Electronic Supplementary Information (ESI)

The impact of structural differences in galactocerebrosides on the behavior of 2D monolayers

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General experimentals: Chemical syntheses

All reactions were performed in flame-dried glassware under an argon atmosphere. The solvents were dried by standard procedures and distilled prior to use. ^{1}H- and ^{13}C-NMR spectra were obtained with 300 MHz and 500 MHz spectrometers using the solvent as internal standard. In the case of solvent mixtures CD_3OD was used as internal standard. Assignments of the respective signals were made by the combination of H,H-COSY, HSQC and HMBC experiments. Unsecured assignments are characterized with the index*. ESI-HRMS mass spectrometry was carried out on a FTICR instrument. IR spectra were measured on a FT/IR-4100 spectrometer with a Pike GladiATR unit. Optical rotations were obtained using a common polarimeter. Dialysis was performed in deionized water using Spectra/Por Float-A-Lyzer G2 with a molecular weight cut off of 500 g/mol. For gel permeation HPLC a system type (recycling system) was used.

General Methods

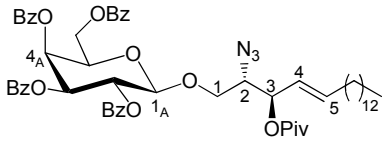
S 1

To a suspension of sphingosine in benzene and water PPh_3 was added and the reaction mixture was stirred at 60 °C for 16 h. The solvents were removed under reduced pressure and the residue was azeotroped with toluene (3 x 1.0 mL). The amine was dried in high vacuum for 1 h and dissolved in dry THF. To a solution of fatty acid in dry THF were added HOBt, EDCI and diisopropylamine at 0 °C. The mixture was stirred at 0 °C for 10 min, then the amine was added dropwise and the solution stirred at r.t. for 16 h. The reaction was stopped by addition of water. The organic layer was washed with sat. NH_4Cl and sat. $NaHCO_3$ solution, dried over Na_2SO_4 and the solvents were removed under reduced pressure.

S 2

To a solution of the protected glycosphingolipids in CH_3OH/CH_2Cl_2 (3:1) NaOMe (1.6 M or 5.2 M in CH_3OH) was added till a pH greater than 12 was reached. The reaction mixture was stirred at r.t. between 16 h and 2 d. The solution was neutralized with amberlite®, filtered and the solvents were removed under reduced pressure.

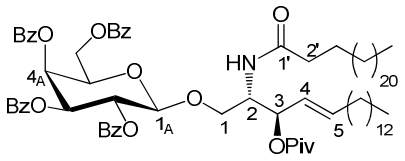
_O_-(2,3,4,6-Tetra-_O_-benzoyl-β-D-galactopyranosyl)-(1→1)-(2_S_,3_R_,4_E_)-2-azido-3-pivaloyl-4-octadecen-1,3-diol (3)



Galactosyl trichloroacetimidate **1** (380 mg, 0.51 mmol, 1.2 eq) and sphingosine **2** (180 mg, 0.44 mmol, 1.0 eq) were dissolved in dry CH_2Cl_2/hexane (6.0 mL), molecular sieves 4 Å were added and the mixture stirred at r.t. for 30 min. Afterwards $BF_3 \cdot OEt_2$ (9 µL, 73 µmol, 0.2 eq) was added at 0 °C and the solution stirred at r.t. for 4 h. After column chromatography on silica gel (pentane/EtOAc, 8:1) 430 mg (99%) of **3** were obtained as a colorless oil.

***R*f**: 0.37 (hexane/EtOAc, 4:1); $[\alpha]_D^{20}$= +41.9° (*c* 0.38, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.8 Hz, 3 H, 18-H_3), 1.17 (s, 9 H, O(CO)C(CH_3)$_3$), 1.14-1.30 (m, 22 H, 7-H_2–17-H_2), 1.85-1.95 (m, 2 H, 6-H_2), 3.61 (dd, *J* = 9.9 Hz, 5.5 Hz, 1 H, 1-H_a), 3.77-3.86 (m, 1 H, 2-H), 3.94 (dd, *J* = 9.9 Hz, 7.0 Hz, 1 H, 1-H_b), 4.33 (dd, *J* = 6.7 Hz, 6.6 Hz, 1 H, 5$_A$-H), 4.41 (dd, *J* = 11.0 Hz, 6.7 Hz, 1 H, 6$_A$-H_a), 4.68 (dd, *J* = 11.0 Hz, 6.6 Hz, 1 H, 6$_A$-H_b), 4.84 (d, *J* = 7.9 Hz, 1 H, 1$_A$-H), 5.26-5.39 (m, 2 H, 3-H, 4-H), 5.61 (dd, *J* = 10.5 Hz, 3.3 Hz, 1 H, 3$_A$-H), 5.62 (dd, *J* = 14.5 Hz, 6.7 Hz, 1 H, 5-H), 5.81 (dd, *J* = 10.5 Hz, 7.9 Hz, 1 H, 2$_A$-H), 5.99 (d, *J* = 3.3 Hz, 1 H, 4$_A$-H), 7.20-7.28 (m, 2 H, Ph-H), 7.34-7.66 (m, 10 H, Ph-H), 7.75-7.81 (m, 2 H, Ph-H), 7.93-8.13 (m, 6 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-18), 22.7, 28.7, 29.0, 29.3, 29.4, 29.6, 29.6, 29.7, 29.7, 31.9 (C-7–inC-17), 27.0 (O(CO)C(*C*H_3)$_3$), 32.2 (C-6), 38.8 (O(CO)*C*(CH_3)$_3$), 61.9 (C-6$_A$), 63.5 (C-2), 68.0 (C-4$_A$), 68.2(C-1), 69.6 (C-2$_A$), 71.4 (C-5$_A$), 71.6 (C-3$_A$), 73.9 (C-3), 101.2 (C-1$_A$), 122.8 (C-4), 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.7, 128.7, 129.0, 129.3, 129.4, 129.8, 129.8, 129.9, 130.0 (C$_{tert}$-Ph), 133.2, 133.3, 133.3, 133.6 (C$_{quart}$-Ph), 138.3 (C-5), 165.0, 165.5, 165.6, 166.0 (O(*C*O)Ph), 176.7 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): ν (cm^{-1}) = 2925, 2853, 2101, 1725, 1602, 1451, 1262, 1144, 1093, 1068; **MS** (ESI): *m*/*z* (%) = 1010.5 $[M+Na]^+$, 1999.0 $[2M+Na]^+$; **HRMS (ESI)** for $C_{57}H_{69}N_3O_{12}$ (988.17): calcd. 1010.4773 $[M+Na]^+$, found1010.4791.

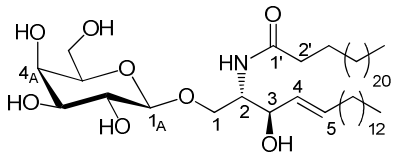
***O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-(tetracosan-amido)-3-*O*-pivaloyl-4-octadecen-1,3-diol (4)**



Reduction of azidosphingosine **3** (50.0 mg, 50.6 µmol, 1.0 eq) in benzene (4.0 mL) and water (19 µL) using PPh_3 (31.0 mg, 116 µmol, 2.3 eq) was performed according to **S1**. The corresponding aminewas dissolved in dry THF (4.0 mL) and HOBt (11.0 mg, 81.4 µmol, 1.6 eq), EDCI (16.0 mg, 83.5 µmol, 1.7 eq), DIPEA (14.0 µL, 82.3 µmol, 1.6 eq) andtetracosanoic acid (30.0 mg, 81.4 µmol, 1.6 eq) dissolved in dry THF (4.0 mL) were added. After column chromatography on silica gel (pentane/EtOAc, 10:1 → 8:1 → 6:1) 44.0 mg (66%) of galactocerebroside **4**were obtained as a colorless oil.

***R*$_f$**: 0.57 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$= +20.0° (*c* 0.30, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 6.4 Hz, 3 H, 18-H_3), 0.88 (t, *J* = 7.0 Hz, 3 H, 24′-H_3), 1.20 (s, 9 H, O(CO)C(CH_3)$_3$), 1.22-1.28 (m, 64 H, 7-H_2–17-H_2, 3′-H_2–23′-H_2), 1.70 (t, *J* = 7.7 Hz, 2 H, 2′-H_2), 1.93-2.04 (m, 2 H, 6-H_2), 3.60 (dd, *J* = 9.7 Hz, 3.7 Hz, 1 H, 1-H_a), 4.19 (dd, *J* = 9.7 Hz, 2.6 Hz, 1 H, 1-H_b), 4.29-4.39 (m, 2 H, 2-H, 5$_A$-H), 4.38 (dd, *J* = 11.2 Hz, 5.2 Hz, 1 H, 6$_A$-H_a), 4.64 (dd, *J* = 11.2 Hz, 6.1 Hz, 1 H, 6$_A$-H_b), 4.79 (d, *J* = 7.4 Hz, 1 H, 1$_A$-H), 5.24-5.43 (m, 2 H, 3-H, 4-H), 5.60 (d, *J* = 9.0 Hz, 1 H, NH), 5.65 (dd, *J* = 10.4 Hz, 3.3 Hz, 1 H, 3$_A$-H), 5.74 (dd, *J* = 10.4 Hz, 7.4 Hz, 1 H, 2$_A$-H), 5.77 (dt, *J* = 15.2 Hz, 6.8 Hz, 1 H, 5-H), 6.00 (d, *J* = 3.3 Hz, 1 H, 4$_A$-H), 7.19-7.28 (m, 2 H, Ph-H), 7.34-7.46 (m, 5 H, Ph-H), 7.47-7.66 (m, 5 H, Ph-H), 7.74-7.81 (m, 2 H, Ph-H), 7.94-8.04 (m, 4 H, Ph-H), 8.08-8.15 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1, 14.1 (C-18, C-24′), 22.6, 25.4, 29.0, 29.1, 29.3, 29.5, 29.5, 29.6, 29.6, 29.6, 29.6, 31.9, 32.2 (C-6–C-17, C-2′–C-23′), 27.0 (O(CO)C(*C*H_3)$_3$), 36.4 (O(CO)*C*(CH_3)$_3$), 50.3 (C-2), 61.9 (C-6$_A$), 67.6 (C-1), 67.9 (C-4$_A$), 70.1 (C-2$_A$), 71.3 (C-5$_A$), 71.3 (C-3$_A$), 73.1 (C-3), 101.3 (C-1$_A$), 125.1 (C-4), 128.2, 128.4, 128.5, 128.6, 128.6, 128.7, 128.9, 129.0, 129.3, 129.7, 129.7, 130.0 (C_{tert}-Ph), 133.3, 133.3, 133.5, 133.6 (C_{quart}-Ph), 136.7 (C-5), 165.3, 165.4, 165.4, 165.9 (O(*C*O)Ph), 172.4 (C-1′), 176.8 (O(*C*O)C(CH_3)$_3$); **IR** (ATR):ν (cm^{-1}) = 2919, 2850, 1726, 1647, 1602, 1542, 1451, 1265, 1154, 1093; **MS** (ESI): *m*/*z* (%) = 1334.9 $[M+Na]^+$, 2647.8 $[2M+Na]^+$; **HRMS (ESI)** for $C_{81}H_{117}NO_{13}$ (1312.79): calcd.1334.8417 $[M+Na]^+$, found1334.8423.

