Electronic Supplementary Information

Borinic acid-catalyzed regio- and stereoselective synthesis of β-glycosylceramides

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Materials and Methods

General

All reactions were stirred using teflon-coated magnetic stir bars at room temperature (23 °C) unless otherwise stated. Stainless steel needles and gas-tight syringes were used to transfer air- and moisture-sensitive liquids. Borosilicate glass vials were obtained from VWR and dried at 140 °C for at least 24 hours prior to use. Flash column chromatography was carried out using neutral silica gel (60 Å, 230-400 mesh) (Silicycle) using reagent grade solvents. Analytical TLC was carried out using aluminium-backed silica gel 60 F_{254} plates (EMD Milipore) and visualized with a UV_{254} lamp or with aqueous basic permanganate stain.

Materials

Where indicated, dry solvents are HPLC grade and purified using a solvent purification system equipped with columns of activated alumina under nitrogen. (Innovative Technology, Inc.) Distilled water was obtained from an in-house supply. Nuclear magnetic resonance (NMR) solvents were obtained from Cambridge Isotope Laboratories. Methanesulfonic anhydride was purchased from Sigma Aldrich and stored prior to use in a −17 °C glove box freezer under nitrogen. *N*-Bromosuccinimide was recrystallized from water and dried under high vacuum prior to use. Carbohydrates were purchased from Carbosynth Ltd. or Sigma Aldrich and used without further purification. All other reagents and solvents otherwise not indicated were purchased from Sigma Aldrich or Caledon and used without further purification.

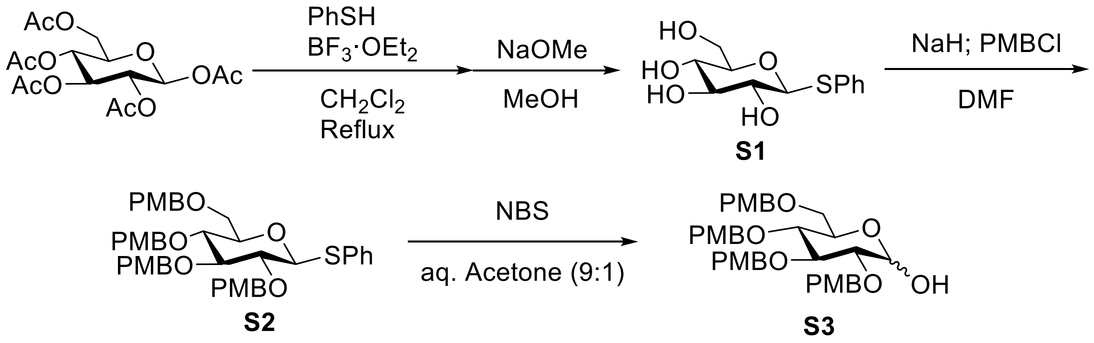
Instrumentation

^{1}H, ^{13}C and 2D NMR spectra were recorded using an Agilent DD2-700 (700 MHz) equipped with an HCN Cold Probe, Agilent DD2-500 (^{13}C, 126 MHz) equipped with a XSens cryoprobe, or Bruker Avance III (400 MHz) instrument at the Centre for Spectroscopic Investigation of Complex Organic Molecules and Polymers (CSICOMP) at the University of Toronto. ^{1}H NMR are reported in parts per million (ppm) relative to tetramethylsilane and referenced to residual protium in the dominant solvent.[1] Spectral features are reported in the following order: chemical shift (δ, ppm); multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, m-complex multiplet, br.-broad); number of protons; coupling constants (*J*, Hz); assignment. Where reported, assignments were made on the basis of coupling constants and 2D (gCOSY/HSQC/HMBC) NMR spectra. High-resolution mass spectra (HRMS) were obtained either on an Agilent 6538 UHD Q-TOF for

ESI$^+$ (electrospray ionization, positive mode) or a JEOL AccuTOF JMS-TL1000LC for DART$^+$ (direct analysis in real time) at the Advanced Instrumentation for Molecular Structure (AIMS) Mass Spectrometry Laboratory at the University of Toronto. Calculated and found mass to charge ratios (*m/z*) are corrected for the mass of the electron (0.00054 Da).

Preparation of Glycosyl Hemiacetal Donors

S3 –2,3,4,6-Tetra-*O*-4-methoxybenzyl-D-glucopyranose



Phenyl β-D-thioglucopyranoside (**S1**) was prepared according to a patent procedure from 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside (Sigma Aldrich).[2] Spectral features are in agreement with those previously reported.[3]

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 7.58–7.52 (m, 2H), 7.33–7.22 (m, 3H), 4.59 (d, *J* = 9.8 Hz, 1H), 3.87 (dd, *J* = 12.1, 2.0 Hz, 1H), 3.67 (dd, *J* = 12.1, 5.4 Hz, 1H), 3.38 (dd, *J* = 9.0, 8.6 Hz, 1H), 3.35–3.26 (m, 2H), 3.21 (dd, *J* = 9.8, 8.6 Hz, 1H).

S1 (817 mg, 3.0 mmol, 1 eq.) was added to an oven-dried 50 mL Schlenk flask purged with argon, followed by dry DMF (20 mL). The solution was cooled to 0 °C using an ice bath and sodium hydride (60 % in mineral oil) (720 mg, 18 mmol, 6.0 eq.) was added in a single portion. The ice bath was removed and the mixture allowed to stir for 20 minutes, after which time the ice bath was replaced. 4-methoxybenzyl chloride (2.45 mL, 18 mmol, 6.0 eq) was added by syringe and the mixture was allowed to stir overnight (16 h). The mixture was then stirred at 40 °C for 8 h, after which time it was quenched with methanol and concentrated by rotary evaporation. The residue was resuspended in ethyl acetate/water. The organic and aqueous phases were partitioned, and the organic phase was washed with saturated aq. sodium chloride. The organic layer was dried over magnesium sulfate, filtered, and concentrated to give a solid residue that was purified by flash chromatography on silica gel (25 % to 35 % ethyl acetate in hexanes) to give 989 mg (44 %) of **S2** as a white crystalline solid. Spectral features are in agreement with those previously reported.[4]

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.63 – 7.52 (m, 2H), 7.38 – 7.29 (m, 2H), 7.29 – 7.20 (m, 7H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.91 – 6.78 (m, 8H), 4.87 – 4.77 (m, 3H), 4.74 (d, *J* = 10.5 Hz, 1H), 4.67 (d, *J* = 10.0 Hz, 1H), 4.64 (d, *J* = 9.8 Hz, 1H), 4.55 (d, *J* = 11.6 Hz, 1H), 4.52 – 4.44

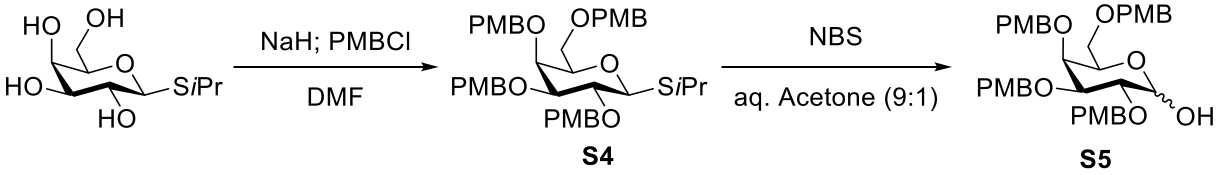
(m, 2H), 3.83 – 3.78 (m, 12H), 3.72 (d, *J* = 2.0 Hz, 1H), 3.70 – 3.62 (m, 2H), 3.58 (dd, *J* = 9.4, 9.2 Hz, 1H), 3.51 – 3.42 (m, 2H).

S2 (753 mg, 1.0 mmol, 1 eq) was added to a 50 mL round-bottomed flask under ambient conditions. It was dissolved in acetone/water (9:1) (10 mL) and *N*-bromosuccinimide (178 mg, 1.0 mmol, 1.0 eq.) was added. An orange colour was immediately observed that dissipated within a minute. Another portion of *N*-bromosuccinimide (178 mg, 1.0 mmol, 1.0 eq.) was added after 10 minutes. After 10 minutes of further stirring, the mixture was concentrated by rotary evaporation until turbidity arose. The mixture was redissolved in ethyl acetate/water, and the organic and aqueous phases were partitioned. The organic phase was washed with saturated aq. sodium bicarbonate, followed by saturated aq. sodium chloride, dried over magnesium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography on silica gel (50% ethyl acetate/hexanes) to give 464 mg (70 %) of **S3** as a white solid whose spectral features are in agreement with those previously reported.[5]

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.32 – 7.18 (m, 8.4H), 7.03 (m, 2.9H), 6.90 – 6.76 (m, 11.3H), 5.16 (d, *J* = 3.6 Hz, 1H), 4.88 – 4.82 (m, 1.74H), 4.78 – 4.66 (m, 4.6H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.58 – 4.34 (m, 4.34H), 3.98 (ddd, *J* = 9.9, 4.0, 2.2 Hz, 1H), 3.92 – 3.86 (m, 1.31H), 3.82 – 3.75 (m, 16.8H), 3.67 – 3.62 (m, 1.41H), 3.60 – 3.51 (m, 3.83H), 3.50 – 3.46 (m, 0.73H), 3.34 (dd, *J* = 9.1, 7.7 Hz, 0.42H).

α:β = 2.4:1

S5 –2,3,4,6-Tetra-*O*-4-methoxybenzyl-D-galactopyranose



Isopropyl β-D-thiogalactopyranoside (477 mg, 2.0 mmol, 1 eq.) (Carbosynth Ltd.) was added to an oven-dried 50 mL Schlenk flask purged with argon, followed by dry DMF (10 mL). The solution was cooled to 0 °C using an ice bath and sodium hydride (60 % in mineral oil) (480 mg, 12 mmol, 6.0 eq.) was added in a single portion. The mixture was stirred for 30 minutes, after which time the ice bath was removed. Once warmed to room temperature, 4-methoxybenzyl chloride (1.6 mL, 12 mmol, 5.9 eq) was added by syringe. The mixture was stirred vigorously overnight (17 h). The resulting solution was quenched with methanol and concentrated by rotatory evaporation. The residue obtained was resuspended in ethyl acetate/diethyl ether/water. The organic and aqueous phases were partitioned, and the organic phase was washed with saturated aq. sodium chloride. The organic layer was dried over magnesium sulfate, filtered, and concentrated to give a white solid residue that was purified by flash chromatography on silica gel (7% to 60 % ethyl acetate in hexanes) to give 1.37 g (95 %) of **S4** as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.35 – 7.26 (m, 4H, Ar-H), 7.25 – 7.15 (m, 4H, Ar-H), 6.93 – 6.78 (m, 8H, Ar-H), 4.84 (d, *J* = 11.4 Hz, 1H, $ArCH_2$), 4.78 (d, *J* = 9.8 Hz, 1H, $ArCH_2$), 4.69 (d, *J* = 9.8 Hz, 1H, $ArCH_2$), 4.64 (s, 2H, $ArCH_2$), 4.54 (d, *J* = 11.4 Hz, 1H, $ArCH_2$), 4.45 (d, *J* = 9.7 Hz, 1H, H-1), 4.38 (d, *J* = 11.3 Hz, 1H, $ArCH_2$), 4.31 (d, *J* = 11.3 Hz, 1H, $ArCH_2$), 3.87 (dd, *J* = 2.9, 1.2 Hz, 1H, H-4), 3.81 (s, 3H, Ar-OCH_3), 3.79 (s, 6H, Ar-OCH_3), 3.79 (s, 3H, Ar-OCH_3), 3.74 (dd, *J* = 9.7, 9.3 Hz, 1H, H-2), 3.56 – 3.46 (m, 4H, H-3, H-5, H-6), 3.20 (heptet, *J* = 6.7 Hz, 1H, $C\underline{H}$(Me)2), 1.31 (d, *J* = 6.7 Hz, 6H, $CH(C\underline{H}_3)_2$).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 159.5, 159.4, 159.2, 159.0, 131.1, 130.9, 130.8, 130.2, 130.2, 129.9, 129.7, 129.3, 113.9, 113.9, 113.7, 85.2, 84.1, 78.5, 77.4, 75.5, 74.0, 73.3, 73.2, 73.2, 72.6, 68.9, 55.4, 55.4, 55.4, 55.4, 35.4, 24.1, 24.0.

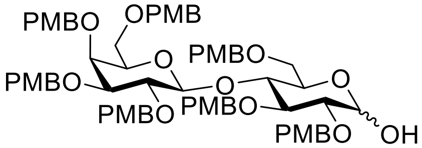
HRMS (DART$^+$, *m/z*): calculated for $C_{41}H_{54}NO_9S$ $[M+NH_4]^+$: 736.35193, found 736.35095.

S4 (1.08 g, 1.50 mmol, 1 eq) was added to a 50 mL round-bottomed flask under ambient conditions. It was dissolved in acetone/water (9:1) (10 mL) and *N*-bromosuccinimide (254 mg, 1.42 mmol, 0.95 eq.) was added in a single portion with stirring. An orange colour was immediately observed that dissipated within a minute. After 30 minutes of stirring, the mixture was concentrated by rotary evaporation until turbidity arose. The mixture was redissolved in ethyl acetate/water, and the organic and aqueous phases were partitioned. The organic phase was washed with saturated aq. sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated. The resulting solid residue was purified by flash chromatography on silica gel (50% to 60 % ethyl acetate/hexanes) to give 842 mg (85 %) of **S5** as a white solid whose spectral features are in agreement with those previously reported.[6]

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.31 – 7.22 (m, 5.7H), 7.18 (dd, *J* = 7.4, 5.6 Hz, 5.3H), 6.90 – 6.75 (m, 10.7H), 5.19 (d, *J* = 3.6 Hz, 1H), 4.85 – 4.78 (m, 2H, 1.73H), 4.75 – 4.70 (m, 1.34H), 4.68 – 4.57 (m, 4.11H), 4.54 – 4.45 (m, 1.40H), 4.42 – 4.36 (m, 1.35H), 4.34 – 4.26 (m, 1.37H), 4.09 (dd, *J* = 6.5, 6.4, Hz, 1H), 3.95 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.87 (dd, *J* = 2.9, 1.2 Hz, 1H), 3.82 (m, 1.37H), 3.80 – 3.72 (m, 16.4H), 3.68 (dd, *J* = 9.6, 7.4 Hz, 0.38H), 3.55 – 3.49 (m, 0.72H), 3.49 – 3.37 (m, 2.71H), 3.33 (d, *J* = 6.4 Hz, 0.33H), 3.07 (br. s, 1H).

