DOI: 10.1002/chem.200501586

Synthesis of Sulfated Galactocerebrosides from an Orthogonal β-D-Galactosylceramide Scaffold for the Study of CD1–Antigen Interactions

Federica Compostella,*[a] Silvia Ronchi,[a] Luigi Panza,[b] Sabrina Mariotti,[c] Lucia Mori,[c] Gennaro De Libero,[c] and Fiamma Ronchetti[a]

Abstract: CD1a protein binds sulfatide (3-*O*-sulfo-β-D-galactosylceramide) to form an antigen complex that interacts with T cell receptors and activates T cells. To assess the role of the position of the sulfate in T cell activation, the synthesis of three β-D-galactosylceramides, variously bearing a sulfate at position 2, 4, or 6 of galactose, has been planned and carried out. The compounds were synthesized by an orthogonal sulfation strategy from a common β-D-galactosylceramide scaffold, which was in turn obtained through an efficient glycosylation reaction between a fully orthogonally protected galactosyl imidate and 3-*O*-benzoylazidosphingosine. Immunological evaluation of the three sulfated compounds in CD1a-mediated T cell activation, in comparison with natural sulfatide, provided evidence of the influence of the sulfate position in the recognition event between the antigen, the CD1 protein and the T cell receptor.

Keywords: carbohydrate scaffold · galactosylceramides · glycolipids · protecting groups · sulfatide

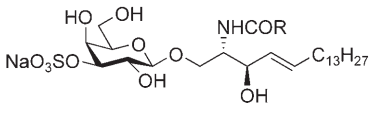
Introduction

The availability of biologically active molecules with well defined chemical and structural properties is a fundamental requirement for structure–function studies in biochemical systems. The basic recognition motif of the ligand, identified through structure elucidation and biological activity studies, can in many cases be used as a template for the preparation of a large number of related derivatives. This approach is useful to assess different biological questions, such as the identification of functionalities capable of modulating the binding of the ligand to biological receptors, the study of potential to induce specific responses of structurally different antigen–receptor complexes, or the development of derivatives for biological tagging, such as with fluorescent probes. In this context, we have recently been involved in the study of sulfatide–protein interactions.[1] Sulfatide is a mammalian glycolipid antigen that participates in intracellular signalling mechanisms and is presented to the immune system by the CD1 family of antigen-presenting surface proteins. The biological mechanism involving sulfatide consists of T cell activation after recognition between the T cell receptor and the CD1–antigen complex; sulfatide is a particularly promiscuous ligand that binds all human CD1 molecules and is presented by the different CD1 isoforms to specific T cells.[2] Structurally it is a β-D-galactosylceramide bearing a sulfate group at position 3 in galactose. The solved 3D structures of CD1s reveal that these molecules each possess a narrow and deep hydrophobic antigen-binding groove, specially tailored to recognize and bind the long alkyl chains of lipid antigens such as sulfatide.[3] In particular, the atomic structure of CD1a with bound sulfatide shows that the protein has two hydrophobic pockets that extend out of the centre of the binding groove, each of which accommodates an alkyl chain,

[a] Dr. F. Compostella, Dr. S. Ronchi, Prof. F. Ronchetti
Dipartimento di Chimica, Biochimica e Biotecnologie per la Medicina, Università di Milano, Via Saldini 50
20133-Milano (Italy)
Fax: (+39) 02-5031-6036
E-mail: federica.compostella@unimi.it

[b] Prof. L. Panza
Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale
Via Bovio 6, 28100-Novara (Italy)

[c] Dr. S. Mariotti, Dr. L. Mori, Prof. G. De Libero
Experimental Immunology, Department of Research
Basel University Hospital, Hebelstrasse 20, 4031-Basel (Switzerland)



Sulfatide

while the charged and polar headgroups of the bound antigen stick out from the middle of the lipid-binding groove of CD1a for T cell receptor recognition. The galactose moiety and the 3-sulfate group of sulfatide (that is, the polar part of the antigen) form contacts with the charged and polar residue of the protein, located at the intersection of the two hydrophobic pockets.[3c] From the consideration that these contacts are important for the orientation of the bound antigen and its presentation to T cell receptor, it is interesting to evaluate how the position of the sulfate on the galactose influences the spatial arrangement of the antigen bound to the CD1a protein and hence the potential of the complex presented to T cells.

To address this problem, the availability of a β-D-galactosylceramide scaffold with a set of mutually orthogonal protecting groups on galactose[4]—through selective deprotection at the desired hydroxy group, sulfation and final removal of the remaining protections—could allow the preparation of a family of sulfated galactosylceramides differing in the position of the sulfate on galactose.

Here we report the synthesis of the β-D-galactosylceramide **1** (Scheme 1) fully orthogonally protected at the sugar moiety. The versatility of this glycolipid scaffold has been investigated through the synthesis of a family of position isomers of sulfatide:[5] that is, a series of β-D-galactosylceramides bearing the sulfate group at positions 2 or 4 or 6 in galactose. These compounds have been tested for activation of a sulfatide-specific and CD1a-restricted T cell clone to assess the role of the position of the sulfate in T cell activation.

Results and Discussion

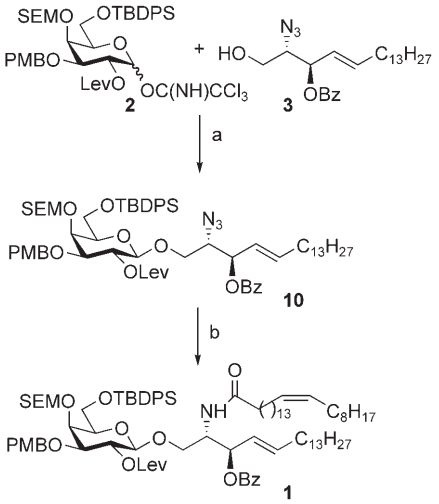
Synthesis: The design of the β-D-glycosylceramidic scaffold **1** involved the incorporation of a levulinate moiety at C-2, a *p*-methoxybenzyloxy group at C-3, and a [β-(trimethylsilyl)ethoxy]methyl (SEM) acetal, together with a *tert*-butyldiphenylsilyl ether, at C-4 and C-6, respectively. The four protecting groups on galactose are orthogonal and are not constrained to any particular deprotection sequence for the introduction of the desired functionalities. Furthermore, although the three ether groups can be simultaneously removed under acid conditions, they are at the same time stable enough to acidic glycosidation procedures. In addition, we were not interested in having selective access to the hydroxy group on ceramide, which was thus protected as a benzoate. As a consequence, the protecting groups on galactose are not only orthogonal between themselves, but also towards the benzoate, which, in contrast, is not orthogonal with respect to levulinate.

The preparation of compound **1** requires quantities of the galactosyl imidate **2** and azidosphingosine **3**, to drive the glycosidation reaction to the production of the glycolipid template (Scheme 1). To date the preparation of glycosylceramides through the Schmidt "azidosphingosine glycosylation procedure" remains the most efficient approach for their synthesis.[6] This procedure has been widely explored in our laboratories, where we have developed efficient large-scale syntheses of (2*S*,3*R*,4*E*)-3-*O*-benzoylazidosphingosine (**3**).[1b,7]

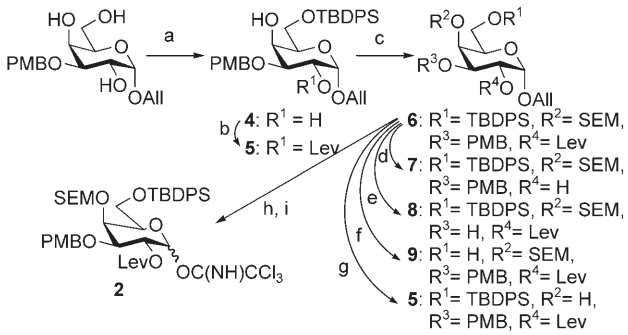
Allyl α-D-galactopyranoside derivative **6** (Scheme 2) was chosen as the precursor of imidate **2** to allow selective deprotection of the anomeric position, thus making compound **6** a fully orthogonally protected derivative.

The synthesis started from allyl 3-*O*-*p*-methoxybenzyl-α-D-galactopyranoside,[8] which was selectively protected at the primary hydroxy group as the *tert*-butyldiphenylsilyl ether to yield compound **4**, which was in turn subsequently acylated at position 2 by treatment with levulinic acid and a condensing agent. Finally, protection of the free 4-OH group was accomplished by use of [β-(trimethylsilyl)ethoxy]-methyl (SEM) chloride and Hünig base to give galactopyranoside **6**.

At this point it was essential to verify the complete orthogonality of this attractive set of protecting groups on this



Scheme 1. a) TESOTf, CH_2Cl_2, m.s., −50 °C, 86 %; b) nervonic acid, Bu_3P, EDCI, CH_2Cl_2, 60 %.



Scheme 2. a) TBDPSCl, CH_2Cl_2/Py, 87 %; b) Levulinic acid, EDCI, DMAP, CH_2Cl_2, 62 %; c) SEMCl, DIPEA, CH_2Cl_2, 95 %; d) NH_2NH_2·AcOH, CH_2Cl_2/MeOH, 89 %; e) DDQ, CH_2Cl_2/H_2O, 85 %; f) TBAF, THF/AcOH, 91 %; g) $MgBr_2$·Et_2O, Et_2O/CH_3NO_2, 87 %; h) Ir catalyst, THF, then NBS, THF/H_2O, 85 %; i) Cl_3CCN, DBU, CH_2Cl_2, 85 %. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, Lev = levulinyl, NBS = *N*-bromosuccinimide, PMB = *p*-methoxybenzyl, SEM = [β-(trimethylsilyl)ethoxy]methyl, TBDPS = *tert*-butyldiphenylsilyl.

