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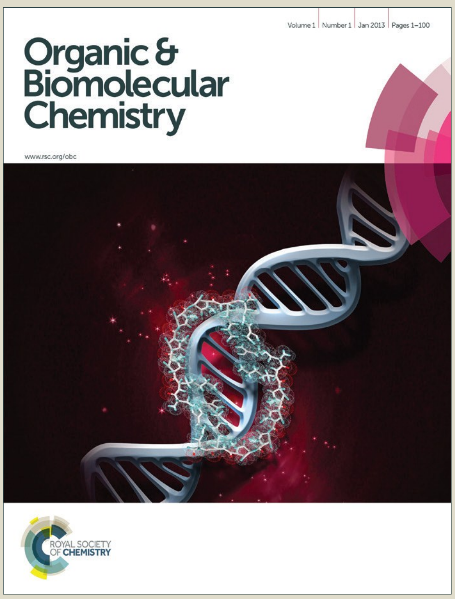

Design, Synthesis and *in vitro* Evaluation of D-Glucose-Based Cationic Glycolipids for Gene Delivery

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Abstract

The cationic lipid consists of hydrophilic headgroup, backbone and hydrophobic tails which have an immense influence on the transfection efficiency of the lipid. In this paper, two novel series of cationic cyclic glycolipids with quaternary ammonium headgroup and different-length hydrophobic tails (dodecyl, tetradecyl, hexadecyl) have been designed and synthesized for gene delivery. One contains lipids **1-3** with two hydrophobic alkyl chains linked to glucose cycle directly in ether. The other contains lipids **4-6** with two hydrophobic chains in the positively charged nitrogen atoms. All of the lipids were characterized for their ability to bind to DNA, size, ζ-potential, and toxicity. The atomic force microscope showed that the lipids and DNA-lipid complexes were sphere-like forms. The lipids were used to transfer enhanced green fluorescent protein (EGFP-C3) to HEK293 cells without helper lipid, the results indicated that lipids **4-6** have better transfection efficiency, especially the lipids **5-6** have similar or better efficiency, compared with commercial transfection reagent lipofectamine 2000.

1. Introduction

It has been proved that gene therapy has shown a prodigious potential to the treatment of a variety of human diseases such as cancer, AIDS, viral infection, cardiovascular [1-4] and retinal disease [5-7]. However, one of the most important requirements for gene therapy is to develop a safe and efficient gene delivery system. Viral and non-viral vectors were developed as two major types of carriers used *in vitro* and *in vivo* gene transfection. Viral vectors comprise retroviruses [8-10], adenoviruses [11-12] and adeno-associated [13]. Viruses have shown high transfection efficiency and have been applied to many clinical trials, but their safety has always been worried. In addition, viral vectors have a low insert-size limit for the therapeutic genes they can pack inside [14]. Consequently, increasing attention has been focused on non-viral vectors, including cationic lipids [15], polymers [16-17], dendrimers [18-20] and nanoparticles [21]. However, unlike viral analogues, the non-viral vectors have not evolved means to overcome cellular barriers and immune defense mechanisms. The vitro cellular barriers for cellular uptake contain (a) adsorption of the lipid-DNA complex to the cell surface [22]; (b) uptake of the complex, mainly by endocytosis into the cells [23-24]; (c) release of DNA from the endosomes [25]; (d) the half-life of DNA in the cytoplasm [26]; and (e) delivery of the DNA into the nucleus [27]. Because of the impedance of numerous extracellular and intracellular obstacles, non-viral gene carriers consistently exhibit significantly reduced transfection efficiency. However, biocompatibility and potentiality of large-scale substances binding make these non-virus vectors increasingly attractive for gene therapy. Among these arsenals of non-viral transfection vectors, cationic lipids hold promise for: (a) their

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reproducibility and simplicity in preparation, (b) their non-immunogenic nature and (c) their efficiency in forming stable injectable complexes even with large DNA [28].

Since the first cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium (DOTMA) was synthetized and applied to gene transfection as novel non-viral reagents reported by Felgner et al. in 1987 [29], a large number of cationic lipids have been synthesized and investigated, including cholesterol-based lipids [30-31], amino acids-based lipids [32-34], glycerol based lipids [35-36], tocopherol based lipids [37-38], and sugar based lipids [39-43]. In recent years, it has become more and more active to develop novel cationic glycolipids as gene transfection reagent and investigate their capacity of deliver nucleic acids into the cells. Previous studies have focused mainly on the relationship between transfection efficiency and structure of cationic glycolipids. Primarily, R. Banerjee, et al. prepared and evaluated open-cycle D-xylose and D-arabinose-based glycolipids with quaternary ammonium cationic headgroup, and have found that additional hydroxyl groups attached to the hydrophilic terminal of the cationic glycolipids can improve the transfection efficiency and decrease the cell cytotoxicity [39]. Posteriorly, Mahidhar V. Y. and coworkers discovered that the transfection efficiency of the open-form galactosylated cationic glycolipids was strikingly dependent on the spacer-arm between the open-form galactose and the positively charged nitrogen atom in the headgroup region [40]. Similarly, Mukthavaram R., et al. synthesized two series of cationic glycolipids with cyclic and open D-galactose heads containing varying spacer-arm lengths between the sugar and positively charged nitrogen atoms, and it was indicated that cationic glycolipids with cyclic sugar-head require longer spacer arms than their acyclic sugar-head counterparts for efficient gene transfection [41]. Then, Maslov M. A. synthesized novel cationic cholesteryl glucosides with different heterocyclic headgroups, and the outcomes revealed that lipids/DNA complexes contained more fingerprint structures possessing higher transfection activity both in the presence and in the absence of serum [42]. Apart from the monosaccharide-based cationic glycolipids mentioned above, polysaccharide-based cationic lipids were developed for nucleic acid delivery. Eliz Amar-Lewis furnished starch-based lipids and applied them to delivery siRNA to NAR cells. The results reflected that these lipids showed high cell uptake during a 24-hour study, which also suggested that intracellular siRNA delivery barriers governed the kinetics of siRNA transfection [17]. Chae S. Y., et al. developed deoxycholic acid-conjugated chitosan oligosaccharide nanoparticles for transferring plasmid DNA to Hek 293 cells with high efficiency and low cytotoxicity. More than 20 and 100 times enhanced gene transfections were observed by these nanoparticles mediated gene delivery than that of polylysine (PLL) in the absence and presence of FBS, respectively [43]. The results from literatures [39-43] indicated that the sugar-based cationic glycolipids have high transfection efficiency, low toxicity and higher biodegradability. Besides those merits reminded above, glycolipids can deliver gene to target site by lectin-carbohydrate interactions [44-47]. Mariana Magalhães and coworkers developed a glycolipid-based nanosystem incorporating a galactose, which can promote the nanosystem interaction with the asialoglycoprotein receptor (ASGP-R) and finally increase the gene delivery efficiency and specificity to HepG2 cells (human HCC) [44]. Based on similar principles, Ivanova E. A. et al. furnished a bivalent galactose-containing neutral lipid system for targeted gene delivery system to the hepatocytes, this lipid system showed high agglutination of cationic liposomes by ricinus communis lectin (RCA120). Furthermore, lots of glycolipid-based targeting gene delivery systems and the type of targeted cells determined by carbohydrate terminal of glycolipid were comprehensive reviewed [46-47].

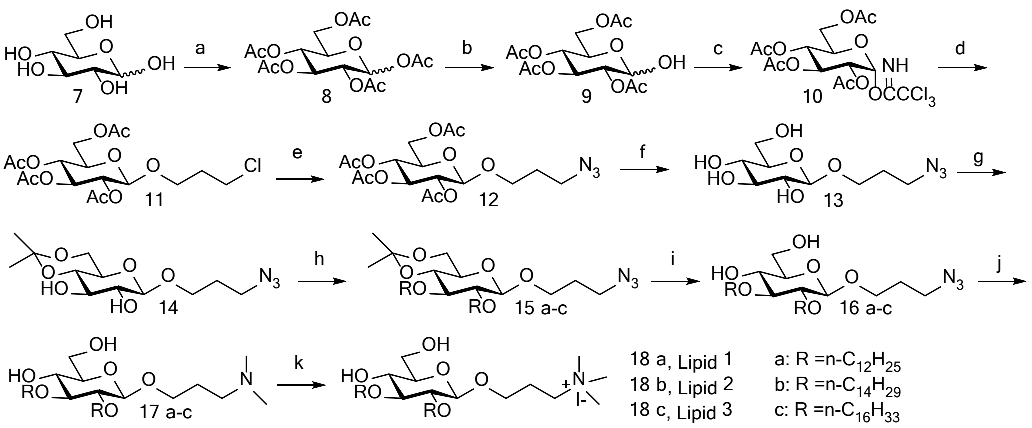
Pioneering research indicates that specific deliver gene to tumor cells can be accomplished by targeting cell surface lectins. However, different carbohydrate terminal of glycolipid only can be recognized by specific lectin caused to target delivery. Therefore, we designed and synthesized two novel series of cationic cyclic glycolipids containing quaternary ammonium headgroup and varying hydrophobic alkyl tails for gene delivery, and to evaluate its efficacy and specificity. The two novel series of cationic glycolipids were distinguished by hydrophobic tail attached to the different positions of lipids. One serial (lipids **1-3**, Scheme 1) was directly bonded to glucose in ether, while the other serial (lipids **4-6**, Scheme 1) was attached to the positively charged nitrogen atoms. The size and zeta potential of lipids and complexes were determined by DLS, and the ability of DNA-banding as well as the cytotoxicity of the lipids were measured via agarose gel electrophoresis and MTT respectively. Furthermore, the morphology of the lipids and complexes were analyzed by atomic force microscopy (AFM). All the lipids were applied to transfer pEGFP into the HEK293 cells. The transfection efficiencies of those lipids were also assayed, and the relationship between structure and activity was discussed.

