The UGent Institutional Repository is the electronic archiving and dissemination platform for all UGent research publications. Ghent University has implemented a mandate stipulating that all academic publications of UGent researchers should be deposited and archived in this repository. Except for items where current copyright restrictions apply, these papers are available in Open Access.

This item is the archived peer-reviewed author-version of:

**Title:**

Synthesis and in vitro evaluation of alpha-GalCer epimers

**Author(s):** Trappeniers, M (Trappeniers, Matthias); Goormans, S (Goormans, Stijn); Van Beneden, K (Van Beneden, Katrien); Decruy, T (Decruy, Tine); Linclau, B (Linclau, Bruno); Al-Shamkhani, A (Al-Shamkhani, Aymen); Elliott, T (Elliott, Tim); Ottensmeier, C (Ottensmeier, Christian); Werner, JM (Werner, Joern M.); Elewaut, D (Elewaut, Dirk); Van Calenbergh, S (Van Calenbergh, Serge)

**Source:** CHEMMEDCHEM (2008), 3 (7), 1061-1070; **DOI:** 10.1002/cmdc.200800021
Synthesis and in vitro evaluation of α-GalCer epimers

Matthias Trappeniers, Stijn Goormans, Katrien Van Beneden, Bruno Linclau, Aymen Al-Shamkhani, Tim Elliot, Christian Ottenmeier, Joern Werner, Dirk Elewaut, and Serge Van Calenbergh

[a] M. Trappeniers, S. Goormans, Prof. Dr. Serge Van Calenbergh
Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University
Harelbekestraat 72, 9000 Gent (Belgium)
Fax: (+32) 9-264 81 46
E-mail: serge.vancalenbergh@ugent.be

[b] Dr. Katrien Van Beneden, Prof. Dr. Dirk Elewaut
Laboratory for Molecular Immunology and Inflammation, Department of Rheumatology,
Ghent University Hospital, Ghent University, De Pintelaan 185, 9000 Ghent (Belgium)

[c] Prof. Dr. Bruno Linclau
School of Chemistry, University of Southampton,
Highfield, Southampton SO17 1BJ (UK)

[d] Prof. Dr. Aymen Al-Shamkhani Prof. Dr. Christian Ottenmeier Prof. Dr. Tim Elliot
Cancer Sciences Division, School of Medicine, University of Southampton, Somers Cancer
Research Building, MP824, Tremona Road, Southampton SO16 6YD (UK)

[e] Prof. Dr. Joern Werner
School of Biological Sciences, University of Southampton
Bassett Crescent East, Southampton SO16 7PX (UK)

[+] These authors contributed equally to this work.
Abstract

α-GalCer (also known as KRN 7000) is an immunomodulatory glycolipid known to potently activate invariant natural killer T (NKT) cells upon CD1d-mediated stimulation. Since Th1 and Th2 cytokines, 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 (2) and two α-Gal-L-lyxo-Cer analogues, one with the natural acyl chain (3), the other with a truncated one (4).
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. An extensively studied exogenous glycolipid activator of NKT cells is α-GalCer (also known as KRN7000, 1a, Figure 1). α-GalCer was originally generated by the Kirin group from structure-activity studies of glycolipids, isolated from the marine sponge Agelas mauritianus.

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 anti-tumour, anti-viral and anti-bacterial effects of α-GalCer, while immunomodulatory Th2 cytokines such as IL-4 delay or prevent the onset of autoimmune diseases like type 1 diabetes. Since 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 complexed with α-GalCer was elucidated and unravelled the specific binding mode of α-GalCer to CD1d. The acyl chain of α-GalCer fits into the A’ pocket by adopting a counterclockwise circular curve, while 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 Thr-154, the 2’-OH of the galactose ring forms a hydrogen bond to Asp-151 and the 3-OH on the sphingosine chain forms the third hydrogen bond to Asp-80. These bonds are
assumed to anchor $\alpha$-GalCer in a proper orientation for recognition by the TCR of NKT cells.

In another crystallographic study of mouse CD1d complexed with an OCH analogue, Asp-80 was found to interact with both secondary hydroxyl groups (i.e., 3-OH and 4-OH) of the sphingosine backbone.\[7\] In addition, Arg-79 is oriented in a way that it may participate in hydrogen bonding with the same 3-OH. More recently, Wong et al. reported the crystal structure of mCD1d charged with the Sphingomonas glycolipid $\alpha$-galacturonosyl ceramide (GalA-GSL), characterized by a shorter fatty acyl chain, the absence of a 4-OH on the sphingosine moiety and the presence of a 6'-COOH 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 oxygens of Asp-80. As a consequence, the hydrogen bond between Arg-79 and the 3-OH of the sphinganine backbone is lost and Arg-79 is now oriented differently, possibly affecting 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 bound to the potent NKT-cell agonist $\alpha$-GalCer.\[9\] Consistent with the previously determined structures,\[6,7\] $\alpha$-GalCer protrudes minimally from the CD1d cleft with only the galactosyl head group exposed for recognition by the NKT TCR, interacting solely with the CDR1$\alpha$ and CDR3$\alpha$ loops. The galactose ring is sandwiched between Trp-153 of CD1d and the aliphatic moiety of Arg-95$\alpha$, 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 Gly-96$\alpha$, Ser-30$\alpha$ and Phe-29$\alpha$, respectively, located on the invariant TCR $\alpha$-chain. This mode of binding is consistent with the fine specificity the NKT TCR exhibits for $\alpha$-GalCer and its closely related analogues.
Most reported analogues of α-GalCer result from modifications of the acyl chain,\cite{10,11} the glycosidic linkage,\cite{12,13} or the glycosyl residue\cite{7,14-17} while relatively few alterations of the phytosphingosine part have been explored.\cite{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.\cite{19,20} Other studies reported the synthesis of a 4-deoxy-α-GalCer congener\cite{2,5,21,22} or analogues in which the phytoceramide moiety was replaced by a ceramide moiety, characterized by a double bound between C(4) and C(5).\cite{23,24} Interestingly, both types of analogues retained their ability to stimulate NKT cells, suggesting 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 and co-workers\cite{25} and more recently by Wong et al.\cite{26}

These studies illustrate the importance of exploring the stereochemistry at C(3) and C(4) 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\cite{27-29} or a serine-derived 1,5-dioxaspiro[3.2]hexane\cite{30} as the chiral template, by an aldol condensation between an iminoglycinate bearing a chiral auxiliary and an appropriate aldehyde\cite{31} or by asymmetric dihydroxylation of an (E)-α,β-unsaturated ester as the chiral induction stage.\cite{32} Remarkably, till now no attempts to alter the stereochemistry of the secondary hydroxyl groups (i.e., 3-OH and 4-OH) of the phytosphingosine moiety of α-GalCer have been published. Recent work by Sanghee Kim et al. on the efficient synthesis of D-xylo, L-lyxo- and L-arabino-phytosphingosine from the natural D-ribo-phytosphingosine\cite{33} prompt 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 (3), the other with a truncated one (4) (Fig. 1).

→ FIGURE 1

2. Results and discussion

2.1 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 hydroxyl groups at C(1) and C(3) afforded the 1,3-di-tert-butyl silylene derivative 7.[34] Benzyl protection of the remaining 4-OH group with freshly prepared benzyl 2,2,2-trichloroacetimidate, followed by silylene 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, after final deprotection of the trityl group, the desired D-xylo-phytosphingosine acceptor 14.

→ SCHEME 1

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, after final deprotection of the trityl group, the desired L-lyxo-phytosphingosine acceptor 20.
For the glycosidation step the 4,6-benzylidene-protected trichloroacetimidate 21 was used as the galactosyl donor (Scheme 3). Reaction with the L-lyxo-phytosphingosine acceptor 20 using BF$_3$.Et$_2$O as the promoter afforded the desired α-glycoside 23 in 58% yield. The glycosidation of the D-xylo-phytosphingosine acceptor 14 was performed with TMSOTf, as 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 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 C(3) and C(4) was established by preparing three bicyclic compounds 27, 28 and 31 and subjecting these to $^1$H NMR 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 cyclisation 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 hydroxyl groups at C(1) and C(3) afforded the 1,3-di-tert-butyl silylene derivative 30. Reduction of the azide and simultaneous deprotection of the 4-OH group gave, after cyclisation with triphosgene, the desired cis-fused bicyclic compound 31.

