Amide Analogs of CD1d Agonists Modulate iNKT cell-Mediated Cytokine Production

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Synthesis of new CD1d Ligands

α-GalCer and α-GalCer Analogues 8–10: Our first approach to α-GalCer 1 and its ureido analogue 9 is summarized in Scheme 1. Nishida and Kobayashi’s dehydrative glycosylation methodology was used to install the α-glycosidic linkage;\textsuperscript{1,2} thus reaction of 2,3,4,6-tetra-O-benzyl-galactose 14\textsuperscript{3,4} with CBr\textsubscript{4}/PPh\textsubscript{3} afforded the corresponding galactosyl bromide, which was reacted in situ with acceptor 15,\textsuperscript{5} in the presence of tetramethylurea (TMU) and Bu\textsubscript{4}NBr, to provide the desired galactoside 16 as a single α-anomer (Scheme 1). Staudinger reduction of the azide in 16 with PMe\textsubscript{3} in wet THF\textsuperscript{6,7} afforded amine 17, which reacted with hexacosanoyl chloride\textsuperscript{8,9} to provide amide 18. Formation of the corresponding urea 19 from amine 17 required the synthesis of an appropriate isocyanate, which would be accessed by a Curtius rearrangement on the corresponding acid azide. Since the hydrophobic A’ binding pocket in CD1d optimally accommodates an acyl chain length containing 26 carbon atoms,\textsuperscript{10} we chose to use tetracosanoic acid as our starting material as this would be provide a urea product containing 25 atoms in the acyl chain (24 carbons and one nitrogen). Since the α-GalCer analogue containing a C\textsubscript{24} acyl chain displays similar biological activity to α-GalCer containing a C\textsubscript{26} chain,\textsuperscript{11} differences in biological activity between a ureido analogue containing 25 atoms in the acyl chain (i.e. 9), and α-GalCer 1, would be a attributable to an amide–urea switch and not the slightly truncated alkyl chain length. Tricosanoyl isocyanate was duly prepared from tetracosanoic acid following a procedure from Várová and co-workers,\textsuperscript{12} and used, without purification, in a reaction with amine 17 to provide urea 19 in 68% yield. Hydrogenolysis of the benzyl groups in amide 18 and urea 19 effected global deprotection and afforded our first target, urea 9, alongside α-GalCer 1, which would serve as the control in our biological studies (Scheme 1).
Scheme 1. Synthesis of $\alpha$-GalCer 1 and urea 9. (a) 14, PPh$_3$, CBr$_4$, CH$_2$Cl$_2$, r.t., 3 h; then Me$_2$NC(O)NMe$_2$, Bu$_4$NBr, CH$_2$Cl$_2$; then 15, CH$_2$Cl$_2$, 3 Å MS, r.t., 3 d, 62%. (b) PMe$_3$, THF, r.t., 4 h, then H$_2$O, 1 h, 72%. (c) CH$_3$(CH$_2$)$_{25}$C(O)Cl, Et$_3$N, CH$_2$Cl$_2$, 0 °C to r.t., 8 h, 18 (54%). (d) CH$_3$(CH$_2$)$_{22}$NCO, toluene, reflux, 8 h, 19 (68%). (e) Pd(OH)$_2$/C, H$_2$, THF, r.t., 22 h: 1 (68% from 17); 9 (73% from 17).

Since one of the benzyl ethers in the phytosphingosine unit of amide 18 and urea 19 proved to be particularly stubborn to remove, we investigated a phytosphingosine acceptor in which the internal 1,2-diol was protected as an isopropylidene acetal.$^{13}$ Galactoside 20 was consequently accessed under our standard conditions in good yield and once again with complete $\alpha$-stereoselectivity (Scheme 2). Subsequent Staudinger reduction provided amine 21, which was acylated as before to provide amide 22. Alternatively, reaction with a mixed carbonate, prepared from 1-tetracosanol and $N,N'$-disuccinimidyl carbonate,$^{14}$ provided carbamate 23. A two-step acetal hydrolysis / debenzylaion sequence on 22 and 23 proceeded uneventfully in both cases, to provide $\alpha$-GalCer 1 and carbamate
derivative 10, respectively. Finally the thioamide 8 was prepared from α-GalCer in a three-step sequence, involving peracetylation to provide 24, thionation of the amide with Lawesson’s reagent to afford thioamide 25, followed by deacetylation under Zemplen conditions (Scheme 2).

**Scheme 2.** Improved synthesis of α-GalCer 1, and synthesis of carbamate 10 and thioamide 8. (a) PMe₃, THF, 3 h, r.t., then H₂O, 1 h, 93%. (b) CH₃(CH₂)₂₄C(O)Cl, Et₃N, CH₂Cl₂, 0 °C to r.t., 12 h, 22 (85%). (c) N-succinimidyl-tetracosanoyl carbonate, Et₃N, CH₂Cl₂, r.t., 4 h, 23 (82%). (d) from 22: (i) TFA, CH₂Cl₂–H₂O, 10:1, 2 h, r.t.; (ii) Pd(OH)₂ / C, H₂, THF, 6 h, 1 (75%). (e) from 23: (i) TFA, CH₂Cl₂–MeOH, 2:1, 2 h, r.t.; (ii) Pd(OH)₂ / C, H₂, THF, 6 h, 10 (75%). (f) Ac₂O, pyridine, r.t., 10 h, 94%. (g) Lawesson’s reagent, toluene, 80 °C, 4 h, 85%. (h) NaOMe, MeOH, r.t., 2 h, 90%.

**ThrCer and ThrCer analogues 11–13:** Our attention turned to the synthesis of ThrCer 2 and its three analogues 11, 12 and 13. Ready access to an advanced intermediate, namely amine 26, using a slight modification of our previously established methodology, alongside that developed for generating the three α-GalCer analogues, provided straightforward access to the corresponding ThrCer analogues as summarized in Scheme 3. ThrCer 2 was synthesized from amine 26 in a threestep sequence involving acylation, followed by silyl ether deprotection and acetal hydrolysis.
Thionation of the acylation product 27 provided thioamide 28, which underwent the same two deprotection steps to afford our first ThrCer target, namely thioamide analogue 11. Alternatively, treatment of amine 26 with the mixed carbonate derived from the reaction of 1-tetracosanol with $N,N'$-disuccinimidyl carbonate, provided carbamate 29, and with tricosanyl isocyanate, furnished urea 30, and thence our final two targets, carbamate 13 and urea 12, after silyl deprotection and acetal hydrolysis (Scheme 3).

Scheme 3. Synthesis of ThrCer 2 and thioamide, urea and carbamate analogues. (a) CH$_3$(CH$_2$)$_{24}$C(O)Cl, Et$_3$N, CH$_2$Cl$_2$, 0 °C to r.t., 12 h, 85%. (b) Lawesson’s reagent, toluene, 80 °C, 5 h, 88%. (c) Bu$_4$NF, THF, r.t., 4 h. (d) TFA, CH$_2$Cl$_2$–MeOH (10:1), r.t.; 2 (74% from 27); 11 (73% from 28); 12 (72% from 30); 13 (70% from 29). (e) $N$-succinimidyl-tetracosanyl carbonate, Et$_3$N, CH$_2$Cl$_2$, r.t., 5 h, 29 (86%). (f) CH$_3$(CH$_2$)$_{24}$NCO, toluene, reflux, 8 h, 30 (80%).
General Experimental

Infra-red spectra were recorded neat as thin films. The intensity of each band is described as s (strong), m (medium) or w (weak) and with the prefix v (very) and suffix br (broad) where appropriate. $^1$H–NMR and $^{13}$C–NMR spectra were recorded in the solvent specified, at 500 and 125 MHz, 400 and 100 MHz, or 300 and 75 MHz, respectively. Chemical shifts are reported as $\delta$ values (ppm) referenced to the following solvent signals: CHCl$_3$, $\delta$$_H$ 7.26; CDCl$_3$, $\delta$$_C$ 77.0; CH$_3$OD, $\delta$$_H$ 3.34; CD$_3$OD, $\delta$$_C$ 49.9. The term, 'stack' is used to describe a region where resonances arising from non-equivalent nuclei are coincident, and multiplet, m, to describe a resonance arising from a single nucleus (or equivalent nuclei) in which coupling constants cannot be readily assigned. In analyzing AB systems, where the resonance pattern forms two well-separated groups, each of two lines, these are separately reported as "A of AB" and "B of AB", along with $J_{A-B}$. Connectivities were deduced from COSY90, HSQC and HMBC experiments. Mass spectra were recorded on a liquid chromatography time-of-flight (LCT) spectrometer utilizing electrospray ionization with a methanol mobile phase and are reported as ($m/z$ (%)). HRMS were recorded on a LCT spectrometer using a lock mass incorporated into the mobile phase. Melting points were determined using open capillaries and are uncorrected.

Reactions were monitored by thin layer chromatography using pre-coated glass-backed silica plates (60A F$_{254}$) and visualized by UV detection (at 254 nm) or by staining with ammonium molybdate(IV)–cerium(IV) sulfate staining dip, or 5% phosphomolybdic acid in EtOH (MPA spray), or 1% $\alpha$-naphthol, 5% H$_2$SO$_4$ in EtOH. Column chromatography was performed on silica gel (particle size 40–63 $\mu$m mesh) using standard glass columns or using pre-packed cartridges (silica, particle size 40 $\mu$m) [1 g (6 mL) cartridge size for purifying <30 mg of product, 2 g (12 mL) cartridge size for purifying 25–50 mg, 5 g (20 mL) cartridge size for purifying 50–100 mg of product].
All reactions were conducted in oven-dried (140 °C) or flame-dried glassware under a N₂ atmosphere, and at ambient temperature (20 to 25 °C) unless specified otherwise, with magnetic stirring. Volumes of 1 mL or less were measured and dispensed with gastight syringes. Evaporation and concentration under reduced pressure was performed at 50–500 mbar at 40 °C. Residual solvent was removed under high vacuum (1 mbar).

All reagents were obtained from commercial sources and used without further purification unless specified otherwise. Toluene and CH₂Cl₂ was freshly distilled under N₂ from CaH₂. THF were freshly distilled under N₂ from sodium benzophenone ketyl. Dry MeCN was purchased as puriss., absolute grade, over 4 Å molecular sieves (H₂O ≤0.001%), ≥99.5% (GC) and used without further purification. All solutions are aqueous and saturated unless specified otherwise. Pyridine and Et₃N were distilled from KOH and stored over 4 Å molecular sieves.

\[(2S,3S,4R)-3,4-Di-O-benzyl-1-O-(2',3',4',6'-tetra-O-benzyl-\alpha-D-galactopyranosyl)-2-(tricosylanaminocarbonylamino)octadecane-1,3,4-triol (19)\]

