3), 1.85-1.80 (m, 2 H, OCH2CH2CH2N(CH3)3), 1.50-1.40 (m, 4 H, N(CH2CH2CH2N(CH3)3), 1.37-1.21 (m, 44 H, N(CH2CH2CH2N(CH3)3)), 0.89 (t, 6 H, J = 7.0 Hz, N(CH2CH2CH2N(CH3)3)); ^13C NMR (125 MHz, CDCl3): δ (ppm) 103.1 (1 C, C-1), 76.6 (1 C, C-3), 76.0 (1 C, C-5), 73.5 (1 C, C-2), 70.0 (1 C, C-4), 68.2 (1 C, OCH2CH2CH2N(CH2CH2CH2N(CH3)3), 66.0 (1 C, C-6), 52.8, 52.8 (2 C, N(CH2CH2CH2N(CH3)3), 50.7 (1 C, OCH2CH2CH2N(CH2CH2CH2N(CH3)3), 31.9, 29.7, 29.6, 29.5, 29.3, 27.4, 26.3, 24.9, 22.6 (25 C some signals were overlapped, N(CH2CH2CH2N(CH3)3), OCH2CH2CH2N(CH2CH2CH2N(CH3)3), 14.1, 14.1 (2 C, N(CH2CH2CH2N(CH3)3)).

**3’-[(N,N-di-tetradecylamino)-N-methyl]-propyl-β-D-glucopyranoside (21b, lipid5)**

The mixture of compound 20b (220.0 mg, 0.32 mmol) and iodomethane (180.0 mg, 1.28 mmol, 79.0 µL), THF (5.0 ml) was stirring at the room temperature until TLC (ethyl acetate : methanol = 5 : 1) showed the starting material was disappeared. The reaction mixture was evaporated to dryness and a solid was precipitated when the acetone (10.0 mL) was drop to the syrup. The mixture was filtered, and the filter cake was washed with acetone (5.0 mL \times 3) and dried by vacuum to give white solid 21b (160.0 mg, 59.2%). ^1H NMR (500 MHz, CDCl3): δ (ppm) 5.11 (s, 1 H, OH), 4.89 (s, 2 H, 2 OH), 4.44 (d, 1 H, J1,2 = 7.5 Hz, H-1), 4.10-4.00 (m, 2 H, OH, OCH2CH2CH2N(CH3)3), 3.84-3.73 (m, 3 H, H-6, OCH2CH2CH2N(CH3)3, C13H30), 3.74-3.50 (m, 4 H, H-3, H-4, OCH2CH2CH2N(CH3)3, C14H29), 3.44-3.28 (m, 6 H, H-2, H-5, CH3N(CH2CH2CH2C11H22), 3.20 (s, 3 H, CH3N(CH2CH2CH2C11H22), 2.20-2.10 (m, 2 H, OCH2CH2CH2N(CH3)3, C14H29), 1.72-1.60 (m, 2 H, CH3N(CH2CH2CH2C11H22)),1.40-1.20 (m, 44 H, CH3N(CH2CH2CH2C11H22)), 0.85 (t, 6 H, J = 6.5 Hz, CH3N(CH2CH2CH2C11H22)); ^13C NMR (125 MHz, CDCl3): δ (ppm) 102.7 (1 C, C-1), 76.2 (1 C, C-3), 75.9 (1 C, C-5), 73.2 (1 C, C-2), 69.8 (1 C, C-4), 66.2 (1 C, OCH2CH2CH2N(CH3)3, C14H29), 61.2, 61.2, 61.2 (3 C, OCH2CH2CH2N(CH3)3, C11H22)
CH$_3$)$_2$), 60.8 (1 C, C-6), 49.4 (1 C, (CH$_3$)$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 26.3, 23.5, 22.5 (25 C, some signals were overlapped, (CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$, OCH$_2$CH$_2$CH$_2$N(CH$_3$(C$_{12}$H$_{25}$))$_2$, 14, 14.0 (2 C, (CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$). ESI-MS: $m/z$ = 644.8, in agreement with the calculated mass for [M]$^+$ = C$_{38}$H$_{78}$NO$_6^+$. 

Synthesis of lipid 6

3'-[N,N-di-hexadecylamino]-propyl-β-D-glucopyranoside (20c)

The mixture of compound 20b (1.2 g, 5.1 mmol), anhydrous K$_2$CO$_3$ (1.4 g, 10.2 mmol), CH$_3$OH (20.0 mL), CH$_3$CH$_2$OH (20.0 mL) was refluxed at 75°C until TLC (methanol) showed the starting material was disappeared. The mixture was diluted with DCM (30.0 mL), washed with water for two times. The organic layer was dried by anhydrous Na$_2$SO$_4$ and concentrated to dryness. The residue was purified by silica gel column chromatography with EtOAc : methanol = 4 : 1 as the eluent to give compound 20c (1.1 g, 32.4 %) as a syrup.

