Supporting Information for:

Conformationally Rigid Pyrazoloquinazoline α-Amino Acids: One- and Two-Photon Induced Fluorescence

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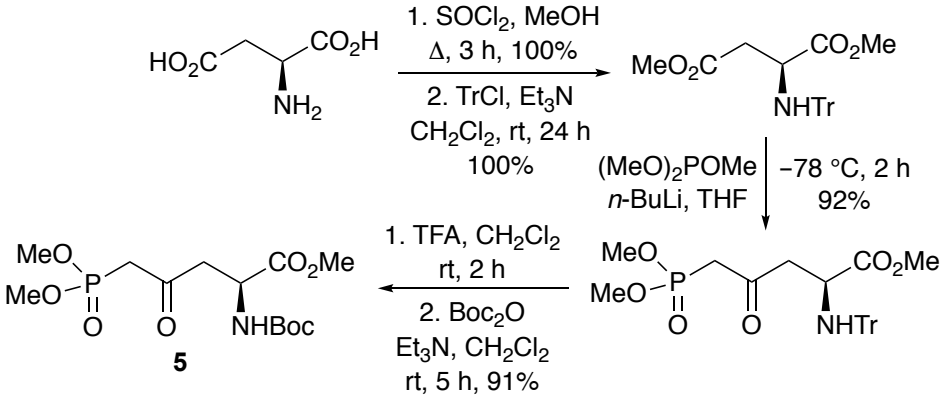
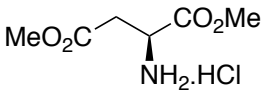
1. General Experimental

All reagents and starting materials were obtained from commercial sources and used as received. Dry solvents were purified using a PureSolv 500 MD solvent purification system. All reactions were performed under an atmosphere of argon unless otherwise mentioned. Brine refers to a saturated solution of sodium chloride. Flash column chromatography was carried out using Fisher matrix silica 60. Macherey-Nagel aluminium-backed plates pre-coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and visualised by staining with $KMnO_4$, vanillin or ninhydrin. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 or 500 spectrometer with chemical shift values in ppm relative to TMS (δ_H 0.00 and δ_C 0.0), or residual chloroform (δ_H 7.26 and δ_C 77.2), dimethylsulfoxide (δ_H 2.50 and δ_C 39.5) or methanol (δ_H 3.31 and δ_C 49.0) as standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Mass spectra were obtained using a JEOL JMS-700 spectrometer for EI and CI or Bruker Microtof-q for ESI. Infrared spectra were obtained neat using a Shimadzu IR Prestige-21 spectrometer. Melting points were determined on a Reichert platform melting point apparatus. Optical rotations were determined as solutions irradiating with the sodium D line (λ= 589 nm) using an Autopol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}.

2. Experimental Procedures and Spectroscopic Data for all Compounds



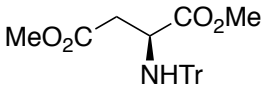
Dimethyl (2*S*)-2-aminobutandioate hydrochloride[1]



To a suspension of L-aspartic acid (5.00 g, 37.6 mmol) in methanol (100 mL) at 0 °C under argon was added thionyl chloride (3.80 mL, 52.6 mmol). The mixture was warmed to room temperature and stirred under reflux for 3 h. The solution was cooled to room temperature and concentrated *in vacuo* to give

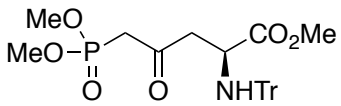
dimethyl (2*S*)-2-aminobutandioate hydrochloride (7.41 g, 100%) as a white solid. Mp 115–116 °C (lit.[1] Mp 114–115 °C); $[\alpha]_D^{24}$ +22.0 (*c* 1.0, MeOH); δ_H (400 MHz, DMSO-d_6) 2.99 (1H, dd, *J* 18.0, 5.5 Hz, 3-*H*H), 3.05 (1H, dd, *J* 18.0, 5.5 Hz, 3-H*H*), 3.66 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 4.35 (1H, t, *J* 5.5 Hz, 2-H), 8.72 (3H, s, $CHNH_3^+$); δ_C (100 MHz, DMSO-d_6) 34.0 (CH_2), 48.4 (CH), 52.2 (CH_3), 53.0 (CH_3), 168.7 (C), 169.6 (C); *m/z* (CI) 162 (MH^+, 100%), 148 (5), 102 (20).

Dimethyl (2*S*)-2-(tritylamino)butandioate[2]



To a solution of dimethyl (2*S*)-2-aminobutandioate hydrochloride (7.46 g, 37.7 mmol) in dichloromethane (150 mL) at 0 °C was added dropwise triethylamine (11.0 mL, 75.4 mmol) and triphenylmethyl chloride (10.5 g, 37.7 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was washed with 2 M citric acid (100 mL), water (100 mL), brine (100 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil. Purification by column chromatography (elution with 50% diethyl ether in petroleum ether) gave dimethyl (2*S*)-2-(tritylamino)butandioate as a colourless solid (15.2 g, 100%). Mp 71–72 °C (lit.[2] 70–71 °C); $[\alpha]_D^{24}$ +36.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.51 (1H, dd, *J* 14.7, 7.0 Hz, 3-*H*H), 2.66 (1H, dd, *J* 14.7, 5.4 Hz, 3-H*H*), 2.93 (1H, d, *J* 10.1 Hz, NH), 3.25 (3H, s, OMe), 3.67 (3H, s, OMe), 3.68–3.73 (1H, m, 2-H), 7.15–7.20 (3H, m, ArH), 7.23–7.28 (6H, m, ArH), 7.46–7.51 (6H, m, ArH); δ_C (101 MHz, $CDCl_3$) 39.0 (CH_2), 50.5 (CH), 50.7 (CH_3), 52.4 (CH_3), 69.9 (C), 125.2 (3 × CH), 126.6 (6 × CH), 127.5 (6 × CH), 144.4 (3 × C), 169.7 (C), 172.6 (C); *m/z* (EI) 403 (M^+, 1%), 326 (35), 243 (100), 165 (30), 83 (70).

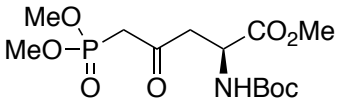
Methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate[2]



A solution of dimethyl methylphosphonate (3.00 mL, 27.3 mmol) in tetrahydrofuran (50 mL) was cooled to –78 °C under an argon atmosphere. *n*-Butyl lithium (2.5 M in hexane, 11.0 mL, 28.6 mmol) was added dropwise and the reaction mixture stirred for 1 h. In a separate reaction vessel, a solution of dimethyl (2*S*)-2-(tritylamino)butandioate (5.00 g, 12.4 mmol) in tetrahydrofuran (100 mL) was cooled to –78 °C and then the dimethyl methylphosphonate/*n*-butyl lithium solution was cannulated into the flask and the reaction mixture stirred at –78 °C for 2 h to give a yellow solution. The reaction was quenched with a saturated solution of ammonium chloride (3 mL) and allowed to warm to room

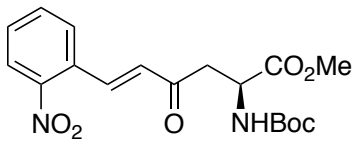
temperature. The mixture was concentrated *in vacuo*. The resulting residue was diluted with ethyl acetate (100 mL), washed with water (2 × 100 mL), brine (100 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. Purification by column chromatography (elution with 75% ethyl acetate in petroleum ether) gave methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate as a colourless solid (5.65 g, 92%). Mp 117–118 °C (lit.[2] 117–118.5 °C); $[\alpha]_D^{24}$ +31.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 16.7, 6.9 Hz, 3-*H*H), 2.85–2.95 (2H, m, 3-H*H* and NH), 3.06 (2H, d, $J_{H–C–P}$ 22.7 Hz, 5-H_2), 3.29 (3H, s, OMe), 3.65–3.73 (1H, m, 2-H), 3.76 (3H, s, OMe), 3.79 (3H, s, OMe), 7.15–7.21 (3H, m, ArH), 7.26 (6H, t, *J* 7.7 Hz, ArH), 7.47 (6H, d, *J* 7.7 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 41.8 (d, $^1J_{C–P}$ 128 Hz, CH_2), 48.8 (CH_2), 52.0 (CH_3), 52.9 (CH_3), 53.0 (CH_3), 53.1 (CH), 71.3 (C), 126.6 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 145.7 (3 × C), 174.0 (C), 199.3 (C); *m/z* (CI) 496 (MH^+, 1%), 301 (5), 254 (90), 243 (100), 237 (55), 167 (45).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (5)



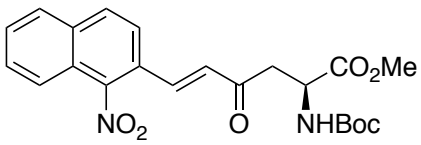
To a solution of methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (1.95 g, 3.94 mmol) in dichloromethane (40 mL) was added trifluoroacetic acid (3.02 mL, 39.4 mmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The resulting residue was dissolved in water (50 mL) and washed with diethyl ether (2 × 50 mL). The aqueous layer was concentrated *in vacuo* to give the TFA salt. This was dissolved in dichloromethane (40 mL), cooled to 0 °C and, di-*tert*-butyl dicarbonate (1.81 mL, 7.87 mmol) and triethylamine (1.12 mL, 7.87 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with dichloromethane (10 mL), washed with 1 M aqueous hydrochloric acid (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography using silica gel, eluting with 2% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) as a clear oil (1.27 g, 91%). ν_{max}/cm^{-1} (neat) 3291 (NH), 2957 (CH), 1713 (C=O), 1506, 1368, 1252, 1165, 1026; $[\alpha]_D^{22}$ +25.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.09 (2H, d, *J* 22.7 Hz, 5-H_2), 3.13 (1H, dd, *J* 18.4, 4.3 Hz, 3-*H*H), 3.29 (1H, dd, *J* 18.4, 4.7 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.50 (1H, ddd, *J* 8.4, 4.7, 4.3 Hz, 2-H), 5.45 (1H, d, *J* 8.4 Hz, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 41.6 (d, J_{C-P} 128 Hz, CH_2) 45.9 (CH_2), 49.5 (CH), 52.7 (CH_3), 53.2 (d, J_{C-O-P} 5.4 Hz, CH_3), 53.3 (d, J_{C-O-P} 5.1 Hz, CH_3), 80.2 (C), 155.5 (C), 171.7 (C), 199.9 (C); *m/z* (ESI) 376.1121 (MNa^+. $C_{13}H_{24}NNaO_8P$ requires 376.1132).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (6a)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) (0.100 g, 0.283 mmol), 2-nitrobenzaldehyde (0.0855 g, 0.566 mmol) and potassium carbonate (0.0430 g, 0.311 mmol) were stirred in anhydrous acetonitrile (3 mL) at 50 °C under argon for 16 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 30% ethyl acetate in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) as a yellow solid (0.0836 g, 78%). Mp 84–86 °C; ν_{max}/cm^{-1} (neat) 3560 (NH), 2981 (CH), 1720 (C=O), 1694 (C=O), 1621, 1605, 1525, 1339, 1159, 1091, 979, 747; $[\alpha]_D^{29}$ +37.7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.22 (1H, dd, *J* 18.0, 4.4 Hz, 3-*H*H), 3.42 (1H, dd, *J* 18.0, 4.4 Hz, 3-H*H*), 3.70 (3H, s, OCH_3), 4.60 (1H, dt, *J* 8.8, 4.4 Hz, 2-H), 5.55 (1H, d, *J* 8.8 Hz, NH), 6.55 (1H, d, *J* 16.2 Hz, 5-H), 7.54 (1H, ddd, *J* 8.5, 7.0, 2.0 Hz, 4′-H), 7.60–7.66 (2H, m, 5′-H and 6′-H), 7.98 (1H, d, *J* 16.2 Hz, 6-H), 8.02 (1H, dd, *J* 8.5, 0.9 Hz, 3′-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.5 (CH_2), 49.5 (CH), 52.7 (CH_3), 80.0 (C), 125.1 (CH), 129.1 (CH), 130.1 (CH), 130.5 (C), 130.8 (CH), 133.8 (CH), 139.3 (CH), 148.4 (C), 155.5 (C) 171.9 (C), 197.3 (C); *m/z* (ESI) 401.1304 (MNa^+. $C_{18}H_{22}N_2NaO_7$ requires 401.1319).

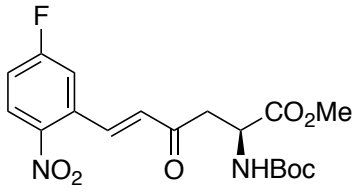
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (6b)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**6b**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) (0.0830 g, 0.235 mmol), 1-nitro-2-naphthaldehyde (0.0944 g, 0.469 mmol) and potassium carbonate (0.0436 g, 0.260 mmol) in anhydrous acetonitrile (3 mL). Purification by flash chromatography using silica gel, eluting with 50% to 80% diethyl ether in petroleum ether gave methyl

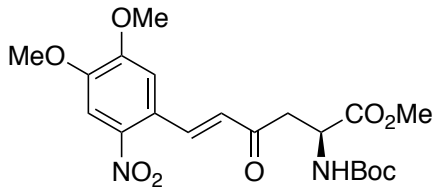
(2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**6b**) as a yellow solid (0.0757 g, 75%). Mp 88–90 °C; ν_{max}/cm^{-1} (neat) 3373 (NH), 2982 (CH), 1735 (C=O), 1692 (C=O), 1669 (C=O), 1523, 1367,1298, 1167, 747; $[\alpha]_D^{26}$ +30.2 (*c* 0.7, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 3.24 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.45 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.65 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.55 (1H, d, *J* 8.6 Hz, NH), 6.85 (1H, d, *J* 16.1 Hz, 5-H), 7.62 (1H, d, *J* 16.1 Hz, 6-H), 7.63–7.70 (2H, m, 6′-H and 7′-H), 7.72 (1H, d, *J* 8.7 Hz, 3′-H), 7.75–7.81 (1H, m, 5′-H), 7.90–7.95 (1H, m, 8′-H), 8.00 (1H, d, *J* 8.7 Hz, 4′-H); δ_C (126 MHz, $CDCl_3$) 28.5 (3 × CH_3), 43.2 (CH_2), 49.6 (CH), 52.9 (CH_3), 80.3 (C), 122.4 (CH), 122.5 (CH), 123.7 (C), 124.6 (C), 128.3 (CH), 128.9 (CH), 129.5 (CH), 130.3 (CH), 131.3 (CH), 134.7 (C), 135.8 (CH), 148.9 (C), 155.7 (C), 171.9 (C), 196.9 (C); *m/z* (ESI) 451.1457 (MNa^+. $C_{22}H_{24}N_2NaO_7$ requires 451.1476).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (6c)



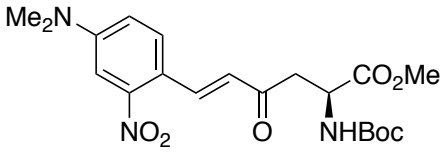
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**6c**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) (0.100 g, 0.313 mmol), 5-fluoro-2-nitrobenzaldehyde (0.0960 g, 0.566 mmol) and potassium carbonate (0.0430 g, 0.311 mmol) in anhydrous acetonitrile (3 mL). Purification by flash chromatography using silica gel, eluting with 50% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**6c**) as a yellow solid (0.0470 g, 42%). Mp 83–86 °C; ν_{max}/cm^{-1} (neat) 2921 (CH), 1748 (C=O), 1701 (C=O), 1614, 1583, 1526, 1343, 1161, 729; $[\alpha]_D^{30}$ +24.2 (*c* 1.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 3.30 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.67 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.64 (1H, d, *J* 8.6 Hz, NH), 6.62 (1H, d, *J* 16.1 Hz, 5-H), 7.29 (1H, ddd, *J* 9.2, 6.9, 2.8 Hz, 4′-H), 7.36 (1H, dd, *J* 8.8, 2.8 Hz, 6′-H), 8.04 (1H, d, *J* 16.1 Hz, 6-H), 8.19 (1H, dd, *J* 9.2, 5.0 Hz, 3′-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.7 (CH_2), 49.5 (CH), 52.7 (CH_3), 80.1 (C), 116.1 (d, $^2J_{CF}$ 24.6 Hz, CH), 117.5 (d, $^2J_{CF}$ 23.4 Hz, CH), 128.1 (d, $^3J_{CF}$ 10.0 Hz, CH), 130.9 (CH), 133.9 (d, $^3J_{CF}$ 9.2 Hz, C), 138.4 (CH), 144.3 (d, $^4J_{CF}$ 2.9 Hz, C), 155.5 (C), 164.9 (d, $^1J_{CF}$ 258.0 Hz, C), 171.8 (C), 197.0 (C); *m/z* (ESI) 419.1215 (MNa^+. $C_{18}H_{21}FN_2NaO_7$ requires 419.1225).

Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (6d)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**6d**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) (0.321 g, 0.909 mmol), 6-nitroveratraldehyde (0.384 g, 1.82 mmol) and potassium carbonate (0.138 g, 0.999 mmol) in anhydrous acetonitrile (9 mL). Purification by flash chromatography using silica gel, eluting with 50% to 80% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**6d**) as a yellow solid (0.275 g, 69%). Mp 75–78 °C; ν_{max}/cm^{-1} (neat) 3384 (NH), 2981 (CH), 1747 (C=O), 1707 (C=O), 1670 (C=O), 1521, 1331, 1280, 1218, 1165, 1066; $[\alpha]_D^{29}$ +28.8 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.37 (9H, s, 3 × CH_3), 3.21 (1H, dd, *J* 17.8, 4.3 Hz, 3-*H*H), 3.39 (1H, dd, *J* 17.8, 4.0 Hz, 3-H*H*), 3.68 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.58–4.59 (1H, m, 2-H), 5.53 (1H, d, *J* 8.7 Hz, NH), 6.46 (1H, d, *J* 16.1 Hz, 5-H), 6.93 (1H, s, 6′-H), 7.58 (1H, s, 3′-H), 8.05 (1H, d, *J* 16.1 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 42.1 (CH_2), 49.5 (CH), 52.6 (CH_3), 56.5 (CH_3) 56.6 (CH_3), 79.9 (C), 108.0 (CH), 109.8 (CH), 124.9 (C), 129.1 (CH), 140.1 (CH), 141.3 (C), 150.2 (C), 153.2 (C), 155.5 (C), 171.9 (C), 197.4 (C); *m/z* (ESI) 461.1517 (MNa^+. $C_{20}H_{26}N_2NaO_9$ requires 461.1531).

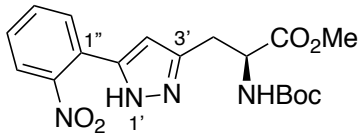
Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4'-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (6e)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (**6e**) was synthesised as described for methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-5-(dimethoxyphosphoryl)-4-oxopentanoate (**5**) (0.697 g, 1.97 mmol), 4-(dimethylamino)-2-nitrobenzaldehyde (0.767 g, 3.95 mmol) and potassium carbonate (0.300 g, 2.17 mmol) in anhydrous acetonitrile (10 mL). The reaction was stirred at 50 °C under argon for 72 h. Purification by flash

chromatography using silica gel, eluting with 60% to 100% diethyl ether in petroleum ether gave methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (**6e**) as a red solid (0.603 g, 82%). Mp 102–104 °C; ν_{max}/cm^{-1} (neat) 3378 (NH), 2953 (CH), 1750 (C=O), 1717 (C=O), 1590, 1541, 1368, 1168; $[\alpha]_D^{25}$ +49.1 (*c* 0.4, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.04 (6H, s, 2 × CH_3), 3.15 (1H, dd, *J* 17.8, 4.3 Hz, 3-*H*H), 3.38 (1H, dd, *J* 17.8, 4.3 Hz, 3-H*H*), 3.69 (3H, s, OCH_3), 4.57 (1H, dt, *J* 8.8, 4.3 Hz, 2-H), 5.55 (1H, d, *J* 8.8 Hz, NH), 6.47 (1H, d, *J* 16.0 Hz, 5-H), 6.80 (1H, dd, *J* 8.9, 2.7 Hz, 5'-H), 7.08 (1H, d, *J* 2.7 Hz, 3'-H), 7.51 (1H, d, *J* 8.9 Hz, 6'-H), 7.83 (1H, d, *J* 16.0 Hz, 6-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 40.1 (2 × CH_3), 42.1 (CH_2), 49.6 (CH), 52.6 (CH_3), 79.9 (C), 106.7 (CH), 115.3 (C), 115.5 (CH), 125.4 (CH), 129.2 (CH), 138.8 (CH), 150.6 (C), 151.4 (C), 155.5 (C), 172.1 (C), 197.3 (C); *m/z* (ESI) 444.1725 (MNa^+. $C_{20}H_{27}N_3NaO_7$ requires 444.1741).