A screw-capped glass tube containing a solution of tetracosanoic acid (450 mg, 1.22 mmol) in (COCl)₂ (2.0 mL, 23 mmol) was closed tightly and heated at 70 °C for 2 h. The volatiles were then evaporated under a stream of argon and the tube then placed on a high vacuum line for at least 2 h to remove the residual volatiles. The resulting tetracosanoyl chloride was used directly in the next step without further purification: a solution of freshly prepared tetracosanoyl chloride (450 mg, 1.22
mmol) in THF (5 mL) was added dropwise over 10 min to a solution of NaN₃ (300 mg, 4.62 mmol) in H₂O (0.5 mL) at 0 ºC. The reaction mixture was stirred at r.t. for 5 h. The organic phase was then extracted with cold (~10 ºC) THF (5 mL) and dried over Na₂SO₄. The drying agent was removed by filtration. The solvent was removed under reduced pressure to provide tetracosanoyl azide as a white solid, which was used immediately in the next step: a solution of tetracosanoyl azide (481 mg, 1.22 mmol (assuming quantitative conversion in the previous step)) in toluene (5 mL) was heated under reflux for 4 h, after which time, the reaction mixture was cooled to r.t. The resulting solution of tricosanyl isocyanate product was used directly without further purification in the next step: a solution of amine 17 (150 mg, 0.147 mmol) in toluene (5 mL) was added to a solution of tricosanyl isocyanate (1.22 mmol (assuming quantitative conversion)) in toluene (5 mL) at r.t. The reaction mixture was heated under reflux for 8 h and then concentrated under reduced pressure. Purification of the residue by flash column chromatography (20% EtOAc in hexane) afforded urea 19 as a pale yellow oil (140 mg, 68% based on amine): Rₛ = 0.3 (20% EtOAc in hexane); [α]²⁰°D = +25.2 (c 1, CHCl₃); νₑₒₓₑₒₓₑₒₓₑₒ (cm⁻¹) 3363m (N–H), 1670m (C=O); δₜ (500 MHz, CDCl₃) 0.89 (t, J 7.0, 6H, 2 × CH₂CH₃), 1.10–1.39 (stack, 65H, alkyl chain methylenes), 1.39–1.51 (m, 1H, alkyl chain CH₃H₈ₐ), 1.56–1.64 (m, 1H, C(5)H₈ₐH₈ₖ), 1.64–1.73 (m, 1H, C(5)H₈ₐH₈ₖ), 2.90–3.06 (stack, 2H, CH₃H₈ᵦNH), 3.33 (dd, J 9.5, 5.0, 1H, C(6’)H₈ₐH₈ₕ), 3.55 (dd, J 9.5, 7.0, 1H, C(6’)H₈ₐH₈ₕ), 3.59–3.64 (m, 1H, H₄), 3.73 (dd, J 10.8, 3.1, 1H, C(1)H₈ₐH₈ₖ), 3.80–3.88 (stack, 3H, H₂–H₃, H₄), 3.89 (dd, J 10.1, 2.7, 1H, H₃), 3.98 (app. t, J 6.0, 1H, H₅), 4.05 (dd, J 10.1, 3.6, 1H, H₂), 4.11 (br dd, J 10.8, 4.5, 1H, C(1)H₈ₐH₈ₕ), 4.38 (A of AB, Jₐ–ₐ'B 12.0, 1H, C(6’)OCH₃H₈ₕH₈ₕ), 4.45–4.48 (stack, 2H, C(6’)OCH₃H₈ₕH₈ₕ), 4.54 (A of AB, Jₐ–ₐ'B 11.3, 1H, C(3)OCH₃H₈ₕH₈ₕ), 4.57 (A of AB, Jₐ–ₐ'B 11.8, 1H, C(4’)OCH₃H₈ₕH₈ₕ), 4.61 (B of AB, Jₐ–ₐ'B 12.6, 1H, C(4)OCH₃H₈ₕH₈ₕ), 4.66 (A of AB, Jₐ–ₐ'B 11.7, 1H, C(2’)OCH₃H₈ₕH₈ₕ), 4.75 (A of AB, Jₐ–ₐ'B 11.8, 1H, C(3’)OCH₃H₈ₕH₈ₕ), 4.78 (B of AB, Jₐ–ₐ'B 11.3, 1H, C(3)OCH₃H₈ₕH₈ₕ), 4.79 (B of AB, Jₐ–ₐ'B 11.7, 1H, C(2’)OCH₃H₈ₕH₈ₕ), 4.81 (B of AB, Jₐ–ₐ'B 11.8, 1H, C(3’)OCH₃H₈ₕH₈ₕ), 4.87 (d, J 3.6, 1H, H₁), 4.93 (B of AB, Jₐ–ₐ'B 11.8, 1H, C(4’)OCH₃H₈ₕH₈ₕ), 5.00 (d, J
7.8, 1H, C(2)NH); 7.23–7.38 (stack, 30H, 6 × Ph), CH2NH not observed; δ (125 MHz, CDCl3) 14.1 (CH3, 2 × CH₂CH₃), [22.7, 26.0, 26.9, 29.4, 29.7, 29.9, 30.3, 31.9 (CH₂, alkyl chain methylenes, resonance overlap)], 40.3 (CH₂, CH₂NH), 51.7 (CH, C-2), 69.9 (CH₂, C-6’), 70.1 (CH, C-5’), 71.0 (CH₂, C-1), 71.9 (CH₂, C(4)OCH₂Ph), 73.1 (CH₂, C(3’)OCH₂Ph), 73.4 (CH₂, C(2’)OCH₂Ph), 73.6 (CH₂, C(6’)OCH₂Ph), 73.8 (CH₂, C(3)OCH₂Ph), 74.6 (CH₂, C(4’)OCH₂Ph), 75.0 (CH, C-3), 76.8 (CH, C-2’), 78.8 (CH, C-3’), 79.9 (CH, C-4’), 80.3 (CH, C-4), 99.8 (CH, C-1’), [127.5, 127.6, 127.67, 127.70, 127.84, 127.88, 127.9, 128.25, 128.28, 128.34, 128.37, 128.5 (CH, Ph, resonance overlap)], 137.39 (C, ipso Ph), 138.42 (C, ipso Ph), 138.48 (C, ipso Ph), 138.67 (C, ipso Ph), 138.72 (C, ipso Ph), 138.8 (C, ipso Ph), 158.8 (C, C=O); MS (TOF ES+) m/z 1408.0 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₉₀H₁₃₂N₂O₉Na [M + Na]⁺ 1407.9831, found 1407.9844. The unreacted amine was also recovered (40 mg, 83%).

**General procedure for catalytic hydrogenolysis:** Pd(OH)₂/C or Pd/C (0.05 eq per benzyl group) was added to a solution of the benzylated compound in THF (0.01 M). H₂ gas was bubbled through the stirred suspension. The progress of the reaction was monitored by TLC. On completion, the reaction mixture was filtered through a plug of Celite, washed with THF and then CHCl₃/MeOH (90/10, v/v), and concentrated under reduced pressure to provide the crude product, which was purified by flash column chromatography.
(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(tricosylaminocarbonylamino)octadecane-1,3,4-triol (9)

Urea 9 was prepared from perbenzylated urea 19 (115 mg, 0.083 mmol) and Pd/C (26.5 mg, 10% wet) in THF (10 mL) according to the general procedure for catalytic hydrogenolysis. After 22 h, work-up and purification by flash column chromatography (6% MeOH in CHCl₃) afforded urea 9 as an amorphous, white solid (51 mg, 73%): \( R_f = 0.23 \) (6% MeOH in CHCl₃); [\( \alpha \)]\(_D\) = +28.0 (c 0.5, CHCl₃); mp 154 – 155 °C; \( \nu_{\text{max}} \) (film) / cm\(^{-1}\) 3363m (N–H), 1670m (C=O); \( \delta_H \) (500 MHz, CDCl₃:CD₃OD, 3:1) 0.68 (t, \( J = 6.5 \), 6H, \( 2 \times \text{CH}_2\text{C}_3\)), 1.06–1.10 (stack, 62H, alkyl chain methylenes), 1.22–1.27 (stack, 2H), 1.33–1.36 (m, 1H), 1.48–1.52 (m, 1H), 1.67–1.69 (stack, 2H), 2.83–2.96 (stack, 2H, \( \text{CH}_2\text{NH} \)), 3.28–3.31 (m, 1H, H-3), 3.33–3.36 (m, 1H, H-4), 3.49–3.63 (stack, 6H, H-3’, 2 \( \times \) H-6’, H-2’, H-5’, \( \text{C}(1)\text{H}_3\text{H}_b \)), 3.66 (dd, \( J = 10.5, 4.5 \), 1H, \( \text{C}(1)\text{H}_2\text{H}_b \)), 3.72 (br d, \( J = 2.5, 1 \)H, H-4’), 3.97 (dd, \( J = 9.0, 4.5 \), 1H, H-2), 4.67 (d, \( J = 3.5 \), 1H, H-1’); \( \delta_C \) (125 MHz, CDCl₃:CD₃OD, 3:1) 13.5 (\( \text{CH}_3 \), 2 \( \times \) \( \text{CH}_2\text{C}_3 \)), [22.3, 25.1, 25.5, 26.6, 29.0, 29.1, 29.3, 29.4, 29.8, 31.5, 32.7 (\( \text{CH}_2 \), alkyl chain methylenes, resonance overlap)], 39.8 (\( \text{CH}_2 \), \( \text{CH}_2\text{NH} \)), 50.6 (CH, C-2), 61.5 (\( \text{CH}_2 \), C-6’), 67.6 (\( \text{CH}_2 \), C-1), 68.6 (CH, C-2’), 69.4 (CH, C-4’), 70.0 (CH, C-3’), 70.5 (CH, C-5’), 72.1 (CH, C-4), 75.2 (CH, C-3), 99.4 (CH, C-1’), 158.9 (C, C=O); MS (TOF ES+) \( m/z \) 867.9 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for \( \text{C}_{48}\text{H}_{96}\text{N}_2\text{O}_9\text{Na} \) [M + Na]⁺ 867.7014, found 867.7026.
(2S,3S,4R)-2-Amino-3,4-O-isopropylidene-1-O-(2',3',4',6'-tetra-O-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (21)

PMe₃ (930 µL of a 1.0 M soln in THF, 0.93 mmol) was added dropwise over 5 min to a solution of azide 20 (700 mg, 0.77 mmol) in THF (7 mL). The reaction mixture was stirred at r.t. for 3 h, after which time, H₂O (0.5 mL) was added. The reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The residual H₂O was removed by co-evaporation with toluene (3 × 3 mL) to provide the crude product. Purification by flash column chromatography (25% EtOAc in hexane) afforded amine 21 as a colorless oil (632 mg, 93%): Rₕ = 0.2 (25% EtOAc in hexane); [α]²⁰ = +35.6 (c 1, CHCl₃); νₓₓₓₓₓxx̂xx (film) / cm⁻¹ 3372 br (N-H); δₓₓ (300 MHz, CDCl₃) 0.87 (t, J 7.0, 3H), 1.20–1.43 (stack, 24H), 1.37 (s, 3H), 1.42–1.57 (stack, 5H), 2.99–3.08 (m, 1H), 3.37 (dd, J 10.1, 7.6, 1H), 3.48–3.57 (stack, 2H), 3.81–3.98 (stack, 5H), 4.02–4.12 (stack, 2H), 4.38 (A of AB, Jₐ–ₐ 11.8, 1H), 4.46 (B of AB, Jₐ–ₐ 11.8, 1H), 4.57 (d, J 11.5, 1H), 4.68 (d, J 11.8, 1H), 4.71–4.83 (stack, 3H), 4.91–4.93 (stack, 2H), 7.22–7.39 (stack, 20H), NH₂ not observed; δₓ (100 MHz, CDCl₃) 14.1 (CH₃), 22.7 (CH₂), 25.9 (CH₃), 26.2 (CH₂), 28.3 (CH₃), [29.3, 29.7, 29.8 (CH₂, resonance overlap)], 31.9 (CH₂), 50.7 (CH), 69.0 (CH₂), 69.5 (CH), 72.3 (CH₃), 73.0 (CH₂), 73.3 (CH₂), 73.5 (CH₂), 74.8 (CH₂), 75.0 (CH), 76.8 (CH), 77.9 (CH), 79.0 (CH), 79.1 (CH), 99.0 (CH), 107.8 (C), [127.4, 127.5, 127.6, 127.7, 127.8 128.2, 128.3 (CH, resonance overlap)], 138.0 (C), [138.7, 138.8 (C, resonance overlap)]; MS (TOF ES⁺) m/z 880.8 ([M + H]⁺, 100%); HRMS (TOF ES⁺) calcd for C₅₅H₇₈NO₈ [M + H]⁺ 880.5727, found 880.5721.
Carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester

