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Synthetic studies on glycosphingolipids from Protostomia phyla: total syntheses of glycosphingolipids from the parasite, *Echinococcus multilocularis*

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Abstract—Efficient and systematic syntheses of four neutral glycosphingolipids that have been isolated from the metacestodes of *Echinococcus multilocularis* have been achieved. A key step is the direct glycosylation of galactosyl donors using thioglycosides with benzoyl ceramide in the presence of *N*-iodosuccinimide (NIS)/TfOH, which gave the desired oligosaccharide derivatives. The fully protected glycosides **13**, **20**, **22** and **25** were deprotected to give four target glycosphingolipids (**1**–**4**).

Keywords: Glycosphingolipids; *Echinococcus multilocularis*; Chemical synthesis; Benzoyl ceramide

1. Introduction

In our continuing studies to elucidate the mechanism of host–parasite interaction, we have synthesized glycosphingolipids from various parasites.[1–4] Persat et al.[5–7] found some novel glycosphingolipids, β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)-Cer (**1**), α-L-Fuc*p*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)-Cer (**2**), β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)-Cer (**3**) and β-D-Gal*p*-(1→6)-[α-L-Fuc*p*-(1→3)]-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)-Cer (**4**) in metacestodes of the parasite, *Echinococcus multilocularis*, which belongs to the class Cestoda. It is particularly interesting to note that these glycosphingolipids contain the unique structures of a β-D-Gal*p*-(1→6)-β-D-Gal*p*-core and a fucose residue and point to the functional importance of glycolipids in parasitism that are recognized by serum antibodies from different patients with alveolar hydatid disease.

In one of our previous papers we reported[1] the synthesis of four glycosphingolipid analogues containing 2-branched fatty alkyl residues in place of ceramide. Therefore, at this time we report the total synthesis of four glycosphingolipids. Excellent pioneering studies of the coupling of oligosaccharides with sphingolipids have been reported by Ogawa,[8–11] Schmidt[12–15] and Hasegawa.[16–19] Reactions to couple the oligosaccharide and the sphingolipid employ a glycosyl imidate as the donor and an azidosphingosine as the acceptor in the presence of TMSOTf or BF_3OEt_2.[12–19] The coupling with azidosphingosine is now popularly employed because of the good yield for coupling and the ability to accept a series of fatty acids as components of ceramide, although the resulting glycosyl azidosphingosine necessitates further transformation. Furthermore, the coupling of glycosyl fluorides as a donor with sphingolipids as an acceptor have been also reported by Ogawa[11] and Nicolaou.[20] However, no one has reported the coupling of a thioglycoside with sphingolipids except the instance with azidosphingosine by Wong[21] and Danishefsky.[22] We synthesized the four

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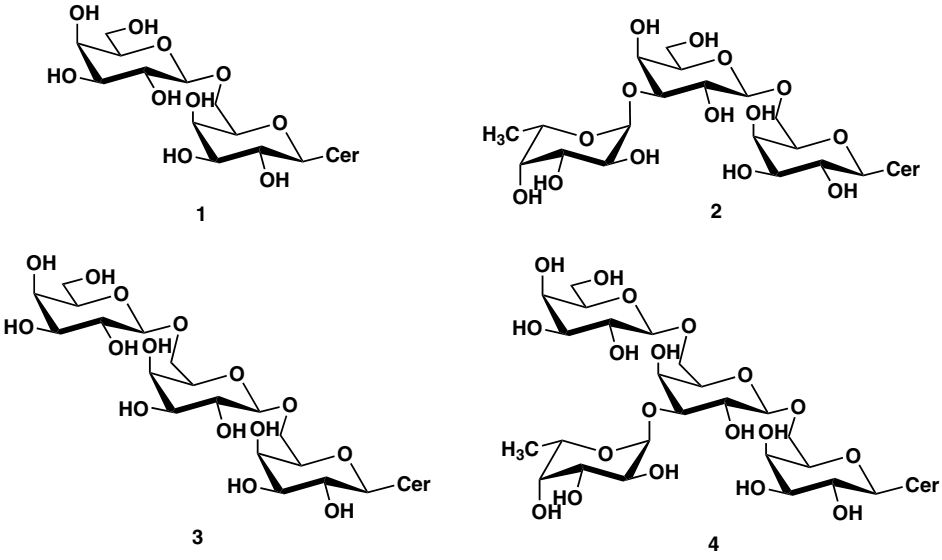
target glycolipids **1**–**4**, as a systematic, integrated approach for the synthesis of tetrasaccharides using thioglycoside derivatives at the reducing end of the oligosaccharide moiety. This is the first report of the glycosylation of a thioglycoside with sphingolipids.



2. Results and discussion

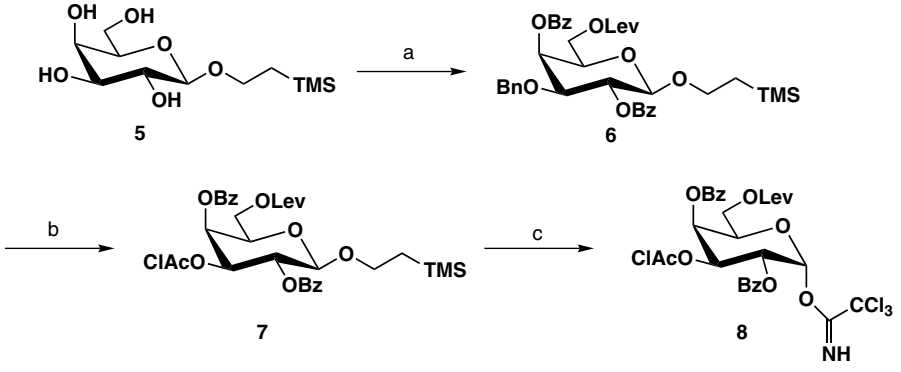
2.1. Syntheses of monosaccharide derivatives

Syntheses of the additional galactopyranosyl building blocks **8** were carried out as depicted in Scheme 1. 2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-β-D-galactopyranoside (**6**) was prepared from known 2-(trimethylsilyl)ethyl β-D-galactopyranoside (**5**)[23] by regioselective benzylation of the in situ prepared stannylidene derivative with benzyl bromide and subsequent regioselective levulinoylation with levulinic acid, followed by benzoylation. Removal of the benzyl groups from **6** by catalytic hydrogenolysis over 10% Pd–C and subsequent chloroacetylation gave compound **7**. For selective removal of the 2-(trimethylsilyl)ethyl (SE) group, **7** was treated[23] with trifluoroacetic acid in dichloromethane to give the 1-hydroxy compound, which, on further treatment[24] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane, gave only the corresponding α-trichloroacetimidate **8**.

