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Glycosidation of Alkylamino-alkan-1-ol. A Simple and Convenient Synthesis of Glycosylated Cationic Lipids

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Abstract—Starting from long chain alkylamino-alkan-1-ol a series of amino glycolipids were synthesized. This procedure allows a short and convenient preparation of glycosylated cationic lipids for gene delivery. © 2002 Published by Elsevier Science Ltd.

Long chain dialkylamines like ditetradecylamine or dioctadecylamine find increasing applications in fields such as liposomes, cationic lipids, or lipid chelating agents.[1] Connection of dialkylamine with an hydrophylic compound at the end of the aliphatic chains would allow the introduction of new properties like targeting, aggregation prevention or charge masking.[2,3]

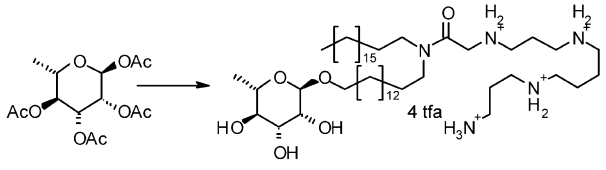
We have recently reported[4] the preparation of glycolipidic polyamimes and the studies of their physico-chemical and transfecting properties. This synthesis has required a sequence of height steps affording the glycolipidic vector in 20% overall yield (Scheme 1).

As we need to prepare a large amount of these new transfecting agents, we envisioned a shorter synthesis of the glycosylated alkylamine by the glycosylation of a long chain alkylamino-alkan-1-ol (Scheme 2).

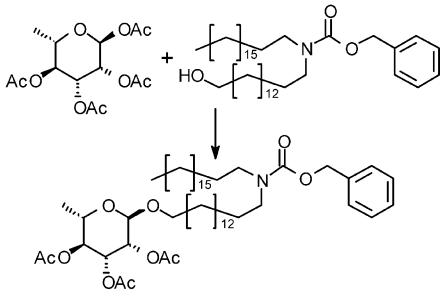
Recent reports have described the glycosidation of carbohydrates with long chain diols using enolates,[5] trichloroacetamidates[6] or anhydro sugars[7] as glycosyl donors. We were attracted by another procedure, that is, the method described by Banoub and Bundle[8] for the glycosylation of a peracetylated carbohydrate with a hydroxy ester.

The synthesis of the 15-(benzyloxycarbonyl-octadecyl-amino)-pentadecyl-ß-D-2,3,4,6-tetra-*O*-acetyl-manno-pyranoside is shown in Scheme 3. The pentadecalone **1** was treated with 2N sodium methoxide in methanol to yield the methyl pentadecanoate **2** in 80% yield. The methyl ester was fused with octadecylamine at 148 °C to give the amide **3** quantitatively.

Reduction of **3** with lithium aluminium hydride afforded the octadecylamino-pentadecan-1-ol **4** (yield 97%). Formation of the benzyloxy derivative **5** was performed by the reaction of **4** with benzylchloroformate in the presence of triethylamine (yield 70%). Treatment of the penta-*O*-acetyl galactopyranose **6** with 1.5 equiv of



Scheme 1. Synthesis of a glycolipidic polyamime.



Scheme 2. Glycosylation of long chain alkylamino-alkan-1-ols.

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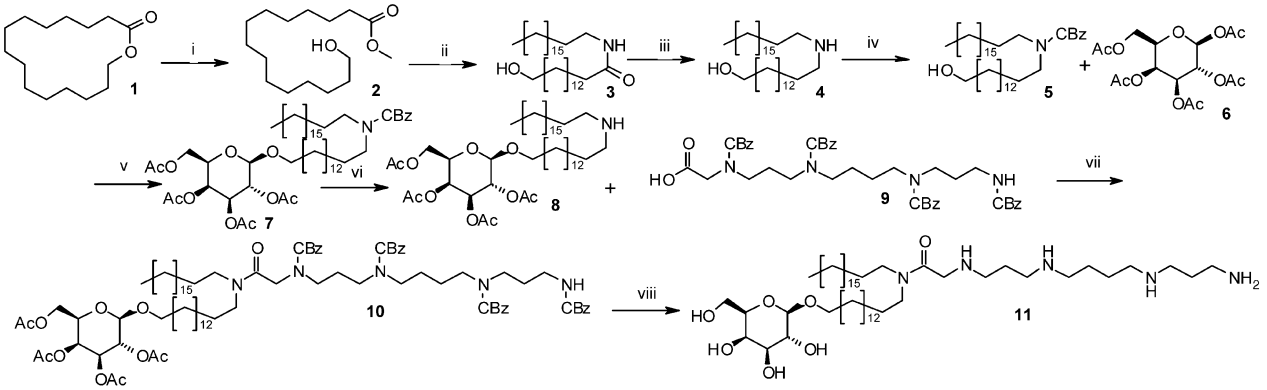

