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Synthesis of some aglycon analogs of globotriosylceramide

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Abstract

Seven aglycon analogs of globotriosylceramide were synthesized by glycosylation of suitable functionalized alcohols with peracetylated globotriose trichloroacetimidate, followed by further transformations of the aglycon and removal of the protecting groups. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Globotriosylceramide aglycon analogs; Protection groups; Functionalized alcohols

1. Introduction

Globotriosylceramide [Gal-α-(1→4)Gal-β-(1→4)Glc-β-(1→Cer, Gb3] is present on the surface of virtually all mammalian cells, where it functions inter alia as receptor for microbes and toxins [1]. Of special interest here is the binding of *Escherichia coli*-derived verotoxin b subunits to Gb3 prior to toxin action by the a subunit [2]. In the preceding paper [3] we described the synthesis of some aminodeoxy globotriosides, designed to fit the receptor site of the verotoxin b subunit. However, since simple globotriosides do not bind verotoxin strongly enough to inhibit its binding to immobilized Gb3 in the microwell and thin-layer plate assays [4] (although its binding to the verotoxin b subunit could be demonstrated by microcalorimetry [5]), globotriosides with alternative aglycons might do so. The objectives of the present investigation were to find simple analogs of Gb3 that inhibit verotoxin binding at low concentrations and that are more soluble than Gb3 in aqueous solution. We report here the synthesis of some novel globotriosides (**1**–**7**, Fig. 1), the structural design being based on the preliminary observation [6] that semisynthetic **2** is an efficient inhibitor of verotoxin binding.

The exploitation of **1**–**7** as inhibitors of globotriose-binding proteins will be reported in due course.

2. Results and discussion

Synthesis of compounds **1**–**7**.—The syntheses of **1** and **3** were based on glycosylation of the corresponding alcohols with peracetylated globotriosyl trichloroacetimidate, whereas **2**, **4**, **5**, and **6** were obtained by N-acylation of 2-aminoethyl globotriosides with suitably activated carboxylic acids.

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Synthesis of aglycon alcohols.—1-Adamantaneacetic acid was activated by reaction with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC), and then treated with ethanolamine to furnish the crystalline compound **8** in 75% yield (Scheme 1).

Treatment of 1,3-bis-mercaptomethylbenzene with methyl acrylate in the presence of triethylamine gave the mono-S-alkylated ester **9** (70%), together with 10–15% of the corresponding di-S-alkylated product. Attempted alkylation with Cs_2CO_3 as base instead of triethylamine gave mainly the dialkylated product, probably due to low solubility in dimethyl formamide (DMF) of the initially formed cesium salt of di-1,3-mercaptomethylbenzene; the solubility of the monoalkylated product is higher, and consequently, it reacts faster with methyl acrylate. Compound **9** was then S-alkylated with 3-bromopropionic acid in the presence of Cs_2CO_3, to yield the ester–acid **10** (58%). Condensation of **10** with *N*-hydroxysuccinimide (NHS) in the presence of EDC furnished the NHS–ester **11** (79%), suitable for N-acylation of compounds **17** and **19** (Scheme 3).

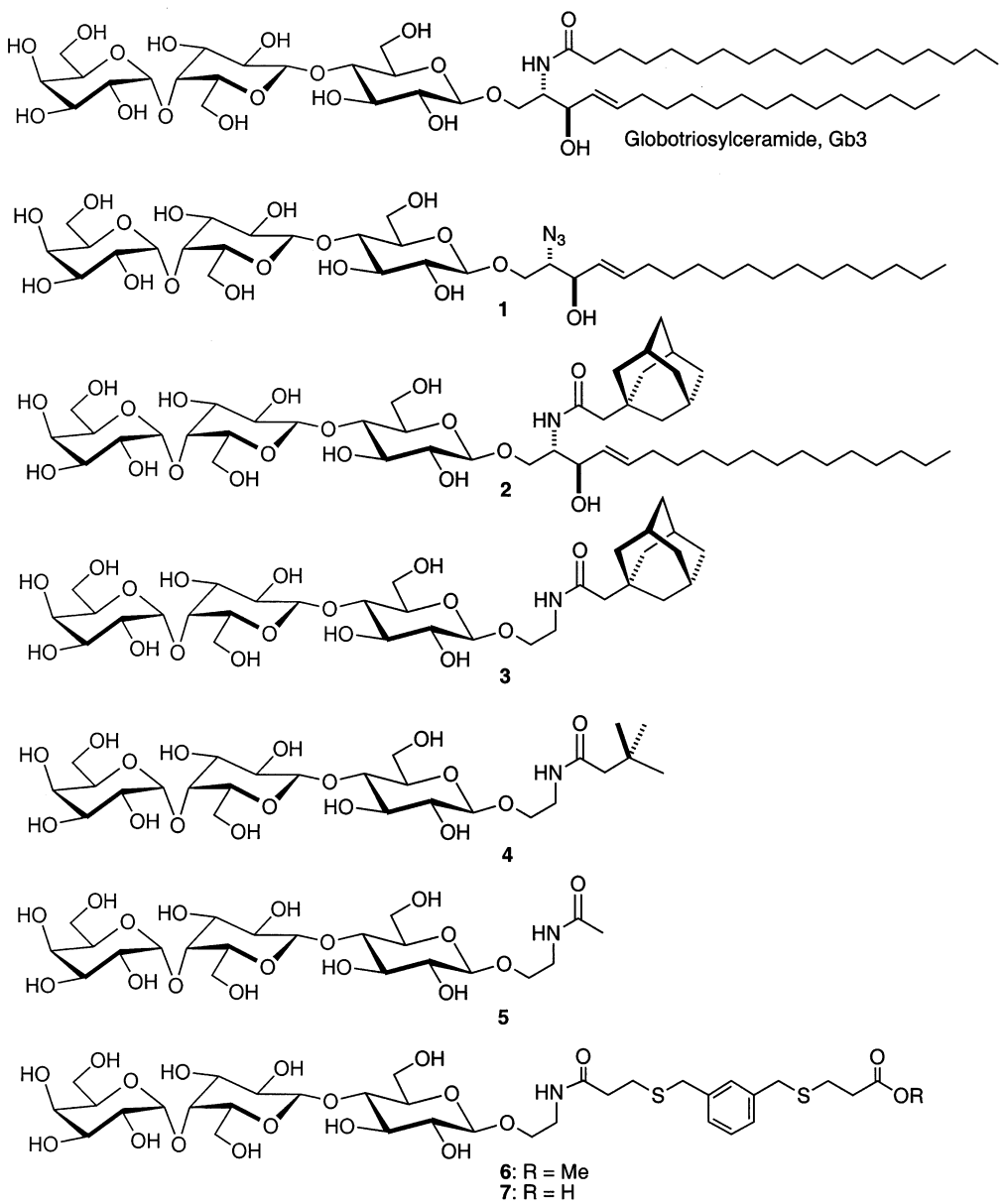
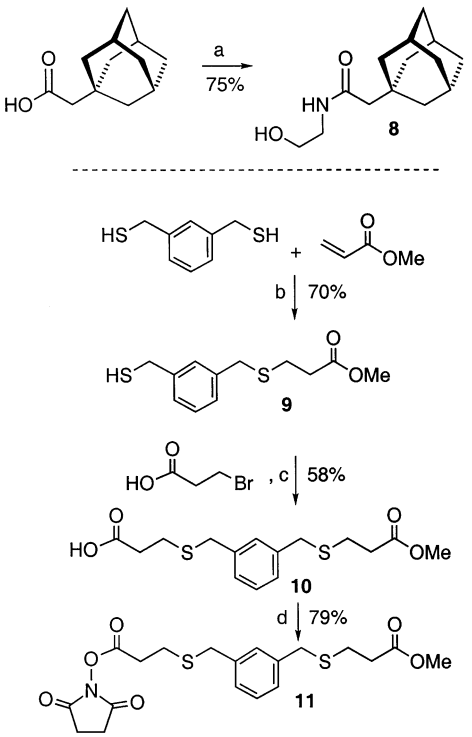


