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Synthesis and in vitro Evaluation of α-GalCer Epimers

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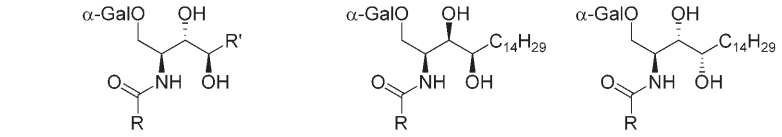
α-GalCer (also known as KRN7000) is an immunomodulatory glycolipid that is known to potently activate invariant natural killer T (NKT) cells upon CD1d-mediated stimulation. Because Th1 and Th2 cytokines, which are released after α-GalCer presentation, antagonize each other's effects, α-GalCer analogues that induce a biased Th1/Th2 response are highly awaited. In this context, we report the synthesis and in vitro evaluation of α-Gal-D-xylo-Cer and two α-Gal-L-lyxo-Cer analogues, one with the natural acyl chain, the other with a truncated chain.

Introduction

Natural killer T (NKT) cells are a unique subset of T cells that express an invariant T cell antigen receptor (TCR). Unlike other T cells, NKT cells recognize glycolipid antigens when presented by the major histocompatibility complex (MHC) class I-like molecule CD1d.[1] An extensively studied exogenous glycolipid activator of NKT cells is α-GalCer (also known as KRN7000, **1a**, Figure 1).[2–4] α-GalCer was originally generated by the Kirin group from structure–activity studies of glycolipids that were isolated from the marine sponge *Agelas mauritianus*.



(2*S*,3*S*,4*R*)-**1a**: R=$C_{25}H_{51}$; R'=$C_{14}H_{29}$
(2*S*,3*S*,4*R*)-**1b**: R=$C_{23}H_{47}$; R'=C_5H_{11}
(2*S*,3*R*,4*R*)-**2**: R=$C_{25}H_{51}$
(2*S*,3*S*,4*S*)-**3**: R=$C_{25}H_{51}$
(2*S*,3*S*,4*S*)-**4**: R=C_7H_{15}

Figure 1. Structure of α-GalCer (**1a**, KRN7000), OCH (**1b**), α-Gal-D-*xylo*-Cer (**2**), and two α-Gal-L-*lyxo*-Cer analogues, one with the natural acyl chain (compound **3**) and the other with a truncated one (compound **4**).

Stimulation of NKT cells by CD1d-mediated α-GalCer presentation leads to rapid release of T helper 1 (Th1) and T helper 2 (Th2) cytokines. Proinflammatory Th1 cytokines such as IFN-γ mediate antitumor, antiviral and antibacterial effects of α-GalCer, while immunomodulatory Th2 cytokines such as IL-4 delay or prevent the onset of autoimmune diseases like type 1 diabetes.[5] Because Th1 and Th2 cytokines antagonize each other's effects, α-GalCer analogues that induce a biased Th1/Th2 response are highly awaited.

In 2005, the crystal structure of human CD1d in a complex with α-GalCer was elucidated, and it revealed the specific binding mode of α-GalCer to CD1d.[6] The acyl chain of α-GalCer fits into the A′ pocket by adopting a counterclockwise circular curve, and the sphingosine chain adopts an extended conformation to fit into the F′ pocket and to reach the end of the binding groove. The galactose ring is well ordered and extends above the surface of the lipid-binding groove. The crystal structure revealed three hydrogen bonds between human CD1d and α-GalCer. The glycosidic linkage 1′-O is hydrogen bonded to Thr154, the 2′-OH group of the galactose ring forms a hydrogen bond to Asp151, and the 3-OH group on the sphingosine chain forms the third hydrogen bond to Asp80. These bonds are assumed to anchor α-GalCer in a proper orientation for recognition by the TCR of NKT cells.

In another crystallographic study of mouse CD1d in a complex with an OCH analogue, Asp80 was found to interact with both secondary hydroxy groups (i.e., 3-OH and 4-OH) of the sphingosine backbone.[7] In addition, Arg79 is oriented in such a way that it can participate in hydrogen bonding with the same 3-OH. More recently, Wong and co-workers reported the crystal structure of mCD1d that was charged with the *Sphingomonas* glycolipid α-galacturonosyl ceramide (GalA-GSL), which is characterized by a shorter fatty acyl chain, the absence of a 4-OH on the sphingosine moiety, and the presence of a 6′-

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CO_2H group on the galactose unit.[8] The absence of the 4-OH causes the sphinganine backbone to shift slightly deeper in the F′ groove to establish an optimal hydrogen bond with the terminal oxygen atoms of Asp80. As a consequence, the hydrogen bond between Arg79 and the 3-OH of the sphinganine backbone is lost, and Arg79 is now oriented differently; this possibly affects the interaction of GalA-GSL with CD1d and the TCR.

Recently, Borg et al. reported the structure of a human NKT TCR in complex with CD1d that was bound to the potent NKT-cell agonist α-GalCer.[9] Consistent with the previously determined structures,[6,7] α-GalCer protrudes minimally from the CD1d cleft; only the galactosyl head group is exposed for recognition by the NKT TCR, and it interacts solely with the CDR1α and CDR3α loops. The galactose ring is sandwiched between Trp153 of CD1d and the aliphatic moiety of Arg95α, the side-chain of which also hydrogen bonds to the 3-OH on the sphingosine chain. The 2′-OH, 3′-OH and 4′-OH of the galactose ring form hydrogen bonds to Gly96α, Ser30α, and Phe29α, respectively, which are located on the invariant TCR α-chain. This mode of binding is consistent with the fine specificity that the NKT TCR exhibits for α-GalCer and its closely related analogues. Most reported analogues of α-GalCer result from modifications of the acyl chain,[10,11] the glycosidic linkage,[12,13] or the glycosyl residue,[7,14–17] whereas relatively few alterations of the phytosphingosine part have been explored.[11,18]

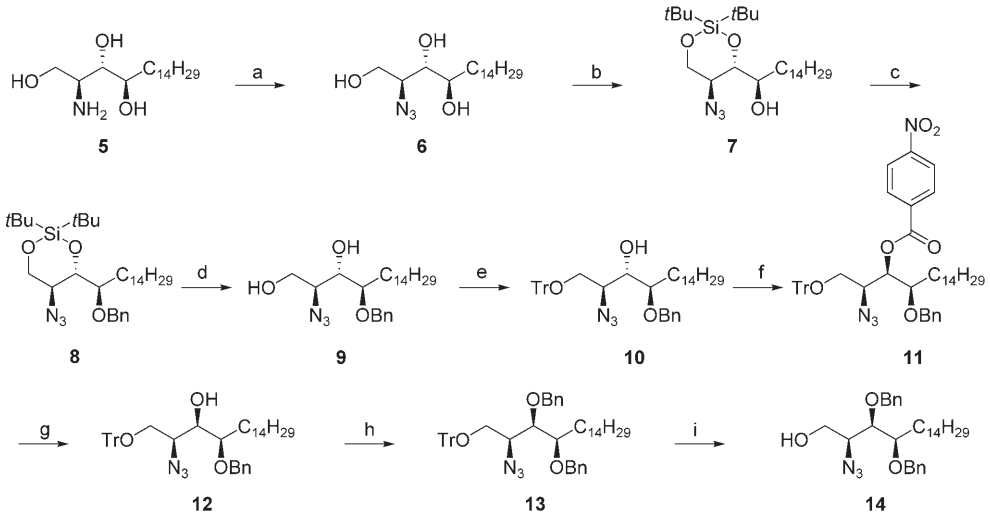
With the synthesis of OCH (**1b**, Figure 1) it was demonstrated that analogues with a truncated sphingosine base tend to predominantly trigger the production of Th2 cytokines.[19,20] Other studies reported the synthesis of a 4-deoxy-α-GalCer congener,[2,5,21,22] or analogues in which the phytoceramide moiety was replaced by a ceramide moiety, characterized by a double bond between C4 and C5.[23,24] Interestingly, both types of analogues retained their ability to stimulate NKT cells; this suggests that the 4-OH is not critical for CD1d presentation. An overview of these modifications and the influence of the glycolipid structure on NKT cell response have been published by Savage et al.,[25] and more recently by Wong and co-workers.[26]

These studies illustrate the importance of exploring the stereochemistry at C3 and C4 of the phytosphingosine moiety. A few strategies are reported for the synthesis of D-*xylo*- and L-*lyxo*-phytosphingosine. The desired stereochemistry of the three chiral centers has been realized by using D-(−)-tartaric acid[27–29] or a serine-derived 1,5-dioxaspiro[3.2]hexane[30] as the chiral template, by an aldol condensation between an iminoglycinate that bears a chiral auxiliary and an appropriate aldehyde,[31] or by asymmetric dihydroxylation of an (*E*)-α,β-unsaturated ester as the chiral induction stage.[32] Remarkably, until now, no attempts to alter the stereochemistry of the secondary hydroxy groups (i.e., 3-OH and 4-OH) of the phytosphingosine moiety of α-GalCer have been published. Recent work by Kim et al.[33] on the efficient synthesis of D-*xylo*-, L-*lyxo*-, and L-*arabino*-phytosphingosine from the natural D-*ribo*-phytosphingosine has prompted us to report the synthesis and in vitro evaluation of α-Gal-D-*xylo*-Cer (**2**) and two α-Gal-L-*lyxo*-Cer analogues, one with the natural acyl chain (compound **3**), the other with a truncated one (compound **4**) (Figure 1).

