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Carbohydrate Research 315 (1999) 148–158

CARBOHYDRATE RESEARCH

A simple synthesis of 8-(methoxycarbonyl)octyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside and derivatives and their use in the preparation of neoglycoconjugates

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Received 23 October 1998; accepted 9 December 1998

Abstract

8-(Methoxycarbonyl)octyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**10**) was synthesized in 54% yield by regioselective diglycosylation of unprotected mannoside **4**, employing the trichloroacetimidate donor **1**, followed by debenzoylation. Derivatives of compounds **4** and **10** were used to prepare conjugates containing fluorochromes for the study of carbohydrate–lectin interactions, as well as conjugates with phospholipids for the preparation of liposomes. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Mannotrioside; Neoglycoconjugates; Glycolipid; Synthesis

1. Introduction

Terminal α-D-mannopyranosyl residues are ubiquitous features of glycoproteins of bacterial pathogens, parasites, yeasts and virus envelopes as well as certain endogenous glycoproteins such as lysosomal enzymes. They are recognized by various C-type lectins, particularly the mannose receptors of macrophages [1] and dendritic cells [2,3], and the collectins, soluble circulating proteins such as the 'mannose-binding proteins' and the lung surfactant proteins [4]. The collectins are oligomeric proteins that require complex multidentate mannose-containing ligands of rather specific geometry and spatial orientation for strong binding [4]. In contrast, the monomeric mannose receptors appear to bind some relatively simple mannose-containing oligosaccharides quite effectively [5]. For this reason, there has been interest in the potential of using synthetic mannose-containing neoglycoconjugates of drugs and antigens as vehicles for their delivery to the mannose receptors on macrophages and dendritic cells [6–8]. For example, the present enzyme replacement therapy for Gaucher's disease relies upon this concept for specific cellular uptake of the enzyme [9].

The mannose receptor of human macrophages binds branched mannose oligosaccharide derivatives such as methyl 3,6-di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside specifically and with relatively high

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PII: S0008-6215(98)00330-9

affinity, in comparison with more simple multivalent structures, such as a permannosylated lysyl–serine decapeptide, which carries an array of single mannosyl residues [5]. The 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranosyl structure is part of the common core structure of *N*-glycans and is found as a constituent of microbial polysaccharides, such as mannans in yeast cell walls [10]. The specificity of the mannose receptor on dendritic cells has not yet been studied in detail.

An efficient synthesis of neoglycoconjugates based on 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranosides was required in connection with biological studies. Several formal routes to glycosides of this trisaccharide have been reported, depending on stepwise glycosylation of a mannopyranoside derivative protected at all positions except O-3 [11], or diglycosylation of 2,4-di-*O*-benzyl- [12,13] or 2,4-di-*O*-benzoyl- [14] D-mannopyranosides or of a partially stannylated mannoside [15]. Additionally, and of particular relevance to the present work, a direct approach to glycosides of the mannotriose has been applied by Kaur and Hindsgaul using octyl β-D-mannopyranoside [16] and Verez-Bencomo and co-workers using 5-azido-3-oxapentyl α-D-mannopyranoside [17,18], who obtained 17 and 34% yields, respectively, of 3,6-linked mannotriosides with tetra-*O*-acetyl-α-D-mannopyranosyl bromide as glycosylating agent. In the former work, the 2,6- and 4,6-linked trisaccharides, formed together with the required product, were elegantly destroyed by periodate oxidation, and thus the isolation of the required trimer derivative was specifically facilitated.

We now report a simple and efficient synthesis of novel α-glycosidic derivatives of the (1→3),(1→6)-linked mannotriose suitable for the preparation of neoglycoconjugates which employs a minimal *O*-protection strategy with 8-(methoxycarbonyl)octyl α-D-mannopyranoside (**4**) as acceptor and the previously unreported 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**1**) as glycosyl donor.

2. Results and discussion

Under reaction conditions favouring the formation of the thermodynamic products, the synthesis of mannopyranosides normally proceeds with high α-stereoselectivity; formation of β-glycosides is not observed, even with non-participating protecting groups at O-2, because of a strong anomeric effect [19,20]. Nevertheless, participating ester protecting groups in the glycosylating donors are often preferred, because protection and deprotection can be achieved readily and efficiently. However, if 2-*O*-acetyl-protected donors are employed, orthoesters may be obtained rather than the desired glycosides [12,15,21]. In some cases, with careful control of reaction conditions, such orthoesters may be converted in situ to glycosides, but in other instances the orthoesters have to be isolated and converted in a separate reaction step. Rearrangement of orthoesters to glycosides is often accompanied by side reactions such as hydrolysis, acetyl migration from O-2 of the donor to a free hydroxy group on the acceptor, or formation of isomeric glycosides [22,23], and it is therefore often expedient to avoid their participation in the reactions.

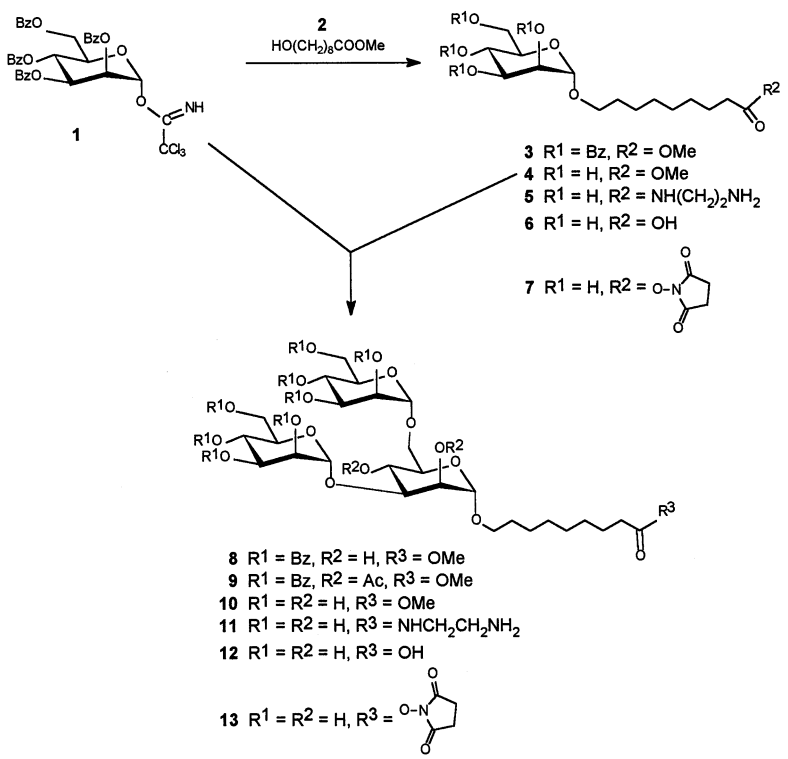
O-Benzoyl-protected donors seem to be less prone to orthoester formation, and especially the donor 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide [24] has been used for the high-yielding preparations of α-mannosides without formation of orthoesters [14,25]. This advantage, however, is offset by the requirement for stoichiometric amounts of silver salts for its activation, as these are expensive and inconvenient to use in large-scale preparations.

