SUPPORTING INFORMATION

Synthesis and Biological Evaluation of (-)-Kainic Acid Analogues as Phospholipase D-Coupled Metabotropic Glutamate Receptor Ligands

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LIST OF CONTENTS

General Information

^{1}H (400.13 MHz), ^{13}C (100.58 MHz) and ^{19}F (376.45 MHz) NMR spectra were recorded on a Bruker ADVANCE III spectrometer. ^{1}H NMR chemical shifts are reported relative to TMS, and the solvent resonance was employed as the internal standard ($CDCl_3$ δ = 7.26, CD_3OD δ = 3.31, D_2O δ = 4.79). ^{13}C NMR spectra were recorded with complete proton decoupling, and the chemical shifts are reported relative to TMS with the solvent resonance as the internal standard ($CDCl_3$, δ = 77.0, CD_3OD δ = 49.00). ^{19}F NMR spectra were recorded with complete proton decoupling. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, dd = doublet-doublet, ddd = doublet-doublet-doublet, dt = doublet-triplet, t = triplet, td = triplet-doublet, q = quartet, m = multiplet, bs = broad singlet, bq = broad quartet. All chemical shifts (δ) are expressed in parts per million and coupling constant (*J*) are given in Hertz. LC-MS experiments were performed on an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by a ESI ionization source and a Single Quadrupole mass selective detector using an Analytical C18 RP Column (Phenomenex Luna, C18, 250x4.60 mm, 5 μ, 100 Å). HPLC purifications were performed on the Agilent 1200 system using a semi preparative C18 RP Column (Phenomenex Luna, 250x10.00 mm, 5 μ, 100 Å). A CEM Discover® System was used to perform reaction with microwaves. Optical rotation values were measured on an AA-65 Angular Scale automatic polarimeter (Optical Activity Limited) with a 1dm cell at the sodium D line. All reactions were carried out in oven- or flame-dried glassware under nitrogen atmosphere, unless stated otherwise. All commercially available reagents were used as received. Reactions were magnetically stirred and monitored by TLC on silica gel (60 F254 pre-coated glass plates, 0.25 mm thickness). Visualization was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molibdate or $KMnO_4$ solution. Flash chromatography was performed on silica gel (60 Å, particle size 0.040–0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise. Abbreviations used: DCM for dichloromethane, EtOAc for ethyl acetate, Et_2O for diethyl ether, PfBr for 9-Bromo-9-phenylfluorene, DMSO for dimethyl sulfoxide, NBS for *N*-bromosuccinimide, DIBAL-H for diisobutylaluminium hydride, DME for dimethoxyethane, DIPEA *N*,*N*-diisopropylethylamine, THF for tetrahydrofuran, MeOH for methanol, TEA for triethylamine, EDC for 1-(3-dimethylaminiopropyl) -3-ethylcarbodimide, DMF for *N*,*N*-dimethylformamide.

Synthetic Procedures and Compounds Characterizations

Methyl (2S, 4R)-4-hydroxypyrrolidine-2-carboxylate hydrochloride

Thionyl chloride (4.00 mL, 30.5 mmol) was added to methanol (20.0 mL) at 0°C and the reaction was allowed to warm to r.t. 4-*trans*-L-hydroxyproline (4.00 g, 54.9 mmol) was added, the mixture was heated at 45°C and stirred overnight. The solvent was removed under reduced pressure and the solid was treated with Et_2O (3 x 10 mL) followed by evaporation. Recrystallization of the solid from MeOH-Et_2O gave the desired methyl ester (8.97 g, 90%) as a withe solid, used without further purification. ESI MS *m/z*: $[M+H]^+$ calcd for $C_6H_{12}NO_3$ 146.07, found (relative intensity) 146.1 (100) $[M+H]^+$. (For the complete characterization refer to D.S. Kemp, P. Curran, M.D. William, J. G. Boyd, C. Muendel, *J. Org. Chem.* **1991**, *56*, 6672-6682 and J.E. Baldwin, S. J. Bamford, A.M. Fryer, M.P. W. Rudolph, M.E. Wood, *Tetrahedron*, **1997**, *53*, 5233-5254).

Methyl (2S, 4R)-1-benzoyl-4-hydroxypyrrolidine-2-carboxylate

To a solution of methyl (2*S*, 4*R*)-4-hydroxypyrrolidine-2-carboxylate hydrochloride (5.45 g, 30.0 mmol) in H_2O/THF (1:1, 20 mL) $NaHCO_3$ (5.48 g, 63.0 mmol) was slowly added and the mixture was cooled to 0°C. A solution of benzoyl chloride (3.83 mL, 33.0 mmol) in THF (8.8 mL) was added drop wise and the mixture was stirred at r.t for 1.5 h. The solvent was removed under vacuum, $CHCl_3$ (50 mL) was added, the organic layers were washed with brine dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was recrystallized in EtOAc to give the desired benzoyl amide (7.33 mg, 98%) as a withe crystalline solid, used without further purification. R*f* 0.21 (DCM/EtOAc 1:1); ESI MS *m/z*: $[M+H]^+$ calcd for $C_{13}H_{16}NO_4$ 250.10, $[2M+Na]^+$ calcd for $C_{26}H_{30}N_2O_8Na$ 521.19, found (relative intensity) 250.1 (100) $[M+H]^+$, 521.1 (100) $[M+H]^+$. (For the complete characterization refer to D.S. Kemp, P. Curran, M.D. William, J. G. Boyd, C. Muendel, *J. Org. Chem.* **1991**, *56*, 6672-6682).

Methyl (2S)-1-benzoyl-4-oxopyrrolidine-2-carboxylate (2)

To a solution of methyl (2*S*, 4*R*)-1-benzoyl-4-hydroxypyrrolidine-2-carboxylate (7.30 g, 29.0 mmol) in a mixture of CH_3CN (32 mL), CCl_4 (32 mL) and H_2O (48 mL), $NaIO_4$ (116.0 mmol, 24.8 g) and ruthenium trichloride hydrate (300 mg, 1.45 mmol) were added. The

mixture was vigorously stirred for 4 h at r.t. then DCM (160 mL) was added and the aqueous phase was further extracted with DCM (3 x 50 mL). The organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (DCM/ EtOAc 6:4) to give ketone **2** (5.74 g, 80%) as a light brown oil. R*f* 0.50 (DCM/EtOAc 1:1); ESI MS *m/z*: $[M+H]^+$ calcd for $C_{13}H_{14}NO_4$ 248.08, found (relative intensity) 248.1 (100) $[M+H]^+$. (For the complete characterization refer to D.S. Kemp, P. Curran, M.D. William, J. G. Boyd, C. Muendel, *J. Org. Chem.* **1991**, *56*, 6672-6682).

Methyl (2S,3R)-1-benzoyl-3-[2-(tert-butoxy)-2-oxoethyl]-4-oxopyrrolidine-2-carboxylate (3)

A solution of *n*BuLi (1.6 N in hexane, 1.83 mL, 2.92 mmol) was added dropwise to a stirred solution of ketone **2** (687.9 mg, 2.79 mmol) in THF (5.3 mL) and dry HMPA (1.1 mL) at -78°C. The resulting mixture was stirred at -78°C for 30 minutes and then rapidly transferred with a cannula to a solution of NaI (251.0 mg, 1.67 mmol) and *t*buthylbromoacetate (1.23 mL, 8.37 mmol) in THF (5.3 mL) at -60°C. The reaction was allowed to warm to -42°C, stirred for an additional hour and treated with a 30% aqueous solution of H_3PO_4 (5.3 mL). H_2O (5.3 mL) was added and the resulting mixture was extracted with EtOAc, the organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 7:3) to give compound **3** (276.6 mg, 30%, single diastereoisomer) as a white solid. R*f* 0.25 (Hexane/EtOAc 7:3); $[\alpha]^{25}_D$ -36.1 (c: 1.0, $CHCl_3$); ESI MS *m/z*: $[M+H]^+$ calcd for $C_{19}H_{24}NO_6$ 361.15, $[M+Na]^+$ calcd for $C_{19}H_{23}NO_6Na$ 384.14, found (relative intensity) 362.1 (100) $[M+H]^+$, 384.1 (55) $[M+Na]^+$. (For the complete characterization refer to D.S. Kemp, P. Curran, M.D. William, J. G. Boyd, C. Muendel, *J. Org. Chem.* **1991**, *56*, 6672-6682).

5 Methyl (2S)-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate

A solution of methyl (2*S*, 4*R*)-4-hydroxypyrrolidine-2-carboxylate hydrochloride (3.0 g, 16.5 mmol) and chlorotrimethylsilane (5.2 mL, 41.3 mmol) in DCM (40 mL) at 0°C was treated with TEA (8.0 mL, 57.8 mmol) and allowed to reach r.t. The mixture was stirred at reflux for 1 h, cooled to 0°C, treated with MeOH (1.0 mL) in DCM (4.5 mL), allowed to warm to r.t. for 1 h and then treated with PfBr (6.9 g, 21.45 mmol) and $Pb(NO_3)_2$ (4.9 g, 14.9 mmol). The mixture was stirred at r.t. for 96 h, filtered and evaporated. The remaining solid was dissolved in MeOH (54 mL) and citric acid (5.5 g) was added. The solution was vigorously stirred for 1

additional hour, solvent was evaporated under reduce pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 1:1) to give the Pf protected compound (5.7 g, 90%) as a white foam. R*f* 0.42 (Hexane/EtOAc 1:1); $[\alpha]^{25}_{D}$ -139 (c: 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.79 (ddd, 1H, *J* = 5.6, 8.9, 13.0 Hz), 1.98 (dt, 1H, *J* = 5.6, 12.6 Hz), 2.92 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.24 (s, 3H), 3.29 (dd, 1H, *J* = 5.3, 8.8 Hz), 3.57 (dd, 1H, *J* = 5.3, 10.0 Hz), 4.43-4.58 (m, 1H), 7.16 (td, 1H, *J* = 1.1, 7.5 Hz), 7.21-7.35 (m, 6H), 7.43 (td, 1H, *J* = 1.1, 7.5 Hz), 7.50-7.59 (m, 3 H), 7.65 (dd, 1H, *J* = 0.7, 6.8 Hz), 7.74 (dd, 1H, *J* = 0.8, 6.8 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 40.0, 51.3, 56.8, 59.3, 70.4, 76.1, 119.8, 120.1, 126.4, 127.1, 127.2, 127.3 (x 2), 127.4, 127.6, 128.3 (x 2), 128.4, 128.8, 139.9, 141.5, 142.7, 146.1, 147.2, 175.8; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{25}H_{24}NO_3$ 385.17, $[M+Na]^+$ calcd for $C_{25}H_{23}NO_3Na$ 408.16, found (relative intensity) 385.2 (100) $[M+H]^+$, 408.2 (70) $[M+Na]^+$.

6 Methyl (2S)-4-oxo-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (4)

DMSO (2.3 mL, 29.5mmol) was added to a solution of oxalyl chloride (2M in DCM, 14.8 mmol, 7.4 mL) at -78°C. After 10 minutes a solution of methyl (2*S*)-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (2.2 g, 5.68 mmol) in DCM (38 mL) was added. The mixture was stirred at -78°C for 1 h, treated with TEA (18.2 mL) and allowed to reach r.t. A saturated aqueous solution of $NaHCO_3$ (82 mL) was added and the aqueous layer was extracted with DCM (3 x 150 mL). The combined organic layers were extracted, washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 8:2) to give compound **4** (1.96 g, 90%) as a white solid. R*f* 0.32 (Hexane/EtOAc 7:3); $[\alpha]^{25}_{D}$ -60 (c: 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.29 (dd, 1H, *J* = 2.6, 18.4 Hz), 2.44 (ddd, 1H, *J* = 0.7, 8.6, 18.4 Hz), 3.21 (s, 3H), 3.48 (dt, 1H, *J* = 1.0, 17.9 Hz), 3.76 (d, 1H, *J* = 17.9 Hz), 3.76 (dd, 1H, *J* = 2.9, 8.6 Hz), 7.22-7.32 (m, 6H), 7.36-7.40 (m, 2H), 7.40-7.48 (m, 3H), 7.69-7.74 (m, 2 H); ^{13}C NMR (100MHz, $CDCl_3$) d: 41.7, 51.5, 55.3, 58.2, 76.0, 120.1, 120.3, 125.5, 126.9 (x 2), 127.0, 127.6, 127.7, 128.1, 128.6 (x 2), 128.9, 129.0, 140.4, 140.9, 141.9, 145.4, 146.5, 173.1, 212.9; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{25}H_{22}NO_3$ 383.15, $[M+Na]^+$ calcd for $C_{25}H_{21}NO_3Na$ 406.14, found (relative intensity) 383.1 (100) $[M+H]^+$, 406.1 (60) $[M+Na]^+$.

7 Methyl (2S, 3R)-3-[2-tert-butoxy)-2-oxoethyl]-4-oxo-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (5a)

A solution of *n*BuLi (1.6 N in hexane, 3 mL, 4.8 mmol) was added drop wise to a stirred solution of ketone **4** (1.69 g, 4.4 mmol) in THF (9 mL) and dry HMPA (2 mL) at -78°C. The resulting mixture was stirred at -78°C for 30 minutes and then rapidly transferred with a cannula to a solution of NaI (396 mg, 2.6 mmol) and *t*buthylbromoacetate (1.95 mL, 13.2 mmol) in THF (9 mL) at -60°C. The reaction was allowed to warm to -42°C, stirred for an additional hour and treated with a 30% aqueous solution of H_3PO_4 (9 mL) H_2O (9 mL) was added and the resulting mixture was extracted with EtOAc, the organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 7:3) to give compound **5a** (1.7 g, 80%, dr 16:1) as a white solid. R*f* 0.58 (Hexane/EtOAc 6:4); $[\alpha]^{25}_D$ -51.4 (c: 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (s, 9 H), 2.46 (dd, 2H, *J* = 3.6, 6.3 Hz), 2.87 (dd, 1H, *J* = 5.8, 11.7 Hz), 3.14 (s, 3H), 3.49 (d, 1H, *J* = 17.7 Hz), 3.50 (d, 1H, *J* = 6.1 Hz), 3.82 (d, 1H, *J* = 17.7 Hz), 7.22-7.33 (m, 5H); 7.40 (t, 2H, *J* = 7.2 Hz), 7.47 (d, 1H, *J* = 7.4 Hz), 7.54 (d, 3H, *J* = 7.0 Hz), 7.73 (d, 2H, *J* = 7.4 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.9 (x 3), 34.3, 49.3, 51.5, 55.7, 63.7, 75.8, 81.4, 120.0, 120.2, 126.0, 127.0 (x 2), 127.3, 127.7, 127.9, 128.0, 128.5 (x 2), 128.9 (x 2), 140.5, 140.1, 141.5, 144.5. 146.1, 169.6, 172.9, 211.8; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{31}H_{32}NO_5$ 498.22, $[M+Na]^+$ calcd for $C_{31}H_{31}NO_5Na$ 520.21, found (relative intensity) 498.2 (10) $[M+H]^+$, 520.2 (100) $[M+Na]^+$.

Methyl-(2S, 3S)-3-[2-(tert-butoxy)-2-oxoethyl]-4-oxo-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (5b)

R*f* 0.60 (Hexane/EtOAc 6:4); $[\alpha]^{25}_D$ -89.2 (c: 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (s, 9 H), 1.83 (dd, 1H, *J* = 8.9, 17.4 Hz), 2.57 (dd, 1H, *J* = 5.2, 17.4 Hz), 3.00-3.11 (m, 1H), 3.15 (s, 3H), 3.58 (dd, 1H, *J* = 1.0, 17.6 Hz), 3.80 (d, 1H, *J* = 17.6 Hz), 4.02 (d, 1H, *J* = 8.2 Hz), 7.22-7.28 (m, 6H), 7.30-7.38 (m, 2H), 7.38-7.44 (m, 3H), 7.68 (d, 1H, *J* = 7.5 Hz), 7.73 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.9 (x 3), 31.1, 48.0, 51.1, 53.7, 61.9, 75.4, 81.1, 120.2 (x 2), 125.4, 126.7, 126.8 (x 2), 127.6, 127.7, 128.1, 128.6 (x 2), 128.9, 129.0, 140.0, 141.2, 141.8, 145.3, 146.9, 170.2, 171.8, 212.1; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{31}H_{32}NO_5$ 498.22, $[M+Na]^+$ calcd for $C_{31}H_{31}NO_5Na$ 520.21, found (relative intensity) 498.2 (20) $[M+H]^+$, 520.2 (100) $[M+Na]^+$.

Methyl-(2S,3R,4S)-3-[2-(tert-butoxy)-2-oxoethyl]-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)-4-(trifluoromethyl)pyrrolidine-2-carboxylate (6)

To a solution of ketone **5a** (100 mg, 0.27 mmol), in DMF (4.2 mL), were added trimethylsilyl trifluoromethane (0.2 mL, 1.15 mmol) and Cs_2CO_3 (105 mg, 0.34 mmol). After stirring for 2 h TBAF (1M in THF, 1.6 mL) was added. After 30 minutes the mixture was diluted with H_2O (9 mL) and extracted with EtOAc (3 x 20 mL). Combined organic layer were washed with H_2O, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 1:1) to give compound **6** (56 mg, 48%) as a white solid. R*f* 0.55 (Hexane/EtOAc 1:1); $[\alpha]^{25}_D$ +142.2 (c: 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (s, 9 H), 1.73 (dd, 1H, *J* = 10.7, 16.1 Hz), 2.35 (dd, 1H, *J* = 4.3, 16.1 Hz), 2.55-2.69 (m, 1H), 2.90 (d, 1H, *J* = 3.4 Hz), 3.27 (s, 3H), 3.36 (d, 1H, *J* = 10.0 Hz), 3.55 (d, 1H, *J* = 10.0 Hz), 5.96 (s, OH), 7.19 (td, 1H, *J* = 1.1, 7.5 Hz), 7.22-7.38 (m, 5H), 7.40-7.46 (m, 1H), 7.51 (dd, 2H, *J* = 4.3, 10.8 Hz), 7.55-7.61 (m, 2H), 7.67 (d, 1H, *J* = 7.5 Hz), 7.80 (dd, 1H, *J* = 1.8, 6.6 Hz); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: -74.6; ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.8 (x 3), 35.7, 48.0, 52.4, 54.4, 64.5, 75.4, 79.8 (q, $J_{C\text{-}F}$ = 28.8 Hz), 81.5, 120.0, 120.3, 124.6 (q $J_{C\text{-}F}$ = 283.0 Hz), 126.3, 127.0, 127.3, 127.4 (x 2), 127.8, 128.0, 128.6 (x 2), 128.8, 129.2, 139.7, 140.8, 141.5, 144.7, 146.7, 170.1, 177.0; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{32}H_{33}F_3NO_5$ 568.22, $[M+Na]^+$ calcd for $C_{32}H_{32}F_3NO_5Na$ 590.21, found (relative intensity) 568.2 (80) $[M+H]^+$, 590.2 (100) $[M+Na]^+$.

(2S,3R,4S)-3-(carboxymethyl)-4-hydroxy-4-(trifluoromethyl)pyrrolidine-2-carboxylic acid (1a)

To a solution of compound **6** (50.0 mg, 0.089 mmol) in toluene (1 mL) at r.t. was added drop wise aqueous phosphoric acid (85 wt %, 0.02 mL, 0.45 mmol). The mixture was stirred for 2 h and the progress was checked by mass analysis {ESI MS *m/z*: $[M+H]^+$ calcd for $C_9H_{13}F_3NO_5$ 272.07, $[2M+Na]^+$ calcd for $C_{18}H_{24}$ $F_6N_2O_{10}Na$ 565.13, found (relative intensity) 272.1 (100) $[M+H]^+$, 565.1 (30) $[2M+Na]^+$}. Once the reaction was completed, water was added (5 mL), and the mixture was washed with hexane (3 x 10 mL). The pH of the aqueous layer was adjusted to 7 (adding a solution of LiOH 0.5 N in water) and the solvent was evaporated under reduce pressure in order to obtain a pale yellow solid. The crude product was diluted with water (2 mL) and an aqueous solution of LiOH is added (0.5 N, 0.35 mL, 0.18 mmol). The mixture was stirred for 6 h at r.t. (progress was checked by mass analysis) then the pH is neutralised by addition of aqueous HCl (1N in water) and the

solvent was evaporated under reduced pressure. The crude product was purified by HPLC (Semi-preparative C18 Luna column, Eluent A: H_2O in isocratic condition, 5mL/min, Retention Time: 9.5 min) in order to obtain compound **1a** (11 mg, 48% in two steps) as a white solid. $[\alpha]^{25}_D$ +20.2 (c: 0.7, H_2O); ^{1}H NMR (400 MHz, D_2O) δ: 2.70 (dd, 1H, *J* = 7.2, 17.4 Hz), 2.80 (ddd, 1H, *J* = 1.4, 7.0, 17.4 Hz), 3.11 (q, 1H, *J* = 6.8 Hz), 3.59 (d, 1H, *J* = 13.2 Hz), 3.79 (d, 1H, *J* = 13.2 Hz), 4.07 (d, 1 H, *J* = 6.3 Hz); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: -75.7; ^{13}C NMR (100MHz, D_2O) δ: 34.9, 46.7, 50.2, 64.7, 80.1(q, $J_{C\text{-}F}$ = 30.0 Hz), 124.0 (q, $J_{C\text{-}F}$ = 284.2 Hz), 171.9, 175.5; ESI MS *m/z*: $[M+H]^+$ calcd for $C_8H_{11}F_3NO_5$ 258.05, found (relative intensity) 258.0 (100) $[M+H]^+$; HRMS calcd. for $C_8H_{11}F_3NO_5$: 258.0511, found: 258.0586.

Methyl-(2S,3R,4S)-3-[2-(tert-butoxy)-2-oxoethyl]-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (7)

To a stirred solution of ketone **5a** (433 mg, 0.87 mmol) in MeOH (14.0 mL), $NaBH_4$ (32.4 mg, 0.95 mmol) was added. After 2 h the mixture was poured into a saturated aqueous solution of NH_4Cl (20 mL) and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with aqueous HCl (0.1N in water), brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 8:2) to give compound **7** (391 mg, 90%) as a white solid and single diastereoisomer. R*f* 0.40 (Hexane/EtOAc 6:4); $[\alpha]^{25}_D$ +191 (c: 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (s, 9 H), 1.73 (dd, 1H, *J* = 8.4, 15.6 Hz), 1.90 (dd, 1H, *J* = 7.4, 15.6 Hz), 2.33 (t, 1H, *J* = 7.9 Hz), 2.70 (d, 1H, *J* = 2.8 Hz), 3.20 (dd, 1H, *J* = 4.1, 10.2 Hz), 3.32 (s, 3H), 3.44 (dd, 1H, *J* = 1.3, 10.2 Hz), 3.83 (ddd, 1H, *J* = 2.0, 3.9, 8.4 Hz), 4.46 (d, OH, *J* = 10.4 Hz), 7.15 (td, 1H, *J* = 1.1, 7.5 Hz), 7.22-7.35 (m, 5H), 7.39 (td, 1H, *J* = 1.1 Hz), 7.49 (t, 2H, *J* = 7.1 Hz), 7.58 (d, 2H, *J* = 6.9 Hz) 7.65 (d, 1H, *J* = 7.5 Hz), 7.79 (dd, 1H, *J* = 1.6, 6.9 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.9 (x 3), 38.5, 48.3, 51.9, 55.2, 64.1, 75.4, 75.2, 81.1, 120.0, 120.2, 126.2, 126.9, 127.2, 127.4 (x 2), 127.5, 127.8, 128.5 (x 3), 128.9, 139.3, 141.7, 141.8, 145.2, 147.8, 170.5, 177.6; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{31}H_{34}NO_5$ 500.24, found (relative intensity) 500.3 (100) $[M+H]^+$.

