Insight into *ortho*-boronoaldehyde conjugation via a FRET-based reporter assay

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Supplementary figures and tables

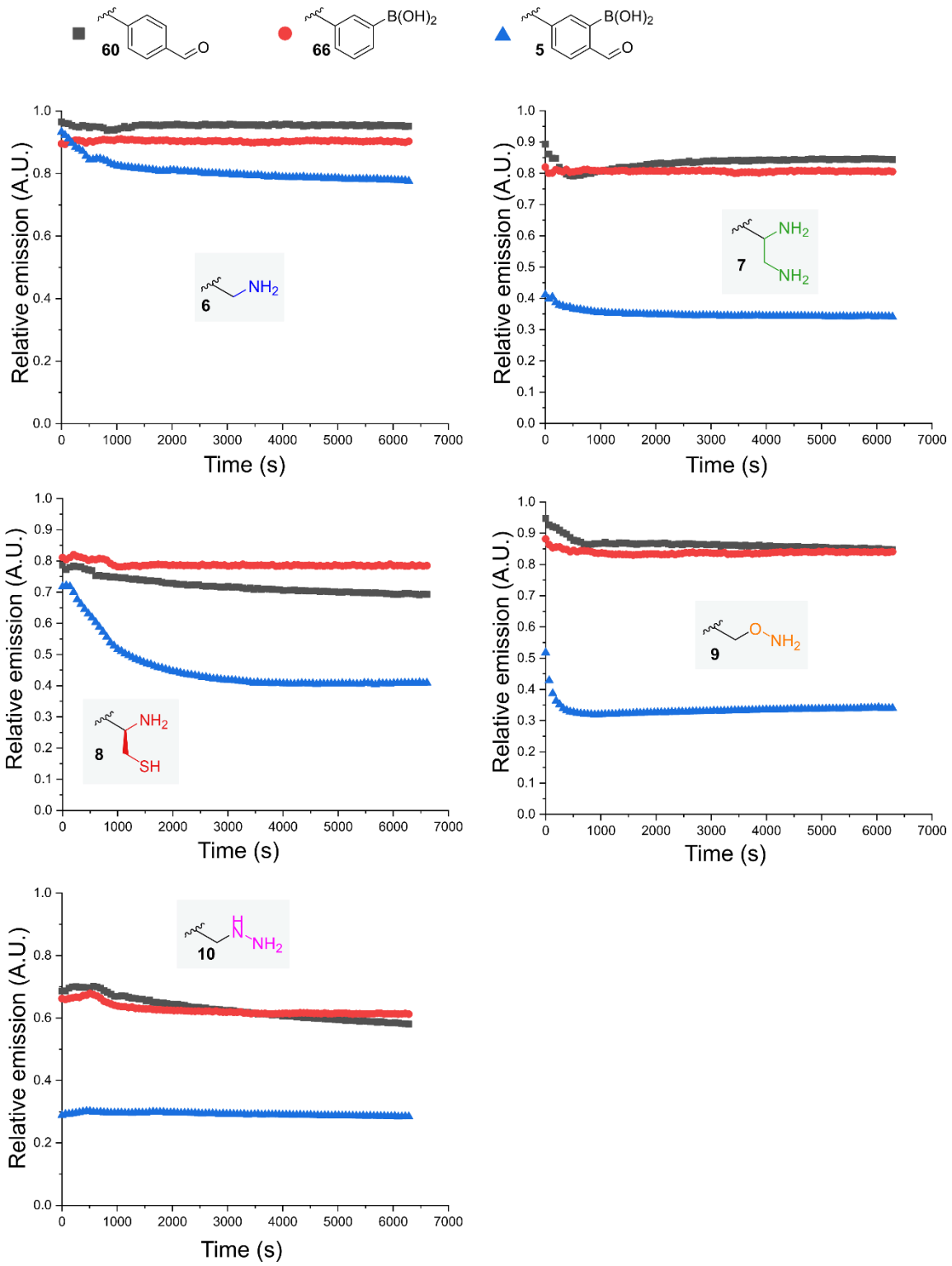


Figure S1: Plots of emission at 580 nm over time following the addition of Cy5-nucleophiles **6**-**10** (50 µM) to Cy3-substrates (5 µM) **5** (*o*BA) and controls **60** (benzaldehyde) and **66** (phenylboronic acid), relative to the emissions of controls containing either Cy3 or Cy5 substrate alone.

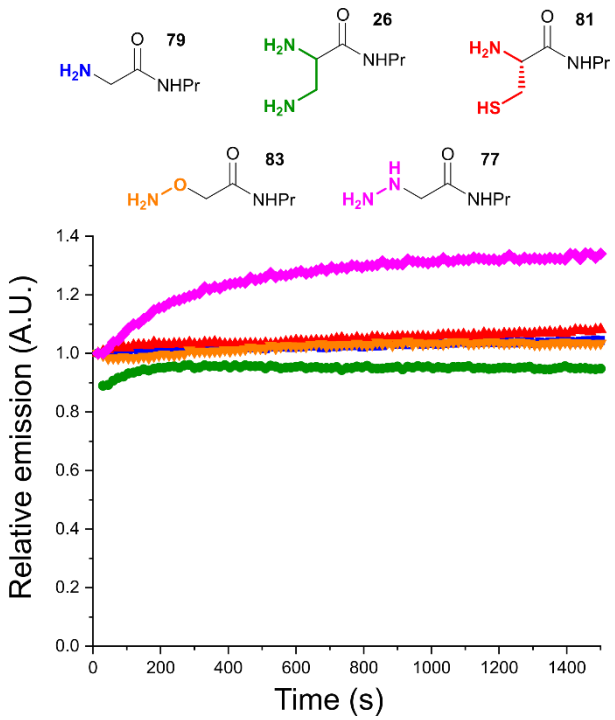


Figure S2: Plots of emission at 560 nm over time following the addition of propylamide-capped nucleophiles **26**, **76**, **78**, **80**, or **82** (50 µM) to Cy3-*o*BA **5** (5 µM), normalised to the emission at t = 3 seconds (first measurement). In this experiment, the absence of a Cy5-acceptor should mean that no drop in Cy3 emission is observed upon *o*BID formation. Although an *increase* in emission is observed for the addition of hydrazine **76**, the kinetics of this process are negligible relative to the rate of hydrazone/DAB formation.

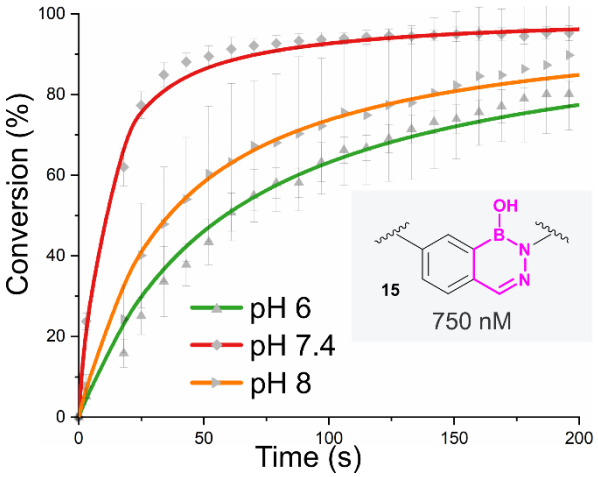


Figure S3: Plot of reaction conversion against time for the formation of *o*BID **15** and from Cy3-*o*BA **5** and Cy5-hydrazine **10**, at a reduced concentration of 750 nM under second-order conditions. Fits are based on second-order irreversible model, with errors based on the standard deviation of experiments run in triplicate.

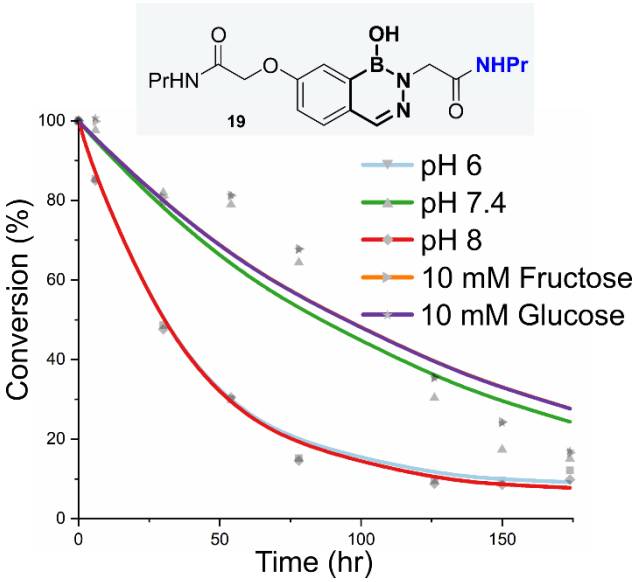


Figure S4: Plot of cleavage against time for propyl amide-DAB **19** (370 µM) following addition of methyl amide-hydrazine **21** (3700 µM) in the stated buffer. Fits are based on the model described in SI Section 10. Nb. Data in the presence of 10 mM glucose and fructose are very similar, leading to overlap of the fits.

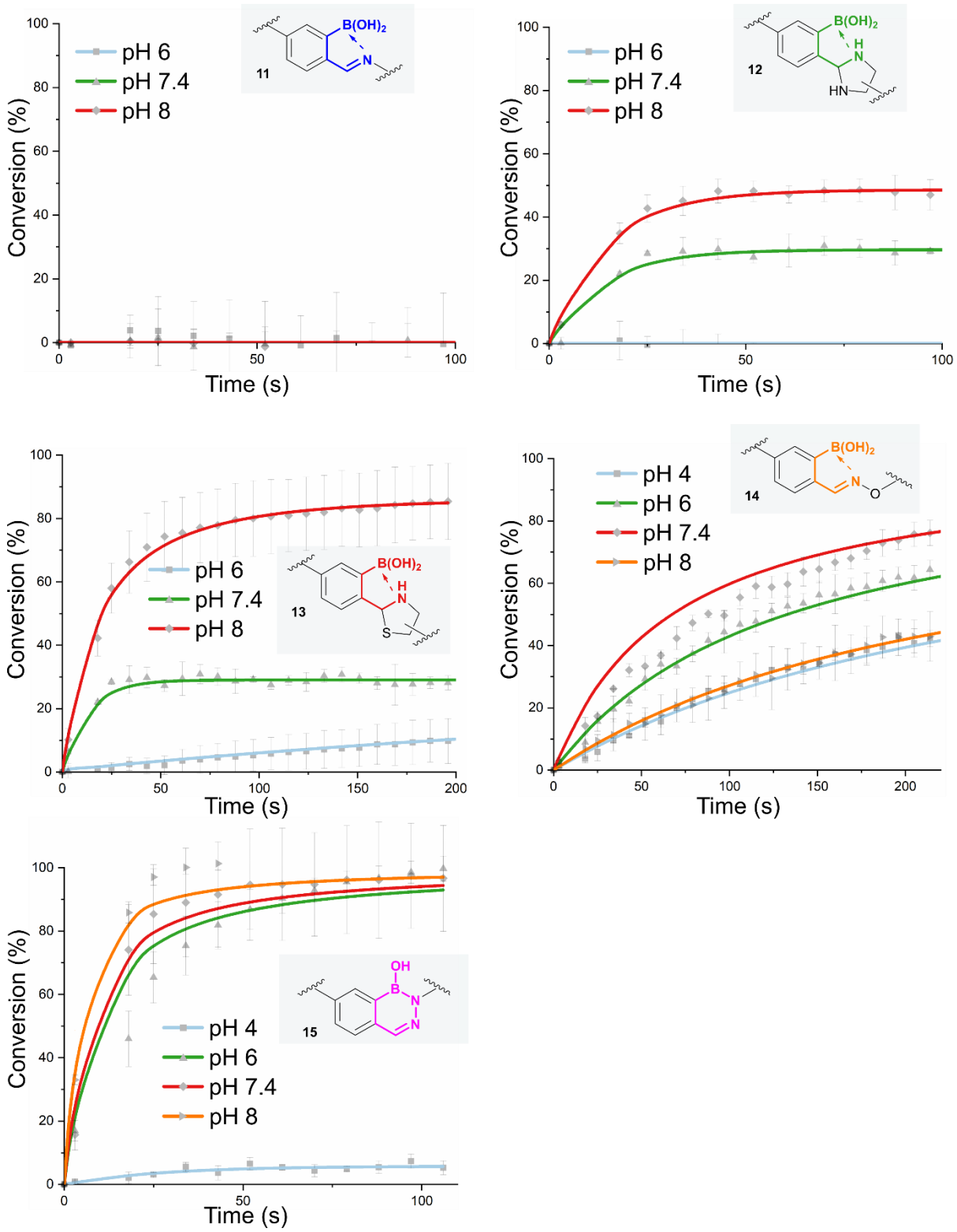


Figure S5: Conversion data grouped by nucleophile/structure formed, across range of pHs.

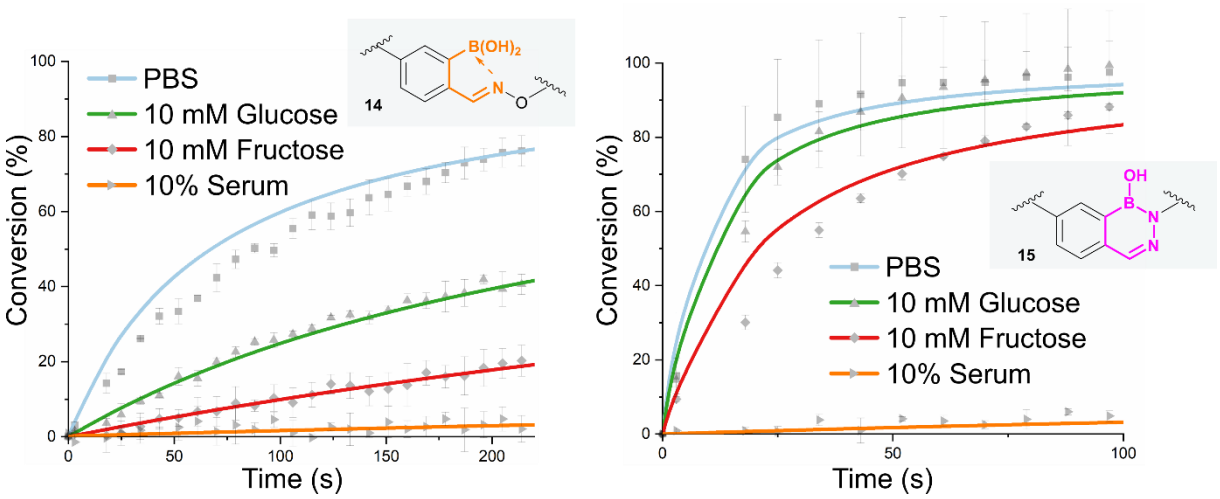


Figure S6: Plot of reaction conversion against time for the formation of *o*BIDs **14** and **15** from Cy3-*o*BA **5** and the relevant Cy5-nucleophile, in pH 7.4 PBS containing the stated additive. Reactions were run at a concentration of 2.5 µM under second-order conditions. Fits are based on second-order irreversible, or reversible models, with errors based on the standard deviation of experiments run in triplicate.

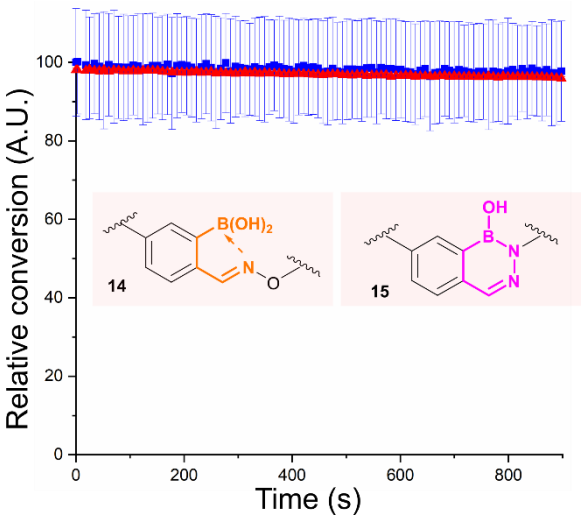


Figure S7: Plot of relative stability of **14** and **15** (2.5 µM), pre-formed in PBS, over time following the addition of 10% bovine serum. The absence of suitable references means it is not possible to calculate absolute conversions, and data is therefore based on changes in FRET ratio over time relative to *o*BA **5**.

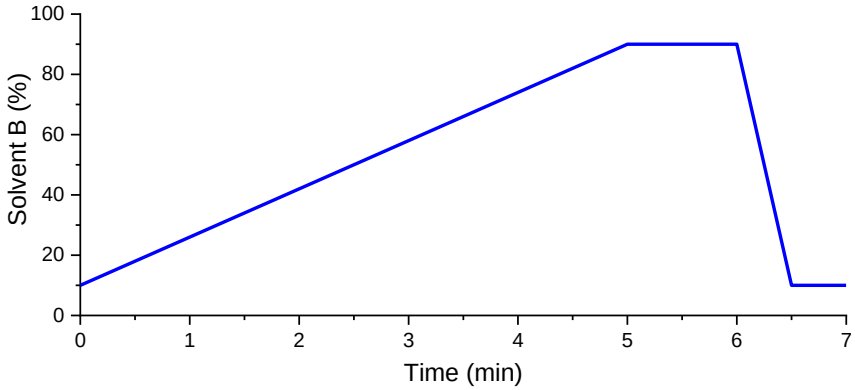
General considerations

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR respectively) spectra were recorded on a Jeol ECX-400 (400 MHz) or Bruker AVIIIHD (500 MHz) spectrometer. NMR shifts were assigned using COSY, HSQC and HMBC spectra. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^{1}H NMR: $CDCl_3$ = 7.26; MeOD = 3.31; D_2O = 4.69; DMSO-d_6 = 2.50 and ^{13}C NMR: $CDCl_3$ = 77.16, MeOD = 49.00, DMSO-d_6 = 39.52). Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent, br = broad. Melting points (m.p.) were recorded on a Gallenkamp melting point apparatus. Infrared (IR) spectra were recorded on a Perkin Elmer UATR Two FT-IR spectrometer. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}). UV-Vis spectra were recorded on a Shimadzu UV-1800 UV spectrophotometer in a glass cuvette, using a 480/30 nm excitation filter and a 580/10 nm emission filter, a pathlength of 1 cm, and a sampling interval of 1 nm. 96-well plate fluorescence measurements were recorded on a PerkinElmer VICTOR Nivo Multimode Plate Reader. Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer in a glass fluorescence cuvette with a pathlength of 1 cm, a sampling interval of 1 nm, and excitation and emission slit widths of 5 nm. High resolution electrospray ionisation (ESI) mass spectra (HRMS) were recorded on a Bruker Compact TOF-MS or a Jeol AccuTOF GCx-plus spectrometer. Nominal and exact *m/z* values are reported in Daltons.

Thin layer chromatography (TLC) was carried out using aluminium backed sheets coated with 60 F_{254} silica gel (Merck). Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254, 302, or 366 nm), and/or ammonium molybdate (5% in 2M H_2SO_4), and/or potassium permanganate (5% $KMnO_4$ in 1M NaOH with 5% potassium carbonate), and/or ninhydrin (1.5% ninhydrin, 3% AcOH in *n*-butanol), and/or bromocresol green (0.4% bromocresol green in ethanol, basified till blue with 0.1 M NaOH). Flash column chromatography was carried out using Geduran Si 60 (40-63 μm) (Merck). Mobile phases are reported as ratios of more polar solvent to less polar solvent. Anhydrous solvents were dried over a PureSolv MD 7 Solvent Purification System. Deionized water was used for chemical reactions. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reagents were purchased from Sigma-Aldrich and used as supplied, unless otherwise

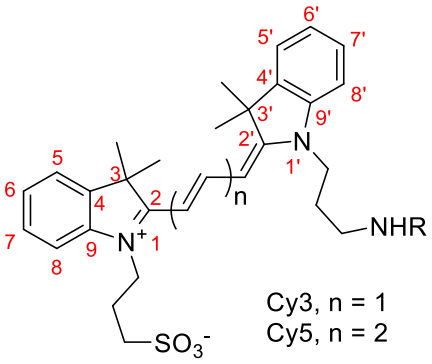
indicated. Brine refers to a saturated solution of sodium chloride. Petrol refers to the fraction of petroleum ether boiling in the range 40-60 ºC. Anhydrous magnesium sulfate ($MgSO_4$) was used as the drying agent after reaction workup unless otherwise stated.

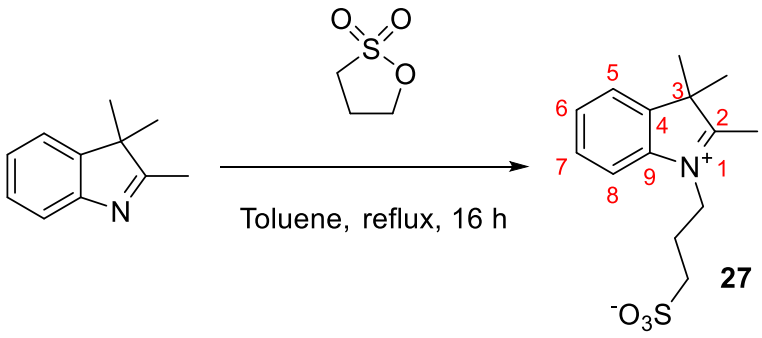
Liquid chromatography-mass spectrometry (LC-MS) was performed on a HCTultra ETD II ion trap spectrometer, coupled to an Ultimate300 HPLC using an Accucore C18 column (150 × 2.1 mm, 2.6 µm particle size). Water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid, were used as the mobile phase at a flow rate of 0.3 mL min^{-1}. LC traces were measured via UV absorption at 220, 270, and 280. The gradient was programmed as shown below:



1. Core dye synthesis

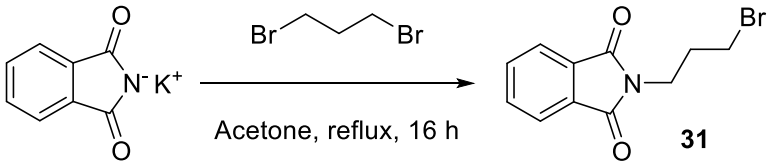
Numbering system for Cy3/5 NMR assignments





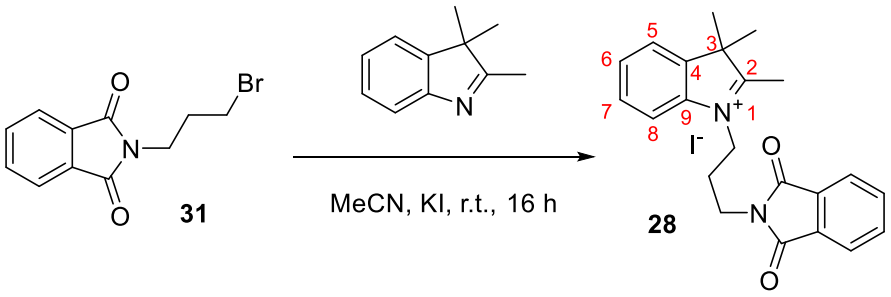
A mixture of 2,3,3-trimethylindolenine (2.00 mL, 12.7 mmol) and 1,3-propanesultone (1.55 g, 12.7 mmol) in toluene (50 mL) was refluxed for 20 h, during which time a dark red precipitate formed. After cooling to r.t., the reaction mixture was concentrated under reduced pressure. The residue was redissolved in dichloromethane (5 mL) and the solution added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (50 mL), and dried in air to yield a red oil (3.10 g, 11.0 mmol, 87%). Data were consistent with those previously reported.[1]

^{1}H NMR (400 MHz, CD_3OD) δ = 8.01-7.93 (m, 1H, H5), 7.78-7.70 (m, 1H, H7), 7.68-7.59 (m, 2H, H6, H8), 4.78-4.67 (m, 2H, $PhCH_2$), 3.03-2.93 (m, 2H, $CH_2SO_3^-$), 2.43-2.26 (m, 2H, $CH_2CH_2SO_3^-$), 1.58 (s, 6H, 2 × CH_3); **HRMS**: m/z (ESI^+) calc. for $C_{14}H_{19}NO_3S$ $[M+H]^+$: 282.1158: Obs.: 282.1162; ν_{max}: (FT-ATR)/cm^{-1}: 3426, 2989, 1641, 1460, 1212, 1160, 1035, 758, 522.



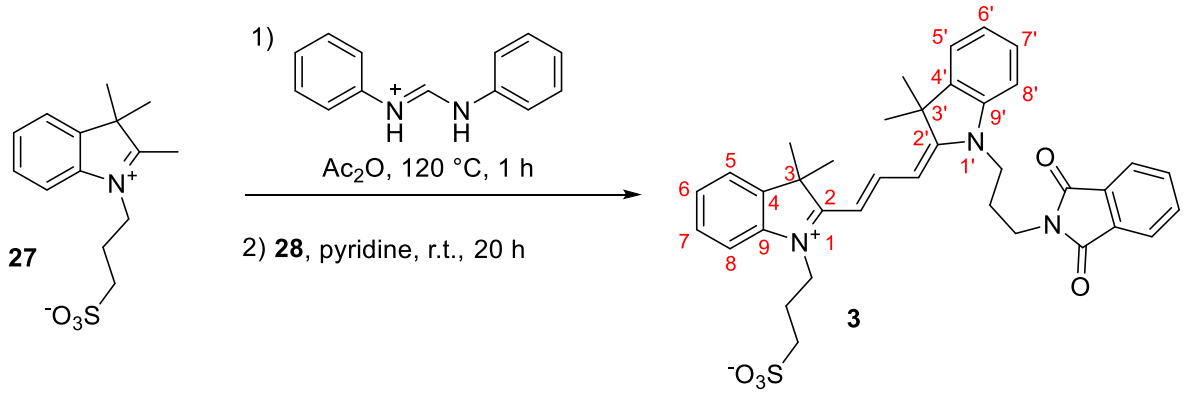
Potassium phthalimide (1.85 g, 10.0 mmol) was added in portions over 5 min to a stirred solution of 1,3-dibromopropane (1.00 mL, 10.0 mmol) in acetone (50 mL). The solution was then refluxed for 18 h. After cooling to r.t., the reaction mixture was filtered under vacuum, and the filtrate concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:petrol (3:8). Fractions containing the product were concentrated under reduced pressure to provide a white solid (1.17 g, 4.38 mmol, 44%). Data were consistent with those previously reported.[2]

R$_f$: 0.21 (2:8, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 7.91-7.79 (m, 2H, PhthH2), 7.78-7.66 (m, 2H, PhthH3), 3.83 (t, *J* = 6.8 Hz, 2H, CH_2N), 3.41 (t, *J* = 6.8 Hz, 2H, CH_2Br), 2.25 (tt, $J_1 = J_2$ = 6.8 Hz, 2H, CH_2CH_2Br); **HRMS**: m/z (ESI^+) calc. for $C_{11}H_{10}{}^{79}BrNO_2$ $[^{79}M+Na]^+$: 289.9787; Obs.: 289.9774; ν_{max}: (FT-ATR)/cm^{-1}: 3454, 2985, 1765, 1705, 1442, 1406, 1375, 1230, 1055, 966, 870, 723; **m.p.:** 71-74 °C.



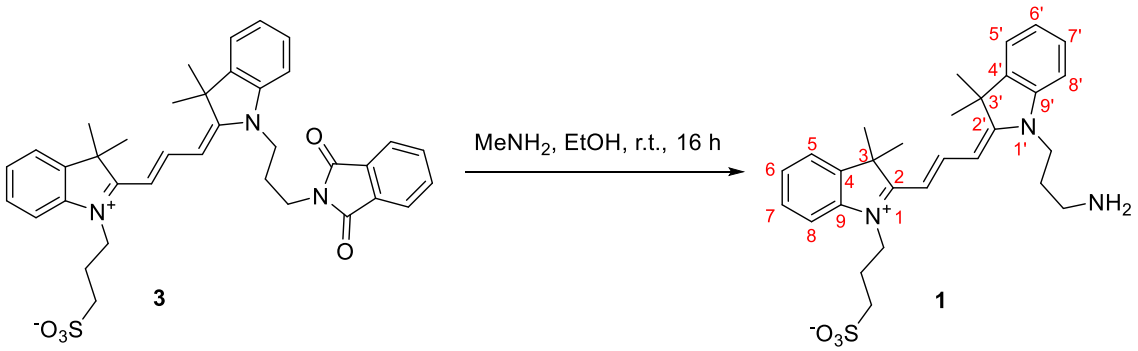
Potassium iodide (744 mg, 4.48 mmol) was added to a stirred solution of 2,3,3-trimethylindolenine (710 µL, 4.48 mmol), and **31** (1.00 g, 3.73 mmol) in anhydrous acetonitrile (20 mL) under an argon atmosphere. The resulting mixture was refluxed for 5 h. After cooling to r.t., the reaction was filtered under vacuum and the filtrate concentrated under reduced pressure. The residue was then redissolved in acetone (10 mL) and the solution added dropwise to diethyl ether (200 mL). The resultant brown precipitate was collected by filtration, washed with diethyl ether (30 mL) and dried in air. The solid was then redissolved in acetone (10 mL) and concentrated under reduced pressure to afford the product as a brown solid (878 mg, 2.53 mmol, 68%).

***R*$_f$**: 0.29 (1:9, MeOH:CH_2Cl_2, UV active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 7.89-7.85 (m, 1H, H5), 7.84-7.80 (m, 2H, PhthH2), 7.79-7.73 (m, 3H, PhthH3, H7), 7.64-7.55 (m, 2H, H8, H6), 4.64 (t, *J* = 7.0 Hz, 2H, CH_2N^+), 3.88 (t, *J* = 7.0 Hz, 2H, CH_2NPhth), 2.37 (tt, $J_1 = J_2$ = 7.0 Hz, 2H, CH_2CH_2N), 1.61 (s, 6H, 2 × CH_3); **^{13}C NMR** (400 MHz, CD_3OD) δ = 168.5 (PhthCON), 142.0 (C9), 141.2 (C4), 134.2 (PhthC2), 132.0 (PhthC1), 129.9 (C7), 129.2 (C6), 123.4 (PhthC3), 123.0 (C5), 115.1 (C8), 46.1 (CH_2NPhth), 34.8 ($CH_2CH_2CH_2$NPhth), 26.5 (CH_2CH_2NPhth), 21.5 (CyCH_3); **HRMS**: m/z (ESI$^+$) calc. for $C_{22}H_{22}N_2O_2$ $[M]^+$: 347.1754; Obs.: 347.1761; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3441, 2976, 1769, 1707, 1608, 1463, 1398, 765, 721, 530; **m.p.:** 176-179 °C.



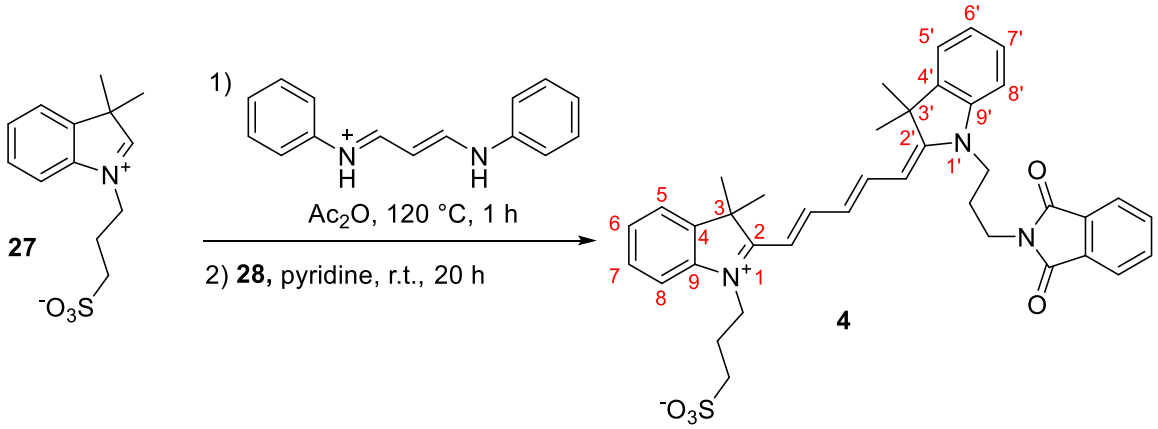
A mixture of **27** (3.10 g, 11.0 mmol) and *N*,*N*'-diphenylformamidine (2.17 g, 11.0 mmol) in acetic anhydride (10 mL) was heated to 120 °C for 1 h. After the reaction mixture was cooled to r.t., a solution of **28** (3.44 g, 9.91 mmol) in pyridine (10 mL) was added and the mixture stirred at r.t. for a further 20 h. After this time, the mixture was added dropwise to diethyl ether (500 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then redissolved in methanol (20 mL) and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a pink powder (2.65 g, 4.16 mmol, 42%).

R_f: 0.16 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 8.32 (dd, J_1 = J_2 = 13.4 Hz, 1H, C<u>H</u>CHCN), 7.84-7.75 (m, 4H, Phth<u>H</u>2, Phth<u>H</u>3), 7.62-7.54 (m, 3H, <u>H</u>5, <u>H</u>5', <u>H</u>8/<u>H</u>8'), 7.45-7.35 (m, 3H, <u>H</u>7, <u>H</u>7', <u>H</u>8/<u>H</u>8'), 7.25 (ddd, J_1 = J_2 = 7.5 Hz, J_3 = 2.8 Hz, 2H, <u>H</u>6, <u>H</u>6'), 6.50 (d, J = 13.4 Hz, 2H, C<u>H</u>CN), 4.26-4.18 (m, 4H, C<u>H</u>$_2$CH$_2$CH$_2$SO$_3^-$, C<u>H</u>$_2$CH$_2$CH$_2$NPhth), 3.69 (t, J = 7.1 Hz, 2H, C<u>H</u>$_2$NPhth), 2.52 (t, J = 7.4 Hz, 2H, <u>CH</u>$_2$SO$_3^-$), 2.08 (tt, J_1 = J_2 = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_2$NPhth), 2.00 (tt, J_1 = J_2 = 7.4 Hz, 2H, C<u>H</u>$_2$CH$_2$SO$_3^-$), 1.67 (s, 12H, CyC<u>H</u>$_3$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 174.9 (<u>C</u>HCHCN), 174.6 (<u>C</u>2, <u>C</u>2'), 168.4 (Phth<u>C</u>ON), 151.1 (<u>C</u>3, <u>C</u>3'), 141.9 (<u>C</u>9, <u>C</u>9'), 140.9 (<u>C</u>4, <u>C</u>4'), 134.1 (Phth<u>C</u>2), 132.1 (Phth<u>C</u>1), 128.7 (<u>C</u>7, <u>C</u>7'), 125.6 (<u>C</u>6, <u>C</u>6'), 123.9 (Phth<u>C</u>3), 122.2 (<u>C</u>5, <u>C</u>5'), 111.3 (<u>C</u>8/<u>C</u>8'), 111.0 (<u>C</u>8/<u>C</u>8'), 103.8 (<u>C</u>HCN), 103.7 (<u>C</u>HCN), 49.4 (<u>C</u>H$_2$SO$_3^-$), 42.7 (<u>C</u>H$_2$CH$_2$CH$_2$SO$_3^-$), 41.6 (<u>C</u>H$_2$NPhth), 35.1 (<u>C</u>H$_2$CH$_2$CH$_2$NPhth), 27.6 (<u>C</u>H$_2$CH$_2$NPhth), 26.1 (Cy<u>C</u>H$_3$), 22.8 (<u>C</u>H$_2$CH$_2$SO$_3^-$); **HRMS**: m/z (ESI$^+$) calc. for $C_{37}H_{39}N_3O_5S$ [M+H]$^+$: 638.2683; Obs.: 638.2695; ***ν*_max**: (FT-ATR)/cm^{-1}: 3443, 2975, 2930, 1709, 1555, 1428, 1373, 1152, 1037, 929, 759, 723; **m.p.:** 272-276 °C.



A mixture of methylamine (40% in methanol, 30 mL) and **3** (500 mg, 0.78 mmol) in methanol (5 mL) was stirred at r.t. for 16 h. The reaction mixture was then concentrated under reduced pressure to ~5 mL, and the solution added dropwise to diethyl ether (400 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL) and dried in air. The solid was then redissolved in methanol (20 mL) and concentrated under reduced pressure to give a pink solid (498 mg, 0.78 mmol, quantitative yield).

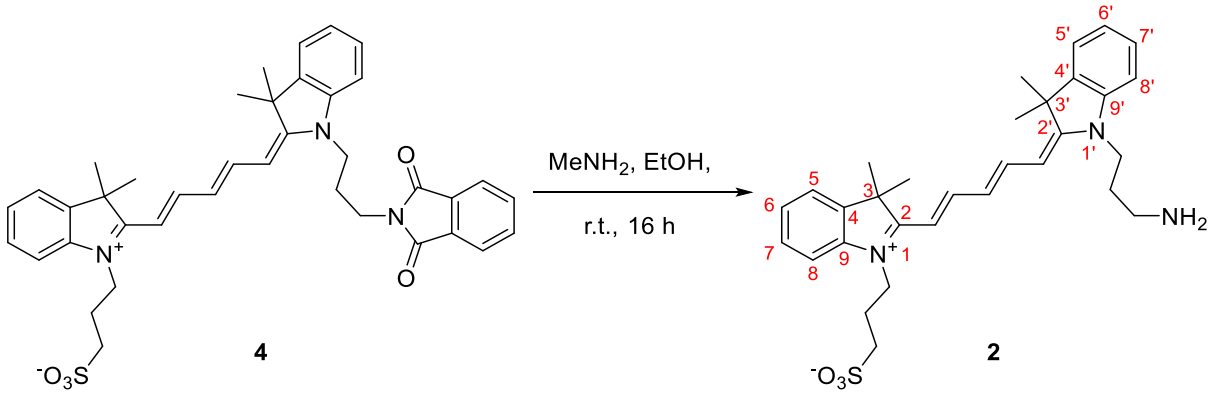
***R*$_f$**: 0.18 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.52 (dd, $J_1 = J_2$ = 13.5 Hz, 1H, CHCHCN), 7.53 (d, J = 7.5 Hz, 2H, H5, H5'), 7.45-7.30 (m, 4H, H7, H7', H8, H8'), 7.34-7.22 (m, 2H, H6, H6'), 6.82 (d, J = 13.5 Hz, 1H, CHCN), 6.60 (d, J = 13.5 Hz, 1H, CHCN), 4.41 (t, J = 7.7 Hz, 2H, $CH_2CH_2CH_2SO_3^-$), 4.27 (t, J = 7.7 Hz, 2H, $CH_2CH_2CH_2NH_2$), 3.21 (t, J = 7.7 Hz, 2H, CH_2NH_2), 3.02 (t, J = 7.7 Hz, 2H, $CH_2SO_3^-$), 2.29-2.23 (m, 2H, $CH_2CH_2NH_2$), 2.20-2.16 (m, 2H, $CH_2CH_2SO_3^-$), 1.73 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 174.8 (CHCHCN), 174.5 (C2, C2'), 151.0 (C3, C3'), 141.9 (C9, C9'), 140.9 (C4, C4'), 128.8 (C7, C7'), 125.5 (C6, C6'), 122.3 (C5, C5'), 111.3 (C8/C8'), 111.1, (C8/C8'), 103.1 ($CHCN^+$), 102.8 (CHCN), 49.3 ($CH_2SO_3^-$), 42.6 ($CH_2CH_2CH_2SO_3^-$), 41.5 (CH_2NH_2), 37.9 ($CH_2CH_2CH_2NH_2$), 28.1 ($CH_2CH_2NH_2$), 27.0 ($CyCH_3$), 23.0 ($CH_2CH_2SO_3^-$); **HRMS**: m/z (ESI^+) calc. for $C_{29}H_{36}N_3O_3S$ $[M+H]^+$: 508.2636; Obs.: 508.2636; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3437, 2975, 1711, 1556, 1429, 1207, 1147, 1037, 971, 930, 758; **m.p.:** >325 °C.



A mixture of **27** (2.00 g, 7.12 mmol) and malonaldehyde bis(phenylimine) monohydrochloride (1.75 g, 7.83 mmol) in acetic anhydride (10 mL) was heated to 120 °C for 1.5 h. After cooling to r.t, a solution of **28** (2.25 g, 6.48 mmol) in pyridine (10 mL) was added and stirring was continued at r.t. for a further 16 h. The reaction mixture was then concentrated under reduced pressure to ~5 mL, and the remaining solution

added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then redissolved in methanol (10 mL) and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue solid (1.35 g, 2.04 mmol, 29%).

***R*$_f$**: 0.29 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR (**400 MHz, DMSO-d_6) δ = 8.28 (dd, *J* = 13.2, 10.1 Hz, 2H, 2 × CHCHCN), 7.85-7.75 (m, 4H, PhthH2, PhthH3), 7.56 (d, *J* = 7.4 Hz, 1H, H5/H5'), 7.53 (d, *J* = 7.4 Hz, 1H, H5/H5'), 7.46 (d, *J* = 7.9 Hz, 1H, H8/H8'), 7.38-7.32 (m, 2H, H7, H7'), 7.29 (d, *J* = 7.9 Hz, 1H, H8/H8'), 7.20 (dd, *J* = 7.4 Hz, 1H, H6/H6'), 7.15 (dd, *J* = 7.4 Hz, 1H, H6/H6'), 6.45-6.33 (m, 2H, CHCHCHCN, CHCN), 6.20 (d, *J* = 13.2 Hz, 1H, CHCN), 4.34-4.22 (m, 2H, $CH_2CH_2CH_2SO_3^-$), 4.17 (t, *J* = 7.2 Hz, 2H, $CH_2CH_2CH_2$NPhth), 3.67 (t, *J* = 7.2 Hz, 2H, CH_2NPhth), 2.57 (t, *J* = 6.8 Hz, 2H, $CH_2SO_3^-$), 2.05-1.95 (m, 4H, CH_2CH_2NPhth, $CH_2CH_2SO_3^-$), 1.62 (s, 12H, CyCH_3); **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 173.8 (C2, C2'), 172.5 (C3, C3'), 168.5 (CON), 155.1 (CHCHCN), 154.3 (CHCHCN), 142.6 (C9/C9'), 142.5 (C9/C9'), 141.7 (C4/C4'), 141.5 (C4/C4'), 134.9 (PhthC2), 132.3 (PhthC1), 129.0 (C7/C7'), 128.9 (C7/C7'), 126.1 (CHCHCHCN), 125.5 (C6/C6'), 125.0 (C6/C6'), 123.6 (PhthC3), 123.0 (C5, C5'), 111.9 (C8/C8'), 111.3 (C8/C8'), 104.3 (CHCN), 103.3 (CHCN), 48.4 ($CH_2SO_3^-$), 43.3 ($CH_2CH_2CH_2SO_3^-$) 41.5 ($CH_2CH_2CH_2$NPhth), 35.6 (CH_2NCO), 27.6 (CyCH_3), 26.5 (CH_2CH_2NPhth), 24.0 ($CH_2CH_2SO_3^-$); **HRMS**: m/z (ESI$^+$) calc. for $C_{39}H_{41}N_3O_5S$ $[M+H]^+$: 664.2840; Obs.: 664.2858; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3442, 2973, 1770, 1709, 1492, 1455, 1381, 1337, 1132, 1108, 1034, 1017, 927, 795, 721, 530; **m.p.:** 264-269 °C.

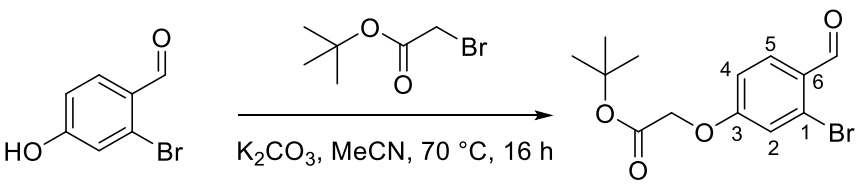


Methylamine (40% in water, 30 mL) was added to a solution of **4** (200 mg, 0.30 mmol) in ethanol (5 mL) and the solution stirred at r.t. for 16 h. The reaction mixture was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (400 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL) and dried in air. The solid was then redissolved in methanol (20 mL) and concentrated under reduced pressure to give the product as a blue solid (159 mg, 0.298 mmol, 99%).

Nb. Attempts to cleave the phthalimide with traditional hydrazinolysis were unsuccessful, with a loss of colour over a period of 1 h indicating a loss of conjugation/dye structure.

***R*$_f$**: 0.17 (1:9, MeOH:CH_2Cl_2, visible light active).**^{1}H NMR** (400 MHz, CD_3OD) δ = 8.21 (d, *J* = 13.2 Hz 2H, CHCHCN), 7.49-7.43 (m, 2H, H5, H5'), 7.41-7.34 (m, 3H, H7, H7', H8/H8'), 7.27-7.17 (m, 3H, H6, H6', H8/H8'), 6.69-6.65 (m, 1H, CHCHCHCN), 6.62-6.58 (m, 1H, CHCN), 6.33-6.28 (m, 1H, CHCN), 4.35 (t, *J* = 8.0 Hz, 2H, $CH_2CH_2CH_2SO_3^-$), 4.22-4.10 (m, 2H, $CH_2CH_2CH_2NH_2$), 3.02-2.94 (m, 4H, CH_2NH_2, $CH_2SO_3^-$), 2.24-2.18 (m, 2H, $CH_2CH_2SO_3^-$), 2.10-2.00 (m, 2H, $CH_2CH_2NH_2$), 1.66 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 174.7 (C2, C2'), 170.9 (C3/C3'), 170.3 (C3/C3'), 155.1 (CHCHCN), 154.1 (CHCHCN), 142.8 (C9/C9'), 142.1 (C9/C9'), 141.4, (C4, C4'), 128.5 (C7, C7'), 125.9 (CHCHCHCN), 125.0 (C6, C6'), 123.3 (C5, C5'), 122.1 (C7, C7'), 110.8 (C8, C8'), 103.4 (CHCN), 102.9 (CHCN), 49.1 ($CH_2SO_3^-$), 42.7 ($CH_2CH_2CH_2SO_3^-$), 41.7 ($CH_2CH_2CH_2NH_2$), 36.7 (CH_2NH_2), 28.8 ($CyCH_3$), 26.6 ($CH_2CH_2NH_2$), 22.9 ($CH_2CH_2SO_3^-$); **HRMS**: m/z (ESI$^+$) calc. for $C_{31}H_{39}N_3O_3S$ $[M+H]^+$: 534.2785; Obs.: 534.2803; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3438, 2968, 2937, 1573, 1482, 1454, 1381, 1338, 1136, 1105, 1035, 1017, 927, 800, 752, 709, 525; **m.p.:** 252-256 °C.

2. Synthesis of reactive handles

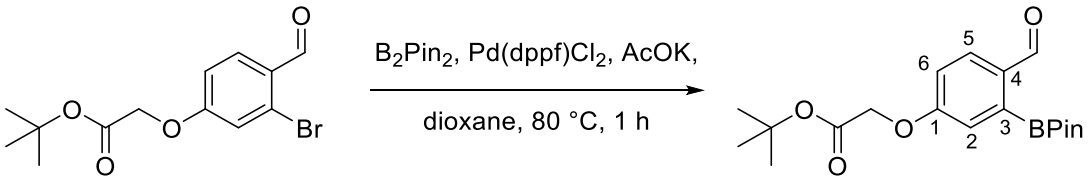


32

A mixture of 2-bromo-4-hydroxybenzaldehyde (2.07 g, 10.4 mmol), *tert*-butyl bromoacetate (1.53 mL, 10.4 mmol) and potassium carbonate (2.43 g, 17.6 mmol) in

acetonitrile (30 mL) was stirred for 16 h at 70 °C. The mixture was then cooled to r.t. and diluted with water (150 mL). The aqueous mixture was extracted with ethyl acetate (3 × 70 mL), and the combined organics washed with brine (2 × 200 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (15:85). Fractions containing the product were concentrated under reduced pressure to provide a white solid (3.23 g, 10.3 mmol, 99%). Data were consistent with those previously reported.[3]

***R*$_f$**: 0.35 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.19 (s, 1H, C<u>H</u>O), 7.87 (d, *J* = 8.6 Hz, 1H, Ph<u>H</u>5), 7.11 (d, *J* = 2.5 Hz, 1H, Ph<u>H</u>2), 6.91 (dd, *J* = 8.6, 2.5 Hz, 1H, Ph<u>H</u>4), 4.57 (s, 2H, C<u>H</u>$_2$O), 1.47 (s, 9H, ^{t}Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 190.6 (<u>C</u>HO), 166.8 (<u>C</u>OO), 162.8 (Ph<u>C</u>4), 131.5 (Ph<u>C</u>5), 128.7 (Ph<u>C</u>1), 127.7 (Ph<u>C</u>3), 119.5 (Ph<u>C</u>2), 114.5 (Ph<u>C</u>6), 83.3 (<u>C</u>Me$_3$), 65.8 (<u>C</u>H$_2$O), 28.1 (^{t}Bu); **HRMS**: m/z (ESI$^+$) calc. for $C_{13}H_{15}{}^{79}BrO_4$ [^{79}M+Na]$^+$: 337.0053; Obs.: 337.0046; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 2979, 2863, 1746, 1685, 1590, 1486, 1368, 1310, 1218, 1152, 1071, 1028, 843, 613; **m.p.:** 87-89 °C.

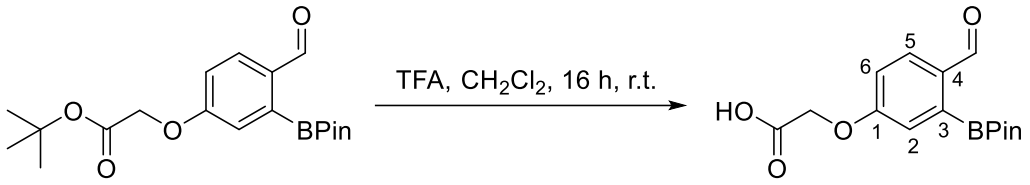


32 **33**

32 (2.15 g, 6.85 mmol), bis(pinacolato)diboron (4.52 g, 17.8 mmol), 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (500 mg, 0.685 mmol) and potassium acetate (3.62 g, 37.0 mmol) were placed under a nitrogen atmosphere, and anhydrous dioxane (25 mL) was added. Nitrogen was bubbled through the reaction mixture for 10 min, which was then stirred at 80 °C for 1 h. After cooling to r.t., the reaction was concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (15:85). Fractions containing the product were concentrated under reduced pressure to yield a white solid (1.87 g, 5.16 mmol, 75%). Data were consistent with those previously reported.[3]

***R*$_f$**: 0.38 (15:85, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.39 (s, 1H, C<u>H</u>O), 7.93 (d, *J* = 8.8 Hz, 1H, Ph<u>H</u>5), 7.26 (d, *J* = 2.5 Hz, 1H, Ph<u>H</u>2), 7.03 (dd, *J* = 8.8, 2.5 Hz, 1H, Ph<u>H</u>6), 4.59 (s, 2H, C<u>H</u>$_2$O), 1.47 (s, 9H, ^{t}Bu), 1.36 (s, 12H, C(C<u>H</u>$_3$)$_2$);

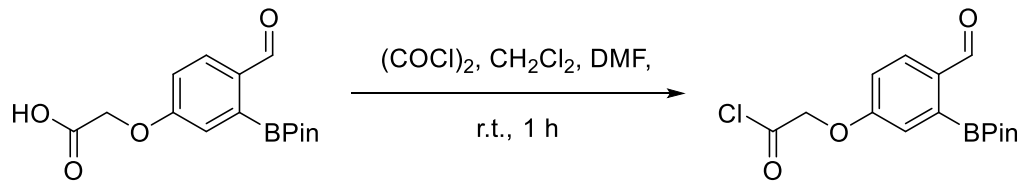
^{13}C NMR (101 MHz, $CDCl_3$) δ = 193.2 (CHO), 167.4 (COO), 161.5 (PhC1), 135.5 (PhC3), 132.0 (PhC4), 130.4 (PhC5), 120.5 (PhC2), 117.2 (PhC6), 84.6 ($OC(CH_3)_2$), 82.9 (CMe_3), 65.6 (CH_2O), 28.1 (^{t}Bu), 25.1 ($C(CH_3)_2$); **HRMS**: m/z (ESI^+) calc. for $C_{19}H_{27}BO_6$ $[M+H]^+$: 363.1977; Obs.: 363.1977; ν_{max}: (FT-ATR)/cm^{-1}: 2979, 2933, 1752, 1686, 1589, 1420, 1340, 1323, 1211, 1147, 1123, 1077, 1052, 964, 849, 734; **m.p.:** 80-83 °C.



33 **34**

Trifluoroacetic acid (3.0 mL) was added dropwise to a solution of **33** (1.00 g, 2.76 mmol) in dichloromethane (15 mL), and the mixture was stirred at r.t. for 16 h. The reaction mixture was then concentrated under reduced pressure and azeotroped with dichloromethane (4 × 20 mL) to obtain a white powder. (810 mg, 2.65 mmol, 96%). Data were consistent with those previously reported.[3]

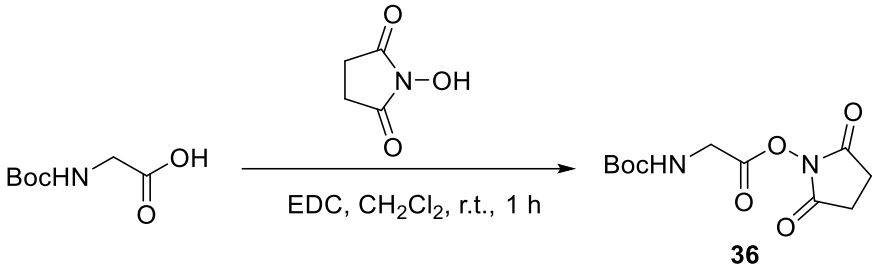
***R*$_f$**: 0.24 (4:6, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 10.10 (s, 1H, CHO), 7.85 (d, *J* = 8.5 Hz, 1H, PhH5), 7.12 (dd, *J* = 8.5, 2.8 Hz, 1H, PhH6), 7.09 (d, *J* = 2.8 Hz, 1H, PhH2), 4.80 (s, 2H, CH_2O), 1.30 (s, 12H, $C(CH_3)_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 194.4 (CHO), 168.4 (COOH), 161.4 (PhC1), 135.2 (PhC3), 131.3 (PhC4), 130.8 (PhC5), 121.0 (PhC2), 116.7 (PhC6), 85.0 ($OC(CH_3)_2$), 64.5 (CH_2O), 25.0 ($C(CH_3)_2$); **HRMS**: m/z (ESI^+) calc. for $C_{15}H_{18}BO_6$ $[M+H]^+$: 307.1350; Obs.: 307.1350; ν_{max}: (FT-ATR)/cm^{-1}: 2979, 2937, 1763, 1561, 1418, 1371, 1343, 1283, 1203, 1174, 1125, 1072, 960, 850, 691; **m.p.:** 169-172 °C.



34 **35**

Oxalyl chloride (61 µL, 0.71 mmol) was added to a solution of **34** (72 mg, 0.24 mmol), dichloromethane (3 mL) and dimethylformamide (1 drop), and stirred at r.t. for 1 h. Excess oxalyl chloride and dichloromethane were removed under reduced pressure to

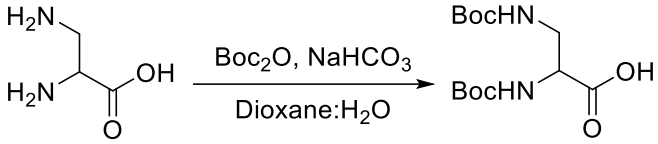
give the crude product as a brown oil, which was carried forward without further purification.



36

A mixture of *N*-(tert-butoxycarbonyl)glycine (100 mg, 0.571 mmol), *N*-hydroxy succinimide (99 mg, 0.857 mmol), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (164 mg, 0.86 mmol) in dichloromethane (5 mL) was stirred at r.t. for 1 h. Dichloromethane (30 mL) was then added and the organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure to give a white solid (165 mg, 0.61 mmol, 75%). Data were consistent with those previously reported.[4]

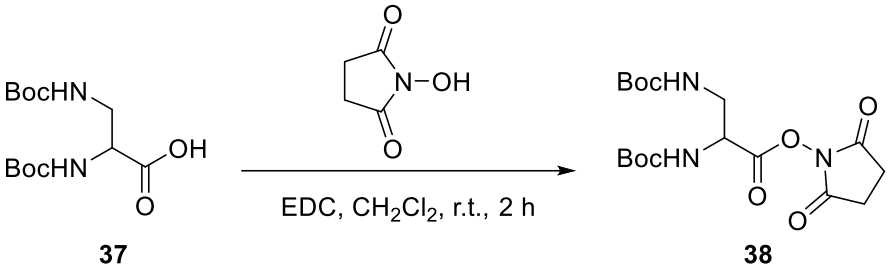
***R*$_f$**: 0.27 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.97 (app br s, 1H, NH), 4.28 (d, *J* = 5.9 Hz, 2H, CH_2N), 2.84 (s, 4H, OSu), 1.44 (s, 9H, Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_{11}H_{16}N_2O_6$ [M+Na]$^+$: 295.0901; Obs.: 295.0901; **m.p.:** 156-159 °C.



37

Di-*tert*-butyl dicarbonate (3.05 g, 14 mmol) was added to a solution of 2,3-diaminopropionic acid (500 mg, 3.5 mmol) and sodium bicarbonate (2.94 g, 10 mmol) in a mixture of dioxane (15 mL) and water (15 mL), and the reaction was stirred at r.t. for 18 h. The mixture was then diluted with water (50 mL) and washed with dichloromethane (2 × 15 mL). The aqueous layer was acidified with hydrochloric acid (1 M) to pH ~2, and then extracted with dichloromethane (3 × 30 mL). The combined organic extracts of the acidified aqueous fraction were combined, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to yield a colourless oil (210 g, 0.7 mmol, 20%). Data were consistent with those previously reported.[5]

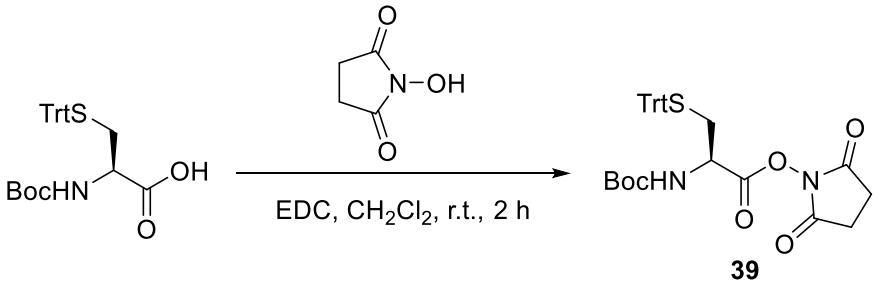
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.71 (br s, 1H, NH), 5.19 (br s, 1H, NH), 4.22-4.31 (m, 1H, H_α), 3.46-3.75 (m, 2H, H_β), 1.43 (s, 18H, 2 × Boc).



37 **38**

A mixture of **37** (29 mg, 95 µmol), *N*-hydroxysuccinimide (16 mg, 0.143 mmol), and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (28 mg, 0.143 mmol) in dichloromethane (1.0 mL) was stirred at r.t. for 2 h. Dichloromethane (10 mL) was then added and the organics were washed with water (2 × 15 mL) and brine (15 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a pink foam (28 mg, 70 µmol, 74%). The product was used immediately without any further purification or analysis.

***R*$_f$**: 0.28 (1:9, EtOAc:Petrol); **HRMS**: m/z (ESI^+) calc. for $C_{17}H_{27}N_3O_8$ $[M+H]^+$: 402.1871; Obs.: 402.1874; **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 2.82 (m, 4H, NHS-CH_2), 1.42 (s, 18H, 2 × Boc).

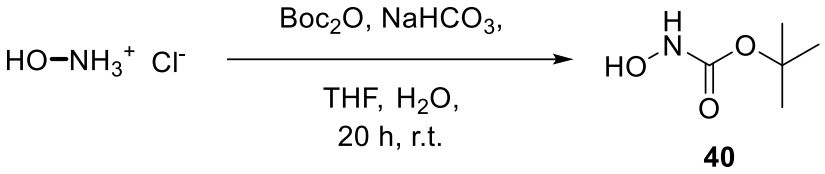


39

A mixture of Boc-Cys-(Trt)-OH (1.00 g, 2.16 mmol), *N*-hydroxysuccinimide (372 mg, 3.23 mmol), and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (620 mg, 3.23 mmol) in dichloromethane (20 mL) was stirred at r.t. for 2 h. Dichloromethane (30 mL) was then added and the organics were washed with water (2 × 50 mL) and brine (50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a white foam. (1.16 g, 0.207 mmol, quantitative yield).

***R*$_f$**: 0.24 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.43 (dd, *J* = 7.5, 1.7 Hz, 6H, PhH2), 7.29 (t, *J* = 7.5 Hz, 6H, PhH3), 7.24-7.18 (t, *J* = 7.5, 1.7 Hz,

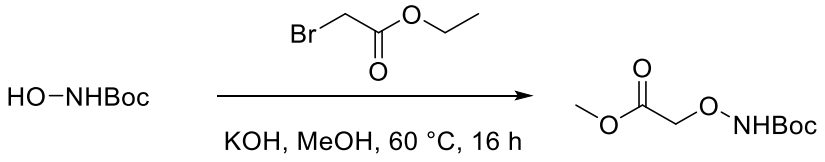
3H, PhH4), 4.86 (d, *J* = 8.3 Hz, 1H, CHNHBoc), 2.79 (s, 4H, OSu), 2.81-2.76 (m, 1H, CH_2STrt), 2.71-2.66 (m, 1H, CH_2STrt), 1.42 (s, 9H, Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_{29}H_{30}N_2O_6S$ $[M+H]^+$: 536.1658; Obs.: 536.1658; $\boldsymbol{\nu}_{\mathbf{max}}$: (FT-ATR)/cm^{-1}: 3426, 2978, 1707, 1491, 1444, 1393, 1368, 1217, 1162, 1052, 852, 744, 700, 675, 620, 505; **m.p.:** 71-74 °C.



40

A solution of sodium hydrogen carbonate (2.44 g, 29.0 mmol) in water (30 mL) was added dropwise to a mixture of hydroxylamine hydrochloride (1.00 g, 14.5 mmol) and di-*tert*-butyl dicarbonate (3.16 g, 14.5 mmol) in tetrahydrofuran (20 mL), and the reaction stirred at r.t. for 20 h. Water (150 mL) was then added, and the aqueous was extracted with ethyl acetate (2 × 150 mL). The combined organics were washed with water (30 mL) and brine (2 × 30 mL), dried with $MgSO_4$, filtered, and concentrated to afford a colourless oil (1.71 g, 12.9 mmol, 89%). Data were consistent with those previously reported.[6,7]

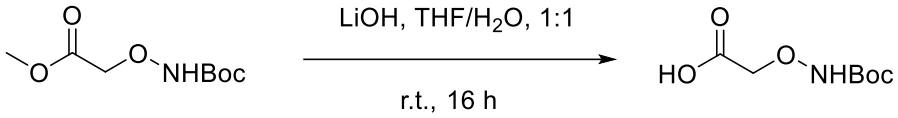
***R*$_f$**: 0.30 (1:9, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.02 (s, 1H, NH), 1.46 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 158.8 (C=O), 82.3 (CMe_3), 28.3 (Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_5H_{10}NO_3$ $[M+Na]^+$: 156.0633; Obs.: 156.0633.



41

A mixture of ethyl bromoacetate (1.40 mL, 12.6 mmol), **UK/NR/037** (1.68 g, 12.6 mmol), and potassium hydroxide (0.71 g, 12.6 mmol) in methanol (15 mL) was stirred at 60 °C for 16 h. The reaction mixture was then concentrated under reduced pressure. Water (30 mL) was added to residue and the aqueous was extracted with dichloromethane (4 × 30 mL). The combined organics were washed with brine (50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:petrol (2:8). Pure fractions were concentrated under reduced pressure to provide a yellow solid (1.36 g, 6.63 mmol, 53%). Data were consistent with those previously reported.[8]

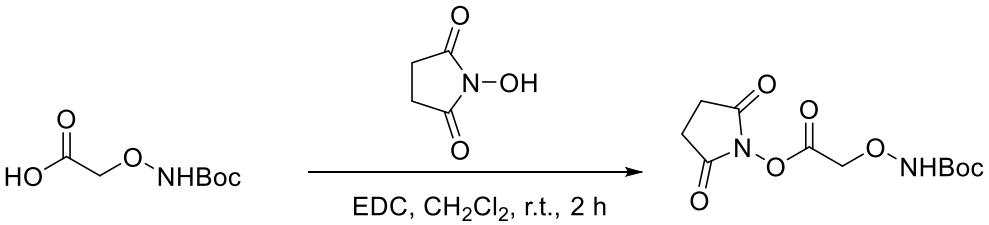
***R*$_f$**: 0.33 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.75 (s, 1H, NH), 4.43 (s, 2H, CH_2O), 3.77 (s, 3H, OCH_3), 1.48 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.2 (CO), 156.3 (COONH), 82.3 (CMe_3), 72.6 (CONH), 52.2 (CH_3O), 28.2 (Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_8H_{14}NO_5$ $[M+Na]^+$: 228.0842; Obs.: 228.0841; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3305, 2979, 1737, 1439, 1368, 1216, 1165, 1117, 995, 848, 776, 713, 589; **m.p.:** 55-57 °C.



41 **42**

Lithium hydroxide (0.40 g, 16.6 mmol) was added to a solution of **41** (1.36 g, 6.63 mmol) in a mixture of tetrahydrofuran (5 mL) and water (5 mL), and the reaction was stirred at r.t. for 16 h. The tetrahydrofuran was then removed under reduced pressure and hydrochloric acid (1 M, 30 mL) was added. The aqueous was extracted with ethyl acetate (3 × 50 mL) and the combined organics dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a cream-white solid (972 mg, 5.09 mmol, 77%).

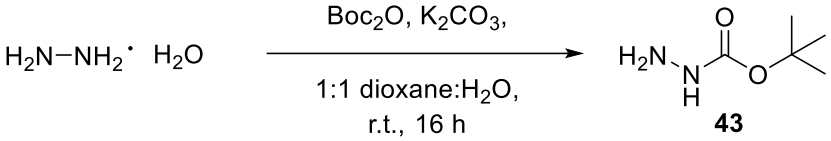
***R*$_f$**: 0.22 (1:1, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 11.02 (s, 1H, OH), 8.21 (s, 1H, NH), 4.46 (s, 2H, CH_2O), 1.47 (s, 9H, Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_7H_{11}NO_5$ $[M+H]^+$: 190.0721; Obs.: 190.0716; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3266, 2981, 2936, 1721, 1479, 1395, 1370, 1251, 1163, 1122, 1054, 979, 847, 777, 675; **m.p.:** 102-105 °C.



41 **42**

A mixture of **41** (920 mg, 4.82 mmol), *N*-hydroxysuccinimide (831 mg, 7.23 mmol), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1.39 g, 7.23 mmol) in dichloromethane (20 mL) was stirred at r.t. for 2 h. Dichloromethane (30 mL) was then added and the organics were washed with water (2 × 30 mL) and brine (30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a colourless oil (1.12 g, 3.89 mmol, 81%).

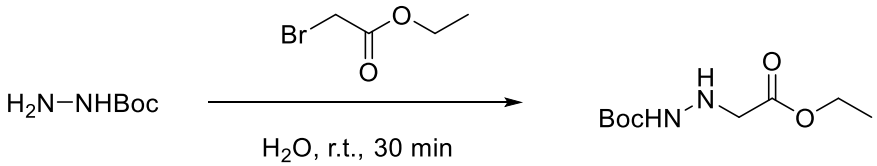
***R*$_f$**: 0.35 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.99 (s, 1H, N<u>H</u>), 4.71 (s, 2H, C<u>H</u>$_2$O), 2.81 (s, 4H, C<u>H</u>$_2$CO), 1.41 (s, 9H, Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_{11}H_{15}N_2O_7$ [M+Na]$^+$: 311.0850; Obs.: 311.0840; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3230, 2981, 1702, 1395, 1370, 1215, 1162, 1120, 1080, 997, 815, 86, 716, 655; **m.p.:** 110-114 °C.



43

A solution of di-*tert*-butyl dicarbonate (3.52 g, 16.2 mmol) in dioxane (30 mL) was added dropwise to a stirred solution of hydrazine monohydrate (3.20 mL, 66.4 mmol) and potassium carbonate (9.28 g, 66.4 mmol) in water (30 mL), and the mixture stirred at r.t for 16 h. The reaction mixture was then extracted with diethyl ether (3 × 50 mL), and the combined organics dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a white solid (2.08 g, 15.8 mmol, 97%).

***R*$_f$**: 0.18 (8:2, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.79 (s, 1H, N<u>H</u>), 1.44 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 135.9 (<u>C</u>ONH), 80.6 (<u>C</u>Me$_3$), 28.4 (Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_5H_{12}N_2O_2$ [M+Na]$^+$: 155.0791; Obs.: 155.0792; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3333, 2978, 2933, 1701, 1489, 1366, 1287, 1161, 1061, 870, 768; **m.p.:** 40-43 °C.

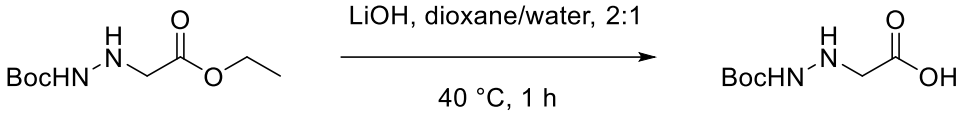


43 **44**

Ethyl bromoacetate (840 µL, 7.58 mmol) was added to a stirred solution of **43** (1.50 g, 11.3 mmol) in water (15 mL) and stirred at r.t. for 1 h. The reaction mixture was then extracted with diethyl ether (3 × 40 mL), and the combined organics were washed with brine (2 × 50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (4:6). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (1.12 g, 5.14 mmol, 68%).

***R*$_f$**: 0.27 (4:6, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.47 (s, 1H, N<u>H</u>), 4.23-4.13 (m, 2H, C<u>H</u>$_2$CH$_3$), 3.66-3.59 (m, 2H, C<u>H</u>$_2$O), 1.42 (s, 9H, Boc), 1.25 (t, *J* = 7.1 Hz,

3H, $CH_2\underline{C}H_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.2 ($\underline{C}OCH_2$), 135.9 ($\underline{C}ONH$), 80.8 ($\underline{C}Me_3$), 61.1 ($\underline{C}H_2CH_3$), 52.9 ($\underline{C}H_2NH$), 28.4 (Boc), 14.3 ($CH_2\underline{C}H_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_9H_{18}N_2O_4$ [M+Na]$^+$: 241.1159; Obs.: 241.1160; ***ν*max**: (FT-ATR)/cm^{-1}: 3290, 2971, 2926, 2854, 1675, 1557, 1456, 1429, 1151, 1114, 795.

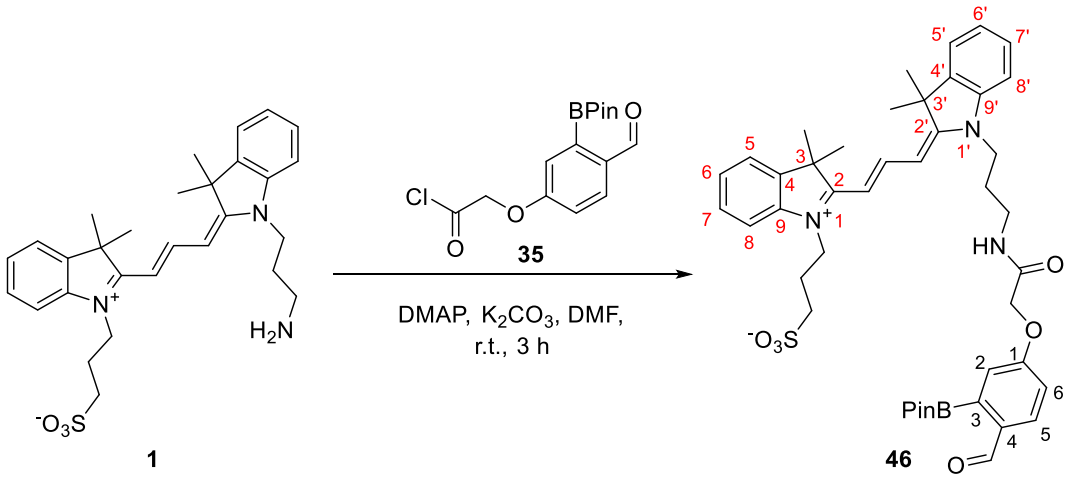


44 **45**

A solution of lithium hydroxide (749 mg, 31.2 mmol) in water (5 mL) was added to asolution of **44** (680 mg, 3.12 mmol) in dioxane (10 mL), and the mixture stirred at 40 °C for 1 h. The reaction was then cooled to r.t. and acidified to pH ~4 by addition of potassium bisulphate (1 M). The aqueous mixture was then extracted with dichloromethane (3 × 20 mL), and the combined organics were washed with brine (40 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a white solid (256 mg, 1.35 mmol, 43%).

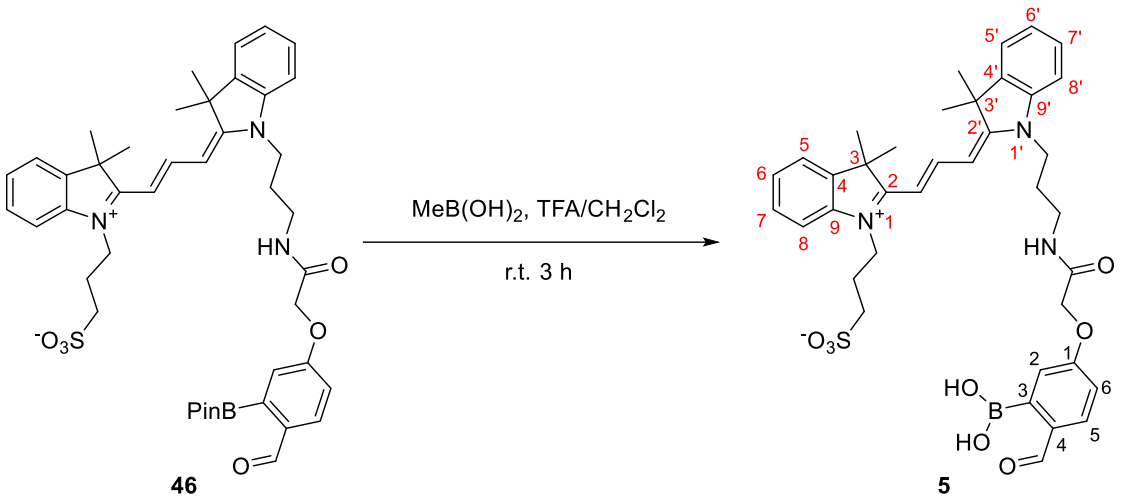
***R*f**: 0.11 (9:1, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 3.34-3.23 (m, 2H, $C\underline{H}_2O$), 1.42 (s, 9H, Boc); **^{13}C NMR** (101 MHz, CD_3OD) δ = 172.9 ($\underline{C}OCH_2$), 135.9 ($\underline{C}ONH$), 83.3 ($\underline{C}Me_3$), 51.9 ($\underline{C}H_2$), 27.3 (Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_7H_{14}N_2O_4$ [M+Na]$^+$: 213.0846; Obs.: 213.0846; ***ν*max**: (FT-ATR)/cm^{-1}: 3252, 2978, 2964, 1701, 1536, 1368, 1247, 1148, 1058, 803, 736. **m.p**: 143-145 °C.

3. Reactive dye synthesis



4-Dimethylaminopyridine (75 mg, 0.62 mmol) was added to a mixture of **1** (104 mg, 0.21 mmol), **35** (77 mg, 0.24 mmol), and potassium carbonate (85 mg, 0.62 mmol) in anhydrous dichloromethane (5 mL) and stirred at r.t. for 2 h. The reaction mixture was then precipitated in diethyl ether (400 mL). The solid was then collected by filtration, washed with diethyl ether (30 mL), and dried in air to give a pink powder. The residue was purified via flash column chromatography on silica gel eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a pink oil, which was redissolved in dichloromethane (30 mL). The organics were washed with hydrochloric acid (0.1 M, 2 × 10 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure, to give a pink oil (30 mg, 38 µmol, 18%).

***R*$_f$**: 0.16 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD, NMR data is provided for the acetal) δ = 8.48 (dd, J_1 = J_2 = 13.4 Hz, 1H, C<u>H</u>CHCN), 7.53-7.48 (m, 2H, <u>H</u>5, <u>H</u>5'), 7.43-7.36 (m, 3H, <u>H</u>7, <u>H</u>7', <u>H</u>8/<u>H</u>8'), 7.35-7.22 (m, 4H, Ph<u>H</u>5, <u>H</u>6, <u>H</u>6', <u>H</u>8/<u>H</u>8',), 7.00 (dd, *J* = 8.1, 2.6 Hz, 1H, Ph<u>H</u>6), 6.85 (d, *J* = 2.6 Hz, 1H, Ph<u>H</u>2), 6.66 (dd, *J* = 13.4, 5.2 Hz, 1H, C<u>H</u>CN), 6.41 (dd, *J* = 13.4, 5.2 Hz, 1H, C<u>H</u>CN), 5.47 (s, 1H, C<u>H</u>$(OR)_2$), 4.52 (s, 2H, C<u>H</u>$_2$O), 4.26 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$$CH_2CH_2SO_3$), 4.12 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$$CH_2CH_2NH$), 3.45 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$NH), 2.97 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$$SO_3$), 2.25-2.20 (m, 2H, C<u>H</u>$_2$$CH_2SO_3$), 2.11-2.01 (m, 2H, C<u>H</u>$_2$$CH_2NH$), 1.72 (s, 12H, CyC<u>H</u>$_3$), 1.21 (s, 12H, C(C<u>H</u>$_3$)$_2$); **^{13}C NMR** (101 MHz, CD_3OD, NMR data is provided for the acetal) δ = 174.7 (<u>C</u>HCHCN), 174.6 (<u>C</u>2, <u>C</u>2'), 170.2 (<u>C</u>ONH), 157.4 (Ph<u>C</u>1), 150.9 (<u>C</u>3, <u>C</u>3'), 141.9 (<u>C</u>9, <u>C</u>9'), 140.8 (<u>C</u>4, <u>C</u>4'), 134.1 (Ph<u>C</u>4), 130.7 (Ph<u>C</u>5), 128.8 (<u>C</u>7/<u>C</u>7'), 128.7 (<u>C</u>7/<u>C</u>7'), 125.5 (<u>C</u>6, <u>C</u>6'), 125.4 (Ph<u>C</u>3), 122.2 (<u>C</u>5, <u>C</u>5') , 115.9 (Ph<u>C</u>2), 114.4 (Ph<u>C</u>6), 111.2 (<u>C</u>8/<u>C</u>8'), 111.0 (<u>C</u>8/<u>C</u>8'), 102.9 (<u>C</u>HCN), 102.8 (<u>C</u>HCN), 74.5 ((<u>C</u>H$_3$)$_2$O), 66.9 (<u>C</u>H$_2$CO), 49.3 (<u>C</u>H$_2$$SO_3$), 42.6 (<u>C</u>H$_2$$CH_2CH_2SO_3$), 41.6 (<u>C</u>H$_2$$CH_2CH_2NH$), 36.1 (<u>C</u>H$_2$NH), 27.0 (Cy<u>C</u>H$_3$), 23.9 (<u>C</u>H$_2$$CH_2NH_2$), 23.7 (C(<u>C</u>H$_3$)$_2$), 22.9 (<u>C</u>H$_2$$CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{44}H_{54}BN_3O_8S$ $[M+Na]^+$: 818.3636; Obs.: 818.3636; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3415, 3076, 2915, 1645, 1556, 1454, 1427, 1217, 1149, 1113, 1036, 926, 795, 756, 731, 680, 527.

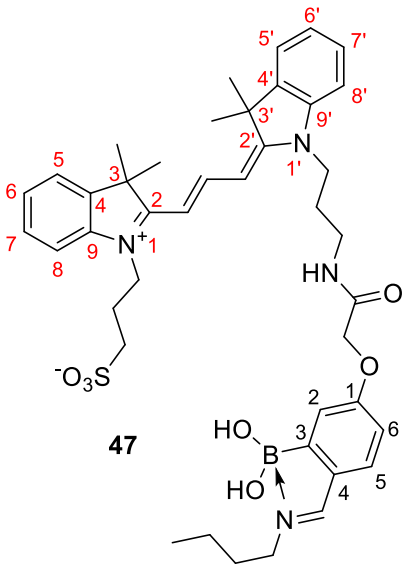


Trifluoroacetic acid (0.5 mL) was added to a solution of **46** (30 mg, 38 µmol) and methylboronic acid (23 mg, 377 µmol) in dichloromethane (5 mL), and the mixture stirred at r.t for 3 h. The reaction was azeotroped with dichloromethane (3 × 20 mL), and hydrochloric acid (0.1 M, 2 × 10 mL) was added, and concentrated under reduced pressure. The residue was then suspended in water (10 mL) and lypophilised to yield a pink solid. (26 mg, 38 µmol, quantitative yield).

***R*$_f$**: 0.12 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD, NMR data is provided for the acetal) δ = 8.53 (dd, J_1 = J_2 = 13.4 Hz, 1H, CHCHCN), 7.55 (dd, J = 7.4, 2.8 Hz, 2H, H5, H5'), 7.47-7.39 (m, 3H, H7, H7', H8/H8'), 7.37-7.27 (m, 4H, PhH5, H6, H6', H8/H8'), 7.04 (dd, J = 8.1, 2.7 Hz, 1H, PhH6), 6.90 (d, J = 2.7 Hz, 1H, PhH2), 6.62 (d, J = 13.4 Hz, 1H, CHCN), 6.46 (d, J = 13.4 Hz, 1H, CHCN), 5.41 (s, 1H, $CH(OR)_2$), 4.57 (s, 2H, CH_2O), 4.39-4.27 (m, 2H, $CH_2CH_2CH_2SO_3$), 4.16 (t, J = 7.2 Hz, 2H, $CH_2CH_2CH_2NH$), 3.49 (t, J = 7.2 Hz, 2H, CH_2NH), 3.02 (t, J = 6.9 Hz, 2H, CH_2SO_3), 2.29-2.25 (m, 2H, $CH_2CH_2SO_3$), 2.10 (tt, J_1 = J_2 = 7.2 Hz, 2H, CH_2CH_2NH), 1.77 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD, NMR data is provided for the acetal) δ = 174.7 (CHCHCN), 174.5 (C2, C2'), 170.2 (CONH), 157.4 (PhC1), 150.8 (C3, C3'), 141.8 (C9, C9'), 140.8 (C4, C4'), 134.1 (PhC4), 130.7 (PhC5), 128.7 (C7, C7'), 125.4 (C6, C6'), 125.3 (PhC3), 122.1 (C5, C5'), 115.9 (PhC2), 114.4 (PhC6), 111.2 (C8/C8'), 111.0 (C8/C8'), 102.9 ($CHCN^+$), 102.7 (CHCN), 66.9 ($(CH_3)_2O$), 49.2 (CH_2CO), 46.8 (CH_2SO_3), 42.6 ($CH_2CH_2CH_2SO_3$), 41.5 ($CH_2CH_2CH_2NH$), 36.1 (CH_2NH), 26.9 ($CyCH_3$), 26.6 ($CH_2CH_2NH_2$), 22.8 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{44}BN_3O_8S$ $[M+Na]^+$: 736.2834; Obs.: 736.2834; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}:

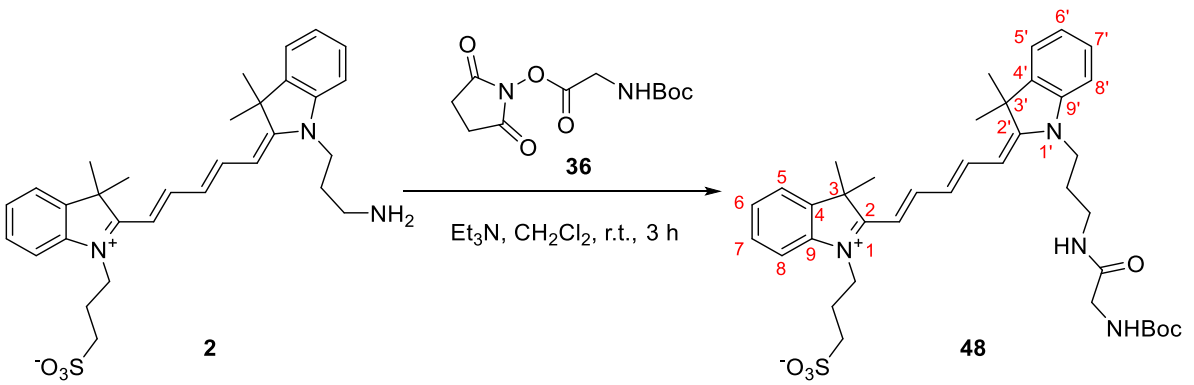
3289, 2926, 1676, 1558, 1456, 1429, 1373, 1232, 1151, 1115, 1037, 927, 756, 681; **m.p**: 315-320 °C;

Evidence for the formation of **5** was further provided by incubating 5 mg with 1.5 equiv. of *n*-butylamine in MeOD for 30 min. After this time, exclusive formation of boronoimine **47** was observed.



47

^{1}H NMR (400 MHz, CD_3OD, NMR data is provided for the acetal) δ = 8.62 (s, 1H, -CHNCH$_2$), 8.57 (dd, J_1 = J_2 = 13.4 Hz, 1H, CHCHCN), 7.63 (d, J = 8.3 Hz, 1H, PhH5), 7.58-7.54 (m, 2H, H5, H5'), 7.48-7.41 (m, 3H, H7, H7', H8/H8'), 7.35-7.30 (m, 3H, PhH5, H6, H6', H8/H8'), 7.15 (d, J = 2.4 Hz, 1H, PhH2), 6.99 (dd, J = 8.3, 2.4 Hz, 1H, PhH6), 6.63 (d, J = 13.4 Hz, 1H, CHCN), 6.50 (d, J = 13.4 Hz, 1H, CHCN), 4.64 (s, 2H, CH$_2$O), 4.39-4.33 (m, 2H, CH$_2$CH$_2$CH$_2$SO$_3$), 4.22 (t, J = 7.5 Hz, 2H, CH$_2$CH$_2$CH$_2$NH), 3.57 (t, J = 7.7 Hz, 2H, CH$_2$NH), 3.48 (t, J = 7.0 Hz, 2H, -CHNCH$_2$), 3.00 (t, J = 6.7 Hz, 2H, CH$_2$SO$_3$), 2.29-2.23 (m, 2H, CH$_2$CH$_2$SO$_3$), 2.15-2.10 (m, 2H, CH$_2$CH$_2$NH), 1.79 (s, 12H, CyCH$_3$), 1.52-1.38 (m, 2H, -CHNCH$_2$CH$_2$CH$_2$), 1.01 (t, 3H, -CH$_3$).

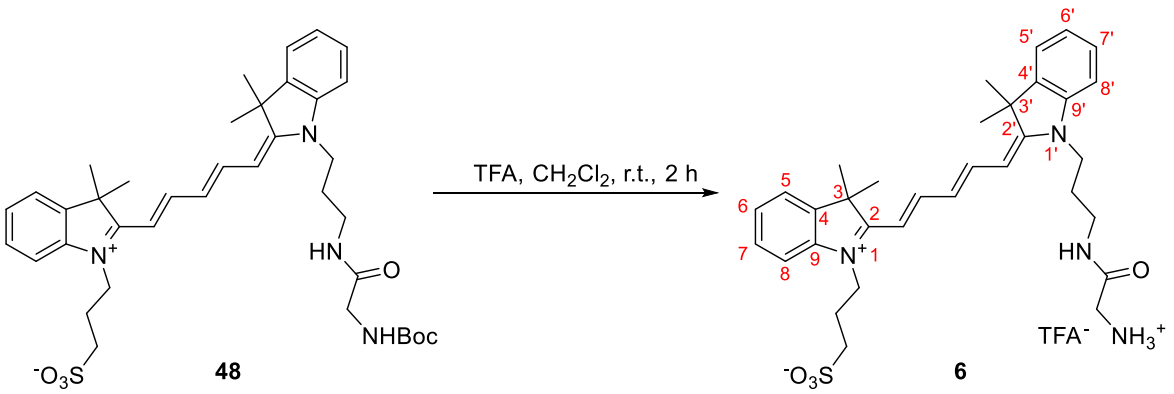


2 **36** **48**

Et$_3$N, CH_2Cl_2, r.t., 3 h

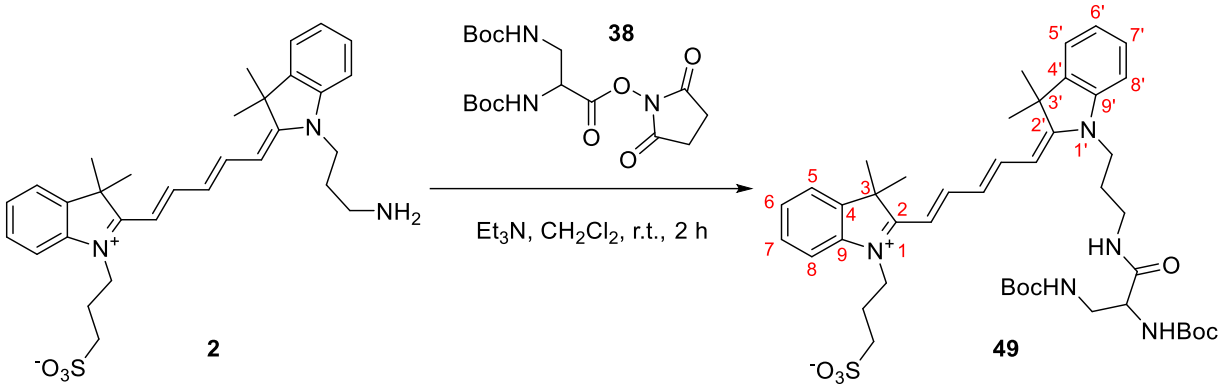
A mixture of **2** (30 mg, 56 µmol), **36** (31 mg, 0.112 mmol), and triethylamine (39 µL, 0.280 mmol) in dichloromethane (3 mL) was stirred at r.t. for 3 h. The reaction was then concentrated under reduced pressure and the residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue oil (30 mg, 44 µmol, 77%).

