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Total synthesis of glyconononaosyl ceramide with a sialyl dimeric Le^x sequence*

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The first total synthesis of glyconononaosyl ceramide with a sialyl dimeric Le^x sequence 1 is described. Regio- and stereo-selective glycosylations of sialyl donors 6,7,8 with the suitably protected Le^x trisaccharide acceptors 9,10β were performed to give the expected tetrasaccharides 15 and 21, which were converted into the corresponding donors 20 and 22. Boron trifluoride etherate-promoted glycosylation of 20 with pentasaccharide acceptor 11 afforded regioselectively the expected nonasaccharide 23. After replacing benzyl groups of 23 by acetyl groups, the anomeric acetate was transformed into the α-trichloroacetimidate 27. The crucial coupling between 27 and (2S, 3R, 4E-3-*O*-benzoyl-2-*N*-tetracosanoylsphingenine 3 was executed to afford completely protected β-glycoside 28. Finally, selective cleavage of the methyl ester and N,O-deprotection of 28 gave the target ganglioside 1.

Keywords: E-selectin; P-selectin, sialyl dimeric Le^x, glycolipid, synthesis

Introduction

In 1984, Fukushi and Hakomori reported the isolation and characterization of sialyl dimeric Le^x glycolipid 1 from human colonic adenocarcinomas by methylation analyses, direct probe mass spectrometry, and monoclonal antibodies scrutiny [1]. Sialyl dimeric Le^x glycolipids which were prepared from precursors derived from bovine erythrocytes exhibit high potency [2] for the E-selectin (endothelial-leukocyte adhesion molecule-1) and P-selectin (formerly called granule membrane protein 140 or GMP-140) binding molecules [3] but no one has synthesized it yet due to its molecular complexity, although the carbohydrate portion was synthesized by Nicolaou [4]. Aiming at further biochemical studies of **1**, an efficient chemical synthesis is required. As part of our project on the synthesis of glycosphingolipids with biological significance, we describe herein a stereocontrolled first total synthesis of sialyl dimeric Le^x glyconononaosyl ceramide **1** [5].

* Dedicated to Professor Sen-itiroh Hakomori on the occasion of his 65th birthday.

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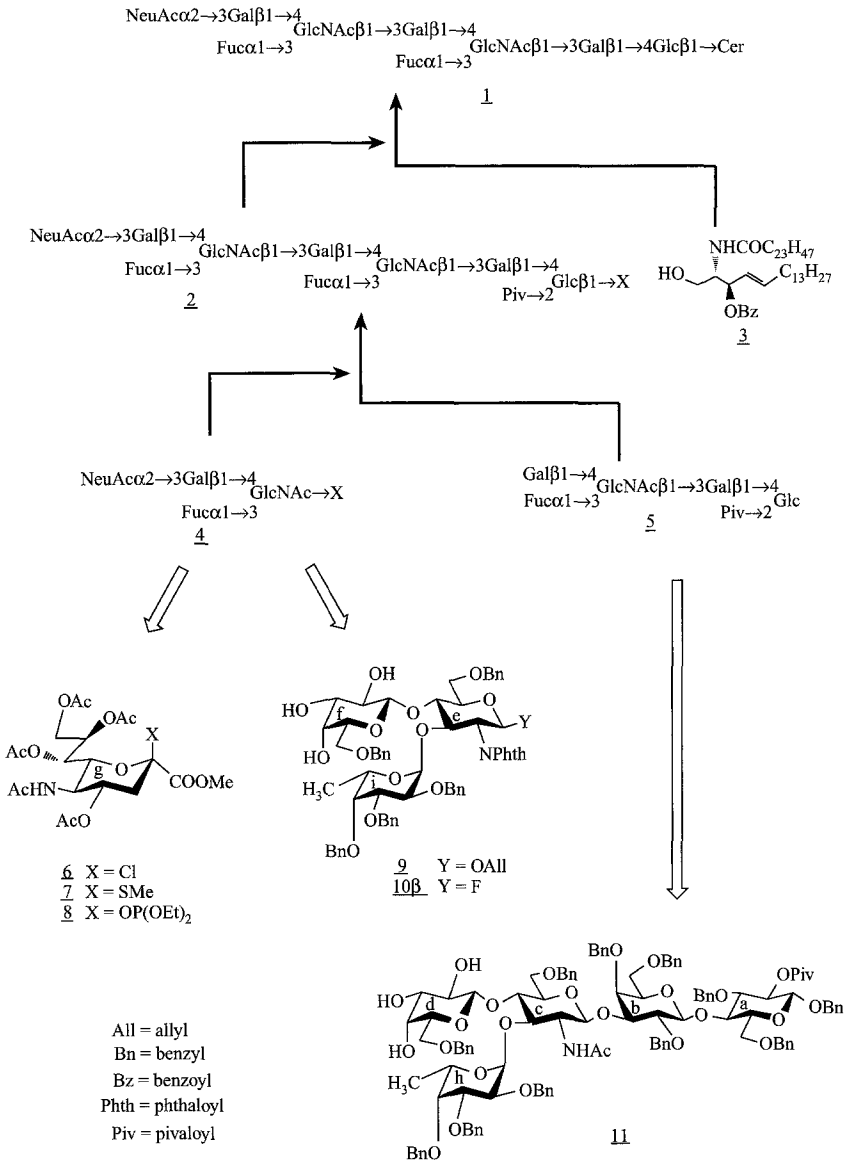
Results and discussion