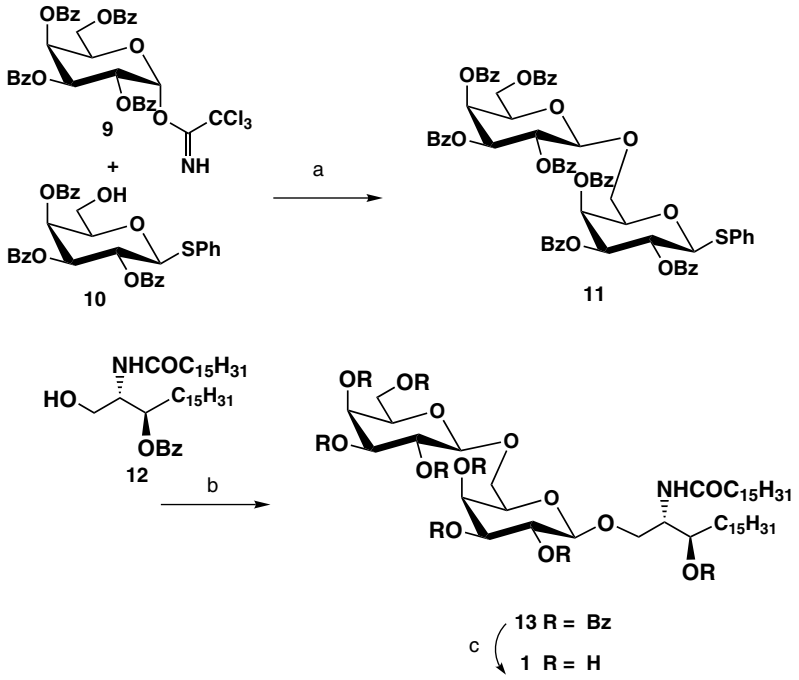
2.2. Systematic syntheses of the each of the glycosphingolipids

In this work, four kinds of target glycolipids **1**–**4** were synthesized by stepwise condensation of suitably protected monosaccharide units. A systematic plan for synthesis of these compounds was designed as shown in Schemes 1–5.

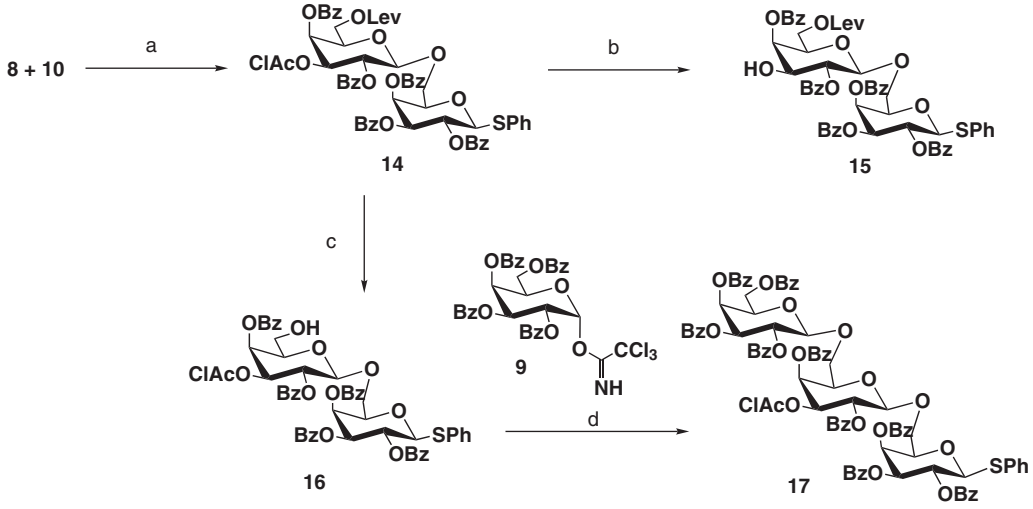
First, we chose compounds **11**, **17**, **19** and **24**, which were phenyl thioglycoside derivatives, as the intermedi-



Scheme 1. Reagents: (a) (i) Bu_2SnO, benzene, Bu_4NBr, BnBr, benzene; (ii) DCC, DMAP, LevOH, CH_2Cl_2; (iii) BzCl, Pyr., 71% three steps; (b) (i) H_2, Pd–C, AcOH, 2:1 THF–MeOH; (ii) $ClCH_2COCl$, Pyr., CH_2Cl_2, 88%; (c) (i) CF_3COOH, CH_2Cl_2; (ii) CCl_3CN, DBU, CH_2Cl_2, 74%.

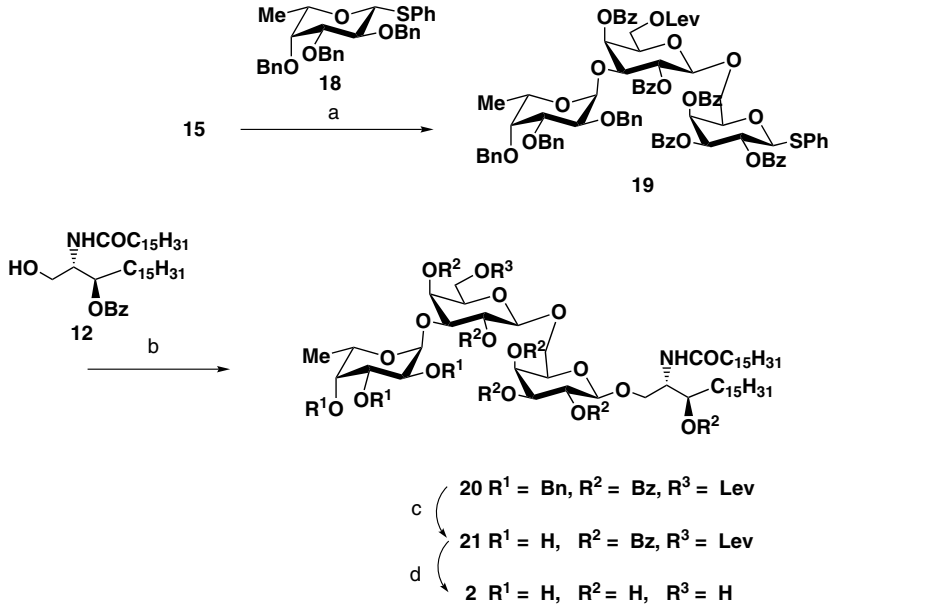


Scheme 2. Reagents: (a) TMSOTf, CH_2Cl_2, 91%; (b) NIS, TfOH, CH_2Cl_2, 44%; (c) NaOMe, 1:1 dioxane–MeOH, quant.

