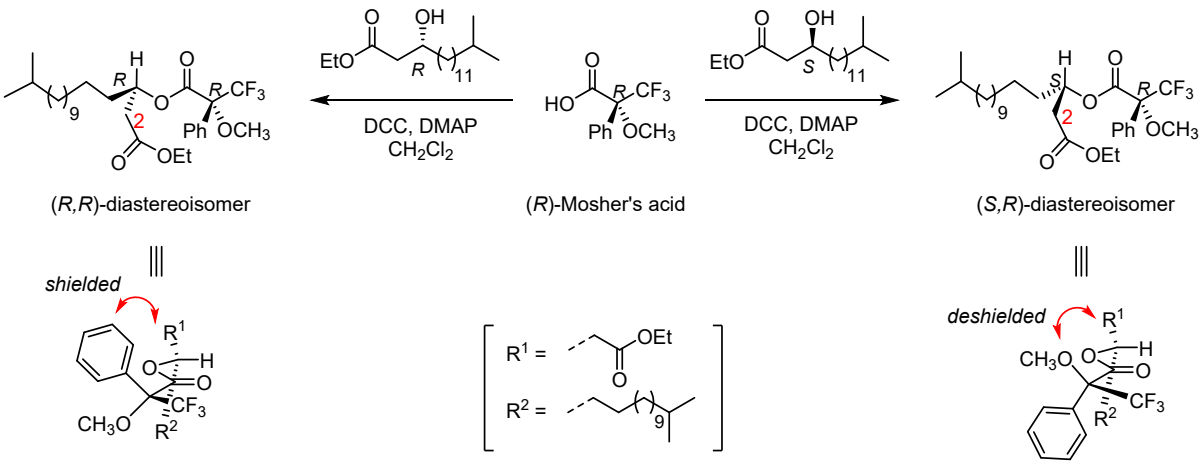
SUPPORTING INFORMATION

Glycolipids from the gut symbiont *Bacteroides fragilis* are agonists for NKT cells and induce their regulatory differentiation

Garth Cameron, Tram Nguyen, Marcin Ciula, Spencer J. Williams* and Dale I. Godfrey*

Contents



Compound	δ (H2a) ppm	δ (H2b) ppm	OCH_3
S,R	2.67	2.54	3.59
R,R	2.73	2.58	3.66

Figure S1. Mosher ester analysis of β-hydroxyesters from Noyori catalyst reduction of β-keto ester.

Inset table: summary of chemical shift data for individual diasteroisomers.

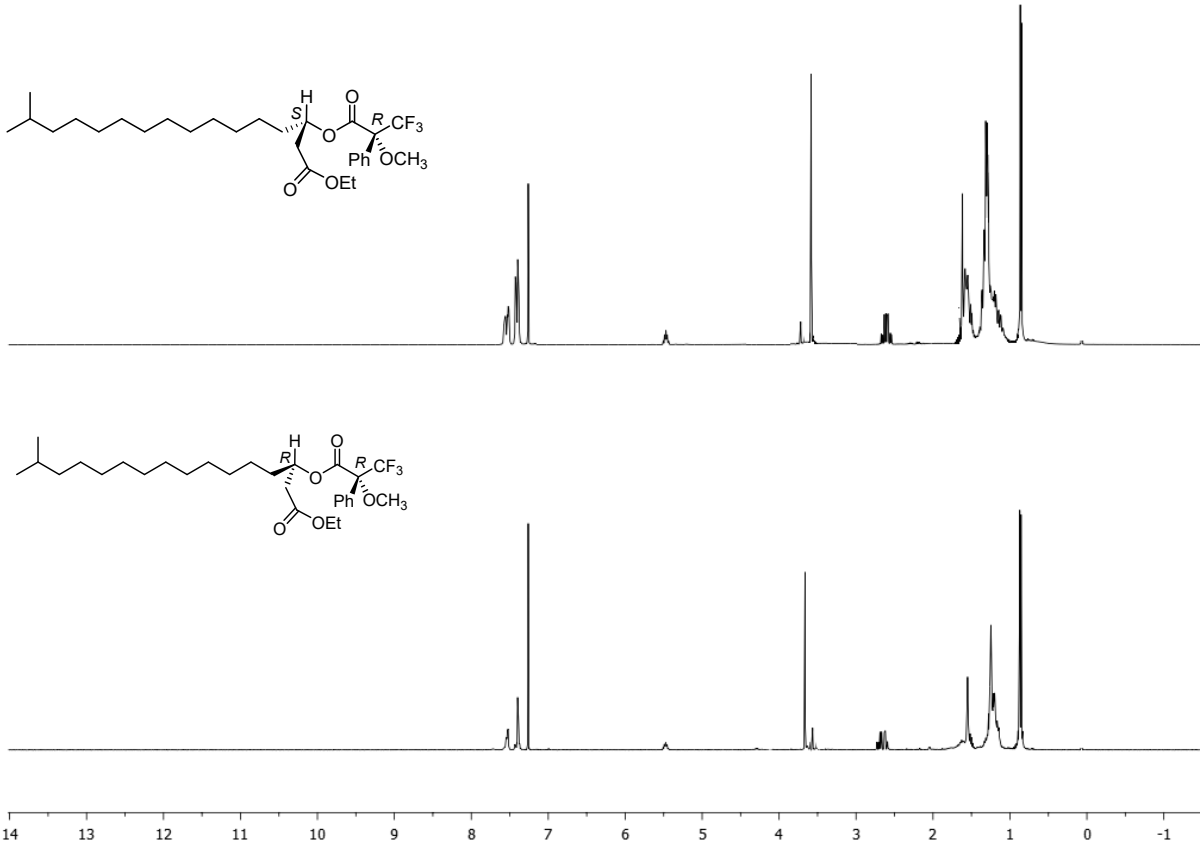


Figure S2. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of individual Mosher ester products in $CDCl_3$.

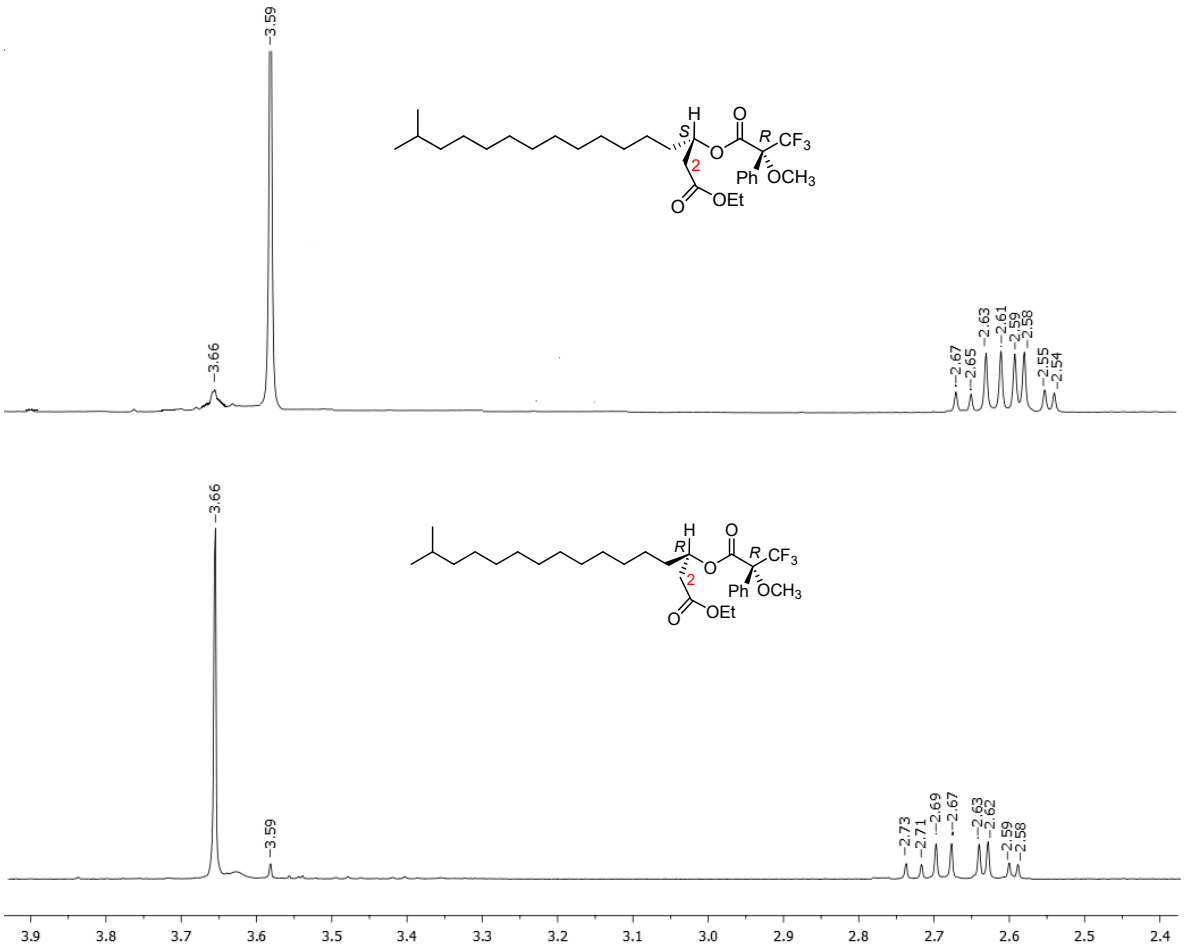


Figure S3. Expansion of ^{1}H NMR spectra (400 MHz, $CDCl_3$) of individual Mosher ester products in $CDCl_3$, in the region surrounding the signal for the methyl ester group and H2.

