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Total synthesis of globotriaosylceramide (Gb_3) and lysoglobotriaosylceramide (lysoGb$_3$)

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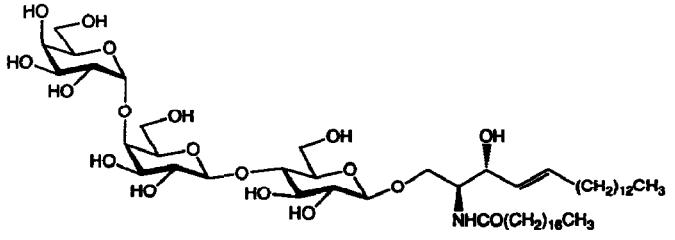
ABSTRACT

We have recently reported a highly efficient and stereocontrolled synthesis of globotriaosylceramide (Gb_3, **1**) in optically pure form[1]. Key to our synthetic strategy was the implementation of the two-stage activation of thioglycosides for formation of the glycosidic bonds and the utilization of (2*S*, 3*S*, 4*E*)-2-azido-3-*O*-(*tert*-butyldimethylsilyl)-4-octadecen-1,3-diol (**9**) as a sphingosine equivalent. The syntheses of Gb_3 (**1**) and lysoGb$_3$ (**2**) were achieved by stereocontrolled coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl fluoride (**15**) with phenyl O-(6-*O*-benzoyl-2,3-di-*O*-pivaloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-pivaloyl-1-thio-β-D-glucopyranoside (**14**) to form the P^k antigen trisaccharide masked as a phenyl 1-thioglycoside at the reducing end. Thioglycoside **16** was converted into glycosyl fluoride **19**, which was coupled to **9** in high yield. The coupled product **20** was converted into the title compounds **1** and **2** in four and three steps, respectively. This article presents the total synthesis of **1** and **2** in full experimental detail.

INTRODUCTION

It is well established that glycosphingolipids are molecules of considerable biological significance within the area of cellular recognition[2]. Recently, lysosphingolipids have been recognized as molecules possessing novel biological activities not exhibited by glycosphingolipids[3]. It is, therefore, highly desirable that further investigations with these compounds be carried out. Unfortunately, many important glycosphingolipids are only available through difficult isolation procedures from rare natural sources, such as human cancer tissues, and then only in heterogenous form. Recently, we have developed a strategy capable of delivering glyco- and lyso-sphingolipids stereospecif-



1 : Gb_3

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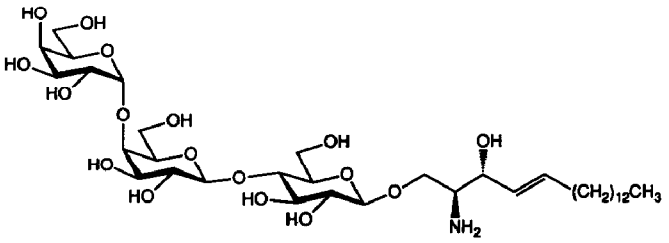


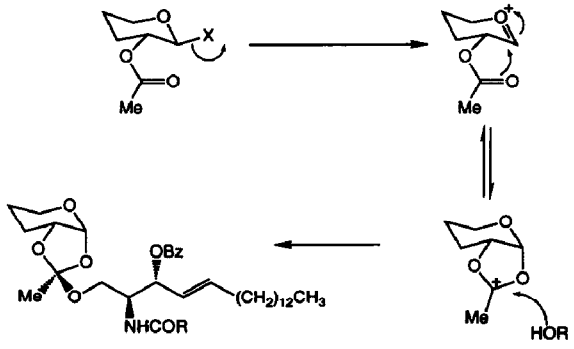
2 : lysoGb$_3$

ically and in optically pure form[1]. The following syntheses of globotriaosylceramide (Gb_3, **1**) and globotriaosylsphingosine (lysoGb$_3$, **2**) are illustrative of this method.

Gb_3, an important member of the glycosphingolipid class of marker molecules, is highly expressed in most Burkitt lymphoma cell-lines[4], human teratocarcinoma[5], human embryonal carcinoma[6], and other types of tumor cells[7]. This remarkable molecule is also a constituent of human erythrocytes[8], and has been shown to provide a cell-surface receptor for verotoxin, as well as to play a role in the affinity of the α interferon receptors[9]. Gb_3 is closely linked to Fabry's disease[10] in which patients lack an α-D-galactosidase, thus causing a breakdown in the catabolic pathway of this molecule. This genetic disorder leads to an accumulation of Gb_3 in critical organs and tissues, impairing their function; this may ultimately result in death. Recently lysoGb$_3$ (**2**) has been shown to be active in the lysis of human neutrophils, an activity which Gb_3 itself does not possess[3].

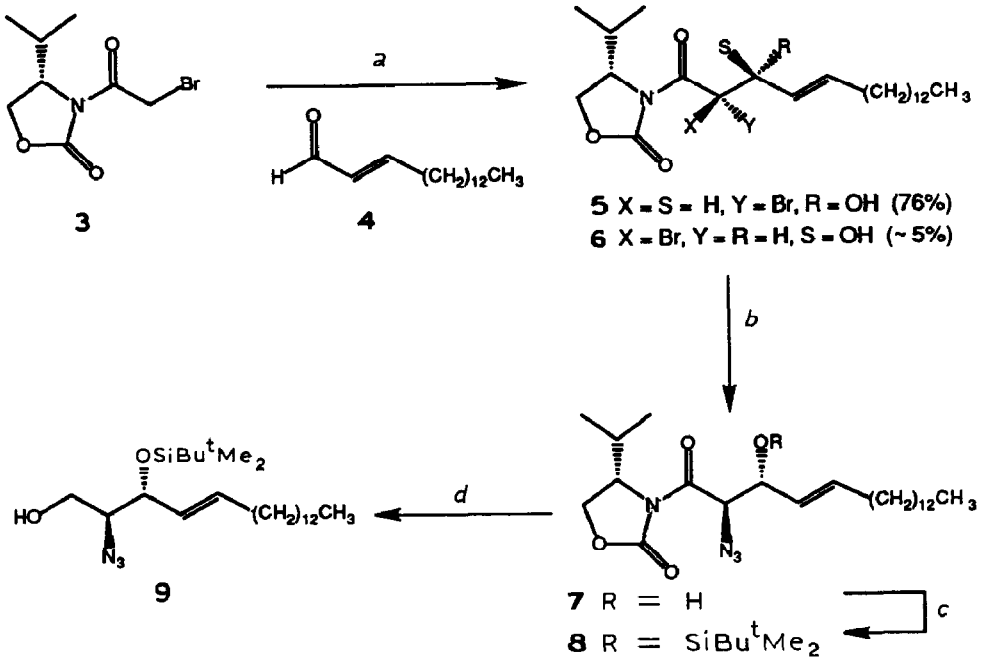
Naturally, a molecule of such biological significance has been the focus of considerable synthetic attention. The synthesis of the trisaccharide unit of Gb_3, also known as the P^k antigen, has been pursued by several groups[11]. The synthesis of the complete glycosphingolipid (Gb_3) has been accomplished by two groups[12,13]. In both syntheses, the crucial coupling of the trisaccharide donor to a 3-*O*-protected ceramide unit proceeded in only low to moderate yields.

Glycosidation of the 3-*O*-benzoylceramide unit has been a classical problem in



Scheme 1. Orthoester formation.

most glycosphingolipid syntheses. Results from our own and other laboratories led us to conclude that this problem was attributable to two effects: (*a*) the potential of the 3-*O*-benzoylceramide unit to bind the glycosidation catalyst, and (*b*) preferential nucleophilic attack of the ceramide unit to the acetoxy carbon of the acetoxonium ion intermediate over C-1 glycosidation, leading to an orthoester (Scheme 1). Potential solutions to these problems may rely on (*a*) masking the amide of the ceramide unit as some functionality not capable of binding the glycosidation catalyst, and (*b*) the use of a β-directing group at C-2 of the saccharide donor that is not susceptible to attack at the acetoxy carbon. Excellent studies along these lines have been reported from the laboratories of Schmidt[14] and Ogawa[15].