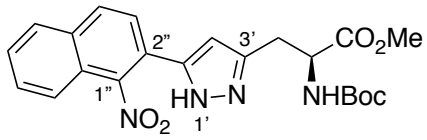
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (7a)



Methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(2′-nitrophenyl)-4-oxohex-5-enoate (**6a**) (0.277 g, 0.733 mmol) was dissolved in methanol (15 mL) and hydrazine monohydrate (65%, 0.176 mL, 3.67 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 4 h, poured into 1 M aqueous hydrochloric acid (50 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and concentrated *in vacuo* to give the dihydropyrazole as a yellow solid (0.247 g, 86%). The dihydropyrazole (0.194 g, 0.494 mmol) was dissolved in anhydrous dichloromethane (20 mL), cooled to −15 °C and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.112 g, 0.494 mmol) was added. After stirring at −15 °C for 3 h, the reaction mixture was concentrated. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2′′-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) as a yellow solid (0.126 g, 65%). Mp 88–90 °C; ν_{max}/cm^{-1} (neat) 3298 (NH), 2923 (CH), 1743 (C=O), 1694 (C=O), 1528, 1366, 1160, 752; $[\alpha]_D^{29}$ +12.4 (*c* 1.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.19 (1H, dd, *J* 14.8, 5.5 Hz, 3-*H*H), 3.25 (1H, dd, *J* 14.8, 5.5 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.54–4.70 (1H, m, 2-H), 5.42 (1H, d, *J* 8.1 Hz, NH), 6.23 (1H, s, 4′-H), 7.46 (1H, td, *J* 8.0, 1.4 Hz, 4′′-H), 7.59 (1H, td, *J* 8.0, 1.2 Hz, 5′′-H), 7.68 (1H, dd, *J* 8.0, 1.4 Hz, 6′′-H), 7.72 (1H, dd, *J* 8.0, 1.2 Hz, 3′′-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 29.4 (CH_2), 52.7 (CH_3), 53.1 (CH), 80.4 (C), 104.7 (CH), 123.6 (CH), 126.5 (C), 128.7 (CH), 130.8 (CH), 132.0 (CH), 141.6

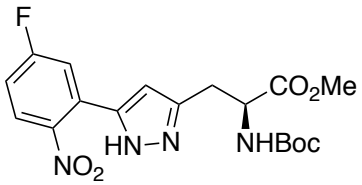
(C), 145.4 (C), 149.0 (C), 155.5 (C), 172.1 (C); *m/z* (ESI) 413.1423 (MNa^+. $C_{18}H_{22}N_4NaO_6$ requires 413.1432).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1″-nitronaphthalen-2″-yl)-1′*H*-pyrazol-3′-yl]propanoate (7b)



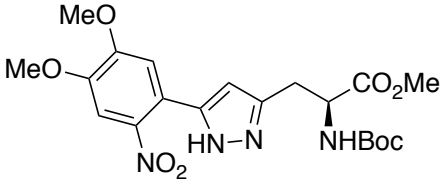
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(2″-nitronaphthalenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7b**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) using methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(1′-nitronaphthalen-2′-yl)-4-oxohex-5-enoate (**6b**) (0.469 g, 1.10 mmol) and hydrazine monohydrate (0.264 mL, 5.48 mmol) in methanol (15 mL). This gave the dihydropyrazole as a yellow solid (0.490 g, 100%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.122 g, 0.538 mmol) was added to a solution of the dihydropyrazole (0.238 g, 0.538 mmol) in dichloromethane (20 mL) at −40 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(1″-nitronaphthalen-2″-yl)-1′*H*-pyrazol-3′-yl]propanoate (**7b**) as a yellow solid (0.0581 g, 25%). Mp 86–90 °C; ν_{max}/cm^{-1} (neat) 3312 (NH), 2922 (CH), 1741 (C=O), 1697 (C=O), 1530, 1368, 1162, 751, 732; $[\alpha]_D^{20}$ +26.0 (*c* 0.6, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.24 (1H, dd, *J* 15.2, 6.0 Hz, 3-*H*H), 3.32 (1H, dd, *J* 15.2, 5.3 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.67 (1H, br s, 2-H), 5.46 (1H, d, *J* 6.9 Hz, NH), 6.41 (1H, s, 4′-H), 7.53–7.66 (2H, m, 6″-H and 7″-H), 7.70 (1H, d, *J* 8.4 Hz, 5″-H), 7.83 (1H, d, *J* 8.6 Hz, 3″-H), 7.88 (1H, d, *J* 8.0 Hz, 8″-H), 7.95 (1H, d, *J* 8.6 Hz, 4″-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.4 (CH_2), 52.9 (CH_3), 53.2 (CH), 80.7 (C), 104.6 (CH), 121.7 (CH), 124.8 (C), 125.6 (CH), 127.6 (CH), 128.1 (CH), 128.9 (CH), 130.4 (CH), 133.2 (2 × C), 141.5 (C), 145.5 (C), 145.7 (C), 155.6 (C), 172.1 (C); *m/z* (ESI) 463.1573 (MNa^+. $C_{22}H_{24}N_4NaO_6$ requires 463.1588).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5″-fluoro-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (7c)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5″-fluoro-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7c**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) using (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(5′-fluoro-2′-nitrophenyl)-4-oxohex-5-enoate (**7c**) (0.479 g, 1.21 mmol) and hydrazine monohydrate (0.291 mL, 6.05 mmol) in methanol (15 mL). This gave the dihydropyrazole as a yellow solid (0.474 g, 95%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.0320 g, 0.141 mmol) was added to a solution of the dihydropyrazole (0.0579 g, 0.141 mmol) in dichloromethane (10 mL) at −40 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5″-fluoro-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7c**) as a yellow solid (0.0260 g, 45%). Mp 82–84 °C; ν_{max}/cm^{-1} (neat) 3287 (NH), 2981 (CH), 1741 (C=O), 1696 (C=O), 1529, 1366, 1161, 887, 734; $[\alpha]_D^{26}$ +20.2 (*c* 0.8, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.18 (1H, dd, *J* 15.0, 5.1 Hz, 3-*H*H), 3.26 (1H, dd, *J* 15.0, 5.1 Hz, 3-H*H*), 3.76 (3H, s, OMe), 4.64 (1H, br s, 2-H), 5.44 (1H, d, *J* 5.0 Hz, NH), 6.22 (1H, s, 4′-H), 7.12 (1H, ddd, *J* 8.9, 7.3, 2.6 Hz, 4″-H), 7.41 (1H, dd, *J* 9.0, 2.6 Hz, 6″-H), 7.76 (1H, dd, *J* 8.9, 5.0 Hz, 3″-H); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.5 (CH_2), 52.9 (CH_3), 53.1 (CH), 80.8 (C), 104.9 (CH), 115.6 (d, $^2J_{CF}$ 23.5 Hz, CH), 117.8 (d, $^2J_{CF}$ 24.5 Hz, CH), 126.5 (d, $^3J_{CF}$ 9.7 Hz, CH), 130.1 (d, $^3J_{CF}$ 9.2 Hz, C), 141.1 (C), 145.2 (d, $^4J_{CF}$ 2.7 Hz, C), 145.4 (C), 155.6 (C), 163.9 (d, $^1J_{CF}$ 254.6 Hz, C), 172.0 (C); *m/z* (ESI) 431.1321 (MNa^+. $C_{18}H_{21}FN_4NaO_6$ requires 431.1337).

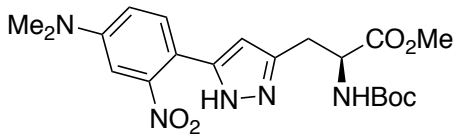
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4″,5″-dimethoxy-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (7d)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4″,5″-dimethoxy-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7d**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) using methyl (2*S*,5*E*)-2-(*tert*-

butoxycarbonylamino)-6-(4′,5′-dimethoxy-2′-nitrophenyl)-4-oxohex-5-enoate (**6d**) (0.573 g, 1.31 mmol) and hydrazine monohydrate (0.315 mL, 6.55 mmol) in methanol (15 mL). This gave the dihydropyrazole as a yellow solid (0.532 g, 90%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.145 g, 0.637 mmol) was added to a solution of the dihydropyrazole (0.288 g, 0.637 mmol) at −15 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 0% to 20% acetonitrile in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4″,5″-dimethoxy-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7d**) as a yellow solid (0.0955 g, 34%). Mp 79–82 °C; ν_{max}/cm^{-1} (neat) 3395 (NH), 2925 (CH), 1711 (C=O), 1522, 1337, 1268, 1221, 1163, 1045, 790; $[\alpha]_D^{29}$ +9.1 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.10–3.32 (2H, m, 3-H_2), 3.76 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.65 (1H, br s, 2-H), 5.51 (1H, d, *J* 8.1 Hz, NH), 6.18 (1H, s, 4′-H), 7.04 (1H, s, 6″-H), 7.44 (1H, s, 3″-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 29.7 (CH_2), 52.7 (CH_3), 53.1 (CH), 56.4 (CH_3), 56.5 (CH_3), 80.4 (C), 105.4 (CH), 107.7 (CH), 113.0 (CH), 121.3 (C), 141.3 (C), 142.1 (C), 145.5 (C), 148.6 (C), 152.2 (C), 155.5 (C), 172.1 (C); *m/z* (ESI) 473.1632 (MNa^+. $C_{20}H_{26}N_4NaO_8$ requires 473.1643).

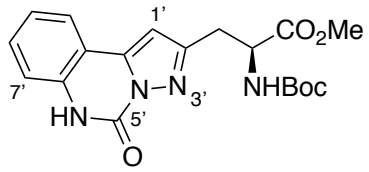
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (7e)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**7e**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) using methyl (2*S*,5*E*)-2-(*tert*-butoxycarbonylamino)-6-(4′-dimethylamino-2′-nitrophenyl)-4-oxohex-5-enoate (**6e**) (0.291 g, 0690 mmol) and hydrazine monohydrate (0.166 mL, 3.45 mmol) in methanol (10 mL). This gave the dihydropyrazole as a yellow solid (0.263 g, 88%). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.695 g, 3.06 mmol) was added to a solution of the dihydropyrazole (1.33 g, 3.06 mmol) in dichloromethane (60 mL) at −40 °C and stirred for 3 h. Purification by flash chromatography using silica gel, eluting with 3% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4''-dimethylamino-2''-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**7e**) as a yellow solid (0.960 g, 72%). Mp 107–110 °C; ν_{max}/cm^{-1} (neat) 3301 (NH), 2983 (CH), 1713 (C=O), 1625 (C=O), 1531, 1367, 1163; $[\alpha]_D^{26}$ +24.6 (*c* 0.7, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.02 (6H, s, 2 × CH_3), 3.18 (2H, d, *J* 5.2 Hz, 3-H_2), 3.73 (3H, s, OCH_3), 4.61 (1H, br s, 2-H), 5.50 (1H, d, *J* 6.5, NH), 6.11 (1H, s, 4′-H), 6.82 (1H, dd, *J* 8.8, 2.7 Hz, 5″-H), 6.95 (1H, d, *J* 2.7 Hz, 3"-H), 7.43 (1H, d, *J* 8.8 Hz, 6"-H); δ_C

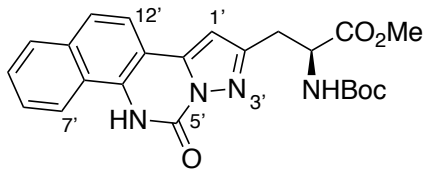
(101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 29.9 (CH_2), 40.3 (2 × CH_3), 52.7 (CH_3), 53.2 (CH), 80.3 (C), 104.6 (CH), 106.5 (CH), 112.5 (C), 115.1 (CH), 131.7 (CH), 143.6 (C), 149.9 (C), 150.3 (2 × C), 155.6 (C), 172.3 (C); *m/z* (ESI) 456.1840 (MNa^+. $C_{20}H_{27}N_5NaO_6$ requires 456.1854).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-5′-yl)propanoate (8a)



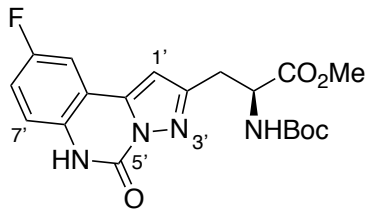
To a solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino-3-[5′-(2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7a**) (0.0871 g, 0.223 mmol) in methanol (5 mL) was added 10% palladium on carbon (0.0120 g, 0.0112 mmol). The reaction mixture was purged with hydrogen for 0.5 h and then stirred under an atmosphere of hydrogen at room temperature for 16 h. The reaction mixture was filtered through Celite®, which was washed with methanol. The filtrate was concentrated *in vacuo* and the resulting aniline was dissolved in dichloromethane (10 mL). Triphosgene (0.0265 g, 0.089 mmol) and triethylamine (0.063 mL, 0.0446 mmol) was added at −10 °C. After stirring at room temperature for 1 h, the reaction mixture was diluted with dichloromethane (40 mL), washed with 1 M aqueous hydrochloric acid (50 mL), water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by flash chromatography using silica gel, eluting with 90% ethyl acetate in petroleum ether gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) as a white solid (0.0448 g, 52%). Mp 123–126 °C; ν_{max}/cm^{-1} (neat) 3248 (NH), 2978 (CH), 1715 (C=O), 1598, 1491, 1343, 1162, 734; $[\alpha]_D^{30}$ +13.4 (*c* 1.3, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.38 (9H, s, 3 × CH_3), 3.32 (1H, dd, *J* 14.8, 7.0 Hz 3-*H*H), 3.40 (1H, dd, *J* 14.8, 5.0 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.67–4.80 (1H, m, 2-H), 5.44 (1H, d, *J* 8.3 Hz, NH), 6.75 (1H, s, 1′-H), 7.24 (1H, t, *J* 7.4 Hz, 9′-H), 7.36–7.48 (2H, m, 7′-H and 8′-H), 7.72 (1H, d, *J* 7.4 Hz, 10′-H), 11.24 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.5 (CH_2), 52.7 (CH_3), 53.1 (CH), 80.2 (C), 101.0 (CH), 112.7 (C), 116.6 (CH), 123.8 (CH), 124.1 (CH), 130.5 (CH), 133.8 (C), 142.0 (C), 146.6 (C), 154.3 (C), 155.5 (C), 172.3 (C); *m/z* (ESI) 409.1467 (MNa^+. $C_{19}H_{22}N_4NaO_5$ requires 409.1482).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (8b)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate **(8b)** was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(2″-nitronaphthalenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7b**) (0.133 g, 0.301 mmol) with 10% palladium on carbon (0.0160 g, 0.0151 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0357 g, 0.120 mmol) and triethylamine (0.0854 mL, 0.602 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 30% to 60% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate **(8b**) as a white solid (0.0391 g, 30%). Mp 140–142 °C; ν_{max}/cm^{-1} (neat) 3278 (NH), 2975 (CH), 1709 (C=O), 1344, 1165; $[\alpha]_D^{23}$ +17.6 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.31–3.47 (2H, m, 3-H), 3.82 (3H, s, OCH_3), 4.72–4.86 (1H, m, 2-H), 5.60 (1H, d, *J* 8.3 Hz, NH), 6.72 (1H, s, 1′-H), 7.51–7.61 (3H, m, 9′-H, 11′-H and 12′-H), 7.70 (1H, t, *J* 8.0 Hz, 8′-H), 7.82 (1H, d, *J* 8.0 Hz, 10′-H), 8.39 (1H, d, *J* 8.0 Hz, 7′-H), 10.50 (1H, s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.4 (CH_2), 52.8 (CH_3), 53.1 (CH), 80.2 (C), 100.9 (CH), 108.5 (C), 120.4 (CH), 120.9 (CH), 121.7 (C), 124.6 (CH), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.7 (C), 133.9 (C), 142.4 (C), 146.0 (C), 154.7 (C), 155.6 (C), 172.5 (C); *m/z* (ESI) 459.1634 (MNa^+. $C_{23}H_{24}N_4NaO_5$ requires 459.1639).

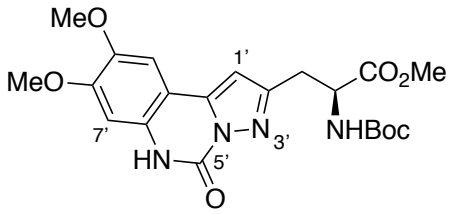
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (8c)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8c**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) using

methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(5″-fluoro-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7c**) (0.142 g, 0.347 mmol) with 10% palladium on carbon (0.0185 g, 0.0174 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0412 g, 0.139 mmol) and triethylamine (0.0982 mL, 0.694 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 50% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8c**) as a white solid (0.105 g, 75%). Mp 135–137 °C; ν_{max}/cm^{-1} (neat) 3250 (NH), 2979 (CH), 1720 (C=O), 1497, 1367, 1268, 1169; $[\alpha]_D^{26}$ +16.2 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.32 (1H, dd, *J* 14.7, 7.3 Hz, 3-*H*H), 3.41 (1H, dd, *J* 14.7, 5.0 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.69–4.78 (1H, m, 2-H), 5.41 (1H, d, *J* 8.3 Hz, NH), 6.75 (1H, s, 1′-H), 7.20 (1H, td, *J* 8.7, 2.5 Hz, 8′-H), 7.37–7.42 (1H, m, 10′-H), 7.46 (1H, dd, *J* 8.7, 4.2 Hz, 7′-H), 11.30 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 31.4 (CH_2), 52.6 (CH_3), 53.0 (CH), 80.2 (C), 101.6 (CH), 109.6 (d, $^2J_{CF}$ 24.6 Hz, CH), 113.6 (d, $^3J_{CF}$ 9.2 Hz, C), 118.3 (d, $^2J_{CF}$ 23.4 Hz, CH), 118.4 (d, $^3J_{CF}$ 8.5 Hz, CH), 130.1 (d, $^4J_{CF}$ 1.7 Hz, C), 141.0 (C), 146.3 (C), 154.4 (C), 155.4 (C), 158.9 (d, $^1J_{CF}$ 244.5 Hz, C), 172.1 (C); *m/z* (ESI) 427.1378 (MNa^+. $C_{19}H_{21}FN_4NaO_5$ requires 427.1388).