Results and Discussion

Chemistry

The synthesis of the D-*xylo*-phytosphingosine acceptor **14** was started from the commercially available D-*ribo*-phytosphingosine **5** (Scheme 1). Conversion of the primary amine to an azide, and subsequent protection of the hydroxy groups at C1 and C3 afforded the 1,3-di-*tert*-butylsilylene derivative **7**.[34] Benzyl protection of the remaining 4-OH group with freshly prepared benzyl 2,2,2-trichloroacetimidate, followed by silylene



Scheme 1. Reagents and conditions: a) N_3Tf, K_2CO_3, $CuSO_4$, CH_2Cl_2, CH_3OH, H_2O, RT, overnight, 98%; b) $(tBu)_2Si(OTf)_2$, DMF, pyridine, −20 °C, 4 h, 96%; c) benzyltrichloroacetimidate, TfOH (cat.), Et_2O, 0 °C→RT, 48 h, 58%; d) HF·pyridine, THF/pyridine (1:1), 0 °C, 0.5 h, 98%; e) TrCl, DMAP, pyridine, 70 °C, overnight, 87%; f) *p*-nitrobenzoic acid, DIAD, PPh_3, THF, RT, overnight, 71%; g) NaOMe, MeOH, RT, 1 h, 89%; h) BnBr, NaH, DMF, 0 °C→RT, overnight, 94%; i) $ZnBr_2$, CH_2Cl_2/*i*PrOH (85:15), RT, overnight, 73%.

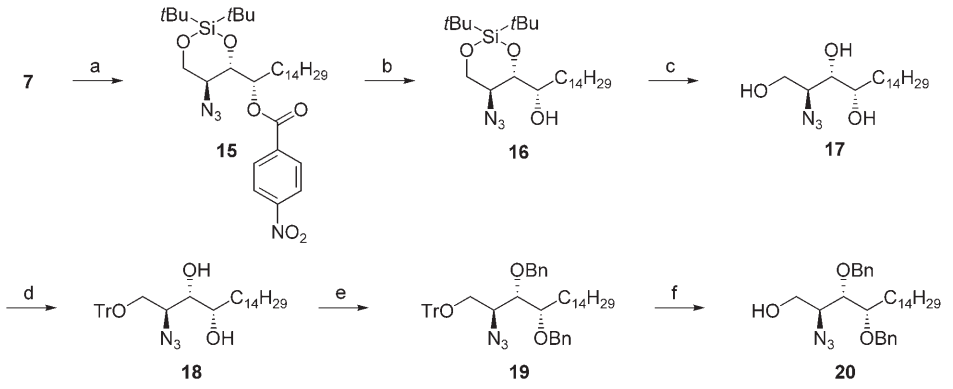
deprotection gave diol **9**. The primary alcohol was then tritylated, and inversion of the 3-OH group under Mitsunobu conditions afforded the *p*-nitrobenzoic ester derivative **11**. Solvolysis of the activated ester and subsequent protection of the inverted 3-OH group as a benzyl ether gave the desired D-*xylo*-phytosphingosine acceptor **14** after final deprotection of the trityl group.

For the synthesis of the L-*lyxo*-phytosphingosine acceptor **20**, we started from intermediate **7** (Scheme 2). Inversion of the 4-OH group under Mitsunobu conditions afforded the *p*-nitrobenzoic ester intermediate **15**. Solvolysis of the activated ester and subsequent deprotection of the silylene gave triol **17**. Selective tritylation of the primary alcohol, followed by a dibenzylation step, afforded the desired L-*lyxo*-phytosphingosine acceptor **20** after final deprotection of the trityl group.

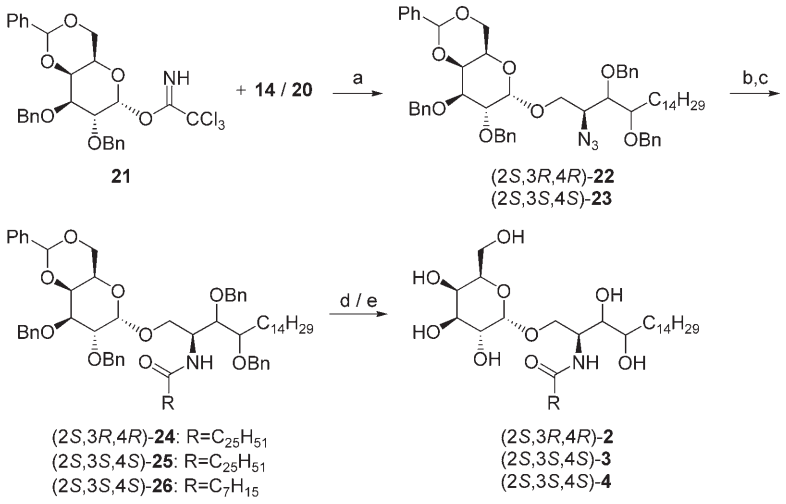
For the glycosidation step, the 4,6-benzylidene-protected trichloroacetimidate **21** was used as the galactosyl donor (Scheme 3).[35] Reaction with the L-*lyxo*-phytosphingosine acceptor **20** by using boron trifluoride diethyl etherate as the promoter afforded the desired α-glycoside **23** in 58% yield. The glycosidation of the D-*xylo*-phytosphingosine acceptor **14** was performed with trimethylsilyl triflate[36] because it became clear during the course of this project that this promoter gave improved coupling yields. The desired α-glycoside **22** was isolated in 70% yield. Staudinger reduction, followed by acylation with the appropriate acid and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as the coupling reagent gave the protected compounds **24**–**26**. Final hydrogenolysis afforded the desired analogues **2**–**4**.

Confirmation of the inversion of the stereochemistry at C3 and C4 was established by preparing three bicyclic compounds **27**, **28** and **31**, and subjecting these to ^{1}H NMR spectroscopic selective decoupling experiments. The synthesis of the *trans*-fused bicyclic compounds **27** and **28** started from intermediates **7** and **16**, respectively (Scheme 4). Staudinger reduction of the azide, followed by cyclization with triphosgene afforded the desired compounds **27** and **28**. For the synthesis of the *cis*-fused bicyclic compound **31**, intermediate **12** was used. Detritylation of the primary alcohol, followed by protection of the hydroxy groups at C1 and C3 afforded the 1,3-di-*tert*-butylsilylene derivative **30**. Reduction of the azide and simultaneous deprotection of the 4-OH group gave the desired *cis*-fused bicyclic compound **31** after cyclization with triphosgene.

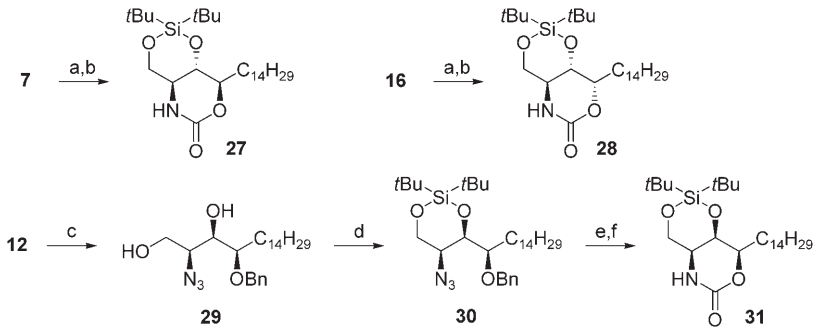
A ^{1}H NMR spectrum that was obtained by selective decoupling of H2 permitted us to determine the coupling constant between H3 and H4 (Figure 2). For the *trans*-fused derivative **27**, we found a $^3J_{3,4}$ value of 9.4 Hz, which confirms the transdiaxial position of H3 and H4. Irradiation of H2 in compound **28** gave rise to a $^3J_{3,4}$ value of 5.0 Hz, and compound **31** furnished a $^3J_{3,4}$ value of 2.1 Hz. According to the Karplus equation, these values indicate a dihedral angle between 60° and 90°; this confirms the inversion of the stereochemistry at C3 (compound **31**) and C4 (compound **28**).



Scheme 2. Reagents and conditions: a) *p*-nitrobenzoic acid, DIAD, PPh_3, toluene, RT, overnight, 78%; b) NaOMe, MeOH, RT, 1 h, 90%; c) HF·pyridine, THF/pyridine (1:1), 0 °C, 0.5 h, 96%; d) TrCl, DMAP, pyridine, 70 °C, 4 h; e) BnBr, NaH, DMF, 0 °C→RT; f) $ZnBr_2$, CH_2Cl_2/*i*PrOH (85:15), RT, overnight, 63% over three steps.



Scheme 3. Reagents and conditions: a) **14**, TMSOTf, THF, 4 Å MS, −20 °C, 2 h, 70% or **20**, BF_3·Et_2O, Et_2O/THF, 4 Å MS, −20 °C, 3 h, 58%; b) 1) PMe_3, THF, RT, 2 h, 2) 1 M NaOH, RT, 2 h; c) $C_{25}H_{51}COOH$ or $C_7H_{15}COOH$, EDC, CH_2Cl_2, RT, 18 h, 76% (**24**), 60% (**25**) and 64% (**26**); d) H_2, Pd black, $CHCl_3$/EtOH (1:3), 4 h, 68% (**2**); e) H_2, Pd/C, EtOAc, 43% (**3**) and 75% (**4**).



Scheme 4. Reagents and conditions: a) 1) PMe_3, THF, RT, 2 h, 2) 1 M NaOH, RT, 2 h; b) triphosgene, iPr_2EtN, CH_2Cl_2, RT, 16 h, 64% (**27**), 83% (**28**) over two steps; c) $ZnBr_2$, CH_2Cl_2/*i*PrOH (85:15), RT, overnight, 89%; d) $(tBu)_2Si(OTf)_2$, DMF, pyridine, −20 °C, 4 h, 95%; e) H_2, Pd black, $CHCl_3$/EtOH (1:3), 7 h; f) triphosgene, iPr_2EtN, CH_2Cl_2, RT, 16 h, 43% (**31**) over two steps.