α:β = 2.8:1

S6 – 4-*O*-(2′,3′,4′,6′-Tetra-*O*-4-methoxybenzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-4-methoxybenzyl-D-glucopyranose



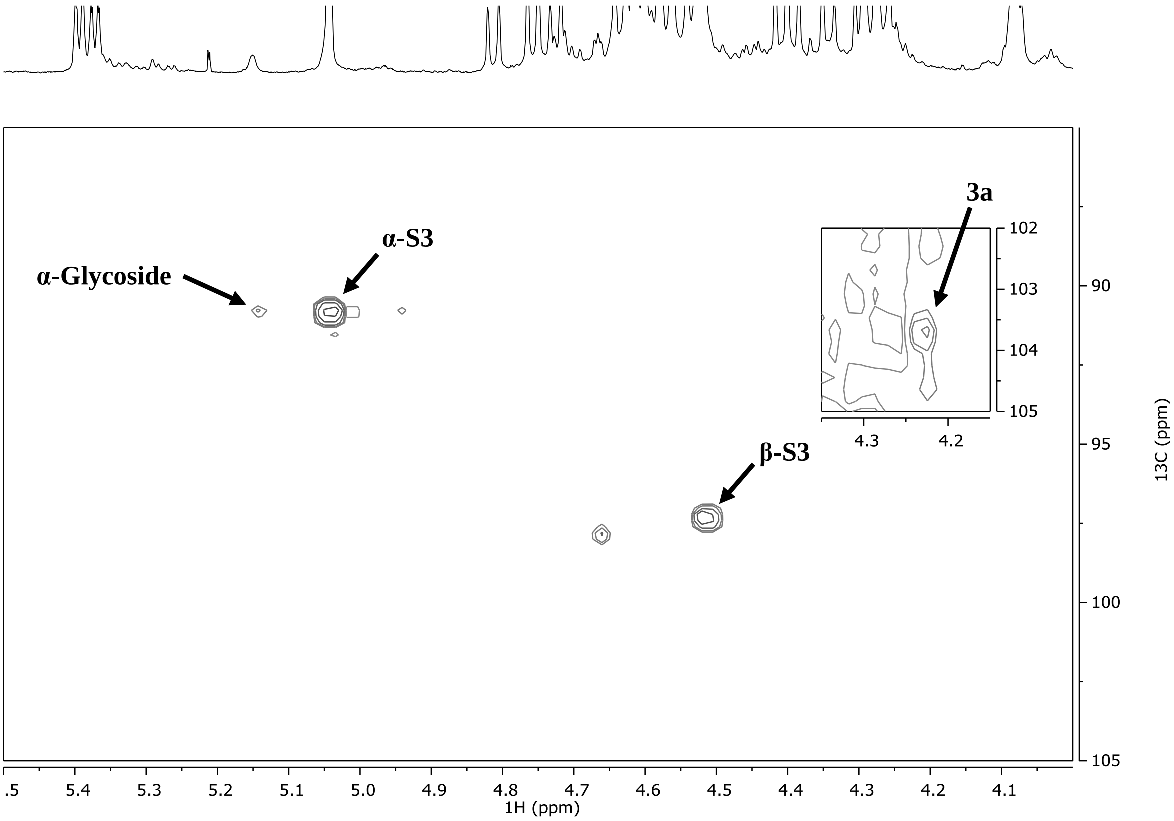
S6

Prepared from α-lactose monohydrate as previously reported.[7]

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.32–7.09 (m, 26H), 6.90–6.73 (m, 21H), 6.71–6.66 (m, 2.7H), 5.11 (d, *J* = 3.9 Hz, 1H, H-1α), 5.02–4.82 (m, 3.5H), 4.81–4.53 (m, 13.3H), 4.53–4.41 (m, 3.8H), 4.39–4.18 (m, 7.1H), 3.99–3.63 (m, 49H), 3.63–3.21 (m, 12H), 3.08–2.82 (m, 1.55H, OH).

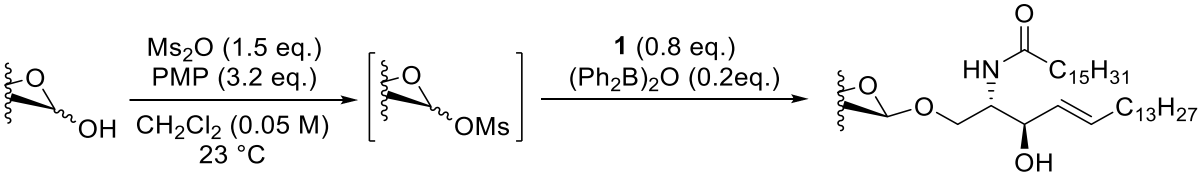
Synthesis of Glycosylceramides

Figure S1 – Expansion of HSQC spectrum of crude reaction mixture from reaction of methanesulfonate 2a with *N*-palmitoyl-D-sphingosine in the absence of catalyst



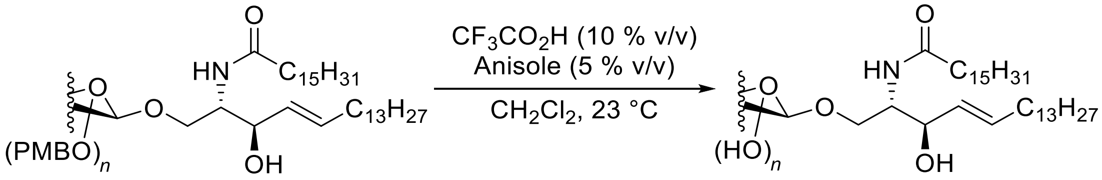
Vertical scaling in the inset has been increased to display the labelled peak corresponding to trace **3a**. Noise is evident.

General Procedure A – Ceramide Glycosylation using Glycosyl Methanesulfonates



To an oven-dried 1 dram vial was added the glycosyl hemiacetal (0.05 mmol, 1.0 eq.). A septum was placed on the vial and it was thrice evacuated and purged with argon. Dry dichloromethane (0.5 mL) was added, followed by pentamethylpiperidine (PMP) (29 μL, 0.16 mmol, 3.2 eq.). In a glove box, a separate oven-dried 1 dram vial was charged with methanesulfonic anhydride (Ms_2O) (20.9 mg, 0.12 mmol), which was then removed from the glove box and dissolved in dry dichloromethane (0.8 mL) to give a 0.15 M solution of Ms_2O.[*] To the solution of the hemiacetal, 0.5 mL of 0.15 M Ms_2O in dichloromethane (0.075 mmol, 1.5 eq.) was added.[†] The solution was stirred for at least 10 minutes, or until a persistent colouration (very light yellow to yellow) was observed. A third oven-dried 1 dram vial was charged with *N*-palmitoyl-D-sphingosine (**1**) (21.5 mg, 0.040 mmol, 0.8 eq.) (Sigma Aldrich) and diphenylborinic anhydride.[8] (2.8 mg, 0.008 mmol, 0.2 eq.) The vial was capped with a septum and thrice evacuated and purged with argon. The previously prepared 0.05 M solution of glycosyl methanesulfonate (1 mL, 0.05 mmol, 1 eq.) was then added. The septum was quickly replaced with a plastic cap, the vial sealed with Teflon tape, and the mixture stirred overnight for at least 15 hours. Volatiles were evaporated by air stream and the resulting residue purified by flash chromatography on silica gel to afford the desired glycosylceramide.[‡]

General Procedure B – Deprotection of PMB-Protected Glycosylceramides



In a 1 dram vial under ambient conditions, the PMB-protected glycosylceramide was dissolved in HPLC-grade dichloromethane (850 μL), to which anisole (50 μL), and then trifluoroacetic acid (100 μL) were added. The solution was stirred for at least 1 hour, after which time it was

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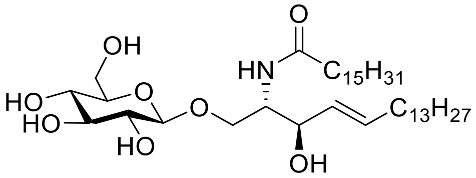
[*] We have found that dichloromethane solutions of methanesulfonic anhydride are not stable for prolonged periods of time at room temperature and should be prepared fresh prior to use.

[†] An exotherm has been observed on occasion during addition of methanesulfonic anhydride. Care should be taken to add the Ms_2O solution slowly to minimize decomposition of the glycosyl methanesulfonate when the procedure is performed on larger scales.

[‡] Yields of glycoside products are based on the mass of acceptor used.

diluted with toluene and concentrated by rotary evaporation. The resulting residue was coevaporated with toluene three times to give a white solid that was purified by flash chromatography on silica gel to afford the desired glycosylceramide.

4a – β-D-Glucosyl *N*-palmitoyl-D-*erythro*-sphingosine



The intermediate PMB-protected glycosylceramide **3a** was prepared according to General Procedure A from hemiacetal **S3** (33.0 mg, 0.050 mmol, 1.0 eq.) and purified by flash chromatography on silica gel (80 % diethyl ether in hexanes) to give a white waxy solid. (36.3 mg, 77 %) The following characterization data were obtained for intermediate **3a**:

^{1}H NMR (700 MHz, $CDCl_3$): δ (ppm) = 7.27 – 7.21 (m, 6H, Ar-H), 7.06 – 7.03 (m, 2H, Ar-H), 6.88 – 6.83 (m, 6H, Ar-H), 6.82 – 6.80 (m, 2H, Ar-H), 6.20 (d, *J* = 7.7 Hz, 1H, N-H), 5.71 (dddd, *J* = 15.0, 6.8, 6.8 1.2 Hz, 1H, H-5), 5.50 (dddd, *J* = 15.4, 6.7, 1.5, 1.5 Hz, 1H, H-4), 4.83 (d, *J* = 10.6 Hz, 1H, $ArCH_2$), 4.76 – 4.66 (m, 4H, $ArCH_2$), 4.51 (d, *J* = 11.7 Hz, 1H, $ArCH_2$), 4.44 (d, *J* = 11.7 Hz, 1H, $ArCH_2$), 4.40 (d, *J* = 10.5 Hz, 1H, $ArCH_2$), 4.31 (d, *J* = 7.8 Hz, 1H, H-1′), 4.14 (dddd, *J* = 6.2, 6.1, 6.1, 1.2 Hz, 1H, H-3), 4.06 (dd, *J* = 10.9, 4.8 Hz, 1H, H-1a), 4.00 (dddd, *J* = 7.7, 4.8, 4.8, 3.3 Hz, 1H, H-2), 3.84 (dd, *J* = 10.8, 3.3 Hz, 1H, H-1b), 3.81 – 3.77 (m, 13H, 3-OH, $Ar\text{-}OCH_3$), 3.64 (dd, *J* = 10.5, 2.1 Hz, 1H, H-6′a), 3.59 – 3.54 (m, 2H, H-3′, H-6′b), 3.48 (dd, *J* = 9.8, 8.9 Hz, 1H, H-4′), 3.40 (ddd, *J* = 9.9, 5.4, 2.1 Hz, 1H, H-5′), 3.37 (dd, *J* = 9.1, 7.8 Hz, 1H, H-2′), 2.05 – 1.99 (m, 4H, α-C=O, H-7), 1.55 – 1.49 (m, 2H, β-C=O), 1.38 – 1.32 (m, 2H, H-8), 1.32 – 1.19 (m, 44H, aliphatic CH_2), 0.90 – 0.86 (m, 6H, ω-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 174.0, 159.5, 159.5, 159.4, 133.9, 130.8, 130.4, 130.2, 129.8, 129.8, 129.8, 129.8, 129.6, 129.2, 114.0, 114.0, 114.0, 104.2, 84.6, 82.0, 77.6, 75.5, 74.8, 74.8, 73.4, 73.3, 69.6, 68.6, 55.4, 55.4, 55.4, 54.6, 36.7, 32.6, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.4, 25.9, 14.3.

HRMS (ESI$^+$, *m/z*): calculated for $C_{72}H_{110}NO_{12}$ [M+H]$^+$: 1180.8023 found: 1180.8014.

Glycosylceramide **4a** was prepared from intermediate **3a** (33.7 mg, 0.029 mmol) according to General Procedure B and purified by flash chromatography on silica gel (12.5 % methanol in chloroform) to give a white solid. (14.3 mg, 72 %)

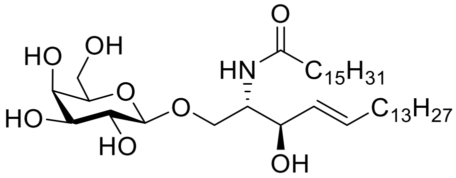
^{1}H NMR (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.7 mg/250 μL): δ (ppm) = 5.65 (dddd, *J* = 15.5, 6.7, 6.7, 1.2 Hz, 1H, H-5), 5.40 (dddd, *J* = 15.6, 7.1, 1.7, 1.5 Hz, 1H, H-4), 4.20 (d, *J* = 7.8 Hz, 1H, H-1′), 4.05 (ddd, *J* = 6.7, 6.7, 1.2 Hz, 1H, H-3), 4.02 (dd, *J* = 10.4, 5.2 Hz, 1H, H-1a), 3.96

(ddd, *J* = 6.7, 5.2, 3.5 Hz, 1H, H-2), 3.79 (dd, *J* = 12.0, 3.0 Hz, 1H, H-6′a), 3.67 (dd, *J* = 12.0, 4.8 Hz, 1H, H-6′b), 3.58 (dd, *J* = 10.4, 3.5 Hz, 1H, H-1b), 3.40 – 3.33 (m, 2H, H-3′, H-4′), 3.23 (ddd, *J* = 8.7, 4.8, 3.0 Hz, 1H, H-5′), 3.20 (dd, *J* = 7.8, 7.8 Hz, 1H, H-2′), 2.14 – 2.07 (m, 2H, α-C=O), 1.99 – 1.92 (m, 2H, H-7), 1.56 – 1.49 (m, 2H, β-C=O), 1.32 – 1.25 (m, 2H, H-8), 1.25 – 1.09 (m, 44H, aliphatic CH_2), 0.86 – 0.77 (m, 6H, ω-CH_3).

^{13}C NMR (126 MHz, 9:1 $CDCl_3$/CD_3OD, 23 °C, 1.7 mg/250 μL): δ (ppm) = 174.5, 134.5, 128.9, 103.1, 77.4, 76.9, 76.3, 76.0, 73.5, 72.4, 70.0, 68.7, 61.5, 53.3, 36.6, 32.4, 29.7, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 25.9, 14.1.

HRMS (ESI$^+$, *m/z*): calculated for $C_{40}H_{78}NO_8$ $[M+H]^+$: 700.5722 found: 700.5727.

4b – β-D-Galactosyl *N*-palmitoyl-D-*erythro*-sphingosine



The intermediate PMB-protected glycosylceramide **3b** was prepared according to General Procedure A from hemiacetal **S5** (33.0 mg, 0.050 mmol, 1.0 eq.) and purified by flash chromatography on silica gel (80 % diethyl ether in hexanes) to give a white solid. (36.3 mg, 77 %) The following characterization data were obtained for intermediate **3b**:

^{1}H NMR (700 MHz, $CDCl_3$): δ (ppm) = 7.28 – 7.25 (m, 4H, Ar-H), 7.21 – 7.17 (m, 4H, Ar-H), 6.88 – 6.82 (m, 8H, Ar-H), 6.19 (d, *J* = 7.9 Hz, 1H, N-H), 5.70 (dddd, *J* = 14.9, 6.7, 6.7, 1.2 Hz, 1H, H-5), 5.47 (dddd, *J* = 15.4, 6.6, 1.4, 1.4 Hz, 1H, H-4), 4.83 (d, *J* = 11.3 Hz, 1H, $ArCH_2$), 4.75 (d, *J* = 10.4 Hz, 1H, $ArCH_2$), 4.70 (d, *J* = 10.4 Hz, 1H, $ArCH_2$), 4.66 – 4.62 (m, 2H, $ArCH_2$), 4.52 (d, *J* = 11.3 Hz, 1H, $ArCH_2$), 4.40 (d, *J* = 11.4 Hz, 1H, $ArCH_2$), 4.32 (d, *J* = 11.5 Hz, 1H, $ArCH_2$), 4.26 (d, *J* = 7.7 Hz, 1H, H-1′), 4.15 – 4.11 (m, 1H, H-3), 4.07 (dd, *J* = 10.9, 4.4 Hz, 1H, H-1a), 4.00 (dddd, *J* = 7.9, 4.6, 3.4, 3.2 Hz, 1H, H-2), 3.83 – 3.77 (m, 13H, H-4′, Ar-OCH_3), 3.77 – 3.73 (m, 2H, H-1b, H-2′), 3.52 (dd, *J* = 8.7, 5.8 Hz, 1H, H-6′a), 3.49 – 3.41 (m, 3H, H-3′, H-4′, H-6′b), 2.03 – 1.98 (m, 2H, H-7), 2.00 – 1.90 (m, 2H, α-C=O), 1.53 – 1.48 (m, 2H, β-C=O), 1.39 – 1.14 (m, 46H, aliphatic CH_2), 0.91 – 0.86 (m, 6H, ω-CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 173.9, 159.5, 159.4, 159.4, 159.4, 133.8, 130.7, 130.7, 130.5, 130.1, 129.9, 129.8, 129.8, 129.3, 129.2, 114.0, 114.0, 113.9, 113.8, 104.7, 82.3, 79.2, 75.2, 74.2, 73.7, 73.5, 73.4, 72.9, 72.8, 69.8, 68.6, 55.4, 55.4, 55.4, 54.3, 36.7, 32.5, 29.9, 29.9, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.4, 25.9, 14.3.