A $^1$H NMR spectrum obtained by selective decoupling of H(2) permitted us to determine the coupling constant between H(3) and H(4) (Fig. 2). For the trans-fused derivative 27 we found
a $^3J_{3,4}$ value of 9.4 Hz, which confirms the transdiaxial position of H(3) and H(4). Irradiation of H(2) in compound 28 gave rise to a $^3J_{3,4}$ value of 5.0 Hz, while 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°, thus confirming the inversion of the stereochemistry at C(3) (compound 31) and C(4) (compound 28).

FIGURE 2

2.2 Biological evaluation

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-$\gamma$ and IL-4 production in a dose dependent manner. Remarkably, epimer 4 activated NKT cells to induce similar levels of both IFN-$\gamma$ and IL-4 compared to $\alpha$-GalCer, while the level of IFN-$\gamma$ was significantly lower when splenocytes were cultured at the lowest concentrations with either 2 or 3. A comparable level of IFN-$\gamma$ was observed after culture of splenocytes with 1a, 2, or 3 at the highest concentration of 250 ng/ml. In addition, no cytokine production was observed when splenocytes from either J$\alpha$18$^{-/-}$ or CD1d$^{-/-}$ mice were cultured with $\alpha$-GalCer or $\alpha$-GalCer-analogues, indicating that these glycolipids induce CD1d-dependent TCR activation of NKT cells (Fig. 3).

FIGURE 3

3. Conclusions

This paper describes the synthesis and in vitro evaluation of $\alpha$-Gal-D-xylo-Cer (2) and two $\alpha$-Gal-L-lyxo-Cer analogues, one with the natural acyl chain (3), the other with a truncated one
(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**

*Synthesis*

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 DMSO-d$_6$ 2.54 ppm for $^1$H and 40.5 ppm for $^{13}$C, in the case of CDCl$_3$ 7.26 ppm for $^1$H and 77.4 ppm for $^{13}$C and in the case of pyridine-d$_5$ 7.18 ppm, 7.56 ppm and 8.71 ppm for $^1$H and 123.5 ppm, 135.5 ppm and 149.9 ppm for $^{13}$C. All signals assigned to hydroxyl groups were exchangeable with D$_2$O. 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, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 µL/min. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under UV light at 254 nm and revealed by sulphuric acid-anisaldehyde spray. Column chromatography was performed on ICN silica gel (63-200 µm, ICN, Asse Relegem, Belgium).

*(2S,3S,4R)-2-azidoctadecane-1,3,4-triol (6)*
A mixture of NaN₃ (20.48 g, 315 mmol), CH₂Cl₂ (50 ml) and H₂O (50 ml) was cooled at 0°C and Tf₂O (11.25 ml, 63 mmol) was added slowly. After 2 h stirring at 0°C, the reaction mixture was separated. The aqueous layer was extracted with CH₂Cl₂ (2 x 25 ml) and the combined organic layers were washed with H₂O (2 x 100 ml).

To a mixture of phytosphingosine 5 (10 g, 31.5 mmol), K₂CO₃ (8.7 g, 62.9 mmol), CuSO₄ (50 mg, 0.32 mmol), H₂O (200 ml) and MeOH (600 ml) was added the freshly prepared TfN₃-solution (100 ml in CH₂Cl₂). The reaction mixture was stirred overnight and evaporated in vacuo to remain 200 ml of a white slurry. The precipitate was filtered, washed with H₂O (5 x 100 ml) and lyophilised to yield compound 6 (10.60 g, 98 %) as a white solid.

**1H NMR** (300 MHz, pyridine-d₅): δ 0.86 (3H, t, J= 6.7), 1.18-1.36 (22H, m), 1.60-1.74 (1H, m), 1.84-1.90(2H, m), 2.12-2.24 (1H, m), 4.20-4.27 (1H, m), 4.32 (1H, dd, J= 4.4 and 6.7) 4.43 (1H, ddd, J= 3.5, 4.1 and 7.6), 4.57 (1H, dd, J= 7.5 and 11.4), 4.69 (1H, dd, J= 3.5 and 11.4), 6.38 (1H, br s), 7.08 (2H, br s).

**13C NMR** (75 MHz, pyridine-d₅): δ 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.

**Exact mass** (ESI-MS) for C₁₈H₃₇N₃O₃ [M+Na]⁺ found, 366.2725; calcd, 366.2733.

**(2S,3S,4R)-2-azido-1,3-O-di-(tert-butyl)silane-yl-octadecane-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 at -20°C and (tBu)₂Si(OTf)₂ (10.37 ml, 32.02 mmol) was added dropwise during 1 h.
After additional stirring for 1 h at -20°C, the reaction mixture was quenched with H₂O (800 ml). The aqueous layer was extracted with EtOAc (3 x 250 ml) and the combined organic layers were washed with a 1 M HCl solution (150 ml) and H₂O (2 x 150 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 95:5), affording 7 (13.47 g, 96%) as a colorless oil.

\[ ^1H \text{ NMR} \quad (300 \text{ MHz, CDCl}_3): \delta 0.87 \text{ (3H, t, } J = 6.7), 1.00 \text{ (9H, s), 1.04 (9H, s), 1.20-1.44 (23H, m), 1.48-1.62 (3H, m), 2.09 (1H, d, } J = 8.5), 3.51 \text{ (1H, app dt, } J = 4.7 \text{ and 10.0), 3.71-3.79 (1H, m), 3.92 (1H, dd, } J = 6.0 \text{ and 10.0), 3.94 (1H, dd, } J = 10.0 \text{ and 10.1), 4.22 (1H, dd, } J = 4.7 \text{ and 10.0).} \]

\[ ^13C \text{ NMR} \quad (75 \text{ MHz, CDCl}_3): \delta 14.34, 20.48, 22.92, 22.93, 25.88, 27.23, 27.71, 29.59, 29.83, 29.86, 29.89, 29.90, 29.92, 29.93, 31.10, 32.15, 58.99, 66.56, 73.15, 79.22. \]

**Exact mass** (ESI-MS) for C₂₆H₅₃N₃O₃Si₁ [M-H]⁺ found, 482.3780; calcd, 482.3783.

**A solution of benzyl alcohol (12.3 ml, 118.85 mmol) in Et₂O (45 ml) was cooled at 0°C and NaH (1.19 g, 29.71 mmol) was added. After 30 minutes stirring at 0°C, Cl₃CCN (11.92 ml, 118.85 mmol) was added, and the solution was allowed to stir at room temperature for 1 h. The reaction mixture was quenched with NaHCO₃ (50 ml) and the aqueous layer was extracted with Et₂O (3 x 50 ml). The combined organic layers were dried over MgSO₄, filtered and evaporated to dryness to afford benzyl 2,2,2-trichloroacetimidate as a brown oil.**
To a mixture of 7 (11.5 g, 23.77 mmol) and freshly prepared benzyl 2,2,2-trichloroacetimidate (118.85 mmol) in Et₂O (55 ml), TfOH (208 µl, 2.38 mmol) was added dropwise. The brown reaction mixture was stirred at room temperature for 48 h and quenched with NaHCO₃ (100 ml). The aqueous layer was extracted with Et₂O (3 x 100 ml) and the combined organic layers were washed with H₂O (50 ml), dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 99:1 + 1 V% Et₃N) to afford compound 8 (7.9 g, 58%) as a colorless oil.

1H NMR (300 MHz, CDCl₃): δ 0.82 (3H, t, J = 6.7), 0.92 (9H, s), 0.98 (9H, s), 1.20-1.44 (23H, m), 1.48-1.62 (3H, m), 3.45 (1H, m), 3.52 (1H, m), 3.82 (1H, dd, J = 9.9 and 10.9), 3.98 (1H, dd, J = 3.1 and 8.9), 4.12 (1H, dd, J = 4.4 and 10.9), 4.52 (1H, d, J = 12.0), 4.57 (1H, d, J = 12.0), 7.20-7.30 (5H, m).

13C NMR (75 MHz, CDCl₃): δ 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.

Exact mass (ESI-MS) for C₃₃H₅₉N₃O₃Si₁ [M+Na]⁺ found, 596.5014; calcd, 596.4223.

