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CD1a-binding glycosphingolipids stimulating human autoreactive T-cells: synthesis of a family of sulfatides differing in the acyl chain moiety

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Abstract—Native sulfatide (a mixture of 3-sulfated β-D-galactopyranosylceramides with different fatty acids at the ceramide moiety) is an antigen presented by CD1a proteins. Herein the preparation of four sulfatides, which are constituents of the natural mixture and bear palmitic, stearic, behenic or nervonic fatty acid chains, is described. Azidosphingosine was stereoselectively synthesized through a CuCN-catalyzed allylic alkylation of a hexenitol dimesylate derived from D-xylose; β-glycosylation of azidosphingosine with a suitable D-galactosyl trichloroacetimidate led, after reduction of the azido group, to the galactosylsphingosine skeleton, which was derivatized with the different fatty acids. Final regioselective 3-sulfation gave the desired sulfatides, which were tested for activation of sulfatide-specific and CD1a-restricted T-cell clones.

1. Introduction

T lymphocytes recognize as antigens short peptides (8–12 aminoacids long) associated with the MHC class I and II proteins. These molecules are characterized by a peptide-binding groove and the complex formed by the presentation molecule with the bound peptide is the antigenic complex stimulating T-cells.[1]

Recently, a new family of antigen presenting molecules (APM), the CD1 molecules, have been shown to mediate presentation of lipid and glycolipid antigens to T-cells.[2–7] The CD1 family of APM in humans is composed of five functional proteins: CD1a, CD1b, CD1c, CD1d and CD1e. A number of lipids presented by CD1 molecules have been characterized. The CD1a isoform was shown to present mycobacterial lipid antigens to specific T-cells, although the precise structure(s) of the presented lipid(s) was not reported.[8] Only recently sulfatide **1** (a mixture of 3-sulfated β-D-galactosylceramides containing fatty acid residues of different structure and chain length) was demonstrated to bind to the CD1a molecule,[9] and to be presented by CD1a to sulfatide-specific T-cell clones.[10] In all these studies a mixture of sulfatides purified from bovine and porcine brain and differing in the lipid moiety was used.

Herein is described an account of our studies which has led to the synthesis of a family of 3-sulfated galactosylceramides (sulfatide, **1**), as antigens presented by CD1a; the synthesized compounds differ in the chain length and the structure of the fatty acid amide, and are constituents of the natural mixture. These compounds have been shown to be capable of stimulating sulfatide-specific and CD1a-restricted T-cell clones.

2. Results and discussion

In order to obtain the sulfatide **1** (Fig. 1) by synthesis, it is necessary to have access to the aglycone part, which is the ceramide. In particular, we focused our attention on 3-OH protected azidosphingosine as it represents a better substrate than ceramide in glycosylation reactions.[11]

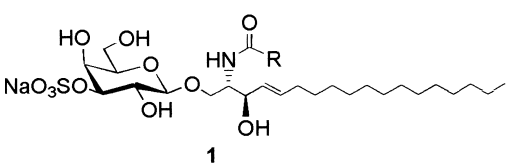

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Figure 1.

Keywords: azidosphingosine; antigens; glycolipids; biologically active compounds.

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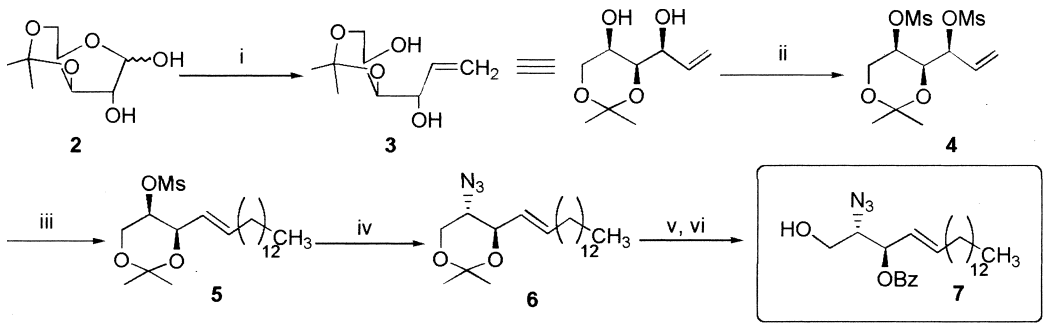