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\(N,N'-\text{Disuccinimidyl carbonate (190 mg, 0.75 mmol) was added to a solution of tetracosan-1-ol (177 mg, 0.50 mmol) and NEt}_3 (210 \mu\text{L, 1.5 mmol) in dry CH}_3\text{CN (2.5 mL) at r.t. The resulting mixture was stirred at r.t. for 4 h and then concentrated under reduced pressure. The residue was diluted with NaHCO}_3 \text{ solution (10 mL) and extracted with EtOAc (2 \times 10 mL). The combined extracts were washed with brine (5 mL) and dried over Na}_2\text{SO}_4. \text{Evaporation of the solvent under reduced pressure provided the corresponding mixed carbonate as a white solid, which was used directly in the next step (248 mg, quant.).}} \quad R_f = 0.3 \text{ (25\% EtOAc in hexane); } \nu_{\max}(\text{film}) / \text{cm}^{-1} 1711\text{m (C=O), } 1693\text{m (C=O); } \delta_\text{H}(300 \text{ MHz, CDCl}_3) 0.88 \text{ (t, } J 7.0, 3\text{H, CH}_2\text{CH}_3), 1.16-1.46 \text{ (stack, 42H, alkyl chain methylenes, 1.51-1.80 (stack, 2H), 2.84 (s, 4H), 4.31 (t, } J 6.6, 2\text{H); } \delta_\text{C}(100 \text{ MHz, CDCl}_3) 14.1 \text{ (CH}_3, \text{ CH}_2\text{CH}_3), 22.7 \text{ (CH}_2), 28.4 \text{ (CH}_2), [29.1, 29.36, 29.42, 29.5, 29.7 \text{ (CH}_2, \text{ alkyl chain, resonance overlap)}], 31.9 \text{ (CH}_2), 71.7 \text{ (CH}_2), 151.6 \text{ (C, OC=O), 168.7 \text{ (C, NC=O); MS (TOF ES+) } m/z 518.5 \text{ ([M + Na]}^+, 100\%); HRMS (TOF ES+) calcd for C}_{29}\text{H}_{53}\text{NO}_5\text{Na } [M + Na]^+ 518.3821, \text{ found 518.3817.}
A solution of carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester (50 mg, 0.10 mmol) in CH$_2$Cl$_2$ (0.5 mL) was added to a stirred solution of amine 21 (60 mg, 0.068 mmol) and NEt$_3$ (24 µL, 0.17 mmol) in CH$_2$Cl$_2$ (1 mL). The resulting mixture was stirred at r.t. until no mixed carbonate remained as determined by TLC (4 h). The mixture was then diluted with CH$_2$Cl$_2$ (8 mL) and washed sequentially with NaHCO$_3$ solution (10 mL) and brine (10 mL). The organic phase was dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure. Purification of the residue by flash column chromatography (5% EtOAc in toluene) provided carbamate 23 as a colorless oil (70 mg, 82%): $R_f = 0.3$ (10% EtOAc in hexane); $[\alpha]_{D}^{20} = +36.8$ (c 1, CHCl$_3$); $\nu_{\text{max}}$(film) / cm$^{-1}$ 1689m (C=O); $\delta_H$(500 MHz, CDCl$_3$) 0.88 (t, $J_6.8$, 6H, 2 × CH$_2$C$_6$H$_5$), 1.24−1.34 (stack, 64H, alkyl chain methylenes, C(C$_6$H$_5$)$_2$), 1.39−1.51 (stack, 8H), 1.57−1.59 (stack, 3H), 1.63−1.67 (m, 1H), 3.45 (dd, $J_9.3$, 6.3, 1H, C(6'H)$_a$H$_b$), 3.52 (dd, $J_9.3$, 6.5, 1H, C(6'H)$_a$H$_b$), 3.66−3.70 (m, 1H, H-3), 3.77−3.84 (m, 1H, H-2), 3.91−3.98 (stack, 6H, H-3', H-4', H-5', C(1)H$_a$aH$_b$, H-4', C(1'')H$_a$aH$_b$), 4.03−4.11 (stack, 3H, H-2', C(1)H$_a$aH$_b$, C(1'')H$_a$aH$_b$), 4.38 (A of AB, $J_{A,B}$ 11.8, 1H, C(6'O)OCH$_{a,H_b}$Ph), 4.49 (B of AB, $J_{B,A}$ 11.8, 1H, C(6'O)OCH$_{a,H_b}$Ph), 4.56 (A of AB, $J_{A,B}$ 11.5, 1H, OCH$_{a,H_b}$Ph), 4.67 (A of AB, $J_{A,B}$ 11.7, 1H, OCH$_{a,H_b}$Ph), 4.74 (A of AB, $J_{A,B}$ 11.7, 1H, C(2'O)OCH$_{a,H_b}$Ph), 4.78 (B of AB, $J_{B,A}$ 11.7, 1H, OCH$_{a,H_b}$Ph), 4.83 (B of AB, $J_{B,A}$ 11.7, 1H, C(2'O)OCH$_{a,H_b}$Ph), 4.92 (B of AB, $J_{B,A}$ 11.5, 1H, OCH$_{a,H_b}$Ph), 4.95 (d, $J_3.6$, 1H, H-1'), 5.32 (br d, $J_9.5$, 1H, NH), 7.23−7.35 (stack, 18H, Ph),
7.38–7.39 (stack, 2H, Ph); δ_C (125 MHz, CDCl_3) 14.1 (CH_3, 2 × CH_2CH_3), 22.7 (CH_2), 25.9 (CH_2, CH_3, resonance overlap), 26.5 (CH_2), 28.2 (CH_2), 28.8 (CH_2), 29.1 (CH_2), [29.3, 29.4, 29.7, 29.8 (CH_2, alkyl chain, resonance overlap)], 32.0 (CH_2), 50.7 (CH), 65.1 (CH_2), 69.3 (CH_2), 69.7 (CH), 69.9 (CH_2), 73.1 (CH_2), 73.2 (CH_2), 73.6 (CH_2), 74.7 (CH_2), 74.9 (CH), 75.5 (CH), 76.8 (CH), 77.8 (CH), 79.0 (CH), 99.2 (CH, C-1'), 107.7 (C, C(CH_3)_2), [127.5, 127.6, 127.8, 127.9 (CH, Ph, resonance overlap)], [128.23, 128.26, 128.34, 128.36, 128.4 (CH, Ph, resonance overlap)], 137.8 (C, ipso Ph), 138.6 (C, 2 × ipso Ph), 138.8 (C, ipso Ph), 155.9 (C, C=O); MS (TOF ES+) m/z 1283.0 ([M + Na]^+, 100%); HRMS (TOF ES+) calcd for C_{80}H_{125}NO_{10}Na [M + Na]^+ 1282.9201, found 1282.9244.

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(tetracosanyloxycarbonylamino)octadecane-1,3,4-triol (10)

TFA (120 µL) was added dropwise over 1 min to a solution of acetal 23 (60 mg, 0.048 mmol) in CH_2Cl_2 / CH_3OH (2:1, 0.6 mL). After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et_2O (3 × 3 mL) to provide the acetal hydrolysis product as a white solid (58 mg, quant.), which was treated with Pd(OH)_2/C (15 mg, 10% wet) and H_2 in THF (6 mL) according to the general hydrogenolysis procedure. After 6 h, work-up and purification by flash column chromatography (8% MeOH in CHCl_3) afforded carbamate 10 as an amorphous white solid (31 mg, 75%): R_f = 0.3 (8% MeOH in CHCl_3); [α]_D^{18} = +46.0 (c 1, CHCl_3); mp 166 – 167 °C; ν_max(neat) / cm⁻¹ 3388s br (OH), 1683m (C=O); δ_H(500 MHz, CDCl_3:CD_3OD, 2:1) 0.63 (t, J 6.8, 6H), 0.91–1.12 (stack, 66H), 1.22–1.48 (stack, 4H), 3.27–3.38 (stack, 2H), 3.39–3.58 (stack, 6H), 3.64–3.75 (stack, 4H), 3.77–3.84 (m, 1H), 7.38–7.39 (stack, 2H, Ph); δ_C (125 MHz, CDCl_3) 14.1 (CH_3, 2 × CH_2CH_3), 22.7 (CH_2), 25.9 (CH_2, CH_3, resonance overlap), 26.5 (CH_2), 28.2 (CH_2), 28.8 (CH_2), 29.1 (CH_2), [29.3, 29.4, 29.7, 29.8 (CH_2, alkyl chain, resonance overlap)], 32.0 (CH_2), 50.7 (CH), 65.1 (CH_2), 69.3 (CH_2), 69.7 (CH), 69.9 (CH_2), 73.1 (CH_2), 73.2 (CH_2), 73.6 (CH_2), 74.7 (CH_2), 74.9 (CH), 75.5 (CH), 76.8 (CH), 77.8 (CH), 79.0 (CH), 99.2 (CH, C-1'), 107.7 (C, C(CH_3)_2), [127.5, 127.6, 127.8, 127.9 (CH, Ph, resonance overlap)], [128.23, 128.26, 128.34, 128.36, 128.4 (CH, Ph, resonance overlap)], 137.8 (C, ipso Ph), 138.6 (C, 2 × ipso Ph), 138.8 (C, ipso Ph), 155.9 (C, C=O); MS (TOF ES+) m/z 1283.0 ([M + Na]^+, 100%); HRMS (TOF ES+) calcd for C_{80}H_{125}NO_{10}Na [M + Na]^+ 1282.9201, found 1282.9244.

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(tetracosanyloxycarbonylamino)octadecane-1,3,4-triol (10)

TFA (120 µL) was added dropwise over 1 min to a solution of acetal 23 (60 mg, 0.048 mmol) in CH_2Cl_2 / CH_3OH (2:1, 0.6 mL). After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et_2O (3 × 3 mL) to provide the acetal hydrolysis product as a white solid (58 mg, quant.), which was treated with Pd(OH)_2/C (15 mg, 10% wet) and H_2 in THF (6 mL) according to the general hydrogenolysis procedure. After 6 h, work-up and purification by flash column chromatography (8% MeOH in CHCl_3) afforded carbamate 10 as an amorphous white solid (31 mg, 75%): R_f = 0.3 (8% MeOH in CHCl_3); [α]_D^{18} = +46.0 (c 1, CHCl_3); mp 166 – 167 °C; ν_max(neat) / cm⁻¹ 3388s br (OH), 1683m (C=O); δ_H(500 MHz, CDCl_3:CD_3OD, 2:1) 0.63 (t, J 6.8, 6H), 0.91–1.12 (stack, 66H), 1.22–1.48 (stack, 4H), 3.27–3.38 (stack, 2H), 3.39–3.58 (stack, 6H), 3.64–3.75 (stack, 4H), 3.77–3.84 (m, 1H), 7.38–7.39 (stack, 2H, Ph); δ_C (125 MHz, CDCl_3) 14.1 (CH_3, 2 × CH_2CH_3), 22.7 (CH_2), 25.9 (CH_2, CH_3, resonance overlap), 26.5 (CH_2), 28.2 (CH_2), 28.8 (CH_2), 29.1 (CH_2), [29.3, 29.4, 29.7, 29.8 (CH_2, alkyl chain, resonance overlap)], 32.0 (CH_2), 50.7 (CH), 65.1 (CH_2), 69.3 (CH_2), 69.7 (CH), 69.9 (CH_2), 73.1 (CH_2), 73.2 (CH_2), 73.6 (CH_2), 74.7 (CH_2), 74.9 (CH), 75.5 (CH), 76.8 (CH), 77.8 (CH), 79.0 (CH), 99.2 (CH, C-1’), 107.7 (C, C(CH_3)_2), [127.5, 127.6, 127.8, 127.9 (CH, Ph, resonance overlap)], [128.23, 128.26, 128.34, 128.36, 128.4 (CH, Ph, resonance overlap)], 137.8 (C, ipso Ph), 138.6 (C, 2 × ipso Ph), 138.8 (C, ipso Ph), 155.9 (C, C=O); MS (TOF ES+) m/z 1283.0 ([M + Na]^+, 100%); HRMS (TOF ES+) calcd for C_{80}H_{125}NO_{10}Na [M + Na]^+ 1282.9201, found 1282.9244.

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(tetracosanyloxycarbonylamino)octadecane-1,3,4-triol (10)
4.65 (d, J 3.4, 1H), OH and NH resonances not observed; \(\delta_c\) (125 MHz, CDCl\(_3\); CD\(_3\)OD, 2:1) 13.4, 22.2, 25.3, 25.4, 28.6, 28.7, 28.9, 29.2, 31.4, 32.1, 51.3, 61.4, 64.8, 67.1, 68.5, 69.4, 69.9, 70.4, 71.5, 74.4, 99.3, 156.9 (significant resonance overlap in the alkyl chain methylene resonances); MS (TOF ES+) m/z 882.4 ([M + Na]\(^+\), 100%); HRMS (TOF ES+) calcd for C\(_{49}\)H\(_{97}\)NO\(_{10}\)Na [M + Na]\(^+\) 882.7010, found 882.7000.

(2S,3S,4R)-3,4-Di-O-acetyl-1-O-(2',3',4',6'-tetra-O-acetyl-\(\alpha\)-D-galactopyranosyl)-2-(hexacosanoylamino)octadecane-1,3,4-triol (24)

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\begin{align*}
\text{Ac}_2\text{O} & \text{ (300 \(\mu\)L, 3.2 mmol) was added dropwise over 1 min to a solution of } \alpha-\text{GalCer 1 (90 mg, 0.11 mmol) in pyridine (2 mL) and the reaction mixture was stirred at r.t. for 10 h, after which time, the volatiles were removed under reduced pressure. The residue was diluted with CH\(_2\)Cl\(_2\) (10 mL), washed sequentially with H\(_2\)O (5 mL), NaHCO\(_3\) solution (10 mL), brine (3 mL) and then dried over Na\(_2\)SO\(_4\). The drying agent was removed by filtration and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (25% EtOAc in hexane) to afford hexa-acetate 24 as a white solid (110 mg, 94%): } R_f = 0.3 (20% EtOAc in hexane); [\(\alpha\)]\(^{20}\)_D = +8.4 (c 0.5, CHCl\(_3\)); mp 43 – 44 °C; \(\nu_{\text{max}}\) (film) / cm\(^{-1}\) 1745s (C=O), 1683w (C=O); \(\delta_h\) (300 MHz, CDCl\(_3\))
\end{align*}
\]

0.87 (t, J 6.7, 6H), 1.12–1.40 (stack, 68H), 1.56–1.72 (stack, 4H), 1.98 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 2.27 (t, J 7.4, 2H), 3.39 (dd, J 10.6, 2.2, 1H), 3.64 (dd, J 10.7, 2.6, 1H), 3.97–4.14 (stack, 4H), 4.31–4.41 (m, 1H), 4.90 (d, J 3.7, 1H), 5.13 (dd, J 10.8, 3.7, 1H), 5.25–5.36 (stack, 2H), 5.44 (d, J 3.1, 1H), 6.39 (d, J 9.7, 1H); \(\delta_c\) (100 MHz, CDCl\(_3\)) 14.1 (CH\(_3\)),
[20.60, 20.66, 20.72, (CH₃), resonance overlap], 20.1 (CH₃), 22.7 (CH₂), 25.6 (CH₂), 25.7 (CH₂), [29.29, 29.35, 29.40, 29.7 (CH₂, resonance overlap)], 31.9 (CH₂), 36.7 (CH₂), 47.8 (CH), 61.8 (CH₂), 66.7 (CH), 67.2 (CH₂), 67.5 (CH), 67.9 (CH), 70.5 (CH), 73.4 (CH), 97.1 (CH), 169.7 (C), 170.1 (C), 170.4 (C), 170.7 (C), 171.1 (C), 172.9 (C), some resonance overlap in C=O region; MS (TOF ES+) m/z 1132.8 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₆₂H₁₁₁NO₁₅Na [M + Na]⁺ 1132.7851, found 1132.7860.

