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Carbohydrate Research 343 (2008) 7–17
Carbohydrate RESEARCH

Synthesis of β-(1→4)-oligo-D-mannuronic acid neoglycolipids

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Received 23 July 2007; received in revised form 5 October 2007; accepted 11 October 2007
Available online 22 October 2007

Abstract—Mammalian Toll-like receptors (TLRs) play important roles in host immune defense. The activation of TLR and downstream signaling pathways have great impact on human physiology. Chemically diverse microbial products as well as synthetic ligands serve as agonists for these receptors. Recently, synthetic TLR ligands are being exploited as useful therapeutic agents for a variety of diseases including infections, inflammatory diseases, and cancers. Alginate polymers and oligosaccharides are strong immune stimulants mediated by TLR2/4, but synthesis of alginate oligomers is rarely studied. Reported here are the design and chemical synthesis of two β-(1→4)-di- and β-(1→4)-tri-D-mannuronic acid neoglycolipids **1** and **2** as potential TLR ligands. By using 4,6-di-*O*-benzylidene-protected 1-thio mannoside **7** as a glycosyl donor, the diastereoselective β-D-mannosylation protocol provides the β-(1→4)-D-mannobiose and β-(1→4)-D-mannotriose derivatives, which upon regioselective oxidation with TEMPO/BAIB oxidation system yield the corresponding β-(1→4)-D-mannuronic acid containing neoglycolipids **1** and **2**.

Keywords: Alginate; D-Mannuronic acid; Oligosaccharide synthesis; Glycolipids; TLR ligand

1. Introduction

Mammalian Toll-like receptors (TLRs) are innate pattern recognition receptors that play critical roles in detecting invading pathogens and triggering of subsequent inflammatory and immune response. To date, thirteen members of TLRs have been found in mice and eleven in humans.[1] Chemically diverse microbial products as well as endogenous ligands such as heat shock protein (HSP) serve as agonists for these receptors.[2] Activation of TLRs and downstream signaling pathways lead to the expression of pro- and anti-inflammatory mediators, which have great impact on human physiology. Conceivably, either naturally occurring or synthetic ligands capable of regulating TLR signaling pathways will be extremely useful in treating various diseases including infection, chronic inflammation, and cancers.[3–5] As part of our synthetic vaccine program,[6] we have previously reported a few lipid A-based synthetic TLR 4 ligands, which are potent immune-stimulatory molecules potentially useful as vaccine adjuvants.[7–9] To continue our studies in synthetic TLR ligands for modulating immune responses, here we report the design and synthesis of two alginate-derived neoglycolipids **1** and **2** as potential ligands for TLRs (Chart 1).

Alginates are linear polysaccharides consisting of β-(1→4)-linked D-mannuronic acid (M) residues and its C-5-epimer α-(1→4)-linked L-guluronic acid (G) residues (Chart 1).[10] In nature, alginates are produced by marine macroalgae and some bacteria belonging to the genera *Azotobacter* and *Pseudomonas*.[11] Their compositions are highly variable depending on the sources from which they are isolated. Three types of polymeric blocks have been reported for natural alginates: (a) poly-M block which contains mainly D-mannuronic acid residues; (b) poly-G block consisting of mainly L-guluronic acid residues; and (c) poly-GM block that contains alternating D-mannuronic acid and L-guluronic acid residues. Alginates from bacteria also display certain degrees of acetylation at the O-2- and O-3-position of D-mannuronic acid residues.[12] Based on their gel-forming properties, alginates have traditionally been widely used as additives in the food industry,[13] and more recently in the biomedical industry as biocompatible materials

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doi:10.1016/j.carres.2007.10.007

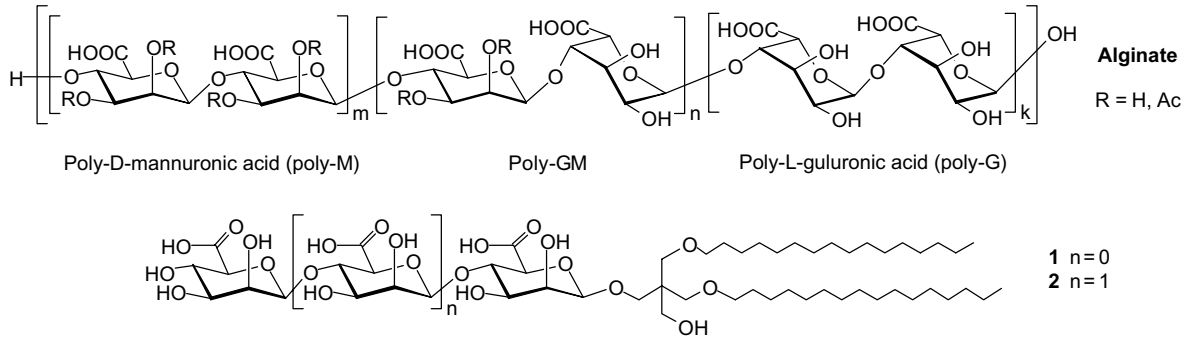


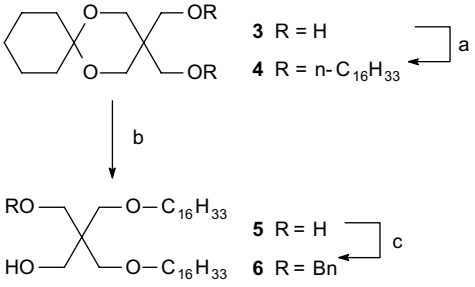
Chart 1. Structures of alginates and neoglycolipids **1** and **2**.

for drug delivery[14] and immobilization of microorganisms and living cells.[15] Recently, alginate polymers[16,17] and alginate oligosaccharides[18,19] have been reported to be potent immune-stimulating agents in eliciting cytokine production by monocytes. The immune-stimulatory potency depends on the content of M and G residues of the polymeric blocks. TLR2 and TLR4 are involved in mediating cytokine production induced by both alginate polymers and alginate oligosaccharides,[17,19] suggesting that short oligosaccharides from alginates may well serve as agonists or antagonists for these receptors.

To better understand the biochemical properties of alginates and the underlying mechanism of TLR-mediated innate immune activation, it is highly desirable to access chemically defined alginate fragments. However, due to the challenge posed by constructing 1,2-cis β-glycosidic linkage of D-mannuronic acid as well as accessing L-guluronic acid building blocks, synthesis of alginate oligomers has rarely been studied. During the course of our investigation toward the synthesis of β-D-mannuronic acid glycosides, van den Bos et al.[20] reported an elegant methodology, albeit unexpectedly, for the stereocontrolled direct synthesis of β-D-mannuronic acid glycosides. They found that 1-thio mannuronic acid derivatives can be activated by either diphenyl sulfoxide–trifluoromethanesulfonic acid anhydride (Ph_2SO–Tf_2O) or *N*-iodosuccinimide–trimethylsilyl trifluoromethanesulfonate (NIS–TMSOTf) to produce β-D-mannuronic acid glycosides in high yield and with excellent β-selectivity. Recently, we have reported the synthesis of two β-(1→4)-di-D-mannuronic acid glycosides through β-D-mannobiose as the precursor.[21] Presented here are the full experimental results for the synthesis of two neoglycolipids **1** and **2** containing β-(1→4)-di- and β-(1→4)-tri-D-mannuronic acid residues, respectively.

