Electronic Supplementary Information

Synthetic preparation and immunological evaluation of β-mannosylceramide and related *N*-acyl analogues

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General Experimental

Experiments requiring anhydrous conditions were performed under a dry nitrogen or argon atmosphere using apparatus heated and dried under vacuum, unless stated otherwise. Anhydrous dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene, acetonitrile (CH_3CN) and methanol (CH_3OH) were dried using the PURE SOLV MD-6 solvent purification system. 6 M HCl in 1,4-dioxane was prepared from 36% aqueous HCl and 1,4-dioxane. All other reagents were purchased as analytical or reagent grade and used without further purification. Aqueous solutions of sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$) and ammonium chloride (NH_4Cl) were saturated. Reactions performed at room temperature (rt) were carried out at approximately 20 °C and reaction temperatures from -78 °C to 0 °C were obtained using the following cooling bath mixtures: acetone/dry ice, -78 °C; acetonitrile/dry ice, -40 °C; NaCl/ice, -15 °C; water/ice, 0 °C. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.2 mm Kieselgel F254 (Merck) silica gel plates using UV light as a visualising agent and then stained and developed with heat using either vanillin in ethanolic sulfuric acid, ammonium heptamolybdate and cerium sulfate in aqueous sulfuric acid, or potassium permanganate and potassium carbonate in aqueous sodium hydroxide. Separation of mixtures was performed by flash chromatography using 0.063–0.1 mm silica gel with the indicated eluent. Infrared (IR) spectra were recorded on a Bruker Optics Alpha FT-IR spectrometer with a diamond Attenuated Total Reflectance (ATR) top plate. No sample preparation was required. Absorption peaks are reported as wavenumbers (ν, cm^{-1}). NMR spectra were recorded on a Varian 400-MR spectrometer operating at 400 MHz for ^1H nuclei and 100 MHz for ^{13}C nuclei at 25 °C, or a Varian 500 MHz AR Premium Shielded Spectrometer at operating at 500 MHz for ^1H nuclei and 125 MHz for ^{13}C nuclei at 25 °C. ^1H NMR chemical shifts are reported in parts per million (ppm) relative to the chloroform ($CDCl_3$, δ 7.26), or dimethyl sulfoxide (DMSO-*d6*, δ 2.50) peak. ^1H NMR values are reported as chemical shifts δ multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet), coupling constant (J, Hz) and relative integral. Coupling constants were taken directly from the spectra. ^{13}C NMR chemical shifts are reported in ppm relative to the chloroform ($CDCl_3$, δ 77.0), or dimethyl sulfoxide (DMSO-*d6*, δ 39.5) peak. ^{13}C NMR values are reported as chemical shifts δ and assignment. Decoupled ^{19}F NMR spectra were recorded on a Varian 400-MR spectrometer operating at 376 MHz at 25 °C and data are expressed in ppm. Assignments were made with the aid of DEPT, gCOSY, gHSQC, and gHMBC experiments. Mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer by electrospray ionisation in

positive and negative mode. High-resolution mass spectra (HRMS) were obtained with a nominal resolution of 5,000 to 10,000.

HPLC analysis

Synthetic purity of compounds for biological testing was assessed by analytical RP-HPLC on an Agilent 1260 Infinity Quaternary HPLC equipped with both an Agilent 1260 Multiple Wavelength Detector and either an Agilent 6130 single quadrupole mass spectroscopic detector using ESI (peak identification) or a Dionex Corona Ultra RS charged aerosol detector (CAD) (synthetic purity). Using a Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm) functionalized silica column. The standard method for purity assessment: line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins).

Methods and Materials for Assays

Cell lines. The iNKT hybridoma cell line, DN32.D3, was obtained from Albert Bendelac (University of Chicago, Chicago, Illinois, USA) and cultured in RPMI 1640 medium (Gibco) supplemented with 10% FCS (Corning), L-glutamine (Gibco), sodium pyruvate (Gibco), nonessential amino acids (Gibco), and 2-mercaptoethanol (5×10^{-5} M) (Gibco). The CT26 colon carcinoma was maintained in RPMI 1640, containing the supplements above except for the 2-mercaptoethanol. Cells were cultured in an atmosphere of 37°C and 5% CO_2.

Mice. Female BALB/c mice were purchased from Animal Production Colonies, Frederick Cancer Research Facility, National Cancer Institute, or The Jackson Laboratory. Female C57BL/6 mice were purchased from Animal Production Colonies, Frederick Cancer Research Facility, National Cancer Institute.

iNKT Cell Hybridoma Stimulation Assay

Splenocytes were harvested from mice, and the single cell suspension was depleted of erythrocytes with ACK Lysis Buffer (Lonza, Basel, Switzerland). T-cells were depleted from splenocytes using CD90.2 magnetic beads (Miltenyi Biotec, San Diego, CA) and autoMACS (Miltenyi Biotec, San Diego, CA). The negative fraction was collected and used as a source

of antigen presenting cells (APCs). APCs (1×10^6 cells/well) were co-cultured with the hybridoma clone (5×10^4 cells/well) in 96- well flat-bottom plate in the presence of an analogue of β-ManCer, β-ManCer, α-GalCer, or vehicle. After an 18h incubation at 37 ºC 5% CO_2, supernatants were collected and IL-2 concentrations were determined by ELISA (eBioScience, San Diego, CA) according to the manufacturer's instructions.

In vivo lung metastasis assay

CT26 cells (5×10^5 cells) in 0.2 ml PBS were injected i.v. into the tail vein of BALB/c mice. The analogues of β-ManCer, β-ManCer, or vehicle (0.00025% Tween 20 in PBS) were injected i.p. (in 0.2 ml PBS) within 1 hour after tumour challenge. Mice were sacrificed 14–16 days after the tumour challenge. The lungs were stained with 10% India Ink and fixed with Fekete's solution. The number of lung nodules were macroscopically enumerated. All animal experiments were performed in accordance with NIH policies and approved by the NCI Animal Care and Use Committee.

Experimental

1-((2'*S*,3'*S*,4'*R*)-2'-Azido-3',4'-di-*O*-benzyl-1'-octadecanyl)-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (16)

Sodium methoxide solution (2.5M, 5 mL, 12.5 mmol) was added dropwise to a solution of glycoside **15** (346 mg, 0.347 mmol) in methanol (10 mL). The reaction was stirred overnight at rt then neutralised with amberlite IR-120 (H^+ form). The solution was filtered through Celite washed with methanol and the solvent removed *in vacuo* to give the residue. The residue was diluted with EtOAc and washed with 50% aqueous $NaHCO_3$ and the organic concentrated *in vacuo* to afford an analytically pure sample of the *title compound* **16** (318 mg, 0.333 mmol, 96%) as a white solid that was used without further purification. δ_H(400 MHz, $CDCl_3$): 7.44 – 7.27 (25H, m), 7.18 (2H, dd, *J* = 7.2, 2.3 Hz), 4.95 (1H, d, *J* = 11.3 Hz), 4.84 (2H, d, *J* = 11.2 Hz), 4.72 – 4.65 (2H, m), 4.61 – 4.52 (4H, m), 4.49 (1H, d, *J* = 12.2 Hz), 4.26 – 4.22 (1H, m, H-1), 4.19 (1H, dd, *J* = 10.5, 6.6 Hz), 3.88 (1H, dd, *J* = 10.6, 3.2 Hz), 3.79 – 3.69 (4H, m), 3.64 (2H, td, *J* = 6.8, 5.5, 3.0 Hz), 3.60 – 3.56 (2H, m), 3.42 (1H, dt, *J* = 9.6, 3.2 Hz), 2.41 (1H, br s), 1.75 – 1.20 (26H, m), 0.89 (3H, t, *J* = 6.6 Hz); δ_C(101 MHz, $CDCl_3$): 138.80, 138.42, 138.26, 138.25, 138.09, 128.59, 128.57, 128.53, 128.51, 128.48, 128.26, 128.25, 128.13,

128.10, 128.06, 127.93, 127.89, 127.88, 127.82, 127.72, 102.80 (d, $^1J_{CH}$ = 162.54 Hz, C-1), 84.53, 79.37, 78.96, 77.56, 75.42, 75.26 , 75.15 , 74.89, 73.93 , 73.61 , 72.20 , 69.54, 68.90, 61.82, 32.08 , 30.02 , 29.92 , 29.86 , 29.85 , 29.83 , 29.82, 29.78, 29.76, 29.52, 25.49, 22.85, 14.28; HRMS (ESI-pos): calcd for $C_{59}H_{77}O_8N_3Na$ $[M + Na]^+$ *m/z* 978.5603, found *m/z* 978.5626.

1-((2'*S*,3'*S*,4'*R*)-2'-azido-3',4'-di-*O*-benzyl-1'-octadecanyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (17)

A solution of glucoside **16** (318 mg, 0.333 mmol) in anhydrous CH_2Cl_2 (5 mL) at rt under Ar was treated with Dess-Martin periodinane (460 mg, 1.030 mmol) in one portion. The resulting suspension was stirred for 16 hrs then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with 50% sat. aqueous $NaHCO_3$. The combined organic layers were dried and concentrated *in vacuo* to give the residue which was triturated with di-isopropyl ether to remove excess Dess-Martin related materials and filtered. The filtrate was concentrated *in vacuo* to afford the intermediate ulose that was used directly in the next step. L-Selectride (1M in THF, 700 µL, 0.700 mmol) was added to a solution of the crude ulose (318 mg, 0.333 mmol) in anhydrous THF (1 mL, 3 mL per mmol) at -78 °C under Ar. After 10 min the reaction was stopped by the addition of acetic acid. The reaction was warmed to rt, diluted with $CH_2Cl_2$and washed with 2M HCl, followed by sat. aq. $NaHCO_3$. The combined organic extracts were concentrated *in vacuo* to afford the crude residue which was purified by column chromatography (PE to 7:1 PE/EtOAc) to afford a single diastereomer of the *title compound* **17** (140 mg, 0.146 mmol, 44% over two steps) as a colourless oil. δ_H(500 MHz, $CDCl_3$): 7.42 – 7.25 (22H, m), 7.23 (3H, ddd, *J* = 9.5, 7.0, 3.1 Hz), 4.90 (1H, d, *J* = 10.8 Hz), 4.78 (1H, d, *J* = 11.9 Hz), 4.70 – 4.65 (3H, m), 4.60 (1H, d, *J* = 12.2 Hz), 4.57 – 4.50 (4H, m), 4.33 (1H, s, H-1), 4.16 (1H, dd, *J* = 10.5, 7.4 Hz), 4.08 (1H, d, *J* = 3.1 Hz), 3.91 (1H, t, *J* = 9.4 Hz), 3.85 (1H, dd, *J* = 10.5, 3.4 Hz), 3.81 (1H, ddd, *J* = 7.5, 5.4, 3.4 Hz), 3.74 – 3.71 (2H, m), 3.65 (1H, t, *J* = 5.0 Hz), 3.61 (1H, dt, *J* = 7.0, 4.1 Hz), 3.52 (1H, dd, *J* = 9.0, 3.1 Hz), 3.36 (1H, ddd, *J* = 9.7, 4.3, 2.8 Hz), 2.34 (1H, b, OH), 1.74 - 1.52 (2H, m), 1.45 - 1.10 (23H, m), 0.89 (3H, t, *J* = 6.9 Hz); δ_C(126 MHz, $CDCl_3$): 138.46, 138.41, 138.05, 138.01, 128.68, 128.62, 128.56, 128.52, 128.51, 128.47, 128.42, 128.36, 128.25, 128.21, 128.08, 128.05, 128.00, 127.98, 127.95, 127.90, 127.86, 127.80, 127.67, 99.73 (d, $^1J_{CH}$ = 157.18 Hz, C-1), 81.44, 79.25, 79.21, 75.62, 75.31, 74.27, 73.89, 73.60, 72.15, 71.47, 69.45, 69.21, 68.26, 61.93, 32.08, 30.09 ,

29.95, 29.86, 29.83, 29.82, 29.79, 29.76, 29.52, 25.39, 22.85, 14.28; HRMS (ESI-pos): calcd for $C_{59}H_{77}O_8N_3Na$ $[M + Na]^+$ *m/z* 978.5603, found *m/z* 978.5619.

1-[(2'*S*,3'*S*,4'*R*)-2'-*N*-hexacosanamide-3',4'-di-*O*-benzyl-1'-octadecanyl]-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (19)

To a solution of azide **8** (α/β = 5:95) (160 mg, 0.168 mmol) in anhydrous THF (5 mL) at 0 °C under argon was added trimethylphosphine (1M in THF, 1100 µL, 1.100 mmol). After warming to rt over 1 hr. the solution was then stirred at rt for 4 hrs before the addition of NaOH (2M, 4 mL). The mixture was stirred at rt overnight to hydrolyse the imino-phosphorane, then the reaction was diluted with CH_2Cl_2, washed with water and the volatiles were removed *in vacuo* to give the crude amine intermediate **18** as a colourless oil. HRMS (ESI-pos): calcd for $C_{59}H_{78}O_8N$ $[M + H]^+$ *m/z* 928.5722, found *m/z* 928.5728. EDCI·HCl (100 mg, 0.518 mmol) and HOBt (80 mg, 0.579 mmol) were added to a stirred suspension of hexacosanoic acid (99 mg, 0.236 mmol) in CH_2Cl_2/DMF (10:4, 14 mL) at rt. After 30 min a solution of crude amine **18** (0.168 mmol) in anhydrous CH_2Cl_2 (5 mL) was added followed by DIPEA (200 µL, 1.142 mmol) and the reaction stirred at rt overnight. The reaction was diluted with CH_2Cl_2, washed with water and the aqueous layer re-extracted with EtOAc. The combined organic extracts were concentrated *in vacuo* to give the crude material which was purified by column chromatography (PE, to 5:1 PE:EtOAc) to give the *title compound* **19** (178 mg, 0.136 mmol, 81% over two steps) both as white solids. R_f = 0.36 (5:1 PE/EtOAc); δ_H(500 MHz, $CDCl_3$): 7.55 – 7.27 (25H, m), 5.67 (1H, d, *J* = 8.3 Hz), 5.62 (1H, s), 4.91 (1H, d, *J* = 12.2 Hz), 4.82 (1H, d, *J* = 12.3 Hz), 4.77 (1H, d, *J* = 11.5 Hz), 4.71 (1H, d, *J* = 12.4 Hz), 4.65 – 4.52 (4H, m), 4.45 – 4.35 (2H, m), 4.26 (1H, dd, *J* = 10.4, 4.8 Hz), 4.19 (1H, t, *J* = 9.6 Hz), 4.08 (1H, dd, *J* = 10.3, 7.8 Hz), 3.96 – 3.84 (2H, m), 3.83 – 3.74 (2H, m, *H*-1), 3.57 (2H, m), 3.26 (1H, td, *J* = 9.7, 4.8 Hz), 2.22 – 1.88 (2H, m), 1.76 – 1.60 (2H, m), 1.35 – 1.20 (70H, m), 0.90 (6H, t, *J* = 6.8 Hz,); δ_C(126 MHz, $CDCl_3$): 172.91, 138.63, 138.61, 138.57, 138.42, 137.66, 128.98, 128.55, 128.50, 128.43, 128.41, 128.29, 128.03, 127.96, 127.90, 127.79, 127.69, 127.67, 126.18, 102.17 (d, $^1J_{CH}$ = 155.29 Hz C-1), 101.56, 80.57, 79.72, 78.76, 77.95, 76.06, 74.81, 73.69, 72.55, 72.17, 68.71, 68.67, 67.66, 49.88, 36.89, 32.06, 30.52, 29.94, 29.86, 29.83, 29.79, 29.69, 29.54, 29.52, 29.51, 29.49, 26.04, 25.76, 22.83, 14.26; HRMS (ESI-pos): calcd for $C_{85}H_{127}O_9NNa$ $[M + Na]^+$ *m/z* 1328.9403 found *m/z* 1328.9420; Anal. Calcd for $C_{85}H_{127}NO_9$: C, 78.12; H, 9.80; N, 1.07. Found C, 78.06; H, 9.99; N, 1.15.

_N_-[(1_S_,2_S_,3_R_)-2,3-dihydroxy-1-[(β-D-mannopyranosyloxy)methyl]heptadecyl] hexacosanamide (β-ManCer, 2)

To a solution of glycolipid **19** (251 mg, 0.192 mmol) in CH_2Cl_2/MeOH (1:1, 20 mL) was added 10% palladium on carbon (52 mg, 0.049 mmol). The stirred reaction mixture was degassed under vacuum and backfilled with hydrogen three times then left to stir overnight under a balloon of hydrogen. The hydrogen atmosphere was removed and the grey precipitate that formed during the reaction was dissolves on dilution with hot EtOH. The solution was then filtered through Celite and washed with excess hot EtOH. The solvents were removed *in vacuo* to give the crude material which was precipitated by dissolving in hot ethanol and triturating out of solution with dropwise addition of water, once the precipitate formed the mixture was heated to re-dissolve. The resulting solution was allowed to cool slowly to rt and then cooled in the freezer (-20°C), the resulting precipitate was filtered through a Hirsch funnel and dried to a constant weight affording the known[1, 2] title compound **2** (151 mg, 0.176 mmol, 92%) as a white solid. R_f = 0.41 (15% MeOH:$CHCl_3$); δ_H(500 MHz, 2:1 $CDCl_3$:CD_3OD): 4.47 (1H, s), 4.17 (1H, dt, *J* = 7.1, 4.1 Hz), 4.04 (1H, dd, 10.3, 3.9 Hz), 3.86 (2H, dd, *J* = 12.3, 2.7 Hz), 3.71 (2H, td, *J* = 11.9, 11.0, 5.0 Hz), 3.62 – 3.50 (3H, m), 3.43 (1H, dd, *J* = 9.4, 3.3 Hz), 3.20 (1H, ddd, *J* = 8.9, 5.9, 2.5 Hz), 2.16 (2H, t, *J* = 7.6 Hz), 1.65 – 1.46 (4H, m), 1.40 – 1.18 (75H, s), 0.84 (6H, t, *J* = 6.8 Hz); δ_C(126 MHz, 2:1 $CDCl_3$:CD_3OD): 174.82, 100.49 (d, $^1J_{CH}$ = 158.16 Hz, C-1), 77.02, 74.47, 74.25, 72.40, 71.23, 68.53, 67.71, 62.04, 50.66 , 36.88, 32.43, 32.32, 30.18, 30.16, 30.12, 30.10, 30.08, 30.06, 30.03, 29.96, 29.83, 29.77, 29.76, 29.74, 26.34, 26.31, 23.05, 14.26; HRMS (ESI-pos): calcd for $C_{50}H_{99}O_9NNa$ $[M + Na]^+$ *m/z* 880.7212 found *m/z* 880.7227; Anal. Calcd for $C_{50}H_{99}NO_9 \cdot H_2O$: C, 68.53; H, 11.62; N, 1.60. Found C, 68.41; H, 11.75; N, 1.59.

1-((2'_S_,3'_S_,4'_R_)-2'-azido-3',4'-di-_O_-benzyl-1'-octadecanyl)-2-_O_-benzoyl-3,4,6-tri-_O_-benzyl-α-D-mannopyranoside (22)

Triflic acid (5 µL, 0.057 mmol) was added to a mixture of **7** (80 mg, 0.153 mmol), thioglycoside **21** (121 mg, 0.186 mmol), and NIS (60 mg, 0.253 mmol) in anhydrous CH_2Cl_2 (5 mL) containing pre-activated 4Å MS at -10°C under argon. The reaction was warmed slowly to rt and after ca. 4 hours was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and $Na_2S_2O_3$ solutions then concentrated *in vacuo* to give the crude material which was purified by column chromatography (PE to 6:1 PE/EtOAc) to afford the *title compound* **22** (120 mg, 0.113 mmol, 74%) as a colourless oil. δ_H(500 MHz, $CDCl_3$): 8.14 – 7.98 (2H, m), 7.56 (1H, td, 7.4, 1.3 Hz), 7.43 – 7.16 (27H, m), 5.65 (1H, d, 2.0 Hz, *H*-2), 4.96 (1H, d, 1.9 Hz, *H*-1), 4.88 (1H, d, *J* = 10.8 Hz), 4.78 (1H, d, *J* = 11.4 Hz), 4.69 (2H, dd, *J* =

11.6, 4.8 Hz), 4.64 – 4.45 (6H, m), 4.14 – 4.08 (3H, m), 3.86 – 3.80 (2H, m), 3.78 (1H, ddd, *J* = 8.3, 5.4, 2.8 Hz), 3.65 (2H, dd, *J* = 9.5, 2.1 Hz), 3.60 (2H, td, *J* = 10.3, 9.5, 6.0 Hz), 1.72 – 1.62 (1H, m), 1.50 – 1.60 (3H, m), 1.37 – 1.47 (1H, m), 1.21 – 1.35 (21H,m), 0.89 (3H, t, *J* = 6.6 Hz); δ_C(126 MHz, $CDCl_3$): 165.77, 138.55, 138.41, 138.11, 137.95, 133.27 , 130.12 , 130.01 , 128.56 , 128.55 , 128.53 , 128.47 , 128.43 , 128.25 , 128.20 , 128.11 , 127.98 , 127.82 , 127.74 , 127.66 , 127.62 , 98.54 (d, $^1J_{CH}$ = 166.32 Hz, *C*-1), 79.39, 79.27, 78.17, 75.40, 74.26, 73.87, 73.57, 72.29, 72.09, 71.71, 68.97, 68.90, 62.15, 32.08, 30.08, 29.93, 29.86, 29.83, 29.82, 29.80, 29.76, 29.52, 25.48, 22.85, 14.28; HRMS (ESI-pos): calcd for $C_{66}H_{81}O_9N_3Na$ $[M + Na]^+$ *m/z* 1082.5865, found *m/z* 1082.5837.

1-((2'*S*,3'*S*,4'*R*)-2'-Amino-1',3',4'-octadecanyl)-α-D-mannopyranoside-2,2,2-trifluoroacetate (α-ManPhs, 23)

To a solution of glycoside **22** (120 mg, 0.113 mmol) in MeOH (10 mL) was added freshly prepared sodium methoxide solution (2.5M in MeOH, 80 µL, 0.100 mmol) and the reaction mixture was stirred at rt for 3 hours. The reaction was diluted with CH_2Cl_2 and concentrated *in vacuo*. The resulting white solid was dissolved in CH_2Cl_2 and washed with brine. The solvent was removed *in vacuo* to give a white solid which was used directly in the next step. This was dissolved in CH_2Cl_2/MeOH (1:1, 6 mL) along with 1M hydrochloric acid (400 µL, 0.400 mmol). 10% palladium on carbon (11 mg) was added and the mixture was stirred hydrogen overnight. The hydrogen atmosphere was removed, the reaction diluted with hot EtOH, and filtered through Celite. The solvents were removed *in vacuo* to give the crude material as its hydrochloride salt. The residue was purified by C18 reverse phase chromatography (Isolute C-18(EC) with a gradient of H_2O (0.05% TFA) and MeCN 80:20 to 0:100 eluting around 60:40). The fractions containing the target molecule were combined and freeze-dried overnight to give the *title compound* **23** (33 mg, 0.055 mmol, 49%) as a lyophilized white solid. δ_H(500 MHz, 2:1, $CDCl_3$:CD_3OD): 4.75 (1H, d, *J* = 1.7 Hz), 4.13 – 3.95 (1H, m), 3.88 – 3.79 (2H, m), 3.68 (2H, ddd, *J* = 11.4, 5.7, 2.4 Hz), 3.63 – 3.53 (3H, m), 3.53 – 3.45 (2H, m), 3.42 – 3.35 (1H, m), 1.75 (1H, br t, *J* = 10.0 Hz), 1.57 – 1.45 (1H, m), 1.35 – 1.15 (24H, s), 0.84 (3H, t, *J* = 6.8 Hz); δ_C(126 MHz, 2:1, $CDCl_3$:CD_3OD): 101.00 (d, $^1J_{CH}$ = 171.36 Hz *C*-1), 73.67, 72.70, 71.99, 71.43, 70.41, 67.73, 64.00, 62.12, 54.04, 34.64 , 32.22 , 29.98 , 29.94 , 29.65 , 25.54 , 22.95 , 14.20; δ_F(376 MHz, 2:1, $CDCl_3$:CD_3OD): -75.98; HRMS (ESI-pos): calcd for $C_{24}H_{50}O_8N$ $[M + H]^+$ *m/z* 480.3531 found *m/z* 480.3515.

_N_-[(1_S_,2_S_,3_R_)-2,3-dihydroxy-1-[(α-D-mannopyranosyloxy)methyl]heptadecyl] hexacosanamide (α-ManCer, 20)

TEA (200 µL, 1.424 mmol) then isopropyl chloroformate solution (70 µL, 0.070 mmol) was added to a mixture of cerotic acid (21 mg, 0.066 mmol) in anhydrous CH_2Cl_2 (5mL) under an argon atmosphere, and the resulting solution was stirred at rt for 50 min. This was added to a solution of **23** (31 mg, 0.052 mmol) in anhydrous DMF (5 mL) at 0 °C under argon and with the exclusion of light. After 5 min the ice bath was removed and the reaction was warmed to rt overnight. The reaction was diluted with MeOH and the solvents were removed *in vacuo* to give the residue which was absorbed onto silica gel from a mixture of warm MeOH:$CHCl_3$ and purified by column chromatography ($CHCl_3$ to 40% MeOH:$CHCl_3$) to give the known[3] title compound **20** (42 mg, 0.049 mmol, 94%) as a white solid. δ_H(500 MHz, 2:1 $CDCl_3$:CD_3OD): 7.29 (1H, d, *J* = 8.8 Hz), 4.73 (1H, d, *J* = 1.7 Hz), 4.18 – 4.11 (1H, m), 3.87 – 3.75 (3H, m), 3.70 (1H, dd, *J* = 11.9, 5.1 Hz), 3.66 (1H, dd, *J* = 9.3, 3.2 Hz), 3.64 – 3.56 (2H, m), 3.53 – 3.42 (3H, m), 2.16 (2H, t, *J* = 7.6 Hz), 1.73 – 1.42 (5H, m,), 1.30 – 1.18 (67H, m), 0.84 (6H, t, *J* = 6.8 Hz); δ_C(126 MHz, 2:1 $CDCl_3$:CD_3OD): 175.07, 100.90 (d, $^1J_{CH}$ = 158.16 Hz, *C*-1), 74.93, 73.24, 72.49, 71.57, 70.85, 67.68, 67.06, 61.97, 51.37, 36.73, 32.70, 32.21, 30.04, 30.00, 29.96, 29.92, 29.85, 29.71, 29.64, 26.21, 26.14, 22.94, 14.21; HRMS (ESI-pos): calcd for $C_{50}H_{99}O_9NNa$ $[M + Na]^+$ *m/z* 880.7212 found *m/z* 880.7194.

1-[(2'_S_,3'_S_,4'_R_)-2'-[11''-(4-Fluorophenyl)-undecamidyl]-3',4'-di-_O_-benzyl-1'-octadecanyl-2,3-di-_O_-benzyl-4,6-_O_-benzylidene-β-D-mannopyranoside (26)

To a solution of azide **8** (α/β = 5:95) (149 mg, 0.156 mmol) in anhydrous THF (10 mL) at 0 °C under argon was added trimethylphosphine (1M in THF, 1.100 mL, 1.100 mmol). The mixture was warmed to rt over 1 hr. and then stirred at rt for 4 hrs. NaOH (2M, 4 mL) was added to the reaction mixture and the resulting solution stirred at rt overnight. The reaction was diluted with CH_2Cl_2, washed with water and solvents were removed *in vacuo* to give amine intermediate **18** as a colourless oil. R_f = 0.55 (10% MeOH in $CHCl_3$); HRMS (ESI-pos): calcd for $C_{59}H_{78}O_8N$ $[M + H]^+$ *m/z* 928.5722, found *m/z* 928.5728. EDCI·HCl (95 mg, 0.498 mmol) and HOBt (78 mg, 0.568 mmol) were added to a stirred suspension of 11-(4-fluorophenyl)undecanoic acid **24**[4, 5] (60 mg, 0.213 mmol) in anhydrous CH_2Cl_2/DMF (10:4, 7 mL) at rt under argon. After 30 min a solution of **18** (0.156 mmol) in CH_2Cl_2 (5 mL) was added followed by DIPEA (190 µL, 1.085 mmol) and the reaction stirred at rt overnight. The reaction was diluted with CH_2Cl_2, washed twice with 2M aqueous NaOH (to remove excess acid) and brine. The combined organic extracts were concentrated *in vacuo* to give the residue which was purified by column chromatography (PE, to 6:1 PE:EtOAc) the *title compound* **26** (129 mg, 0.108 mmol, 70%) as

a colourless oil. δ_H(500 MHz, $CDCl_3$): 7.53 – 7.21 (25H, m), 7.11 (2H, ddd, *J*= 8.7, 5.8, 3.2 Hz), 6.95 (2H, td, *J*= 8.7, 2.2 Hz), 5.65 (1H, d, *J*= 8.4 Hz), 5.60 (1H, s), 4.89 (1H, d, *J*= 12.3 Hz), 4.80 (1H, d, *J*= 12.2 Hz), 4.75 (1H, d, *J*= 11.5 Hz), 4.69 (1H, d, *J*= 12.5 Hz), 4.61 (1H, d, *J*= 9.4 Hz), 4.59 (1H, d, *J*= 10.5 Hz), 4.56 (1H, d, *J*= 6.9 Hz), 4.53 (1H, d, *J*= 6.9 Hz), 4.41 – 4.35 (2H, m), 4.25 (1H, dd, *J*= 10.4, 4.8 Hz), 4.17 (1H, t, *J*= 9.6 Hz), 4.06 (1H, dd, *J*= 10.3, 7.9 Hz), 3.90 – 3.83 (2H, m), 3.80 – 3.74 (2H, m), 3.59 – 3.52 (2H, m), 3.24 (1H, td, *J*= 9.7, 4.9 Hz), 2.55 (2H, t, *J*= 8.0 Hz), 1.93 (2H, dtd, *J*= 23.1, 14.7, 6.9 Hz), 1.70 – 1.45 (8H, m), 1.33 – 1.20 (2634H, m), 0.88 (3H, t, *J*= 6.9 Hz); δ_C(126 MHz, $CDCl_3$): 172.92, 161.25 ($^1J_{CF}$ = 242.7 Hz), 138.65, 138.64 (d, $^4J_{CF}$ = 3.1 Hz), 138.56, 138.44, 137.67, 129.76 (d, $^3J_{CF}$ = 7.7 Hz), 129.02, 128.59, 128.57, 128.53, 128.52, 128.45, 128.34, 128.31, 128.05,127.98, 127.82, 127.69, 126.20, 115.03 (d, $^2J_{CF}$ = 21.0 Hz), 102.21 (d, $^1J_{CH}$ = 155.50 Hz, *C*-1), 101.59, 80.63, 79.72, 78.78, 77.95, 76.06, 74.82, 73.71, 72.57, 72.20, 68.70, 67.68, 49.93, 36.89, 35.27, 32.08, 31.76, 30.57, 29.96, 29.87, 29.85, 29.82, 29.72, 29.65, 29.62, 29.58, 29.52, 29.35, 26.06, 25.75, 22.84, 14.27; δ_F(376 MHz, $CDCl_3$): -118.66 (tt, *J*= 9.4, 5.6 Hz); HRMS (ESI-pos): calcd for $C_{76}H_{100}O_9NFNa$ $[M + Na]^+$ *m/z* 1212.7274 found *m/z* 1212.7249.

11-(4-Fluorophenyl)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-[(β-D-mannopyranosyloxy)methyl]heptadecyl]undecanamide (28)

To a solution of **26** (87 mg, 0.073 mmol) in CH_2Cl_2/MeOH (1:1, 20 mL) was added 10% palladium on carbon (18 mg, 0.017 mmol). The stirred reaction mixture was degassed under vacuum and backfilled with hydrogen three times then left to stir overnight under a balloon of hydrogen. The hydrogen atmosphere was removed and after dilution with MeOH the solution was filtered through Celite and washed with CH_2Cl_2/MeOH followed by approx. 50 mL of hot EtOH. The solvents were removed *in vacuo* to give the crude material which was precipitated by dissolving in hot ethanol and triturating out of solution by dropwise addition of water. Once a precipitate formed the mixture was heated to re-dissolve and the resulting solution was allowed to cool slowly to rt and then cooled in ice. The resulting precipitate was filtered through a Hirsch funnel and dried to a constant weight affording the *title compound* **28** (45 mg, 0.061 mmol, 83%) as a white solid. δ_H(500 MHz, 2:1 $CDCl_3$:CD_3OD): 7.39 (1H, d, *J*= 9.0 Hz), 7.11 – 7.06 (2H, m), 6.90 (2H, tt, *J*= 9.0, 2.1 Hz), 4.46 (1H, d, *J*= 1.1 Hz, H-1), 4.17 (1H, dqd, *J*= 5.9, 4.0, 2.8, 1.7 Hz), 4.03 (1H, dd, *J*= 10.3, 3.8 Hz), 3.90 – 3.81 (2H, m, *H*-6), 3.74 – 3.66 (2H, m), 3.59 – 3.48 (3H, m), 3.42 (1H, dd, *J*= 9.4, 3.3 Hz), 3.20 (1H, ddd, *J*= 9.7, 5.8, 2.5 Hz), 2.53 (2H, t, *J*= 7.9 Hz), 2.16 (2H, t, *J*= 7.7 Hz), 1.47 – 1.65 (6H,m), 1.32 – 1.14 (40H, m), 0.84 (3H, t, *J*= 6.9 Hz,); δ_C(126 MHz, 2:1 $CDCl_3$:CD_3OD): 174.66 , 161.48 (d, $^1J_{CF}$ = 242.2 Hz), 138.81 (d, $^4J_{CF}$ = 3.3 Hz), 129.96 (t, $^3J_{CF}$ = 8.0 Hz), 115.09 (d, $^2J_{CF}$ = 21.0 Hz), 100.39 (d,

$^1J_{CH}$ = 157.4 Hz, *C*-1), 76.87, 74.39, 74.14, 72.35, 71.11, 68.49, 67.52, 61.85, 50.50, 36.79, 35.40, 32.36, 32.23, 31.94, 30.10, 30.07, 30.03, 30.01, 29.98, 29.96, 29.88, 29.82, 29.81, 29.77, 29.76, 29.71, 29.68, 29.66, 29.49, 26.26, 26.19, 22.96,14.22; δ_F(376 MHz, 2:1 $CDCl_3$:CD_3OD): -119.07 (tt, *J*= 9.1, 5.3 Hz); HRMS (ESI-pos): calcd for $C_{41}H_{72}O_9NFNa$ $[M + Na]^+$ *m/z* 764.5083 found *m/z* 764.5110; Anal. Calcd for $C_{41}H_{72}NO_9 \cdot 2H_2O$: C, 66.37; H, 9.78; N, 1.89. Found C, 66.13; H, 9.49; N, 1.93.