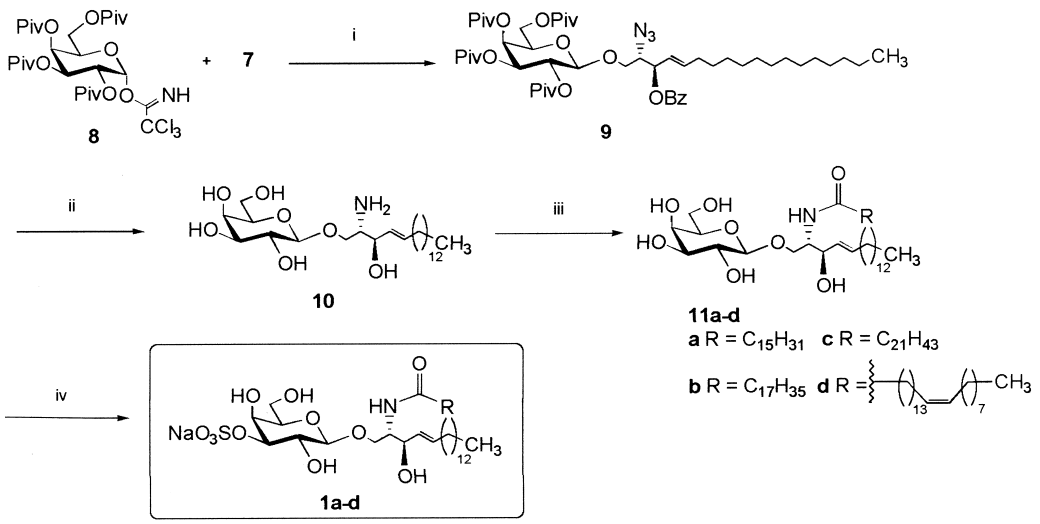
Scheme 1. (i): (a) Me_3SiCH_2MgCl, THF, 70°C, 3 h, (b) KH, THF, 50°C, 3 h, 58% (two steps); (ii): MsCl, DMAP, collidine, 0°C to rt, 3 h, 74%; (iii) *n*-$C_{12}H_{25}MgBr$, CuCN, THF, 0°C, 1 h, 41% *trans*, 22% *cis*; (iv): Bu_4NN_3, toluene, 80°C, 30 h, 90%; (v): (a) HCl 6N, THF/H_2O, rt, 30 h, 80%; (vi): see Ref. 18.

The synthesis of azidosphingosine requires the installation of both the proper stereochemistry and the (*E*)-double bond, and a considerable body of work has been reported in the literature.[12–15] We decided to use a synthetic approach which exploits a starting material from the chiral pool, namely D-xylose, through a S_N2'-type reaction for the attachment of the long chain and introduction of the (*E*)-double bond.[16] The published procedure utilizes benzyl ethers as protecting groups for the xylose hydroxyl groups at positions 3 and 5. However, benzyl-protecting groups are not suitable for our purposes as they are incompatible with the azide and the alkene functions in deprotection steps. We therefore decided to use the known 3,5-*O*-isopropylidene-D-xylofuranose **2** as starting material, which can be easily obtained[17] from D-xylose, although only in moderate yield. Attempts to perform the Wittig reaction on compound **2** with tetradecyliden-triphenylphosphorane gave quite low and poorly reproducible yields. Thus, compound **2** was converted into the protected hexenitol **3** (58% yield) through a Peterson olefination, by condensation between **2** and the Grignard reagent derived from chloromethyltrimethylsilane, followed by treatment of the β-silylalcohol with potassium hydride. Treatment of diol **3** with methanesulfonyl chloride smoothly afforded the dimesylate **4** in excellent yield. Allylic displacement of the mesyl group in position 3 was effected with *n*-dodecylmagnesium bromide in the presence of catalytic copper cyanide. To our surprise, and differently from the result described in the literature,[16] besides the expected product **5**, a significant amount of the *cis* isomer was also formed (*E*/*Z* 2:1). Although this result is not easy to explain, it is apparent that changing the protecting groups on position 1 and 3 from benzyl to isopropylidene influences the stereochemistry of the double bond formation during the allylic displacement. After careful chromatographic separation of the double bond isomers, compound **5** was treated with freshly prepared tetrabutylammonium azide in toluene to allow substitution of the second mesylate with an azido group. Compound **6** was then deprotected at positions 1 and 3, and was transformed into the desired 3-*O*-benzoylazidosphingosine **7** through standard protection–deprotection reactions[18] (Scheme 1).

Stereoselective β-glycosylation of acceptor **7** with 2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl trichloroacetimidate **8**[19] in the presence of boron trifluoride–diethyl ether complex gave compound **9** in very good yield.

In order to introduce the fatty acid and, finally, perform the sulfation of the position 3 of the sugar moiety, the galactosyl sphingosine precursor **9** was transformed[19] into compound **10** by Zèmplen reaction followed by reduction of the azido group.

The crude amine **10** was used directly for the subsequent



Scheme 2. (i): $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C to rt, 88%; (ii): see Ref. 19; (iii): RCOCl, 50% aq. AcONa, THF, rt, 3 h (for **11a**–**c**, 71–80%), or nervonic acid, EDCI, CH_2Cl_2, reflux, 2 h (for **11d**, 64%); (iv): (a) Bu_2SnO, MeOH, reflux, 2 h, (b) $Me_3N{\cdot}SO_3$, THF, rt, 2–20 h, 70–75%.

Table 1. Activation of T-cells clone by sulfatides

Compound	Fatty acid substituent	Released TNFα (pg/mL)[a]
1a	16:0	330
1b	18:0	345
1c	22:0	1425
1d	24:1	3354
Bovine sulfatide	Natural mixture	4580
Medium	–	298

[a] T cell clone A124.

acylation step. The saturated fatty acid chains were introduced by treatment of the amine with the appropriate acid chloride in a 1:1 mixture of THF and 50% aqueous sodium acetate,[19] and the amides **11a–c**, derived from palmitoyl, stearoyl or behenoyl chlorides were obtained in yields ranging from 60 to 70%. A different procedure was used in the case of the nervonoyl derivative **11d** which was obtained by treatment of compound **10** with nervonic acid and EDCI in dichloromethane.