(2S,3S,4R)-2-azido-4-O-benzyl-octadecane-1,3,4-triol (9)

To a solution of 9 (7.5 g, 13.08 mmol) in THF (65 ml) and pyridine (65 ml) at 0°C, a HF solution in pyridine (3.99 ml, 28.78 mmol, 65-70 %) was added dropwise. After 30 minutes the reaction mixture was diluted with EtOAc (150 ml), the organic layer was washed with a 1 M HCl solution (3 x 50 ml) and H₂O (3 x 50 ml), dried on Na₂SO₄, filtered and evaporated to
dryness. The residue was purified by column chromatography (hexane/EtOAc 7:3) to yield compound 9 (5.56 g, 98%) as colorless oil.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.84 (3H, t, $J= 6.7$), 1.20-1.38 (23H, m), 1.42-1.56 (2H, m), 1.62-1.68 (1H, m), 2.40-2.58 (2H, br s), 3.53 (1H, m), 3.62 (1H, m), 3.84 (1H, dd, $J= 5.0$ and 11.7), 3.93 (1H, m), 3.95 (1H, dd, $J= 4.4$ and 11.7), 4.59 (2H, s), 7.20-7.30 (5H, m).

$^{13}$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.

Exact mass (ESI-MS) for C$_{25}$H$_{43}$N$_3$O$_3$ [M+H]$^+$ found, 434.3372; calcd, 434.3377.

(2S,3S,4R)-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 tritylchloride (3.90 g, 13.70 mmol) in pyridine (125 ml) was stirred overnight at 70°C. The reaction mixture was quenched with NaHCO$_3$ and extracted with CH$_2$Cl$_2$ (3 x 150 ml). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 9:1) to yield compound 10 (7.33 g, 87%) as a yellow oil.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (3H, t, $J= 6.6$), 1.20-1.38 (23H, m), 1.42-1.52 (2H, m), 1.54-1.60 (1H, m), 2.32 (1H, d, $J= 4.1$), 3.34-3.42 (2H, m), 3.53-3.64 (2H, m), 3.80 (1H, ddd, $J= 4.4$, 4.7 and 6.9), 4.28 (1H, d, $J= 11.3$), 4.43 (1H, d, $J= 11.3$), 7.19-7.35 (14H, m), 7.44-7.50 (6H, m).
\[^{13}\text{C}\text{ NMR}\ (75\text{ MHz, CDCl}_3)\]: \(\delta\ 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.\]

**Exact mass** (ESI-MS) for C_{44}H_{57}N_{3}O_{3} [M+Na]^+ found, 698.4277; calcd, 698.4298.

(2S,3R,4R)-2-azido-4-O-benzyl-3-O-(4-nitro)-benzoyl-1-O-trityloctadecane-1,3,4-triol (11)

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, DIAD (5.39 ml, 26.04 mmol) was added. After stirring for 1 h, compound 10 (7.0 g, 10.42 mmol) in THF (40 ml) was added and the reaction mixture was stirred overnight at room temperature. The next day, THF was removed \textit{in vacuo} and the residue was purified by column chromatography (hexane/EtOAc 97:3) to afford 11 (6.07 g, 71%) as a yellow oil.

\[^{1}\text{H NMR}\ (300\text{ MHz, CDCl}_3)\]: \(\delta\ 0.84\ (3\text{H, t, } J= 6.7), 1.20-1.38\ (24\text{H, m}), 1.44-1.52\ (2\text{H, m}), 3.27\ (1\text{H, dd, } J= 6.1\text{ and } 10.1), 3.45-3.55\ (2\text{H, m}), 3.75\ (1\text{H, ddd, } J= 4.2, 5.9\text{ and } 5.9), 4.05\ (1\text{H, d, } J= 11.5), 4.43\ (1\text{H, d, } J= 11.3), 5.51\ (1\text{H, ddd, } J= 4.7\text{ and } 5.8), 7.06-7.12\ (2\text{H, m}), 7.20-7.30\ (12\text{H, m}), 7.40-7.46\ (6\text{H, m}), 8.15\ (2\text{H, d, } J= 9.1), 8.27\ (2\text{H, d, } J= 9.1).\]

\[^{13}\text{C NMR}\ (75\text{ MHz, CDCl}_3)\]: \(\delta\ 14.35, 22.92, 25.37, 29.60, 29.69, 29.75, 29.87, 29.89, 29.93, 29.94, 30.27, 32.16, 61.75, 63.15, 72.60, 74.76, 78.13, 87.58, 123.77, 127.47, 127.73, 127.87, 128.17, 128.51, 128.83, 131.22, 135.28, 138.10, 143.45, 150.86, 164.09.\)
Exact mass (ESI-MS) for C_{51}H_{60}N_{4}O_{6} [M+Na]^+ found, 847.3873; calcd, 847.4411.

(2S,3R,4R)-2-azido-4-\textit{O}-benzyl-1-\textit{O}-trityloctadecane-1,3,4-triol (12)

To a solution of 11 (5.8 g, 7.02 mmol) in MeOH (70 ml) at room temperature, NaOMe (400 mg, 7.02 mmol) was added. The white suspension was stirred for 1 h, quenched with a saturated NH_{4}Cl solution and extracted with CH_{2}Cl_{2} (3 x 100 ml). The combined organic layers were washed with brine (1 x 100 ml), dried over MgSO_{4}, filtered and evaporated to dryness. The crude was purified by column chromatography (hexane/EtOAc 9:1) to yield compound 12 (4.23 g, 89%) as a yellow solid.

{superscript}1\text{H} NMR (300 MHz, CDCl\textsubscript{3}): \delta 0.82 (3H, t, \textit{J}= 6.7), 1.20-1.38 (24H, m), 1.42-1.56 (2H, m), 2.14 (1H, d, \textit{J}= 5.6), 3.24-3.42 (4H, m), 3.62 (1H, dd, \textit{J}= 5.2 and 9.7), 4.14 (1H, d, \textit{J}= 11.1), 4.44 (1H, d, \textit{J}= 11.3), 7.10-7.26 (18H, m), 7.35-7.40 (2H, m).

{superscript}13\text{C} NMR (75 MHz, CDCl\textsubscript{3}): \delta 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.

Exact mass (ESI-MS) for C_{44}H_{57}N_{3}O_{3} [M+Na]^+ found, 698.4279; calcd, 698.4298.

(2S,3R,4R)-2-azido-3,4-di-\textit{O}-benzyl-1-\textit{O}-trityloctadecane-1,3,4-triol (13)
To a solution of 12 (3.0 g, 4.43 mmol) in DMF (44 ml) at 0°C, NaH (266 mg, 6.65 mmol) was added. After 30 minutes stirring at 0°C, benzyl bromide (806 µl, 6.65 mmol) was added and the reaction mixture was stirred overnight at room temperature. H2O (200 ml) was added and the aqueous layer was extracted with CH2Cl2 (3 x 200 ml). The combined organic layers were washed with brine (3 x 100 ml), dried over MgSO4, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 97:3) to yield compound 13 (3.19 g, 94%) as a colorless oil.

\[
\text{1H NMR (300 MHz, CDCl}_3\text{: }\delta \text{ 0.82 (3H, t, } J = 6.7\text{), 1.12-1.24 (24H, m), 1.42-1.52 (2H, m), 3.25-3.32 (2H, m), 3.35-3.45 (2H, m), 3.48-3.54 (2H, m), 4.28 (1H, d, } J = 11.4\text{), 4.36 (1H, d, } J = 11.4\text{), 4.45 (1H, d, } J = 11.4\text{), 4.58 (1H, d, } J = 11.5\text{), 7.08-7.48 (25H, m).}
\]

\[
\text{13C NMR (75 MHz, CDCl}_3\text{: }\delta \text{ 14.35, 22.93, 25.74, 29.60, 29.85, 29.94, 30.75, 30.77, 32.16, 62.40, 63.55, 73.27, 74.70, 79.83, 80.12, 127.17, 127.24, 127.37, 127.75, 127.83, 128.06, 128.12, 128.23, 128.48, 128.50, 128.86, 128.96, 138.33, 138.70, 143.83.}
\]

