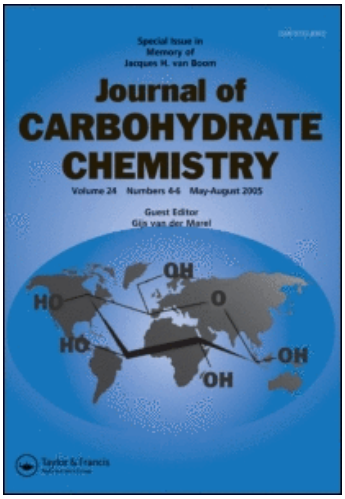

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Synthesis of a (4E, 8Z)-Sphingadienine Moiety Containing Cerebroside from *Tetragonia Tetragonoides* with Antiulcerogenic Activity[1,2]

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SYNTHESIS OF A (4E,8Z)-SPHINGADIENINE MOIETY CONTAINING CEREBROSIDE FROM *TETRAGONIA TETRAGONOIDES* WITH ANTIULCEROGENIC ACTIVITY [1,2]

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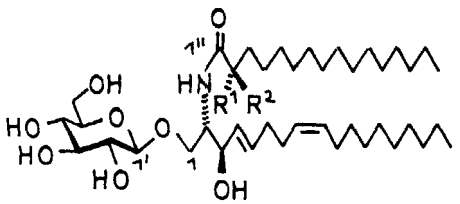
ABSTRACT

The natural cerebroside 1a possessing antiulcerogenic activity and its diastereomer 1b were readily synthesized via the azidosphingosine glycosylation method. The required (4E,8Z)-azidosphingadienine 13 was obtained from (4Z)-tetradecenyl bromide (8) and 2,4-O-benzylidene-D-threose (10) via Wittig reaction, subsequent azide introduction and benzylidene group removal. For the glucosylation the 3-O-benzoyl derivative 16 was prepared; it provided, with the O-(tetra-O-acetyl-ß-D-glucopyranosyl)trichloroacetimidate (17), the desired O-glucosyl derivative 18 in high yield. Deprotection and azido group reduction afforded compound 20 with a free amino group. N-Acylation with both enantiomers of α-hydroxy palmitic acid (using the derivatives 21a,b) yielded, after deacylation, compounds 1a and 1b, respectively.

INTRODUCTION

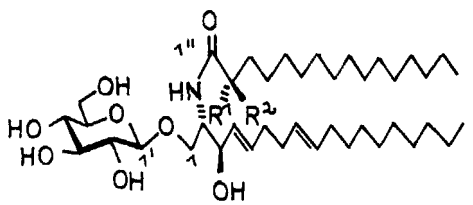
A large variety of different cerebrosides and glycosphingolipids in general were found as membrane constituents in natural sources.[3-5] The variations are identified on the carbohydrate, the fatty acid, and the sphingosine level. Many important biological functions have been recently assigned to these compounds generating interest in the synthesis of structurally homogeneous material for the required biological and pharmacological studies.[3,4]

SCHEME 1



1 a : $R^1 = H$, $R^2 = OH$

b : $R^1 = OH$, $R^2 = H$



2 a : $R^1 = H$, $R^2 = OH$

b : $R^1 = OH$, $R^2 = H$

During a survey of neurotropic components of oriental crude drugs Okuyama and Yamazaki recognized that the principles of *Tetragonia tetragonoides* possessing antiulcerogenic activity are due to cerebrosides to which they assigned the ß-D-glucopyranosyl ceramide structures 1 and 2 (SCHEME 1).[2] The configuration of the (4E,8Z)- and the (4E,8E)-sphingadienine moieties were suggested to be D-erythro; the configuration of the α-hydroxypalmitoyl residue was not assigned.

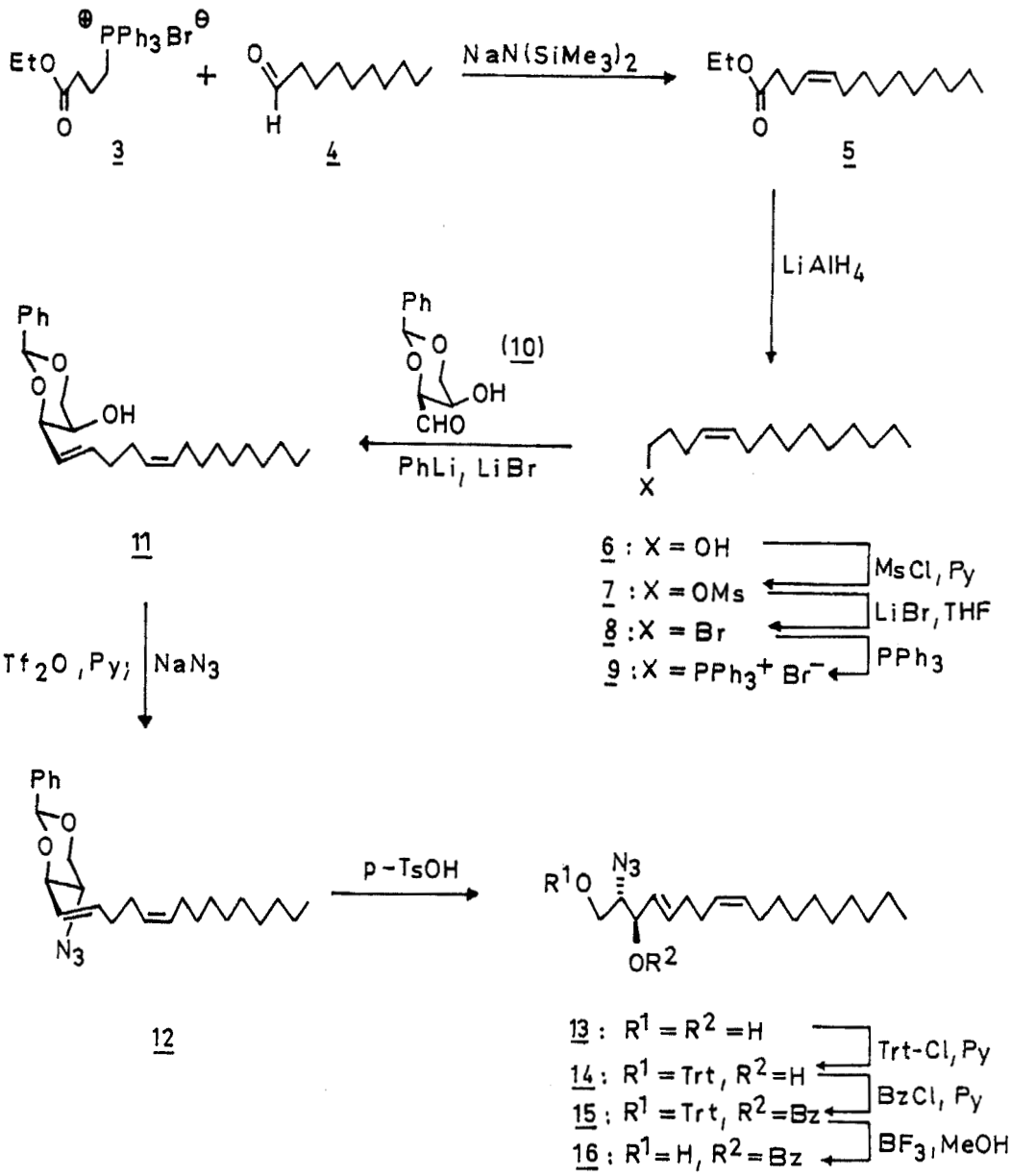
Our interest in developing efficient glycosphingolipid syntheses[3,6-10] led us to apply our recently introduced "azidosphingosine glycosylation method"[7] to the preparation of the diastereomers 1a and 1b. Compounds 2a,b were also synthesized by us via another route.[10] Due to previous findings[11] compound 1a with (R)-configuration of the α-hydroxy palmitoyl residue was expected to be the natural product. A short communication, published now by Nakagawa et al.[12], describing a different approach for the synthesis of compounds 1a,b confirms this suggestion. In their paper our previously introduced "ceramide glycosylation method" is applied.[6]