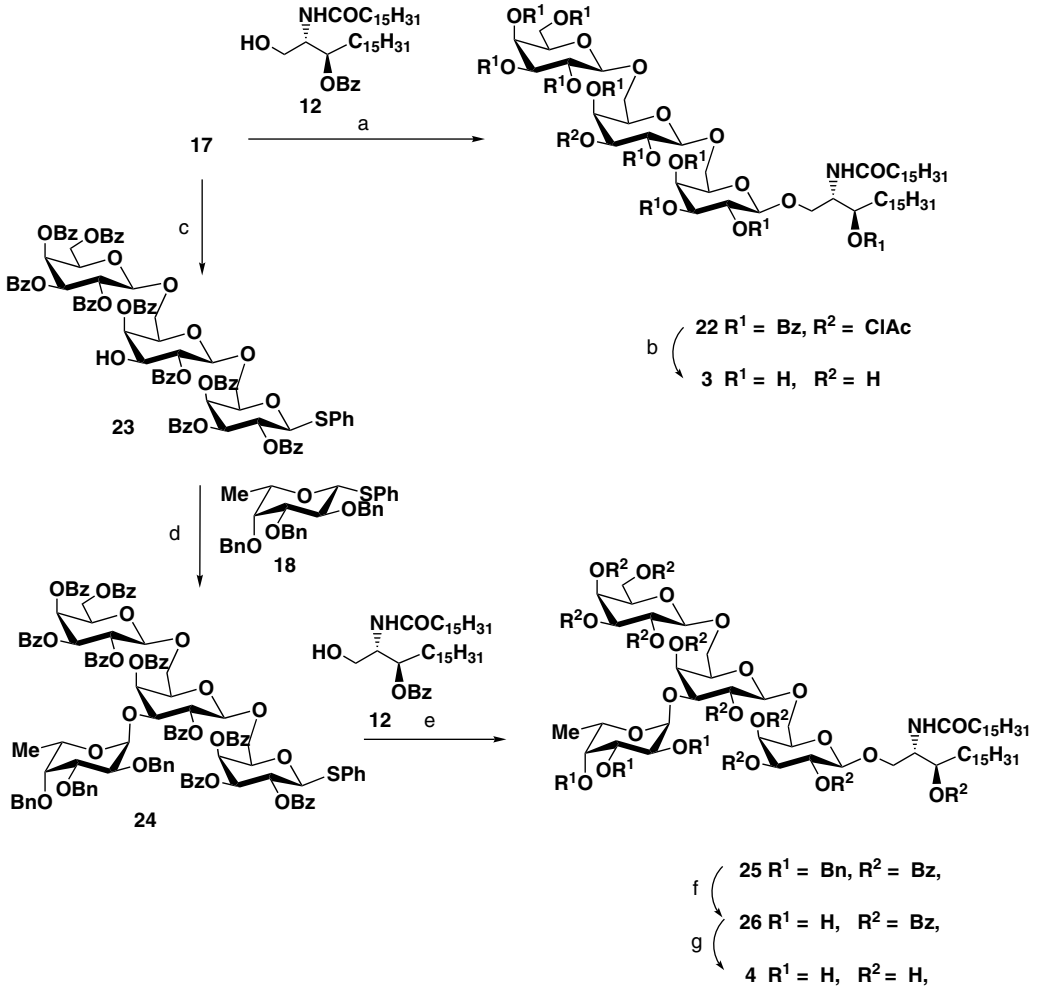


Scheme 3. Reagents: (a) TMSOTf, CH_2Cl_2, quant.; (b) thiourea, 6:1 pyr.–EtOH, 75%; (c) hydrazine acetate, 10:1 THF–MeOH, 88%; (d) TMSOTf, CH_2Cl_2, 92%.

ates. Glycosylation of **10**[25] with **9**[26] in the presence of trimethylsilyl triflate TMSOTf (cat.)[27] and 4A MS in dichloromethane gave the desired disaccharide **11** (91%) as evidenced by ^{1}H NMR spectroscopy (H-1′ 4.91 ppm, J 7.9 Hz). For the other targets, the common donor **8** was chosen for compounds **17**, **19** and **24**, which were condensed with **10** under the agency of TMSOTf (cat) to yield disaccharide **14** (quant) as evidenced by ^{1}H NMR spectroscopy (H-1′ 4.42 ppm, J 8.0 Hz). Selective removal of the chloroacetyl group in **14** with thiourea and 6:1 pyridine–ethanol gave the partially protected disaccharide derivative **15**. Glycosylation of **15** with phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside **18**[28] in the presence of NIS and TfOH[29] as the glycosyl promoter and 4A MS in dichloromethane gave the desired α-glycoside **19** in 72% yield. Significant signals of the fucose unit in the ^{1}H NMR spectrum showed a signal for the anomeric hydrogen atoms at δ 5.05 (d, J 2.0 Hz). The α-L configuration of the newly formed glycosidic bond was also indicated



Scheme 4. Reagents: (a) NIS, TfOH, CH_2Cl_2, 72%; (b) NIS, TfOH, CH_2Cl_2, 54%; (c) H_2, Pd–C, 2:1 THF–MeOH, 84%; (d) NaOMe, 1:1 dioxane–MeOH, 73%.



Scheme 5. Reagents: (a) NIS, TfOH, CH_2Cl_2, 33%; (b) NaOMe, 1:1 dioxane–MeOH, quant.; (c) thiourea, 6:1 Pyr.–EtOH, 84%; (d) NIS, TfOH, CH_2Cl_2, 91%; (e) NIS, TfOH, CH_2Cl_2, 27%; (f) H_2, Pd–C, 2:1 THF–MeOH, 98%; (g) NaOMe, 1:1 dioxane–MeOH, 94%.

by the $J_{C,H}$ value[30] of 169.7 Hz in the ^{13}C NMR spectrum.

Compound **14** was converted by *O*-delevulinoylation using hydrazine acetate and condensed with **9** in the presence of TMSOTf as described for compound **11** to give the trisaccharide **17** (92%), as evidenced by ^1H NMR spectroscopy (H-1″ 4.63 ppm, *J* 7.9 Hz). Removal of the 3′-*O*-chloroacetyl group from **17** by thiourea gave the acceptor **23** (84%) for the tetrasaccharide derivative. Furthermore the glycosylation of **23** with **18** in the presence of NIS/TfOH as described for **19** gave the tetrasaccharide derivative **24** (91%), as evidenced by ^1H NMR spectroscopy (H-1 of Fuc 5.06 ppm, *J* 3.7 Hz).

Next, compounds **11**, **17**, **19** and **24** were used as glycosyl donors without further conversion. Glycosylation of benzoylceramide **12**[31] with the each glycosyl donors, which was carried out in the presence of NIS/TfOH and 4A MS, afforded the desired β-glycosides **13** (44%), **20** (54%), **22** (33%) and **25** (27%). Removal of the benzyl groups from **20** and **25** by catalytic hydrogenolysis over 10% Pd–C in THF–MeOH gave **21** and **26**, and subsequent deacylation of **13**, **21**, **22** and **26** with sodium methoxide in 1:1 methanol–1,4-dioxane afforded the desired target glycolipids **1**–**4**.

In conclusion, a stereocontrolled, efficient route to the *Echinococcus* glycosphingolipids was exploited by use of phenyl thioglycosides as key glycosyl donors.

Biological testing results from studies of the sera from patients with alveolar hydatid disease for these compounds will be reported in detail elsewhere.

3. Experimental

3.1. General

Optical rotations were determined with a Jasco digital polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded with a 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. High-resolution mass spectra were recorded on a JEOL JMS-700 under FAB conditions. 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 β-D-galactopyranoside (**5**), 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**9**), phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**10**) and phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**18**) were prepared by literature methods.[23,25,26,28] Benzoylceramide **12** was prepared from *N*-palmitoyl-D-erythro-sphingosine, which was purchased from Acros Organics (Belgium) by the conventional four-step procedure.[31]

3.2. 2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-β-D-galactopyranoside (6)