2. Results and discussion

2.1. Synthesis of cationic glucolipids

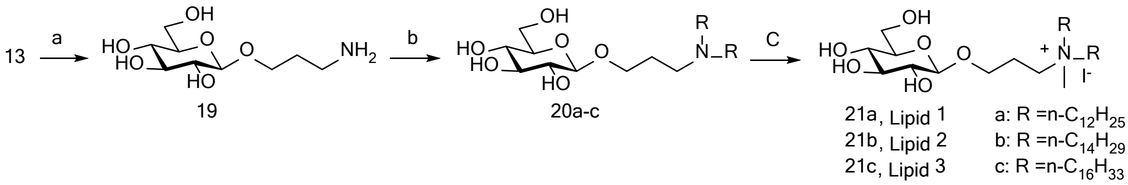
Synthetic routes adopted for preparing the cationic glycolipids **1-3** and glycolipids **4-6** are shown in Scheme 1 and Scheme 2 respectively. The key intermediate 3'-Azidopropyl β-D-glucopyranoside (**13**) was prepared conventionally in five steps. Briefly, intermediate **9** was synthesized by peracetylation of D-glucose (**7**) with acetic anhydride and perchloric acid and followed by selective anomeric deacetylation with ammonia in methanol. Treated intermediate **9** with trichloroacetonitrile in the presence of potassium carbonate in anhydrous dichloromethane provided the glycosyl donor **10**. Glycosyl donor **10** was coupled with 3-chloro-1-propanol in anhydrous dichloromethane catalyzed by TMSOTf to give *β*-glycoside **11**. Then the key intermediate **13** was furnished by azidation of compound **11** with sodium azide in *N*,*N*-dimethylformamide and followed by deacetylation with ammonia in methanol. Intermediate **13** was selectively protected by isopropylidination to yield compound **14**, which was transferred into compound **15** by Williamson ether reaction with alkyl bromide in the presence of sodium cyanide in anhydrous *N*,*N*-dimethylformamide. Treated compound **15** with 2% acetyl chloride in methanol, compound **16** was afforded. Glycolipids **1-3** were then prepared by one-pot reduction of azide to tertiary amine **17** followed by quaterisation reaction. Cationic glycolipids **4-6** were synthesized from compound **13** by Staudinger reduction, followed tertiary amination with alkyl bromines in the presence of potassium carbonate and quaterisation reaction with methyl iodine. The structures of the important intermediates and lipids **1-6** were characterized and confirmed by ^{1}H NMR, ^{13}C NMR, gCOSY and HSQC. The detail procedure for synthesis of the target lipids (**1** and **4**) and NMR data were included in section 3.2 in this paper, the other lipids' were introduced in the supporting information.

Scheme 1: Synthesis of Cationic Glycolipids 1-3



Reagents and conditions: (a) Ac_2O, $HClO_4$, 0-20 ^oC, 10 h, 94.8%; (b) NH_3(g), CH_3OH, 1,4-Dioxane, 1 h, 73.9%; (c) CCl_3CN, K_2CO_3, DCM, 12 h, 85.1%; (d) $HOCH_2CH_2CH_2Cl$, TMSOTf, DCM, -20 ^oC to rt, 4 h, 50.7%; (e) NaN_3, DMF, 75 ^oC, 10 h, 84.9%; (f) NH_3(g), CH_3OH, 9 h, 95.0%; (g) $H_3CC(OCH_3)_2CH_3$, concd H_2SO_4, 6 h, 46.3%; (h) Alkyl Bromide, NaH, DMF, 24 h, 41.5%; (i) CH_3COCl, CH_3OH, 2 h, 61.7%; (j) H_2, 5% Pd/C, HCHO, CH_3OH, 24 h, 48.8%; (k) CH_3I, THF, 20 h, 32.5%.

Scheme 2: Synthesis of Cationic Glycolipids 4-6



Reagents and conditions: (a) PPh_3, H_2O, THF, reflux, 5 h, 85.4%.; (b) Alkyl Bromide, CH_3OH, CH_3CH_2OH, reflux, 48 h, 41.3%; (c) CH_3I, THF, 20 h, 86.8%.

2.2. In vitro transfection biology

High transfection efficiency is a prerequisite for an applicable non-viral gene vector. To investigate the gene transfection efficiencies of the synthesized glycolipids, the fluorescence microscopy assay was carried out using pEGFP-C3 plasmid DNA as the reporter gene in HEK293 cells, and lipo2000 was used as the positive control. Five levels of N/P ratios (2, 4, 6, 8 and 10) were set for each lipid and corresponding enhanced green fluorescent protein expression in HEK293 cells were observed by an inverted fluorescent microscope. The highest density of transfected cells for each glycolipid was obtained and corresponding fluorescence image was shown in Fig. 1. The fluorescence microscope images of HEK293 cells transfected by glycolipid/DNA complexes in the other four levels of N/P ratios were displayed in supporting information (Figure S1). The glycolipids **1-2** (Fig. 1, **B** and **C**) with the hydrophobic tails linked to glucose cycle directly in ether were found to be inefficient in transfecting HEK293 cells compared to lipo2000 (Fig. 1, **A**). The transfection efficiency of glycolipids **5-6** with the hydrophobic tails in the positively charged nitrogen atoms (Fig. 1, **F** and **G**) were as good as or better than lipo2000. However, glycolipid **3** and **4** (Fig. 1, **D, E**; Figure S1) did not have any transfection efficiency at all N/P ratios. In order to reflect the transfection efficiency between lipo2000 and lipids **5-6** accurately, the cell counting of lipo2000 (2 μL) and lipids **5-6** (N / P = 4, 6, 8, 10) in HEK293 cells were shown in Fig. 2. As shown in Fig. 2, the transfection efficiency of lipid **5** and **6** were increased with the lipid/DNA charge ratios from 4 to 10, and they got the maximum efficiency at N/P 10, 8 respectively, which were better than lipo2000. Obviously, the lipid **6** with double C16 saturated chains was more efficient than lipid **5** with double C14 saturated chains. In a word, the transfection results indicated that the position and the length of the hydrophobic tails in the glucose-based lipids had a significant influence on transfection efficiency.

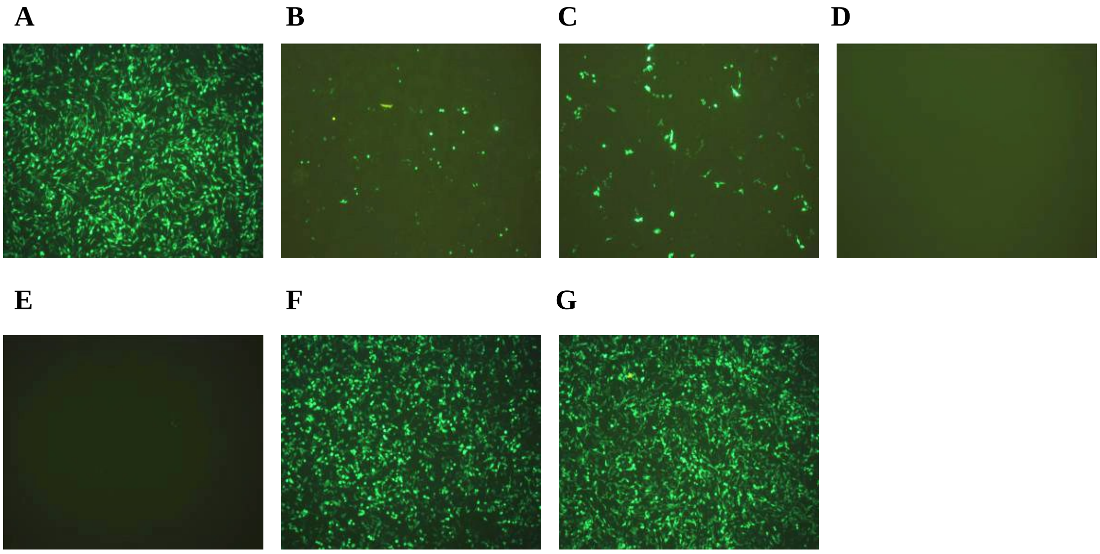


Fig. 1. Fluorescence microscope images of HEK293 cells transfected by glycolipid/DNA complexes. **A**: lipo2000 (2 μL), **B**: lipid **1** (N/P=10), **C**: lipid **2** (N/P=8), **D**: lipid **3** (N/P=8), **E**: lipid **4** (N/P=6), **F**: lipid **5** (N/P=8), **G**: lipid **6** (N/P=8).