"model" compound (Scheme 2), so the Lev ester was removed by treatment with hydrazine acetate in dichloromethane/methanol to give the 2-deprotected compound **7**. Oxidative cleavage of the 3-*O*-*p*-methoxybenzyl group of **6**, on the other hand, was easily accomplished by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane/water to give **8**. The *tert*-butyldiphenylsilyl ether at C-6 was instead removed by treatment with tetrabutylammonium fluoride buffered with acetic acid; these conditions did not affect the levulinate at position 2, which was otherwise partially hydrolyzed if acetic acid was not present. The ether protection at C-4, the SEM group, was cleaved with magnesium bromide in a diethyl ether/nitromethane system, yielding compound **5**. These mild cleavage conditions did not affect the other ether groups on compound **6**. All the deprotection conditions, as tested on allyl galactopyranoside **6**, should also be compatible when the sugar is linked to ceramide, in which there are no groups unstable to these procedures. The allyl group of compound **6** was hydrolysed by smooth isomerization of the allyloxy group, followed by hydrolysis of the enol ether. In conclusion, this particular set of protecting groups should offer an exciting opportunity to obtain a range of well defined structures in a fast and facile manner.

The 1-*O*-unprotected derivative was converted into the trichloroacetimidate donor **2** by treatment with trichloroacetonitrile in dichloromethane in the presence of DBU as the base (Scheme 2).

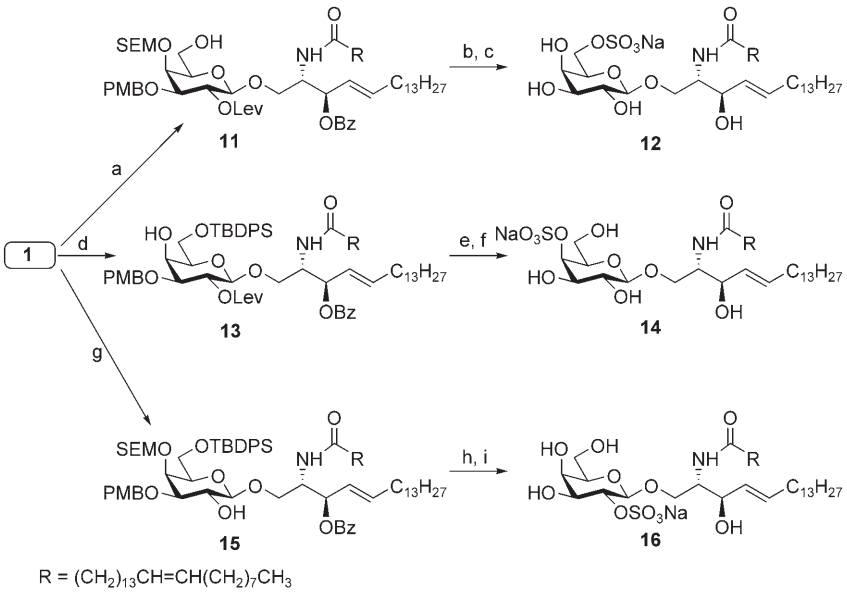
The glycosylation reaction to give **10** (Scheme 1) was successfully accomplished by treating imidate **2** with (2*S*,3*R*,4*E*)-3-*O*-benzoylazidosphingosine (**3**)[1b,7] in dichloromethane at low temperature with catalysis by triethylsilyl triflate. The conditions proved to be compatible with the acid-sensitive ether protecting groups on galactose, provided that the reaction was carried out at −50 °C. The glycosylation product was recovered in high yield and with good stereoselectivity, as confirmed by the characteristic 8.0 Hz value of the *trans*-diaxial $J_{1',2'}$ coupling constant found in the ^{1}H NMR spectrum. The synthesis of β-D-galactosylceramide derivative **1** was completed by reduction of the azido functionality in a Staudinger reaction with concomitant formation of the desired amide bond through condensation with nervonic acid[1a] in the presence of soluble carbodiimide.

With scaffold **1** to hand, the desired 6-*O*-, 4-*O*- and 2-*O*-sulfo-β-D-galactosylceramides were prepared as described in Scheme 3. Glycolipid **1** was first selectively deprotected in high yield at position 6 of galactose and was then subjected to sulfation at the primary hydroxy group by treatment with sulfur trioxide/trimethylamine in DMF. The sulfated compound was fully deprotected by simultaneous removal of ether protections with 5% trifluoroacetic acid in dichloromethane, followed by Zemplén transesterification of the benzoate and levulinate. The desired 6-*O*-sulfated **12** was recovered as its sodium salt after loading onto a cation-exchange resin column, followed by flash chromatography.

On the other hand, treatment of galactosylceramide **1** with magnesium bromide afforded a high yield of 4-*O*-unprotected **13**, which was then sulfated. The sodium salt of 4-*O*-sulfated **14** was recovered after treatment with trifluoroacetic acid, followed by removal of the silyl ether with tetrabutylammonium fluoride, ester deprotection and standard purifications. Finally, compound **1** was selectively deprotected at the 2-position of galactose by hydrolysis of the Lev ester with hydrazine acetate. The free hydroxy group was smoothly sulfated under standard conditions, but a longer reaction time was needed. The desired 2-*O*-sulfated **16** was obtained in good yield after removal of the protecting groups and final purification.

Although it should in principle also be possible to obtain the 3-*O*-sulfate from scaffold **1**, it was not synthesized as it had been prepared by us previously.[1a]

Biology: We evaluated the biological activity of the synthetic sulfated galactosylceramides, differing in the positions of their sulfate groups, by a T cell antigen presentation assay. A CD1a-restricted human T cell clone reactive to natural β-



Scheme 3. a) TBAF, THF/AcOH, 86%; b) $SO_3{\cdot}Me_3N$, DMF, 40 °C, 73%; c) 1) CF_3COOH 5% in CH_2Cl_2, 2) MeONa, $MeOH/CH_2Cl_2$, 3) Dowex Na^+ form, 70%; d) $MgBr_2$, Et_2O/CH_3NO_2, 84%; e) $SO_3{\cdot}Me_3N$, DMF, 40 °C, 68%; f) 1) CF_3COOH 5% in CH_2Cl_2, 2) TBAF, THF, 3) MeONa, $MeOH/CH_2Cl_2$, 4) Dowex Na^+ form, 66%; g) $NH_2NH_2{\cdot}AcOH$, CH_2Cl_2/MeOH 82%; h) $SO_3{\cdot}Me_3N$, DMF, 40 °C, 62%; i) see f), 75%.

D-galactosylceramides bearing the sulfate group in position 3 of galactose was selected for these studies because desulfation experiments had clearly demonstrated the importance of the sulfate group for TCR recognition.[9] The clone was stimulated with two types of CD1a-expressing human antigen-presenting cells (APCs): monocyte-derived dendritic cells (DCs) (Figure 1A), or CD1a-transfected promyelocytic

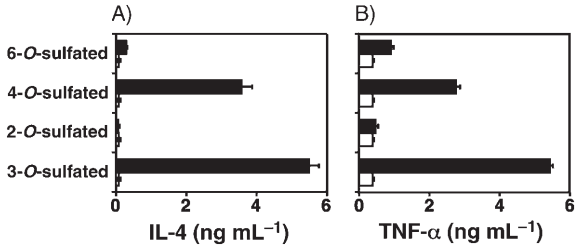


Figure 1. The position of the sulfate group influences the glycolipid immunogenicity. Monocyte-derived DCs (A) or CD1a-transfected human promyelocitic cell line THP-1 (B) were preincubated with (black bars) or without (white bars) the indicated sulfated-β-D-galactosylceramides prior to the addition of sulfatide-specific CD1a-restricted T cell clone K34B9.1. IL-4 or TNF-α released were measured. Data are expressed as means ± SDs (ng mL^{-1}) of triplicates.

THP-1 cells (Figure 1B) preincubated with the various compounds. T cell activation was detected by measuring released IL-4 or TNF-α in the culture supernatant and was compared with the optimal response obtained with the 3-*O*-sulfated galactosylceramide. The 4-*O*-sulfated compound (**14**) and to a lesser extent the 6-*O*-sulfated compound (**12**) were active with both types of APC, whereas the 2-*O*-sulfated analogue (**16**) did not stimulate T cells.

We chose CD1a-restricted presentation of sulfatide as our experimental model in order to exclude the possibility that presentation of sulfatide analogues might be influenced by late-endosomal resident proteins. As CD1a recycles in early endosomes and not in late endosomes,[2] its loading with glycolipid antigens is not influenced by lipid transfer proteins, and nor by sulfatases, which reside in late endosomes. In addition, CD1a can be directly loaded on the surface of antigen-presenting cells without internalization and recombinant CD1a can associate in vitro with sulfatide in the absence of other proteins.[9] These unique characteristics of CD1a loading with sulfatide make it unlikely that differences in antigen presentation would be responsible for the observed reduced responses to the tested analogues.

The published crystal structure of CD1a–sulfatide complex shows the exact position of the galactose, partially protruding out of the CD1a α helices.[3c] In this structure, the sulfate in position 3 is in an optimal orientation to make direct contact with the TCR, thus participating in the establishment of a high-affinity TCR–CD1a–sulfatide interaction, sufficient for T cell stimulation. Our findings with sulfatide analogues are in agreement with this hypothesis and show that the exact position of sulfate is important for optimal stimulation of tested sulfatide-specific T cells.

Moreover, these data also confirm the discriminatory capacity of the T cell receptor, which is influenced by the precise position of the polar moiety of the glycolipid antigen.[10] Indeed, other experimental models have shown the importance for immunogenicity of the anomeric form of the glycosidic bond to the ceramide,[11] of the type of sugars present in the CD1-associated ligand[12,13] and of the presence of additional sugar moieties, which are not cleaved during antigen processing and facilitate or inhibit antigen recognition.[13]

Conclusion

Activation of T cells is triggered by contact between the CD1a–sulfatide antigen complex and specific T cell receptors. Shedding light on the chemical requirements underlying this process may be significant for understanding of the molecular determinants of immune response.