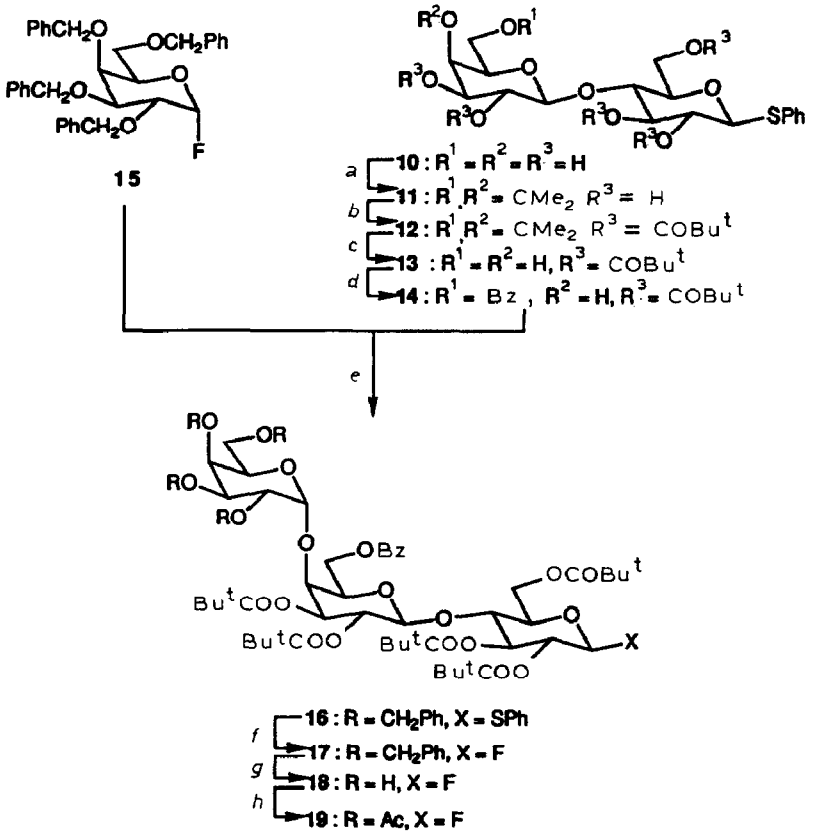


Scheme 2. Synthesis of sphingosine equivalent. Reagents and conditions. (*a*) 1.2 equiv. of Bu_2BOTf, 1.4 equiv. of Et_3N, −78°, 30 min then for 2 h at 20°, add 0.75 equiv. of **4** at −78°, 2 h, then H_2O_2–MeOH–ether, 0° 1 h, 75%; (*b*) 2.0 equiv. of NaN_3, Me_2SO, 25°, 2 h, 92%; (*c*) 1.5 equiv. of Bu^tMe_2SiOTf, 2.0 equiv. of 2,6-lutidine, CH_2Cl_2, 0°, 1.5 h, 97%; (*d*) 3.0 equiv. of $LiBH_4$, THF 0°; 2 h, 81%.

Using these principles as our guide, we embarked on a synthesis based on coupling the sphingosine equivalent **9** (in which an azide functionality serves as a masked amide (Scheme 2), with the trisaccharide fluoride **19** (Scheme 4) (containing a pivalate group at C-2 in order to avoid orthoester formation).

RESULTS AND DISCUSSION

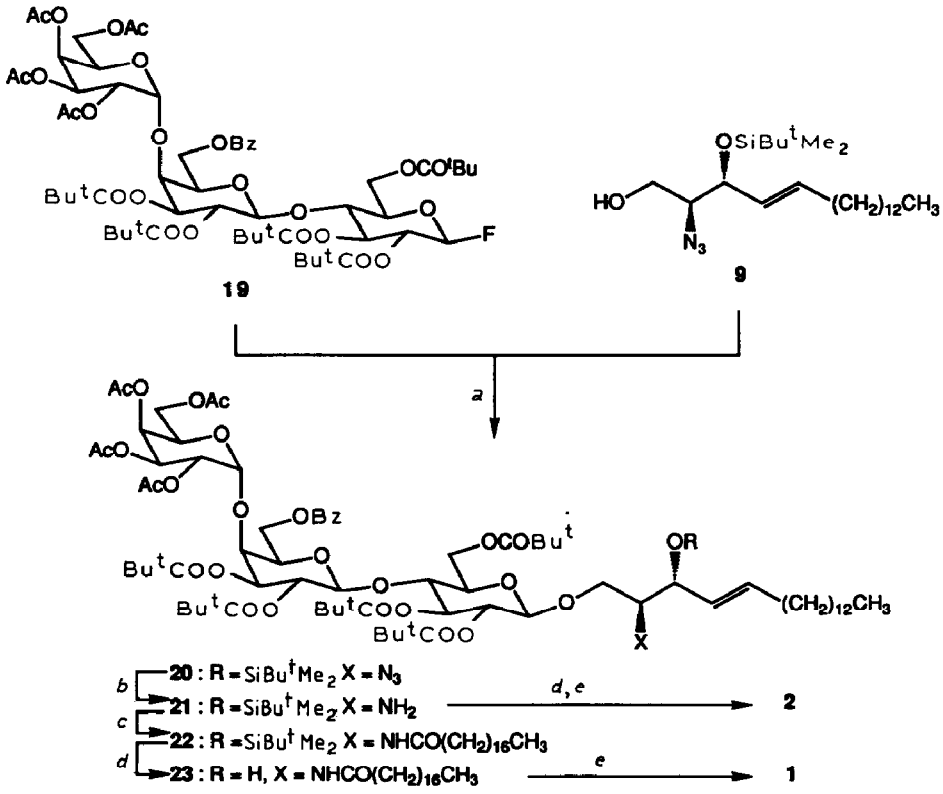
We recognized that the requisite 2-azidosphingosine equivalent (**9**) could be synthesized in enantiomerically pure form by utilizing the principles set forth by Evans *et al.*[16] and Pridgen *et al.*[17]. The following strategy was thus designed and executed. The α-bromoacetoxy oxazolidinone[17] (**3**) derived from L-valine was converted into its boron enolate via the addition of freshly prepared dibutylboron triflate[18] and triethylamine. Condensation of the boron enolate with (*E*)-2-hexadecenal (**4**) in ether provided **5** as the major product (75%), along with a second product, presumably the other *syn*-diastereomer of **5** (compound **6**, 5%). ^{1}H-N.m.r. spectroscopic analysis of the crude product-mixture indicated a ratio of 9:1 for the two diastereoisomers formed in the aldol condensation reaction. The use of freshly prepared boron triflate for enolate formation proved crucial to the success of the aldol condensation, as the use of a commercially



Scheme 3. Synthesis of trisaccharide fluoride (**19**). Reagents and conditions. (*a*) $Me_2C(OMe)_2$, cat. TsOH, DMF, 25°, 2 h; (*b*) 8.0 equiv. of Bu^{t}COCl, cat. DMAP, pyridine, 75°, 40 h, 78% overall from **10**; (*c*) CF_3CO_2H:THF:H_2O, 3:2:1, 0°, 1.5 h, (*d*) 1.1 equiv. of PhCOCN, 1.3 equiv. of Et_3N, DMF, −20 to −10°, 0.5 h, 93% from **12**; (*e*) 2.0 equiv. of **15**, 2.0 equiv. of AgOTf, 2.0 equiv. of $SnCl_2$, 4 Å mol. sieves, Et_2O, 0°C, 2 h, 73%; (*f*) 1.2 equiv. of NBS, excess HF·pyridine, CH_2Cl_2, −35 to 0°, 1 h, 89%; (*g*) H_2, $Pd(OH)_2$–C, EtOH, 25°, 24 h; (*h*) 5.0 equiv. of Ac_2O, 5.0 equiv. of pyridine, cat. DMAP, CH_2Cl_2, 0 to 25°, 13 h, 91% from **17**.

available solution yielded none of the desired product. Displacement of the bromide in **5** with sodium azide in Me_2SO led to the azide **7** in 92% yield with complete inversion of stereochemistry at the reaction center. Silylation of the hydroxy group of **7** by the action of *tert*-butyldimethylsilyl triflate gave the silyl ether **8** in 97% yield. The latter compound (**8**) was smoothly reduced with an excess of lithium borohydride in THF at 20° to afford the requisite primary alcohol **9** in 82% isolated yield.

