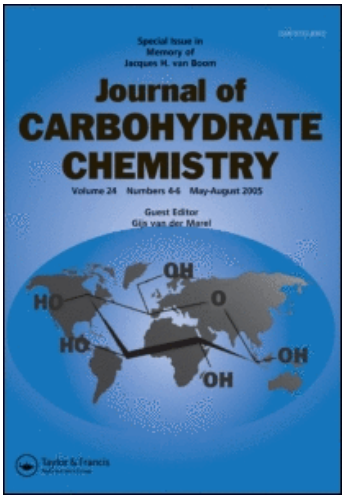

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Synthesis of Neoglycolipids Containing Oligosaccharides Based on 3,6-Branched-α-D-Mannopyranosides as the Carbohydrate Moieties

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SYNTHESIS OF NEOGLYCOLIPIDS CONTAINING OLIGOSACCHARIDES BASED ON 3,6-BRANCHED-α-D-MANNOPYRANOSIDES AS THE CARBOHYDRATE MOIETIES[1]

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ABSTRACT

Several oligosaccharides containing 3,6-branched-α-D-mannopyranosides were obtained by selective glycosylation of 5-azido-3-oxapentyl α-D-mannopyranoside with acetobromosugars. After deacetylation and reduction of the spacer azido group, the oligosaccharides were coupled with the activated hemisuccinate derivatives of cholesterol and 1,2-di-*O*-alkylglycerol. The neoglycolipids so obtained were characterized by NMR spectroscopy and will be used as liposome coating molecules for targeting entrapped antigens.

INTRODUCTION

Gregoriadis[2] suggested the possibility of inducing a T-dependent response against a B-cell epitope peptide, by coadministering it together with a T-cell epitope peptide in a liposome. In our first experiment in this area,[3] we coated a meningococcal outer membrane proteoliposome (a source of T-epitope) with neoglycolipid containing the H-1 oligosaccharide. The response against the oligosaccharide was very low, so this

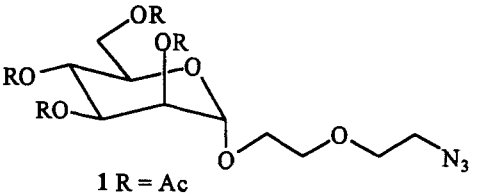
approach offered the possibility of using the neoglycolipid construct to deliver antigens or drugs entrapped in liposomes without any important undesired anti-neoglycolipid immune response.

Many cells and tissues express carbohydrate-specific receptor lectins and could be accessible to neoglycolipid-coated liposomes.[4,5,6] The addition of glycolipids to liposomes can also induce a change in the uptake by the mononuclear phagocyte system.[7]

Recently,[8] a neoglycolipid containing the Man-α-(1-3)-[Man-α-(1-6)]-Man trisaccharide was found to induce cellular immunity against a specific antigen. This result inspired us to initiate a project aimed at modulating the immune response against carbohydrate-conjugated antigens by entrapping them in liposomes coated with artificial neoglycolipids. In the present paper, we describe the synthesis of neoglycolipids via the preparation of 3,6-branched mannose-containing oligosaccharide derivatives and their coupling through a diethylenglycol bridge with two different lipid anchors.

RESULTS AND DISCUSSION

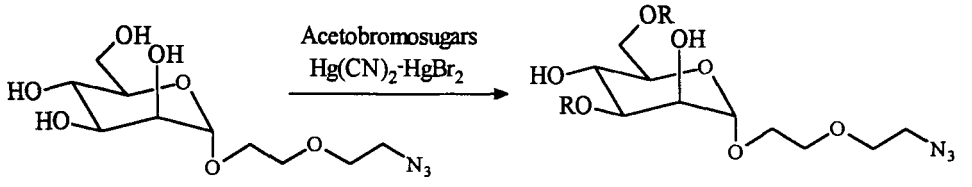
The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide with 5-azido-3-oxa-1-pentanol[9,10] in the presence of mercury(II) cyanide and mercury(II) bromide gave mannoside **1** that, after deacetylation using Zemplen conditions, afforded acceptor **2** in good yield.



1 R = Ac
2 R = H

The selective diglycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide was performed as previously reported by Kaur and Hindsgaul.[11] The reaction proceeded surprisingly well, affording, after column chromatography, the 3,6-branched trisaccharide as the sole component of the trisaccharide fraction. The 34% yield was higher that obtained (17%)[11] when the acceptor was octyl β-D-mannopyranoside. The structure of **3** was ascertained by NMR spectroscopy. Typical[12] singlets were observed for the anomeric protons at 5.13, 4.98 and 4.85 ppm in the ^{1}H NMR spectra thus indicating that the configurations around the

anomeric centers were α; in the ^{13}C NMR spectra, the signals corresponding to C-3 and C-6 of the central mannopyranose were deshielded, appearing at 81.5 and 66.4 ppm, respectively. The selectivity of the reaction was the same for the acetobromo derivatives of D-galactose (→ **4**, 55 %), D-glucose (→ **5**, 28 %), L-rhamnose (→ **6**, 50 %) and for that of the disaccharide lactose (→ **7**, 28 %). The specificity was confirmed in all cases by the deshielding in the ^{13}C NMR spectra of the signals corresponding to C-3 of the central mannose units (83.9, 83.6, 78.6 and 83.6 ppm, respectively).

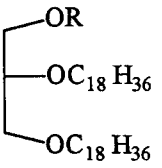


2

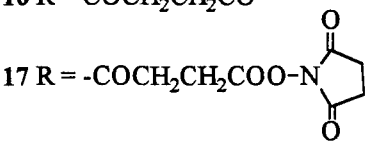
3 R = $(OAc)_4$-α-D-Manp-
4 R = $(OAc)_4$-β-D-Galp-
5 R = $(OAc)_4$-β-D-Glcp-
6 R = $(OAc)_3$-α-L-Rhap-
7 R = $(OAc)_4$-β-D-Galp-(1-4)-$(OAc)_3$-β-D-Glcp-

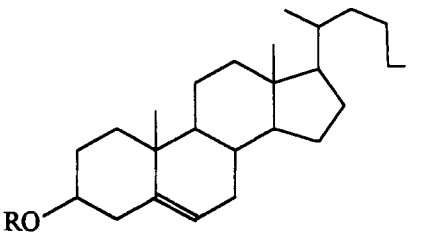
The trisaccharides was deacetylated with sodium methoxide in methanol and the azido groups were reduced (H_2, Pd/C 10 %) to afford oligosaccharides **8-12**. The presence of the spacer amino groups was established for each compound by the observation of a triplet located at 3.40 ppm in the ^1H NMR spectrum and a signal at δ 40.7 ppm in the ^{13}C NMR spectrum corresponding to CH_2NH_2.