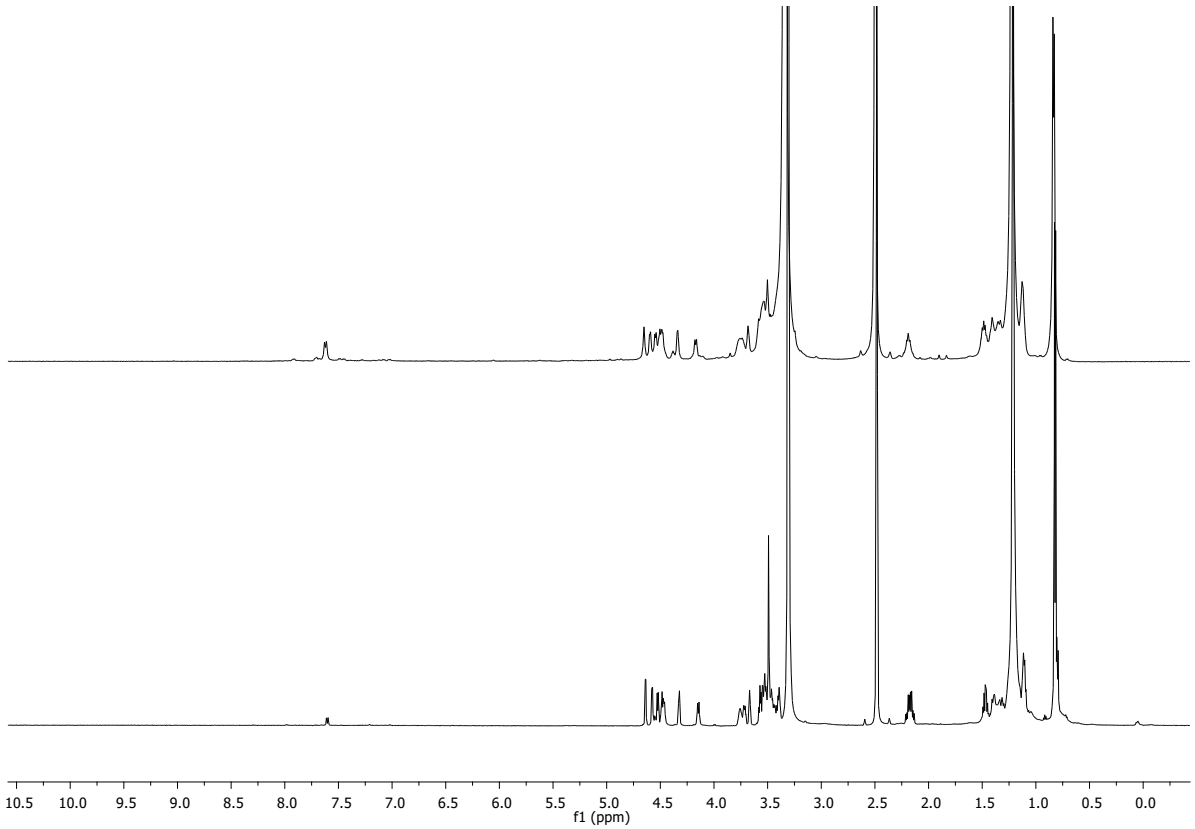
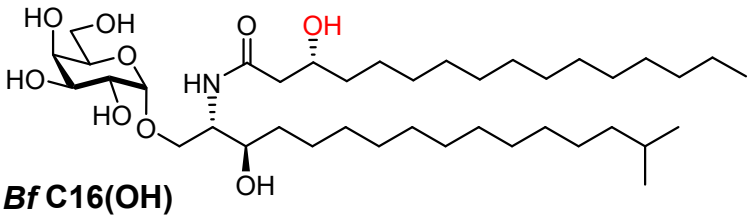
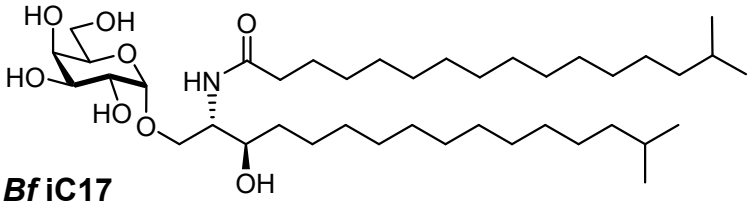


Figure S4. Comparison of high resolution ^{1}H NMR spectra of synthetic and natural *Bf* αGC.

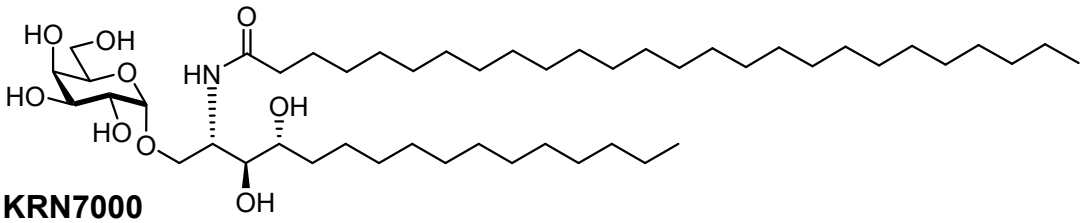
(Top) Synthetic material and (bottom) purified α-GalCer$_{Bf\text{-}716}$ extracted from *B. fragilis*, courtesy of Prof. John Clardy, Harvard University. Both spectra are at 600 MHz in d_6-DMSO.



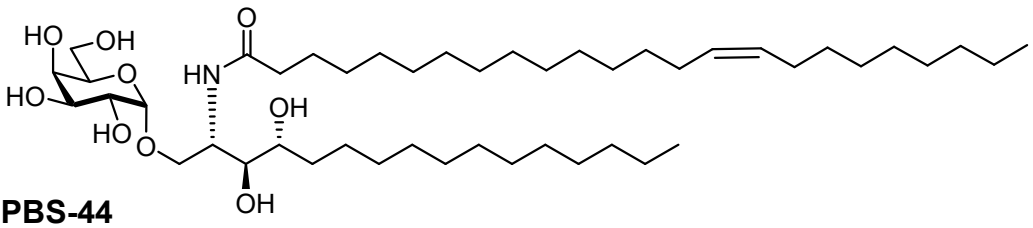
***Bf* C16(OH)**



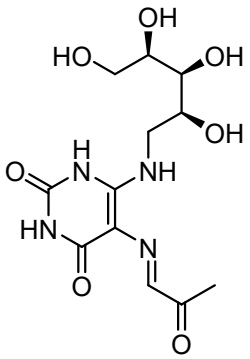
***Bf* iC17**



KRN7000



PBS-44



5-OP-RU

Figure S5. Structures of αGC analogues and 5-OP-RU.

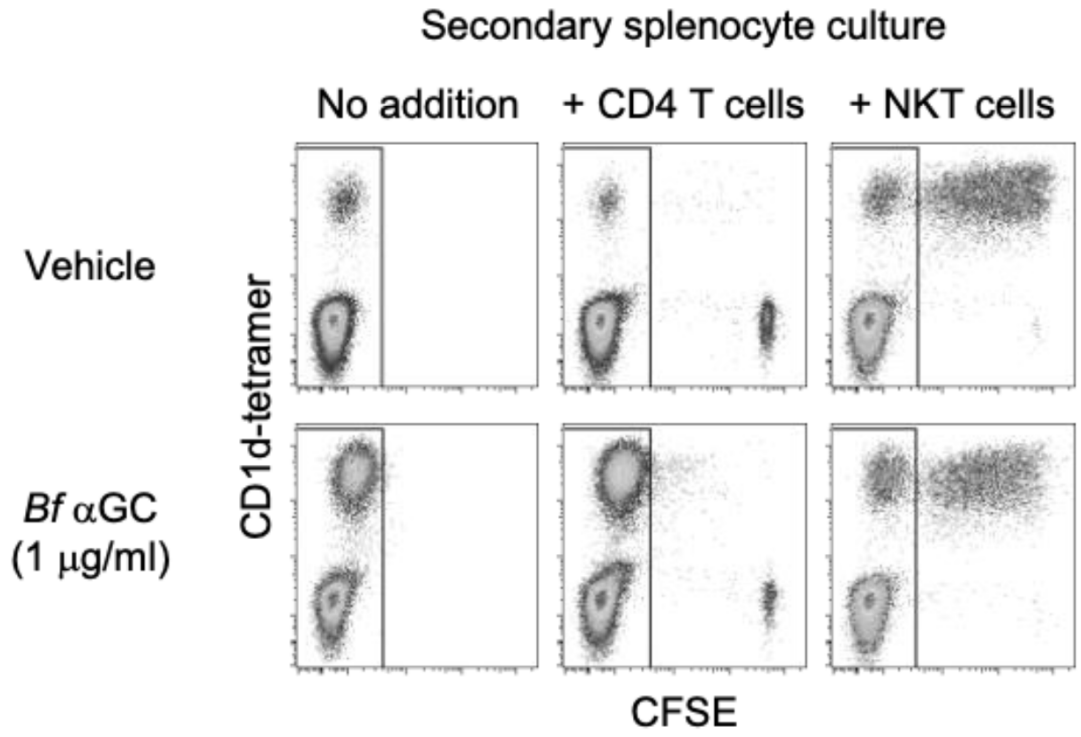


Figure S6. Gating strategy to exclude *Bf* αGC-experienced cells.

Table S1. Assignment and comparison of NMR data (600 MHz, d_6-DMSO) of synthetic *Bf* αGC and natural material extracted from *B. fragilis*.

Data from natural *Bf* αGC courtesy of Prof. John Clardy, Harvard University.