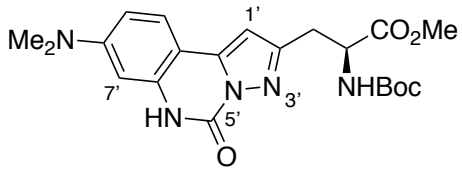
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (8d)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8d**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5′-(4″,5″-dimethoxy-2″-nitrophenyl)-1′*H*-pyrazol-3′-yl]propanoate (**7d**) (0.0280 g, 0.0622 mmol) with 10% palladium on carbon (0.00330 g, 0.00311 mmol) in methanol (5 mL). The resulting aniline was reacted with triphosgene (0.00740 mg, 0.0249 mmol) and triethylamine (0.0176 mL, 0.124 mmol) in dichloromethane (5 mL). Purification by flash chromatography using silica gel, eluting first with 60% ethyl acetate in dichloromethane and then with 3% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8d**) as a white solid (0.0156 g, 58%). Mp 128–130 °C; ν_{max}/cm^{-1} (neat) 3250 (NH), 2929 (CH), 1719 (C=O), 1509, 1278, 1226, 1165, 1021; $[\alpha]_D^{28}$ +1.6 (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.29 (1H, dd, *J* 14.8, 7.4 Hz, 3-

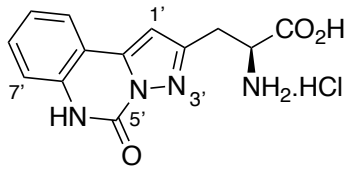
*H*H), 3.37 (1H, dd, *J* 14.8, 4.9 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.68–4.80 (1H, m, 2-H), 5.42 (1H, d, *J* 8.3 Hz, NH), 6.59 (1H, s, 1′-H), 6.83 (1H, s, 7′-H), 7.04 (1H, s, 10′-H), 11.05 (1H, s, NH); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.6 (CH_2), 52.7 (CH_3), 53.1 (CH), 56.5 (CH_3), 56.7 (CH_3), 80.2 (C), 99.2 (CH), 99.3 (CH), 105.0 (CH), 105.3 (C), 128.7 (C), 142.2 (C), 146.6 (C), 146.7 (C), 152.1 (C), 154.4 (C), 155.6 (C), 172.4 (C); *m/z* (ESI) 469.1676 (MNa^+. $C_{21}H_{26}N_4NaO_7$ requires 469.1694).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (8e)



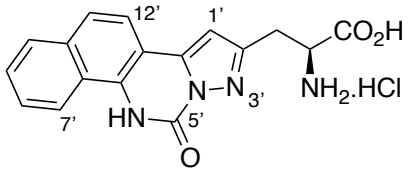
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**8e**) was synthesised as described for methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[5'-(4"-dimethylamino-2"-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoate (**7e**) (0.0814 g, 0.188 mmol) with 10% palladium on carbon (0.0100g, 00.00940 mmol) in methanol (10 mL). The resulting aniline was reacted with triphosgene (0.0223 g, 0.0752 mmol) and triethylamine (0.0532 mL, 0.376 mmol) in dichloromethane (10 mL). Purification by flash chromatography using silica gel, eluting with 80% to 100% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**8e**) as a yellow solid (0.0384 g, 48%). Mp 174–176 °C; ν_{max}/cm^{-1} (neat) 3271 (NH), 2978 (CH), 1705 (C=O), 1628 (C=O), 1528, 1373, 1157, 910, 725; $[\alpha]_D^{27}$ +6.0 (*c* 0.7, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.39 (9H, s, 3 × CH_3), 3.01 (6H, s, 2 × CH_3), 3.26 (1H, dd, *J* 14.4, 7.2 Hz, 3-*H*H), 3.33 (1H, dd, *J* 14.4, 4.8 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.64–4.77 (1H, m, 2-H), 5.44 (1H, d, *J* 8.2 Hz, NH), 6.36–6.46 (2H, m, 1'-H and 7'-H), 6.56 (1H, dd, *J* 8.8, 1.5 Hz, 9'-H), 7.47 (1H, d, *J* 8.8 Hz, 10'-H), 10.44 (1H, s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 31.4 (CH_2), 40.4 (2 × CH_3), 52.6 (CH_3), 53.1 (CH), 80.0 (C), 96.9 (CH), 98.0 (CH), 102.0 (C), 109.3 (CH), 124.8 (CH), 135.6 (C), 142.9 (C), 146.8 (C), 151.9 (C), 154.1 (C), 155.6 (C), 172.5 (C); *m/z* (ESI) 452.1900 (MNa^+. $C_{21}H_{27}N_5NaO_5$ requires 452.1904).

(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4a)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8a**) (0.0417 g, 0.108 mmol) was dissolved in methanol (3.5 mL) and a solution of caesium carbonate (0.0457 g, 0.140 mmol) in water (1.5 mL) was added. The reaction mixture was stirred at room temperature for 16 h before being concentrating *in vacuo*. The resulting residue was dissolved in water (20 mL), acidified to pH 1 with 1 M aqueous hydrochloric acid and extracted with dichloromethane (3 × 30 mL). The organic layers were combined and washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in 2 M aqueous hydrochloric acid (5 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and the resulting residue was purified by recrystallisation from methanol and diethyl ether to give (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4a**) as a white solid (0.0268 g, 80%). Mp 280–283 °C (decomposition); ν_{max}/cm^{-1} (neat) 3373 (NH), 2479 (CH), 1701 (C=O), 1599, 1490, 1355, 1119, 973; $[\alpha]_D^{27}$ –16.6 (*c* 0.5, MeOH); δ_H (500 MHz, CD_3OD) 3.43 (1H, dd, *J* 16.3, 8.3 Hz, 3-*H*H), 3.56 (1H, dd, *J* 16.3, 3.9 Hz, 3-H*H*), 4.44 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 7.02 (1H, s, 1′-H), 7.25–7.36 (2H, m, 7′-H and 9′-H), 7.48–7.54 (1H, m, 8′-H), 7.93 (1H, d, *J* 7.8 Hz, 10′-H); δ_C (126 MHz, CD_3OD) 30.0 (CH_2), 53.4 (CH), 102.2 (CH), 113.7 (C), 117.0 (CH), 125.1 (CH), 125.3 (CH), 132.0 (CH), 135.6 (C), 143.5 (C), 147.0 (C), 154.3 (C), 171.3 (C); *m/z* (ESI) 295.0795 (MNa^+. $C_{13}H_{12}N_4NaO_3$ requires 295.0802).

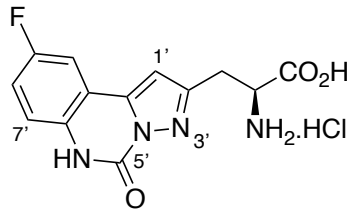
(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4b)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8b**) (0.0293 g, 0.0671 mmol) was suspended in 6 M aqueous hydrochloric acid (5 mL). The reaction mixture was stirred under reflux for 16 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* and recrystallised from methanol and

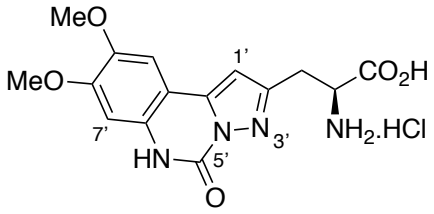
diethyl ether to give (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2-yl)propanoic acid hydrochloride (**4b**) as an off-white solid (0.0089 g, 37%). Mp 278–279 °C (decomposition); ν_{max}/cm^{-1} (neat) 3178 (NH), 2975 (CH), 1725 (C=O), 1509, 1350, 1177, 801; $[\alpha]_D^{23}$ –17.0 (*c* 0.2, MeOH); δ_H (500 MHz, CD_3OD) 3.48 (1H, dd, *J* 16.4, 8.3 Hz, 3-*H*H), 3.61 (1H, dd, *J* 16.4, 3.9 Hz, 3-H*H*), 4.55 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 7.10 (1H, s, 1′-H), 7.64–7.72 (2H, m, 8′-H and 9′-H), 7.77 (1H, d, *J* 8.7 Hz, 11′-H), 7.92 (1H, d, *J* 8.7 Hz, 12′-H), 7.95 (1H, dd, *J* 8.1, 1.0 Hz, 10′-H), 8.50 (1H, d, *J* 8.1 Hz, 7′-H); δ_C (126 MHz, CD_3OD) 29.9 (CH_2), 53.1 (CH), 102.1 (CH), 109.6 (C), 121.7 (CH), 122.5 (CH), 123.3 (C), 125.9 (CH), 128.6 (CH), 129.1 (CH), 130.1 (CH), 131.7 (C), 135.7 (C), 144.0 (C), 147.4 (C), 154.6 (C), 170.9 (C); *m/z* (ESI) 345.0948 (MNa^+. $C_{17}H_{14}N_4NaO_3$ requires 345.0958).

(2*S*)-2-Amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4c)



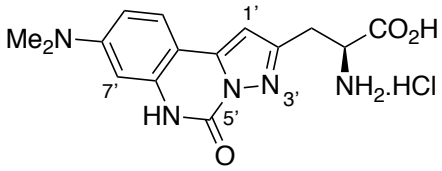
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazole[1′,5′-c]quinazolin-2′-yl)propanoate (**8c**) (0.0469 g, 0.116 mmol) was suspended in 6 M aqueous hydrochloric acid (5 mL). The reaction mixture was stirred under reflux for 16 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* and recrystallised from methanol and diethyl ether to give (2*S*)-2-amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4c**) as a white solid (0.0150 g, 40%). Mp 275–276 °C (decomposition); ν_{max}/cm^{-1} (neat) 3175 (NH), 2950 (CH), 1710 (C=O), 1506, 1358, 1268, 1191, 828; $[\alpha]_D^{27}$ −14.5 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 3.19–3.45 (2H, m, 3-H_2), 4.34 (1H, t, *J* 6.5 Hz, 2-H), 7.18 (1H, s, 1′-H), 7.35–7.47 (2H, m, 8′-H and 10′-H), 7.94 (1H, dd, *J* 8.8, 1.6 Hz, 7′-H), 8.35 (2H, br s, NH_2), 11.93 (1H, s, NH); δ_C (101 MHz, DMSO-d_6) 29.0 (CH_2), 51.6 (CH), 102.4 (CH), 109.7 (d, $^2J_{CF}$ 24.9 Hz, CH), 113.0 (d, $^3J_{CF}$ 9.6 Hz, C), 117.8 (d, $^3J_{CF}$ 8.9 Hz, CH), 118.0 (d, $^2J_{CF}$ 24.9 Hz, CH), 131.0 (d, $^4J_{CF}$ 1.4 Hz, C), 140.6 (d, $^4J_{CF}$ 3.3 Hz, C), 143.9 (C), 151.6 (C), 157.8 (d, $^1J_{CF}$ 239.5 Hz, C), 169.9 (C); *m/z* (ESI) 313.0706 (MNa^+. $C_{13}H_{11}FN_4NaO_3$ requires 313.0707).

(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4d)



(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4d**) was synthesised as described for (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoate (**8d**) (0.0226 g, 0.0507 mmol) and caesium carbonate (0.0215 g, 0.0658 mmol). Recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4d**) as a white solid (0.0152 g, 82%). Mp 268–270 °C (decomposition); ν_{max}/cm^{-1} (neat) 3375 (NH), 2919 (CH), 1715 (C=O), 1507, 1284, 1229, 1011, 791; $[\alpha]_D^{25}$ −17.6 (*c* 0.4, MeOH); δ_H (500 MHz, CD_3OD) 3.45 (1H, dd, *J* 16.0, 9.3 Hz, 3-*H*H), 3.59 (1H, dd, *J* 16.0, 3.5 Hz, 3-H*H*), 3.86 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.57 (1H, dd, *J* 9.3, 3.5 Hz, 2-H), 6.56 (1H, s, 1′-H), 6.89 (1H, s, 10′-H), 7.25 (1H, s, 7′-H); δ_C (126 MHz, CD_3OD) 30.1 (CH_2), 53.6 (CH), 56.5 (CH_3), 56.9 (CH_3), 99.4 (CH), 100.8 (CH), 105.8 (C), 106.4 (CH), 130.1 (C), 143.4 (C), 146.7 (C), 147.8 (C), 153.4 (C), 154.0 (C), 171.0 (C); *m/z* 355.0998 (MNa^+. $C_{15}H_{16}N_4NaO_5$ requires 355.1013).

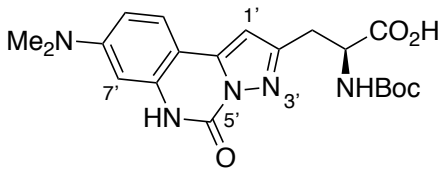
(2*S*)-2-Amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4e)



(2*S*)-2-Amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4e**) was synthesised as described for (2*S*)-2-amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4a**) using methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**8e**) (0.188 g, 0.438 mmol) and caesium carbonate (0.187 g, 0.575 mmol). An alternative work-up procedure was used for the ester hydrolysis reaction: The resulting residue was dissolved in

water (50 mL) and acidified to pH 1 with 1 M aqueous hydrochloric acid. Solid sodium chloride was added and extracted with dichloromethane (3 × 50 mL). The organic layers were combined and washed with brine (50 mL). The brine was re-extracted with dichloromethane (30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in 2 M aqueous hydrochloric acid (5 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and the resulting residue was purified by recrystallisation from methanol and diethyl ether to give (2*S*)-2-amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (**4e**) as a yellow solid (0.108 g, 78%). Mp 208–210 °C (decomposition); ν_{max}/cm^{-1} (neat) 3423 (NH), 2854 (CH), 1713 (C=O), 1612, 1497, 1335, 1211, 810; $[\alpha]_D^{32}$ –10.7 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) 3.11 (6H, s, 2 × CH_3), 3.40 (1H, dd, *J* 16.6, 8.3 Hz, 3-*H*H), 3.52 (1H, dd, *J* 16.6, 3.9 Hz, 3-H*H*), 4.49 (1H, dd, *J* 8.3, 3.9 Hz, 2-H), 6.66 (1H, d, *J* 2.3 Hz, 7'-H), 6.80 (1H, s, 1'-H), 6.91 (1H, dd, *J* 8.9, 2.2 Hz, 9'-H), 7.80 (1H, d, *J* 8.9 Hz, 10'-H); δ_C (126 MHz, CD_3OD) 29.9 (CH_2), 43.9 (2 × CH_3), 53.1 (CH), 103.8 (C), 109.1 (C), 113.9 (CH), 127.1 (CH), 136.9 (CH), 137.0 (CH), 143.2 (C), 146.9 (C), 149.3 (C), 154.4 (C), 170.8 (C); *m/z* 338.1224 (MNa^+. $C_{15}H_{17}N_5NaO_3$ requires 338.1224).

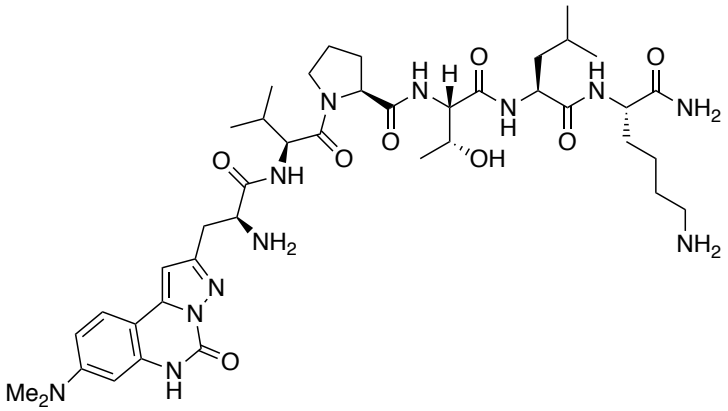
(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (9)



Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8′-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoate (**8e**) (0.188 g, 0.438 mmol) was dissolved in methanol (8.5 mL) and a solution of caesium carbonate (0.187 g, 0.575 mmol) in water (3.5 mL) was added. The reaction mixture was stirred at room temperature for 20 h and then concentrated *in vacuo*. The resulting residue was dissolved in water (50 mL) and acidified to pH 1 with 1 M aqueous hydrochloric acid. Solid sodium chloride was added and extracted with dichloromethane (3 × 50 mL). The organic layers were combined and washed with brine (50 mL). The brine was re-extracted with dichloromethane (30 mL). The organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (**9**) as a yellow solid (0.143 g, 78%). Mp 295–297 °C; ν_{max}/cm^{-1} (neat) 3414 (NH), 2976 (CH), 1708 (C=O), 1629 (C=C), 1533, 1387, 1163 cm^{-1}; $[\alpha]_D^{23}$ +15.3 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, CD_3OD) 1.39 (9H, s, 3 ×

CH_3), 3.03 (6H, s, 2 × CH_3), 3.13 (1H, dd, *J* 14.6, 9.2 Hz, 3-*H*H), 3.28–3.33 (1H, m, 3-H*H*), 4.52 (1H, dd, *J* 9.2, 4.8 Hz, 2-H), 6.41 (1H, d, *J* 2.3 Hz, 7′-H), 6.65 (1H, s, 1′-H), 6.69 (1H, dd, *J* 8.9, 2.3 Hz, 9′-H), 7.61 (1H, d, *J* 8.9 Hz, 10′-H); δ_C (101 MHz, CD_3OD) 28.7 (3 × CH_3), 31.9 (CH_2), 40.3 (2 × CH_3), 54.7 (CH), 80.6 (C), 97.4 (CH), 99.1 (CH), 103.0 (C), 110.3 (CH), 125.9 (CH), 137.3 (C), 143.9 (C), 147.2 (C), 153.5 (C), 156.0 (C), 157.9 (C), 175.2 (C); *m/z* 438.1738 (MNa^+. $C_{20}H_{25}N_5NaO_5$ requires 438.1748).

Hexapeptide 10



The pentapeptide was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using a Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using TentaGel™ S RAM resin. The resin-bound peptide was synthesised by first loading Fmoc-Lys(Boc)-OH to the resin and by introducing the amino acids (4 equivalents) successively with a combination of 0.5 M 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) in DMF (4 equivalents) and 2 M diisopropylethylamine in NMP (8 equivalents). Fmoc groups were removed using 20% piperidine in DMF. The resin-bound pentapeptide (0.296 g, 0.100 mmol) was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was filtered, and the resin-bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin-bound peptide was suspended in DMF (2 mL) followed by addition of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(8'-dimethylamino-5'-oxo-5'-6'-dihydropyrazolo[1',5'-c]quinazolin-2'-yl)propanoic acid (**9**) (0.0834 g, 0.200 mmol), diisopropylethylamine (0.0700 mL, 0.400 mmol) and 0.5 M HCTU in DMF (0.400 mL, 0.200 mmol). The mixture was shaken for 18 h. The solution was filtered, and the resin-bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin-bound peptide was treated with trifluoroacetic acid/water/triisopropylsilane (2 mL, 95:2.5:2.5) and shaken for 2 h. The cleavage cocktail was evaporated using a stream of nitrogen and peptide **10** was precipitated from a solution of ice-cold diethyl ether (2 mL), centrifuged at 4500 rpm for 5 minutes and washed with ice-cold diethyl ether (3 × 2 mL). Peptide **10** was purified on a reverse-phase Dionex

HPLC system equipped with Dionex P680 pumps and a Dionex UVD170U UV-Vis detector (monitoring at 214 nm and 280 nm), using a Phenomenex, Gemini, C18, 5 µm, 250 × 21.2 mm column. Gradients were run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA), and collected fractions were lyophilised on a Christ Alpha 2-4 LO plus freeze dryer to give a yellow solid (0.0614 g, 0.0720 mmol, 72%). Peptide **10** was analysed on a Shimadzu reverse-phase HPLC (RP-HPLC) system equipped with Shimadzu LC-20AT pumps, a Shimadzu SIL-20A autosampler and a Shimadzu SPD-20A UV-Vis detector (monitoring at 214 nm and 280 nm) using a Phenomenex, Aeris, 5 µm, peptide XB-C18, 150 × 4.6 mm column at a flow rate of 1 mL/minute. RP-HPLC gradients were run using a solvent system consisting of solution A (5% MeCN in H_2O + 0.1% TFA) and B (5% H_2O in MeCN + 0.1% TFA). Two gradients were used to characterise peptide **10**, a gradient from 0–100% solution B over 20 minutes (Figure 1) and a gradient from 0–100% solution B over 50 minutes (Figure 2). Analytical RP-HPLC data is reported as column retention time (T_R) in minutes (Table 1). High-resolution mass spectrometry (HRMS) was performed on a Bruker microTOF-Q II (ESI+). HRMS data are reported as mass to charge ratio (*m/z*) = observed / MW.

20 Minute gradient		**50 Minute gradient**		**Calculated MW**	**Observed MW**
T_R (min)	Purity (%)	T_R (min)	Purity (%)	853.5043 $[M+H]^+$	853.5045 $[M+H]^+$
11.56	99	20.25	98		

Table 1: RP-HPLC gradients and HRMS data.