Scheme 3. (i) MeOH rt MeONa (2N in MeOH) 80%; (ii) octadecylamine 148 °C 100%; (iii) $LiAlH_4$ THF 10 h rt 97%; (iv) Benzylchloroformate, CH_2Cl_2 triethylamine rt 70%; v $SnCl_4$ 0 °C, 30 min then rt 3 h, 69%; (vi) Pd/C 10% H_2, MeOH, 100%; (vii) DIPEA, BOP, $CHCl_3$ (83%); (viii) (a) 2 N MeONa in MeOH, (b) Pd/C 10% H_2, MeOH, quantitative.

Table 1. Glycosidation of peracetylated hexopyranose

Entry	Peracetylated glycosides	15-alkylamino-pentadecyl glycosides	Yield (%)[a]
1	6	7	69
2	12	16	50
3	13	17	54
4	14	18	24
5	15	19	26

[a]All yields referred to isolated products.

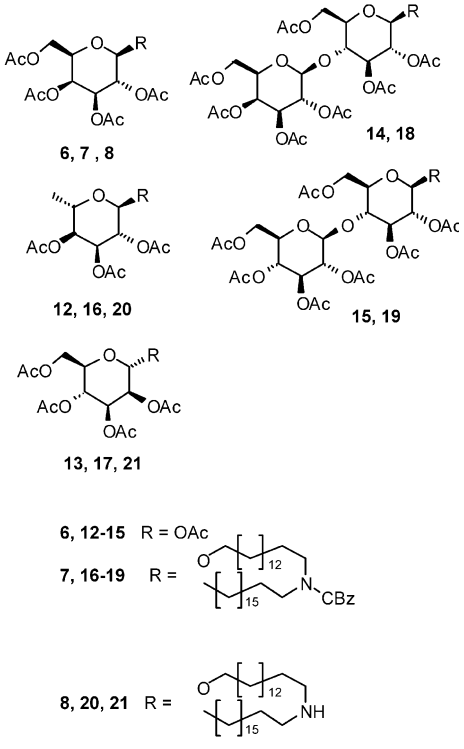

Figure 1.

tin(IV) chloride for 30 min at 0 °C followed by the addition of **5** afforded only the 15-(benzyloxy-carbonyl-octadecyl-amino)-pentadecyl-ß-D-2,3,4,6-tetra-*O*-acetyl-manno-pyranoside **7** in 69% yield.[9] In the same fashion the peracetylated glycosides **12**–**15** were reacted with **5** and the obtained results were outlined in Table 1. For

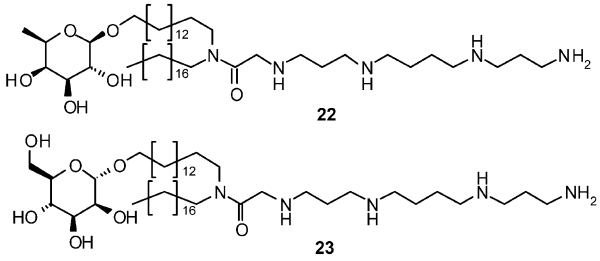

Figure 2.

the monosaccharides the yields range from 50 to 69%. For the disaccharides (Table entries 4–5), the glycosidation led only to modest yields due to longer reaction times that probably favored the cleavage of the glycosidic linkage procedure. However, these yields are superior to those recorded for a classical procedure and led again to a more effective condensation. With the alkylamino-alkyloxy glycosides in hand the synthesis of the polycationic glycolipid was straightforward (Scheme 3). Removal of the benzyloxycarbonyl yielded quantitatively the glycosides **8**, **20**, **21**. Condensation of the amine **8** with the CBz carboxyspermine **9** afforded the protected polycationic glycolipid **10** in 70% yield. Finally, treatment of **10** with sodium methoxide (2N in methanol) followed by the hydrogenation (Pd/C) of the resulting mixture gave the polyamino glycolipid **11**[10] (yield 100%). In the same fashion, the reaction of carboxyspermine **9** with amino-glycosides **20** and **21** afforded respectively the fucosylated and the mannosylated polyamino lipids **22**[11] and **23**[12] (Figs. 1 and 2).

