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Synthesis of targetable cationic amphiphiles

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Abstract

Cationic amphiphiles **1a–1c**, with monosaccharide at the ω-position of the hydrocarbon tails, were synthesized by utilization of Schmidt's trichloroacetimidate procedure for the glycosylation step and application of the commercially available 3,5-dihydroxybenzyl alcohol as a scaffold for the attachment of double hydrocarbon tails. Application of the Zemplén condition for *O*-deacetylation in the presence of base-sensitive benzyl bromide was found to be efficient. The synthetic route provides an entry for the synthesis of versatile quaternary ammonium amphiphiles having the cell targeting glycosyl ligands.

Increasing interest in the nonviral-mediated DNA delivery system has led to the synthesis of various structural cationic lipids to deliver genes into cells.[1] These synthetic gene transfer molecules are designed to form DNA/cationic lipid complexes through electrostatic interaction between cationic lipids and polyanion DNA. These positively charged particles nonspecifically bind to the negatively charged cell surface. This triggers endocytosis and endosomal membrane disruption. The inserted gene is then expressed. The nonspecific binding between positively charged DNA/lipid particles and the negatively charged cell surface is a concern for targeted gene delivery. To solve this problem, cell targeting ligands were introduced to the synthetic vectors[2] based on the concept of receptor-mediated endocytosis.[3] As part of our program dealing with cationic lipid-mediated gene delivery and cell targeting,[4] we initially introduced the galactosyl, mannosyl ligand to the quaternary ammonium type of cationic amphiphiles. The rationale for our design is that the quaternary ammonium residue should spontaneously compact the DNA via electrostatic interaction to form lipid-coated DNA; the hydrocarbon chains provide a hydrophobic domain which will prevent the DNA from early degradation and facilitate the transfer of DNA from the endosome to the cytoplasm. Meanwhile, the glycosides at the ω-position of the hydrocarbon tails serve as molecular signals for cell targeting and at the same time prevent DNA/cationic lipid complex aggregation.

The synthesis of cationic amphiphiles **1a,1b** is outlined in Scheme 1. The synthesis was started by chemoselective removal of anomeric acetyl group in pentaacetate D-galactose **7a** or pentaacetate D-glucose **7b** by hydrazine acetate.[5] Subsequent treatment of the resulting hemiacetal with a large excess