With the sphingosine equivalent **9** in hand we turned our attention to the synthesis of trisaccharide **19** (Scheme 3). To this end, phenyl *O*-(*β*-D-galactopyranosyl)-(1→4)-1-thio-*β*-D-glucopyranoside **10** (ref. 19) was selectively transformed into its terminal 4,6-isopropylidene acetal by the action of 2,2-dimethoxypropane and catalytic *p*-toluenesulfonic acid. The crude product was treated with pivaloyl chloride and DMAP in pyridine at 75° to afford the pentapivalate **12** possessing the crucial C-2 pivalate group (78% yield). Deacetonation of **12** by graded acid hydrolysis followed by monobenzoylation led to the desired lactosyl derivative **14** in 93% yield via diol **13**.



Scheme 4. Synthesis of globotriaosylceramide (Gb_3, **1**) and globotriaosylsphingosine (lysoGb$_3$, **2**). Reagents and conditions. (*a*) 4.7 equiv. of **9** 2 equiv. of $AgClO_4$, 2.0 equiv. of $SnCl_2$, 1.0 equiv. of 2,6-lutidine, CH_2Cl_2, 0°, 12 h, 80%; (*b*) 2.0 equiv. of Ph_3P, 10 equiv. of H_2O, benzene, 45°, 11 h, 90%; (*c*) 1.2 equiv. of octadecanoyl chloride, 1.5 equiv. of Et_3N, cat. DMAP, CH_2Cl_2, 0°, 1 h, 97%; (*d*) 1.5 equiv. of Bu_4NF, THF, 0–25°C, 2 h, 95%; (*e*) 1.0 equiv. of NaOMe, MeOH, 60°, 24 h, 90%.

Disaccharide **14** was suitably protected for coupling to per-*O*-benzylgalactopyranosyl fluoride (**15**), which was prepared by the action of HF·pyridine complex and NBS on phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[20].

Intermediates **14** and **15** were coupled under the influence of silver triflate and stannous chloride in ether to afford trisaccharide **16** in 73% yield (based on **14**). Silver triflate proved to be a superior glycosidation catalyst as compared to the standard Mukaiyama conditions ($AgClO_4$ and $SnCl_2$)[21] which, in the present case, resulted in a 67% yield of **16**. As expected from stereoelectronic effects and the presence of a benzyl group at C-2 of the galactosyl fluoride (**15**), the newly generated glycosidic bond in **16** had the desired *α* configuration. Signal overlap made determination of stereochemistry by simple ^{1}H-n.m.r. spectroscopy rather difficult, and a 2D ^{1}H–^{13}C heterocorrelation experiment was carried out on compound **16** (500–125 MHz, $CDCl_3$).

The three anomeric carbons resonated at δ 86.1 (C-1), 100.1 (C-1′), and 100.4 (C-1″), whereas the anomeric protons resonated at δ 4.42 (d, 1 H, J 9.3 Hz, H-1′), 4.61 (d, 1 H, J 9.8 Hz, H-1), and 4.61 (d, 1 H, $J < 5.0$ Hz, H-1″). The latter coupling constant established the *α* configuration of the terminal saccharide residue.

At this juncture, we considered it prudent to remove the benzyl groups from the terminal residue rather than face their removal in the presence of the ceramide double-bond at a later stage in the synthetic sequence. The instability of phenyl 1-thioglycosides to benzyl ether cleavage-conditions prompted us to replace the phenylthio group with fluoride and then attempt hydrogenolysis of the benzyl ethers. To this end, **16** was carefully reacted with HF·pyridine complex in the presence of NBS at $-35 \rightarrow 0°$ in dichloromethane and quenched at 0° giving the β-fluoride **17** in 89% yield with no *α*-fluoride detected. Selective formation of the β-fluoride was deemed important to the success of the next coupling, since earlier studies had shown that *α* fluorides of this type were unreactive to the coupling conditions. Quenching the reaction at 0° was crucial, since higher temperatures caused cleavage of the acid-labile terminal glycosidic bond. Hydrogenolysis of **17** over palladium hydroxide on carbon, followed by acylation under standard conditions, provided **19** in 91% yield over the two steps. Coupling of trisaccharide fluoride **19** with an excess of **9** under the influence of silver perchlorate and stannous chloride in the presence of one equivalent of 2,6-lutidine afforded compound **20** in 80% yield. The coupling reaction proceeded with complete stereocontrol at the newly formed glycosidic bond in the desired sense, as expected from the β-directing ability of the pivalate group. Furthermore, no orthoester formation was observed under these conditions.

The remainder of the synthesis required reduction of the azido group to an amine, producing the key intermediate **21** which was to be carried on to both Gb_3 (**1**) and lysoGb$_3$ (**2**). Transformation of azide **20** to amine **21** by reduction with triphenylphosphine–water was achieved in 90% yield. Compound **21** was then subjected to amide formation with octadecanoyl chloride and DMAP to afford **22** in 97% yield. Desilylation of **22** using TBAF proceeded smoothly to produce **23** in 95% yield. Finally, removal of the ester groups by reaction of **23** with sodium methoxide in refluxing methanol yielded globotriaosylceramide (Gb_3, **1**) in 90% yield. By similar desilylation

and deacylation procedures, **21** was also converted into globotriaosylsphingosine (lysoGb$_3$, **2**) in 63% overall yield.

The described total synthesis of Gb$_3$ (**1**) and lysoGb$_3$ (**2**) demonstrates the power of the developed strategy for the synthesis of complex glyco- and lyso-sphingolipids and renders these compounds readily available in pure form for further biological investigations. Also, the uncovered stability of glycosyl fluorides to hydrogenolysis conditions should considerably expand the utility of these intermediates in oligosaccharide synthesis, or as partners to phenyl 1-thioglycosides in the two-stage activation procedure.

EXPERIMENTAL

General methods. — N.m.r. spectra were recorded on a Bruker AM-500 spectrometer. I.r. spectra were recorded on a Perkin–Elmer Model 781 spectrophotometer. Optical rotations were determined with a Perkin–Elmer Model 241 polarimeter.

All reactions were monitored by thin-layer chromatography on 0.25 mm E. Merck silica gel plates (60F-254) with u.v. light and 7% ethanolic phosphomolybdic acid–heat as developing agents. Flash column chromatography was performed on E. Merck silica gel (60, particle size 0.040–0.063 mm).

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H-n.m.r.) homogeneous materials, unless otherwise noted.

(4S)-3-[(2'S,3'R,4'E)-2'-Bromo-3'-hydroxy-4-(isopropyl)-4'-octadecenoyl]-2-oxazolidinone (**5**). — To a stirred solution of (4*S*)-3-(bromoacetyl)-4-(isopropyl)-2-oxazolidinone[17] (11.0 g, 44.00 mmol) in 150 mL of dry ether at −78° was added Et_3N (8.6 mL, 62 mmol). After 5 min, dibutylboron triflate (11.3 mL, 44.9 mmol) was added slowly via syringe. After 15 min, the −78° bath was removed and the mixture was stirred for 2 h at ambient temperature. At the end of this time the mixture was slowly cooled back down to −78° and a solution of *E*-hexadec-2-enal (6.66 g, 27.9 mmol) in 130 mL of dry ether was added via cannula. The mixture was stirred for 45 min at −78°, and then for 1.5 h at 0°. At the end of this time, the reaction was diluted with 250 mL of ether and poured over 150 mL of M aqueous $NaHSO_4$. The layers were separated and the organic layer was washed with 150 mL of M aq. $NaHSO_4$ followed by a washing with 150 mL of brine. The ether layer was evaporated *in vacuo* and the resulting residue was redissolved in 150 mL of ether and cooled to 0°. To this stirred mixture was slowly added 150 mL of a 1:1 mixture of MeOH and 30% aq. H_2O_2. After the addition, the mixture was stirred for 1 h at 0°. At the end of this time, the reaction was diluted with 200 mL of ether and poured over 200 mL of saturated aq. $NaHCO_3$. The layers were separated and the aq. layer was extracted twice with 100 mL of ether. The combined ether extracts were washed twice with 150 mL of saturated aq. $NaHCO_3$ followed by 150 mL of brine. The ether solution was then dried ($MgSO_4$) and concentrated. Flash chromatography (silica, 15% EtOAc in petroleum ether) afforded 10.21 g (75%) of **5**; R_F 0.32 (silica, 15%

