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Immunomodulating glycosphingolipids: an efficient synthesis of a 2′-deoxy-α-galactosyl-GSL

Valeria Costantino,* Ernesto Fattorusso, Concetta Imperatore and Alfonso Mangoni

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", via D. Montesano 49, 80131 Naples, Italy

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Abstract—A new and efficient approach to the total synthesis of 2′-deoxy-α-galactosyl glycosphingolipids was accomplished. Commercially available 3,4,6-tri-*O*-acetylgalactal was used as the chiral starting material for both the sugar and phytosphingosine building blocks required for the synthesis of 1-*O*-(2-deoxy-α-D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol. The key step of the synthetic strategy was the stereoselective α-glycosidation of the azido precursor of sphingosine. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

As part of our continuing study of the chemistry of sponges belonging to genera *Agelas* and *Axinella*, we discovered a new class of glycolipids (α-galactosyl glycosphingolipids) characterized by the presence of an α-galactose as the first sugar of the saccharide chain.[1] The simplest α-galactosyl glycosphingolipid, agelasphin, was discovered by a Japanese group.[2] This compound showed an interesting immunostimulating activity, which is related to its in vivo antitumor properties. Further studies[3,4] demonstrated that (i) the stereochemistry of the glycosidic linkage strongly affects the bioactivity of these compounds, α-glycosides being more active than β-glycosides, and (ii) the bioactivity is highest when the first sugar linked to the ceramide chain is a galactose.

Eight new α-galactosyl glycosphingolipids have been discovered in our laboratory. Some of these compounds showed immunostimulating properties, being able to induce proliferation of T-cells in vitro, others resulted to be inactive. By a comparison of the structure of active and inactive glycolipids, we proposed a structure–activity relationship for the immunomodulating activity: only glycosphingolipids with a non-glycosylated OH at position 2 of the first sugar are immunostimulating agents.[1]

To perform more detailed structure–activity studies, we planned to synthesize some analogues of natural α-Gal-GSLs. Our first target was the total synthesis of 1-*O*-(2-deoxy-α-D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol (**1**), where the key hydroxyl group at position 2′ is not present at all. The synthesis of a 2′-deoxygalactosylceramide has never been performed before, except for a report by Koezuka's group[5] that described a procedure involving the coupling of a bromide donor with a ceramide, leading to the desired compound in unspecified yield and stereoselectivity.

2. Results and discussion

2.1. Synthetic strategy

From a retrosynthetic point of view, the target compound **1** is composed of two main building blocks: the saccharide unit and the phytosphingosine unit (that is acylated with a fatty acid to give the ceramide). Both these units can be derived from the commercially available 3,4,6-tri-*O*-acetyl-D-galactal (**2**) that possesses the D-*arabino* configuration required for the synthesis of natural phytosphingosine (amination leads to inversion of configuration at C-2). To prepare the ceramide moiety, we based our strategy on the synthesis of phytosphingosine proposed by Schmidt in 1995,[6] starting from tri-*O*-benzyl-D-galactal, that was suitably modified to meet our needs.

In our synthetic scheme, selective deprotection of the primary OH of phytosphingosine was required before glycosylation; therefore, the easily removable TIPS group was introduced from the beginning of the synthesis to protect the OH at position 6 of galactal (the incoming position 1 of phytosphingosine). Because the TIPS group is acid labile, an alternative way was required for the preparation of the protected 2-deoxygalactose that is usually obtained by acid-catalyzed hydration of the corresponding galactal. For this purpose, we used the recently reported[7] reaction with *N*-iodosuccinimide (NIS) in CH_3CN–H_2O and subsequent removal of the iodine atom with $Na_2S_2O_4$.

Keywords: galactal; α-glycosidation; glycosphingolipids; immunomodulating.

* Corresponding author. Tel.: +39-81-678-504; fax: +39-81-678-552; e-mail: costanti@unina.it

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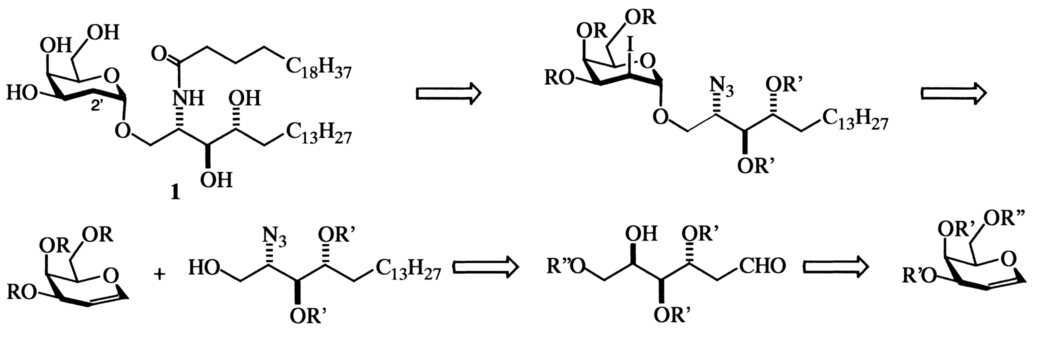


Scheme 1. Structure and retrosynthetic analysis of (2*S*,3*S*,4*R*)-1-*O*-(2-deoxy-α-D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol (**1**).

The key point of our strategy was to find out a suitable way to couple the two building blocks with a highly selective α-glycosidation. Stereoselective α-glycosidation is a difficult task in carbohydrate chemistry. The possibility of utilizing glycals as glycosyl donors in the synthesis of 2-deoxyglycosides in an iodonium-mediated coupling with a suitable acceptor has been exploited in the pioneering research by Thiem.[8] The stereochemistry of this reaction is governed by *trans*-diaxial addition and the α-linked glycoside is produced with high yield and stereoselectivity. Many kinds of α-2-deoxyglycosides have been prepared in this way, but the reaction has never been used in the synthesis of glycolipids. We found that the iodonium-mediated coupling of glycals can be conveniently used for the synthesis of glycosphingolipids, provided that a proper acceptor is chosen.

