Synthetic Studies on Glycosphingolipids from Protostomia Phyla: Synthesis of Neogala-Series Glycolipid Analogues Containing a Mannose Residue from the Earthworm *Pheretima hilgendorfi*

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Two kinds of glycosphingolipid analogues from the earthworm *Pheretima hilgendorfi* were synthesized as follows: the trisaccharide 2-(tetradecyl)hexadecyl α-D-mannopyranosyl-(1→4)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (13) and the tetrasaccharide 2-(tetradecyl) hexadecyl α-D-galactopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→4)]-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (20) were synthesized by stepwise condensation of suitably protected monosaccharide units. A 2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside derivative (5) was used as the glycosyl acceptor and thiophenyl derivatives of D-galactose and D-mannose were used as donors respectively.

Key words glycosphingolipids; *Pheretima hilgendorfi*; chemical synthesis; stepwise condensation

Glycolipids are known to be involved in a variety of functions, including extracellular recognition, cell–cell interaction, differentiation, oncogenesis and immunity.[1)] Especially sialic acid-containing glycosphingolipids, such as gangliosides in mammalian membranes have been recognized to play important roles as above. Therefore a number of sialyloligosaccharides and their mimetics have been synthesized by many carbohydrate chemists to obtain a systematic understanding of structure–function relationships of sialyloligosaccharides at the molecular level.[2)] On the other hand, synthetic studies on oligosaccharides from invertebrate animal species that do not have gangliosides have been neglected, in spite of their having interesting structures.[3)]

We have been interested in the relationship between the structure and biological function of glycolipids from invertebrate animal species and have so far synthesized oligosaccharides from various protostomia phyla.[4)] In our previous paper we reported[5)] the synthesis of four glycosphingolipids analogues from *Echinococcus multilocularis*, in the neogala series whose structures have a β-D-Gal*p*-(1→6)-β-D-Gal*p*-core, and a fucose residue,[6)] suggesting the functional importance of glycolipids in parasitism. Recently, Sugita *et al*. found and characterized a new neogala series of glycosphingolipids containing a mannose, glucose and cholinephosphoryl residue from the earthworm *Pheretima* (*P.*) *hilgendorfi*.[7)] In the present study, we attempted the synthesis of the new neogala series of glycosphingolipids containing the mannose residue.

The disaccharide derivative **6** was obtained by condensation of 2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**5**)[5)] with phenyl 4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**2**), which was benzylidenated and benzoylated from thiophenyl β-D-galactopyranoside (**1**) (Chart 1), in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH).[8)] It is clearly seen in the ^{1}H-NMR spectrum of **6** that the newly introduced anomeric proton signal showed a doublet at δ 4.77 with a coupling constant of 7.9 Hz. Reductive ring-opening of the benzylidene acetal in **6** with sodium cyanoborohydride–hydrogen chloride in dry diethylether afforded compound **7**. Compound **7** was condensed with phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (**4**), which was benzoylated and thioglycosylated using tin(IV) chloride from D-mannose, in the presence of NIS and TfOH, to give the trisaccharide derivative **8** in 76% yield. In the ^{13}C-NMR the $J_{C,H}$ value of 171.3 Hz supported the α configuration of the newly formed glycosidic bond.[9)] **8** was converted by *O*-debenzylation to **9** and subsequent acetylation gave the per-*O*-acylated trisaccharide **10**. On the other hand, the trisaccharide alcohol **9** was glycosylated by the galactosyl donor **14**[10)] with NIS and AgOTf at −78 °C. After work-up, the protected tetrasaccharide was obtained as a mixture of anomers **15** (67%) and **16** (23%). The anomeric hydrogen atom of the α-D-galactose unit of compound **15** in the ^{1}H-NMR spectrum showed a signal at δ 5.18 (d, J=3.1 Hz). The α-D configuration of the newly formed glycosidic bond was also indicated by the $J_{C,H}$ value of 171.7 Hz in the ^{13}C-NMR spectrum. Removal of the benzyl groups from **15** by catalytic hydrogenolysis over 10% Pd–C, and subsequent acetylation gave the per-*O*-acylated tetrasaccharide **17**.

Next, for the selective removal of the 2-(trimethylsilyl)-ethyl group, the fully acylated oligosaccharides **10** and **17** were treated[11)] with trifluoroacetic acid in dichloromethane for 1—2 h at 0 °C to give the 1-hydroxy compounds, which, on further treatment[12)] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in di-