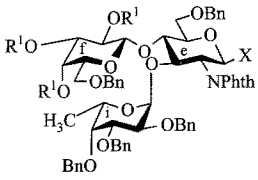
A retrosynthetic analysis of **1** (Scheme 1) led us to design the putative glycosyl donor 2 that could be coupled with ceramide derivative 3 [6]. The glycosyl donor 2 was expected to be constructed from tetrasaccharide donor 4 and pentasaccharide acceptor 5. Compound 4 was further dissected into sialic acid donors 6, 7, 8, and trisaccharide acceptors 9, 10β. Compound 11 [7] was available being designed for pentasaccharide acceptor 5. The efficiency of the pivaloyl auxiliary group at 0–2a of 11 has been established in previous studies [8]. In order to construct putative tetrasaccharide donor 4, triol 9 which was designed to afford a better coupling yield for the α-sialylation at 3f-OH according to Hasegawa *et al.* [9] was obtained from known compound 12 (Scheme 2) [7] by Zemplén O-deacetylation with a 97% yield. Glycosylation of 6 (3 molar equivalent) [10] with 9 in CH_3CN was performed by the action of $HgBr_2$-$Hg(CN)_2$ [11] to afford a 44% yield (based on acceptor) of the desired α-(2→3)-linked tetrasaccharide 15 accompanied by a 20% yield of its β-isomer 16. α-isomer 15 was easily separated from β-isomer 16 by recrystallization from MeOH. Glycosylation



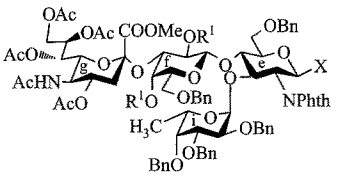
Scheme 1

of diethyl phosphite 8 with 9 (1.5 molar equivalent) under the agency of a catalytic amount of Me_3SiOTf gave 15 (40%), in high conversion yield (based on donor) and α-selectivity, which is consistent with previous data [12]. The structures of 15 and 16 were confirmed as follows. Two step acetylation of 15 and 16: (i) acetic anhydride, pyridine, at room temperature for 2 days; (ii) add DMAP, at room temperature for 5 h afforded completely acetylated 17 and 18 respectively. Homonuclear Hartmann-Hahn (HOHAHA) NMR spectra showed that H-2f and H-4f in 17 and 18 were deshielded to δ 4.754, 5.058 and δ 5.085, 5.503 respectively, indicating that the sialyl residue was unambiguously introduced at C-3f of the galactose residue. The configurations at C-2g in 17 and 18 were assigned as α and β, respectively based on the ^{1}H-NMR data. The signals for H-4g in 17 and 18 were observed at δ 4.908 and δ 5.031, and the $J_{7g,8g}$ values were observed to be 9.5 and 2.6 Hz, respectively, in good consistency with previous observations [13]. Compound 17 was converted into trichloroacetimidate 20 as follows. Deallylation of 17 with (i) $[Ir(COD)(PMeph_2)_2]PF_6$ [14] in THF and; (ii) I_2 in aq THF, afforded hemiacetal 19 in 84% yield. 19 was transformed into β-trichloroacetimidate 20 at an 80% yield in the presence of CCl_3CN and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) [15].

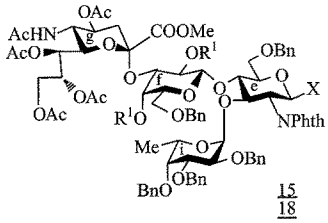
Next, we examined the synthesis of another glycosyl donor tetrasaccharide 22. After deallylation of 12, thus obtained hemiacetal 13 was treated with DAST (diethylaminosulfurtrifluoride), [16] at −10 °C to give fluorides 14 (α:β = 1:2.5). Separation of these anomers was not



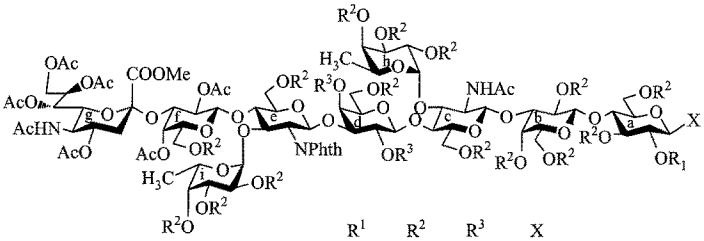
	R^1	X
9	H	β - OAll
10α	H	α - F
10β	H	β -F
12	Ac	β - OAll
13	Ac	β - OH
14	Ac	αβ - F



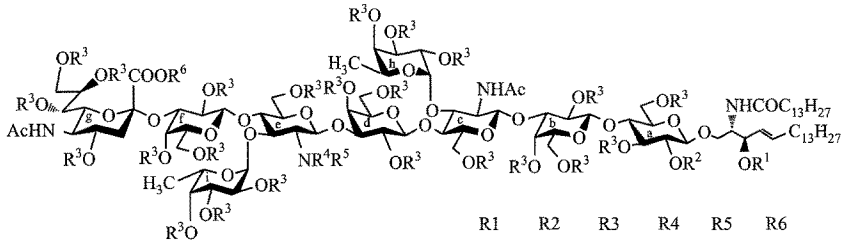
	R^1	X
15	H	β - OAll
17	Ac	β - OAll
19	Ac	β -OH
20	Ac	β - $OCNHCCl_3$
21	H	β - F
22	Ac	β - F



	R^1	X
15	H	β - OAll
18	Ac	β - OAll



	R^1	R^2	R^3	X
23	Piv	Bn	H	β - OBn
24	Piv	Bn	Ac	β - OBn
25	Piv	Ac	Ac	αβ - OAc
26	Piv	Ac	Ac	αβ -OH
27	Piv	Ac	Ac	α - $OCNHCCl_3$



	R1	R2	R3	R4	R5	R6
28	Bz	Piv	Ac	Phth		Me
29	Bz	Piv	Ac	Phth		Li
1	H	H	H	H	Ac	Na

Scheme 2

achieved at this stage, subsequent deacetylation into 10α and 10β allowed easy separation by silica gel chromatography. To our delight, glycosylation of fluoride 10β and thioglycoside 7 [9] was smoothly proceeded under the agency of PhSeOTf [17] in CH_3CN at –40 °C to give a desired 21 in 37% yield without affecting the anomeric fluoride. No self-condensed product nor β-isomer was detected. This result may be regarded as one of the further examples of recently proposed orthogonal glycosylation strategy [18]. The regiochemistry of newly introduced glycosidic linkage of 21 was similarly deduced by converting 21 into acetate 22 which showed in the ^{1}H-NMR spectrum newly deshielded signals for H-2f and H-4f at δ 4.909, δ 5.054, respectively. The observed chemical shifts of H-4g (δ 4.910) and coupling constant of $J_{7g,8g}$ 9.5 Hz are characteristic of the α-glycosidic configuration of NeuAc.