**Exact mass** (ESI-MS) for C51H63N3O3 [M+Na]+ found, 788.4368; calcd, 788.4767.

(2S,3R,4R)-2-azido-3,4-di-O-benzyloctadecane-1,3,4-triol (14)

A solution of ZnBr2 (7.7 g, 33.59 mmol, 1M) in CH2Cl2/iPrOH 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. H2O (50 ml) was added and the aqueous layer was extracted with CH2Cl2 (3 x 75 ml). The combined organic layers were washed with brine (1 x 100 ml), dried over MgSO4, filtered and evaporated to dryness. The yellow crude was purified by
column chromatography (hexane/EtOAc 9:1) to afford compound 14 (792 mg, 73%) as a colorless oil.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3) : \delta 0.82 (3H, t, J= 6.7), 1.14-1.28 (24H, m), 1.42-1.56 (2H, m), 2.18 (1H, br s), 3.47-3.55 (3H, m), 3.57-3.64 (2H, m), 4.46 (2H, s), 4.57 (1H, d, J= 11.5), 4.62 (1H, d, J= 11.5), 7.18-7.32 (10H, m). \]

\[ ^13C \text{ NMR (75 MHz, CDCl}_3) : \delta 14.35, 22.92, 26.24, 29.60, 29.78, 29.85, 29.90, 29.94, 30.18, 32.16, 62.69, 64.56, 73.22, 74.39, 79.44, 80.05, 128.20, 128.23, 128.33, 128.47, 128.72, 128.74, 138.02, 138.09. \]

**Exact mass** (ESI-MS) for C\(_{32}H_{49}N_3O_3\) [M+H]\(^+\) found, 524.3839; calcd, 524.3846.

(2S,3S,4S)-2-azido-4-O-(4-nitro)-benzoyl-1,3-O-di-(tert-butyl)silanediy1-octadecane-1,3,4-triol (15)

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, DIAD (8.78 ml, 44.60 mmol) was added. After stirring for 1 h, compound 7 (8.63 g, 17.84 mmol) in toluene (20 ml) was added and the reaction mixture was stirred overnight at room temperature. The next day, toluene was removed \textit{in vacuo} and the residue was purified by column chromatography (hexane/EtOAc 97:3) to afford 15 (8.83 g, 78%) as a yellow oil.
\textbf{1H NMR} (300 MHz, CDCl\textsubscript{3}): \(\delta 0.86 \text{ (3H, t, } J = 6.6\), 1.04 (9H, s), 1.06 (9H, s), 1.20-1.42 (24H, m), 1.84-1.94 (2H, m), 3.54 (1H, app td, \(J = 5.0\) and 10.4), 3.91 (1H, t, \(J = 10.8\)), 3.94 (1H, \(J = 1.8\) and 10.0), 4.22 (1H, dd, \(J = 5.0\) and 10.8) 5.43 (1H, app td, \(J = 1.5\) and 7.0), 8.23 (2H, d, \(J = 9.1\)), 8.29 (2H, d, \(J = 9.1\)).

\textbf{13C NMR} (75 MHz, CDCl\textsubscript{3}): \(\delta 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.

\textbf{(2S,3S,4S)-2-azido-1,3-O-di-( tert-butyl)silanediyl-octadecane-1,3,4-triol (16)}

To a solution of 15 (8.0 g, 12.64 mmol) in MeOH (100 ml) at room temperature, NaOMe (680 mg, 12.64 mmol) was added. The white suspension was stirred for 1 h, quenched with a saturated NH\textsubscript{4}Cl solution and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 100 ml). The combined organic layers were washed with brine (1 x 100 ml), dried over MgSO\textsubscript{4}, filtered and evaporated to dryness. The crude was purified by column chromatography (hexane/EtOAc 9:1) to yield compound 16 (5.52 g, 90%) as a colorless oil.

\textbf{1H NMR} (300 MHz, pyridine-d\textsubscript{5}): \(\delta 0.97 \text{ (3H, t, } J = 7.0\), 1.11 (9H, s), 1.13 (9H, s), 1.22-1.42 (22H, m), 1.50-1.62 (2H, m), 1.86-2.10 (2H, m), 3.97 (1H, dd, \(J = 1.8\) and 9.4), 4.05 (1H, t, \(J = 12.3\)), 4.10-4.17 (1H, m), 4.32-4.44 (2H, m), 5.83 (1H, d, \(J = 7.0\)).

\textbf{13C NMR} (75 MHz, pyridine-d\textsubscript{5}): \(\delta 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.

\textbf{Exact mass} (ESI-MS) for C\textsubscript{26}H\textsubscript{53}N\textsubscript{3}O\textsubscript{3} [M-H]\textsuperscript{+} found, 482.3781; calcd, 482.3778.
(2S,3S,4S)-2-azidoctadecane-1,3,4-triol (17)

To a solution of 16 (1.20 g, 2.48 mmol) in THF (10 ml) and pyridine (10 ml) at 0°C, a HF solution in pyridine (140 µl, 1.01 mmol, 65-70 %) was added dropwise. After 30 minutes the reaction mixture was diluted with EtOAc (50 ml), the organic layer was washed with a 1 M HCl solution (3 x 50 ml) and H2O (3 x 50 ml), dried on Na2SO4, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 1:1) to yield compound 17 (818 mg, 96%) as a white powder.

1H NMR (300 MHz, pyridine-d5): δ 0.86 (3H, t, J = 6.7), 1.21-1.40(22H, m), 1.48-1.62 (1H, m), 1.66-1.78 (1H, m), 1.81-1.92 (1H, m), 1.98-2.12 (1H, m), 4.02 (1H, app td, J = 1.8 and 7.9), 4.21-4.32 (2H, m), 4.36-4.46 (1H, m), 4.65 (1H, ddd, J = 3.2, 5.0 and 11.4), 6.02 (1H, d, J = 6.7), 6.44 (1H, d, J = 7.9), 6.85 (1H, t, J = 5.6).

13C NMR (75 MHz, pyridine-d5): δ 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.

Exact mass (ESI-MS) for C18H37N3O3 [M+Na]+ found, 366.2724; calcd, 366.2733.

(2S,3S,4S)-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 tritylchloride (842 mg, 1.56 mmol) in pyridine (5 ml) was stirred overnight at 70°C. The reaction mixture was diluted with EtOAc (20 ml) and washed with an ice cold 0.1 M HCl solution (3 x 20 ml), H2O
(3 x 20 ml) and brine (1 x 20 ml). The organic layer was dried over Na₂SO₄, filtered and co-evaporated twice with toluene.

To a solution of the crude compound 18 in DMF (10 ml) at 0°C, NaH (643 mg, 16.08 mmol) was added. After 30 minutes stirring at 0°C, benzyl bromide (1.92 ml, 16.08 mmol) was added and the reaction mixture was stirred overnight at room temperature. H₂O (40 ml) was added and the aqueous layer was extracted with CH₂Cl₂ (3 x 75 ml). The combined organic layers were washed with brine (3 x 50 ml), dried over MgSO₄, filtered and evaporated to dryness.

A solution of ZnBr₂ (13.8 g, 60.0 mmol, 1M) in CH₂Cl₂/iPrOH 85:15 (60 ml) was added to the crude compound 19 and the resulting yellow reaction mixture was stirred overnight at room temperature. The reaction mixture was evaporated to dryness and resolubilised in CH₂Cl₂ (20 ml). The solution was washed with H₂O (3 x 20 ml) and brine (1 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The crude was purified by column chromatography (hexane/EtOAc 8:2) to afford compound 20 (665 mg, 63% over 3 steps) as a colorless oil.

**¹H NMR** (300 MHz, pyridine-d₅): δ 0.86 (3H, t, J= 6.7), 1.20-1.34 (22H, m), 1.42-1.61 (2H, m), 1.82-1.92 (2H, m), 3.92 (1H, app td, J= 3.8 and 6.2), 4.05 (1H, dd, J= 3.8 and 7.0), 4.12 (1H, app td, J= 3.2 and 6.7), 4.26-4.35 (1H, m), 4.42 (1H, ddd, J= 2.9, 4.7 and 11.4), 7.01 (1H, t, J= 5.28), 7.26-7.40 (6H, m), 7.50-7.56 (4H, m).