2. Results and discussion

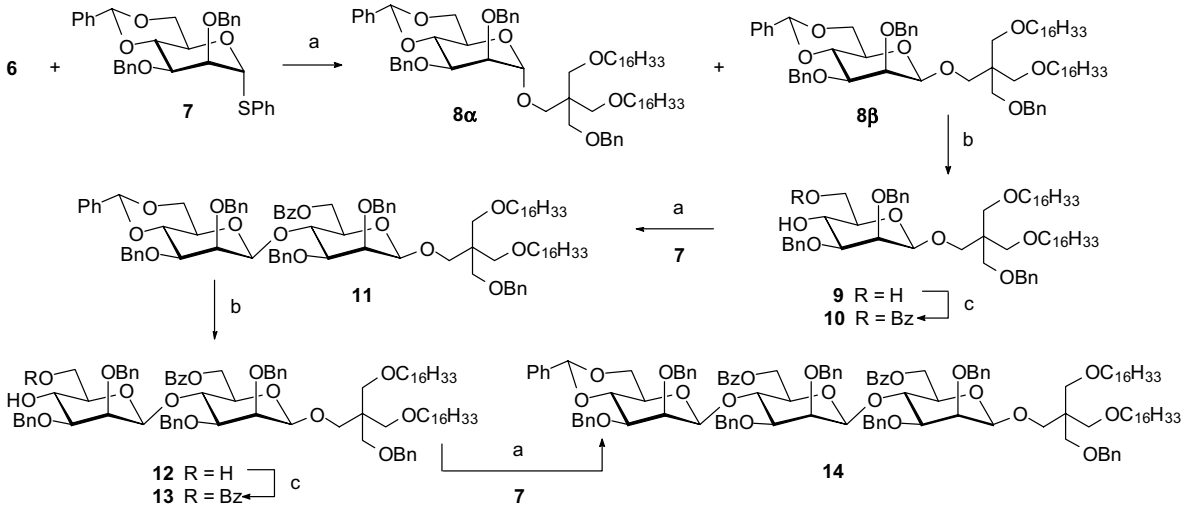
Naked small carbohydrate molecules are rapidly cleared away from the biological system and therefore are deemed to be poor drug candidates.[22] Here we have designed both compounds **1** and **2** as glycolipids in which the conjugation with lipids may enhance the stability and bioavailability of small carbohydrates in the biological system. Glycolipids are also suitable for liposome formulation, a drug delivery system proven to be efficient in reducing toxicity and side effects.[23] As the lipid anchor capable of incorporating multiple lipid chains, we have chosen the multifunctional pentaerythritol, which has recently been used as a core to build multivalent ligands.[24,25] Thus, starting from the readily available pentaerythritol ketal derivative **3**,[26] the lipidated pentaerythritol derivative **6** is prepared as the aglycone (Scheme 1). Lipidation of **3** is effected by the treatment with 1-bromohexadecane and sodium hydride in DMF to give **4** in 59% yield. The removal of the cyclohexylidene group to liberate diol **5** is incomplete when **4** is treated under these conditions: (a) 10:1:0.2 EtOAc–MeOH–H_2O in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) under reflux; and (b) 2:1:0.3 ExOAc–hexane–ethylene glycol in the presence of *p*-TsOH as the catalyst at room temperature. Gratifyingly, diol **5** is obtained in 95% yield through a trans-ketal reaction with neopentyl glycol in anhyd CH_2Cl_2 in the presence of camphorsulfonic acid (CSA). Diol **5** is then converted to the mono-benzylated lipid aglycone **6** in 70% yield by treatment with dibutyltin oxide (Bu_2SnO), followed by benzyl bromide and tetrabutylammonium bromide.[27]



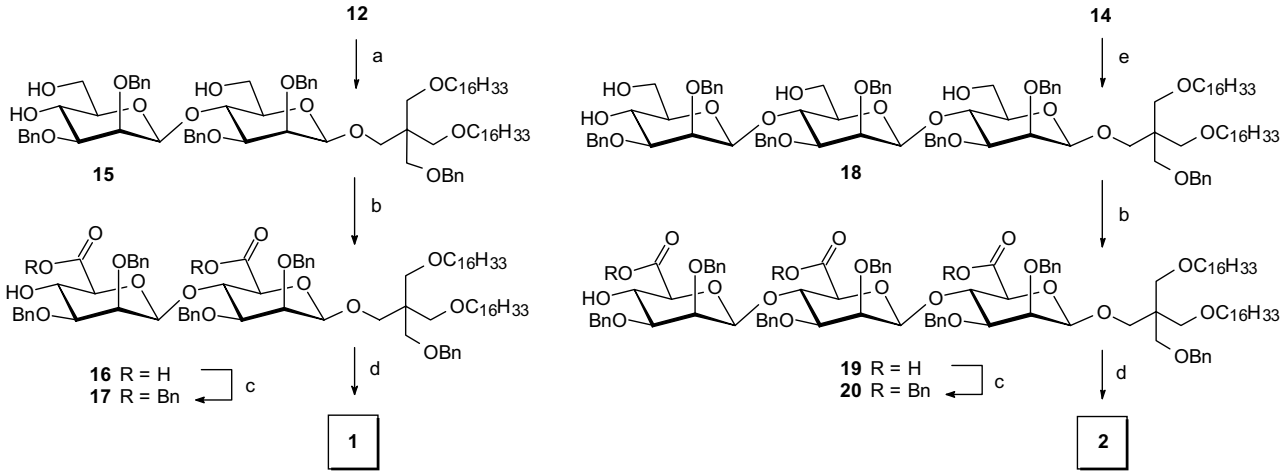
Scheme 1. Reagents and conditions: (a) *n*-$C_{16}H_{33}Br$, DMF, NaH, 50 °C, 59%; (b) neopentyl glycol, CSA, CH_2Cl_2, 35 °C, 95%; (c) (i) Bu_2SnO, benzene, 90 °C; (ii) BnBr, Bu_4NBr, 90 °C, 70%.

The strategy we employ to make the β-(1→4)-di- and β-(1→4)-tri-D-mannuronic acid glycolipids **1** and **2** is to first synthesize their corresponding β-(1→4)-D-mannosides as precursors, and then to convert all the mannose residues to mannuronic acid residues through an oxidation step. Diastereoselective synthesis of β-D-mannopyranoside has been a long-standing challenge in carbohydrate chemistry.[28] The methodology recently developed by the group of Crich and co-workers[29,30] by employing 4,6-di-*O*-benzylidene-protected 1-thio and 1-sulfoxide mannoside donors offers an elegant solution for the introduction of β-D-mannoside linkage. Thus, by using the 1-thio mannoside **7**[29] as the glycosylation donor and 1-benzenesulfinyl piperidine (BSP) and Tf_2O in the presence of the hindered base, 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the promoting system, the β-linked (1→4)-di-D-mannoside **11** and (1→4)-tri-D-mannoside **14** can be prepared in a straightforward manner (Scheme 2). Activation of donor **7** in dichloromethane at −60 °C followed by the addition of the glycosylation acceptor **6** at −78 °C, gives the desired β-D-mannoside **8β** in 85% yield. α-Glycoside **8α** is also formed in 8% yield. The β-glycosidic linkage in **8β** is confirmed by the characteristic H-5 signal[29] (δ 3.30, ddd, 1H, *J* 10.0, 10.0, 4.5 Hz) of its ^1H NMR spectrum and the $^1J_{C,H}$ coupling constant of the anomeric carbon[29,31] (δ 101.55, $^1J_{C,H} = 154.6$ Hz and 103.44, $^1J_{C,H} = 155.8$ Hz, C-1 and the acetal carbon of the benzylidene group). The α-linkage in **8α** is evidenced by the $^1J_{C,H}$ coupling constant of the anomeric carbon (δ 99.61, $^1J_{C,H} = 167.1$ Hz and 101.66, $^1J_{C,H} = 157.5$ Hz). Compound **8β** is then converted to diol **9** in 90% yield by treating with neopentyl glycol in the presence of CSA in anhydrous dichloromethane. Regioselective benzoylation of **9** with benzoyl chloride and pyridine in the presence of 4-dimethylaminopyridine (DMAP) affords 6-*O*-benzoyl derivative **10** in 70% yield.

Using the same glycosylation protocol as described for the synthesis of **8α/8β**, monosaccharide **10** is glycosylated with 1-thio mannoside donor **7** to give disaccharide **11** in 67%. The newly formed β-linkage in **11** is confirmed by the characteristic H-5 signal[29] (δ 3.05, ddd, 1H, *J* = 10.0, 10.0, 4.5 Hz,) of the sugar residue bearing the 4,6-di-*O*-benzylidene group, as well as the presence of three acetal carbons (two anomeric carbons and one in benzylidene group) with $^1J_{C,H}$ coupling constants less than 160 Hz (δ 101.57, $^1J_{C,H} = 157.5$, Hz; 102.14, $^1J_{C,H} = 153.7$ Hz; 102.48, $^1J_{C,H} = 155.4$ Hz).[29,31] A minor product formed in about 10% yield shows characteristic signals of the α-isomer of the disaccharide in its ^1H and ^{13}C NMR spectra, but its structure is not confirmed since the analytically pure compound has not been obtained. Following the same sequence of reaction steps as described for the transformation of **8**β to **10**, disaccharide **11** is first converted to diol **12** (90%) and then to 6-*O*-monobenzoate **13** in 94% yield. Using the same glycosylation protocol, disaccharide **13** is reacted with donor **7** to give the desired trisaccharide **14** in 51% yield (with 10% unreacted **13** recovered). The newly formed β-linkage in **14** is similarly confirmed by the characteristic H-5 signal of the 4,6-di-*O*-benzylidene-bearing mannose residue (δ 2.97, ddd, *J* 9.5, 9.5, 5.0 Hz, 1H) and by the $^1J_{C,H}$ coupling constant value of the anomeric carbons (δ 101.51, $^1J_{C,H} = 153.1$ Hz; 101.57, $^1J_{C,H} = 151.6$ Hz; 102.11, $^1J_{C,H} = 152.3$ Hz, 102.49, $^1J_{C,H} = 156.5$ Hz). Again, a minor product (possibly the α-isomer) has been detected, but its structure is not established. Repeated chromatographic purification of the trisaccharide **14** partly accounts for its relatively low yield.