Having prepared the designed tetrasaccharide donors 20 and 22, and the pentasaccharide acceptor 11 in hand, crucial glycosylation was examined. Boron trifluoride etherate-promoted glycosylation [19] between 20 and 11 in CH_3CN at –40 °C was performed in a regio and

stereocontrolled manner to afford nonasaccharide 23 in 52% yield. But in the case of trimethylsilyl triflate-promoted glycosylation [20], the coupling yield was decreased to 30%. The configuration of C-le was expected to be β, due to the presence of the N-2 phthaloyl group in 20, which favours the formation of 1,2-trans stereochemistry. Indeed, the ^{1}NMR spectral data showed the anomeric proton of H-le at δH 5.346 (d J 8.4 Hz), thus confirming the β configuration. The regiochemistry of 23 was deduced by converting 23 into 24 by two steps acetylation which showed in the HOHAHA-NMR spectrum newly deshielded signals for H-4d and H-2d at δ 5.470 and 4.616 respectively. To our great disappointment, the glycosylation between 22 and 11 in the presence of either Cp_2HfCl_2-AgOTf [21] or $SnCl_2$-AgOTf [22] in 1,2-dichloroethane failed and we just recovered intact starting materials.

The transformation of 24 into glycosyl donor 27 was performed as follows. Hydrogenolysis of 24 by Perlman's catalyst, and subsequent acetylation afforded 25 in 47% yield. Chemoselective cleavage of the anomeric acetate of 25 with hydrazinium acetate [23] in DMF afforded hemiacetal 26 in 91% yield. 26 was converted into α-trichloroacetimidate 27 in 87% yield. The crucial coupling between 27 and 3 was achieved in freshly distilled $CHCl_3$ in the presence of boron trifluoride etherate at −15 °C to afford a 39% yield of the desired 28. The newly introduced glycosidic linkage was rigorously confirmed to be β (δH 4.403 J 7.7 Hz) as revealed in the HOHAHA experiment. Further conversion into the target glycolipid 1 was executed as follows. Compound 28 was refluxed for 1 day with a large excess of LiI in pyridine [24] to give 93% yield of the lithium salt 29. Subsequent treatment of 29 with (i) NH_2NHMe in refluxing EtOH [25]; (ii) Ac_2O in MeOH; (iii) aq NaOH in 1:1 MeOH-THF, afforded the target compound 1 in 51% yield, after gel filtration through Sephadex LH-20 using 5:5:1 $CHCl_3$-MeOH-H_2O. The ^{1}H-NMR data of synthetic 1 was good consistency with those reported for natural glycolipid [26]. The biological properties of 1 are currently being studied.

In summary, an unambiguous total synthesis of sialyl dimeric Le^x glycononaosyl ceramide was exploited for the first time in a regio- and stereo-controlled manner by use of glycononaosyl trichloroacetimidate 27 as a key glycosyl donor.

Experimental

General methods

Melting points were recorded with a BUCHI 510 and are uncorrected. Optical rotations were determined for solutions in $CHCl_3$ at 22 ± 3 °C with a JASCO Model DIP-370 polarimeter, unless otherwise stated. All reactions were monitored by high-performance thin-layer chromatography on Kieselgel 60 F_{254} (Merck) with detection by UV light and/or by charring with 5% sulfuric acid in ethanol. Flash chromatography was performed on columns of Wakogel C-300 (200 ~ 300 mesh). ^{1}H-NMR spectra were recorded with a JNM-GX 500 Fourier-transform instrument. The values of δH are expressed in p.p.m. downfield from internal Me_4Si, for solutions in $CDCl_3$ at 25 °C unless otherwise noted. Fast atom bombardment (FAB) and electrospray ionization (ESI) mass spectroscopy were recorded on a Finnigan MAT TSQ 700 triple stage quadrupole mass spectrometer equipped with an Ion Tech FAB gun or electrospray ion source. Powdered molecular sieves (3A or 4A; GL sciences Inc.Japan) and lithium iodide were heated to 250 °C under vacuum overnight. All reactions except hydrogenation were performed under atmospheres of dry nitrogen. 1,2-Dichloroethane, dichloromethane, CH_3CN, EtCN, $CHCl_3$, were distilled from CaH_2.

Allyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-(fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 9

To a solution of 12 (16.00 g, 13.0 mmol) in dry MeOH (200 ml) was added 28% MeONa (1.28 ml), and the mixture was stirred for 6 h at room temperature, neutralized with Amberlyst 15E (H^+) resin, and filtered. The resin was washed with MeOH, and the combined filtrate was evaporated in vacuo. Chromatography of the residue on SiO_2 in 20:1 $CHCl_3$-MeOH afforded 9 (13.5 g, 93.8%); $[\alpha]_D$ −13.2° (c 0.3); R_f 0.61 (12:1 $CHCl_3$-MeOH). ^{1}H-NMR data ($CDCl_3$): δ 5.660 (m, 1H, $-CH_2CH=CH_2$), 5.129 (d, 1H, J 8.4 Hz, H-le), 4.839 (d, 1H, J 3.3 Hz, H-li), 4.555 (d, 1H, J 7.7 Hz, H-1f) 4.290 (dd, 1H, J 8.4, 10.6 Hz, H-3e), 4.103 (dd, 1H, J 3.3, 11.0 Hz, H-3i), 3.873 (dd, 1H, J 1.5, 11.0 Hz, H-2i), 3.651 (dd, 1H, J 3.3, 10.3 Hz, H-3f), 3.521 (dd, 1H J 7.7, 9.5 Hz, H-2f), 1.126 (d, 3H, J 6.6 Hz, H-6i).