1-[(2'*S*,3'*S*,4'*R*)-2'-[11''-(4-(4-Fluorophenoxy)phenyl)-undecamidyl]-3',4'-di-*O*-benzyl-1'-octadecanyl]-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (27)

To a solution of azide **8** (α/β = 5:95) (148 mg, 0.154 mmol) in anhydrous THF (10 mL) at 0 °C under argon was added trimethylphosphine (1M in THF, 1.100 mL, 1.100 mmol). The solution was warmed to rt over 1 hr then stirred at rt for an additional 4 hrs. NaOH (2M, 4 mL) was added to the reaction mixture and the resulting solution stirred at rt overnight to hydrolyse the imino-phosphorane. The reaction was diluted with CH_2Cl_2, washed with water and the volatiles were removed *in vacuo* to give amine intermediate **18** as a colourless oil. EDCI·HCl (94 mg, 0.490 mmol) and HOBt (81 mg, 0.585 mmol) were added to a stirred suspension of 11-(4-(4-fluorophenoxy)phenyl)undecanoic acid **25** (82 mg, 0.220 mmol) in anhydrous CH_2Cl_2/DMF (10:4, 7 mL) at rt under argon. After 30 min a solution of **18** (143 mg, 0.154 mmol) in anhydrous CH_2Cl_2 (5 mL) was added followed by DIPEA (190 µL, 1.085 mmol) and the reaction stirred at rt overnight. The reaction was diluted with CH_2Cl_2, washed with water and the aqueous layer re-extracted with EtOAc. The combined organics were concentrated *in vacuo* to give the crude which was purified by column chromatography (PE, to 5:1 PE:EtOAc) to give the *title compound* **27** as a mixture of anomers (α/β = 5:95) as a colourless oil. Data for β-**27**: δ_H(500 MHz, $CDCl_3$): *inter alia* 7.53 – 7.22 (25H, m), 7.14 – 7.09 (2H, m), 7.03 – 6.93 (4H, m), 6.91 – 6.85 (2H, m), 5.67 (1H, d, *J*= 8.3 Hz), 5.60 (1H, s), 4.89 (1H, d, *J*= 12.2 Hz), 4.80 (1H, d, *J*= 12.2 Hz), 4.75 (1H, d, *J*= 11.5 Hz), 4.69 (1H, d, *J*= 12.4 Hz), 4.61 (1H, d, *J*= 9.3 Hz), 4.59 (1H, d, *J*= 10.4 Hz), 4.56 (1H, d, *J*= 7.2 Hz), 4.53 (1H, d, *J*= 7.4 Hz), 4.42 – 4.35 (2H, m), 4.25 (1H, dd, *J*= 10.4, 4.9 Hz), 4.17 (1H, t, *J*= 9.5 Hz), 4.05 (1H, dd, *J*= 10.3, 7.9 Hz), 3.89 – 3.83 (2H, m), 3.80 – 3.74 (2H, m,), 3.55 (2H, m), 3.24 (1H, td, *J*= 9.7, 4.8 Hz), 2.56 (2H, t, *J*= 7.8 Hz), 1.94 (2H, qt, *J*= 14.6, 7.6 Hz), 1.72 – 1.55 (4H, m), 1.53 – 1.45 (2H, m), 1.33 – 1.21 (36H, m), 0.88 (3H, t, *J*= 6.8 Hz,); δ_C(126 MHz, $CDCl_3$): 172.94 , 158.73 (d, $^1J_{CF}$ = 241.2 Hz), 155.51 , 153.52 (d, $^4J_{CF}$ = 2.4 Hz), 138.65 , 138.62 , 138.58 , 138.43 , 138.01 , 137.66 , 129.70 , 128.59 , 128.57 , 128.52 , 128.45 , 128.32 , 128.04 , 127.99 , 127.82 , 127.70 , 126.20 , 120.20 (d, $^3J_{CF}$ = 8.1 Hz), 118.48 , 116.29 (d, $^2J_{CF}$ = 23.3 Hz), 102.20 (d, $^1J_{CH}$ = 156.28 Hz *C*-1), 101.59, 80.63, 79.71, 78.77, 77.94, 76.07, 74.83, 73.70, 72.58, 72.20, 68.70, 67.68,

49.94, 36.89, 35.35, 32.08, 31.78, 30.57, 29.96, 29.87, 29.85, 29.82, 29.74, 29.67, 29.65, 29.61, 29.53 , 29.46, 26.05, 25.76, 22.84, 14.27; δ_F(376 MHz, $CDCl_3$): -121.12 (tt, *J*= 8.7, 4.7 Hz); HRMS (ESI-pos): calcd for $C_{82}H_{104}O_{10}NFNa$ $[M + Na]^+$ *m/z* 1304.7530 found *m/z* 1304.7536.

1-[(2'*S*,3'*S*,4'*R*)-2'-[11''-(*p*-(4-Fluorophenoxy)phenyl)-undecamidyl]-3',4'-di-*O*-acetyl-1'-octadecanol]-2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (30)

To a solution of glycolipid **27** (134 mg, 0.104 mmol) in CH_2Cl_2/MeOH (1:1, 20 mL) was added 10% palladium on carbon (32 mg, 0.030 mmol). The stirred reaction mixture was degassed under vacuum and backfilled with hydrogen three times then left to stir overnight under a balloon of hydrogen. The hydrogen atmosphere and solvents were removed *in vacuo* to give the crude material which was precipitated by dissolving in hot ethanol and triturating out of solution with dropwise addition of water. Once a precipitate formed the mixture was heated to re-dissolve and the resulting solution was allowed to cool slowly to rt and then cooled in ice. The resulting precipitate was filtered through a Hirsch funnel and dried to a constant weight affording glycolipid **29** (82 mg, 0.098 mmol, 94%) as a white solid which contained traces of the α-anomer. Acetic anhydride (1 mL, 10.58 mmol) was added dropwise to a stirred solution of **29** in pyridine (1 mL, 12.41 mmol) at rt. The reaction was stirred at rt overnight, after which a standard aqueous workup was performed using EtOAc as the organic solvent. Additional washes of the organic extract were performed with 2M aqueous HCl and sat. aqueous $NaHCO_3$ solutions. The combined organic extracts were concentrated *in vacuo* to give the crude material which was purified by column chromatography (PE, to 3:1 PE:EtOAc) to give the *title compound* **30** (79 mg, 0.073 mmol, 74%) as a colourless oil. δ_H(500 MHz, $CDCl_3$): 7.14 – 7.08 (2H, m), 7.03 – 6.92 (4H, m), 6.91 – 6.83 (2H, m), 6.27 (1H, dd, *J*= 9.6, 3.5 Hz), 5.40 (1H, d, *J*= 3.4 Hz), 5.22 (1H, t, *J*= 9.9 Hz), 5.03 (2H, td, *J*= 9.9, 9.4, 2.9 Hz,), 4.82 (1H, dt, *J*= 10.2, 2.9 Hz), 4.54 (1H, s), 4.35 (1H, tt, *J*= 9.5, 2.9 Hz), 4.21 (1H, dd, *J*= 12.2, 5.3 Hz), 4.15 (1H, dd, *J*= 12.2, 2.7 Hz), 3.96 (1H, dd, *J*= 9.5, 2.8 Hz), 3.60 (1H, ddd, *J*= 10.1, 5.5, 2.7 Hz), 3.44 (1H, dd, *J*= 9.5, 2.7 Hz), 2.56 (2H, t, *J*= 7.8 Hz), 2.22 (2H, t, *J*= 7.7 Hz), 2.20 (3H, s), 2.08 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.00 (6H, s), 1.60 (6H, dp, *J*= 20.9, 6.9 Hz), 1.38 – 1.17 (36H, m), 0.87 (3H, t, *J*= 6.4 Hz); δ_C(126 MHz, $CDCl_3$): 173.21, 171.34, 171.15, 170.64, 170.09, 169.72, 169.67, 158.69 (d, $^1J_{CF}$ = 241.1 Hz), 155.46 , 153.53 (d, $^4J_{CF}$ = 2.4 Hz), 138.04, 129.70, 120.17 (d, $^3J_{CF}$ = 8.2 Hz), 118.46 , 116.26 (d, $^2J_{CF}$ = 23.2 Hz), 98.26 (d, $^1J_{CH}$ = 158.42 Hz *C*-1), 73.48, 72.50, 71.27, 70.76, 68.63, 68.17, 66.19, 62.55, 47.41, 36.77, 35.33, 32.05 , 31.77, 29.82, 29.78, 29.74, 29.69, 29.65, 29.62, 29.49, 29.47, 29.43, 27.73, 25.77, 25.74, 22.81, 21.15, 21.12, 20.83, 20.81, 20.79 (CH_3), 20.75, 20.73, 20.70, 14.24; δ_F(376 MHz, $CDCl_3$): -

121.17 (tt, *J*= 8.4, 4.7 Hz); HRMS (ESI-pos): calcd for $C_{59}H_{88}O_{16}NFNa$ $[M + Na]^+$ *m/z* 1108.5959 found *m/z* 1108.5979.

11-(4-(4-Fluorophenoxy)phenyl)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-[(β-D-mannopyranosyloxy)methyl]heptadecyl] undecanamide (29)

To a solution of peracetate **30** (69 mg, 0.064 mmol) in CH_2Cl_2/MeOH (2:4 mL) was added sodium methoxide solution (2.5M in MeOH, 40 µL, 0.100 mmol) and the reaction mixture was stirred at rt for 3 hrs. The reaction was diluted with CH_2Cl_2/MeOH (1:1, 20 mL) and the volatiles removed *in vacuo*. The resulting white solid was dissolved in hot EtOH, cooled to rt and placed in the freezer (-18°C) for an hr. The resulting precipitate was filtered and dried to a constant weight to afford the *title compound* **29** (45 mg, 0.054 mmol, 85%) as a white solid. δ_H(500 MHz, 2:1 $CDCl_3$:CD_3OD): 7.42 (1H, d, *J*= 8.7 Hz), 7.09 (2H, dt, *J*= 8.4, 2.7 Hz), 7.00 – 6.89 (4H, m), 6.86 – 6.82 (2H, m), 4.46 (1H, s), 4.17 (1H, dt, *J*= 6.4, 4.0 Hz), 4.04 (1H, dd, *J*= 10.3, 3.7 Hz), 3.89 – 3.81 (2H, m), 3.74 – 3.67 (2H, m), 3.62 – 3.50 (3H, m), 3.43 (1H, dd, *J*= 9.4, 3.3 Hz), 3.20 (1H, ddd, *J*= 9.6, 5.7, 2.5 Hz), 2.54 (2H, t, *J*= 7.8 Hz), 2.16 (2H, t, *J*= 7.6 Hz,), 1.68 – 1.47 (6H, m), 1.40 – 1.19 (42H, m), 0.84 (3H, t, *J*= 6.9 Hz); δ_C(126 MHz, 2:1 $CDCl_3$:CD_3OD): 174.68 , 158.97 (d, $^1J_{CF}$ =240.9 Hz), 155.69 , 153.79 (d, $^4J_{CF}$ =2.4 Hz), 138.29, 129.91, 120.33 (d, $^3J_{CF}$ =8.2 Hz), 118.66 , 116.41 (d, $^2J_{CF}$ =23.4 Hz), 100.44 (d, $^1J_{CH}$ = 158.99 Hz *C*-1), 76.85, 74.37, 74.06, 72.39, 71.08, 68.65, 67.29, 61.58, 50.36, 36.77, 35.49, 32.34, 32.24, 31.97, 30.11, 30.08, 30.04, 30.02, 29.97, 29.92, 29.87, 29.82, 29.75, 29.71, 29.67, 29.60, 26.29, 26.22, 22.97, 14.22; δ_F(376 MHz, 2:1 $CDCl_3$:CD_3OD): -121.42 (tt, *J*= 8.3, 4.4 Hz); HRMS (ESI-pos): calcd for $C_{47}H_{76}O_{10}NFNa$ $[M + Na]^+$ *m/z* 856.5345 found *m/z* 856.5348.

1-((2'*S*,3'*S*,4'*R*)-2'-[*N*-*tert*-butoxycarbonylamino]-3',4'-di-*O*-benzyl-1'-octadecanol)-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (31)

To a solution of azide **8** (α/β = 5:95) (237 mg, 0.249 mmol) in anhydrous THF (10 mL) at 0 °C under argon was added trimethylphosphine (1M in THF, 1.100 mL, 1.100 mmol). It was warmed to rt over 1 hr and then stirred at rt for 4 hrs. NaOH (2M, 4 mL) was added to the reaction and the resulting solution stirred at rt. The reaction was diluted with CH_2Cl_2, washed with water and the volatiles were removed *in vacuo* to give a colourless oil. Di-*tert*-butyl dicarbonate (70 mg, 0.321 mmol) and DIPEA (260 µL, 1.49 mmol) was added to a stirred solution of the intermediate in DMF (5mL) at rt. The reaction was heated to 50 °C overnight then allowed to cool to rt. The reaction was diluted with EtOAc and a standard aqueous workup was preformed using EtOAc as the organic solvent. The solvents were removed *in vacuo* to

afford a residue that was purified by column chromatography (PE, to 4:1 PE:EtOAc) to give the *title compound* **31** 199 mg, 0.194 mmol, 78%) as a colourless oil.

δ_H(500 MHz, $CDCl_3$): 7.56 – 7.44 (4H, m), 7.42 – 7.26 (21H, m), 5.61 (1H, s), 4.96 (1H, d, *J*= 12.2 Hz), 4.86 (1H, d, *J*= 12.2 Hz), 4.80 (1H, d, *J*= 11.1 Hz), 4.71 (1H, d, *J*= 12.5 Hz), 4.66 – 4.58 (3H, m), 4.51 (1H, d, *J*= 11.6 Hz,), 4.39 (1H, s), 4.25 (1H, dd, *J*= 10.4, 4.8 Hz), 4.19 (1H, t, *J*= 9.6 Hz), 4.11 (1H, dd, *J*= 9.9, 6.5 Hz), 4.03 – 3.95 (1H, m), 3.92 (1H, d, *J*= 3.0 Hz), 3.87 (1H, t, *J*= 10.3 Hz), 3.77 (1H, dd, *J*= 7.0, 3.0 Hz), 3.66 (1H, dd, *J*= 10.1, 3.5 Hz), 3.56 (2H, td, *J*= 8.2, 7.0, 3.2 Hz), 3.26 (1H, td, *J*= 9.7, 4.8 Hz), 1.75 – 1.63 (2H, m, CH_2), 1.43 (9H, s), 1.22 – 1.23 (24H, d, *J*= 4.5 Hz), 0.90 (3H, t, *J*= 6.8 Hz); δ_C(126 MHz, $CDCl_3$): 155.44, 138.68, 138.66, 138.57, 138.47, 137.7, 128.99, 128.60, 128.53, 128.48, 128.46, 128.33, 128.31, 128.28, 128.26, 128.13, 128.06, 127.79, 127.74, 127.71, 127.69, 126.20, 102.16 (d, $^1J_{CH}$ = 155.94 Hz *C*-1), 101.56, 79.92, 79.80, 79.46, 78.81, 77.97, 75.83, 74.80, 74.06, 72.57, 71.99, 69.14, 68.70, 67.72, 51.16, 32.07, 29.88, 29.86, 29.84, 29.81, 29.51, 28.54, 26.05, 22.84, 14.27; HRMS (ESI-pos): calcd for $C_{64}H_{85}O_{10}NNa$ $[M + Na]^+$ *m/z* 1050.6066 found *m/z* 1050.6088.

1-((2'*S*,3'*S*,4'*R*)-2'-Amino-1',3',4'-octadecanyl)-β-D-mannopyranoside.TFA salt (32)

To a solution of *N*-Boc glycoside **31** (199 mg, 0.194 mmol) in CH_2Cl_2/MeOH (1:1, 20 mL) was added 10% palladium on carbon (60 mg, 0.056 mmol). The stirred reaction mixture was degassed under vacuum and backfilled with hydrogen three times then left to stir overnight under a balloon of hydrogen. After dilution with hot EtOH the solution was filtered through Celite and washed with hot EtOH (50 mL). The solvents were removed *in vacuo*, the residue was dissolved in CH_2Cl_2 (5 mL). Trifluoroacetic acid (1 mL, 13.07 mmol) was added and the reaction was stirred at rt overnight before being diluted with CH_2Cl_2. The solvents removed *in vacuo* to give a residue which was purified by C18 reverse phase chromatography (Isolute C-18(EC)) with a gradient of H_2O and MeCN 80:20 to 0:100 eluting around 60:40). The fractions containing the target molecule were combined and lyophilized to give the *title compound* **32** (89 mg, 0.147 mmol, 77% over two steps) as a white solid. δ_H(500 MHz, 2:1, $CDCl_3$:CD_3OD): 4.54 (1H, d, *J*= 0.9 Hz), 4.11 – 3.96 (2H, m), 3.93 (1H, d, *J*= 3.1 Hz), 3.90 (1H, dd, *J*= 11.9, 2.4 Hz), 3.71 (1H, dd, *J*= 11.9, 5.8 Hz), 3.62 (1H, dt, *J*= 8.7, 4.0 Hz), 3.58 – 3.52 (2H, m, *H*-4), 3.47 (1H, dd, *J*= 9.4, 3.2 Hz), 3.42 (1H, td, *J*= 8.4, 2.7 Hz), 3.23 (1H, ddd, *J*= 9.6, 5.8, 2.4 Hz), 1.76 (1H, ddd, *J*= 13.5, 7.1, 2.9 Hz), 1.43 -1.55 (1H, m), 1.42 – 1.15 (26H, m), 0.85 (3H, t, *J*= 6.9 Hz); δ_C(126 MHz, 2:1, $CDCl_3$:CD_3OD): 100.24 (d, $^1J_{CH}$ = 158.29 Hz *C*-1), 77.38, 74.15, 72.95, 72.30, 71.42, 67.56, 65.77, 61.90, 54.42, 34.79, 32.45, 30.23, 30.20, 30.19, 30.16,

29.86, 25.74, 23.16, 14.29; δ_F(376 MHz, 2:1, $CDCl_3$:CD_3OD): -76.15; HRMS (ESI-pos): calcd for $C_{24}H_{50}O_8N$ $[M + H]^+$ *m/z* 480.3531 found *m/z* 480.3508.

1-[(2'*S*,3'*S*,4'*R*)-2'-*N*-[(11*Z*,14*Z*)-1''-oxo-11'',14''-eicosadien-1''-yl]-3',4'-di-*O*-acetyl-1'-octadecanyl]-2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (34)

To a mixture of cis-11,14-elicosadienoic acid (42 mg, 0.133 mmol) in anhydrous CH_2Cl_2 (5mL) under an argon atmosphere and with the exclusion of light, was added TEA (300 µL, 2.137 mmol) then isopropyl chloroformate solution (130 µL, 0.130 mmol) and the resulting solution was stirred at rt for 50 min to give the crude mixed carbonic anhydride. This was added to a solution of **33** (62 mg, 0.104 mmol) in anhydrous DMF (5 mL) at 0 °C under argon and with the exclusion of light. After 5 min the ice bath was removed and the reaction was warmed to rt. When TLC indicated loss of starting material and formation of the amide intermediate (approx. 3 hrs), acetic anhydride (600 µL, 6.35 mmol) and DMAP (1 crystal) were added to the reaction mixture and the reaction was stirred at rt overnight. The reaction was diluted with CH_2Cl_2, washed with water and brine and concentrated *in vacuo* to give the residue that was purified by column chromatography (PE to 2:1 PE/EtOAc) to give the *title compound* **34** (91 mg, 0.089 mmol, 86%) as a colourless oil. δ_H(500 MHz, acetone-d_6): 7.18 (1H, d, *J*= 9.4 Hz, NH), 5.45 – 5.31 (5H, m), 5.19 (1H, t, *J*= 9.9 Hz), 5.12 (2H, dd, *J*= 10.1, 3.2 Hz), 4.96 (1H, dt, *J*= 10.6, 2.9 Hz), 4.92 (1H,), 4.40 (1H, ddd, *J*= 14.1, 6.9, 4.3 Hz), 4.27 (1H, dd, *J*= 12.1, 5.5 Hz), 4.15 (1H, dd, *J*= 12.2, 2.5 Hz), 4.00 (1H, dd, *J*= 10.0, 4.7 Hz), 3.88 (1H, ddd, *J*= 9.7, 5.5, 2.6 Hz), 3.59 (1H, dd, *J*= 10.0, 2.9 Hz), 2.83 (2H, t, *J*= 7.0 Hz), 2.25 – 2.16 (2H, m), 2.14 (3H, s), 2.08 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.98 (3H, s), 1.95 (3H, s), 1.83 – 1.74 (1H, m), 1.70 – 1.57 (3H, m), 1.26 – 1.44 (46H,m), 0.91 (6H, app q, *J*= 6.5 Hz); δ_C(126 MHz, Acetone-d_6): 172.95, 170.98, 170.75, 170.72, 170.20, 170.17, 170.15, 130.70, 130.68, 128.78, 128.77 (*C*-12''), 98.73 (d, $^1J_{CH}$ = 160.16 Hz *C*-1), 73.56, 72.91, 72.50, 71.82, 69.54, 69.11, 66.95, 63.16, 48.18, 36.72, 32.66, 32.25, 30.45, 30.40, 30.37, 30.32, 30.28, 30.25, 30.10, 30.07, 30.06, 29.94, 28.47, 27.87, 27.81, 26.46, 26.31, 26.23, 23.35, 23.23, 20.99, 20.83, 20.71, 20.68, 20.54, 14.38, 14.36; HRMS (ESI-pos): calcd for $C_{56}H_{95}O_{15}NNa$ $[M + Na]^+$ *m/z* 1044.6594 found *m/z* 1044.6604.

***N*-[(1*S*,2*S*,3*R*)-2,3-Dihydroxy-1-[(β-D-mannopyranosyloxy)methyl]heptadecyl]-11*Z*, 14*Z*-eicosadienamide (33)**

To a solution of glycolipid **34** (32 mg, 0.032 mmol) in CH_2Cl_2/MeOH (2:4, 10 mL) under argon with the exclusion of light was added sodium methoxide solution (2.5M in MeOH, 30.0 µL,

0.075 mmol) and the reaction mixture was stirred at rt for 3 hours. The reaction was diluted with CH_2Cl_2/MeOH (1:1, 20 mL) and the volatiles removed *in vacuo*. The residue was dissolved in minimal ethanol and transferred to a 14 mL centrifuge tube and 10 mL water was added causing a milky white suspension to form. The tube was spun at 5200 rpm for 25 min before the aqueous layer was decanted leaving behind a white solid. The solid was re-suspended in 10 mL water and spun again to further wash the solid, again decanting the aqueous layer. The combined aqueous layers were evaporated to dryness and the procedure repeated to obtain further solid. The solids were dissolved in ethanol, transferred to a round bottom flask, concentrated *in vacuo*, and dried to a constant weight to afford the *title compound* **33** (22 mg, 0.028 mmol, 88%) as a white solid. δ_H(500 MHz, 2:1 $CDCl_3$:CD_3OD): 5.39 – 5.23 (4H, m), 4.46 (1H, s), 4.17 (1H, dt, *J*= 7.0, 3.9 Hz), 4.06 (1H, dd, *J*= 10.2, 3.6 Hz), 3.88 (1H, d, *J*= 3.1 Hz), 3.83 (1H, dd, *J*= 12.0, 2.5 Hz), 3.71 (2H, ddd, *J*= 17.9, 11.1, 4.6 Hz), 3.62 – 3.49 (3H, m), 3.43 (1H, dd, *J*= 9.3, 3.2 Hz), 3.19 (1H, ddd, *J*= 9.6, 5.2, 2.5 Hz), 2.73 (2H, t, *J*= 6.8 Hz), 2.16 (2H, t, *J*= 7.7 Hz), 2.01 (4H, q, *J*= 7.0 Hz), 1.63 – 1.46 (4H, m), 1.40 – 1.17 (48H, m), 0.85 (6H, q, *J*= 6.7 Hz); δ_C(126 MHz, 2:1 $CDCl_3$:CD_3OD): 174.68, 130.44, 130.37, 128.29, 128.25, 100.41 (d, $^1J_{CH}$ = 156.97 Hz, *C*-1), 76.90, 74.41, 74.16, 72.36, 71.13, 68.51, 67.53, 61.87, 50.52, 36.81, 32.38, 32.25, 31.85, 30.12, 30.09, 30.05, 30.03, 30.02, 29.98, 29.90, 29.87, 29.77, 29.73, 29.68, 29.66, 29.65, 27.54, 27.50, 26.28, 26.22, 25.92, 22.98, 22.87, 14.23, 14.20. HRMS (ESI-pos): calcd for $C_{44}H_{83}O_9NNa$ $[M + Na]^+$ *m/z* 792.5960 found *m/z* 792.5940.

Figures for Supporting Information

Figure SI-1 Stacked ^{1}H NMR partial spectra of α-ManCer (20)(top) and β-ManCer (2) (bottom)

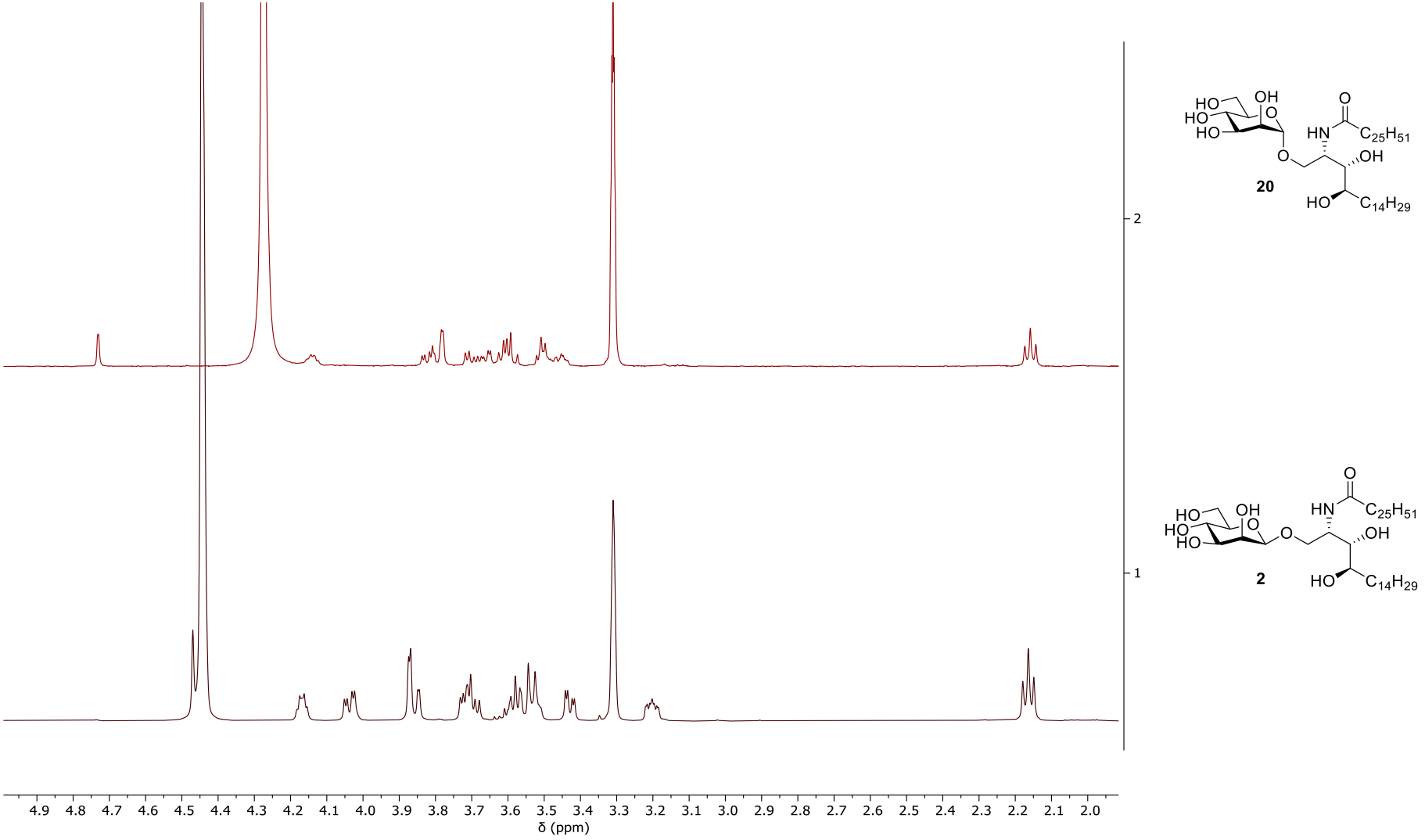
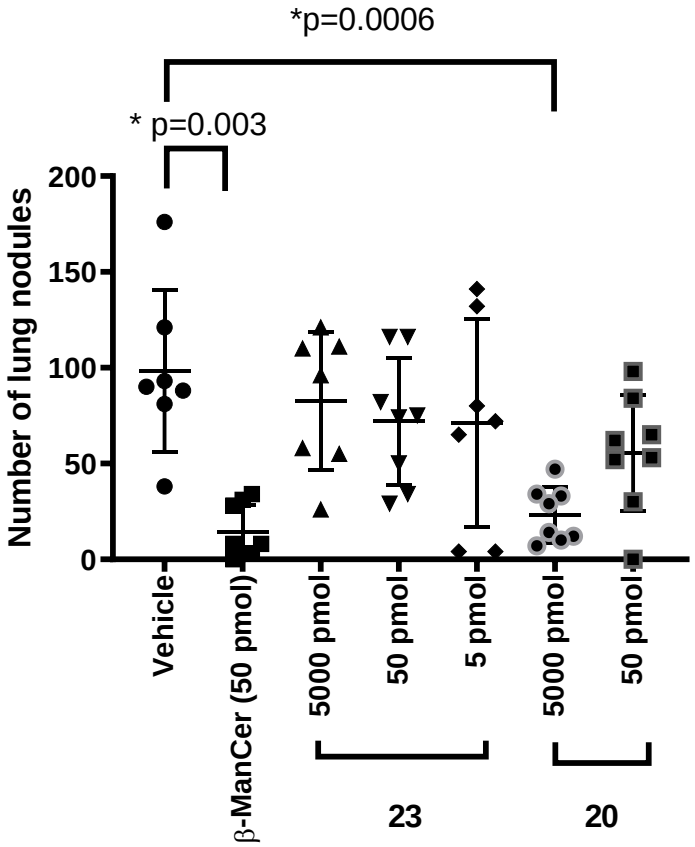


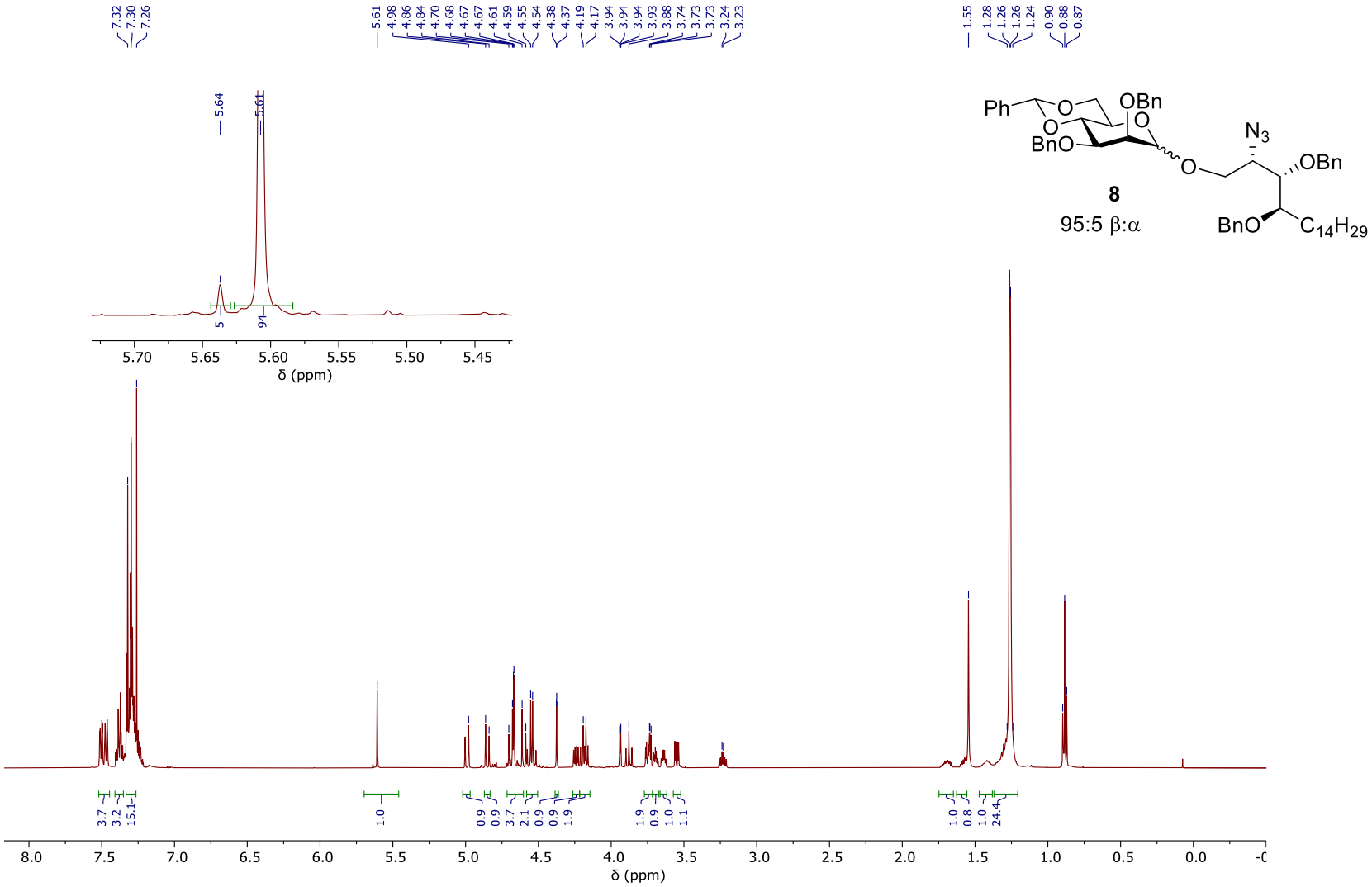
Figure SI-2.



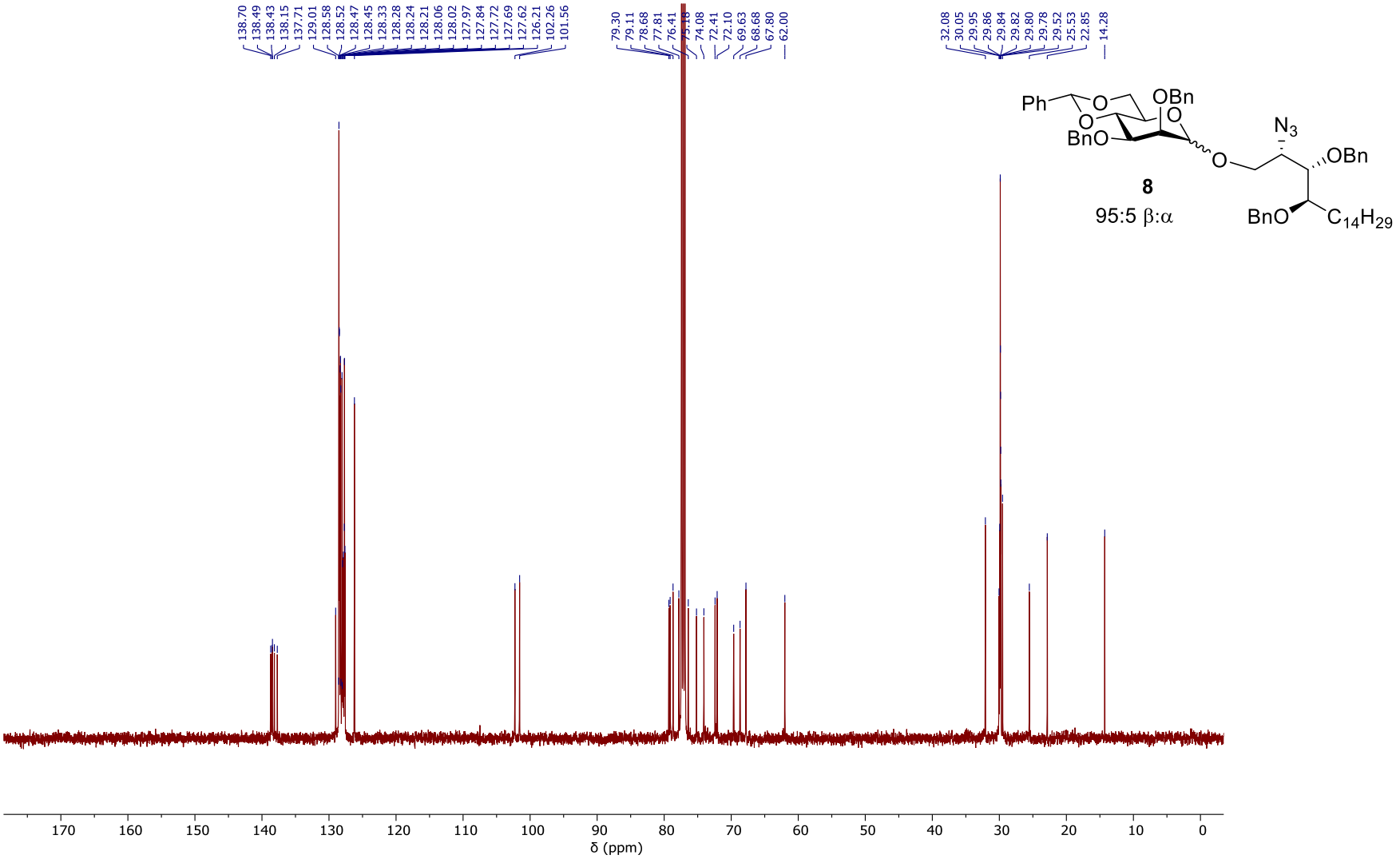
Effects of α-ManCer (**20**) and α-ManPhyt (**23**) compared to vehicle (PBS/TWEEN20) and β-ManCer at 5000 and 50 pmol. (N=8 mice/group) *p<0.05 compared with vehicle control (Mann-Whitney).