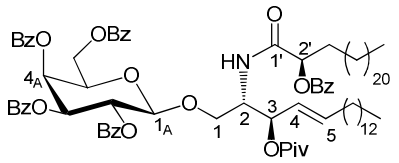
***O*-(β-D-Galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-(tetracosanamido)-4-octadecen-1,3-diol (GAlCer C 24:0)** [1]



Global deprotection of **4**(44.0 mg, 33.5 µmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (2.0 mL) using NaOMe was performed according to **S2**. The reaction mixture was stirred at r.t. for 16 h. After dialysis for 2 d and lyophilisation 14.0 mg (51%) of **GalCer C 24:0**were obtained as a white solid.

***R*f**: 0.52 (CH_2Cl_2/CH_3OH, 5:1); **^{1}H-NMR** (300 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 0.86 (t, *J* = 6.9 Hz, 3 H, 18-H_3), 0.87 (t, *J* = 7.1 Hz, 3 H, 24′-H_3), 1.22-1.32 (m, 64 H, 7-H_2–17-H_2, 4′-H_2–23′-H_2), 1.95-2.05 (m, 2 H, 6-H_2), 2.15 (t, *J* = 7.2 Hz, 2 H, 3′-H_2)*, 3.44-3.59 (m, 3 H), 3.67-3.81 (m, 2 H), 3.82-3.86 (m, 1 H), 3.92-4.00 (m, 1 H), 4.04-4.11 (m, 1 H), 4.14-4.23 (m, 1 H), 4.28-4.34 (m, 1 H), 5.37-5.47 (m, 1 H, 4-H), 5.67 (dt, *J* = 15.1 Hz, 7.6 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 14.4, 14.4 (C-18, C-24′), 23.4, 26.7, 30.1, 30.1, 30.2, 30.2, 30.3, 30.3, 30.4, 30.4, 30.4, 30.5, 30.5, 32.7, 32.7, 33.1, 37.3 (C-6–C-17, C-2′–C-23′), 54.5 (C-2), 62.3, 69.6, 70.0, 72.3, 72.9, 74.5, 76.2 (C-1, C-3, C-2_A, C-3_A, C-4_A, C-5_A, C-6_A)*, 104.8 (C-1_A), 130.5 (C-4), 134.8 (C-5), 175.6 (C-1′); **HRMS (ESI)** for $C_{48}H_{93}NO_8$ (812.25): calcd.834.6793 $[M+Na]^+$, found834.6796.

***O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*,2′*R*)-2-(2′-benzoyl-oxy-tetracosanamido)-3-*O*-pivaloyl-4-octadecen-1,3-diol (5)**

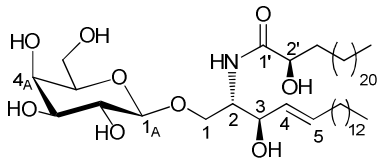


Reduction of azidosphingosine **3** (66.9 mg, 67.7 µL, 1.0 eq) in benzene (4.0 mL) and water (25 µL) using PPh_3 (40.9 mg, 156 µmol, 2.3 eq) was performed according to **S1**. The corresponding amine was dissolved in dry THF (2.7 mL) and HOBt (15.0 mg, 108 µmol, 1.6 eq), EDCI (21.0 mg, 110 µmol, 1.6 eq), DIPEA (18.0 µL, 108 µmol, 1.6 eq) and α-hydroxylated tetracosanoic acid [2] (53.0 mg, 108 µmol, 1.6 eq) dissolved in dry THF (6.0 mL) were added. After column chromatography on silica gel (pentane/EtOAc, 6:1) 66.0 mg (68%) of galactocerebroside **5** were obtained as a colorless oil.

***R*f**: 0.55 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$= +38.3° (*c* 0.57, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.85 (t, *J* = 6.4 Hz, 3 H, 18-H_3), 0.85 (t, *J* = 6.1 Hz, 3 H, 24′-H_3), 1.10 (s, 9 H, O(CO)C(CH_3)$_3$), 1.12-1.27 (m, 64 H, 6-H_2–17-H_2, 4′-H_2–23′-H_2), 1.75-1.88 (m, 2 H, 3′-H_2), 3.67 (dd, *J* = 10.3 Hz, 5.4 Hz, 1 H, 1-H_a), 4.00-4.07 (m, 1 H, 1-H_b), 4.24 (dd, *J* = 7.0 Hz,

6.3 Hz, 1 H, 5_A-H), 4.30-4.44 (m, 2 H, 2-H, 6_A-H_a), 4.57 (dd, *J* = 11.2 Hz, 6.3 Hz, 1 H, 6_A-H_b), 4.78 (d, *J* = 8.0 Hz, 1 H, 1_A-H), 5.17 (dd, *J* = 5.9 Hz, 5.8 Hz, 1 H, 2′-H), 5.27-5.38 (m, 2 H, 3-H, 4-H), 5.51-5.66 (m, 1 H, 5-H), 5.54 (dd, *J* = 10.2 Hz, 3.2 Hz, 1 H, 3_A-H), 5.71 (dd, *J* = 10.2 Hz, 8.0 Hz, 1 H, 2_A-H), 5.94 (d, *J* = 3.2 Hz, 1 H, 4_A-H), 6.42 (d, *J* = 8.9 Hz, 1 H, NH), 7.14-7.63 (m, 15 H, Ph-H), 7.68-7.89 (m, 4 H, Ph-H), 7.91-8.09 (m, 6 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1, 14.1 (C-18, C-24′), 22.6, 24.6, 28.8, 29.1, 29.3, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 31.4, 32.1 (C-6–C-17, C-4′–C-23′), 26.9 (O(CO)C(CH_3)$_3$), 31.8 (C-3′), 38.7 (O(CO)*C*(CH_3)$_3$), 50.9 (C-2), 61.8 (C-6_A), 66.9 (C-1), 67.9 (C-4_A), 69.6 (C-2_A), 71.3 (C-5_A), 71.5 (C-3_A), 73.0 (C-3), 74.4 (C-2′), 100.9 (C-1_A), 124.2 (C-4), 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 129.0, 129.1, 129.3, 129.3, 129.6, 129.7, 129.8, 129.9 (C_{tert}-Ph), 133.1, 133.1, 133.4, 133.5 (C_{quart}-Ph), 136.6 (C-5), 165.0, 165.1, 165.4, 165.4, 165.8 (O(*C*O)Ph), 169.3 (C-1′), 176.8 (O(*C*O)C(CH_3)$_3$); **IR** (ATR):ν (cm^{-1}) = 2922, 2852, 1726, 1602, 1451, 1262, 1150, 1093, 1068; **MS** (ESI): *m/z* (%) = 1454.9 $[M+Na]^+$, 2887.8 $[2M+Na]^+$; **HRMS (ESI)** for $C_{88}H_{121}NO_{15}$ (1432.90): calcd.1454.8628 $[M+Na]^+$, found1454.8642.

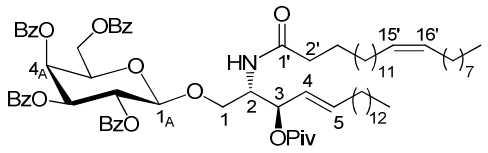
***O*-(β-D-Galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*,2′*R*)-2-(2′-hydroxytetracosanamido)-4-octadecen-1,3-diol (GalCer C 24:0 (2-OH))** [3]



Global deprotection of **5** (62.0 mg, 43.3 μmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (12 mL) using NaOMe was performed according to **S2**. The reaction mixture was stirred at r.t. for 2 d. After dialysis for 2 d and lyophilisation 20.0 mg (56%) of **GalCer C 24:0 (2-OH)** were obtained as a white solid.

***R*$_f$**: 0.48 (CH_2Cl_2/CH_3OH, 5:1); **^{1}H-NMR** (300 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 0.85 (t, *J* = 6.9 Hz, 3 H, 18-H_3), 0.85 (t, *J* = 6.0 Hz, 3 H, 24′-H_3), 1.16-1.27 (m, 64 H, 6-H_2–17-H_2, 4′-H_2–23′-H_2), 1.92-2.02 (m, 2 H, 3′-H_2), 3.44-3.50 (m, 3 H), 3.65-3.73 (m, 2 H), 3.74-3.80 (m, 1 H), 3.81-3.85 (m, 1 H), 3.93-4.00 (m, 2 H), 4.01-4.12 (m, 1 H), 4.17 (d, *J* = 6.7 Hz, 1 H, 1_A-H), 5.35-5.45 (m, 1 H, 4-H), 5.69 (dt, *J* = 14.6 Hz, 7.3 Hz, 1 H, 5-H); **^{13}C-NMR**(126 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 14.4, 14.4 (C-18, C-24′), 23.1, 25.7, 29.7, 29.8, 29.8, 29.9, 30.1, 30.1, 30.1, 30.2, 30.2, 30.2 (C-6–C-17, C-3′–C-23′), 53.8 (C-2), 62.1, 68.9, 69.6, 71.8, 72.5, 72.6, 74.0, 75.7 (C-1, C-3, C-2′, C-2_A, C-3_A, C-4_A, C-5_A, C-6_A)*, 104.2 (C-1_A), 129.4 (C-4), 134.8 (C-5), 176.5(C-1′); **HRMS (ESI)** for $C_{48}H_{93}NO_9$ (828.25): calcd. 850.6743 $[M+Na]^+$, found850.6744.