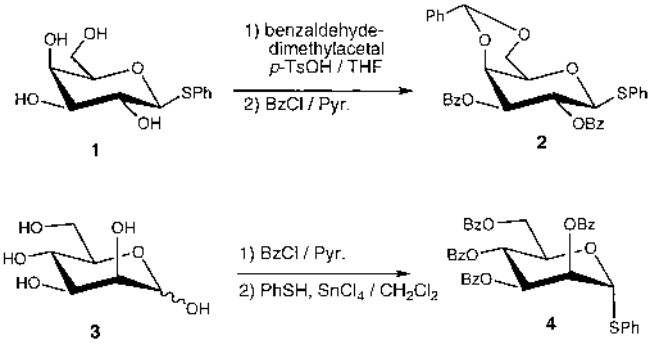

Chart 1

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chloromethane for 2 h at 0 °C, gave the corresponding receptor carbohydrates **11** and **18**. Glycosylation[13] of 2-(tetradecyl)hexadecanol[14] with each of the glycosyl donors, which was carried out in the presense of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and molecular sieves (MS)4A for 2 h at 0 °C, afforded the desired *β*-glycosides **12** (62%) and **19** (32%), respectively. Finally, removal of all acyl groups with sodium methoxide in 1 : 1 methanol/1,4-dioxane for 5 h at room temperature afforded the desired two glycolipid analogues **13** and **20**. (Chart 2, Chart 3)

In summary, the systematic approach to the mannose containing neogala series of glycosphingolipid analogues **13** and **20** and the synthesis of other oligosaccharides from *P. hilgendorfi* was successfully carried out.

Experimental

Optical rotations were determined with a JASCO digital polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with JNM A 500 FT NMR spectrometer with Me_4Si as the internal standard for solutions in $CDCl_3$. MALDI-TOFMS was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on Silica gel 60 F_{254} (E. Merck) with detection by quenching of UV fluorescence and by spraying with 10% H_2SO_4. Column chromatography was carried out on Silica gel 60 (E. Merck). 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-*β*-D-galactopyranoside (**5**), phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-*β*-D-galactopyranoside (**14**) were prepared by literature methods.[5,10]

Phenyl 4,6-*O*-Benzylidene-2,3-di-*O*-benzoyl-1-thio-*β*-D-galactopyranoside (2) To a solution of phenyl 1-thio-*β*-D-galactopyranoside (**1**) (3.0 g, 11.02 mmol) in tetrahydrofuran (THF 50 ml) were added benzaldehyde dimethylacetal (2 ml) and *p*-TsOH (170 mg) at 0 °C. The reaction mixture was stirred at room temperature for 12 h, then neutralized with Et_3N. After evaporation the residues were diluted with $CHCl_3$, washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 30 : 1 $CHCl_3$–MeOH as eluent to give phenyl 4,6-*O*-benzylidene-1-thio-*β*-D-galactopyranoside (3.86 g, 96.4%). To a solution of this compound (3.86 g, 10.71 mmol) in pyridine (30 ml) was added benzoyl chloride (5 ml), and the mixture was stirred for 5 h at 0 °C. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 4 : 1 hexane–ethyl acetate as eluent to give **2** (5.60 g, 92.1%), $[\alpha]_D^{24}$ = +60.6° (*c* = 1.7, $CHCl_3$). ^{1}H-NMR *δ*: 7.98—7.23 (20H, m, 4×Ph), 5.81(1H, t, $J_{1,2}$ = $J_{2,3}$ = 10.4 Hz, H-2), 5.51 (1H, s, benzylidene methine), 5.36 (1H, dd, $J_{3,4}$ = 3.1 Hz, H-3), 4.96 (1H, d, H-1), 4.59 (1H, d, H-4), 4.44 (1H, dd, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.8 Hz, H-6a), 4.08 (1H, dd, $J_{5,6b}$ = 1.8 Hz, H-6b), 3.75 (1H, br s, H-5). MALDI-TOFMS: Calcd for $C_{33}H_{28}O_7S$ *m/z*: 568.2. Found *m/z*: 591.8 $(M+Na)^+$.

