Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 13 (2005) 2907–2916

Synthesis and human NKT cell stimulating properties of 3-*O*-sulfo-α/β-galactosylceramides

Guo-Wen Xing,[a] Douglass Wu,[a] Michael A. Poles,[b,c] Amir Horowitz,[b,c] Moriya Tsuji,[b,c,*] David D. Ho[c,*] and Chi-Huey Wong[a,*]

[a]*Department of Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*
[b]*Department of Medical and Molecular Parasitology, New York University School of Medicine, 341 E. 25th Street, New York, NY 10010, USA*
[c]*Aaron Diamond AIDS Research Center, The Rockefeller University, 455 1st Avenue, New York, NY 10016, USA*

Accepted 8 February 2005

Abstract—Two novel hybrid molecules 3-*O*-sulfo-α/β-galactosylceramide **3** and **4**, which are derived from an immunostimulatory agent α-GalCer **1** and self-glycolipid ligand sulfatide **2**, were designed and synthesized. Compound **3** was shown to efficiently stimulate human NKT cells to secret IL-4 and IFN-γ, with activities similar to **1**, suggesting that modification of the 3″-OH position of the galactose moiety with sulfate has no significant effect on NKT cell stimulation. As a comparison, the β-isomer **4** has no affinity to NKT cells, which demonstrates that the α-glycosidic bond of galactosylceramide is crucial to the NKT cells activation.

1. Introduction

A marine-sponge-derived glycolipid α-galactosylceramide (α-GalCer, **1**) was found to exhibit antitumor activities.[1] Further investigation showed that **1** was a highly potent immunostimulatory agent and was identified to act as a specific ligand presented by CD1d to the invariant mouse $V_\alpha14$ or human $V_\alpha24$ antigen receptor of natural killer T-cells (NKT cells) to activate the immune system.[2,3] Injection of **1** to tumor-bearing mice was shown to be efficacious against tumor growth and metastasis in a CD1d-mediated NKT cell-dependent manner.[4] Moreover, studies have demonstrated that NKT cells can produce various immunoregulatory cytokines such as IL-4 and IFN-γ, and play a major role in immunoregulation, tumor immunity, and the prevention of autoimmune disease.[5,6]

Sulfatide, **2**, broadly exists in the serum of mammals.[7] From its chemical structure, sulfatide is similar to α-galactosylceramide with the exception of a β anomeric linkage to the ceramide, a sulfate group at 3″-OH of the galactose moiety, and a *trans*-olefin instead of an OH at the 4 position of the sphingosine base. Its ceramide portion is composed of hydroxy or nonhydroxy fatty acid, which has lengths varying from C-14 to C-26.[8] It has been reported that **2** can be presented by CD1a, CD1b, and CD1c to specific T-cells or is capable of stimulating sulfatide-specific CD1a-restricted T-cell clones.[9,10] In addition, **2** is sufficient to prevent antigen-induced experimental autoimmune encephalomyelitis through suppressing both Th1 (IFN-γ) and Th2 (IL-4) cytokine secretion by pathogenic myelin protein-reactive T-cells.[11]

Recent efforts have been directed toward the design and synthesis of new α-GalCer analogs. However, aside from a C-linked analog of α-GalCer, few compounds were reported to possess more efficient bioactivity than α-GalCer.[12,13] Furthermore, despite the clear activity of α-GalCer against experimental mice tumors and their metastases, a recent phase I trial with 24 patients bearing solid tumors performed in The Netherlands indicates that α-GalCer did not display antitumor activity.[14] Sulfatide **2** is a self-glycolipid ligand recognized by a population of CD1d-restricted T cells that is distinct from the

Keywords: Glycolipid; Immunology; NKT cell activation.
* Corresponding authors. Tel.: +1 858 784 2487; fax: +1 858 784 2409 (C.-H.W.); fax: +1 212 263 8116 (M.T.); fax: +1 212 725 1126 (D.D.H.); e-mail addresses: moriya.tsuji@med.nyu.edu; dho@adarc.org; wong@scripps.edu

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.02.018

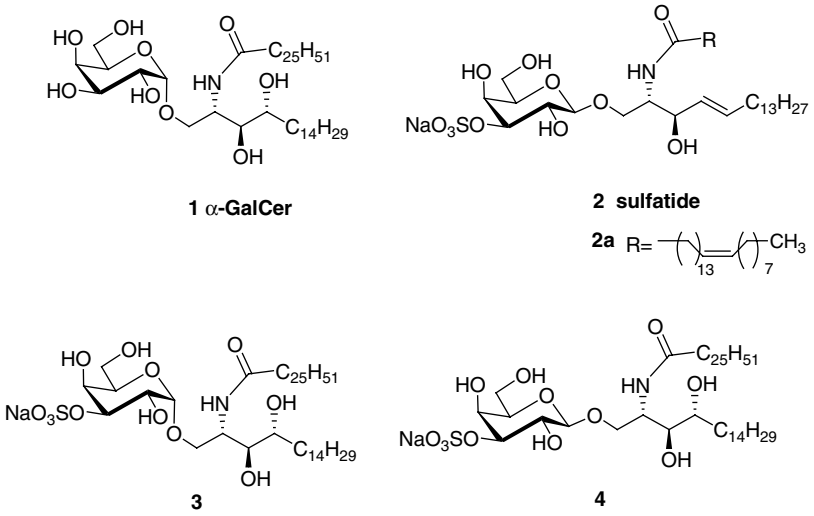


Figure 1. Structures of α-galactosylceramide (**1**), sulfatide (**2**) and (**2a**), and 3-*O*-sulfo-α/β-galactosylceramides (**3**) and (**4**).

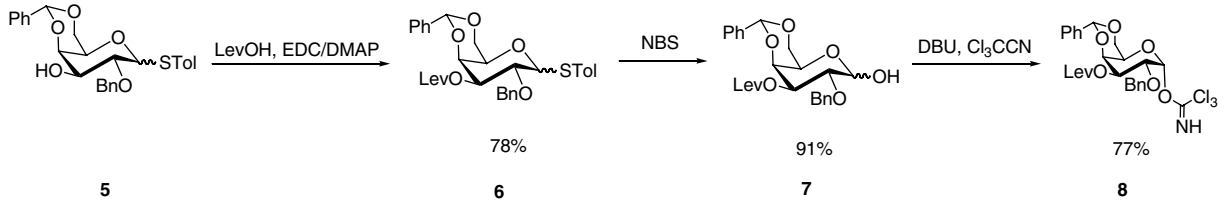
α-GalCer-reactive NKT lymphocytes.[11] On the basis of these findings and considering the molecular similarity of α-GalCer and sulfatide **2**, we have designed and synthesized two hybrid molecules of **1** and **2**, which are sulfate derivatives 3-*O*-sulfo-α/β-galactosylceramides **3** and **4**, and studied their human NKT cell stimulating properties (Fig. 1). We have found that **3** has immunostimulatory activity equivalent to, or better than, that of **1**.