HRMS (ESI^+, *m/z*): calculated for $C_{72}H_{110}NO_{12}$ $[M+H]^+$: 1180.8023 found: 1180.8019.

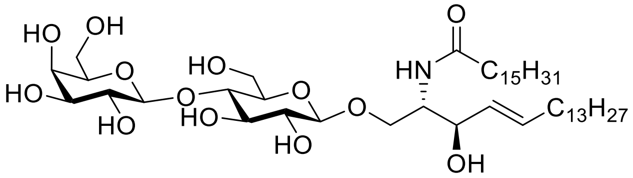
Glycosylceramide **4b** was prepared from intermediate **3b** (35.6 mg, 0.030 mmol) according to General Procedure B and purified by flash chromatography on silica gel (15 % methanol in chloroform) to give a white solid. (11.6 mg, 55 %)

^{1}H NMR (700 MHz, 9:1 $CDCl_3$/CD_3OD, 40 °C, 1.6 mg/250 µL): δ (ppm) = 5.64 (dddd, *J* = 15.4, 7.0, 6.6, 1.4 Hz, 1H, H-5), 5.40 (ddd, *J* = 15.4, 7.0, 1.6, 1.4 Hz, 1H, H-4), 4.15 (d, *J* = 7.6 Hz, 1H, H-1′), 4.08 – 4.03 (m, 2H, H-1a, H-3), 3.96 (ddd, *J* = 6.8, 5.2, 3.5 Hz, 1H, H-2), 3.85 (dd, *J* = 2.9, 1.2 Hz, 1H, H-4′), 3.79 (dd, *J* = 11.7, 6.4 Hz, 1H, H-6′a), 3.71 (dd, *J* = 11.7, 4.7 Hz, 1H, H-6′b), 3.58 (dd, J = 10.4, 3.5 Hz, 1H, H-1b), 3.49 (dd, J = 9.3, 9.2 Hz, 1H, H-2′), 3.46 – 3.41 (m, 2H, H-3′, H-5′), 2.13 – 2.07 (m, 2H, α-C=O), 1.99 – 1.92 (m, 2H, H-7), 1.56 – 1.49 (m, 2H, β-C=O), 1.32 – 1.26 (m, 2H, H-8), 1.26 – 1.14 (m, 44H, aliphatic CH_2), 0.84 – 0.79 (m, 6H, ω-CH_3).

^{13}C NMR (126 MHz, 9:1 $CDCl_3$/CD_3OD, 23 °C, 1.6 mg/250 μL): δ (ppm) = 174.5, 134.4, 128.9, 103.7, 74.8, 73.4, 72.3, 71.3, 69.2, 68.8, 61.8, 53.3, 36.6, 32.4, 29.7, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.4, 29.3, 25.9, 14.1.

HRMS (ESI$^+$, *m/z*): calculated for $C_{40}H_{78}NO_8$ $[M+H]^+$: 700.5722 found: 700.5720.

4c – β-D-Lactosyl *N*-palmitoyl-D-*erythro*-sphingosine



The intermediate PMB-protected glycosylceramide **3c** was prepared according to General Procedure A from hemiacetal **S6** (59.2 mg, 0.050 mmol, 1.0 eq.) and partially purified by flash chromatography on silica gel (80 % diethyl ether in hexanes) to give a white solid (54.2 mg). Hemiacetal **S6** could not be separated from intermediate **3c** and was carried forward to the deprotection step. The following partial characterization data were obtained for intermediate **3c**:

^{1}H NMR (700 MHz, $CDCl_3$): δ (ppm) = 6.20 (d, *J* = 7.8 Hz, 1H, N-H), 5.70 (dddd, *J* = 14.9, 6.9, 6.7, 1.2 Hz, 1H, H-5), 5.48 (dddd, *J* = 15.4, 6.6, 1.6, 1.4 Hz, 1H, H-4), 4.33 (d, *J* = 7.9 Hz, 1H, H-1″), 4.30 (d, *J* = 8.1 Hz, 1H, H-1′), 4.13 – 4.07 (m, 2H, H-1a, H-3), 3.99 (dddd, *J* = 7.8, 4.2, 4.2, 3.0 Hz, 1H, H-2).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 173.9, 133.9, 129.1, 104.2, 103.0, 54.3, 36.6, 32.6, 25.9, 14.3.

HRMS (ESI$^+$, *m/z*): calculated for $C_{102}H_{144}NO_{20}$ $[M+H]^+$: 1703.0276 found: 1703.0257.

Glycosylceramide **4c** was prepared from the mixture of intermediate **3c** and residual hemiacetal **S6** (51.3 mg) according to General Procedure B and purified by flash chromatography on silica gel (20 % methanol in chloroform) to give a white grainy solid. (12.3 mg, 38 % over two steps)

^{1}H NMR (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.5 mg/250 μL): δ (ppm) = 5.64 (dddd, *J* = 15.3, 6.8, 6.8, 1.5 Hz, 1H, H-5), 5.39 (dddd, *J* = 15.3, 7.0, 1.2, 1.2 Hz, 1H, H-4), 4.31 (d, *J* = 7.8 Hz, 1H, H-1″), 4.22 (d, *J* = 7.8 Hz, 1H, H-1′), 4.08 – 4.03 (m, 2H, H-1a, H-3), 3.95 (ddd, *J* = 6.6, 4.1, 4.1 Hz, 1H, H-2), 3.84 (dd, *J* = 3.3, 2.0 Hz, 1H, H-4″), 3.83 – 3.75 (m, 3H, H-6′, H-6″a), 3.71 (dd, *J* = 11.8, 4.1 Hz, 1H, H-6″b), 3.59 – 3.49 (m, 5H, H-1b, H-3′, H-4′, H-2″, H-5″), 3.45 (dd, *J* = 9.6, 3.3 Hz, 1H, H-3″), 3.34 – 3.30 (m, 1H, H-5′), 3.27 (dd, *J* = 8.2, 7.8 Hz, 1H, H-2′), 2.12 – 2.08 (m, 2H, α-C=O), 1.97 – 1.93 (m, 2H, H-7), 1.55 – 1.49 (m, 2H, β-C=O), 1.31 – 1.26 (m, 2H, H-8), 1.26 – 1.14 (m, 44H, aliphatic CH_2), 0.84 – 0.79 (m, 6H, ω-CH_3).

^{13}C NMR (126 MHz, 9:1 $CDCl_3/CD_3OD$, 23 °C, 1.5 mg/250 μL): δ (ppm) = 174.5, 134.5, 129.0, 103.5, 102.9, 79.1, 75.3, 75.0, 74.7, 73.4, 73.2, 72.3, 71.0, 69.3, 68.7, 61.8, 60.9, 53.2, 36.5, 32.4, 29.7, 29.7, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 25.9, 14.1.

HRMS (ESI$^+$, *m/z*): calculated for $C_{46}H_{88}NO_{13}$ $[M+H]^+$: 862.6250 found: 862.6237.

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<u>^{1}H, ^{13}C and 2D NMR Spectra</u>

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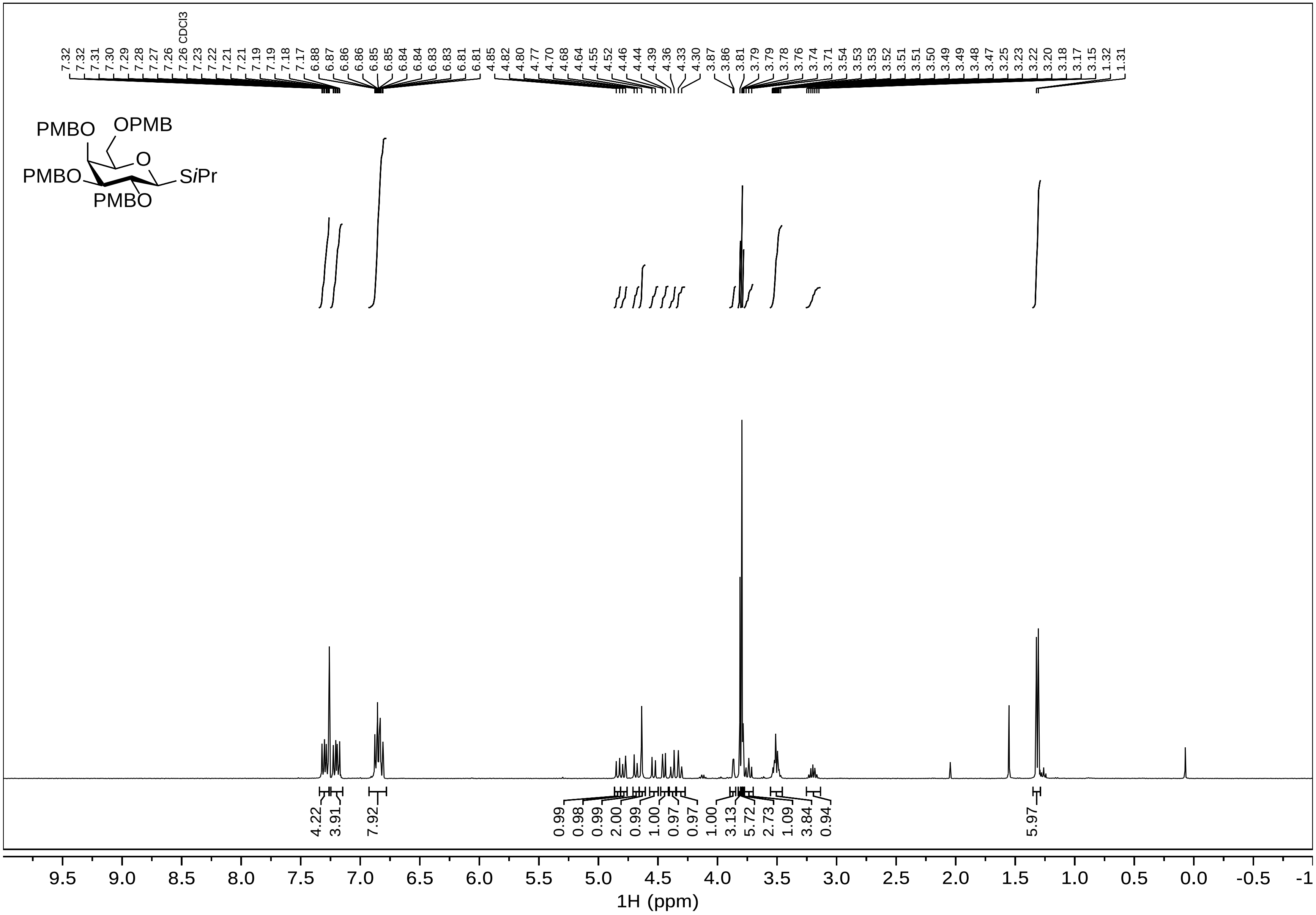
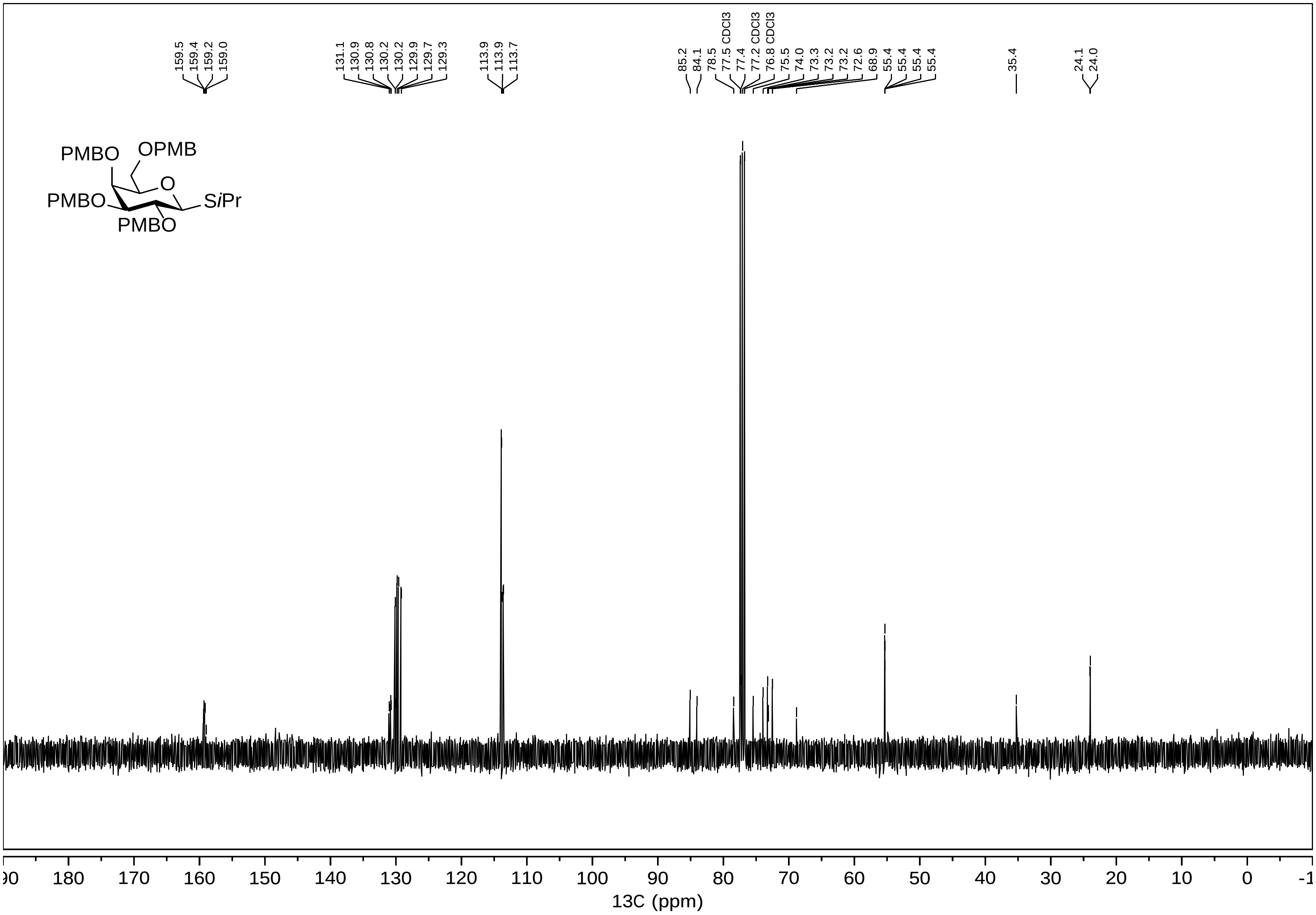
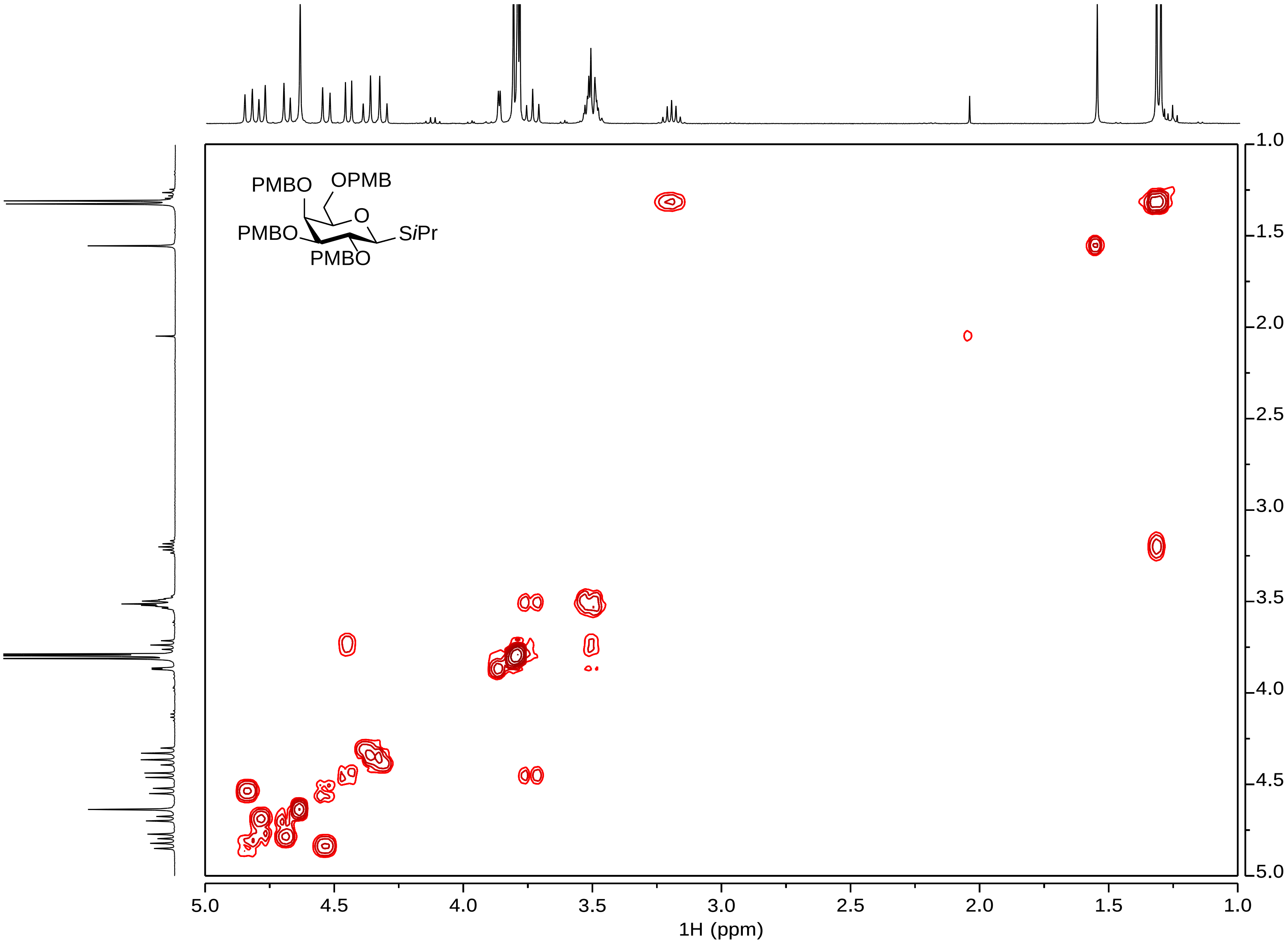
S4 – ^{1}H NMR (400 MHz, CDCl$_3$)