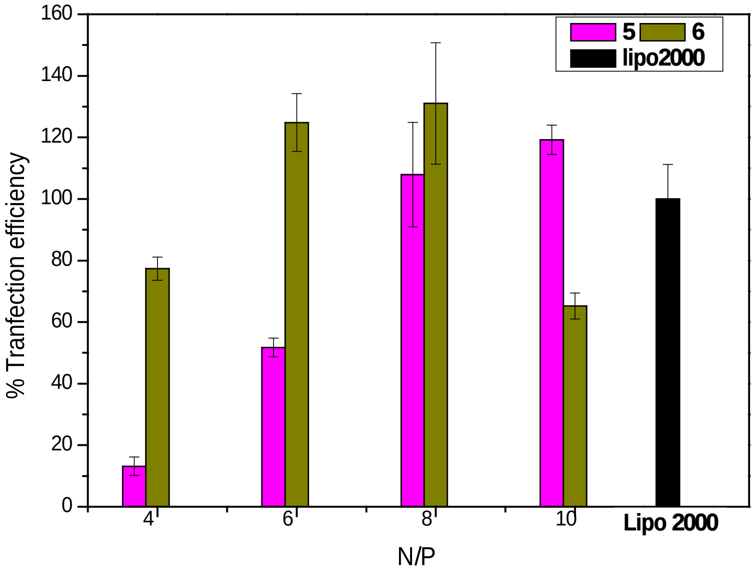


Fig 2. *In vitro* gene delivery efficiencies of lipids **5-6** in HEK293 cells at various glycolipid/DNA charge ratios (4:1 - 10:1). Lipo2000 (2 μL) was used as the positive control. ($p<0.05$)

Besides HEK 293 cells, we transfected other three cells including Hela, SW480 and HepG2 to further investigate the different transfection performances of the designed glycolipids. As the fluorescence microscope images shown in supporting information (Figure S2), Lipid **2** showed very weak gene expression in Hela cells at all the levels of N/P ration. However, lipid **3** showed gradually decreased transfection efficiency in Hela cells with the increase of N/P rations. Lipid **5** and 6 displayed relatively much lower transfection efficiency in HepG2 cells and SW480 cells respectively. But lipid **6** showed slightly enhanced transfection efficiency in HepG2 cells compared to lipid **5**. The transfection efficiency of the designed glycolipids in above-mentioned three cells was different and relatively lower than HEK 293 cells, which was due to the fact that the *in vitro* transfection capabilities are cell-line-dependent and the transfection efficiency of nonviral vectors depends on various parameters such as cytotoxicity, DNA condensation and protection, serum stability, cellular uptake efficiency, or intracellular trafficking.

2.3. Sizes and zeta potentials (ζ)

Physicochemical properties of lipids and their complexes with nucleic acids are very important characteristics that can influence the transfection efficiency. The sizes and surface potentials of net lipids and complexes formed with pEGFP-C3 at N/P charge ratios (2 : 1, 4 : 1, 6 : 1, 8 : 1, 10 : 1) were evaluated by using the dynamic light scattering (DLS). As shown in table 1 and table 2, the average sizes of lipids **1-6** and their complexes were nano-sized particles smaller than 250 nm, which had a potential for transferring nucleic acids into the cells in the absence of serum [48]. The sizes of lipids **1-6** were smaller than 100 nm except lipid **4** (247 nm). After lipids/DNA complexes were formed, the sizes of the complexes formed from lipids **1-2** were not significantly changed. However, the sizes of lipids **3-4**/DNA complexes decreased and lipids **5-6**/DNA complexes increased compared with the corresponding net lipids. Size distribution also became little narrower after complexation. As shown in Fig. 1., the lipids **5-6** showed higher transfer efficiency than other lipids. This may be attributed to their larger sizes, because large particles taken up by cells lead to the formation of large intracellular vesicles, which disrupt and release DNA into the cytoplasm more easily [49]. Though the lipid **4**/DNA complex had a large size, it had low transfection efficiency probably owing to its high cell toxicity (Fig. 5). For the interaction between lipids and DNA, lipoplexes and anionic cell membranes are electrostatic; the positive charged complexes could encourage electrostatic interaction with the anionic plasma membrane for sufficient cellular uptake. So Zeta potential was an important factor in characterizing the surface charges of liposomes and lipoplexes. As shown in table 1, 2, all glycolipids and lipids/DNA complexes exhibited positive zeta potentials. The zeta potentials of lipids **1-6** were 35-70 mV and their complexes decreased to 7-40 mV after DNA complexation.

Table 1. The average size and zeta potential of lipids **1-6**.

Glycolipids	Zeta potential (mV)	Average size (nm)	PDI
lipid **1**	+46.0 ± 0.5	90.3 ± 8.5	0.318 ± 0.021
lipid **2**	+36.2 ± 0.6	63.4 ± 10.1	0.501 ±0.080
lipid **3**	+37.4 ± 0.4	109.7 ± 9.4	0.466 ± 0.074
lipid **4**	+69.5 ± 0.3	247.1 ± 8.7	0.333 ± 0.032
lipid **5**	+42.5 ± 0.5	80.4 ± 9.0	0.429 ± 0.056
lipid **6**	+40.0 ± 0.4	75.0 ± 8.8	0.388 ± 0.041

Table 2. The average size and zeta potential of lipids/DNA complexes.

Lipids/DNA complexes	N/P ratios	Zeta potential (mV)	Average size (nm)	PDI
	2:1	+11.2 ± 1.2	130.9 ± 4.9	0.507 ± 0.054
	4:1	+13.5 ± 1.3	133.0 ± 0.8	0.487 ± 0.047
lipid **1**/DNA	6:1	+19.9 ± 2.2	136.5 ± 7.2	0.565 ± 0.058
	8:1	+15.9 ± 3.3	123.3 ± 5.7	0.597 ± 0.063
	10:1	+15.9 ± 1.3	91.4 ± 8.2	0.217 ± 0.049
	2:1	+14.5 ± 2.7	144.9 ± 2.8	0.368 ± 0.087
	4:1	+18.8 ± 1.9	86.1 ± 1.9	0.323 ± 0.071
lipid **2**/DNA	6:1	+16.7 ± 1.4	86.1 ± 5.1	0.377 ± 0.082
	8:1	+7.1 ± 1.6	66.4 ± 9.0	0.341 ± 0.064
	10:1	+16.2 ± 1.5	77.1 ± 2.3	0.367 ± 0.078
	2:1	+10.8 ± 3.8	173.3 ± 23.5	0.557 ± 0.091
	4:1	+24.8 ± 3.3	148.5 ± 16.2	0.482 ± 0.052
lipid **3**/DNA	6:1	+26.6 ± 2.2	140.3 ± 15.3	0.534 ± 0.088
	8:1	+20.8 ± 1.6	92.8 ± 11.5	0.603 ± 0.097
	10:1	+29.1 ± 2.1	115.7 ± 11.5	0.472 ± 0.085
	2:1	+15.9 ± 1.6	190.3 ± 16.7	0.490 ± 0.079
	4:1	+17.4 ± 2.2	188.4 ± 9.7	0.470 ± 0.065
lipid **4**/DNA	6:1	+41.2 ± 2.4	173.0 ± 9.3	0.320 ± 0.050
	8:1	+30.0 ± 3.9	121.2 ± 14.3	0.425 ± 0.066
	10:1	+21.7 ± 4.7	114.1 ± 10.5	0.313 ± 0.054
	2:1	+12.7 ± 2.5	147.3 ± 16.9	0.417 ± 0.068
	4:1	+15.3 ± 2.8	176.0 ± 10.4	0.533 ± 0.081
lipid **5**/DNA	6:1	+18.4 ± 1.8	194.6 ± 24.5	0.505 ± 0.093
	8:1	+15.7 ± 2.7	183.3 ± 20.6	0.599 ± 0.086
	10:1	+27.2 ± 1.7	147.2 ± 10.8	0.526 ± 0.070
	2:1	+10.2 ± 3.7	186.0 ± 4.5	0.280 ± 0.058
	4:1	+17.9 ± 3.5	207.9 ± 10.6	0.308 ± 0.046
lipid **6**/DNA	6:1	+24.7 ± 2.9	249.0 ± 16.4	0.407 ± 0.083
	8:1	+32.8 ± 3.3	129.0 ± 8.4	0.284 ± 0.037
	10:1	+29.2 ± 3.5	167.2 ± 3.8	0.173 ± 0.028

2.4 Atomic force microscopy (AFM)

Further characterization was done in order to reach a better understanding of the morphology of the lipids and complexes, which were analyzed by atomic force microscopy (AFM). As opposed to DLS, the AFM scanning was done on a dried mica sheet. Although the dried form of the complexes in AFM does not give an accurate information about the complexes' size as in a suspension (as was done in DLS), it can give visual insight into the lipids' and complexes' morphology and size. Firstly, the samples of lipids **2** and **6** were scanned in order to visualize the glycolipids before complexation. It seemed that the lipid **2** spread well on the mica and irregular spherical particles were formed with a diameter of 100-200 nm (Fig. 3. **A**). However, lipid **6** formed spherical particles (about 200 nm) or aggregated particles (>400 nm) (Fig. 3. **C**). Lipoplexes formed at N/P ratio of 8 : 1 can be observed in a regular spherical form with its typical diameter of approximately 50-200 nm (Fig. 3. **B** and **D**). AFM images also indicated lipoplexes are much better dispersed than the net lipids.