Several lipid tails have been used for the construction of spacered neoglycolipids.[13] We selected the dioctadecylglyceryl and cholesteryl hemisuccinates (**16** and **19**, respectively) activated for coupling to the spacer amino function through their *N*-succinimidyl esters (**17** and **20**, respectively). The synthesis of **17** was achieved starting from 1-*O*-benzylglycerol that was octadecanylated in 77% yield. The benzyl group was hydrogenolyzed and the remaining free hydroxyl group was succinylated with succinic anhydride in pyridine-chloroform in the presence of DMAP (→ **15**, 70%). The carboxylic functions of both compounds **15** and **18** were activated by the reaction with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide.[14]

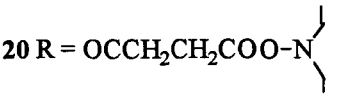


13 R = Bn
14 R = H
15 R = $COCH_2CH_2COOH$
16 R = $COCH_2CH_2CO^-$



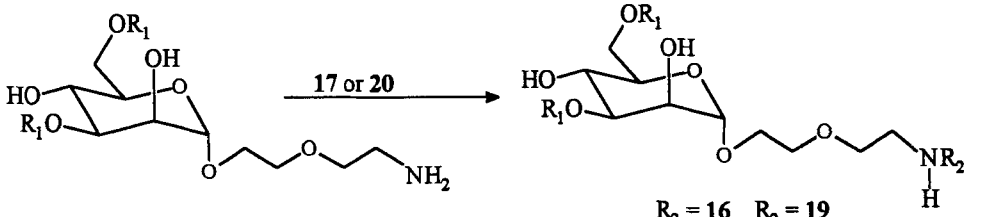


18 R = $OCCH_2CH_2COOH$
19 R = $OCCH_2CH_2CO^-$



The coupling between **8** and the lipid derivatives **17** and **20** was accomplished in dimethyl sulfoxide in the presence of triethylamine to afford the corresponding neoglycolipids **21** and **22** in 65 and 63% yields, respectively.

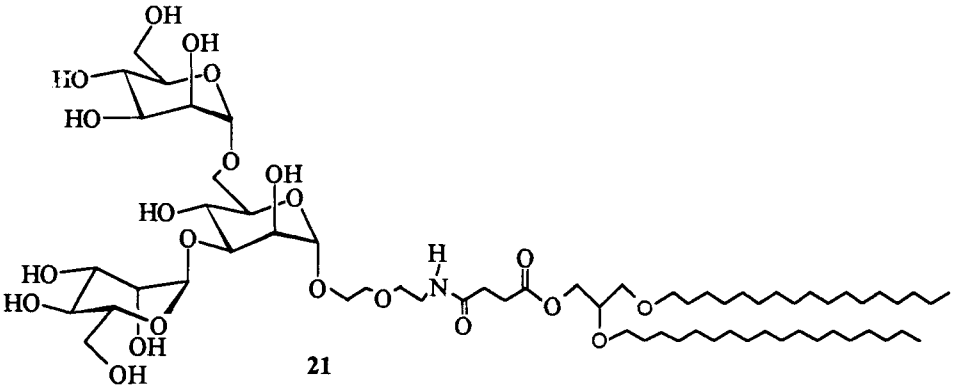
The reaction between **9-12** and the lipid derivatives **17** and **20** proceeded in a similar fashion giving the corresponding neoglycolipids in 60-65% yields.



8 R_1 = α-D-Manp-
9 R_1 = β-D-Galp-
10 R_1 = β-D-Glcp-
11 R_1 = α-L-Rhap-
12 R_1 = β-D-Galp-(1-4)-β-D-Glcp-

	R_2 = 16	R_2 = 19
8 →	**21**	**22**
9 →	**23**	**24**
10 →	**25**	**26**
11 →	**27**	**28**
12 →	**29**	**30**

Preliminary results indicated that coating liposomes with **21** gave rise to an IgG1→IgG2 shift of the immune response against entrapped antigens. Further characterization of the change induced by neoglycolipids **21-30** on the immune response against entrapped antigens will be reported in due course.



21

EXPERIMENTAL

General procedures. Optical rotations were measured at 25 °C with a POLAMAT A automatic polarimeter, using a 5 cm 5 mL cell. NMR spectra were recorded at 25 °C with a BRUKER AC-250F spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal tetramethylsilane for ^{1}H NMR spectra and indirectly to the central line of $CDCl_3$, δ 77.03, for ^{13}C NMR spectra. Assignments were made on the basis of homonuclear and heteronuclear correlation experiments. The following notation was used to define the NMR signals: **a** for the central mannose, **b** and **c** for the (1-6)- and (1-3) linked monosaccharides respectively; **b'** and **c'** for the galactose unit of lactose in compounds **7**, **12**, **29** and **30** and **g** and **ch** for glycerol and cholesterol backbones, respectively.

All compounds were purified by column chromatography on Kieselgel 60 (Fluka, < 230 mesh ASTM) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by charring with sulfuric acid after examination under UV light. Evaporations were conducted under reduced pressure at 40 °C (bath). Elemental analyses were performed at the National Center for Scientific Research (CNIC).

5-Azido-3-oxapentyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (1). A solution of freshly prepared 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (1.05 g, 2.58 mmol) in dry acetonitrile (10 mL) was added to a stirred mixture of 5-azido-3-oxa-1-pentanol (0.37 mL, 2.82 mmol), mercury(II) cyanide (650 mg, 2.56 mmol), mercury(II) bromide (925 mg, 2.56 mmol) and 0.3 nm powdered molecular sieves in acetonitrile (8 mL). After the mixture had been stirred for 1 h at rt, dichloromethane (20 mL) was

added and the resulting mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The syrupy residue was extracted with dichloromethane (4 x 10 mL), the combined extracts were filtered, and the resulting solution was washed with 10 % aqueous potassium iodide, saturated aqueous bicarbonate and water, dried, filtered and concentrated. The residual syrup was chromatographed using dichloromethane/acetone 20:1 (v/v) as eluant to afford **1** as a colorless syrup (860 mg, 75%); $[\alpha]_D$ +24.3° (*c* 1.03, chloroform); R_f 0.51 (ethyl acetate/hexane 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.32 (m, 3H, H-2, 3, 4), 4.89 (s, 1H, H-1), 4.30 (dd, 1H, $J_{6,6a}$ = 10.1 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.08 (m, 1H, H-5), 3.85 (dd, 1H, $J_{5,6b}$ = 4.2 Hz, H-6b), 3.48 (m, 6H, 3CH_2O), 3.34 (t, 2H, J = 5.1 Hz, CH_2N_3), 2.10-1.98 (4s, 12H, 4xCH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.5, 169.4, 169.3 (C=O), 97.2 (C-1), 69.0 (C-5), 68.2 (C-2), 66.8 (C-3), 65.6 (C-4), 62.0 (C-6), 50.2 (CH_2N_3), 20.4, 20.3, 20.2 (CH_3CO).

Anal. Calcd for $C_{18}H_{27}N_3O_{11}$ (461.42): H, 5.90; C, 46.85; N, 9.11. Found : C, 46.61; H, 6.01; N, 9.03.

5-Azido-3-oxapentyl 3,6-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (3). Compound **1** (860 mg, 1.86 mmol) was dissolved in a freshly prepared solution of methanolic sodium methoxide (0.01 M). After being stirred for 30 min at rt, the solution was neutralized with Amberlite IR 120 (H^+), filtered and concentrated to afford *5-azido-3-oxapentyl α-D-mannopyranoside* (**2**) as a colorless syrup (530 mg, 97%); R_f = 0.42 (ethyl acetate/methanol 3:1 v/v). A mixture of **2** (200 mg, 0.68 mmol), mercury(II) bromide (615 mg, 1.7 mmol), mercury(II) cyanide (430 mg, 1.7 mmol) and 0.3 nm powdered molecular sieves (2 g) in acetonitrile (10 mL) was stirred for 30 min at rt. A solution of freshly prepared 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (700 mg, 1.7 mmol) in dry acetonitrile (10 mL) was then added dropwise and the mixture was further stirred for 1 h. The suspension was then filtered through Celite, the solid material rinsed with methanol (20 mL) and the filtrate was evaporated to dryness. The syrupy residue was extracted with dichloromethane (15 mL x 4), the combined extracts were then filtered and the filtrate was successively washed with 10 % aqueous potassium iodide (20 mL), saturated sodium bicarbonate (20 mL) and water (20 mL). The organic solution was dried using $MgSO_4$, filtered and concentrated. Column chromatography of the residue using dichloromethane/acetone 5:1 (v/v) as eluent afforded **3** as a colorless foam (215 mg, 34%); $[\alpha]_D$ = +69° (*c* 1.45, chloroform);