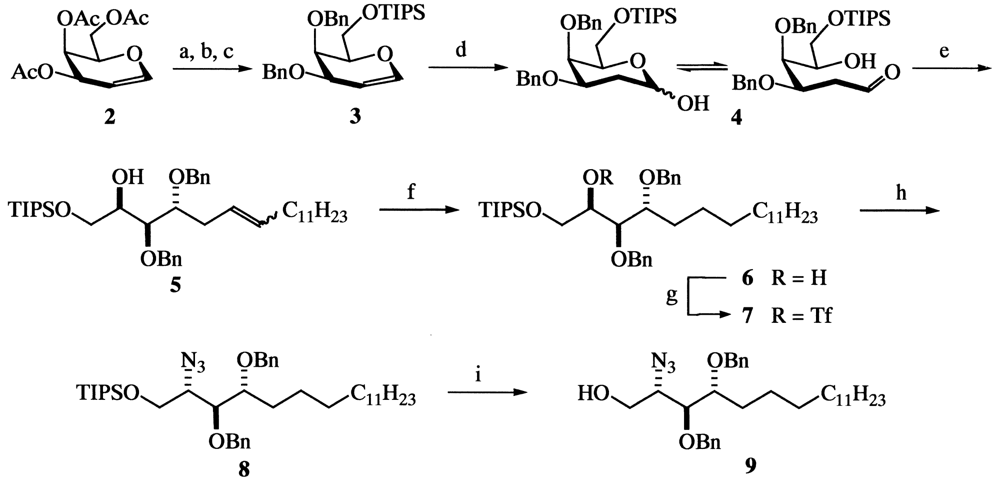
As shown in Scheme 1, the glycosidation reaction succeeded when the azidosphingosine triol **9** was used as acceptor, while any attempt failed when the 2,3-*O*-dibenzylated ceramide was used. In fact, it has been reported that the primary hydroxyl group of a ceramide shows a much lower nucleophilicity than that expected for such a group;[9] in contrast, the corresponding group of the azidosphingosine is a much better nucleophile.

After glycosylation, the synthesis of the target compound **1** was easily completed with reductive removal of the iodine atom with simultaneous reduction to amine of the azido group, followed by acylation and deprotection.

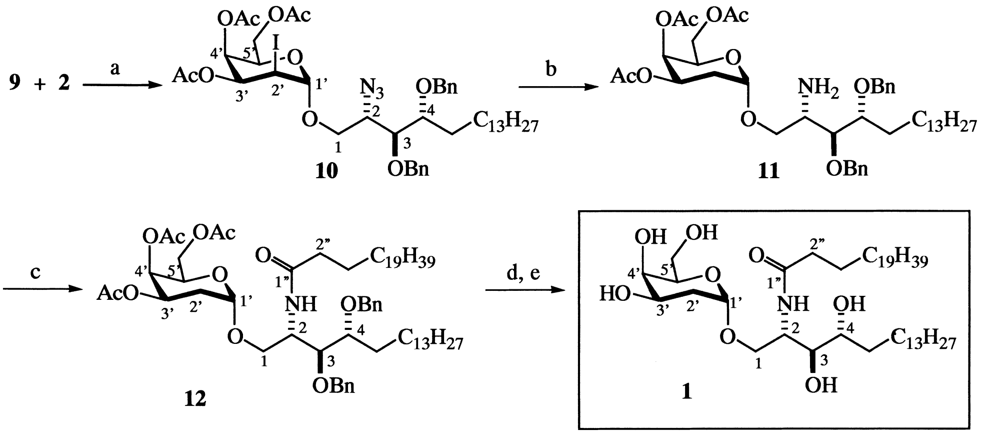
2.2. Synthetic procedure

As outlined in Scheme 2, the azidosphingosine triol **3** was prepared starting from the commercially available 3,4,6-tri-*O*-acetylgalactal. 3,4,6-Tri-*O*-acetylgalactal **2** was converted into 3,4-di-*O*-benzyl-6-*O*-TIPS-galactal **3** in three steps, and the corresponding 2-deoxysugar (**4**) was obtained in 93% yield by treatment of **3** with NIS in CH_3CN–H_2O 95:5 followed by removal of the iodide group with $Na_2S_2O_4$.[7]

For the chain extension, the Wittig reaction was used.[6] We have recently demonstrated that the Wittig ylide causes benzylic alcohol elimination from the benzyl-protected 2-deoxysugar, unless catalytic amounts of a triphenyl- or trialkyltin halide are added.[10] Therefore, treatment of compound **4** with 4 equiv. of the Wittig ylide and 0.5 equiv. of triphenyltin chloride gave the desired (2*R*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-TIPS-6-octadecene-1,2,3,4-tetrol **5** in 84% yield (*E*/*Z* mixture). This chiral precursor was converted in the azidosphingosine **9** as follows. The double bond was selectively reduced with diimine (prepared in situ from *p*-toluenesulfonyl hydrazide and



Scheme 2. Synthesis of (2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (**9**). *Reagents*: (a) MeONa, MeOH; (b) TIPSCl, DMF, imidazole (80%, 2 steps); (c) NaH, DMF, 0°C, then BnBr (90%); (d) (i) *N*-iodosuccinimide, CH_3CN–H_2O 95:5, 0°C, (ii) $Na_2S_2O_4$, DMF–H_2O 1:1, $NaHCO_3$ (94%); (e) $C_{12}H_{25}PPh_3Br$, BuLi, Ph_3SnCl, CH_2Cl_2–toluene (84%); (f) $TsNHNH_2$, AcONa, EtOH (85%); (g) Tf_2O, $CHCl_2$–pyridine; (h) NaN_3, DMF (71%, 2 steps); (i) TBAF, THF (82%).