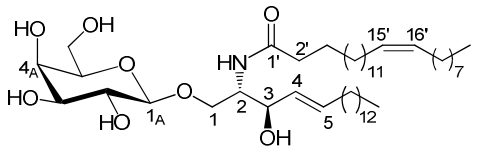
***O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*,15′*Z*)-2-(15′-tetracosenamido)3-*O*-pivaloyl-4-octadecen-1,3-diol (6)**



Reduction of azidosphingosine **3** (66.9 mg, 67.7 µL, 1.0 eq) in benzene (5.0 mL) and water (25 µL) using PPh_3 (40.9 mg, 156 µmol, 2.3 eq) was performed according to **S1**. The corresponding amine was dissolved in dry THF (2.7 mL) and HOBt (15.0 mg, 108 µmol, 1.6 eq), EDCI (21.0 mg, 110 µmol, 1.6 eq), DIPEA (18.0 µL, 108 µmol, 1.6 eq) nervonic acid (40.0 mg, 109 µmol, 1.6 eq) dissolved in dry THF (6.0 mL) were added. After column chromatography on silica gel (pentane/EtOAc, 10:1 → 8:1 → 6:1) 60.0 mg (68%) of galactocerebroside **6** were obtained as a colorless oil.

***R*$_f$**: 0.57 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$= +39.5° (*c* 0.21, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.7 Hz, 3 H, 18-H_3), 0.88 (t, *J* = 7.3 Hz, 3 H, 24′-H_3), 1.19 (s, 9 H, O(CO)C(CH_3)$_3$), 1.20-1.34 (m, 58 H, 7-H_2–17-H_2, 2′-H_2–13′-H_2, 18′-H_2–23′-H_2), 1.64-1.74 (m, 2 H, 6-H_2)*, 1.92-2.06 (m, 4 H, 14′-H_2, 17′-H_2)*, 3.59 (dd, *J* = 9.7 Hz, 3.9 Hz, 1 H, 1-H_a), 4.18 (dd, *J* = 9.7 Hz, 2.9 Hz, 1 H, 1-H_b), 4.28-4.43 (m, 2 H, 2-H, 5$_A$-H), 4.38 (dd, *J* = 11.0 Hz, 6.5 Hz, 1 H, 6$_A$-H_a), 4.63 (dd, *J* = 11.0 Hz, 6.0 Hz, 1 H, 6$_A$-H_b), 4.78 (d, *J* = 7.8 Hz, 1 H, 1$_A$-H), 5.23-5.41 (m, 4 H, 3-H, 4-H, 15′-H, 16′-H), 5.58 (d, *J* = 9.5 Hz, 1 H, NH), 5.64 (dd, *J* = 10.4 Hz, 3.5 Hz, 1 H, 3$_A$-H), 5.73 (dd, *J* = 10.4 Hz, 7.8 Hz, 1 H, 2$_A$-H), 5.77 (dt, *J* = 14.9 Hz, 6.5 Hz, 1 H, 5-H), 5.99 (d, *J* = 3.5 Hz, 1 H, 4$_A$-H), 7.18-7.29 (m, 2 H, Ph-H), 7.32-7.67 (m, 10 H, Ph-H), 7.72-7.82 (m, 2 H, Ph-H), 7.90-8.04 (m, 4 H, Ph-H), 8.06-8.14 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1, 14.1 (C-18, C-24′), 22.7, 22.7, 25.5, 27.2, 29.0, 29.2, 29.2, 29.3, 29.3, 29.3, 29.4, 29.5, 29.5, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 29.8, 31.9, 31.9, 32.3 (C-6–C-17, C-2′–C-14′, C-17′–C-23′), 27.1 (O(CO)C(*C*H_3)$_3$), 36.4 (O(CO)*C*(CH_3)$_3$), 50.3 (C-2), 62.0 (C-6$_A$), 67.7 (C-1), 67.9 (C-4$_A$), 70.1 (C-2$_A$), 71.3 (C-5$_A$), 71.3 (C-3$_A$), 73.2 (C-3), 101.3 (C-1$_A$), 125.1 (C-4), 128.3, 128.5, 128.5, 128.7, 128.7, 129.0, 129.1, 129.3, 129.7, 129.8, 129.9, 129.9, 130.0 (C_{tert}-Ph), 129.7, 129.7 (C-15′, C-16′),133.3, 133.3, 133.5, 133.6 (C_{quart}-Ph), 136.8 (C-5), 165.4, 165.5, 165.5, 170.0 (O(*C*O)Ph), 172.4 (C-1′), 176.9 (O(*C*O)C(CH_3)$_3$); **IR** (ATR):ν (cm^{-1}) = 2922, 2852, 1727, 1675, 1602, 1584, 1451, 1263, 1152, 1093; **MS** (ESI): *m*/*z* (%) = 1332.9 $[M+Na]^+$, 2643.8 $[2M+Na]^+$; **HRMS (ESI)** for $C_{81}H_{115}NO_{13}$ (1310.78): calcd. 1332.8261 $[M+Na]^+$, found1332.8262.

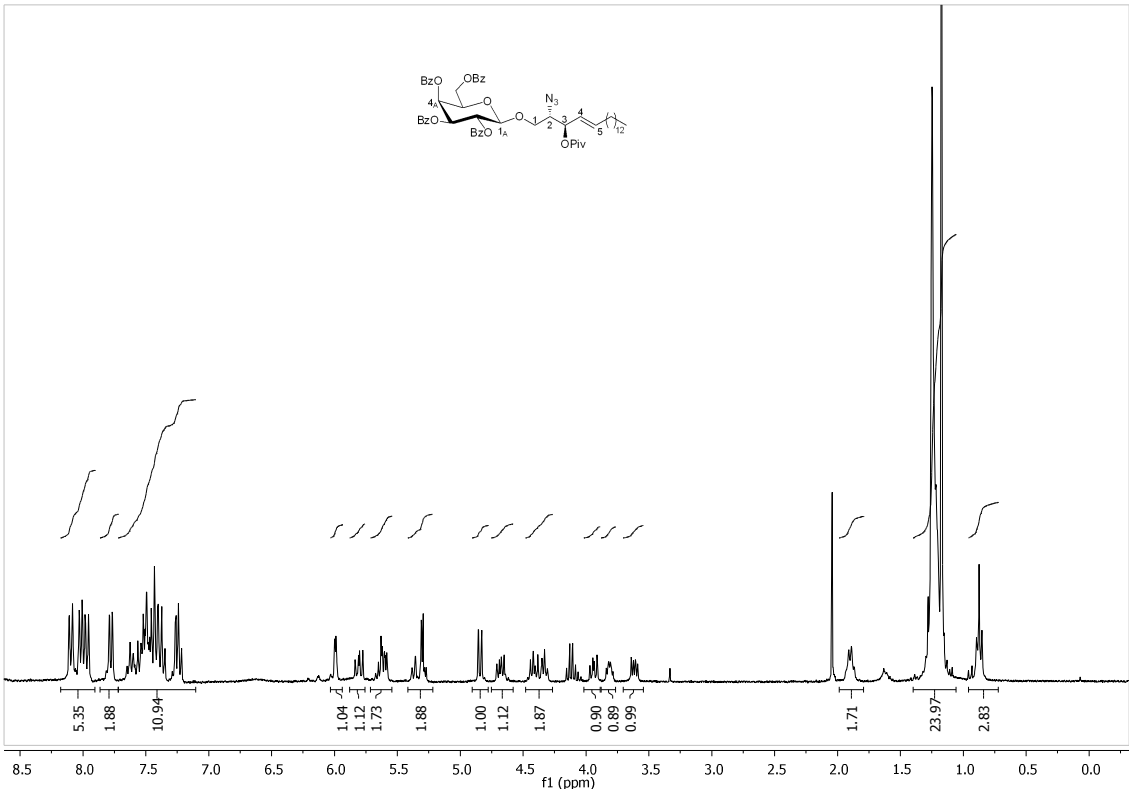
***O*-(β-D-Galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*,15′*Z*)-2-(15′-tetracosenamido)-4-octadecen-1,3-diol (GalCer C 24:1)** [4]