Methyl (2S,3R,4S)-3-[2-(tert-butoxy)-2-oxoethyl]-4-methoxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (8)

To an ice cooled solution of alcohol **7** (50 mg, 0.10 mmol), in DMF (1 mL), NaH (60% in mineral oil, 8.8 mg, 0.22 mmol) was added. The mixture was allowed to stir for 30 minutes at 0 °C, and then MeI (0.007 mL, 0.11 mmol) was added. The resulting solution was stirred for 3h at r.t. then was quenched with a few drops of H_2O and 5 mL of a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with Et_2O (2 x 10 mL), the combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 8:2) to give compound **8** (36 mg, 70%) as a white solid. R*f* 0.45 (Hexane/EtOAc 6:4); $[\alpha]^{25}_D$ +180 (c: 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (s, 9 H), 2.03 (d, 2H, *J* = 7.1 Hz), 2.51-2.61 (m, 1H), 2.74 (d, 1H, *J* = 6.8 Hz), 3.27 (s, 3H), 3.31 (s, 3H), 3.35-3.49 (m. 3H), 7.15 (td, 1H, *J* = 1.1, 7.5 Hz), 7.22-7.38 (m, 6H), 7.44 (ddd, 2H, *J* = 3.2, 7.0, 8.5 Hz), 7.58 (d, 2H, *J* = 6.9 Hz), 7.64 (d, 1H, *J* = 7.4 Hz), 7.76 (d, 1H, *J* = 7.5 Hz);^{13}C NMR (100MHz, $CDCl_3$) δ: 27.9 (x 3) , 31.6, 37.8, 45.8, 51.3, 53.4, 57.5, 65.2, 76.5, 80.6, 83.0, 119.7, 120.2, 125.8, 127.1, 127.2 (x 2), 127.3, 127.5, 128.3, 128.4 (x 2), 128.8, 139.5, 141.8, 143.1, 146.3, 147.1, 170.5, 174.6; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{32}H_{36}NO_5$ 513.25, found (relative intensity) 513.2 (100) $[M+H]^+$.

(2S,3R,4S)-3-(carboxymethyl)-4-methoxypyrrolidine-2-carboxylic acid (1b)

To a solution of compound **8** (79.3 mg, 0.15 mmol) in toluene (2 mL) at r.t. was added drop wise aqueous phosphoric acid (85 wt %, 0.03 mL, 0.76 mmol). The mixture was stirred for 2 h and the progress was checked by mass analysis {ESI MS *m/z*: $[M+H]^+$ calcd for $C_9H_{16}NO_5$ 218.10, found (relative intensity) 218.1 (100) $[M+H]^+$}. Once the reaction was completed, water was added (8 mL), and the mixture was washed with hexane (3 x 15 mL). The pH of the aqueous layer was adjusted to 7 (adding a solution of LiOH 0.5 N in water) and the solvent was evaporated under reduce pressure in order to obtain a pale yellow solid. The crude product was diluted with water (2 mL) and an aqueous solution of LiOH is added (0.5 N, 0.60 mL, 0.30 mmol). The mixture was stirred for 4 h at r.t. (progress was checked by mass analysis) then the pH is neutralised by addition of aqueous HCl (1N in water) and the solvent was evaporated under reduced pressure. The crude product was purified by HPLC (Semi-preparative C18 Luna column, Eluent A: H_2O in isocratic condition, 5mL/min, Retention Time: 13.2 min) in order to obtain compound **1b** (18 mg, 60% in two steps) as a white solid.$[\alpha]^{25}_D$ +16.2 (c: 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ: 2.29 (dd, 1H, *J* = 9.9, 15.3 Hz), 2.50 (dd, 1H, *J* = 6.1, 15.4 Hz), 3.05-3.10 (m, 1H), 3.31 (s, 3H), 3.54 (dd, 1H, *J* =

4.1, 13.3 Hz), 3.61 (d, 1H, *J* = 13.3 Hz), 3.94 (d, 1H, *J* = 3.0 Hz), 3.96 (d, 1H, *J* = 4.0 Hz);^{13}C NMR (100MHz, D_2O) δ: 39.4, 44.6, 49.2, 56.0, 64.6, 82.8, 173.4, 178.8; ESI MS *m/z*: $[M+H]^+$ calcd for $C_8H_{14}NO_5$ 204.08, found (relative intensity) 204.1 (100) $[M+H]^+$; HRMS calcd. for $C_8H_{12}NO_5$: 202.0794, found: 202.0723.

Methyl (2S,3R,4R)-3-[2-(tert-butoxy)-2-oxoethyl]-4-ethynyl-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (9)

To a solution of (trimethylsilyl)acetylene (0.31 mL, 2.2 mmol) in THF (8.0 mL), *n*BuLi (1.6 N in hexane, 1.4 mL, 2.4 mmol) was added dropwise at -10°C and the resulting mixture was stirred for 1 h. Ketone **5a** (1.0 g, 2.0 mmol) in THF (4.0 mL), was added, after 4 h at -10°C, the temperature was raised to 0°C and NaOH (208 mg, 5.2 mmol) in MeOH (4.0 mL) was added. The mixture was warmed to r.t., the pH was adjusted to 7 adding concentrated acetic acid and the solution was poured into H_2O (53 mL). The aqueous layer was extracted with EtOAc (3 x 100mL), the combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 7:3) to give compound **9** (1.5 g, 70%) as a white solid. R*f* 0.45 (Hexane/EtOAc 7:3); $[\alpha]^{25}_D$ +180.2 (c: 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (s, 9 H), 1.49 (dd, 1H, *J* = 10.0, 15.5 Hz), 2.32-2.40 (m, 1H), 2.41 (s, 1H), 2.43 (dd, 1H, *J* = 2.5, 15.5 Hz), 2.73 (d, 1H, *J* = 2.5 Hz), 3.14 (d, 1H, *J* = 9.9 Hz), 3.16 (s, 3H), 3.52 (d, 1H, *J* = 9.7 Hz), 5.42 (bs, OH), 7.05 (td, 1H, *J* = 0.9, 7.5 Hz), 7.12-7.24 (m, 5H), 7.32 (t, 1H, *J* = 7.5 Hz), 7.41 (dd, 2H, *J* = 6.2, 13.6 Hz), 7.48 (d, 2H, *J* = 6.9 Hz), 7.55 (d, 1H, *J* = 7.5 Hz), 7.70 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.8 (x 3), 38.4, 50.9, 52.2, 59.8, 64.0, 74.6, 75.2, 75.3, 81.0, 81.1, 120.0, 120.2, 126.4, 127.0, 127.2, 127.4 (x 2), 127.6, 127.8, 128.5 (x 2), 128.6, 129.0, 139.4, 141.0, 141.7, 144.5, 147.3, 170.2, 177.4; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{33}H_{34}NO_5$ 524.24, found (relative intensity) 524.2 (100) $[M+H]^+$.

Methyl (2S,3R,4S)-3-[2-(tert-butoxy)-2-oxoethyl]-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)-4-(1H-1,2,3-triazol-4-yl)pyrrolidine-2-carboxylate (10)

To solution of alkyne **9** (570 mg, 1.0 mmol) and trimethylsilyl azide (0.17 mL, 1.2 mmol) in DMF/H_2O (4:1, 8.0 mL), $CuSO_4$ (8.0 mg, 0.05 mmol) and sodium ascorbate (79.3 mg, 0.4 mmol) were added. The reaction mixture was placed in a microwave reactor and irradiated for 30 minutes at 120 °C. The solution was cooled, ice was added, and it was extracted with EtOAc (3 x 10 mL). The organic layers were washed with H_2O, brine, dried over Na_2SO_4,

filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 1:1) to give compound **10** (397 mg, 70%) as a white solid. R*f* 0.20 (Hexane/EtOAc 1:1); $[\alpha]^{25}_{D}$ +101 (c: 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.11 (s, 9 H), 1.42 (dd, 1H, *J* = 10.4, 15.9 Hz), 1.69 (dd, 1H, *J* = 5.3, 15.9 Hz), 2.42 (dd, 1H, *J* = 5.3, 10.4 Hz), 2.75 (d, 1H, *J* = 1.6 Hz), 3.24 (s, 3H), 3.53 (d, 1H, *J* = 9.7 Hz), 3.67 (d, 1H, *J* = 9.7 Hz), 5.89 (s, OH), 7.06 (td, 1H, *J* = 7.5 Hz), 7.12-7.23 (m, 4 H), 7.26 (d, 1H, *J* = 7.5 Hz), 7.37 (td, 1H, *J* = 1.2, 7.5 Hz), 7.44 (td, 1H, *J* = 1.1, 7.5 Hz), 7.53 (dd, 4H, *J* = 1.9, 7.1 Hz), 7.57 (d, 1H, *J* = 7.5 Hz), 7.65 (s, 1H), 7.73 (d, 1H, *J* = 7.0 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ: 27.7 (x 3), 38.3, 51.4, 52.3, 57.5, 64.5, 75.4, 78.0, 80.8, 120.0, 120.3, 126.5, 127.0, 127.2, 127.5 (x 2), 127.6, 128.0, 128.5 (x 2), 128.6, 129.0, 130.4, 130.8, 139.3, 141.0, 141.9, 144.4, 147.6, 169.9, 178.3; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{33}H_{35}N_4O_5$ 566.25, found (relative intensity) 566.2 (100) $[M+H]^+$.

(2S,3R,4S)-3-(carboxymethyl)-4-hydroxy-4-(1H-1,2,3-triazol-4-yl)pyrrolidine-2-carboxylic acid (1c)

To a solution of compound **10** (124.0 mg, 0.22 mmol) in toluene (5 mL) at r.t. was added drop wise aqueous phosphoric acid (85 wt %, 0.05 mL, 1.11 mmol). The mixture was stirred for 2 h and the progress was checked by mass analysis {ESI MS *m/z*: $[M+H]^+$ calcd for $C_{10}H_{14}N_4O_5$ 271.10, found (relative intensity) 271.1 (100) $[M+H]^+$}. Once the reaction was completed, water was added (10 mL), and the mixture was washed with hexane (3 x 20 mL). The pH of the aqueous layer was adjusted to 7 (adding a solution of LiOH 0.5 N in water) and the solvent was evaporated under reduce pressure in order to obtain a pale yellow solid. The crude product was diluted with water (4 mL) and an aqueous solution of LiOH is added (0.5 N, 0.86 mL, 0.44 mmol). The mixture was stirred for 6 h at r.t. (progress was checked by mass analysis) then the pH is neutralised by addition of aqueous HCl (1N in water) and the solvent was evaporated under reduced pressure. The crude product was purified by HPLC (Semi-preparative C18 Luna column, Eluent A: H_2O in isocratic condition, 5mL/min, Retention Time: 3.0 min) in order to obtain compound **1c** (37 mg, 65% in two steps) as a white solid.$[\alpha]^{25}_{D}$ -16.3 (c: 1.1, D_2O); ^{1}H NMR (400 MHz, D_2O) δ: 2.44 (dd, 1H, *J* = 7.8, 17.1 Hz), 2.53 (dd, 1H, *J* = 7.0, 17.1 Hz), 3.38 (td, 1H, *J* = 4.2, 7.2 Hz), 3.80 (d, 1H, *J* = 12.6 Hz), 4.12 (d, 1H, *J* = 12.6 Hz), 4.56 (d, 1H, *J* = 3.8 Hz), 8.01 (s, 1H); ^{13}C NMR (100MHz, D_2O) δ: 39.6, 54.2, 58.7, 68.6, 82.5, 131.0, 149.1, 175.9, 179.0; MS (ESI), calculated m/z

$C_9H_{12}N_4O_5$ 257.08 $[M+H]^+$, found m/z (relative intensity) 257.0 (100) [M+H]+; HRMS calcd. for $C_9H_{12}N_4O_5$: 257.0808, found: 257.0884.

5-[(3aS,4S,6aR)-2-oxo-hexahydrothieno[3,4-d]imidazolidin-4-yl]-N-(20-azido 3,6,9,12,15,18-hexaoxaicosan-1-yl)pentanamide (12)

To a solution of D-(+)-biotin (100 mg, 0.40 mmol) and *N*-hydroxysuccinimide (51 mg, 0.44mmol) in DMF (5.0 mL) was added EDC·HCl (92 mg, 0.2 mmol). After being stirred for 24 h at room temperature, the reaction solution was concentrated to give white solid. The solid was washed with methanol several times, and excess solvent was evaporated under reduced pressure. The crude product **11** was characterized by mass {ESI MS *m/z*: $[M+H]^+$ calcd for $C_{14}H_{19}N_3O_5S$ 341.10, found (relative intensity) 341.0 (100) $[M+H]^+$} and submitted to the next step without further purification. To a solution of crude *N*-hydroxysuccinimido biotin (0.40 mmol) and *O*-(2-Aminoethyl)-*O′*-(2-azidoethyl)pentaethylene glycol (70 mg, 0.40 mmol) in DMF (2.0 mL) was added TEA (0.06 mL, 0.40 mmol). After being stirred for 24 h at room temperature, the solvent was evaporated under reduced pressure and the resulting residue was diluted in DCM (30 mL). The organic layer was washed with H_2O, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (MeOH/ DCM 5:95) to give compound **12** (76.3 mg, 98%) as a white solid. R*f* 0.52 (MeOH/ DCM 10:90); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.40-1.52 (m, 2H), 1.55-1.82 (m, 4H), 2.24 (t, 2H, *J* = 7.4 Hz), 2.73 (d, 1H, *J* = 12.7 Hz), 2.95 (dd, 1H, *J* = 5.0, 12.7 Hz), 3.23 (ddd, 1H, *J* = 4.6, 5.8, 9.0 Hz), 3.39 (dd, 4H, *J* = 5.4, 10.7 Hz), 3.56 (t, 2H, *J* = 5.6 Hz), 3.60-3.74 (m, 22H), 4.33 (dd, 1H, *J* = 4.5, 7.9 Hz), 4.51 (ddd, 1H, *J* = 0.7, 4.9, 7.9 Hz); ^{13}C NMR (100MHz, CD_3OD) δ: 25.4, 28.1, 28.4, 35.3, 39.0, 39.7, 50.4, 55.6, 60.2, 62.0, 69.2, 69.7, 69.9, 70.1, 70.2 (x 6), 70.3 (x 2), 164.7, 174.7; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{24}H_{45}N_6O_8S$ 576.29, $[M+Na]^+$ calcd for $C_{24}H_{44}N_6O_8S$ Na 599.28, found (relative intensity) 577.3 (45) $[M+H]^+$, 599.3 (100) $[M+Na]^+$.

Methyl (2S,3R,4S)-4-[1-(20-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazolidin-4-yl]pentanamido}-3,6,9,12,15,18-hexaoxaicosan-1-yl)-1H-1,2,3-triazol-4-yl]-3-[2-(tert-butoxy)-2-oxoethyl]-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate (13)

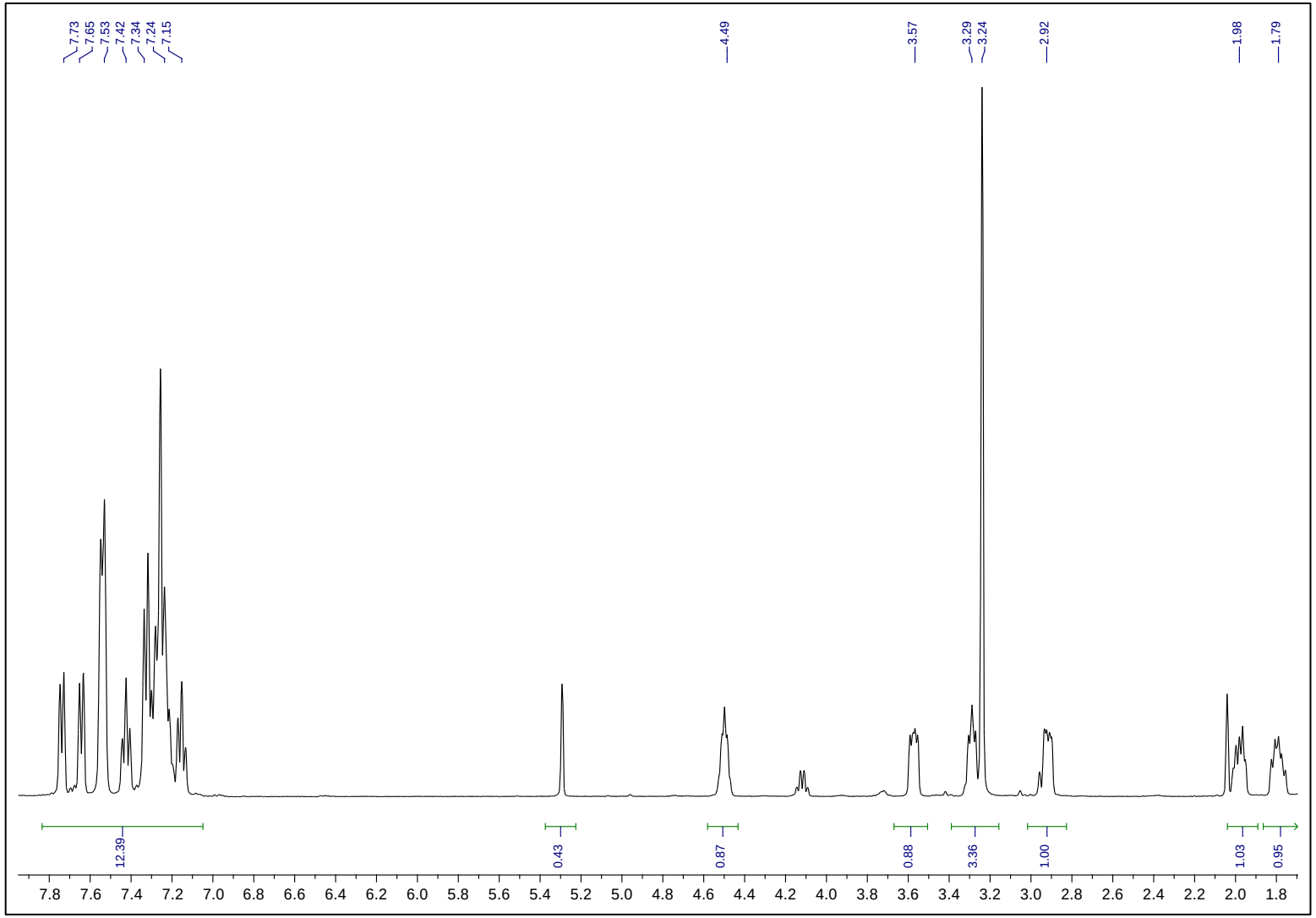
To a solution of biotin azide **12** (54.7 mg, 0.13 mmol) and alkyne **9** (50.0 mg, 0.095 mmol) in *t*BuOH-H_2O (1:1, 0.5 mL) and THF (0.5 mL) at r.t. were added $CuSO_4$ (3.0 mg, 0.012 mmol) and sodium ascorbate (1.0 M in H_2O, 3 drops). The reaction mixture was stirred at r.t. for 36 h and then the solvent was evaporated. The residue was diluted with H_2O (2 mL) and the mixture was extracted with DCM (3 x 5 mL). The combined organic extract was washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (MeOH/ DCM 5:95) to give compound **13** (87.7 mg, 83%) as a white solid. R*f* 0.54 (MeOH/ DCM 10:90); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.23 (s, 9 H), 1.39-1.52 (m, 3H), 1.54-1.81 (m, 5H), 2.23 (t, 2H, *J* = 6.1 Hz), 2.46 (dd, 1H, *J* = 4.5, 11.2 Hz), 2.71 (d, 1H, *J* = 12.7 Hz), 2.87 (d, 1H, *J* = 1.7 Hz), 2.93 (dd, 1H, *J* = 5.0, 12.7 Hz), 3.17-3.26 (m, 1H), 3.35-3.42 (m, 4 H), 3.49-3.73 (m, 24 H), 3.86 (d, 1H, *J* = 9.6 Hz), 3.88 (t, 2H, *J* = 5.0 Hz), 4.30 (dd, 1H, *J* = 4.6, 7.9 Hz), 4.49 (dd, 1H, *J* = 4.6, 8.2 Hz) 4.55 (dd, 2H, *J* = 4.1, 5.6 Hz) 7.18 (dd, 1H, *J* = 4.6, 7.9 Hz), 7.23-7.38 (m, 5H), 7.50 (td, 1H, *J* = 1.2, 7.5 Hz), 7.57 (td, 1H, *J* = 1.2, 7.5 Hz), 7.61 (d, 2H, *J* = 7.2 Hz), 7.74 (t, 2H, *J* = 7.1 Hz), 7.92 (d, 1H, *J* = 7.1 Hz), 8.02 (s, 1H); ^{13}C NMR (100MHz, CD_3OD) δ: 25.4, 26.7 (x 3), 28.1, 28.4, 35.3, 37.7, 39.0, 39.6, 50.1, 50.4, 51.4, 51.6, 55.6, 56.9, 60.2, 62.0, 64.6, 68.8, 69.2, 69.7, 69.9, 70.0, 70.2 (x 6), 75.5, 77.7, 80.5, 119.8, 120.1, 124.04, 126.7 (x 2), 126.3, 127.2 (x 2), 127.3, 127.7, 128.2 (x 2), 128.5, 129.0, 139.3, 141.5, 142.1, 144.4, 147.0, 147.7, 164.7, 170.1, 174.7, 178.3; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{57}H_{78}N_7O_{13}S$ 1099.53, $[M+Na]^+$ calcd for $C_{57}H_{77}N_7O_{13}SNa$ 1122.52, found (relative intensity) 1100.4 (100) $[M+H]^+$, 1122.4 (80) $[M+Na]^+$.

