Synthesis of a Fluorescent Sulfatide for the Study of CD1 Antigen Binding Properties

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A fluorescent derivative of sulfatide, labeled with a dansyl probe at position 6 of galactose, was prepared through a glycosylation reaction between a 6-*O*-functionalized galactose donor and 3-*O*-benzoyl-azidosphingosine. The sphingoid acceptor was obtained in high yield through exploitation of a highly stereoselective nucleophilic addition of pentadecyne to 2,3-*O*-cyclohexylidene-D-glyceraldehyde. The fluorescent sulfatide binds to soluble recombinant human CD1a and is a valuable tool for the study of CD1 antigen binding properties.

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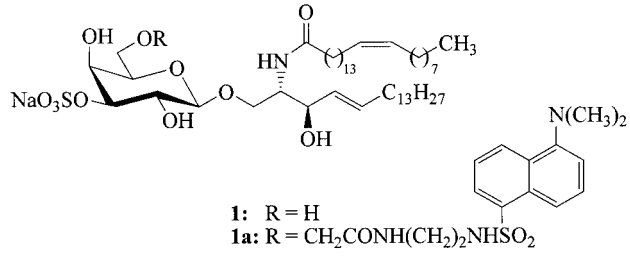
Introduction

CD1 proteins are antigen-presenting molecules that bind foreign and native lipids and stimulate specific T-cell responses.[1−3] In humans, five CD1 proteins are expressed, encoded by different genes on chromosome 1 and classified into two distinct groups according to their sequence homology.[4] At present, different classes of lipid antigens, all containing a common motif of a polar head group linked to a hydrophobic tail of one or more alkyl chains, are known to be presented by CD1 proteins to T cells.[1−3] As has emerged from recently solved crystal structures, these lipid antigens bind to the CD1-hydrophobic binding groove through their hydrophobic alkyl chains and initiate CD1-dependent, lipid-specific T cell responses.[5−8]

Sulfatide (a mixture of 3-sulfated β-D-galactosylceramides with different fatty acids in the ceramide moiety; Figure 1 shows the structure of the nervonoyl derivative **1**, one of the most represented in the natural mixture) is one of the characterized glycolipid antigens that bind group I CD1 molecules.[9,10] The crystal structure of CD1a in complex with sulfatide[7] shows that the lipid-binding region of CD1a is suited for binding the alkyl chains of sulfatide. The various parameters affecting lipid binding to the different CD1 isoforms remain largely unexplored, however; in the case of a promiscuous ligand such as sulfatide, for example, direct measurement of the on-rate and off-rate of the ligand with CD1 proteins could yield information on the stability of the different sulfatide-CD1 complexes. Moreover, a labeled sulfatide would provide a means to quantify its association with CD1 proteins and to probe the antigen-binding properties of different CD1 isoforms (wild types and mutants). Fluorescent labeling is currently an extremely useful tool to address this point as it allows observation of chemical species involved in the biochemical process at low concentrations. It has been widely used for studies on cellular distributions of bioactive compounds, including glycolipids,[11] and for assisting structure-based studies of CD1 function.[12]



1: R = H
1a: R = $CH_2CONH(CH_2)_2NHSO_2$

Figure 1. Structures of compounds **1** and **1a**

In order to minimize inaccurate results it is important that the addition of the fluorescent probe should not interfere in the association of the ligand with its host; in the case

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of sulfatide and CD1 proteins, the presence of the fluorophore on the sugar part should in principle not interfere with antigen loading, since only the two alkyl chains are docked in the hydrophobic binding region while the polar head group protrudes from the surface of the protein binding groove for T cell recognition.[13]

On the basis of these considerations, compound **1a** (Figure 1) − a fluorescent derivative of sulfatide with a dansyl probe linked to the 6-position of galactose via a spacer − was designed; this is the first example in which a fluorophore has been attached on the sugar part of sulfatide,[14] providing an analogue of the natural compound suitable for studies on antigen loading rates.

The synthesis of β-D-glycosylceramides through glycosylation reactions generally exploits 3-*O*-benzoyl-D-*erythro*-azidosphingosine (**4**) as one of the best acceptors to prepare compounds such as **1a**.[15] During recent years we have been involved in β-D-galactosylceramide syntheses, and particular attention has been addressed to the development of new and efficient procedures for the preparation of compound **4**.[16,17] Recently we reported a new route to this compound, the key steps in which were a nucleophilic addition of the Grignard reagent of 1-pentadecyne to 2,3-*O*-cyclohexylidene-D-glyceraldehyde and an enzymatic resolution of the mixture of diastereomeric propargylic alcohols (*syn*/*anti* 4:6) followed by Mitsunobu inversion on the wrong *anti* diastereoisomer.[16] We have now developed a significant improvement in the synthesis of compound **4** through a highly stereoselective *syn* addition of pentadecyne to D-glyceraldehyde, which allows the steps for the conversion of the wrong diastereoisomer to be avoided and makes this method a good practical procedure for larger scale preparations.

This paper describes the synthesis and activity of compound **1a** and a new strategy for the preparation of 3-*O*-benzoyl-D-*erythro*-azidosphingosine (**4**).

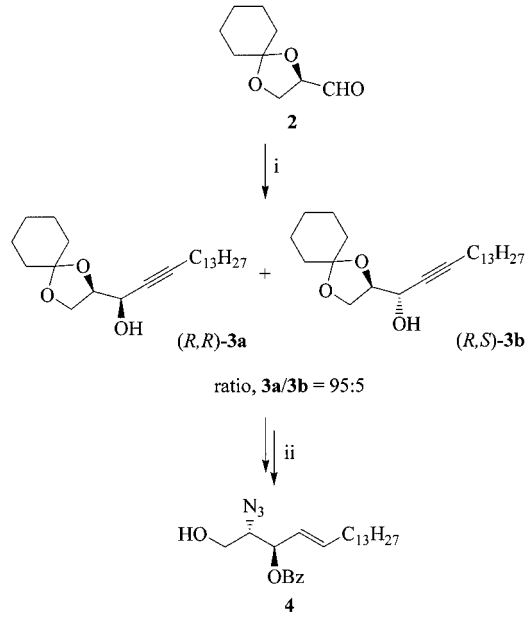
Results and Discussion

The fluorescent sulfatide derivative **1a** is labeled with a dansyl probe linked through a spacer to the 6-position of galactose. The choice of the spacer was made in order to link the probe to the galactosylceramide through condensation between a dansyl-ethylenediamino group and a carboxylic functionality on the sugar. Thus, the β-D-galactosylceramide **11**, functionalized with a carboxymethyl group at the 6-position of galactose, was devised as an intermediate for the introduction of the fluorescent probe. This compound was in turn obtained through a glycosylation reaction between 3-*O*-benzoyl-D-*erythro*-azidosphingosine (**4**) and the galactosyl donor **7**.