EtOAc in petroleum ether); $[\alpha]_D^{22}$ 0.50° (*c* 0.24, $CHCl_3$); ν_{max}^{neat} 3500 (bm), 2960 (s), 2925 (s), 1785 (s), 1705 (s), 1387 (s), and 1202 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$); δ 5.83 (dt, 1 H, *J* 6.8, 15.4 Hz, C=C–*H*), 5.68 (d, 1 H, *J* 5.2 Hz, C*H*Br), 5.45 (dd, 1 H, *J* 6.5, 15.5 Hz, C=C–*H*), 4.49–4.42 (m, 2 H, C*H*O, C_2H), 4.33–4.23 (m, 2 H, C_2H and C_1H), 3.03 (d, 1 H, *J* 2.9 Hz, O*H*), 2.41 (m, 1 H, C*H*Me_2), 2.02 (dt, 2 H, *J* 6.8, 7.1 Hz, C=C–CH_2), 1.35–1.23 (m, 22 H, CH_2), 0.92 (d, 6 H, *J* 7.0 Hz, CH_3), 0.90 (t, 3 H, *J* 8.3 Hz, CH_3); ^{13}C-n.m.r. (125 MHz, $CDCl_3$: δ 169.0, 152.8, 136.3, 126.7, 71.5, 63.4, 58.3, 49.2, 32.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8, 27.9, 22.7, 17.8, 17.7, 14.7, 14.6, and 14.1; *m/z* calc. for $C_{24}H_{46}N_2BrO_4$ 507,262 ($M+NH_4^+$); found 507,259.

(4S)-3-[(2′R,3′R,4′E)-2′-azido-3′-hydroxy-4′-octadecenoyl]-4-(isopropyl)-2-oxazolidinone (**7**). — To a stirred solution of **5** (10.21 g, 20.88 mmol) in dry Me_2SO (53 mL was added NaN_3 (2.67 g, 41.1 mmol). The reaction was complete in 2 h as determined by t.l.c. analysis. The mixture was diluted with ether (300 mL), washed with water (3 × 100 mL) and brine (100 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 20% EtOAc in petroleum ether) yielded 8.65 g of **7** (92%) as a light yellow oil; R_F 0.41 (silica, 20% EtOAc in petroleum ether); $[\alpha]_D^{22}$ +20.7° (*c* 2.42, $CHCl_3$); $\nu_{max}^{CCl_4 smear}$ 3520 (bm), 2960 (s), 2925 (s), 2125 (s), 1785 (s), 1705 (s), 1395 (s), and 1212 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 5.89 (dt, 1 H, *J* 7.1, 15.4 Hz, C=C–*H*), 5.60 (dd, 1 H, *J* 7.2, 15.4 Hz, C=C–*H*), 5.10 (d, 1 H, *J* 7.5 Hz, C*H*N_3), 4.50 (ddd, 1 H, *J* 7.2, 7.4, 7.6 Hz, C*H*OH), 4.46 (m, 1 H, C_2H), 4.35 (dd, 1 H, *J* 8.4, 9.0 Hz, C_2H), 4.26 (dd, 1 H, *J* 3.1, 9.2 Hz, C_1H), 2.50 (d, 1 H, *J* 7.6 Hz, O*H*), 2.38 (m, 1 H, C*H*Me_2), 2.08 (dt, 2 H, *J* 7.0, 7.2, Hz, C=C–CH_2), 1.41–1.24 (m, 22 H, CH_2), 0.93 (d, 3 H, *J* 7.9 Hz, CH_3), 0.89 (t, 3 H, *J* 11.3 Hz, CH_3), and 0.88 (d, 3 H, *J* 11.5 Hz, CH_3); ^{13}C-n.m.r. (125 MHz, $CDCl_3$): δ 169.5, 154.0, 136.4, 127.5, 73.7, 73.6, 62.6, 58.9, 32.2, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8, 28.4, 22.6, 17.9, 14.6, and 14.0; *m/z* calc. for $C_{24}H_{46}N_5O_4$ 468,355 (M + NH_4); found 468.347.

*(4S)-3-[(2′R, 3′R, 4′E)-2′-azido-3-*O*-tert-butyldimethylsilyl-3′-hydroxy-4′-octadecenoyl)-4-(isopropyl)-2-oxazolidinone* (**8**). — To a stirred solution of **7** (6.81 g, 15.1 mmol) in dry THF (75 mL) at 0° was added 2,6-lutidine (3.5 mL) followed by *tert*-butyldimethylsilyl triflate (5.2 mL). After 0.5 h, the ice bath was removed and the mixture was stirred for 1 h at ambient temperature. The mixture was diluted with EtOAc (250 mL), washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 7% EtOAc in petroleum ether) yielded 8.27 g of **8** (97%) as an oil; R_F 0.36 (silica, 10% EtOAc in petroleum ether); $[\alpha]_D^{22}$ −1.63° (*c* 4.0, $CHCl_3$); ν_{max}^{neat} 2970 (s,) 2940 (s), 2865 (s), 2110 (s), 1795 (s), 1395 (s), and 2110 (s), cm^{-1}; ^{1}H-n.m.r. (500 MH, $CDCl_3$) δ 5.74 (dt, 1 H, *J* 7.3, 15.4 Hz, C=C–*H*), 5.54 (dd, 1 H, *J* 6.2, 15.4 Hz, C=C–*H*), 5.22 (d, 1 H, *J* 6.4 Hz, C*H*N_3), 4.65 (dd, 1 H, *J* 6.7, 7.0 Hz, C*H*OSi), 4.50 (m, 1 H, C_1H), 4.33 (dd, 1 H, *J* 8.0, 9.0 Hz, C_2H), 4.25 (dd, 1 H, *J* 3.0, 9.0 Hz, C_2H), 2.31 (m, 1 H, C*H*Me_2), 2.06 (dt, 2 H, *J* 5.3, 7.0 Hz, C=CH_2), 1.39–1.27 (m, 22 H, CH_2), 0.94 (d, 3 H, *J* 7.0 Hz, CH_3), 0.91–0.88 (m, 15 H, SiCMe_3) and 2 × CH_3), 0.10 and 0.07 (singlets, 3 H each, OSiCH_3); ^{13}C-n.m.r. (125 MHz, $CDCl_3$): δ 167.3, 153.5, 135.8, 128.2, 74.4, 64.7, 63.4, 58.5, 32.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.2, 25.7, 22.7, 18.0, 17.9, 14.6, and 14.1; *m/z* calc for $C_{30}H_{57}N_4O_4$ 565.437 (M + H); found 565.4122.

*(2S,3S,4E)-2-azido-3-O-(*tert*-butyldimethylsilyl)-4-octadecen-1,3-diol* (**9**). — To a stirred solution of **8** (7.43 g, 13.2 mmol) in dry THF at 0° (66 mL) was added $LiBH_4$ (859 mg) in three portions. The mixture was stirred for 1.5 h at 0° and then for 0.5 h at ambient temperature. The reaction was cooled back down to 0°, diluted with EtOAc (100 mL) and the excess $LiBH_4$ was quenched by the slow addition of saturated aq. NH_4Cl (100 mL). The layers were separated and the aq. layer was extracted with EtOAc (2 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated. Flash chromatography (silica, 10% EtOAc in petroleum ether) yielded 4.74 g of **9** (82%) as an oil; R_F 0.33 (silica, 2% ether in CH_2Cl_2); $[\alpha]_D^{22}$ −40.5° (*c* 4.5, $CHCl_3$); ν_{max}^{neat} 3510 (bm), 2940 (s), 2865 (s), 1215 (s), 1210 (s), and 1095 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 5.65 (dt, 1 H, *J* 7.0, 15.4 Hz, C=C–*H*), 5.42 (dd, 1 H, *J* 6.8, 15.4 Hz, C=C–*H*), 4.12 (dd, 1 H, *J* 5.6, 6.7 Hz, C*H*OSi), 3.66–3.58 (m, 2 H, CH_2OH), 3.36 (ddd, 1 H, C*H*N_3), 2.52 (bs, 1 H, O*H*), 2.01 (dt, 2 H, *J* 7.0, 7.1 Hz, CH_2C=C), 1.35–1.22 (m, 22 H, CH_2), 0.91–0.84 (m, 12 H, CH_3), and $OSiCMe_3$, 0.06 and 0.01 (singlets, 3 H each, $OSiCMe_2$); ^{13}C-n.m.r. (125 MHz, $CDCl_3$): δ 134.6, 129.0, 75.0, 68.0, 67.9, 32.2, 29.6, 29.4, 29.3, 28.9, 25.7, 22.6, 17.9, and 14.0; *m/z* calc. for $C_{24}H_{53}N_4O_2Si$ 457.3922 ($M + NH_4$); found 457.3961.

