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Sphingosine and Phytosphingosine from D-Threose Synthesis of a 4-Keto-Ceramide

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Abstract: Reaction of 2,4-O-benzylidene-D-threose **3** with tetradecyl magnesium bromide furnished D-*arabino*- and L-*xylo*-octadecane-1,2,3,4-tetrols **5a,x**. Regioselective oxidation of the 4-OH group gave 4-keto-D-*erythro*-derivative **6** which can be reduced with acetaldehyde in a SmI_2-catalyzed Tishtshenko reaction to afford exclusively **5a**. Regioselective 2-O-mesylation of **5a** (→ **7a**) and then acid catalyzed debenzylation afforded exclusively 2-O-mesyl-tetrol **9a**. Reaction with NaN_3 and ensuing azide reduction furnished D-*ribo*-C_{18}-phytosphingosine (**2**) in high overall yield. Treatment of 2-O-mesyl derivatives **7a,x** with NaN_3 and then with 4-nitrobenzenesulfonyl chloride in pyridine afforded **11r,l**. Elimination with DBU or, alternatively, by treatment with phenylselenide and then with H_2O_2, gave known 1,3-O-benzylidene protected azidosphingosine **14**, which can be readily converted into sphingosine (**1**). Transformation of **2** into ceramide **15**, selective 1,3-O-silyl protection, oxidation of the 4-OH group (→ **17**) and then desilylation afforded the 4-keto ceramide **18** found in a marine sponge. Reduction of **17** offers a convenient possibility for radioactive labelling of ceramides with tritium.

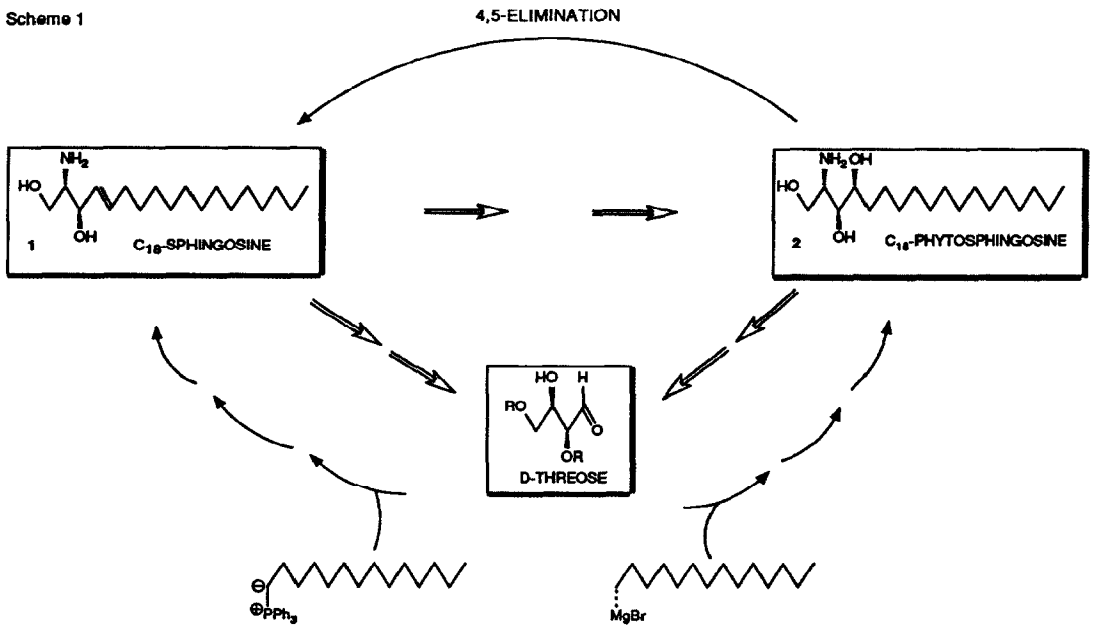
Introduction

Sphingosine (Scheme 1, **1**) and related long chain bases, as for instance phytosphingosine (**2**), are building blocks of sphingolipids (glycosphingolipids, sphingomyelins, etc.) which are important membrane constituents.[1] The sphingosine base most frequently found in tissue is C_{18}-sphingosine (**1**). Large amounts of the corresponding C_{20}-homologue were detected in the gangliosides of the brain, while especially in the skin[2] C_{18}-phytosphingosine (**2**) is encountered in addition to sphingosine. Interest in these intermediates of the sphingolipid metabolism increased not only in synthetic approaches to glycosphingolipids, which mediate cell recognition events,[3] but also when sphingosine itself was found to inhibit strongly and specifically protein kinase C, a pivotal regulatory enzyme in cell growth.[4] In contrast, other results demonstrated that sphingosine, at low concentrations, stimulates DNA synthesis and cell proliferation of quiescent cultures of Swiss 3T3 fibroblasts in a protein kinase C independent way.[5] Thus, a dual role can be ascribed to these membrane constituents.[6]

Several syntheses for sphingosine and phytosphingosine have been reported.[7-10] We have developed via an *erythro*-specific aldol reaction of N,O-persilylated glycine with α,β-unsaturated aldehydes a two-step synthesis of racemic sphingosines.[8] This efficient route also provided ceramides very readily.[11] The required racemate resolution and the 3-O-protection for glycosphingolipid synthesis could be easily combined.[11] However, a stereocontrolled synthesis from the chiral pool seemed to be desirable. Therefore, we developed the "azidosphingosine glycosylation procedure"[12] which is based on azidosphingosine synthesis.[9] This method starts from 2,4-di-O-protected D-threose (see retro synthetic Scheme 1) which gives in a *trans*-selective Wittig-

reaction directly the required D-*threo*-4-octadecene-1,2,3-triol and then via azidosphingosine target molecule **1**. Additionally, 2,4-di-O-protected D-threose can be transformed via Grignard-reaction into a octadecane-1,2,3,4-tetrol required for the synthesis of phytosphingosine[10] (**2**). However, this reaction was found not to be D-*arabino*-specific in order to exclusively provide, via S_N2 nitrogen introduction at C-2, the required D-*ribo*-configuration of **2**. We report now a method for the stereoselective transformation of the Grignard addition products into the D-*arabino*-stereoisomer. Thus, also the synthesis and detailed structural assignment of a phytosphingosine based 4-keto-ceramide found in the marine sponge *Ircina variabilis*[13] was accomplished. The combination Grignard addition and *trans*-selective 4,5-elimination (Scheme 1) offered also a convenient route to sphingosine (**1**).