Finally, compounds **11a–d** were selectively sulfated to the desired sulfatides **1a–d** in about 70% yield, according to Flitsch et al.,[20] by activation of the sugar hydroxyls in position 3 and 4 with dibutyltin oxide followed by treatment of the dibutylstannylene acetal with $Me_3N{\cdot}SO_3$ as sulfation reagent (Scheme 2).

The synthetic compounds **1a–d** were tested for activation of sulfatide-specific T-cells and compared to a commercial sample of sulfatide isolated from bovine brain and containing the natural fatty acids mixture on ceramide moiety.[21] The activity of the synthetic and natural sulfatides was studied through the release of TNFα as T-cell activation read out.[10] The results (Table 1) show that the analyzed T-cells are differently activated by the synthetic sulfatides. Thus, the tested synthetic compounds have a biological activity, which is modulated by the type and the length of acyl chain, as shown in Table 1.

3. Conclusion

In conclusion, a series of sulfatides bearing different fatty acids have been synthesized and evaluated for T-cells activation when presented by CD1a molecules. More detailed studies to evaluate the marked influence of the fatty acid in binding to CD1 molecules and in forming immunogenic complexes recognized by T-cells, are in progress and will be described in due course.

4. Experimental

4.1. General methods

Optical rotations were determined on a Perkin–Elmer 241 polarimeter in a 1 dm cell at 20°C. IR spectra were recorded on a Perkin–Elmer 1420 spectrophotometer; NaCl crystal windows. Mass spectrometry was carried out on a quadrupolar Hewlett Packard HP 5988A or Thermo Quest Finningan LCQ™DECA mass spectrometers using chemical ionization (CI/NH_3) and negative electrospray (ES^-) as indicated. All NMR spectra were recorded at 303 K with a Bruker AM-500 spectrometer equipped with an Aspect-3000 computer, a process controller, and an array processor; chemical shifts of NMR spectra are reported as δ (ppm) relative to tetramethylsilane as internal standard. Solvents were purified and dried in the usual way. All reactions were monitored by TLC on Silica Gel 60 F-254 plates (Merck) with detection by spraying with 50% H_2SO_4 solution and heating at 110°C, or by dipping in an ammonium molybdate solution followed by heating. Flash column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck). All evaporations were carried out under reduced pressure at 40°C. Compound **2** was obtained from D-xylose as described in Ref. 17. EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Bovine sulfatide was purchased from Fluka (Buchs, Switzerland).

4.1.1. (2*R*,3*R*,4*S*)-1,3-*O*-Isopropylidene-5-hexen-1,2,3,4-tetrol (3). Mg (1.54 g, 63.8 mmol) was covered with a 13 mL portion of a solution of 8.8 mL (63.8 mmol) of chloromethyltrimethylsilane in 53 mL of dry THF. To this mixture were first added few drops of 1,2-dibromoethane (evolution of ethylene), then, dropwise at slight reflux, the rest of the chloromethyltrimethylsilane solution. The mixture was stirred until complete consumption of Mg. To this solution of the Grignard reagent, a solution of compound **2** (2.42 g, 12.8 mmol) in 53 mL of dry THF was added via cannula. The reaction was heated at 70°C for 3 h, and then quenched by the careful addition of a saturated aqueous NH_4Cl solution; THF was removed under reduced pressure, and the aqueous phase extracted with AcOEt (5×50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give the crude β-silylalcohol as a white solid. This adduct, without further purification, was diluted in 55 mL of dry THF. KH was slowly added to this solution under Ar until the appearance of a precipitate. The mixture was heated at 50°C for 3 h, turning from yellow to brick-red, and then quenched by the slow addition of water. After evaporation of the THF, the aqueous phases were extracted with AcOEt (6×50 mL), and the combined organic phases were dried and concentrated. Flash chromatography (hexane/AcOEt, from 4:6 to 2:8) of the residue furnished compound **3** (1.39 g, 58%) as an oil. $[\alpha]_D=-10.2$ (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): 1.43 (s, 3H, CH_3); 1.44 (s, 3H, CH_3); 2.68 (*br* s, 1H, OH); 2.86–2.91 (m, 1H, OH); 3.44–3.48 (m, 1H, H-2); 3.61 (dd, 1H, $J_{3,4}=7.5$ Hz, $J_{2,3}=1.5$ Hz, H-3); 3.77 (dd, 1H, $J_{1a,1b}=12.5$ Hz, $J_{1a,2}=2.0$ Hz, H-1a); 3.97 (dd, 1H, $J_{1a,1b}=12.5$ Hz, $J_{1b,2}=1.5$ Hz, H-1b); 4.29–4.34 (m, 1H, H-4); 5.25 (ddd, 1H, $J_{5,6a}=10.5$ Hz, $J_{6a,6b}=1.0$ Hz, $J_{4,6a}=1.0$ Hz, H-6a); 5.44 (ddd, 1H, $J_{5,6b}=17.0$ Hz, $J_{6a,6b}=1.0$ Hz, $J_{4,6b}=1.0$ Hz, H-6b); 5.82 (ddd, 1H, $J_{5,6b}=17.0$ Hz, $J_{5,6a}=10.5$ Hz, $J_{4,5}=6.5$ Hz, H-5). ^{13}C NMR ($CDCl_3$): 19.1; 30.2; 64.4; 66.5; 73.3; 75.9; 100.2; 119.0; 135.6. ν_{max} (liquid film): 3400, 3090, 2990, 2950, 1645, 1380 cm^{-1}. CI-MS: *m/z* 206 $[M+NH_4]^+$. $C_9H_{16}O_4$: calcd C 57.43, H 8.57; found C 57.21, H 8.45.

