Design, Synthesis and Functional Activity of Labeled CD1d Glycolipid Agonists

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Chemistry

General Experimental

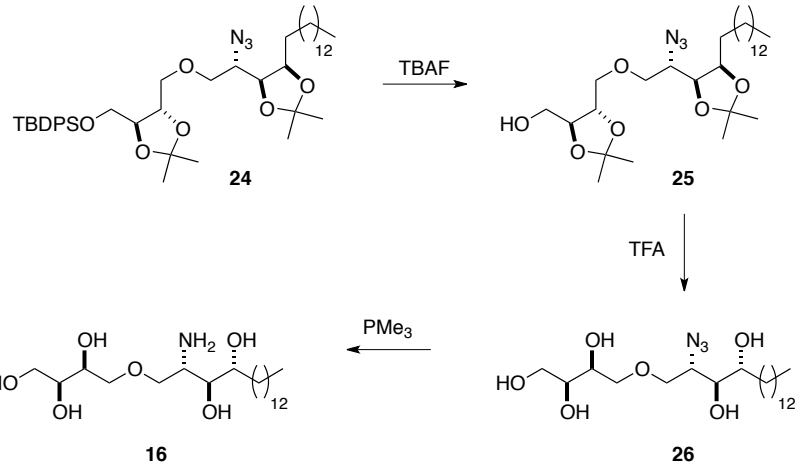
Melting points were determined using open capillaries and are uncorrected. Infrared spectra were recorded either neat or as thin films between NaCl discs. The intensity of each band is described as s (strong), m (medium) or w (weak), and with the prefix v (very) and suffix br (broad) where appropriate. ^{1}H-NMR spectra were recorded at 500 MHz, 400 MHz, or 300 MHz. ^{13}C-NMR spectra were recorded at 125 MHz, 100 MHz, or 75 MHz. 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_3OH, 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) but where coupling constants cannot be readily assigned. Mass spectra were recorded utilizing electrospray ionization (and a MeOH mobile phase), and are reported as (*m*/*z* (%)). HRMS were recorded using a lock mass incorporated into the mobile phase.

All reagents were obtained from commercial sources and used without further purification unless stated otherwise. Anhydrous solvents were stored over 4 Å molecular sieves and under an Ar atmosphere. All solutions are aqueous and saturated unless stated otherwise.

All reactions were monitored by TLC using pre-coated aluminum-backed ICN silica plates (60A F_{254}) and visualized by UV detection (at 254 nm) and staining with 5% phosphomolybdic acid in EtOH (MPA spray). Column chromatography was performed on silica gel (particle size 40-63 μm mesh).

Experimental Procedures and compound characterization

Synthesis of Amine 16:



(2*S*, 3*S*, 4*R*)-2-Azido-1-*O*-[2', 3'-*O*-isopropylidene-L-threitol]-3, 4-*O*-isopropylidene-octadecane-1, 3, 4-triol (25)

Bu_4NF (1.0 M solution in THF, 1.1 mL, 1.1 mmol) was added to a solution of silyl ether **24**[1] (750 mg, 0.98 mmol) in THF (10 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 and the residue was purified by flash column chromatography (25% EtOAc in hexanes) to provide alcohol **25** as a colorless oil (490 mg, 95%); R_f = 0.35 (30% EtOAc in hexane); $[\alpha]_D^{21}$ = 22.0 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 3487br (O–H), 2098s (N_3); δ_H(300 MHz, $CDCl_3$) 0.87 (t, *J* 6.2, 3H), 1.22–1.34 (stack, 25H), 1.40–1.45 (stack, 9H), 1.46–1.65 (stack, 4H), 1.99 (dd, *J* 7.8, 4.8, 1H), 3.51–4.18 (broad stack, 11H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 25.6 (CH_3), 26.4 (CH_2), 27.0 (CH_3, resonance overlap), 28.1 (CH_3), [29.4, 29.6 (CH_2, some resonance overlap)], 31.9

(CH_2), 59.9 (CH), 62.3 (CH_2), 71.9 (CH_2), 72.9 (CH_2), 75.6 (CH), 76.0 (CH), 77.8 (CH), 79.4 (CH), 108.3 (C), 109.4 (C); MS (TOF ES+) *m/z* 550.5 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{28}H_{53}N_3O_6Na$ $[M + Na]^+$ 550.3832, found 550.3853.

(2*S*, 3*S*, 4*R*)-2-Azido-1-*O*-[L-threitol]octadecane-1, 3, 4-triol (26)

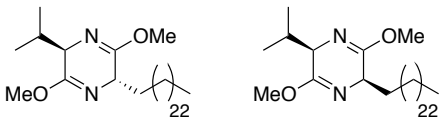
TFA (2.00 mL) was added over 1 min to alcohol **25** (400 mg, 0.76 mmol). After stirring for 1 h at r.t., the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et_2O (3 × 10 mL) to provide crude pentaol **26** as a white solid (315 mg, quant.), which was sufficiently pure to be used in the next step without further purification; R_f = 0.23 (10% MeOH in $CHCl_3$); $[\alpha]_D^{21}$ = +29.2 (*c* 1, $CHCl_3$); ν_{max}(film) / cm^{-1} 3306s br (O–H), 2095s (N_3); δ_H(300 MHz, $CDCl_3$) 0.85 (t, *J* 6.6, 3H), 1.13–1.41 (stack, 24H), 1.44–1.70 (stack, 2H), 3.52–3.70 (stack, 8H), 3.71–3.91 (stack, 3H), exchangeable hydrogens not observed; δ_C(100 MHz, $CDCl_3$) 14.3 (CH_3), 23.1 (CH_2), 26.2 (CH_2), [29.8, 30.1, 32.6, 32.7 (CH_2, some resonance overlap)], 62.6 (CH), 63.9 (CH_2), 70.7 (CH), 71.2 (CH_2), 72.2 (CH), 72.3 (CH), 73.0 (CH_2), 74.5 (CH); MS (TOF ES+) *m/z* 470.1 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{22}H_{45}N_3NaO_6$ $[M + Na]^+$ 470.3206, found 470.3197.

(2*S*, 3*S*, 4*R*)-2-Amino-1-*O*-[L-threitol]octadecane-1, 3, 4-triol (16)

PMe_3 (0.29 mL of a 1.0 M solution in THF, 0.29 mmol) was added dropwise over 5 min to a solution of azide **26** (100 mg, 0.24 mmol) in THF/H_2O (3 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 × 10 mL) to provide amine **16** as a white solid (88 mg, 94%), which was used directly in the next step without further purification.