Scheme 2. Reagents and conditions: (a) BSP, DTBMP, Tf_2O, CH_2Cl_2, −60 °C then −78 °C to 0 °C, 8% for **8α** and 85% for **8β**; 67% for **11**; and 51% for **14**; (b) neopentyl glycol, CSA, CH_2Cl_2, 35 °C, 90% for both **9** and **12**; (c) BzCl, pyridine, DMAP, CH_2Cl_2, −25 °C to 0 °C, 70% for **10** and 94% for **13**.



Scheme 3. Reagents and conditions: (a) NaOMe, 1:1 MeOH–CH_2Cl_2, rt, 100%; (b) BAIB, TEMPO, 2:1 CH_2Cl_2–H_2O, rt, 95% for **16** and 70% for **19**; (c) BnBr, KF, DMF, rt, 78% for **17** and 80% for **20**; (d). H_2, Pd/C, 4:1 THF–H_2O, rt, 90% for both **1** and **2**; (e) (i) neopentyl glycol, CSA, CH_2Cl_2, 35 °C; (ii) NaOMe, 1:1 MeOH–CH_2Cl_2, rt, two steps 83%.

With the desired β-(1→4)-D-mannobiose and β-(1→4)-D-mannotriose on hand, the next step is to convert the mannose residues to the mannuronic acid residues. Thus, compounds **12** and **14** are transformed to their 6-hydroxyl derivatives **15** and **18**, respectively, through standard protecting group manipulation (Scheme 3). The primary hydroxyl groups in **15** and **18** are then regioselectively oxidized to carboxylic acid functional groups by the combination of 2,2,6,6-tetramethylpiperidinyloxy free radical (TEMPO) and [bis(acetoxy)iodo]benzene (BAIB),[32] providing the corresponding dicarboxylic acid **16** and tricarboxylic acid **19** in 95% and 70% yield, respectively. For structural confirmation, both acids **16** and **19** are converted into their benzyl esters **17** and **20** by the treatment with benzyl bromide and potassium fluoride in DMF in 78% and 80% yield, respectively. The TEMPO/BAIB oxidation system provides a good yield in the simultaneous oxidation of multiple primary hydroxyl groups to carboxylic acid functions in the presence of secondary hydroxyl groups. Hydrogenolytic debenzylation of **17** and **20** in the presence of palladium-on-charcoal under a hydrogen atmosphere gives the target glycolipids **1** and **2** in 90% yield. The structures of **1** and **2** have been confirmed by mass spectroscopy data.

In conclusion, an efficient synthesis of β-(1→4)-di- and β-(1→4)-tri-D-mannuronic acid neoglycolipids **1** and **2** has been reported. The strategy incorporates the diastereoselective synthesis of β-(1→4)-D-mannobiose and β-(1→4)-D-mannotriose using a 4,6-di-*O*-benzylidene-protected 1-thio D-mannoside as the glycosyl donor, followed by simultaneous regioselective oxidation of all the mannose residues to the mannuronic acid residues by the TEMPO/BAIB oxidation system. The method described here shall be applicable for synthesizing glycoconjugates containing larger β-(1→4)-oligo-D-mannuronic acid fragments. Ongoing research is devoted to the synthesis of other types of alginate oligomers and the biological investigation of these neoglycoconjugates in respect to their immune-stimulating and/or -modulating properties mediated with TLR2/4.

3. Experimental

3.1. General methods

All air- and moisture-sensitive reactions have been performed under nitrogen atmosphere. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Aldrich Chemical Co., and other dry solvents were prepared in accordance with standard procedures. ACS grade solvents were purchased from Fisher Scientific Co. and used for chromatography without distillation. TLC plates (Silica Gel $60F_{254}$, thickness 0.25 mm) and Silica Gel 60 (40–63 μm) for flash column chromatography were purchased from Silicycle, Inc., Canada. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500-MHz spectrometer, and tetramethylsilane (TMS, δ 0.00 ppm) and chloroform (δ 77.23 ppm) were used as internal standards for ^1H and ^{13}C chemical shifts, respectively. Multiplicity of proton signals are indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Sugar units are designated as I, II, and III (as superscripts), beginning at the reducing end of the molecule, according to the Whelan system (Rule 2-Carb-37.2).[33] Optical rotations were measured on a Perkin–Elmer 241 polarimeter at room temperature (20–22 °C). Elemental analysis was carried out on a CEC (SCP) 240-XA Analyzer at Lakehead University Instrumentation Laboratory (LUIL). Low-resolution MALDI mass spectra were obtained from a Biflex-IV

MALDI linear/reflector instrument at the University of Manitoba, Canada, and high-resolution electrospray-ionization (HRESI) mass spectra were measured on the Applied Biosystems Mariner Biospectrometry Workstation at the University of Alberta, Canada.

3.2. 3,3-Dihexadecyloxymethyl-1,5-dioxaspiro[5,5] undecane (4)

To a completely dissolved solution of 1-bromohexadecane (26.20 mL, 85.44 mmol) in anhyd DMF (60 mL) was carefully added sodium hydride (2.60 g, 102.65 mmol), followed by dropwise addition of **3** (9.25 g, 42.77 mmol, dissolved in 20 mL of DMF). The mixture was heated at 50 °C and stirred for 16 h. The mixture was then poured into ice-water (400 mL), extracted with EtOAc (300 mL × 3), and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by flash chromatography (30:1 and then 20:1 hexane–EtOAc) to provide **4** (14.31 g, 59%) as a colored syrup. R_f 0.41 (20:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (t, 6H, J 6.5 Hz, $2CH_3$), 1.20 (m, 56H, $26CH_2$), 1.30 (m, 2H, CH_2), 1.40 (m, 8H, $4CH_2$), 3.30 (m, 8H, $4CH_2O$), 3.60 (s, 4H, $2CH_2O$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.40, 22.83, 22.96, 25.98, 26.43, 29.64, 29.77, 29.82, 29.91, 29.93, 29.98, 32.19, 32.87, 39.23, 62.30, 70.61, 71.88, 98.21. Anal. Calcd for $C_{43}H_{84}O_4{\cdot}0.5H_2O$ (665.10): C, 76.60; H, 12.70. Found: C, 76.97; H, 12.33.

3.3. 2,2-Dihexadecyloxymethyl-1,3-propanediol (5)

To a solution of **4** (14.31 g, 21.52 mmol) and neopentyl glycol (9.0 g, 86.1 mmol) in anhyd CH_2Cl_2 (200 mL) was added camphorsulfonic acid (CSA, 1.0 g, 4.3 mmol). The mixture was stirred at 35 °C under an N_2 atmosphere for 16 h. The mixture was then washed with satd aq $NaHCO_3$ (100 mL), and the organic extract was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (2:1 hexane–EtOAc) to give **5** (12.1 g, 95%) as a white powder. R_f 0.45 (2:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, $2CH_3$), 1.26 (m, 52H, $26CH_2$), 1.55 (m, 4H, $2CH_2$), 2.80 (t, 2H, J 6.0 Hz, 2OH), 3.42 (t, 4H, J 6.0 Hz, $2CH_2O$), 3.51 (s, 4H, $2CH_2O$), 3.64 (d, 4H, J 6.0 Hz, $2CH_2OH$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.14, 22.71, 29.38, 29.45, 29.52, 29.61, 29.64, 29.68, 29.70, 29.72, 31.94, 31.98, 44.50, 65.48, 72.06, 73.23. Anal. Calcd for $C_{37}H_{76}O_4$ (585.10): C, 75.97; H, 13.09. Found: C, 75.85; H, 12.87.

