Synthesis of α-Galactosyl Ceramide, a Potent Immunostimulatory Agent

Oliver Plettenburg,† Vera Bodmer-Narkevitch, and Chi-Huey Wong*

Department of Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, California 92067

wong@scripps.edu

Received March 7, 2002

α-Galactosyl ceramide has been identified to be a potent stimulatory agent for a novel immunological process, mediated by CD1 molecules. This paper describes a short and efficient synthesis of α-galactosyl ceramide. Starting from commercially available 2-deoxy galactose, a suitable sphingosine derivative was obtained in nine steps and 36% overall yield, which was subsequently glycosylated to give the target molecule. This flexible route will provide various glycolipids for further exploration of this interesting biological process.

Introduction

CD1 molecules represent a new class of highly conserved, antigen-presenting cell surface proteins.[1] They recognize and bind glycolipid antigens through lipid−protein interactions and present the sugar moiety of the antigen to a receptor on natural killer T-cells (NKT cells) to activate the immune system. In humans, five different isoforms of CD1 have been detected so far. Since the recognition event is highly specific for glycolipids and no carrier proteins are required, this novel defense mechanism has gained considerable interest in the past years, with the hope that a new type of vaccine may be developed in the future. Since the discovery of α-galactosyl ceramide **1** in 1993 from the marine sponge *Agelas mauritianus*,[2] it has been shown that **1** exhibits tumor growth inhibition activities.[3] Further examination of the pharmaceutical potential of this class of compounds has led to the discovery that **1** is capable of initiating a CD1-mediated activation of NKT cells[4] and preventing autoimmune diseases such as type I diabetes.[5] Another derivative, whose sphingoside chain is truncated, seems to prevent autoimmune encephalomyelitis.[6] It is worth noting that, although *β*-galactosyl ceramides are frequently found in mammalian tissues and do possess a number of functions,[7,8] α-linked galactosyl ceramides have not been detected in normal mammalian cells so far. They have been found in cancer[9] or fetal[10] cell lines, however. In addition to the unusual α-linkage between galactose and ceramide, the ceramide portion of **1** also differs from the normally found ceramide in which a *trans*-olefin instead of a dihydroxy geometry is in place.

A few syntheses of **1** have been published so far.[11,12] As a part of our effort to understand the mechanism of CD1-mediated T-cell activation and to develop novel glycolipid vaccines, we are interested in developing a general and effective strategy for the synthesis of this compound and related structures.

Results and Discussion

Our synthesis starts from 2-deoxy galactose (Scheme 1), a relatively inexpensive starting material, which possesses all necessary stereochemical information found in compound **1**.

By Wittig reaction of a suitably protected 2-deoxy galactose derivative, it should be possible to introduce the long chain. The absence of an electron-withdrawing hydroxy group at C2 position should facilitate the opening of the pyranose ring. The single free hydroxy group generated in the Wittig reaction can be converted to an azide, serving as a protected form of the amide group.

* To whom correspondence should be addressed.

† Current address: Aventis Pharma Deutschland GmbH, DI&A Chemistry, D-65926 Frankfurt, Germany.

(1) (a) Burdin, M.; Kronenberg, M. *Curr. Opinion Immunol.* **1999**, *11*, 326−331. (b) Park, S.-H.; Bendelac, A. *Nature* **2000**, *406*, 788−792.

(2) (a) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron Lett.* **1993**, *34*, 5591−5592. (b) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771−2784.

(3) Koezuka, Y.; Kazuhiro, M.; Sakai, T.; Takenori, N. *Recent Res. Dev. Cancer* **1999**, *1*, 341−354.

(4) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626−1629.

(5) (a) Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q.-S.; Sondhi, J.; Naidenko, O. V.; Kronenberg, M.; Koezuka, Y.; Delovitch, T. L. *Nature Med.* **2001**, *7*, 1057−1062. (b) Hong, S.; Wilson, M. T.; Serizawa, I.; Wu, L.; Nagendra, S.; Naidenko, O.; Miura, T.; Haba, T.; Scherer, D. C.; Wie, J.; Kronenberg, M.; Koezuka, Y.; van Kaer, L. *Nature Med.* **2001**, *7*, 1052−1056.

(6) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531−534.

(7) Harouse, J. M.; Bhat, S.; Spitalnik, S. L.; Laughlin, M.; Stefano, K.; Silberberg, D. H.; Gonzales-Scarano F. *Science* **1991**, *253*, 320−323.

(8) (a) Leeden, R. *J. Neurosci. Res.* **1984**, *12*, 147−159. (b) Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500−507.

(9) Watanabe, K.; Matsubara, T.; Hakomori, S. *J. Biol. Chem.* **1976**, *251*, 2385−2387.

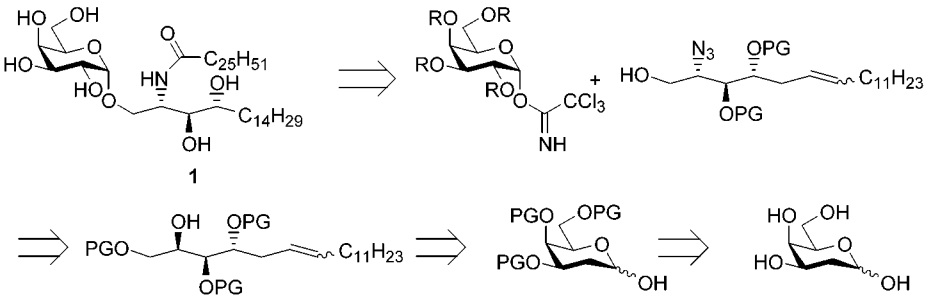
(10) Nishimura, K.; Nakamura, A. *J. Biochem.* **1985**, *98*, 1247−1254.

(11) (a) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176−2187. (b) Morita, M.; Natori, T.; Akimoto, K.; Osawa, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 699−704. (c) Figueroa-Pérez, S.; Schmidt, R. R. *Carbohydr. Res.* **2000**, *328*, 95−102. (d) Takikawa, H.; Muto, S−e.; Mori, K. *Tetrahedron* **1998**, *54*, 3141−3150.