2. Results and discussion

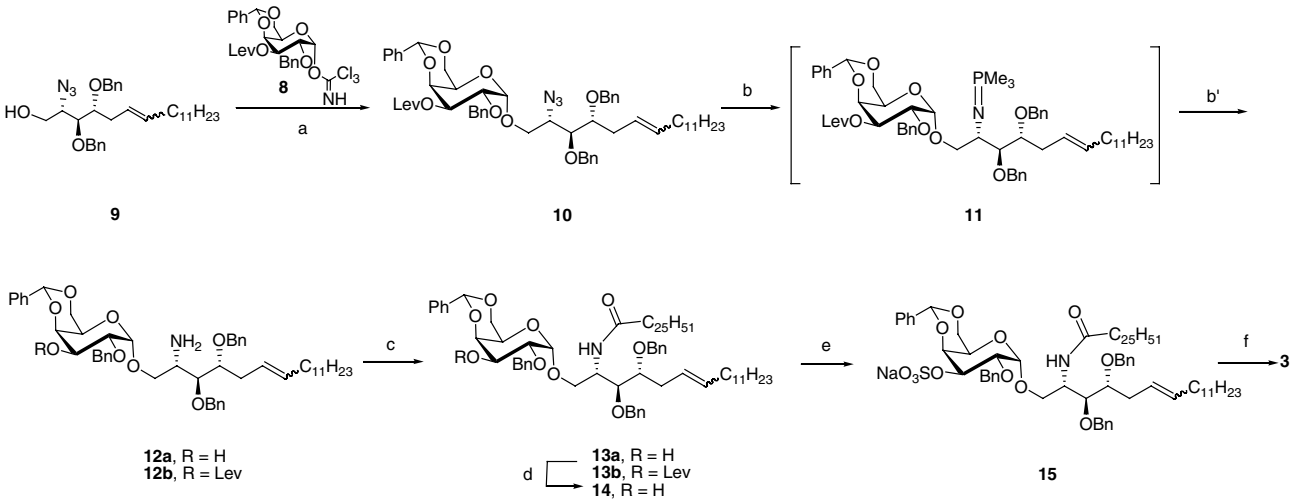
Selective sulfation at 3″-OH of galactose moiety is the key step for the synthesis of **3**. Traditionally, regioselective sulfation of the 3-hydroxyl of sugar ring utilizes dibutylstannylene acetals as activated intermediates. However, this method can only be applied to β-galactosides; for α-galactosides, the dibutylstannylene acetal can form a complex between the 2-hydroxyl and the anomeric oxygen to give the 2″-*O*-derivative by reaction with an electrophile.[15] Addressing this question, we prepared a 2″-benzyl-4″,6″-benzylidene-3″-levulinoyl-galactosyl trichloroacetimidate donor **8**. The orthogonal levulinoyl (Lev) protecting group can be selectively removed after glycosylation in the presence of hydrazine. The benzyl and benzylidene groups at the 2, and 4,6 positions, respectively, direct the next α-glycosidic bond formation.[16]

As shown in Scheme 1 the preparation of **8** started with the known thioglycoside **5**[17] in 55% yield over three steps. The sphingosine building block **9** was employed in this synthesis, which has been synthesized in the preparation of α-GalCer from our previous study.[16b] Donor **8** was coupled to acceptor **9** with TMSOTf as promoter to give the expected α-glycoside **10** in a moderate yield. During the Staudinger reduction of **10** with PMe_3, 1 M NaOH was used to hydrolyze the imino-phosphorane intermediate **11**. However, the Lev group was partially labile under this condition and approximately 50% of the Lev group was cleaved to give an amine mixture of **12a** and **12b** (1:1) as determined by ^1H NMR. Since **12a** had already possessed a free C-3 hydroxyl, it was crucial to choose a selective coupling reagent in the condensation between amine **12a** and the fatty acid. It was reported that DEPBT [3-(diethoxyphosphoryloxy)-(1,2,3)-benzotriazin-4(3*H*)-one] was used in a similar case, and can selectively form an amide bond in the presence of unprotected hydroxyl groups.[18] For this purpose, DEPBT was used for the reaction between mixture **12a**,**b**, and hexacosanoic acid to give **13a** and **13b**, followed by deprotection of the remaining Lev groups using hydrazine to provide the desired galactosylceramide **14** in 56% yield over three steps. Treating the 3″-OH free glycolipid **14** with Py·SO_3 led to the sulfate derivative **15** in high yield,[19] which gave **3** upon hydrogenation with palladium black and neutralization with $NaHCO_3$ aqueous solution in 78% yield (Scheme 2).

For the synthesis of **4**, we firstly tried the glycosylation between perbenzoylated trichloroacetimidate donor **16**



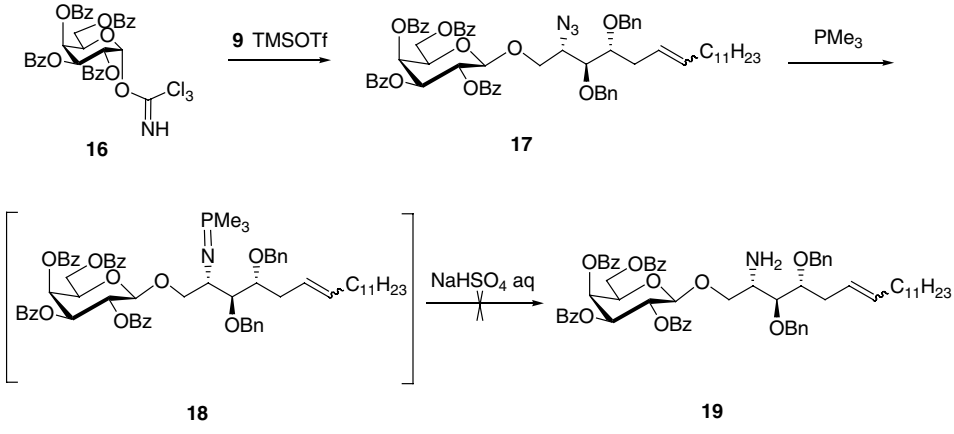
Scheme 1. Preparation of donor **8**.



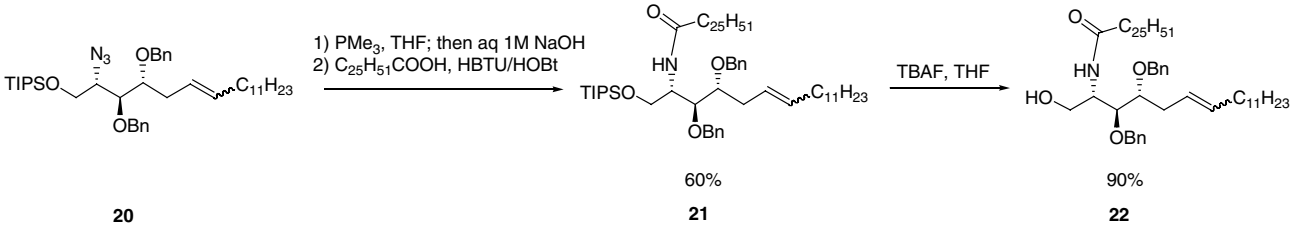
Scheme 2. Synthesis of 3-*O*-sulfo-α-D-galactosylceramide, sodium salt, **3**. Reagents and conditions: (a) TMSOTf/THF, 46%; (b) PMe_3, THF; (b′) aq 1 M NaOH; (c) $C_{25}H_{51}COOH$, DEPBT, CH_2Cl_2; (d) NH_2NH_2/HOAc/Py, 56% (three steps); (e) Py·SO_3, Py; then aq $NaHCO_3$, 90%; (f) H_2, Pd/C, HOAc/MeOH; then aq $NaHCO_3$, 83%.

and sphingosine acceptor **9** and obtained the desired β-galactosylceramide derivative **17**. After the Staudinger reduction of **17**, a complex mixture was produced and the expected amine **19** not isolated (Scheme 3). Since the perbenzoylated galactosylceramide is sensitive to basic conditions, a $NaHSO_4$ solution instead of a NaOH solution was used for the reduction workup procedure to decompose the imino-phosphorane intermediate **18**. However, hydrolysis of **18** into **19** was very slow, and in turn, the longer reaction time led to the degradation of glycosidic bond and formation of numerous side products.