O-(2,3,4-Tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose 13

To a solution of (1,5-cyclooctadiene) bis (methylphenylphosphine) iridium hexafluorophosphate (287 mg, 0.24 mmol) which was activated by H_2 in THF (15 ml) was added a solution of 12 (3.00 g, 2.43 mmol) in the THF (15 ml). The mixture was stirred for 1 h at room temperature, then I_2 (3.64 g) and H_2O(70 ml) was added and stirred at room temperature for 1 h, diluted with $CHCl_3$, washed successively with aq. sodium thiosulfate, aq. $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on SiO_2 in 1.5:1 toluene-AcOEt to afford 13 (2.35 g, 81%); Rf 0.38 (1:1 toluene-EtOAc): ^{1}H-NMR data ($CDCl_3$): δ 7.40–6.96 (m, 25H 5XBn), 5.363 (d, 1H, J 3.1 Hz, H-4f), 5.256 (t, 1H, J 8.7 Hz, H-1e), 4.975 (dd, 1H, J 8.3, 10.3 Hz, H-2f), 4.812

(d, 1H, J 3.7 Hz, H-li), 4.757 (t, 1H, J 8.8 Hz, H-3e), 4.743 (dd, 1H, J 3.4, 10.5 Hz, H-3f), 4.618 (d, 1H, J 8.3 Hz, H-1f), 4.288 (dd, 1H, J 8.7, 10.4 Hz, H-2e), 4.129 (t, 1H, J 9.3 Hz, H-4e), 3.847 (dd, 1H, J 3.5, 10.0 Hz, H-3i), 3.781 (dd, 1H, J 3.5, 10.0 Hz, H-2i), 3.558 (br.dd, 1H, J 3.0, 8.2 Hz, H-5e), 3.536 (br.s, 1H, J H-4i), 3.466 (dd, 1H, J 5.6, 8.0 Hz, H-6e), 3.320 (t, 1H, J 8.5 Hz, H-6é), 3.224 (br.s, 1H, OH-le), 1.983, 1.946, 1.754 (3s, 9H 3Ac), 1.172 (d, 3H, J 5.4 Hz, H-6i).

O-(2,3,4-Tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl fluoride 14

To a solution of 13 (2.34 g, 1.96 mmol) in $(ClCH_2)_2$ (4 ml) was added diethylamino-sulfur trifluoride (1 ml, 7.57 mmol) at –15 °C. The reaction mixture was stirred for 40 min at –10 °C, diluted with EtOAc, and evaporated in vacuo. Chromatography of the residue on SiO_2 in 2:1 toluene-EtOAc afforded 14 (2.30 g, 98%) as a 1:2.5 mixture of α and β anomers; R_f 0.38 (1:1 toluene-EtOAc); ^{1}H-NMR data ($CDCl_3$): δ 5.822 (dd, 0.71H, J 7.7, 54.6 Hz, H-leβ), 5.545 (dd, 0.29H, J 2.9, 54.6 Hz, H-leα), 5.378 (d, 0.29H, J 3.3 Hz, H-4fα), 5.361 (d, 0.71H, J 3.3 Hz, H-4fβ), 5.010 (dd, 0.29H, J 8.0, 9.9 Hz, H-2fα), 4.989 (dd, 0.71H, J 8.2, 10.1 Hz, H-2fβ), 4.659 (d, 0.71H, J 8.2 Hz, H-1fβ), 2.004, 1.997, 1.956, 1.950, 1.775, 1.752, (6s, 18H, 6Ac), 1.156 (d, 0.29H, J 6.2 Hz, H-6iα), 1.225 (d, 0.71H, J 6.6 Hz, H-6iβ).

O-(6-O-Benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-α-D-glucopyranosyl fluoride 10α and β-D-glucopyranosyl fluoride 10β

To a solution of 14 (414 mg, 0.35 mmol) in MeOH (15 ml) was added 28% CH_3ONa (34 μl), and the mixture was stirred for 2 h at –10 °C, neutralized with Amberlyst 15E (H^+) resin, and filtered. The resin was washed with MeOH, and the combined filtrate was evaporated in vacuo. Chromatography of the residue on SiO_2 in 2:3 toluene-EtOAc afforded 10_α (23 mg, 6.2%), 10_β (184 mg, 50%).

10α had $[\alpha]_D$ +10.7° (c 1.0); R_f 0.45 (1:2 toluene-EtOAc); ^{1}H-NMR data ($CDCl_3$): δ 5.603 (dd, 1H, J 2.9, 54.6 Hz, H-le), 4.937 (d, 1H, J 3.3 Hz, H-li), 0.968 (d, 3H, J, 6.6 Hz, H-6i).

10β had $[\alpha]_D$ +4.6° (c 1.0); R_f 0.40 (1:2 toluene-EtOAc); ^{1}H-NMR data ($CDCl_3$): δ 5.909 (dd, 1H, J 7.7, 54.6 Hz, H-le), 4.815 (d, 1H, J 3.3 Hz, H-li), 4.521 (d, 1H, J 7.7 Hz, H-1f), 1.042 (d, 3H, J 6.6 Hz, H-6i). Anal. Calcd for $C_{61}H_{64}O_{15}NF$; C, 68.46; H, 6.03 N, 1.31 Found: C, 68.46, H, 6.13, N, 1.27.

Allyl O-(methyl 5-acetamido-4,7,8,9-O-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 15Allyl O-(methyl 5-acetimodo-4,7,8,9-O-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 16

To a stirred mixture of 1:1 $HgBr_2$-$Hg(CN)_2$ (337 mg), and 1:2 3A-4A molecular sieves (500 mg) was added a solution of 9 (100 mg, 0.090 mmol) in CH_3CN (2 ml), the mixture was stirred for 1 h at room temperature. A solution of 6 (207 mg, 0.41 mmol) in CH_3CN was added, and this was stirred for 1 day at room temperature. The mixture was quenched with Et_3N, diluted with EtOAc, and filtered through a Celite bed. The filtrate was washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by successive chromatography, first on Bio-Beads S-X3 in toluene and then on SiO_2 in 1:2 toluene-EtOAc, to give 15 (63 mg, 44%) and 16 (29 mg, 20.3%).