**¹³C NMR** (75 MHz, pyridine-d₅): 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.
**Exact mass** (ESI-MS) for C\textsubscript{32}H\textsubscript{49}N\textsubscript{3}O\textsubscript{3} [M+H]\textsuperscript{+} found, 524.3870; calcd, 524.3846.

**(2S,3R,4R)-2-azido-3,4-di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene-\alpha-D-galactopyranosyl)octadecane-1,3,4-triol (22)**

To a mixture of 21 (545 mg, 0.92 mmol) and powdered 4Å molecular sieves in THF (5 ml), a solution of 14 (401 mg, 0.77 mmol) in THF (5 ml) was added. The reaction 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 reaction mixture was neutralized with Et\textsubscript{3}N and filtered through celite. The filtrate was evaporated to dryness and the resulting residue was purified by column chromatography (hexane/EtOAc 5:1 + 1 V% Et\textsubscript{3}N) to afford compound 22 (508 mg, 70%) as a white solid.

**\textsuperscript{1}H NMR** (300 MHz, CDCl\textsubscript{3}): δ 0.74 (3H, t, \textit{J} = 6.3), 1.14-1.20 (22H, m), 1.30-1.46 (4H, m), 3.37 (1H, m), 3.40-3.50 (4H, m), 3.63 (1H, dd, \textit{J} = 3.1 and 9.9), 3.74 (1H, dd, \textit{J} = 1.6 and 12.4), 3.80 (1H, dd, \textit{J} = 3.3 and 10.1), 3.88-4.00 (2H, m), 4.38 (2H, s), 4.46 (1H, d, \textit{J} = 11.7), 4.50 (1H, d, \textit{J} = 11.8), 4.52 (1H, t, \textit{J} = 2.3), 4.57 (1H, d, \textit{J} = 12.0), 4.60 (1H, d, \textit{J} = 12.4), 4.64 (1H, d, \textit{J} = 12.5), 4.68 (1H, d, \textit{J} = 12.0), 4.73 (1H, d, \textit{J} = 3.5), 5.30 (1H, s), 7.10-7.25 (23H, m), 7.34-7.38 (2H, m).

**\textsuperscript{13}C NMR** (75 MHz, CDCl\textsubscript{3}): δ 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.
Exact mass (ESI-MS) for C\textsubscript{59}H\textsubscript{75}N\textsubscript{3}O\textsubscript{8} [M+Na]\textsuperscript{+} found, 976.5492; calcd, 976.5452.

(2S,3S,4S)-2-azido-3,4-di-\textit{O}-benzyl-1-\textit{O}-(2,3-di-\textit{O}-benzyl-4,6-\textit{O}-benzylidene-\textalpha-D-galactopyranosyl)octadecane-1,3,4-triol (23)

To a mixture of \textbf{21} (960 mg, 1.62 mmol), \textbf{20} (570 mg, 1.09 mmol) and powdered 4 Å molecular sieves in Et\textsubscript{2}O/THF 13:1 (20 ml) cooled to –20°C, BF\textsubscript{3}.Et\textsubscript{2}O (280 µl, 2.18 mmol) was added dropwise. The mixture was stirred for 2 h at –20°C and an additional portion of compound \textbf{21} (960 mg, 1.62 mmol) was added. After 1 h, the reaction mixture was diluted with EtOAc (50 ml) and filtered through celite. The filtrate was washed with a saturated NaHCO\textsubscript{3} solution (2 x 50 ml) and brine (1 x 50 ml), dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 7:1) to afford compound \textbf{23} (578 mg, 56%) as a white powder.

\textbf{1H NMR} (300 MHz, CDCl\textsubscript{3}): \textit{δ} 0.81 (3H, \textit{t}, \textit{J} = 6.7), 1.12-1.26 (22H, \textit{m}), 1.44-1.56 (4H, \textit{m}), 3.46 (1H, app td, \textit{J} = 3.1 and 6.3), 3.53-3.58 (2H, \textit{m}), 3.62 (1H, dd, \textit{J} = 3.2 and 7.7), 3.71 (1H, dd, \textit{J} = 5.0 and 10.6), 3.90 (1H, dd, \textit{J} = 1.7 and 12.5), 3.92-3.99 (2H, \textit{m}), 4.04 (1H, dd, \textit{J} = 3.1 and 10.1), 4.08-4.13 (2H, \textit{m}), 4.42-4.47 (3H, \textit{m}), 4.58 (1H, d, \textit{J} = 11.6), 4.59 (1H, d, \textit{J} = 11.7), 4.67 (1H, d, \textit{J} = 12.3), 4.74 (1H, d, \textit{J} = 12.4), 4.79 (1H, d, \textit{J} = 11.7), 4.90 (1H, d, \textit{J} = 3.2), 5.40 (1H, s), 7.13-7.35 (23H, \textit{m}), 7.43-7.47 (2H, \textit{m}).

\textbf{13C NMR} (75 MHz, CDCl\textsubscript{3}): \textit{δ} 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,
Exact mass (ESI-MS) for C_{59}H_{75}N_{3}O_{8} [M+Na]^+ found, 976.5516; calcd, 976.5452.

(2S,3R,4R)-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)

To a solution of 22 (508 mg, 0.53 mmol) in THF (5.4 ml) at room temperature, a solution of trimethylphosphine in THF (2.66 ml, 2.66 mmol, 1M) was added dropwise. After stirring for 2 h at room temperature, a NaOH solution (10 ml, 1M) was added and the reaction mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 ml), washed with H_{2}O (3 x 20 ml) and brine (1 x 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_{2}Cl_{2} (8 ml) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH_{2}Cl_{2} (20 ml), washed with H_{2}O (3 x 20 ml) and brine (1 x 20 ml), dried over MgSO_{4}, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 4:1) to afford compound 24 (532 mg, 76%) as a yellow oil.

{\textsuperscript{1}H NMR (300 MHz, CDCl}_{3}): \(\delta\) 0.88 (6H, t, \(J= 6.9\)), 1.18-1.34 (68H, m), 1.40-1.62 (4H, m), 2.10 (2H, t, \(J= 7.3\)), 3.42-3.52 (3H, m), 3.57 (1H, dd, \(J= 7.3\) and 9.8), 3.72 (1H, dd, \(J= 1.3\) and 7.6), 3.83 (1H, dd, \(J= 1.6\) and 11.7), 3.87 (1H, dd, \(J= 3.5\) and 10.4), 4.01-4.10 (3H, m), 4.38 (1H, app q, \(J= 7.9\)), 4.48 (1H, d, \(J= 11.4\)), 4.55 (1H, d, \(J= 11.4\)), 4.61 (1H, d, \(J= 12.3\)), 4.64
(1H, d, J= 12.0), 4.70 (1H, d, J= 12.9), 4.76 (1H, d, J= 12.3), 4.82 (1H, d, J= 12.0), 4.83 (1H, d, J= 11.7), 4.90 (1H, d, J= 3.5), 5.42 (1H, s), 5.76 (1H, d, J= 9.1), 7.18-7.40 (23H, m), 7.47-7.52 (2H, m).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 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.

**Exact mass** (ESI-MS) for C$_{85}$H$_{127}$N$_1$O$_9$ [M-H]$^-$ found, 1329.0897; calcd, 1328.9408.

(2S,3S,4S)-3,4-di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25)

To a solution of 23 (360 mg, 0.38 mmol) in THF (4 ml) at room temperature, a solution of trimethylphosphine in THF (1.90 ml, 1.90 mmol, 1M) was added dropwise. After stirring for 2 h at room temperature, a NaOH solution (1.9 ml, 1M) was added and the reaction mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 ml), washed with H$_2$O (3 x 20 ml) and brine (1 x 20 ml), dried over Na$_2$SO$_4$, 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), hexacosanoic acid (465 mg, 1.14 mmol) in CH$_2$Cl$_2$ (5 ml) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (20 ml), washed with H$_2$O (3 x 20 ml) and brine (1 x 20 ml), dried over
Na₂SO₄, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 4:1) to afford compound 25 (298 mg, 60%) as a white solid.