***R*$_f$**: 0.38 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.20 (dd, J_1 = J_2 = 13.5 Hz, 2H, CHCHCN), 7.43 (dd, J = 7.9, 2.6 Hz, 2H, H5, H5'), 7.37-7.30 (m, 3H, H7, H7', H8/H8'), 7.30-7.27 (m, 1H, H8/H8'), 7.19 (dd, J = 7.9, 2.8 Hz, 2H, H6, H6'), 6.67 (dd, J_1 = J_2 = 13.5 Hz, 1H, CHCHCHCN), 6.38 (d, J = 13.5 Hz, 1H, CHCN), 6.27 (d, J = 13.5 Hz, 1H, CHCN), 4.34-4.26 (m, 2H, $CH_2CH_2CH_2SO_3$), 4.08 (t, J = 7.6 Hz, 2H, $CH_2CH_2CH_2NH$), 3.70 (s, 2H, CH_2NHBoc), 3.36 (t, J = 7.6 Hz, 2H, CH_2NH), 2.97 (t, J = 7.5 Hz, 2H, CH_2SO_3), 2.22 (tt, J_1 = J_2 = 7.5 Hz, 2H, $CH_2CH_2SO_3$), 1.95 (tt, J_1 = J_2 = 7.6 Hz, 2H, CH_2CH_2NH), 1.65 (s, 12H, $CyCH_3$), 1.42 (s, 9H, Boc); **^{13}C NMR** (101 MHz, CD_3OD) δ = 173.1 (C2, C2'), 171.8 (C3, C3'), 169.9 (CON), 157.2 (CHCHCN), 154.4 (CHCHCN), 142.2 (C9/C9'), 142.0 (C9/C9'), 141.3 (C4/4'), 141.2 (C4/C4'), 130.1 (C7, C7'), 129.9 (C7, C7'), 126.0 (CHCHCHCN), 124.9 (C6/C6'), 124.8 (C6/C6'), 122.1 (C5, C5'), 110.8 (C8/C8'), 110.7 (C8/C8'), 103.3 (CHCN), 103.1 (CHCN), 79.4 (CH_2NHBoc), 79.1, (CMe_3), 49.2 (CH_2SO_3), 42.4 ($CH_2CH_2CH_2SO_3$), 41.5 ($CH_2CH_2CH_2NH$), 36.4 (CH_2NH), 27.4 (Boc), 26.9 (CH_2CH_2NH), 26.6 ($CyCH_3$), 22.8 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{50}N_4O_6$ [M+Na]$^+$: 713.3343; Obs.: 713.3351; **ν_{max}**: (FT-ATR)/cm^{-1}:3300, 2968, 2924, 2852, 1702, 1659, 1492, 1482, 1453, 1378, 1338, 1216, 1138, 1102, 1035, 925, 709, 522.



Trifluoroacetic acid (1.0 mL) was added to a solution of **48** (30 mg, 44 µmol) in dichloromethane (5 mL) and stirred at r.t. for 2 h. The reaction mixture was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then dissolved in methanol (30 mL) and concentrated under reduced pressure to give a blue oil (19 mg, 32 µmol, 73%).

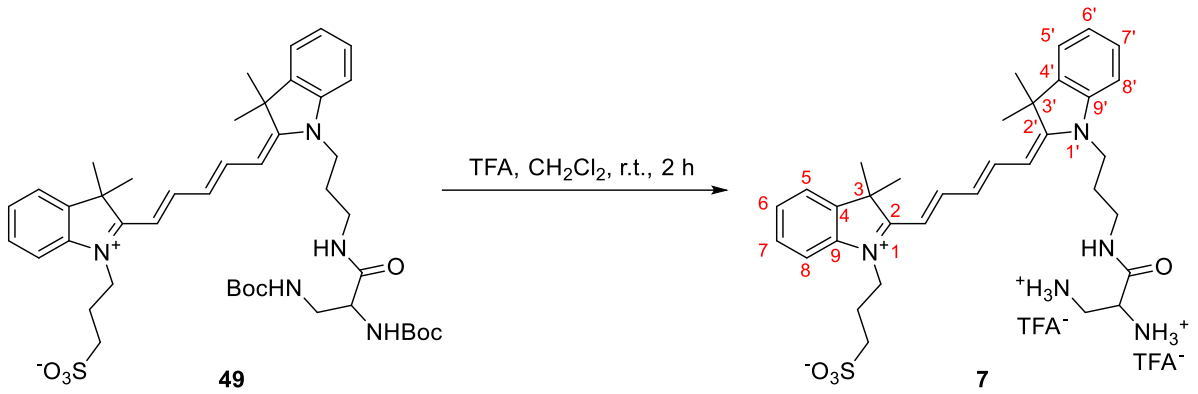
***R*$_f$**: 0.21 (5:95, CH_2Cl_2:MeOH, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.10 (d, *J* = 13.6 Hz, 2H, CHCHCN), 7.40 (dd, $J_1 = J_2$ = 7.5 Hz, 2H, H5, H5'), 7.34 (t, *J* = 7.5 Hz, 2H, H7, H7'), 7.29 (dd, *J* = 7.9, 2.5 Hz, 2H, H8, H8'), 7.15 (dd, $J_1 = J_2$ = 7.5 Hz, 2H, H6, H6'), 6.61 (dd, $J_1 = J_2$ = 13.6 Hz, 1H, CHCHCHCN), 6.48 (d, *J* = 13.6 Hz, 1H, CHCN), 6.17 (d, *J* = 13.6 Hz, 1H, CHCN), 4.32 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_2SO_3$), 4.13 (t, *J* = 7.4 Hz, 2H, $CH_2CH_2CH_2NH$), 3.81 (s, 2H, $CH_2NH_3^+$), 3.42 (t, *J* = 7.4 Hz, 2H, CH_2NH), 3.03 (t, *J* = 7.5 Hz, 2H, CH_2SO_3), 2.23 (tt, $J_1 = J_2$ = 7.5 Hz, 2H, $CH_2CH_2SO_3$), 1.96 (tt, $J_1 = J_2$ = 7.4 Hz, 2H, CH_2CH_2NH), 1.59 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 173.5 (C2, C2'), 172.7 (C3, C3'), 166.4 (CO), 154.2 (CHCHCN), 153.7 (CHCHCN), 142.0 (C9, C9'), 141.3 (C4, C4'), 128.4 (C7, C7'), 126.1 (CHCHCHCN), 125.0 (C6, C6'), 122.1 (C5, C5'), 110.7 (C8, C8'), 103.9 (CHCN), 49.1 (CH_2NH_3), 47.5 (CH_2SO_3), 45.3, 41.4 ($CH_2CH_2CH_2SO_3$), 40.4 ($CH_2CH_2CH_2NH$), 36.5 (CH_2NH), 26.9 (CH_2CH_2NH), 26.6 ($CyCH_3$), 22.8 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{33}H_{42}N_4O_4S$ $[M+H]^+$: 591.3014; Obs.: 591.3000; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 2918, 2856, 1683, 1495, 1461, 1388, 1145, 1106, 1034, 928, 799, 752, 710.



A mixture of **2** (19 mg, 36 µmol), **38** (28 mg, 70 µmol), and triethylamine (24 µL, 0.18 mmol) in dichloromethane (1 mL) was stirred at r.t. for 2 h. The reaction mixture was then concentrated under reduced pressure, and the residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue oil. (11 mg, 13 µmol, 38%).

***R*$_f$**: 0.32 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.22 (dd, *J* = 13.0 Hz, 2H, 2 × CHCHCN), 7.44 (d, *J* = 7.3 Hz, 2H, H5, H5'), 7.40-7.36 (m, 2H, H7, H7'), 7.36-7.28 (m, 2H, H8, H8'), 7.20 (dd, $J_1 = J_2$ = 7.3 Hz, 2H, H6, H6'), 6.67 (dd, $J_1 = J_2$ = 13.0 Hz, 1H, CHCHCHCN), 6.38 (d, *J* = 13.0 Hz, 1H, CHCN), 6.29 (t, *J*

= 13.0 Hz, 1H, CHCN), 4.40-4.24 (m, 2H, $CH_2CH_2CH_2SO_3$), 4.13-4.07 (m, 3H, $CH_2CH_2CH_2NH$, CHCO), 3.40-3.33 (m, 2H, CH_2NH), 3.30-3.23 (m, 2H, CH_2NHBoc), 2.98 (t, J = 6.9 Hz, 2H, CH_2SO_3), 2.23 (tt, J_1 = J_2 = 6.9 Hz, 2H, $CH_2CH_2SO_3$), 1.97 (tt, J_1 = J_2 = 7.0 Hz, 2H, CH_2CH_2NH), 1.66 (s, 12H, $CyCH_3$), 1.40 (s, 18H, 2 × Boc); **^{13}C NMR** (101 MHz, CD_3OD) δ = 173.1 (C2, C2'), 172.2 (C3, C3'), 167.8 (CON), 157.4 (CHCHCN), 156.5 (CHCHCN), 142.2 (C9/C9'), 142.1 (C9/C9'), 141.3 (C4/C4'), 141.2 (C4/C4'), 128.5 (C7, C7'), 126.1 (CHCHCHCN), 124.8 (C6, C6'), 122.1 (C5, C5'), 110.8 (C8, C8'), 103.1 (CHCN), 103.4 (CHCN), 80.9 (CMe_3), 80.6 (CMe_3), 56.0 (CH_2NHBoc), 49.3 (CHCO), 48.3 (CH_2SO_3), 42.7 ($CH_2CH_2CH_2SO_3$), 41.5 ($CH_2CH_2CH_2NH$), 36.6 (CH_2NH), 27.4 ($CyCH_3$), 26.6 (Boc), 26.6 (CH_2CH_2NH), 22.8 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI^+) calc. for $C_{44}H_{61}N_5O_8S$ $[M+H]^+$: 820.4314; Obs.: 820.4341; ν_{max}: (FT-ATR)/cm^{-1}: 3655, 2981, 2927, 1707, 1481, 1453, 1381, 1138, 1101, 1035, 926, 803, 753, 709, 552; **m.p.:** 168-171 °C.

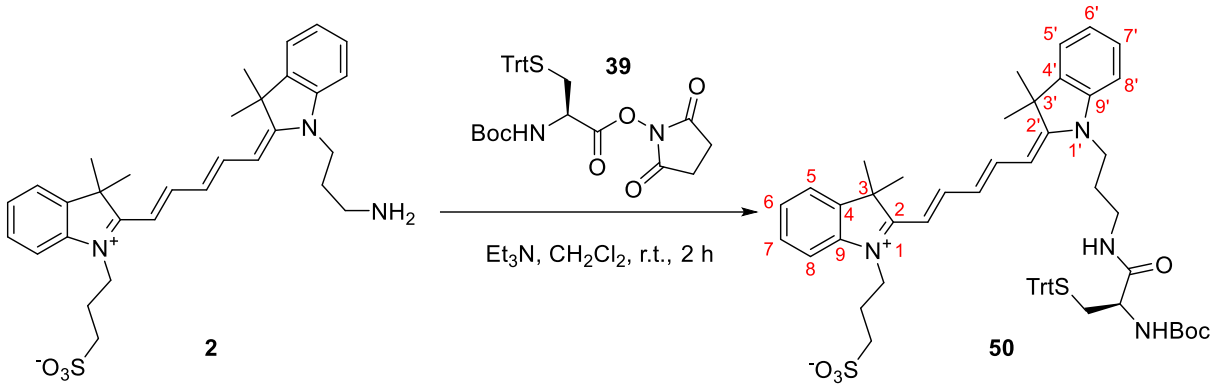


49 **7**

Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **49** (11 mg, 13 µmol) in dichloromethane (9 mL) and the mixture was stirred at r.t. for 2 h. The reaction was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then dissolved in methanol (10 mL) and concentrated under reduced pressure to afford a blue oil. (10 mg, 13 µmol, quantitative yield).

R_f: 0.15 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.13 (dd, J_1 = J_2 = 13.6 Hz, 2H, 2 × CHCHCN), 7.48-7.40 (m, 2H, H5, H5'), 7.38-7.34 (m, 2H, H7, H7'), 7.32-7.25 (m, 2H, H8, H8'), 7.25-7.12 (m, 2H, H6, H6'), 6.66 (dd, J_1 = J_2 = 13.6 Hz, 1H, CHCHCHCN), 6.52 (d, J = 13.6 Hz, 1H, CHCN), 6.21 (d, J = 13.6 Hz, 1H, CHCN), 4.36-4.32 (m, 3H, $CH_2CH_2CH_2SO_3$/CHCO), 4.19-4.15 (m, 2H,

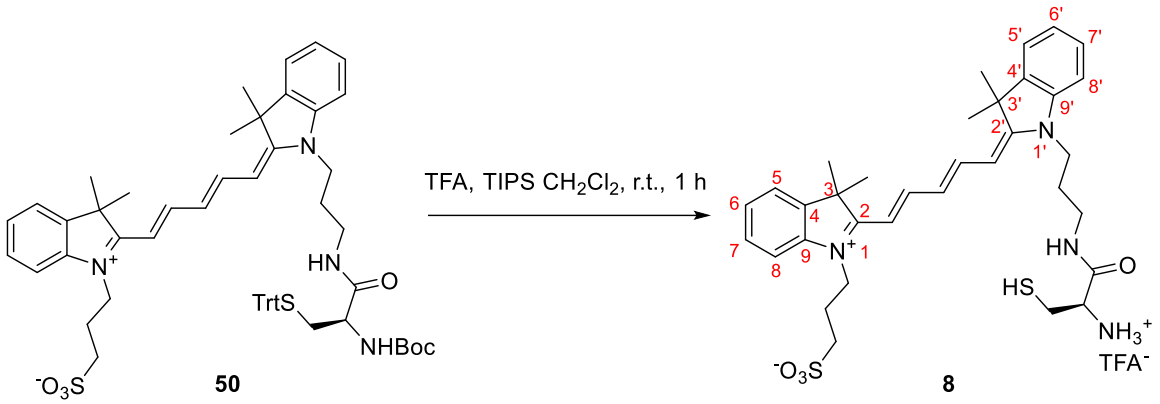
C$\underline{H}_2$CH$_2$CH$_2$NH), 3.60-3.54 (m, 2H, C$\underline{H}_2$NH), 3.44-3.38 (m, 2H, C$\underline{H}_2$SO$_3$), 3.08 (t, *J* = 7.0 Hz, 2H, C$\underline{H}_2$NH$_3^+$), 2.24 (tt, J_1 = J_2 = 7.5 Hz, 2H, C$\underline{H}_2$CH$_2$SO$_3$), 2.02 (tt, J_1 = J_2 = 7.0 Hz, 2H, CH$_2$C$\underline{H}_2$NH), 1.62 (s, 12H, CyC$\underline{H}_3$); **^{13}C NMR** (101 MHz, CD$_3$OD) δ = 174.9 (C2, C2'), 174.2 (C3, C3'), 168.5 (CON), 155.5 (CHCHCN), 154.9 (CHCHCN), 143.4 (C9, C9'), 142.7 (C4, C4'), 129.3 (C7, C7'), 126.9 (CHCHCHCN), 126.3 (C6, C6'), 123.6, (C5, C5'), 111.9 (C8, C8'), 105.5 (CHCN), 104.4 (CHCN), 57.4 (CH$_2$NH$_3^+$), 50.6 (CHCO), 50.5 (CH$_2$SO$_3$), 44.3 (CH$_2$CH$_2$CH$_2$SO$_3$), 42.8 (CH$_2$CH$_2$CH$_2$NH), 38.4 (CH$_2$NH), 28.4 (CyCH$_3$), 24.5, (CH$_2$CH$_2$NH), 24.2 (CH$_2$CH$_2$SO$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{34}H_{45}N_5O_4S$ $[M+H]^+$: 620.3265; Obs.: 620.3265; ***ν*max**: (FT-ATR)/cm^{-1}: 2978, 2929, 1680, 1488, 1456, 1384, 1145, 1038 1018, 995, 926, 800; **m.p.:** 178-181 °C.



A mixture of **2** (30 mg, 56 µmol), **39** (90 mg, 0.169 mmol), and triethylamine (29 µL, 0.280 mmol) in dichloromethane (2 mL) was stirred at r.t. for 2 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH$_2$Cl$_2$ (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue solid (45 mg, 46 µmol, 84%). The product was carried into the next step without further characterisation.

***R*$_f$**: 0.32 (5:95, MeOH:CH$_2$Cl$_2$, visible light active); **^{1}H NMR** (400 MHz, CD$_3$OD) δ = 8.24 (dd, *J* = 13.5, 13.0 Hz, 2H, 2 × CHCHCN), 7.45 (dd, *J* = 7.3, 2.2 Hz, 2H, H5, H5'), 7.42-7.38 (m, 2H, H7, H7'), 7.34 (d, *J* = 7.7 Hz, 6H, TrtPhH2), 7.31-7.26 (m, 2H, H8, H8'), 7.26-7.20 (m, 8H, TrtPhH3 / H6, H6'), 7.17 (t, *J* = 7.7 Hz, 3H, TrtPhH4), 6.63 (dd, J_1 = J_2 = 13.0 Hz, 1H, CHCHCHCN), 6.34 (d, *J* = 13.5 Hz, 1H, CHCN), 6.25 (d, *J* = 13.5 Hz, 1H, CHCN), 4.30 (t, *J* = 8.3 Hz, 2H, CH$_2$CH$_2$CH$_2$SO$_3$), 4.04 (t, *J* = 6.9 Hz, 2H, CH$_2$CH$_2$CH$_2$NH), 3.96 (t, *J* = 6.9 Hz, 1H, CHCO), 3.33 (d, *J* = 6.9 Hz, 2H, CH$_2$NH), 2.95 (t, *J* = 7.0 Hz, 2H, CH$_2$STrt), 2.57-2.42 (m, 2H, CH$_2$SO$_3$), 2.24-2.17 (m, 2H,

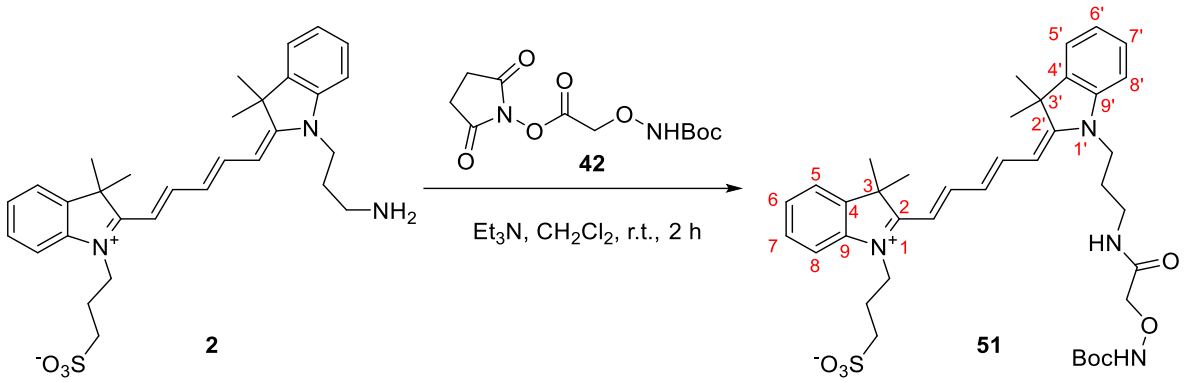
C<u>H</u>$_2$CH$_2$SO$_3$), 1.99-1.92 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 1.68 (s, 12H, CyC<u>H</u>$_3$), 1.40 (s, 9H, Boc); **HRMS**: m/z (ESI$^+$) calc. for $C_{58}H_{66}N_4O_6S_2$ $[M+H]^+$: 979.4497; Obs.: 979.4542; ***ν*max**: (FT-ATR)/cm^{-1}: 3419, 2925, 2859, 1711, 1489, 1455, 1382, 1338, 1216, 1140, 1104, 1036, 1018, 926, 750, 707; **m.p.:** 167-154 °C.



Trifluoroacetic acid (2 mL) was added dropwise to a stirred solution of **50** (40 mg, 41 µmol) and triisopropylsilane (43 µL, 0.210 mmol) in dichloromethane (10 mL), and the mixture stirred at r.t. for 1 h. The reaction was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then dissolved in methanol (10 mL) and concentrated under reduced pressure to afford a blue oil. (22 mg, 35 µmol, 86%).

***R*$_f$**: 0.29 (1:9, MeOH:CH$_2$Cl$_2$, visible light active); **^{1}H NMR** (400 MHz, CD$_3$OD) δ = 8.13 (dd, *J* = 13.5, 13.0 Hz, 2H, 2 × C<u>H</u>CHCN), 7.41 (dd, *J* = 7.4, 1.1 Hz, 2H, <u>H</u>5, <u>H</u>5'), 7.38-7.33 (m, 2H, <u>H</u>7, <u>H</u>7'), 7.33-7.26 (m, 2H, <u>H</u>8, <u>H</u>8'), 7.17 (ddd, J_1 = J_2 = 7.5, J_3 = 1.0 Hz, 2H, <u>H</u>6, <u>H</u>6'), 6.64 (dd, J_1 = J_2 = 13.0 Hz, 1H, C<u>H</u>CHCHCN), 6.48 (d, *J* = 13.5 Hz, 1H, C<u>H</u>CN), 6.21 (d, *J* = 13.5 Hz, 1H, C<u>H</u>CN), 4.34 (t, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$CH$_2$CH$_2$SO$_3$), 4.18-4.10 (m, 3H, C<u>H</u>$_2$CH$_2$CH$_2$NH, C<u>H</u>CO), 3.53-3.40 (m, 2H, C<u>H</u>$_2$NH), 3.17-3.11 (m, 1H, C<u>H</u>$_2$SH), 3.09-3.00 (m, 3H, C<u>H</u>$_2$SO$_3$, C<u>H</u>$_2$SH), 2.26-2.20 (m, 2H, C<u>H</u>$_2$CH$_2$SO$_3$), 2.05-1.93 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 1.61 (s, 12H, CyC<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD$_3$OD) δ = 173.5 (<u>C</u>2, <u>C</u>2'), 172.7 (<u>C</u>3, <u>C</u>3'), 167.5 (<u>C</u>ONH), 154.3 (<u>C</u>HCHCN), 153.7 (<u>C</u>HCHCN), 142.0 (<u>C</u>9/<u>C</u>9'), 141.3 (<u>C</u>9/<u>C</u>9'), 142.0 (<u>C</u>4/<u>C</u>4'), 141.3 (<u>C</u>4/<u>C</u>4'), 128.4 (<u>C</u>7, <u>C</u>7'), 126.2 (<u>C</u>HCHCHCN), 125.0 (<u>C</u>6/<u>C</u>6'), 124.8 (<u>C</u>6/<u>C</u>6'), 122.1 (<u>C</u>5, <u>C</u>5'), 110.7 (<u>C</u>8/<u>C</u>8'), 110.4 (<u>C</u>8/<u>C</u>8'), 103.9 (<u>C</u>HCN), 102.9 (<u>C</u>HCN), 54.9 (<u>C</u>H$_2$SH), 49.1 (<u>C</u>HCO), 48.3 (<u>C</u>H$_2$SO$_3$), 42.6 (<u>C</u>H$_2$CH$_2$CH$_2$SO$_3$), 41.5 (<u>C</u>H$_2$CH$_2$CH$_2$NH), 36.8 (<u>C</u>H$_2$NH), 26.9

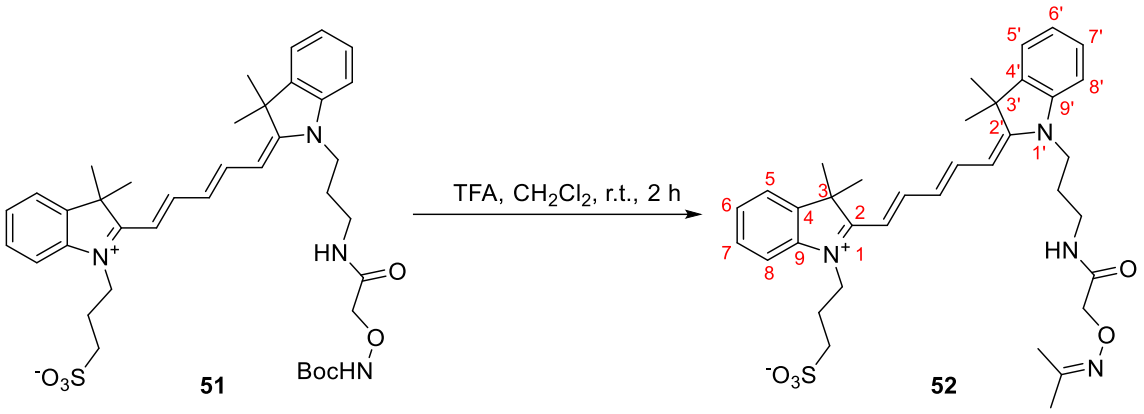
(Cy$\underline{C}$H$_3$), 26.6 ($\underline{C}$H$_2$CH$_2$NH), 22.8 ($\underline{C}$H$_2$CH$_2$SO$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{34}H_{44}N_4O_4S_2$ [M+H]$^+$: 637.2877; Obs.: 637.2873; ν_{max}: (FT-ATR)/cm^{-1}: 3412, 2966, 1677, 1490, 1455, 1381, 1338, 1202, 1139, 1101, 1035, 1016,925, 798, 751, 709; **m.p.:** 194-197 °C.



A mixture of **2** (30 mg, 56 µmol), **42** (49 mg, 0.170 mmol), and triethylamine (29 µL, 0.28 mmol) in dichloromethane (2 mL) was stirred for at r.t. for 2 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue oil (15 mg, 21 µmol, 38%).

***R*$_f$**: 0.24 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.00 (s, 1H, N$\underline{H}$Boc), 7.86-7.78 (m, 2H, 2 × C$\underline{H}$CHCN) 7.39-7.32 (m, 2H, $\underline{H}$5, $\underline{H}$5'), 7.31 (d, *J* = 7.8 Hz, 2H, $\underline{H}$7, $\underline{H}$7'), 7.24-7.18 (m, 1H, $\underline{H}$8/$\underline{H}$8'), 7.20-7.12 (m, 2H, $\underline{H}$8/$\underline{H}$8', $\underline{H}$6/$\underline{H}$6'), 7.10 (d, *J* = 7.8 Hz, 1H, $\underline{H}$6/$\underline{H}$6'), 7.08-7.03 (m, 1H, C$\underline{H}$CN), 6.92-6.66 (m, 1H, 2 × C$\underline{H}$CN), 6.05 (dd, $J_1 = J_2 =$ 13.4 Hz, 1H, C$\underline{H}$CHCHCN), 4.48 (s, 2H, C$\underline{H}_2$O), 4.46-4.40 (m, 2H, C$\underline{H}_2$CH$_2$CH$_2$SO$_3$), 4.07 (t, *J* = 7.8 Hz, 2H, C$\underline{H}_2$CH$_2$CH$_2$NH), 3.45 (t, *J* = 7.8 Hz, 2H, C$\underline{H}_2$NH), 3.03 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2$SO$_3$), 2.32-2.21 (m, 2H, C$\underline{H}_2$CH$_2$SO$_3$), 2.09-1.98 (m, 2H, C$\underline{H}_2$CH$_2$NH), 1.64 (s, 12H, CyC$\underline{H}_3$), 1.37 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 174.7 (C2/C2'), 174.3 (C2/C2'), 172.3 (C3/C3'), 172.1 (C3/C3'), 160.6 (CON), 155.3 (CHCHCN), 155.0 (CHCHCN), 143.5 (C9, C9'), 142.2 (C4, C4'), 130.4 (C7/C7'), 130.3 (C7/C7'), 128.9 (CHCHCHCN), 126.8 (C6/C6'), 126.4 (C6/C6'), 123.7 (C5/C5'), 123.6 (C5/C5'), 112.3 (C8/C8'), 111.9 (C8/C8'), 104.6 (CHCN), 104.5 (CHCN), 80.8 (CMe_3), 77.5 (CH_2O), 48.9 ($CH_2SO_3^-$), 47.6 ($CH_2CH_2CH_2SO_3^-$), 43.3 ($CH_2CH_2CH_2NH$), 37.2 (CH_2NH), 29.7 (Boc), 29.0 (CH_2CH_2NH), 26.8 (CyCH_3), 24.9

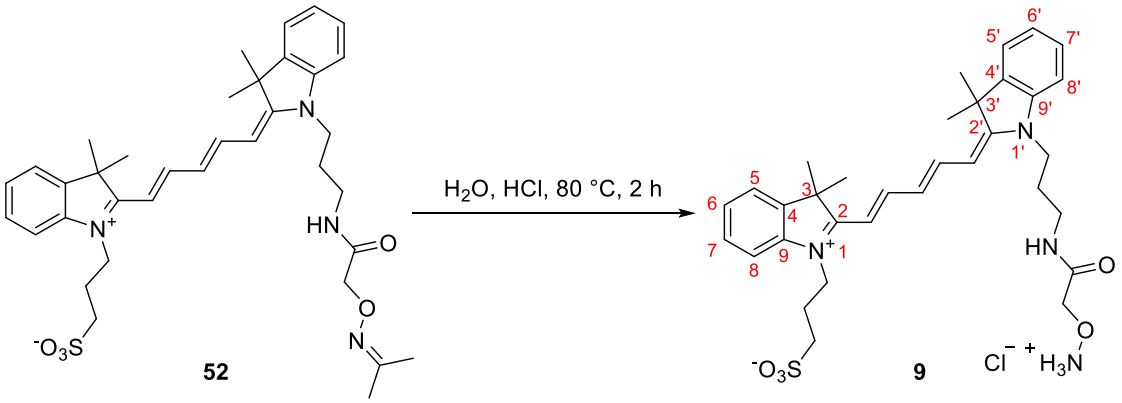
($\underline{C}H_2CH_2SO_3^-$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{50}N_4O_7S$ $[M+H]^+$: 707.3473; Obs.: 707.3492.



Trifluoroacetic acid (4 mL) was added dropwise to a stirred solution of **51** (30 mg, 42 µmol) in dichloromethane (4 mL) and the mixture was stirred at r.t. for 2 h. The reaction was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (400 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was dissolved in methanol (10 mL) and concentrated under reduced pressure to afford a blue oil (20 mg, 31 µmol, 74%).

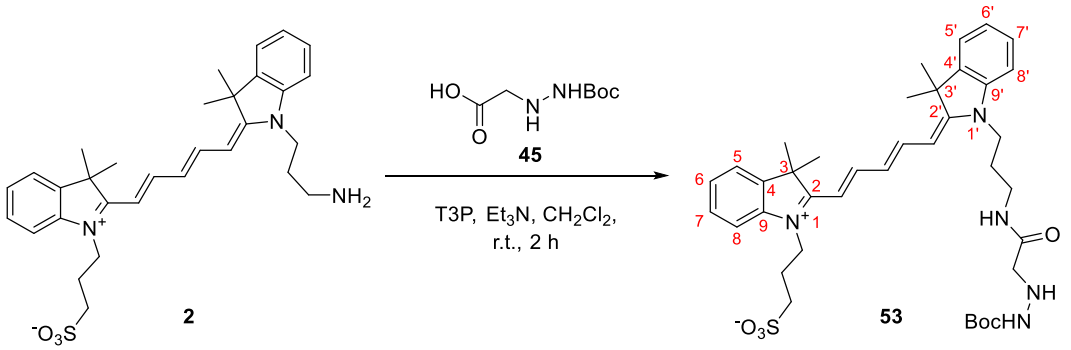
***R**f*: 0.21 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.24 (dd, $J_1 = J_2$ = 13.5 Hz, 2H, 2 × C$\underline{H}$CHCN), 7.98 (t, J = 6.1 Hz, 1H, N$\underline{H}$CO), 7.45 (d, J = 7.5 Hz, 2H, $\underline{H}$5, $\underline{H}$5'), 7.41-7.33 (m, 3H, $\underline{H}$7, $\underline{H}$7', $\underline{H}$8/$\underline{H}$8'), 7.31-7.17 (m, 3H, $\underline{H}$6, $\underline{H}$6', $\underline{H}$8/$\underline{H}$8'), 6.64 (dd, $J_1 = J_2$ = 13.0 Hz, 1H, C$\underline{H}$CHCHCN), 6.40 (d, J = 13.5 Hz, 1H, C$\underline{H}$CN), 6.26 (d, J = 13.5 Hz, 1H, C$\underline{H}$CN), 4.43 (s, 2H, C$\underline{H}_2$O), 4.32 (t, J = 7.2 Hz, 2H, C$\underline{H}_2$CH$_2$CH$_2$SO$_3$), 4.09 (t, J = 7.6 Hz, 2H, C$\underline{H}_2$CH$_2$CH$_2$NH), 3.43-3.36 (m, 2H, C$\underline{H}_2$NH), 2.97 (t, J = 7.2 Hz, 2H, C$\underline{H}_2$SO$_3$), 2.22 (tt, $J_1 = J_2$ = 7.2 Hz, 2H, C$\underline{H}_2$CH$_2$SO$_3$), 2.01 (tt, $J_1 = J_2$ = 7.6 Hz, 2H, C$\underline{H}_2$CH$_2$NH), 1.94 (s, 3H, C$\underline{H}_3$), 1.83 (s, 3H, C$\underline{H}_3$), 1.68 (s, 12H, CyC$\underline{H}_3$). **^{13}C NMR** (101 MHz, CD_3OD) δ = 174.5 (C2/C2'), 173.7 (C2/C2'), 173.5 (C3/C3'), 173.1 (C3/C3'), 172.0 (CON), 157.8 (CHCHCN), 154.6 (CHCHCN), 142.2 (C9/C9'), 142.1 (C9/C9'), 141.3 (C4/C4'), 141.2 (C4/C4'), 128.5 (C7/C7'), 128.4 (C7/C7'), 125.8 (CHCHCHCN), 125.0 (C6/C6'), 124.8 (C6/C6'), 122.1 (C5/C5'), 122.1 (C5/C5'), 110.8 (C8/C8'), 110.5 (C8/C8'), 103.4 (CHCN), 102.9 (CHCN), 71.8 (CH$_2$O), 49.3 (C=N), 49.2 (CH$_2$SO$_3$), 42.7 (CH$_2$CH$_2$CH$_2$SO$_3$), 41.3 (CH$_2$CH$_2$CH$_2$NH), 36.2 (CH$_2$NH), 27.6 (CyCH$_3$), 26.9 (CH$_2$CH$_2$NH), 26.6 (CH$_3$), 26.5 (CH$_3$), 22.8

($C\underline{H}_2CH_2SO_3$); **HRMS**: m/z (ESI^+) calc. for $C_{36}H_{45}N_4O_5S$ $[M+H]^+$: 647.3261: Obs.: 647.3261;



52 (15 mg, 23 µmol) was dissolved in hydrochloric acid (0.1 M 3 mL,) and stirred at 80 °C for 2 h. The reaction mixture was then lyophilised to give the product as a blue oil (14 mg, 23 µmol, 99%). Due to the reactivity of the oxime, the product was used directly in the next experiments without further analysis.

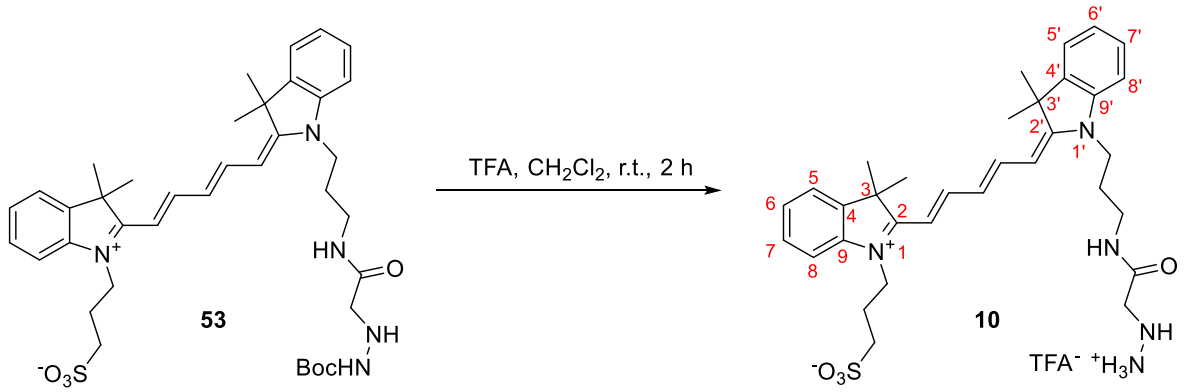
R_f: 0.14 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.30-8.24 (m, 2H, 2 × C<u>H</u>CHCN), 7.49-7.43 (m, 2H, <u>H</u>5, <u>H</u>5'), 7.46-7.39 (m, 3H, <u>H</u>7, <u>H</u>7', <u>H</u>8/<u>H</u>8'), 7.33-7.24 (m, 3H, <u>H</u>6, <u>H</u>6', <u>H</u>8/<u>H</u>8'), 6.68 (dd, J_1 = J_2 = 12.4 Hz, 1H, C<u>H</u>CHCHCN), 6.59 (d, J = 13.7 Hz, 1H, C<u>H</u>CN), 6.24 (d, J = 13.7 Hz, 1H, C<u>H</u>CN), 4.64 (s, 2H, $C\underline{H}_2O$), 4.41-4.35 (m, $C\underline{H}_2CH_2CH_2SO_3$), 4.18-4.10 (m, 2H, $C\underline{H}_2CH_2CH_2NH$), 3.46-3.42 (m, 2H, $C\underline{H}_2NH$), 3.05-2.98 (m, 2H, $C\underline{H}_2SO_3$), 2.24-2.30 (m, 2H, $CH_2C\underline{H}_2SO_3$), 2.10-2.02 (m, 2H, $C\underline{H}_2CH_2NH$), 1.76 (s, 12H, $CyC\underline{H}_3$); **HRMS**: m/z (ESI^+) calc. for $C_{33}H_{42}N_4O_5S$ $[M+H]^+$: 607.2949: Obs.: 607.2964.



Propylphosphonic anhydride solution (50% w/w in EtOAc, 44 µL, 140 µmol) was added to a stirred solution of **2** (30 mg, 56 µmol), **45** (21 mg, 112 µmol), and triethylamine (39 µL, 281 µmol) in dichloromethane (3 mL) dropwise at 0 °C. The solution was then stirred at r.t. for 16 h. The reaction was concentrated under reduced pressure and the

residue purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a blue oil (18 mg, 18 µmol, 45%).

***R*$_f$**: 0.28 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.18 (dd, *J* = 13.3, 12.5 Hz, 2H, 2 × CHCHCN), 7.43 (dd, *J* = 7.6, 2.3 Hz 2H, H5, H5'), 7.39-7.31 (m, 3H, H7, H7', H8/H8'), 7.31-7.25 (m, 1H, H8/H8'), 7.25-7.16 (m, 2H, H6, H6'), 6.60 (dd, J_1 = J_2 = 12.5 Hz, 1H, CHCHCHCN), 6.52 (d, *J* = 13.3 Hz, 1H, CHCN), 6.16 (d, *J* = 13.3 Hz, 1H, CHCN), 5.47 (s, 1H, CH_2NH), 4.34 (t, *J* = 7.6 Hz, 2H, $CH_2CH_2CH_2SO_3$), 4.14 (t, *J* = 7.8 Hz, 2H, $CH_2CH_2CH_2NH$), 3.54 (s, 2H, $CH_2NHNHBoc$), 3.38 (t, *J* = 7.8 Hz 2H, CH_2NH), 2.98 (t, *J* = 7.6 Hz, 2H, CH_2SO_3), 2.22 (tt, J_1 = J_2 = 7.6 Hz, 2H, $CH_2CH_2SO_3$), 2.00 (tt, J_1 = J_2 = 7.8 Hz, 2H, CH_2CH_2NH), 1.64 (s, 12H, $CyCH_3$), 1.45 (s, 9H, Boc); **^{13}C NMR** (101 MHz, CD_3OD) δ = 172.9 (C2, C2'), 172.8 (C3, C3'), 163.5 (CON), 154.3 (CHCHCN), 153.7 (CHCHCN), 142.0 (C9, C9'), 141.3 (C4/C4'), 141.2 (C4/C4'), 128.5 (C7/C7'), 128.4 (C7/C7'), 125.0 (CHCHCHCN), 124.8 (C6, C6'), 122.1 (C5, C5'), 110.8 (C8/C8'), 110.5 (C8/C8'), 103.8 (CHCN), 102.9 (CHCN), 79.3, (CMe_3), 54.9 ($CH_2NHNHBoc$), 46.5 (CH_2SO_3), 42.6 ($CH_2CH_2CH_2SO_3$), 41.2 ($CH_2CH_2CH_2NH$), 36.2 (CH_2NH), 27.3 (Boc), 26.9 ($CyCH_3$), 26.6 (CH_2CH_2NH), 22.7 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{51}N_5O_6S$ $[M+Na]^+$: 728.3488,; Obs.: 728.3488; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3267, 2974, 2929, 1705, 1658, 1492, 1456, 1338, 1141, 1104, 1018, 926, 802, 756.

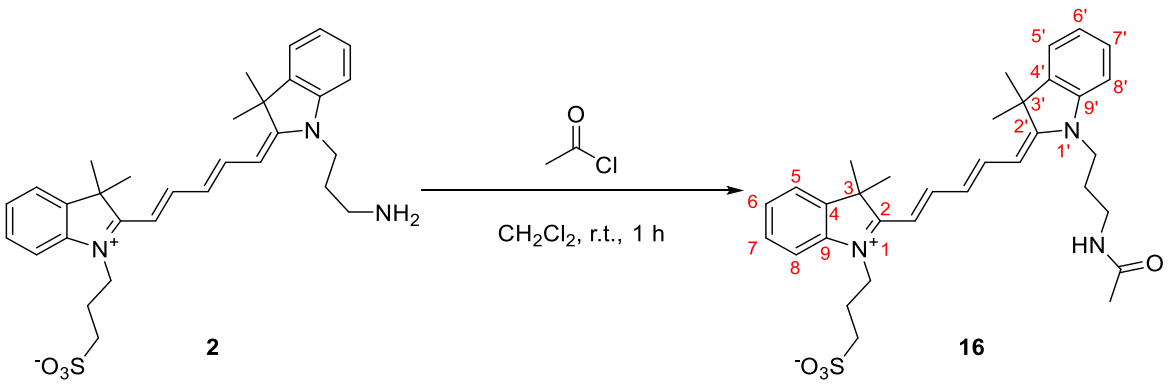


Trifluoroacetic acid (1 mL) was added to a solution of **53** (13 mg, 18 µmol) in dichloromethane (5 mL) and stirred at r.t. for 2 h. The reaction mixture was then added dropwise into diethyl ether (400 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then

dissolved in methanol (30 mL) and concentrated under reduced pressure to give a blue oil (11 mg, 18 µmol, quantitative yield).

***R*$_f$**: 0.13 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.19 (dd, *J* = 13.3, 12.5 Hz, 2H, 2 × CHCHCN), 7.44 (dd, *J* = 7.3, 2.0 Hz, 2H, H5, H5'), 7.41-7.33 (m, 3H, H7, H7', H8/H8'), 7.28 (d, *J* = 7.9 Hz, 1H, H8/H8') 7.25-7.16 (m, 2H, H6, H6'), 6.63 (dd, J_1 = J_2 = 12.5 Hz, 1H, CHCHCHCN), 6.52 (d, *J* = 13.3 Hz, 1H, CHCN), 6.18 (d, *J* = 13.3 Hz, 1H, CHCN), 4.35 (t, 2H, *J* = 7.0 Hz, $CH_2CH_2CH_2SO_3$), 4.13 (t, *J* = 6.9 Hz, 2H, $CH_2CH_2CH_2NH$), 3.75 (s, 2H, CH_2NHNH_2), 3.39 (t, *J* = 6.9 Hz, 2H, CH_2NH), 3.01 (t, *J* = 7.0 Hz, 2H, CH_2SO_3), 2.23 (tt, J_1 = J_2 = 7.0 Hz, 2H, $CH_2CH_2SO_3$), 1.99 (tt, J_1 = J_2 = 6.9 Hz, 2H, CH_2CH_2NH), 1.66 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 173.6 (C2, C2'), 172.8 (C3, C3'), 163.2 (CON), 154.4 (CHCHCN), 153.8 (CHCHCN), 142.0 (C9, C9'), 141.2 (C4, C4'), 128.5 (C7, C7'), 125.0 (CHCHCHCN), 124.8 (C6, C6'), 122.1 (C5, C5'), 110.7 (C8/C8'), 110.4 (C8/C8'), 104.0 (CHCN), 102.6 (CHCN), 49.2 (CH_2NHNH_2), 47.2 (CH_2SO_3), 42.4 ($CH_2CH_2CH_2SO_3$), 41.2 ($CH_2CH_2CH_2NH$), 36.3 (CH_2NH), 26.9 ($CyCH_3$), 26.6 (CH_2CH_2NH), 22.7 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{33}H_{43}N_5O_4S$ $[M+H]^+$: 606.3109; Obs.: 606.3122; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3294, 2923, 2853, 1678, 1495, 1458, 1385, 1338, 1144, 1105, 1038, 926, 751.

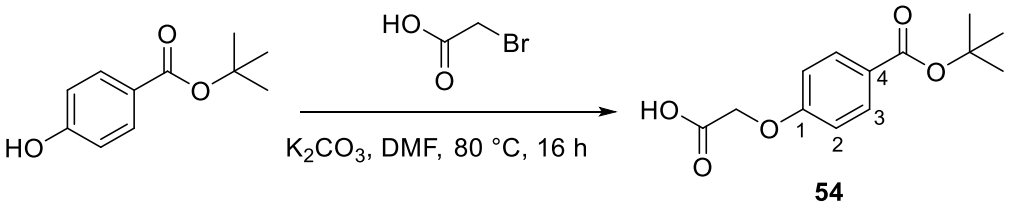
4. Synthesis of control substrates for FRET studies



Acetyl chloride (7 µL, 99 µmol) was added to a solution of **2** (10 mg, 19 µmol) in dichloromethane (3 mL) and stirred at r.t. for 1 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing

the product were concentrated under reduced pressure to provide a blue oil (7 mg, 12 µmol, 64%).

***R*$_f$**: 0.29 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.30 (dd, J_1 = J_2 = 13.1 Hz, 2H, C<u>H</u>CHCN), 7.53-7.47 (m, 2H, <u>H</u>5, <u>H</u>5'), 7.45-7.41 (m, 3H, <u>H</u>7, <u>H</u>7', <u>H</u>8/<u>H</u>8'), 7.31-7.25 (m, 3H, <u>H</u>6, <u>H</u>6', <u>H</u>8/<u>H</u>8'), 6.68 (dd, J_1 = J_2 = 13.1 Hz, 1H, C<u>H</u>CHCHCN), 6.47 (d, J = 13.1 Hz, 1H, C<u>H</u>CN), 6.30 (d, J = 13.1 Hz, 1H, C<u>H</u>CN), 4.37 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_2$CH$_2$SO$_3$), 4.15 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_2$CH$_2$NH$_2$), 3.34-3.30 (m, 2H, C<u>H</u>$_2$NH$_2$), 3.01 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$SO$_3$), 2.27 (tt, J_1 = J_2 = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_2$SO$_3$), 2.03 (tt, J_1 = J_2 = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_2$NH$_2$), 1.99 (s, 3H, COC<u>H</u>$_3$), 1.74 (s, 12H, CyC<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 173.5 (<u>C</u>2, <u>C</u>2'), 172.9 (<u>C</u>3/<u>C</u>3'), 172.2 (<u>C</u>3/<u>C</u>3'), 164.7 (<u>C</u>ON), 154.6 (<u>C</u>HCHCN), 154.1 (<u>C</u>HCHCN), 142.1 (<u>C</u>9, <u>C</u>9'), 141.3 (<u>C</u>4, <u>C</u>4'), 128.4 (<u>C</u>7, <u>C</u>7'), 125.8 (<u>C</u>HCHCHCN), 125.0 (<u>C</u>6, <u>C</u>6'), 122.0 (<u>C</u>5, <u>C</u>5'), 110.6 (<u>C</u>8, <u>C</u>8'), 103.1 (<u>C</u>HCN), 102.8 (<u>C</u>HCN), 49.2 (<u>C</u>H$_2$SO$_3$), 46.5 (<u>C</u>H$_2$CH$_2$CH$_2$SO$_3$), 41.9 (<u>C</u>H$_2$CH$_2$CH$_2$NH$_2$), 36.5 (<u>C</u>H$_2$NH), 26.7 (Cy<u>C</u>H$_3$), 26.4 (<u>C</u>H$_3$), 26.5 (<u>C</u>H$_2$CH$_2$NH$_2$), 22.7 (<u>C</u>H$_2$CH$_2$SO$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{33}H_{41}N_3O_4S$ [M+H]$^+$: 576.2891; Obs.: 576,2905; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3288, 3054, 2921, 2850, 1657, 1492, 1452, 1377, 1337, 1217, 1137, 1099, 1016, 926, 796, 708, 593.

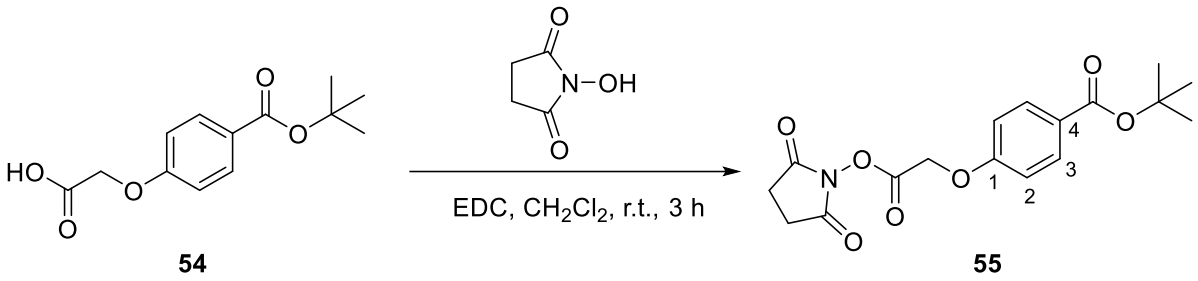


54

A mixture of *tert*-butyl 4-hydroxybenzoate (1.00 g, 5.15 mmol), bromoacetic acid (1.08 g, 7.73 mmol) and potassium carbonate (1.92 g, 13.9 mmol) in dimethylformamide (10 mL) was stirred for 16 h at 80 °C. The mixture was then cooled to r.t. and diluted with water (100 mL). The aqueous was extracted with ethyl acetate (3 × 70 mL) and the combined organic layers washed with brine (2 × 70 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (15:85). Fractions containing the product were concentrated under reduced pressure to provide a white foam (77 mg, 0.31 mmol, 6%).

***R*$_f$**: 0.22 (15:85, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 7.82 (d, J = 8.4 Hz, 2H, Ph<u>H</u>3), 6.80 (d, J = 8.4 Hz, 2H, Ph<u>H</u>2), 4.45 (s, 2H, C<u>H</u>$_2$), 1.52 (s, 9H, ^{t}Bu);

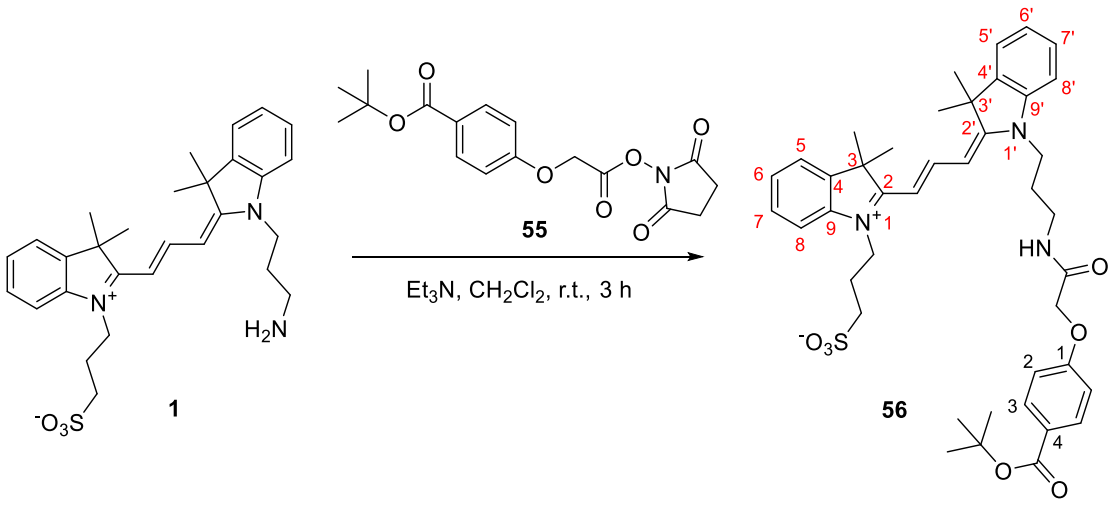
^{13}C NMR (101 MHz, CD_3OD) δ = 166.1 (COOH) 165.5 (COOC), 160.8 (PhC4), 131.7 (PhC3), 131.5 (PhC3), 125.5 (PhC1), 115.2 (PhC2), 114.2 (PhC2), 81.0 (CCH_3) , 65.1 (CH_2), 28.3 (^{t}Bu); **HRMS**: m/z (ESI$^+$) calc. for $C_{13}H_{16}O_5$ $[M+H]^+$: 253.1080; Obs.: 253.1080; ν_{max}: (FT-ATR)/cm^{-1}: 3296, 2979, 2932, 1674, 1605, 1589, 1514, 1442, 1317, 1280, 1226, 1154, 1102, 849, 774, 700, 618, 520, 499; **m.p.:** 185-204 °C.



54 **55**

A mixture of **54** (77 mg, 0.31 mmol), *N*-hydroxysuccinimide (53 mg, 0.46 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (88 mg, 0.46 mmol) in dichloromethane (3 mL) was stirred at r.t. for 3 h. Dichloromethane (20 mL) was then added and the organics were washed with water (2 × 30 mL) and brine (30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a colourless oil (61 mg, 0.18 mmol, 56%).

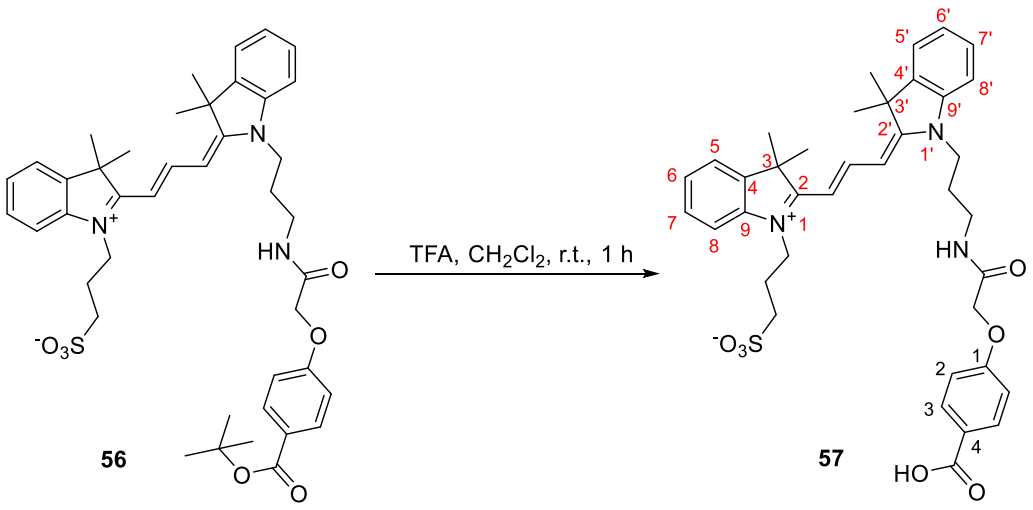
R_f: 0.31 (1:9, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 7.95-7.91 (m, 2H, PhH3), 6.94-6.90 (m, 2H, PhH2), 4.99 (s, 2H, CH_2O), 2.82 (s, 4H, OSu), 1.54 (s, 9H, ^{t}Bu); **^{13}C NMR** (101 MHz, CD_3OD) δ = 169.3 (CON), 168.8 (CON), 165.3 (COO^{t}Bu), 164.3 (COON), 160.4, (PhC4), 131.6 (PhC3), 131.4 (PhC3), 126.3 (PhC1), 114.2, (PhC2), 114.2 (PhC2), 80.8 (CCH_3) , 65.2 (CH_2O), 28.3 (^{t}Bu), 25.6 (OSu), 25.5 (OSu); **HRMS**: m/z (ESI$^+$) calc. for $C_{17}H_{19}NO_7$ $[M+Na]^+$: 372.1054; Obs.: 372.1054.



1 **56**

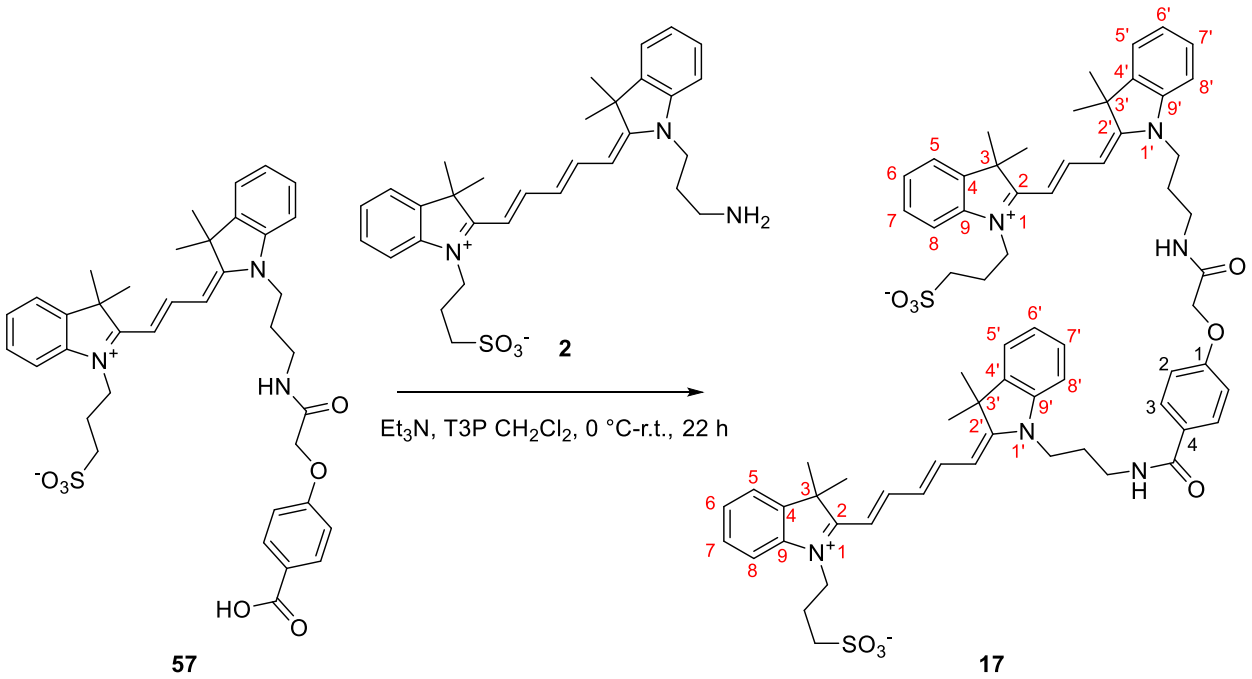
A mixture of **1** (67 mg, 0.13 mmol), **55** (60 mg, 0.17 mmol) and triethylamine (91 µL, 0.66 mmol) in dichloromethane (3 mL) was stirred for 3 h. The reaction mixture was then concentrated under reduced pressure, and the residue purified via flash column chromatography on silica gel eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a pink oil (29 mg, 39 µmol, 30%).

***R*$_f$**: 0.36 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.51 (dd, J_1 = J_2 = 13.5 Hz, 1H, CHCHCN), 7.92-7.84 (m, 2H, PhH3), 7.53-7.49 (m, 2H, H5, H5'), 7.46-7.34 (m, 3H, H7, H7', H8/H8'), 7.34-7.19 (m, 3H, H6, H6', H8/H8'), 7.09-7.00 (m, 2H, PhH2), 6.60 (dd, *J* = 13.5, 3.6 Hz, 1H, CHCN), 6.44 (dd, *J* = 13.5, 3.6 Hz, 1H, CHCN), 4.61 (s, 2H, CH_2O), 4.30 (t, *J* = 7.2 Hz, 2H, $CH_2CH_2CH_2SO_3$), 4.14 (t, *J* = 7.4 Hz, 2H, $CH_2CH_2CH_2NH$), 3.45 (t, *J* = 7.0 Hz, 2H, CH_2NH), 2.96 (t, *J* = 7.2 Hz, 2H, CH_2SO_3), 2.21 (tt, J_1 = J_2 = 7.2 Hz, 2H, $CH_2CH_2SO_3$), 2.06 (tt, J_1 = J_2 = 7.4 Hz, 2H, CH_2CH_2NH), 1.74 (s, 12H, $CyCH_3$), 1.52 (s, 9H, ^{t}Bu); **^{13}C NMR** (101 MHz, CD_3OD) δ = 176.3 (CONH), 176.1 (CHCHCN), 171.0 (C2, C2'), 165.4 (COO^tBu), 162.2 (PhC4), 152.4 (C3, C3'), 143.4 (C9, C9'), 142.6 (C4, C4'), 132.6 (PhC3), 130.2 (C7/C7'), 130.1 (C7/C7'), 127.1 (C6/C6'), 126.9 (C6/C6'), 125.5 (PhC1), 123.8 (C5/C5'), 123.6 (C5/C5'), 115.4 (PhC2), 112.6 (C8/C8'), 112.4 (C8/C8'), 104.5 (CHCN), 104.2 (CHCN), 81.0 (CCH_3), 68.4 (CH_2CO), 50.8 (CH_2SO_3), 44.1 ($CH_2CH_2CH_2SO_3$), 43.1 ($CH_2CH_2CH_2NH$), 37.7 (CH_2NH), 28.5 ($CyCH_3$), 28.3 (^{t}Bu), 28.3 ($CH_2CH_2NH_2$), 24.3 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{42}H_{50}N_3O_7S$ $[M+H]^+$: 742.3530; Obs.: 742.3530; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3410, 2979, 2934, 1752, 1686, 1589, 1450, 1339, 1211, 1145, 1076, 964, 850, 734, 674.



Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **56** (15 mg, 20 µmol) in dichloromethane (4 mL) and the mixture stirred at r.t. for 1 h. The reaction was then added dropwise to diethyl ether (200 mL). The precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was redissolved in methanol (10 mL) and concentrated under reduced pressure to afford a pink oil (10 mg, 15 µmol, 73%).

***R*$_f$**: 0.23 (1:9, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.51 (dd, *J* = 13.5, 4,3 Hz, 1H, C<u>H</u>CHCN), 7.95 (d, *J* = 8.4 Hz, 2H, Ph<u>H</u>3), 7.54-7.48 (m, 2H, <u>H</u>5, <u>H</u>5'), 7.45-7.34 (m, 3H, <u>H</u>7, <u>H</u>7', <u>H</u>8/<u>H</u>8'), 7.30-7.22 (m, 3H, <u>H</u>6, <u>H</u>6', <u>H</u>8/<u>H</u>8'), 7.06 (d, *J* = 8.4 Hz, 2H, Ph<u>H</u>2), 6.58 (dd, *J* = 13.5, 4.3 Hz 1H, C<u>H</u>CN), 6.41 (dd, *J* = 13.5, 4.3 Hz, 1H, C<u>H</u>CN), 4.62 (s, 2H, C<u>H</u>$_2$O), 4.37-4.26 (m, 2H, C<u>H</u>$_2$CH$_2$CH$_2$SO$_3$), 4.15-4.11 (m, 2H, C<u>H</u>$_2$CH$_2$CH$_2$NH), 3.52-3.39 (m, 2H, C<u>H</u>$_2$NH), 3.01-2.94 (m, 2H, C<u>H</u>$_2$SO$_3$), 2.25-2.31 (m, 2H, C<u>H</u>$_2$CH$_2$SO$_3$), 2.09-2.03 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 1.73 (s, 12H, CyC<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 176.4 (<u>C</u>ONH), 176.1 (<u>C</u>HCHCN), 171.1 (<u>C</u>2, <u>C</u>2'), 169.5 (<u>C</u>OOH), 163.1 (Ph<u>C</u>4), 152.4 (<u>C</u>3, <u>C</u>3'), 143.4 (<u>C</u>9/<u>C</u>9'), 143.3 (<u>C</u>9/<u>C</u>9'), 142.4 (<u>C</u>4, <u>C</u>4'), 133.1 (Ph<u>C</u>3), 130.2 (<u>C</u>7/<u>C</u>7'), 130.1 (<u>C</u>7/<u>C</u>7'), 127.0 (<u>C</u>6/<u>C</u>6'), 126.9 (<u>C</u>6/<u>C</u>6'), 125.4 (Ph<u>C</u>1), 123.7 (<u>C</u>5/<u>C</u>5'), 123.6 (<u>C</u>5/<u>C</u>5'), 115.8 (Ph<u>C</u>2), 112.7 (<u>C</u>8/<u>C</u>8'), 112.4 (<u>C</u>8/<u>C</u>8'), 104.5 (<u>C</u>HCN), 104.2 (<u>C</u>HCN), 68.4 (<u>C</u>H$_2$CO), 50.8 (<u>C</u>H$_2$SO$_3$), 44.1 (<u>C</u>H$_2$CH$_2$CH$_2$SO$_3$), 43.1 (<u>C</u>H$_2$CH$_2$CH$_2$NH), 37.7 (CH$_2$NH), 28.5 (Cy<u>C</u>H3), 28.3 (<u>C</u>H$_2$CH$_2$NH$_2$), 24.3 (<u>C</u>H$_2$CH$_2$SO$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{42}N_3O_7S$ $[M+H]^+$: 686.2917; Obs.: 686.2917; ***ν*$_{max}$**; (FT-ATR)/cm^{-1}: 2979, 2929, 1709, 1558, 1457, 1430, 1275, 1153, 1114, 928, 750.



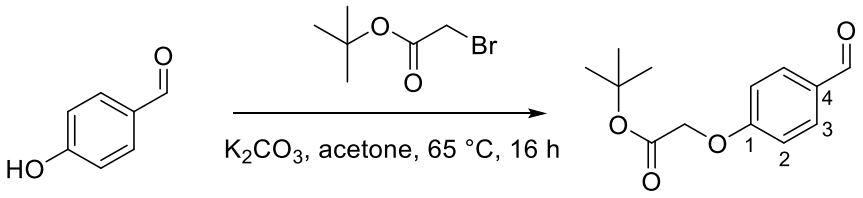
57 2 17

To a solution of **57** (10 mg, 15 µmol), **2** (12 mg, 22 µmol), and triethylamine (10 µL, 73 µmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 13 µL, 37 µmol) at 0 °C and the mixture stirred at r.t. for 16 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a purple oil (12 mg, 10 µmol, 10, 66%).

R_f: 0.32 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.50 (dd, J_1 = J_2 = 13.5 Hz, 1H, Cy3-C<u>H</u>CHCN), 8.22-8.16 (m, 2H, C<u>H</u>CHCN), 7.89-7.84 (m, 2H, Ph<u>H</u>3), 7.52-7.48 (m, 2H, <u>H</u>7, <u>H</u>7'), 7.48-7.44 (m, 2H, <u>H</u>7, <u>H</u>7'), 7.42-7.35 (m, 6H, <u>H</u>5, <u>H</u>5', <u>H</u>5, <u>H</u>5', <u>H</u>8, <u>H</u>8'), 7.30-7.21 (m, 6H, <u>H</u>8, <u>H</u>8', 2 × <u>H</u>6, 2 × <u>H</u>6'), 7.19-7.15 (m, 2H, Ph<u>H</u>2), 6.57 (d, *J* = 13.5 Hz, 1H, Cy3-C<u>H</u>CN), 6.46 (d, *J* = 13.5 Hz, 1H, Cy3-C<u>H</u>CN), 6.40 (t, *J* = 12.5 Hz, 1H, C<u>H</u>CHCHCN), 6.31 (d, *J* = 13.5 Hz, 1H, Cy5-C<u>H</u>CN), 6.18 (d, *J* = 13.5 Hz, 1H, Cy5-C<u>H</u>CN), 4.65 (s, 2H, C<u>H</u>$_2$O), 4.36-4.26 (m, 4H, C<u>H</u>$_2$CH$_2$CH$_2$SO$_3$), 4.21-4.11 (m, 4H, C<u>H</u>$_2$CH$_2$CH$_2$NH), 3.44-3.50 (m, 4H, C<u>H</u>$_2$NH), 3.01-2.95 (m, 4H, C<u>H</u>$_2$SO$_3$), 2.19-2.11 (m, 4H, C<u>H</u>$_2$CH$_2$SO$_3$), 2.16-2.04 (m, 4H, C<u>H</u>$_2$CH$_2$NH), 1.80-1.56 (m, 24H, CyC<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 176.3 (Cy3-<u>C</u>2/<u>C</u>2'), 176.1 (Cy3-<u>C</u>2/<u>C</u>2'), 174.9 (Cy3-<u>C</u>HCHCN), 174.6 (Cy3-<u>C</u>3/<u>C</u>3'), 174.4 (Cy3-<u>C</u>3/<u>C</u>3'), 171.1 (Cy5-<u>C</u>2, <u>C</u>2'), 169.8 (Cy3-<u>C</u>ON), 162.1, 155.8, 152.4, 143.6 (<u>C</u>9, <u>C</u>9'),

143.3 (C9/C9'), 142.8 (C9/C9'), 142.7 (C4/C4'), 142.4 (C4/C4'), 130.6 (PhC2), 130.2 (PhC1), 130.0 (PhthC3), 129.9 (C7/C7'), 128.8 (C7/C7'), 126.9 (C7/C7'), 126.9 (C7/C7'), 126.4 (C6, C6'), 126.4 (CHCHCHCN)), 124.2 (C6/C6'), 123.7 (C6/C6'), 123.6 (PhthC3), 123.6 (C5, C5'), 116.1 (C5, C5'), 112.7 (C8/C8'), 112.5 (C8/C8'), 112.3 (C8/C8'), 112.1 (C8/C8'), 104.4 (CHCN), 104.2 (CHCN), 71.6 (CH_2O), 50.1 ($CH_2SO_3^-$), 48.1 ($CH_2SO_3^-$), 44.1 ($CH_2CH_2CH_2SO_3^-$), 43.1 ($CH_2CH_2CH_2SO_3^-$), 42.8 ($CH_2CH_2CH_2NCO$), 38.3 ($CH_2CH_2CH_2NCO$), 37.7 (CH_2NCO), 28.5 ($CyCH_3$), 28.4 ($CyCH_3$), 28.2 (CH_2CH_2NCO), 28.1 (CH_2CH_2NCO), 24.8 ($CH_2CH_2SO_3^-$), 24.4 ($CH_2CH_2SO_3^-$); **HRMS**: m/z (ESI$^+$) calc. for $C_{69}H_{80}N_6O_9S_2$ $[M+Na]^+$: 1223.5320; Obs.: 1223.5322; ν_{max}: (FT-ATR)/cm^{-1}: 3358, 2922, 2852, 1659, 1633, 1556, 1487, 1429, 1454, 1377, 1140, 1035, 925, 797, 750, 708, 552.

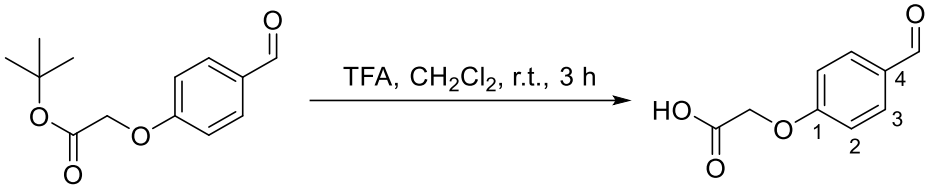
5. Synthesis of Cy3 negative controls



58

A mixture of *tert*-butyl bromoacetate (1.20 mL, 8.20 mmol), 4-hydroxybenzaldehyde (1.00 g, 8.20 mmol), and potassium carbonate (1.92 g, 14.0 mmol) in acetone (15 mL) was stirred at 65 °C for 16 h. The mixture was then cooled to r.t. and diluted with water (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 70 mL), and the combined organics washed with brine (2 × 70 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:petrol (15:85). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (1.43 g, 6.06 mmol, 74%). Data were consistent with those previously reported.[9]

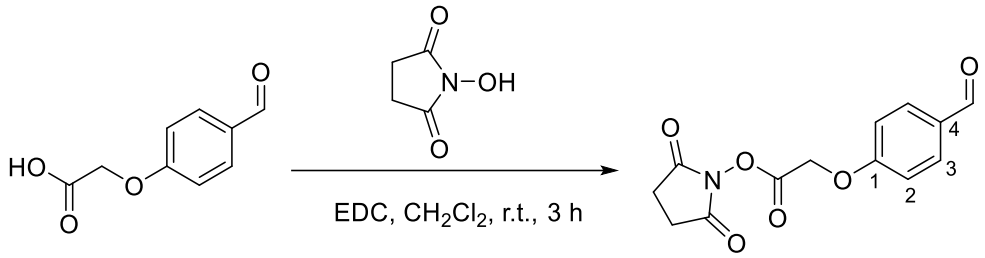
R_f: 0.26 (15:85, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.91 (s, 1H, CHO), 7.86 (d, *J* = 8.0 Hz, 2H, PhH3), 7.04 (d, *J* = 8.0 Hz, 2H, PhH2), 4.77 (s, 2H, CH_2), 0.06 (s, 9H, ^{t}Bu); **HRMS**: m/z (ESI$^+$) calc. for $C_{13}H_{16}O_4$ $[M+H]^+$: 237.1121; Obs.: 237.1119; ν_{max}: (FT-ATR)/cm^{-1}: 2980, 1748, 1691, 1598, 1509, 1368, 1308, 1216, 1148, 1071, 944, 831, 746, 608, 513; **m.p.:** 191-194 °C.



58 **59**

Trifluoroacetic acid (10 mL) was added dropwise to a stirred solution of **58** (1.43 g, 6.06 mmol) in dichloromethane (10 mL) and the mixture was stirred at r.t. for 1 h. The reaction mixture was then concentrated under reduced pressure and the residue was azeotroped with dichloromethane (3 × 10 mL) to afford a yellow powder (1.08 g, 6.00 mmol, 99%). Data were consistent with those previously reported.[10]

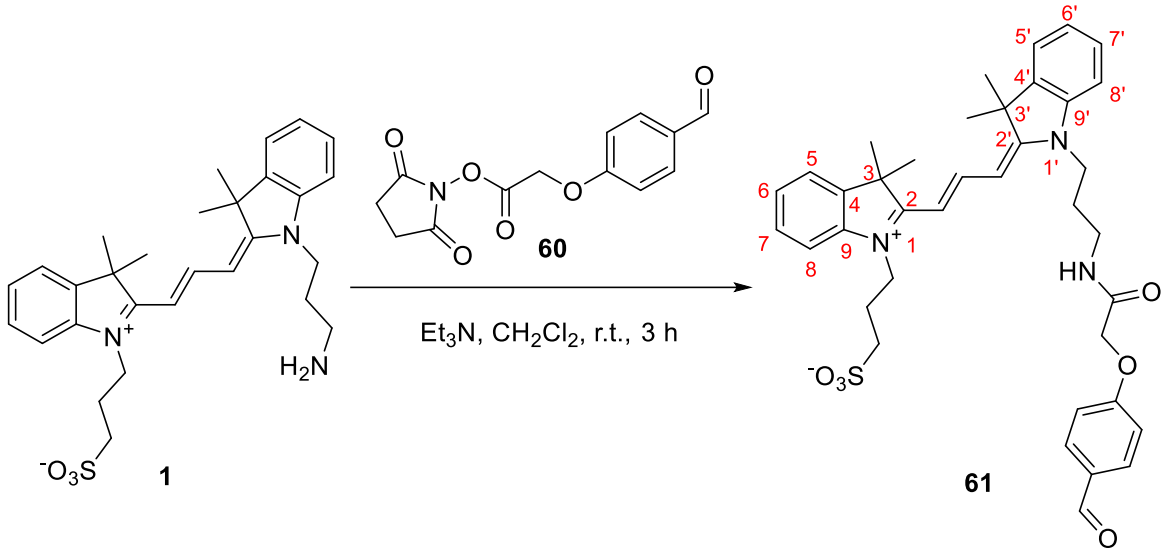
***R*$_f$**: 0.28 (2:8, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 13.13 (s, 1H, OH), 9.83 (s, 1H, CHO), 7.82 (d, *J* = 7.9 Hz, 2H, PhH3), 7.06 (d, *J* = 7.9 Hz, PhH2), 4.79 (s, 2H, CH_2); **HRMS**: m/z (ESI$^+$) calc. for $C_9H_8O_4$ [M-H]$^-$: 179.0350; Obs.: 179.0351; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3660, 2982, 1598, 1385, 1259, 1166, 1074, 954, 750; **m.p.:** 191-194 °C;



59 **60**

A reaction mixture of **59** (100 mg, 0.56 mmol), *N*-hydroxysuccinimide (96 mg, 0.83 mmol), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (150 mg, 0.83 mmol) in dichloromethane (2 mL) was stirred at r.t. for 2 h. Dichloromethane (20 mL) was then added and the organic layer was washed with water (2 × 30 mL) and brine (30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a pink foam which was used in the subsequent step without further analysis or purification (72 mg, 0.26 mmol, 46%).

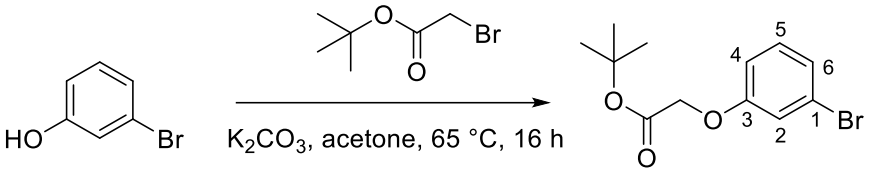
***R*$_f$**: 0.29 (1:9, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD for aldehyde) δ = 9.90 (s, 1H, CHO), 7.87 (d, *J* = 8.0 Hz, 2H, PhH3), 7.05 (d, *J* = 8.0 Hz, 2H, PhH2), 6.08 (s, CH_2O), 2.87 (s, 4H, OSu); **HRMS**: m/z (ESI$^+$) calc. $C_{13}H_{11}NO_6$ [M+H]$^+$: 278.0652; Obs.: 278.0652; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 2978, 1824, 1785, 1737, 1600, 1508, 1427, 1207, 1165, 1074, 834, 646; **m.p.:** 142-146 °C.



A reaction mixture of **1** (40 mg, 79 µmol), **60** (22 mg, 79 µmol), and triethylamine (42 µL, 0.395 µmol) in dichloromethane (2 mL) was stirred at r.t. for 3 h. The reaction mixture was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a pink oil (18 mg, 27 µmol, 45%).

***R**f*: 0.17 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD; in this solvent, a mix of aldehyde and methanol hemi-acetal were formed. Data is provided for the aldehyde form) δ = 9.82 (s, 1H, CHO), 8.53 (dd, J_1 = J_2 = 13.4 Hz, 1H, CHCHCN), 7.86 (d, J = 8.5 Hz, 2H, PhH3), 7.54-7.50 (m, 2H, H5, H5'), 7.45-7.33 (m, 3H, H7, H7', H8/H8'), 7.31-7.25 (m, 3H, H8/H8', H6, H6'), 7.17 (d, J = 8.5 Hz 2H, PhH2), 6.60 (d, J = 13.4 Hz, 1H, CHCN), 6.44 (d, J = 13.4 Hz, 1H, CHCN), 4.55 (s, 2H, CH_2O), 4.32 (t, J = 7.1 Hz, 2H, $CH_2CH_2CH_2SO_3$), 4.16 (t, J = 7.3 Hz, 2H, $CH_2CH_2CH_2NH$), 3.45 (t, J = 7.3 Hz, 2H, CH_2NH), 2.96 (t, J = 7.1 Hz, 2H, CH_2SO_3), 2.27-2.21 (m, 2H, $CH_2CH_2SO_3$), 2.11-2.07 (m, 2H, CH_2CH_2NH), 1.76 (s, 12H, $CyCH_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 192.8 (CHO), 176.3 (CONH), 176.0 (PhC1), 175.9 (CHCHCN), 171.4 (C2, C2'), 159.3 (PhC4), 152.3 (C3, C3'), 143.2(C9, C9'), 142.2 (C4, C4'), 133.1 (PhC3), 130.1 (PhC3), 129.3 (C7, C7'), 126.8 (C6, C6'), 123.5 (C5, C5'), 116.4 (PhC2), 115.5 (PhC2), 112.6 (C8/C8'), 112.3 (C8/C8'), 104.3 (CHCN), 104.1 (CHCN), 68.3 (CH_2CO), 48.0 (CH_2SO_3), 44.0 ($CH_2CH_2CH_2SO_3$), 42.9 ($CH_2CH_2CH_2NH$), 37.5 (CH_2NH), 28.1 ($CH_2CH_2NH_2$), 26.3 (CyCH3), 24.2 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{38}H_{43}N_3O_6S$ $[M+H]^+$ requires 670.2945,

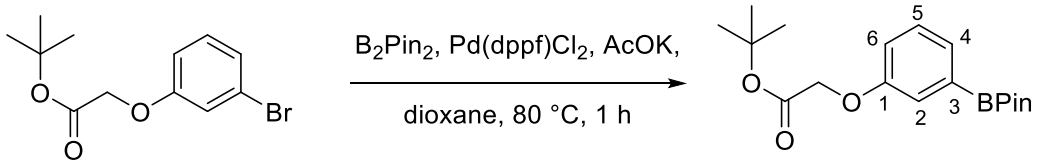
found 670.2928; ν_{max}: (FT-ATR)/cm^{-1}: 3378, 2925, 2854, 1713, 1600, 1557, 1457, 1430, 1373, 1218, 1153, 1115, 1037, 928, 795.



62

A mixture of 3-bromophenol (2.00 g, 11.6 mmol), *tert*-butyl bromoacetate (1.71 mL, 11.6 mmol), and potassium carbonate (2.71 g, 19.7 mmol) in acetone (20 mL) was stirred at 65 °C for 16 h. After cooling to r.t., water (100 mL) was added, and the aqueous was extracted with ethyl acetate (3 × 70 mL). The combined organics were washed with brine (2 × 70 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:petrol (1:9). Fractions containing the product were concentrated under reduced pressure to provide a red oil. (3.32 g, 11.5 mmol, 99%).

***R*$_f$**: 0.23 (1:9, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.14-7.10 (m, 1H, PhH5), 7.11-7.08 (m, 1H, PhH4), 7.03 (d, *J* = 2.1 Hz, 1H, PhH2), 6.81 (dd, *J* = 7.8, 2.1, 1H, PhH6), 4.48 (s, 2H, CH_2O), 1.47 (s, 9H, ^{t}Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.6 (C=O), 158.7 (PhC1), 130.7 (PhC5), 124.8 (PhC4), 122.9 (PhC3), 118.1 (PhC2), 113.7 (PhC6), 82.8 (CMe_3), 65.8 (CH_2O), 28.1 ^{t}Bu); **HRMS**: m/z (ESI$^+$) calc. for $C_{12}H_{15}{}^{79}BrO_3$ [M+Na]$^+$: 309.0097; Obs.: 309.0096; ν_{max}: (FT-ATR)/cm^{-1}: 2979, 2933, 1750, 1575, 1474, 1368, 1304, 1215, 1150, 1078, 834, 767.

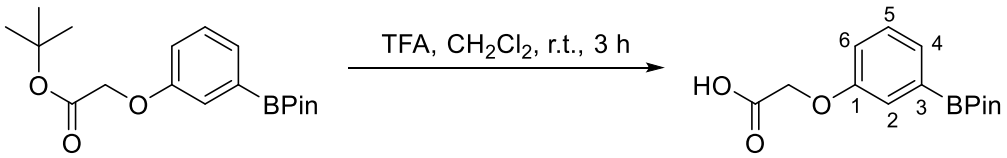


62 **63**

62 (1.77 g, 6.17 mmol), bis(pinacolato)diboron (2.35 g, 9.25 mmol), 1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (452 mg, 0.617 mmol) and potassium acetate (3.26 g, 33.3 mmol) were placed under a nitrogen atmosphere, and anhydrous dioxane (5 mL) was added. Nitrogen was bubbled through the reaction mixture for 10 min, which was then stirred at 80 °C for 1 h. After cooling to r.t., water (70 mL) was added, and the aqueous was extracted with ethyl acetate (3 × 50 mL).

The combined organics were washed with brine (2 × 50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:petrol (15:85). Fractions containing the product were concentrated under reduced pressure to provide a white solid (1.76 g, 5.27 mmol, 88%).

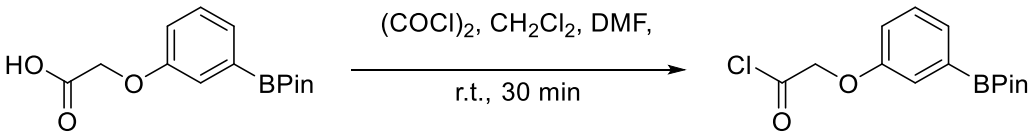
***R*$_f$**: 0.22 (15:85, EtOAc:petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41 (dd, J_1 = J_2 = 8.2 Hz, 1H, PhH5), 7.29 (d, J = 8.2 Hz, 1H, PhH4), 7.27 (d, J = 2.8 Hz, 1H, PhH2), 7.03 (dd, J = 8.2, 2.8 Hz, 1H, PhH6), 4.53 (s, 2H, CH_2O), 1.47 (s, 9H, ^{t}Bu), 1.31 (s, 12H, $C(CH_3)_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.2 (C=O), 157.4 (PhC1), 129.1 (PhC4), 128.1 (PhC5), 120.0 (PhC3), 119.4 (PhC2), 118.9 (PhC6), 83.9 ($C(CH_3)_2$), 82.3 (CMe_3), 65.8 (CH_2O), 28.1 (^{t}Bu), 24.9 ($C(CH_3)_2$); **HRMS**: m/z (ESI$^+$) calc. for $C_{18}H_{27}BO_5$ [M+Na]$^+$: 357.1855; Obs.: 357.1844; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 2979, 2993, 1754, 1576, 1428, 1355, 1317, 1213, 1147, 1085, 065, 852, 775, 705, 673, 599; **m.p.:** 65-69 °C.



63 **64**

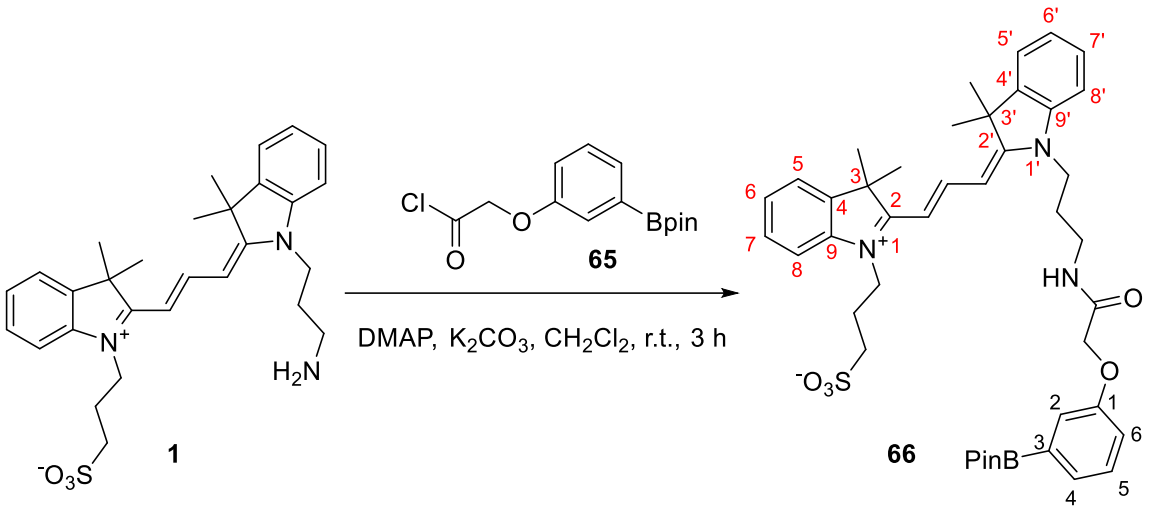
Trifluoroacetic acid (5 mL) was added dropwise to a solution of **63** (500 mg, 1.50 mmol) in dichloromethane (15 mL) and the mixture was stirred at r.t. for 3 h. The reaction mixture was then concentrated under reduced pressure and azeotroped with dichloromethane (3 × 20 mL) to afford a white powder. (344 mg, 1.24 mmol, 83%).