$^1$H NMR (500 MHz, CDCl$_3$): δ (ppm): 4.30 (d, 1 H, J$_{1,2}$ = 7.0 Hz, H-1), 3.93-3.87 (m, 1 H, OCH/HCH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 3.86-3.78 (m, 2 H, H-6), 3.60-3.51 (m, 3 H, OCH/HCH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$, 3.30-3.25 (m, 1 H, H-5), 2.78-2.57 (m, 2 H, OCH$_2$CH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 2.57 (t, J = 6.5 Hz, 4 H, N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 1.83-1.73 (m, 2 H, OCH$_2$CH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 1.50-1.40 (m, 4 H, N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 1.26-1.14 (m, 3 H, CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$). 13C NMR (125 MHz, CDCl$_3$): δ (ppm): 103.1 (1 C, C-1), 76.5 (1 C, C-3), 76.0 (1 C, C-5), 73.5 (1 C, C-2), 70.0 (1 C, C-4), 68.4 (1 C, OCH$_2$CH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 61.6 (1 C, C-6), 53.1, 31.9, 29.7, 29.6, 29.5, 29.3, 27.6, 26.7, 25.3, 22.7 (29 C, some signals were overlapped, N(CH$_2$(C$_{14}$H$_{29}$)CH$_2$)$_2$, OCH$_2$CH$_2$CH$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 14.1, 14.1 (2 C, N(CH$_2$(C$_{14}$H$_{29}$)CH$_2$)$_2$).

3'-[N,N-di-hexadecyl-N-methylammonium iodine]-propyl-β-D-glucopyranoside (21c, lipid6)

The mixture of compound 20c (200.0 mg, 0.3 mmol) and iodomethane (170.0 mg, 1.2 mmol, 75.0 µL) THF (5.0 mL) was stirring at the room temperature until TLC (ethyl acetate : methanol = 5 : 1) showed the starting material was disappeared. The reaction mixture was evaporated to dryness and a solid was precipitated when the acetone (10.0 mL) was drop to the syrup. The mixture was filtered, and the filter cake was washed with acetone (5.0 mL × 3) and dried by vacuum to give white solid 21c (150.0 mg, 60.4%). $^1$H NMR (500 MHz, CDCl$_3$): δ (ppm): 4.33 (d, 1 H, J$_{1,2}$ = 7.5 Hz, H-1), 4.03-3.97 (m, 1 H, OCH/HCH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$)), 3.93-3.90 (m, 1 H, H-6), 3.82-3.75 (m, 1 H, OCH/HCH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$))), 3.73-3.66 (m, 1 H, H-6b), 3.35-3.49 (m, 2 H, OCH$_2$CH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$))), 3.43-3.26 (m, 7 H, OCH$_2$CH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$))CH$_2$C$_{12}$H$_{25}$(C$_{12}$H$_{25}$)CH$_2$), 3.22 (dd, 1 H, J$_{2,1}$ = 7.5 Hz, J$_{2,3}$ = 4.0 Hz, H-2), 3.09 (s, 3 H, (CH$_3$)$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 2.14-2.05 (m, 2 H, OCH$_2$CH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$))), 1.82-1.75 (m, 4 H, (CH$_3$)$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 1.50-1.20 (m, 1 H, (CH$_3$)$_2$N(CH$_2$CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$), 0.93 (t, 6 H, J = 6.5 Hz, (CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{25}$)CH$_2$)$_2$). 13C NMR (125 MHz, CDCl$_3$): δ (ppm): 103.0 (1 C, C-1), 76.6, 76.6 (2 C, C-3, C-5), 73.6 (1 C, C-2), 70.0 (1 C, C-4), 65.9 (1 C, OCH$_2$CH$_2$CH$_2$N(CH$_2$(C$_{14}$H$_{29}$))), 61.4, 61.4, (2 C, N(CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{25}$)CH$_2$)), 61.3 (1 C, C-6), 59.4 (1 C, OCH$_2$CH$_2$CH$_2$N(CH$_3$)(C$_{14}$H$_{29}$)), 48.1 (1 C, (CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{25}$))CH$_2$), 31.6, 29.3, 29.2, 29.1, 28.7, 26.0, 22.8, 22.3, 21.7 (29 C, some signals were overlapped, (CH$_3$)$_2$N(CH$_2$(C$_{14}$H$_{28}$)CH$_2$)$_2$, OCH$_2$CH$_2$CH$_2$N(CH$_3$)(C$_{14}$H$_{29}$)), 13.0, 13.0 (2 C, (CH$_3$)$_2$N(CH$_2$(C$_{12}$H$_{28}$)CH$_2$)$_2$). ESI-MS: $m/z$ = 700.9, in agreement with the calculated mass for [M]$^+$ = C$_{42}$H$_{84}$NO$_6^+$. 