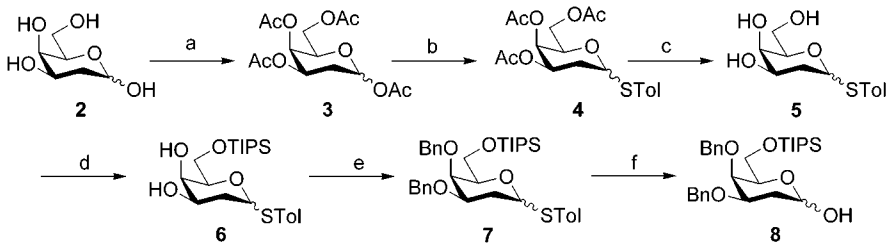
(12) Several derivatives have been reported: (a) NBD-derivative: Sakai T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359−361. (b) biotinylated derivative: Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1995**, *38*, 1836−1841.

10.1021/jo0201530 CCC: $22.00
Published on Web 05/10/2002

Scheme 1. Retrosynthetic Analysis of α-Galactosyl Ceramide 1

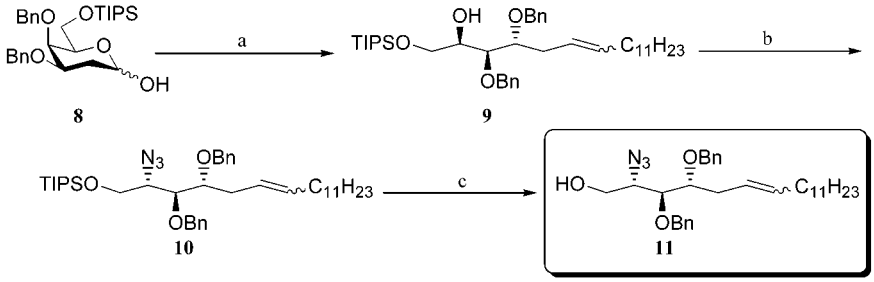


Scheme 2 Toward the Synthesis of the Sphingoine Derivative 11[a]



[a] (a) Ac_2O, py, quant; (b) TolSH, $BF_3{\cdot}OEt_2$, 83%; (c) NaOMe in MeOH; 99.5%; (d) TIPSCl, imidazole, THD, 86%; (e) BnBr, NaH, DMF, 88%; (f) NBS, acetone, 77%.

Scheme 3. Preparation of the Sphingosine Derivative 11[a]



[a] (a) *n*-$C_{12}H_{25}PPh_3Br$, BuLi, THF, 92%; (b) HN_3, PPh_3, DEAD, THF, 93%; (c) TBAF, THF, 91%.

An ideal strategy is to have an easily and selectively cleavable protecting group at the primary hydroxy group and another type of mildly cleavable protecting group on the hydroxy groups at C3 and C4 positions. We decided to use benzyl ethers as the protection groups since this would allow simultaneous conversion of an azide to amine and reduction of the double bond while liberating the molecule in the final stage of the synthesis.

The route described in Scheme 2 involves the use of thiotoluene as a selective protecting group for the anomeric center. Free 2-deoxy-galactose was peracetylated and then reacted with thiocresol under BF_3 etherate catalysis to give thioglycoside **4**.

Subsequent cleavage of the acetates by sodium methoxide in methanol, followed by selective silylation of the 6-OH of **5** and benzylation of the hydroxy groups, gave **7**. The choice of the silyl protection group turned out to be important, since use of TBDMS or TBDPS led to cleavage or migration of the silyl group during benzylation.

Cleavage of the thiotoluene group was performed with NBS. The reaction is complete within minutes. The subsequent Wittig reaction was then carried out using 3 equiv of the Wittig reagent and 2.8 equiv of BuLi. The reaction proceeds very fast; after 2 h, little starting material can be detected by TLC. The *E*/*Z* ratio of the isolated olefinic product was determined to be 3.7:1. Our attempt to introduce the azide group by mesylation of the free hydroxy group in **9** and substitution by azide gave either no conversion at low temperature, or decomposition at high temperature. This was surprising to some extent, since a successful conversion was described recently for a similar molecule.[13] Changing the solvent from DMF to DMSO did not make any difference.[14] Also, chloromesylation did not give the desired product.[15]

However, reaction with a freshly prepared HN_3 solution[16] under Mitsonobu conditions led to the desired compound **10** in 93% yield in a very clean reaction. After desilylation with TBAF, the sphingosine derivative **11** was obtained in 91% yield (Scheme 3).

Overall, the sphingosine building block **11** was prepared in nine steps starting from commercially available 2-deoxy galactose in an overall yield of 36%. Our further strategy featured simultaneous deprotection after glycosylation by simple and mild hydrogenation, thereby cleaving all benzyl groups, removing the double bond, and reducing the azide to an amine. Amide formation using

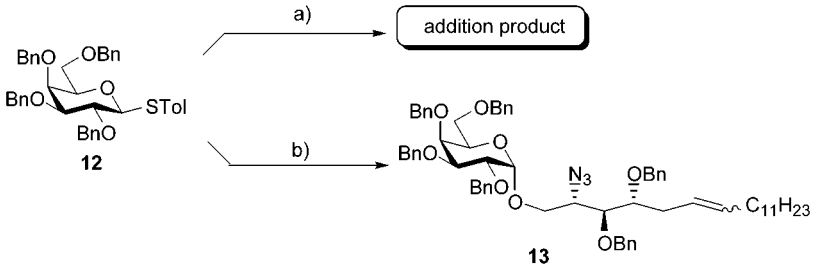
(13) Ohlsson, J.; Magnusson, G. *Carbohydr. Res.* **2001**, *331*, 91−94.

(14) Alvarez, S. G.; Alvarez, M. T. *Synthesis* **1997**, 413−414.

(15) Saotome, C.; Kanie, Y.; Kanie, O.; Wong, C.-H. *Bioorg. Med. Chem.* **2000**, *8*, 2249−2261.