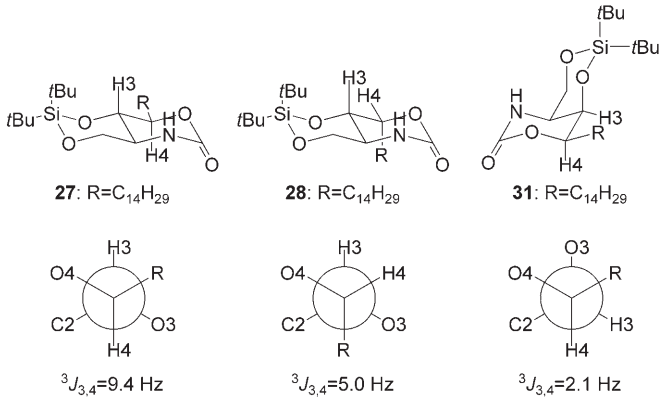


Figure 2. Determination of the coupling constant between H3 and H4 of bicyclic compounds **27**, **28**, and **31**.

In vitro stimulation of NKT cells

To investigate whether these analogues could elicit NKT cell activation, splenocytes from B6 mice were cultured with different concentrations of glycolipids. Figure 3 demonstrates that all compounds induced significant IFN-γ and IL-4 production in a dose-dependent manner. Remarkably, epimer **4** activated NKT cells to induce similar levels of both IFN-γ and IL-4 compared with α-GalCer, while the level of IFN-γ was significantly lower when splenocytes were cultured at the lowest concentrations with either **2** or **3**. A similar level of IFN-γ was observed after culturing the splenocytes with **1a**, **2**, or **3** at the highest concentration of 250 ng mL^{-1}. In addition, no cytokine production was observed when splenocytes from either *Jα18*$^{-/-}$ or *CD1d*$^{-/-}$ mice were cultured with α-GalCer or α-GalCer analogues; this indicates that these glycolipids induce CD1d-dependent TCR activation of NKT cells (Figure 3).

Conclusions

Herein we describe the synthesis and in vitro evaluation of α-Gal-D-*xylo*-Cer (**2**) and two α-Gal-L-*lyxo*-Cer analogues, one with the natural acyl chain (compound **3**), the other with a truncated one (compound **4**). The in vitro data demonstrate that these compounds showed significant biological activities through activation of NKT cells in both a TCR and CD1d-dependent manner. Furthermore, our data show evidence that a single modification by alteration of the stereochemistry of either the 3-OH or 4-OH in the phytosphingosine chain, causes the induction of differential cytokine levels. This observation can be probably explained by suggesting that these modifications might induce changes in the affinity for CD1d.

Experimental Section

General: NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, California, USA) or a Bruker Avance II 700 spectrometer. Chemical shifts are given in ppm (δ) relative to residual solvent peak, in the case of [D_6]DMSO: $\delta = 2.54$ ppm for ^{1}H and $\delta = 40.5$ ppm for ^{13}C, in the case of $CDCl_3$: $\delta = 7.26$ ppm for ^{1}H and $\delta = 77.4$ ppm for ^{13}C, and in the case of [D_5]pyridine $\delta =$ 7.18 ppm, 7.56 ppm and $\delta = 8.71$ ppm for ^{1}H and $\delta = 123.5$, 135.5 and 149.9 ppm for ^{13}C. All signals that were assigned to hydroxy groups were exchangeable with D_2O. Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qT of 2, Micromass, Manchester, UK) that was equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL min^{-1}. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under UV light at 254 nm and stained by sulfuric acid–anisaldehyde spray. Column chromatography was performed on ICN silica gel (63–200 μm, ICN, Asse Relegem, Belgium).

(2*S*,3*S*,4*R*)-2-Azidooctadecane-1,3,4-triol (6): A mixture of NaN_3 (20.48 g, 315 mmol), CH_2Cl_2 (50 mL) and H_2O (50 mL) was cooled to 0 °C, and Tf_2O (11.25 mL, 63 mmol) was added slowly. After 2 h stirring at 0 °C, the mixture was separated. The aqueous layer was extracted with CH_2Cl_2 (2×25 mL) and the combined organic layers were washed with H_2O (2×100 mL). This freshly prepared TfN_3 solution (100 mL in CH_2Cl_2) was added to a mixture of phytosphingosine **5** (10 g, 31.5 mmol), K_2CO_3 (8.7 g, 62.9 mmol), $CuSO_4$ (50 mg, 0.32 mmol), H_2O (200 mL) and MeOH (600 mL). The mixture was stirred overnight and evaporated in vacuo to 200 mL of a white slurry. The precipitate was filtered, washed with H_2O (5×100 mL) and lyophilized to yield compound **6** (10.60 g, 98%) as a white solid. ^{1}H NMR (300 MHz, [D_5]pyridine): $\delta = 0.86$ (t, $J = 6.7$, 3H), 1.18–1.36 (m, 22H), 1.60–1.74 (m, 1H), 1.84–1.90 (m, 2H), 2.12–2.24 (m, 1H), 4.20–4.27 (m, 1H), 4.32 (dd, $J = 4.4$ and 6.7, 1H), 4.43 (ddd, $J =$ 3.5, 4.1 and 7.6, 1H), 4.57 (dd, $J = 7.5$ and 11.4, 1H), 4.69 (dd, $J = 3.5$ and 11.4, 1H), 6.38 (br s, 1H), 7.08 ppm (br s 2H); ^{13}C NMR (75 MHz, [D_5]pyridine): $\delta = 14.20$, 22.86, 26.31, 29.53, 29.84, 29.89, 29.99, 30.15, 32.04, 34.12, 61.93, 66.67, 72.33, 75.96 ppm; HRMS (ESI-MS): *m/z*: for $C_{18}H_{37}N_3O_3$: calcd: 366.2733 [*M*+Na]$^+$, found: 366.2725.

(2*S*,3*S*,4*R*)-2-Azido-1,3-*O*-di-(*tert*-butyl)silanediyloctadecane-1,3,4-triol (7): A solution of **6** (10 g, 29.11 mmol) in DMF (200 mL) and pyridine (2.6 mL, 32.02 mmol) was cooled to −20 °C and

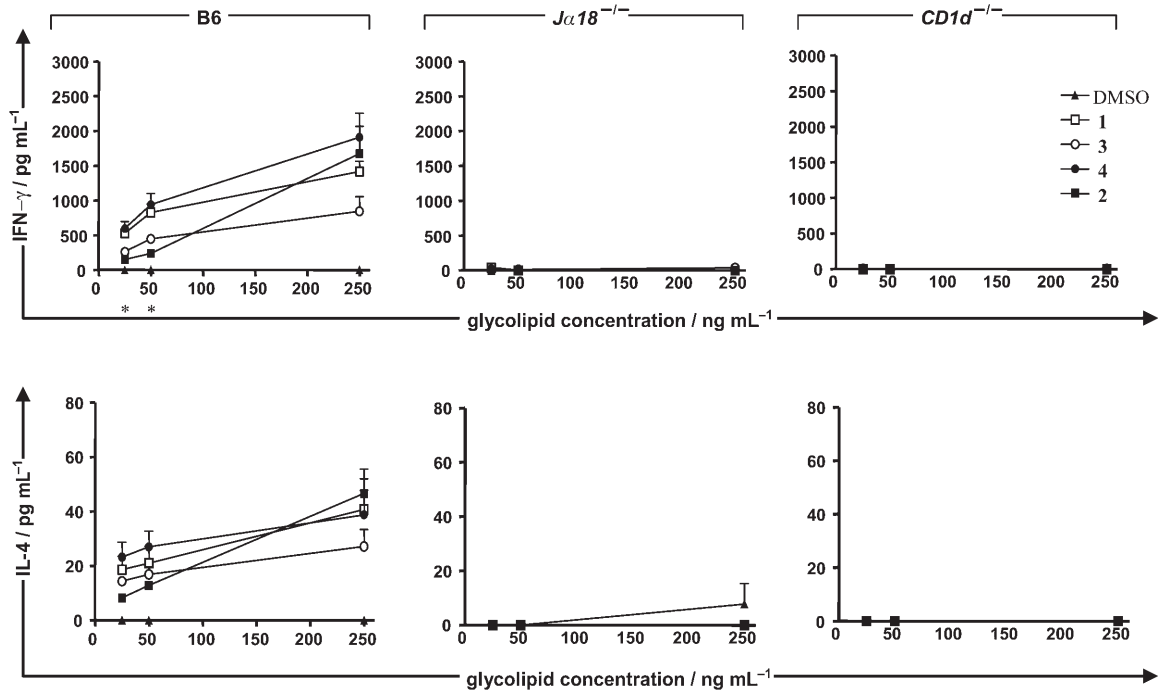


Figure 3. Properties of glycolipids to activate the TCR of NKT cells in a CD1d-dependent manner. Splenocytes of B6, *Jα18*$^{-/-}$, and *CD1d*$^{-/-}$ mice were cultured with various concentrations of **1a** (□), **2** (■), **3** (○), **4** (●), or DMSO (▲). After 72 h, supernatants were harvested, and both IFN-γ (top row) and IL-4 (bottom row) levels were measured by ELISA. Data represent the mean ±SEM of 6–8 wells that were pooled from two experiments. (*$P < 0.05$ for both compounds **2** and **3** versus **1a** at glycolipid concentrations of 50 ng mL^{-1} and 25 ng mL^{-1}; Mann–Whitney *U* test).