Synthesis of 3-*O*-Benzoyl-D -*erythro*-azidosphingosine (4)

As already outlined in the Introduction, we developed a synthesis of compound **4** based on a nucleophilic addition of the Grignard reagent of 1-pentadecyne to 2,3-*O*-cyclohexylidene-D-glyceraldehyde.[16] The only drawback of this otherwise straightforward procedure was the low selectivity of the nucleophilic addition, which prompted us to look for a more stereoselective approach. In particular, our attention was drawn towards Carreira's very efficient technique for enantioselective addition of terminal alkynes to aldehydes under mild conditions in the presence of a chiral additive.[18]

An equimolar amount of 2,3-*O*-cyclohexylidene-D-glyceraldehyde (**2**) was therefore added at room temperature to the zinc alkynylide of 1-pentadecyne, generated in situ by treatment of the alkyne with zinc triflate, triethylamine, and (−)-(1*R*,2*S*)-*N*-methylephedrine in toluene (Scheme 1). The addition product **3** was recovered in acceptable yields (61%) and with excellent selectivity; the mixture of diastereomeric alcohols was in fact obtained in a *syn*/*anti* ratio of 95:5.



Scheme 1. *Reagents and conditions*: (i) 1-pentadecyne, $Zn(OTf)_2$, TEA, (−)-*N*-methylephedrine, toluene, room temp. (ii) ref.[16]

When the reaction was repeated with use of 2 equivalents of pentadecyne under the previously reported conditions, we were delighted to find that the product was obtained in a very high yield (91%) and in the same diastereomeric ratio.

After this clean construction of the C-18 skeleton, we were able to obtain 3-*O*-benzoyl-D-*erythro*-azidosphingosine very efficiently from the diastereomeric mixture of compound **3** by following our previous procedure,[16] thus avoiding the tedious steps needed in recycling of the wrong isomer.

The small amount of the *anti* adduct **3b** was easily separated by chromatography after its selective enzymatic transacetylation. Protecting group manipulations, followed by efficient introduction of the azide through use of chloromesylate as leaving group, yielded compound **4**.[16] The process was scaled up to 5 g of the starting 2,3-*O*-cyclohexylidene-D-glyceraldehyde (**2**); the overall yield from **2** to

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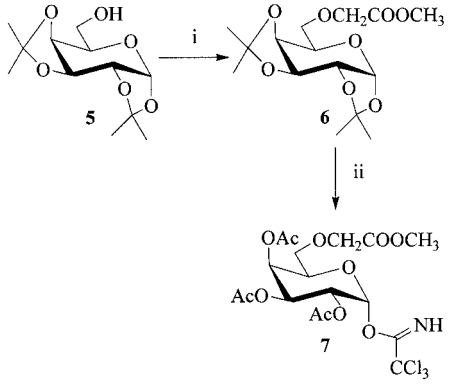
the final azidosphingosine **4** was 38%, illustrating the efficiency of the sequence.

Synthesis of the Galactosyl Donor 7

Commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) was alkylated[19] at position 6 by treatment with NaH, chloroacetic acid, and CH_3I (Scheme 2), affording compound **6** in high yield. Protecting groups were then exchanged from ketals to esters in order to introduce a participating group at the 2-position. Removal of the acetonides, promoted by aqueous TFA, caused partial hydrolysis of the methyl ester, which was re-introduced by treatment with diazomethane. Acetylation with Ac_2O and pyridine afforded an α,β anomeric mixture of the peracetylated compound. Finally, the trichloroacetimidate donor **7** was obtained after regioselective removal of the anomeric acetates,[20] followed by treatment with trichloroacetonitrile and DBU,[15] with an overall yield from compound **5** of 32%.



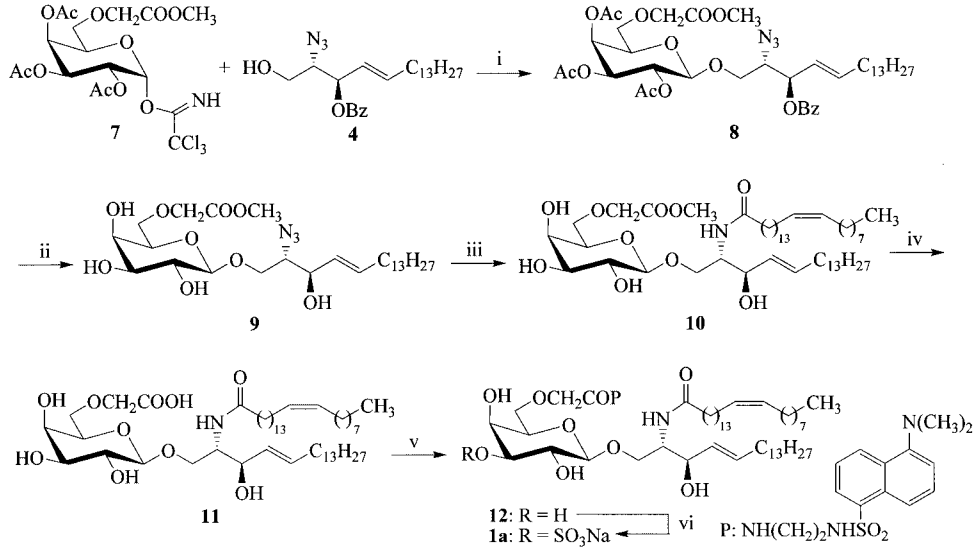
Scheme 2. *Reagents and conditions*: (i): NaH, $ClCH_2COOH$, CH_3I, DMF, 40°C to room temp., 92%; (ii): (a) 90% aq. TFA, 0°C, (b) MeOH, CH_2N_2, Et_2O, 0°C, (c) Ac_2O, Py, room temp., (d) ethylenediamine, glacial acetic acid, THF, room temp., (e) CCl_3CN, DBU, CH_2Cl_2, room temp., 35% (five steps)

Synthesis of the Fluorescent Sulfatide 1a

The galactosyl donor **7** was treated with 3-*O*-benzoyl azidosphingosine **4** in hexane/dichloromethane, with use of triethylsilyl trifluoromethanesulfonate as catalyst, and the desired β-D-galactosylceramide precursor **8** was obtained in 55% yield, accompanied by a small quantity of the 1-OAc derivative of compound **4** (Scheme 3). Compound **8** was subjected to Zemplén reaction conditions to yield compound **9**, which was recovered in 75% yield. After reduction of the azido group of compound **9** with hydrogen sulfide in pyridine/water,[15] the nervonoyl fatty acid chain was introduced by treatment with nervonic acid, hydroxybenzotriazole, and EDCI, affording the galactosylceramide derivative **10** in 57% yield. To conjugate the fluorescent probe to the 6-position of galactose, the carboxymethyl derivative **11**, obtained after hydrolysis of compound **10** with sodium hydroxide in ethanol, was coupled with dansyl-ethylenediamine[21] in the presence of hydroxybenzotriazole and EDCI; the reaction gave the fluorescent compound **12** in 86% yield. Finally, the sulfate group at the 3-position of galactose was introduced as reported by Flitsch et al.,[22] affording the target compound **1a** in 70% yield.

