SUPPORTING INFORMATION

PART 1

Synthesis of a Versatile Building Block for the Preparation of 6-*N*-Derivatized α-Galactosyl Ceramides: Rapid Access to Biologically Active Glycolipids

Peter J. Jervis,[†] Liam R. Cox,[‡,] Gurdyal S. Besra[†,]**

[‡]School of Chemistry, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom. [†] School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

Table of Contents

Supporting Information Part 1 (S1-S24) [General experimental details, additional experimental procedures and characterization data, References]

Supporting Information Part 2 (S25-S56) [Scanned ^{1}H NMR and ^{13}C NMR spectra]

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 $CDCl_3$ unless specified otherwise, 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; C_6H_6 δ_H 7.26; C_6D_6, δ_C 128.6; 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 region where resonances arising from a single nucleus (or equivalent nuclei) are coincident but 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 $J_{A\text{-}B}$. In analyzing ABX (and similar) systems, where the resonance pattern forms two, clearly separated groups of lines (two sets of four lines for an ABX system), these are reported as “A of ABX” and “B of ABX”, along with $J_{A\text{-}B}$, which can be directly measured from the spectrum. Whilst $J_{A\text{-}X}$ cannot strictly be measured directly from the spectrum, the value obtained from the spectrum is sufficiently close to the actual value for it still to be useful; however it is acknowledged that the value quoted for $J_{A\text{-}X}$ is not the true value. 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*

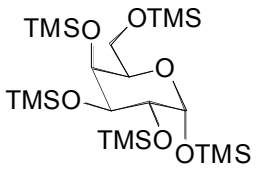
(%)). HRMS were recorded on a LCT spectrometer using a lock mass incorporated into the mobile phase. Melting points were determined using open capillaries and are uncorrected.

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).

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).

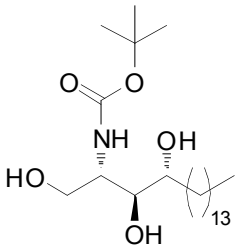
All reagents were obtained from commercial sources and used without further purification unless specified otherwise. CH_2Cl_2 was freshly distilled under N_2 from CaH_2. THF were freshly distilled under N_2 from sodium benzophenone ketyl. All solutions are aqueous and saturated unless specified otherwise. Pyridine was distilled from KOH and stored over 4 Å molecular sieves.

Trimethylsilyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-α-D-galactopyranoside (**13**) [1,2]



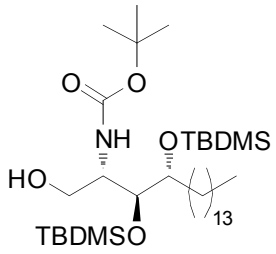
Hexamethydisilazane (HMDS) (100 mL, 0.480 mol) and TMSCl (50 mL, 0.39 mol) were added sequentially to a solution of D-galactose (10.0 g, 55.5 mmol) in pyridine (500 mL). The solution was stirred at 75 ºC for 1 h under an Ar atmosphere and then cooled to rt. The mixture was poured into ice-water (500 mL) and extracted with hexane (3 × 300 mL). The combined organic extracts were washed with H_2O (3 × 300 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford per-silylated galactose **13** as a viscous, colorless oil (94%): R_f = 0.25 (4% EtOAc in hexane); $[\alpha]^{20}_D$ +69.7 (*c* 0.50, $CHCl_3$) (lit.[1] +66.2 (*c* 0.37, $CHCl_3$)); ν_{max}(film)/cm^{-1} 2957s, 2933s, 2874s, 1242s; δ_H(300 MHz) 0.10 (s, 9H), 0.11 (s, 9H), 0.12 (s, 9H), 0.13 (s, 9H), 0.14 (s, 9H), 3.53 (A of ABX, $J_{A\text{-}B}$ 9.5, $J_{A\text{-}X}$ 5.7, 1H), 3.62 (B of ABX, $J_{B\text{-}A}$ 9.5, $J_{B\text{-}X}$ 7.5, 1H), 3.79-3.83 (stack, 2H), 3.87-3.94 (stack, 2H), 5.04 (d, *J* 1.8, 1H); δ_C(75 MHz) –0.5 (CH_3), 0.2 (CH_3), 0.3 (CH_3), 0.4 (CH_3), 0.6 (CH_3), 61.2 (CH_2), 70.0 (CH), 70.5 (CH), 71.1 (CH), 72.3 (CH), 94.6 (CH); MS (TOF ES+) *m*/*z* 564.1 ($[M + Na]^+$, 100%).

