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Concise Synthesis of the Unnatural Sphingosine and Psychosine Enantiomer

Archana R. Parameswar,[a] Jacqueline A. Hawkins,[b] Laurel K. Mydock,[a] Mark S. Sands,[b] and Alexei V. Demchenko*[a]

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The accumulation of psychosine (galactosyl sphingosine) has been associated with the pathogenesis of Krabbe disease; however, the exact mechanism of its cytotoxicity remains unclear. Herein, we describe the synthesis of the unnatural enantiomer of erythro-sphingosine, psychosine, and related derivatives thereof that would allow for the mechanistic elucidation of the toxicity of psychosine.

Introduction

Psychosine (galactosyl sphingosine) is a natural glycosphingolipid found in a variety of mammalian cells. While it typically exists in very low concentrations, elevated levels of psychosine are linked to the pathogenesis of Globoid Cell Leukodystrophy (GLD) or Krabbe disease.[1,2] In GLD, the abnormal accumulation of psychosine is due to a genetic deficiency in galactosylceramide-β-galactosidase (also referred to as galactosyl ceramidase) activity, a degradative lysosomal enzyme.[3] Additionally, psychosine is highly cytotoxic[4] and is known to lead to the death of oligodendrocytes, the myelin-producing cells in the nervous system.[5] As a consequence, patients with GLD suffer from a number of neurological deficits and typically die before the age of five.[2] Despite its obvious relevance to the pathogenesis of GLD,[6] very little is understood about the mechanisms underlying the toxicity of psychosine. Initially, it was presumed that the interactions between psychosine and specific protein partners were solely responsible for the resultant toxicity.[7] However, more recently it has been demonstrated that the accumulation of psychosine in the central nervous system (CNS) may also interfere with membrane lipid raft (LR) function, significantly disrupting cellular signaling processes.[8] As such, it remains unclear which of these general mechanisms is responsible for the toxicity of psychosine, or whether both pathways are involved during different stages of the disease.

In order to resolve this fundamental question, we needed to discriminate between the two plausible mechanisms of toxicity. As such, an investigation of the enantiomer of natural psychosine (*ent*-psychosine, **1**) was seen as a reliable approach whereby the two differing pathways could be distinguished. Because of the high stereochemical specificity of protein interactions, it can be assumed that the *ent*-psychosine would no longer be able to bind to any partner proteins. If psychosine toxicity is due to protein interactions, *ent*-psychosine would not exhibit any cytotoxic effect. However, if the toxicity of psychosine is mediated through LRs within the cell membrane, the hydrophobic interactions between *ent*-psychosine and the achiral lipid membrane will be preserved, along with toxicity. Previously, Covey and others have made considerable progress elucidating the mechanisms of various signaling pathways by synthesizing and applying unnatural *ent*-steroids.[9] Hence, the synthesis of unnatural *ent*-psychosine (**1**) and the investigation of its mode of action appealed to us as an important first step toward the elucidation of the psychosine toxicity pathways in GLD.

In spite of the considerable progress that has been made toward the synthesis of various glycosphingolipids and analogues thereof,[10] there still remains a challenge in ob-

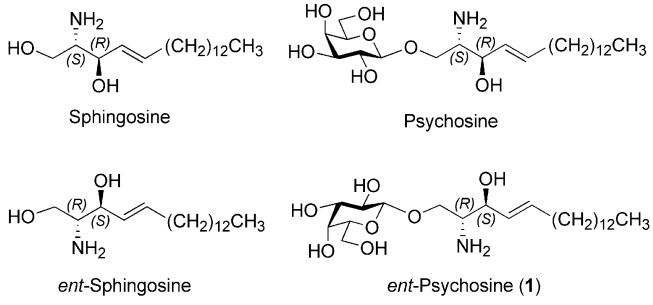


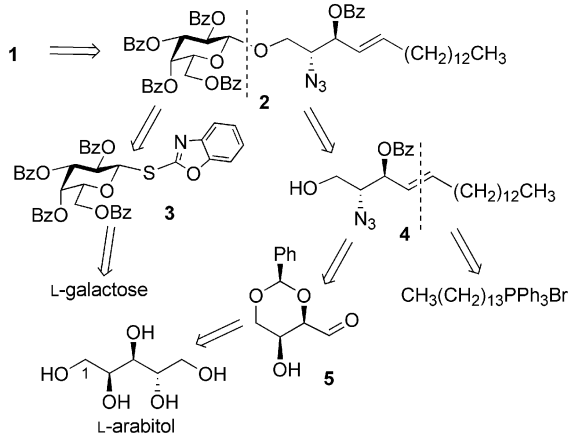
Figure 1. Sphingosine, Psychosine, and enantiomers thereof.

[a] Department of Chemistry and Biochemistry, University of Missouri – St. Louis,
One University Boulevard, St. Louis, Missouri 63121, USA
Fax: +1-314-516-5342
E-mail: demchenkoa@umsl.edu
[b] Department of Internal Medicine and Department of Genetics, Washington University School of Medicine,
St. Louis, Missouri 63110, USA

taining such targets in high yields, with minimal synthetic steps and with complete stereochemical purity. These characteristics are essential, as the interpretation of biological toxicity studies often hinges on the differential action of enantiomers. Herein, we report the synthesis of unnatural *ent*-psychosine (**1**) from the chirally pure starting material (Figure 1). Unlike a number of previously reported approaches to the synthesis of enantio- and diastereomeric sphingosine analogues,[11] our synthetic target is derived from a readily available carbohydrate precursor of the L-series containing predetermined stereocenters.