Fig. 1. Inhibitors of globotriose-binding proteins.



Scheme 1. (a) $HOCH_2CH_2NH_2$, EDC, CH_2Cl_2, N_2, 22 °C, 14 h. (b) Et_3N, DMF, 22 °C, 2.5 h. (c) Cs_2CO_3, DMF, 22 °C, 14 h. (d) NHS, EDC, CH_2Cl_2, N_2, 22 °C, 14 h.

Synthesis of compounds **1**–**3**.—Treatment of 3-*O*-benzoylated azidosphingosine (**13**) [7] with the trisaccharide trichloroacetimidate **12** [8] in the presence of $BF_3{\cdot}OEt_2$ gave the glycoside **14** in 66% yield (Scheme 2). The synthesis of **14** has been reported previously, but without full experimental details [9]. Similarly, treatment of compound **8** with trichloroacetimidate **12** in the presence of $BF_3{\cdot}OEt_2$ gave globotrioside **16** in 70% yield.

Reduction of the azido group of **14** by hydrogen sulfide gave the corresponding crude amine, which was then acylated with 1-adamantaneacetic acid in the presence of EDC, to give **15** in 79% yield.

O-Deacylation of **14**, **15**, and **16** with sodium methoxide–methanol gave compounds **1**–**3** in 95, 90, and 85% yield, respectively.

Synthesis of compounds **4**–**7**.—The known aminoethyl globotrioside **17** [8] (Scheme 3) was N-acylated with the NHS ester of *t*-butylacetic acid to give compound **4** (60%), with acetic anhydride to give **5** (100%), and with the NHS–ester **11** to give **6** (62%). Hydrolysis of the methyl ester **6** with aqueous sodium hydroxide then furnished the carboxylic acid **7** (98%).

An alternative route to compound **6** was also investigated. Catalytic hydrogenation of the azido group of the known [8] globotrioside **18** in a mixture of ethanol and one equivalent of hydrochloric acid, furnished the amine **19** in 78% yield. The presence of hydrochloric acid is crucial in such hydrogenations because the amine must be protonated as formed in order to avoid poisoning of the catalyst [3]. N-Acylation of **19** with the NHS–ester **11**, followed by O-deacetylation of the crude material with sodium methoxide–methanol, gave compound **6** in 79% yield.

The yield in N-acylations with the NHS–ester **11** was lower for the unprotected compound **17** than for O-acetylated **19** (62 versus 79%). The difference may be attributable to the difference in solubility, which is especially pronounced for larger saccharides. We therefore suggest that N-acylations of larger sugars with moderate solubility should be performed with the O-protected derivatives whenever this is convenient.