***R*$_f$**: 0.22 (1:9, EtOAc:petrol, UV active); **^{1}H NMR** (600 MHz, DMSO-d_6) δ = 7.32 (dd, J = 8.1, 7.2 Hz, 1H, PhH5), 7.27 (ddd, J = 7.2, 2.8, 1.2 Hz, 1H, PhH4), 7.11 (dd, J = 2.8, 1.2 Hz, 1H, PhH2), 7.05 (ddd, J = 8.1, 2.8, 1.2 Hz, 1H, PhH6), 4.69 (s, 2H, CH_2O), 1.30 (s, 12H, $C(CH_3)_2$); **13C NMR** (101 MHz, DMSO-d_6) δ = 170.7 (C=O), 157.8 (PhC1), 129.7 (PhC5), 127.7 (PhC4), 120.0 (PhC3), 119.6 (PhC2), 118.6 (PhC6), 84.2 ($C(CH_3)_2$), 64.8 (CH_2O), 25.1 ($C(CH_3)_2$); **HRMS**: m/z (ESI$^-$) calc. for $C_{14}H_{18}BO_5$ [M-H]$^-$: 277.1263; Obs.:277.1263; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3059, 2979, 2932, 1737, 1575, 1428, 1356, 1143, 1064, 964, 705; **m.p.:** 155-158 °C.



64 **65**

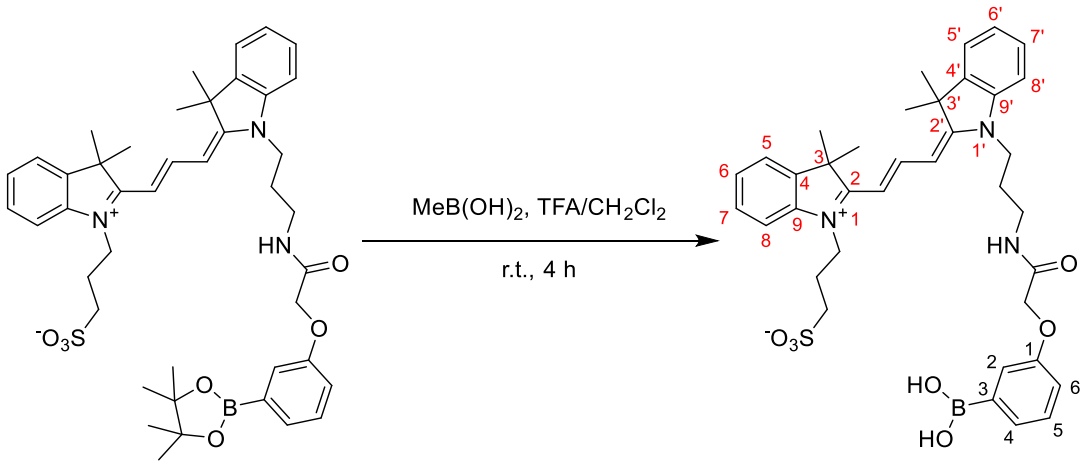
Oxalyl chloride (46 µL, 0.540 mmol) was added to a solution of **64** (50 mg, 0.180 mmol), and dimethylformamide (1 drop) in dichloromethane (3 mL), and the mixture was stirred at r.t. for 30 min. Excess oxalyl chloride and dichloromethane were removed under reduced pressure to give the crude product as an orange oil, which was carried forward without further purification.



4-Dimethylaminopyridine (75 mg, 0.62 mmol) was added to a mixture of **1** (77 mg, 0.15 mmol), **65** (77 mg, 0.24 mmol), and potassium carbonate (62 mg, 0.44 mmol) in anhydrous dichloromethane (5 mL) and the reaction stirred at r.t. for 3 h. The reaction mixture was then added dropwise to diethyl ether (400 mL), and the resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air to give a pink powder. The precipitate was then purified via flash column chromatography on silica gel eluting with MeOH:CH_2Cl_2 (5:95). Fractions containing the product were concentrated under reduced pressure to provide a pink oil. The residue was then redissolved in dichloromethane (30 mL), and the organics were washed with hydrochloric acid (0.1 M, 2 × 10 mL) to remove co-ordinating 4-dimethylaminopyridine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure, to give a pink oil (5 mg, 6 µmol, 4%).

***R*$_f$**: 0.34 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.54 (d, J_1 = J_2 = 13.5 Hz, 1H, C<u>H</u>CHCN), 7.57-7.53 (m, 2H, <u>H</u>5, <u>H</u>5'), 7.49-7.37 (m, 4H, <u>H</u>7,

H7', H8, H8'), 7.34-7.31 (m, 2H, H6, H6'), 7.31-7.25 (m, 2H, PhH5, PhH4), 7.25-7.21 (m, 1H, PhH6), 7.11-7.07 (m, 1H, PhH2), 6.57 (d, *J* = 13.5 Hz, 1H, CHCN), 6.46 (d, *J* = 13.5 Hz, 1H, CHCN), 4.56 (s, 2H, CH_2O), 4.33-4.26 (m, 2H, $CH_2CH_2CH_2SO_3$), 4.18 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_2NH$), 3.47 (t, *J* = 7.5 Hz, 2H, CH_2NH), 3.00 (t, *J* = 7.8 Hz, 2H, CH_2SO_3), 2.25 (tt, $J_1 = J_2$ = 7.8 Hz, 2H, $CH_2CH_2SO_3$), 2.11 (tt, $J_1 = J_2$ = 7.5 Hz, 2H, CH_2CH_2NH), 1.79 (s, 12H, $CyCH_3$); **^{13}C NMR** (151 MHz, CD_3OD) δ = 176.3 (CONH), 174.7 (CHCHCN), 170.2 (C2, C2'), 157.2 (PhC1), 150.8 (C3, C3'), 141.8 (C9, C9'),140.8 (C4, C4'), 129.8 (PhC5), 128.7 (C7, C7'), 127.1 (PhC4), 126.5 (PhC3), 125.4 (C6, C6'), 122.1 (C5, C5'), 119.1 (PhC2), 116.0 (PhC6), 111.2 (C8/C8'), 110.9 (C8/C8'), 102.7 (CHCN), 102.6 (CHCN), 66.9 (CH_2CO), 46.8 (CH_2SO_3), 42.5 ($CH_2CH_2CH_2SO_3$), 41.4 ($CH_2CH_2CH_2NH$), 36.0 (CH_2NH), 26.9 ($CyCH_3$), 26.6 ($CH_2CH_2NH_2$), 22.8 ($CH_2CH_2SO_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{43}H_{54}BN_3O_7S$ $[M+Na]^+$: 790.3668; Obs.: 790.3712; ***ν*max**: (FT-ATR)/cm^{-1}: 3378, 2921, 2850, 1558, 1457, 1430, 1372, 1152, 1114, 749.



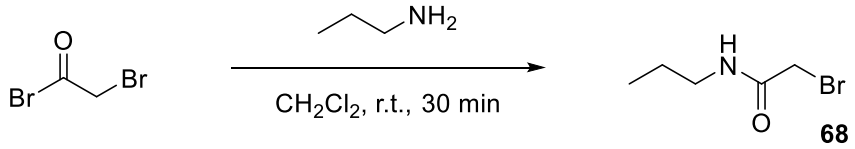
66 **67**

A solution of **66** (5 mg, 6.5 µmol) and methylboronic acid (4 mg, 65.3 µmol) in a mixture of dichloromethane (5 mL) and trifluoroacetic acid (0.5 mL) was stirred at r.t. for 4 h. The reaction mixture was then concentrated under reduced pressure. The residue was azeotroped with hydrochloric acid (0.1 M, 2 × 10 mL) to give a pink oil (4 mg, 6.5 µmol, quantitative yield).

***R*f**: 0.29 (5:95, MeOH:CH_2Cl_2, visible light active); **^{1}H NMR** (600 MHz, CD_3OD) δ = 8.54 (dd, $J_1 = J_2$ = 13.4 Hz, 1H, CHCHCN), 7.57-7.53 (m, 2H, H5, H5'), 7.49-7.40 (m, 3H, H7, H7', H8/H8'), 7.34 – 7.27 (m, 3H H6, H6', H8/H8'), 6.62-6.52 (m, 1H, CHCN), 6.48-6.42 (m, 1H, CHCN), 4.56 (s, 2H, CH_2O), 4.29 (d, *J* = 7.2 Hz, 2H, $CH_2CH_2CH_2SO_3$),

4.18 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_2$CH$_2$NH), 3.48 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$NH), 2.99 (t, *J* = 7.2 Hz, 2H, C<u>H</u>$_2$SO$_3$), 2.25 (tt, $J_1 = J_2$ = 7.2 Hz, 2H, C<u>H</u>$_2$CH$_2$SO$_3$), 2.11 (tt, $J_1 = J_2$ = 7.5 Hz, 2H, CH$_2$C<u>H</u>$_2$CH$_2$NH), 1.78 (s, 12H, CyC<u>H</u>$_3$); **^{13}C NMR** (151 MHz, CD$_3$OD) δ = 174.6 (<u>C</u>ONH), 174.5 (<u>C</u>HCHCN), 157.3 (<u>C</u>2, <u>C</u>2'), 150.8 (<u>C</u>3, <u>C</u>3'), 141.8 (<u>C</u>9, <u>C</u>9'), 140.8 (<u>C</u>4, <u>C</u>4'), 128.7 (Ph<u>C</u>5), 128.6 (<u>C</u>7, <u>C</u>7'), 125.4 (Ph<u>C</u>4), 125.3 (Ph<u>C</u>3), 122.1 (<u>C</u>6, <u>C</u>6'), 122.1 (<u>C</u>5, <u>C</u>5'), 119.1 (Ph<u>C</u>2), 116.7 (Ph<u>C</u>6), 111.2 (<u>C</u>8/<u>C</u>8'), 110.9 (<u>C</u>8/<u>C</u>8'), 102.9 (<u>C</u>HCN), 102.7 (<u>C</u>HCN), 66.9 (<u>C</u>H$_2$CO), 49.2 (<u>C</u>H$_2$SO$_3$), 42.5 (<u>C</u>H$_2$CH$_2$CH$_2$SO$_3$), 41.4 (<u>C</u>H$_2$CH$_2$CH$_2$NH), 36.0 (<u>C</u>H$_2$NH), 27.0 (Cy<u>C</u>H$_3$), 26.9 (<u>C</u>H$_2$CH$_2$NH$_2$), 22.7 (<u>C</u>H$_2$CH$_2$SO$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{37}H_{44}BN_3O_7S$ [M+Na]$^+$: 708.2882; Obs.: 708.2885; $\boldsymbol{\nu}_{\mathbf{max}}$: (FT-ATR)/cm^{-1}: 3321, 2923, 1663, 1559, 1429, 1373, 1229, 1151, 1113, 1039, 756;

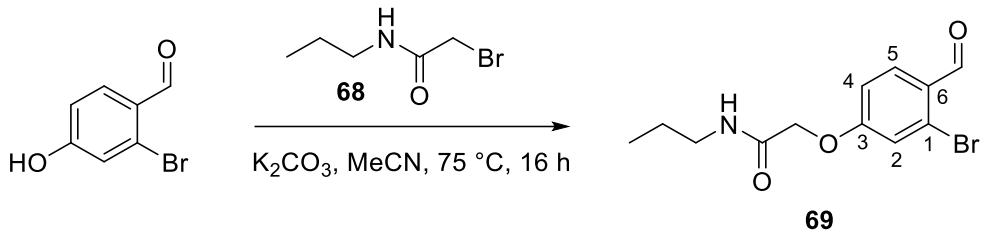
5. Synthesis of OBA substrates for NMR and LC-MS studies



68

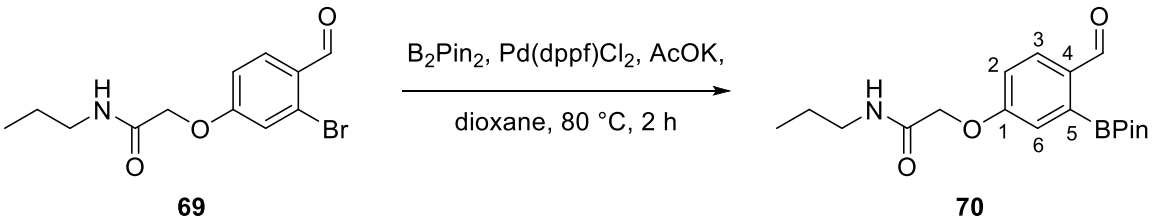
Propylamine (3.78 mL, 45.4 mmol) was added to a solution of bromoacetyl bromide (2.00 mL, 22.6 mmol) in dichloromethane (40 mL) and stirred at r.t. for 30 min. The mixture was then diluted with water (150 mL) and the aqueous extracted with dichloromethane (3 × 70 mL). The combined organics were washed with brine (2 × 200 mL), dried with MgSO$_4$, filtered, and concentrated under reduced pressure to provide a colourless oil (3.40 g, 19.0 mmol, 84%).

R$_f$: 0.32 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CDCl$_3$) δ = 6.67 (s, 1H, N<u>H</u>), 3.88 (s, 2H, C<u>H</u>$_2$Br), 3.28-3.15 (m, 2H, C<u>H</u>$_2$N), 2.00-1.91 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.92 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CDCl$_3$) δ = 165.8 (<u>C</u>ON), 42.0 (<u>C</u>H$_2$NH), 29.4 (<u>C</u>H$_2$Br), 22.6 (<u>C</u>H$_2$CH$_3$), 11.3 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_5H_{10}{}^{79}BrNO$ [M+H]$^+$: 181.0019; Obs.: 181.0020; $\boldsymbol{\nu}_{\mathbf{max}}$: (FT-ATR)/cm^{-1}: 3265, 3073, 2965, 2934, 2876, 1738, 1650, 1550, 1460, 1437, 1313, 1211, 1150, 953, 651, 550.



A mixture of 2-bromo-4-hydroxybenzaldehyde (1.56 g, 8.57 mmol), **68** (1.72 g, 8.57 mmol) and potassium carbonate (2.01 g, 14.6 mmol) in acetonitrile (30 mL) was stirred at 75 °C for 16 h. The mixture was then cooled to r.t. and diluted with water (150 mL). The aqueous was extracted with ethyl acetate (3 × 70 mL), and the combined organics washed with brine (2 × 200 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to provide an orange oil (2.50 g, 8.32 mmol, 97%). Data were consistent with those previously reported.[3]

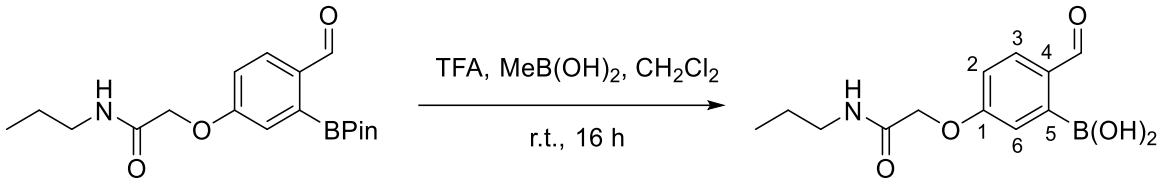
***R*f**: 0.30 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$); δ = 10.20 (s, 1H, PhCOH), 7.89 (d, *J* = 8.7 Hz, 1H, PhH5), 7.17 (d, *J* = 2.5 Hz, 1H, PhH2), 6.96 (dt, *J* = 8.7, 2.5 Hz, 1H, PhH4), 6.49 (s, 1H, NH), 4.54 (s, 2H, CH_2O), 3.30 (t, *J* = 7.4 Hz, 2H, CH_2N), 1.60-1.52 (m, 2H, CH_2CH_3), 0.91 (t, *J* = 7.4 Hz, 3H, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$)) δ = 190.5 (PhCOH), 166.6 (CON), 161.8 (PhC3), 131.8 (PhC5), 128.8 (PhC6), 128.3 (PhC1), 119.6 (PhC2), 114.5 (PhC4), 67.6 (CH_2O), 41.0 (CH_2N), 22.9 (CH_2CH_3), 11.4 (CH_3); **HRMS**: m/z (ESI$^+$) calc. for $C_{12}H_{14}NO_3$ $[M+Na]^+$: 324.0028; Obs.: 324.0028; ***ν*max**: (FT-ATR)/cm^{-1}: 3319, 2974, 2934, 2876, 1679, 1590, 1540, 1412, 1336, 1215, 1142, 965, 852, 831, 675, 578.



69 (300 mg, 1.00 mmol), bis(pinacolato)diboron (660 mg, 2.60 mmol), 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (146 mg, 0.20 mmol), and potassium acetate (530 mg, 5.40 mmol) were placed under a nitrogen atmosphere, and anhydrous dioxane (20 mL) was added. The reaction was degassed under a constant flow of nitrogen for 10 min, and then stirred at 80 °C for 16 h. After cooling to r.t., the reaction was concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (2:8).

Fractions containing the product were concentrated under reduced pressure to yield a colourless oil (34 mg, 95 µmol, 9%).

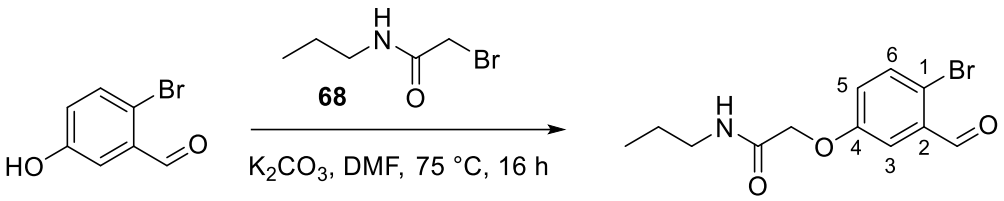
***R*$_f$**: 0.25 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.29 (s, 1H, PhCOH), 7.86 (d, *J* = 8.6 Hz, 1H, PhH3), 7.00 (dd, *J* = 8.6, 2.8 Hz, 1H, PhH2), 6.80 (t, *J* = 2.8 Hz, 1H, PhH6), 6.51 (s, 1H, NH), 4.51 (s, 2H, CH_2O), 3.22 (dt, $J_1 = J_2$ = 6.6 Hz, 2H, CH_2N), 1.51-1.45 (m, 2H, CH_2CH_3), 1.30 (s, 12H, Pin), 0.84-0.79 (m, 3H, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 193.0 (COH), 167.8 (CON), 160.7 (PhC1), 135.6 (PhC4), 130.7 (PhC5), 122.3 (PhC2) 121.0 (PhC3), 116.6 (PhH6), 84.6 ($C(CH_3)_2$), 82.8 ($C(CH_3)_2$), 67.1 (CH_2O), 40.9 (CH_2N), 24.8 (Pin), 22.7 (CH_2CH_3), 11.3 (CH_3); **HRMS**: m/z (ESI^+) calc. for $C_{18}H_{26}BNO_5$ $[M+Na]^+$: 370.1796; Obs.: 370.1802; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3315, 2966, 2933, 2875, 1657, 1542, 1422, 1336, 1213, 1141, 1060, 964, 812, 675, 578.



70 **25**

Trifluoroacetic acid (2 mL) was added to a solution of **70** (34 mg, 95 µmol) and methylboronic acid (57 mg, 0.95 mmol) in dichloromethane (10 mL), and the mixture stirred at r.t for 16 h. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3 × 20 mL), then hydrochloric acid (0.1 M, 2 × 10 mL), to give a brown oil (25 mg, 95 µmol, quantitative yield).

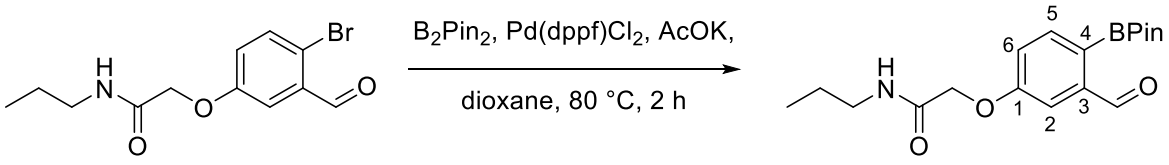
***R*$_f$**: 0.18 (3:7, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, 100 mM deuterated PBS + 10% DMSO-d_6) δ = 9.84 (s, 1H, -CHO), 7.97-8.01 (m, 1H, PhH3), 7.21-7.14 (m, 2H, PhH6 and PhH2), 4.75 (s, 2H, CH_2O), 3.25 (t, *J* = 6.9 Hz, 2H, CH_2N), 1.54 (tt, $J_1 = J_2$ = 6.9 Hz, 2H, CH_2CH_3), 0.88 (t, *J* = 6.9 Hz, 3H, CH_3); **^{13}C NMR** (101 MHz, 100 mM deuterated PBS + 10% DMSO-d_6) δ = 195.2 (-CHO), 170.3 (CON), 161.9 (PhC1), 136.1 (PhC4), 132.4 (PhC5), 118.2 (PhC2), 115.3 (PhC3), 114.56 (PhH6), 66.6 (CH_2O), 40.9 (CH_2N), 21.9 (CH_2CH_3), 10.6 (CH_3); **HRMS**: m/z (ESI^+) calc. for $C_{12}H_{16}BNO_5$ $[M-H]^+$: 288.1014; Obs.: 288.1018; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3329, 3010, 2996, 2980, 1690, 1592, 1555, 1456, 1320, 1286, 1130, 911, 750, 512.



71

A mixture of 2-bromo-4-hydroxybenzaldehyde (800 mg, 4.00 mmol), bromoacetate **68** (864 mg, 4.80 mmol) and potassium carbonate (1.10 g, 8.00 mmol) in dimethylformamide (30 mL) was stirred at 75 °C for 16 h. The mixture was then cooled to r.t. and diluted with water (150 mL). The aqueous mixture was extracted with ethyl acetate (3 × 70 mL), and the combined organics washed with brine (2 × 200 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a white solid (700 mg, 2.34 mmol, 59%).

***R*$_f$**: 0.32 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.29 (s, 1H, C<u>H</u>O), 7.59 (d, *J* = 8.6 Hz, 1H, Ph<u>H</u>6), 7.45 (d, *J* = 1.6 Hz, 1H, Ph<u>H</u>3), 7.07 (dd, *J* = 8.6, 1,6 Hz, 1H, Ph<u>H</u>5), 6.50 (s, 1H, N<u>H</u>), 4.50 (s, 2H, C<u>H</u>$_2$O), 3.36-3.27 (m, 2H, C<u>H</u>$_2$N), 1.59-1.53 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.94 (t, *J* = 7.6 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 191.4 (<u>C</u>HO), 167.1 (<u>C</u>ON), 156.9 (Ph<u>C</u>4), 135.1 (Ph<u>C</u>6), 134.4 (Ph<u>C</u>1), 122.4 (Ph<u>C</u>5), 119.2 (Ph<u>C</u>2), 114.9 (Ph<u>C</u>3), 67.7 (<u>C</u>H$_2$O), 40.9 (<u>C</u>H$_2$N), 22.9 (<u>C</u>H$_2$CH$_3$), 11.4 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{12}H_{14}{}^{79}BrNO_3$ [M+H]$^+$: 300.0231; Obs.: 300.0231; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3350, 3075, 2963, 2870, 1667, 1540, 1285, 1227, 1068, 959, 824, 695, 597.

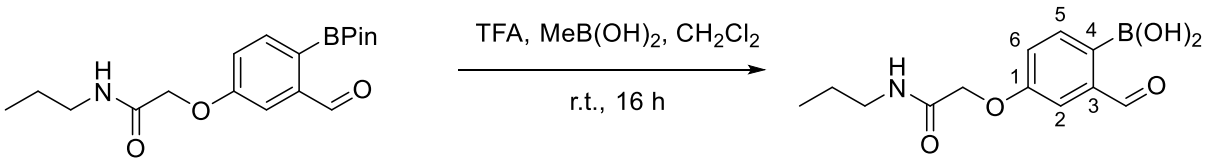


71 **72**

71 (200 mg, 0.66 mmol), bis(pinacolato)diboron (205 mg, 0.80 mmol), 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (24 mg, 0.03 mmol) and potassium acetate (194 mg, 2.00 mmol) were placed under a nitrogen atmosphere, and anhydrous dioxane (5 mL) was added. Nitrogen was bubbled through the reaction mixture for 10 min, which was then stirred at 80 °C for 2 h. After cooling to r.t., the

reaction was concentrated under reduced pressure. The residue was then dissolved in ethyl acetate (100 mL), and washed with water (2 × 70 mL) and brine (2 × 70 mL), and dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to yield a colourless oil (190 mg, 0.548 mmol, 83%).

***R*$_f$**: 0.34 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.64 (s, 1H, C<u>H</u>O), 7.90 (d, *J* = 8.4 Hz, 1H, Ph<u>H</u>5), 7.51 (d, *J* = 2.2 Hz, 1H, Ph<u>H</u>2), 7.13 (dd, *J* = 8.4, 2.2 Hz, 1H, Ph<u>H</u>6), 6.55 (s, 1H, N<u>H</u>), 4.54 (s, 2H, C<u>H</u>$_2$O), 3.30 (t, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$N), 1.62-1.51 (m, 2H, C<u>H</u>$_2$CH$_3$), 1.36 (s, 12H, Pin), 0.92 (t, *J* = 7.6 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 194.4 (<u>C</u>HO), 167.4 (<u>C</u>ON), 159.5 (Ph<u>C</u>1), 143.8 (Ph<u>C</u>4), 138.5 (Ph<u>C</u>5), 119.3 (Ph<u>C</u>6), 119.2 (Ph<u>C</u>3), 112.4 (Ph<u>C</u>2), 84.5 (<u>C</u>(CH$_3$)$_2$), 67.3 (<u>C</u>H$_2$O), 40.9 (<u>C</u>H$_2$N), 25.0 (Pin), 22.9 (<u>C</u>H$_2$CH$_3$), 11.4 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{18}H_{26}BNO_5$ [M+H]$^+$: 348.1977; Obs.: 348.1981; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3323, 2975, 1685, 1663, 1596, 1538, 1378, 1344, 1269, 1243, 1112, 1042, 962, 857, 652, 579.

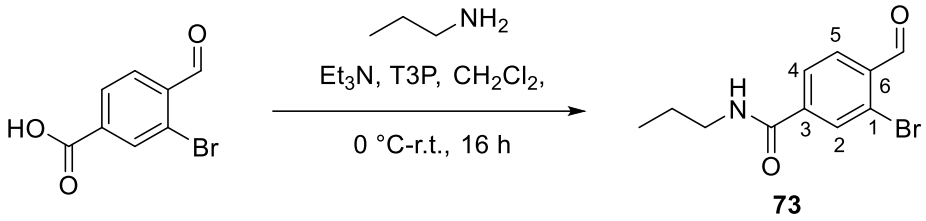


72 **22**

Trifluoroacetic acid (2.0 mL) was added to a solution of **72** (132 mg, 0.42 mmol) and methylboronic acid (216 mg, 3.60 mmol) in dichloromethane (10 mL), and the mixture stirred at r.t for 16 h. The reaction was azeotroped with dichloromethane (3 × 20 mL), and hydrochloric acid (0.1 M, 2 × 10 mL) was added, and concentrated under reduced pressure to give a colourless oil (98 mg, 0.42 mmol, quantitative yield).

***R*$_f$**: 0.21 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, 100 mM deuterated PBS + 10% DMSO-*d*$_6$) δ = 9.97 (s, 1H, -C<u>H</u>O), 7.69-7.59 (m, 1H, Ph<u>H</u>6), 7.54 (d, *J* = 2.4 Hz, 1H, Ph<u>H</u>2), 7.32 (d, *J* = 8.3 Hz, 1H, Ph<u>H</u>5), 4.70 (s, 2H, C<u>H</u>$_2$O), 3.24 (t, *J* = 6.9 Hz, 2H, C<u>H</u>$_2$N), 1.57-1.47 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.85 (t, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD, for acetal) δ = 169.6 (<u>C</u>ON), 158.1 (Ph<u>C</u>1), 143.5 (Ph<u>C</u>4), 131.8 (Ph<u>C</u>5), 114.2 (Ph<u>C</u>6), 111.9 (Ph<u>C</u>3), 102.3 (Ph<u>C</u>2), 66.9 (<u>C</u>H$_2$O), 40.6 (<u>C</u>H$_2$N), 22.4 (<u>C</u>H$_2$CH$_3$), 10.4 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{12}H_{16}BNO_5$ [M+Na]$^+$: 288.1014; Obs.:

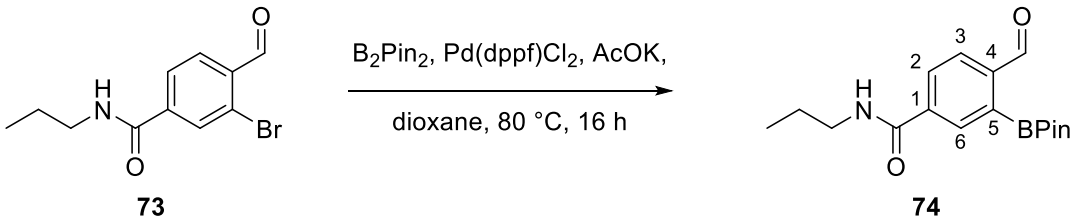
288.1016; ν_{max}: (FT-ATR)/cm^{-1}: 3395, 2968, 2938, 2875, 1661, 1548, 1426, 1348, 1274, 1230, 1148, 807.



73

To a solution of 3-bromo-4-formylbenzoic acid (1.00 g, 4.37 mmol), propylamine (430 µL, 5.24 mmol) and triethylamine (2.79 mL, 21.8 mmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 3.47 mL, 10.9 mmol) at 0 °C and the mixture was warmed to r.t. and stirred for 16 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (1:9). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (547 mg, 2.03 mmol, 46%).

***R*$_f$**: 0.28 (1:9, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.36 (s, 1H, C<u>H</u>O), 8.04 (d, *J* = 1.6 Hz, 1H, Ph<u>H</u>2), 7.93 (d, *J* = 8.0 Hz, 1H, Ph<u>H</u>5), 7.74 (dd, *J* = 8.0, 1.6 Hz, 1H, Ph<u>H</u>4), 6.25 (s, 1H, N<u>H</u>), 3.44-3.38 (m, 2H, C<u>H</u>$_2$N), 1.67-1.61 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.98 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 191.3 (<u>C</u>HO), 165.2 (<u>C</u>ON), 141.0 (Ph<u>C</u>4), 135.2 (Ph<u>C</u>3), 132.8 (Ph<u>C</u>2), 130.1 (Ph<u>C</u>1), 127.2 (Ph<u>C</u>4), 126.1 (Ph<u>C</u>5), 42.2 (<u>C</u>H$_2$N), 22.9 (<u>C</u>H$_2$CH$_3$), 11.5 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{11}H_{12}{}^{79}BrNO_2$ [M+H]$^+$: 270.1260; Obs.: 270.0124; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3313, 3075, 2965, 2934, 2875, 1698, 1642, 1545, 1467, 1441, 1313, 1288, 1202, 1039, 891, 846, 758, 653.

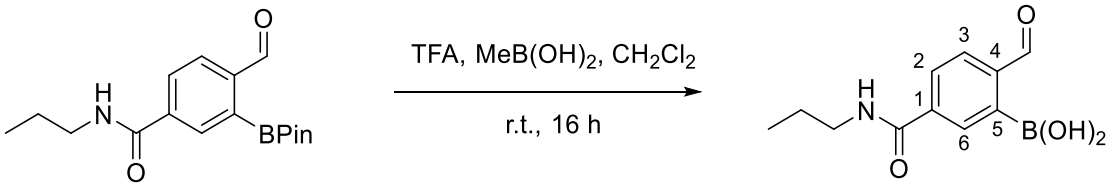


73 **74**

73 (265 mg, 0.981 mmol), bis(pinacolato)diboron (648 mg, 2.55 mmol), 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (144 mg, 0.196 mmol) and potassium acetate (520 mg, 5.30 mmol) were placed under a nitrogen atmosphere, and anhydrous dioxane (15 mL) was added. Nitrogen was bubbled through the reaction mixture for 10 min, which was then stirred at 80 °C for 16 h. After cooling to

r.t., the reaction was concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to yield a colourless oil (206 mg, 0.619 mmol, 24%).

***R*$_f$**: 0.37 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 10.52 (s, 1H, C<u>H</u>O), 8.15 (d, *J* = 1.4 Hz, 1H, Ph<u>H</u>6), 7.94-7.88 (m, 2H, Ph<u>H</u>2, Ph<u>H</u>3), 6.70 (t, *J* = 5.9 Hz, 1H, N<u>H</u>), 3.38-3.31 (m, 2H, C<u>H</u>$_2$N), 1.58 (dt, J_1 = J_2 = 7.5 Hz, 2H, C<u>H</u>$_2$CH$_3$), 1.32 (s, 12H, Pin), 0.91 (t, *J* = 7.5 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 194.2 (<u>C</u>HO), 166.8 (<u>C</u>ON), 143.1 (Ph<u>C</u>4), 138.6 (Ph<u>C</u>1), 134.0 (Ph<u>C</u>6), 133.9 (Ph<u>C</u>5), 129.7 (Ph<u>C</u>2), 128.0 (Ph<u>C</u>3), 83.2 (<u>C</u>(CH$_3$)$_2$), 83.0 (<u>C</u>(CH$_3$)$_2$), 42.0 (<u>C</u>H$_2$N), 24.6 (Pin), 22.9 (<u>C</u>H$_2$CH$_3$), 11.5 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^-$) calc. for $C_{11}H_{13}BNO_4$ [M-Pin]$^-$: 234.0943; Obs.: 234.0948; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3358, 2977, 1643, 1535, 1452, 1371, 1341, 1141, 982, 851, 673, 578,.

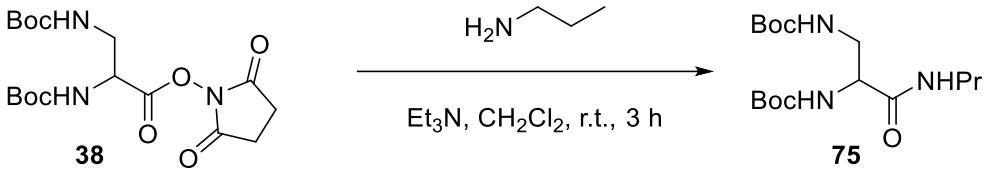


74 **24**

Trifluoroacetic acid (1.0 mL) was added to a solution of **74** (70 mg, 0.22 mmol) and methylboronic acid (132 mg, 2.21 mmol) in dichloromethane (5 mL), and the mixture stirred at r.t for 16 h. The reaction was azeotroped with dichloromethane (3 × 20 mL), and hydrochloric acid (0.1 M, 2 × 10 mL) was added, and concentrated under reduced pressure to give a yellow oil (52 mg, 0.22 mmol, quantitative yield).

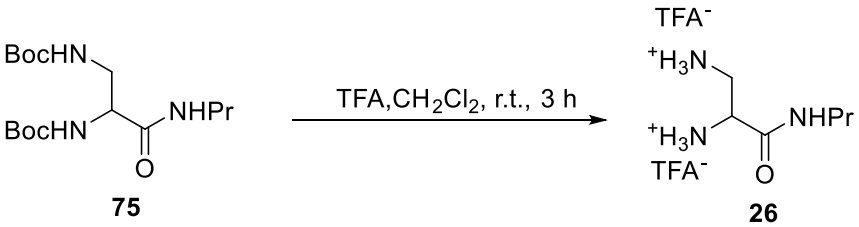
***R*$_f$**: 0.29 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD, for acetal) δ = 7.76-7.66 (m, 2H, Ph<u>H</u>6, Ph<u>H</u>2), 7.37 (d, *J* = 8.0 Hz, 1H, Ph<u>H</u>3), 5.45 (s, 1H, C<u>H</u>(OMe)$_2$), 3.27-3.19 (m, 2H, C<u>H</u>$_2$N), 1.53 (dt, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$CH$_3$), 0.86 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD, for acetal) δ = 168.8 (<u>C</u>ON), 144.3 (Ph<u>C</u>4), 133.7 (Ph<u>C</u>1), 133.0 (Ph<u>C</u>6), 128.6 (Ph<u>C</u>5), 126.9 (Ph<u>C</u>2), 125.6 (Ph<u>C</u>3), 102.3 (<u>C</u>H(OMe)$_2$), 84.1 (<u>C</u>(CH$_3$)$_2$), 41.5 (<u>C</u>H$_2$N), 23.7 (Pin), 22.4 (<u>C</u>H$_2$CH$_3$), 10.5 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{11}H_{13}BNO_4$ [M-H]$^+$: 234.0943; Obs.: 234.0945; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3314, 2967, 2932, 1693, 1639, 1540, 1343, 1316, 1206, 1141, 1066, 964, 851, 813, 760.

6. Synthesis of nucleophiles for NMR and LC-MS studies, and FRET controls



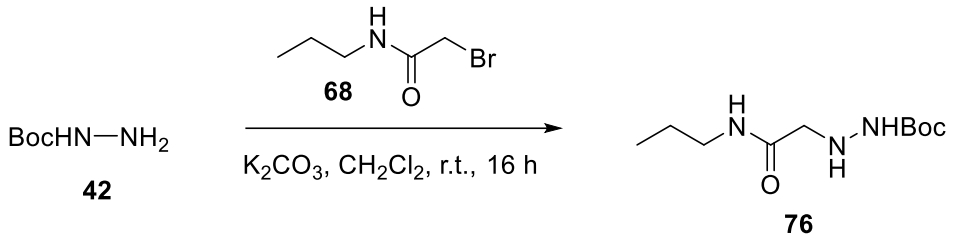
A mixture of **38** (154 mg, 0.38 mmol), propylamine (96 µL, 1.15 µmol), and triethylamine (266 µL, 1.92 mmol) in dichloromethane (5 mL) was stirred at r.t. for 3 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (35 mg, 0.10 mmol, 27%).

***R*$_f$**: 0.38 (3:7, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.72 (s, 1H, N<u>H</u>Pr), 5.84 (s, 1H, BocN<u>H</u>CH), 5.26 (s, 1H, BocN<u>H</u>CH_2), 4.16 (dt, J_1 = J_2 = 6.1 Hz, 1H, C<u>H</u>NHBoc), 3.49-3.40 (m, 2H, C<u>H</u>$_2$NHBoc), 3.22-3.15 (m, 2H, C<u>H</u>$_2$$CH_2CH_3$), 1.49 (q, J = 7.5 Hz, 2H, C<u>H</u>$_2$$CH_3$), 1.43-1.39 (m, 18H, 2 × Boc), 0.88 (s, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.6 (<u>C</u>ONH), 157.2 (<u>C</u>OBoc), 156.3 (<u>C</u>OBoc), 80.3 (<u>C</u>$(CH_3)_3$), 80.0 (<u>C</u>$(CH_3)_3$), 55.7 (<u>C</u>HNHBoc), 42.5 (<u>C</u>H_2NHBoc), 41.2 (<u>C</u>$H_2CH_2CH_3$), 28.4 (Boc), 28.4 (Boc), 22.8 (<u>C</u>H_2CH_3), 11.4 (<u>C</u>H_3); **HRMS**: m/z (ESI$^+$) calc. for $C_{16}H_{32}N_3O_5$ $[M+H]^+$: 346.2336; Obs.: 346.2335; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3330, 2975, 2933, 2876, 1690, 1654, 1518, 1365, 1249, 1163, 1078, 868, 780, 644.



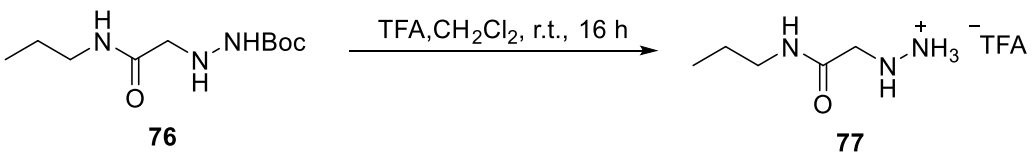
Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **74** (30 mg, 87 µmol) in dichloromethane (5 mL) and the solution was stirred at r.t. for 3 h. The reaction mixture was concentrated under reduced pressure and azeotroped with dichloromethane (4 × 30 mL) to obtain a colourless oil. (13 mg, 87 µmol, quantitative yield).

***R*$_f$**: 0.24 (3:7, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 4.21 (t, *J* = 7.2 Hz, 1H, C<u>H</u>CO), 3.47–3.26 (m, 3H, CH_2NH, CONHC<u>H</u>$_2$), 3.12 (dt, *J* = 13.7, 7.2 Hz, 1H, ONHC<u>H</u>$_2$), 1.55 (tq, J_1 = J_2 = 7.4 Hz, 2H, C<u>H</u>$_2$CH$_3$), 0.93 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 165.3 (<u>C</u>ON), 50.8 (<u>C</u>HNH$_3^+$), 41.5 (CONH<u>C</u>H$_2$), 39.7 (<u>C</u>H$_2$NH$_3^+$) , 21.9 (<u>C</u>H$_2$CH$_3$), 10.3 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_6H_{15}N_3O$ $[M+H]^+$: 146.1288; Obs.: 146.1288; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3287, 2968, 2938, 2879, 1649, 1553, 1462, 1200, 1136, 837, 800, 722.



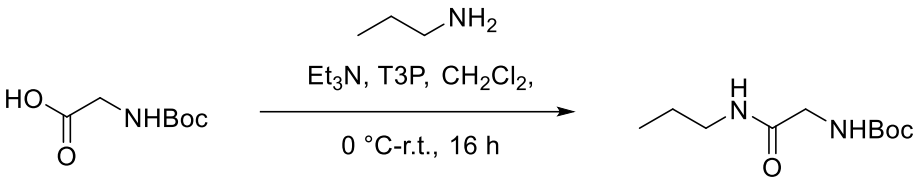
A mixture of **42** (371 mg, 2.81 mmol), **68** (603 mg, 3.37 mmol), and potassium carbonate (776 mg, 5.62 mmol) in dimethylformamide (5 mL) was stirred at 80 °C for 30 min. The mixture was then cooled to r.t. and diluted with water (50 mL). The aqueous was extracted with ethyl acetate (3 × 50 mL), and the combined organics washed with brine (2 × 30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (532 mg, 2.30 mmol, 82%).

***R*$_f$**: 0.36 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.56 (s, 1H, NHPr), 6.40 (s, 1H, N<u>H</u>Boc), 4.19 (s, 1H, N<u>H</u>NHBoc), 3.46 (s, 2H, C<u>H</u>$_2$CO), 3.20 (t, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$NH), 1.55-1.49 (m, 2H, C<u>H</u>$_2$CH$_3$), 1.41 (s, 9H, Boc), 0.90 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.1 (<u>C</u>ON), 156.8 (<u>C</u>OBoc), 81.0 (<u>C</u>(CH$_3$)$_3$), 55.5 (<u>C</u>H$_2$O), 41.0 (<u>C</u>H$_2$NH), 28.3 (Boc), 22.8 (<u>C</u>H$_2$CH$_3$), 11.5 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{10}H_{21}N_3O_3$ $[M+Na]^+$: 254.1475; Obs.: 254.1469; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3298, 2969, 2934, 2877, 1714, 1650, 1545, 1460, 1367, 1282, 1250, 1159, 1046. 1022, 849, 754, 593.



Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **76** (70 mg, 0.142 mmol) in dichloromethane (5 mL), and the solution was stirred at r.t. for 16 h. The reaction mixture was then concentrated under reduced pressure and azeotroped with dichloromethane (4 × 30 mL) to obtain a colourless oil. (40 mg, 0.142 mmol, quantitative yield).

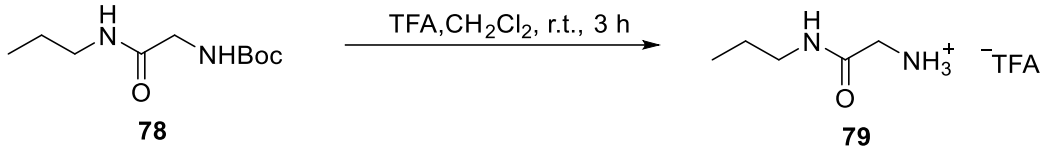
***R*$_f$**: 0.24 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 3.62 (s, 2H, C<u>H</u>$_2$NHNH$_3^+$), 3.16 (t, *J* = 7.3 Hz, 2H, C<u>H</u>$_2$NHCO), 1.58-1.48 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.91 (t, *J* = 7.3 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.8 (<u>C</u>ON), 50.4 (<u>C</u>H$_2$ONHNH$_3^+$), 40.8 (<u>C</u>H$_2$NH), 22.2 (<u>C</u>H$_2$CH$_3$), 10.3 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_5H_{13}N_3O$ [M+Na]$^+$: 132.1131; Obs.: 132.1130; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3300, 2965, 2934, 2876, 1651, 1543, 1460, 1201, 1146, 721.



78

To a solution of Boc-Gly-OH (150 mg, 0.857 mmol), propylamine (178 µL, 2.14 mmol) and triethylamine (593 µL, 4.29 mmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 681 µL, 2.14 mmol) at 0 °C and the mixture stirred at r.t. for 16 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (132 mg, 0.71 mmol, 49%).

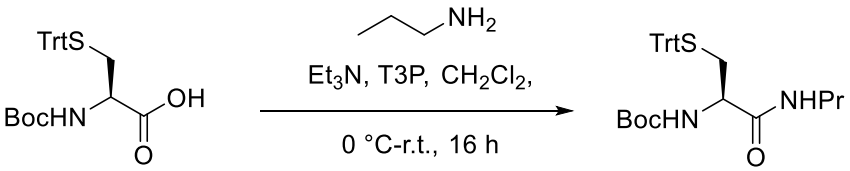
***R*$_f$**: 0.32 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.22 (s, 1H, N<u>H</u>CO), 5.21 (s, 1H, N<u>H</u>Boc), 3.75 (s, 2H, C<u>H</u>$_2$CO), 3.21 (t, *J* = 7.2 Hz, 2H, C<u>H</u>$_2$NH), 1.54-1.46 (m, 2H, C<u>H</u>$_2$CH$_3$), 1.43 (s, 9H, Boc), 0.90 (t, *J* = 7.2, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.5 (<u>C</u>ON), 156.0 (<u>C</u>OBoc), 80.4 (<u>C</u>H$_2$O), 44.6 (<u>C</u>(CH$_3$)$_3$), 41.2 (<u>C</u>H$_2$N), 28.4 (Boc), 22.8 (<u>C</u>H$_2$CH$_3$), 11.4 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{10}H_{20}N_2O_3$ [M+Na]$^+$: 239.1366; Obs.: 239.1367; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3312, 2968, 2933, 2876, 1656, 1512, 1365, 1248, 1164, 1049, 940, 864, 735, 551, 462.



78 **79**

Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **78** (90 mg, 0.42 mmol) in dichloromethane (5 mL) and the solution was stirred at r.t. for 3 h. The reaction mixture was concentrated under reduced pressure and azeotroped with dichloromethane (4 × 30 mL) to obtain a colourless oil. (48 mg, 0.42 mmol, quantitative yield).

***R*$_f$**: 0.32 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 8.29 (m, 1H, NH), 3.68 (s, 2H, CH_2CO), 3.16 (t, *J* = 7.3 Hz, 2H, CH_2NH), 1.51 (dt, J_1 = J_2 = 7.3 Hz, 2H, CH_2CH_3), 0.89 (t, *J* = 7.3 Hz, 3H, CH_3); **^{13}C NMR** (101 MHz, CD_3OD) δ = 165.8 (CON), 41.0 (CH_2O), 40.2 (CH_2N), 22.8 (CH_2CH_3), 10.29 (CH_3); **HRMS**: m/z (ESI^+) calc. for $C_5H_{12}N_2O$ $[M+H]^+$: 117.1022; Obs.: 117.1022; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3305, 2926, 1663, 1576, 1436, 1275, 1130, 840, 916, 798, 723, 518.

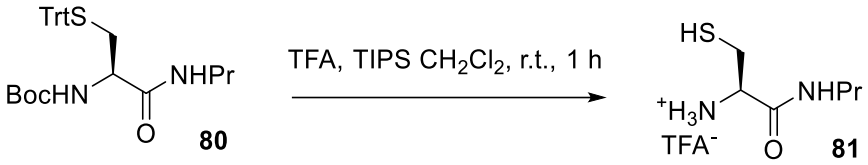


80

To a solution of Boc-Cys(Trt)-OH (200 mg, 0.431 mmol), propylamine (54 µL, 0.647 mmol), and triethylamine (300 µL, 2.16 mmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 343 µL, 1.08 mmol) at 0 °C and the mixture stirred at r.t. for 16 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (205 mg, 0.43 mmol, 99%).

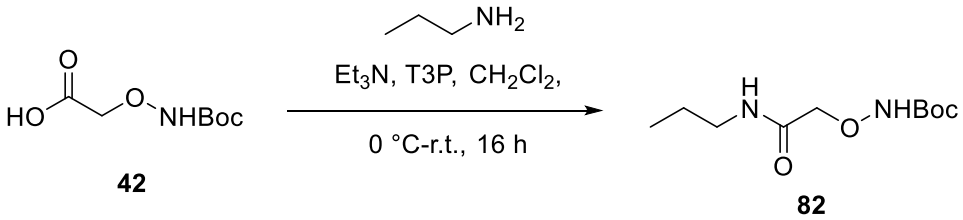
***R*$_f$**: 0.29 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.44-7.38 (m, 6H, PhH2), 7.31-7.25 (m, 6H, PhH3), 7.23-7.18 (m, 3H, PhH4), 5.94 (s, 1H, NHBoc), 4.80-4.64 (m, 1H, NHPr), 3.80 (d, *J* = 6.4 Hz, 1H, CHNHBoc), 3.16-3.09 (m, 2H, CH_2NH), 2.74-2.66 (m, 1H, CH_2STrt), 2.53-2.45 (m, 1H, CH_2STrt), 1.50-1.42 (m, 2H, CH_2CH_3), 1.40 (s, 9H, Boc), 0.86 (t, *J* = 7.4 Hz, 3H, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.4 (CON), 144.5 (COBoc), 129.7 (PhC2), 128.2 (PhC3), 127.0 (PhC4), 82.3 (CHNHBoc), 67.3 (CH_2STrt), 41.3 (CH_2NH), 28.4 (Boc), 22.8 (CH_2CH_3), 11.4 (CH_3);

HRMS: m/z (ESI$^+$) calc. for $C_{30}H_{36}N_2O_3S$ [M+Na]$^+$: 527.2335; Obs.: 527.2335; ν_{max}: (FT-ATR)/cm^{-1}: 3300, 3058, 2967, 2931, 2875, 1655, 1526, 1489, 1366, 1248, 1165, 1047, 865, 739, 698, 621, 505.



Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **80** (195 mg, 0.41 mmol) and triisopropylsilane (439 µL, 2.04 mmol) in dichloromethane (5 mL), and the mixture stirred at r.t. for 16 h. The reaction was then concentrated under reduced pressure to ~5 mL, and the remaining solution added dropwise to diethyl ether (200 mL). The resultant precipitate was collected by filtration, washed with diethyl ether (30 mL), and dried in air. The solid was then dissolved in methanol (10 mL) and concentrated under reduced pressure to afford a colourless oil. (67 mg, 0.41 mmol, quantitative yield).

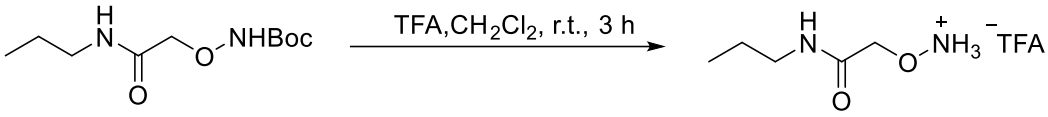
***R*$_f$**: 0.18 (2:8, EtOAc:Petrol, UV active); **^{1}H NMR** (400 MHz, CD_3OD) δ = 3.98-3.90 (m, 1H, C<u>H</u>NH$_3^+$), 3.29-3.15 (m, 2H, C<u>H</u>$_2$NH), 3.04-2.87 (m, 2H, C<u>H</u>$_2$STrt), 1.59-1.48 (m, 2H, C<u>H</u>$_2$CH$_3$), 0.92 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 166.9 (<u>C</u>ON), 54.8 (<u>C</u>HNH$_3^+$), 41.2 (<u>C</u>H$_2$NH), 25.0 (<u>C</u>H$_2$SH), 22.1 (<u>C</u>H$_2$CH$_3$), 10.4 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_6H_{14}N_2OS$ [M+Na]$^+$: 185.0719; Obs.: 185.0720; ν_{max}: (FT-ATR)/cm^{-1}: 3286, 3090, 2966, 1655, 1571, 1265, 1181, 1133, 838, 798, 722, 517.



To a solution of **42** (115 mg, 0.602 mmol), propylamine (125 µL, 1.51 mmol) and triethylamine (416 µL, 3.01 mmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 479 µL, 1.51 mmol) at 0 °C and the mixture was warmed to r.t. and stirred for 16 h. The reaction was then concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the

product were concentrated under reduced pressure to provide a colourless oil (65 mg, 0.28 mmol, 46%).

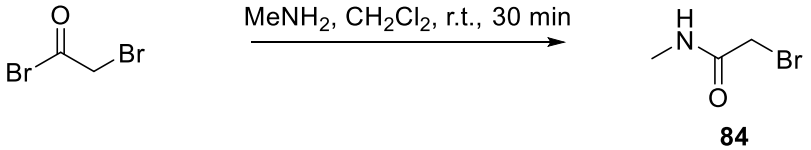
***R*$_f$**: 0.36 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.15 (s, 1H, N<u>H</u>), 4.24 (s, 2H, C<u>H</u>$_2$O), 3.20 (t, *J* = 7.3 Hz, 2H, C<u>H</u>$_2$NH), 1.51 (dt, *J* = 7.3 Hz, 2H, C<u>H</u>$_2$CH$_3$), 1.42 (s, 9H, Boc), 0.88 (t, *J* = 7.3 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.1 (<u>C</u>ONH), 158.0 (<u>C</u>OBoc), 82.8 (C(CH$_3$)$_3$), 76.1 (<u>C</u>H$_2$CO), 40.9 (<u>C</u>H$_2$NH), 28.2 (Boc), 22.6 (<u>C</u>H$_2$CH$_3$), 11.5 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_{10}H_{20}N_2O_4$ [M+Na]$^+$: 255.1315; Obs.: 255.1311; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3285, 2969, 2934, 2877, 1724, 1650, 1552, 1459, 1368, 1252, 1162, 1110, 776, 586.



82 **83**

Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **82** (60 mg, 0.26 mmol) in dichloromethane (5 mL) and the solution was stirred at r.t. for 3 h. The reaction mixture was concentrated under reduced pressure and azeotroped with dichloromethane (4 × 30 mL) to obtain a colourless oil. (34 mg, 0.26 mmol, quantitative yield).

***R*$_f$**: 0.27 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 4.20 (s, 2H, C<u>H</u>$_2$CO), 2.92 (t, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$NH), 1.26 (dt, J_1 = J_2 = 7.4 Hz, 2H, C<u>H</u>$_2$CH$_3$), 0.64 (t, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 168.5 (<u>C</u>ONH), 71.4 (<u>C</u>H$_2$CO), 40.6 (<u>C</u>H$_2$NH), 22.2 (<u>C</u>H$_2$CH$_3$), 10.3 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_5H_{12}N_2O_2$ [M+H]$^+$: 133.0972; Obs.: 133.0967; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3288, 3089, 2966, 2877, 1654, 1544, 1460, 1201, 1084, 833, 580.

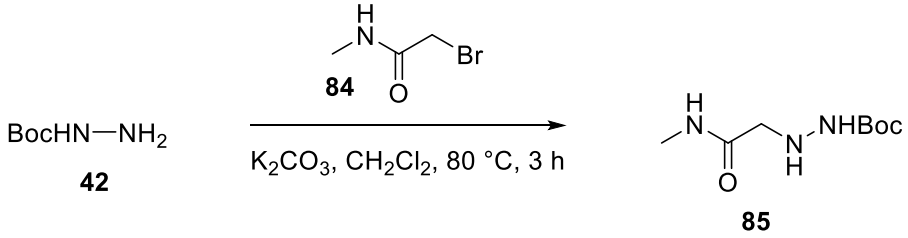


84

Methylamine (1.00 mL, 22.6 mmol) was added to a solution of bromoacetyl bromide (1.00 mL, 11.3 mmol) in dichloromethane (30 mL) and stirred at r.t. for 30 min. The mixture was then diluted with water (70 mL) and the aqueous was extracted with ethyl acetate (3 × 70 mL). The combined organics were washed with brine (2 × 100 mL),

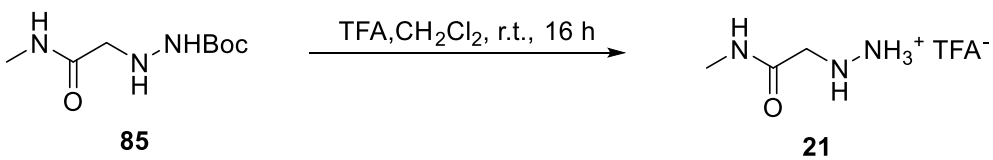
dried with $MgSO_4$, filtered, and concentrated under reduced pressure to provide a colourless oil (1.56 g, 1.03 mmol, 91%).

***R*$_f$**: 0.29 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.80 (s, 2H, C<u>H</u>$_2$), 3.73 (s, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.8 (<u>C</u>ON), 53.2 (<u>C</u>H$_2$), 25.7 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for C_3H_6NO $[M+Na]^+$: 193.9525; Obs.: 193.9525; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 2956, 1736, 1437, 1280, 1165, 1113, 1006, 884, 708, 670, 549.



A mixture of **42** (500 mg, 3.79 mmol), **84** (732 mg, 4.55 mmol), and potassium carbonate (1.05 g, 7.58 mmol) in dimethylformamide (10 mL) was stirred at 80 °C for 3 h. The mixture was then cooled to r.t. and diluted with water (70 mL). The aqueous was extracted with ethyl acetate (3 × 50 mL), and the combined organics washed with brine (2 × 50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography on silica gel, eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a colourless oil (50 mg, 0.25 mmol, 6%).

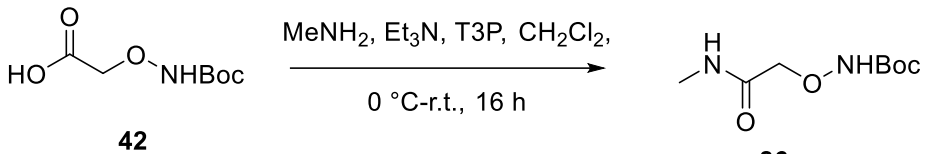
***R*$_f$**: 0.26 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$, data provided for major rotamer) δ = 6.53 (s, 1H, NHMe), 3.78-3.68 (m, 3H, C<u>H</u>$_3$), 3.60 (s, 2H, C<u>H</u>$_2$), 1.40 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$, data provided for major rotamer) δ = 171.9 (<u>C</u>ON), 162.9 (<u>C</u>OBoc), 81.0 (<u>C</u>(CH$_3$)$_3$), 53.0 (C<u>H</u>$_2$), 52.2 (C<u>H</u>$_3$), 28.6 (C(<u>C</u>H$_3$)$_3$); **HRMS**: Product was not observed via HRMS; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}: 3320, 2978, 1714, 1438, 1367, 1209, 1149, 1049, 1017, 779.



Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **84** (43 mg, 0.21 mmol) in dichloromethane (5 mL) and the solution was stirred at r.t. for 16 h. The reaction mixture was then concentrated under reduced pressure and azeotroped with

dichloromethane (4 × 30 mL) to obtain a colourless oil (22 mg, 0.21 mmol, quantitative yield).

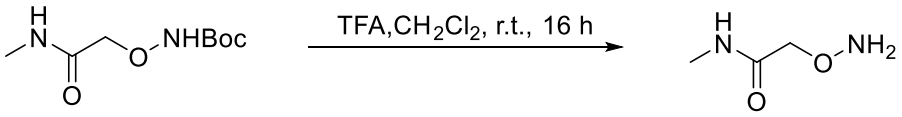
R_f: 0.18 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 3.82-3.76 (m, 2H, C<u>H</u>$_2$), 3.78-3.70 (m, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 171.8 (<u>C</u>ON), 52.9 (<u>C</u>H$_2$), 50.6 (<u>C</u>H$_3$); **HRMS**: Product was not observed via HRMS; ***ν*_{max}**: (FT-ATR)/cm^{-1}: 2959, 1730, 1438, 1205, 1154, 1047, 1005, 907, 761.



42 **86**

To a solution of **42** (300 mg, 1.57 mmol), methylamine (140 µL, 3.14 mmol), and triethylamine (1.09 mL, 7.85 mmol) in dichloromethane (5 mL), was added propylphosphonic anhydride solution (50% w/w in EtOAc, 1.25 mL, 3.93 mmol) at 0 °C, and the mixture was then stirred at r.t. for 16 h. The reaction was concentrated under reduced pressure and the residue was purified via flash column chromatography on silica gel eluting with EtOAc:Petrol (2:8). Fractions containing the product were concentrated under reduced pressure to provide a white solid (145 mg, 0.71 mmol, 45%).

R_f: 0.31 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.03 (s, 1H, N<u>H</u>Boc), 7.60 (s, 1H, N<u>H</u>Me), 4.33 (s, 2H, C<u>H</u>$_2$), 2.86 (s, 3H, C<u>H</u>$_3$), 1.48 (s, 9H, Boc); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.4 (<u>C</u>ONH), 157.9 (<u>C</u>OBoc), 83.4 (<u>C</u>(CH$_3$)$_3$), 76.6 (<u>C</u>H$_2$CO), 28.2 (<u>C</u>(CH$_3$)$_3$), 25.8 (<u>C</u>H$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_8H_{16}N_2O_4$ [M+Na]$^+$: 227.1002; Obs.: 227.1008; ***ν*_{max}**: (FT-ATR)/cm^{-1}: 3287, 2979, 2939, 1723, 1658, 1559, 1480, 1369, 1280, 1253, 1163, 1112, 977, 583.



86 **20**

Trifluoroacetic acid (1 mL) was added dropwise to a stirred solution of **86** (60 mg, 0.26 mmol) in dichloromethane (5 mL) and stirred at r.t. for 3 h. The reaction mixture was concentrated under reduced pressure and azeotroped with dichloromethane (4 × 30 mL) to obtain a colourless oil. (34 mg, 0.26 mmol, quantitative yield).

***R*$_f$**: 0.22 (2:8, EtOAc:Petrol); **^{1}H NMR** (400 MHz, CD_3OD) δ = 4.50 (s, 2H, C<u>H</u>$_2$), 2.80 (s, 3H, C<u>H</u>$_3$); **^{13}C NMR** (101 MHz, CD_3OD) δ = 170.1 (<u>C</u>ON), 72.7 (C<u>H</u>$_2$), 25.9 (C<u>H</u>$_3$); **HRMS**: m/z (ESI$^+$) calc. for $C_3H_8N_2O_2$ $[M+H]^+$: 105.0659; Obs.: 105.0652; ***ν*$_{max}$**: (FT-ATR)/cm^{-1}:3313, 2924, 1654, 1553, 1414, 1199, 1135, 1085, 834, 800, 722, 577.

7. Determination of substrate concentration via UV-Vis analysis

Due to the low amounts of Cy3 and Cy5 substrates synthesised, and the potential for errors in mass calculations that could result, the concentrations of stock solutions of each substrate were calculated from a calibration curve of **1** or **2** of known concentrations. Briefly, stock dilutions of **1** or **2** were made in water to concentrations in the range of 0.1-400 µM (at least 6 data points). Absorbance spectra were then recorded in the range 400-600 nm, and the absorbance at the λ_{max} plotted as a function of concentration (Cy3: 543 nm; Cy5: 641 nm).

Aliquots of each substrate were then serially diluted in water to generate samples for measurement. Concentrations were then determined for appropriately dilute samples for which absorbance at λ_{max} fell within the linear range of the calibration curves.

8. Initial screening of Cy3 quenching

General procedure: A solution of Cy5-nucleophile (50 µL, 100 µM) in PBS buffer was added to a solution Cy3-*o*BA **5** (50 µL, 10 µM) in PBS buffer in a 96-well plate, to give final Cy3 and Cy5 concentrations of 5 µM and 50 µM respectively (pseudo-first order). Single-point fluorescence emission intensities ($\lambda_{excitation}$ = 480 nm; $\lambda_{emission}$ = 580 nm) in the Cy3 channel were then recorded every 1 min for a period of 100 min.

Negative control: Run as for the general procedure, using Cy5-NHAc **16** (50 µL, 100 µM)

Positive control: The emission of a solution of Cy3-Cy5 covalent control **17** (100 µL, 5 µM) was recorded over time as described above.

Data processing: Emission at 580 nm was plotted as a function of time, relative to the negative (100%) and positive (0%) controls.

Cy3 controls: Run as for the general procedure, using either Cy3-benzaldehyde **61** or Cy3-phenylboronic acid **67** (50 µL, 10 µM).

Controls to validate quenching via FRET: Run as for the general procedure, using PrNH-capped nucleophiles **26**, **77**, **79**, **81**, or **83** (50 µL, 100 µM) in place of the Cy5-nucleophile.

9. FRET studies

General procedure: FRET studies were performed in a 700 µL fluorescence cuvette under second-order conditions. A solution of Cy5-nucleophile (300 µL, 5 µM) in the stated buffer was added to a solution of Cy3-*o*BA **5** (300 µL, 5 µM) in the same buffer and rapidly mixed by pipetting up and down. Fluorescence emission spectra between 520-700 nm were recorded immediately after mixing, and then subsequently ever 15 seconds for a total of 100 measurements. The delay between mixing and the measurement of the first spectra was ~3 seconds. All measurements were performed in triplicate.

Negative control: Run as for the general procedure, using Cy5-NHAc **16** (300 µL, 5 µM).

Positive control: The emission of a solution of Cy3-Cy5 covalent control **17** (600 µL, 2.5 µM) was recorded over time as described above.

Data processing: The ratio of the emission at the λ_{max} of Cy3 ($Emiss_{560}$) and Cy5 ($Emiss_{657}$) was used to determine the FRET ratio ($Emiss_{560}/Emiss_{657}$). As the initial spectra were recorded at t = 3 sec, a plot of $1/[Emiss_{560/657}]$ against time and linear regression analysis was used to determine $Emiss_{560/657}$ at t = 0 (intercept of linear regression). A minimum of 4 data points that lay within the initial linear region of this plot were included in this analysis. $Emiss_{700}$ was used as a background measurement and subtracted from $Emiss_{560/657}$ prior to analysis.

Conversion of FRET ratios to conversion: Data from the positive and negative controls was used to account for drift in the system and to calculate the expected FRET ratio for 0% and 100% conjugation at t = x, averaged across three triplicates:

i) *0% conjugation*: Changes in $Emiss_{560/657}$ from the negative control over the period of the measurement were fitted to a linear regression analysis, generating the gradients of drift $a_{560/657}$. The 0% conjugation FRET reference, *A*, then equals:

$$A = \frac{{}^{0}Emiss_{560} + (a_{560} \times x)}{{}^{0}Emiss_{657} + (a_{657} \times x)}$$

ii) *100% conjugation*: Changes in $Emiss_{560/657}$ from the positive control over the period of the measurement were fitted to a linear regression analysis, generating the gradients of drift $b_{560/657}$ and the emissions at t = 0, ${}^{0}c_{560/657}$. The 100% conjugation FRET reference, *B*, then equals:

$$B = \frac{{}^{0}c_{560} + (b_{560} \times x)}{{}^{0}c_{657} + (b_{657} \times x)}$$

Conversion can then be calculated from:

$$Conversion = \frac{A - FRET}{A - B} \times 100$$

Conversions over time were then averaged over the three triplicates and standard deviations at each time point calculated

Data fitting: Data were fit to a second order reversible kinetic model in Copasi 4.34.251. k_1 and k_{-1} were estimated using the evolutionary programming method built into the software, with 200 generations and a population size of 20. Parameters were restricted within the confines of: k_1 10^{-6}-10^{7} M^{-1} s^{-1}; k_{-1} 10^{-8}-10^{3} s^{-1}.

10. LC-MS reversibility studies

General procedure: Stock solutions of **25** (3 µL, 38 mM, 114 nmol) and propyl amide nucleophile **83** or **77** (6 µL, 38 mM, 228 nmol) in methanol were added sequentially to the relevant buffer (300 µL, final *o*BA concentration 370 µM) and shaken for 30 min. At this point, a stock solution of the analogous methyl amide nucleophile **20** or **21** (30 µL, 38 mM, 1140 nmol) was added and the mixture incubated at room temperature. Aliquots were analysed via LC-MS analysis every 24 h for 1 week.

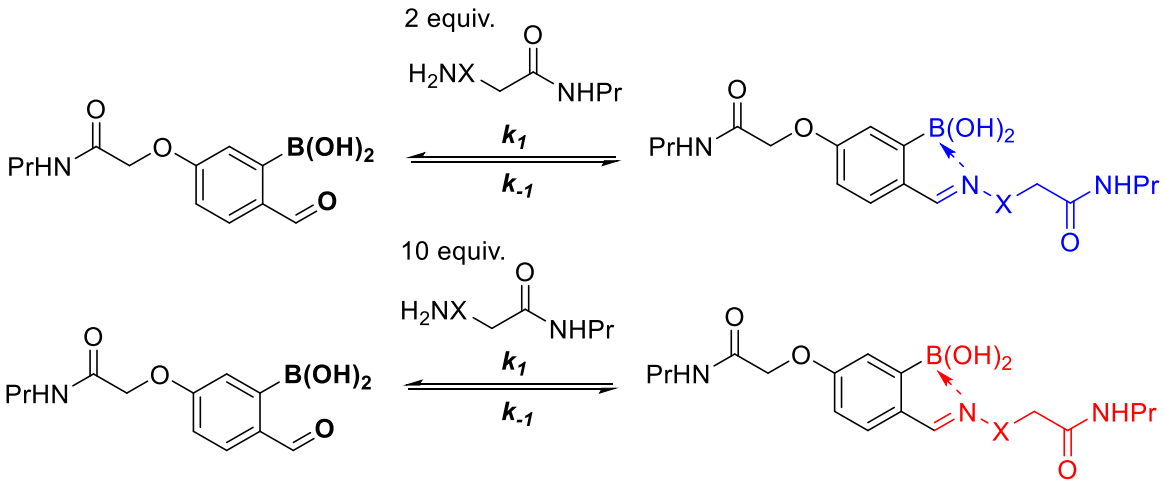
Negative control: Run as described above, but methanol (30 µL) was added in place of the methyl amide nucleophile.

Positive control: Run as described above, but methanol (6 µL) was added in place of the propyl amide nucleophile.

Data analysis: The absorbance at 280 nm at time t = x (A_x) was integrated for peaks relating to propyl-*o*BID (elution time: 2.90 min) products. A plot of ln(Integration) was used to determine the integration at t = 0 (intercept of linear regression). Data from the positive controls was used to calculate the expected integration at 100% exchange (B). Conversions were then calculated from:

$$Conversion = \frac{A_0 - A_x}{A_0 - B} \times 100$$

Data fitting: Data were fit to a two-reaction reversible kinetic model using Copasi 4.34.251, based on the following reactions:



k_1 calculated from the FRET studies were used to estimate k_{-1}, using the evolutionary programming method built into the software, with 200 generations and a population size of 20, and with the assumption that rates of reactions were the same for propyl- and methyl-amide nucleophiles. Parameters were restricted within the confines of: k_{-1} 10^{-10}-10^{-1} s^{-1}.

11. NMR studies of pH dependent DAB-hydrazone exchange

Solutions of *o*BA **25** (5 mg, 19 µmol) in DMSO-d_6 (25 µL) and propyl-amide hydrazine **77** (4.6 mg, 19 µmol) in DMSO-d_6 were added sequentially to deuterated buffers (450 µL, 100 mM; pH 4 – acetate buffer, pH 5, 6, 7.4, 8 – phosphate buffer; prepared by evaporating standard buffer and then redissolving the residue in D_2O three times) and

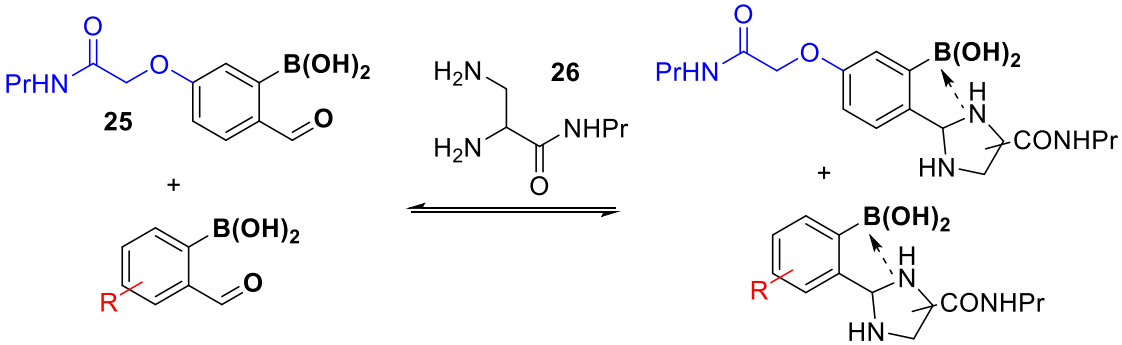
incubated for 30 min. The sample was then analysed by NMR and the ratio of DAB to hydrazone determined. In all cases, only signals from the cyclic DAB were observed, indicated by a singlet at δ ~ 8.6, as previously reported by Gu *et al*.[11]

12. NMR studies of sugar binding

Solutions of *o*BA **25** (2.5 mg, 9 µmol) in DMSO-d_6 (25 µL) and either propyl-amide hydrazine **77** or hydroxylamine **83** (9 µmol) in DMSO-d_6 were added sequentially to deuterated PBS (450 µL, 100 mM; prepared by evaporating standard buffer and then redissolving the residue in D_2O three times) and incubated for 30 min. Glucose or fructose (9 µmol) was then added and the samples incubated for a further 30 min. After this time the samples were analysed by NMR and shifts in the oxime/DAB peaks used to determine the extent of sugar binding. In all cases, no shift in signal was observed indicating no sugar binding was taking place under these conditions.

13. NMR studies of analogue reaction equilibria

General procedure: *o*BA **25** (2 mg, 7.5 µmol) and another *o*BA analogue, **22**-**24** (7.5 µmol), were dissolved in a mixture of deuterated PBS (0.5 mL, 100 mM; prepared by evaporating standard PBS and then redissolving the residue in D_2O three times) and DMSO-d_6 (50 µL). A solution of 1,2-diamine **26** (1.1 mg, 7.5 µmol) in deuterated PBS (0.5 mL) was then added and the mixture incubated for 2 hrs, prior to NMR analysis.