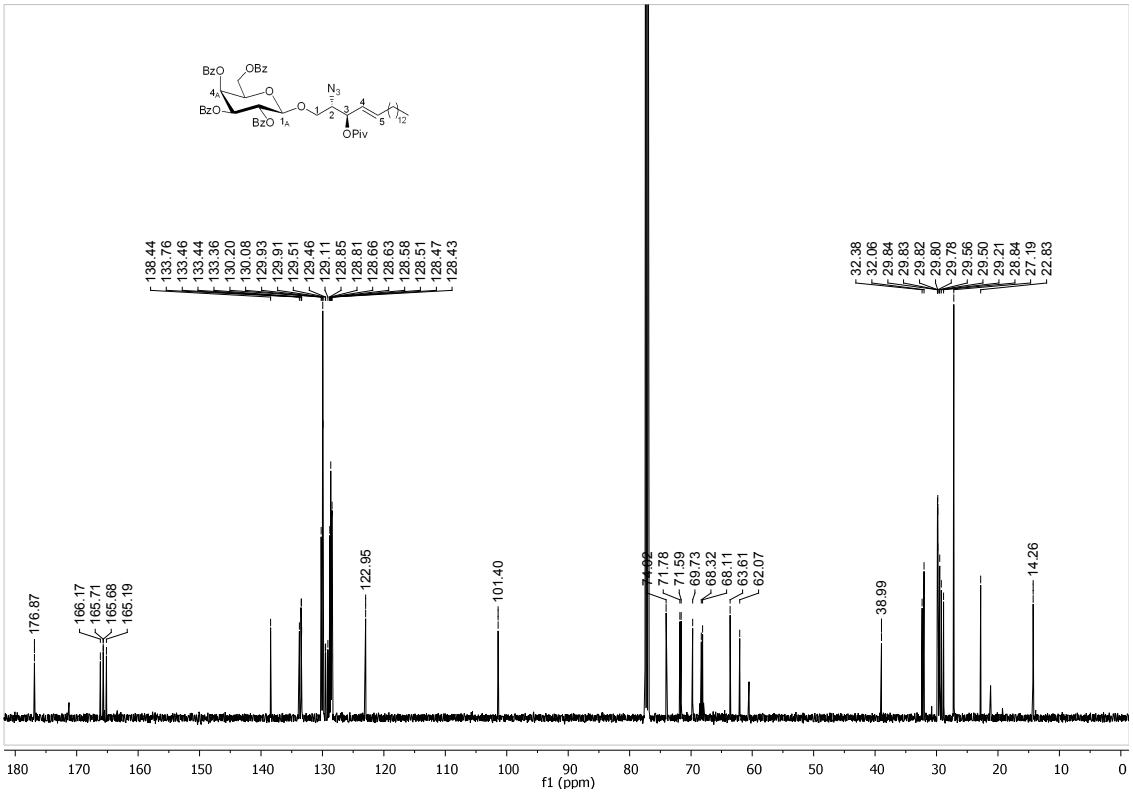
Global deprotection of **6** (60.0 mg, 46.0 μmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (4.0 mL) using NaOMe was performed according to **S2**. The reaction mixture was stirred at r.t. for 2 d. After dialysis for 2 d and lyophilisation 10.0 mg (27%) of **GalCer C 24:1** were obtained as a white solid.

***R*f**: 0.56 (CH_2Cl_2/CH_3OH, 5:1); **^{1}H-NMR** (300 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 0.84 (t, *J* = 6.9 Hz, 3 H, 18-H_3), 0.84 (t, *J* = 6.5 Hz, 3 H, 24′-H_3), 1.18-1.35 (m, 58 H, 7-H_2 – 17-H_2, 2′-H_2–13′-H_2, 18′-H_2–23′-H_2), 1.92-2.03 (m, 4 H, 14′-H_2, 17′-H_2)*, 2.09-2.16 (m, 2 H, 6-H_2)*, 3.43-3.54 (m, 3 H), 3.54 (dd, *J* = 10.3 Hz, 3.2 Hz, 1 H), 3.69 (dd, *J* = 11.6 Hz, 4.9 Hz, 1 H, 6_A-H_a), 3.78 (dd, *J* = 11.6 Hz, 6.8 Hz, 1 H, 6_A-H_b), 3.84 (d, *J* = 2.8 Hz, 1 H, 4_A-H), 3.91-3.99 (m, 1 H), 4.06 (dd, *J* = 7.4 Hz, 6.7 Hz, 1 H), 4.11 (dd, *J* = 10.1 Hz, 4.1 Hz, 1 H), 4.17 (d, *J* = 7.1 Hz, 1 H, 1_A-H), 5.28-5.33 (m, 2 H, 15′-H, 16′-H), 5.35-5.45 (m, 1 H, 4-H), 5.65 (dt, *J* = 15.5 Hz, 6.8 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 14.3, 14.3 (C-18, C-24′), 26.3, 27.7, 27.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.8, 29.8, 29.9, 29.9, 30.0, 30.0, 30.0, 30.0, 30.1, 30.1, 30.1, 32.2, 32.3, 32.7, 36.9 (C-6–C-17, C-2′–C-14′, C-17′–C-23′), 53.7 (C-2), 61.9 ($C\text{-}6_A$), 69.1, 69.4, 71.7, 72.5, 73.8, 75.3 (C-1, C-3, $C\text{-}2_A$, $C\text{-}3_A$, $C\text{-}4_A$, $C\text{-}5_A$), 104.0 ($C\text{-}1_A$), 129.5 (C-4), 130.2, 130.2 (C-15′, C-16′), 134.6 (C-5), 174.9 (C-1′); **HRMS (ESI)** for $C_{48}H_{91}NO_8$ (810.24): calcd.832.6637 $[M+Na]^+$, found832.6644.

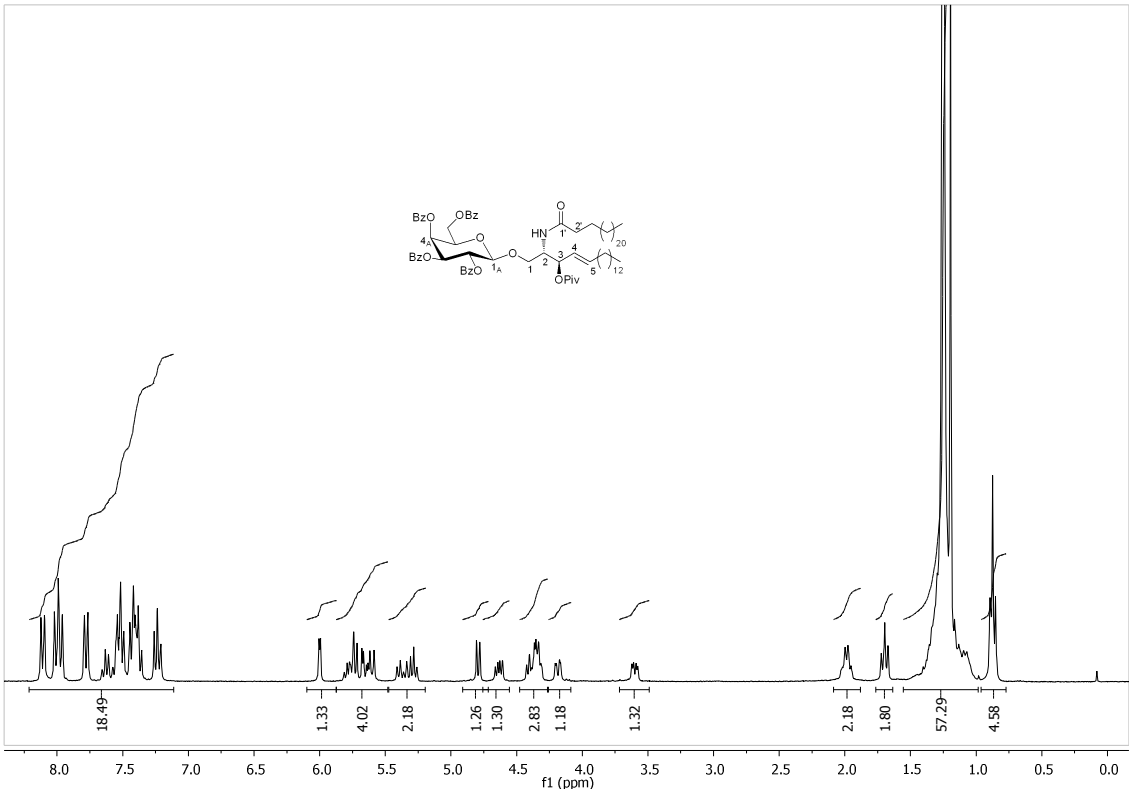
^{1}H-NMR of **3** (300 MHz, $CDCl_3$)



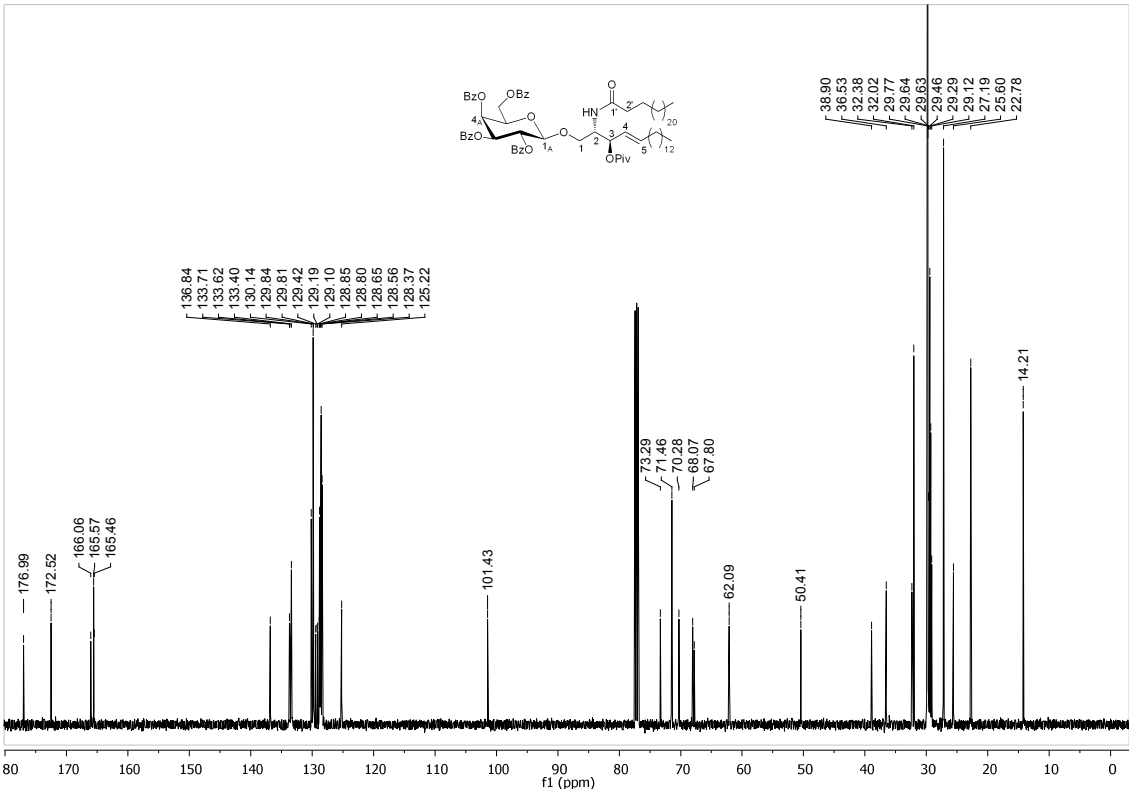
^{13}C-NMR of **3** (126 MHz, $CDCl_3$)