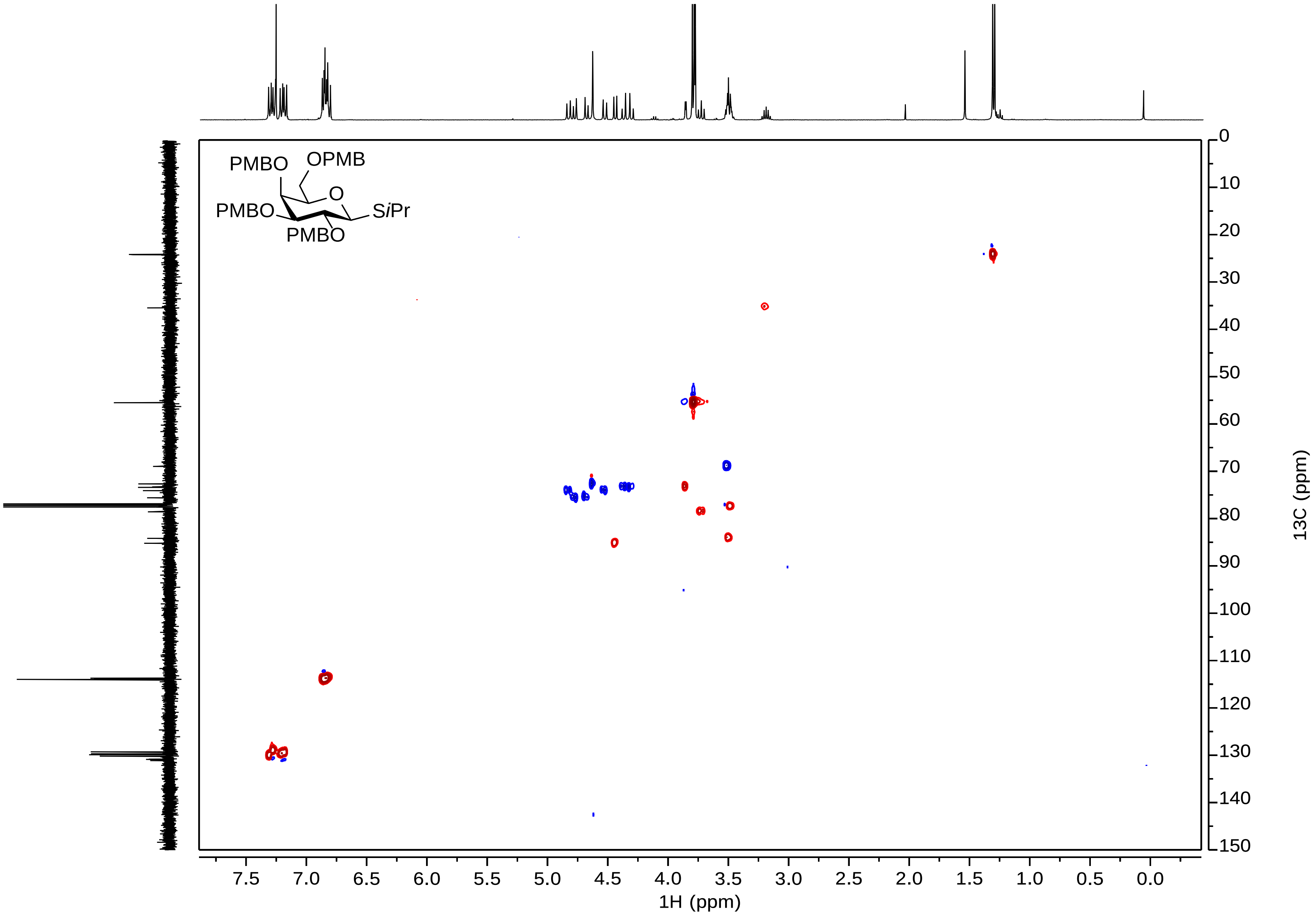
S4 – ^{13}C NMR (101 MHz, $CDCl_3$)



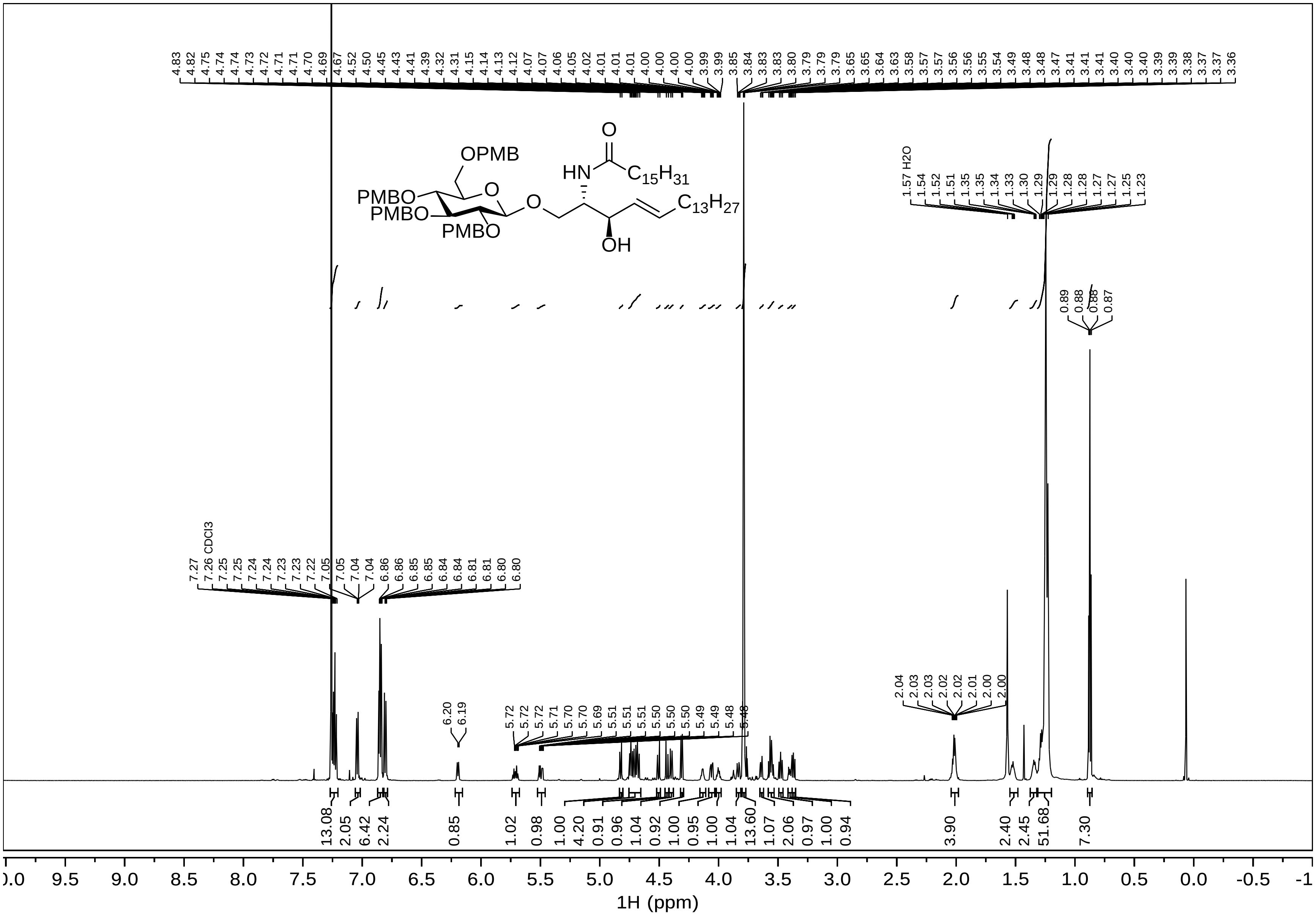
S4 – gCOSY (400 MHz, $CDCl_3$)



S4 – HSQC (400 MHz, CDCl$_3$)



3a – ^{1}H NMR (700 MHz, $CDCl_3$)



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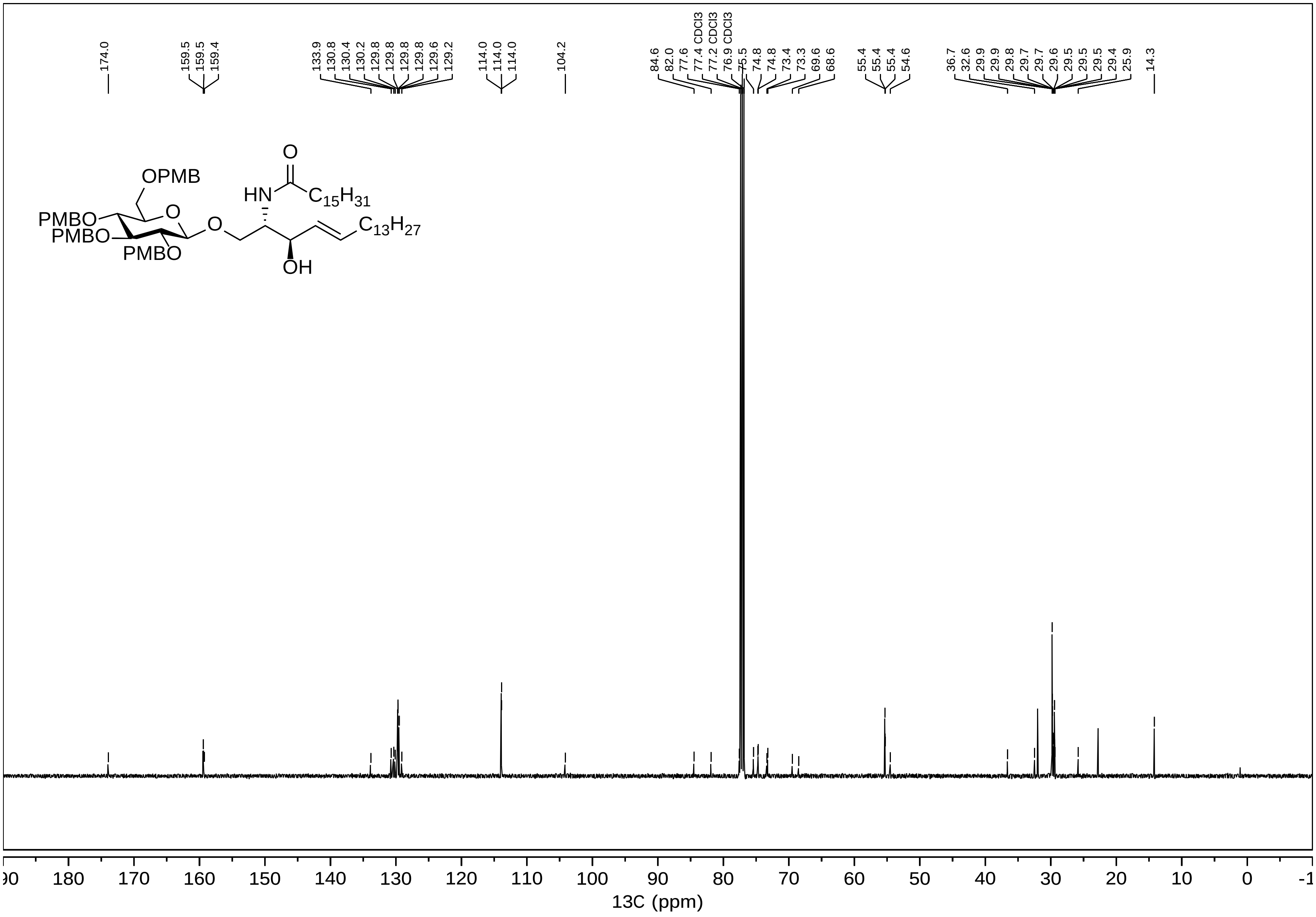