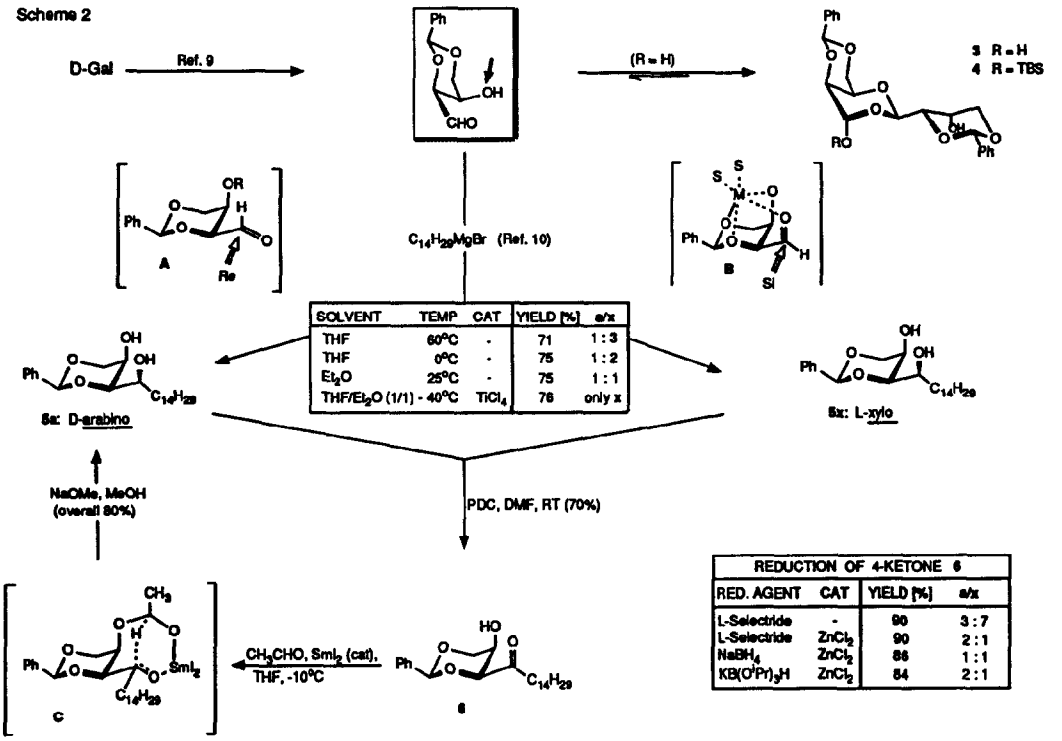
Scheme 1



Synthesis of Phytosphingosine

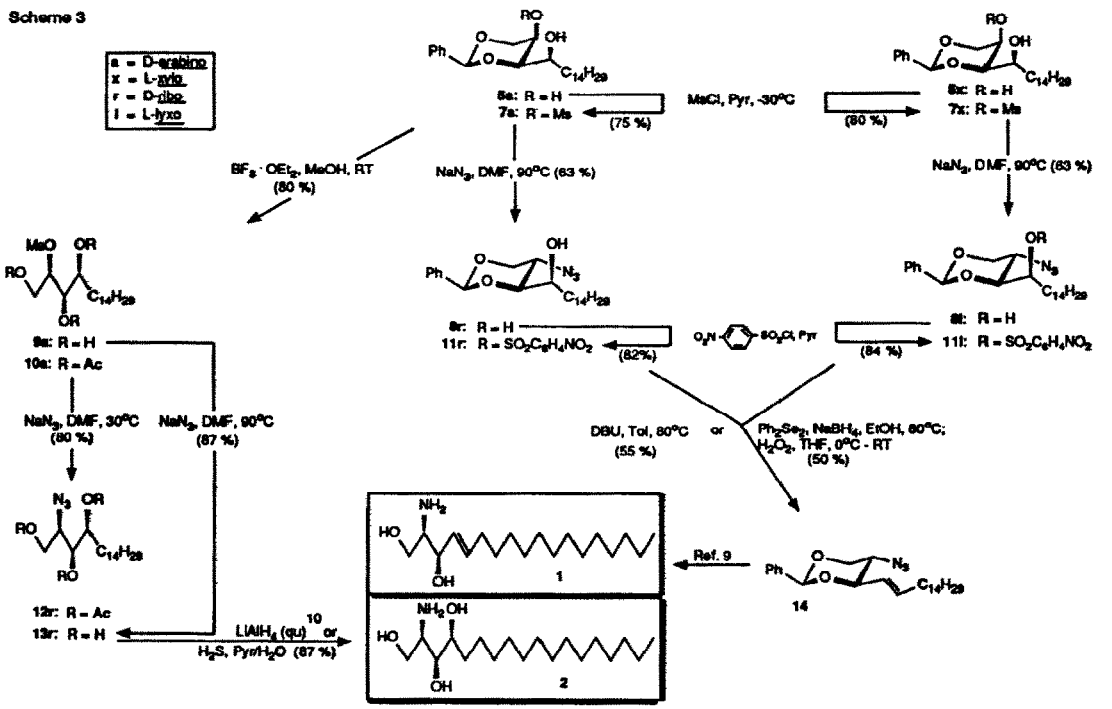
As 2,4-di-O-protected D-threose, 2,4-O-benzylidene-D-threose (Scheme 2, **3**) was chosen, which is readily available in two steps from D-galactose.[9,14] **3** was found to exist essentially in dimeric form,[14,15] therefore, diastereocontrol of the Grignard reaction with tetradecyl magnesium bromide turned out to be very difficult.[10,16] Variations of the solvent, the temperature, and the catalyst led finally exclusively to the undesired D-*xylo*-derivative **5x**, due to *Si*-face addition in **B**;[10] however, the ratio in favor of the desired D-*arabino*-isomer **5a** never exceeded 1:1 (some results of our previous investigations are compiled in Scheme 1).[10] Therefore, it was hoped that with the help of bulky silyl protection at the 3-hydroxy group the monomer of **3** could be received, which should favor *Re*-face addition via **A**. However, treatment of **3** with *tert*-butyldimethylsilyl chloride (R = TBS in **A**) in the presence of imidazole afforded only the monosilylated derivative **4** of the dimer. Therefore, regioselective oxidation of the 4-hydroxy group in **5a,x** and ensuing D-*arabino*-selective reduction of the 4-keto compound was investigated. Surprisingly, the first goal could be

readily reached with the help of pyridinium dichlorochromate (PDC) in DMF affording from both diastereomers pure 4-keto derivative **6** in 70% yield. However, D-*arabino*-selective reduction of **6** with various reducing agents (see some selected results in Scheme 2)[16] led, if at all, only to modest diastereoselection. Therefore, the SmI_2-assisted Tishtshenko reaction[17] with the help of acetaldehyde as reducing agent was applied to this problem and, indead, desired **5a** was obtained exclusively via the 2-O-acetyl derivative as intermediate, which gave with NaOMe/MeOH the target molecule. Complex C seems to be the decisive transition state leading to Si-selective hydride transfer from acetaldehyde.



The transformation of **5a** into **2**, described already previously,[10] could be further improved (Scheme 3). Thus, regioselective 2-O-mesylation of **5a** provided **7a** (and from **5x** also **7x**) in high yields, indicating higher reactivity for the 2-hydroxy group; this phenomenon is presumably based on increased acidity due to hydrogen bond formation and/or enhanced nucleophilicity due to lone pair orbital repulsion with the 1-O- and 3-O-atoms. Acid catalyzed debenzylidenation afforded **9a** which furnished upon treatment with acetic anhydride/pyridine the tri-O-acetyl derivative **10a**. Reaction with NaN_3 in DMF at 90 °C afforded O-acetyl-protected azidophytosphingosine **12r** in 80% yield; treatment with NaOMe/MeOH furnished known, O-unprotected azidophytosphingosine **13r**.[10] As found already earlier by us,[18] the same compound can be also directly obtained from **9r** by treatment with NaN_3 in DMF in 87% yield;[19] obviously, the unprotected hydroxy groups do not interfer with azide introduction. Reduction of **13r** with LiAlH[10] or with H_2S in pyridine/water furnishes

2 in essentially quantitative yield. Thus, **2** can be readily obtained from D-galactose via **3** in eight highyielding, convenient steps.