(2*R*, 5*S*)-2-Isopropyl-3, 6-dimethoxy-5-tetracosanyl-2, 5-dihydropyrazine (13a) and (2*R*, 5*R*)-2-isopropyl-3, 6-dimethoxy-5-tetracosanyl-2, 5-dihydropyrazine (13b)



13a (2*R*, 5*S*) **13b** (2*R*, 5*R*)

A solution of BuLi (2.5 M in hexane, 52 μL, 0.13 mmol) was added dropwise over 1 min to a stirred solution of commercially available (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine **12** (23 μL, 0.13 mmol) in THF (1 mL) at –78 ºC. Stirring was continued at this temperature for 45 min before a solution of 1-iodotetracosane[2] (60 mg, 0.13 mmol) in THF (1 mL) was added over 5 min. The reaction mixture was then stirred for 24 h, slowly warming to r.t. The reaction was quenched by the addition of NH_4Cl solution (5 mL) and EtOAc (5 mL) and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a mixture of dihydropyrazine diastereoisomers ((2*R*, 5*S*)-diastereoisomer (**13a**) : (2*R*, 5*R*)-diastereoisomer (**13b**), 15:1). Purification of this mixture by flash column chromatography (3% EtOAc in hexane) afforded, in order of elution, the major (2*R*, 5*S*)-dihydropyrazine, **13a**, as a white solid (60 mg, 87%); R_f = 0.29 (3% EtOAc in hexane); $[\alpha]_D^{21}$ = –2.3 (*c* 1, $CHCl_3$); mp 78 – 79 ºC; ν_{max}(film) / cm^{-1} 1694s (C=N); δ_H(300 MHz, $CDCl_3$) 0.68 (d, *J* 6.9, 3H), 0.88 (t, *J* 6.8, 3H), 1.05 (d, *J* 6.9, 3H), 1.17–1.40 (stack, 44H), 1.61–

1.86 (stack, 2H), 2.27 (septet of doublets, *J* 6.9, 3.3, 1H), 3.68 (s, 3H), 3.69 (s, 3H), 3.93 (app. t, *J* 3.3, 1H), 4.03 (dt, *J* 5.7, 3.3, 1H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 16.5 (CH_3), 19.1 (CH_3), 19.1 (CH_2), 22.7 (CH_2), 24.4 (CH_2), [29.4, 29.5, 29.6, 29.7, 31.9, 34.1 (CH_2, some resonance overlap)], 31.6 (CH), 52.28 (CH_3), 52.33 (CH_3), 55.5 (CH), 66.7 (CH), 163.4 (C), 164.0 (C); MS (TOF ES+) *m/z* 521.5 ([M + H]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{33}H_{65}N_2O_2$ [M + H]$^+$ 521.5046, found 521.5050; and then the minor (2*R*, 5*R*)-dihydropyrazine, **13b**, as a white solid (4 mg, 6%); R_f = 0.24 (3% EtOAc in hexane); $[\alpha]_D^{21}$ = –12.7 (*c* 1, $CHCl_3$); mp 74 – 75 °C; ν_{max}(film) / cm^{-1} 1694s (C=N); δ_H(300 MHz, $CDCl_3$) 0.74 (d, *J* 6.9, 3H), 0.88 (t, *J* 6.8, 3H), 1.06 (d, *J* 6.9, 3H), 1.18–1.36 (stack, 42H), 1.37–1.52 (stack, 3H), 1.81–1.90 (m, 1H), 2.21 (septet of doublets, *J* 6.9, 3.0, 1H), 3.68 (s, 6H), 3.90–4.12 (stack, 2H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 17.6 (CH_3), 19.6 (CH_3), 22.7 (CH_2), 26.1 (CH_2), [29.4, 29.5, 29.7, 31.9, 36.6 (CH_2, some resonance overlap)], 31.5 (CH), 52.4 (CH_3, resonance overlap), 55.8 (CH), 60.9 (CH), quaternary carbon resonances were not observed; MS (TOF ES+) *m/z* 521.5 ([M + H]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{33}H_{65}N_2O_2$ [M + H]$^+$ 521.5046, found 521.5048.

(2*S*)-Methyl 2-aminohexacosanoate (14)

Hydrochloric acid (0.64 mL, 1.0 M solution, 0.64 mmol) was added to a vigorously stirred solution of dihydropyrazine **13a** (55 mg, 0.105 mmol) in THF (2 mL) at r.t. After 4 h, the mixture was cooled to 0 ºC and quenched with NaOH solution (0.32 mL, 2.0 M solution, 0.64 mmol). EtOAc (5 mL) and H_2O (5 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% MeOH in $CHCl_3$) to afford amino ester **14** as a white solid (32 mg, 72%); R_f = 0.22 (5% MeOH in

$CHCl_3$); $[\alpha]_D^{21}$ = +24.3 (*c* 1, $CHCl_3$); mp 95 – 97 ºC; ν_{max}(film) / cm^{-1} 3370s br (NH_2), 1746s (C=O); δ_H(300 MHz, $CDCl_3$) 0.86 (t, *J* 6.6, 3H), 1.15–1.42 (stack, 44H), 1.49–1.62 (m, 1H), 1.51–1.78 (m, 1H), 3.43 (dd, *J* 7.2, 5.4, 1H), 3.71 (s, 3H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 25.6 (CH_2), [29.37, 29.44, 29.5, 29.7, 31.9, 35.0 (CH_2, some resonance overlap)], 51.8 (CH_3), 54.5 (CH), 176.7 (C); MS (TOF ES+) *m/z* 426.4 ($[M + H]^+$, 100%); HRMS (TOF ES+) calcd for $C_{27}H_{56}NO_2$ $[M + H]^+$ 426.4311, found 426.4313.

(2*S*)-Methyl 2-azidohexacosanoate

Amino ester **14** (32 mg, 0.075 mmol) was dissolved in MeOH/CH_2Cl_2/H_2O (4 mL, 2:1:1). K_2CO_3 (79 mg, 0.74 mmol), $CuSO_4$ (168 μg, 1.1 μmol) and imidazole-1-sulfonyl azide[3] (88 mg, 0.42 mmol) were added sequentially. The reaction mixture was stirred overnight and then diluted with EtOAc (15 mL) and H_2O (15 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (60 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (3% EtOAc in hexane) provided (2*S*)-methyl 2-azidohexacosanoate as a white solid (26 mg, 77%); R_f = 0.27 (3% EtOAc in hexane); $[\alpha]_D^{21}$ = +17.8 (*c* 1, $CHCl_3$); mp 69 – 70 ºC; ν_{max}(film) / cm^{-1} 2106s (N_3), 1745s (C=O); δ_H(300 MHz, $CDCl_3$) 0.88 (t, *J* 6.6, 3H), 1.16–1.49 (stack, 44H), 1.68–1.90 (stack, 2H), 3.79 (s, 3H), 3.80–3.86 (m, 1H); δ_C(100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 25.7 (CH_2), [29.0, 29.3, 29.5, 29.7, 31.4, 31.9 (CH_2, some resonance overlap)], 52.5 (CH_3), 62.0 (CH), 171.2 (C); MS (TOF ES+) *m/z* 474.4 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{27}H_{53}N_3NaO_2$ $[M + Na]^+$ 474.4035, found 474.4031.