(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]octadecane-1,3,4-triol (**18**) [3-5]



NEt_3 (5.30 mL, 37.8 mmol) and Boc_2O (7.60 g, 33.0 mmol) were added sequentially to a vigorously stirred suspension of phytosphingosine **17** (10.0 g, 31.5 mmol) in THF (250 mL). After 18 h, the reaction mixture was concentrated under reduced pressure. Recrystallization of the residue from EtOAc (200 mL) yielded carbamate **18** as white crystals (12.5 g, 88%): R_f = 0.50 (50% EtOAc in hexane); mp 86–88 °C (lit.[3] 89.2–90.4 °C); $[\alpha]^{20}_D$ +10.2 (*c* 1.00, $CHCl_3$) (lit.[4] +7.9 (*c* 1.00, $CHCl_3$)); ν_{max}(film)/cm^{-1} 3262s br (O–H), 2918s, 2851s, 1684s (C=O); δ_H(300 MHz) 0.87 (t, *J* 6.9, 3H), 1.19-1.37 (stack, 22H), 1.44 (s, 9H), 1.44-1.76 (stack, 4H), 3.59-3.75 (stack, 3H), 3.76-3.92 (stack, 2H), 5.43 (d, *J* 7.8, 1H), O*H* resonances not observed; δ_C(75 MHz) 14.1 (CH_3), 22.7 (CH_2), 25.9 (CH_2), 28.4 (CH_3), [29.3, 29.6, 29.7, 31.9, 33.1 (CH_2, resonance overlap)], 53.0 (CH), 62.0 (CH_2), 72.9 (CH), 76.9 (CH), 80.1 (C), 156.5 (C); MS (TOF ES+) *m*/*z* 440.3 ([M + Na]$^+$, 100%).

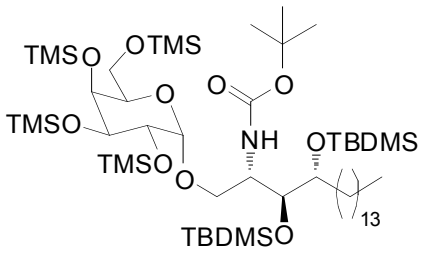
(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]-3,4-di-*tert*-butyldimethylsilyloxy-octadecan-1-ol (**16**) [5,6]



A solution of triol **18** (12.0 g, 28.8 mmol) in CH_2Cl_2 (400 mL) at 0 °C was treated sequentially with TBDMSOTf (33.0 mL, 144.7 mmol) and 2,6-lutidine (50 mL). The reaction mixture was stirred at 0 °C for 30 min, and then warmed to 25 °C and stirred at this temperature for 20 h, after which time, CH_3OH (100 mL) was added and stirring continued for 10 min. The solvent was then removed under reduced pressure and the residue taken up in Et_2O (200 mL) and washed sequentially with H_2O (200 mL), $NaHCO_3$ solution (200 mL) and brine (200 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to give a residue which was purified by flash chromatography to afford trisilyl ether **19** as a colorless oil (17.95 g, 82%), which was used directly in the next step. A solution of **19** (17.95 g, 22.7 mmol) in THF (350 mL) under a N_2 atmosphere at 0 °C was treated with HF·pyridine (4.10 mL of a 70% solution, 158.0 mmol) in THF–pyridine (25 mL, 65:35). The reaction mixture was stirred for 30 min at 0 °C and then allowed to warm to rt. After 1 h, the mixture was quenched by the addition of $NaHCO_3$ solution (15 mL) and stirred for 10 min. The reaction mixture was extracted with EtOAc (2 × 200 mL) and the organic phases were washed with brine (200 mL) and then filtered. Removal of the volatiles under reduced pressure and purification of the residue by flash column chromatography afforded primary alcohol **16**

as a colorless oil (12.14 g, 86%): R_f = 0.27 (10% EtOAc in hexane); $[\alpha]^{20}_D$ –8.3 (*c* 1.00, $CHCl_3$) (lit.[6] –11.6 (*c* 1.93, $CHCl_3$)); ν_{max}(film)/cm^{-1} 3445s br (O–H), 2926s, 2855s, 1697s (C=O); δ_H(300 MHz) 0.08 (s, 6H), 0.11 (s, 6H), 0.88 (t, *J* 6.9, 3H), 0.90 (s, 9H), 0.92 (s, 9H), 1.20-1.48 (stack, 22H), 1.43-1.61 (stack, 13H, including [1.44 (s, 9H)]), 2.85 (br s, 1H), 3.62 (dd, *J* 11.1, 3.9, 1H), 3.69-3.80 (stack, 2H), 3.82-3.86 (m, 1H), 4.12 (dd, *J* 11.4, 2.4, 1H), 5.23 (d, *J* 8.4, 1H); δ_C(75 MHz) –5.1 (CH_3), –4.6 (CH_3), –4.1 (CH_3), 3.8 (CH_3), 14.1 (CH_3), 18.2 (C), 22.7 (CH_2), 25.8 (CH_2), 25.95 (CH_3), 26.03 (CH_3), 28.4 (CH_3), [29.3, 29.6, 29.7, 31.9, 34.1 (CH_2, resonance overlap)], 52.2 (CH), 63.4 (CH_2), 75.4 (CH), 77.4 (CH), 79.2 (C), 155.4 (C); MS (TOF ES+) *m*/*z* 645.5 ($[M + Na]^+$, 100%).

