Amide Analogs of CD1d Agonists Modulate *i*NKT cell-Mediated Cytokine Production

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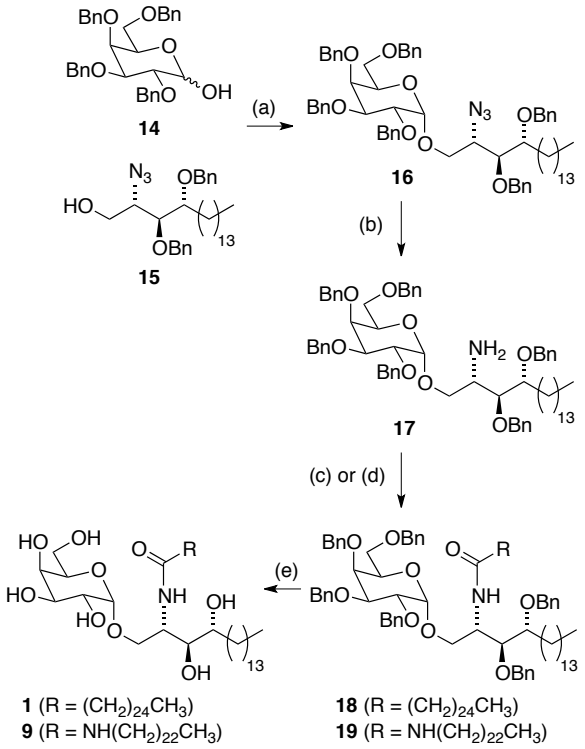
Supporting Information I

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;[1,2] thus reaction of 2,3,4,6-tetra-*O*-benzyl-galactose **14**[3,4] with CBr_4/PPh_3 afforded the corresponding galactosyl bromide, which was reacted in situ with acceptor **15**,[5] in the presence of tetramethylurea (TMU) and Bu_4NBr, to provide the desired galactoside **16** as a single α-anomer (Scheme 1). Staudinger reduction of the azide in **16** with PMe_3 in wet THF[6,7] afforded amine **17**, which reacted with hexacosanoyl chloride[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,[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_{24} acyl chain displays similar biological activity to α-GalCer containing a C_{26} chain,[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. Tricosanyl isocyanate was duly prepared from tetracosanoic acid following a procedure from Várová and co-workers,[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).

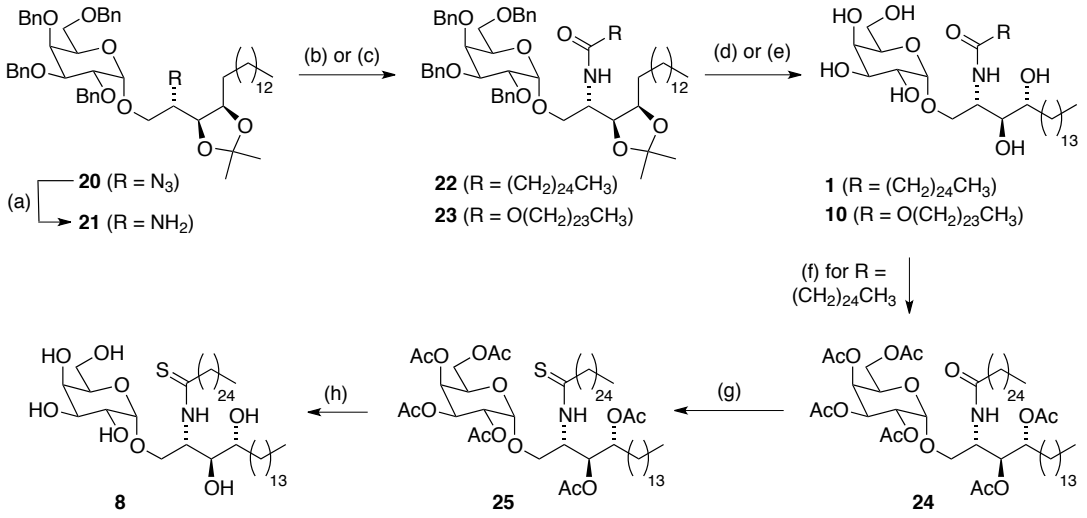
Scheme 1. Synthesis of α-GalCer **1** and urea **9**. (a) **14**, PPh_3, CBr_4, CH_2Cl_2, r.t., 3 h; then $Me_2NC(O)NMe_2$, Bu_4NBr, CH_2Cl_2; then **15**, CH_2Cl_2, 3 Å MS, r.t., 3 d, 62%. (b) PMe_3, THF, r.t., 4 h, then H_2O, 1 h, 72%. (c) $CH_3(CH_2)_{24}C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C to r.t., 8 h, **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 α-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 / debenzylation sequence on **22** and **23** proceeded uneventfully in both cases, to provide α-GalCer **1** and carbamate

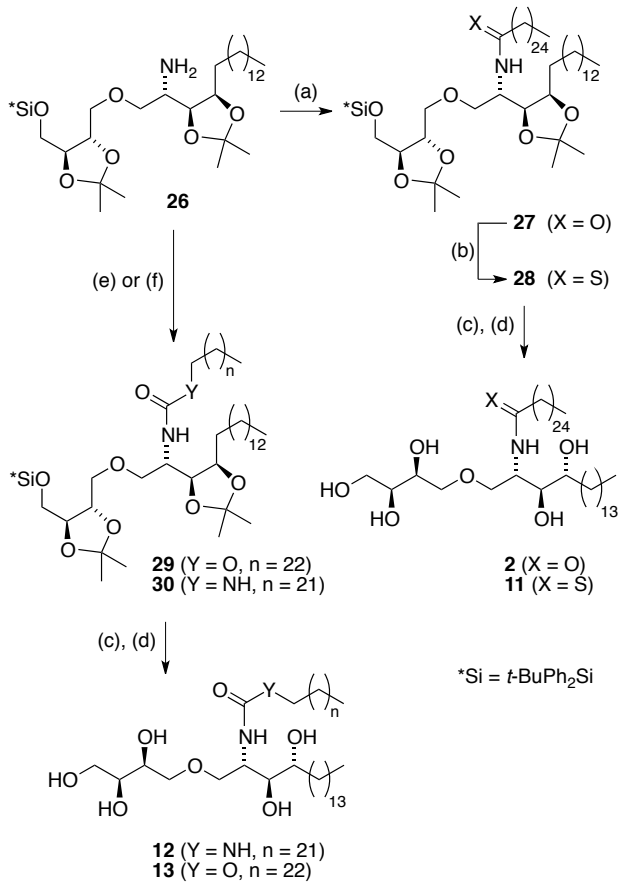
derivative **10**, respectively. Finally the thioamide **8** was prepared from α-GalCer in a three-step sequence, involving peracetylation to provide **24**, thionation of the amide[15] with Lawesson's reagent to afford thioamide **25**, followed by deacetylation under Zémplen conditions (Scheme 2).

Scheme 2. Improved synthesis of α-GalCer **1**, and synthesis of carbamate **10** and thioamide **8**. (a) PMe_3, THF, 3 h, r.t., then H_2O, 1 h, 93%. (b) $CH_3(CH_2)_{24}C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C to r.t., 12 h, **22** (85%). (c) *N*-succinimidyl-tetracosanyl carbonate, Et_3N, CH_2Cl_2, r.t., 4 h, **23** (82%). (d) from **22**: (i) TFA, CH_2Cl_2–H_2O, 10:1, 2 h, r.t.; (ii) $Pd(OH)_2$ / C, H_2, THF, 6 h, **1** (75%). (e) from **23**: (i) TFA, CH_2Cl_2–MeOH, 2:1, 2 h, r.t.; (ii) $Pd(OH)_2$ / C, H_2, THF, 6 h, **10** (75%). (f) Ac_2O, 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,[16] 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 three-step 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_3N, CH_2Cl_2, 0 °C to r.t., 12 h, 85%. (b) Lawesson's reagent, toluene, 80 °C, 5 h, 88%. (c) Bu_4NF, THF, r.t., 4 h. (d) TFA, CH_2Cl_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_3N, CH_2Cl_2, r.t., 5 h, **29** (86%). (f) $CH_3(CH_2)_{24}NCO$, toluene, reflux, 8 h, **30** (80%).