R_f 0.55 (dichloromethane/acetone 4:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.30 (m, 6H, H-2b, 3b, 4b, 2c, 3c, 4c), 5.13 (s, 1H, H-1c), 4.98 (s, 1H, H-1b), 4.85 (s, 1H, H-1a), 4.35 (m, 1H, H-5c), 4.23 (m, 4H, H-6c, 6`c, 6b, 6`b), 4.14 (m, 2H, H-5b, 2a), 3.95 (m, 1H, H-4a), 3.86 (m, 4H, H-3a, 5a, 6a, 6`a), 3.66 (m, 6H, 3xCH_2O), 3.38 (t, 2H, J = 5.1 Hz, CH_2N_3), 3.29 (d, J = 3.5 Hz, 1H, O*H*-4a), 2.95 (d, J = 5.6 Hz, 1H, O*H*-2a), 2.18-2.03 (8s, 24H, 8xCH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.5-169.7 (C=O), 99.8 (C-1a), 99.1 (C-1c), 97.2 (C-1b), 81.5 (C-3a), 71.5 (C-5a), 69.9 (C-2a), 69.6, 69.3, 69.1, 68.9 (C-2b, 2c, 3b, 3c), 68.9 , 68.3 (C-5b, 5c), 66.4 (C-6a), 66.0 (C-4b, 4c), 65.6 (C-4a), 62.7, 62.4 (C-6b, 6c), 50.2 (CH_2N_3), 20.8-20.2 (CH_3CO).

Anal. Calcd for $C_{38}H_{55}O_{25}N_3$ (953.86): C, 47.85; H, 5.81; N, 4.41. Found: C, 47.46; H, 5.91; N, 4.28.

5-Azido-3-oxapentyl 3,6-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-mannopyranoside (4). Crystalline 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (700 mg, 1.7 mmol) was added to a solution of **2** (200 mg, 0.68 mmol), mercury(II) cyanide (200 mg, 0.68 mmol), mercury(II) bromide (615 mg, 1.7 mmol), and 0.3 nm molecular sieves in acetonitrile and the reaction was performed as previously described for the preparation of **3** to give **4** as a foam (347 mg, 55%); $[\alpha]_D$ = +9.3° (*c* 1.05, chloroform); R_f 0.54 (dichloromethane/acetone 4:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.40 (m, 2H, H-4b, 4c), 5.20 (m, 2H, H-2b, 2c), 5.05 (m, 2H, H-3b, 3c), 4.85 (d, 1H, J = 1.3 Hz, H-1a), 4.58, 4.55 (2d, 2H, J = 7.8 Hz, H-1b, 1c), 4.29 (s, 1H, O*H*-4), 4.25 (s, 1H, O*H*-2), 4.17 (m, 4H, H-6b, 6'b, 6c, 6'c), 4.15-3.85 (m, 3H, H-5b, 5c, 2a), 3.80-3.62 (m, 10H, H-3a, 4a, 5a, 6a, 6`a, CH_2O), 3.40 (t, 2H, J = 5.1 Hz, CH_2N_3), 2.18-2.03 (m, 24H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.6-169.7 (C=O), 101.7 (C-1b, 1c), 98.9 (C-1a), 83.9 (C-3a), 71.1, 70.4 (C-5b, 5c), 70.8 (C-5a), 70.6, 70.3 (C-3b, 3c), 69.5 (C-2a), 68.8, 68.7 (C-2b, 2c), 66.8, 66.6 (C- 4b, 4c), 65.8 (C-6a), 65.6 (C-4a), 61.4, 61.0 (C-6b, 6c), 50.4 (CH_2N_3), 20.6-20.4 (CH_3CO).

Anal. Calcd for $C_{38}H_{55}O_{25}N_3$ (953.86): C, 47.85; H, 5.81; N, 4.41. Found: C, 48.05; H, 5.25; N, 4.35.

5-Azido-3-oxapentyl 3,6-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-mannopyranoside (5). Crystalline 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (700 mg, 1.7 mmol) was added to a solution of **2** (200 mg, 0.68 mmol), mercury(II) cyanide (430 mg, 1.7 mmol), mercury(II) bromide (615 mg, 1.7 mmol) and

0.3 nm molecular sieves in acetonitrile and the reaction was performed as previously described for the preparation of **3** to give **5** as a syrup (177 mg, 28%); $[\alpha]_D$ = +8.5° (*c* 1.00, chloroform); R_f 0.54 (dichloromethane/acetone 4:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.20 (dd, $J_{2,3}$ = 9 Hz, $J_{3,4}$ = 7.8 Hz, 2H, H-3b, 3c), 5.11- 4.97 (m, 4H, H-2b, 2c, 4b, 4c), 4.85 (d, J = 1.2 Hz, 1H, H-1a), 4.62, 4.59 (2d, J = 7.8 Hz, 2H, H-1b, 1c), 4.26- 4.10 (m, 4H, H-6c, 6'c, 6b, 6'b), 3.40 (t, 2H, J = 5.1 Hz, C*H*$_2$N$_3$), 2.18-2.03 (m, 24H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$) δ 170.6- 169.7 (C=O), 101.1 (C-1b, 1c), 99.0 (C-1a), 83.6 (C-3a), 72.2 72.0 (C-3b, 3c), 71,8. 71.5 (C-5b, 5c), 71.1 (C-2b, 2c), 70.9 (C-5a), 69.5 (C-2a), 68.3, 68.2 (C-4b, 4c), 66.0 (C-6a), 65.4 (C-4a), 61.7, 61.6 (C-6b, 6c), 50.4 (*C*H$_2$N$_3$), 20.6-20.4 (*C*H$_3$CO).

Anal. Calcd for $C_{38}H_{55}O_{25}N_3$ (953.86): C, 47.85; H, 5.81; N, 4.41. Found: C, 48.66; H, 5.92; N, 4.31.

5-Azido-3-oxapentyl 3,6-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-mannopyranoside (6). A solution of 2,3,4,6-tetra-*O*-acetyl-α-L-rhamnopyranosyl bromide (564 mg, 1.7 mmol) in acetonitrile was added to a solution of **2** (200 mg, 0.68 mmol), mercury(II) cyanide (430 mg, 1.7 mmol), mercury(II) bromide (615 mg, 1.7 mmol) and 0.3 nm molecular sieves in acetonitrile (10 mL) and the reaction was performed as previously described for the preparation of **3** to give **6** as a syrup (272 mg, 50%); $[\alpha]_D$ = -10.1° (*c* 1.00, chloroform); R_f 0.57 (dichloromethane/acetone 4:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.30 (m, 4H, H-2b, 2c, 3b, 3c), 5.18 (m, 2H, H-4b, 4c), 4.96 (s, 1H, H-1b or 1c), 4.90 (s, 2H, H-1a, 1b or 1c), 4.24 (m, 1H, H-5b or 5c), 4.06-3.82 (m, 4H, H-2a, 5b or 5c, 3a, 4a), 3.40 (t, 2H, CH_2N_3), 2.20-1.98 (m, 18H, C*H*$_3$CO), 1.25 (d, 6H, H-6b, 6c); ^{13}C NMR ($CDCl_3$) δ 170.3-169.7 (C=O), 99.4 (C-1a), 97.8, 95.2 (C-1c, 1b), 78.3 (C-3a), 71.5 (C-5a), 70.7-70.5 (C-4b, 4c), 69.0, 68.9 (C-2b, 2c, 3b, 3c), 67.5 (C-2a), 66.7, 66.2 (C-5b, 5c), 66.4 (C-6a), 65.7 (C-4a), 50.4 (*C*H$_2$N$_3$), 20.7-20.5 (*C*H$_3$CO), 17.2 (C-6b, 6c).