(tBu)$_2$Si(OTf)$_2$ (10.37 mL, 32.02 mmol) was added dropwise over 1 h. After additional stirring for 1 h at −20 °C, the mixture was quenched with H_2O (800 mL). The aqueous layer was extracted with EtOAc (3×250 mL) and the combined organic layers were washed with 1 M HCl (150 mL) and H_2O (2×150 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (95:5), to afford **7** (13.47 g, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 6.7$, 3H), 1.00 (s, 9H), 1.04 (s, 9H), 1.20–1.44 (m, 23H), 1.48–1.62 (m, 3H), 2.09 (d, $J = 8.5$, 1H), 3.51 (app dt, $J = 4.7$ and 10.0, 1H), 3.71–3.79 (m, 1H), 3.92 (dd, $J = 6.0$ and 10.0, 1H), 3.94 (dd, $J = 10.0$ and 10.1, 1H), 4.22 ppm (dd, $J = 4.7$ and 10.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.34$, 20.48, 22.92, 22.93, 25.88, 27.23, 27.71, 29.59, 29.83, 29.86, 29.89, 29.90, 29.92, 29.93, 31.10, 32.15, 58.99, 66.56, 73.15, 79.22 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{26}H_{53}N_3O_3Si_1$: 482.3783 $[M-H]^+$, found: 482.3780.

(2*S*,3*S*,4*R*)-2-Azido-4-*O*-benzyl-1,3-*O*-di-(*tert*-butyl)silanediyloctadecane-1,3,4-triol (8): A solution of benzyl alcohol (12.3 mL, 118.85 mmol) in Et_2O (45 mL) was cooled to 0 °C and NaH (1.19 g, 29.71 mmol) was added. After 30 min stirring at 0 °C, Cl_3CCN (11.92 mL, 118.85 mmol) was added, and the solution was allowed to stir at room temperature for 1 h. The mixture was quenched with $NaHCO_3$ (50 mL) and the aqueous layer was extracted with Et_2O (3×50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness to afford benzyl 2,2,2-trichloroacetimidate as a brown oil. TfOH (208 μL, 2.38 mmol) was added dropwise to a mixture of **7** (11.5 g, 23.77 mmol) and freshly prepared benzyl 2,2,2-trichloroacetimidate (118.85 mmol) in Et_2O (55 mL). The brown mixture was stirred at room temperature for 48 h and quenched with $NaHCO_3$ (100 mL). The aqueous layer was extracted with Et_2O (3×100 mL), and the combined organic layers were washed with H_2O (50 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography with 1% Et_3N (*v/v*) in hexanes/EtOAc (99:1) to afford compound **8** (7.9 g, 58%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.82$ (t, $J = 6.7$, 3H), 0.92 (s, 9H), 0.98 (s, 9H), 1.20–1.44 (m, 23H), 1.48–1.62 (m, 3H), 3.45 (m, 1H), 3.52 (m, 1H), 3.82 (dd, $J = 9.9$ and 10.9, 1H), 3.98 (dd, $J = 3.1$ and 8.9, 1H), 4.12 (dd, $J = 4.4$ and 10.9, 1H), 4.52 (d, $J = 12.0$, 1H), 4.57 (d, $J = 12.0$, 1H), 7.20–7.30 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.35$, 20.57, 22.86, 22.92, 25.76, 27.24, 27.76, 29.59, 29.83, 29.86, 29.89, 29.90, 29.92, 29.94, 32.16, 59.37, 66.57, 72.18, 77.17, 81.15, 127.81, 128.19, 128.55, 138.73 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{33}H_{59}N_3O_3Si_1$: 596.4223 $[M+Na]^+$, found: 596.5014.

(2*S*,3*S*,4*R*)-2-Azido-4-*O*-benzyloctadecane-1,3,4-triol (9): A solution of HF in pyridine (3.99 mL, 28.78 mmol, 65–70%) was added dropwise to a solution of **8** (7.5 g, 13.08 mmol) in THF (65 mL) and pyridine (65 mL) at 0 °C. After 30 min the mixture was diluted with EtOAc (150 mL), the organic layer was washed with 1 M HCl (3× 50 mL) and H_2O (3×50 mL), dried on Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (7:3) to yield compound **9** (5.56 g, 98%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, $J = 6.7$, 3H), 1.20–1.38 (m, 23H), 1.42–1.56 (m, 2H), 1.62–1.68 (m. 1H), 2.40–2.58 (br s, 2H), 3.53 (m, 1H), 3.62 (m, 1H), 3.84 (dd, $J = 5.0$ and 11.7, 1H), 3.93 (m, 1H), 3.95 (dd, $J = 4.4$ and 11.7, 1H), 4.59 (s, 2H), 7.20–7.30 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.35$, 22.91, 25.53, 29.00, 29.59, 29.79, 29.83, 29.89, 29.93, 29.94, 29.95, 32.15, 62.97, 63.13, 72.23, 72.51, 79.76, 128.24, 128.26, 128.79, 138.02 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{25}H_{43}N_3O_3$: 434.3377 $[M+H]^+$, found: 434.3372.

(2*S*,3*S*,4*R*)-2-Azido-4-*O*-benzyl-1-*O*-trityloctadecane-1,3,4-triol (10): A solution of **9** (5.4 g, 12.45 mmol), DMAP (384 mg, 3.11 mmol) and trityl chloride (3.90 g, 13.70 mmol) in pyridine

(125 mL) was stirred overnight at 70 °C. The mixture was quenched with $NaHCO_3$ and extracted with CH_2Cl_2 (3×150 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (9:1) to yield compound **10** (7.33 g, 87%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, J = 6.6, 3H), 1.20–1.38 (m, 23H), 1.42–1.52 (m, 2H), 1.54–1.60 (m, 1H), 2.32 (d, J = 4.1, 1H), 3.34–3.42 (m, 2H), 3.53–3.64 (m, 2H), 3.80 (ddd, J = 4.4, 4.7 and 6.9, 1H), 4.28 (d, J = 11.3, 1H), 4.43 (d, 1H, J = 11.3), 7.19–7.35 (m, 14H), 7.44–7.50 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.36, 22.93, 25.22, 28.78, 29.60, 29.81, 29.86, 29.90, 29.93, 29.94, 29.99, 32.16, 62.67, 64.32, 71.38, 71.99, 79.65, 87.71, 127.39, 127.99, 128.00, 128.17, 128.65, 128.90, 138.31, 143.77 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{44}H_{57}N_3O_3$: 698.4298 $[M+Na]^+$, found: 698.4277.

(2*S*,3*R*,4*R*)-2-Azido-4-*O*-benzyl-3-*O*-(4-nitro)benzoyl-1-*O*-trityloctadecane-1,3,4-triol (11): DIAD (5.39 mL, 26.04 mmol) was added to a solution of triphenylphosphine (6.83 g, 26.04 mmol) and *p*-nitrobenzoic acid (4.34 g, 26.04 mmol) in THF (60 mL) at room temperature. After stirring for 1 h, compound **10** (7.0 g, 10.42 mmol) in THF (40 mL) was added, and the mixture was stirred overnight at room temperature. The next day, the THF was removed in vacuo, and the residue was purified by column chromatography with hexanes/EtOAc (97:3) to afford **11** (6.07 g, 71%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (t, J = 6.7, 3H), 1.20–1.38 (m, 24H), 1.44–1.52 (m, 2H), 3.27 (dd, 1H, J = 6.1 and 10.1), 3.45–3.55 (m, 2H), 3.75 (ddd, J = 4.2, 5.9 and 5.9, 1H), 4.05 (d, J = 11.5, 1H), 4.43 (d, J = 11.3, 1H), 5.51 (dd, J = 4.7 and 5.8, 1H), 7.06–7.12 (m, 2H), 7.20–7.30 (m, 12H), 7.40–7.46 (m, 6H), 8.15 (d, J = 9.1, 2H), 8.27 ppm (d, J = 9.1, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.92, 25.37, 29.60, 29.69, 29.75, 29.87, 29.89, 29.93, 29.94, 30.27, 32.16, 61.75, 63.15, 72.60, 74.76, 78.13, 87.58, 123.77, 127.47, 127.73, 127.87, 128.17, 128.51, 128.83, 131.22, 135.28, 138.10, 143.45, 150.86, 164.09 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{51}H_{60}N_4O_6$: 847.4411 $[M+Na]^+$, found: 847.3873.

(2*S*,3*R*,4*R*)-2-Azido-4-*O*-benzyl-1-*O*-trityloctadecane-1,3,4-triol (12): NaOMe (400 mg, 7.02 mmol) was added to a solution of **11** (5.8 g, 7.02 mmol) in MeOH (70 mL) at room temperature. The white suspension was stirred for 1 h, quenched with a saturated NH_4Cl solution, and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with brine (1×100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (9:1) to yield compound **12** (4.23 g, 89%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82 (t, J = 6.7, 3H), 1.20–1.38 (m, 24H), 1.42–1.56 (m, 2H), 2.14 (d, J = 5.6, 1H), 3.24–3.42 (m, 4H), 3.62 (dd, J = 5.2 and 9.7, 1H), 4.14 (d, J = 11.1, 1H), 4.44 (d, J = 11.3, 1H), 7.10–7.26 (m, 18H), 7.35–7.40 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.93, 25.30, 29.60, 29.82, 29.94, 30.09, 30.20, 31.92, 32.16, 63.53, 63.95, 72.42, 72.59, 79.55, 127.40, 127.49, 128.00, 128.03, 128.14, 128.68, 128.87, 138.23, 143.79 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{44}H_{57}N_3O_3$: 698.4298 $[M+Na]^+$, found: 698.4279.