Scheme 3. Synthesis of compound **1**. *Reagents*: (a) *N*-iodosuccinimide, CH_2Cl_2 (69% based on consumed **9**); (b) Ph_3SnH, AIBN, benzene, reflux (65%); (c) $C_{21}H_{43}COCl$, pyridine–CH_2Cl_2 (82%); (d) H_2, $Pd(OH)_2$–C, EtOH–AcOH, 40°C; (e) MeOH–Et_3N 8:2, 40°C (73%, 2 steps).

sodium acetate) giving compound **6** (85% yield). Compound **6** was then transformed in the 2-azido derivative **8** in a one-pot reaction by treatment with trifluoromethansulfonic anhydride, giving the 2-*O* triflate derivative **7**, followed by reaction with sodium azide in DMF with inversion of configuration at C-2 (71% yield based on **6**). Finally, the TIPS protecting group was selectively removed by treating compound **8** with a 1 M solution of tetra-butylammoniumfluoride (TBAF) in THF to give the azidosphingosine **9** (82% yield).

Stereoselective 2-deoxygalactosidation of acceptor **9** (Scheme 3) was then accomplished using 4 equiv. of tri-*O*-acetylgalactal **2**, and 5 equiv. of NIS in dry CH_2Cl_2. The I^+ addition to the double bond of the galactal **2** is followed by the nucleophilic attack of the azidosphingosine **9** in a one-pot reaction that was allowed to proceed overnight. This reaction confirmed to have a high proclivity for *trans*-diaxial addition and provided only the desired α-galactoside **10**, bearing an axial iodine atom at position 2 of galactose, in 59% yield and with 14% recovery of unreacted compound **9** (69% yield based on consumed **9**).

The axial orientation of the iodine atom was indicated by the small coupling constant (4.9 Hz) of H-2′ with H-3′ in the ^1H NMR spectrum. Due to the equatorial orientation of H-2, the small (<2 Hz) coupling constant between H-1′ and H-2′ was not diagnostic for the α configuration of the glycosidic bond. This was later demonstrated by the proton spectrum of the deiodinated compound **11** (described later) whose anomeric proton resonated as a broad doublet (J=2.7 Hz) at δ 4.91.

After SiO_2 chromatography, compound **10** was subjected to reduction to remove the iodine atom and to convert the azido group to amine. This was obtained in one step using Ph_3SnH and AIBN in benzene for 20 h at room temperature followed by 1 h under reflux. The resulting compound **11** was purified on SiO_2 column using eluents containing 1% of pyridine, and then treated with docosanoyl chloride in pyridine–CH_2Cl_2 at room temperature for 18 h, affording 1-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-2-docosanoylamino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (**12**) in 82% yield. Finally, the benzyl protecting groups were removed by hydrogenolysis using $Pd(OH)_2$ in EtOH–AcOH 9:1, while the acetyl groups were removed in MeOH–Et_3N 8:2 to give the target compound **1**.

In conclusion, the total synthesis of compound **1** was successfully achieved starting from commercially available tri-*O*-acetylgalactal in only 12 steps. The overall yield was 7.6% based on tri-*O*-acetylgalactal **2** used for the preparation of the azidosphingosine **9**. The biological activity of this compound is currently being evaluated and will be reported elsewhere.

3. Experimental

3.1. General methods

EI and FAB mass spectra were recorded on a VG Prospec-Autospec (Fisons) mass spectrometer. FAB spectra were performed in glycerol (positive ion mode) or triethanolamine (negative ion mode) matrices. ESI mass spectra were recorded on a Finnigan LCQ ion-trap mass spectrometer, using $CHCl_3$–MeOH (1:1) as solvent. Optical rotations were measured at 589 nm on a Perkin–Elmer 192 polarimeter using a 10 cm microcell. ^1H and ^{13}C NMR spectra were determined on a Bruker AMX-500 spectrometer at 500.13 and 125.77 MHz, respectively; chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H=7.26, δ_C=77.0; CD_5N: δ_H=8.73, 7.58, and 7.21, δ_C=149.8, 135.3, and 123.4). The spectra of new compounds were assigned thanks to COSY, HMQC, and, when needed, HMBC two-dimensional NMR experiments. The reverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded by using a pulse sequence with a BIRD pulse 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C; the interpulse delays were adjusted for an average $^1J_{CH}$ of 142 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiment was optimized for a $^3J_{CH}$ of 8.3 Hz.