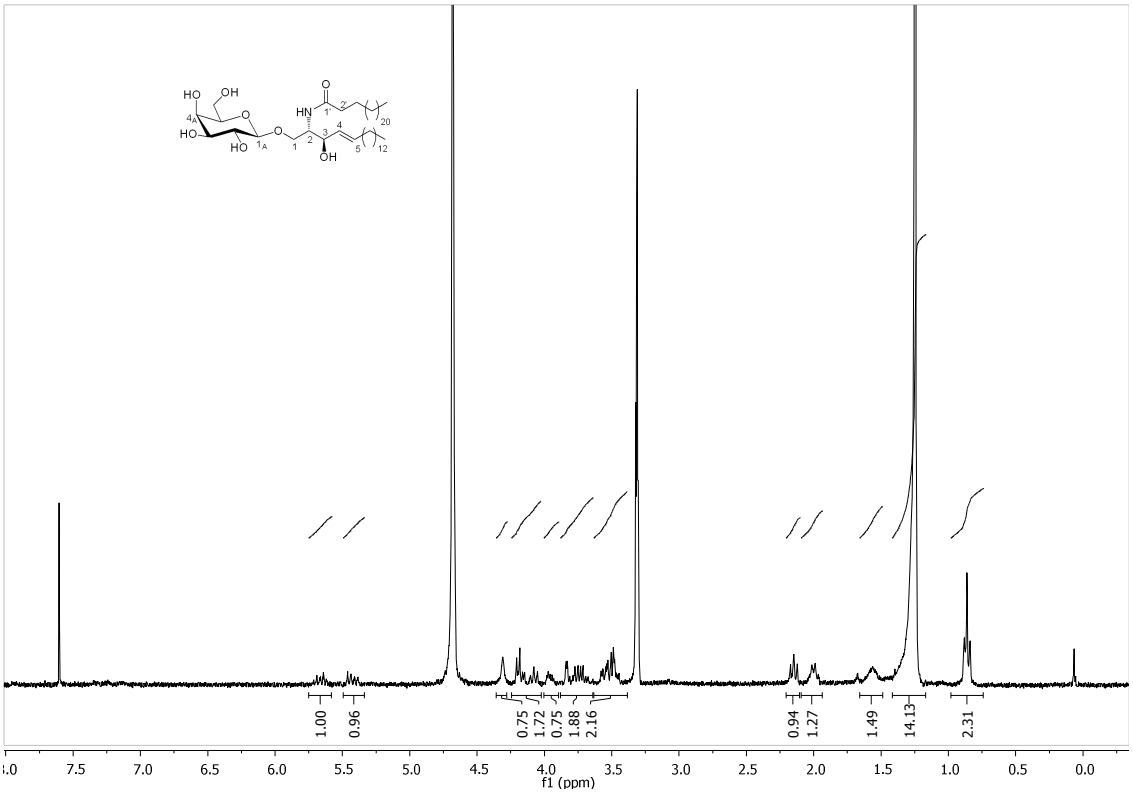
^{1}H-NMR of **4** (300 MHz, $CDCl_3$)



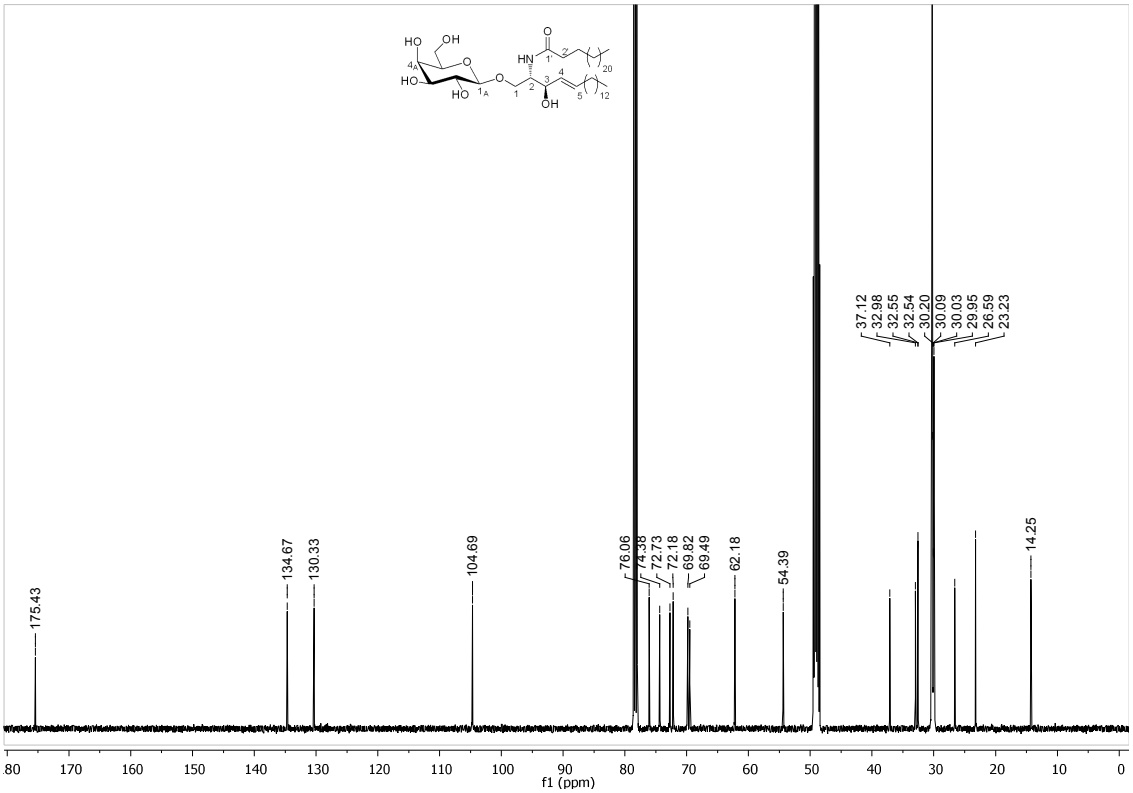
^{13}C-NMR of **4** (126 MHz, $CDCl_3$)



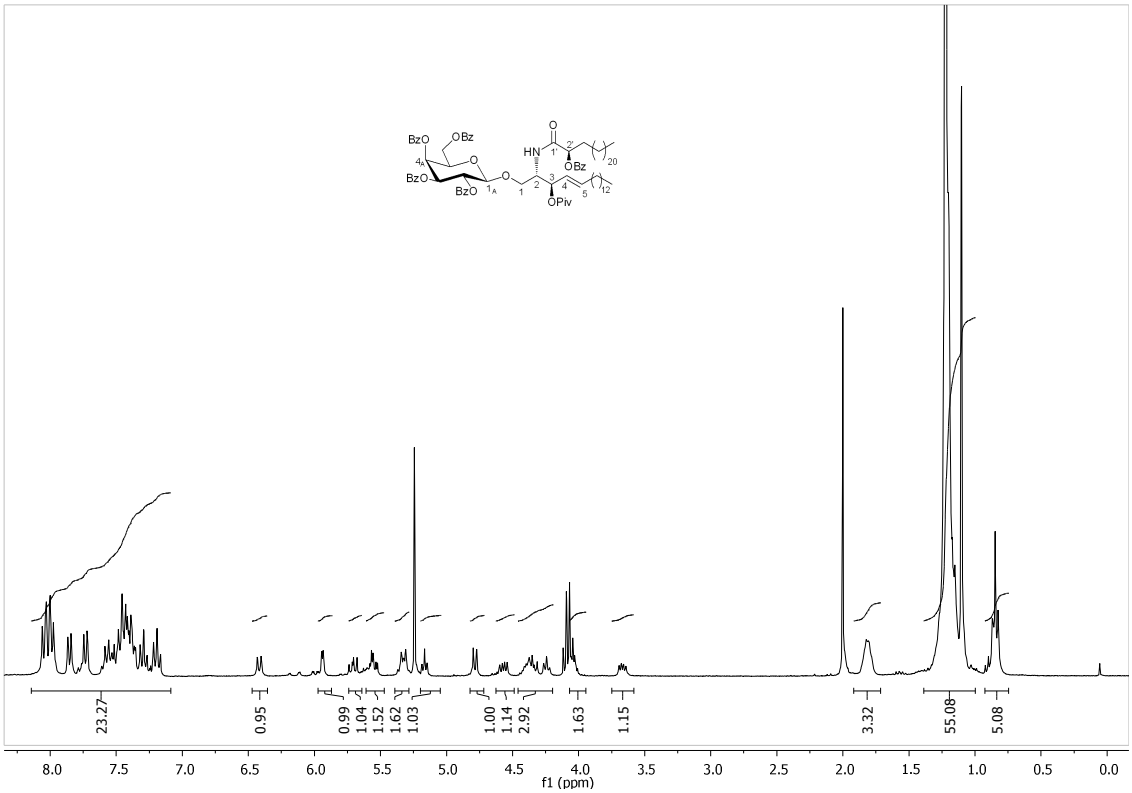
^{1}H-NMR of **GalCer C 24:0** (300 MHz, $CDCl_3/CD_3OD$, 3:1)