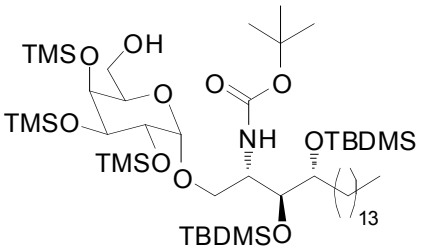
(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]-3,4-di-*tert*-butyldimethylsilyloxy-1-*O*-(2,3,4,6-tetrakis-*O*-trimethylsilyl-α-D-galactopyranosyl)octadecane (**20**)



TMSI (1.42 mL, 10.5 mmol) was added to a solution of per-silylated galactose **13** (5.70 g, 10.5 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The reaction mixture was stirred under an Ar atmosphere for 15 min before benzene (40 mL) was added. The solvent was removed under reduced pressure and the resulting glycosyl iodide intermediate **15** was dissolved in CH_2Cl_2 (40 mL) and kept under an Ar atmosphere. In a separate flask, a mixture of activated 4 Å molecular sieves (7.20 g), *n*-Bu_4NI (7.76 g, 21.0 mmol), *i*-Pr_2NEt (6.9 mL,

15.8 mmol) and alcohol **16** (2.26 g, 3.50 mmol) in CH_2Cl_2 (40 mL) was prepared and stirred under an Ar atmosphere at rt for 15 min. The solution of glycosyl iodide **15** in CH_2Cl_2 was then added dropwise over 5 min to this mixture and the resulting mixture was stirred overnight. After removal of the solvent under reduced pressure, Et_2O (50 mL) and H_2O (50 mL) were added and the phases were separated. The organic phase was dried ($MgSO_4$) and then concentrated under reduced pressure. The resulting yellow solid was purified by flash column chromatography (3% EtOAc in hexane) to afford glycoside **20** as a colorless oil (3.26 g, 75%, α-anomer only): R_f = 0.26 (3% EtOAc in hexane); $[\alpha]^{20}_D$ +32.1 (*c* 0.50, $CHCl_3$); ν_{max}(film)/cm^{-1} 2972s, 2901s, 1719m (C=O); δ_H(300 MHz) 0.04 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.098 (s, 3H), 0.104 (s, 9H), 0.11 (s, 9H), 0.14 (s, 9H), 0.15 (s, 9H), 0.88 (t, *J* 6.9, 3H), 0.89 (s, 9H), 0.90 (s, 9H), 1.20-1.36 (stack, 22H), 1.40-1.55 (stack, 4H), 1.49 (s, 9H), 3.53 (A of ABX, $J_{A\text{-}B}$ 9.0, $J_{A\text{-}X}$ 5.4, 1H), 3.58-3.81 (stack, 7H), 3.82-3.88 (stack, 3H), 4.66 (d, *J* 3.6, 1H), 5.19 (d, *J* 5.1, 1H); δ_C(100 MHz) –4.8 (CH_3), –4.7 (CH_3), –3.8 (CH_3), –3.2 (CH_3), –0.5 (CH_3), 0.2 (CH_3), 0.5 (CH_3), 0.6 (CH_3), 14.1 (CH_3), 18.2 (C), 18.4 (C), 22.7 (CH_2), 25.9 (CH_2), 26.1 (CH_3), 26.2 (CH_3), 28.4 (CH_3), [29.4, 29.7, 29.9, 31.9, 33.0 (CH_2, resonance overlap)], 52.7 (CH), 60.4 (CH_2), 68.7 (CH_2), 69.5 (CH), 70.8 (CH), 71.5 (CH), 71.9 (CH), 75.4 (CH), 75.6 (CH), 78.6 (C), 101.9 (CH), 155.7 (C); MS (TOF ES+) *m*/*z* 1118.5 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{53}H_{117}NO_{10}Si_6Na$ [M + Na]$^+$ 1118.7191, found 1118.7189.

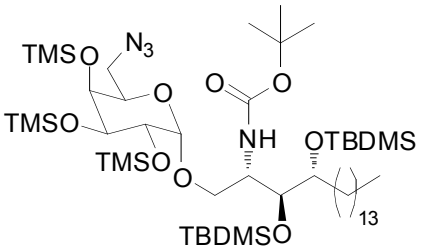
(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]-3,4-di-*tert*-butyldimethylsilyloxy-1-*O*-(2,3,4-tri-*O*-trimethylsilyl-α-D-galactopyranosyl)octadecane (**21**)



AcOH (0.17 mL, 3.0 mmol) was added to a solution of galactoside **20** (1.72 g, 1.57 mmol) in acetone (3.50 mL) and MeOH (4.70 mL) at 0 °C. The reaction mixture was allowed to warm to rt. After 8 h, the reaction was quenched by the addition of $NaHCO_3$ (0.50 g, 5.95 mmol) and then filtered. Concentration of the filtrate under reduced pressure and purification of the resulting oil by flash column chromatography (6% EtOAc in hexane) afforded alcohol **21** as a colorless oil (1.32 g, 78%): R_f = 0.28 (6% EtOAc in hexane); $[\alpha]^{20}_D$ +22.8 (*c* 0.50, $CHCl_3$); ν_{max}(film)/cm^{-1} 3442s br, 2956s, 2925s, 2855s, 1697m (C=O); δ_H(300 MHz) 0.04 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.10 (s, 3H), 0.11 (s, 9H), 0.14 (app. s, 18H), 0.88 (t, *J* 7.2, 3H), 0.89 (s, 9H), 0.91 (s, 9H), 1.20-1.35 (stack, 22H), 1.39-1.63 (stack, 4H), 1.51 (s, 9H), 3.56-3.65 (m, 1H), 3.66-3.73 (m, 1H), 3.74-3.80 (stack, 5H), 3.81-3.86 (m, 1H), 3.87-3.95 (stack, 3H), 4.76 (d, *J* 3.6, 1H), 5.23 (d, *J* 7.2, 1H), O*H* not observed; δ_C(100 MHz) –5.0 (CH_3), –4.7 (CH_3), –3.9 (CH_3), –3.7 (CH_3), 0.2 (CH_3), 0.5 (CH_3), 0.6 (CH_3), 14.1 (CH_3), 18.2 (C), 18.3 (C), 22.7 (CH_2), 26.0 (CH_3), 26.2 (CH_3), 28.4 (CH_3), [29.6, 29.7, 30.1, 31.8, 31.9, 33.1 (CH_2, resonance overlap)], 52.8 (CH), 62.9 (CH_2), 69.4 (CH), 70.85 (CH_2), 70.90 (CH), 71.9 (CH), 73.4 (CH), 75.3 (CH), 76.3 (CH), 79.1 (C), 103.0 (CH), 155.7 (C); MS (TOF ES+) *m*/*z* 1046.7