An alternative synthetic strategy to first reduce the azide and then couple the fatty acid before the glycosylation step was employed. Compound **22** was prepared from the sphingosine derivative **20**[16b] in 54% yield over two steps (Scheme 4). In the presence of TMSOTf as promoter, the ceramide acceptor **22** was reacted with donor **16** to give the β-glycoside **23** in 54% yield. After deben-



Scheme 3. Preparation of amine **19**.



Scheme 4. Preparation of acceptor **22**.

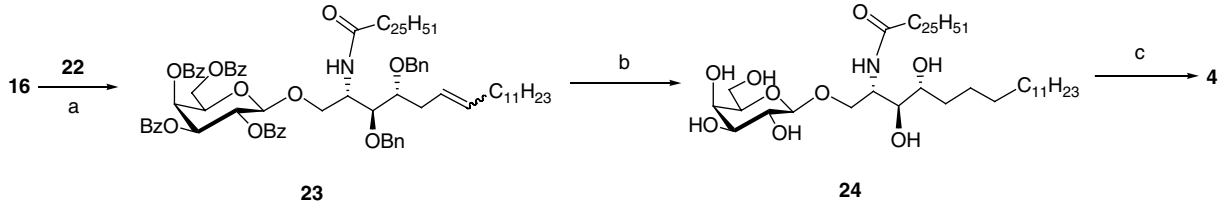
zoylation and hydrogenation of **23**, the β-galactosylceramide **24** was obtained in quantitative yield. Compound **24** was finally sulfated by $Bu_2SnO/Me_3N{\cdot}SO_3$ and subsequently neutralized by $NaHCO_3$ to give the expected **4** in 80% yield (Scheme 5).[10]

Various glycolipids were used to determine their immunostimulatory activities in this study. The structures of compounds **25**–**34** were shown in Figure 2. As illustrated in Figure 3a–c, the response of human NKT cells to glycolipid presented by $CD14^+$ DCs, 3-sulfo-α-GalCer **3** was found to be similar to or even better than α-GalCer at 10–20 μg/mL. Other glycolipids including sulfatide **2a** and 3-sulfo-β-GalCer **4** had weak or no affinity to NKT cells. Compound **3** can efficiently stimulate IL-4 and IFN-γ secretion, suggesting that modification of 3″-OH position of galactose moiety with sulfate is helpful to NKT cells stimulation. These results were confirmed by the studies using a human NKT cell line, in which the stimulatory activity of 3-sulfo-α-GalCer **3** against human NKT cells to secrete IFN-γ and IL-4 was similar to that of α-GalCer regardless of the dosage, that is, 10, 2, 0.4, and 0.08 μg/mL (Fig. 4a and b). Figure 4a and b further demonstrate that the reactivity of compound **3** with human NKT cells requires CD1d molecules, because Hela cells transfected with a human CD1d gene but not wild-type Hela cells lacking the CD1d, were able to stimulate a human NKT cell line. These results confirm the binding of compound **3** to the CD1d molecules.

NKT cell activation is sensitive to the configuration of the anomeric carbon of the glycolipid antigen molecule.[2] 3-Sulfo-β-GalCer **4** has no affinity to NKT lymphocytes due to the β-linkage of the glycosidic bond, indicating that an α-linkage is essential to NKT cell recognition even despite having binding, even the helpful sulfate group existing at the 3″ position of the sugar ring.

3. Conclusion

In summary, two novel hybrid molecules 3-*O*-sulfo-α/β-galactosylceramides **3** and **4**, which are derived from an immunostimulatory agent α-GalCer **1** and self-glyco-



Scheme 5. Synthesis of 3-*O*-sulfo-β-D-galactosylceramide, sodium salt, **4**. Reagents and conditions: (a) $TMSOTf/CH_2Cl_2$, 54%; (b) $H_2/Pd(OH)_2$/EtOAc; then $NaOMe/MeOH–CH_2Cl_2$, quantitative (two steps); (c) Bu_2SnO/MeOH; $Me_3N{\cdot}SO_3$/THF, then aq $NaHCO_3$/MeOH, 80% (two steps).

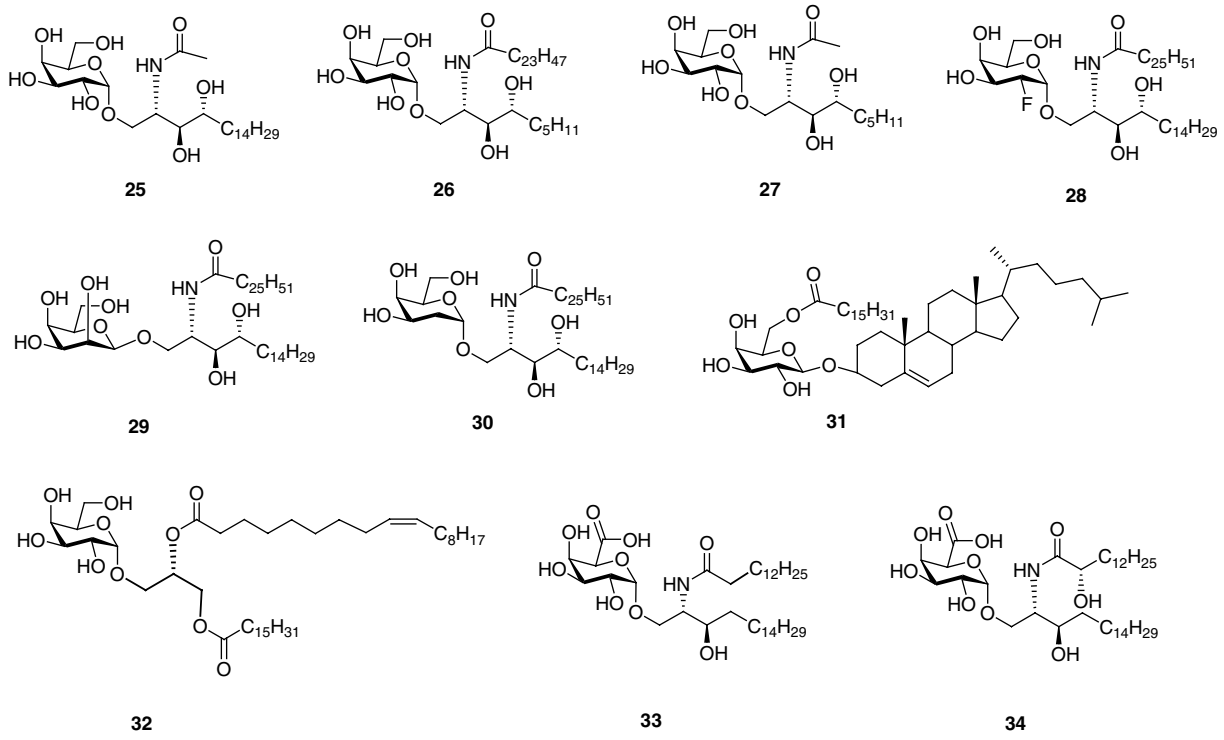


Figure 2. Structures of additional glycolipid compounds examined for immunostimmulatory activity.