(2*S*,3*R*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-trityloctadecane-1,3,4-triol (13): NaH (266 mg, 6.65 mmol) was added to a solution of **12** (3.0 g, 4.43 mmol) in DMF (44 mL) at 0 °C. After 30 min stirring at 0 °C, benzyl bromide (806 μL, 6.65 mmol) was added, and the mixture was stirred overnight at room temperature. H_2O (200 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3× 200 mL). The combined organic layers were washed with brine (3× 100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (97:3) to yield compound **13** (3.19 g, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82 (t, 3H, J = 6.7), 1.12–1.24 (m, 24H), 1.42–1.52 (m, 2H), 3.25–3.32 (m, 2H), 3.35–3.45 (m, 2H), 3.48–3.54 (m, 2H), 4.28 (d, J = 11.4, 1H), 4.36 (d, J = 11.4, 1H), 4.45 (d, J = 11.4, 1H), 4.58 (d, J = 11.5, 1H), 7.08–7.48 ppm (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.93, 25.74, 29.60, 29.85, 29.94, 30.75, 30.77, 32.16, 62.40, 63.55, 73.27, 74.70, 79.83, 80.12, 127.17, 127.24, 127.37, 127.75, 127.83, 128.06, 128.12, 128.23, 128.48, 128.50, 128.86, 128.96, 138.33, 138.70, 143.83 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{51}H_{63}N_3O_3$: 788.4767 $[M+Na]^+$; found: 788.4368.

(2*S*,3*R*,4*R*)-2-Azido-3,4-di-*O*-benzyloctadecane-1,3,4-triol (14): A solution of $ZnBr_2$ (7.7 g, 33.59 mmol, 1 M) in CH_2Cl_2/*i*PrOH 85:15 (33.6 mL) was added to compound **13** (1.59 g, 2.07 mmol), and the resulting yellow reaction mixture was stirred overnight at room temperature. H_2O (50 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3×75 mL). The combined organic layers were washed with brine (1×100 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The yellow crude was purified by column chromatography with hexanes/EtOAc (9:1) to afford compound **14** (792 mg, 73%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82 (t, J = 6.7, 3H), 1.14–1.28 (m, 24H), 1.42–1.56 (m, 2H), 2.18 (br s, 1H), 3.47–3.55 (m, 3H), 3.57–3.64 (m, 2H), 4.46 (s, 2H), 4.57 (d, 1H, J = 11.5), 4.62 (d, 1H, J = 11.5), 7.18–7.32 ppm (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.92, 26.24, 29.60, 29.78, 29.85, 29.90, 29.94, 30.18, 32.16, 62.69, 64.56, 73.22, 74.39, 79.44, 80.05, 128.20, 128.23, 128.33, 128.47, 128.72, 128.74, 138.02, 138.09 ppm. HRMS (ESI-MS): *m/z*: calcd for $C_{32}H_{49}N_3O_3$: 524.3846 $[M+H]^+$, found: 524.3839.

(2*S*,3*S*,4*S*)-2-Azido-4-*O*-(4-nitro)benzoyl-1,3-*O*-di-(*tert*-butyl)silanediyloctadecane-1,3,4-triol (15): DIAD (8.78 mL, 44.60 mmol) was added to a solution of triphenylphosphine (11.70 g, 44.60 mmol) and *p*-nitrobenzoic acid (7.45 g, 44.60 mmol) in toluene (60 mL) at room temperature. After stirring for 1 h, compound **7** (8.63 g, 17.84 mmol) in toluene (20 mL) was added, and the mixture was stirred overnight at room temperature. The next day, toluene was removed in vacuo, and the residue was purified by column chromatography with hexanes/EtOAc (97:3) to afford **15** (8.83 g, 78%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.6, 3H), 1.04 (s, 9H), 1.06 (s, 9H), 1.20–1.42 (m, 24H), 1.84–1.94 (m, 2H), 3.54 (app td, J = 5.0 and 10.4, 1H), 3.91 (t, J = 10.8, 1H), 3.94 (dd, 1H, J = 1.8 and 10.0), 4.22 (dd, J = 5.0 and 10.8, 1H) 5.43 (app td, J = 1.5 and 7.0, 1H), 8.23 (d, J = 9.1, 2H), 8.29 ppm (d, J = 9.1, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.92, 25.37, 29.60, 29.69, 29.75, 29.87, 29.89, 29.93, 29.94, 30.27, 32.16, 61.75, 63.15, 72.60, 74.76, 78.13, 87.58, 123.77, 127.47, 127.73, 127.87, 128.17, 128.51, 128.83, 131.22, 135.28, 138.10, 143.45, 150.86, 164.09 ppm.

(2*S*,3*S*,4*S*)-2-Azido-1,3-*O*-di-(*tert*-butyl)silanediyloctadecane-1,3,4-triol (16): NaOMe (680 mg, 12.64 mmol) was added to a solution of **15** (8.0 g, 12.64 mmol) in MeOH (100 mL) at room temperature. The white suspension was stirred for 1 h, quenched with a saturated NH_4Cl solution and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with brine (1×100 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The crude was purified by column chromatography (hexanes/EtOAc 9:1) to yield compound **16** (5.52 g, 90%) as a colorless oil. ^{1}H NMR (300 MHz, [D_5]pyridine): δ = 0.97 (t, J = 7.0, 3H), 1.11 (s, 9H), 1.13 (s, 9H), 1.22–1.42 (m, 22H), 1.50–1.62 (m, 2H), 1.86–2.10 (m, 2H), 3.97 (dd, J = 1.8 and 9.4, 1H), 4.05 (t, J = 12.3, 1H), 4.10–4.17 (m, 1H), 4.32–4.44 (m, 2H), 5.83 ppm (d, J = 7.0, 1H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 14.17, 20.39, 22.79, 22.84, 26.30, 27.11, 27.56, 29.51, 29.79, 29.82, 29.84, 29.87, 30.05, 32.03, 33.76, 58.77, 66.90,

70.44, 79.27 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{26}H_{53}N_3O_3$: 482.3778 $[M-H]^+$; found: 482.3781.

(2*S*,3*S*,4*S*)-2-Azidooctadecane-1,3,4-triol (17): A solution of HF in pyridine (140 μL, 1.01 mmol, 65–70%) was added dropwise to a solution of **16** (1.20 g, 2.48 mmol) in THF (10 mL) and pyridine (10 mL) at 0 °C. After 30 min the mixture was diluted with EtOAc (50 mL), the organic layer was washed with 1 M HCl (3×50 mL) and H_2O (3×50 mL), dried on Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (1:1) to yield compound **17** (818 mg, 96%) as a white powder. ^{1}H NMR (300 MHz, [D$_5$]pyridine): $\delta=0.86$ (t, $J=6.7$, 3H), 1.21–1.40(m, 22H), 1.48–1.62 (m, 1H), 1.66–1.78 (m, 1H), 1.81–1.92 (m, 1H), 1.98–2.12 (m, 1H), 4.02 (app td, $J=1.8$ and 7.9, 1H), 4.21–4.32 (m, 2H), 4.36–4.46 (m, 1H), 4.65 (ddd, $J=3.2$, 5.0 and 11.4, 1H), 6.02 (d, $J=6.7$, 1H), 6.44 (d, 1H, $J=7.9$), 6.85 ppm (t, 1H, $J=5.6$); ^{13}C NMR (75 MHz, [D$_5$]pyridine): $\delta=14.18$, 22.85, 26.56, 29.52, 29.83, 29.86, 29.88, 29.95, 30.09, 32.04, 34.89, 63.14, 66.26, 70.92, 73.09 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{18}H_{37}N_3O_3$: 366.2733 $[M+Na]^+$, found: 366.2724.

(2*S*,3*S*,4*S*)-2-Azido-3,4-di-*O*-benzyloctadecane-1,3,4-triol (20): A solution of **17** (690 mg, 2.01 mmol), DMAP (61 mg, 0.50 mmol), and trityl chloride (842 mg, 1.56 mmol) in pyridine (5 mL) was stirred overnight at 70 °C. The mixture was diluted with EtOAc (20 mL) and washed with ice-cold HCl (0.1 M, 3×20 mL), H_2O (3×20 mL), and brine (1×20 mL). The organic layer was dried over Na_2SO_4, filtered and co-evaporated twice with toluene. NaH (643 mg, 16.08 mmol) was added to a solution of the crude compound **18** in DMF (10 mL) at 0 °C. After 30 min stirring at 0 °C, benzyl bromide (1.92 mL, 16.08 mmol) was added, and the mixture was stirred overnight at room temperature. H_2O (40 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3×75 mL). The combined organic layers were washed with brine (3×50 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. A solution of $ZnBr_2$ (13.8 g, 60.0 mmol, 1 M) in CH_2Cl_2/*i*PrOH (85:15, 60 mL) was added to the crude compound **19** and the resulting yellow mixture was stirred overnight at room temperature. The mixture was evaporated to dryness and dissolved in CH_2Cl_2 (20 mL). The solution was washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (8:2) to afford compound **20** (665 mg, 63% over three steps) as a colorless oil. ^{1}H NMR (300 MHz, [D$_5$]pyridine): $\delta=0.86$ (t, $J=6.7$, 3H), 1.20–1.34 (m, 22H), 1.42–1.61 (m, 2H), 1.82–1.92 (m, 2H), 3.92 (app td, $J=3.8$ and 6.2, 1H), 4.05 (dd, $J=3.8$ and 7.0, 1H), 4.12 (app td, $J=3.2$ and 6.7, 1H), 4.26–4.35 (m, 1H), 4.42 (ddd, $J=2.9$, 4.7 and 11.4, 1H), 7.01 (t, $J=5.28$, 1H), 7.26–7.40 (m, 6H), 7.50–7.56 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_5$]pyridine): $\delta=14.35$, 22.92, 26.24, 29.60, 29.78, 29.85, 29.90, 29.94, 30.18, 32.16, 62.69, 64.56, 73.22, 74.39, 79.44, 80.05, 128.20, 128.23, 128.33, 128.47, 128.72, 128.74, 138.02, 138.09 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{32}H_{49}N_3O_3$: 524.3846 $[M+H]^+$, found: 524.3870.