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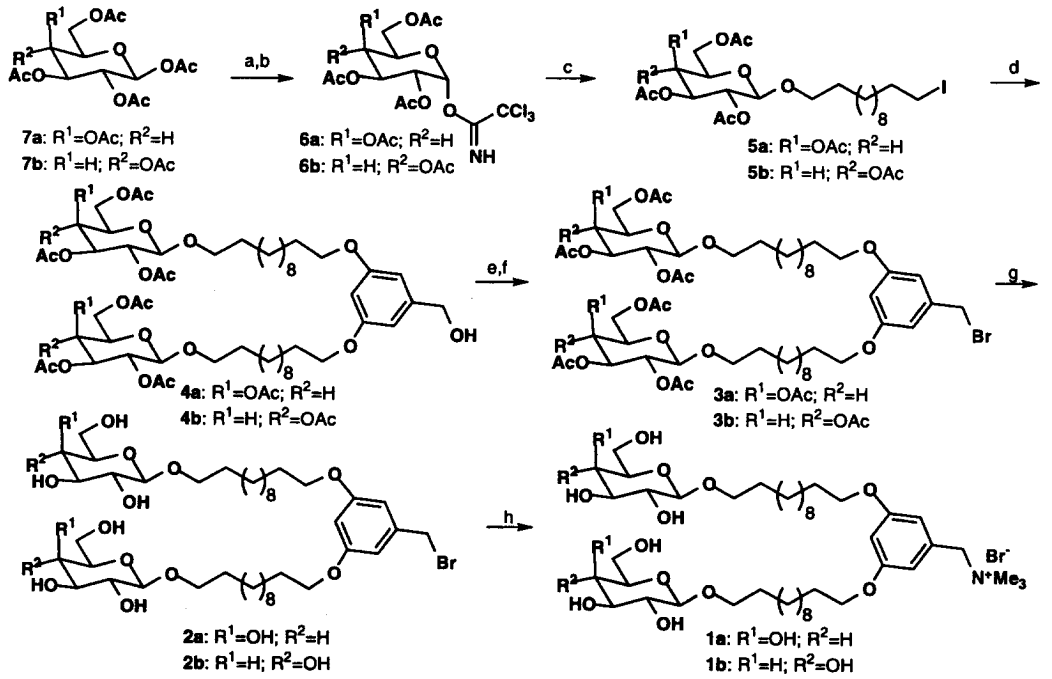
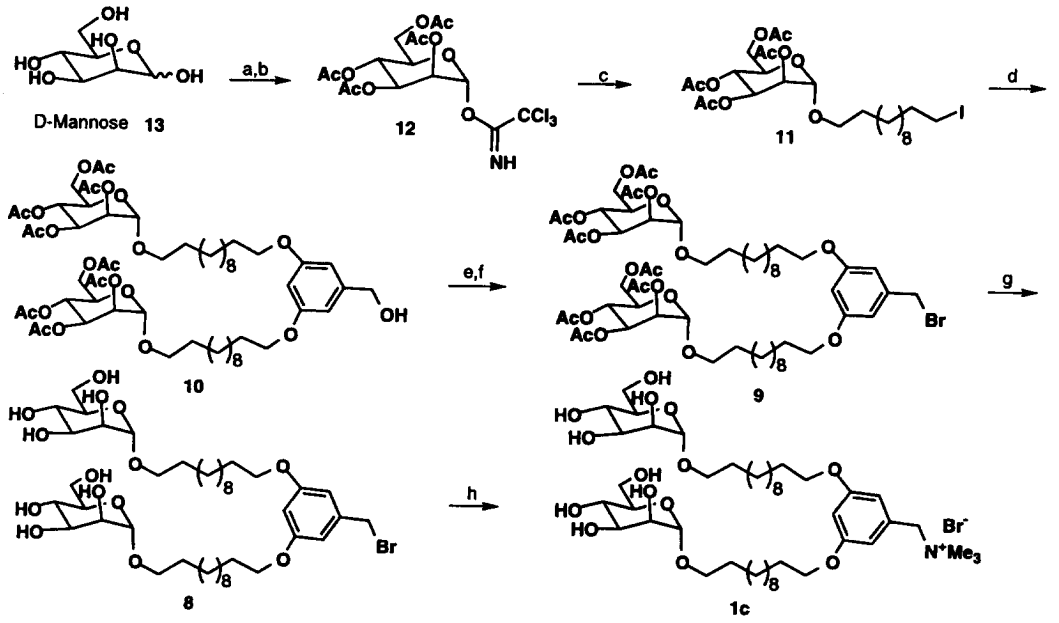


Scheme 1. *Reagents and conditions*: (a) NH_2NH_2-AcOH (1.1 equiv.), DMF, 55°C, 15 min, 100%; (b) CCl_3CN-CH_2Cl_2 (1:4=v/v), cat. DBU, 0°C, 1.5 h, 95%; (c) $HO(CH_2)_{12}I$ (1.2 equiv.), 4 Å MS, cat. $BF_3 \cdot Et_2O$, −78°C to 0°C, 2 h, 53% for **5a**, 63% for **5b**; (d) 3,5-dihydroxybenzyl alcohol (0.35 equiv.), K_2CO_3 (1.1 equiv.), 18-crown-6 (0.2 equiv.), 4 Å MS, dry MeCN, reflux, 8 h, 60% for **4a**, 48% for **4b**; (e) MsCl (1.25 equiv.), Et_3N (1.5 equiv.), CH_2Cl_2, 0°C, 30 min, 100%; (f) LiBr (5 equiv.), MEK, reflux, 30 min, 92%; (g) 0.02 M NaOMe-MeOH, rt, 30 min, 98%; (h) 25% (W/W) Me_3N-MeOH, reflux, 1.5 h, 83% for **1a**, 85% for **1b**. DBU=1,8-diazabicyclo[5,4,0]undec-7-ene, MsCl=methanesulfonyl chloride, MEK=methyl ethyl ketone