(2S,3R,4S)-4-[1-(20-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazolidin-4-yl]pentanamido}-3,6,9,12,15,18-hexaoxaicosan-1-yl)-1H-1,2,3-triazol-4-yl]-3-(carboxymethyl)-4-hydroxypyrrolidine-2-carboxylic acid (14)

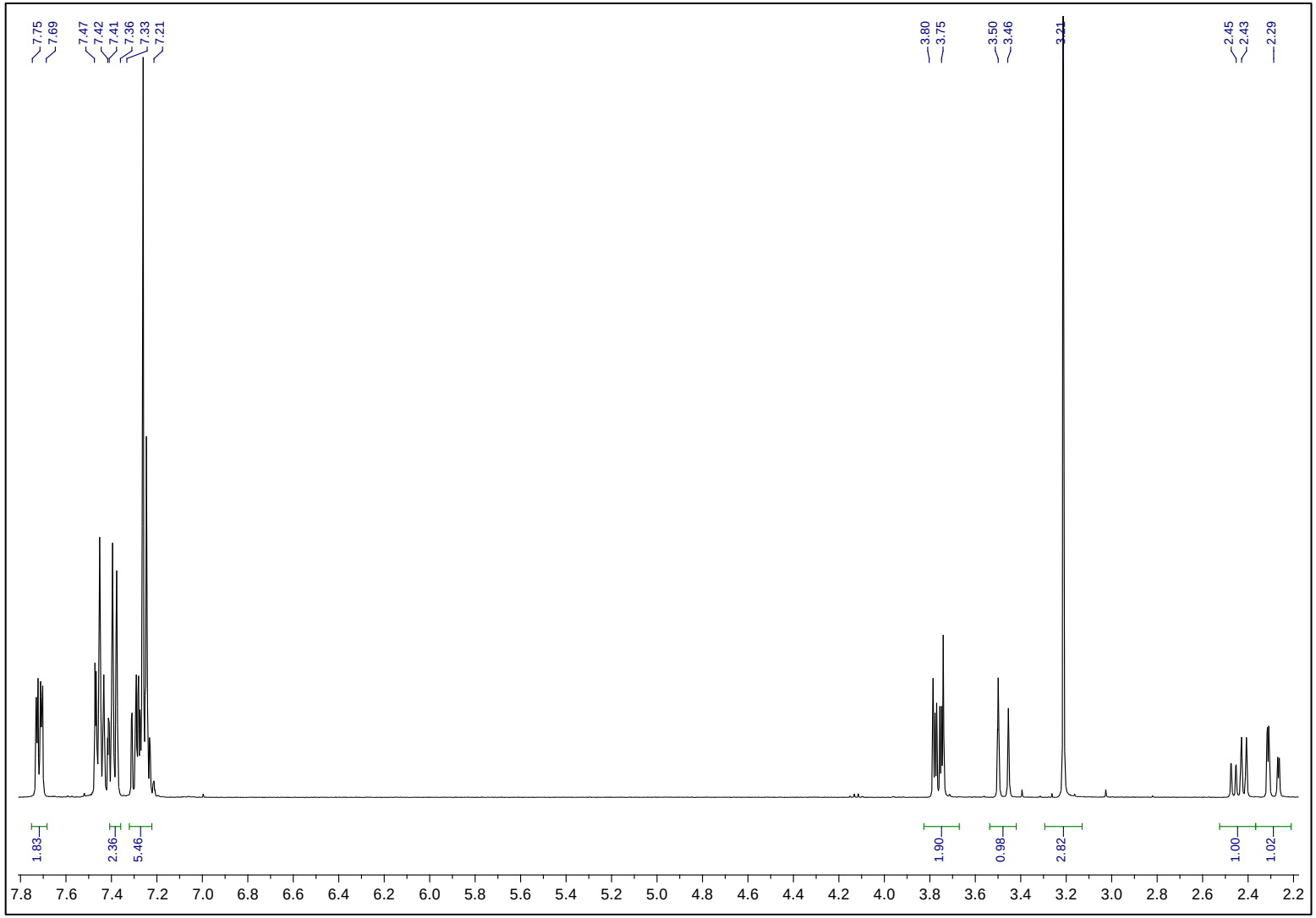
To a solution of compound **13** (80.0 mg, 0.07 mmol) in DCM (1 mL) at r.t. was added drop wise aqueous phosphoric acid (85 wt %, 0.016 mL, 0.35 mmol). The mixture was stirred for 24 h and the progress was checked by mass analysis {ESI MS *m/z*: $[M+H]^+$ calcd for $C_{34}H_{58}N_7O_{13}S$ 803.37, found (relative intensity) 804.3 (100) $[M+H]^+$}. Once the reaction was completed, water was added (2 mL), and the mixture was washed with hexane (3 x 5 mL). The pH of the aqueous layer was adjusted to 7 (adding a solution of LiOH 0.5 N in water) and the solvent was evaporated under reduce pressure in order to obtain a pale yellow solid. The crude product was diluted with water (1 mL) and an aqueous solution of LiOH is added

(0.5 N, 0.27 mL, 0.14 mmol). The mixture was stirred for 12 h at r.t. (progress was checked by mass analysis) then the pH is neutralised by addition of aqueous HCl (1N in water) and the solvent was evaporated under reduced pressure. The crude product was purified by HPLC (Semi-preparative C18 Luna column, Eluent A: H_2O, Eluent B: CH_3CN, method, linear gradient from 2% B to 40% B in 15 min, 5mL/min, Retention time: 11.7 min) in order to obtain compound **14** (31.5 mg, 50% in two steps) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ: 1.29-1.39 (m, 2H), 1.44-1.72 (m, 4H), 2.20 (t, 2H, *J* = 7.3Hz), 2.32 (dd, 1H, *J* = 8.6, 16.9 Hz), 2.43 (dd, 1H, *J* = 5.9, 16.9 Hz), 2.70 (d, 1H, *J* = 13.0 Hz), 2.92 (dd, 1H, *J* = 5.0, 13.1 Hz), 3.11-3.19 (m, 1H), 3.21-3.29 (m, 1H), 3.31 (t, 2H, *J* = 5.3 Hz), 3.55 (t, 2H, *J* = 5.3 Hz), 3.51-3.64 (m, 20H), 3.67 (d, 1H, *J* = 12.6 Hz), 3.90 (t, 2H, *J* = 5.0 Hz), 3.98 (d, 1H, *J* = 12.6 Hz), 4.09 (d, 1H, *J* = 4.6 Hz), 4.35 (dd, 1H, *J* = 4.5, 7.9 Hz), 4.53 (dd, 1H, *J* = 4.5, 7.9 Hz), 4.57 (t, 2H, *J* = 5.0 Hz), 8.03 (s, 1H); ^{13}C NMR (100MHz, D_2O): 25.1, 27.7, 27.9, 35.4, 35.8, 38.9, 39.7, 49.8, 50.1, 53.6, 55.3, 60.2, 62.1, 65.1, 68.8, 68.9, 69.4, 69.6 (x 8), 69.7, 77.7, 124.9, 146.2, 165.3, 172.8, 174.8, 176.9; ESI MS *m/z*: $[M+H]^+$ calcd for $C_{33}H_{56}N_7O_{13}S$ 790.36, found (relative intensity) 790.3 (100) $[M+H]^+$; HRMS calcd. for $C_{33}H_{56}N_7O_{13}S$: 790.3657, found: 790.3632.

^{1}H NMR of ***5 Methyl (2S)-4-hydroxy-1-(9-phenyl-9H-fluoren-9-yl)pyrrolidine-2-carboxylate***



^{1}H NMR of **4**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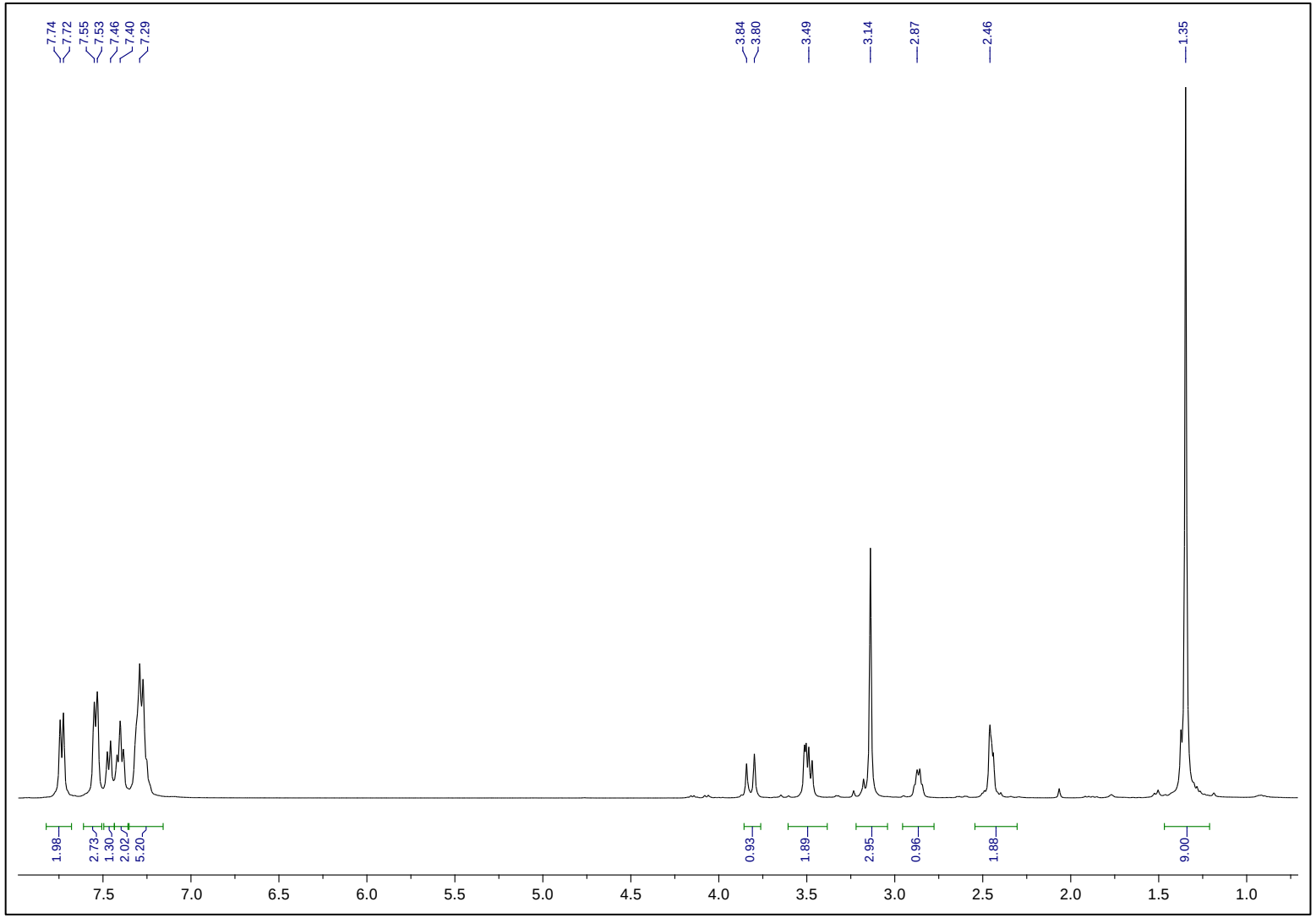
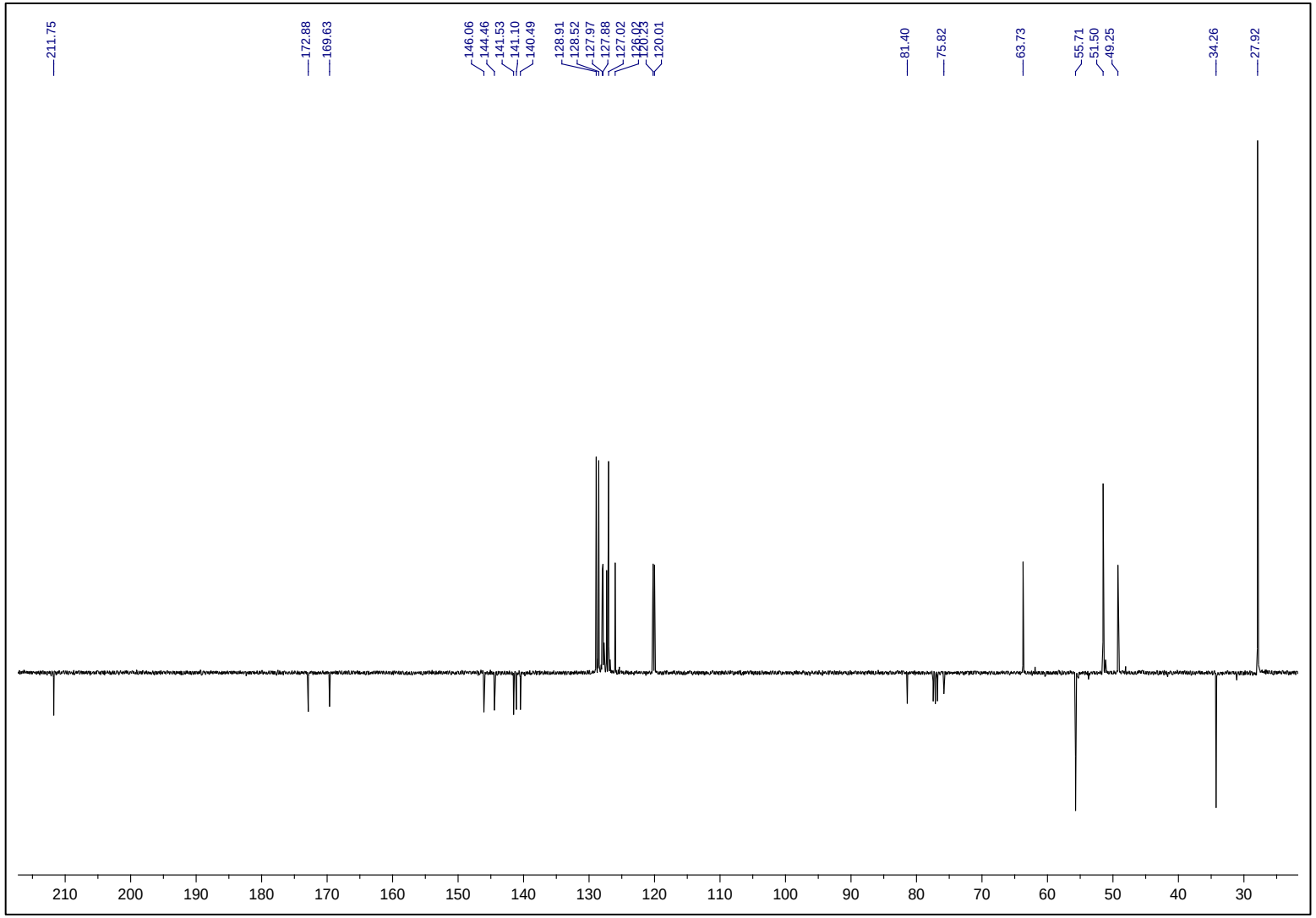
1.00
1.02
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2

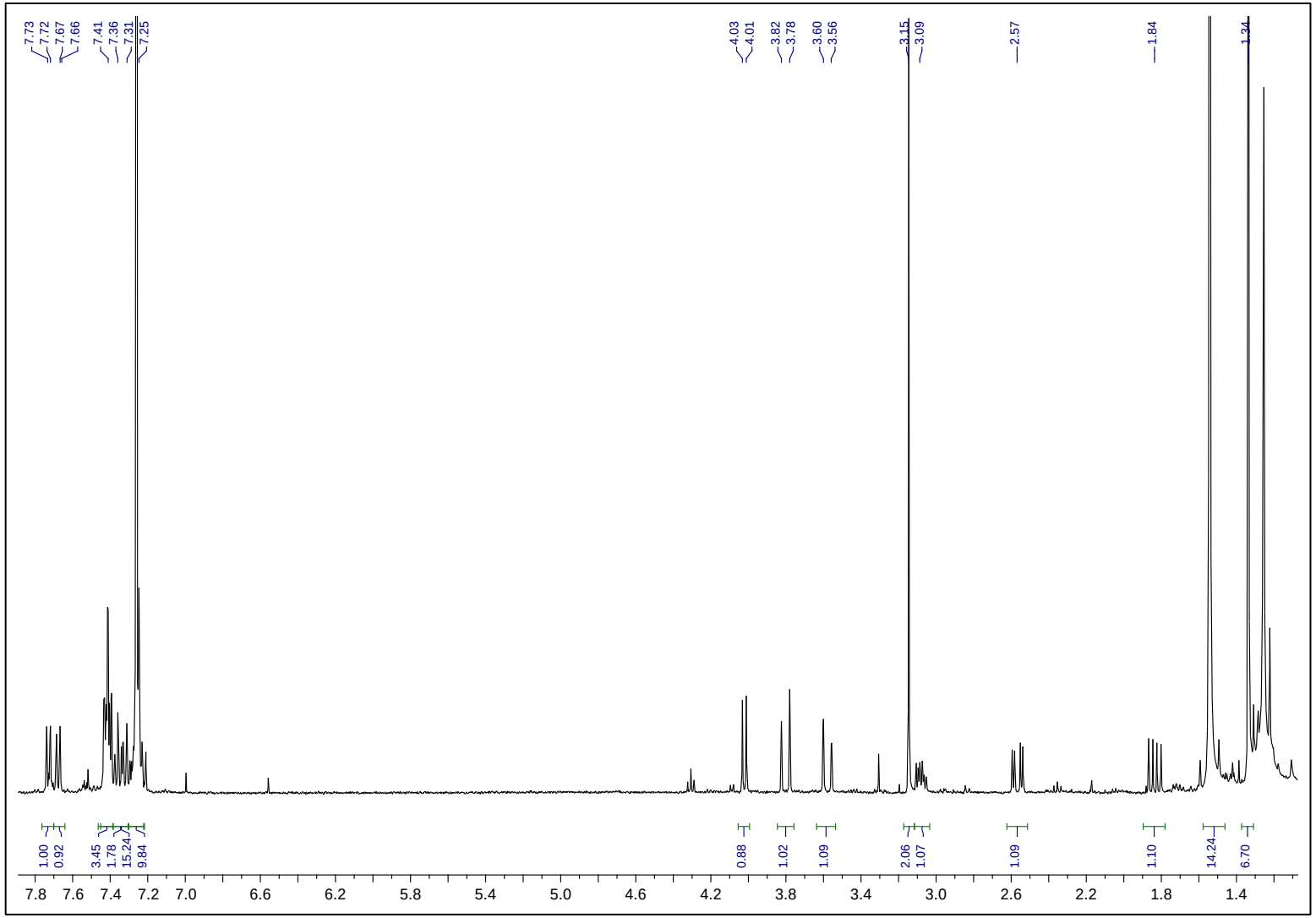
^{1}H NMR of **5a**


7.74
7.72
7.55
7.53
7.46
7.40
7.29
3.84
3.80
3.49
3.14
2.87
2.46
1.35
1.98
2.73
1.30
2.02
5.20
0.93
1.89
2.95
0.96
1.88
9.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

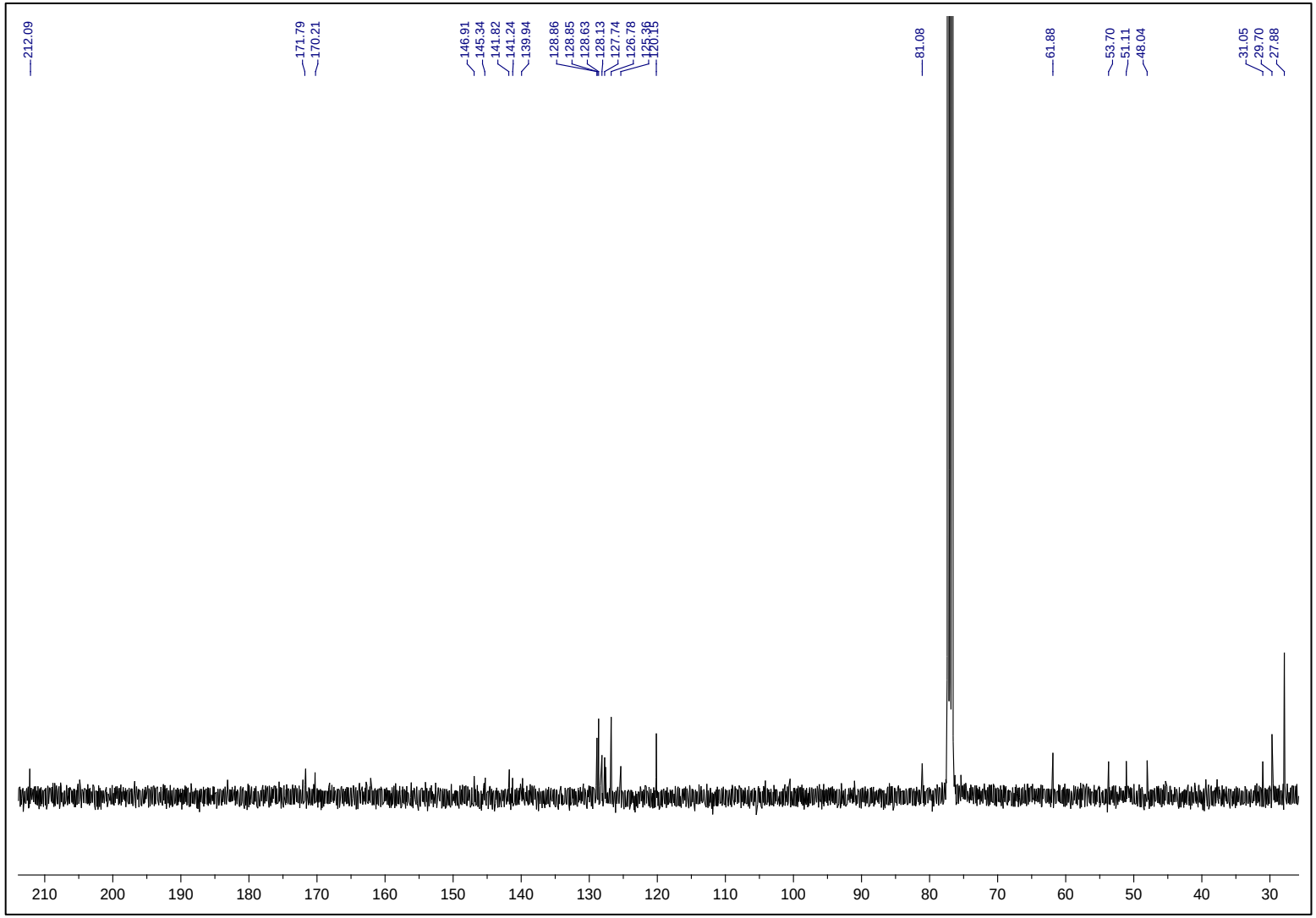
^{13}C NMR of **5a**


211.75
172.88
169.63
146.06
144.46
141.53
141.10
140.49
128.91
128.52
127.97
127.88
127.02
120.23
120.01
81.40
75.82
63.73
55.71
51.50
49.25
34.26
27.92
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