4.1.2. (2*R*,3*R*,4*S*)-1,3-*O*-Isopropylidene-2,4-di-*O*-methylsulfonyl-5-hexen-1,2,3,4-tetrol (4). 2,4,6-Collidine (3,05 mL, 23.07 mmol), DMAP (0.05 g) and methanesulfonyl chloride (1.65 mL, 21.3 mmol) were added to a solution

at 0°C of compound **3** (1.34 g, 7.1 mmol) in dry CH_2Cl_2 (32 mL). The reaction mixture was slowly warmed to rt and stirred for about 3 h at this temperature, then the solvent was evaporated off. The residue was diluted with AcOEt (30 mL) and washed with a saturated aqueous $NaHCO_3$ solution (30 mL). The aqueous phase was separated and extracted with AcOEt (3×25 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (hexane/AcOEt, 6:4), afforded compound **4** (1.81 g, 74%) as a foam. $[\alpha]_D=-25.8$ (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): 1.44 (s, 3H, CH_3); 1.45 (s, 3H, CH_3); 3.02 (s, 3H, CH_3SO_2); 3.12 (s, 3H, CH_3SO_2); 3.99–4.04 (m, 2H, H-1a,3); 4.23 (dd, 1H, $J_{1a,1b}=13.8$ Hz, $J_{1b,2}=2.0$ Hz, H-1b); 4.48–4.51 (m, 1H, H-2); 5.08 (dd, 1H, $J_{3,4}=7.0$ Hz, $J_{4,5}=6.5$ Hz, H-4); 5.52 (d, 1H, $J_{5,6a}=10.5$ Hz, H-6a); 5.70 (d, 1H, $J_{5,6b}=17.0$ Hz, H-6b); 5.91 (ddd, 1H, $J_{5,6b}=17.0$ Hz, $J_{5,6a}=10.5$ Hz, $J_{4,5}=6.5$ Hz, H-5). ^{13}C NMR ($CDCl_3$): 19.5; 29.4; 39.4; 40.5; 62.9; 71.7; 72.4; 82.7; 100.3; 124.0; 129.9. ν_{max} (liquid film): 2995, 2940, 1640, 1380, 1310, 1210, 1070, 790 cm^{-1}. CI-MS: *m/z* 362 $[M+NH_4]^+$. $C_{11}H_{20}O_8S_2$: calcd C 38.36, H 5.85; found C 38.47, H 5.72.

4.1.3. (2*R*,3*R*,4*E*)-1,3-*O*-Isopropylidene-2-*O*-methylsulfonyl-4-octadecen-1,2,3-triol (5). A flame-dried flask was charged with freshly prepared CuCN[22] (0.044 g, 0.49 mmol). After the flask had been flushed with argon, dry THF (15 mL) was added, and the mixture was cooled to 0°C. A solution of freshly prepared *n*-$C_{12}H_{25}MgBr$ (9.88 mmol) in dry Et_2O (60 mL) was added, and the mixture stirred for 10 min. A solution of compound **4** (1.70 g, 4.94 mmol) in dry THF (60 mL) was then slowly added via cannula, and stirring was continued for 1 h. The reaction was quenched with saturated aqueous NH_4Cl (40 mL), and the resulting mixture extracted with Et_2O (3×30 mL). The combined organic phases were washed with brine, dried and concentrated. Purification by flash chromatography (hexane/AcOEt, 9:1) gave first the *cis*-adduct (0.43 g, 20%), then compound **5** (0.848 g, 41%) as a foam. $[\alpha]_D=-33.9$ (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): 0.86 (t, 3H, CH_3); 1.16–1.32 (m, 20H, 10 CH_2); 1.33–1.41 (m, 2H, CH_2); 1.45 (s, 3H, CH_3); 1.47 (s, 3H, CH_3); 2.01–2.09 (m, 2H, 2 H-6); 3.04 (s, 3H, CH_3SO_2); 3.98–4.13 (m, 2H, 2 H-1); 4.41–4.47 (m, 2H, H-2,3); 5.51 (dd, 1H, $J_{3,4}=6.5$ Hz, $J_{4,5}=15.5$ Hz, H-4); 5.82 (dt, 1H, $J_{4,5}=15.5$ Hz, $J_{5,6}=6.7$ Hz, H-5). ^{13}C NMR ($CDCl_3$): 14.8; 19.6; 23.4; 29.5–30.3 (10C); 32.6; 33.0; 39.5; 63.7; 72.1; 75.2; 99.7; 125.8; 136.8. ν_{max} (liquid film): 2990, 2950, 1460, 1380, 1180, 960 cm^{-1}. CI-MS: *m/z* 436 $[M+NH_4]^+$. $C_{22}H_{42}O_5S$: calcd C 63.12, H 10.11; found C 65.30, H 11.92.

