New mannotriosides and trimannosides as potential ligands for mannose-specific binding proteins

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Abstract: The α-D-mannopyranosyl and 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl neoglycolipids **3**–**6** and the branched and cluster trimannosidic acids **38** and **41** have been made in connection with studies of liposomes as transporters of antigens to dendritic cells.

Key words: mannotrioside, trimannoside, neoglycoconjugate, glycolipid, synthesis.

Résumé : Dans le cadre d'études sur les liposomes comme transporteurs d'antigènes dans des cellules dendritiques, on a préparé les néoglycolipides α-D-mannopyranosyl- et 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyle (**3**–**6**) ainsi que les acides trimannosidiques ramifié et en agrégat (**38** et **41**).

Mots clés : mannotrioside, trimannoside, néoglycoconjugué, glycolipide, synthèse.

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Introduction

Terminal α-D-mannopyranosyl units commonly occur in the glycoproteins of pathogenic bacteria, yeasts, viruses, and various parasites and are recognised by receptors of macrophages (1) and dendritic cells (2, 3). Because the branched trisaccharide 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl ligand binds with higher affinity to the mannose receptors of human macrophages and dendritic cells than do mono- and linear oligo-mannosyl ligands (4), we began studies of synthetic neoglycoconjugates and liposomes containing this trimer with a view to using the liposomes to transport antigens to dendritic cells.

In the first report of this work (5), we exploited the earlier finding (6, 7) that various α-D-mannopyranosides could be di-*O*-mannosylated concurrently and with some selectivity at O-3 and O-6 by use of tetra-*O*-acetyl-α-D-mannopyranosyl bromide to give the required branched-chain mannotriose derivatives, and we introduced the new tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate as glycosylating agent. By its use 8-(methoxycarbonyl)octyl α-D-mannopyranoside was converted to the 3,6-bis-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-mannopyranoside in 55% yield, and the neoglycolipids **1** and **2** (Scheme 1) were made (5). More recently the 3,6-di-*O*-α-D-mannosylation of *p*-nitrophenyl α-D-mannopyranoside in 42% yield by use of tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate as glycosylating agent has been described (4).

We now report (Scheme 1) the preparation of the neoglycolipids **3**–**6** which differ from analogues **1** and **2** in having four-carbon (compounds **3** and **5**) and six-carbon (compounds **4** and **6**) as opposed to nine-carbon atom spacer groups. Additionally, and with further neoglycolipids in view, the polyoxyethylene glycosides **30** and **34** and the mannotriose-containing acid **38** were made, as well as the alternative cluster type of trimannosyl glycosidic (8) acid **41** (Scheme 2). Considerable effort has gone into the study of this last kind of trimannoside (9).

Results and discussion

The neoglycolipids **3** and **4** (Scheme 1) were made following glycosylations of methyl 4-hydroxybutanoate and methyl 6-hydroxyhexanoate, respectively, by use of tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate. The first products **7** and **11** were converted successively to the required neoglycolipids by the previously used processes (5), that is, via the debenzoylated glycosides **8** and **12**, the carboxylic acids **9** and **13** and the *N*-succinimido derivatives **10** and **14**, respectively. The final step involved coupling of the activated acids **10** and **14** with dipalmitoyl-1-α-phosphatidyl ethanolamine (DPPE) to give products **3** and **4**.

Selective bis-(tetra-*O*-benzoyl-α-D-mannosylation) of glycosides **8** and **12**, by use of the glycosylating agent mentioned above, afforded the trisaccharide derivatives **15** and **21** which were isolated following chromatographic separation of their derived diacetates **16** (23% from **8**) and **22** (33% from **12**), respectively. Compounds **16** and **22** were used to prepare the neoglycolipids **5** and **6**, respectively, by the routes used for compounds **3** and **4**, i.e., **16**→**18**→**19**→**20**→**5** and **22**→**24**→**25**→**26**→**6** as also outlined in Scheme 1. The 1→3-, 1→6-linked trisaccharide

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Dedicated to the memory of Professor Raymond U. Lemieux.

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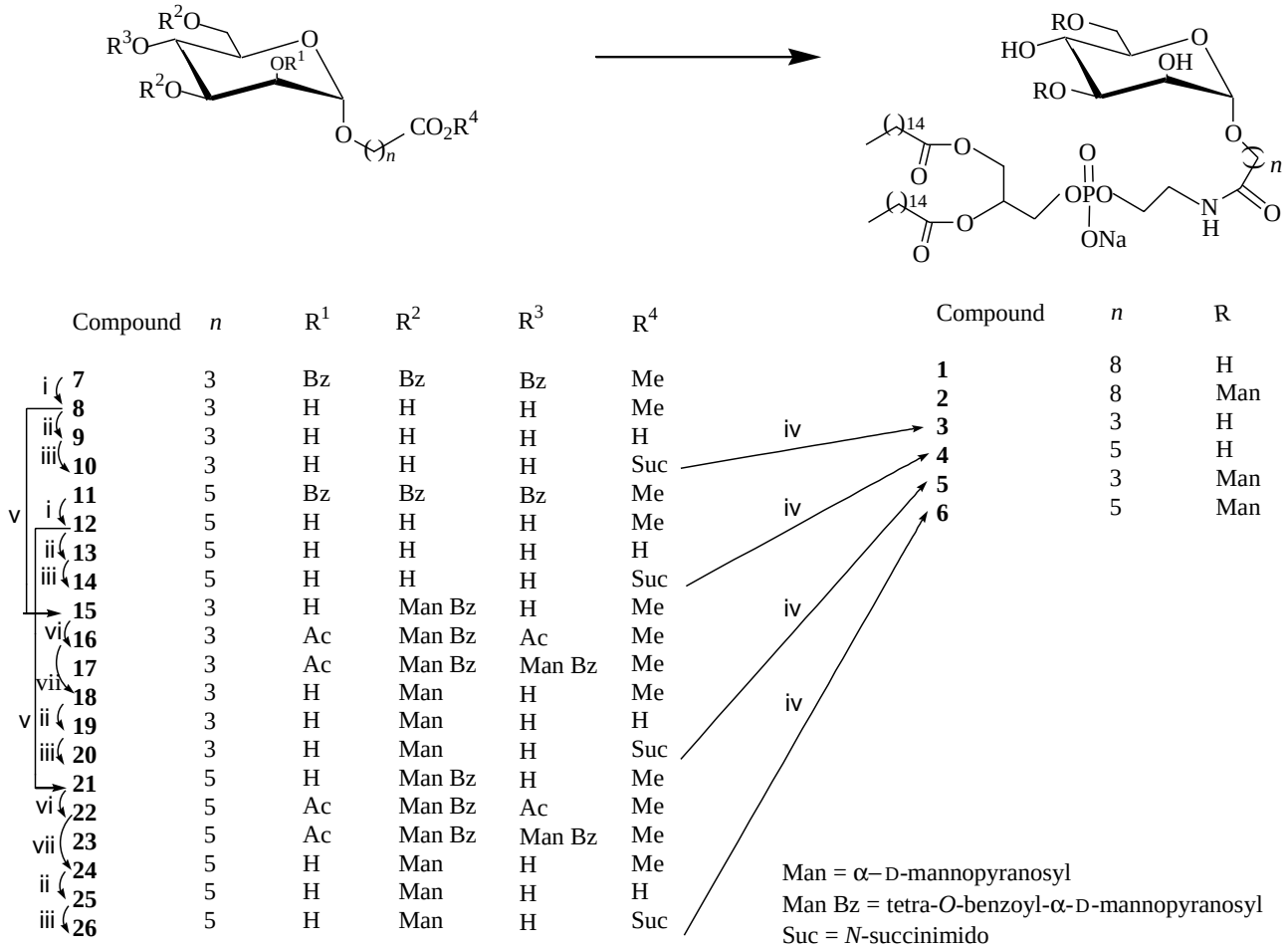
Scheme 1.



Reagents: i, K_2CO_3, MeOH; ii, NaOH (aq.); iii, *N*-hydroxysuccinimide; DCC; iv, 1,2-hexadecanoyl-sn-glycero-3-phosphoethanolamine, $NaHCO_3$; v, tetra-*O*-benzoyl-D-mannopyranosyl trichloroacetimidate; vi, Ac_2O, Py; vii, NaOMe, MeOH

structures of the products **15** and **21** followed from the ^{1}H NMR characteristics of their diacetates **16** and **22**, the H-2 and H-4 resonances being ca. 1 ppm deshielded with respect to those of H-3, and the $J_{2,3}$ and $J_{3,4}$ coupling constant values being 3.4 and 9.8 Hz, respectively. These data compare closely with those obtained for the corresponding trisaccharide octa-*O*-benzoyl diacetate prepared in the course of making compound **2** (5).