([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{109}NO_{10}Si_5Na$ [M + Na]$^+$ 1046.6796, found 1046.6818.

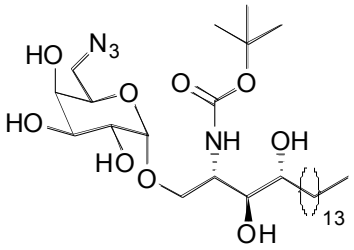
(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]-3,4-di-*tert*-butyldimethylsilyloxy-1-*O*-(6-deoxy-6-azido-2,3,4-tri-*O*-trimethylsilyl-α-D-galactopyranosyl)octadecane (**22**)



PPh_3 (490 mg, 1.86 mmol), followed by DIAD (365 μL, 1.86 mmol) and then DPPA (400 μL, 1.86 mmol) were added sequentially to a solution of alcohol **21** (950 mg, 0.90 mmol) in THF (20 mL) at 0 °C. The mixture was allowed to warm to rt and then stirred overnight. Concentration under reduced pressure followed by purification of the residue by flash column chromatography (2% EtOAc in hexane) afforded azide **22** as a colorless oil (821 mg, 87%): R_f = 0.23 (2% EtOAc in hexane); $[\alpha]^{20}_D$ –8.1 (*c* 0.50, $CHCl_3$); ν_{max}(film)/cm^{-1} 2916s, 2847s, 2116s (N_3), 1699s (C=O); δ_H(300 MHz) 0.05 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.10 (s, 3H), 0.11 (s, 9H), 0.15 (s, 9H), 0.16 (s, 9H), 0.88 (t, *J* 6.9, 3H), 0.898 (s, 9H), 0.903 (s, 9H), 1.19-1.37 (stack, 22H), 1.38-1.59 (stack, 4H), 1.43 (s, 9H), 3.30 (A of ABX, $J_{A\text{-}B}$ 12.0, $J_{A\text{-}X}$ 6.9, 1H), 3.47 (B of ABX, $J_{B\text{-}A}$ 12.0, $J_{B\text{-}X}$ 6.9, 1H), 3.76-3.94 (stack, 9H), 4.68 (d, *J* 3.3, 1H), 5.14 (d, *J* 5.4, 1H); δ_C(100 MHz) –4.8 (CH_3), –4.7 (CH_3), –3.9 (CH_3), –3.8 (CH_3), 0.2 (CH_3), 0.5 (CH_3), 0.6 (CH_3), 14.1 (CH_3), 18.2 (C), 18.4 (C), 22.7 (CH_2), 26.0 (CH_2), 26.1 (CH_3), 26.2 (CH_3), 28.4 (CH_3), [29.4,

29.7, 29.9, 31.9, 33.0 (CH_2, resonance overlap)], 51.1 (CH_2), 52.7 (CH), 69.1 (CH), 69.5 (CH_2), 70.2 (CH), 70.5 (CH), 72.7 (CH), 75.5 (CH), 75.7 (CH), 78.7 (C), 102.0 (CH), 155.6 (C); MS (TOF ES+) *m*/*z* 1071.8 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{108}N_4O_9Si_5Na$ [M + Na]$^+$ 1071.6860, found 1071.6873.

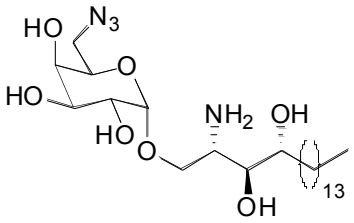
(2*S*, 3*S*, 4*R*)-2-[(*N*-*tert*-Butoxycarbonyl)amino]-1-*O*-(6-deoxy-6-azido-α-D-galactopyranosyl)octadecane-3,4-diol (**9**)