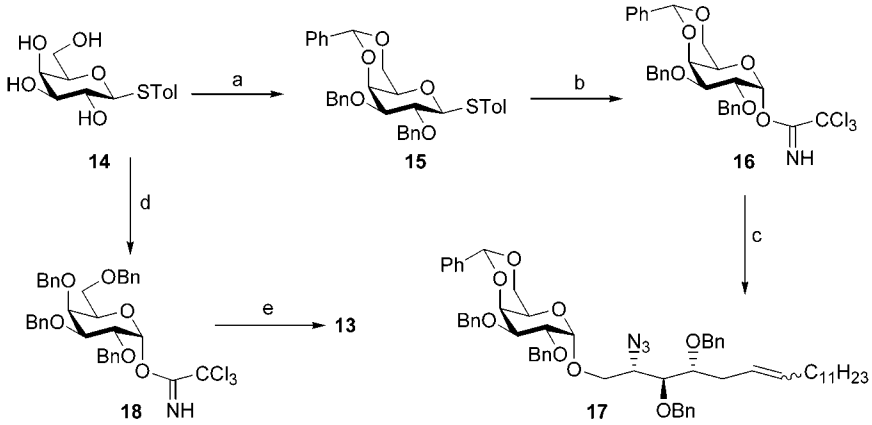
(16) The preparation and handling of hydrazoic acid solutions are described: Wolff, H. *Org. React.* **1946**, *3*, 307−336.

Scheme 4. Preparation of α-Galactosyl Ceramide 13[a]



[a] (a) **11**, NIS, AgOTf; (b) **11**, DMTST, 39%.

Scheme 5. Preparation of α-Galactosyl Ceramides 13 and 17[a]



[a] (a) See ref 19; (b) (1) NBS, acetone, 95%; (2) CCl_3CN, DBU, 93%; (c) **11**, $BF_3{\cdot}OEt_2$, 68%; (d) see ref 20; (e) **11**, $BF_3{\cdot}OEt_2$, 50%.

standard techniques should yield the desired galactosyl lipid. This simple reaction sequence should facilitate the synthesis of glycolipids and related structures.

To simplify the deprotection of final products, a suitable glycosylation donor should be used so that the protected glycolipid product can be completely deprotected by hydrogenation. We first turned our attention to thioglycosides. In a first experiment, NIS and AgOTf were used as a promotor system and perbenzylated thiogalactose **12** as the donor, a system that was used successfully in preliminary experiments.[17] Unfortunately in this case, the reaction led to the formation of several byproducts resulting from addition of electrophiles to the double bond (Scheme 4). Therefore, DMTST[18] was used as another promotor to avoid the production of electrophilic iodine species. The reaction led to glycoside **13**, containing the desired α-linkage and whose exact mass corresponds to MNa − N_2. The observed isomerization of the double bond was deemed insignificant, since subsequent hydrogenation of either isomer would furnish the desired product. No *β*-anomer formation was observed. Further optimization allowed isolation of the galactosyl ceramide **13** in 39% yield.

On the other hand, Schmidt[11c] had demonstrated in a similar case the benefits of using the 4,6-benzylidene-protected trichloroacetimidate **16**. **16** can be obtained easily from thiogalactose **14**[19] (Scheme 5). BF_3 etherate was chosen as a mild promotor, delivering the galactosyl ceramide **17** in a clean reaction in 68% yield. A similar reaction on perbenzylated trichloroacetimidate **18**[20] yielded the desired α-galactosyl ceramide **13** in 50% yield along with other products, the α/*β* ratio being 2.9:1. In both **13** and **17** *E*/*Z* ratio was found to be unchanged compared to the starting ceramide (3.7:1).

Our further investigations emphasized the hydrogenation of both **13** and **17** using Pearlmann's catalyst. The yields thus obtained were, however, relatively low, and we noticed that the reactions were accompanied by decomposition upon longer reaction times. An alternative synthetic strategy (Scheme 6) was therefore tried on galactosyl ceramide **17**. Staudinger reduction, followed by coupling of the free amine **19** with hexacosanoic acid gave the expected galactosyl ceramide **20** in 69% yield, which gave **1** upon hydrogenation with palladium black.

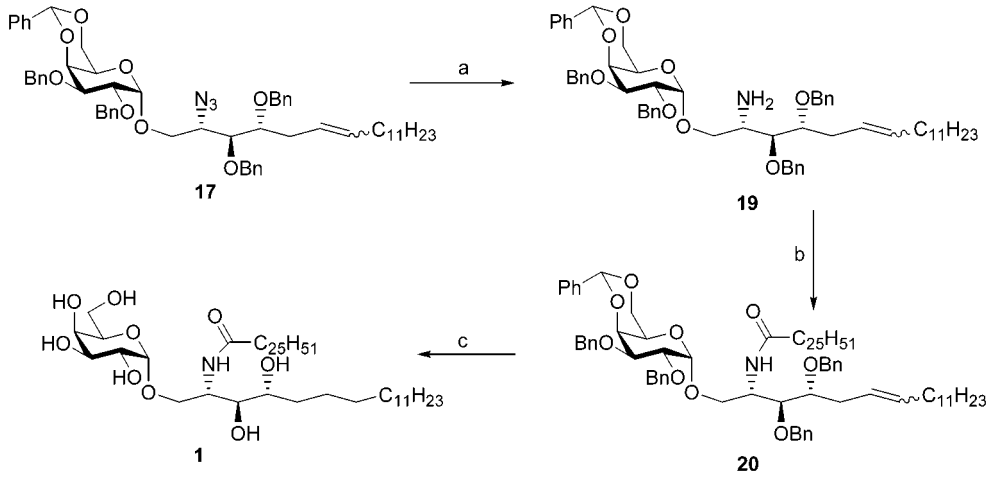
In summary, the new strategy described here for the synthesis of **1** and related glycolipids should provide access to glycolipid libraries for exploitation of their immunostimulating activities. The amide as well as the ceramide chain can be easily modified. In addition, the stereochemistry of the ceramide part can be altered by use of different starting sugars.

Experimental Section

General Methods. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium/benzophenone, and methylene chloride (CH_2Cl_2) over calcium chloride. Reagents of commercial quality were purchased and used without further purification. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Glycosylation experiments were performed by using molecular sieves, which were flame-dried right before the reaction under high vacuum. Analytical thin-layer chromatography was performed using silica gel 60 F_{254} glass plates, compound spots were visualized

(17) Plettenburg, O.; Wong, C.-H, unpublished results.
(18) Ye, X.-S., Wong, C.-H. *J. Org. Chem.* **2000**, 65, 2410−2431.
(19) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734−753.