(2*S*)-2-Azidohexacosanoic acid [(*S*)-15]

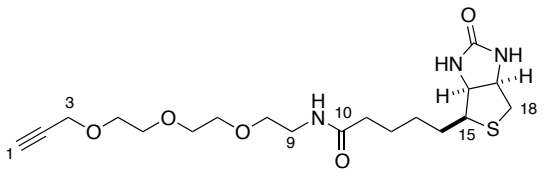
$LiOH{\cdot}H_2O$ (9.2 mg, 0.22 mmol) was added to a vigorously stirred solution of (2*S*)-methyl 2-azidohexacosanoate (25 mg, 0.055 mmol) in THF/H_2O (1:1, 2 mL) at r.t. After stirring for 1 h, the mixture was cooled to 0 ºC and quenched by the addition of hydrochloric acid (10 mL, 0.5 M solution). EtOAc (10 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 10 mL) and the combined organic phases were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (60% EtOAc in hexane) afforded carboxylic acid **(*S*)-15** as a white solid (22 mg, 93%); R_f = 0.2 (30% EtOAc in hexane); $[\alpha]_D^{21}$ = –15.5 (*c* 1, $CHCl_3$); mp 77 – 78 °C; ν_{max}(neat) / cm^{-1} 3300-2600w v br (O–H), 2124m (N_3), 1717m (C=O); δ_H(300 MHz, $CDCl_3$) 0.88 (t, *J* 6.9, 3H), 1.19–1.40 (stack, 41H), 1.41–1.54 (stack, 3H), 1.72–1.96 (stack, 2H), 3.89 (dd, *J* 8.3, 5.2, 1H), O*H* resonance not observed; δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 25.7 (CH_2), 29.1 (CH_2), [29.4, 29.5, 29.7 (CH_2, significant resonance overlap)], 31.3 (CH_2), 32.0 (CH_2), 61.9 (CH), 176.6 (C); MS (TOF ES–) *m/z* 436.4 ($[M - H]^-$, 100%); HRMS (TOF ES–) calcd for $C_{26}H_{50}N_3O_2$ $[M - H]^-$ 436.3903, found 436.3920.

(2*S*, 3*S*, 4*R*, 2''*S*)-2-[2''-Azidohexacosanoylamino]-1-*O*-[L-threitol]octadecane-1, 3, 4-triol [(*S*)-17]

A solution of azido acid **(*S*)-15** (20 mg, 0.046 mmol) in $(COCl)_2$ (1.00 mL) was stirred at 70 °C for 2 h, after which time, the solution was cooled to r.t, 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 mL) and added, with vigorous stirring, to a solution of amine **16** (18 mg,

0.046 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (2 mL, 1:1). 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 × 1 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (10% MeOH in $CHCl_3$) afforded amide **(*S*)-17** as a white solid (29 mg, 74%); R_f = 0.30 (10% MeOH in $CHCl_3$); the poor solubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; mp 109 – 111 °C; ν_{max}(film) / cm^{-1} 3305br m (O–H, N–H), 2103m (N_3), 1653m (C=O); δ_H(300 MHz, $CDCl_3/CD_3OD$, 2:1) 0.84 (t, *J* 6.9, 6H), 1.17–1.44 (stack, 70H), 1.71–1.89 (stack, 2H), 3.47–3.64 (stack, 8H), 3.68–3.83 (stack, 3H), 4.18 (app. q, *J* 4.4, 1H), 7.69 (d, *J* 9.0, 1H), O*H*s not observed; δ_C(100 MHz, $CDCl_3/CD_3OD$, 2:1) 14.2 (CH_3), 23.0 (CH_2), 25.9 (CH_2), 26.2 (CH_2), [29.6, 29.7, 29.8, 30.0, 32.2, 32.9 (CH_2, some resonance overlap)], 50.5 (CH), 63.8 (CH_2), 63.9 (CH), 70.5 (CH_2), 70.6 (CH), 72.2 (CH), 72.6 (CH), 73.2 (CH_2), 74.9 (CH), 170.8 (C); MS (TOF ES+) *m/z* 864.0 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{48}H_{96}N_4NaO_7$ $[M + Na]^+$ 863.7177, found 863.7213.

3-(2'-(2''-(2'''-Amide-D-biotin-ethoxy)ethoxy)ethoxy)prop-1-yne (18)



PMe_3 (0.34 mL of a 1.0 M solution in THF, 0.34 mmol) was added dropwise over 5 min to a solution of 3-(2'-(2''-(2'''-azidoethoxy)ethoxy)ethoxy)prop-1-yne[4] (60 mg, 0.28 mmol) in THF/H_2O (3 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 × 10 mL) to provide the corresponding amine as a white solid. The crude amine was dissolved in DMF (3 mL) before (+)-biotin *N*-hydroxysuccinimide ester (96 mg, 0.28 mmol) was added. The reaction mixture was stirred for 12 h at r.t. and then the volatiles were removed under reduced pressure. Purification of the residue by flash column chromatography (5% MeOH in $CHCl_3$) provided the amide **18** as a colorless paste (90 mg, 78%); R_f = 0.36 (5% MeOH in $CHCl_3$); ν_{max}(film) / cm^{-1} 3306s (N–H / ≡C–H), 2251w (C≡C), 1701s (C=O), 1654m (C=O); δ_H(500 MHz, $CDCl_3$) 1.38 (app. quintet, *J* 7.2, 2H, C(13)H_2), 1.57–1.73 (stack, 4H, C(14)H_2, C(12)H_2), 2.17 (t, *J* 7.6, 2H, C(11)H_2), 2.43 (t, *J* 2.2, 1H, C(1)*H*), 2.68 (app. d, *J* 12.7, 1H, C(18)H_aH_b), 2.84 (dd, *J* 12.7, 4.8, 1H, C(18)H_aH_b), 3.08–3.15 (m, 1H, C(15)*H*), 3.37–3.41 (m, 2H, C(9)H_2), 3.51 (t, *J* 5.1, 2H, C(8)H_2), 3.56–3.59 (stack, 4H, C(6)H_2, C(7)H_2), 3.60–3.65 (stack, 4H, C(5)H_2, C(4)H_2), 4.14 (d, *J* 2.2, 2H, C(3)H_2), 4.24–4.26 (m, 1H, C(16)*H*), 4.43–4.46 (m, 1H, C(17)*H*), 6.05 (s, 1H, C(17)N*H*), 6.81 (s, 1H, C(16)N*H*), 6.85 (t, *J* 5.4, 1H, C(9)N*H*); δ_C(125 MHz, $CDCl_3$) 25.5 (CH_2, *C*(12)), 28.0 (CH_2, *C*(14)), 28.2 (CH_2, *C*(13)), 35.8 (CH_2, *C*(11)), 39.0 (CH_2, *C*(9)), 40.4 (CH_2, *C*(18)), 55.6 (CH, *C*(15)), 58.2 (CH_2, *C*(3)), 60.1 (CH, *C*(17)), 61.6 (CH, *C*(16)), 68.9 (CH_2, *C*(4)), 69.8 (CH_2, *C*(8)), 69.9 (CH_2, *C*(7)), 70.1 (CH_2, *C*(5)), 70.2 (CH_2, *C*(6)), 74.6 (CH, *C*(1)), 79.4 (C, *C*(2)), 164.2 (C, NH*C*=ONH), 173.3 (C, *C*(10)); MS (TOF ES+) *m/z* 436.1 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{19}H_{31}N_3NaO_5S$ $[M+Na]^+$ 436.1882, found 436.1869.