During the isolation of the acetylated trisaccharide derivatives **16** and **22** the (3,4,6-tri-*O*-mannosyl)mannoside by-products **17** and **23**, containing small proportions of other oligosaccharide derivatives, were also obtained. The ^{1}H NMR spectra of monoacetates **17** and **23** showed δ values for the H-2 resonances of the central mannose residues of 5.34 and 5.43, respectively. The less-deshielding acetyl groups are consequently concluded to be substituted at O-2 since, for both of the compounds, the nine protons at the secondary benzoylated ring positions resonated within the range δ 5.79–6.27. The tetrasaccharide by-products are therefore 3,4,6-tris-*O*-(tetra-*O*-benzoyl-mannopyranosyl)-mannopyranosides.

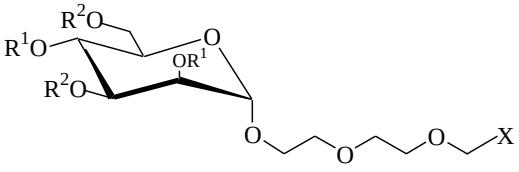
In all the above cases the glycosylation reactions gave no observable orthoester by-products.

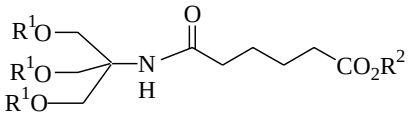
En route to alternative types of neoglycolipids the mono- and trisaccharide glycosides **27** and **35** (Scheme 2) were made by standard condensations of tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate with the corresponding alcohols whereas, in our hands, the corresponding acetylglycosyl bromide with mercury(II) cyanide was more effective for the preparation of triglycoside **39** from [tris(hydroxymethyl)]methyl-(5-methoxycarbonyl)pentanoamide (8).

From **27** and **35** the acids **30** and **38** were made by standard methods, and from **27** the extended-chain compound **34** was produced via the azide **31**. The dimannosylation of compound **29** gave the 1→3-,1→6-linked trisaccharide derivative **35** in 40% yield, the positions of substitution being determined from the ^{1}H,^{1}H coupling constants of the H-2 and H-4 resonances of the derived diacetate **36**. De-esterification of the substituted triglycoside **39** gave acid **41** via methyl ester **40**.

The biological evaluation of compounds described above will be reported separately.

Scheme 2.





Compound	R^1	R^2	X
27	Bz	Bz	Cl
28	Bz	Bz	CN
29	H	H	CN
30	H	H	CO_2H
31	Bz	Bz	N_3
32	Bz	Bz	NH_2
33	Bz	Bz	$NHCO(CH_2)_4CO_2Me$
34	H	H	$NHCO(CH_2)_4CO_2Me$
35	H	Man Bz	CN
36	Ac	Man Bz	CN
37	H	Man	CN
38	H	Man	CO_2H

Compound	R^1	R^2
39	Man Ac	Me
40	Man	Me
41	Man	H

Man Ac = tetra-*O*-acetyl-α-D-mannopyranosyl.
For other abbreviations see Scheme 1.

Reagents: i, KCN, DMSO; ii, K_2CO_3, MeOH; iii, NaOH (aq.); iv, NaN_3, DMF; v, $NaBH_4$, $NiCl_2$, EtOH; vi, methyl adipate, 2-ethoxy-1-ethoxycarbonyl-1,2-didydroquinoline ; vii, tetra-*O*-benzoyl-D-mannopyranosyl trichloroacetimidate; viii, Ac_2O, Py; ix, NaOMe, MeOH.

Experimental

General methods

All glycosylation reactions were performed under argon in dry CH_2Cl_2. TLC was performed on silica gel HF-254 and column chromatography on silica gel 230–400 mesh (Merck). ^1H NMR spectra were recorded with a Bruker Avance 300 MHz NMR spectrometer equipped with a 5 mm o.d. Quattro nucleus probe or (when indicated) a Varian Unity 500 MHz spectrometer equipped with 5 mm Universe probe. Resonance assignments were by use of DEPT, H,H-COSY, inverse C,H-COSY, and H,H-TOCSY experiments. All spectra were consistent with those expected for the structures assigned to the respective compounds; unresolved resonances and those of substituent groups and carbonyl groups (^{13}C spectra) are frequently not listed. High resolution mass spectra (HR-MS) were measured with a VG70-250S double focussing magnetic sector mass spectrometer or a Mariner 8105 electrospray TOF mass spectrometer. Optical rotations were measured with a PerkinElmer 241 automatic polarimeter. 2-[2-(2-Chloroethoxy)ethoxy]ethanol was purchased from Aldrich.

*3-(Methoxycarbonyl)propyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (7)*

A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (5) (4.45 g, 6.0 mmol) and methyl 4-hydroxybutanoate (10) (760 mg, 6.4 mmol) in CH_2Cl_2 (30 mL) was stirred for 30 min at room temperature over molecular sieves (4 Å, 700 mg, finely ground). The solution was cooled in an ice bath and TmsOTf (60 μL) was added. After 20 min the reaction was quenched by addition of Et_3N (0.5 mL), and the solvent was evaporated in vacuo. Column chromatography (hexane–EtOAc, 2:1) gave the title compound **7** (3.60 g, 92%, contaminated with 10% of inseparable methyl 4-hydroxybutanoate): $[\alpha]_D^{20}$ –47 (*c* 1.0, $CHCl_3$, corrected for the impurity). ^1H NMR ($CDCl_3$) δ: 6.10 (t, $J_{3,4}$, $J_{4,5}$ = 10 Hz, 1H, H-4), 5.88 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.69 (dd, $J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.08 (d, 1H, H-1), 4.69 (dd, $J_{5,6}$ = 2.5 Hz, $J_{6,6'}$ = 12.0 Hz, 1H, H-6), 4.50 (dd, $J_{5,6'}$ = 4.4 Hz, 1H, H-6′), 4.42 (m, 1H, H-5), 3.90 (dt, *J* = 9.8, 6.2 Hz, 1H, OC*H*H), 3.73 (s, 3H, Me), 3.61 (dt, *J* = 9.8, 6.3 Hz, 1H, OCH*H*), 2.53 (m, 2H, CH_2), 2.06 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 97.65 (C-1), 70.43, 70.09, 68.93, 67.48 (CH_2), 66.91, 62.87 (CH_2), 51.69 (CH_3), 30.78 (CH_2), 24.73 (CH_2). FAB-HR-MS calcd. for $C_{39}H_{37}O_{12}$ $[M + H]^+$ (*m*/*z*): 697.2285; found: 697.2330.

3-(Methoxycarbonyl)propyl α-D-mannopyranoside (8)

To a solution of compound **7** (3.6 g) in methanol (60 mL) K_2CO_3 (300 mg) was added and the mixture was stirred overnight then neutralized with Amberlyst 15 resin (H^+), filtered and the filtrate was evaporated to dryness. Column chromatography ($CHCl_3$–MeOH, 3:1) of the residue gave

8 (1.03 g, 71% after two debenzoylation treatments): $[\alpha]_D^{20}$ +54.2 (*c* 0.59, MeOH). ^{1}H NMR (CD_3OD) δ: 4.74 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 3.68 (s, Me), 2.44 (t, 2H, CH_2), 1.91 (m, 2H, CH_2). ^{13}C NMR (CD_3OD) δ: 176.03 (C=O), 101.97 (C-1), 75.07, 73.04, 72.56, 69.02, 67.92 (CH_2), 63.33 (CH_2), 52.47 (CH_3), 32.19 (CH_2), 26.40 (CH_2). FAB-HR-MS calcd. for $C_{11}H_{21}O_8$ $[M + H]^+$: 281.1236; found: 281.1243.

3-Carboxypropyl* α-D-*mannopyranoside (9)

NaOH (0.75 mL, 3M aq) was added to a solution of the methyl ester **8** (517 mg, 1.84 mmol) in THF (15 mL) and water (7 mL), The reaction mixture was stirred for 24 h, neutralised with Amberlyst 15 resin (H^+), filtered, concentrated in vacuo and freeze-dried to afford the acid **9** (440 mg, 90%): $[\alpha]_D^{20}$ +46.1 (*c* 0.41, water). ^{1}H NMR (D_2O) δ: 4.78 (d, $J_{1,2}$ = 1.2 Hz, 1H, H-1), 3.46–3.87 (m, 8H), 2.42 (t, 2H, CH_2), 1.86 (m, 2H, CH_2). ^{13}C NMR (D_2O) δ: 178.73 (C=O), 100.11 (C-1), 73.12, 71.00, 70.44, 67.22 (CH_2), 67.15, 61.31 (CH_2), 31.36 (CH_2), 24.55 (CH_2). FAB-HR-MS calcd. for $C_{10}H_{19}O_8$ $[M + H]^+$: 267.1080; found: 267.1084.