A mixture of 2-(trimethylsilyl)ethyl β-D-galactopyranoside (**5**) (2.00 g, 7.13 mmol), dibutyltin oxide (1.93 g, 7.75 mmol) and 150 mL of dry benzene was stirred under reflux for 5 h. Benzene (75 mL) was distilled off, and the solution was cooled to 60 °C and treated with Bu_4NBr (2.86 g, 8.87 mmol) and BnBr (1.73 mL, 14.7 mmol). The reaction mixture was stirred for 4.5 h, and the solution was concentrated. Purification of the residue by column chromatography (20:1 $CHCl_3$–MeOH) on silica gel gave 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside (2.60 g, quant). To a solution of this compound (201 mg, 0.54 mmol) in CH_2Cl_2 (2 mL) was added levulinic acid (61 μL, 0.60 mmol), DCC (202 mg, 0.98 mmol) and DMAP (20 mg, 0.16 mmol). The reaction mixture was stirred for 3 h at rt, then extracted with $CHCl_3$, washed with 5% HCl, dried ($MgSO_4$), and concentrated. The crude product was purified by silica gel column chromatography using 35:1 $CHCl_3$–MeOH as eluent to give 2-(trimethylsilyl)ethyl 3-*O*-benzyl-6-*O*-levulinoyl-β-D-galactopyranoside (223 mg, 87.7%). To a solution of this compound (223 mg, 0.48 mmol) in pyridine (2 mL) was added benzoyl chloride (0.22 mL, 1.90 mmol), and the mixture was stirred for 2 h, then extracted with $CHCl_3$, washed with 5% HCl, aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 20:1 toluene–acetone as eluent to give compound **6** (264 mg, 82.0%). $[\alpha]_D^{24}$ +83.5 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.24–7.14 (m, 15H, Ph×3), 5.92 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.57 (dd, 1H $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.8 Hz, *H*-2), 4.77 and 4.60 (each d, 2H, J_{gem} 12.8 Hz, benzyl methylene), 4.68 (d, 1H, *H*-1), 4.44 (dd, 1H, $J_{5,6a}$ 6.7 Hz, $J_{6a,6b}$ 11.0 Hz, *H*-6a), 4.27 (dd, 1H, $J_{5,6b}$ 6.7 Hz, *H*-6b), 4.11–4.06 (m, 1H, –OCH_2CH$_2$–), 4.03 (t, 1H, *H*-5), 3.85 (dd, 1H, *H*-3), 3.68–3.62 (m, 1H, –OCH_2CH$_2$–), 2.85 (t, 2H, –OCOCH_2CH$_2$COCH$_3$), 2.65 (t, 2H, –OCOCH$_2$CH_2-COCH$_3$), 2.29 (s, 3H, –OCOCH$_2$CH$_2$COCH_3), 1.02–0.93 (m, 2H, –OCH$_2$CH_2–), −0.09 (s, 9H, –Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$): δ 206.6, 172.3, 165.9, 137.3, 133.3, 130.1, 129.9, 129.4, 128.5, 128.2, 128.0, 127.6, 101.0, 76.1, 71.2, 70.9, 70.7, 67.5, 66.4, 62.1, 38.0, 29.8, 27.9, 17.9. MALDI-TOFMS: Calcd for $C_{37}H_{44}O_{10}Si$: *m*/*z* 676. Found: *m*/*z* 699 $[M+Na]^+$. HRFABMS: Calcd for $C_{37}H_{44}O_{10}SiNa$ $[M+Na]^+$: *m*/*z* 699.2601. Found: *m*/*z* 699.2648.

3.3. 2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-6-*O*-levulinoyl-β-D-galactopyranoside (7)

A solution of **6** (1.41 g, 2.08 mmol) in 2:1 THF–MeOH (15 mL) was hydrogenolyzed in the presence of 10% Pd–C (500 mg) for 4 h at rt, then filtered and concentrated. The product was purified by silica gel column

chromatography using 5:1 toluene–acetone as eluent to give 2-(trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranoside (1.20 g, quant). To a solution of this compound (349 mg, 0.595 mmol) in CH_2Cl_2 (1 mL) was added $ClCH_2COCl$ (0.10 mL, 1.26 mmol) and pyridine (0.10 mL). The reaction mixture was stirred for 2 h at rt, then extracted with $CHCl_3$, washed with 5% HCl, aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 30:1 toluene–acetone as eluent to give compound **7** (354 mg, 89.7%). $[\alpha]_D^{24}$ +37.6 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.19–7.33 (m, 10H, Ph×2), 5.77 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.63 (dd, 1H, $J_{1,2}$ 7.9 Hz, *H*-2), 5.45 (dd, 1H, $J_{2,3}$ 10.4 Hz, *H*-3), 4.80 (d, 1H, *H*-1), 4.44 (dd, 1H, $J_{5,6a}$ 6.7 Hz, $J_{6a,6b}$ 11.6 Hz, *H*-6a), 4.24 (dd, 1H, $J_{5,6b}$ 6.7 Hz, *H*-6b), 4.17 (t, 1H, *H*-5), 4.13–4.07 (m, 1H, $-OCH_2CH_2-$), 3.97–3.88 (each d, 2H, J_{gem} 15.3 Hz, $ClCH_2CO-$), 3.72–3,67 (m, 1H, $-OCH_2CH_2-$), 2.79 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.61 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.24 (s, 3H, $-OCOCH_2CH_2COCH_3$), 1.03–0.92 (m, 2H, $-OCH_2CH_2-$), −0.09 (s, 9H, $-Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 206.3, 172.1, 166.8, 165.9, 165.0, 133.7, 133.3, 130.0, 129.7, 129.3, 128.7, 128.6, 128.4, 100.9, 73.0, 70.7, 69.4, 67.8, 67.6, 61.5, 40.4, 37.8, 29.7, 27.8, 18.0. MALDI-TOFMS: Calcd for $C_{32}H_{39}ClO_{11}Si$: *m*/*z* 662. Found: *m*/*z* 685 $[M+Na]^+$. HRFABMS: Calcd for $C_{32}H_{39}ClO_{11}SiNa$ $[M+Na]^+$: *m*/*z* 685.1848. Found: *m*/*z* 685.1855.

3.4. 2,4-Di-*O*-benzoyl-3-*O*-chloroacetyl-6-*O*-levulinoyl-α-D-galactopyranosyl trichloroacetimidate (8)

To a solution of **7** (84 mg, 0.13 mmol) in CH_2Cl_2 (1 mL) cooled to 0 °C was added CF_3COOH (1 mL), and the mixture was stirred for 2 h at rt and concentrated. EtOAc and toluene (1:2) were added and then evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (2 mL) cooled at 0 °C were added trichloroacetonitrile (0.27 mL, 2.69 mmol) and DBU (30.0 μL, 0.20 mmol). The mixture was stirred for 1 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (30:1 toluene–acetone) of the residue on silica gel gave **8** (66 mg, 73.7%) as an amorphous mass. $[\alpha]_D^{24}$ +122.0 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.65 (s, 1H, N*H*), 8.11–7.26 (m, 10H, Ph×2), 6.84 (d, 1H, $J_{1,2}$ 7.9 Hz, *H*-1), 5.87 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.84 (dd, 1H, $J_{2,3}$ 11.0 Hz, *H*-3), 5.74 (dd, 1H, *H*-2), 4.64 (t, 1H, $J_{5,6b}$ $J_{5,6a}$ 6.1 Hz, *H*-5), 4.29 (dd, 1H, $J_{6a,6b}$ 11.0 Hz, *H*-6a), 4.24 (dd, 1H, *H*-6b), 3.96–3.88 (each d, 2H, J_{gem} 15.3 Hz, $ClCH_2CO-$), 2.69 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.53 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.15 (s, 3H, $-OCOCH_2CH_2COCH_3$); ^{13}C NMR ($CDCl_3$): δ 206.2, 172.1, 166.7, 165.8, 165.5, 160.6, 134.0, 133.8, 130.0, 129.9, 128.8, 128.7, 128.6, 93.5 (C-1), 69.6 (C-2), 69.3 (C-5), 68.0 (C-4), 67.5 (C-3), 61.6 (C-6), 40.4, 37.8, 29.8, 27.7. HRFABMS: Calcd for $C_{29}H_{27}Cl_4NO_{11}Na$ $[M+Na]^+$: *m*/*z* 728.0236. Found: *m*/*z* 728.0266.