Herein the synthesis of a series of sulfo-β-D-galactosylceramides, each bearing a sulfate ester variously at position 2, 4, or 6 of galactose—that is, a family of position isomers of mammalian sulfatide—through the use of an orthogonally protected galactosylceramide scaffold has been described. The isomers of the natural sulfatide were used to investigate the role of the position of the sulfate ester in determining the antigen–CD1a protein interaction. In this context, our study has confirmed the discriminatory capacity of the T cell receptor, which depends on a molecular recognition process, influenced by the precise position of the polar moiety of the glycolipid antigen.

Moreover, to the best of our knowledge, this is the first example of the preparation of a galactosylceramidic scaffold. We believe that this scaffold and the approach we have presented can be used to obtain new series of cerebroside derivatives, bearing various functionalities at different positions.

Experimental Section

General: Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 20 °C. ^{1}H and ^{13}C NMR spectra were recorded at 298 K with a Bruker AVANCE 500 spectrometer operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are reported on the δ (ppm) scale and are relative to TMS as internal reference. MS spectra were recorded in the negative or positive mode on a Thermo Quest Finnigan LCQ DECA spectrometer by electrospray ionization as indicated. All reactions were monitored by TLC on silica gel 60 F-254 plates (Merck), 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 60 (230–400 mesh, Merck). 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 and diethyl ether were distilled from sodium, whilst dichloromethane and pyridine were distilled from calcium hydride. DMF and methanol were dried on molecular sieves (4 Å). EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-(4-methoxybenzyl)- α-D-galactopyranoside (4): TBDPSCl (4.60 mL, 17.69 mmol) was added at 0 °C to a solution of allyl 3-*O*-(4-methoxybenzyl)-α-D-galactopyranoside[8] (3.00 g,

8.81 mmol) in CH_2Cl_2/Py (2:1, 150 mL). The reaction mixture was allowed to warm slowly to room temperature, and stirring was continued for 20 h. The solution was diluted with CH_2Cl_2 (300 mL), washed with HCl (2 N, 2×150 mL) and water (2×150 mL) and dried, and the solvent was removed under reduced pressure. Flash chromatography (hexane/AcOEt 4:6) afforded **4** as an amorphous white solid (4.44 g, 87%). R_f = 0.48 (hexane/AcOEt 4:6); $[\alpha]_D^{20}$ = +67.7 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = 1.04 (s, 9H; $(CH_3)_3CSi$), 2.05 (d, $J_{2,OH}$ = 8.0 Hz, 1H; OH), 2.41 (s, 1H; OH), 3.60 (dd, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.0 Hz, 1H; H-3), 3.74–3.85 (m, 5H; H-5, -6a and OCH_3), 3.88 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 9.5 Hz, 1H; H-6b), 3.93–4.03 (m, 2H; H-2 and $OCH_aH_bCH{=}CH_2$), 4.05 (br s, 1H; H-4), 4.13–4.19 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.62 (d, J = 11.7 Hz, 1H; OCH_aH_bPh), 4.68 (d, J = 11.7 Hz, 1H; OCH_aH_bPh), 4.94 (d, $J_{1,2}$ = 3.7 Hz, 1H; H-1), 5.15–5.20 (m, 1H; $CH{=}CH_aH_b$), 5.21–5.29 (m, 1H; $CH{=}CH_aH_b$), 5.84–5.94 (m, 1H; $CH{=}CH_aH_b$), 6.85–6.89 (m, 2H; arom.), 7.27–7.43 (m, 8H; arom.), 7.63–7.68 ppm (m, 4H; arom.); ^{13}C NMR ($CDCl_3$): δ = 19.0, 26.6 (3×C), 55.1, 63.0, 66.7, 68.2, 68.4, 70.1, 71.6, 78.2, 97.3, 113.8 (2×C), 117.7, 127.5–135.4 (16×C), 159.2 ppm; ESI-MS (positive-ion mode): m/z (%): 601.3 (30) $[M+Na]^+$, 1179.0 (100) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{42}O_7Si$ (578.76): C 68.48, H 7.31; found: C 68.67, H 7.40.

Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (5)

*From compound **4***: EDCI (4.37 g, 22.80 mmol) and DMAP (1.86 g, 15.20 mmol) were added under argon to a solution of compound **4** (4.40 g, 7.60 mmol) and levulinic acid (1.32 g, 11.4 mmol) in dichloromethane (100 mL). The reaction mixture was stirred at room temperature overnight (TLC: dichloromethane/AcOEt 8:2; R_f of **5** = 0.60), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexane/AcOEt 6:4) to give **5** (3.20 g, 62%) as an oil.

*From compound **6***: $MgBr_2$ (0.46 g, 1.80 mmol) was treated with dry diethyl ether (0.50 mL), and $MeNO_2$ (0.10 mL) was added to this two-phase solution. The resulting (one-phase) solution was added under argon to a stirred solution of compound **6** (0.10 g, 0.12 mmol) in diethyl ether (1 mL). The reaction mixture was stirred for 3 h at room temperature and was then diluted with AcOEt (10 mL) and washed with water (10 mL). The aqueous layer was extracted with AcOEt (2×10 mL), and the combined organic layers were washed with brine (2×10 mL), dried and concentrated. The crude product was purified by flash chromatography (hexane/AcOEt 1:1) to afford **5** (0.073 g, 87%) as an oil. R_f = 0.50 (hexane/AcOEt 1:1); $[\alpha]_D^{20}$ = +59.0 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = 1.02 (s, 9H; $(CH_3)_3CSi$), 2.16 (s, 3H; CH_3CO), 2.50 (br s, 1H; OH), 2.53–2.78 (m, 4H; $OCOCH_2CH_2$), 3.76–3.91 (m, 7H; H-3, -5, -6a, -6b and OCH_3), 3.92–3.98 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.04 (br d, $J_{3,4}$ = 3.0 Hz, 1H; H-4), 4.07- 4.13 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.56 (d, J = 11.7 Hz, 1H; OCH_aCH_bPh), 4.61 (d, J = 11.7 Hz, 1H; OCH_aCH_bPh), 5.01 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1), 5.13 (dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.0 Hz, 1H; H-2), 5.14–5.18 (m, 1H; $CH{=}CH_aH_b$), 5.20–5.27 (m, 1H; $CH{=}CH_aH_b$), 5.81–5.91 (m, 1H; $CH{=}CH_aH_b$), 6.84–6.88 (m, 2H; arom.), 7.23–7.27 (m, 2H; arom.), 7.32–7.43 (m, 6H; arom.), 7.63–7.69 ppm (m, 4H; arom.); ^{13}C NMR ($CDCl_3$): δ = 19.9, 27.5 (3×C), 28.7, 30.5, 38.6, 55.9, 63.8, 68.1, 68.9, 70.7, 71.2, 72.8, 76.0, 95.9, 114.6 (2×C), 118.3, 128.4–136.3 (16×C), 160.1, 172.9, 207.0 ppm; ESI-MS (positive-ion mode): m/z (%): 699.2 (75) $[M+Na]^+$, 1375.0 (100) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{38}H_{48}O_9Si$ (676.87): C 67.43, H 7.15; found: C 67.58, H 7.26.

Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-α-D-galactopyranoside (6): Compound **5** (3.15 g, 4.65 mmol) was dissolved in dry dichloromethane (50 mL). *N*,*N*-Diisopropylethylamine (6.37 mL, 37.20 mmol) and [β-(trimethylsilyl)ethoxy]methyl chloride (3.28 mL, 18.60 mmol) were successively added to this solution, which was then heated at reflux for 15 h under argon, during which an additional quantity of SEMCl (0.80 mL) was added. The solvent was removed under reduced pressure and pure **6** (3.56 g, 95%) was recovered after flash chromatography (hexane/AcOEt 7.5:2.5) as an oil. R_f = 0.63 (hexane/AcOEt 6:4); $[\alpha]_D^{20}$ = +36.7 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.14 (s, 9H; $(CH_3)_3Si$), 0.64–0.76 (m, 2H; $SiCH_2$), 1.03 (s, 9H; $(CH_3)_3CSi$), 2.14 (s, 3H; CH_3CO), 2.52–2.77 (m, 4H; $OCOCH_2CH_2$), 3.43–3.54 (m, 2H; CH_2OCH_2O), 3.75–3.83 (m, 6H; H-5, -6a, -6b and OCH_3), 3.83 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.7 Hz, 1H; H-3), 3.89–3.94 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.03–4.09 (m, 2H; H-4 and $OCH_aH_bCH{=}CH_2$), 4.53 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.61 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.70 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.86 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 5.04 (d, $J_{1,2}$ = 3.7 Hz, 1H; H-1), 5.11–5.23 (m, 3H; H-2 and $CH{=}CH_2$), 5.78–5.87 (m, 1H; $CH{=}CH_2$), 6.83–6.87 (m, 2H; arom.), 7.21–7.26 (m, 2H; arom.), 7.32–7.42 (m, 6H; arom.), 7.62–7.66 ppm (m, 4H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 18.4, 19.9, 27.5 (3×C), 28.7, 30.5, 38.6, 55.9, 64.0, 66.3, 68.8, 71.8, 72.1, 72.8, 73.2, 76.6, 95.8, 96.2, 114.5 (2×C), 118.1, 128.4–136.2 (16×C), 159.9, 172.8, 207.0 ppm; ESI-MS (positive-ion mode): m/z (%): 829.3 (79) $[M+Na]^+$, 1635.7 (100) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{44}H_{62}O_{10}Si_2$ (807.13): C 65.48, H 7.74; found: C 65.39, H 7.80.

Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-α-D-galactopyranoside (7): A solution of hydrazine acetate (0.033 g, 0.36 mmol) in methanol (0.10 mL) was added to a stirred solution of compound **6** (0.10 g, 0.12 mmol) in dichloromethane (10 mL) and the reaction mixture was kept stirring at room temperature for 3 h. Water (10 mL) was added, and after separation the aqueous layer was extracted with dichloromethane (2×10 mL). The combined organic layers were dried, concentrated and purified by flash chromatography (dichloromethane/AcOEt 9:1) to yield **7** (0.076 g, 89%) as an oil. R_f = 0.44 (dichloromethane/AcOEt 9:1); $[\alpha]_D^{20}$ = +85.8 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.14 (s, 9H; $(CH_3)_3Si$), 0.68–0.79 (m, 2H; $SiCH_2$), 1.04 (s, 9H; $(CH_3)_3CSi$), 2.07 (d, $J_{2,OH}$ = 7.5 Hz, 1H; OH), 3.47–3.55 (m, 3H; H-5 and CH_2OCH_2O), 3.59 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 2.7 Hz, 1H; H-3), 3.74–3.82 (m, 5H; H-6a, -6b and OCH_3), 3.93–3.99 (m, 1H; $OCH_aH_bCH{=}CH_2$), 3.99–4.05 (m, 1H; H-2), 4.08–4.14 (m, 2H; H-4 and $OCH_aH_bCH{=}CH_2$), 4.57 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.69 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.71 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.88 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.96 (d, $J_{1,2}$ = 3.7 Hz, 1H; H-1), 5.12–5.18 (m, 1H; $CH{=}CH_aH_b$), 5.19–5.25 (m, 1H; $CH{=}CH_aH_b$), 5.81–5.90 (m, 1H; $CH{=}CH_2$), 6.84–6.89 (m, 2H; arom.), 7.26–7.43 (m, 8H; arom.), 7.62–7.68 ppm (m, 4H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 18.4, 19.9, 27.5 (3×C), 56.0, 64.0, 66.3, 68.9, 69.6, 72.3 (3×C), 79.3, 96.1, 98.0, 114.6 (2×C), 118.5, 128.4–136.2 (16×C), 159.9 ppm; ESI-MS (positive-ion mode): m/z (%): 709.2 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{39}H_{56}O_8Si_2$ (709.03): C 66.06, H 7.96; found: C 66.28, H 8.00.

Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-α-D-galactopyranoside (8): DDQ (0.035 g, 0.16 mmol) was added to a mixture of compound **6** (0.10 g, 0.12 mmol) in dichloromethane (1.5 mL) containing water (5%) and the mixture was stirred vigorously for 3 h, diluted with dichloromethane (15 mL) and washed successively with saturated $Na_2S_2O_3$ (1×10 mL), $NaHCO_3$ (1×10 mL) and brine (1×10 mL). The organic phase was dried and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/AcOEt 7:3) to give compound **8** (0.070 g, 85%) as an oil. R_f = 0.30 (hexane/AcOEt 7:3); $[\alpha]_D^{20}$ = +41.2 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.02 (s, 9H; $(CH_3)_3Si$), 0.88–0.96 (m, 2H; $SiCH_2$), 1.03 (s, 9H; $(CH_3)_3CSi$), 2.15 (s, 3H; CH_3CO), 2.60–2.82 (m, 4H; $OCOCH_2CH_2$), 3.46–3.54 (m, 1H; $CH_aH_bOCH_2O$), 3.71 (dd, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 10.0 Hz, 1H; H-6a), 3.80 (dd, $J_{5,6b}$ = 8.0, $J_{6a,6b}$ = 10.0 Hz, 1H; H-6b), 3.82–3.89 (m, 1H; $CH_aH_bOCH_2O$), 3.89–3.99 (m, 3H; H-3, -5 and $OCH_aH_bCH{=}CH_2$), 4.01 (br d, $J_{3,4}$ = 3.0 Hz, 1H; H-4), 4.03–4.08 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.57 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.75 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.92 (d, $J_{1,2}$ = 3.7 Hz, 1H; H-1), 4.98 (dd, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.3 Hz, 1H; H-2), 5.12–5.17 (m, 1H; $CH{=}CH_aH_b$), 5.21–5.27 (m, 1H; $CH{=}CH_aH_b$), 5.79–5.88 (m, 1H; $CH{=}CH_2$), 7.33–7.44 (m, 6H; arom.), 7.60–7.65 ppm (m, 4H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 18.7, 19.9, 27.5 (3×C), 28.8, 30.5, 38.7, 62.4, 67.5, 68.1, 69.2, 71.1, 72.6, 81.5, 96.6, 97.8, 118.1, 128.5–136.2 (13×C), 173.1, 207.1 ppm; ESI-MS (positive-ion mode): m/z (%): 709.1 (41) $[M+Na]^+$, 1395.8 (100) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{36}H_{54}O_9Si_2$ (686.98): C 62.94, H 7.92; found: C 62.85, H 7.78.

Allyl 2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-α-D-galactopyranoside (9): Tetrabutylammonium fluoride (0.18 mL, 1 M in THF) was added to a solution of compound **6** (0.10 g, 0.12 mmol) in tetrahydrofuran/acetic acid (4:1, 2 mL) and the solution was stirred at room temperature for five days, during which additional aliquots of TBAF were added (overall 5 equiv). The solution was diluted with ethyl acetate (20 mL), washed with $NaHCO_3$ (2×10 mL) and NaCl (1×10 mL), dried and concentrated. The residue was purified by flash chromatography (hexane/AcOEt 1:1) to give compound **9** (0.062 g, 91%) as an oil. R_f = 0.32 (hexane/AcOEt 1:1); $[\alpha]_D^{20}$ = +41.0 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.01 (s, 9H; $(CH_3)_3Si$), 0.86–0.98 (m, 2H; $SiCH_2$), 2.14 (s, 3H; CH_3CO), 2.52–2.77 (m, 4H; $OCOCH_2CH_2$), 3.02 (t, $J_{6,OH}$ = 7.5 Hz, 1H; OH), 3.47–3.53 (m, 1H; $CH_aH_bOCH_2O$), 3.64–3.72 (m, 2H; H-6a, -6b), 3.74–3.82 (m, 4H; $CH_aH_bOCH_2O$ and OCH_3), 3.87–3.94 (m, 2H; H-3, -5), 3.96–4.01 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.06 (br d, $J_{3,4}$ = 3.0 Hz, 1H; H-4), 4.10–4.15 (m, 1H; $OCH_aH_bCH{=}CH_2$), 4.56 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.60 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.76 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.81 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 5.04 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1), 5.15 (dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.5 Hz, 1H; H-2), 5.15–5.20 (m, 1H; $CH{=}CH_aH_b$), 5.22–5.29 (m, 1H; $CH{=}CH_aH_b$), 5.82–5.92 (m, 1H; $CH{=}CH_2$), 6.83–6.88 (m, 2H; arom.), 7.21–7.26 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 18.8, 28.7, 30.5, 38.6, 56.0, 61.3, 67.2, 69.3, 70.4, 71.8, 73.4, 74.5, 76.0, 96.3, 97.6, 114.6 (2×C), 118.3, 129.9 (2×C), 130.9, 134.5, 160.0, 172.8, 206.9 ppm; ESI-MS (positive-ion mode): m/z (%): 591.2 (100) $[M+Na]^+$, 1159.5 (40) $[2M+Na]^+$; elemental analysis calcd (%) for $C_{28}H_{44}O_{10}Si$ (568.73): C 59.13, H 7.80; found: C 58.90, 7.57.

6-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-α,β-D-galactopyranosyl trichloroacetimidate (2): Bis(methyldiphenyl phosphine)cyclooctadiene-iridium(I) hexafluorophosphate (0.098 g, 0.12 mmol) was suspended in dry tetrahydrofuran (25 mL) under Ar, and hydrogen was bubbled through the mixture for 15 min. The resulting clear solution was then added dropwise to a stirred solution of **6** (3.10 g, 3.84 mmol) in dry tetrahydrofuran (40 mL) under Ar. After 3 h, additional tetrahydrofuran (200 mL), water (15 mL), and *N*-bromosuccinimide (1.03 g, 5.77 mmol) were added. After 15 min, the mixture was concentrated, dichloromethane was added (200 mL), and the organic layer was washed with saturated $NaHCO_3$ solution (2× 100 mL), dried and evaporated. The galactopyranose product (2.51 g, 85%) was recovered after flash chromatography (hexane/AcOEt 6:4; R_f=0.22), and dissolved in dry dichloromethane (45 mL). Trichloroacetonitrile (3.28 mL, 32.70 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.10 mL) were added and the mixture was stirred for 2 h at room temperature. The solvent was evaporated, and the residue was purified by flash chromatography (hexane/AcOEt 7:3, 1% triethylamine) to yield **2** (2.53 g, 85%) as an oil. R_f = 0.30 (hexane/AcOEt 7:3); $[\alpha]_D^{20}$ = +20.9 (c= 1 in chloroform); ^{1}H NMR ($CDCl_3$) (selected signals): δ = 3.48 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.0 Hz, 0.3H; H-3$_\beta$), 3.91 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 2.5 Hz, 0.7H; H-3$_\alpha$), 5.38 (dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.5 Hz, 0.7H; H-2$_\alpha$), 5.47 (dd, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.0 Hz, 0.3H; H-2$_\beta$), 5.67 (d, $J_{1,2}$ = 8.5 Hz, 0.3H; H-1$_\beta$), 6.47 (d, $J_{1,2}$ = 3.5 Hz, 0.7H; H-1$_\alpha$), 8.47 (s, 0.7H; α-NH), 8.56 ppm (s, 0.3H; β-NH); ESI-MS (positive-ion mode): m/z (%): 932.0 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{43}H_{58}Cl_3NO_{10}Si_2$ (911.45): C 56.66, H 6.41; found: C 56.49, H 6.36.