NMR Spectra and HPLC Chromatograms

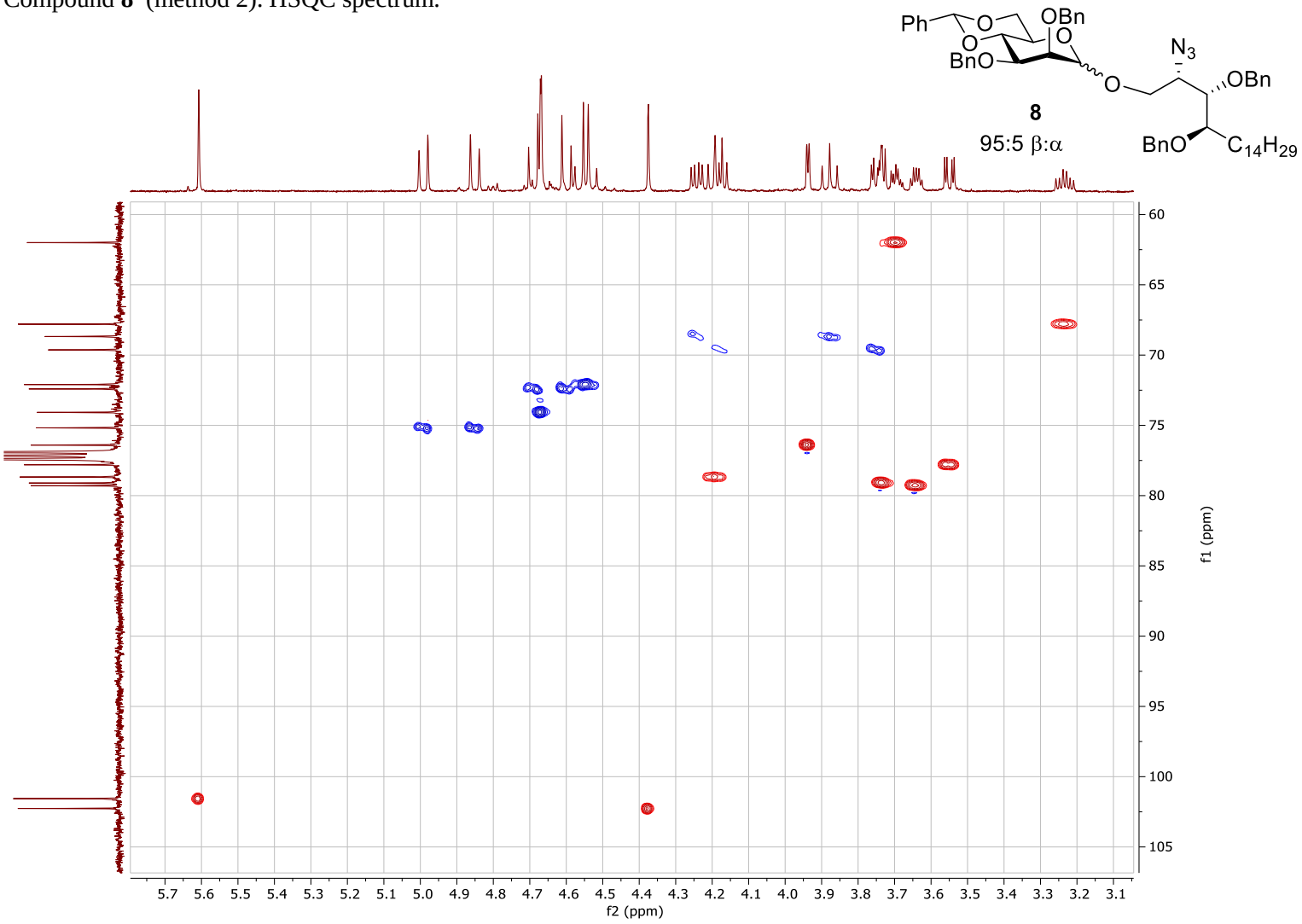
Compound **8** (method 2). ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



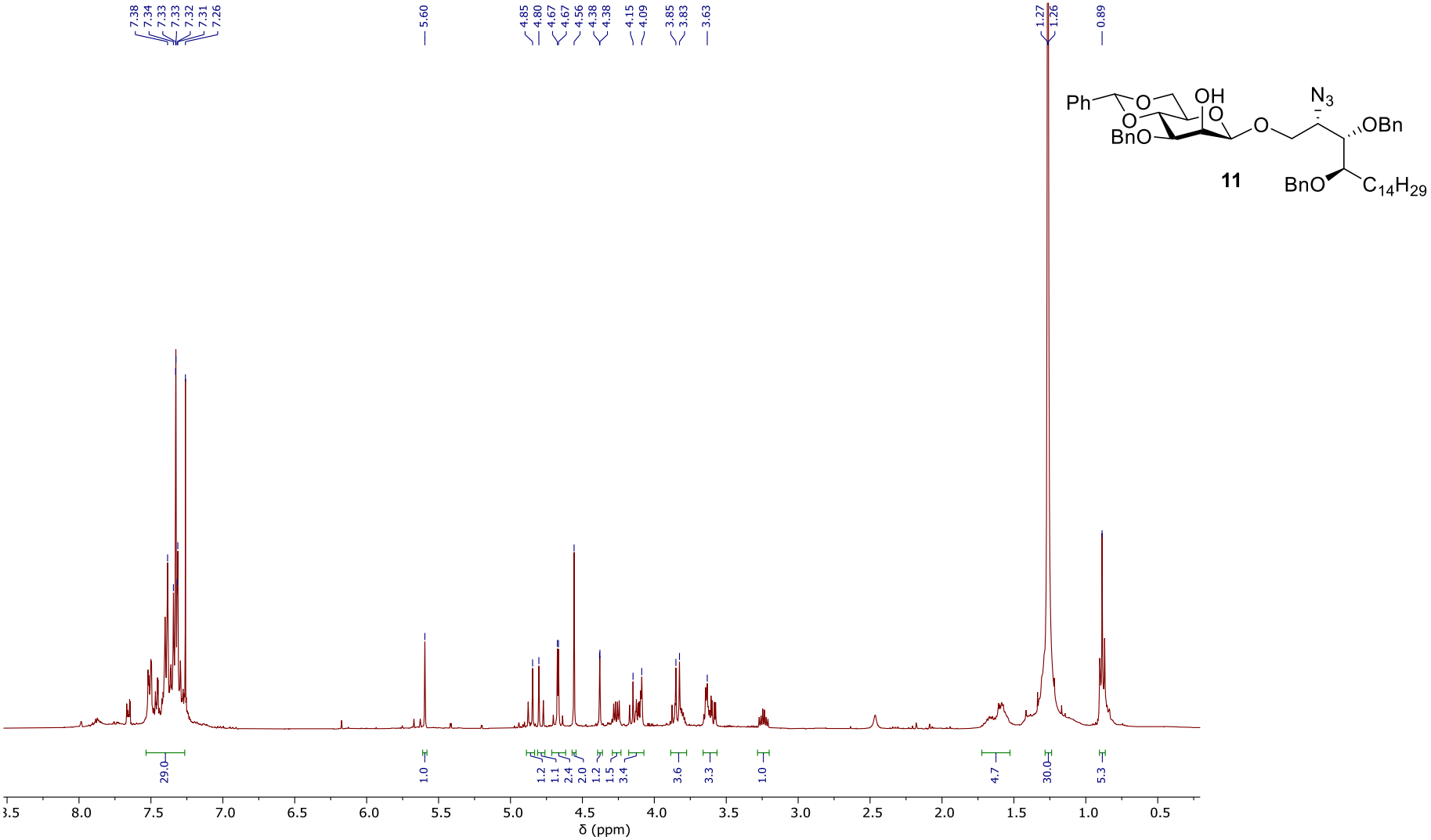
Compound **8** (method 2) ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



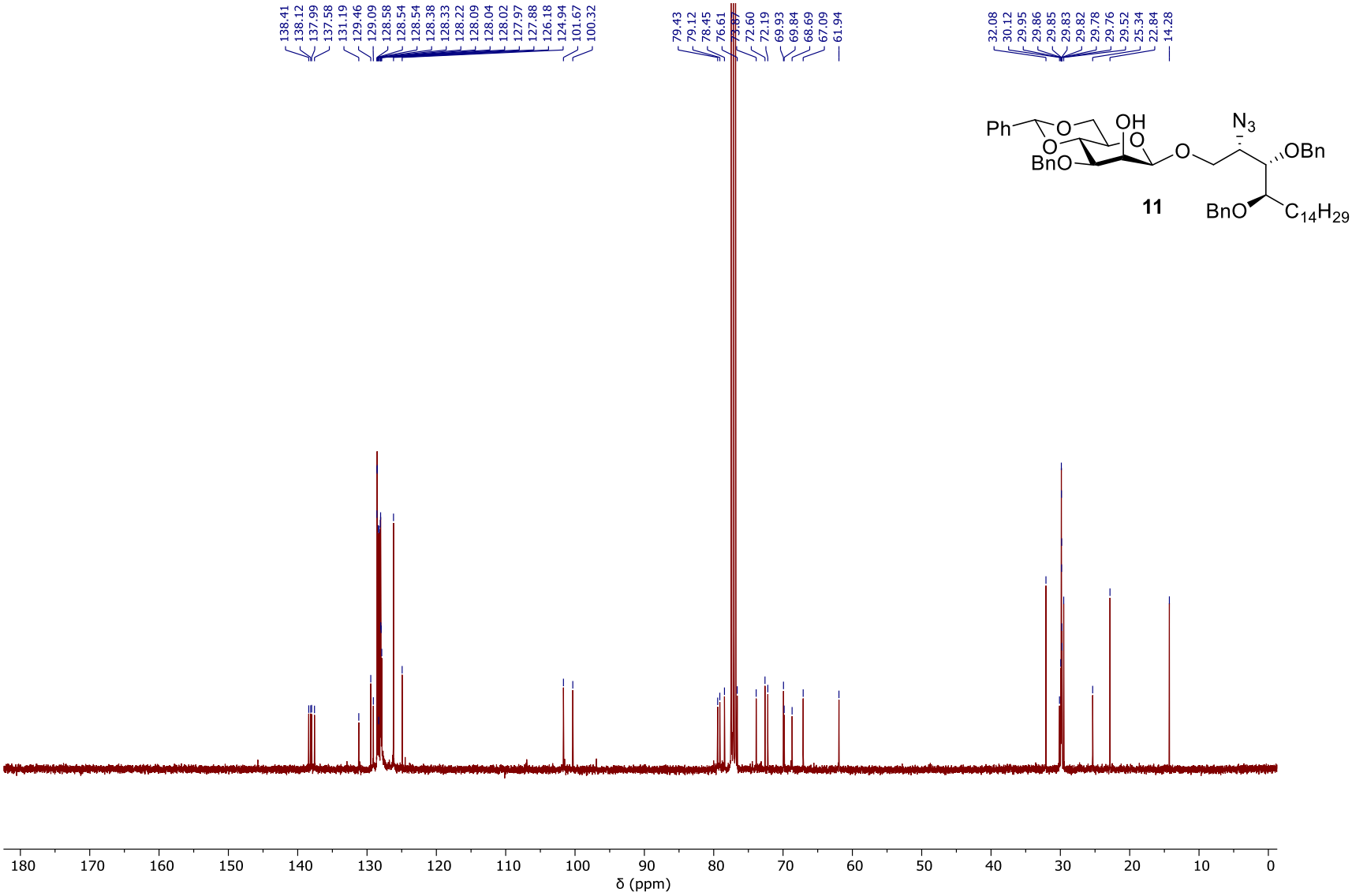
Compound **8** (method 2). HSQC spectrum.



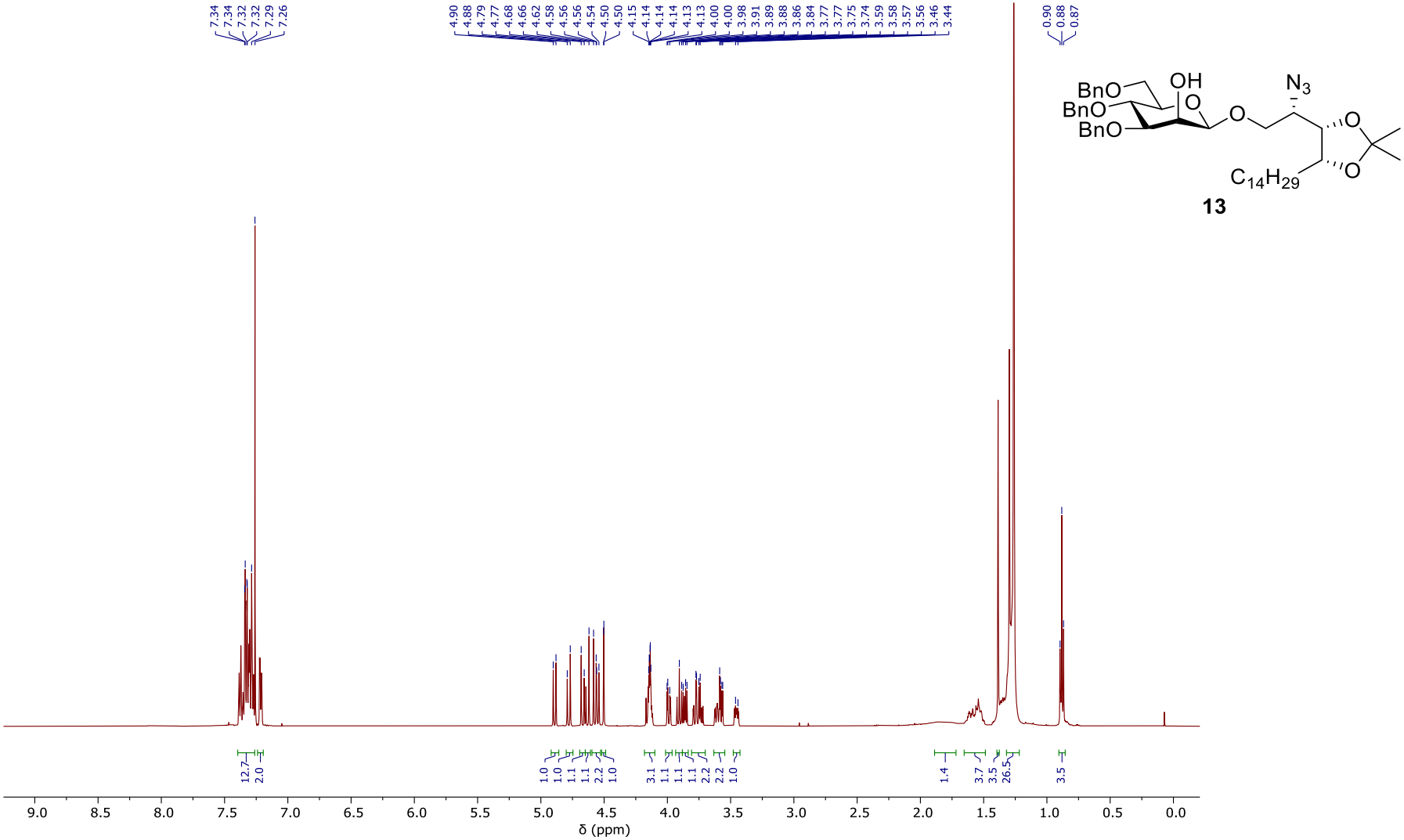
Compound **11**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



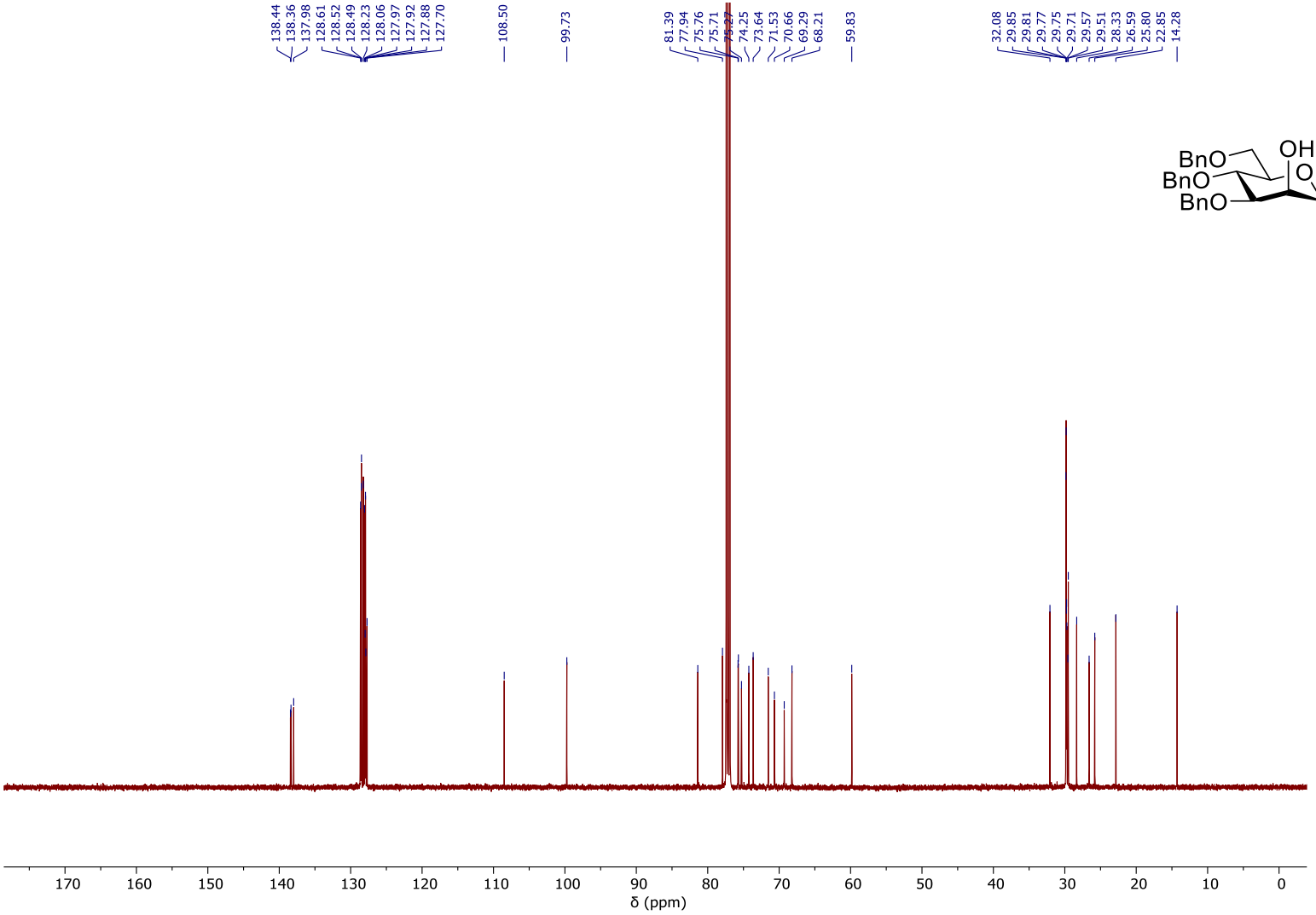
Compound **11** ^{13}C NMR spectrum (125 MHz, $CDCl_3$).

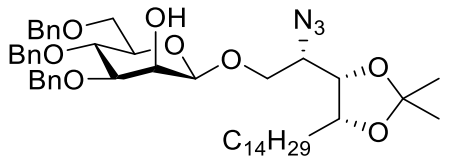


Compound **13**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



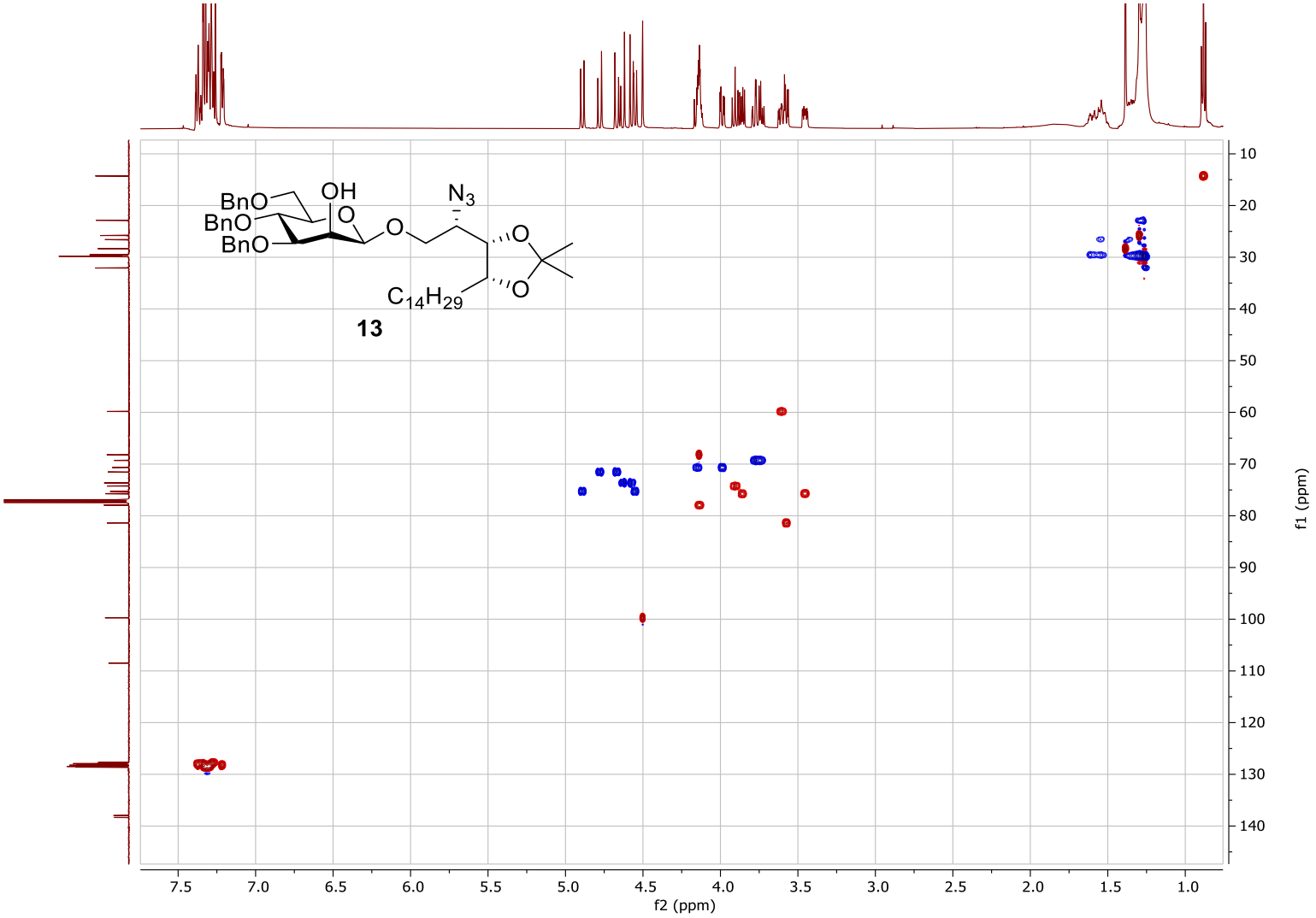
Compound **13** ^{13}C NMR spectrum (125 MHz, $CDCl_3$). δ (ppm)



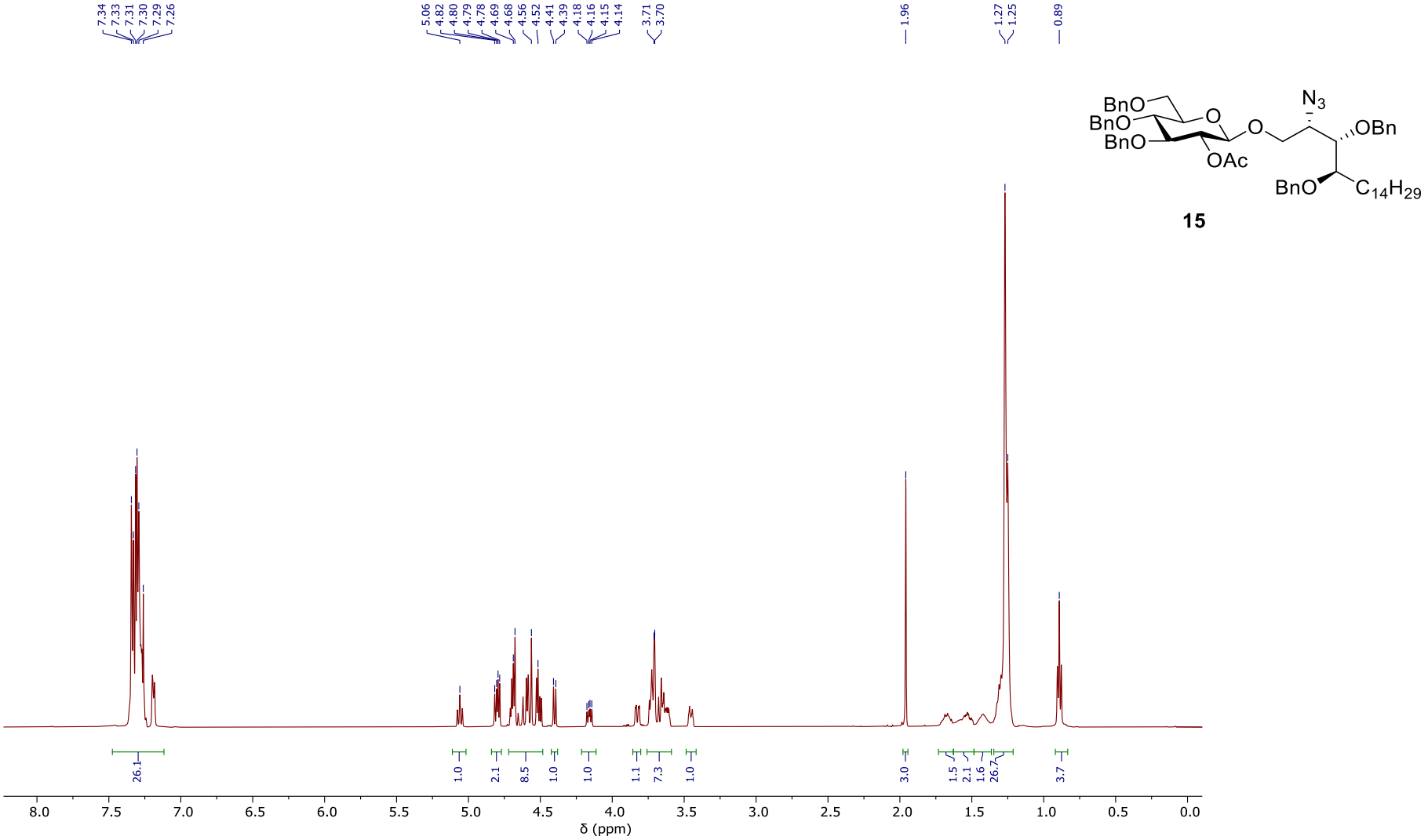


13

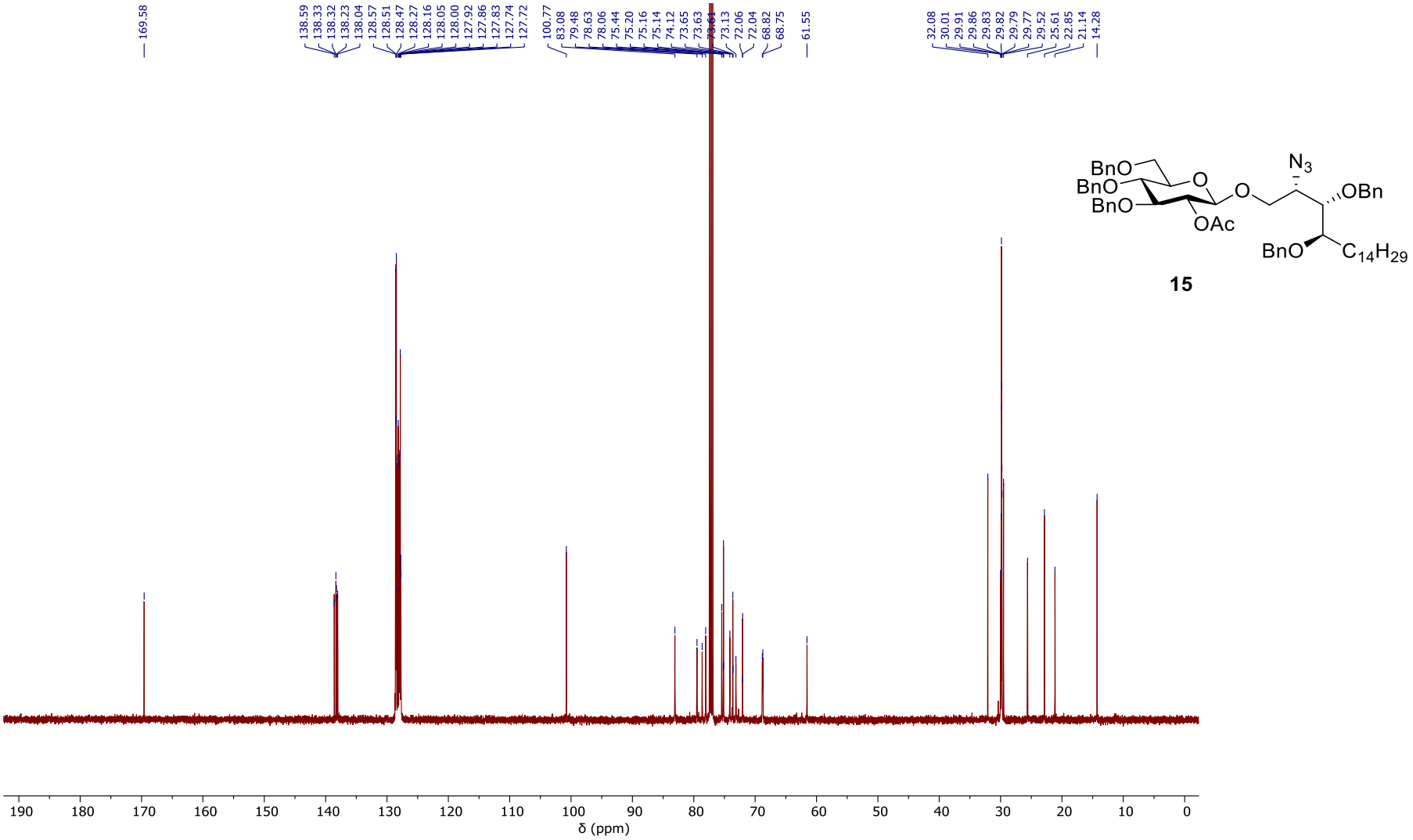
Compound **13**. HSQC spectrum.