Our experience correlates with that of others regarding the merits of glycosyl imidates as donors compared with corresponding halogenoses [20]: their higher shelf stability yet greater reactivity on activation with catalytic amounts of a simple Lewis acid, and their efficiency confer on them real advantages. The syrupy imidate **1** was obtained in 68% yield from D-mannose by reaction of its 2,3,4,6-tetrabenzoate [26] with trichloroacetonitrile in dichloromethane in the presence of potassium carbonate. Reaction with the 'Lemieux spacer'

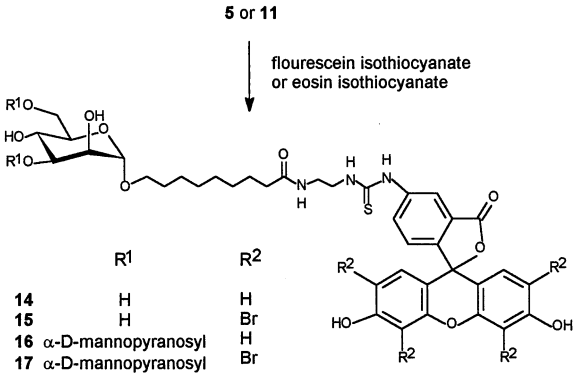
alcohol 8-(methoxycarbonyl)octanol (**2**) [27–30], with Me_3SiOTf as catalyst afforded the α-mannoside **3** in 86% yield. The formation of orthoesters as intermediates or as byproducts was not observed. The acceptor **2** was made (21%) in one step from 1,9-nonanediol by calcium hypochlorite-mediated oxidation in the presence of acetic acid in methanol [31]. Deprotection of benzoate **3** with potassium carbonate in methanol gave crystalline glycoside **4** in 85% yield [20,25,30,32].

On the grounds that the primary group can confidently be expected to be the most reactive of the hydroxyl functions of α-mannopyranosides and that those at C-2 and C-4 will be least reactive (axial and inherently unreactive, respectively [33]), it can be predicted that disubstitution will lead preferentially to the 3,6-linked products. Accordingly, unprotected mannoside **4**, treated with two equiv of trichloroacetimidate **1** with Me_3SiOTf as catalyst, afforded the 3,6-branched trisaccharide glycoside **8**, which was isolated very readily in 55% yield on a 5-g scale by silica gel column chromatography (Scheme 1). The elution profile from gel-filtration chromatography on lipophilic Sephadex LH-20 size-exclusion gel indicated that other products present were eluted appreciably earlier or later than the required mannotrioside **8**. From earlier eluting fractions the tetrasaccharide glycoside 8-(methoxycarbonyl)octyl 3,4,6-tri-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside dodecabenzoate was obtained in 5% yield, while the 4,6- and 2,6-linked trisaccharides derivatives were not detected; neither were orthoesters. It was important to use only two equivalents of trichloroacetimidate **1**. Otherwise, substantial proportions of higher glycosylated products were obtained. Activation of the glycosylating agent **1** by zinc triflate–triflic acid again gave compound **8** as the major product in similar yield to that obtained with Me_3SiOTf, but the reaction mixture was more heterogeneous (TLC), and purification of the main product was consequently more difficult.

A sample of mannotrioside **8** was acetylated to give the diacetate **9** for characterisation by NMR methods. The specific movements to lower field positions of the ^1H NMR signals for H-2 and H-4 of compound **9** proved that the glycosidic linkages were at O-3 and O-6. The ^1H-coupled ^{13}C NMR spectrum showed C-1–H-1 coupling constants of 168–172 Hz



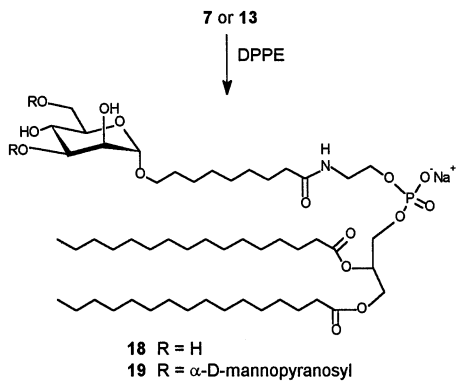
Scheme 1.



Scheme 2.

for the three anomeric carbon atoms, which is indicative of α-glycosides, β-anomers giving values that are 10 Hz smaller [34–36].

Treatment of product **8** with potassium carbonate in methanol gave the unprotected mannotrioside **10** quantitatively, and reaction of compounds **4** or **10** with 1,2-diaminoethane [37] afforded amides **5** and **11**, respectively, which are suitable for the preparation of conjugates with amine-reactive probes. Alkaline hydrolysis of the ester groups of compounds **4** and **10**, followed by acidification, afforded the free acids **6** and **12**, which were converted to the *N*-acyloxysuccinimide compounds **7** and **13**. Coupling of amides **5** and **11** with fluorescein isothiocyanate and eosin isothiocyanate gave the fluorochromes **14**, **15** and **16**, **17**, respectively (Scheme 2), which were required for the study of carbohydrate–lectin interactions, especially with the mannose receptor. Coupling the activated compounds of **7** or **13** with dipalmitoyl-l-α-phosphatidyl ethanolamine (DPPE) afforded the neoglycolipids **18** and **19** (Scheme 3), which were required for



Scheme 3.

the preparation of liposomes to be used for drug delivery [38–40].

In summary, a short and efficient synthesis of novel 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosides and the preparation from them, and from the corresponding α-D-mannopyranosides, of neoglycoconjugates have been demonstrated.

3. Experimental

General methods.—TLC was performed on Silica Gel 60 F254 (E. Merck) with detection by UV absorption and/or by charring after spraying with $EtOH–H_2O–H_2SO_4$ (14:4:1, v/v). Column chromatography was performed on Silica Gel S32–63 μm (230–400 mesh, Riedel-de Haën) at medium pressure (4–15 bar) and for reversed-phase work on LiChroprep RP-18 25–40 μm (E. Merck) at normal pressure. Lipophilic Sephadex LH-20 (Sigma) was used for gel filtration. NMR spectra were recorded for ^1H at 300 or 500 MHz and for ^{13}C at 75.5 or 125 MHz, using a Bruker AC300E or a Varian Unity 500 spectrometer. Me_4Si was used as internal standard, unless otherwise stated. Assignments of ^1H and ^{13}C resonances were based on 2D-experiments ($^1H–^1H$ COSY, $^1H–^1H$ TOCSY, $^1H–^{13}C$ HMQC, $^1H–^{13}C$ HMBC) and polarisation-transfer experiments (DEPT). HMBC experiments were optimized for the detection of long-range couplings (5–7 Hz). High-resolution FABMS was performed in the positive-ion mode for compounds **16** and **17** in a 1:5 dithioerythritol–dithiothreitol ('magic bullet') matrix on a VG70-250S mass spectrometer (VG Analytical) equipped with a standard liquid secondary caesium ion gun, by Dr John Allen (Hort Research Ltd, Palmerston North, New Zealand). All other samples were examined in a 3-nitrobenzyl alcohol matrix (except for amide **5**: glycerol matrix) on an MS 80 RFA spectrometer (Kratos), equipped with a TECH ZN11 NF FAB ion gun with xenon as bombarding atom by Dr Bruce Clark, University of Canterbury, New Zealand. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. Fluorescein isothiocyanate isomer I was purchased from Aldrich Chemi-

cal Co., eosin isothiocyanate from Molecular Probes, and DPPE from Sigma Chemical Co.