Anal. Calcd for $C_{34}H_{55}O_{21}N_3$ (837.79): C, 48.74; H, 6.14; N, 5.02. Found: C, 48.60; H, 6.35; N, 5.16.

5-Azido-3-oxapentyl 3,6-Di-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-β-D-glucopyranosyl)-α-D-mannopyranoside (7). Crystalline 2,2',3,3',4',6,6'-hepta-*O*-acetyl-α-lactosyl bromide (896 mg, 1.7 mmol) was added to a solution of **2** (200 mg, 0.68 mmol), mercury(II) cyanide (430 mg, 1.7 mmol), mercury(II)

bromide (615 mg, 1.7 mmol) and 0.3 nm molecular sieves in acetonitrile (15 mL) and the reaction was performed as previously described for the preparation of **3** to give **7** as a foam (228 mg, 26%); $[\alpha]_D$ = +9.4° (*c* 1.00, chloroform); R_f 0.37 (dichloromethane/ acetone 4:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.36 (m, 2H, H-4b',4c'), 5.23 (dd, 2H, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 7.9 Hz, H-3b, 3c), 5.11 (m, 2H, H-2b', 2c'), 4.99 (m, 2H, H-3b', 3c'), 4.93 (m, 4H, H-2b, 2c), 4.86 (d, 1H, J = 1.1 Hz, H-1a), 4.61 (m, 2H, H-1b, 1c), 4.56 (m, 2H, H-1b', 1c'), 4.26-4.10 (m, 8H, H-6b, 6'b, 6b', 6'b', 6c, 6'c, 6c', 6'c'), 3.94 (m, 1H, H-5b, 5b'), 3.90 (m, 1H , H-2a), 3.80-3.62 (m, 10H, H-4a, 5a, 6a, 4b, 4c, CH_2O), 3.40 (t, 2H, J = 5.1 Hz, CH_2N_3), 2.20-1.90 (m, 24H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.6-169.7 (C=O), 100.9, 100.7 (C-1b', 1c', 1b, 1c), 98.9 (C-1a), 83.6 (C-3a), 75.9 (C-4a, 4b), 72.3 71.9 (C-3b, 3c), 71.5, 71.4 (C-2b, 2c), 70.4 (C-3b', 3c'), 70.3 (C-2a), 68.8 (C-2b', 2c'), 66.4 (C-4b', 4c'), 65.9 (C-6a), 65.2 (C-4a), 61.8, 61.4, 60.6 (C-6b, 6c, 6b', 6c'), 50.4 (CH_2N_3), 20.6-20.2 (CH_3CO).

Anal. Calcd for $C_{62}H_{87}O_{41}N_3$ (1530.34): C, 48.66; H, 5.17; N, 2.74. Found: C, 48.51; H, 5.32; N, 2.83.

5-Amino-3-oxapentyl 3,6-Di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (8). Compound **3** (215 mg, 0.34 mmol) was dissolved in a freshly prepared solution of methanolic sodium methoxide (0.01 M, 5 mL). After being stirred for 30 min at rt, the solution was neutralized with Amberlite IR 120 (H^+) resin, filtered and the resin washed with methanol (5 mL). The combined solutions were hydrogenolized over 10% Pd/C (100 mg) for 12 h. The catalyst was removed by filtration and rinsed with water (10 mL), then the combined filtrate and washings were concentrated to afford **8** as a colorless powder (190 mg, 95%), which was used in the next step without further purification; R_f 0.35 (methanol); ^{1}H NMR (D_2O) δ 5.15 (s, 1H, H-1c), 4.94 (s, 1H, H-1b), 4.90 (s, 1H, H-1a), 4.17 (dd, 1H, H-2a), 4.11 (dd, 1H, H-2c), 4.05 (m, 1H, H-6a), 4.01 (dd, 1H, H-2b), 3.95 (m, 1H, H-3a), 3.25 (t, 2H, J = 4.8 Hz, CH_2NH_2); ^{13}C NMR (D_2O) δ 103.5 (C-1c), 101.2 (C-1a), 100.4 (C-1b), 79.7 (C-3a), 74.5, 73.8 (C-5b, 5c), 72.2 (C-5a), 71.8 (C-3c), 71.5(C-3a), 70.7 (C-3b), 71.2 (C-2c), 71.1 (C-2b), 70.7 (C-2a), 68.0, 67.8 (C-4b, 4c), 66.7 (C-4a), 66.3 (C-6a), 62.2, 62.1 (C-6b, 6c), 40.3 (CH_2NH_2).

Anal. Calcd. for $C_{26}H_{56}NO_{17}$ (654.72): H, 8.62; C, 47.70; N, 2.14. Found: H, 8.73; C, 47.51; N, 2.10.

5-Amino-3-oxapentyl 3,6-Di-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (9). Compound **4** was deacetylated and hydrogenolyzed as described above for the

preparation of **8** to give **9** as a colorless powder; R_f 0.34 (methanol); ^{1}H NMR (D_2O) δ 4.90 (d, J = 1.3 Hz, 1H, H-1a), 4.57, 4.51 (2d, J = 7.9 Hz, 2H, H-1b, 1c), 4.17 (dd, 1H, H-2a), 4.05 (m, 1H, H-6a), 3.35 (t, 2H, J = 5.1 Hz, CH_2N_3); ^{13}C NMR ($CDCl_3$) δ 104.1 (C-1c), 101.3 (C-1a), 100.3 (C-1b), 79.8 (C-3a), 76.5, 76.0 (C-5b, 5c), 73.9, 73.7 (C-3b, 3c), 71.9, 70.7 (C-2b, 2c), 71.3 (C-5a), 70.1 (C-2a), 69.8 (C-4a, 4b), 67.2 (C-4a), 66.0 (C-6a), 61.2, 61.1 (C-6b, 6c), 40.1 (CH_2N_3).

Anal. Calcd. for $C_{26}H_{56}NO_{17}$ (654.72): H, 8.62; C, 47.70; N, 2.14. Found: H, 8.75; C, 47.46; N, 2.05.

5-Amino-3-oxapentyl 3,6-Di-*O*-(β-D-glucopyranosyl)-α-D-mannopyranoside (10). Compound **5** was deacetylated and hydrogenolyzed as described above for the preparation of **8** to give **10** as a colorless powder; R_f 0.34 (methanol); ^{1}H NMR (D_2O) δ 4.95 (s, 1H, H-1a), 4.58, 4.52 (2d, 2H, J = 8.1 Hz, H-1b, 1c), 4.17 (dd, 1H, H-2a), 4.05 (m, 1H, H-6a), 3.95 (m, 1H, H-3a), 3.82-3.60 (m, 18H, 4a, 5a, 6'a, CH_2O), 3.22 (t, 2H, J = 4.7 Hz, CH_2NH_2); ^{13}C NMR (D_2O) δ 103.9 (C-1c), 101.4 (C-1a), 100.7 (C-1b), 79.2 (C-3a), 77.1, 77.0, 76.7 (C-3a, 3b, 5b, 5c), 74.3, 74.0 (C-2b, 2c), 72.9 (C-5a), 70.7 (C-3a ,2a, 4a, 4b), 68.0, 67.7 (C-4a), 66.8 (C-6a), 61.8 (C-6b, 6c), 40.2 (CH_2NH_2).