(2*S*,3*R*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)octadecane-1,3,4-triol (22): A solution of **14** (401 mg, 0.77 mmol) in THF (5 mL) was added to a mixture of **21** (545 mg, 0.92 mmol) and powdered 4 Å molecular sieves in THF (5 mL). The mixture was cooled to −20 °C, and TMSOTf (21 μL, 0.11 mmol) was added dropwise. After stirring for 1 h at −20 °C, the mixture was neutralized with Et_3N and filtered through celite. The filtrate was evaporated to dryness, and the resulting residue was purified by column chromatography (hexanes/EtOAc 5:1 + 1 V% Et_3N) to afford compound **22** (508 mg, 70%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.74$ (t, $J=6.3$, 3H), 1.14–1.20 (m, 22H), 1.30–1.46 (m, 4H), 3.37 (m, 1H), 3.40–3.50 (m, 4H), 3.63 (dd, $J=3.1$ and 9.9, 1H), 3.74 (dd, $J=1.6$ and 12.4, 1H), 3.80 (dd, $J=3.3$ and 10.1, 1H), 3.88–4.00 (m, 2H), 4.38 (s, 2H), 4.46 (d, 1H, $J=11.7$), 4.50 (d, $J=11.8$, 1H), 4.52 (t, $J=2.3$, 1H), 4.57 (d, $J=12.0$, 1H), 4.60 (d, $J=12.4$, 1H), 4.64 (d, $J=12.5$, 1H), 4.68 (d, $J=12.0$, 1H), 4.73 (d, $J=3.5$, 1H), 5.30 (s, 1H), 7.10–7.25 (m, 23H), 7.34–7.38 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=14.28$, 22.85, 25.80, 29.53, 29.77, 29.83, 29.87, 29.94, 30.50, 32.09, 61.83, 63.18, 68.51, 69.51, 72.26, 72.84, 73.15, 73.67, 74.53, 74.85, 75.64, 75.78, 77.37, 79.63, 79.95, 99.39, 101.21, 126.48, 127.73, 127.87, 127.91, 127.93, 128.15, 128.27, 128.39, 128.46, 128.47, 128.54, 128.65, 129.04, 137.95, 138.35, 138.54, 138.89 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{59}H_{75}N_3O_8$: 976.5452 $[M+Na]^+$; found: 976.5492.

(2*S*,3*S*,4*S*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)octadecane-1,3,4-triol (23): $BF_3<$**M.**$>Et_2O$ (280 μL, 2.18 mmol) was added dropwise to a mixture of **21** (960 mg, 1.62 mmol), **20** (570 mg, 1.09 mmol) and powdered 4 Å molecular sieves in Et_2O/THF 13:1 (20 mL) at −20 °C. The mixture was stirred for 2 h at −20 °C, and an additional portion of compound **21** (960 mg, 1.62 mmol) was added. After 1 h, the mixture was diluted with EtOAc (50 mL) and filtered through celite. The filtrate was washed with a saturated $NaHCO_3$ solution (2×50 mL) and brine (1×50 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (7:1) to afford compound **23** (578 mg, 56%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=$ 0.81 (t, $J=6.7$, 3H), 1.12–1.26 (m, 22H), 1.44–1.56 (m, 4H), 3.46 (app td, $J=3.1$ and 6.3, 1H), 3.53–3.58 (m, 2H), 3.62 (dd, $J=3.2$ and 7.7, 1H), 3.71 (dd, $J=5.0$ and 10.6, 1H), 3.90 (dd, $J=1.7$ and 12.5, 1H), 3.92–3.99 (m, 2H), 4.04 (dd, $J=3.1$ and 10.1, 1H), 4.08–4.13 (m, 2H), 4.42–4.47 (m, 3H), 4.58 (d, $J=11.6$, 1H), 4.59 (d, $J=11.7$, 1H), 4.67 (d, $J=12.3$, 1H), 4.74 (d, $J=12.4$, 1H), 4.79 (d, $J=11.7$, 1H), 4.90 (d, $J=3.2$, 1H), 5.40 (s, 1H), 7.13–7.35 (m, 23H), 7.43–7.47 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=14.36$, 22.93, 26.09, 29.60, 29.82, 29.89, 29.90, 29.92, 29.95, 30.01, 30.32, 32.16, 61.16, 63.30, 68.21, 69.64, 72.20, 72.66, 73.93, 74.50, 74.82, 75.54, 76.14, 77.44, 78.18, 79.16, 99.26, 101.32, 126.58, 127.73, 127.78, 127.89, 127.94, 127.99, 128.27, 128.35, 128.35, 128.46, 128.52, 128.53, 128.56, 129.11, 138.07, 138.22, 138.64, 138.92, 138.95 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{59}H_{75}N_3O_8$: 976.5452 $[M+Na]^+$, found: 976.5516.

(2*S*,3*R*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (24): A solution of trimethylphosphine in THF (2.66 mL, 2.66 mmol, 1 M) was added dropwise to a solution of **22** (508 mg, 0.53 mmol) in THF (5.4 mL) at room temperature. After stirring for 2 h at room temperature, a NaOH solution (10 mL, 1 M) was added and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over $MgSO_4$, filtered, and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (204 mg, 1.06 mmol), hexacosanoic acid (333 mg, 0.80 mmol) in CH_2Cl_2 (8 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **24** (532 mg, 76%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.88$ (t, $J=6.9$, 6H), 1.18–1.34 (m, 68H), 1.40–1.62 (m, 4H), 2.10 (t, $J=7.3$, 2H), 3.42–3.52 (m, 3H), 3.57 (dd, $J=7.3$ and 9.8, 1H), 3.72 (dd, $J=1.3$ and 7.6, 1H), 3.83 (dd, $J=1.6$ and 11.7, 1H), 3.87 (dd, $J=3.5$ and 10.4, 1H), 4.01–4.10 (m, 3H), 4.38 (app q, $J=7.9$,

1H), 4.48 (d, $J=11.4$, 1H), 4.55 (d, $J=11.4$, 1H), 4.61 (d, $J=12.3$, 1H), 4.64 (d, $J=12.0$, 1H), 4.70 (d, $J=12.9$, 1H), 4.76 (d, $J=12.3$, 1H), 4.82 (d, $J=12.0$, 1H), 4.83 (d, $J=11.7$, 1H), 4.90 (d, $J=3.5$, 1H), 5.42 (s, 1H), 5.76 (d, $J=9.1$, 1H), 7.18–7.40 (m, 23H), 7.47–7.52 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=14.36$, 22.93, 25.24, 26.07, 29.59, 29.61, 29.67, 29.83, 29.89, 29.94, 29.97, 30.11, 31.00, 32.16, 32.17, 37.12, 49.03, 63.16, 68.87, 69.56, 72.27, 73.37, 73.63, 74.99, 75.67, 75.82, 77.44, 79.82, 80.89, 99.49, 101.26, 126.58, 127.80, 127.84, 127.93, 127.95, 128.02, 128.06, 128.32, 128.55, 128.60, 128.64, 129.08, 138.05, 138.86, 138.91, 138.94, 172.89 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{85}H_{127}N_1O_9$: 1328.9408 $[M-H]^+$, found: 1329.0897.

(2*S*,3*S*,4*S*)-3,4-Di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25): A solution of trimethylphosphine in THF (1.90 mL, 1.90 mmol, 1 M) was added dropwise to a solution of **23** (360 mg, 0.38 mmol) in THF (4 mL) at room temperature. After stirring for 2 h at room temperature, a 1 M NaOH solution (1.9 mL) was added, and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4, filtered and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (225 mg, 1.14 mmol), and hexacosanoic acid (465 mg, 1.14 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **25** (298 mg, 60%) as a white solid. ^{1}H NMR (700 MHz, [D_5]pyridine): $\delta=0.84$–0.90 (m, 6H), 1.20–1.34 (m, 65H), 1.36–1.41 (m, 1H), 1.43–1.49 (m, 1H), 1.54–1.61 (m, 1H), 1.78–1.89 (m, 2H), 1.92–1.98 (m, 2H), 2.41 (t, $J=7.4$, 2H), 3.97 (dd, $J=5.8$ and 10.6, 1H), 4.02 (s, 1H), 4.10 (dd, $J=5.6$ and 10.2, 1H), 4.18 (d, $J=12.2$, 1H), 4.22 (t, $J=5.8$, 1H), 4.35 (dd, $J=4.4$ and 10.2, 1H), 4.39–4.44 (m, 3H), 4.64 (d, $J=2.2$, 1H), 4.73 (d, $J=10.9$, 1H), 4.77 (d, $J=12.4$, 1H), 4.80 (d, $J=11.2$, 1H), 4.86–4.93 (m, 4H), 4.97 (d, $J=11.4$, 1H),5.06 (m, 1H), 5.42 (d, $J=2.1$, 1H), 5.81 (s, 1H), 7.26–7.40 (m, 16H), 7.48 (d, $J=7.6$, 3H), 7.53 (d, $J=7.5$, 2H), 7.59 (d, $J=7.5$, 2H), 7.78 (d, $J=7.3$, 2H), 8.35 ppm (d, $J=7.9$, 1H); ^{13}C NMR (75 MHz, [D_5]pyridine): $\delta=14.20$, 22.86, 22.88, 25.72, 26.30, 29.53, 29.57, 29.73, 29.76, 29.84, 29.88, 29.95, 29.97, 30.26, 30.85, 32.05, 32.06, 36.78, 50.64, 63.58, 67.86, 69.63, 71.48, 72.63, 73.62, 74.41, 74.49, 76.61, 77.10, 80.15, 80.51, 99.36, 100.97, 126.85, 127.74, 127.76, 127.80, 127.82, 127.85, 127.96, 128.10, 128.35, 128.40, 128.58, 128.64, 128.65, 129.00, 139.30, 139.44, 139.45, 139.53, 139.60, 172.80 ppm.