3.4. 3-Benzyloxy-2,2-dihexadecyloxymethyl-1-propanol (6)

A solution of **5** (5.85 g, 10.0 mmol) and dibutyltin oxide (2.49 g, 10.0 mmol) in benzene (100 mL) was kept at reflux at 90 °C for 16 h with removal of water by a Dean–Stark trap. Then Bu_4NBr (3.22 g, 10.0 mmol) and $PhCH_2Br$ (2.38 mL, 20.0 mmol) were added, and the mixture was stirred under reflux for 5 h. The solvent was then removed in vacuum, and the residue was redissolved in EtOAc (150 mL) and washed with satd aq $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated in vacuum. The residue was purified by flash chromatography (20:1 and then 12:1 hexane–EtOAc) to provide **6** (4.72 g, 70%) as a syrup. R_f 0.36 (10:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, J 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.52 (m, 5H, $2CH_2$, OH), 3.38 (t, 4H, J 6.5 Hz, $2CH_2O$), 3.47 (s, 4H, $2CH_2O$), 3.52 (s, 2H, CH_2OH), 3.73 (s, 2H, CH_2O), 4.50 (s, 2H, CH_2Ph), 7.27–7.35 (m, 5H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.38, 22.95, 26.42, 29.62, 29.74, 29.83, 29.89, 29.91, 29.93, 29.96, 32.19, 45.10, 66.84, 71.01, 71.88, 72.05, 73.71, 126.60, 127.71, 128.54, 138.76. Anal. Calcd for $C_{44}H_{82}O_4$ (675.13): C, 78.28; H, 12.24. Found: C, 77.92; H, 12.14.

3.5. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (8α) and 3-benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-mannopyranoside (8β)

To a solution of **7**[29] (1.63 g, 3.01 mmol) in anhyd CH_2Cl_2 (50 mL) were added 1-benzenesulfinylpiperidine (BSP, 0.69 g, 3.3 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 1.26 g, 6.12 mmol), and molecular sieves (4 Å, 2.0 g). The mixture was stirred at room temperature under N_2 atmosphere for 1 h. Trifluoromethanesulfonic acid anhydride (Tf_2O, 0.56 mL, 3.30 mmol) was added dropwise at −60 °C, and the mixture was stirred for 5 min. The mixture was cooled to −78 °C, and a solution of **6** (1.35 g, 2.00 mmol, dissolved in 10 mL of dry CH_2Cl_2) was added dropwise. The mixture was kept stirring at −78 °C for 2 h and then warmed up to room temperature before quenched with satd aq $NaHCO_3$ (30 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layer was dried over Na_2SO_4, and concentrated, and the residue was purified by flash chromatography (20:1 and then 10:1 hexane–EtOAc) to provide compound **8β** (2.10 g, 85%) as a syrup. The minor isomer was further purified by flash chromatography (12:1 hexane–EtOAc) to give analytical pure compound **8α** (190 mg, 8%). For **8α**: R_f 0.39 (8:1 hexane–EtOAc); $[\alpha]_D^{22}$ +15.8 (c 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, J 6.5 Hz, $2CH_3$), 1.26 (m, 52H, $26CH_2$), 1.51 (m, 4H, $2CH_2$), 3.28–3.35 (m, 9H), 3.39 (s, 2H), 3.73 (m, 2H), 3.79 (ddd, 1H, J 10.0, 10.0, 4.5 Hz, H-5), 3.85 (dd, 1H, J 9.5, 9.5 Hz), 3.88 (dd, 1H, J 9.5, 3.5 Hz, H-2), 4.21–4.25 (m, 2H), 4.43 (d,

1H, *J* 12.0 Hz, PhC*H*H), 4.47 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.63 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.72 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.76 (d, 1H, *J* 1.5 Hz, H-1), 4.79 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.83 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.63 (s, 1H, C*H*Ph), 7.23–7.50 (m, 20H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.39, 22.96, 26.48, 29.63, 29.80, 29.90, 29.93, 29.98, 32.18, 45.25, 64.32, 66.41, 66.87, 69.12, 69.47, 71.86, 73.45, 73.53, 73.57, 76.56, 76.90, 79.44, 99.61 ($^1J_{C,H}$ 167.1 Hz), 101.66 ($^1J_{C,H}$ 157.5 Hz), 126.35, 127.55, 127.59, 127.75, 127.92, 127.96, 128.16, 128.34, 128.49, 128.59, 128.96, 138.10, 138.44, 138.90, 139.05. HRE-SIMS: $[M+Na]^+$ calcd for $C_{71}H_{108}O_9Na$, 1127.7885; found (positive-ion mode): 1127.7884. For **8β**: R_f 0.35 (8:1 hexane–EtOAc); $[\alpha]_D^{22}$ −15.7 (*c* 0.66, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.52 (m, 4H, $2CH_2$), 3.30 (ddd, 1H, *J* 10.0, 10.0, 4.5 Hz, H-5), 3.32–3.38 (m, 4H, $2CH_2O$), 3.39–3.54 (m, 8H, $4CH_2O$), 3.83 (d, 1H, *J* 3.0 Hz, H-2), 3.92 (dd, 1H, *J* 10.0, 10.0 Hz), 3.99 (d, 1H, *J* 10.0 Hz), 4.30 (dd, 1H, *J* 10.0, 4.5 Hz), 4.37 (s, 1H, H-1), 4.41 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.50 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.57 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.68 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.79 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.95 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.60 (s, 1H, C*H*Ph), 7.21–7.50 (m, 20H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.36, 22.93, 26.49, 29.61, 29.77, 29.87, 29.90, 29.92, 29.95, 32.18, 45.46, 67.76, 68.83, 69.81, 69.88, 69.90, 70.65, 71.80, 71.81, 72.62, 73.50, 74.81, 76.04, 78.28, 78.87, 101.55 ($^1J_{C,H}$ 154.6 Hz), 103.44 ($^1J_{C,H}$ 155.8 Hz), 126.26, 127.55, 127.57, 127.71, 127.72, 128.35, 128.43, 128.49, 128.75, 129.00, 137.86, 138.61, 138.72, 139.07. Anal. Calcd for $C_{71}H_{108}O_9$ (1105.63): C, 77.13; H, 9.85. Found: C, 76.93; H, 9.81.

3.6. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-β-D-mannopyranoside (9)

To a solution of **8β** (2.10 g, 1.92 mmol) and neopentyl glycol (0.80 g, 7.68 mmol) in anhyd CH_2Cl_2 (20 mL) was added camphorsulfonic acid (0.30 g, 1.15 mmol). The mixture was stirred at 35 °C under an N_2 atmosphere for 16 h, and the reaction was quenched with satd aq $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuum, and the residue was purified by flash chromatography (5:1 and then 2:1 hexane–EtOAc) to give **9** (1.74 g, 90%) as a syrup. R_f 0.36 (5:4 hexane–EtOAc); $[\alpha]_D^{22}$ −40.4 (*c* 0.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.52 (m, 4H, $2CH_2$), 2.34 (dd, 1H, *J* 6.0, 6.0 Hz, OH), 3.25 (dd, 1H, *J* 9.5, 3.0 Hz, H-3), 3.29 (m, 1H, H-5), 3.34–3.54 (m, 11H), 3.80 (dd, 1H, *J* 9.5, 4.5 Hz), 3.84 (d, 1H, *J* 3.0 Hz, H-2), 3.90 (m, 2H), 3.99 (d, 1H, *J* 9.0 Hz), 4.24 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.39 (s, 1H, H-1), 4.44 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.45 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.52 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.69 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.91 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.23–7.39 (m, 15H, Ar-H). Anal. Calcd for $C_{64}H_{104}O_9$ (1017.52): C, 75.55; H, 10.30. Found: C, 75.07; H, 10.13.