Data analysis: Peaks relating to *o*BID formation for both **25** and the *o*BA analogue competitor were identified via prior control reactions in which each *o*BA was incubated with **26** alone. Integration of peaks that fell within unique regions of the spectra relating

to *o*BID formation were used to determine the ratio of products formed. This ratio was then used to calculate K_d for each analogue, as follows:

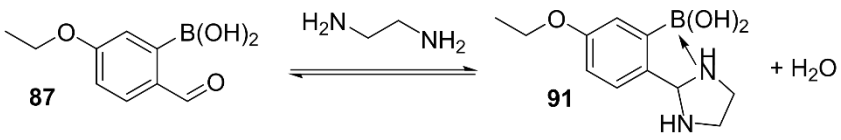
$$K_d(\mathbf{25}) = \frac{[\mathbf{A}][\mathbf{B}]}{[\mathbf{C}]} \qquad K_d(\boldsymbol{X}) = \frac{[\mathbf{D}][\mathbf{B}]}{[\mathbf{E}]}$$

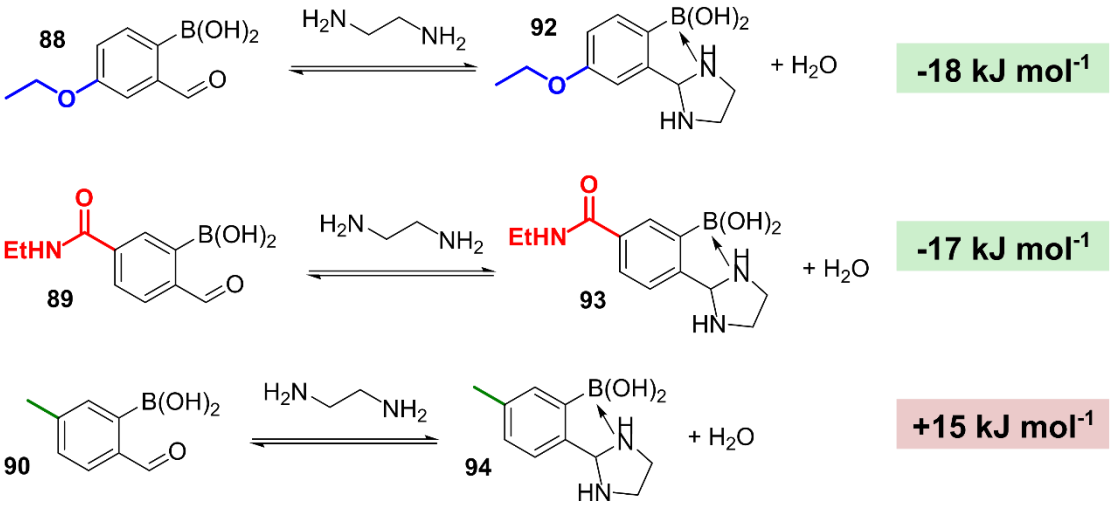
Assuming K_d(**25**) = K_d(**5**), which is known from our FRET studies, these equations can be rearranged to:

$$[B] = \frac{[\mathbf{C}]K_d(\mathbf{5})}{[\mathbf{A}]} \qquad K_d(\boldsymbol{X}) = \frac{[\mathbf{D}][\mathbf{C}]}{[\mathbf{E}][\mathbf{A}]}K_d(\mathbf{5})$$

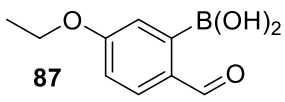
14. DFT

Calculations were performed to identify the relative energies of the imidazolidino-boronates **91**-**95** formed between model substrates **87**-**90** and ethylenediamine. The relative energies of intermediates and transition states were determined using the TURBOMOLE V6.4 package using the resolution of identity (RI) approximation.[12–19] Initial optimisations were performed at the (RI-)BP86/SV(P) level, followed by frequency calculations at the same level. All minima were confirmed as such by the absence of imaginary frequencies. Single-point energies were then performed on the (RI-)BP86/SV(P) optimised geometries using the hybrid PBE0 functional and the flexible def2-TZVPP basis set. Energies, xyz coordinates and the first 50 lines of the vibrational spectra are presented. Solvation effects were modelled using COMSO[20] using the dielectronic constant of 78.2 for water and energies were corrected for dispersion using Grimme's D3-method with Becke-Johnson dampening.[21]





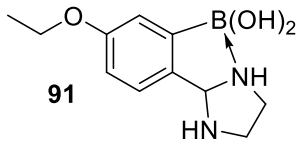
Differences in energy between aldehydes **88**-**90** and imidazolidino-boronates **92**-**94**, relative to the energy difference between aldehyde **87** and imidazolidino-boronate **91**.




```
SCF Energy (au)BP86/SV(P)                          -674.9440225343
SCF Energy (au)PBE0/def2-TZVPP -674.9308469654
SCF Energy (au)PBE0/def2-TZVPP -674.9532019095 (H2O Correction)
Zero Point Energy (au)                             0.1888044
Chemical Potential (kJ mol-1)  386.35
Dispersion Correction (au) PBE0/def2-TZVPP      -0.02324058

xyz coordinates

Energy = -674.9440225343
O     0.1229231    0.2566308    2.2691099
C    -0.3831042    0.2529079    1.0117610
C    -1.7247219    0.6933151    0.8880434
H    -2.2607038    1.0042750    1.7982604
C    -2.3279417    0.7147554   -0.3673593
H    -3.3758318    1.0485868   -0.4677091
C    -1.6123663    0.3084333   -1.5190090
C    -2.2472719    0.3208490   -2.8451379
C    -0.2648622   -0.1323970   -1.4130654
C     0.3340958   -0.1565716   -0.1407737
H     1.3725067   -0.5090888   -0.0449698
C     1.4652611   -0.1905523    2.4973762
C     1.7428751   -0.0918841    3.9895334
H     2.1752183    0.4442802    1.9157179
H     1.5757650   -1.2413014    2.1387234
```

```
B     0.5657355   -0.5979504   -2.6935615
O     1.3233065    0.2628537   -3.4641737
O     0.7193913   -1.9454335   -2.8970460
H     1.2582170   -2.0933026   -3.7054397
H     1.0912610    1.2007071   -3.3093381
H     1.6343672    0.9559426    4.3428952
H     2.7789774   -0.4302677    4.2062575
H     1.0376194   -0.7290728    4.5649349
H    -3.3268260    0.6586063   -2.8730183
O    -1.6738907   -0.0043208   -3.8820117


$vibrational spectrum
#  mode     symmetry     wave number   IR intensity    selection rules
#                         cm**(-1)        km/mol          IR     RAMAN
     1                        0.00         0.00000        -       -
     2                        0.00         0.00000        -       -
     3                        0.00         0.00000        -       -
     4                        0.00         0.00000        -       -
     5                        0.00         0.00000        -       -
     6                        0.00         0.00000        -       -
     7        a              32.60         0.29671       YES     YES
     8        a              59.47         0.14740       YES     YES
     9        a              85.73         1.16228       YES     YES
    10        a             103.79         0.56832       YES     YES
    11        a             116.68         5.39791       YES     YES
    12        a             122.86         3.87764       YES     YES
    13        a             137.78        10.50889       YES     YES
    14        a             213.73         2.31304       YES     YES
    15        a             217.02         3.50938       YES     YES
    16        a             261.80         0.16675       YES     YES
    17        a             280.86         4.72200       YES     YES
    18        a             290.73         2.40361       YES     YES
    19        a             319.04         2.92983       YES     YES
    20        a             359.36         7.26941       YES     YES
    21        a             401.72         1.43017       YES     YES
    22        a             455.23        68.91350       YES     YES
    23        a             466.39        35.73710       YES     YES
    24        a             493.82        13.51565       YES     YES
    25        a             544.15        21.64311       YES     YES
    26        a             554.12        21.30955       YES     YES
    27        a             617.06         8.35809       YES     YES
    28        a             624.34        30.34825       YES     YES
    29        a             632.83        67.02284       YES     YES
    30        a             686.74         5.27673       YES     YES
    31        a             735.21         1.98373       YES     YES
    32        a             811.49        36.01370       YES     YES
    33        a             813.08         0.11214       YES     YES
    34        a             817.54        23.26148       YES     YES
    35        a             867.51        13.75104       YES     YES
    36        a             876.10         4.05614       YES     YES
    37        a             937.92        21.01572       YES     YES
    38        a             943.69         0.37931       YES     YES
    39        a             968.15       134.73842       YES     YES
    40        a             985.95         0.63291       YES     YES
    41        a            1003.86       138.29590       YES     YES
    42        a            1041.32        18.16669       YES     YES
    43        a            1057.69       143.17251       YES     YES
    44        a            1106.83        18.69413       YES     YES
    45        a            1113.53        11.41937       YES     YES
```

46	a	1142.58	3.88275	YES	YES
47	a	1211.72	84.69479	YES	YES
48	a	1244.38	63.71511	YES	YES
49	a	1266.50	2.30874	YES	YES
50	a	1276.66	353.78756	YES	YES



91

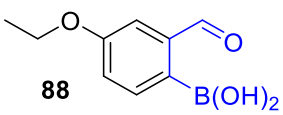
SCF Energy (au)BP86/SV(P) -788.9406342711
SCF Energy (au)PBE0/def2-TZVPP -788.9253276879
SCF Energy (au)PBE0/def2-TZVPP -788.9473947863 (H_2O Correction)
Zero Point Energy (au) 0.2758400
Chemical Potential (kJ mol^{-1}) 603.64
Dispersion Correction (au) PBE0/def2-TZVPP -0.03331502

xyz coordinates
34

O	0.9899580	-0.0449146	3.3529898
C	0.4667609	-0.1027417	2.0925335
C	-0.8984918	0.2339073	1.9683906
H	-1.4525093	0.5217452	2.8763760
C	-1.5185551	0.1976461	0.7144989
H	-2.5810070	0.4666748	0.5975692
C	-0.8066750	-0.1839085	-0.4392565
C	-1.4717173	-0.1617649	-1.8098950
C	0.5640050	-0.5310371	-0.3327214
C	1.1896539	-0.4712039	0.9370550
H	2.2543873	-0.7437827	1.0181176
N	-2.9288865	0.1600810	-1.7913571
N	-0.8367965	0.8554651	-2.6638820
C	-1.8581630	1.1625053	-3.6741741
H	-1.8344296	0.3713715	-4.4588992
H	-1.6531834	2.1381534	-4.1639778
C	2.3556563	-0.4001573	3.5558619
C	2.6573487	-0.2780990	5.0427110
H	3.0187859	0.2752676	2.9611927
H	2.5357294	-1.4436400	3.1994127
B	1.4453406	-0.9639174	-1.5728429
O	2.6967897	-0.4186876	-1.7997094
O	1.0418140	-1.9838088	-2.3996487
H	1.7071870	-2.1138094	-3.1109314
H	2.8643027	0.3329171	-1.1944079
C	-3.1939919	1.1114965	-2.8896169
H	-3.4855202	-0.6961625	-1.8976481
H	-3.4462559	2.1228081	-2.4871024
H	-4.0556896	0.7873835	-3.5156344
H	-0.6665468	1.6941016	-2.0851981
H	-1.3061406	-1.1517411	-2.2998667
H	2.4860790	0.7619611	5.3946299
H	3.7168728	-0.5480603	5.2426811
H	2.0038884	-0.9560485	5.6327499

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
1		0.00	0.00000	-	-
2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a	32.46	1.32549	YES	YES
8	a	40.97	2.68258	YES	YES
9	a	51.66	0.11918	YES	YES
10	a	75.95	0.36585	YES	YES
11	a	95.13	0.28409	YES	YES
12	a	98.26	0.14764	YES	YES
13	a	102.32	0.24753	YES	YES
14	a	128.55	2.21866	YES	YES
15	a	131.79	2.47801	YES	YES
16	a	202.27	0.33551	YES	YES
17	a	213.48	0.64153	YES	YES
18	a	252.74	1.99361	YES	YES
19	a	262.36	0.46050	YES	YES
20	a	281.69	4.01542	YES	YES
21	a	312.62	4.84903	YES	YES
22	a	323.17	3.21589	YES	YES
23	a	390.64	4.09645	YES	YES
24	a	411.73	3.00280	YES	YES
25	a	436.81	0.50164	YES	YES
26	a	493.47	25.43168	YES	YES
27	a	508.77	113.08135	YES	YES
28	a	534.05	10.23991	YES	YES
29	a	545.71	37.10191	YES	YES
30	a	582.58	46.20626	YES	YES
31	a	603.54	29.32661	YES	YES
32	a	613.23	2.51359	YES	YES
33	a	623.82	15.41473	YES	YES
34	a	667.15	58.97105	YES	YES
35	a	690.21	6.87422	YES	YES
36	a	740.82	4.20967	YES	YES
37	a	788.89	12.36679	YES	YES
38	a	806.29	36.88200	YES	YES
39	a	815.21	3.71629	YES	YES
40	a	841.54	12.53565	YES	YES
41	a	854.07	33.43152	YES	YES
42	a	859.27	10.26141	YES	YES
43	a	871.22	11.46102	YES	YES
44	a	892.07	37.04017	YES	YES
45	a	905.67	8.55998	YES	YES
46	a	937.16	21.95969	YES	YES
47	a	945.86	36.11721	YES	YES
48	a	971.76	11.93884	YES	YES
49	a	972.52	78.17454	YES	YES
50	a	984.94	6.11996	YES	YES



88

SCF Energy (au)BP86/SV(P) -674.9406753105

SCF Energy (au)PBE0/def2-TZVPP -674.9275964242
SCF Energy (au)PBE0/def2-TZVPP -674.9498077236 (H_2O Correction)
Zero Point Energy (au) 0.1887753
Chemical Potential (kJ mol^{-1}) 385.49
Dispersion Correction (au) PBE0/def2-TZVPP -0.02309721

xyz coordinates
25

C	0.8692490	-0.2257113	0.7959236
C	-0.4933351	0.1470849	0.7817398
O	-1.2467293	0.3952855	1.8872224
C	-1.1462022	0.2753851	-0.4623468
H	-2.2125705	0.5562666	-0.4742417
C	-0.4484443	0.0409857	-1.6599565
C	-1.1696590	0.1687176	-2.9471273
C	0.9268066	-0.3308772	-1.6705095
C	1.5508787	-0.4593147	-0.4161465
H	2.6117724	-0.7592731	-0.3633153
B	1.7364544	-0.6019414	-3.0137117
O	2.3881120	0.3873014	-3.7246896
O	1.9662966	-1.9047792	-3.3751826
H	2.4899450	-1.9267254	-4.2065726
H	2.1209506	1.2835536	-3.4374479
H	-2.2637207	0.4468256	-2.8688666
O	-0.6509369	-0.0052088	-4.0423814
H	1.4116867	-0.3426130	1.7458226
C	-0.6582688	0.2460222	3.1812092
H	-0.2756138	-0.7962551	3.3033303
C	-1.7256349	0.5569297	4.2200851
H	0.2109718	0.9401257	3.2840675
H	-2.1002831	1.5962815	4.1009766
H	-1.3046897	0.4495908	5.2429648
H	-2.5870355	-0.1376568	4.1191539

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
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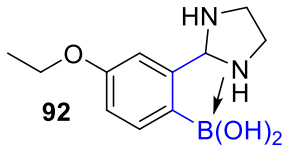
2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a	31.64	0.24155	YES	YES
8	a	43.04	0.40078	YES	YES
9	a	82.54	0.56006	YES	YES
10	a	106.67	0.52903	YES	YES
11	a	112.90	1.19890	YES	YES
12	a	126.18	4.91741	YES	YES
13	a	141.32	8.38718	YES	YES
14	a	205.15	3.52309	YES	YES
15	a	226.48	4.78505	YES	YES
16	a	257.07	3.30743	YES	YES
17	a	258.98	3.85558	YES	YES
18	a	279.68	2.29754	YES	YES
19	a	339.90	5.23091	YES	YES
20	a	379.61	0.90233	YES	YES
21	a	402.91	2.40395	YES	YES

22	a	449.69	11.90479	YES	YES
23	a	467.58	50.36263	YES	YES
24	a	471.87	80.36941	YES	YES
25	a	555.14	28.28682	YES	YES
26	a	561.10	15.75117	YES	YES
27	a	589.64	3.77702	YES	YES
28	a	632.43	33.65381	YES	YES
29	a	669.46	24.59020	YES	YES
30	a	697.17	27.98071	YES	YES
31	a	734.85	1.72734	YES	YES
32	a	772.46	34.88571	YES	YES
33	a	806.35	15.56160	YES	YES
34	a	827.09	5.52891	YES	YES
35	a	874.01	13.17085	YES	YES
36	a	881.59	26.93655	YES	YES
37	a	942.82	1.69313	YES	YES
38	a	962.79	20.66050	YES	YES
39	a	964.17	112.17912	YES	YES
40	a	990.31	0.11234	YES	YES
41	a	1005.40	161.89850	YES	YES
42	a	1045.66	75.63712	YES	YES
43	a	1077.47	29.76333	YES	YES
44	a	1109.99	26.70062	YES	YES
45	a	1129.81	33.18926	YES	YES
46	a	1144.78	4.42337	YES	YES
47	a	1155.94	25.49348	YES	YES
48	a	1240.86	34.94524	YES	YES
49	a	1261.88	0.47023	YES	YES
50	a	1284.18	494.84043	YES	YES



92

SCF Energy (au)BP86/SV(P) -788.9425095482
SCF Energy (au)PBE0/def2-TZVPP -788.9272939722
SCF Energy (au)PBE0/def2-TZVPP -788.9492048317 (H_2O Correction)
Zero Point Energy (au) 0.2759082
Chemical Potential (kJ mol^{-1}) 604.22
Dispersion Correction (au) PBE0/def2-TZVPP -0.03346174

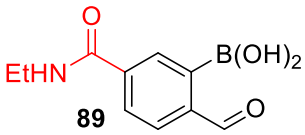
xyz coordinates
34

C	1.6317658	-0.3479865	2.2473479
C	0.2665208	-0.0278803	2.0810459
O	-0.4062145	0.3462255	3.2065425
C	-0.3122739	-0.1072353	0.7965076
H	-1.3660726	0.1413393	0.5999420
C	0.4587230	-0.5164703	-0.3050478
C	-0.1706303	-0.5285419	-1.6957723
C	1.8297725	-0.8568570	-0.1601645
C	2.3873852	-0.7453958	1.1388462
H	3.4505926	-1.0037102	1.2967715
N	-1.6314178	-0.2156127	-1.7264191
N	0.4908565	0.4719760	-2.5510039
C	-0.5133563	0.8045091	-3.5692147

```
H    -0.5042715    0.0154072   -4.3567411
H    -0.2806843    1.7766286   -4.0538044
B     2.7425595   -1.3411831   -1.3517439
O     4.0306815   -0.8647663   -1.5279718
O     2.3240682   -2.3397690   -2.2000745
H     3.0147252   -2.5039665   -2.8792582
H     4.2162361   -0.1236691   -0.9151972
C    -1.8531340    0.7815326   -2.7927318
H    -2.1704221   -1.0710785   -1.9058403
H    -2.0624831    1.7884914   -2.3565248
H    -2.7279886    0.5111897   -3.4248166
H     0.6855723    1.3077032   -1.9766280
H     0.0083855   -1.5313798   -2.1510942
H     2.0721549   -0.2848673    3.2552423
C    -1.7910747    0.6792012    3.1077210
H    -2.3614873   -0.1871967    2.6942842
H    -1.9265405    1.5345195    2.4025335
C    -2.2865812    1.0401763    4.5010088
H    -3.3643880    1.3095001    4.4657631
H    -1.7208149    1.9054300    4.9087122
H    -2.1601641    0.1837363    5.1977803

$vibrational spectrum
#  mode     symmetry     wave number   IR intensity    selection rules
#                         cm**(-1)        km/mol         IR     RAMAN
    1                        0.00         0.00000        -       -
     2                       0.00          0.00000        -       -
     3                       0.00          0.00000        -       -
     4                       0.00          0.00000        -       -
     5                       0.00          0.00000        -       -
     6                       0.00          0.00000        -       -
     7        a              37.95         0.69293       YES     YES
     8        a              38.84         0.46599       YES     YES
     9        a              48.39         2.60176       YES     YES
    10        a              79.45         0.58012       YES     YES
    11        a              93.99         0.23451       YES     YES
    12        a             103.32         0.63507       YES     YES
    13        a             105.62         0.22877       YES     YES
    14        a             116.11         1.73820       YES     YES
    15        a             147.07         1.51618       YES     YES
    16        a             208.88         0.51054       YES     YES
    17        a             236.65         1.98296       YES     YES
    18        a             257.76         1.30882       YES     YES
    19        a             260.10         2.31876       YES     YES
    20        a             263.29         0.51744       YES     YES
    21        a             293.91         1.24063       YES     YES
    22        a             338.92         6.93296       YES     YES
    23        a             381.96         5.02205       YES     YES
    24        a             420.43         6.02983       YES     YES
    25        a             439.96         7.25825       YES     YES
    26        a             500.81         8.47909       YES     YES
    27        a             507.87       121.18499       YES     YES
    28        a             525.93        36.83331       YES     YES
    29        a             553.06        29.36751       YES     YES
    30        a             579.75        14.87910       YES     YES
    31        a             608.79         2.80337       YES     YES
    32        a             625.73        14.53982       YES     YES
    33        a             643.18        16.37811       YES     YES
    34        a             658.54        61.05687       YES     YES
```

35	a	681.57	17.97435	YES	YES
36	a	741.62	6.06715	YES	YES
37	a	771.86	16.26398	YES	YES
38	a	805.38	19.76878	YES	YES
39	a	814.11	3.29819	YES	YES
40	a	822.52	19.82781	YES	YES
41	a	829.72	65.21726	YES	YES
42	a	879.30	2.31600	YES	YES
43	a	895.71	24.60431	YES	YES
44	a	901.86	4.59493	YES	YES
45	a	907.46	27.34382	YES	YES
46	a	929.34	11.72127	YES	YES
47	a	944.29	2.70749	YES	YES
48	a	964.76	105.19476	YES	YES
49	a	980.14	10.17753	YES	YES
50	a	984.42	18.46665	YES	YES



89

SCF Energy (au)BP86/SV(P) -768.3662804744
SCF Energy (au)PBE0/def2-TZVPP -768.3458665976
SCF Energy (au)PBE0/def2-TZVPP -768.3756837592 (H_2O Correction)
Zero Point Energy (au) 0.2106700
Chemical Potential (kJ mol^{-1}) 433.00
Dispersion Correction (au) PBE0/def2-TZVPP -0.02729908

xyz coordinates
28

C	0.0815768	0.0436788	0.1620131
C	-1.2543376	0.4694664	-0.0023776
C	-1.8297388	0.4787211	-1.2795124
H	-2.8712530	0.8192015	-1.4165020
C	-1.0790225	0.0648810	-2.3992879
C	-1.6915726	0.0838783	-3.7472164
C	0.2720443	-0.3604746	-2.2631661
C	0.8292976	-0.3476902	-0.9722863
H	1.8760597	-0.6505322	-0.8046681
B	1.1343219	-0.8500432	-3.5134552
O	1.9893301	-0.0232462	-4.2127994
O	1.1755455	-2.1944022	-3.7709400
H	1.7584923	-2.3662090	-4.5430413
H	1.8803724	0.9198741	-3.9763774
H	-2.7778383	0.3976095	-3.7896502
O	-1.0891597	-0.2076672	-4.7721562
C	0.7912857	0.0306121	1.4995650
N	-0.0181142	-0.1486585	2.5991530
O	2.0140463	0.1634793	1.5761011
C	0.4980592	-0.1741438	3.9621896
H	-1.0054440	-0.3691893	2.4505331
C	-0.0734348	0.9441371	4.8426513
H	1.5995304	-0.0800184	3.8651824
H	0.2876611	-1.1697421	4.4188334
H	0.1796869	1.9445177	4.4294523
H	0.3446522	0.8777397	5.8713307

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H    -1.1817634    0.8752183    4.9263331
H    -1.8402839    0.8290020    0.8600983

$vibrational spectrum
#  mode     symmetry     wave number   IR intensity    selection rules
#                         cm**(-1)        km/mol         IR     RAMAN
     1                        0.00         0.00000        -       -
     2                        0.00         0.00000        -       -
     3                        0.00         0.00000        -       -
     4                        0.00         0.00000        -       -
     5                        0.00         0.00000        -       -
     6                        0.00         0.00000        -       -
     7        a              24.87         0.73453       YES     YES
     8        a              36.10         2.24005       YES     YES
     9        a              47.86         3.70088       YES     YES
    10        a              67.34         1.22314       YES     YES
    11        a              82.65         0.89780       YES     YES
    12        a             101.28         2.24117       YES     YES
    13        a             113.66         3.75005       YES     YES
    14        a             130.81         4.40072       YES     YES
    15        a             163.93         8.95849       YES     YES
    16        a             201.74         0.66020       YES     YES
    17        a             240.55         4.70702       YES     YES
    18        a             258.98         7.88328       YES     YES
    19        a             282.03         5.04865       YES     YES
    20        a             299.91         5.90835       YES     YES
    21        a             313.93        11.23654       YES     YES
    22        a             339.20         9.53129       YES     YES
    23        a             384.21         3.56646       YES     YES
    24        a             446.48         1.20622       YES     YES
    25        a             459.07        83.62511       YES     YES
    26        a             471.70        45.41283       YES     YES
    27        a             496.92        49.46255       YES     YES
    28        a             511.15        62.50418       YES     YES
    29        a             548.62        15.98943       YES     YES
    30        a             560.11         9.20575       YES     YES
    31        a             587.34         6.07136       YES     YES
    32        a             631.28        37.54256       YES     YES
    33        a             673.14        17.65288       YES     YES
    34        a             712.23         8.34670       YES     YES
    35        a             727.61         5.37663       YES     YES
    36        a             757.91         9.14752       YES     YES
    37        a             766.19        26.77140       YES     YES
    38        a             826.67         9.04013       YES     YES
    39        a             831.07        56.65390       YES     YES
    40        a             885.58         9.11821       YES     YES
    41        a             903.16         1.39390       YES     YES
    42        a             936.17         8.12480       YES     YES
    43        a             946.62         0.72619       YES     YES
    44        a             963.95       140.66609       YES     YES
    45        a             990.29         0.08891       YES     YES
    46        a            1002.37       135.59014       YES     YES
    47        a            1044.67         2.05876       YES     YES
    48        a            1062.00        20.47711       YES     YES
    49        a            1082.01         9.56451       YES     YES
    50        a            1123.54         4.40527       YES     YES
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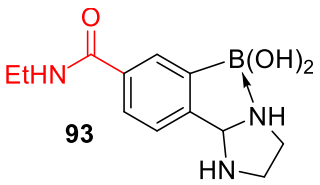

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SCF Energy (au)BP86/SV(P) -882.3692975849
SCF Energy (au)PBE0/def2-TZVPP -882.3468880601
SCF Energy (au)PBE0/def2-TZVPP -882.3757328395 (H_2O Correction)
Zero Point Energy (au) 0.2981772
Chemical Potential (kJ mol^{-1}) 653.54
Dispersion Correction (au) PBE0/def2-TZVPP -0.03751637

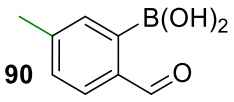
xyz coordinates
37

C	0.1198952	0.8785706	1.2542675
C	-1.0885955	0.2080747	0.9759599
H	-1.9750041	0.3558618	1.6171557
C	-1.1980916	-0.6178452	-0.1544076
H	-2.1397636	-1.1343117	-0.4013854
C	-0.1059423	-0.7994209	-1.0192777
C	-0.2521202	-1.6390043	-2.2828730
C	1.1277891	-0.1379215	-0.7604647
C	1.2021339	0.7077888	0.3652670
H	2.1291059	1.2597408	0.5984070
N	-1.5701461	-2.3215501	-2.4341617
N	-0.0826139	-0.7797205	-3.4684177
C	-0.8108216	-1.4823147	-4.5336593
H	-0.1647862	-2.2964048	-4.9359968
H	-1.0550161	-0.7909063	-5.3676636
B	2.4113694	-0.2958537	-1.6747118
O	3.1737027	0.7901072	-2.0561657
O	2.8670510	-1.5444099	-2.0222533
H	3.6749555	-1.4581833	-2.5751683
H	2.7619573	1.6360566	-1.7825120
C	-2.0457096	-2.0747283	-3.8104243
H	-1.4695759	-3.3276750	-2.2552984
H	-2.8847130	-1.3370189	-3.8138548
H	-2.4298981	-3.0076395	-4.2789514
H	-0.5454925	0.1241911	-3.2800709
H	0.5693856	-2.3949179	-2.2951885
C	0.3235276	1.8122350	2.4232215
N	-0.5257680	1.6256879	3.4937738
O	1.1950966	2.6859690	2.4105236
C	-0.4218421	2.4128428	4.7149741
H	-1.1024710	0.7814055	3.5133983
H	-1.4417422	2.7421954	5.0207096
H	0.1594940	3.3175387	4.4414215
C	0.2651732	1.6673816	5.8676258
H	0.3068949	2.3090475	6.7756886
H	1.3052133	1.3889726	5.5919263
H	-0.2826317	0.7361590	6.1385868

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
1		0.00	0.00000	-	-

2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a	33.23	1.92012	YES	YES
8	a	39.26	0.21237	YES	YES
9	a	44.62	1.48425	YES	YES
10	a	47.53	1.03714	YES	YES
11	a	62.55	5.34562	YES	YES
12	a	79.56	0.96542	YES	YES
13	a	86.40	0.16151	YES	YES
14	a	100.11	1.23173	YES	YES
15	a	118.25	0.79332	YES	YES
16	a	127.65	1.60168	YES	YES
17	a	167.56	2.21721	YES	YES
18	a	210.76	1.05532	YES	YES
19	a	240.80	3.10806	YES	YES
20	a	243.91	2.67756	YES	YES
21	a	272.02	2.30307	YES	YES
22	a	290.54	7.23765	YES	YES
23	a	315.85	0.90137	YES	YES
24	a	325.10	17.16460	YES	YES
25	a	377.42	5.93944	YES	YES
26	a	404.47	2.20545	YES	YES
27	a	432.62	3.28190	YES	YES
28	a	472.68	19.64407	YES	YES
29	a	484.85	4.97184	YES	YES
30	a	512.19	49.18796	YES	YES
31	a	515.62	183.46617	YES	YES
32	a	534.83	13.62593	YES	YES
33	a	563.17	13.53597	YES	YES
34	a	586.91	17.31830	YES	YES
35	a	600.59	34.08771	YES	YES
36	a	609.84	1.53027	YES	YES
37	a	656.05	56.84228	YES	YES
38	a	657.05	19.13621	YES	YES
39	a	717.18	1.23204	YES	YES
40	a	739.49	0.90394	YES	YES
41	a	749.62	17.34563	YES	YES
42	a	778.78	12.71984	YES	YES
43	a	802.54	21.86722	YES	YES
44	a	810.80	25.39209	YES	YES
45	a	848.15	10.94579	YES	YES
46	a	855.33	56.44561	YES	YES
47	a	882.84	0.49062	YES	YES
48	a	891.59	19.53127	YES	YES
49	a	898.51	6.57716	YES	YES
50	a	905.10	10.25409	YES	YES



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SCF Energy (au)BP86/SV(P) -560.4967517160
SCF Energy (au)PBE0/def2-TZVPP -560.4829583873
SCF Energy (au)PBE0/def2-TZVPP -560.5031469908 (H_2O Correction)

Zero Point Energy (au) 0.1562139
Chemical Potential (kJ mol^{-1}) 307.75
Dispersion Correction (au) PBE0/def2-TZVPP -0.02008636

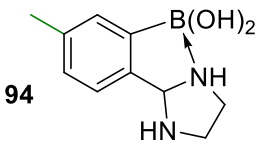
xyz coordinates
21

C	0.7124482	0.1671435	3.1277351
C	0.0769321	0.1724504	1.7565363
C	-1.2561549	0.6003831	1.5729546
H	-1.8525741	0.9166407	2.4456734
C	-1.8248592	0.6174581	0.2933383
H	-2.8708807	0.9439486	0.1552711
C	-1.0701973	0.2108811	-0.8266783
C	-1.6747167	0.2225142	-2.1737977
C	0.2753983	-0.2272526	-0.6784133
C	0.8156764	-0.2383285	0.6198758
H	1.8525537	-0.5891937	0.7694565
B	1.1527588	-0.7100252	-1.9190795
O	1.9354869	0.1371922	-2.6781784
O	1.2998109	-2.0599849	-2.1060684
H	1.8737796	-2.2220887	-2.8871601
H	1.7164661	1.0792363	-2.5304242
H	-2.7546499	0.5570557	-2.2246473
O	-1.0767488	-0.0967469	-3.1951920
H	1.3435907	1.0741882	3.2773115
H	1.3761470	-0.7140295	3.2658553
H	-0.0502671	0.1585581	3.9356313

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
1		0.00	0.00000	-	-
2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a	33.78	0.26919	YES	YES
8	a	55.15	0.38611	YES	YES
9	a	86.50	2.44503	YES	YES
10	a	108.26	1.38162	YES	YES
11	a	127.47	8.19956	YES	YES
12	a	182.64	5.40546	YES	YES
13	a	197.50	8.01195	YES	YES
14	a	271.87	0.90310	YES	YES
15	a	288.02	7.23366	YES	YES
16	a	346.83	0.50713	YES	YES
17	a	353.06	4.14536	YES	YES
18	a	412.58	4.98028	YES	YES
19	a	449.17	16.06998	YES	YES
20	a	463.19	104.39600	YES	YES
21	a	534.46	32.98871	YES	YES
22	a	552.02	15.21097	YES	YES
23	a	581.27	6.67493	YES	YES
24	a	612.42	8.25378	YES	YES
25	a	634.42	59.27584	YES	YES
26	a	689.77	5.43340	YES	YES
27	a	743.64	1.12778	YES	YES

28	a	790.94	45.77967	YES	YES
29	a	807.00	19.23278	YES	YES
30	a	891.04	11.51409	YES	YES
31	a	901.19	1.97278	YES	YES
32	a	946.60	0.77056	YES	YES
33	a	964.52	129.20301	YES	YES
34	a	986.18	13.29851	YES	YES
35	a	990.04	0.40928	YES	YES
36	a	1004.46	130.90090	YES	YES
37	a	1025.59	12.33435	YES	YES
38	a	1065.49	35.77171	YES	YES
39	a	1126.65	8.08611	YES	YES
40	a	1207.27	44.74838	YES	YES
41	a	1210.43	34.83124	YES	YES
42	a	1254.05	27.96557	YES	YES
43	a	1330.27	171.49387	YES	YES
44	a	1351.68	88.75970	YES	YES
45	a	1362.08	15.12464	YES	YES
46	a	1375.55	2.46360	YES	YES
47	a	1394.99	30.06201	YES	YES
48	a	1401.08	285.33565	YES	YES
49	a	1426.04	7.40076	YES	YES
50	a	1435.19	4.52209	YES	YES



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SCF Energy (au)BP86/SV(P) -674.4962885969
SCF Energy (au)PBE0/def2-TZVPP -674.4801110801
SCF Energy (au)PBE0/def2-TZVPP -674.4998723066 (H_2O Correction)
Zero Point Energy (au) 0.2433506
Chemical Potential (kJ mol^{-1}) 525.27
Dispersion Correction (au) PBE0/def2-TZVPP -0.03018458

xyz coordinates
30

C	0.9615971	-0.1561163	2.7473288
C	-0.4085181	0.1444622	2.6102510
H	-0.9937709	0.4318756	3.5015820
C	-1.0390199	0.0894534	1.3564603
H	-2.1070343	0.3353991	1.2390795
C	-0.3207677	-0.2774020	0.2062477
C	-0.9878706	-0.2716974	-1.1645320
C	1.0613721	-0.5931271	0.3076675
C	1.6721986	-0.5090029	1.5797057
H	2.7482331	-0.7482663	1.6753500
N	-2.4488151	0.0332180	-1.1507084
N	-0.3557693	0.7462093	-2.0223312
C	-1.3872611	1.0751530	-3.0153648
H	-1.3856417	0.2926450	-3.8092351
H	-1.1770939	2.0539622	-3.4967019
B	1.9467905	-1.0179769	-0.9322428
O	3.1889318	-0.4565013	-1.1674352
O	1.5542112	-2.0482050	-1.7520142

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H     2.2204495   -2.1754228   -2.4629407
H     3.3558295    0.2930965   -0.5596097
C    -2.7086486    1.0299855   -2.2084708
H    -2.9910716   -0.8239167   -1.3102878
H    -2.9236182    2.0327217   -1.7649889
H    -3.5928104    0.7497969   -2.8233841
H    -0.1660244    1.5790662   -1.4417190
H    -0.8138082   -1.2653397   -1.6433218
C     1.6391251   -0.1348581    4.1005527
H     1.1996457    0.6386337    4.7677941
H     2.7287778    0.0666656    4.0110273
H     1.5303821   -1.1145110    4.6222420

$vibrational spectrum
#  mode     symmetry     wave number   IR intensity    selection rules
#                         cm**(-1)        km/mol          IR     RAMAN
     1                        0.00         0.00000        -       -
     2                        0.00         0.00000        -       -
     3                        0.00         0.00000        -       -
     4                        0.00         0.00000        -       -
     5                        0.00         0.00000        -       -
     6                        0.00         0.00000        -       -
     7        a              37.19         1.07755       YES     YES
     8        a              42.72         1.92848       YES     YES
     9        a              45.10         1.04826       YES     YES
    10        a              78.11         0.37988       YES     YES
    11        a              85.88         0.18659       YES     YES
    12        a             114.96         0.90027       YES     YES
    13        a             118.33         1.35459       YES     YES
    14        a             165.56         0.49163       YES     YES
    15        a             210.24         1.45287       YES     YES
    16        a             264.41         1.63196       YES     YES
    17        a             298.33         9.64356       YES     YES
    18        a             312.15         2.07755       YES     YES
    19        a             321.25         3.53982       YES     YES
    20        a             349.83         1.60179       YES     YES
    21        a             410.33         2.89567       YES     YES
    22        a             481.43        12.55252       YES     YES
    23        a             499.07        83.02366       YES     YES
    24        a             520.16        67.73934       YES     YES
    25        a             539.77        21.57590       YES     YES
    26        a             581.96        21.28512       YES     YES
    27        a             588.36        20.64614       YES     YES
    28        a             592.14        25.51628       YES     YES
    29        a             609.98         1.93984       YES     YES
    30        a             659.85        72.13826       YES     YES
    31        a             690.41         2.07992       YES     YES
    32        a             744.81         5.57087       YES     YES
    33        a             788.48        20.19874       YES     YES
    34        a             800.94        29.39020       YES     YES
    35        a             837.46         9.88888       YES     YES
    36        a             852.03        45.88054       YES     YES
    37        a             875.17         8.16490       YES     YES
    38        a             891.16        25.43808       YES     YES
    39        a             897.36         3.00083       YES     YES
    40        a             904.60         7.74601       YES     YES
    41        a             944.41        19.71977       YES     YES
    42        a             967.99        86.85103       YES     YES
    43        a             972.36         0.71430       YES     YES
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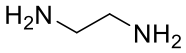
44	a	984.13	23.87868	YES	YES
45	a	985.59	9.21871	YES	YES
46	a	1010.34	119.89112	YES	YES
47	a	1028.34	10.58650	YES	YES
48	a	1042.10	64.72899	YES	YES
49	a	1071.27	19.34589	YES	YES
50	a	1083.57	11.05166	YES	YES



SCF Energy (au)BP86/SV(P) -190.3560477141
SCF Energy (au)PBE0/def2-TZVPP -190.3692189326
SCF Energy (au)PBE0/def2-TZVPP -190.3826064451 (H_2O Correction)
Zero Point Energy (au) 0.1067987
Chemical Potential (kJ mol^{-1}) 207.67
Dispersion Correction (au) PBE0/def2-TZVPP -0.00604782

xyz coordinates
12

N	-1.1330603	1.2252625	0.8145257
C	0.1691229	-0.8078177	0.2452857
H	-0.7747596	-1.3939372	0.1383079
N	1.1992336	-1.4579361	-0.5637413
C	-0.1182786	0.6709942	-0.0824277
H	-2.0474290	0.7819880	0.6331013
H	0.8223445	1.2525489	0.0623127
H	-0.3629794	0.7448803	-1.1809071
H	-1.2610430	2.2327847	0.6398534
H	0.4530204	-0.8811665	1.3189967
H	2.0984138	-0.9624036	-0.4600138
H	0.9554147	-1.4051973	-1.5652936

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
1		0.00	0.00000	-	-
2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a	143.62	1.42402	YES	YES
8	a	238.53	21.17081	YES	YES
9	a	284.76	9.86618	YES	YES
10	a	321.97	65.34488	YES	YES
11	a	459.49	17.85979	YES	YES
12	a	770.49	11.50482	YES	YES
13	a	803.12	200.13669	YES	YES
14	a	822.11	128.91349	YES	YES
15	a	945.89	19.96233	YES	YES
16	a	990.00	18.01493	YES	YES
17	a	1074.81	15.70973	YES	YES
18	a	1092.00	4.86136	YES	YES
19	a	1122.72	2.30425	YES	YES
20	a	1233.03	8.03994	YES	YES
21	a	1280.34	10.26362	YES	YES

22	a	1322.06	7.30029	YES	YES
23	a	1348.02	2.15091	YES	YES
24	a	1383.18	3.55631	YES	YES
25	a	1428.85	2.41996	YES	YES
26	a	1453.69	1.96652	YES	YES
27	a	1607.04	22.89082	YES	YES
28	a	1614.36	20.10656	YES	YES
29	a	2796.50	130.04398	YES	YES
30	a	2930.46	29.66559	YES	YES
31	a	2954.77	47.09360	YES	YES
32	a	2999.09	38.15234	YES	YES
33	a	3319.14	2.94822	YES	YES
34	a	3325.98	2.44491	YES	YES
35	a	3402.76	0.32029	YES	YES
36	a	3410.43	0.17064	YES	YES

SCF Energy (au)BP86/SV(P) -76.34519822147
SCF Energy (au)PBE0/def2-TZVPP -76.379978209
SCF Energy (au)PBE0/def2-TZVPP -76.3919088408 (H_2O Correction)
Zero Point Energy (au) 0.0199820
Chemical Potential (kJ mol^{-1}) 5.89
Dispersion Correction (au) PBE0/def2-TZVPP -0.00027693

xyz coordinates
3

O	0.0000000	0.0000000	0.4047790
H	-0.7707436	0.0000000	-0.2023895
H	0.7707436	0.0000000	-0.2023895

$vibrational spectrum

# mode	symmetry	wave number cm**(-1)	IR intensity km/mol	selection rules IR	RAMAN
1		0.00	0.00000	-	-
2		0.00	0.00000	-	-
3		0.00	0.00000	-	-
4		0.00	0.00000	-	-
5		0.00	0.00000	-	-
6		0.00	0.00000	-	-
7	a1	1604.11	62.01507	YES	YES
8	a1	3526.34	0.10597	YES	YES
9	b1	3640.63	16.46475	YES	YES

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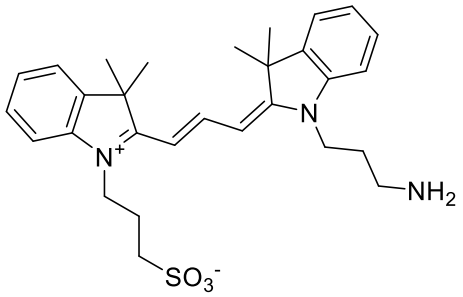

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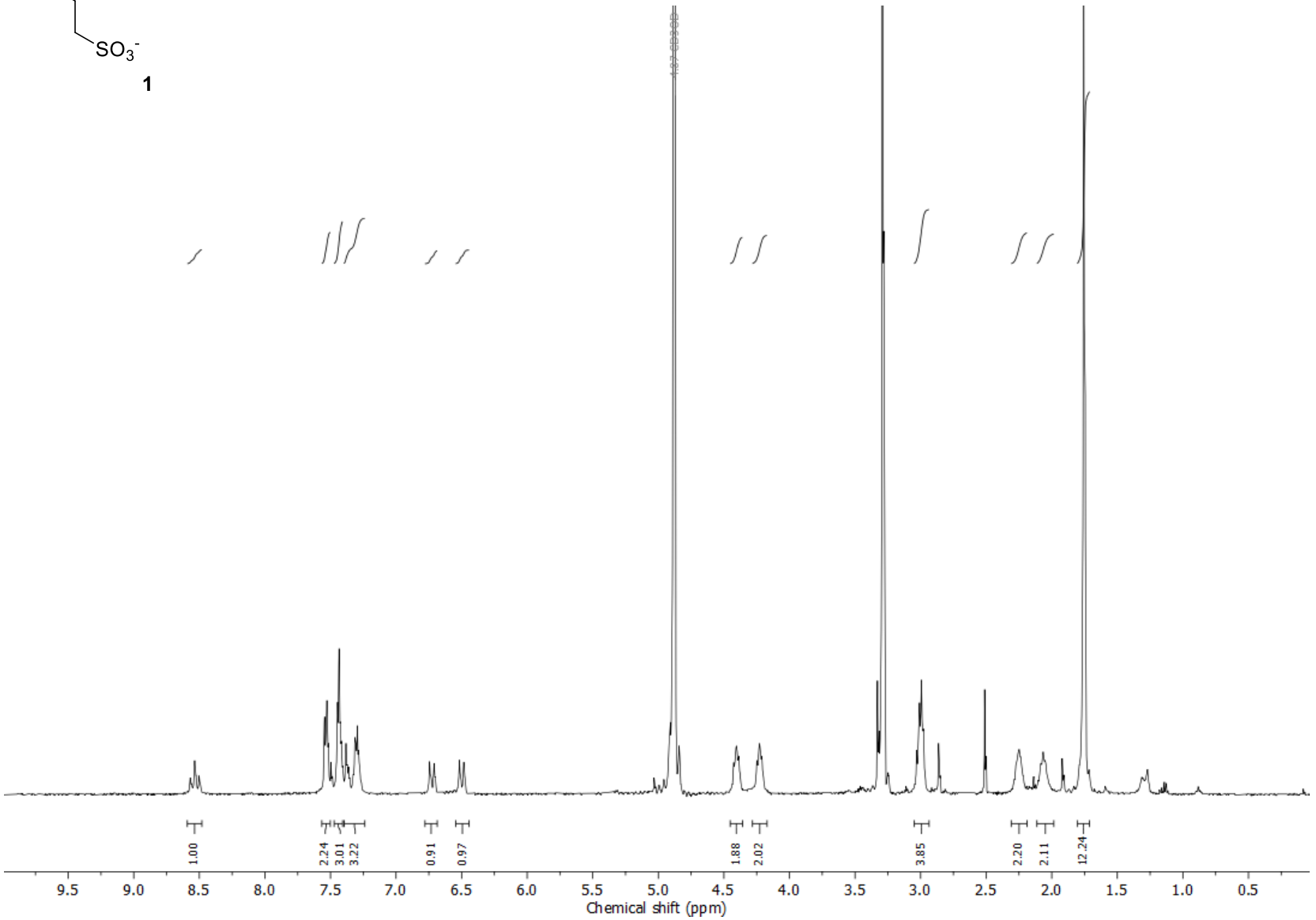

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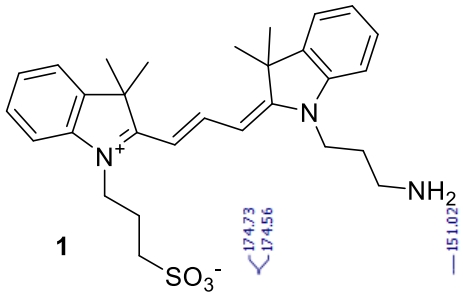

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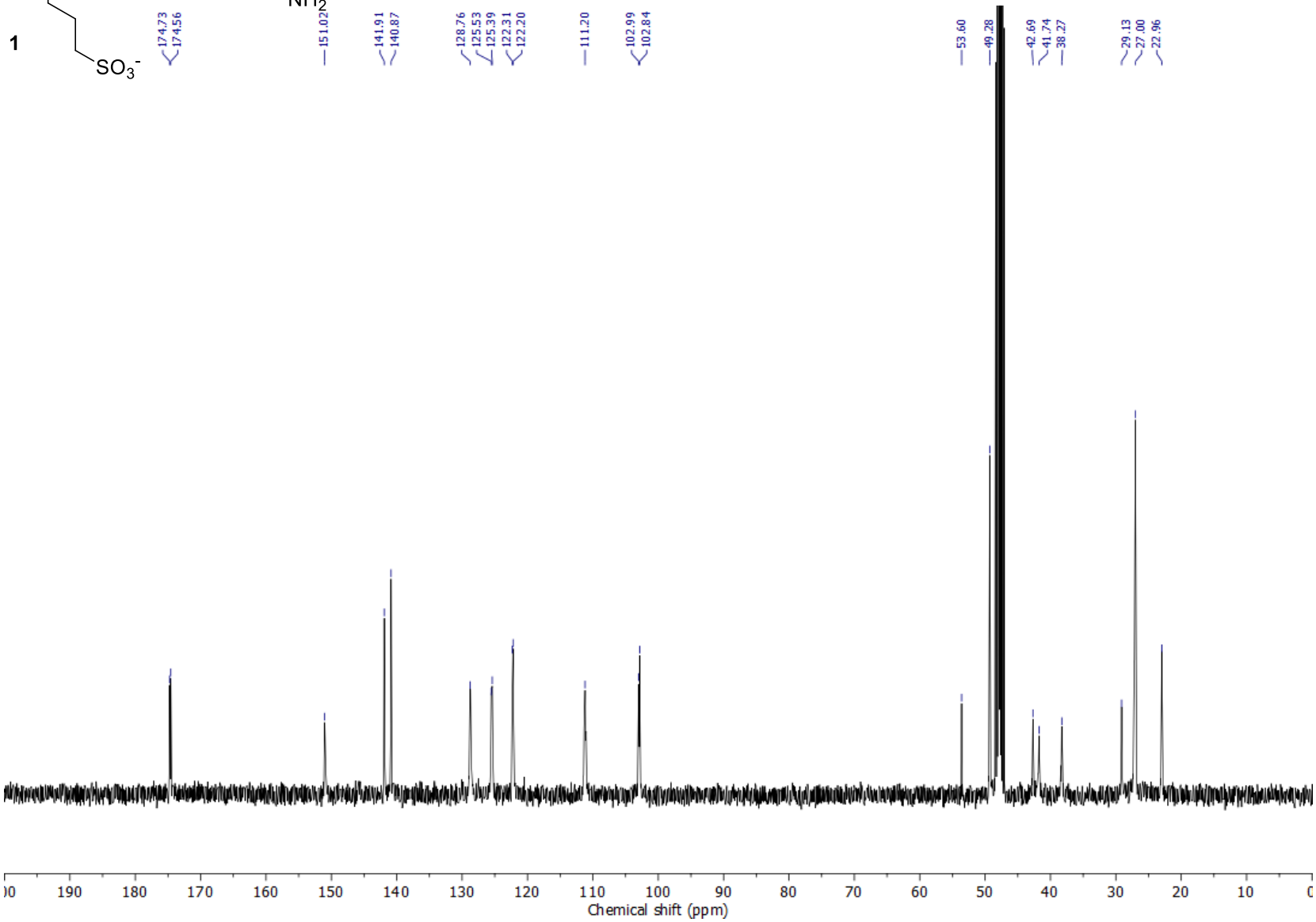

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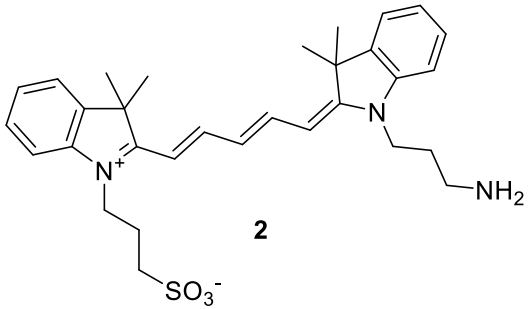

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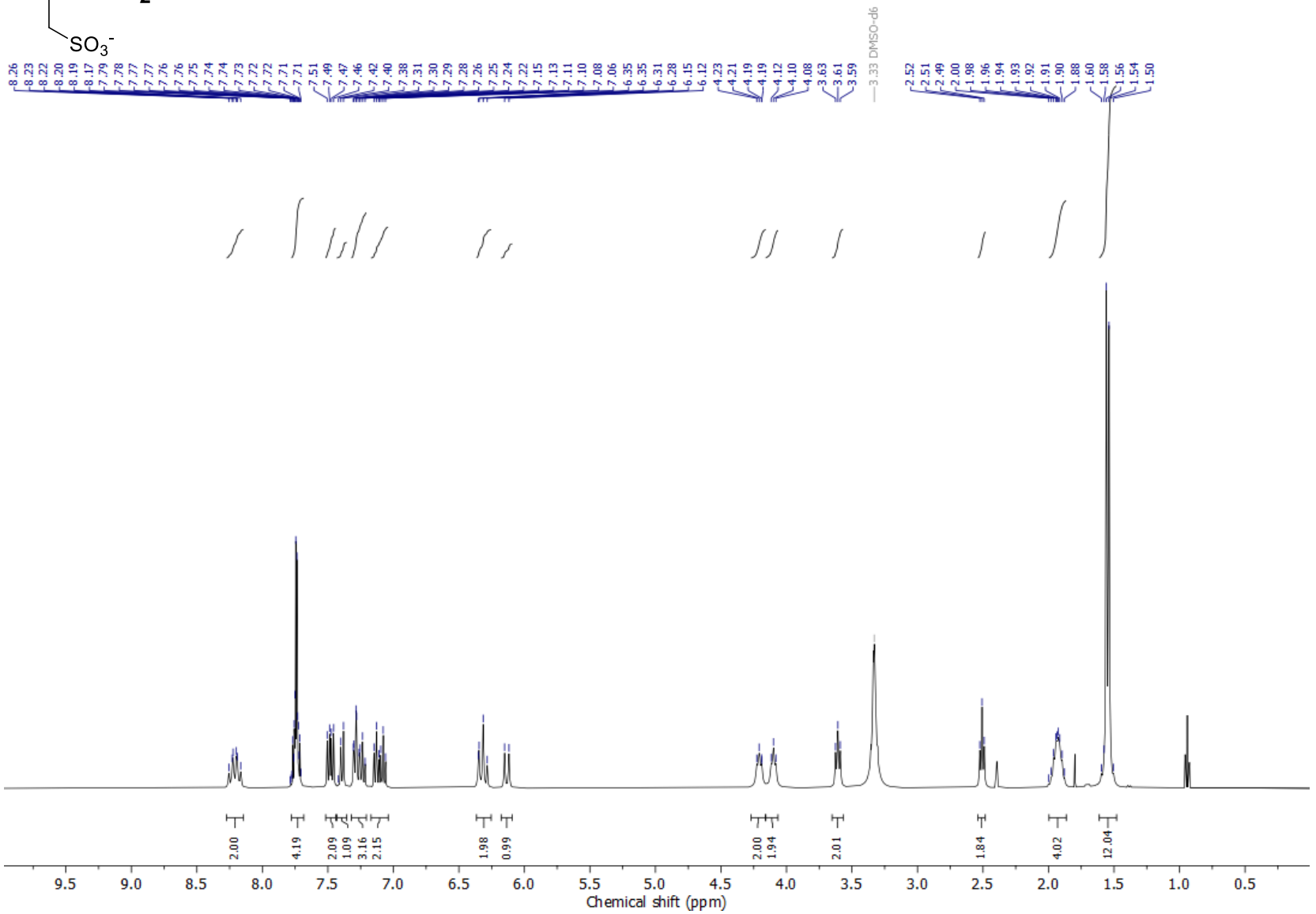

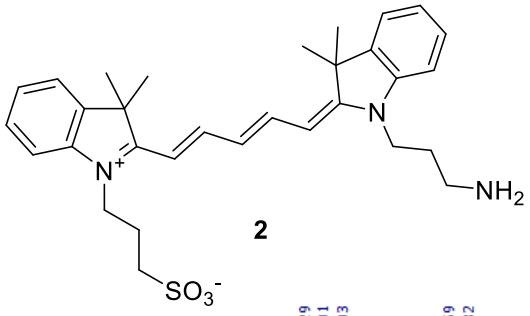

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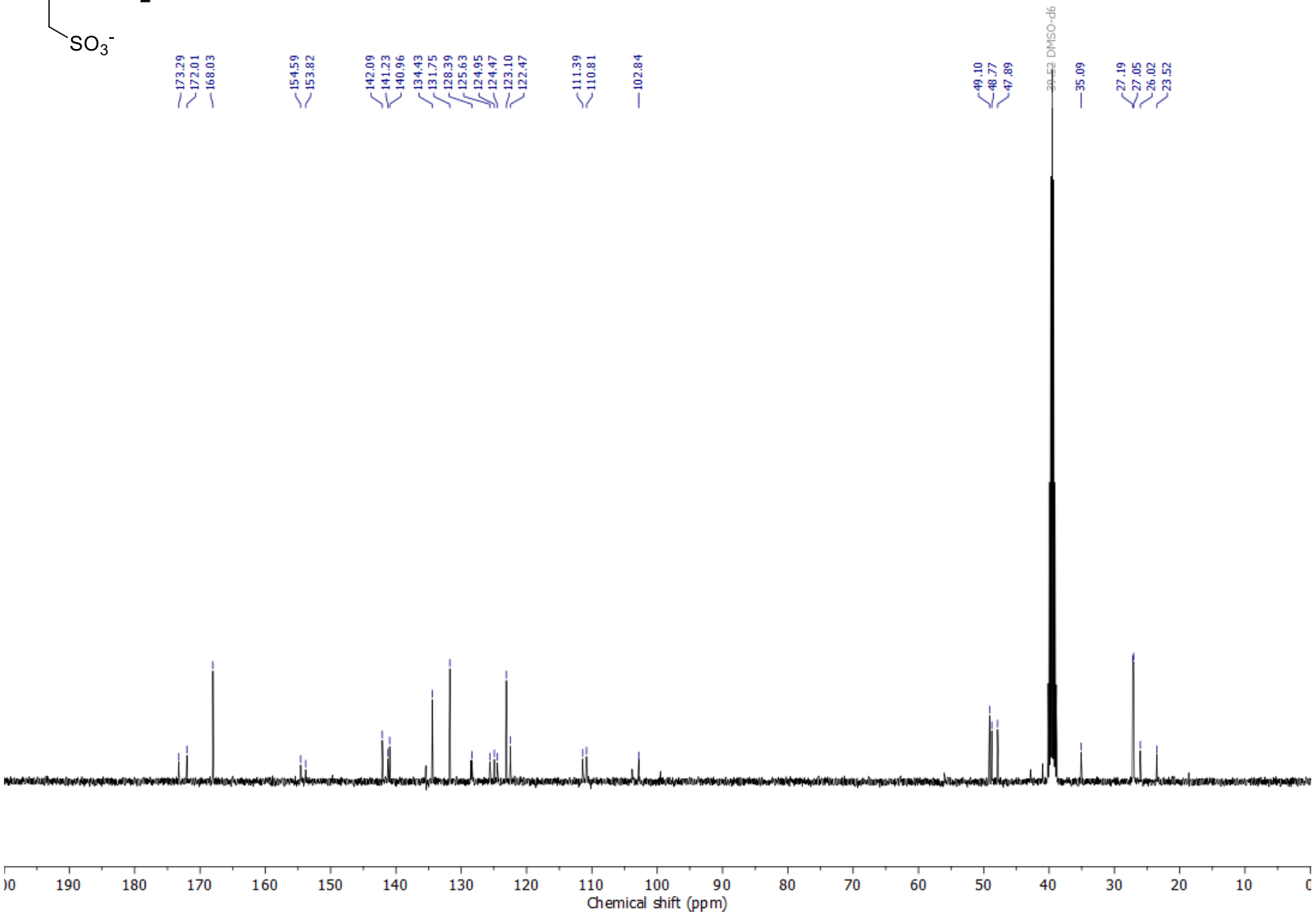

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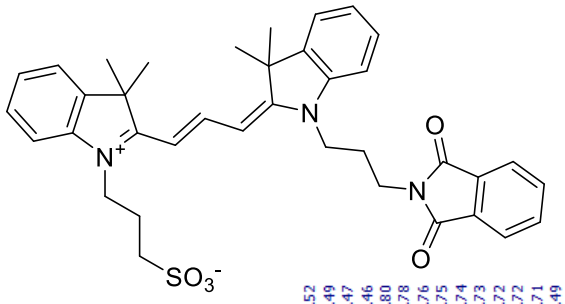

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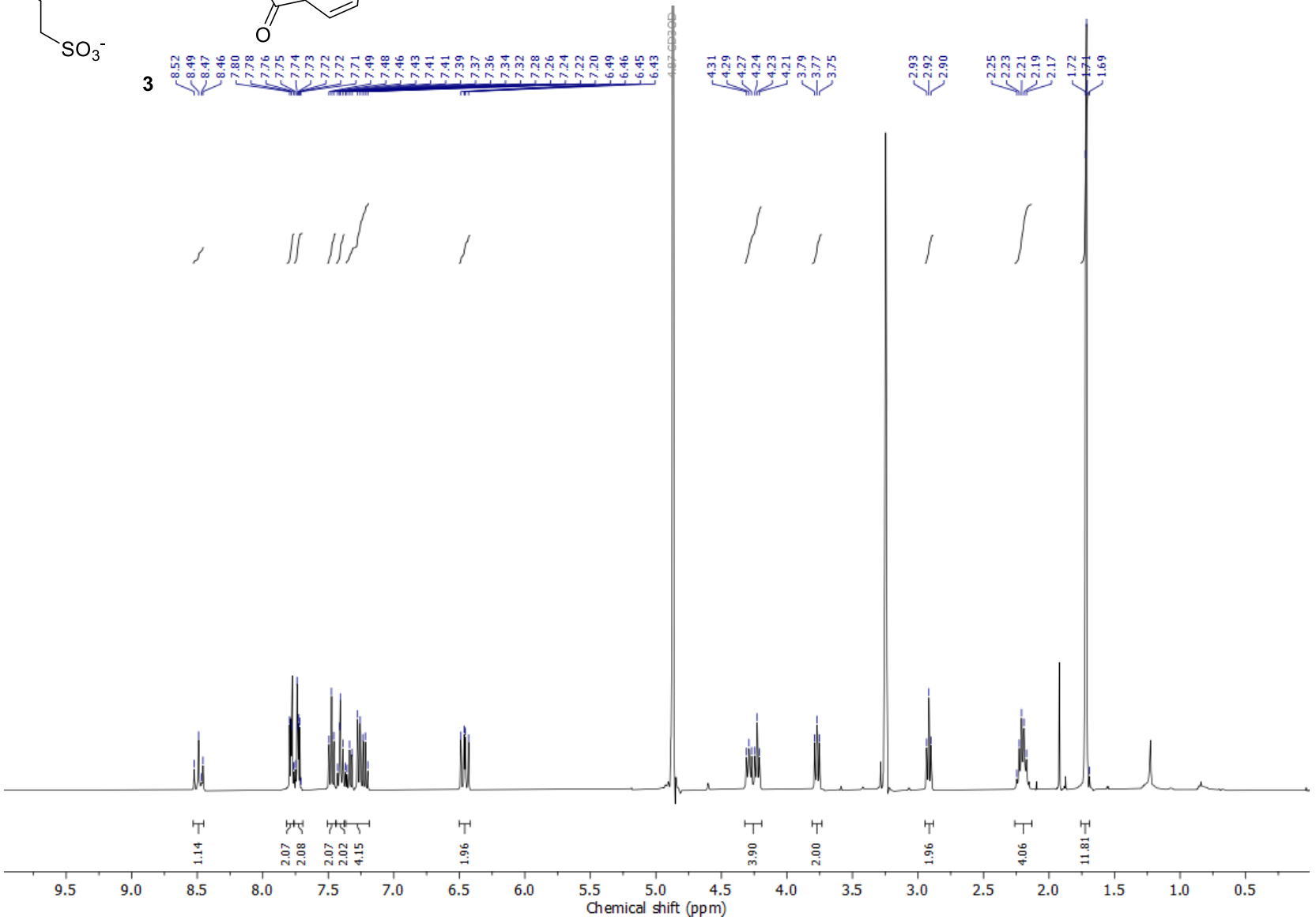

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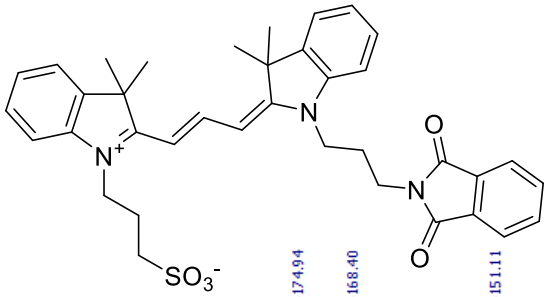

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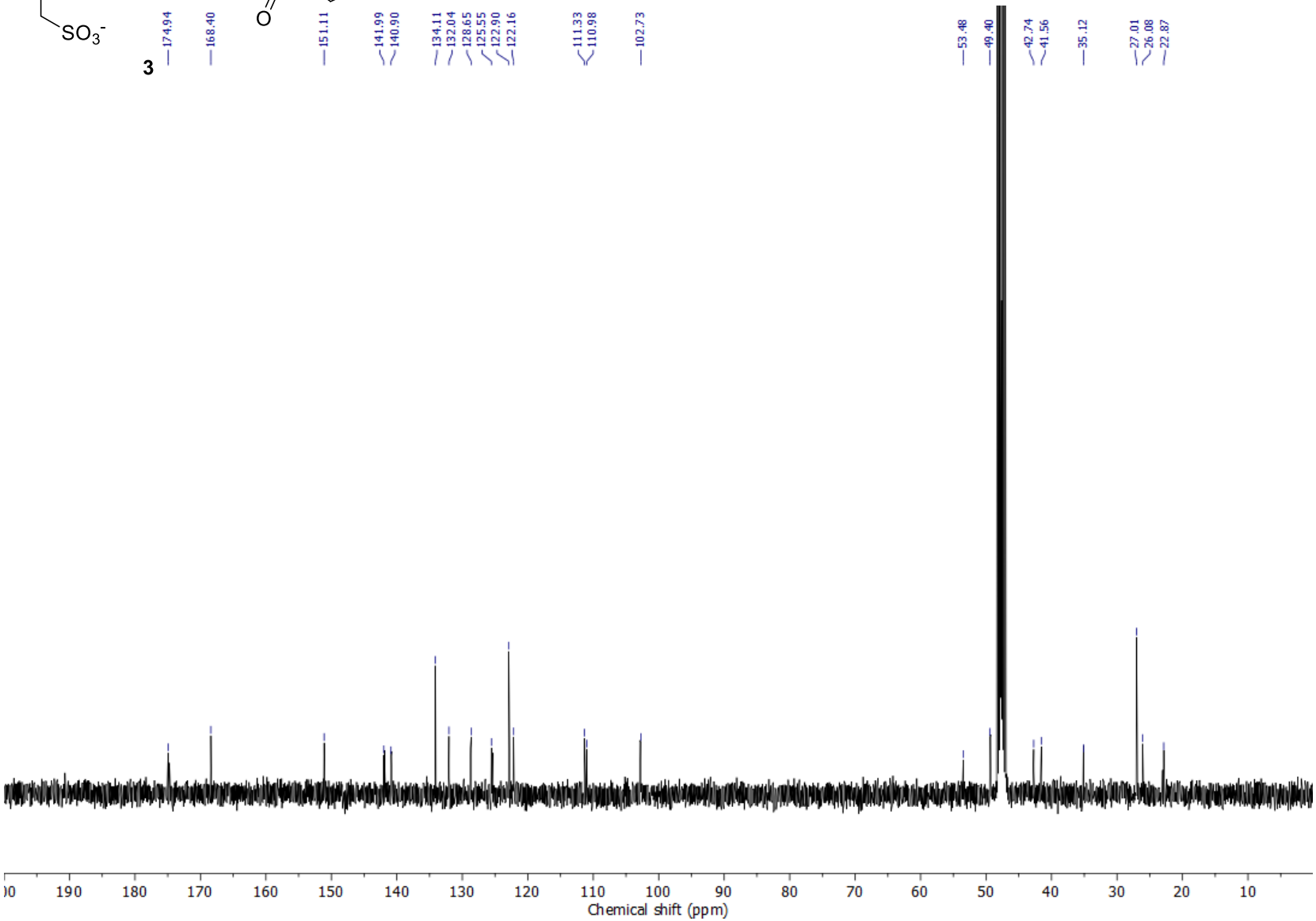

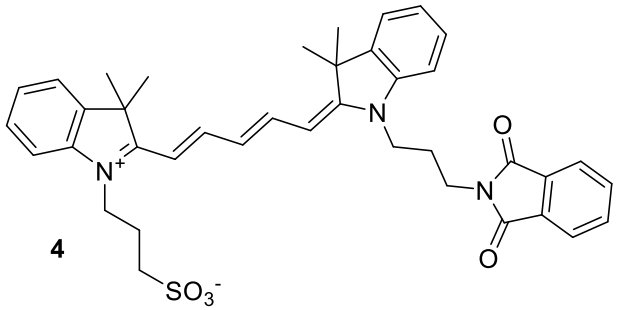

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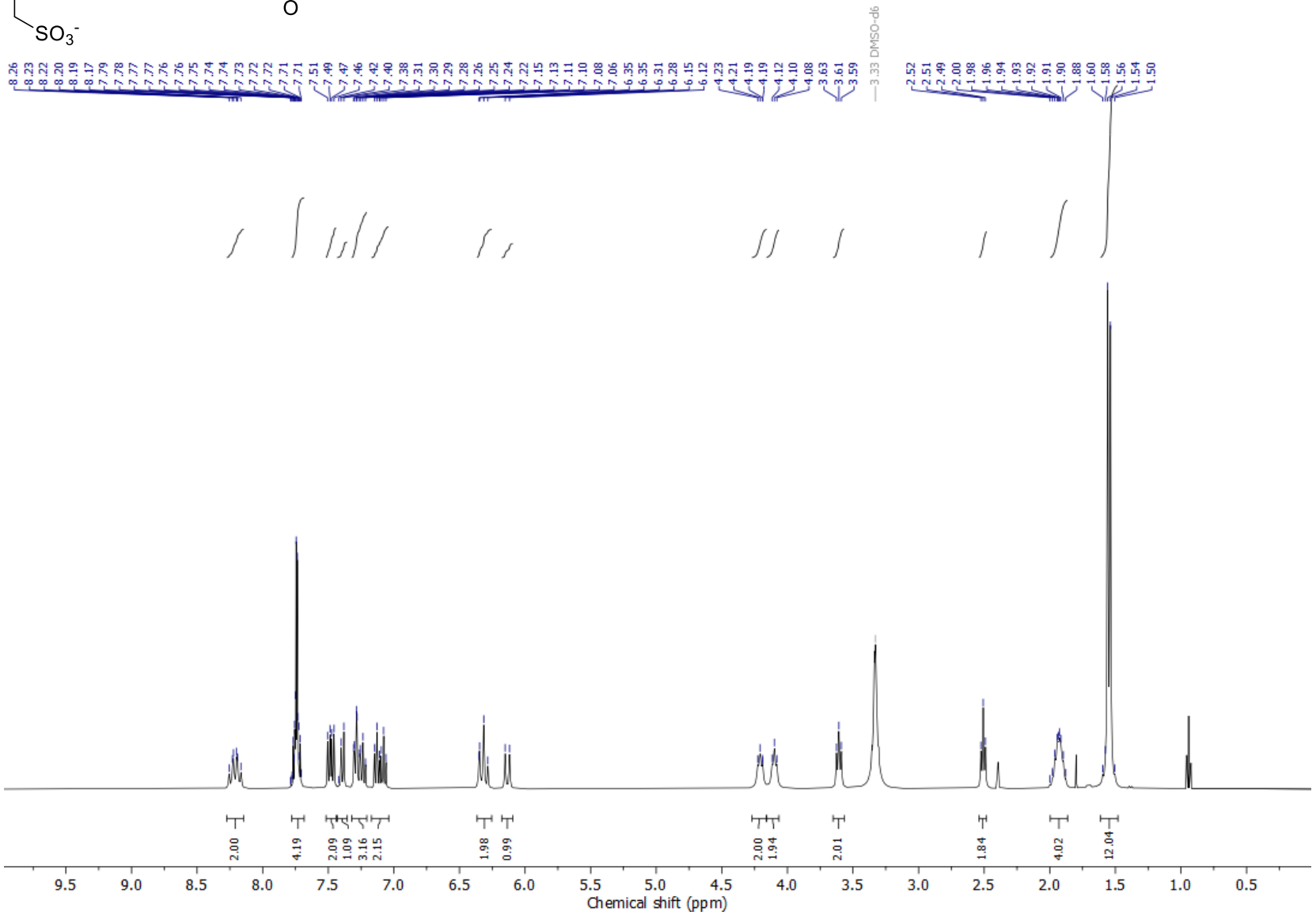

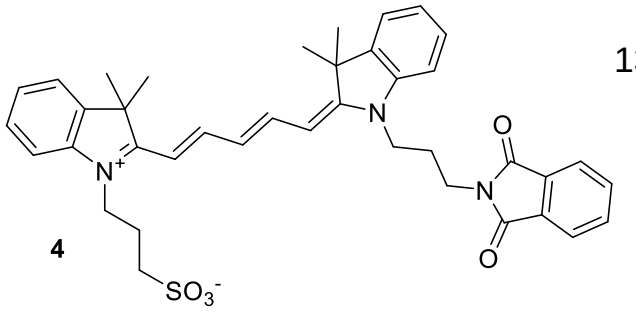

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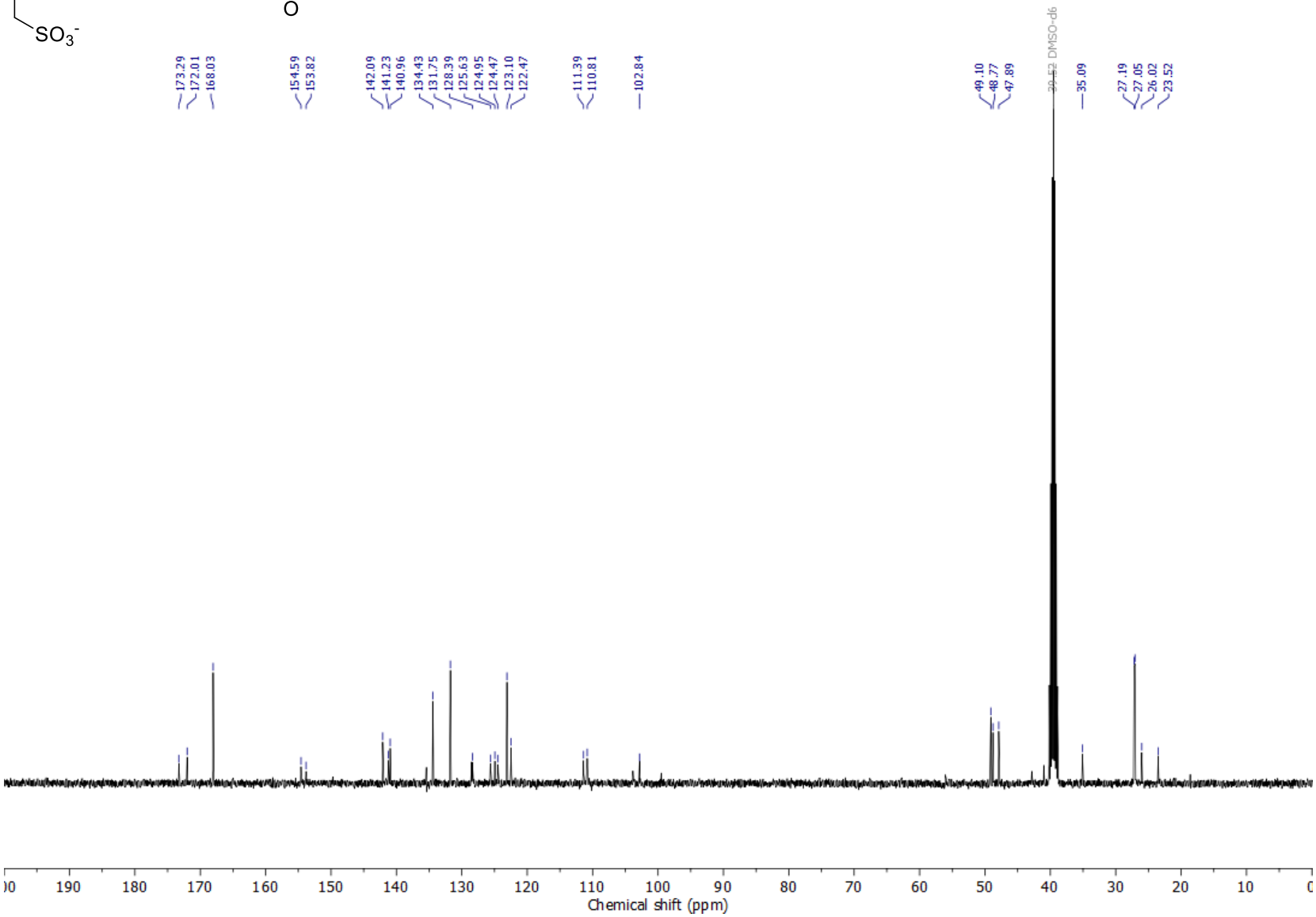

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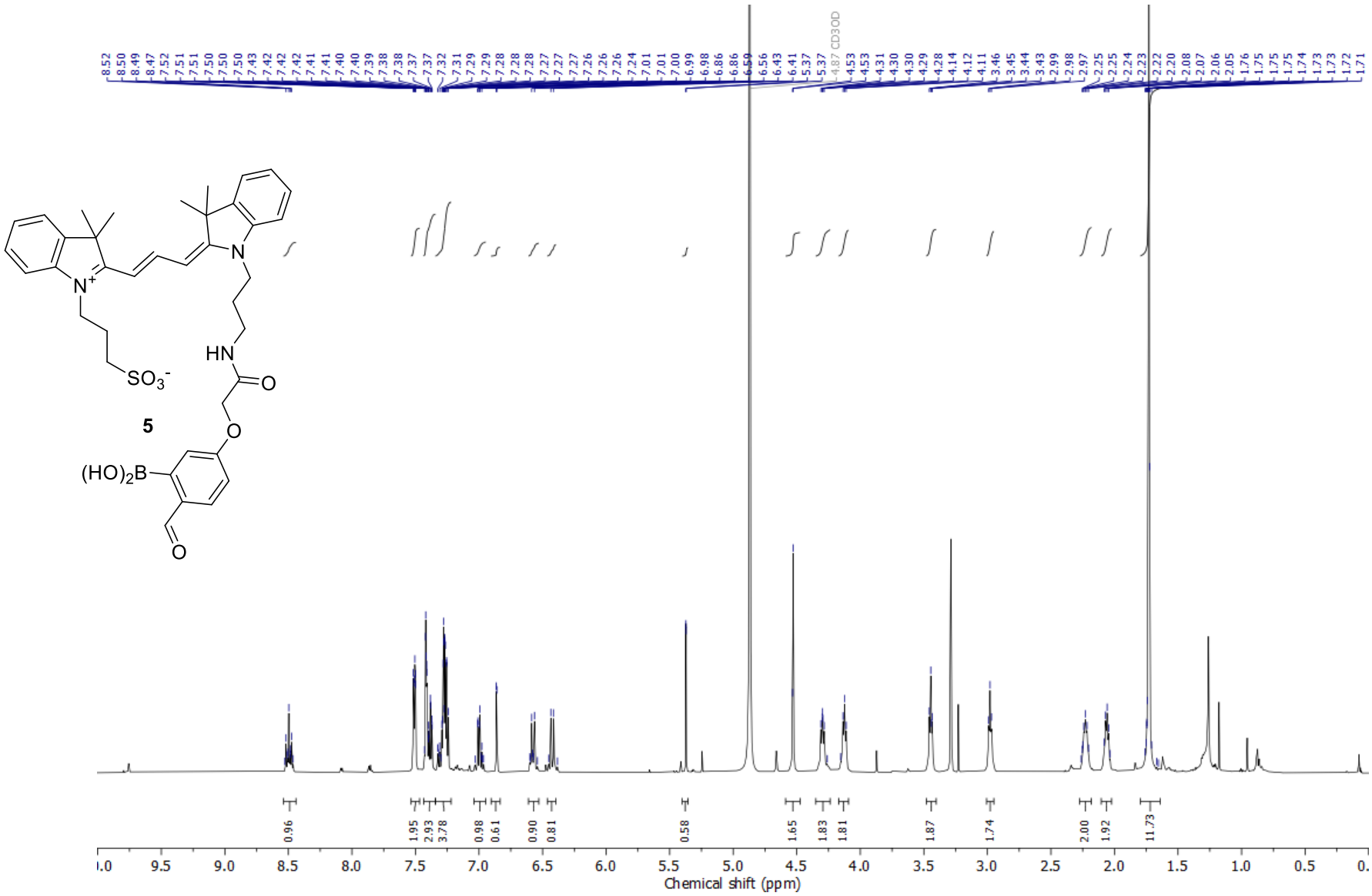

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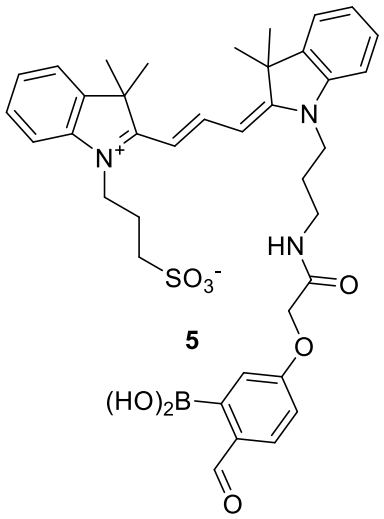

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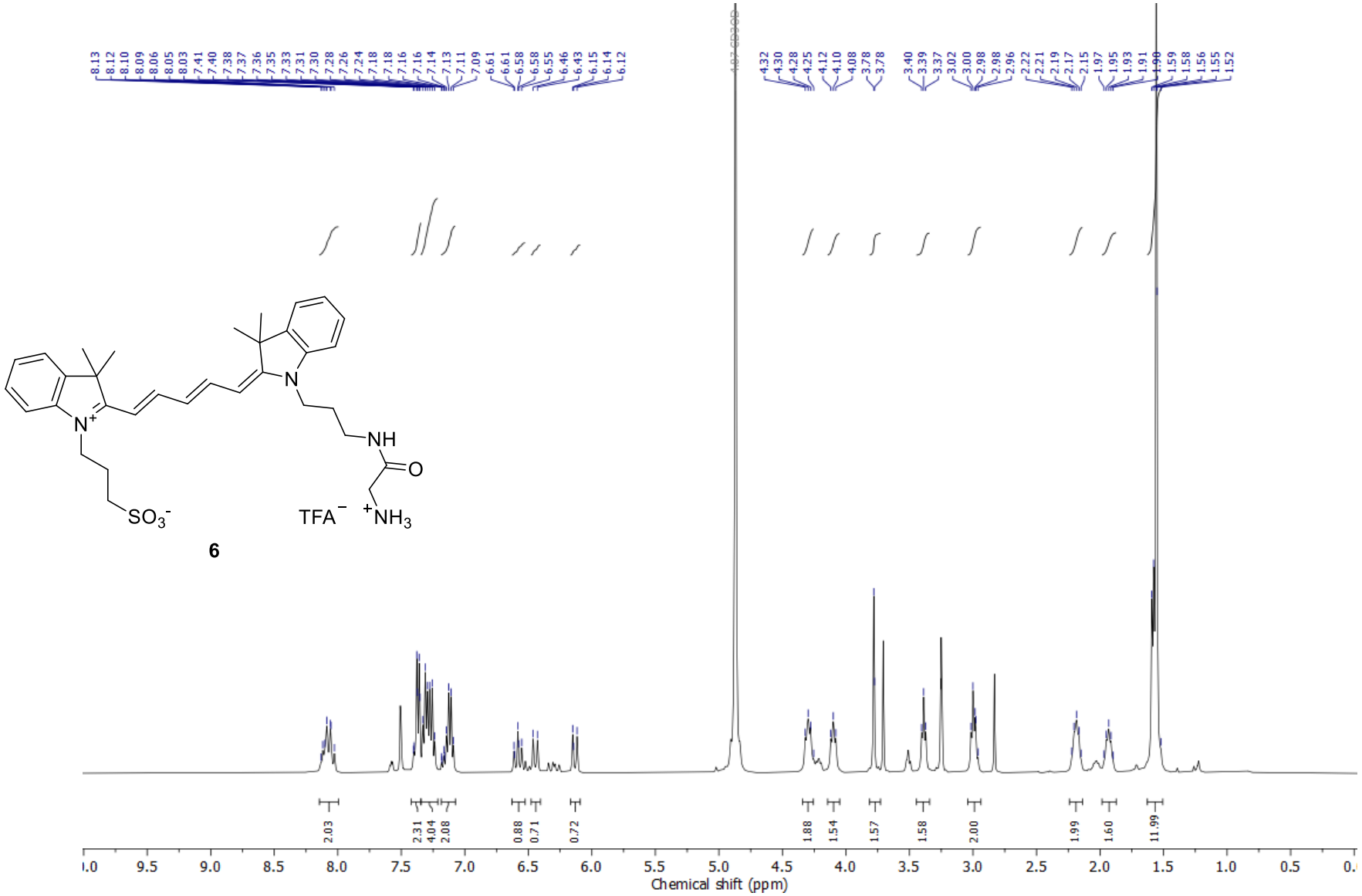

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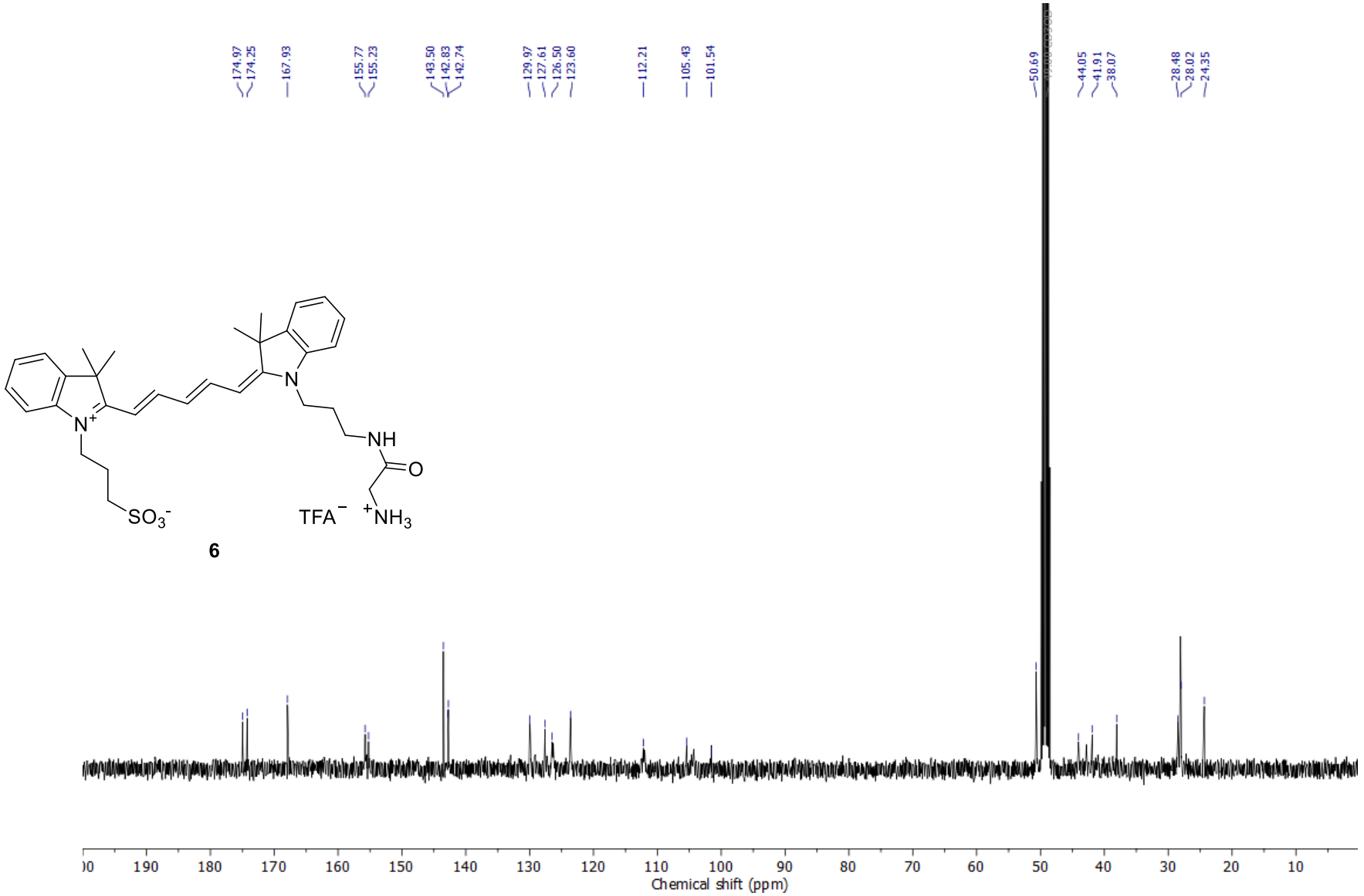

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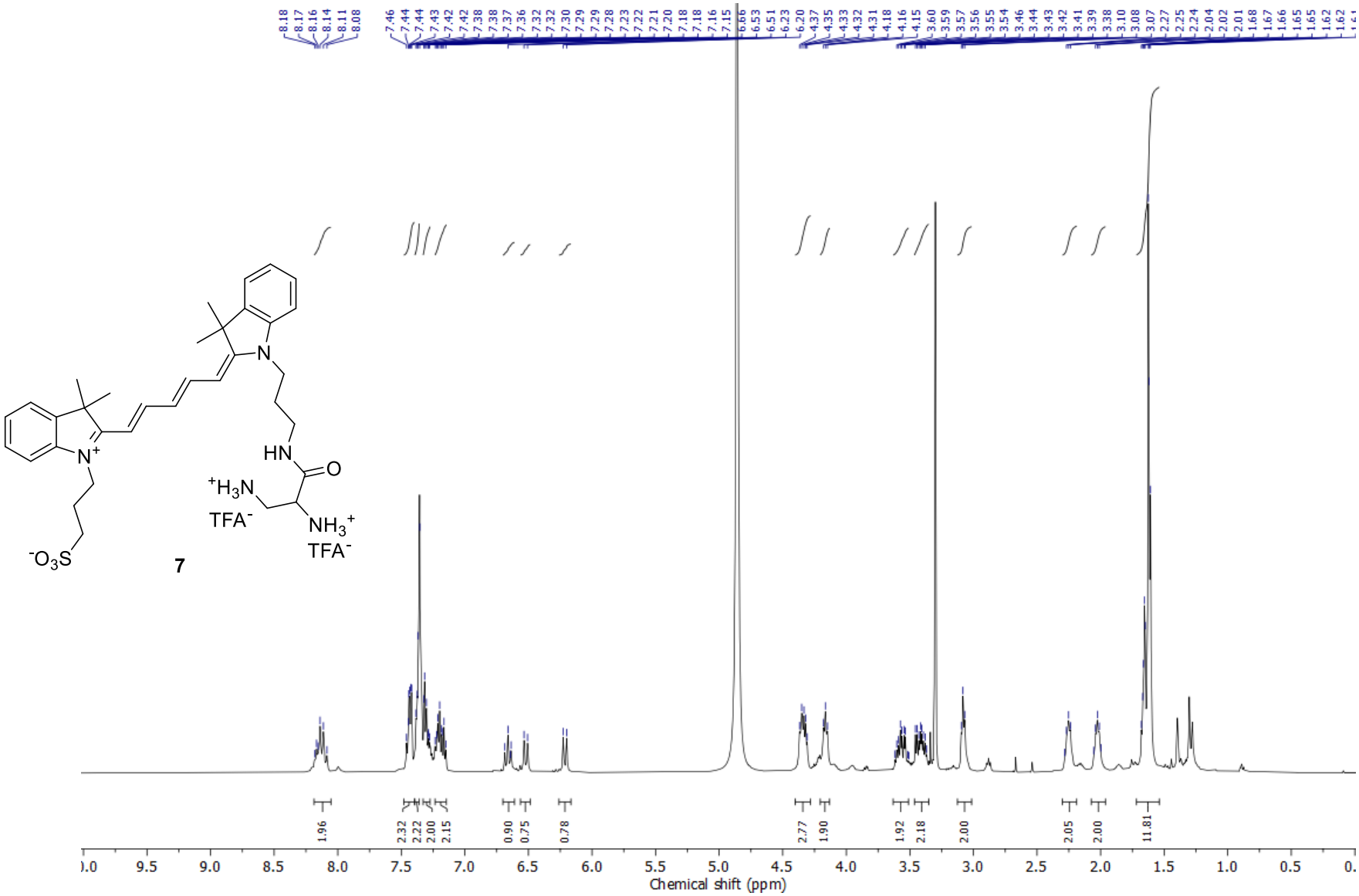

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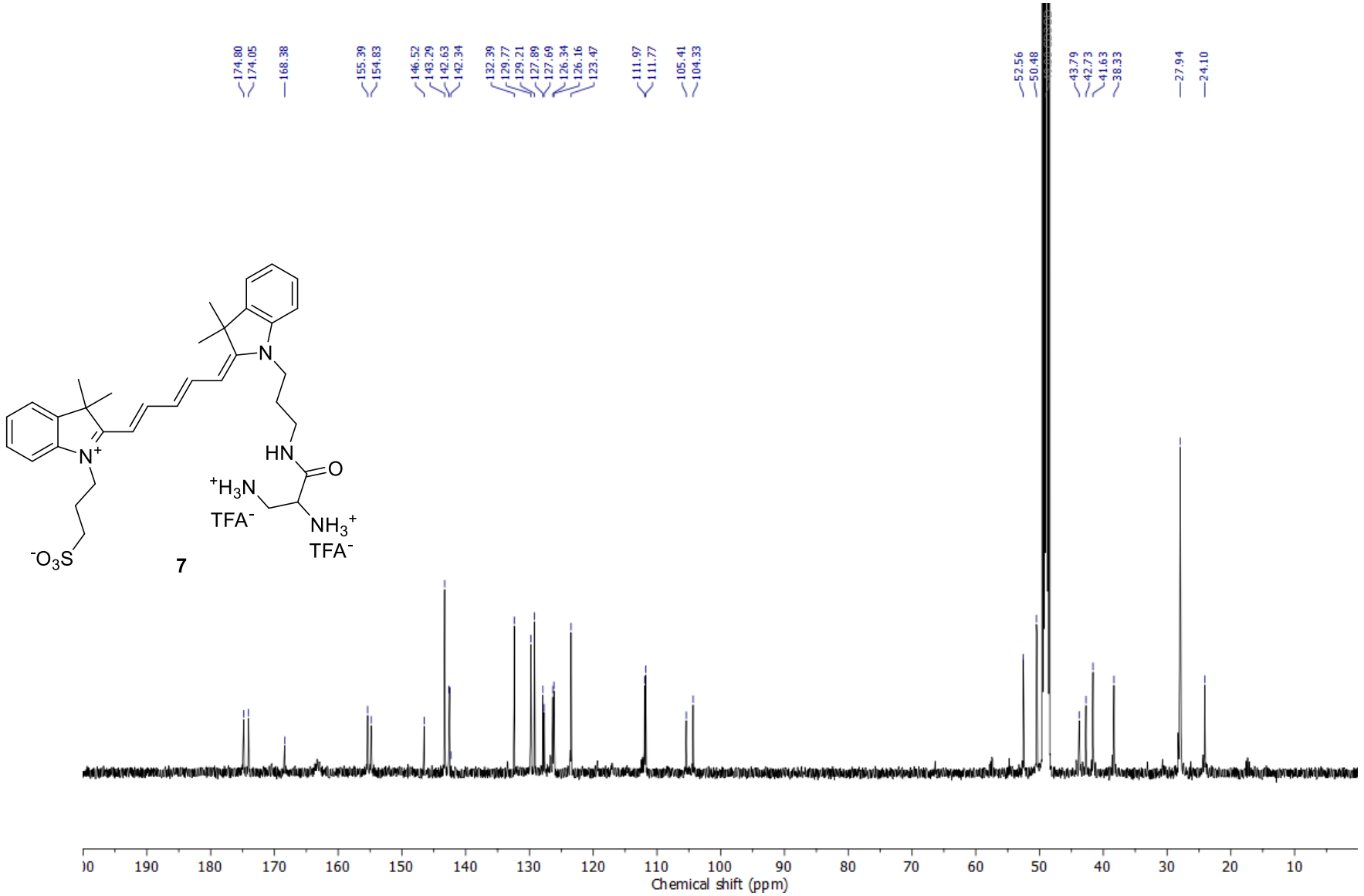

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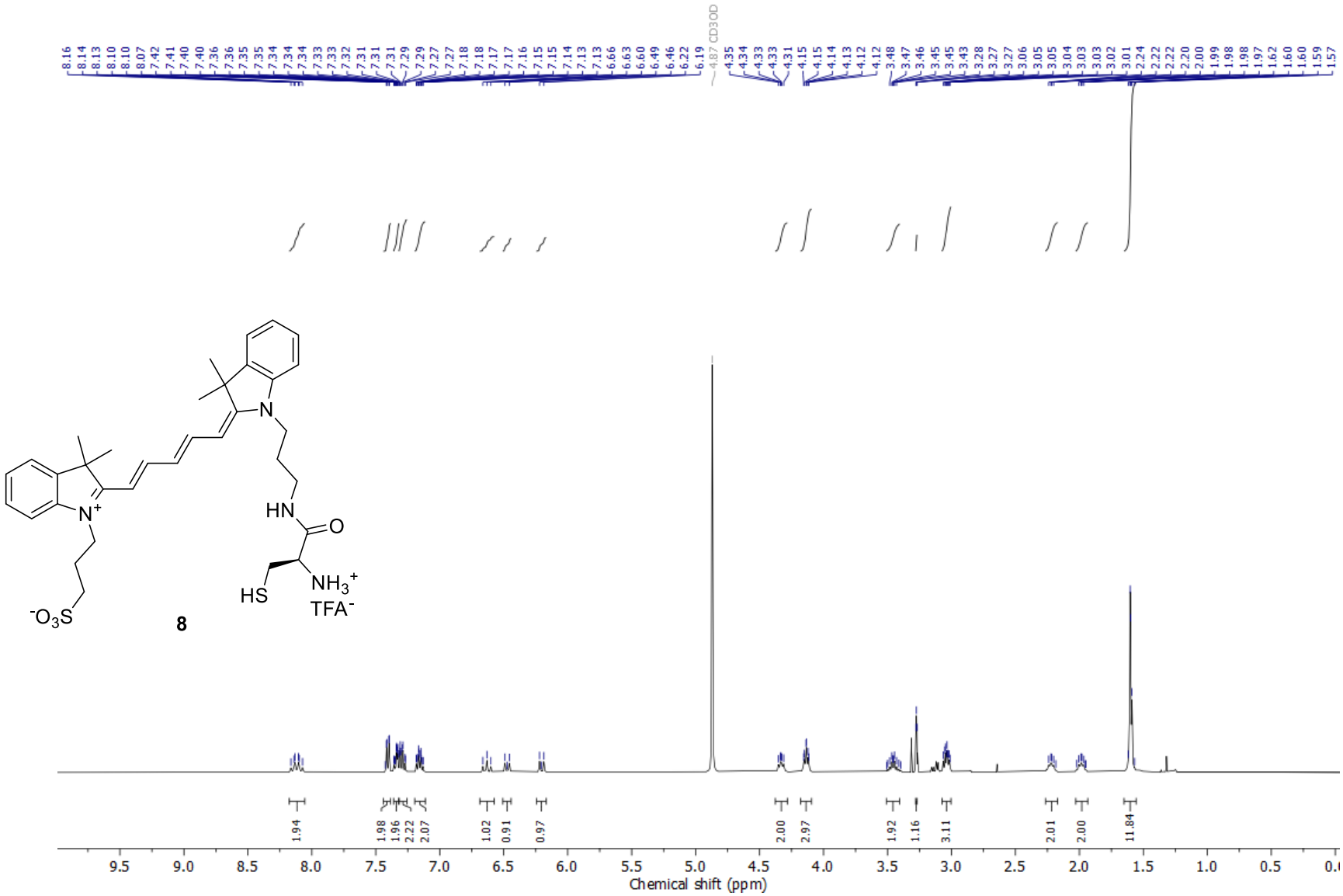