RESULTS AND DISCUSSION

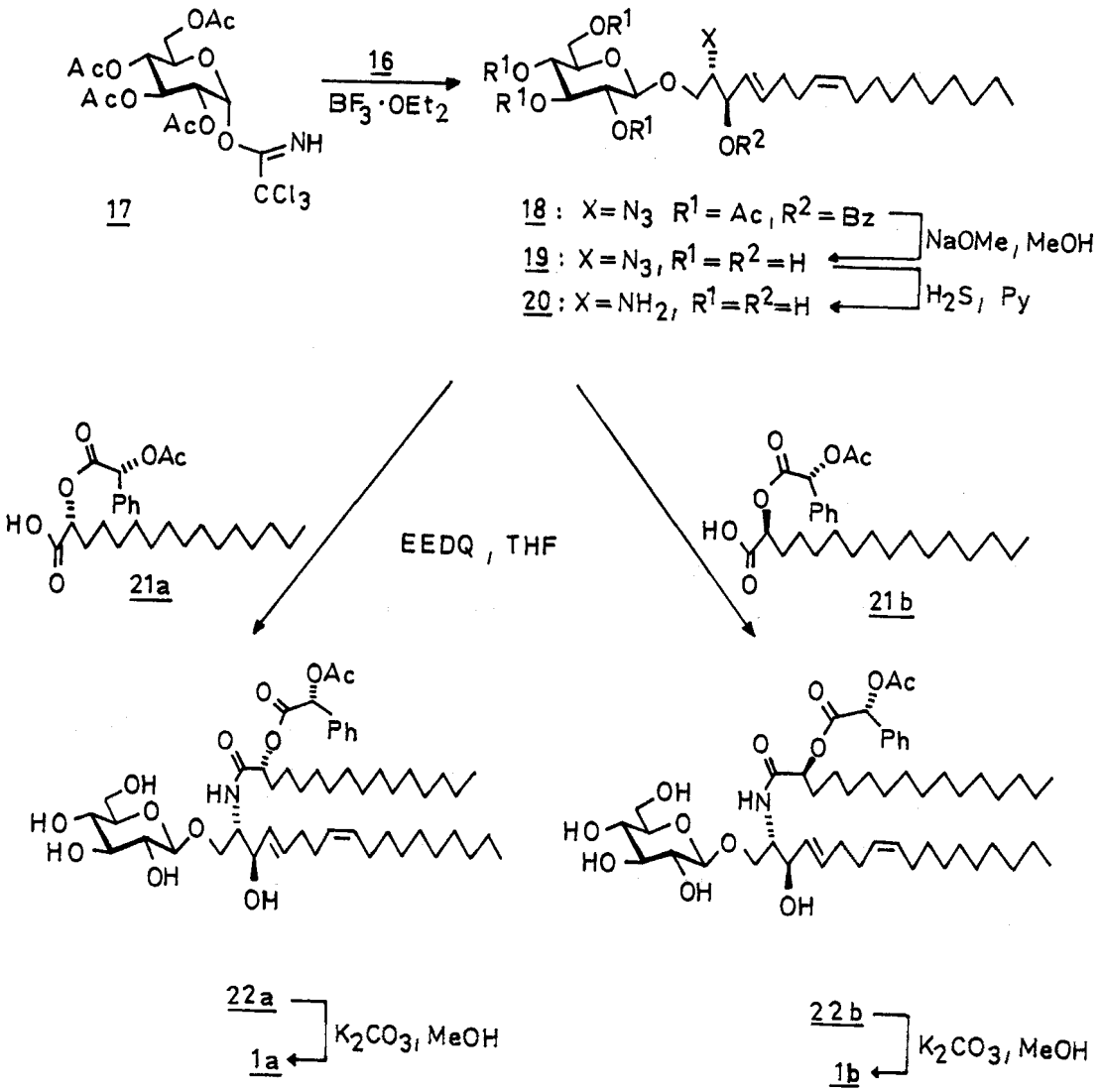
Starting from 2,4-di-O-protected D-threose derivatives, readily obtained from D-galactose or D-xylose,[13] a high yielding three step synthesis of the azido derivative of (4E)-D-erythro-sphingosines could be developed.[13,14] The 3-O-benzoyl derivative of these compounds gave excellent yields in 1-O-glycosylation and subsequent transformations into the derived glycosphingolipids.[7-9] The application of this "azidosphingosine glycosylation method" to the synthesis of compounds 1a and 1b required the preparation of (4E,8Z)-azidosphingosine 13 as an intermediate (SCHEME 2). For this aim ethyl 4-bromobutyrate was transformed into the phosphonium salt 3 and then treated with sodium bis-(trimethylsilyl)amide to generate a lithium salt free Wittig reagent according to a procedure of Bestmann and coworkers.[15] Reaction with n-decanal (4)[16] furnished exclusively the *cis*-product 5. Reduction of the ester moiety with lithium aluminium hydride gave (4Z)-tetradecenol 6, which was prepared already via other routes.[2,17] Following a general procedure of Wenkert and coworkers,[18] O-mesylation of this compound with methanesulfonyl chloride in pyridine afforded the O-mesyl (Ms) derivative 7 which, after treatment with lithium bromide in tetrahydrofuran, furnished the corresponding bromide 8. Heating with triphenylphosphine led to the phosphonium salt 9. Ylide formation with phenyllithium in the presence of excess lithium bromide in molecular disperse form[14] provided with 2,4-O-benzylidene-D-threose (10)[13,14] cleanly the (4E,8Z)-octadecadiene-triol derivative 11. This procedure for the generation of the (4E)-CC-double bond proved to be successful in other sphingosine syntheses.[7-10,13,14] Azide introduction in the 2-position was carried out via activation of the unprotected 2-hydroxy group with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of pyridine and treatment of the generated triflate with sodium azide at room temperature. The azido derivative 12 was then deprotected with p-toluene sulfonic acid (p-TsOH) as an acid catalyst providing the desired azidosphingosine 13.