of trichloroacetonitrile[6] in the presence of a catalytic amount of DBU[7] for 1.5 h at 0°C, gave only the thermodynamically more stable α-trichloroacetimidate in an excellent yield. The corresponding β-anomer was not detected by NMR. The anomeric hydrogen of α-galactose trichloroacetimidate **6a** appeared as a doublet with a coupling constant of 3.3 Hz and centered at 6.60 ppm, while the anomeric hydrogen of α-glucose trichloroacetimidate **6b** appeared as a doublet with a coupling constant of 3.6 Hz and centered at 6.57 ppm. These coupling constants are in the range of axial–equatorial configuration of H-2 and H-1 and therefore an α-trichloroacetimidate is assigned. $BF_3 \cdot Et_2O$ promoted glycosylation gave the corresponding β-glycoside derivatives **5a**,**5b** in 53 and 63% isolated yields, respectively. The stereochemistries of compounds **5a**,**5b** were confirmed by their ^1H NMR spectra. The anomeric proton of compound **5a** gave a doublet with a coupling constant of 8.0 Hz centered at 4.45 ppm. Similarly, the anomeric proton of compound **5b** resonated at 4.49 ppm with a coupling constant of 8.0 Hz. The preference for the formation of β-isomer can be rationalized in terms of competition between substitution at the sterically less hindered position (β) versus stabilization from the anomeric effect (α).[8] It is interesting to note that 12-iodododecyl acetate was obtained as a byproduct (ca. 5–10% yield, not shown in Scheme 1) in these Schmidt's type glycosylations due to the transesterfication of the acyl group from the glycosyl donor to the glycosyl acceptor.[9] With the compounds **5a**,**5b** in hand, selective dialkylation of the commercially available 3,5-dihydroxybenzyl alcohol was conducted under anhydrous basic conditions to yield the corresponding benzyl alcohols **4a**,**4b** in modest separated yields,[10] combined with monoalkylated benzyl alcohol (not shown in Scheme 1) in ca. 10% yield. In this

reaction, the degassed anhydrous acetonitrile was used as solvent to prevent the occurrence of the phenol oxidation and *O*-deacetylation. The dialkylated benzyl alcohols **4a,4b** were then easily converted into the corresponding benzyl bromides **3a,3b** by a two-step transformation (mesylation and bromination). Removal of the acetyl groups of compounds **2a,2b** was achieved by short treatment of compounds **3a,3b** with the Zemplén method (0.02 M NaOMe/MeOH)[11] while leaving the base-sensitive benzyl bromide intact. Benzyl bromides **2a,2b** were subsequently refluxed with 25% (W/W) trimethylamine in methanol (Menshutkin's quaternarization) for 1.5 h to give the desired quaternary ammonium compounds **1a,1b** in good yields.[12]



Scheme 2. *Reagents and conditions*: (a) Ac_2O (5.5 equiv.), Py, cat. DMAP, 0°C to rt, 3 h; then NH_2NH_2-AcOH (1.1 equiv.), DMF, 55°C, 15 min, 100%; (b) CCl_3CN-CH_2Cl_2 (1:4=v/v), cat. DBU, 0°C, 1.5 h, 92%; (c) 4 Å MS, $HO(CH_2)_{12}I$ (1.2 equiv.), cat. $BF_3 \cdot Et_2O$, −78°C to 0°C, 2h, 57%; (d) 3,5-dihydroxybenzyl alcohol (0.35 equiv.), K_2CO_3 (1.1 equiv.), 18-crown-6 (0.2 equiv.), 4 Å MS, dry MeCN, reflux, 8 h, 45%; (e) MsCl (1.25 equiv.), Et_3N (1.5 equiv.), CH_2Cl_2, 0°C, 30 min, 100%; (f) LiBr (5 equiv.), MEK, reflux, 30 min, 92%; (g) 0.02 M NaOMe-MeOH, rt, 30 min, 95%; (h) 25% (W/W) Me_3N-MeOH, reflux, 1.5 h, 88%

The synthesis of cationic amphiphile **1c** was accomplished similarly as discussed above. Briefly, treatment of D-mannose **13** with Ac_2O in pyridine in the presence of a catalytic amount of DMAP gave the pentaacetate D-mannose (α:β isomer=3:1 based on ^1H NMR) in quantitative yield (Scheme 2). Selective removal of the anomeric acetyl group and treatment of the resulting hemiacetal with trichloroacetonitrile furnished the α-mannose trichloroacetimidate **12** exclusively. The anomeric proton resonated at 6.28 ppm as a doublet with $^3J_{1,2}$=1.6 Hz which confirmed that the H-1 and H-2 were in equatorial–equatorial fashion. Stereoselective glycosylation led to sole α-D-mannoside **11** in 57% yield, again 12-iodododecyl acetate as an acyl migration byproduct was obtained in ca. 10% yield. The stereochemistry of α-D-mannoside **11** was established by its ^1H NMR spectrum, which showed an anomeric proton at 4.80 ppm as a doublet with $^3J_{1,2}$=1.2 Hz. At this stage, both the steric and anomeric factors favor the formation of α-anomer with the retention of configuration during glycosylation. With compound **11** in hand, the synthesis of the desired ammonium salt **1c** was straightforward. Namely, dialkylation of the 3,5-dihydroxybenzyl alcohol generated the corresponding benzyl alcohol **10**.[10] A two-step transformation