3.5. Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (11)

To a solution of **9** (190 mg, 0.26 mmol) and **10** (100 mg, 0.17 mmol) in dry CH_2Cl_2 (3 mL) was added powdered 4A MS (300 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. TMSOTf (46 μL, 0.25 mmol) was added to the mixture, which was stirred for 1 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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 70:1 toluene–acetone as eluent to give **11** (181 mg, 91.0%). $[\alpha]_D^{24}$ +235.3 (*c* 0.3, 1 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.11–7.20 (m, 40H, Ph×8), 5.95 (d, 1H, $J_{3',4'}$ 3.7 Hz, *H*-4′), 5.91 (d, 1H, $J_{3,4}$ 3.7 Hz, *H*-4), 5.79 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, *H*-2′), 5.68 (t, 1H, $J_{1,2}$ 10.4 Hz, $J_{2,3}$ 10.4 Hz, *H*-2), 5.57 (dd, 1H, *H*-3′), 5.51 (dd, 1H, *H*-3), 4.91 (d, 1H, *H*-1′), 4.89 (d, 1H, *H*-1), 4.41 (dd, 1H, $J_{5',6'b}$ 6.1 Hz, $J_{6'a,6'b}$ 11.0 Hz, *H*-6′a), 4.27–4.21 (m, 3H, *H*-5, *H*-5′, *H*-6′b), 4.09 (dd, 1H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 11.0 Hz, *H*-6a), 3.96 (dd, 1H, $J_{5,6b}$ 4.9 Hz, *H*-6b); ^{13}C NMR ($CDCl_3$): δ 165.9, 165.5, 165.4, 165.2, 165.1, 134.1, 133.6, 133.5, 133.5, 133.4, 133.2, 133.1, 130.9, 130.1, 130.1, 129.8, 129.8, 129.3, 129.0, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 101.2 (C-1′), 85.3 (C-1), 76.8 (C-5), 73.0 (C-3), 71.7 (C-3′), 71.3 (C-5′), 69.7 (C-2′), 68.5 (C-4), 67.9 (C-6), 67.8 (C-4′), 67.7 (C-2), 61.7 (C-6′). MALDI-TOFMS: Calcd for $C_{67}H_{54}O_{17}S$: *m*/*z* 1162. Found: *m*/*z* 1185 $[M+Na]^+$.

3.6. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecanamido-octadecane-1,3-diol (13)

To a solution of **11** (50 mg, 43.0 μmol) and **12** (33 mg, 51.2 μmol) in dry CH_2Cl_2 (1.5 mL) was added powdered 4A MS (150 mg), and the mixture was stirred for 2 h at rt, then cooled to −40 °C. NIS (15 mg, 66.7 μmol) and TfOH (1.5 μL, 17.0 μmol) were added to the mixture, which was stirred for 2 h at −40 °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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 20:1 toluene–EtOAc as eluent to give **13** (32 mg, 43.9%). $[\alpha]_D^{24}$

+69.2 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 4.55 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.41(d, 1H, $J_{1,2}$ 7.9 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{102}H_{119}NO_{21}$: *m*/*z* 1693. Found: *m*/*z* 1716 $[M+Na]^+$.

3.7. β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-2-hexadecanamido-octadecane-1,3-diol (1)

To a solution of **12** (32 mg, 18.9 μmol) in 1:1 MeOH–1,4-dioxane (2 mL) was added NaOMe (40 mg), and the mixture was stirred overnight at rt, 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 **1** (16 mg, quant). $[\alpha]_D^{24}$ +6.8 (*c* 0.6, 1:1 $CHCl_3$–MeOH); ^1H NMR (49:1 DMSO-d_6–D_2O) δ: 4.15 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.07 (d, 1H, $J_{1,2}$ 7.3 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{46}H_{89}NO_{13}$: *m*/*z* 863. Found: *m*/*z* 886 $[M+Na]^+$. HRFABMS: Calcd for $C_{46}H_{89}NO_{13}Na$ $[M+Na]^+$: *m*/*z* 886.6232. Found: *m*/*z* 886.6153.

3.8. Phenyl 2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (14)

To a solution of **8** (140 mg, 0.20 mmol) and **10** (77 mg, 0.13 mmol) in dry CH_2Cl_2 (2 mL) was added powdered 4A MS (300 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. TMSOTf (7.2 μL, 39.8 μmol) was added to the mixture, which was stirred for 30 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 water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 15:1 toluene–acetone as eluent to give **14** (145 mg, quant). $[\alpha]_D^{24}$ +96.3 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.14–7.20 (m, 30H, Ph×6), 5.84 (d, 1H, $J_{3,4}$ 3.7 Hz, *H*-4), 5.67–5.64 (m, 2H, H-2, *H*-4′), 5.58 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, *H*-2′), 5.46 (dd, 1H, $J_{2,3}$ 9.8 Hz, *H*-3), 5.35 (dd, 1H, *H*-3′), 4.90 (d, 1H, $J_{1,2}$ 9.8 Hz, *H*-1), 4.82 (d, 1H, *H*-1′), 4.27 (dd, 1H, $J_{5',6a'}$ 4.9 Hz, $J_{6a',6b'}$ 7.32 Hz, *H*-6a′), 4.12–3.94 (m, 5H, *H*-5, *H*-5′, *H*-6a, *H*-6b, *H*-6b′), 3.91–3.83 (each d, 2H, J_{gem} 15.3 Hz, ClCH_2CO–), 2.72 (t, 2H, –OCO$CH_2$$CH_2$COCH$_3$), 2.49 (dd, 2H, –OCOCH$_2CH_2$COCH$_3$), 2.17 (s, 3H, –OCOCH$_2CH_2COCH_3$); ^{13}C NMR ($CDCl_3$): δ 147.3, 134.0, 133.8, 133.5, 133.3, 130.2, 129.9, 129.8, 129.8, 129.4, 129.1, 128.9, 128.8, 128.5, 128.4, 128.2, 113.0, 109.3, 101.4 (C-1′), 91.8, 85.4 (C-1), 76.8 (C-5), 73.1 (C-3), 72.9 (C-3′), 71.0 (C-5′), 69.3 (C-2′), 68.7 (C-4), 68.5 (C-6), 67.9 (C-4′), 67.6 (C-2), 61.5 (C-6′), 52.2, 48.3, 40.4, 38.0, 30.9, 29.7, 27.9, 22.7, 22.0, 11.9. MALDI-TOFMS: Calcd for $C_{60}H_{53}ClO_{18}S$: *m*/*z* 1128. Found: *m*/*z* 1151 $[M+Na]^+$. HRFABMS: Calcd for $C_{60}H_{54}ClO_{18}S$ $[M+H]^+$: *m*/*z* 1129.2719. Found: *m*/*z* 1129.2684.

3.9. Phenyl 2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (15)

To a solution of **14** (50 mg, 44.2 μmol) in 6:1 pyridine–EtOH (3.5 mL) was added thiourea (34 mg, 0.45 mmol), and the mixture was stirred for 1 h at rt. The mixture was diluted with $CHCl_3$, washed with $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 7:1 toluene–acetone as eluent to give **15** (35 mg, 75.1%). $[\alpha]_D^{24}$ +132.0 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.13–7.16 (m, 30H, Ph×6), 5.82 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.64 (t, 1H, $J_{1,2}$ 9.8 Hz, $J_{2,3}$ 10.4 Hz, *H*-2), 5.57 (d, 1H, $J_{3',4'}$ 3.7 Hz, *H*-4′), 5.45 (dd, 1H, *H*-3), 5.29 (d, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, *H*-2′), 4.92 (d, 1H, *H*-1), 4.76 (d, 1H, *H*-1′), 4.31 (dd, 1H, *H*-5), 4.12–3.90 (m, 6H, *H*-3, *H*-5′, *H*-6a, *H*-6b, *H*-6a′, *H*-6b′), 2.70 (t, 2H, –OCOCH_2CH$_2$COCH$_3$), 2.49–2.41 (m, 2H, –OCOCH$_2$CH_2COCH$_3$), 2.13 (d, 1H, OH), 2.10 (s, 3H, –OCOCH$_2$CH$_2$COCH_3); ^{13}C NMR ($CDCl_3$): δ 172.4, 167.0, 166.2, 165.4, 165.2, 133.6, 133.4, 133.3, 133.1, 131.4, 130.1, 130.0, 129.9, 129.8, 129.7, 129.4, 129.0, 128.8, 128.6, 128.5, 128.4, 128.4, 128.20, 101.3 (C-1′), 85.4 (C-1), 76.7 (C-5), 73.7 (C-5′), 73.1 (C-3), 72.0 (C-3′), 71.3 (C-6), 70.4 (C-4′), 68.7 (C-2′,C-4), 67.9 (C-2), 63.2 (C-6′), 38.0, 29.7, 27.9. MALDI-TOFMS: Calcd for $C_{58}H_{52}O_{17}S$: *m*/*z* 1052. Found: *m*/*z* 1075 $[M+Na]^+$. HRFABMS: Calcd for $C_{58}H_{53}O_{17}S$ $[M+H]^+$: *m*/*z* 1053.3003. Found: *m*/*z* 1053.3073.