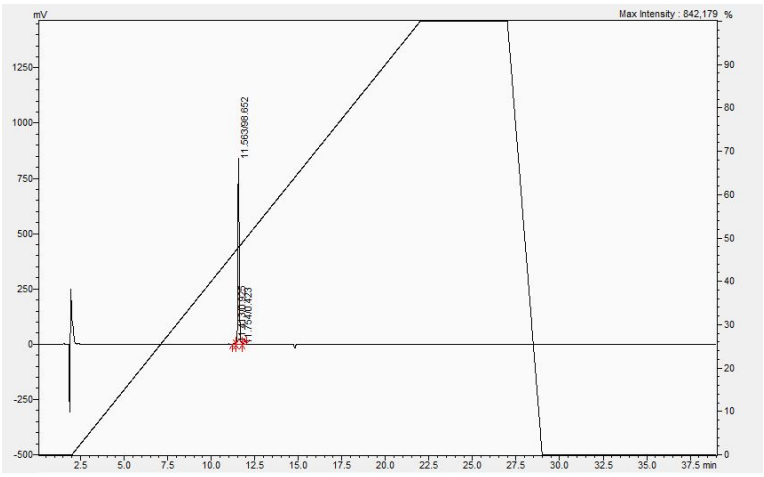


Figure 1: Analytical HPLC 20-minute gradient.

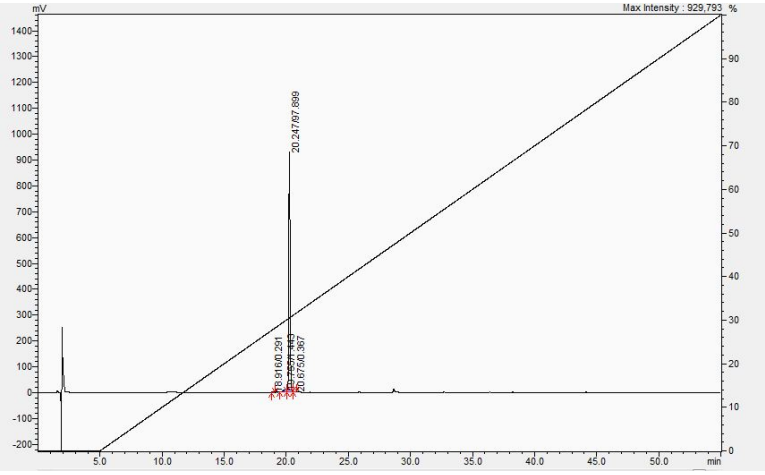


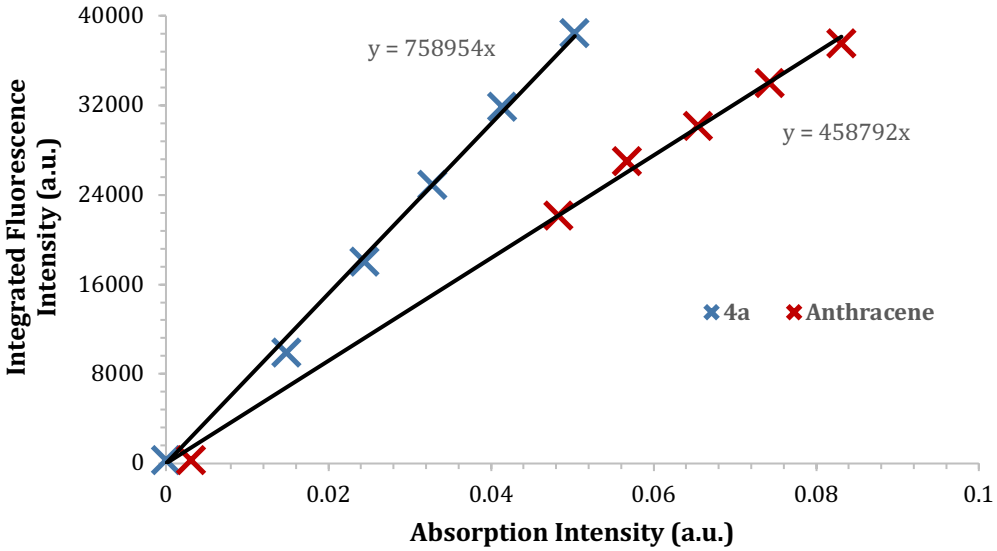
Figure 2: Analytical HPLC 50-minute gradient.

3. Absorbance and Emission Spectra for all Compounds

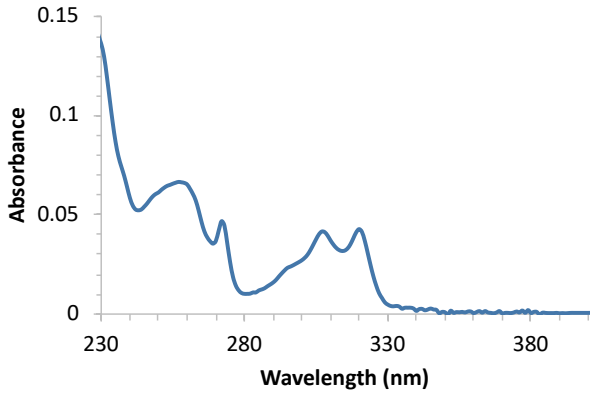
UV-Vis spectra were recorded on a Pekin Elmer Lamda 25 instrument. Fluoresence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Emission data were measured as 0.5 × 10^{-5} M solutions, using an excitation slit width of 3 nm and emission slit width of 3 nm. Quantum yields were determined using a comparative method against two standards, anthracene (Φ = 0.27, ethanol) and L-tryptophan (Φ = 0.14, water).[3–5] The integrated fluorescence intensity of each compound was determined from the emission spectra given. Measurements were performed at five different concentrations. Concentrations were chosen to ensure the absorption value was below 0.1 to avoid re-absorption effects. Integrated fluorescence intensity was plotted as a function of the measured absorbance and a linear fit is calculated with an intercept of zero. The resultant gradient was then used to calculate the quantum yield.

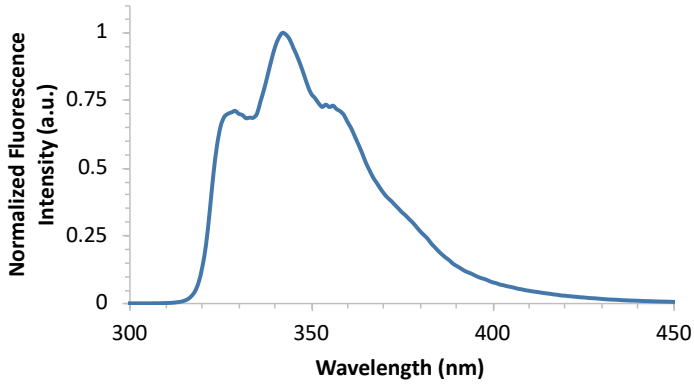
$$\Phi_x = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_x^2}{\eta_{ST}^2}\right)$$

Calculation of quantum yield: Subscript *ST* signifies the quantities associated with the quantum yield standard. Subscript *X* signifies the quantities associated with the novel compound. $Grad_X$ is the determined gradient associated with the novel compound. $Grad_{ST}$ is the determined gradient associated with quantum yield standard. η is the refractive index of the solvent used in the fluorescence measurements. η = 1.333 for water, 1.361 for ethanol and 1.331 for methanol.[6,7]

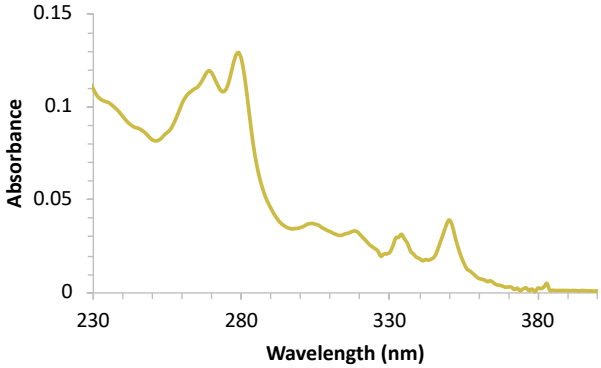


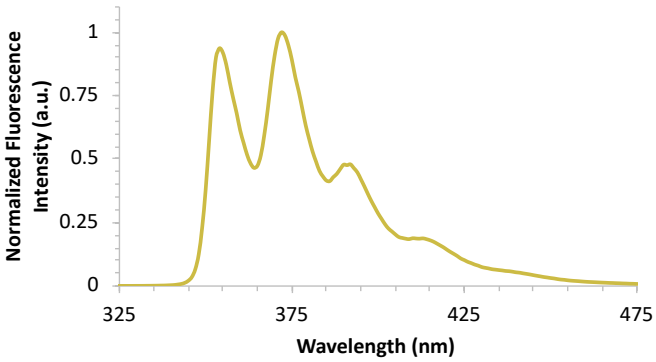
(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4a)



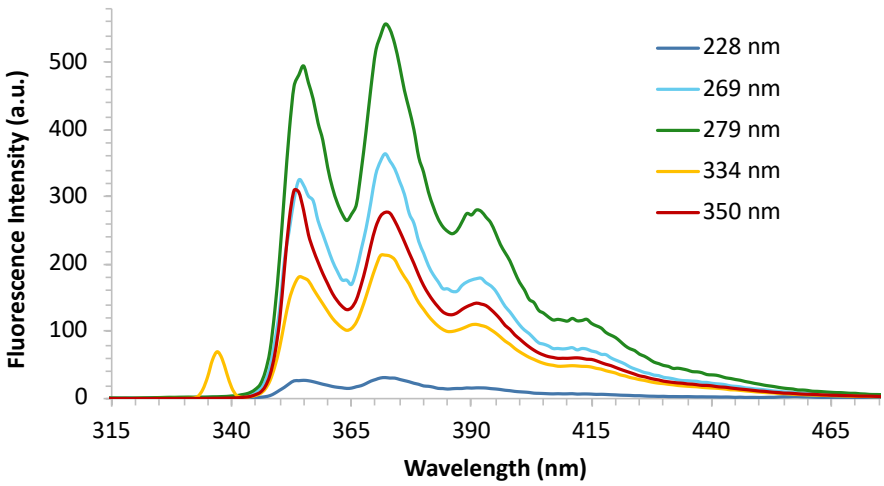


(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4b)

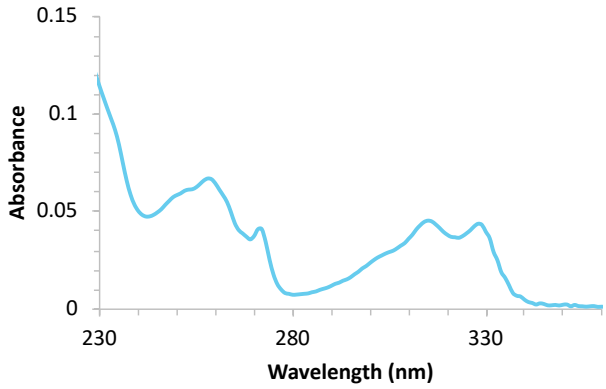


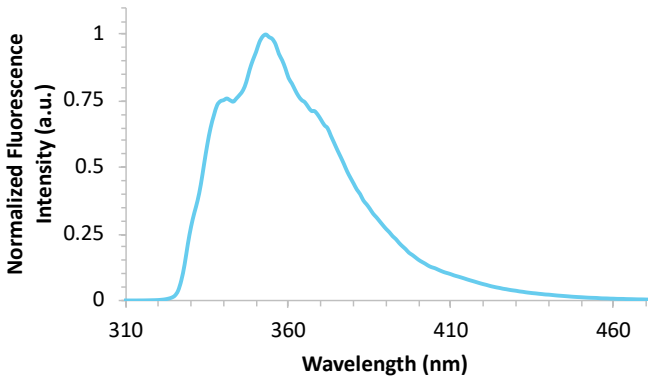


Emission spectra of 4b following excitation at various wavelengths

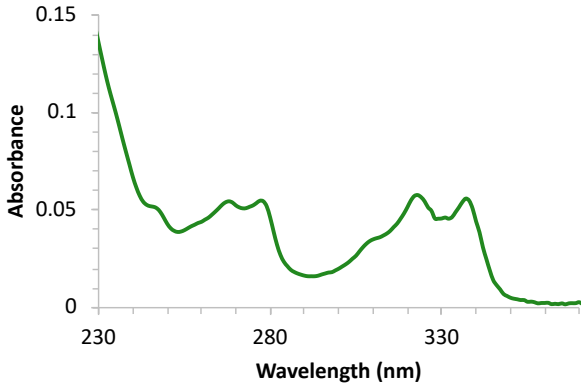


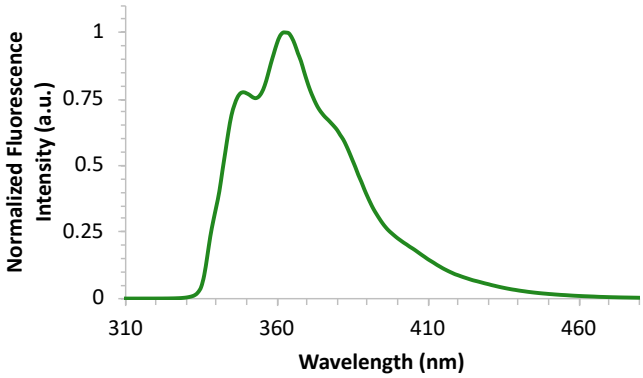
(2*S*)-2-Amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4c)



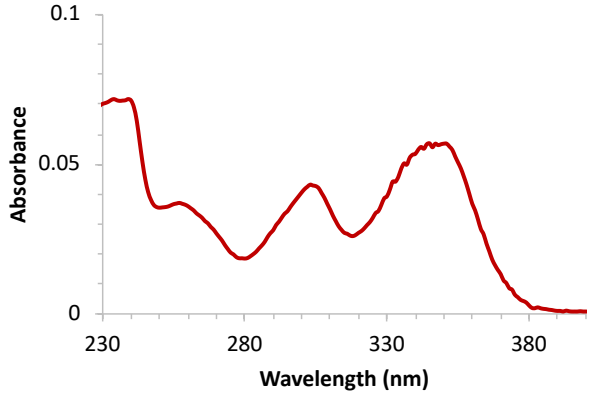


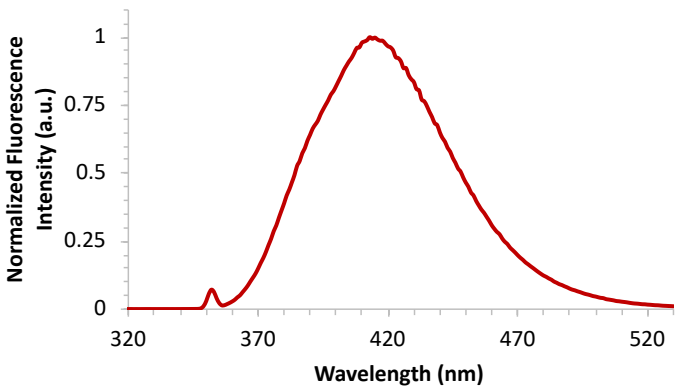
(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4d)



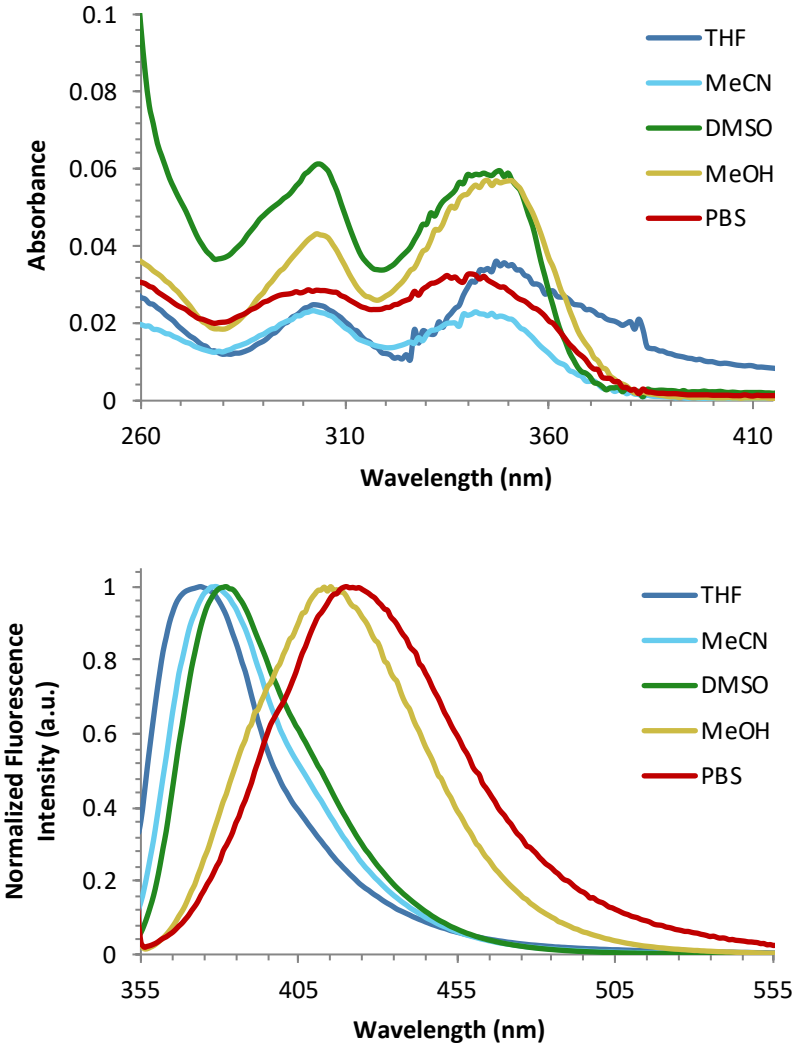


(2*S*)-2-Amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4e)



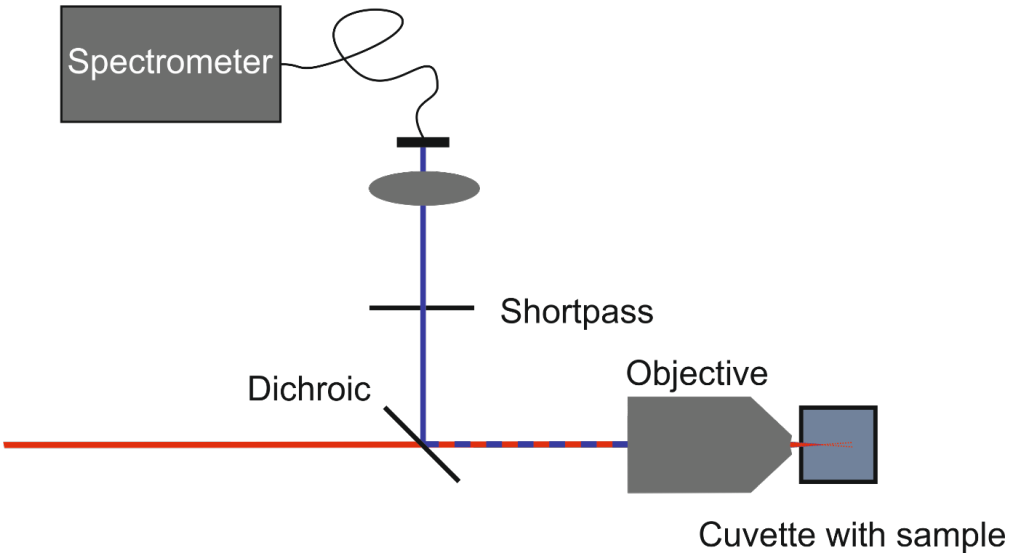


Absorbance and emission spectra of 4e in various solvents



4. Two-Photon Calculations, Experimental Details and Spectra

Setup: A schematic of the two-photon spectroscopy setup can be found below. The excitation source is a broadband Ti:Sapphire laser with a repetition rate of 80 MHz (Vitara UBB, Coherent). The compressed pulses from the oscillator have a duration of 15 fs. The laser spectrum is either attenuated by a combination of 2 neutral density filters in series (Thorlabs) to obtain different excitation powers (power dependence) or filtered with a band pass filter for the cross-section measurement (Thorlabs 700 nm, 10 nm FWHM). The light passes through a dichroic mirror (Semrock FF652-Di01) and is focused by an objective (Nikon Plan Fluor 10×) into a cuvette with the sample. The emitted light is collected by the same objective and reflected by the dichroic mirror onto a short-pass filter (Semrock, FF01-650/SP) to filter out any residual laser light. Subsequently it is focused by a lens (Edmund PCX UV 25×38, coating UV-VIS CTD TS) onto an optical fibre and guided into the spectrometer (AvaSpec ULS2048L-USB2 with the grating VA from Avantes).