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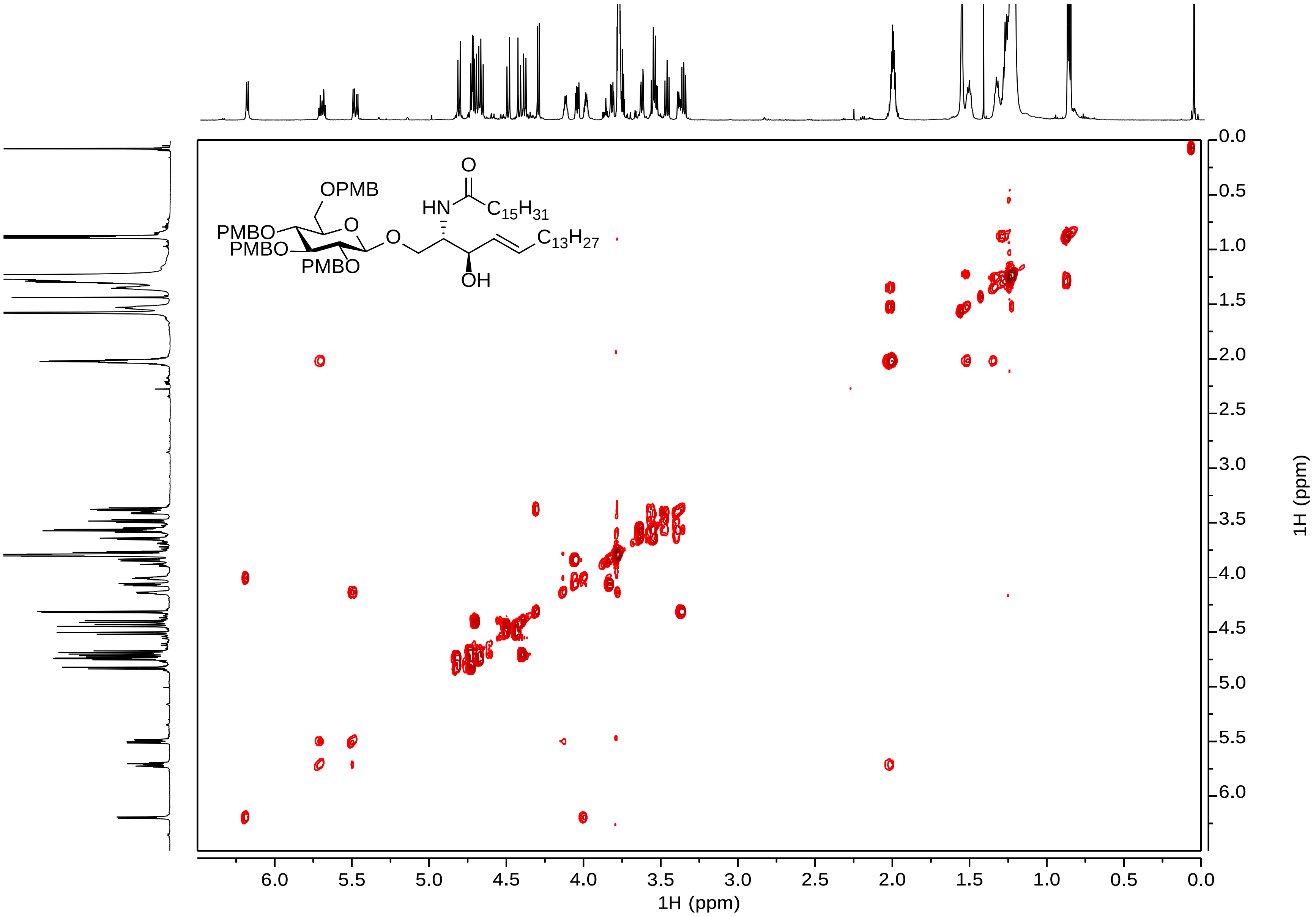

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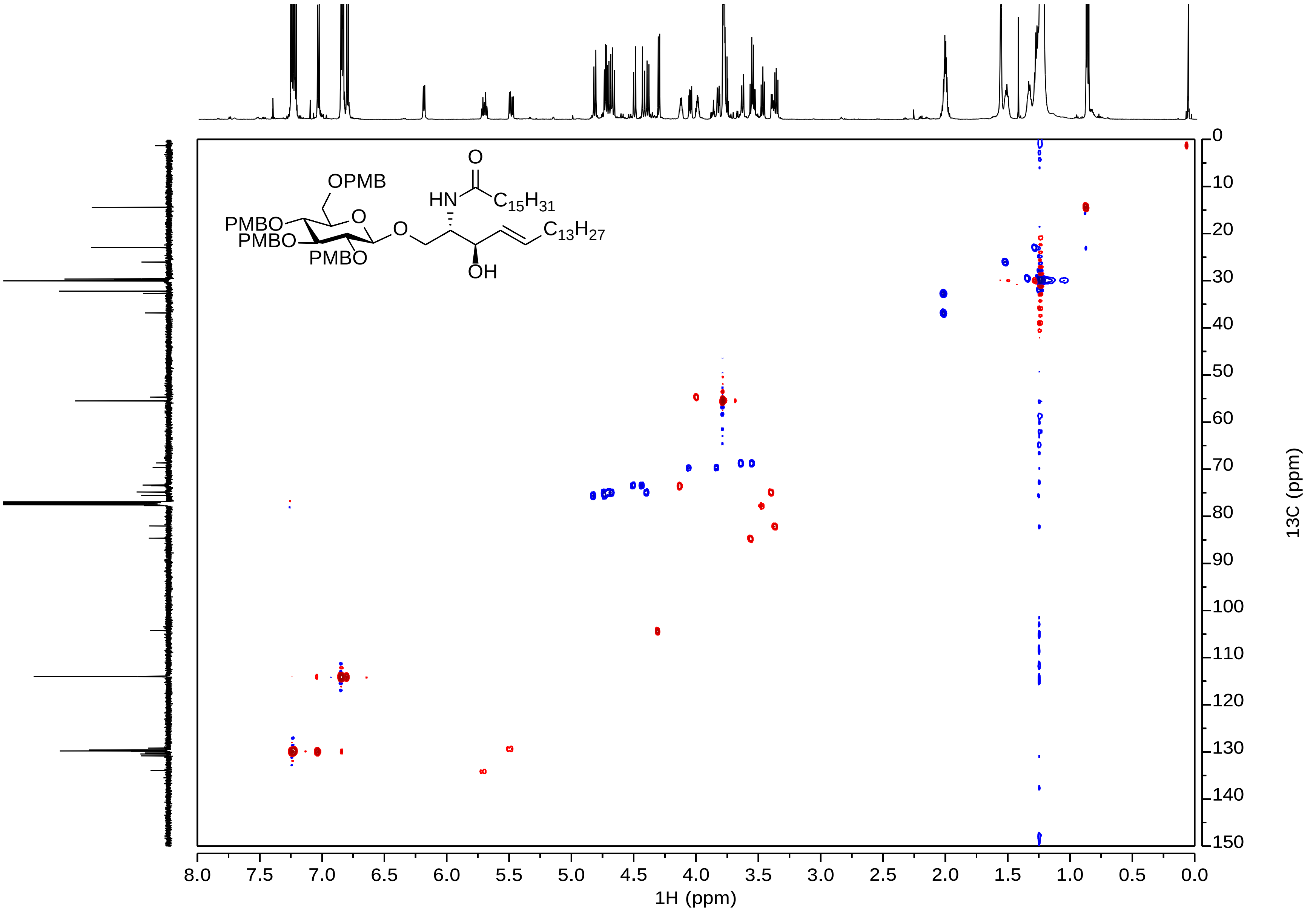

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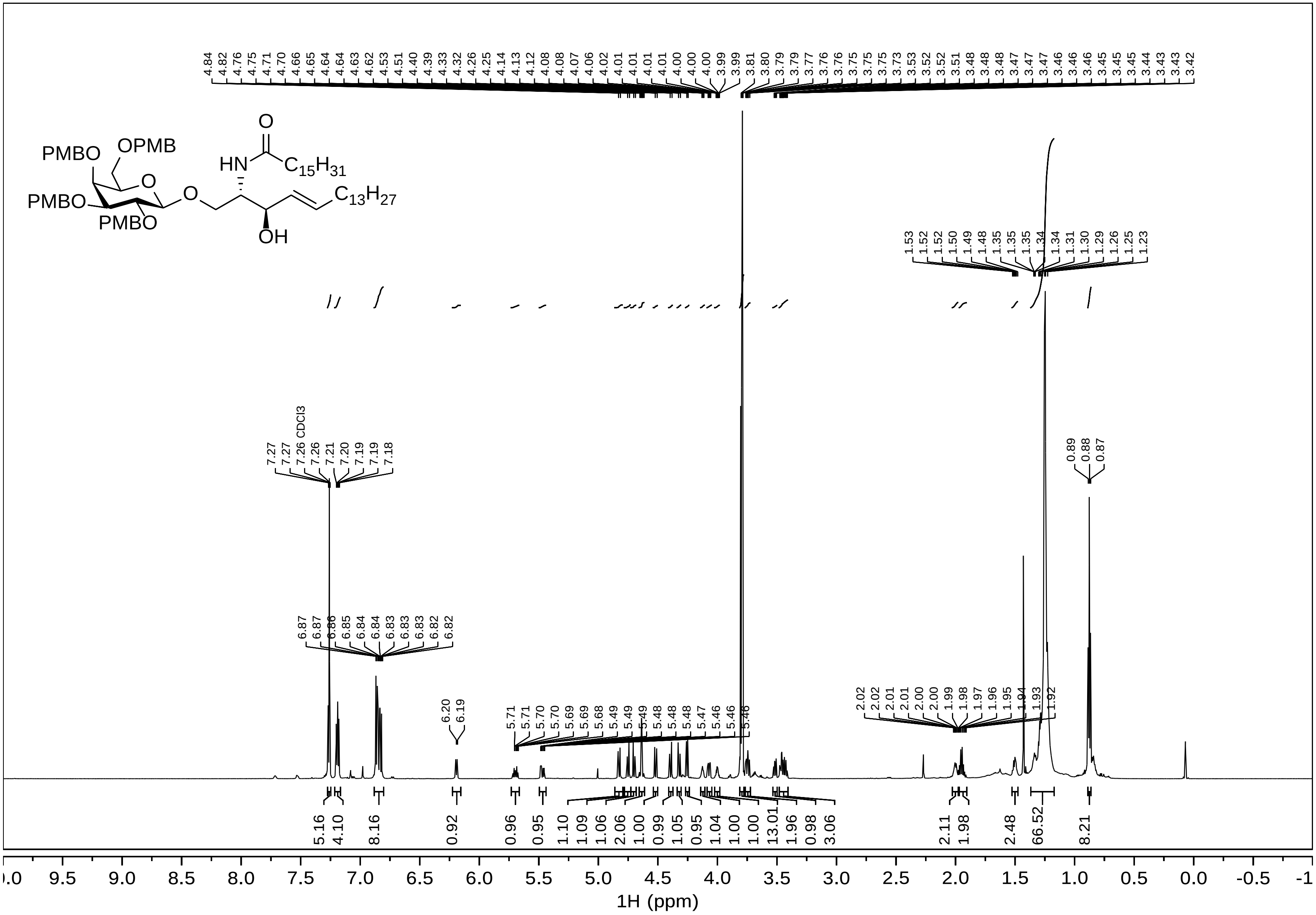

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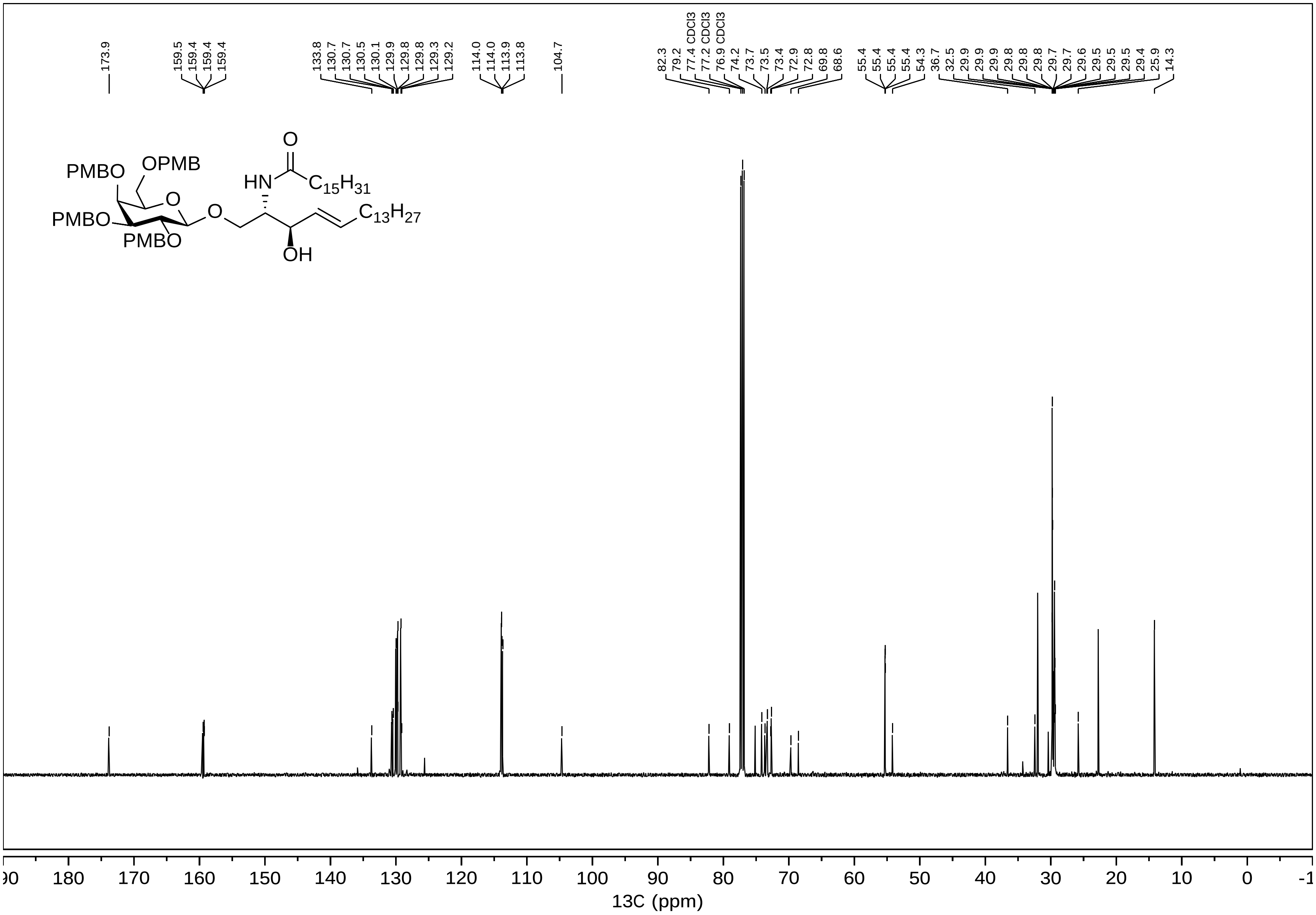

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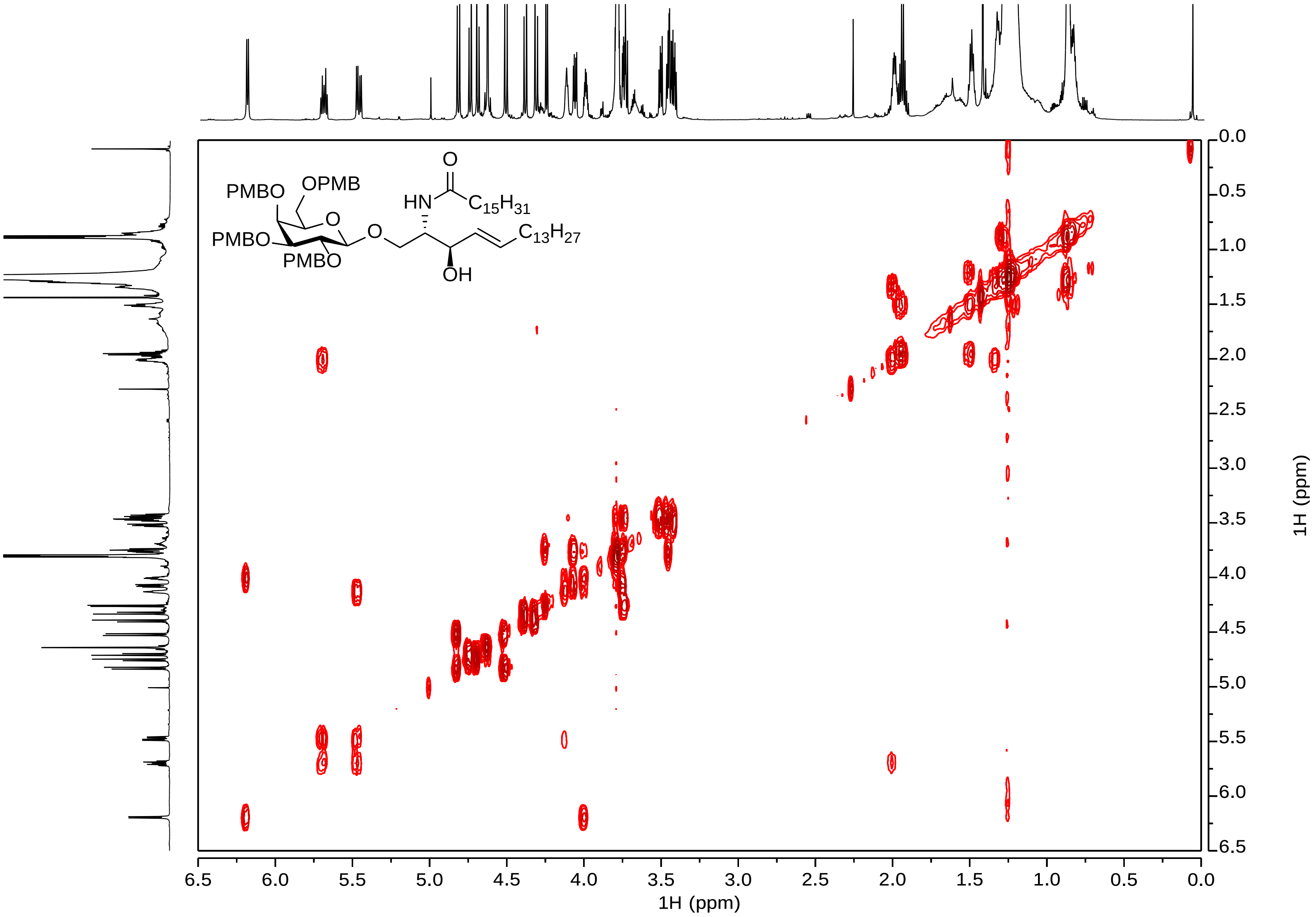