3.7. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside (10)

A solution of **9** (480 mg, 0.48 mmol) and 4-(dimethylamino)pyridine (DMAP, 7.6 mg, 0.048 mmol) in dry pyridine (5 mL) was cooled to −25 °C and benzoyl chloride (0.061 mL, 0.53 mmol, dissolved in 5 mL of anhyd CH_2Cl_2) was added dropwise. The reaction mixture was kept to stir for 2 h at −25 °C and then warmed up to room temperature and stirred at room temperature for 16 h. The mixture was then diluted with EtOAc (100 mL) and washed successively with 4 M HCl (30 mL), ice-water (30 mL), and satd aq $NaHCO_3$ (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by flash chromatography (10:1 and then 4:1 hexane–EtOAc) to give compound **10** (370 mg, 70%) as a syrup. R_f 0.63 (2:1 hexane–EtOAc); $[\alpha]_D^{22}$ −35.0 (*c* 0.38, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.26 (m, 52H, $26CH_2$), 1.49 (m, 4H, $2CH_2$), 2.90 (br s, 1H, OH), 3.30–3.56 (m, 13H), 3.85 (d, 1H, *J* 3.0 Hz, H-2), 4.01–4.04 (m, 2H), 4.39 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.46 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.49 (s, 1H, H-1), 4.52 (dd, 1H, *J* 12.0, 6.0 Hz, H-6), 4.59 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.61 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.66 (dd, 1H, *J* 12.0, 3.5 Hz, H-6), 4.68 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.92 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.20–7.48 (m, 18H, Ar-H), 8.05 (d, 2H, *J* 8.0 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.30, 22.85, 26.38, 29.52, 29.68, 29.77, 29.82, 29.85, 29.87, 32.09, 45.35, 64.35, 66.95, 69.77, 69.84, 69.86, 70.18, 71.35, 71.69, 71.71, 73.39, 73.53, 73.94, 74.45, 81.56, 102.86 ($^1J_{C,H}$ 154.6 Hz, C-1), 126.99, 127.40, 127.47, 127.85, 127.96, 128.19, 128.27, 128.32, 128.34, 128.58, 129.96, 132.91, 137.89, 138.93, 138.99, 166.82 (CO). Anal. Calcd for $C_{71}H_{108}O_{10}$ (1121.63): C, 76.03; H, 9.71. Found: C, 75.85; H, 9.49.

3.8. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside (11)

To a solution of **7**[29] (245 mg, 0.41 mmol) in anhyd CH_2Cl_2 (10 mL) were added 1-benzenesulfinylpiperidine (BSP, 103 mg, 0.45 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 187 mg, 0.83 mmol), and molecular sieves (4 Å, 1.0 g). The mixture was stirred at room temperature under an N_2 atmosphere for 30 min and then

cooled to −60 °C followed by the addition of trifluoromethanesulfonic acid anhydride (0.08 mL, 0.45 mmol). After stirring at −60 °C for 5 min, the mixture was cooled to −78 °C, and a solution of **10** (330 mg, 0.27 mmol, dissolved in 3 mL of CH_2Cl_2) was added dropwise. The mixture was kept to stir at −78 °C for an additional 2 h and then warmed up to room temperature. The reaction was quenched by adding satd aq $NaHCO_3$ (10 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuum and the residue was purified by flash chromatography (10:1 and then 5:1 hexane–EtOAc,) to provide compound **11** (291 mg, 67%) as a syrup. R_f 0.64 (5:2 hexane–EtOAc); $[\alpha]_D^{22}$ −26.8 (*c* 0.40, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.46 (m, 4H, $2CH_2$), 3.05 (ddd, 1H, *J* 9.5, 9.5, 4.5 Hz, H-5^{II}), 3.26–3.32 (m, 4H, $2OCH_2$), 3.36–3.43 (m, 4H, $2CH_2O$), 3.43–3.52 (m, 6H), 3.54 (dd, 1H, *J* 9.5, 3.0 Hz, H-3), 3.59 (dd, 1H, *J* 9.5, 3.0 Hz, H-3), 3.69 (dd, 1H, *J* 10.0, 10.0 Hz), 3.83 (d, 1H, *J* 3.0 Hz, H-2), 3.93 (d, 1H, *J* 3.0 Hz, H-2), 3.99 (dd, 1H, *J* 10.0, 4.5 Hz, H-6^{II}), 4.01 (d, 1H, *J* 10.0 Hz), 4.10 (dd, 1H, *J* 9.0, 9.0 Hz, H-4), 4.16 (dd, 1H, *J* 9.0, 9.0 Hz, H-4), 4.38 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.42 (s, 1H, H-1), 4.43 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.50 (m, 2H, 2H-6^{I}), 4.55 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.57 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.60 (s, 1H, H-1), 4.66 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.67 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.69 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.83 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.88 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.89 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.51 (s, 1H, PhC*H*), 7.23–7.52 (m, 32H, Ar-H), 7.53 (t, 1H, *J* 7.5 Hz, Ar-H), 8.03 (d, 2H, *J* 8.0 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.40, 22.95, 26.45, 29.63, 29.79, 29.89, 29.92, 29.95, 29.97, 32.18, 45.43, 64.09, 67.63, 68.74, 69.86, 69.89, 70.23, 71.81, 71.83, 72.10, 72.56, 73.47, 73.54, 73.98, 74.78, 75.39, 76.16, 77.32, 77.47, 78.55, 78.67, 80.03, 101.57 ($^1J_{C,H}$ 157.5, Hz), 102.14 ($^1J_{C,H}$ 153.7 Hz), 102.48 ($^1J_{C,H}$ 155.4 Hz), 126.32, 127.40, 127.49, 127.52, 127.68, 127.72, 127.75, 128.02, 128.31, 128.37, 128.43, 128.54, 128.55, 128.63, 129.05, 130.01, 130.17, 133.24, 137.79, 138.54, 138.77, 138.82, 139.10, 139.12, 166.55 (CO). Anal. Calcd for $C_{98}H_{134}O_{15}\cdot H_2O$ (1552.13): C, 74.97; H, 8.73. Found: C, 74.98; H, 9.07.

3.9. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside (12)

In a similar way as described for the preparation of **9**, compound **11** (2.92 g, 1.84 mmol) was treated with neopentyl glycol (783 mg, 7.54 mmol) in dry CH_2Cl_2 (20 mL) at 35 °C under an N_2 atmosphere for 16 h. Usual workup and flash chromatographic purification (2:1 and then 5:4 hexane–EtOAc) provided **12** (2.64 g, 96%) as a syrup. R_f 0.38 (5:3 hexane–EtOAc); $[\alpha]_D^{22}$ −39.0 (*c* 0.34, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.48 (m, 4H, $2CH_2$), 3.07 (m, 1H, H-5), 3.20 (dd, 1H, *J* 9.5, 3.5 Hz, H-3), 3.25–3.35 (m, 4H, $2OCH_2$), 3.38–3.42 (m, 4H, $2OCH_2$), 3.44–3.56 (m, 6H), 3.62–3.66 (m, 2H), 3.72 (dd, 1H, *J* 12.5, 3.5 Hz), 3.86 (d, 1H, *J* 3.0 Hz, H-2), 3.88 (dd, 1H, *J* 9.5, 9.5 Hz), 3.92 (d, 1H, *J* 3.0 Hz, H-2), 4.00 (d, 1H, *J* 9.5 Hz), 4.23 (dd, 1H, *J* 9.5, 9.5 Hz), 4.31 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.40 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.42 (s, 1H, H-1), 4.47 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.48 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.50 (m, 1H, H-6^{I}), 4.54 (s, 1H, H-1), 4.55 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.65 (dd, *J* 12.0, 3.0 Hz, H-6^{I}), 4.68 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.72 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.76 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.83 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.92 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.22–7.55 (m, 27H, Ar-H), 7.54 (t, 1H, *J* 7.5 Hz, Ar-H), 8.05 (d, 2H, *J* 8.5 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.36, 22.93, 26.43, 29.59, 29.75, 29.85, 29.88, 29.91, 29.93, 32.15, 45.44, 61.42, 62.65, 64.17, 67.07, 69.75, 69.80, 70.14, 71.37, 71.74, 71.76, 72.35, 73.39, 73.58, 74.10, 74.44, 74.60, 75.74, 75.18, 75.94, 79.55, 81.94, 101.41 ($^1J_{C,H}$ 155.7), 102.57 ($^1J_{C,H}$ 157.2 Hz), 127.49, 127.52, 127.58, 127.66, 127.76, 127.85, 128.00, 128.16, 128.29, 128.36, 128.41, 128.42, 128.56, 128.71, 128.75, 129.98, 130.06, 133.42, 137.80, 138.48, 138.74, 138.99, 139.11, 166.66 (CO). HRESIMS: $[M+Na]^+$ calcd for $C_{91}H_{130}O_{15}Na$, 1485.9301; found (positive-ion mode): 1485.9301. Anal. Calcd for $C_{91}H_{130}O_{15}$: C, 74.66; H, 8.95. Found: C, 74.46; H, 8.74.