Preliminary experiments in direct glycoside bond formation with compound 13 exhibited an insufficient selectivity for the primary hydroxy group.[19] Therefore we transformed this compound

SCHEME 2



SCHEME 3



in high yielding reactions, via selective 1-O-tritylation with trityl (Trt) chloride/pyridine, into compound 14. Subsequent 3-O-benzoylation with benzoyl (Bz) chloride/pyridine into compound 15, and detritylation with boron trifluoride/methanol into the 1-O-unprotected 3-O-benzoyl derivative 16.

1-O-Glycosylation of the azidosphingosine derivative 16 with the O-acetylated trichloroacetimidate 17[20] at room temperature, in the presence of boron trifluoride·diethyl ether as a catalyst, afforded the derived ß-D-glucopyranoside 18 in 80% yield (SCHEME 3).[21] Deacylation with sodium methoxide/methanol provided the fully deprotected glucoside 19 which gave, after azido group reduction with hydrogen sulfide in pyridine, the ß-D-glucopyranosyl sphingosine 20. For the attachment of the enantiomers of α-hydroxy palmitic acid, the racemate was separated with the help of (D)-O-acetyl mandelic acid as chiral auxiliary using a known procedure.[22] The diastereomers 21a and 21b obtained were attached to the free amino group of compound 20 with 2-ethoxy-1-ethyloxycarbonyl-1,2-dihydroquinoline (EEDQ) in tetrahydrofuran as a condensating agent yielding the cerebrosides 22a and 22b, respectively. Deacylation with potassium carbonate in methanol afforded the desired unprotected cerebrosides 1a and 1b. As expected, compound 1a was identical with compound B_{1-b} isolated from Tetragonia tetragonoides.[5,10]

EXPERIMENTAL

General Procedures. Melting points are uncorrected. Analytical thin layer chromatography was performed on Kieselgel plates (0.25 mm thickness) obtained from E. Merck AG, Darmstadt (BRD). Preparative chromatography was performed on Kieselgel 60 (0.062 - 0.20 mm), obtained from Merck. Medium pressure liquid chromatography was performed on Kieselgel "LiChroprep" Si 60, 15-25 µm, using a refractive index detector. In all cases, small samples were finally purified through medium pressure liquid chromatography. Specific rotations were determined with a PERKIN-ELMER 241 MC polarimeter. ^{1}H NMR spectra were recorded with a "JEOL-GX 400" (400 MHz) and a BRUKER "WM 250 Cryospec" (250 MHz) spectrometers, using TMS as internal standard. While citing ^{1}H NMR data the following abbreviations have been used:

s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), br s (broad singlet), dd (double doublet) and dt (double triplet). Peak positions have been assigned, either on the basis of coupling constants, or on the basis of interrelationship observed from decoupling experiments.

Ethyl (4Z)-4-tetradecanoate (5). (a) **Synthesis of compound** 3, not reported in ref [15]. To a solution of ethyl 4-bromobutyrate (90 g, 0.46 mol) in dry toluene (500 mL) under nitrogen, was added triphenylphosphine (125 g, 0.47 mol). The reaction mixture was heated to reflux for 36 h, cooled, and the solvent decanted. To the solid residue was added dry tetrahydrofuran to form a suspension of the solid. Filtering and drying of the solid under vacuum afforded 147.5 g (70%) of the title product 2, mp 160 °C (ref [15], mp 160°).

(b) (5): Ethyl 1-(triphenylphosphonium bromide)-4-butyrate (50 g, 109 mmol) on reaction with sodium bis-(trimethylsilyl) amide gave the corresponding ylide, which on further reaction with decanal 4[16] (17.1 g, 109 mmol) at -78 °C under conditions reported in the lit.[15] and work up afforded 31 g of crude reaction product. This on purification (first through a column of silica using petroleum ether/ethyl acetate (9:1) as eluents and then on distillation) gave 17.0 g (61%) of the title product as a liquid, having R_F 0.54 in petroleum ether/ethyl acetate (9:1). ^1H NMR ($CDCl_3$, 400 MHz), δ 5.41 (dt, 1H, J = 10.7, 6.8 Hz, H-4), 5.32 (dt, 1H, J = 10.7, 6.8 Hz, H-5), 4.12 (q, 2H, J = 7.1 Hz, $-COOC\underline{H}_2CH_3$), 2.34 (m, 4H, 2H-2, 2H-3), 2.11 (m, 2H, 2H-6), 1.26 (m, 14H, 7x $-C\underline{H}_2$ aliphatics), 0.88 (t, 3H, $-CH_3$).