A **B**

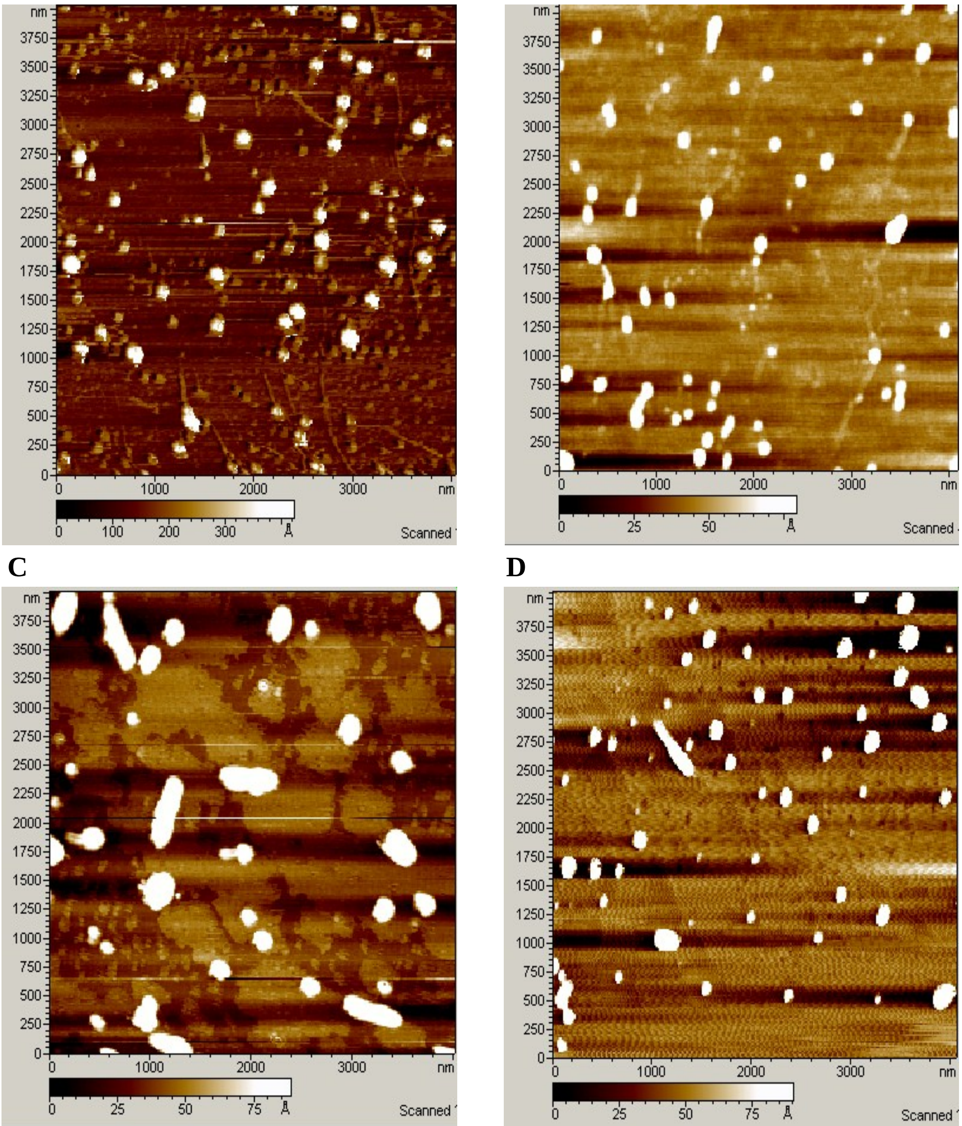


Fig. 3. Atomic Force Microscopy (AFM) images of lipid **2** (50 μmol/L) (**A**), lipid **2**/DNA complex at N/P=8 (80 μmol/L) (**B**), lipid **6** (100 μmol/L) (**C**), and lipid **6**/DNA complex at N/P=8 (100 μmol/L) (**D**).

2.5 Lipid : DNA binding assay

DNA binding affinity was considered as an important parameter in the formation of lipids/DNA lipoplexes. Herein, DNA binding affinities of glycolipids were evaluated by agarose gel retardation assay. As shown in Fig. 4, the intensity of free DNA migrating bands gradually decreased with the addition of the percentage of glycolipids. It could be seen that DNA was completely retarded with the addition of lipids **1-3** at N/P 1-2, while weaker DNA retardation of lipids **4-6** was observed at N/P 2-3. The results indicated that the lipids **1-3** with the hydrophobic tails linked to glucose cycle directly in ether possessed higher DNA binding affinity than that of lipids **4-6** with the hydrophobic tails in the positively charged nitrogen atoms, and the high DNA binding capability could be attributed to the combination effect of the electrostatic and hydrophobic interactions. Although the lipids **1-3** had a better capacity for DNA-binding relative to lipid **4-6**, the disparity was little. In short, the glycolipids had an excellent capability for

DNA-binding, because all of them could combine with DNA completely at low N/P ratios (2 or 3).

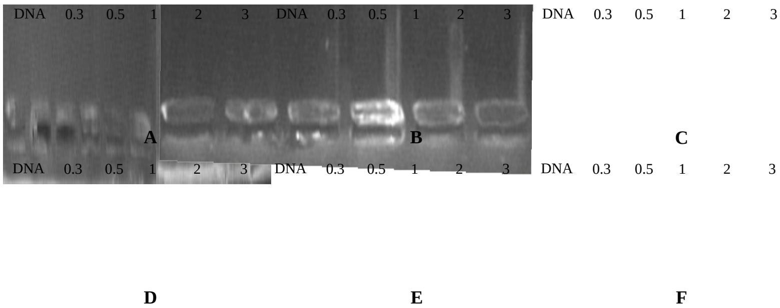


Fig. 4. Electrophoretic gel patterns for lipoplex-associated DNA in gel retardation assay. (**A**)–(**F**) refer to lipids **1-6**, respectively. The lipid/DNA charge ratios are indicated at the top of each lane. The details of the treatment are as described in the text.

2.6 Toxicity studies

Cytotoxicity was considered as a key issue in gene delivery, because favorable gene carriers should be low cytotoxic. Thus, the correlations between molecular structure and cytotoxicity of cationic lipids were important to elucidate. MTT-based cell viabilities of lipids **1-6** were evaluated in HEK293 cells used for *in vitro* transfection across the entire range of lipid/DNA charge ratios used in the actual transfection experiments and commercially available lipo2000 was utilized as the positive control. As shown in Fig. 5, the cells incubated with lipids **1-3** showed higher cell viabilities (95-140%) than that of lipids **4-6** (50-110%) within the N/P ratios from 2 to 10. The result indicated that the lipids with the hydrophobic tails in the positively charged nitrogen atoms had more cytotoxicity related to the lipids with the hydrophobic tails linked to glucose cycle directly in ether. Especially, the lipid **4** with dodecyl hydrophobic chains had the highest cell toxicity among all the lipids, and its cell viability decreased with the increase of N/P ratios and merely half part of lipo2000 when the N/P ratio reached to 10:1. According to the Fig. 1, the lipid **4** did not have any transfection efficiency possibly owing to its lowest cell viability. However, the cell viabilities of lipids **5-6**, which had the highest transfection efficiency, increased with the lengthening of the hydrophobic chains, closed to the lipo2000.