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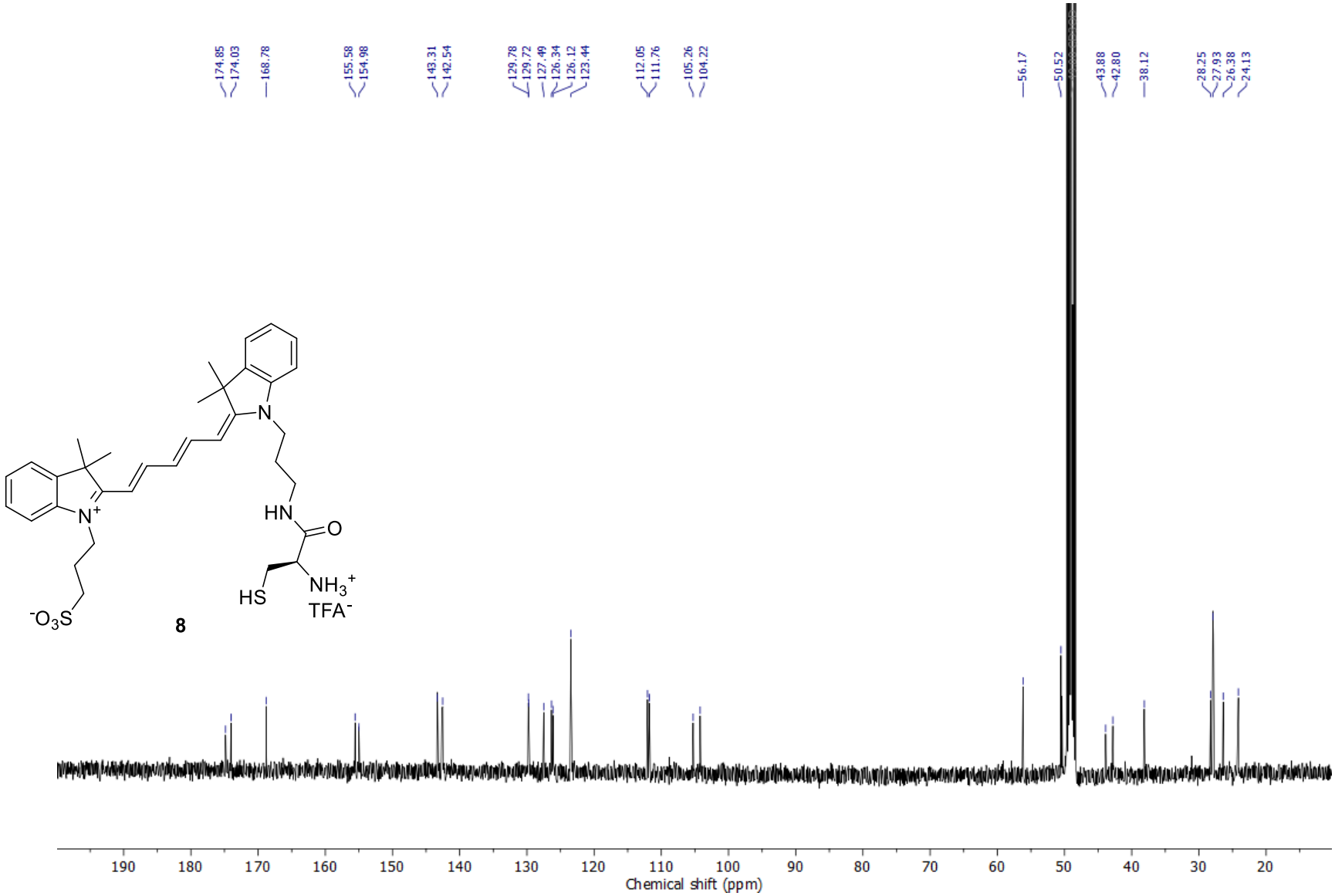

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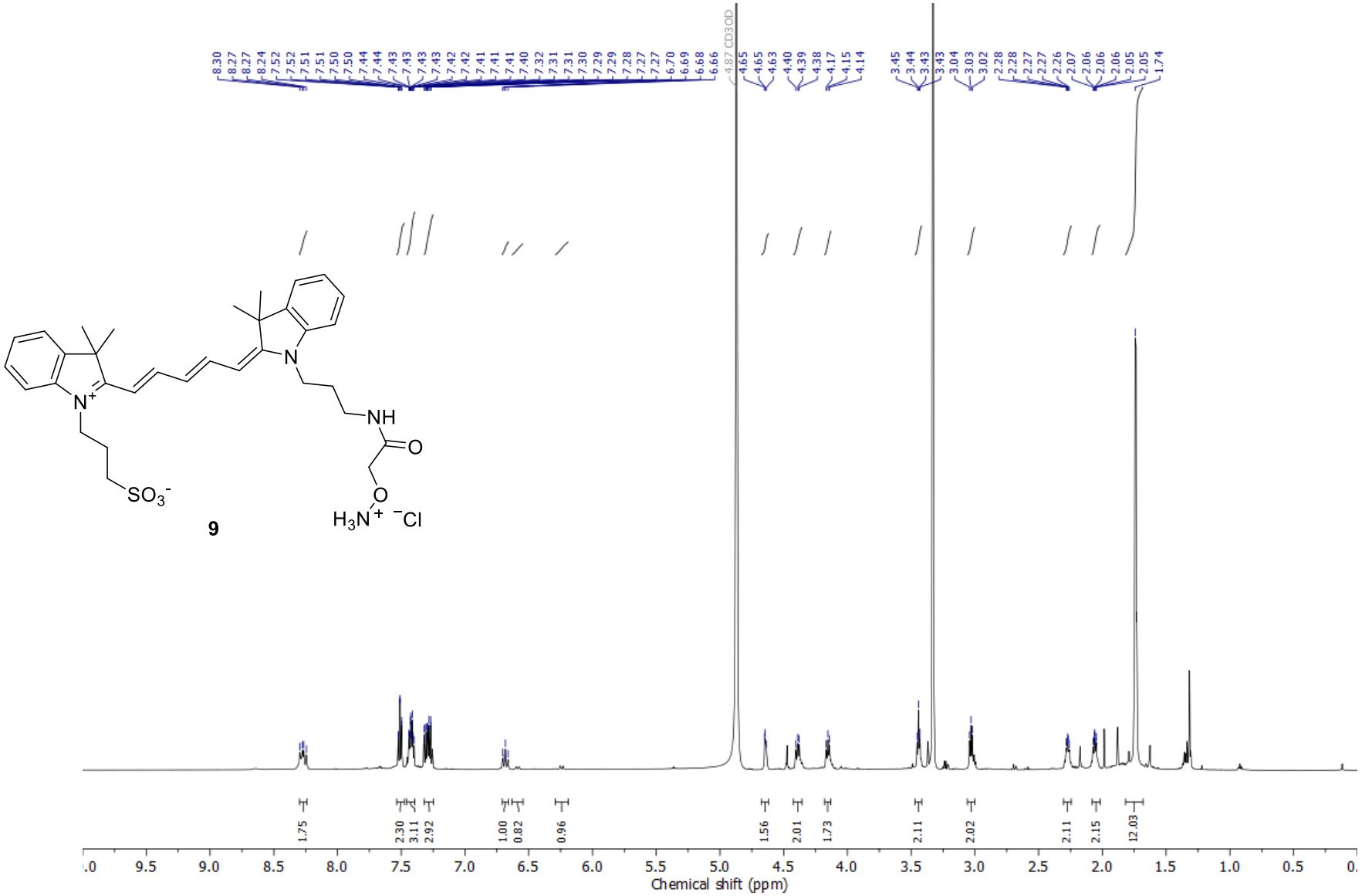

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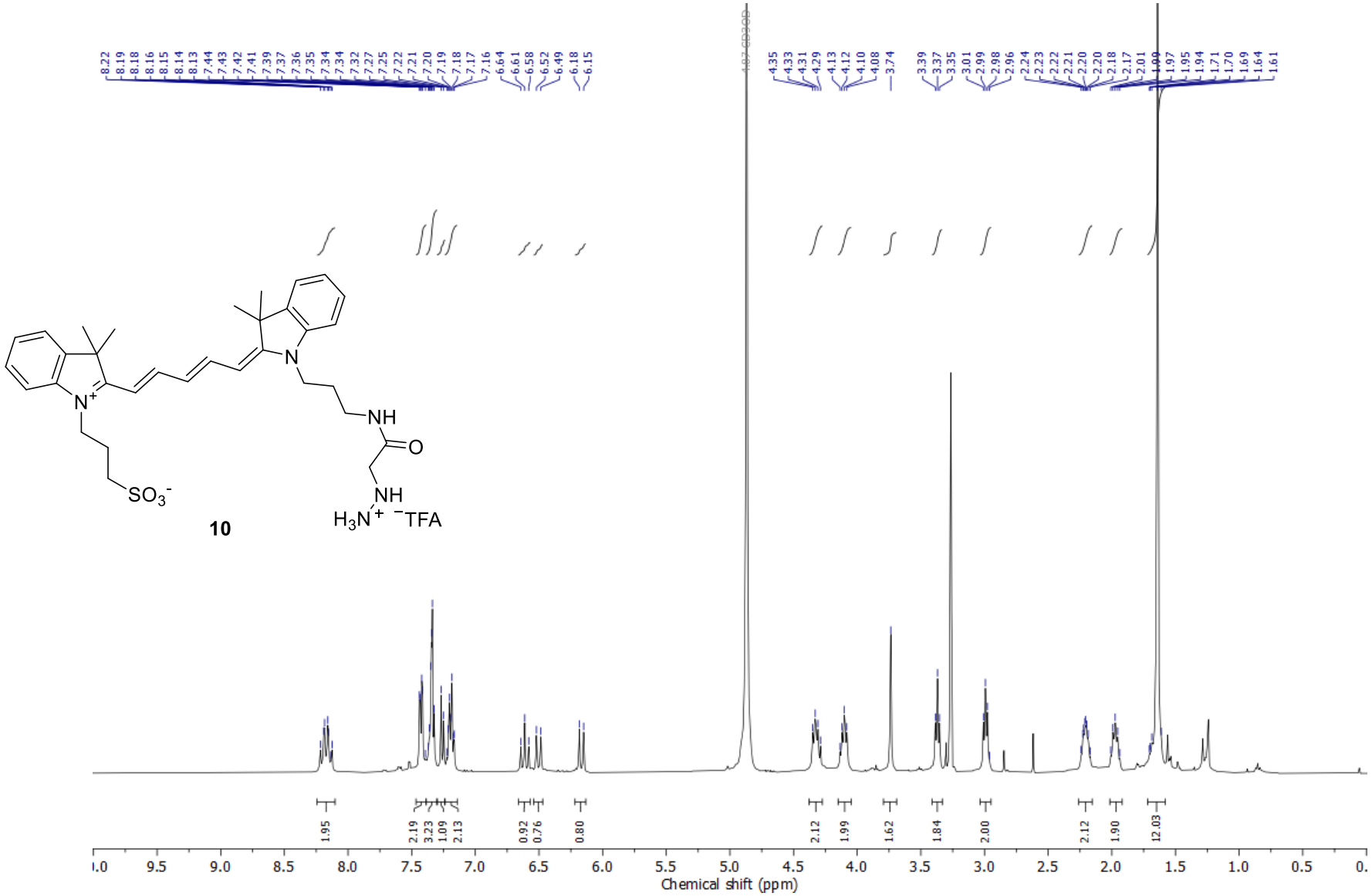

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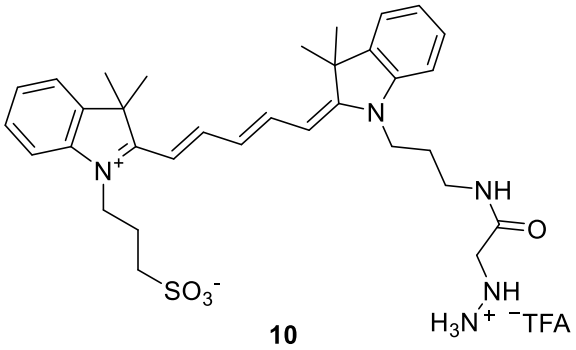

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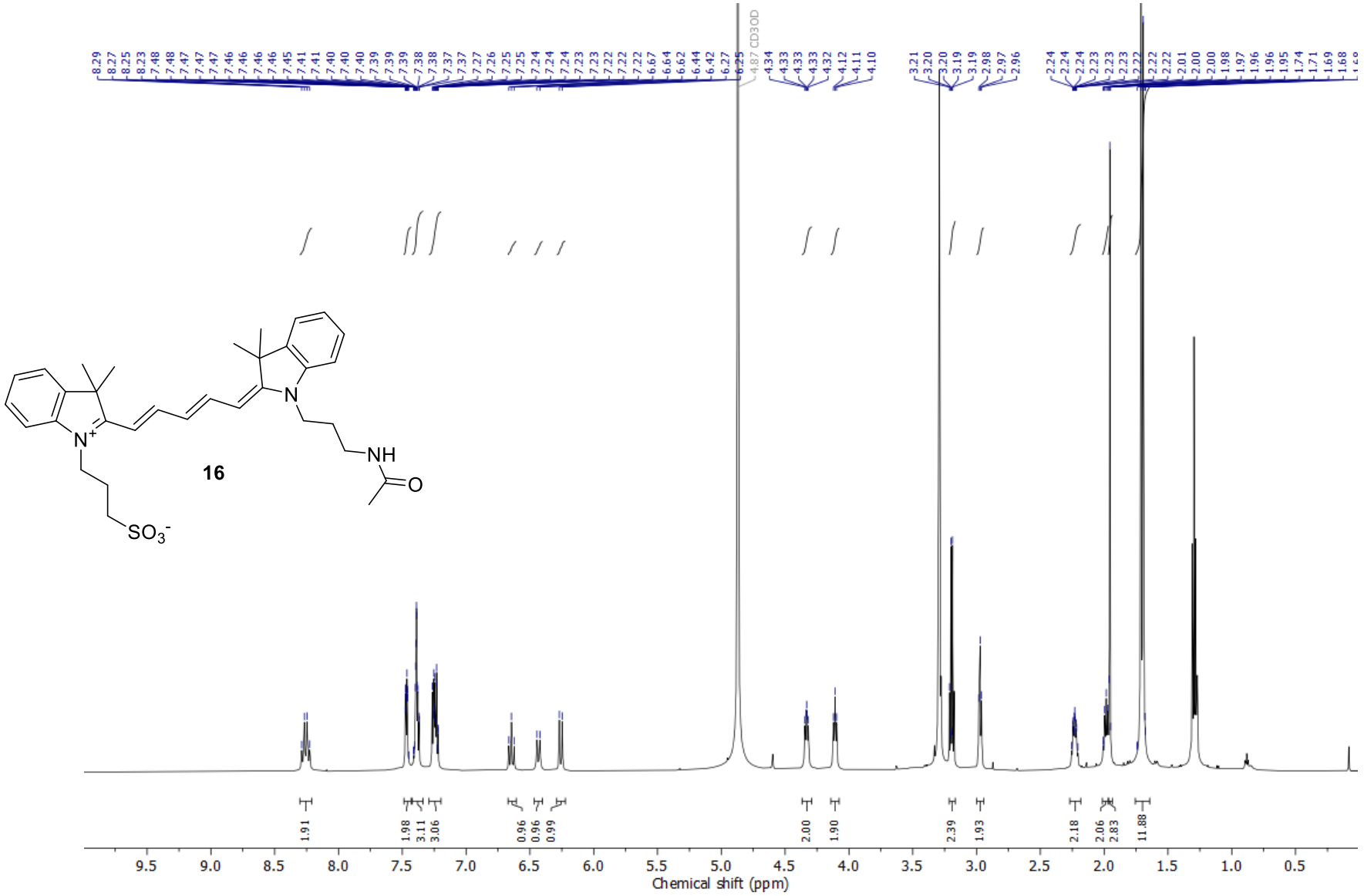

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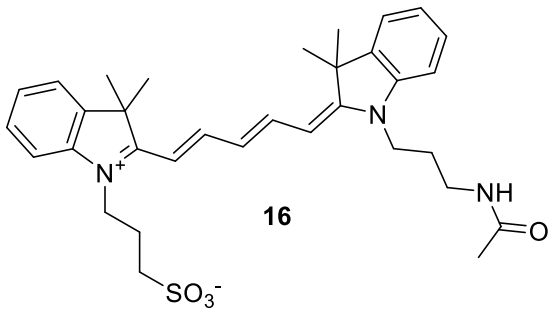

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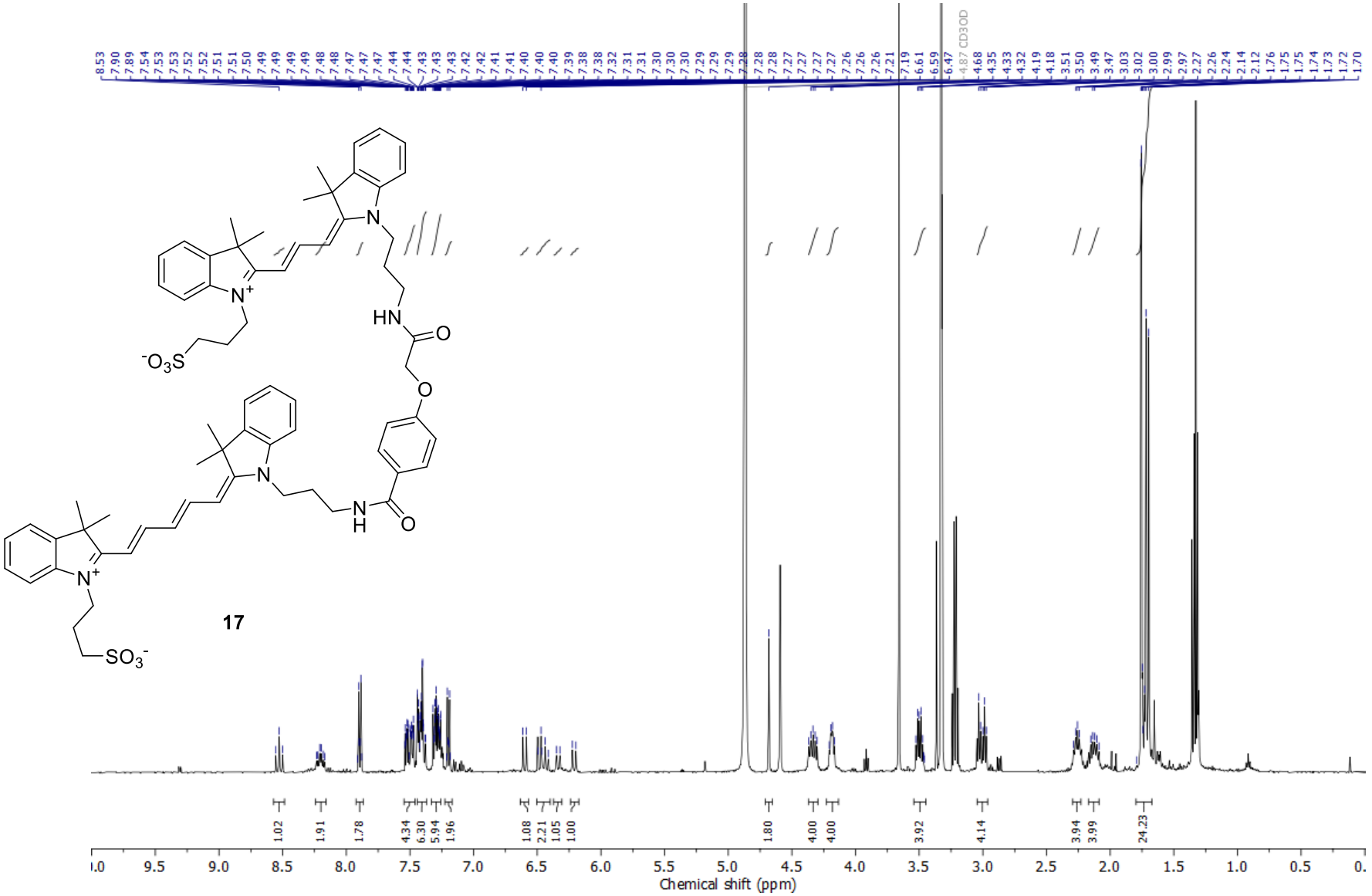

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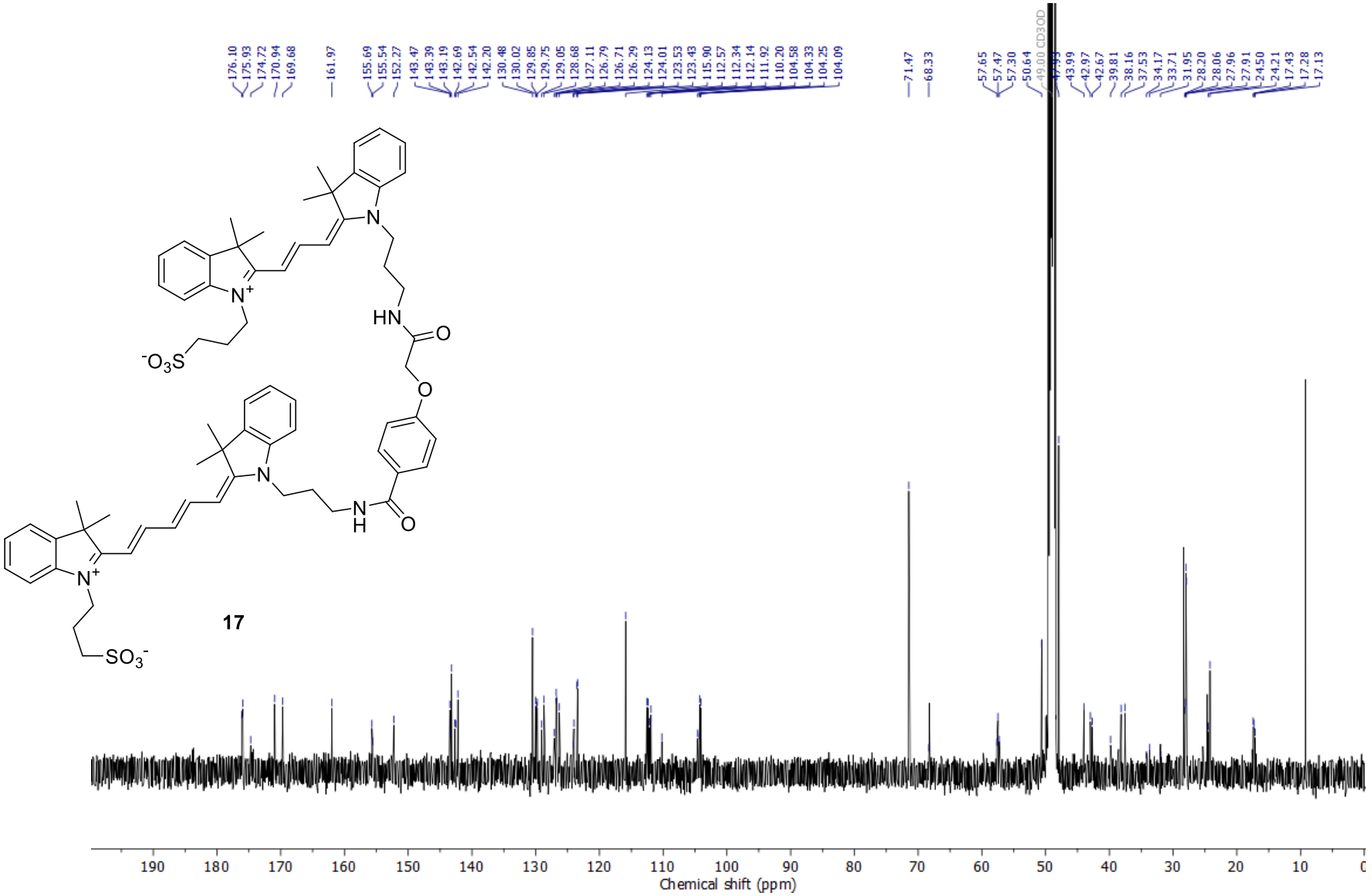

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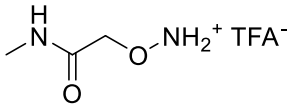

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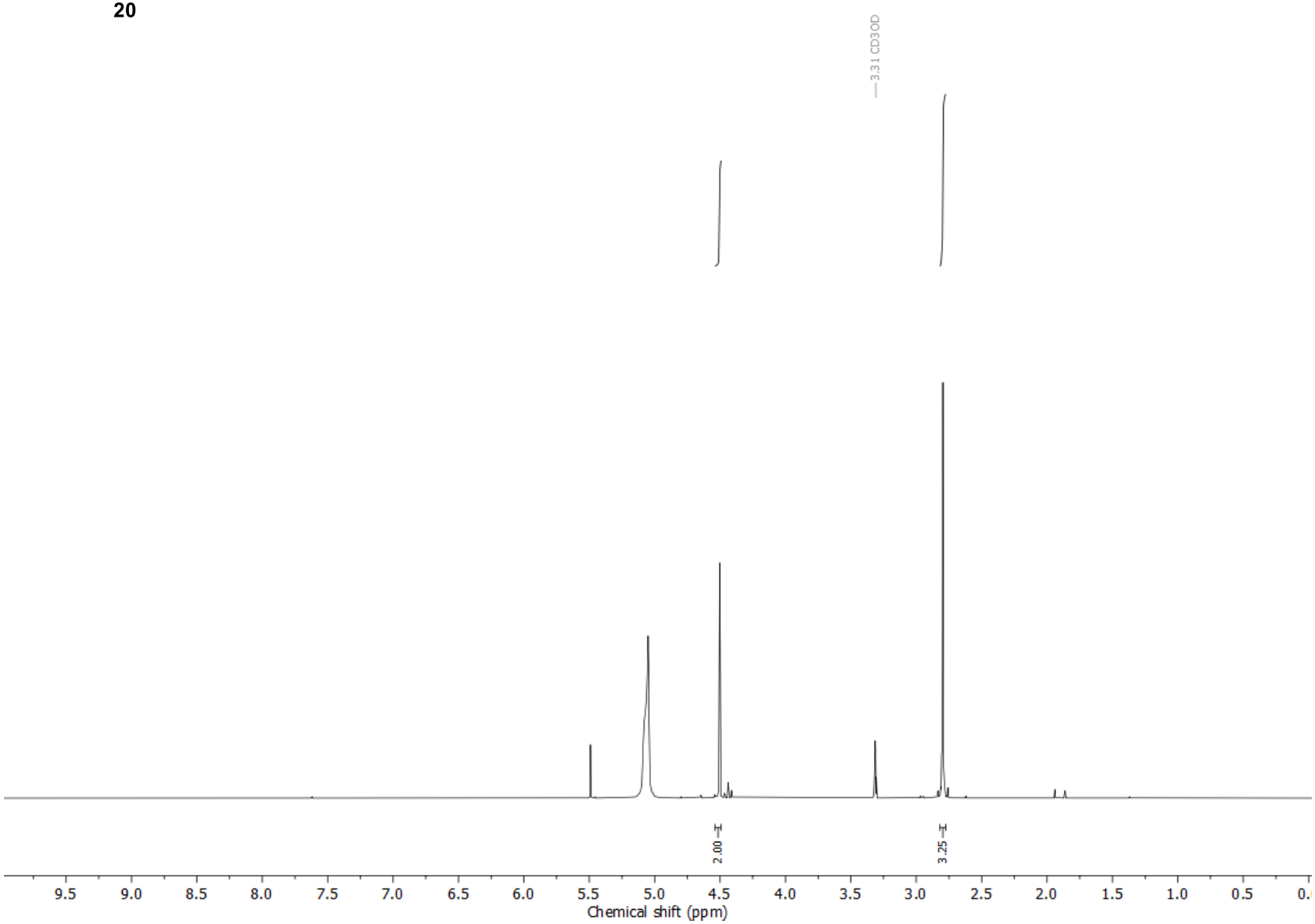

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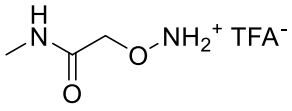

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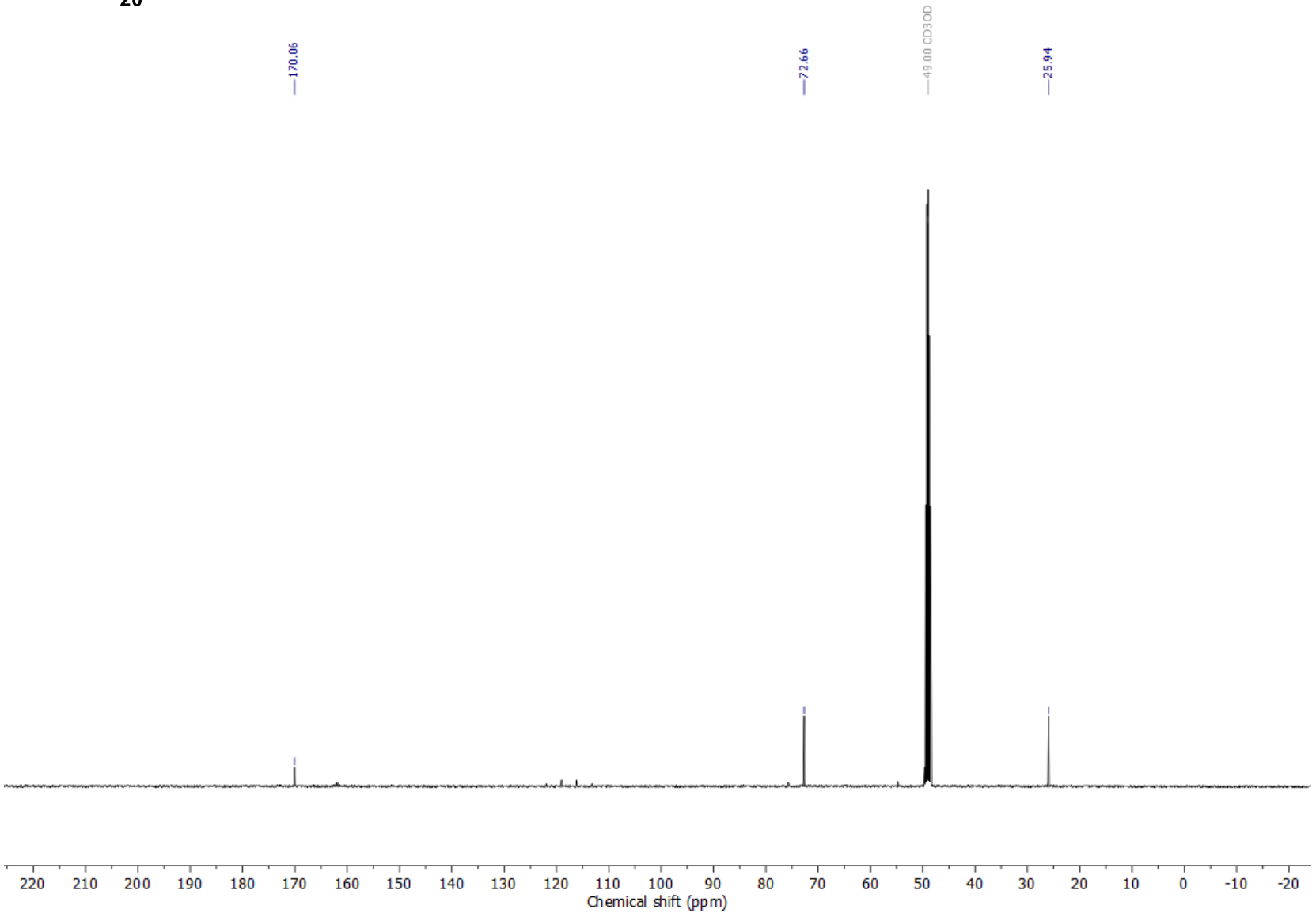

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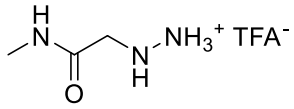

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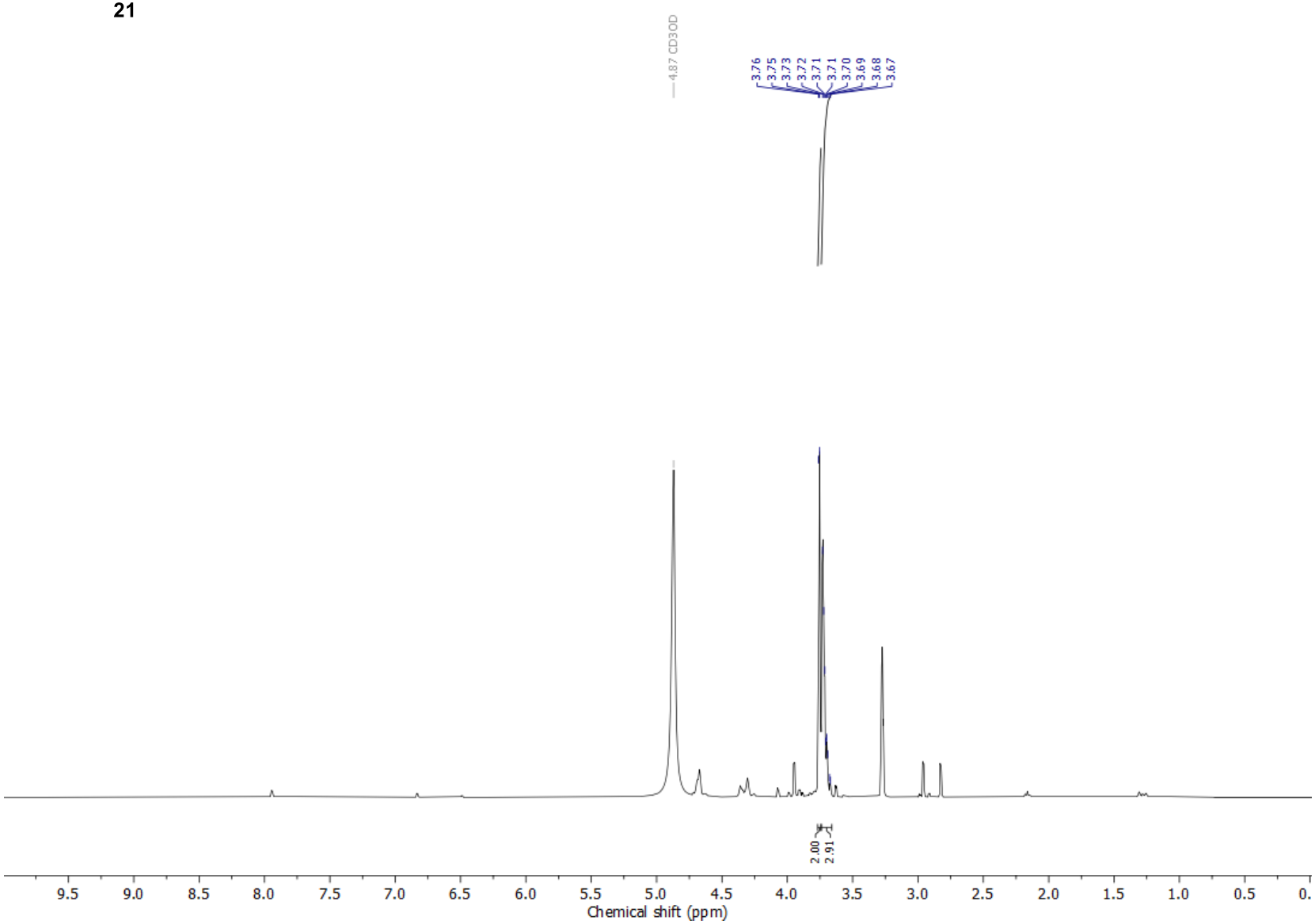

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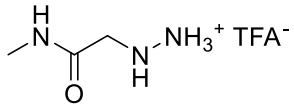

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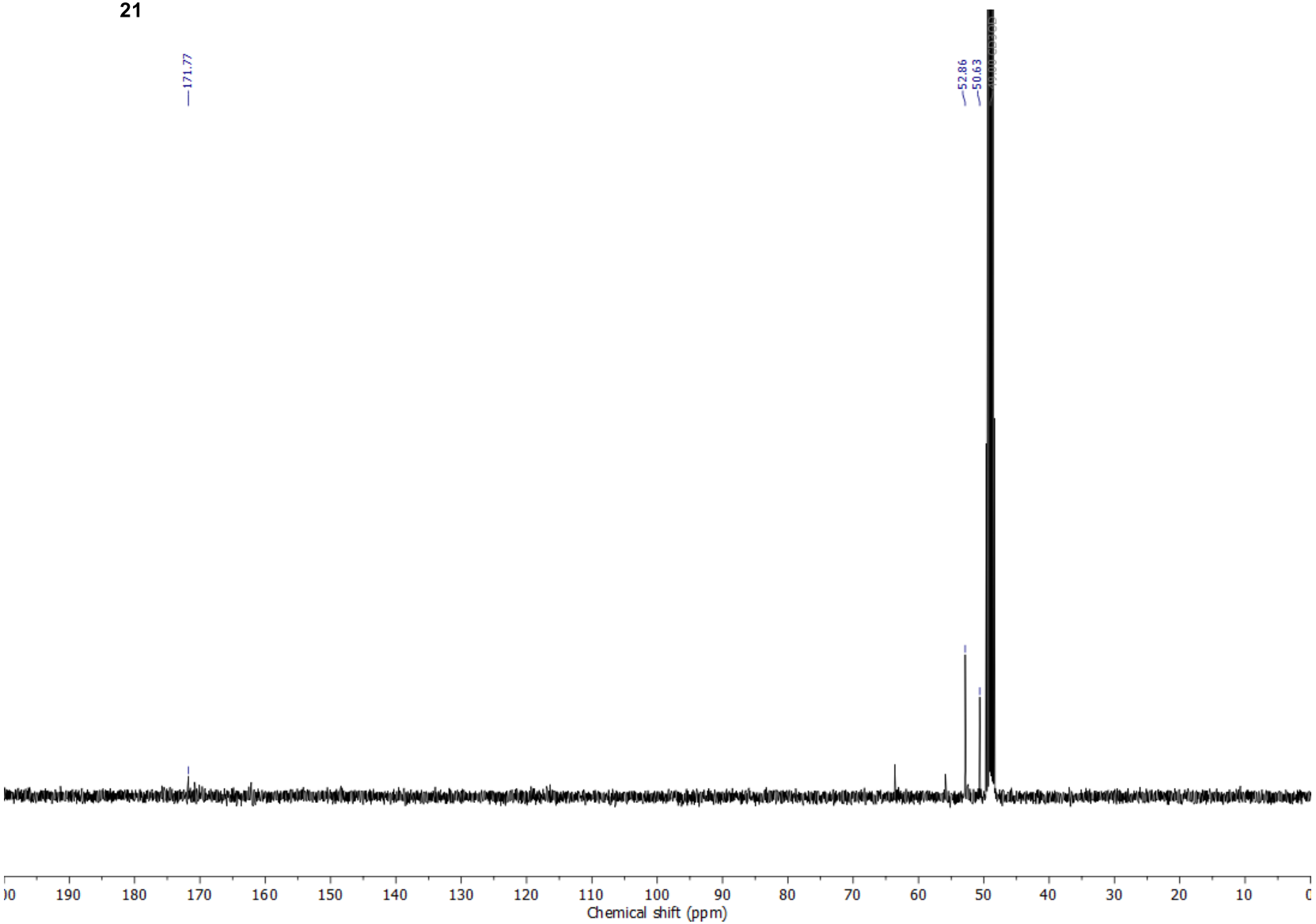

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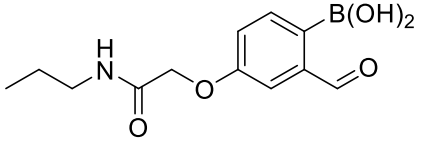

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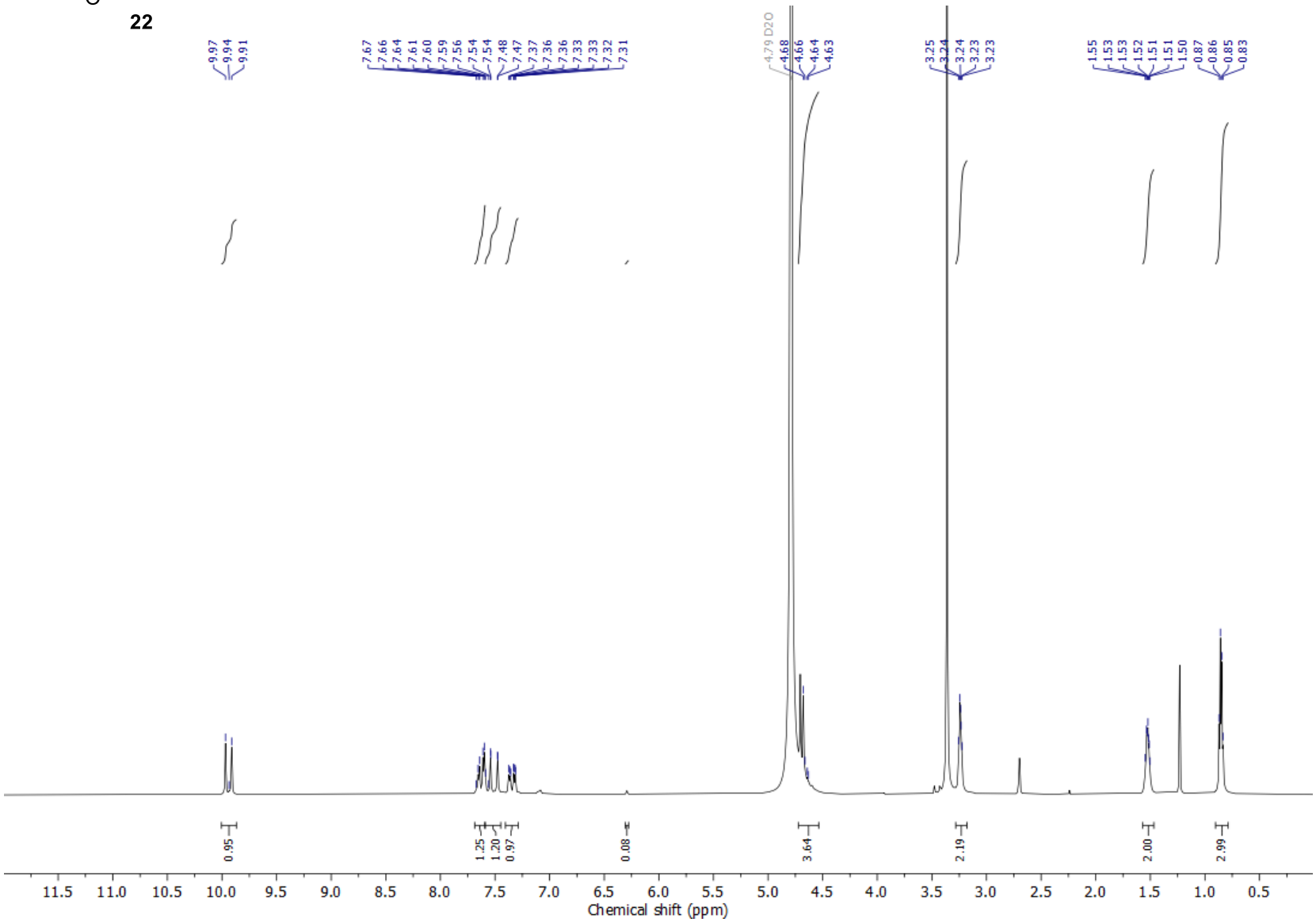
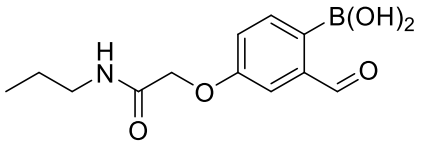


^{13}C NMR (100 MHz, MeOD)



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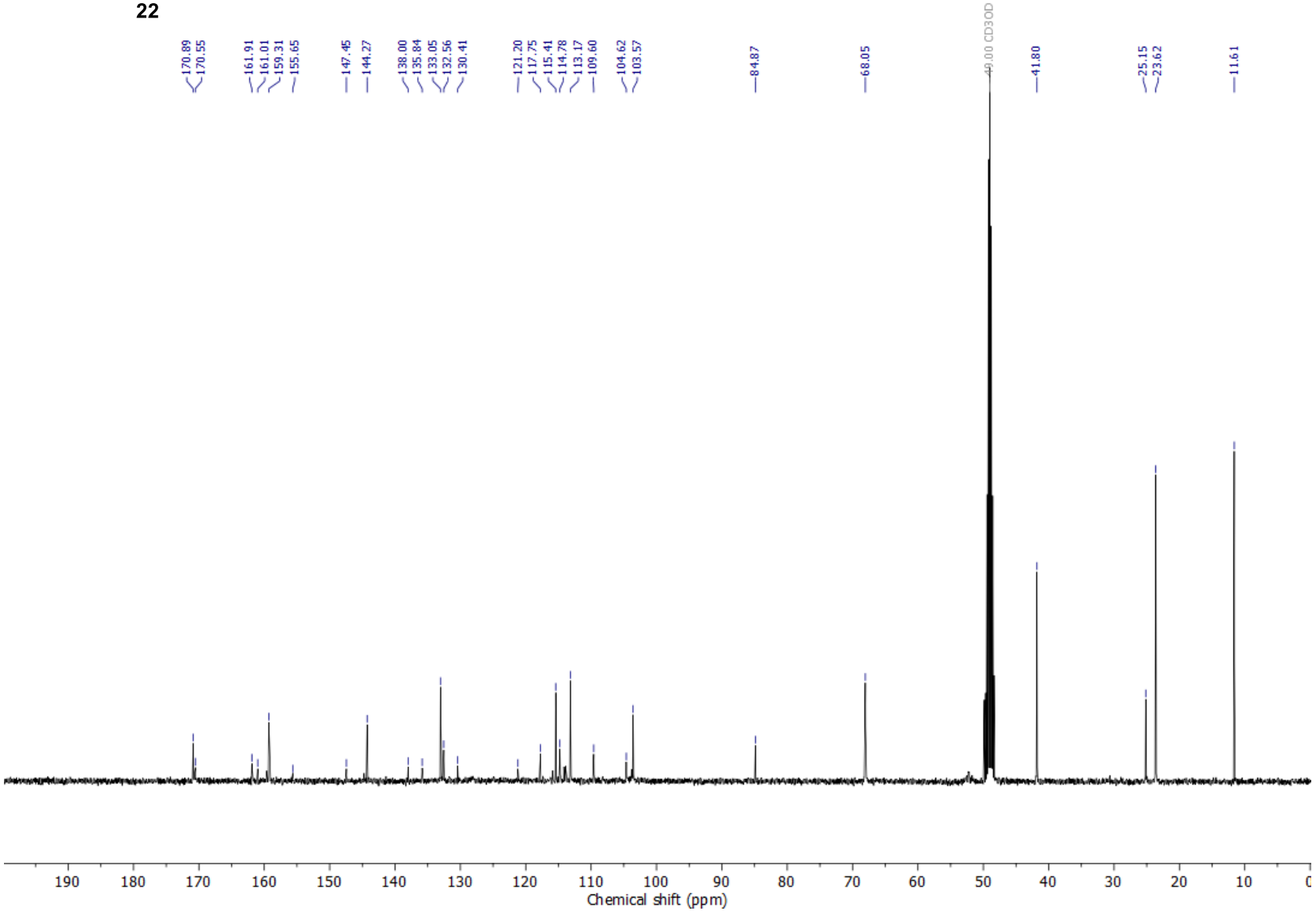
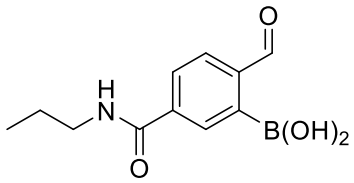


^{1}H NMR (400 MHz, MeOD)



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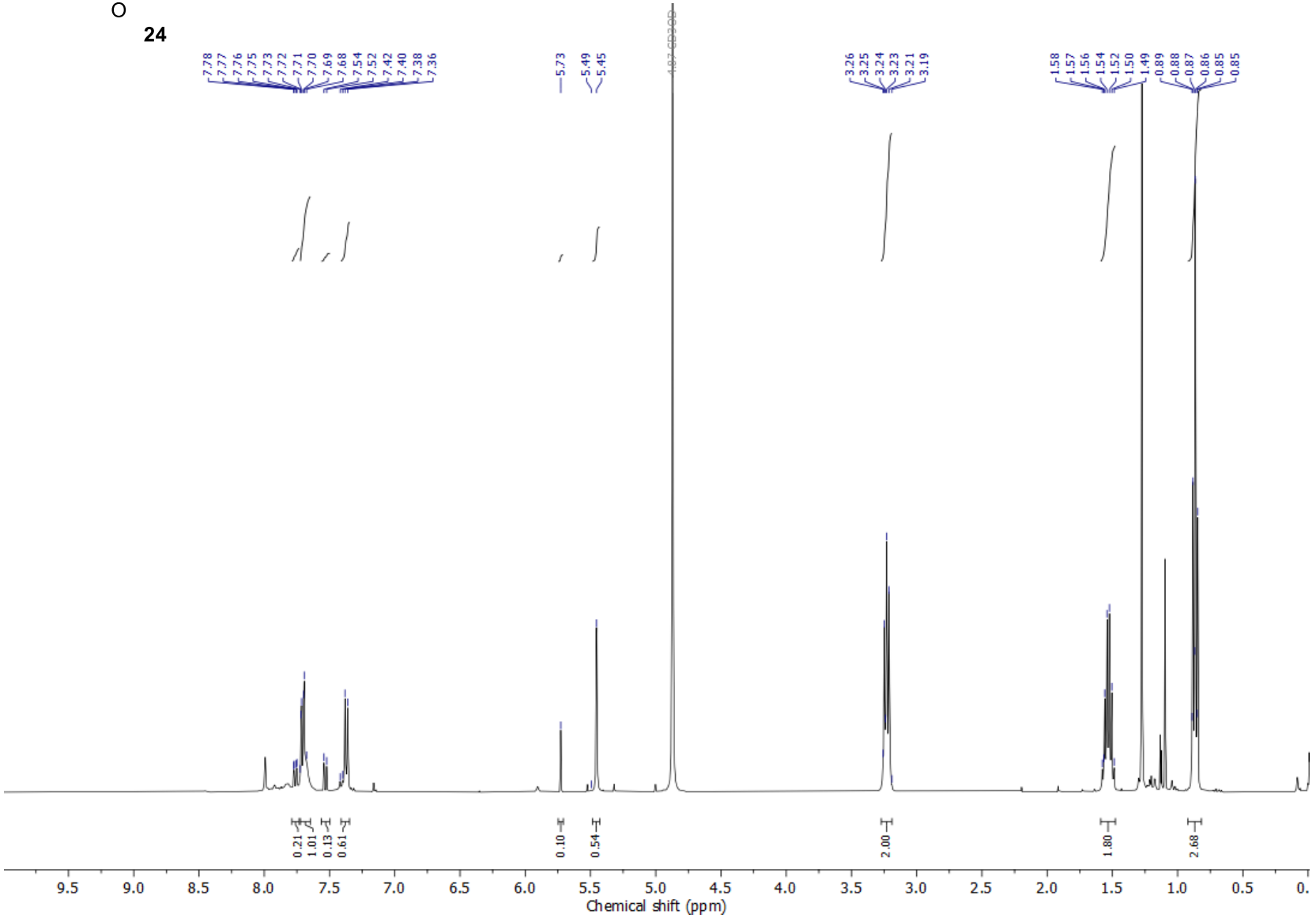
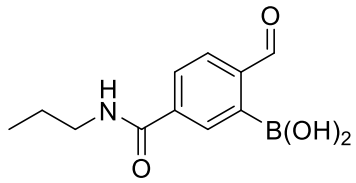


^{13}C NMR (100 MHz, MeOD)



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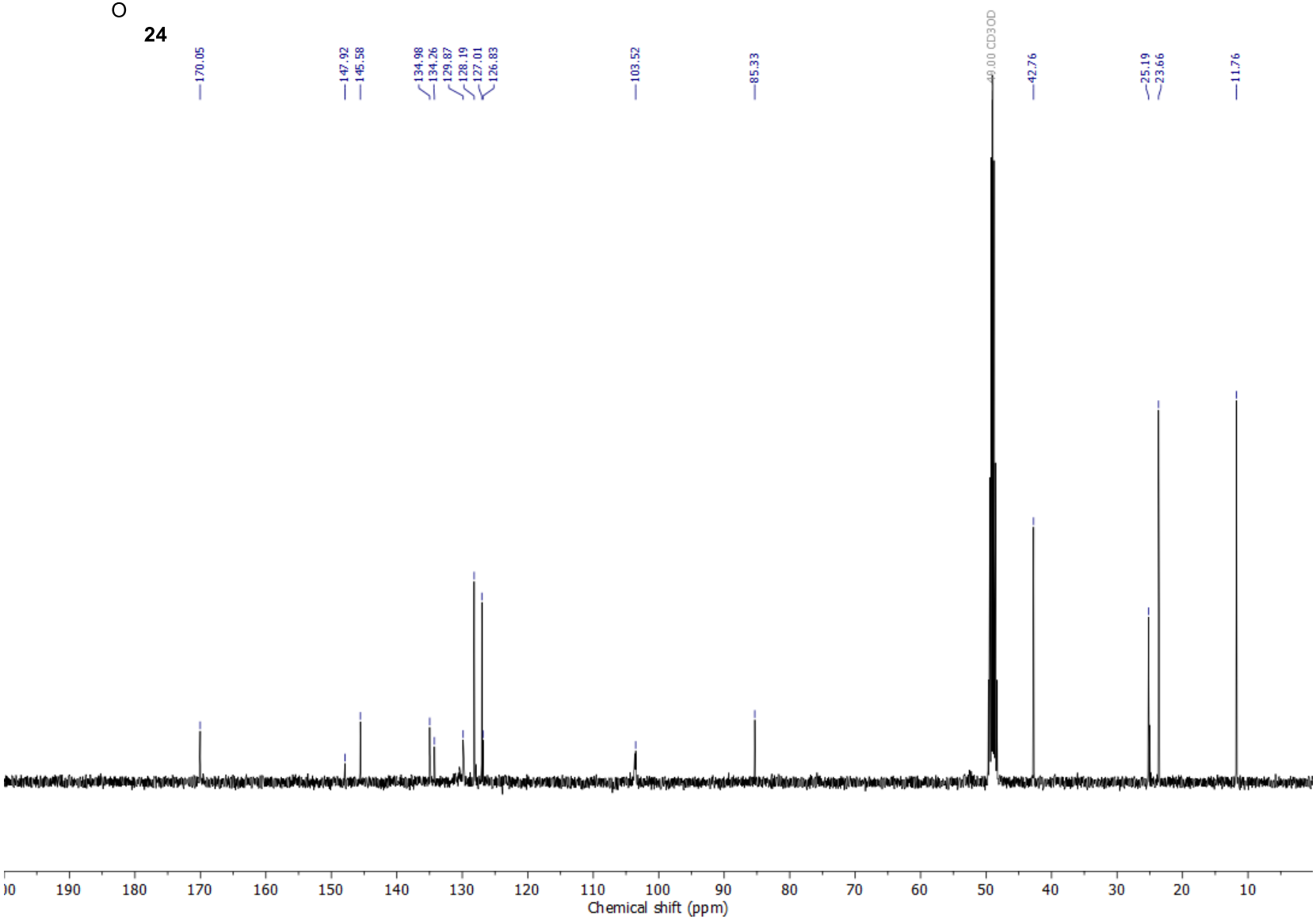
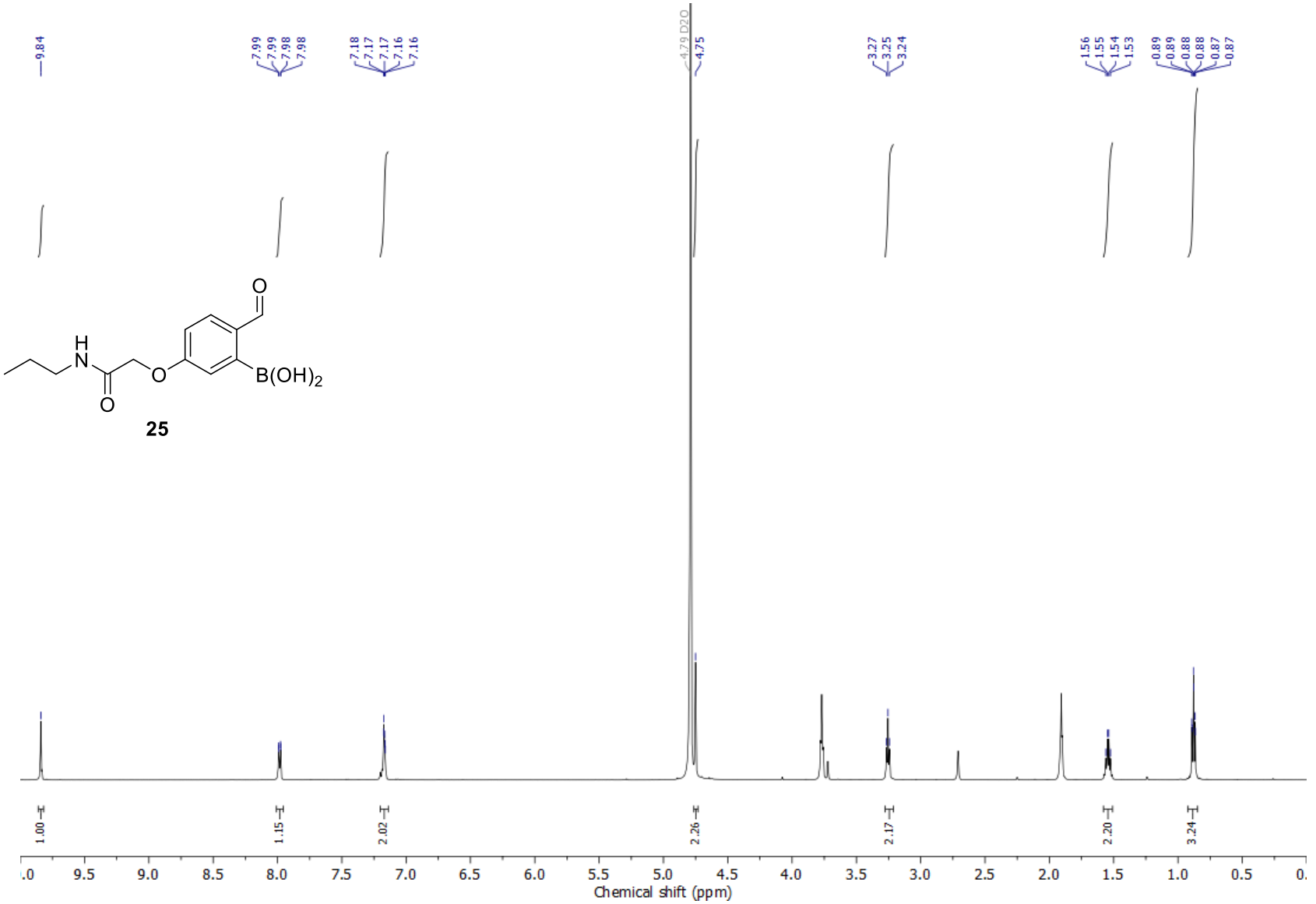
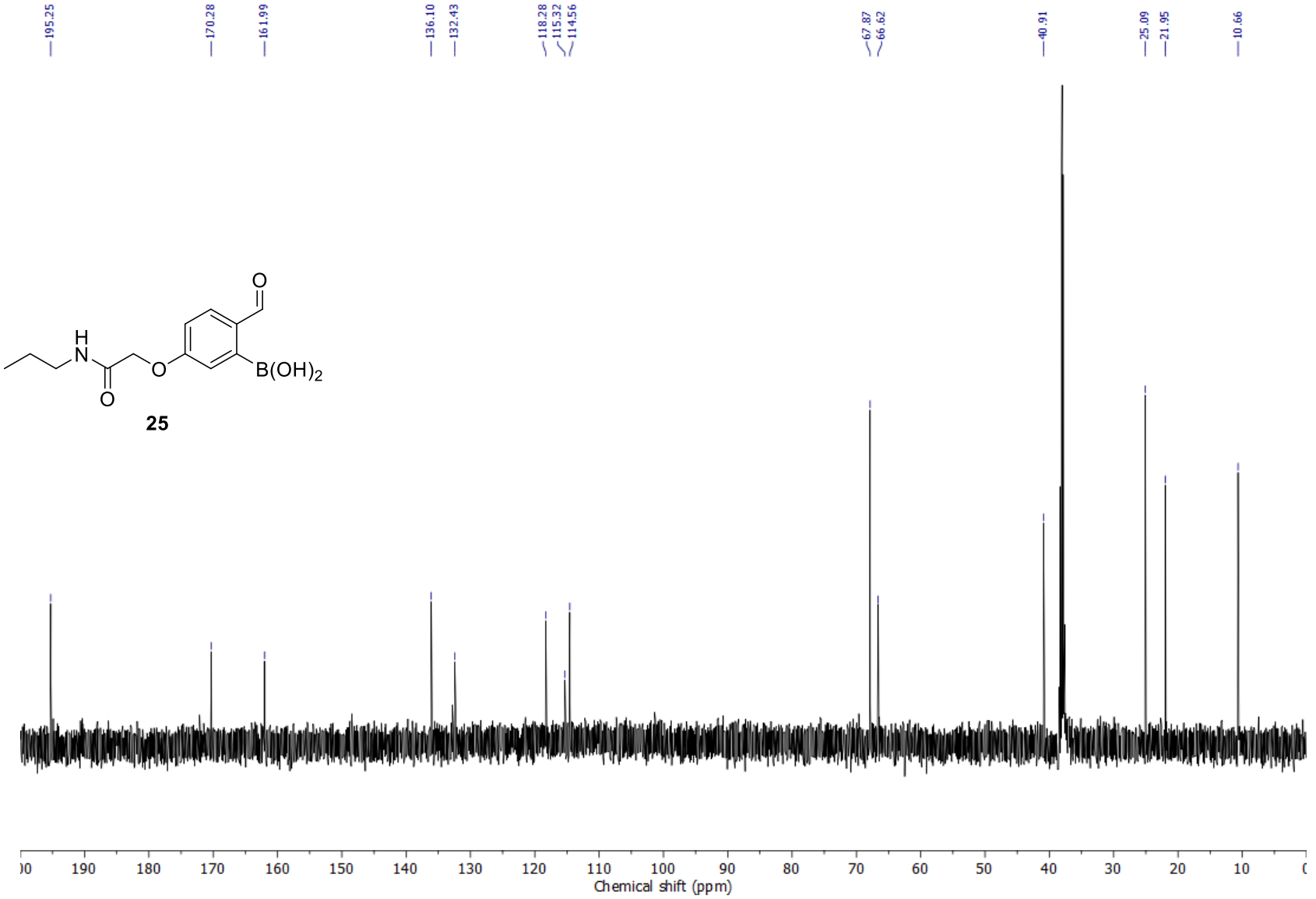


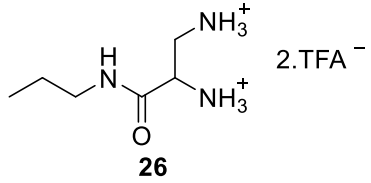
^{1}H NMR (400 MHz, 100 mM deuterated PBS + 10% DMSO-d_6)



^{13}C NMR (100 MHz, 100 mM deuterated PBS + 10% DMSO-d_6)



^{1}H NMR (400 MHz, MeOD)



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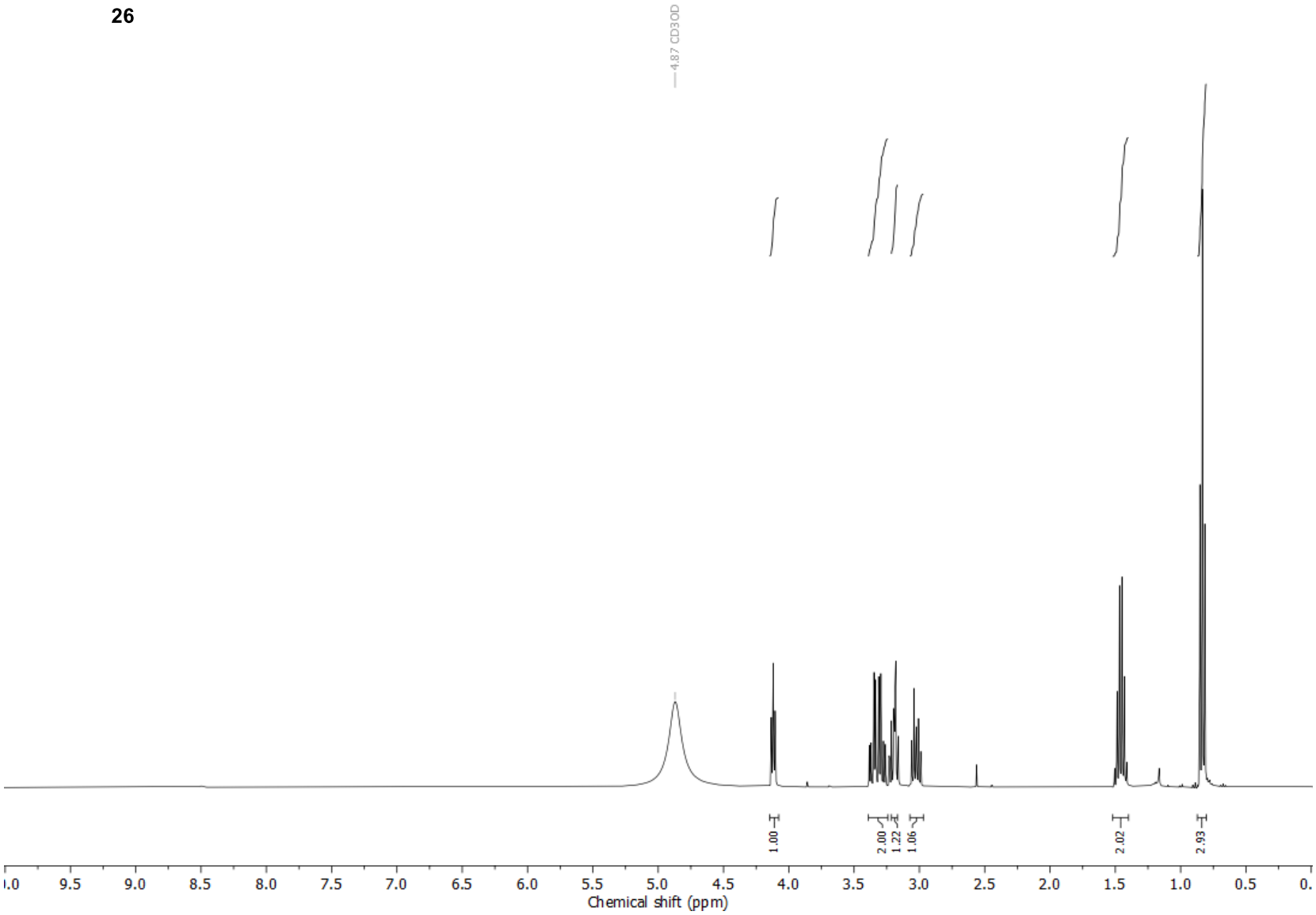
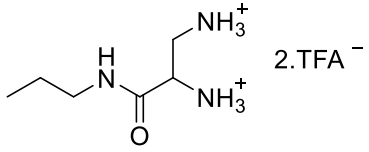


^{13}C NMR (100 MHz, MeOD)



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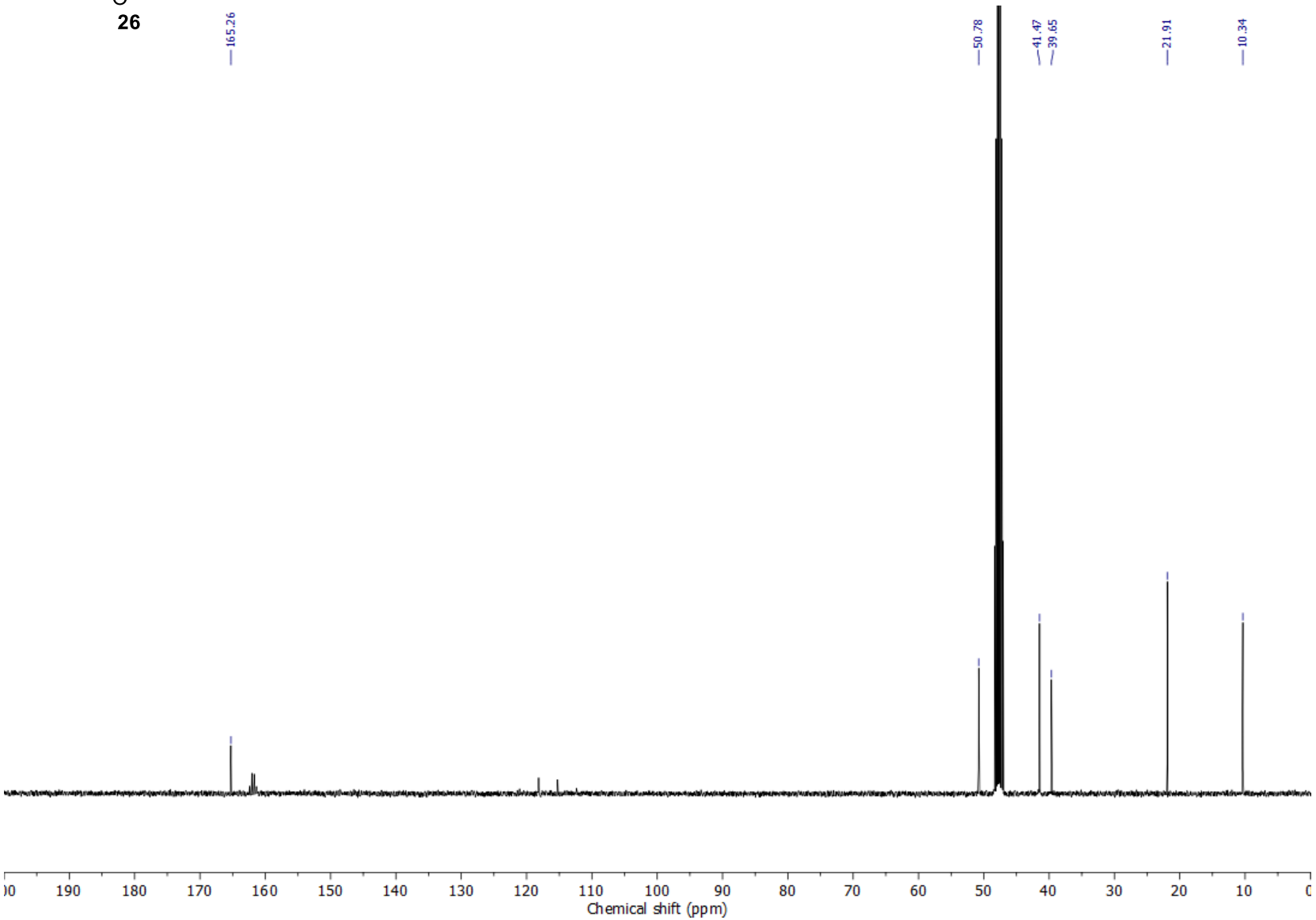
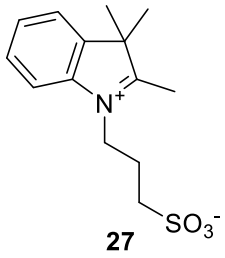
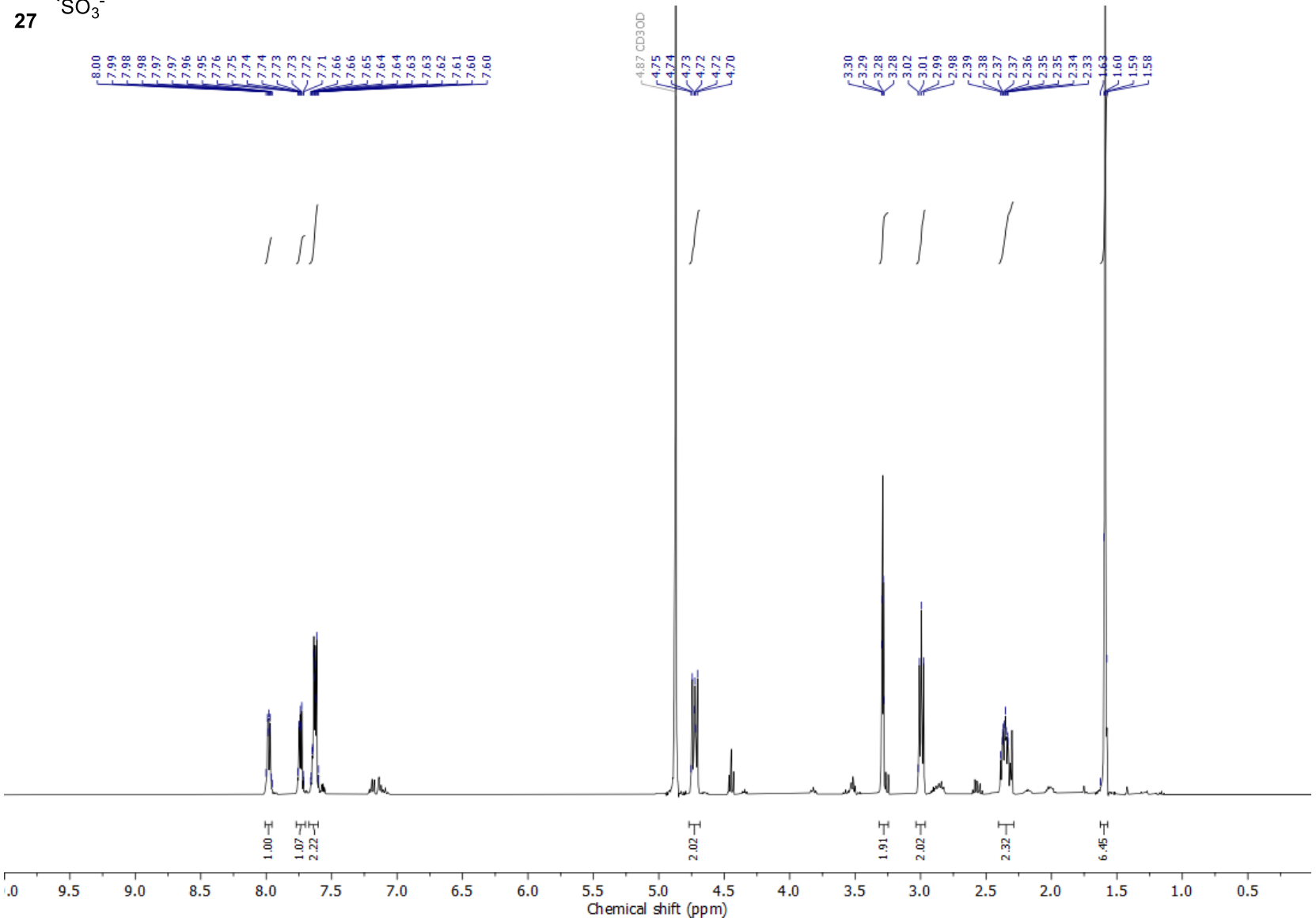
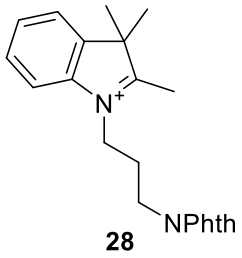




27

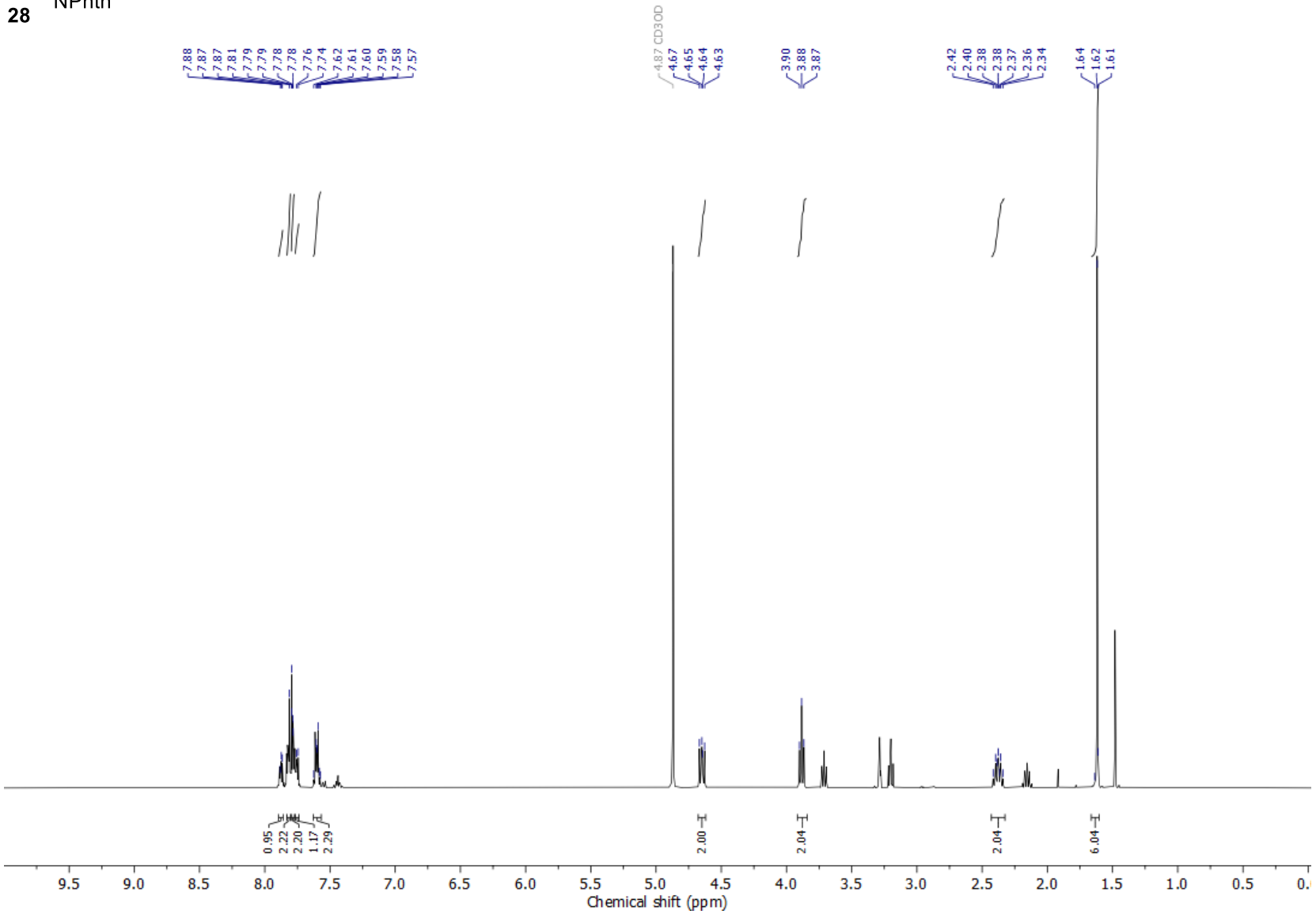
^{1}H NMR (400 MHz, MeOD)

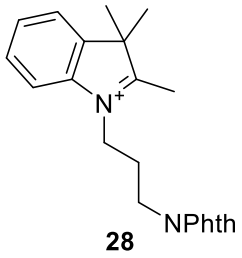




28

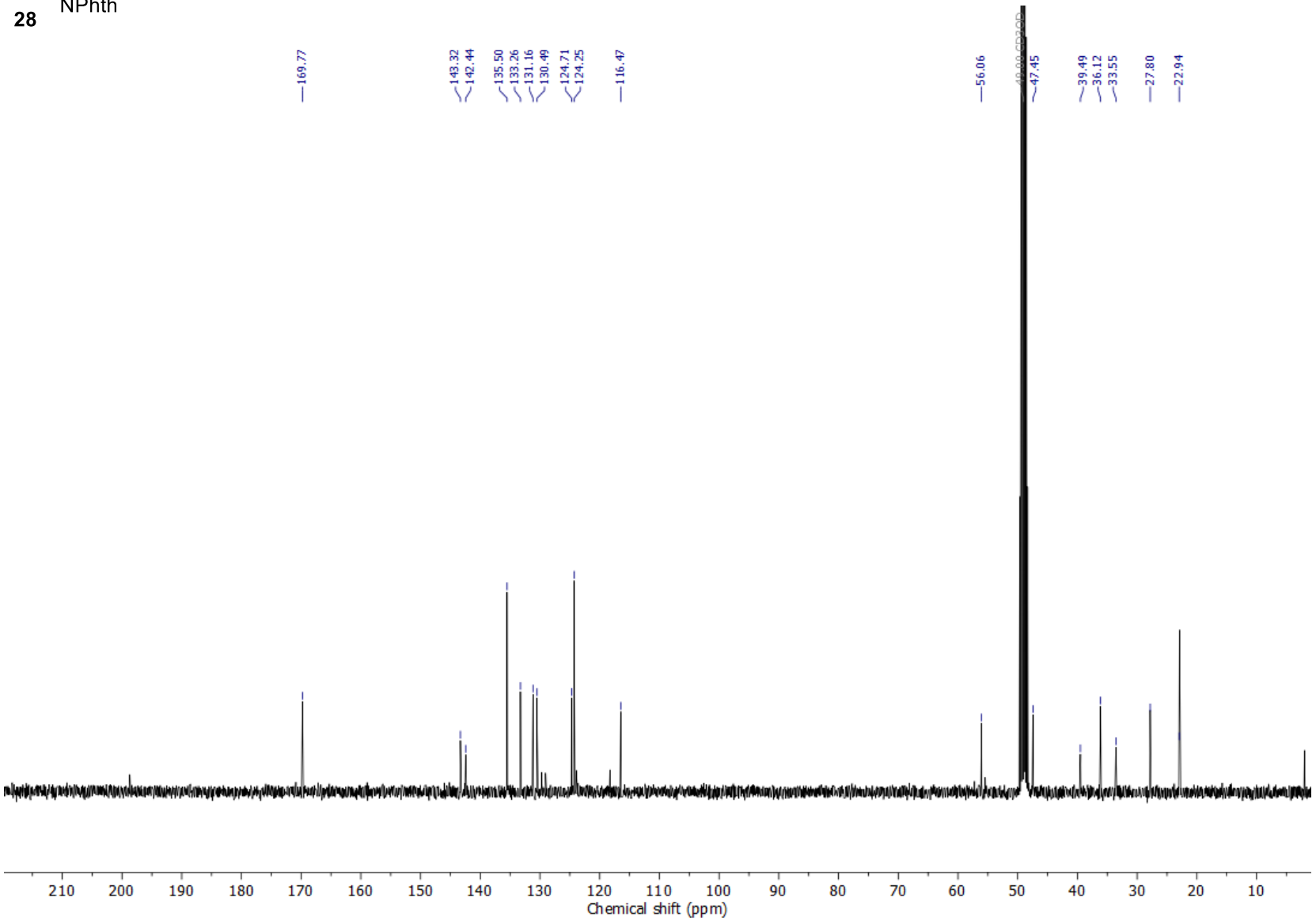
^{1}H NMR (400 MHz, MeOD)



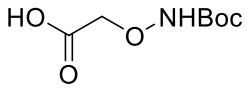


28

^{13}C NMR (100 MHz, MeOD)



^{1}H NMR (400 MHz, $CDCl_3$)



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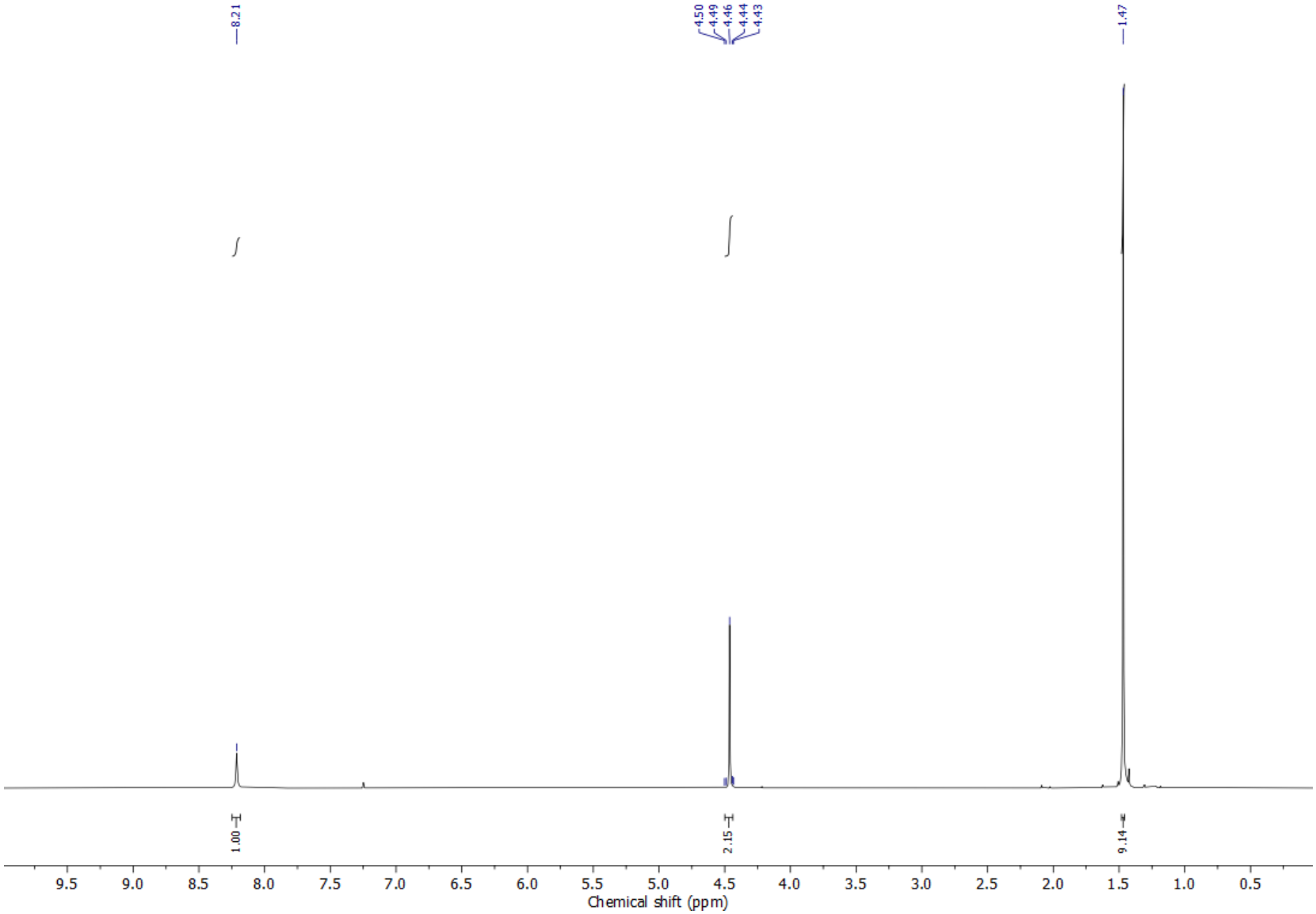
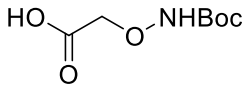


^{13}C NMR (100 MHz, $CDCl_3$)



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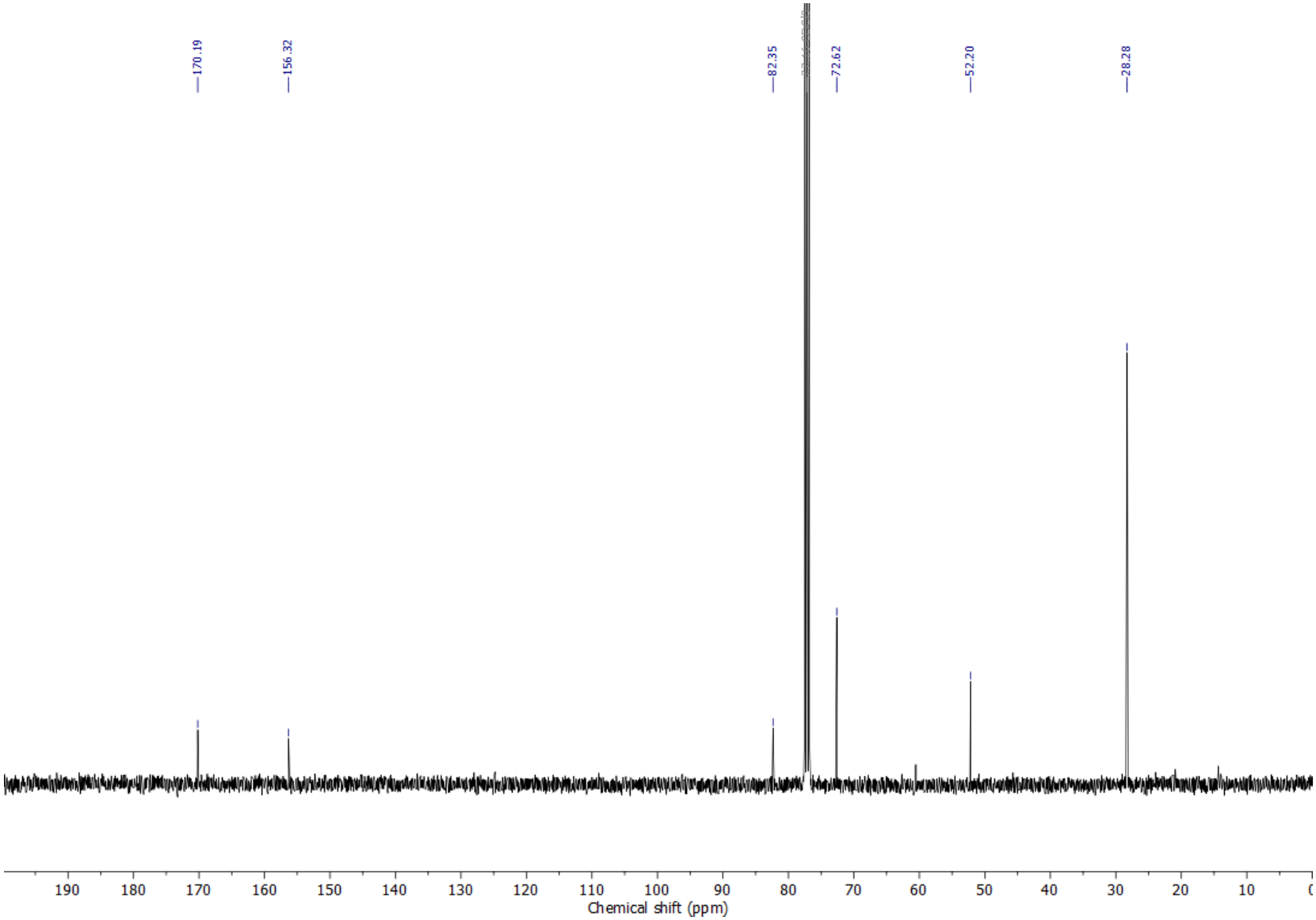
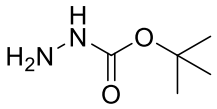