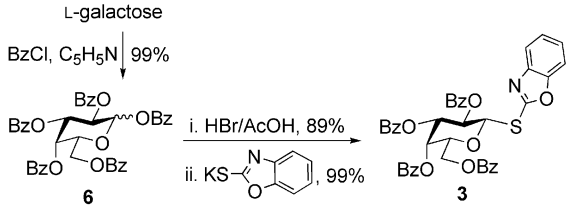
Results and Discussion

ent-Psychosine (**1**) is a complex glycosphingolipid that is comprised of the sphingosine enantiomer (*ent*-sphingosine) and L-galactose moieties (Figure 1). Synthetically, we envisaged that fully protected precursor **2** could be assembled from a suitable L-galactose donor, such as *S*-benzoxazolyl (SBox) L-galactoside **3** (Scheme 1), whereas partially protected enantiomeric sphingosine derivative **4** could serve as a suitable glycosyl acceptor. Although glycosyl donor **3** could be obtained in three steps from L-galactose, as described previously for the synthesis of its counterpart of the D-galacto series,[12,13] the synthesis of acceptor **4** required careful retrosynthetic analysis. As depicted in Scheme 1, we decided that the most suitable option would be to base the synthesis on the inexpensive L-arabitol, which contains a predetermined stereochemistry at the C-2 and C-3 carbon atoms that would be appropriate for our synthesis of *ent*-sphingosine. Subsequent regioselective 1,3-*O*-benzylidene protection, followed by oxidative cleavage with sodium periodate, could then provide desired precursor **5**.



Scheme 1. Retrosynthetic analysis of *ent*-psychosine (**1**).

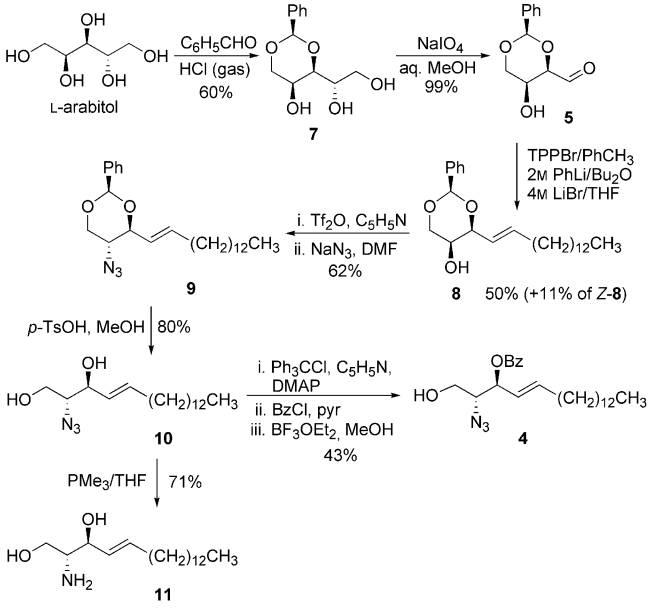
To initiate this route, we first synthesized L-galactosyl donor **3** as shown in Scheme 2. Benzoylation of L-galactose resulted in the formation of perbenzoate **6**, which was subjected to sequential anomeric bromination and the introduction of the SBox leaving group by the protocol developed in our laboratory for D-sugars (Scheme 2).[12] Resulting glycosyl donor **3** was obtained in 88% overall yield.



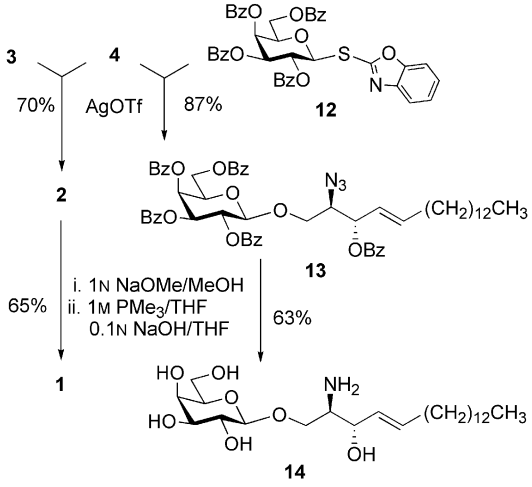
Scheme 2. Synthesis of perbenzoylated SBox L-galactosyl donor **3**.