(2''*S*)-Biotinylated ThrCer [(*S*)-10]

$CuSO_4$ solution (12 μL of a 0.5 M solution, 6 μmol) and sodium ascorbate solution (26 μL of a 1.0 M solution, 26 μmol) were added to a solution of azide **(*S*)-17** (25 mg, 0.029 mmol) and alkyne **18** (13 mg, 0.029 mmol) in *t*-BuOH/H_2O (1 mL, 1:1) at r.t. The reaction mixture was heated for 10 h at 50 °C

and then diluted with $CHCl_3$ (10 mL), and washed with brine (3 mL). The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 5 mL). The combined organic layers were dried over Na_2SO_4 and the volatiles were removed under reduced pressure. Purification of the residue by flash column chromatography (10% MeOH in $CHCl_3$) afforded triazole **(*S*)-10** as a white paste (28 mg, 77%); R_f = 0.20 (10% CH_3OH in $CHCl_3$); the poor solubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; ν_{max}(film) / cm^{-1} 3332br (O–H, N–H), 1672s (C=O); δ_H(500 MHz, $CDCl_3/CD_3OD$, 2:1) 0.84 (t, *J* 6.9, 6H, 2 × CH_2CH_3), 1.17–1.36 (stack, 70H), 1.38–1.46 (stack, 2H), 1.52–1.75 (stack, 4H, C(12''')H_2 (middle of stack), C(14''')H_aH_b (LHS of stack), C(14''')H_aH_b (RHS of stack)), 2.01–2.09 (stack, 1H, C(3")H_aH_b), 2.10–2.22 (stack, 3H, C(3")H_aH_b, C(11''')H_2), 2.71 (d, *J* 12.8, 1H, C(18''')H_aH_b), 2.89 (dd, *J* 12.8, 5.0, 1H, C(18''')H_aH_b), 3.13–3.19 (m, 1H, C(15''')*H*), 3.33–3.38 (stack, 2H, C(9''')H_2), 3.47–3.71 (stack, 18H, including C(1)H_aH_b, C(4''')H_2), 3.73 (dd, *J* 10.0, 5.0, 1H, C(1)H_aH_b), 3.75–3.79 (m, 1H), 4.17–4.22 (m, 1H, C(2)*H*), 4.30 (d, *J* 7.8, 4.3, 1H, C(16''')*H*), 4.48 (dd, *J* 7.8, 4.8, 1H, C(17''')*H*), 4.65 (s, 2H, C(3''')H_2), 5.27 (dd, *J* 8.5, 6.5, 1H, C(2")*H*), 7.99 (s, 1H, C(1''')*H*), exchangeable hydrogens not observed; δ_C(125 MHz, $CDCl_3/CD_3OD$, 2:1) 14.3 (CH_3, *C*(18), *C*(26") (resonance overlap)), 23.2 (CH_2), 26.1 (CH_2, *C*(12''')), 26.2 (CH_2), 26.4 (CH_2), 28.8 (CH_2, *C*(14''')), [29.1, 29.5, 29.87, 29.90, 30.1, 30.19, 30.22, 32.5 (CH_2, alkyl chain, some resonance overlap)], 36.2 (CH_2, *C*(11''')), 39.8 (CH_2, *C*(9''')), 40.8 (CH_2, *C*(18''')), 51.3 (CH, *C*(2)), 56.3 (CH, *C*(15''')), 60.9 (CH, *C*(17''')), 62.6 (CH, *C*(16''')), 63.9 (CH_2, *C*(4')), 64.7 (CH, *C*(2")), 64.8 (CH_2, *C*(3''')), 70.17 (CH_2, *C*(4''')), 70.24 (CH_2), 70.5 (CH_2), 70.6 (CH_2), 70.8 (CH), 70.99 (CH_2), 71.03 (CH_2), 72.5 (CH), 72.8 (CH), 73.4 (CH_2, *C*(1')), 75.0 (CH, *C*(3)), 123.3 (CH, *C*(1''')), 145.3 (C, *C*(2''')), 165.1 (C, NH*C*=ONH), 169.1 (C, *C*(1")), 175.2 (C, *C*(10''')); MS (TOF ES+) *m/z* 1276.8 ($[M + Na]^+$, 100%), 649.9 (7), 398.1 (5); HRMS (TOF ES+) calcd for $C_{67}H_{127}N_7O_{12}SNa$ $[M + Na]^+$ 1276.9161, found 1276.9166.

(*rac*)-2-Azidohexacosanoic acid (*rac*-15)

A mixture of hexacosanoic acid (600 mg, 1.51 mmol) and red phosphorus (47 mg, 1.5 mmol) was heated to 95 °C and Br_2 (270 μL, 5.29 mmol) was added dropwise over 2 min at this temperature with stirring. The mixture was stirred for 6 h and then cooled to r.t., diluted with Et_2O (30 mL) and washed with H_2O (30 mL). The aqueous phase was extracted with Et_2O (2 × 30 mL), and the combined organic fractions were washed with brine (10 mL), dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure to yield 2-bromohexacosanoic acid as a white solid, which was recrystallized from hexane (481 mg, 67%); R_f = 0.35 (30% EtOAc in hexane); mp 73 – 74 °C (from hexane); ν_{max}(film) / cm^{-1} 3300–2600w v br (O–H), 1715m (C=O), 1697s (C=O); δ_H (300 MHz, $CDCl_3$) 0.88 (t, *J* 6.8, 3H), 1.21–1.33 (stack, 44H), 1.95–2.21 (m, 2H), 4.24 (app t, *J* 7.6, 1H), O*H* not observed; δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 22.7 (CH_2), 27.2 (CH_2), 28.8 (CH_2), [29.3, 29.4, 29.5, 29.7, 29.8 (CH_2, resonance overlap)], 32.0 (CH_2), 34.7 (CH_2), 45.4 (CH), 176.0 (C); MS (TOF ES–) *m/z* 475.3 ([M(^{81}Br isotopomer) – H]$^-$, 100%), 473.3 (100, [M(^{79}Br isotopomer) – H]$^-$); HRMS (TOF ES–) calcd for $C_{26}H_{50}{}^{79}BrO_2$ [M – H]$^-$ 473.2994, found 473.2989.

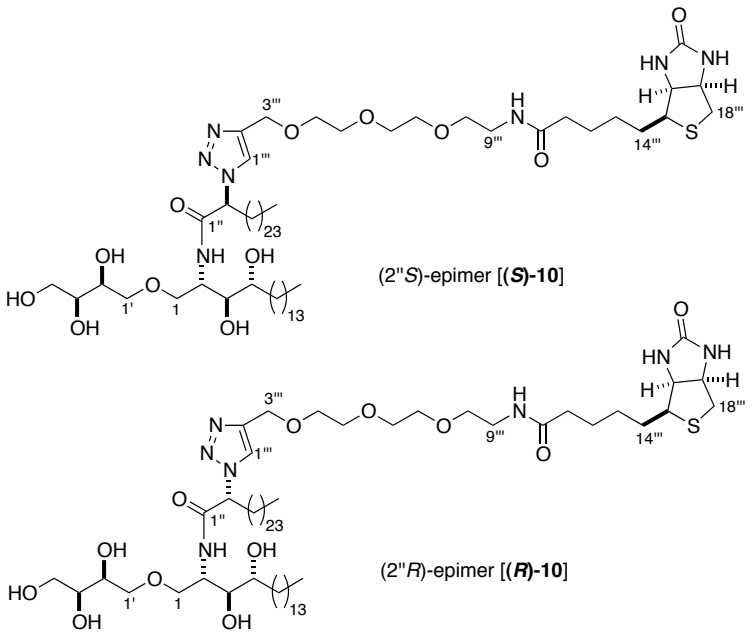
NaN_3 (690 mg, 10.6 mmol) was added to a solution of 2-bromohexacosanoic acid (481 mg, 1.01 mmol) in DMF (5 mL). The reaction mixture was stirred vigorously at 60 °C. After 48 h, the reaction mixture was cooled to r.t., diluted with Et_2O (20 mL) and washed with hydrochloric acid (50 mL, 1 M). The phases were separated and the aqueous phase was extracted with Et_2O (2 × 25 mL). The combined organic fractions were washed with brine (20 mL) and dried with $MgSO_4$. Removal of the volatiles under reduced pressure and purification of the residue by flash column chromatography (15% EtOAc in toluene) afforded racemic α-azido acid **15** as a white solid (394 mg, 60% over 2 steps from

hexacosanoic acid). The ^{1}H NMR, ^{13}C NMR, IR spectroscopic and MS data were identical to those obtained for (2*S*)-2-azidohexacosanoic acid [**(*S*)-15**] (*vide supra*).