General Experimental

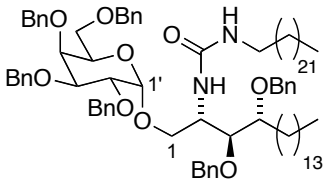
Infra-red spectra were recorded neat as thin films. The intensity of each band is described as s (strong), m (medium) or w (weak) and with the prefix v (very) and suffix br (broad) where appropriate. ^{1}H–NMR and ^{13}C–NMR spectra were recorded in the solvent specified, at 500 and 125 MHz, 400 and 100 MHz, or 300 and 75 MHz, respectively. Chemical shifts are reported as δ values (ppm) referenced to the following solvent signals: $CHCl_3$, δ_H 7.26; $CDCl_3$, δ_C 77.0; CH_3OD, δ_H 3.34; CD_3OD, δ_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% α-naphthol, 5% H_2SO_4 in EtOH. Column chromatography was performed on silica gel (particle size 40–63 μm mesh) using standard glass columns or using pre-packed cartridges (silica, particle size 40 μm) [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 N_2 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 CH_2Cl_2 was freshly distilled under N_2 from CaH_2. THF were freshly distilled under N_2 from sodium benzophenone ketyl. Dry MeCN was purchased as puriss., absolute grade, over 4 Å molecular sieves (H_2O ≤0.001%), ≥99.5% (GC) and used without further purification. All solutions are aqueous and saturated unless specified otherwise. Pyridine and Et_3N were distilled from KOH and stored over 4 Å molecular sieves.

(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(tricosanylaminocarbonylamino)octadecane-1,3,4-triol (19)



19

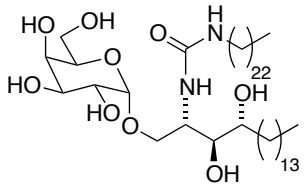
A screw-capped glass tube containing a solution of tetracosanoic acid (450 mg, 1.22 mmol) in $(COCl)_2$ (2.0 mL, 23 mmol) was closed tightly and heated at 70 ºC for 2 h. The volatiles were then evaporated under a stream of argon and the tube then placed on a high vacuum line for at least 2 h to remove 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 NaN_3 (300 mg, 4.62 mmol) in H_2O (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 Na_2SO_4. 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): R_f = 0.3 (20% EtOAc in hexane); $[\alpha]^{20}_{D}$ = +25.2 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 3363m (N–H), 1670m (C=O); δ_H(500 MHz, $CDCl_3$) 0.89 (t, *J* 7.0, 6H, 2 × CH_2CH_3), 1.10–1.39 (stack, 65H, alkyl chain methylenes), 1.39–1.51 (m, 1H, alkyl chain C$H_a$$H_b$), 1.56–1.64 (m, 1H, C(5)$H_a$$H_b$), 1.64–1.73 (m, 1H, C(5)$H_a$$H_b$), 2.90–3.06 (stack, 2H, C$H_a$$H_b$NH), 3.33 (dd, *J* 9.5, 5.0, 1H, C(6')$H_a$$H_b$), 3.55 (dd, *J* 9.5, 7.0, 1H, C(6')$H_a$$H_b$), 3.59–3.64 (m, 1H, H-4), 3.73 (dd, *J* 10.8, 3.1, 1H, C(1)$H_a$$H_b$), 3.80–3.88 (stack, 3H, H-2, H-3, H-4'), 3.89 (dd, *J* 10.1, 2.7, 1H, H-3'), 3.98 (app. t, *J* 6.0, 1H, H-5'), 4.05 (dd, *J* 10.1, 3.6, 1H, H-2'), 4.11 (br dd, *J* 10.8, 4.5, 1H, C(1)$H_a$$H_b$), 4.38 (A of AB, $J_{A\text{-}B}$ 12.0, 1H, C(6')OC$H_a$$H_b$Ph), 4.45–4.48 (stack, 2H, C(6')OC$H_a$$H_b$Ph, C(4)OC$H_a$$H_b$Ph), 4.54 (A of AB, $J_{A\text{-}B}$ 11.3, 1H, C(3)OC$H_a$$H_b$Ph), 4.57 (A of AB, $J_{A\text{-}B}$ 11.8, 1H, C(4')OC$H_a$$H_b$Ph), 4.61 (B of AB, $J_{B\text{-}A}$ 12.6, 1H, C(4)OC$H_a$$H_b$Ph), 4.66 (A of AB, $J_{A\text{-}B}$ 11.7, 1H, C(2')OC$H_a$$H_b$Ph), 4.75 (A of AB, $J_{A\text{-}B}$ 11.8, 1H, C(3')OC$H_a$$H_b$Ph), 4.78 (B of AB, $J_{B\text{-}A}$ 11.3, 1H, C(3)OC$H_a$$H_b$Ph), 4.79 (B of AB, $J_{B\text{-}A}$ 11.7, 1H, C(2')OC$H_a$$H_b$Ph), 4.81 (B of AB, $J_{B\text{-}A}$ 11.8, 1H, C(3')OC$H_a$$H_b$Ph), 4.87 (d, *J* 3.6, 1H, H-1'), 4.93 (B of AB, $J_{B\text{-}A}$ 11.8, 1H, C(4')OC$H_a$$H_b$Ph), 5.00 (d, *J*

7.8, 1H, C(2)N*H*), 7.23–7.38 (stack, 30H, 6 × Ph), CH_2NH not observed; δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, 2 × CH_2CH_3), [22.7, 26.0, 26.9, 29.4, 29.7, 29.9, 30.3, 31.9 (CH_2, alkyl chain methylenes, resonance overlap)], 40.3 (CH_2, CH_2NH), 51.7 (CH, C-2), 69.9 (CH_2, C-6'), 70.1 (CH, C-5'), 71.0 (CH_2, C-1), 71.9 (CH_2, C(4)OCH_2Ph), 73.1 (CH_2, C(3')OCH_2Ph), 73.4 (CH_2, C(2')OCH_2Ph), 73.6 (CH_2, C(6')OCH_2Ph), 73.8 (CH_2, C(3)OCH_2Ph), 74.6 (CH_2, C(4')OCH_2Ph), 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 $C_{90}H_{132}N_2O_9Na$ $[M + Na]^+$ 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). H_2 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 $CHCl_3$/MeOH (90/10, v/v), and concentrated under reduced pressure to provide the crude product, which was purified by flash column chromatography.

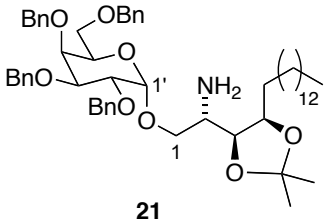
(2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(tricosanylaminocarbonylamino)octadecane-1,3,4-triol (9)



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 $CHCl_3$) afforded urea **9** as an amorphous, white solid (51 mg, 73%): R_f = 0.23 (6% MeOH in $CHCl_3$); $[\alpha]^{18}_D$ = +28.0 (*c* 0.5, $CHCl_3$); mp 154 – 155 °C; ν_{max}(film) / cm^{-1} 3363m (N–H), 1670m (C=O); δ_H(500 MHz, $CDCl_3$:CD_3OD, 3:1) 0.68 (t, *J* 6.5, 6H, 2 × CH_2CH_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, CH_2NH), 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 × H-6', H-2', H-5', C(1)H_aH_b), 3.66 (dd, *J* 10.5, 4.5, 1H, C(1)H_aH_b), 3.72 (br d, *J* 2.5, 1H, H-4'), 3.97 (dd, *J* 9.0, 4.5, 1H, H-2), 4.67 (d, *J* 3.5, 1H, H-1'); δ_C(125 MHz, $CDCl_3$:CD_3OD, 3:1) 13.5 (CH_3, 2 × CH_2CH_3), [22.3, 25.1, 25.5, 26.6, 29.0, 29.1, 29.3, 29.4, 29.8, 31.5, 32.7 (CH_2, alkyl chain methylenes, resonance overlap)], 39.8 (CH_2, CH_2NH), 50.6 (CH, C-2), 61.5 (CH_2, C-6'), 67.6 (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 $C_{48}H_{96}N_2O_9Na$ [M + Na]$^+$ 867.7014, found 867.7026.