**¹H NMR** (700 MHz, pyridine-d₅): δ 0.84-0.90 (6H, m), 1.20-1.34 (65H, m), 1.36-1.41 (1H, m), 1.43-1.49 (1H, m), 1.54-1.61 (1H, m), 1.78-1.89 (2H, m), 1.92-1.98 (2H, m), 2.41 (2H, t, J= 7.4), 3.97 (1H, dd, J= 5.8 and 10.6), 4.02 (1H, s), 4.10 (1H, dd, J= 5.6 and 10.2), 4.18 (1H, d, J= 12.2), 4.22 (1H, t, J= 5.8), 4.35 (1H, dd, J= 4.4 and 10.2), 4.39-4.44 (3H, m), 4.64 (1H, d, J= 12.2), 4.73 (1H, d, J= 10.9), 4.77 (1H, d, J= 12.4), 4.80 (1H, d, J= 11.2), 4.86-4.93 (4H, m), 4.97 (1H, d, J= 11.4), 5.06 (1H, m), 5.42 (1H, d, J= 2.1), 5.81 (1H, s), 7.26-7.40 (16H, m), 7.48 (3H, d, J= 7.6), 7.53 (2H, d, J= 7.5), 7.59 (2H, d, J= 7.5), 7.78 (2H, d, J= 7.3), 8.35 (1H, d, J= 7.9).

**¹³C NMR** (75 MHz, pyridine-d₅): δ 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.

(2S,3S,4S)-3,4-di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-2-octanoylamino-octadecane-1,3,4-triol (26)

To a solution of 23 (210 mg, 0.22 mmol) in THF (1 ml) at room temperature, a solution of trimethylphosphine in THF (1.10 ml, 1.10 mmol, 1M) was added dropwise. After stirring for 2 h at room temperature, a NaOH solution (1.1 ml, 1M) was added and the reaction mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 ml),
washed with H₂O (3 x 20 ml) and brine (1 x 20 ml), dried over Na₂SO₄, 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 CH₂Cl₂ (3 ml) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH₂Cl₂ (20 ml), washed with H₂O (3 x 20 ml) and brine (1 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 4:1) to afford compound 26 (145 mg, 64%) as a white solid.

**¹H NMR** (700 MHz, pyridine-d₅): δ 0.81 (3H, t, J= 6.9), 0.86 (3H, t, J= 7.0), 1.15-1.35 (30H, m), 1.43-1.48 (1H, m), 1.54-1.59 (1H, m), 1.76-1.84 (2H, m), 1.92-1.97 (2H, m), 2.38 (2H, t, J= 7.3), 3.97 (1H, dd, J= 5.9 and 10.6), 4.02 (1H, s), 4.10 (1H, dd, J= 5.6 and 10.2), 4.18 (1H, dd, J= 1.4 and 11.6), 4.22 (1H, dd, J= 4.7 and 6.0), 4.35 (1H, dd, J= 4.4 and 10.2), 4.40-4.44 (3H, m), 4.64 (1H, d, J= 2.2), 4.73 (1H, d, J= 11.4), 4.77 (1H, d, J= 12.3), 4.78 (1H, d, J= 12.0), 4.80 (1H, d, J= 11.4), 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 (1H, m), 5.42 (1H, d, J= 2.6), 5.80 (1H, s), 7.26-7.39 (15H, m), 7.47 (4H, dd, J= 1.4 and 7.6), 7.54 (2H, d, J= 7.5), 7.59 (2H, d, J= 7.5), 7.78 (2H, d, J= 7.1), 8.33 (1H, d, J= 7.9).

**¹³C NMR** (75 MHz, pyridine-d₅): δ 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.

**Exact mass** (ESI-MS) for C₆₇H₉₁N₁O₉ [M+H]⁺ found, 1054.6716; calcd, 1054.6772.
1-O-(α-D-Galactopyranosyl)-2-hexacosylamino-D-xylo-1,3,4-octadecanetriol (2)

A solution of 24 (485 mg, 0.37 mmol) in CHCl₃/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 (CH₂Cl₂/MeOH 9:1) to afford compound 2 (215 mg, 68%) as a white powder.

^1H NMR (300 MHz, pyridine-d₅): δ 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 (1H, d, J= 8.7).

^13C NMR (75 MHz, pyridine-d₅): δ 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.

Exact mass (ESI-MS) for C₅₀H₉₀N₁O₉ [M+H]^+ found, 858.6768; calcd, 858.7392.

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 (CH₂Cl₂/MeOH 9:1) to afford compound 3 (77 mg, 43%) as a white powder.

**1H NMR** (700 MHz, pyridine-**d₅**): δ 0.88 (6H, m), 1.18-1.43 (66H, m), 1.53-1.60 (1H, m), 1.72-1.79 (2H, m), 1.80-1.86 (2H, m), 2.07-2.13 (1H, m), 2.45 (2H, t, \( J = 7.4 \)), 4.12-4.17 (2H, m), 4.38-4.41 (1H, dd, \( J = 5.6 \) and 10.9), 4.43-4.44 (1H, dd, \( J = 6.0 \) and 10.5), 4.44-4.46 (1H, dd, \( J = 3.1 \) and 9.8), 4.48-4.53 (3H, m), 4.55 (1H, d, \( J = 3.0 \)), 4.66 (1H, dd, \( J = 3.8 \) and 9.9), 4.87-4.97 (1H, m), 5.49 (1H, d, \( J = 3.8 \)), 5.62 (1H, br s), 6.06 (1H, br s), 6.28 (1H, br s), 6.40 (3H, br s), 8.57 (1H, d, \( J = 8.8 \)).

**13C NMR** (75 MHz, pyridine-**d₅**): δ 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.

**Exact mass** (ESI-MS) for C₅₀H₉₉N₁O₉ [M+H]⁺ found, 858.7355; calcd, 858.7398.

**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 (CH₂Cl₂/MeOH 95:15) to afford compound 4 (50 mg, 75%) as a white powder.

**¹H NMR** (700 MHz, pyridine-d₅): δ 0.79 (3H, t, J = 7.0), 0.85 (3H, t, J = 7.1), 1.10-1.18 (5H, m), 1.20-1.38 (25H, m), 1.52-1.59 (1H, m), 1.71-1.78 (3H, m), 1.80-1.85 (1H, m), 2.06-2.12 (1H, m), 2.42 (2H, t, J = 7.5), 4.12-4.17 (2H, m), 4.37-4.41 (1H, dd, J = 6.5 and 11.1), 4.42-4.44 (1H, dd, J = 6.5 and 11.1), 4.44-4.46 (1H, dd, J = 3.4 and 10.1), 4.48-4.53 (3H, m), 4.55 (1H, d, J = 3.1), 4.66 (1H, dd, J = 3.8 and 10.0), 4.86-4.91 (1H, m), 5.48 (1H, d, J = 3.8), 5.91 (6H, br s), 8.56 (1H, d, J = 8.6).

**¹³C NMR** (75 MHz, pyridine-d₅): 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.

**Exact mass** (ESI-MS) for C₃₂H₆₃N₁O₉ [M+H]⁺ found, 606.4557; calcd, 606.4581.

**(2S,3S,4R)-1,3-O-di-( tert-buty l)silaned iyl-(2-N,4-O)-oxazinanone-octadecane-1,3,4-tri o l** (27)

To a solution of 7 (220 mg, 0.45 mmol) in THF (5 ml) at 0°C, a solution of trimethylphosphine in THF (2.25 ml, 2.25 mmol, 1M) was added dropwise. After stirring for 15 minutes at room temperature, a NaOH solution (5 ml, 1M) was added and the reaction mixture was allowed to stir for an additional h. The solution was diluted with EtOAc (20 ml), washed with H₂O (2 x 20 ml) and brine (2 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness to afford the amine.
To a solution of the crude amine in CH₂Cl₂ (2 ml), DIPEA (160 µl, 0.90 mmol) and triphosgene (270 mg, 0.90 mmol) were added at 0°C. The reaction 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 H₂O (2 x 20 ml) and brine (2 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The crude was purified by column chromatography (hexane/EtOAc 2:1) to afford compound 27 (140 mg, 64%) as a colorless wax.

\(^1\)H NMR (300 MHz, CDCl₃): δ 0.86 (3H, t, \( J = 6.7 \)), 0.95 (9H, s), 1.03 (9H, s), 1.18-1.36 (22H, m), 1.40-1.48 (1H, m), 1.52-1.66 (2H, m), 1.84-1.95(1H, m), 3.43 (1H, ddd, \( J = 4.5, 9.2 \) and 10.5), 3.73 (1H, t, \( J = 9.1 \)), 3.79 (1H, t, \( J = 10.4 \)), 4.03-4.10 (1H, m), 4.11 (1H, dd, \( J = 4.7 \) and 10.2), 7.17 (1H, br s).