(2*S*,3*S*,4*S*)-3,4-Di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-octanoylaminooctadecane-1,3,4-triol (26): A 1 M solution of trimethylphosphine in THF (1.10 mL, 1.10 mmol) was added dropwise to a solution of **23** (210 mg, 0.22 mmol) in THF (1 mL) at room temperature. After stirring for 2 h at room temperature, a 1 M NaOH solution (1.1 mL) was added, and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (127 mg, 0.66 mmol), octanoic acid (105 μL, 0.66 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **26** (145 mg, 64%) as a white solid. ^{1}H NMR (700 MHz, [D_5]pyridine): $\delta=0.81$ (t, $J=6.9$, 3H), 0.86 (t, $J=7.0$, 3H), 1.15–1.35 (m, 30H), 1.43–1.48 (m, 1H), 1.54–1.59 (m, 1H), 1.76–1.84 (m, 2H), 1.92–1.97 (m, 2H), 2.38 (t, $J=7.3$, 2H), 3.97 (dd, $J=5.9$ and 10.6, 1H), 4.02 (s, 1H), 4.10 (dd, $J=5.6$ and 10.2, 1H), 4.18 (dd, $J=1.4$ and 11.6, 1H), 4.22 (dd, $J=4.7$ and 6.0, 1H), 4.35 (dd, $J=4.4$ and 10.2, 1H), 4.40–4.44 (m, 3H), 4.64 (d, $J=2.2$, 1H), 4.73 (d, $J=11.4$, 1H), 4.77 (d, $J=12.3$, 1H), 4.78 (d, $J=12.0$, 1H), 4.80 (d, $J=11.4$, 1H), 4.83 (1H, d, $J=10.6$), 4.87 (1H, d, $J=10.6$), 4.92 (1H, d, $J=11.7$), 4.97 (1H, d, $J=11.7$), 5.01 (m, 1H), 5.42 (d, $J=2.6$, 1H), 5.80 (s, 1H), 7.26–7.39 (m, 15H), 7.47 (dd, $J=1.4$ and 7.6, 4H), 7.54 (d, $J=7.5$, 2H), 7.59 (d, $J=7.5$, 2H), 7.78 (d, $J=7.1$, 2H), 8.33 ppm (d, $J=7.9$, 1H); ^{13}C NMR (75 MHz, [D_5]pyridine): $\delta=14.15$, 14.21, 22.81, 22.87, 25.71, 26.26, 27.81, 28.37, 29.32, 29.55, 29.62, 29.87, 29.94, 29.50, 30.25, 30.84, 31.89, 32.05, 50.62, 63.59, 67.88, 69.62, 71.47, 72.62, 73.61, 74.41, 74.49, 76.60, 77.09, 80.17, 80.51, 99.36, 100.98, 127.75, 127.80, 127.82, 127.84, 127.96, 128.09, 128.35, 128.40, 128.58, 128.60, 128.64, 128.65, 129.00, 139.30, 139.44, 139.53, 139.59, 172.79 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{67}H_{91}N_1O_9$: 1054.6772 $[M+H]^+$; found: 1054.6716.

1-*O*-(α-D-Galactopyranosyl)-2-hexacosylamino-D-*xylo*-1,3,4-octadecanetriol (2): A solution of **24** (485 mg, 0.37 mmol) in $CHCl_3$/EtOH (1:3, 12 mL) was hydrogenated under atmospheric pressure for 4 h in the presence of Pd black (50 mg). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with CH_2Cl_2/MeOH (9:1) to afford compound **2** (215 mg, 68%) as a white powder. ^{1}H NMR (300 MHz, [D_5]pyridine): $\delta=0.86$ (6H, m), 1.18–1.42 (66H, m), 1.62–1.90 (4H, m), 2.00 (2H, m), 2.44 (2H, t, $J=7.5$), 4.08–4.18 (2H, m), 4.25–4.32 (1H, m), 4.34–4.50 (4H, m), 4.50–4.58 (2H, m), 4.60–4.68 (1H, m), 4.98–5.08 (1H, m), 5.48 (1H, d, $J=3.5$), 5.93 (1H, d, $J=5.0$), 6.26 (1H, d, $J=4.4$), 6.30 (1H, d, $J=3.9$), 6.37 (1H, d, $J=7.2$), 6.46 (1H, t, $J=5.5$), 6.53 (1H, d, $J=5.74$), 8.25 ppm (1H, d, $J=8.7$); ^{13}C NMR (75 MHz, [D_5]pyridine): $\delta=14.44$, 23.07, 23.09, 26.59, 26.76, 29.74, 29.78, 29.87, 29.98, 30.05, 30.12, 30.17, 30.23, 30.32, 32.25, 32.28, 34.02, 36.91, 51.09, 62.83, 69.32, 70.55, 71.11, 71.80, 72.55, 73.08, 74.32, 101.35, 173.95 ppm; HRMS (ESI-MS): *m/z*: calcd for (ESI-MS) for $C_{50}H_{99}N_1O_9$: 858.7392 $[M+H]^+$, found: 858.6768.

1-*O*-(α-D-Galactopyranosyl)-2-hexacosylamino-L-*lyxo*-1,3,4-octadecanetriol (3): A solution of **25** (275 mg, 0.21 mmol) in EtOAc (20 mL) was hydrogenated under atmospheric pressure for 16 h in the presence of 10% Pd/C (55 mg). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with CH_2Cl_2/MeOH (9:1) to afford compound **3** (77 mg, 43%) as a white powder. ^{1}H NMR (700 MHz, [D_5]pyridine): $\delta=0.88$ (m, 6H), 1.18–1.43 (m, 66H), 1.53–1.60 (m, 1H), 1.72–1.79 (m, 2H), 1.80–1.86 (m, 2H), 2.07–2.13 (m, 1H), 2.45 (t, $J=7.4$, 2H), 4.12–4.17 (m, 2H), 4.38–4.41 (dd, $J=5.6$ and 10.9, 1H), 4.43–4.44 (dd, $J=6.0$ and 10.5, 1H), 4.44–4.46 (dd, $J=3.1$ and 9.8, 1H), 4.48–4.53 (m, 3H), 4.55 (d, $J=3.0$, 1H), 4.66 (dd, $J=3.8$ and 9.9, 1H), 4.87–4.97 (m, 1H), 5.49 (d, $J=3.8$, 1H), 5.62 (brs, 1H), 6.06 (brs, 1H), 6.28 (brs, 1H), 6.40 (brs, 3H), 8.57 ppm (d, $J=8.8$, 1H); ^{13}C NMR (75 MHz, [D_5]pyridine): $\delta=14.22$, 22.88, 26.30, 26.85, 29.55, 29.57, 29.65, 29.75, 29.85, 29.88, 29.94, 29.96, 30.07, 30.20, 52.88, 62.59, 69.91, 70.52, 70.80, 70.90, 71.49, 73.05, 73.59, 102.19, 174.62 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{50}H_{99}N_1O_9$: 858.7398 $[M+H]^+$; found: 858.7355.

1-*O*-(α-D-Galactopyranosyl)-2-octanoylamino-L-*lyxo*-1,3,4-octadecanetriol (4): A solution of **26** (120 mg, 0.11 mmol) in EtOAc (15 mL) was hydrogenated under atmospheric pressure for 16 h in the presence of 10% Pd/C (24 mg). The solution was diluted with

pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with CH_2Cl_2/MeOH (95:5) to afford compound **4** (50 mg, 75%) as a white powder. ^{1}H NMR (700 MHz, [D$_5$]pyridine): δ = 0.79 (t, J = 7.0, 3H), 0.85 (t, J = 7.1, 3H), 1.10–1.18 (m, 5H), 1.20–1.38 (m, 25H), 1.52–1.59 (m, 1H), 1.71–1.78 (m, 3H), 1.80–1.85 (m, 1H), 2.06–2.12 (m, 1H), 2.42 (t, J = 7.5, 2H), 4.12–4.17 (m, 2H), 4.37–4.41 (dd, J = 5.6 and 10.9, 1H), 4.42–4.44 (dd, J = 6.5 and 11.1, 1H), 4.44–4.46 (dd, J = 3.4 and 10.1, 1H), 4.48–4.53 (m, 3H), 4.55 (d, J = 3.1, 1H), 4.66 (dd, J = 3.8 and 10.0, 1H), 4.86–4.91 (m, 1H), 5.48 (d, J = 3.8, 1H), 5.91 (brs, 6H), 8.56 ppm (d, J = 8.6, 1H); ^{13}C NMR (75 MHz, [D$_5$]pyridine): δ = 14.14, 14.21, 22.78, 22.87, 26.25, 26.84, 29.28, 29.52, 29.54, 29.85, 29.92, 29.94, 30.04, 30.20, 52.90, 62.59, 69.87, 70.54, 70.83, 70.90, 71.51, 73.06, 73.60, 102.21, 174.60 ppm; HRMS (ESI-MS): m/z: calcd for $C_{32}H_{63}N_1O_9$: 606.4581 $[M+H]^+$; found: 606.4557.