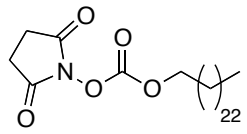
(2*S*,3*S*,4*R*)-2-Amino-3,4-*O*-isopropylidene-1-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (21)



21

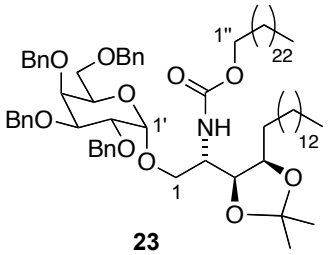
PMe_3 (930 μL of a 1.0 M soln in THF, 0.93 mmol) was added dropwise over 5 min to a solution of azide **20** (700 mg, 0.77 mmol) in THF (7 mL). The reaction mixture was stirred at r.t. for 3 h, after which time, H_2O (0.5 mL) was added. The reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The residual H_2O 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%): R_f = 0.2 (25% EtOAc in hexane); $[\alpha]^{20}_D$ = +35.6 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 3372 br (N–H); δ_H(300 MHz, $CDCl_3$) 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, $J_{A\text{-}B}$ 11.8, 1H), 4.46 (B of AB, $J_{B\text{-}A}$ 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), NH_2 not observed; δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 25.9 (CH_3), 26.2 (CH_2), 28.3 (CH_3), [29.3, 29.7, 29.8 (CH_2, resonance overlap)], 31.9 (CH_2), 50.7 (CH), 69.0 (CH_2), 69.5 (CH), 72.3 (CH_2), 73.0 (CH_2), 73.3 (CH_2), 73.5 (CH_2), 74.8 (CH_2), 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 $C_{55}H_{78}NO_8$ $[M + H]^+$ 880.5727, found 880.5721.

Carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester



N,*N*′-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 μL, 1.5 mmol) in dry CH_3CN (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 $NaHCO_3$ solution (10 mL) and extracted with EtOAc (2 × 10 mL). The combined extracts were washed with brine (5 mL) and dried over Na_2SO_4. 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.): R_f = 0.3 (25% EtOAc in hexane); ν_{max}(film) / cm^{-1} 1711m (C=O), 1693m (C=O); δ_H(300 MHz, $CDCl_3$) 0.88 (t, *J* 7.0, 3H, CH_2CH_3), 1.16–1.46 (stack, 42H, alkyl chain methylenes), 1.51–1.80 (stack, 2H), 2.84 (s, 4H), 4.31 (t, *J* 6.6, 2H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 28.4 (CH_2), [29.1, 29.36, 29.42, 29.5, 29.7 (CH_2, alkyl chain, resonance overlap)], 31.9 (CH_2), 71.7 (CH_2), 151.6 (C, O*C*=O), 168.7 (C, N*C*=O); MS (TOF ES+) *m*/*z* 518.5 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{29}H_{53}NO_5Na$ $[M + Na]^+$ 518.3821, found 518.3817.

(2*S*,3*S*,4*R*)-3,4-*O*-Isopropylidene-1-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(tetracosanyloxycarbonylamino)octadecane-1,3,4-triol (23)

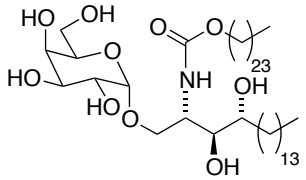


23

A solution of carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester (50 mg, 0.10 mmol) in CH_2Cl_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_2Cl_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_2Cl_2 (8 mL) and washed sequentially with $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_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); $[\alpha]^{20}_D$ = +36.8 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 1689m (C=O); δ_H(500 MHz, $CDCl_3$) 0.88 (t, *J* 6.8, 6H, 2 × CH_2CH_3), 1.24–1.34 (stack, 64H, alkyl chain methylenes, $C(CH_3)_2$), 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_aH_b), 3.52 (dd, *J* 9.3, 6.5, 1H, C(6')H_aH_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_aH_b, H-4', C(1")H_aH_b), 4.03–4.11 (stack, 3H, H-2', C(1)H_aH_b, C(1")H_aH_b), 4.38 (A of AB, $J_{A\text{-}B}$ 11.8, 1H, C(6')OCH_aH_bPh), 4.49 (B of AB, $J_{B\text{-}A}$ 11.8, 1H, C(6')OCH_aH_bPh), 4.56 (A of AB, $J_{A\text{-}B}$ 11.5, 1H, OCH_aH_bPh), 4.67 (A of AB, $J_{A\text{-}B}$ 11.7, 1H, OCH_aH_bPh), 4.74 (A of AB, $J_{A\text{-}B}$ 11.7, 1H, C(2')OCH_aH_bPh), 4.78 (B of AB, $J_{B\text{-}A}$ 11.7, 1H, OCH_aH_bPh), 4.83 (B of AB, $J_{B\text{-}A}$ 11.7, 1H, C(2')OCH_aH_bPh), 4.92 (B of AB, $J_{B\text{-}A}$ 11.5, 1H, OCH_aH_bPh), 4.95 (d, *J* 3.6, 1H, H-1'), 5.32 (br d, *J* 9.5, 1H, N*H*), 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_3), 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.

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

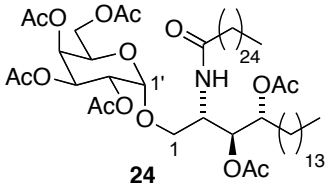


10

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 $CHCl_3$); $[\alpha]^{18}_D$ = +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),

4.65 (d, *J* 3.4, 1H), O*H* and N*H* resonances not observed; δ_C(125 MHz, $CDCl_3$:CD_3OD, 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.

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

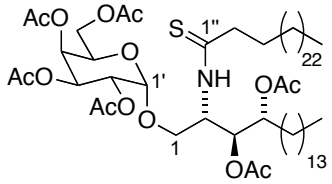


24

Ac_2O (300 μL, 3.2 mmol) was added dropwise over 1 min to a solution of α-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_2Cl_2 (10 mL), washed sequentially with H_2O (5 mL), $NaHCO_3$ solution (10 mL), brine (3 mL) and then dried over Na_2SO_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; ν_{max}(film) / cm^{-1} 1745s (C=O), 1683w (C=O); δ_H(300 MHz, $CDCl_3$) 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); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3),

[20.60, 20.66, 20.72, (CH_3), resonance overlap], 20.1 (CH_3), 22.7 (CH_2), 25.6 (CH_2), 25.7 (CH_2), [29.29, 29.35, 29.40, 29.7 (CH_2, resonance overlap)], 31.9 (CH_2), 36.7 (CH_2), 47.8 (CH), 61.8 (CH_2), 66.7 (CH), 67.2 (CH_2), 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 $C_{62}H_{111}NO_{15}Na$ [M + Na]$^+$ 1132.7851, found 1132.7860.

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

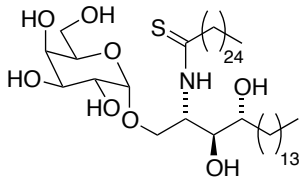


25

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 ºC for 4 h and then the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (10 mL), washed sequentially with H_2O (5 mL), $NaHCO_3$ solution (10 mL), brine (2 mL) and then dried over Na_2SO_4. 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%): R_f = 0.3 (15% EtOAc in hexane); $[\alpha]^{20}_D$ = +46.0 (*c* 1, $CHCl_3$); mp 47 – 48 °C; ν_{max}(film) / cm^{-1} 1747s (C=O); δ_H(500 MHz, $CDCl_3$) 0.87 (t, *J* 6.8, 6H, 2 × CH_2CH_3), 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)CH_3), 2.00 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 2.08 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.66–2.78 (stack, 2H, H-2"), 3.40 (dd, *J* 10.7, 1.9, 1H,

C(1)H_aH$_b$), 3.65 (dd, *J* 10.7, 2.7, 1H, C(1)H$_a$$H_b$), 3.97–4.07 (stack, 2H, C(6')H_aH$_b$, H-5'), 4.08–4.14 (m, 1H, C(6')H$_a$$H_b$), 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*); δ_C(125 MHz, $CDCl_3$) 14.11 (CH_3, 2 × CH_2*C*H$_3$), [20.50, 20.54, 20.62, 20.66, 20.95 (CH_3, C(O)*C*H$_3$), resonance overlap], [22.6, 25.5, 27.5, 28.9, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9 (CH_2, alkyl chain methylenes, resonance overlap)], 47.0 (CH_2, C-2"), 53.7 (CH, C-2), 61.7 (CH_2, C-6'), 65.3 (CH_2, 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 $C_{62}H_{111}NO_{14}SNa$ [M + Na]$^+$ 1148.7623, found 1148.7631.