\(^{13}\)C NMR (75 MHz, CDCl₃): 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.

Exact mass (ESI-MS) for C₂₇H₅₃N₁O₄ [M+H]^⁺ found, 484.3806; calcd, 484.3822.

\((2S,3S,4S)-1,3-O\text{-di(tert-butyl)silanediyI-(2-N,4-O)-oxazinanone-octadecane-1,3,4-triol}\) (28)

To a solution of 16 (110 mg, 0.23 mmol) in THF (2.5 ml) at 0°C, a solution of trimethylphosphine in THF (1.16 ml, 1.16 mmol, 1M) was added dropwise. After stirring for 1 h at room temperature, a NaOH solution (1.16 ml, 1M) was added and the reaction mixture was allowed to stir for an additional h. The solution was diluted with EtOAc (20 ml), washed
with H₂O (2 x 20 ml) and brine (2 x 20 ml), dried over Na₂SO₄, filtered en evaporated to dryness to afford the amine.

To a solution of the crude amine in CH₂Cl₂ (2 ml), DIPEA (80 µl, 0.46 mmol) and triphosgene (137 mg, 0.46 mmol) were added at 0°C. The reaction 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 H₂O (2 x 20 ml) and brine (2 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The crude was purified by column chromatography (hexane/EtOAc 2:1) to afford compound 28 (91 mg, 83%) as a colorless oil.

**1H NMR** (300 MHz, CDCl₃): δ 0.87 (3H, t, J= 6.7), 0.97 (9H, s), 1.03 (9H, s), 1.18-1.38 (22H, m), 1.41-1.59 (2H, m), 1.62-1.74 (1H, m), 1.80-1.88 (1H, m), 3.53 (1H, app td, J= 4.4 and 10.1), 3.80 (1H, t, J= 10.2), 4.14 (1H, dd, J= 5.1 and 10.5), 4.19 (1H, dd, J= 5.3 and 9.6), 4.33 (1H, ddd, J= 1.9, 5.1 and 11.9), 7.10 (1H, br s).

**13C NMR** (75 MHz, CDCl₃): 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.

**Exact mass** (ESI-MS) for C₂₇H₅₃N₁O₄ [M+H]⁺ found, 484.3806; calcd, 484.3822.

**(2S,3R,4R)-2-azido-4-O-benzyloctadecane-1,3,4-triol (29)**

A solution of ZnBr₂ (4.14 g, 18.76 mmol, 1M) in CH₂Cl₂/iPrOH 85:15 (18.8 ml) was added to compound 12 (794 mg, 1.17 mmol) and the resulting yellow reaction mixture was stirred overnight at room temperature. H₂O (25 ml) was added and the aqueous layer was extracted with CH₂Cl₂ (3 x 50 ml). The combined organic layers were washed with brine (1 x 50 ml),
dried over MgSO₄, filtered and evaporated to dryness. The yellow crude was purified by column chromatography (hexane/EtOAc 3:1) to afford compound 29 (434 mg, 89%) as a colorless oil.

**¹H NMR** (300 MHz, CDCl₃): δ 0.88 (3H, t, J= 6.4), 1.23-1.34 (22H, m), 1.34-1.44 (2H, m), 1.52-1.70 (2H, m), 2.33 (1H, t, J= 5.9), 2.68 (1H, d, J= 4.7), 3.42 (1H, app q, J= 4.7), 3.58 (1H, app q, J= 5.9), 3.74 (1H, m), 3.83 (2H, m), 4.50 (1H, d, J= 11.1), 4.69 (1H, d, J= 11.1), 7.31-7.41 (5H, m).

**¹³C NMR** (75 MHz, CDCl₃): 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.

**Exact mass** (ESI-MS) for C₂₅H₄₃N₃O₃ [M+Na]⁺ found, 456.3254; calcd, 456.3202.

(2S,3R,4R)-2-azido-1,3-O-di-(tert-butyl)silanediyl-octadecane-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)₂Si(OTf)₂ (563 µl, 1.74 mmol) was added dropwise. After stirring for 1 h at -20°C, the reaction mixture was quenched with H₂O (20 ml). The aqueous layer was extracted with EtOAc (3 x 25 ml) and the combined organic layers were washed with a 1 M HCl solution (20 ml) and H₂O (2 x 20 ml), dried over Na₂SO₄, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 9:1), affording 30 (540 mg, 95%) as a colorless oil.
\[^1\text{H} \text{NMR}\] (300 MHz, CDCl\(_3\)): \(\delta\) 0.88 (3H, t, \(J = 6.4\)), 1.08 (9H, s), 1.10 (9H, s), 1.20-1.34 (22H, m), 1.40-1.52 (4H, m), 3.54 (1H, m), 3.62 (1H, app q, \(J = 1.8\)), 4.18 (1H, dd, \(J = 1.8\) and 7.6), 4.32 (1H, dd, \(J = 1.8\) and 12.9), 4.37 (1H, dd, \(J = 2.4\) and 12.9), 4.61 (1H, d, \(J = 10.8\)), 4.92 (1H, d, \(J = 10.8\)), 7.24-7.40 (5H, m).

\[^{13}\text{C} \text{NMR}\] (75 MHz, CDCl\(_3\)): \(\delta\) 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.

Exact mass (ESI-MS) for \(\text{C}_{33}\text{H}_{59}\text{N}_{3}\text{O}_{3}\text{Si}\) [M+Na]\(^+\) found, 596.4490; calcd, 596.4223.

\((2S,3R,4R)-1,3-\text{O-di-(tert-butyl)silanediyl-(2-N,4-O)-oxazinanone-octadecane-1,3,4-triol}\) (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.

To a solution of the crude amine in CH\(_2\)Cl\(_2\) (2 ml), DIPEA (52 µl, 0.31 mmol) and triphosgene (92 mg, 0.31 mmol) were added at 0°C. The reaction 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\(_2\)O (2 x 20 ml) and brine (2 x 20 ml), dried over Na\(_2\)SO\(_4\), filtered and evaporated to dryness. The crude was purified by column chromatography (CH\(_2\)Cl\(_2\)/MeOH 95:5) to afford compound 31 (73 mg, 43%) as a yellow oil.
\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 0.86 (3H, t, \(J = 6.6\)), 0.99 (9H, s), 1.05 (9H, s), 1.20-1.40 (22H, m), 1.40-1.52 (2H, m), 1.68-1.90 (2H, m), 3.47 (1H, dd, \(J = 2.1\) and 4.6), 4.04 (1H, dd, \(J = 1.5\) and 12.8), 4.13 (1H, app t, \(J = 6.7\)), 4.25 (1H, m), 4.32 (1H, dd, \(J = 1.8\) and 12.8), 6.54 (1H, br s).

\(^{13}\)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.

Exact mass (ESI-MS) for C\(_{27}\)H\(_{53}\)N\(_1\)O\(_4\)Si \([\text{M+Na}]^+\) found, 506.3619; calcd, 506.3642.

Animals

C57BL/6J (B6) mice were originally purchased from The Jackson Laboratory, CD1d\(^{-/-}\) and J\(\alpha\)18\(^{-/-}\), 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 \(\alpha\)-GalCer and \(\alpha\)-GalCer-analogues

Stock solutions of \(1, 2, 3\) and \(4\) were prepared in 100 % DMSO at a concentration of 1 mg/ml. Before use, the solutions were diluted with phosphate buffered saline (pH 7.4) to obtain a final concentration of 10 \(\mu\)g/ml.
**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 NH₄Cl, the remaining lymphocytes were washed three times with Dulbecco’s PBS. Cells were counted with trypan blue to exclude dead cells. Splenocytes were suspended in DMEM medium supplemented with 10 % fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.03 % glutamine, and 5 x 10⁻⁵ M 2-ME (all obtained from Life Technologies, Paisly, UK). This medium will be further referred to as complete DMEM. Splenocytes were cultured in flat bottom 96-well plates at 2 x 10⁶ cells/ml per well in 200 µl with a final concentration of glycolipids of 250 ng/ml, 50 ng/ml, or 25 ng/ml glycolipid. After culture for 72 h, supernatants was 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 using purified capture and biotin-conjugated detection monoclonal antibodies and standards. After incubation with avidin-peroxidase, ELISA’s were developed with TMB substrate, followed by evaluation using a microplate reader.
Acknowledgements

M.T. is indebted to the “Fund for Scientific Research-Flanders (Belgium) (F.W.O.-Vlaanderen) for a position as Aspirant. The authors are indebted to the Fund for Scientific Research-Flanders (Belgium) (F.W.O.-Vlaanderen) and Cancer Research Technology for financial support.¹
Keywords: α-galactosylceramide, epimers, glycolipids, synthesis, cytokines, NKT cells
References

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 (3), the other with a truncated one (4).