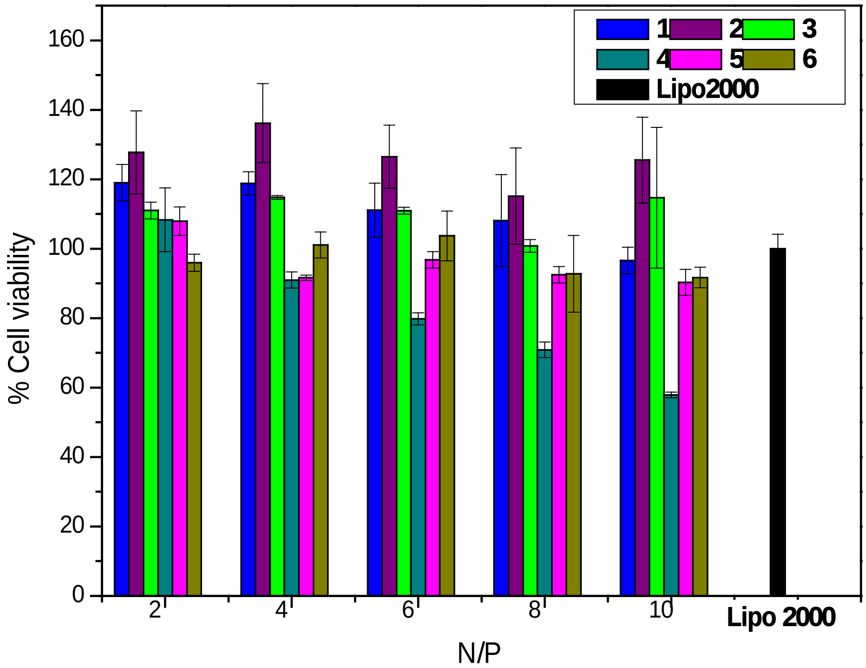


Fig. 5. Representative percent cell viabilities of lipids **1-6** in HEK293 cells using MTT assay. The toxicity assays were performed as described in the text. The data presented are the average values of three independent experiments (n = 3, $p<0.05$).

3. Materials and methods

3.1 Materials

Lauryl bromide, Myristyl bromide, Cetyl bromide, 2,2-Dimethoxypropane, 33% aqueous Formaldehyde, trimethylsilyl trifluoromethanesulfonate(TMSOTf), Sodium azide, 5%Pd/C, Trichloroacetonitrile and 1-Chloro-3-hydroxypropane were purchased from Shanghai Bangcheng Chemical Co. Ltd. Sodium cyanide was purchased from Tianjing Chemical Reagent Resarch Institute. Glucose was purchased from sinoparm Chemical Reagent Co. Ltd. Triphenylphosphine was purchased from Hunan Huihong Chemical Regent Co. Ltd. Iodomethane was purchased from Xiya Reagent. Column chromatography was performed using 200-300 mesh silica gel.

Biowest agarose was purchased from Gene Tech, Shanghai. Lipofectamine 2000 was received from Invitrogene Life Technology, USA. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Amresco. Penicillin-Streptomycin Solution and Dulbecco's Modified Eagle Media (DMEM) was purchased from HyClone. Trypsin and fetal bovine serum (FBS) were purchased from the Gbico. 96-well and 24-well cultivation plates and 50 mL cell cultivation flasks were purchased from Corning Co. Ltd., USA. Plasmid DNA and cells line were kindly provided by the lab of Prof. Shuanglin Xiang, School of Life Sciences, Hunan Normal University. In this work, all the other reagents and solvents were analytical grade and were utilized without further purification. ^{1}H NMR, gCOSY and HSQC spectra were recorded on a Bruker 500MHz and ^{13}C NMR spectra were recorded on a Bruker 125MHz. 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.

3.2 Syntheses of cationic lipids 1-6.

As representative details, syntheses and spectral characterization of lipids **1** and **4** and all their synthetic intermediates shown in Schemes 1 and Schemes 2 were provided. Lipids **2-3** and lipids **5-6** were synthesized following essentially the same protocols adopted for preparing lipids **1** and **4**, respectively. The detailed procedure for synthesis of the target lipids **2**, **3**, **5**, **6**, NMR datas and NMR spectrums were included in the supporting information.

3.2.1 Synthesis of Cationic Glycolipids 1 (**Scheme 1**)

1,2,3,4,6-Penta-*O*-acetyl-α,β-D-glucopyranose (8)

The acetic anhydride (393.5 mL, 4.2 mol) in 1.0 L round flask was cooled to 0^{o}C under stirring. Then perchloric acid (2.0 mL) was added dropwise. The D-glucose (**7**, 100.0 g, 55.5 mmol) was added in partition under the temperature was not over 20^{o}C. 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. DCM (500.0 mL) was added, and the mixture was put into a flask with ice water. The mixture was dispensed, and the organic layer was dried by anhydrous Na_2SO_4 and concentrated to dryness. The residue was recrystallized with petroleum ether-ethyl acetate to give compound **8** as a white solid (205.4 g, 94.8%).

2,3,4,6-Tetra-*O*-acetyl-α,β-D-glucopyranose (9)

Ammolonia was bubbled to the solution of compound **8** (50.0 g, 128.2 mmol) in methanol / 1,4-dioxane (250.0 mL, $V_{methanol}$: $V_{dioxane}$ = 2 : 5). The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material was almost disappeared. Then the mixture was concentrated and the residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 2 : 1 as the eluent to give compound **9** (33.0 g, 73.9%) as a syrup.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (10)

Anhydrous K_2CO_3 (14.0 g, 101.4 mmol) and trichloroacetonitrile (22.6 mL, 225.4 mmol) were added to the solution of compound **9** (33.0 g, 94.7 mmol) in dry DCM (200.0 mL). The mixture was stirring at room temperature until TLC (petroleum ether : ethyl acetate = 3 : 1) showed the starting material was disappeared. The mixture was filtered and concentrated to dryness. The residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 2 : 1 as the eluent to give compound **10** (39.7 g, 85.1%) as a syrup.

3'-Chloropropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (11)

The compound **10** (32.0 g, 65.0 mmol) and 4Ǻ MS (2.0 g) were dried at 60 ^{o}C under vacuum for 2 h, then 3-chloro-1-propanol (13.0 mL, 155.5 mmol) and dry DCM (250.0 mL) were added. The mixture was stirred and cooled to -20^{o}C, and TMSOTf (106.0 μL, 0.6 mmol) was added dropwise. The mixture was stirred for 2 h, during which time the temperature was gradually raised to ambient temperature. The mixture was quenched with Et_3N (0.2 mL) and washed with water and extracted with DCM for three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 4 : 1 as the eluent to give a syrup, and the syrup recrystallized with petroleum ether and ethyl acetate to give compound **11** (14.0 g, 50.7%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 5.19 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.06 (dd, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4), 4.95 (dd, 1 H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 4.90 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.25 (dd, 1 H, $J_{6a,5} = 3.5$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.13 (dd, 1 H, $J_{6b,5} = 2.5$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 3.99-3.95 (m, 1 H, $OCH_2CH_2C\mathit{H}HCl$), 3.70-3.65 (m, 2 H, H-5, $OCH_2CH_2CH\mathit{H}Cl$), 3.59-3.56 (m, 2 H, $OC\mathit{H}_2CH_2CH_2Cl$), 2.06 (s, 3 H, $C\mathit{H}_3CO$), 2.03 (s, 3 H, $C\mathit{H}_3CO$), 2.00 (s, 3 H, $C\mathit{H}_3CO$), 1.98 (s, 3 H, $C\mathit{H}_3CO$), 1.97-1.88 (m, 2 H, $OCH_2C\mathit{H}_2CH_2Cl$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 170.6 (1 C, $CH_3\mathit{C}O$), 170.2 (1 C, $CH_3\mathit{C}O$), 169.4, 169.4 (2 C, 2 $CH_3\mathit{C}O$), 101.0 (1 C, C-1), 72.7 (1 C, C-3), 71.8 (1 C, C-2), 71.3 (1 C, C-5), 68.4 (1 C, C-4), 66.4 (1 C, $OCH_2CH_2\mathit{C}H_2Cl$), 61.9 (1 C, C_6), 41.3 (1 C, $O\mathit{C}H_2CH_2CH_2Cl$), 32.2 (1 C, $OCH_2\mathit{C}H_2CH_2Cl$), 20.7(1 C, $\mathit{C}H_3CO$), 20.6(1 C, $\mathit{C}H_3CO$), 20.5,20.5 (2 C, $\mathit{C}H_3CO$).