Synthesis of Sphingosine

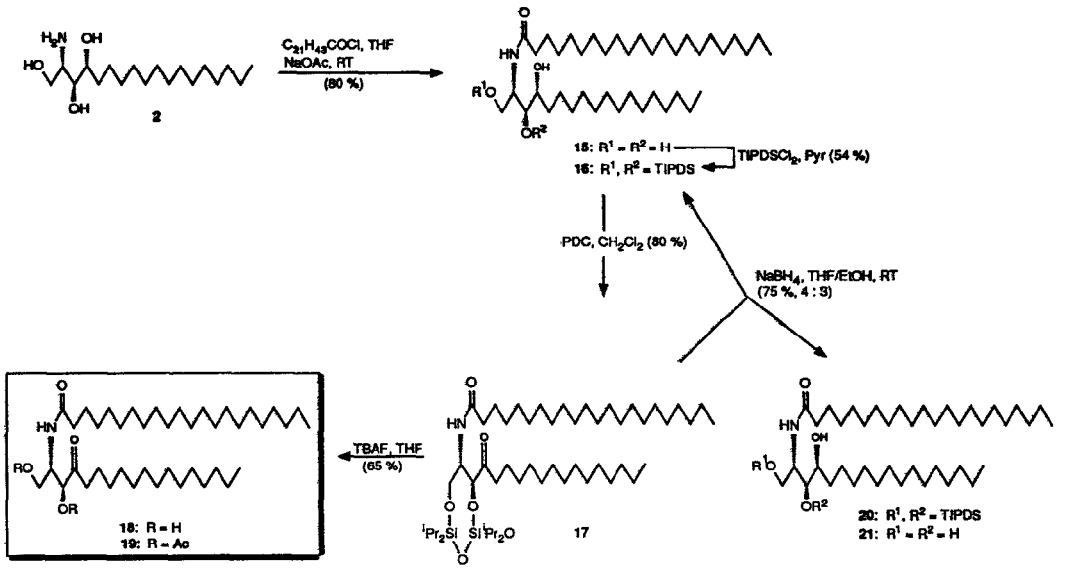
Compounds **7a,x** can be also employed for immediate azide introduction (Scheme 3); thus, with NaN_3 in DMF at 90 °C the D-*ribo*- and the L-*lyxo*-derivative **8r** and **8l**, respectively, were obtained in good yields. For the generation of a 4,5-*trans* CC-double bond various leaving groups were attached to the 4-hydroxy group. The best results were gained with the p-nitrobenzenesulfonate moiety. The required compounds **11r** and **11l** were readily obtained from **8r,l** by treatment with p-nitrobenzenesulfonyl chloride in pyridine. Elimination with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene at 80 °C afforded, independent of the starting material, practically exclusively known *trans*-elimination product **14**. The *cis*-elimination product and the 3,4-elimination product were under these conditions only very minor byproducts.[16] Reaction of **11r** or **11l** with phenylselenide, which was generated in situ from diphenyl diselenide and sodium borohydride, and subsequent oxidation with hydrogen peroxide at 0 °C and then raising the temperature to 20 °C gave only *trans*-product **14** in good yield; **14** can be conveniently transformed in two steps into **1**[9]. Thus, Grignard addition to D-threose **3** and then elimination based synthesis of sphingosine is a straightforward alternative to the Wittig reaction based approach described earlier.[9]

Synthesis of a 4-Keto-ceramide

A large number of sphingosine, ceramide, and glycosphingolipid analogues has been recently found, especially in marine organisms.[21] Cafieri und Fattorusso[13] isolated from the marine sponge *Ircina variabilis* a 4-keto-ceramide derivative to which based on MS and NMR data structure **18** (Scheme 4) was assigned. However, the configurations could be only tentatively deduced from the NMR data. Therefore, **2** was transformed by us with behenoyl chloride in the presence of NaOAc, following Shapiro's method,[22] into ceramide **15**, which is also a constituent of skin ceramides.[2] Regioselective protection of the 1- and 3-hydroxy groups could be accomplished with 1,3-dichloro 1,1,3,3-tetra-isopropyl-disiloxane ($TIPDSCl_2$) in the presence of pyridine furnishing **16** in good yield. Oxidation with PDC in dichloromethane afforded 1,3-O-protected 4-keto derivative **17**. Treatment with tetra-n-butylammonium fluoride (TBAF) in THF gave the desired D-*erythro*-4-keto ceramide **18**, which was transformed with acetic anhydride in pyridine into the 1,3-di-O-acetyl derivative **19**. Comparison of the optical rotation and the NMR data with the isolated material[13] confirmed the identity.

Compound **17** (and **18**) exhibited also the potential to introduce, for instance via reduction, a label at the 4-position.[16,23] To this aim, sodium borohydride reduction was investigated providing in THF/ethanol a 4:3 ratio of **16** and **20**, which could be separated; desilylation with TBAF afforded the D-*ribo*- and D-*lyxo*-ceramides **15** and **21**, respectively. Thus, it is also confirmed, that in **17** the configuration at C-3, next to the carbonyl group, is not changed during the oxidation process.

Scheme 4



Experimental

Solvents were purified and dried in the usual way; boiling range of the petroleum ether used: 35-65 °C. ^{1}H NMR spectra: Bruker AC 250, internal standard tetramethylsilane (TMS). Flash chromatography: Silica gel 60 (Baker, 30-60 μm) at a pressure of 0.3 bar. Thin-layer chromatography (TLC): Silica gel 60 F_{254} (Merck; plastic plates, layer thickness 0.2 mm), detection by treatment with a solution of 20 g of ammonium molybdate and 0.4 g of cerium(IV) sulfate in 400 mL of 10% sulfuric acid and heating at 120 °C. Optical rotations: Perkin-Elmer polarimeter 241/MS, 1-dm cell.

Compound **4**: To a solution of **3**[9] (750 mg, 36 mmol) in dry dichloromethane (50 mL) maintained at 0 °C was added imidazole (490 mg, 7.2 mmol) and TBDMSCl (624 mg, 4.14 mmol); thereafter the reaction mixture was brought to room temperature and stirred at this temperature for 24 h. Subsequently it was washed with water, extracted with dichloromethane, the organic phase was dried over anhydrous $MgSO_4$ and concentrated. Purification of the residue by column chromatography over silica gel with petroleum ether/ethyl acetate (6:4) furnished **4** (590 mg, 62%) as a white foam. TLC (silica gel; petroleum ether/ethyl acetate, 6:4), R_f = 0.2, m.p. 75-78 °C, $[\alpha]^{23}$ = +25 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.15, 0.16 (2 s, 6 H, 2 CH_3), 0.92 (s, 9 H, -C(CH_3)$_3$), 2.99 (d, $J_{3',3'\text{-OH}}$ = 9.3 Hz, 1 H, 3'-OH), 3.62-3.64 (m, 1 H, 3-H), 3.80-3.85 (m, 1 H, 2-H), 3.93-3.97 (m, 1 H, 3'-H), 4.01 (dd, $J_{3',4'a}$ = 0.9 Hz, $J_{4'a,4'b}$ = 12.0 Hz, 1 H, 4'-H_a), 4.04 (dd, $J_{3,4a}$ = 1.8 Hz, $J_{4a,4b}$ = 13.0 Hz, 1 H, 4-H_a), 4.13 (dd, $J_{2',3'}$ = 0.9 Hz, $J_{1',2'}$ = 6.6 Hz, 1 H, 2'-H), 4.21 (dd, $J_{3,4'b}$ = 1.7 Hz, $J_{4'a,4'b}$ = 12.0 Hz, 1 H, 4'-H_b), 4.35 (dd, $J_{3,4b}$ = 0.9 Hz, $J_{4a,4b}$ = 13.0 Hz, 1 H, 4-H_b), 4.98 (d, $J_{1',2'}$ = 6.6 Hz, 1 H, 1'-H), 5.04 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1-H), 5.57, 5.58 (2 s, 2 H, 2 C*H*Ph), 7.32-7.58 (2 m, 10 H, 2 Ph).