4.1.4. (2*S*,3*R*,4*E*)-2-Azido-1,3-*O*-isopropylidene-4-octadecen-1,3-diol (6). Tetrabutylammonium azide (0.97 g, 4.41 mmol) was added to a solution of **5** (0.57 g, 1.36 mmol) in dry toluene (30 mL). The reaction was stirred at 80°C for 30 h. Water (100 mL) was added, the mixture was extracted with AcOEt (3×80 mL), and the combined organic layers were dried and concentrated to dryness. Purification by flash chromatography (hexane/AcOEt, 97:3) afforded pure **6** (0.45 g, 90%) as an oil. $[\alpha]_D=-41.5$ (*c* 1.5, $CHCl_3$) (Lit.:[18] −42.6 (*c* 1.5, $CHCl_3$)) All the physical data were in agreement with those reported in Ref. 18.

4.1.5. (2*S*,3*R*,4*E*)-2-Azido-3-benzoyloxy-4-octadecen-1-ol (7). 6N HCl (2 mL) was added dropwise to a solution of compound **6** (0.45 g, 1.23 mmol) in THF/H_2O 9:1 (20 mL), and the reaction was stirred for 30 h. The solution was neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with chloroform (3×40 mL). The combined organic extracts were washed with brine, dried and concentrated. Purification by flash chromatography (hexane/ AcOEt, 7:3) gave the known *(2S,3R,4E)-2-azido-octadec-4-ene-1,3-diol*[18] (0.32 g, 80%) as an oil. The diol was transformed into the title compound **7** (0.32 g, 75% three steps) according to Ref. 18. $[\alpha]_D=-46.5$ (*c* 4, $CHCl_3$) (Lit.:[18] −46.0 (*c* 4, $CHCl_3$)).

4.1.6. (2*S*,3*R*,4*E*)-2-Amino-1-(β-D-galactopyranosyloxy)-3-hydroxy-4-octadecene (10). Boron trifluoride diethyl ether in dry CH_2Cl_2 (0.1 M solution, 4.7 mL) was added dropwise to a solution at 0°C under argon of imidate **8**[19] (0.77 g, 1.16 mmol) and **7** (0.20 g, 0.47 mmol) in dry CH_2Cl_2 (6 mL). The reaction was warmed to rt, and after 30 min an additional amount of compound **8** (0.30 g) was added. After 1 h the reaction mixture was diluted with CH_2Cl_2 and treated with sat. $NaHCO_3$ solution (40 mL). After separation, the aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (hexane/AcOEt, 9:1) gave the known *(2S,3R,4E)-2-azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-pivaloyl-β-D-galacto-pyranosyloxy)-octadec-4-ene* (**9**)[19] (0.38 g, 88%) as an oil. Compound **9** was transformed, according to Ref. 19, into the title amino derivative **10** (0.14 g, 76%), by Zèmplen reaction followed by reduction of the azido group with hydrogen sulfide in pyridine/water. Crude **10** was used directly in the next step.

4.1.7. (2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(hexadecanoylamino)-3-hydroxy-4-octadecene (11a). Compound **10** (0.030 g, 0.063 mmol) was dissolved in THF (7.5 mL) and treated with aqueous sodium acetate (50%, 6.5 mL) and hexadecanoyl chloride (0.023 mL, 0.076 mmol); the mixture was vigorously stirred for 3 h. The aqueous phase was extracted with THF (2×10 mL). The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to give compound **11a** (0.035 g, 80%) as a foam. $[\alpha]_D=-5.0$ (*c* 1, pyridine) (Lit.:[19] −5.2 (*c* 1, pyridine)). Physical data of compound **11a** were in agreement with those reported in Ref. 19.

4.1.8. (2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(octadecanoylamino)-3-hydroxy-4-octadecene (11b). Compound **11b** was obtained from the amino derivative **10** (0.027 g, 0.059 mmol) as described for compound **11a**. The reaction mixture was purified by flash-chromatography (CH_2Cl_2/MeOH, 85:15) to yield compound **11b** (0.030 g, 71%) as a foam. $[\alpha]_D=-4.9$ (*c* 1, Py). ^1H NMR ($CDCl_3$/CD_3OD, 1:1): 0.86 (t, 6H, $J=7.5$ Hz, 2 CH_3); 1.20–1.40 (m, 50H, 25 CH_2); 1.52–1.62 (m, 2H, CH_2); 1.96–2.02 (m, 2H, 2 H-6); 2.14 (t, 2H, $J=7.5$ Hz, $COCH_2$); 3.44–3.50 (m, 2H, H-3′,5′); 3.53 (dd, 1H, $J_{1',2'}=7.5$ Hz, $J_{2',3'}=9.0$ Hz, H-2′); 3.56 (dd, 1H, $J_{1a,1b}=10.0$ Hz, $J_{1a,2}=3.0$ Hz, H-1a); 3.71 (dd, 1H, $J_{5',6'a}=5.0$ Hz, $J_{6'a,6'b}=11.5$ Hz, H-6′a); 3.78 (dd, 1H, $J_{6'a,6'b}=11.5$ Hz, $J_{5',6'b}=6.6$ Hz, H-6′b); 3.84 (br d, 1H,