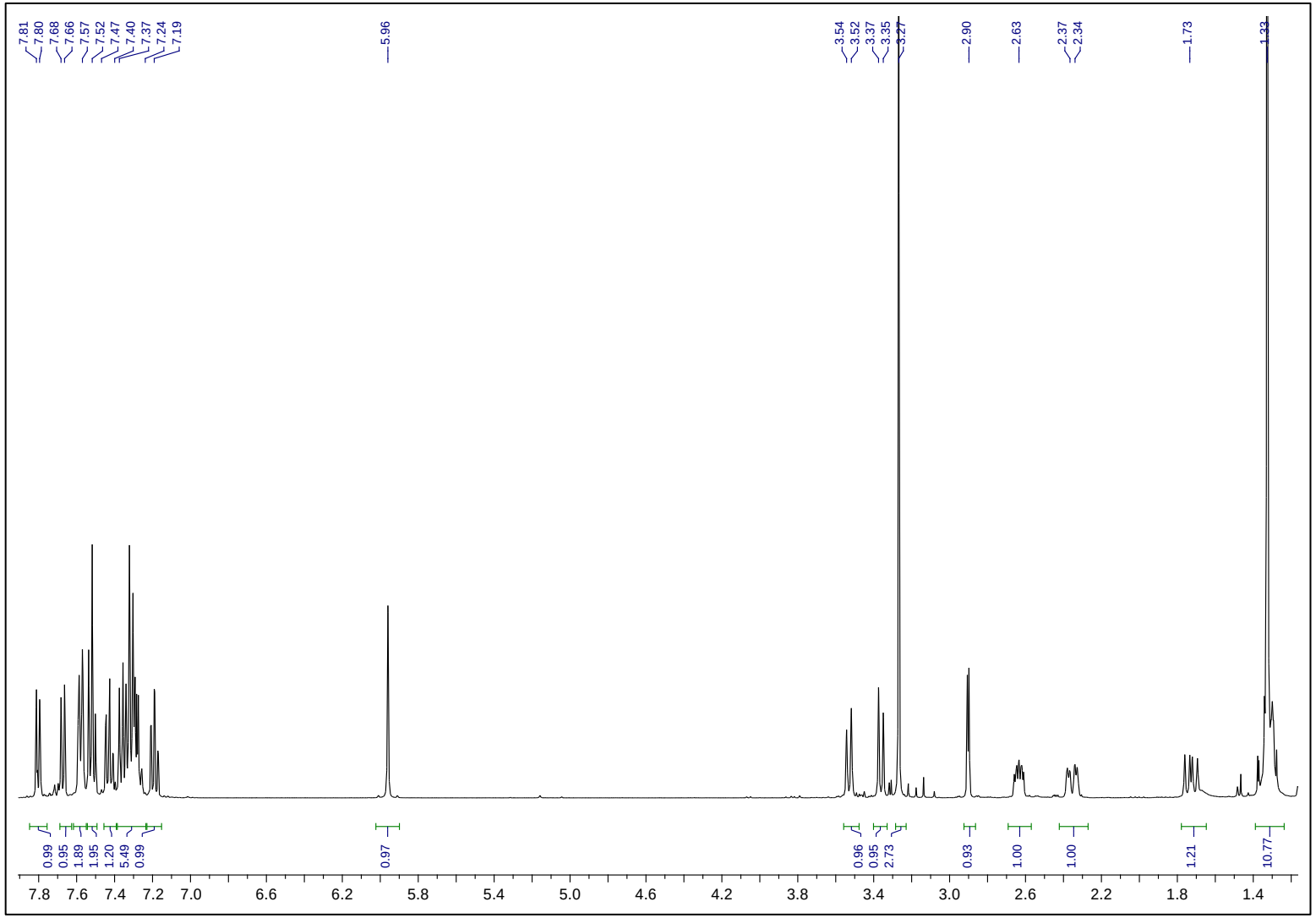
^{1}H NMR of **5b**


7.73
7.72
7.67
7.66
7.41
7.36
7.31
7.25
4.03
4.01
3.82
3.78
3.60
3.56
3.15
3.09
2.57
1.84
1.34
1.00
0.92
3.45
1.78
15.24
9.84
0.88
1.02
1.09
2.06
1.07
1.09
1.10
14.24
6.70
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4

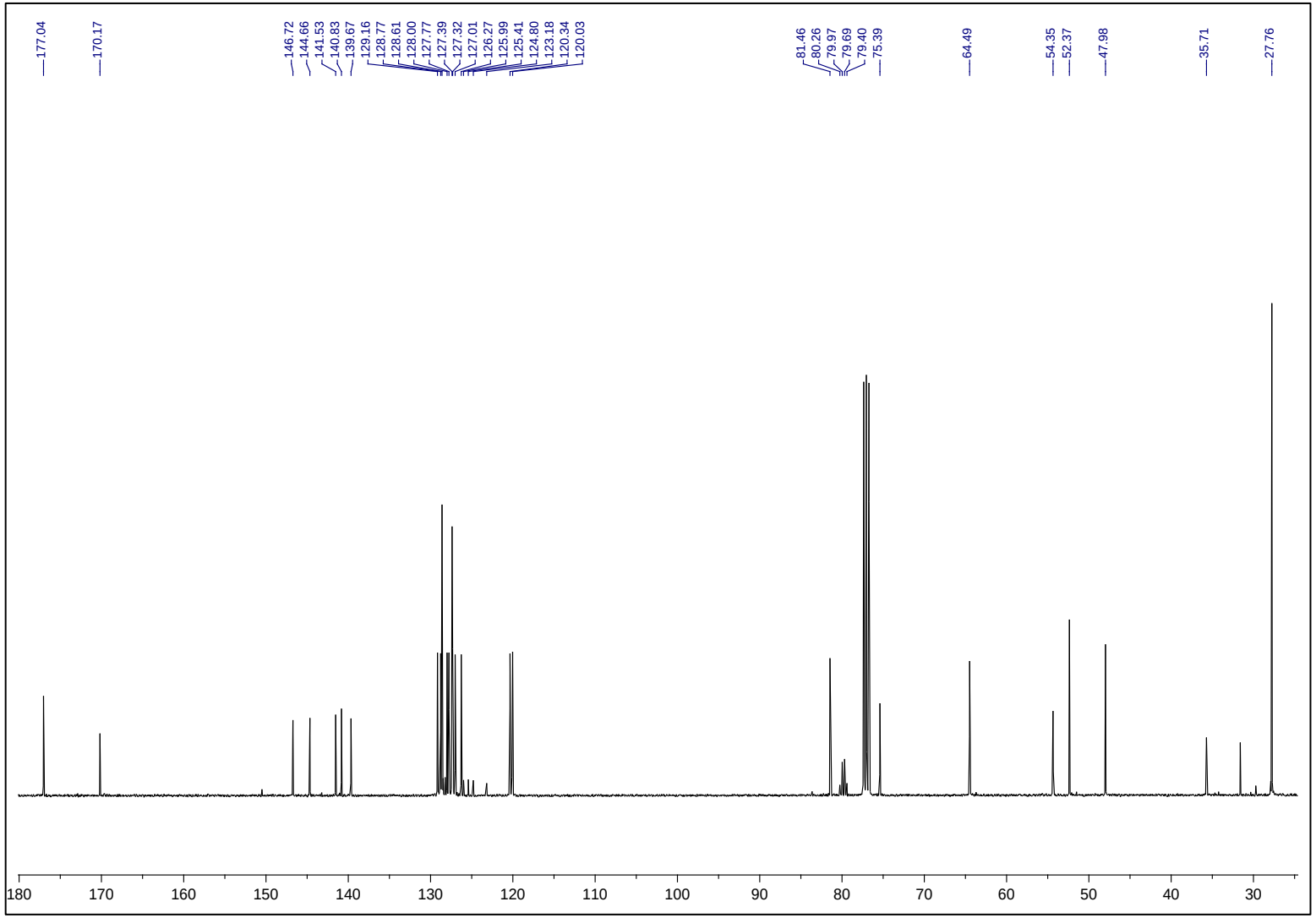
^{13}C NMR of **5b**


212.09
171.79
170.21
146.91
145.34
141.82
141.24
139.94
128.86
128.85
128.63
128.13
127.74
126.78
120.15
81.08
61.88
53.70
51.11
48.04
31.05
29.70
27.88
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

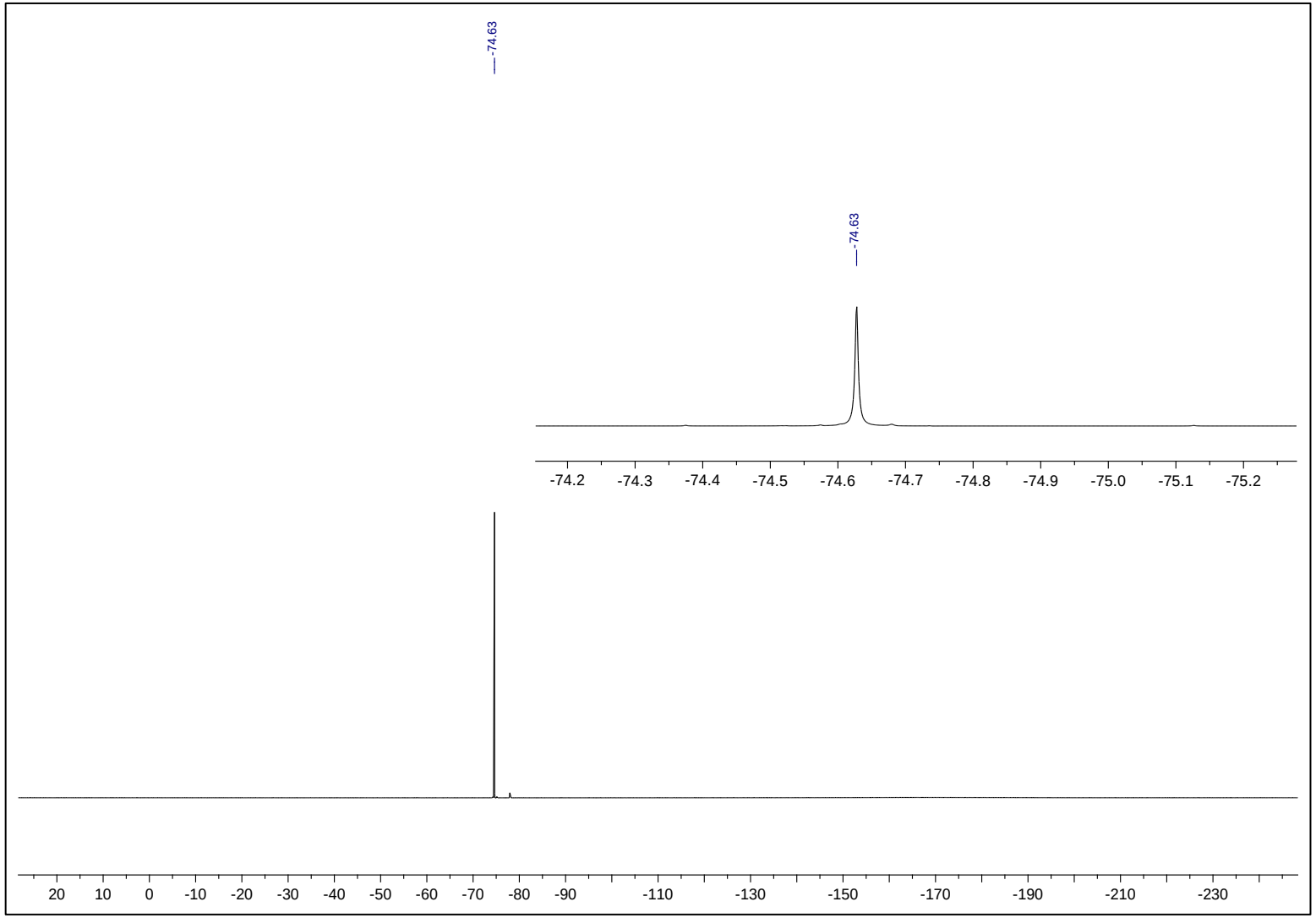
^{1}H NMR of **6**


7.81
7.80
7.68
7.66
7.57
7.52
7.47
7.40
7.37
7.24
7.19
5.96
3.54
3.52
3.37
3.35
3.27
2.90
2.63
2.37
2.34
1.73
1.33
0.99
0.95
1.89
1.95
1.20
5.49
0.99
0.97
0.96
0.95
2.73
0.93
1.00
1.00
1.21
10.77
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4

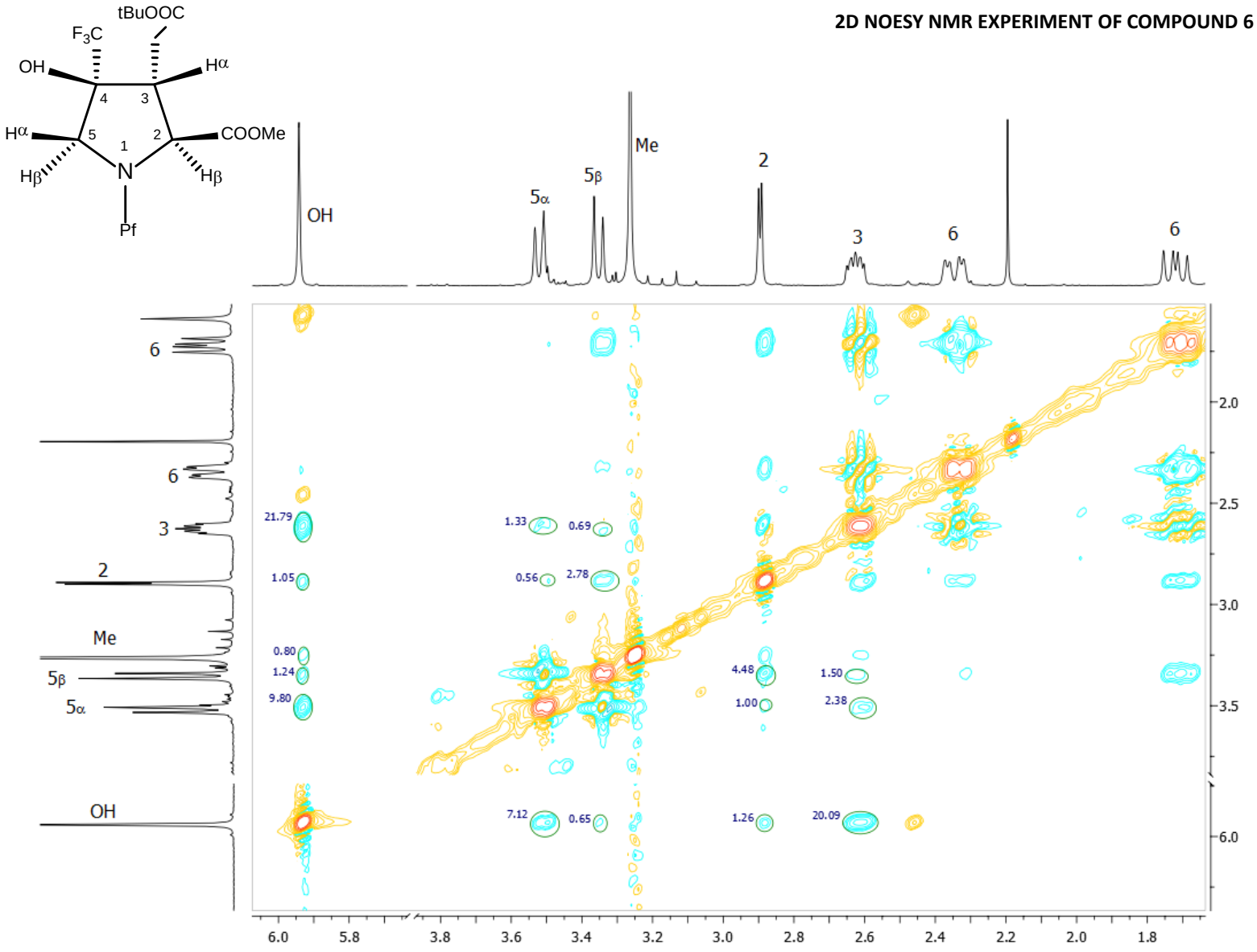
^{13}C NMR of **6**


177.04
170.17
146.72
144.66
141.53
140.83
139.67
129.16
128.77
128.61
128.00
127.77
127.39
127.32
127.01
126.27
125.99
125.41
124.80
123.18
120.34
120.03
81.46
80.26
79.97
79.69
79.40
75.39
64.49
54.35
52.37
47.98
35.71
27.76
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

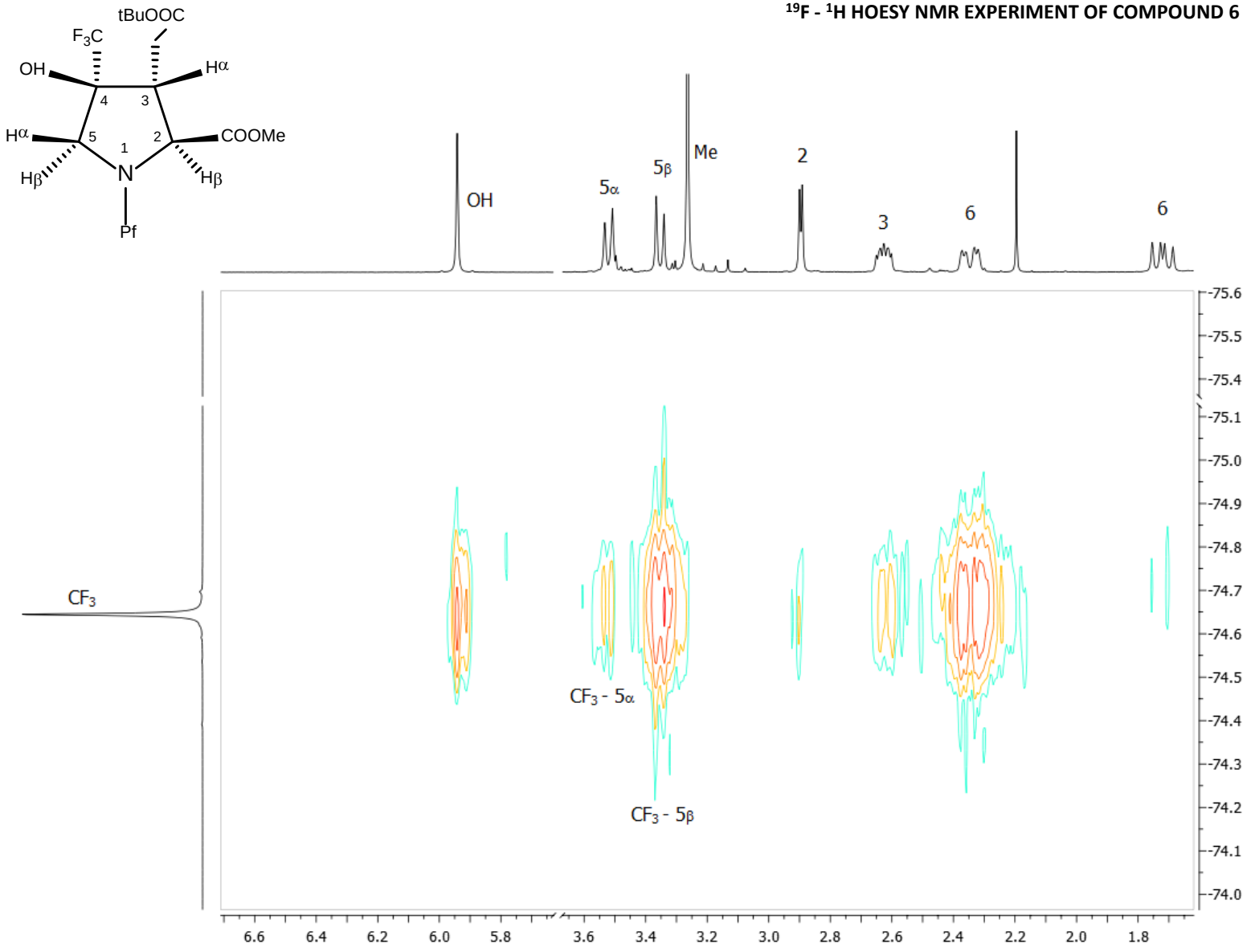
^{19}F NMR of **6**


-74.63
-74.63
-74.2
-74.3
-74.4
-74.5
-74.6
-74.7
-74.8
-74.9
-75.0
-75.1
-75.2
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-110
-130
-150
-170
-190
-210
-230