(Z)-4-Tetradecen-1-ol (6). A slurry of lithium aluminium hydride (1.9 g) in anhydrous ether (300 mL) was cooled to -60 °C. Compound 5 (17.0 g, 67 mmol) dissolved in anhydrous ether (200 ml) was added in 15 min. Following the addition, the reaction was warmed to -10 °C and stirred for 16 h. The reaction was quenched with water at 0 °C and worked up as usual with ether. The resulting reaction product (14.0 g) was purified on silica (petroleum ether/ethyl acetate; 9:1) and gave 13.4 g (90%) of the title product, as a liquid, having R_F 0.23 in petroleum ether/ethyl acetate (9:1). ^1H NMR data is in accordance with (Z)-isomer reported in the lit.[17]. ^1H NMR ($CDCl_3$,

250 MHz) δ 5.41 (dt, 1H, J = 10.7, 6.4 Hz, H-4), 5.36 (dt, 1H, J = 10.7, 6.4 Hz, H-5), 3.65 (t, 2H, J = 6.4 Hz, 2H-1), 2.12 (q, 2H, J = 6.4 Hz, 2H-3), 2.03 (q, 2H, J = 6.6 Hz, 2H-6), 1.63 (p, 2H, J = 6.6 Hz, 2H-2), 1.26 (m, 14H, 7 x $C\underline{H}_2$ aliphatics), 0.88 (t, 3H, J = 6.6 Hz, 3H-14)

Anal. Calcd for $C_{14}H_{28}O \cdot 0.25\ H_2O$: C, 77.53; H, 13.01. Found: C, 77.51; H, 12.96.

(Z)-4-Tetradecen-1-methanesulfonate (7) **and bromide** (8). Preparation of the title products 7 and 8 were carried out according to the general procedure reported in the lit.[18]. 7: ^1H NMR ($CDCl_3$, 250 MHz) δ 5.43 (dt, 1H, J = 10.7, 7.0 Hz, H-4), 5.31 (dt, 1H, J = 10.7, 7.0, H-5), 4.21 (t, 2H, J = 6.4 Hz, 2H-1), 2.99 (s, 3H, $-OSO_2CH_3$), 2.16 (q, 2H, J = 7.0 Hz, 2H-3), 2.00 (q, 2H, J = 6.7 Hz, 2H-6), 1.80 (p, 2H, J = 7.0 Hz, 2H-2), 1.26 (m, 14H, 7 x $-CH_2$ aliphatics), 0.88 (t, 3H, J = 6.7 Hz, 3H-14). 8: ^1H NMR ($CDCl_3$, 250 MHz), δ 5.45 (dt, 1H, J = 10.7, 7.3 Hz, H-4), 5.30 (dt, 1H, J = 10.7, 7.3 Hz, H-5), 3.42 (t, 2H, J = 6.8 Hz, 2H-1), 2.20 (q, 2H, J = 7.1 Hz, 2H-3), 2.05 (q, 2H, J = 6.8 Hz, 2H-6), 1.92 (p, 2H, J = 7.1 Hz, 2H-2), 1.27 (m, 14H, 7 x $-CH_2$ aliphatics), 0.89 (t, 3H, J = 7.1 Hz, 3H-14).

Anal. Calcd for $C_{14}H_{27}Br$: C, 61.09; H, 9.82. Found: C, 60.92; H, 9.58.

(Z)-4-Tetradecen-1-triphenylphosphoniumbromide (9). A neat mixture of triphenylphosphine (16.6 g, 63.36 mmol) and compound 8 (17.4 g, 63.3 mmol) was heated at 140 °C under nitrogen for 24 h. This material after cooling was directly used for the next reaction.

(2S,3R,4E,8Z)-1,3-O-Benzylidene-1,2,3-trihydroxy-4,8-octadecadiene (11). To a suspension of compound 9 (crude product obtained above) in dry toluene (700 mL) under nitrogen, was added a freshly prepared solution of phenyllithium from lithium (4.44 g, 633 mmol) and bromobenzene (49.7 g, 316 mmol) in anhydrous ether (140 mL)) dropwise at room temp. After 15 min the reaction mixture was cooled to -30° and a solution of 2,4-O-benzylidene-D-threose 10[13,14] (13.21 g, 63.5 mmol) in dry THF (200 mL) was added dropwise in 20 min. After stirring at room temp for 15 min, the reaction mixture was quenched, firstly

with methanol (75 mL) and then with water (75 mL), extracted the organic layer, diluted with toluene (300 mL), washed with water (2 x 200 mL) and dried ($MgSO_4$). Evaporation of the solvent resulted in the isolation of 48 g of the crude reaction product. This product was purified on chromatography over silica, using petroleum ether/ethyl acetate (9:1) as eluents giving 11 g (45%) of the title product as an oil, having R_F = 0.43 in petroleum ether/ethyl acetate (4:1): $[\alpha]_D^{20}$ -2.32° (c = 1.42, $CHCl_3$)), ^1H NMR ($CDCl_3$, 250 MHz) δ 7.52, 7.36 (2m, 5H, aromatics), 5.87 (dt, 1H, J = 15.4, 6.6 Hz, H-5), 5.66 (dd, 1H, J = 15.4, 6.1 Hz, H-4), 5.62 (s, 1H, Ph-C<u>H</u>), 5.37 (m, 2H, H-8, H-9), 4.41 (d, 1H, J = 6.1 Hz, H-3), 4.25 (dd, 1H, $J_{a,b}$ = 11.9, $J_{a,x}$ = 1.8 Hz, H_a-1), 4.08 (dd, 1H, $J_{a,b}$ = 11.9, $J_{b,x}$ = 0.9 Hz, H_b-1), 3.52 (m, 1H, H-2), 2.68 (br s, 1H, -O<u>H</u>), 2.0 (m, 6H, 2H-6, 2H-7, 2H-10), 1.25 (m, 14H, 7 x $-CH_2$ aliphatic protons) 0.88 (t, 3H, J = 6.5 Hz, 3H-18).