$J_{3',4'}$=3.0 Hz, H-4′); 3.93–3.99 (m, 1H, H-2); 4.08 (dd, 1H, $J_{2,3}=J_{3,4}$=7.5 Hz, H-3); 4.17 (dd, 1H, $J_{1a,1b}$=10.0 Hz, $J_{1b,2}$=4.3 Hz, H-1b); 4.19 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′); 5.42 (ddt, 1H, $J_{4,5}$=15.5 Hz, $J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.66 (dt, $J_{4,5}$=15.5 Hz, $J_{5,6}$=7.0 Hz, H-5); 7.57 (d, 1H, $J_{2,NH}$=9.0 Hz, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1): 15.0 (2C); 23.9 (2C); 26.3; 27.3; 30.6–30.9 (20C); 33.2 (2C); 33.6; 37.7; 54.7; 62.7; 70.0; 70.3; 72.7; 73.2; 74.8; 76.5; 105.1; 130.8; 135.5; 176.0. ν_{max} (nujol): 3450, 1645 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 726.7 [M−H]$^-$. $C_{42}H_{81}NO_8$: calcd C 69.28, H 11.21, N 1.92; found C 68.95, H 11.35, N 2.03.

4.1.9. (2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(docosanoylamino)-3-hydroxy-4-octadecene (11c). Compound **11c** was obtained from the amino derivative **10** (0.027 g, 0.059 mmol) as described for compound **11a**. The reaction mixture was purified by flash-chromatography (CH_2Cl_2/MeOH, 9:1) to yield compound **11c** (0.032 g, 71%) as a foam. $[\alpha]_D$=−7.2 (*c* 1, Py). ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1): 0.86 (t, 6H, *J*=7.5 Hz, 2 CH_3); 1.20–1.40 (m, 58H, 29 CH_2); 1.52–1.62 (m, 2H, CH_2); 1.96–2.02 (m, 2H, 2 H-6); 2.14 (t, 2H, *J*=7.5 Hz, $COCH_2$); 3.44–3.50 (m, 2H, H-3′,5′); 3.53 (dd, 1H, $J_{1',2'}$=7.5 Hz, $J_{2',3'}$=9.0 Hz, H-2′); 3.56 (dd, 1H, $J_{1a,1b}$=10.0 Hz, $J_{1a,2}$=3.0 Hz, H-1a); 3.71 (dd, 1H, $J_{5',6'a}$=5.0 Hz, $J_{6'a,6'b}$=11.5 Hz, H-6′a); 3.78 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6'b}$=6.8 Hz, H-6′b); 3.84 (br d, 1H, $J_{3',4'}$=2.8 Hz, H-4′); 3.93–3.98 (m, 1H, H-2); 4.08 (dd, 1H, $J_{2,3}=J_{3,4}$=7.5 Hz, H-3); 4.17 (dd, 1H, $J_{1a,1b}$=10.0 Hz, $J_{1b,2}$=4.0 Hz, H-1b); 4.19 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′); 5.42 (ddt, 1H, $J_{4,5}$=15.5 Hz, $J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.66 (dt, $J_{4,5}$=15.5 Hz, $J_{5,6}$=6.5 Hz, H-5); 7.57–7.59 (m, 1H, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1): 15.0 (2C); 23.9 (2C); 26.0; 27.3; 30.6–30.9 (24C); 33.2 (2C); 33.6; 37.7; 54.7; 62.7; 70.0; 70.3; 72.7; 73.2; 74.8; 76.5; 105.1; 130.8; 135.5; 176.0. ν_{max} (nujol): 3400, 1650 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 782.7 [M−H]$^-$. $C_{46}H_{89}NO_8$: calcd C 70.45, H 11.44, N 1.79; found C 70.17, H 11.53, N 1.85.

4.1.10. (2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(15(*Z*)-tetracosenoylamino)-3-hydroxy-4-octadecene (11d). Nervonic acid (0.036 g, 0.097 mmol) and EDCI (0.025 g, 0.13 mmol) were added to a solution of compound **10** (0.030 g, 0.065 mmol) in dry CH_2Cl_2 (4 mL) under argon. The reaction mixture was heated at reflux temperature for 2 h, then the solvent was removed under reduced pressure. The crude was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) affording compound **11d** (0.034 g, 64%) as a foam. $[\alpha]_D$=−3.0 (*c* 1, Py). ^{1}H NMR ($CDCl_3/CD_3OD$, 1:2): 0.87 (t, 6H, *J*=7.5 Hz, 2 CH_3); 1.20–1.40 (m, 54H, 27 CH_2); 1.52–1.61 (m, 2H, CH_2); 1.97–2.09 (m, 6H, 3 CH=CHCH_2); 2.15 (t, 2H, *J*=7.5 Hz, $COCH_2$); 3.47 (dd, 1H, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=3.5 Hz, H-3′); 3.49 (dd, 1H, $J_{5',6a'}$=7.0 Hz, $J_{5',6b'}$=5.2 Hz, H-5′); 3.54 (dd, 1H, $J_{2',3'}$=9.5 Hz, $J_{1',2'}$=7.5 Hz, H-2′); 3.57 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1a,2}$=3.2 Hz, H-1a); 3.71 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6a'}$=7.0 Hz, H-6′a); 3.78 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6b'}$=5.2 Hz, H-6′b); 3.84 (br d, 1H, H-4′); 3.95–3.99 (m, 1H, H-2); 4.08 (dd, 1H, $J_{2,3}$=8.0 Hz, $J_{3,4}$=7.5 Hz, H-3); 4.17 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1b,2}$=4.5 Hz, H-1b); 4.20 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′); 5.31 (m, 2H, CH_2CH=C*H*CH_2);5.43 (ddt, 1H, $J_{4,5}$=15.5 Hz, $J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.67 (dt, $J_{4,5}$=15.5 Hz, $J_{5,6}$=7.0 Hz, H-5); 7.67 (d, 1H, $J_{2,NH}$=9.2 Hz, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:2): 14.9 (2C); 23.9 (2C); 27.2; 28.3 (2C); 30.6–31.3 (27C); 33.1 (2C); 33.6; 37.6; 54.8; 62.6; 70.0; 70.3; 72.7; 73.1; 74.8; 76.6; 105.2; 131.0 (3C); 135.3; 176.0. ν_{max} (nujol): 3445, 1650 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 808.7 [M−H]$^-$. $C_{48}H_{91}NO_8$: calcd C 71.15, H 11.32, N 1.73; found C 70.90, H 11.10, N 1.85.