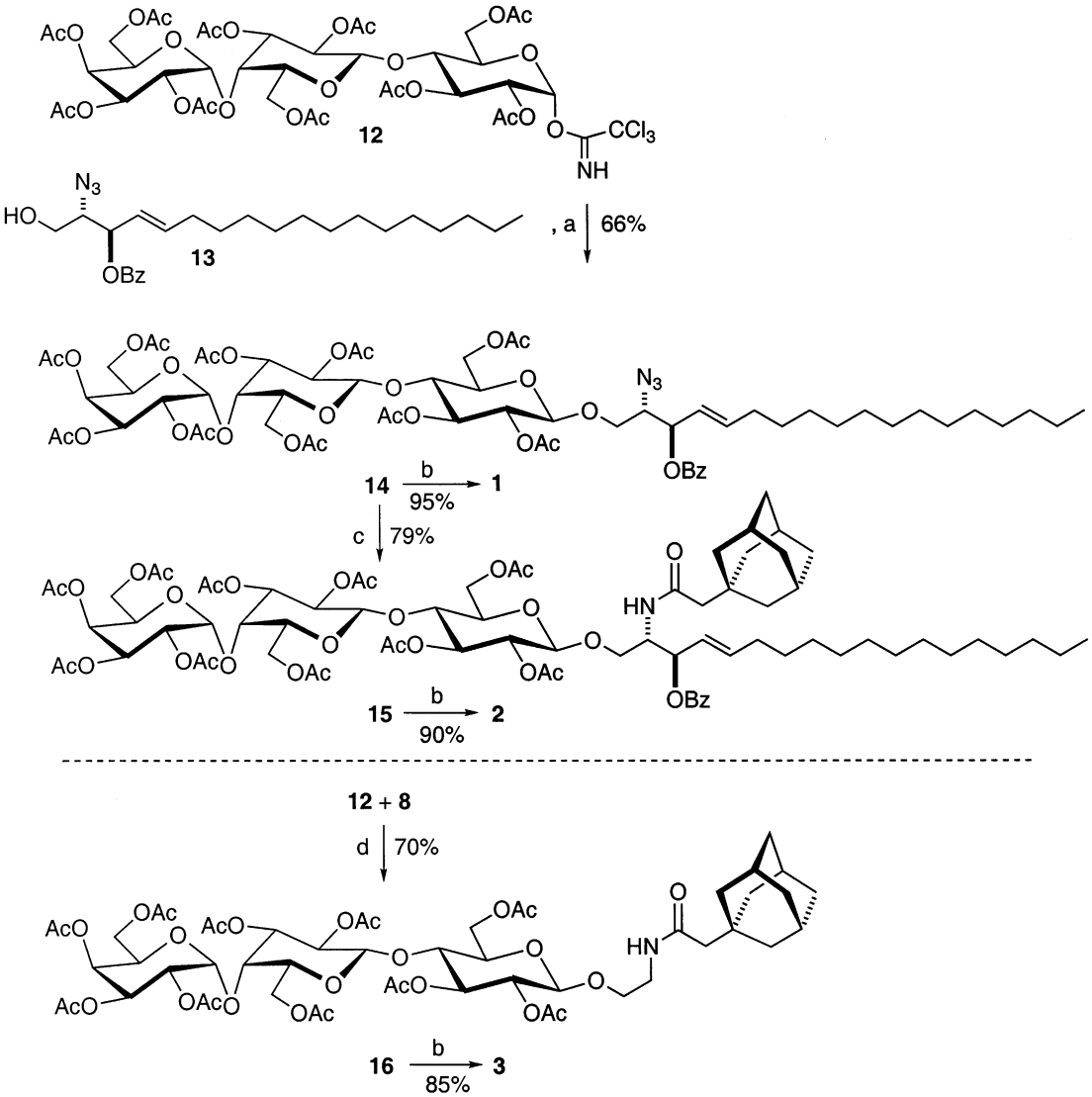
3. Experimental

General experimental procedures and methods were as described previously [3]. The compounds **12** [3,10], **13** [7], **14** [11], **17** [12], and **18** [3] have been reported.

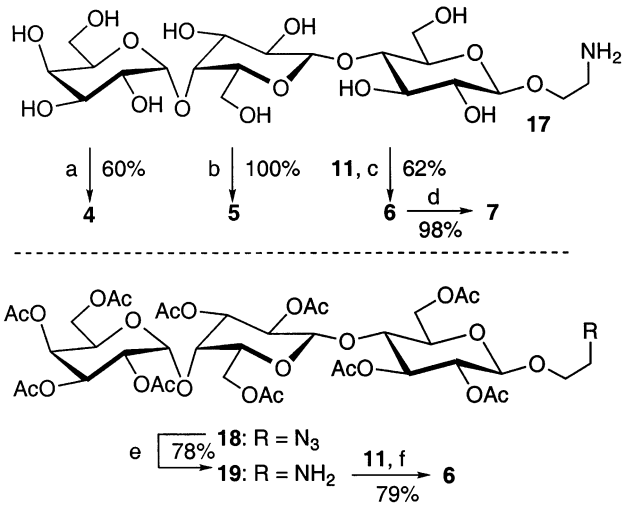
*(2*S*,3*R*,4*E*)-2-Azido-3-hydroxyoctadec-4-enyl (α-*D*-galactopyranosyl)-(1→4)-(β-*D*-gal-actopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**1**).—Compound **14** (7 mg, 0.005 mmol) was treated with 0.5 M NaOMe–MeOH for 16 h. The reaction mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18 (water–MeOH 1:0→4:1→3:2→2:3→1:4→0:1, 5 mL of each) to give **1** (4 mg, 95%); $[\alpha]_D^{22}$ +29° (*c* 0.4, MeOH); ^1H NMR (CD_3OD–D_2O 3:1): δ (assignments of aglycon protons are shown in italic) 5.73–5.83 (m, 1 H, *H-5*), 5.50 (dd, 1 H, *J* 7.7, 15.4 Hz, *H-4*), 4.93 (br s, 1 H, H-1″), 4.46 (d, 1 H, *J* 7.6 Hz, H-1′), 4.36 (d, 1 H, *J* 7.8 Hz,

H-1), 4.30 (br t, 1 H, *J* 6.3 Hz, H-5″), 4.17 (br t, 1 H, *J* 6.7 Hz, *H-3*), 4.00 (d, 1 H, *J* 2.8 Hz, H-4′), 3.79–3.97 (m, 8 H, incl. H-2″), 3.51–3.77 (m, 9 H, incl. H-2′,3′,3), 3.42–3.49 (m, 1 H), 3.30 (H-2 hidden under solvent peak), 2.00–2.11 (m, 2 H, CH_2CH=CH), 1.20–1.45 (m, 24 H, CH_2–), 0.89 (br t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (CD_3OD–D_2O 3:1): δ 136.6, 129.3, 105.1, 104.1, 102.4, 80.6, 79.4, 76.7, 76.4, 76.2, 74.6, 74.3, 73.5, 72.6, 71.1, 70.8, 70.4, 70.3, 67.1, 62.4, 61.6, 33.3, 33.0, 30.6, 30.5, 30.4, 30.1, 30.0, 23.7, 14.6; HRMS calcd for $C_{36}H_{65}N_3NaO_{17}$ [M + Na]: 834.4212. Found: 834.4207.