3-[Carboxy-2-(1,2-dihexadecanoyl*-sn-*glycero-3-phospho)ethanolamido]propyl* α-D-*mannopyranoside sodium salt (3)

A mixture of acid **9** (119 mg, 0.45 mmol), DCC (248 mg, 1.5 mmol), *N*-hydroxysuccinimide (139 mg, 1.5 mmol) in THF (6.5 mL) – $CHCl_3$ (3 mL) – methanol (6.5 mL) was stirred at room temperature for 24 h. The solvent was removed and column chromatography ($CHCl_3$–MeOH, 4:1) of the residue gave the crude amide **10** (115 mg, 71%). FAB-HR-MS calcd. for $C_{14}H_{12}NO_{10}$ $[M + H]^+$: 364.1244; found: 364.1225.

To a suspension of 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (DPPE, 200 mg, 0.284 mmol) in $CHCl_3$–MeOH (2:1, 6 mL), water (0.4 mL), and $NaHCO_3$ (120 mg, 1.42 mmol) were added, and the mixture was stirred at 30°C for 30 min. A solution of **10** (94 mg, 0.27 mmol) in $CHCl_3$–MeOH (2:1, 6 mL) was added and stirring was continued at 30°C overnight. The solvents were evaporated and column chromatography ($CHCl_3$–MeOH, 3:1) of the residue gave glycolipid **3**, 125 mg (47%): $[\alpha]_D^{20}$ +17.1 (*c* 0.38, $CHCl_3$–MeOH, 3:1). ^{13}C NMR ($CDCl_3$–CD_3OD, 3:1) δ: 174.33, 173.93, 173.61 (C=O), 100.09 (C-1), 72.59, 71.39, 71.15, 70.75, 70.50, 66.55, 66.55 (CH_2), 64.39 (CH_2), 63.80 (CH_2), 62.66 (CH_2), 61.89 (CH_2), 61.18 (CH_2), 60.89 (CH_2), 57.62 (CH_2), 51.60, 40.36 (CH_2), 34.26 (CH_2), 34.11 (CH_2), 31.94 (CH_2), 29.70 (CH_2), 29.36 (CH_2), 29.22 (CH_2), 25.34 (CH_2), 24.94(CH_2), 22.67 (CH_2), 13.96 (CH_3). FAB-HR-MS calcd. for $C_{47}H_{89}NO_{15}P$ $[M]^+$: 938.5970; found: 938.6012.

5-(Methoxycarbonyl)pentyl 2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranoside (11)

Glycosylation of methyl 6-hydroxyhexanoate (11) (0.94 g, 6.4 mmol) was carried out with the glycosylating agent (4.45 g, 6.0 mmol) used in the preparation of compound **7** and gave 5.16 g of crude title compound contaminated with 6-hydroxyhexanoate. A chromatographically purified sample had: ^{1}H NMR ($CDCl_3$) δ: 6.10 (t, $J_{3,4}$, $J_{4,5}$ = 10 Hz, 1H, H-4), 5.92 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.69 (dd, $J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.08 (d, 1H, H-1), 4.69 (dd, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$ = 12.0 Hz, 1H, H-6), 4.50 (dd, $J_{5,6'}$ = 4.5 Hz, 1H, H-6′), 4.42 (m, 1H, H-5), 3.84 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OC*H*H), 3.68 (s, 3H, CH_3), 3.58 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OCH*H*), 2.36 (t, 2H, CH_2), 1.71 (m, 4H, 2 × CH_2), 1.46 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 98.08 (C-1), 71.02, 70.55, 69.28, 68.83 (CH_2), 67.46, 63.37 (CH_2), 51.87 (CH_3), 34.32 (CH_2), 29.46 (CH_2), 26.08 (CH_2) 25.05 (CH_2). FAB- HR-MS calcd. for $C_{41}H_{41}O_{12}$ $[M + H]^+$: 725.2598; found: 725.2582.

5-(Methoxycarbonyl)pentyl* α-D-*mannopyranoside (12)

Debenzoylation was carried out on compound **11** (5.16 g) as with the ester **7** to give glycoside **12** (1.735 g, 93%, two treatments): $[\alpha]_D^{20}$ +44.1 (*c* 0.76, MeOH). ^{1}H NMR (CD_3OD) δ: 4.74 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 3.84 (dd, $J_{5,6}$ = 2.3 Hz, $J_{6,6'}$ = 11.7 Hz, 1H, H-6), 3.80 (dd, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 3.68–3.77 (m, 3H, H-3, H-6′, OC*H*H), 3.67 (s, 3H, CH_3), 3.62 (t, $J_{3,4}$, $J_{4,5}$ = 9.2 Hz, 1H, H-4), 3.54 (m, 1H, H-5), 3.43 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OCH*H*), 2.35 (t, *J* = 7.4 Hz, 2H, CH_2), 1.64 (m, 4H, 2 × CH_2), 1.44 (m, 2H, CH_2). ^{13}C NMR (CD_3OD) δ: 176.32 (C=O), 101.98 (C-1), 75.02, 73.09, 72.67, 69.08, 68.73(CH_2), 63.36 (CH_2), 52.38 (CH_3), 35.12 (CH_2), 30.62 (CH_2), 27.27 (CH_2), 26.19 (CH_2). FAB-HR-MS calcd. for $C_{13}H_{25}O_8$ $[M + H]^+$: 309.1549; found: 309.1540.

5-Carboxypentyl* α-D-*mannopyranoside (13)

Ester hydrolysis of **12** (785 mg, 2.54 mmol) was carried out as for compound **8** and gave **13** (750 mg, 100%): $[\alpha]_D^{20}$ +53.2 (*c* 0.34, MeOH). ^{1}H NMR (CD_3OD) δ: 4.76 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 3.33–3.89 (m, 8H), 2.28 (t, 2H, CH_2), 1.65 (m, 4H, 2 × CH_2), 1.42 (m, 2H, CH_2). ^{13}C NMR (CD_3OD) δ: 179.48 (C=O), 101.95 (C-1), 74.91, 73.05, 72.65, 69.01, 68.85 (CH_2), 63.17 (CH_2), 36.42 (CH_2), 30.71 (CH_2), 27.43(CH_2), 26.68 (CH_2). FAB-HR-MS calcd. for $C_{12}H_{23}O_8$ $[M + H]^+$: 295.1393; found: 295.1398.

5-(*N*-Succinimidyloxycarbonyl)pentyl* α-D-*mannopyranoside (14)

Amide formation was effected with acid **13** (148 mg, 0.5 mmol) as for analogue **9** and gave amide **14** (170 mg, 89%). ^{13}C NMR (CD_3OD) δ: 101.97 (C-1), 75.00, 73.08, 72.66, 69.09, 68.70 and 63.35 (CH_2), 52.73, 32.55 (CH_2), 30.53 (CH_2), 27.12 (CH_2), 26.92 (CH_2), 25.94 (CH_2). FAB-HR-MS calcd. for $C_{16}H_{26}NO_{10}$ $[M + H]^+$: 392.1557; found: 392.1542.

5-[Carboxy-2-(1,2-dihexadecanoyl*-sn-*glycero-3-phospho)ethanolamido]pentyl* α-D-*mannopyranoside sodium salt (4)

The title glycolipid (170 mg, 87%) was made from amide **14** (77 mg, 0.19 mmol) in like manner to its analogue **3**: $[\alpha]_D^{20}$ +13.1 (*c* 0.58, $CHCl_3$–MeOH, 2:1). ^{13}C NMR ($CDCl_3$–CD_3OD) δ: 101.94, 74.76, 73.12, 72.63, 69.05, 68.82 (CH_2), 63.34 (CH_2), 52.62, 50.11, 35.44 (CH_2), 35.25 (CH_2), 33.51 (CH_2), 31.20 (CH_2), 31.01 (CH_2), 30.92 (CH_2), 30.81 (CH_2), 30.66 (CH_2), 27.22 (CH_2), 26.50 (CH_2), 24.19 (CH_2), 15.09 (CH_3). FAB-HR-MS calcd. for $C_{49}H_{93}NNaO_{15}P$ $[M]^+$: 989.6180; found: 989.6157.