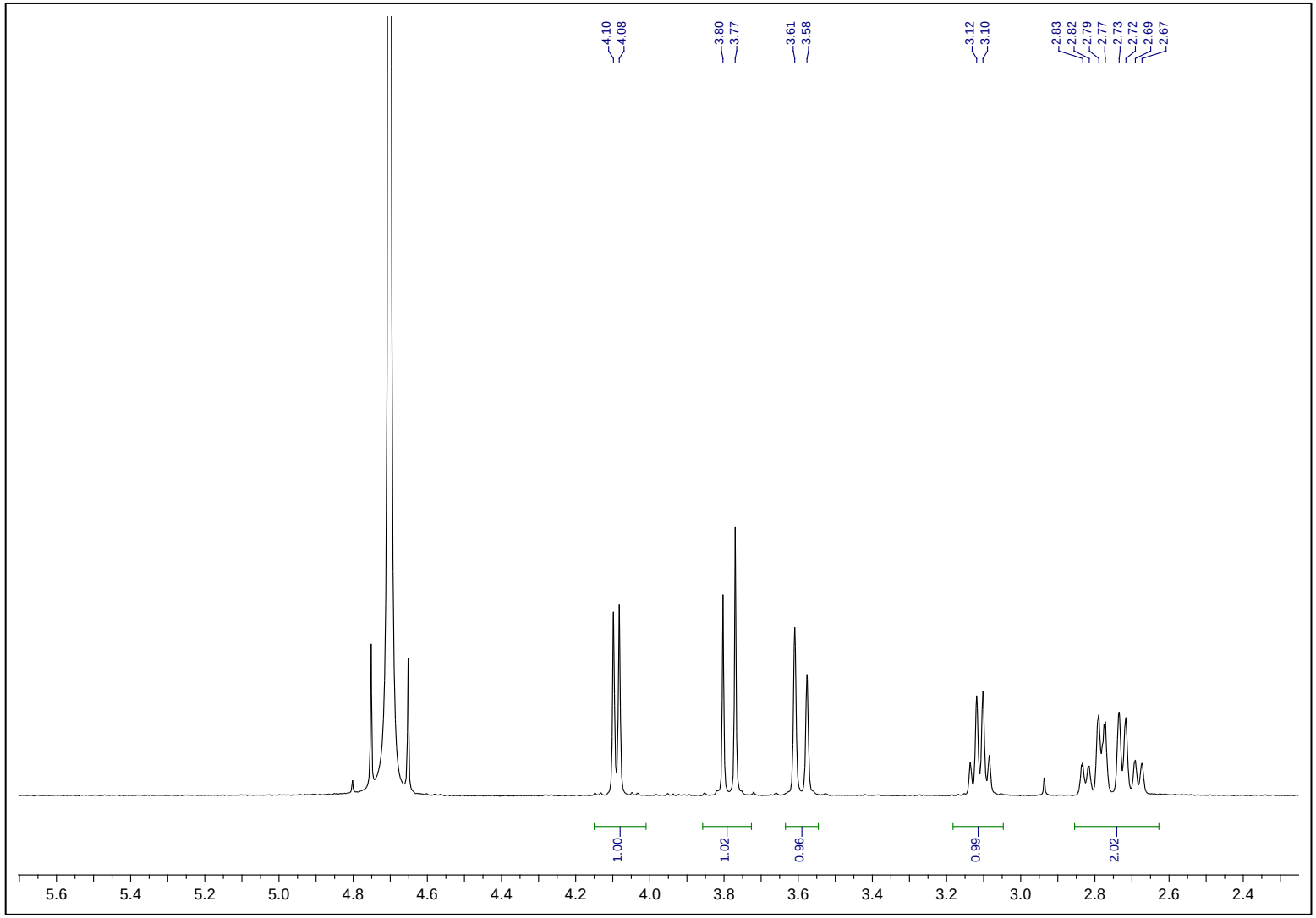
2D NOESY NMR EXPERIMENT OF COMPOUND 6



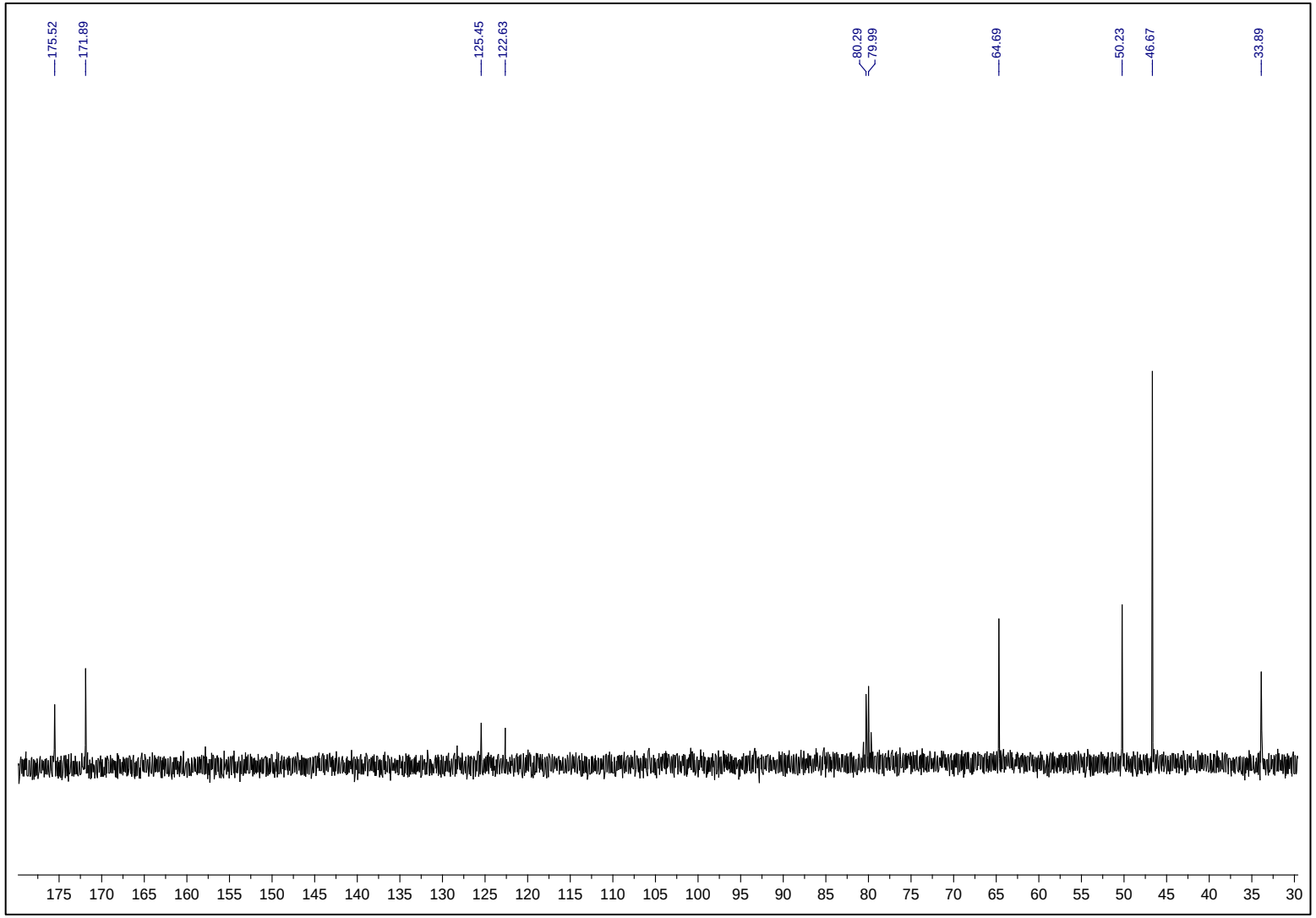
^{19}F - ^{1}H HOESY NMR EXPERIMENT OF COMPOUND 6



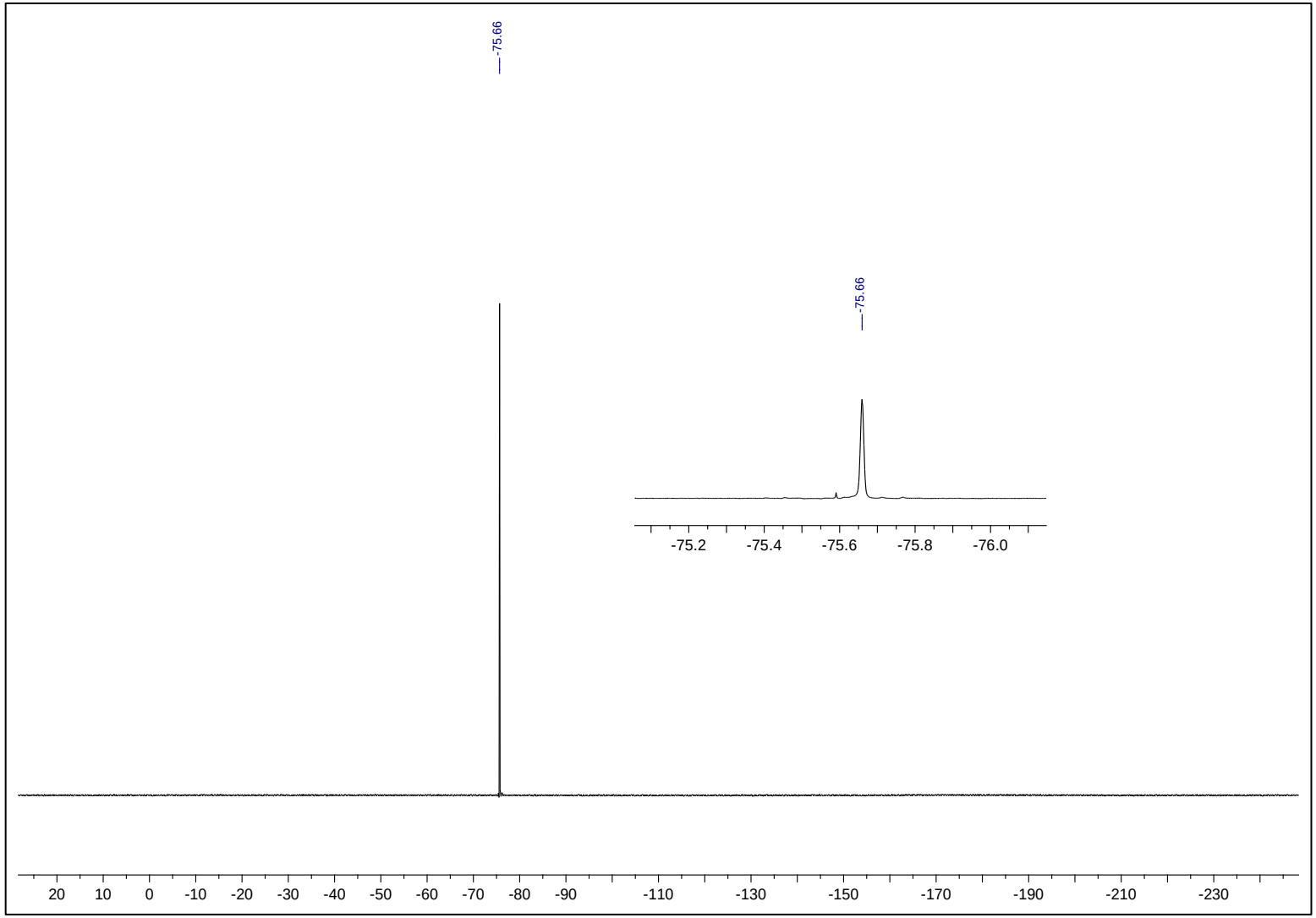
^{1}H NMR of **1a**


4.10
4.08
3.80
3.77
3.61
3.58
3.12
3.10
2.83
2.82
2.79
2.77
2.73
2.72
2.69
2.67
1.00
1.02
0.96
0.99
2.02
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4

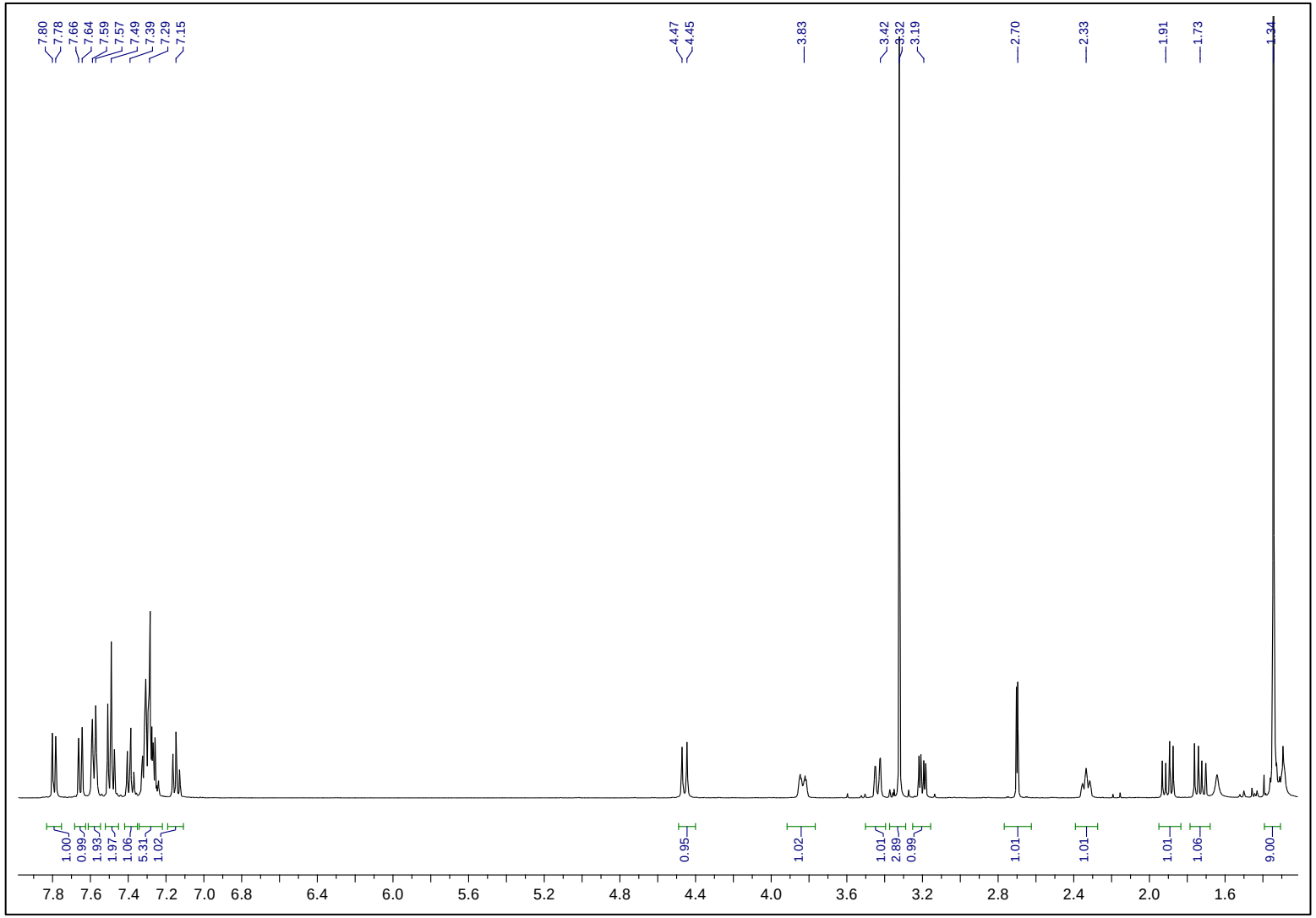
^{13}C NMR of **1a**


175.52
171.89
125.45
122.63
80.29
79.99
64.69
50.23
46.67
33.89
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30

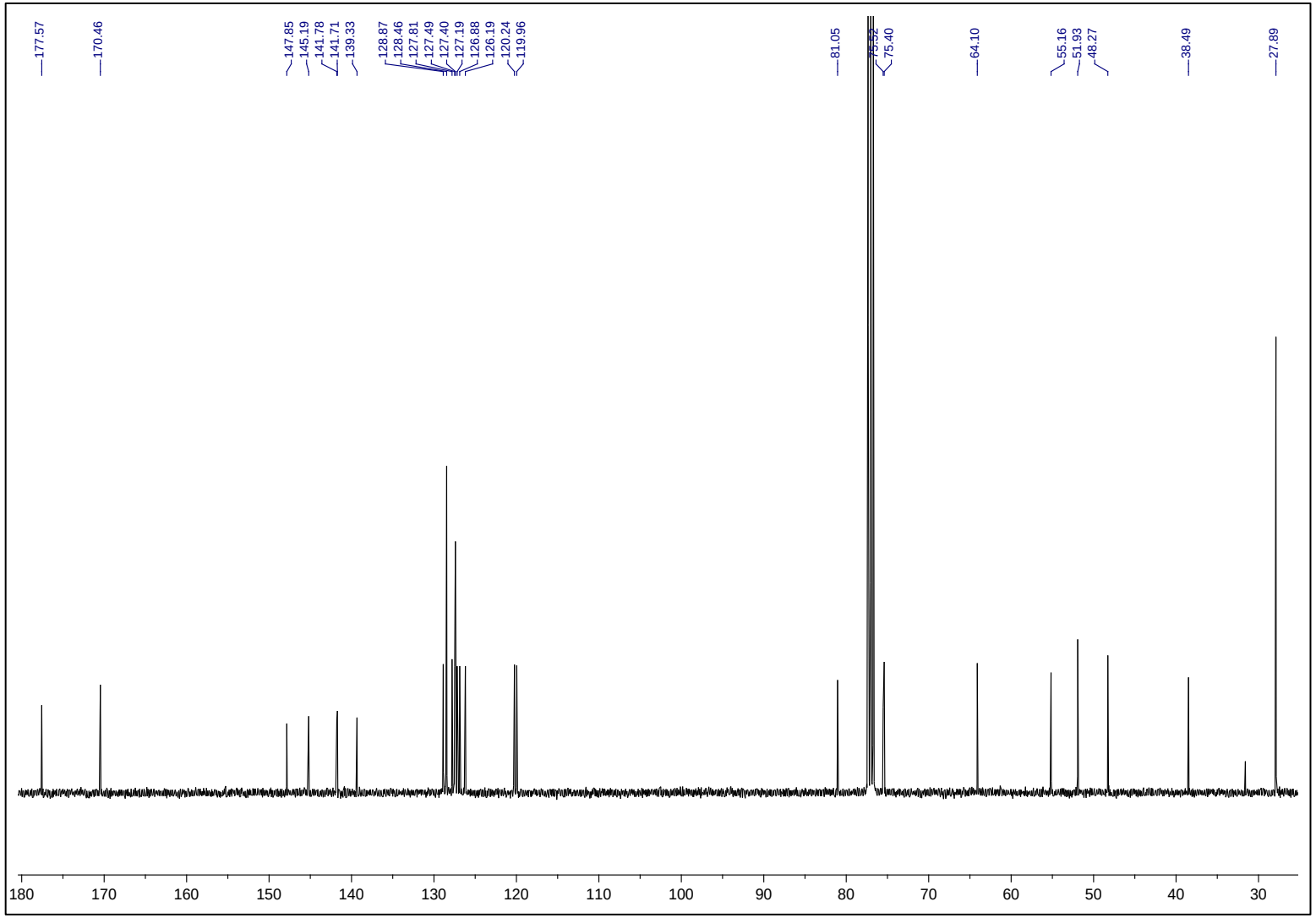
^{19}F NMR of **1a**


-75.66
-75.66
-75.2
-75.4
-75.6
-75.8
-76.0
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-110
-130
-150
-170
-190
-210
-230

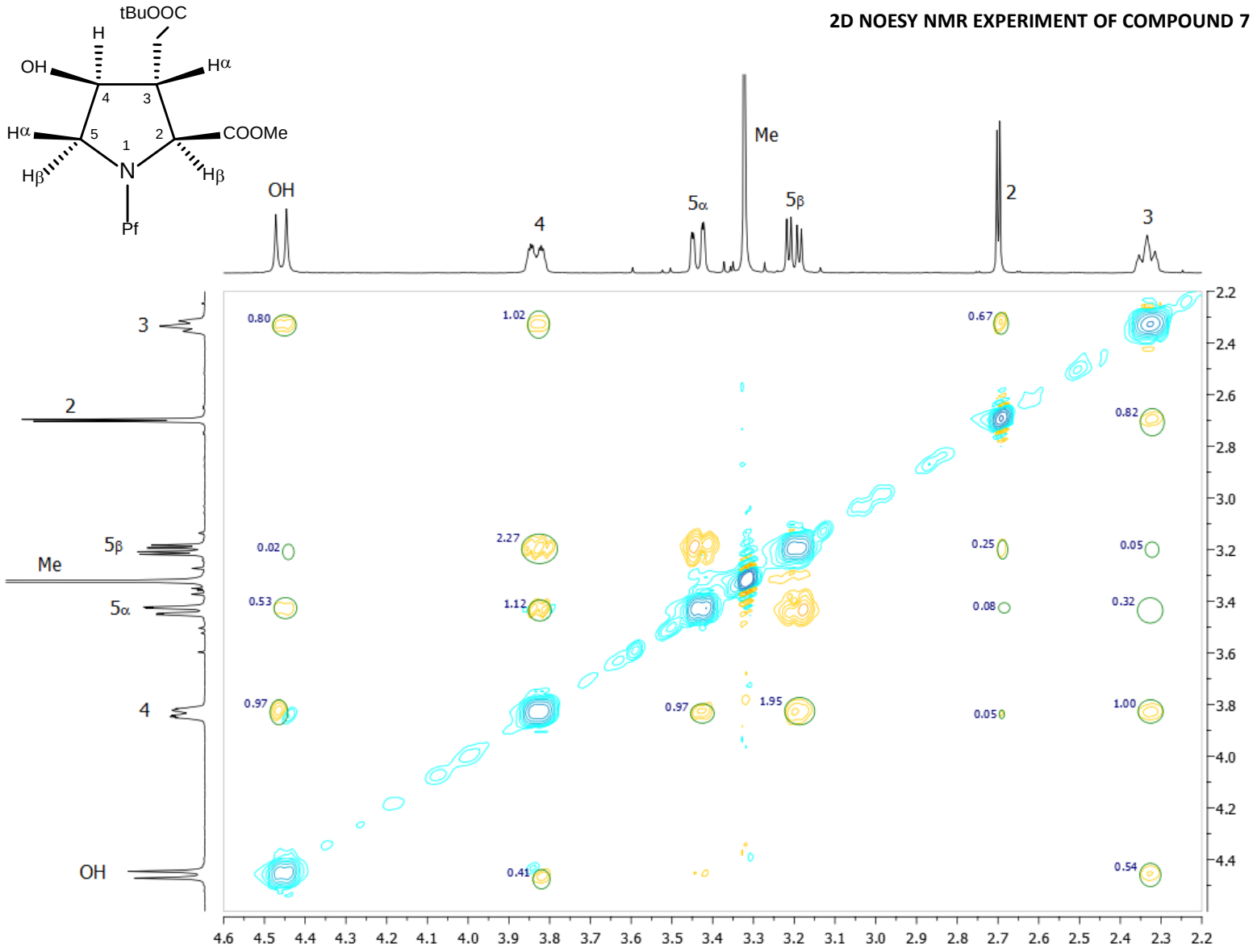
^{1}H NMR of **7**


7.80
7.78
7.66
7.64
7.59
7.57
7.49
7.39
7.29
7.15
4.47
4.45
3.83
3.42
3.32
3.19
2.70
2.33
1.91
1.73
1.34
1.00
0.99
1.93
1.97
1.06
5.31
1.02
0.95
1.02
1.01
2.89
0.99
1.01
1.01
1.01
1.06
9.00
7.8
7.6
7.4
7.2
7.0
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6

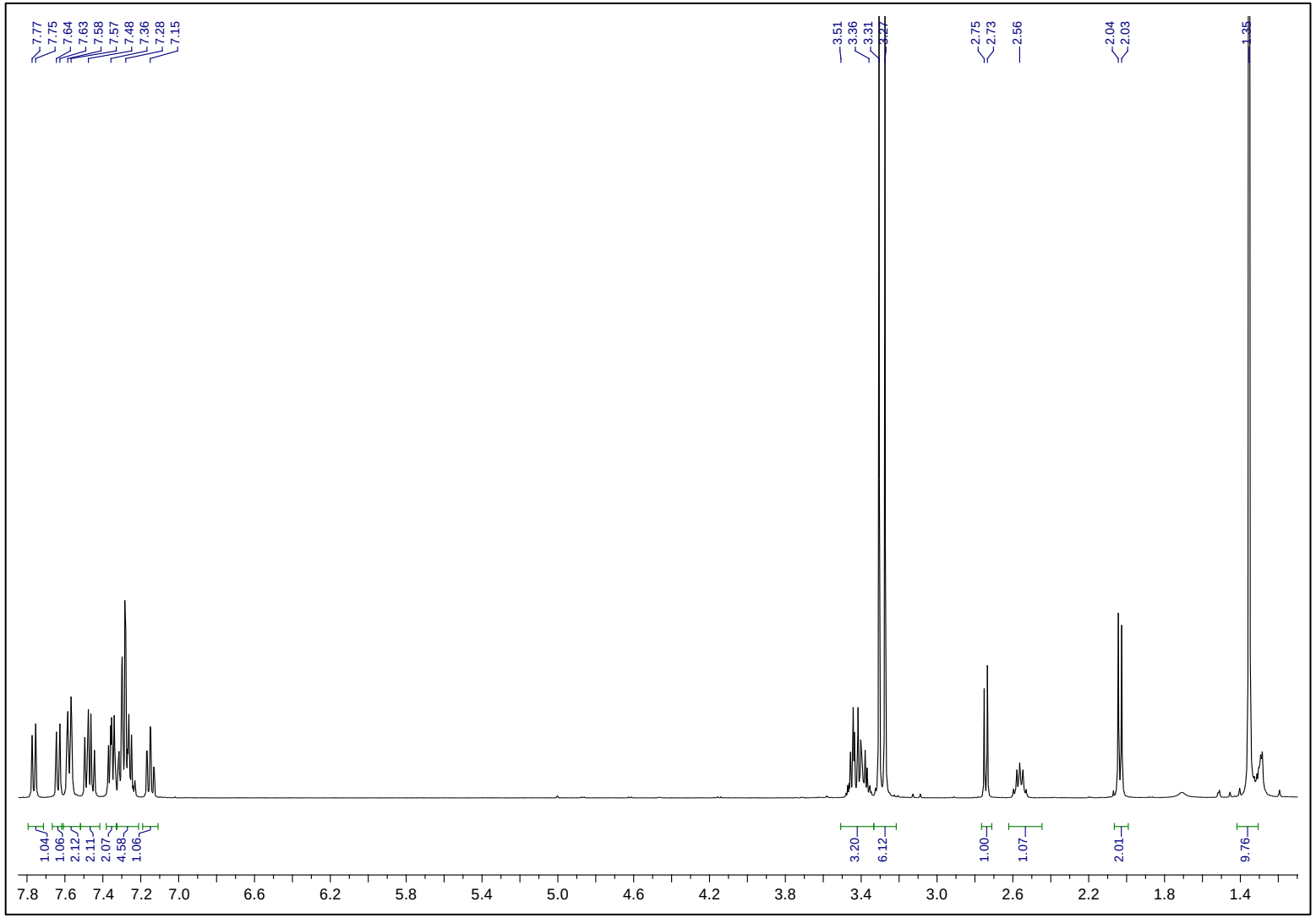
^{13}C NMR of **7**


177.57
170.46
147.85
145.19
141.78
141.71
139.33
128.87
128.46
127.81
127.49
127.40
127.19
126.88
126.19
120.24
119.96
81.05
76.52
75.40
64.10
55.16
51.93
48.27
38.49
27.89
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