(2S,3S,4R)-3,4-Di-O-acetyl-1-O-(2',3',4',6'-tetra-O-acetyl-α-D-galactopyranosyl)-2-(hexacosanethioylamino)octadecane-1,3,4-triol (25)

Lawesson’s reagent (60 mg, 0.15 mmol) was added to a solution of amide 24 (110 mg, 0.10 mmol) in toluene (2 mL) at r.t. The reaction mixture was stirred at 80 °C for 4 h and then the solvent was removed under reduced pressure. The residue was diluted with CH₂Cl₂ (10 mL), washed sequentially with H₂O (5 mL), NaHCO₃ solution (10 mL), brine (2 mL) and then dried over Na₂SO₄. The drying agent was removed by filtration and the filtrate was concentrated under reduced pressure. Purification of the residue by flash column chromatography (20% EtOAc in hexane) provided thioamide 25 as a pale yellow solid (96 mg, 85%): Rₛ = 0.3 (15% EtOAc in hexane); [α]²₁₀ = +46.0 (c 1, CHCl₃); mp 47 – 48 °C; νₛₒₜₐₜ (film) / cm⁻¹ 1747s (C=O); δᵢₜ (500 MHz, CDCl₃) 0.87 (t, J 6.8, 6H, 2 × CH₂CH₃), 1.21–1.37 (stack, 68H, alkyl chain methylenes), 1.58–1.69 (stack, 2H, H-5), 1.71–1.82 (stack, 2H, H-3”), 1.99 (s, 3H, C(O)CH₃), 2.00 (s, 3H, C(O)CH₃), 2.03 (s, 3H, C(O)CH₃), 2.07 (s, 3H, C(O)CH₃), 2.08 (s, 3H, C(O)CH₃), 2.12 (s, 3H, C(O)CH₃), 2.66–2.78 (stack, 2H, H-2”), 3.40 (dd, J 10.7, 1.9, 1H,
C(1)H₆H₆, 3.65 (dd, J 10.7, 2.7, 1H, C(1)H₆H₆), 3.97–4.07 (stack, 2H, C(6’)H₆H₆, H-5’), 4.08–4.14 (m, 1H, C(6’)H₆H₆), 4.75–4.81 (m, 1H, H-4), 4.93 (d, J 3.6, 1H, H-1’), 5.08–5.16 (stack, 2H, (including 5.10 (dd, J 10.8, 3.7, 1H, H-2’)), H-2’, H-2), 5.37 (dd, J 10.8, 3.4, 1H, H-3’), 5.40–5.42 (m, 1H, H-4’), 5.49 (dd, J 10.0, 2.4, 1H, H-3), 8.66 (d, J 9.2, 1H, N-H); δc(125 MHz, CDCl₃) 14.11 (CH₃, 2 × CH₂CH₃), [20.50, 20.54, 20.62, 20.66, 20.95 (CH₃, C(O)CH₃, resonance overlap), [22.6, 25.5, 27.5, 28.9, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9 (CH₂, alkyl chain methylenes, resonance overlap)], 47.0 (CH₂, C-2”), 53.7 (CH, C-2), 61.7 (CH₂, C-6’), 65.3 (CH₂, C-1), 67.0 (CH, C-5’), 67.3 (CH, C-3’), 67.8 (CH, C-2’), 68.0 (CH, C-4’), 69.8 (CH, C-3), 73.6 (CH, C-4), 96.8 (CH, C-1’), 169.7 (C, C(3)C=O), 170.0 (C, C(4’)C=O), 170.3 (C, C(3’)C=O), 170.4 (C, C(6’)C=O), 170.5 (C, C(2’)C=O), 171.5 (C, C(4)C=O), 207.0 (C, C=S); MS (TOF ES+) m/z 1148.9 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₆₂H₁₁₁NO₁₄SNa [M + Na]⁺ 1148.7623, found 1148.7631.

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(hexacosanethiolamino)octadecane-1,3,4-triol (8)

NaOMe (10 µL of a 0.5 M soln in MeOH, 0.005 mmol) was added to a solution of hexa-acetate 25 (25 mg, 0.022 mmol) in MeOH (2.5 mL). After stirring at r.t. for 2 h, the reaction mixture was neutralized by the addition of acidic ion-exchange resin (Dowex H CR-S, pre-washed with MeOH (100 mL) and CHCl₃ (50 mL)). The solution was filtered and the resin washed with MeOH (25 mL) and CHCl₃/MeOH (25 mL, 9:1). The filtrate was concentrated under reduced pressure. Purification of the residue by flash column chromatography (8% MeOH in CHCl₃) provided thioamide 8 as a pale
yellow solid (17 mg, 90%): $R_f = 0.2$ (8% MeOH in CHCl$_3$); $[\alpha]^{20}_D = +43.2$ (c 1, CHCl$_3$:CH$_3$OH, 2:1); mp 136 – 137 °C; $\nu_{\text{max}}$(film) / cm$^{-1}$ 3368m br (O–H); $\delta_t$(400 MHz, CDCl$_3$:CD$_3$OD, 2:1) 0.84 (t, $J$ 6.9, 6H), 1.14–1.44 (stack, 69H), 1.45–1.78 (stack, 3H), 2.56-2.66 (stack, 2H), 3.50–3.58 (m, 1H), 3.65–3.83 (stack, 8H), 3.90 (d, $J$ 2.9, 1H), 3.96 (dd, $J$ 10.9, 4.3, 1H), 4.85 (app. q, $J$ 4.3, 1H) 4.94 (d, $J$ 3.7, 1H), OH resonances not observed; $\delta_c$(100 MHz, CDCl$_3$:CD$_3$OD, 2:1) 14.3 (CH$_3$), 23.1 (CH$_2$), 26.4 (CH$_2$), 29.6 (CH$_2$), [29.9, 30.0, 30.1, 30.21 (CH$_2$, resonance overlap)], 32.4 (CH$_2$), 32.7 (CH$_2$), 47.1 (CH$_3$), 56.8 (CH), 62.3 (CH$_2$), 66.8 (CH$_2$), 69.5 (CH), 70.4 (CH), 70.8 (CH), 71.4 (CH), 72.6 (CH), 73.9 (CH), 100.2 (CH), 206.1 (C); MS (TOF ES+) $m/z$ 896.8 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{50}$H$_{99}$NO$_8$SNa [M + Na]$^+$ 896.6989, found 896.6998.

First-Generation Approach to $\alpha$-GalCer 1

(a) (i) 14, PPh$_3$, CBr$_4$, CH$_2$Cl$_2$, r.t., 3 h; (ii) Me$_2$NC(O)NMe$_2$, Bu$_4$NBr, CH$_2$Cl$_2$, then 15, CH$_2$Cl$_2$, 3 Å MS, r.t., 3 d, 62%. (b) PMe$_3$, THF, r.t., 4 h, then H$_2$O, 1 h, 72%. (c) CH$_3$(CH$_2$)$_{24}$C(O)Cl, Et$_3$N, CH$_2$Cl$_2$, 0 °C to r.t., 8 h, 18 (54%). (d) Pd / C, H$_2$, THF, r.t., 22 h, 1 (68% from 17).
(2S,3R,4R)-2-Azido-3,4-di-O-benzyl-1-O-(2’,3’,4’,6’-tetra-O-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (16)\textsuperscript{17}

Galactoside 16 was prepared according to a slightly modified procedure to that reported in the literature:\textsuperscript{18} PPh\textsubscript{3} (1.46 g, 5.55 mmol) and CBr\textsubscript{4} (1.84 g, 5.55 mmol) were added sequentially to a solution of 2,3,4,6-tetra-O-benzyl-α-D-galactose 14\textsuperscript{4,19} (1.00 g, 1.85 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) at r.t. The reaction mixture was stirred for 3 h. In separate flasks, a solution of tetramethyl urea (TMU) (1.2 mL) and Bu\textsubscript{4}NBr (1.79 g, 5.55 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL), and a solution of azide 15\textsuperscript{5} (1.46 g, 2.78 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL), were stirred over activated 3 Å MS for 30 min, after which time, these solutions were added dropwise (15 min) via syringe sequentially (TMU/Bu\textsubscript{4}NBr solution first) to the solution containing the glycosyl donor. The reaction mixture was stirred at r.t. for 3 d until the donor was no longer being consumed (as judged by TLC). The reaction mixture was then filtered through a silica plug, washed with CH\textsubscript{2}Cl\textsubscript{2} (1.2 L) and concentrated under reduced pressure to provide the crude product, which was purified by flash column chromatography (8% EtOAc in hexane) to provide glycoside 16 as a colorless oil (1.21 g, 62%, α-anomer only): $R_f = 0.3$ (8% EtOAc in hexane); $[\alpha]_D^{18} = +22$ (c 1.4, CH\textsubscript{2}Cl\textsubscript{2}) (lit.\textsuperscript{17} $[\alpha]_D^{20} = +26$ (c 1.4, CH\textsubscript{2}Cl\textsubscript{2})); $\nu_{\text{max}}$(film)/cm\textsuperscript{-1} 2097\text-superscript{m} (N\textsubscript{3}); $\delta$H (500 MHz, CDCl\textsubscript{3}) 0.90 (t, J\textsubscript{6.9} 6.9, 3H, CH\textsubscript{2}CH\textsubscript{3}), 1.20-1.35 (stack, 23H, alkyl chain methylenes), 1.35-1.45 (m, 1H, alkyl chain CH\textsubscript{a}H\textsubscript{b}), 1.50-1.58 (m, 1H, C(5)H\textsubscript{a}H\textsubscript{b}), 1.63-1.71 (m, 1H, C(5)H\textsubscript{a}H\textsubscript{b}), 3.47-3.54 (stack, 2H, C(6’)H\textsubscript{a}H\textsubscript{b}), 3.60-3.63 (m, 1H, H-4), 3.71-3.76 (stack, 3H, C(1)H\textsubscript{a}H\textsubscript{b}, H-2, H-3), 3.94-3.98 (stack, 2H, H-4’, H-5’), 3.98-4.04 (stack, 2H, H-3’, C(1)H\textsubscript{a}H\textsubscript{b}), 4.08 (dd, J\textsubscript{10.0} 10.0, 3.5, 1H, H-2’), 4.37 (A of AB, $J_{A-B}$ 11.8, 1H, C(6’)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.45 (B of AB, $J_{B-A}$ 11.8, 1H, C(6’)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.48 (A of AB, $J_{A-B}$ 11.6, 1H, C(4)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.57-4.59 (stack, 2H, C(4)OCH\textsubscript{b}H\textsubscript{b}Ph, C(4’)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.63 (A of AB, $J_{A-B}$ 11.3, 1H, C(3)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.67 (B of AB, $J_{B-A}$ 11.3, 1H, C(3)OCH\textsubscript{b}H\textsubscript{b}Ph), 4.69 (A of AB, $J_{A-B}$...
12.0, 1H, C(2')OCH$_2$H$_n$Ph), 4.74 (A of AB, $J_{A-B}$ 11.5, 1H, C(3')OCH$_2$H$_n$Ph), 4.81 (B of AB, $J_{B-A}$ 12.0, 1H, C(2')OCH$_2$H$_n$Ph), 4.84 (B of AB, $J_{B-A}$ 11.5, 1H, C(3')OCH$_2$H$_n$Ph), 4.91 (d, $J$ 3.5, 1H, H-1'), 4.95 (d, $J$ 11.5, 1H, C(3')OCH$_2$H$_n$Ph), 7.22-7.40 (stack, 30H, 6×Ph); $\delta$ (125 MHz, CDCl$_3$) 14.1 (CH$_3$, CH$_2$CH$_3$), [22.6, 25.3, 29.3, 29.6, 29.9, 31.9 (CH$_2$, alkyl chain, resonance overlap]), 61.9 (CH, C-2), 68.4 (CH$_2$, C-1), 68.9 (CH$_2$, C-6'), 69.6 (CH, C-5'), 71.9 (CH$_2$, C(4')OCH$_2$Ph), 73.0 (CH$_2$, C(3')OCH$_2$Ph), 73.1 (CH$_2$, C(2')OCH$_2$Ph), 73.3 (CH$_2$, C(6')OCH$_2$Ph), 73.6 (CH$_2$, C(3)OCH$_2$Ph), 74.7 (CH$_2$, C(4')OCH$_2$Ph), 75.0 (CH, C(4')), 76.3 (CH, C(2')), 78.7 (CH, C(3')), 78.8 (CH, C(3)), 79.2 (CH, C(4)), 98.6 (CH, C(1')), [127.3, 127.4, 127.56, 127.60, 127.7, 127.8, 128.1, 128.2 (CH, Ph, resonance overlap)], 137.9 (C, ipso Ph), 138.0 (C, ipso Ph), 138.3 (C, ipso Ph), 138.58 (C, ipso Ph), 138.63 (C, ipso Ph), 138.7 (C, ipso Ph); MS (TOF ES+) m/z 1068.8 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{66}$H$_{83}$N$_3$O$_8$Na [M + Na]$^+$ 1068.6078, found 1068.6063; and then unreacted azide 15 (445 mg, 45%). Data for 16 were in agreement with those reported in the literature for 16 prepared by a different route.$^{17}$