3.10. Phenyl 2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (16)

To a solution of **14** (50 mg, 44.3 μmol) in 2:1 MeOH–THF (1.7 mL) was added hydrazine acetate (4.08 mg, 44.3 μmol), and the mixture was stirred for 3 h at rt. The mixture was diluted with $CHCl_3$, was washed with aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 20:1 toluene–acetone as eluent to give **16** (40 mg, 87.6%). $[\alpha]_D^{24}$ +97.4 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.07–7.12 (m, 30H, Ph×6), 5.87 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.59–5.51 (m, 3H, *H*-2, *H*-2′, *H*-4′), 5.41 (dd, 1H, $J_{2,3}$ 9.8 Hz, *H*-3), 5.30 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.1 Hz, *H*-3′), 4.79 (d, 1H, $J_{1,2}$ 9.8 Hz, *H*-1), 4.70 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.10 (t, 1H, $J_{5,6b}$ 6.1 Hz, $J_{5,6a}$ 5.5 Hz, *H*-5), 3.91 (dd, 1H, $J_{6a,6b}$ 11.0 Hz, *H*-6a) 3.87–3.74 (m, 4H, *H*-5′, *H*-6b, ClCH_2CO–), 3.55

(dd, 1H, $J_{5',6a'}$ 6.7 Hz, $J_{6a',6b'}$ 11.6 Hz, *H*-6a′), 3.38 (dd, 1H, $J_{5',6b'}$ 5.5 Hz, *H*-6b′), 2.09 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 166.8, 166.6, 165.5, 165.3, 165.2, 165.1, 134.4, 133.9, 133.5, 133.5, 133.3, 133.2, 130.6, 130.2, 129.9, 129.7, 129.3, 129.1, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.2, 101.3 (C-1′), 85.3 (C-1), 76.8 (C-5), 73.9 (C-5′), 73.1 (C-3), 72.9 (C-3′), 69.6 (C-2′), 68.5 (C-4), 68.2 (C-4′), 67.9 (C-2), 67.8 (C-6), 60.7 (C-6′), 40.4, 29.7. MALDI-TOFMS: Calcd for $C_{55}H_{47}ClO_{16}S$: *m*/*z* 1030. Found: *m*/*z* 1053 $[M+Na]^+$. HRFABMS: Calcd for $C_{55}H_{48}ClO_{16}S$ $[M+H]^+$: *m*/*z* 1031.2352. Found: *m*/*z* 1031.2356.

3.11. Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)- 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (17)

To a solution of **16** (150 mg, 0.14 mmol) and **9** (162 mg, 0.22 μmol) in dry CH_2Cl_2 (3 mL) was added powdered 4A MS (300 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. TMSOTf (7.8 μL, 43.1 μmol) was added to the mixture, which was stirred for 1 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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 80:1 toluene–acetone as eluent to give **17** (215 mg, 91.8%). $[\alpha]_D^{24}$ +93.3 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.12–7.20 (m, 50H, Ph×10), 5.89 (d, 1H, $J_{3,4}$ 3.1 Hz, *H*-4), 5.87 (d, 1H, $J_{3',4'}$ 3.1 Hz, *H*-4′), 5.78 (d, 1H, $J_{3'',4''}$ 3.1 Hz, *H*-4″), 5.67–5.32 (m, 6H, *H*-2, *H*-2′, *H*-2″, *H*-3, *H*-3′, *H*-3″), 4.88 (d, 1H, $J_{1,2}$ 9.8 Hz, *H*-1), 4.69 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.63 (d, 1H, $J_{1'',2''}$ 7.3 Hz, *H*-1″), 4.15–3.54 (m, 11H, *H*-5, *H*-5′, *H*-5″, *H*-6a, *H*-6b, *H*-6′a, *H*-6′b, *H*-6″a, *H*-6″b, $ClCH_2CO$–); ^{13}C NMR ($CDCl_3$): δ 166.6, 165.7, 165.5, 165.4, 165.3, 165.1, 165.0, 163.4, 134.0, 133.6, 133.3, 133.2, 130.9, 130.1, 130.0, 129.9, 129.8, 129.7, 129.7, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 100.9, 100.5, 76.7, 73.0, 72.8, 71.9, 71.5, 71.0, 69.8, 69.4, 68.4, 67.8, 67.7, 67.2, 65.6, 61.2, 40.5. MALDI-TOFMS: Calcd for $C_{89}H_{73}ClO_{25}S$: *m*/*z* 1608. Found: *m*/*z* 1631 $[M+Na]^+$.

3.12. Phenyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (19)

To a solution of **15** (100 mg, 86.7 μmol) and **18** (100 mg, 190 μmol) in dry CH_2Cl_2 (2 mL) was added powdered 4A MS (200 mg), and the mixture was stirred for 2 h at rt, then cooled to −65 °C. NIS (70 mg, 0.31 μmol) and TfOH (1.7 μL, 19 μmol) were added to the mixture, which was stirred for 2 h at −65 °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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 4:3 hexane–EtOAc as eluent to give **19** (100 mg, 78.5%). $[\alpha]_D^{24}$ +68.1 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.15–6.94 (m, 45H, Ph×9), 5.83 (d, 1H, *H*-4), 5.70–5.60 (m, 3H, *H*-2′, *H*-2, *H*-4′), 5.45 (dd, 1H, $J_{2,3}$ 9.8 Hz, *H*-3), 5.05 (d, 1H, $J_{1'',2''}$ 2.0 Hz, *H*-1″), 4.89 (d, 1H, $J_{1,2}$ 9.8 Hz, *H*-1), 4.81 and 4.49 (each d, 2H, J_{gem} 11.6 Hz, benzylic methylene), 4.76 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.38–3.85 (m, 12H, benzylic methylene×2, *H*-6a, *H*-6b, *H*-6a′, *H*-6b′, *H*-3′, *H*-5, *H*-5′, *H*-5″), 3.78 (dd, 1H, $J_{2'',3''}$ 10.4 Hz, *H*-2″), 3.63 (dd, 1H, *H*-3″), 3.49 (s, 1H, *H*-4″), 2.74 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.52 (t, 2H, $-OCOCH_2CH_2COCH_3$), 2.13 (s, 3H, $-OCOCH_2CH_2COCH_3$), 1.01 (d, 3H, H-6″); ^{13}C NMR ($CDCl_3$): δ 206.8, 172.6, 166.3, 165.4, 165.3, 165.1, 164.8, 139.0, 138.6, 138.5, 133.8, 133.4, 133.4, 133.3, 133.1, 133.1, 130.2, 129.9, 129.9, 129.8, 129.7, 129.5, 129.4, 129.1, 128.9, 128.6, 128.5, 128.4, 128.4, 128.4, 128.2, 128.2, 128.1, 128.0, 127.5, 127.3, 127.2, 127.1, 101.9 (C-1′), 98.8 (C-1″), 85.4 (C-1), 78.9 (C-3″), 78.0 (C-4″), 76.8 (C-5) 75.2 (C-2″), 75.1 (C-3′), 74.7 (benzylic methylene), 73.3 (benzylic methylene), 73.1 (C-3), 72.0 (benzylic methylene), 71.9, 71.6, 69.9, 68.9 (C-4), 68.7 (C-6), 68.0 (C-2′), 67.7 (C-2), 62.5 (C-6′), 38.1, 29.7, 28.0, 16.4. MALDI-TOFMS: Calcd for $C_{85}H_{80}O_{21}S$: *m*/*z* 1468. Found: *m*/*z* 1491 $[M+Na]^+$.