Compound 15 had: $[\alpha]_D$ –4.9° (c 0.3); R_f 0.14 (10:1 toluene-MeOH); ^{1}H-NMR data ($CDCl_3$): δ 5.671 (m, 1H, CH_2-*CH*=CH_2), 5.447 (m, 1H, H-8g), 5.327 (dd, 1H, J 1.8, 8.8 Hz, H-7g), 5.175 (d, 1H, J 8.8 Hz, H-le), 4.954 (m, 1H, H-4g), 4.919 (d, 1H, J 3.0 Hz, H-li), 4.659 (d, 1H, J 7.3 Hz, H-1f), 3.753 (s, 3H, OMe), 3.152 (d, 1H, J 1.1 Hz, OH), 2.671 (dd, 1H, J 4.8, 13.2 Hz, H-$3g_{eq}$), 2.430 (d, 1H, J 4.0 Hz, OH), 2.110, 2.099, 2.036, 1.999, 1.901 (5s, 15H, 5Ac), 1.066 (d, 3H, J 6.6 Hz, H-6i). Anal. Calcd for $C_{84}H_{96}O_{28}N_2$; C, 63.79; H, 6.12; N, 1.77. Found: C, 63.77; H, 6.13; N, 1.67.

Compound 16 had: R_f 0.19 (10:1 toluene-MeOH): ^{1}H-NMR data ($CDCl_3$): δ 5.695 (m, 1H, CH_2-*CH*=CH_2, 5.452 (d, 1H, J 9.9 Hz, NH) 5.356 (dd, 1H, J 2.2, 4.0 Hz, H-7g), 5.349 (m, 1H, H-4g), 5.242 (m, 1H, H-8g), 5.188 (d, 1H J 8.8 Hz, H-le), 3.707 (s, 3H, OMe), 3.138 (d, 1H, J 4.0 Hz, OH), 2.599 (dd, 1H, J 4.4, 13.6 Hz, H-$3g_{eq}$), 2.353 (s, 1H, OH), 2.123, 2.005, 1.967, 1.938, 1.822 (5s, 15H, 5Ac), 1.076 (d, 3H, J 6.6 Hz, H-6i).

Allyl O-(methyl5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 17

To a solution of 15 (1.264 g, 0.799 mmol) in pyridine (10 ml) was added acetic anhydride (10 ml), and the mixture was stirred for 2 days at 25 °C. 4-dimethylaminopyridine (50 mg) was added to the reaction mixture and stirred for 5 h at 25 °C, and coevaporated with toluene. Chromatography of the residue over SiO_2 in 9:1 toluene-MeOH afforded 17 (1.154 g, 87%); $[\alpha]_D$ –9.1° (c 0.2); R_f 0.25 (9:1 toluene-MeOH); ^{1}H-NMR data ($CDCl_3$): δ 5.640

(m, 1H, -CH_2-*CH*=CH_2), 5.580 (m, 1H, H-8g), 5.391 (dd, 1H, J 2.6, 9.5 Hz, H-7g), 5.119 (d, 1H, J 8.4 Hz, H-le), 5.058 (d, 1H, J 3.3 Hz, H-4f), 4.908 (m, 1H, H-4g), 4.754 (dd, 1H, J 8.8, 10.2 Hz, H-2f), 2.542 (dd, 1H, J 4.8, 12.5 Hz, H-3g_{eq}), 2.224, 2.081, 2.059, 2.004, 1.996, 1.854, 1.784 (7s, 21H, 7Ac), 1.207 (d, 3H, J 6.2 Hz, H-6i). Anal. Calcd for $C_{88}H_{100}O_{30}N_2$: C, 63.4; H, 6.05; N, 1.68 Found: C, 63.38; H, 6.13; N, 1.52.

Allyl O-(methyl 5-acetimido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 18

To a solution of 18 (5.2 mg, 3.3 μmol) in pyridine (1 ml) was added acetic anhydride (1 ml), and the mixture was stirred for 1 day at room temperature. 4-Dimethylaminopyridine was added to the reaction mixture and stirred for 1 day at room temperature, and coevaporated with toluene. Chromatography of the residue over SiO_2 in 1:3 toluene-AcOEt afforded 18 (5.4 mg, 99%); $[\alpha]_D$ −3.1° (c 0.3); R_f 0.46 (1:3 toluene-AcOEt); ^{1}H-NMR data ($CDCl_3$: δ 5.681 (d, 1H, J 10.3 Hz NH), 5.503 (d, 1H, J 3.6 Hz, H-4f), 5.146 (dd, 1H, J 2.6, 12.1 Hz, H-7g), 5.085 (t, 1H, J 9.5 Hz, H-2f), 5.077 (d, 1H, J 8.4 Hz, H-le), 5.031 (m, 1H, H-4g), 4.846 (d, 1H, J 3.7 Hz, H-li), 4.782 (d, 1H, J 8.4 Hz, H-lf), 4.738 (dd, 1H, J 2.9, 9.9 Hz, H-3f), 4.728 (t, 1H, J 9.9 Hz, H-2e), 4.139 (t, 1H, J 9.5 Hz, H-4e), 3.855 (s, 3H, OMe), 2.139, 2.091, 2.006, 1.956, 1.940, 1.913, 1.182, (7s, 21H, 7Ac), 1.182 (d, 3H, J, 6.6 Hz, H-6i).