(2*S*,3*S*,4*R*)-1,3-*O*-Di-(*tert*-butyl)silanediyl-(2-*N*,4-*O*)-oxazinano-neoctadecane-1,3,4-triol (27): A solution of trimethylphosphine in THF (2.25 mL, 2.25 mmol, 1 M) was added dropwise to a solution of **7** (220 mg, 0.45 mmol) in THF (5 mL) at 0 °C. After stirring for 15 min at room temperature, a 1 M NaOH solution (5 mL) was added, and the mixture was allowed to stir for an additional hour. The solution was diluted with EtOAc (20 mL), washed with H_2O (2× 20 mL) and brine (2×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness to afford the amine. iPr$_2$EtN (160 μL, 0.90 mmol) and triphosgene (270 mg, 0.90 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0 °C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL) and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude was purified by column chromatography (hexanes/EtOAc 2:1) to afford compound **27** (140 mg, 64%) as a colorless wax. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.7, 3H), 0.95 (s, 9H), 1.03 (s, 9H), 1.18–1.36 (m, 22H), 1.40–1.48 (m, 1H), 1.52–1.66 (m, 2H), 1.84–1.95 (m, 1H), 3.43 (ddd, J = 4.5, 9.2 and 10.5, 1H), 3.73 (t, J = 9.1, 1H), 3.79 (t, J = 10.4, 1H), 4.03–4.10 (m, 1H), 4.11 (dd, J = 4.7 and 10.2, 1H), 7.17 ppm (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.33, 20.08, 22.90, 23.02, 24.30, 27.11, 27.57, 29.57, 29.66, 29.69, 29.76, 28.87, 29.89, 29.90, 29.91, 30.94, 32.14, 53.70, 67.50, 72.18, 80.94, 154.52 ppm; HRMS (ESI-MS): m/z: calcd for $C_{27}H_{53}N_1O_4$: 484.3822 $[M+H]^+$, found: 484.3806.

(2*S*,3*S*,4*S*)-1,3-*O*-Di-(*tert*-butyl)silanediyl-(2-*N*,4-*O*)-oxazinanone-octadecane-1,3,4-triol (28): A 1 M solution of trimethylphosphine in THF (1.16 mL, 1.16 mmol) was added dropwise to a solution of **16** (110 mg, 0.23 mmol) in THF (2.5 mL) at 0 °C. After stirring for 1 h at room temperature, a 1 M NaOH solution (1.16 mL) was added, and the mixture was allowed to stir for an additional hour. The solution was diluted with EtOAc (20 mL), washed with H_2O (2× 20 mL) and brine (2×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness to afford the amine. iPr$_2$EtN (80 μL, 0.46 mmol) and triphosgene (137 mg, 0.46 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0 °C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL), and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (2:1) to afford compound **28** (91 mg, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, J = 6.7, 3H), 0.97 (s, 9H), 1.03 (s, 9H), 1.18–1.38 (m, 22H), 1.41–1.59 (m, 2H), 1.62–1.74 (m, 1H), 1.80–1.88 (m, 1H), 3.53 (apptd, J = 4.4 and 10.1, 1H), 3.80 (t, J = 10.2, 1H), 4.14 (dd, J = 5.1 and 10.5, 1H), 4.19 (dd, J = 5.3 and 9.6, 1H), 4.33 (ddd, J = 1.9, 5.1 and 11.9, 1H), 7.10 ppm (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.34, 20.27, 22.91, 23.06, 25.87, 27.22, 27.58, 28.72, 29.58, 29.67, 29.69, 29.77, 29.86, 29.90, 29.92, 32.14, 49.53, 67.86, 70.84, 79.43, 153.82 ppm; HRMS (ESI-MS): m/z: calcd for $C_{27}H_{53}N_1O_4$: 484.3822 $[M+H]^+$, found: 484.3806.

(2*S*,3*R*,4*R*)-2-azido-4-*O*-benzyloctadecane-1,3,4-triol (29): A 1 M solution of $ZnBr_2$ (4.14 g, 18.76 mmol) in CH_2Cl_2/iPrOH (85:15, 18.8 mL) was added to compound **12** (794 mg, 1.17 mmol), and the resulting yellow mixture was stirred overnight at room temperature. H_2O (25 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with brine (1×50 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The yellow crude was purified by column chromatography with hexanes/EtOAc (3:1) to afford compound **29** (434 mg, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.4, 3H), 1.23–1.34 (m, 22H), 1.34–1.44 (m, 2H), 1.52–1.70 (m, 2H), 2.33 (t, J = 5.9, 1H), 2.68 (d, J = 4.7, 1H), 3.42 (appq, J = 4.7, 1H), 3.58 (appq, J = 5.9, 1H), 3.74 (m, 1H), 3.83 (m, 2H), 4.50 (d, J = 11.1, 1H), 4.69 (d, J = 11.1, 1H), 7.31–7.41 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.35, 22.92, 25.19, 29.59, 29.76, 29.81, 29.87, 29.89, 29.90, 29.92, 29.93, 30.08, 30.13, 32.15, 63.58, 63.77, 72.52, 73.81, 79.52, 128.24, 128.31, 128.84, 138.01 ppm; HRMS (ESI-MS): m/z: calcd for $C_{25}H_{43}N_3O_3$: 456.3202 $[M+Na]^+$, found: 456.3254.

(2*S*,3*R*,4*R*)-2-Azido-1,3-*O*-di-(*tert*-butyl)silanediyloctadecane-1,3,4-triol (30): A solution of **29** (430 mg, 0.99 mmol) in DMF (3 mL) and pyridine (97 μL, 1.19 mmol) was cooled at −20 °C and (tBu)$_2$Si(OTf)$_2$ (563 μL, 1.74 mmol) was added dropwise. After stirring for 1 h at −20 °C, the mixture was quenched with H_2O (20 mL). The aqueous layer was extracted with EtOAc (3×25 mL), and the combined organic layers were washed with 1 M HCl (20 mL) and H_2O (2×20 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (9:1) to afford **30** (540 mg, 95%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.4, 3H), 1.08 (s, 9H), 1.10 (s, 9H), 1.20–1.34 (m, 22H), 1.40–1.52 (m, 4H), 3.54 (m, 1H), 3.62 (appq, J = 1.8, 1H), 4.18 (dd, J = 1.8 and 7.6, 1H), 4.32 (dd, J = 1.8 and 12.9, 1H), 4.37 (dd, J = 2.4 and 12.9, 1H), 4.61 (d, J = 10.8, 1H), 4.92 (d, J = 10.8, 1H), 7.24–7.40 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.36, 20.71, 22.93, 23.60, 25.67, 27.29, 28.02, 29.60, 29.83, 29.85, 29.90, 29.93, 29.94, 30.92, 32.16, 60.07, 65.50, 75.51, 79.61, 81.41, 127.74, 128.38, 128.52, 139.40 ppm; HRMS (ESI-MS): m/z: calcd for $C_{33}H_{59}N_3O_3Si$: 596.4223 $[M+Na]^+$; found: 596.4490.

(2*S*,3*R*,4*R*)-1,3-*O*-Di-(*tert*-butyl)silanediyl-(2-*N*,4-*O*)-oxazinano-neoctadecane-1,3,4-triol (31): A solution of **30** (200 mg, 0.35 mmol) in $CHCl_3$/EtOH (1:3, 12 mL) was hydrogenated under atmospheric pressure for 7 h in the presence of Pd black (200 mg) and formic acid (13 μL, 0.35 mmol). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene to afford the crude amine as a colorless oil. iPr$_2$EtN (52 μL, 0.31 mmol) and triphosgene (92 mg, 0.31 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0 °C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL), and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude was purified by column chromatography with CH_2Cl_2/MeOH (95:5) to afford compound **31** (73 mg, 43%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.6, 3H), 0.99 (s, 9H), 1.05 (s, 9H), 1.20–1.40 (m, 22H), 1.40–1.52 (m, 2H), 1.68–1.90 (m, 2H), 3.47 (dd, J = 2.1 and 4.6, 1H), 4.04 (dd, J = 1.5 and 12.8, 1H), 4.13 (appt, J =

6.7, 1H), 4.25 (m, 1H), 4.32 (dd, $J=1.8$ and 12.8, 1H), 6.54 ppm (br s 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=14.33$, 20.66, 22.90, 23.65, 25.00, 27.06, 28.07, 29.57, 29.63, 29.68, 29.73, 29.85, 29.86, 29.88, 29.89, 29.91, 30.24, 32.14, 53.14, 65.91, 66.27, 80.47, 154.82 ppm; HRMS (ESI-MS): *m/z*: calcd for $C_{27}H_{53}N_1O_4Si$: 506.3642 $[M+Na]^+$, found: 506.3619.

In vivo experiments: C57BL/6J (B6) mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME, USA), and *CD1d*$^{-/-}$ and *Jα18*$^{-/-}$, both on B6 background, were kindly provided by Dr. François Trottein (Lille, France). Mice were bred in our breeding facility, and treated and used in agreement with the institutional guidelines. All animal procedures were approved by the Institutional Animal Care and Ethics Committee.

Dissolution of α-GalCer and α-GalCer-analogues: Stock solutions of **1**, **2**, **3** and **4** were prepared in 100% DMSO at a concentration of 1 mg mL^{-1}. Before use, the solutions were diluted with phosphate buffered saline (pH 7.4) to obtain a final concentration of 10 μg mL^{-1}.

In vitro stimulation with α-GalCer-analogues: Spleens from 8- to 12-week-old mice were removed and teased apart. After lysis of the erythrocytes with 0.17 M NH_4Cl, the remaining lymphocytes were washed three times with Dulbecco's phosphate-buffered saline (PBS). Cells were counted with trypan blue to exclude dead cells. Splenocytes were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 0.03% glutamine, and 5×10^{-5} M 2-ME (all obtained from Life Technologies, Paisley, UK). Splenocytes were cultured in flat-bottomed 96-well plates at 2×10^6 cells mL^{-1} per well in 200 μL, with a final concentration of glycolipids of 250 ng mL^{-1}, 50 ng mL^{-1}, or 25 ng mL^{-1}. After culture for 72 h, supernatants were harvested for determination of cytokine levels.

ELISA: The level of both IFN-γ and IL-4 in cell culture supernatants was measured by standard sandwich ELISA by using purified-capture and biotin-conjugated detection monoclonal antibodies and standards. After incubation with avidin peroxidase, ELISAs were developed with TMB substrate, followed by evaluation with a microplate reader.

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