Analysis calcd. for $C_{28}H_{38}O_8Si$: C, 63.37; H, 7.21. Found: C, 63.01; H, 7.17.

(2R,3R,4R)-1,3-O-Benzylidene-1,2,3,4-octadecanetetrol (**5a**) and *(2R,3R,4S)-1,3-O-Benzylidene-1,2,3,4-octadecanetetrol* (**5x**): a) From compound **3**: The Grignard reagent $C_{14}H_{29}MgBr$ was prepared by the usual method from magnesium (10.8 g, 444 mmol) and n-tetradecyl bromide (123.6 g, 444 mmol) in dry THF (180 mL). Subsequently the reaction mixture was warmed to 60 °C and a solution of **3**[9] (30 g, 144 mmol) in dry THF (120 mL) was slowly dropped into it; as followed by TLC the reaction was complete in 4 h at 60 °C. The reaction mixture was thereafter cooled and washed with ice-cold saturated NH_4Cl solution for hydrolysis; on addition of brine and an additional amount of THF two phases were obtained. The aqueous phase was extracted once again with THF (3 x 150 mL), the combined organic phases were dried over anhydrous $MgSO_4$ and concentrated in vacuo; for complete removal of solvent the yellowish crystalline residue was dried under high vacuum. The diastereomers **5a** and **5x** were separated by chromatography over silica gel with petroleum ether/ethyl acetate (7:3); but when separation of the diastereomers was not necessary, the residue was purified by crystallization from n-hexane (800 mL). Yield **5a** (10.5 g, 18%); white solid; TLC (silica gel; petroleum ether/ethyl acetate, 1:1), R_f = 0.32; m.p. 117 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.2 Hz, 3 H, CH_3), 1.10-1.45 (m, 24 H, 12 CH_2), 1.60-1.70 (m, 2 H, 5-H_a, H_b), 2.33 (d, $J_{2,2\text{-OH}}$ = 4.5 Hz, 1 H, 2-OH), 3.26 (d, $J_{4,4\text{-OH}}$ = 9.0 Hz, 1 H, 4-OH), 3.67 (dd, $J_{2,3}$ = 1.3 Hz, $J_{3,4}$ = 6.7 Hz, 1 H, 3-H), 3.85-3.95 (m, 2 H, 2-H, 4-H), 4.02 (dd, $J_{1a,2}$ = 1.3 Hz, $J_{1a,1b}$ = 12.2 Hz, 1 H, 1-H_a), 4.25 (dd, $J_{1b,2}$ = 1.8 Hz, $J_{1a,1b}$ = 12.2 Hz, 1 H, 1-H_b), 5.57 (s, 1 H, C*H*Ph), 7.36-7.53 (2 m, 5 H, Ph). Yield **5x** (30.5 g, 52%); white solid; TLC (silica gel, petroleum ether/ethyl acetate, 1:1), R_f = 0.79; m.p. 71 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.2 Hz, 3 H, CH_3,

1.12-1.42 (m, 24 H, 12 CH_2), 1.45-1.52 (m, 2 H, 5-H_b), 2.54 (d, $J_{2,2\text{-OH}}$ = 3.3 Hz, 1 H, 2-OH), 3.05 (d, $J_{4,4\text{-OH}}$ = 9.5 Hz, 1 H, 4-OH), 3.68-3.71 (m, 2 H, 2-H, 3-H), 3.88-3.97 (m, 1 H, 4-H), 4.04 (dd, $J_{1a,2}$ = 1.2 Hz, $J_{1a,1b}$ = 12.0 Hz, 1 H, 1-H_a), 4.23 (dd, $J_{1b,2}$ = 1.9 Hz, $J_{1a,1b}$ = 12.0 Hz, 1 H, 1-H_b), 5.60 (s, 1 H, C*H*Ph), 7.38-7.53 (2 m, 5 H, Ph).

b) From compound **6** by reduction with L-selectride: To a solution of **6** (30 g, 74.15 mmol) in dry THF (600 mL), maintained at room temperature and under a nitrogen atmosphere was added dry $ZnCl_2$ (20.74 g, 150 mmol). After stirring the mixture for 15 min a solution of L-selectride (150 mL, 1 M in THF) was slowly dropped into it. The reaction was complete in 4 h, subsequently it was hydrolysed under ice-cooling with 10% NaOH solution and 30% H_2O_2 and extracted with THF (3 x 400 mL). The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and concentrated. The crude product (mixture of **5a** and **5x**) on crystallization, twice from ethyl acetate afforded the pure diastereomer **5a**; the concentrated mother liquor furnished the enriched diastereomer **5x**. Yields **5a**: (18.1 g, 60%, white solid); **5x**: (9.0g, 30%, white solid).

c) From compound **6** by reduction with KB [$OCH(CH_3)_2)_3H$]: To a solution of **6** (500 mg, 1.23 mmol) in dry THF (10 mL) maintained at room temperature and under an nitrogen atmosphere was added dry $ZnCl_2$ (0.345 g, 2.5 mmol). After stirring the above mixture for 15 min a solution of KB [$OCH(CH_3)_2)_3H$ (2.5 mL, 1 M in THF) was slowly dropped into it. The reaction was complete in 4 h, subsequently it was hydrolysed under ice-cooling with 10% NaOH solution and 30% H_2O_2. The remaining work-up was the same as mentioned above. Yields **5a**: (280 mg, 56%); **5x**: (140 mg, 28%).

d) Compound **5a** from **6** via an intramolecular Tishtshenko-reduction: Preparation of the samarium iodide solution (0.04 M):[24] Samarium metal powder (3 g, 0.02 mol) was placed under a nitrogen atmosphere in an 500 mL capacity 2-necked flask fitted with a dropping funnel; into this was slowly dropped a solution of 1,2-diiodoethane (2.82 g, 0.01 mol) in dry THF (250 mL) (the excess samarium powder can be reused). The above THF-solution was stirred for 2 h at room temperature, thus an intensive blue-green SmI_2 solution (0.04 M in THF) was obtained. The SmI_2 solution could be stored for a longer time under inert conditions when small amounts of metallic samarium was present. For reproducible results, the SmI_2 solution once prepared must be used within a few days.

Freshly distilled acetaldehyde (3.45 mL, 61.8 mmol) was added to a solution of **6** (5 g, 12.36 mmol) in dry THF (75 mL) maintained under a nitrogen atmosphere. The reaction flask was wrapped up in an aluminium foil in order to protect it from light and was cooled to -10 °C. The SmI_2 solution (0.04 M in THF, 31 mL, ~ 1.24 mmol) was slowly dropped into it and the reaction mixture was stirred for 2 h at -10 °C. Subsequently it was worked up, water (~ 30 mL) was added and warmed to room temperature; for better phase separation additional THF and brine were added. The organic phase was washed successively with saturated $NaHCO_3$ solution (30 mL), $Na_2S_2O_3$ solution (30 mL) and brine, dried ($MgSO_4$), filtered, concentrated and subjected to high vacuum for complete removal of residual solvents. The glassy solid obtained was taken up in dry CH_2Cl_2 (100 mL) and NaOMe solution (5 mL from 0.023 g sodium and 10 mL dry MeOH) was added and stirred. After 12 h it was neutralised with ion exchange resin (Amberlite IR 120, H^+). The solution was filtered, concentrated in vacuo and the white residue was crystalised from ethyl acetate (~ 100 mL) to furnish **5a** (4.02 g, 80%) as a white solid.