(2S,3S,4R)-2-Amino-3,4-di-O-benzyl-1-O-(2',3',4',6'-tetra-O-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (17)$^{17}$

Amine 17 was prepared according a different procedure to that reported in the literature:$^{17}$ PMe$_3$ (455 µL of a 1.0 M soln in THF, 0.46 mmol) was added dropwise over 5 min to a solution of azide 16 (433 mg, 0.414 mmol) in THF (3.5 mL). The reaction mixture was stirred at r.t. for 4 h, after which time, H$_2$O (3 mL) was added. The reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The residual H$_2$O was removed by co-evaporation with toluene (3 × 3 mL) to provide the
crude product, which was purified by flash column chromatography (35% EtOAc in hexane) to afford amine 17 as a white solid (300 mg, 72%), which was used directly without further purification. Selected data: $R_f = 0.3$ (35% EtOAc in hexane); MS (TOF ES+) $m/z$ 1020.5 ([M + H]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{66}$H$_{86}$NO$_8$ [M + H]$^+$ 1020.6353, found 1020.6357. Data for 17 were in agreement with those reported in the literature for 17 prepared by a different route.$^{17}$

(2S,3S,4R)-3,4-Di-O-benzyl-1-O-(2′,3′,4′,6′-tetra-O-benzyl-α-D-galactopyranosyl)-2-(hexacosanoylamino)octadecane-1,3,4-triol (18)$^{20}$

A screw-capped glass tube containing a solution of hexacosanoic acid (240 mg, 0.580 mmol) in (COCl)$_2$ (2.0 mL, 23 mmol) was closed tightly and heated at 70 °C. After 2 h, the volatiles were removed under a stream of N$_2$ and the residual solvent removed on the vacuum line (1 h) to provide hexacosanoyl chloride as a pale yellow oil, which was used directly in the next step without further purification (265 mg, quant.): a solution of freshly prepared hexacosanoyl chloride (265 mg, 0.58 mmol) in CH$_2$Cl$_2$ (1.5 mL) was added dropwise over 2 min to an ice-cooled solution of amine 17 (500 mg, 0.49 mmol) and NEt$_3$ (136 µL, 0.98 mmol) in CH$_2$Cl$_2$ (3.5 mL) at 0 °C. The reaction mixture was stirred at r.t. for 8 h and then diluted with CH$_2$Cl$_2$ (20 mL), washed sequentially with NaHCO$_3$ solution (20 mL), brine (4 mL) and then dried over Na$_2$SO$_4$. The drying agent was removed by filtration and the filtrate concentrated under reduced pressure. Purification of the residue by flash column chromatography (12% EtOAc in hexane) afforded amide 18 as a white solid (370 mg, 54%): $R_f = 0.3$ (15% EtOAc in hexane); $[\alpha]^{20}_D = +31.2$ (c 1, CHCl$_3$) (lit.$^{20}$ $[\alpha]^{24}_D = +18.8$ (c 0.9, CHCl$_3$); mp 75 – 76 °C (lit.$^{20}$ mp 74 – 75 °C); $\nu_{\text{max}}$(film)/cm$^{-1}$ 1647m (C=O); $\delta_{\text{Ht}}$(300 MHz, CDCl$_3$) 0.88 (t, $J$ 6.9, 6H), 1.16-
1.34 (stack, 69H), 1.37-1.57 (stack, 2H), 1.58-1.72 (m, 1H), 1.85-2.00 (stack, 2H), 3.36-3.43 (m, 1H), 3.44-3.53 (stack, 2H), 3.69-3.76 (m, 1H), 3.83-3.96 (stack, 4H), 3.99-4.08 (stack, 2H), 4.09-4.20 (m, 1H), 4.32-4.47 (stack, 2H), 4.48-4.67 (stack, 4H), 4.70-4.86 (stack, 6H), 4.92 (d, J 11.7, 1H), 6.12 (d, J 8.7, 1H), 7.20-7.37 (stack, 30H); δ_C (75 MHz, CDCl_3) 14.1 (CH_3), 14.7 (CH_3), [22.7, 25.7, 26.1, 29.3, 29.4, 29.7, 31.9, 36.7 (CH_2, resonance overlap)], 50.3 (CH), 69.6 (CH_2), 69.96 (CH_2), 70.05 (CH), 71.7 (CH_2), 71.8 (CH_2), 72.9 (CH_2), 73.4 (CH_2), 73.6 (CH_2), 73.7 (CH), 74.8 (CH_2), 74.9 (CH), 78.6 (CH), 78.9 (CH), 80.1 (CH), 99.6 (CH), [127.4, 127.6, 127.8, 128.2, 128.3 (CH, resonance overlap)], [137.5, 138.4, 138.5, 138.6 (C, resonance overlap)], 172.8 (C); MS (TOF ES+) m/z 1421.6 ([M + Na]^+, 100%). Data for 18 were in agreement with those reported in the literature for 18 prepared by a different route.20

(2S,3S,4R)-1-O-α-D-galactopyranosyl-2-(hexacosanoylamino)octadecane-1,3,4-triol (α-GalCer) (1)^21,22

α-GalCer (1) was prepared according a slightly modified procedure to that reported in the literature:21 Amide 1 (α-GalCer) was prepared from perbenzylated amide 18 (180 mg, 0.129 mmol) and Pd/C (85 mg, 10% wet) in THF (10 mL) according to the general procedure. After 22 h, work-up and purification by flash column chromatography (8% MeOH in CHCl_3) afforded amide 1 as an amorphous white solid (75 mg, 68%): R_f = 0.3 (10% MeOH in CHCl_3); [α]_D^20 = +15.2 (c 1, CHCl_3:CH_3OH, 2:1) (lit. [α]_D^20 = +43.6 (c 1, pyridine); mp 188 – 189 °C (lit. mp 189 – 190 °C); ν_{max} (film) / cm^{-1} 3313br (OH), 1642m (C=O); δ_H (400 MHz, CDCl_3:CD_3OD, 2:1) 0.83 (t, J 6.7, 6H, 2 × CH_3CH_2), 1.16-1.40 (stack, 68H, alkyl chain methylenes), 1.45-1.63 (stack, 4H), 2.16 (app. t, J 7.8, 2H), 3.46-3.57 (stack, 2H), 3.60-3.79 (stack, 6H), 3.80-3.87 (m, 1H), 3.90 (d, J 2.5, 1H), 4.11-4.18
Second-Generation Approach to α-GalCer (1)

(a) (i) 14, PPh₃, CBr₄, CH₂Cl₂, r.t., 3 h; (ii) Me₂NC(O)NMe₂, Bu₄NBr, CH₂Cl₂, then acceptor, CH₂Cl₂, 3 Å MS, r.t., 3 d, 71%. (b) PMe₃, THF, r.t., 3 h, then H₂O, 1 h, 93%. (c) CH₅(CH₂)₂C(O)Cl, Et₃N, CH₂Cl₂, 0 °C to r.t., 12 h, 85%. (d) (i) TFA, CH₂Cl₂-H₂O, 10:1, 2 h, r.t.; (ii) Pd(OH)₂ / C, H₂, THF, 8 h, 75%.
(2S,3S,4R)-2-Azido-3,4-O-isopropylidene-1-O-(2’3’,4’,6’-tetra-O-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (20)\textsuperscript{22}

Glycoside 20 was prepared according a different procedure to that reported in the literature:\textsuperscript{22} Ph\textsubscript{3}P (1.46 g, 5.55 mmol) and CBr\textsubscript{4} (1.84 g, 5.55 mmol) were added sequentially to a solution of 2,3,4,6-tetra-O-benzyl-α-D-galactose 14\textsuperscript{419} (1.00 g, 1.85 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) at r.t. The reaction mixture was stirred for 3 h. In separate flasks, a solution of tetramethylurea (TMU) (1.2 mL) and Bu\textsubscript{4}NBr (1.79 g, 5.55 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL), and a solution of (2S,3S,4R)-2-azido-3,4-O-isopropylidene-octadecane-1,3,4-triol\textsuperscript{624} (1.07 g, 2.78 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL) were stirred over activated 3 Å MS for 30 min, after which time, these solutions were added dropwise (15 min) and sequentially (TMU/Bu\textsubscript{4}NBr solution first) to the solution containing the glycosyl donor. The reaction mixture was stirred at r.t. for 3 d until there was no evidence by TLC that the donor was still being consumed. The reaction mixture was then filtered through a silica plug, washing with CH\textsubscript{2}Cl\textsubscript{2} (1.2 L) and then concentrated under reduced pressure. Purification of the residue by flash column chromatography (10% EtOAc in hexane) afforded glycoside 20 as a colorless oil (1.56 g, 71%, α-anomer only): \(R_f = 0.2\) (10% EtOAc in hexane); [\(\alpha\)]\textsubscript{D}\textsuperscript{22} = +24.8 (c 1, CHCl\textsubscript{3}); lit.\textsuperscript{22} [\(\alpha\)]\textsubscript{D}\textsuperscript{22} = +32.1 (c 2.0, CHCl\textsubscript{3}); \(\nu\)\textsubscript{max}(film) / cm\textsuperscript{-1} 2099s (N\textsubscript{3}); \(\delta\)\textsubscript{H}(500 MHz, CDCl\textsubscript{3}) 0.87 (t, \(J\) 6.8, 3H, CH\textsubscript{2}CH\textsubscript{3}), 1.20-1.42 (stack, 29H, alkyl chain methylenes, C(CH\textsubscript{3})\textsubscript{2}), 1.47-1.64 (stack, 3H), 3.44-3.54 (stack, 3H, C(6’)H\textsubscript{2}, H-2), 3.71 (dd, \(J\) 10.8, 6.7, 1H, C(1)H\textsubscript{b}H\textsubscript{a}), 3.91-3.94 (m, 1H, H-4’), 3.95-4.12 (stack, 6H, H-2’, H-3’, H-5’, C(1)H\textsubscript{a}H\textsubscript{b}, H-3, H-4), 4.39 (A of AB, \(J\)\textsubscript{A-B} 11.9, 1H, OCH\textsubscript{a}H\textsubscript{b}Ph), 4.47 (B of AB, \(J\)\textsubscript{B-A} 11.9, 1H, OCH\textsubscript{a}H\textsubscript{b}Ph), 4.56 (A of AB, \(J\)\textsubscript{A-B} 11.5, 1H, OCH\textsubscript{a}H\textsubscript{b}Ph), 4.70 (A of AB, \(J\)\textsubscript{A-B} 12.0, 1H, OCH\textsubscript{a}H\textsubscript{b}Ph), 4.79 (B of AB, \(J\)\textsubscript{B-A} 12.0, 1H, OCH\textsubscript{a}H\textsubscript{b}Ph).
4.71 (A of AB, $J_{AB} 11.8, 1H, OCH_2CH_2Ph$), 4.79 (B of AB, $J_{BA} 12.0, 1H, OCH_2CH_2Ph$), 4.84 (B of AB, $J_{BA} 11.8, 1H, OCH_2CH_2Ph$), 4.94 (B of AB, $J_{BA} 11.5, 1H, OCH_2CH_2Ph$), 7.22-7.33 (stack, 16H, Ph), 7.36-7.38 (stack, 4H, Ph); δc(125 MHz, CDCl$_3$) 14.1 (CH$_3$, CH$_2$CH$_3$), 22.7 (CH$_2$), 25.7 (CH$_3$, 1 × C(CH$_3$)$_2$), 26.6 (CH$_3$), 28.1 (CH$_3$, 1 × C(CH$_3$)$_2$), [29.3, 29.60, 29.65, 29.69 (CH$_2$, alkyl chain, resonance overlap)], 31.9 (CH$_2$), 59.8 (CH, C-2), 69.1 (CH$_2$, C-6’), 69.6 (CH$_2$, C-1), 69.9 (CH), 72.9 (CH$_2$, CH$_2$Ph), 73.3 (CH$_2$, CH$_2$Ph), 73.4 (CH$_2$, CH$_2$Ph), 74.7 (CH$_2$, CH$_2$Ph), 75.3 (CH, C-4’), 75.4 (CH), 76.6 (CH), 77.8 (CH, C-4), 78.7 (CH), 98.8 (CH, C-1’), 108.2 (C, C(CH$_3$)$_2$), [127.4, 127.5, 127.60, 127.64, 127.7 (CH, Ph, resonance overlap)], [128.20, 128.25, 128.29, 128.35 (CH, Ph, resonance overlap)], 138 (C, ipso Ph), 138.7 (C, ipso Ph), 138.9 (C, 2 × ipso Ph, resonance overlap); MS (TOF ES+) m/z 928.7 ([M+Na]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{55}$H$_{75}$O$_8$Na [M+Na]$^+$ 928.5452, found 928.5470. The unreacted azide was also recovered (394 mg, 37%). Data for 20 were in agreement with those reported in the literature for 20, prepared by a different route.$^{22}$