In summary, we herein report a short and high yield preparation of cationic glycolipids. The key step of this synthesis was the direct connection of a long chain secondary amino alcohol to a peracetylated carbohydrate. This methodology will be useful for a wide range of application in lipids and liposomes chemistry.

Acknowledgements

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le Cancer (ARC) the MENRT and the Région Ile-de-France (SESAME).

References and Notes

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9. Typical experimental for the glycosidation of peracetylated carbohydrates by alkylamino-1alkan-1-ol: To a solution of **6** (1.5 g, 4.52 mmol) in dry acetonitrile (50 mL) was added tin(IV) chloride (0.63 mL 5.41 mmol) at 0 °C. The solution was stirred for 30 min then (15-hydroxy-pentadecyl)-octadecyl carbamic acid benzyl ester **5** (3.13 g, 4.97 mmol) was added. After 5 h all **6** has disappeared and the reacting mixture was diluted with diethyl ether and quenched with sodium hydrogen phosphate. The aqueous phase was extracted with diethyl ether and the combined organic extracts were washed with sodium hydrogen carbonate and brine, dried ($MgSO_4$). After removal of the solvent the resulting oil was purified (flash chromatography, heptane–ethyl acetate 6:4) to give **7** in 69% yield. **7** $[\alpha]_D = -12$ (*c* 0.66 CH_2Cl_2); MS (CI), *m/e* 918 ($M + NH_4+$); ^1H NMR ($CDCl_3$) δ ppm 0.87 (t, 3H, *J* = 6.96 Hz, H-33), 1.2 (d, 3H, *J* = 6.51 Hz, H-6), 1.25 (m, 54H, $(CH_2)27$), 1.52 (m, 6H, $OCH_2\underline{CH_2}$, H-14 and H-17), 1.95, 2.05 and 2.15 (s, 3H, $OCO\underline{CH_3}$), 3.14–3.25 (m, 4H, H-15 and H-16), 3.44 (m, 1H, $OCHaCH_2$), 3.63 (m, 1H, $OCHbCH_2$), 3.79 (m, 1H, H-5), 4.41 (d, 1H, *J* = 7.98 Hz, H-1), 4.99 (dd, 1H, *J* = 3.52 and 10.46 Hz, H-3), 5.09 (s, 2H, $O\underline{CH_2}$Phe), 5.16 (dd, 1H, *J* = 7.98 and 10.46 Hz, H-2), 5.23 (dd, *J* = 3.52 and 3.31 Hz, H-4), 7.32 (m, 5H, Phe).
^{13}C RMN ($CDCl_3$) δ (ppm) 14.7 (C-33), 17.3 (C-2), 20.7 ($\underline{CH_3}COO$), 27.3 (C-6), 29.72 ($(CH_2)27$), 25.9–32 ($OCH_2\underline{CH_2}$, C-14, C-17), 47.2–48 (C-15 and C-16), 66.9 ($\underline{CH_2}$Phe), 69.6 ($O\underline{CH_2}CH_2$), 69.4 (C-2), 70.6 (C-5), 70.8 (C-4), 71.4 (C-3), 96.2 (C-1), 128.4 (Phe), 156.2 and 171.3 (CO). Anal. calcd. for $C_{53}H_{91}NO_{10}$: C, 70.55; H, 10.17; N, 1.55. Found: C, 70.67; H, 10.23; N, 1.71.
10. 2-{3-[4-(3-Amino-propyl-amino)-butylamino]-propylamino}-N-octadecyl-N-[15-pentadecyl-(ß-D-galactopyranosyl)]-acetamide (**11**).
$[\alpha]_D = 0$ (*c* 0.1 MeOH); ^1H NMR ($CDCl_3$) δ ppm 0.88 (t, 3H, m, 1H, H-18 octadecyl), 1.21 (m, 48H, H-3, 13 pentadecyl and H-4.16 octadecyl), 1.26 (m, 4H, H-13 pentadecyl and H-3 octadecyl), 1.28 (m, 2H, H-17 octadecyl), 1.43 (m, 2H, H-14 pentadecyl), 1.46 (m, 6H, H-2 octadecyl, H-2 and H-3 butyl), 1.51 (m, 2H, H-2 pentadecyl), 1.60 (m, 2H, H-2 propyl), 1.64 (m, 2H, H-2 propyl), 2.62 (m, 2H, H-3-propyl), 2.63 (m, 4H, H-1 propyl and H-4 butyl), 2.64 (m, 2H, H-1 butyl), 2.72 (m, 2H, H-3 propyl), 2.74 (m, 2H, H-1 propyl), 3.14 (m, 2H, H-15 pentadecyl), 3.32 (m, 2H, $NHC\underline{H_2}CO$), 3.35 (m, 2H, H-1 pentadecyl and H-1 octadecyl), 3.53 (m, 1H, H-2 gal), 3.58 (m, 1H, H-5 gal), 3.60 (m, 1H, H-4 gal), 3.72 (m, 2H, H-6 gal), 4.03 (m, 1H, H-3 gal), 4.17 (m, 2H, H-1 gal).