(2*S*, 3*S*, 4*R*, 2''*R,S*)-2-[2''-Azidohexacosanoylamino]-1-*O*-[L-threitol]octadecane-1, 3, 4-triol (17) (1:1 mixture of epimers, epimeric at C(2''))

A solution of *rac*-**15** (61 mg, 0.14 mmol) in $(COCl)_2$ (2.00 mL) was stirred at 70 °C for 2 h, after which time, the solution was cooled to r.t., and the remaining $(COCl)_2$ 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 (1.5 mL) and added, with vigorous stirring, to a solution of amine **16** (54 mg, 0.14 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (1:1, 3 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 × 1.5 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (10% MeOH in $CHCl_3$) afforded amide **17** as a white solid (85 mg, 72%, 1:1 mixture of 2''-epimers). Data on mixture unless stated otherwise: R_f = 0.30 (10% MeOH in $CHCl_3$); mp 109 – 110 °C; ν_{max}(film) / cm^{-1} 3303br m (O–H, N–H), 2101m (N_3), 1655m (C=O); δ_H(300 MHz, $CDCl_3$/CD_3OD, 2:1) 0.85 (t, *J* 6.9, 6H), 1.17–1.54 (stack, 70H), 1.71–1.89 (stack, 2H), 3.46–3.64 (stack, 8H), 3.67–3.88 (stack, 3H), 4.15–4.26 (m, 1H); δ_C(100 MHz, $CDCl_3$/CD_3OD, 2:1) 14.2 (CH_3), 23.0 (CH_2), 25.9 (CH_2), 26.0 (CH_2), 26.3 (CH_2), [29.6, 29.7, 29.8, 30.1, 32.3, 32.8 (CH_2, some resonance overlap)], 50.7 (CH), 63.8 (CH_2), [63.9 (CH of (2''*S*)-epimer), 64.1 (CH of (2''*R*)-epimer)], 70.5 (CH_2), 70.6 (CH), 72.3 (CH), 72.6 (CH), [73.20 (CH_2 of (2''*S*)-epimer), 73.25 (CH_2 of (2''*R*)-epimer)], 74.9 (CH), 170.9 (C); MS (TOF ES–) *m/z* 839.7 ([M – H]$^-$, 100%), 812.0 (70); HRMS (TOF ES–) calcd for $C_{48}H_{95}N_4O_7$ [M – H]$^-$ 839.7201, found 839.7195.

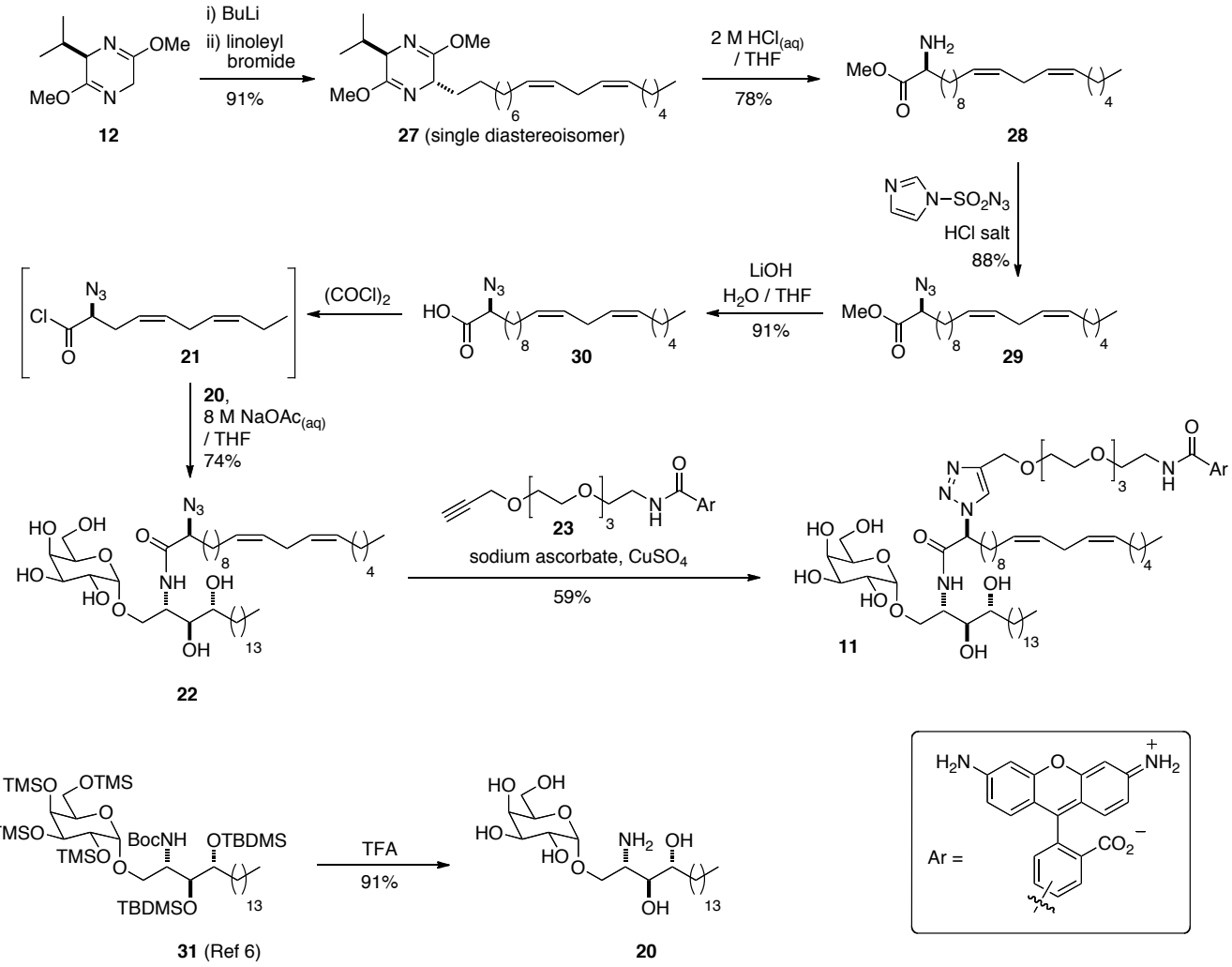
Biotinylated ThrCer (10) [1:1 mixture of epimers, epimeric at C(2'')]