Scheme 6. Preparation of α-Galactosyl Ceramide 1[a]



[a] (a) PMe_3, THF; then aq 1 M NaOH; (b) $C_{25}H_{51}COOH$, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 69%; (c) H_2, Pd/EtOAc, quant.

by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash chromatography was performed on silica gel 60 Geduran (35−75 μm, EM Science).

1,3,4,6-Tetra-*O*-acetyl 2-Deoxy-D-galactopyranoside (3). 2-Deoxygalactose (10 g, 60.9 mmol) was dissolved in a mixture of 50 mL of pyridine and 50 mL of acetic anhydride. The mixture was stirred overnight. The solvent was removed in vacuo, and the resulting syrup was taken up in 200 mL of chloroform and extracted with sat. sodium bicarbonate solution (three to four times) and with brine (1×). The organic layer was dried over sodium sulfate, and the solvent was evaporated to give **3** (20.0 g, quant.) as a yellow oil. It was used for the next step without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.75 (dd, 1H, J = 2.6, 10.1 Hz); 5.25 (d, 1H, J = 2.9 Hz); 5.07 (ddd, 1H, J = 12.5, 5.0, 3.1 Hz); 4.13 (dd, 1H, J = 11.0, 6.6 Hz); 4.07 (dd, 1H, J = 11.4, 6.6 Hz); 3.90 (dt, J = 6.6, 1.3 Hz); 2.11 (s, 3H); 2.09 (s, 3H); 2.00−2.07 (m, 2H); 2.00 (s, 3H); 1.96 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.37; 170.13; 169.80; 168.71; 91.59; 71.88; 67.93; 65.03; 61.51; 30.00; 20.91; 20.68; 20.62 (2C). HRMS: Calcd. ($C_{14}H_{20}O_9Na^+$): 355.0999, found 355.0996.

***p*-Methylphenyl 3,4,6-Tri-*O*-acetyl 2-deoxy-1-thio-D-galactopyranoside (4).** **3** (21.0 g, 53 mmol) was dissolved in 350 mL of dry dichloromethane. Thiocresol (13.22 g, 106 mmol, 2 equiv) was added. The reaction mixture was cooled to −40° C. $BF_3{\bullet}OEt_2$ (11.9 mL) was added slowly. The reaction mixture was allowed to warm to 0 °C and stirred for 1 h at this temperature. The reaction was monitored closely by TLC (hexanes:ethyl acetate 1:1). After almost all starting material was consumed, the reaction was quenched by addition of saturated sodium bicarbonate solution. The organic layer was extracted with sat. $NaHCO_3$ (3×) and brine (1×), dried over sodium sulfate, and evaporated. The resulting oil was chromatographed on silica gel (hexane:ethyl acetate 4:1) to give **4** as an oil (20.8 g, 83%). The α/β ratio was estimated from the ^{1}H NMR to be 2.5:1. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.41 (d, 2H-β, J = 7.9 Hz); 7.35 (d, 2H-α, J = 7.9 Hz); 7.10 (d, 2H-β, J = 7.9 Hz); 7.09 (d, 2H-α, J = 7.9 Hz); 5.66 (d, 1H-α, J = 5.7 Hz); 5.36 (d, 1H-α, J = 2.6 Hz); 5.28−5.23 (m, 1H-α + 1H-β); 4.98 (ddd, 1H-β, J = 9.9, 7.2, 2.9 Hz); 4.75 (dd, 1H-β, J = 8.3, 5.7 Hz); 4.69 (t, 1H-β, J = 6.6 Hz); 4.16 (dd, 1H-β, J = 11.2, 7.2 Hz); 4.11−4.06 (m, 2H-α+2H-β); 3.80 (dt, 1H-β, J = 0.9, 7.0 Hz); 2.44 (dt, 1H-α, J = 5.6, 12.8 Hz); 2.32 (s, 3H-β); 2.30 (s, 3H-α); 2.11 (s, 3H-α); 2.10 (s, 3H-β); 2.09−2.02 (m, 1H-α+1H-β); 2.02 (s, 3H-β); 1.982 (s, 3H-α); 1.978 (s, 3H-α); 1.97 (s, 3H-β). ^{13}C NMR ($CDCl_3$, 110 MHz): α-isomer: δ = 170.43; 170.20; 169.88; 137.75; 132.26; 130.04; 129.72; 84.01; 67.44; 66.68; 66.66; 62.42; 30.57; 21. 09; 20.81; 20.64 (2C); β-isomer: δ = 170.27; 170.25; 169.96; 138.07; 132.55; 129.59; 129.48; 83.04; 74.61; 69.37; 65.35; 62.12; 31.46; 21.11; 20.77; 20.68. HRMS calcd ($C_{19}H_{24}O_7SNa^+$) 419.1135, found 419.1141.

***p*-Methylphenyl 2-Deoxy-1-thio-D-galactopyranoside (5).** **4** (1.4 g, 3.53 mmol) was dissolved in 75 mL of dry methanol. The suspension was cooled in an ice bath, and 3 mL of a solution of sodium methoxide in methanol was added. The reaction was allowed to warm to room temperature and stirred for 3 h. Amberlyst 15 (H^+-form) was added to neutralize the sodium methoxide, the mixture was then diluted with methanol, and the exchange resin was filtered off. The resin was washed thoroughly and the filtrate was concentrated to give 950 mg (99.5%) of a white solid. ^{1}H NMR (MeOD, 600 MHz): δ = 7.29 (d, 2H-α+ 2H-β, J = 8.3 Hz); 7.00 (d, 2H-α+2H-β, J = 7.9 Hz); 5.46 (d, 1H-α, J = 5.5 Hz); 4.68 (dd, 1H-β, J = 11.0, 3.1 Hz); 4.18 (1, 1H-α, J = 6.1 Hz); 3.82 (ddd, 1H-α, J = 12.3, 4.8, 3.1 Hz); 3.74 (d, 1H-α, J = 2.6 Hz); 3.67−3.55 (m, 1H-α +3H-β); 3.35 (dd, 1H-β, J = 11.2, 7.2 Hz); 3.21 (m, 1H, OH); 2.27 (dt, 1H-β + 1H-α, J = 2.0, 12.7 Hz); 2.20 (s, 3H-α, 3H-β); 1.86−1.81 (m, 1H-α+1H-β). ^{13}C NMR (MeOD, 110 MHz): α-isomer δ = 138.55; 133.54; 132.66; 130.55; 86.22; 73.09; 69.54; 67.44; 62.74; 34.37; 21.10. β-isomer δ = 138.26; 132.47; 132.28; 130.52; 84.31; 80.69; 70.73; 68.44; 62.92; 35.52; 21.06. HRMS calcd ($C_{13}H_{18}O_4SNa^+$) 293.0818, found 293.0820.