(2S,3S,4R)-2-Hexacosanoylamino-3,4-O-isopropylidene-1-O-(2',3',4',6'-tetra-O-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (22)$^{22}$

Amide 22 was prepared according a different procedure to that reported in the literature.$^{22}$ A screw-capped glass tube containing a solution of hexacosanoic acid (100 mg, 0.25 mmol) in (COCl)$_2$ (2.0 mL, 23 mmol) was closed tightly and heated at 70 °C. After 2 h, the volatiles were evaporated under a stream of argon and the tube then placed on a high vacuum line for at least 2 h to remove the residual
volatiles. The resulting hexacosanoyl chloride was used directly without further purification: a solution of freshly prepared hexacosanoyl chloride (105 mg, 0.25 mmol) in CH₂Cl₂ (0.5 mL) was added dropwise over 2 min to a solution of amine 21 (132 mg, 0.15 mmol) and NEt₃ (42 µL, 0.30 mmol) in CH₂Cl₂ (1.0 mL) at 0 ºC. The reaction mixture was stirred at r.t. for 12 h and then diluted with CH₂Cl₂ (10 mL), washed sequentially with NaHCO₃ solution (10 mL), brine (2 mL) and then dried over Na₂SO₄. The drying agent was removed by filtration and the filtrate concentrated under reduced pressure. Purification of the residue by flash column chromatography provided amide 22 as a white solid (160 mg, 85%): Rₙ = 0.3 (10% EtOAc in hexane); [α]²⁰⁰D = +41.6 (c 1, CHCl₃); lit.²² [α]¹¹D = +44.2 (c 0.85, CHCl₃); mp 87 – 88 ºC; νmax (film) / cm⁻¹ 1648m (C=O); δH(500 MHz, CDCl₃) 0.87 (t, J 6.9, 6H, CH₃CH₂), 1.15-1.34 (stack, 71H, alkyl chain methylenes, 1 × C(CH₃)₂), 1.39 (s, 3H, 1 × C(CH₃)₂), 1.40-1.47 (stack, 2H, including C(3’’)-H₃H₆), 1.48-1.56 (stack, 2H), 1.93-2.01 (m, 1H, C(2’’)-H₆H₈), 2.01-2.09 (m, 1H, C(2’’)-H₈H₆), 3.37 (dd, J 9.4, 5.7, 1H, C(6’)-H₆H₈), 3.54 (dd, J 9.4, 7.0, 1H, C(6’)-H₆H₈), 3.60 (br d, J 9.7, 1H, C(1)H₆H₈), 3.88-3.93 (stack, 3H, H-3’, H-4’, H-3 or H-4), 3.97 (app t, J 6.3, 1H, H-5’), 4.01-4.12 (stack, 4H, H-2’, C(1)H₆H₈, H-2, H-3 or H-4), 4.36 (A of AB, Jₐₐ 11.8, 1H, C(6’)-OCH₃H₆Ph), 4.47 (B of AB, Jₐ₋ₐ 11.8, 1H, C(6’)-OCH₃H₈Ph), 4.57 (A of AB, Jₐ₋ₐ 11.6, 1H, C(6’)-OCH₃H₈Ph), 4.65 (A of AB, Jₐ₋ₐ 11.5, 1H, C(2’)-OCH₃H₈Ph), 4.73 (A of AB, Jₐ₋ₐ 11.8, 1H, C(3’)-OCH₃H₈Ph), 4.79 (B of AB, Jₐ₋ₐ 11.5, 1H, C(2’)-OCH₃H₈Ph), 4.80 (B of AB, Jₐ₋ₐ 11.8, 1H, C(3’)-OCH₃H₈Ph), 4.89 (d, J 3.7, 1H, H-1’), 4.91 (B of AB, Jₐ₋ₐ 11.6, 1H, C(4’)-OCH₃H₈Ph), 6.24 (d, J 8.9, 1H, NH), 7.21-7.38 (stack, 20H, Ph); δC(125 MHz, CDCl₃) 14.1 (CH₃, CH₂CH₃), 22.6 (CH₃), 25.6 (CH₂), 25.9 (CH₃, 1 × C(CH₃)₂), 26.5 (CH₂), 28.2 (CH₃, 1 × C(CH₃)₂), 28.9 (CH₂), [29.31, 29.37, 29.45, 29.55, 29.57, 29.62, 29.66, 29.68 (CH₂, alkyl chain, resonance overlap)], 31.9 (CH₂), 36.7 (CH₂, C-2’’), 48.7 (CH, C-2), 69.5 (CH₂, C-6’), 69.9 (CH, C-5’), 70.6 (CH₂, C-1), 73.0 (CH₂, C(3’)-OCH₂Ph), 73.46 (CH₂, C(2’)-OCH₂Ph), 73.54 (CH₂, C(6’)-OCH₂Ph), 74.6 (CH₂, C(4’)-OCH₂Ph), 74.7 (CH, C-4’), 75.4 (CH, C-3 or C-4), 76.8 (CH, C-2’), 77.8 (CH, C-3 or C-4), 78.9 (CH, C-3’), 99.7 (CH, C-1’), 107.8 (C, (CH₃)₂C), [127.4, 127.5, 127.7, 127.8, 127.85, 127.91 (CH, Ph, some
resonance overlap], [128.2, 128.31, 128.34, 128.36, 128.40 (CH, Ph, some resonance overlap)], 137.5 (C, ipso Ph on C-6'), 138.3 (C, ipso Ph), 138.4 (C, ipso Ph), 138.6 (C, ipso Ph on C-3'), 172.4 (C, C=O); MS (TOF ES+) m/z 1281.0 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₈₁H₁₂₇NO₉Na [M + Na]⁺ 1280.9409, found 1280.9417. Data for 22 were in agreement with those reported in the literature for 22 which had been prepared using different reagents.²²

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(hexacosanoylamino)octadecane-1,3,4-triol

(α-GalCer) (1)²¹,²²

α-GalCer (1) was prepared from a different precursor to that reported in the literature.²¹ TFA (150 µL) was added dropwise over 1 min to a solution of acetal 22 (120 mg, 0.095 mmol) in CH₂Cl₂ / H₂O (10:1, 0.9 mL). After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et₂O (3 × 3 mL) to provide the crude acetal hydrolysis product as a white solid (116 mg, quant.), which was treated with Pd(OH)₂/C (30 mg, 10% wet) in THF (10 mL) according to the general hydrogenolysis procedure. After 8 h, work-up and purification by flash column chromatography (8% MeOH in CHCl₃) afforded amide 1 as a white solid (61 mg, 75%). Data for 1 were in agreement with those reported for this compound prepared from 22 and also with those reported in the literature.²²
(2S,3S,4R)-2-Amino-1-O-[4’-O-tert-butyldiphenylsilyl-2’,3’-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-octadecane-1,3,4-triol (26)

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\text{\[2S,3S,4R\]-2-Amino-1-O[4’-O-tert-butyldiphenylsilyl-2’,3’-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-octadecane-1,3,4-triol (26)}
\]

PMe$_3$ (0.7 mL of a 1.0 M soln in THF, 0.7 mmol) was added dropwise over 2 min to a solution of (2S,3S,4R)-2-azido-1-O-[4’-O-tert-butyldiphenylsilyl-2’,3’-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-octadecane-1,3,4-triol (500 mg, 0.65 mmol) in THF / H$_2$O (7 mL, 15:1). The reaction mixture was stirred at r.t. for 4 h and then concentrated under reduced pressure. The residual H$_2$O was removed by co-evaporation with toluene (3 x 3 mL) to provide the crude amine product. Purification of the residue by flash column chromatography (30% EtOAc in hexane) afforded amine 26 as a colorless oil (433 mg, 90%): $R_f = 0.3$ (30% EtOAc in hexane); $[\alpha]_{D}^{20} = +45.6$ (c 1, CHCl$_3$); $\nu_{\text{max}}$(film) / cm$^{-1}$ 3076w, 1113s, 1083s, 702s; $\delta$$_H$(300 MHz, CDCl$_3$) 0.88 (t, J 7.0, 3H), 1.08 (s, 9H), 1.20–1.43 (stack, 35H), 1.49–1.63 (stack, 3H), 3.58–3.75 (stack, 4H), 3.76–3.88 (stack, 3H), 3.91–3.99 (stack, 2H), 4.10–4.24 (stack, 2H), 7.34–7.47 (stack, 6H), 7.64–7.72 (stack, 4H); $\delta$$_C$(100 MHz, CDCl$_3$): 14.1 (CH$_3$), 19.2 (CH$_3$), 22.7 (CH$_2$), 25.7 (CH$_3$), 26.4 (CH$_2$), [26.8, 27.0, 27.1, 28.2 (CH$_3$, some resonance overlap], [29.3, 29.7 (CH$_2$, some resonance overlap)], 31.9 (CH$_2$), 60.0 (CH), 64.2 (CH$_2$), 72.4 (CH$_2$), 80.0 (CH$_2$), 75.8 (CH), 77.8 (CH), 77.9 (2 × CH), 108.2 (C), 109.4 (C), [127.7, 129.7 (CH, some resonance overlap)], 133.2 (C), 135.6 (CH, some resonance overlap); MS (TOF ES+) $m/z$ 740.6 ([M + H]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{44}$H$_{74}$NO$_6$Si [M + H]$^+$ 740.5285, found 740.5293.
A screw-capped glass tube containing a solution of hexacosanoic acid (163 mg, 0.41 mmol) in (COCl)$_2$ (2.0 mL, 23 mmol) was closed tightly and heated at 70 °C for 2 h. The volatiles were then evaporated under a stream of argon and the tube then placed on a high vacuum line for at least 2 h to remove the residual volatiles. The resulting hexacosanoyl chloride was used directly without further purification: a solution of freshly prepared hexacosanoyl chloride (187 mg, 0.41 mmol) in CH$_2$Cl$_2$ (1 mL) was added dropwise over 2 min to a solution of amine 26 (250 mg, 0.34 mmol) and NEt$_3$ (95 µL, 0.68 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C. The reaction mixture was stirred at r.t. for 12 h and then diluted with CH$_2$Cl$_2$ (10 mL), washed sequentially with NaHCO$_3$ solution (10 mL), brine (2 mL) and then dried over Na$_2$SO$_4$. The drying agent was removed by filtration and the filtrate concentrated under reduced pressure. Purification of the residue by flash column chromatography provided amide 27 as a colorless oil (323 mg, 85%): $R_f = 0.3$ (10% EtOAc in hexane); $[\alpha]^{30}_D = +11.8$ (c 1, CHCl$_3$); $\nu_{\text{max}}$(film) / cm$^{-1}$ 1646m (C=O); $\delta_H$(300 MHz, CDCl$_3$) 0.88 (t, $J$ 7.0, 6H), 1.06 (s, 9H), 1.16–1.36 (stack, 69H, including (1.32 (s, 3H)), 1.40 (s, 3H), 1.41 (s, 3H), 1.42 (s, 3H), 1.45–1.66 (stack, 6H), 2.05–2.18 (stack, 2H), 3.49–3.67 (stack, 3H), 3.74–3.89 (stack, 4H), 4.00–4.23 (stack, 4H), 5.71 (br d, $J$ 9.2, 1H), 7.34–7.48 (stack, 6H), 7.63–7.72 (stack, 4H); $\delta_C$(100 MHz, CDCl$_3$) 14.1 (CH$_3$), 22.7 (CH$_2$), 25.7 (CH$_2$), 25.8 (CH$_3$), 26.5 (CH$_2$), 26.9 (CH$_3$), 27.1 (CH$_3$), 27.2 (CH$_3$), 28.1 (CH$_3$), 29.1 (CH$_2$), [29.33, 29.37, 29.38, 29.43, 29.67, 29.68, 29.69, 29.71, 29.73 (CH$_2$, some resonance overlap)], 31.9 (CH$_2$),...
(2S,3S,4R)-1-O-[4'-O-tert-Butyldiphenylsilyl-2',3'-O-isopropylidene-L-threitol]-2-hexacosanethioylamino-3,4-O-isopropylidene-octadecane-1,3,4-triol (28)