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl fluoride (**15**). — To a solution of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[20] (5.98 g, 9.44 mmol) in dry CH_2Cl_2 (47 mL) at −30° was added HF–pyridine complex (4.5 mL) followed by N-bromosuccinimide (2.35 g). The mixture was stirred for 1 h at −30° and then slowly allowed to warm over 3 h to 0°. The mixture was then diluted with EtOAc (200 mL) and quenched by the slow addition of a saturated aq. $NaHCO_3$ (50 mL). The layers were separated and the organic layer was washed with saturated aq. $NaHCO_3$ (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 15% EtOAc in petroleum ether) afforded 4.90 g of **15** (96%) as a white foam; R_F 0.55 (silica 30% ether in petroleum ether); $[\alpha]_D^{22}$ +4.40° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3040 (s), 3020 (s), 2930 (s), 2880 (s), 1955 (w), 1885 (w), 1815 (w), 1750 (w), 1372 (s), and 1345 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 7.52–7.38 (m, 20 H, aromatic), 5.59 (dd, 1 H, *J* 53.7, 2.7 Hz, H-1), 4.94 (d, 1 H, *J* 11.4 Hz, CH_2-Ph), 4.84 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.81 (d, 1 H, *J* 11.8 Hz CH_2-Ph), 4.74 (d, 1 H, *J* 11.8 Hz, CH_2-Ph), 4.72 (d, 1 H, *J* 11.8 Hz, CH_2-Ph), 4.57 (d, 1 H, *J* 11.4 Hz, CH_2-Ph), 4.48 (d, 1 H, *J* 11.9 Hz, CH_2-Ph), 4.41 (d, 1 H, *J* 11.9 Hz, CH_2-Ph). 4.10 (t, 1 H, *J* 6.5 Hz, H-5), 4.04 (ddd, 1 H, *J* 25.2, 10.0, 2.7 Hz, H-2), 4.02 (d, 1 H, *J* 2.7 Hz, H-4), 3.94 (dd, 1 H, *J* 10.0, 2.7 Hz, H-3), and 3.54 (d, 2 H, *J* 6.5 Hz, H-6); *m/z* calc. for $C_{34}H_{35}FO_5$ 542.247 (M^+); found 542.251.

Phenyl O-*(4,6-O-isopropylidene-2,3-di-O-pivaloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-pivaloyl-1-thio-β-D-glucopyranoside* (**12**). — To a stirred solution of phenyl O-(β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside[22] (3.96 g, 9.13 mmol) in dry *N,N*-dimethylformamide (20 mL) at ambient temperature was added 2,2-dimethoxypropane (1.7 mL) and *p*-toluenesulfonic acid (100 mg). After 2 h, the mixture was quenched by the addition of Et_3N (1 mL), then concentrated and dried over P_2O_5 (vacuum, 12 h). The residue was redissolved in dry pyridine (50 mL) and 4-dimethylaminopyridine (500 mg) followed by trimethylacetyl chloride (9 mL) were

added. The mixture was heated for 40 h at 75° and then was allowed to cool to room temperature, diluted with EtOAc (200 mL), washed with water (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. Excess pyridine was removed by azeotroping repeatedly with PhMe. Flash chromatography (silica, 10% EtOAc in petroleum ether) afforded 6.08 g (78%) of **12** as a white foam; R_F 0.54 (silica, 20% EtOAc in petroleum ether); $[\alpha]_D^{22}$ +11.2° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3015 (w), 2980 (s), 2875 (s), 1745 (s), 1488 (s), and 1285 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 7.44–7.42 (m, 3 H, aromatic *H*), 7.27–7.25 (m, 2 H, aromatic *H*), 5.26–5.21 (m, 2 H, H-3, H-2'), 4.89 (dd, 1 H, *J* 9.5, 9.7 Hz, H-2), 4.68 (d, 1 H, *J* 9.5 Hz, H-1), 4.65 (m, 1 H, C*H*O), 4.47 (dd, 1 H, *J* 1.8, 10.8 Hz, H-3'), 4.36 (d, 1 H, *J* 8.1 Hz, H-1'), 4.31 (m, 1 H, C*H*O), 4.18 (dd, 1 H, *J* 5.8, 11.8 Hz, C*H*O), 3.94 (m, 2 H, C*H*O), 3.83 (dd, 1 H, *J* 9.6, 9.7 Hz, C*H*O), 3.58 (m, 1 H, H-5), 3.26 (bs, 1 H, H-5'), 1.30 and 1.28 (singlets, 3 H each, C*Me*$_2$), 1.20, 1.19, 1.18 (singlets, 9 H each, 3 × C*Me*$_3$), and 1.11 (singlets, 18 H, 2 × C*Me*$_3$); *m/z* calc. for $C_{45}H_{67}O_{15}S$ (M − CH_3); 879.420; found 879.423.

Phenyl O-*(6-*O*-benzoyl-2,3-di-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-pivaloyl-1-thio-β-*D*-glucopyranoside* (**14**). — To a cold (0°) solution of **12** (4.88 g, 5.45 mmol) in tetrahydrofuran (10 mL) was added water (5 mL) followed by CF_3CO_2H (15 mL). After stirring for 1.5 h at 0°, the mixture was poured into a mixture of EtOAc (200 mL) and saturated aq. $NaHCO_3$ (150 mL). The layers were separated and the organic layer was washed with brine (50 mL), dried ($MgSO_4$), and evaporated. The crude product was azeotroped with benzene (2 × 50 mL), redissolved in dry *N,N*-dimethylformamide (40 mL) and cooled to −20°. Triethylamine (0.94 mL) and PhCN (0.68 mL) were added and the reaction was allowed to warm to 0° over 0.5 h. At the end of this time, the reaction was quenched by the addition of MeOH (1 mL). The mixture was diluted with EtOAc (150 mL), washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 10% EtOAc in petroleum ether) afforded 4.42 g (93%) of **14** as a white foam; R_F 0.35 (silica, 40% ether in petroleum ether); $[\alpha]_D^{22}$ −4.2° (*c* 3.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3500 (w), 2980 (s,) 1740 (s), 1403 (w), 1375 (w), and 1285 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.02–7.26 (m, 10 H, aromatic), 5.29 (t, 1 H, *J* 9.5 Hz, H-3), 5.22 (dd, 1 H, *J* 10.2, 8.0 Hz, H-2'), 4.90 (dd, 1 H, *J* 10.1, 9.5 Hz, H-2), 4.89 (dd, 1 H, *J* 10.2, 3.5 Hz, H-3'), 4.69 (d, 1 H, *J* 10.1 Hz, H-1), 4.65 (dd, 1 H, *J* 11.6, 5.2 Hz, H-6'a), 4.58 (dd, 1 H, *J* 12.0, 1.6 Hz, H-6a), 4.49 (d, 1 H, *J* 8.0 Hz, H-1'); 4.46 (dd, 1 H, *J* 11.6, 7.4 Hz, H-6'b), 4.14 (dd, 1 H, *J* 12.0, 5.1 Hz, H-6b), 4.07 (bt, 1 H, *J* 4.2 Hz, H-4'), 3.91 (t, 1 H, *J* 9.5 Hz, H-4), 3.85 (dd, 1 H, *J* 7.4, 5.2 Hz, H-5'), 3.63 (ddd, 1 H, *J* 9.5, 5.1, 1.6 Hz, H-5), 2.36 (d, 1 H, *J* 5.2 Hz, O*H*), 1.18, 1.18, 1.17, 1.15, and 1.13 (singlets, 9 H each, C*Me*$_3$); *m/z* calc. for $C_{50}H_{71}O_{16}S$ 959.4462 (M + H); found 959.4558.