*(2*S*,3*R*,4*E*)-2-(1-Adamantaneacetamido)-3-hydroxy-octadec-4-enyl (α-*D*-galactopyranosyl)-(1→4)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**2**).—Compound **15** (18 mg, 0.012 mmol) was treated with 0.5 M NaOMe–MeOH for 16 h. The mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, water–MeOH 4:1 → 3:2 → 1:1 → 2:3 → 3:7 → 1:4 → 0:1, 4 mL of each) to give **2** (10.4 mg, 90%); $[\alpha]_D^{22}$ + 36° (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD–D_2O 2:1): δ (assignments of aglycon protons are shown



Scheme 2. (a) Molecular sieves, CH_2Cl_2, 30 min, then $BF_3{\cdot}OEt_2$, N_2, 22 °C, 1.5 h. (b) NaOMe, MeOH. (c) H_2S, pyridine, water, 0 °C, 1 h, then 22 °C, 48 h, then EDC, 1-adamantaneacetic acid, CH_2Cl_2, 22 °C, 8 h. (d) Molecular sieves, CH_2Cl_2, 20 min, then $BF_3{\cdot}OEt_2$, 22 °C, 1.5 h.



Scheme 3. (a) Me_3CCH_2COOH, NHS, EDC, CH_2Cl_2, 22 °C, 14 h. (b) Ac_2O, MeOH, 22 °C, 14 h. (c) DMF, pyridine, 60 °C, 4.5 h. (d) NaOH, water, 22 °C, 5.5 h. (e) H_2, Pd–C, EtOH, aq HCl, 2 h. (f) Et_3N, CH_2Cl_2, 22 °C, 3 h, then NaOMe–MeOH, 22 °C, 6 h.

in italic) 7.82 (d, 1 H, *J* 9.0 Hz, NH), 5.69–5.78 (m, 1 H, *H-5*), 5.46 (br dd, 1 H, *J* 7.8, 15.3 Hz, *H-4*), 4.45 (d, 1 H, *J* 7.7 Hz, H-1′), 4.35 (d, 1 H, *J* 7.8 Hz, H-1), 4.30 (br t, 1 H, *J* 6.6 Hz, H-5″), 4.17 (dd, 1 H, *J* 4.9, 10.4 Hz), 4.09 (t, 1 H, *J* 8.4 Hz, *H-3*), 3.79–4.02 (m, 8 H), 3.44–3.75 (m, 9 H), 1.90–2.05 (m, 7 H), 1.59–1.75 (m, 12 H), 1.20–1.41 (m, 24 H), 0.88 (t, 3 H, *J* 6.8 Hz, *CH*$_3$); ^{13}C NMR (CD_3OD–D_2O 2:1): δ 174.1, 136.1, 131.0, 105.1, 104.3, 102.3, 80.7, 79.4, 76.7, 76.3, 76.0, 74.7, 74.2, 72.6, 71.0, 70.7, 70.3, 62.3, 61.6, 54.9, 52.1, 43.7, 37.9, 34.0, 33.3, 33.0, 30.6, 30.3, 30.1, 30.0, 23.7, 14.6; HRMS calcd for $C_{48}H_{83}NNaO_{18}$ [M + Na]: 984.5508. Found: 984.5485.

*2-(1-Adamantaneacetamido)ethyl (α-*D*-galactopyranosyl)-(1→4)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**3**).—Compound **16** (20 mg, 0.018 mmol) was treated with 0.5 M NaOMe–MeOH for 14 h. The mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, water–MeOH 1:0→9:1→4:1→7:3→3:2→1:1→2:3, 5 mL of each) to give **3** (10.8 mg, 85%); $[\alpha]_D^{25}$ + 51° (*c* 1.0, water); ^{1}H NMR (D_2O): δ 4.81 (d, 1 H, *J* 3.9 Hz, H-1″), 4.36 (d, 2 H, *J* 7.9 Hz, H-1,1′), 4.22 (br t, 1 H, *J* 6.5 Hz, H-5″), 3.15–3.92 (m, 21 H), 1.88 (s, 2 H, CH_2CO), 1.77–1.85 (m, 3 H), 1.42–1.61 (m, 12 H); ^{13}C NMR (D_2O): δ 175.3, 103.6, 102.5, 100.7, 79.1, 77.7, 75.8, 75.1, 74.7, 73.3, 72.5, 71.24, 71.15, 69.5, 69.3, 68.9, 68.8, 60.8, 60.7, 60.5, 51.0, 42.5, 39.4, 36.5, 32.8, 28.7; HRMS calcd for $C_{32}H_{53}NNaO_{17}$ [M + Na]: 746.3211. Found: 746.3243.

*2-(*t*-Butylacetamido)ethyl (α-*D*-galactopyranosyl)-(1→4)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**4**).—*t*-Butylacetic acid (0.064 mL, 0.5 mmol) was dissolved in CH_2Cl_2 (6 mL), *N*-hydroxysuccinimide (NHS, 288 mg, 2.5 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC, 480 mg, 2.5 mmol) were added, and the reaction mixture was stirred at room temperature (rt) for 14 h. The mixture was washed with water, dried, and concd. The residue was chromatographed (SiO_2, 2:1 EtOAc–heptane) to give the NHS–ester of *t*-BuCH_2CO (102 mg, 96%); ^{1}H NMR ($CDCl_3$): δ 2.80 (m, 4 H, $COCH_2CH_2CO$), 2.44 (s, 2 H, *t*-Bu*CH*$_2$CO), 1.09 (s, 9 H, CMe_3); ^{13}C NMR ($CDCl_3$): δ 169.7, 167.3, 44.9, 31.6, 29.8, 26.0. The NHS–ester (8.2 mg, 0.038 mmol) and **17** (7 mg, 0.013 mmol) were dissolved in dry pyridine (2 mL) at 65 °C. The mixture was stirred for 12 h and concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, 1:0→9:1→4:1→7:3→3:2→1:1 water–MeOH, 5 mL of each) to give **4** (5 mg, 60%); $[\alpha]_D^{22}$ + 35° (*c* 0.4, water); ^{1}H NMR (D_2O): δ 4.81 (d, 1 H, *J* 3.9 Hz, H-1″), 4.36, 4.35 (d, 1 H each, *J* 7.7 and 8.0 Hz, H-1 and H-1′), 4.22 (t, 1 H, *J* 6.6 Hz, H-5″), 3.15–3.93 (m, 21 H), 2.00 (s, 2 H, CH_2CO), 0.85 (s, 9 H, CMe_3); ^{13}C NMR (D_2O): δ 176.1, 103.6, 102.5, 100.7, 79.0, 77.7, 75.8, 75.1, 74.7, 73.2, 72.5, 71.23, 71.15, 69.5, 69.3, 68.9, 60.8, 60.7, 60.4, 49.7, 39.4, 30.6, 29.4; HRMS calcd for $C_{26}H_{47}NNaO_{17}$ [M + Na]: 668.2742. Found: 668.2722.