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

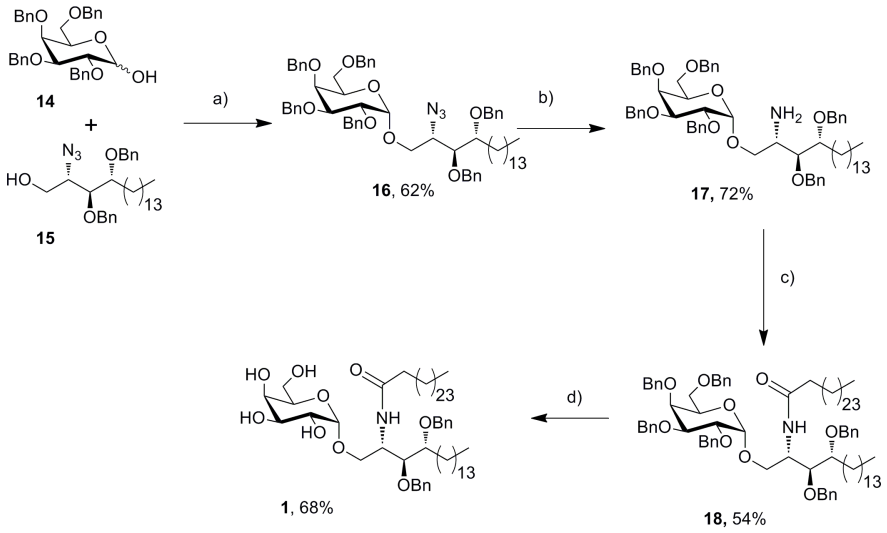


8

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 $CHCl_3$ (50 mL)). The solution was filtered and the resin washed with MeOH (25 mL) and $CHCl_3$/MeOH (25 mL, 9:1). The filtrate was concentrated under reduced pressure. Purification of the residue by flash column chromatography (8% MeOH in $CHCl_3$) provided thioamide **8** as a pale

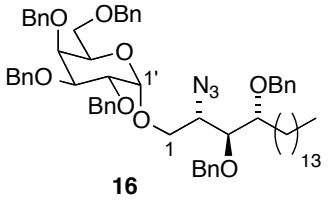
yellow solid (17 mg, 90%): R_f = 0.2 (8% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ = +43.2 (*c* 1, $CHCl_3$:CH_3OH, 2:1); mp 136 – 137 °C; ν_{max}(film) / cm^{-1} 3368m br (O–H); δ_H(400 MHz, $CDCl_3$:CD_3OD, 2:1) 0.84 (t, *J* 6.9, 6H), 1.14–1.44 (stack, 69H), 1.45–1.78 (stack, 3H), 2.56-2.66 (stack, 2H), 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), O*H* resonances not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3), 23.1 (CH_2), 26.4 (CH_2), 29.6 (CH_2), [29.9, 30.0, 30.1, 30.17, 30.21 (CH_2, resonance overlap)], 32.4 (CH_2), 32.7 (CH_2), 47.1 (CH_2), 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 $C_{50}H_{99}NO_8SNa$ $[M + Na]^+$ 896.6989, found 896.6998.

First-Generation Approach to α-GalCer 1



(a) (i) **14**, PPh_3, CBr_4, CH_2Cl_2, r.t., 3 h; (ii) $Me_2NC(O)NMe_2$, Bu_4NBr, CH_2Cl_2, then **15**, CH_2Cl_2, 3 Å MS, r.t., 3 d, 62%. (b) PMe_3, THF, r.t., 4 h, then H_2O, 1 h, 72%. (c) $CH_3(CH_2)_{24}C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C to r.t., 8 h, **18** (54%). (d) Pd / C, H_2, THF, r.t., 22 h, **1** (68% from **17**).

(2*S*,3*R*,4*R*)-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)[17]

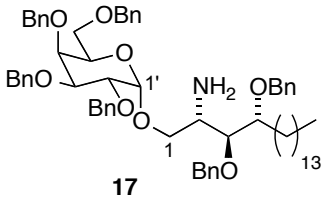


16

Galactoside **16** was prepared according a slightly modified procedure to that reported in the literature:[18] PPh_3 (1.46 g, 5.55 mmol) and CBr_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**[4,19] (1.00 g, 1.85 mmol) in CH_2Cl_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_4NBr (1.79 g, 5.55 mmol) in CH_2Cl_2 (5 mL), and a solution of azide **15**[5] (1.46 g, 2.78 mmol) in CH_2Cl_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_4NBr 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_2Cl_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); $[\alpha]^{18}_D$ = +22 (*c* 1.4, CH_2Cl_2) (lit.[17] $[\alpha]^{20}_D$ = +26 (*c* 1.4, CH_2Cl_2)); ν_{max}(film)/cm^{-1} 2097m (N_3); δ_H(500 MHz, $CDCl_3$) 0.90 (t, *J* 6.9, 3H, CH_2CH_3), 1.20-1.35 (stack, 23H, alkyl chain methylenes), 1.35-1.45 (m, 1H, alkyl chain CH_aH$_b$), 1.50-1.58 (m, 1H, C(5)H_aH$_b$), 1.63-1.71 (m, 1H, C(5)H$_a$$H_b$), 3.47-3.54 (stack, 2H, C(6')H_aH_b), 3.60-3.63 (m, 1H, H-4), 3.71-3.76 (stack, 3H, C(1)H_aH$_b$, H-2, H-3), 3.94-3.98 (stack, 2H, H-4', H-5'), 3.98-4.04 (stack, 2H, H-3', C(1)H$_a$$H_b$), 4.08 (dd, *J* 10.0, 3.5, 1H, H-2'), 4.37 (A of AB, $J_{A\text{-}B}$ 11.8, 1H, C(6')OCH_aH$_b$Ph), 4.45 (B of AB, $J_{B\text{-}A}$ 11.8, 1H, C(6')OCH$_a$$H_b$Ph), 4.48 (A of AB, $J_{A\text{-}B}$ 11.6, 1H, C(4)OCH_aH$_b$Ph), 4.57-4.59 (stack, 2H, C(4)OCH$_a$$H_b$Ph, C(4')OC$H_aH_b$Ph), 4.63 (A of AB, $J_{A\text{-}B}$ 11.3, 1H, C(3)OCH_aH$_b$Ph), 4.67 (B of AB, $J_{B\text{-}A}$ 11.3, 1H, C(3)OCH$_a$$H_b$Ph), 4.69 (A of AB, $J_{A\text{-}B}$

12.0, 1H, C(2')OCH_aH$_b$Ph), 4.74 (A of AB, $J_{A\text{-}B}$ 11.5, 1H, C(3')OCH_aH$_b$Ph), 4.81 (B of AB, $J_{B\text{-}A}$ 12.0, 1H, C(2')OCH$_a$$H_b$Ph), 4.84 (B of AB, $J_{B\text{-}A}$ 11.5, 1H, C(3')OCH$_a$$H_b$Ph), 4.91 (d, *J* 3.5, 1H, H-1'), 4.95 (d, *J* 11.5, 1H, C(4')OCH$_a$$H_b$Ph), 7.22-7.40 (stack, 30H, 6 × Ph); δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, CH_2CH_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_2Ph), 73.0 (CH_2, C(3')OCH_2Ph), 73.1 (CH_2, C(2')OCH_2Ph), 73.3 (CH_2, C(6')OCH_2Ph), 73.6 (CH_2, C(3)OCH_2Ph), 74.7 (CH_2, C(4')OCH_2Ph), 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 $C_{66}H_{83}N_3O_8Na$ [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]