(2*S*,3*R*,4*E*)-2-Azido-3-benzoyloxy-1-[6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-β-D-galactopyranosyloxy]-octadec-4-ene (10): Triethylsilyl trifluoromethanesulfonate in dry CH_2Cl_2 (0.1 M solution, 2.1 mL) was added dropwise at −50 °C under argon to a mixture of imidate **2** (2.29 g, 2.52 mmol), 3-*O*-benzoylazidosphingosine (**3**,[7] 0.90 g, 2.10 mmol) and molecular sieves (4 Å, 1.80 g) in dry CH_2Cl_2 (70 mL). After 30 min the reaction mixture was quenched by addition of saturated $NaHCO_3$ solution and filtered over a pad of celite. After separation, the aqueous layer was extracted with CH_2Cl_2 (3×50 mL) and the combined organic layers were dried and concentrated. Purification by flash chromatography (hexane/AcOEt 8:2) gave pure **10** (2.12 g, 86%) as a colourless oil. R_f = 0.38 (hexane/AcOEt 8:2); $[\alpha]_D^{20}$ = −21.0 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.12 (s, 9H; $(CH_3)_3Si$), 0.70–0.76 (m, 2H; $SiCH_2$), 0.86 (t, J = 6.0 Hz, 3H; CH_3), 1.03 (s, 9H; $(CH_3)_3CSi$), 1.11–1.38 (m, 22H; CH_2), 1.96–2.04 (m, 2H; 2×H-6), 2.14 (s, 3H; CH_3CO), 2.54–2.85 (m, 4H; $OCOCH_2CH_2$), 3.33–3.39 (m, 2H; H-3′, -5′), 3.42 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 6.3 Hz, 1H; H-1a), 3.46–3.57 (m, 2H; CH_2OCH_2O), 3.75–3.94 (m, 7H; H-1b, -2, -6a′, -6b′ and OCH_3), 4.04 (br d, $J_{3',4'}$ = 2.5 Hz, 1H; H-4′), 4.26 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.47 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 4.62 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 4.69 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.85 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 5.23 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 5.44–5.54 (m, 2H; H-3, -4), 5.78–5.89 (m, 1H; H-5), 6.84–6.90 (m, 2H; arom.), 7.20–7.26 (m, 2H; arom.), 7.32–7.68 (m, 13H; arom.), 7.98–8.04 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 14.8, 18.5, 19.9, 23.4, 27.5 (3×C), 28.6–38.6 (14×C), 55.9, 63.6, 64.3, 66.3, 68.0, 71.2, 71.9, 72.0, 75.7, 76.5, 79.3, 96.1, 101.8, 114.5 (2×C), 123.4, 128.5–139.4 (22×C), 160.0, 165.8, 172.0, 207.3 ppm; ESI-MS (positive-ion mode): m/z (%): 1200.3 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{66}H_{95}N_3O_{12}Si_2$ (1178.64): C 67.26, H 8.12, N 3.57; found: C 67.39, H 8.21, N 3.62.

(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-[6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (1): Bu_3P (0.47 mL, 1.91 mmol) was added under argon to a solution of compound **10** (1.5 g, 1.27 mmol) and nervonic acid (0.70 g, 1.91 mmol) in dry dichloromethane (25 mL). After consumption of the starting material (3 h), as shown by TLC (hexane/AcOEt 7:3), EDCI (0.73 g, 3.81 mmol) was added and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated off and pure **1** (1.14 g, 60%) was recovered as a colourless oil after purification of the crude product by flash chromatography (hexane/AcOEt from 8:2 to 7:3). R_f = 0.50 (hexane/AcOEt 7:3); $[\alpha]_D^{20}$ = −13.9 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ= −0.12 (s, 9H; $(CH_3)_3Si$), 0.70–0.76 (m, 2H; $SiCH_2$), 0.86 (t, J = 6.5 Hz, 6H; 2×CH_3), 1.01 (s, 9H; $(CH_3)_3CSi$), 1.12–1.36 (m, 54H; 27×CH_2), 1.51–1.60 (m, 2H; CH_2), 1.88–2.04 (m, 9H; 3×$CH_2C{=}C$ and CH_3CO), 2.04–2.18 (m, 2H; CH_2CONH), 2.34–2.42 and 2.47–2.59 and 2.73–2.82 (3×m, 4H; $OCOCH_2CH_2$), 3.29–3.35 (m, 2H; H-3′, -5′), 3.46–3.59 (m, 3H; H-1a and CH_2OCH_2O), 3.73–3.78 (m, 2H; 2×H-6′), 3.78 (s, 3H; OCH_3), 4.03 (br d, $J_{3',4'}$ = 2.5 Hz, 1H; H-4′), 4.07 (dd, $J_{1a,1b}$ = 10.5, $J_{1b,2}$= 3.7 Hz, 1H; H-1b), 4.21 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.36–4.45 (m, 2H; H-2 and OCH_aH_bPh), 4.61 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 4.70 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.84 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 5.23 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 5.27–5.37 (m, 2H; CH=CH), 5.38–5.48 (m, 2H; H-3, -4), 5.78 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5), 6.60 (d, $J_{2,NH}$ = 9.3 Hz, 1H; NH), 6.84–6.87 (m, 2H; arom.), 7.18–7.22 (m, 2H; arom.), 7.28–7.64 (m, 13H; arom.), 7.93–7.97 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 14.8 (2×C), 18.4, 19.8, 23.4–38.5 (38×C), 51.5, 55.9, 63.3, 66.3, 67.8, 71.1, 71.9 (2×C), 75.3, 76.2, 79.5, 96.1, 102.3, 114.5 (2×C), 125.6–137.5 (25×C), 159.9, 166.0, 171.9, 173.8, 207.5 ppm; ESI-MS (positive-ion mode): m/z (%): 1522.6 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{90}H_{141}NO_{13}Si_2$ (1501.25): C 72.00, H 9.47, N 0.93; found: C 72.15, H 9.25, N 0.96.

(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-[2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (11): Tetrabutylammonium fluoride (1.33 mL, 1 M in THF) was added to a solution of compound **1** (0.20 g, 0.13 mmol) in tetrahydrofuran/acetic acid (4:1, 7 mL) and the solution was stirred at room temperature for 5 days, during which additional aliquots of TBAF were added (overall 5 equiv). The solution was diluted with ethyl acetate (20 mL), washed with saturated $NaHCO_3$ (2×10 mL) and NaCl (1×10 mL), dried and concentrated. The residue was purified by flash chromatography (hexane/AcOEt 1:1) to give compound **11** (0.14 g, 86%) as a colourless oil. R_f = 0.29 (hexane/AcOEt 1:1); $[\alpha]_D^{20}$ = −21.9 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = 0.00 (s, 9H; $(CH_3)_3Si$), 0.82–0.98 (m, 8H; 2×CH_3 and $SiCH_2$), 1.15–1.38 (m, 54H; 27×CH_2), 1.52–1.61 (m, 2H; CH_2), 1.94–2.04 (m, 9H; 3×$CH_2C{=}C$ and CH_3CO), 2.06–2.18 (m, 2H; CH_2CONH), 2.36–2.44 and 2.46–2.54 and 2.56–2.64 and 2.71–2.80 (4 m, 4H; $OCOCH_2CH_2$), 3.18 (br s, 1H; OH), 3.40–3.53 (m, 3H; H-3′, 5′ and $CH_aH_bOCH_2O$), 3.56–3.70 (m, 3H; H-1a and 2×H-6′), 3.72–3.80 (m, 4H; OCH_3 and $CH_aH_bOCH_2O$), 3.97–4.02 (m, 2H; H-1b, 4′), 4.27 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.39–4.47 (m, 2H; H-2 and OCH_aH_bPh), 4.56 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 4.76 (d, J= 7.0 Hz, 1H; $CH_2OCH_aH_bO$), 4.81 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_bO$),

5.20 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.3 Hz, 1H; H-2′), 5.27–5.37 (m, 2H; CH=CH), 5.45 (br dd, $J_{3,4}$ = 7.5, $J_{4,5}$ = 15.0 Hz, 1H; H-4), 5.53 (t, $J_{2,3}$ = $J_{3,4}$ = 7.5 Hz, 1H; H-3), 5.83 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5), 6.44 (d, $J_{2,NH}$ = 9.0 Hz, 1H; NH), 6.83–6.87 (m, 2H; arom.), 7.17–7.21 (m, 2H; arom.), 7.38–7.44 (m, 2H; arom.), 7.49–7.55 (m, 1H; arom.), 7.97–8.02 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 14.8 (2×C), 18.8, 23.4–38.5 (35×C), 51.5, 55.9, 61.3, 67.1, 68.2, 71.9, 72.4, 72.6, 75.2 (2×C), 79.2, 97.2, 102.2, 114.5 (2×C), 125.6–137.9 (13×C), 160.1, 166.1, 172.0, 173.8, 207.3 ppm; ESI-MS (positive-ion mode): *m/z* (%): 1284.5 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{74}H_{123}NO_{13}Si$ (1262.85): C 70.38, H 9.82, N 1.11; found: C 70.49, H 9.91, N 1.18.