Two-photon cross section: The two-photon cross section of the sample (σ_2^S) can be calculated using a standard with a known cross section σ_2^R (see references 8 and 9):

$$\frac{\sigma_2^S \phi^S}{\sigma_2^R \phi^R} = \frac{\eta^R \left(n^S_{\lambda emission}\right)^2 n^R_{\lambda excitation} C^R F^S \langle P^R \rangle^2}{\eta^S \left(n^R_{\lambda emission}\right)^2 n^S_{\lambda excitation} C^S F^R \langle P^S \rangle^2}$$

Where ϕ is the quantum yield of the fluorescence, η accounts for the wavelength dependence of the detection efficiency, n is the refractive index of the solvent, C is the concentration, F is the integrated fluorescence signal and P is the excitation power. The superscripts R and S denote reference and sample respectively.

By making the simplifications $n_{\lambda emission} = n_{\lambda excitation} = n$ and $\eta^R = \eta^S$ this can be written as:

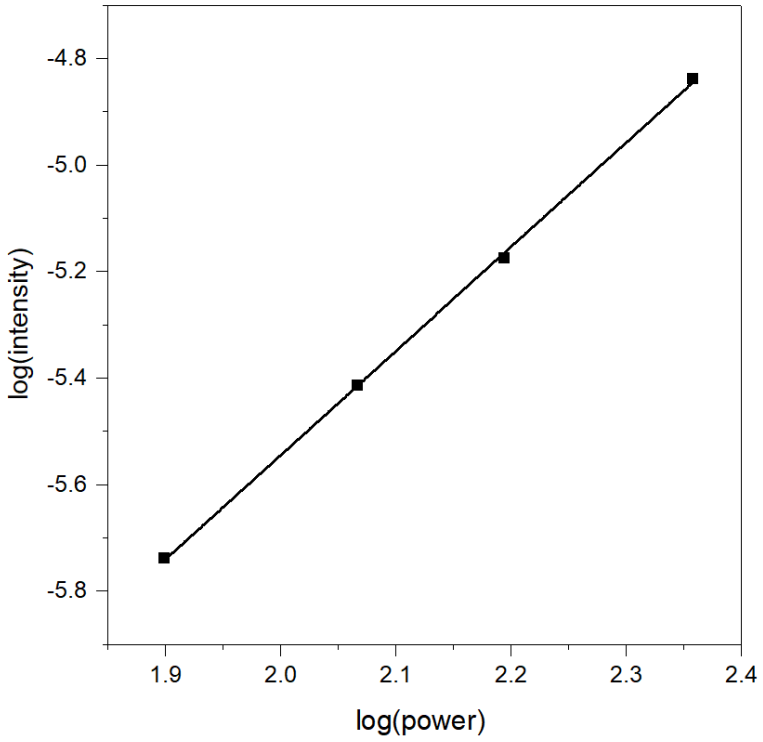
$$\frac{\sigma_2^S \phi^S}{\sigma_2^R \phi^R} = \frac{C^R F^S \langle P^R \rangle^2}{C^S F^R \langle P^S \rangle^2}$$

The constants used are:

- Extinction coefficient Rh B: 110,000 $cm^{-1}M^{-1}$ (see reference 10)
- Extinction coefficient **4e**: 12000 $cm^{-1}M^{-1}$
- σ_2^R (700 nm) = 240 GM (see reference 10)
- ϕ^R = 0.7 (see reference 11)
- ϕ^S = 0.43

Power dependence:

To confirm two photon excitation of the sample, the emission intensity in dependence on the excitation power was recorded. The result is shown in figure 2. The slope of the linear fit to the logarithmic plot is 1.96±0.03, which is in good agreement with a two-photon excitation.



5. Time-Correlated Single-Photon Counting (TCSPC)

Lifetimes were recorded for **4e** in ethanol (6.8 μM) using a FluoTime 300 fluorescence spectrometer (PicoQuant, Berlin, Germany). The excitation light was provided by a Fianium WhiteLase super-continuum laser (NKT Photonics, Birkerød, Denmark) and passed through a SuperChrome filter (NKT Photonics, Birkerød, Denmark) before arriving at the sample holder. The FWHM of the instrument response function was ≈ 120 ps at 10 MHz rep rate. The excitation and emission wavelengths were 390 nm and 430 nm, respectively. The excitation bandpass was 10 nm, while the emission bandpass was 2.7 nm. All of the fluorescence measurements were carried out under magic-angle conditions to avoid fluorescence polarization artefacts. The time/channel was 8 ps. The measurements were recorded until they reached five thousand counts in the peak channel. The fluorescence decays were best fit to tri-exponential decay functions. The fitting was carried out using the FluoFit data analysis software (PicoQuant, Berlin, Germany). Decays were fitted by iterative re-convolution, assuming a multi-exponential function, given in eq 1.

$$I(t) = \sum_{i=1}^{n} A_i \exp\left(\frac{-t}{\tau_i}\right) \qquad (1)$$

where I is the fluorescence intensity as a function of time, t, (normalised to the intensity at t=0); τ_i is the fluorescence lifetime of the ith decay component and A_i is the fractional amplitude (A-factor) of that component. The χ^2 value was 0.993 and the residuals were randomly distributed around zero.

The average lifetime, $<\tau>$, of the emitting population is given by eq 2.

$$<\tau> = \frac{\sum_{i=1}^{n} A_i \tau_i}{\sum_{i=1}^{n} A_i} \qquad (2)$$

6. References

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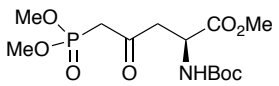
7. ^{1}H and ^{13}C NMR Spectra for all Novel Compounds