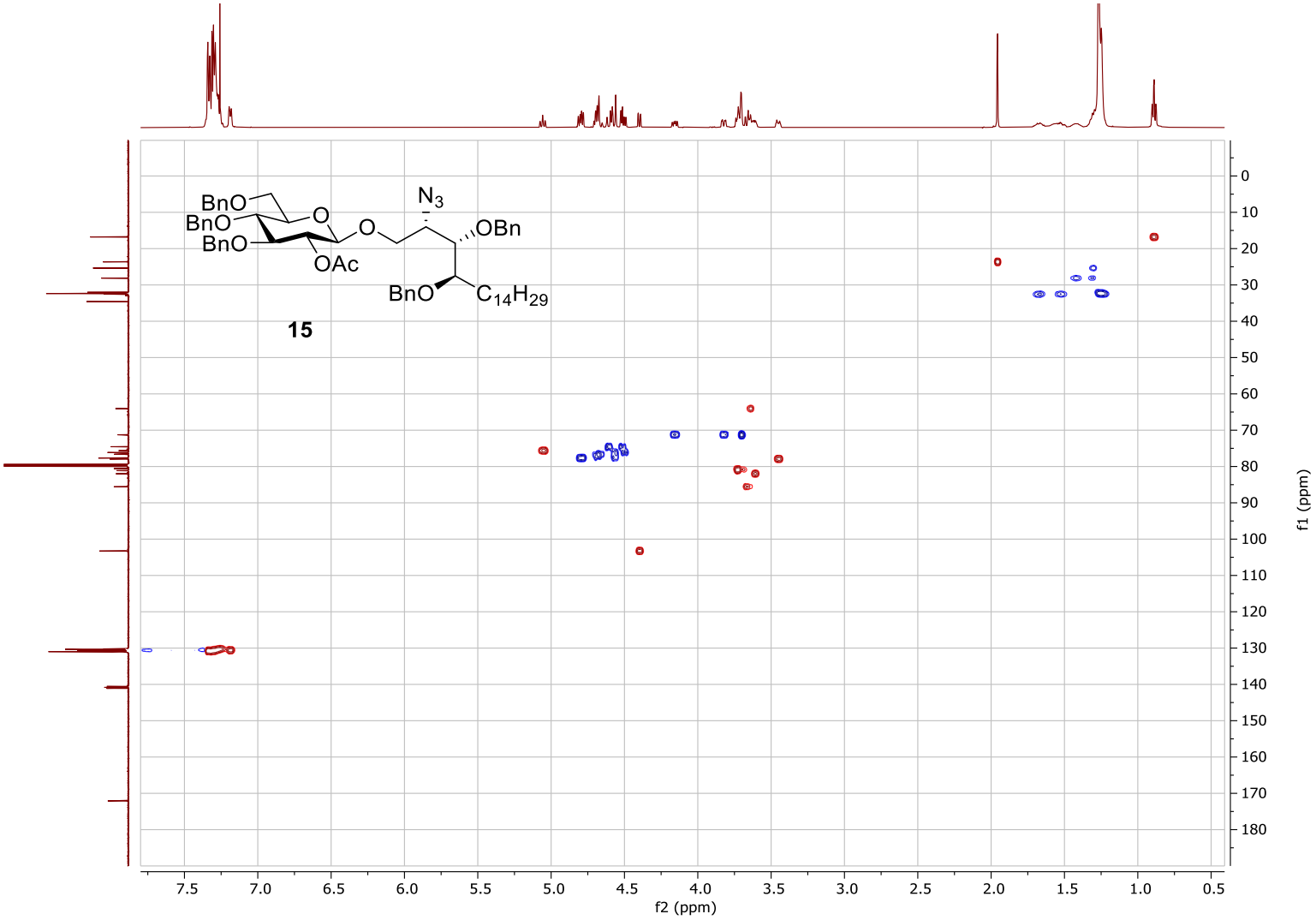
Compound **15** ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



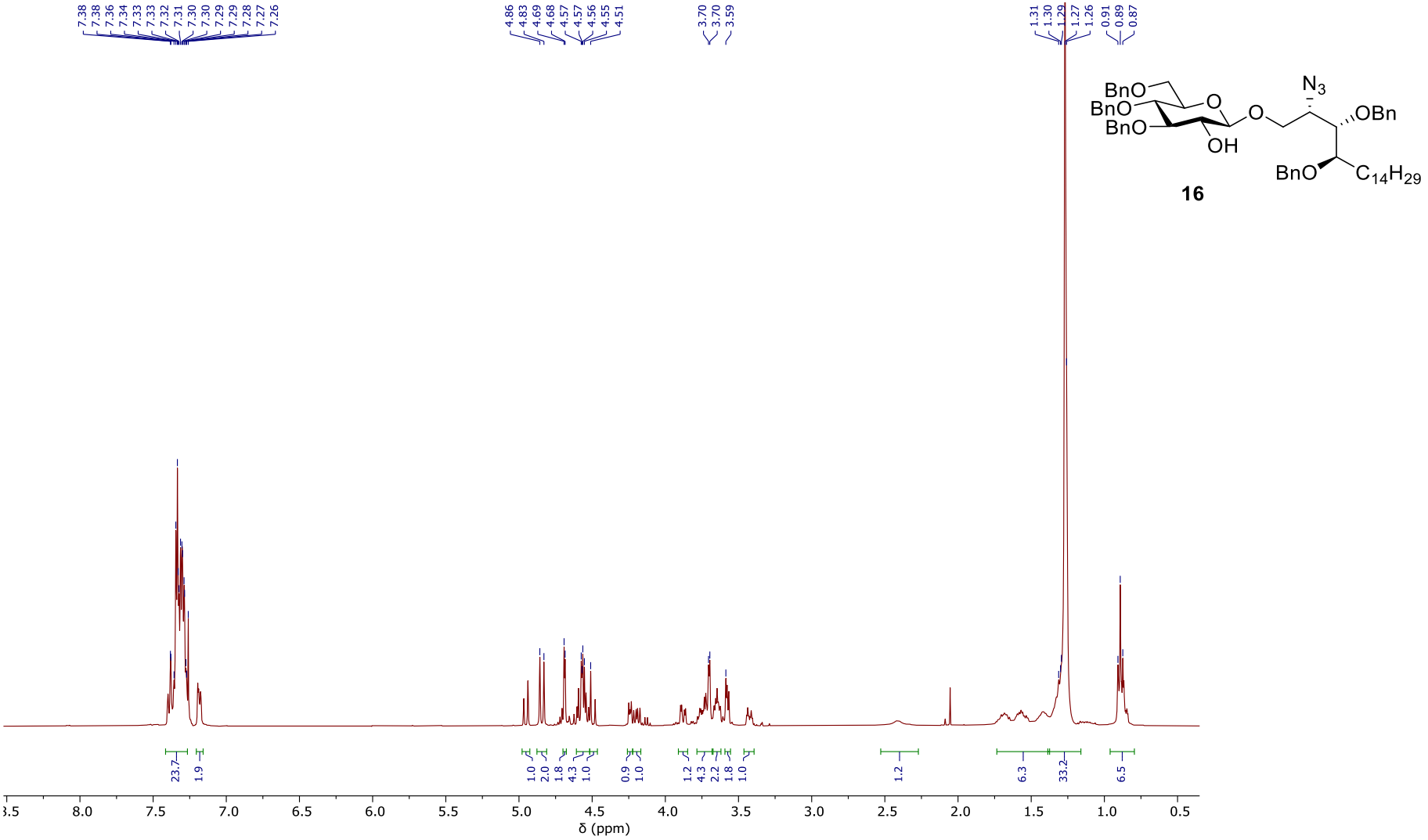
Compound **15**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



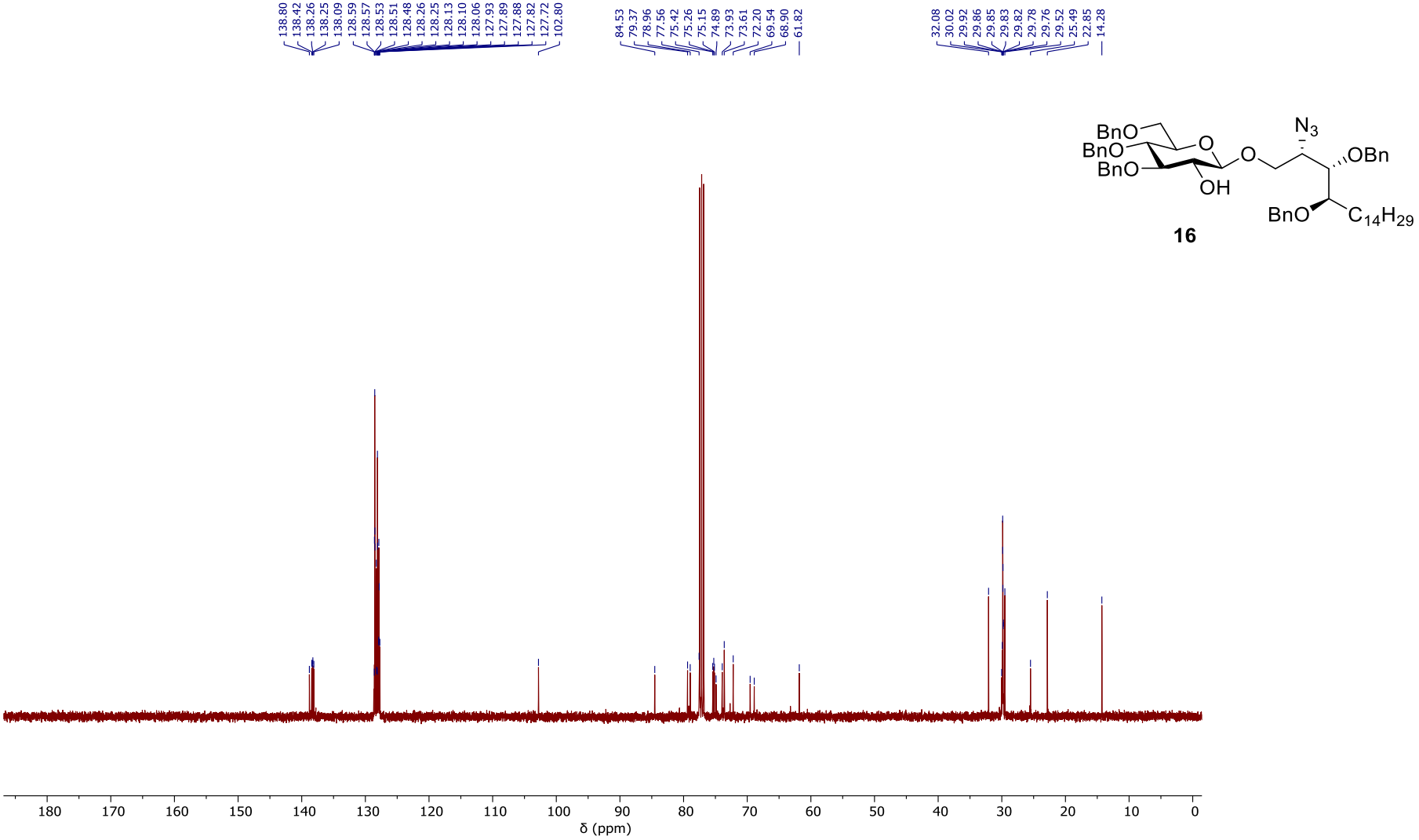
Compound **15**. HSQC spectrum.



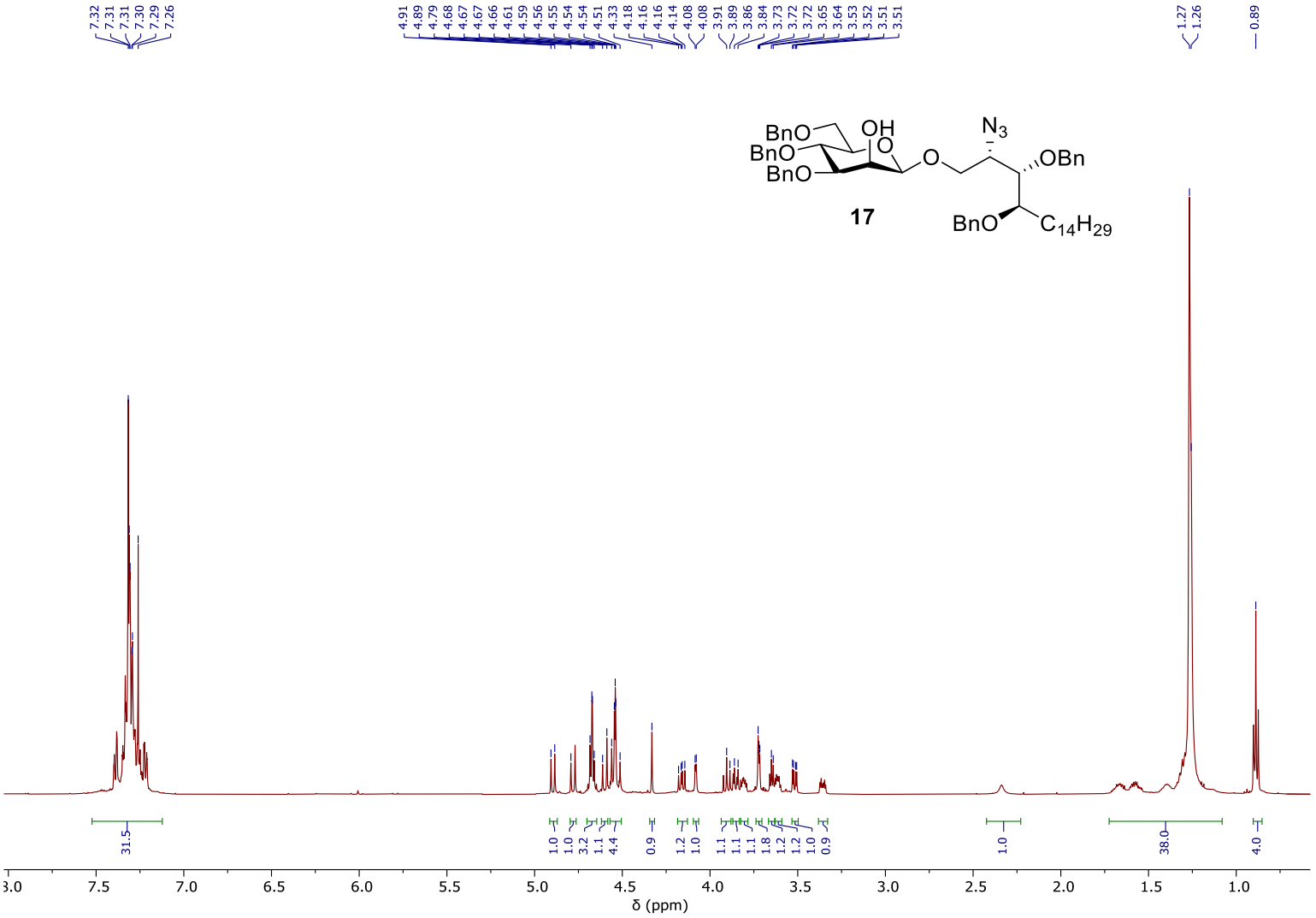
Compound **16**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



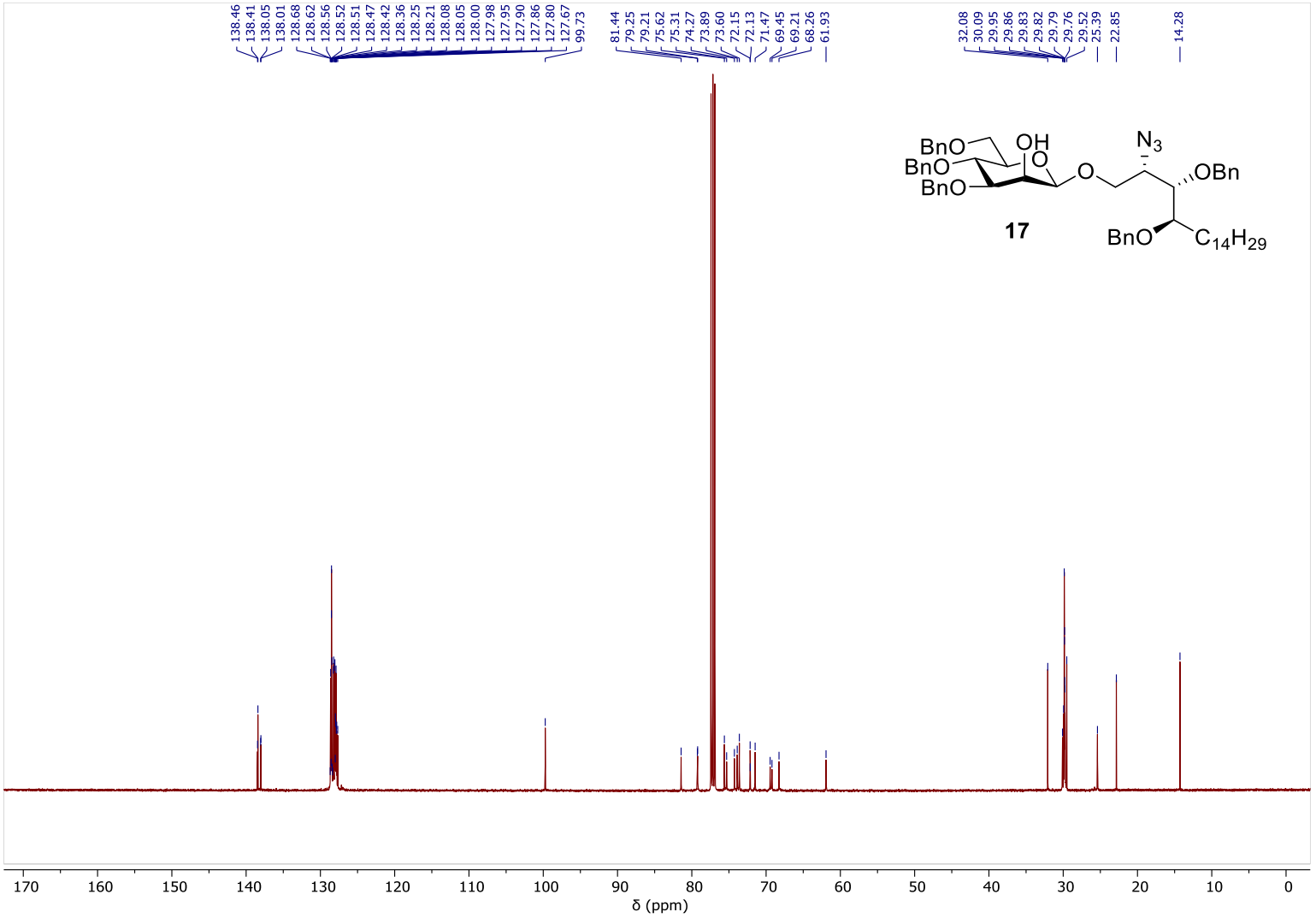
Compound **16**. ^{13}C NMR spectrum (500 MHz, $CDCl_3$).



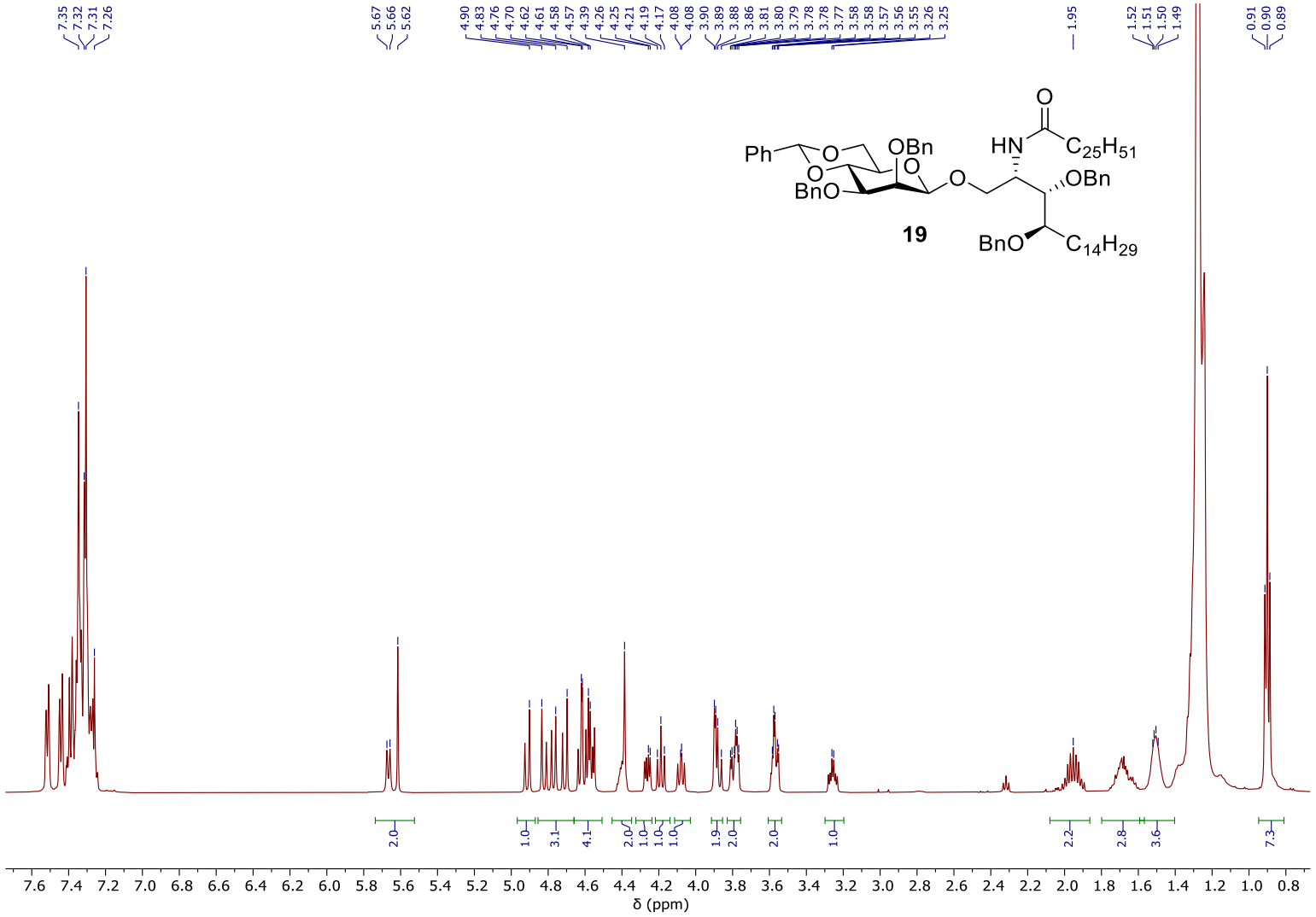
Compound **17**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



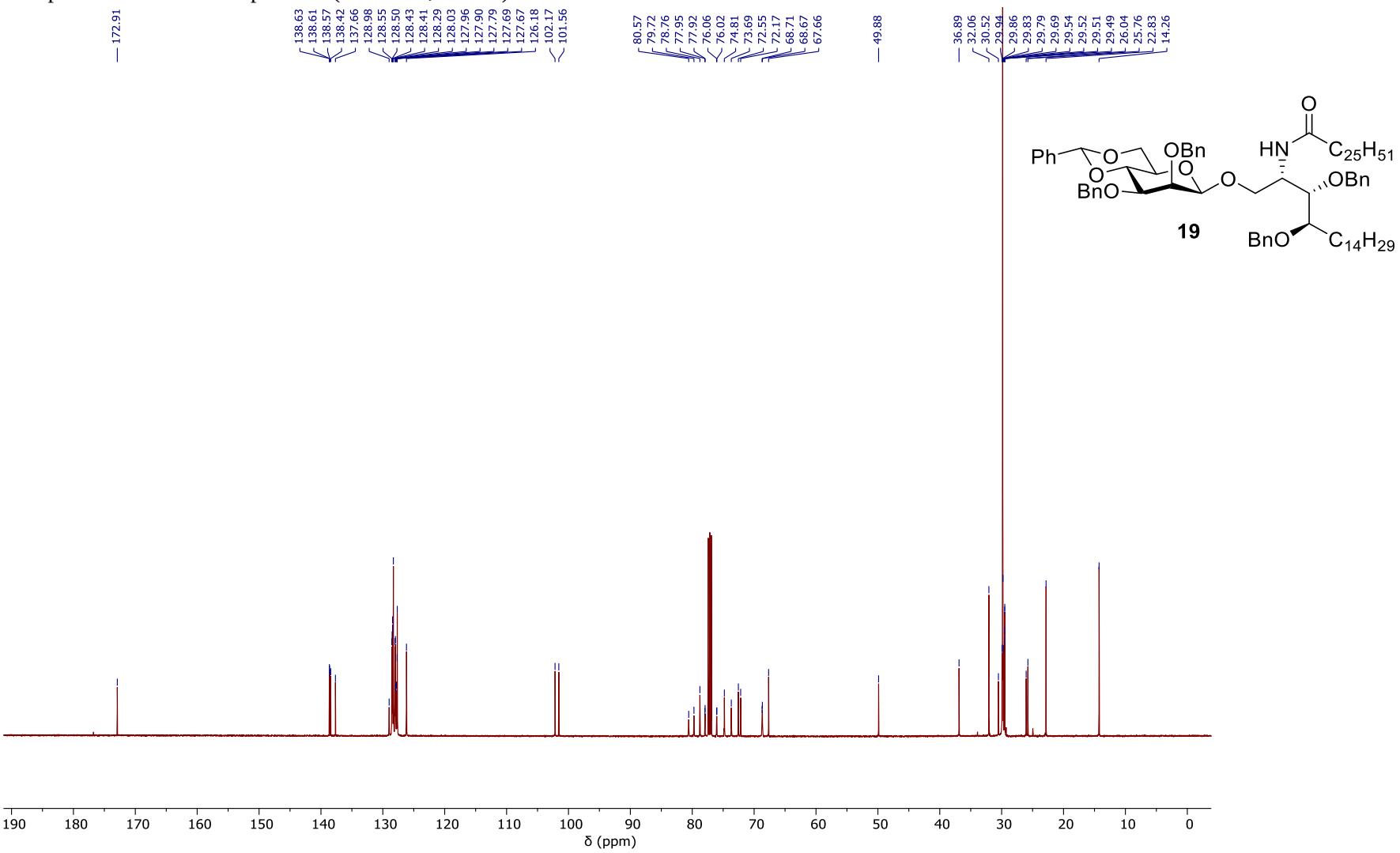
Compound **17**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



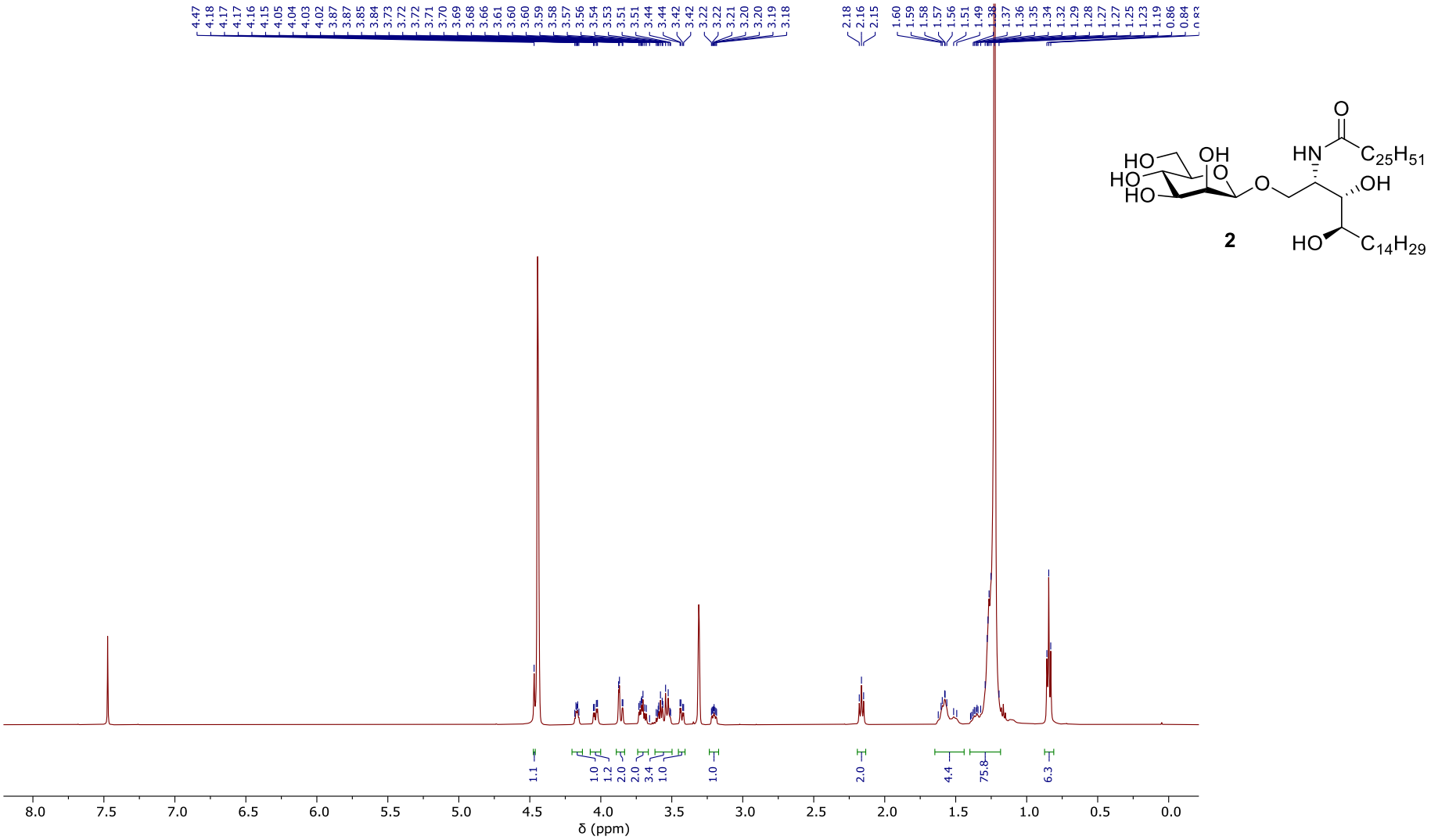
Compound **19**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



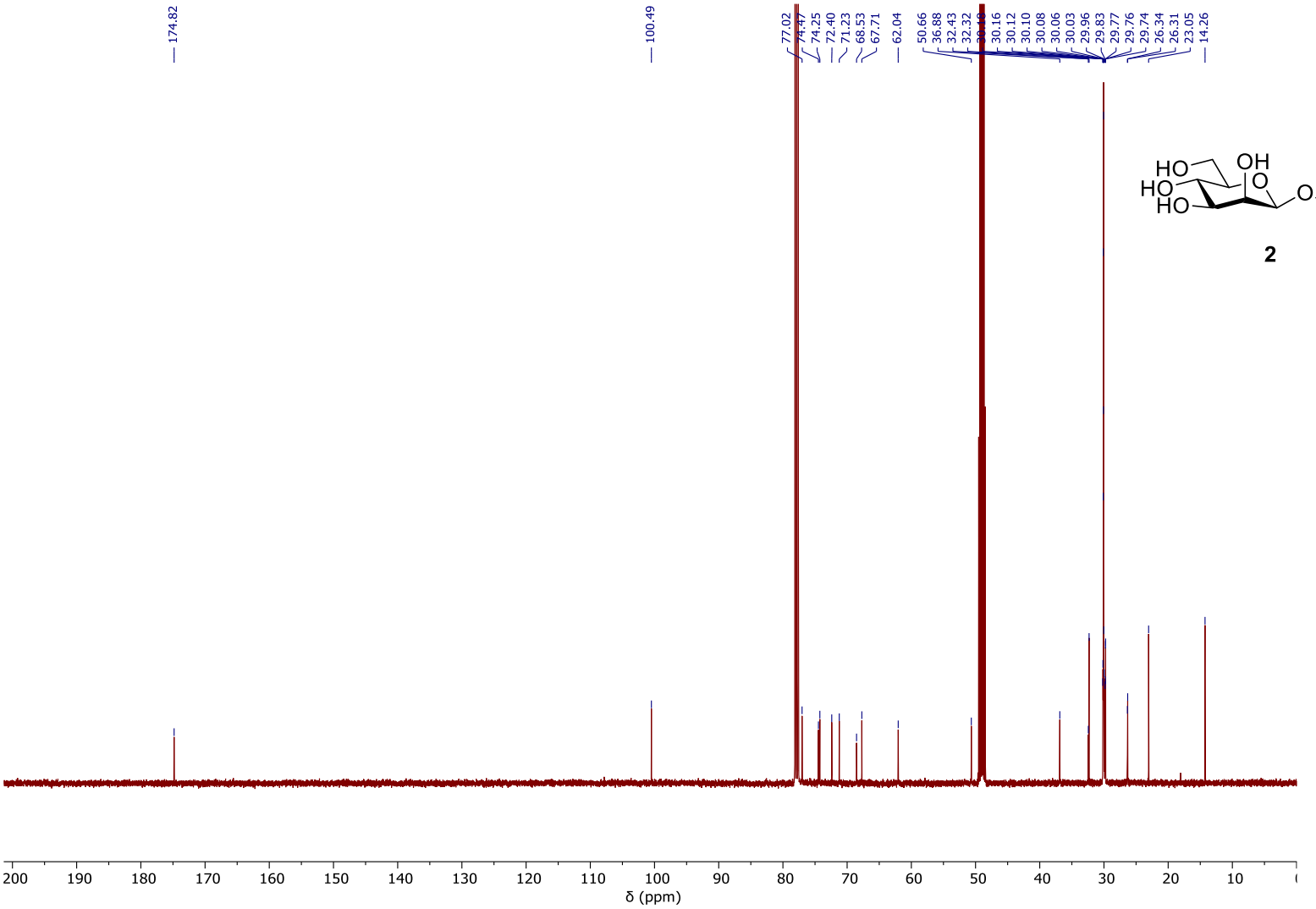
Compound **19**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



Compound **2**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **2**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **2**. HSQC spectrum.

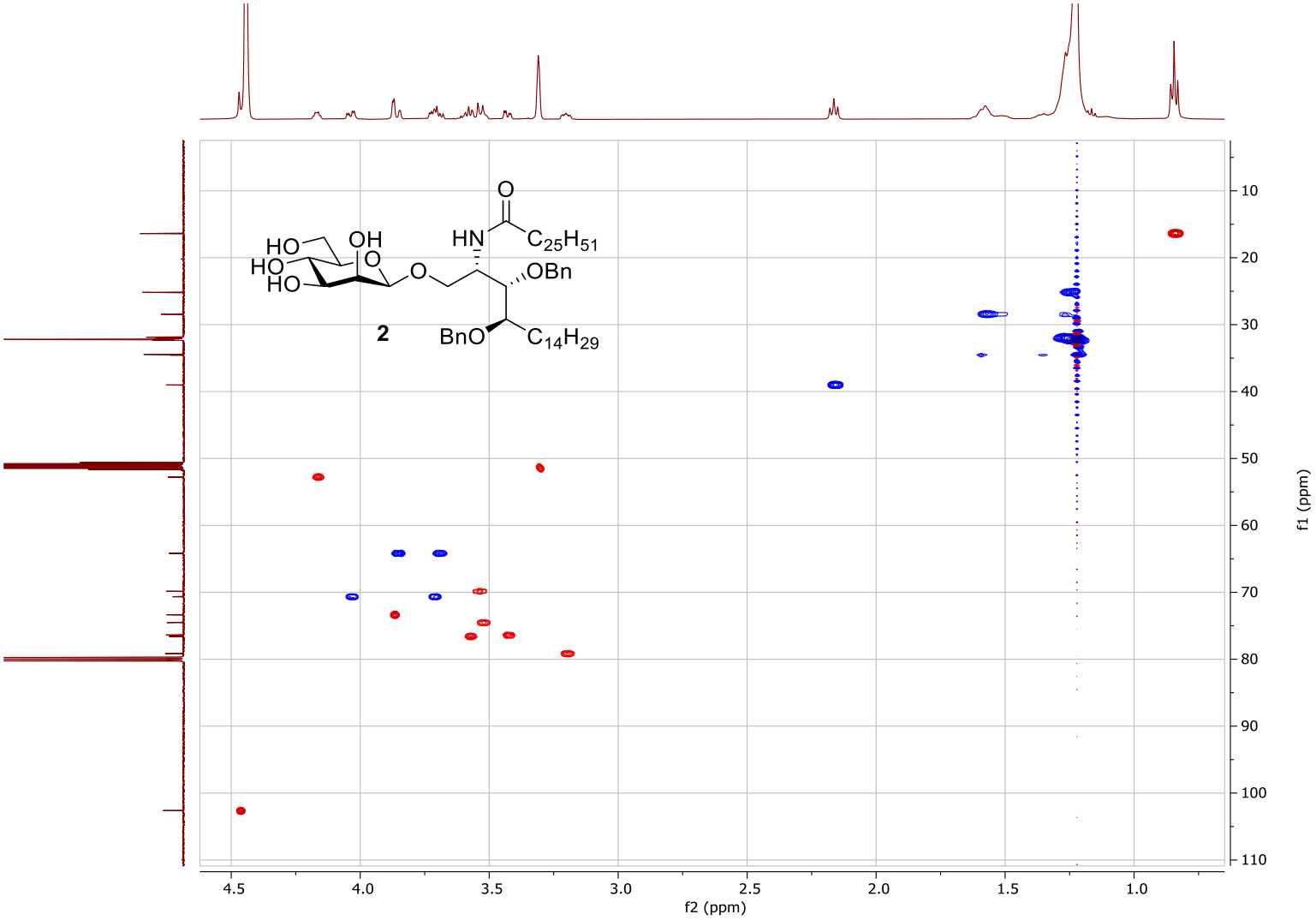
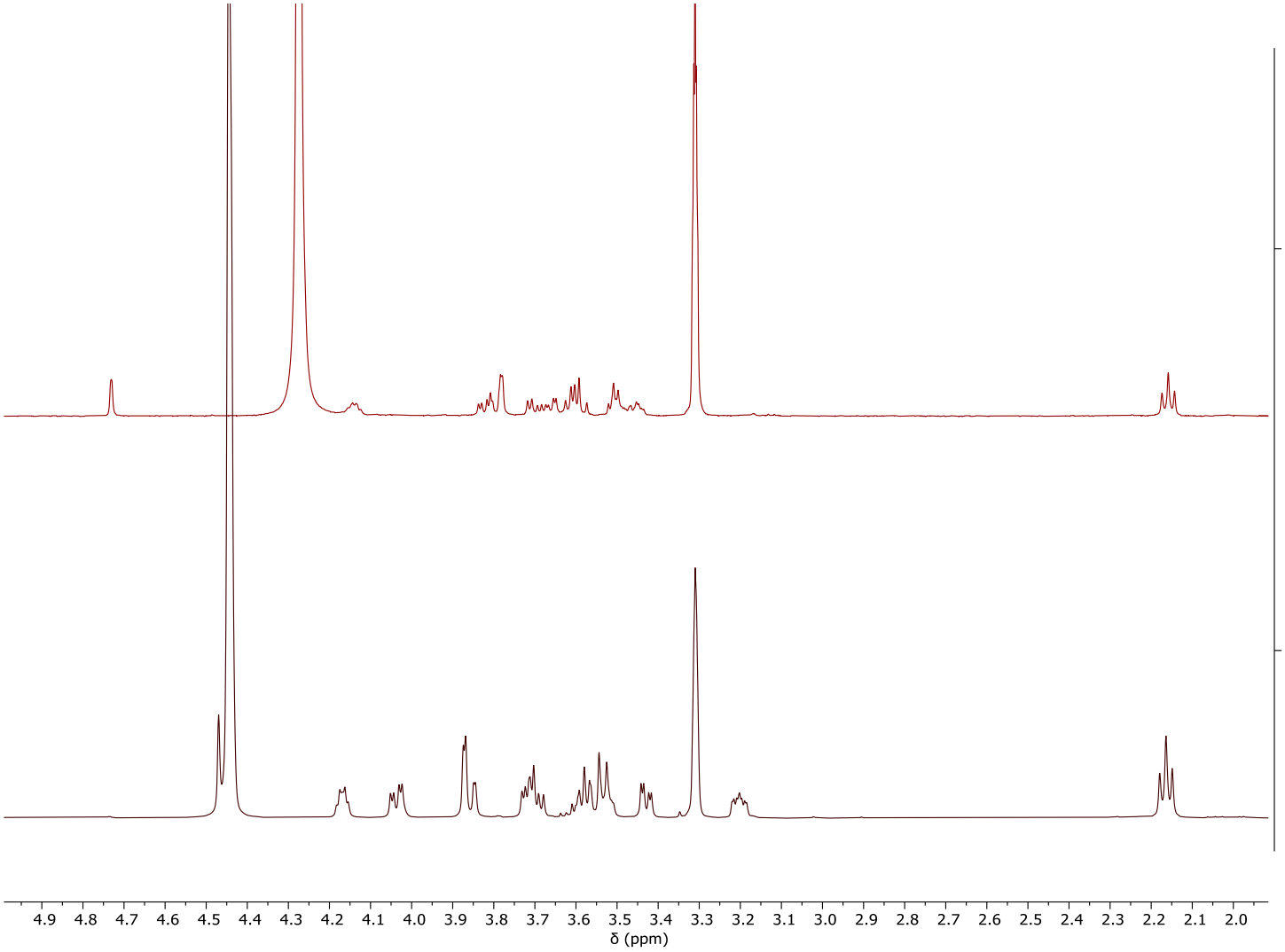
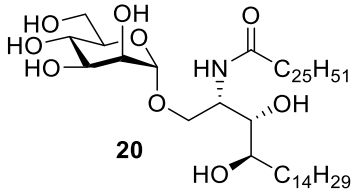
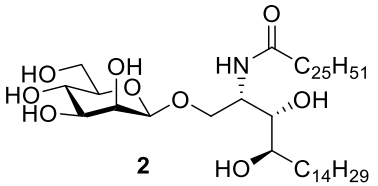