***p*-Methylphenyl 2-Deoxy-1-thio-6-*O*-triisopropylsilyl-D-galactopyranoside (6).** **5** (553 mg, 2.05 mmol) was dissolved in 5 mL of dry THF. Imidazole (181 mg, 2.7 mmol) was added, and the reaction mixture was cooled to 0 °C. TIPSCl (0.49 mL, 2.25 mmol, 1.1 equiv) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2.5 h. The reaction was monitored by TLC and after completion quenched by addition of saturated NH_4Cl. The reaction mixture was diluted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel (hexanes:ethyl acetate 2:1) to give 750 mg (86%) of **6**. Since α/β assignment of the protons is difficult, integrals are given as absolute values, referring to the signal at 7.31 ppm as 2. Due to chromatography, α/β values changed-to-a ratio of about 1:1.4. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.39 (d, 1.4H, J = 7.5 Hz); 7.32 (d, 2H; J = 7.9 Hz); 7.06 (2d, 3.4H); 5.61 (d, 1H, J = 5.3 Hz); 4.64 (dd, 0.7H, J = 11.8, 2.2 Hz); 4.22 (t, 1H, J = 4.8 Hz); 4.04 (s, 1H); 4.00−3.87 (m, 5.3H); 3.68 (b−s, 0.7H, OH); 3.62 (d, 1H, J = 3.1 Hz); 3.38 (t, 0.7H, J = 4.8 Hz); 3.24 (d, 0.7H, J = 5.3 Hz); 2.95 (bt, 3.0H, J = 10.1 Hz); 2.35−2.27 (m, 1.5H); 2.30 (s, 2.1H); 2.29 (s, 3H); 2.09−2.02 (m, 1.3H); 1.89−1.81 (m, 0.7 H); 1.13−0.98 (m, 37.9 H). ^{13}C NMR ($CDCl_3$, 110 MHz): δ = 137.43; 136.91; 132.10; 131.40; 131.27; 130.05; 129.49; 129.45; 84.38; 82.96; 78.26; 70.18; 69.69; 69.33; 67.93; 66.46; 66.36; 63.76; 34.75; 33.59;

21.02; 20.97; 17.85; 17.83; 17.80; 17.77; 11.72; 11.69. calcd ($C_{22}H_{38}O_4SSiNa^+$) 449.2152, found 449.2158.

***p*-Methylphenyl 3,4-Di-*O*-benzyl-2-deoxy-1-thio-6-*O*-triisopropylsilyl-D-galactopyranoside (7). 6** (700 mg, 1.58 mmol) was dissolved in 10 mL of DMF. The reaction mixture was cooled to −10 °C (ice bath/NaCl). Benzyl bromide (0.48 mL, 3.95 mmol, 2.5 equiv) was added, followed by 84 mg (3.32 mmol, 2.1 equiv) of sodium hydride (95%). The reaction was monitored by TLC (hexanes:ethyl acetate 2:1) and completed after 40 min at −10 °C. The reaction was quenched by addition of solid NH_4Cl, diluted with water, and the reaction mixture was extracted with ethyl acetate (3×). The combined organic layer was extracted with water (3×) and brine, dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 15:1) to give 880 mg (89%) of **7.** Ratio of the isomers after chromatography: approx. 1:0.6. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.45−7.25 (m, 24.6H); 7.09 (d, 2H, J = 8.3 Hz); 7.06 (d, 1.2H, J = 7.9 Hz); 5.68 (d, 1H, J = 5.7 Hz); 4.99 (d, 1.6H, J = 11.4 Hz); 4.72 (dd, 2.3H, J = 11.8, 3.1 Hz); 4.69−4.58 (m, 4.2H); 4.29 (t, 1H, J = 6.6 Hz); 4.05 (s, 1H); 3.97 (ddd, 1H, J = 12.2, 4.1, 2.5 Hz); 3.93 (s, 0.6 H); 3.89−3.84 (m, 2.1H); 3.74 (dd, 1H, J = 9.7, 5.7 Hz); 3.62 (ddd, 0.6H, J = 11.7, 4.3, 2.3 Hz); 3.44 (t, 0.6H, J = 6.6 Hz); 2.63 (dt, 1H, J = 12.7 Hz, 5.7 Hz); 2.33 (s, 3H); 2.325 (s, 2.1H); 2.20 (dd, 1H, J = 12.9, 4.6 Hz); 1.16−1.01 (m, 37H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 139.12; 138.94; 138.31; 138.20; 137.08; 136.94; 132.03; 131.57; 131.11; 130.55; 129.50; 129.39; 128.37; 128.32; 128.10; 127.98; 127.73; 127.69; 127.54; 127.33; 127.30; 127.24; 127.13; 84.88; 82.96; 79.71; 78.51; 75.43; 74.46; 74.03; 73.08; 72.35; 71.48; 70.40; 70.10; 62.36; 32.52; 31.86; 21.02; 17.96; 11.81. HRMS: calcd ($C_{36}H_{50}O_4SSiNa^+$) 629.3091, found 629.3075.