^{1}H NMR (400 MHz, $CDCl_3$)



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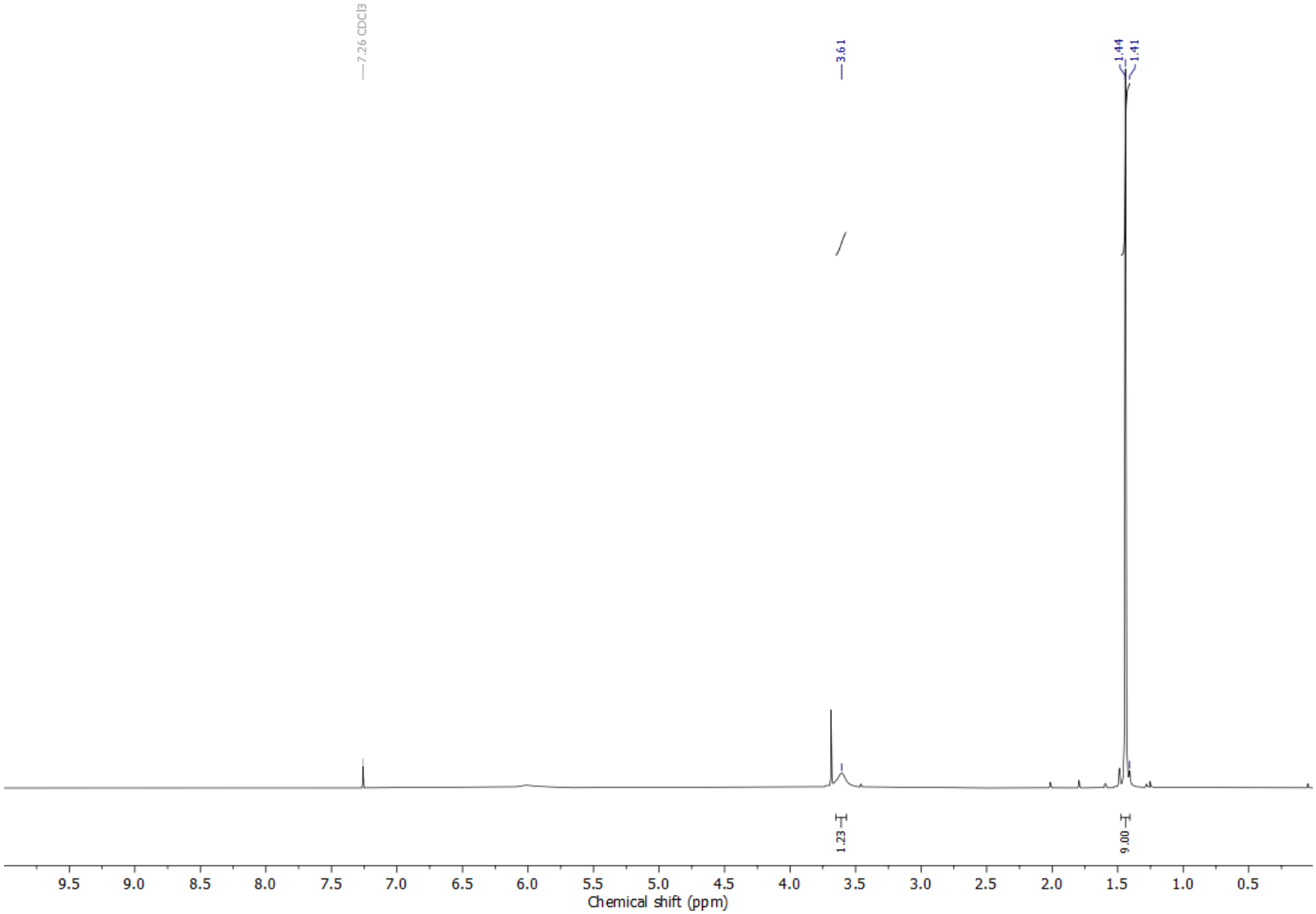
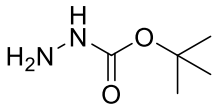


^{13}C NMR (100 MHz, $CDCl_3$)



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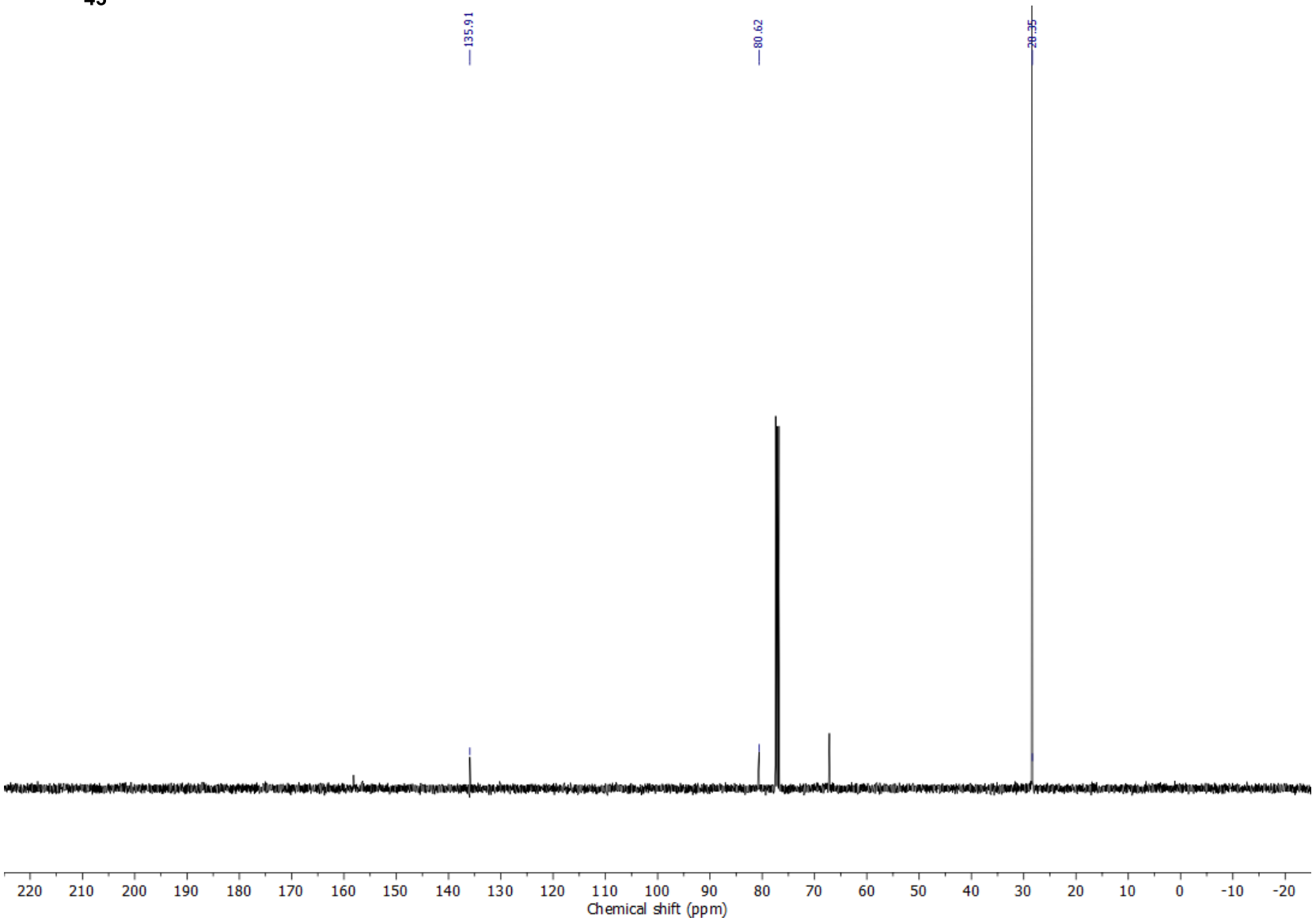
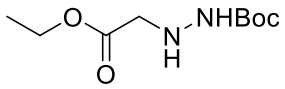


^{1}H NMR (400 MHz, $CDCl_3$)



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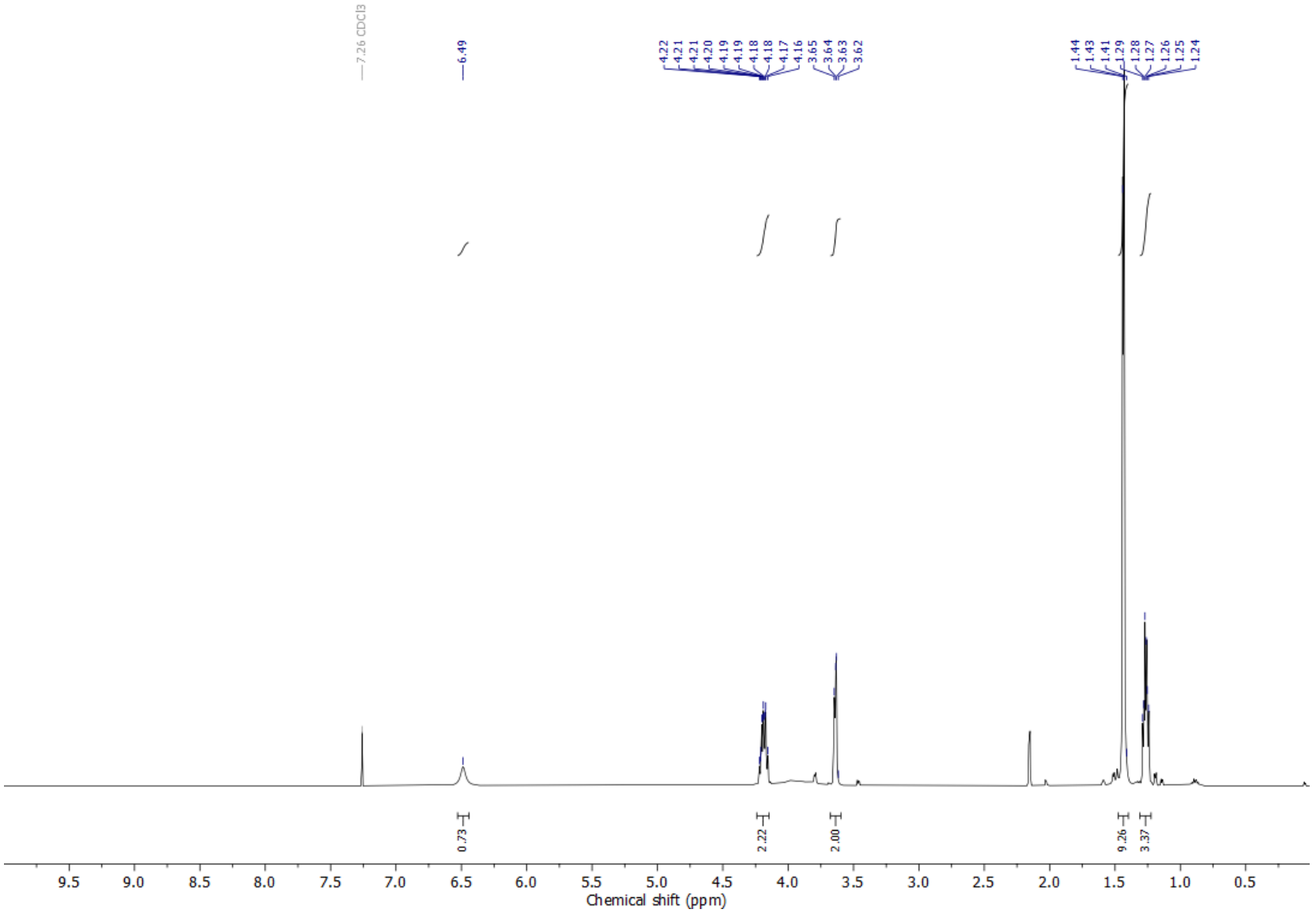
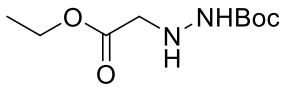


^{13}C NMR (100 MHz, $CDCl_3$)



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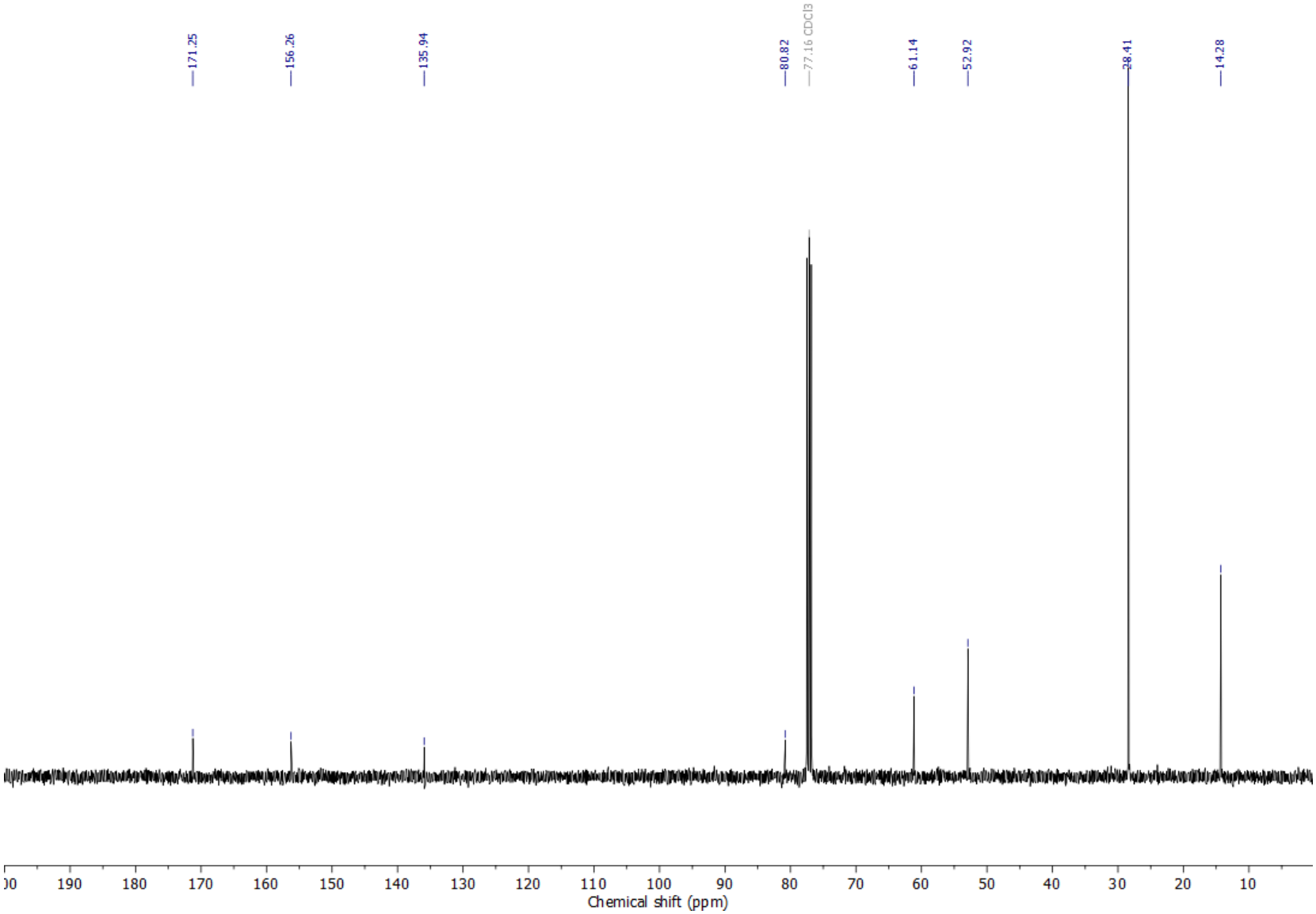
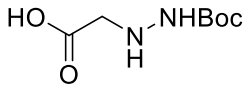


^{1}H NMR (400 MHz, MeOD)



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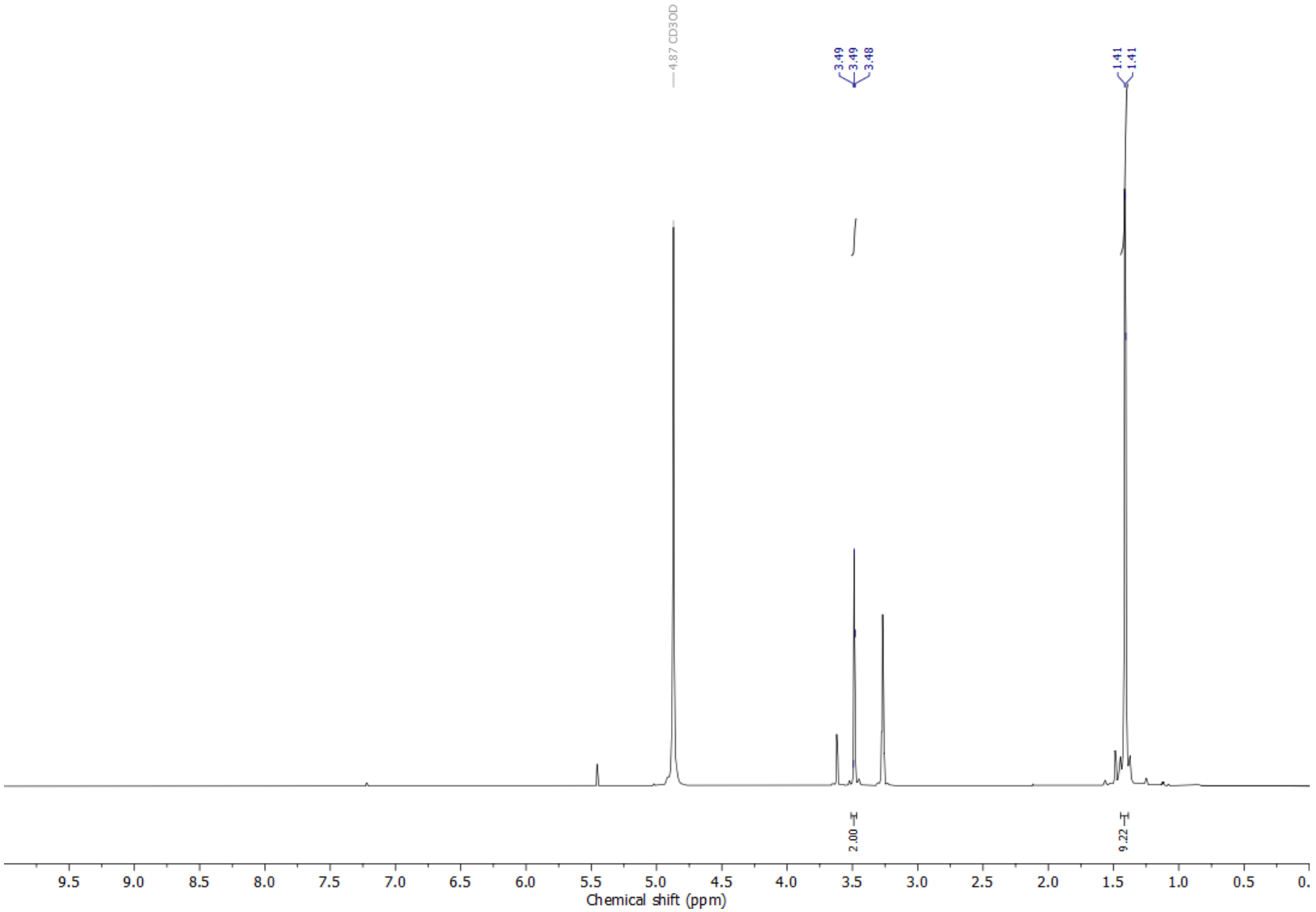
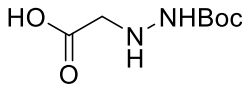


^{13}C NMR (100 MHz, MeOD)



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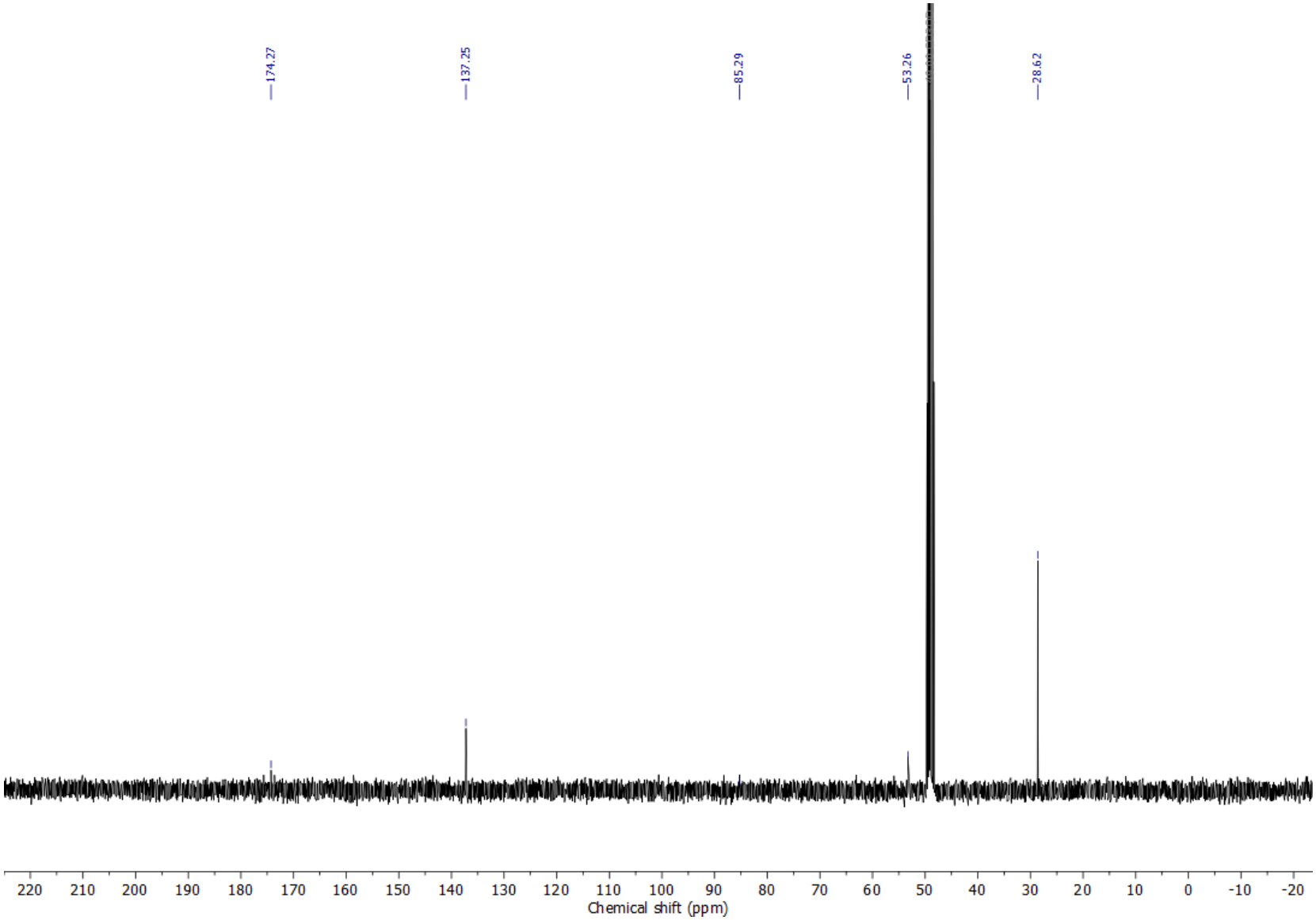
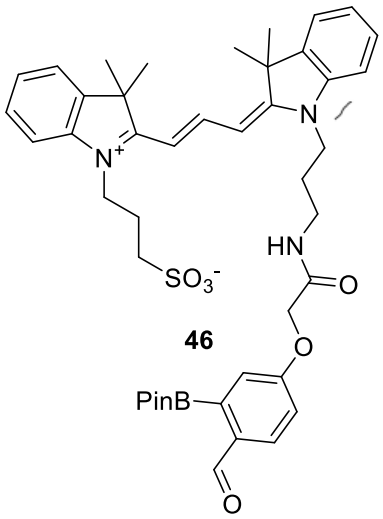
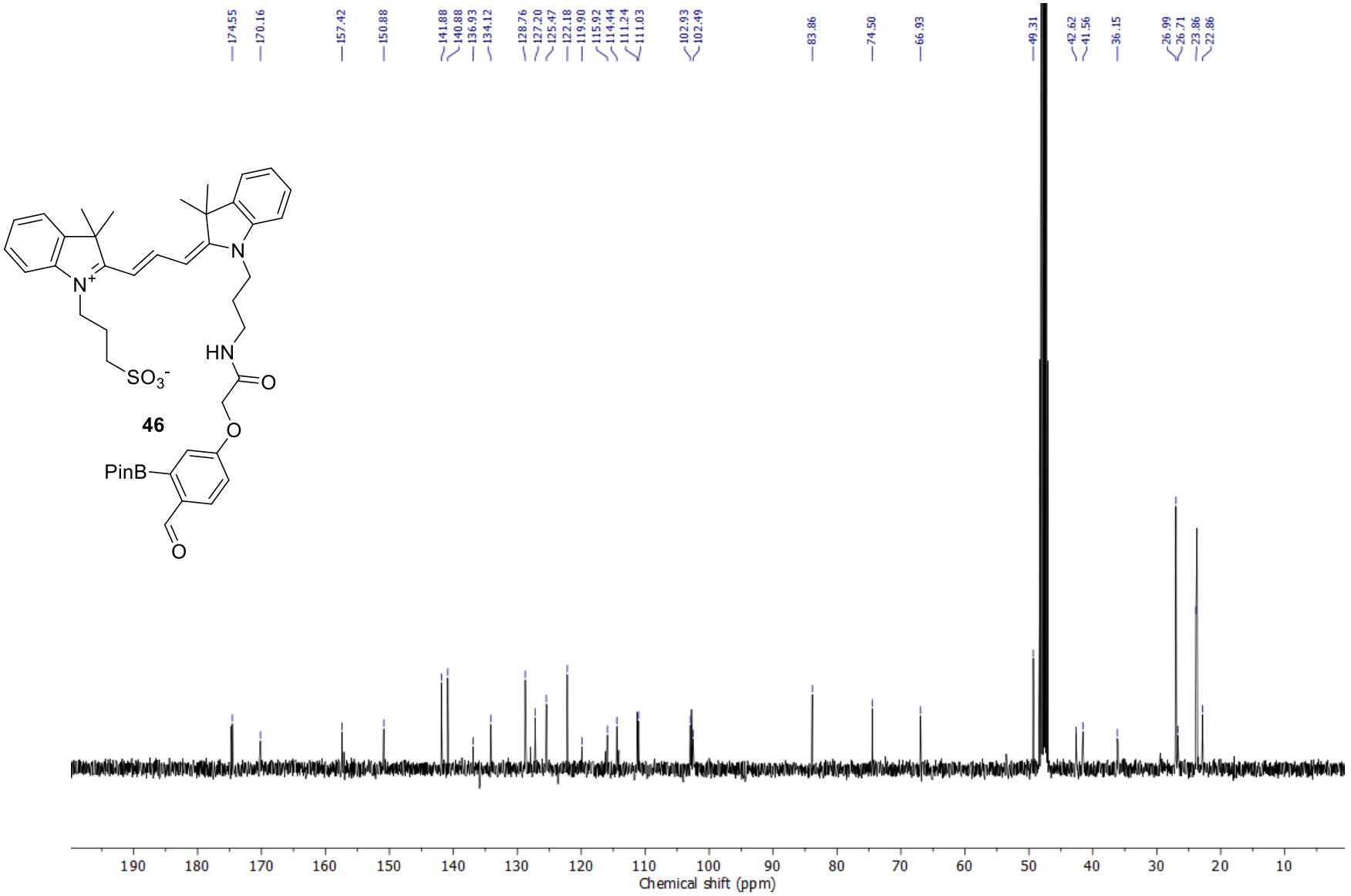


^{1}H NMR (600 MHz, MeOD)

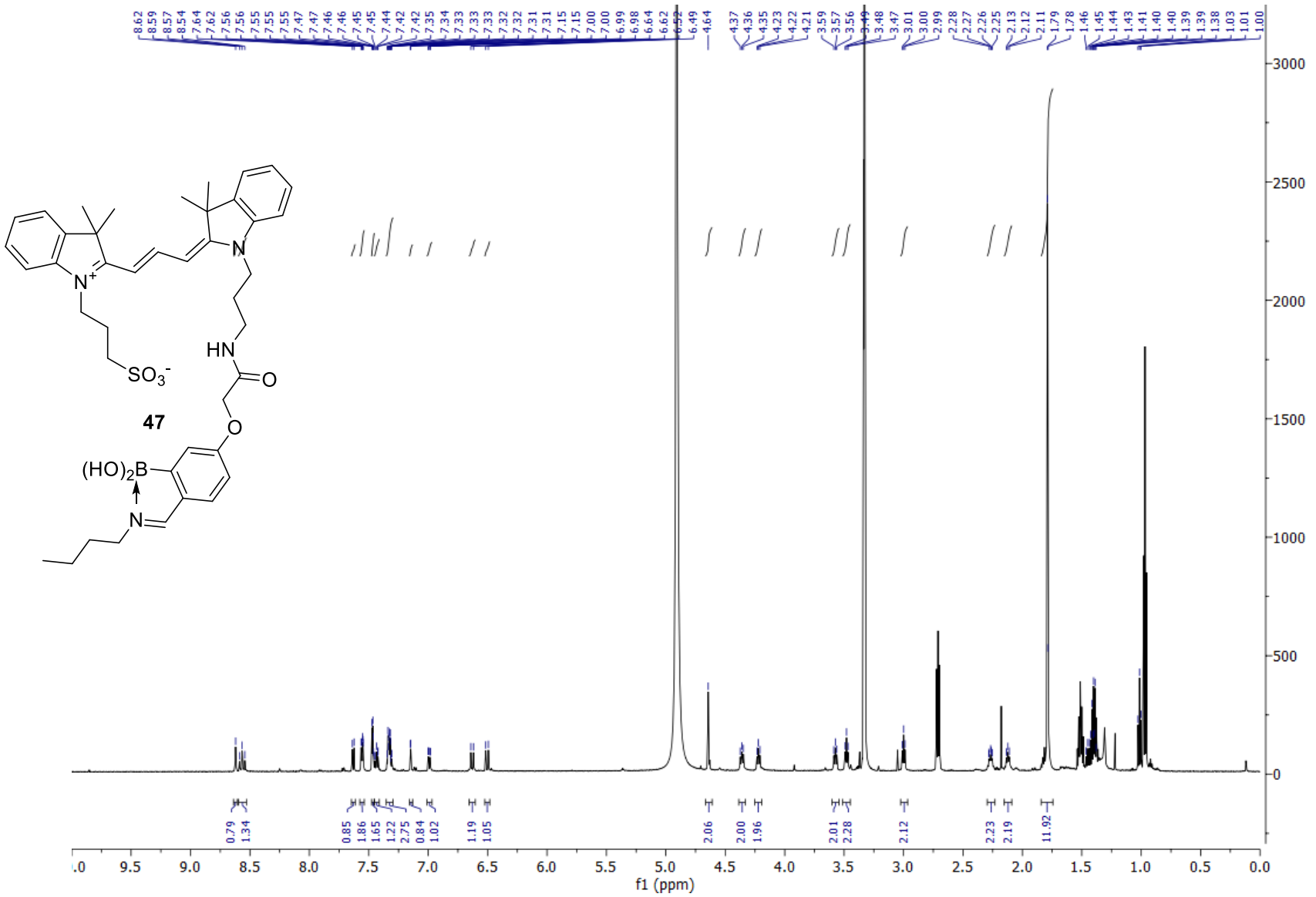


46

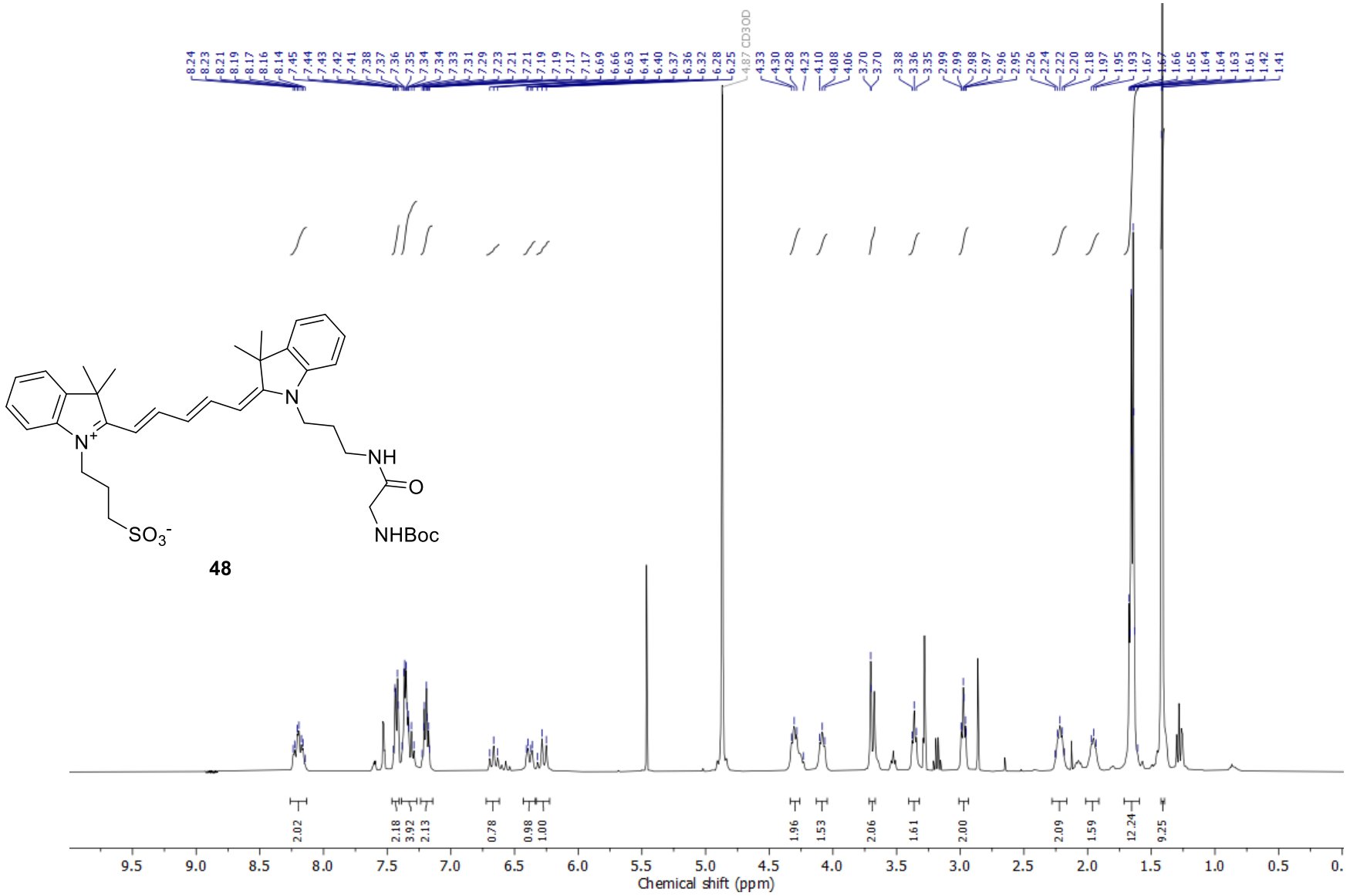
^{13}C NMR(600 MHz, MeOD)



^{1}H NMR (400 MHz, MeOD)

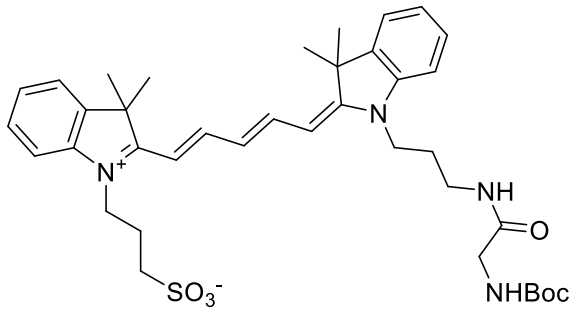


^{1}H NMR (400 MHz, MeOD)



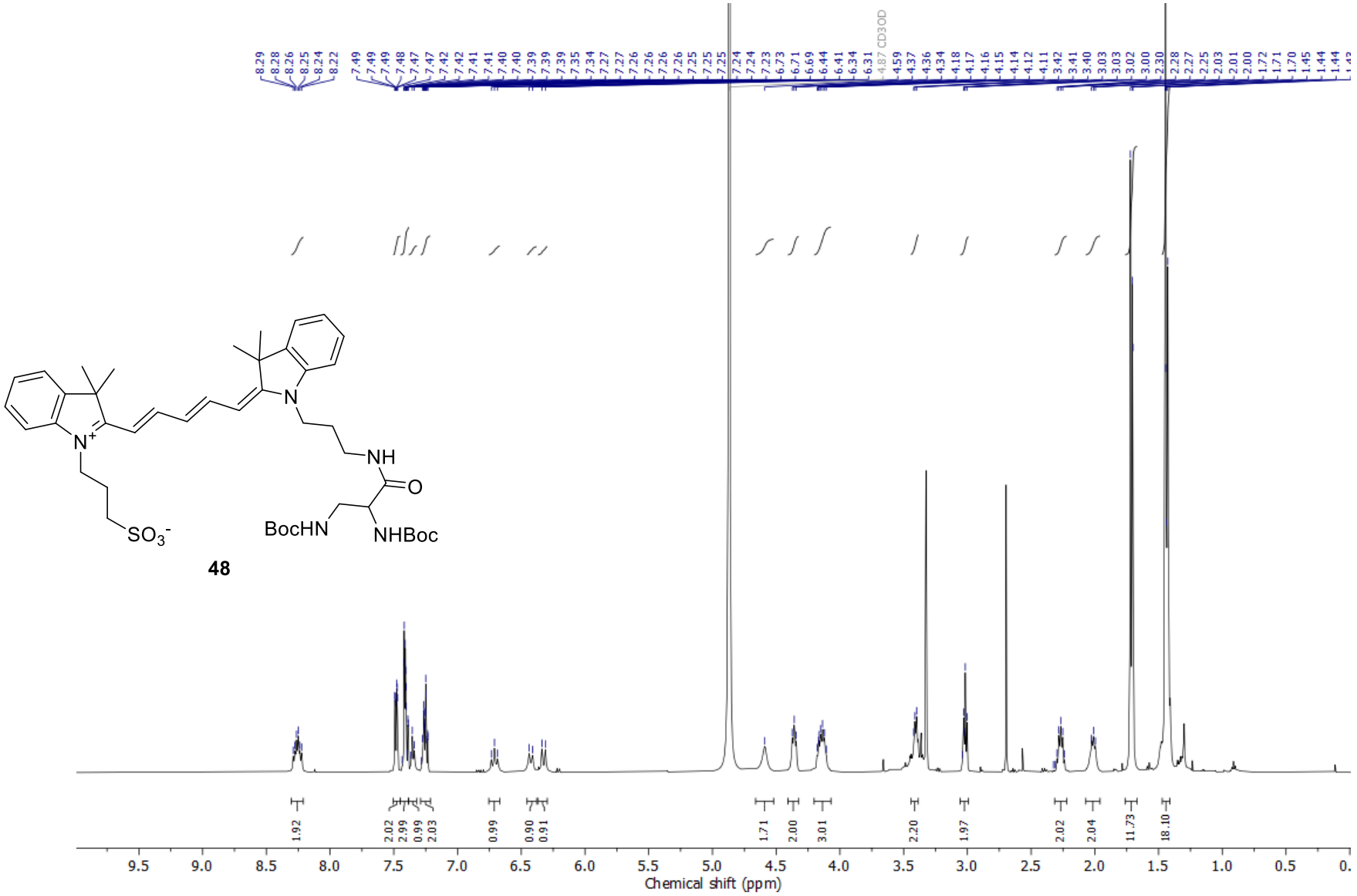
48

^{13}C NMR (100 MHz, MeOD)



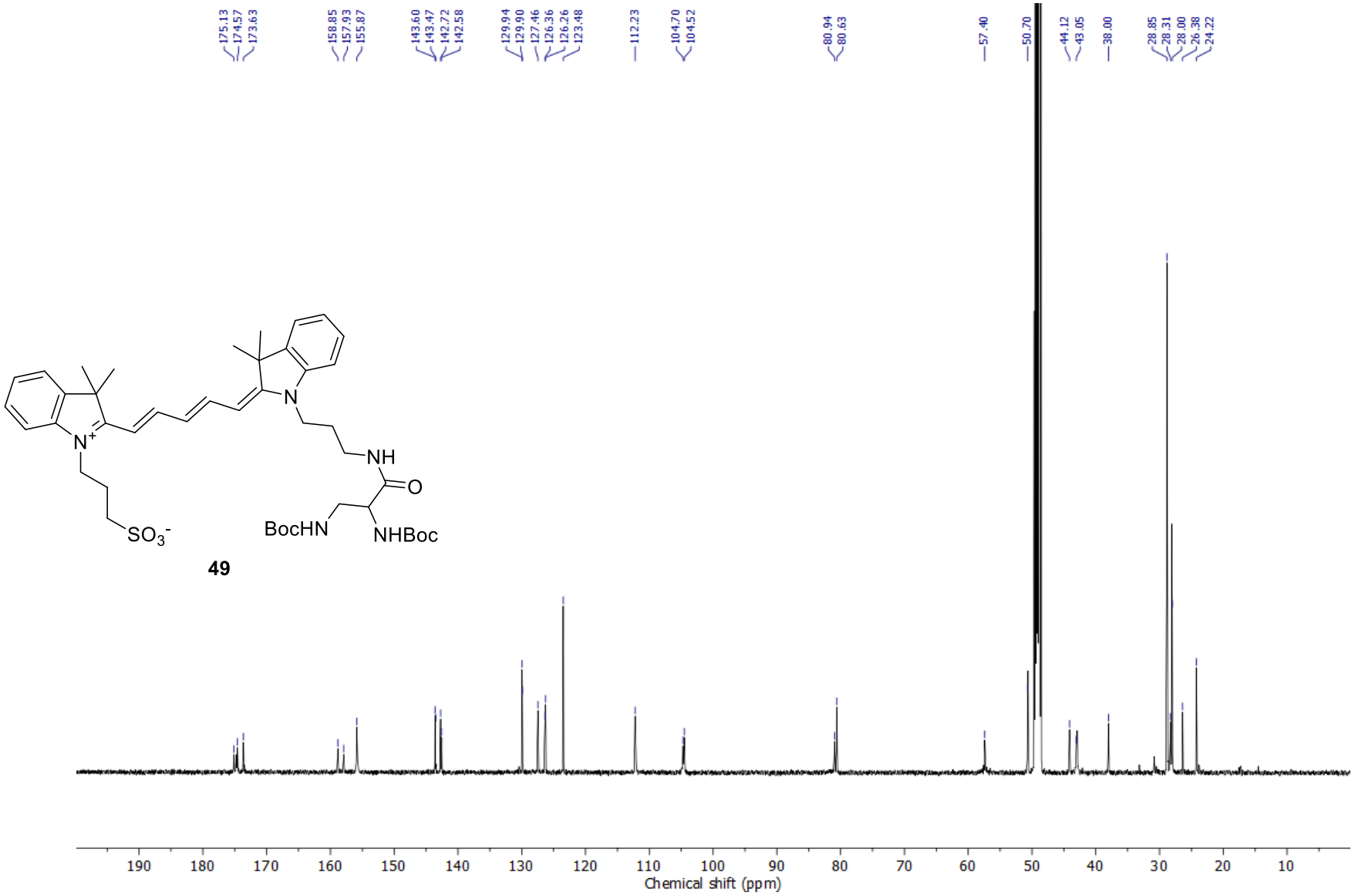
48

^{1}H NMR (400 MHz, MeOD)

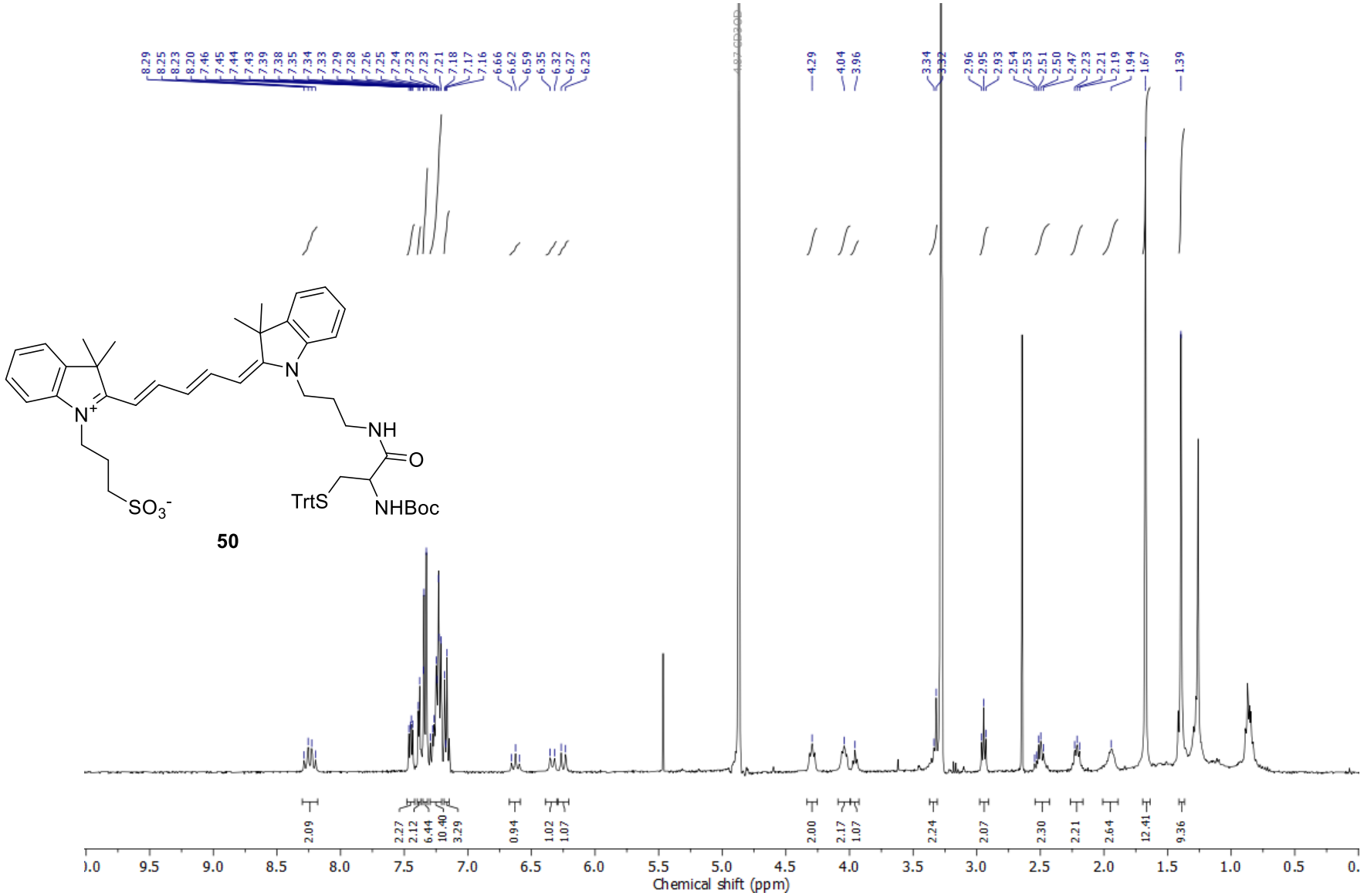


48

^{13}C NMR (100 MHz, MeOD)

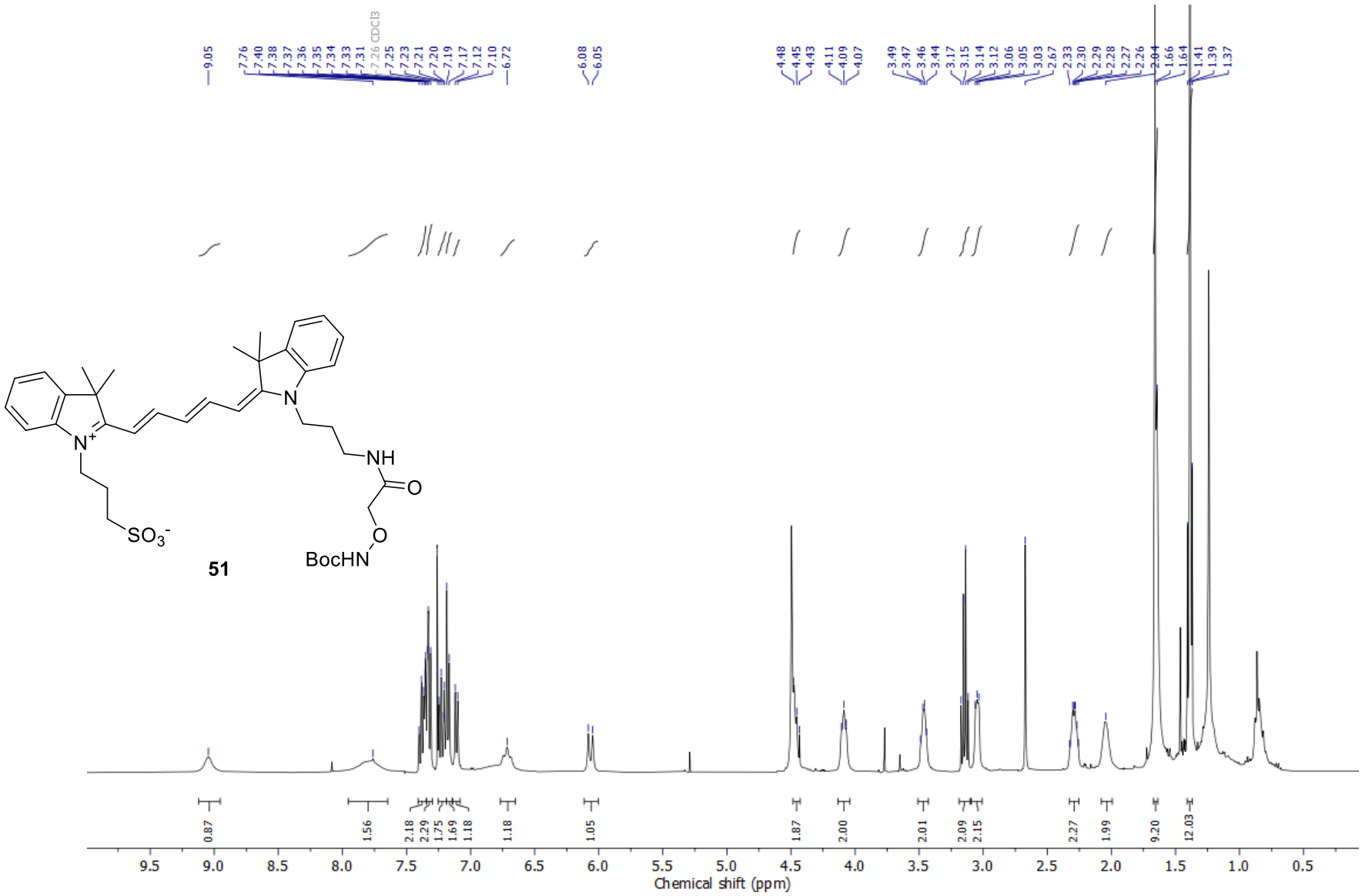


^{1}H NMR (400 MHz, MeOD)

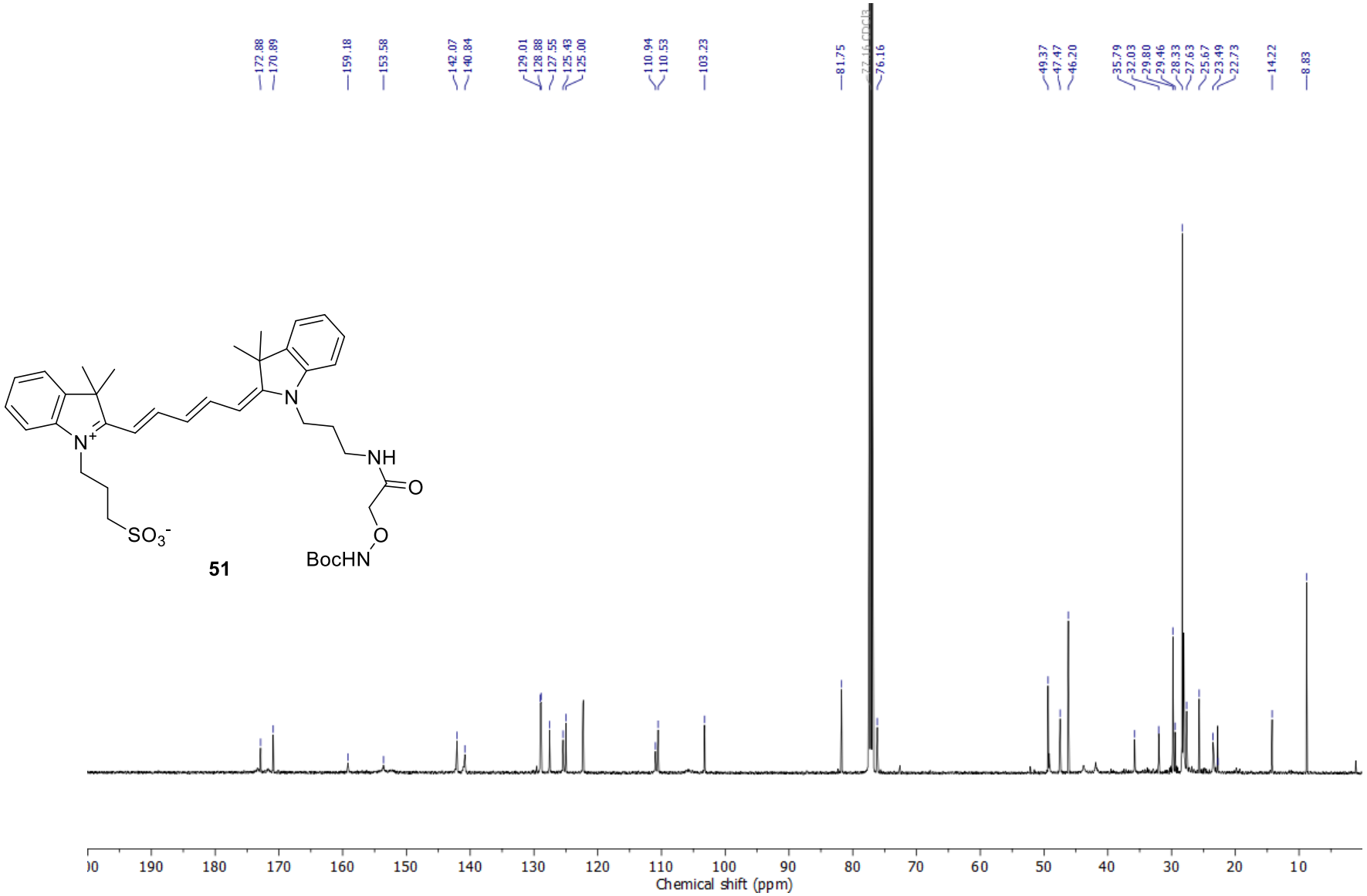


50

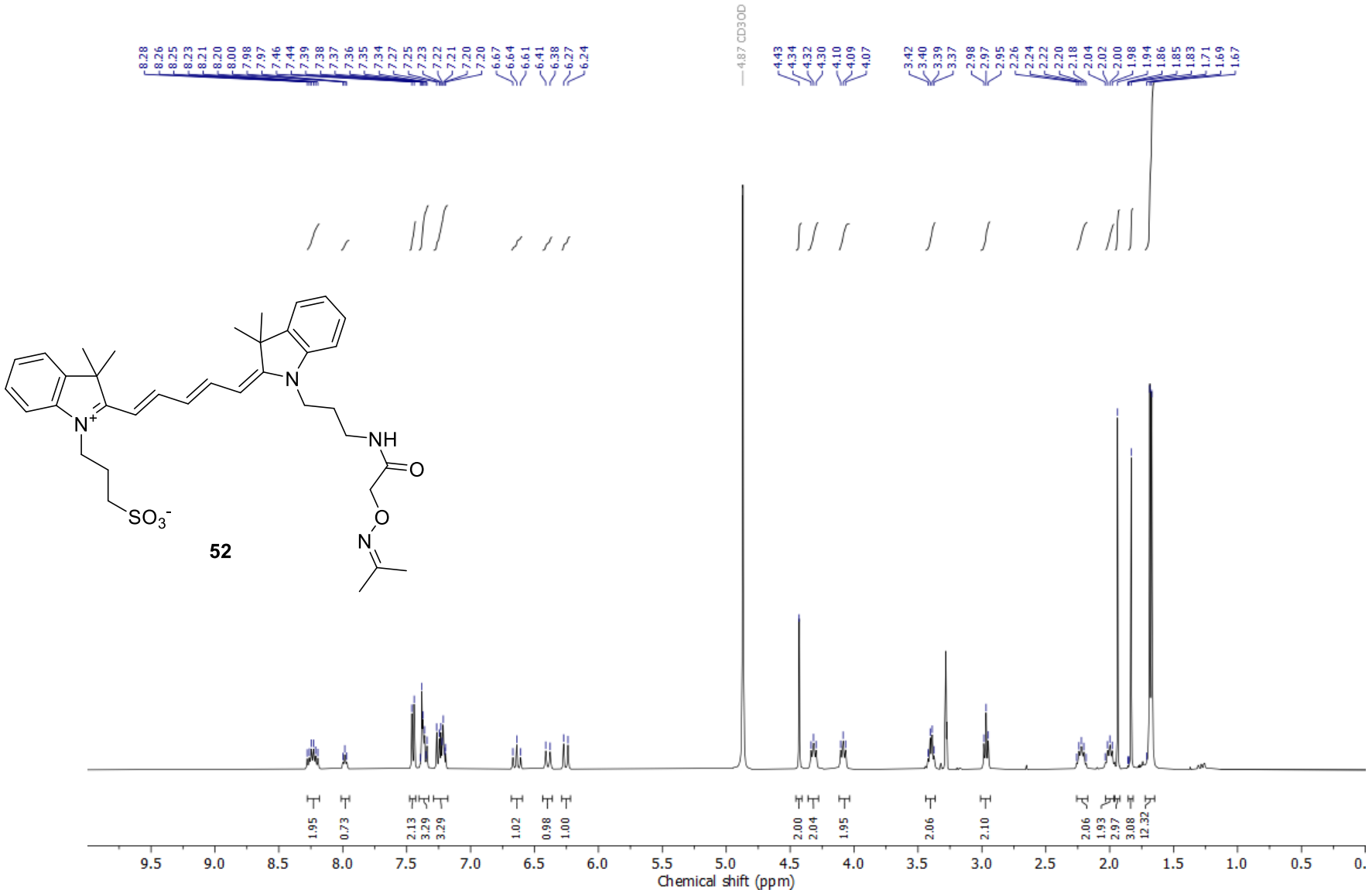
^{1}H NMR (400 MHz, MeOD)



^{13}C NMR (100 MHz, MeOD)

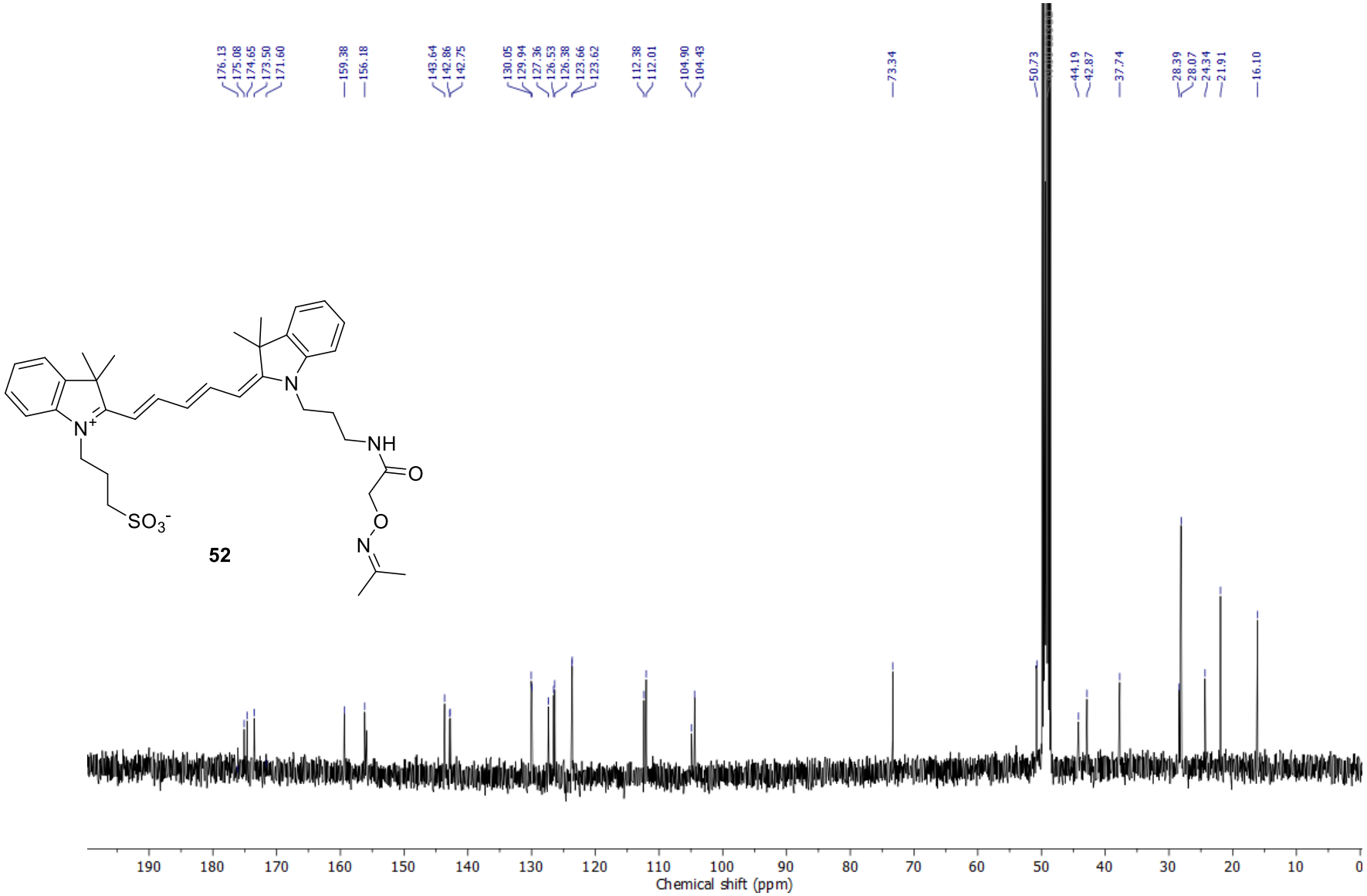


^{1}H NMR (400 MHz, MeOD)

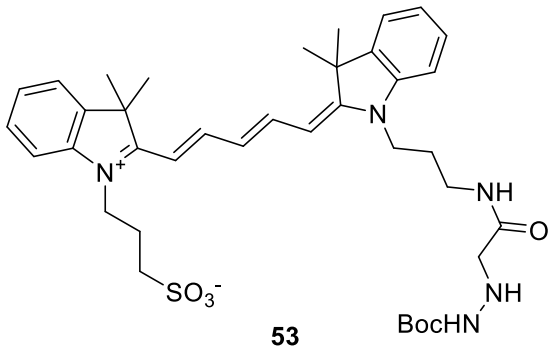


52

^{13}C NMR (100 MHz, MeOD)

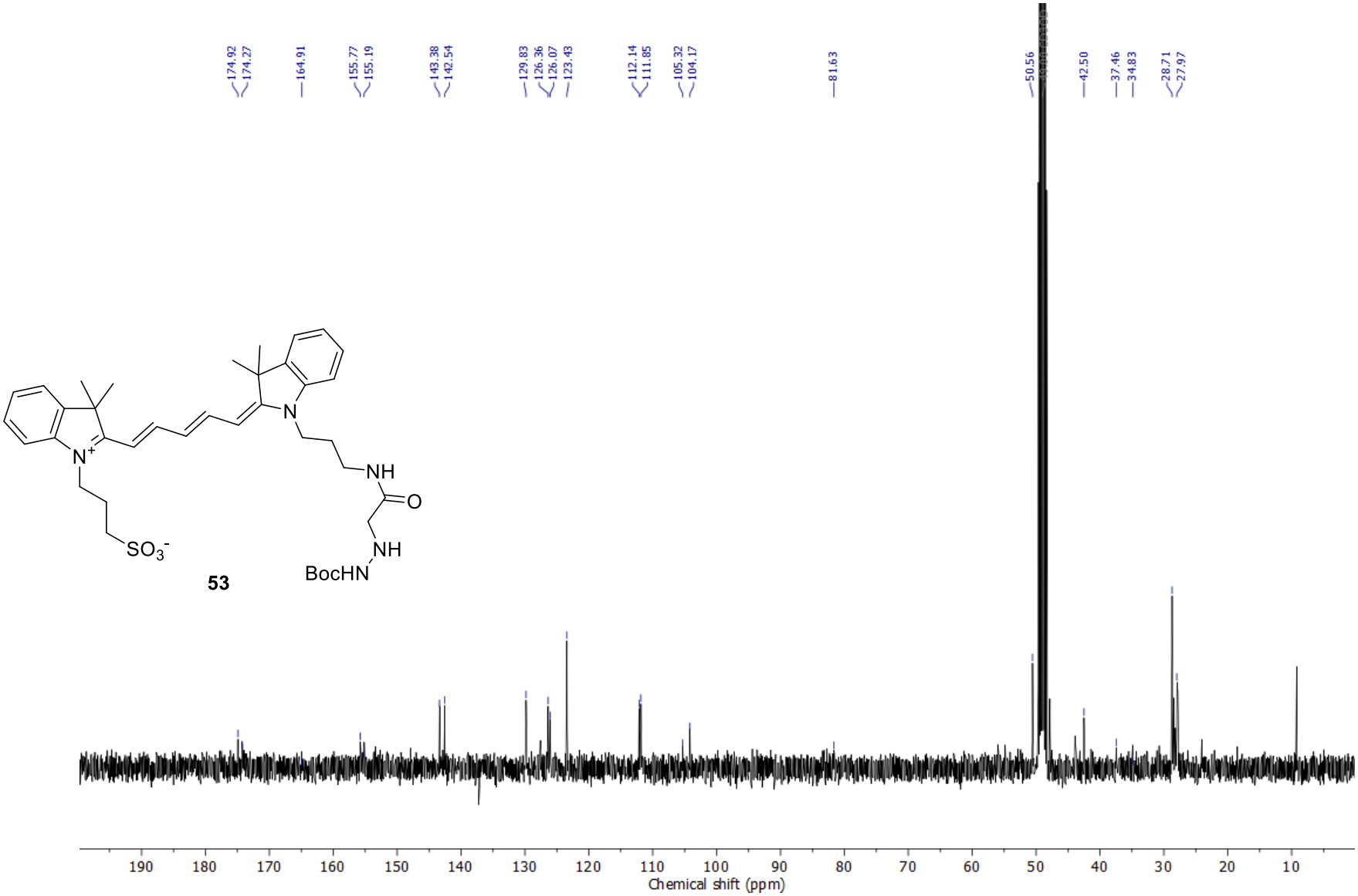


^{1}H NMR (400 MHz, MeOD)



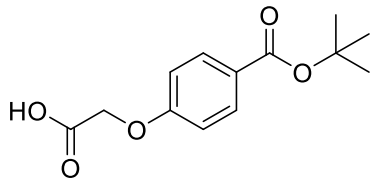
53

^{13}C NMR (100 MHz, MeOD)



53

^{1}H NMR (400 MHz, $CDCl_3$)



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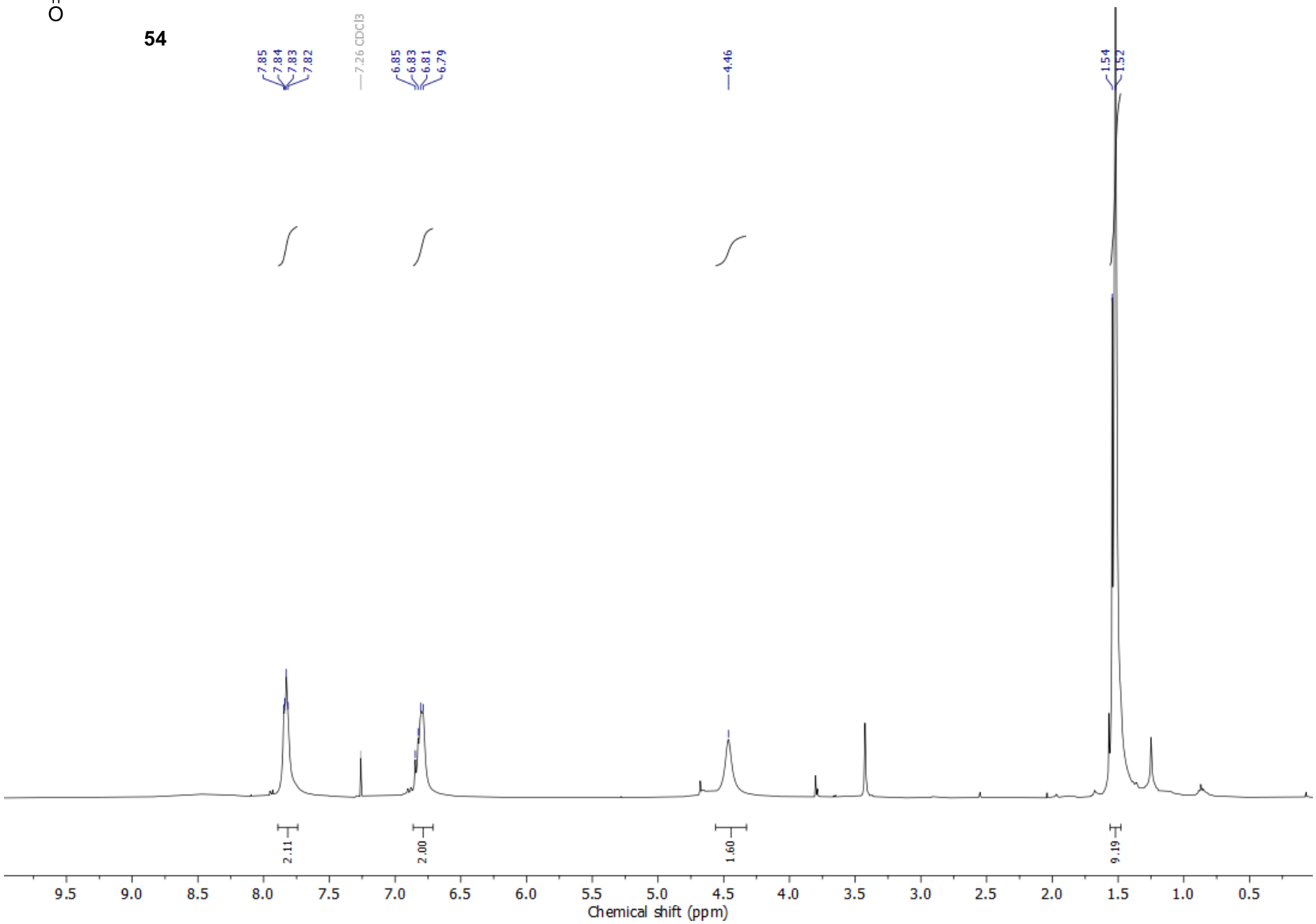
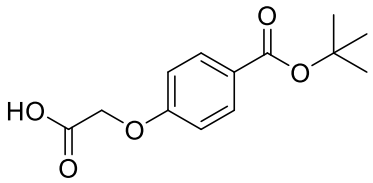


^{13}C NMR (100 MHz, $CDCl_3$)



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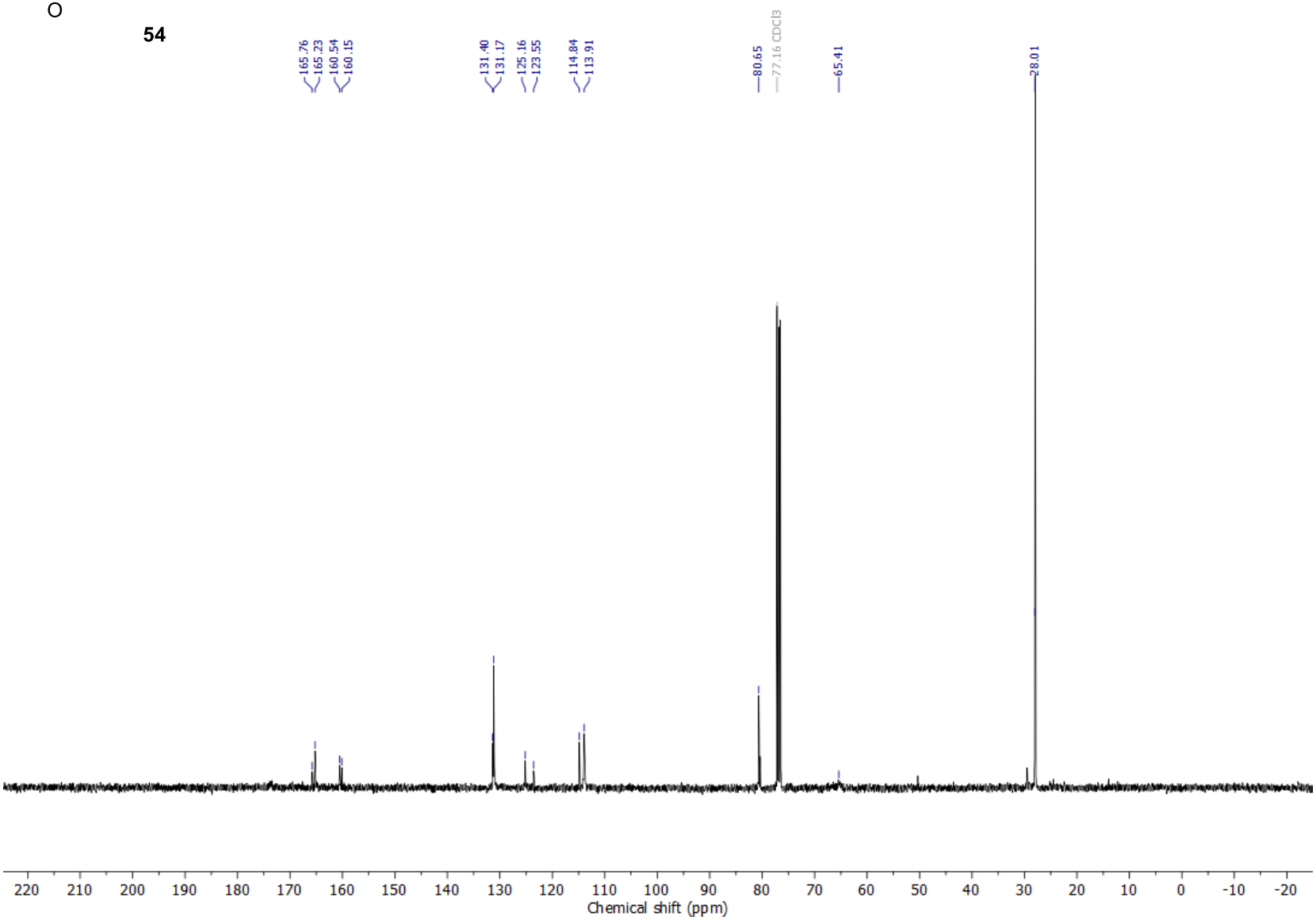
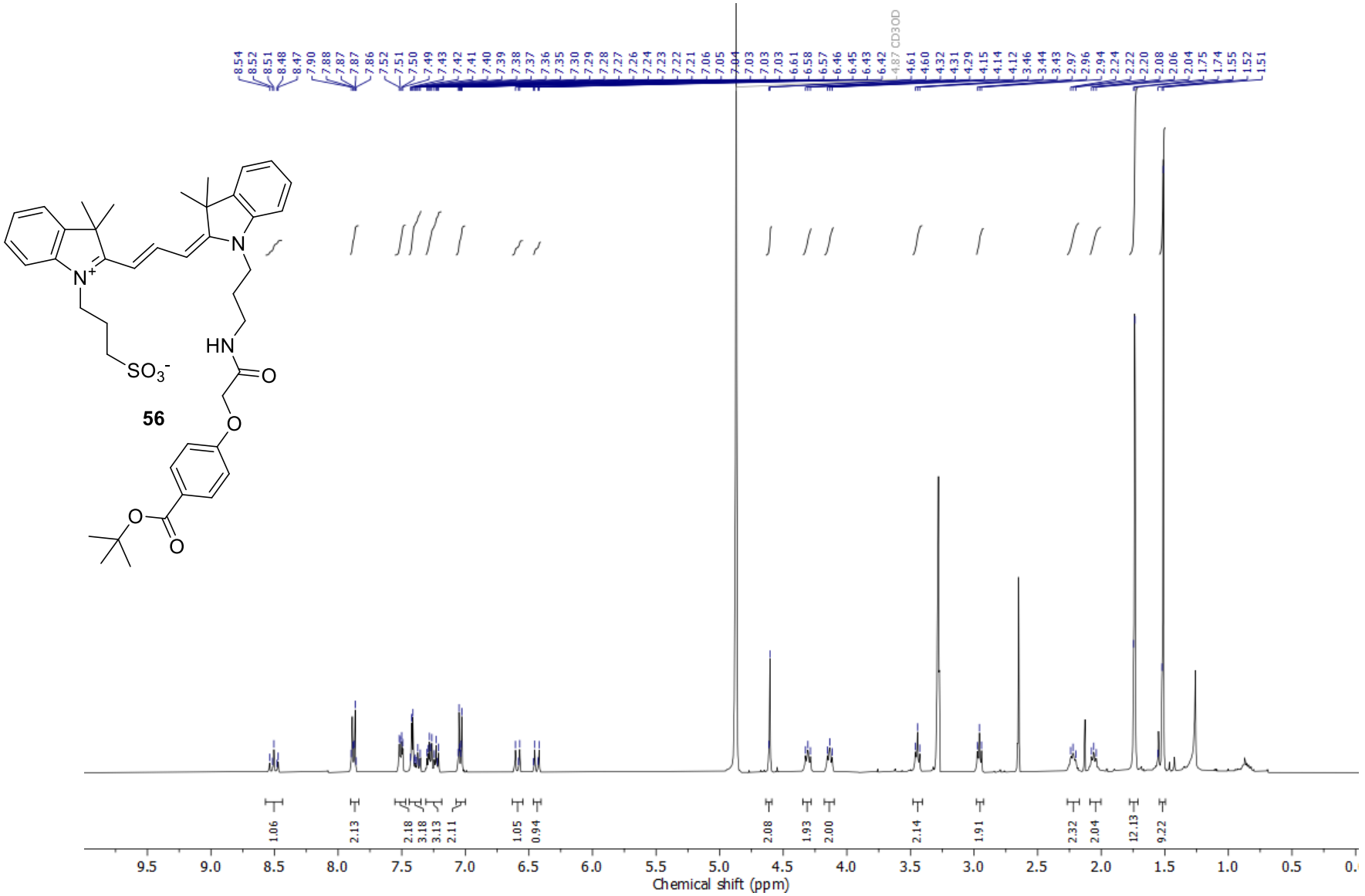
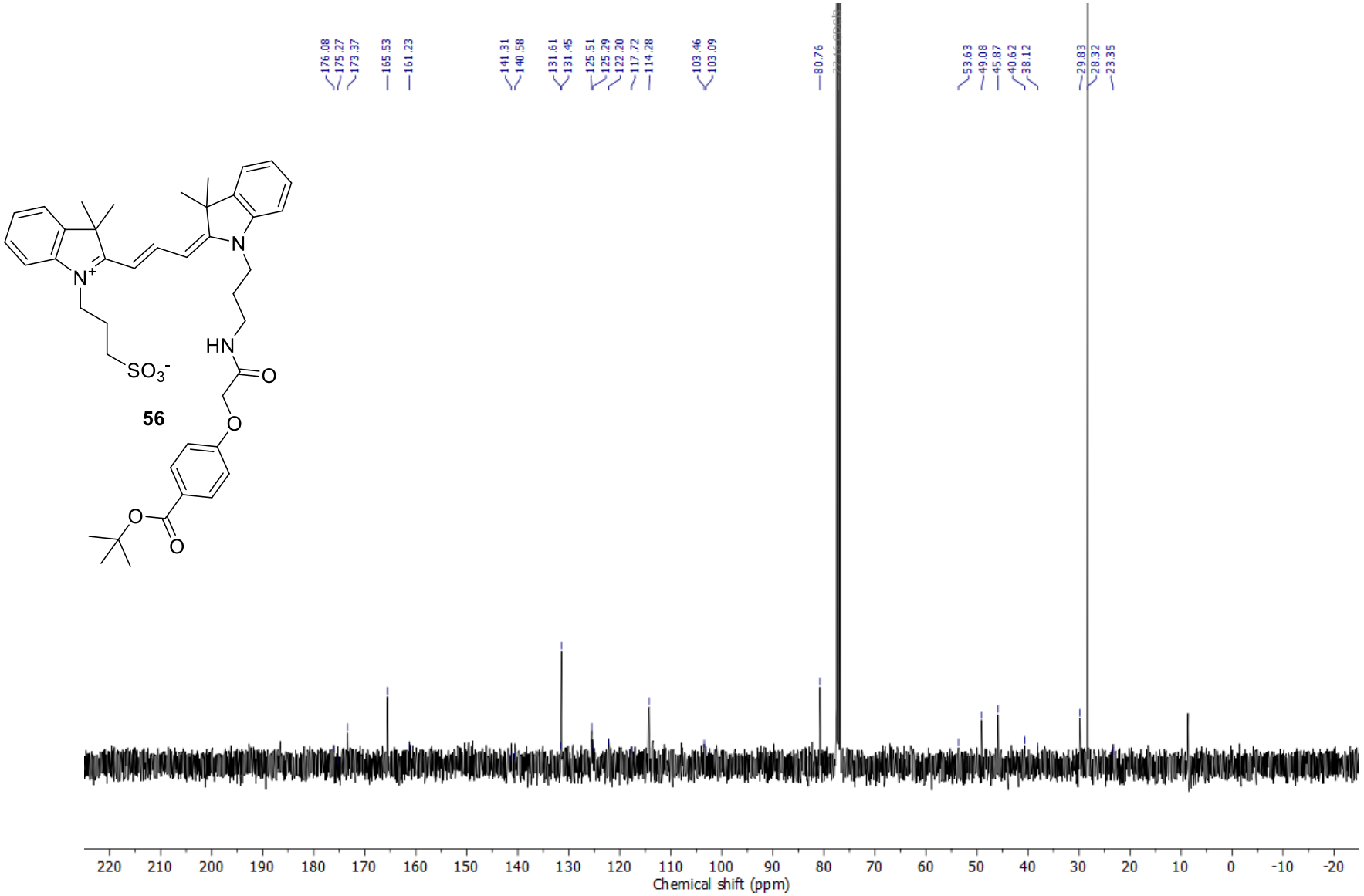


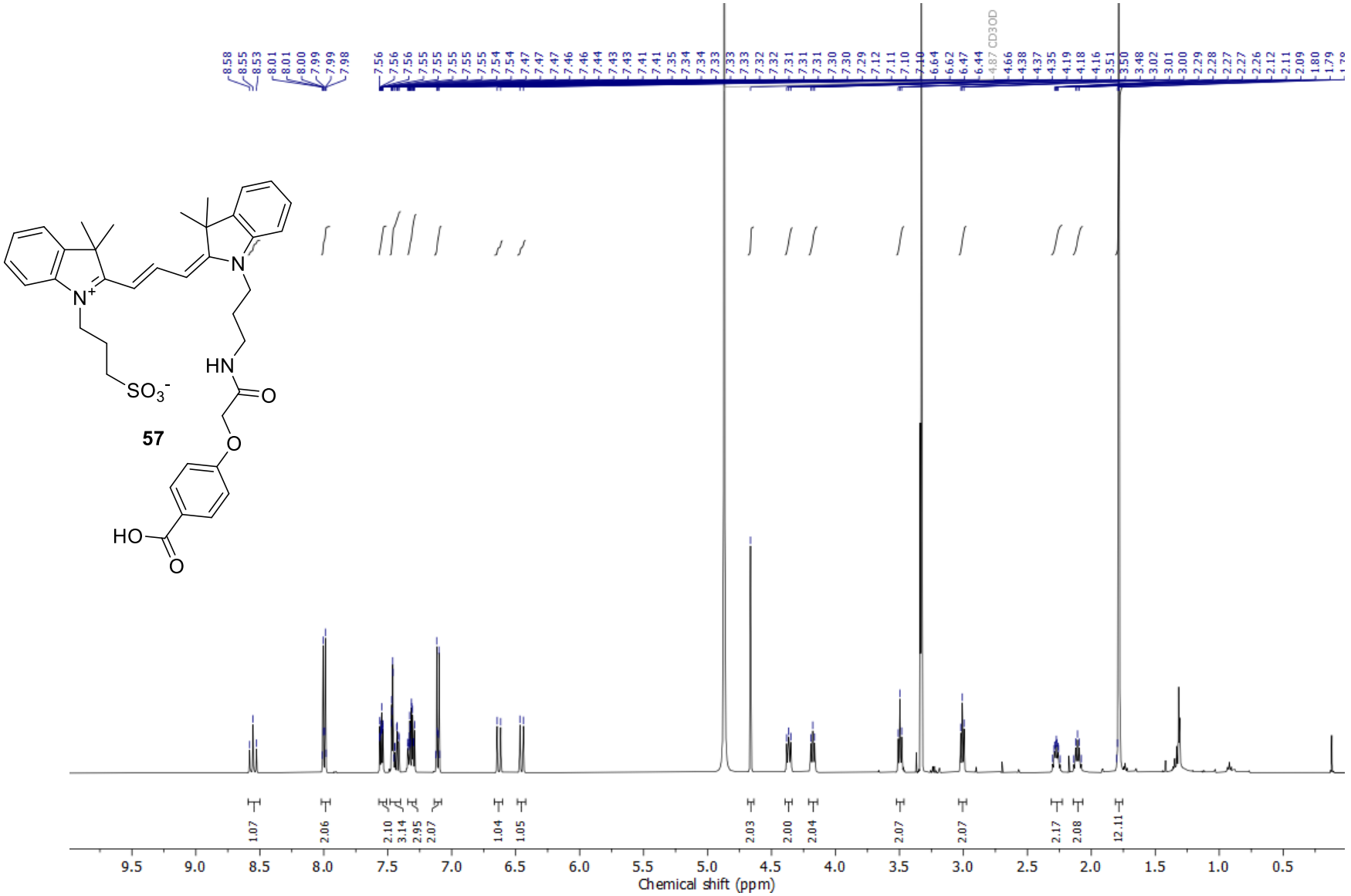
^{1}H NMR (600 MHz, MeOD)