Figure SI-### Stacked ^{1}H NMR partial spectra of α-ManCer **20**(top) and β-ManCer **2** (bottom)



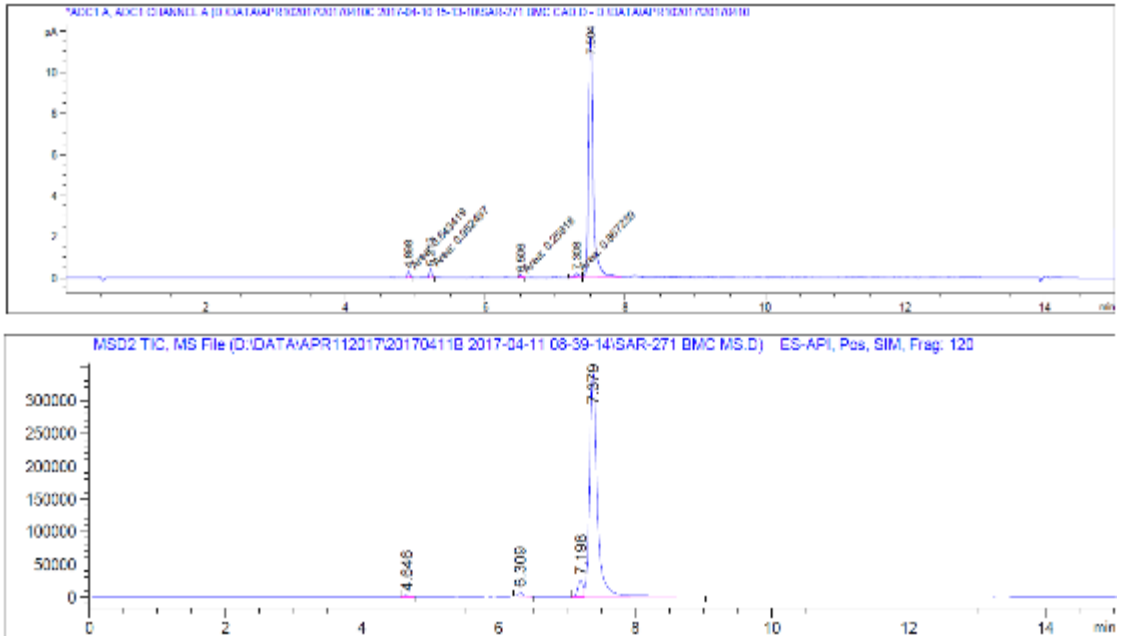


20



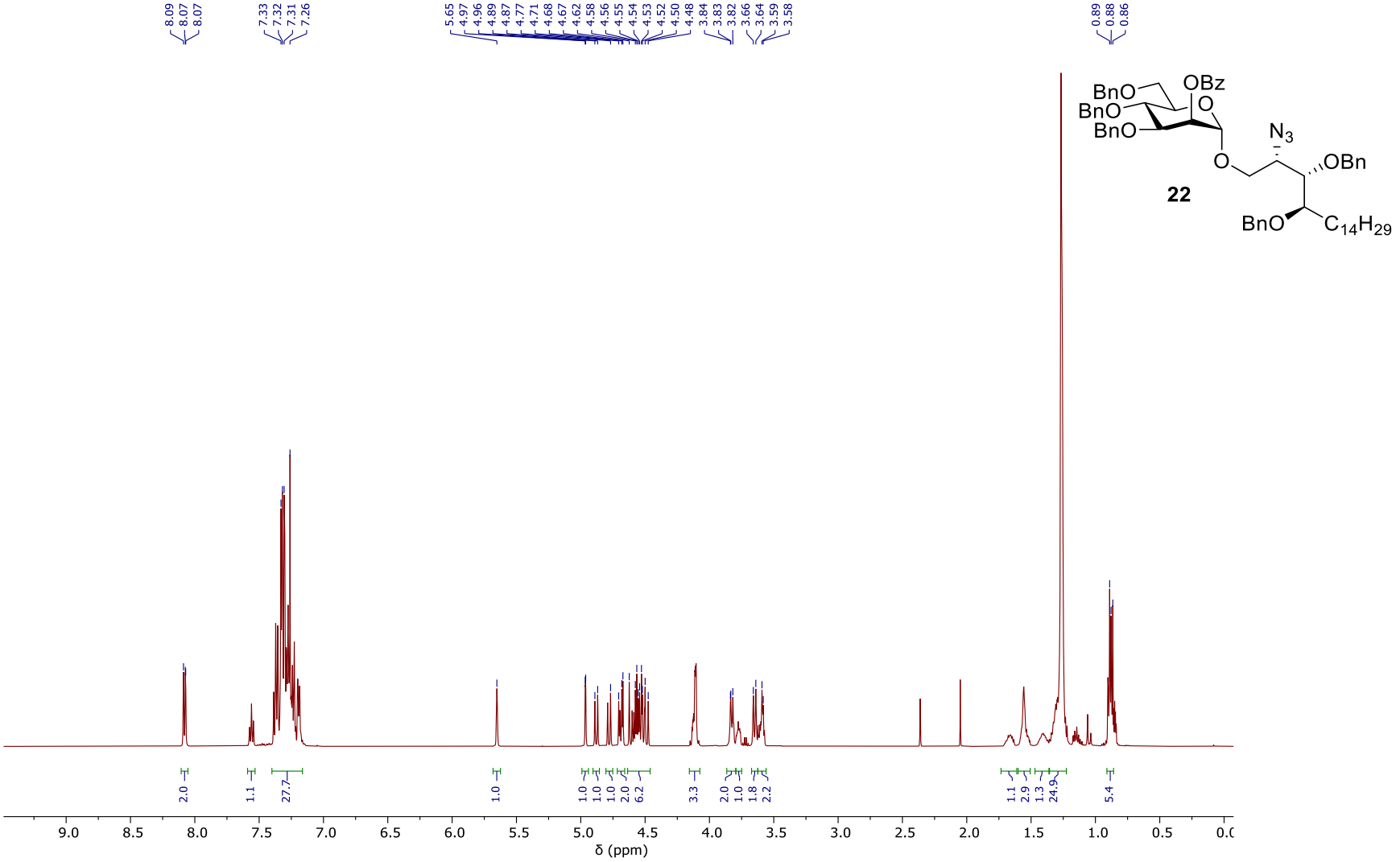
2

HPLC chromatogram of **2**

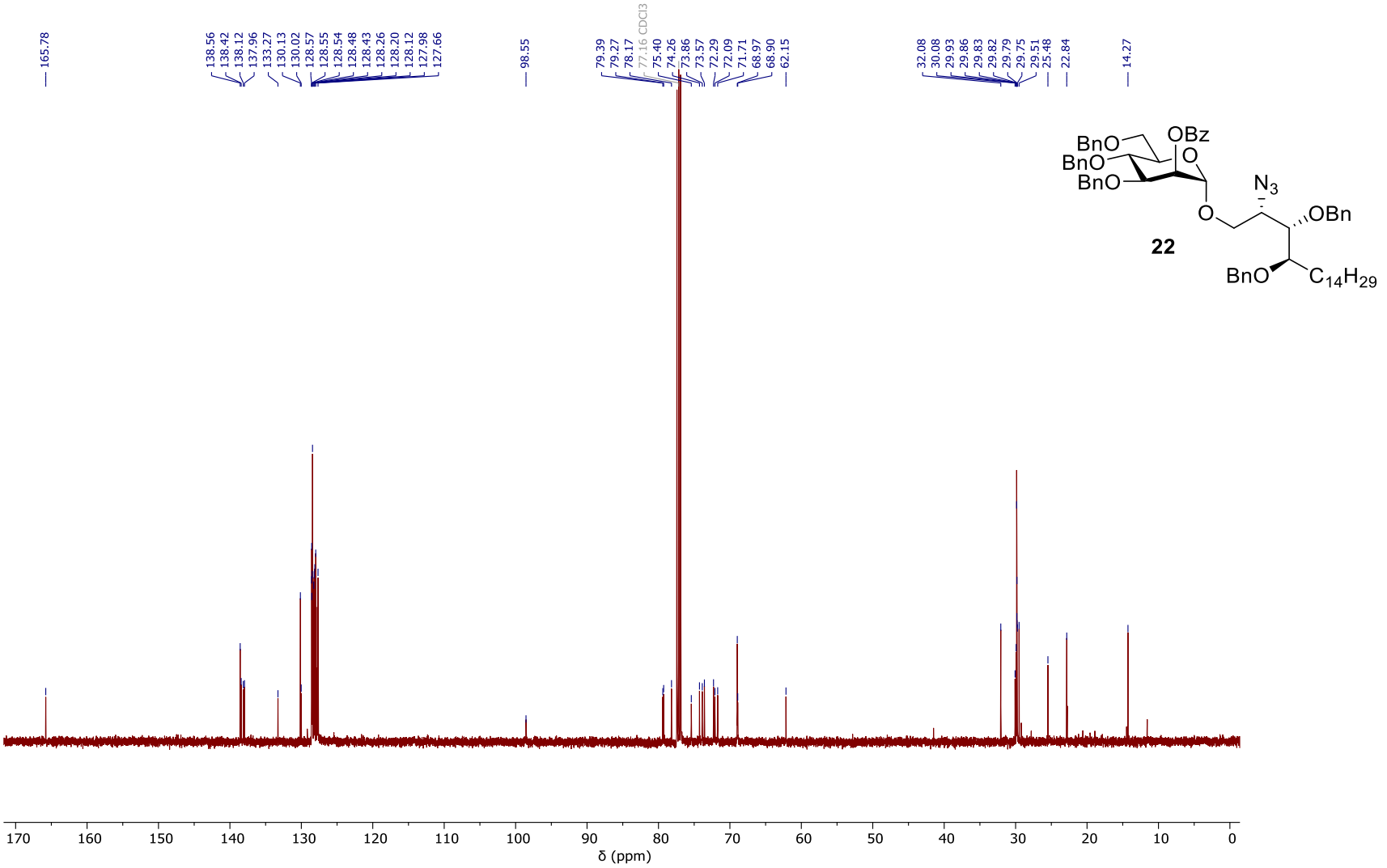


Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), t_R =7.504 min (95.1%).

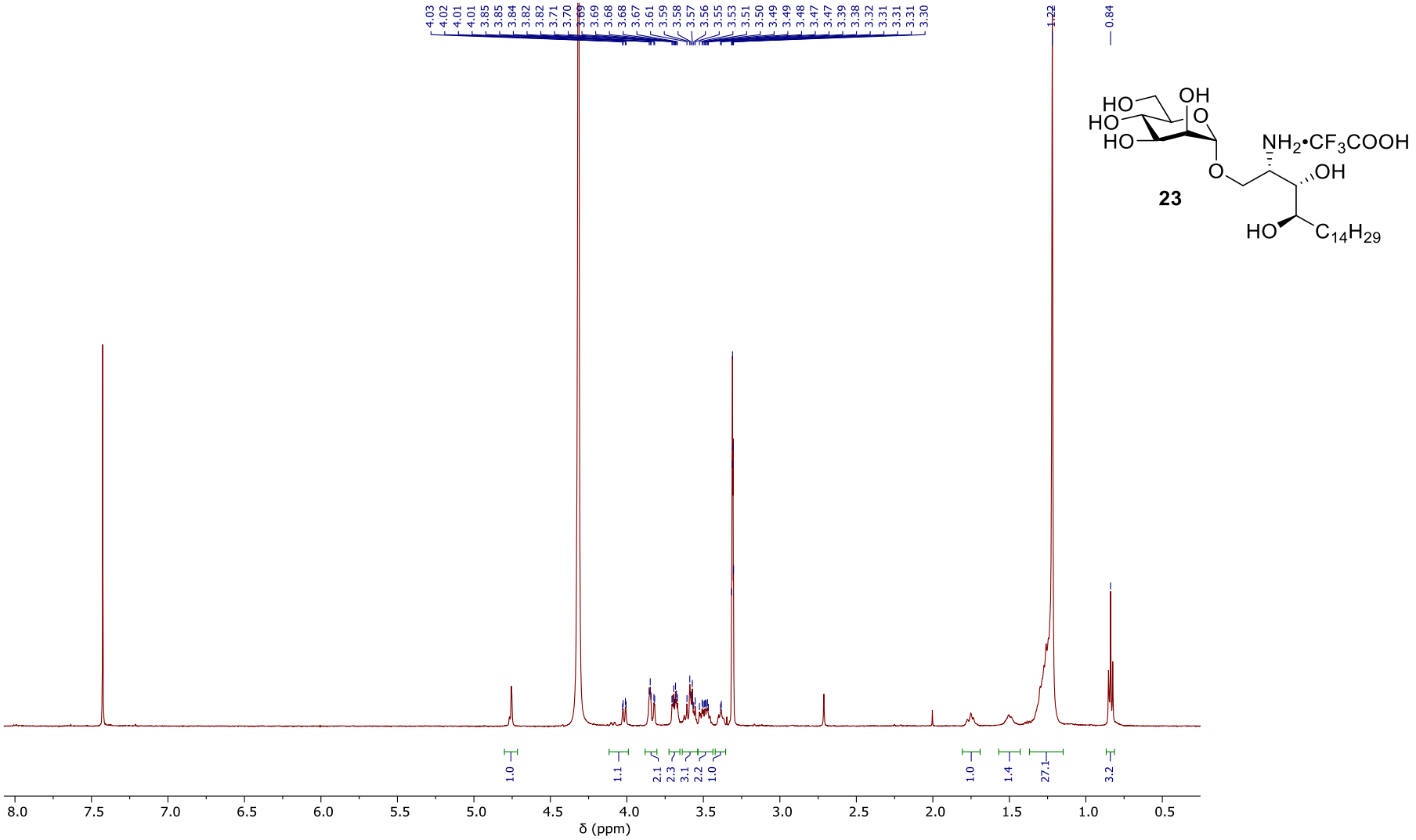
Compound **22**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



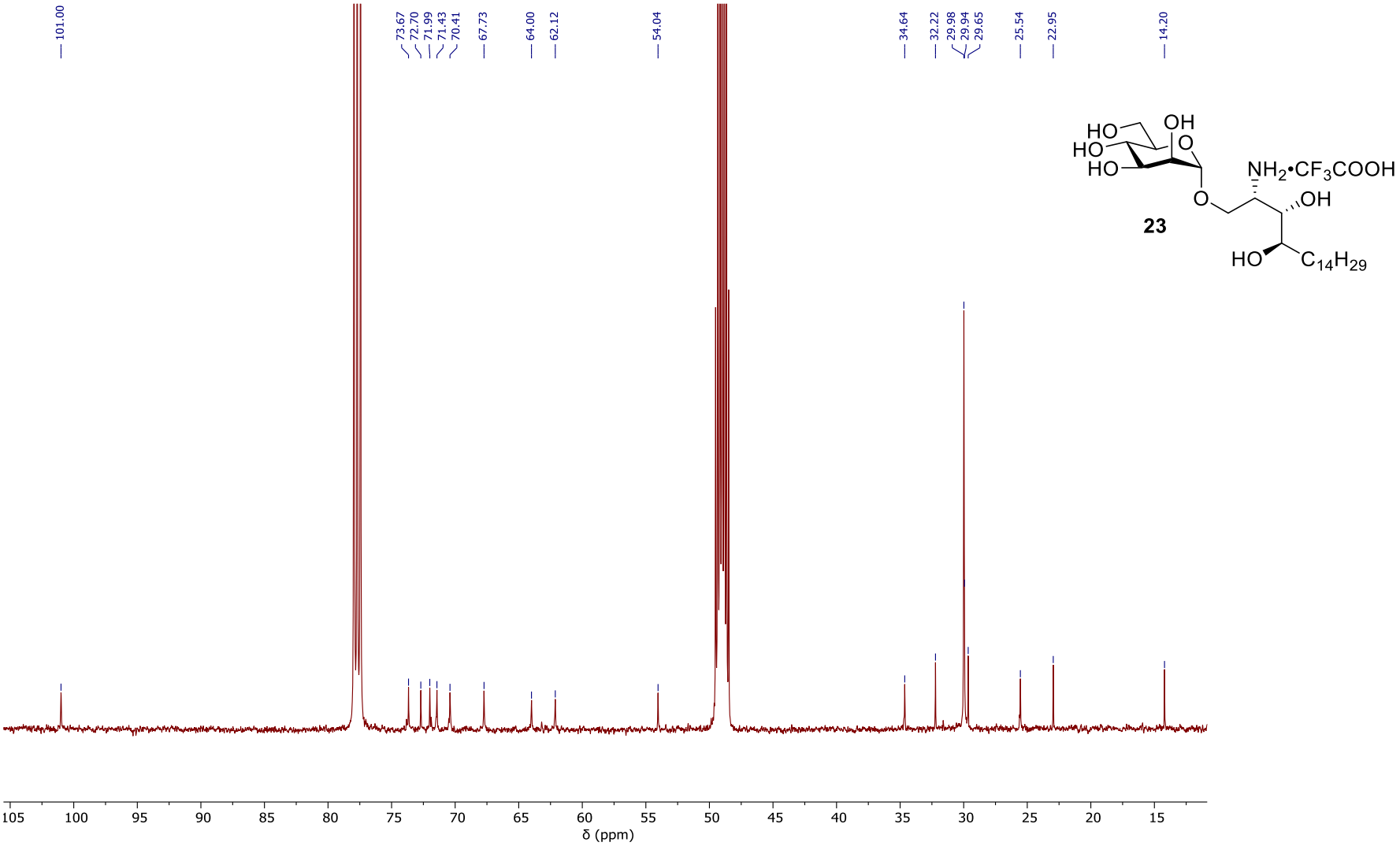
Compound **22**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



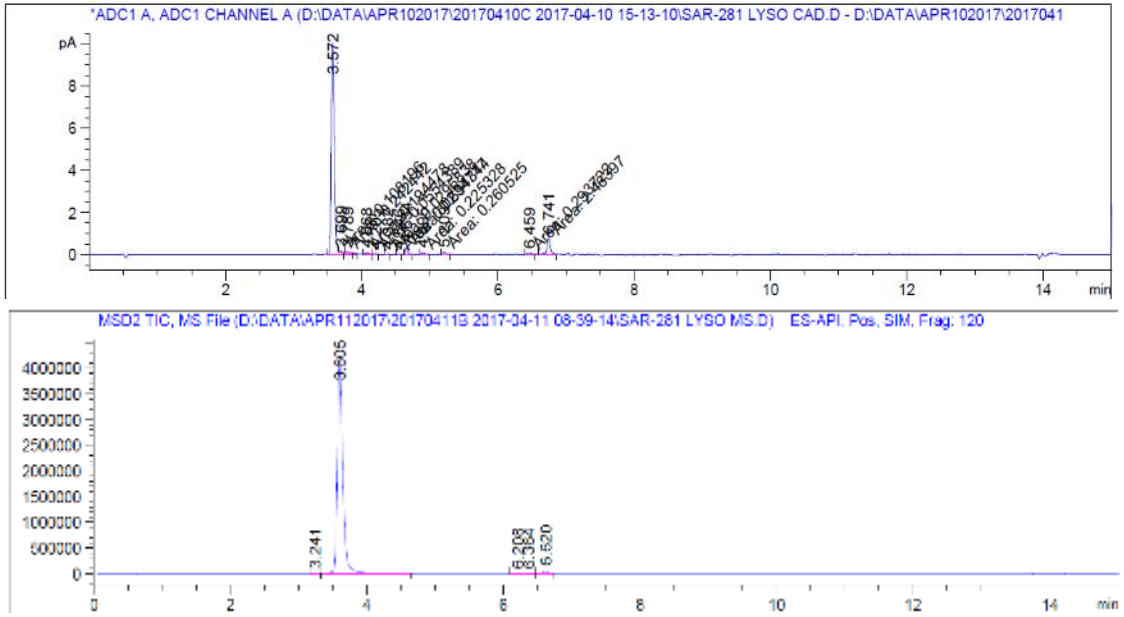
Compound **23**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **23**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).

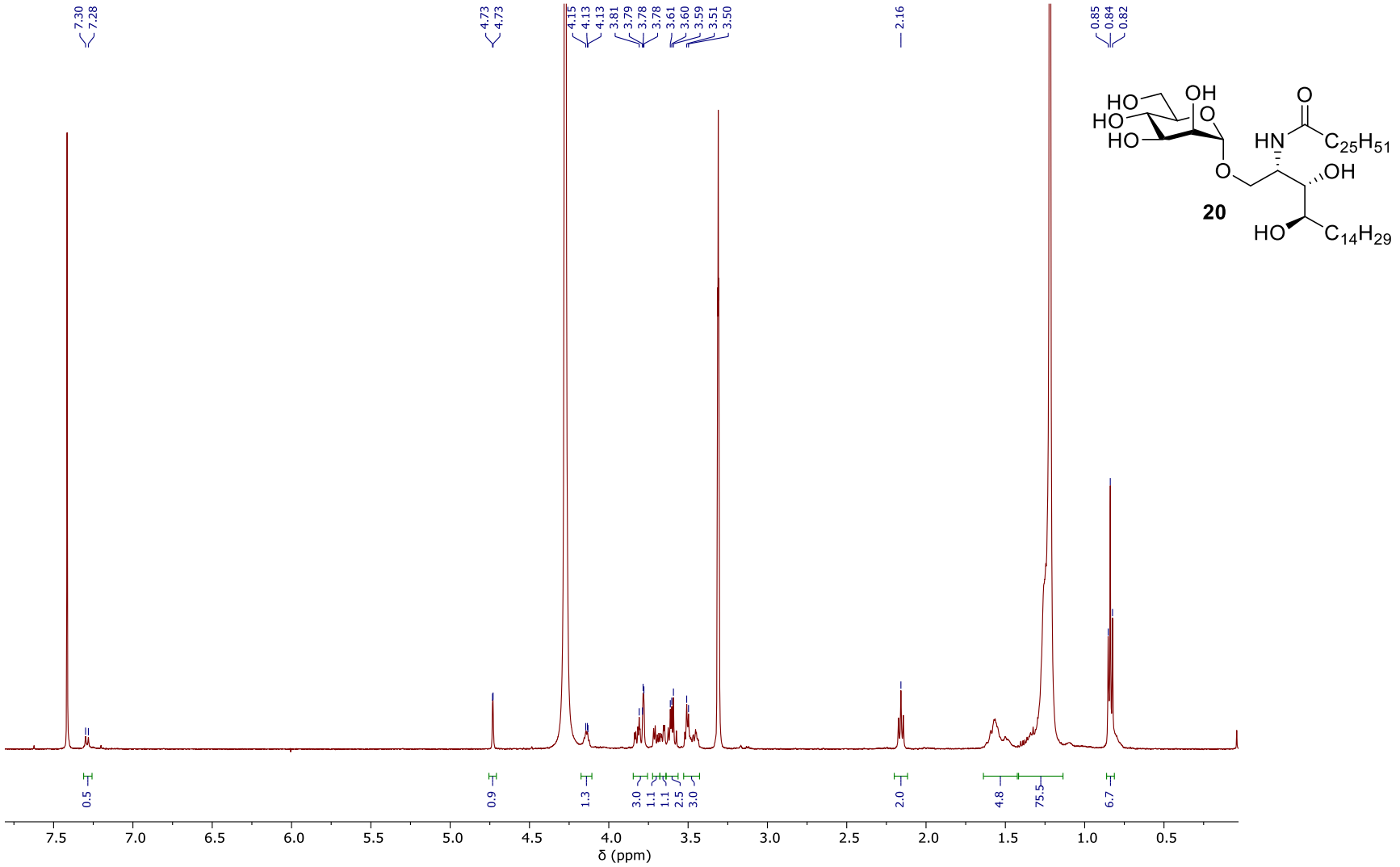


HPLC chromatogram of **23**

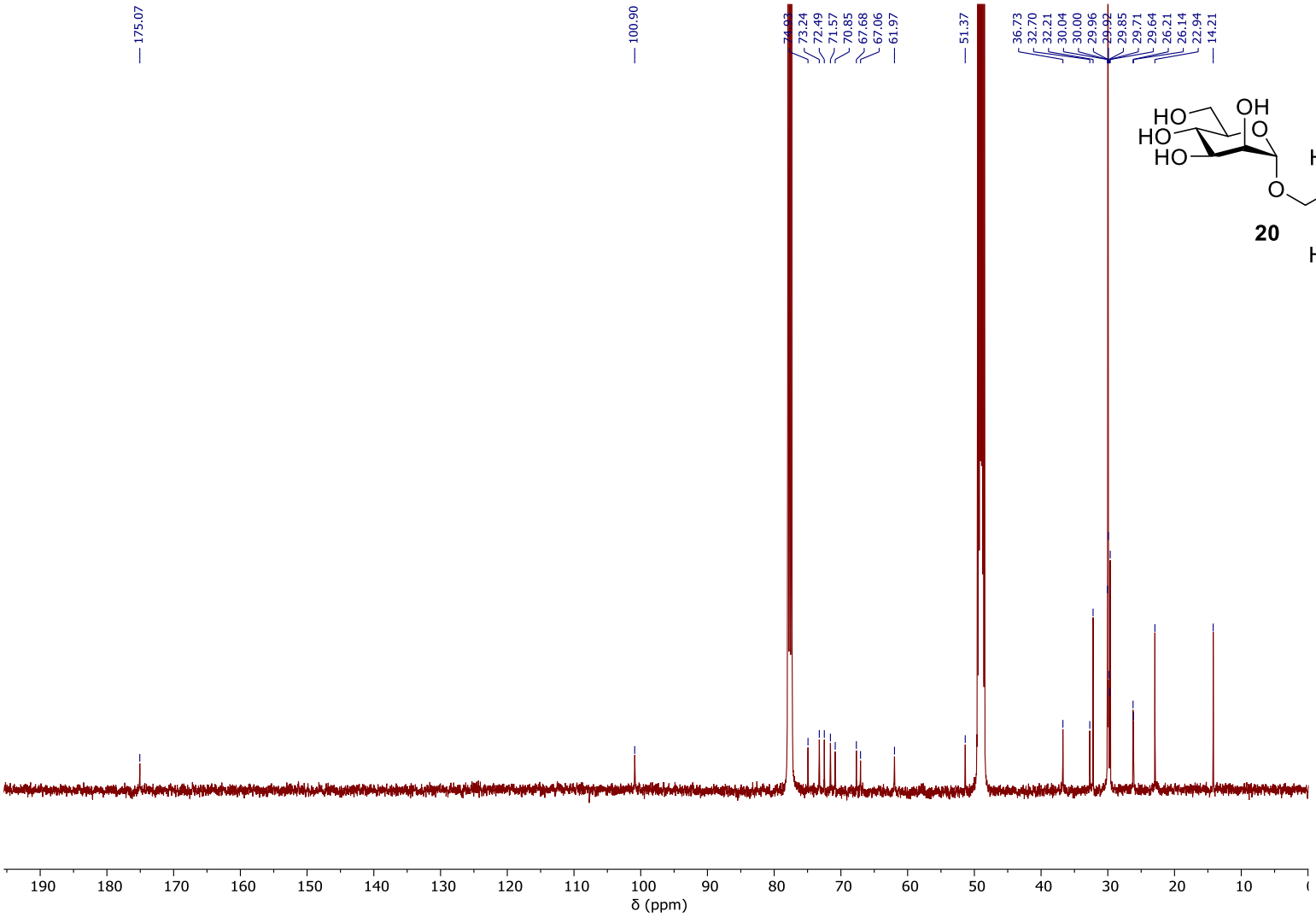


Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), t_R =3.57 min (91.3%).

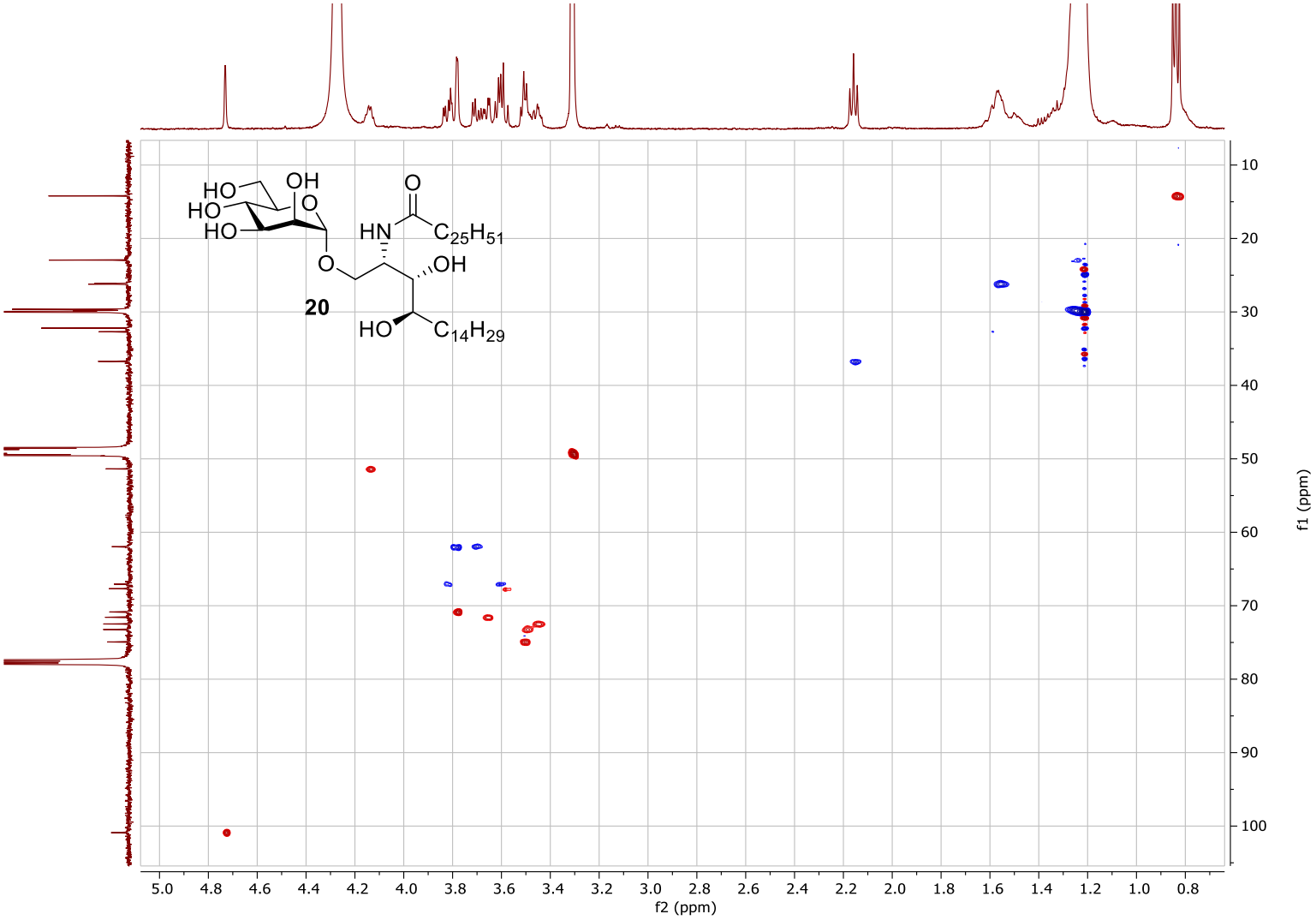
Compound **20**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).