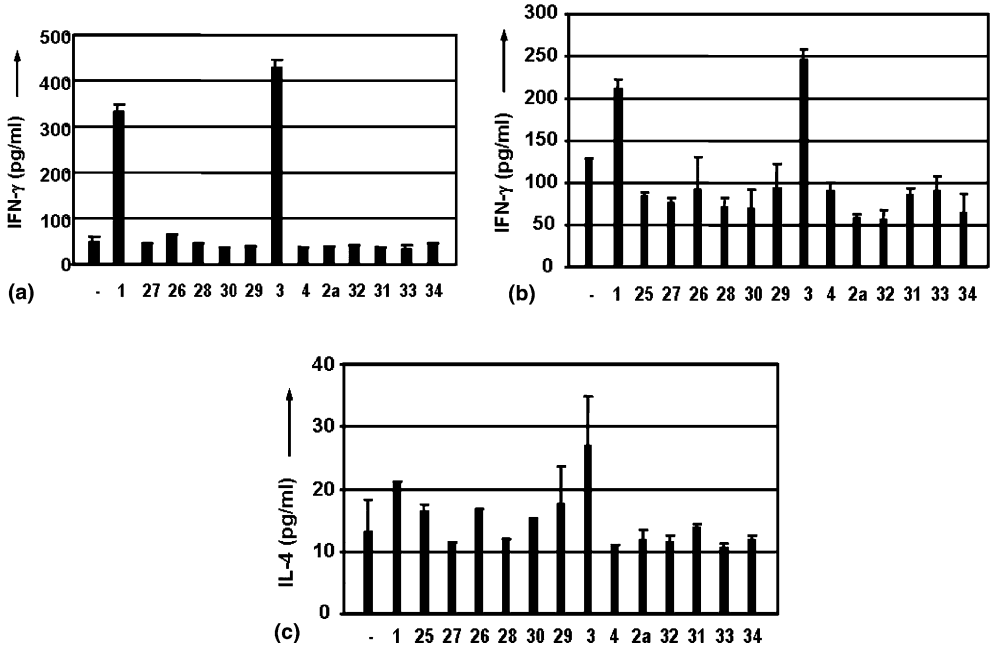


Figure 3. (a) In vitro IFN-γ secretion by human CD161$^+$ NK + NKT cells (4×10^5/well) in the presence of CD14$^+$ DCs (2×10^5/well) and 10 μg/mL of various glycolipids. (b) In vitro IFN-γ secretion by human CD161$^+$ NK + NKT cells (2×10^5/well) in the presence of CD14$^+$ DCs (4×10^5/well) and 20 μg/mL of various glycolipids. (c) In vitro IL-4 secretion by human CD161$^+$ NK + NKT cells (2×10^5/well) in the presence of CD14$^+$ DCs (4×10^5/well) and 20 μg/mL of various glycolipids.

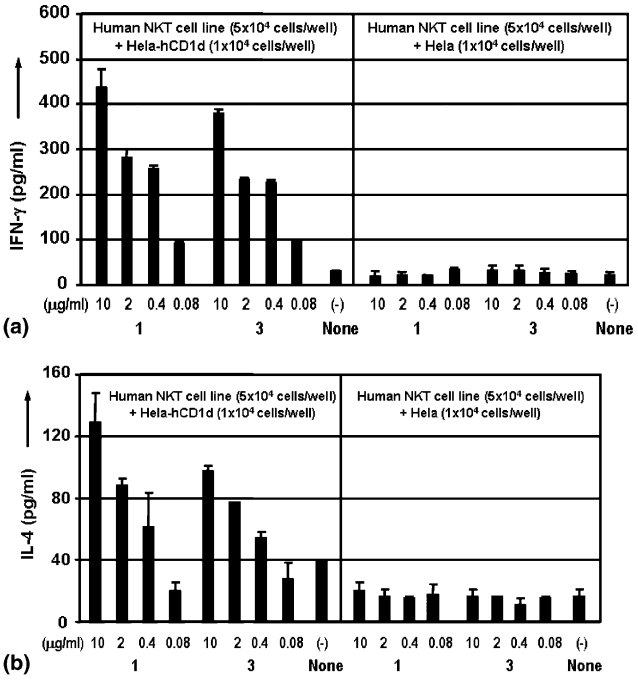


Figure 4. (a) In vitro IFN-γ secretion by human NKT cell line co-cultured with Hela-hCD1d in the presence of compounds **1** and **3**. (b) In vitro IL-4 secretion by human NKT cell line co-cultured with Hela-hCD1d in the presence of compounds **1** and **3**.

lipid ligand sulfatide **2**, were devised and synthesized in this research. Compound **3** was prepared from a newly designed 2″-benzyl-4″,6″-benzylidene-3″-levulinoyl protected trichloroacetimidate donor **8** and the known sphingosine acceptor **9** in 19% overall yield by a linear six-step procedure. DEPBT was utilized for the key coupling between hydroxy amine derivative **12a** and hexacosanoic acid to yield the amide bond of the ceramide moiety. For the synthesis of **4**, we employed an alternative synthetic strategy to first accomplish the ceramide portion and then perform the glycosylation. Compound **4** was efficiently prepared in 23% total yield over seven steps. The human NKT cell stimulating activity of 3-sulfo-α-GalCer **3** was found to be as effective as, or better than, that of **1**. As a comparison, 3-sulfo-β-GalCer **4** has no affinity to NKT cells. The development described herein should accelerate further understanding of CD1d-mediated NKT cell activation and development of new therapeutics.

4. Experimental

4.1. General methods

All chemicals were purchased as reagent grade and used without further purification. Dichloromethane (CH_2Cl_2, DCM) was distilled over calcium hydride and tetrahydrofuran (THF) over sodium/benzophenone. Anhydrous methanol (MeOH) and pyridine (Py) were purchased from a commercial source. Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel 60 F_{254} glass plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Flash column chromatography was performed on silica gel 60 Geduran (35–75 μm EM Science). ^{1}H NMR spectra were recorded on a 400, 500, or 600 Hz NMR spectrometer at 20 °C. Chemical shift (in ppm) was determined relative to tetramethylsilane (δ 0 ppm) in deuterated solvents. Coupling

constant(s) in hertz (Hz) were measured from one-dimensional spectra. ^{13}C Attached Proton Test (C-Apt) spectra were obtained with the NMR-400, -500, or -600 spectrometer (100, 125, or 150 Hz) and were calibrated with either $CDCl_3$ (δ 77.23 ppm) or Py-d_5 (δ 123.87 ppm).

4.2. *p*-Methylphenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-levulinoyl-1-thio-D-galactopyranoside, 6

Compound **5** (3 g, 6.45 mmol) was dissolved in DCM. LevOH (0.9 mL, 1.35 equiv), EDC (1.6 g, 1.3 equiv), and DMAP (197 mg, 0.25 equiv) were added. The reaction was allowed to proceed overnight while covered in foil. The reaction was then diluted with DCM, washed with water, saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. After removal of the solvent the mixture was purified by column chromatography (hexanes–EtOAc–DCM 3:1:1) to give 2.83 g of **6** in 78% yield.

^1H NMR ($CDCl_3$, 500 MHz): δ 7.61–7.03 (m, 14H), 5.48 (s, 1H), 4.98 (dd, 1H, J = 3.7, 9.6 Hz), 4.77 (d, 1H, J = 11.0 Hz), 4.63 (d, 1H, J = 9.5 Hz), 4.51 (d, 1H, J = 11.0 Hz,), 4.36–4.32 (m, 2H), 3.99–3.97 (m, 1H), 3.90–3.86 (m, 1H), 3.51 (s, 1H), 2.56–2.50 (m, 2H), 2.46–2.40 (m, 2H), 2.31 (s, 3H), 2.09, (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.05, 172.09, 138.18, 137.76, 137.64, 133.11, 129.61, 128.98, 128.57, 128.22, 128.01, 127.68, 127.57, 126.45, 100.83, 86.53, 75.41, 75.05, 73.77, 73.71, 69.09, 37.70, 29.60, 27.99; HRMS (MALDI-FTMS) calcd for $C_{32}H_{34}O_7SNa$ $[M+Na]^+$ 585.1923. Found: 585.1900.