2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**1**).—A solution of 2,3,4,6-tetra-*O*-benzoyl-α,β-D-mannopyranose [26] (30 g, 50.3 mmol) in CH_2Cl_2 (200 mL) was treated with KCO_3 (10 g) and trichloroacetonitrile (20 mL, 199 mmol) under vigorous stirring at room temperature (rt) overnight. The reaction mixture was filtered and the volatiles were evaporated in vacuo. Chromatography on silica gel with 4:1 petroleum ether–EtOAc afforded compound **1** (28 g, 37.8 mmol, 81%) as a syrup: $[\alpha]_D^{20} -28.2°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (s, 1 H, NH), 8.08 (m, 4 H, Bz), 7.97 (m, 2 H, Bz), 7.84 (m, 2 H, Bz), 7.65–7.23 (m, 12 H, Bz), 6.57 (br s, 1 H, H-1), 6.23 (dd, 1 H, $J_{3,4} \sim J_{4,5}$ 10.0 Hz, H-4), 5.98 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.94 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.73 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.63 (ddd, 1 H, $J_{5,6b}$ 4.1 Hz, H-5), 4.52 (dd, 1 H, H-6b); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 94.757 (d, $J_{C\text{-}1,H\text{-}1}$ 180.5 Hz, C-1). Anal. Calcd for $C_{36}H_{28}Cl_3NO_{10}$: C, 58.35; H 3.80. Found: C, 58.18; H 3.87.

8-(Methoxycarbonyl)octanol (**2**).—See Refs. [27–30]. 1,9-Nonanediol (12 g, 75 mmol) was dissolved in MeOH (80 mL) and MeCN (300 mL). Molecular sieves (4Å, powdered, 30 g), calcium hypochlorite (44 g, 0.31 mol) and acetic acid (36 mL), were added, and the mixture was mechanically stirred overnight in the dark at rt [41]. The mixture was filtered, evaporated in vacuo, and the residue was dissolved in CH_2Cl_2. The solution was washed with aq sodium thiosulfate (50 g/200 mL), then brine, dried ($MgSO_4$), and the solvent was evaporated in vacuo. Kugelrohr distillation of the residue afforded a mixture of 1,9-nonanediol and product, which was separated by chromatography on silica gel with 4:1 petroleum ether–EtOAc to afford ester **2** (3 g, 16 mmol, 21%) as an oil: ^{1}H NMR data were identical to those reported [28].

8-(Methoxycarbonyl)octyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (**3**).—Trichloroacetimidate **1** (9 g, 12.1 mmol) and 8-(methoxycarbonyl)octanol **2** (2.5 g, 12.4 mmol) were dissolved in CH_2Cl_2 (100 mL) and stirred for 30 min at rt over molecular sieves (4Å, pearled). A solution of Me_3SiOTf (50 μL, 0.26 mmol) in CH_2Cl_2 (2 mL) was added dropwise. After 2 h the reaction mixture was treated with triethylamine (0.3 mL) and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL) and petroleum ether (100 mL) was added. Precipitated trichloroacetamide was removed by filtration, and the filtrate was evaporated in vacuo. Chromatography on silica gel with 5:1 petroleum ether–EtOAc afforded glycoside **3** (8.0 g, 10.4 mmol, 86%) as a syrup: $[\alpha]_D^{20} -45.3°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (m, 4 H, Bz), 7.96 (m, 2 H, Bz), 7.83 (m, 2 H, Bz), 7.63–7.23 (m, 12 H, Bz), 6.11 (dd, 1 H, $J_{3,4} \sim J_{4,5}$ 10.0 Hz, H-4), 5.93 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.71 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-2), 5.10 (d, 1 H, H-1), 4.72 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.51 (dd, 1 H, $J_{5,6b}$ 4.5 Hz, H-6b), 4.44 (ddd, 1 H, H-5), 3.83 (m, 1 H, CH_2O-spacer), 3.66 (s, 3 H, CH_3), 3.58 (m, 1 H, CH_2O-spacer), 2.32 (t, 2 H, *J* 7.5 Hz, CH_2COO), 1.75–1.50 (m, 4 H, CH_2), 1.45–1.25 (m, 8 H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 97.664 (d, $J_{C\text{-}1,H\text{-}1}$ 168.8 Hz, C-1); FABMS: *m/z* Calcd for $C_{44}H_{47}O_{12}$ $(M+H)^+$: 767.3068. Found: 767.3144.

8-(Methoxycarbonyl)octyl α-D-mannopyranoside (**4**).—See Refs. [25,30,32]. Compound **3** (5.9 g, 7.7 mmol) in MeOH (100 mL) was stirred with KCO_3 (500 mg) overnight then neutralized with ion-exchange resin (Amberlite IR 120, H^+-form). Removal of the resin and the solvents gave a solid (2.7 g, 100%). Chromatography on silica gel with 10:1 $CHCl_3$–MeOH afforded glycoside **4** (3.0 g, 8.6 mmol, 85%): mp 84 °C (petroleum ether–EtOAc) (lit. [25] 81–82 °C), $[\alpha]_D^{20} +51.5°$ (*c* 1.0, MeOH) (lit. [32] +52.7°, *c* 0.4, MeOH); the ^{1}H NMR (D_2O) spectrum was identical to that reported [30].

8-[N-(2-Aminoethyl)carboxamido]octyl α-D-mannopyranoside (**5**).—Compound **4** (107 mg, 0.31 mmol) was dissolved in 1,2-diaminoethane (2 mL) and heated to 60 °C for 36 h [37]. The solution was evaporated in vacuo, and the residue was dissolved in water and loaded on a C-18 SepPak cartridge. Absorbed amide **5** was washed with water, eluted

with 1:1 H_2O–MeCN, and the solvent was evaporated to afford amide **5** (110 mg, 0.29 mmol, 95%) as a syrup: $[\alpha]_D^{20} + 40.0°$ (*c* 1.0, H_2O); ^1H NMR (300 MHz, D_2O, HDO = 4.8 ppm): δ 4.91 (br s, 1 H, H-1), 3.98 (m, 1 H, H-2), 3.95–3.65 (m, 6 H, H-3, 4, 5, 6a, 6b, CH_2O-spacer), 3.59 (m, 1 H, CH_2O-spacer), 3.33 (t, 2 H, *J* 6.2 Hz, CH_2NH), 2.82 (t, 2 H, *J* 6.2 Hz, CH_2NH_2), 2.31 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.72–1.57 (m, 4 H, CH_2), 1.45–1.30 (m, 8 H, CH_2); FABMS: *m/z* Calcd for $C_{17}H_{35}N_2O_7$ $(M + H)^+$: 379.2444. Found: 379.2453.