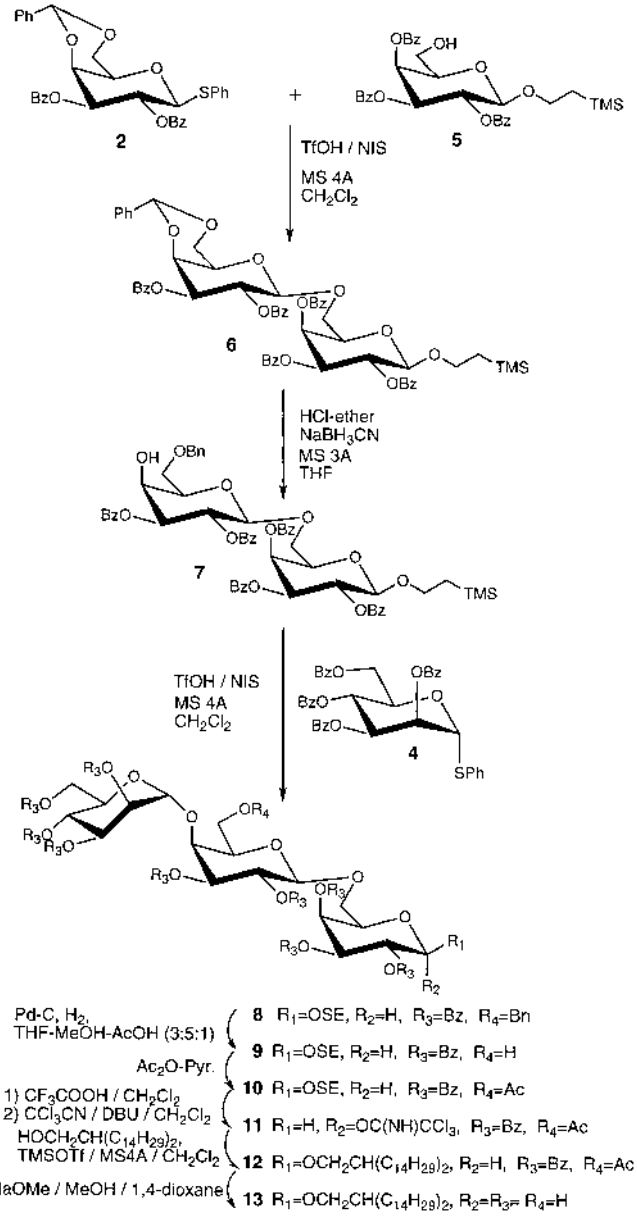

Chart 2

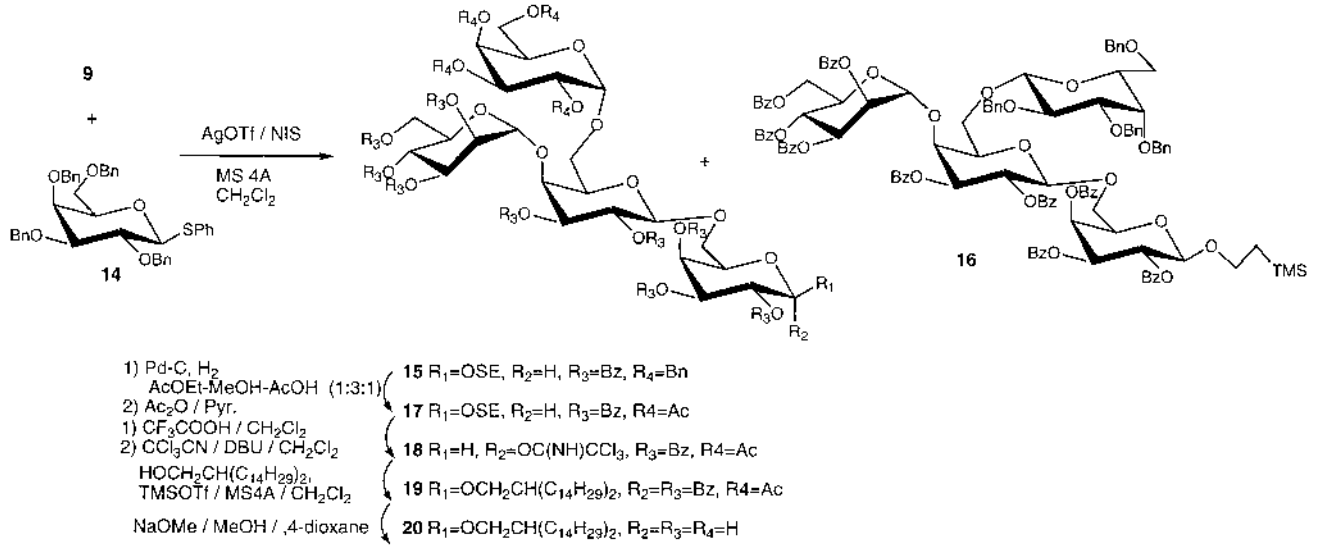

Chart 3

Phenyl 2,3,4,6-Tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (4) To a solution of D-mannose (3 g, 16.65 mmol) in pyridine (100 ml) was added benzoyl chloride (20 ml), and the mixture was stirred for 4 h at 0 °C. MeOH (2 ml) was added to the mixture, and this was concentrated, then extracted with CH_2Cl_2. The extract was successively washed with 5% HCl, aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to give 1,2,3,4,6-penta-*O*-benzoyl-β-D-mannopyranose quantitatively. To a solution of this compound (1.5 g, 2.14 mmol) in CH_2Cl_2 (7 ml) cooled to 0 °C, were added thiophenol (0.44 ml, 4.28 mmol) and tin(IV) chloride (250 μl, 1.71 mmol), and the mixture was stirred for 3 h at room temperature. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 6 : 1 hexane–ethyl acetate as eluent to give **4** (980 mg, 66.5%), $[\alpha]_D^{24}=+20.0°$ (c=2.9, $CHCl_3$). ^{1}H-NMR δ: 8.10—7.16 (25H, m, 5×Ph), 6.11 (1H, t, $J_{3,4}=J_{4,5}$=10.0 Hz, H-4), 5.98 (1H, dd, $J_{1,2}$=1.5 Hz, $J_{2,3}$=3.5 Hz, H-2), 5.87 (1H, dd, H-3), 5.78 (1H, d, H-1), 5.01 (1H, ddd, $J_{5,6a}$=2.5 Hz, $J_{5,6b}$=5.5 Hz, H-5), 4.66 (1H, dd, $J_{6a,6b}$=12.2 Hz, H-6a), 4.56 (1H, dd, H-6b). MALDI-TOFMS: Calcd for $C_{40}H_{32}O_9S$ *m/z*: 688.3. Found *m/z*: 711.6 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 4,6-*O*-Benzylidene-2,3-di-*O*-benzoyl-β-D-galacto-pyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (6) To a solution of **2** (1.0 g, 1.76 mmol) and compound **5** (871 mg, 1.47 mmol) in dry CH_2Cl_2 (5 ml) was added powdered 4A MS (3 g), and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. NIS (594 mg, 2.64 mmol) and TfOH (31.4 μl, 0.35 mmol) were added to the mixture, which was stirred for 20 min at 0 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. The product was chromatographed on silica gel using 3 : 2 hexane–ethyl acetate as eluent to give **6** (1.38 g, 89.3%), $[\alpha]_D^{24}=+144.1°$ (c=1.0 $CHCl_3$), ^{1}H-NMR δ: 8.05—7.12 (30H, m, 6×Ph), 5.97 (1H, d, $J_{3,4}$= 3.7 Hz, H-4), 5.90 (1H, dd, $J_{1',2'}$=7.9 Hz, $J_{2',3'}$=10.4 Hz, H-2′), 5.70 (1H, dd, $J_{1,2}$=8.6 Hz, $J_{2,3}$=10.4 Hz, H-2), 5.50 (1H, s, benzylidene methine) 5.48 (1H, dd, H-3), 5.34 (1H, dd, $J_{3',4'}$=3.7 Hz, H-3′), 4.83 (1H, d, H-1′), 4.65 (1H, d, H-1), 4.58 (1H, d, H-4′), 4.46 (1H, dd, H-6′a), 4.22 (1H, br t, H-5), 4.12 (1H, dd, H-6′b), 4.04 (1H, dd, H-6a), 3.98 (1H, dd, H-6b), 3.89 (1H, dt, –C<u>H</u>$_2$CH$_2$–Si), 3.70 (1H, s, H-5′), 3.42 (1H, dt, –C<u>H</u>$_2$CH$_2$–), 0.80—0.65 (2H, m, –CH$_2$C<u>H</u>$_2$–Si), −0.12 (9H, s, Si(CH$_3$)$_3$). ^{13}C-NMR ($CDCl_3$) δ: 101.4 (C-1′), 100.8 (C-1), 100.8 (benzylidene methine), 73.5 (C-5), 73.4 (C-4′), 72.7 (C-3′), 72.1 (C-3), 69.9 (C-2), 69.0 (C-4), 68.9 (C-6′), 68.7 (C-2′), 68.0 (C-6), 67.5 (–O<u>C</u>H$_2$CH$_2$–), 66.5 (C-5′), 17.7 (–OCH$_2$<u>C</u>H$_2$–), −1.4 (SiMe$_3$). MALDI-TOFMS: Calcd for $C_{59}H_{58}O_{16}Si$ *m/z*: 1050.4. Found *m/z*: 1073.5 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 6-*O*-Benzyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (7) To a solution of compound **6** (674 mg, 0.64 mmol) and sodium cyanoborohydride (402 mg, 6.41 mmol) in dry THF (20 ml) was added powdered 3A MS (3 g), and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. Hydrogen chloride in diethyl ether was added until the solution was acidic (pH paper, gas evolution). After 1 h, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 20 : 1 benzene–acetone as eluent to give **7** (534 mg, 79.1%), $[\alpha]_D^{24}=+100.0°$ (c=3.6, $CHCl_3$). ^{1}H-NMR δ: 8.07—7.20 (30H, m, 6×Ph), 5.93 (1H, d, $J_{3,4}$=3.1 Hz, H-4), 5.75 (1H, dd, $J_{1',2'}$=7.9 Hz, $J_{2',3'}$=10.4 Hz, H-2′), 5.69 (1H, dd, $J_{1,2}$=7.9 Hz, $J_{2,3}$=10.4 Hz, H-2), 5.49 (1H, dd, $J_{3,4}$=3.7 Hz, H-3), 5.23 (1H, dd, $J_{3',4'}$=3.1 Hz, H-3′), 4.72 (1H, d, H-1′), 4.69 (1H, d, H-1), 4.45 (1H, each d, J_{gem}=11.2 Hz, benzyl methylene), 4.34 (1H, s, H-4′), 4.13 (1H, m, H-5), 4.06 (1H, dd, H-6b), 3.89 (2H, m, H-6a, O-C<u>H</u>$_2$), 3.77 (1H, br t, H-5′), 3.66 (1H, dd, $J_{5',6'a}$=6.1 Hz, $J_{6'a,6'b}$=10.3 Hz, H-6′a), 3.52 (1H, dd, H-6′b), 3.46 (1H, m, O–CH$_2$–), 2.84 (1H, br s, OH). MALDI-TOFMS: Calcd for $C_{59}H_{60}O_{16}Si$ *m/z*: 1052.4. Found *m/z*: 1075.4 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (8) To a solution of **4** (392 mg, 0.57 mmol) and compound **7** (400 mg, 0.38 mmol) in dry CH_2Cl_2 (5 ml) was added powdered 4A MS (1 g) , and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. NIS (171 mg, 0.76 mmol) and TfOH (17 μl, 0.20 mmol) were added to the mixture, which was stirred for 3 h at 0 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. The product was chromatographed on silica gel using 2 : 1 hexane–ethyl acetate as eluent to give **8** (503 mg, 81.2%), $[\alpha]_D^{24}=+42.9°$ (c=3.8, $CHCl_3$). ^{1}H-NMR δ: 8.14—7.15 (50H, m, 10×Ph), 6.19 (1H, t, $J_{3'',4''}=J_{4'',5''}$=9.8 Hz, H-4″), 6.03 (1H, dd, $J_{2'',3''}$=3.7 Hz, H-3″), 5.93 (1H, d, $J_{3,4}$=3.7 Hz, H-4), 5.89 (1H, dd, $J_{1'',2''}$=1.3 Hz, H-2″), 5.87 (1H, dd, $J_{1',2'}$=7.3 Hz, $J_{2',3'}$=11.0 Hz, H-2′), 5.73 (1H, dd, $J_{1,2}$=7.9 Hz, $J_{2,3}$=9.8 Hz, H-2), 5.54 (1H, dd, H-3), 5.37 (1H, dd, $J_{3',4'}$=3.7 Hz, H-3′), 5.28 (1H, d, H-1″), 4.82 (1H, d, H-1′), 4.78 (1H, d, H-1), 4.68 (1H, br t, H-5″), 4.67 (1H, each d, J=11.6 Hz,benzyl methylene), 4.63 (1H, d, H-4′), 4.51 (1H, each d, benzyl methylene), 4.24 (1H, dd, H-5), 4.11 (1H, m, H-6a), 3.93 (3H, m, H-5′, 6b, 6′a), 3.79 (2H, m, H-6′b, 6″a), 3.72 (1H, dd, H-6″b). ^{13}C-NMR ($CDCl_3$) δ: 101.4 (C-1′), 100.7 (C-1), 99.1 (C-1 of Man), 75.1 (C-4′), 73.4 (C-5), 73.3 (C-3′), 73.3 (benzyl methylene), 72.8 (C-5′), 72.0 (C-3), 70.4 (C-2 of Man), 70.0 (C-3 of Man), 69.9 (C-2), 69.8 (C-2′), 69.2 (C-5 of Man), 69.0 (C-4), 68.4 (C-6), 67.3 (–O<u>C</u>H$_2$CH$_2$–), 66.6 (C-6′), 66.2 (C-4 of Man), 61.9 (C-6 of Man), 17.6 (–OCH$_2$<u>C</u>H$_2$–), −1.5 (SiMe$_3$). MALDI-TOFMS: Calcd for $C_{93}H_{86}O_{25}Si$ *m/z*: 1630.6. Found *m/z*: 1653.8 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (9) A solution of **8** (84.8 mg, 0.06 mmol) in MeOH (5 ml), THF (3 ml) and AcOH (1 ml) was hydrogenated over 10% Pd–C (250 mg) for 18 h at room temperature, then filtered through Celite and the residue was washed with methanol and concentrated. The product was chromatographed on silica gel using 5 : 1 benzene–acetone as eluent to give **9** (52.4 mg, 66.4%), $[\alpha]_D^{24}=+91.4°$ (c=1.3, $CHCl_3$). ^{1}H-NMR δ: 5.31 (1H, s, H-1″), 4.84 (1H, d, J=7.3 Hz, H-1′), 4.79 (1H, d, J=7.9 Hz, H-1), 2.67 (1H, br d, OH). MALDI-TOFMS: Calcd for $C_{86}H_{80}O_{25}Si$ *m/z*: 1540.7. Found *m/z*: 1563.7 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (10) Compound **9** (105 mg, 0.07 mmol) was acetylated with Ac_2O (2 ml) in pyridine (3 ml) for 5 h at room temperature. Work-up as described for **2**, the product was purified by silica gel column chromatography using 1 : 1 hexane–ethyl acetate as an eluent to give **10** (105 mg, 96.8%), $[\alpha]_D^{24}=+68.4°$ (c=2.6, $CHCl_3$). ^{1}H-NMR δ: 5.22 (1H, d, J=1.2 Hz, H-1″), 4.87 (1H, d, J=7.3 Hz, H-1′), 4.77 (1H, d, J=8.0 Hz, H-1), 2.08 (3H, s, OAc). MALDI-TOFMS: Calcd for $C_{88}H_{82}O_{26}Si$ *m/z*: 1582.6. Found *m/z*: 1605.9 $(M+Na)^+$.