3.10. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside (13)

In a similar way as described for the preparation of **10**, compound **12** (310 mg, 0.21 mmol) was treated with 4-(dimethylamino)pyridine (DMAP, 13.8 mg, 0.11 mmol) and benzoyl chloride (0.08 mL, 0.69 mmol) in dry pyridine (5 mL) at −25 °C. Usual workup and flash chromatographic purification (8:1 and then 5:1 hexane–EtOAc) afforded compound **13** (310 mg, 94%) as a syrup. R_f 0.72 (5:3 hexane–EtOAc); $[\alpha]_D^{22}$ −58.7 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.23–1.30 (m, 52H, $26CH_2$), 1.46 (m, 4H, $2CH_2$), 3.20–3.23 (m, 1H), 3.24–3.46 (m, 12H), 3.59 (dd, 1H, *J* 9.5, 4.0 Hz, H-3), 3.65 (m, 1H), 3.80 (d, 1H, *J* 4.0 Hz, H-2), 3.92 (d, 1H, *J* 4.0 Hz, H-2), 4.00 (m, 2H), 4.24 (dd, 1H, *J* 9.5, 9.5 Hz), 4.33 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.36 (s, 1H, H-1), 4.37 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.44 (m, 3H, 3H-6), 4.51 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.58 (d, 1H, *J* 12.0 Hz, PhC*H*H),

4.59 (s, 1H, H-1), 4.60 (m, 1H, H-6), 4.61 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.64 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.68 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.75 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.84 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.86 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.19–7.39 (m, 29H, Ar-H), 7.44 (t, 1H, *J* 7.5 Hz, Ar-H), 7.51 (t, 1H, *J* 7.5 Hz, Ar-H), 7.95 (d, 2H, *J* 7.5 Hz, Ar-H) 8.04 (d, 2H, *J* 7.5 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.35, 22.92, 26.41, 29.59, 29.75, 29.85, 29.88, 29.91, 29.93, 32.15, 45.41, 61.64, 63.99, 64.22, 66.38, 69.84, 69.87, 70.14, 71.45, 71.79, 71.81, 72.01, 73.45, 73.57, 73.89, 74.49, 74.54, 74.65, 74.79, 75.64, 79.27, 81.67, 101.54 ($^1J_{C,H}$ 156.4 Hz, C-1), 102.40 ($^1J_{C,H}$ 158.2 Hz, C-1), 127.47, 127.48, 127.62, 127.75, 127.94, 128.05, 128.07, 128.17, 128.29, 128.31, 128.40, 128.46, 128.52, 128.61, 128.75, 133.12, 133.24, 137.75, 138.60, 138.99, 139.05, 139.10, 166.57 (CO), 166.92 (CO). Anal. Calcd for $C_{98}H_{134}O_{16}$ (1568.13): C, 75.06; H, 8.64. Found: C, 74.94; H, 8.86.

3.11. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside (14)

Using the same glycosylation protocol as described for the preparation of **8α/8β**, the 1-thio donor **7**[29] (70.3 mg, 0.13 mmol) was activated by BSP (29.3 mg, 0.14 mmol) and Tf_2O (0.08 mL, 0.45 mmol) in the presence of DTBMP (55.4 mg, 0.27 mmol) at −60 °C for 5 min, followed by addition of the glycosylation acceptor **13** (110 mg, 0.07 mmol) at −78 °C. The crude product was purified by repeated flash chromatography (5:1 hexane–EtOAc; 7:1 hexane–EtOAc; and 5:0.8:0.5 hexane–EtOAc–CH_2Cl_2) to give **14** (71.7 mg, 51%) along with unreacted **13** (11 mg, 10%). The yield of **14** was 61% based on the amount of **13** that reacted. R_f 0.26 (5:0.7:0.5 hexane–EtOAc–CH_2Cl_2); $[\alpha]_D^{22}$ −23.6 (*c* 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.24 (m, 52H, $26CH_2$), 1.46 (m, 4H, $2CH_2$), 2.97 (ddd, 1H, *J* 9.5, 9.5, 5.5 Hz, H-5^{III}), 3.26–3.45 (m, 14H), 3.54–3.61 (m, 4H,), 3.78 (d, 1H, *J* 3.0 Hz, H-2), 3.86 (d, 1H, *J* 3.0 Hz, H-2), 3.89 (d, 1H, *J* 3.0 Hz, H-2), 3.89 (dd, 1H, *J* 9.5, 9.5 Hz), 3.97 (d, 1H, *J* 10.0 Hz), 4.06 (dd, 1H, *J* 9.5, 9.5 Hz), 4.16 (m, 2H), 4.30 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.33 (m, 2H), 4.36 (s, 1H, H-1), 4.36 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.44 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.47 (s, 1H, H-1), 4.50 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.54 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.55 (m, 1H), 4.56 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.63 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.64 (m, 1H), 4.65 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.69 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.77 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.78 (s, 1H, H-1), 4.79 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.82 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.87 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.48 (s, 1H, C*H*Ph), 7.04–7.43 (m, 39 H, Ar-H), 7.47 (t, 1H, *J* 7.5 Hz, Ar-H), 7.51 (t, 1H, *J* 7.5 Hz, Ar-H), 7.94 (d, 2H, *J* 7.5 Hz, Ar-H), 8.04 (d, 2H, *J* 7.5 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.38, 22.93, 26.42, 29.62, 29.78, 29.88, 29.91, 29.94, 29.96, 32.15, 45.41, 63.68, 64.13, 67.57, 68.66, 69.85, 70.18, 71.80, 71.81, 72.10, 72.15, 72.46, 73.45, 73.48, 73.55, 73.55, 73.95, 74.57, 74.81, 75.36, 75.53, 75.89, 75.99, 77.20, 78.44, 78.56, 80.15, 101.51 ($^1J_{C,H}$ 153.1 Hz), 101.57 ($^1J_{C,H}$ 151.6 Hz), 102.11 ($^1J_{C,H}$ 152.3 Hz), 102.49 ($^1J_{C,H}$ 156.5 Hz), 126.3, 127.42, 127.44, 127.47, 127.50, 127.66, 127.68, 127.71, 127.85, 128.06, 128.26, 128.30, 128.33, 128.41, 128.48, 128.51, 128.63, 129.89, 130.00, 133.26, 133.29, 137.81, 138.56, 138.65, 138.78, 138.79, 139.11, 139.12, 139.19, 166.43 (CO), 166.52 (CO). HRESIMS: $[M+Na]^+$ calcd for $C_{125}H_{160}O_{21}Na$, 2020.1344; found (positive-ion mode): 2020.1345. Anal. Calcd for $C_{125}H_{160}O_{21}$ (1998.63): C, 75.12; H, 8.07. Found: C, 75.38; H, 8.22.

3.12. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-benzyl-β-D-mannopyranoside (15)

Compound **12** (370 mg, 0.253 mmol) was dissolved in 2:1 MeOH–CH_2Cl_2 (30 mL), and NaOMe in MeOH was added until pH 10. The mixture was kept to stir for 17 h and then neutralized with weakly acidic resin (IRC-50, H^+ form). The crude product was purified by flash chromatography (1:1 and then 1:2 hexane–EtOAc) to provide **15** (319 mg, 92%) as a white power. R_f 0.21 (1:1 hexane–EtOAc); $[\alpha]_D^{22}$ −42.0 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.51 (m, 4H, $2CH_2$), 2.24 (br s, 3H, 3OH), 3.19 (m, 1H), 3.29–3.51 (m, 13H), 3.69–3.74 (m, 2H), 3.81–3.88 (m, 2H), 3.91–3.96 (m, 2H), 4.11 (dd, 1H, *J* 9.5, 9.5 Hz), 4.15 (dd, 1H, *J* 9.5, 9.5 Hz), 4.37 (s, 1H, H-1), 4.38 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.43 (d, 1H, *J* 12.0 Hz), 4.49 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.50 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.56 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.59 (s, 1H, H-1), 4.67 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.73 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.78 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.85 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.88 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.24–7.34 (m, 25H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.37, 22.93, 26.47, 29.61, 29.77, 29.87, 29.90, 29.93, 29.95, 32.16, 45.55, 62.09, 63.01, 67.56, 69.68, 69.72, 71.54, 71.89, 72.43, 73.02, 73.52, 74.20, 74.49, 74.52, 74.83, 75.37, 75.71, 75.76, 76.64, 80.29, 82.20, 101.60 (C-1), 102.56 (C-1), 127.21, 127.43, 127.52, 127.61, 127.63, 127.72, 127.73, 127.78, 127.80, 127.92, 128.19, 128.23, 128.36, 128.38, 128.46, 128.47, 128.54, 128.78, 137.88, 138.68, 138.75, 139.06. Anal. Calcd for $C_{84}H_{126}O_{14}\cdot H_2O$ (1359.92): C, 73.70; H, 9.34. Found: C, 73.65; H, 9.16.