Among a plethora of available approaches[14] for the synthesis of *ent*-sphingosine precursor **4**, we adapted a very effective strategy developed by Schmidt et al. for the synthesis of natural sphingosine from D-arabitol.[15] Accordingly, L-arabitol was first converted into 1,3-*O*-benzylidene arabitol **7** in 60% yield by using benzaldehyde in the presence of HCl gas (Scheme 3).[16] Compound **7** was then subjected to oxidative cleavage by using sodium periodate. Resulting aldehyde **5**, which was found to exist in the dimeric form (as reported for similar analogous compounds),[17] was obtained in 99% yield. Next, Wittig olefination of aldehyde **5** was accomplished by using (1-tetradecyl)triphenylphosphonium bromide (TPPBr) in the presence of 2 M PhLi/Bu_2O and 4 M LiBr/THF to yield predominantly (*E*)-**8**. It was found that the addition of LiBr can benefit the preferential formation of *E* isomers[18] and proved essential in obtaining a good isolated yield of 50% for compound **8**. Nevertheless, this reaction also yielded (*Z*)-**8** in 11% yield; however, the stereoisomers were easily separable by column chromatography. The free hydroxy group in compound **8** was then converted into an azide by sequential trifluoromethanesulfonation with triflic anhydride in pyridine, followed by treatment with sodium azide in DMF. Resulting compound **9** was isolated in 62% yield over two steps. Acetal cleavage of **9** was carried out by using *p*-toluenesulfonic acid in methanol, and resulting diol intermediate **10** was obtained in 80% yield. Lastly, compound **4** was obtained in 43% yield through a three-step protocol involving sequential tritylation with trityl chloride, benzoylation with benzoyl bromide, and detritylation with $BF_3{\cdot}Et_2O$. Intermediate **10** was also deprotected by using PMe_3 in THF to afford *ent*-sphingosine (**11**) in 71% yield.

Having obtained protected *ent*-sphingosine precursor **4**, we investigated its capability as a glycosyl acceptor. For this purpose, it was subjected to glycosylation with SBox L- and D-galactosyl donors **3** and **12** (Scheme 4). These glycosylations were promoted with silver(I) triflate to afford the corresponding glycosides **2** and **13** in 70 and 87% yield, respectively. Complete deprotection of psychosine precursors **2** and **13** was accomplished by deacylation in the presence of 1 N NaOMe in MeOH, followed by azide reduction by using PMe_3 in THF. This sequence yielded target *ent*-psychosine (**1**) in 65% yield and its D-galactose diastereomer **14** in 63% yield. It should be noted that the use of PMe_3 for the azide reduction step was found to be the most advantageous,[19] as other methods that used H_2S, $HS(CH_2)_3SH$, PPh_3, or $NaBH_4$ either failed or yielded the unprotected derivatives in lower yields.



Scheme 3. Synthesis of *ent*-sphingosine acceptor **4**.



Scheme 4. Synthesis of *ent*-psychosine (**1**) and its D-galactose analogue **14**.

Conclusions

In conclusion, we developed an efficient protocol for the synthesis of enantiomeric sphingosine and psychosine derivatives. The approach is based on sugar precursors, which eliminates the need for a stereocontrolled synthesis and ensures complete enantiomeric purity of the end product. It is expected that the developed route would be suitable for the synthesis and application of other classes of natural and unnatural glycosphingolipids.[20] With the successful synthesis of *ent*-psychosine (**1**) and its D-galactose analogue **14**, the next step will be the biological evaluation of these compounds, wherin the elucidation of the toxicity pathways of psychosine in GLD afflicted mammalian nervous systems will be determined. This research is currently under way in our laboratories and will be reported in due course.

Experimental Section

General: Column chromatography was performed on silica gel 60 (70–230 mesh); reactions were monitored by TLC on Kieselgel 60 F254. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. $ClCH_2CH_2Cl$ was distilled from CaH_2 directly prior to application. Methanol was dried by heating at reflux with magnesium methoxide, distilled, and stored under an argon atmosphere. Toluene was distilled from CaH_2 under an argon atmosphere and was heated at reflux for 2 h before application. THF was distilled from metallic sodium by using benzophenone as indicator under an argon atmosphere and was heated at reflux for 2 h before use. Anhydrous pyridine, anhydrous DMF, and redistilled benzaldehyde were obtained from Sigma–Aldrich and used as is. (1-Tetradecyl)triphenylphosphonium bromide and L-galactose were purchased from TCI chemicals. Molecular sieves (3 Å) were crushed and activated in vacuo at 390 °C during 8 h in the first instance and then for 2–3 h at 390 °C directly prior to application. AgOTf was coevaporated with toluene (3 × 10 mL) and dried in vacuo for 2–3 h directly prior to application. Optical rotations were measured with a Jasco P-1020 polarimeter. Melting points were measured with a Thomas Hoover capillary melting point apparatus. Unless noted otherwise, ^1H NMR spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Avance); ^{13}C NMR spectra and 2D experiments were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) or at 125 MHz (Bruker ARX-500). HRMS determinations were made with a JEOL MStation (JMS-700) Mass Spectrometer.