3,4-Di-*O*-benzyl-2-deoxy-6-*O*-triisopropylsilyl-D-galactopyranoside (8). **7** (820 mg, 1.35 mmol) was dissolved in 50 mL of acetone. The reaction mixture was cooled to 0 °C, and NBS (281 mg, 1.59 mmol, 1.18 equiv) was added. The reaction mixture turned orange immediately. After 10 min. the reaction was quenched by addition of solid NH_4Cl. The mixture was diluted with water and ethyl acetate, and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 4:1) to give 520 mg (77%) of **8**. Ratio of α/β isomers: 1:0.4. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.40−7.22 (m, 14H); 5.42 (d, 1H, J = 2.2 Hz); 4.96 (d, 1.4H, J = 11.4 Hz); 4.70 (dd, 1.8H, J = 11.6, 5.5 Hz); 4.67−4.57 (m, 2.8H); 4.02−3.97 (m, 3H); 3.94 (s−b, 0.4 H); 3.90 (t, 0.4H, J = 9.2 Hz); 3.81 (dd, 1H, J = 9.7, 7.5 Hz); 3.80 (m, 0.4H); 3.73 (dd, 1H, J = 9.7, 5.7 Hz); 3.57 (ddd, 0.4H, J = 12.1, 4.2, 2.6 Hz); 3.52 (s−b, 0.4H); 3.40 (dd, 0.4H, J = 7.9, 5.7 Hz); 3.39 (s−b, 1H); 2.20 (dt, J = 12.5, 3.5 Hz); 2.15 (dt, 0.4 Hz, J = 12.3, 2.1 Hz); 2.00 (m, 0.4H); 1.98 (dd, 1H, J = 12.7, 4.0 Hz); 1.15−1.03 (m, 31H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 139.00; 138.89; 138.55; 138.21; 128.40; 128.34; 128.11; 128.04; 127.60; 127.36; 127.28; 94.82; 92.62; 77.14; 75.86; 74.53; 74.40; 74.26; 72.91; 71.76; 71.43; 70.36; 70.20; 62.38; 61.63; 34.90; 31.12; 17.98; 17.96; 14.14; 11.86; 11.84. HRMS calcd ($C_{29}H_{44}O_5SiNa^+$) 523.285, found 523.284.

3,4-Di-*O*-benzyl-1-*O*-triisopropylsilyl-octadec-6-ene-2-ol (9). Dodecyl triphenylphosphonium bromide (1.53 g, 3 mmol, 3 equiv) was suspended in 20 mL of dry THF. The mixture was cooled to −10°. BuLi (1.75 mL, 2.8 mmol, 2.8 equiv) (1.6 M solution in hexanes) was added dropwise. The orange solution was stirred for 30 min, and then the starting material **8** (510 mg, 0.99 mmol in 15 mL of dry THF) was added slowly. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was monitored by TLC using hexanes:ethyl acetate as a solvent system. For workup, the reaction mixture was quenched with methanol. Afterward 80% methanol in water was added and the reaction mixture was extracted with hexanes (4×). The combined hexanes layer was dried over sodium sulfate and evaporated, and the residue was subjected to column chromatography (hexanes:ethyl acetate 10:1) to yield 610 mg (92%) of **9**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.33−7.25 (m, 10H); 5.57−5.33 (m, 2H); 4.69 (d, 1H, J = 11.4 Hz); 4.60 (m, 3H); 3.94 (m, 1H); 3.72 (m, 4H); 3.07 (d, 1H, J = 4.8 Hz); 2.45 (dt, 1H, J = 15.4, 7.0 Hz); 2.39 (dt, 1H, J = 15.2, 5.6 Hz); 1.99 (q, 2H, J = 7.0 Hz); 1.33−1.20 (m, 19H); 1.12−0.99 (m, 21H); 0.86 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.29; 133.64; 132.33; 128.34; 128.30; 128.00; 127.77; 127.70; 127.57; 124.99; 80.06; 78.14; 73.69; 72.71; 71.30; 63.86; 31.91; 29.67; 29.64; 29.57; 29.38; 29.35; 29.01; 27.54; 22.68; 17.99; 14.10; 14.10; 11.87. HRMS calcd ($C_{42}H_{70}O_4SiNa^+$) 675.4779, found 675.4780.

2-Azido-3,4-di-*O*-benzyl-1-*O*-triisopropylsilyl-octadec-6-ene (10). Sodium azide (6.5 g) was dispersed in 6.5 mL of water and 40 mL of toluene. The suspension was cooled to about 0 °C. Concentrated sulfuric acid (1.35 mL) was added dropwise. While the internal temperature was kept below 10 °C, the reaction mixture was stirred slowly for another 30 min. The toluene layer was decanted into a cooled conical flask (0 °C), dried over sodium sulfate, and stored in a freezer. To determine the concentration, 0.3 mL of the solution and 0.3 mL of water were stirred in a vial and one drop of a phenolphthalein-solution (1% in MeOH) was added, and the solution was titrated with 0.25 N NaOH. Triphenylphosphine (482 mg, 1.84 mmol, 4 equiv) was dissolved in 15 mL of dry THF under argon. The solution was cooled to −78 °C. DEAD (0.29 mL, 320 mg, 1.84 mmol, 4 equiv) and 1.3 mL (c = 1.4 M) of the previously prepared HN_3 solution were added. After stirring for 1 min, a solution of 300 mg (0.46 mmol) of **9** in 7 mL of THF was added rapidly. The reaction was stirred overnight. The reaction progress was checked by TLC (hexanes:ethyl acetate 9:1). The reaction was quenched by the addition of methanol (color changes from orange to light yellow), concentrated in vacuo, and chromatographed on silica gel (hexanes:ethyl acetate 20:1) to yield 263 mg (84%) of **10**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.37−7.26 (m, 10H); 5.55−5.44 (m, 2H); 4.74−4.53 (m, 4H); 4.06 (dd, 1H, J = 10.5, 3.1 Hz); 3.84 (dd, 1H, J = 10.5, 7.9 Hz); 3.74−3.70 (m, 2H); 3.63 (dd, 1H, J = 6.1, 4.2 Hz); 2.48 (t, 1H, J = 5.5 Hz); 2.06 (m, 1H); 1.38−1.23 (m, 19H); 1.11−1.02 (m, 21H); 0.90 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.32; 138.11; 132.34;128.30; 128.27; 127.90; 127.67; 127.51; 125.14; 79.48; 78.89; 73.59; 72.05; 64.65; 64.62; 31.92; 29.68; 29.65; 29.60; 29.57;29.40; 29.35; 27.90; 27.56; 22.68; 17.91; 14.11; 11.85. HRMS calcd ($C_{42}H_{70}N_3O_3Si^+$) 678.5024, found 678.5054.