Binding of Synthetic Fluorescent Sulfatide 1a to Soluble Recombinant Human CD1a

Sulfatide binds to CD1a and forms a complex that stimulates specific T cells.[9] We have developed a binding assay of lipids to human recombinant soluble CD1a (sCD1a) immobilized on magnetic beads and have demonstrated that these complexes are biologically active and stimulate the re-



Scheme 3. *Reagents and conditions*: (i) TESOTf, hexane/CH_2Cl_2, (8:2), 0°C to room temp., 0.5 h, 55%; (ii) (a) MeONa, MeOH, room temp., 44 h; (b) CH_2N_2, MeOH, Et_2O, 0°C, 75% (two steps); (iii) (a) H_2S, pyridine, water, room temp., 48 h; (b) nervonic acid, EDCI, HOBt, CH_2Cl_2, reflux, 4 h, 57% (two steps); (iv) NaOH, EtOH, room temp., 2 h; (v) dansyl-ethylenediamine, EDCI, HOBt, CH_2Cl_2, reflux, 3 h, 67% (two steps); (vi) (a) Bu_2SnO, MeOH, reflux, 2 h; (b) $Me_3N{\cdot}SO_3$, THF, room temp., 2 h, 70%

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sponse of specific T cells (manuscript in preparation). This in vitro assay was used to test the capacity of fluorescent sulfatide to bind to CD1a. Figure 2 shows that fluorescent sulfatide binds to sCD1a. The binding depends on the presence of CD1a, because sulfatide does not bind the beads when sCD1a is not present. Furthermore, the binding of fluorescent sulfatide is specific, because it is displaced by unlabelled sulfatide and not by tripalmitin, a lipid unable to bind to CD1a. These results show that the method described here for the synthesis of fluorescent sulfatide generates molecules that retain the capacity to interact and bind to CD1a. Whether this binding is influenced by lipid-binding proteins will be matter of future studies.

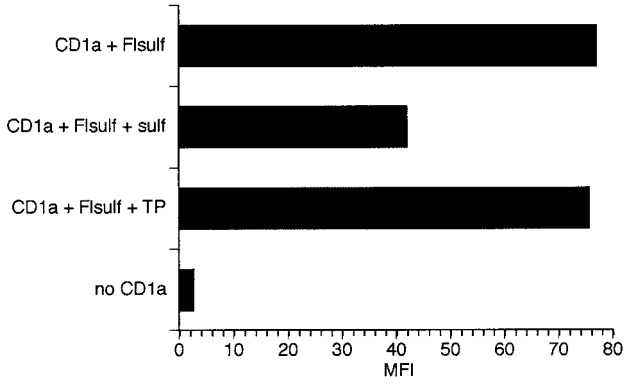

Figure 2. Fluorescent sulfatide binding to soluble recombinant CD1a; fluorescent sulfatide (Flsulf, 30.76 μM) binds to sCD1a and is displaced by unlabelled sulfatide (sulf) but not by tripalmitin (TP); results are expressed as median fluorescent intensity (MFI) and are representative of two independent experiments

Conclusion

The fluorescent sulfatide derivative **1a** with a dansyl probe linked through a spacer to the 6-position of galactose has been synthesized by a synthetic route that exploits a new stereoselective procedure for the preparation of the 3-*O*-benzoyl azidosphingosine. Compound **1a** binds to soluble recombinant human CD1a and is a valuable tool for the study of CD1 antigen binding properties. Further immunological characterization is under study, and will be described in due course.

Experimental Section

General: Optical rotations were measured in $CHCl_3$ solutions with a 241 Perkin−Elmer polarimeter at 20 °C. ^1H NMR and ^{13}C spectra were recorded with a Bruker AM-500 spectrometer. Chemical shifts are reported on the δ (ppm) scale and are relative to TMS as internal reference. The diastereoisomeric ratio of compound **3** was determined by integration of well separated signals in ^1H NMR spectra; in particular, signals relating to 1a-H for **3a** (3.84 ppm) and 3-H for **3b** (4.48 ppm) were integrated. The spectroscopic data for **3a** and **3b** are reported in ref.[16]

IR spectra were recorded on a Perkin−Elmer 1420 spectrophotometer; NaCl crystal windows. MS spectra were recorded in the negative or positive modes on a Thermo Quest Finnigan LCQ$^{\text{DECA}}$ spectrometer with use of electrospray ionization as indicated. All reactions were monitored by TLC on silica gel plates (Merck 60 F-254), spots being developed with 5% sulfuric acid in methanol/water (1:1), or with phosphomolybdate-based reagent. Flash column chromatography was performed on silica gel (230−400 mesh, Merck 60). Organic solutions were dried over sodium sulfate. All evaporations were carried out under reduced pressure at 40°C. Dry solvents and liquid reagents were distilled prior to use: THF, *n*-hexane, and diethyl ether were distilled from sodium; dichloromethane and pyridine were distilled from calcium hydride. DMF, toluene, and methanol were dried on 4Å molecular sieves; triethylamine was distilled from KOH. *Candida antarctica* lipase SP 435L, immobilized on a macroporous acrylic resin (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S. (−)-*N*-Methylephedrine and 1-pentadecyne were purchased from Fluka, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and $Zn(OTf)_2$ from Aldrich. Nervonic acid was purchased from Sigma. Dansyl-ethylenediamine was prepared as in ref.[21] and was obtained pure after crystallization from methanol/water (10:3) (mp, 149−152 °C). NaH was washed three times with hexane prior to use. EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