(2*S*,3*R*,4*E*)-1-[6-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (12): A solution of compound **11** (0.13 g, 0.10 mmol) in dry DMF (2 mL) was treated with sulfur trioxide/trimethylamine complex (0.042 g, 0.30 mmol), and the resulting mixture was stirred at 40 °C for 1 h. The mixture was concentrated to a few microlitres under reduced pressure (high vacuum pump) and purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to afford the 6-sulfated product (0.098 g, 73%) as a colourless oil. R_f = 0.34 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$/CD_3OD 2:1): δ = −0.05 (s, 9H; $(CH_3)_3Si$), 0.75–0.92 (m, 8H; 2×CH_3 and $SiCH_2$), 1.16–1.36 (m, 54H; 27×CH_2), 1.50–1.60 (m, 2H; CH_2), 1.94–2.22 (m, 11H; 3×CH_2C=C and CH_3CO and CH_2CONH), 2.38–2.82 (m, 4H; OCOCH_2CH_2), 3.48 (dd, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.0 Hz, 1H; H-3′), 3.56–3.68 (m, 3H; H-1a and CH_2OCH_2O), 3.75–3.85 (m, 4H; H-5′ and OCH_3), 3.98–4.18 (m, 4H; H-1b, 4′, 6a′, 6b′), 4.28–4.42 (m, 3H; H-1′, -2 and OC$H_a$$H_b$Ph), 4.62 (d, J = 11.5 Hz, 1H; OCH_aH_bPh), 4.72 (d, J = 7.0 Hz, 1H; $CH_2OCH_aH_b$O), 4.85 (d, J= 7.0 Hz, 1H; $CH_2OCH_aH_b$O), 5.17 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 5.27–5.36 (m, 2H; CH=CH), 5.41–5.52 (m, 2H; H-3, -4), 5.81 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 7.0 Hz, 1H; H-5), 6.81–6.89 (m, 2H; arom.), 7.16–7.25 (m, 2H; arom.), 7.38–7.58 (m, 4H; 3×H arom. and NH), 7.93–8.02 ppm (m, 2H; arom.); ESI-MS (negative-ion mode): *m/z* (%): 1340.8 (100) $[M-H]^-$.

The 6-sulfated compound (0.080 g, 0.060 mmol) was treated with trifluoroacetic acid in dichloromethane (5% 2 mL) at room temperature for 10 min., and the solvent was then evaporated under reduced pressure. The residue was dissolved in dry dichloromethane/methanol (1:1, 1.5 mL) and sodium methoxide in dry methanol (0.05 M solution, 1.48 mL) was added. The reaction mixture was stirred overnight at room temperature and was then neutralized with an ion-exchange resin (Dowex 50×8, H^+ form), filtered and concentrated. The solvent was removed under reduced pressure, and the residue was then 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 and subjected to flash chromatography (CH_2Cl_2/MeOH 8:2) to give compound **12** (0.038 g, 70%) as an amorphous white solid. R_f = 0.20 (CH_2Cl_2/MeOH 8:2); $[\alpha]_D^{20}$ = −4.4 (c = 0.5 in chloroform/methanol 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 1:1): δ = 0.88 (t, J = 6.5 Hz, 6H; 2×CH_3), 1.20–1.45 (m, 54H; 27×CH_2), 1.53–1.63 (m, 2H; CH_2), 1.97–2.08 (m, 6H; 3×CH_2C=C), 2.17 (t, J = 7.5 Hz, 2H; CH_2CO), 3.50–3.60 (m, 3H; H-1a, -2′, -3′), 3.75–3.80 (m, 1H; H-5′), 3.94 (d, $J_{3',4'}$ = 2.5 Hz, 1H; H-4′), 3.98–4.02 (m, 1H; H-2), 4.09 (t, $J_{2,3}$ = $J_{3,4}$ = 7.5 Hz, 1H; H-3), 4.14 (dd, $J_{1a,1b}$ = 10.5, $J_{1b,2}$ = 4.7 Hz, 1H; H-1b), 4.16–4.24 (m, 3H; H-1′, -6a′, -6b′), 5.29–5.37 (m, 2H; CH=CH), 5.43 (ddt, $J_{3,4}$ = 7.5, $J_{4,5}$ = 15.0, J_{all} = 1 Hz, 1H; H-4), 5.69 ppm (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1): δ = 13.6 (2×C), 22.5–36.3 (32×C), 53.4, 65.8, 68.2, 69.0, 71.2, 71.9, 72.8, 73.0, 103.8, 129.3, 129.7 (2×C), 134.1, 174.8 ppm; ESI-MS (negative-ion mode): *m/z* (%): 888.9 (100) $[M-Na]^-$; elemental analysis calcd (%) for $C_{48}H_{90}NNaO_{11}S$ (912.28): C 63.19, H 9.94, N 1.54; found: C 63.03, H 9.80, N 1.50.

(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-[6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-levulinyl-3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (13): $MgBr_2$ (0.50 g, 1.95 mmol) was treated with dry diethyl ether (1 mL), and $MeNO_2$ (0.20 mL) was added to this biphasic solution. The resulting (one-phase) solution was added under argon to a stirred solution of compound **1** (0.20 g, 0.13 mmol) in diethyl ether (2 mL). The reaction mixture was stirred for 3 h at room temperature and was then diluted with AcOEt (20 mL) and washed with water (20 mL). The aqueous layer was extracted with AcOEt (3×15 mL), and the combined organic layers were washed with brine (2×20 mL), dried and concentrated. The crude product was purified by flash chromatography (hexane/AcOEt 7:3) to afford **13** (0.15 g, 84%) as an oil. R_f = 0.35 (hexane/AcOEt 7:3); $[\alpha]_D^{20}$ = −1.4 (c = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, J = 6.5 Hz, 6H; 2×CH_3), 1.00 (s, 9H; $(CH_3)_3CSi$), 1.12–1.36 (m, 54H; 27×CH_2), 1.51–1.64 (m, 2H; CH_2), 1.88–2.21 (m, 11H; 3×CH_2C=C, CH_3CO and CH_2CONH), 2.35–2.43 and 2.46–2.63 and 2.75–2.84 (3×m, 5H; OCOCH_2CH_2 and OH), 3.36–3.43 (m, 2H; H-3′, -5′), 3.53 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 3.5 Hz, 1H; H-1a), 3.67 (dd, $J_{6a',6b'}$ = 10.3, $J_{5',6a'}$ = 5.5 Hz, 1H; H-6a′), 3.76–3.83 (m, 4H; H-6b′ and OCH_3), 4.00 (br d, $J_{3',4'}$ = 3.0 Hz, 1H; H-4′), 4.06 (dd, $J_{1a,1b}$ = 10.5, $J_{1b,2}$ = 3.7 Hz, 1H; H-1b), 4.26 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.38–4.44 (m, 1H; H-2), 4.50 (d, J = 12.0 Hz, 1H; OC$H_a$$H_b$Ph), 4.57 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 5.17 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 5.29–5.36 (m, 2H; CH=CH), 5.38–5.50 (m, 2H; H-3, 4), 5.78 (br dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5), 6.63 (d, $J_{2,NH}$ = 9.3 Hz, 1H; NH), 6.83–6.87 (m, 2H; arom.), 7.18–7.64 (m, 15H; arom.), 7.91–7.95 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = 14.8 (2×C), 19.8–38.5 (39×C), 51.5, 55.9, 62.6, 66.4, 67.4, 71.6, 72.0, 75.1, 75.3, 79.0, 101.7, 114.6 (2×C), 125.6–137.6 (25×C), 165.5, 166.0, 172.1, 173.9, 207.6 ppm; ESI-MS (positive-ion mode): *m/z* (%): 1392.7 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{84}H_{127}NO_{12}Si$ (1370.99): C 73.59, H 9.34, N 1.02; found: C 73.70, H 9.40, N 1.05.

(2*S*,3*R*,4*E*)-1-[4-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (14): A solution of compound **13** (0.14 g, 0.10 mmol) in dry DMF (2 mL) was treated with sulfur trioxide/trimethylamine complex (0.042 g, 0.30 mmol), and the resulting mixture was stirred at 40 °C for 3 h. The mixture was concentrated to a few microlitres under reduced pressure (high vacuum pump) and purified by flash chromatography (CH_2Cl_2/MeOH 9.5:0.5) to afford the 4-sulfated product (0.098 g, 68%) as a colourless oil. R_f = 0.15 (CH_2Cl_2/MeOH 9.5:0.5); ^{1}H NMR ($CDCl_3$/CD_3OD 2:1): δ = 0.84 (t, J = 6.5 Hz, 6H; 2×CH_3), 0.97 (s, 9H; $(CH_3)_3CSi$), 1.12–1.36 (m, 54H; 27×CH_2), 1.48–1.62 (m, 2H; CH_2), 1.90–2.15 (m, 11H; 3×CH_2C=C, CH_3CO and CH_2CONH), 2.35–2.43 and 2.46–2.55 and 2.59–2.68 and 2.75–2.84 (4×m, 4H; OCOCH_2CH_2), 3.39–3.43 (m, 2H; H-3′, -5′), 3.48 (dd, $J_{1a,1b}$ = 10.0, $J_{1a,2}$ = 3.5 Hz, 1H; H-1a), 3.76 (s, 3H; OCH_3), 3.84 (dd, $J_{6a',6b'}$ = 11.5, $J_{5',6a'}$ = 7.4 Hz, 1H; H-6a′), 3.99 (dd, $J_{6a',6b'}$ = 11.5, $J_{5',6b'}$ = 4.0 Hz, 1H; H-6b′), 4.07 (dd, $J_{1a,1b}$ = 10.0, $J_{1b,2}$ = 4.5 Hz, 1H; H-1b), 4.25 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.31–4.39 (m, 2H; H-2 and OC$H_a$$H_b$Ph), 4.72 (d, J = 12.0 Hz, 1H; OCH_aH_bPh), 4.79 (d, $J_{3',4'}$ = 3.0 Hz, 1H; H-4′), 4.94 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 5.26–5.34 (m, 2H; CH=CH), 5.35–5.45 (m, 2H; H-3, -4), 5.79 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5), 6.80–6.86 (m, 2H; arom.), 7.18–7.65 (m, 15H; arom.), 7.89–7.95 (m, 3H; arom. and NH) ppm; ESI-MS (negative-ion mode): *m/z* (%): 1448.8 (100) $[M-H]^-$.