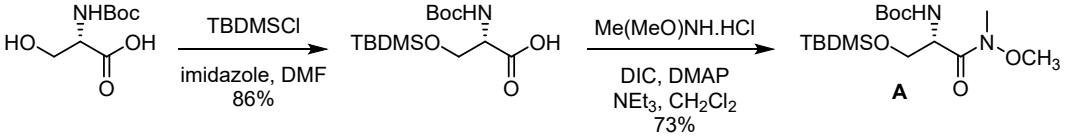
Hydrogen	^{1}H NMR data: δ ppm (multiplicity, *J*, integration)	
	Natural (Lit)[2]	Synthetic
NH	7. 60 (d, *J* = 9.1 Hz, 1 H)	7.61-7.63 (d, *J* = 9.0 Hz, 1 H)
a	4.64 (d, *J* = 3.3 Hz, 1 H)	4.65-4.66 (d, *J* = 3.2 Hz, 1 H)
3'-OH	4.58 (d, *J* = 4.9 Hz, 1 H)	4.59-4.60 (d, *J* = 4.7 Hz, 1 H)
3'-OH	4.52 (d, *J* = 6.4 Hz, 1 H)	4.53-4.54 (d, *J* = 6.3 Hz, 1 H)
d-OH	4.47 (d, *J* = 5.4 Hz, 1 H)	4.48-4.57 (d, *J* = 5.6 Hz, 1 H)
f-OH	4.48 (d, *J* = 5.4 Hz, 1 H)	4.48-4.57 (d, *J* = 4.9 Hz, 1 H)
c-OH	4.33 (d, *J* = 4.2 Hz, 1 H)	4.33-4.34 (d, *J* = 3.9 Hz, 1 H)
b-OH	4.15 (d, *J* = 7.6 Hz, 1 H)	4.15-4.17 (d, *J* = 7.4 Hz, 1 H)
b, c, d, e, f 1, 2, 3 3'	3.79-3.37 (m, 9 H)	3.41-3.76 (m, 9 H)
2'	2.17 (ddd, *J* = 26.3, 13.8, 6.6 Hz, 2 H)	2.15-2.24 (qd, *J* = 13.8, 6.5 Hz, 2 H)
4, 4'	1.38-1.43	1.46-1.52 (m, 4 H)
15, 15'	1.42 (m, 2 H)	1.41 (m, 2 H)
lipid	1.17-1.24 (m, 36 Hz)	1.12-1.19 (m, 36 H),
16, 16'	0.82 (d, *J* = 6.6 Hz, 12 H)	0.83-0.85 (d, *J* = 6.6 Hz, 12 H)

Chemistry Methods

General

Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al*.[2] Reactions were monitored using thin layer chromatography, performed with silica gel 60 F254. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, 10% phosphomolybdic acid in EtOH, and/or visualizing with UV light. Flash chromatography was performed using silica gel 60 according to the method of Still *et al*.[3] NMR experiments were conducted on 400 and 600 MHz instruments, with chemical shifts referenced relative to residual protiated solvent, and are in ppm. $^{1}H-^{1}H$ COSY spectra were used to confirm proton assignments. Mass spectra were acquired using an ESI quadrupole orbitrap.

1. Synthesis of Weinreib amide A



(2*S*)-2-*tert*-Butoxycarbonylamino-3-(*tert*-butyldimethylsilanyloxy)propionic acid

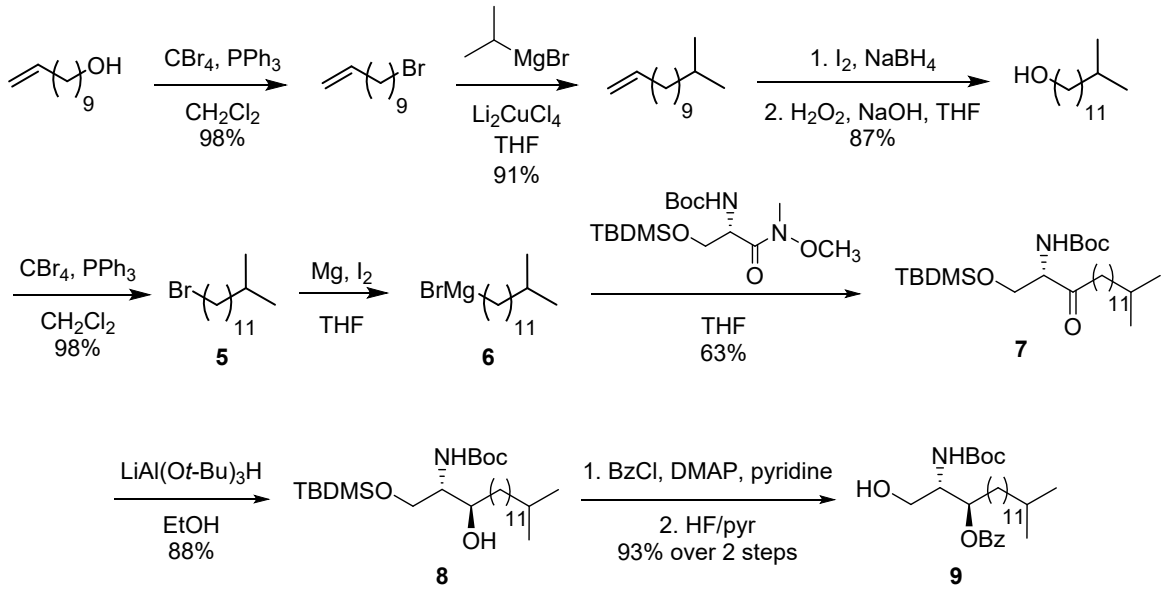
tert-Butyldimethylsilyl chloride (2.26 g, 15.0 mmol) was added to a mixture of (*S*)-*N*-(*tert*-butoxycarbonyl)serine (1.03 g, 5.00 mmol) and imidazole (1.02 g, 15.0 mmol) in dry DMF (25 mL) at 0 ºC and stirred overnight. The mixture was extracted with EtOAc, washed with water, aq. brine and dried ($MgSO_4$). The combined organic solvents were evaporated under reduced pressure and purified by flash chromatography (EtOAc) to afford (2*S*)-2-*tert*-butoxycarbonylamino-3-(*tert*-butyldimethylsilanyloxy)propionic acid as a colourless oil (1.39 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.32 (1 H, d, *J* = 8.0 Hz), 4.36 (1 H, bs), 4.11-4.09 (1 H, d, *J* = 7.5 Hz), 3.84-3.80 (1 H, dd, *J* = 10.0, 4.4 Hz), 1.46 (9 H, s), 0.88 (s, 9 H), 0.07 (3 H, s), 0.06 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 155.8, 80.5, 63.4, 55.2, 28.5, 26.0, 18.4, -5.4; HRMS (ESI) *m/z* calcd. for $[C_{14}H_{29}NO_4Si+H]^+$: 320.1889, obsd: 320.1887.

Methyl (2*S*)-*N*-methyl-2-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsilanyloxy)propanohydroxamate

DIC (1.01 mL, 0.82 g, 6.53 mmol) was added into a mixture of (2*S*)-2-*tert*-butoxycarbonylamino-3-(*tert*-butyldimethylsilanyloxy)propionic acid (1.39 g, 4.35 mmol),

Me(MeO)NH.HCl (0.64 g, 6.53 mmol), DMAP (0.11 g, 0.87 mmol) and NEt_3 (0.91 mL, 0.66 mg, 6.53 mmol) in dry CH_2Cl_2 (15 mL) at r.t and stirred overnight. The mixture was then filtered, the organic layer was extracted with EtOAc, washed with water, aq. brine, and dried ($MgSO_4$). The organic layer was concentrated and purified by flash chromatography (EtOAc) to afford methyl (2*S*)-*N*-methyl-2-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsilanyloxy)propanohydroxamate as the colourless oil (1.15 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.33 (1 H, d, *J* = 8.6 Hz), 4.73 (1 H, s), 3.82-3.83 (1 H, dd, *J* = 9.9, 7.4 Hz), 3.78-3.77 (1 H, dd, *J* = 10.0, 5.2 Hz), 3.73 (3 H, s), 3.19 (3 H, s), 1.41 (9 H, s), 0.84 (9 H, s), 0.01 (3 H, s), 0.00 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 155.5, 79.6, 63.6, 61.6, 52.6, 32.3, 28.5, 25.9, 18.4, -5.4; HRMS (ESI) *m/z* calcd. for $[C_{16}H_{35}N_2O_5Si+H]^+$: 363.2310, obsd: 363.2311.

2. Synthesis of protected sphinganine 9



10-Bromo-1-tridecene

A mixture of triphenylphosphine (7.87 g, 30.0 mmol), carbon tetrabromide (7.96 g, 24.0 mmol) and 9-decen-1-ol (3.41 g, 4.01 mL, 20.0 mmol) in CH_2Cl_2 (100 mL) was stirred at 0 ºC for 2 h. The solvent was evaporated under reduced pressure and triphenylphosphine oxide was precipitated with pet. spirits. The precipitate was removed by filtration and the filter cake was washed with pet. spirits. The combined organic solvents were concentrated and purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford 10-bromo-1-tridecene as a colourless oil (4.57 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86-5.76 (1 H, ddt, *J* = 17.0, 10.2, 6.7 Hz), 5.02-4.92 (2 H, dd, *J* = 17.0, 1.5 Hz), 3.42 (3 H, t, *J* = 6.9 Hz), 2.06-2.01 (2 H, q, *J* =

6.9 Hz), 1.88-1.81 (3 H, m), 1.44-1.36 (4 H, m), 1.29 (8 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 114.3, 34.2, 33.9, 33.0, 29.5, 29.2, 29.1, 28.9, 28.3.

11-Methyl-1-tridecene

A mixture of 10-bromo-1-tridecene (4.57 g, 19.6 mmol) in THF (100 mL) was cooled to -78 ºC. Isopropyl magnesium bromide (2.0 M in THF; 58.8 mmol, 29.4 mL) then Li_2CuCl_4 (0.1 M in THF; 0.23 mmol, 2.31 mL) were added dropwise into the reaction mixture. After the addition, the reaction was warmed to r.t and stirred overnight. Saturated aqueous NH_4Cl was added to quench the excess of the reagents. The organic layer was extracted with pet. spirits, washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The combined organic layers were evaporated under reduced pressure and the crude material was purified by flash chromatography (pet. spirits) to afford 11-methyl-1-tridecene as a colourless oil (3.50 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87-5.77 (1 H, ddt, *J* = 16.9, 10.2, 6.7 Hz), 5.02-4.92 (2 H, dd, *J* = 17.1, 1.4 Hz), 2.07-2.02 (2 H, dd, *J* = 14.2, 6.9 Hz), 1.54-1.49 (1 H, m), 1.38 (1 H, m), 1.27-1.26 (12 H, m), 1.16-1.14 (2 H, m), 0.87-0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.4, 114.2, 39.2, 34.0, 30.1, 29.9, 29.8, 29.7, 29.3, 29.1, 28.1, 27.6, 22.8.

12-Methyl-1-tridecanol

Iodine (5.42 g, 21.4 mmol) in dry THF (40 mL) was added dropwise into a stirring mixture of $NaBH_4$ (1.68 g, 44.5 mmol) in dry THF (20 mL) at 0 ºC, which was kept at this temperature for 2.5 h. The mixture was then heated under reflux for 1 h until the brown colour disappeared. 11-Methyl-1-dodecene (3.50 g, 17.8 mmol) in dry THF (20 mL) was added dropwise into the colourless mixture at 0 ºC. The reaction was warmed to r.t and stirred overnight. The mixture was then cooled to 0 ºC again, and a mixture of 30% aq. H_2O_2/ 3 N aq. NaOH (1:1) (60 mL) was carefully added in portions with continued cooling. **Caution:** each addition released a large volume of gas in an exothermic reaction. The mixture was extracted with EtOAc (3 ×), washed with water, aq. $NaHCO_3$, aq. brine, dried ($MgSO_4$) and concentrated under reduced pressure to afford a light-yellow liquid. Purification by flash chromatography (EtOAc:pet. spirits, 20:80) afforded 12-methyl-1-tridecanol as the light yellow liquid (3.40 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.61 (2 H, t, *J* = 6.6 Hz), 1.57-1.47 (3 H, m), 1.25 (16 H, m), 1.15 (2 H, m), 0.85-0.86 (6 H, d, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.3, 39.2, 33.0, 30.1, 29.9, 29.8, 29.77, 29.76, 29.6, 28.1, 27.6, 29.5, 22.8.