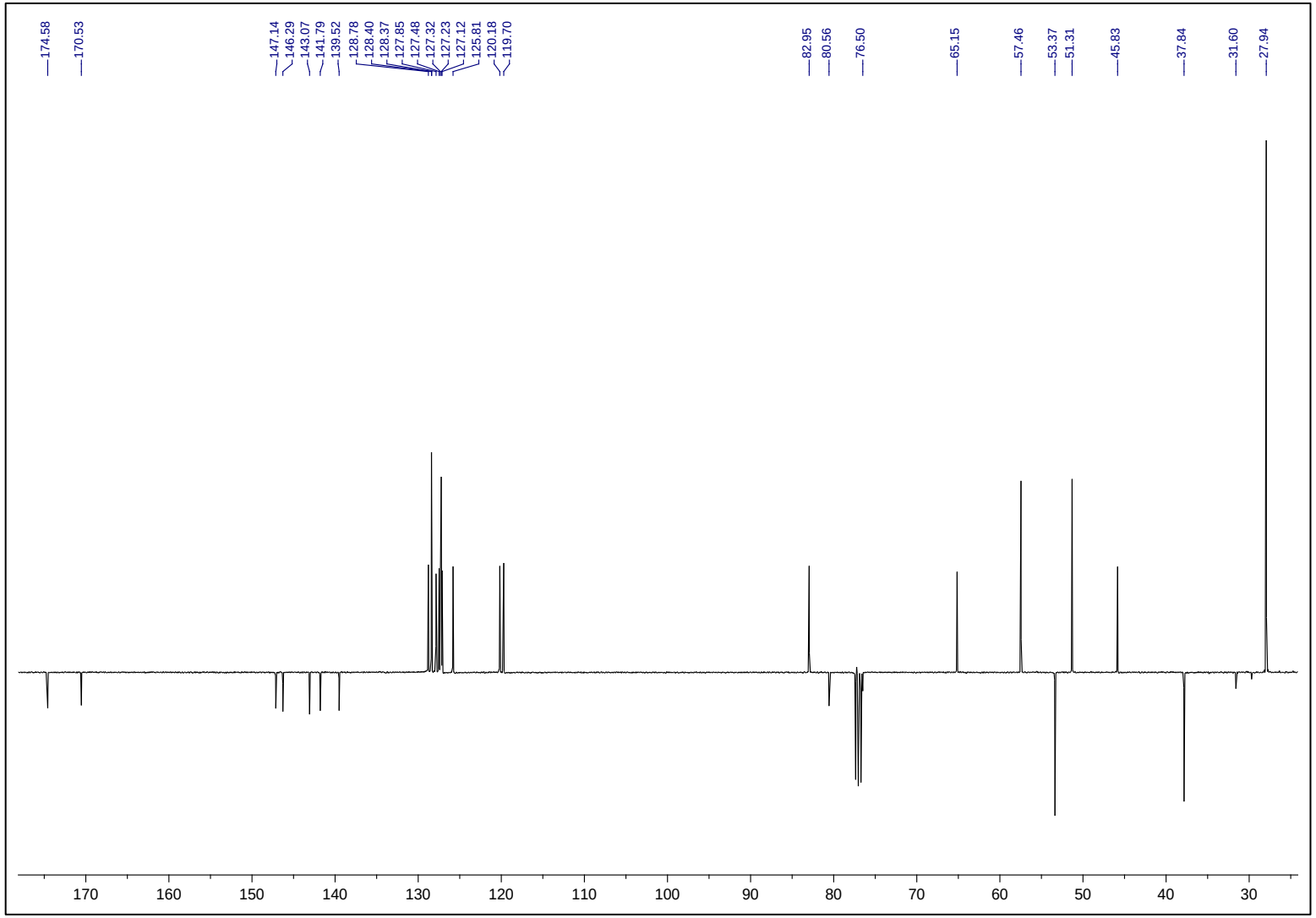
2D NOESY NMR EXPERIMENT OF COMPOUND 7



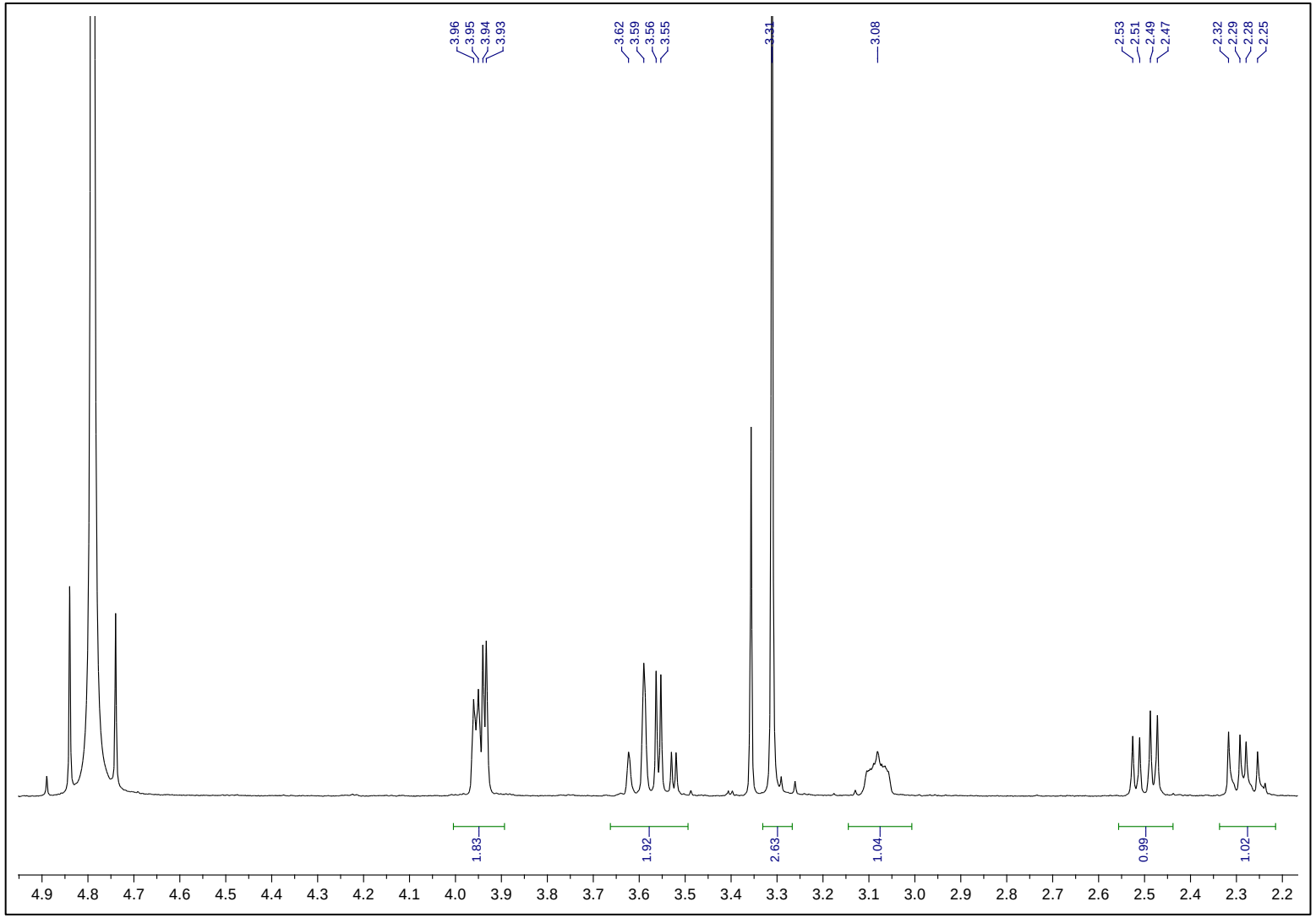
^{1}H NMR of **8**


7.77
7.75
7.64
7.63
7.58
7.57
7.48
7.36
7.28
7.15
3.51
3.36
3.31
3.27
2.75
2.73
2.56
2.04
2.03
1.35
1.04
1.06
2.12
2.11
2.07
4.58
1.06
3.20
6.12
1.00
1.07
2.01
9.76
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4

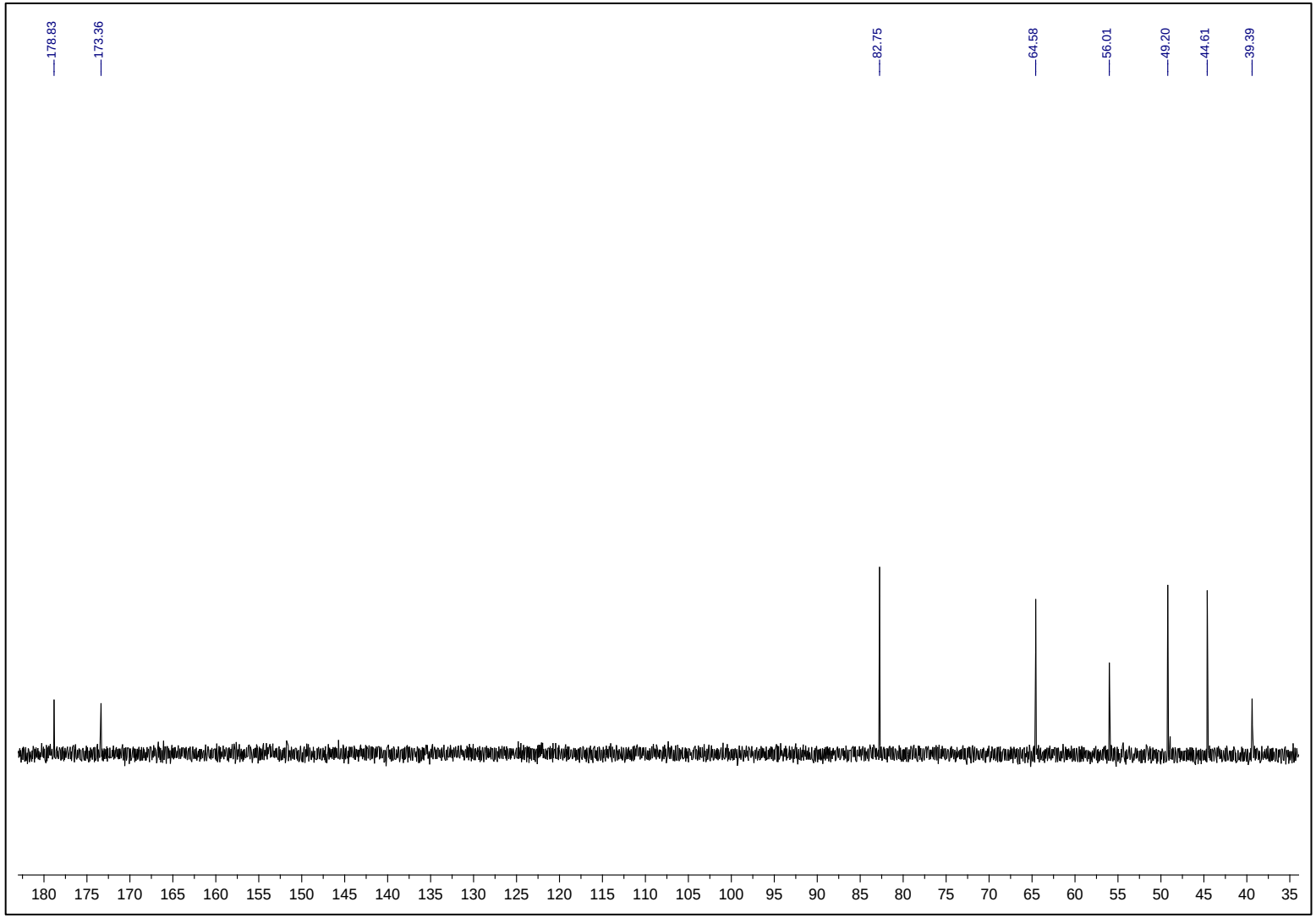
^{13}C NMR of **8**


174.58
170.53
147.14
146.29
143.07
141.79
139.52
128.78
128.40
128.37
127.85
127.48
127.32
127.23
127.12
125.81
120.18
119.70
82.95
80.56
76.50
65.15
57.46
53.37
51.31
45.83
37.84
31.60
27.94
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

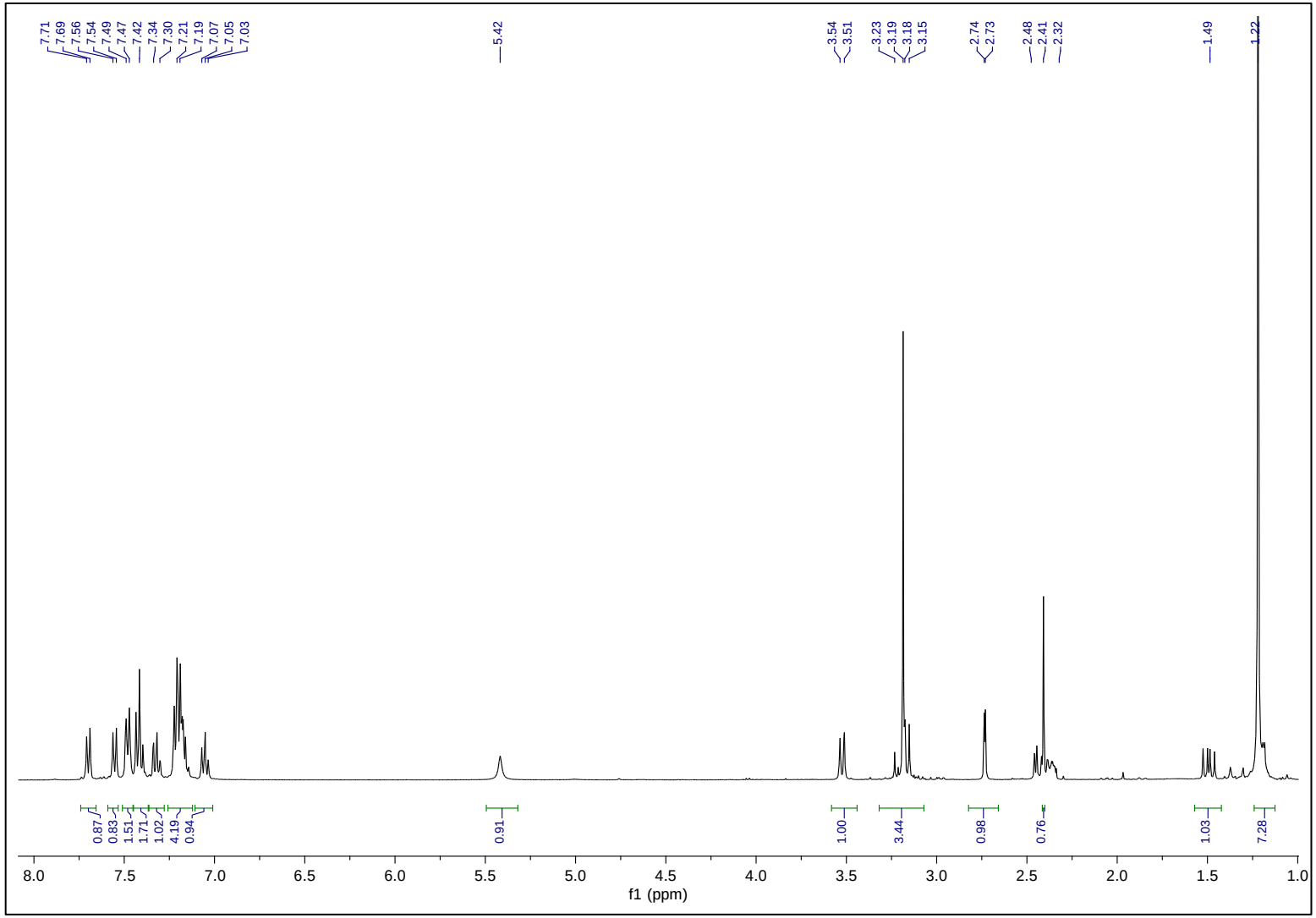
^{1}H NMR of **1b**


3.96
3.95
3.94
3.93
3.62
3.59
3.56
3.55
3.31
3.08
2.53
2.51
2.49
2.47
2.32
2.29
2.28
2.25
1.83
1.92
2.63
1.04
0.99
1.02
4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2

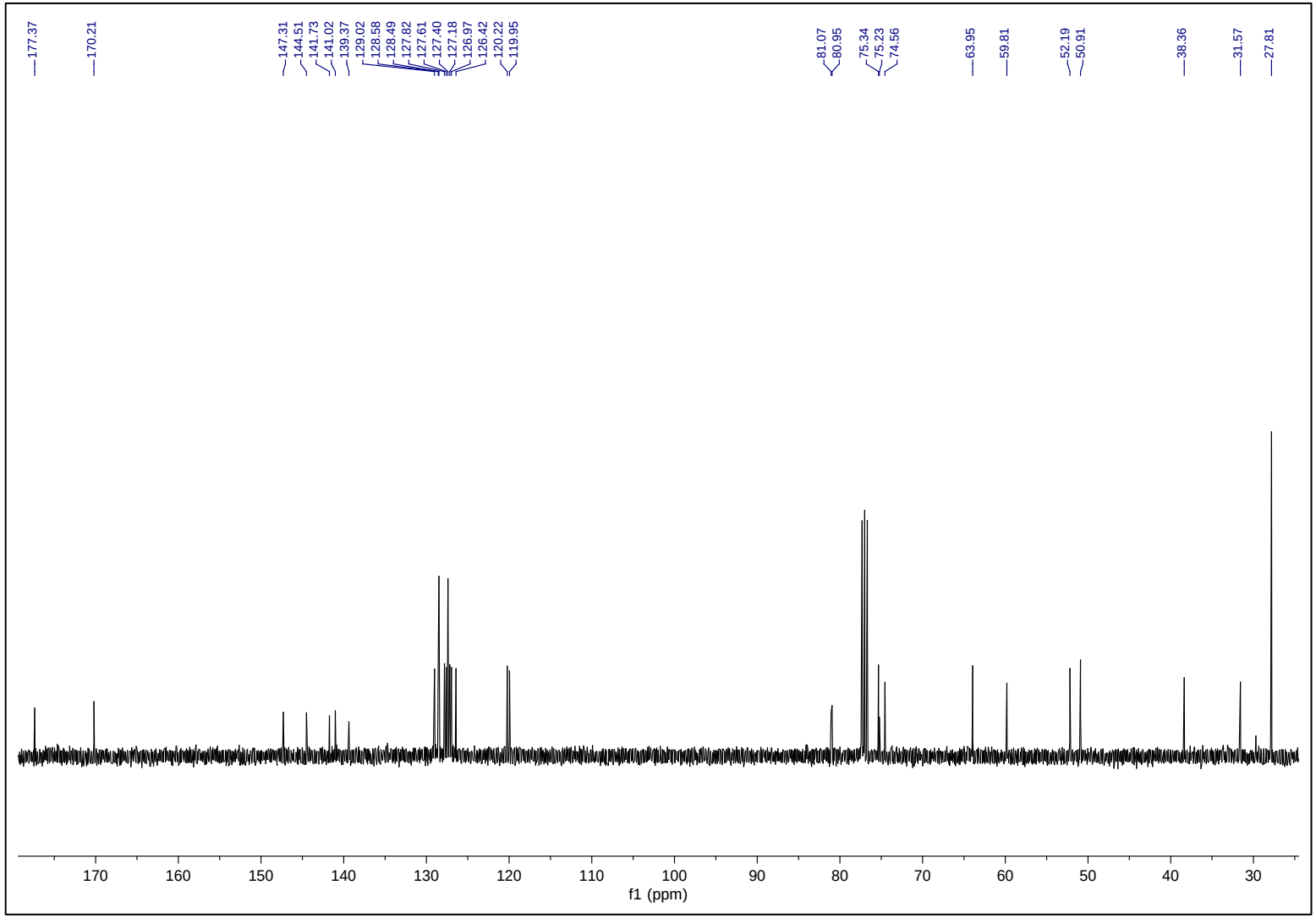
^{13}C NMR of **1b**


178.83
173.36
82.75
64.58
56.01
49.20
44.61
39.39
180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35