The 4-sulfated compound (0.080 g, 0.055 mmol) was treated with trifluoroacetic acid in dichloromethane (5%, 2 mL) at room temperature for 10 min., and the solvent was then evaporated under reduced pressure. Tetrabutylammonium fluoride (0.21 mL, 1 M in THF) was added to a solution of the residue in dry THF (2 mL) and the solution was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in dry dichloromethane/methanol (1:1, 1.5 mL) and sodium methoxide in dry methanol (0.05 M solution, 1.6 mL) was added. The reaction mixture was stirred overnight at room temperature and was then neutralized with an ion-exchange resin (Dowex 50×8, H^+ form), filtered and concentrated. The solvent was removed under reduced pressure, and the residue was then 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 and subjected to flash chromatography (CH_2Cl_2/MeOH 9.5:0.5) to give compound **14** (0.033 g, 66%) as an amorphous white solid. R_f = 0.26 (CH_2Cl_2/MeOH 8:2); $[\alpha]_D^{20}$ = −1.6 (c = 0.75 in chloroform/methanol 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 1:1): δ = 0.87 (t, J = 6.5 Hz, 6H; 2×CH_3), 1.18–1.43 (m, 54H; 27×CH_2), 1.53–1.63 (m, 2H; CH_2), 1.97–2.06 (m, 6H; 3×CH_2C=C), 2.18 (t, J = 7.5 Hz, 2H; CH_2CO), 3.54–3.60 (m, 2H; H-1a, -2′), 3.62–3.68 (m, 2H; H-3′, -5′), 3.72 (dd, $J_{5',6a'}$ = 6.5, $J_{6a',6b'}$

= 11.0 Hz, 1H; H-6a′), 3.83 (dd, $J_{5',6b'}$ = 7.5 Hz, $J_{6a',6b'}$ = 11.0 Hz, 1H; H-6b′), 3.95–4.00 (m, 1H; H-2), 4.08 (t, $J_{2,3} = J_{3,4}$ = 7.5 Hz, 1H; H-3), 4.15 (dd, $J_{1a,1b}$ = 10.0, $J_{1b,2}$ = 4.5 Hz, 1H; H-1b), 4.24 (d, $J_{1',2'}$ = 7.5 Hz, 1H; H-1′), 4.73 (br d, $J_{3',4'}$ = 3.0 Hz, 1H; H-4′), 5.29–5.37 (m, 2H; CH=CH), 5.43 (ddt, $J_{3,4}$ = 7.5, $J_{4,5}$ = 15.0, J_{all} = 1 Hz, 1H; H-4), 5.69 ppm (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.7 Hz, 1H; H-5); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1): δ = 13.7 (2×C), 22.5–36.3 (32×C), 53.3, 60.0, 68.9, 71.6, 71.7, 72.5, 74.0, 74.8, 103.6, 129.3, 129.7 (2×C), 134.1, 174.7 ppm; ESI-MS (negative-ion mode): *m/z* (%): 888.9 (100) $[M-Na]^-$; elemental analysis calcd (%) for $C_{48}H_{90}NNaO_{11}S$ (912.28): C 63.19, H 9.94, N 1.54; found: C 63.30, H 9.99, N 1.58.

(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-[6-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-(4-methoxybenzyl)-4-*O*-{[β-(trimethylsilyl)ethoxy]methyl}-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (15): A solution of hydrazine acetate (0.060 g, 0.65 mmol) in methanol (0.39 mL) was added to a stirred solution of compound **1** (0.20 g, 0.13 mmol) in dichloromethane (13 mL) and the reaction mixture was kept stirring at room temperature for 6 h (TLC: dichloromethane/AcOEt 9:1). Water (15 mL) was added and, after separation, the aqueous layer was extracted with dichloromethane (2×15 mL). The combined organic layers were dried, concentrated and purified by flash chromatography (hexane/AcOEt 7.5:2.5) to yield **15** (0.15 g, 82%) as an oil. R_f = 0.25 (hexane/AcOEt 7.5:2.5); $[\alpha]_D^{20}$ = −8.0 (*c* = 1 in chloroform); ^{1}H NMR ($CDCl_3$): δ = −0.09 (s, 9H; $(CH_3)_3Si$), 0.74–0.81 (m, 2H; $SiCH_2$), 0.86 (t, *J* = 6.5 Hz, 6H; 2×CH_3), 1.02 (s, 9H; $(CH_3)_3CSi$), 1.17–1.37 (m, 54H; 27×CH_2), 1.50–1.60 (m, 2H; CH_2), 1.92–2.04 (m, 6H; 3×CH_2C=C), 2.04–2.14 (m, 2H; CH_2CONH), 3.23 (dd, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 2.5 Hz, 1H; H-3′), 3.32 (t, $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz, 1H; H-5′), 3.46–3.53 (m, 1H; CH_aH$_b$OCH$_2$O), 3.55–3.64 (m, 2H; H-1a and CH$_a$$H_bOCH_2$O), 3.74–3.82 (m, 6H; H-2′, -6a′, -6b′ and OCH_3), 4.03–4.08 (m, 2H; H-1b, -4′), 4.13 (d, $J_{1',2'}$=7.7 Hz, 1H; H-1′), 4.39–4.46 (m, 1H; H-2), 4.55 (d, *J* = 12.0 Hz, 1H; OCH_aH$_b$Ph), 4.69 (d, *J* = 7.0 Hz, 1H; CH$_2$OCH_aH$_b$O), 4.73 (d, *J*= 12.0 Hz, 1H; OCH$_a$$H_b$Ph), 4.84 (d, *J* = 7.0 Hz, 1H; CH$_2$OCH$_a$$H_b$O), 5.29–5.37 (m, 2H; CH=CH), 5.39–5.46 (br dd, $J_{3,4}$ = 7.5, $J_{4,5}$ = 15.5 Hz, 1H; H-4), 5.49 (t, $J_{2,3} = J_{3,4}$ = 7.5 Hz, 1H; H-3), 5.80 (dt, $J_{4,5}$ = 15.5, $J_{5,6}$=6.5 Hz, 1H; H-5), 6.26 (d, $J_{2,NH}$ = 9.5 Hz, 1H; NH), 6.84–6.88 (m, 2H; arom.), 7.25–7.42 (m, 10H; arom.), 7.47–7.53 (m, 1H; arom.), 7.57–7.64 (m, 4H; arom.), 7.95–8.00 ppm (m, 2H; arom.); ^{13}C NMR ($CDCl_3$): δ = −0.8 (3×C), 14.8 (2×C), 18.5–37.6 (37×C), 52.1, 55.9, 63.4, 66.1, 69.7, 71.0, 71.8, 72.1, 75.4, 76.0, 81.4, 96.0, 105.4, 114.6 (2×C), 125.4–137.7 (25×C), 160.0, 166.1, 174.0 ppm; ESI-MS (positive-ion mode): *m/z* (%): 1424.7 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{85}H_{135}NO_{11}Si_2$ (1403.15): C 72.76, H 9.70, N 1.00; found: C 72.90, H 9.78, N 1.03.

(2*S*,3*R*,4*E*)-1-[2-*O*-(Sodium oxysulfonyl)-β-D-galactopyranosyloxy]-2-((*Z*)-tetracos-15-enoylamino)-octadec-4-ene (16): A solution of compound **15** (0.14 g, 0.10 mmol) in dry DMF (2 mL) was treated with sulfur trioxide/trimethylamine complex (0.042 g, 0.30 mmol), and the resulting mixture was stirred at 40°C for 4 days. The mixture was concentrated to a few microlitres under reduced pressure (high vacuum pump) and purified by flash chromatography (CH_2Cl_2/MeOH 9.5:0.5) to afford the 2-sulfated product (0.092 g, 62%) as a colourless oil. ^{1}H NMR ($CDCl_3$/CD_3OD 2:1): δ = −0.16 (s, 6H; $(CH_3)_2SiCH_3$), 0.07 (s, 3H; $(CH_3)_2SiCH_3$), 0.60–0.74 (m, 2H; $SiCH_2$), 0.87 (t, *J* = 6.5 Hz, 6H; 2×CH_3), 0.96 (s, 9H; $(CH_3)_3CSi$), 1.15–1.36 (m, 54H; 27×CH_2), 1.55–1.65 (m, 2H; CH_2), 1.86–1.96 (m, 2H; CH_2C=C), 1.97–2.05 (m, 4H; 2×CH_2C=C), 2.22–2.32 (m, 2H; CH_2CONH), 3.28–3.46 (m, 5H; H-1a, -3′, -5′ and CH_2OCH$_2$O), 3.65–3.73 (m, 2H; H-6a′, 6b′), 3.77 (s, 3H; OCH_3), 3.82 (br d, $J_{3',4'}$ = 3.0 Hz, 1H; H-4′), 4.16–4.23 (m, 2H; H-1b, -1′), 4.33–4.38 (m, 1H; H-2), 4.48 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.0 Hz, 1H; H-2′), 4.59 (d, *J* = 7.0 Hz, 1H; CH$_2$OCH_aH$_b$O), 4.62 (d, *J* = 12.0 Hz, 1H; OCH_aH$_b$Ph), 4.75 (d, *J* = 7.0 Hz, 1H; CH$_2$OCH$_a$$H_b$O), 4.79 (d, *J* = 12.0 Hz, 1H; OCH$_a$$H_b$Ph), 5.29–5.42 (m, 3H; CH=CH and H-4), 5.58 (t, $J_{2,3} = J_{3,4}$ = 7.7 Hz, 1H; H-3), 5.83 (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5), 6.83–6.88 (m, 2H; arom.), 7.16–7.60 (m, 15H; arom.), 7.90 (d, $J_{2,NH}$ = 9.5 Hz, 1H; NH), 7.99–8.04 (m, 2H; arom.) ppm; ESI-MS (negative-ion mode): *m/z* (%): 1480.9 (100) $[M-H]^-$.