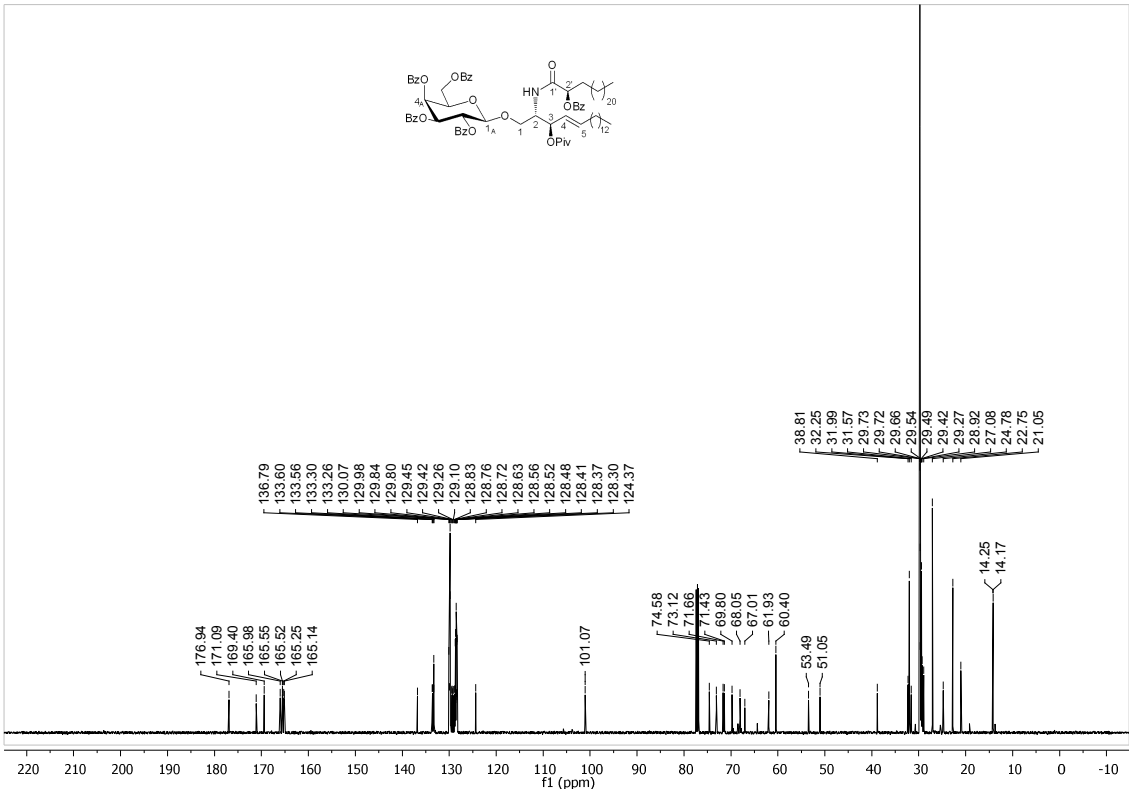
^{13}C-NMR of **GalCer C 24:0** (126 MHz, $CDCl_3/CD_3OD$, 3:1)



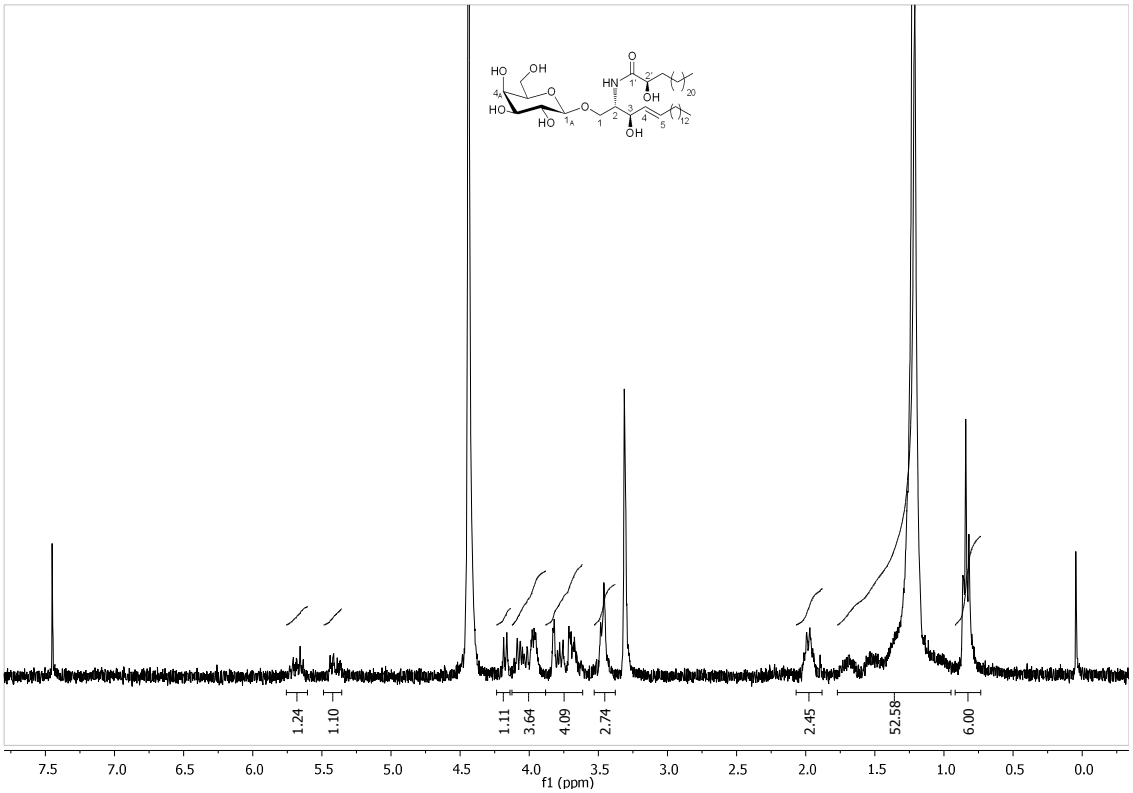
^{1}H-NMR of **5** (300 MHz, $CDCl_3$)



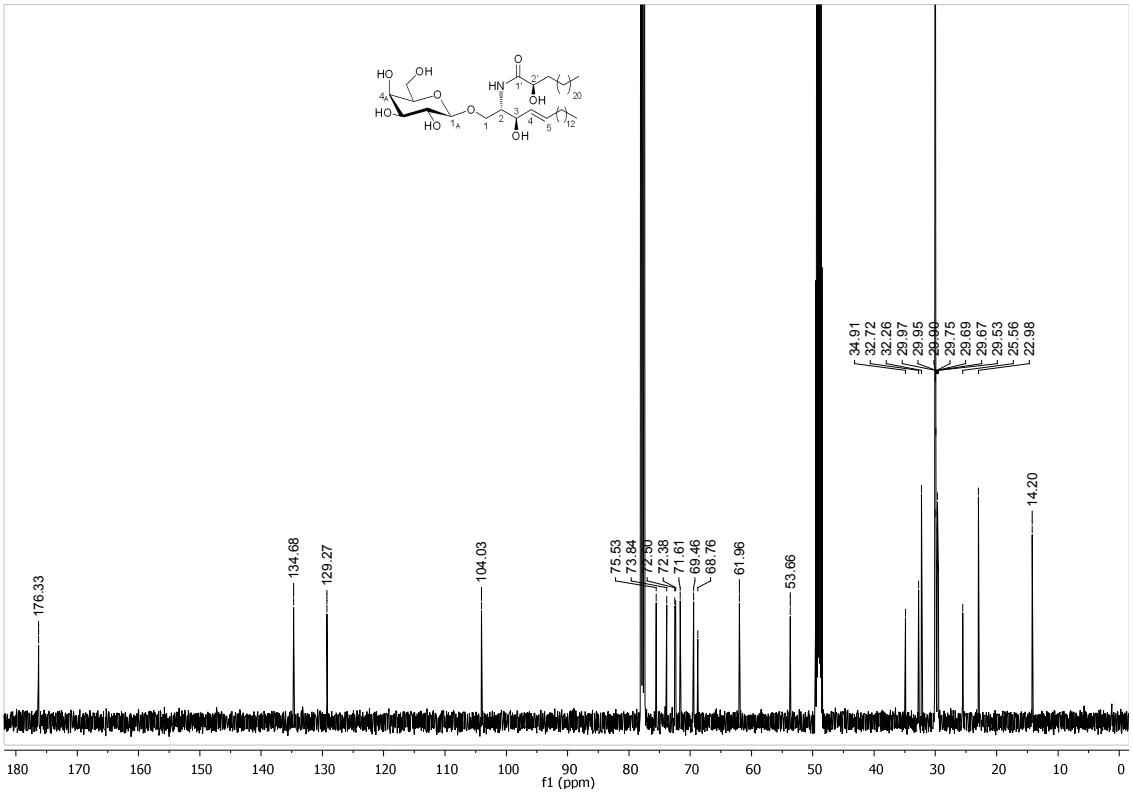
^{13}C-NMR of **5** (126 MHz, $CDCl_3$)



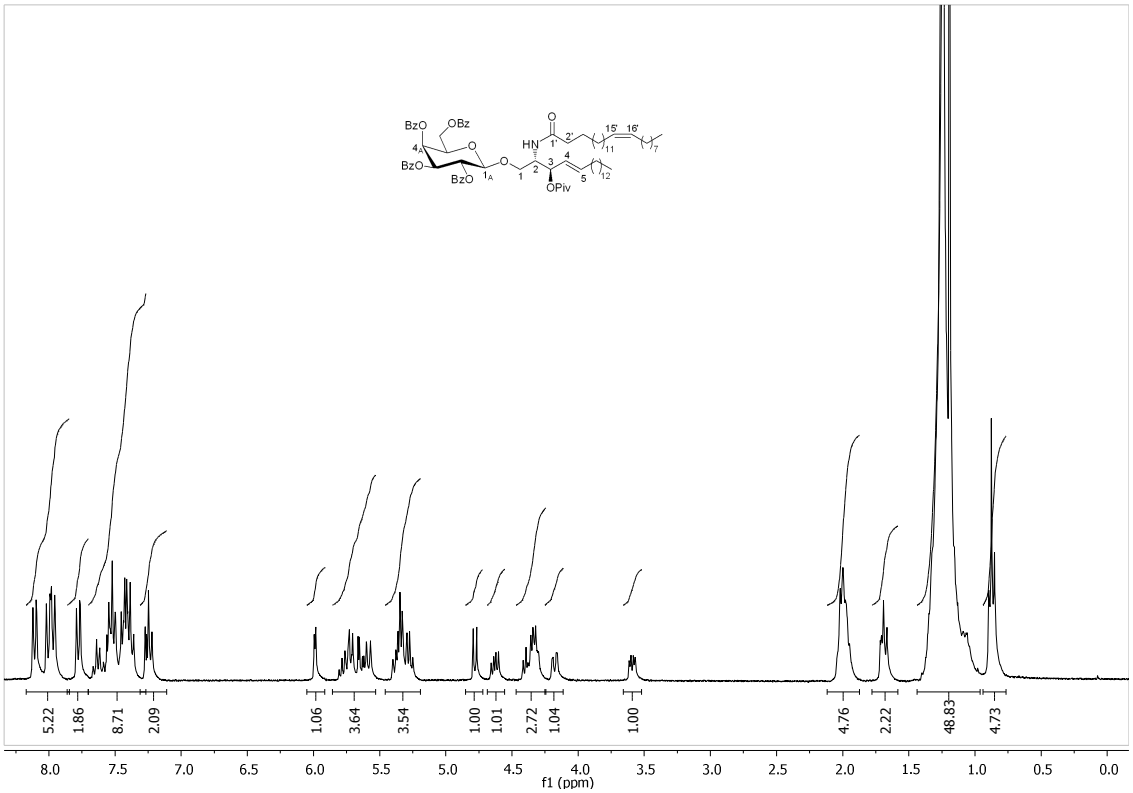
^{1}H-NMR of **GalCer C 24:0(2-OH)** (300 MHz, $CDCl_3/CD_3OD$, 3:1)