3'-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (12)

The mixture of compound **11** (3.0 g, 7.1 mmol), dry DMF (5.0 mL), and NaN_3 (2.8 g, 42.6 mmol) was stirred at 75^{o}C until TLC (petroleum ether : ethyl acetate = 4 : 1) showed the starting material was disappeared. Then the reaction mixture was filtered, and the filter was diluted with DCM (150.0 mL), washed with water and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated in vacuo to dyness. The residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 4 : 1 as the eluent to give compound **12** (2.6 g, 84.9%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 5.19 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.07 (dd, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4), 4.97 (dd, 1 H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 4.90 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.25 (dd, 1 H, $J_{6a,5} = 4.5$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.14 (dd, 1 H, $J_{6b,5} = 3.6$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 3.94-3.92 (m, 1 H, $OCH_2CH_2C\mathit{H}HN_3$), 3.70-3.67 (m, 1 H, $OCH_2CH_2CH\mathit{H}N_3$), 3.61-3.58 (ddd, 1 H, $J_{5,4} = 9.5$ Hz, $J_{5,6a} = 4.5$ Hz, $J_{5,6b} = 3.6$ Hz, H-5), 3.34-3.37 (m, 2 H, $OC\mathit{H}_2CH_2CH_2Cl$), 2.07 (s, 3 H, $C\mathit{H}_3CO$), 2.04 (s, 3 H, $C\mathit{H}_3CO$), 2.01 (s, 3 H, $C\mathit{H}_3CO$), 1.99 (s, 3 H, $C\mathit{H}_3CO$), 1.80-1.86 (m, 2 H, $OCH_2C\mathit{H}_2CH_2Cl$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 170.6 (1 C, $CH_3\mathit{C}O$), 170.2 (1 C, $CH_3\mathit{C}O$), 169.3 (1 C, $CH_3\mathit{C}O$), 169.2, 169.2 (2 C, $CH_3\mathit{C}O$), 100.8 (1 C, C-1), 72.8 (1 C, C-3), 71.8 (1 C, C-2), 71.3 (1 C, C-5), 68.4 (1 C, C-4), 66.4 (1 C, $OCH_2CH_2\mathit{C}H_2Cl$), 61.9 (1 C, C-6), 47.9 (1 C, $O\mathit{C}H_2CH_2CH_2Cl$), 28.9 (1 C, $OCH_2\mathit{C}H_2CH_2Cl$), 20.7 (1 C, $\mathit{C}H_3CO$), 20.6 (1 C, $\mathit{C}H_3CO$), 20.5, 20.5 (2 C, $\mathit{C}H_3CO$).

3'-Azidopropyl β-D-glucopyranoside (13)

Ammonia was bubbled to the solution of compound **12** (3.0 g, 7.1 mmol) in methanol (20.0 mL). The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material disappeared. Then the mixture was concentrated and the residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 2 : 1 as the eluent to give compound **13** (1.5 g, 95.0%) as a syrup.

3'-Azidopropyl 4,6-*O*-isopropylidene-β-D-glucopyranoside (14)

The mixture of compound **13** (1.5 g, 5.7 mmol) and 2,2-dimethoxypropane (10 mL, 81.3 mmol) was stirred at the room temperature, and four drops of conc. sulfuric acid was added. The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 3 : 1) showed the starting material disappeared. The mixture was neutralized with K_2CO_3, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography with petroleum ether : EtOAc = 3 : 1 as the eluent to give compound **14** (0.8 g, 46.3%) as a syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 4.34 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 3.98-3.90 (m, 2 H, H-6a, $OCH_2CH_2C\mathit{H}HN_3$), 3.78 (dd, 1 H, $J_{6b,5}$ = 5.4 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.68-3.64 (m, 2 H, H-3, $OCH_2CH_2CH\mathit{H}N_3$), 3.57 (dd, 1 H, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.46-3.41 (m, 2 H, H-2, $OC\mathit{H_2}CH_2CH_2N_3$), 3.27 (ddd, 1 H, $J_{5,4}$ = 9.5 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 5.4 Hz, H-5), 1.91-1.86 (m, 2 H, $OCH_2C\mathit{H_2}CH_2N_3$), 1.50 (s, 3 H, $C(C\mathit{H_3})_2$), 1.43 (s, 3 H, $C(C\mathit{H_3})_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 103.2 (1 C, C-1), 99.8 (1 C, $\mathit{C}(CH_3)_2$), 74.6 (1 C, C-2), 73.5 (1 C, C-3), 73.0 (1 C, C-4), 67.3 (1 C, C-5), 67.0 (1 C, C-6), 62.0 (1 C, $OCH_2CH_2\mathit{C}H_2N_3$), 48.3 (1 C, $O\mathit{C}H_2CH_2CH_2N_3$), 29.0 (1 C, $OCH_2\mathit{C}H_2CH_2N_3$), 28.9, 19.0 (2 C, $C(\mathit{C}H_3)_2$).

3'-Azidopropyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-β-D-glucopyranoside (15a)

NaH (100.0 mg, 4.0 mmol) was added slowly to the solution of compound **14** (0.2 g, 0.65 mmol) in dry dimethylformamide (2.0 mL) and then 1-bromododecane (0.65 mL, 2.6 mmol) was added dropwise. The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed no change occurred. The mixture was diluted with DCM (10 mL), and washed with water for three times. The organic layer was dried by anhydrous Na_2SO_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 **15a** (170.0 mg, 41.5 %) as a syrup. ^{1}H NMR (500MHz, $CDCl_3$): δ (ppm) 4.30 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 3.93-3.86 (m, 2 H, $OCH_2CH_2C\mathit{H}HN_3$, H-6a), 3.75-3.70 (m, 3 H, $OC\mathit{H_2}(CH_2)_{10}CH_3$, H-6b), 3.66-3.58 (m, 3 H, $OCH_2CH_2CH\mathit{H}N_3$, $OC\mathit{H_2}(CH_2)_{10}CH_3$), 3.53 (dd, 1 H, $J_{4,3}$ = 8.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.89-3.41 (m, 2 H, $OC\mathit{H_2}CH_2CH_2N_3$), 3.25 (dd, 1 H, $J_{3,2}$ = 9.0 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.16 (ddd,1 H, $J_{5,4}$ = 9.5 Hz, $J_{5,6a}$ = 5.5 Hz, $J_{5,6b}$ = 3.6 Hz, H-5), 3.06 (dd, 1 H, $J_{2,1}$ = 7.5 Hz, $J_{2,3}$ = 9.0 Hz, H-2), 1.87-1.84 (m, 2 H, $OCH_2C\mathit{H_2}CH_2N_3$), 1.55-1.51 (m, 4 H, 2 $OCH_2C\mathit{H_2}(CH_2)_9CH_3$), 1.46 (s, 3 H, $C(C\mathit{H_3})_2$), 1.38 (s, 3 H, $C(C\mathit{H_3})_2$), 1.38-1.20 (m, 36 H, 2 $OCH_2CH_2(C\mathit{H_2})_9CH_3$), 0.88 (t, 6 H, J = 6.5 Hz, 2 $OCH_2CH_2(CH_2)_9C\mathit{H_3}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 104.0 (1 C, C-1), 99.2 (1 C, $\mathit{C}(CH_3)_2$), 82.2 (1 C, C-2), 81.7 (1 C, C-3), 73.9 (1 C, C-4), 73.6 (1 C, $O\mathit{C}H_2(CH_2)_{10}CH_3$), 73.2 (1 C, $O\mathit{C}H_2(CH_2)_{10}CH_3$), 66.9 (1 C, C-5), 66.7 (1 C, $OCH_2CH_2\mathit{C}H_2N_3$), 62.2 (1 C, C-6), 48.2 (2 C, $O\mathit{C}H_2CH_2CH_2N_3$), 31.9, 30.3, 30.2, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 26.4 (21 C, some signals were overlapped, 2 $OCH_2(\mathit{C}H_2)_{10}CH_3$, $O\mathit{C}H_2\mathit{C}H_2CH_2N_3$), 22.6 (1 C, $C(\mathit{C}H_3)_2$), 19.0 (1 C, $C(\mathit{C}H_3)_2$), 14.0, 14.0 (2 C, $2OCH_2CH_2(CH_2)_9\mathit{C}H_3$).

3'-Azidopropyl 2,3-di-*O*-dodecyl-β-D-glucopyranoside (16a)

The mixture of compound **15a** (170.0 mg, 0.27 mmol) and dry methanol (10.0 mL) was cooled to 0^oC under stirring, and then the acetyl chloride (0.2 mL, 2.4 mmol) was added dropwise. The reaction mixture was stirring until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material 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 **16a** (100.0 mg, 61.7%) as a white oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.30 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1), 3.96-3.87 (m, 1 H, $OCH_2CH_2CH\mathit{H}N_3$), 3.88-3.87 (m, 2 H, $OC\mathit{H}H(CH_2)_{10}CH_3$, H-6a), 3.79-3.73 (m, 2 H, $OC\mathit{H}H$ $(CH_2)_{10}CH_3$, H-6b), 3.64-3.56 (m, 3 H, $OC\mathit{H_2}(CH_2)_{10}CH_3$,

$OCH_2CH_2CHHN_3$), 3.47-3.40 (m, 3 H, $OCH_2CH_2CH_2N_3$, H-4), 3.34-3.28 (m, 1 H, H-5), 3.19 (dd,1 H, $J_{3,2} = J_{3,4} = 9.0$ Hz, H-3), 3.06 (dd, 1 H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.0$ Hz, H-2), 1.88-1.85 (m, 2 H, $OCH_2CH_2CH_2N_3$), 1.57-1.55 (m, 4 H, 2 $OCH_2CH_2(CH_2)_9CH_3$), 1.24 (m, 36 H, 2 $OCH_2CH_2(CH_2)_9CH_3$), 0.87 (t, 6 H, $J = 6.5$ Hz, 2 $OCH_2CH_2(CH_2)_9CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 103.7 (1 C, C-1), 84.2 (1 C, C-2), 82.1 (1 C, C-3), 74.8 (1 C, C-4), 73.6 (1 C, $OCH_2(CH_2)_{10}CH_3$), 73.0 (1 C, $OCH_2(CH_2)_{10}CH_3$), 70.4 (1 C, C-5), 66.7 (1 C, $OCH_2CH_2CH_2N_3$), 62.8 (1 C, C-6), 48.2 (2 C, $OCH_2CH_2CH_2N_3$), 31.9, 30.4, 30.3, 29.6, 29.5, 29.3, 29.2, 26.2, 26.1, 22.7 (21 C, some signals were overlapped, 2 $OCH_2(CH_2)_{10}CH_3$, $OCH_2CH_2CH_2N_3$), 14.1, 14.1 (2 C, 2 $OCH_2CH_2(CH_2)_9CH_3$).