^{13}C RMN ($CDCl_3$) δ 14.09 (C-18 octadecyl), 22.69 (C-17 octadecyl), 25.99 (C-3 pentadecyl), 27.57 (C-3 octadecyl), 27.75 (C-13 pentadecyl), 29.3--30.2 (C-2, C-4, 16 octadecyl, C-4, 12 pentadecyl, C-14 pentadecyl), 32.70 (C-16 octadecyl), 40.23 (C-1 propyl), 46.24 (C-1 octadecyl), 47.02 (C-15 pentadecyl), 47.63 (C-3-propyl), 48.01 (C-3 propyl), 48.25 (C-1 propyl), 49.64 (C-4 butyl), 50.25 (C-1 butyl), 50.60 ($CO\underline{CH_2}NH$), 61.30 (C-1 pentadecyl), 68.85 (C-6 gal), 70.00 (C-3 gal), 71.51 (C-4 gal), 73.93 (C-5 gal), 74.71 (C-2 gal), 103.64 (C-1 gal), 170.54 (NHCO). Anal. calcd. for $C_{51}H_{105}N_5O_7$, 2 H_2O C 65.45, H 11.65, N 7.48. Found C 65.68, H 11.32, N 7.32.
Purification Supelco C-8 reverse phase cartrige (100% water to 100% acetonitrile then acetonitrile-MeOH 90--10).
11. 2-{3-[4-(3-Amino-propylamino)-butylamino]-propyl-amino}-*N*-octadecyl-*N*-[15-pentadecyl(ß-D-manno-pyranosyl)]-acetamide (**22**).
$[\alpha]_D = 0$ (*c* 0.1 MeOH); ^1H NMR (pyridine-d_6) δ ppm 0.77 (m, 3H, H-18 octadecyl), 1.22 (m, 46H, H-3, 12 pentadecyl and H-4, 17 octadecyl) 1.26 (m, 4H, H-13 pentadecyl and H-3 octadecyl), 1.42 (m, 2H, H-14 pentadecyl), 1.49 (m, 2H, H-2 pentadecyl), 1.52 (m, 2H, H-2 octadecyl), 1.83 (m, 6H, H-2 butyl, H-2 propyl and H-3 butyl), 1.87 (m, 2H, H-2 propyl), 2.71 (m, 4H, H-1 and H-4 butyl), 2.72 (m, 2H, H-3-propyl), 2.88 (m, 4H, H-1 and H-3 propyl), 3.03 (m, 2H, H-1 propyl), 3.18 (m, 2H, H-15 pentadecyl), 3.4 (m, 2H, H-1 pentadecyl), 3.43 (m, 2H, H-1 octadecyl), 3.56 ($NHC\underline{H_2}CO$), 3.84 (m, 2H, H-1 pentadecyl), 4.33 (m, 2H, H-6 man), 4.44 (m, 2H, H-6 man), 4.19 (m, 2H, H-4 man), 4.48 (m, 2H, H-2 and H-5 man), 4.57 (m, 2H, H-3 man), 5.24 (m, 2H, H-1 man).
^{13}C NMR (pyridine-d_6) δ ppm 14.14 (C-18 octadecyl), 22.79 (C-17 octadecyl), 26.45 (C-3 pentadecyl), 27.03 (C-3 octadecyl), 27.26 (C-13 pentadecyl), 29.20–30.2 (C-2, C-4, 15 octadecyl, C-4, 12 pentadecyl, C-14 pentadecyl), 31.71 (C-2 pentadecyl), 31.99 (C-16 octadecyl), 40.40 (C-1 propyl), 46.97 (C-1 octadecyl), 47.61 (C-15 pentadecyl), 47.85 (C-3-propyl), 48.13 (C-3 propyl), 48.46 (C-1 propyl), 48.78 (C-4 butyl), 48.98 (C-1 butyl), 50.55 ($CO\underline{CH_2}NH$), 62.94 (C-1 pentadecyl), 62.94 (C-1 pentadecyl), 67.38 (C-6 man), 67.38 (C-6 man), 68.99 (C-3 man), 72.05 (C-4 man), 72.98 (C-5 man), 75.10 (C-2 man), 101.42 (C-1 man), 170.98 NHCO). Anal. calcd. for $C_{51}H_{105}N_5O_7$, 0.5 H_2O C; 67.40, H; 11.67, N; 7.70. Found C; 67.11, H; 11.32, N; 7.09.
Purification Supelco C-8 reverse phase cartrige (100% water to 100% acetonitrile then acetonitrile-MeOH 90–10).
12. 2-{3-[4-(3-Amino-propylamino)-butylamino]-propyl-amino}-*N*-octadecyl-*N*-[15-pentadecyl-(α-L-6-deoxy-galactopyranosyl)]-acetamide(**23**).
$[\alpha]_D = 0$ (*c* 0.1 MeOH); ^1H NMR ($CDCl_3$) δ ppm 0.81 (m, 3H, H-18 octadecyl), 1.18 (m, 6H, H-3 octadecyl, H-17 octadecyl and H-13 pentadecyl), 1.2 (m, 48H, H-3, 13 pentadecyl and H-4, 16 octadecyl), 1.22 (m, 2H, H-3 pentadecyl), 1.32 (m, 3H, H-6 fuc), 1.5 (m, 2H, H-2 pentadecyl), 2.51 (m, 2H, H-4 butyl), 2.51 (m, 2H, H-1 butyl), 2.58 (m, 2H, H-3-propyl), 2.59 (m, 2H, H-1 propyl), 2.73 (m, 2H, H-1 propyl), 3.08 (m, 2H, H-3 propyl), 3.09 (m, 2H, H-1 octadecyl), 3.14 (m, 2H, H-15 pentadecyl), 3.26 (m, 2H, $NHC\underline{H_2}CO$), 3.41 (m, 2H, H-1 pentadecyl), 3.48 (m, 2H, H-3 fuc), 3.48 (m, 2H, H-4 fuc), 3.62 (m, 2H, H-5 fuc), 3.76 (m, 2H, H-1 pentadecyl), 3.94 (m, 2H, H-2 fuc), 4.22 (m, 2H, H-1 fuc).