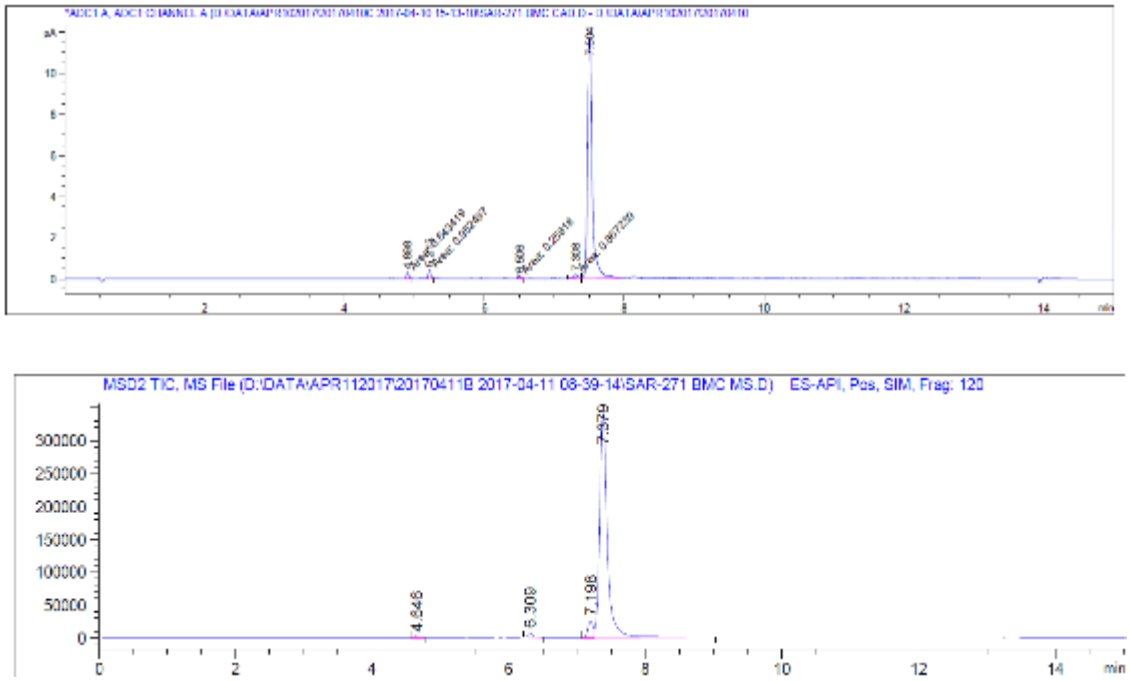
Compound **20**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **20**. HSQC spectrum

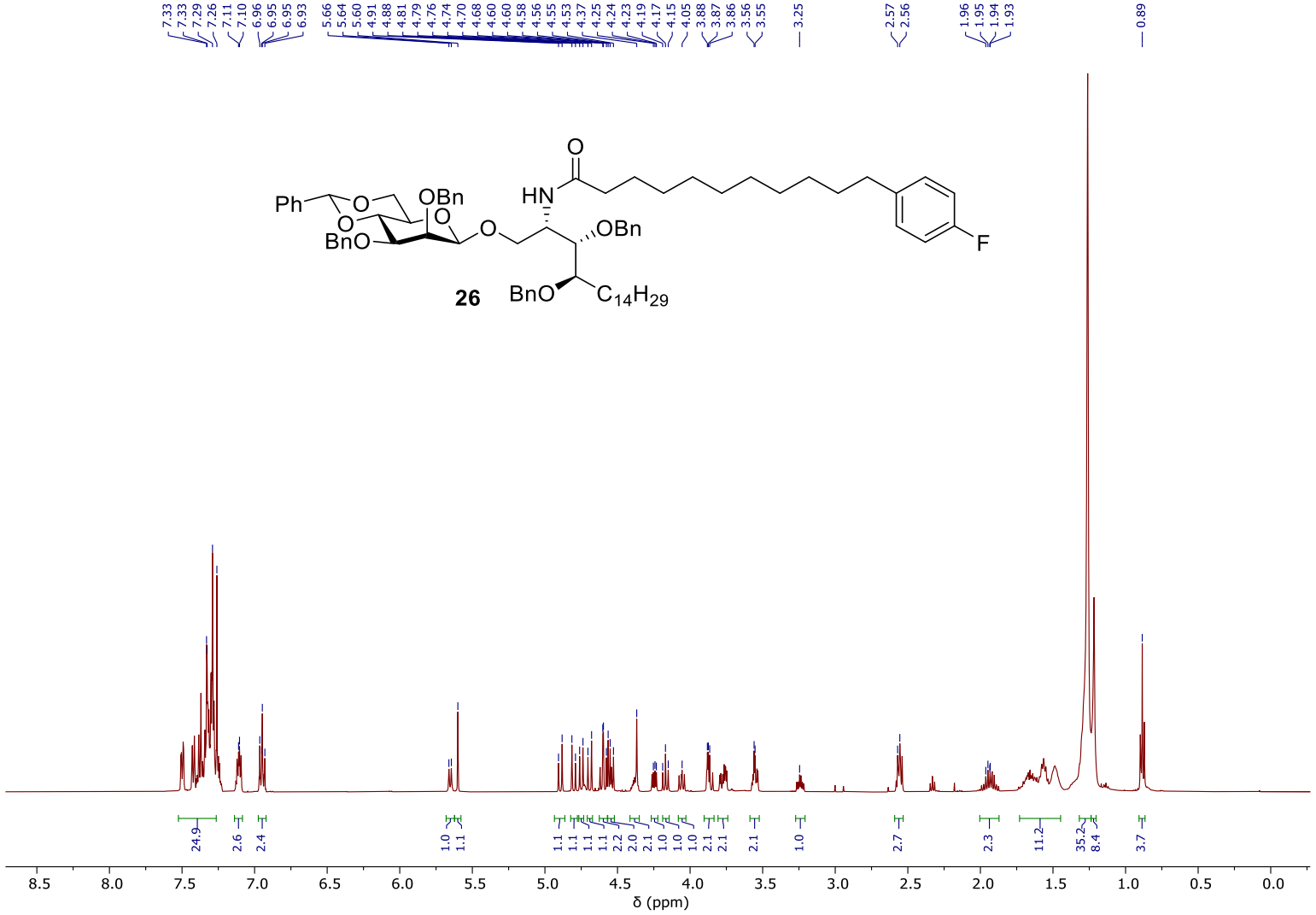


HPLC chromatogram of **20**

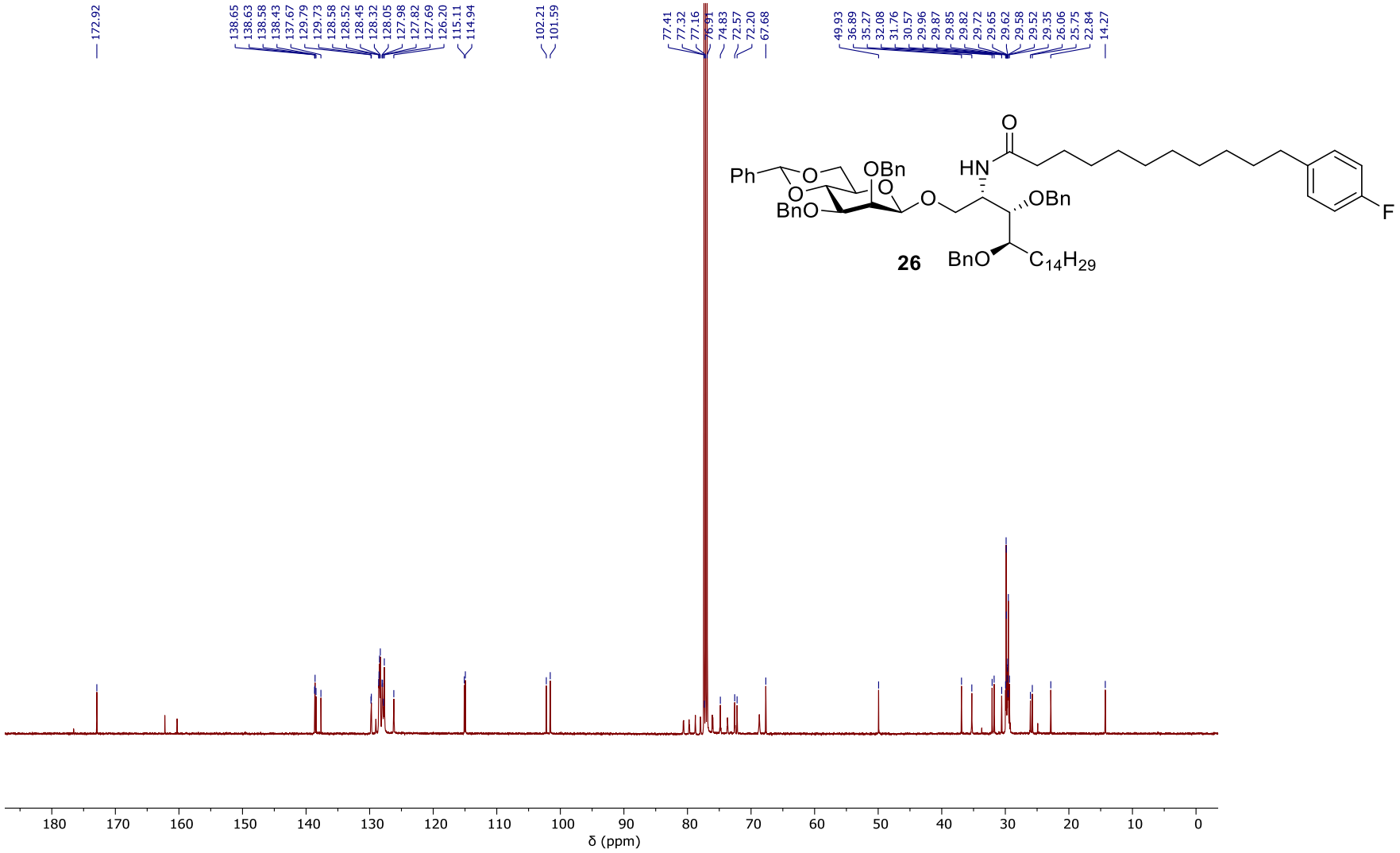


Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), t_R =7.379 min (96.6%).

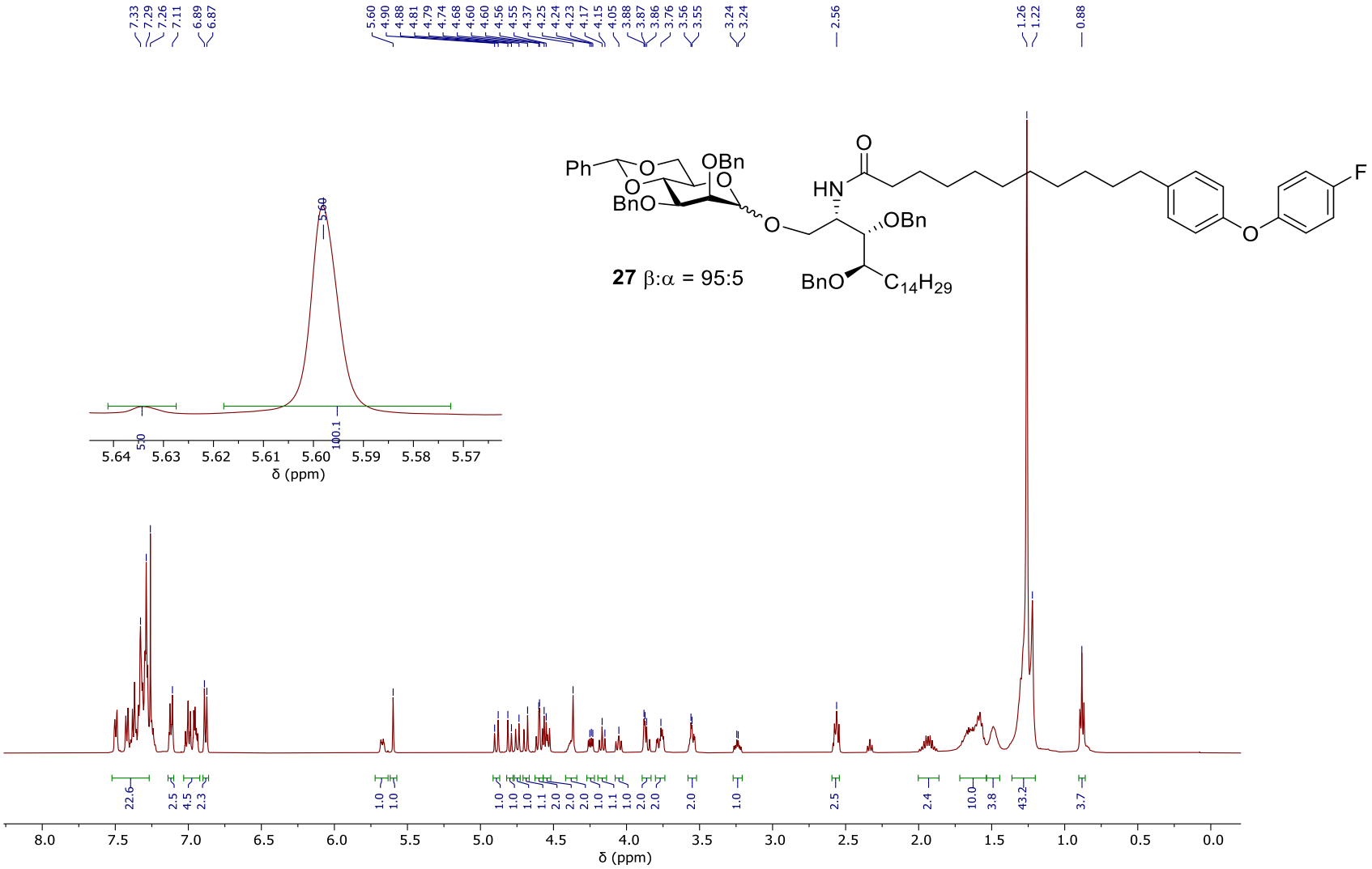
Compound **26**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



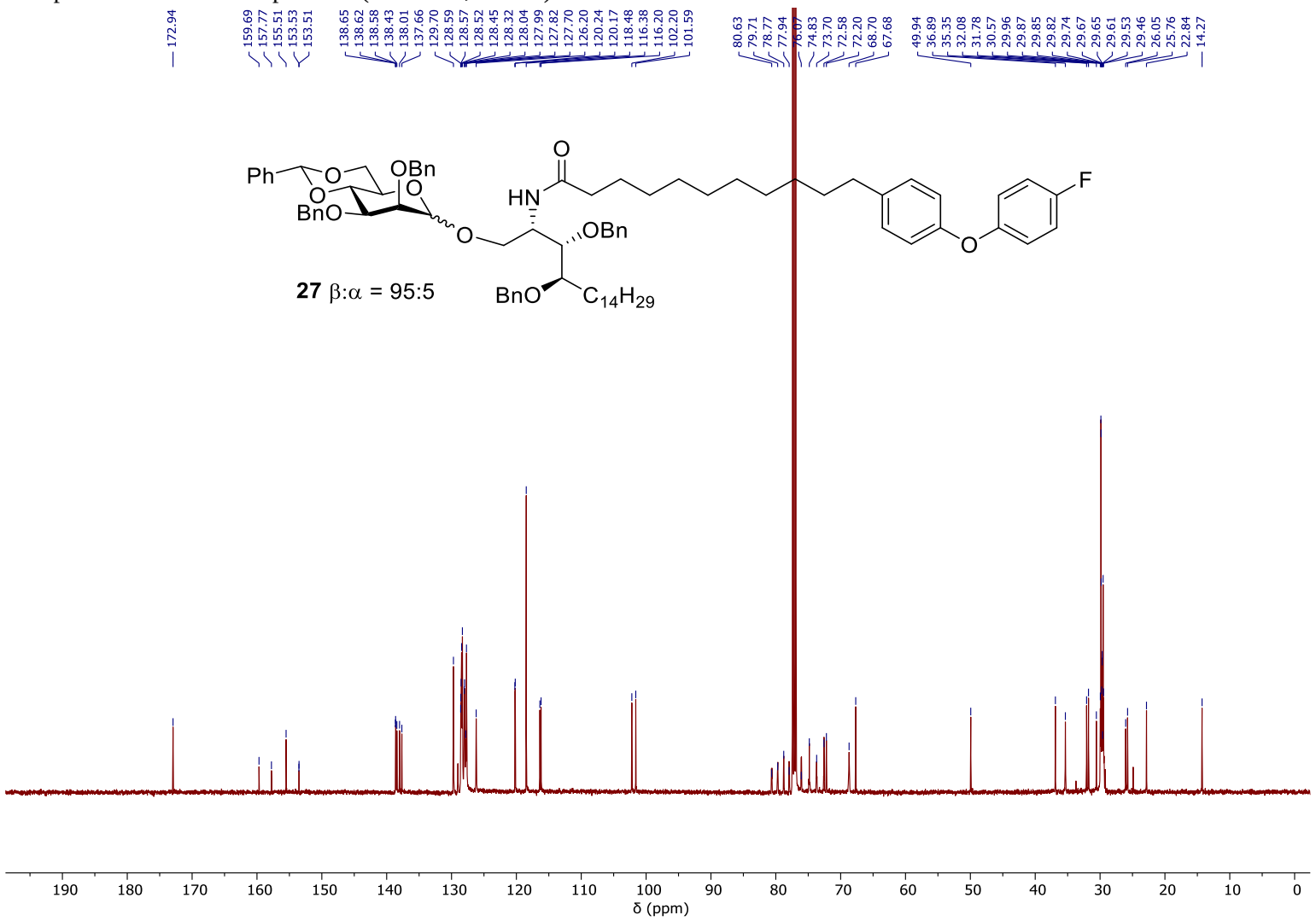
Compound **26**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



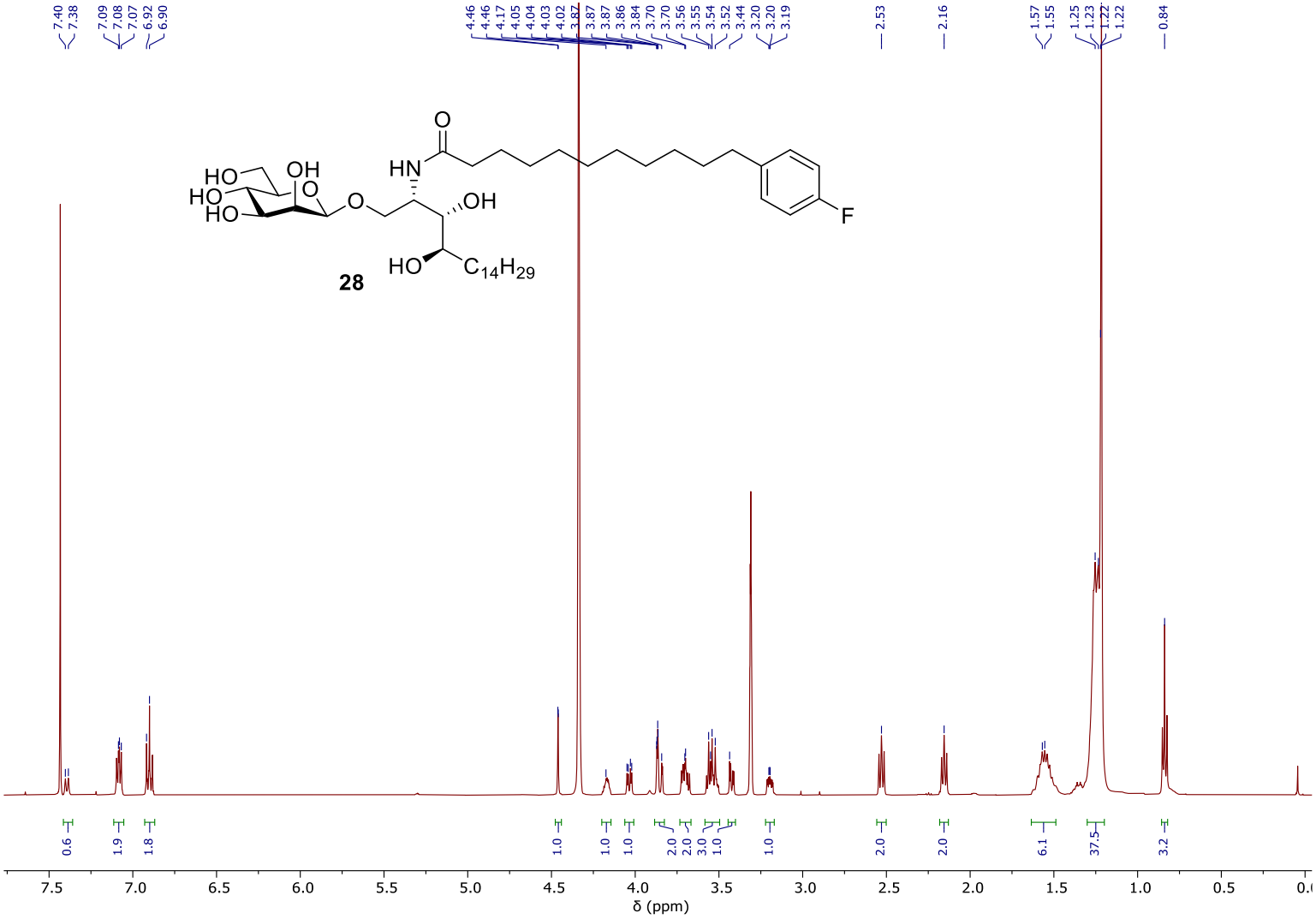
Compound **27**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$). β:α = 95:5



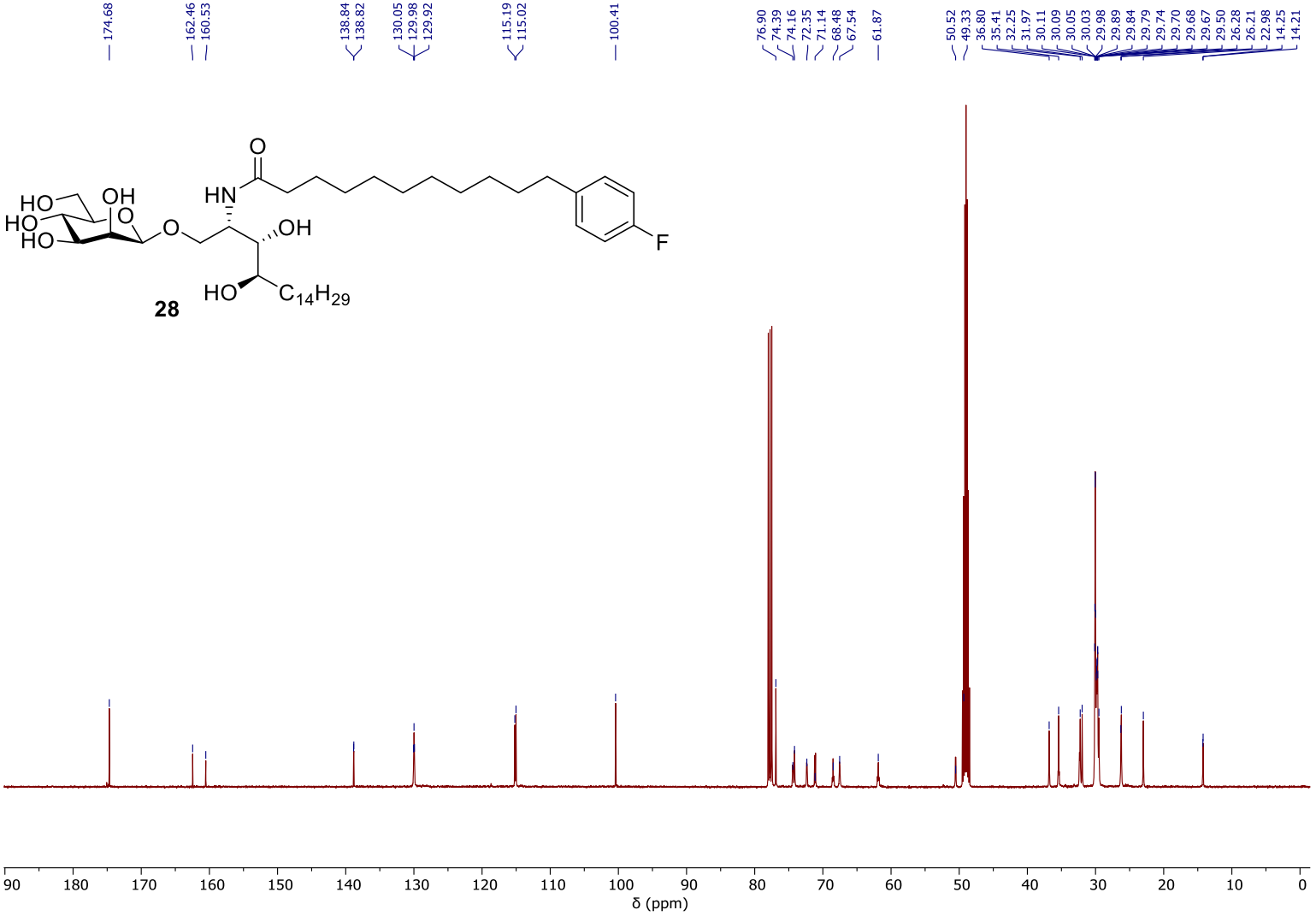
Compound **27**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



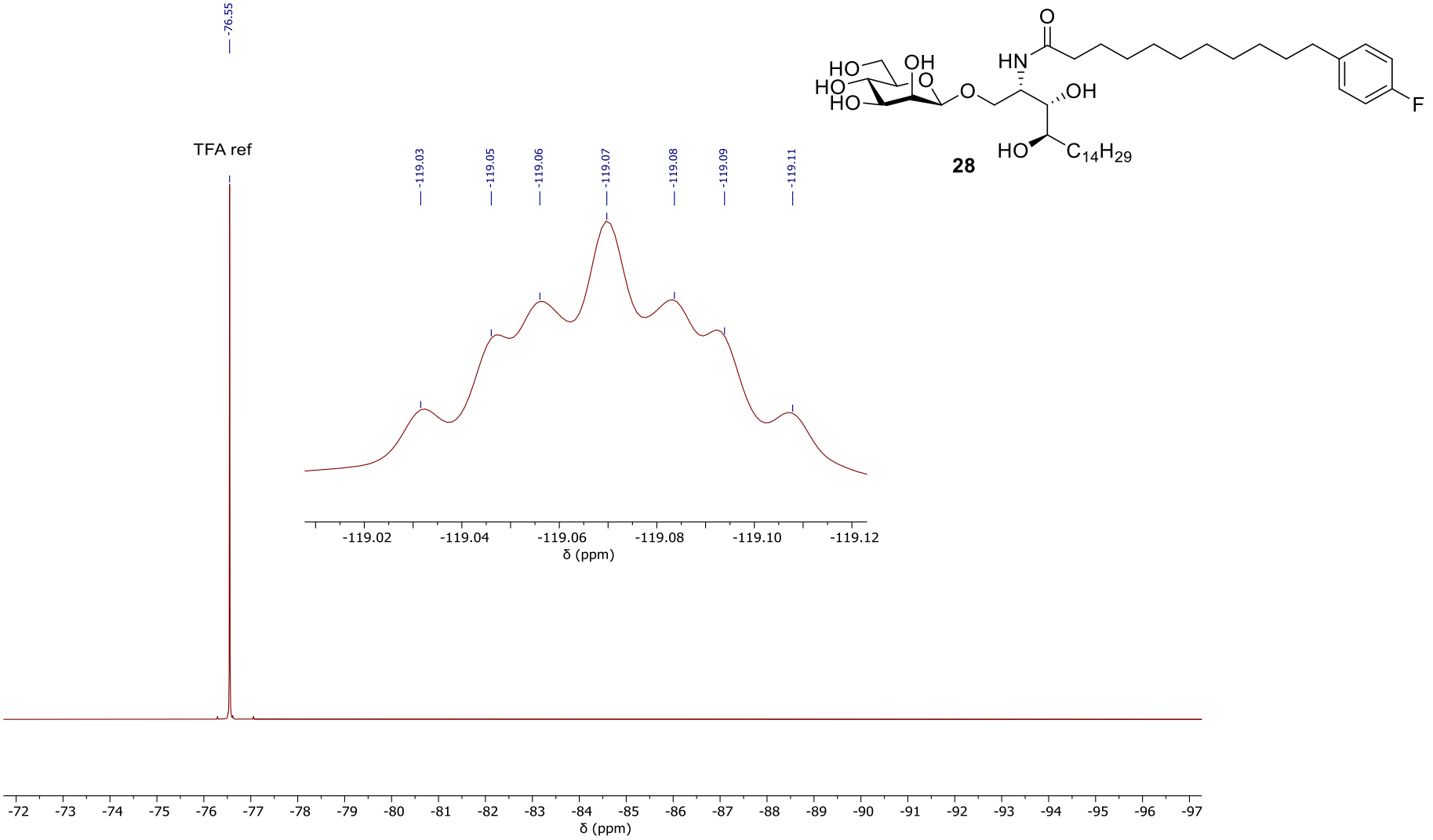
Compound **28**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **28**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **28**. ^{19}F NMR spectrum (376 MHz, 2:1 $CDCl_3$:CD_3OD)



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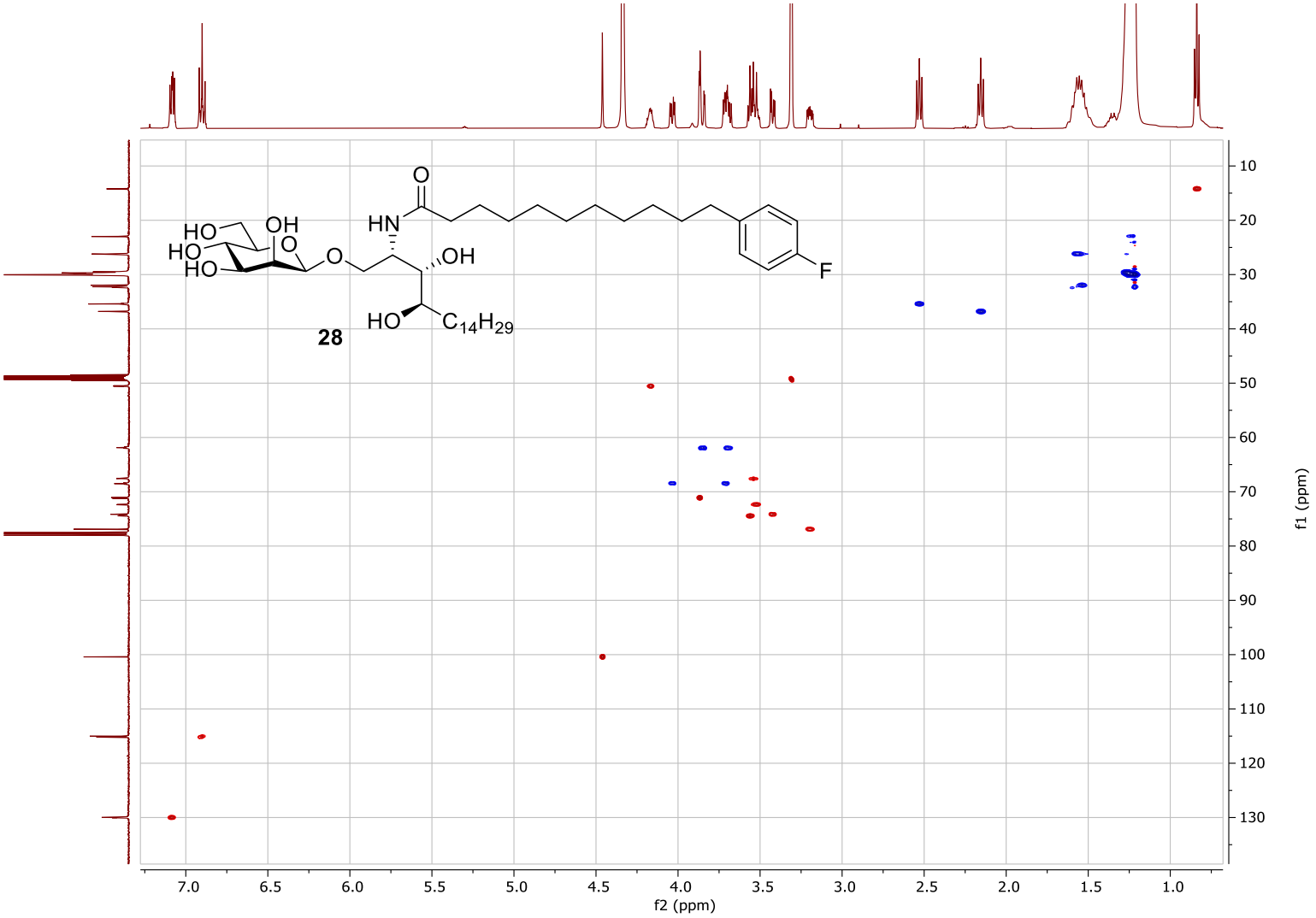
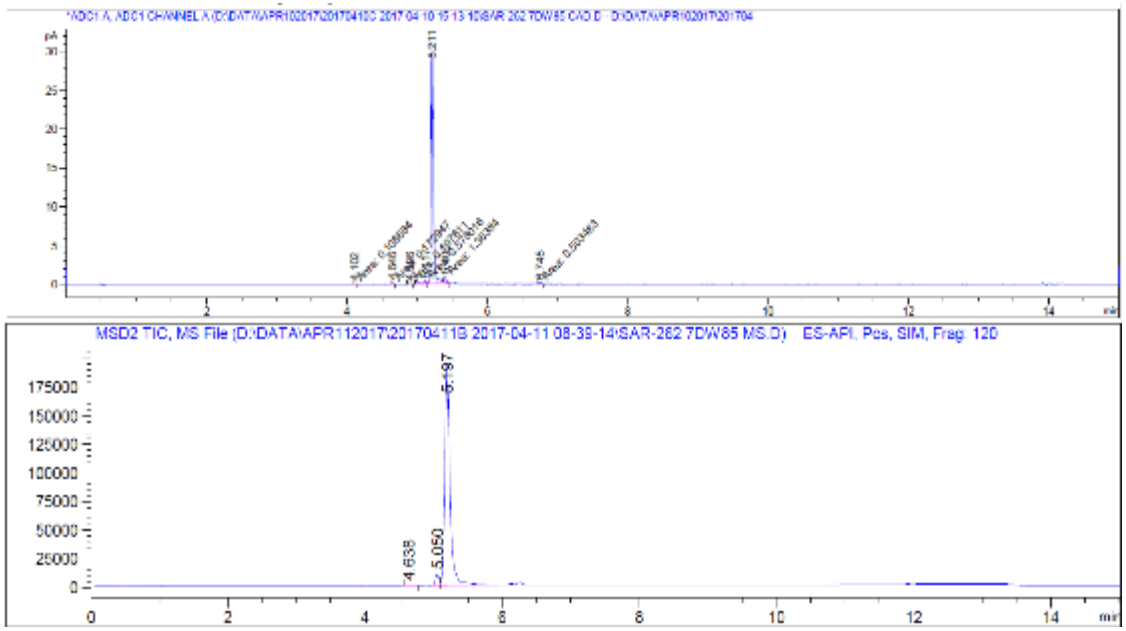
Compound **28**. HSQC spectrum

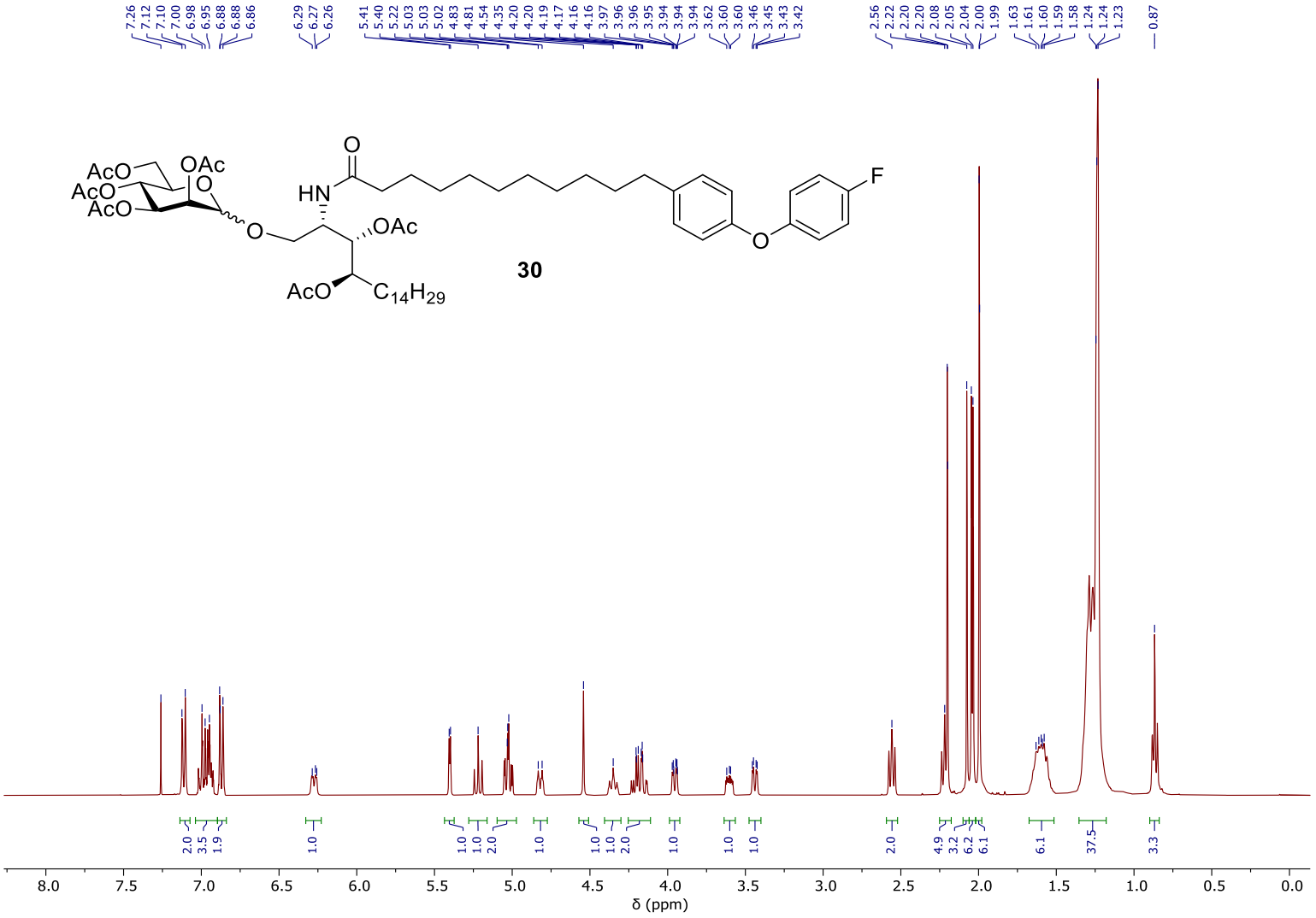


HPLC chromatogram of **28**

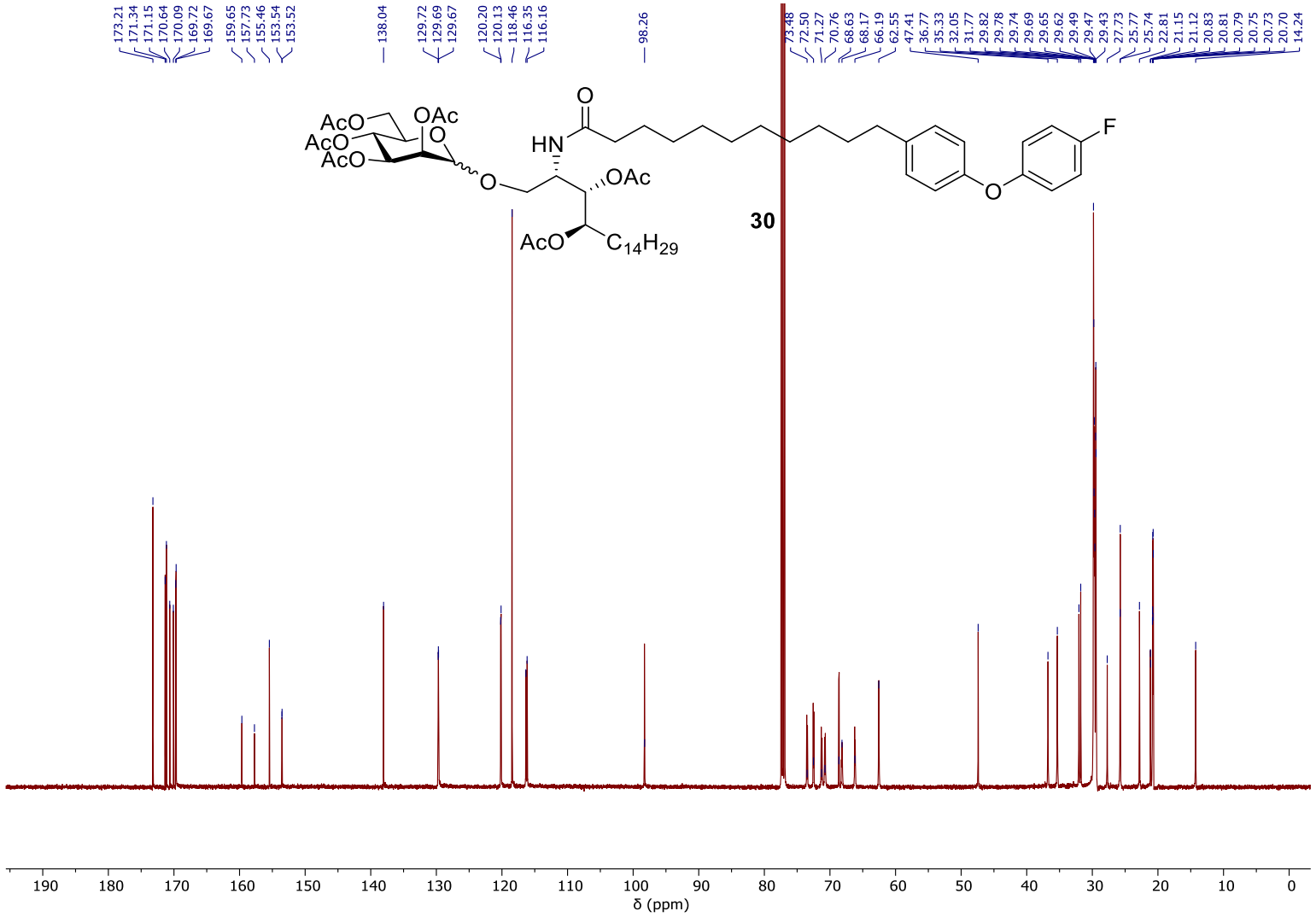


Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), , t_R =5.211 min (96.2%)