3.2. Synthesis

3.2.1. 3,4-Di-*O*-benzyl-6-*O*-TIPS-D-galactal (3). 3,4,6-Tri-*O*-acetyl-D-galactal (1.37 g, 5.0 mmol) was dissolved in a solution of MeONa in MeOH (0.01 M, 20 ml), and kept stirring for 24 h at room temperature. After removal of the solvent, 0.73 g of D-galactal was obtained, used in the subsequent reaction without any further purification. D-Galactal was dissolved in dry DMF (5 ml), and imidazole (1.0 g, 14.7 mmol) and triisopropylsilyl chloride (TIPSCl, 0.96 ml, 1.06 g, 5.5 mmol) were added. After stirring for 12 h, water (100 ml) was added and the reaction mixture was extracted with EtOAc (3×200 ml). The combined organic layers were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (solvent *n*-hexane–EtOAc 6:4) to give 1.2 g (4.0 mmol, 80%) of 6-*O*-TIPS-D-galactal as a colorless oil. It was dissolved in dry DMF (50 ml) and cooled to 0°C, and NaH (238 mg, 9.9 mmol) was slowly added. After a few minutes, benzyl bromide (0.99 ml, 7.9 mmol) was added dropwise. After stirring for 12 h at room temperature, water (100 ml) was added and the reaction mixture was extracted three times with EtOAc (300 ml). The combined organic layers were washed with water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (solvent *n*-hexane–EtOAc 9:1) to give 1.72 g (3.57 mmol, 90%) of compound **3**: colorless oil, $[\alpha]_D=-28.4$ ($CHCl_3$, c=2.8); IR: ν_{max} ($CHCl_3$) 2951, 2872, 1646, 1455, 1092 cm^{-1}; ESIMS (positive ions): *m/z* 505 ($[M+Na]^+$; ^1H NMR ($CDCl_3$): δ 7.36–7.33 (10H, m, aromatic protons), 6.40 (1H, d, J=6.2 Hz, H-1), 4.87 (1H, d, J=11.4 Hz, benzylic proton), 4.85 (1H, dd, J=6.2, 2.7 Hz, H-2), 4.77 (1H, d, J=11.4 Hz, benzylic proton), 4.65 (1H, d, J=11.4 Hz, benzylic proton), 4.59 (1H, d, J=11.4 Hz, benzylic proton), 4.22 (1H, m, H-5), 4.05 (1H, dd, J=11.3, 3.4 Hz, H-6a), 4.01 (1H, dd, J=11.3, 2.5 Hz, H-6b), 3.94 (2H, H-3 and H-4 overlapped), 1.10 (3H, m, TIPS methine protons), 1.07 (18H, d, J=6.5 Hz, TIPS methyl protons); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.2 (CH, C-1), 138.7 (C, aromatic carbon), 138.6 (C, aromatic carbon), 128.3–127.4 (CH, aromatic carbons), 100.1 (CH, C-2), 77.7 (CH, C-3 or C-4), 73.7 (CH_2, benzylic methylene group), 71.4 (CH, C-5), 71.1 (CH, C-4 or C-3), 70.8 (CH_2, benzylic methylene group), 61.6 (CH_2, C-6), 18.0 (CH_3, TIPS methyl groups), 11.9 (CH, TIPS methine groups).

3.2.2. 3,4-Di-*O*-benzyl-6-*O*-TIPS-2-deoxy-D-galactose (4). The protected galactal **3** (965 mg, 2 mmol) was dissolved in CH_3CN *m/z* H_2O (95:5, 15 ml) and cooled to 0°C. Then, the solution was treated with *N*-iodosuccinimide (495 mg, 2.2 mmol), allowed to warm to room temperature and left to stir for 15 min. The solvent was then removed in vacuo. The residue was dissolved in DMF (20 ml) and a solution of $H_2O–HCO_3^-$ (10 mol equiv., 20 ml) was then added followed by solid $Na_2S_2O_4$ (1.537 g, 8 mmol). The reaction was allowed to proceed under stirring for 5 h at room temperature. After that, the reaction mixture was diluted with 800 ml of AcOEt and washed with water (3 times, 400 ml each), followed by brine (400 ml). The organic layer was purified by column chromatography (*n*-hexane–EtOAc 7:3) to give 940 mg of compound **4** (1.88 mmol, 94% yield) as mixture of anomers, identified by comparison of its ^1H NMR spectrum and optical rotation with literature data:[7] $[\alpha]_D=+32$ ($CHCl_3$, c=0.2); ^1H NMR ($CDCl_3$, α anomer): δ 7.42–7.22 (10H, m, aromatic protons), 5.45 (1H, br s, H-1), 4.96 (1H, d, J=11.4 Hz, benzylic proton), 4.70 (1H, d, J=11.4 Hz, benzylic proton), 4.62 (2H, s, benzylic protons), 4.01 (1H, br s, H-4), 3.99 (2H, H-3 and H-5 overlapped), 3.84 (1H, dd, J=9.7, 8.0 Hz, H-6a), 3.72 (1H, dd, J=9.7, 5.9 Hz, H-6b), 2.21 (1H, m, H-2eq), 2.02 (1H, m, H-2ax), 1.10 (3H, m, TIPS methine protons), 1.05 (18H, d, J=6.5 Hz, TIPS methyl protons).

3.2.3. (2*R*,3*S*,4*R*)-1-*O*-TIPS-3,4-di-*O*-benzyl-6-octadecene-1,2,3,4-tetrol (5). Ph_3SnCl (0.9 mmol, 347 mg) was dissolved in dry CH_2Cl_2 (15 ml) and added to the deoxysugar **4** (940 mg, 1.88 mmol). Then, the ylide was prepared from *n*-BuLi (4.7 ml of a 1.6 M solution in *n*-hexane, 7.52 mmol) and *n*-dodecyltriphenylphosphonium bromide (4.81 g, 9.40 mmol) in dry toluene (15 ml). The solution of 2-deoxysugar was then added dropwise to the ylide solution, and allowed to react at room temperature for 2 h. The reaction mixture was then diluted with CH_2Cl_2 (400 ml) and washed with water (3 times, 400 ml each), followed by brine (400 ml). The organic layer was purified by column chromatography (*n*-hexane–EtOAc 8:2) to give 1.02 g of compound **5** (84% yield) as mixture of *E*/*Z* stereoisomers, identified by comparison of its ^1H, ^{13}C NMR spectra and optical rotation with literature data:[10] $[\alpha]_D=-3.7$ ($CHCl_3$, c=0.9); ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.28 (10H, m, aromatic protons), 5.56–5.38 (2H, m, H-6 and H-7), 4.70 (1H, d, J=11.8 Hz, benzylic proton), 4.61 (2H, s, benzylic protons), 4.60 (1H, d, J=11.8 Hz, benzylic protons), 3.97 (1H, m, H-2), 3.79–3.70 (4H, m, H_2-1, H-2, and H-4), 3.11 (1H, br s, 2-OH), 2.51–2.37 (2H, m, H_2-5), 2.03–1.96 (2H, m, H_2-8), 1.25 (alkyl chain CH_2 protons), 1.08 (TIPS methine protons), 1.05 (TIPS methyl protons), 0.88 (3H, t, J=7.0 Hz, H_3-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 132.2 (CH, C-7), 128.7–127.6 (CH, aromatic carbons), 124.4 (CH, C-6), 80.1 (CH, C-3), 78.1 (CH, C-4), 73.7 (CH_2, benzylic methylene group), 72.5 (CH_2, benzylic methylene group), 71.3 (CH, C-2), 63.8 (CH_2, C-1), 34.2 (CH_2, C-5, *E* isomer), 32.7 (CH_2, C-8, *E* isomer), 29.7–29.2 (CH_2, alkyl chains CH_2 groups), 29.0 (CH_2, C-5, *Z* isomer), 27.6 (CH_2, C-8, *Z* isomer), 18.0 (CH_3, TIPS methyl groups), 14.1 (CH_3, C-18), 11.8 (CH, TIPS methine groups).