TFA (0.50 mL, 6.62 mmol) was added dropwise over 5 min to a solution of azide **22** (200 mg, 0.19 mmol) in CH_2Cl_2 (5.0 mL) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure to afford pentaol **9** as a colorless oil (115 mg, quant.): R_f = 0.22 (15% $CHCl_3$ in MeOH); $[\alpha]^{20}_D$ +36.3 (*c* 0.50, $CHCl_3$); ν_{max}(film)/cm^{-1} 3282s br (O–H), 2916s, 2847s, 2114s (N_3), 1696m (C=O); δ_H(300 MHz, $CDCl_3$:CD_3OD, 2:1) 0.85 (t, *J* 6.0, 3H), 1.18-1.39 (stack, 22H), 1.40-1.71 (stack, 4H), 1.44 (s, 9H), 3.26 (A of ABX, $J_{A\text{-}B}$ 12.6, $J_{A\text{-}X}$ 4.9, 1H), 3.51-3.64 (stack, 3H), 3.65-3.98 (stack, 7H), 4.89 (d, *J* 3.3, 1H), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3), 23.2 (CH_2), 26.4 (CH_2), 28.6 (CH_3), 51.7 (CH), 51.8 (CH_2), 68.3 (CH_2), 69.3 (CH), 70.4 (CH), 70.6 (2 × CH, resonance overlap), 72.5 (CH), 75.3 (CH), 80.1 (C), 100.2

(CH), 156.8 (C); MS (TOF ES+) *m*/*z* 627.3 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{29}H_{56}N_4O_9Na$ [M + Na]$^+$ 627.3945, found 627.3956.

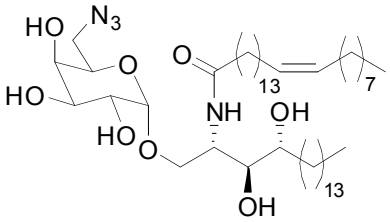
(2*S*, 3*S*, 4*R*)-2-Amino-1-*O*-(6-deoxy-6-azido-α-D-galactopyranosyl)octadecane-3,4-diol (**10**)



From azide 22: TFA (1.0 mL, 13.2 mmol) was added dropwise over 5 min to azide **22** (400 mg, 0.38 mmol) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure. The resulting colorless oil was used in the next step without further purification (192 mg, quant.).

From azide 9: TFA (0.50 mL, 13.2 mmol) was added dropwise over 5 min to azide **9** (114 mg, 0.19 mmol) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure. The resulting colorless oil was used in the next step without further purification (96 mg, quant.).

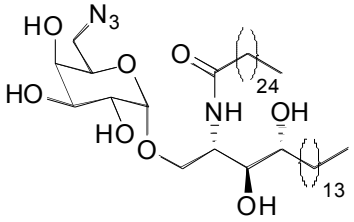
(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-azido-α-D-galactopyranosyl)-2-[(*N*-nervonoyl)amino]octadecane-3,4-diol (**23**)



A solution of nervonic acid (33 mg, 0.09 mmol) in $(COCl)_2$ (2.0 mL) was stirred at 70 °C for 2 h, after which time, the solution was cooled to rt, and the $(COCl)_2$ was removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (1.0 mL) and added, with vigorous stirring, to a solution of amine **10** (40 mg, 0.08 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (1:1, 2.0 mL). Vigorous stirring was maintained for 2 h, after which time, the mixture was left to stand and the phases were separated. The aqueous phase was extracted with THF (2 × 2.0 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (gradient: $CHCl_3$ to 15% MeOH in $CHCl_3$) afforded amide **23** as a white solid (55 mg, 81%): R_f = 0.20 (15% $CHCl_3$ in MeOH); mp 162–164 °C; the very poor solubility of this amphiphilic compound at rt prevented us from obtaining reliable optical rotation data; ν_{max}(film)/cm^{-1} 3402s br, 3095w, 2919s, 2103s (N_3), 1736s (C=O), 1639m (C=C); δ_H(400 MHz, $CDCl_3$:CD_3OD, 2:1) 0.85 (app. t, *J* 6.8, 6H, 2 × CH_2CH_3), 1.18-1.41 (stack, 56H, alkyl chain), 1.45-1.69 (stack, 4H, alkyl chain), 2.00 (app. q, *J* 6.1, 4H, CH_2CH=$CHCH_2$), 2.20 (stack, 2H, $NHCOCH_2$), 3.26 (dd, *J* 12.8, 4.4, 1H, 6-H_a), 3.49-3.60 (stack, 3H, 4-*H*, 6-H_b, 4'-*H*), 3.63-3.81 (stack, 4H, 2-*H*, 3-*H*, 1'-H_a, 3'-*H*), 3.83-3.93 (stack, 2H, 5-*H*, 1'-

H_b), 4.12-4.20 (m, 1H, 2'-*H*), 4.89 (d, *J* 4.0, 1H, 1-*H*), 5.20-5.40 (stack, 2H, C*H*=C*H*), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3, 2 × CH_2CH_3), [23.1, 26.4, 27.6, 29.7, 29.8, 29.9, 30.0, 30.1, 32.4, 32.6 (CH_2, alkyl chain, resonance overlap)], 36.9 (CH_2, NHCOCH_2), 50.6 (CH, *C*-2'), 51.7 (CH_2, *C*-6), 67.9 (CH_2, *C*-1'), 69.1 (CH), 70.2 (CH), 70.5 (2 × CH, including *C*-5, resonance overlap), 72.5 (CH), 74.8 (CH), 100.1 (CH, *C*-1), 130.3 (CH, 2 × *C*H=*C*H), 174.8 (C, *C*=O); MS (TOF ES+) *m*/*z* 875.8 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{48}H_{92}N_4O_8Na$ [M + Na]$^+$ 875.6813, found 875.6839.