(2*R*,3*R*)-1,2-*O*-Cyclohexylidene-4-octadecyne-1,2,3-triol (3a): A flask was charged under argon with $Zn(OTf)_2$ (4.27 g, 11.75 mmol) and (−)-*N*-methylephedrine (2.11 g, 11.75 mmol), which were diluted with toluene (20 mL, dried over molecular sieves). Triethylamine (1.64 mL) was added, and the mixture was stirred at room temperature for 3 h, after which 1-pentadecyne (3.08 mL, 11.75 mmol) was added in one portion. After the system had been stirred for 30 min, a solution of the aldehyde **2**[23] (1.00 g, 5.88 mmol) in toluene (10 mL) was added by cannula. After 15 min the reaction was quenched by addition of satd. NH_4Cl (70 mL) and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried and evaporated at reduced pressure. Purification of the material by flash chromatography (*n*-hexane/EtOAc, 9:1) afforded a mixture of addition products **3a** and **3b** (2.03 g, 91%), the diastereomeric ratio of which was established by ^1H NMR analysis. Lipase from *Candida antarctica* (2.00 g) and vinyl acetate (0.64 mL, 6.97 mmol) were added to a solution of this mixture in cyclohexane (30 mL), and the mixture was shaken at 40 °C for 8 h. The enzyme was filtered off and washed with cyclohexane. After evaporation of the solvent, the residue was subjected to flash chromatography (petroleum ether/EtOAc, 9:1), affording pure **3a** (1.79 g, 80% overall yield) as unconverted starting material. The ^1H NMR and $[\alpha]_D$ data were in agreement with those reported in ref.[16] Compound **3a** was transformed into **4** as in ref. 16 in 47% yield.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-methoxycarbonylmethyl-α-D-galactopyranose (6): A mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) (2.00 g, 7.69 mmol) and NaH (2.08 g, 69.2 mmol) in dry DMF (40 mL) was stirred at 40 °C for 10 min, and chloroacetic acid (1.85 g, 19.5 mmol) was then added in small portions at room temperature. Stirring was continued at room temperature until TLC (*n*-hexane/EtOAc, 6:4) showed the disappearance of the starting material. After 24 h, iodomethane (4 mL, 65.0 mmol) was added, the mixture was stirred at room temperature for 20 h, and the reaction was then quenched with MeOH. The mixture was taken up in EtOAc (470 mL), washed with brine (3 × 200 mL), and dried, and the solvents were evaporated. Flash chromatography of the residue on silica gel (*n*-hexane/EtOAc, 7:3) gave the title compound **6** as a colorless oil (2.35 g, 92%), $[\alpha]_D = -73.4$ ($c = 1$, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 1.31, 1.32, 1.42, 1.52 (4s, 12 H, 4

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CH_3), 3.64 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6a}$ = 7.0 Hz, 1 H, 6a-H), 3.72 (s, 3 H, CH_2COOCH_3), 3.82 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6b}$ = 5.0 Hz, 1 H, 6b-H), 4.02 (ddd, $J_{5,6a}$ = 7.0, $J_{5,6b}$ = 5.0, $J_{4,5}$ = 1.5 Hz, 1 H, 5-H), 4.12 (d, J = 16.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.21 (d, J = 16.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.25 (dd, $J_{3,4}$ = 7.5, $J_{4,5}$ = 1.5 Hz, 1 H, 4-H), 4.29 (dd, $J_{1,2}$ = 5.0, $J_{2,3}$ = 2.5 Hz, 1 H, 2-H), 4.58 (dd, $J_{3,4}$ = 7.5, $J_{2,3}$ = 2.5 Hz, 1 H, 3-H), 5.51 ppm (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1-H). ^{13}C NMR ($CDCl_3$): δ = 25.1, 25.6, 26.6, 26.7, 52.5, 67.7, 69.5, 71.1, 71.3, 71.4, 71.8, 97.0, 109.3, 110.0, 171.6 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 1750, 1210, 1070, 1000 cm^{-1}. ESI-MS (positive-ion mode): m/z = 355.1 [M + Na]$^+$.$C_{15}H_{24}O_8$ (332.346) calcd. C 54.21, H 7.28; found C 54.43, H 7.12.

2,3,4-Tri-*O*-acetyl-6-*O*-methoxycarbonylmethyl-α-D-galactopyranosyl Trichloroacetimidate (7): Compound **6** (1.10 g, 3.30 mmol) was dissolved at 0°C in trifluoroacetic acid/water (9:1, 10 mL), and the solution was stirred at this temperature for 10 min, concentrated, and then subjected to azeotropic distillation with toluene (2 × 10 mL). The crude product was diluted with anhydrous MeOH (10 mL), a freshly prepared solution of CH_2N_2 in Et_2O was added dropwise at 0°C until the solution had turned pale yellow, a few drops of acetic acid were added to destroy the excess of CH_2N_2, and the solution was concentrated. The crude product was acetylated with pyridine (10 mL) and acetic anhydride (3 mL); after 24 hours MeOH was added dropwise to quench the excess of acetic anhydride. The solution was diluted with CH_2Cl_2 (50 mL), washed with HCl (0.1 M, 50 mL) and satd. $NaHCO_3$ (50 mL), dried, and the solvents were evaporated. Chromatography of the residue on silica gel (*n*-hexane/EtOAc, 6:4) provided the peracetylated compound as an α,β-mixture (0.74 g, 54%). A solution of this compound in THF (15 mL) was cannulated into a suspension previously prepared by addition of glacial acetic acid (0.145 mL, 2.48 mmol) to a solution of ethylenediamine (0.13 mL, 2.13 mmol) in THF (6 mL) (the addition of AcOH results in an immediate formation of a precipitate, which remains present until aqueous workup). The mixture was stirred overnight, and was then diluted with CH_2Cl_2 (50 mL) and washed with water (1 × 10 mL). After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the organic layers were combined, dried, and concentrated. After flash chromatography (*n*-hexane/EtOAc, 6:4) the product (0.46 g, 68%) was dissolved in CH_2Cl_2 (9 mL). Trichloroacetonitrile (1.21 mL, 12.1 mmol) and DBU (0.036 mL, 0.24 mmol) were added to this solution at room temp. After 2 hours the solution was concentrated and purified by flash chromatography (*n*-hexane/EtOAc/TEA, 10:10:0.1), affording the title compound **7** (0.60 g, 95%) as a colorless oil. [α]D = +80.3 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.99, 2.00, 2.16 (3s, 9 H, $COCH_3$), 3.59 (dd, $J_{6a,6b}$ = 11.5, $J_{5,6a}$ = 7.5 Hz, 1 H, 6a-H), 3.64 (dd, $J_{6a,6b}$ = 11.5, $J_{5,6b}$ = 8.0 Hz, 1 H, 6b-H), 3.70 (s, 3 H, CH_2COOCH_3), 4.00 (d, J = 20.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.08 (d, J = 20.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.41 (dd, $J_{5,6a}$ = 7.5, $J_{5,6b}$ = 8.0 Hz, 1 H, 5-H), 5.34 (dd, $J_{2,3}$ = 13.0, $J_{1,2}$ = 4.2 Hz, 1 H, 2-H), 5.42 (dd, $J_{2,3}$ = 13.0, $J_{3,4}$ = 4.0 Hz, 1 H, 3-H), 5.60 (br. d, $J_{3,4}$ = 4.0 Hz, 1 H, 4-H), 6.57 (d, $J_{1,2}$ = 4.2 Hz, 1 H, 1-H), 8.61 ppm (s, 1 H, NH). ^{13}C NMR ($CDCl_3$): δ = 21.2−21.3 (3 C), 52.5, 67.8, 68.3, 68.5, 69.0, 69.7, 70.9, 94.4, 161.7, 170.6−170.9 ppm (4 C). IR (nujol): $\tilde{\nu}_{max}$ = 1750, 1670 cm^{-1}. ESI-MS (positive-ion mode): m/z = 543.9 [M + Na]$^+$. $C_{17}H_{22}Cl_3NO_{11}$ (522.716) calcd. C 39.06, H 4.24, N 2.68; found C 39.19, H 4.40, N 2.49.