Analysis calcd. for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found C, 78.81; H, 10.47.

(2R,3S)-1,3-O-Benzylidene-1,2,3-octadecanetriol-4-one (**6**): A (1:3) mixture of compounds **5a** and **5x** (74 g, 182 mmol) was dissolved in dry DMF (375 mL) and maintained at 0 °C; to this was added PDC (138.8 g, 369 mmol) in small portions. Subsequently the reaction mixture was warmed to room temperature and stirred for 3 d. As monitored by TLC when the reaction was complete it was poured into water (~ 2 L) and extracted with diethyl ether (5 x 1 L). The combined organic phases were washed successively with saturated $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and concentrated. Crystallization from ethanol yielded **6** (52 g, 70%) as a white solid; TLC (silica gel; toluene/acetone, 7:1), R_f = 0.46, m.p. 111 °C, $[\alpha]^{20}$ = -55 (c = 2, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.3 Hz, 3 H, CH_3), 1.15-1.40 (m, 18 H, 9 CH_2), 1.55-1.65 (m, 2 H, 6-H_a, H_b), 2.58-2.76 (m, 2 H, 5-H_a, H_b), 2.74 (d, $J_{2,2\text{-OH}}$ = 10.7 Hz, 1 H, 2-OH), 3.99-4.04 (m, 1 H, 2-H), 4.09 (dd, $J_{1a,2}$ = 1.3 Hz, $J_{1a,1b}$ = 12.0 Hz, 1 H, 1-H_a), 4.23 (dd, $J_{1b,2}$ = 1.8 Hz, $J_{1a,1b}$ = 12.0 Hz, 1 H, 1-H_b), 4.32-4.34 (m, 1 H, 3-H), 5.60 (s, 1 H, C*H*Ph), 7.36-7.56 (2 m, 5 H, Ph).

Analysis calcd. for $C_{25}H_{40}O_4$: C, 74.22; H, 9.96. Found C, 74.55; H, 9.55

Compounds **7a, 7x, 8r** and **8l** were synthesized according to the procedure by Schmidt and Maier.[10]

(2R,3R,4R)-2-O-Methanesulfonyl-1,2,3,4-octadecanetetrol (**9a**): **7a** (10 g, 20.6 mmol) was suspended in dry methanol (250 mL), to this was added $BF_3 \cdot Et_2O$ (2 mL) and the reaction mixture was stirred for 3-5 d at room temperature. Subsequently the reaction was worked up, at first saturated $NaHCO_3$ solution (25 mL) was added and the quantity of solvent was reduced to one-third, then another 250 mL of saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with ethyl acetate. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. Purification by crystallization from methanol (80-120 mL) gave **9a** (6.54 g, 80%, white solid). TLC (silica gel; dichloromethane/methanol, 9:1), R_f = 0.52, m.p. 126 °C, $[\alpha]^{20}$ = +10 (c = 1, $CHCl_3$/MeOH, 1:1). ^1H NMR (250 MHz, DMSO-d_6): δ = 0.84 (t, J = 6.3 Hz, 3 H, CH_3), 1.15-1.70 (m, 26 H, 13 CH_2), 3.15 (s, 3 H, Ms), 3.34-3.37 (m, 2 H, 3-H, 4-H), 3.59-3.65 (m, 2 H, 1-H_a, H_b), 4.45 (d, $J_{4,4\text{-OH}}$ = 6.2 Hz, 1 H, 4-OH), 4.70-4.74 (m, 1 H, 2-H), 4.89 (d, $J_{3,3\text{-OH}}$ = 6.8 Hz, 1 H, 3-OH), 4.99 (t, $J_{1,1\text{-OH}}$ = 5.7 Hz, 1 H, 1-OH).

Analysis calcd. for $C_{19}H_{10}O_6S$: C, 57.54; H, 10.17. Found C, 57.51; H, 10.25.

(2R,3R,4R)-1,3,4-Tri-O-acetyl-2-O-methanesulfonyl-1,3,4-octadecanetriol (**10a**): A solution of **9a** (5.68 g, 14.07 mmol) in dry pyridine (30 mL) and acetic anhydride (10 mL) was stirred for 12 h at room temperature, subsequently the solvent was removed under vacuo and the solid residue was crystallized from a small amount of petroleum ether to give **9a** (7.11 g, 97%, white solid); TLC (silica gel; toluene/acetone, 7:1), R_f = 0.42, m.p. 73-74 °C, $[\alpha]^{20}$ = +25 (c = 1, $CHCl_3$): δ = 0.86 (t, J = 6.4 Hz, 3 H, CH_3), 1.15-1.35 (m, 24 H, 12 CH_2), 1.50-1.60 (m, 2 H, 5-H_a, H_b), 2.06, 2.09, 2.11 (3 s, 9 H, 3 CH_3CO), 3.07 (s, 3 H, Ms), 4.14 (dd, $J_{1a,2}$ = 7.3 Hz, $J_{1a,1b}$ = 12.3 Hz, 1 H, 1-H_a), 4.32 (dd, $J_{1b,2}$ = 4.0 Hz, $J_{1a,1b}$ = 12.3 Hz, 1 H, 1-H_b), 4.95-5.05 (m, 2 H, 2-H, 4-H), 5.24 (dd, J = 4.2 Hz, J = 6.6 Hz, 1-H, 3-H).

Analysis calcd. for $C_{25}H_{46}O_9S$: C, 57.45; H, 8.87. Found C, 57.48; H, 8.85.

(2S,3S,4R)-2-Azido-1,3-O-benzylidene-4-(4-nitrobenzenesulfonyloxy)-1,3-octadecanediol (**11r**): A premixed solution of **8r** (250 mg, 0.58 mmol), dry toluene (5 mL), dry pyridine (0.2 mL) and p-nitrobenzene sulfonyl chloride (167 mg, 0.75 mmol) was stirred for 24 h at 60 °C. Subsequently the solvent was removed in vacuo and the residue was chromatographed over silica gel with petroleum ether/ethyl acetate (10:1) to give

11r (294 mg, 82%, yellow oil); TLC (silica gel, petroleum ether/ethyl acetate, 9:1), R_f = 0.42, $[\alpha]^{20}$ = +31.8 (c = 2, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.3 Hz, 3 H, CH_3), 1.26-1.54 (m, 24 H, 12 CH_2), 1.74-2.08 (m, 2 H, 5-H_a, H_b), 3.52-3.60 (m, 1 H, 2-H), 3.64 (dd, $J_{1a,2} = J_{1a,1b}$ = 10.0 Hz, 1 H, 1-H_a), 3.79 (dd, $J_{3,4}$ = 1.5 Hz, $J_{2,3}$ = 9.5 Hz, 1 H, 3-H), 4.37 (dd, $J_{1b,2}$ = 4.3 Hz, $J_{1a,1b}$ = 10.0 Hz, 1 H, 1-H_b), 4.92 (ddd, $J_{3,4}$ = 1.5 Hz, $J_{4,5a}$ = 4.9 Hz, $J_{4,5b}$ = 4.9 Hz, 1 H, 4-H), 5.32 (s, 1 H, C*H*Ph), 7.23-7.40 (2 m, 5 H, Ph), 8.00-8.12 (2 m, 4 H, pNO_2Ph).

Analysis calcd. for $C_{31}H_{44}N_4O_7S$: C, 60.37; H, 7.19; N, 9.08. Found C, 60.55; H, 7.30; N, 8.62.