9-(α-D-Mannopyranosyloxy)nonanoic acid (**6**).—Compound **4** (109 mg, 0.31 mmol) was dissolved in 2:1 oxolane–H_2O (4.5 mL). Aqueous NaOH (15%, 50 μL) was added, and the reaction mixture was left for 2 days at rt. Strongly acidic ion-exchange resin (Amberlite IR 120, H^+-form) was added with stirring and the pH was brought to 3.8. The mixture was filtered, evaporated in vacuo, and the crude product was freeze-dried to afford acid **6** (100 mg, 0.30 mmol, 96%) as a syrup: $[\alpha]_D^{20} + 67.3°$ (*c* 1.0, MeOH); ^1H NMR (300 MHz, MeOD): δ 4.63 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.72 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.73–3.55 (m, 4 H, H-2, 3, 6b, CH_2O-spacer), 3.51 (dd, 1 H, $J_{3,4} \sim J_{4,5} \sim 9.2$ Hz, H-4), 3.42 (ddd, 1 H, $J_{5,6b}$ 5.5 Hz, H-5), 3.31 (m, 1 H, CH_2O-spacer), 2.18 (t, 2 H, *J* 7.4 Hz, CH_2COO), 1.55–1.45 (m, 4 H, CH_2), 1.35–1.17 (m, 8 H, CH_2); FABMS: *m/z* Calcd for $C_{15}H_{29}O_8$ $(M + H)^+$: 337.1862. Found: 337.1855.

N-[9-(α-D-Mannopyranosyloxy)nonanoyloxy]succinimide (**7**).—Carboxylic acid **6** (30 mg, 89 μmol), *N*,*N*′-dicyclohexylcarbodiimide (26 mg, 126 μmol) and *N*-hydroxysuccinimide (12 mg, 104 μmol), were dissolved in 2:1 oxolane–$CHCl_3$ (3 mL) and stirred overnight at rt. Chromatography on silica gel with 10:1 $CHCl_3$–MeOH afforded compound **7** (30 mg, 69 μmol, 78%) as a syrup: $[\alpha]_D^{20} + 43.6°$ (*c* 1.0, oxolane); ^1H NMR (300 MHz, 10:1 $CDCl_3$–MeOD): δ 4.80 (br s, 1 H, H-1), 3.92–3.72 (m, 5 H, H-2, 3, 4, 6a, 6b), 3.67 (m, 1 H, CH_2O-spacer), 3.57 (m, 1 H, H-5), 3.41 (m, 1 H, CH_2O-spacer), 2.85 (s, 4 H, CH_2CON), 2.62 (t, 2 H, *J* 7.4 Hz, CH_2COO), 1.78 (m, 2 H, CH_2CH_2COO), 1.66–1.50 (m, 2 H, CH_2CH_2O), 1.50–1.20 (m, 8 H, CH_2).

8-(Methoxycarbonyl)octyl (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 (**8**).—Acceptor **4** (1.0 g, 2.85 mmol), after dissolution in toluene and removal of the solvent (×3), was dissolved in CH_2Cl_2 (60 mL) and stirred for 1 h at rt over molecular sieves (4 Å, pearled). The solution was cooled to −40 °C and Me_3SiOTf (180 μL, 0.9 mmol) was added. Stirring was applied for 5 min, and trichloroacetimidate **1** (4.22 g, 5.7 mmol) in CH_2Cl_2 (15 mL) was added dropwise with stirring over 30 min. The solution was stirred for a further 60 min, during which time the temperature rose to −18 °C. $NaHCO_3$ (0.50 g) was added, and the mixture was stirred for 30 min. The solids were filtered off, and the solvents were evaporated. Chromatography on silica gel with 5:1 toluene–EtOAc afforded mannotrioside **8** (2.36 g, 1.57 mmol, 55%), as a syrup: $[\alpha]_D^{20} -$ 22.8° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$; the assignments of the Man′ and Man″ residue resonances could be interchanged): δ 8.17–7.80 (m, 16 H, Bz), 7.60–7.15 (m, 24 H, Bz), 6.17 (dd, 1 H, $J_{3',4'}$ 10.3 Hz, $J_{4',5'}$ 9.7 Hz, H-4′), 6.15 (dd, 1 H, $J_{3'',4''}$ 10.1 Hz, $J_{4'',5''}$ 9.8 Hz, H-4″), 6.06 (dd, 1 H, $J_{2',3'}$ 2.8 Hz, H-3′), 6.00 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-3″), 5.91 (br s, 1 H, H-2′), 5.81 (br s, 1 H, H-2″), 5.49 (br s, 1 H, H-1′), 5.36 (br s, 1 H, H-1″), 4.96 (m, 1 H, H-5″), 4.78–4.50 (m, 6 H, H-1, 5′, 6a′, 6b′, 6a″, 6b″), 4.30–4.18 (m, 3 H, H-2, 4, 6a), 4.09–3.99 (m, 2 H, H-3, 6b), 3.90 (m, 1 H, H-5), 3.73 (m, 1 H, CH_2O-spacer), 3.62 (s, 3 H, CH_3O), 3.44 (br s, 1 H, OH), 3.38 (m, 1 H, CH_2O-spacer), 2.96 (br s, 1 H, OH), 2.23 (t, 2 H, *J* 7.6 Hz, CH_2COO), 1.60–1.47 (m, 4 H, CH_2), 1.40–1.18 (m, 8 H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, selective data, coupling constants from HMBC): δ 99.973 (d, $J_{C\text{-}1,H\text{-}1}$ 168 Hz, C-1), 99.823 (d, $J_{C\text{-}1',H\text{-}1'}$ 172 Hz, C-1′), 97.557 (d, $J_{C\text{-}1'',H\text{-}1''}$ 172 Hz, C-1″); FABMS: *m/z* Calcd for $C_{84}H_{82}O_{26}$ Cs $(M + Cs)^+$: 1639.4149. Found: 1639.4182.

8-(Methoxycarbonyl)octyl 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 (**9**).—Compound **8** (50 mg, 0.03 mmol) was dissolved in 3:1 pyridine–Ac_2O (4 mL) and left for 1 day at rt in the dark. The solution was diluted with CH_2Cl_2 (20 mL), washed with aq $NaHCO_3$, M HCl, phosphate buffer (pH 7), and water, dried ($MgSO_4$), and evaporated in vacuo to afford diacetate **9** (50 mg, 0.03 mmol, 95%) as a syrup: $[\alpha]_D^{20} - 19.3°$ (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.15–7.80 (m, 16 H, Bz), 7.63–7.12 (m, 24 H, Bz), 6.19 (dd, 1 H, $J_{3'',4''} \sim J_{4'',5''}$ 10.1 Hz, H-4″), 6.14 (dd, 1 H, $J_{3',4'} \sim J_{4',5'}$ 10.0 Hz, H-4′), 5.94 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-3″), 5.82 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.76 (dd, 1 H, $J_{1'',2''}$ 1.7 Hz, H-2″), 5.53 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, H-2′), 5.47–5.41 (m, 2 H, H-2, 4), 5.38 (d, 1 H, H-1′), 5.16 (d, 1 H, H-1″), 4.87 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.76–4.46 (m, 6 H, H-5′, 5″, 6a′, 6b′, 6a″, 6b″), 4.39 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.7 Hz, H-3), 4.07–3.97 (m, 2 H, H-5, 6a), 3.83 (m, 1 H, CH_2O-spacer), 3.72 (m, 1 H, H-6b), 3.61 (s, 3 H, CH_3O), 3.51 (m, 1 H, CH_2O-spacer), 2.34 (s, 3 H, CH_3CO), 2.28 (s, 3 H, CH_3CO), 2.22 (t, 2 H, *J* 7.7 Hz, CH_2COO), 1.65–1.47 (m, 4 H, CH_2), 1.43–1.18 (m, 8 H, CH_2); FABMS: *m/z* Calcd for $C_{88}H_{86}O_{28}Cs$ $(M + Cs)^+$: 1723.4360. Found: 1723.4409.