1-Bromo-12-methyltridecane (5)

A mixture of triphenylphosphine (5.74 g, 21.9 mmol), tetrabromomethane (5.80 g, 17.5 mmol) and 12-methyl-1-tridecanol (3.40 g, 15.9 mmol) in dry CH_2Cl_2 (100 mL) was stirred at 0 ºC for 2 h. The solvent was concentrated and triphenylphosphine oxide was precipitated with pet. spirits. The mixture was filtered, and the filtercake was washed with pet. spirits. The combined organic layers were concentrated and the residue was purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford 1-bromo-12-methyltridecane **5** as the colourless oil (4.32 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.42-3.39 (2 H, t, *J* = 6.9 Hz), 1.89-1.82 (2 H, m), 1.53-1.49 (1 H, m), 1.42 (2 H, m), 1.26 (15 H, m), 1.16 (2 H, m), 0.85-0.87 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.2, 34.2, 33.0, 30.1, 29.8, 29.8, 29.7, 29.6, 28.9, 28.4, 28.1, 27.6, 22.8.

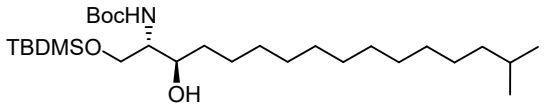
2-(*S*)-*tert*-Butyloxycarbonylamino-1-((*tert*-butyldimethylsilyl)oxy)-15-methyl-3-oxohexadecane (7)

(i) 12-Methyltridecane magnesium bromide (6)

1-Bromo-12-methyltridecane **5** (3.97 g, 14.3 mmol) in dry THF was added slowly to a mixture of Mg (3.47 g, 0.143 mol; pre-activated by stirring overnight with I_2 (100 mg)), at such a rate as to minimize a temperature rise. The mixture was stirred at r.t for 1 h then heated under reflux for 2 h. The mixture was used immediately in the next reaction.

(ii) (*S*)-2-*tert*-Butyloxycarbonylamino-1-((*tert*-butyldimethylsilyl)oxy)-15-methyl-3-oxohexadecane (7)

Weinreb amide **A** (1.04 g, 2.86 mmol) in dry THF (28 mL) was added slowly to the mixture of freshly prepared 12-methyltridecanyl magnesium bromide **6** at -15 ºC. Reaction was slowly warmed to rt and then was stirred overnight. Aq. HCl (1 M) was added dropwise to quench excess Grignard reagent. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The combined organic layers were concentrated and purified by flash chromatography (EtOAc:pet. spirits, 5:95) to afford **7** as a colourless oil (0.901 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49-5.48 (1 H, d, *J* = 7.3 Hz), 4.27-4.06 (1 H, bs), 4.05-4.03 (1 H, dd, *J* = 10.3, 2.6 Hz), 3.83-3.80 (1 H, dd, *J* = 10.3, 3.8 Hz), 2.59-2.54 (1 H, ddt, *J* = 4.2, 17.3, 7.4 Hz), 2.49-2.44 (1 H, ddt, *J* = 4.2, 17.3, 7.4 Hz), 1.57-1.50 (3 H, m), 1.45 (9 H, s), 1.25 (18 H, m), 1.14-1.15 (2 H, m), 0.85-0.87 (6 H, d, *J* = 6.1 Hz), 0.85 (9 H, s), 0.02 (6 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 155.5, 79.9, 63.6, 61.4, 40.3, 39.2, 30.1, 29.86, 29.81, 29.76, 29.61, 29.56, 29.4, 28.5, 28.1, 27.6, 25.9, 23.5, 22.8, 18.3, -5.4; HRMS (ESI) *m/z* calcd. for $[C_{28}H_{57}NO_4Si+H]^+$: 500.4130, obsd: 500.4138.



(2*S*,3*R*)-2-*tert*-Butyloxycarbonylamino-1-((*tert*-butyldimethylsilyl)oxy)-15-methyl-3-hydroxyhexadecane (8)

Lithium tri-*tert*-butoxyaluminium hydride (1.01 g, 3.96 mmol) was added slowly into a solution of *tert*-butyl-(*S*)-(1-(((*tert*-butyldimethosilyl)oxy)-15-methyl-3-oxohexadecan-2-yl) carbamate **7** (0.901 g, 1.80 mmol) in dry EtOH (18 mL) at -78 ºC. The reaction was warmed to r.t and stirred for 4 h. The mixture was filtered, diluted with EtOAc, washed with aq. brine and dried ($MgSO_4$). The combined organic layers were concentrated and purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford the secondary alcohol **8** as a colourless oil (0.796 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33-5.31 (1 H, d, *J* = 7.8 Hz), 3.97-3.94 (1 H, dd, *J* = 10.6, 2.7 Hz), 3.83 (1 H, d, *J* = 10.1 Hz). 3.64-3.62 (1 H, dd, *J* = 8.4, 4.2 Hz), 3.50 (1 H, bs), 3.04-3.02 (1 H, d, *J* = 8.8 Hz), 1.53-1.50 (4 H, m), 1.45 (9 H, s), 1.25 (19 H, m), 1.15 (2 H, m), 0.90 (9 H, s), 0.87-0.85 (6 H, d, *J* = 6.6 Hz), 0.08 (6 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 79.5, 74.5, 63.7, 53.9, 39.2, 35.1, 30.1, 29.9, 29.8, 29.81, 29.75, 29.72, 28.6, 28.1, 27.6, 26.1, 26.0, 22.8, 18.3, -5.4, -5.0; HRMS (ESI) *m/z* calcd. for $[C_{28}H_{59}NO_4Si+H]^+$: 502.4286, obsd: 502.4293.

(2*S*,3*R*)-2-*tert*-Butoxycarbonylamino-1-(*tert*-butyldimethylsilyl)oxy-15-methylhexadecan-3-yl benzoate

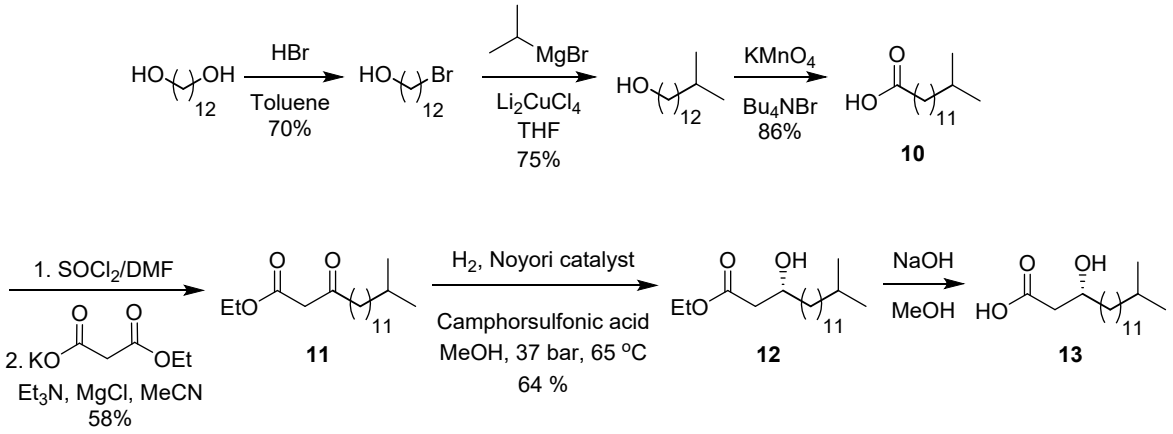
Benzoyl chloride (0.891 g, 6.34 mmol, 0.736 mL) was added to a solution of (2*S*,3*R*)-2-*tert*-Butyloxycarbonylamino-1-((*tert*-butyldimethylsilyl)oxy)-15-methyl-3-hydroxyhexadecane **8** (0.796 g, 1.59 mmol) and DMAP (97.1 mg, 0.80 mmol) in dry pyridine (2 mL) at r.t and stirred over 3 h. The reaction was extracted with EtOAc, washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The combined organic layers were concentrated and the residue was purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford the benzoate as a colourless oil (0.944 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04-8.03 (2 H, d, *J* = 7.8 Hz), 7.57-7.54 (1 H, t, *J* = 7.4 Hz), 7.45-7.42 (2 H, d, *J* = 7.7 Hz), 5.20 (1 H, s), 4.93-4.91 (2 H, d, *J* = 9.1 Hz), 3.97 (1 H, s), 3.75-3.73 (1 H, dd, *J* = 10.2, 3.5 Hz), 3.69-3.66 (1 H, dd, *J* = 10.2, 4.3 Hz), 1.53-1.47 (1 H, m), 1.44 (9 H, s), 1.25-1.22 (21 H, m), 1.16-1.13 (2 H, m), 0.87 (9 H, s), 0.86-0.85 (6 H, d, *J* = 6.6 Hz), 0.00 (3 H, s), -0.02 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 155.7, 133.0, 130.6, 129.8. 128.5, 79.6, 74.4, 62.1, 53.9, 39.2, 31.3, 30.1, 29.9, 29.80, 29.77, 29.73, 29.6, 29.5, 28.5,

28.1, 27.6, 26.0, 25.4, 22.8, 18.4, 14.1, -5.4, -5.5; HRMS (ESI) *m/z* calcd. for $[C_{35}H_{63}NO_5Si+H]^+$: 606.4548, obsd: 606.4557.