3'- (*N*,*N*-Dimethylamino)propyl 2,3-di-*O*-dodecyl-β-D-glucopyranoside (17a)

The mixture of compound **16a** (1.0 g, 1.7 mmol) and formaldehyde (36%, 1.1 mL, 13.6 mmol), Pd/C (5%, 300 mg), methanol (60.0 mL) was stirred at the room temperature under H_2 atmosphere. The reaction mixture was stirring until TLC (ethyl acetate : methanol = 5 : 2) showed the starting material 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 **17a** (0.5 g, 48.8%) as a colorless oil.

3'-(*N*,*N*,*N*-Trimethylaminonium iodine)propyl 2,3-di-*O*-dodecyl-β-D-glucopyranoside (18a, lipid 1)

The mixture of compound **17a** (240.0 mg, 0.4 mmol) and iodomethane (48.0 µL, 1.6 mmol), THF (3.0 mL) was stirred at the room temperature. The reaction mixture was stirring until TLC (ethyl acetate : methanol = 2 : 1) showed the starting material 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 lipid **1** (100.0 mg, 32.5%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.31 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 3.92-3.85 (m, 4 H, $OCH_2CH_2CH_2N(CH_3)_3$, H-6a, OH), 3.78-3.64 (m, 6 H, $OCHH(CH_2)_{10}CH_3$, $OCH_2(CH_2)_{10}CH_3$, $OCH_2CH_2CH_2N(CH_3)_3$, H-6b), 3.57-3.52 (m, 1 H, $OCHH(CH_2)_{10}CH_3$), 3.46-3.32 (m, 11 H, $OCH_2CH_2CH_2N(CH_3)_3$, H-4, H-5), 3.25 (s,1 H, OH), 3.19 (dd, 1 H, $J_{3,2} = 8.5$ Hz , $J_{3,4} = 8.0$ Hz, H-3), 3.01 (dd, 1 H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 8.5$ Hz, H-2), 2.27-2.19 (m, 1 H, $OCH_2CHHCH_2N(CH_3)_3$), 2.06-2.02 (m, 1 H, $OCH_2CHHCH_2N(CH_3)_3$), 1.56-1.48 (m, 4 H, 2 $OCH_2CH_2(CH_2)_9CH_3$), 1.24 (m, 36 H, 2 $OCH_2CH_2(CH_2)_9CH_3$), 0.87 (t, 6 H, $J = 7.0$ Hz, 2 $OCH_2CH_2(CH_2)_9CH_3$) ; ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 103.8 (1 C, C-1), 84.1 (1 C, C-3), 81.8 (1 C, C-2), 75.6 (1 C, C-5), 73.6 (1 C, $OCH_2(CH_2)_{10}CH_3$), 73.0 (1 C, $OCH_2(CH_2)_{10}CH_3$), 79.9 (1 C, C-4), 66.5 (1 C, $OCH_2CH_2CH_2N(CH_3)_3$), 64.7 (1 C, $OCH_2CH_2CH_2N(CH_3)_3$), 61.4 (1 C, C-6), 53.9, 59.9, 53.9 (3 C, $OCH_2CH_2CH_2N(CH_3)_3$), 31.8, 30.8, 30.4, 29.7, 29.6, 29.3, 26.2, 26.1, 24.4, 22.7 (21 C, some signals were overlapped, 2 $OCH_2(CH_2)_{10}CH_3$, $OCH_2CH_2CH_2N(CH_3)_3$), 14.0, 14.0 (2 C, 2 $OCH_2CH_2(CH_2)_9CH_3$). ESI-MS: *m*/*z* = 616.8, in agreement with the calculated mass for $[M]^+ = C_{36}H_{74}NO_6^+$.

3.2.2 Synthesis of Cationic Glycolipids 4 (Scheme 2)

3'-aminopropyl-β-D-glucopyranoside (19)

The mixture of compound **13** (3.9 g, 14.8 mmol), THF (50.0 mL), H_2O (5.0 mL), and PPh_3 (7.8 g, 29.6 mmol) was refluxed at 75°C until TLC (petroleum ether : ethyl acetate = 2 : 1) showed the starting material disappeared. The reaction mixture was evaporated to dryness, H_2O (50.0 mL) was dropped to it, and a white solid would be given, then the mixture was filtered and a yellow syrup **19** (3.0, 85.4%) would be given when the filtrate was evaporated to dryness.

3'-[(*N*,*N*-di-dodecylamino]-propyl-β-D-glucopyranoside (20a)

The mixture of compound **18** (1.2 g, 5.1 mmol), 1-bromododecane (5.1 g, 20.4 mmol), anhydrous K_2CO_3 (1.4 g, 10.2 mmol), CH_3OH (20.0 mL), CH_3CH_2OH (20.0 mL) was refluxed at 75°C until TLC (methanol) showed the starting material disappeared. The mixture was diluted with DCM (30.0 mL), and washed with water for two times. The organic layer was dried by anhydrous Na_2SO_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 **20a** (1.2 g, 41.3 %) as a syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 4.30 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 3.95-3.85 (m, 1 H, $OCHHCH_2CH_2N(CH_2CH_2(C_9H_{18})CH_3)_2$), 3.88-3.70 (m, 2 H, H-6), 3.64-3.50 (m, 3 H, $OCHHCH_2$ $CH_2N(CH_2CH_2(C_9H_{18})CH_3)_2$), H-3, H-4), 3.39 (dd, 1 H, $J_{2,1}$ = 7.5 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 3.30-3.28 (m, 1 H, H-5), 2.58-2.50 (m, 2 H, $OCH_2CH_2CH_2N(CH_2CH_2(C_9H_{18})CH_3)_2$), 2.44 (t, J = 6.5 Hz, 4 H, $N(CH_2CH_2(C_9H_{18})CH_3)_2$), 1.89-1.75 (m, 2 H, $OCH_2CH_2CH_2N(CH_2CH_2$ $(C_9H_{18})CH_3)_2$), 1.49-1.40 (m, 4 H, $N(CH_2CH_2(C_9H_{18})CH_3)_2$), 1.37-1.21 (m, 36 H, $N(CH_2CH_2$ $(C_9H_{18})CH_3)_2$), 0.89 (t, 6 H, J = 6.5 Hz, $N(CH_2CH_2(C_9H_{18})CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 103.1 (1 C, C-1), 76.5 (1 C, C-3), 75.8 (1 C, C-5), 73.5 (1 C, C-2), 69.6 (1 C, C-4), 68.7 (1 C, $OCH_2CH_2CH_2N(CH_2CH_2(C_9H_{18})CH_3)_2$), 61.4 (1 C, C-6), 53.4, 53.4 (2 C, $N(CH_2CH_2(C_9H_{18})$ $CH_3)_2$), 50.7 (1 C, $OCH_2CH_2CH_2N(CH_2CH_2(C_9H_{18})CH_3)_2$), 31.9, 29.8, 29.7, 29.6, 29.3, 27.7, 27.2, 25.7, 22.6 (21 C, some signals were overlapped, $N(CH_2(C_{10}H_{20})CH_3)_2$), $OCH_2CH_2CH_2N$ $(CH_2CH_2(C_9H_{18})CH_3)_2$), 14.1, 14.1 (2 C, $N(CH_2(C_{10}H_{20})CH_3)_2$).