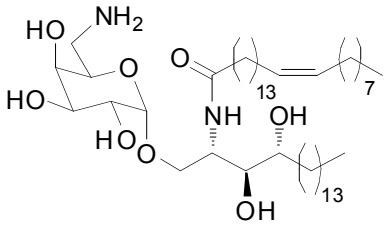
(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-azido-α-D-galactopyranosyl)-2-[(*N*-hexacosanoyl)amino]octadecane-3,4-diol (**24**)



A solution of hexacosanoic acid (44 mg, 0.11 mmol) in $(COCl)_2$ (2.00 mL) was stirred at 70 °C for 2 h, after which time, the solution was cooled to rt, and the $(COCl)_2$ was removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (1.25 mL) and added, with vigorous stirring, to a solution of amine **10** (50 mg, 0.10 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (1:1, 2.5 mL). Vigorous stirring was maintained for 2 h, after which time, the mixture was left to stand and the phases were separated. The aqueous phase was

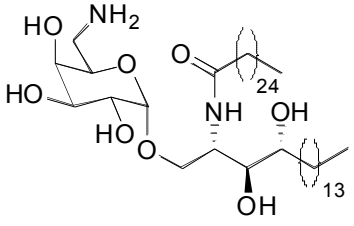
extracted with THF (2 × 2.5 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (gradient: $CHCl_3$ to 15% MeOH in $CHCl_3$) afforded amide **24** as a white solid (67 mg, 76%): R_f = 0.23 (15% MeOH in $CHCl_3$); mp 156–158 °C; the very poor solubility of this amphiphilic compound at rt prevented us from obtaining reliable optical rotation data; ν_{max}(film)/cm^{-1} 3345s br, 2917s, 2850s, 2102m (N_3), 1708w, 1641m (C=O); δ_H(300 MHz, $CDCl_3$:CD_3OD, 2:1) 0.84 (app. t, *J* 6.8, 6H, 2 × CH_2CH_3), 1.17-1.33 (stack, 68H, alkyl chain), 1.50-1.67 (stack, 4H, alkyl chain), 2.12-2.28 (stack, 2H, $NHCOCH_2$), 3.26 (dd, *J* 12.8, 4.8, 1H, 6-H_a), 3.49-3.58 (stack, 3H, including 6-H_b, 3'-*H*), 3.66-3.71 (stack, 2H, including 1'-H_a), 3.76 (dd, *J* 10.0, 3.6, 1H, 2-*H*), 3.78-3.81 (m, 1H), 3.86-3.94 (stack, 2H, 5-*H*, 1'-H_b), 4.16 (app. q, *J* 4.4, 1H, 2'-*H*), 4.88 (d, *J* 3.6, 1H, 1-*H*), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 13.5 (CH_3, 2 × CH_2CH_3), [22.3, 25.5, 29.0, 29.1, 29.3, 31.5, 32.0 (CH_2, alkyl chain, resonance overlap)], 36.1 (CH_2, $NHC(O)CH_2$), 49.7 (CH, *C*-2'), 50.9 (CH_2, *C*-6), 67.3 (CH_2, *C*-1'), 68.3 (CH), 69.3 (CH), 69.7 (CH), 71.1 (2 × CH, resonance overlap), 74.1 (CH), 99.3 (CH, *C*-1), 173.9 (C, *C*=O); MS (TOF ES+) *m*/*z* 905.6 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{98}N_4O_8Na$ $[M + Na]^+$ 905.7282, found 905.7290.

(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-amino-α-D-galactopyranosyl)-2-[(*N*-nervonoyl)amino]octadecane-3,4-diol (**28**) (Staudinger product from **23**)



PMe_3 (71 µL of a 1.0 M solution in THF, 0.071 mmol) was added to a solution of azide **23** (50 mg, 0.059 mmol) in THF (2.5 mL) at rt. The resulting solution was stirred for 5 h. H_2O (5.0 µL, 0.28 mmol) was then added and the mixture was stirred for 1 h before being concentrated under reduced pressure. The residual H_2O was removed by azeotropic distillation with toluene (2 × 10 mL). The residue was then subjected to high vacuum at 60 °C for 24 h to remove the Me_3PO by-product. The resulting amine **28** was obtained as a white powder (48 mg, quant.) and used in the next step without further purification.

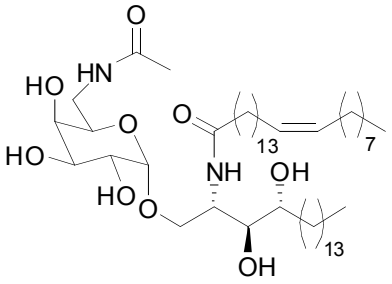
(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-amino-α-D-galactopyranosyl)-2-[(*N*-hexacosanoyl)amino]octadecane-3,4-diol (**29**) (Staudinger product from **24**)



PMe_3 (136 µL of a 1.0 M solution in THF, 0.136 mmol) was added to a solution of azide **24** (100 mg, 0.113 mmol) in THF (5.0 mL) at rt. The resulting solution was stirred for 5

h. H_2O (10 μL, 0.56 mmol) was added and the mixture was stirred for 1 h before being concentrated under reduced pressure. The residual H_2O was removed by azeotropic distillation with toluene (2 × 10 mL). The residue was then subjected to high vacuum at 60 °C for 24 h to remove the Me_3PO by-product. The resulting amine **29** was obtained as a white powder (97 mg, quant.) and used in the next step without further purification.