(2*S*,3*R*)-2-*tert*-Butoxycarbonylamino-1-hydroxy-15-methylhexadecan-3-yl benzoate (9)

A solution of (2*S*,3*R*)-2-*tert*-butoxycarbonylamino-1-(*tert*-butyldimethylsilyl)oxy-15-methylhexadecan-3-yl benzoate (0.944 g, 1.56 mmol) in dry THF (16 mL) was added dropwise into a solution of HF/pyr (70:30 v/v; 82.7 mmol, 2.15 mL), in dry pyridine (9.27 mL) at 0 ºC and stirred overnight. The reaction was quenched by slowly addition of aq. $NaHCO_3$, extracted with EtOAc, washed with aq. brine and dried ($MgSO_4$). The combined organic layers were concentrated and purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford sphinganine alcohol **9** as a colourless oil (0.729 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06-8.04 (2 H, d, *J* = 7.8 Hz), 7.62-7.58 (1 H, t, *J* = 7.4 Hz), 7.49-7.45 (2 H, t, *J* = 7.7 Hz), 5.23-5.21 (1 H, d, *J* = 9.1 Hz), 5.08-5.04 (1 H, dd, *J* = 13.5, 7.1 Hz), 3.82 (1 H, m), 3.64 (2 H, bs), 2.84 (1 H, bs), 1.77-1.81 (2 H, m), 1.50 (1 H, m), 1.45 (9 H, s), 1.29-1.22 (18 H, bs), 1.14-1.13 (2 H, m), 0.86-0.85 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 156.0, 133.7, 130.0, 129.6, 128.7, 80.0, 74.4, 61.9, 54.6, 39.2, 31.5, 30.1, 29.9, 29.79, 29.77, 29.68, 29.55, 29.50, 28.5, 28.1, 27.6, 25.7, 22.8, 14.0; HRMS (ESI) *m/z* calcd. for $[C_{29}H_{49}NO_5+H]^+$: 492.3684, obsd: 492.3689.

3. Synthesis of hydroxy-fatty acid 13



12-Bromododecan-1-ol

Aqueous HBr (48% w/v, 3.58 mL, 31.6 mmol) was added to a solution of 1,12-dodecanediol (4.00 g, 19.8 mmol) in toluene (100 mL). The mixture was heated under reflux for 72 h. The solution was cooled to r.t and diluted with Et_2O, washed with aq. NaOH (2 M), followed by aq. HCl (10%), water and aq. brine, dried ($MgSO_4$) and concentrated. Purification of the residue

by flash chromatography (EtOAc:pet. spirits, 20:80) afforded 12-bromododecan-1-ol as a white solid (3.62 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.63 (2 H, t, *J* = 6.6 Hz), 3.42-3.39 (2 H, t, *J* = 6.9 Hz), 1.87-1.83 (2 H, m), 1.58-1.54 (2 H, m), 1.44-1.39 (2 H, m), 1.36-1.24 (14 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.2, 34.2, 32.98, 32.95, 29.70, 29.67, 29.65, 29.6, 28.9, 28.3, 25.9.

13-Methyltetradecan-1-ol

Isopropylmagnesium bromide (2 M in THF; 9.42 mmol, 4.70 mL) and dilithium tetrachlorocuprate (0.1 M in THF; 0.0220 mmol, 0.220 mL) was added into a stirred mixture of 12-bromododecan-1-ol (0.500 g, 1.89 mmol) in dry Et_2O (5 mL) at -78 ºC. The mixture was warmed to r.t and stirred overnight. The mixture was then quenched with sat. NH_4Cl and extracted with EtOAc. The combined organic extracted were washed with water, aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The organic layer was concentrated under reduced pressure to give a colourless oil. Purification of the oil by flash chromatography (EtOAc:pet. spirits, 20:80) afforded 13-methyltetradecan-1-ol as a colourless oil (0.324 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.63 (2 H, t, *J* = 6.6 Hz), 1.58-1.50 (3 H, m), 1.32-1.22 (18 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.2, 39.1, 32.9, 30.0, 29.70, 29.74, 29.73, 29.71, 29.66, 29.6, 29.5, 28.0, 27.5, 25.8, 22.7.

13-Methyltetradecanoic acid (10)

A mixture of 13-methyltetradecan-1-ol (0.324 g, 1.42 mmol), tetrabutylammonium bromide (0.229 g, 0.710 mmol), $KMnO_4$ (1.11 g, 7.02 mmol), AcOH (4.00 ml), water (14.0 mL) and CH_2Cl_2 (14.0 mL) was heated under reflux for 16 h. The dark purple mixture was cooled to r.t then acidified by the addition of aq. HCl (3 M). Small portions of Na_2SO_3 were added carefully to discharge the colour. The organic phase was collected and the remaining aqueous phase was extracted with CH_2Cl_2 and dried ($MgSO_4$), filtered and evaporated under reduced pressure. Purification of the residue by flash chromatography (EtOAc:pet. spirits, 20:80) afforded 13-methylpentadodecanoic acid **10** as a white solid (0.288 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35-2.33 (2 H, t, *J* = 7.5 Hz), 1.64 (2 H, m), 1.50 (1 H, m), 1.24-1.26 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5, 39.2, 34.1, 30.1, 29.85, 29.80, 29.75, 29.7, 29.4, 29.2, 28.1, 27.6, 24.8, 22.8.

13-Methyltetradecanoyl chloride

A mixture of 13-methyltetradecanoic acid **10** (0.288 g, 1.19 mmol), DMF (0.25 ml), and thionyl chloride (3.00 ml) was heated under reflux for 2 h. Thionyl chloride was removed by evaporation to afford 13-methyltetradecanoyl chloride as a colourless oil that was used immediately without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89-2.86 (2 H, t, *J* = 7.1 Hz), 1.64 (2 H, m), 1.50 (1 H, m), 1.26-1.24 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz).

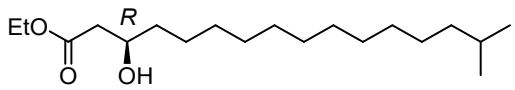
Ethyl 15-methyl-3-oxohexadecanoate (11)

Et_3N (0.133 g, 0.183 mL, 1.31 mmol) and $MgCl_2$ (0.283 g, 2.98 mmol) was added to a solution of potassium ethyl malonate (0.425 mg, 2.50 mmol) in dry MeCN (11.9 mL) stirred under nitrogen. Stirring was continued at r.t for 2 h and then the solution was cooled to 0 ºC and 13-methyltetradecanoyl chloride (0.310 g, 1.19 mmol) in dry MeCN (11.9 mL) was added dropwise. The mixture was warmed to r.t and stirred for 16 h. It then was cooled to 0 ºC, diluted with aq. HCl (1 M) and extracted with Et_2O (3 times). The combined organic layers were washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The solvent was evaporated and purified by flash chromatography (EtOAc:pet. spirits, 20:80) to afford the title compound as a white solid (0.215 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21-4.17 (2 H, q, *J* = 7.1 Hz), 3.42 (2 H, s), 2.54-2.51 (2 H, t, *J* = 7.4 Hz), 1.64 (2 H, m), 1.50 (1 H, m), 1.24-1.26 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 170.4, 61.2, 49.3, 43.2, 38.2, 30.1, 29.9, 29.82, 29.81, 29.72, 29.67, 28.1, 27.6, 25.6, 22.8.

Procedure for stereoselective ketone reduction with Noyori catalyst

Chloro[2,2'-bis(diphenylphosphino)-1,1'-binaphthyl](*p*-cymene) ruthenium(II) chloride (0.01 eq) and camphorsulfonic acid (1 eq) were added to a solution of ethyl 15-methyl-3-oxohexadecanoate (**11**) (1 eq) in dry methanol (10 mL mmol^{-1}). The solution was placed under H_2 at 39 atm, and stirred at 60 ºC for 3 d. The solvent was evaporated and the crude material was purified by flash chromatography (EtOAc:pet. spirits, 10:90) to afford the alcohol.

Ethyl-(*R*)-3-hydroxy-15-methylhexadecanoate (12)



Applying the general procedure for reduction of ethyl-15-methyl-3-oxohexadecanoate **11** (0.100 g, 0.320 mmol) with chloro[(*R*)-2,2'-bis(diphenylphosphino)-1-1'-binaphthyl](*p*-cymene) ruthenium(II) chloride) (0.0030 g, 0.0032 mmol) after flash chromatography afforded

ethyl (*R*)-3-hydroxy-15-methylhexadecanoate **12** as a white solid (0.0644 g, 64%). $[\propto]_D^{25°C}$ = - 11.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (1 H, m), 3.72 (2 H, s), 2.54-2.50 (1 H, dd, *J* = 16.4, 3.1 Hz), 2.44-2.39 (1 H, dd, *J* = 16.4, 9.0 Hz), 1.50 (2 H, m), 1.43 (2 H, m), 1.24-1.26 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 68.2, 51.9, 41.2, 39.2, 36.7, 30.1, 29.9, 29.82, 29.81, 29.72, 29.67, 28.1, 27.6, 25.6, 22.8.

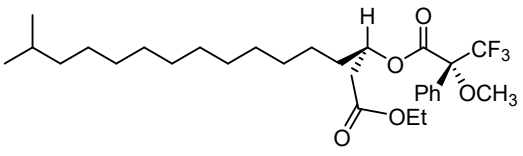
Ethyl-(*S*)-3-hydroxy-15-methylhexadecanoate

Applying the general procedure for reduction of ethyl-15-methyl-3-oxohexadecanoate **11** (0.078 g, 0.248 mmol) and chloro[(*S*)-2,2'-bis(diphenylphosphino)-1-1'-binaphthyl](*p*-cymene) ruthenium(II) chloride) (0.0023 g, 0.0025 mmol) after flash chromatography afforded ethyl-(*S*)-3-hydroxy-15-methylhexadecanoate as a white solid (0.0500 g, 64%). $[\propto]_D^{25°C}$ = + 11.3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (1 H, m), 3.71 (2 H, s), 2.53-2.49 (1 H, dd, *J* = 16.4, 3.1 Hz), 2.37-2.43 (1 H, dd, *J* = 16.4, 9.0 Hz), 1.50 (2 H, m), 1.43 (2 H, m), 1.24-1.26 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 68.3, 51.9, 41.3, 39.3, 36.7, 30.3, 29.9, 29.82, 29.81, 29.72, 29.67, 28.1, 27.6, 25.6, 22.8.

General procedure for synthesis of Mosher's ester

A mixture of (*R*)- or (*S*)-hydroxyl ester (1 eq), (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (1.1 eq), DCC (1.1 eq) and DMAP (1.1 eq) in dry CH_2Cl_2 (10 mL mmol^{-1}) was stirred at r.t for 17 h. Aq. HCl (1 M) was added dropwise to the stirred mixture, which was extracted with CH_2Cl_2 (3 times), washed with water, aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure and purification by flash chromatography (EtOAc:pet. spirits, 10:90) gave a white solid product.