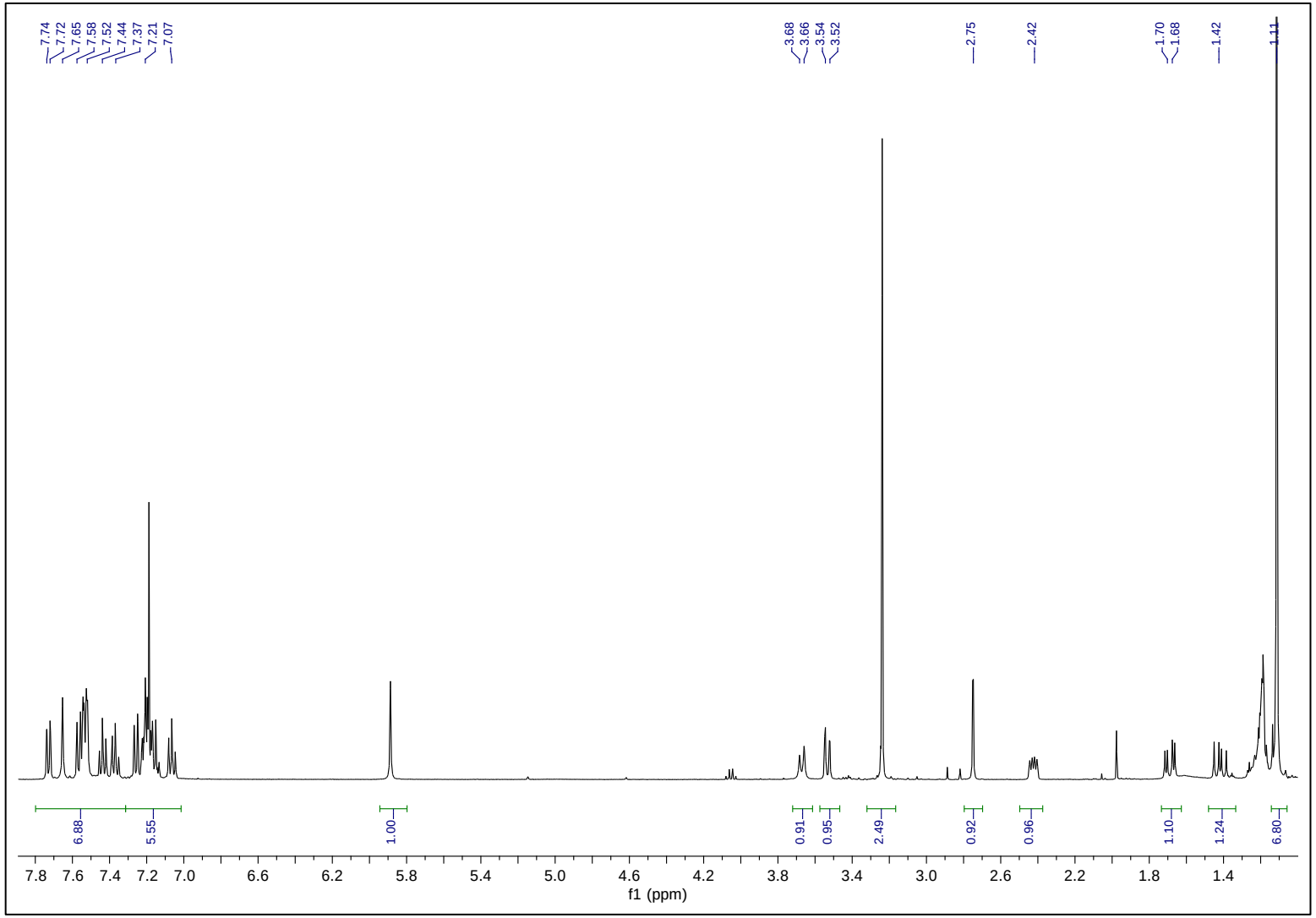
^{1}H NMR of **9**


7.71
7.69
7.56
7.54
7.49
7.47
7.42
7.34
7.30
7.21
7.19
7.07
7.05
7.03
5.42
3.54
3.51
3.23
3.19
3.18
3.15
2.74
2.73
2.48
2.41
2.32
1.49
1.22
0.87
0.83
1.51
1.71
1.02
4.19
0.94
0.91
1.00
3.44
0.98
0.76
1.03
7.28
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

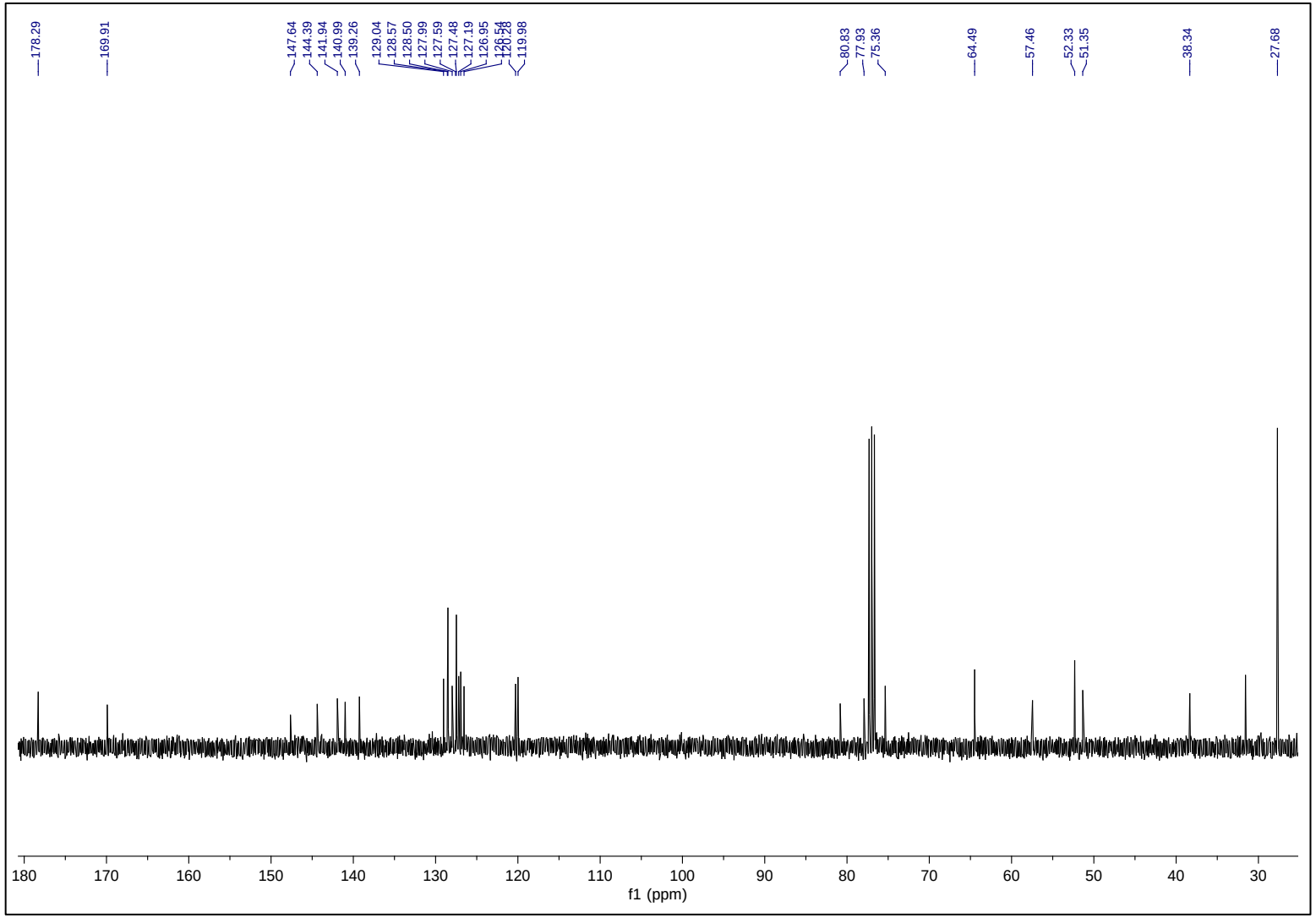
^{13}C NMR of **1b**


177.37
170.21
147.31
144.51
141.73
141.02
139.37
129.02
128.58
128.49
127.82
127.61
127.40
127.18
126.97
126.42
120.22
119.95
81.07
80.95
75.34
75.23
74.56
63.95
59.81
52.19
50.91
38.36
31.57
27.81
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

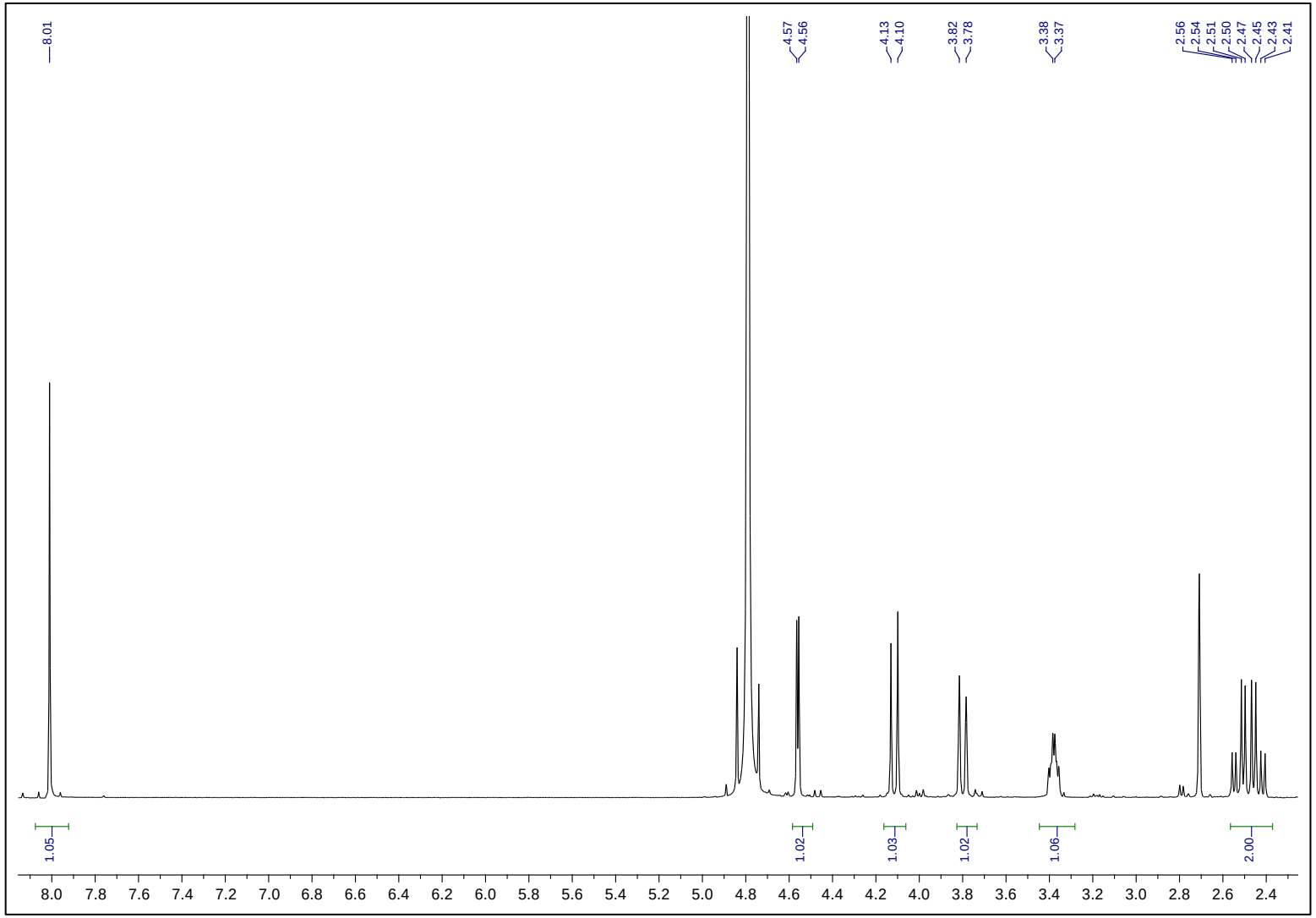
^{1}H NMR of **10**


7.74
7.72
7.65
7.58
7.52
7.44
7.37
7.21
7.07
3.68
3.66
3.54
3.52
2.75
2.42
1.70
1.68
1.42
1.11
6.88
5.55
1.00
0.91
0.95
2.49
0.92
0.96
1.10
1.24
6.80
7.8 7.6 7.4 7.2 7.0 6.6 6.2 5.8 5.4 5.0 4.6 4.2 3.8 3.4 3.0 2.6 2.2 1.8 1.4
f1 (ppm)

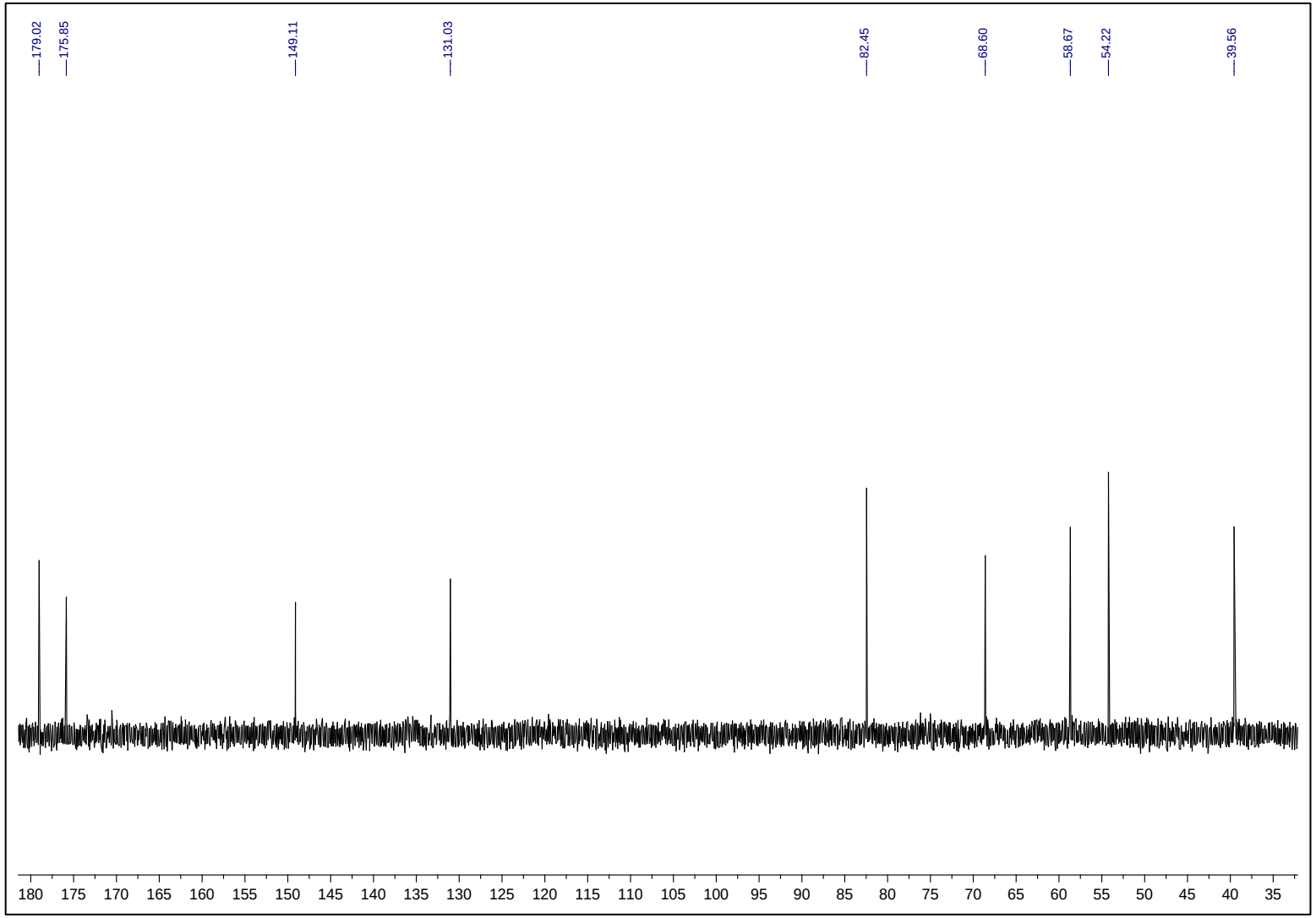
^{13}C NMR of **10**


178.29
169.91
147.64
144.39
141.94
140.99
139.26
129.04
128.57
128.50
127.99
127.59
127.48
127.19
126.95
126.54
120.28
119.98
80.83
77.93
75.36
64.49
57.46
52.33
51.35
38.34
27.68
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

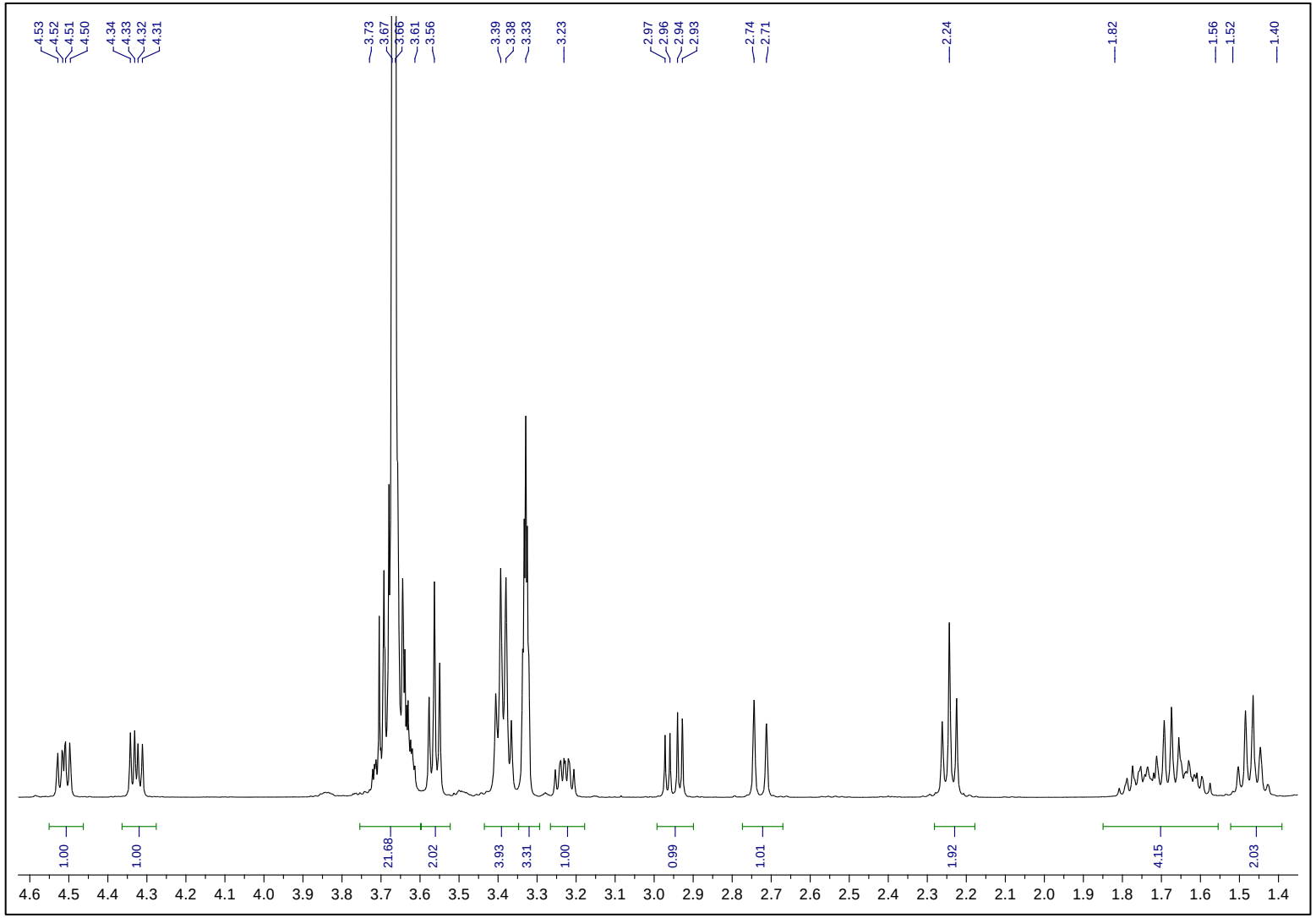
^{1}H NMR of **1c**


8.01
4.57
4.56
4.13
4.10
3.82
3.78
3.38
3.37
2.56
2.54
2.51
2.50
2.47
2.45
2.43
2.41
1.05
1.02
1.03
1.02
1.06
2.00
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4

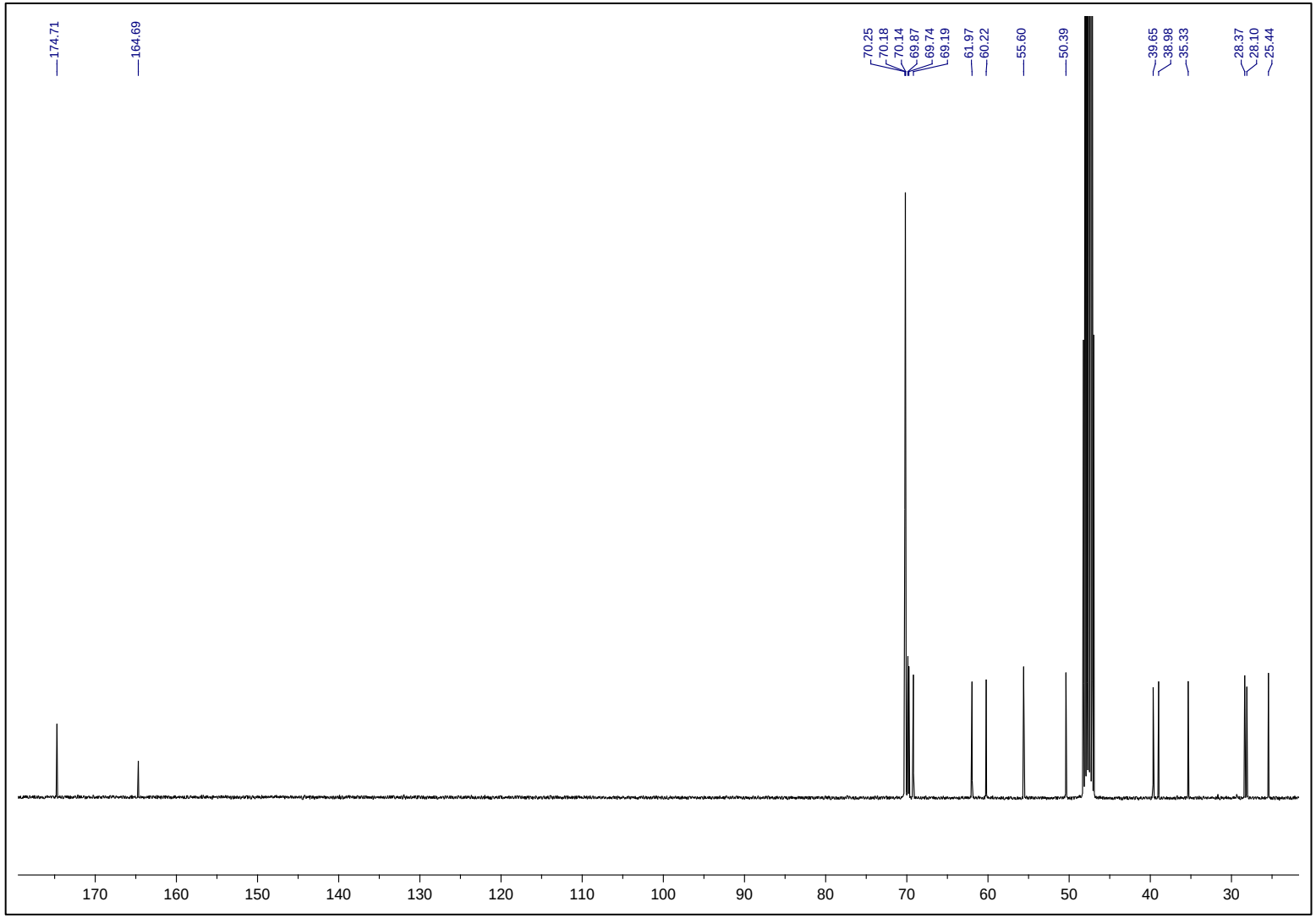
^{13}C NMR of **1c**


179.02
175.85
149.11
131.03
82.45
68.60
58.67
54.22
39.56
180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35