<u>Anal.</u> Calcd for $C_{25}H_{38}O_3$: C, 77.72; H, 9.84. Found: C, 77.16; H, 9.85.

(2S,3R,4E,8Z)-2-Azido-1,3-O-benzyliden-1,3-dihydroxy-4,8-octadecadiene (<u>12</u>). To a solution of compound <u>11</u> (4.0 g, 10.36 mmol) in dry dichloromethane (50 mL), was added dry pyridine (1.5 mL). The mixture was cooled to -15 °C under nitrogen and trifluoromethanesulfonic anhydride (3.39 g, 12 mmol) was added dropwise in 20 min. After stirring for 15 min at -15°C was added dry DMF (175 mL) and then sodium azide (6.0 g, 92.3 mmol). This reaction mixture was brought to room temp. and stirred for 5 h, diluted with dichloromethane (300 mL) and washed several times with water, satd aq sodium chloride and dried ($MgSO_4$). Filtering, evaporating, and chromatography over silica using petroleum ether/ethyl acetate (9:1) as eluents, resulted in the isolation of 2.16 g (51%) of the title product, having R_F 0.63 in petroleum ether/ethyl acetate (9:1), $[\alpha]_D^{20}$ -8° (c = 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.4 (m, 5H, aromatics), 5.97 (dt, 1H, J = 15.3, 6.7 Hz, H-5), 5.56 (dd, 1H, J = 15.3, 7.0 Hz, H-4), 5.47 (s, 1H, Ph-C<u>H</u>), 5.38 (m, 2H, H-8, H-9), 4.31 (dd, 1H, J = 10.7, 4.9 Hz, H-3), 4.04 (t, 1H, J = 8.8 Hz, H-1), 3.52 (m, 2H, H-1, H-2), 2.10, 1.97 (2m, 6H, 2H-6, 2H-7, 2H-10), 1.26 (m, 14H, 7 x $-CH_2$ aliphatic protons), 0.88 (t, 3H, 3H-18).

Anal. Calcd for $C_{25}H_{37}N_3O_2$: C, 72.95; H, 9.06; N, 10.21. Found: C, 72.92; H, 8.94; N, 10.0.

(2S,3R,4E,8Z)-2-Azido-1,3-dihydroxy-4,8-octadecadiene (13). To a solution of compound 12 (2.0 g, 4.86 mmol) in dry methanol (100 mL), was added 100 mg of p-toluenesulfonic acid. The reaction mixture was stirred for 48 h at room temp, neutralized with solid sodium bicarbonate, the solvent removed under vacuum, and the residue dissolved in ether (300 mL). The ethereal layer was washed with a satd soln of $NaHCO_3$, water and dried ($MgSO_4$) followed by filtration of the salts, solvent evaporation and chromatography of the residue over silica, using CH_2Cl_2/CH_3OH (95:5) as eluents resulting in the isolation of 1.2 g (76%) of the title product as an oil, having R_F = 0.38, CH_2Cl_2/CH_3OH (95:5); $[\alpha]_D^{20}$ -24.49° (c = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 5.81 (dt, 1H, J = 15.6, 6.4 Hz, H-5), 5.52 (dd, 1H, J = 15.6, 7.3 Hz, H-4), 5.39 (m, 2H, H-8, H-19), 4.25 (dd, 1H, $J_1 = J_2$ = 5.2 Hz, H-3), 3.78 (d, 2H, J = 5.2 Hz, 2H-1), 3.50 (dt, 1H, J = 5.2 Hz, H-2), 2.35 (br s, 2H, 2 x OH), 2.06 (m, 6H, 2H-6, 2H-7, 2H-10), 1.26 (m, 14H, 7 x $-CH_2$ aliphatic protons), 0.88 (t, 3H, 3H-18)

Anal. Calcd for $C_{18}H_{33}N_3O_2$: C, 66.83; H, 10.28; N, 12.99. Found: C, 66.55; H, 10.25; N, 13.00.

(2S,3R,4E,8Z)-2-Azido-3-hydroxy-1-triphenylmethyloxy-4,8-octadecadiene (14). To a solution of 13 (1.1 g, 3.4 mmol) in dry pyridine/chloroform/tetrahydrofuran (1:1:1, 45 mL), was added 1.37 g (4.9 mmol) of trityl chloride with stirring, at room temp. After 48 h of stirring at room temp, the solvents were removed under water vacuum, and the residue was dissolved in diethyl ether (200 mL). Usual work up was performed. Chromatography of the reaction product over silica using gradient elution with petroleum ether/ethyl acetate (95:5) to (80:20) afforded 1.73 g (90%) of the title product as a semi solid material, having R_F 0.38 in petroleum ether/ethyl acetate (9:1): $[\alpha]_D^{20}$ -5.1° (c = 0.23, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.45, 7.3 (m, 15H, aromatic protons), 5.64 (dt, 1H, J = 15.4, 6.6 Hz, H-5), 5.34 (m, 3H, H-4, H-8, H-9), 4.20 (m, 1H, H-3), 3.52 (dt, 1H, J = J = 5.4 Hz, H-2), 3.30 (d, 2H, J = 5.4 Hz, 2H-1), 1.99 (m, 7H, 2H-6, 2H-7, 2H-10, OH), 1.25 (m, 14H, 7 x CH_2 aliphatic protons), 0.88 (t, 3H, 3H-18).

Anal. Calcd for $C_{37}H_{47}N_3O_2$: C, 78.52; H, 8.37; N, 7.45. Found: C, 78.17; H, 8.31; N, 7.5.