(2*S*,3*R*,4*E*)-2-Azido-3-benzoyloxy-1-(2,3,4-tri-*O*-acetyl-6-*O*-methoxycarbonylmethyl-β-D-galactopyranosyloxy)-4-octadecene (8): A solution of triethylsilyl trifluoromethanesulfonate in CH_2Cl_2 (0.08 M, 0.94 mL) was added under argon at 0°C to a solution of the imidate **7** (0.54 g, 1.02 mmol) and 3-*O*-benzoylazidosphingosine **4** (0.188 g, 0.44 mmol) in *n*-hexane/CH_2Cl_2 (8:2, 9.5 mL). The reaction mixture was kept at 0°C for 10 min and then allowed to warm to room temp.; after 30 min the solution was diluted with CH_2Cl_2 (40 mL) and washed with satd. $NaHCO_3$ (20 mL). After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried and concentrated. Flash chromatography (*n*-hexane/EtOAc from 8:2 to 7:3) first gave the acceptor **4** as the 1-*O*-Ac derivative (0.060 g, 0.13 mmol) and then the title compound **8** (0.19 g, 55%) as an oil. [α]D = −15.0 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, J = 7.0 Hz, 3 H, CH_3), 1.20−1.40 (m, 22 H, 11 CH_2), 1.96, 2.08, 2.14 (3s, 9 H, $COCH_3$), 2.02−2.12 (m, 2 H, 2 6-H), 3.53−3.64 (m, 3 H, 6′a-H, 6′b-H, 1a-H), 3.70 (s, 3 H, CH_2COOCH_3), 3.86−3.96 (m, 3 H, 5′-H, 1b-H, 2-H), 4.00 (d, J = 17.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.05 (d, J = 17.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.47 (d, $J_{1',2'}$ = 7.5 Hz, 1 H, 1′-H), 5.00 (dd, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5 Hz, 1 H, 3′-H), 5.22 (dd, $J_{2',3'}$ = 10.5, $J_{1',2'}$ = 7.5 Hz, 1 H, 2′-H), 5.42 (br. d, 1 H, $J_{3',4'}$ = 3.5 Hz, 4′-H), 5.53 (ddt, $J_{4,5}$ = 15.0, $J_{3,4}$ = 7.5, $J_{4,6}$ = 1.0 Hz, 1 H, 4-H), 5.58 (dd, $J_{3,4}$ = 7.5, $J_{2,3}$ = 3.5 Hz, 1 H, 3-H), 5.91 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 7.0 Hz, 1 H, 5-H), 7.41−7.46 (m, 2 H, Ph), 7.55 (m, 1 H, Ph), 8.01−8.05 ppm (m, 2 H, Ph). ^{13}C NMR ($CDCl_3$): δ = 14.8, 21.3, 21.4, 21.5, 23.3, 29.4−30.4 (9 C), 32.6, 33.1, 52.5, 64.2, 68.1, 68.7, 69.2, 69.4, 70.0, 71.7, 73.1, 75.5, 101.7, 123.3, 129.1 (2 C), 130.4 (2 C), 130.7, 133.9, 139.8, 165.8, 170.1, 170.8, 171.0, 171.1 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 2110, 1760, 1730, 1260, 1240, 1070 cm^{-1}. ESI-MS (positive-ion mode): m/z = 812.2 [M + Na]$^+$. $C_{40}H_{59}N_3O_{13}$ (789.909) calcd. C 60.82, H 7.53, N 5.32; found C 60.69, H 7.33, N 5.48.

(2*S*,3*R*,4*E*)-2-Azido-1-(6-*O*-methoxycarbonylmethyl-β-D-galactopyranosyloxy)octadec-4-en-3-ol (9): Sodium methoxide in dry methanol (0.05 M solution, 2.9 mL) was added to a stirred solution of compound **8** (0.19 g, 0.24 mmol) in dry CH_2Cl_2 (5 mL) and the solution was stirred at room temp. overnight. The solution was neutralized with an ion-exchange resin (Dowex 50 × 8, H^+ form), filtered, and concentrated. Because of partial hydrolysis of the methyl ester, the residue was diluted in MeOH (7 mL) and a freshly prepared solution of CH_2N_2 in Et_2O was added dropwise at 0°C until the solution had turned pale yellow; after 10 min a few drops of acetic acid were added to destroy the excess of CH_2N_2 and the solution was concentrated. Flash chromatography (CH_2Cl_2/MeOH, 8:2) yielded compound **9** (0.10 g, 75%) as an oil. [α]D = −14.2 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1): δ = 0.86 (t, J = 7.0 Hz, 3 H, CH_3), 1.20−1.40 (m, 22 H, 11 CH_2), 2.02−2.08 (m, 2 H, 2 6-H), 3.47 (dd, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.5 Hz, 1 H, 3′-H), 3.52−3.58 (m, 2 H, 1a-H, 2′-H), 3.65−3.80 (m, 7 H, 5′-H, 6′a-H, 6′b-H, 2-H, CH_2COOCH_3), 3.86 (br. d, $J_{3',4'}$ = 3.5 Hz, 1 H, 4′-H), 3.91 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 6.5 Hz, 1 H, 1b-H), 4.13 (d, J = 17.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.17 (t, $J_{2,3}$ = $J_{3,4}$ = 7.5 Hz, 1 H, 3-H), 4.19 (d, J = 17.0 Hz, 1 H, $CH_aH_bCOOCH_3$), 4.22 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, 1′-H), 5.48 (ddt, $J_{4,5}$ = 15.0, $J_{3,4}$ = 7.5, $J_{4,6}$ = 1.0 Hz, 1 H, 4-H), 5.75 ppm (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 7.0 Hz, 1 H, 5-H). ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1): δ = 15.0, 23.9, 30.3−30.9 (9 C), 33.1, 33.6, 52.9, 67.1, 69.7, 70.1 (2 C), 71.8, 72.4, 73.4, 74.7, 75.1, 104.9, 129.4, 137.4, 172.9 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 3450, 2100, 1710, 1270 cm^{-1}. ESI-MS (positive-ion mode): m/z = 582.2 [M + Na]$^+$ (100%), 1140.7 [2 M + Na]$^+$ (75%). $C_{27}H_{49}N_3O_9$ (559.693) calcd. C 57.94, H 8.82, N 7.51; found C 57.73, H 8.63, N 7.72.