*2-(Acetamido)ethyl (α-*D*-galactopyranosyl)-(1→4)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**5**).—To a solution of **17** (3 mg, 0.006 mmol) in MeOH (2 mL) was added Ac_2O (0.1 mL). The mixture was stirred at rt for 14 h, and the mixture was concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, water–MeOH 1:0→9:1→4:1→7:3→3:2→

1:1, 5 mL of each) to give **5** (3.2 mg, 100%); $[\alpha]_D^{23}$ +35° (*c* 0.3, water); ^{1}H NMR (D_2O): δ 4.79 (d, 1 H, *J* 3.8 Hz, H-1″), 4.35, 4.34 (d, 1 H each, *J* 7.8 and 8.0 Hz, H-1 and H-1′), 4.20 (br t, 1 H, *J* 6.5 Hz, H-5″), 3.39–3.90 (m, 19 H), 3.24–3.30 (m, 1 H, CH_2NH), 3.13–3.19 (m, 1 H, CH_2NH), 1.84 (s, 3 H, CH_3CO); ^{13}C NMR (D_2O): δ 174.5, 103.6, 102.5, 100.6, 78.9, 77.7, 75.8, 75.1, 74.7, 73.2, 72.5, 71.2, 71.1, 69.4, 69.2, 68.9, 60.8, 60.7, 60.3, 39.7, 23.6, 22.2; HRMS calcd for $C_{22}H_{39}NNaO_{17}$ [M + Na]: 612.2116. Found: 612.2122.

5-[3-(4-Methoxycarbonyl-2-thiabutyl)-phenyl]-4-pentanecarboxamidoethyl (α-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (**6**).—(a) The NHS–ester **11** (7.4 mg, 0.016 mmol) and compound **17** (10 mg, 0.018 mmol) were dissolved in a mixture of freshly distilled DMF (1 mL) and pyridine (1 mL). The reaction mixture was stirred at 60 °C for 4.5 h and the mixture was concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, 1:0→9:1→4:1→7:3→3:2→1:1 water–MeOH, 5 mL of each) to give **6** (8.5 mg, 62%).

(b) Compound **19** (11 mg, 0.011 mmol) and the NHS–ester **11** (6.4 mg, 0.015 mmol) were dissolved in CH_2Cl_2 (3 mL) and Et_3N (0.020 mL). The mixture was stirred at rt for 3 h and concd. The residue was chromatographed (3:1:1 EtOAc–heptane–MeOH) to give O-acetylated **6** (12 mg), part of which (11 mg, 0.009 mmol) was then deacetylated with 0.5 M NaOMe–MeOH. After 6 h, the mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concd. The residue was chromatographed on a reversed-phase column (Varian Mega Bond Elut C18, 1:0→9:1→4:1→7:3→3:2→1:1 water–MeOH, 5 mL of each) to give **6** (6.3 mg, 79% based on **19**); $[\alpha]_D^{23}$ +34° (*c* 0.8, water); ^{1}H NMR (D_2O): δ 7.12–7.30 (4 H, Ar), 4.82 (d, 1 H, *J* 3.7 Hz, H-1″), 4.36, 4.35 (d, 1 H each, *J* 7.8 Hz, H-1 or H-1′), 4.23 (br t, 1 H, *J* 6.6 Hz, H-5″), 3.15–3.93 (m, 24 H), 2.53–2.62 (m, 4 H), 2.49, 2.39 (br t, 4 H each, *J* 6.6 Hz); ^{13}C NMR (D_2O): δ 175.5, 174.9, 139.2, 129.6, 129.5, 128.1, 103.6, 102.5, 100.7, 79.0, 77.7, 75.8, 75.1, 74.7, 73.2, 72.5, 71.22, 71.15, 69.5, 69.2, 68.9, 68.8, 60.8, 60.7, 60.4, 52.6, 39.7, 35.7, 35.3, 34.2, 26.9, 26.0; HRMS calcd for $C_{35}H_{56}NO_{19}S_2$ [M + H]: 858.2888. Found: 858.2900.

5-[3-(4-Carboxy-2-thiabutyl)-phenyl]-4-pentanecarboxamidoethyl (α-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (**7**).—To a solution of **6** (6 mg, 0.007 mmol) in water (1 mL) was added aq 0.5 M NaOH (0.07 mL, 0.035 mmol). The mixture was stirred at rt for 5.5 h and the mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concd to give **7** (5.8 mg, 98%); $[\alpha]_D^{23}$ +16° (*c* 0.7, water); ^{1}H NMR (D_2O): δ 7.11–7.25 (m, 4 H, Ar), 4.79 (d, 1 H, *J* 3.9 Hz, H-1″), 4.33, 4.32 (d, 1 H each, *J* 7.7 and 8.0 Hz, H-1 and H-1′), 4.20 (br t, 1 H, *J* 6.4 Hz, H-5″), 3.12–3.91 (m, 21 H), 2.48–2.61 (m, 6 H), 2.30–2.39 (m, 6 H); ^{13}C NMR (D_2O): δ 175.0, 139.3, 139.2, 129.7, 129.5, 128.1, 128.0, 103.6, 102.5, 100.7, 79.0, 77.7, 75.8, 75.1, 74.6, 73.2, 72.5, 72.4, 71.2, 71.1, 68.4, 69.2, 68.9, 68.8, 62.8, 60.8, 60.7, 39.7, 35.7, 35.3, 35.2, 27.1, 26.9; HRMS calcd for $C_{34}H_{54}NO_{19}S_2$ [M + H]: 844.2731. Found: 844.2715; HRMS calcd for $C_{34}H_{53}NNaO_{19}S_2$ [M − H + Na]: 866.2551. Found: 866.2539.