of the benzyl alcohol **10** afforded the benzyl bromide **9**. Unmasking of the acetate protective group in the presence of base-sensitive benzyl bromide moiety cleanly gave bromide **8**. To this end, Menshutkin's type quaternarization of the bromide **8** afforded the desired cationic amphiphile **1c** in 88% yield.[12]

In summary, we have developed an efficient route toward the synthesis of monosaccharide-linked cationic amphiphiles **1a–1c** using Schmidt's trichloroacetimidate procedure for glycosylation step and application of the commercially available 3,5-dihydroxybenzyl alcohol as a scaffold for the attachment of double hydrophobic tails. Application of the Zemplén condition for *O*-deacetylation in the presence of base-sensitive benzyl bromide was found to be efficient. The synthetic route provides an entry for the synthesis of versatile quaternary ammonium amphiphiles having the cell targeting glycosyl ligands. Application of these amphiphiles to deliver DNA into the targeted cells in vitro and in vivo is now underway.

Acknowledgements

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10. Compound **4a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (d, *J*=2.0 Hz, 2H, Ar), 6.37 (t, *J*=2.0 Hz, 1H, Ar), 5.39 (d, *J*=3.3 Hz, 2H, H-4), 5.20 (dd, *J*=10.4, 8.0 Hz, 2H, H-2), 5.01 (dd, *J*=10.4, 3.3 Hz, 2H, H-3), 4.61 (s, 2H, $ArCH_2$), 4.44 (d, *J*=7.9 Hz, 2H, H-1), 4.19 (dd, *J*=11.4, 6.6 Hz, 2H, H-6a), 4.12 (dd, *J*=11.4, 6.8 Hz, 2H, H-6b), 3.93 (t, *J*=6.6 Hz, 4H, 2×$ArOCH_2$), 3.87 (m, 4H, H-5 and alkyl chain H-1′), 3.49 (m, 2H, alkyl chain H-1′), 2.15 (s, 6H, 2×Ac), 2.05 (s, 12H, 4×Ac), 1.99 (s, 6H, 2×Ac), 1.76 (m, 4H, 2×CH_2), 1.57 (m, 4H, 2×CH_2), 1.27 (m, 32H, 16×CH_2) ppm. Compound **4b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (d, *J*=2.0 Hz, 2H, Ar), 6.37 (t, *J*=2.0 Hz, 1H, Ar), 5.20 (t, *J*=9.4 Hz, 2H, H-3), 5.08 (t, *J*=9.5 Hz, 2H, H-4), 4.98 (dd, *J*=9.4, 7.9 Hz, 2H, H-2), 4.61 (s, 2H, $ArCH_2$), 4.49 (d, *J*=7.9 Hz, 2H, H-1), 4.26 (dd, *J*=12.4, 4.6 Hz, 2H, H-6a), 4.13 (dd, *J*=12.4, 2.3 Hz, 2H, H-6b), 3.93 (t, *J*=6.6 Hz, 4H, 2×$ArOCH_2$), 3.86 (m, 2H, alkyl chain H-1′), 3.68 (ddd, *J*=9.5, 4.6, 2.3 Hz, 2H, H-5), 3.48 (m, 2H, alkyl chain H-1′), 2.22 (s, 6H, 2×Ac), 2.04 (s, 6H, 2×Ac), 2.02 (s, 6H, 2×Ac), 2.01 (s, 6H, 2×Ac), 1.76 (m, 4H, 2×CH_2), 1.57 (m, 4H, 2×CH_2), 1.27 (m, 32H, 16×CH_2) ppm. Compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (d, *J*=2.0 Hz, 2H, Ar), 6.37 (t, *J*=2.0 Hz, 1H, Ar), 5.35 (dd, *J*=9.9, 3.3 Hz, 2H, H-3), 5.27 (t, *J*=9.7 Hz, 2H, H-4), 5.23 (dd, *J*=3.3, 1.0 Hz, 2H, H-2), 4.80 (d, *J*=1.0 Hz, 2H, H-1), 4.62 (s, 2H, $ArCH_2$), 4.28 (dd, *J*=12.2, 5.2 Hz, 2H, H-6a), 4.11 (dd, *J*=12.2, 2.2 Hz, 2H, H-6b), 3.97 (ddd, *J*=9.7, 5.2, 2.2 Hz, 2H, H-5), 3.93 (t, *J*=6.6 Hz, 4H, 2×$ArOCH_2$), 3.68 (m, 2H, alkyl chain H-1′), 3.45 (m, 2H, alkyl chain H-1′), 2.16 (s, 6H, 2×Ac), 2.11 (s, 6H, 2×Ac), 2.05 (s, 6H, 2×Ac), 1.99 (s, 6H, 2×Ac), 1.76 (m, 4H, 2×CH_2), 1.58 (m, 4H, 2×CH_2), 1.26 (m, 32H, 16×CH_2) ppm.