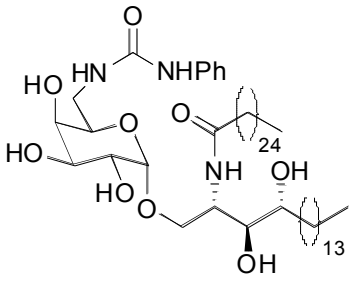
(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-acetamido-α-D-galactopyranosyl)-2-[(*N*-nervonoyl)amino]octadecane-3,4-diol (**6**) [7]



A solution of AcCl (4.3 μL, 0.06 mmol) in THF (1.0 mL) and added, with vigorous stirring, to a solution of amine **28** (41 mg, 0.05 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (1:1, 2.0 mL). Vigorous stirring was maintained for 2 h, after which time, the mixture was left to stand and the phases were separated. The aqueous phase was extracted with THF (2 × 2 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (gradient: $CHCl_3$ to 15% MeOH in $CHCl_3$) afforded amide **6** as a white solid (39 mg, 89%): R_f = 0.25 (15% MeOH in $CHCl_3$); mp 148–150 °C; the very poor solubility of this amphiphilic compound at rt prevented us from obtaining reliable optical rotation data; ν_{max}(film)/cm^{-1} 3285s br (O–H), 2918s, 2850s, 1652s (C=O), 1619s (C=O), 1553m (C=C); δ_H(300 MHz,

$CDCl_3$:CD_3OD, 2:1) 0.85 (app. t, *J* 6.8, 6H, 2 × CH_2CH_3), 1.17-1.40 (stack, 56H, alkyl chain), 1.48-1.67 (stack, 4H, alkyl chain), 1.95 (s, 3H, NHC(O)CH_3), 1.95-2.01 (stack, 4H, CH_2CH=$CHCH_2$), 2.17 (app. t, *J* 7.6, 2H, NHC(O)CH_2), 3.20 (dd, *J* 14.0, 7.2, 1H, 6-H_a), 3.47-3.55 (stack, 3H, including 6-H_b), 3.62 (dd, *J* 10.8, 4.8, 1H, 1'-*H*), 3.64-3.71 (m, 1H, 2'-*H*), 3.72-3.77 (stack, 3H, including 5-*H*), 3.81 (dd, *J* 10.8, 4.4, 1H, 1'-H_b), 4.14 (app. q, *J* 4.8, 1H, 2'-*H*), 4.84 (d, *J* 3.6, 1H, 1-*H*), 5.26-5.35 (stack, 2H, C*H*=C*H*), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 13.3 (CH_3, 2 × CH_2CH_3), 21.7 (CH_3, NHC(O)CH_3), [22.1, 25.37, 25.44, 26.7, 28.79, 28.85, 28.95, 28.99, 29.1, 29.2, 31.4, 32.0 (CH_2, alkyl chain, resonance overlap)], 35.9 (CH_2, NHC(O)CH_2), 39.5 (CH_2, *C*-6), 50.1 (CH, *C*-2'), 66.5 (CH_2, *C*-1'), 68.4 (CH), 68.5 (CH), 69.0 (CH), 69.5 (CH), 71.5 (CH), 74.1 (CH), 99.2 (CH, *C*-1), 129.4 (CH, 2 × *C*H=*C*H), 172.2 (C, *C*=O), 174.2 (C, *C*=O); MS (TOF ES+) *m*/*z* 893.0 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{50}H_{96}N_2O_9Na$ $[M + Na]^+$ 891.7014, found 891.7046.

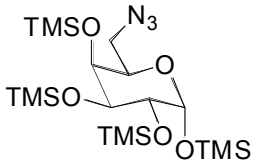
(2*S*, 3*S*, 4*R*)-1-*O*-(6-Deoxy-6-phenylaminocarbonylamino-α-D-galactopyranosyl)-2-[(*N*-hexacosanoyl)amino]octadecane-3,4-diol (**8**) [8]



Phenyl isocyanate (6.5 μL, 0.06 mmol) was added dropwise over 5 min to solution of amine **29** (45 mg, 0.05 mmol) in DMF (1.0 mL) at rt. After stirring for 1 h, the DMF was