3-(Methoxycarbonyl)propyl 2,4-di-O-acetyl-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (16) and 3-(methoxycarbonyl)propyl 2-O-acetyl-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→4)]-{[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]}-α-D-mannopyranoside (17)

A solution of glycoside **8** (832 mg, 3.0 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature over molecular sieves (4 Å, 500 mg, finely ground) for 30 min. The solution was cooled to −40°C and TmsOTf (200 μL) was added, followed by 2,3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (4.41 g, 5.95 mmol) in CH_2Cl_2 (30 mL) dropwise over 30 min. The solution was stirred for further 30 min, treated with Et_3N (0.5 mL), and evaporated to dryness. Column chromatography (hexane–EtOAc, 1.5:1, then 1:1) afforded a mixture of trisaccharide (**15**) (FAB-HR-MS calcd. for $C_{79}H_{72}O_{26}Cs$ $[M + Cs]^+$: 1569.3366; found 1569.3367) and tetrasaccharide derivatives (2.863 g). Acetylation of this crude mixture under standard conditions followed by column chromatography (hexane–EtOAc, 1:1) gave the following title compounds.

16:

(1.055 g, 23%): $([\alpha]_D^{20}$ −21.3 (*c* 0.65, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 6.18 (t, $J_{3'',4''}$, $J_{4'',5''}$ = 10 Hz, 1H, H-4″), 6.13 (t, $J_{3',4'}$, $J_{4',5'}$ = 10 Hz, 1H, H-4′), 5.95 (dd, $J_{2',3'}$ = 3.3 Hz, 1H, H-3′), 5.82 (dd, $J_{2'',3''}$ = 3.2 Hz, 1H, H-3″), 5.75 (dd, 1H, H-2′), 5.53 (dd, 1H, H-2″), 5.44 (m, 2H, H-2,4), 5.37 (d, $J_{1'',2''}$ = 1.7 Hz, 1H, H-1″), 5.16 (d, $J_{1',2'}$ = 1.7 Hz, 1H, H-1′), 4.88 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.71 (m, 2H, H-6′a, H-6″a), 4.59 (m, 2H, H-5′, H-5″), 4.52 (m, 2H, H-6′b, H-6″b), 4.37 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, 1H, H-3), 4.02 (m, 2H, H-5,6a), 3.89 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OC*H*H), 3.69 (m, 1H, H-6b), 3.63 (s, 3H, CH_3), 3.57 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OCH*H*), 2.45 (m, 2H, CH_2), 2.32, 2.27 (2s, 2 × 3 H, OAc), 2.00 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 99.46 (C-1″), 97.87 (C-1′), 97.80 (C-1), 75.39 (C-3), 71.39 (C-2), 71.19 (C-2″), 70.86 (C-2′), 70.39 (C-3′), 70.10, 70.07 (C-5, C-5″), 69.78 (C-3″), 69.41 (C-5′), 68.89 (C-4), 67.70 (OCH_2), 67.29 (C-6, C-4′), 66.93 (C-4″), 63.35, 63.02 (C-6′, C-6″), 51.95 (CH_3), 31.19 (CH_2), 25.08 (CH_2), 21.41 (*C*H_3CO), 21.22 (*C*H_3CO). FAB-HR-MS calcd. for $C_{83}H_{76}O_{28}Cs$ $[M + Cs]^+$: 1653.3577; found: 1653.3583.

17:

(1.670 g, 27%, containing small proportions of other oligosaccharide derivatives). ^1H NMR (500 MHz, $CDCl_3$) δ: 6.21 (t, $J_{3'',4''}$, $J_{4'',5''}$ = 10.1 Hz, 1H, H-4″), 6.12, 6.10 (dt, 2H, H-4′, H-4‴), 6.04 (m, 2H, H-2′, H-3′), 5.95 (dd, $J_{2'',3''}$ = 2.7 Hz, 1H, H-3″), 5.91 (dd, $J_{2''',3'''}$ = 3.2 Hz, $J_{3''',4'''}$ = 10.2 Hz, 1H, H-3‴), 5.88 (m, 1H, H-2″), 5.79 (m, 1H, H-2‴), 5.68 (d, $J_{1'',2''}$ = 1.7 Hz, 1H, H-1″), 5.51 (d, $J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.34 (dd, $J_{2,3}$ = 3.0 Hz, 1H, H-2), 5.26 (d, $J_{1''',2'''}$ = 1.7 Hz, 1H, H-1‴), 4.83 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.43–4.73 (m, 9H, H-5′, 6′a, 6′b, H-5″, 6″a, 6″b, H-5‴, 6‴a, 6‴b), 4.38 (m, 2H, H-3, H-4), 4.23 (dd, $J_{5,6a}$ = 5 Hz, $J_{6a,6b}$ = 12 Hz, 1H, H-6a), 4.18 (dd, $J_{5,6b}$ = 1.5 Hz, 1H, H-6b), 4.00 (m, 1H, H-5), 3.82, 3.48 (2m, 2H, OC$H_2$$CH_2$), 3.58 (s, 3H, CH_3), 2.38 (m, 2H, CH_2), 2.18 (s, 3H, Ac), 1.92 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 100.68 (C-1′), 100.43 (C-1″), 98.53 (C-1‴), 97.14 (C-1), 79.61 (C-3), 76.52 (C-4), 72.29 (C-2), 71.86 (C-2″); 71.66 (C-5), 71.33 (C-2′), 70.78 (C-2‴), 70.48 (C-3‴), 70.27, 70.16, 69.56 (C-5′, C-5″, C-5‴), 69.77, 69.73 (C-3′, C-3″); 68.80 (CH_2), 68.80, 67.25, 67.24 (C-4′, C-4″, C-4‴), 66.97 (C-6), 63.49, 63.08, 62.95 (C-6′, C-6″, C-6‴), 51.95 (CH_3), 31.11 (CH_2), 25.12 (CH_2), 21.38 (*C*H_3CO). FAB-HR-MS calcd. for $C_{115}H_{100}O_{36}Cs$ $[M + Cs]^+$: 2189.5049; found: 2189.5031.

3-(Methoxycarbonyl)propyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (18)

To a solution of the perester **16** (1.050 g, 0.65 mmol) in MeOH (30 mL) was added NaOMe (8 mmol, 1 M in MeOH), and the mixture was stirred at room temperature for 24 h, taken to pH ~ 5 with Amberlyst 15 (H^+), filtered, and evaporated to dryness to afford the title trisaccharide glycoside **18** (420 mg, 100%): $[\alpha]_D^{20}$ +74.7 (*c* 0.38, H_2O). ^1H NMR (D_2O) δ: 5.07 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.86 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.77 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 3.43–4.03 (m, 26H), 2.46 (t, 2H, CH_2), 1.93 (m, 2H, CH_2). ^{13}C NMR (D_2O) δ: 177.24 (C=O), 102.77, 100.32, 99.83 (C-1), 79.00, 73.74, 73.11, 71.51, 71.03, 70.81, 70.49, 70.39, 70.08, 67.35 (CH_2), 67.16, 66.12, 65.74 (CH_2), 61.38 (2 × CH_2), 52.65 (CH_3), 31.19 (CH_2) and 24.54 (CH_2). FAB-HR-MS calcd. for $C_{23}H_{41}O_{18}$ $[M + H]^+$: 605.2293; found: 605.2321.

3-Carboxypropyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (19)

To a solution of glycoside **18** (121 mg, 0.20 mmol) in H_2O (3 mL), NaOH (0.5 mL, 3 M, aq) was added. The reaction mixture was stirred for 2 days, neutralized with Amberlyst 15 resin (H^+), filtered, and freeze-dried to afford the acid **19** (120 mg, 101%): $[\alpha]_D^{20}$ +85.5 (*c* 0.31, MeOH). ^{13}C NMR (CD_3OD) δ: 170.34 (C=O), 104.27, 102.17, 101.81 (C-1), 81.13, 75.32, 74.76, 73.90, 73.06, 72.89, 72.48 (2×CH), 71.81, 69.22, 69.01, 68.17 (CH_2), 67.89, 67.68 (CH_2), 63.29 (2 × CH_2), 32.27 (CH_2), 26.44 (CH_2). FAB-HR-MS calcd. for $C_{22}H_{39}O_{18}$ $[M + H]^+$: 591.2136; found: 591.2159.