***O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl Trichloroacetimidate (11)** To a solution of **10** (90 mg, 0.06 mmol) in CH_2Cl_2 (2 ml) cooled to 0 °C, was added CF_3COOH (2 ml), and the mixture was stirred for 1 h at room temperature and concentrated. Ethyl acetate and toluene (1 : 2) were added and then removed to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (1 ml) cooled at 0 °C were added trichloroacetonitrile (171 μl, 1.71 mmol) and DBU (8.6 μl, 0.06 mmol). The mixture was stirred for 2 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (20:1 benzene–acetone) of the residue on silica gel gave **11** (89 mg, 96%), $[\alpha]_D^{24}=+77.3°$ (c=2.2, $CHCl_3$). ^{1}H-NMR δ: 8.26 (1H, s, NH), 6.77 (1H, d, J=3.7 Hz, H-1), 5.20 (1H, br s, H-1″), 4.86 (1H, d, J=8.0 Hz, H-1′). MALDI-TOFMS: Calcd for $C_{85}H_{70}Cl_3NO_{26}$ *m/z*: 1626.8. Found *m/z*: 1649.7 $(M+Na)^+$.

2-(Tetradecyl)hexadecyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (12) To a solution of the trichloroacetimidate **11** (89 mg, 55 μmol) and 2-(tetradecyl)hexadecanol (36 mg, 82 μmol) in CH_2Cl_2 (1 ml) were added molecular sieves 4A (500 mg) and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. TMSOTf (10 μl, 55 μmol) was added and the mixture was stirred for 2 h at 0 °C then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 15 : 1 benzene–acetone as eluent to give **12** (64 mg, 61.7%), ^{1}H-NMR ($CDCl_3$) δ: 5.21 (1H, d, J=1.2 Hz, H-1″), 4.88 (1H, d, J=7.9 Hz, H-1′), 4.67 (1H, d, J=7.9 Hz, H-1), 3.92 (1H, dd, –OCH$_2$–), 3.33 (1H, dd, –OCH$_2$–), 1.34 (52H, br s, 2×CH$_2$), 0.96 (6H, t, 2×–CH$_2$C<u>H</u>$_3$). MALDI-TOFMS: Calcd for $C_{113}H_{130}O_{26}$ *m/z*: 1902.9. Found *m/z*: 1925.6 $(M+Na)^+$.

2-(Tetradecyl)hexadecyl α-D-Mannopyranosyl-(1→4)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (13) To a solution of **12** (39 mg, 20.5 μmol) in 1 : 1 MeOH/1,4-dioxane (2 ml) was added NaOMe (20 mg) and the mixture was stirred for 3 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed

with 1 : 1 $CHCl_3$–MeOH. The filtrate and washings were combined and concentrated. Column chromatography (1 : 1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave **13** (18 mg, 95%), $[\alpha]_D^{24}$ −12.4° (c=0.5, 1 : 1 $CHCl_3$–MeOH). ^{1}H-NMR (2 : 1 $CDCl_3$–CD_3OD) δ: 4.85 (1H, d, J=1.8 Hz, H-1 of Man), 4.34 (1H, d, J=7.3 Hz, H-1′ of Gal), 4.20 (1H, d, J=7.3 Hz, H-1 of Gal). MALDI-TOFMS: Calcd for $C_{48}H_{92}O_{16}$ *m/z*: 924.6. Found *m/z*: 947.8 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (15) and 2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (16) To a solution of **9** (187 mg, 0.12 mmol) and **14** (116 mg, 0.18 mmol) in dry CH_2Cl_2 (1.5 ml) was added powdered 4A MS (500 mg) , and the mixture was stirred for 2 h at room temperature, then cooled to −78 °C. NIS (81 mg, 0.24 mmol) and AgOTf (138 mg, 0.36 mmol) were added to the mixture, which was stirred for 1 h at −78 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 15 : 1 benzene–ethyl acetate as eluent to give **15** (165 mg, 66.6%) and **16** (58 mg, 23.4%). **15**: $[\alpha]_D^{24}$ +60.1° (c=0.3, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.27 (1H, br s, H-1 of Man), 5.08 (1H, d, J=3.1 Hz, H-1″ of Gal), 4.71 (1H, d, J=7.9 Hz, H-1′ of Gal), 4.68 (1H, d, J=7.9 Hz, H-1 of Gal). ^{13}C-NMR ($CDCl_3$) δ: 101.1 ($J_{C,H}$=163.5 Hz, C-1′ of Gal), 100.7 ($J_{C,H}$=161.4 Hz, C-1 of Gal), 98.8 ($J_{C,H}$=173.8 Hz, C-1 of Man), 98.2 ($J_{C,H}$=171.7 Hz, C-1″ of Gal). MALDI-TOFMS: Calcd for $C_{120}H_{114}O_{30}Si$ *m/z*: 2062.8. Found *m/z*: 2085.6 $(M+Na)^+$. **16**: $[\alpha]_D^{24}$ +59.7° (c=0.8, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.31 (1H, br s, H-1 of Man), 4.75 (1H, d, J=7.9 Hz, H-1′ of Gal), 4.73 (1H, d, J=7.9 Hz, H-1 of Gal), 4.48 (1H, d, J=7.9 Hz, H-1″ of Gal). ^{13}C-NMR ($CDCl_3$) δ: 104.0 ($J_{C,H}$=159.3 Hz, C-1″ of Gal), 101.2 ($J_{C,H}$=163.5 Hz, C-1′ of Gal), 100.7 ($J_{C,H}$=161.4 Hz, C-1 of Gal), 98.9 ($J_{C,H}$=171.7 Hz, C-1 of Man). MALDI-TOFMS: Calcd for $C_{120}H_{114}O_{30}Si$ *m/z*: 2062.8. Found *m/z*: 2085.7 $(M+Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (17) A solution of **15** (145 mg, 0.07 mmol) in MeOH (3 ml), EtOAc (1 ml) and AcOH (1 ml) was hydrogenated over 10% Pd–C (100 mg) for 18 h at room temperature, then filtered through Celite and the residue was washed with methanol and concentrated. The residue was acetylated with Ac_2O (1.5 ml) in pyridine (2 ml) for 5 h at room temperature. Work-up as described for **2**, the product was purified by silica gel column chromatography using 20 : 1 benzene–acetone as an eluent to give **17** (80 mg, 60.9%), $[\alpha]_D^{24}$ +90.8° (c=2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.29 (1H, br s, H-1 of Man), 5.25 (1H, d, J=3.7 Hz, H-1″ of Gal), 4.84 (1H, d, J=7.9 Hz, H-1′of Gal), 4.77 (1H, d, J=8.0 Hz, H-1 of Gal). MALDI-TOFMS: Calcd for $C_{100}H_{98}O_{34}Si$ *m/z*: 1870.7. Found *m/z*: 1893.7 $(M+Na)^+$.

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl Trichloroacetimidate (18) To a solution of **17** (80 mg, 43 mmol) in CH_2Cl_2 (1.5 ml) cooled to 0 °C, was added CF_3COOH (1.5 ml), and the mixture was stirred for 2 h at room temperature and concentrated. Ethyl acetate and toluene (1 : 2) were added and then removed to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (1 ml) cooled at 0 °C were added trichloroacetonitrile (128 μl, 1.28 mmol) and DBU (6.5 μl, 43 μmol). The mixture was stirred for 2 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (20 : 1 benzene–acetone) of the residue on silica gel gave **18** (65 mg, 79.3%), $[\alpha]_D^{24}$=+116.8° (c=1.6 $CHCl_3$). ^{1}H-NMR δ: 8.30 (1H, s, NH), 6.77 (1H, d, J=3.7 Hz, H-1 of Gal), 5.32 (1H, br s, H-1 of Man), 5.25 (1H, d, J=3.1 Hz, H-1″ of Gal), 4.84 (1H, d, J=7.9 Hz, H-1′ of Gal). MALDI-TOFMS: Calcd for $C_{97}H_{86}Cl_3NO_{34}$ *m/z*: 1914.8. Found *m/z*: 1937.7 $(M+Na)^+$.

(Tetradecyl)hexadecyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (19) To a solution of the trichloroacetimidate **18** (65 mg, 34 μmol) and 2-(tetradecyl)hexadecanol (22 mg, 51 μmol) in CH_2Cl_2 (1 ml) were added molecular sieves 4A (300 mg) and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. TMSOTf (6.5 μl, 30 μmol) was added and mixture was stirred for 2 h at 0 °C then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 15 : 1 benzene–acetone as eluent to give **19** (24 mg, 32%), $[\alpha]_D^{24}$= +70.2° (c=0.8 $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.29 (1H, br s, H-1 of Man), 5.25 (1H, d, J=3.7 Hz, H-1″ of Gal), 4.84 (1H, d, J=7.9 Hz, H-1′ of Gal), 4.68 (1H, d, J=7.9 Hz, H-1 of Gal), 3.92 (1H, dd, $-OCH_2-$), 3.33 (1H, dd, $-OCH_2-$), 1.34 (52H, br s, $2\times CH_2$), 0.96 (6H, t, $2\times-CH_2C\underline{H}_3$). MALDI-TOFMS: Calcd for $C_{125}H_{146}O_{34}$ *m/z*: 2190.9. Found *m/z*: 2214.3 $(M+Na)^+$.