removed under reduced pressure. Purification of the residue by flash column chromatography afforded urea **8** as a white solid (35 mg, 71%): R_f = 0.26 (15% MeOH in $CHCl_3$); mp 145–147 °C; the very poor solubility of this amphiphilic compound at rt prevented us from obtaining reliable optical rotation data; ν_{max}(film)/cm^{-1} 3330s br, 2918s, 2851s, 1638m (C=O), 1600m, 1549m; δ_H(300 MHz, $CDCl_3$:CD_3OD, 2:1); 0.85 (app. t, *J* 6.9, 6H), 1.16-1.36 (stack, 68H), 1.42-1.68 (stack, 4H), 2.10-2.19 (stack, 2H), 3.43-3.58 (stack, 3H), 3.61-3.88 (stack, 7H), 4.10-4.19 (m, 1H), 4.86 (d, *J* 3.6, 1H), 6.95 (t, *J* 7.2, 1H), 7.18-7.25 (m, 2H), 7.32 (d, *J* 7.5, 2H), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 13.3 (CH_3), [22.1, 25.4, 28.8, 29.2, 31.4, 31.9 (CH_2, resonance overlap)], 35.9 (CH_2), 39.8 (CH_2), 49.9 (CH), 66.6 (CH_2), 68.2 (CH), 69.1 (CH), 69.4 (CH), 69.6 (CH), 71.5 (CH), 74.0 (CH), 99.2 (CH), 118.7 (CH), 122.1 (CH), 128.3 (CH), 138.8 (C), 156.9 (C), 174.3 (C); MS (TOF ES+) *m*/*z* 998.9 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{57}H_{105}N_3O_9Na$ [M + Na]$^+$ 998.7749, found 998.7754.

Trimethylsilyl 6-deoxy-6-azido-2,3,4-tri-*O*-trimethylsilyl-α-D-galactopyranoside (**25**)



AcOH (0.17 mL, 3.0 mmol) was added to a solution of galactoside **13** (1.72 g, 1.57 mmol) in acetone (3.5 mL) and MeOH (4.7 mL) at 0 °C. The reaction mixture was allowed to warm to rt. After 8 h, the reaction was quenched by the addition of $NaHCO_3$

(0.50 g, 5.95 mmol) and then filtered. After concentration of the filtrate under reduced pressure, the resulting oil was purified by flash column chromatography (6% EtOAc in hexane) to afford alcohol **26** as a colorless oil [(1.25 g, 1.22 mmol, 78%): R_f = 0.28 (6% EtOAc in hexane)], which was dissolved in THF (25 mL) and cooled to 0 °C. PPh_3 (641 mg, 2.44 mmol) was added, followed by DIAD (0.54 mL, 2.44 mmol) and then DPPA (0.52 mL, 2.44 mmol). The mixture was allowed to warm to rt and then stirred overnight. Concentration under reduced pressure followed by purification of the residue by flash column chromatography (5% EtOAc in hexane) afforded azide **25** as a colorless oil (2.87 g, 87%): R_f = 0.30 (5% EtOAc in hexane); $[\alpha]^{20}_D$ +42.7 (*c* 0.50, $CHCl_3$); ν_{max}(film)/cm^{-1} 2957m, 2916m, 2101s (N_3), 1671w; δ_H(300 MHz) 0.10 (s, 9H), 0.140 (s, 9H), 0.143 (s, 9H), 0.16 (s, 9H), 3.17 (A of ABX, $J_{A\text{-}B}$ 12.3, $J_{A\text{-}X}$ 6.0, 1H), 3.44 (B of ABX, $J_{B\text{-}A}$ 12.3, $J_{B\text{-}X}$ 7.5, 1H), 3.76-3.79 (m, 1H), 3.80-3.83 (stack, 2H), 4.02 (t with unresolved fine coupling, *J* 6.8, 1H), 5.06 (d, *J* 2.1, 1H); δ_C(100 MHz) 0.0 (CH_3), 0.2 (CH_3), 0.4 (CH_3), 0.6 (CH_3), 51.4 (CH_2), 69.5 (CH), 69.9 (CH), 70.2 (CH), 73.2 (CH), 94.4 (CH); MS (TOF ES+) *m*/*z* 516.2 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{18}H_{43}N_3O_5Si_4Na$ $[M + Na]^+$ 516.2178, found 516.2180.

References

1. Acree, T. E.; Shallenberger, R. S.; Mattick, L. R. *Carbohydr. Res*. **1968**, *6*, 498-502.

2. Bhat, A. S.; Gervay-Hague, J. *Org. Lett.* **2001**, *3*, 2081-2084.

3. Lu, X.; Bittman R. *Tetrahedron Lett.* **2005**, *46*, 3165-3168.

4. van den Berg, R. J. H. N.; Boltje, T. J.; Verhagen C. P.; Litgens, R. E. J. N.; van der Marel, G. A.; Overkleeft, H. S. *J. Org. Chem*. **2006**, *71*, 836-839.

5. Blauvelt, M. L.; Khalili, M.; Jaung, W.; Paulsen, J.; Anderson, A. C.; Wilson, S. B.; Howell, A. R. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 6374-6376.

6. Mormeneo, D.; Casas, J.; Llebaria, A.; Delgado, A. *Org. Biomol. Chem.* **2007**, *5*, 3769-3777.

7. Liu, Y.; Goff, R. D.; Zhou, D.; Mattner, J.; Sullivan, B. A.; Khurana, A.; Cantu, C., III; Ravkov, E. V.; Ibegbu, C. C.; Altman, J. D.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Immunol. Meth*. **2006**, *312*, 34–39.

8. Trappeniers, M.; Van Beneden, K.; Decruy, T.; Hillaert, U.; Linclau, B.; Elewaut, D.; Van Calenbergh, S. *J. Am. Chem. Soc.* **2008**, *130*, 16468–16469.