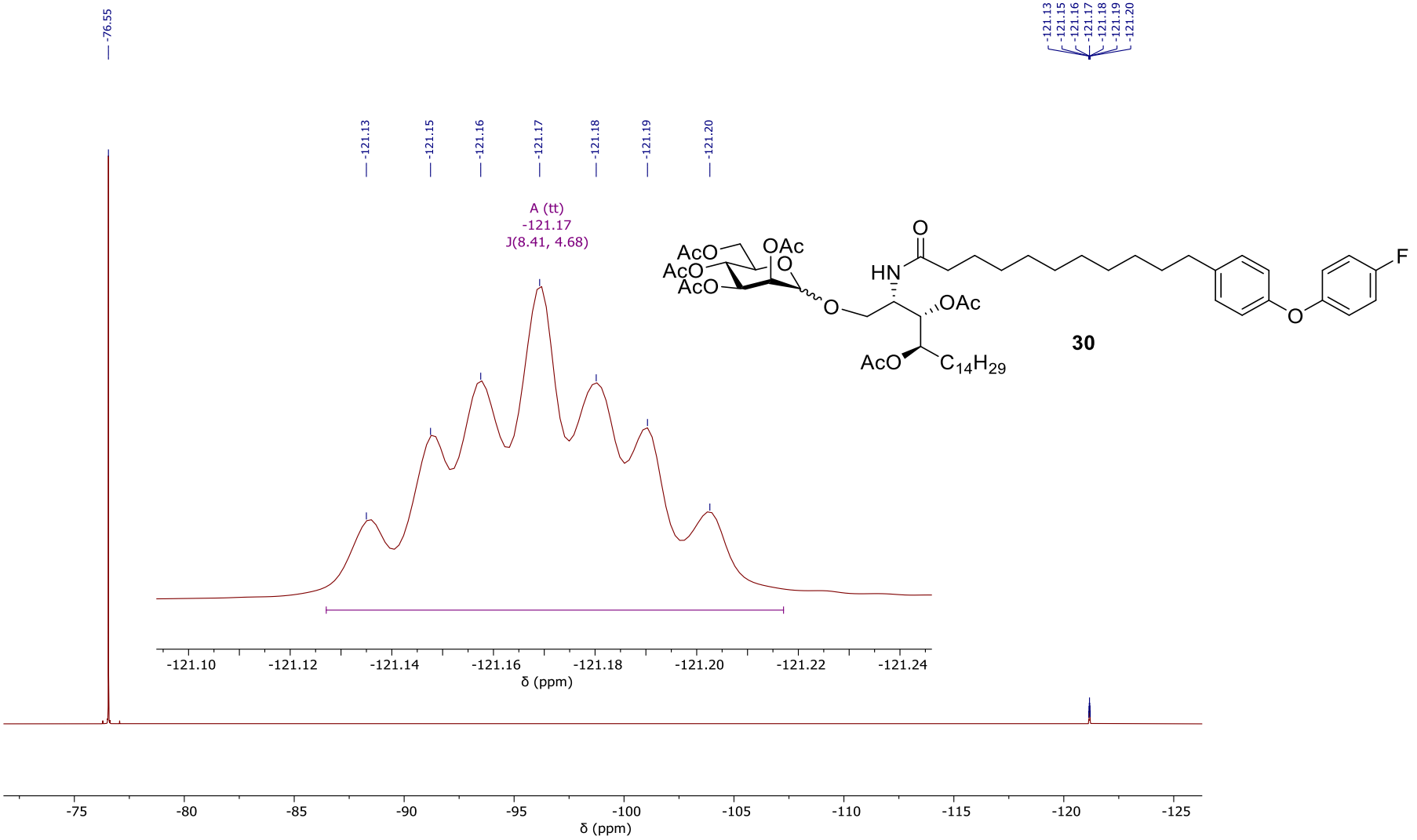
Compound **30**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



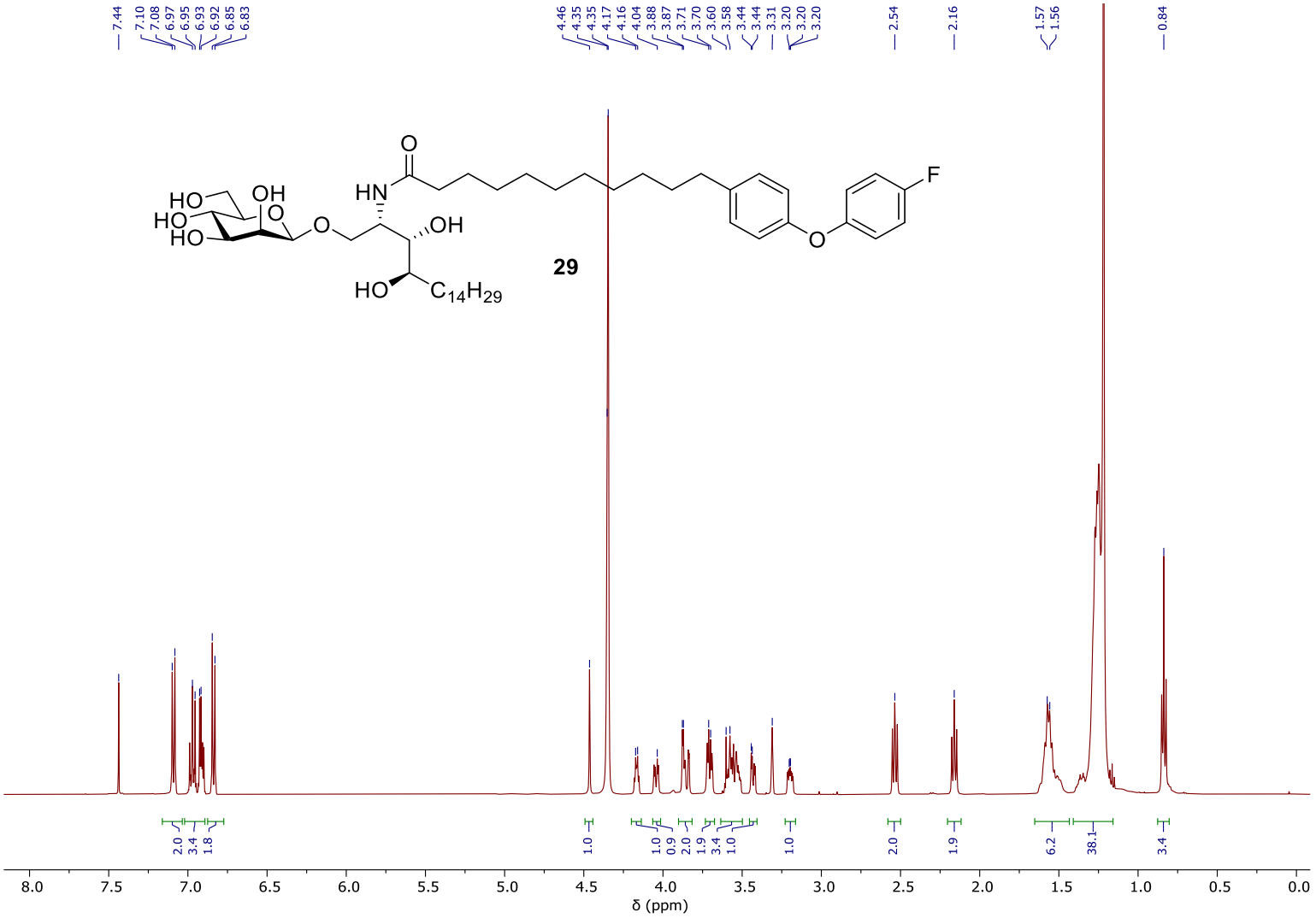
Compound **30**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



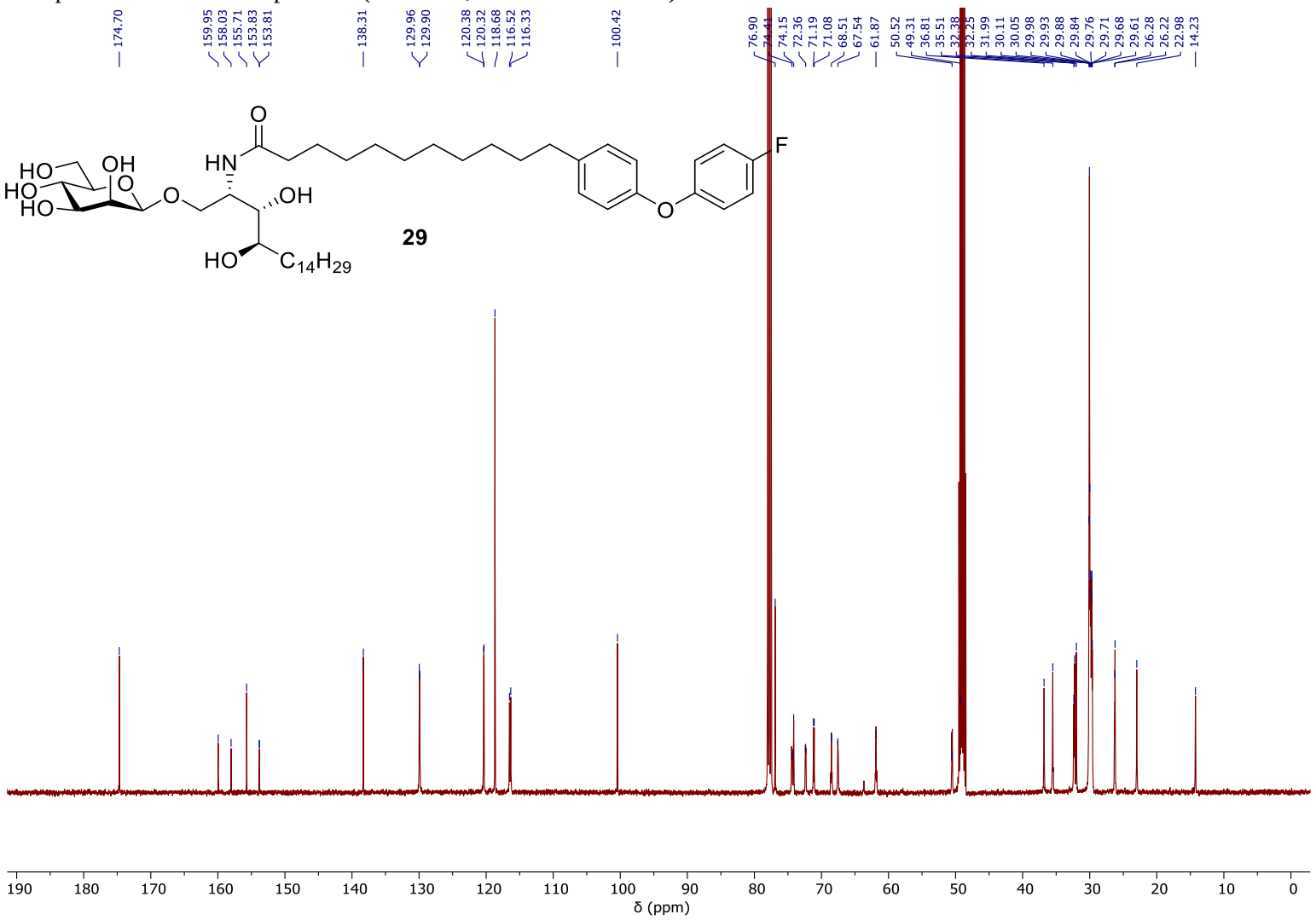
Compound **30**. ^{19}F NMR spectrum (376 MHz, $CDCl_3$).



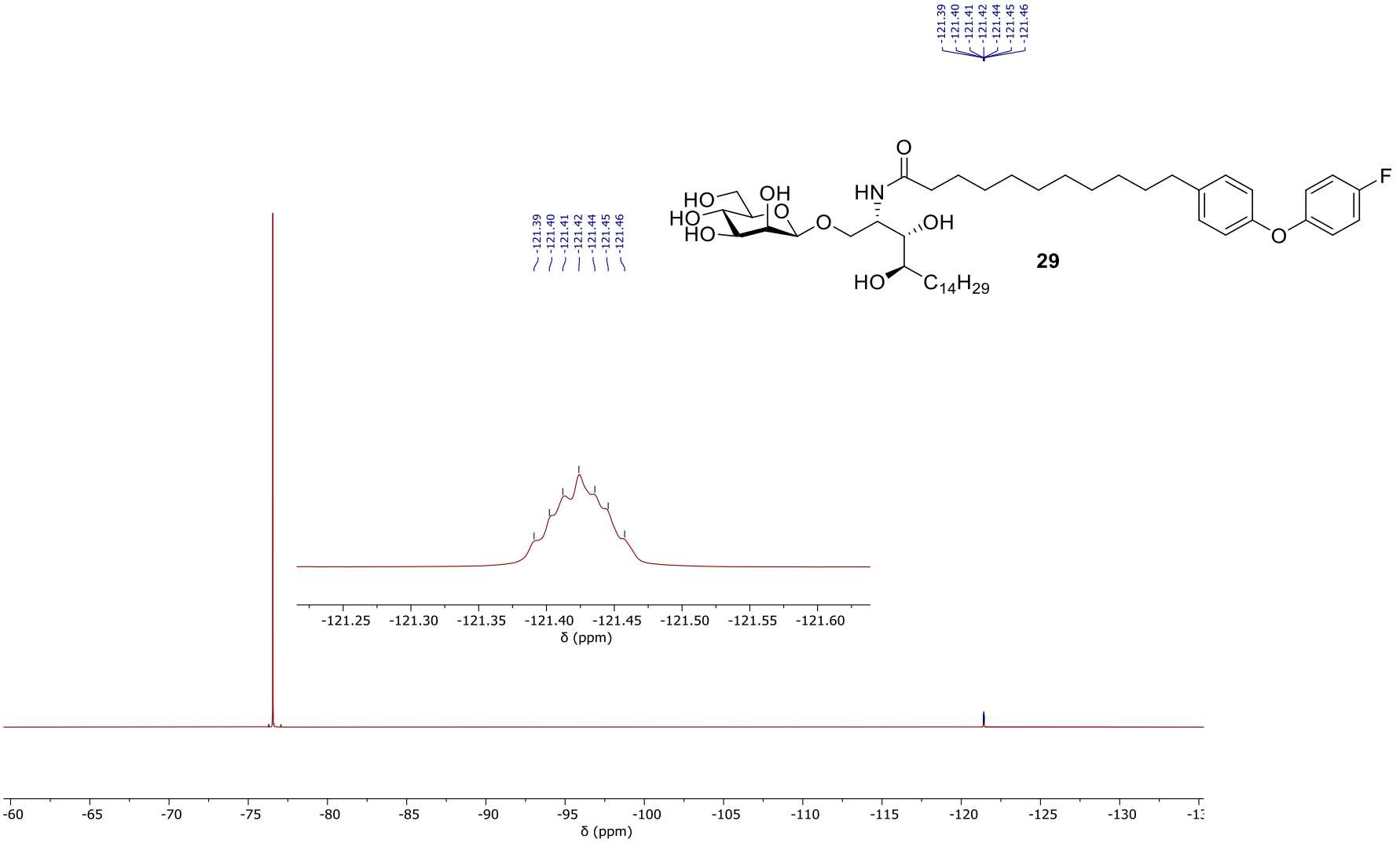
Compound **29**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).



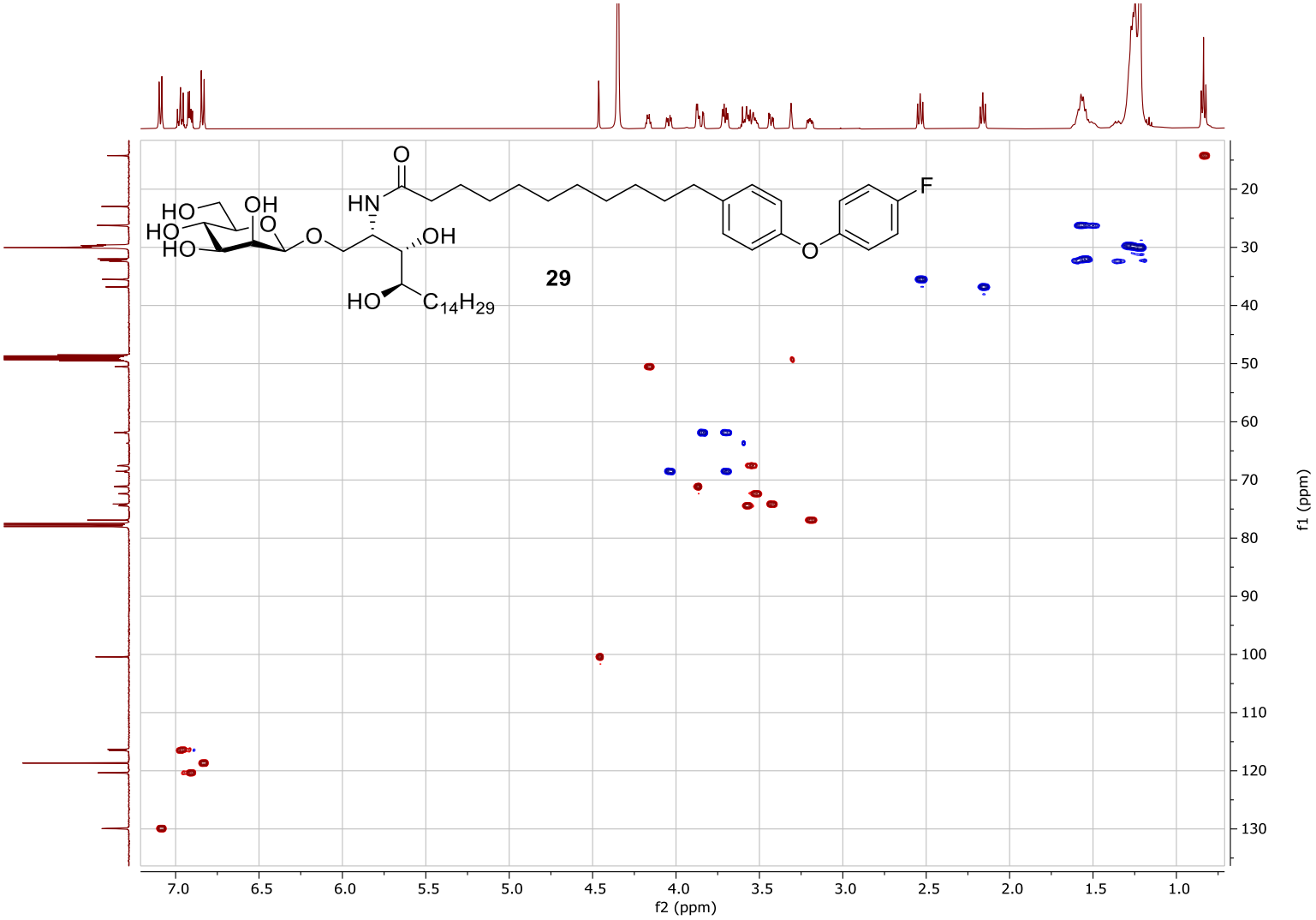
Compound **29**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



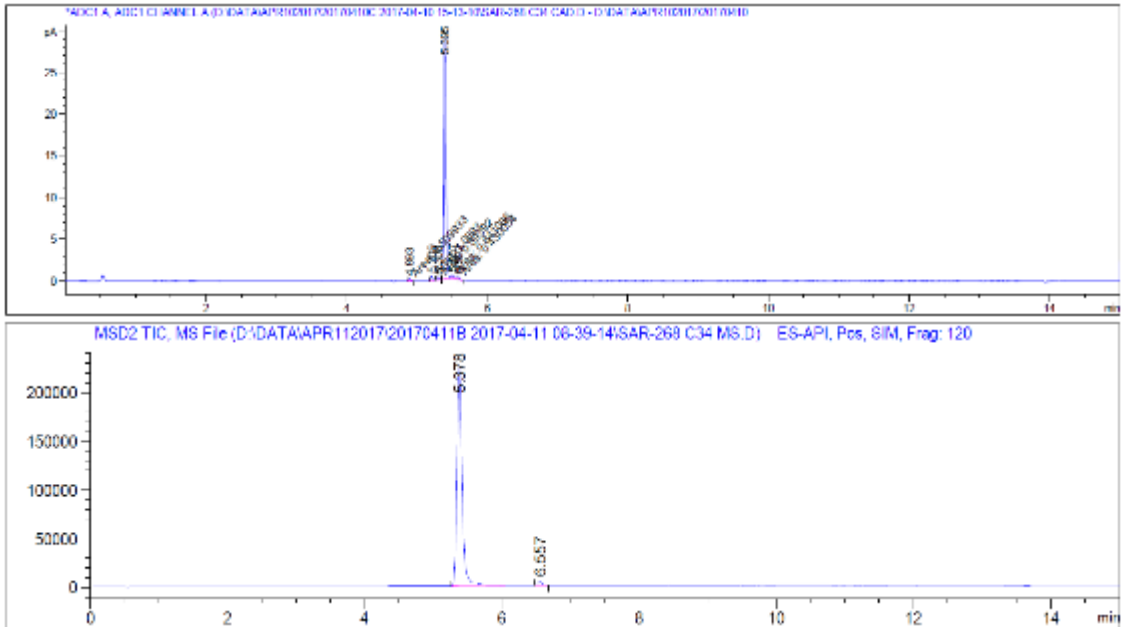
Compound **29**. ^{19}F NMR spectrum (376 MHz, 2:1 $CDCl_3$:CD_3OD)



Compound **29**. HSQC spectrum

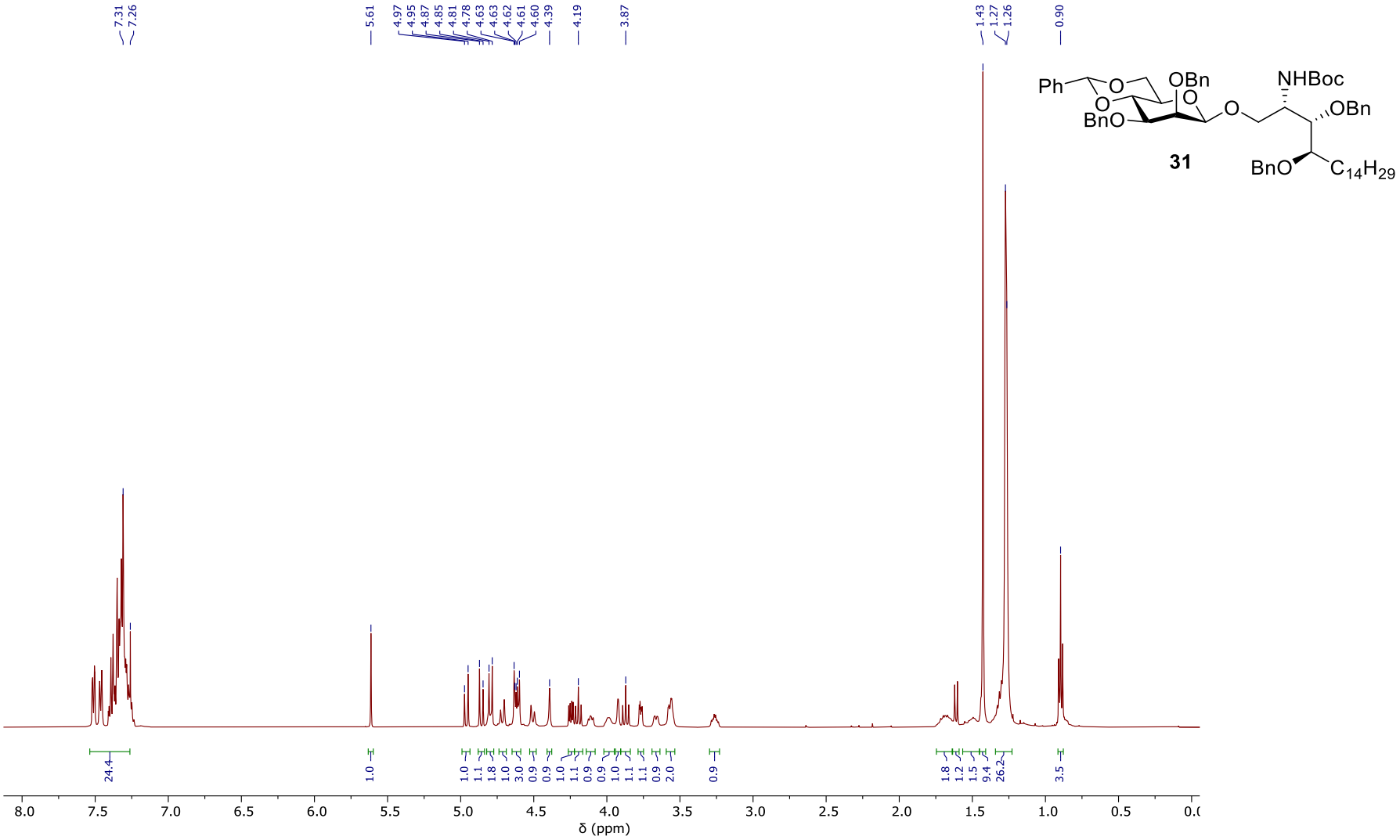


HPLC chromatogram of **29**

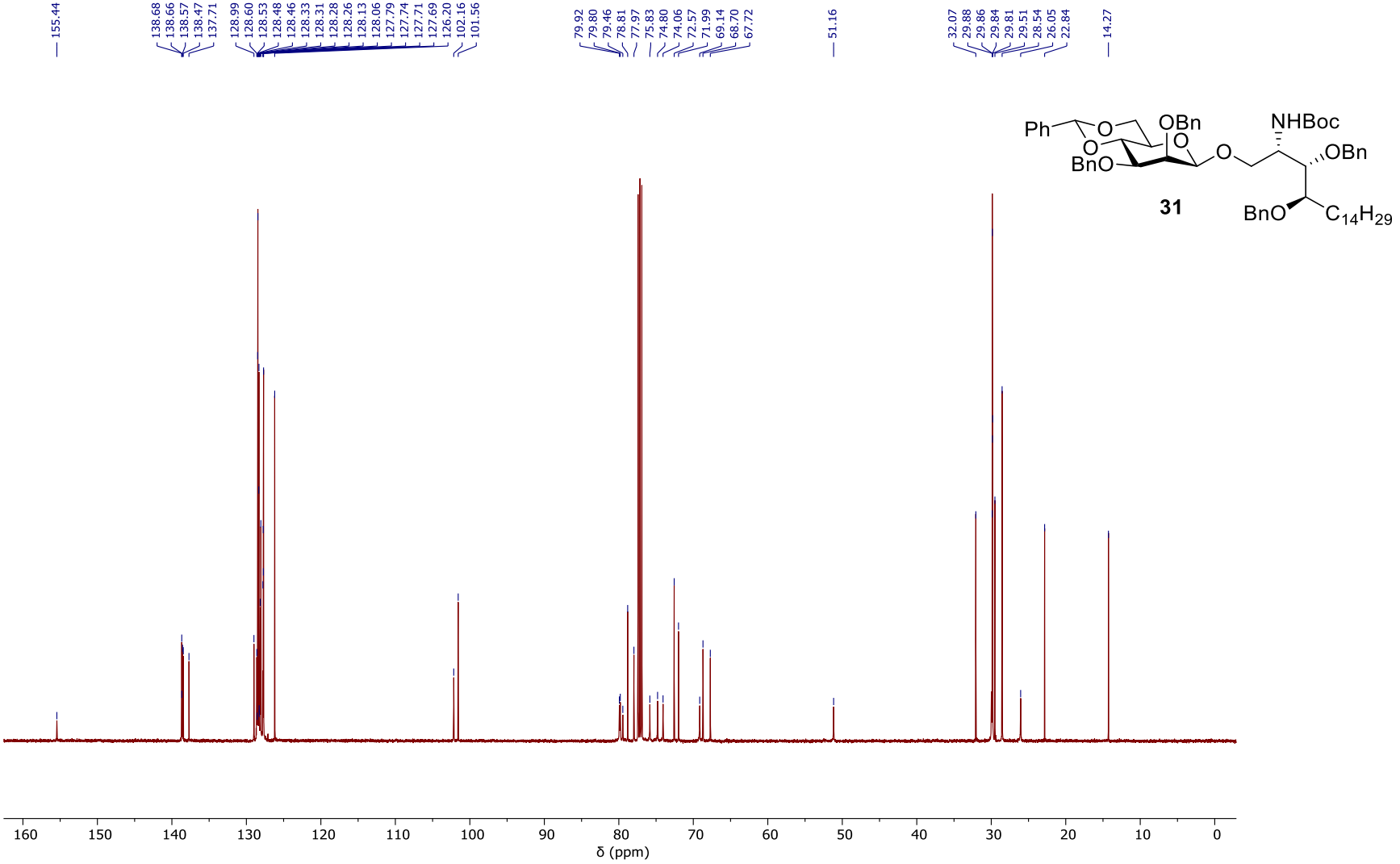


Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), t_R =5.40 min (94.8%).