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12. Amphiphile **1a**: ^{1}H NMR (300 MHz, CD_3OD) δ 6.68 (d, *J*=2.0 Hz, 2H, Ar), 6.63 (t, *J*=2.0 Hz, 1H, Ar), 4.45 (s, 2H, $ArCH_2$), 4.20 (d, *J*=6.8 Hz, 2H, H-1), 3.98 (t, *J*=6.3 Hz, 4H, 2×$ArOCH_2$), 3.90 (m, 2H, alkyl chain H-1′), 3.74 (d, *J*=2.6 Hz, 2H, H-4), 3.73 (m, 4H, H-6a and H-6b), 3.54–3.43 (m, 8H, H-2, H-3, H-5 and alkyl chain H-1′), 3.13 (s, 9H, N^+Me_3), 1.75 (m, 4H, 2×CH_2), 1.58 (m, 4H, 2×CH_2), 1.31 (m, 32H, 16×CH_2) ppm. FABMS (positive mode, MNBA as matrix): *m/z* 874 (M^+–Br^-). Amphiphile **1b**: ^{1}H NMR (300 MHz, CD_3OD) δ 6.59 (d, *J*=2.0 Hz, 2H, Ar), 6.58 (t, *J*=2.0 Hz, 1H, Ar), 4.36 (s, 2H, $ArCH_2$), 4.17 (d, *J*=7.7 Hz, 2H, H-1), 3.92 (t, *J*=6.3 Hz, 4H, 2×$ArOCH_2$), 3.90–3.76 (m, 4H, H-6a and alkyl chain H-1′), 3.58 (dd, *J*=11.8, 4.8 Hz, 2H, H-6b), 3.45 (m, 2H, alkyl chain H-1′), 3.30–3.10 (m, 8H, H-2, H-3, H-4 and H-5), 3.08 (s, 9H, N^+Me_3), 1.70 (m, 4H, 2×CH_2), 1.53 (m, 4H, 2×CH_2), 1.24 (m, 32H, 16×CH_2) ppm. FABMS (positive mode, MNBA as matrix): *m/z* 874 (M^+–Br^-). Amphiphile **1c**: ^{1}H NMR (300 MHz, CD_3OD) δ 6.61(d, *J*=2.0 Hz, 2H, Ar), 6.55 (t, *J*=2.0 Hz, 1H, Ar), 4.65 (d, *J*=1.3 Hz, 2H, H-1), 4.38 (s, 2H, $ArCH_2$), 3.92 (t, *J*=6.3 Hz, 4H, 2×$ArOCH_2$), 3.74 (dd, *J*=11.8, 2.3 Hz, 2H, H-3), 3.70 (m, 2H, H-2), 3.66–3.58 (m, 6H, H-6a, H-6b and alkyl chain H-1′), 3.53 (t, *J*=10.6 Hz, 2H, H-4), 3.45 (m, 2H, H-5), 3.32 (m, 2H, alkyl chain H-1′), 3.07 (s, 9H, N^+Me_3), 1.67 (m, 4H, 2×CH_2), 1.58 (m, 4H, 2×CH_2), 1.24 (m, 32H, 16×CH_2) ppm. FABMS (positive mode, MNBA as matrix): *m/z* 874 (M^+–Br^-).