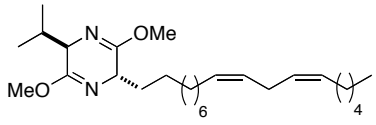
$CuSO_4$ solution (12 μL of a 0.5 M solution, 6 μmol) and sodium ascorbate solution (26 μL of a 1.0 M solution, 26 μmol) were added to a solution of azide **17** (1:1 mixture of 2''-epimers, (55 mg, 0.065 mmol) and alkyne **18** (27 mg, 0.065 mmol) in *t*-BuOH/H_2O (1 mL, 1:1) at r.t. The reaction mixture was heated for 10 h at 50 °C and then diluted with $CHCl_3$ (10 mL), and washed with brine (3 mL). The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 5 mL). The combined organic layers were dried over Na_2SO_4 and the volatiles were removed under reduced pressure. Purification of the residue by flash column chromatography (10% CH_3OH in $CHCl_3$) afforded biotinylated ThrCer **10** (64 mg, 78%, 1:1 mixture of 2''-epimers). Careful re-purification of a small sample of this mixture by flash column chromatography (gradient 3 – 8% CH_3OH in $CHCl_3$) afforded, in order of elution, **(2''*R*)-10** as a white paste; R_f = 0.30 (10% CH_3OH in $CHCl_3$); ν_{max}(film) / cm^{-1} 3333br (O–H, N–H), 1672s (C=O); δ_H(500 MHz, $CDCl_3/CD_3OD$, 2:1) 0.84 (t, *J* 6.9, 6H, 2 × CH_2CH_3), 1.17–1.35 (stack, 70H), 1.36–1.45 (stack, 2H), 1.48–1.73 (stack, 4H, C(12''')H_2 (middle of stack),

C(14''')H_aH$_b$ (LHS of stack), C(14''')H$_a$$H_b$ (RHS of stack)), 1.97–2.07 (stack, 1H, C(3")H_aH$_b$), 2.09–2.21 (stack, 3H, C(3")H$_a$$H_b$, C(11''')$H_2$), 2.70 (d, *J* 12.8, 1H, C(18''')H_aH$_b$), 2.89 (dd, *J* 12.8, 4.9, 1H, C(18''')H$_a$$H_b$), 3.12–3.18 (m, 1H, C(15''')*H*), 3.32–3.38 (m, 2H, C(9''')H_2), 3.43–3.74 (stack, 20H), 4.12–4.17 (m, 1H, C(2)*H*), 4.30 (dd, *J* 8.0, 4.8, 1H, C(16''')*H*), 4.48 (dd, *J* 8.0, 4.8, 1H, C(17''')*H*), 4.65 (s, 2H, C(3''')H_2), 5.27 (dd, *J* 8.5, 6.5, 1H, C(2")*H*), 8.00 (s, 1H, C(1''')*H*); δ_C(125 MHz, $CDCl_3$/CD_3OD, 2:1) 14.2 (CH_3, *C*(18), *C*(26") (resonance overlap)), 23.0 (CH_2), 25.9 (CH_2, *C*(12''')), 25.9 (CH_2), 26.1 (CH_2), 26.3 (CH_2), 28.6 (CH_2, *C*(14''')), [28.9, 29.4, 29.73, 29.75, 30.05, 30.09, 30.18, 30.24, 32.3, 32.7, 33.4 (CH_2, alkyl chain, some resonance overlap)], 36.1 (CH_2, *C*(11''')), 39.7 (CH_2, *C*(9''')), 40.6 (CH_2, *C*(18''')), 51.1 (CH, *C*(2)), 56.1 (CH, *C*(15''')), 60.8 (CH, *C*(17''')), 62.5 (CH, *C*(16''')), 63.8 (CH_2, *C*(4')), 64.5 (CH, *C*(2")), 64.6 (CH_2, *C*(3''')), 70.0 (CH_2, *C*(4''')), 70.1 (CH_2), 70.5 (2 × CH_2, resonance overlap), 70.7 (CH), 70.8 (CH_2), 70.9 (CH_2), 72.2 (CH), 72.7 (CH), 73.2 (CH_2, *C*(1')), 74.8 (CH, *C*(3)), 123.2 (CH, *C*(1''')), 145.2 (C, *C*(2''')), 164.8 (C, NH*C*=ONH), 168.7 (C, *C*(1")), 175.2 (C, *C*(10''')) MS (TOF ES+) *m/z* 1277.1 ([M + Na]$^+$, 100%), 650.0 (8); HRMS (TOF ES+) calcd for $C_{67}H_{127}N_7O_{12}SNa$ [M + Na]$^+$ 1276.9161, found 1276.9182; and then **(2''*S*)-10** as a white paste: R_f = 0.20 (10% CH_3OH in $CHCl_3$). The physical data obtained for **(2''*S*)-10** isolated from this epimeric mixture were identical to those obtained for the **(2''*S*)-10** obtained from the reaction of epimerically pure (***S***)-**17** with alkyne **18**.

Synthesis of labeled glycolipid 11:

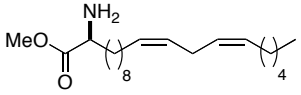


(2*R*, 5*S*, 9'*Z*, 12'*Z*)-Isopropyl-3, 6-dimethoxy-5-(octadeca-9', 12'-dienyl)-2, 5-dihydropyrazine (27)



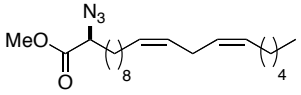
A solution of BuLi (2.5 M in hexane, 52 μL, 0.13 mmol) was added dropwise over 1 min to a stirred solution of commercially available (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine **12** (23 μL, 0.13 mmol) in THF (1 mL) at –78 °C. Stirring was continued at this temperature for 45 min before a solution of linoleyl bromide[5] (42 mg, 0.13 mmol) in THF (1 mL) was added over 5 min. The reaction mixture was then stirred for 24 h, slowly warming to r.t. The reaction mixture was quenched by the addition of NH_4Cl solution (5 mL) and EtOAc (5 mL) and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 5 mL) and then the combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography to afford dihydropyrazine **27** as a colorless oil (51 mg, 91%); R_f = 0.27 (3% EtOAc in hexane); $[\alpha]_D^{20}$ –15.3 (*c* = 1 in $CHCl_3$); ν_{max}(film) / cm^{-1} 1693s (C=N), 1634m (C=C); δ_H(300 MHz, $CDCl_3$) 0.68 (d, *J* 6.8, 3H), 0.88 (t, *J* 6.8, 3H), 1.04 (d, *J* 6.8, 3H), 1.14–1.43 (stack, 18H), 1.60–1.90 (stack, 2H), 1.98–2.11 (stack, 4H), 2.26 (septet of doublets, *J* 6.9, 3.3, 1H), 2.76 (app t, *J* 6.0, 2H), 3.67 (s, 3H), 3.68 (s, 3H), 3.92 (dd, *J* 6.9, 3.3, 1H), 4.01 (dt, *J* 5.7, 4.2, 1H), 5.26–5.44 (stack, 4H); δ_C(100 MHz, $CDCl_3$) 14.0 (CH_3), 16.5 (CH_3), 19.0 (CH_3), 22.5 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 27.2 (CH_2), [29.27, 29.32, 29.7, 31.5, 34.1 (CH_2, some resonance overlap)], 31.6 (CH), 52.2 (CH_3, resonance overlap), 55.5 (CH), 60.6 (CH), 127.9 (CH), 128.0 (CH), 129.99 (CH), 130.11 (CH), 163.3 (C), 164.0 (C); MS (TOF ES+) *m/z* 433.4 ($[M + H]^+$, 100%); HRMS (TOF ES+) calcd for $C_{27}H_{49}N_2O_2$ $[M + H]^+$ 433.3794, found 433.3791.