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

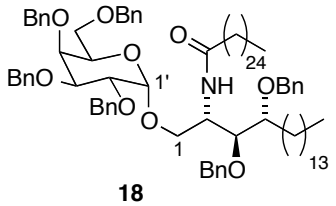


17

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_2O (3 mL) was added. The reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The residual H_2O 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]

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

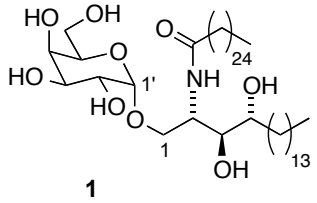


18

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 ºC. 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_2Cl_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_2Cl_2 (3.5 mL) at 0 ºC. The reaction mixture was stirred at r.t. for 8 h and then diluted with CH_2Cl_2 (20 mL), washed sequentially with $NaHCO_3$ solution (20 mL), brine (4 mL) and then dried over Na_2SO_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 ºC (lit.[20] mp 74 – 75 ºC); ν_{max}(film)/cm^{-1} 1647m (C=O); δ_H(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]

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

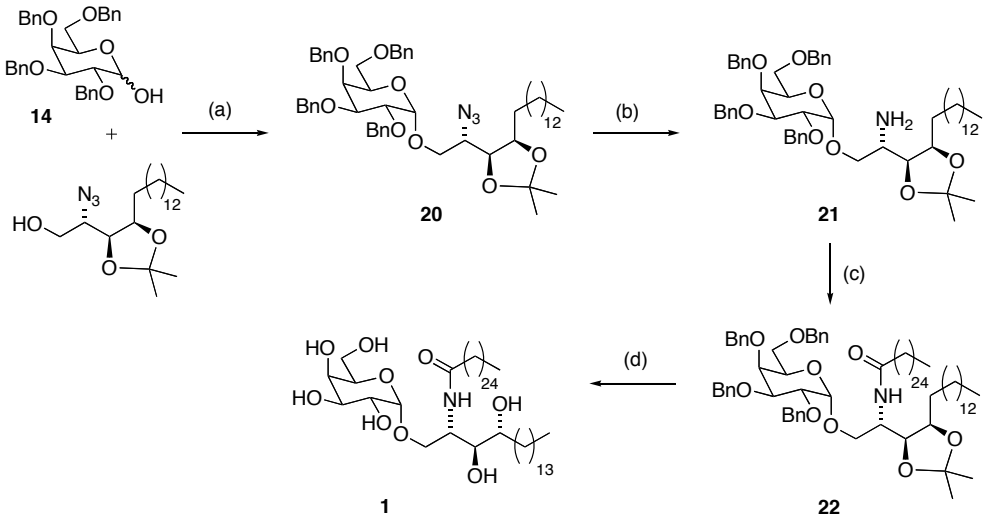


1

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 $CHCl_3$); $[\alpha]^{20}_D$ = +15.2 (*c* 1, $CHCl_3$:CH_3OH, 2:1) (lit.[23] $[\alpha]^{23}_D$ = +43.6 (*c* 1, pyridine); mp 188 – 189 °C (lit.[22] 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_2CH_3), 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

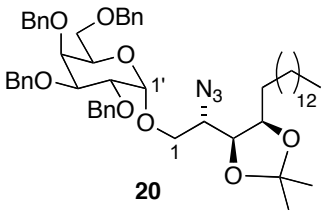
(m, 1H), 4.86 (d, *J* 3.7, 1H), O*H* and N*H* resonances not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3), 23.1 (CH_2), 26.29 (CH_2), 26.32 (CH_2), [29.8, 29.9, 30.0, 30.09, 30.13, 30.2 (CH_2, resonance overlap)], 32.4 (CH_2), 32.9 (CH_2), 36.9 (CH_2), 50.9 (CH), 62.3 (CH_2), 67.8 (CH_2), 69.4 (CH), 70.3 (CH), 70.8 (CH), 71.2 (CH), 72.5 (CH), 75.1 (CH), 100.2 (CH), 175.1 (C); MS (TOF ES+) *m/z* 880.7 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{99}NO_9Na$ [M + Na]$^+$ 880.7218, found 880.7198. Data for **1** were in agreement with those reported for this compound prepared from **22** and also with those reported in the literature.[22]

Second-Generation Approach to α-GalCer (1)



(a) (i) **14**, PPh_3, CBr_4, CH_2Cl_2, r.t., 3 h; (ii) $Me_2NC(O)NMe_2$, Bu_4NBr, CH_2Cl_2, then acceptor, CH_2Cl_2, 3 Å MS, r.t., 3 d, 71%. (b) PMe_3, THF, r.t., 3 h, then H_2O, 1 h, 93%. (c) $CH_3(CH_2)_{24}C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C to r.t., 12 h, 85%. (d) (i) TFA, CH_2Cl_2-H_2O, 10:1, 2 h, r.t.; (ii) $Pd(OH)_2$ / C, H_2, THF, 8 h, 75%.

(2*S*,3*S*,4*R*)-2-Azido-3,4-*O*-isopropylidene-1-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol (20)[22]

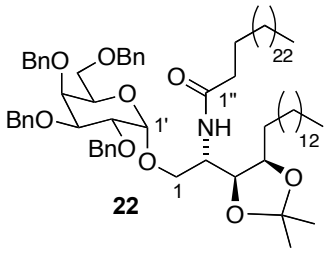


20

Glycoside **20** was prepared according a different procedure to that reported in the literature:[22] Ph_3P (1.46 g, 5.55 mmol) and CBr_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**[4,19] (1.00 g, 1.85 mmol) in CH_2Cl_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_4NBr (1.79 g, 5.55 mmol) in CH_2Cl_2 (5 mL), and a solution of (2*S*,3*S*,4*R*)-2-azido-3,4-*O*-isopropylidene-octadecane-1,3,4-triol[6,24] (1.07 g, 2.78 mmol) in CH_2Cl_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_4NBr 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_2Cl_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); $[\alpha]^{22}_D$ = +24.8 (*c* 1, $CHCl_3$); lit.[22] $[\alpha]^{r.t.}_D$ = +32.1 (*c* 2.0, $CHCl_3$); ν_{max}(film) / cm^{-1} 2099s (N_3); δ_H(500 MHz, $CDCl_3$) 0.87 (t, *J* 6.8, 3H, CH_2CH_3), 1.20-1.42 (stack, 29H, alkyl chain methylenes, $C(CH_3)_2$), 1.47-1.64 (stack, 3H), 3.44-3.54 (stack, 3H, C(6')H_2, H-2), 3.71 (dd, *J* 10.8, 6.7, 1H, C(1)H_aH_b), 3.91-3.94 (m, 1H, H-4'), 3.95-4.12 (stack, 6H, H-2', H-3', H-5', C(1)H_aH_b, H-3, H-4), 4.39 (A of AB, $J_{A\text{-}B}$ 11.9, 1H, OCH_aH$_b$Ph), 4.47 (B of AB, $J_{B\text{-}A}$ 11.9, 1H, OCH$_a$$H_b$Ph), 4.56 (A of AB, $J_{A\text{-}B}$ 11.5, 1H, OCH_aH$_b$Ph), 4.70 (A of AB, $J_{A\text{-}B}$ 12.0, 1H, OCH_aH$_b$Ph),