(2S,3S,4S)-2-Azido-1,3-O-benzylidene-4-(4-nitrobenzenesulfonyloxy)-1,3-octadecanediol (**11l**): **11l** was obtained from **8l** (300 mg, 0.695 mmol) and p-nitrobenzene sulfonylchloride (200 mg, 0.9 mmol) by the same procedure as was mentioned for **11r**. Yield (360 mg, 84%, colourless oil), TLC (silica gel; petroleum ether/ethyl acetate, 9:1), R_f = 0.36, $[\alpha]^{20}$ = +2.1 (c = 0.76, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.3 Hz, 3 H, CH_3), 1.15-1.35 (m, 24 H, 12 CH_2), 1.70-1.95 (m, 2 H, 5-H_a, H_b), 3.58-3.76 (m, 3 H, 1-H_a, H_b, 3-H), 4.41-4.46 (m, 1 H, 2-H), 5.09 (ddd, $J_{3,4}$ = 2.0 Hz, $J_{4,5a} = J_{4,5b}$ = 7.1 Hz, 1 H, 4-H), 5.41 (s, 1 H, C*H*Ph), 7.34-7.43 (m, 5 H, Ph), 8.10-8.38 (2 m, 4 H, pNO_2Ph).

Analysis calcd. for $C_{31}H_{44}N_4O_7S$: C, 60.37; H, 7.19; N, 9.08. Found C, 60.46; H, 7.21; N, 9.00.

(2S,3S,4R)-1,3,4-Tri-O-acetyl-2-azido-1,3,4-octadecanetriol (**12r**): Dry sodium azide (12.77 g, 196.23 mmol) was added to a solution of **10a** in dry DMF (200 mL) and the reaction mixture was stirred for 2 d at 90-100 °C. Subsequently it was cooled to room temperature, dichloromethane (400 mL) was added and excess sodium azide was filtered off. The filtrate was concentrated in vacuo at 40-50 °C; purification of the residue by column chromatography over silica gel whith petroleum ether/ethyl acetate (9:1) furnished **12r** (16.0 g, 87%, white solid); TLC (silica gel; petroleum ether/ethyl acetate, 9:1), R_f = 0.18; m.p. 35 °C, $[\alpha]^{20}$ = +16 (c = 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.83 (t, J = 6.9 Hz, 3 H, CH_3), 1.10-1.35 (m, 24 H, 12 CH_2), 1.50-1.65 (m, 2 H, 5-H_a, H_b), 2.02, 2.03, 2.05 (3 s, 9 H, 3 CH_3CO), 3.71-3.78 (m, 1 H, 2-H), 4.13 (dd, $J_{1a,2}$ = 7.8 Hz, $J_{1a,1b}$ = 11.8 Hz, 1 H, 1-H_a), 4.33 (dd, $J_{1b,2}$ = 3.2 Hz, $J_{1a,1b}$ = 11.8 Hz, 1 H, 1-H_b), 5.03-5.15 (m, 2 H, 3-H, 4-H).

Analysis calcd. for $C_{24}H_{43}O_6N_3$: C, 61.38; H, 9.33; N, 8.95. Found C, 61.87; H, 9.23; N, 9.00.

(2S,3S,4R)-2-Azido-1,3,4-octadecanetriol (**13r**): a) From **12r**: Sodium methoxide solution (50 mL, 0.2 M) was added to a solution of **12r** (16.0 g, 34.1 mmol) in dry methanol (200 mL). After 2 h, when the reaction was complete it was neutralized with ion-exchange resin Amberlite IR 120 (H^+), the resin was filtered off and the solvent evaporated. The white crystalline substance obtained was **13r** (11.7 g, quantitative) and could be used without further purification in the next step. TLC (silica gel, dichloromethane/methanol, 9:1), R_f = 0.49, m.p. 92-93 °C, $[\alpha]^{20}$ = +17 (c = 0.25, $CHCl_3$/MeOH, 1:1). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.4 Hz, 3 H, CH_3), 1.15-1.60 (m, 26 H, 13 CH_2), 2.23 (d, br, 1 H, OH), 2.55 (d, br, 1 H, OH), 2.72 (t, br, 1 H, 1-OH), 3.65-4.02 (m, 5 H, 1-H_a, H_b, 2,3,4-H).

Analysis calcd. for $C_{18}H_{37}N_3O_3$: C, 62.94; H, 10.85; N, 12.23. Found C, 62.64; H, 10.75; N, 12.07.

b) From **9a**: Sodium azide (2.46 g, 37.8 mmol) was added to a solution of **9a** (3 g, 7.56 mmol) in DMF (30 mL) and the reaction mixture was stirred for 48 h at 90 °C. Subsequently it was cooled to room temperature, dichloromethane (120 mL) was added, the excess sodium azide was filtered off and the filtrate was concentrated in vacuo. Column chormatography of the residue over silica gel with dichloromethane/methanol (15:1) gave **13r** (2.08 g, 80%) as a white solid.

(2S,3R,4E)-2-Azido-1,3-O-benzylidene-4-octadecene-1,3-diol (**14**): a) From **11r/11l** in toluene: DBU (121 µl, 0.810 mmol) was added to a solution of **11r/11l** (200 mg, 0.324 mmol) in dry toluene (3 mL) and the mixture was stirred for 15 h at 70-80 °C. The solvent was then evaporated and the residue chromatographed over silica gel with petroleum ether/ethyl acetate (95:5) to give **14** as a colourless oil. Yields: from **11r** (74 mg, 55%) and from **11l** (48 mg, 36%).

b) From a (1:3) mixture of **11r** and **11l** through selenoxide fragmentation: Diphenyldiselenide (63 mg, 0.2 mmol) was placed in dry ethanol (3 mL) along with sodium borohydride (15.5 mg, 0.41 mmol) and the mixture was stirred for 15 min at room temperature; subsequently a (1:3) mixture of **11r** and **11l** (200 mg, 0.324 mmol) was added to it. This solution was refluxed for 3.5 h and then cooled to 0 °C, it was diluted with THF (3 mL) and H_2O_2 (0.350 mL, 30%) was added slowly. The cooling was removed and the reaction mixture was stirred for another 1 h at room temperature before workup. The reaction mixture was washed with saturated $NaHCO_3$ solution and extracted several times with petroleum ether; the combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated. Chromatography over silica gel with petroleum ether/ethyl acetate (9:1) furnished **14** (66 mg, 50%). TLC (silica gel; petroleum ether/ethyl acetate, 9:1), $R_f = 0.80$, $[\alpha]^{20} = -11.7$ (c = 3, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 0.87$ (t, J = 6.4 Hz, 3 H, CH_3), 1.10-1.50 (m, 22 H, 11 CH_2), 2.11 (m, 2 H, 6-H_a, H_b), 3.48 (ddd, $J_{1b,2} = 4.8$ Hz, $J_{2,3} = 9.1$ Hz, $J_{1a,2} = 11.0$ Hz, 1 H, 2-H), 3.61 (dd, $J_{1a,1b} = J_{1a,2} = 11.0$ Hz, 1 H, 1-H_a), 4.05 (dd, $J_{3,4} = 7.3$ Hz, $J_{2,3} = 9.1$ Hz, 1 H, 3-H), 4.33 (dd, $J_{1b,2} = 4.8$ Hz, $J_{1a,1b} = 11.0$ Hz, 1 H, 1-H_b), 5.49 (s, 1 H, C*H*Ph), 5.58 (dd, $J_{3,4} = 7.3$ Hz, $J_{4,5} = 15.6$ Hz, 1 H, 4-H), 5.97 (m, 1 H, 5-H), 7.37-7.48 (2 m, 5 H, Ph).