3.13. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosyluronate-(1→4)-benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosiduronate (17)

3.13.1. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-β-D-mannopyranosyluronic acid-(1→4)-2,3-di-*O*-benzyl-β-D-mannopyranuronic acid (16). To a flask charged with **15** (300.0 mg, 0.22 mmol), 2,2,6,6-tetramethylpiperidinyloxy free radical (TEMPO) (21.0 mg, 0.132 mmol) and [bis(acetoxy)iodo]benzene (BAIB) (425.0 mg, 1.32 mmol) was added 2:1 CH_2Cl_2–water (6 mL), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with 10% aq $Na_2S_2O_3$ (20 mL), and the mixture was extracted with CH_2Cl_2 (20 mL × 3). The organic layer was washed with water, dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by flash column chromatography (3:1:0.1:0.2 hexane–EtOAc–MeOH –HOAc) to give **16** (291 mg, 95%). R_f 0.32 (3:1:0.1:0.2 hexane–EtOAc–MeOH –HOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 6.5 Hz, $2CH_3$), 1.25 (m, 52H, $26CH_2$), 1.48 (m, 4H, $2CH_2$), 3.31–3.47 (m, 12H), 3.57 (d, 1H, *J* 9.5 Hz, H-5), 3.71 (d, 1H, *J* 9.5 Hz, H-5), 3.79 (br s, 1H, H-2), 3.91–3.96 (m, 2H, H-3^{I}, H-3^{II}), 3.97 (d, 1H, *J* 3.0 Hz, H-2), 4.19 (dd, 1H, *J* 9.5, 9.5 Hz, H-4), 4.34 (dd, 1H, *J* 9.5, 9.5 Hz, H-4), 4.41 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.48 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.48 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.49 (s, 1H, H-1), 4.53 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.58 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.64 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.66 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.73 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.77 (s, 1H, H-1), 4.78 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.80 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.12–7.38 (m, 25H, Ar-H), 10.20 (s, 2H, 2COOH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.32, 22.89, 26.40, 29.65, 29.73, 29.83, 29.85, 29.89, 29.91, 32.12, 45.67, 68.53, 69.11, 69.25, 70.35, 71.94, 71.97, 72.54, 72.79, 73.21, 73.53, 74.11, 74.29, 74.56, 74.98, 75.06, 76.21, 77.93, 80.62, 100.97 ($^1J_{C,H}$ 153.4 Hz, C-1), 101.99 ($^1J_{C,H}$ 156.7 Hz, C-1), 127.61, 127.64, 127.68, 127.82, 128.00, 128.03, 128.23, 128.33, 128.45, 128.53, 128.55, 128.57, 137.46, 138.07, 138.25, 138.71, 138.79, 172.02 (CO), 176.93 (CO).

3.13.2. Benzyl esterification of 16. To a solution of **16** (266 mg, 0.19 mmol) and potassium fluoride (220 mg, 3.80 mmol) in dry DMF (5 mL) was added $PhCH_2Br$ (0.23 mL, 1.90 mmol). The mixture was kept to stir for 16 h until TLC indicated complete reaction. The DMF was removed under high vacuum, and water (25 mL) was added. The mixture was extracted with CH_2Cl_2 (25 mL × 3), and the organic layer was dried over Na_2SO_4. The residue was purified by flash chromatography (5:1 and then 7:2 hexane–EtOAc) to give compound **17** (234 mg, 78%) as a syrup. R_f 0.69 (2:1:0.05:0.05 hexane–EtOAc–MeOH–HOAc); $[\alpha]_D^{22}$ −76.5 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 7.0 Hz, $2CH_3$), 1.25 (br s, 52H, $26CH_2$), 1.48 (m, 4H, $2CH_2$), 2.77 (d, 1H, *J* 2.5 Hz, OH), 3.10 (dd, 1H, *J* 10.0, 3.0 Hz, H-3), 2.28–3.49 (m, 13H), 3.64 (d, 1H, *J* 3.0 Hz, H-2), 3.65 (d, 1H, *J* 3.0 Hz, H-2), 3.81 (d, 1H, *J* 9.5 Hz, H-5), 4.00 (d, 1H, *J* 9.5 Hz, H-5), 4.18 (ddd, 1H, *J* 10.0, 9.5, 2.5 Hz, H-4^{II}), 4.32 (s, 1H, H-1), 4.40 (m, 3H), 4.45 (m, 2H), 4.46 (s, 1H, H-1), 4.47 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.49 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.64 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.68 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.71 (d, 1H, *J* 12.0 Hz, PhC*H*H), 4.85 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.00 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.01 (d, 1H, *J* 12.0 Hz, PhC*H*H), 5.10 (d, 1H, *J* 12.0 Hz), 5.20 (d, 1H, *J* 12.0 Hz, PhC*H*H), 7.25 (m, 35H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 26.2, 29.3, 29.5, 29.6, 29.7, 31.9, 45.2, 66.9, 67.1, 68.0, 69.6, 70.5, 71.6, 72.3, 73.2, 73.7, 74.2, 74.6, 74.8, 75.0, 75.1, 79.4, 80.1, 102.1 (C-1), 102.9 (C-1), 127.2, 127.3, 127.4, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2, 128.27, 128.3, 128.4, 128.5, 128.6, 135.1, 135.3, 138.0, 138.6, 138.7, 138.9, 168.1 (CO), 169.1 (CO). HRESIMS: $[M+Na]^+$ calcd for $C_{98}H_{134}O_{16}Na$, 1589.9564; found (positive-ion mode): 1589.9565. Anal. Calcd for $C_{98}H_{134}O_{16}$: C, 75.06; H, 8.61. Found: C, 74.79; H, 8.67.

3.14. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-benzyl-β-D-mannopyranoside (18)

To a solution of **14** (220 mg, 0.11 mmol) and neopentyl glycol (75 mg, 0.72 mmol) in anhyd CH_2Cl_2 (10 mL) was added camphorsulfonic acid (37.5 mg, 0.16 mmol). The mixture was stirred at 35 °C under an N_2 atmosphere for 16 h and then washed with satd aq $NaHCO_3$ (10 mL). The aqueous layer was further extracted with CH_2Cl_2 (10 mL × 2), and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuum. The residue was dried under high vacuum to give the diol intermediate (400 mg) that was then treated with a catalytic amount of NaOMe in 2:1 MeOH–CH_2Cl_2 (30 mL, pH 10.0) for 17 h. The reaction mixture was neutralized with a weakly acidic resin (IRC-50, H^+ form) and the crude product was purified by flash chromatography (1:1:0.5 hexane–EtOAc–CH_2Cl_2) to afford **18** (155 mg, 83%, two steps) as a white powder. R_f 0.38 (1:2:0.5 hexane–EtOAc–CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 7.0 Hz, $2CH_3$), 1.25 (br s, 52H, $26CH_2$), 1.51 (m, 4H, $2CH_2$), 2.30 (br s, 4H, 4OH), 3.19 (m, 2H), 3.24 (m, 1H), 3.28 (dd, 1H, *J* 9.5, 3.0 Hz, H-3), 3.34–3.50 (m, 14H), 3.62–3.66 (m, 2H), 3.70–3.72 (m, 2H), 3.80–3.84 (m, 3H), 3.86–3.88 (m, 2H), 3.96 (d, 1H, *J* 9.5 Hz), 4.08 (dd, 1H, *J* 9.0, 9.0 Hz, H-4), 4.13 (d, 1H, *J* 9.0, 9.0 Hz, H-4), 4.34 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.37 (s, 1H, H-1), 4.44 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.49

(d, 1H, *J* 12.5 Hz, PhC*H*H), 4.50 (s, 1H, H-1), 4.50 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.54 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.55 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.59 (s, 1H, H-1), 4.73 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.74 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.75 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.79 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.80 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.83 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.87 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.89 (d, 1H, *J* 12.5 Hz, PhC*H*H), 7.24–7.40 (m, 35H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.38, 22.93, 26.42, 29.62, 29.78, 29.88, 29.91, 29.95, 29.96, 32.15, 45.41, 62.11, 62.23, 63.03, 67.56, 69.69, 69.73, 70.21, 71.55, 71.91, 72.45, 73.03, 73.54, 74.22, 74.50, 74.55, 74.84, 75.39, 75.39, 75.70, 75.73, 75.73, 75.78, 76.65, 80.13, 80.30, 82.22, 101.20 (C-1), 101.61 (C-1), 102.58 (C-1), 127.23, 127.45, 127.53, 127.63, 127.65, 127.73, 127.75, 127.79, 127.81, 127.94, 128.20, 128.25, 128.37, 128.40, 128.47, 128.49, 128.55, 128.56, 128.80, 137.88, 138.72, 138.78, 138.82, 138.85, 138.90, 139.06. Anal. Calcd for $C_{104}H_{148}O_{19}\cdot 0.5H_2O$ (1702.31): C, 72.99; H, 8.77. Found: C, 72.88; H, 8.51.