S5
$^1$H-$^1$H COSY (CDCl$_3$, 500Hz)

AcO

OAc

O

Cl

OAc
$^1$H NMR (CDCl₃, 500 Hz)

\[ \text{AcO} \rightarrow O \rightarrow \text{N₃} \]

S10
$^{13}$C NMR (CDCl$_3$, 125 Hz)

H$_3$C
H$_3$C
O
O
N$_3$

OH

OH
$^1$H$^{13}$C HSQC (CDCl$_3$, 500Hz)

H$_3$C

H$_3$C

O

O

O

O

N$_3$

H

O

O

N$_3$
$\text{H}_3\text{C}^1\text{H NMR (CDCl}_3, 500 \text{ Hz)}$

$\text{H}_3\text{C}$

$\text{O}$

$\text{O}$

$\text{O}$

$\text{N}_3$

$\text{R=C}_1\text{H}_{25}^n$
$\text{H}_3\text{C}\text{ }{^1}\text{H}-{^1}\text{H COSY (CDCl}_3, 500\text{Hz)}$

$\text{H}_3\text{C}$

$\begin{align*}
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{N}_3 \\
\text{RO} & \quad \text{OR} & \quad \text{OR} & \quad \text{R} = \text{C}_{12}\text{H}_{25-H}
\end{align*}$
$R = \text{C}_{12}\text{H}_{25} - n$
$^1$H NMR (CDCl$_3$, 500 Hz)

\[ \text{R} = \text{C}_{12}\text{H}_{25-n} \]
$^{1}H^{1}H$ COSY (CDCl$_3$, 500Hz)

$\text{O} \hspace{1cm} \text{O}$

$\text{H}$

$\text{C}$

$\text{N}_3$

$\text{O} \hspace{1cm} \text{O} \hspace{1cm} \text{N}_3$

$\text{R} = \text{C}_{12} \text{H}_{25-n}$
$^{1}{H}^{12}{C}$ HSQC (CDCl$_3$, 500Hz)

$R = C_{12}H_{25-n}$
\( ^{13}\text{C NMR (CDCl}_3, 125 \text{ Hz}) \)

\[
\begin{array}{cccc}
\text{HO} & \text{O} & \text{O} & \text{H}_3\text{C} \\
\text{RO} & \text{OR} & \text{N} & \text{CH}_3 \\
\text{R=C}_{12}\text{H}_{25-n} \\
\end{array}
\]
$^1$H-$^{13}$C HSQC (CDCl$_3$, 500Hz)

H$_2$C

R = C$_{12}$H$_{25}$n

\[
R = \text{C}_{12}\text{H}_{25}n
\]
The document contains an NMR spectrum of compound with assigned chemical shifts. The structure is shown below the spectrum.
$R = C_{14}H_{29-n}$
$^{1}H$ NMR (CDCl$_3$, 500 Hz)

R$=\text{C}_{14}\text{H}_{29}^{n}$
$^{13}$C NMR (CDCl$_3$, 125 Hz)

$\text{R} = \text{C}_{14}\text{H}_{29-n}$
$^{1}H-^{1}H$ COSY (CDCl$_3$, 500Hz)

$R = C_{14}H_{29-n}$
$^1$H-$^1$C HSQC (CDCl$_3$, 500Hz)

R = C$_{14}$H$_{29-n}$
\[ R = C_{14}H_{25-n} \]
$^{13}$C NMR (CDCl$_3$, 125 Hz)

$R = C_{14}H_{29-n}$
$^{1}H-^{1}H$ COSY (CDCl$_{3}$, 500Hz)