4.3. 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-levulinoyl-D-galactopyranoside, 7

Compound **6** (600 mg, 1.07 mmol) was dissolved in 50 mL of acetone. The reaction mixture was cooled to 0 °C, and NBS (228 mg, 1.28 mmol, 1.2 equiv) was added. The reaction mixture turned orange immediately. After 10 min the reaction was quenched by addition of solid NH_4Cl. The mixture was diluted with water and ethyl acetate, and the aqueous layer was extracted with ethyl acetate (3 ×). The combined organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes–EtOAc–DCM 1:1:1) to give 442 mg (91%) of **7**.

^1H NMR ($CDCl_3$, 500 MHz): δ 7.50–7.25 (m, 10H), 5.48 (d, 1H, J = 4.8 Hz), 5.38 (s, 1H), 5.32 (dd, 1H, J = 3.7, 10.3 Hz), 4.94–4.90 (m, 1H), 4.73–4.62 (m, 3H), 4.36 (d, 1H, J = 3.3 Hz), 4.05 (dd, 1H, J = 3.3, 10.3 Hz), 4.00–3.98 (m, 2H), 3.93, (s, 1H), 3.52–3.51 (m, 1H), 2.71–2.53 (m, 4H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.43, 177.73, 172.35, 172.24, 138.41, 137.78, 137.63, 137.57, 128.89, 128.85, 128.38, 128.21, 128.03, 127.75, 127.67, 127.51, 126.15, 126.12, 100.61, 97.50, 91.98, 77.57, 74.68, 74.10, 73.82, 73.56, 73.38, 73.28, 70.55, 69.17, 68.93, 66.24, 62.18, 37.82, 37.79, 29.67, 28.38, 28.11, 28.04; HRMS (MALDI-FTMS) calcd for $C_{25}H_{29}O_8$ $[M+H]^+$ 457.1862. Found: 457.1856.

4.4. *O*-(2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-levulinoyl-D-galactopyranosyl)trichloroacetimidate, 8

To a solution of **7** (188.5 mg, 0.46 mmol) dissolved in 4 mL of DCM was added CCl_3CN (0.46 mL, 4.62 mmol) and DBU (31 μL, 0.21 mmol). After 2 h at room temperature, the dark solution was concentrated and then purified by flash chromatography hexanes–EtOAc (2:1) and 1% triethylamine to yield **8** (211 mg, 77%).

^1H NMR ($CDCl_3$, 500 MHz): δ 7.59–7.34 (m, 10H), 5.61 (s, 1H), 5.45 (dd, 1H, J = 3.2, 10.7 Hz), 4.80–4.72 (m, 2H), 4.60 (d, 2H, J = 3.3 Hz), 4.38–4.33 (m, 2H), 4.13–4.10 (dd, 1H, J = 1.8, 12.5 Hz), 4.05 (s, 1H), 2.79–2.72 (m, 2H), 2.65 (m, 2H), 2.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.43, 177.73, 172.35, 172.27, 138.41, 137.78, 137.63, 137.57, 128.89, 128.85, 128.38, 128.21, 128.03, 127.86, 127.75, 127.67, 127.51, 126.15, 126.12, 100.61, 97.50, 91.98, 77.57, 74.68, 74.10, 73.56, 73.38, 73.28, 70.55, 69.17, 68.93, 66.24, 62.18, 37.82, 37.79, 29.67, 29.38, 28.11, 28.04.

4.5. 2-Azido-3,4-di-*O*-benzyl-1-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-levulinoyl-α-D-galactopyranosyl)-D-*ribo*-octadeca-6-ene-1-ol, 10

A solution of trichloroacetimidate **8** (150 mg, 0.25 mmol, 1.5 equiv) and sphingosine derivative **9** (86 mg, 0.16 mmol) in 2.5 mL of anhydrous THF was added over freshly dried powdered AW-300 molecular sieves and cooled to −20 °C. TMSOTf (23 μL, 0.8 equiv) was slowly added to the solution, and the mixture was warmed up to 0 °C in 2.5 h. The reaction was quenched by addition of Et_3N (0.1 mL), and the mixture was diluted with EtOAc and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (hexanes–EtOAc 6:1) to furnish **10** (57 mg, 46% based on consumed acceptor **9**) as a syrup, and recover **9** (18 mg).

^1H NMR ($CDCl_3$, 400 MHz): δ 7.49–7.23 (m, 20H), 5.56–5.45 (m, 3H), 5.32 (dd, 1H, J = 3.5, 10.5 Hz), 4.98 (d, 1H, J = 3.1 Hz), 4.70–4.51 (m, 6H), 4.38 (m, 1H), 4.13–3.82 (m, 5H), 3.71–3.62 (m, 4H), 2.75–2.40 (m, 6H), 2.08 (s, 3H), 2.06–1.97 (m, 2H), 1.25 (br s, 18H), 0.88 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.30, 172.25, 138.21, 137.93, 137.67, 132.60, 128.89, 128.37, 128.35, 128.33, 129.29, 128.08, 127.27, 128.08, 127.78, 127.73, 127.69, 127.63, 127.60, 127.17, 124.69, 100.65, 98.61, 79.41, 78.95, 74.06, 73.65, 73.41, 73.10, 71.94, 70.79, 69.02, 68.21, 62.41, 61.97, 37.93, 31.89, 29.71–29.32, 28.19, 27.58, 22.66, 14.10; ESI-MS (positive-ion mode): m/z 982.4 $[M+Na]^+$.

4.6. Di-*O*-benzyl-1-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-hexacosylamino-D-*ribo*-octadeca-6-ene-1-ol, 14

The azide **10** (57 mg, 0.059 mmol) was dissolved in 2.0 mL of anhydrous THF and cooled to 0 °C. PMe_3

(0.4 mL of 1.0 M in toluene, 0.4 mmol) was added to the solution, and the reaction was warmed up to room temperature and stirred overnight. After the almost disappearance of the starting material, 0.8 mL of aq 1 M NaOH was added to the mixture and stirred for 5 h. CH_2Cl_2 was then added to dilute the solution, and the mixture was washed with brine, dried over Na_2SO_4, and concentrated. The residue was used for the next step without further purification. Hexacosanoic acid (35 mg, 0.088 mmol, 1.5 equiv) was suspended in CH_2Cl_2 (2.0 mL), and then DEPBT (26 mg, 0.087 mmol, 1.5 equiv) and DIEA (15 μL, 1.5 equiv) were added. The mixture was vigorously shaken for 1 h to give a clear light-yellow solution in which the above crude amine mixture **12a** and **12b** was added subsequently. The solution was stirred overnight at room temperature and then diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated to afford a solid (**13a** and **13b**, 57 mg), which was dissolved in 2 mL Py–HOAc solution (3:1 v/v, contains 0.30 M $NH_2NH_2{\cdot}HOAc$) and stirred for 1.5 h at room temperature. After the usual workup similarly as above, the residue was purified by column chromatography on silica gel (hexanes–EtOAc 2:1) to furnish **14** (40 mg, 56% over three steps) as a solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47–7.23 (m, 20H), 5.67 (d, 1H, J = 8.6 Hz), 5.51–5.44 (m, 3H), 4.95 (d, 1H, J = 2.7 Hz), 4.77–4.49 (m, 6H), 4.40 (m, 1H), 4.21 (d, 1H, J = 2.7 Hz), 4.12–4.07 (m, 2H), 3.94–3.58 (m, 8H), 2.45 (m, 2H), 2.08–1.88 (m, 4H), 1.49 (m, 2H), 1.25 (br s, 62H), 0.88 (t, 6H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.14, 138.50, 138.33, 137.74, 132.57, 129.32, 128.62, 128.40, 128.08, 127.95, 127.85, 126.45, 125.20, 101.37, 99.04, 79.97, 79.22, 76.29, 73.48, 73.41, 71.79, 69.50, 68.86, 68.19, 62.94, 50.26, 36.96, 32.13, 29.91–29.56, 28.14, 27.78, 25.93, 22.90, 14.34; HRMS (MALDI-FTMS) calcd for $C_{78}H_{119}NO_9Na$ $[M+Na]^+$ 1236.8777. Found: 1236.8741.