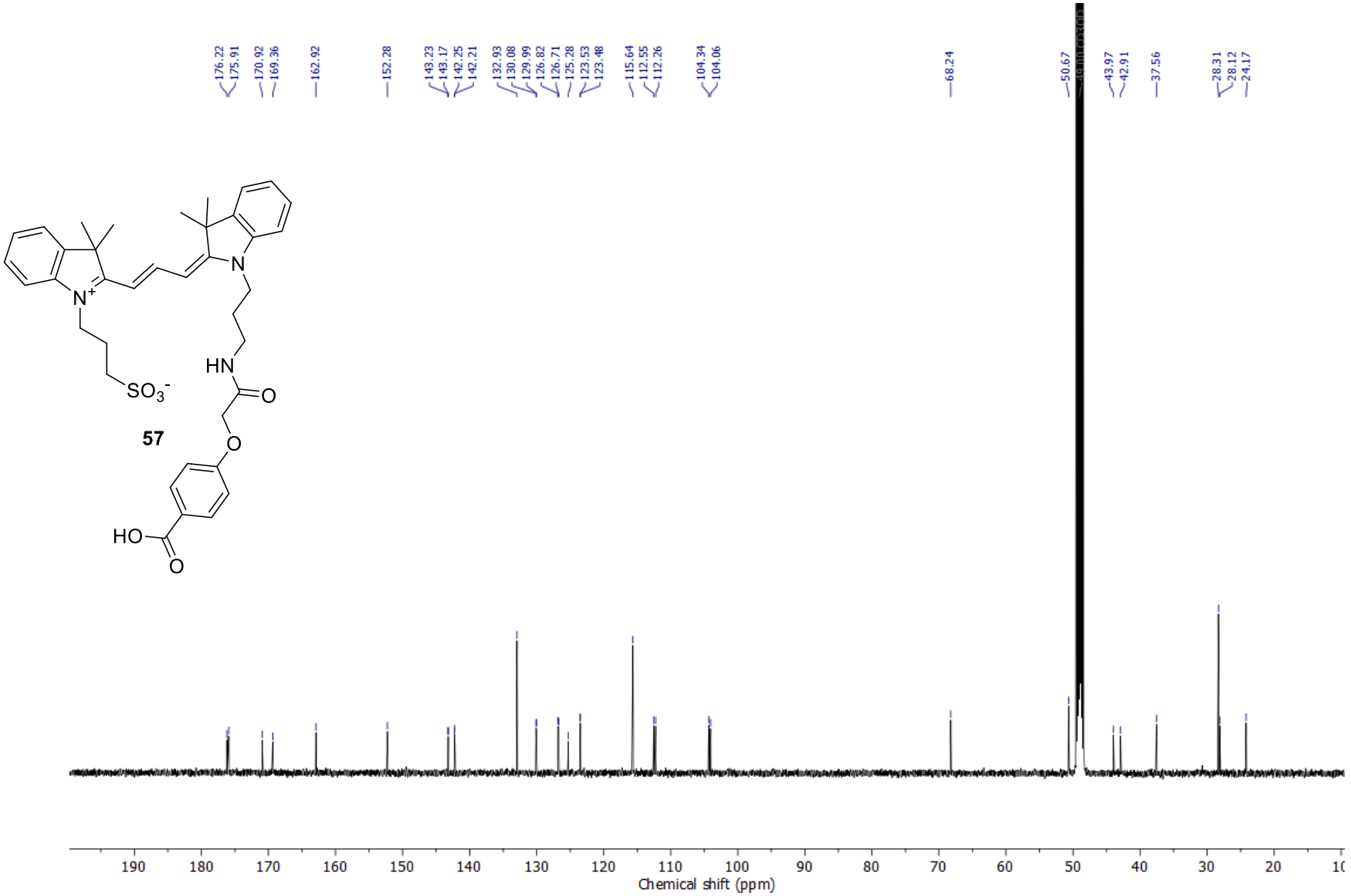
^{13}C NMR(600 MHz, MeOD)



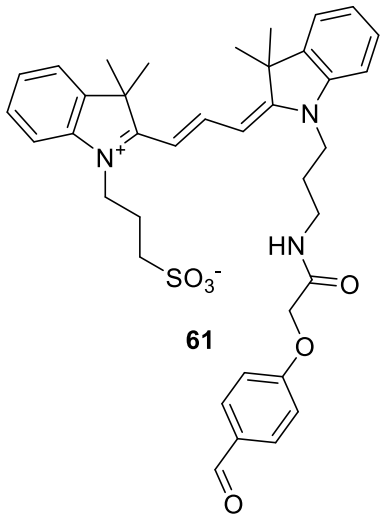
^{1}H NMR (600 MHz, MeOD)



^{13}C NMR(600 MHz, MeOD)

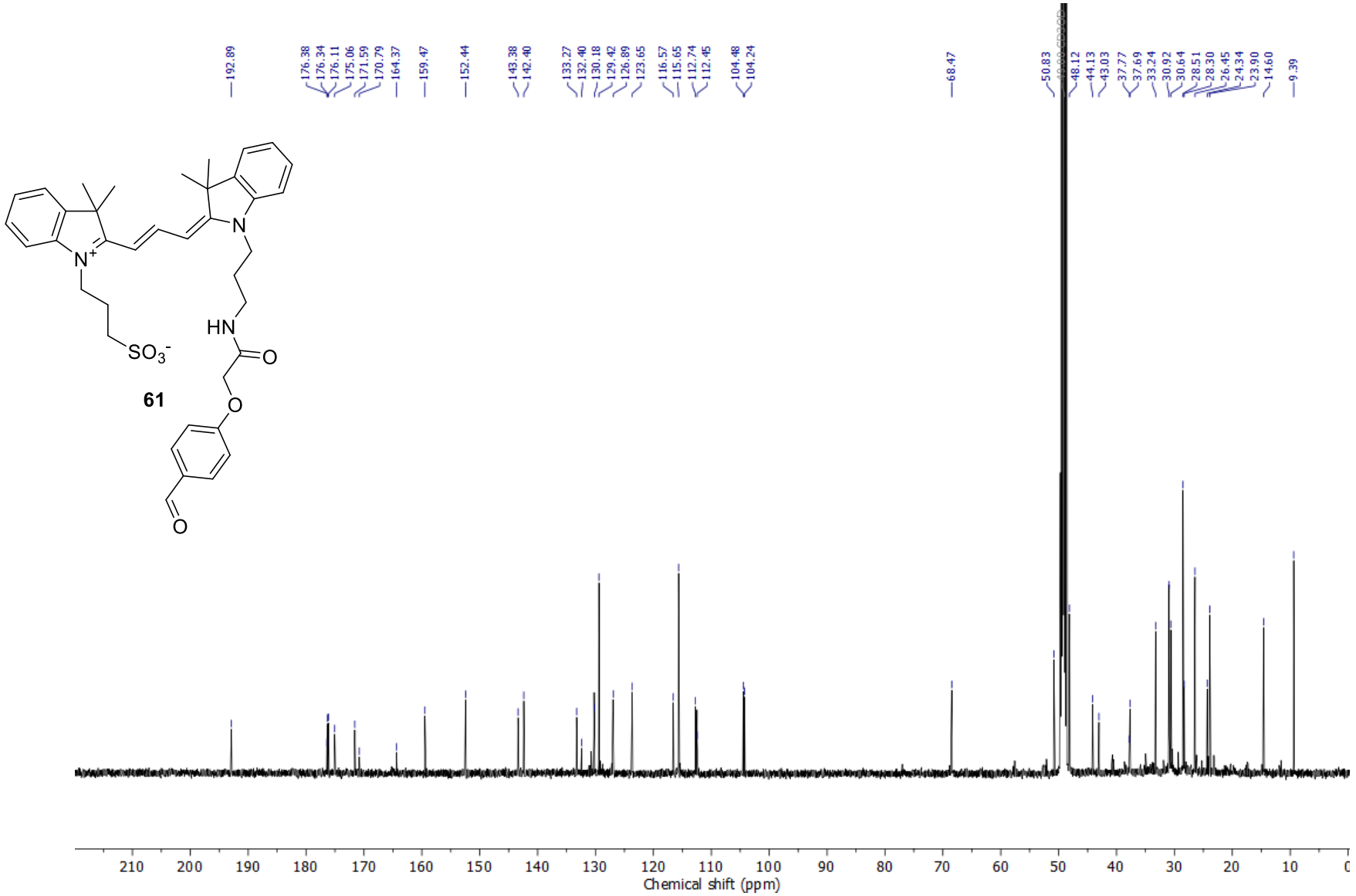


^{1}H NMR (500 MHz, MeOD)

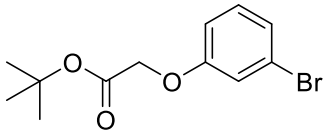


61

^{13}C NMR(500 MHz, MeOD)



^{1}H NMR (400 MHz, $CDCl_3$)



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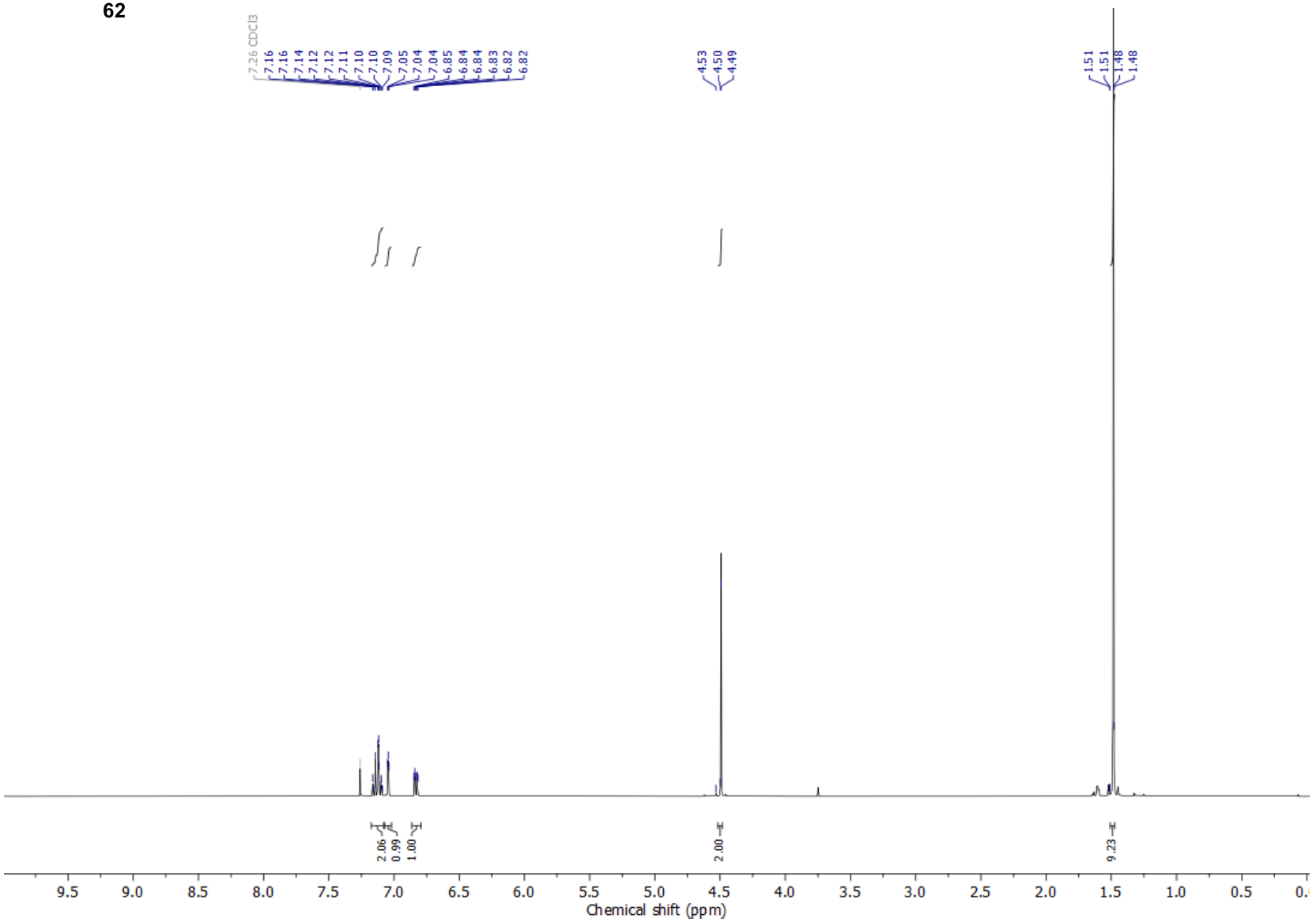
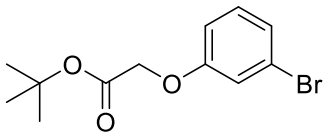


^{13}C NMR (100 MHz, $CDCl_3$)



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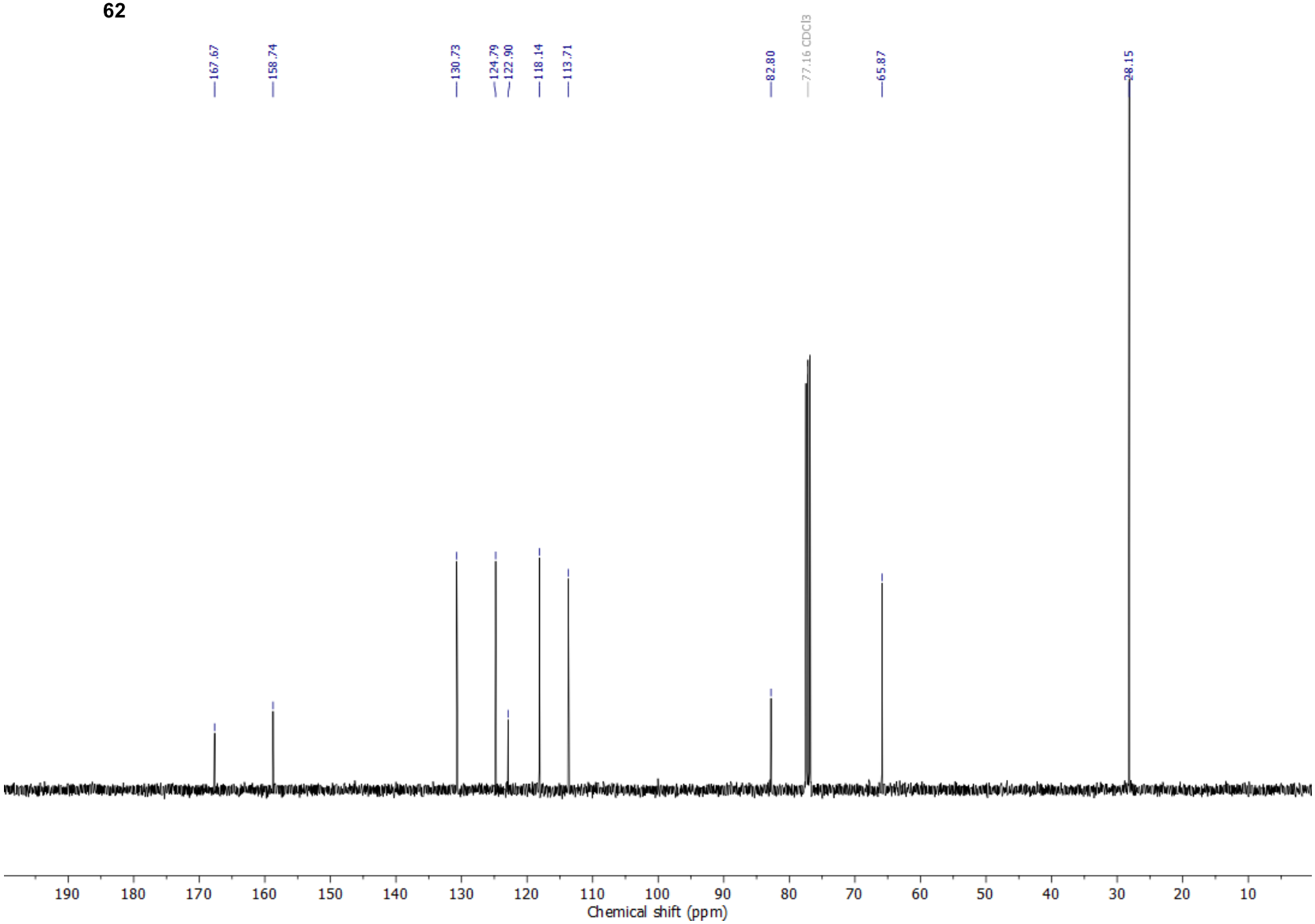
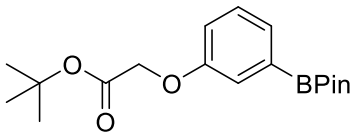


^{1}H NMR (400 MHz, $CDCl_3$)



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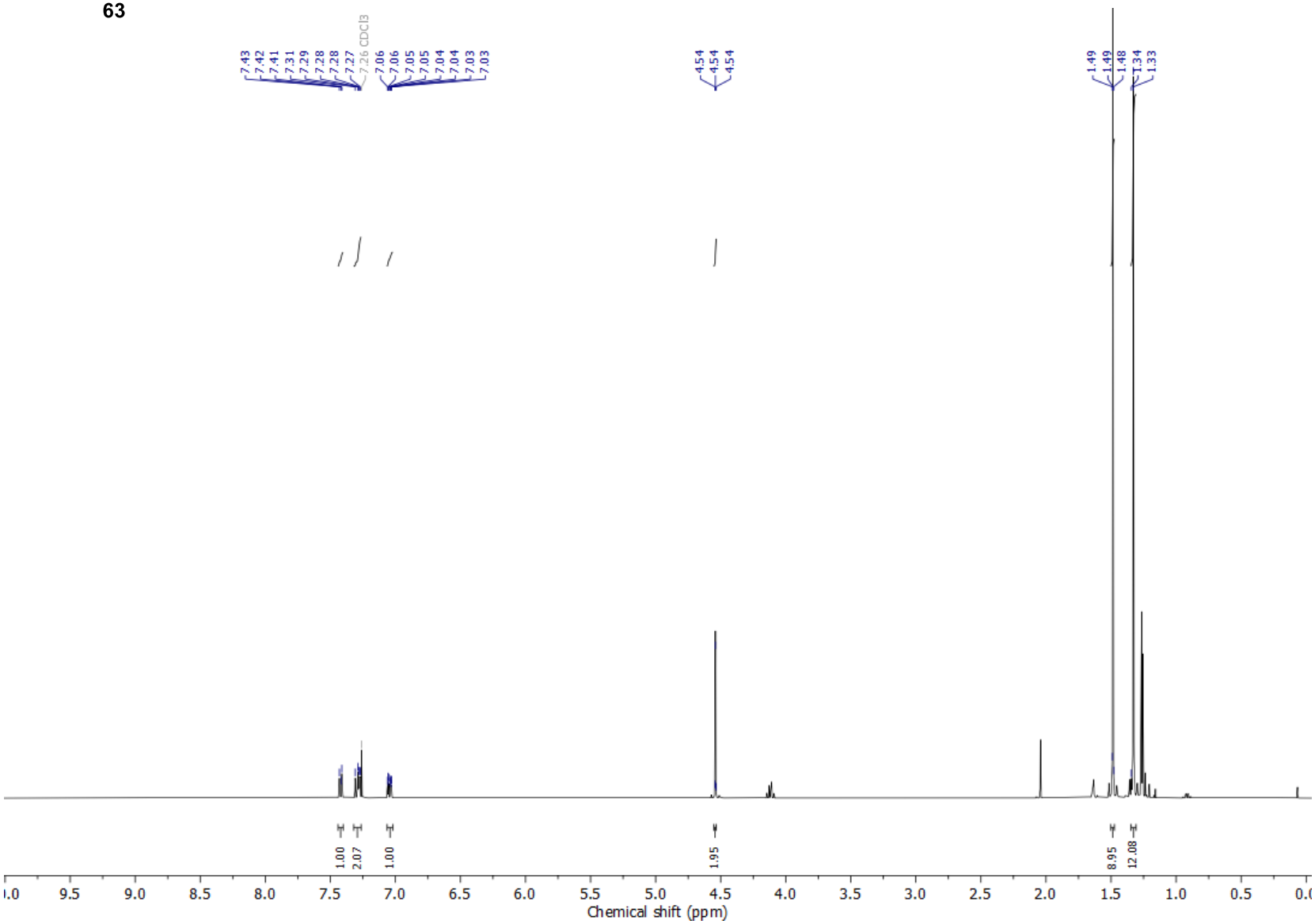
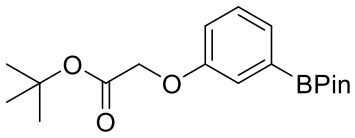


^{13}C NMR (100 MHz, $CDCl_3$)



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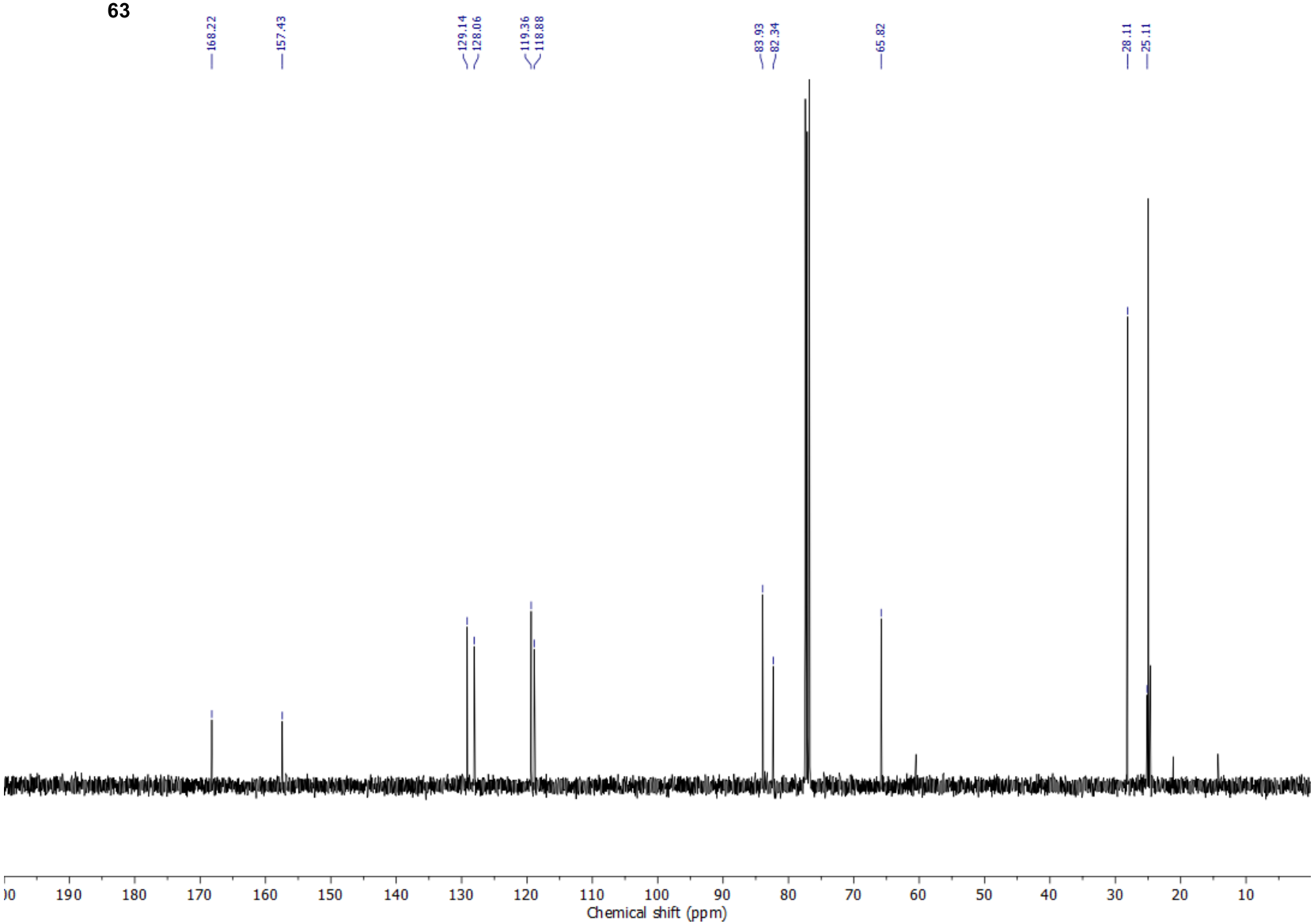
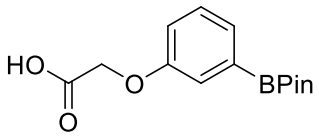


^{1}H NMR (400 MHz, MeOD)



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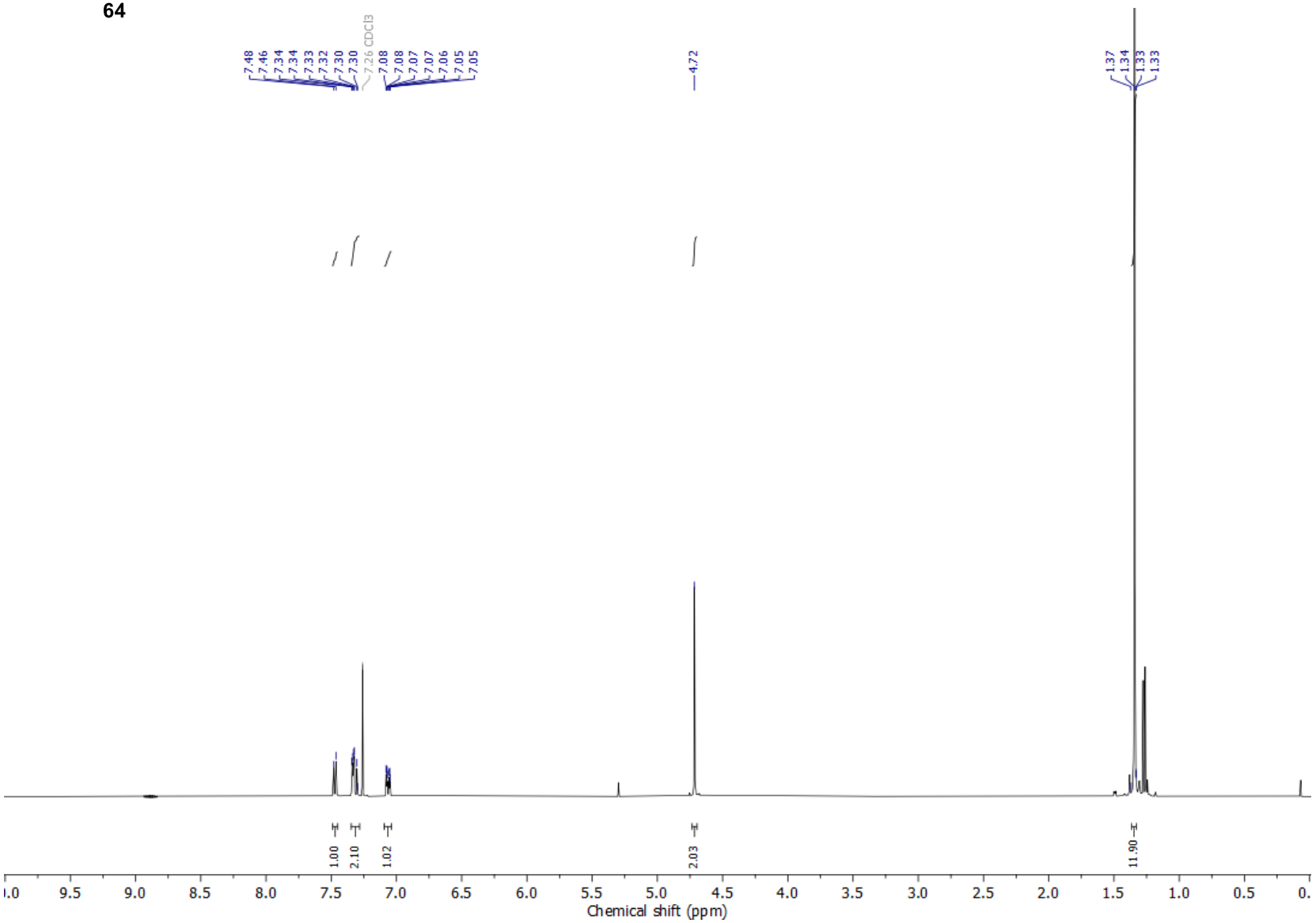
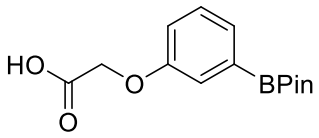


^{13}C NMR (100 MHz, MeOD)



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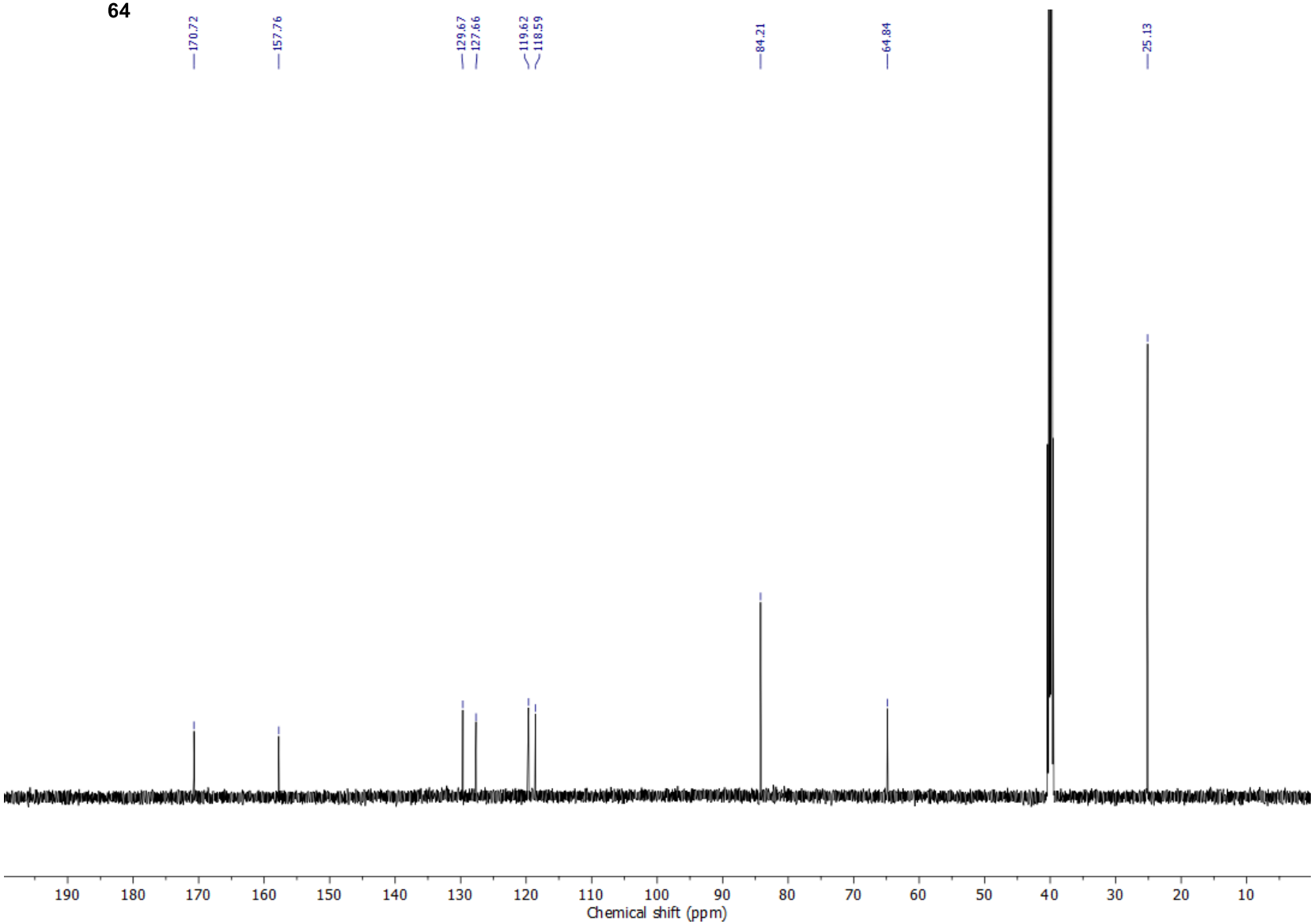
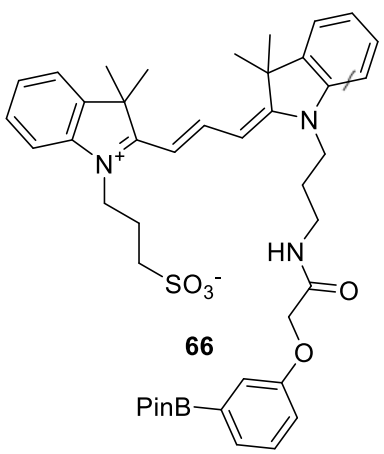


^{1}H NMR (500 MHz, MeOD)



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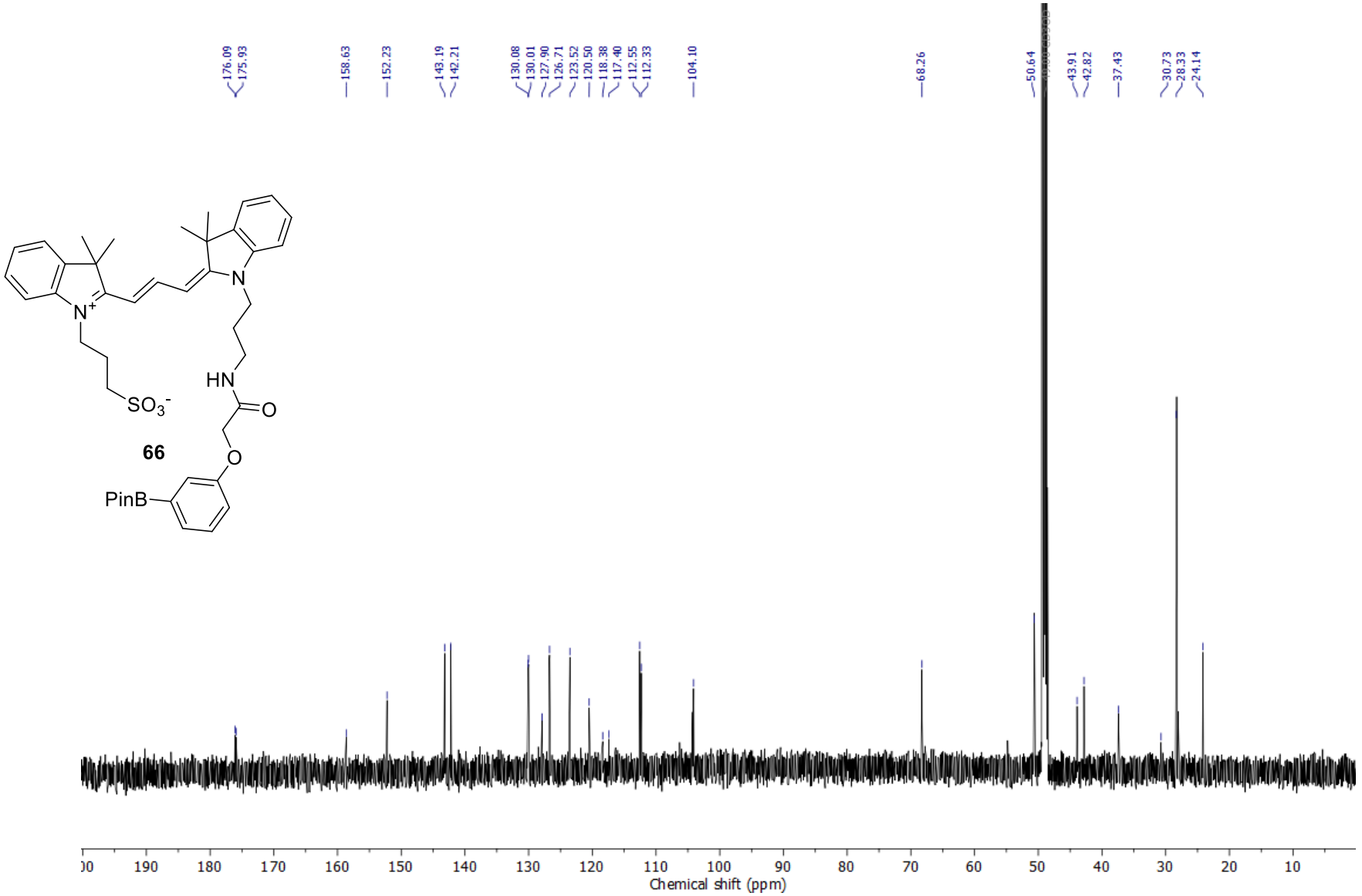
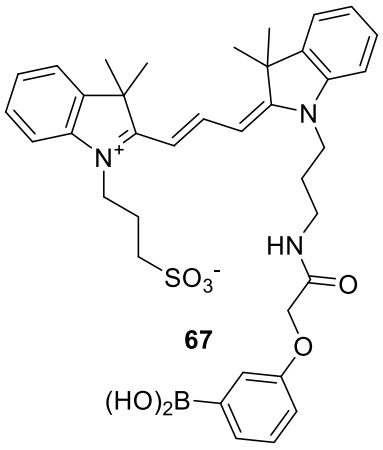
^{13}C NMR(500 MHz, MeOD)

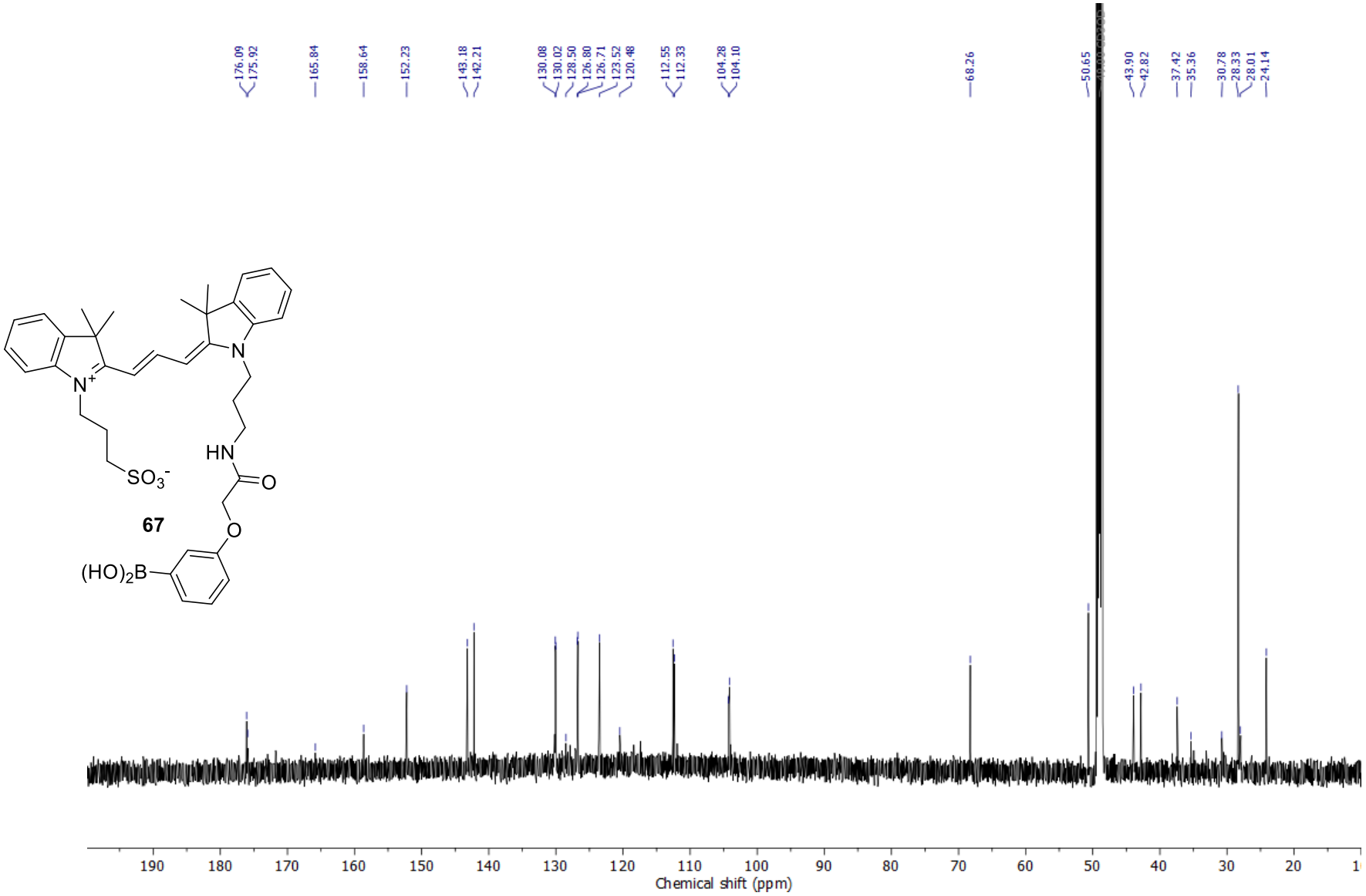


^{1}H NMR (500 MHz, MeOD)

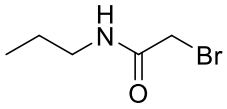


67

^{13}C NMR(500 MHz, MeOD)



^{1}H NMR (400 MHz, $CDCl_3$)



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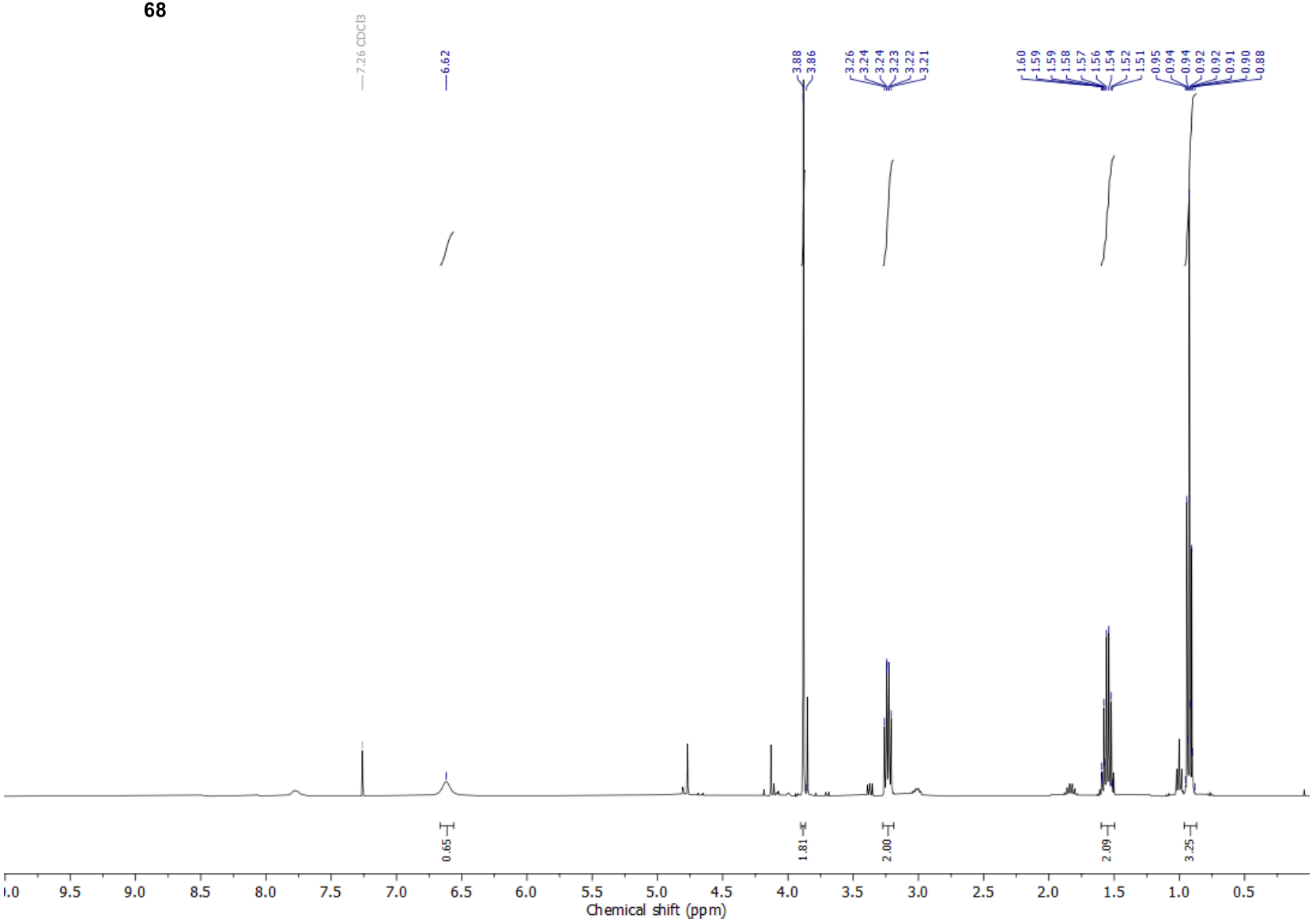
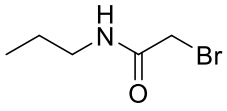


^{13}C NMR (100 MHz, $CDCl_3$)



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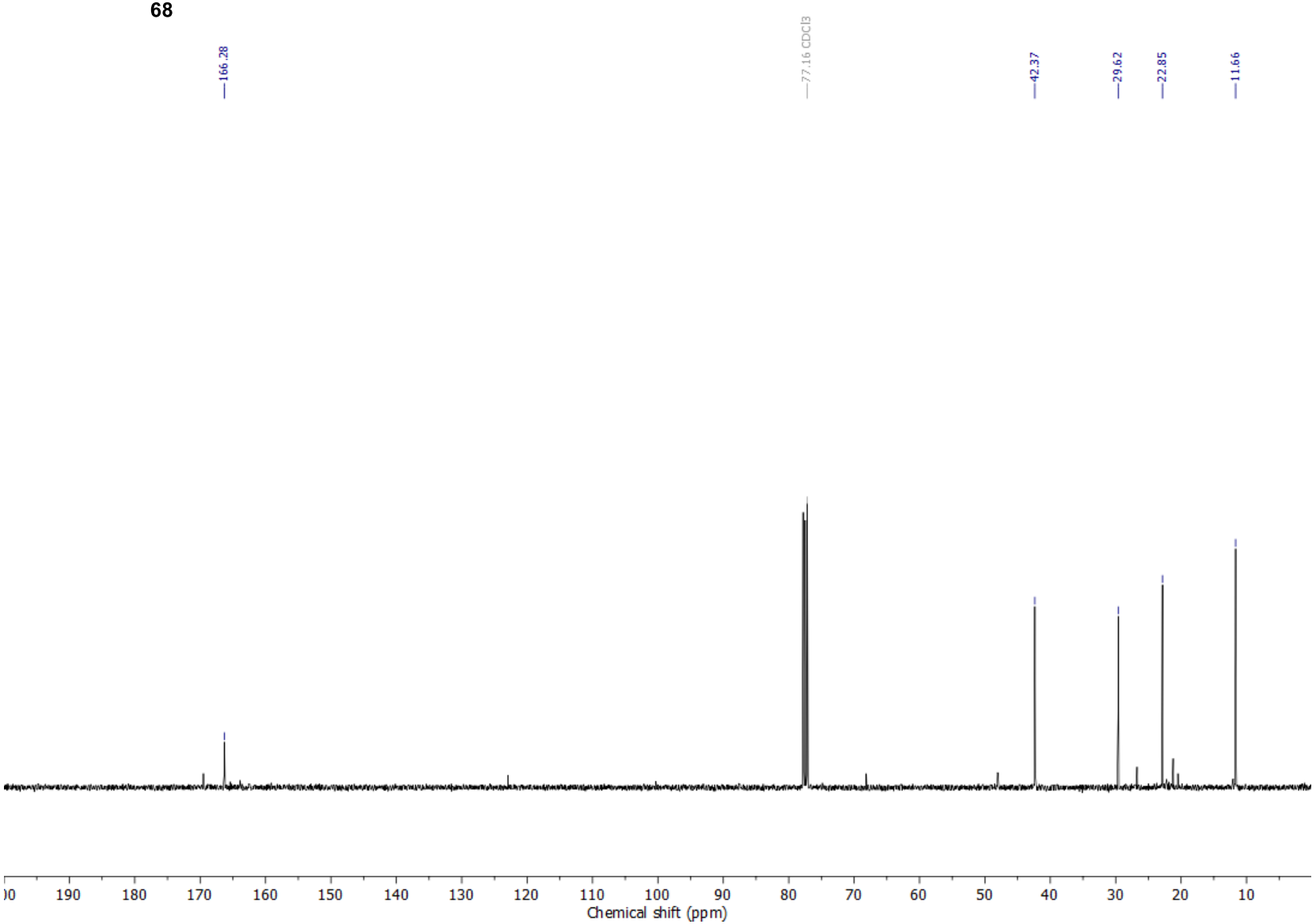
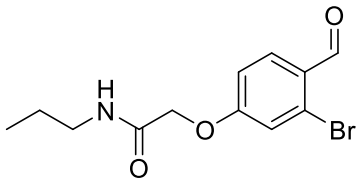


^{1}H NMR (400 MHz, $CDCl_3$)



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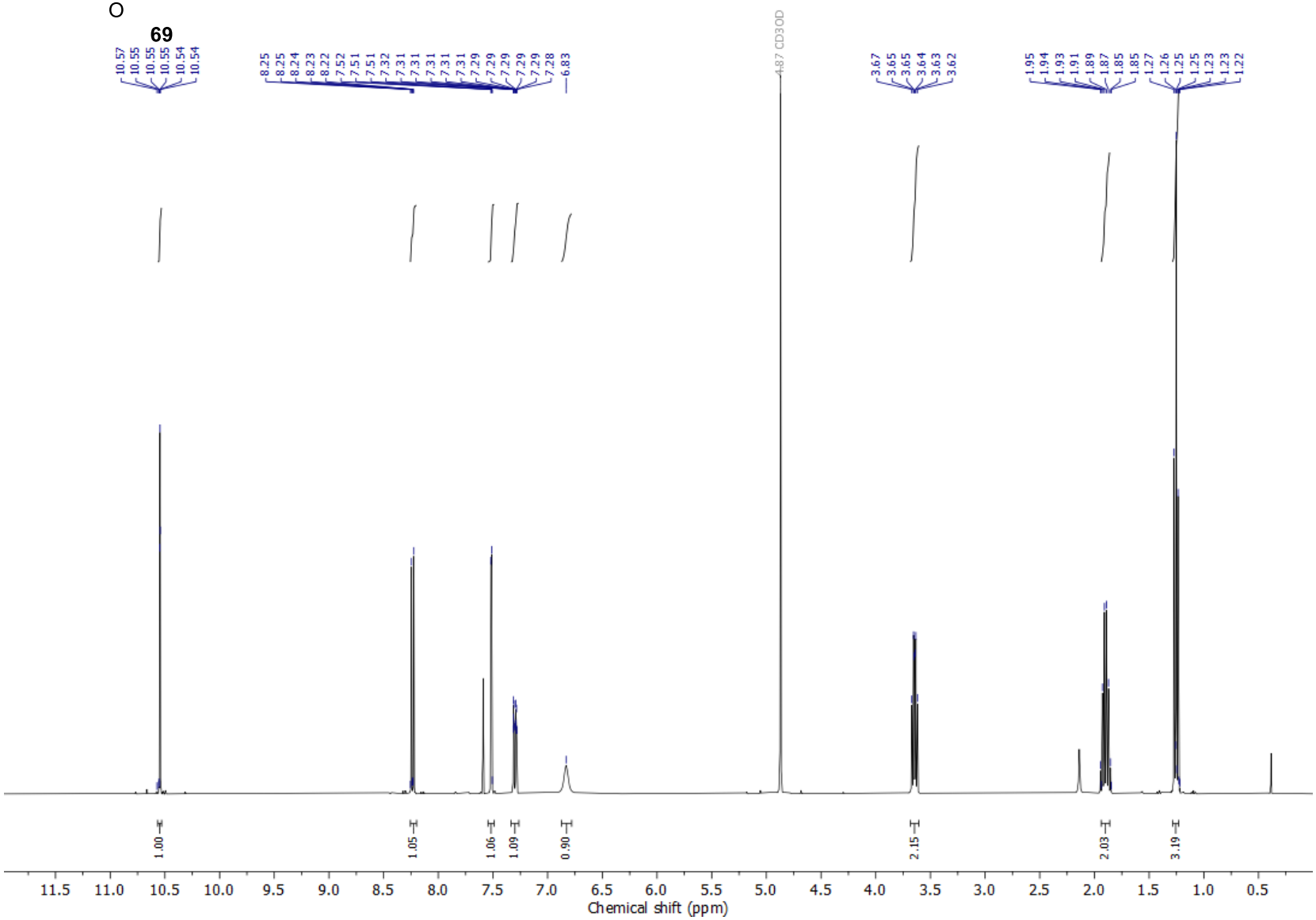
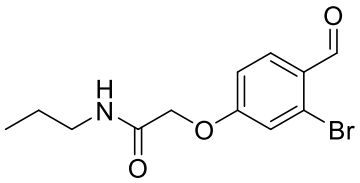


^{13}C NMR (100 MHz, $CDCl_3$)



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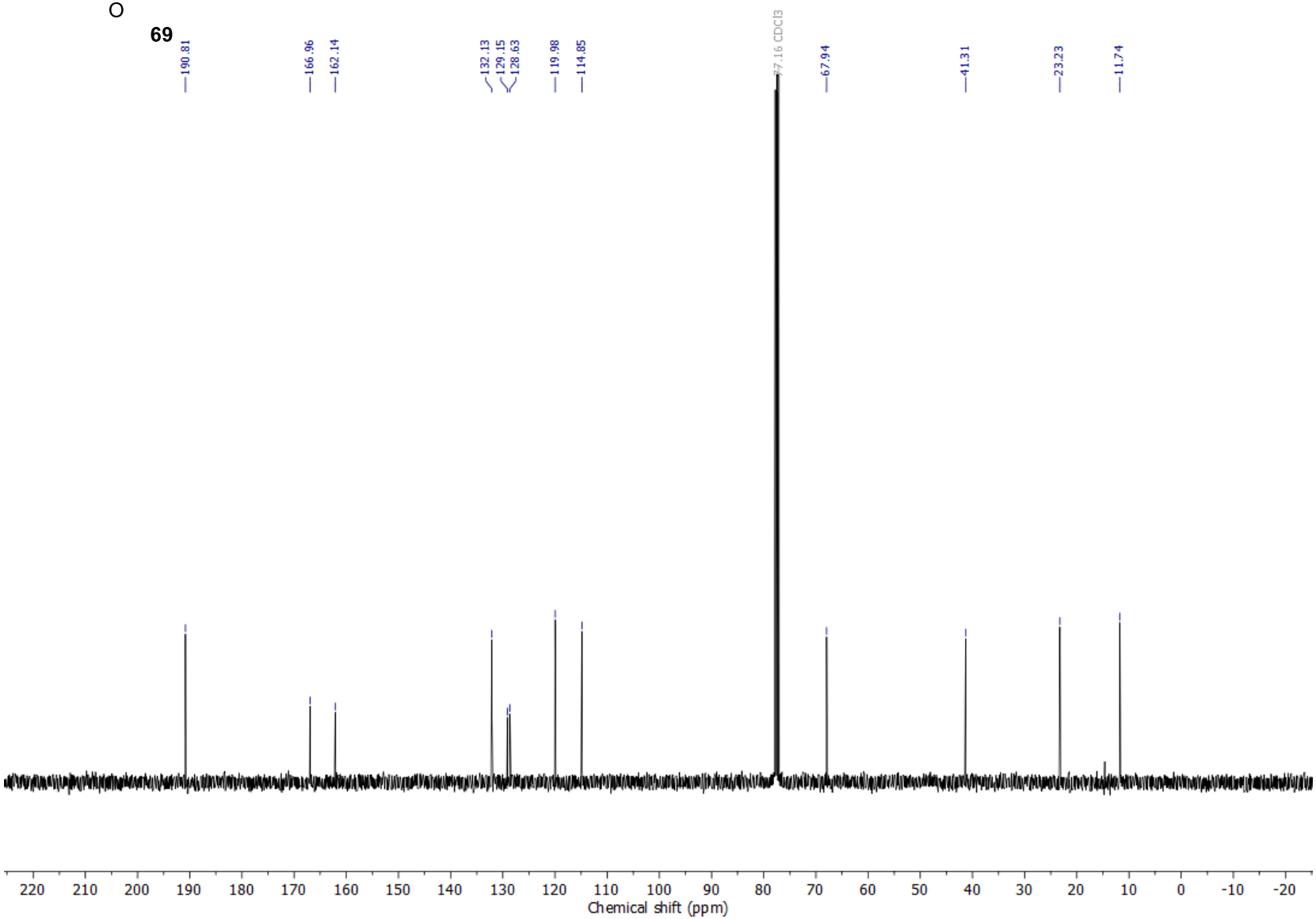

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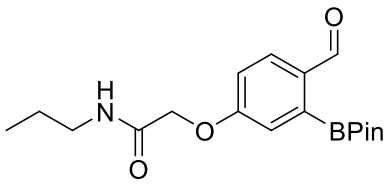

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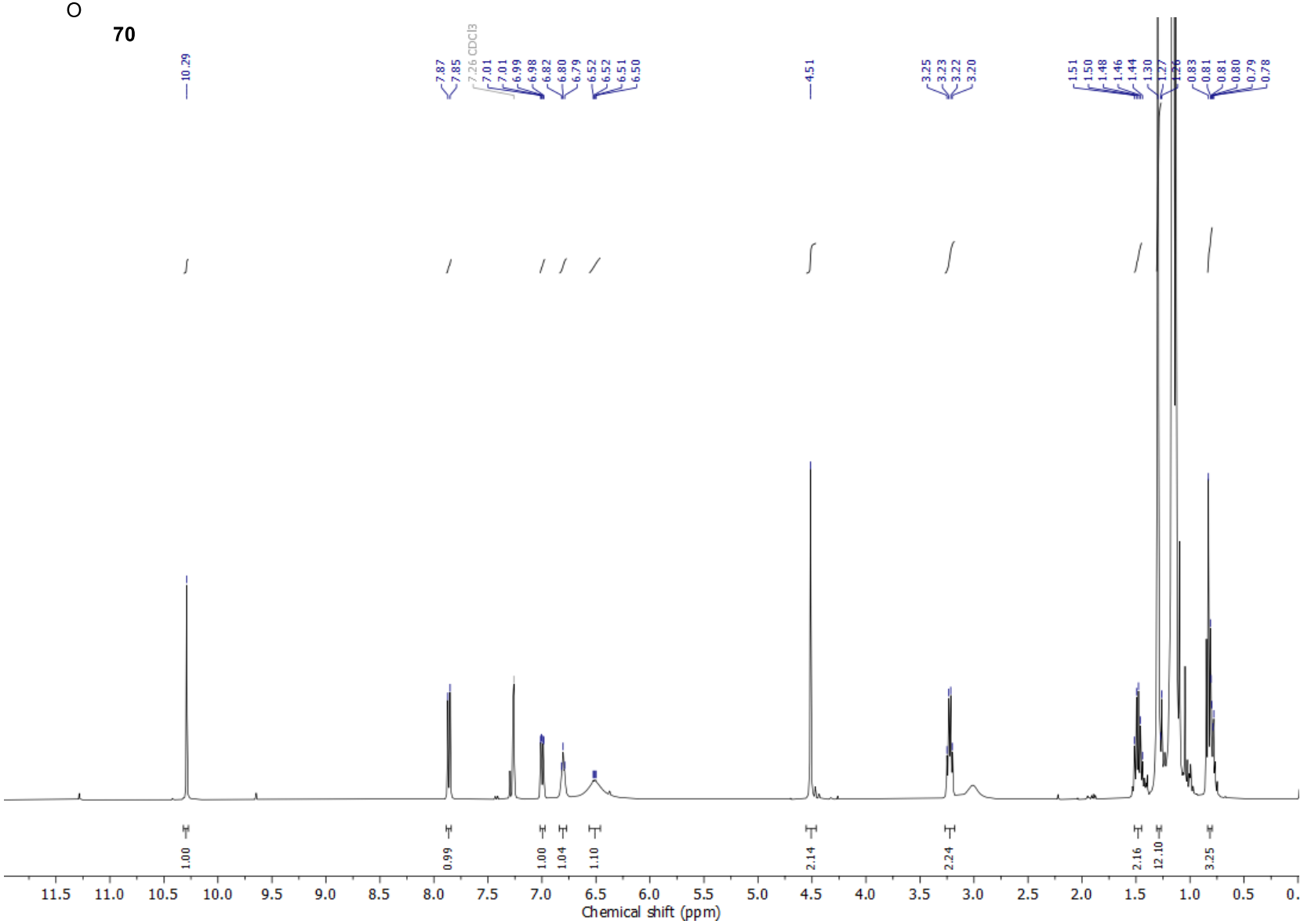
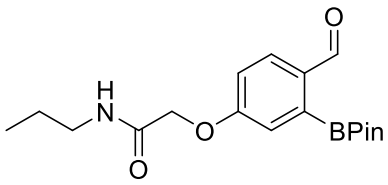


^{13}C NMR (100 MHz, $CDCl_3$)



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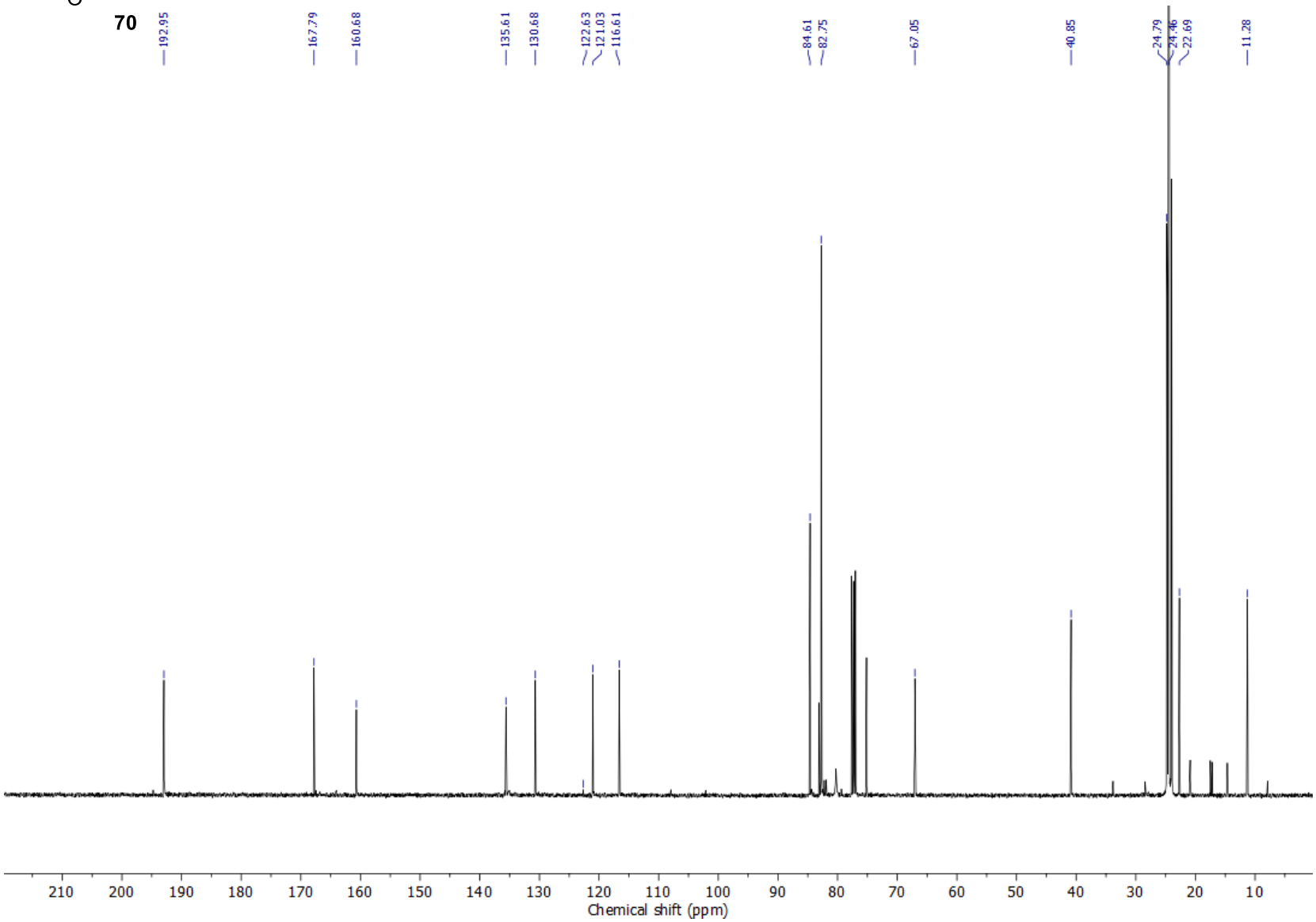
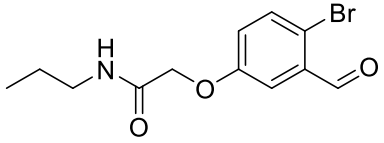


^{1}H NMR (400 MHz, $CDCl_3$)



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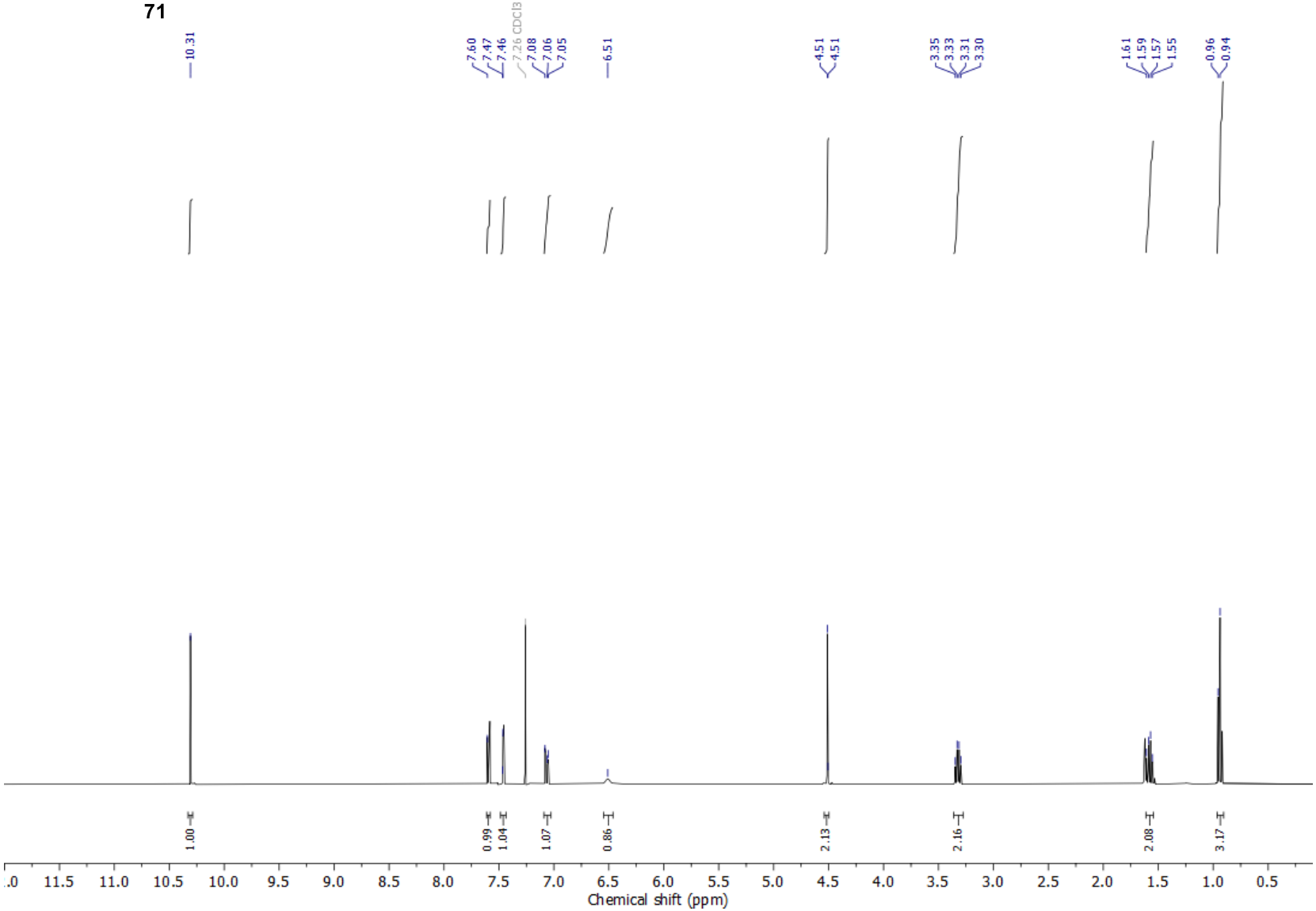
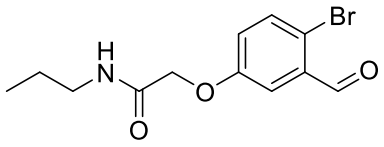


^{13}C NMR (100 MHz, $CDCl_3$)



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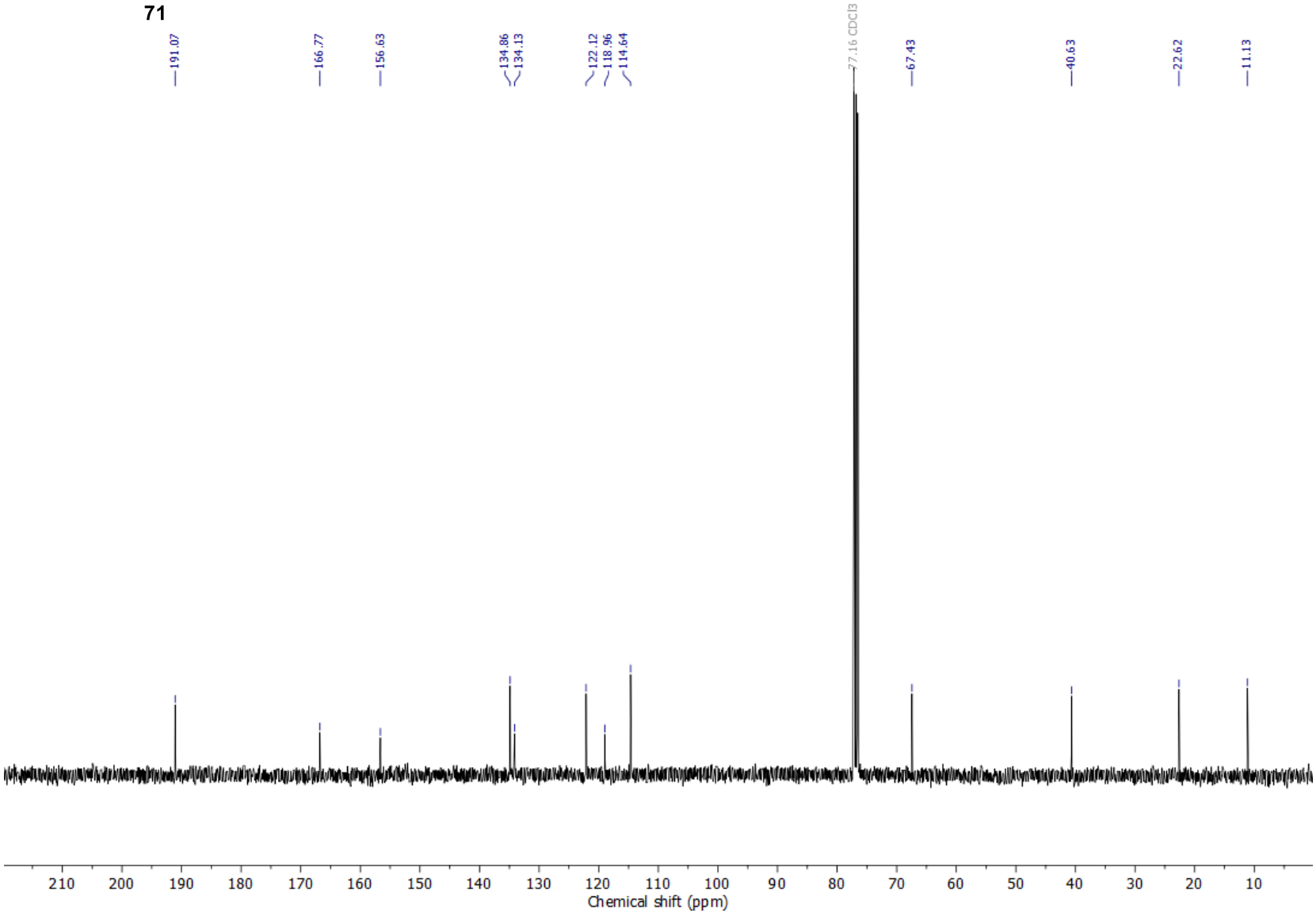
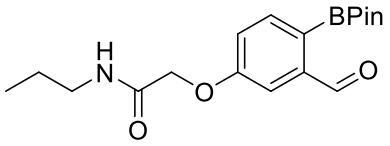


^{1}H NMR (400 MHz, $CDCl_3$)



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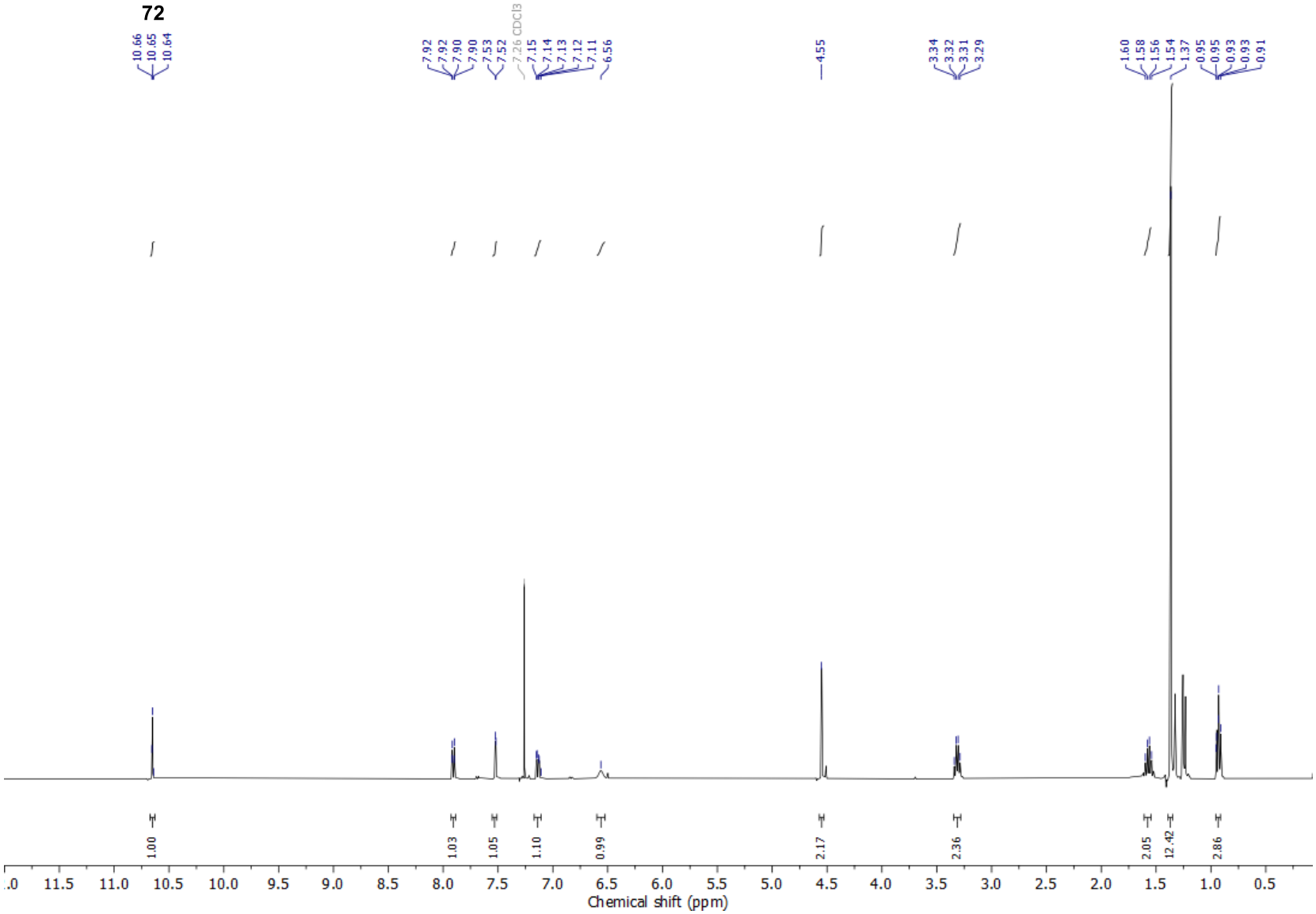
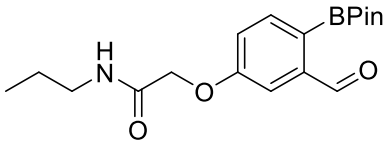


^{13}C NMR (100 MHz, $CDCl_3$)



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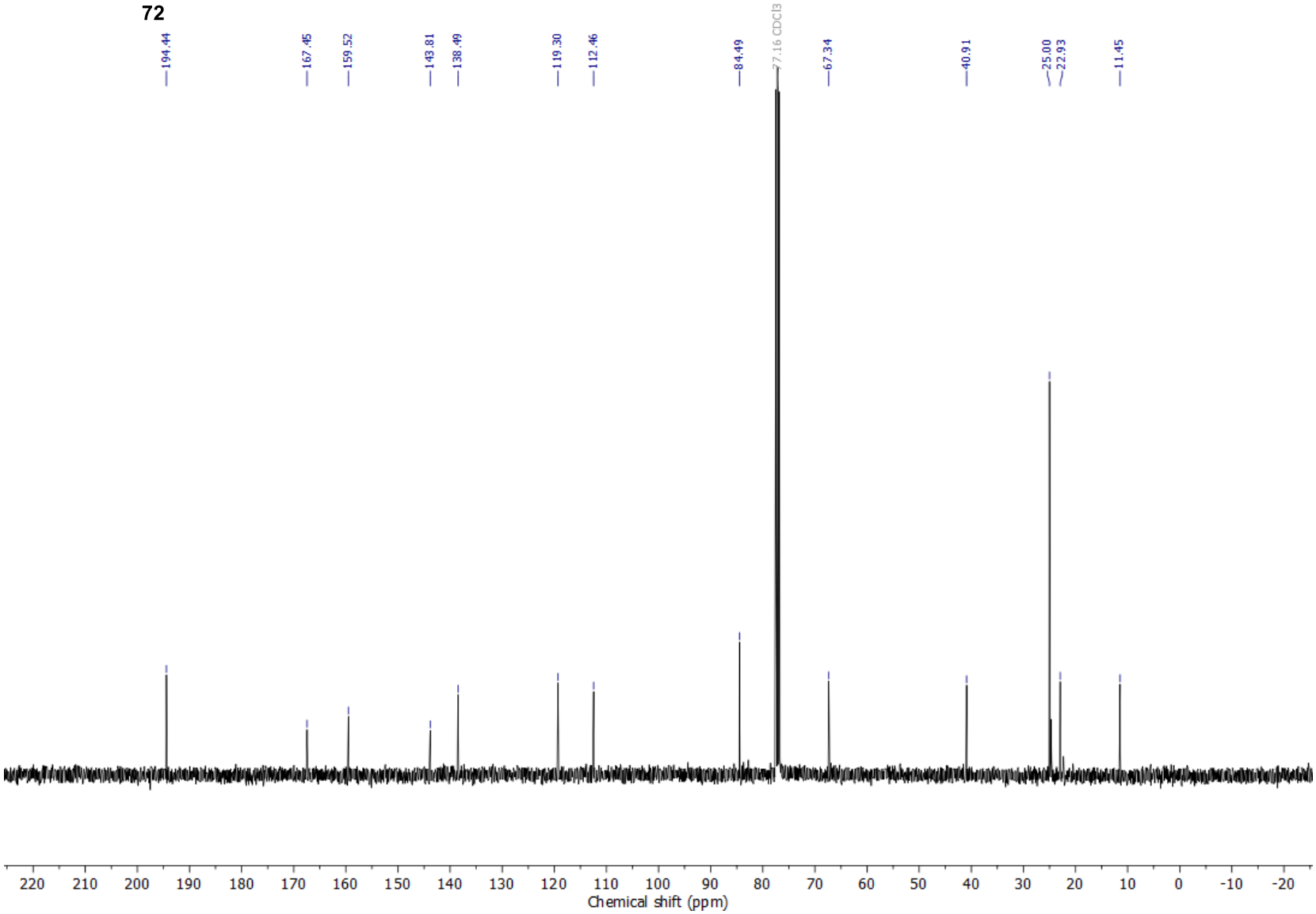
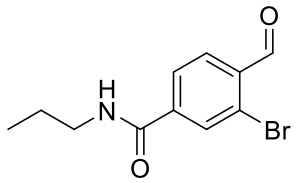


^{1}H NMR (400 MHz, MeOD)



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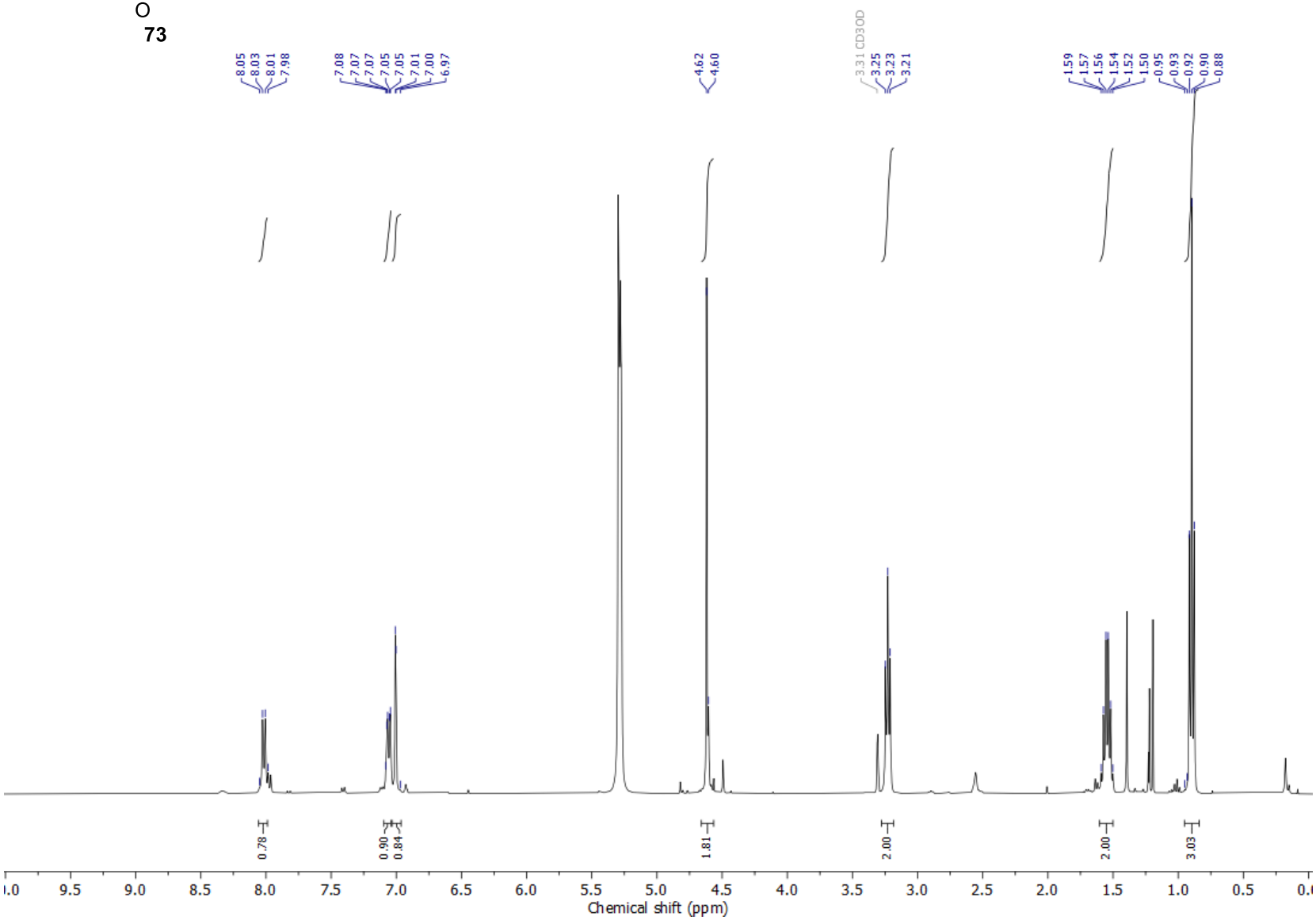
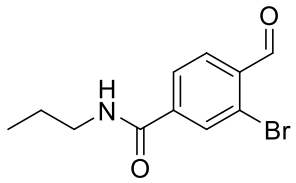


^{13}C NMR (100 MHz, MeOD)



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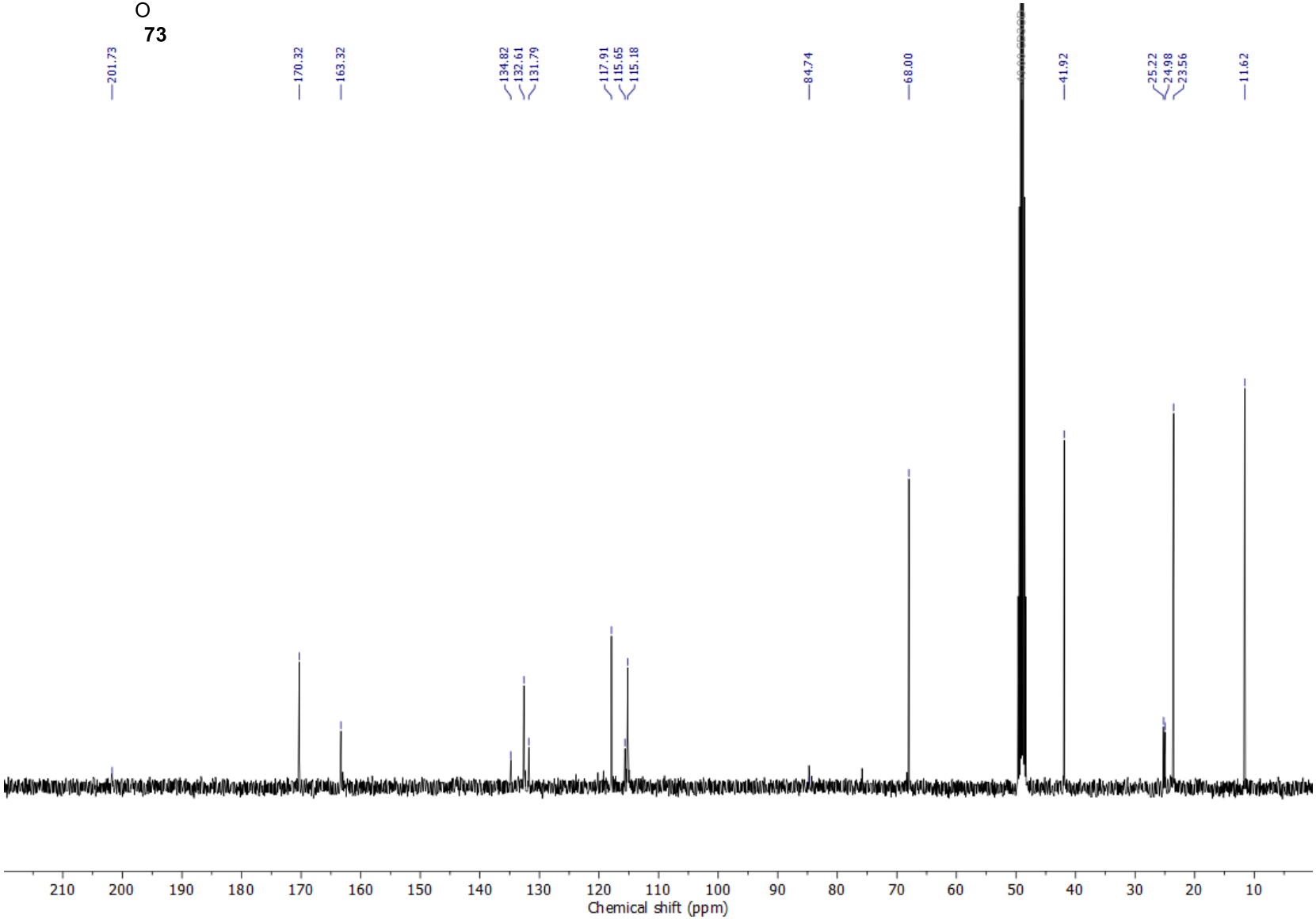
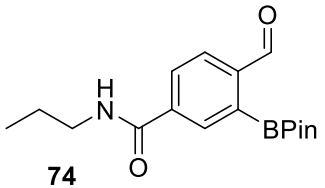


^{1}H NMR (400 MHz, $CDCl_3$)