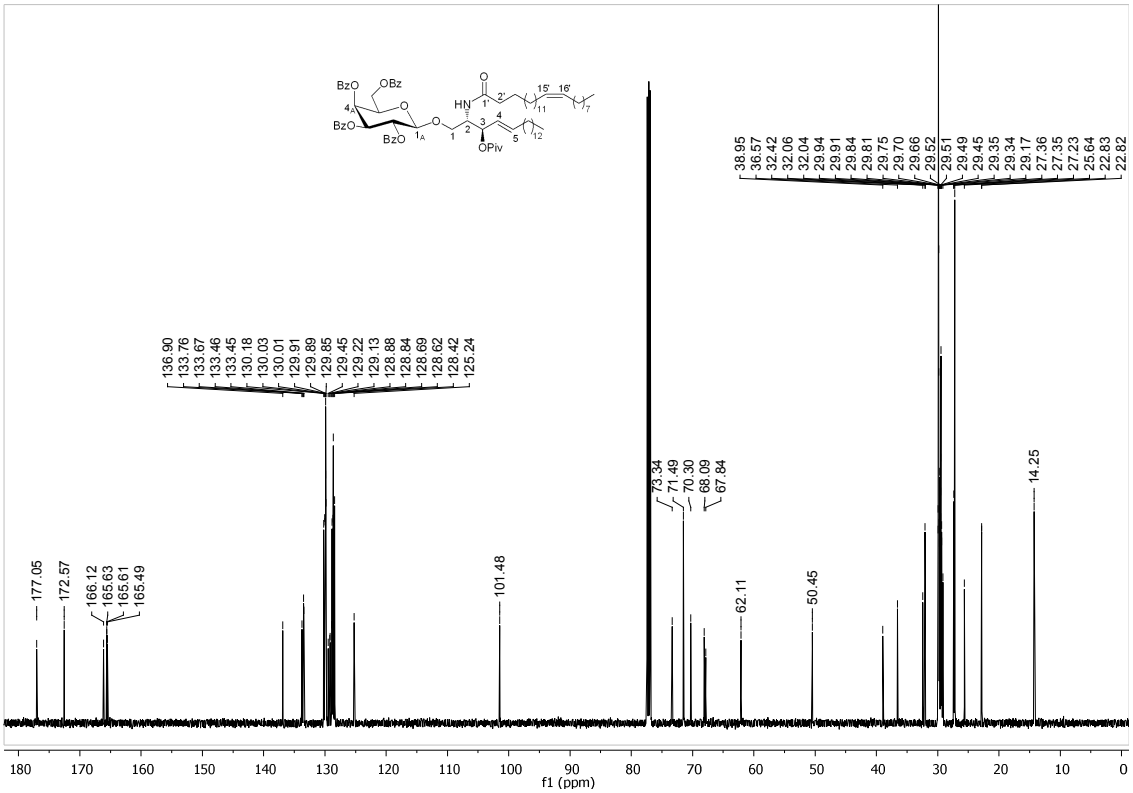
^{13}C-NMR of **GalCer C 24:0(2-OH)** (126 MHz, $CDCl_3/CD_3OD$, 3:1)



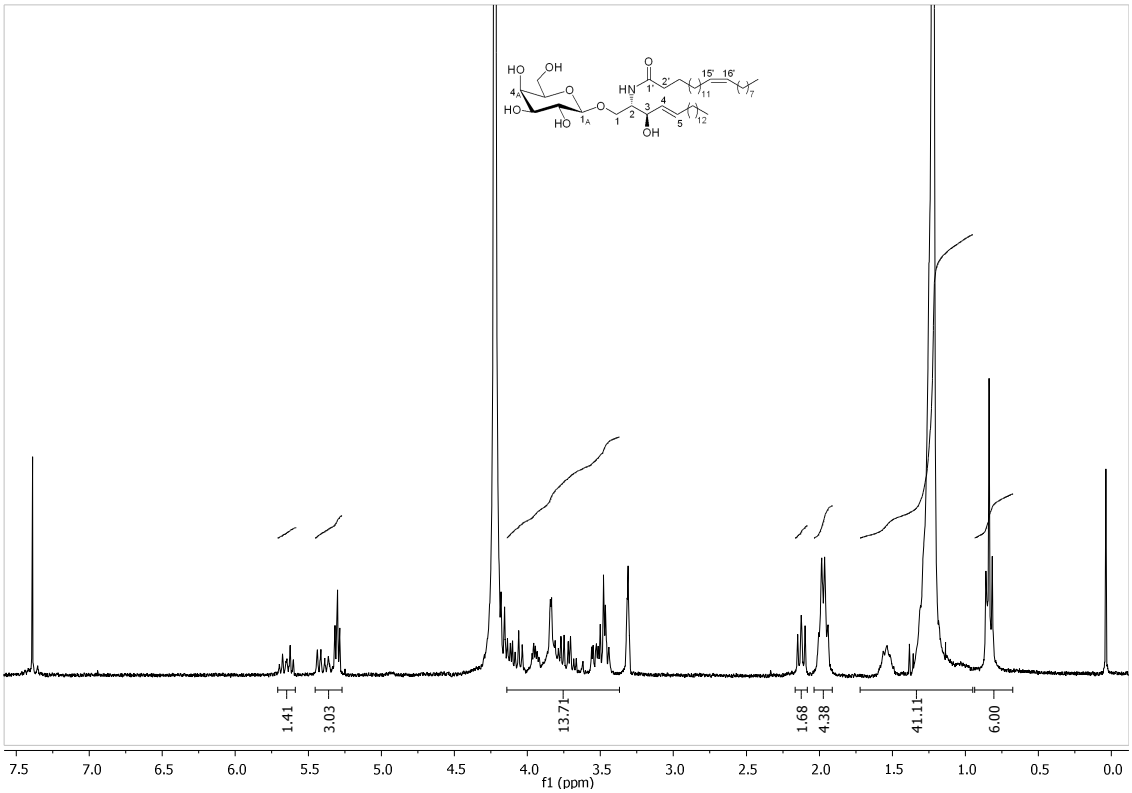
^{1}H-NMR of **6** (300 MHz, $CDCl_3$)



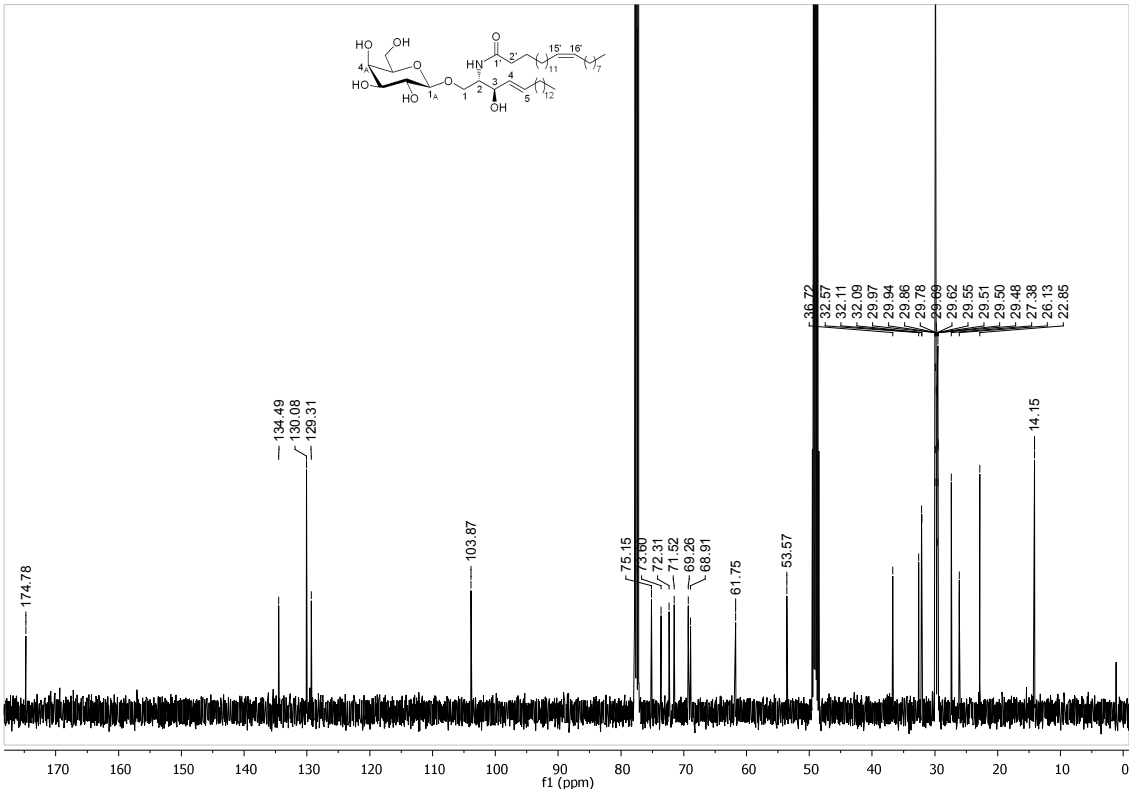
^{13}C-NMR of **6** (126 MHz, $CDCl_3$)