(2S,3R,4E,8Z)-2-Azido-3-benzoyloxy-1-triphenylmethyloxy-4,8-octadecadiene (15). To a solution of compound 14 (1.7 g, 3.0 mmol) in dry toluene/pyridine (4:1, 20 mL), was added benzoyl chloride (1.05 g, 9.3 mmol) dropwise. The reaction mixture was stirred at room temp for 12 h. Usual work up with ether and chromatography of the resulting reaction product with petroleum ether/ethyl acetate (95:5) gave 1.81 g (90%) of the title product as an oil, having R_F 0.5 in petroleum ether/ethyl acetate (9:1), $[\alpha]_D^{20}$ -13.33° (c = 0.105, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.94, 7.56, 7.43, 7.25 (4m, 20H, aromatic protons), 5.80 (dt, 1H, J = 15.2, 7.8, 6.8 Hz, H-5), 5.63 (dd, 1H, J = 7.8, 4.9 Hz, H-3), 5.40 (m, 3H, H-4, H-8, H-9), 3.86 (dt, 1H, J = 6.8, 4.9 Hz, H-2), 3.28 (dd, 1H, J = 9.8, 6.8 Hz, H-1), 3.20 (dd, 1H, J = 9.8, 4.9 Hz, H-1), 1.96 (m, 6H, 2H-6, 2H-7, 2H-10), 1.25 (m, 14H, 7 x $-CH_2$ aliphatic protons), 0.88 (t, 3H, 3H-18).

Anal. Calcd for $C_{44}H_{51}N_3O_2$: C, 78.86; H, 7.67; N, 6.29. Found: C, 78.77; H, 7.70; N, 6.0.

(2S,3R,4E,8Z)-2-Azido-3-benzoyloxy-1-hydroxy-4,8-octadecadiene (16). To a solution of compound 15 (1.8 g, 2.69 mmol) in dry toluene/methanol (3:1) 20 mL, was added dropwise 0.4 mL of pure boron trifluoride·diethyl ether, under nitrogen, in 5 min. After stirring for 5 h, the reaction mixture was neutralized with solid sodium bicarbonate. Usual work up was performed with ether. Chromatography of the resulting reaction product with petroleum ether/ethyl acetate (9:1) as eluents gave 1.03 g (90%) of the title product as a semi solid material, having R_F 0.37 in petroleum ether/ethyl acetate (4:1), $[\alpha]_D^{20}$ -13.62° (c = 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 8.05, 7.58, 7.46 (3m, 5H, aromatic protons), 5.95 (dt, 1H, J = 14.4, 6.8 Hz, H-5), 5.61 (m, 2H, H-4, H-3), 5.38 (m, 2H, H-8, H-9), 3.78, 3.63 (2m, 3H, 2H-1, H-2), 2.06 (m, 6H, 2H-6, 2H-7, 2H-10), 1.25 (m, 14H, 7 x $-CH_2$ aliphatic protons), 0.87 (t, 3H, 3H-18).

Anal. Calcd for $C_{25}H_{37}N_3O_3$: C, 70.22; H, 8.72; N, 9.87. Found: C, 70.01; H, 8.65; N, 9.5.

(2S,3R,4E,8Z)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyloxy)-4,8-octadecadiene (18). To a solution of compound 16 (600 mg, 1.40 mmol), in hexane (5 mL) was added O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyloxy) trichloroacetimidate 17[20] (1.37 g, 2.80 mmol). Activated molecular sieves (4A, 200 mg) were added, and the mixture was stirred for 15-20 min. Then 2 mL of 0.1 M boron trifluoride·diethyl ether in dichloromethane was added dropwise. After 12 h of stirring at room temp, only one drop of pure boron trifluoride·diethyl ether was added very carefully. The reaction mixture was further stirred for 15 h, and neutralized with solid sodium bicarbonate. Usual work up with dichloromethane, and chromatography over silica with petroleum ether/ethyl acetate (4:1) gave 16 (0.15 g) and title product 0.640 g (80%, based on 16 consumed) as a semi solid material having R_F 0.22 in petroleum ether/ethyl acetate (4:1): $[\alpha]_D^{20}$ -35.6° (c = 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) 8.05, 7.57, 7.45 (3m, 5H, aromatic protons), 5.93 (dt, 1H, J = 14.4, 6.8 Hz, H-5), 5.56 (m, 2H, H-3, H-4), 5.37 (m, 2H, H-8, H-9), 5.20 (dd, 1H, J = J = 9.5 Hz, H-3'), 5.10 (dd, 1H, J = J = 9.5 Hz, H-4'), 5.03 (dd, 1H, J = 9.5, 8.0 Hz, H-2'), 4.54 (d, 1H, J = 8.0 Hz, H-1'), 4.22 (dd, 1H, J = 12.3, 4.9 Hz, H-6'), 4.12 (dd, 1H, J = 12.3, 2.2 Hz, H-6'), 3.92 (m, 2H, H-2, H-1), 3.69 (m, 1H, H-5'), 3.59 (dd, 1H, J = 9.5, 5.1 Hz, H-1), 2.02 (m, 18H, 4 x $-CH_3$, 2H-6, 2H-7, 2H-10), 1.25 (m, 14H, 7 x $-CH_2$ aliphatic protons), 0.88 (t, 3H, 3H-18).

Anal. Calcd for $C_{39}H_{55}N_3O_{12}$: C, 61.81; H, 7.31; N, 5.54. Found: C, 61.56; H, 7.29; N, 5.44.

(2S,3R,4E,8Z)-2-Azido-3-hydroxy-1-ß-D-glucopyranosyloxy-4,8-octadecadiene (19). To a solution of compound 18 (0.600 g, 0.79 mmol) in dry methanol (20 mL), was added dropwise, a solution of sodium methoxide (0.5 M) in methanol, during 15 min. The reaction mixture was stirred for 16 h at room temp. and was neutralized with ion exchange resin (Amberlite JR 120 H^+-form). Following filtration of the resin evaporation of solvent, and chromatography of the resulting residue with chloroform/methanol (85:15) gave 0.308 g (80%) of the title product, as a viscous material, having an R_F 0.26 in chloroform/methanol (85:15); $[\alpha]_D^{20}$ -25.2° (c = 4.3, CH_3OH); ^1H NMR (DMSO-d_6, 250 MHz) δ 5.65 (dt, 1H, J = 15.4, 6.6 Hz, H-5), 5.43

(dd, 1H, J = 15.6, 7.1 Hz, H-4), 5.36 (m, 2H, H-8, H-9), 5.26 (d, 1H, J = 4.9 Hz, O<u>H</u>), 5.01 (d, 1H, J = 4.9 Hz, O<u>H</u>), 4.93 (dd, 2H, J = 11.5, 4.9 Hz, 2 x O<u>H</u>), 4.50 (t, 1H, J = 5.9 Hz, CH_2O<u>H</u>), 4.12 (m, 2H, H-1', H-3) 3.67, 3.47, 3.35, 3.07, 2.95 (5m, 9H, 2H-1, H-2, H-2', H-3', H-4', H-5', 2H-6'), 1.99 (m, 6H, 2H-6, 2H-7, 2H-10), 1.24 (m, 14H, 7 x $C\underline{H}_2$ aliphatic protons), 0.86 (t, 3H, 3H-18).