3.13. 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecan-amido-octadecane-1,3-diol (20)

To a solution of **19** (96 mg, 65 μmol) and **12** (87 mg, 0.14 μmol) in dry CH_2Cl_2 (2 mL) was added powdered AW300 MS (200 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. NIS (23 mg, 102 μmol) and TfOH (3 μL, 34 μmol) were added to the mixture, which 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 aq $Na_2S_2O_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 3:2 hexane–EtOAc as eluent to give **20** (70 mg, 53.5%). $[\alpha]_D^{24}$ +38.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.08 (d, 1H, $J_{1'',2''}$ 3.7 Hz, *H*-1″), 4.55 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.20 (d, 1H, $J_{1,2}$ 7.9 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{120}H_{147}NO_{25}$: *m*/*z* 2002. Found: *m*/*z* 2025 $[M+Na]^+$.

3.14. α-L-Fucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecanamido-octadecane-1,3-diol (21)

A solution of **20** (70 mg, 35 μmol) in 2:1 THF–MeOH (4.5 mL) was hydrogenolyzed in the presence of 10% Pd–C (120 mg) overnight at rt, then filtered and concentrated. The product was purified by silica gel column chromatography using 60:1 $CHCl_3$–MeOH as eluent to give **21** (51 mg, 84.2%). $[\alpha]_D^{24}$ +91.1 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.93 (d, 1H, $J_{1'',2''}$ 3.7 Hz, *H*-1″), 4.64 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.45 (d, 1H, $J_{1,2}$ 6.7 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{99}H_{129}NO_{25}$: *m*/*z* 1731. Found: *m*/*z* 1754 $[M+Na]^+$.

3.15. α-L-Fucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-2-hexadecanamido-octadecane-1,3-diol (2)

To a solution of **21** (26 mg, 15 μmol) in 1:1 MeOH–1,4-dioxane (2 mL) was added NaOMe (20 mg), and the mixture was stirred overnight at rt, 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 **2** (11 mg, 72.6%). $[\alpha]_D^{24}$ +7.0 (*c* 0.3, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (49:1 DMSO-d_6–D_2O): δ 4.91 (d, 1H, $J_{1'',2''}$ 3.7 Hz, *H*-1″), 4.24 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.07 (d, 1H, $J_{1,2}$ 7.3 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{52}H_{99}NO_{17}$: *m*/*z* 1009. Found: *m*/*z* 1032 $[M+Na]^+$. HRFABMS: Calcd for $C_{52}H_{99}NO_{17}Na$ $[M+Na]^+$: *m*/*z* 1032.6811. Found: *m*/*z* 1032.6766.

3.16. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecanamido-octadecane-1,3-diol (22)

To a solution of **17** (100 mg, 62.1 μmol) and **12** (56 mg, 87 μmol) in dry CH_2Cl_2 (1.5 mL) was added powdered 4A MS (200 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. NIS (28 mg, 0.12 mmol) and TfOH (2.8 μL, 32 μmol) were added to the mixture, which 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 aq $Na_2S_2O_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 20:1 toluene–EtOAc as eluent to give **22** (44 mg, 33.0%). $[\alpha]_D^{24}$ +37.6 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.62 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.54 (d, 1H, $J_{1'',2''}$ 7.9 Hz, *H*-1″), 4.18 (d, 1H, $J_{1,2}$ 7.9 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{124}H_{140}ClNO_{29}$: *m*/*z* 2141. Found: *m*/*z* 2164 $[M+Na]^+$.

3.17. β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-2-hexadecanamido-octadecane-1,3-diol (3)

To a solution of **22** (35 mg, 16.3 μmol) in 1:1 MeOH–1,4-dioxane (1 mL) was added NaOMe (40 mg), and the mixture was stirred overnight at rt, 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 **3** (17 mg, quant). $[\alpha]_D^{24}$ +9.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR (49:1 DMSO-d_6–D_2O): δ 4.17 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.15 (d, 1H, $J_{1'',2''}$ 6.7 Hz, *H*-1″), 4.07 (d, 1H, $J_{1,2}$ 7.3 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{52}H_{99}NO_{18}$: *m*/*z* 1025. Found: *m*/*z* 1048 $[M+Na]^+$. HRFABMS: Calcd for $C_{52}H_{99}NO_{18}Na$ $[M+Na]^+$: *m*/*z* 1048.6760. Found *m*/*z* 1048.6816.

3.18. Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (23)

To a solution of **17** (256 mg, 159 μmol) in 6:1 pyridine–EtOH (3 mL) was added thiourea (60 mg, 788 μmol), and the mixture was stirred for 1 h at 60 °C. The mixture was was diluted with $CHCl_3$, washed with $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 20:1 toluene–acetone as eluent to give **23** (204 mg, 83.7%). $[\alpha]_D^{24}$ +86.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.13–7.14 (m, 50H, Ph×10), 5.89 (d, 2H, $J_{3,4}$ 3.1 Hz, $J_{3'',4''}$ 3.1 Hz, *H*-4, *H*-4″), 5.72–5.50 (m, 5H, *H*-2, *H*-2″, *H*-3, *H*-3″, *H*-4′), 5.26 (dd, 1H, $J_{1',2'}$ 9.8 Hz, $J_{2',3'}$ 7.9 Hz, *H*-2′), 4.91 (d, 1H, $J_{1,2}$ 9.8 Hz, *H*-1), 4.65 (d, 1H, $J_{1'',2''}$ 9.8 Hz, *H*-1″), 4.63 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.24–3.51 (m, 10H, *H*-3′, *H*-5, *H*-5′, *H*-5″, *H*-6a, *H*-6b, *H*-6′a, *H*-6′b, *H*-6″a, *H*-6″b); ^{13}C NMR ($CDCl_3$): δ 166.6, 166.3, 165.8, 165.5, 165.3, 165.1, 165.0, 133.8, 133.6, 133.4, 133.3, 133.1, 131.2, 130.1, 129.9, 129.8, 129.7, 129.5, 129.3, 129.3, 129.0, 128.9, 128.79, 128.7, 128.6, 128.4, 128.4, 128.2, 128.2, 101.0, 100.8, 85.5, 76.7, 73.4, 73.0, 72.4, 71.8, 71.5, 71.1, 70.2, 69.8, 68.5, 67.9, 67.5, 66.8, 61.5. MALDI-TOFMS: Calcd for $C_{87}H_{72}O_{24}S$: *m*/*z* 1532. Found: *m*/*z* 1555 $[M+Na]^+$.