Benzoxazolyl 2,3,4,6-Tetra-*O*-benzoyl-1-thio-β-L-galactopyranoside (3): Benzoyl chloride (1.14 mL, 9.9 mmol) was added dropwise to a stirring mixture of L-galactose (0.255 g, 1.41 mmol) in dry pyridine (3.0 mL) under an argon atmosphere at room temperature. Upon completion (≈16 h), the reaction mixture was quenched by the addition of methanol (≈5 mL), evaporated, and coevaporated with toluene (3 × 25 mL). The residue was diluted with CH_2Cl_2 (50 mL), washed with water (20 mL), 1 N HCl (20 mL), water (20 mL), $NaHCO_3$ (2 × 20 mL), and water (2 × 20 mL). The organic layer was separated, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford 1,2,3,4,6-penta-*O*-benzoyl-L-galactopyranoside (**6**; 0.98 g, 99%) as a white foam. HBr (30% v/v) in glacial AcOH (1.0 mL) was added to a stirred solution of pentabenzoate **6** (0.98 g, 1.4 mmol) in CH_2Cl_2 (5 mL) at room temperature. Upon completion (≈2 h), the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed successively with ice-cold water (20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and cold water (3 × 20 mL). The organic layer was separated, dried, and concentrated in vacuo. The crude residue was purified by crystallization from anhydrous diethyl ether and hexanes to afford 2,3,4,6-tetra-*O*-benzoyl-α-L-galactopyranosyl bromide (0.83 g, 89%) as white crystals. Potassium benzoxazole-2-thiolate[12] (0.36 g, 1.9 mmol) was added to the stirring solution of the freshly prepared 2,3,4,6-tetra-*O*-benzoyl-α-L-galactopyranosyl bromide (0.83 g, 1.25 mmol) in dry acetone (8.0 mL) under an argon atmosphere at room temperature. Upon completion (≈3 h), the mixture was diluted with CH_2Cl_2 (30 mL) and washed successively with 1% aqueous NaOH (2 × 15 mL), saturated aqueous $NaHCO_3$ (15 mL), and water (3 × 15 mL). The organic layer was separated, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc gradient elution) to afford title compound **3** (0.92 g, 99%). R_f = 0.49 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{23}$ = –130.77 (*c* = 1, $CHCl_3$). ^1H NMR: δ = 4.46 (dd, 1 H, 6a-

H), 4.54–4.61 (m, 2 H, 5-H, 6b-H), 5.79 (dd, $J_{2,3}$ = 8.8 Hz, $J_{3,4}$ = 3.4 Hz, 1 H, 3-H), 5.97–6.07 (m, 3 H, 1-H, 2, 4), 7.20–7.51 (m, 16 H, aromatic), 7.77 (dd, 2 H, aromatic), 7.90 (m, 4 H, aromatic), 8.08 (dd, 2 H, aromatic) ppm. ^{13}C NMR: δ = 68.4, 68.6, 72.8, 84.4, 110.2, 119.0, 124.7 (2 C), 128.6 (4 C), 128.7 (2 C), 128.8 (2 C), 128.9, 129.0, 129.4, 129.9 (3 C), 130.0 (2 C), 130.1 (2 C), 130.2 (2 C), 133.2, 133.5, 133.7, 133.8, 141.5, 152.0, 161.1, 165.4 (2 C), 165.6, 166.1 ppm. HRMS (FAB): calcd. for $C_{41}H_{32}NO_{10}S$ [M + H]$^+$ 730.1747; found 730.1740.

1,3-*O*-Benzylidene-L-arabitol (7): Hydrogen chloride gas (generated by the dropwise addition of H_2SO_4 to NaCl) was bubbled through a mixture of L-arabitol (5.0 g, 32.0 mmol) and freshly distilled benzaldehyde (4.0 mL, 39 mmol) for 1 h until L-arabitol was completely dissolved. The reaction mixture was kept for an additional 16 h; after that, the resulting solidified mass was broken up and placed in a desiccator containing KOH and H_2SO_4 and dried for 24 h in vacuo. The resulting solid was then triturated with diethyl ether (≈10 mL) and mixed with 20% aqueous $NaHCO_3$ (≈20 mL). The solid containing compound **7** was filtered off and rinsed with water (10 mL) and diethyl ether (10 mL). The resulting solid was recrystallized from 2-propanol to afford title compound **7** (4.5 g, 60%) as white crystals. Analytical data for **7** was essentially the same as reported previously.[21] ^{1}H NMR (CD_3OD): δ = 3.56 (m, 1 H), 3.69 (br. s, 1 H), 3.71 (br. s, 1 H), 3.80 (br. s, 2 H), 4.00–4.13 (m, 2 H), 5.50 (s, 1 H, PhC*H*), 7.21–7.26 (m, 3 H, aromatic), 7.41–7.44 (m, 2 H, aromatic) ppm. ^{13}C NMR (CD_3OD): δ = 64.1, 64.2, 71.1, 73.9, 80.2, 102.7, 127.6 (2 C), 129.1, 129.9, 140.1 ppm.