O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose 19

To a solution of (1,5-cyclooctadiene)bis(methylphenylphosphine)iridium hexafluorophosphate (10.6 mg, 9.0 μmol) which was activated by H_2 in THF (5 ml) was added a solution of 17 (150 mg, 90 μmol) in THF (5 ml). The mixture was stirred for 1.5 h at room temperature, then I_2 (136 mg, 1.1 mmol) and H_2O (2.3 ml) was added and stirred at room temperature for 0.5 h, diluted with $CHCl_3$, washed successively with aq sodium thiosulfate, aq $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on SiO_2 in 5:1 toluene-MeOH to afford 19 (122 mg, 84%); $[\alpha]_D$ −1.6° (c 0.5); R_f 0.37 (5:1 toluene-MeOH); ^{1}H-NMR data ($CDCl_3$): δ 5.583 (m, 1H, H-8g), 5.391 (dd, 1H, 2.6, 9.2 Hz, H-7g), 5.063 (d, 1H, 2.9 Hz, H-4f), 4.950-4.902 (m, 2H, H-1f and H-2f), 4.869 (d, 1H, J 3.7 Hz, H-li), 4.643 (q, 1H, J 6.6 Hz, H-5i), 4.281 (dd, 1H, J 8.4, 10.6 Hz, H-2e), 3.832 (s, 3H, OMe), 2.541 (dd, 1H, J 4.8, 12.8 Hz, H-3g_{eq}), 2.227, 2.067, 2.064, 2.007, 1.987, 1.856, 1.778 (7s, 21H, 7Ac), 1.225 (d, 3H, J 6.2 Hz, H-6i). Anal. Calcd for $C_{85}H_{96}O_{30}N_2$: C, 62.80; H, 5.95; N, 1.72 Found: C, 62.67; H, 5.96; N, 1.82.

O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate 20

A solution of 19 (246 mg, 151 μmol), CCl_3CN (236 μl, 2.35 mmol), and DBU (16 μl, 106 μmol) in $(ClCH_2)_2$ (2 ml) was stirred for 2 h at 0 °C. The reaction mixture was directly chromatographed on a SiO_2 in 1:3 toluene-EtOAc to afford 20 (214 mg, 80%); R_f 0.33 (3:1 $CHCl_3$-acetone); ^{1}H-NMR data ($CDCl_3$): δ 8.518 (s, 1H, C = NH), 6.394 (d, 1H, J 8.8 Hz, H-le), 5.577 (m, 1H, H-8g), 5.388 (dd, 1H, J 2.9, 9.5 Hz, H-7g), 4.887 (d, 1H, J 4.0 Hz, H-li), 4.842 (d, 1H, J 8.8, 10.6 Hz, H-2e), 4.680 (dd, 1H, J 8.8, 10.6 Hz, H-2f), 3.822 (s, 3H, OMe), 2.233, 2.080, 2.071, 2.007, 1.986, 1.795, 1.855 (7s, 21H, 7Ac).

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl) fluoride 21

A mixture of 10β (100 mg, 93.4 μmol), 7 (65.4 mg, 125.4 μmol) and 3A molecular sieves (500 mg) in MeCN (2 ml) was stirred for 15 min at room temperature under N_2. After cooling to −40 °C, a solution of AgOTf (83.8 mg, 326 μmol) in MeCN (1 ml), and PhSeCl (62.4 mg, 32.6 μmol) were added. After stirring for 2 h at −40 °C, the reaction mixture was quenched with Et_3N, diluted with EtOAc and filtered through a Celite bed. The filtrate was washed with aq $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by successive chromatography, first on Bio-Beads S-X3 in toluene and then on SiO_2 1:3 toluene-EtOAc, to give 21 (47.4 mg, 36.7%); $[\alpha]_D$ +1.2° (c 1.0); R_f 0.13 (1:1 THF-hexane); ^{1}H-NMR data ($CDCl_3$): δ 5.915 (dd, 1H, J 7.7, 54.6 Hz, H-le), 5.456 (m, 1H, H-8g), 5.324 (dd, 1H, J 1.8, 9.2 Hz, H-7g), 5.186 (d, 1H, J 9.9 Hz, NH), 4.953 (m, 1H, H-4g), 4.885 (d, 1H, J 3.3 Hz, H-li), 3.755 (s, 3H, OMe), 2.675 (dd, 1H, J 4.8, 13.2 Hz, H-3g_{eq}), 2.113, 2.105, 2.037, 1.993, 1.900 (5s, 15H, 5Ac), 1.066 (d, 3H, J 6.6 Hz, H-6i). Anal Calcd for $C_{81}H_{91}O_{27}N_2F$: C, 63.03; H, 5.94; N, 1.81 Found: C, 62.74; H, 6.06; N, 1.72.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl) fluoride 22

To a solution of 21 (40.6 mg, 26.3 μmol) in pyridine

(2 ml) was added acetic anhydride (2 ml), and the mixture was stirred for 1 day at room temperature. A catalytic amount of DMAP was added to the reaction mixture and stirred for another day at room temperature, and coevaporated with toluene. Chromatography of the residue on SiO_2 in 1:4 toluene-EtOAc afforded 22 (36.5 mg, 87.5%); $[\alpha]_D$ –3.4° (c 1.0); R_f 0.25 (1:3 toluene-EtOAc); ^{1}H-NMR data ($CDCl_3$): δ 5.844 (dd, 1H, J 7.7, 54.6 Hz, H-1e), 5.586 (m, 1H, H-8g), 5.389 (dd, 1H, J 2.6, 9.5 Hz, H-7g), 5.054 (d, 1H, J 2.9 Hz, H-4f), 4.931–4.889 (m, 3H, H-1f, H-2f and H-4g), 4.842 (d, 1H, J 3.7 Hz, H-1i), 4.757 (dd, 1H, J 8.8, 10.6 Hz, H-2e), 4.530 (t, 1H, J 8.8 Hz, H-3e), 4.296 (t, 1H, J 8.8 Hz, H-4e), 3.836 (s, 3H, OMe), 2.544 (dd, 1H, J 4.8, 12.5 Hz, H-3g_{eq}), 2.230, 2.085, 2.073, 2.007, 1.986, 1.856, 1.787 (7s, 21H, 7Ac), 1.711 (t, 1H, J 12.5 Hz, H-3g_{ax}), 1.194 (d, 3H, J 6.6 Hz, H-6i).

Benzyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 23

To a stirred mixture of 11 (82 mg, 43 μmol) and powdered molecular sieves (3A, 400 mg) in dry CH_3CN (1 ml) was added a solution of 20 (113 mg, 64 μmol) in dry CH_3CN (1 ml) at –40 °C. After stirring for 10 min at –40 °C, boron trifluoride etherate (8.5 μl, 64 μmol) was added, and stirred for 1 h. The mixture was diluted with EtOAc, filtered through Celite and the filtrate was washed successively with aq $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated in vacuo, Chromatography of the residue on SiO_2 in 5:1 $CHCl_3$-acetone and gel filtration over Bio-Beads S-Xl in toluene afforded 23 (78 mg, 52%); $[\alpha]_D$ –22.8° (c 1.0) R_f 0.48 (3:1 $CHCl_3$-acetone); ^{1}H-NMR data ($CDCl_3$): δ 5.508 (m, 1H, H-8g), 5.399 (dd, 1H, J 2.6, 9.2 Hz, H-7g), 5.346 (d, 1H, J 8.4 Hz, H-1e), 4.896 (d, 1H, J 3.7 Hz, H-1i or H-1h), 3.835 (s, 3H, OMe), 2.531 (dd, 1H, J 4.8, 12.5 Hz, H-3g_{eq}), 2.246, 2.079, 2.066, 2.007, 2.006, 1.969, 1.855, 1.788 (8s, 24H, 8Ac), 1.114 (s, 9H, ^{t}Bu), 0.796 (d, 3H, J 6.2 Hz, H-6i or H-6h). Anal. Calcd for $C_{199}H_{223}O_{53}N_3$: C, 67.58; H, 6.35; N, 1.19. Found: C, 67.62; H, 6.69; N, 1.01.

Benzyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 24

To a solution of 23 (105 mg, 30 μmol) in pyridine (2 ml) was added acetic anhydride (2 ml), and the mixture was stirred for 2 days at room temperature. A catalytic amount of DMAP was added to the reaction mixture and stirred for another 2 days at room temperature, and coevaporated with toluene. Chromatography of the residue on SiO_2 in 2:3 toluene-EtOAc afforded 24 (92 mg, 85.6%): $[\alpha]_D$ –19.2° (c 0.8): R_f 0.71 (3:1 $CHCl_3$-acetone); ^{1}H-NMR data ($CDCl_3$): δ 5.470 (d, 1H, J 4.0 Hz, H-4d), 5.390 (dd, 1H, J 2.6, 9.2 Hz, H-7g), 5.015 (d, 1H, J 3.7 Hz, H-1i or H-1h), 4.942 (d, 1H, J 2.9 Hz, H-4f), 4.616 (dd, 1H, J 8.1, 9.2 Hz, H-2d), 3.844 (s, 3H, OMe), 3.599 (dd, 1H, J 10.6, 2.9 Hz, H-3d or H-3f), 2.229, 2.132, 2.058, 2.007, 1.981, 1.967, 1.856, 1.777, 1.754, 1.607 (10s, 30H, 10Ac), 1.215 (d, 3H, J 6.6 Hz, H-6h), 1.121 (s, 9H, ^{t}Bu), 0.882 (d, 3H, J 6.2 Hz, H-6i). Anal. Calcd for $C_{203}H_{227}O_{57}N_3$: C, 67.34; H, 6.32; N, 1.16 Found: C, 67.52; H, 6.78; N, 1.16.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,-4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,-4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-pivaloyl-D-glucopyranosyl acetate 25

A mixture of 24 (92 mg, 25 μmol) and 20% $Pd(OH)_2$-C (92 mg) in 4:1 MeOH-H_2O (10 ml) was stirred under H_2 for 23 h at room temperature, diluted with 4:1 MeOH-H_2O and filtered through a Celite bed. The filtrate was coevaporated with toluene. To a solution of the residue in pyridine (3 ml) was added Ac_2O (3 ml) and a catalytic amount of DMAP. The mixture was stirred for 3 days at room temperature and then coevaporated with toluene in vacuo. Chromatography of the residue on Sephadex LH-20 in 1:1 $CHCl_3$-MeOH afforded 25 (34 mg, 47%) a 1:1 mixture of α and β anomers; R_f 0.36 (20:1 $CHCl_3$-MeOH) ^{1}H-NMR data ($CDCl_3$): δ 6.284 (d, 0.5H, J 3.7 Hz, H-1aα), 5.688 (d, 0.5H, J 8.1 Hz, H-1aβ), 3.860 (s, 3H, OMe), 1.179 (d, 3H, J 6.6 Hz, H-6h), 1.129, 1.116 (2s, 18H, ^{t}Bu), 0.821 (d, 3H, J 6.6 Hz, H-6i).

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,-4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,-

4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-pivaloyl-D-glucopyranose 26

A mixture of 25 (34 mg, 12 μmol) and hydrazinium acetate (2.2 mg, 24 μmol) in DMF (1 ml) was stirred for 2 h at room temperature. The mixture was chromatographed on Sephadex LH-20 in MeOH to afford 26 (31 mg, 91%); R_f 0.24 (20:1 $CHCl_3$-MeOH); ^{1}H-NMR data ($CDCl_3$): δ 3.862 (s, 3H, OMe), 1.177 (d, 3H, J 7.0 Hz, H-6h), 1.170(s, 9H, ^{t}Bu), 0.822 (d, 3H, J 6.2 Hz, H-6i).