(2S,3S,4R)-2-Amino-1,3,4-octadecanetriol (**2**): **13r** (11.71 g, 34.09 mmol) was suspended in a mixture of water and pyridine (150 mL each); the reaction mixture was saturated with H_2S and stirred for 5-7 d at room temperature. Subsequently the solvents were removed under vacuo and the residual yellowish-brown solid was purified by chromatography over silica gel with chloroform/methanol/2 M ammonia (40:10:1) to give **2** (9.4 g, 87%) as a white solid; TLC (silica gel, chloroform/methanol/2 M ammonia, 40:10:1), $R_f = 0.21$, m.p. 95 °C, $[\alpha]^{20} = +7.7$ (c = 1, pyridine). The physical data were in agreement with that reported in literature.[10,25]

Analysis calcd. for $C_{18}H_{39}NO_3$: C, 68.09; H, 12.38; N, 4.41. Found: C, 68.09; H, 11.94; N, 4.07.

(2S,3S,4R)-2-Docosanoylamino-1,3,4-octadecanetriol (**15**): a) Preparation of $C_{21}H_{43}COCl$: A mixture of the acid $C_{21}H_{43}COOH$ and $SOCl_2$ in a molar ratio of 1:1.5 was refluxed under dry conditions until the evolution of gas ceased. The excess of $SOCl_2$ was distilled out and the acid chloride so obtained was used in the preparation of ceramide without further purification.

b) Preparation of ceramide **15**: Compound **2** (9.4 g, 29.61 mmol) was dissolved in THF (250 mL), to this was added sodium acetate solution (250 mL, 50%) and subsequently under vigorous stirring was added portionwise the above prepared acid chloride (10.63 g, 29.61 mmol). After the reaction was complete (~ 2-3 h) the reaction mixture was diluted with THF and brine was added to it for better phase separation. The organic phase on separation was washed with water, dried over anhydrous $MgSO_4$ and concentrated. Crystallization of the residue from methanol (~ 1 L) furnished **15** (15.16 g, 80%) as a white solid; TLC (silica gel, chloroform/methanol, 9:1), $R_f = 0.46$; m.p. 121-123 °C, $[\alpha]^{20} = +7.2$ (c = 0.25, $CHCl_3$/MeOH, 1:1). ^1H NMR (250 MHz, $CDCl_3$/MeOD d_4): $\delta = 0.78$ (t, J = 6.2 Hz, 6 H, 2 CH_3), 1.10-1.60 (m, 62 H, 31 CH_2), 2.14 (t, J = 7.2

Hz, 2 H, NCO-CH_2), 3.46-3.51 (m, 2 H, 3-H, 4-H), 3.58 (dd, $J_{1a,2}$ = 5.7 Hz, $J_{1a,1b}$ = 11.6 Hz, 1 H, 1-H_a), 3.72 (dd, $J_{1b,2}$ = 3.3 Hz, $J_{1a,1b}$ = 11.6 Hz, 1 H, 1-H_b), 3.95-4.05 (m, 1 H, 2-H).

Analysis calcd. for $C_{40}H_{81}NO_4$: C, 75.06; H, 12.76; N, 2.18. Found C, 75.06; H, 12.77; N, 2.24.

(2S,3S,4R)-2-Docosanoylamino-1,3-O-(1,1,3,3-tetraisopropyl disiloxan-1,3-diyl)-1,3,4-octadecanetriol (**16**): To a solution of **15** (400 mg, 0.625 mmol) in dry pyridine (12 mL) maintained under a nitrogen atmosphere at 0 °C was added slowly 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane (0.23 mL, 0.75 mmol). Subsequently the reaction mixture was warmed to room temperature. After 6 h, when the reaction was complete, it was taken up in dichloromethane (~ 30 mL), washed successively with saturated $NaHCO_3$ solution and water. The organic phase was dried over anhydrous $MgSO_4$, concentrated and the residue was purified by chromatography over silica gel with petroleum ether/ethyl acetate (95:5) to give **16** (300 mg, 54%) as a white solid; TLC (silica gel; petroleum ether/ethyl acetate, 9:1), R_f = 0.45, m.p. 40 °C, $[\alpha]^{20}$ = +9.3 (c = 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.3 Hz, 6 H, 2 CH_3), 0.92-1.60 [m, 62 H, 31 CH_2, 28 H, 4 (CH_3)$_2$C*H*-], 2.17 (dt, J = 3.3 Hz, J = 7.6 Hz, 2 H, NCO-CH_2), 2.47 (d, $J_{4,4\text{-OH}}$ = 8.9 Hz, 1 H, 4-OH), 3.52-3.67 (m, 2 H, 3-H, 4-H), 3.71 (dd, $J_{1a,2}$ = 1.4 Hz, $J_{1a,1b}$ = 10.0 Hz, 1 H, 1-H_a), 3.99-4.07 (m, 1 H, 2-H), 4.18 (dd, $J_{1b,2}$ = 1.2 Hz, $J_{1a,1b}$ = 10.0 Hz, 1 H, 1-H_b), 5.90 (d, $J_{2,2\text{-NH}}$ = 9.4 Hz).

Analysis calcd. for $C_{52}H_{107}NO_2Si_2$: C, 70.77; H, 12.22; N, 1.59. Found C, 70.69; H, 12.29; N, 1.91.

(2S,3S)-2-Docosanoylamino-1,3-O-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-1,3-octadecanediol-4-one (**17**): To a solution of **16** (290 mg, 0.328 mmol) in dry dichloromethane (7 mL) maintained under a nitrogen atmosphere, was added PDC (370 mg, 0.984 mmol) and the reaction mixture was stirred for 3-5 d at room temperature. Subsequently the solvent was evaporated and the residue was taken up in ethyl acetate; the excess PDC was separated by filtration; further purification by chromatography over silica gel with petroleum ether/ethyl acetate (95:5) furnished **17** (232 mg, 80%) as a white wax; TLC (silica gel, petroleum ether/ethyl acetate, 9:1), R_f = 0.52, m.p. 40-41 °C, $[\alpha]^{20}$ = +9 (c = 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.82-1.15 [m, 6 H, 2 CH_3, 28 H, 4 (CH_3)$_2$C*H*-], 1.15-1.65 (m, 62 H, 31 CH_2), 2.11 (t, J = 7.2 Hz, 2 H, NCO-CH_2), 2.52-2.78 (m, 2 H, 5-H_a, H_b), 3.69 (dd, $J_{1a,2}$ = 1.2 Hz, $J_{1a,1b}$ = 10.1 Hz, 1 H, 1-H_a), 3.86 (d, $J_{2,3}$ = 10.4 Hz, 1 H, 3-H), 4.09-4.20 (m, 2 H, 1-H_b, 2-H), 5.68 (d, $J_{2,2\text{-NH}}$ = 9.4 Hz, 1 H, N*H*).

Analysis calcd. for $C_{52}H_{105}NO_5Si_2$: C, 70.93; H, 12.03; N, 1.59. Found: C, 70.94; H, 11.98; N, 1.58.