(2S,3R,4E,8Z)-2-Amino-1-ß-D-glucopyranosyloxy-3-hydroxy-4,8-octadecadiene (<u>20</u>). To a solution of compound <u>19</u> (0.3 g, 0.62 mmol) in pyridine/water (1:1, 20 mL), was bubbled hydrogen sulfide gas for 15 min. The reaction mixture was stirred at room temp. for 48 h. Solvents were removed with special care (from frothing). Chromatography of the residue with chloroform/methanol (7:3), gave the title product 0.28 g in quantitative yield, as a jelly, having R_F 0.57 in $CHCl_3/CH_3OH/H_2O$ (5:4:1). This product was immediately used for the next reaction.

(R)-2-[(2R)-2-Acetoxyphenylacetoxy]hexadecanoic acid[23] (<u>21a</u>). Preparation of this compound was carried out as explained below in steps (a)-(d).

(a) **Benzyl (R,S)-2-hydroxyhexadecanoate.** A mixture of (R,S)-2-hydroxyhexadecanoic acid (5.0 g, 18.38 mmol) and benzyl alcohol saturated with HCl gas was stirred for 24 h at room temperature. HCl gas and excess benzyl alcohol were removed in vacuo. The residue was subjected to chromatography over silica (petroleum ether/ethyl acetate, 9:1) and gave 6.18 g (93%) of the title product as a crystalline solid: mp 43 °C; R_F 0.54 in petroleum ether/ethyl acetate (8:2).

(b) **Benzyl (R,S)-2-[(2R)-2-Acetoxyphenylacetoxy]hexadecanoate.** This compound was prepared from benzyl(R,S)-2-hydroxyhexadecanoate according to the literature procedure.[22]

(c) **Benzyl (R)-2-[(2R)-2-Acetoxyphenylacetoxy]hexadecanoate.** This compound was separated from its (R,S)-mixture obtained above in (b), using medium pressure liquid chromatography (toluene/acetone, 200:1). The title product has an R_F 0.29 in toluene/acetone (200:1) and $[\alpha]_D^{20}$ -24.7° (<u>c</u> = 1, $CHCl_3$).

(d) **Compound** <u>21a</u>: To a solution of the compound obtained above in step (c) (500 mg, 0.93 mmol), in ethyl acetate (10 mL)

was added a catalytic ammount of Pd/C (10%) and the mixture was shaken under hydrogen atmosphere for 1 h. After removal of the catalyst by filtration and evaporation of solvent, the title product was obtained in quantitative yield, having an R_F 0.51 in chloroform/methanol (9:1) and $[\alpha]_D^{20}$ -26.7° (c = 1.1, $CHCl_3$).

(S)-2-[(2R)-2-Acetoxyphenylacetoxy]hexadecanoic acid (21b). (a) **Benzyl (S)-2-[(2R)-2-acetoxyphenylacetoxy]hexadecanoate.** This ester was separated from the corresponding (RS)-mixture, as given above. The title product has an R_F 0.21 in toluene/acetone (200:1) and $[\alpha]_D^{20}$ -48.5° (c = 1, $CHCl_3$).

(b) **Compound** 21b: Procedure as given above; R_F 0.50 in toluene/acetone (200:1), $[\alpha]_D^{20}$ -53.5° (c = 1.5, $CHCl_3$).

(c) **Structure proof**: Small samples of compounds 21a and 21b were deacylated using K_2CO_3 in CH_2Cl_2/CH_3OH (10:1) to provide the corresponding (R)- and (S)-2-hydroxyhexadecanoic acid. Sodium salts of these two hydroxy acids were prepared and their optical rotations were found to be in accordance with their literature values.[22]

(2S,3R,4E,8Z)-2-{(2R)-2-[(2R)-2-Acetoxy-phenylacetoxy)] hexadecanoylamino}-3-hydroxy-1-ß-D-glucopyranosyloxy-4,8-octadecadiene (22a). To a solution of compound 20 (0.120 g, 0.26 mmol) and (2R)-2-[(2R)-2-acetoxyphenylacetoxy)]hexadecanoic acid (21a) (135 mg, 0.30 mmol) in THF (5 mL) was added EEDQ (75 mg, 0.30 mmol) and the mixture was stirred at room temperature for 12-15 h. After evaporation of solvent the residue was subjected to chromatography on silica using chloroform/methanol (9:1) as eluents resulting in the isolation of 0.140 g (60%) of the title product as a thin film, having an R_F 0.30 in chloroform/methanol (9:1): $[\alpha]_D^{20}$ -9.4° (c = 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$ + CD_3OD, 400 MHz) δ 7.46, 7.35 (2m, 5H, aromatic protons), 6.36 (br s, 1H, -NH), 5.93 (s, 1H, -C-CH(OAc)Ph), 5.65 (dt, 1H, J = 14.6, 6.6 Hz, H-5), 5.40 (m, 3H, H-4, H-8, H-9), 5.13 (dd, 1H, J = 7.6, 4.2 Hz, -CO-CH-CH_2-OCO-), 4.25 (d, 1H, J = 7.8 Hz, H-1'), 3.98 (m, 3H, H-5', 2H-6'), 3.87 (dd, 1H, J = 12.0, 2.9 Hz, H-3'), 3.76, 3.61, 3.45 (3m, 5H, H-4', 2H-1, H-2, H-4), 3.29 (m, 1H, H-2'), 2.25 (s, 3H, -$COCH_3$), 2.00 (m, 6H, 2H-6, 2H-7, 2H-10), 1.26 (m, 40H, 20 x -CH_2 aliphatic protons), 0.88 (t, 6H, 2 x -CH_3).