The 2-sulfated compound (0.080 g, 0.054 mmol) was deprotected by the procedure used to synthesize compound **14** and afforded the title compound (0.037 g, 75%) as a white amorphous solid after purification by flash chromatography (CH_2Cl_2/MeOH 9:1). R_f = 0.05 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20}$ = −2.2 (*c* = 0.5 in chloroform/methanol 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD 1:1): δ = 0.87 (t, *J* = 6.5 Hz, 6H; 2×CH_3), 1.17–1.47 (m, 54H; 27×CH_2), 1.51–1.63 (m, 2H; CH_2), 1.93–2.08 (m, 6H; 3×CH_2C=C), 2.16–2.34 (m, 2H; CH_2CO), 3.46 (dd, $J_{1a,1b}$ = 9.5, $J_{1a,2}$ = 3 Hz, 1H; H-1a), 3.50 (t, $J_{5',6a'} = J_{5',6b'}$ = 5.8 Hz, 1H; H-5′), 3.67–3.86 (m, 3H; H-3′, -6a′, -6b′), 3.88–3.93 (m, 1H; H-2), 3.95 (d, $J_{3',4'}$ = 3.0 Hz, 1H; H-4′), 4.12 (t, $J_{2,3} = J_{3,4}$ = 7.5 Hz, 1H; H-3), 4.32–4.41 (m, 3H; H-1b, -1′, -2′), 5.29–5.37 (m, 2H; CH=CH), 5.42 (ddt, $J_{3,4}$ = 7.5, $J_{4,5}$ = 15.0, J_{all} = 1 Hz, 1H; H-4), 5.69 ppm (dt, $J_{4,5}$ = 15.0, $J_{5,6}$ = 6.5 Hz, 1H; H-5); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1): δ = 13.5 (2×C), 22.3–36.3 (32×C), 52.8, 61.2, 68.8, 68.9, 71.1, 72.5, 74.3, 77.8, 101.9, 129.3, 129.7 (2×C), 133.8, 174.7 ppm; ESI-MS (negative-ion mode): *m/z* (%): 888.9 (100) $[M-Na]^-$; elemental analysis calcd (%) for $C_{48}H_{90}NNaO_{11}S$ (912.28): C 63.19, H 9.94, N 1.54; found: C 63.30, H 9.98, N 1.56.

Biological assay: All experiments were performed in RPMI 1640 medium containing glutamine (2 mM), Na pyruvate (1 mM) and non-essential amino acids (1%) (all from Cambrex, Verviers, Belgium), Kanamycin (Gibco, 100 μg mL^{-1}) and AB human serum (5%, Swiss Red Cross, Bern).

The sulfatide-specific CD1a-restricted K34B9.1 T cell clone was established and maintained as previously described.[9]

Human promyelocytic cell line THP-1 expressing CD1a was obtained by transfection with human CD1a cDNA. The surface expression of CD1a molecules was tested with a specific mAb (OKT6, ATCC CRL8019, American Type Culture Collection).

DCs from healthy donors were isolated from peripheral blood mononuclear cells by culturing in the presence of GM-CSF and IL-4. Each preparation of DC was tested for the surface expression of CD1a molecules with the OKT6 mAb.

Synthetic compounds for antigen presentation assays were firstly dissolved in a chloroform/methanol 1:1 mixture and then dried under stream of nitrogen. The dried material was re-dissolved in water and sonicated.

DCs (2×10^4 per well) or CD1a-transfected THP-1 cells (4×10^4 per well) were preincubated for 2 h at 37°C with sonicated antigens (15 μg mL^{-1}) before addition of K34B9.1 T cell clone (5×10^4 per well in triplicate). Supernatants were harvested after 36 h and released cytokines were measured by ELISA. TNF-α was detected by use of sandwich ELISA kits according to manufacturer's instruction (Instrumentation Laboratory, Schlieren, CH) and IL-4 was detected by use of anti-IL-4 mAbs (BD PharMingen). Data are expressed as means ± SDs (ng mL^{-1}) of triplicates. All experiments were repeated at least three times.

Acknowledgements

This work was supported by MIUR-Italy (COFIN 2004: "Structure and synthesis of glycolipids"; FIRB 2001: "Development of new immune response modifiers and of peptide and DNA vaccines for tuberculosis immunotherapy"), the Swiss National Foundation (grant No. 3100 A0–109918/1) and the Swiss Multiple Sclerosis Society.

[1] a) F. Compostella, L. Franchini, G. De Libero, G. Palmisano, F. Ronchetti, L. Panza, *Tetrahedron* **2002**, *58*, 8703–8708; b) L. Franchini, F. Compostella, A. Donda, L. Mori, D. Colombo, G. De Libero, P. Matto, F. Ronchetti, L. Panza, *Eur. J. Org. Chem.* **2004**, 4755–4761.
[2] G. De Libero, L. Mori, *Nat. Rev. Immunol.* **2005**, *5*, 485–496.
[3] a) Z. Zeng, A. R. Castano, B. W. Segelke, E. A. Stura, P. A. Peterson, I. A. Wilson, *Science* **1997**, *277*, 339–345; b) 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; c) D. M. Zajonc, M. A. Elsliger, L. Teyton, I. A. Wilson, *Nat. Immunol.* **2003**, *4*, 808–815; d) 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; e) M. Koch, V. S. Stronge, D. Shepherd, S. D. Gadola, B. Mathew, G. Ritter, A. R. Fersht, G. S. Besra, R. R. Schmidt, E. Y. Jones, V. Cerundolo, *Nat. Immunol.* **2005**, *6*, 819–826.

[4] Examples of multifunctional scaffolds based on carbohydrates: a) T. Wunberg, C. Kallus, T. Opatz, S. Henke, W. Schmidt, H. Kunz, *Angew. Chem.* **1998**, *110*, 2620–2622; *Angew. Chem. Int. Ed.* **1998**, *37*, 2503–2505; b) C.-H. Wong, X.-S. Ye, Z. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 7137–7138; c) C. Kallus, T. Opatz, T. Wunberg, W. Schmidt, S. Henke, H. Kunz, *Tetrahedron Lett.* **1999**, *40*, 7783–7786; d) M. Ghosh, R. G. Dulina, R. Kakarla, M. J. Sofia, *J. Org. Chem.* **2000**, *65*, 8387–8390; e) F. Peri, F. Nicotra, C. P. Leslie, F. Micheli, P. Seneci, C. Marchioro, *J. Carbohydr. Chem.* **2003**, *22*, 57–71; f) T. Opatz, C. Kallus, T. Wunberg, W. Schmidt, S. Henke, H. Kunz, *Eur. J. Org. Chem.* **2003**, 1527–1536; g) U. Hünger, J. Ohnsmann, H. Kunz, *Angew. Chem.* **2004**, *116*, 1125–1127; *Angew. Chem. Int. Ed.* **2004**, *43*, 1104–1107; h) S. Castoldi, M. Cravini, F. Micheli, E. Piga, G. Russo, P. Senesi, L. Lay, *Eur. J. Org. Chem.* **2004**, 2853–2862; i) R.-H. Fan, J. Achkar, J. M. Hernández-Torres, A. Wei, *Org. Lett.* **2005**, *7*, 5095–5098; j) F. Sicherl, V. Wittmann, *Angew. Chem.* **2005**, *117*, 2133–2136; *Angew. Chem. Int. Ed.* **2005**, *44*, 2096–2099.

[5] A. Marinier, A. Martel, J. Banville, C. Bachand, R. Remillard, P. Lapointe, B. Turmel, M. Menard, W. E. Harte, Jr., J. J. K. Wright, G. Todderud, K. M. Tramposch, J. Bajorath, D. Hollenbaugh, A. Aruffo, *J. Med. Chem.* **1997**, *40*, 3234–3247.

[6] Y. D. Vankar, R. R. Schmidt, *Chem. Soc. Rev.* **2000**, *29*, 201–216.

[7] F. Compostella, L. Franchini, G. Giovenzana, L. Panza, D. Prosperi, F. Ronchetti, *Tetrahedron: Asymmetry* **2002**, *13*, 867–872.

[8] L. Lay, L. Panza, L. Poletti, D. Prosperi, S. Canevari, M. E. Perico, *Eur. J. Org. Chem.* **2001**, 4331–4336.

[9] A. Shamshiev, H.-J. Gober, A. Donda, Z. Mazorra, L. Mori, G. De Libero, *J. Exp. Med.* **2002**, 195, 1013–1021.

[10] D. B. Moody, D. M. Zajonc, I. A. Wilson, *Nat. Rev. Immunol.* **2005**, *5*, 1–14.

[11] T. Kawano, J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, H. Koseki, M. Taniguchi, *Science* **1997**, *278*, 1626–1629.

[12] D. B. Moody, B. B. Reinhold, M. R. Guy, E. M. Beckman, D. E. Frederique, S. T. Furlong, S. Ye, V. N. Reinhold, P. A. Sieling, R. L. Modlin, G. S. Besra, S. A. Porcelli, *Science* **1997**, *278*, 283–286.

[13] A. Shamshiev, A. Donda, T. I. Prigozy, L. Mori, V. Chigorno, C. A. Benedict, L. Kappos, S. Sonnino, M. Kronenberg, G. De Libero, *Immunity* **2000**, *13*, 255–264.

Received: December 17, 2005
Revised: February 8, 2006
Published online: April 25, 2006