3.15. 3-Benzyloxy-2,2-dihexadecyloxymethylpropyl benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosyluronate-(1→4)-benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosyluronate-(1→4)-benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosiduronate (20)

3.15.1. Oxidation of 18. Compound **18** (155 mg, 0.091 mmol), TEMPO (11.5 mg, 0.074 mmol), and BAIB (232 mg, 0.72 mmol) were dissolved in 2:1 CH_2Cl_2–MeOH (6 mL), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (40 mL) and washed successively with 10% aq $Na_2S_2O_3$ (20 mL) and ice-water (10 mL). The organic layer was dried through Na_2SO_4 and concentrated in vacuum. The residue was purified by flash chromatography (6:1:0.1:0.2 hexane–EtOAc–MeOH–HOAc) to give tricarboxylic acid **19** (111 mg, 70%). R_f 0.28 (3:1:0.1:0.2 hexane–EtOAc–MeOH–HOAc).

3.15.2. Benzyl esterification of 19. To a solution of **19** (101 mg, 0.058 mmol) in dry DMF (8 mL) were added potassium fluoride (158 mg, 2.7 mmol) and $PhCH_2Br$ (0.19 mL, 1.6 mmol). The mixture was kept to stir at room temperature for 16 h and worked up in a similar way as described for the preparation of compound **17**. Chromatographic purification (7:1 hexane–EtOAc) afforded **20** (93 mg, 80%) as a colorless syrup. R_f 0.70 (3:1:0.1:0.2 hexane–EtOAc–MeOH–HOAc); $[\alpha]_D^{22}$ −39.6 (*c* 0.66, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* 7.0 Hz, $2CH_3$), 1.25 (br s, 52H, $26CH_2$), 1.48 (m, 4H, $2CH_2$), 2.74 (d, 1H, *J* 2.5 Hz, OH), 3.03 (dd, 1H, *J* 9.0, 2.5 Hz, H-3), 3.22 (dd, 1H, *J* 9.0, 2.5 Hz, H-3), 3.28–3.48 (m, 13H), 3.62 (d, 1H, *J* 9.5 Hz, H-5), 3.62 (d, 1H, *J* 3.0 Hz, H-2), 3.68 (d, 2H, *J* 3.0 Hz, 2H-2), 3.75 (d, 1H, *J* 9.5 Hz, H-5), 4.00 (d, 1H, *J* 9.5 Hz, H-5), 4.13 (ddd, 1H, *J* 9.5, 9.5, 2.5 Hz, H-4^{III}), 4.26 (dd, 1H, *J* 9.5, 9.5 Hz, H-4), 4.28 (s, 1H, H-1), 4.31 (s, 1H, H-1), 4.34 (dd, 1H, *J* 9.5, 9.5 Hz, H-4), 4.38 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.39 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.40 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.41 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.42 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.45 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.46 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.57 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.65 (s, 1H, H-1), 4.67 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.69 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.71 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.72 (dd, 1H, *J* 12.5 Hz, PhC*H*H), 4.75 (dd, 1H, *J* 12.5 Hz, PhC*H*H), 4.79 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.85 (d, 1H, *J* 12.5 Hz, PhC*H*H), 4.92 (dd, 1H, *J* 12.5 Hz, PhC*H*H), 4.98 (d, 1H, *J* 12.5 Hz, PhC*H*H), 5.03 (dd, 1H, *J* 12.5 Hz, PhC*H*H), 5.07 (d, 1H, *J* 12.5 Hz, PhC*H*H), 5.16 (d, 1H, *J* 12.5 Hz, PhC*H*H), 7.12–7.35 (m, 50H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.39, 22.94, 26.47, 29.62, 29.80, 29.89, 29.93, 29.95, 29.97, 32.18, 45.46, 66.97, 67.06, 67.14, 68.12, 69.68, 69.72, 69.75, 70.66, 71.71, 71.72, 71.51, 72.51, 72.72, 73.36, 73.84, 74.62, 74.69, 74.93, 74.96, 75.08, 75.17, 76.11, 77.25, 77.29, 77.36, 79.50, 79.75, 80.24, 102.25 ($^1J_{C,H}$ 157.7, C-1), 102.45 ($^1J_{C,H}$ 155.6, C-1), 103.21 ($^1J_{C,H}$ 155.3 Hz, C-1), 127.49, 127.53, 127.56, 127.64, 127.82, 127.96, 128.02, 128.19, 128.29, 128.34, 128.40, 128.53, 128.64, 128.66, 128.70, 128.74, 128.78, 128.83, 128.86, 135.39, 135.48, 135.49, 138.26, 138.81, 139.10, 139.15, 139.20, 139.21, 168.17 (CO), 168.22 (CO), 169.28 (CO). Anal. Calcd for $C_{125}H_{160}O_{22}\cdot 0.5H_2O$ (2014.63): C, 74.19; H, 8.01. Found: C, 74.12; H, 7.89.

3.16. 3-Hydroxy-2,2-dihexadecyloxymethylpropyl β-D-mannopyranosyluronic acid-(1→4)-β-D-mannopyranosiduronic acid (1)

Compound **17** (14 mg, 8.92 μmol) was dissolved in freshly distilled THF (16 mL), and palladium-on-charcoal (20 mg) was added. The mixture was stirred under an H_2 atmosphere for 2 h, and then water (4 mL) was added. The mixture was stirred under an H_2 atmosphere for an additional 16 h. The solid was then filtered off and washed with 1:1 THF–MeOH (20 mL). The filtrate was concentrated in vacuum to give **1** (7.5 mg, 90%) as white powder. R_f 0.46 (4:2:0.4:0.4 $CHCl_3$–CH_3OH–H_2O–HOAc); $[\alpha]_D^{22}$ −116.0 (*c* 0.05, 2:1 MeOH–$CHCl_3$); MALDITOF MS: calcd for $C_{49}H_{92}O_{16}$, 936.63 $[M]^+$; found, 959.63 $[M+Na]^+$, 981.62 $[M-H+2Na]^+$.

3.17. 3-Hydroxy-2,2-dihexadecyloxymethylpropyl β-D-mannopyranosyluronic acid-(1→4)-β-D-mannopyranosyluronic acid-(1→4)-β-D-mannopyranosiduronic acid (2)

Compound **20** (14 mg, 6.95 μmol) was dissolved in THF (32 mL) and treated with palladium on charcoal (48 mg)

under an H_2 atmosphere for 2 h. Then water (6 mL) was added, and the mixture was stirred for an additional 24 h under an H_2 atmosphere. The catalyst was filtered off and washed with THF–MeOH (15 mL). The filtrate was passed through a short filtration silica gel column and concentrated in vacuum. The residue was freeze-dried from 1,4-dioxane to give **2** (7.0 mg, 90%) as a white powder. R_f 0.38 (7:2:0.1 2-PrOH–water–NH_4OH); $[\alpha]_D^{22}$ +47.3 (*c* 0.08, 2:1 MeOH–$CHCl_3$). MALDITOF MS: calcd for $C_{55}H_{100}O_{22}$, 1112.67 $[M]^+$; found, 1136.05 $[M+Na]^+$, 1158.06 $[M-H+2Na]^+$.

Acknowledgments

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC 312630) and Lakehead University. R.X. is grateful to the Ontario Ministry of Training, Colleges and Universities for the Ontario Graduate Scholarship (OGS) during the course of his graduate studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.10.007.

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