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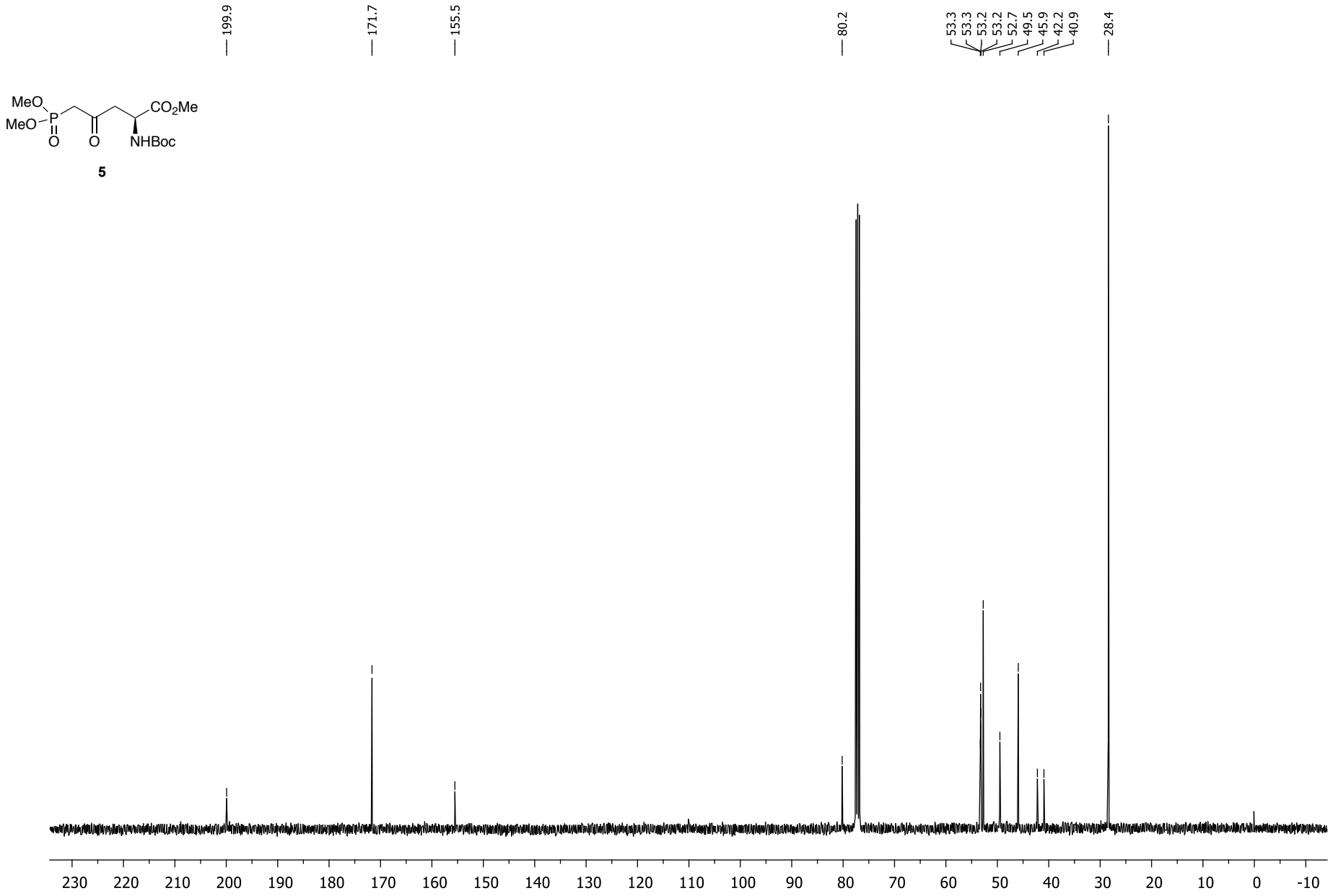
199.9
171.7
155.5
80.2
53.3
53.3
53.2
53.2
52.7
49.5
45.9
42.2
40.9
28.4
MeO
MeO
P
O
O
O
CO2Me
NHBoc
5
230
220
210
200
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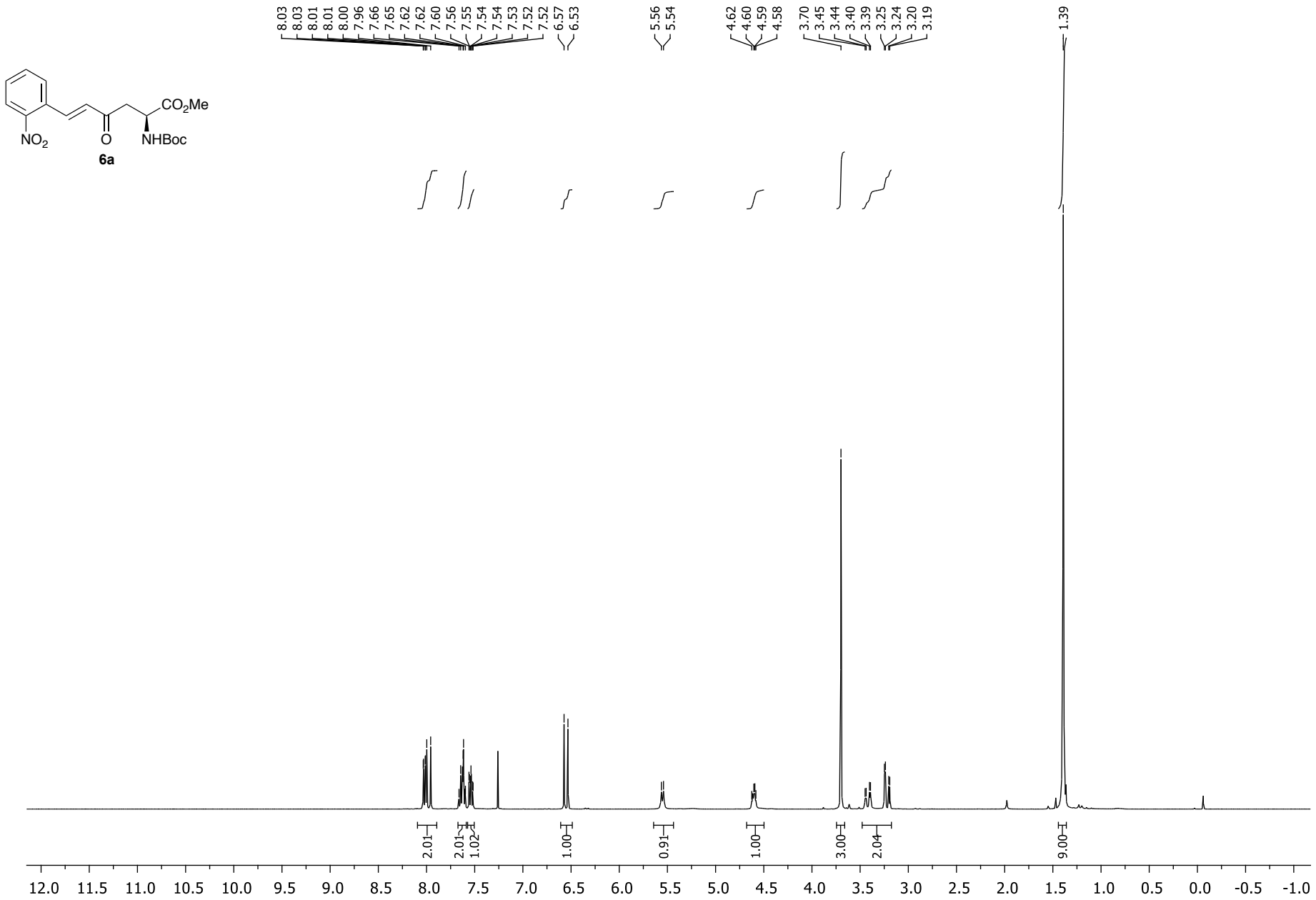
0
-10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8.03
8.01
8.01
8.00
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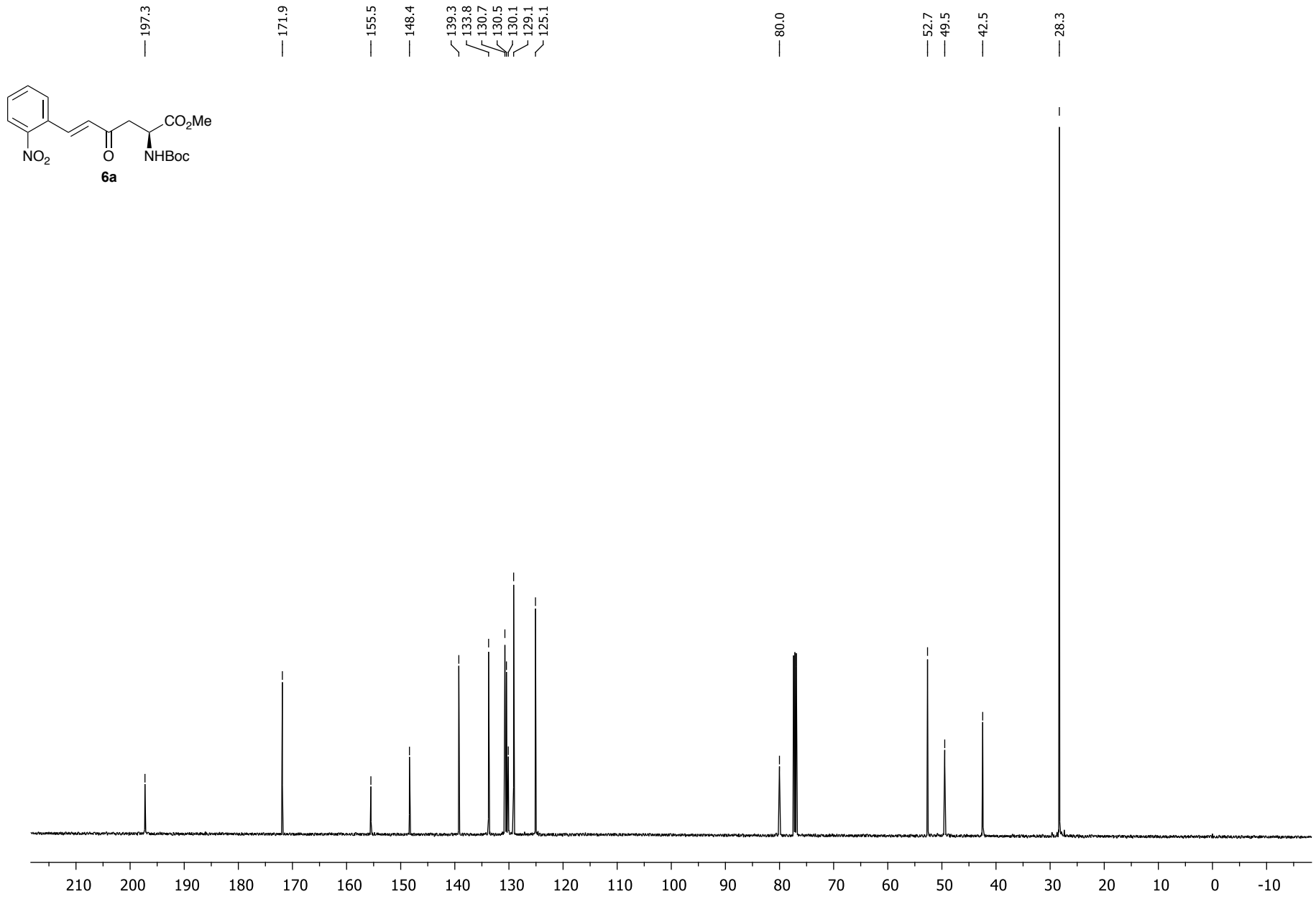
7.66
7.65
7.62
7.62
7.60
7.56
7.55
7.54
7.54
7.53
7.52
7.52
6.57
6.53
5.56
5.54
4.62
4.60
4.59
4.58
3.70
3.45
3.44
3.40
3.39
3.25
3.24
3.20
3.19
1.39
CO2Me
NO2
O
NHBoc
6a
2.01
2.01
1.02
1.00
0.91
1.00
3.00
2.04
9.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0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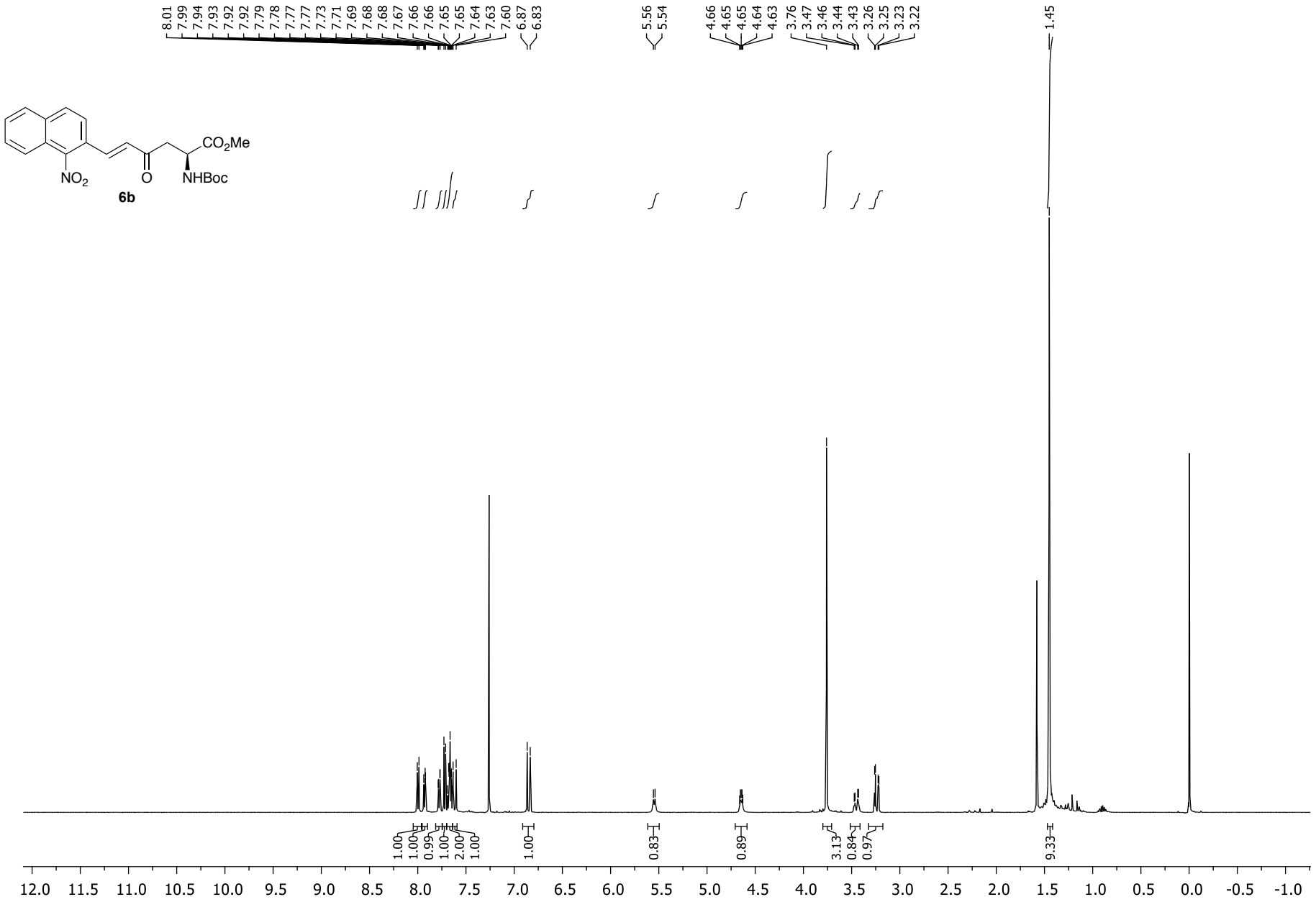
171.9
155.5
148.4
139.3
133.8
130.7
130.5
130.1
129.1
125.1
80.0
52.7
49.5
42.5
28.3
CO2Me
NO2
O
NHBoc
6a
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10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7.99
7.94
7.93
7.92
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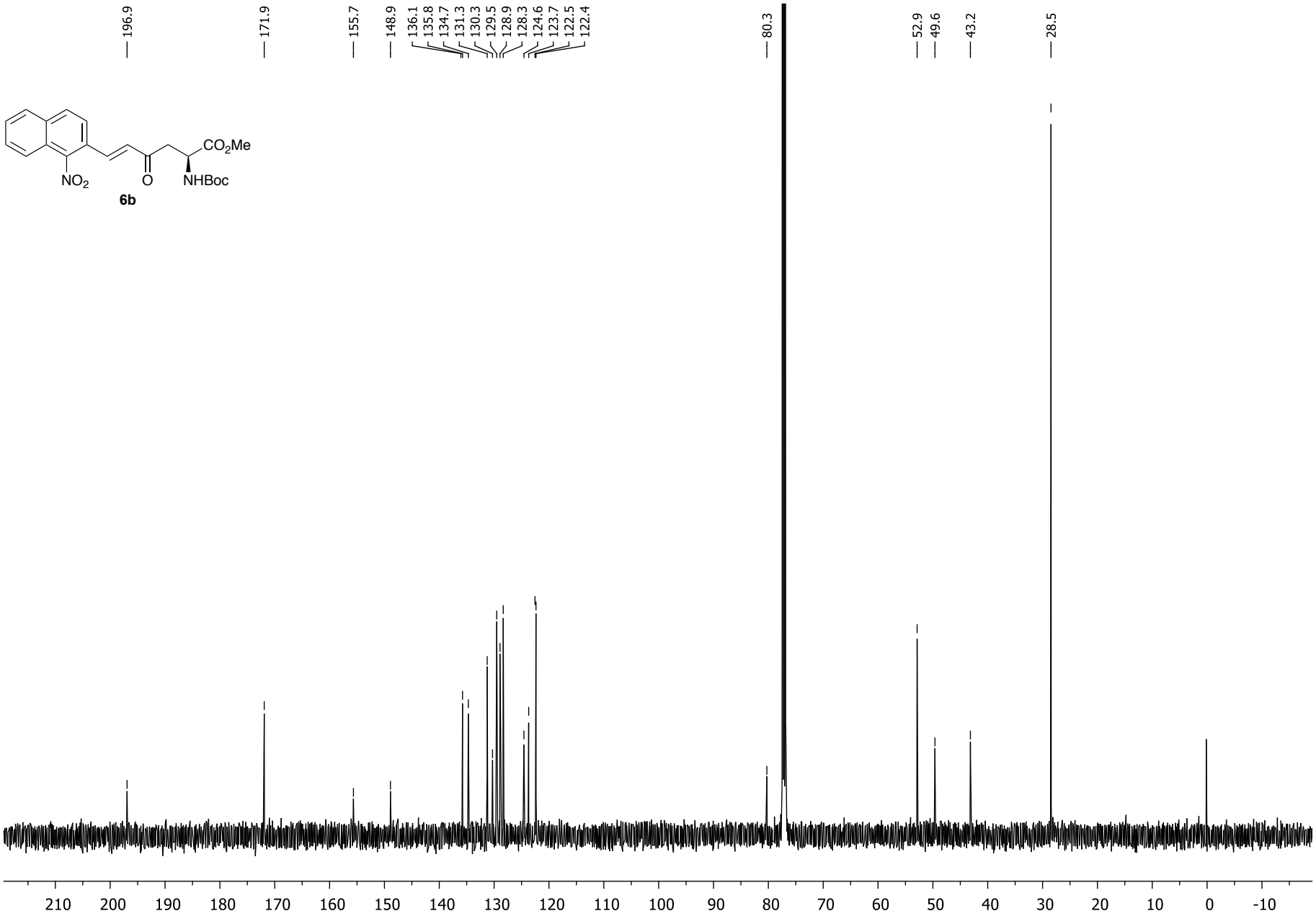
7.79
7.78
7.77
7.77
7.73
7.71
7.69
7.68
7.68
7.67
7.66
7.66
7.65
7.65
7.64
7.63
7.60
6.87
6.83
5.56
5.54
4.66
4.65
4.65
4.64
4.63
3.76
3.47
3.46
3.44
3.43
3.26
3.25
3.23
3.22
1.45
CO2Me
NO2
O
NHBoc
6b
1.00
1.00
0.99
1.00
2.00
1.00
1.00
0.83
0.89
3.13
0.84
0.97
9.33
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0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148.9
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135.8
134.7
131.3
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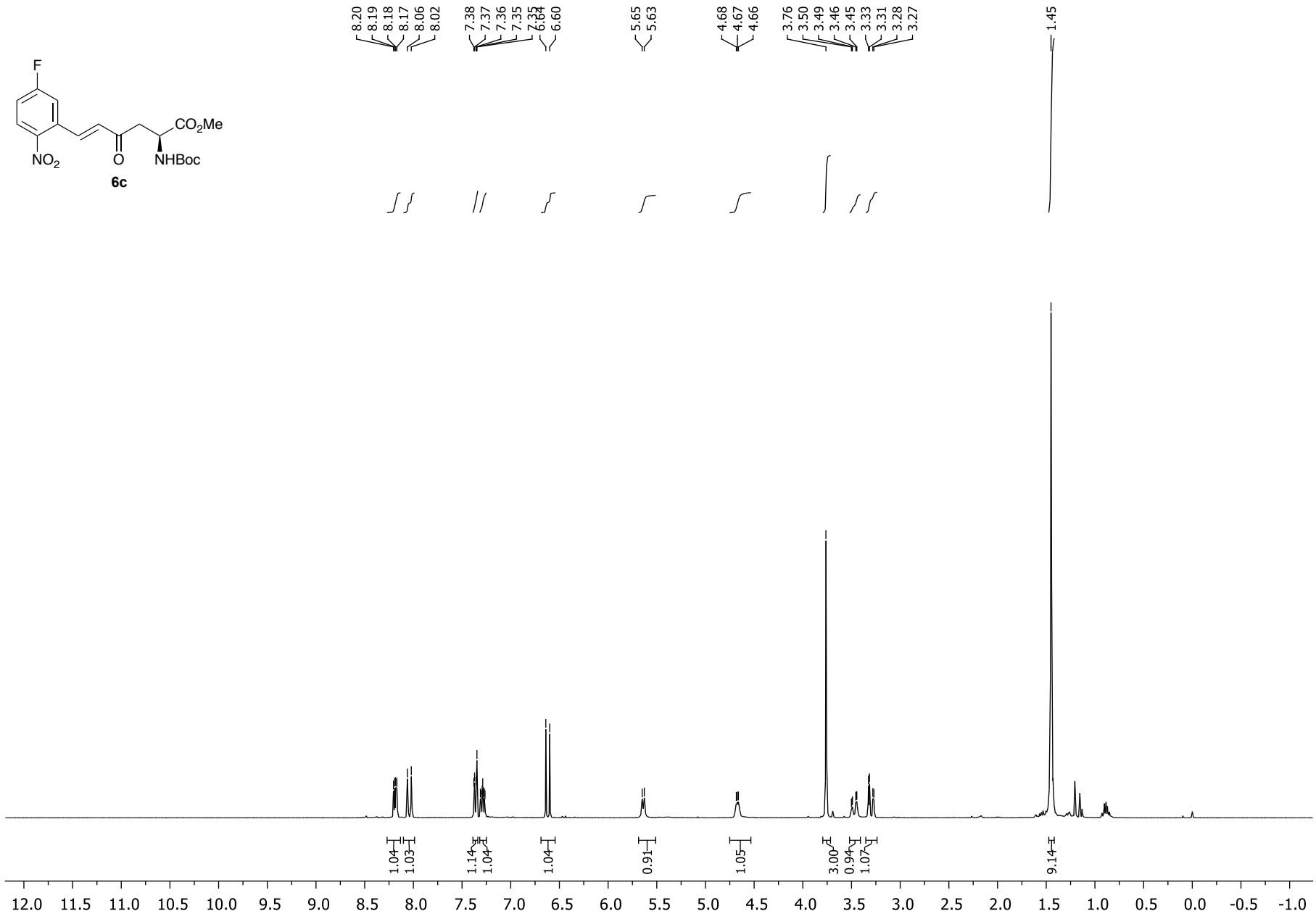
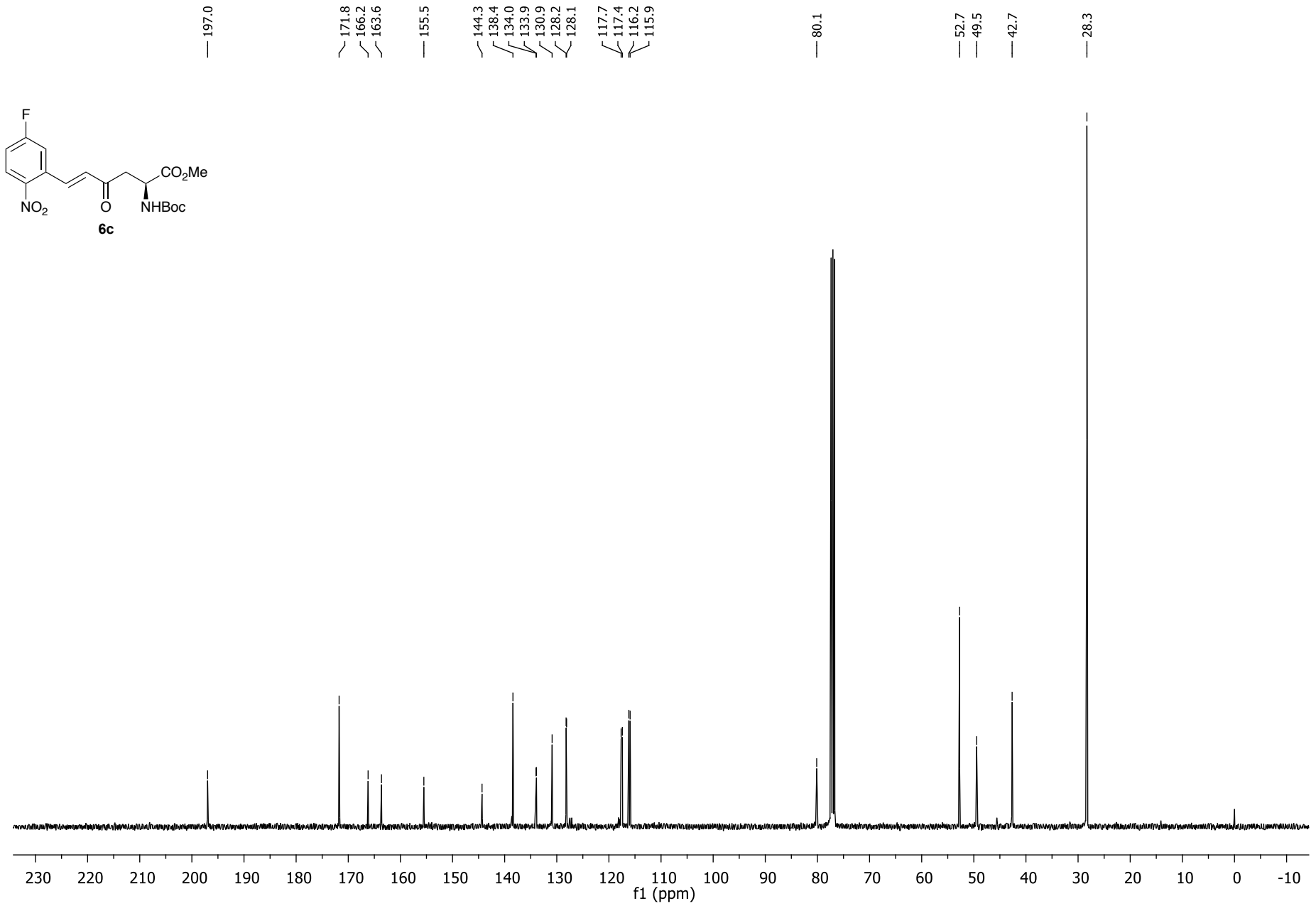
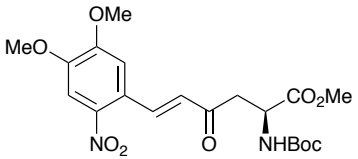
129.5
128.9
128.3
124.6
123.7
122.5
122.4
80.3
52.9
49.6
43.2
28.5
CO2Me
NO2
O
NHBoc
6b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

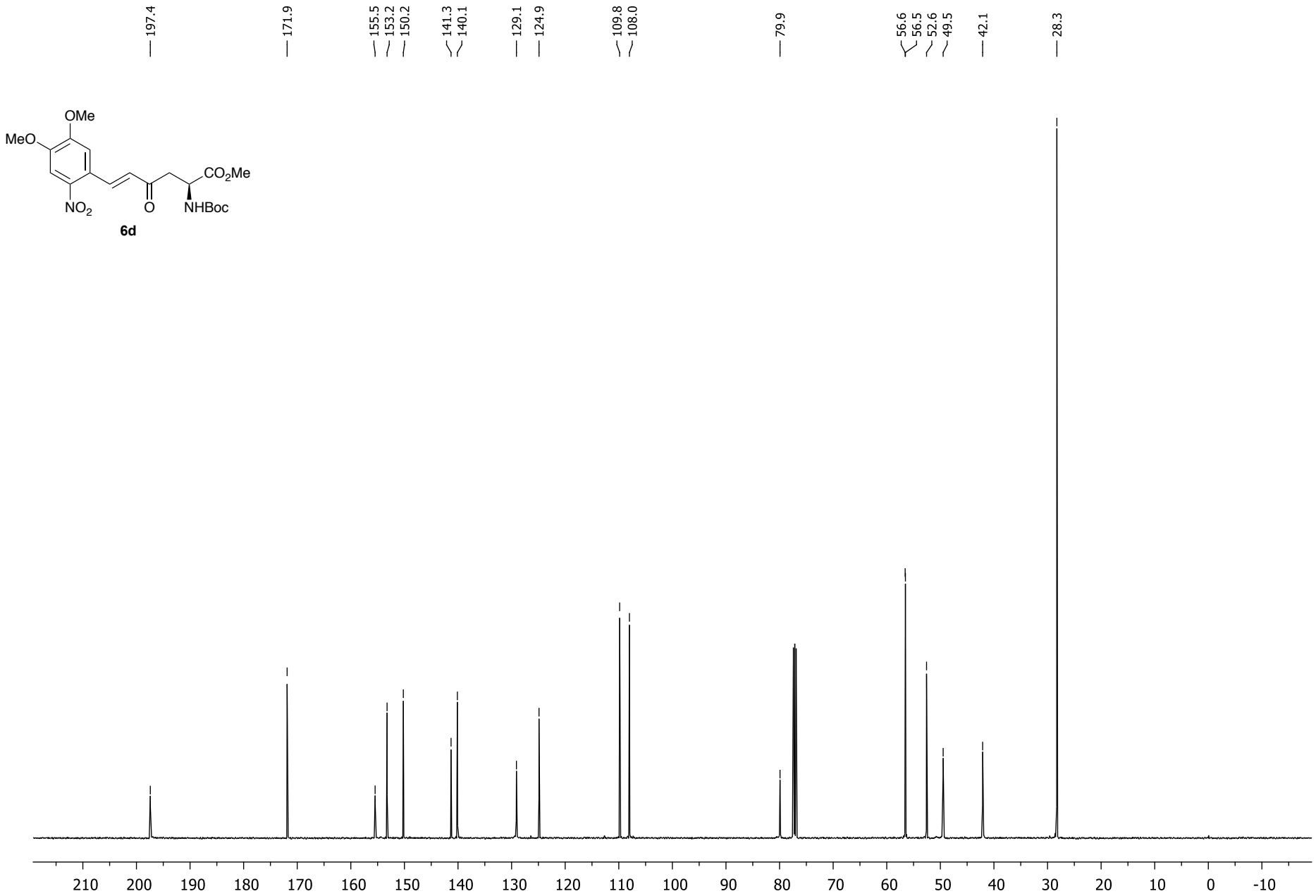

F
CO2Me
NO2
O
NHBoc
6c
8.20
8.19
8.18
8.17
8.06
8.02
7.38
7.37
7.36
7.35
6.64
6.60
5.65
5.63
4.68
4.67
4.66
3.76
3.50
3.49
3.46
3.45
3.33
3.31
3.28
3.27
1.45
1.04
1.03
1.14
1.04
1.04
0.91
1.05
3.00
0.94
1.07
9.14
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0