4.7. 3,4-Di-*O*-benzyl-1-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-sulfo-α-D-galactopyranosyl)-2-hexacosylamino-D-*ribo*-octadeca-6-ene-1-ol, sodium salt, 15

To a solution of **14** (40 mg, 0.033 mmol) in Py (2.5 mL) was added $SO_3{\cdot}$Py complex (79 mg, 0.5 mmol, 15 equiv). The mixture was stirred at room temperature for 2.5 h. Water solution (2.5 mL) of $NaHCO_3$ (62 mg) was added to quench the reaction. The reaction mixture was diluted with CH_2Cl_2, and washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 15:1) to give **15** (39 mg, 90%) as a solid.

^{1}H NMR ($CDCl_3$–CD_3OD 1:1, 400 MHz): δ 7.87 (d, 1H, J = 8.9 Hz), 7.58–7.17 (m, 20H), 5.59 (s, 1H), 5.43 (m, 2H), 4.96 (m, 3H), 4.82 (m, 1H), 4.73 (d, 1H, J = 2.3Hz), 4.62–4.58 (m, 2H), 4.52–4.44 (m, 2H), 4.19–3.99 (m, 5H), 3.78 (br s, 2H), 3.66 (br s, 1H), 3.56 (m, 1H), 2.47 (m, 1H), 2.34 (m, 1H), 2.13 (t, 2H, J = 7.0 Hz), 2.01 (m, 2H), 1.54 (br s, 2H), 1.27 (br s, 62H), 0.89 (t, 6H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 100 MHz): δ 173.92, 138.30, 137.66, 137.57, 131.42, 128.43, 128.01–127.03, 125.95, 125.66, 100.59, 98.99, 80.16, 79.75, 75.04, 74.84, 73.97, 73.60, 73.49, 71.09, 68.74, 67.00, 62.70, 49.71, 49.62, 31.56, 29.29–29.02, 27.01, 25.59, 22.27, 13.44; HRMS (MALDI-FTMS) calcd for $C_{78}H_{118}NO_{12}SNaK$ $[M+K]^+$ 1354.7909. Found: 1354.7933.

4.8. 2-Hexacosylamino-1-*O*-(3-*O*-sulfo-α-D-galactopyranosyl)-D-*ribo*-1,3,4-octadecantriol, sodium salt, 3

Compound **15** (39 mg, 0.030 mmol) was dissolved in HOAc–MeOH (1:1 v/v, 6 mL). Palladium black (80 mg) was added and the reaction solution was saturated with hydrogen by a balloon. After stirring at room temperature for 20 h, the catalyst was removed by filtration over Celite and washed with CH_2Cl_2–MeOH (1:1) thoroughly. Evaporation of the solvent gave a residue which was dissolved in CH_2Cl_2–MeOH (1:1) mixed solvent again and then saturated $NaHCO_3$ (3 mL) was added to stir at room temperature for 0.5 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 6:1) to give **3** (24 mg, 83%) as a light-yellow solid.

^{1}H NMR ($CDCl_3$–CD_3OD 1:1, 400 MHz): δ 4.95 (d, 1H, J = 3.5 Hz), 4.49 (dd, 1H, J = 2.7, 10.2 Hz), 4.35 (m, 1H), 4.17 (m, 1H), 4.02 (dd, 1H, J = 2.7, 9.8 Hz), 3.88–3.85 (m, 2H), 3.80–3.72 (m, 4H), 3.69–3.65 (m, 2H), 3.61–3.57 (m, 1H), 2.24 (t, 2H, J = 7.4 Hz), 1.59 (m, 4H), 1.27 (br s, 68H), 0.89 (t, 6H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$–CD_3OD 1:1, 100 MHz): δ 174.31, 99.08, 77.57, 73.42, 71.64, 70.44, 67.81, 67.19, 66.48, 61.28, 49.90, 35.89, 31.51, 31.32, 29.29–28.94, 25.53, 22.22, 13.34; HRMS (MALDI-FTMS) calcd for $C_{50}H_{98}NO_{12}SNa_2$ $[M+Na]^+$ 982.6599. Found: 982.6585.

4.9. 3,4-Di-*O*-benzyl-2-hexacosylamino-1-*O*-triisopropylsilyl-octadec-6-ene, 21

Compound **20** was reduced to an amine derivative according to the literature.[16b] The amine (26.7 mg, 0.041 mmol) was dissolved in 1 mL of anhydrous DCM and 1,4-dioxane (1:1). Hexacosanoic acid (26 mg, 0.06 mmol, 3 equiv) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (24 mg, 0.062 mmol) were added to the solution. The pH was adjusted with *n*-morpholine (10.1 μL, 0.082 mmol). The mixture was stirred at room temperature for 24 h and then diluted with DCM and washed with water (3 ×) and brine (1 ×). The solution was dried ($MgSO_4$) and concentrated, and the residue was purified by column chromatography on silica gel (toluene–EtOAc 40:1) to furnish **21** (25 mg, 60%) as an amorphous solid.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.33–7.26 (m, 10H), 5.74 (d, 1H, J = 8.8 Hz), 5.50–5.47 (m, 2H), 4.86–4.84 (m, 1H), 4.65–4.62 (m, 1H), 4.56–4.53 (m, 2H), 4.15–4.12 (m, 1H), 4.02–3.99 (m, 1H), 3.82–3.81 (m, 1H), 3.74–3.72 (m, 1H), 3.59–3.58 (m, 1H), 2.55–2.51 (m, 1H), 2.45 (m, 1H), 2.07–2.03 (m, 2H), 1.98–1.96 (m, 3H), 1.54 (m, 2H), 1.25 (m, 64H), 1.04–1.03 (m, 18H), 0.87

(t, 6H, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.35, 138.69, 131.90, 128.30, 128.26, 128.25, 127.82, 127.75, 127.72, 127.53, 127.46, 125.99, 80.25, 78.74, 73.43, 71.96, 62.23, 51.20, 36.90, 31.92, 29.70–29.36, 27.51, 25.62, 22.69, 18.02, 14.11, 11.90; ESI-MS (positive-ion mode): m/z 1031 $[M+H]^+$.

4.10. 3,4-Di-*O*-benzyl-2-hexacosylamino-octadec-6-en-1-ol, 22

Compound **21** (13 mg, 0.0126 mmol) was dissolved in 0.5 mL of dry THF. The reaction mixture was cooled to 0 °C, 25 μL of a 1 M TBAF solution was added, and the reaction mixture was allowed to warm to room temperature. After 10 min, the reaction was controlled by TLC and found to be almost complete. After 20 min, the reaction was stopped by addition of 1 mL saturated $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (4 ×), and the combined organic layer extracted with brine, dried, and evaporated. The residue was chromatographed on silica gel (hexanes–EtOAc 9:1) to yield **22** (10 mg, 0.011 mmol, 90%).