3.2.4. (2*R*,3*S*,4*R*)-1-*O*-TIPS-3,4-di-*O*-benzyl-1,2,3,4-octadecanetetrol (6). The alkene **5** (1.30 g, 1.99 mmol) was dissolved in 95% EtOH (40 ml) and *p*-toluenesulfonyl hydrazide (4.1 g, 22 mmol). The reaction mixture was refluxed for 5 h, and during this time, a solution of sodium acetate (2.9 g, 35.6 mmol) in water (12 ml) was added dropwise. After this, H_2O (100 ml) was added to the reaction mixture, that was subsequently extracted with CH_2Cl_2 (3×200 ml). The combined organic extracts were dried over Na_2SO_4, and the solvent removed. Purification of the residue by column chromatography on SiO_2 (*n*-hexane–EtOAc 95:5) gave 1.11 g (1.69 mmol, 85%) of compound **6**: colorless oil, $[\alpha]_D=-6.9$ ($CHCl_3$, c=2.4); IR: ν_{max} ($CHCl_3$) 3498, 2932, 2872, 1466, 1391, 1114, 1071 cm^{-1}; HREIMS: *m/z* 611.4525 ($[M-i\text{-Pr}]^+$, $C_{38}H_{63}O_4Si$ gives 611.4496); EIMS: *m/z* 611 ($[M-i\text{-Pr}]^+$, 10), 396 (85), 395 (91), 277 (41), 246 (67), 173 (100); ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.27 (10H, aromatic protons), 4.74 (1H, d, J=11.5 Hz, benzylic proton), 4.70 (1H, d, J=11.5 Hz, benzylic proton), 4.66 (1H, d, J=11.5 Hz, benzylic proton), 4.59 (1H, d, J=11.5 Hz, benzylic proton), 3.94 (1H, m, H-2), 3.78–3.70 (4H, overlapping signals, H_2-1, H-3, and H-4), 3.19 (1H, d, *J*=4.2 Hz, OH-2), 1.68 (1H, m, H-5a), 1.60 (1H, m, H-5b), 1.42 (1H, m, H-6a), 1.33–1.23 (alkyl chain CH_2 protons), 1.10 (TIPS methine protons), 1.08 (TIPS methyl protons), 0.90 (3H, t, *J*=7.0 Hz, H_3-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.3 (C, aromatic carbons), 128.7–127.6 (CH, aromatic carbons), 80.5 (CH, C-3), 78.4 (CH, C-4), 73.7 (CH_2, benzylic methylene group), 72.9 (CH_2, benzylic methylene group), 71.5 (CH, C-2), 63.9 (CH_2, C-1), 31.9 (CH_2, C-16), 31.3 (CH_2, C-5), 29.7–29.2 (CH_2, alkyl chains CH_2 groups), 25.8 (CH_2, C-6), 22.7 (CH_2, C-17), 18.0 (CH_3, TIPS methyl groups), 14.1 (CH_3, C-18), 11.8 (CH, TIPS methine groups).

3.2.5. (2*S*,3*S*,4*R*)-1-*O*-TIPS-2-azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (8). Compound **6** (1.41 g, 2.15 mmol) was dissolved in dry CH_2Cl_2 (20 ml) and dry pyridine (2 ml). The solution was cooled to −15°C, triflic anhydride (0.44 ml, 732 mg, 2.59 mmol) was slowly added, and the mixture was stirred for 30 min. After this, dry DMF (100 ml) and solid NaN_3 (750 mg, 11.5 mmol) were added, and the reaction mixture was allowed to react under stirring for 12 h at room temperature. Water (200 ml) was added to the reaction mixture, that was extracted with *n*-hexane (2×300 ml) and EtOAc (2×300 ml). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on SiO_2 (*n*-hexane–EtOAc 95:5) to give 1.04 g (1.53 mmol, 71%) of compound **8**: colorless oil, $[\alpha]_D=+4.7$ ($CHCl_3$, *c*=0.3); IR: ν_{max} ($CHCl_3$) 2936, 2872, 2118, 1114 cm^{-1}; HRFABMS (positive ions): *m/z* 702.4988 ($[M+Na]^+$, $C_{41}H_{69}N_3NaO_3Si$ gives 702.5006); EIMS: *m/z* 636 ($[M-i\text{-}Pr]^+$, 5), 608 (3), 530 (7), 502 (8), 395 (22), 351 (100), 319 (13), 274 (28), 197 (39), 173 (26); ^1H NMR (500 MHz, $CDCl_3$): δ 7.35–7.27 (10H, aromatic protons), 4.74 (1H, d, *J*=11.5 Hz, benzylic proton), 4.60 (1H, d, *J*=11.5 Hz, benzylic proton), 4.59 (1H, d, *J*=11.5 Hz, benzylic proton), 4.53 (1H, d, *J*=11.5 Hz, benzylic proton), 4.07 (1H, dd, *J*=10.5, 2.8 Hz, H-1a), 3.84 (1H, dd, *J*=10.5, 7.5 Hz, H-1b), 3.62 (2H, overlapping signal, H-3 and H-4), 3.58 (1H, m, H-2), 1.68 (1H, m, H-5a), 1.56 (1H, m, H-5b), 1.44 (1H, m, H-6a), 1.33–1.23 (alkyl chain CH_2 protons), 1.10 (TIPS methine protons), 1.08 (TIPS methyl protons), 0.88 (3H, t, *J*=6.9 Hz, H_3-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.3 (C, aromatic carbons), 128.6–127.5 (CH, aromatic carbons), 79.9 (CH, C-3), 78.4 (CH, C-4), 73.7 (CH_2, benzylic methylene groups), 72.6 (CH_2, benzylic methylene groups), 64.7 (CH_2, C-1 and CH, C-2), 31.9 (CH_2, C-16), 31.6 (CH_2, C-5), 30.0–29.4 (CH_2, alkyl chain methylene groups), 25.6 (CH_2, C-6), 22.7 (CH_2, C-17),18.0 (CH_3, TIPS methyl groups), 14.2 (CH_3, C-18), 11.8 (CH, TIPS methine groups).