Anal. Calcd. for $C_{26}H_{56}NO_{17}$ (654.72): H, 8.62; C, 47.70; N, 2.14. Found: H, 8.69; C, 47.58; N, 2.10.

5-Amino-3-oxapentyl 3,6-Di-*O*-(α-L-rhamnopyranosyl)-α-L-mannopyranoside (11). Compound **6** was deacetylated and hydrogenolyzed as described above for the preparation of **8** to give **11** as a colorless powder; R_f 0.38 (methanol); ^{1}H NMR ($CDCl_3$) 5.01 (s, 1H, H-1c), 4.96 (s, 1H, H-1b), 4.95 (s, 1H, H-1a), 4.18 (dd, 1H, H-2a), 4.11-4.02 (m, 2H, H-2b, 2c), 4.05 (m, 1H, H-6a), 3.40 (t, 2H, CH_2NH_2), 1.19 (d, 6H, H-6b, 6c); ^{13}C NMR ($CDCl_3$) δ 101.5 (C-1a), 100.9, 99.8 (C-1b, 1c), 78.1 (C-3a), 73.0, 72.7 (C-4b, 4c), 71.4 (C-5a), 70.7, 70.5 (C-3b, 3c), 69.2, 68.9 (C-2b, 2c), 67.9, 67.3 (C-5b, 5c), 67.3 (C-2a), 67.1 (C-4a), 66.7 (C-6a), 40.7 (CH_2NH_2), 16.7 (C-6b, 6c).

Anal. Calcd. for $C_{26}H_{56}NO_{15}$ (622.73): H, 9.06; C, 50.15; N, 2.25. Found: H, 9.15; C, 50.23; N, 2.19.

5-Amino-3-oxapentyl 3,6-Di-*O*-(4-*O*-[β-D-galactopyranosyl]-β-D-glucopyranosyl)-α-D-mannopyranoside (12). Compound **7** was deacetylated and hydrogenolyzed as described above for the preparation of **8** to give **12** as a colorless powder; R_f 0.27 (methanol); ^{1}H NMR ($CDCl_3$) δ 4.96 (s, 1H, H-1a), 4.63, 4.57 (2d, 2H, J = 7.9 Hz, H-1b,

1c), 4.56 (m, 2H, H-1b', 1c'), 4.18 (dd, 1H, H-2a), 4.05 (m, 1H, H-6a), 3.30 (t, 2H, J = 5.1 Hz, CH_2NH_2); ^{13}C NMR ($CDCl_3$) δ 104.0, 103.9 (C-1b', 1c'), 103.6, 102.1 (C-1b, 1c), 101.2 (C-1a), 80.9 (C-4a, 4b), 79.2 (C-3a), 77.5 (C-3b', 3c'), 77.0, 76.5 (C-5c, 5b), 76.9, 76.2 (C-3b, 3c), 74.5, 73.8 (C-2b, 2c), 71.3 (C-2a), 69.9 (C-2b', 2c'), 68.4 (C- 4b', 4c'), 67.8 (C-4a), 66.9 (C-6a), 61.8, 61.5, 61.3, 60.9 (C-6b, 6c, 6b', 6c'), 40.2 (CH_2NH_2).

Anal. Calcd. for $C_{40}H_{82}NO_{27}$ (1009.08): H, 8.19; C, 47.61; N, 1.39. Found: H, 8.27; C, 47.65; N, 1.32.

1-*O*-Benzyl-2,3-di-*O*-octadecylglycerol (13). To a solution of 1-*O*-benzyl-glycerol (2.15 g, 11.78 mmol) and octadecyl bromide (15.7 mL, 47.12 mmol) in benzene (30 mL) was added powdered KOH (2.45 g, 47.12 mmol) and the mixture was refluxed with azeotropic removal of water for 12 h. Then toluene (30 mL) was added and the solution was washed successively with 2% hydrochloric acid (25 mL), saturated aqueous sodium hydrogen carbonate (25 mL) and water (25 mL), dried (Na_2SO_4) and concentrated. Column chromatography of the residue using hexane/ethyl acetate 30:1 (v/v) as eluent afforded **13** as an amorphous solid (5 g, 77.1%); R_f 0.35 (hexane/ ethyl acetate 30:1 v/v); ^1H NMR ($CDCl_3$) δ 7.32 (m, 5H, Ph), 3.72 (m, 1H, H-2), 3.58-3.43 (m, 8H, H-1, H-3, CH_2O), 1.61 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.4 (Ph), 128.2-127.3 (Ph), 77.9 (CH), 73.3 (CH_2Ph), 71.6 (C-3), 70.2, 70.5, 70.7 (C-1, CH_2O), 33.5-22.7 (CH_2), 14.1 (CH_3).

Anal. Calcd. for $C_{46}H_{84}O_3$ (685.17): H, 12.36; C, 80.64. Found: H, 12.48; C, 80.21.

1,2-Di-*O*-octadecylglycerol (14). Compound **13** (5 g, 7.28 mmol) was hydrogenolized in ethyl acetate (20 mL) over 10% Pd/C (500 mg) for 12 h. The catalyst was removed by filtration and rinsed with toluene (30 mL). The combined filtrate and washings were concentrated to afford **14** as an amorphous solid (4.21 g, 97%); R_f 0.45 (hexane/ethyl acetate 10:1 v/v); ^1H NMR ($CDCl_3$) δ 3.72 (m, 1H, H-2), 3.57 (m, 4H, H-1, 3), 3.45 (t, 4H, CH_2O), 2.32 (t, 1H, OH), 1.61 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 78.2 (C-2), 71.8 (C-1), 70.9, 70.4 (CH_2O), 63.1 (C-3), 31.9-22.7 (CH_2), 14.1 (CH_3).

Anal. Calcd. for $C_{39}H_{78}O_3$ (595.04): H, 13.21; C, 78.72. Found: H, 13.42; C, 79.41.

2,3-Di-*O*-octadecyloxypropyl Hydrogen Butanedioate (15). A solution of **14** (4.21 g, 7.06 mmol), succinic anhydride (1.5 g, 15 mmol) and dimethylaminopyridine (50 mg) in ethanol-free chloroform (2.5 mL) and pyridine (2.5 mL) was stirred at 60 °C for 24 h. The solvent was evaporated and the syrupy residue dissolved in dichloromethane (20 mL). The solution was washed successively with 2% hydrochloric acid (3 x 5mL), saturated aqueous sodium bicarbonate (7 mL) and water (5 mL), dried (Na_2SO_4) and concentrated. Column chromatography of the residue in hexane/ethyl acetate 1:1 (v/v) as eluant afforded **15** as a foam (3.42 g, 69.5%); R_f 0.35 (hexane/ethyl acetate 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 4.35 (AB section of ABX pattern, 2H, H-1, H-1`), 3.70 (m, 1H, H-2), 3.60 (m, 2H, H-3, 3`), 3.45 (t, 4H, CH_2O), 2.82 (m, 4H, 2 x CH_2CO), 1.60 (m, 4H, 2 x CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.6, 169.9 (C=O), 78.2 (C-2), 71.8 (C-1), 70.9, 70.4 (*C*H_2O), 63.1 (C-3), 31.9-22.7 (*C*H_2), 14.1 (*C*H_3).

Anal. Calcd. for $C_{43}H_{82}O_6$ (695.12): H, 11.89; C, 74.30. Found: H, 12.01; C, 74.19.