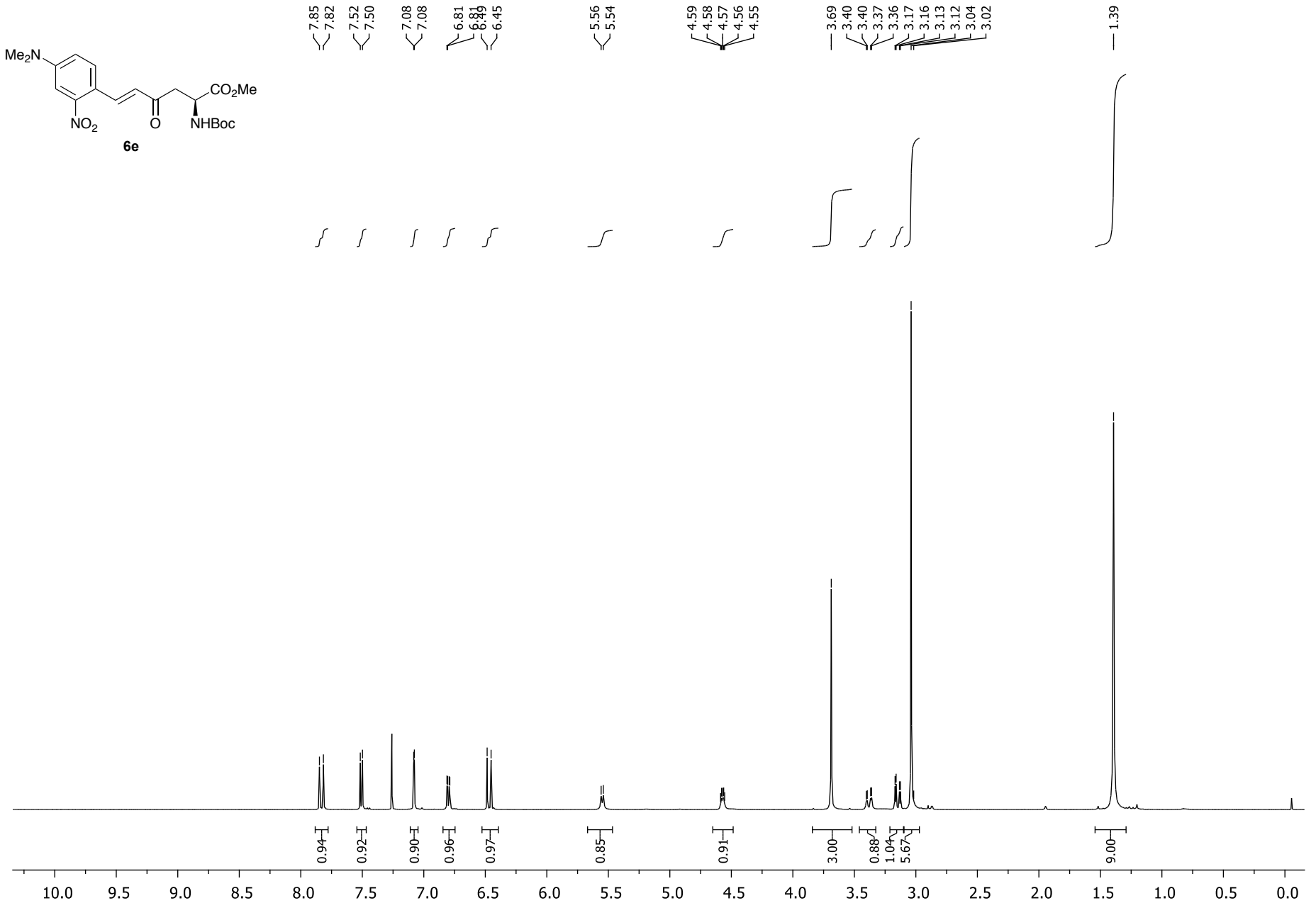

197.0
171.8
166.2
163.6
155.5
144.3
138.4
134.0
133.9
130.9
128.2
128.1
117.7
117.4
116.2
115.9
80.1
52.7
49.5
42.7
28.3
F
CO2Me
NO2
O
NHBoc
6c
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

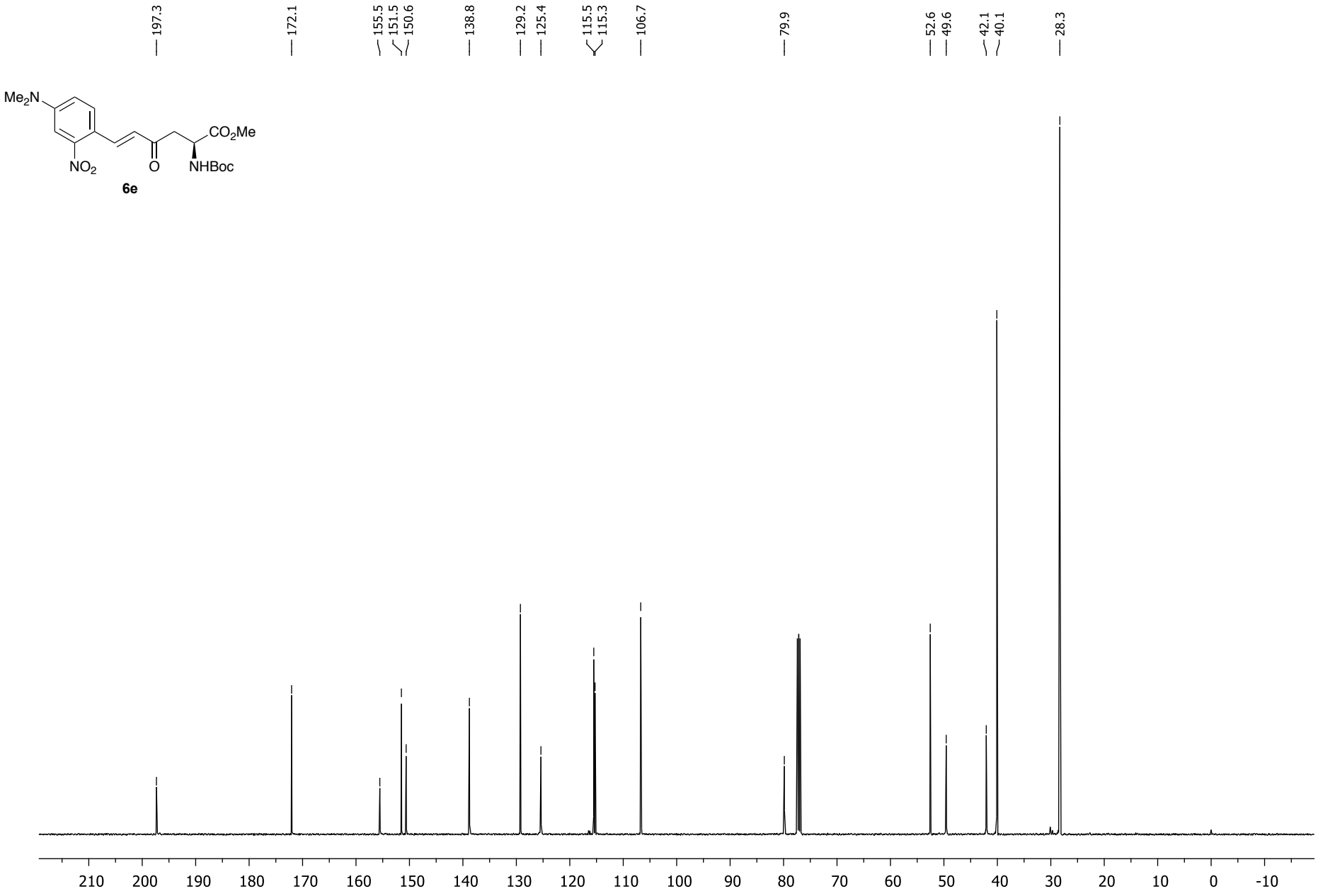

OMe
MeO
CO2Me
NO2
O
NHBoc

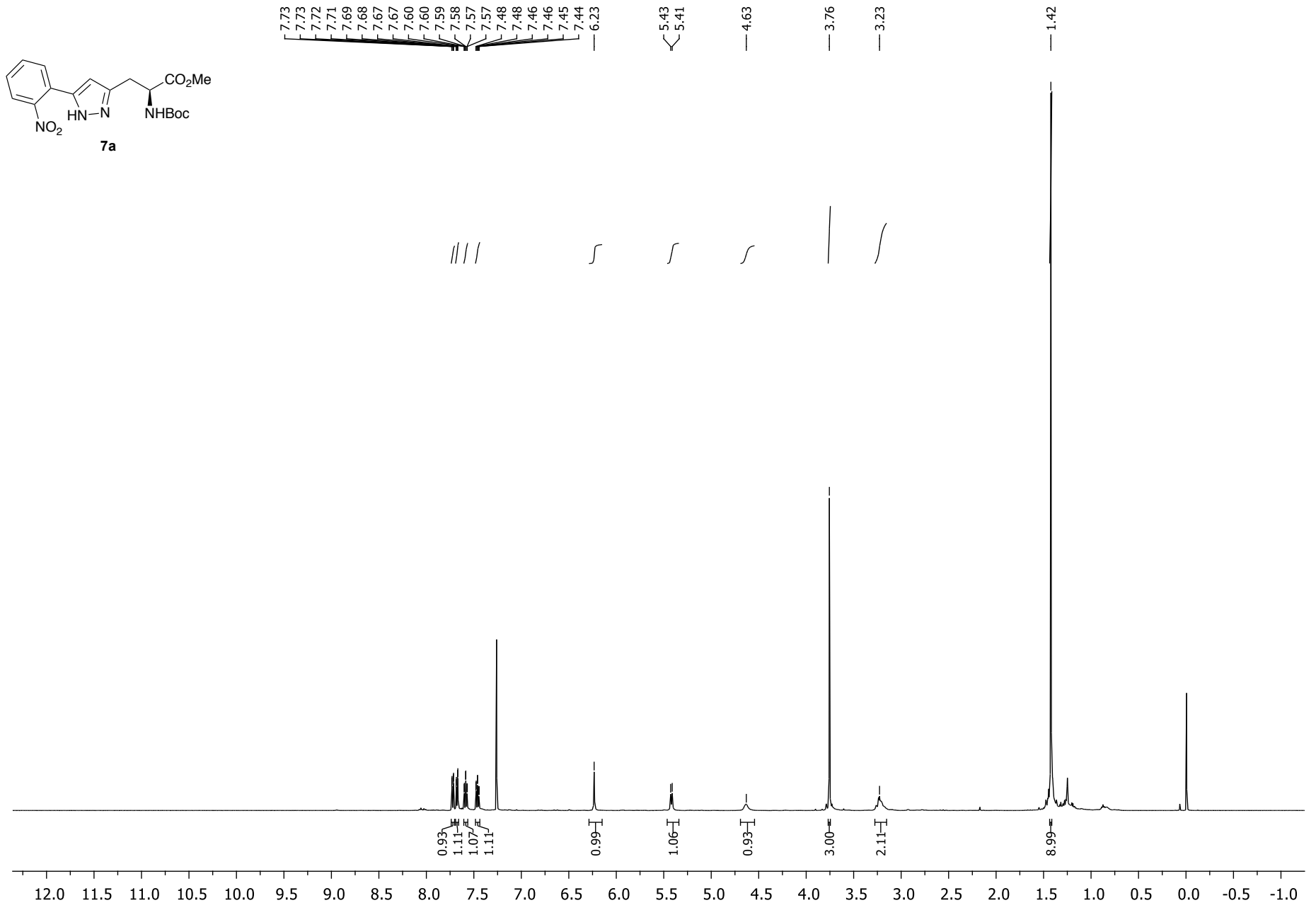
6d

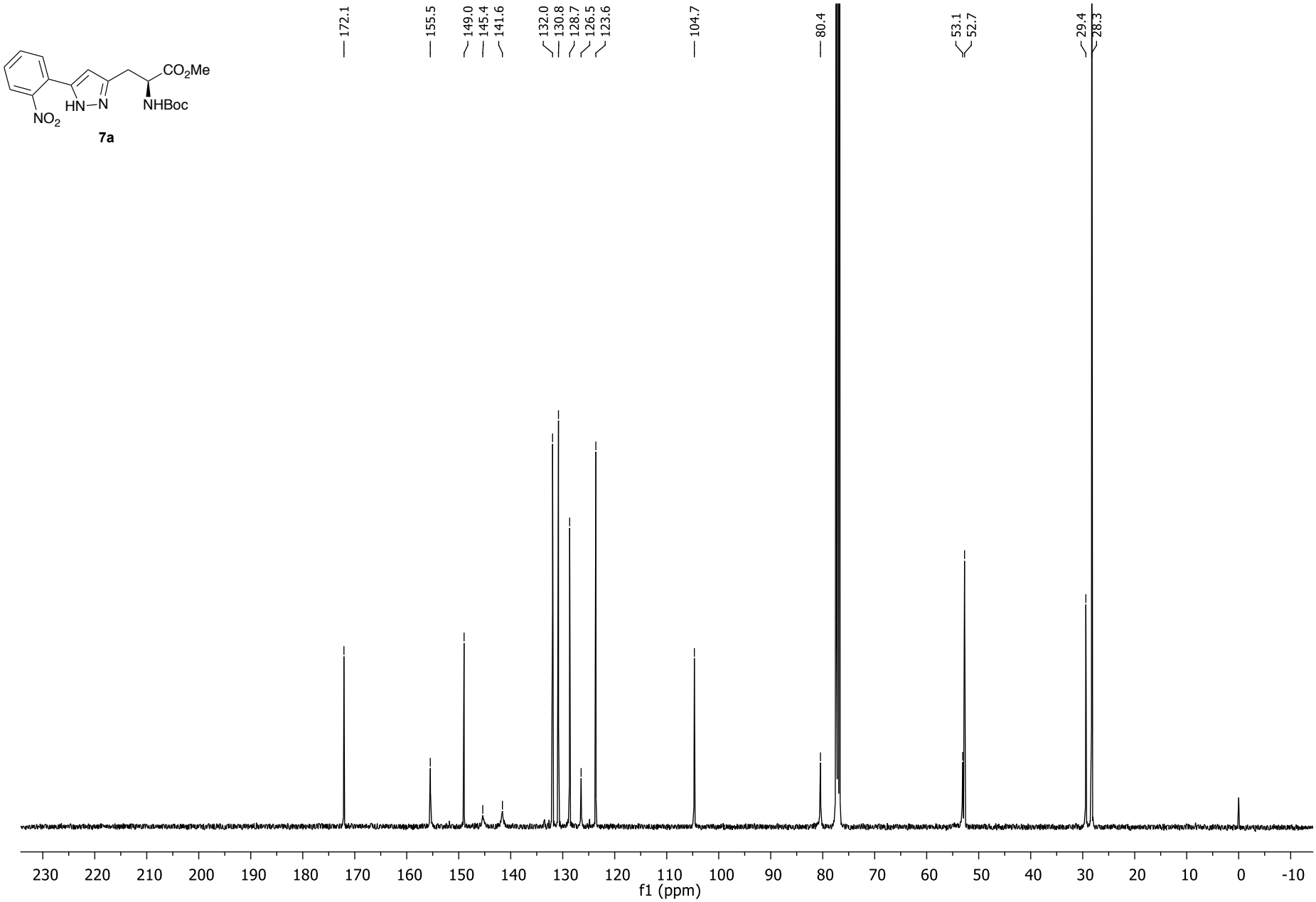
8.07
8.04
7.58
6.93
6.48
6.45
5.54
5.52
4.59
4.58
4.58
3.94
3.92
3.68
3.41
3.38
3.23
3.22
3.20
3.19
1.37
0.91
0.95
0.97
0.94
0.89
0.97
5.74
2.82
0.83
0.95
9.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0

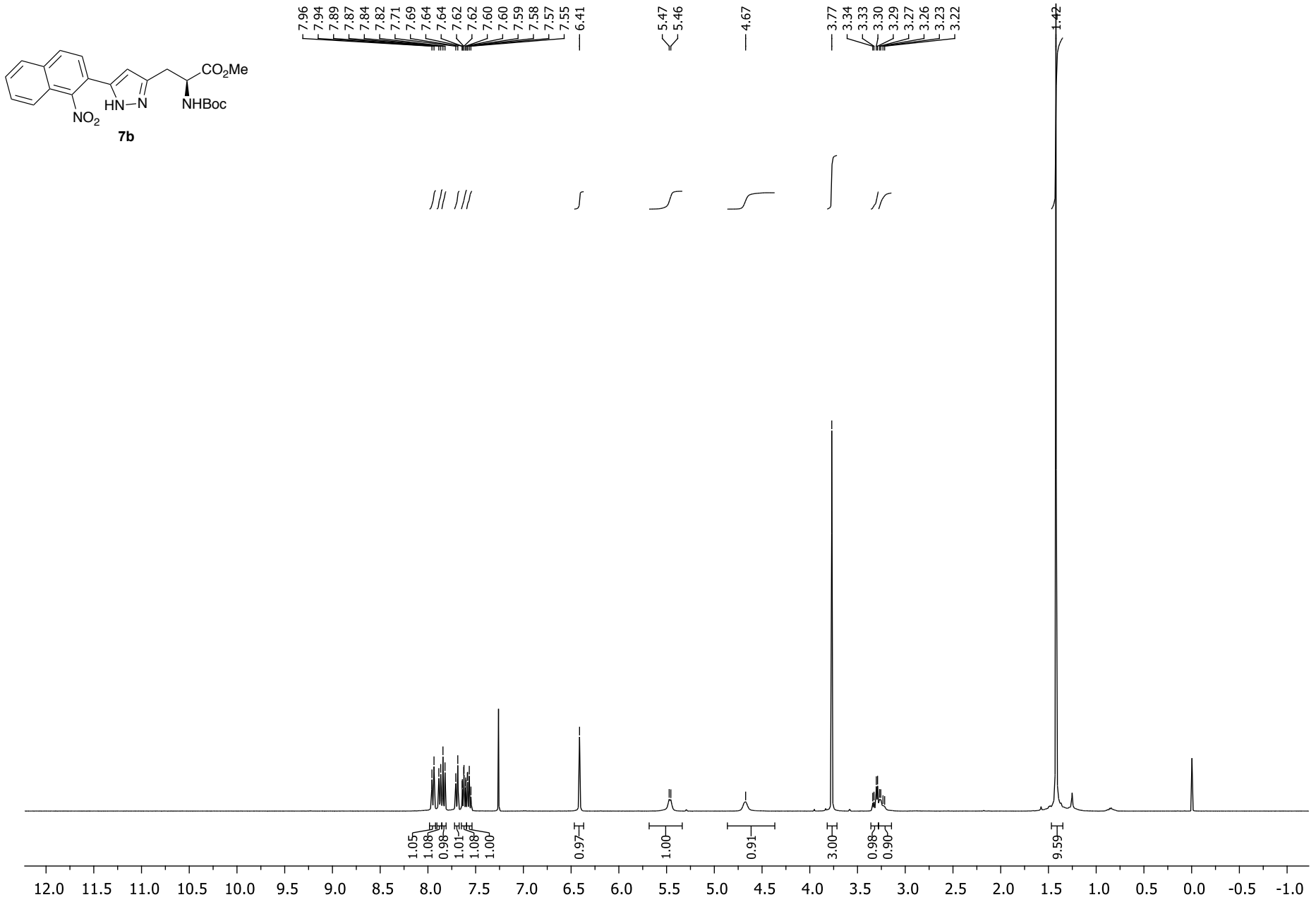

197.4
171.9
155.5
153.2
150.2
141.3
140.1
129.1
124.9
109.8
108.0
79.9
56.6
56.5
52.6
49.5
42.1
28.3
OMe
MeO
CO2Me
NO2
O
NHBoc
6d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


Me2N
NO2
O
NHBoc
CO2Me
6e
7.85
7.82
7.52
7.50
7.08
7.08
6.81
6.45
5.56
5.54
4.59
4.58
4.57
4.56
4.55
3.69
3.40
3.40
3.37
3.36
3.17
3.16
3.13
3.12
3.04
3.02
1.39
0.94
0.92
0.90
0.96
0.97
0.85
0.91
3.00
0.88
1.04
5.67
9.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


197.3
172.1
155.5
151.5
150.6
138.8
129.2
125.4
115.5
115.3
106.7
79.9
52.6
49.6
42.1
40.1
28.3
Me2N
CO2Me
NO2
O
NHBoc
6e
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


7.73
7.73
7.72
7.71
7.69
7.68
7.67
7.67
7.60
7.60
7.59
7.58
7.57
7.57
7.48
7.48
7.46
7.46
7.45
7.44
6.23
5.43
5.41
4.63
3.76
3.23
1.42
CO2Me
HN–N
NHBoc
NO2
7a
0.93
1.11
1.07
1.11
0.99
1.06
0.93
3.00
2.11
8.99
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0