(2S,3S)-2-Docosanoylamino-1,3-octadecanediol-4-one (**18**): TBAF (25 µl, 1.1 M in THF) was added to a solution of **17** (20 mg, 22.7 µmol) in dry THF (1 mL) and the above mixture was stirred for 30 min at room temperature. Subsequently the reaction mixture was worked-up by adding a small amount of water; the solvents were evaporated and chromatography of the residue over silica gel first with toluene/acetone (3:1) as the eluent removed the silyl reagent and then with dichloromethane/methanol (9:1) furnished **18** (9.4 g, 65%) as a white solid; TLC (silica gel, dichloromethane/methanol, 9:1), R_f = 0.56, m.p. 80-82 °C, $[\alpha]^{20}$ = +18 (c = 0.25, $CHCl_3$/MeOH, 1:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.3 Hz, 6 H, 2 CH_3), 1.10-1.30 (m, 58 H, 29 CH_2), 1.45-1.65 (m, 4 H, 2 CH_2), 2.21 (t, J = 7.2 Hz, 2 H, NCO-CH_2), 2.38 (dd, 1 H, $J_{1a,1\text{-OH}}$ = 4.0 Hz, $J_{1b,1\text{-OH}}$ = 7.8 Hz, 1 H, 1-OH), 2.51-2.71 (m, 2 H, 5-H_a, H_b), 3.52-3.72 (m, 2 H, 1-H_a, H_b), 4.24-4.30 (m, 2 H, 3-H, 3-OH), 4.38-4.48 (m, 1 H, 2-H), 6.21 (d, 1 H, $J_{2,2\text{-NH}}$ = 7.9 Hz).

Analysis calcd. for $C_{40}H_{79}NO_4$: C, 75.29; H, 12.47; N, 2.20. Found C, 74.95; H, 12.48; N, 2.50.

(2S,3S)-1,3-Di-O-acetyl-2-docosanoylamino-1,3-octadecanediol-4-one (**19**): TBAF (0.27 mL, 0.3 mmol, 1.1 M in THF) was added to a solution of **17** (131 mg, 0.148 mmol) in dry THF (5 mL) and the above mixture was stirred for 30 min at room temperature. Subsequently to this was added a small amount of water and the reaction mixture was extracted several times with THF, the combined organic phases were dried over anhydrous $MgSO_4$ and concentrated. The residue **18** was acetylated with pyridine/acetic anhydride. Purification by chromatography over silica gel with petroleum ether/ethyl acetate (8:2) gave **19** (93 mg, 87%, white solid). TLC (silica gel, petroleum ether/ethyl acetate, 8:2), R_f = 0.17, m.p. 103-105 °C, $[\alpha]^{20}$ = +3.4 (c = 0.5, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.3 Hz, 6 H, 2 CH_3), 1.15-1.35 (m, 58 H, 29 CH_2), 1.45-1.65 (m, 4 H, 2 CH_2), 2.01 (s, 3 H, CH_3CO), 2.14 (s, 3 H, CH_3CO), 2.14 (t, J = 8.9 Hz, 2 H, NCO-CH_2), 2.42-2.62 (m, 2 H, 5-H_a, H_b), 4.00 (dd, $J_{1a,2}$ = 5.1 Hz, $J_{1a,1b}$ = 11.7 Hz, 1 H, 1-H_a), 4.26 (dd, $J_{1b,2}$ = 6.6 Hz, $J_{1a,1b}$ = 11.7 Hz, 1 H, 1-H_b), 4.66-4.73 (m, 1 H, 2-H), 5.10 (d, $J_{2,3}$ = 4.5 Hz, 1 H, 3-H), 5.77 (d, $J_{2,2\text{-NH}}$ = 8.6 Hz, 1 H, N*H*).

Analysis calcd. for $C_{44}H_{86}NO_6$: C, 73.18; H, 11.58; N, 1.94. Found C, 72.80; H, 11.49; N, 2.20.

(2S,3S,4S)-2-Docosanoylamino-1,3-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-1,3,4-octadecanetriol (**20**): To a solution of **17** (14.5 mg, 16.5 μmol) in freshly distilled THF (0.6 mL) and ethanol (0.2 mL) was added $NaBH_4$ (1 mg, 25 μmol) and the reaction mixture was stirred for 5 h at room temperature, on completion 1-2 drops of water were added and stirring was continued for another 30 min. Subsequently the total reaction mixture was loaded on a preparative TLC-plate (PSC-ready plate, silica gel 60 F_{254}, 1 mm thick, Merck) which was developed in petroleum ether/ethyl acetate (9:1).

The two diastereomers **16** and **20** were eluted out from the silica gel one at a time with warm ethyl acetate (2 x 10-15 mL). Yield: **16** (4.7 mg, 32%, white wax, physical data: already described); **20** (6.3 mg, 43%, colourless oil), TLC (silica gel, petroleum ether/ethyl acetate, 9:1), R_f = 0.37, $[\alpha]^{20}$ = -4 (c = 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.3 Hz, 6 H, 2 CH_3), 0.91-1.70 [3 m, 62 H, 31 CH_2, 28 H, 4 $(CH_3)_2CH$-], 2.21 (dt, J = 2.5 Hz, J = 7.3 Hz, 2 H, NCO-CH_2), 3.33-3.47 (m, 1 H, 4-H), 3.49 (dd, $J_{2,3}$ = 1.1 Hz, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 3.64-3.69 (m, 2 H, 1-H_a, 4-OH), 4.05-4.13 (m, 1 H, 2-H), 4.22 (dd, $J_{1b,2}$ = 1.2 Hz, $J_{1a,1b}$ = 10.1 Hz, 1 H, 1-H_b), 5.98 (d, $J_{2,2\text{-NH}}$ = 9.1 Hz, 1 H, N*H*).

Analysis calcd. for $C_{52}H_{107}NO_5Si_2$: C, 70.77; H, 12.22; N, 1.59. Found: C, 70.65; H, 12.18; N, 1.81.

(2S,3S,4S)-2-Docosanoylamino-1,3,4-octadecanetriol (**21**): TBAF (41 μl, 45.3 μmol, 1.1 M in THF) was added to a solution of **20** (20 mg, 22.6 μmol) in dry THF (4 mL) and the above solution was stirred for 30 min at room temperature. Subsequently 1-2 drops of water were added and it was stirred for another 30 min. The total reaction mixture was then loaded on a preparative TLC plate (PSC-ready plate, silica gel 60 F_{254}, 1 mm thick, Merck) which was developed twice in CH_2Cl_2/THF (1:1) solvent mixture. The ceramide **21** was extracteed out from the silica gel with warm THF (2 x 20-25 mL). Yield: (11.5 mg, 80%, white solid); TLC (silica gel, CH_2Cl_2/THF, 1:1), R_f = 0.66, m.p. 106 °C, $[\alpha]^{20}$ = +7.6 (c = 0.25, $CHCl_3$/MeOH, 1:1). ^1H NMR (250 MHz, $CDCl_3$/MeOD d_4): δ = 0.82 (t, J = 6.3 Hz, 6 H, 2 CH_3), 1.10-1.65 (m, 64 H, 32 CH_2), 2.17 (t, J = 6.7 Hz, 2 H, NCO-CH_2), 3.30 (dd, $J_{3,4}$ = 0.9 Hz, $J_{2,3}$ = 8.8 Hz, 1 H, 3-H), 3.42-3.47 (m, 1 H, 4-H), 3.56 (dd, $J_{1a,2}$ = 3.8 Hz, $J_{1a,1b}$ = 11.4 Hz, 1 H, 1-H_a), 3.65-3.73 (m, 1 H, 2-H), 3.93 (dd, $J_{1b,2}$ = 2.8 Hz, $J_{1a,1b}$ = 11.4 Hz, 1 H, 1-H_b), 6.63 (d, $J_{2,2\text{-NH}}$ = 8.5 Hz, 1 H, N*H*).

Analysis calcd. for $C_{40}H_{81}NO_4$: C, 75.06; H, 12.76; N, 2.19. Found C, 75.13; H, 12.69; N, 2.22.

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