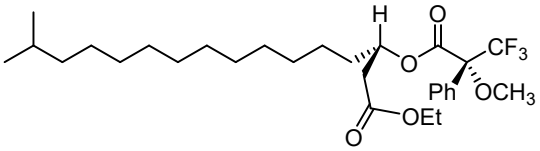
Ethyl (*R*)-15-methyl-3-(((*R*)-α-methoxy-α-trifluoromethylphenylacetyl)oxy)hexadecanoate



Applying the General procedure for Synthesis of Mosher's ester with ethyl (*R*)-3-hydroxy-15-methylhexadecanoate **12** (0.0500 g, 0.159 mmol) and (*R*)-Mosher's acid (40.9 mg, 0.175 mmol) after flash chromatography afforded the (*R*,*R*)-Mosher ester as a white solid (0.0548 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (2 H, m), 7.39-7.37 (3 H, m), 5.47 (1 H, m), 3.66 (2

H, s), 2.72-2.66 (1 H, dd, *J* = 15.9, 8.2 Hz), 2.62-2.57 (1 H, dd, *J* = 15.9, 4.7 Hz), 1.50 (2 H, m), 1.43 (2 H, m), 1.26-1.24 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 166.1, 132.5, 129.7, 128.5, 127.5, 73.5, 55.6, 52.0, 39.2, 38.7, 33.8, 30.1, 29.9, 29.80, 29.75, 29.6, 29.5, 29.3, 28.1, 27.6, 24.8, 22.8.

Ethyl (*S*)-15-methyl-3-(((*R*)α-methoxy-α-trifluoromethylphenylacetoyl)oxy)hexadecanoate

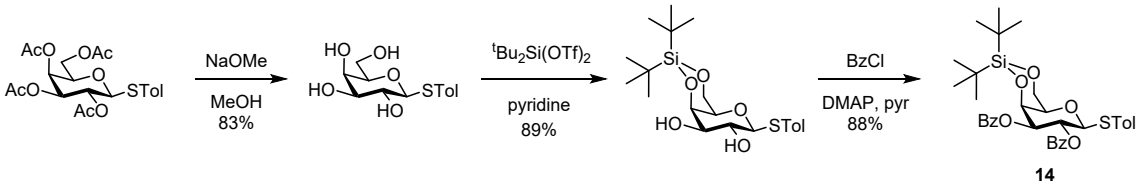


Applying the General procedure for Synthesis of Mosher's ester with ethyl (*S*)-3-hydroxy-15-methylhexadecanoate **315b** (0.0500 g, 0.159 mmol) with the (*R*)-Mosher's acid (40.9 mg, 0.175 mmol) after flash chromatography afforded the (*S*,*R*)-Mosher ester as a white solid (0.0548 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (2 H, m), 7.42-7.38 (3 H, m), 5.47 (1 H, m), 3.58 (2 H, s), 2.65-2.61 (1 H, dd, *J* = 15.9, 8.0 Hz), 2.58-2.55 (1 H, dd, *J* = 15.9, 5.0 Hz), 1.50 (2 H, m), 1.43 (2 H, m), 1.24-1.26 (16 H, m), 1.15 (2 H, m), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 166.0, 129.8, 129.7, 128.9, 128.5, 127.6, 127.0, 73.6, 55.9, 39.2, 38.6, 35.1, 34.1, 33.9, 30.1, 29.8, 29.79, 29.75, 29.62, 29.55, 29.4, 28.1, 27.6, 22.8.

(*R*)-3-Hydroxy-15-methylhexadecanoic acid (13)

Aq. NaOH (1 M, 0.112 mmol, 0.112 mL) was added dropwise into the mixture of ethyl (*R*)-3-hydroxy-15-methylhexadecanoate **12** (0.0353 g, 0.112 mmol) in MeOH (1.1 mL) at 0 ºC and stirred for 4 h. When the reaction was finished, aq. HCl (1 M) was added dropwise to quench excess NaOH. The mixture was extracted with Et_2O (3 ×), washed with aq. brine and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography (EtOAc:pet. spirits, 20:80) afforded (*R*)-3-hydroxy-15-methylhexadecanoic acid **316** as a white solid (32.1 mg, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (1 H, m), 2.58-2.54 (1 H, dd, *J* = 16.6, 3.0 Hz), 2.50-2.47 (1 H, dd, *J* = 16.6, 9.0 Hz), 1.54-1.43 (3 H, m), 1.28-1.26 (20 H, m), 1.15-1.14 (3 H, m) 0.89-0.87 (3 H, t, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 68.0, 41.1, 39.1, 36.5, 30.0, 29.72, 29.68, 29.66, 29.59, 29.57, 29.5, 28.0, 27.4, 25.5, 22.7; HRMS (ESI) *m/z* calcd. for $[C_{17}H_{34}O_3+H]^+$: 287.2581, obsd: 287.2579.

4. Synthesis of galactosyl donor 14



14

4-Methylphenyl 1-thio-β-D-galactopyranoside

NaOMe (25% wt) was added dropwise into the mixture of 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[1] (7.00 g, 15.4 mmol) in dry MeOH (77 mL) to adjust to pH = 10. The reaction was stirred overnight and then Dowex H^+ was added to neutralize the reaction. The mixture was filtered and washed with MeOH. The solvent was evaporated under pressure and the residue was recrystallised with EtOAc containing a small amount of MeOH to afford 4-methylphenyl 1-thio-β-D-galactopyranoside as a white solid (3.66 g, 83%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (2 H, d, *J* = 8.0 Hz), 7.11 (2 H, d, *J* = 8.0 Hz), 4.50 (1 H, d, *J* = 9.6 Hz, H1), 3.88 (1 H, d, *J* = 2.4 Hz, H4), 3.68-3.77 (2 H, m, H6a,6b), 3.51-3.60 (2 H, m, H2,5), 3.48 (1 H, dd, *J* = 9.2, 3.3 Hz, H3), 2.31 (3 H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 138.4, 132.9, 132.1, 130.5, 90.7. 80.6, 76.4, 71.0, 70.4, 62.6, 21.1.

4-Methylphenyl-4,6-*O*-di-*tert*-butylsilylene-1-thio-β-D-galactopyranoside

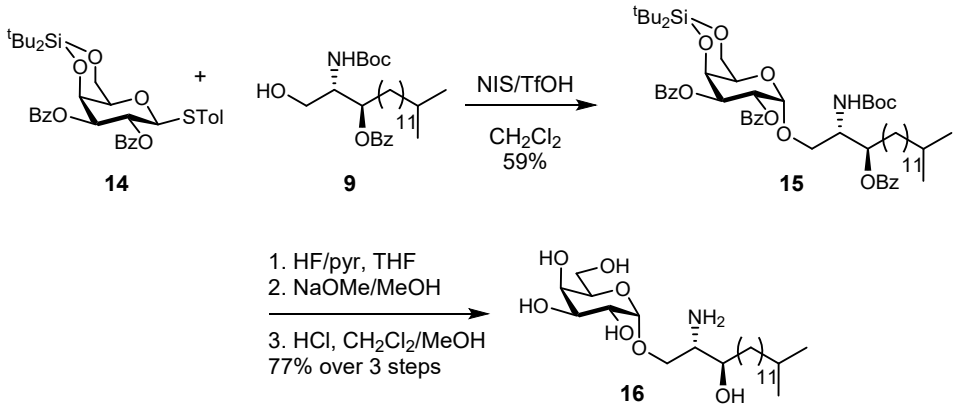
Di-*tert*-butylsilyl-bis(trifluoromethanesulfonate) (1.69 g, 3.84 mmol, 1.25 mL) was added dropwise into the mixture of 4-methylphenyl 1-thio-β-D-galactopyranoside (1.00 g, 3.49 mmol) in dry pyridine (35 mL) at 0 ºC over 5 min and stirring continued for 10 min at same temperature. MeOH was added dropwise to quench excess ^{t}Bu$_2$Si(OTf)$_2$. The solvent was evaporated and the residue was dissolved in EtOAc, washed with water, aq. brine and dried ($MgSO_4$). The combined organic solvents were concentrated and the residue was purified by flash chromatography (EtOAc) to afford the diol as a colourless oil (1.33 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (2 H, d, *J* = 8.0 Hz), 7.09 (2 H, d, *J* = 7.9 Hz), 4.47 (1 H, d, *J* = 9.8 Hz, H1), 4.42 (1 H, d, *J* = 3.2 Hz, H4), 4.25 (2 H, s, H6a,6b), 3.71 (1 H, t, *J* = 9.3 Hz, H2), 3.51 (1 H, dd, *J* = 8.5, 2.5 Hz, H3), 3.42 (1 H, s, H5), 2.34 (3 H, s), 1.05 (9 H, s), 1.02 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 133.5, 129.9, 119.2, 89.6, 75.4, 75.2, 72.7, 70.8, 67.2, 27.7, 27.6, 23.5, 21.3, 20.8; HRMS (ESI) *m/z* calcd. for $[C_{14}H_{27}O_5Si+H]^+$: 427.1969, obsd: 427.1971.

4-Methylphenyl 2,3-*O*-di-benzoyl-4,6-*O*-di-*tert*-butylsilylene-1-thio-β-D-galactopyranoside (14)

DMAP (0.19 g, 1.55 mmol) and benzoyl chloride (2.18 g, 15.5 mmol, 1.80 mL) were added slowly into a mixture of the diol (1.33 g, 3.10 mmol) in dry pyridine (15 mL) and stirred at r.t

over 3 h. The mixture was extracted with EtOAc, washed with water, aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The combined organic solvent was concentrated and the residue was purified by flash chromatography (EtOAc) to afford dibenzoate **14** as the colourless oil (1.73 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (2 H, d, *J* = 7.2 Hz), 7.54-7.49 (2 H, m), 7.37-7.41 (6 H, m), 7.07 (2 H, d, *J* = 7.8 Hz), 5.90 (1 H, t, *J* = 10.0 Hz), 5.18 (1 H, dd, *J* = 9.8, 1.9 Hz), 4.89-4.86 (2 H, m), 4.32-4.30 (2 H, d, *J* = 4.7 Hz), 3.62 (1 H, s), 2.32 (3 H, s), 1.16-1.15 (d, 9 H, *J* = 1.0 Hz), 0.96-0.95 (d, 9 H, *J* = 0.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 165.6, 138.3, 133.4, 133.34, 133.27, 130.1, 129.6, 129.94, 129.90, 129.6, 128.53, 128.48, 88.0, 75.7, 75.1, 70.6, 68.4, 67.3, 27.65, 27.60, 23.4, 21.3, 20.9; HRMS (ESI) *m/z* calcd. for $[C_{28}H_{35}O_7Si+H]^+$: 635.2504, obsd: 635.2496.