(2*S*, 11*Z*, 14*Z*)-Methyl 2-aminoeicosa-(11, 14)-dienoate (28)



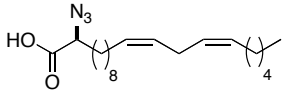
Hydrochloric acid (0.71 mL, 1.0 M solution, 0.71 mmol) was added to a vigorously stirred solution of dihydropyrazine **27** (50 mg, 0.116 mmol) in THF (2 mL) at r.t. After 4 h, the mixture was cooled to 0 °C and quenched with NaOH solution (0.36 mL, 2.0 M solution, 0.72 mmol). EtOAc (5 mL) and H_2O (5 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography afforded amine **28** as a colorless oil (31 mg, 78%); R_f = 0.23 (5% $CHCl_3$ in MeOH); $[\alpha]_D^{20}$ +18.4 (c = 1 in $CHCl_3$); ν_{max}(film) / cm^{-1} 3372s br (NH_2), 1747s (C=O), 1635m (C=C); δ_H(300 MHz, $CDCl_3$) 0.87 (t, J 6.6, 3H), 1.19–1.40 (stack, 16H), 1.41–1.60 (stack, 3H), 1.60–1.75 (m, 1H), 2.01 (app. q, J 6.6, 4H), 2.73 (app. t, J 6.0, 2H), 3.40 (app. t, J 6.6, 1H), 3.68 (s, 3H), 5.21–5.42 (stack, 4H), exchangeable hydogens not observed; δ_C(100 MHz, $CDCl_3$) 13.9 (CH_3), 22.5 (CH_2), 25.5 (CH_2), 27.1 (CH_2), [29.2, 29.28, 29.33, 29.5, 31.4, 34.9 (CH_2, some resonance overlap)], 51.7 (CH_3), 54.4 (CH), 127.8 (CH), 127.9 (CH), 130.0 (CH), 130.1 (CH), 176.6 (C); MS (TOF ES+) m/z 338.3 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{21}H_{40}NaNO_2$ $[M + Na]^+$ 338.3059, found 338.3063.

(2*S*, 11*Z*, 14*Z*)-Methyl 2-azidoeicosa-(11, 14)-dienoate (29)



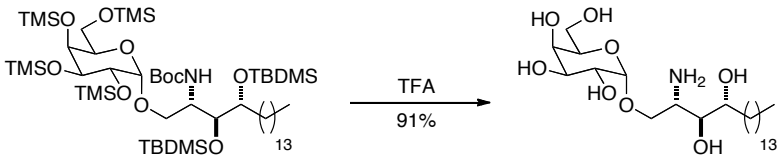
Amine **28** (28 mg, 0.083 mmol) was dissolved in MeOH:CH_2Cl_2:H_2O (2:1:1, 4 mL). K_2CO_3 (62 mg, 0.58 mmol), $CuSO_4$ (133 μg, 0.87 μmol) and imidazole-1-sulfonyl azide hydrochloride[3] (70 mg, 0.33 mmol) were added sequentially. The reaction mixture was stirred overnight and then diluted with EtOAc (15 mL) and H_2O (15 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (60 mmol), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (3% EtOAc in hexane) provided azide **29** as a colorless oil (27 mg, 88%); R_f = 0.24 (3% EtOAc in hexane); $[\alpha]_D^{20}$ –3.6 (c = 1 in $CHCl_3$); ν_{max}(film) / cm^{-1} 2108s (N_3), 1746s (C=O), 1632m (C=C); δ_H(300 MHz, $CDCl_3$) 0.88 (app. t, J 6.8, 3H), 1.21–1.49 (stack, 18H), 1.68–1.90 (stack, 2H), 2.00–2.10 (stack, 4H), 2.77 (app. t, J 6.0, 2H), 3.79 (s, 3H), 3.81 (dd, J 8.4, 5.4, 1H), 5.27–5.44 (stack, 4H); δ_C(100 MHz, $CDCl_3$) 14.0 (CH_3), 22.5 (CH_2), 25.6 (CH_2), 25.7 (CH_2), 27.2 (CH_2), [29.0, 29.2, 29.27, 29.33, 29.6, 31.3, 31.5 (CH_2, some overlapping resonances)], 52.5 (CH_3), 62.0 (CH), 127.9 (CH), 128.0 (CH), 130.0 (CH), 130.2 (CH), 171.2 (C); MS (TOF ES+) m/z 386.3 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{21}H_{37}N_3NaO_2$ $[M + Na]^+$ 386.2783, found 386.2778.

(2*S*, 11*Z*, 14*Z*)-2-Azidoeicosa-(11, 14)-dienoic acid (30)



$LiOH{\cdot}H_2O$ (11.5 mg, 0.28 mmol) was added to a vigorously stirred solution of azide **29** (25 mg, 0.069 mmol) in THF/H_2O (1:1, 2 mL) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C and quenched by the addition of hydrochloric acid (10 mL, 0.5 M solution). EtOAc (10 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 10 mL) and the combined organic extracts were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (60% EtOAc in hexane) afforded carboxylic acid **30** as a colorless oil (22 mg, 91%); R_f = 0.31 (5% $CHCl_3$ in MeOH); $[\alpha]_D^{20}$ –17.6 (*c* = 1 in $CHCl_3$); ν_{max}(film) / cm^{-1} 3300–2600w v br (O–H), 2126m (N_3), 1716m (C=O), 1635m (C=C); δ_H(300 MHz, $CDCl_3$) 0.89 (t, *J* 6.9, 3H), 1.23–1.57 (stack, 18H), 1.71–1.98 (stack, 2H), 2.05 (app. q, *J* 6.6, 4H), 2.78 (app. t, *J* 6.0, 2H), 3.89 (dd, *J* 8.4, 5.4, 1H), 5.27–5.45 (stack, 4H); δ_C(100 MHz, $CDCl_3$) 14.0 (CH_3), 22.5 (CH_2), 25.6 (CH_2), 25.7 (CH_2), 27.2 (CH_2), [29.0, 29.2, 29.3, 29.4, 29.6, 31.3, 31.5 (CH_2, some overlapping resonances)], 61.8 (CH), 127.9 (CH), 128.0 (CH), 130.0 (CH), 130.2 (CH), 176.7 (C); MS (TOF ES+) *m/z* 372.3 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{20}H_{35}N_3NaO_2$ [M + Na]$^+$ 372.2627, found 372.2630.

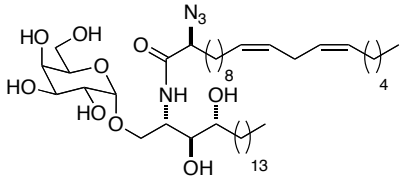
α-D-Galactopyranosyl-1-*O*-phytosphingosine (20)



31 (Ref 6) **20**

TFA (1.0 mL, 13.2 mmol) was added dropwise over 5 min to glycoside **31**[6] (80 mg, 0.072 mmol) at r.t. After 30 min, the reaction mixture was concentrated under reduced pressure to afford amine **20** as a colorless oil (32 mg, 91%). This material was used in the next step without further purification.