4.71 (A of AB, $J_{A\text{-}B}$ 11.8, 1H, OCH_aH$_b$Ph), 4.79 (B of AB, $J_{B\text{-}A}$ 12.0, 1H, OCH$_a$$H_b$Ph), 4.84 (B of AB, $J_{B\text{-}A}$ 11.8, 1H, OCH$_a$$H_b$Ph), 4.93 (d, *J* 3.6, 1H, H-1'), 4.94 (B of AB, $J_{B\text{-}A}$ 11.5, 1H, OCH$_a$$H_b$Ph), 7.22-7.33 (stack, 16H, Ph), 7.36-7.38 (stack, 4H, Ph); δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, CH_2*C*H$_3$), 22.7 (CH_2), 25.7 (CH_3, 1 × C(*C*H$_3$)$_2$), 26.6 (CH_2), 28.1 (CH_3, 1 × C(*C*H$_3$)$_2$), [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, *C*H$_2$Ph), 73.3 (CH_2, *C*H$_2$Ph), 73.4 (CH_2, *C*H$_2$Ph), 74.7 (CH_2, *C*H$_2$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 $C_{55}H_{75}N_3O_8Na$ [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]

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



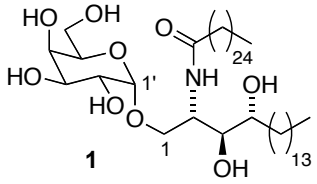
22

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 ºC. 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 CH_2Cl_2 (0.5 mL) was added dropwise over 2 min to a solution of amine **21** (132 mg, 0.15 mmol) and NEt_3 (42 μL, 0.30 mmol) in CH_2Cl_2 (1.0 mL) at 0 ºC. The reaction mixture was stirred at r.t. for 12 h and then diluted with CH_2Cl_2 (10 mL), washed sequentially with $NaHCO_3$ solution (10 mL), brine (2 mL) and then dried over Na_2SO_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 **22** as a white solid (160 mg, 85%): R_f = 0.3 (10% EtOAc in hexane); $[\alpha]^{20}_D$ = +41.6 (*c* 1, $CHCl_3$); lit.[22] $[\alpha]^{r.t.}_D$ = +44.2 (*c* 0.85, $CHCl_3$); mp 87 – 88 °C; ν_{max}(film) / cm^{-1} 1648m (C=O); δ_H(500 MHz, $CDCl_3$) 0.87 (t, *J* 6.9, 6H, CH_2CH_3), 1.15-1.34 (stack, 71H, alkyl chain methylenes, 1 × $C(CH_3)_2$), 1.39 (s, 3H, 1 × $C(CH_3)_2$), 1.40-1.47 (stack, 2H, including C(3'')H_aH_b), 1.48-1.56 (stack, 2H), 1.93-2.01 (m, 1H, C(2'')H_aH_b), 2.01-2.09 (m, 1H, C(2'')H_aH_b), 3.37 (dd, *J* 9.4, 5.7, 1H, C(6')H_aH_b), 3.54 (dd, *J* 9.4, 7.0, 1H, C(6')H_aH_b), 3.60 (br d, *J* 9.7, 1H, C(1)H_aH_b), 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)H_aH_b, H-2, H-3 or H-4), 4.36 (A of AB, J_{A-B} 11.8, 1H, C(6')OCH_aH_bPh), 4.47 (B of AB, J_{B-A} 11.8, 1H, C(6')OCH_aH_bPh), 4.57 (A of AB, J_{A-B} 11.6, 1H, C(4')OCH_aH_bPh), 4.65 (A of AB, J_{A-B} 11.5, 1H, C(2')OCH_aH_bPh), 4.73 (A of AB, J_{A-B} 11.8, 1H, C(3')OCH_aH_bPh), 4.79 (B of AB, J_{B-A} 11.5, 1H, C(2')OCH_aH_bPh), 4.80 (B of AB, J_{B-A} 11.8, 1H, C(3')OCH_aH_bPh), 4.89 (d, *J* 3.7, 1H, H-1'), 4.91 (B of AB, J_{B-A} 11.6, 1H, C(4')OCH_aH_bPh), 6.24 (d, *J* 8.9, 1H, N*H*), 7.21-7.38 (stack, 20H, Ph); δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, CH_2CH_3), 22.6 (CH_2), 25.6 (CH_2), 25.9 (CH_3, 1 × $C(CH_3)_2$), 26.5 (CH_2), 28.2 (CH_3, 1 × $C(CH_3)_2$), 28.9 (CH_2), [29.31, 29.37, 29.45, 29.55, 29.57, 29.62, 29.66, 29.68 (CH_2, alkyl chain, resonance overlap)], 31.9 (CH_2), 36.7 (CH_2, C-2''), 48.7 (CH, C-2), 69.5 (CH_2, C-6'), 69.9 (CH, C-5'), 70.6 (CH_2, C-1), 73.0 (CH_2, C(3')OCH_2Ph), 73.46 (CH_2, C(2')OCH_2Ph), 73.54 (CH_2, C(6')OCH_2Ph), 74.6 (CH_2, C(4')OCH_2Ph), 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, $(CH_3)_2C$), [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 $C_{81}H_{127}NO_9Na$ [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]

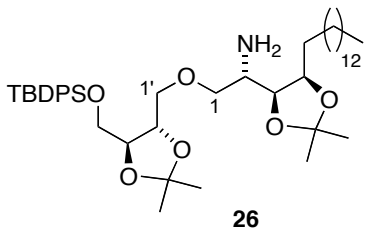
(2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(hexacosanoylamino)octadecane-1,3,4-triol (α-GalCer) (1)[21,22]



1

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 CH_2Cl_2 / H_2O (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 Et_2O (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 $CHCl_3$) 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]

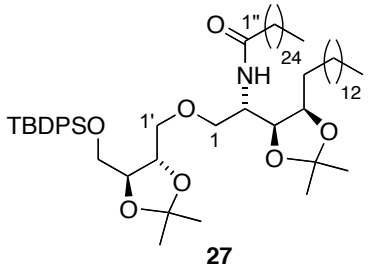
(2*S*,3*S*,4*R*)-2-Amino-1-*O*-[4'-*O*-*tert*-butyldiphenylsilyl-2',3'-*O*-isopropylidene-L-threitol]-3,4-*O*-isopropylidene-octadecane-1,3,4-triol (26)



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 (2*S*,3*S*,4*R*)-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_2O (7 mL, 15:1). The reaction mixture was stirred at r.t. for 4 h and then concentrated under reduced pressure. The residual H_2O was removed by co-evaporation with toluene (3 × 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); $[\alpha]^{20}_D$ = +45.6 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 3076w, 1113s, 1083s, 702s; δ_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); δ_C(100 MHz, $CDCl_3$): 14.1 (CH_3), 19.2 (CH_2), 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 $C_{44}H_{74}NO_6Si$ [M + H]$^+$ 740.5285, found 740.5293.

(2*S*,3*S*,4*R*)-1-*O*-[4'-*O*-*tert*-Butyldiphenylsilyl-2',3'-*O*-isopropylidene-L-threitol]-2-hexacosanoylamino-3,4-*O*-isopropylidene-octadecane-1,3,4-triol (27)

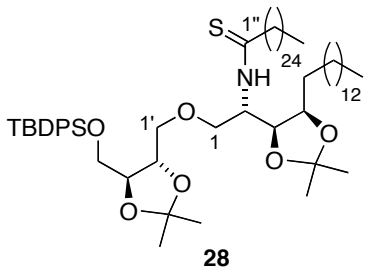


27

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 ºC 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_2Cl_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_2Cl_2 (3 mL) at 0 ºC. The reaction mixture was stirred at r.t. for 12 h and then diluted with CH_2Cl_2 (10 mL), washed sequentially with $NaHCO_3$ solution (10 mL), brine (2 mL) and then dried over Na_2SO_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); $[\alpha]^{20}_D$ = +11.8 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 1646m (C=O); δ_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); δ_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),

37.0 (CH_2), 48.2 (CH), 64.2 (CH_2), 71.3 (CH_2), 72.6 (CH_2), 76.0 (CH), 77.8 (CH), 77.9 (CH), 78.1 (CH), 107.9 (C), 109.5 (C), [127.75, 127.76, 129.79, 129.82, (CH, some resonance overlap)], 133.1 (C), [135.61, 135.62 (CH, some resonance overlap)], 172.4 (C); MS (TOF ES+) *m/z* 1140.5 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{70}H_{123}NO_7Na$ [M + Na]$^+$ 1140.8967, found 1140.8977.