(2-Hydroxyethyl)-1-adamantaneacetamide (**8**).—1-Adamantaneacetic acid (100 mg, 0.515 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and EDC (200 mg, 1.03 mmol) was added at 0 °C under N_2. The mixture was stirred for 1 h and $EtONH_2$ (0.085 mL, 0.64 mmol) was added. The reaction mixture was stirred at rt for 14 h, filtered, and concd. The residue was chromatographed (10:1 EtOAc–MeOH) to give **8** (92 mg, 75%); mp 106–109 °C (EtOAc–heptane); ^{1}H NMR ($CDCl_3$): δ 6.10 (br s, 1 H, NH), 3.72 (br t, 2 H, *J* 4.9 Hz, CH_2O), 3.42 (br q, 2 H, *J* 5.2 Hz, CH_2NHCO), 3.32 (br s, 1 H, OH), 1.94–2.00 (m, 5 H), 1.59–1.74 (m, 12 H); ^{13}C NMR ($CDCl_3$): δ 172.9, 63.0, 52.1, 43.0, 42.9, 37.1, 33.2, 29.0; HRMS calcd for $C_{14}H_{24}NO_2$ [M + H]: 238.1807. Found: 238.1802.

1-Mercaptomethyl-3-(4-methoxycarbonyl-2-thiabutyl)-benzene (**9**).—A mixture of methyl acrylate (0.048 mL, 0.53 mmol), Et_3N (0.074 mL, 0.53 mmol) and DMF (0.9 mL) was added dropwise (2 h) to a mixture of 1,3-bismercaptomethylbenzene (0.087 mL, 0.587 mmol) and DMF (1 mL). The mixture was

stirred for 30 min, water (3 mL) was added, and the reaction mixture was extracted with Et_2O (2 × 10 mL). The organic phase was dried and concd and the residue was chromatographed (1:5 EtOAc–heptane) to give **9** (95 mg, 70%); ^{1}H NMR ($CDCl_3$): δ 7.17–7.31 (m, 4 H, Ar), 3.72–3.76 (m, 4 H, ArCH_2S), 3.69 (s, 3 H, OCH_3), 2.70 (dt, 2 H, *J* 1.1, 7.4 Hz, CH_2CH$_2$), 2.57 (dt, 2 H, *J* 1.1, 7.2 Hz, CH_2CH$_2$), 1.78 (t, 1 H, *J* 7.6 Hz, SH); ^{13}C NMR ($CDCl_3$): δ 172.7, 142.0, 139.0, 129.3, 128.9, 128.0, 127.3, 52.3, 36.6, 34.7, 29.3, 26.7. The dialkylated diester was isolated as a byproduct in 10–15% yield. Selected data: ^{1}H NMR ($CDCl_3$): δ 7.18–7.30 (m, 4 H, Ar), 3.72 (s, 4 H, ArCH_2S), 3.69 (s, 6 H, OCH_3), 2.69, 2.56 (t, 4 H each, *J* 7.0 Hz, CH_2CH$_2$); ^{13}C NMR ($CDCl_3$): δ 172.7, 138.9, 129.7, 129.2, 128.1, 52.2, 36.6, 34.7, 26.7

1-(4-Carboxy-2-thiabutyl)-3-(4-methoxycarbonyl-2-thiabutyl)-benzene (**10**).—A mixture of **9** (90 mg, 0.35 mmol), 3-bromopropionic acid (80 mg, 0.52 mmol) and Cs_2CO_3 (320 mg, 0.99 mmol) in dry DMF (3 mL) was stirred at rt for 14 h, and AcOH (1 mL) and CH_2Cl_2 (20 mL) were added. The mixture was washed with water (3 × 5 mL) and the organic phase was dried and concd. The residue was chromatographed (7:7:2 toluene–EtOAc–heptane → 2:1 toluene–EtOAc + 2% AcOH) to give **10** (67 mg, 58%); ^{1}H NMR ($CDCl_3$): δ 7.18–7.31 (m, 4 H, Ar), 3.75 (s, 2 H, ArCH_2S), 3.73 (s, 2 H, ArCH_2S), 3.70 (s, 3 H, OCH_3), 2.66–2.72 (m, 4 H, CH_2CH$_2$), 2.54–2.63 (m, 4 H, CH_2CH$_2$); ^{13}C NMR ($CDCl_3$): δ 177.1, 173.0, 138.9, 138.8, 129.7, 129.3, 128.13, 128.08, 52.3, 36.6, 34.7, 34.6, 26.6, 26.3; HRMS calcd for $C_{15}H_{21}O_4S_2$ [M + H]: 329.0881. Found: 329.0893.

1-(4-Succinimidoxycarbonyl-2-thiabutyl)-3-(4-methoxycarbonyl-2-thiabutyl)-benzene (**11**).—The monoester **10** (7.3 mg, 0.022 mmol) was dissolved in dry CH_2Cl_2 (2 mL), and NHS (5.7 mg, 0.05 mmol) and EDC (9.2 mg, 0.048 mmol) were added. The reaction mixture was stirred at rt under N_2 for 14 h, CH_2Cl_2 (10 mL) was added, and the mixture was washed with water (3 mL). The organic phase was dried and concd and the residue was chromatographed (2:1 EtOAc–heptane) to give **11** (7.4 mg, 79%); ^{1}H NMR ($CDCl_3$): δ 7.20–7.32 (m, 4 H, Ar), 3.77, 3.74 (s, 2 H each, ArCH_2S), 3.69 (s, 3 H, OCH_3), 2.75–2.88 (m, 8 H), 2.66–2.72 (m, 2 H, CH_2CH$_2$), 2.54–2.60 (m, 2 H, CH_2CH$_2$).