3'-[(*N*,*N*-di-dodecyl-*N*-methyl)aminonium iodine]-propyl-β-D-glucopyranoside (21a, lipid 4)

The mixture of compound **20a** (250.0 mg , 0.44 mmol) and iodomethane (250.0 mg, 1.76 mmol, 109.0 µL), THF (5.0 ml) was stirring at the room temperature until TLC (ethyl acetate : methanol = 5 : 1) showed the starting material disappeared. The reaction mixture was evaporated to dryness and a solid was precipitated when the acetone (10.0 mL) was dropped 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 **lipid 4** (270.0 mg, 86.8%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 5.09 (s, 1 H, O*H*), 4.88 (s, 2 H, 2 O*H*), 4.46 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 4.10-4.00 (m, 2 H, O*H*, $OCHHCH_2CH_2N(CH_3)(C_{12}H_{25})_2$), 3.86-3.75 (m, 3 H, H-6, $OCHHCH_2CH_2N(CH_3)(C_{12}H_{25})_2$), 3.74-3.50 (m, 4 H, H-3, H-4, $OCH_2CH_2CH_2N(CH_3)$ $(C_{12}H_{25})_2$), 3.44-3.30 (m, 6 H, H-2, H-5, $(CH_3)N(CH_2CH_2(C_9H_{18})CH_3)_2$), 3.21 (s, 3 H, $(CH_3)N(CH_2CH_2(C_9H_{18})CH_3)_2$), 2.22-2.14 (m, 2 H, $OCH_2CH_2CH_2N(CH_3)(C_{12}H_{25})_2$), 1.75-1.67 (m, 2 H, $(CH_3)N(CH_2CH_2(C_9H_{18})CH_3)_2$,) 1.40-1.20 (m, 36 H, $(CH_3)N(CH_2CH_2(C_9H_{18})CH_3)_2$), 0.86 (t, 6 H, J = 6.5 Hz, $(CH_3)N(CH_2CH_2$ $(C_9H_{18})CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 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, $OCH_2CH_2CH_2N(CH_3)(C_{12}H_{25})_2$), 61.2, 61.2, 61.2 (3 C, $OCH_2CH_2CH_2N(CH_3)(CH_2CH_2(C_9H_{18})CH_3)_2$), 60.8 (1 C, C-6), 49.4 (1 C, $(CH_3)N(CH_2CH_2(C_9H_{18})CH_3)_2$), 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 26.3, 23.5, 22.6 (21 C, some signals were overlapped, $(CH_3)N(CH_2(C_{10}H_{20})CH_3)_2$), $OCH_2CH_2CH_2N(CH_3)(C_{12}H_{25})_2$), 14.1, 14.1 (2 C, $(CH_3)N(CH_2(C_{10}H_{20})CH_3)_2$). ESI-MS: *m*/*z* = 588.7, in agreement with the calculated mass for $[M]^+ = C_{34}H_{70}NO_6^+$.

3.3 Preparation of liposomes and DNA-Lipid Complexes. Each glycolipid was disappeared into sterile double distilled water. The mixture was ultrasonaticed at 37°C for 30 min in a closed vial to give a clarity solution, and filtered with syringe filter (0.45μm) to form the solution of liposome. The concentration of liposome was 0.5 mmol/L for lipids **1-3**, 0.25 mmol/L for lipids **4-6**. Lipoplexs with different N/P ratio were prepared by mixed the plasmid DNA (1μg) with various amounts of liposomal solution.

3.4 Gel Retardation. To examine the complexation of DNA with cationic lipid suspensions at different lipid-DNA ratios, we prepared lipid-DNA complexes at different charge ratios varying from 0.3:1 to 3:1(lipid/DNA) to measure the DNA binding ability. After 30 min of incubation, these complexes were electrophoretically run on a 1.0% agarose gel containing (0.2 μg/mL) ethidium bromide for DNA staining. The gel was placed in a horizontal electrophoresis apparatus (DYY-6C, Beijing Liuyi Biotechnology Co. Ltd.) containing ×1 Tris acetate EDTA (TAE) buffer solution (prepared in our lab as ×5 stock solution), exposed to an electric field (320 V) for 20 min, and then visualized by Gel image system (Tanon 2500, Shanghai Tianneng science and Technology Co. Ltd).

3.5 Lipoplex and lipids particle sizes and zeta potentials. The average particle size and charge of the different lipids and various N/P ratios were determined by quasielastic laser light scattering with a Malvern Zetasizer (Nano-ZS, Malvern Instruments). Each sample was analyzed in triplicate, and the results were reported as the means ± standard deviation. The lipoplex particle solution was first prepared by mixing liposomes and pEGFP (1 μg/μL) under diverse N/P charge ratios in 1 mL deionized water.

3.6 Morphology study by atomic force microscopy. The samples for AFM measurements were prepared by mixing lipids and 0.8 μg pEGFP in 100 μL of distilled water under the best N/P ratios and incubated for 20 min, and the solution mixture were dropped onto a mica slice, and then the solvent was completely evaporated in the air under the room temperature prior to AFM measurements. Morphologies of the lipids/pEGFP lipoplexes were characterized at room temperature on a NanoScope IV atomic force microscopy (AFM, Veeco Instruments, Co. Ltd.) with a tapping mode.

3.7 Cell Culture. Cells (Hek293) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin-streptomycin, 10,000 U mL^{-1}) in T55 culture dish and were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cells were regularly passaged by trypsinization with 0.1% trypsin (EDTA 0.02%, dextrose 0.05%, and trypsin 0.1%) in PBS (pH = 7.2).

3.8 Transfection of Cells. Transfection of the cells was done essentially as described earlier. In order to obtain about 80% confluent cultures at the time of transfection, 24-well plates were seeded with 100,000 cell/well in 500 μL mediated 24 h before transfection. For the preparation of lipoplexes applied to cells, various amounts of liposomes and DNA were serially diluted separately in serum-free DMEM culture medium; then, the DNA solutions were added into liposome solutions drop by drop and vortexed gently, after which the mixtures were incubated at room temperature for about 30 min to obtain lipoplexes. The DNA was used at a concentration of 0.8 μg/well unless otherwise noted. After 30 min of complexation, old cell culture medium was removed from the wells, cells were washed once with PBS, and the above lipoplexes was added to each well. The plates were then incubated for 4 h at 37°C in a humidified atmosphere containing 5% CO_2. At the end of incubation period, medium was removed and 500 μL of fresh DMEM culture medium containing 10% FBS was added to each well. Plates were further incubated for a period of 24 h before checking the reporter gene expression. For fluorescent microscopy assays, cells were transfected by complexes containing pEGFP-C3. After 24 h incubation, the microscopy images were obtained at the magnification of 20 and recorded using IpWin51C image analysis system. Control transfection was performed in each case using a commercially available transfection reagent Lipofectamine 2000 based on the standard conditions specified by the manufacture.

Cell count of transfection efficiency [49]. After the old cell culture medium was removed from the wells and washed with PBS, 50 uL of parenzyme was introduced to the 24-well plates. When the pates were incubation for 2-3 min at 37°C in a humidified atmosphere containing 5% CO_2, 200 µL of DMEM was added and the well-distributed suspension was afforded by scattering the cells. Then, 100 µL of cell suspension was removed to the centrifugal tube and used to cell count by diluting with another 100 µL of DMEM. Afterwards, 10 µL of the diluted suspension was introduced to serum counting plate, white and fluorescence images were given by fluorescent microscopy in the same position. Three samples of per well were taken out, five white and fluorescence images were afforded by fluorescent microscopy for each sample. Finally, count cells in the white and fluorescence images, and then the y% transfection efficiency of glycolipids were calculated as (cell numbers in the fluorescence images / cell numbers in the white images) × 100. In the same way, x% transfection efficiency of lipo2000 (as a positive control) was obtained. Then the relative transfection efficiency of glycolipid to lipo2000 was calculated according to the formula: $\frac{y\%}{x\%} \times 100$.

3.9 Cytotoxicity. Toxicity of each cationic lipid formulation toward Hek293 cells was determined using 3-(4,5-dimethylthiaz-ole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as described earlier [51-52]. In order to obtain accurate results, cytotoxicity levels of the lipid formulations optimal for transfection experiments were found out under conditions exactly similar to transfection conditions. About 30 000 cells/well were plated in 96 well plates. After 24 h, various N/P ratios of lipids were added to the cells in the absence of serum. After 4 h of incubation, 20 µL of MTT solution was added. Blue Formosan crystals were seen when checked under microscope. Media was removed and 150 µL of DMSO was added per well. The absorbance was measured using a microtiter plate reader. The % viability was then calculated as [{A490 (treated cells) - background]/[A490 (untreated cells) - background}] × 100.

4. Conclusions

In this study, two series of glucose-based cationic lipids were synthesized, and their utility in a pEGFP-encapsulating liposome mediated gene delivery system were investigated. The size, ζ-potential, toxicity and the capacity of DNA-banding of glycolipids were also detected. The transfect efficiency of glycolipids with hydrophobic tails linked to the positively charged nitrogen atoms were higher than that of glycolipids with hydrophobic tails linked to the glucose cyclic directly. Most importantly, among of six glycolipids, lipid **6** with the hexadecyl as hydrophobic tail linked to the positively charged nitrogen atoms was observed the highest gene expression efficiency and lower cytotoxicity in HEK293 cells. We are currently focusing our efforts on performing a detailed structure/activity relationship analysis to gain a better insight and understanding of these glucose-based cationic lipids and will apply it to disease models based on siRNA therapeutics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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