5. Synthesis of galactosyl sphinganine 16



(2*S*,3*R*)-3-(Benzoyloxy)-2-((*tert*-butoxycarbonyl)amino)-15-methylhexadecanyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-β-D-galactopyranoside (15)

Thioglycoside **14** (0.586 g, 1.14 mmol) and the sphinganine alcohol **9** (0.469 g, 0.954 mmol) were co-evaporated twice with dry toluene (2.00 mL) and then dissolved in dry CH_2Cl_2. Activated molecular sieves (3 Å) was added and the mixture was stirred at r.t for 30 min. *N*-Iodosuccinimide (300 mg, 1.34 mmol) was added at r.t. After 5 min, the mixture was cooled to 0 ºC before TfOH (42.9 mg, 0.286 mmol, 25 μL) was added dropwise; the mixture slowly turned dark pink and red. The reaction was stirred at 0 ºC for 2 h, then was diluted with CH_2Cl_2, and quenched with aq. $Na_2S_2O_3$, aq. $NaHCO_3$, washed with water, aq. brine and dried ($MgSO_4$). The organic layers were combined and concentrated. The residue was purified by flash chromatography (EtOAc:toluene, 5:95) to afford **15** as a colourless oil (0.564 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-7.96 (6 H, m), 7.55-7.48 (3 H, m), 7.33-7.42 (6 H, m), 5.60 (1 H, dd, *J* = 3.7, 10.6 Hz), 5.53 (1 H, dd, *J* = 3.1, 10.6 Hz), 5.25 (1 H, d, *J* = 3.2 Hz), 5.17 (1 H, m), 4.98 (1 H, d, *J* = 8.9 Hz), 4.88 (1 H, d, *J* = 2.9 Hz), 4.21-4.15 (2 H, m), 4.12-4.07 (1 H, m),

3.90 (1 H, s), 3.82 (1 H, dd, *J* = 4.6, 10.5 Hz), 3.61 (1 H, dd, *J* = 10.7, 4.9 Hz), 1.49-1.51 (1 H, m), 1.43 (9 H, s), 1.21-1.18 (19 H, m), 1.14-1.13 (3 H, m), 1.11 (9 H, s), 0.95 (9 H, s), 0.86 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 166.2, 166.0, 155.5, 133.3, 133.2, 130.1, 130.0, 129.89, 129.85, 129.80, 129.1, 128.8, 128.57, 128.51, 128.47, 97.8, 80.0, 79.4, 74.8, 71.3, 71.1, 68.5, 67.8, 67.4, 67.0, 39.2, 30.1, 29.9, 29.82, 29.78, 29.7, 29.63, 29.59, 28.5, 28.1, 27.64, 27.57, 27.4, 25.5, 23.4, 22.8, 20.9, 14.3; HRMS (ESI) *m/z* calcd. for $[C_{57}H_{83}NO_{12}Si+H]^+$: 1002.5757, obsd: 1002.5765.

(2*S*,3*R*)-3-(Benzoyloxy)-2-((*tert*-butoxycarbonyl)amino)-15-methylhexadecanyl 2,3-di-*O*-benzoyl-β-D-galactopyranoside

HF/pyr (70:30 v/v; 70.4 mmol, 1.83 mL) was added dropwise into a stirred mixture of compound **15** (0.564 g, 0.563 mmol) in dry THF (5.6 mL) at 0 ºC and stirring continued for 4 h. The reaction was quenched by slow addition of aq. $NaHCO_3$, diluted with EtOAc, washed with water, aq. brine and dried ($MgSO_4$). The organic layer was concentrated and the residue was purified by flash chromatography (EtOAc:CH_2Cl_2, 30:70) to afford the product as a colourless oil (0.412 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00-7.94 (6 H, m), 7.57-7.47 (3 H, m), 7.44-7.31 (6 H, m), 5.71 (1 H, dd, *J* = 10.7, 3.3 Hz), 5.65 (1 H, dd, *J*-10.7, 2.6 Hz), 5.30 (1 H, d, *J* = 3.0 Hz), 5.20-5.17 (1 H, m), 5.05 (1 H, d, *J* = 9.2 Hz), 4.44 (1 H, s), 4.14 (1 H, s), 4.07 (1 H, m), 3.96-3.93 (1 H, m), 3.87-3.82 (2 H, m), 3.73-5.69 (1 H, m), 2.76 (1 H, s), 2.47 (1 H, m), 1.73-1.70 (2 H, m), 1.54-1.49 (1 H, m), 1.44 (9 H, s), 1.21-1.19 (20 H, m), 0.85 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.98, 165.95, 155.7, 133.6, 133.29, 133.33, 130.0, 130.0, 129.9, 129.8, 129.5, 128.63, 128.59, 128.5, 98.3, 74.9, 71.3, 69.9, 69.6, 68.7, 63.2, 61.7, 53.0, 39.2, 31.6, 30.1, 29.9, 29.82, 29.78, 29.7, 29.6, 29.5, 28.1, 27.6, 25.5, 22.8; HRMS (ESI) *m/z* calcd. for $[C_{49}H_{67}NO_{12}+H]^+$: 862.4736, obsd: 862.4716.

(2*S*,3*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-hydroxy-15-methylhexadecanyl β-D-galactopyranoside

NaOMe (25% wt in MeOH) was added dropwise into a stirred mixture of dibenzoate (0.412g, 0.478 mmol) dissolved in MeOH to adjust to pH = 10. The mixture was stirred at r.t overnight then Dowex 50 (H^+) was added to neutralise the mixture. The mixture was filtered, the resin washed with MeOH and the solvent was evaporated. The crude residue was purified by flash chromatography (MeOH:$CHCl_3$, 20:80) to afford product as a colourless oil (0.202 g, 89%).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (1 H, s), 4.96 (1 H, s), 4.10 (1 H, s), 3.96 (2 H, m), 3.84 (3 H, m), 3.79 (1 H, m), 3.69 (3 H, m), 3.05 (1 H, s), 2.70 (1 H, s), 2.47 (1 H, s), 2.34 (1 H, s), 1.49

(1 H, m), 1.45 (9 H, s), 1.25 (20 H, m), 1.15 (2 H, m), 0.87-0.86 (6 H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, D_2O) δ 158.2, 101.2, 80.20, 72.4, 71.9, 71.5, 71.71, 71.0, 79.4, 68.8, 62.7, 56.6, 40.2, 34.9, 31.0, 30.8, 30.7, 29.1, 28.8, 28.5, 26.7, 23.0; HRMS (ESI) *m/z* calcd. for $[C_{28}H_{55}NO_9+H]^+$: 550.3950, obsd: 550.3956.

(2*S*,3*R*)-2-Amino-3-hydroxy-15-methylhexadecanyl β-D-galactopyranoside (16)

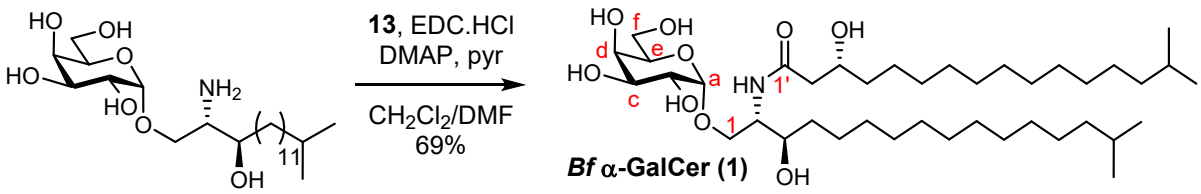
Aqueous HCl (3 M, 1.22 mL) was added dropwise into the mixture of galactosyl Boc-sphinganine (0.202 g, 0.367 mmol) dissolved in CH_2Cl_2/MeOH (1:1, 7.34 mL) at 0 ºC. The mixture was warmed to r.t and stirred until all the starting material was consumed. The solvent was evaporated and the residue was co-evaporated with toluene (0.6 mL, 3 ×) to remove the water. The crude material was advanced to the next reaction without any purification. HRMS (ESI) *m/z* calcd. for $[C_{23}H_{47}NO_7+H]^+$: 450.3425, obsd: 450.3425.

6. Synthesis of αGalCer analogues

General protocol for the coupling with hydroxy acid

DMAP (1 eq) and EDC.HCl (2 eq) were added respectively into the mixture of acid lipid (2 eq) in dry CH_2Cl_2 (5 mL mmol^{-1}) at 0 ºC and stirred at r.t for 15 mins. At the same time, pyridine (1 eq) was added to the mixture of amine (1 eq) in dry DMF: CH_2Cl_2 (1:1, 10 mL mmol^{-1}), and then transferred into the mixture of acid lipid at 0 ºC. The resulted mixture was warmed up to r.t and stirred for 6 h. The solvent was evaporated and the crude material was purified immediately by flash chromatography (MeOH:EtOAc, 5:95) to afford a yellow solid that was purified once more time with reverse column (H_2O:MeOH, 20:80) to afford product as the white solid.