2-Azido-3,4-di-*O*-benzyl-octadec-6-en-1-ol (11). **10** (250 mg, 0.37 mmol) was dissolved in 5 mL of dry THF. The reaction mixture was cooled to 0 °C, 1 mL of a 1 M TBAF solution was added, and the reaction mixture was allowed to warm to room temperature. After 10 min, the reaction was controlled by TLC and found to be almost complete. After 20 min, the reaction was stopped by addition of 5 mL saturated $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (4×), and the combined organic layer extracted with brine, dried, and evaporated. The residue was chromatographed on silica gel (hexanes:ethyl acetate 9:1) to yield 175 mg (91%) of **11**.^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.39−7.26 (m, 10H); 5.51−5.48 (m, 1H); 5.48−5.38 (m, 1H); 4.70−4.61 (m, 3H), 4.56 (d, 1H, J = 11.4 Hz); 3.85 (dd, 1H, J = 11.4, 4.8 Hz); 3.80−3.68 (m, 4H); 2.46 (m, 2H); 2.03 (m, 2H); 1.37−1.22 (m, 18H); 0.88 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 137.81; 137.58; 132.81; 128.44; 128.39; 127.97; 127.92; 127.88; 127.79; 124.40; 80.38; 78.57; 73.59; 72.26; 63.16; 62.24; 31.89; 29.65; 29.61; 29.54; 29.52; 29.36; 29.32; 28.11; 27.57; 22.66; 17.66; 14.09. HRMS calcd ($C_{33}H_{50}N_3O_3^+ - N_2$): 494.3629, found 494.3643

2-Azido-3,4-di-*O*-benzyl-1-*O*-(2,3,4,5-tetra-O-benzyl-α-D-galactopyranosyl)-D-ribo-octadeca-6-en-1-ol (13). A solution of thioglycoside **12** (73 mg, 0.11 mmol) and sphingosine **11** (50 mg, 0.096 mmol) in 1.5 mL of anhyd CH_2Cl_2 was added over freshly dried powdered 4 Å molecular sieves and cooled to 0 °C. Meanwhile, methyl disulfide (34.5 μL, 4 equiv) and methyl trifluoromethanesulfonate (43.9 μL, 4 equiv) were mixed in a separate vial to form solids. The solids in dry CH_2Cl_2 (0.5 mL) were added to the above solution, and the mixture was stirred at 0 °C for 2.5 h. The reaction was quenched by the addition of 3 drops of saturated aqueous $Na_2S_2O_3$, diluted

with CH_2Cl_2, and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (hexanes:EtOAc 7:1) to furnish **13** (39 mg, 39%) as an oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.44−7.19 (m, 30H); 5.48−5.37 (m, 2H); 4.94 (d, 1H, J = 11.4 Hz); 4.89 (d, 1H, J = 3.2 Hz); 4.86−4.32 (m, 11H); 4.05 (dd, 1H, J = 10.0, 3.5 Hz); 3.96 (dd, 1H, J = 10.0, 2.0 Hz); 3.96−3.92 (m, 3H), 3.86−3.81 (m, 1H); 3.71−3.64 (m, 3H); 3.47 (m, 2H); 2.42 (m, 2H); 2.00 (m, 2H); 1.25 (m, 18H); 0.87 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 138.82; 138.76; 138.65; 138.30; 138.09; 137.98; 132.42; 128.35; 128.34; 128.33; 128.31; 128.28; 128.22; 128.19; 127.79; 127.76; 127.75; 127.73; 127.72; 127.70; 127.64; 127.53; 127.51, 127.49; 127.43, 125.50; 98.60; 79.33; 78.89; 78.81; 76.41; 75.08; 73.68; 73.45; 73.14; 73.12; 73.08; 71.92; 69.02; 68.37; 62.24; 62.15; 31.92; 29.70; 29.68; 29.65; 29.60; 29.55, 29.48; 29.35; 27.95; 27.58; 22.68; 14.11. HRMS calcd ($C_{66}H_{81}N_3O_8H^+ - N_2$): 1016.6040, found 1016.6043.

***O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-galactopyranosyl) Trichloroacetimidate (16). 15** (600 mg, 1.35 mmol) was dissolved in 45 mL of acetone. The reaction mixture was cooled to −20 °C, and NBS (231 mg, 1.29 mmol, 1.2 equiv) was added. After 45 min, the reaction was quenched by addition of solid NH_4Cl. The mixture was diluted with water and ethyl acetate, and the aqueous layer was extracted with ethyl acetate (4×). The combined organic layer was extracted with brine (2×), dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 1:1) to furnish 461 mg (95%) of intermediate deprotected product, which was subsequently solubilized in 10 mL of dry CH_2Cl_2. CCl_3CN (1.028 mL, 10.28 mmol 10 equiv) and DBU (77 μL, 0.51 mmol) were added to the solution, and the mixture was stirred at room temperature for 2 h. The dark solution was then concentrated and purified by column chromatography on silica gel (hexanes:EtOAc 2:1 containing 1% NEt_3) to furnish **16** (567 mg, 93%) as a white foam. NMR and mass spectroscopic data correspond to the data given in the literature.[21]

2-Azido-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-D-ribo-octadeca-6-ene-1-ol (17). A solution of trichloroacetimidate **16** (50 mg, 0.084 mmol, 1.1 equiv)) and sphingosine derivative **11** (40 mg, 0.077 mmol) in 1.3 mL of anhyd Et_2O and 0.2 mL of anhyd THF was added over freshly dried powdered 4 Å molecular sieves and cooled to −20 °C. $BF_3{\cdot}OEt_2$ (19.5 μL, 2 equiv) was added to the solution, and the mixture was stirred at −20 °C for 1.5 h. Trichloroacetimidate **16** (22 mg, 0.036 mmol) was then added. Stirring was continued for 30 min followed by addition of another 12 mg (0.02 mmol) of donor. After 30 min, the mixture was diluted with EtOAc and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (hexanes: EtOAc 7:1) to furnish **17** (50 mg, 68%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.52−7.25 (m, 25H); 5.45−5.41 (m, 3H); 4.92 (d, 1H, J = 3.5 Hz); 4.82 (d, 1H, J = 11.7 Hz); 4.79−4.49 (m, 7H); 4.11 (dm, 1H, J = 3.3 Hz); 4.05 (dd, 1H, J = 9.9, 3.3 Hz); 4.01−3.92 (m, 3H), 3.83−3.77 (m, 2H); 3.68−3.64 (m, 3H); 3.50 (m, 1H); 2.41 (m, 2H); 2.00 (m, 2H); 1.22 (m, 18H); 0.85 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.72; 138.21; 137.95; 137.78; 137.77; 132.51; 128.35; 128.31; 128.24; 128.23; 128.21; 128.08; 127.80; 127.73; 127.68; 127.65; 127.64; 127.58; 127.50; 127.49; 127.44; 126.32; 101.02; 99.05; 79.20; 79.09; 75.71; 75.44; 74.65; 73.72; 73.44; 72.01; 71.96; 69.25; 68.29; 62.91; 61.91; 31.90; 29.67; 29.64; 29.58; 29.57; 29.39; 29.38; 29.33; 27.98; 27.57; 22.66; 14.11. HRMS calcd ($C_{59}H_{873}N_3O_8Na^+$): 974.5295, found 974.5306.