8-(Methoxycarbonyl)octyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (**10**).—Compound **8** (2.11 g, 1.40 mmol) was dissolved in MeOH (50 mL) and stirred with KCO_3 (500 mg) overnight at rt. The mixture was neutralized with strongly acidic ion-exchange resin (Amberlite IR 120, H^+-form), filtered, and evaporated in vacuo. The residue was dissolved in water and freeze-dried. Freeze-drying was repeated once to afford mannotrioside **10** (936 mg, 1.39 mmol, 99%) as an amorphous solid: $[\alpha]_D^{20} + 93.9°$ (*c* 0.94, MeOH); ^1H NMR (500 MHz, D_2O, HDO = 4.8 ppm): δ 5.18 (br s, 1 H, H-1′), 4.97 (br s, 1 H, H-1″), 4.90 (br s, 1 H, H-1), 4.16 (br s, 1 H, H-2), 4.14 (m, 1 H, H-2′), 4.08–4.04 (m, 2 H, H-2″, 6a), 4.01–3.71 (m, 15 H, H-3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6b, 6a′, 6b′, 6a″, 6b″, CH_2O-spacer), 3.76 (s, 3 H, CH_3O), 3.63 (m, 1 H, CH_2O-spacer), 2.46 (t, 2 H, *J* 7.5 Hz, CH_2COO), 1.75–1.64 (m, 4 H, CH_2CH_2O, CH_2CH_2COO), 1.50–1.36 (m, 8 H, CH_2); ^{13}C NMR (125 MHz, D_2O, CH_3O = 52.9 ppm, selective data, coupling constants from HMBC): δ 103.175 (d, $J_{C\text{-}1',H\text{-}1'}$ 172 Hz, C-1′), 100.681 (d, $J_{C\text{-}1,H\text{-}1}$ 172 Hz, C-1), 100.244 (d, $J_{C\text{-}1'',H\text{-}1''}$ 171 Hz, C-1″), 79.443 (C-3), 74.150 (C-2′), 73.513 (C-2″), 71.928 (C-2); FABMS: *m/z* Calcd for $C_{28}H_{50}O_{18}Na$ $(M + Na)^+$: 697.2895. Found: 697.2895.

8-[N-(2-Aminoethyl)carboxamido]octyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (**11**).—Compound **10** (100 mg, 0.148 mmol) was dissolved in 1,2-diaminoethane (5 mL) and heated to 60 °C for 36 h [38]. The solution was then evaporated in vacuo, diluted with phosphate buffer (pH 6), and desalted by gel-filtration chromatography on a Sephadex G-10 column with water as eluant. The eluate was passed through a C-18 SepPak cartridge, and absorbed amide **11** was washed with water, eluted with a 3:1→1:1 gradient of H_2O–MeCN and the solvent was evaporated to afford amide **11** (80 mg, 0.113 mmol, 77%) as a syrup: $[\alpha]_D^{20} + 73.2°$ (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O, HDO = 4.8 ppm): δ 5.16 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1′), 4.95 (d, 1 H, $J_{1'',2''}$ 1.7 Hz, H-1″), 4.88 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.14 (m, 1 H, H-2), 4.12 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, H-2′), 4.06–4.02 (m, 1 H, H-6a), 4.03 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-2″), 3.97–3.69 (m, 15 H, H-3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6b, 6a′, 6b′, 6a″, 6b″, CH_2O-spacer), 3.61 (m, 1 H, CH_2O-spacer), 3.40 (t, 2 H, *J* 6.0 Hz, CH_2NH), 2.94 (t, 2 H, *J* 6.0 Hz, CH_2NH_2), 2.32 (t, 2 H, *J* 7.6 Hz, CH_2CON), 1.65 (m, 4 H, CH_2CH_2O, CH_2CH_2CON), 1.46–1.34 (m, 8 H, CH_2); FABMS: *m/z* Calcd for $C_{29}H_{55}N_2O_{17}$ $(M + H)^+$: 703.3501. Found: 703.3510.

{9-O-α-D-Mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyloxy}nonanoic acid (**12**).—Compound **10** (216 mg, 0.32 mmol) was dissolved in 2:1 oxolane–H_2O (6 mL) and aq NaOH (0.2 mL, 15%) was added. After 3 days at rt, strongly acidic ion-exchange resin (Amberlite IR 120, H^+-form) was added and the pH was brought

to 3.8. The mixture was filtered, the filtrate was evaporated in vacuo, and the crude product was freeze-dried from water to afford carboxylic acid **12** (212 mg, 0.32 mmol, 100%) as an amorphous solid: $[\alpha]_D^{20} + 81.4°$ (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, D_2O, HDO = 4.8 ppm): δ 5.18 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1′), 4.97 (d, 1 H, $J_{1'',2''}$ 1.7 Hz, H-1″), 4.89 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.15 (m, 1 H, H-2), 4.14 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.08–4.04 (m, 2 H, H-2″, 6a), 3.99–3.71 (m, 15 H, H-3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6b, 6a′, 6b′, 6a′, 6b″, CH_2O-spacer), 3.63 (m, 1 H, CH_2O-spacer), 2.46 (t, 2 H, *J* 7.6 Hz, C*H*$_2$COO), 1.75–1.64 (m, 4 H, C*H*$_2$CH$_2$O, C*H*$_2$CH$_2$COO), 1.49–1.38 (m, 8 H, CH_2); FABMS: *m*/*z* Calcd for $C_{27}H_{48}O_{18}Na$ $(M + Na)^+$: 683.2738. Found: 683.2745.

N-{*9-[(α-*D*-Mannopyranosyl)-(1→3)-[(α-*D*-mannopyranosyl)-(1→6)]-α-*D*-mannopyranosyloxy)]nonanoyloxy}succinimide* (**13**).—Compound **12** (49 mg, 74 mmol), *N*,*N*′-dicyclohexylcarbodiimide (22 mg, 107 μmol), and *N*-hydroxysuccinimide (10 mg, 87 μmol) were dissolved in 2:1:2 oxolane–$CHCl_3$–MeOH (6 mL) and stirred overnight at rt. The solvents were evaporated in vacuo and gel filtration of the residue on lipophilic Sephadex LH-20 with 1:1 $CHCl_3$–MeOH afforded compound **13** (42 mg, 55 μmol, 75%) as a syrup: $[\alpha]_D^{20} + 64.5°$ (*c* 0.8, 3:1 $CHCl_3$–MeOH); ^{1}H NMR (500 MHz, 3:1 $CDCl_3$–MeOD): δ 5.09 (br s, 1 H, H-1′), 4.86 (br s, 1 H, H-1″), 4.73 (br s, 1 H, H-1), 4.08–3.34 (m, 20 H, H-2, 2′, 2″, 3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6a, 6b, 6a′, 6b′, 6a″, 6b″, CH_2O-spacer), 2.87 (s, 4 H, CH_2CON), 2.63 (t, 2 H, *J* 7.5 Hz, CH_2COO), 1.79–1.72 (m, 2 H, C*H*$_2$CH$_2$COO), 1.63–1.54 (m, 2 H, C*H*$_2$CH$_2$O), 1.44–1.30 (m, 8 H, CH_2).