Phenyl O-*(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzoyl-2,3-di-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-pivaloyl-1-thio-β-*D*-glucopyranoside* (**16**). — To a stirred mixture of silver triflate (107 mg) and $SnCl_2$ (79 mg), azeotropically dried with benzene (2 × 10 mL) in dry ether (5 mL), flame-dried 4 Å molecular sieves (300 mg) were added. The mixture was cooled to 0° and a solution of **14** (200 mg, 0.208 mmol) in dry ether (2 mL) was added *via* syringe. After 5 min, a solution

of **15** (339 mg) in dry ether (2.5 mL) was added *via* syringe. After 2 h at 0° the mixture was diluted with EtOAc (50 mL) and filtered through a pad of Celite. The filtrate was washed with saturated aq. $NaHCO_3$ (2 × 25 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 20% ether in petroleum ether) afforded 226 mg (73%) of **16** as a white foam; R_F 0.42 (silica, 30% ether in petroleum ether); $[\alpha]_D^{22}$ +10.7° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3070 (w), 2990 (s), 2890 (s), 1750 (s), and 1275 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.06–7.17 (m, 30 H, aromatic), 5.23 (t, 1 H, *J* 9.4 Hz, H-3), 5.16 (dd, 1 H, *J* 10.3, 7.8 Hz, H-2'), 4.92–4.72 (m, 7 H, C*H*-O), 4.65–4.57 (m, 5 H, H-1, H-1'', C*H*-O), 4.49–4.42 (m, 3 H, H-1', C*H*-O), 4.28–4.05 (m, 4 H, C*H*-O), 3.96–3.84 (m, 4 H, C*H*-O), 3.73–3.50 (m, 4 H, C*H*-O), 1.20, 1.12, 1.09, 1.08, and 1.07 (singlets, 9 H each, $C(Me_3)$; ^{13}C n.m.r. (125 MHz, $CDCl_3$): δ 177.9, 177.5, 176.9, 176.5, 175.6, 100.4, 100.1, 86.1, 78.5, 77.4, 77.1, 75.1, 74.8, 74.1, 73.3, 73.0, 72.7, 72.0, 70.0, 69.9, 68.9, 67.6, 64.1, and 61.7.

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→4)-O-(6-O-benzoyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-pivaloyl-β-D-glucopyranosyl fluoride (**17**). —To a stirred solution of **16** (830 mg, 0.559 mmol) in dry CH_2Cl_2 (5.6 mL) at −35° was added HF·pyridine complex (0.56 mL) followed by *N*-bromosuccinimide (110 mg).The mixture was allowed to warm to 0° over a period of 1 h and then worked up as already described for compound **15**. Flash chromatography (silica, 10% EtOAc in petroleum ether) afforded **17** (690 mg, 89%) as a white foam; R_F 0.43 (silica, 30% ether in petroleum ether); $[\alpha]_D^{22}$ +30.2° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3050 (w), 2980 (s), 2880 (s), and 1750 (s), 1380 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.08–8.06 (m, 2 H, aromatic), 7.59–7.18 (m, 23 H, aromatic) 5.30 (dd, 1 H, *J* 5.6, 52.6, Hz), 5.21 (dd, 1 H, *J* 7.8, 10.2, Hz, H-2'), 5.15 (dd, 1 H, *J* 7.2, 8.2 Hz, H-3), 4.95–4.87 (m, 3 H, C*H*O), 4.83–4.77 (m, 5 H, C*H*O), 4.66–4.58 (m, 5 H, C*H*O), 4.51–4.44 (m, 3 H, C*H*O), 4.21–4.11 (m, 3 H, C*H*O), 4.06 (bs, 1 H, C*H*O), 4.00–3.92 (m, 2 H, C*H*O), 3.88–3.86 (m, 1 H, C*H*O), 3.76–3.65 (m, 3 H, C*H*O), 1.17, 1.15, 1.10, 1.09, and 1.08 (singlets, 9 H each, C*Me*$_3$); *m/z* calc for $C_{78}H_{99}FO_{21}$ 1390, 666 (M^+); found 1390.673.

O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1→4)-O-(6-O-benzoyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-pivaloyl-β-D-glycopyranosyl fluoride (**19**). — A flask containing a solution of **17** (690 mg, 0.50 mmol) in EtOH (7.5 mL) and 10% $Pd(OH)_2$ on carbon (354 mg) was repeatedly evacuated and flushed with H_2. The reaction was stirred under hydrogen for 24 h at ambient temperature. At the end of this period the reaction was diluted with MeOH and filtered through a pad of Celite and evaporated. The residual white solid was dried azeotropically with benzene (2 × 15 mL) and dissolved in dry pyridine (5 mL). 4-Dimethylaminopyridine (10 mg) and Ac_2O (283 μL) were added and the mixture was stirred overnight. The mixture was worked up as for compound **12**. Flash chromatography (silica, 25% EtOAc in petroleum ether) yielded **19** (543 mg, 91%) as a white foam; R_F 0.58 (silica, 30% EtOAc in petroleum ether); $[\alpha]_D^{22}$ +62.8° (*c* 1.0, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3040 (w), 2980 (s), 1750 (s), and 1375 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.08–7.49 (m, 5 H, aromatic), 5.46 (d, 1 H, *J* 1.9 Hz, H-4''), 5.35 (dd, 1 H, *J* 53.2, 5.0 Hz, H-1), 5.32–5.16 (m, 4 H, C*H*-O), 5.05 (d, 1 H, *J* 3.1 Hz, H-1''), 5.00–4.60 (m, 4 H, C*H*-O), 4.56 (d, 1 H, *J* 7.8 Hz, H-1'), 4.53–3.75 (m, 9 H,

C*H*-O), 2.14, 2.13, 2.07, 1.98 (singlets, 3 H each, acetates), 1.26, 1.21, 1.18, 1.15, and 1.07 (singlets, 9 H each, C*Me*$_3$).