4.1.11. (2*S*,3*R*,4*E*)-1-[3-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-(hexadecanoylamino)-3-hydroxy-4-octadecene (1a). Compound **11a** (0.035 g, 0.049 mmol) and Bu_2SnO (0.018 g, 0.073 mmol) were stirred in MeOH (1 mL) at reflux under argon for 2 h. The solvent was evaporated off under reduce pressure and the dibutylstannylene complex was treated with $Me_3N{\cdot}SO_3$ (0.013 g, 0.098 mmol) in THF at rt for 6 h. The solvent was removed under reduced pressure, than the residue was dissolved in $CHCl_3$/MeOH 1:1 (1 mL) and loaded onto a cation exchange resin column (Dowex 50X8, Na^+ form, 0.5× 4 cm). The mixture was eluted with $CHCl_3$/MeOH 1:1, concentrated in vacuo and subjected to flash chromatography (CH_2Cl_2/MeOH, 85:15) to give compound **1a** (0.029 g, 74%) as a foam. $[\alpha]_D$=+2.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1): 0.86 (t, 6H, *J*=6.5 Hz, 2 CH_3); 1.15–1.40 (m, 46H, 23 CH_2); 1.51–1.60 (m, 2H, CH_2); 1.96–2.02 (m, 2H, 2 H-6); 2.16 (t, 2H, *J*=7.5 Hz, $COCH_2$); 3.55 (dd, 1H, $J_{5',6a}$=5.5 Hz, $J_{5',6b}$=6.5 Hz, H-5′); 3.61 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1a,2}$=3.0 Hz, H-1a); 3.73 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6a}$=5.5 Hz, H-6′a); 3.74 (dd, 1H, $J_{1',2'}$=7.7 Hz, $J_{2',3'}$=9.5 Hz, H-2′); 3.79 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6'b}$=6.5 Hz, H-6′b); 3.95–3.99 (m, 1H, H-2); 4.08 (dd, 1H, $J_{2,3}=J_{3,4}$=7.5 Hz, H-3); 4.15 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1b,2}$=4.5 Hz, H-1b); 4.24 (br d, 1H, $J_{3',4'}$=3.3 Hz, H-4′); 4.26 (dd, 1H, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=3.3 Hz, H-3′); 4.33 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′); 5.42 (ddt, 1H, $J_{4,5}$=15.5 Hz, $J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.67 (dt, 1H, $J_{4,5}$=15.5 Hz, $J_{5,6}$=6.5 Hz, H-5); 7.69 (d, 1H, $J_{2,NH}$=11.5 Hz, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1): 15.0 (2C); 23.9 (2C); 27.3; 30.6–31.0 (18C); 33.2 (3C); 33.6; 37.7; 54.7; 62.6; 68.7; 70.2; 70.9; 73.1; 76.1; 81.7; 104.7; 130.7; 135.5; 176.2. ν_{max} (nujol): 3440, 1650, 1550, 1250, 1090 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 779.0 [M−Na]$^-$. $C_{40}H_{76}NNaO_{11}S$: calcd C 59.90, H 9.55, N 1.75; found C 60.05, H 9.38, N 1.82.