3-(N-Succinimidyloxycarbonyl)propyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (20)

By use of the procedure described for making amide **10**, and using $CHCl_3$ – MeOH – H_2O (5:4:1) as eluent for column chromatography, acid **19** (115 mg, 0.19 mmol) was converted to amide **20** (85 mg, 64%): $[\alpha]_D^{20}$ +87.8 (*c* 0.23, MeOH). ^{13}C NMR ($CDCl_3$) δ: 104.36, 102.28 and 101.81 (C-1, C-1′, C-1″), 81.13, 75.32, 74.76, 73.90, 73.06, 72.89, 72.48, 69.22, 69.01, 67.89, 67.68 (2×CH_2), 63.29 (2×CH_2), 32.27 (CH_2), 30.16, (CH_2), 28.69 (CH_2), 26.17 (CH_2). FAB-HR-MS calcd. for $C_{26}H_{42}NO_{20}$ $[M + H]^+$: 688.2300; found: 688.2317.

3-[Carboxy-2-(1,2-dihexadecanoyl-sn-glycero-3-phospho)ethanolamido]propyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside sodium salt (5)

From amide **20** (83 mg, 0.12 mmol), according to the procedure described for making glycolipid **3**, the analogue **5**

(105 mg, 70%, containing small proportions of 1,2-hexadecanoyl-*sn*-glycero-3-phosphoethanolamine) was obtained following column chromatography with $CHCl_3$–MeOH–H_2O (5:4:1) as eluent: $[\alpha]_D^{20}$ +26.4 (*c* 0.25, $CHCl_3$–MeOH, 3:1). ^{13}C NMR ($CDCl_3$) δ: 106.47, 104.85 and 104.04 (C-1, C-1′, C-1″), 83.33, 77.55, 76.92, 75.90, 75.30, 75.00, 74.65, 71.32, 71.10, 70.17, 69.78, 68.63, 68.04, 66.85, 66.01, 65.27, 44.72, 38.48, 38.33, 36.92, 36.15, 33.93, 33.58, 33.40, 29.53, 29.14, 26.88 and 18.17. FAB-HR-MS calcd. for $C_{59}H_{110}NNaO_{25}P$ $[M]^+$: 1286.7002; found: 1286.6958.

5-(Methoxycarbonyl)pentyl 2,4-di-O-acetyl-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (22) and 5-(methoxycarbonyl)pentyl 2-O-acetyl-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→4)]-{[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]}-α-D-mannopyranoside (23)

A solution of glycoside **12** (917 mg, 2.97 mmol) in CH_2Cl_2 (50 mL) was mannosylated as for the analogue **8**, and column chromatography (hexane–EtOAc, 1.5:1, then 1:1) afforded two main fractions (692 mg) and (2.73 g) (in order of elution), both as mixtures of products mainly containing trisaccharide derivative **21** and a tetrasaccharide analogue. Acetylation of the second fraction under standard conditions followed by column chromatography (hexane–EtOAc, 1:1) gave the trisaccharide glycoside perester **22** and **23**. The latter contained small proportions of impurities — probably tri- and tetra-saccharide isomers of the main products.

22:

(1.543 g, 33% from **12**): $[\alpha]_D^{20}$ –17.7 (*c* 0.52, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 6.18 (t, $J_{3'',4''}$, $J_{4'',5''}$ = 10.0 Hz, 1H, H-4″), 6.12 (t, $J_{3',4'}$, $J_{4',5'}$ = 10.0 Hz, 1H, H-4′), 5.94 (dd, $J_{2',3'}$ = 3.4 Hz, 1H, H-3′), 5.81 (dd, $J_{2'',3''}$ = 3.2 Hz, 1H, H-3″), 5.74 (dd, $J_{1',2'}$ = 1.7 Hz, 1H, H-2′), 5.52 (dd, $J_{1'',2''}$ = 1.7 Hz, 1H, H-2″), 5.43 (m, 2H, H-2,4), 5.37 (d, 1H, H-1″), 5.16 (d, 1H, H-1′), 4.87 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.71 (dd, $J_{5',6'a}$ = 2.3 Hz, $J_{6'a,6'b}$ = 12.1 Hz, 1H, H-6′a), 4.68 (dd, $J_{5'',6''a}$ = 2.3 Hz, $J_{6''a,6''b}$ = 12.3 Hz, 1H, H-6″a), 4.58 (m, 2H, H-5′, H-5″), 4.53 (dd, $J_{5'',6''b}$ = 3.7 Hz, 1H, H-6″b), 4.51 (dd, $J_{5',6'b}$ = 3.8 Hz, 1H, H-6′b), 4.38 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, 1H, H-3), 4.01 (m, 2H, H-5,6a), 3.84 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OC*H*H), 3.69 (m, 1H, H-6b), 3.59 (s, 3H, CH_3), 3.50 (dt, *J* = 6.6, 6,6, 9.6 Hz, 1H, OCH*H*), 2.29 (m, 8H, CH_2, 2 × Ac), 1.66 (m, 4H, 2 × CH_2), 1.42 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 99.36 (C-1″), 97.71, 97.67 (C-1, C-1′), 75.44 (C-3), 71.41 (C-2), 71.10 (C-2″), 70.74 (C-2′), 70.30 (C-3′), 69.95 (C-5, C-5″), 69.71 (C-3″), 69.26 (C-5′), 68.78 (C-4), 68.33 (CH_2), 67.24, 67.21 (C-4′, C-6), 66.82 (C-4″), 63.25 (C-6′), 62.92 (C-6″), 51.65 CH_3), 34.18 (CH_2), 29.29 (CH_2), 26.04 (CH_2), 24.99 (CH_2), 21.35 (*C*H_3CO), 21.13 (*C*H_3CO). FAB-HR-MS calcd. for $C_{85}H_{80}O_{28}Cs$ $[M + Cs]^+$: 1681.3890; found: 1681.3899.

23:

(1.033 g, 17% from **12**). ^1H NMR (500 MHz, $CDCl_3$) δ: 6.27 (t, $J_{3'',4''}$, $J_{4'',5''}$ = 10.1 Hz, 1H, H-4″), 6.17 (2t, 2H, H-4′, H-4‴), 6.12 (m, 2H, H-2′, H-3′), 6.07 (dd, 1H, $J_{2'',3''}$ = 2.9 Hz, H-3″), 5.99 (dd, $J_{2''',3'''}$ = 3.4 Hz, $J_{3''',4'''}$ = 10.0 Hz, 1H, H-3‴), 5.96 (m, 1H, H-2″), 5.86 (dd, 1H, $J_{1''',2'''}$ = 1.7 Hz, H-2‴), 5.76 (d, $J_{1'',2''}$ = 1.7 Hz, 1H, H-1″), 5.59 (d, $J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.43 (dd, $J_{2,3}$ = 2.9 Hz, 1H, H-2), 5.35 (d, 1H, H-1‴), 4.90 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.52–4.82 (m, 9H, H-5′, 6′a, 6′b, H-5″, 6″a, 6″b, H-5‴, 6‴a, 6‴b), 4.50 (dd, 1H, H-3), 4.42 (t, *J* = 9.8 Hz, 1H, H-4), 4.31 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 11.8 Hz, 1H, H-6a), 4.17 (dd, $J_{5,6b}$ = 1.5 Hz, 1H, H-6b), 4.07 (m, 1H, H-5), 3.81, 3.47 (2m, 2H, CH_2), 3.61 (s, 3H, CH_3), 2.34 (m, 2H, CH_2), 2.27 (s, 3H, Ac), 1.69 (m, 4H, 2 × CH_2), 1.45 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 100.68 (C-1′), 100.46 (C-1″), 98.53 (C-1‴), 97.10 (C-1), 79.77 (C-3), 76.57 (C-4), 72.45 (C-2), 71.89 (C-2″), 71.57 (C-5), 71.37 (C-2′), 70.76 (C-2‴), 70.51 (C-3‴), 70.30, 70.26, 69.79, 69.76 and 69.53 (C-3′, C-3″, C-5′, C-5″, C-5‴), 68.65 (CH_2), 67.84, 67.44, 67.28 (C-4′, C-4″, C-4‴), 67.02 (C-6), 63.52, 63.10, 62.97 (C-6′, C-6″, C-6‴), 51.77 (CH_3), 34.33 (CH_2), 29.49 (CH_2), 26.12 (CH_2), 25.14 (CH_2), 21.43 (*C*H_3CO). FAB-HR-MS calcd. for $C_{117}H_{104}O_{36}Cs$ $[M + Cs]^+$: 2217.5362; found: 2217.5339.