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,-4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,-4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-pivaloyl-α-D-glucopyranosyl trichloroacetimidate 27

A solution of 26 (31 mg, 11 μmol), CCl_3CN (17 μl, 165 μmol), and DBU (3.6 μl, 23 μmol) in $(ClCH_2)_2$ (1 ml) was stirred for 2 h at 0 °C. The reaction mixture was directly chromatographed on SiO_2 in 35:1 $CHCl_3$-MeOH to afford 27 (28 mg, 87%). R_f 0.33 (25:1 $CHCl_3$-MeOH); ^{1}H-NMR data ($CDCl_3$): δ 8.651 (s, 1H, NH), 6.498 (d, 1H, J 3.7 Hz, H-la), 5.560 (t, 1H, J 9.9 Hz, H-3a) 5.448 (dd, 1H, J 2.9, 9.9 Hz, H-7g), 5.383 (d, 1H, J 3.7 Hz, H-4b or H-4d), 5.290 (d, 1H, J 2.9 Hz, H-4h or H-4i), 5.272 (d, 1H, J 3.7 Hz, H-4d or H-4b), 5.068 (d, 1H, J 8.0 Hz, H-le), 5.033 (dd, 1H, J 3.7, 9.9 Hz, H-2a), 4.961 (d, 1H, J 3.7 Hz, H-4f), 4.652 (t, 1H, J 9.2 Hz, H-2e), 4.545 (dd, 1H, J 3.7, 10.3 Hz, H-3f), 4.355 (d, 1H, J 8.1 Hz, H-1b or H-1d), 4.312 (d, 1H, J 8.4 Hz, H-1d, or H-1b), 3.864 (s, 3H, OMe), 3.666 (dd, 1H, J 2.6, 11.7 Hz, H-3d or H-3b), 1.179 (s, 3H, J 6.6 Hz, H-6h), 1.125 (s, 9H, ^{t}Bu), 0.824 (d, 3H, J 6.6 Hz, H-6i).

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,-4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(6-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,-4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-O-acetyl-2-O-pivaloyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzyl-2-N-tetracosanoylsphingenine 28

To a stirred mixture of 27 (28 mg, 9.4 μmol) and 3 (14.2 mg, 18.8 μmol) and 4A molecular sieves (500 mg) in $CHCl_3$ (1 ml) was added boron trifluoride etherate (3.4 μl, 37.6 μmol) at −15 °C. The mixture was stirred for 30 min at gradually warmed up temperature, diluted with $CHCl_3$ and filtered through a Celite bed. The filtrate was washed successively with aq $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated in vacuo. Chromatography of the residue over SiO_2 in 2:1 toluene-acetone afforded 28 (13 mg, 39%). $[\alpha]_D$ −30.6° (c 1.0); R_f 0.38 (1:1 toluene-acetone); ^{1}H-NMR data ($CDCl_3$): δ 5.870 (dt, 1H, J 7.0, 15.0 Hz, 5Cer), 5.716 (d, 1H, J 9.2 Hz, NH), 5.385 (br-S, 1H, H-4b or H-4d), 5.289 (br-S, 1H, H-4h or H-4i), 5.256 (br-S, 1H, H-4d or H-4b), 5.215 (br-S, 1H, H-4i or H-4h), 5.170 (t, 1H, J 9.5 Hz, H-2a), 5.068 (d, 1H, J 8.1 Hz, H-1e), 4.963 (br-S, 1H, H-4f), 4.652 (t, 1H, J 9.2 Hz, H-2e), 4.403 (d, 1H, J 7.7 Hz, H-1a), 4.304 (d, 1H, J 8.4 Hz, H-1b), 3.865 (S, 3H, OMe), 1.180 (d, 3H, J 6.6 Hz, H-6h), 1.136 (s, 9H, ^{t}Bu), 0.823 (d, 3H, J 6.6 Hz, H-6i).

O-(Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulpyranosylonate)-(2→3)-O-β-D-galactopyranosyl-(1→4)-O-[α-L-fucopyranosyl-(1→3)]-O-(2-acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→4)-O-[α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→1)-(2S,3R,4E)-2-N-tetra-cosanoylsphingenine 1

A solution of 28 (13 mg, 3.7 μmol) in pyridine (1 ml) was added dropwise onto LiI (13 mg, 97 μmol, dried at 200 °C for 1 day in vacuo), and the mixture was heated for 7 h at reflux under Ar. The reaction mixture was chromatographed first on Sephadex LH-20 in 1:2 $CHCl_3$-MeOH and then on SiO_2 in 10:1 $CHCl_3$-MeOH to afford 29 (12 mg, 93%). A solution of 29 (12 mg, 3.4 μmol) in EtOH (5 ml) was added methylhydrazine (2.5 ml), and the mixture was stirred for 18 h at 80 °C, and then evaporated in vacuo. The residue was purified over Sephadex LH-20 in 5:5:1 $CHCl_3$-MeOH-H_2O to afford amino derivative. To the solution of amino derivative in 2:2:1 MeOH-THF-CH_2Cl_2 (2.5 ml) was added Ac_2O (100 μl), and the mixture was stirred for 1 h at room temperature and evaporated in vacuo. Finally the residue in 1:1 MeOH-THF (0.6 ml) was added 1 N NaOH (0.3 ml), and stirred for 1 day at room temperature. The reaction mixture was chromatographed on a Sephadex LH-20 with 5:5:1 $CHCl_3$-MeOH-H_2O to give 1 (4 mg, 51%). R_f 0.14 (2:1:1 ^{n}BuOH-EtOH-H_2O); ^{1}H-NMR data (50:1 $(CD_3)_2SO$-D_2O) δ; 5.601 (dt, 1H, J 15.4, 7.0 Hz, H-5Cer), 5.410 (dd, 1H, J 15.4, 7.0 Hz, H-4Cer), 4.935 (d, 2H, J 3.3 Hz, H-1h and H-1i), 4.802 (d, 1H, J 7.3 Hz H-1c), 4.788 (d 1H, J 6.6 Hz, H-1e), 4.653 (2q, 2H H-5h and H-5i), 4.404 (d, 1H, J 7.3 Hz, H-1d), 4.360 (d, 1H, J 7.7 Hz, H-1f), 4.334 (d, 1H, J 7.0 Hz, H-1b), 4.220 (d, 1H, J 8.1 Hz, H-1a), 2.818 (dd, 1H, J 5.1, 12.1 Hz, H-3g_{eq}), 1.169 (d, 3H, J 6.6 Hz, H-6h),

1.086 (d, 3H, J 6.6 Hz, H-6i), 1.945, 1.881, 1.875 (3S, 9H, 3NAc), 0.897 (t, 6H, J 7.0 Hz, $2CH_2Me$); ESIMS : m/Z $(M+2Na)^{2+}$ 1177.9 ; FABMS (TEA matrix) : m/Z $(M-Na)^-$ 2287.

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