^1H NMR ($CDCl_3$, 400 MHz): δ 7.38–7.26 (m, 10H), 5.99–5.97 (m, 1H), 5.50–5.49 (m, 1H), 4.70–4.65 (m, 2H), 4.60–4.57 (m, 1H), 4.43–4.40(m, 1H), 4.21–4.20 (m, 1H), 3.89–3.96 (m, 1H), 3.72–3.71 (m, 2H), 3.61–3.60 (m, 1H), 3.07–3.05 (m, 1H) 2.51–2.42 (m, 1H), 2.05–1.90 (m, 4H), 1.51 (m, 2H), 1.25 (m, 64H), 0.88 (t, 6H, $J = 6.4$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.80, 137.96, 134.08, 132.76, 128.66, 128.41, 128.12, 128.07, 127.88, 127.77, 125.36, 124.63, 82.03, 78.56, 73.05, 72.40, 62.94, 50.47, 36.65, 31.89, 29.68–29.27, 28.48, 27.54, 25.60, 22.67, 14.10; HRMS (MALDI-FTMS) calcd $C_{58}H_{99}NO_4Na$ $[M+Na]^+$ 896.7471. Found: 896.7496.

4.11. 3,4-Di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-hexacosylamino-D-*ribo*-octadeca-6-ene-1-ol, 23

A solution of trichloroacetimidate **16** (64 mg, 0.086 mmol, 1.5 equiv) and ceramide derivative **22** (50 mg, 0.057 mmol) in 1 mL of anhydrous CH_2Cl_2 was added over freshly dried powdered AW-300 molecular sieves. TMSOTf (8 μL, 0.8 equiv) was slowly added to the solution, and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was diluted with EtOAc and filtered through Celite. The organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (hexanes–EtOAc 4:1) to furnish **23** (45 mg, 54%) as an oil.

^1H NMR ($CDCl_3$, 400 MHz): δ 8.03–7.22 (m, 30H), 5.98 (d, 1H, $J = 3.5$ Hz), 5.79 (dd, 1H, $J = 7.6$, 10.3 Hz), 5.64 (dd, 1H, $J = 3.2$, 10.2 Hz), 5.51–5.40 (m, 3H), 4.87–4.80 (m, 2H), 4.70 (dd, 1H, $J = 2.9$, 11.1 Hz), 4.60–4.56 (m, 3H), 4.42–4.37 (m, 2H), 4.26 (t, 2H, $J = 6.7$ Hz), 3.83 (dd, 1H, $J = 2.6$, 8.2 Hz), 3.63 (dd, 1H, $J = 3.5$, 9.4 Hz), 3.49–3.40 (m, 1H), 2.52–2.36 (m, 2H), 2.04–1.94 (m, 2H), 1.52 (t, 2H, $J = 7.7$ Hz), 1.25–0.99 (m, 64H), 0.88 (t, 6H, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.53, 166.18, 165.70, 165.65, 138.82, 133.81, 133.54, 132.13, 130.12, 129.96, 129.48–127.75, 126.04, 101.56, 79.83, 78.88, 74.52, 71.59, 71.51, 70.60, 68.84, 68.19, 62.05, 49.47, 36.46, 32.15, 30.50–29.29, 27.84, 27.73, 25.58, 22.92, 14.36; HRMS (MALDI-FTMS) calcd for $C_{92}H_{125}NO_{13}Na$ $[M+Na]^+$ 1474.9043. Found: 1474.9027.

4.12. 1-*O*-(β-D-Galactopyranosyl)-2-hexacosylamino-D-*ribo*-1,3,4-octadecantriol, 24

Compound **23** (45 mg, 0.031 mmol) was dissolved in EtOAc (8 mL). $Pd(OH)_2/C$ (40 mg) was added and the reaction solution was saturated with hydrogen by a balloon. The mixture was stirred at room temperature for 4 h. The catalyst was removed by filtration over Celite and washed with CH_2Cl_2–MeOH (1:1) thoroughly. Evaporation of the solvent gave a solid, which was dissolved in CH_2Cl_2–MeOH (1:1, 3 mL) mixed solvent and then NaOMe methanol solution (50 μL, 25% wt/wt) was added. The reaction was stirred at room temperature for 2 h. Amberlyst 15 (H^+-form) was added to neutralize the sodium methoxide, the mixture was then diluted with CH_2Cl_2–MeOH, and the exchange resin was filtered off. The resin was washed thoroughly and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 8:1) to give **24** (27 mg, quantitative) as a white solid.

The obtained ^1H NMR spectroscopic data is identical to the one reported in the literature.[12a] ^{13}C NMR (Py-d_5, 100 MHz): δ 173.53, 106.30, 77.03, 75.76, 75.36, 72.70, 72.63, 70.81, 70.22, 62.32, 52.26, 36.91, 33.52, 32.15, 30.34–29.65, 26.68, 26.40, 22.97, 14.31; HRMS (MALDI-FTMS) calcd for $C_{50}H_{99}NO_9Na$ $[M+Na]^+$ 880.7212. Found: 880.7241.

4.13. 2-Hexacosylamino-1-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-D-*ribo*-1,3,4-octadecantriol, sodium salt, 4

Compound **24** (24 mg, 0.028 mmol) and Bu_2SnO (10 mg, 0.040 mmol) were stirred in MeOH (2 mL) at reflux under argon for 2 h. The solvent was evaporated off under reduced pressure and the dibutylstannylene complex was treated with $Me_3N{\cdot}SO_3$ (8 mg, 0.057 mmol) in THF (2 mL) at room temperature for 3 h. Methanol (1 mL) was added to quench the reaction. Then water solution (1 mL) of $NaHCO_3$ (25 mg) was added and the mixture was stirred at room temperature for half an hour. The solvent was removed under reduced pressure, and the residue purified by column chromatography on silica gel (CH_2Cl_2–MeOH 7:1) to give **4** (21.6 mg, 80%).

^1H NMR (Py-d_5, 600 MHz): δ 5.32 (d, 1H, $J = 7.5$ Hz), 5.26 (m, 1H), 5.12 (br s, 1H), 5.06 (m, 1H), 4.72–4.66 (m, 2H), 4.53 (m, 1H), 4.32–4.29 (m, 3H), 4.20 (t, 1H, $J = 7.0$Hz), 4.04 (m, 1H), 2.40 (m, 2H), 2.22–2.12 (m, 2H), 1.92 (m, 2H), 1.78–1.66 (m, 4H), 1.45–1.22 (m, 64H), 0.90–0.87 [6H (0.891, t, $J = 7.0$ Hz), 0.875, t, $J = 7.0$ Hz)]; ^{13}C NMR (Py-d_5, 150 MHz): δ 174.96, 105.47, 81.19, 77.38, 76.50, 73.16, 71.21, 70.92, 69.46, 62.77, 52.58, 37.53, 34.32, 32.74, 30.98–30.24, 27.19,

27.02, 23.56, 14.91; HRMS (MALDI-FTMS) calcd for $C_{50}H_{98}NO_{12}SNa_2$ $[M+Na]^+$ 982.6599. Found: 982.6589.