5-(Methoxycarbonyl)pentyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (24)

To a solution of diacetate **22** (1.502 g, 0.97 mmol) in MeOH (30 mL) was added NaOMe (X M in MeOH, 12 mL) and the mixture was stirred at room temperature for 24 h, neutralized to pH ~ 5 with Amberlyst 15 (H^+), filtered, and evaporated to dryness to afford the de-esterified glycoside **24** (575 mg, 94%): $[\alpha]_D^{20}$ +79.7 (*c* 0.33, H_2O). ^1H NMR (D_2O) δ: 5.09 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1′), 4.88 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1″), 4.80 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 3.67 (s, 3H, CH_3), 3.50–4.07 (m, 7H, H-2,3,4,5,6, OC$H_2$$CH_2$), 2.39 (t, *J* = 7.4 Hz, 2H, CH_2), 1.61 (m, 4H, 2 × CH_2), 1.37 (m, 2H, CH_2). ^{13}C NMR (D_2O) δ: 177.98, 102.76, 100.29, 99.82 (C-1, C-1′, C-1″), 79.00, 73.74, 73.12, 71.51, 71.06, 70.79, 70.42, 70.41, 70.13, 68.24 (CH_2), 67.18, 67.17, 66.21, 65.79 (CH_2), 61.39 (2 CH_2), 52.53 (CH_3), 34.02 (CH_2), 28.54 (CH_2), 25.40 (CH_2), 24.40 (CH_2). FAB-HR-MS calcd. for $C_{25}H_{45}O_{18}$ $[M + H]^+$: 633.2606; found: 633.2630.

5-Carboxypentyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (25)

Acid **25** (774 mg, 96%) was obtained from methyl ester **24** (821 mg, 1.30 mmol) by use of the procedure applied to make analogue **9**: $[\alpha]_D^{20}$ +87.3 (*c* 0.75, H_2O). ^{13}C NMR (D_2O) δ: 179.56 (C=O), 102.71, 100.26, 99.78 (C-1, C′-1, C″-1), 79.00, 73.68, 73.06, 71.47, 71.05, 70.79, 70.47, 70.40, 70.13, 68.20 (CH_2), 67.14, 66.20, 65.77 (CH_2), 61.37 (2 CH_2), 34.37 (CH_2), 28.57 (CH_2), 25.41 (CH_2), 24.51 (CH_2). FAB-HR-MS calcd. for $C_{24}H_{43}O_{18}$ $[M + H]^+$: 619.2449; found: 619.2468.

5-[Carboxy-2-(1,2-dihexadecanoyl-sn-glycero-3-phospho)ethanolamido]pentyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside sodium salt (6)

Fom acid **25** (125 mg, 0.202 mmol), following the procedure described to make amide **14**, crude succinimidyl derivative (**26**) was obtained, and was used without further purification, following the method described for making the glycolipid **5**. The sodium salt **6** (132 mg, 50% from acid **25**)

was isolated after column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (5:4:1) as eluent: $[\alpha]_D^{20}$ +37.0 (*c* 0.6, $CHCl_3$–MeOH, 2:1). ^{13}C NMR ($CDCl_3$–CD_3OD) δ: 102.71, 100.26 and 99.78 (C-1, C-1′, C-1″), 79.00, 73.68, 73.06, 71.47, 71.05, 70.79, 70.47, 68.20, 67.14, 66.20, 63.87 (CH_2), 63.35 (CH_2), 62.38 (CH_2), 61.35 (CH_2), 40.03 (CH_2), 35.84 (CH_2), 33.93 (CH_2), 33.78 (CH_2), 33.42 (CH_2), 31.67 (CH_2), 29.40 (CH_2), 29.08 (CH_2), 28.86 (CH_2), 25.69 (CH_2), 25.25 (CH_2), 24.65 (CH_2), 22.36 (CH_2), 13.41 (CH_3). FAB-HR-MS calcd. for $C_{61}H_{113}NNa_2O_{25}P$ $[M + Na]^+$: 1336.7135; found: 1336.7076.

2-[2-(2-Chloroethoxy)ethoxy]ethyl 2,3,4,6-tetra-O-*benzoyl*-α-D-*mannopyranoside (27)*

2-[2-(2-Chloroethoxy)ethoxy]ethanol (1.00 g, 5.95 mmol) was glycosylated according to the procedure described for making glycoside **7**, to give product **27** (4.07 g, 91%): $[\alpha]_D^{20}$ −48.2 (*c* 0.57, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.11 (t, $J_{3,4}$, $J_{4,5}$ = 10.1 Hz, 1H, H-4), 5.94 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.73 (dd, $J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.16 (d, 1H, H-1), 4.70 (m, 1H, H-6), 4.50 (m, 2H, H-5,6′), 4.00–3.61 (m, 12H, spacer protons). ^{13}C NMR ($CDCl_3$) δ: 98.22 (C-1), 71.83 (CH_2), 71.20 (CH_2), 71.14 (CH_2), 70.90, 70.61 (CH_2), 70.47, 69.25, 68.05 (CH_2), 67.39, 63.27 (CH_2), 43.18 (CH_2). FAB-HR-MS calcd. for $C_{40}H_{40}ClO_{12}$ $[M + H]^+$: 747.2208; found: 747.2184.

2-[2-(2-Cyanoethoxy)ethoxy]ethyl 2,3,4,6-tetra-O-*benzoyl*-α-D-*mannopyranoside (28)*

A mixture of chloro-compound **27** (3.98 g, 5.33 mmol), potassium cyanide (3.26 g 50 mmol), and DMSO (40 mL) was stirred under argon at 65°C for 22 h. Water (100 mL) was added, and the mixture was extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with H_2O (20 mL), dried (Na_2SO_4), and evaporated to dryness. Column chromatography (hexane–EtOAc, 1:1) of the residue gave the nitrile **28** (2.77 g, 70%): $[\alpha]_D^{20}$ –37.2 (*c* 0.78, $CHCl_3$). IR (film) (cm^{-1}): 2253. ^1H NMR ($CDCl_3$) δ: 6.11 (t, $J_{3,4}$, $J_{4,5}$ = 10.1 Hz, 1H, H-4), 5.93 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.72 (dd, $J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.16 (d, 1H, H-1), 4.70 (m, 1H, H-6), 4.50 (m, 2H, H-5,6′), 4.00–3.70 (m, 12H, spacer H), 2.63 (t, *J* = 6.4 Hz, 2H, C*H*$_2$CN). ^{13}C NMR ($CDCl_3$) δ: 118.34 (CN), 98.23 (C-1), 71.19 (2 × CH_2), 70.88, 70.64 (CH_2), 70.47, 69.28, 68.02 (CH_2), 67.39, 66.44 (CH_2), 63.26 (CH_2), 19.28 (CH_2). FAB-HR-MS calcd. for $C_{41}H_{40}NO_{12}$ $[M + H]^+$: 738.2550; found: 738.2573.

2-[2-(2-Cyanoethoxy)ethoxy]ethyl α-D-*mannopyranoside (29)*

De-esterification of tetrabenzoate **28** (320 mg, 0.43 mmol), following the procedure described for making glycoside **8**, gave glycoside **29** (128 mg, 92%): $[\alpha]_D^{20}$ +47.0 (*c* 0.61, MeOH). IR (film) (cm^{-1}): 2252.; ^{13}C NMR (CD_3OD) δ: 120.16 (CN), 102.13 (C-1), 74.99, 72.99, 72.51, 72.02 (CH_2), 71.95 (CH_2), 71.85 (CH_2), 69.03, 68.18 (CH_2), 67.51 (CH_2), 63.35 (CH_2), 19.66 (CH_2). FAB-HR-MS calcd. for $C_{13}H_{24}NO_8$ $[M + H]^+$: 322.1502; found: 322.1500.