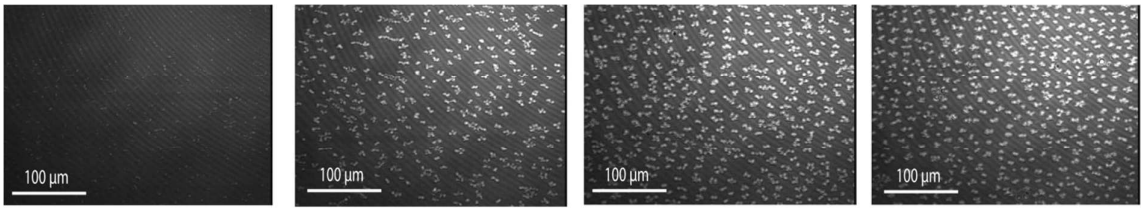


^{1}H-NMR of **GalCer C 24:1** (300 MHz, $CDCl_3/CD_3OD$, 3:1)



^{13}C-NMR of **GalCer C 24:1** (126 MHz, $CDCl_3/CD_3OD$, 3:1)





BAM images obtained for **GalCer C24:1** monolayers on a PBS subphase at 20 °C along the plateau region. Small round domains appear after the hump in the isotherm. Upon compression, the domains increase in number but do not grow substantially. Later, they gather together and form clusters or chains of small domains.

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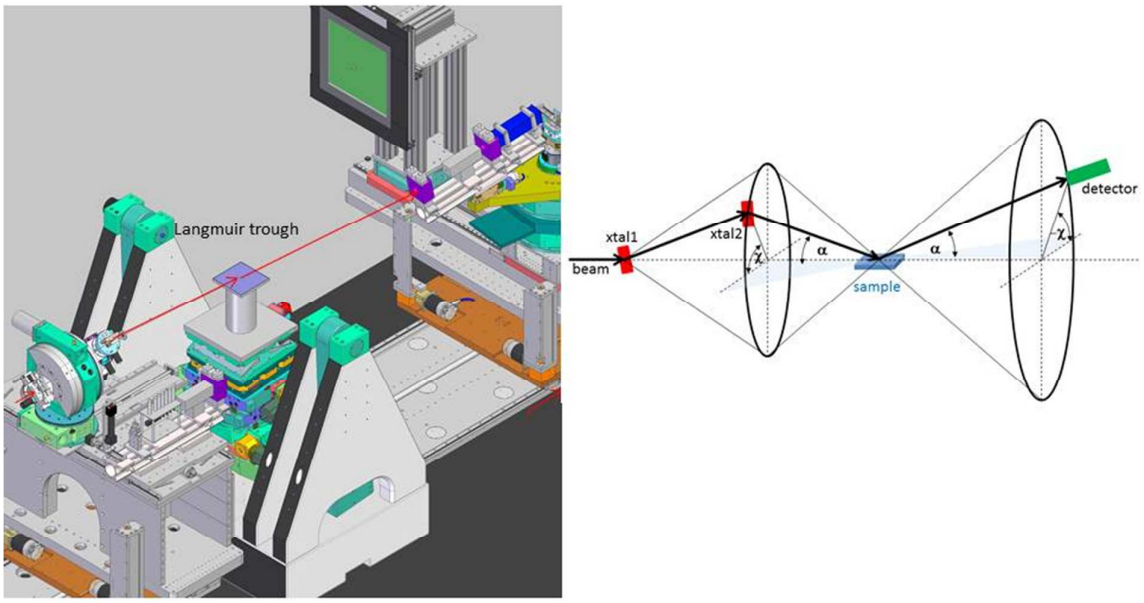
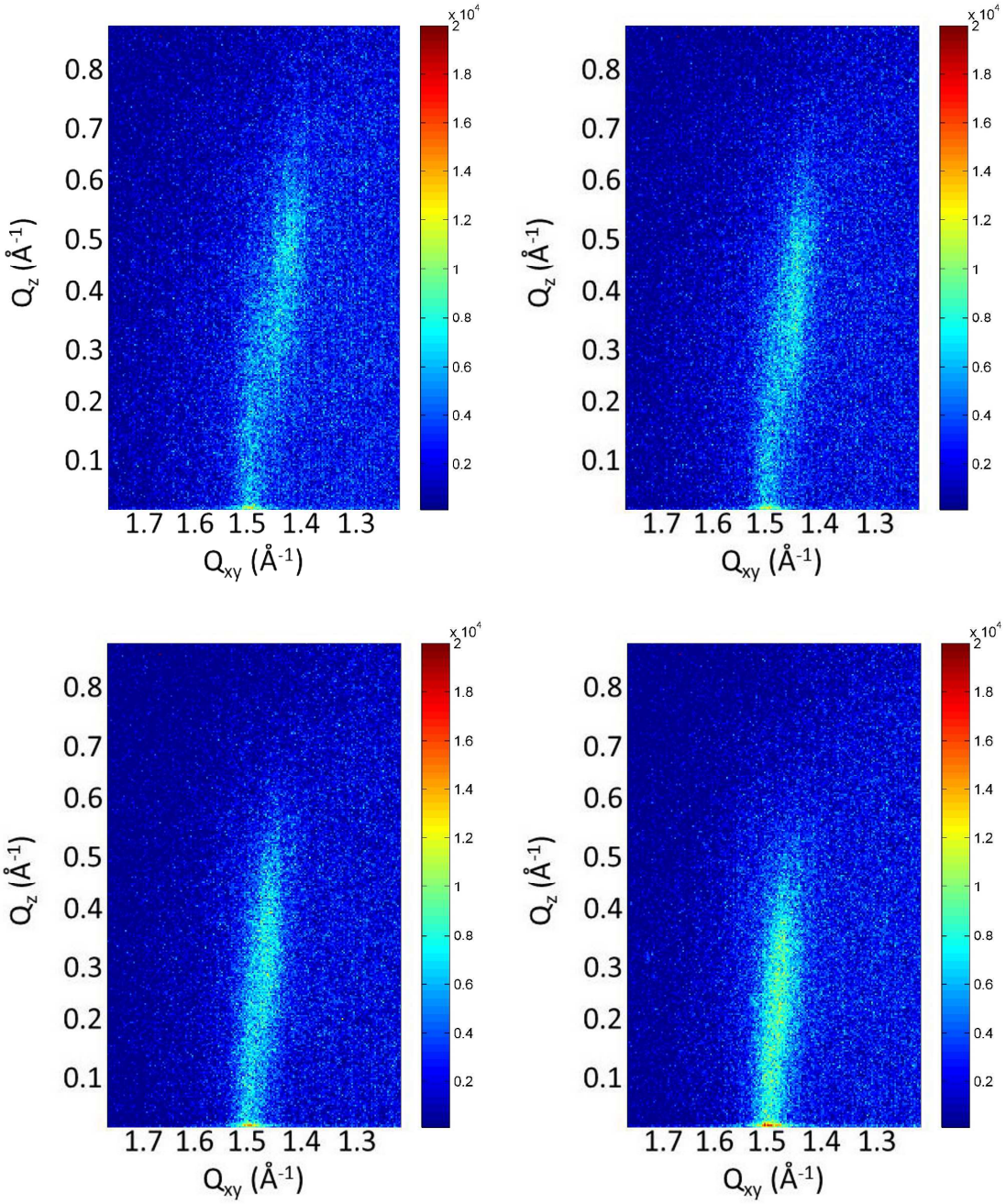
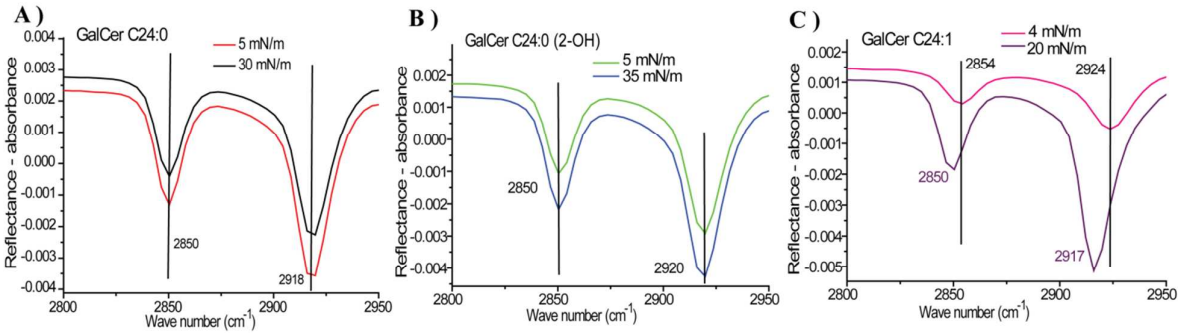


Set-up for scattering on liquid/gas interfaces at the high energy beamline P07 at DESY (Hamburg, Germany)



Diffraction patterns of **GalCer C24:0 (2-OH)** on PBS at 22 °C. Upper row: 4 mN/m and 10 mN/m (from left to right), lower row: 20 mN/m and 30 mN/m (from left to right). The decrease of the tilt angle with increasing pressure can be clearly seen by the shift of the degenerated Bragg peak to lower Q_z and higher Q_{xy} vealues.



IRRA spectra in the CH_2 region measured at different surface pressures for monolayers of **A) GalCer C24:0**, **B) GalCer C24:0 (2-OH)**. **C) GalCer C24:1.** The monolayers are prepared at 20 °C on a PBS subphase (10 mM, pH 7.4, 150 mM NaCl).

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