(2*S*,3*R*,4*E*)-1-(6-*O*-Methoxycarbonylmethyl-β-D-galactopyranosyloxy)-2-[15(*Z*)-tetracosenoylamino]octadec-4-en-3-ol (10): Compound **9** (0.10 g, 0.18 mmol) was dissolved in pyridine/water (1:1, 6 mL). Hydrogen sulfide was bubbled into the solution

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for 20 min, and the solution was stirred at room temp. for 48 h; the reaction mixture was concentrated, and subjected to azeotropic distillation with toluene. Nervonic acid (0.082 g, 0.22 mmol), EDCI (0.072 g, 0.037 mmol), and HOBt (0.030 g, 0.022 mmol) were added under argon to a solution of the crude amine in CH_2Cl_2 (3 mL). The reaction mixture was stirred at reflux temperature for 4h, and the solvent was then removed under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1), affording **10** (0.090 g, 57%) as a foam. $[\alpha]_D = -4.6$ (c = 1, $CHCl_3$/CH_3OH, 1:1). ^1H NMR ($CDCl_3$/CD_3OD, 1:1): δ = 0.86 (t, J = 7.0 Hz, 6 H, 2 CH_3), 1.20−1.61 (m, 56 H, 28 CH_2), 1.94−2.06 (m, 6 H, 3 CH=CHCH_2), 2.15 (t, J = 7.5 Hz, 2 H, NHCOCH_2), 3.48 (dd, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 3.3 Hz, 1 H, 3′-H), 3.53 (dd, $J_{2',3'}$ = 9.5, $J_{1',2'}$ = 7.5 Hz, 1 H, 2′-H), 3.56 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 3.5 Hz, 1 H, 1a-H), 3.65−3.80 (m, 6 H, 5′-H, 2 × 6′-H, CH_2COOCH_3), 3.86 (br. d, $J_{3',4'}$ = 3.3 Hz, 1 H, 4′-H), 3.93−3.99 (m, 1 H, 2-H), 4.07 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 7.5 Hz, 3-H), 4.10−4.23 (m, 4 H, 1b-H, 1′-H, CH_2COOCH_3), 5.31 (m, 2 H, CH_2CH=CHCH_2), 5.42 (ddt, $J_{4,5}$ = 15.0, $J_{3,4}$ = 7.5, $J_{4,6}$ = 1.0 Hz, 1 H, 4-H), 5.66 ppm (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 7.0 Hz, 1 H, 5-H). ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1): δ = 15.0 (2 C), 23.9−37.7 (32 C), 53.0, 54.7, 69.6, 70.2 (2 C), 71.8, 72.6, 73.2, 74.6, 75.0, 105.2, 130.9, 131.1 (2 C), 135.3, 172.9, 176.0 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 3440, 1730, 1650 cm^{-1}. ESI-MS (negative-ion mode): m/z = 880.4 $[M - H]^-$. $C_{51}H_{95}NO_{10}$ (882.301) calcd. C 69.43, H 10.85, N 1.59; found C 69.61, H 10.63, N 1.74.

(2*S*,3*R*,4*E*)-1-[6-*O*-{2-[5-(Dimethylamino)naphthalene-1-sulfonylamino]ethylaminocarbonylmethyl}-β-D-galactopyranosyloxy]-2-[15(*Z*)-tetracosenoylamino]octadec-4-en-3-ol (12): Compound **10** (0.070 g, 0.079 mmol) was treated with NaOH solution in MeOH (1 M, 1.2 mL) at room temp. for 2 h. MeOH was then evaporated and the crude product was diluted with $CHCl_3$ (20 mL) and washed with HCl solution (5%, 10 mL); after separation the organic phase was washed with brine (4 × 20 mL), dried, and concentrated. Crude acid **11**, dansyl-ethylenediamine[21] (0.042 g, 0.14 mmol), EDCI (0.036 g, 0.19 mmol), and HOBt (0.013 g, 0.096 mmol) were diluted with CH_2Cl_2 (4 mL), the mixture was heated at reflux temperature for 3 h, and the solvent was then removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc/MeOH, 9:1), first affording a small amount of unconverted **10** (0.016 g, 0.018 mmol), and then the title compound **12** (0.060 g, 86% based on conversion) as a light green foam. $[\alpha]_D = -2.85$ (c = 1, Py). ^1H NMR ($CDCl_3$/CD_3OD, 1:1): δ = 0.86 (t, J = 7.0 Hz, 6 H, 2 CH_3), 1.18−1.80 (m, 56 H, 28 CH_2), 1.92−2.02 (m, 6 H, 3 CH=CHCH_2), 2.13 (t, J = 7.5 Hz, 2 H, NHCOCH_2), 2.87 [s, 6 H, N(CH_3)$_2$], 2.96−3.02 (m, 2 H, CONHCH_2), 3.22−3.34 (m, 2 H, CH_2NHSO$_2$), 3.52 (dd, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.5 Hz, 1 H, 3′-H), 3.56 (dd, $J_{2',3'}$ = 10.0, $J_{1',2'}$ = 7.5 Hz, 1 H, 2′-H), 3.60 (dd, $J_{1a,1b}$ = 10.0, $J_{1a,2}$ = 3.0 Hz, 1 H, 1a-H), 3.66−3.77 (m, 3 H, 5′-H, 2 × 6′-H), 3.85 (br. d, $J_{3',4'}$ = 3.5 Hz, 1 H, 4′-H), 3.87 (d, J = 15.5 Hz, 1 H, OCH_aH$_b$CONH), 3.92 (d, J = 15.5 Hz, 1 H, OCH$_a$$H_b$CONH), 3.95−4.02 (m, 1 H, 2-H), 4.08 (t, J = 7.5 Hz, 1 H, 3-H), 4.17 (dd, $J_{1a,1b}$ = 10.0, $J_{1b,2}$ = 4.5 Hz, 1 H, 1b-H), 4.24 (d, $J_{1',2'}$ = 7.5 Hz, 1 H, 1′-H), 5.31 (m, 2 H, CH_2CH=CHCH_2), 5.42 (ddt, $J_{4,5}$ = 15.0, $J_{3,4}$ = 7.5, $J_{4,6}$ = 1.0 Hz, 1 H, 4-H), 5.66 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 7.0 Hz, 1 H, 5-H), 7.21 (d, J = 7.5 Hz, 1 H, ArH), 7.50−7.60 (m, 3 H, 2 ArH, NH), 7.73 (m, 1 H, NHSO$_2$), 8.16 (dd, J = 7.5, J = 2.0 Hz, 1 H, ArH), 8.27 (d, J = 8.5 Hz, 1 H, ArH), 8.53 ppm (d, J = 8.5 Hz, 1 H, ArH). ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1): δ = 15.0 (2 C), 23.9 (2 C), 27.3, 28.4 (2 C), 30.6−31.0 (23 C), 33.1, 33.2, 33.6, 37.8, 40.1, 43.3, 46.4 (2 C), 54.7, 70.2, 70.6, 71.5, 72.0, 72.6, 73.2, 74.6, 74.9, 105.5, 116.6, 120.3, 124.5, 129.5, 130.4, 130.9−131.3 (5 C), 131.6, 135.6, 136.3, 153.2, 172.7, 176.0 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 3460, 1680, 1650, 1340, 1170 cm^{-1}. ESI-MS (negative-ion mode): m/z = 1141.8 $[M - H]^-$. $C_{64}H_{110}N_4O_{11}S$ (1143.643) calcd. C 67.21, H 9.69, N 4.90, S 2.80; found C 67.48, H 10.02, N 4.67, S 2.54.