Lawesson’s reagent (81 mg, 0.2 mmol) was added to a solution of the amide 27 (145 mg, 0.13 mmol) in toluene (2 mL) at r.t. The reaction mixture was stirred at 80 °C for 5 h and then the solvent was removed under reduced pressure. The residue was diluted with CH₂Cl₂ (10 mL), washed sequentially with H₂O (5 mL), NaHCO₃ solution (10 mL), brine (2 mL) and then dried over Na₂SO₄. The drying agent was removed by filtration and the filtrate was concentrated under reduced pressure. Purification of the residue by flash column chromatography (15% EtOAc in hexane) provided thioamide 28 as a pale yellow oil (130 mg, 88%): $R_f = 0.3$ (15% EtOAc in hexane); [α]$_D^{20} = +36.8$ (c 0.5, CHCl₃); $ν_{max}$(film) / cm$^{-1}$ 1258s, 1083s, 736s, 702s; $δ_h$(300 MHz, CDCl$_3$) 0.88 (t, $J$ 7.0, 6H), 1.06 (s, 9H), 1.17–1.35 (72H, stack, including (1.31 (s, 3H)), 1.39 (s, 3H), 1.41 (s, 3H), 1.42 (s, 3H), 1.44–1.58 (stack, 3H), 2.49–2.67 (stack, 2H), 3.49–3.58 (m, 1H), 3.61–3.72 (stack, 2H), 3.74–3.91 (stack, 5H), 4.05–4.21 (stack, 2H), 4.28–4.35 (dd, $J$ 7.7, 5.9, 1H), 4.77–4.88 (m, 1H), 7.30–7.49 (stack, 6H), 7.60–7.75 (stack, 4H); $δ_c$(100 MHz, CDCl$_3$) 14.1 (CH₃), 22.7 (CH₂), 25.6 (CH₃), 26.7
(CH₂, 26.9 (CH₃), 27.0 (CH₃), 27.2 (CH₃), 27.7 (CH₃), 29.0 (CH₂), [29.37, 29.42, 29.56, 29.59, 29.61, 29.7 (CH₂, some resonance overlap)], 31.9 (CH₂), 47.6 (CH₂), 54.6 (CH), 64.2 (CH₂), 69.7 (CH₂), 72.6 (CH₂), 75.5 (CH), 77.7 (CH), 77.8 (CH), 77.9 (CH), 108.1 (C), 109.5 (C), [127.8, 129.8, (CH, some resonance overlap)], 133.1 (C), 135.6 (CH, some resonance overlap), 205.5 (C); MS (TOF ES+) m/z 1156.8 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₇₀H₁₂₃NO₆SiNa [M + Na]⁺ 1156.8738, found 1156.8749.

(2S,3S,4R)-2-Hexacosanethioylamino-1-O-[L-threitol]-octadecane-1,3,4-triol (11)

Bu₄F (1.0 M solution in THF, 120 µL, 0.12 mmol) was added to a solution of silyl ether 28 (125 mg, 0.11 mmol) in THF (1 mL) at r.t. After 4 h, NH₄Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL). The solvent was removed under reduced pressure to provide the resulting primary alcohol as a white solid (98 mg, quant.), which was dissolved in CH₂Cl₂ / CH₃OH (10:1, 1.1 mL) and treated with TFA (0.5 mL; dropwise addition over 1 min). After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et₂O (3 × 4 mL). Purification of the residue by flash column chromatography (5% CH₃OH in CHCl₃) afforded pentaol 11 as a pale yellow solid (65 mg, 73%): Rᵣ = 0.3 (8% CH₃OH in CHCl₃); [α]D the insolubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; mp 96 – 97 °C; ν_max(film) / cm⁻¹ 3324s br (O–H); δ_H(500 MHz, CDCl₃:CD₃OD, 2:1) 0.83 (t, J 7.0, 6H, 2 × CH₂CH₃), 1.11–1.41 (stack, 69H, alkyl chain methylenes), 1.43–1.74 (stack, 3H), 2.60 (t, J 8.1, 2H,
C(3")H, 3.48–3.54 (stack, 3H, C(1')H₂, H-4), 3.55–3.63 (stack, 3H, C(4')H₂, H-3'), 3.64–3.72 (stack, 2H, C(1)H₆H₆, H-3), 3.74–78 (m, 1H, H-2'), 3.80–3.86 (m, 1H, C(1)H₆), 4.83–4.88 (m, 1H, H-2); δc(125 MHz, CDCl₃:CD₃OD, 2:1) 14.2 (CH₃, 2×CH₂CH₃), 22.9 (CH₂), 26.2 (CH₂), 29.3 (CH₂), [29.6, 29.7, 29.8, 30.0 (CH₂, alkyl chain, resonance overlap)], 32.2 (CH₂), 32.7 (CH₂, C-5), 46.9 (CH₂, C-2'), 56.1 (CH, C-2), 63.7 (CH₂, C-4'), 69.6 (CH₂, C-1), 70.6 (CH, C-2'), 72.2 (CH, C-3'), 73.0 (CH₂, C-4), 73.2 (CH₂, C-1'), 74.1 (CH, C-3), 205.9 (C, C=S); MS (TOF ES+) m/z 838.7 ([M + Na]+, 100%); HRMS (TOF ES+) calcd for C₅₀H₉₉NO₈SNa [M + Na]+ 838.6934, found 838.6946.

(2S,3S,4R)-1-O-[4’-O-tert-Butyldiphenylsilyl-2’,3’-O-isopropyldiene-1-threitol]-3,4-O-isopropyldiene-2-(tetracosanyloxy carbonylamino)octadecane-1,3,4-triol (29)

A solution of carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester (110 mg, 0.21 mmol) in CH₂Cl₂ (0.5 mL) was added to a stirred solution of amine 26 (104 mg, 0.14 mmol) and NEt₃ (42 µL, 0.3 mmol) in CH₂Cl₂ (1 mL). The resulting mixture was stirred at r.t. until no mixed carbonate remained as determined by TLC (5 h). The mixture was then diluted with CH₂Cl₂ (10 mL) and washed sequentially with NaHCO₃ solution (10 mL) and brine (10 mL). The organic phase was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. Purification of the residue by flash column chromatography (10% EtOAc in toluene) provided carbamate 29 as a colorless oil (135 mg, 86%): $R_f = 0.3$ (10% EtOAc in hexane); $[α]^{22}_D = +28.8$ (c 0.5, CHCl₃); $\nu_{max}$ (film) / cm⁻¹ 1687m (C=O); δd(500 MHz, CDCl₃) 0.88 (t, J 7.1, 6H, 2×CH₂CH₃), 1.06 (s, 9H, C(CH₃)₃), 1.26–1.34 (stack,
70H, alkyl chain methylenes), 1.40 (s, 6H, C(CH₃)₂), 1.42 (s, 3H, C(CH₃)₂), 1.59 (s, 3H, C(CH₃)₂), 3.52–3.65 (stack, 3H, C(1)H₆a, b, C(1’)H₆b), 3.74–3.81 (stack, 3H, C(4’)H₆a, b, C(1)H₆c, d), 3.82–3.90 (stack, 2H, H-3’, H-2), 3.98 (m, 1H, C(1”)H₆a, b), 4.00–4.11 (stack, 3H, H-3, H-4, C(1”)H₆c, d), 4.14–4.20 (m, 1H, H-2’), 4.97 (br d, J 9.5, 1H, NH), 7.36–7.46 (stack, 6H, Ph), 7.66–7.70 (stack, 4H, Ph);

δC(125 MHz, CDCl₃) 14.1 (CH₃, C-18, C-24”), 19.2 (C, (CH₃)₃C), 22.7 (CH₂), 25.8 (CH₃, C(CH₃)₂), 25.9 (CH₂), 26.4 (CH₂), 26.8 (CH₃, C(CH₃)₃), 27.0 (CH₂, C(CH₃)₃), 27.2 (CH₃, C(CH₃)₂), 28.1 (CH₃, C(CH₃)₂), 28.9 (CH₂), 29.1 (CH₂), 29.4 (CH₂), [29.6, 29.7 (CH₂, alkyl chains, some resonance overlap)], 31.9 (CH₂), 50.3 (CH, C-2), 64.1 (CH₂, C-4”), 65.2 (CH₂, C-1”), 71.6 (CH₂, C-1), 72.6 (CH₂, C-1’), 75.9, (CH, C-3), 77.7 (CH, C-2’), 77.8 (CH, C-4), 78.2 (CH, C-3’), 107.8 (C, (CH₃)₂C), 109.4 (C, (CH₂)₂C), [127.7, 129.75, 129.79, (CH, Ph, some resonance overlap)], 133.2 (C, ipso Ph), 135.6 (CH, Ph, some resonance overlap), 156.0 (C, C=O); MS (TOF ES+) m/z 1142.7 ([M + Na]+, 100%); HRMS (TOF ES+) calcd for C₆₉H₁₂₁NO₈SiNa [M + Na]+ 1142.8759, found 1142.8767.

(2S,3S,4R)-2-Tetracosanyloxycarbonylamino-1-O-[L-threitol]-octadecane-1,3,4-triol (13)

Bu₄F (1.0 M solution in THF, 180 µL, 0.18 mmol) was added to a solution of silyl ether 29 (179 mg, 0.16 mmol) in THF (1.5 mL) at r.t. After 4 h, NH₄Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL). The solvent was removed under reduced pressure to provide the crude primary alcohol as a white solid (141 mg, quant.), which was dissolved in CH₂Cl₂ / CH₃OH (10:1, 1.2 mL) and treated with TFA (0.6 mL; dropwise addition over 1 min). After stirring for 2 h at r.t., the reaction mixture was concentrated
under reduced pressure and the residual TFA was removed by co-evaporation with Et₂O (3 × 4 mL) to provide the crude product, which was purified by flash column chromatography (10% CH₃OH in CHCl₃) to afford pentaol 13 as a pale yellow solid (90 mg, 70%): R_f = 0.3 (10% CH₃OH in CHCl₃); [α]_D the insolubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; mp 55 – 56 °C; ν_max (film) / cm⁻¹ 3340m br (O–H), 1683s (C=O); δ_H (400 MHz, CDCl₃:CD₃OD, 2:1) 0.83 (t, J 7.2, 6H), 1.16–1.45 (stack, 65H), 1.46–1.68 (stack, 5H), 3.12-3.20 (m, 1H), 3.49-3.64 (stack, 8H), 3.67–3.79 (stack, 2H), 3.85–4.07 (stack, 3H), OH resonances not observed; δ_C (100 MHz, CDCl₃:CD₃OD, 2:1) 14.2 (CH₃), 20.1 (CH₂), 23.0 (CH₂), 24.1 (CH₂), 26.2 (CH₂), [29.5, 29.7, 30.0, 30.4, (CH₂, some resonance overlap)], 32.3 (CH₂), 32.9 (CH₂), 52.1 (CH), 63.9 (CH₂), 65.8 (CH₂), 70.8 (CH), 71.2 (CH₂), 72.5 (CH), 72.8 (CH), 73.4 (CH₂), 75.5 (CH), 157.7 (C); MS (TOF ES+) m/z 824.8 ([M + Na]^+, 100%); HRMS (TOF ES+) calcd for C₅₀H₉₉NO₈SNa [M + Na]^+ 809.6955, found 824.6940.