4.14. In vitro cytokine secretion assay using CD161$^+$ cells and CD14$^+$ immature dendritic cells

The frequency of human NKT cells that co-express $V_\alpha24$ + T-cell receptor and CD161 are approximately 0.01–0.1% among PBMCs. Therefore, in order to enrich NKT population, we isolated CD161$^+$ cells from leukopaks, using anti-CD161 monoclonal antibody coupled to magnetic beads (Miltenyi biotec, Auburn, CA), and used as a source of responder cells. CD161$^+$ cells represent both NKT and NK cells, and approximately 2–10% of CD161$^+$ cells are NKT cells. We also isolated CD14$^+$ cells from leukopaks, using anti-CD14 monoclonal antibody coupled to magnetic beads (Miltenyi biotec, Auburn, CA) as a source of antigen-presenting cells. Immature dendritic cells were generated from the CD14$^+$ cells after a 2 days incubation in the presence of 300 U/mL GM-CSF (R&D systems, Minneapolis, MN) and 100 U/mL IL-4 (R&D systems, Minneapolis, MN). Following irradiation with 2000 rads, 4×10^5 immature dendritic cells were co-cultured with 5×10^5 syngeneic CD161$^+$ cells in the presence of the glycolipid compounds at the indicated concentrations in a 96-well flat-bottom plate. Twenty-hours later, the culture supernatants were collected and the concentration of IFN- and IL-4 in the supernatants was determined by ELISA (BD Pharmingen, San Diego, CA).

4.15. Generation of $V_\alpha24$+ human NKT cell line

Human NKT cell lines, expressing the $V_\alpha24$+ T-cell receptor as well as CD161, were generated as follows. Anti-CD161 monoclonal antibodies and anti-CD14 monoclonal antibodies, each coupled to magnetic beads (Miltenyi biotec, Auburn, CA), were used sequentially to isolate CD161$^+$ cells and CD14$^+$ cells from leukopaks. Immature dendritic cells were generated from the CD14$^+$ cells after a 2 days incubation in the presence of 300 U/mL GM-CSF (R&D systems, Minneapolis, MN) and 100 U/mL IL-4 (R&D systems, Minneapolis, MN). Following irradiation with 2000 rads, the immature dendritic cells were co-cultured with syngeneic CD161$^+$ cells in the presence of 100 ng/mL of α-galactosylceramide and 10 IU/mL of IL-2 (Invitrogen, Carlsbad CA) for 10–14 days. After stimulating the CD161$^+$ cells a second time with α-galactosylceramide-pulsed, irradiated immature dendritic cells, NKT cell lines were shown to flow cytometrically to express both CD161$^+$ and $V_\alpha24$+ TCR (99% purity).

4.16. In vitro cytokine secretion assay using human NKT cell lines

IFN-γ and IL-4 secretion by the $V_\alpha24$+ human NKT cell line was determined by ELISA (BD Pharmingen, San Diego, CA) after culture for 16 h. For these assays, 5×10^4 $V_\alpha24$+ human NKT cells were co-cultured with 1×10^4 irradiated, Hela cells transfected with human CD1d gene (kind gift from Dr. Mitch Kronenberg), in the presence of the glycolipid compounds at 10 μg/mL in a 96-well flat-bottom plate. Untransfected Hela cells that lack human CD1d molecules were used as a negative control to ensure the necessity of the CD1d for presenting glycolipids to NKT cells.

References and notes

1. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187.
2. Kawano, T.; Cui, J. Q.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629.
3. Kawano, T.; Nakayama, T.; Kamada, N.; Kaneko, Y.; Harada, M.; Ogura, N.; Akutsu, Y.; Motohashi, S.; Iizasa, T.; Endo, H.; Fujisawa, T.; Shinkai, H.; Taniguchi, M. *Cancer Res.* **1999**, *59*, 5102–5105.
4. Reviewed in Brutkiewicz, R. R.; Lin, Y.; Cho, S.; Hwang, Y. K.; Sriram, V.; Roberts, T. J. *Crit. Rev. Immunol.* **2003**, *23*, 403–419.
5. Godfrey, D. I.; MacDonald, H. R.; Kronenberg, M.; Smyth, M. J.; Van Kaer, L. *Nat. Rev. Immunol.* **2004**, *4*, 231–237.
6. Taniguchi, M.; Harada, M.; Kojo, S.; Nakayama, T.; Wakao, H. *Annu. Rev. Immunol.* **2003**, *21*, 483–513.
7. Zhu, X. H.; Hara, A.; Taketomi, T. *J. Biochem. (Tokyo)* **1991**, *110*, 241–245.
8. See the information in http://www.avantilipids.com/NaturalCerebrosideSulfatide.asp.
9. Shamshiev, A.; Gober, H. J.; Donda, A.; Mazorra, Z.; Mori, L.; De Libero, G. *J. Exp. Med.* **2002**, *195*, 1013–1021.
10. Compostella, F.; Franchini, L.; DeLibero, G.; Palmisano, G.; Ronchetti, F.; Panza, L. *Tetrahedron* **2002**, *58*, 8703–8708.
11. Jahng, A.; Maricic, I.; Aguilera, C.; Cardell, S.; Halder, R. C.; Kumar, V. *J. Exp. Med.* **2004**, *199*, 947–957.
12. (a) Morita, M.; Natori, T.; Akimoto, K.; Osawa, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 699–704; (b) Iijima, H.; Kimura, K.; Sakai, T.; Uchimura, A.; Shimizu, T.; Ueno, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1998**, *6*, 1905–1910; (c) Uchimura, A.; Shimizu, T.; Morita, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2245–2249; (d) Motoki, K.; Kobayashi, E.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 705–710; (e) Kobayashi, E.; Motoki, K.; Yamaguchi, Y.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem.* **1996**, *4*, 615–619; (f) Sakai, T.; Morita, M.; Matsunaga, N.; Akimoto, K.; Yokoyama, T.; Iijima, H.; Koezuka, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 697–702; (g) Sakai, T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359–361; (h) Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1999**, *42*, 1836–1841; (i) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631–1641; (j) Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Am. Chem. Soc.* **2004**, *126*, 13602–13603.
13. (a) Zhou, X. T.; Forestier, C.; Goff, R. D.; Li, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *Org. Lett.* **2002**, *4*, 1267–1270; (b) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531–534; (c) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818–3822.

14. Giaccone, G.; Punt, C. J.; Ando, Y.; Ruijter, R.; Nishi, N.; Peters, M.; von Blomberg, B. M.; Scheper, R. J.; van der Vliet, H. J.; van den Eertwegh, A. J.; Roelvink, M.; Beijnen, J.; Zwierzina, H.; Pinedo, H. M. *Clin. Cancer Res.* **2002**, *8*, 3702–3709.
15. Guilbert, B.; Davis, N. J.; Pearce, M.; Aplin, R. T.; Flitsch, S. L. *Tetrahedron: Asymmetry* **1994**, *5*, 2163–2178.
16. (a) Figueroa-Perez, S.; Schmidt, R. R. *Carbohydr. Res.* **2000**, *328*, 95–102; (b) Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C. H. *J. Org. Chem.* **2002**, *67*, 4559–4564.
17. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
18. (a) Liu, P.; Sun, B. Y.; Chen, X. H.; Tian, G. L.; Ye, Y. H. *Synth. Commun.* **2002**, *32*, 473–480; (b) Fan, C. X.; Hao, X. L.; Ye, Y. H. *Synth. Commun.* **1996**, *26*, 1455–1460.
19. Kretzschmar, G.; Toepfer, A.; Sonnentag, M. *Tetrahedron* **1998**, *54*, 15189–15198.