O-*(2,3,4,6-Tetra*-O-*acetyl-α*-D-*galactopyranosyl)-(1→4)*-O-*(6*-O-*benzoyl-2,3-di*-O-*pivaloyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*pivaloyl-β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R,*4*E)-*2-azido-3*-O-tert-*butyldimethylsilyl-4-octadecen-1,3-diol* (**20**). — To a mixture of $AgClO_4$ (62 mg), $SnCl_2$ (57 mg) and 4 Å molecular sieves (300 mg) (prepared as for compound **16**) in CH_2Cl_2 (1 mL) at 0° was added a solution of **19** (179 mg, 0.15 mmol) in CH_2Cl_2 (2.0 mL). After 10 min, a solution of **9** (660 mg) in CH_2Cl_2 (2.0 mL) followed by 2,6-lutidine (17 μL) were added. The mixture was stirred and allowed to warm slowly to ambient temperature over 12 h. Dilution with CH_2Cl_2 (25 mL) followed by processing as for compound **16** and flash chromatography (silica, 10–25% EtOAc in petroleum ether) afforded 194 mg (80%) of **20** as a white foam; R_F 0.45 (silica, 30% EtOAc in petroleum ether); $[a]_D^{23}$ +27.5° (*c* 1.6, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 2980 (m), 2940 (s), 2865 (m), 2110 (m), 1750 (s), and 1485 (m) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.06–7.49 (m, 5 H, aromatic), 5.61 (dt, 1 H, *J* 15.4, 7.7 Hz, C=C-*H*), 5.41 (d, 1 H, *J* 2.3 Hz, H-4″), 5.34 (dd, 1 H, *J* 15.4, 7.3 Hz, C=C-*H*), 5.27 (dd, 1 H, *J* 10.9, 3.3 Hz, H-3″), 5.20 (dd, 1 H, *J* 9.6 Hz, H-3), 5.14 (dd, 1 H, *J* 10.8, 3.5 Hz, H-2″), 5.12 (dd, 1 H, *J* 10.3, 7.9 Hz, H-2′), 5.01 (d, 1 H, *J* 3.4 Hz, H-1″), 4.89 (dd, 1 H, *J* 10.1, 2.5 Hz, H-3′), 4.88 (dd, 1 H, *J* 9.4, 7.9 Hz, H-2), 4.78 (dd, 1 H, *J* 11.9, 3.1 Hz, H-6a″), 4.53 (dd, 1 H, *J* 11.7, 1.5 Hz, H-6a′), 4.49–4.46 (m, 3 H, H-1, H-1′, H-6a), 4.42 (dd, 1 H, *J* 7.0, 7.3 Hz, C*H*–O), 4.24 (dd, 1 H, *J* 10.8, 7.9 Hz, C*H*–O), 4.14–4.06 (m, 3 H, C*H*-O), 4.02 (d, 1 H, *J* 1.8 Hz, H-4′), 3.95 (dd, 1 H, *J* 9.6 Hz, H-4), 3.85 (m, 1 H, C*H*–O), 3.73 (dd, 1 H, *J* 10.4, 6.9 Hz, C*H*–O), 3.51–348 (m, 2 H, C*H*–O), 3.39 (m, 1 H, C*H*–N_3), 2.11, 2.10, 2.05, 1.95 (singlets, 3 H each, acetates), 1.98 (dt, 2 H, *J* 7.6, 6.5 Hz, C=C–CH_2), 1.34–1.03 (m, 67 H, CH_2 and C*Me*$_3$), 0.87–0.82 (m, 12 H, SiCMe$_3$ and CH_3), 0.02 and 0.01 (singlets, 3 H each, Si–CH_3).

O-*(2,3,4,6-Tetra*-O-*acetyl-α*-D-*galactopyranosyl)-(1→4)*-O-*(6*-O-*benzoyl-2,3-di*-O-*pivaloyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*pivaloyl-β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R, *4*E)-*3*-O-tert-*butyldimethylsilylsphingenine* (**21**). — To a stirred solution of **20** (161 mg 0.10 mmol) in benzene (1.0 mL) at 45° was added Ph_3P (52 mg). After 0.5 h, water was added (30 μL) and stirring was continued for 11 h at 45°. The mixture was cooled to room temperature, diluted with EtOAc (30 mL), washed with saturated aq. NH_4Cl (10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography (silica, 20% EtOAc in petroleum ether) afforded **21** (144 mg 90%) as a white foam; R_F 0.32 (5% MeOH in CH_2Cl_2); $[a]_D^{22}$ +38.3° (*c* 0.75, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3300 (w), 2935 (s), 2860 (s), 1745 (s), 1485 (s), and 1375 (s), cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.05–7.48 (m, 5 H, aromatic), 5.58 (dt, 1 H, *J* 7.7, 15.4 Hz, C=C-*H*), 5.41 (d, 1 H, *J* 2.2 Hz, H-4″), 5.28 (dd, 1 H, *J* 7.6, 15.4 Hz, C=C-*H*), 5.27 (dd, 1 H, *J* 3.3, 10.8 Hz, H-3″), 5.20 (dd, 1 H, *J* 9.6, 9.7 Hz, H-3), 5.15 (dd, 1 H, *J* 3.6, 10.7 Hz, H-2″), 5.12 (dd, 1 H, *J* 7.6, 10.3 Hz, H-2′), 5.02 (d, 1 H, *J* 3.5 Hz, H-1″) 4.90 (dd, 1 H, *J* 2.8, 10.1 Hz, H-3′), 4.87 (dd, 1 H, *J* 7.8, 9.8 Hz, H-2), 4.78 (dd, 1 H, *J* 3.1, 11.9 Hz, H-6a″), 4.53 (bd, 1 H, *J* 11.3 Hz, H-6a′), 4.48–4.41 (m, 3 H, H-1, H-1′, H-6a), 4.23 (dd, 1 H, *J* 7.9, 10.8 Hz, C*H*O), 4.14–4.11 (m, 2 H, C*H*O), 4.01 (d, 1 H, *J* 2.2 Hz, H-4′), 3.95 (dd, 1 H *J* 9.3, 9.6 Hz, H-4),

3.90–3.84 (m, 2 H C*H*O), 3.70–3.66 (m, 2 H, C*H*O) 3.61–3.57 (m, 2 H, C*H*O, C*H*N), 3.49–3.45 (m, 1 H, C*H*O), 2.74 (m, 2 H, CH_2), 2.10, 2.07, 2.01, 1.95 (singlets, 3 H each, acetates), 2.00 (dt, 2 H, *J* 6.6, 7.5 Hz, C=C–CH_2), 1.35–1.02 (m, 67 H, CH_2 and C(Me_3), 0.85 (t, 3 H, *J* 7.0 Hz, CH_3), 0.83 (s, 9 H, SiCMe_3), −0.01 and −0.04 (singlets, 3 H each, $Si(Me_2)$; *m/z* calc. for $C_{82}H_{134}NO_{27}Si$ 1592.891 (M + H); found 1592.898.

O-*(2,3,4,6-Tetra*-O-*acetyl-α*-D-*galactopyranosyl)-(1→4)*-O-*(6*-O-benzoyl-2,3-di-O-*pivaloyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*pivaloyl-β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R,*4*E)-*3*-O-tert-*butyldimethylsilyl-2*-N-*octadecanoylsphingenine* (**22**). — To a solution of **21** (70 mg, 44 μmol) in dry CH_2Cl_2 (0.44 mL) was added Et_3N (9.2 μL) and 4-dimethylaminopyridine (1 mg). The mixture was cooled to 0° and octadecanoyl chloride (20 μL) was added *via* syringe with stirring. After 1 h at 0° the mixture was diluted with a cold (0°) mixture of ether (20 mL) and saturated aq. $NaHCO_3$ (10 mL). The layers were separated and the ether phase was washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 20% EtOAc in petroleum ether) afforded **22** (79 mg, 97%) as a white foam; R_F 0.40 (silica, 30% EtOAc in petroleum ether); $[a]_D^{23}$ +27.8° (*c* 0.4, $CHCl_3$); $\nu_{max}^{CCl_4\ smear}$ 3460 (w), 2980 (s), 2940 (s), 2870 (s), 1750 (s), 1690 (m), 1490 (s), and 1285 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.06–7.49 (m, 5 H, aromatic), 5.45 (dt, 1 H, *J* 15.4, 7.6 Hz, C=C–*H*), 5.43–5.38 (m, 2 H, N*H*, H-4″), 5-32 (dd, 1 H, *J* 15.3, 7.5 Hz, C=C–*H*), 5.28 (dd, 1 H, *J* 10.3, 3.3 Hz, H-3″), 5.20 (dd, 1 H, *J* 9.5 Hz, H-3), 5.15–5.10 (m, 2 H, H-2′, H-2″), 5.02 (d, 1 H, *J* 3.1 Hz, H-1″), 4.90–4.84 (m, 2 H, H-2. H-3′), 4.77 (dd, 1 H, *J* 11.4, 3.0 Hz, H-6a″), 4.51–4.41 (m, 5 H, H-1, H-1′, C*H*–O), 4.23 (dd, 1 H, *J* 10.2, 9.5 Hz, C*H*–O), 4.18–4.07 (m, 3 H, C*H*–O), 4.04–4.01 (m, 2 H, C*H*–O), 3.96–3.93 (m, 2 H, C*H*–N and C*H*–O), 3.85 (m, 1 H, *J* 5.7 Hz, C*H*–O), 3.48 (m, 1 H, *J* 8.3 Hz, C*H*–O), 3.43 (m, 1 H, *J* 6.9 Hz, C*H*–O), 2.11, 2.09, 2.05, 1.95 (singlets, 3 H each, acetates), 2.01 (m, 4 H, C(O)CH_2, C=C–CH_2), 1.52 (m, 2 H, C(O)CH_2), 1.26–1.04 (m, 95 H, CH_2, CMe_3) 0.86 (t, 6 H, *J* 6.5 Hz, CH_3), 0.81 (singlet, 9 H, SiCMe_3), −0.41 and −0.51 (singlets, 3 H each, $SiMe_2$).