(2*S*,3*R*,4*E*)-1-[6-*O*-{2-[5-(Dimethylamino)naphthalene-1-sulfonylamino]ethylaminocarbonylmethyl}-3-*O*-(sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-[15(*Z*)-tetracosenoylamino]octadec-4-en-3-ol (1a): Compound **12** (0.040 g, 0.034 mmol) and Bu_2SnO (0.012 g, 0.052 mmol) were stirred in MeOH (2 mL) at reflux under argon for 2h. The solvent was evaporated off under reduced pressure, and the dibutylstannylene complex was treated with $Me_3N{\cdot}SO_3$ (0.010 g, 0.070 mmol) in THF (2 mL) for 2 h. The solvent was removed under reduced pressure, and the residue was then dissolved in $CHCl_3$/MeOH (1:1, 2 mL), loaded onto a cation-exchange resin column (Dowex 50 × 8 Na^+ form, 0.5 × 6 cm), eluted with $CHCl_3$/MeOH (1:1), concentrated under reduced pressure, and subjected to flash chromatography ($CHCl_3$/MeOH, 9:1) to give compound **1a** (70%, 0.030 g) as a foam. $[\alpha]_D = -3.63$ (c = 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$/CD_3OD, 1:1): δ = 0.86 (t, J = 7.0 Hz, 6 H, 2 CH_3), 1.20−1.60 (m, 56 H, 28 CH_2), 1.91−2.03 (m, 6 H, 3 CH=CHCH_2), 2.14 (t, J = 7.5 Hz, 2 H, NHCOCH_2), 2.86 [s, 6 H, N(CH_3)$_2$], 2.93−3.02 (m, 2 H, CONHCH_2), 3.20−3.32 (m, 2 H, CH_2NHSO$_2$), 3.63 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 3.0 Hz, 1 H, 1a-H), 3.66−3.74 (m, 2 H, 2 × 6′-H), 3.74−3.81 (m, 2 H, 2′-H, 5′-H), 3.83 (d, J = 15.5 Hz, 1 H, OCH_aH$_b$CONH), 3.88 (d, J = 15.5 Hz, 1 H, OCH$_a$$H_b$CONH), 3.96−4.02 (m, 1 H, 2-H), 4.07 (t, J = 7.5 Hz, 1 H, 3-H), 4.14 (dd, $J_{1a,1b}$ = 10.5, $J_{1b,2}$ = 4.5 Hz, 1 H, 1b-H), 4.23 (br. d, $J_{3',4'}$ = 3.0 Hz, 1 H, 4′-H), 4.32 (dd, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.0 Hz, 1 H, 3′-H), 4.39 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, 1′-H), 5.31 (m, 2 H, CH_2CH=CHCH_2), 5.41 (ddt, $J_{4,5}$ = 15.5, $J_{3,4}$ = 7.5, $J_{4,6}$ = 1.0 Hz, 1 H, 4-H), 5.66 (dt, $J_{4,5}$ = 15.5, $J_{5,6}$ = 7.0 Hz, 1 H, 5-H), 7.21 (d, J = 7.5 Hz, 1 H, ArH), 7.50−7.61 (m, 2 H, 2 ArH), 8.17 (d, J = 7.5 Hz, 1 H, ArH), 8.28 (d, J = 8.5 Hz, 1 H, ArH), 8.52 ppm (d, J = 8.5 Hz, 1 H, ArH). ^{13}C NMR ($CDCl_3$/CD_3OD, 1:1): δ = 15.0 (2C), 23.9 (2C), 27.3, 28.4 (2C), 30.5−33.7 (26 C), 37.8, 40.2, 43.4, 46.4 (2 C), 54.8, 68.7, 70.8, 70.9, 71.5, 71.9, 73.1, 74.5, 81.3, 104.9, 116.6, 120.4, 124.5, 129.6, 130.5, 130.8, 130.9, 131.1 (2C), 131.3, 131.6, 135.7, 136.3, 153.2, 172.7, 176.2 ppm. IR (nujol): $\tilde{\nu}_{max}$ = 3430, 1660, 1640, 1550, 1250, 1070 cm^{-1}. ESI-MS (negative-ion mode): m/z = 1221.9 $[M - Na]^-$. $C_{64}H_{109}N_4NaO_{14}S_2$ (1245.688) calcd. C 61.71, H 8.82, N 4.50, S 5.15; found C 61.58, H 9.01, N 4.69, S 5.34.