^{1}H NMR of **12**


4.53
4.52
4.51
4.50
4.34
4.33
4.32
4.31
3.73
3.67
3.66
3.61
3.56
3.39
3.38
3.33
3.23
2.97
2.96
2.94
2.93
2.74
2.71
2.24
1.82
1.56
1.52
1.40
1.00
1.00
21.68
2.02
3.93
3.31
1.00
0.99
1.01
1.92
4.15
2.03
4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4

^{13}C NMR of **12**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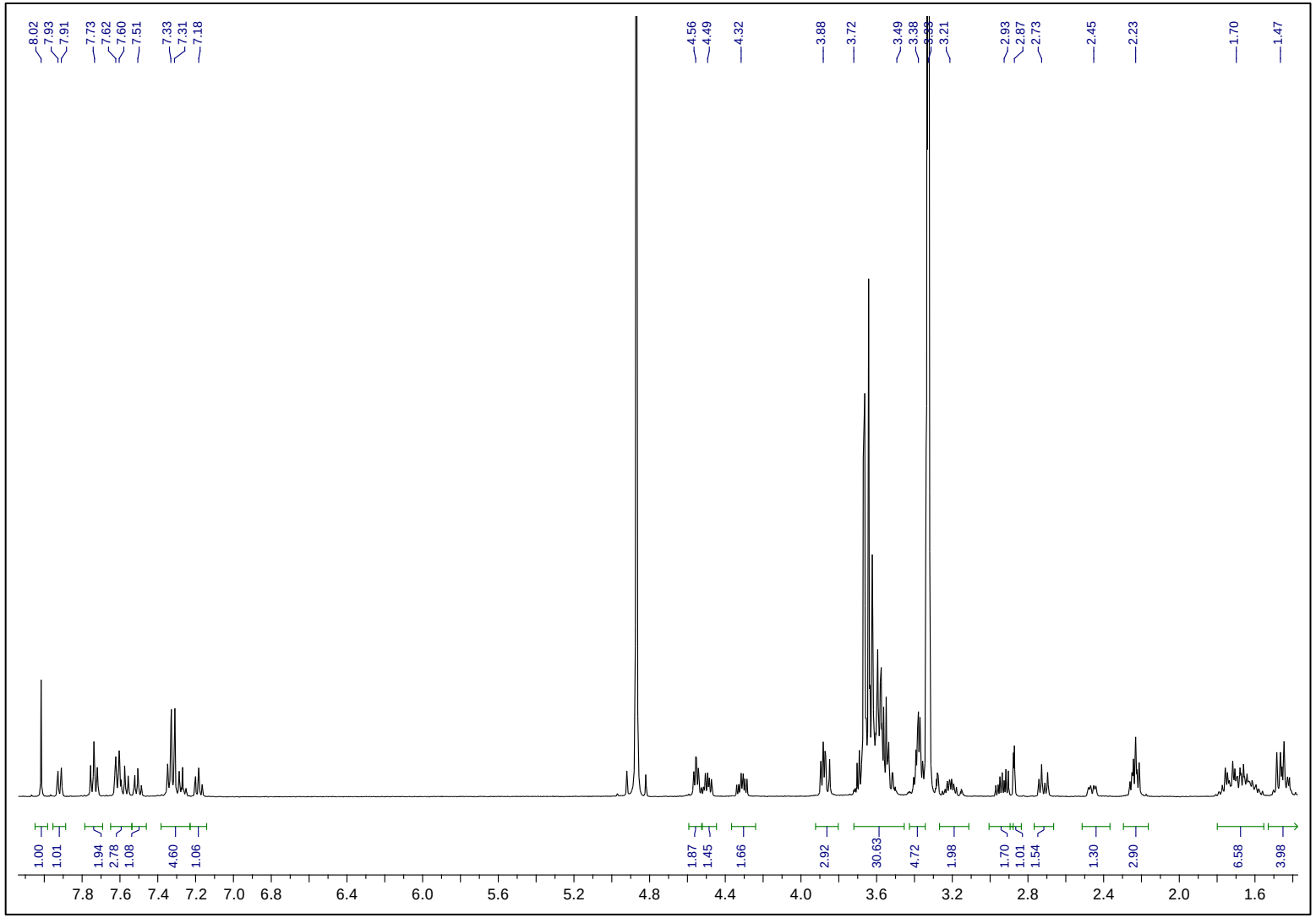
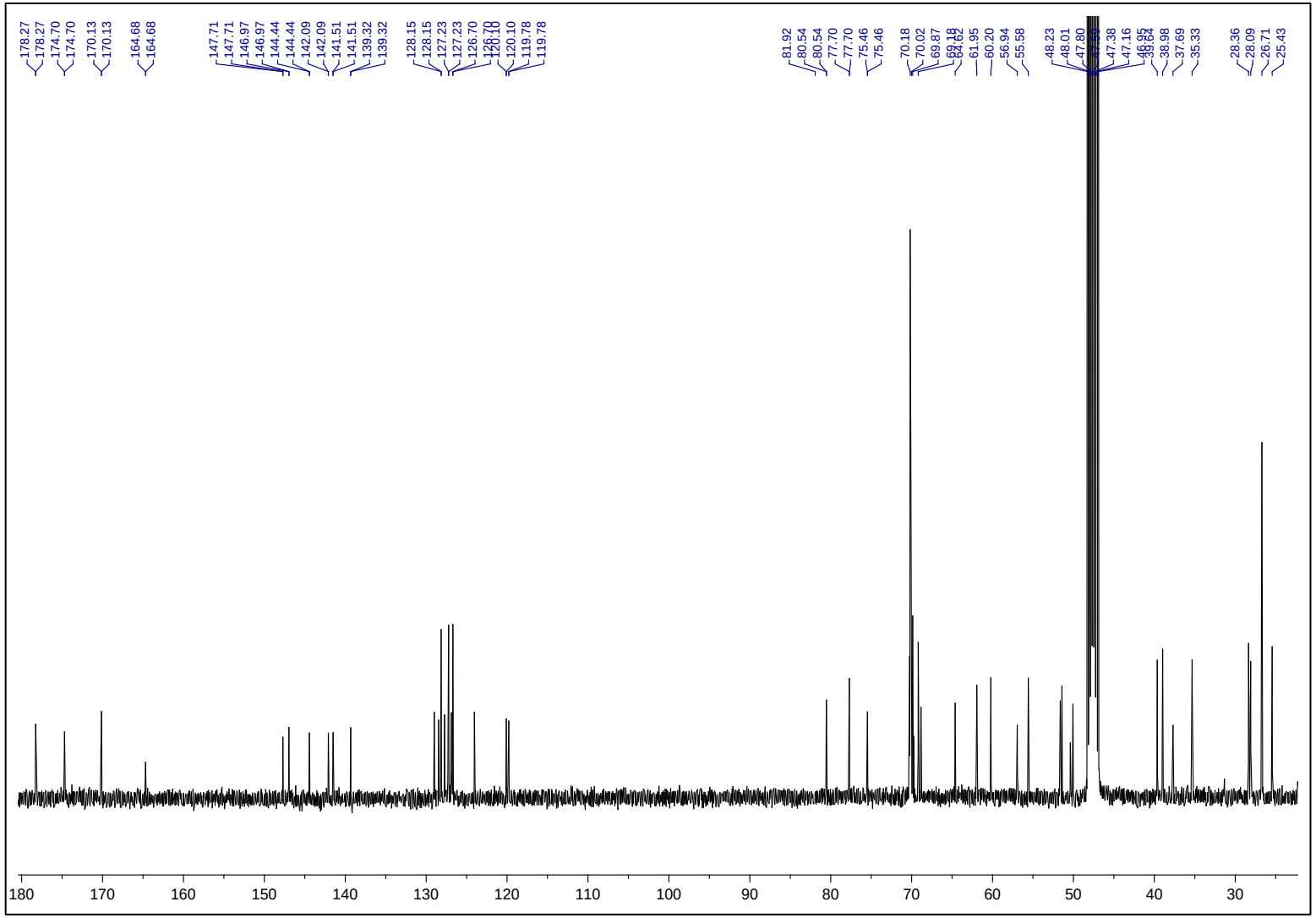
164.69
70.25
70.18
70.14
69.87
69.74
69.19
61.97
60.22
55.60
50.39
39.65
38.98
35.33
28.37
28.10
25.44
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

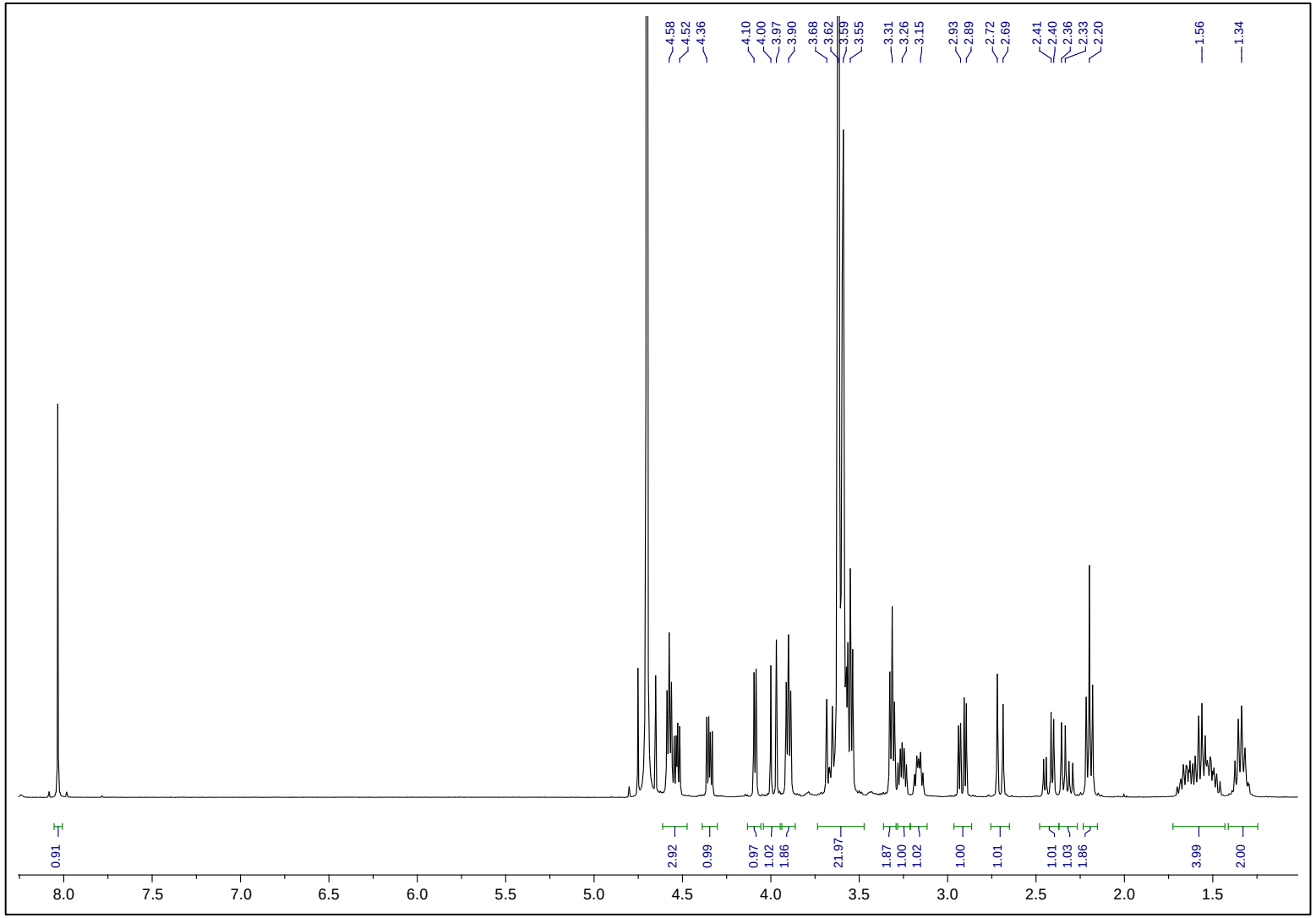
^{1}H NMR of **13**


8.02
7.93
7.91
7.73
7.62
7.60
7.51
7.33
7.31
7.18
4.56
4.49
4.32
3.88
3.72
3.49
3.38
3.21
2.93
2.87
2.73
2.45
2.23
1.70
1.47
1.00
1.01
1.94
2.78
1.08
4.60
1.06
1.87
1.45
1.66
2.92
30.63
4.72
1.98
1.70
1.01
1.54
1.30
2.90
6.58
3.98
7.8
7.6
7.4
7.2
7.0
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6

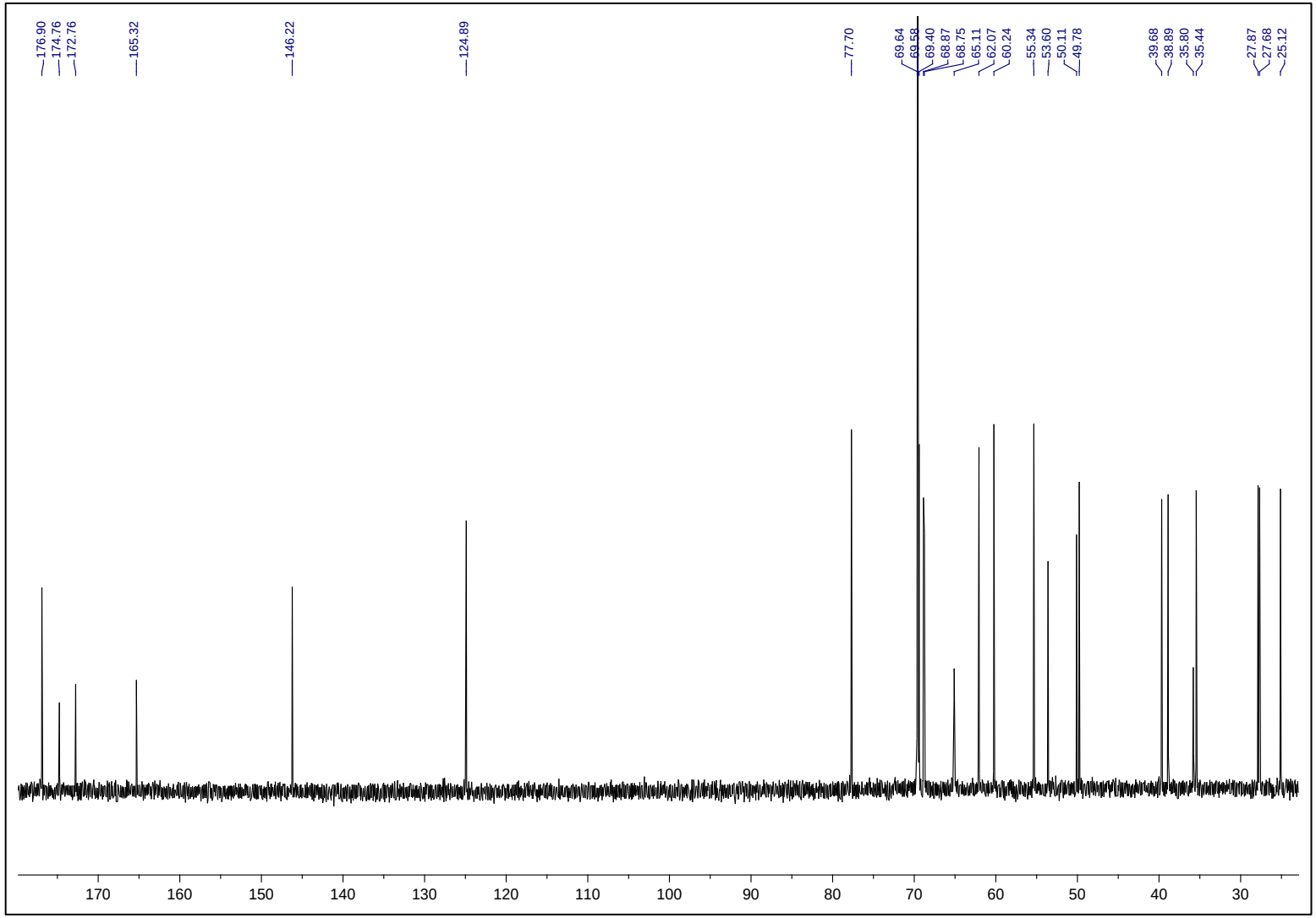
^{13}C NMR of **13**


178.27
178.27
174.70
174.70
170.13
170.13
164.68
164.68
147.71
147.71
146.97
146.97
144.44
144.44
142.09
142.09
141.51
141.51
139.32
139.32
128.15
128.15
127.23
127.23
126.70
126.70
120.10
120.10
119.78
119.78
81.92
80.54
80.54
77.70
77.70
75.46
75.46
70.18
70.02
69.87
61.95
60.20
56.94
55.58
48.23
48.01
47.80
47.38
47.16
38.98
37.69
35.33
28.36
28.09
26.71
25.43
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

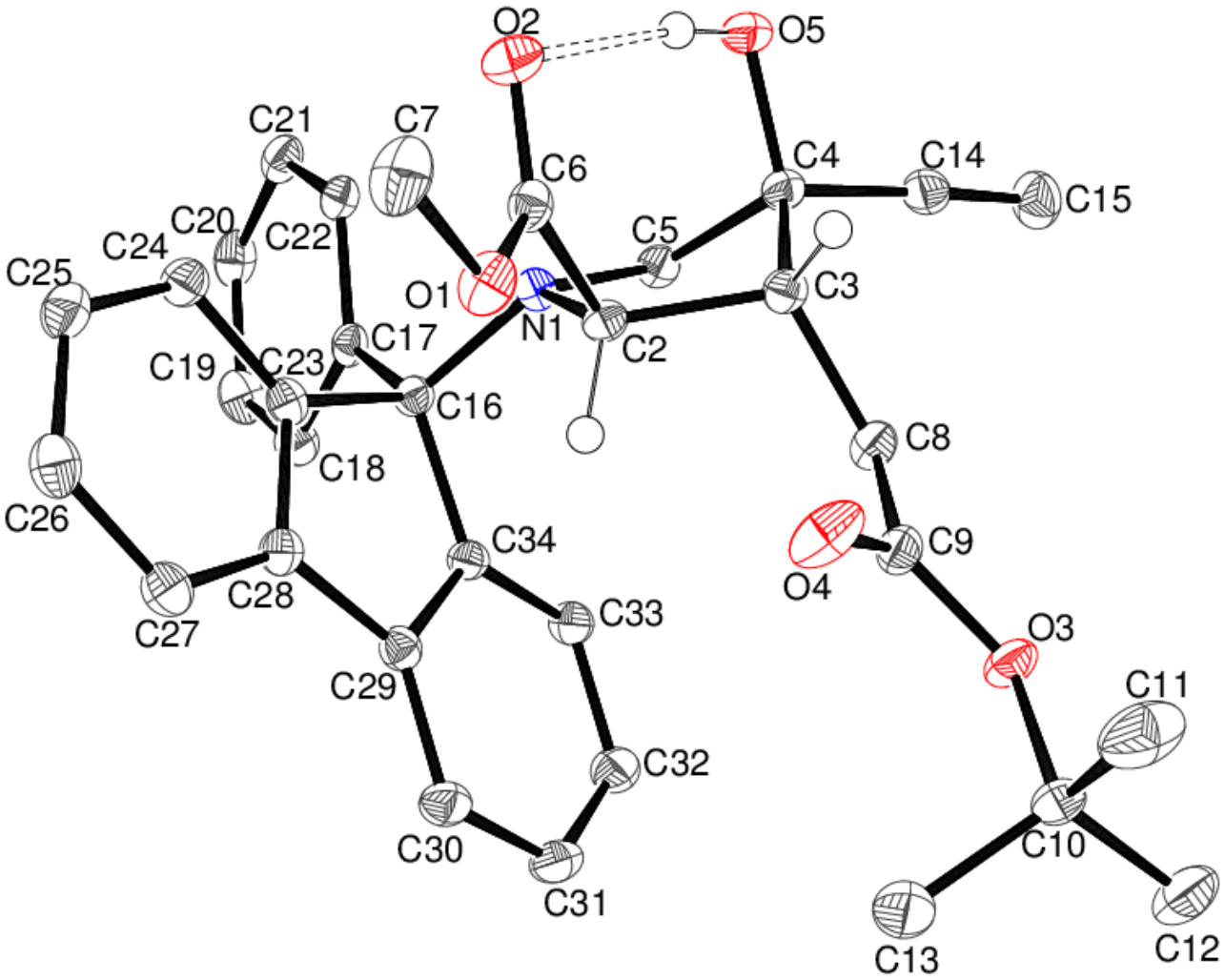
^{1}H NMR of **14**


4.58
4.52
4.36
4.10
4.00
3.97
3.90
3.68
3.62
3.59
3.55
3.31
3.26
3.15
2.93
2.89
2.72
2.69
2.41
2.40
2.36
2.33
2.20
1.56
1.34
0.91
2.92
0.99
0.97
1.02
1.86
21.97
1.87
1.00
1.02
1.00
1.01
1.01
1.03
1.86
3.99
2.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

^{13}C NMR of **14**


176.90
174.76
172.76
165.32
146.22
124.89
77.70
69.64
69.58
69.40
68.87
68.75
65.11
62.07
60.24
55.34
53.60
50.11
49.78
39.68
38.89
35.80
35.44
27.87
27.68
25.12
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

X-RAY OF COMPOUND 9



Biological Testing of the Novel Compounds

Tissue harvest, preparation and recording techniques were as previously published (Bewick et al., 2005; Simon et al., 2010) and are only described here briefly. 4th deep lumbrical nerve-muscle preparations were removed from adult Sprague-Dawley rats (male, 278 – 419 g) after killing in accordance with U.K. Animals (Scientific Procedures) Act, 1986 and associated EU guidelines 2013. Experiments were carried out at room temperature (18 – 21°C) in physiological saline (Liley, 1956).

Muscles received 1mm (~10% muscle length) trapezoidal stretches for 5s and muscle spindle (stretch organ) nerve responses were recorded with silver-wire electrodes above the saline surface. Signals were amplified (A103, Isleworth Electronics, Isleworth, UK and 8102, CF Palmer, HighWycombe UK preamplifiers in series), and recorded using PC software (WCP, Strathclyde Electrophysiology Software, University of Strathclyde, UK). Test muscles were incubated 60 min in cumulative concentrations of ligands, before a drug-free wash. Control muscles had time-matched changes in drug-free saline.

The average stretch-evoked firing frequency was calculated from the first 0.5 s at the stretched length over 3 stretch cycles. The significance of difference (significance threshold P = 0.05) between, conditions was determined by two-way ANOVA with Bonferroni's post-test.