3.2.6. (2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (9). To the TIPS-protected azidosphingosine **8** (1.04 g, 1.53 mmol) dissolved in dry THF (40 ml), a solution of TBAF (1 M in THF, 3.1 ml, 3.1 mmol) was added at 0°C. After 10 min, the solution was allowed to warm at room temperature, and stirred for 90 min. The reaction mixture was treated with water (100 ml) and extracted with EtOAc (4×200 ml). The organic phases were combined, dried, and concentrated under reduced pressure. The crude product was purified by SiO_2 chromatography (*n*-hexane–EtOAc 95:5) column to give 661 mg (1.26 mmol, 82%) of compound **9**: colorless oil, $[\alpha]_D=+3.4$ ($CHCl_3$, *c*=0.35); IR: ν_{max} ($CHCl_3$) 3332, 2936, 2865, 2118, 1104, 1021 cm^{-1}; HRFABMS (positive ions): *m/z* 524.3848 ($[M+H]^+$, $C_{32}H_{50}N_3O_3$ gives 524.3852); ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.29 (10H, aromatic protons), 4.71 (1H, d, *J*=11.5 Hz, benzylic proton), 4.68 (1H, d, *J*=11.5 Hz, benzylic proton), 4.63 (1H, d, *J*=11.5 Hz, benzylic proton), 4.58 (1H, d, *J*=11.5 Hz, benzylic proton), 3.90 (1H, ddd, *J*=11.5, 5.2, 5.2 Hz, H-1a), 3.80 (1H, m, H-1b), 3.71 (1H, m, H-3), 3.68 (1H, m, H-4), 3.65 (1H, m, H-2), 2.54 (1H, t, *J*=6.1 Hz, OH-1), 1.68 (1H, m, H-5a), 1.57 (1H, m, H-5b), 1.42 (1H, m, H-6a), 1.33–1.23 (alkyl chain CH_2 protons), 0.89 (3H, t, *J*=6.8 Hz, H_3-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.6 and 136.8 (C, aromatic carbons), 128.5–127.9 (CH, aromatic carbons), 80.5 (CH, C-3), 79.0 (CH, C-4), 73.7 (CH_2, benzylic CH_2 groups), 72.6 (CH_2, benzylic CH_2 groups), 63.1 (CH, C-2), 62.3 (CH_2, C-1), 32.0 (CH_2, C-16), 30.2 (CH_2, C-5), 29.7–29.4 (CH_2, alkyl chain CH_2 groups), 25.5 (CH_2, C-6), 22.7 (CH_2, C-17), 14.2 (CH_3, C-18).

3.2.7. (2*S*,3*S*,4*R*)-1-*O*-(3,4,6-Tri-*O*-acetyl-2-iodo-2-deoxy-α-D-galactopyranosyl)-2-azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (10). To tri-*O*-acetylgalactal **2** (677 mg, 2.49 mmol) in dry CH_2Cl_2 (5 ml) were added the azidosphingosine **9** (322 mg, 0.62 mmol) in dry CH_2Cl_2 (4 ml) and *N*-iodosuccinimide (698 mg, 3.12 mmol) at 0°C. The reaction mixture was refluxed for 90 min, then left at room temperature for 12 h under stirring. The reaction mixture was treated with a 10% aqueous solution of $Na_2S_2O_3$ until the purple color of the solution disappeared and then with water (50 ml), and extracted with CH_2Cl_2 (4×100 ml). The organic extracts, dried over Na_2SO_4 and concentrated under reduced pressure, were subjected to column chromatography on SiO_2 (*n*-hexane–EtOAc 9:1), giving 45 mg of unreacted **9** (0.08 mmol) and 337 mg (0.37 mmol, 69% based on consumed **9**) of compound **10**: colorless oil, $[\alpha]_D=+6.5$ ($CHCl_3$, *c*=0.3); IR: ν_{max} ($CHCl_3$) 2928, 2856, 2099, 1747, 1374, 1111 cm^{-1}; HREIMS: *m/z* 921.3602 (M^+, $C_{44}H_{64}IN_3O_{10}$ gives 922.3636); EIMS: *m/z* (relative intensity) 921 (M^+, 2), 814 (4), 683 (10), 527 (12), 399 (53), 239 (88), 204 (100), 188 (48), 169 (57); ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.28 (10H, aromatic protons), 5.36 (1H, br s, H-4′), 5.27 (1H, br s, H-1′), 4.90 (1H, dd, *J*=4.7, 3.7 Hz, H-3′), 4.69 (1H, d, *J*=11.5 Hz, benzylic proton), 4.62 (1H, benzylic proton), 4.57 (2H, s, benzylic protons), 4.22 (1H, br d, *J*=4.9 Hz, H-2′), 4.20 (1H, m, H-5′), 4.10 (1H, m, H-6′a), 4.09 (1H, m, H-6′b), 3.99 (1H, dd, *J*=10.5, 2.6 Hz, H-1a), 3.77 (1H, m, H-2), 3.63 (1H, m, H-1b), 3.61 (2H, overlapped signals, H-3 and H-4), 2.18 (3H, s, acetyl protons), 2.08 (3H, s, acetyl protons), 1.95 (3H, s, acetyl protons), 1.67 (1H, m, H-5a), 1.57 (1H, m, H-5b), 1.40 (1H, m, H-5a), 1.25 (alkyl chain CH_2 protons), 0.88 (6H, t, *J*=6.9 Hz, H_3-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.4 (C, C-1″), 170.5–169.5 (C, acetyl CO), 138.1 ad 137.7 (C, aromatic carbons), 128.5–127.8 (CH, aromatic carbons), 103.11 (CH, C-1′), 79.0 (CH, C-4), 78.8 (CH, C-3), 73.7 (CH_2, benzylic CH_2 group), 72.1 (CH_2, benzylic CH_2 group), 68.7 (CH_2, C-1), 66.9 (CH, C-5′), 65.2 (CH, C-4′), 65.0 (CH, C-3′), 62.0 (CH_2, C-6′), 61.7 (CH, C-2), 32.0 (CH_2, C-16), 30.0 (CH_2, C-5), 29.7–29.4 (CH_2, alkyl chain CH_2 groups), 25.2 (CH_2, C-6), 22.7