^{13}C NMR ($CDCl_3$) δ ppm 14.7 (C-18 octadecyl), 16.9 (C-6 fuc), 22.5 (C-17 octadecyl), 26.2 (C-3 pentadecyl), 27.1 (C-3 octadecyl), 27.8 (C-13 pentadecyl), 29.4–30.2, (C-2, C-4.16 octadecyl, C-4.12 pentadecyl, C-14 pentadecyl), 32 (C-2 pentadecyl), 40.6 (C-1 propyl), 46.5 (C-1 octadecyl), 47.2 (C-15 pentadecyl), 47.9 (C-3-propyl), 47.9 (C-3 propyl), 48.3 (C-1 propyl), 48.7 (C-4 butyl), 50 (C-1 butyl), 50.6 ($CO\underline{C}H_2NH$), 69.9 (C-3 fuc), 70.2 (C-1 pentadecyl), 70.2 (C-1 pentadecyl), 70.3 (C-4 fuc), 71.7 (C-5 fuc), 74.5 (C-2 fuc), 104 (C-1 fuc), 171 ($NH\underline{C}O$). Anal. calcd for $C_{51}H_{105}N_5O_6$, 0.5 H_2O C; 68.60, H; 11.88, N; 7.84. Found C; 68.49, H; 11.48, N; 7.03.

Purification Supelco C-8 reverse phase cartrige (100% water to 100% acetonitrile then acetonitrile–MeOH 90:10).