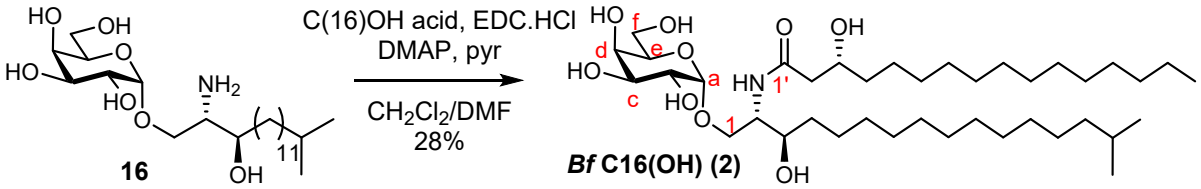
(2*S*,3*R*)-3-Hydroxy-2-((*R*)-3-hydroxy-15-methylhexadecanoyl)amino-15-methylhexadecanyl β-D-galactopyranoside (*Bf* αGC; 1)



The General Protocol for coupling between the amine **16** (0.020 g, 0.045 mmol) and (*R*)-3-hydroxy-15-methylhexadecanoic acid **13** (0.0258 g, 0.090 mmol). After purification, the product **1** was isolated as white solid (0.022 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62

(1 H, d, *J* = 9.0 Hz, NH), 4.65 (1 H, d, *J* = 2.5 Hz, Ha), 4.59 (1 H, d, *J* = 4.7 Hz, 3'-OH), 4.53 (1 H, d, *J* = 6.3 Hz, 3-OH), 4.48 (1 H, d, *J* = 4.9 Hz, d-OH), 4.50 (1 H, d, *J* = 5.6 Hz, f-OH), 4.33 (1 H, d, *J* = 3.9 Hz, c-OH), 4.16 (1 H, d, *J* = 7.4 Hz, b-OH), 3.76-3.41 (9 H, m, Hb,c,d,e,f,1,2,3,3'), 2.24-2.15 (1 H, qd, *J* = 13.8, 6.5 Hz, H2'), 1.52-1.46 (4 H, m, H4,4'), 1.41 (2 H, m, H15,15'), 1.19-1.12 (36 H, m, lipid chain), 0.84 (12 H, d, *J* = 6.6 Hz, H16,16'); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 99.9, 71.3, 69.8, 69.7, 69.2, 68.9, 68.7, 67.9, 67.5, 60.8, 53.3 44.4, 38.5, 36.6, 34.0, 31.2, 29.3, 29.3, 29.2, 29.1, 29.0, 28.9, 28.7, 28.6, 28.5, 28.3, 27.4, 26.8, 25.1, 22.5, 22.1, 14.0; HRMS (ESI) *m/z* calcd. for $[C_{40}H_{80}NO_9+H]^+$: 718.5828, obsd: 718.5827.

(2*S*,3*R*)-3-Hydroxy-2-((*R*)-3-hydroxyhexadecanoyl)amino-15-methylhexadecanyl β-D-galactopyranoside (*Bf* C16(OH); 2)



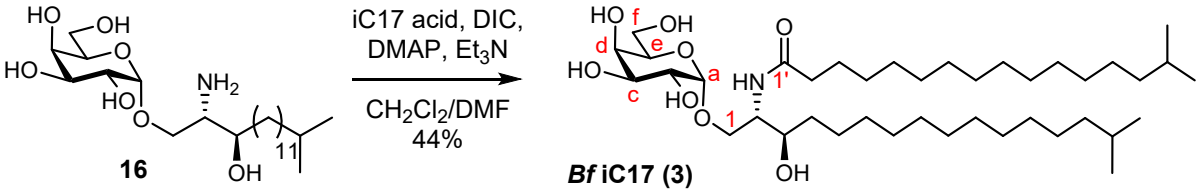
The General Protocol for coupling between the amine **16** (0.020 g, 0.045 mmol) and (*R*)-3-hydroxyhexadecanoic acid (0.0245 g, 0.090 mmol). After purification, the product **2** was isolated as white solid (0.0088 g, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (1 H, d, *J* = 9.1 Hz, NH), 4.64 (1 H, d, *J* = 3.3 Hz, Ha), 4.59 (1 H, d, *J* = 4.8 Hz, 3'-OH), 4.52 (1 H, d, *J* = 6.4 Hz, 3-OH), 4.47 (1 H, d, *J* = 5.1 Hz, d-OH), 4.49 (1 H, d, *J* = 5.5 Hz, f-OH), 4.33 (1 H, d, *J* = 4.0 Hz, c-OH), 4.15 (1 H, d, *J* = 7.5 Hz, b-OH), 3.41-3.76 (9 H, m, Hb,c,d,e,f,1,2,3,3'), 2.20 (1 H, qd, *J* = 13.8, 6.5 Hz, H2'), 1.52-1.46 (4 H, m, H4,4'), 1.41 (2 H, m, H15,15'), 1.19-1.12 (36 H, m, lipid chain), 0.86-0.83 (3 H, m, H16'), 0.84 (6 H, d, *J* = 6.6 Hz, H16); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 99.5, 71.2, 69.8, 69.7, 69.2, 68.9, 68.7, 67.5, 60.6, 53.0, 44.1, 38.4, 36.6, 33.6, 31.2, 29.3, 29.3, 29.2, 29.1, 29.0, 28.9, 28.7, 28.6, 28.50, 28.3, 27.4, 26.8, 25.1, 22.5, 22.1, 14.0; HRMS (ESI) *m/z* calcd. for $[C_{39}H_{77}NO_9+H]^+$: 704.5671, obsd: 704.5672.

General protocol of the coupling with palmitic acid and iso-C17 acid

DMAP (2 eq) and DIC (2 eq) were added respectively into the mixture of acid lipid (1.1 eq) in dried CH_2Cl_2 (10 mL mmol^{-1}) at 0 ºC and stirred at r.t for 15 mins. At the same time, Et_3N (1.2 eq) was added to the mixture of amine (1 eq) in dry DMF, and then transferred into the mixture of acid lipid at 0 ºC. The resulted mixture was warmed up to r.t and stirred overnight. The solvent was evaporated and the crude material was purified immediately by flash

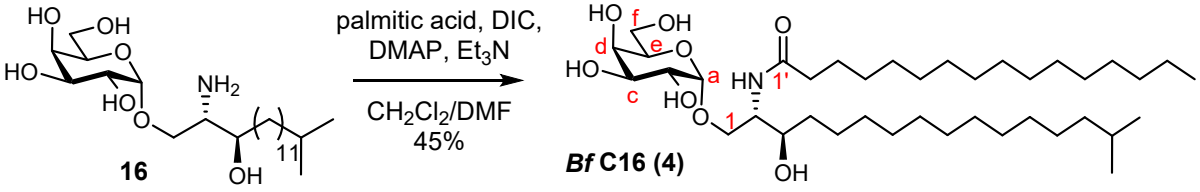
chromatography (MeOH:EtOAc, 5:95) to afford yellow solid which was purified once more time with RC (H_2O:MeOH, 20:80) to afford product as the white solid.

(2*S*,3*R*)-3-Hydroxy-2-(15-methylhexadecanoyl)amino-15-methylhexadecanyl β-D-galactopyranoside (*Bf* iC17; 3)



The General Protocol for coupling between the amine **16** (0.020 g, 0.045 mmol) and 15-methylhexadecanoic acid (0.0134 g, 0.0495 mmol). After purification, the product **3** was isolated as white solid (0.014 g, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (1 H, d, *J* = 8.9 Hz, NH), 4.65 (1 H, d, *J* = 3.2 Hz, Ha), 4.54 (1 H, d, *J* = 6.5 Hz, OH-f), 4.50-4.51 (2 H, m, OH-d, OH-f), 4.35 (1 H, d, *J* = 4.1 Hz, OH-c), 4.16 (1 H, d, *J* = 7.1 Hz, OH-b), 3.71 (1 H, m, H2), 3.68 (1 H, m, Hc), 3.45-3.59 (8 H, m, Hb,d,e,f,f',1a,1b,3), 2.06 (1 H, td, *J* = 13.6, 7.1 Hz, H2'), 1.46-1.51 (2 H, m, H15,15'), 1.41 (1 H, m, H4a), 1.23 (41 H, m, lipid chain), 1.12-1.13 (4 H, m, H14,14'), 0.83 (12 H, d, *J* = 6.6 Hz, H16,16'); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 100.0, 71.3, 69.9, 69.5, 69.1, 68.9, 68.7, 60.9, 53.4, 44.3, 38.5, 36.6, 33.6, 31.3, 29.4, 29.2, 29.1, 29.0, 28.9, 28.8, 28.6, 28.5, 28.4, 27.4, 26.9, 24.9, 22.5, 22.0, 14.1; HRMS (ESI) *m/z* calcd. for $[C_{40}H_{79}NO_8+H]^+$: 702.5878, obsd: 702.5888.

(2*S*,3*R*)-3-Hydroxy-2-palmitoylamino-15-methylhexadecanyl β-D-galactopyranoside (*Bf* C16; 4)



The General Protocol for coupling between the amine **16** (0.020 g, 0.045 mmol) and palmitic acid (0.0125 g, 0.049 mmol). After purification, the product **4** was isolated as white solid (0.014 g, 45 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (1 H, d, *J* = 9.2 Hz, NH), 4.65 (1 H, d, *J* = 2.3 Hz, Ha), 4.54 (1 H, d, *J* = 6.4 Hz, 3-OH), 4.50-4.51 (2 H, m, d-OH, f-OH), 4.35 (1 H, d, *J* = 3.6 Hz, c-OH), 4.15 (1 H, d, *J* = 7.8 Hz, b-OH), 3.72 (1 H, m, H2), 3.68 (1 H, m, H3), 3.58-3.45 (8 H, m, Hb,d,e,f',1a,1b,3), 2.06 (1 H, dq, *J* = 13.7, 6.7 Hz, H2'), 1.52-1.46 (3 H, m, H15,15'), 1.41 (2 H, m, H4), 1.23 (41 H, m, lipid chain), 1.13 (4 H, m, H14,14'), 0.86-0.83 (3

H, m, H16'), 0.84-0.83 (6 H, d, J = 6.6 Hz, H16); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 99.9, 71.2, 69.8, 69.5, 69.3, 68.9, 68.7, 60.6, 53.4, 44.1, 38.4, 36.6, 33.6, 31.3, 29.3, 29.2, 29.1, 29.0, 28.9, 28.7, 28.6, 28.5, 28.3, 27.4, 26.9, 25.0, 22.5, 22.1, 14.0; HRMS (ESI) *m/z* calcd. for $[C_{39}H_{77}NO_8+H]^+$: 688.5722, obsd: 688.5728.

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1. Wang; Wang, Z.; Zhou, L.; El Boubbou, K.; Ye, X.-s.; Huang, X., *J. Org. Chem.* **2007,** *72*, 6409-6420.

2. Brown, L. C. W.; Penaranda, C.; Kashyap, P. C.; Williams, B. B.; Clardy, J.; Kronenberg, M.; Sonnenburg, J. L.; Comstock, L. E.; Bluestone, J. A.; Fischbach, M. A., *PLOS Biol.* **2013,** *11*, 9.