(2*S*,3*S*,4*E*)-1,3-*O*-Benzylidene-4-octadecen-1,2,3-triol (8): Compound **7** (2.7 g, 11 mmol) was dissolved in methanol (80 mL), and the solution was cooled to 0 °C. An aqueous solution of $NaIO_4$ (2.4 g, 11.0 mol) in water (30 mL) was added dropwise, and the resulting mixture was stirred for 45 min. The reaction mixture was then concentrated in vacuo, and the residue was coevaporated with ethanol (3 × 20 mL). The residue was extracted with warm (≈40 °C) ethyl acetate (3 × 150 mL), and the combined organic extract was washed with water (100 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated in vacuo to yield 2,4-*O*-benzylidene-L-threose (**5**; 2.3 g, 99%), which was used for the subsequent step without further purification. To a stirred suspension of 1-tetradecyl triphenylphosphonium bromide (12.2 g, 22.6 mmol) in dry toluene (100 mL) was added a solution of PhLi (2 M in dibutyl ether, 27 mL) followed by a solution of LiBr (4 M in THF, 14 mL) at –10 °C under an argon atmosphere. The resulting orange-red solution was stirred for 30 min at room temperature; after that it was cooled to –35 °C, and a solution of compound **5** (3.8 g, 18.0 mmol) in dry THF (27 mL) was added. The stirring reaction mixture was allowed to gradually warm to 0 °C over a period of 3 h. After that, methanol (≈10 mL) was added, the resulting mixture was poured in water (50 mL), and the resulting emulsion was vigorously stirred for 20 min, transferred into a separatory funnel, and extracted with CH_2Cl_2 (2 × 250 mL). The combined organic extract was washed with water (100 mL), separated, dried, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (ethyl acetate/CH_2Cl_2 gradient elution). Title compound **8** was obtained as a white solid (3.5 g, 50%). *Z*-isomer of **8** was also isolated in 11% yield. Analytical data for **8**: R_f = 0.56 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{23}$ = +3.21 (c = 1, $CHCl_3$). ^{1}H NMR: δ = 0.85 (t, 3 H, CH_3), 1.20–1.36 [m, 22 H, $(CH_2)_{11}CH_3$], 2.04 (m, 2 H, CH=CHCH_2), 2.71 (d, $J_{CH,OH}$ = 10.0 Hz, 1 H, OH), 3.47 (br. d, 1 H, C*H*OH), 4.00 (dd, J = 11.8, 1.2 Hz, 1 H, 0.5 OCH_2), 4.18 (d, J = 11.8, 1.8 Hz, 1 H, 0.5 OCH_2), 4.34 (d, J = 6.0 Hz, 1 H, C*H*CH=CH), 5.56–5.65 (m, 2 H, PhC*H*, CHC*H*=CH), 5.78–5.85 (m, 1 H, CHCH=C*H*), 7.25–7.33 (m, 3 H, aromatic), 7.47–7.50 (m, 2 H, aromatic) ppm. ^{13}C NMR: δ = 14.3, 22.8, 29.1, 29.4, 29.5, 29.6, 29.7, 29.8 (4 C), 32.1, 32.6, 66.5, 72.5, 80.8, 101.5, 126.1 (2 C), 126.2 (2 C), 128.4, 129.1, 135.2, 138.1 ppm. HRMS (FAB): calcd. for $C_{25}H_{40}O_3Na$ [M + Na]$^+$ 411.2875; found 411.2852.

(2*R*,3*S*,4*E*)-2-Azido-1,3-*O*-benzylidene-4-octadecen-1,3-diol (9): To a stirred solution of compound **8** (1.5 g, 3.86 mmol) in CH_2Cl_2 (10.5 mL) was added pyridine (0.75 mL) followed by triflic anhydride (0.8 mL, 4.6 mmol) at –20 °C under an argon atmosphere. When TLC showed complete disappearance of the starting material (≈5 min), DMF (35 mL) was added followed by NaN_3 (0.75 g, 0.011 mmol). The external cooling was removed, and the reaction mixture was stirred for 3 h at room temperature. After that, the reaction mixture was extracted with ethyl acetate (3 × 150 mL), and the combined organic extract was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford title compound **9** (0.98 g, 62%) as a colorless oil. R_f = 0.55 (ethyl acetate/hexanes, 1:9). $[\alpha]_D^{24}$ = +8.45 (c = 1, $CHCl_3$). ^{1}H NMR: δ = 0.86 (t, 3 H, CH_3), 1.20–1.42 [m, 22 H, $(CH_2)_{11}CH_3$], 2.10 (m, 2 H, CH=CHCH_2), 3.45–3.50 (m, 1 H, CHN_3), 3.60 (dd, J = 11.8 Hz, 1 H, 0.5 OCH_2), 4.05 (m, 1 H, C*H*CH=CH), 4.30–4.35 (m, 1 H, 0.5 OCH_2), 5.48–5.62 (m, 2 H, PhC*H*, CHC*H*=CH), 5.94–6.01 (m, 1 H, C*H*CH=CH), 7.34–7.38 (m, 3 H, aromatic), 7.47–7.49 (m, 2 H, aromatic) ppm. ^{13}C NMR: δ = 14.3, 22.9, 28.9, 29.4, 29.6, 29.7, 29.8, 29.9 (4 C), 32.2, 32.7, 57.7, 69.3, 81.9, 101.3, 126.1, 126.6 (2 C), 128.5 (2 C), 129.2, 137.6, 137.9 ppm. HRMS (FAB): calcd. for $C_{25}H_{39}N_3O_2Na$ [M + Na]$^+$ 436.2940; found 436.2929.