(2*S*,3*S*,4*R*)-1-*O*-[4'-*O*-*tert*-Butyldiphenylsilyl-2',3'-*O*-isopropylidene-L-threitol]-2-hexacosanethioylamino-3,4-*O*-isopropylidene-octadecane-1,3,4-triol (28)

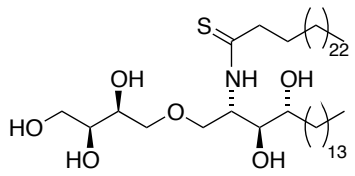


28

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 ºC for 5 h and then the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (10 mL), washed sequentially with H_2O (5 mL), $NaHCO_3$ solution (10 mL), brine (2 mL) and then dried over Na_2SO_4. 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); $[\alpha]^{20}_D$ = +36.8 (*c* 0.5, $CHCl_3$); 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 (CH_3), 22.7 (CH_2), 25.6 (CH_3), 26.7

(CH_2), 26.9 (CH_3), 27.0 (CH_3), 27.2 (CH_3), 27.7 (CH_3), 29.0 (CH_2), [29.37, 29.42, 29.56, 29.59, 29.61, 29.7 (CH_2, some resonance overlap)], 31.9 (CH_2), 47.6 (CH_2), 54.6 (CH), 64.2 (CH_2), 69.7 (CH_2), 72.6 (CH_2), 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 $C_{70}H_{123}NO_6SiSNa$ [M + Na]$^+$ 1156.8738, found 1156.8749.

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

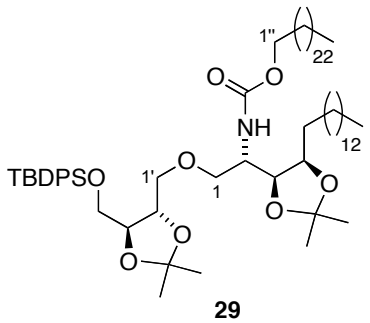


11

Bu_4F (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, NH_4Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (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 CH_2Cl_2 / CH_3OH (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 Et_2O (3 × 4 mL). Purification of the residue by flash column chromatography (5% CH_3OH in $CHCl_3$) afforded pentaol **11** as a pale yellow solid (65 mg, 73%): R_f = 0.3 (8% CH_3OH in $CHCl_3$); $[\alpha]_D$ 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, $CDCl_3$:CD_3OD, 2:1) 0.83 (t, *J* 7.0, 6H, 2 × CH_2CH_3), 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_2), 3.48–3.54 (stack, 3H, C(1')H_2, H-4), 3.55–3.63 (stack, 3H, C(4')H_2, H-3'), 3.64–3.72 (stack, 2H, C(1)H_aH$_b$, H-3), 3.74–78 (m, 1H, H-2'), 3.80–3.86 (m, 1H, C(1)H$_a$$H_b$), 4.83–4.88 (m, 1H, H-2); δ_C(125 MHz, $CDCl_3$:CD_3OD, 2:1) 14.2 (CH_3, 2 × CH_2CH_3), 22.9 (CH_2), 26.2 (CH_2), 29.3 (CH_2), [29.6, 29.7, 29.8, 30.0 (CH_2, alkyl chain, resonance overlap)], 32.2 (CH_2), 32.7 (CH_2, C-5), 46.9 (CH_2, C-2"), 56.1 (CH, C-2), 63.7 (CH_2, C-4'), 69.6 (CH_2, C-1), 70.6 (CH, C-2'), 72.2 (CH, C-3'), 73.0 (CH_2, C-4), 73.2 (CH_2, 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 $C_{50}H_{99}NO_8SNa$ [M + Na]$^+$ 838.6934, found 838.6946.

(2*S*,3*S*,4*R*)-1-*O*-[4'-*O*-*tert*-Butyldiphenylsilyl-2',3'-*O*-isopropylidene-L-threitol]-3,4-*O*-isopropylidene-2-(tetracosanyloxycarbonylamino)octadecane-1,3,4-triol (29)

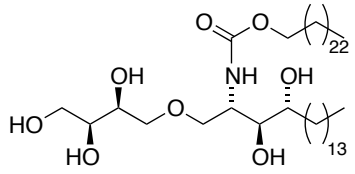


29

A solution of carbonic acid, 2,5-dioxo-1-pyrrolidinyl tetracosanyl ester (110 mg, 0.21 mmol) in CH_2Cl_2 (0.5 mL) was added to a stirred solution of amine **26** (104 mg, 0.14 mmol) and NEt_3 (42 μL, 0.3 mmol) in CH_2Cl_2 (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 CH_2Cl_2 (10 mL) and washed sequentially with $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4 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); $[\alpha]^{22}_D$ = +28.8 (*c* 0.5, $CHCl_3$); ν_{max}(film) / cm^{-1} 1687m (C=O); δ_H(500 MHz, $CDCl_3$) 0.88 (t, *J* 7.1, 6H, 2 × CH_2CH_3), 1.06 (s, 9H, C(CH_3)$_3$), 1.26–1.34 (stack,

70H, alkyl chain methylenes), 1.40 (s, 6H, C(CH_3)$_2$), 1.42 (s, 3H, C(CH_3)$_2$), 1.59 (s, 3H, C(CH_3)$_2$), 3.52–3.65 (stack, 3H, C(1)H_aH$_b$, C(1')H_aH_b), 3.74–3.81 (stack, 3H, C(4')H_aH_b, C(1)H$_a$$H_b$), 3.82–3.90 (stack, 2H, H-3', H-2), 3.98 (m, 1H, C(1")H_aH$_b$), 4.00–4.11 (stack, 3H, H-3, H-4, C(1")H$_a$$H_b$), 4.14–4.20 (m, 1H, H-2'), 4.97 (br d, *J* 9.5, 1H, N*H*), 7.36–7.46 (stack, 6H, Ph), 7.66–7.70 (stack, 4H, Ph); δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, C-18, C-24"), 19.2 (C, (CH_3)$_3$*C*Si), 22.7 (CH_2), 25.8 (CH_3, C(*C*H$_3$)$_2$), 25.9 (CH_2), 26.4 (CH_2), 26.8 (CH_3, C(*C*H$_3$)$_3$), 27.0 (CH_3, C(*C*H$_3$)$_2$), 27.2 (CH_3, C(*C*H$_3$)$_2$), 28.1 (CH_3, C(*C*H$_3$)$_2$), 28.9 (CH_2), 29.1 (CH_2), 29.4 (CH_2), [29.6, 29.7 (CH_2, alkyl chains, some resonance overlap)], 31.9 (CH_2), 50.3 (CH, C-2), 64.1 (CH_2, C-4'), 65.2 (CH_2, C-1"), 71.6 (CH_2, C-1), 72.6 (CH_2, 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, (CH_3)$_2$*C*), 109.4 (C, (CH_3)$_2$*C*), [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 $C_{69}H_{121}NO_8SiNa$ [M + Na]$^+$ 1142.8759, found 1142.8767.

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

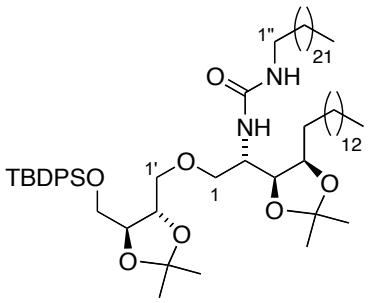


13

Bu_4F (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, NH_4Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (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 CH_2Cl_2 / CH_3OH (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 Et_2O (3 × 4 mL) to provide the crude product, which was purified by flash column chromatography (10% CH_3OH in $CHCl_3$) to afford pentaol **13** as a pale yellow solid (90 mg, 70%): R_f = 0.3 (10% CH_3OH in $CHCl_3$); $[\alpha]_D$ 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, $CDCl_3$:CD_3OD, 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), O*H* resonances not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.2 (CH_3), 20.1 (CH_2), 23.0 (CH_2), 24.1 (CH_2), 26.2 (CH_2), [29.5, 29.7, 30.0, 30.4, (CH_2, some resonance overlap)], 32.3 (CH_2), 32.9 (CH_2), 52.1 (CH), 63.9 (CH_2), 65.8 (CH_2), 70.8 (CH), 71.2 (CH_2), 72.5 (CH), 72.8 (CH), 73.4 (CH_2), 75.5 (CH), 157.7 (C); MS (TOF ES+) *m/z* 824.8 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{99}NO_8SNa$ [M + Na]$^+$ 809.6955, found 824.6940.