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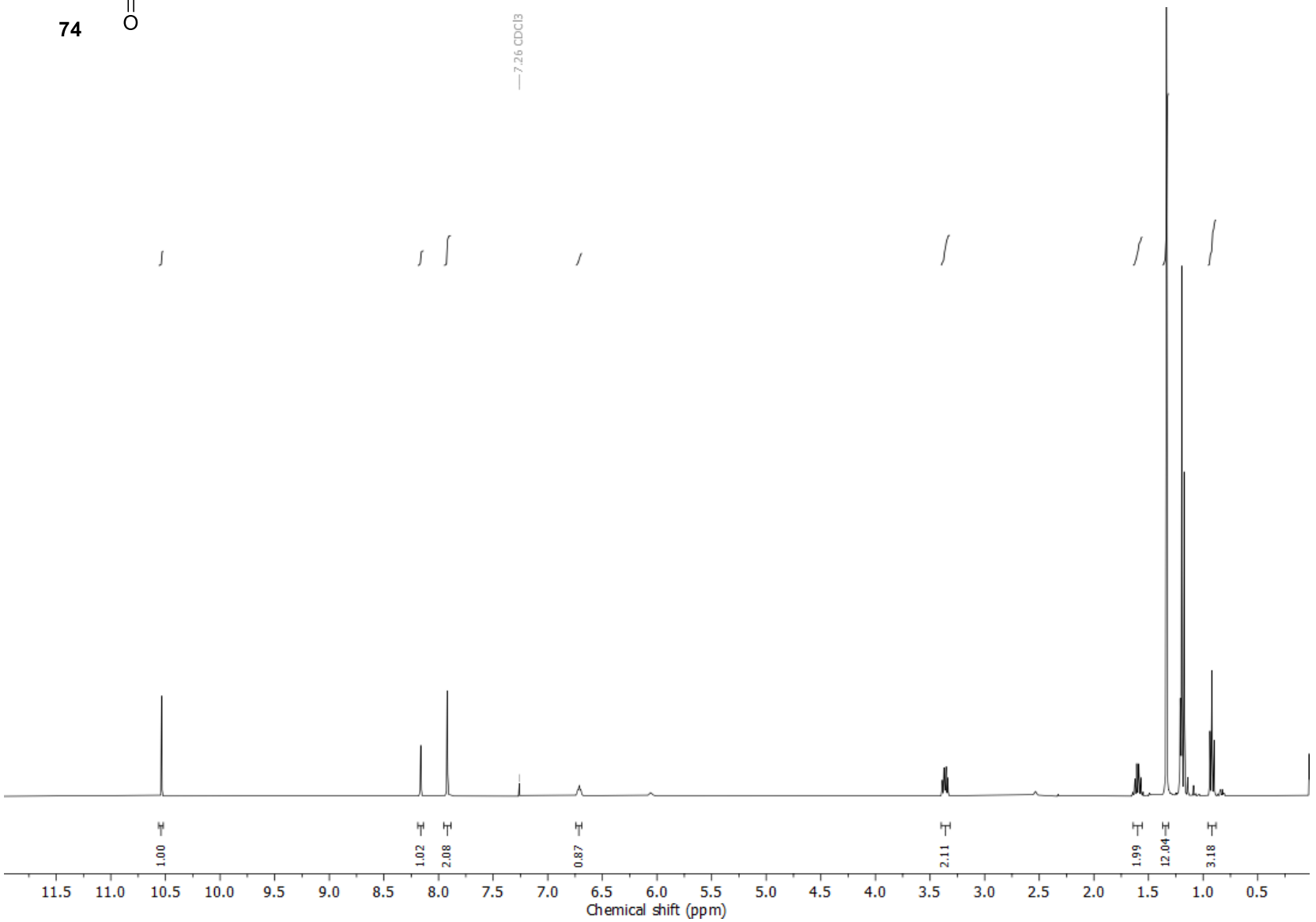
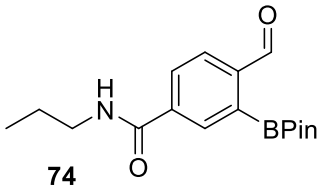


^{13}C NMR (100 MHz, $CDCl_3$)



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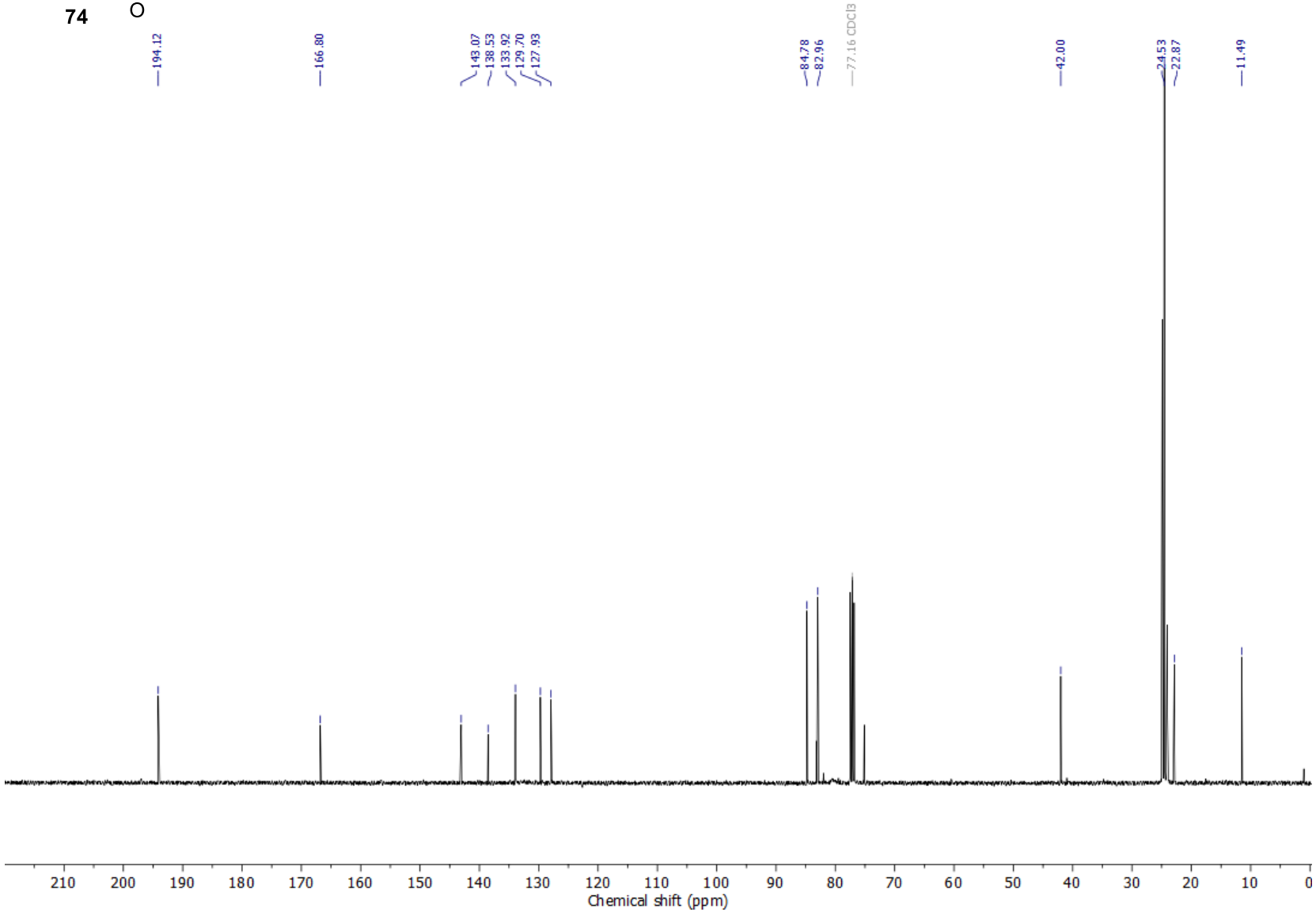
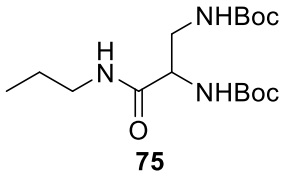


^{1}H NMR (400 MHz, $CDCl_3$)



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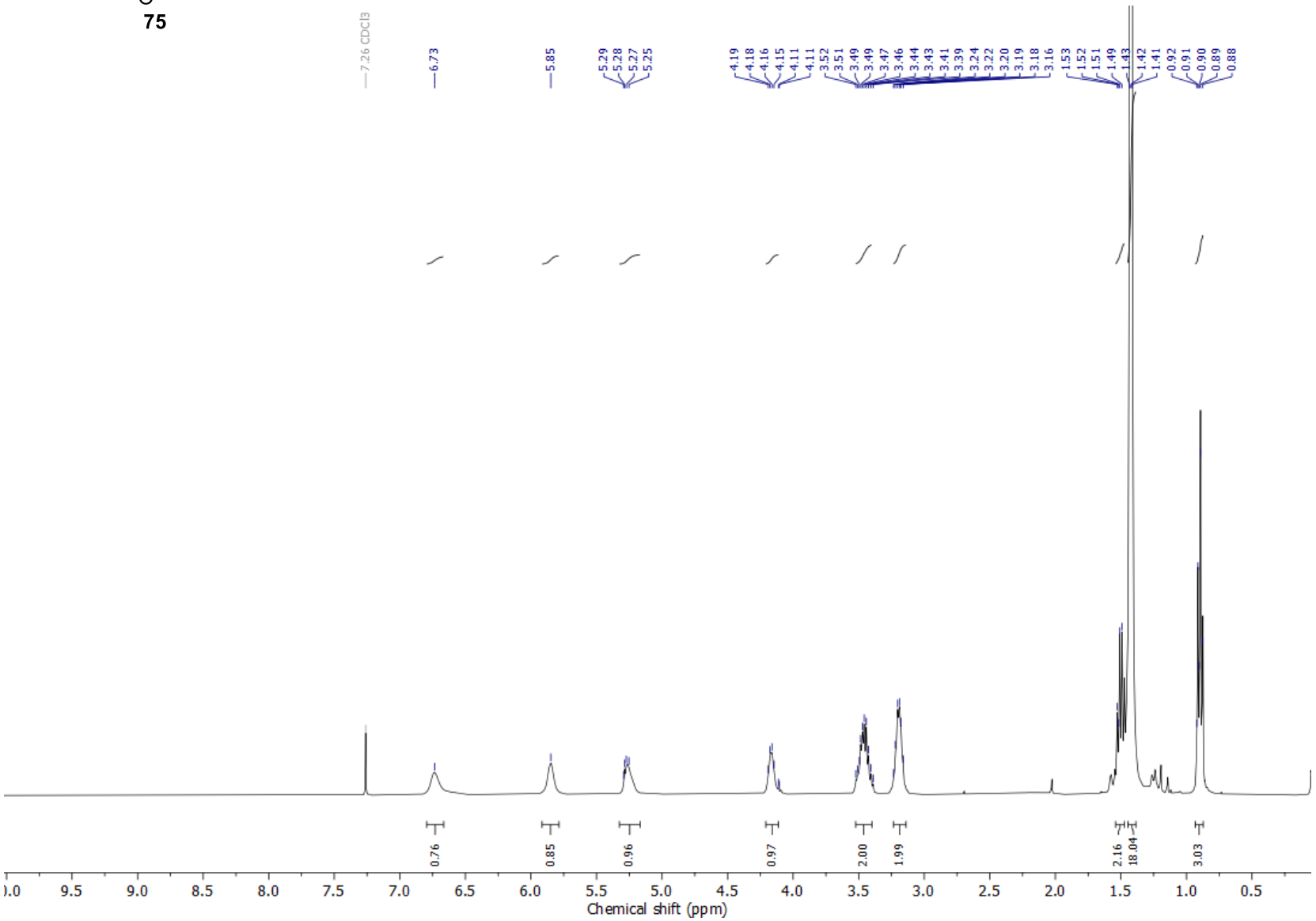
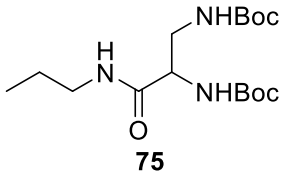


^{13}C NMR (100 MHz, $CDCl_3$)



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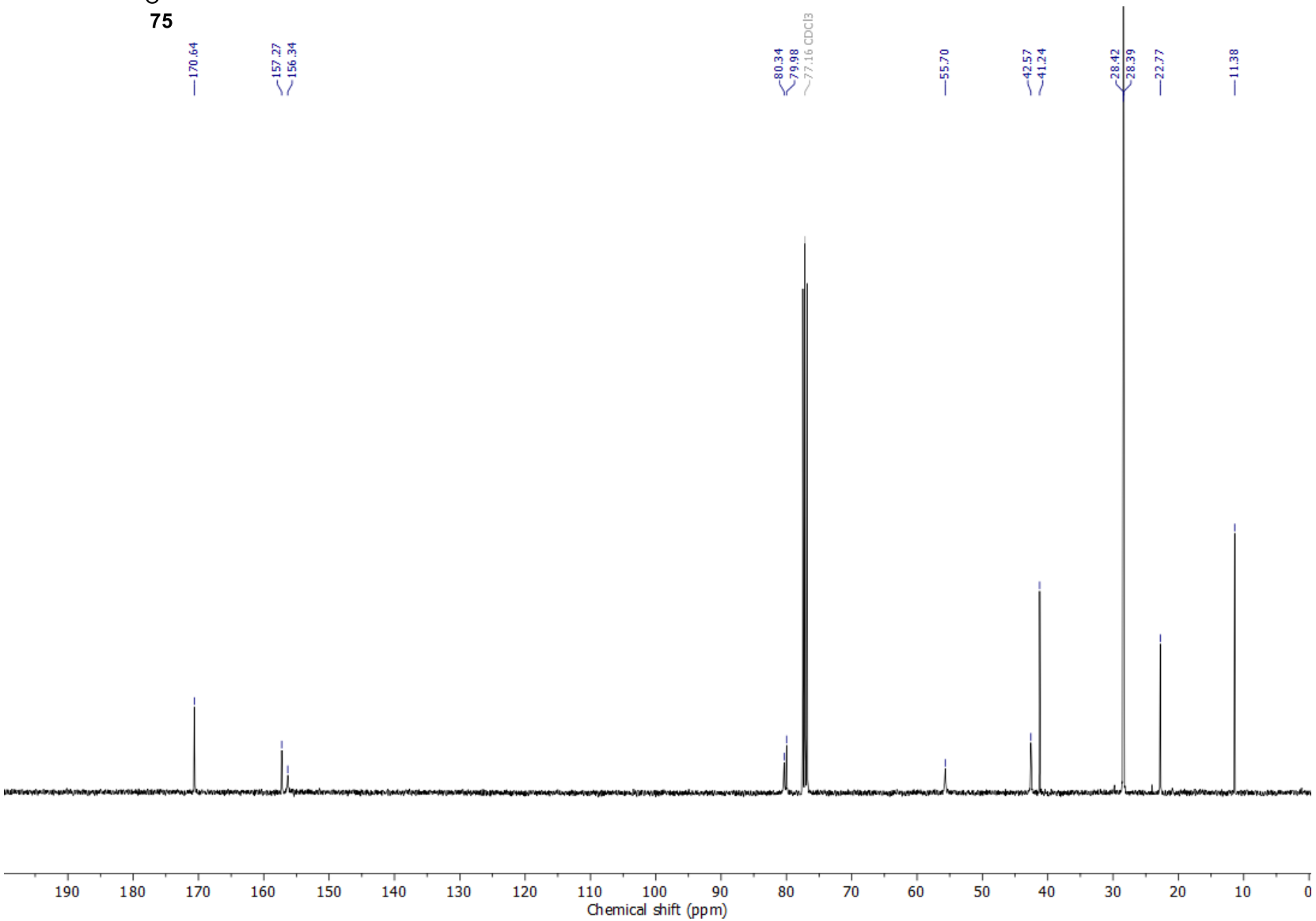
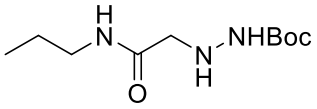


^{1}H NMR (400 MHz, $CDCl_3$)



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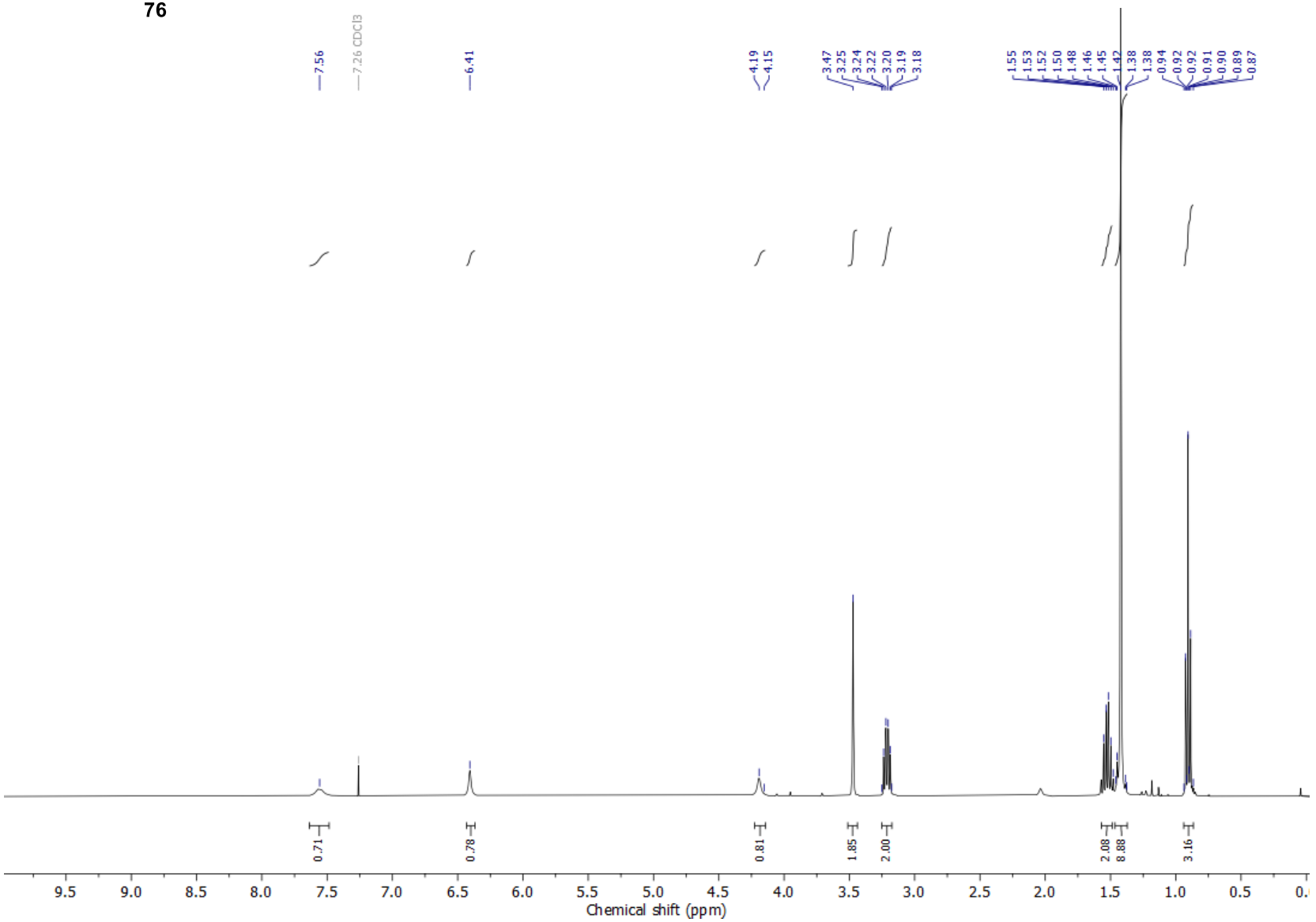
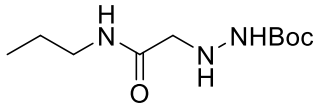


^{13}C NMR (100 MHz, $CDCl_3$)



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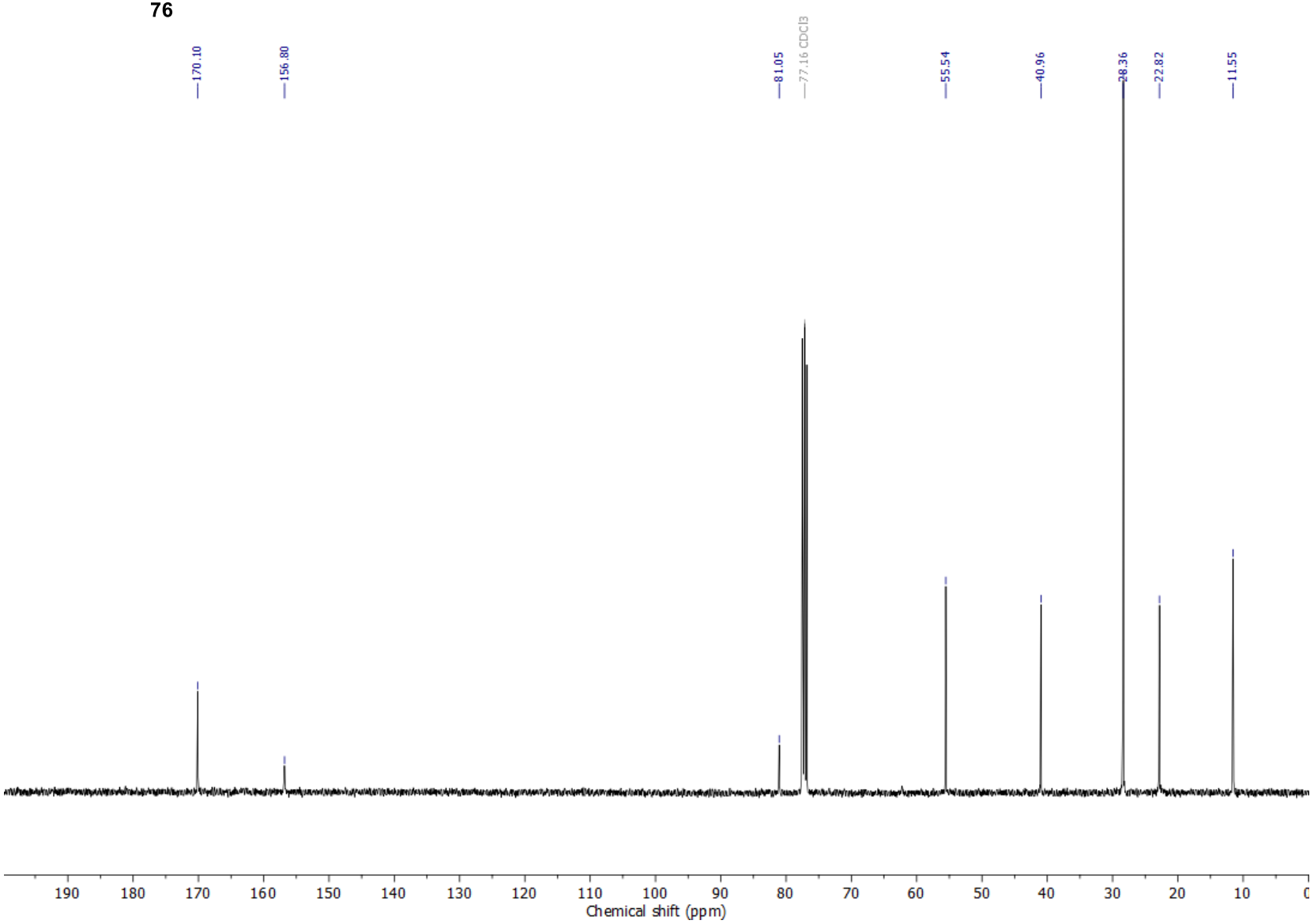
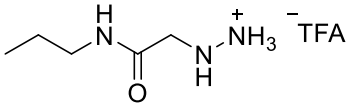


^{1}H NMR (400 MHz, MeOD)



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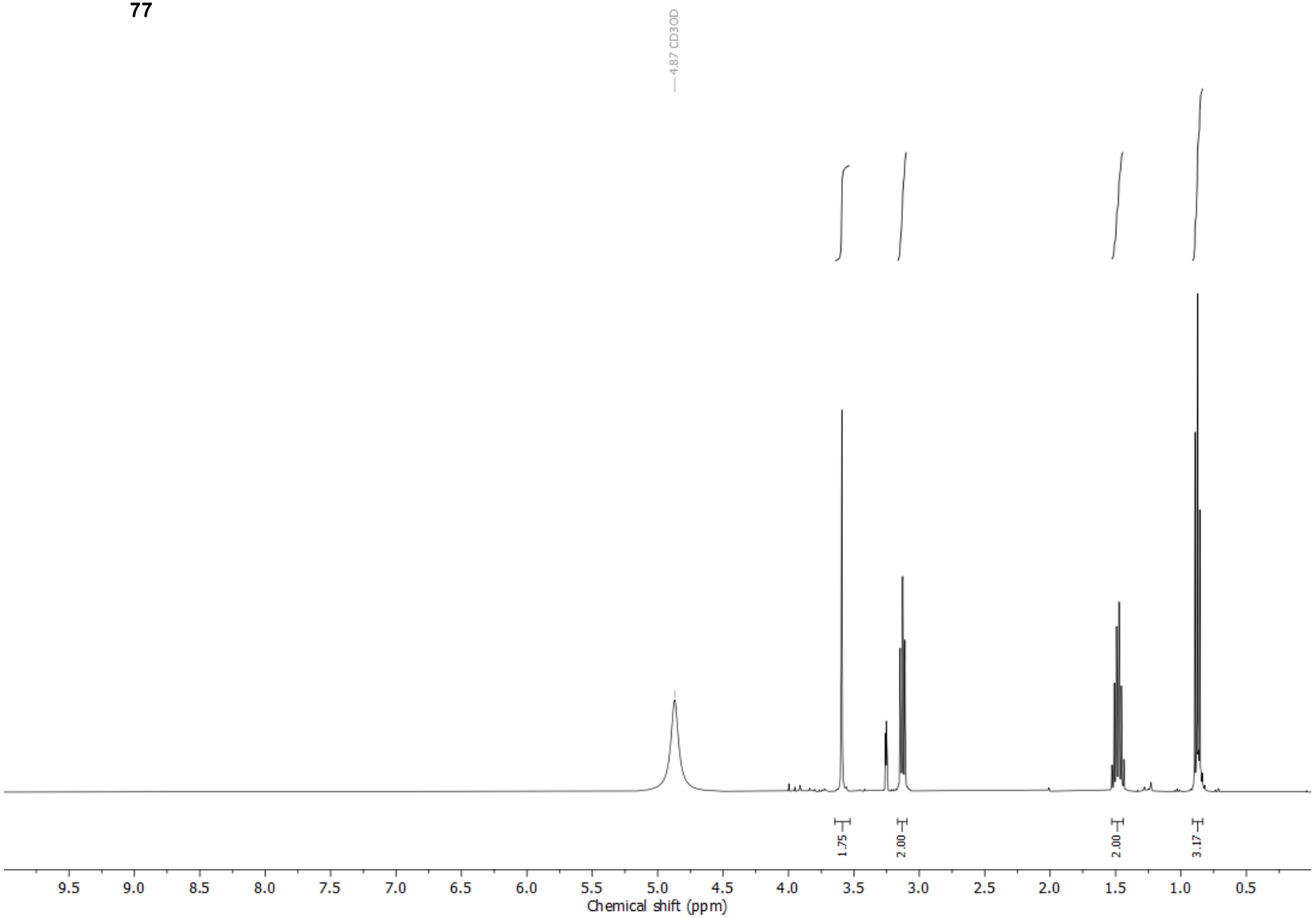
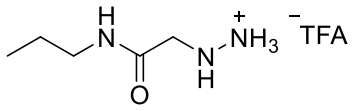


^{13}C NMR (100 MHz, MeOD)



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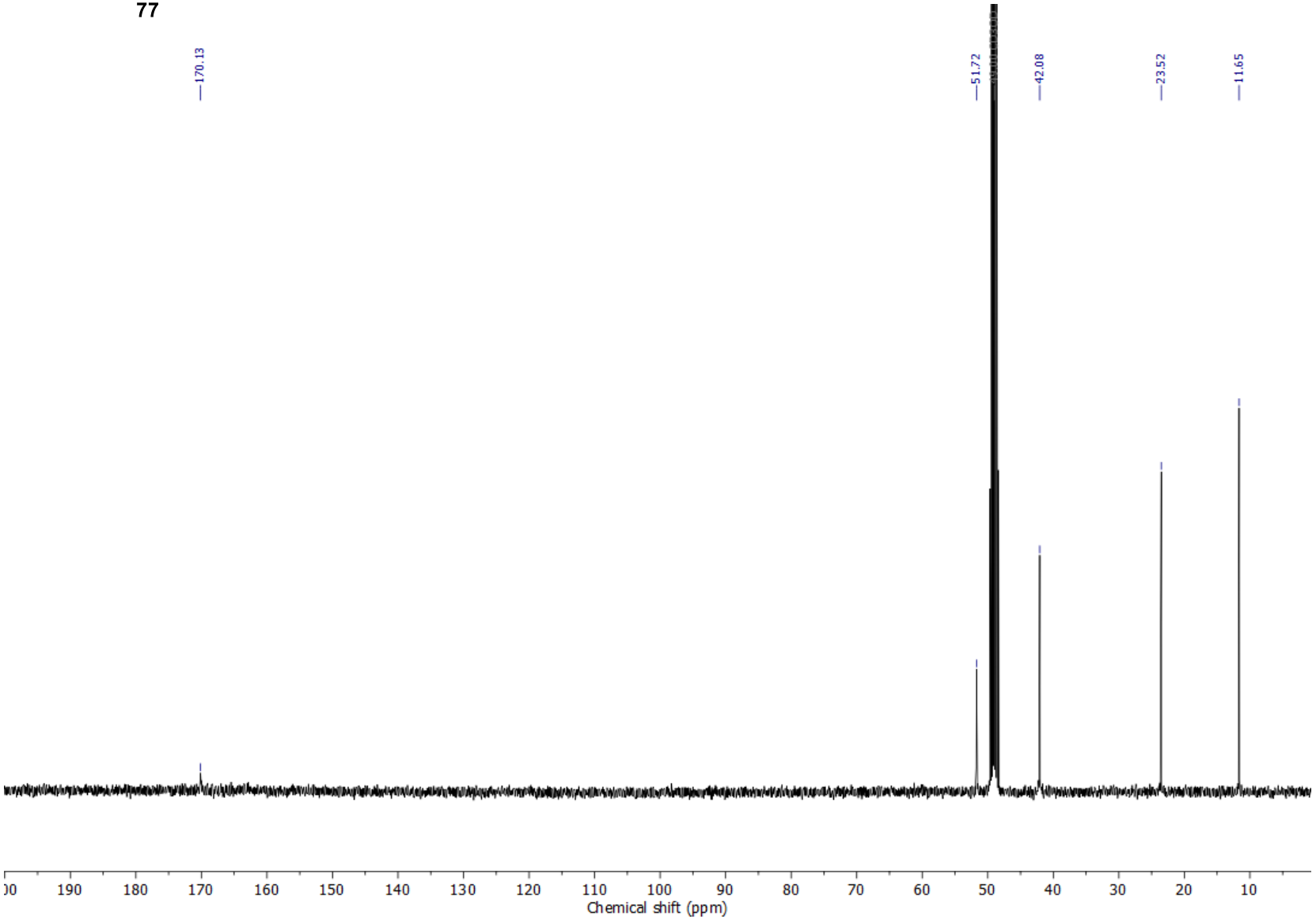
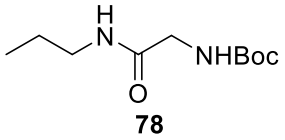


^{1}H NMR (400 MHz, $CDCl_3$)



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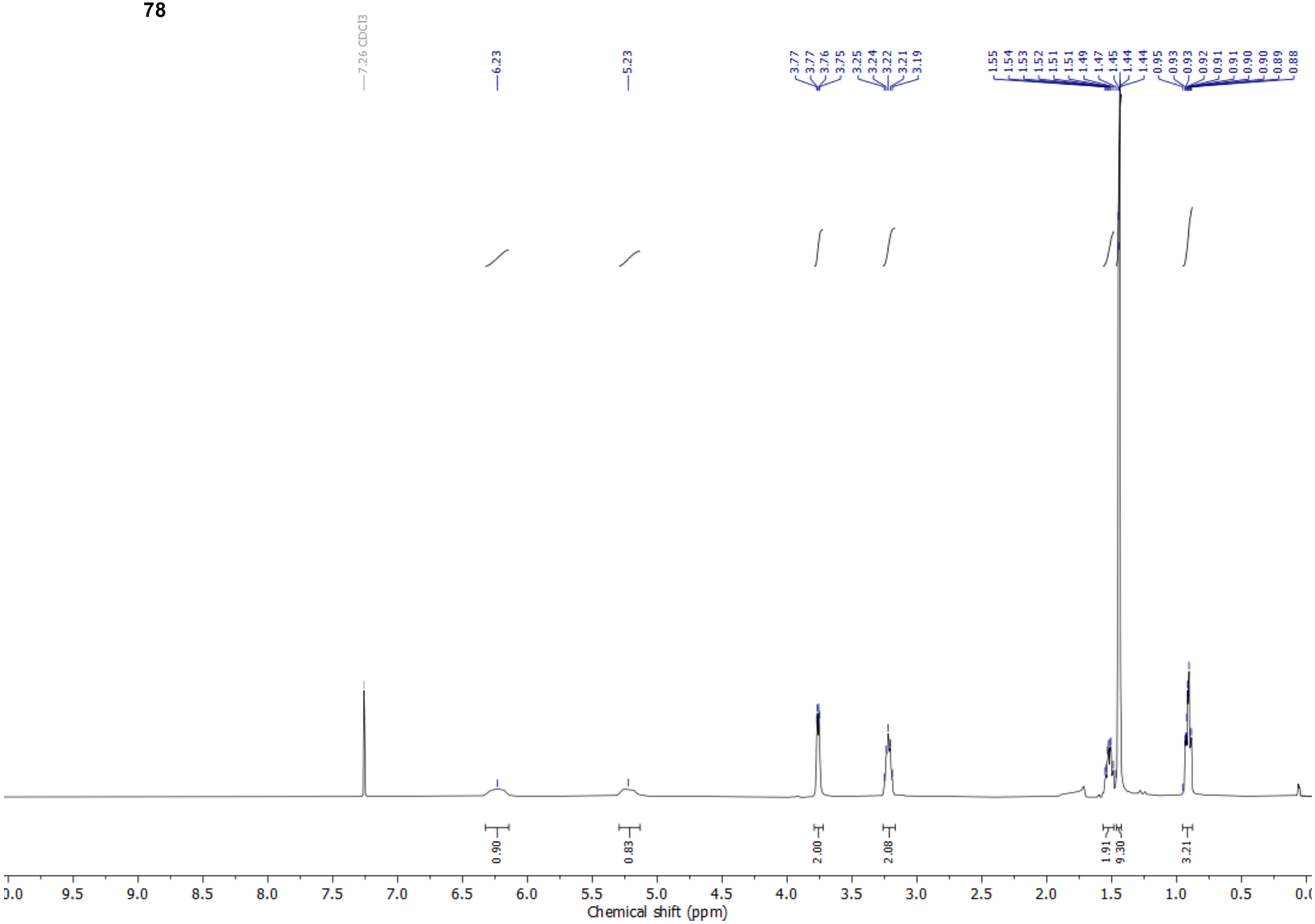
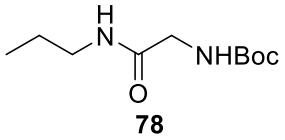


^{13}C NMR (100 MHz, $CDCl_3$)



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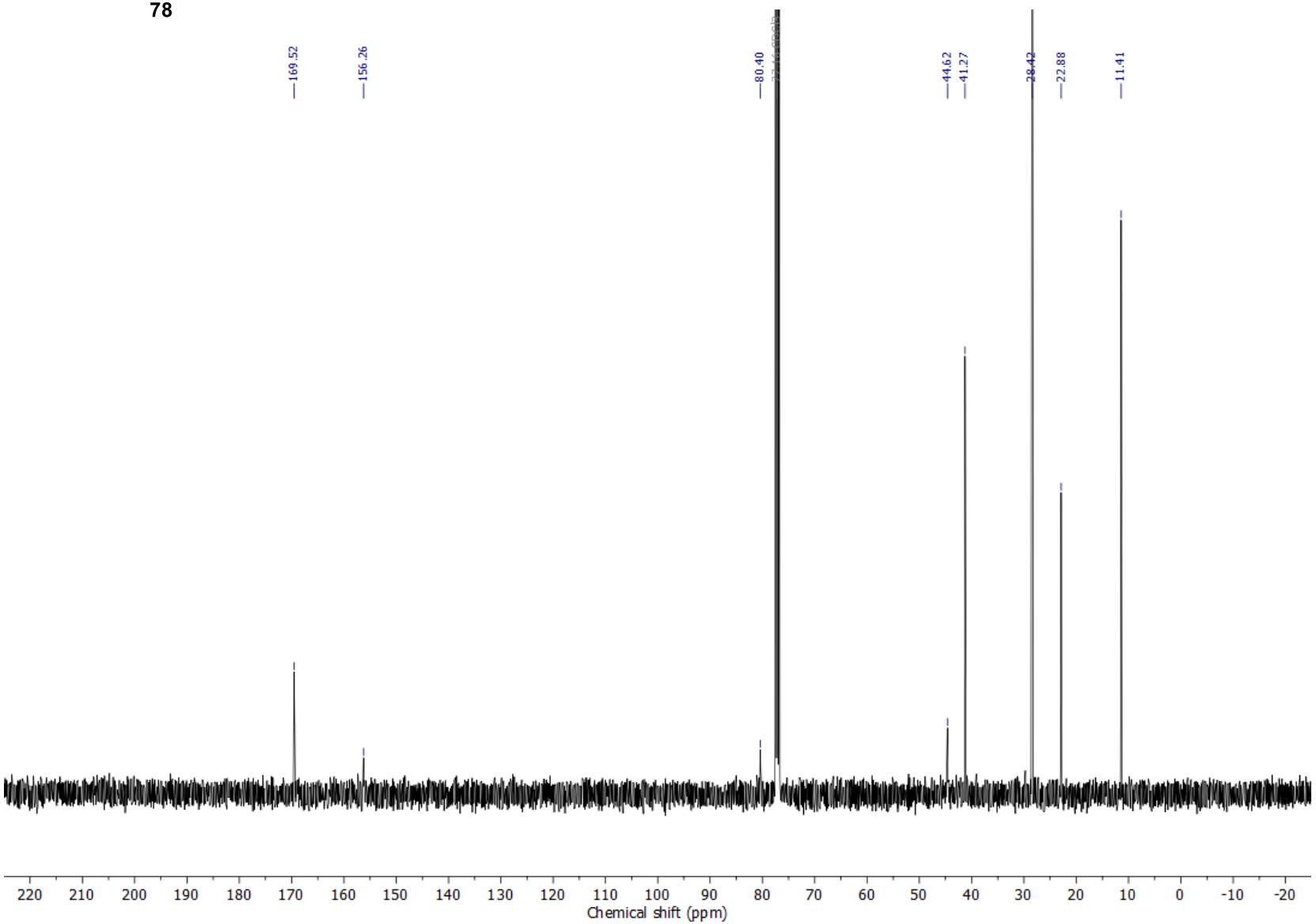
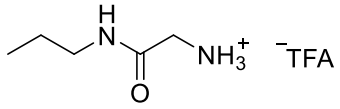


^{1}H NMR (400 MHz, MeOD)



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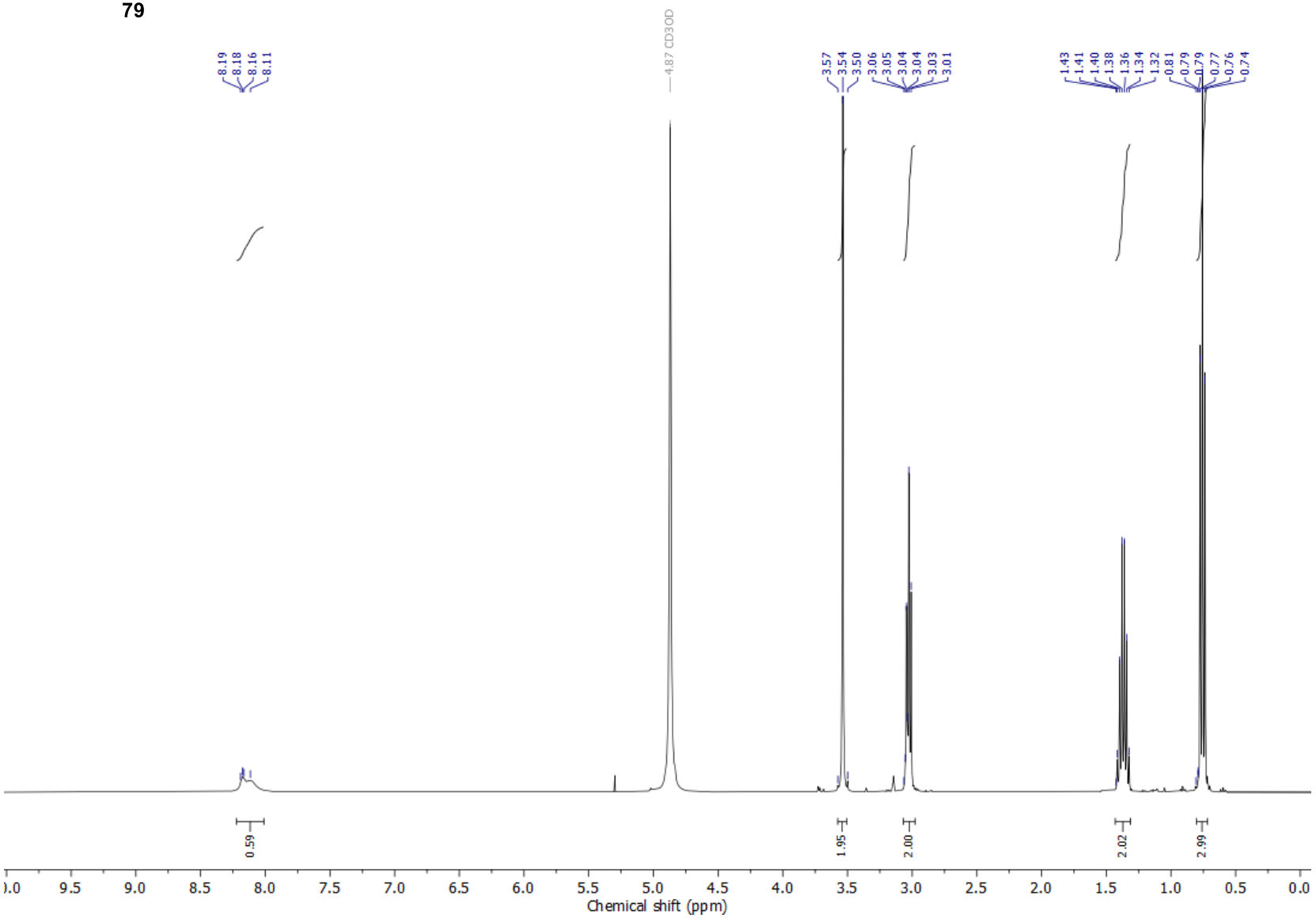
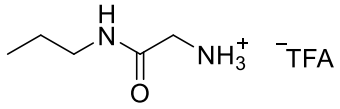


^{13}C NMR (100 MHz, MeOD)



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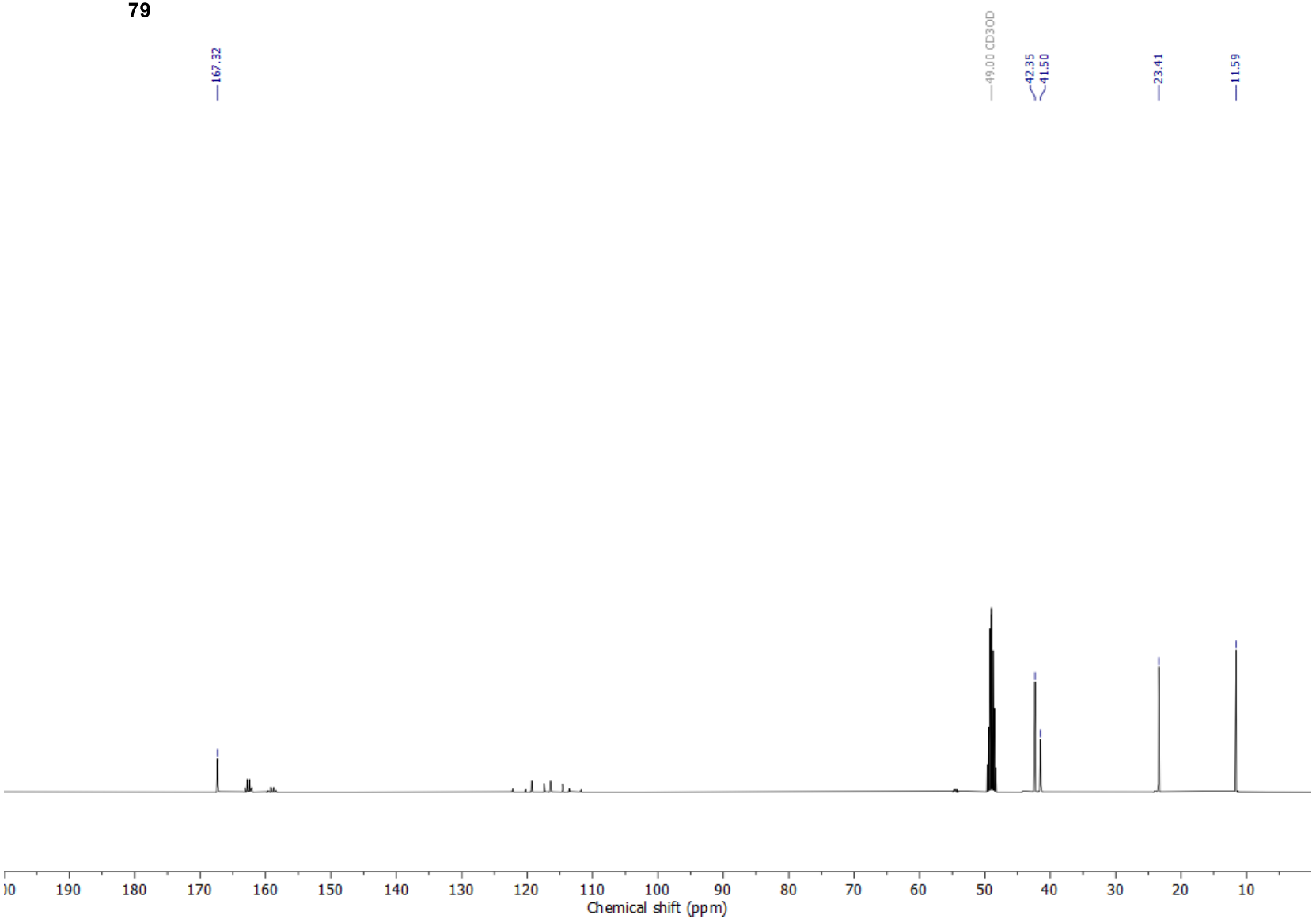
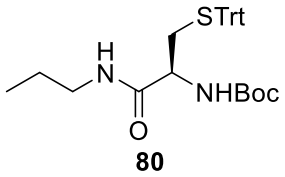


^{1}H NMR (400 MHz, $CDCl_3$)



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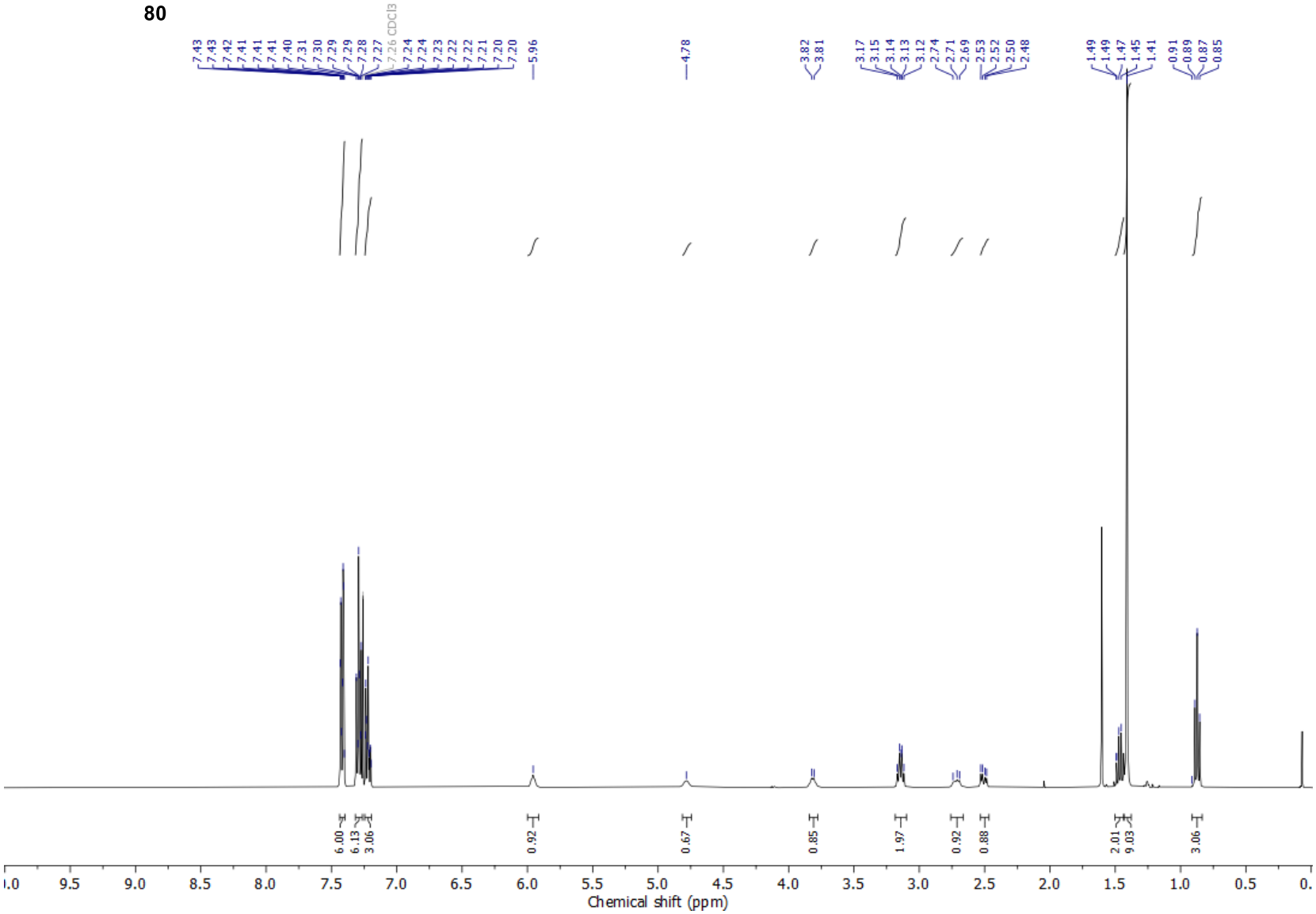
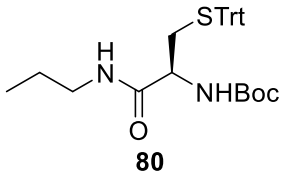


^{13}C NMR (100 MHz, $CDCl_3$)



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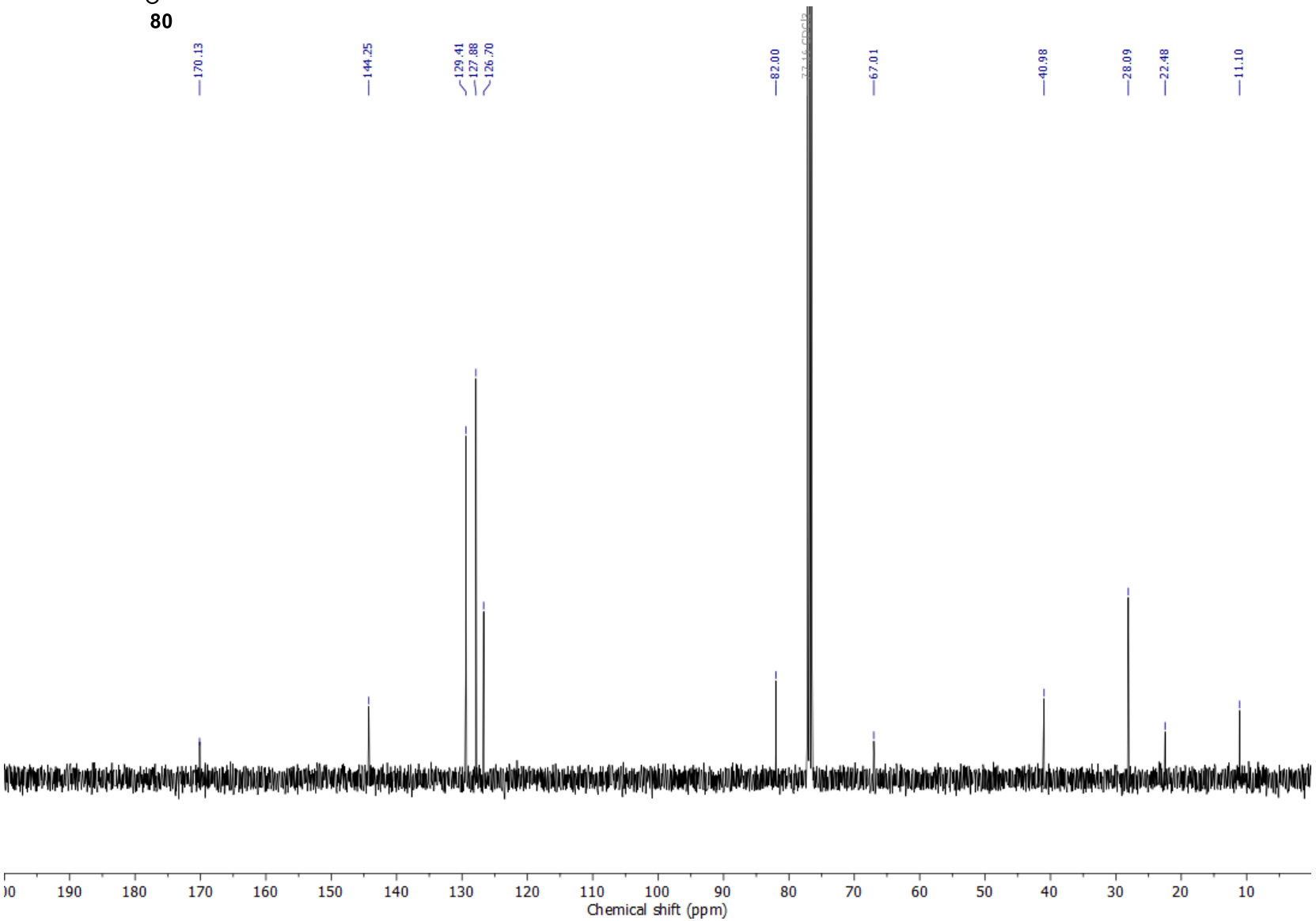
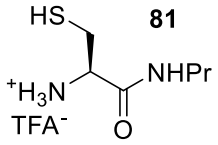
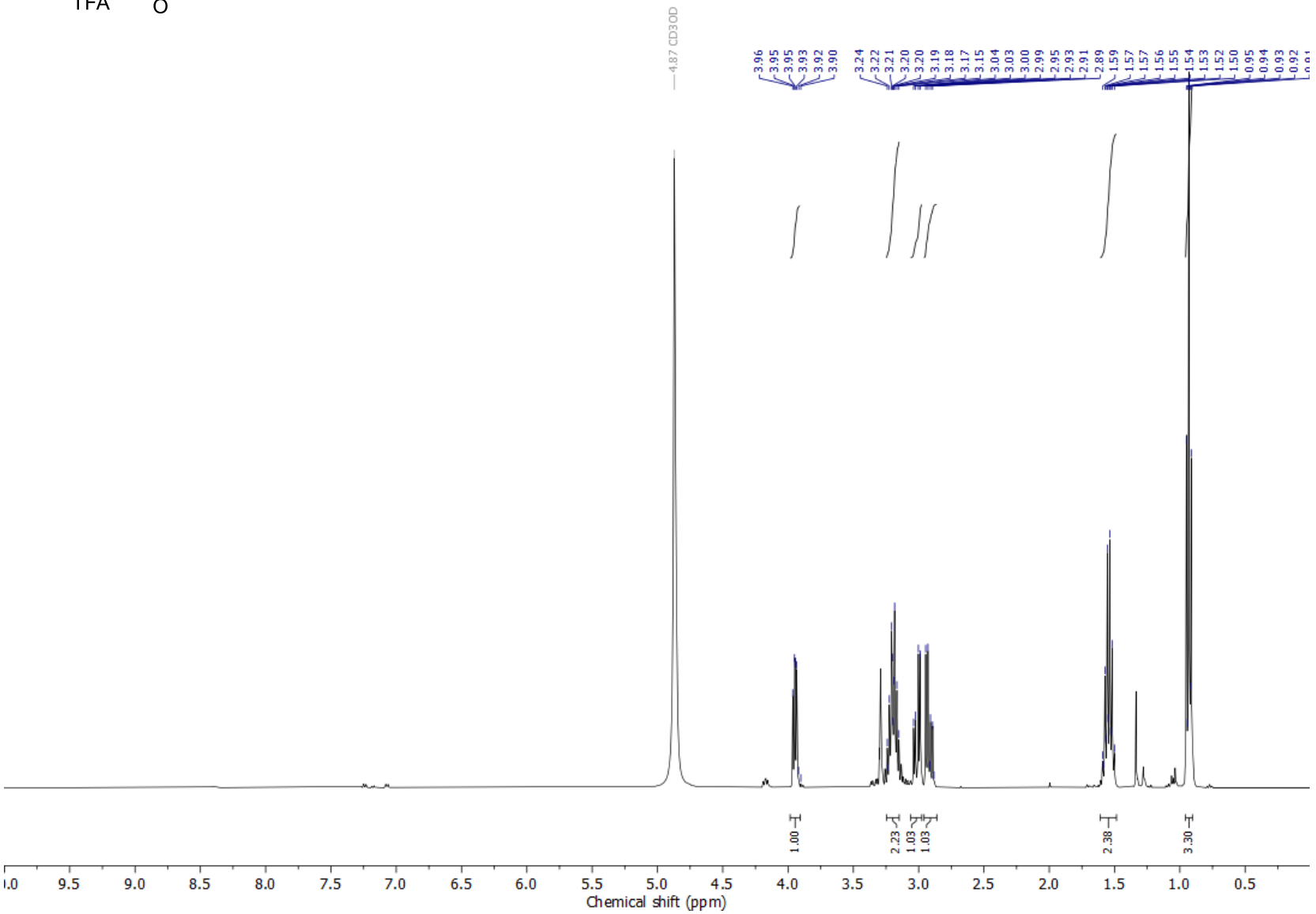
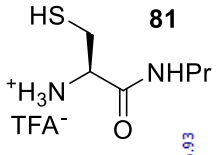


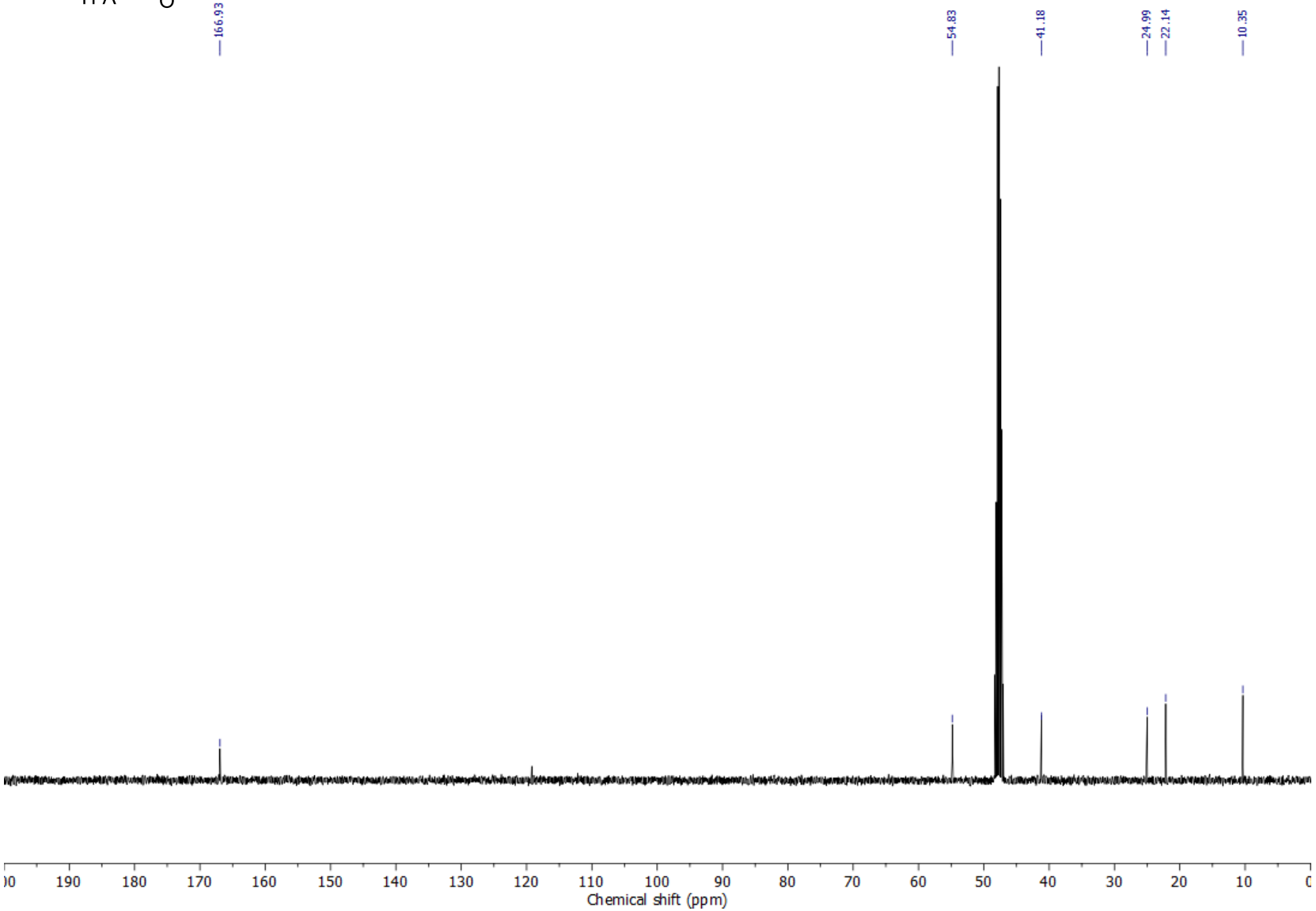
^{1}H NMR (400 MHz, MeOD)



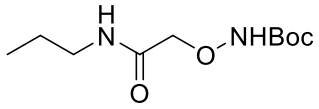


^{13}C NMR (100 MHz, MeOD)





^{1}H NMR (400 MHz, $CDCl_3$)



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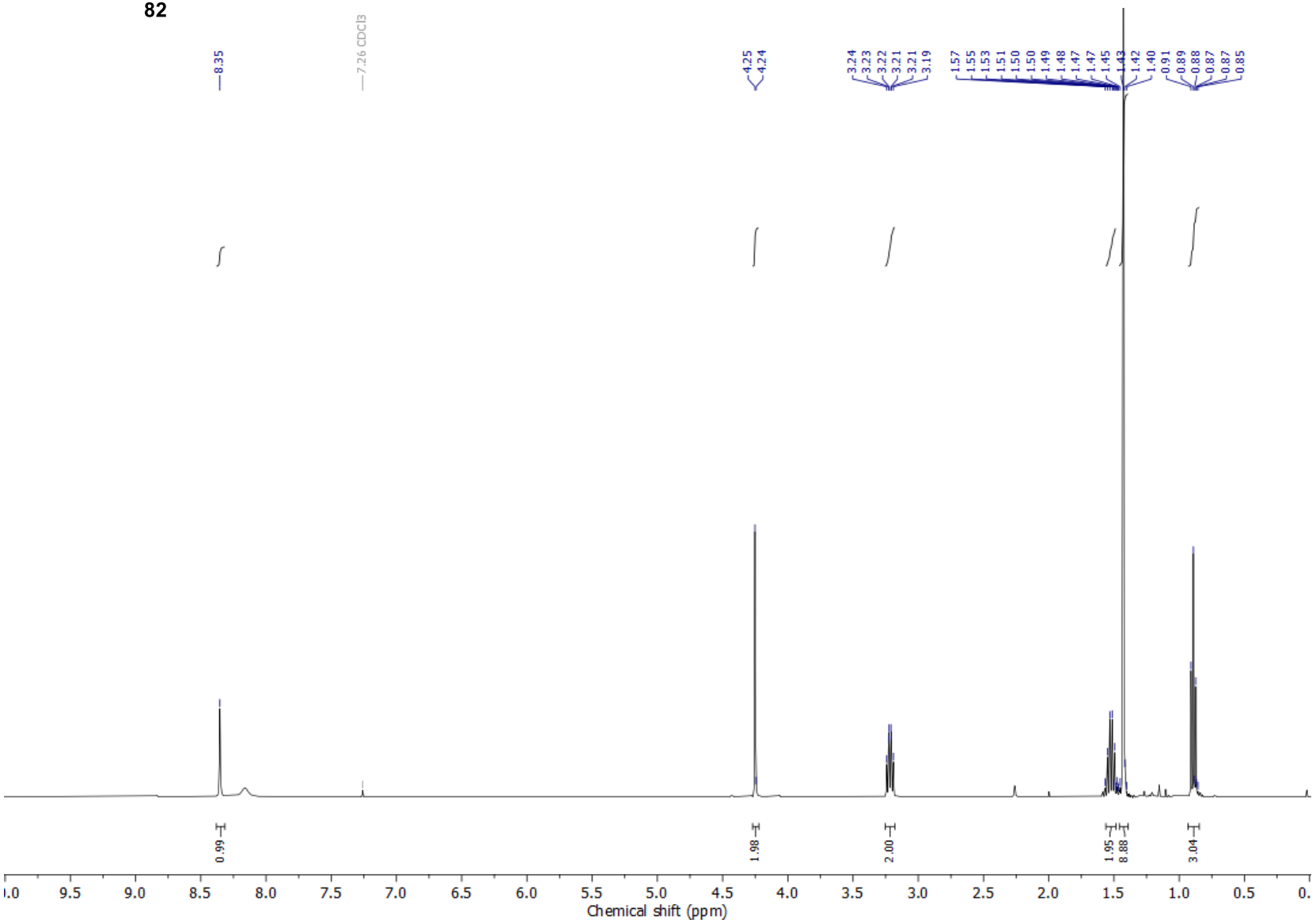
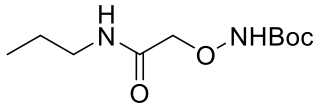


^{13}C NMR (100 MHz, $CDCl_3$)



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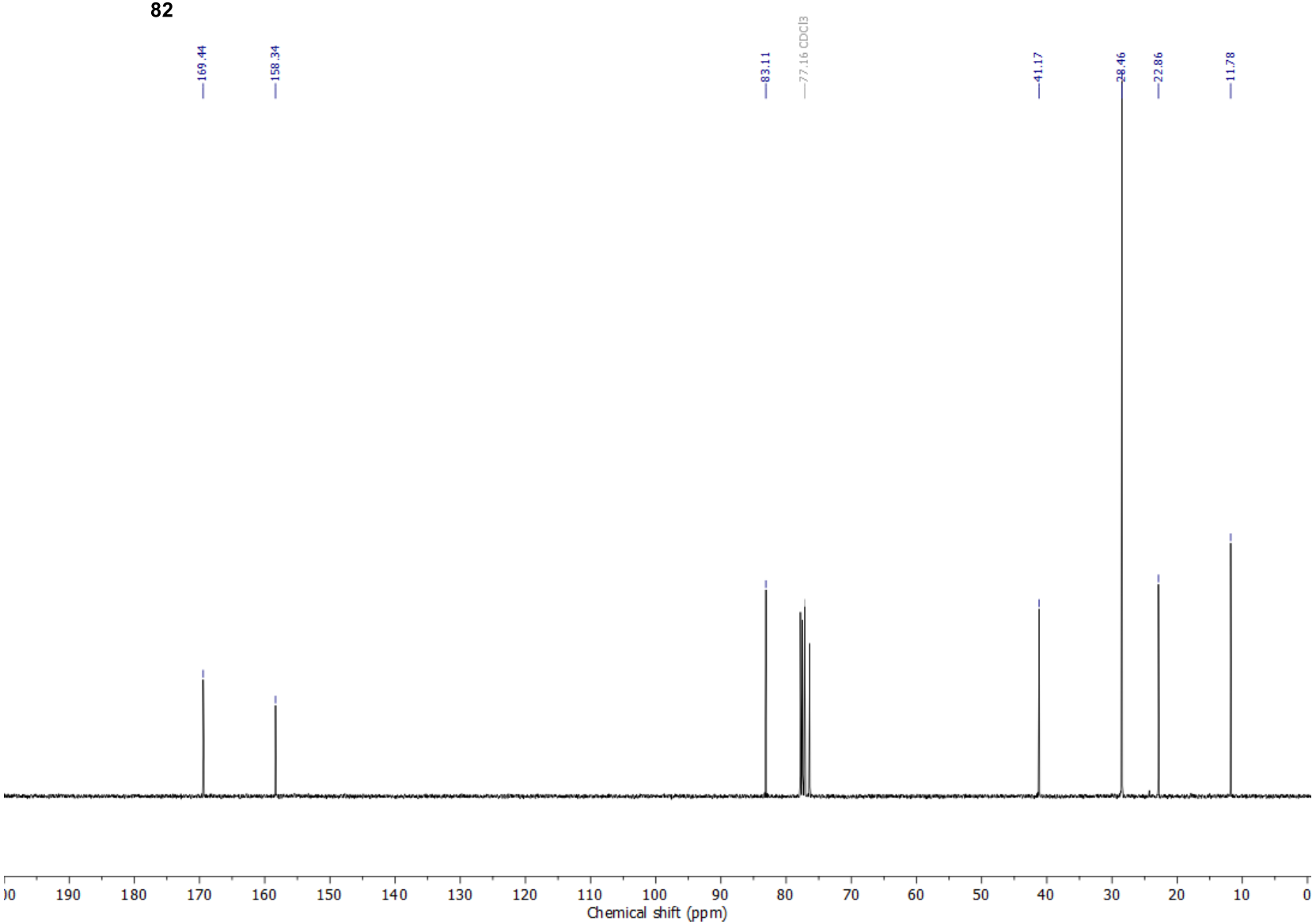
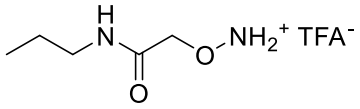


^{1}H NMR (400 MHz, MeOD)



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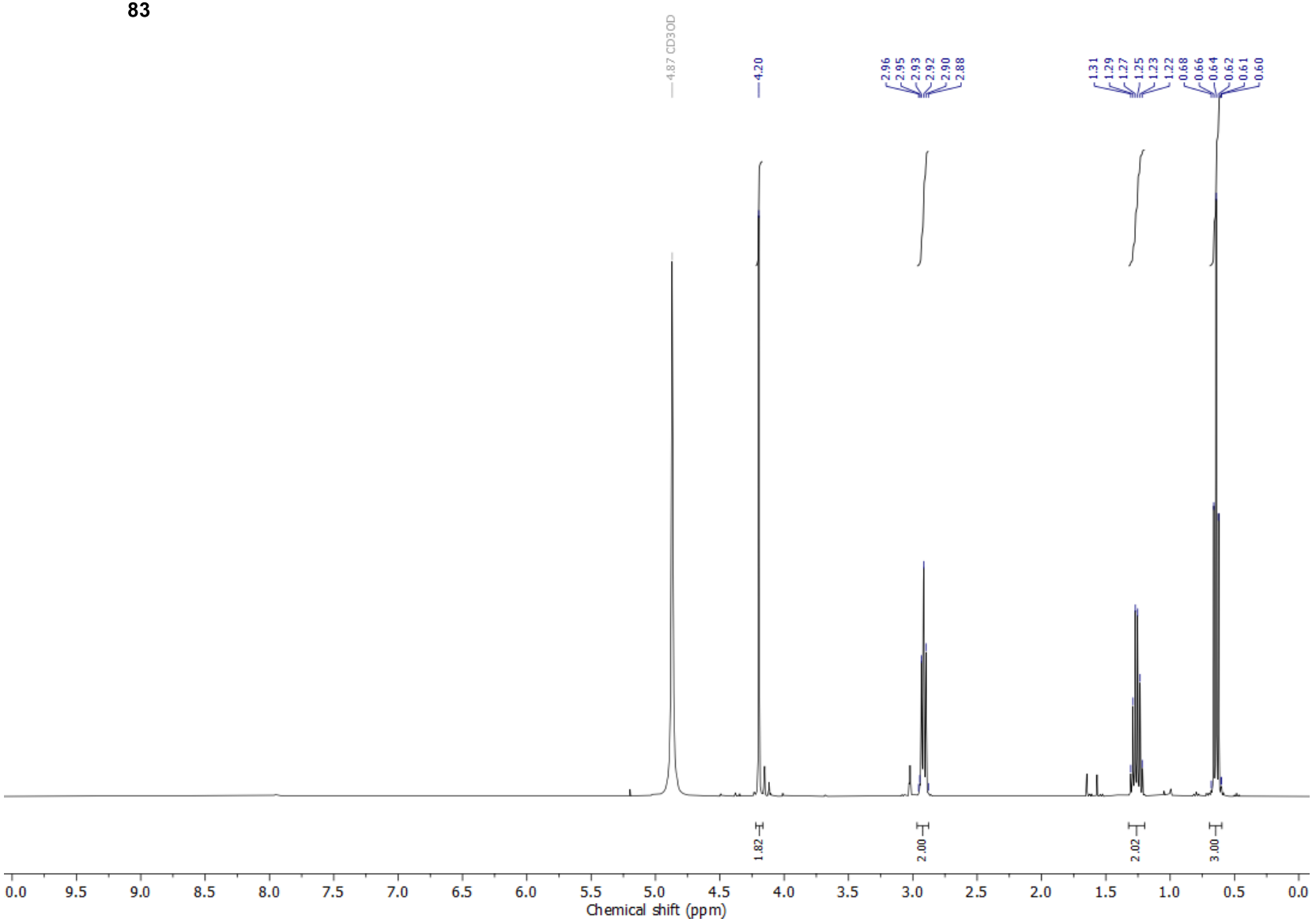
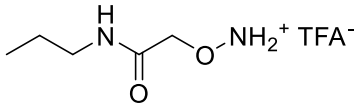


^{13}C NMR (100 MHz, MeOD)



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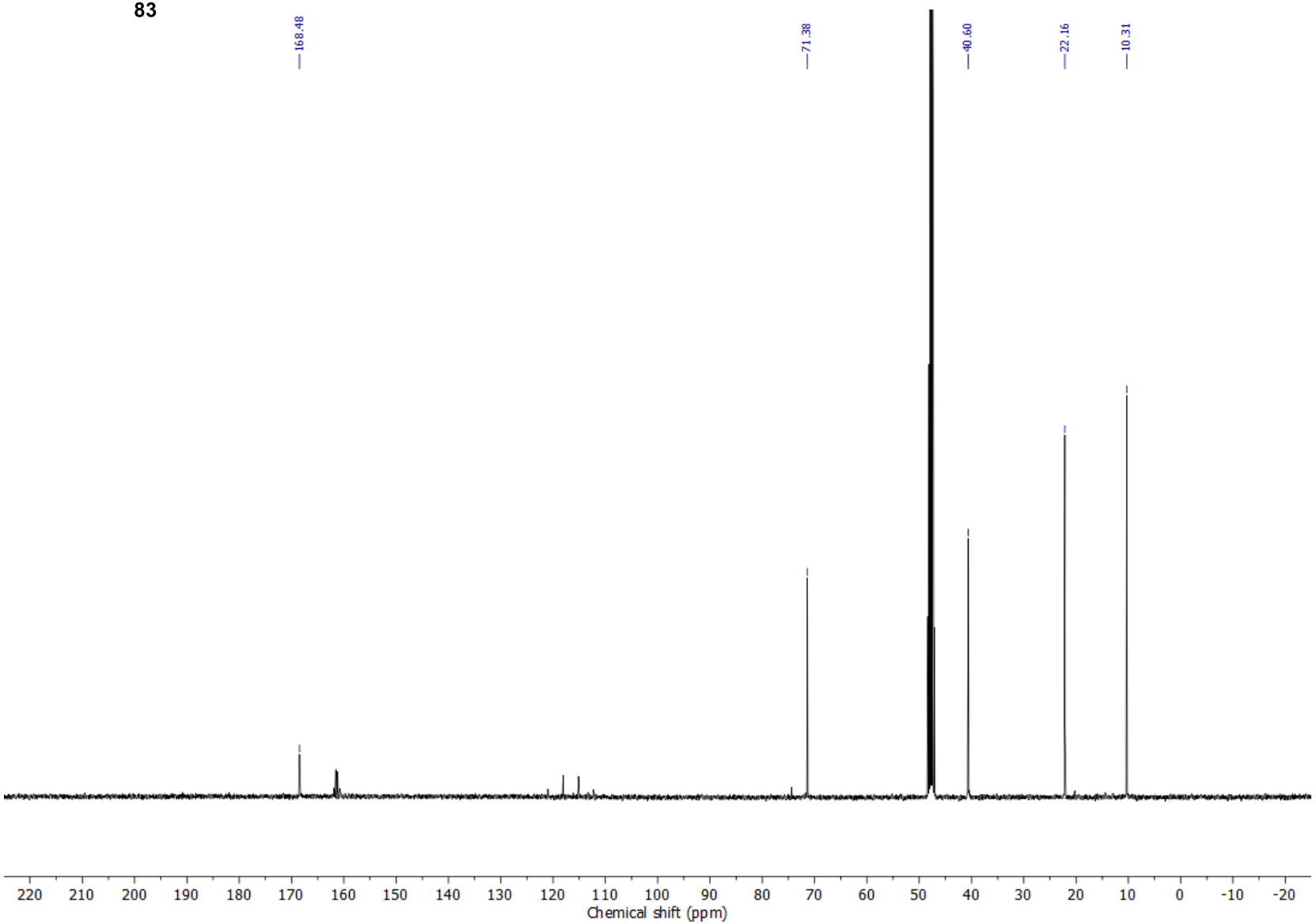
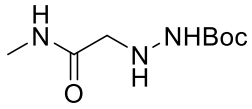


^{1}H NMR (400 MHz, $CDCl_3$)



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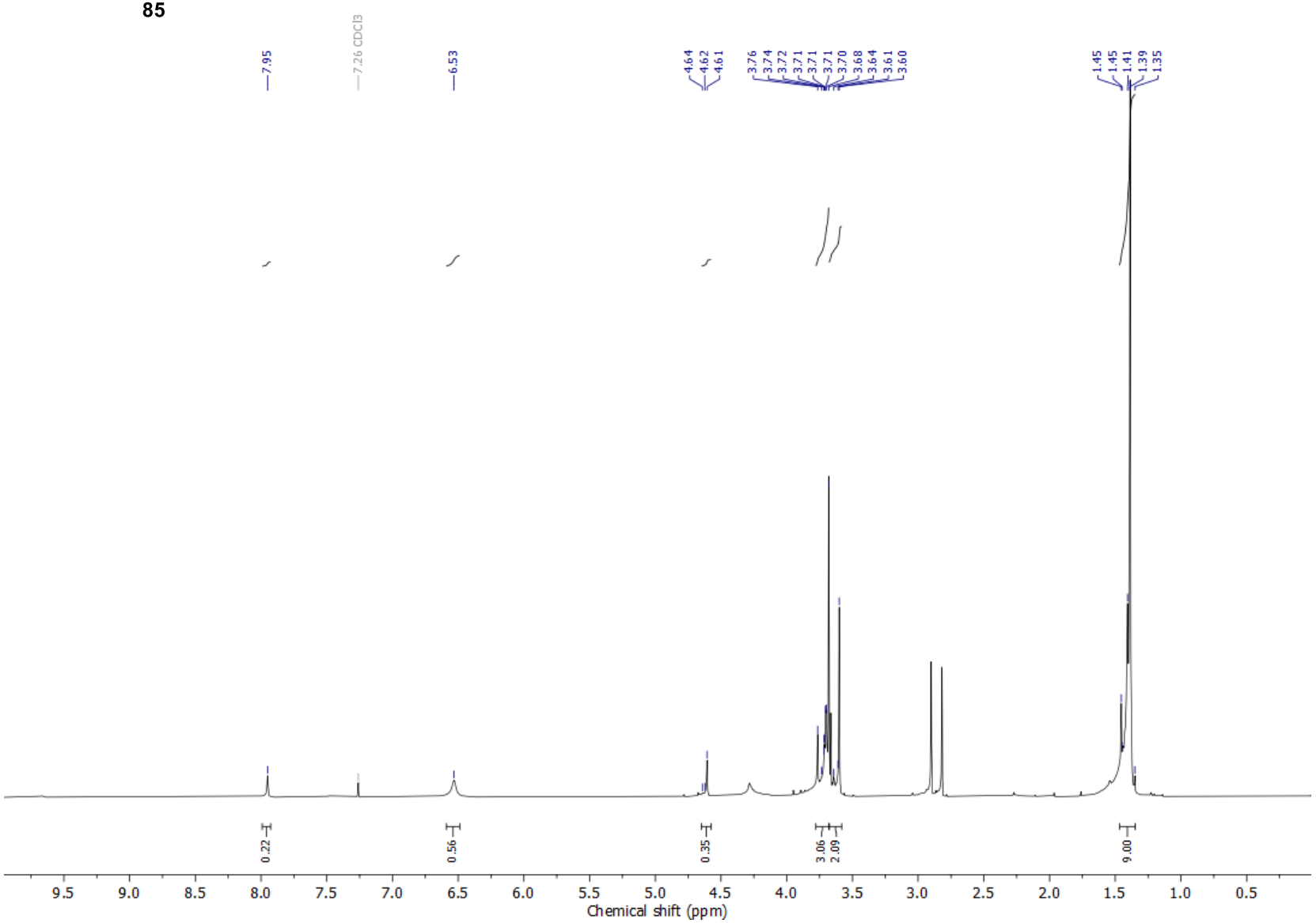

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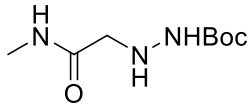

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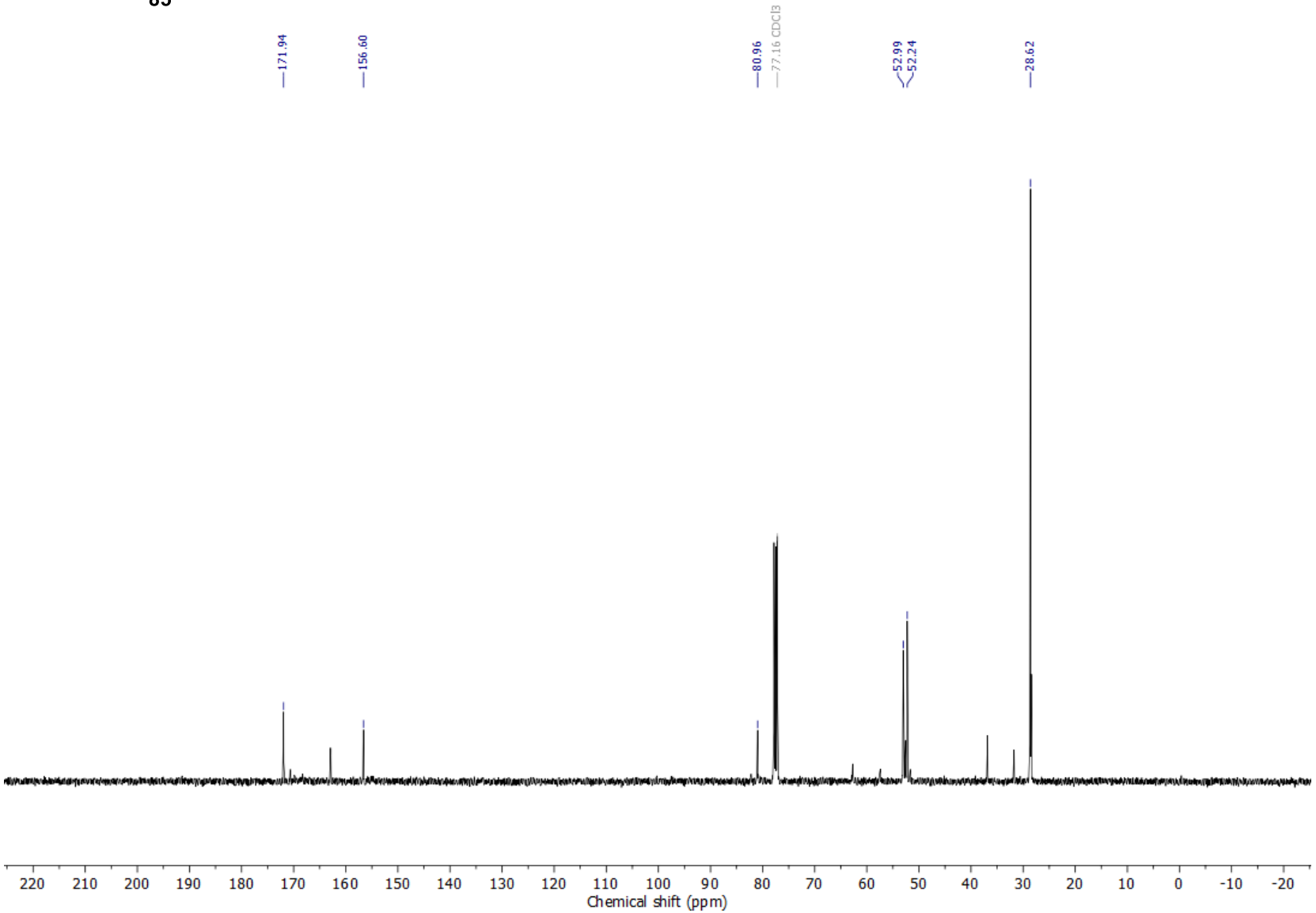

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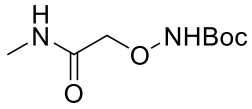

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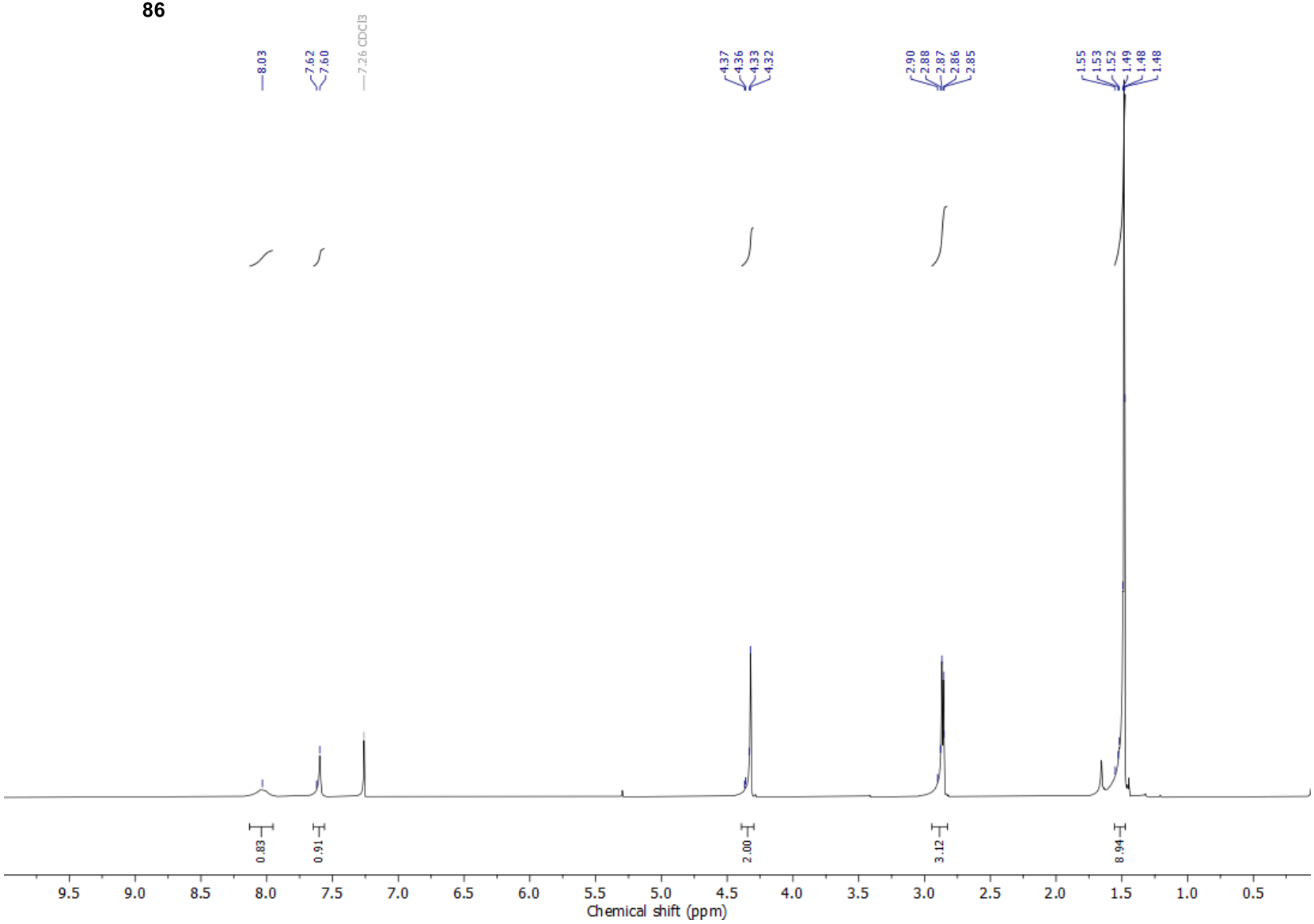
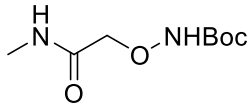


^{13}C NMR (100 MHz, $CDCl_3$)



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