(2*R*,3*S*,4*E*)-2-Azido-4-octadecen-1,3-diol (10): *p*-TsOH monohydrate (31 mg) was added to a stirred solution of compound **9** (0.98 g, 2.37 mmol) in a mixture of methanol (25 mL) and DCM (10 mL) at room temperature. Upon completion (≈24 h), solid $NaHCO_3$ was added until neutral pH (≈7); the solid was filtered off and the filtrate was concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford title compound **10** (0.6 g, 80% yield). R_f = 0.41 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{24}$ = +39.34 (c = 1, $CHCl_3$). ^{1}H NMR: δ = 0.85 (t, 3 H, CH_3), 1.20–1.36 [m, 22 H, $(CH_2)_{11}CH_3$], 2.02–2.14 (m, 2 H, CH=CHCH_2), 2.29 (br. s, 2 H, 2 OH), 3.44–3.50 (m, 1 H, CHN_3), 3.76 (br. s, 2 H, OCH_2), 4.23 (m, 1 H, C*H*CH=CH), 5.46–5.54 (m, 1 H, CHC*H*=CH), 5.75–5.84 (m, 1 H, CHCH=C*H*) ppm. ^{13}C NMR: δ = 14.3, 22.9, 29.1, 29.4, 29.5, 29.7, 29.8, 29.9 (4 C), 32.1, 32.5, 62.7, 66.9, 73.8, 128.2, 136.2 ppm. HRMS (FAB): calcd. for $C_{18}H_{35}N_3O_2Na$ [M + Na]$^+$ 348.2627; found 348.2626.

(2*R*,3*S*,4*E*)-2-Azido-3-benzoyloxy-4-octadecen-1-ol (4): Trityl chloride (0.34 g, 1.22 mmol) was added to a stirring solution of compound **9** (0.305 g, 0.937 mmol) in pyridine (3.0 mL). The reaction mixture was stirred at room temperature for 16 h. Additional trityl chloride (0.3 g, 1.2 mmol), pyridine (1.0 mL), and DMAP (50 mg) were added, and the reaction was stirred for 24 h. The resulting mixture was concentrated under reduced pressure, and the residue was diluted with CH_2Cl_2 (125 mL), washed with water (50 mL), $NaHCO_3$ (2 × 50 mL), and water (3 × 50 mL). The organic layer was separated, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford (2*R*,3*S*,4*E*)-2-azido-1-trityloxy-4-octadecen-3-ol (0.60 g) as a white solid. The latter compound (0.54 g, 0.95 mmol) was dissolved in pyridine (4 mL) and benzoyl chloride (0.22 mL, 1.9 mmol) was added dropwise; the resulting reaction mixture was stirred under an argon atmosphere for 18 h at

room temperature. After that, methanol (≈10 mL) was added, the mixture was concentrated in vacuo, and the residue was coevaporated with toluene (2 × 25 mL). The residue was then diluted with CH_2Cl_2 (100 mL), washed with water (40 mL), 1 N HCl (40 mL), water (40 mL), $NaHCO_3$ (2 × 40 mL), and water (2 × 40 mL). The organic layer was separated, dried, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford (2*R*,3*S*,4*E*)-2-azido-3-benzoyloxy-1-trityloxy-4-octadecene (0.38 g, 60%) as a colorless syrup. The latter compound (0.32 g, 4.76 mmol) was dissolved in methanol (4.0 mL) and $BF_3{\cdot}OEt_2$ (62 μL) was added dropwise; the resulting mixture was stirred for 15 h at room temperature. After that, the reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with water (10 mL), $NaHCO_3$ (10 mL), and water (2 × 10 mL). The organic layer was separated, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes gradient elution) to afford title compound **4** (0.15 g, 72%) as a colorless syrup. R_f = 0.56 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{23}$ = +51.04 (*c* = 1, $CHCl_3$). ^{1}H NMR: δ = 0.85 (t, 3 H, CH_3), 1.20–1.36 [m, 22 H, $(CH_2)_{11}CH_3$], 2.02–2.10 (m, 2 H, CH=CHCH_2), 3.58–3.64 (m, 1 H, 0.5 OCH_2), 3.71–3.80 (m, 2 H, 0.5 OCH_2, CHN_3), 5.54–5.61 (m, 2 H, C*H*CH=CH, CHC*H*=CH), 5.89–5.94 (m, 1 H, CHCH=C*H*), 7.40–7.54 (m, 3 H, aromatic), 8.02–8.05 (m, 2 H, aromatic) ppm. ^{13}C NMR: δ = 14.3, 22.9, 28.9, 29.3, 29.5, 29.6, 29.7, 29.8 (4 C), 32.1, 32.6, 62.2, 66.4, 74.8, 123.4, 128.7 (2 C), 129.9 (3 C), 133.5, 139.0, 165.7 ppm. HRMS (FAB): calcd. for $C_{25}H_{39}N_3O_3Na$ [M + Na]$^+$ 452.2889; found 452.2939.