*2-[2-(2-*α-D-*Carboxyethoxy)ethoxy]ethyl* α-D-*mannopyranoside (30)*

A mixture of nitrile **29** (79 mg, 0.24 mmol) in NaOH (1 mL, 3 M) was stirred at room temperature for 24 h, neutralized with Amberlyst 15 (H^+) resin, filtered, and freeze-dried to afford acid **30** (81 mg, 100%): $[\alpha]_D^{20}$ +38.0 (*c* 0.41, H_2O). ^{13}C NMR (D_2O) δ: 176.89 (C=O), 100.37 (C-1), 73.16, 72.14 (CH_2), 70.92, 70.37, 69.98 (CH_2), 67.18, 66.87 (CH_2), 61.35 (CH_2), 60.81 (CH_2) 57.80 (CH_2), 37.34 (CH_2). FAB-HR-MS calcd. for $C_{13}H_{25}O_{10}$ $[M + H]^+$: 341.1448; found: 341.1449.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-O-*benzoyl*-α-D-*mannopyranoside (31)*

A mixture of chloro-compound **27** (2.785 g, 3.72 mmol), sodium azide (2.6 g 40 mmol), and DMF (40 mL) was stirred under argon at 65°C for 19 h. Water (60 mL) was added, and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with water (10 mL), dried (Na_2SO_4), and evaporated to dryness. Column chromatography (hexane–EtOAc, 1:1) of the residue gave of azide **31** (2.655 g, 95%): $[\alpha]_D^{20}$ –43.7 (*c* 0.6, $CHCl_3$). IR (film) (cm^{-1}): 2105. ^1H NMR ($CDCl_3$) δ: 6.11 (t, $J_{3,4}$, $J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.95 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.73 (dd, $J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.16 (d, 1H, H-1), 4.70 (m, 1H, H-6), 4.50 (m, 2H, H-5,6′), 4.0–3.6 (m, 10H, spacer H), 3.38 (t, *J* = 5.0 Hz, 2H, CH_2N_3). ^{13}C NMR ($CDCl_3$) δ: 98.23 (C-1), 71.28 (CH_2), 71.15 (CH_2), 70.90, 70.63 (CH_2), 70.50, 69.25, 68.06 (CH_2), 67.40, 63.26 (CH_2), 51.10 (CH_2), 14.57 (CH_2). FAB-HR-MS calcd. for $C_{40}H_{39}N_3O_{12}Cs$ $[M + Cs]^+$: 886.1588; found: 886.1538.

2-{2-[2-(5-Methoxycarbonylpentanoylamino)ethoxy]ethoxy}ethyl α-D-*mannopyranoside (34)*

To a solution of azide **31** (1.30 g, 1.72 mmol) in EtOH (25 mL), $NaBH_4$ (325 mg, 8.5 mmol) was added followed by a solution of $NiCl_2$ in EtOH (1.5 mL, 0.2 M), and the mixture was stirred at room temperature for 45 min, filtered through a pad of silica gel (thickness ~ 5 cm) and washed with $CHCl_3$–MeOH (2:1). The filtrate and washings were taken to dryness and purified by column chromatography ($CHCl_3$–MeOH, 3:1) to give the amine **32** (660 mg, 53%). ^{13}C NMR (CD_3OD) δ: 99.53 (C-1), 72.15, 71.99 (CH_2), 71.90, 71.64 (CH_2), 71.49 (CH_2), 70.50, 68.91 (CH_2), 68.41, 68.33 (CH_2), 64.00 (CH_2), 41.05 (CH_2). A solution of amine **32** (200 mg, 0.27 mmol), monomethyl adipate (48 mg, 0.3 mmol), and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (12) (74 mg, 0.3 mmol) in EtOH (10 mL) was heated under reflux for 3 h and taken to dryness in vacuo. Column chromatography ($CHCl_3$–MeOH, 50:1) of the residue gave amide **33** (244 mg, 100%). ^{13}C NMR ($CDCl_3$) δ: 98.23 (C-1), 71.04, 70.90 (CH_2), 70.72 (CH_2), 70.55, 70.49 (CH_2), 70.33 (CH_2), 69.28, 68.06 (CH_2), 67.35, 63.26 (CH_2), 51.84 (CH_3), 39.75 (CH_2), 36.51 (CH_2), 34.11 (CH_2), 25.46 (CH_2), 24.81 (CH_2). A mixture of amide **33** (1.615 g, 18.6 mmol) and K_2CO_3 (200 mg) in MeOH (20 mL) was stirred overnight, neutralized with Amberlyst 15 (H^+), filtered, and taken to dryness in vacuo. Column chromatography ($CHCl_3$–MeOH, 3:1) of the residue gave the de-esterified glycoside **34** (530 mg, 63%): $[\alpha]_D^{20}$ +32.1 (*c* 0.57, MeOH). ^{13}C NMR (CD_3OD) δ: 102.17 (C-1), 75.03, 73.01, 72.54, 72.02 (CH_2), 71.82 (CH_2), 71.71 (CH_2), 71.06 (CH_2), 69.07, 68.13 (CH_2), 63.40 (CH_2), 52.43 (CH_3), 40.79 (CH_2), 37.00 (CH_2), 34.86 (CH_2), 26.77 (CH_2), 25.91 (CH_2). FAB-

HR-MS calcd. for $C_{19}H_{36}NO_{11}$ [M + H]$^+$: 454.2288; found: 454.2326.

2-[2-(2-Cyanoethoxy)ethoxy]ethyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (35)

Glycoside **29** (580 mg, 1.8 mmol) and 2,3,4,6-tetra-*O*-benzoyl α-D-mannopyranosyl trichloroacetimidate (2.667 g, 3.6 mmol) were treated according to the procedure described for making compound **21**, and gave mixed products (1.585 g). Column chromatography (hexane–EtOAc, 1:1) afforded the title trisaccharide derivative **35** (1.070 g, 40%): $[\alpha]_D^{20}$ –24.4 (*c* 0.36, $CHCl_3$). IR (film) (cm^{-1}): 2252.; ^{1}H NMR ($CDCl_3$) δ: 6.12 (t, *J* = 10 Hz, 2H, H-4′, H-4″), 6.00 (m, 2H, H-3′, H-3″), 5.86 (dd, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.0 Hz, 1H, H-2′), 5.79 (dd, $J_{1'',2''}$ = 1.7 Hz, $J_{2'',3''}$ = 3.2 Hz, 1H, H-2″), 5.44 (d, 1H, H-1″), 5.33 (d, 1H, H-1′), 4.83 (d, 1H, H-1), 3.90–4.95 (m, 12H), 3.60–3.72 (m, 10H, spacer H), 2.58 (t, *J* = 6.4 Hz, 2H, C*H*$_2$CN). ^{13}C NMR ($CDCl_3$) δ: 118.05 (CN), 100.07, 99.75, 97.62 (C-1,1′,1″), 82.54, 71.41, 70.73, 70.56, 70.22, 69.49, 68.91, 67.50, 66.98, 66.87, 65.90, 63.26, 62.96 (CH_2), 18.85 (*C*H_2CN); (other CH_2 carbon resonances not identified). FAB-HR-MS calcd. for $C_{81}H_{76}NO_{26}$ [M + H]$^+$: 1478.4656; found: 1478.4629.

2-[2-(2-Cyanoethoxy)ethoxy]ethyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (37)

Standard acetylation of an analytical sample of the trisaccharide derivative **35** yielded 2-[2-(2-cyanoethoxy)ethoxy]ethyl 2,4-di-*O*-acetyl-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (**36**): $[\alpha]_D^{20}$ –20.2 (*c* 0.54, $CHCl_3$). IR (film) (cm^{-1}): 2252. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.19 (t, $J_{3'',4''}$, $J_{4'',5''}$ = 10.1 Hz, 1H, H-4″), 6.12 (t, $J_{3',4'}$, $J_{4',5'}$ = 10.1 Hz, 1H, H-4′), 5.92 (dd, $J_{2',3'}$ = 3.3 Hz, 1H, H-3′), 5.81 (dd, $J_{2'',3''}$ = 3.3 Hz, 1H, H-3″), 5.73 (dd, $J_{1',2'}$ = 1.7 Hz, 1H, H-2′), 5.51 (dd, $J_{1'',2''}$ = 1.7 Hz, 1H, H-2″), 5.47 (dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 5.43 (t, $J_{3,4}$, $J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.36 (d, 1H, H-1″), 5.17 (d, 1H, H-1′), 4.94 (d, 1H, H-1), 4.69–4.75 (m, 2H, H-6′a, H-6″a), 4.55–4.63 (m, 2H, H-5′, H-5″), 4.49 (m, 2H, H-6′b, H-6″b), 4.40 (dd, 1H, H-3), 4.10 (m, 1H, H-5), 4.01 (dd, $J_{5,6}$ = 6.8 Hz, $J_{6a,6b}$ = 10.7 Hz, 1H, H-6a), 3.60–3.73 (m, 11H, H-6b and spacer H), 2.55 (t, *J* = 6.5 Hz, 2H, C*H*$_2$CN). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 99.40 (C-1″), 97.87 (C-1), 97.73 (C-1′), 75.33 (C-3), 71.17 (C-2), 71.08 (C-2″, spacer CH_2), 70.90 (spacer CH_2), 70.73 (C-2′), 70.49 (C-3′, CH_2), 70.02 (C-5″), 69.85 (C-5), 69.77 (C-3″), 69.38 (C-5′), 68.90 (C-4), 67.43, 67.30 (C-6, spacer CH_2), 67.24 (C-4′), 66.87 (C-4″), 66.31 (spacer CH_2), 63.29 (C-6′), 62.91 (C-6″), 21.42 (Ac), 21.21 (Ac), 19.16 (*C*H_2CN).