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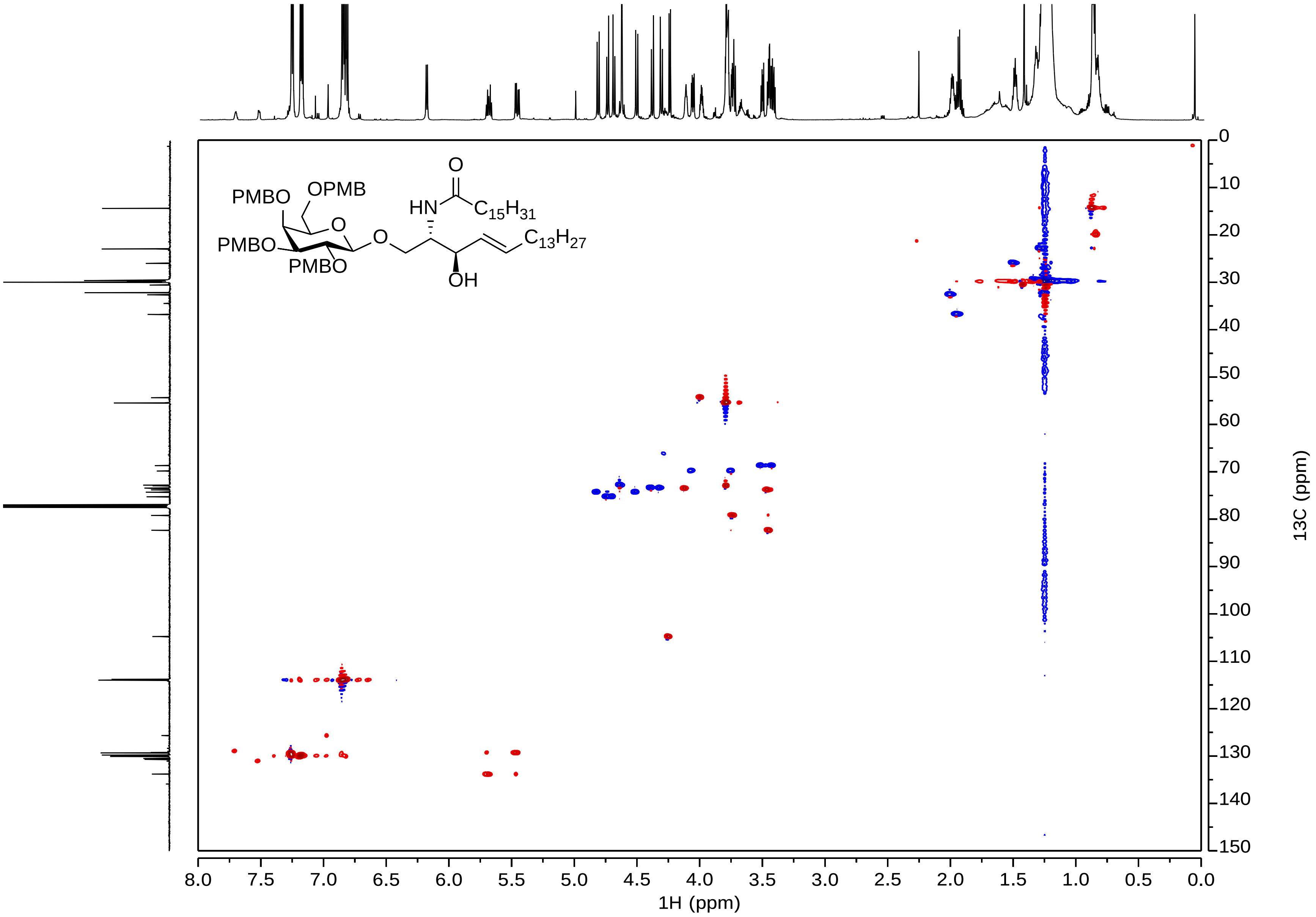
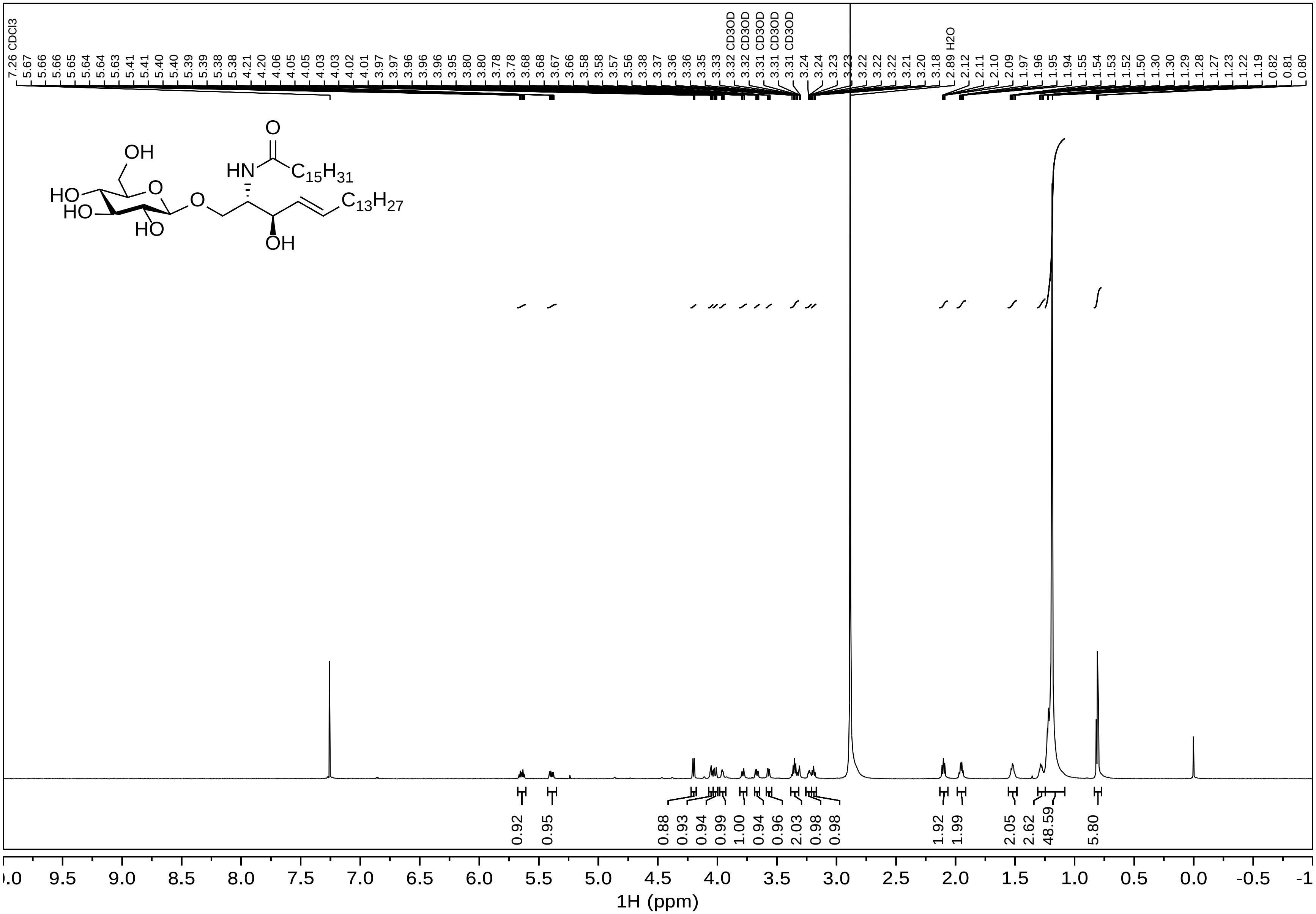
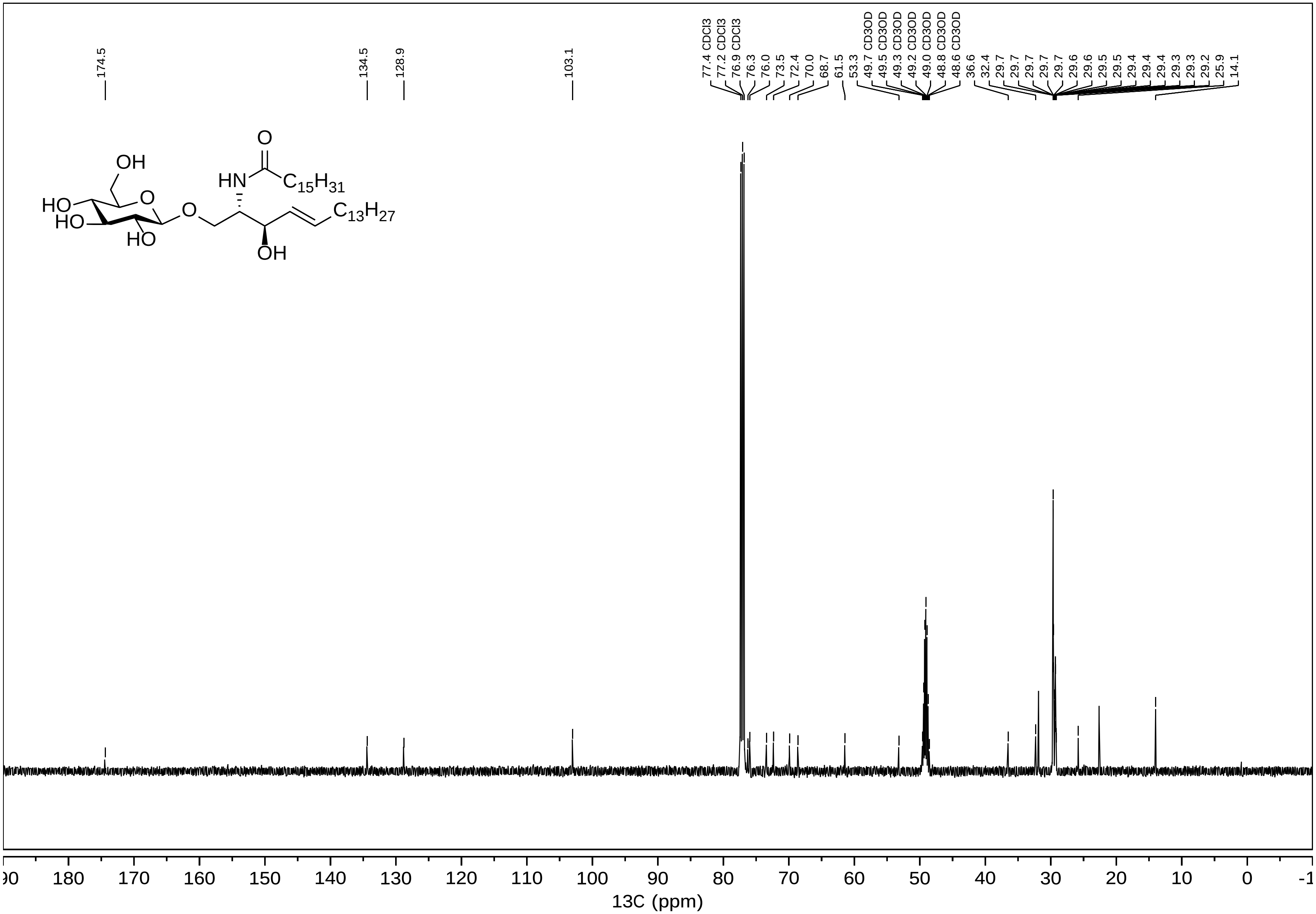


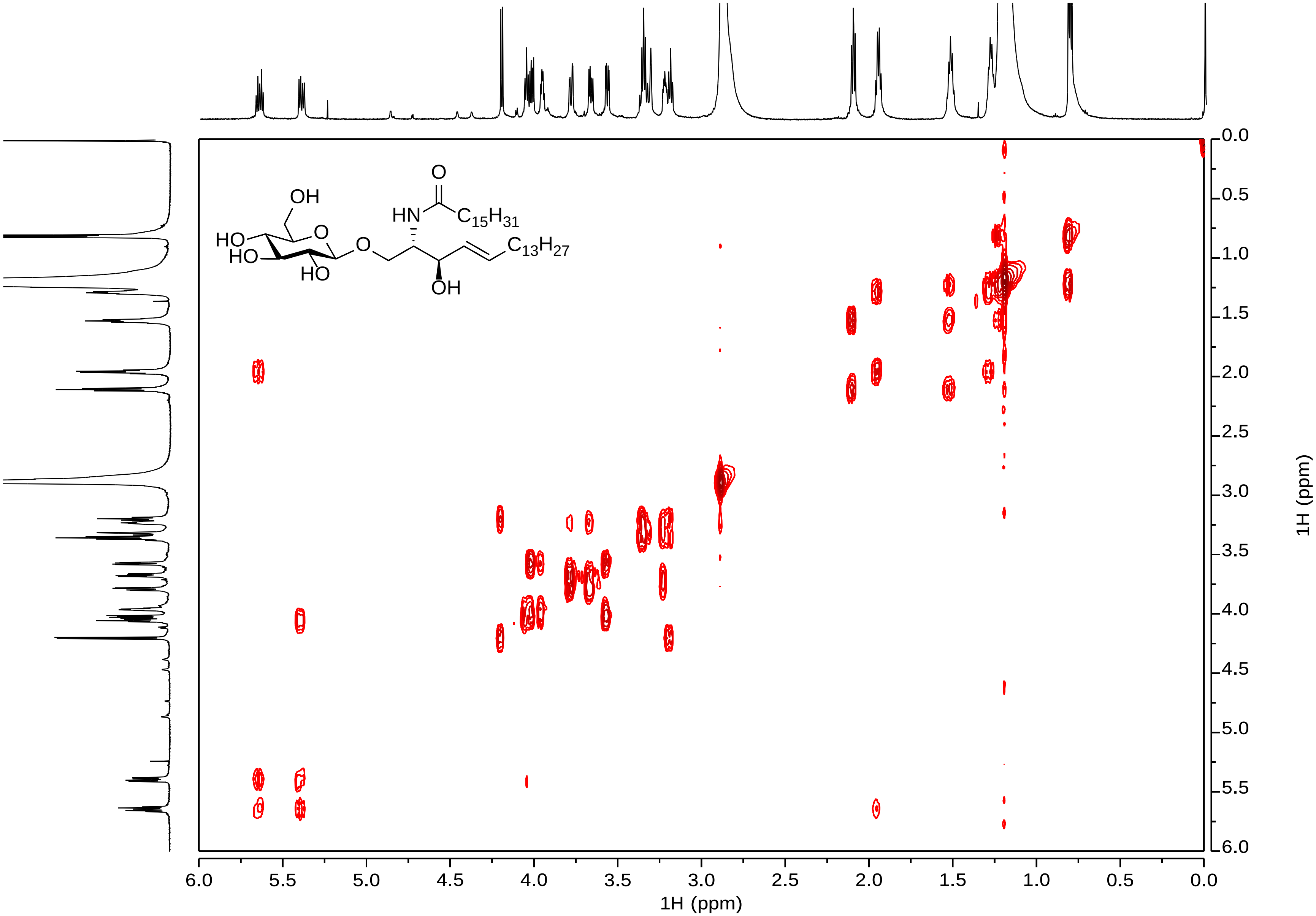
4a – ^{1}H NMR (700 MHz, 9:1 $CDCl_3$/CD_3OD, 40 °C, 1.7 mg/250 µL)