(2*R*,3*S*,4*E*)-2-Amino-4-octadecen-1,3-diol (11): Compound **10** (48 mg, 0.15 mmol) was dissolved in THF (7.0 mL) and 0.1 M NaOH (1 mL) was added; the resulting mixture was stirred for 20 min. A solution of PMe_3 (1 M in THF, 0.15 mL) was then added dropwise, and the reaction mixture was stirred for 16 h at room temperature. After that, it was neutralized with 0.1 N aqueous HCl (≈0.5 mL) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel [packed in 5% solution of methanol/ammonia (40/1 wt/wt) in DCM] to afford title compound **11** (31 mg, 70%) as a white solid. $[\alpha]_D^{25}$ = –9.23 (*c* = 1, CH_3OH). ^{1}H NMR (CD_3OD): δ = 0.83 (t, 3 H, CH_3), 1.15–1.36 [m, 22 H, $(CH_2)_{11}CH_3$], 1.98–2.05 (m, 2 H, CH=CHCH_2), 2.68–2.80 (br. m, 1 H, CHN), 3.41–3.63 (br. m, 2 H, OCH_2), 3.92–3.96 (m, 1 H, C*H*CH=CH), 5.38–5.45 (m, 1 H, CHC*H*=CH), 5.63–5.72 (dd, 1 H, CHCH=C*H*) ppm. ^{13}C NMR (CD_3OD): δ = 14.6, 23.9, 30.5 (2 C), 30.6, 30.7, 30.8 (2 C), 30.9 (3 C), 33.2, 33.6, 58.2, 63.8, 74.6, 130.6, 135.6 ppm. HRMS (FAB): calcd. for $C_{18}H_{38}NO_2$ [M + Na]$^+$ 300.2897; found 300.2899.

(2*R*,3*S*,4*E*)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-benzoyl-β-L-galactopyranosyl)oxy-4-octadecene (2): A mixture of glycosyl donor **3** (0.22 g, 0.31 mmol), glycosyl acceptor **4** (0.12 g, 0.28 mmol), and freshly activated molecular sieves (3 Å, 0.67 g) in $ClCH_2CH_2Cl$ (2.0 mL) was stirred under an argon atmosphere for 1.5 h. Freshly conditioned AgOTf (0.24 g, 0.92 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature. After that, it was diluted with CH_2Cl_2 (15 mL), the solid was filtered off, and the residue was washed with CH_2Cl_2 (3 × 5 mL). The combined filtrate (35 mL) was washed with saturated aqueous $NaHCO_3$ (15 mL) and water (2 × 15 mL), and the organic phase was separated, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford title compound **2** (0.2 g, 70%) as a white foam. R_f = 0.55 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{27}$ = –44.49 (*c* = 1, $CHCl_3$). ^{1}H NMR: δ = 0.83 (t, 3 H, CH_3), 1.20–1.30 [m, 22 H, $(CH_2)_{11}CH_3$], 1.85–1.90 (m, 2 H, CH=CHCH_2), 3.62–3.72 (m, 1 H, 0.5 OCH_2), 3.92–4.05 (m, 2 H, 0.5 OCH_2, CHN_3), 4.29–4.40 (m, 2 H, 6a, 6b-H), 4.45–4.65 (m, 1 H, 5-H), 4.85 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1-H), 5.39–5.50 (m, 1 H, CHC*H*=CH), 5.55–5.65 (m, 2 H, 3-H, C*H*CH=CH), 5.66–5.74 (m, 1 H, CHCH=C*H*), 5.75–5.87 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.3 Hz, 1 H, 2-H), 5.97 (br. d, $J_{3,4}$ = 3.1 Hz, 1 H, 4-H), 7.12–8.11 (m, 25 H, aromatic) ppm. ^{13}C NMR: δ = 14.3 (2 C), 22.9 (2 C), 28.8, 29.3, 29.4 (2 C), 29.5, 29.7, 29.8 (4 C), 32.0 (2 C), 32.5, 62.0, 63.6, 68.1 (2 C), 69.8, 71.5, 71.7, 74.8, 101.4, 122.8, 128.3 (2 C), 128.4 (2 C), 128.6 (3 C), 128.8, 128.9, 129.0, 129.1, 129.3, 129.5, 129.8 (2 C), 129.9 (5 C), 130.1, 130.2, 133.2, 133.3, 133.4, 133.7, 139.1, 165.1 (2 C), 165.7 (2 C), 166.1 ppm. HRMS (FAB): calcd. for $C_{59}H_{65}N_3O_{12}Na$ [M + Na]$^+$ 1030.4466; found 1030.4497.