(2S,3S,4R)-1-O-[4′-O-tert-Butyldiphenylsilyl-2′,3′-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-2-(tricosanylaminocarbonylamino)octadecane-1,3,4-triol (30)

![Chemical Structure](image)

A solution of amine 26 (155 mg, 0.21 mmol) in toluene (1.5 mL) was added to a solution of tricosanyl isocyanate (121 mg, 0.33 mmol; prepared as reported above in the synthesis of urea 19) in toluene (1 mL) at r.t. The reaction mixture was heated under reflux for 8 h and then concentrated under reduced pressure to provide the crude product. Purification of the residue by flash column
chromatography (15% EtOAc in hexane) afforded urea \textbf{30} as a pale yellow oil (186 mg, 80% based on amine): $R_f = 0.3$ (10% EtOAc in hexane); $[\alpha]^{22}_D = +48.8$ (c 1, CHCl$_3$); $\nu_{\text{max}}$ (film) / cm$^{-1}$ 1632m (C=O); $\delta_{\text{H}}$(500 MHz, CDCl$_3$) 0.88 (t, $J$ 7.1, 6H, 2 $\times$ CH$_2$CH$_3$), 1.06 (s, 9H, C(CH$_3$)$_3$), 1.21–1.32 (stack, 68H, alkyl chain methylenes), 1.39 (s, 3H, 1 $\times$ C(CH$_3$)$_3$), 1.40 (s, 3H, 1 $\times$ C(CH$_3$)$_3$), 1.41 (s, 3H, 1 $\times$ C(CH$_3$)$_3$), 1.61 (s, 3H, 1 $\times$ C(CH$_3$)$_3$), 1.61 (s, 3H, 1 $\times$ C(CH$_3$)$_3$), 3.03–3.17 (stack, 2H, C(1")H$_a$H$_b$), 3.53–3.60 (stack, 2H, C(1')H$_a$H$_b$, C(1)H$_a$H$_b$), 3.65 (dd, $J$ 10.5, 3.2, 1H, C(1")H$_a$H$_b$), 3.72–3.80 (stack, 3H, C(4")H$_a$H$_b$, C(1)H$_a$H$_b$), 3.82–3.87 (m, 1H, H-3'), 3.92–3.97 (m, 1H, H-2), 4.04–4.11 (stack, 2H, H-3, H-4), 4.13–4.18 (m, 1H, H-2'), 4.31 (br s, 1H, CH$_2$NH), 4.49 (br d, $J$ 9.2, 1H, CHNH), 7.37–7.46 (stack, 6H, Ph), 7.64–7.70 (stack, 4H, Ph); $\delta_c$(125 MHz, CDCl$_3$) 14.1 (CH$_3$, 2 $\times$ CH$_2$CH$_3$), 19.2 (C, C(CH$_3$)$_3$), 22.7 (CH$_2$), 25.8 (CH$_3$, 1 $\times$ C(CH$_3$)$_3$), 26.4 (CH$_2$), 26.8 (CH$_3$, C(CH$_3$)$_3$), 26.9 (CH$_2$), 27.1 (CH$_3$, 1 $\times$ C(CH$_3$)$_3$), 27.2 (CH$_3$, 1 $\times$ C(CH$_3$)$_3$), 28.1 (CH$_3$, 1 $\times$ C(CH$_3$)$_3$), 29.1 (CH$_2$), [29.4, 29.7 (CH$_2$, alkyl chain, resonance overlap)], 30.2 (CH$_3$), 31.9 (CH$_2$), 40.6 (CH$_2$, C-1''), 49.7 (CH, C-2), 64.3 (CH$_2$, C-4'), 72.0 (CH$_2$, C-1), 72.7 (CH$_2$, C-1''), 76.3 (CH, C-3), 77.3 (CH, C-2'), 77.9 (CH, C-4), 78.2 (CH, C-3'), 107.8 (C, C(CH$_3$)$_2$), 109.5 (C, C(CH$_3$)$_2$), 127.8 (CH, Ph), 129.8 (CH, Ph), 133.1 (C, ipso Ph), 135.6 (CH, Ph), 157.4 (C, C=O); MS (TOF ES+) $m/z$ 1127.7 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for C$_{68}$H$_{120}$N$_2$O$_7$SiNa [M + Na]$^+$ 1127.8763, found 1127.8737.
(2S,3S,4R)-1-O-[1-Threitol]-2-(tricosanylaminocarbonylamino)octadecane-1,3,4-triol (12)

Bu₄F (1.0 M solution in THF, 170 µL, 0.17 mmol) was added to a solution of silyl ether 30 (167 mg, 0.15 mmol) in THF (1.5 mL) at r.t. After 4 h, NH₄Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL). The solvent was removed under reduced pressure to provide a white solid (primary alcohol product, 130 mg, quant.), which was dissolved in CH₂Cl₂ / CH₃OH (10:1, 1.2 mL). TFA (0.6 mL) was added dropwise over 1 min. After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et₂O (3 × 4 mL) to provide the crude product, which was purified by flash column chromatography (10% CH₃OH in CHCl₃) to afford the pentaol 12 as a white solid (85 mg, 72%): Rᵣ = 0.3 (10% CH₃OH in CHCl₃); [α]₀ the insolubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; mp 131 – 132 °C; vₓₓₓₓ (film) / cm⁻¹ 3344s br (O–H), 1607s (C=O); δₓₓ (400 MHz, CDCl₃:CD₃OD, 2:1) 0.84 (t, J 6.9, 6H), 1.15–1.55 (stack, 67H), 1.58–1.69 (m, 1H), 2.99–3.15 (stack, 2H), 3.46–3.66 (stack, 8H), 3.71–3.80 (stack, 2H), 3.93–3.99 (m, 1H), OH and NH resonances not observed; δₓ (100 MHz, CDCl₃:CD₃OD, 2:1) 14.3 (CH₃), 23.2 (CH₂), 26.4 (CH₂), 27.4 (CH₂), [29.8, 29.9, 30.1, 30.2, 30.3 (CH₂, resonance overlap)], 30.7 (CH₂), 32.4 (CH₂), 33.5 (CH₂), 40.7 (CH₂), 51.2 (CH), 64.1 (CH₂), 70.7 (CH), 72.1 (CH₂), 72.5 (CH), 73.5 (CH), 73.9 (CH₂), 75.9 (CH), 159.9 (C); MS (TOF ES+) m/z 809.8 ([M + Na]⁺, 100%); HRMS (TOF ES+) calcd for C₅₀H₉₅NO₈SNa [M + Na]⁺. 809.6959, found 809.6950.
The synthesis of ThrCer (2) has been reported previously;\textsuperscript{6,25} however the literature routes employ slightly different protecting group strategies. The synthesis of 2 from 27 is therefore described below.

\textbf{(2S,3S,4R)-1-O-[L-Threitol]-2-(hexacosanoylamino)octadecane-1,3,4-triol (2)}\textsuperscript{6}

![Chemical Structure](image)

Bu₄F (1.0 M solution in THF, 120 µL, 0.12 mmol) was added to a solution of silyl ether 27 (123 mg, 0.11 mmol) in THF (1 mL) at r.t. After 4 h, \( \text{NH}_4\text{Cl} \) solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with \( \text{CH}_2\text{Cl}_2 \) (3 × 10 mL). The solvent was removed under reduced pressure to provide the corresponding primary alcohol as a white solid (97 mg, quant.), which was dissolved in \( \text{CH}_2\text{Cl}_2 / \text{CH}_3\text{OH} \) (10:1, 1.1 mL) and treated with TFA (0.5 mL; dropwise addition over 1 min). After stirring for 2 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with \( \text{Et}_2\text{O} \) (3 × 4 mL). Purification of the residue by flash column chromatography (5% \( \text{CH}_3\text{OH} \) in \( \text{CHCl}_3 \)) afforded ThrCer 2 as a white yellow solid (65 mg, 74%). Data for 2 were in agreement with those reported in the literature.\textsuperscript{6}
(2S,3S,4R)-2-Azido-1-O-[4’-O-tert-butyldiphenylsilyl-2’,3’-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-octadecane-1,3,4-triol

Tf₂O (235 µL, 1.40 mmol) was added dropwise over 10 min to a solution of 1-O-tert-butyldiphenylsilyl-2,3-O-isopropylidene-L-threitol²⁶,²⁷ (561 mg, 1.40 mmol) and 2,6-di-tert-butylpyridine (346 µL, 1.54 mmol) in CH₂Cl₂ (14 mL) at 0 °C. After 30 min, the reaction mixture was diluted with CH₂Cl₂ (15 mL) and the resulting solution washed sequentially with cold H₂O (2 x 30 mL) and brine (10 mL), dried (Na₂SO₄) and filtered. Removal of the solvent under reduced pressure provided the corresponding triflate, 1-O-tert-butyldiphenylsilyl-2,3-O-isopropylidene-4-O-trifluoromethanesulfonyl-L-threitol, as a colorless oil [Rᵥ = 0.7 (15% EtOAc in hexanes)], which was used immediately in the next etherification step: A solution of (2S,3S,4R)-2-azido-3,4-O-isopropylidene-octadecane-1,3,4-triol⁹ (505 mg, 1.32 mmol) in THF (10 mL) was treated with NaH (60% in mineral oil, 56.0 mg, 1.40 mmol) at 0 °C. After 1 h, a solution of the triflate (assuming 100% conversion, 1.40 mmol) in THF (5 mL) was added dropwise over 5 min. The resulting solution was stirred at this temperature for 1 h and then at r.t. for 12 h. The reaction was then quenched by the addition of MeOH (2 mL) followed by NaHCO₃ solution (10 mL). The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic fractions were washed with brine (15 mL) and dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (eluent: 5% EtOAc in hexanes) to provide (2S,3S,4R)-2-azido-1-O-[4’-O-tert-butyldiphenylsilyl-2’,3’-O-isopropylidene-L-threitol]-3,4-O-isopropylidene-octadecane-1,3,4-triol as a colorless oil (819 mg, 81%): Rᵥ = 0.6 (10% EtOAc in
hexane); $[\alpha]_{D}^{21} = +10.0 \ (c \ 1, \ CHCl_3)$; $\nu_{\max} \ (\text{film}) / \text{cm}^{-1}$ 2098s (N$_2$); $\delta_{\text{H}} (300 \text{ MHz, CDCl}_3) \ 0.88 \ (t, \ J \ 7.0, \ 3H), \ 1.08 \ (s, \ 9H), \ 1.20-1.43 \ (\text{stack, } 35H), \ 1.49-1.63 \ (\text{stack, } 3H), \ 3.58-3.75 \ (\text{stack, } 4H), \ 3.76-3.88 \ (\text{stack, } 3H), \ 3.91-3.99 \ (\text{stack, } 2H), \ 4.10-4.24 \ (\text{stack, } 2H), \ 7.34-7.47 \ (\text{stack, } 6H), \ 7.64-7.72 \ (\text{stack, } 4H); 

$\delta_{\text{C}} (100 \text{ MHz, CDCl}_3) \ 14.1 \ (\text{CH}_3), \ 19.2 \ (\text{C}), \ 22.7 \ (\text{CH}_2), \ 25.7 \ (\text{CH}_3), \ 26.4 \ (\text{CH}_2), \ 26.8 \ (\text{CH}_3), \ 27.0 \ (\text{CH}_3), \ 27.1 \ (\text{CH}_3), \ 28.2 \ (\text{CH}_3), \ [29.3, \ 29.7 \ (\text{CH}_2, \ \text{some resonance overlap})], \ 31.9 \ (\text{CH}_2), \ 60.0 \ (\text{CH}), \ 64.2 \ (\text{CH}_2), \ 72.4 \ (\text{CH}_2), \ 80.0 \ (\text{CH}_2), \ 75.8 \ (\text{CH}), \ 77.8 \ (\text{CH}), \ 77.9 \ (2 \times \text{CH}), \ 108.2 \ (\text{C}), \ 109.4 \ (\text{C}), \ 127.7 \ (\text{CH}), \ 129.7 \ (\text{CH}), \ 133.2 \ (\text{C}), \ 135.6 \ (\text{CH}); \ MS \ (\text{TOF ES}+) \ m/z \ 788.7 \ (\text{[M + Na]}^+, \ 100\%); \ HRMS \ (\text{TOF ES}+) \ \text{calcd for } C_{44}H_{71}N_3O_6SiNa \ \text{[M + Na]}^+ \ 788.5010, \ \text{found}\ 788.5015.
**Transactivation of NK cells**

The production of IFN-γ by NK cells was determined following i.v. delivery of 1 µg lipids to C57 BL/6 mice, as previously described. Single cell suspensions were generated from the spleen and liver of mice at either 24 h or 48 h post injection. Abs for flow cytometry were from eBioscience (NK1.1, DX5, TCRβ, B220, IFN-γ) and intracellular cytokine staining was carried out according to the manufacturer’s protocol. Flow cytometry was performed on a CyAn (Dako) and analysed using FlowJo software.

![Graph showing transactivation of NK cells in spleen, liver, and blood](image)

Supplementary Figure 1. Wildtype C57 BL/6 mice (n = 3/group) were injected i.v. with 1 µg ThrCer, ThrCer-thioamide (11) or ThrCer-carbamate (13). The transactivation of NK cells in the spleen and liver were determined by IFN-γ intracellular cytokine staining using FACS. In addition, IFN-γ levels in blood serum were determined by ELISA at 24 h and 33 h post-injection.

**Statistical Analysis**

All statistical analyses were preformed using Graphpad Prism software version 5.0. Student’s t-test with two-tailed analysis was used to compare the level of significance between data sets. All p-values <0.05 were considered significant.
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