(CH_2, C-17), 21.1–20.7 (CH_3, acetyl methyl groups), 20.6 (CH, C-2′), 14.2 (CH_3, C-18).

3.2.8. (2*S*,3*S*,4*R*)-1-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-2-amino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (11). To a solution of the azide **10** (337 mg, 0.37 mmol) in benzene (10 ml), Ph_3SnH (950 μl, 1.31 g, 3.72 mmol) and a small amount of AIBN were added, and the resulting solution was allowed to react at room temperature for 24 h and subsequently for 1 h under reflux. The solution was cooled to room temperature and concentrated under reduced pressure. Column chromatography on SiO_2 (*n*-hexane–EtOAc–pyridine 66:39:1) gave 183 mg (0.24 mmol, 65%) of **11**: colorless oil, $[\alpha]_D=+25.3$ ($CHCl_3$, $c=0.75$); IR: ν_{max} ($CHCl_3$) 3390, 2917, 2856, 1746, 1431, 1383, 1372, 1111, 1075 cm^{-1}; HRFABMS (positive ions): *m/z* 770.4855 ($[M+H]^+$, $C_{44}H_{68}NO_{10}$ gives 770.4843); FABMS: *m/z* 770 $[M+H]^+$, 498 $[M+H\text{-galactosyl}]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 7.35–7.27 (10H, aromatic protons), 5.30 (1H, br s, H-4′), 5.27 (1H, ddd, $J=12.5$, 4.8, 2.9 Hz, H-3′), 4.91 (1H, br d, $J=2.7$ Hz, H-1′), 4.76 (1H, d, $J=11.6$ Hz, benzylic proton), 4.64 (1H, d, $J=11.6$ Hz, benzylic proton), 4.56 (1H, d, $J=11.6$ Hz, benzylic proton), 4.54 (1H, d, $J=11.6$ Hz, benzylic proton), 4.09 (1H, m, H-5′), 4.04 (1H, m, H-6′a), 3.99 (1H, m, H-6′b), 3.88 (1H, dd, $J=9.5$, 2.8 Hz, H-1a), 3.74 (1H, ddd, $J=7.8$, 3.4, 3.4 Hz, H-4), 3.51 (1H, dd, $J=6.7$, 3.4 Hz, H-3), 3.36 (1H, dd, $J=9.5$, 7.4 Hz, H-1b), 3.12 (1H, ddd, $J=7.0$, 7.0, 2.7 Hz, H-2), 2.13 (3H, s, acetyl protons), 2.05 (1H, ddd, $J=12.5$, 12.5, 3.4 Hz, H-2′a), 1.99 (3H, s, acetyl protons), 1.95 (3H, s, acetyl protons), 1.82 (1H, br dd, $J=12.5$, 5.0 Hz, H-2′b), 1.71 (1H, m, H-5a), 1.59 (1H, m, H-5b), 1.25 (alkyl chain CH_2 protons), 0.88 (3H, t, $J=6.9$ Hz, H_3-18).