O-*(2,3,4,6-Tetra*-O-*acetyl-α*-D-*galactopyranosyl)-(1→4)*-O-*(6*-O-*benzoyl-2,3-di*-O-*pivaloyl-β*-D-*galactopyranosyl-(1→4)*-O-*(2,3,6-tri*-O-*pivaloyl-β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R,*4*E)-*2*-N-*octadecanoylsphingenine* (**23**). — To a solution of **22** (74 mg, 40 μmol) in dry tetrahydrofuran (0.40 mL) was added a solution of Bu_4NF in THF (1.1 M, 44 μL). After stirring for 2 h, the reaction was diluted with EtOAc (20 mL) washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (silica, 15–30% EtOAc in petroleum ether) afforded **23** (66 mg, 95%) as a white foam; R_F 0.38 (silica, 30% EtOAc in petroleum ether); $[a]_D^{22}$ 34.5° (*c* 0.4, $CHCl_3$); $\nu_{max}^{CHCl_3\ smear}$ 3700 (w), 3540 (w), 3460 (w), 1745 (s), 1665 (m), 1485 (s), and 1275 (s) cm^{-1}; ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.07–7.50 (m, 5 H, aromatic) 5.84 (d, 1 H, *J* 6.0 Hz, N*H*), 5.62 (dt, 1 H, *J* 7.7, 15.4 Hz, C=C–*H*), 5.42 (d, 1 H, *J* 2.1 Hz, H-4″), 5.40 (dd, 1 H, 7.8, 15.4 Hz, C=C–*H*), 5.28 (dd, 1 H, *J* 3.2, 10.8 Hz, H-3″), 5.22 (dd, 1 H, *J* 9.6, 9.7 Hz, H-3), 5.17–5.12 (m, 2 H, H-2′, H-2″), 5.03 (d, 1 H, *J* 3.3 Hz, H-1″), 4.93 (dd, 1 H, *J* 2.5, 10.5 Hz, H-3′), 4.83 (dd, 1 H, *J* 7.9, 9.8 Hz, H-2), 4.78 (dd, 1 H, *J* 3.2, 12.1 Hz, H-6a″), 4.71 (bd, *J* 12.5 Hz, 1 H, C*H*O), 4.53 (d, 1 H, *J* 7.9 Hz, H-1′), 4.49 (m, 1 H, C*H*O), 4.44–4.41 (m, 1 H, C*H*O), 4.41 (d, 1 H, *J* 7.9 Hz, H-1), 4.23 (dd, 1 H, *J* 8.0, 10.8 Hz,

CHO), 4.15–4.08 (m, 3 H, CHO), 4.03–3.93 (m, 4 H, CHN CHO), 3.86 (m, 1 H, CHO), 3.56 (dd, 1 H, *J* 3.0, 9.7 Hz, CHO), 3.47 (m, 1 H, CHO), 3.01 (d, 1 H, *J* 7.6 Hz, OH), 2.11, 2.10, 2.05, 1.95 (singlets, 3 H each, acetates), 2.07 (m, 4 H, C=C–CH_2 and NC(O)CH_2), 1.52 (m, 2 H, NC(O)CCH_2), 1.31–0.98 (m, 95 H, CH_2, CMe_3), 0.86 (t, 6 H, *J* 7.0 Hz, CH_3); *m/z* calc. for $C_{44}H_{153}NO_{28}$ 1727.055 (M − OH); found 1727.051.

Globotriaosylceramide (Gb_3, **1**). — To a solution of **23** (60 mg, 34 μmol) in dry MeOH (0.69 mL) was added a freshly prepared solution of NaOMe in MeOH (0.5M, 70 μL). The reaction flask was fitted with a reflux condenser and heated for 24 h to 60°. The mixture was allowed to cool to ambient temperature, diluted with MeOH (1 mL), neutralized with Amberlyst 15-ion exchange resin (50 mg), filtered through a pad of Celite, concentrated, and azeotroped with toluene (2 × 5 mL). Flash chromatography (silica, 60:25:4 $CHCl_3$–MeOH–water, afforded 33 mg (91%) of **1** as an amorphous white powder; R_F 0.35 (silica $CHCl_3$–MeOH–water, 60:25:4); $[a]_D^{23}$ +24.1° (*c* 0.44, pyridine); ^{1}H-n.m.r. (500 MHz, 98:2, Me_2SO-d_6–D_2O): δ 5.53 (dt, 1 H, *J* 15.3, 7.7 Hz, C=C–H), 5.33 (dd, 1 H, *J* 15.4, 7.1 Hz, C=CH), 4.78 (d, 1 H, *J* 3.7 Hz, H-1″), 4.25 (d, 1 H, *J* 7.6 Hz, H-1′), 4.15 (d, 1 H, *J* 7.8 Hz, H-1), 4.05 (t, 1 H, *J* 6.1 Hz, H-5″), 3.93 (dd, 1 H, *J* 10.2, 5.0 Hz, H-1-ceramide), 3.88 (t, 1 H, *J* 7.5 Hz, 1 H, H-3-ceramide), 3.80–3.28 (m, 18 H, CH–O and DHO peak), 3.04 (t, 1 H, *J* 8.0 Hz, H-2), 2.01 (t, 2 H, *J* 7.4 Hz, C=C–CH_2), 1.91 (m, 2 H, COCH_2), 1.43 (m, 2 H, $COCH_2$–CH_2), 1.31–1.11 (m, 50 H, CH_2) and 0.83 (t, 6 H, *J* 6.8 Hz, CH_3); ^{13}C-n.m.r. (125 MHz, Me_2SO-d_6): δ 171.7, 131.5, 131.3, 103.8, 103.5, 100.6, 80.7, 77.1, 75.0 74.8, 74.4, 73.2, 72.8, 71.1, 70.8, 70.7, 69.2, 68.8, 68.6, 60.4, 59.3, 52.9, 39.0, 35.6, 31.8, 29.2, 29.1, 28.8, 28.7, 25.4, 22.2, and 13.8; *m/z* calc. for $C_{54}H_{101}NNaO_{18}$ 1074.492 (M + Na); found 1074.690.

Globotriaosylsphingosine (lysoGb$_3$, **2***).* — To a solution of **21** (60 mg, 37.7 μmol) in dry tetrahydrofuran (0.4 mL) at 0° was added a solution of Bu_4NF (1.1M, 70μL) in tetrahydrofuran. The mixture was then slowly allowed to warm over 3 h to 25° and stirred at that temperature until completion of the reaction (t.l.c). The mixture was worked up as for **23** and azeotroped with PhMe (2 × 10 mL). The crude solid product was dissolved in dry MeOH (1 mL) and freshly prepared solution of NaOMe in MeOH (0.5M, 80 μL) was added. The flask was fitted with a reflux condenser and heated for 24 h at 60°. Flash chromatography (silica, 12:6:1 $CHCl_3$–MeOH–water) afforded 19 mg (63%) of **2** as an amorphous white solid; R_F 0.37 (silica, 12:6:1 $CHCl_3$–MeOH–water,); ^{1}H-n.m.r. (500 MHz, Me_2SO-d_6–D_2O, 98.:2); δ 5.56 (dt, 1 H, *J* 7.6, 15.5 Hz, C=C–H), 5.43 (dd, 1 H, *J* 6.6, 15.5 Hz, C=C–H), 4.79 (d, 1 H, *J* 3.8 Hz, H-1″), 4.24 (d, 1 H, *J* 7.6 Hz, H-1′), 4.16 (d, 1 H, *J* 7.9 Hz, H-1), 4.05 (t, 1 H, *J* 6.5 Hz), 3.82–3.30 (m, 19 H, CH–O), 3.29 (s, DHO), 3.02 (t, 1 H, *J* 8.2 Hz, CH–O), 2.75 (m, 1 H), 1.97 (q, *J* 6.9 Hz, C=C–CH_2), 1.32–1.12 (m, 22 H, CH_2), and 0.84 (t, 3 H, *J* 6.9 Hz, CH_3).

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