Evaluation of the Biological Activity: Soluble CD1a (sCD1a) was produced and purified in our laboratory as described in ref.[9] This sCD1a was engineered with a BirA peptide tag at the carboxyl terminal and was immobilized on BioMag Streptavidin beads (Qiagen, Basel, CH) previously decorated with biotinylated anti-BirA peptide mAb (Bir1.4 hybridoma, obtained in our laboratory). The formation of the complex between sCD1a/biotinylated mAb/streptavidin beads was confirmed by immunofluorescence analysis with use of a CD1a-specific mAb (NA1/34-HLK FITC-conjugated, Serotec, Oxford, UK). The immobilized CD1a complexes were used in in vitro binding assays with fluorescent sulfatide. Briefly, beads loaded with sCD1a (0.4 nmol) were incubated for 5 min at 20 °C with fluorescent sulfatide (2 nmol) and the excess of unbound sulfatide was removed by washing with PBS. Sulfatide binding was detected by flow cytometry on a FACSVantage SE instrument (Becton Dickinson, Allschwil, CH) equipped with a UV laser. The dansyl group was excited at 351 nm, and emitted light was detected with a 530 nm filter.

Displacement of CD1a-bound fluorescent sulfatide was assessed by exposure of beads with CD1a-bound fluorescent sulfatide for 30

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min to a sixfold excess (12 nmol) of unlabelled synthetic nervonoyl sulfatide[17] or tripalmitin (Supelco, Bellefonte, PA, USA). After this incubation beads were washed and analyzed by flow cytometry as above.

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[1] G. De Libero, L. Mori, *News Physiol. Sci.* **2003**, *18*, 71−76.
[2] M. Brigl, M. B. Brenner, *Annu. Rev. Immunol.* **2004**, *22*, 817−890.
[3] D. B. Moody, S. A. Porcelli, *Nature Rev.* **2003**, *3*, 11−22.
[4] F. Calabi, J. M. Jarvis, L. Martin, C. Milstein, *Eur. J. Immunol.* **1989**, *19*, 285−292.
[5] Z. Zeng, A. R. Castano, B. W. Segelke, E. A. Stura, P. A. Peterson, I. A. Wilson, *Science* **1997**, *277*, 339−345.
[6] S. D. Gadola, N. R. Zaccai, K. Harlos, D. Shepherd, J. C. Castro-Palomino, G. Ritter, R. R. Schmidt, E. Y. Jones, V. Cerundolo, *Nat. Immunol.* **2002**, *3*, 721−726.
[7] D. M. Zajonc, M. A. Elsliger, L. Teyton, I. A. Wilson, *Nat. Immunol.* **2003**, *4*, 808−815.
[8] T. Batuwangala, D. Shepherd, S. D. Gadola, K. J. C. Gibson, N. R. Zaccai, A. R. Fersht, G. S. Besra, V. Cerundolo, E. Y. Jones, *J. Immunol.* **2004**, *172*, 2382−2388.
[9] A. Shamshiev, H.-J. Gober, A. Donda, Z. Mazorra, L. Mori, G. De Libero, *J. Exp. Med.* **2002**, *195*, 1013−1021.
[10] M. M. Altamirano, A. Woolfson, A. Donda, A. Shamshiev, L. Briseno-Roa, N. W. Foster, D. B. Veprintsev, G. De Libero, A. R. Fersht, C. Milstein, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3288−3293.
[11] Y. V. Hoang, L. Micouin, C. Ronet, G. Gachelin, M. Bonin, *ChemBioChem* **2003**, *4*, 27−33.
[12] J. S. Im, K. O. A. Yu, P. A. Illarionov, K. P. LeClair, J. R. Storey, W. M. Kennedy, G. S. Besra, S. A. Porcelli, *J. Biol. Chem.* **2004**, *279*, 299−310.
[13] A previous study on α-galactosylceramide, as CD1d-presented antigen, showed that attachment of the label to the C-6 position of the carbohydrate portion, directly or by means of a short spacer, does not alter the stimulating properties of the parent glycolipid. See: X. T. Zhou, C. Forestier, R. D. Goff, C. Li, L. Teyton, A. Bendelac, P. B. Savage, *Org. Lett.* **2002**, *4*, 1267−1270.
[14] For examples of sulfatide, labeled on the ceramide tail, see: [14a] J. P. Vos, M. L. Giudici, L. M. G. Vangolde, A. Preti, S. Marchesini, M. Lopescardozo, *Biochim. Biophys. Acta* **1992**, *1126*, 269−276. [14b] A. Rousseau, S. Gatt, *Febs Lett.* **1990**, *261*, 271−273.
[15] Y. D. Vankar, R. R. Schmidt, *Chem. Soc., Rev.* **2000**, *29*, 201−216.
[16] F. Compostella, L. Franchini, G. Giovenzana, L. Panza, D. Prosperi, F. Ronchetti, *Tetrahedron: Asymmetry* **2002**, *13*, 867−872.
[17] F. Compostella, L. Franchini, G. De Libero, G. Palmisano, F. Ronchetti, L. Panza, *Tetrahedron* **2002**, *58*, 8703−8708.
[18] [18a] D. E. Frantz, R. Fässler, E. M. Carreira, *J. Am. Chem. Soc.* **2000**, *122*, 1806−1807. [18b] D. E. Frantz, R. Fässler, C. S. Tomooka, E. M. Carreira, *Acc. Chem. Res.* **2000**, *33*, 373−381. [18c] D. Boyall, F. López, H. Sasaki, D. E. Frantz, E. M. Carreira, *Org. Lett.* **2000**, *2*, 4233−4236. [18d] H. Sasaki, D. Boyall, E. M. Carreira, *Helv. Chim. Acta* **2001**, *84*, 964−971. [18e] E. El-Sayed, N. Anand, E. M. Carreira, *Org. Lett.* **2001**, *3*, 3017−3020. [18f] D. Boyall, D. E. Frantz, E. M. Carreira, *Org. Lett.* **2002**, *4*, 2605−2606. [18g] R. Sans Diez, B. Adger, E. M. Carreira, *Tetrahedron* **2002**, *58*, 8341−8344. [18h] N. K. Anand, E. M. Carreira, *J. Am. Chem. Soc.* **2001**, *123*, 9687−9688. [18i] S. Reber, T. F. Knöpfel, E. M. Carreira, *Tetrahedron* **2003**, *59*, 6813−6817.
[19] P. L. Kitov, D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1* **2001**, 838−853.
[20] J. Zhang, P. Kovac, *J. Carbohydr. Chem.* **1999**, *18*, 461−469.
[21] B. Ren, F. Gao, Z. Tong, Y. Yan, *Chem. Physics Lett.* **1999**, *307*, 55−61.
[22] B. Guilbert, N. J. Davis, M. Pearce, R. T. Aplin, S. L. Flitsch, *Tetrahedron: Asymmetry* **1994**, *5*, 2163−2178.
[23] A. Chattopadhyay, V. R. Mamdapur, *J. Org. Chem.* **1995**, *60*, 585−587.

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