$R = C_{14}H_{29-π}$

$\begin{align*}
\text{HO} & \text{O} \\
\text{H$_3$C} & \text{CH$_3$} \\
\text{OR} & \text{OR} \\
\text{N} & \text{CH$_3$} \\
\end{align*}$
\[ ^1H-^13C \text{ HSQC (CDCl}_3, 500Hz) \]

\[ \text{HO} \quad \text{O} \quad \text{OR} \quad \text{H}_3\text{C-CH}_3 \quad \text{N-CH}_3 \quad \text{OR} \quad \text{R-C}_{14}\text{H}_{29-4n} \]

\[ \text{ppm} \]

\[ 5.0 \ 4.5 \ 4.0 \ 3.5 \ 3.0 \ 2.5 \ 2.0 \ 1.5 \ 1.0 \ 0.5 \]

\[ 110 \ 100 \ 90 \ 80 \ 70 \ 60 \ 50 \ 40 \ 30 \ 20 \ 10 \]

S40
$\text{H}_3C\quad^{13}\text{C NMR (CDCl}_3\text{, 125 Hz)}$

$\text{H}_3C$

$\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{RO} \\
\text{OR} \\
\text{R=CsH}_{33-n}
\end{array}$

$\text{H}_3C\quad$
\[ \text{H}_3\text{C} - \text{H}^1\text{C} - \text{HSQC (CDCl}_3, 500\text{Hz)} \]

\[
\begin{align*}
\text{OR} & \quad \text{OR} \\
\text{RO} & \quad \text{O} \\
\text{N}_3 & \quad \text{R=CsH}_{13-\pi}
\end{align*}
\]
$^1$H NMR (CDCl$_3$, 500 Hz)

R = C$_{10}$H$_{13}$-$n$
$^{13}$C NMR (CDCl$_3$, 125 Hz)

R=C$_{16}$H$_{33}$-

$$\text{HO} \quad \text{O} \quad \text{O} \quad \text{N}_3 \text{ OR} \quad \text{OR}$$
$^1$H-$^1$H COSY (CDCl$_3$, 500Hz)

$R = C_{10}H_{33}^{-\pi}$
$^3$H-$^1$C HSQC (CDCl$_3$, 500Hz)

\[
\begin{align*}
\text{RO} & \quad \text{OR} \\
\text{HO} & \quad \text{O} \\
\text{HO} & \quad \text{O} \\
\text{N}_3 & \quad \text{OR} \\
R & = \text{C}_{16}\text{H}_{33-n}
\end{align*}
\]
$R = C_{16}H_{13-\Pi}$

$^{1}H$ NMR (CDCl$_3$, 500 Hz)

![NMR Spectrogram](image)

- Resonances at various ppm values corresponding to different chemical shifts.
\[ ^1H \text{NMR (CDCl}_3, \text{ 500 Hz)} \]
$^{1}H$-$^{13}C$ HSQC (CDCl$_3$, 500Hz)
$^{13}$C NMR (CDCl$_3$, 125 Hz)

- OH
- O
- O
- C$_{12}$H$_{25}$
- N$_3$-C$_{12}$H$_{25}$
- CH$_3$
$^{13}$C NMR (CDCl$_3$, 125 Hz)

$\text{HO} - \text{C}_2\text{H}_5\text{N}^+\text{C}_{14}\text{H}_{29}$

$\text{HO}$

$\text{HO}$

$\text{OH}$

$\text{OH}$
$^{1}H^{1}H$ COSY (CDCl$_3$, 500Hz)

\begin{align*}
\text{HO} & \quad \text{O} \\
\text{HO} & \quad \text{O} \\
\text{N} & \quad \text{C}_{14}H_{29} \\
\text{C}_{14}H_{29} & \quad \text{OH}
\end{align*}
$^{1}H$ NMR (CDCl$_3$, 500 Hz)
$^{13}$C NMR (CDCl$_3$, 125 Hz)

\[
\text{HO} - \text{O} - \text{O} - \text{N} - \text{C}_16\text{H}_{33}
\]
$^1$H-13C HSQC (CDCl$_3$, 500Hz)
Fig. S1. The fluorescence microscope images of HEK293 cells transfected by glycolipid/DNA complexes in the other four levels of N/P ratios.
<table>
<thead>
<tr>
<th>Lipopolymer</th>
<th>N/P=2</th>
<th>N/P=4</th>
<th>N/P=6</th>
<th>N/P=8</th>
<th>N/P=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Transfection efficiency of lipid 2/DNA complexes in Hela cells under different N/P ratios.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Transfection efficiency of lipid 3/DNA complexes in Hela cells under different N/P ratios.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Transfection efficiency of lipid 5/DNA complexes in HepG2 cells under different N/P ratios.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d) Transfection efficiency of lipid 6/DNA complexes in cells under different N/P ratios.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e) Transfection efficiency of lipid 6/DNA complexes in SW480 cells under different N/P ratios.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. S2. The fluorescence microscope images of transfected by glycolipid/DNA complexes in Hela, HepG2 and SW480 cells.