2,3-Di-*O*-octadecyloxypropyl Succinimidyl Butanedioate (17). To a stirred solution of compound **15** (3.42 g, 5 mmol) in dichloromethane (5 mL) at 0 °C was added a solution of *N*-hydroxysuccinimide (580 mg, 5 mmol) in *N,N*-dimethylformamide (2 mL), and a solution of dicyclohexylcarbodiimide (1 g, 5 mmol) in dichloromethane (5 mL). After the mixture was stirred overnight, a suspension formed that was filtered through glass wool. The filtrate was concentrated to provide a solid that was further recrystallized from ethyl acetate/ether to afford **17** as a colorless powder (3 g, 79%): mp 72-73 °C; R_f 0.53 (hexane/ ethyl acetate 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 4.35 (ABX, 2H, H-1, 1`), 3.70 (m, 1H, H-2), 3.60 (m, 2H, H-3, 3`), 3.45 (t, 4H, CH_2O), 2.94 (t, 2H, CH_2CO), 2.80 (s, 4H, CH_2CON), 2.70 (t, 2H, CH_2CO), 1.60 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.1, 170.0 (C=O), 78.2 (C-2), 71.8 (C-1), 70.9, 70.4 (*C*H_2O), 63.1 (C-3), 31.9-22.7 (*C*H_2), 14.1 (*C*H_3).

Anal. Calcd. for $C_{47}H_{85}NO_8$ (792.19): H, 10.81; C, 71.26; N, 1.77. Found: H, 11.03; C, 71.17; N, 1.66.

5-Cholesten-3-β-yl Succinimidyl Butanedioate (20). To a stirred solution of compound **18** (200 mg, 0.41 mmol) in dichloromethane (1 mL) at 0 °C was added a solution of N-hydroxysuccinimide (52 mg, 0.45 mmol) in *N,N*-dimethylformamide (0.2

mL) and a solution of dicyclohexylcarbodiimide (93 mg, 0.45 mmol) in dichloromethane (1 mL). After the reaction mixture was stirred overnight, the suspension formed was filtered through glass wool. The filtrate was concentrated to provide a solid that was further recrystallized from ethyl acetate to afford **20** as a colorless powder: mp 145-146 °C; $[\alpha]_D$ -14° (*c* 1.0, chloroform) R_f 0.53 (hexane/ethyl acetate 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.36 (m, 1H, H-6), 4.62 (m, 1H, H-3), 2.94 (t, 2H, CH_2CO), 2.80 (s, 4H, CH_2CON), 2.70 (t, 2H, CH_2CO), 2.30 (d, 2H, H-4), 1.02 (s, 3H, H-19), 0.69 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 170.3, 168.9, 167.7 (C=O), 139.4 (C-5), 122.7 (C-6), 74.8 (C-3).

Anal. Calcd. for $C_{35}H_{53}NO_6$ (583.80): H, 9.15; C, 72.01; N, 2.40. Found: H, 9.21; C, 71.96; N, 2.29.

5-(3-[(2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3,6-Di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (21). To a mixture of **8** (150 mg, 0.25 mmol) and **17** (198 mg, 0.25 mmol), was added dimethyl sulfoxide (2 mL) and triethylamine (35 μL, 0.25 mmol); the solution was vigorously stirred at 60 °C for 6 h. Then water (5 mL) was added and the resulting suspension was centrifuged. The supernatant liquid was discarded and the pellet was dissolved in chloroform/methanol 4:1 (v/v) and chromatographed using the same solvent system to afford **21** as an amorphous solid (209 mg, 65%); R_f 0.33 (chloroform/methanol/H_2O 85:35:1 v/v); ^{1}H NMR ($CDCl_3$/ CD_3OD 1:1) δ 5.15 (d, 1H, H-1c), 4.94 (d, 1H, H-1b), 4.90 (s, 1H, H-1a), 4.18 (AB section of ABX pattern, 2H, H-1g, 1`g), 4.11 (d, 1H, $J_{2,3}$ = 3.2 Hz, H-2a), 4.06 (dd, 1H, H-2c), 4.00 (dd, 1H, H-6a), 3.92 (dd, 1H, H-2b), 2.80 (t, 2H, CH_2CO), 2.60 (t, 2H, CH_2CO), 1.60 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 170.3, 168.9 (C=O), 101.8 (C-1c), 99.9 (C-1a), 99.2 (C-1b), 79.3 (C-3a), 76.0 (C-2g), 72.8, 72.1 (C-5b, 5c), 71.3 (C-3g), 71.2 (C-5a), 71.1 (C-3c), 70.8 (C-3a), 70.6 (C-3b), 70.1 (C-2c, 2b, 2a), 67.0 (C-4b, 4c), 66.7 (C-4a), 66.0 (C-6a), 63.6 (C-1g), 61.2, 61.0 (C-6b, 6c), 38.7 (CH_2NH), 31.9-22.7 (CH_2), 14.1 (CH_3).

Anal. Calcd for $C_{65}H_{123}O_{22}N$ (1270.68): C, 61.44; H, 9.75; N, 1.10. Found: C, 61.38; H, 9.90; N, 1.09.

5-{3-[(5-Cholesten-3β-yl)oxycarbonyl]propanoyl}-5-aza-3-oxapentyl 3,6-Di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (22). To a mixture of **8** (150 mg, 0.25 mmol) and **20** (140 mg, 0.24 mmol), was added dimethyl sulfoxide (2 mL) and

triethylamine (35 μL, 0.25 mmol) and the solution was vigorously stirred at 60 °C for 4 h. Then acetone (5 mL) was added and the suspension was centrifuged, the supernatant liquid was discarded, the pellet was dissolved in chloroform/methanol 4:1 (v/v) and chromatographed in the same system to afford **22** as an amorphous solid (172 mg, 63%); $[\alpha]_D$ +17° (*c* 1.0, chloroform/methanol 2:1 v/v); R_f 0.28 (chloroform/methanol/water 85:35:1 v/v); ^{1}H NMR ($CDCl_3$/ CD_3OD 1:1) δ 5.36 (m, 1H, H-6ch), 5.09 (s, 1H, H-1c), 4.86 (s, 1H, H-1b), 4.79 (s, 1H, H-1a), 4.06-3.92 (m, 5H, H-6a, 2a, 2c, 2b, 3ch), 3.55 (t, 2H, J = 4.5 Hz, C*H*$_2$NH), 2.62 (t, 2H, C*H*$_2$CO), 2.53 (t, 2H, C*H*$_2$CO), 2.31 (d, 2H, J = 7.5 Hz, H-4ch), 1.02 (s, 3H, H-19ch), 0.69 (s, 3H, H-18ch); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 170.3, 168.8 (C=O), 139.4 (C-5ch), 122.7 (C-6ch), 101.8 (C-1c), 100.0 (C-1a), 99.3 (C-1b), 79.2 (C-3a), 74.2 (C-3ch), 72.8, 72.1 (C-5b, 5c), 71.2 (C-5a), 71.1 (C-3c), 70.8 (C-3a), 70.6 (C-3b), 70.1 (C-2c, 2b, 2a), 67.1, 66.8 (C-4b, 4c), 66.1 (C-4a), 65.2 (C-6a), 61.2, 61.1 (C-6b, 6c), 38.9 (*C*H$_2$NH).

Anal. Calcd for $C_{53}H_{89}O_{20}N$ (1060.34): C, 60.03; H, 8.46; N, 1.32. Found: C, 59.96; H, 8.61; N, 1.28.