(2S,3R,4E,8Z)-2-{(2S)-2-[(2R)-2-Acetoxyphenylacetoxy]hexadecanoylamino}-3-hydroxy-1-ß-D-glucopyranosyloxy-4,8-octadecadiene (22b). The above described procedure for 22a was applied, and (2S)-2-[(2R)-2-acetoxyphenylacetoxy]hexadecanoic acid (21b) was used for the N-acylation reaction: yield 66%, R_F 0.44 in chloroform/methanol (9:1); $[\alpha]^{20}$ -48.92° (c = 0.78, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.50, 7.39 (2m, 5H, aromatic protons), 6.89 (d, 1H, J = 8.3 Hz, -NH), 5.83 (s, 1H, -CO-CH(OAc)Ph), 5.72 (dt, 1H, J = 15.6, 6.8 Hz, H-5), 5.44 (dd, 1H, J = 15.6, 6.4 Hz, H-4), 5.38 (m, 2H, H-8, H-9), 5.07 (dd, 1H, J = 9.0, 3.6 Hz, -CO-CH(CH_2)-OCO-), 4.31 (d, 1H, J = 7.8 Hz, H-1'), 4.23, 4.13 (2m, 3H, H-5', 2H-6'), 3.90 (dd, 1H, J = 12.0, 3.0 Hz, H-3'), 3.82 (dd, 1H, J = 12.0, 4.6 Hz, H-4'), 3.73, 3.57, 3.38, 3.32 (4m, 5H, H-2', 2H-1, H-2, H-4), 2.24 (s, 3H, -$COCH_3$), 2.00 (m, 6H, 2H-6, 2H-7, 2H-10), 1.26 (m, 40H, 20 x -CH_2 aliphatic protons), 0.88 (t, 6H, 2 x -CH_3).

(2S,3R,4E,8Z)-3-Hydroxy-2-[(2R)-2-hydroxyhexadecanoylamino]-1-ß-D-glucopyranosyloxy-4,8-octadecadiene (1a). To a solution of compound 22a (100 mg, 0.11 mmol) in dry dichloromethane/methanol (10:1, 10 mL) was added 20-30 mg of anhydrous K_2CO_3. The reaction mixture was stirred at room temperature for 4 h, neutralized with ion exchange resin (Amberlite 120, H^+-form), and filtered. Removal of the solvent and subsequent chromatography on silica using chloroform/methanol (95:5) as eluents afforded 72 mg (90%) of the title product 18a as a solid, mp 183°C, having an R_F 0.15 in chloroform/methanol (9:1): $[\alpha]_D^{20}$ +4° (c = 0.2, CH_3OH); lit.[12]: mp 183 °C; $[\alpha]_D$ +4.6°; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.36 (d, 1H, J = 9.3 Hz, -NH), 5.56 (dt, 1H, J = 15.0, 6.6 Hz, H-5), 5.49 (d, 1H, J = 4.9, -OH), 5.34 (m, 3H, H-4, H-8, H-9), 4.91 (m, 4H, 4 x -OH), 4.49 (t, 1H, J = 5.9 Hz, -CH_2OH), 4.10 (d, 1H, J = 7.8 Hz, H-1'), 3.98, 3.79 (2m, 3H, H-2, H-3, H-2"), 3.9 (dd, 1H, J = 10.2, 5.6 Hz, H-1), 3.65 (dd, 1H, J = 9.8, 5.9 Hz, H-6'), 3.50 (dd, 1H, J = 10.2, 5.9 Hz, H-1), 3.40 (dt, 1H, J = 11.7, 5.9 Hz, H-6'), 3.06 (m, 3H, H-3', H-4', H-5'), 2.95 (m, 1H, H-2), 1.95 (m, 6H, 2H-6, 2H-7, 2H-10), 1.22 (m, 40H, 20 x -CH_2 aliphatic protons), 0.84 (t, 6H, J = 6.6 Hz, 2 x -CH_3).

Anal. Calcd for $C_{40}H_{75}NO_9 . 3.25\ H_2O$: C, 62.18; H, 10.56; N, 1.81. Found: C, 61.96; H, 10.05; N, 1.98.

(2S,3R,4E,8Z)-3-Hydroxy-2-[(2S)-2-hydroxyhexadecanoylamino]-1-β-D-glucopyranosyloxy-4,8-octadecadiene (1b).
Procedure as above from compound 1a: yield = 91.4%; mp 150-151 °C; R_F 0.22 in chloroform/methanol (9:1); $[\alpha]_D^{20}$ -15.5° (c = 0.97, CH_3OH), lit.[12]; mp 151 °C $[\alpha]_D$ -14.7°; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.40 (d, 1H, J = 8.8 Hz, -NH), 5.59 (dt, 1H, J = 15.6, 7.3 Hz, H-5), 5.36 (m, 4H, H-4, H-8, H-9, -OH), 4.95 (m, 4H, 4 x -OH), 4.50 (t, 1H, J = 5.6 Hz, $-CH_2OH$), 4.10 (d, 1H, J = 8.0 Hz, H-1'), 4.05 (m, 1H, H-3), 3.88 (dd, 1H, J = 10.2, 5,6 Hz, H-1), 3.80 (m, 2H, H-2, H-2"), 3.66 (dd, 1H, J = 11.2, 5.9 Hz, H-6'), 3.44 (m, 2H, H-1, H-6'), 3.04-3.13 (m, 3H, H-3', H-4', H-5'), 2.95 (m, 1H, H-2'), 1.94 (m, 6H, 2H-6, 2H-7, 2H-10), 1.23 (m, 40H, 20 x $-CH_2$ aliphatic protons), 0.85 (t, 6H, 2 x $-CH_3$).

Anal. Calcd for $C_{40}H_{75}NO_9 \cdot 1.25\ H_2O$: C, 65.22; H, 10.47; N, 1.89. Found: C, 65.16; H, 10.48; N, 1.98.

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