2-Amino-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-D-ribo-octadeca-6-en-1-ol (19). The azide **17** (20 mg, 0.021 mmol) was dissolved in 0.5 mL of anhyd THF and cooled to 0 °C. PMe_3 (0.11 mL of 1.0 M in THF, 0.11 mmol) was added to the solution, and the reaction was stirred for 45 min at 0 °C and 2 h at room temperature. After complete disappearance of the starting material, 0.2 mL of aq 1 M NaOH was added to the mixture and stirred for 2 h. EtOAc was then added to the solution, and the mixture was washed with water (3×) and brine (1×), dried ($MgSO_4$), and concentrated. The residue was used for the next step without prior purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.53−7.26 (m, 25H); 5.50 (m, 2H); 5.47 (s, 1H); 4.97 (d, 1H, J = 3.3 Hz); 4.85 (d, 1H, J = 11.7 Hz); 4.82−4.56 (m, 7H); 4.18 (dm, 1H, J = 2.3 Hz); 4.10−4.07 (m, 2H); 4.01−3.98 (m, 2H), 3.89 (m, 2H); 3.76 (m, 1H); 3.57 (m, 1H); 3.38 (m, 1H); 3.26 (m, 1H); 2.47 (m, 2H); 2.04 (m, 2H); 1.26 (m, 18H); 0.89 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.73; 138.59; 138.48; 138.05; 137.81; 132.29; 128.84; 128.36; 128.37; 128.32; 128.28; 128.27; 128.09; 127.79; 127.67; 127.66; 127.62; 127.60; 127.54; 126.32; 101.04; 99.23; 79.61; 76.11; 75.72; 74.48; 74.47; 73.66; 73.61; 71.94; 71.79; 69.36; 69.37; 62.65;52.77; 31.91; 29.69; 29.65; 29.64; 29.60; 29.43; 29.42; 29.35; 28.36; 27.61; 22.7; 14.12. HRMS calcd ($C_{59}H_{75}NO_8Na^+$): 948.5385, found 948.5379.

3,4-Di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-hexacosylamino-D-ribo-octadeca-6-en-1-ol (20). The crude amine **19** was dissolved in 0.5 mL of anhyd CH_2Cl_2 and hexacosanoic acid (26 mg, 0.06 mmol, 3 equiv) and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC) (11 mg, 0.063 mmol, 3 equiv) were added to the solution. The mixture was stirred at room temperature for 24 h and then diluted with CH_2Cl_2 and washed with water (3×) and brine (1×). The solution was dried ($MgSO_4$) and concentrated, and the residue was purified by column chromatography on silica gel (hexanes:EtOAc 4:1) to furnish **20** (11 mg, 69%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.52−7.25 (m, 25H); 5.70 (d, 1H, J = 8.2 Hz); 5.47−5.45 (m, 3H); 4.93 (d, 1H, J = 3.5 Hz); 4.83 (d, 1H, J = 11.7 Hz); 4.78−4.48 (m, 7H); 4.38 (m, 1H); 4.16−4.04 (m, 3H), 4.07−4.04 (m, 3H); 3.94−3.87 (m, 2H); 3.77−3.73 (m, 1H); 3.59 (m, 1H); 2.45 (m, 1H); 2.09 (m, 1H); 1.87 (m, 2H); 1.73 (m, 2H); 1.50 (m, 2H); 1.35−1.20 (m, 62H); 0.90 (t, 6H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 172.87; 138.67; 138.56; 138.37; 138.32; 137.81; 132.29; 128.84; 128.44; 128.37; 128.32; 128.31; 128.08; 127.87; 127.84; 127.76; 127.73; 127.66; 127.61; 127.57; 127.54; 126.31; 125.09; 101.00; 99.63; 79.93; 78.99; 76.13; 75.65; 74.40; 73.73; 73.34; 71.76; 71.59; 69.40; 68.24; 62.90; 50.20; 31.90; 29.72−29.61, 29.45; 29.42; 29.36; 27.99; 27.57; 25.71, 22.69; 14.13. HRMS calcd ($C_{85}H_{125}NO_9Na^+$): 1326.9246, found 1326.9248.

1-*O*-(α-D-galactopyranosyl)-2-hexacosylamino-D-ribo-1,3,4-octadecantriol (1). A solution of **20** (11 mg, 0.008 mmol) in EtOAc (1 mL) was added to 3 mg of palladium black suspended in 1.5 mL of EtOAc and saturated with hydrogen. The reaction vessel was purged with hydrogen, and the mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with pyridine, and the catalyst was removed by filtration over Celite. Evaporation of the solvent gave pure **1** (7 mg, 99.5%). The obtained spectroscopic data is identical to the one reported in the literature.[11a] HRMS calcd ($C_{50}H_{99}NO_9Na^+$): 880.7212, found 880.7227.

Acknowledgment. O. P. acknowledges a fellowship of the Deutsche Forschungsgemeinschaft. V. B.-N. acknowledges the Swiss National Chemical Society fellowship. This research is supported by the Skaggs funds.

Supporting Information Available: ^{1}H and ^{13}C NMR spectral data of compounds **3**−**11**, **13**, **17**, **19**, **20**, and **1** is available free of charge via the Internet at http://pubs.asc.org.

JO0201530

(20) (a) Mottawia, M. S.; Olsen, C. E.; Denyer, K.; Smith, A. M.; Moller, B. L. *Carbohydr. Res.* **2001**, *330*, 309−318. (b) Wegmann, B.; Schmidt, R. R. *J. Carbohydr. Chem.* **1987**, *6*, 357−375.

(21) Koeman, F. A. W.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron* **1993**, *49*, 5291−5309.