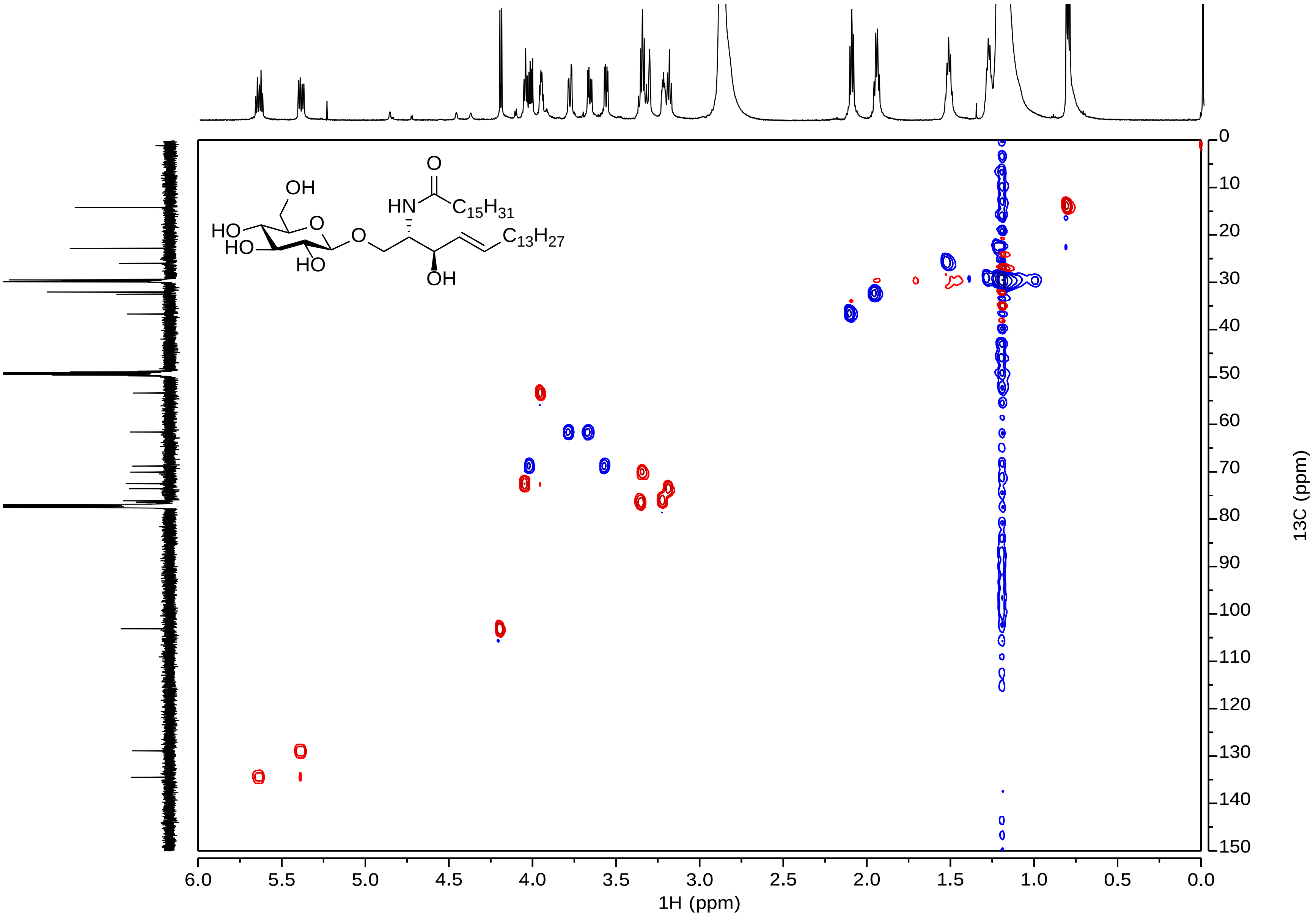
4a – ^{13}C NMR (126 MHz, 9:1 CDCl$_3$/CD$_3$OD, 23 °C, 1.7 mg/250 µL)



4a – gCOSY (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.7 mg/250 μL)



4a – HSQC (700 MHz, 9:1 $CDCl_3$/CD_3OD, 40 °C, 1.7 mg/250 µL)



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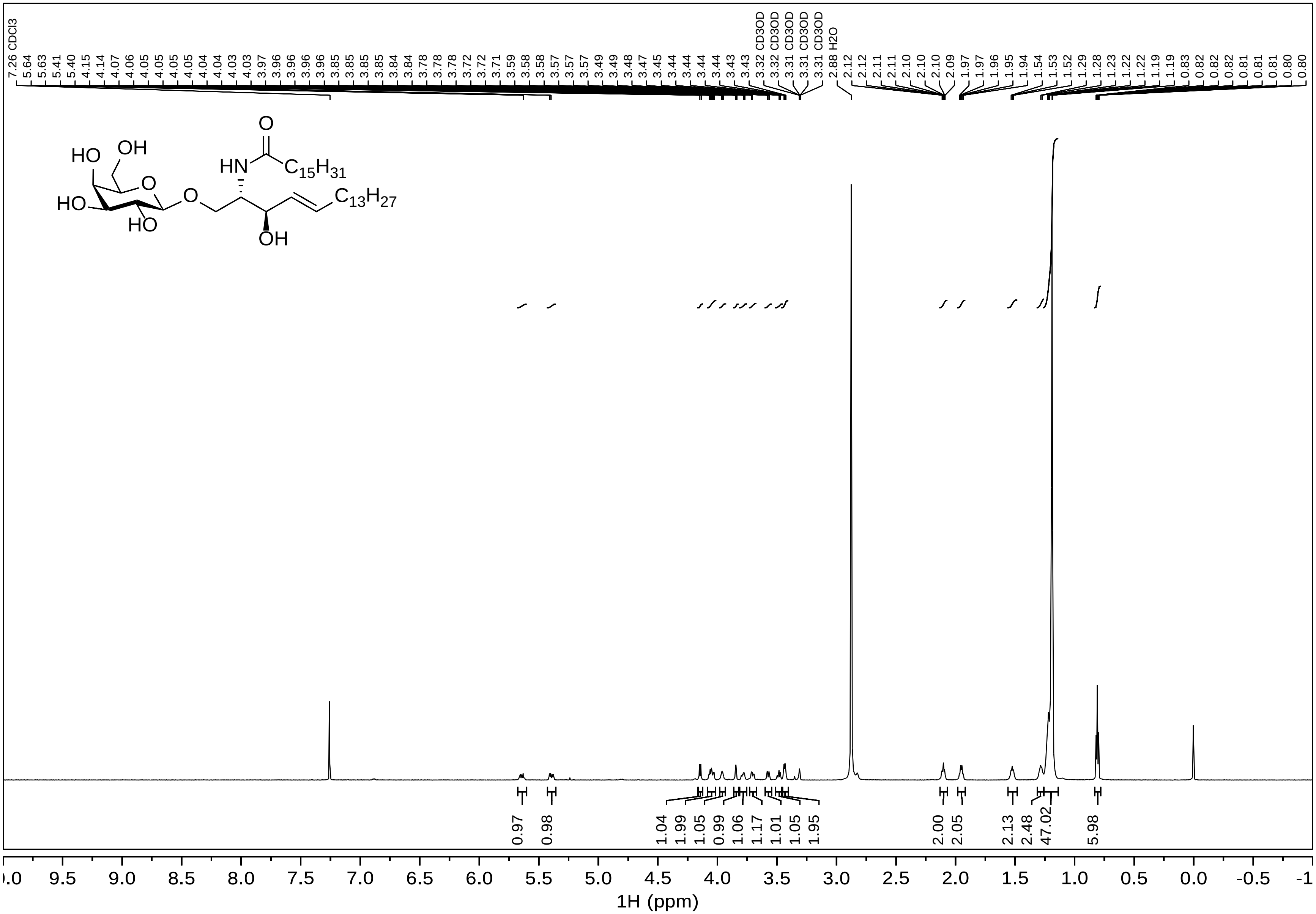
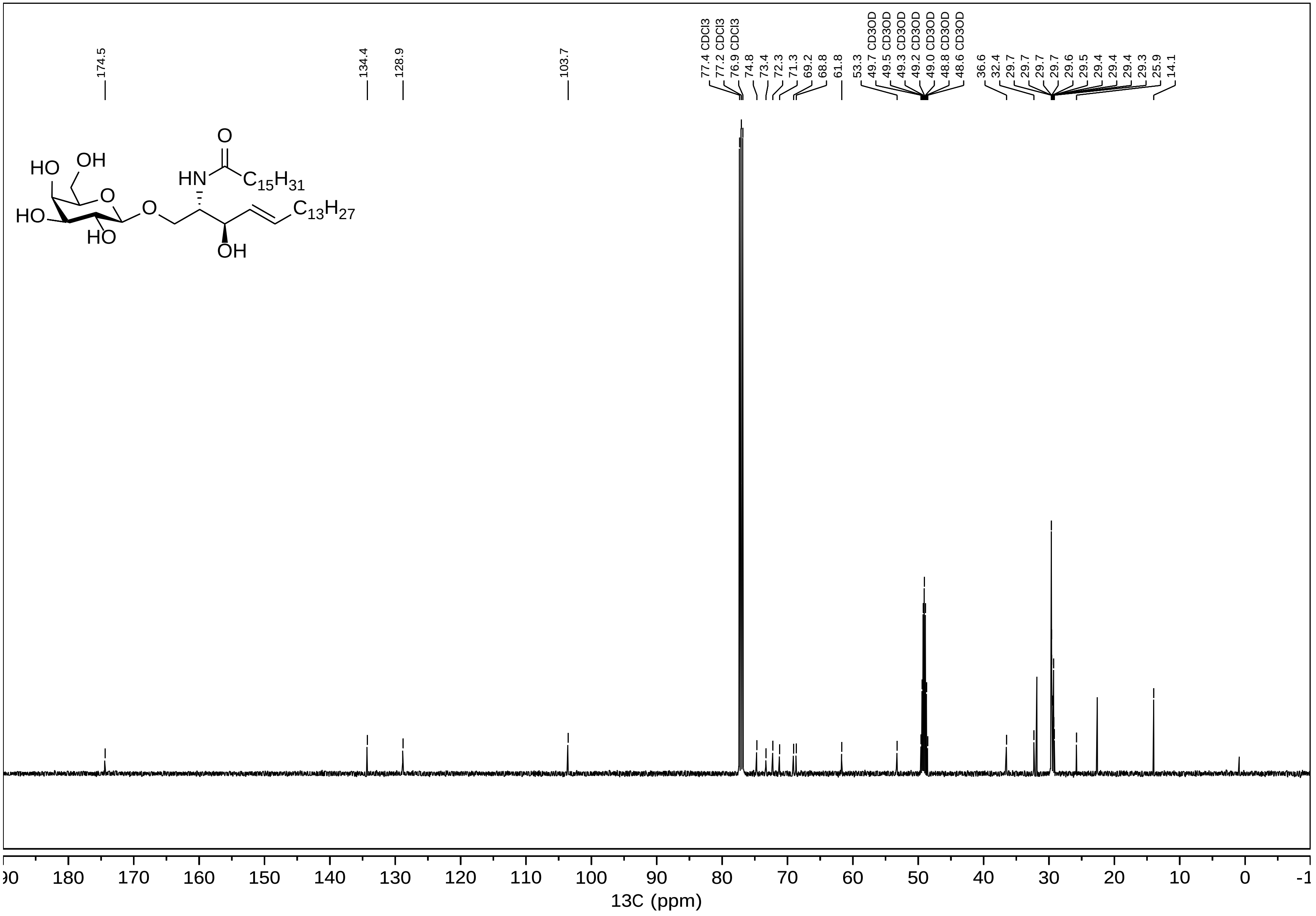
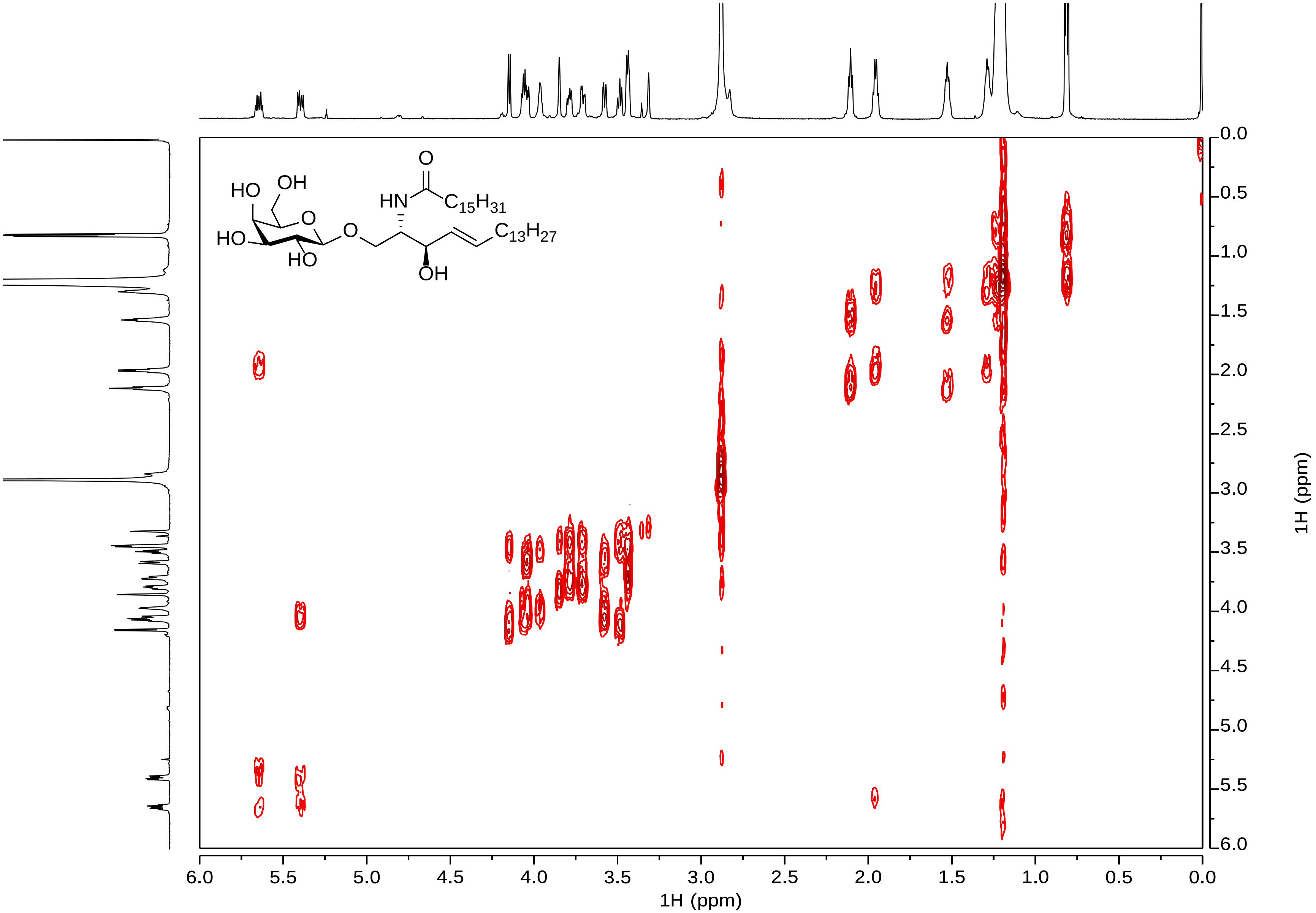