(2*R*,3*S*,4*E*)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)oxy-4-octadecene (13): The title compound was obtained as a white foam in 87% from building blocks **4** and **12**[12] as described for the synthesis of **2**. Analytical data for **13**: R_f = 0.55 (ethyl acetate/hexanes, 3:7). $[\alpha]_D^{24}$ = +80.06 (*c* = 1, $CHCl_3$). ^{1}H NMR: δ = 0.84 (t, 3 H, CH_3), 1.15–1.35 [m, 22 H, $(CH_2)_{11}CH_3$], 1.95–2.04 (m, 2 H, CH=CHCH_2), 3.50–3.62 (m, 1 H, 0.5 OCH_2), 3.90–4.02 (m, 1 H, CHN_3), 4.05–4.18 (m, 1 H, 0.5 OCH_2), 4.32–4.40 (m, 2 H, 6a, 6b-H), 4.60–4.75 (m, 1 H, 5-H), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1-H), 5.42–5.48 (m, 2 H, CHC*H*=CH, C*H*CH=CH), 5.58–5.62 (br. dd, 1 H, 3-H), 5.79–5.85 (m, 2 H, 2-H, CHCH=C*H*), 5.99 (br. d, 1 H, 4-H), 7.15–7.60–8.11 (m, 25 H, aromatic) ppm. ^{13}C NMR: δ = 14.3 (2 C), 22.9 (2 C), 28.8, 29.4, 29.5 (2 C), 29.6, 29.8, 29.9 (4 C), 32.1 (2 C), 32.5, 62.1, 64.5, 68.2, 69.4, 69.8, 71.6, 71.8, 74.8, 102.1, 123.1, 128.5 (2 C), 128.6 (2 C), 128.7 (3 C), 128.8, 128.9, 129.2, 129.4, 129.6, 129.9 (3 C), 130.0 (5 C), 130.2, 133.4 (2 C), 133.5, 133.8, 138.8, 165.3, 165.5, 165.7, 165.8, 166.2 ppm. HRMS (FAB): calcd. for $C_{59}H_{65}N_3O_{12}Na$ [M + Na]$^+$ 1030.4466; found 1030.4497.

(2*R*,3*S*,4*E*)-2-Amino-1-(β-L-galactopyranosyl)oxy-3-hydroxy-4-octadecene (1): To a stirred solution of **2** (173 mg, 0.171 mmol) in dry methanol (3 mL) was added 1 M NaOMe (≈0.4 mL, pH 10), and the resulting mixture was stirred for 20 h at room temperature. After that it was neutralized by the addition of Dowex (H^+), and the resin was filtered off and rinsed successively with methanol (5 × 5 mL). The filtrate was concentrated in vacuo, and the crude residue (93 mg) was dissolved in THF (13.0 mL); 0.1 M NaOH (1.9 mL) was added, and the resulting mixture was stirred for 20 min. A solution of PMe_3 (1 M in THF, 0.19 mL) was then added dropwise, and the reaction mixture was stirred for 16 h at room temperature. After that, it was neutralized with 0.1 N aqueous HCl and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel [packed in 5% solution of methanol/ammonia (40/1 wt/wt) in CH_2Cl_2] to afford title compound **1** (58 mg, 65%) as a white solid. $[\alpha]_D^{26}$ = +1.46 (*c* = 1, CH_3OH). ^{1}H NMR (CD_3OD): δ = 0.82 (t, 3 H, CH_3), 1.20–1.34 [m, 22 H, $(CH_2)_{11}CH_3$], 1.97–2.02 (m, 2 H, CH=CHCH_2), 2.80–2.89 (m, 1 H, CHN_3), 3.35–3.50 (m, 3 H, 2, 4, 5-H), 3.62–3.81 (m, 5 H, 3, 6a, 6b-H, OCH_2), 3.90–3.95 (m, 1 H, C*H*CH=CH), 4.14 (d, $J_{1,2}$ = 7.2 Hz, 1 H, 1-H), 5.38–5.48 (m, 1 H, CHC*H*=CH), 5.65–5.76 (m, 1 H, CHCH=C*H*) ppm. ^{13}C NMR (CD_3OD): δ = 14.6, 23.9, 30.4, 30.5, 30.6, 30.7, 30.8 (2 C), 30.9 (3 C), 33.2, 33.5, 56.7, 62.7, 70.0, 70.5, 72.6, 73.2, 74.9, 76.9, 104.9, 129.9, 136.1 ppm. HRMS (FAB): calcd. for $C_{24}H_{48}NO_7$ [M + H]$^+$ 462.3431; found 462.3434.

(2*R*,3*S*,4*E*)-2-Amino-1-(β-D-galactopyranosyl)oxy-3-hydroxy-4-octadecene (14): The title compound was obtained as a white solid in 63% from **13** as described for the synthesis of **1**. Analytical data for **14**: $[\alpha]_D^{22}$ = –6.20 (*c* = 1, CH_3OH). ^{1}H NMR (CD_3OD): δ = 0.82

(t, 3 H, CH_3), 1.20–1.34 [m, 22 H, $(CH_2)_{11}CH_3$], 1.97–2.04 (m, 2 H, CH=CHCH_2), 2.80–2.90 (m, 1 H, $CHNH_2$), 3.38–3.50 (m, 4 H, 2, 4, 5-H, 0.5 OCH_2), 3.64–3.78 (m, 3 H, 3, 6a, 6b-H), 3.92–4.01 (m, 2 H, C*H*CH=CH, 0.5 OCH_2), 4.14 (d, $J_{1,2}$ = 7.2 Hz, 1 H, 1-H), 5.40–5.45 (m, 1 H, CHC*H*=CH), 5.65–5.76 (m, 1 H, CHCH=C*H*) ppm. ^{13}C NMR (CD_3OD): δ = 14.6, 23.9, 30.4, 30.5, 30.6, 30.7, 30.8 (2 C), 30.9 (3 C), 33.2, 33.5, 56.7, 62.7, 70.0, 70.5, 72.6, 73.2, 74.9, 76.9, 104.9, 129.9, 136.1 ppm. HRMS (FAB): calcd. for $C_{24}H_{48}NO_7$ $[M + H]^+$ 462.3431; found 462.3434.

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