Figure 2. Determination of the coupling constant between H(3) and H(4) of bicyclic compounds 27, 28 and 31.

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 different concentrations of 1a (○), 2 (●), 3 (○), 4 (▲) or DMSO (△). After 72 h, supernatants was harvested and both IFN-γ (A) and IL-4 (B) levels were measured by ELISA. Data represent the mean ± SEM of 6 to 8 wells pooled from 2 experiments. (* P < 0.05 for both compounds 2 and 3 versus 1a at concentrations of 50 ng/ml and 25 ng/ml glycolipid; Mann-Whitney U test)

Scheme 1. Reagents and conditions: (a) N3Tf, K2CO3, CuSO4, CH2Cl2, CH3OH, H2O, rt, overnight, 98%; (b) (t-Bu)2Si(OTf)2, DMF, pyridine, -20°C, 4h, 96%; (c) Bn-trichloroacetimidate, TfOH (cat.), Et2O, 0°C-rt, 48h, 58%; (d) HF.pyridine, THF/pyridine: 1/1, 0°C, 0.5h, 98%; (e) TrCl, DMAP, pyridine, 70°C, overnight, 87%; (f) p-nitrobenzoic acid, DIAD, PPh3, THF, rt, overnight, 71%; (g) NaOMe, MeOH, rt, 1h, 89%; (h) BnBr, NaH, DMF, 0°C-rt, overnight, 94%; (i) ZnBr2, CH2Cl2/iPrOH: 85/15, rt, overnight, 73%

Scheme 2. Reagents and conditions: (a) p-nitrobenzoic acid, DIAD, PPh3, toluene, rt, overnight, 78%; (b) NaOMe, MeOH, rt, 1h, 90%; (c) HF.pyridine, THF/pyridine: 1/1, 0°C, 0.5h, 96%; (d) TrCl, DMAP, pyridine, 70°C, 4h; (e) BnBr, NaH, DMF, 0°C-rt; (f) ZnBr2, CH2Cl2/iPrOH: 85/15, rt, overnight, 63% over 3 steps
Scheme 3. Reagents and conditions: (a) 14, TMSOTf, THF, 4 Å MS, -20°C, 2h, 70% or 20, BF3·Et2O, Et2O/THF, 4 Å MS, -20°C, 3h, 58%; (b) (i) PMe3, THF, rt, 2h; (ii) NaOH 1M, rt, 2h; (c) C23H51COOH or C7H15COOH, EDC, CH2Cl2, rt, 18h, 76% (24), 60% (25) and 64% (26); (d) H2, Pd black, CHCl3/EtOH: 1/3, 4h, 68% (2); (e) H2, Pd/C, EtOAc, 43% (3) and 75% (4).

Scheme 4. Reagents and conditions: (a) (i) PMe3, THF, rt, 2h; (ii) NaOH 1M, rt, 2h; (b) triphosgene, (iPr)2EtN, CH2Cl2, rt, 16h, 64% (27), 83% (28) over 2 steps; (c) ZnBr2, CH2Cl2/iPrOH: 85/15, rt, overnight, 89%; (d) (t-Bu)2Si(OTf)2, DMF, pyridine, -20°C, 4h, 95%; (e) H2, Pd black, CHCl3/EtOH: 1/3, 7h; (f) triphosgene, (iPr)2EtN, CH2Cl2, rt, 16h, 43% (31) over 2 steps.
Probing the immunoregulatory effect of α-GalCer epimers: CD1d-mediated NKT cell activation by α-GalCer analogues like KRN7000 (1) induces immediate release of IL-4 and IFNγ. To assess the stereochemical requirements of the phytosphingosine part of 1, the 3'- (2) and 4'-epimers (3) of KRN7000 were synthesized starting from phytosphingosine.

M. Trappeniers, S. Goormans, K. Van Beneden, B. Linclau, A. Al-Shamkhani, T. Elliot, C. Ottenmeier, J. Werner, D. Elewaut, S. Van Calenbergh*
Figure 1

\[
\begin{align*}
\alpha\text{-GalO} & \quad C_{14}H_{29} \quad OH \\
\alpha\text{-GalO} & \quad C_{14}H_{29} \quad OH \\
\end{align*}
\]

[2S,3S,4R]-1a: \( R = C_{25}H_{51} \); \( R' = C_{14}H_{29} \)
[2S,3S,4R]-1b: \( R = C_{23}H_{47} \); \( R' = C_{5}H_{11} \)
[2S,3S,4S]-3: \( R = C_{25}H_{51} \)
[2S,3S,4S]-4: \( R = C_{7}H_{15} \)

Figure 2

\[
\begin{align*}
\text{H(3)} & \quad \text{H(4)} \\
\text{H(3)} & \quad \text{H(4)} \\
\end{align*}
\]

27: \( R = C_{14}H_{29} \)
28: \( R = C_{14}H_{29} \)
31: \( R = C_{14}H_{29} \)

\[
\begin{align*}
\text{H(3)} & \quad \text{H(4)} \\
\text{H(3)} & \quad \text{H(4)} \\
\end{align*}
\]

\( ^3J_{3,4} = 9.4 \text{ Hz} \)
\( ^3J_{3,4} = 5.0 \text{ Hz} \)
\( ^3J_{3,4} = 2.1 \text{ Hz} \)

Figure 3

\[
\begin{align*}
\text{B6} & \quad \text{CD1d} \\
\text{IFN-\gamma} & \quad \text{IL-4} \\
\end{align*}
\]

- **DMSO**
- 1
- 2
- 3
- 4
Scheme 3

\[ \text{Ph} - \overset{\text{O}}{\text{O}} - \overset{\text{O}}{\text{O}} - \overset{\text{NH}}{\text{O}} - \overset{\text{CCl}_3}{\text{O}} + 14/20 \rightarrow \text{BnO} - \overset{\text{OBn}}{\text{O}} - \overset{\text{OBn}}{\text{O}} - \overset{\text{Ph}}{\text{O}} \]

\[ \text{21} \]

\[ \text{Ph} - \overset{\text{O}}{\text{O}} - \overset{\text{O}}{\text{O}} - \overset{\text{O}}{\text{O}} - \overset{\text{OBn}}{\text{BnO}} - \overset{\text{OBn}}{\text{R}} - \overset{\text{NH}}{\text{O}} \rightarrow \text{BnO} - \overset{\text{OBn}}{\text{O}} - \overset{\text{OBn}}{\text{O}} - \overset{\text{Ph}}{\text{O}} \]

\[ [2S,3R,4R]-22 \]
\[ [2S,3S,4S]-23 \]

\[ [2S,3R,4R]-24: R = \text{C}_{25}H_{51} \]
\[ [2S,3S,4S]-25: R = \text{C}_{25}H_{51} \]
\[ [2S,3S,4S]-26: R = \text{C}_7H_{15} \]

Scheme 4

\[ \text{7} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \]

\[ \text{16} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \]

\[ \text{12} \rightarrow \overset{\text{HO}}{\overset{\text{O}}{\overset{\text{N}}{\text{O}}}} - \overset{\text{O}}{\overset{\text{Bn}}{\text{O}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \]

\[ \text{29} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \]

\[ \text{30} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \rightarrow \overset{\text{t-Bu} - \overset{\text{Si}}{\text{t-Bu}}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{C}_{14}H_{29}}{\overset{\text{HN}}{\text{O}}}}}} \]

\[ \text{31} \]