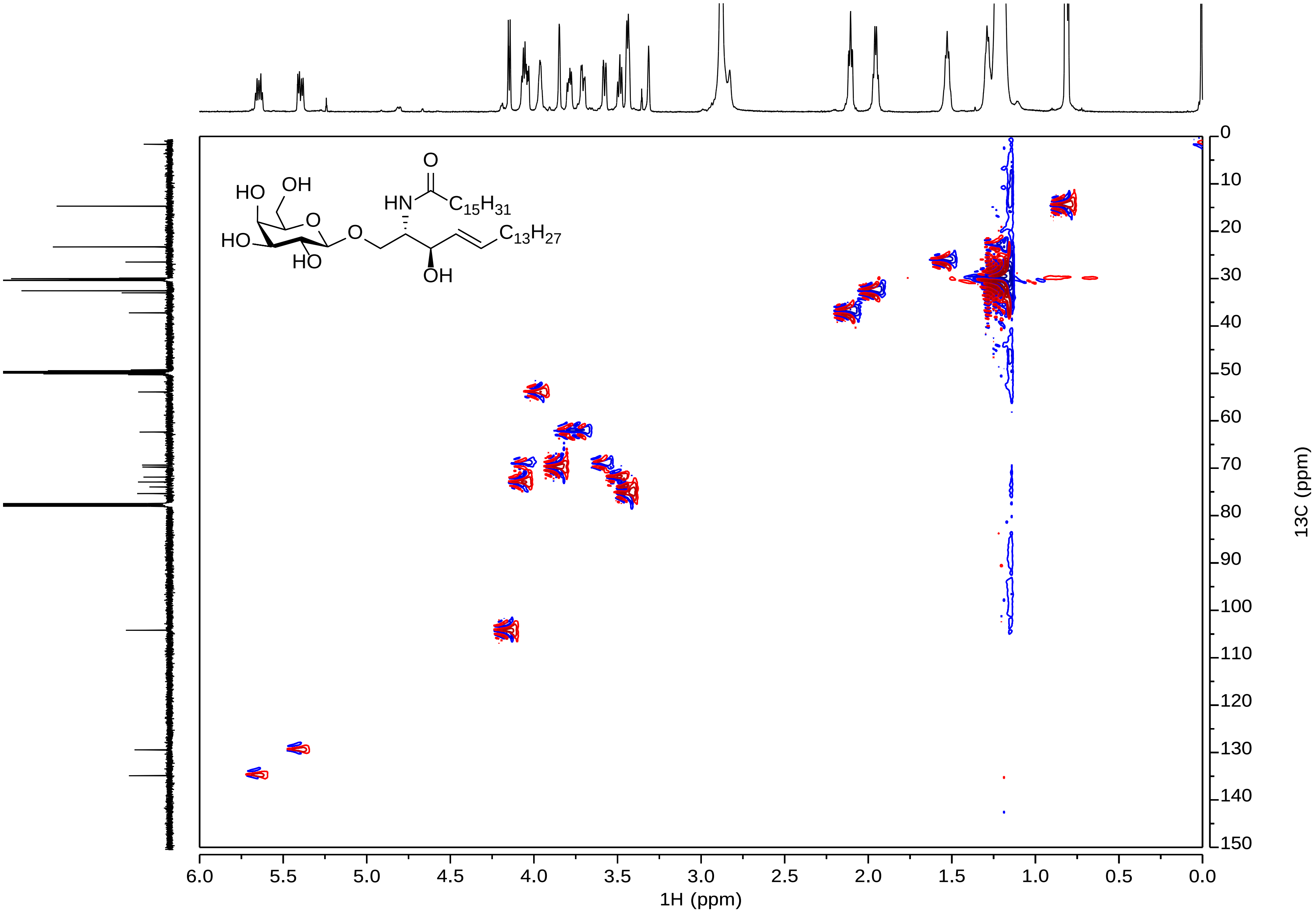
4b – ^{13}C NMR (126 MHz, 9:1 CDCl$_3$/CD$_3$OD, 23 °C, 1.6 mg/250 µL)



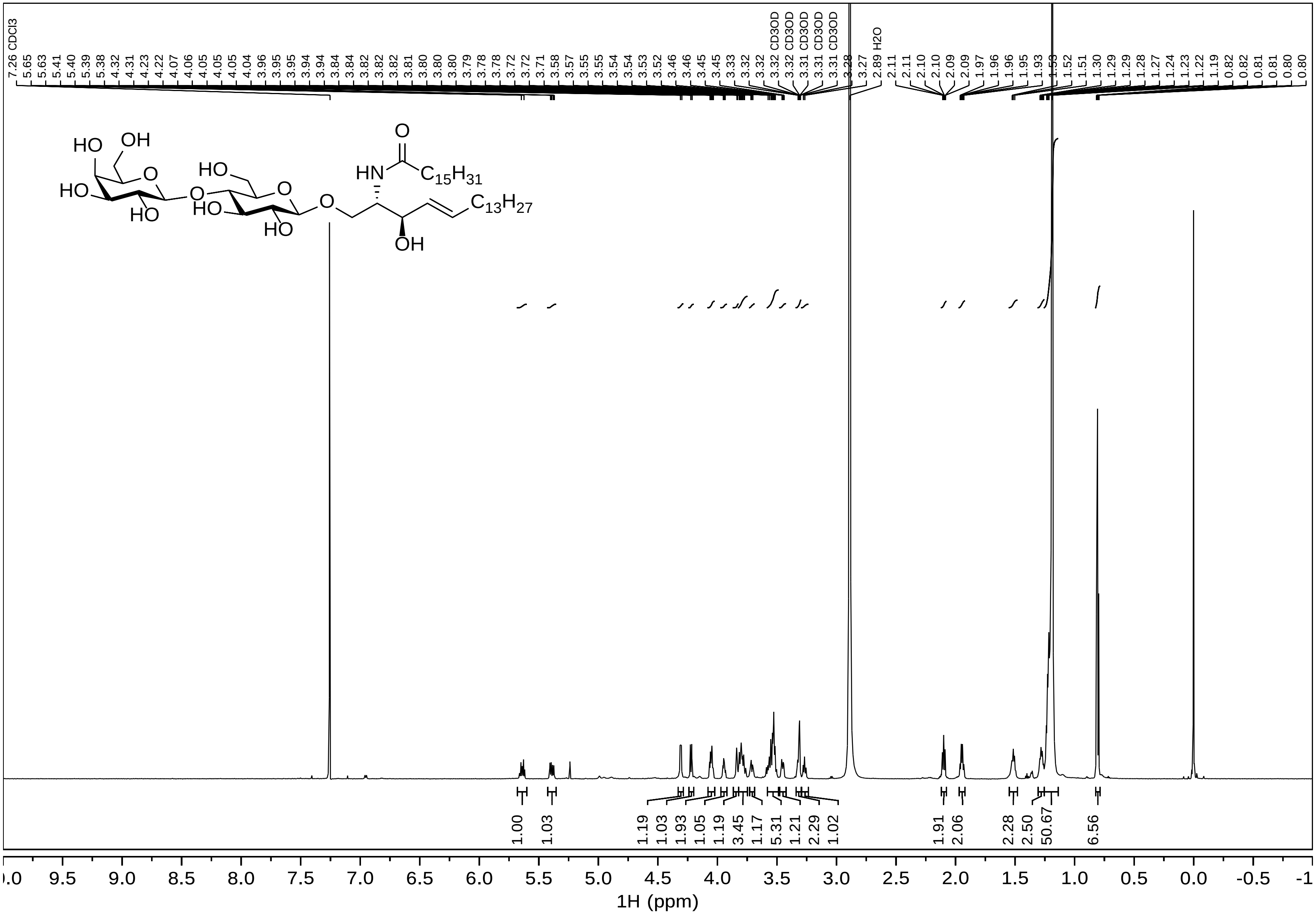
4b – gCOSY (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.6 mg/250 µL)



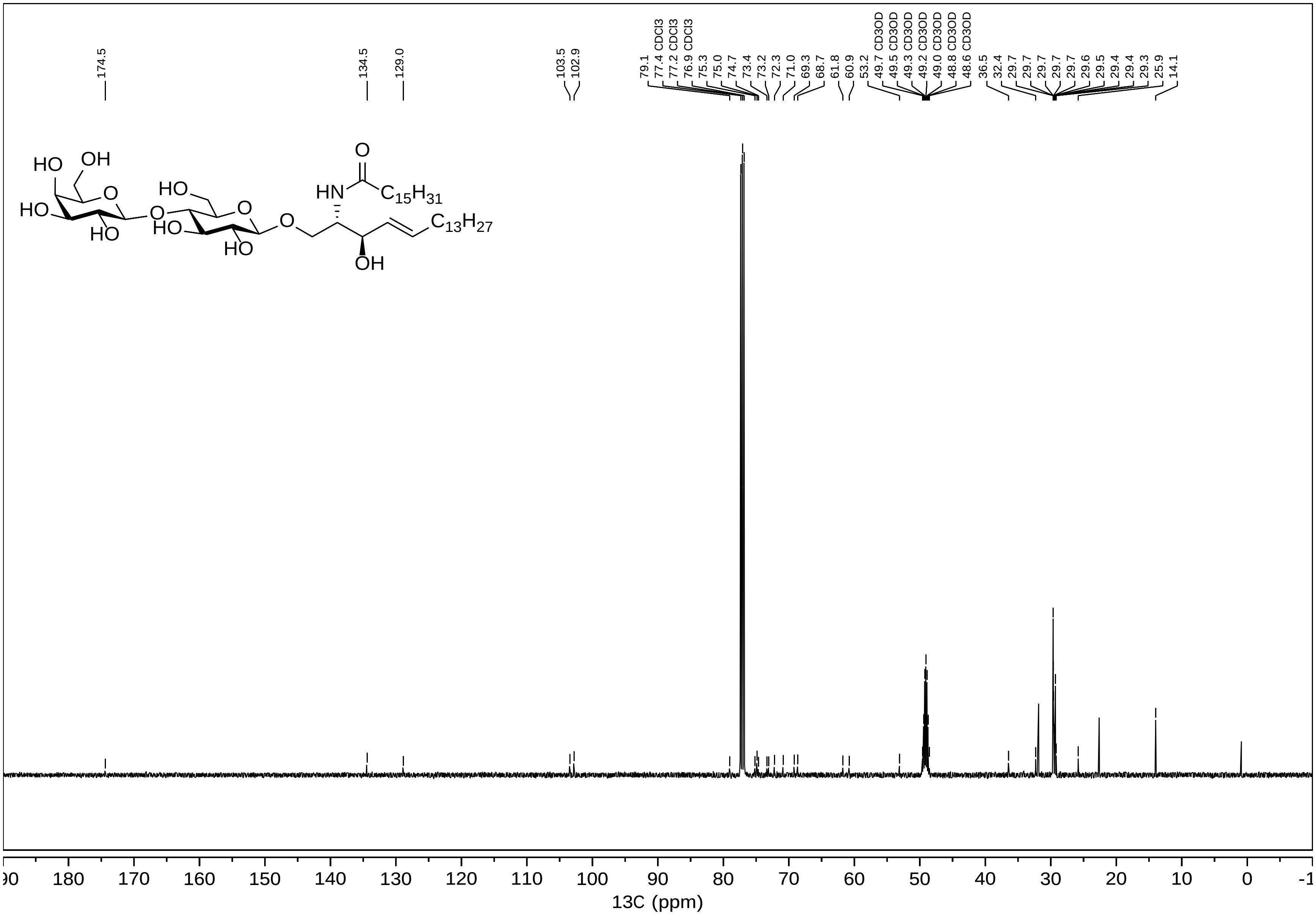
4b – HSQC (700 MHz, 9:1 $CDCl_3$/CD_3OD, 40 °C, 1.6 mg/250 μL)



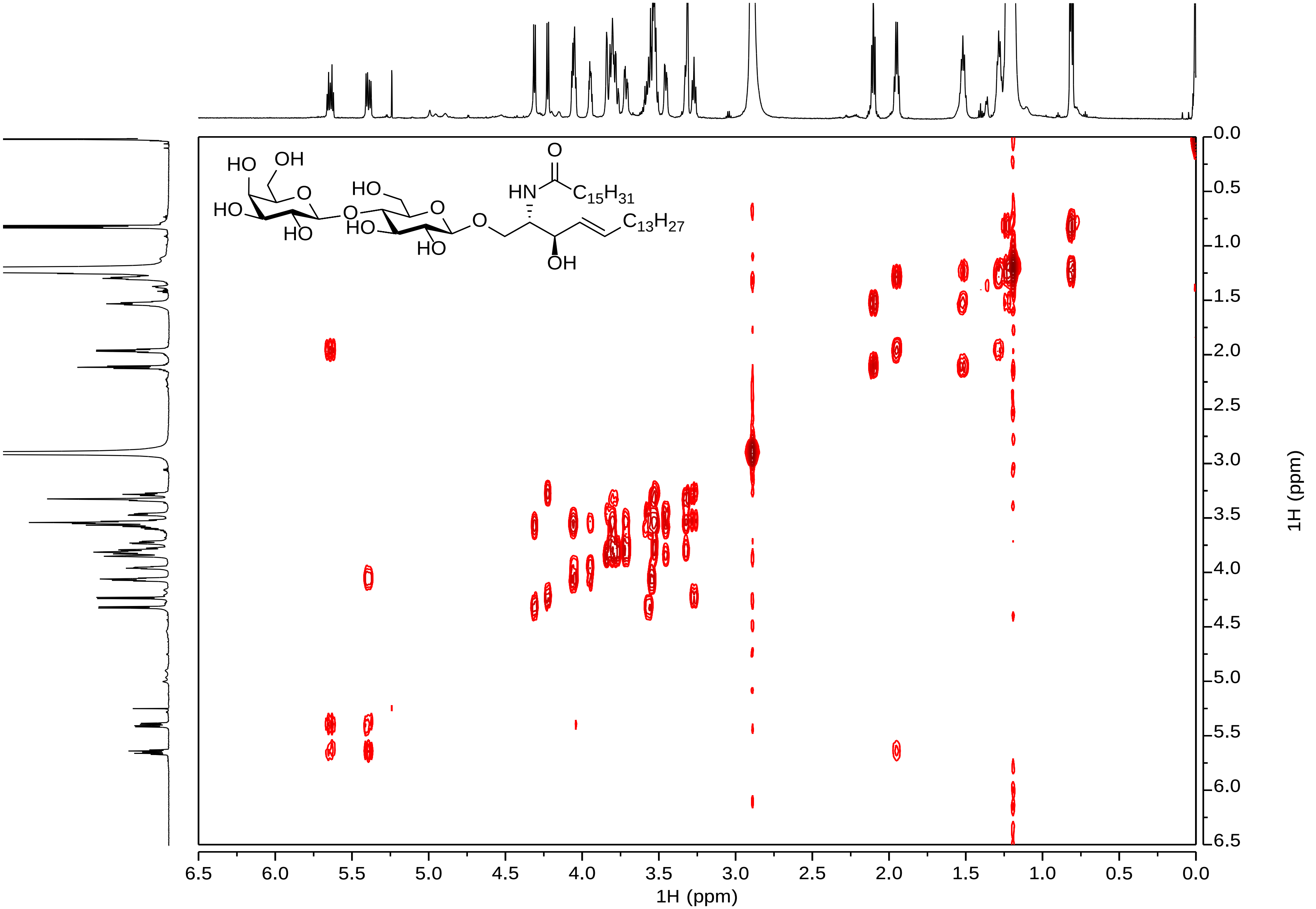
4c – ^{1}H NMR (700 MHz, 9:1 $CDCl_3$/CD_3OD, 40 °C, 1.5 mg/250 µL)



4c – ^{13}C NMR (126 MHz, 9:1 $CDCl_3$/CD_3OD, 23 °C, 1.5 mg/250 μL)



4c – gCOSY (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.5 mg/250 µL)



4c – HSQC (700 MHz, 9:1 $CDCl_3/CD_3OD$, 40 °C, 1.5 mg/250 µL)