*(2*S*,3*R*,4*E*)-2-Azido-3-benzoyloxyoctadec-4-enyl (2,3,4,6-tetra*-O-*acetyl-*α-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*acetyl-*β-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-*β-D-*glucopyranoside* (**14**).—To a solution of trichloroacetimidate **12** [3,10] (30 mg, 0.028 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-benzoyloxy-4-octadecen-1-ol [7] (**13**, 12 mg, 0.028 mmol) in dry CH_2Cl_2 (3 mL) was added molecular sieves (AW-300). The mixture was stirred for 30 min, $BF_3{\cdot}OEt_2$ (0.003 mL, 0.024 mmol) was added, and the reaction mixture was stirred at rt under N_2 for 1.5 h The mixture was filtered through Celite and the filtrate was washed with satd aq $NaHCO_3$, dried, and concd. The residue was chromatographed (1:1 EtOAc–heptane) to give **14** [11] (25 mg, 66%). ^{1}H NMR ($CDCl_3$): δ (assignments of aglycon protons are shown in italic) 8.03–8.08 (m, 2 H, Ar), 7.55–7.62 (m, 1 H, Ar), 7.43–7.49 (m, 2 H, Ar), 5.93 (dt, 1 H, *J* 6.8, 15.0 Hz, *H-5*), 5.50–5.63 (m, 3 H, H-4″, *H-3*, *H-4*), 5.39 (dd, 1 H, *J* 3.3, 11.0 Hz, H-3″), 5.21 (t, 1 H, *J* 9.0 Hz, H-3), 5.19 (dd, 1 H, *J* 3.6, 11.1 Hz, H-2″), 5.11 (dd, 1 H, *J* 7.8, 10.8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.6 Hz, H-1″), 4.93 (dd, 1 H, *J* 7.7, 9.1 Hz, H-2), 4.74 (dd, 1 H, *J* 2.5, 10.9 Hz, H-3′), 4.54 (d, 1 H, *J* 7.6 Hz, H-1), 4.52 (d, 1 H, *J* 7.7 Hz, H-1′), 4.39–4.52 (m, 3 H, H-5″,6′,6), 4.02–4.21 (m, 4 H, incl. H-6,6′), 4.01 (br d, 1 H, *J* 2.0 Hz, H-4′), 3.84–3.97 (m, 2 H, *H-1,2*), 3.83 (t, 1 H, *J* 9.3 Hz, H-4), 3.76 (t, 1 H, *J* 6.8 Hz, H-5′), 3.64 (ddd, 1 H, *J* 2.0, 5.1, 9.9 Hz, H-5), 3.59 (dd, 1 H, *J* 5.8, 10.3 Hz, *H-1)*, 2.135, 2.090, 2.084, 2.072, 2.066, 2.058, 2.042, 2.040, 1.99 (s, 30 H, Ac), 1.19–1.44 (m, 24 H), 0.88 (t, 3 H, *J* 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.1, 170.93, 170.91, 170.8, 170.5, 170.14, 170.08, 170.0, 169.3, 165.5, 139.5, 133.6, 130.3, 130.2, 128.9, 123.0, 101.6, 100.7, 100.1, 77.4, 76.8, 75.1, 73.6, 73.2, 73.0, 72.2, 72.0, 69.4, 69.3, 68.8, 68.3, 67.54, 67.50, 63.9, 62.5, 61.7, 60.7, 32.8, 32.3, 30.10, 30.08, 30.0, 29.82, 29.78, 29.6, 29.1, 23.1, 21.4, 21.2, 21.13, 21.08, 21.0, 20.9, 14.6.

*(2*S*,3*R*,4*E*)-2-(1-Adamantaneacetamido)-3-(benzoyloxy)-octadec-4-enyl (2,3,4,6-tetra*-O-

acetyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**15**). —Hydrogen sufide was bubbled through a solution of **14** (22 mg, 0.0165 mmol) in a pyridine–water mixture (6:1, 6 mL) for 1 h at 0 °C, and the reaction mixture was kept under H_2S at 22 °C for 48 h. Residual H_2S was removed by a stream of N_2 for 1 h, and the mixture was co-concd with toluene. The residue was dissolved in dry CH_2Cl_2 (2 mL) and EDC (20 mg, 0.1 mmol) and 1-adamantaneacetic acid (20 mg, 0.1 mmol) were added. The mixture was stirred at rt for 8 h and concd. The residue was chromatographed (2:1 EtOAc–heptane) to give **15** (19.4 mg, 79%); $[\alpha]_D^{24}$ + 35° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (assignments of aglycon protons are shown in italic) 8.00–8.05, 7.53–7.60, 7.41–7.48 (m, 5 H, Ar), 5.89 (dt, 1 H, *J* 6.7, 15.3 Hz, *H-5*), 5.66 (d, 1 H, *J* 9.0 Hz, NH), 5.56–5.62 (m, 2 H, H-4″, *H-3*), 5.45–5.54 (m, 1 H, *H-4*), 5.39 (dd, 1 H, *J* 3.4, 11.0 Hz, H-3″), 5.15–5.23 (m, 2 H, H-2″,3), 5.10 (dd, 1 H, *J* 7.8, 10.8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.6 Hz, H-1″), 4.91 (dd, 1 H, *J* 7.8, 9.3 Hz, H-2), 4.73 (dd, 1 H, *J* 2.5, 10.8 Hz, H-3′), 4.49 (d, 1 H, *J* 7.7 Hz, H-1′), 4.48 (d, 1 H, *J* 7.7 Hz, H-1), 4.40–4.53 (m, 3 H, H-5″,6′, *H-2*), 4.36 (br dd, 1 H, *J* 1.8, 12.0 Hz, H-6), 4.07–4.21 (m, 3 H, H-6′,6″), 3.95–4.05 (m, 3 H, H-4′,6, *H-1*), 3.73–3.82 (m, 2 H, H-4,5′), 3.56–3.68 (m, 2 H, H-5, *H-1*), 1.85–2.15 (35 H), 1.56–1.72 (m, 12 H), 1.20–1.39 (m, 24 H), 0.89 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.1, 170.91, 170.86, 170.8, 170.5, 170.2, 170.04, 169.96, 169.3, 165.6, 137.9, 133.5, 130.7, 130.0, 128.8, 125.0, 101.6, 100.7, 100.1, 76.8, 74.3, 73.32, 73.26, 73.0, 72.3, 69.3, 68.3, 68.0, 67.6, 67.5, 62.6, 61.8, 60.7, 52.3, 51.3, 43.0, 37.1, 33.2, 32.8, 32.4, 30.1, 30.05, 29.9, 29.8, 29.7, 29.3, 29.0, 23.1, 21.4, 21.24, 21.17, 21.1, 21.0, 20.9, 14.6; HRMS calcd for $C_{75}H_{107}NNaO_{29}$ [M + Na]: 1508.6826. Found: 1508.6818.