8-{Carboxy-[2-(fluoresceinylthioureido)ethyl]amido}octyl α-D-*mannopyranoside* (**14**).—Amide **5** (14 mg, 37 μmol), fluorescein isothiocyanate (25 mg, 64 μmol), and KCO_3 (10 mg, 72 μmol) were dissolved in 1:1 acetone–H_2O (2 mL). The mixture was stirred overnight at rt, then evaporated in vacuo. Gel filtration on Sephadex LH-20 with 5:4:1 $CHCl_3$–MeOH–H_2O afforded compound **14** (25 mg, 33 μmol, 88%) as a syrup, $[\alpha]_D^{20} - 12.5°$ (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, 1:1 $CDCl_3$–MeOD): δ 7.95 (br s, 1 H, H-4^{f1}), 7.81 (br d, 1 H, H-6^{f1}), 7.20–7.18 (m, 3 H, H-7^{fl}, 1′^{fl}, 8′^{fl}), 6.70–6.66 (m, 4 H, H-2′^{f1},4′^{f1},5′^{f1},7′^{f1}), 4.76 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.83 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.85–3.76 (m, 4 H, H-6a, 6b, CH_2NHCS), 3.75 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.71 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.67 (m, 1 H, C*H*$_2$O-spacer), 3.52 (ddd, 1 H, $J_{5,6a} \sim J_{5,6b} \sim 3.0$ Hz, H-5), 3.46 (t, 2 H, *J* 6.0 Hz, CH_2NH), 3.39 (m, 1 H, CH_2O-spacer), 2.20 (t, 2 H, *J* 7.5 Hz, CH_2CON), 1.61–1.52 (m, 4 H, CH_2), 1.39–1.24 (m, 8 H, CH_2); FABMS: *m*/*z* Calcd for $C_{38}H_{46}N_3O_{12}S$ $(M + H)^+$: 768.2802. Found: 768.2825.

8-{Carboxy-[2-(2′,4′,5′,7′-tetrabromofluoresceinylthioureido)ethyl]amido}octyl α-D-*mannopyranoside* (**15**).—Compound **5** (10 mg, 26 μmol) was dissolved in aq $NaHCO_3$ (2 mL, 0.2M, pH 9), and eosin isothiocyanate (20 mg, 28 μmol) was added. The mixture was stirred overnight at rt then evaporated in vacuo. Reversed-phase chromatography (C-18 silica) with 9:1→4:1 H_2O–MeCN, followed by chromatography on silica gel with 5:1 MeCN–H_2O afforded compound **15** (19 mg, 17 μmol, 64%) as a syrup, $[\alpha]_D^{20} - 16.5°$ (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O, HDO = 4.80 ppm): δ 7.83 (br s, 1 H, H-4^{f1}), 7.68 (m, 1 H, H-6^{f1}), 7.55 (brs, 2 H, H-1′^{f1}, 8′^{f1}), 7.12 (m, 1 H, H-7^{fl}), 4.76 (br s, 1 H, H-1), 3.90–3.75 (m, 6 H, H-2, 3, 6a, 6b, CH_2NHCS), 3.69 (dd, 1 H, $J_{3,4} \sim J_{4,5} \sim 9.5$ Hz, H-4), 3.60–3.45 (m, 4 H, H-5, CH_2NH, CH_2O-spacer), 3.25 (m, 1 H, CH_2O-spacer), 2.13 (m, 2 H, CH_2CON), 1.37 (m, 2 H, CH_2CH_2CON), 1.27 (m, 2H, CH_2CH_2O), 1.05–0.85 (m, 8 H, CH_2); FABMS: *m*/*z* Calcd for $C_{38}H_{40}^{79}Br_2^{81}Br_2N_3Na_2O_{12}S$ $(M - H + 2Na)^+$: 1127.8821. Found: 1127.8842.

*8-{Carboxy-[2-(fluoresceinylthioureido)ethyl]amido}octyl (*α-D-*mannopyranosyl)-(1→3)-[(*α-D-*mannopyranosyl)-(1→6)]-*α-D-*mannopyranoside* (**16**).—Compound **11** (60 mg, 85 μmol), fluorescein isothiocyanate (38 mg, 98 μmol), and KCO_3 (17 mg, 123 μmol) were dissolved in 1:1 acetone–H_2O (2 mL) and stirred overnight at rt. The solution was evaporated in vacuo. Gel filtration on Sephadex LH-20 with 4:6:1 $CHCl_3$–MeOH–H_2O, followed by reversed-phase chromatography (C-18 silica) with 15:1→5:1 H_2O–MeCN, afforded compound **16** (65 mg,

60 μmol, 70%) as a syrup, $[\alpha]_D^{20} + 22.0°$ (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O, HDO = 4.80 ppm): δ 7.79 (br s, 1 H, H-4^{fl}), 7.60 (br d, 1 H, H-6^{fl}), 7.28–7.26 (m, 2 H, H-1′^{fl}, 8′^{fl}), 7.21 (d, 1 H, H-7^{fl}), 6.73–6.69 (m, 4 H, H-2′^{fl}, 4′^{fl}, 5′^{fl}, 7′^{fl}, 5.13 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.91 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.77 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.12 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 4.07 (m, 1 H, H-2), 4.01 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-2″), 4.03–4.00 (m, 1 H, H-6a), 3.97–3.66 (m, 16 H, H-3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6b, 6a′, 6b′, 6a″, 6b″, CH_2NHCS), 3.58–3.52 (m, 3 H, CH_2NCO, CH_2O-spacer), 3.37 (m, 1 H, CH_2O-spacer), 2.24 (t, 2 H, *J* 7.0 Hz, CH_2CON), 1.53 (m, 2 H, CH_2CH_2CON), 1.40 (m, 2 H, CH_2CH_2O), 1.21 (m, 2 H, $CH_2CH_2CH_2CON$), 0.97 (m, 6 H, CH_2); FABMS: *m/z* Calcd for $C_{50}H_{66}N_3O_{22}S$ $(M + H)^+$; $C_{50}H_{65}N_3NaO_{22}S$ $(M + Na)^+$: 1092. 3855; 1114.3678. Found: 1092. 3867; 1114.3717.