5-(3-[(2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3,6-Di-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (23). Compound **9** (50 mg, 83 μmol) was condensed with **17** (66 mg, 83 μmol) as described above for the preparation of **21** to give **23** as an amorphous solid (69 mg, 64%); R_f 0.33 (chloroform/ methanol/ H_2O 85:35:1 v/v); ^{1}H NMR ($CDCl_3$/ CD_3OD 1:1 v/v) δ 4.90 (d, 1H, J = 1.3 Hz, H-1a), 4.37, 4.34 (2d, 2H, J = 7.7 Hz, H-1b, 1c), 4.15 (AB section of ABX pattern, 2H, H-1g, 1`g), 4.06 (d, 1H, H-2a), 2.65 (t, 2H, CH_2CO), 2.55 (t, 2H, CH_2CO), 1.60 (m, 4H, C*H*$_2$CH$_2$O), 1.30 (s, 60H, C*H*$_2$), 0.90 (t, 6H, C*H*$_3$); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.3, 172.1 (C=O), 102.1 (C-1c), 100.3 (C-1a), 99.9 (C-1b), 79.7 (C-3a), 76.5 (C-2g), 76.1, 75.8 (C-5b, 5c), 72.9, 72.8 (C-3b, 3c), 70.5, 70.1 (C-2b, 2c), 70.2 (C-5a), 69.6 (C-2a), 69.1 (C-4a, 4b), 67.6 (C-4a), 66.4 (C-6a), 61.1, 61.0 (C-6b, 6c), 38.9 (*C*H$_2$NH), 31.9-22.7 (*C*H$_2$), 14.1 (*C*H$_3$).

Anal. Calcd for $C_{65}H_{123}O_{22}N$ (1270.68): C, 61.44; H, 9.75; N, 1.10. Found: C, 61.49; H, 9.83; N, 1.07.

5-{3-[(5-Cholesten-3β-yl)oxycarbonyl]propanoyl}-5-aza-3-oxapentyl 3,6-Di-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (24). Compound **9** (50 mg, 83 μmol) was condensed with **20** (46 mg, 83 μmol) as described above for the preparation of **22** to

give **24** as an amorphous solid (54 mg, 60%); $[\alpha]_D$ -7° (*c* 0.9, chloroform/methanol 2:1 v/v); R_f 0.28 (chloroform/ methanol/water 85:35:1 v/v); ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1) δ 5.40 (m, 1H, H-6ch), 4.90 (d, J = 1.3 Hz, 1H, H-1a), 4.37, 4.34 (2d, J = 7.9 Hz, 2H, H-1b, 1c), 4.12 (m, 1H, H-3ch), 4.06 (dd, 1H, H-2a), 2.65 (t, 2H, C*H*$_2$CO), 2.55 (t, 2H, C*H*$_2$CO), 2.35 (d, 2H, H-4ch), 1.02 (s, 3H, H-19ch), 0.70 (s, 3H, H-18ch); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.3, 172.1 (C=O), 139.4 (C-5ch), 122.7 (C-6ch), 102.1 (C-1c), 100.3 (C-1a), 99.9 (C-1b), 79.7 (C-3a), 76.1, 75.8 (C-5b, 5c), 74.1 (C-3ch), 72.9, 72.8 (C-3b, 3c), 70.5, 70.1 (C-2b, 2c), 70.2 (C-5a), 69.6 (C-2a), 69.1 (C-4a, 4b), 67.6 (C-4a), 66.4 (C-6a), 61.1, 61.0 (C-6b, 6c), 38.8 (*C*H$_2$NH).

Anal. Calcd for $C_{53}H_{89}O_{20}N$ (1060.34): C, 60.03; H, 8.46; N, 1.32. Found: C, 60.18; H, 8.52; N, 1.39.

5-(3-[2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3,6-Di-*O*-(β-D-glucopyranosyl)-α-D-mannopyranoside (25). Compound **10** (50 mg, 83 μmol) was condensed with **17** (66 mg, 83 μmol) as described above for the preparation of **21** to give **25** as an amorphous solid (65 mg, 61%); R_f 0.33 (chloroform/methanol/ water 85:35:1 v/v); ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1) δ 4.90 (d, 1H, H-1a), 4.48, 4.46 (2d, J = 7.6 Hz, 2H, H-1b, 1c), 4.15 (ABX, 1H, H-1g), 4.06 (dd, 1H, H-2a), 2.65 (t, 2H, C*H*$_2$CO), 2.55 (t, 2H, C*H*$_2$CO), 1.60 (m, 4H, C*H*$_2$CH$_2$O), 1.30 (s, 60H, C*H*$_2$), 0.90 (t, 6H, C*H*$_3$); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1) δ 172.7, 172.3 (C=O), 102.9 (C-1c), 100.9 (C-1a), 99.5 (C-1b), 79.3 (C-3a), 76.5 (C-2g), 76.1, 75.7, 75.6 (C-3a, 3b, 5b, 5c), 77.3, 72.8 (C-2b, 2c), 71.9 (C-5a), 69.6, 69.3 (C-3a ,2a, 4a, 4b), 67.9 (C-4a), 66.2 (C-6a), 63.7 (C-1g), 61.0, 60.6 (C-6b, 6c), 38.7 (*C*H$_2$NH), 31.9-22.7 (C*H*$_2$), 14.1 (C*H*$_3$).

Anal. Calcd for $C_{65}H_{123}O_{22}N$ (1270.68): C, 61.44; H, 9.75; N, 1.10. Found: C, 61.32; H, 9.91; N, 1.13.

5-{3-[(5-Cholesten-3β-yl)oxycarbonyl]propanoyl}-5-aza-3-oxapentyl 3,6-Di-*O*-(β-D-glucopyranosyl)-α-D-mannopyranoside. (26). Compound **10** (50 mg, 83 μmol) was condensed with **20** (46 mg, 83 μmol) as described above for the preparation of **22** to give **26** as an amorphous solid (51 mg, 57%); $[\alpha]_D$ -9° (*c* 0.9, chloroform/methanol 2:1 v/v); R_f 0.28 (chloroform/methanol/water 85:35:1 v/v); ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1) δ 5.40 (m, 1H, H-6ch), 4.90 (d, 1H, H-1a), 4.42, 4.41 (2d, J = 7.8 Hz, 2H, H-1b, 1c), 4.12 (m, 1H, H-3ch), 4.06 (dd, 1H, H-2a), 2.65 (t, 2H, C*H*$_2$CO), 2.55 (t, 2H, C*H*$_2$CO), 2.35 (d, 2H, H-4ch), 1.02 (s, 3H, H-19ch), 0.70 (s, 3H, H-18ch); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1)

δ 172.3, 172.1 (C=O), 139.4 (C-5ch), 122.7 (C-6ch), 102.9 (C-1c), 100.4 (C-1a), 99.5 (C-1b), 79.2 (C-3a), 76.0, 75.7, 75.6 (C-3a, 3b, 5b, 5c), 73.1, 72.8 (C-2b, 2c), 71.9 (C-5a), 69.6, 69.3 (C-3a, 2a, 4a, 4b), 67.9 (C-4a), 66.2 (C-6a), 63.7 (C-1g), 61.0, 60.8 (C-6b, 6c), 38.7 (CH_2NH).

Anal. Calcd for $C_{53}H_{89}O_{20}N$ (1060.34): C, 60.03; H, 8.46; N, 1.32. Found: C, 60.09; H, 8.59; N, 1.39.