2-(1-Adamantaneacetamido)ethyl (2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**16**). —To a solution of **8** (7.3 mg, 0.031 mmol) and **12** (30 mg, 0.028 mmol) in dry CH_2Cl_2 (3 mL) was added molecular sieves (AW-300). The mixture was stirred for 20 min and $BF_3{\cdot}OEt_2$ (0.003 mL, 0.024 mmol) was added. The mixture was stirred for 1.5 h, then filtered through Celite, and CH_2Cl_2 (10 mL) was added. The mixture was washed with a satd aq $NaHCO_3$, dried, and concd. The residue was chromatographed (3:1→4:1 EtOAc–heptane) to give **16** (22.5 mg, 70%); $[\alpha]_D^{23}$ + 37° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.82 (br t, 1 H, *J* 4.9 Hz, NH), 5.59 (br d, 1 H, *J* 3.3 Hz, H-4″), 5.39 (dd, 1 H, *J* 3.4, 11.0 Hz, H-3″), 5.20 (t, 1 H, *J* 9.2 Hz, H-3), 5.18 (dd, 1 H, *J* 3.5, 11.0 Hz, H-2″), 5.11 (dd, 1 H, *J* 7.7, 10.8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.6 Hz, H-1″), 4. 89 (dd, 1 H, *J* 8.0, 9.5 Hz, H-2), 4.74 (dd, 1 H, *J* 2.8, 10.9 Hz, H-3′), 4.52 (d, 1 H, *J* 7.7 Hz, H-1′), 4.48 (d, 1 H, *J* 7.9 Hz, H-1), 4.41–4.55 (m, 3 H, incl. H-5″), 4.06–4.20 (m, 4 H), 4.02 (br d, 1 H, *J* 2.3 Hz, H-4′), 3.60–3.84 (m, 5 H, incl. H-4, OCH_2CH_2), 3.35–3.52 (m, 2 H, CH_2NH), 1.91–2.15 (35 H), 1.58–1.74 (m, 12 H, adamantyl–CH_2); ^{13}C NMR ($CDCl_3$): δ 171.5, 171.1, 170.94, 170.90, 170.88, 170.86, 170.5, 170.3, 170.03, 170.02, 169.3, 101.5, 101.2, 100.1, 77.3, 76.7, 73.4, 73.2, 72.22, 72.19, 69.9, 69.4, 69.3, 68.3, 67.6, 67.5, 62.5, 61.7, 60.7, 52.0, 43.03, 42.99, 39.5, 37.2, 33.1, 29.0, 21.4, 21.3, 21.23, 21.15, 21.11, 21.08, 21.04, 20.95; HRMS calcd for $C_{52}H_{73}NNaO_{27}$ [M + Na]: 1166.4268. Found: 1166.4271.

2-Aminoethyl (2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**19**). —Compound **18** [3] (14 mg, 0.014 mmol) was dissolved in EtOH (2 mL) and a drop of aq 0.1 M HCl was added. The mixture was hydrogenated (H_2, Pd/C, 1 atm) for 2 h, then filtered through Celite and concd. The residue was chromatographed (2:1 EtOAc–MeOH + 5% Et_3N) to give **19** (10.7 mg, 78%); $[\alpha]_D^{22}$ + 51° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$–CD_3OD 1:1): δ 5.27 (br d, 1 H, *J* 3.3 Hz, H-4″), 5.09 (dd, 1 H, *J* 3.4, 11.1 Hz, H-3″), 4.85–4.93 (m, 2 H, H-3,2″), 4.81 (dd, 1 H, *J* 7.8, 10.9 Hz, H-2′), 4.73 (d, 1 H, *J* 3.6 Hz, H-1″), 4.53–4.61 (m, 2 H), 4.21 (br t, 1 H, *J* 6.8 Hz, H-5″), 4.16 (dd, 1 H, *J* 6.4, 11.1 Hz, H-6′ or H-6), 3.76–3.92 (m, 4 H), 3.52–3.70 (m, 3 H), 3.40–3.46 (m, 1 H), 1.85, 1.84, 1.80, 1.79, 1.78, 1.77, 1.760,

1.755, 1.69 (s, 30 H, Ac); ^{13}C NMR (CD_3OD): δ 172.4, 172.3, 172.1, 172.0, 171.94, 171.91, 171.8, 171.5, 171.4, 171.2, 102.1, 101.9, 100.8, 78.8, 77.3, 74.32, 74.30, 74.0, 73.5, 72.8, 70.9, 69.8, 69.5, 69.0, 68.9, 68.5, 63.7, 63.3, 61.8, 47.7, 21.2, 21.0, 20.79, 20.75, 20.7, 20.6, 20.5; HRMS calcd for $C_{40}H_{57}NNaO_{26}$ (M + Na): 990.3067. Found: 990.3066.

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