3.19. Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-[2,3,4-tri-*O*-benzyl-α-L-fuco-pyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (24)

To a solution of **23** (63 mg, 41.1 μmol) and **18** (43 mg, 81.6 μmol) in dry CH_2Cl_2 (1 mL) was added powdered

4A MS (100 mg), and the mixture was stirred for 2 h at rt, then cooled to −60 °C. NIS (28 mg, 124 μmol) and TfOH (0.7 μL, 7.91 μmol) were added to the mixture, which was stirred for 1 h at −60 °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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 10:1 toluene–EtOAc as eluent to give **24** (73 mg, 91.1%). $[\alpha]_D^{24}$ +36.1 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.06 (d, 1H, $J_{1,2}$ 3.7 Hz, Fuc of *H*-1), 4.83 (d, 1H, $J_{1,2}$ 9.2 Hz, *H*-1), 4.68 (d, 1H, $J_{1',2'}$ 7.9 Hz, *H*-1′), 4.57 (d, 1H, $J_{1'',2''}$ 7.9 Hz, *H*-1″); ^{13}C NMR ($CDCl_3$): δ 101.3 (C-1′), 101.1 (C-1″), 98.5 (Fuc of C-1), 85.4 (C-1). MALDI-TOFMS: Calcd for $C_{114}H_{100}O_{28}S$: *m*/*z* 1948. Found: *m*/*z* 1971 $[M+Na]^+$.

3.20. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecanamido-octadecane-1,3-diol (25)

To a solution of **24** (68 mg, 34.9 μmol) and **12** (45 mg, 69.9 μmol) in dry CH_2Cl_2 (1.5 mL) was added powdered 4A MS (150 mg), and the mixture was stirred for 2 h at rt, then cooled to −40 °C. NIS (16 mg, 71.1 μmol) and TfOH (1.6 μL, 18.1 μmol) were added to the mixture, which was stirred for 2 h at −40 °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 ($MgSO_4$) and concentrated. The product was purified by silica gel column chromatography using 10:1 toluene–EtOAc as eluent to give **25** (23 mg, 26.6%). $[\alpha]_D^{24}$ +33.6 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.10 (d, 1H, $J_{1,2}$ 3.7 Hz, Fuc of *H*-1), 4.68 (d, 1H, $J_{1'',2''}$ 7.9 Hz, *H*-1″), 4.52 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.16 (d, 1H, $J_{1,2}$ 7.3 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{149}H_{165}NO_{32}$: *m*/*z* 2482. Found: *m*/*z* 2506 $[M+Na]^+$.

3.21. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-[α-L-fucopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-3-*O*-benzoyl-2-hexadecanamido-octadecane-1,3-diol (26)

A solution of **25** (23 mg, 9.3 μmol) in 2:1 THF–MeOH (1.5 mL) was hydrogenolyzed in the presence of 10% Pd–C (40 mg) for 15 h at rt, then filtered and concentrated. The product was purified by silica gel column chromatography using 30:1 toluene–acetone as eluent to give **26** (20 mg, 97.6%). MALDI-TOFMS: Calcd for $C_{128}H_{149}NO_{32}$: *m*/*z* 2212. Found: *m*/*z* 2235 $[M+Na]^+$.

3.22. β-D-Galactopyranosyl-(1→6)-[α-L-fucopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*)-2-hexadecanamido-octadecane-1,3-diol (4)

To a solution of **26** (20 mg, 9.04 μmol) in 1:1 MeOH–1,4-dioxane (1 mL) was added NaOMe (20 mg), and the mixture was stirred for 14 h at rt, 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 **4** (10.0 mg, 94.4%). $[\alpha]_D^{24}$ −11.0 (*c* 0.3, 1:1 $CHCl_3$–MeOH); ^1H NMR (49:1 DMSO-d_6–D_2O): δ 4.92 (d, 1H, $J_{1,2}$ 4.3 Hz, Fuc of *H*-1), 4.25 (d, 1H, $J_{1'',2''}$ 7.3 Hz, *H*-1″), 4.16 (d, 1H, $J_{1',2'}$ 7.3 Hz, *H*-1′), 4.07 (d, 1H, $J_{1,2}$ 7.3 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{58}H_{109}NO_{22}$: *m*/*z* 1171. Found: *m*/*z* 1194 $[M+Na]^+$. HRFABMS: Calcd for $C_{58}H_{109}NO_{22}Na$ $[M+Na]^+$: *m*/*z* 1194.7339. Found: *m*/*z* 1194.7284.

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References

1. Hada, N.; Hayashi, E.; Takeda, T. *Carbohydr. Res.* **1999**, *316*, 58–70.
2. Hada, N.; Kuroda, M.; Takeda, T. *Chem. Pharm. Bull.* **2000**, *48*, 1160–1165.
3. Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600–604.
4. Ohtsuka, I.; Hada, N.; Sugita, M.; Takeda, T. *Carbohydr. Res.* **2003**, *337*, 2037–2047.
5. Persat, F.; Bouhours, J.-F.; Mojon, M.; Petavy, A.-F. *J. Biol. Chem.* **1992**, *267*, 8764–8769.
6. Persat, F.; Vuncent, C.; Mojon, M.; Petavy, A. F. *Parasitol. Immunol.* **1991**, *13*, 379–389.
7. Persat, F.; Bouhours, J. F.; Mojon, M.; Francoise, A. *Mol. Biochem. Parasitol.* **1990**, *38*, 97–104.
8. Ito, Y.; Numata, M.; Sugimoto, M.; Ogawa, T. *J. Am. Chem. Soc.* **1989**, *111*, 8508–8510.
9. Numata, M.; Sugimoto, M.; Koike, K.; Ogawa, T. *Carbohydr. Res.* **1990**, *203*, 205–217.
10. Numata, M.; Sugimoto, M.; Koike, K.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 209–225.
11. Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43–69.
12. Zimmermann, P.; Greilich, U.; Schmidt, R. R. *Tetrahedron Lett.* **1990**, *31*, 1849–1852.
13. Schmidt, R. R.; Bar, T.; Apell, H. J. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 793–794.
14. Lassaletta, J. M.; Carlsson, K.; Garegg, P. J.; Schmidt, R. R. *J. Org. Chem.* **1996**, *61*, 6873–6880.

15. Lassaletta, J. M.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 4209–4212.
16. Kiso, M.; Nakamura, A.; Tomida, T.; Hasegawa, A. *Carbohydr. Res.* **1986**, *158*, 101–111.
17. Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. A. *Carbohydr. Res.* **1989**, *188*, 71–80.
18. Hotta, K.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1995**, *14*, 491–506.
19. Kameyama, A.; Ehara, T.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1995**, *14*, 507–523.
20. Nicolaou, K. C.; Caulfield, T. J.; Katoaka, H. *Carbohydr. Res.* **1990**, *202*, 177–191.
21. Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C.-H. *J. Org. Chem.* **2002**, *67*, 4559–4564.
22. Deshpande, P. P.; Kim, H. M.; Zatorski, A.; Park, T. K.; Ragupathi, G.; Livingston, P. O.; Live, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1600–1614.
23. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G.; Dahmen, J.; Noori, G.; Stenvall, K. *J. Org. Chem.* **1988**, *53*, 5629–5647.
24. Schmidt, R. R.; Grundler, G. *Synthesis* **1981**, 885–887.
25. Marra, A.; Esnault, J.; Veyrieres, A.; Sinay, P. *J. Am. Chem. Soc.* **1992**, *114*, 6354–6360.
26. Rio, S.; Beau, J.-M.; Jacwuinet, J.-C. *Carbohydr. Res.* **1991**, *219*, 71–90.
27. Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
28. Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833–1847.
29. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
30. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.
31. Koike, K.; Numata, M.; Sugimoto, M.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *158*, 113–123.