8-{Carboxy-[2-(2′,4′,5′,7′-tetrabromofluoresceinylthioureido)ethyl]amido}octyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (**17**).—Compound **11** (20 mg, 28 μmol) was dissolved in aq $NaHCO_3$ (2 mL, 0.2 M, pH 9) and eosin isothiocyanate (20 mg, 28 μmol) dissolved in *N*,*N*-dimethylformamide (1 mL) was added. The mixture was stirred overnight at rt, then evaporated in vacuo. Gel filtration on Sephadex LH-20 with 5:4:1 $CHCl_3$–MeOH–H_2O, followed by reversed-phase chromatography (C-18 silica) with 9:1→2:1 H_2O–MeCN, afforded **17** (29 mg, 20 μmol, 70%) as a syrup: $[\alpha]_D^{20} - 12.6°$ (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O, HDO = 4.80 ppm): δ 7.84 (br s, 1 H, H-4^{fl}), 7.71 (br s, 1 H, H-6^{fl}), 7.60 (s, 2 H, H-1′^{fl}, 8′^{fl}), 7.18 (br s, 1 H, H-7^{fl}), 5.13 (br s, 1H, H-1′), 4.93 (br s, 1 H, H-1″), 4.76 (br s, 1 H, H-1), 4.12 (m, 1 H, H-2′), 4.07 (m, 1 H, H-2), 4.03 (m, 1 H, H-2″), 4.17–3.61 (m, 17 H, H-3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6a, 6b, 6a′, 6b′, 6a″, 6b″, CH_2NHCS), 3.61–3.48 (m, 3 H, CH_2NHCO, CH_2O-spacer), 3.31 (m, 1 H, CH_2O-spacer), 2.19 (m, 2 H, CH_2CON), 1.43 (m, 2 H, CH_2CH_2CON), 1.32 (m, 2 H, CH_2CH_2O), 1.07 (m, 2 H, CH_2CH_2-CH_2CON), 0.96 (m, 6 H, CH_2); FABMS: *m/z* Calcd for $C_{50}H_{61}^{79}Br_3^{81}Br\,KN_3O_{22}S$ $(M + K)^+$; $C_{50}H_{61}^{79}Br_2^{81}Br_2KN_3O_{22}S$ $(M + K)^+$: 1443.9821; 1445.9807. Found: 1443.9815; 1445.9839.

8-[Carboxy-2-(1,2-dihexadecanoyl-sn-glycero-3-sodio-phospho)ethanolamido]octyl α-D-mannopyranoside (**18**).—1,2-Dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) (40 mg, 58 μmol) was suspended in 8:1 oxolane–H_2O (3 mL). $NaHCO_3$ (25 mg, 0.3 mmol) was added, and the mixture was warmed to ~30 °C to obtain a clear solution. Compound **7** (25 mg, 58 μmol), dissolved in oxolane (0.83 mL), was added, and stirring was continued for 1 h at rt, with occasional warming as precipitation occurred. After filtration, the oxolane was evaporated in vacuo, and the residue was freeze-dried. Chromatography on silica gel with 30:15:1 $CHCl_3$–MeOH–H_2O, followed by chromatography on lipophilic Sephadex LH-20 with 2:1→6:1 $CHCl_3$–MeOH, afforded compound **18** (46 mg, 45 μmol, 77%) as a syrup: $[\alpha]_D^{20} + 17.8°$ (*c* 1.0, $CHCl_3$, MeOH 3:1); ^1H NMR (300 MHz, 3:1 $CDCl_3$–MeOD): δ 5.21 (m, 1 H, CHOCOR), 4.74 (br s, 1 H, H-1), 4.38 (dd, 1 H, *J* 12.0 Hz, 3.1 Hz, CH_2OCOR), 4.14 (dd, 1 H, *J* 12.0 Hz, 6.7 Hz, CH_2OCOR), 3.96–3.82 (m, 6 H, 2 × CH_2OP, H-6a, 2), 3.77–3.60 (m, 4 H, H-4, 3, 6b, CH_2O-spacer), 3.46 (m, 1 H, H-5), 3.41 (t, 2H, *J* 5.0 Hz, CH_2N), 3.39 (m, 1 H, CH_2O-spacer), 2.35–2.22 (m, 4 H, 2 × CH_2COO), 2.17 (t, 2 H, *J* 7.7 Hz, CH_2CON), 1.63–1.50 (m, 8 H, CH_2CH_2CON, 2 × CH_2CH_2COO, CH_2CH_2O), 1.33–1.19 (m, 56 H, CH_2), 0.85 (t, 6 H, *J* 6.8 Hz, 2 × CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, MeOD, 3:1): δ 174.495, 173.581, 173.207 (3 × C=O), 100.504 (C-1), 72.956 (C-5), 71.426 (C-3), 71.050 (C-2), 70.759 (*C*HOCO), 67.809 (CH_2O-spacer), 66.541 (C-4), 64.679 (CH_2CH_2OP), 63.914 (CH_2OP-glyc), 62.886 (CH_2OCO), 60.714 (C-6), 40.443 (CH_2N), 36.498 (CH_2CON), 34.522, 34.366 (2 × CH_2COO), 32.185, 30.448, 29.945, 29.793, 29.605, 29.409, 29.207, 28.905, 28.667, 25.954, 25.720, 25.170, 22.917 (32 × CH_2), 14.185 (2 × CH_3); FABMS: *m/z* Calcd for $C_{52}H_{99}NNa_2O_{15}P$ $(M + Na)^+$ 1054.6548. Found: 1054.6574.

*8-[Carboxy-2-(1,2-dihexadecanoyl-*sn*-glycero-3-sodio-phospho)ethanolamido]octyl (α-*D*-mannopyranosyl)-(1→3)-[(α-*D*-mannopyranosyl)-(1→6)]-α-*D*-mannopyranoside* (**19**)

Method A. By use of isolated *N*-oxysuccinimide **13.** DPPE (38 mg, 55 μmol) was dissolved in 2:1 $CHCl_3$–MeOH (5 mL), and $NaHCO_3$ (25 mg, 0.3 mmol) dissolved in water (0.2 mL) was added, followed by compound **13** (35 mg, 46 mmol), dissolved in 2:1 $CHCl_3$–MeOH (1 mL), and the mixture was stirred for 1 h at rt. Chromatography on Sephadex LH-20 with 3:1 $CHCl_3$–MeOH, followed by chromatography on silica gel with 20:16:1 $CHCl_3$–MeOH–H_2O afforded product **19** (43 mg, 32 μmol, 69%) as a syrup: $[\alpha]_D^{20} + 29.0°$ (*c* 0.7, 3:1 $CHCl_3$–MeOH); ^1H NMR (500 MHz, 3:1 $CDCl_3$–MeOD): δ 5.28–5.21 (m, 1 H, CHOCOR), 5.15 (br s, 1 H, H-1′), 4.87 (br s, 1 H, H-1″), 4.74 (br s, 1 H, H-1), 4.46–4.37 (m, 1 H, CH_2OCOR), 4.22–4.30 (m, 1 H, CH_2OCOR), 4.20–3.34 (m, 26 H, H-2, 2′, 2″, 3, 3′, 3″, 4, 4′, 4″, 5, 5′, 5″, 6a, 6b, 6a′, 6b′, 6a″, 6b″, $2 \times CH_2OP$, CH_2O-spacer, CH_2NH), 2.40–2.27 (m, 4 H, CH_2COO), 2.22 (m, 2 H, CH_2CON), 1.70–1.50 (m, 8 H, CH_2CH_2CON, $2 \times CH_2$-CH_2COO, CH_2CH_2O), 1.45–1.20 (m, 56 H, CH_2), 0.88 (t, 6 H, $2 \times CH_3$). FABMS: *m*/*z* Calcd for $C_{64}H_{120}NNaO_{25}P$ $(M+H)^+$; $C_{64}H_{119}NNa_2O_{25}P$ $(M+Na)^+$: 1356.7785; 1378.7604. Found: 1356.7756; 1378.7533.