2-(Tetradecyl)hexadecyl α-D-Mannopyranosyl-(1→4)-[α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl]-(1→6)-β-D-galactopyranoside (20) To a solution of **19** (24 mg, 11 mmol) in 1 : 1 MeOH/1,4-dioxane (2 ml) was added NaOMe (20 mg) and the mixture was stirred for 3 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1 : 1 $CHCl_3$–MeOH. The filtrate and washings were combined and concentrated. Column chromatography (1 : 1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave **20** (10 mg, 82%), $[\alpha]_D^{24}$ +9.2° (c=0.3, 1 : 1 $CHCl_3$–MeOH). ^{1}H-NMR (2 : 1 $CDCl_3$–CD_3OD) δ: 4.88 (1H, br s, H-1 of Man), 4.87 (1H, d, J=3.7 Hz, H-1″ of Gal), 4.34 (1H, d, J=7.9 Hz, H-1′ of Gal), 4.20 (1H, d, J=7.9 Hz, H-1 of Gal). MALDI-TOFMS: Calcd for $C_{54}H_{102}O_{21}$ *m/z*: 1086.7. Found: *m/z* 1109.8 $(M+Na)^+$.

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References

1) Varki A., *Glycobiology*, **3**, (1993) 97—130.
2) *a*) Kameyama A., Ishida H., Kiso M., Hasegawa A., *J. Carbohydr. Chem.*, **10**, 549—560 (1991); b) Tsukida T., Yoshida M., Kurokawa K., Nakai Y., Achiha T., Kiyoi T., Kondo H., *J. Org. Chem*., **62**, 6876—6881 (1997); *c*) Hayashi M., Tanaka M., ItohM., Miyauchi H., *J. Org. Chem*., **61**, 2938—2945 (1996).
3) *a*) Hori T., Sugita M., Ando S., Kuwahara M., Kumauchi K.,Sugie E., Itasaka O., *J. Biol. Chem*., **256**, 10979—10985 (1981); *b*) Levery S. B., Weiss J. B., Salyan M. E. K., Roberts C. E., Hakomori S., Magnani J. L., Strand M., *J. Biol. Chem*., **267**, 5542—5551 (1992).
4) *a*) Kanie O., Takeda T., Ogihara Y., *Carbohydr. Res*., **190**, 53—64 (1989); *b*) Kanie O., Takeda T., Hada N., Ogihara Y., *J. Carbohydr. Chem*., **10**, 561—581 (1991); *c*) Takeda T., Hada N., Ogihara Y., *Chem. Pharm. Bull.*, **40**, 1930—1933 (1992) ; *d*) Takeda T., Hada N., Ogihara Y., *Chem. Pharm. Bull*., **41**, 2058—2060 (1993); *e*) Hada N., Takeda T., Ogihara Y., *Carbohydr. Res*., **258**, 93—104 (1994); *f*) Hada N., Sugita M., Takeda T., Maki T., Ogihara Y., *Yakugakuzasshi*, **114**, 333—341 (1994) .
5) Hada N., Hayashi E., Takeda T., *Carbohydr. Res*., **316**, 58—70 (1999).
6) Persat F., Bouhours J.-F., Mojon M., Petavy A.-F., *J. Biol. Chem*., **267**, 8764—8769 (1992).
7) Sugita M., Mizunoma T., Inagaki F., Suzuki M., Suzuki A., Ohta S., *J. Jpn. Oil Chem. Soc*., **43**, 1052—1061 (1994).
8) *a*) Veeneman G. H., van Leeuwen S. H., van Boom J. H., *Tetrahedron Lett*., **31**, 1331—1334, (1990); *b*) Konradsso P., Udodong U. E., Fraser-Reid B., *Tetrahedron Lett*., **31**, 4313—4316, (1990).
9) Bock K., Pedersen C., *J. Chem. Soc.*, *Perkin Trans. 2*, **1974**, 293—297.
10) Sarkar K. A., Matta L. K., *Carbohydr. Res*., **233**, 245—250 (1992).
11) *a*) Jansson K., Ahlfors S., Frejd T., Kihlberg J., Magnusson G., *J. Org. Chem.*, **53**, 5629—5647 (1988); *b*) Jansson K., Frejd T., Kihlberg J., Magnusson G., *Tetrahedron Lett*., **29**, 361—362 (1988).
12) Schmidt R. R., Grundler G., *Synthesis*, 885—887 (1981).
13) Schmidt R. R., *Angew. Chem*., *Int. Ed. Engl.*, **25**, 212—235 (1986).
14) *a*) Tokuda A., Ariga T., Isogai Y., Komba S., Kiso M., Hasegawa A., Tai T., Yu R. K., *J. Carbohydr. Chem*., **17**, 535—546 (1998); *b*) Ladisch S., Hasegawa A., Li, R., Kiso M., *Biochem. Biophys. Res. Comm*., **203**, 1102—1109 (1994) .