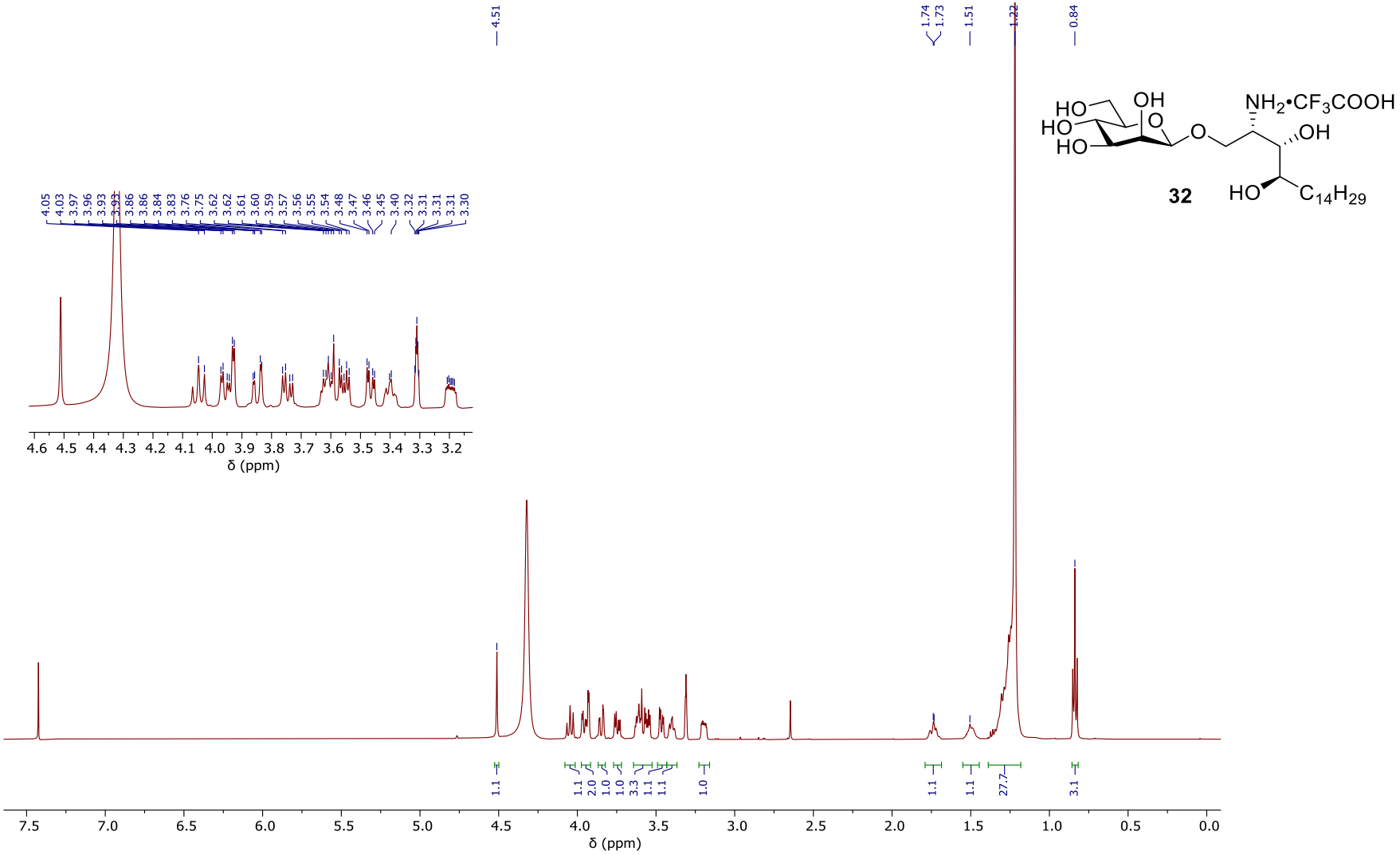
Compound **31**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



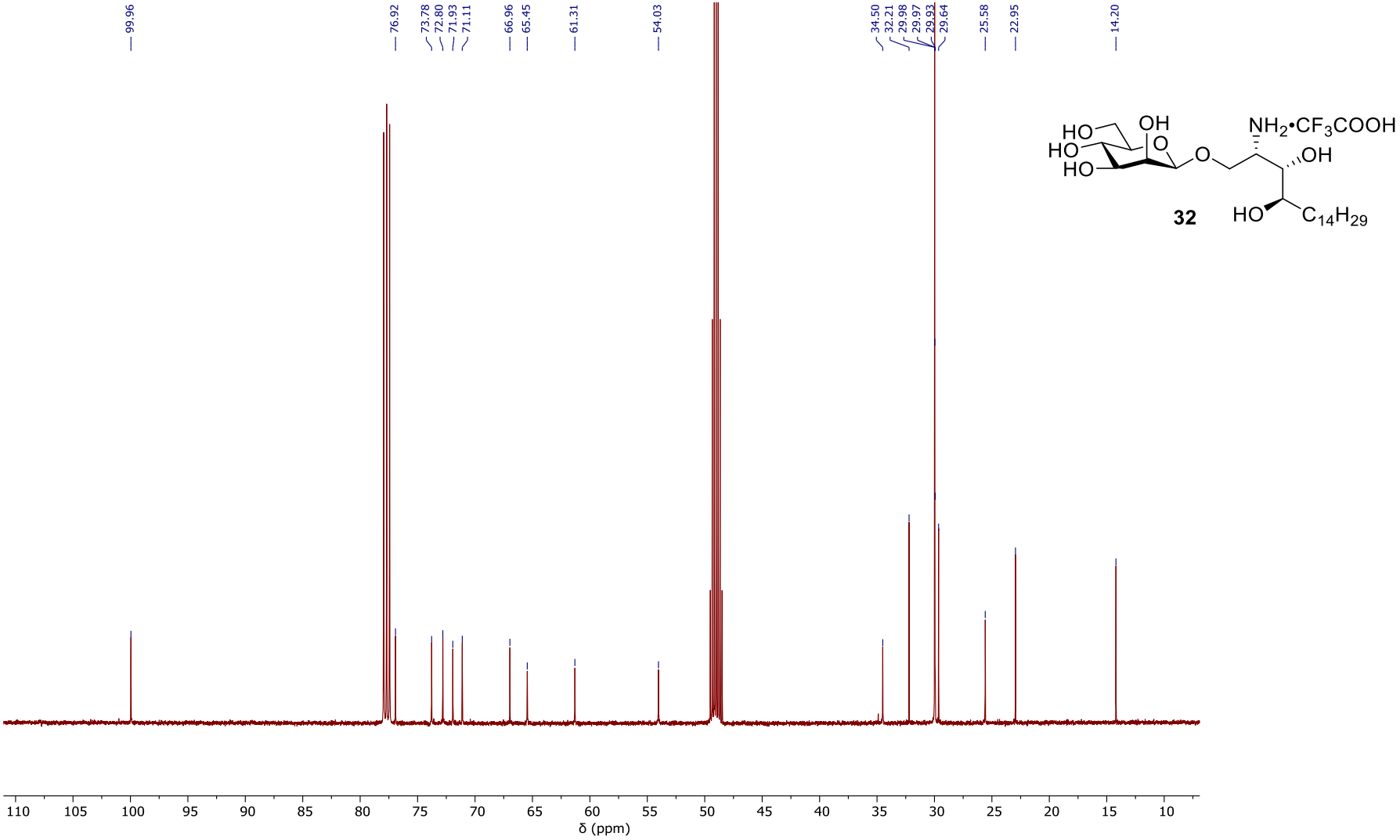
Compound **31**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



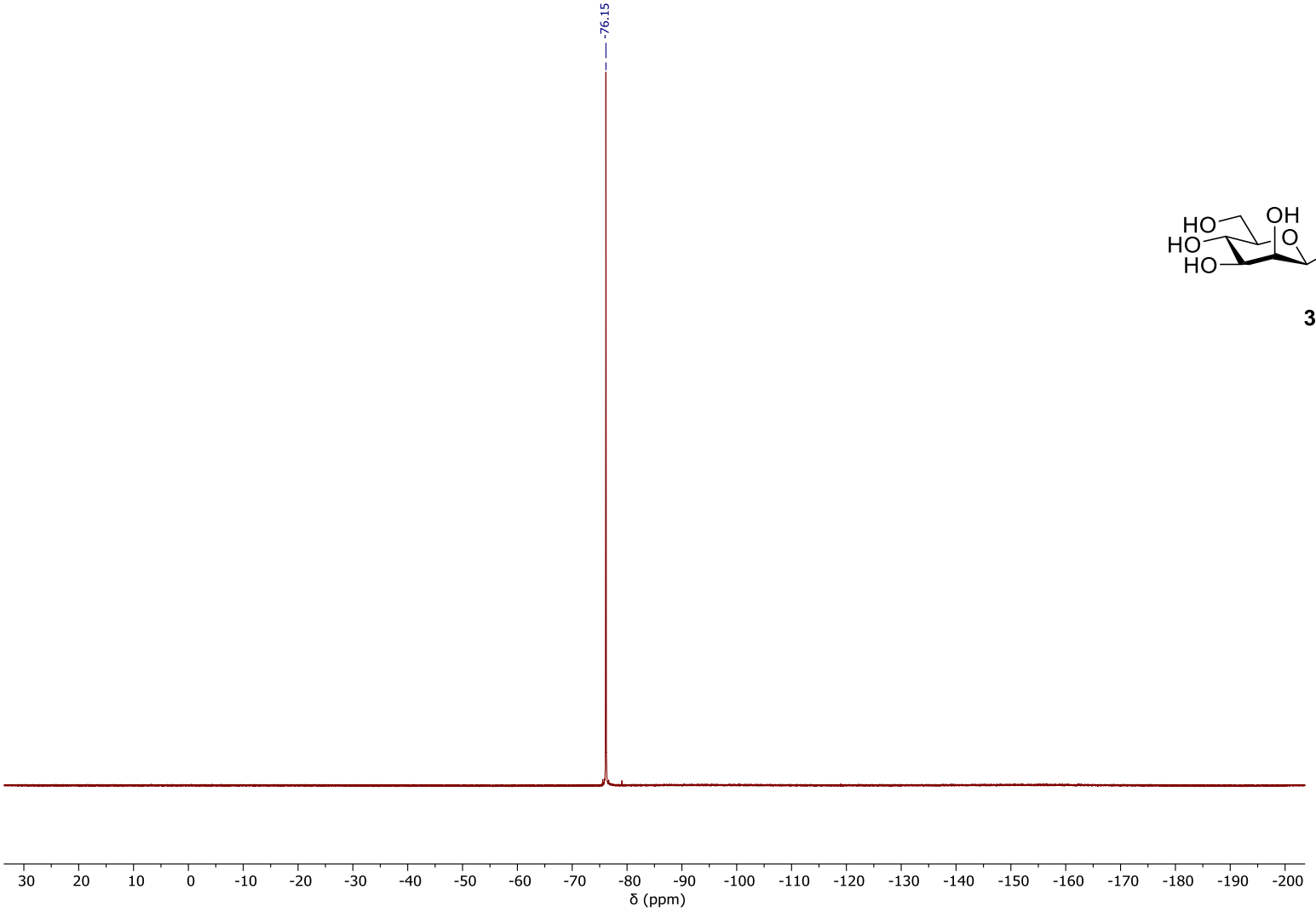
Compound **32**. ^{1}H NMR spectrum (500 MHz, 2:1 $CDCl_3$:CD_3OD).

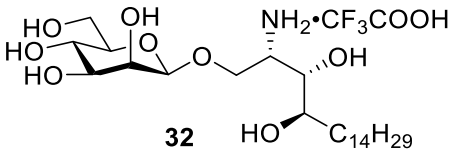


Compound **32**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



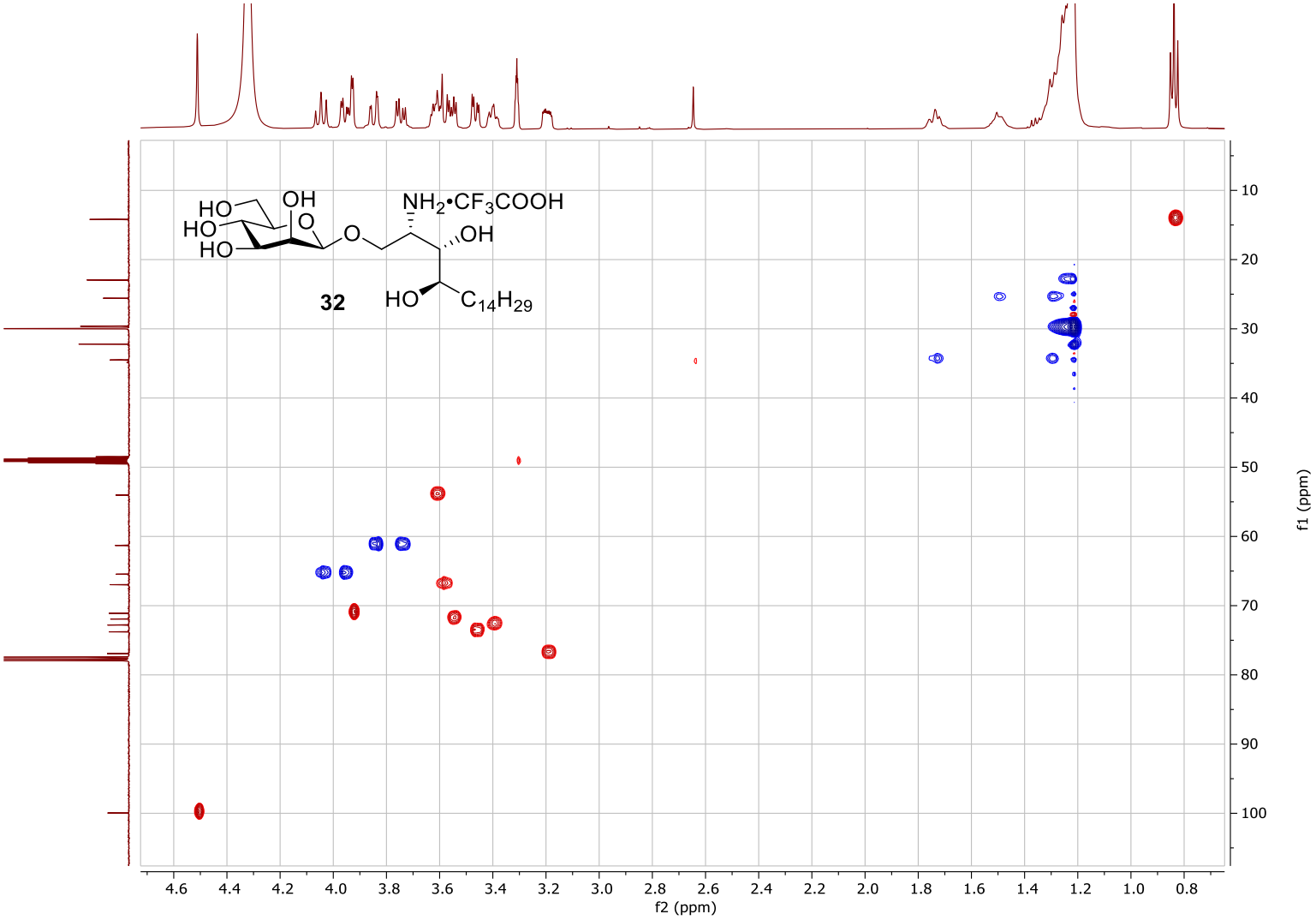
Compound **32**. ^{19}F NMR spectrum (376 MHz, 2:1 $CDCl_3$:CD_3OD)



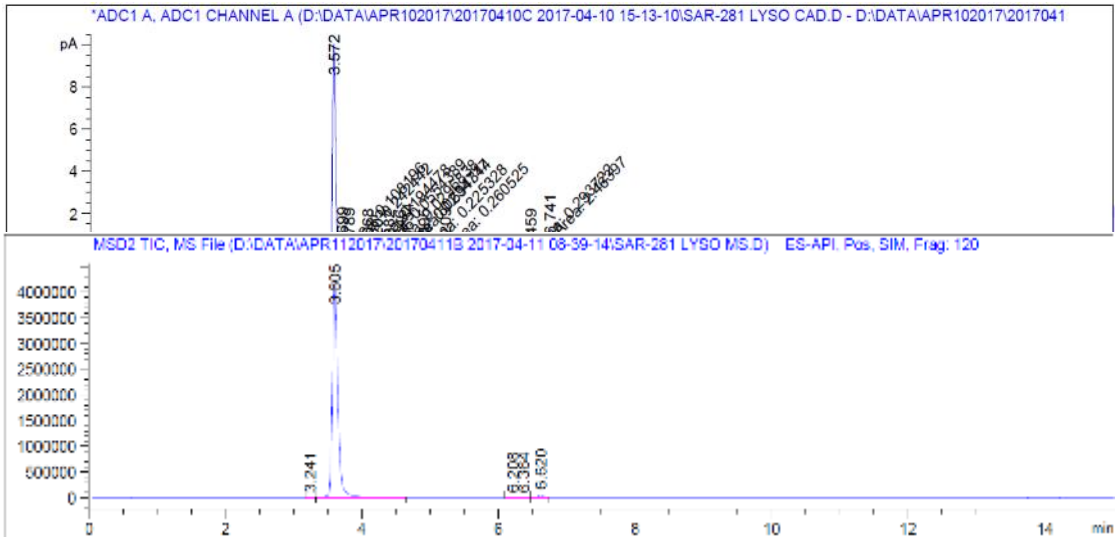


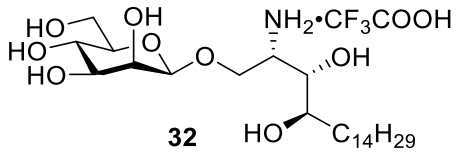
32

Compound **32**. HSQC spectrum



HPLC chromatogram of **32**

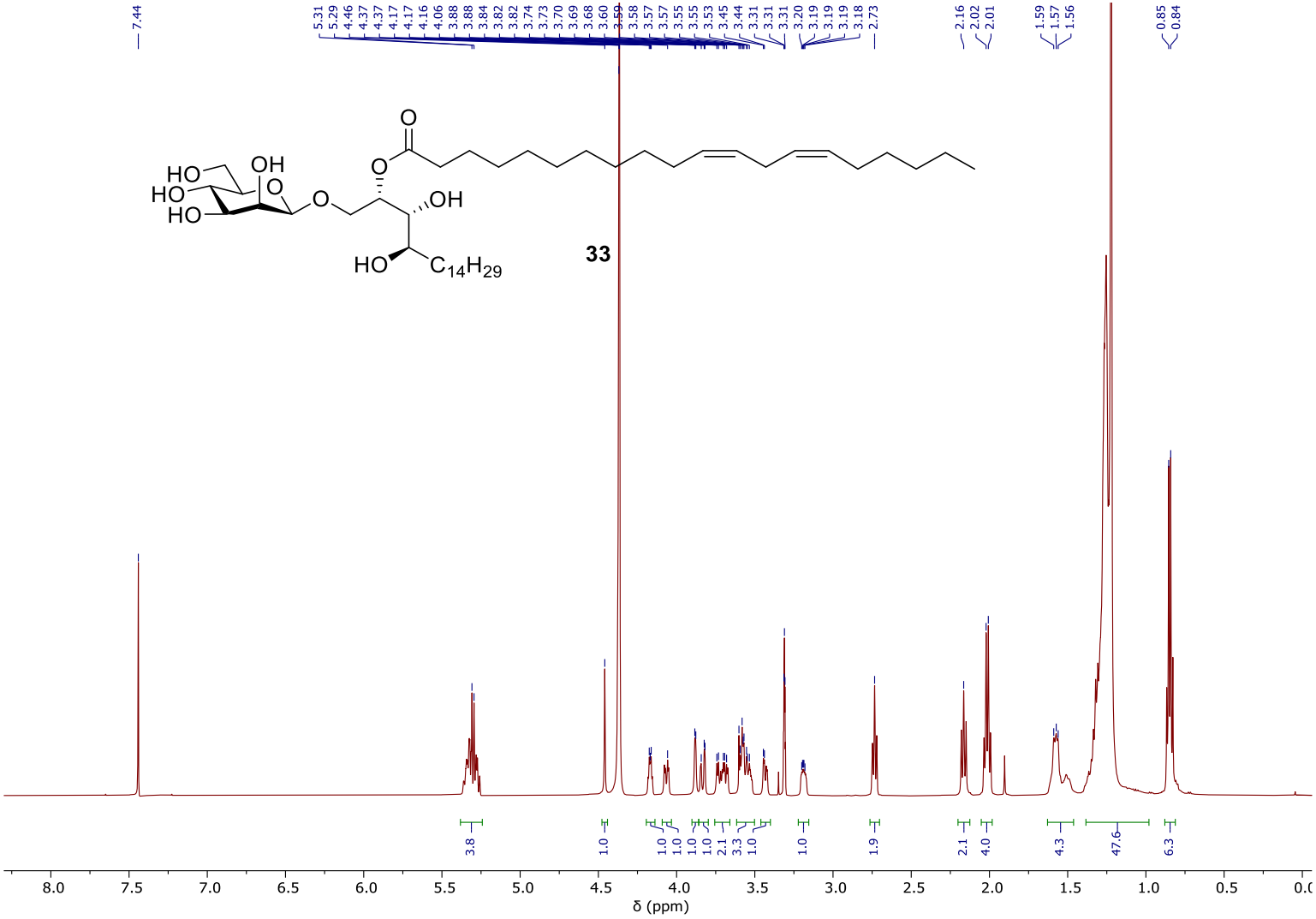




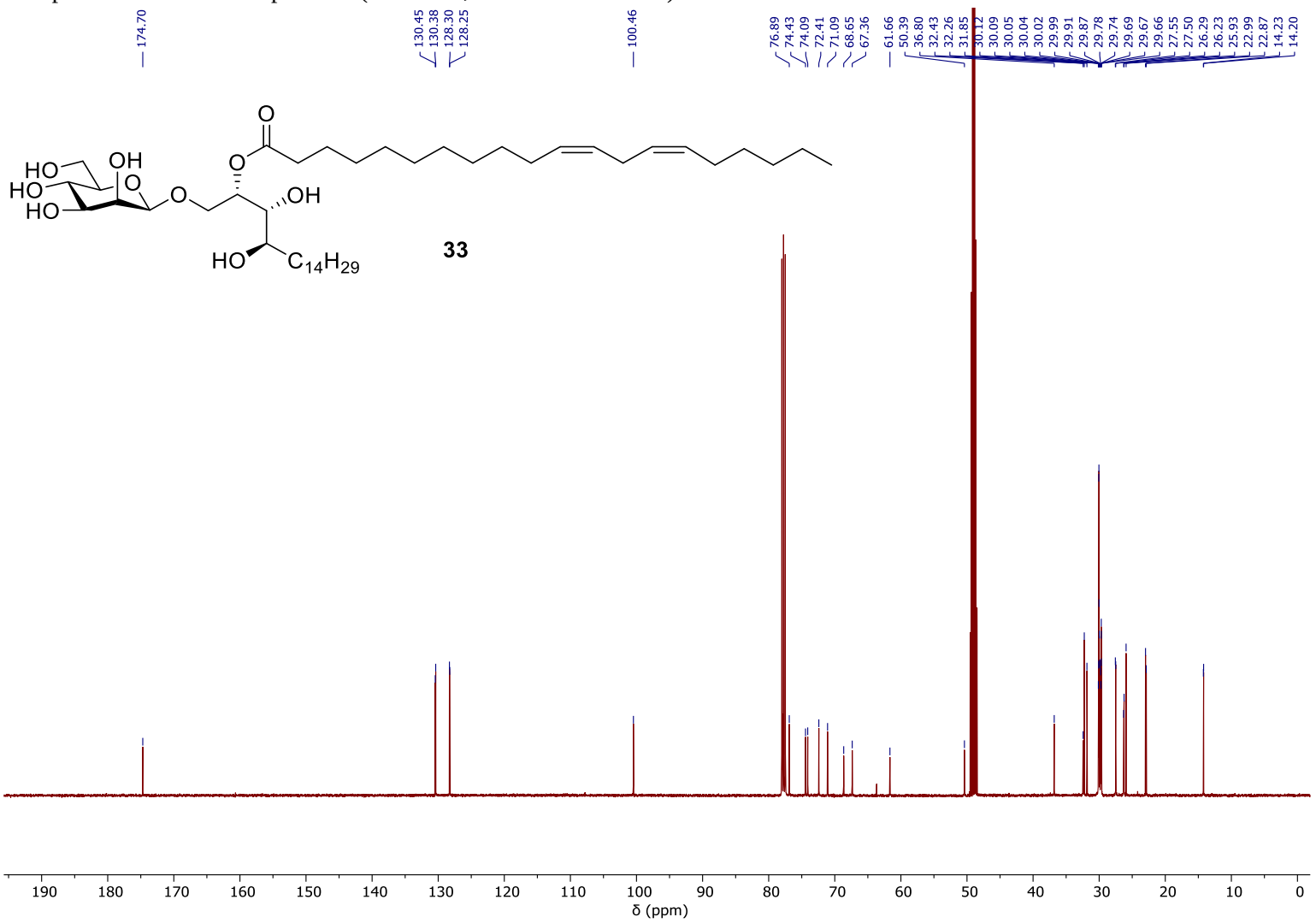
32

Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins),, t_R =3.572 min (87.2492%).

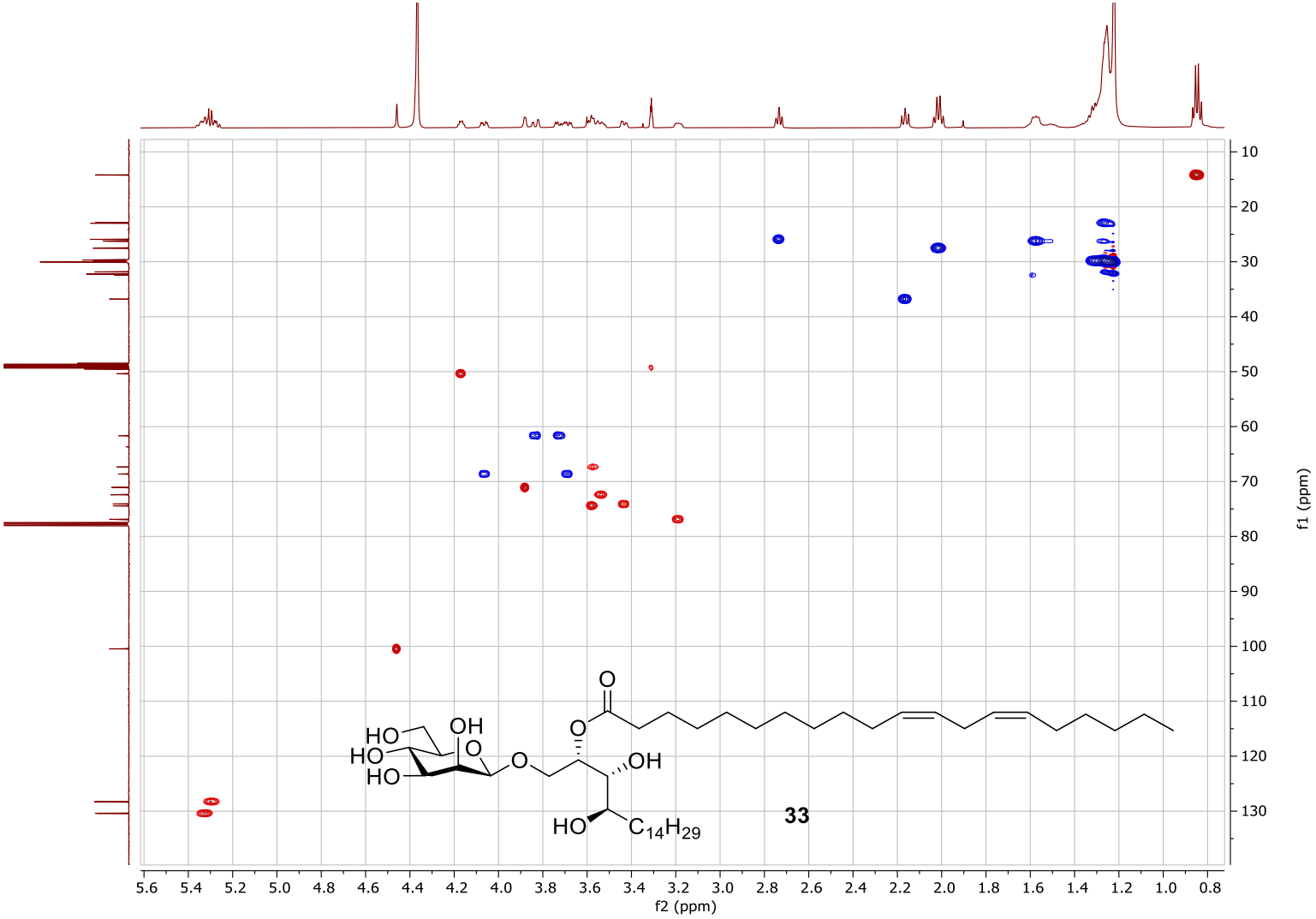
Compound **33**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



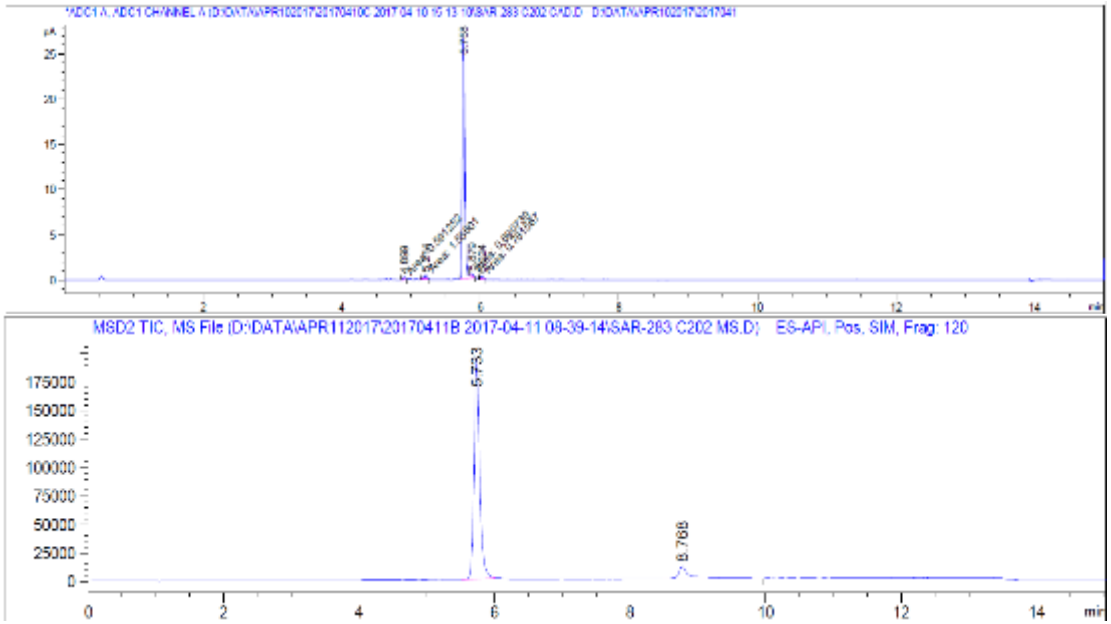
Compound **33**. ^{13}C NMR spectrum (125 MHz, 2:1 $CDCl_3$:CD_3OD).



Compound **33**. HSQC spectrum



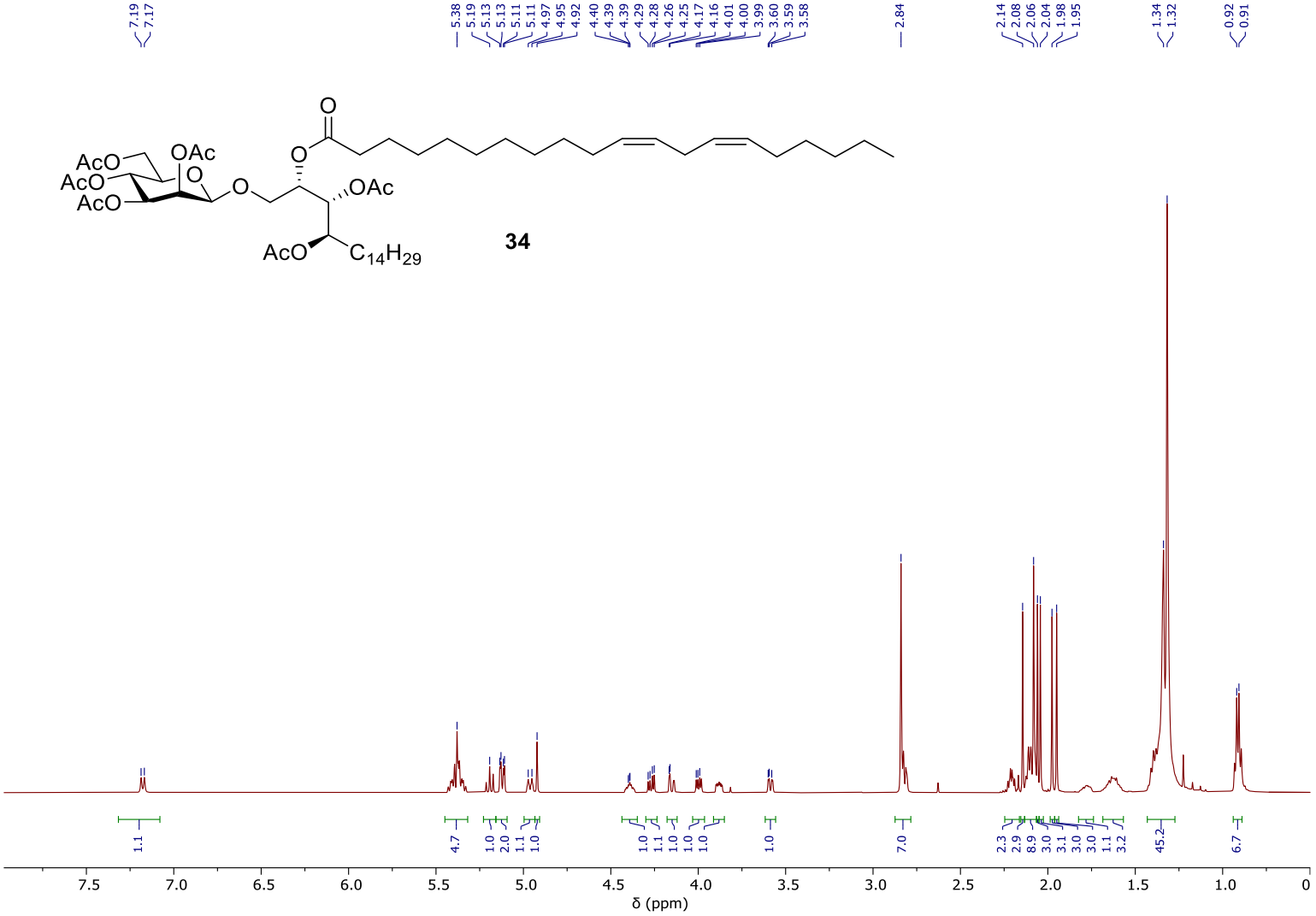
HPLC chromatogram of **33**



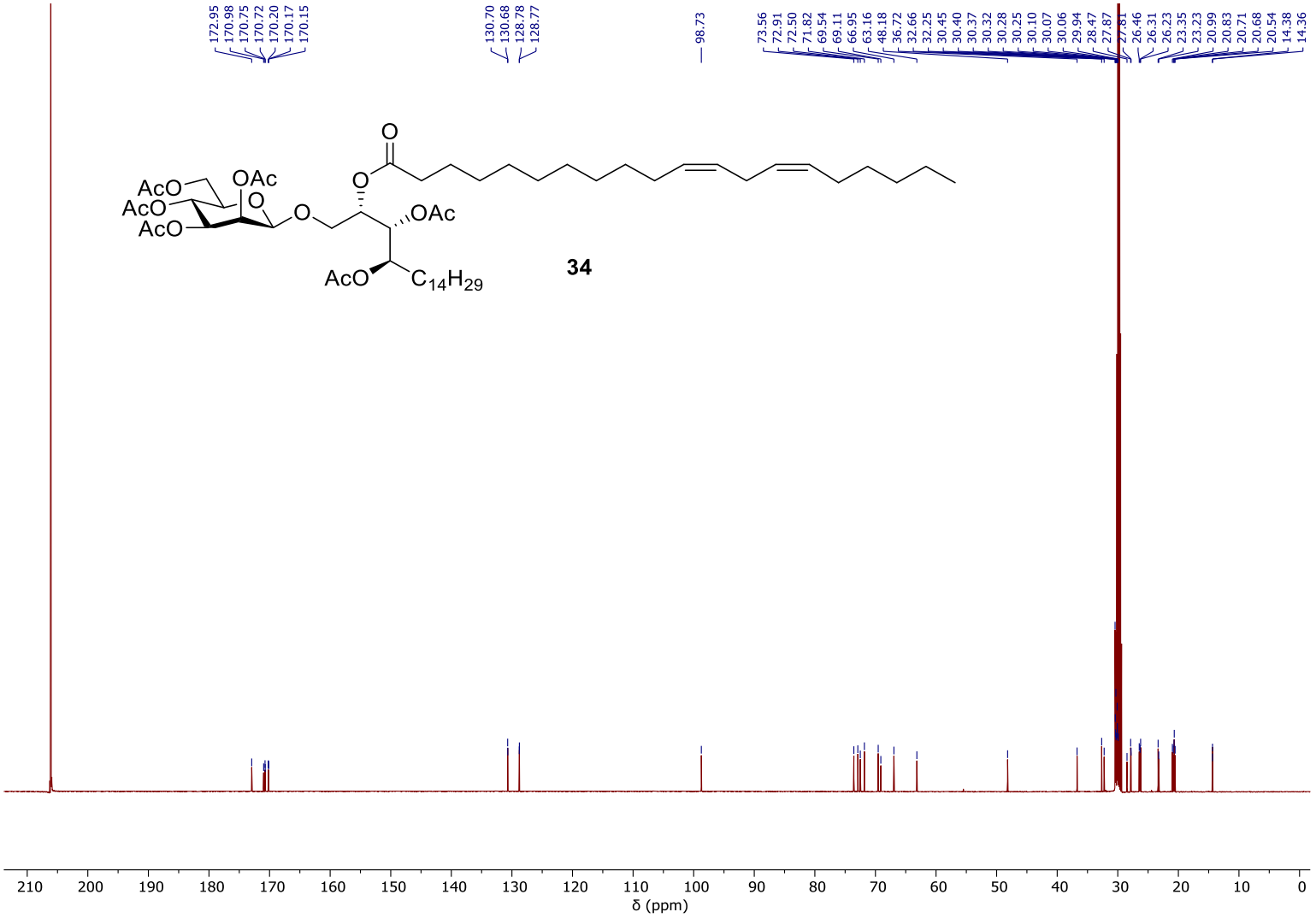
S

Synthetic purity assessment: Analytical HPLC [Phenomenex Kinetex 100Å C18 (2.6 µm, 3.0 x 50 mm), line A = H_2O, line B = CH_3OH, both modified with 0.5% TFA, flow rate = 0.50 mL min^{-1}, gradient = 60% -100% line B (4 mins), 100% line B(8 mins), 100% - 60% line B (1 min), 60% line B (2 mins), t_R =5.758 min (94.7%)

Compound **34**. ^{1}H NMR spectrum (500 MHz, $(CD_3)_2CO$).



Compound **34**. ^{13}C NMR spectrum (125 MHz, $(CD_3)_2CO$).



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1. *Application: US; US Pat.,* 2012-13582612, 8835613, 2014.

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3. N. Okamoto, O. Kanie, Y.-Y. Huang, R. Fujii, H. Watanabe and M. Shimamura, *Chem. Biol.*, 2005, **12**, 677-683.
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5. L. Panza, F. Compostella and D. Imperio, *Carbohydr. Res.*, 2019, **472**, 50-57.