Method B. By in situ generation of *N*-oxysuccinimide **13**. Acid **12** (1.0 g, 1.5 mmol), after dissolution in toluene and evaporation of the solvent (2×50 mL), was dissolved in a mixture of oxolane (45 mL), $CHCl_3$ (25 mL) and MeOH (60 mL), and the solution was stirred over molecular sieves (4Å, pearled) for 1 h. *N*-Hydroxysuccinimide (0.21 g, 1.8 mmol, 1.2 equiv) and *N*,*N*′-dicyclohexylcarbodiimide (0.44 g, 2.1 mmol, 1.4 equiv) were added and the mixture was stirred overnight. Removal of the solids and evaporation of the solvent gave crude compound **13** that was dissolved in $CHCl_3$ (100 mL) and MeOH (50 mL) and added to DPPE (1.0 g, 1.45 mmol) dissolved, together with $NaHCO_3$ (1 g, 12 mmol) in $CHCl_3$ (100 mL), MeOH (50 mL) and water (6 mL) at 35 °C. The mixture was stirred for 24 h at 35 °C, and evaporation of the solvents and chromatography of the crude product on silica gel (15:10:1 $CHCl_3$–MeOH–H_2O) gave compound **19** (0.87 g, 0.64 mmol, 44%), which was identical to the product prepared by method A.

Acknowledgements

The authors thank Dr Herbert Wong for recording NMR spectra and for helpful discussions, Professor Robin Ferrier for assistance with preparing the manuscript, and the New Zealand Foundation for Research Science and Technology for financial support under contracts C08301, C08614 and C08811.

References

[1] R.A.B. Ezekowitz, P.D. Stahl, *J. Cell. Sci. Suppl.*, 9 (1988) 121–133.
[2] F. Sallusto, M. Cella, C. Danieli, A. Lanzavecchia, *J. Exp. Med.*, 182 (1995) 389–400.
[3] W. Jiang, W.J. Swiggard, C. Heufler, M. Peng, A. Mirza, R.M. Steinman, M.C. Nussenzweig, *Nature*, 375 (1995) 151–155.
[4] J. Epstein, Q. Eichbaum, S. Sheriff, R.A.B. Ezekowitz, *Curr. Opin. Immunol.*, 8 (1996) 29–35.
[5] V. Kery, J.J.F. Krepinsky, C.D. Warren, P. Capek, P.D. Stahl, *Arch. Biochem. Biophys.*, 298 (1992) 49–55.
[6] V. Apostolopoulos, G.A. Pietersz, B.E. Loveland, M.S. Sandrin, I.F.C. McKenzie, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 10128–10132.
[7] M.S. Wadhwa, K.G. Rice, *J. Drug Target*, 3 (1995) 111–127.
[8] T. Ferkol, J.C. Perales, F. Mularo, R.W. Hanson, *Proc. Natl. Acad. Sci. USA*, 93 (1996) 101–105.
[9] N.W. Barton, F.S. Furbish, G.J. Murray, M. Garfield, R.O. Brady, *Proc. Natl. Acad. Sci. USA*, 87 (1990) 1913–1916.
[10] R.E. Cohen, W.-J. Zhang, C.E. Ballou, *J. Biol. Chem.*, 257 (1982) 5730–5737.
[11] F.M. Winnik, J.-R. Brisson, J.P. Carver, J.J. Krepinsky, *Carbohydr. Res.*, 103 (1982) 15–28.
[12] H. Paulsen, F. Reck, I. Brockhausen, *Carbohydr. Res.*, 236 (1992) 39–71.
[13] J. Arnarp, J. Lönngren, *Acta Chem. Scand. Ser. B*, 32 (1978) 696–697.
[14] S. Oscarson, A.-K. Tidén, *Carbohydr. Res.*, 247 (1993) 323–328.
[15] T. Ogawa, K. Katano, M. Matsui, *Carbohydr. Res.*, 64 (1978) C3–C9.
[16] K.J. Kaur, O. Hindsgaul, *Glycoconjugate J.*, 8 (1991) 90–94.
[17] R. Roy, D. Pagé, S. Figueroa-Pèrez, V. Verez-Bencomo, *Glycoconjugate J.*, 15 (1998) 251–263.
[18] S. Figueroa-Pèrez, V. Verez-Bencomo, *J. Carbohydr. Chem.*, 17 (1998) 851-868.
[19] R.U. Lemieux, *Pure Appl. Chem.*, 25 (1971) 527–548.
[20] R.R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 50 (1994) 21–123.

[21] D.S. Tsui, P.A.J. Gorin, *Carbohydr. Res.*, 156 (1998) 1–8.
[22] A.F. Bochkov, V.I. Betaneli, N.K. Kochetkov, *Izv. Akad. Nauk. SSSR Ser. Khim.*, 6 (1974) 1379–1386.
[23] N.K. Kochetkov, A.J. Khorlin, A.F. Bochkov, *Tetrahedron*, 23 (1967) 693-707.
[24] R.K. Ness, H.G. Fletcher Jr., C.S. Hudson, *J. Am. Chem. Soc.*, 72 (1950) 2200–2205.
[25] T. Iversen, D.R. Bundle, *Carbohydr. Res.*, 103 (1982) 29–40.
[26] J. Fiandor, M.T. García-López, F.G. de las Heras, P.P. Méndez-Castrillón, *Synthesis*, (1985) 1121–1123.
[27] R.U. Lemieux, D.R. Bundle, D.A. Baker, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
[28] H. Gerlach, P. Künzler, K. Oertle, *Helv. Chim. Acta*, 61 (1978) 1226–1231.
[29] Y. Nagao, K. Kawabata, E. Fujita, *J. Chem. Soc., Chem. Commun.*, (1978) 330.
[30] K. Wada, T. Chiba, Y. Takei, H. Ishihara, H. Hayashi, K. Onozaki, *J. Carbohydr. Chem.*, 13 (1994) 941–965.
[31] C.E. McDonald, L.E. Nice, A.W. Shaw, N.B. Nestor, *Tetrahedron Lett.*, 34 (1993) 2741–2744.
[32] T. Sugawara, K. Irie, H. Iwasawa, T. Yoshikawa, S. Okuno, H.K. Watanabe, T. Kato, M. Shibukawa, Y. Ito, *Carbohydr. Res.*, 230 (1992) 117–149.
[33] A.H. Haines, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 11–109.
[34] K. Bock, C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
[35] K. Bock, I. Lundt, C. Pedersen, *Tetrahedron Lett.*, 13 (1973) 1037–1040.
[36] K. Bock, C. Pedersen, *Acta Chem. Scand. Ser. B*, 29 (1975) 258–264.
[37] J.Y. Zhao, N.J. Dovichi, O. Hindsgaul, S. Gosselin, M.M. Palcic, *Glycobiology*, 4 (1994) 239–242.
[38] S. Medda, S. Mukherjee, N. Das, K. Naskar, S.B. Mahato, M.K. Basu, *Biotechnol. Appl. Biochem.*, 17 (1993) 37–47.
[39] H. Snippe, A.F.M. Verheul, *J. Liposome Res.*, 5 (1995) 453–465.
[40] K. Yachi, H. Kikuchi, H. Yamauchi, S. Hirota, M. Tomikawa, *J. Microencapsul.*, 12 (1995) 377–388.
[41] T. Ouchi, Y. Ohya, *Neoglycoconjugate: Preparation and Application*, Academic Press, San Diego, 1994, pp. 465–498.