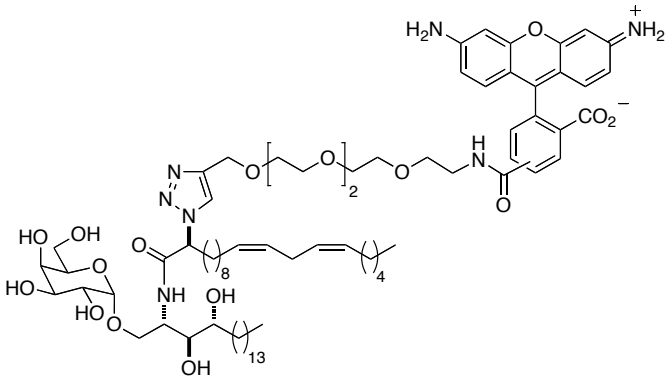
(2*S*, 3*S*, 4*R*, 2'*S*, 11'*Z*, 14'*Z*)-2-[2'-Azidoeicosa-(11', 14')-dienoylamino]-1-*O*-[α-D-galactopyranose]octadecane-1, 3, 4-triol (22)



A solution of carboxylic acid **30** (10 mg, 0.029 mmol) and $(COCl)_2$ (7.4 μL, 0.087 mmol) in CH_2Cl_2 (1.0 mL) was stirred at 70 °C for 3 h, after which time, the solution was cooled to r.t. and the CH_2Cl_2 and unreacted $(COCl)_2$ were removed under a stream of argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride **21** was dissolved in THF (1 mL) and added, with vigorous stirring, to a solution of amine **20** (13.7 mg, 0.029 mmol) in THF / $NaOAc_{(aq)}$ (8 M) (2 mL, 1:1). 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 × 1 mL), and the combined organic phases were concentrated under reduced pressure. Purification of the residue by flash column chromatography (10% MeOH in $CHCl_3$) afforded amide **22** as a colorless paste (17.4 mg, 74%); R_f = 0.23 (5% $CHCl_3$ in MeOH); the poor solubility of this amphiphilic compound at r.t. prevented us from obtaining reliable optical rotation data; ν_{max}(film) / cm^{-1} 3301br s (O–H), 2107 (N_3), 1698m (C=O), 1634s (C=C); δ_H(400 MHz, $CDCl_3$/CD_3OD, 2:1) 0.86 (app. t, *J* 6.8, 6H, 2 × terminal C*H*$_3$), 1.20–1.45 (stack, 42H, alkyl chain), 1.47–1.88 (stack, 4H, alkyl chain), 2.02 (app. q, *J* 6.8, 4H), 2.74 (app. t, *J* 6.4, 2H,), 3.48–3.57 (stack, 2H), 3.63–3.74 (stack, 4H), 3.75–3.80 (stack, 3H), 3.87 (dd, *J* 10.8, 4.8, 1H), 3.89–3.92 (m, 1H), 4.20 (app. q, *J* 4.8, 1H), 4.88 (d, *J* 3.6, 1H, anomeric-*H*), 5.24–5.49 (stack, 4H, 2 × C*H*=C*H*), exchangeable protons not observed; δ_C(100 MHz, $CDCl_3$) 14.3 (CH_3, 2 × terminal *C*H_3), 23.0 (CH_2), 23.1 (CH_2), 26.0 (CH_2), 26.2 (CH_2), 26.3 (CH_2), 27.6 (CH_2), [29.6, 29.7, 29.8, 29.9, 30.2, 31.97, 32.04, 32.3, 32.4, 32.9 (CH_2, alkyl chain, some overlapping resonances)], 51.0 (CH), 62.3 (CH_2), 64.0 (CH), 67.6 (CH_2), 69.4 (CH), 70.3 (CH), 70.8 (CH), 71.4 (CH), 72.3 (CH), 74.9 (CH), 100.3 (CH, *C*-anomeric), 128.4 (CH, *C*H=CH), 128.5 (CH, *C*H=CH), 130.4 (CH, *C*H=CH), 130.5 (CH, *C*H=CH), 171.0 (C, *C*=O); MS (TOF ES+) *m*/*z* 833.6 ([M + Na]$^+$, 100%); HRMS (TOF ES+) calcd for $C_{44}H_{82}N_4NaO_9$ [M + Na]$^+$ 833.5979, found 833.5971.

Fluor 488-labeled α-GalCer (11) [1:1, mixture of regioisomers in the label]



$CuSO_4$ solution (6 μL of a 0.5 M solution, 3 μmol) and sodium ascorbate solution (13 μL of a 1.0 M solution, 13 μmol) were added to a solution of azide **22** (1.4 mg, 1.7 μmol) and alkyne **23** (1.0 mg, 1.7 μmol) in *t*-BuOH / H_2O (0.5 mL, 1:1) at r.t. The reaction mixture was heated for 10 h at 50 °C and then diluted with $CHCl_3$ (5 mL), and washed with brine (1.5 mL). The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 2.5 mL). The combined organic layers were dried over Na_2SO_4 and the volatiles were removed under reduced pressure. Purification of the residue by flash column chromatography ($CHCl_3$/MeOH/H_2O 65:25:4) afforded triazole **11** as a red solid (1.4 mg, 59%, 1:1, mixture of regioisomers in the label); R_f = 0.32 ($CHCl_3$/MeOH/H_2O, 65:25:4); δ_H(400 MHz, $CDCl_3$/CD_3OD, 2:1) 0.83 (t, *J* 6.7, 3H), 0.84 (t, *J* 6.7, 3H), 1.10–1.39 (stack, 44H), 1.40–1.65 (m, 1H), 1.65–1.89 (m, 1H), 1.92–2.03 (stack, 5H), 2.12–2.30 (1H, m), 2.66–2.79 (stack, 2H), 3.46–3.79 (stack, 22H), 3.86 (dd, *J* 10.8, 4.8, 1H), 3.90 (br s, 1H), 4.12–4.22 (m, 1H), 4.55–4.67 (m, 2H), 4.88 (d, *J* 3.6, 1H), 5.21–5.41 (stack, 5H), 6.63–6.77 (stack, 4H), 6.96–7.09 (stack, 2H), 7.31 (d, *J* 9.0, 0.5H), 7.76 (br s, 0.5H), 7.92 (s, 1H), 8.13–8.24 (m, 1H), 8.31 (d, *J* 9.0, 0.5H), 8.72 (br s, 0.5H); MS (TOF ES–) *m/z*

1396.8 ([M – H]$^-$, 10%), 1218.8 (5, [M – galactose]$^-$), 1004.6 (15), 915.6 (25), 850.7 (65), 815.7 (35), 451.5 (95), 423.5 (100), 179.1 (20, [galactose]$^-$).

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Statistical Analysis of Data Summarized in Figure 3

t-test used to compare ThrCer **5** with either (*R*)-**10**, (*S*)-**10** or ThrCer **10** at each concentration of lipids.

Figure 3a

conc'n (ng mL^{-1})	**5** *vs* (*R*)-**10**	**5** *vs* (*S*)-**10**	**5** *vs* **10** (epimeric mixture)
1000	NS	0.0494	NS
500	0.027	NS	NS
250	0.0058	< 0.0001	0.0315
125	0.0159	NS	NS
64	0.0278	0.0083	0.0251
32	0.0046	0.0018	NS
16	0.0009	NS	NS

Figure 3b

conc'n (ng mL^{-1})	**5** *vs* (*R*)-**10**	**5** *vs* (*S*)-**10**	**5** *vs* **10** (epimeric mixture)
1000	0.0037	0.0485	0.0246
100	0.0325	0.0002	0.0002
10	NS	0.0005	0.0005
1	NS	NS	NS
0.1	NS	NS	NS
0.001	NS	NS	NS