3.2.9. (2*S*,3*S*,4*R*)-1-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-2-docosanoylamino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (12). Docosanoic acid (232 mg, 0.68 mmol) dissolved in $SOCl_2$ (1.5 ml, 2.39 g, 20.1 mmol) was refluxed for 90 min, and the excess of $SOCl_2$ removed under reduced pressure. A solution of the amine **11** (171 mg, 0.22 mmol) in dry pyridine (6 ml) and dry CH_2Cl_2 (8 ml) was added to the obtained docosanoyl chloride. After 12 h, the solvents were removed under vacuum, and residue was partitioned between CH_2Cl_2 and a saturated $NaHCO_3$ aqueous solution. The organic phase, dried over Na_2SO_4 and concentrated under reduced pressure, was subjected to column chromatography on SiO_2 (*n*-hexane–EtOAc 7:3), giving 197 mg (0.18 mmol, 82%) of **12**: colorless oil, $[\alpha]_D=+6.5$ ($CHCl_3$, $c=0.4$); IR: ν_{max} ($CHCl_3$) 3210, 2930, 2860, 1745, 1671, 1508, 1463, 1377, 1114 cm^{-1}; HRFABMS (negative ions): *m/z* 1090.7969 ($[M-H]^-$, $C_{66}H_{108}NO_{11}$ gives 1090.7922); FABMS: *m/z* 1090 $[M-H]^-$, 818 $[M-\text{galactosyl}]^-$; ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.27 (10H, aromatic protons), 5.61 (1H, d, $J=9.3$ Hz, NH-2), 5.30 (1H, br d, $J=3.0$ Hz, H-4′), 5.22 (1H, ddd, $J=12.3$, 4.9, 3.0 Hz, H-3′), 4.82 (1H, br d, $J=3.0$ Hz, H-1′), 4.82 (1H, d, $J=11.7$ Hz, benzylic proton), 4.61 (1H, d, $J=11.7$ Hz, benzylic proton), 4.58 (1H, d, $J=11.7$ Hz, benzylic proton), 4.51 (1H, d, $J=11.7$ Hz, benzylic proton), 4.24 (1H, m, H-2), 4.08 (1H, m, H-5′), 4.04 (1H, m, H-6′a), 3.99 (1H, m, H-6′b), 3.73–3.67 (3H, overlapping signals, H_2-1 and H-3), 3.54 (1H, m, H-4), 2.37 (2H, $J=7.1$ Hz, H_2-2″), 2.13 (3H, s, acetyl protons), 2.02 (1H, m, H-2′a), 2.01 (3H, s, acetyl protons), 2.00 (3H, s, acetyl protons), 1.72 (2H, overlapping signals, H-2′b and H-5a), 1.63 (2H, overlapping signals, H_2-3″ and H-5b), 1.25 (alkyl chain CH_2 protons), 0.88 (6H, t, $J=6.9$ Hz, H_3-18 and H_3-22‴); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.5 (C, C-1″), 170.4–170.0 (C, acetyl CO), 138.4 (C, aromatic carbon), 128.5–127.9 (CH, aromatic carbons), 97.9 (CH, C-1′), 80.1 (CH, C-4), 78.4 (CH, C-3), 73.2 (CH_2, benzylic CH_2 group), 72.1 (CH_2, benzylic CH_2 group), 67.2 (CH_2, C-1), 66.8 (CH, C-5′), 66.4 (CH, C-4′), 66.0 (CH, C-3′), 62.2 (CH_2, C-6′), 49.4 (CH, C-2), 34.1 (CH_2, C-2″), 31.9 (CH_2, C-16 and C-20″), 30.2 (CH_2, C-5 and C-2′), 30.0–29.1 (CH_2, alkyl chain CH_2 groups), 24.9 (CH_2, C-3″), 22.6 (CH_2, C-17 and C-21″), 20.8–20.6 (CH_3, acetyl methyl groups), 14.1 (CH_3, C-18 and C-22″).

3.2.10. (2*S*,3*S*,4*R*)-1-*O*-(2-Deoxy-α-D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol (1). The protected glycosphingolipid **12** (197 mg, 0.18 mmol) and $Pd(OH)_2$–C (50 mg, 20% w/w) were suspended in 95% EtOH (45 ml) and AcOH (5 ml). The obtained mixture was hydrogenated at a pressure of 3 bar in a Parr reactor for 48 h at 40°C. The reaction mixture was filtered over celite and the filtrate washed with 95% EtOH, MeOH, and $CHCl_3$. The organic extracts were combined and concentrated to dryness under reduced pressure, dissolved in MeOH (16 ml) and Et_3N (4 ml), and kept at 40°C for 18 h. After removal of the solvents, the crude reaction product was purified by reversed-phase column chromatography on RP-18 silica gel (MeOH–EtOAc 95:5) to give 103 mg (0.13 mmol, 73%) of compound **1**: amorphous solid, $[\alpha]_D=+27.3$ ($CHCl_3$–MeOH 1:1, $c=0.8$); IR: ν_{max} (KBr) 3385, 2926, 2850, 1643, 1542, 1365, 1078 cm^{-1}; HRFABMS (negative ions): *m/z* 784.6703 ($[M-H]^-$, $C_{46}H_{90}NO_8$ gives 784.6666); FABMS: *m/z* 784 $[M-H]^-$, 638 $[M-\text{galactosyl}]^-$; ^1H NMR (500 MHz, pyridine-d_5): δ 8.78 (1H, d, $J=3.0$ Hz, NH-2), 6.64 (1H, d, $J=6.0$ Hz, OH-3), 6.55 (1H, t, $J=5.3$ Hz, OH-6′), 6.24 (1H, d, $J=4.6$ Hz, OH-4′), 6.20 (1H, d, $J=6.9$ Hz, OH-3′), 6.05 (1H, d, $J=6.8$ Hz, OH-4), 5.24 (1H, br d, $J=3.0$ Hz, H-1′), 5.15 (1H, m, H-2), 4.59 (1H, dd, $J=10.3$, 3.5 Hz, H-1a), 5.15 (1H, m, H-3′), 4.42–4.37 (4H, overlapping signals, H-3, H-4′, H-5′, and H-6′a), 4.35 (1H, m, H-6′b), 4.30 (1H, dd, $J=10.3$, 7.3 Hz, H-1b), 4.26 (1H, m, H-4), 2.49 (3H, overlapping signals, H_2-2″ and H-2′a), 2.24 (1H, m, H-5a), 2.18 (1H, br dd, $J=12.5$, 4.8 Hz, H-2′b), 1.94 (2H, m, H-5b and H-6a), 1.84 (2H, quintet, $J=7.3$ Hz, H_2-3″), 1.69 (1H, m, H-6b), 1.47–1.33 (3H, overlapping signals, H-7a and H_2-4″), 1.31–1.20 (alkyl chain CH_2 protons), 0.85 (6H, t, $J=7.3$ Hz, H_3-18 and H_3-22‴); ^{13}C NMR (125 MHz, pyridine-d_5): δ 173.6 (C, C-1″), 99.4 (CH, C-1′), 76.2 (CH, C-3), 72.8 (CH, C-5′), 72.7 (CH, C-4), 69.7 (CH, C-4′), 67.7 (CH_2, C-1), 66.2 (CH, C-3′), 63.2 (CH_2, C-6′), 52.9 (CH, C-2), 36.9 (CH_2, C-2″), 34.3 (CH_2, C-2′), 33.7 (CH_2, C-5), 32.1 (CH_2, C-16 and C-20″), 30.3–29.5 (CH_2, alkyl chain CH_2 groups), 26.6 (CH_2, C-6), 26.5 (CH_2, C-3″), 23.0 (CH_2, C-17 and C-21″), 14.3 (CH_3, C-18 and C-22″).

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