4.1.12. (2*S*,3*R*,4*E*)-1-[3-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-(octadecanoylamino)-3-hydroxy-4-octadecene (1b). Sulfatide **1b** was obtained as a foam (0.017 g, 70%) from compound **11b** (0.021 g, 0.029 mmol) as described for **1a**. After the addition of $Me_3N{\cdot}SO_3$ the reaction was stirred at rt for 4 h. $[\alpha]_D$=+2.2 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1): 0.86 (t, 6H, *J*=7.0 Hz, 2 CH_3); 1.20–1.40 (m, 50H, 25 CH_2); 1.54–1.62 (m, 2H, CH_2); 1.96–2.02 (m, 2H, 2 H-6); 2.16 (t, 2H, *J*=7.5 Hz, $COCH_2$); 3.56 (dd, 1H, $J_{5',6'a}$=5.0 Hz, $J_{5',6'b}$=6.5 Hz, H-5′); 3.62 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1a,2}$=3.0 Hz, H-1a); 3.70–3.76 (m, 2H, H-2′,6′a); 3.79 (dd, 1H, $J_{5',6'b}$=6.5 Hz, $J_{6'a,6'b}$=11.5 Hz, H-6′b); 3.99 (m, 1H, H-2); 4.07 (dd, 1H, $J_{2,3}=J_{3,4}$=7.5 Hz, H-3); 4.12 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1b,2}$=4.5 Hz, H-1b); 4.25 (br d, 1H, $J_{3',4'}$=3.3 Hz, H-4′); 4.28 (dd, 1H, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=3.3 Hz, H-3′); 4.33 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′); 5.41 (ddt, 1H, $J_{4,5}$=15.5 Hz,

$J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.67 (dt, $J_{4,5}$=15.5 Hz, $J_{5,6}$=6.5 Hz, H-5); 7.69 (d, 1H, $J_{2,NH}$=9.0 Hz, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1): 15.0 (2C); 23.9 (2C); 27.3; 30.6–31.0 (21C); 33.2 (2C); 33.7; 37.7; 54.8; 62.6; 68.8; 70.3; 70.9; 73.2; 76.0; 81.6; 104.7; 130.6; 135.6; 176.2. ν_{max} (nujol): 3390, 1640, 1550, 1250, 1085 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 806.9 [M−Na]$^-$. $C_{42}H_{80}NNaO_{11}S$: calcd C 60.77, H 9.71, N 1.69; found C 60.92, H 9.93, N 1.75.

4.1.13. (2*S*,3*R*,4*E*)-1-[3-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-(docosanoylamino)-3-hydroxy-4-octadecene (1c). Sulfatide **1c** (0.022 g, 75%) was obtained as a foam from compound **11c** (0.026 g, 0.033 mmol) as described for **1a**. After the addition of $Me_3N{\cdot}SO_3$ the reaction was stirred at rt for 2 h. $[\alpha]_D$=+2.2 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1): 0.86 (t, 6H, *J*=7.0 Hz, 2 CH_3); 1.20–1.40 (m, 58H, 29 CH_2); 1.54–1.62 (m, 2H, CH_2); 1.96–2.02 (m, 2H, 2 H-6); 2.15 (t, 2H, *J*=7.5 Hz, $COCH_2$); 3.55 (dd, 1H, $J_{5',6'a}$=5.0 Hz, $J_{5',6'b}$=6.5 Hz, H-5′); 3.59 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1a,2}$=3.0 Hz, H-1a); 3.69–3.76 (m, 2H, H-2′,6′a); 3.79 (dd, 1H, $J_{6'a,6'b}$=11.5 Hz, $J_{5',6'b}$=6.5 Hz, H-6′b); 3.94–3.99 (m, 1H, H-2); 4.08 (dd, 1H, $J_{2,3}$=$J_{3,4}$=7.5 Hz, H-3); 4.17 (dd, 1H, $J_{1a,1b}$=10.5 Hz, $J_{1b,2}$=4.5 Hz, H-1b); 4.21–4.27 (m, 2H, H-3′,4′); 4.32 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′); 5.41 (ddt, 1H, $J_{4,5}$=15.5 Hz, $J_{3,4}$=7.5 Hz, $J_{4,6}$=1.0 Hz, H-4); 5.67 (dt, $J_{4,5}$=15.5 Hz, $J_{5,6}$=6.5 Hz, H-5); 7.59 (br s, 1H, NH). ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1): 15.0 (2C); 23.9 (2C); 27.3; 30.6–31.0 (25C); 33.2 (2C); 33.7; 37.7; 54.7; 62.6; 68.7; 70.2; 70.9; 73.1; 76.1; 81.6; 104.7; 130.7; 135.6; 176.1. ν_{max} (nujol): 3440, 1645, 1540, 1245, 1080 cm^{-1}. ESI/MS (negative-ion mode): *m/z* 862.9 [M−Na]$^-$. $C_{46}H_{88}NNaO_{11}S$: calcd C 62.34, H 10.01, N 1.58; found C 62.00, H 9.90, N 1.31.

4.1.14. (2*S*,3*R*,4*E*)-1-[3-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-(15(*Z*)-tetracosenoylamino)-3-hydroxy-4-octadecene (1d). Sulfatide **1d** (0.016 g, 70%) was obtained as a foam from compound **11d** (0.021 g, 0.026 mmol) as described for **1a**. After the addition of $Me_3N{\cdot}SO_3$ the reaction was stirred at rt for 20 h. Compound **1d** was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1). $[\alpha]_D$=+2.7 (*c* 1, MeOH) (Lit.:[20] +2.6 (*c* 1, MeOH)). Physical data of compound **1d** were in agreement with those reported in Ref. 20.

4.1.15. Evaluation of the biological activity. The T-cell clone A124 was isolated from peripheral blood. The assays were performed in triplicates using 10 μg/ml sulfatide, 5×10^4/well T-cells and 3×10^4/well dendritic cells as antigen-presenting cells, and TNFα was measured by ELISA as described in Ref. 10.

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