5-(3-[2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3,6-Di-*O*-(α-L-rhamnopyranosyl)-α-D-mannopyranoside (27). Compound **11** (50 mg, 88 μmol) was condensed with **17** (70 mg, 83 μmol) as described above for the preparation of **21** to give **27** as an amorphous solid (67 mg, 62%); $[\alpha]_D$ -28° (*c* 0.9, chloroform/methanol 2:1 v/v); R_f 0.35 (chloroform/methanol/water 85:35:1 v/v); ^1H NMR δ ($CDCl_3/CD_3OD$ 1:1) 4.96 (d, 1H, H-1c), 4.92 (d, 1H, H-1b), 4.89 (d, 1H, H-1a), 4.13 (ABX, 1H, H-1g), 4.06 (dd, 1H, H-2a), 2.65 (t, 2H, CH_2CO), 2.55 (t, 2H, CH_2CO), 1.60 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.4, 172.2 (C=O), 100.3 (C-1a), 99.6, 98.9 (C-1b, 1c), 78.5 (C-3a), 76.6 (C-2g), 72.1, 72.0 (C-4b, 4c), 71.0 (C-5a), 70.1, 69.9 (C-3b, 3c), 69.0, 68.9 (C-2b, 2c), 67.6, 67.3 (C-5b, 5c), 67.2 (C-2a), 66.8 (C-4a), 66.4 (C-6a), 63.6 (C-1g), 38.8 (CH_2NH_2), 31.9-22.7 (CH_2), 16.5 (C-6b, 6c), 14.1 (CH_3).

Anal. Calcd for $C_{65}H_{123}O_{20}N$ (1238.68): C, 63.02; H, 10.01; N, 1.13. Found: C, 62.94; H, 10.16; N, 1.09.

5-{3-[(5-Cholesten-3β-yl)oxycarbonyl]propanoyl}-5-aza-3-oxapentyl 3,6-Di-*O*-(α-L-rhamnopyranosyl)-α-D-mannopyranoside (28). Compound **11** (50 mg, 88 μmol) was condensed with **20** (49 mg, 83 μmol) as described above for the preparation of **21** to give **28** as an amorphous solid (53 mg, 59%); $[\alpha]_D$ -6° (*c* 0.9, chloroform/ methanol 2:1 v/v); R_f 0.30 (chloroform/methanol/water 85:35:1 v/v); ^1H NMR δ ($CDCl_3/CD_3OD$ 1:1) 5.41 (m, 1H, H-6ch), 4.96 (d, 1H, H-1c), 4.92 (d, 1H, H-1b), 4.89 (d, 1H, H-1a), 4.12 (m, 1H, H-3ch), 4.04 (dd, 1H, H-2a), 2.65 (t, 2H, CH_2CO), 2.55 (t, 2H, CH_2CO), 2.35 (d, 2H, H-4ch), 1.02 (s, 3H, H-19ch), 0.70 (s, 3H, H-18ch); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.4, 172.2 (C=O), 139.4 (C-5ch), 122.7 (C-6ch), 100.3 (C-1a), 99.6, 98.9 (C-1b, 1c), 78.4 (C-3a), 74.0 (C-3ch), 72.1, 71.9 (C-4b, 4c), 71.0 (C-5a), 70.2, 69.9 (C-3b, 3c), 69.1, 68.8 (C-2b, 2c), 67.6, 67.4 (C-5b, 5c), 67.2 (C-2a), 66.9 (C-4a), 66.4 (C-6a), 38.7 (CH_2NH_2), 16.4 (C-6b, 6c).

Anal. Calcd for $C_{53}H_{89}O_{18}N$ (1028.34): C, 61.90; H, 8.73; N, 1.36. Found: C, 61.79; H, 8.88; N, 1.41.

5-(3-[2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3,6-Di-*O*-(4-*O*-[β-D-galactopyranosyl]-β-D-glucopyranosyl)-α-D-mannopyranoside (29). Compound **12** (50 mg, 53 μmol) was condensed with **17** (42 mg, 83 μmol) as described above for the preparation of **21** to give **29** as an amorphous solid (47 mg, 56 %); R_f 0.35 (chloroform/methanol/water 85:35:1 v/v); ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1) δ 4.89 (s, 1H, H-1a), 4.51, 4.49 (2d, J = 7.9 Hz, 2H, H-1b, 1c), 4.42 (m, 2H, H-1b', 1c'), 4.13 (AB section of ABX pattern, 2H, H-1g, 1`g), 4.06 (d, 1H, H-2a), 2.65 (t, 2H, CH_2CO), 2.55 (t, 2H, CH_2CO), 1.60 (m, 4H, CH_2CH_2O), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.4, 172.2 (C=O), 102.3, 102.2 (C-1b', 1c'), 102.3, 100.8 (C-1b, 1c), 100.1 (C-1a), 79.9 (C-4a, 4b), 79.2 (C-3a), 76.6 (C-2g), 76.5 (C-3b', 3c'), 76.3, 75.7 (C-5c, 5b), 75.7, 75.5 (C-3b, 3c), 73.5, 73.1 (C-2b, 2c), 70.2 (C-2a), 69.7 (C-2b', 2c'), 68.7 (C- 4b', 4c'), 67.2 (C-4a), 66.9 (C-6a), 63.6 (C-1g), 60.8, 60.3, 60.3, 60.1 (C-6b, 6c, 6b', 6c'), 38.8 (CH_2NH), 31.9-22.7 (CH_2), 14.1 (CH_3).

Anal. Calcd for $C_{77}H_{143}O_{32}N$ (1593.88): C, 58.20; H, 8.97; N, 0.88. Found: C, 57.98; H, 9.09; N, 0.80.

5-{3-[(5-Cholesten-3β-yl)oxycarbonyl]propanoyl}-5-aza-3-oxapentyl 3,6-Di-*O*-(4-*O*-[β-D-galactopyranosyl]-β-D-glucopyranosyl)-α-D-mannopyranoside (30). Compound **12** (50 mg, 53 μmol) was condensed with **20** (30 mg, 53 μmol) as described above for the preparation of **22**. Yield (36 mg, 51%); $[\alpha]_D$ -5° (*c* 0.9, chloroform/ methanol 2:1 v/v); R_f 0.30 (chloroform/methanol/water 85:35:1 v/v); ^{1}H NMR ($CDCl_3$/ CD_3OD 1:1) δ 5.41 (m, 1H, H-6ch), 4.89 (d, 1H, H-1a), 4.51, 4.49 (2d, J = 7.9 Hz, 2H, H-1b, 1c), 4.42 (m, 2H, H-1b', 1c'), 4.12 (m, 1H, H-3ch), 4.04 (dd, 1H, H-2a), 2.65 (t, 2H, CH_2CO), 2.55 (t, 2H, CH_2CO), 2.35 (d, 2H, H-4ch), 1.02 (s, 3H, H-19ch), 0.70 (s, 3H, H-18ch); ^{13}C NMR ($CDCl_3$/ CD_3OD 1:1) δ 172.4, 172.2 (C=O), 139.4 (C-5ch), 122.7 (C-6ch), 102.3, 102.2 (C-1b', 1c'), 102.3, 100.8 (C-1b, 1c), 100.1 (C-1a), 79.9 (C-4a, 4b), 79.2 (C-3a), 76.6 (C-2g), 76.5 (C-3b', 3c'), 76.3, 75.7 (C-5c, 5b), 75.7, 75.5 (C-3b, 3c), 74.2 (C-3ch), 73.5, 73.1 (C-2b, 2c), 70.2 (C-2a), 69.7 (C-2b', 2c'), 68.7 (C- 4b', 4c'), 67.2 (C-4a), 66.9 (C-6a), 63.6 (C-1g), 60.8, 60.3, 60.3, 60.1 (C-6b, 6c, 6b', 6c'), 38.8 (CH_2NH), 31.9-22.7 (CH_2), 14.1 (CH_3).

Anal. Calcd for $C_{65}H_{109}O_{28}N$ (1352.73): C, 57.71; H, 8.13; N, 1.03. Found: C, 57.59; H, 8.29; N, 1.10.

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