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

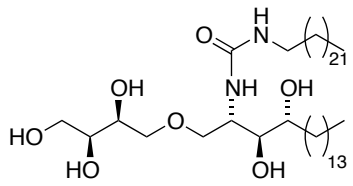


30

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 **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, $CHCl_3$); ν_{max}(film) / cm^{-1} 1632m (C=O); δ_H(500 MHz, $CDCl_3$) 0.88 (t, *J* 7.1, 6H, 2 × CH_2CH_3), 1.06 (s, 9H, $C(CH_3)_3$), 1.21–1.32 (stack, 68H, alkyl chain methylenes), 1.39 (s, 3H, 1 × $C(CH_3)_2$), 1.40 (s, 3H, 1 × $C(CH_3)_2$), 1.41 (s, 3H, 1 × $C(CH_3)_2$), 1.61 (s, 3H, 1 × $C(CH_3)_2$), 3.03–3.17 (stack, 2H, C(1")H_aH_b), 3.53–3.60 (stack, 2H, C(1')H_aH_b, C(1)H_aH_b), 3.65 (dd, *J* 10.5, 3.2, 1H, C(1')H_aH_b), 3.72–3.80 (stack, 3H, C(4')H_aH_b, C(1)H_aH_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_2NH), 4.49 (br d, *J* 9.2, 1H, CHNH), 7.37–7.46 (stack, 6H, Ph), 7.64–7.70 (stack, 4H, Ph); δ_C(125 MHz, $CDCl_3$) 14.1 (CH_3, 2 × CH_2CH_3), 19.2 (C, $C(CH_3)_3$), 22.7 (CH_2), 25.8 (CH_3, 1 × $C(CH_3)_2$), 26.4 (CH_2), 26.8 (CH_3, $C(CH_3)_3$), 26.9 (CH_2), 27.1 (CH_3, 1 × $C(CH_3)_2$), 27.2 (CH_3, 1 × $C(CH_3)_2$), 28.1 (CH_3, 1 × $C(CH_3)_2$), 29.1 (CH_2), [29.4, 29.7 (CH_2, alkyl chain, resonance overlap)], 30.2 (CH_2), 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 $C_{68}H_{120}N_2O_7SiNa$ $[M + Na]^+$ 1127.8763, found 1127.8737.

(2*S*,3*S*,4*R*)-1-*O*-[L-Threitol]-2-(tricosanylaminocarbonylamino)octadecane-1,3,4-triol (12)

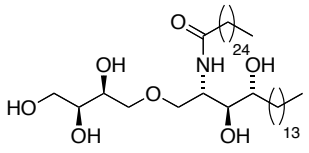


12

Bu_4F (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, NH_4Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (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 CH_2Cl_2 / CH_3OH (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 Et_2O (3 × 4 mL) to provide the crude product, which was purified by flash column chromatography (10% CH_3OH in $CHCl_3$) to afford the pentaol **12** as a white solid (85 mg, 72%): R_f = 0.3 (10% CH_3OH in $CHCl_3$); $[\alpha]_D$ the insolubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; mp 131 – 132 °C; ν_{max}(film) / cm^{-1} 3344s br (O–H), 1607s (C=O); δ_H(400 MHz, $CDCl_3$:CD_3OD, 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), O*H* and N*H* resonances not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3), 23.2 (CH_2), 26.4 (CH_2), 27.4 (CH_2), [29.8, 29.9, 30.1, 30.2, 30.3 (CH_2, resonance overlap)], 30.7 (CH_2), 32.4 (CH_2), 33.5 (CH_2), 40.7 (CH_2), 51.2 (CH), 64.1 (CH_2), 70.7 (CH), 72.1 (CH_2), 72.5 (CH), 73.5 (CH), 73.9 (CH_2), 75.9 (CH), 159.9 (C); MS (TOF ES+) *m/z* 809.8 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{99}NO_8SNa$ $[M + Na]^+$. 809.6959, found 809.6950.

The synthesis of ThrCer (**2**) has been reported previously;[6,25] however the literature routes employ slightly different protecting group strategies. The synthesis of **2** from **27** is therefore described below.

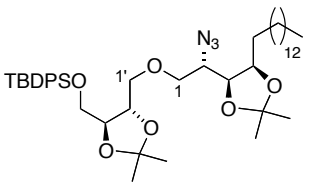
(2*S*,3*S*,4*R*)-1-*O*-[L-Threitol]-2-(hexacosanoylamino)octadecane-1,3,4-triol (2)[6]



2

Bu_4F (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, NH_4Cl solution (10 mL) was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_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 CH_2Cl_2 / CH_3OH (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 Et_2O (3 × 4 mL). Purification of the residue by flash column chromatography (5% CH_3OH in $CHCl_3$) afforded ThrCer **2** as a white yellow solid (65 mg, 74%). Data for **2** were in agreement with those reported in the literature.[6]

(2*S*,3*S*,4*R*)-2-Azido-1-*O*-[4'-*O*-*tert*-butyldiphenylsilyl-2',3'-*O*-isopropylidene-L-threitol]-3,4-*O*-isopropylidene-octadecane-1,3,4-triol

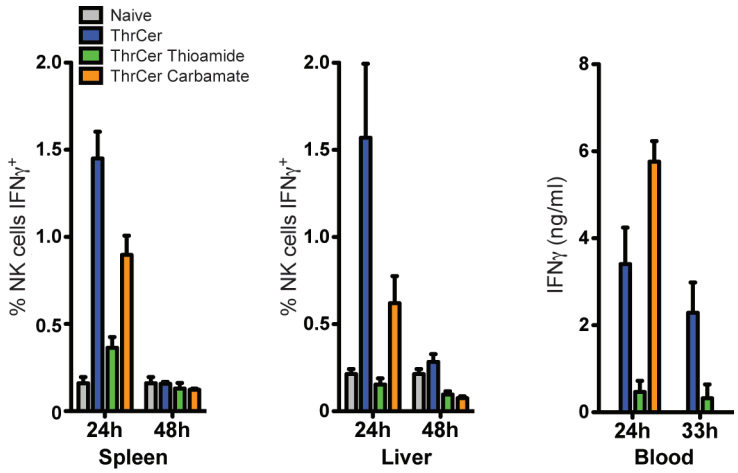


Tf_2O (235 μL, 1.40 mmol) was added dropwise over 10 min to a solution of 1-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene-L-threitol[26,27] (561 mg, 1.40 mmol) and 2,6-di-*tert*-butylpyridine (346 μL, 1.54 mmol) in CH_2Cl_2 (14 mL) at 0 °C. After 30 min, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and the resulting solution washed sequentially with cold H_2O (2 × 30 mL) and brine (10 mL), dried (Na_2SO_4) 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 [R_f = 0.7 (15% EtOAc in hexanes)], which was used immediately in the next etherification step: A solution of (2*S*,3*S*,4*R*)-2-azido-3,4-*O*-isopropylidene-octadecane-1,3,4-triol[6] (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 ºC. 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 $NaHCO_3$ solution (10 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic fractions were washed with brine (15 mL) and dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (eluent: 5% EtOAc in hexanes) to provide (2*S*,3*S*,4*R*)-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%): R_f = 0.6 (10% EtOAc in

hexane); $[\alpha]^{21}_{D} = +10.0$ (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 2098s (N_3); δ_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); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 19.2 (C), 22.7 (CH_2), 25.7 (CH_3), 26.4 (CH_2), 26.8 (CH_3), 27.0 (CH_3), 27.1 (CH_3), 28.2 (CH_3), [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 (CH), 129.7 (CH), 133.2 (C), 135.6 (CH); MS (TOF ES+) *m/z* 788.7 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{44}H_{71}N_3O_6SiNa$ $[M + Na]^+$ 788.5010, 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.[28.] 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, TCRb, 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.



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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