A mixture of perester **35** (895 mg, 0.605 mmol) and K_2CO_3 (250 mg) in MeOH (30 mL) was stirred at room temperature for 22 h, neutralized to pH ~ 5 with Amberlyst 15 (H^+), filtered, and evaporated to dryness to afford title compound **37** (230 mg, 59%): $[\alpha]_D^{20}$ +73.9 (*c* 0.41, H_2O). ^{13}C NMR (D_2O) δ: 120.35 (CN), 102.78, 100.58 and 99.85 (C-1,1′,1″) 79.01, 73.74, 73.11, 71.49, 71.04, 70.81, 70.50, 70.38, 70.09 (CH_2), 69.97 (2 × CH_2), 67.17 (CH, CH_2), 66.10, 65.79 (2 × CH_2), 61.40 (2 × CH_2), 18.52 (*C*H_2CN). FAB-HR-MS calcd. for $C_{25}H_{44}NO_{18}$ [M + H]$^+$: 646.2558; found: 646.2560.

2-[2-(2-Carboxyethoxy)ethoxy]ethyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (38)

Hydrolysis of the nitrile **37** (90 mg) was carried out in sodium hydroxide solution (2 mL, 3 M, aq) over 2 days at 20°C. Water (5 mL) was added and the base was neutralized to pH ~ 5 with Amberlyst resin (H^+). Evaporation of the solvent gave the title acid (95 mg, 102%): $[[\alpha]_D^{20}$ +70.0 (*c* 0.43, H_2O). ^{13}C NMR (D_2O) δ: 105.31, 103.12, 102.38 (C-1, C-1′, C-1″), 81.50, 76,29, 75.66, 74.70 (CH_2), 74.03, 73.57, 73.34, 72.93, 72.53 (2 CH_2), 69.70 (CH_2), 68.65, 68.24 (CH_2), 63.95 (2CH_2), 63.37 (CH_2), 37.92 (CH_2). FAB-HR-MS calcd. for $C_{25}H_{45}O_{20}$ [M$^+$]: 665.2504; found: 665.2508.

N-*[Tris-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxymethyl)]methyl-(5-methoxycarbonyl)pentanoamide (39)*

A mixture of tetra-*O*-acetyl-α-D-mannopyranosyl bromide (3.69 g, 8.97 mmol), *N*-[tris(hydroxymethyl)]methyl-(5-methoxycarbonyl)pentanoamide (8) (590 mg, 2.24 mmol) and $HgCN_2$ (2.3 g, 9.1 mmol) in CH_3NO_2–toluene (1:1 v/v, 60 mL) was heated at 60°C for 3.5 h. The mixture was evaporated in vacuo to a syrup, dissolved in $CHCl_3$ (150 mL), and the organic layer was washed with NaCl solution (3 × 60 mL, sat., aq), H_2O (50 mL), dried (Na_2SO_4), and evaporated to dryness. Column chromatography ($CHCl_3$–MeOH, 30:1) of the residue gave the title compound (1.555 g, 55%): $[\alpha]_D^{20}$ +30.7 (*c* 0.88, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 5.33–5.17 (m, 9H, H-2,3,4), 4.85 (d, $J_{1,2}$ = 1.3 Hz, 3H, H-1), 4.34 (dd, $J_{5,5}$ = 4.9 Hz, $J_{6,6'}$ = 12.3 Hz, 3H, H-6), 4.13 (m, $J_{5,6'}$ = 2.4 Hz, H-6′, 6H, OC*H*HCN), 3.96 (m, 3H, H-5), 3.73 (d, $^2J_{H,H}$ = 10.1 Hz, 3H, OCH*H*CN), 3.66 (s, 3H, CH_3), 2.35 (m, 2H, CH_2), 2.11 (m, 2H, CH_2), 1.71 (m, 4H, CH_2), 2.14, 2.12, 2.04, 1.98 (4s, 36H, Ac). ^{13}C NMR ($CDCl_3$) δ: 103.06 (C-1), 73.84, 73.7, 71.11 (CH_2), 70.52, 66.78 (CH_2), 63.74 (C), 56.19 (CH_3), 41.25 (CH_2), 38.15 (CH_2), 29.72 (CH_2), 28.81 (CH_2), 25.31 (*C*H_3CO). FAB-HR-MS calcd. for $C_{53}H_{76}NO_{33}$ [M + H]$^+$: 1254.4300; found: 1254.4381.

N-*[Tris-(α-D-mannopyranosyloxymethyl)]methyl-(5-methoxycarbonyl)pentanoamide (40)*

To a solution of perester **39** (2.45 g, 1.95 mmol) in methanol (30 mL) was added NaOMe (2 drops, X M in MeOH) and the mixture was stirred at room temperature overnight. Removal of the solvent afforded the triglycoside **40** (1.47 g, 100%): $[\alpha]_D^{20}$ +45.4 (*c* 2.52, MeOH). ^{13}C NMR (CD_3OD) δ: 176.48 and 176.33 (C=O), 102.77 (C-1), 75.24, 73.16, 72.39, 69.22, 67.30 (CH_2), 63.22 (CH_2), 61.03 (C), 50.29 (CH_3), 37.60 (CH_2), 34.85 (CH_2), 26.92 (CH_2), 25.91 (CH_2). FAB-HR-MS calcd. for $C_{29}H_{52}NO_{21}$ [M + H]$^+$: 750.3032; found: 750.3067.

N-*[Tris-(α-D-mannopyranosyloxymethyl)]methyl-5-carboxypentanoamide (41)*

To a solution of methyl ester **40** (1.47 g, 1.95 mmol) in THF (30 mL) and H_2O (15 mL), sodium hydroxide (1.3 mL, 3 M aq) was added. The mixture was stirred for 48 h, neutralized with Amberlyst 15 resin (H^+), filtered, evaporated in vacuo, and freeze-dried to afford acid **41** (1.45 g, 100%):

$[\alpha]_D^{20}$ +42.7 (*c* 0.66, H_2O). ^{13}C NMR (D_2O) δ: 180.43 and 177.00 (C=O), 100.77 (C-1), 73.42, 71.10, 70.37, 67.16, 65.87 (CH_2), 61.25 (CH_2), 59.81 (C), 36.52 (CH_2), 35.00 (CH_2), 25.55 (CH_2), 24.61 (CH_2). FAB-HR-MS calcd. for $C_{28}H_{50}NO_{21}$ $[M + H]^+$: 736.2875; found: 736.2829.

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References

1. R.A.B. Ezekowitz and P.D. Stahl. J. Cell. Sci. Suppl. **9**, 121 (1998).
2. F. Sallusto, M. Cella, C. Danieli, and A. Lanzavecchia. J. Exp. Med. **182**, 389 (1995).
3. W. Jiang, W.J. Swiggard, C. Heufler, M. Peng, A. Mirza, R.M. Steinman, and M.C. Nussenzweig. Nature (London), **375**, 151 (1995).
4. H. Tanaka, Y. Nishida, and K. Kobayashi. J. Carbohydr. Chem. **19**, 413 (2000).
5. B. Becker, R.H. Furneaux, F. Reck, and O.A. Zubkov. Carbohydr. Res. **315**, 148 (1999).
6. K.J. Kaur and O. Hindsgaul. Glycoconjugate J. **8**, 90 (1991).
7. R. Roy, D. Page, S. Figueroa-Perez, and V. Verez-Bencomo. Glycoconjugate J. **15**, 251 (1998).
8. Y.C. Lee. Carbohydr. Res. **67**, 509 (1978).
9. T.K. Lindhorst, S. Kötter, U. Krallman-Wenzel, and S. Ehlers. J. Chem. Soc. Perkin Trans. 1, 823 (2001).
10. C.D. Reeve, D.H.G. Crout, K. Cooper, and M.J. Fray. Tetrahedron: Asymmetry, **3**, 785 (1992).
11. E. Bosone, P. Farina, G. Guazzi, S. Innocenti, V. Marotta, and U. Valcavi. Synthesis, 942 (1983).
12. B. Belleau and G. Malek. J. Am. Chem. Soc. **90**, 1651 (1968).