CO2Me
HN–N
NHBoc
NO2
7a
172.1
155.5
149.0
145.4
141.6
132.0
130.8
128.7
126.5
123.6
104.7
80.4
53.1
52.7
29.4
28.3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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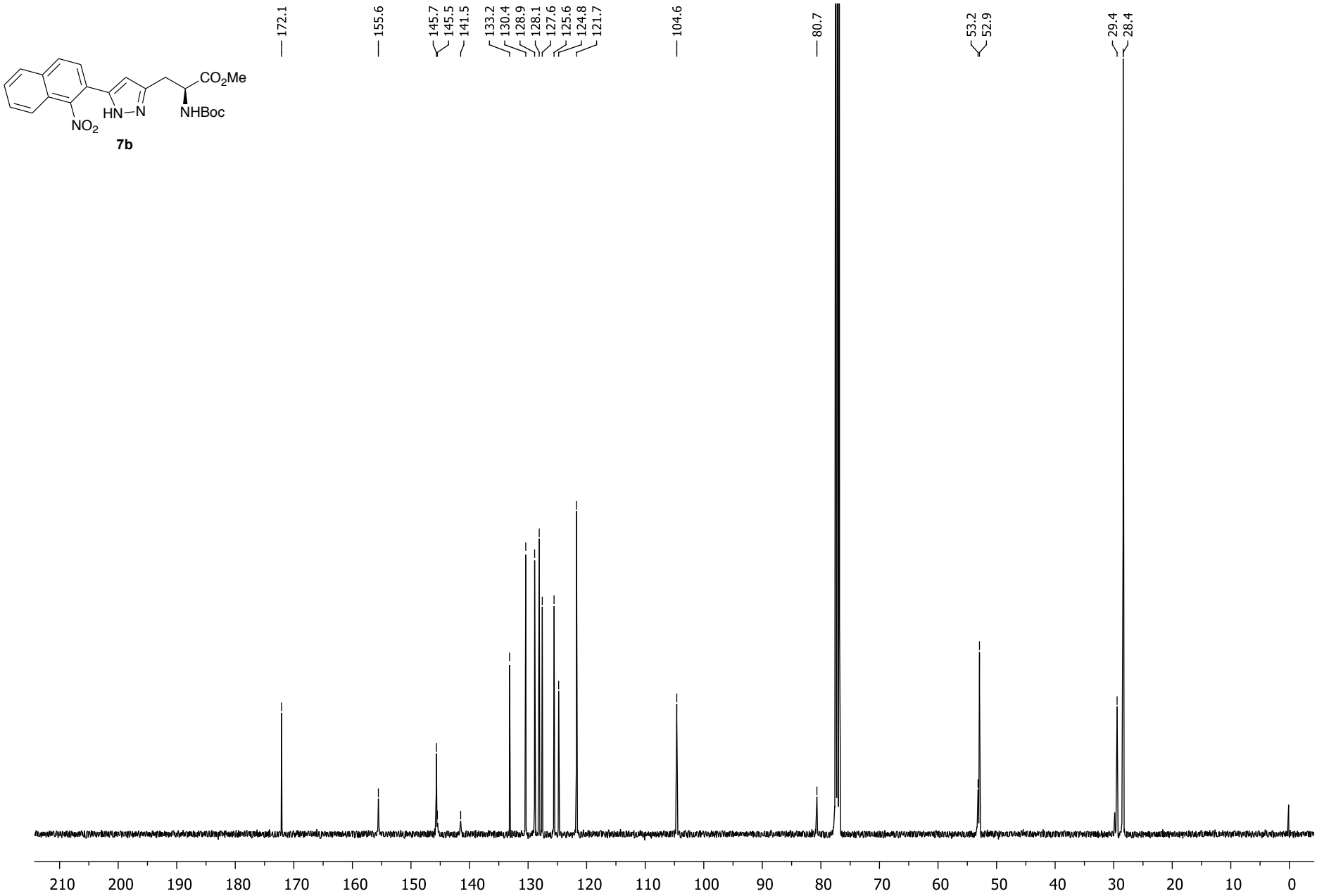
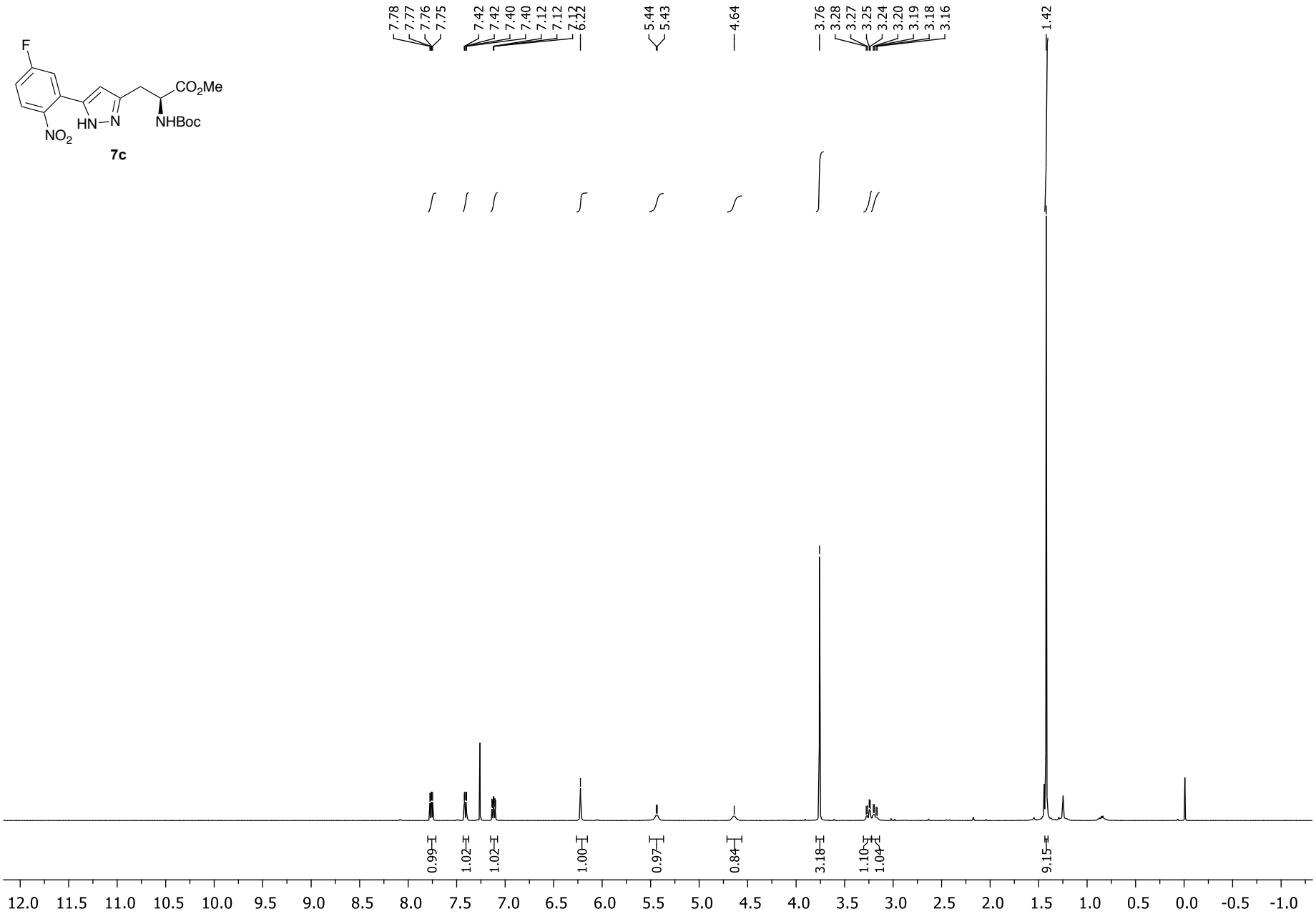
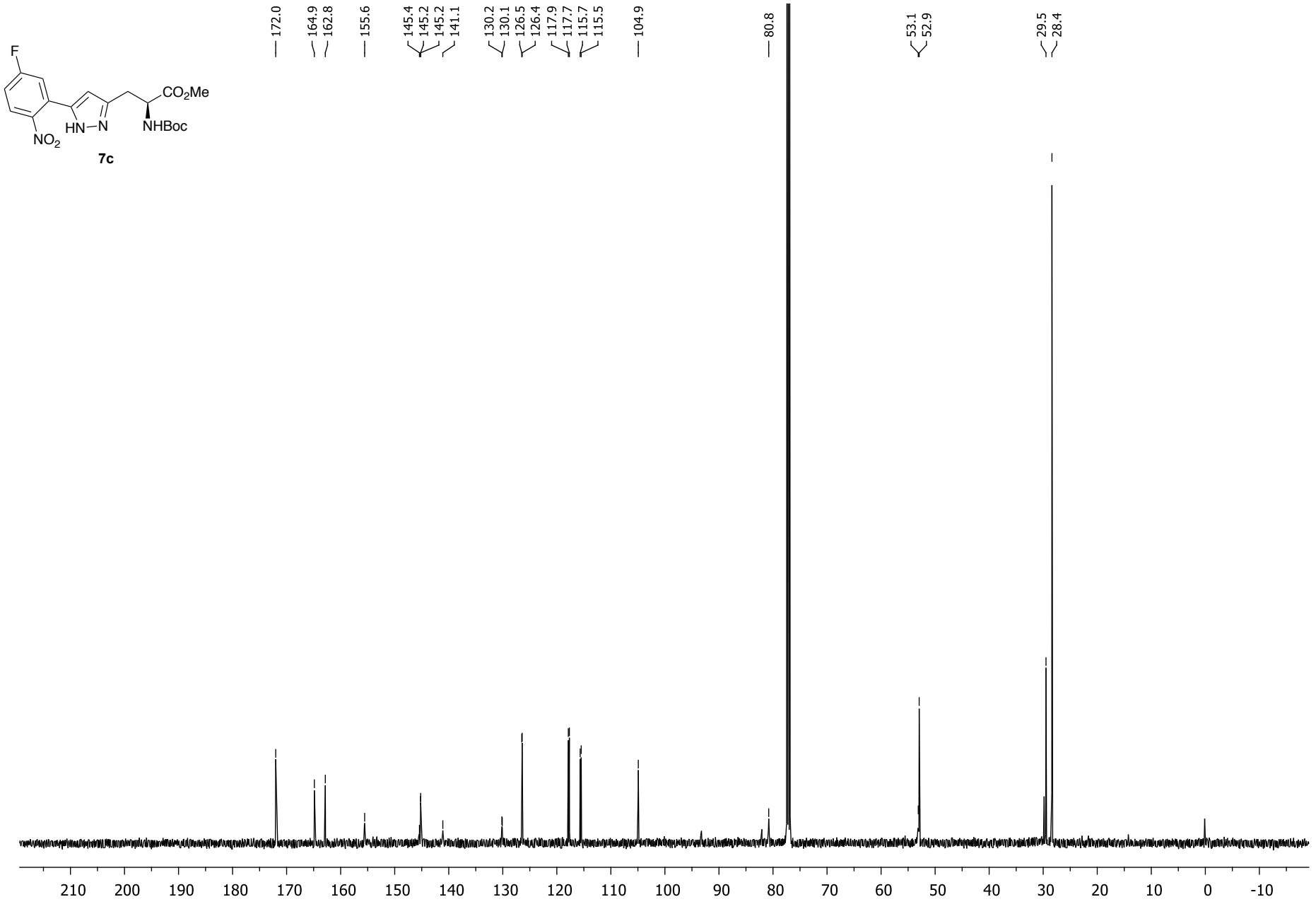
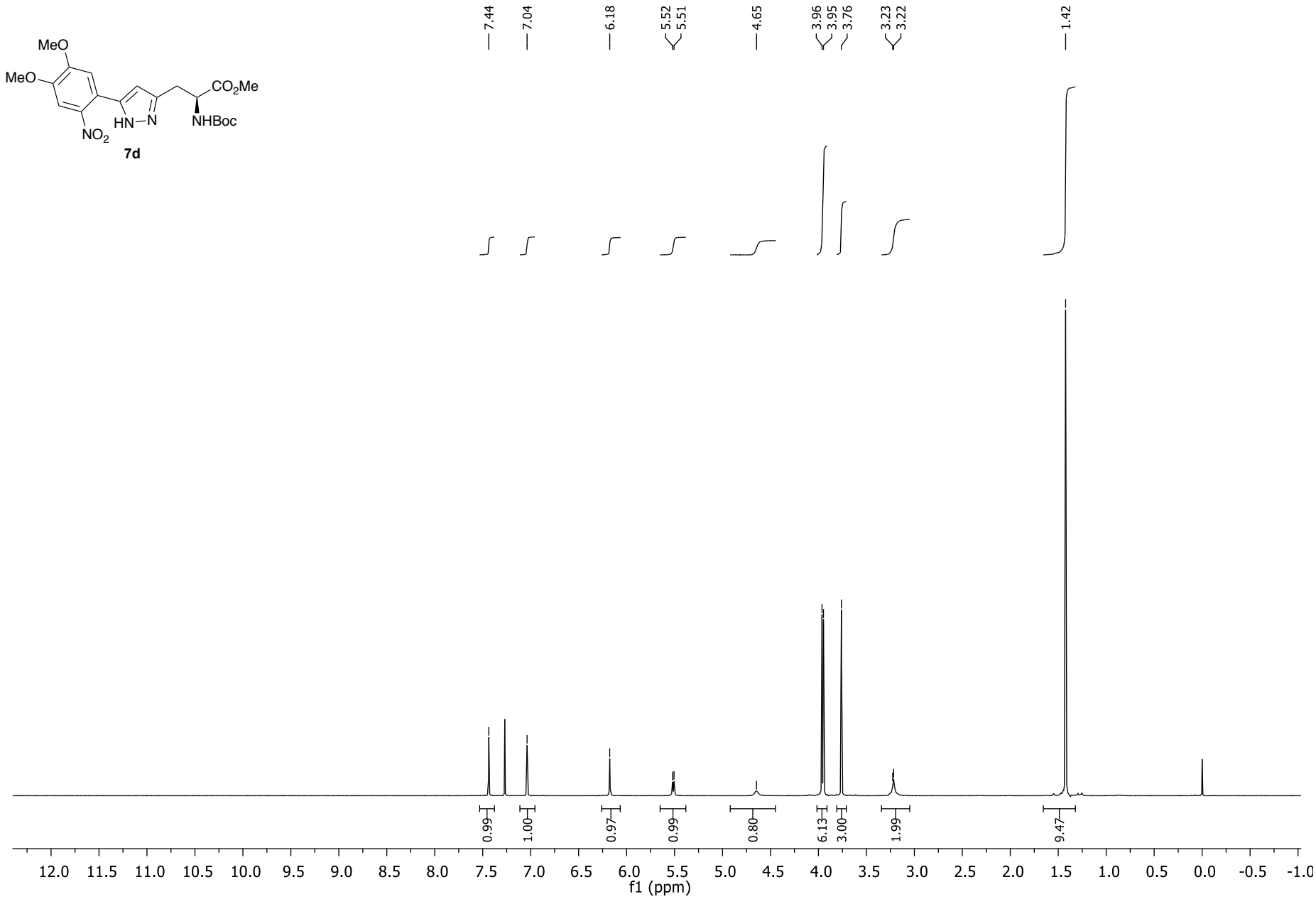
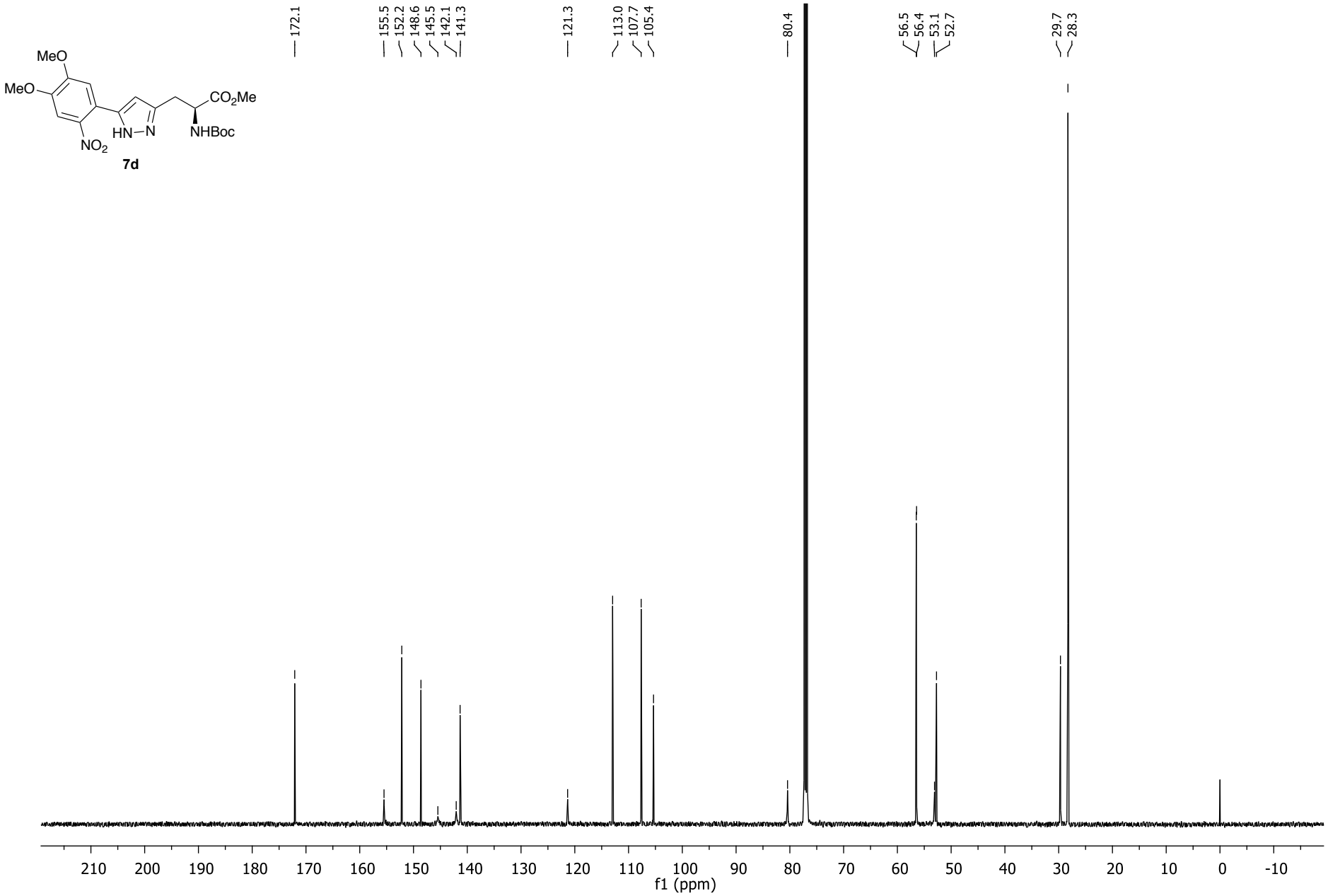
7.94
7.89
7.87
7.84
7.82
7.71
7.69
7.64
7.64
7.62
7.62
7.60
7.60
7.59
7.58
7.57
7.55
6.41
5.47
5.46
4.67
3.77
3.34
3.33
3.30
3.29
3.27
3.26
3.23
3.22
1.42
CO2Me
HN–N
NHBoc
NO2
7b
1.05
1.08
0.98
1.01
1.08
1.00
0.97
1.00
0.91
3.00
0.98
0.90
9.59
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

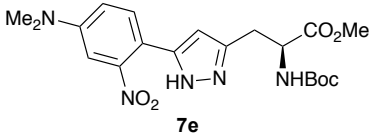

172.1
155.6
145.7
145.5
141.5
133.2
130.4
128.9
128.1
127.6
125.6
124.8
121.7
104.6
80.7
53.2
52.9
29.4
28.4
CO2Me
HN–N
NHBoc
NO2
7b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


F
CO2Me
HN–N
NHBoc
NO2
7c
7.78
7.77
7.76
7.75
7.42
7.42
7.40
7.40
7.12
7.12
6.22
5.44
5.43
4.64
3.76
3.28
3.27
3.25
3.24
3.20
3.19
3.18
3.16
1.42
0.99
1.02
1.02
1.00
0.97
0.84
3.18
1.10
1.04
9.15
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0


F
CO2Me
HN–N
NHBoc
NO2
7c
172.0
164.9
162.8
155.6
145.4
145.2
145.2
141.1
130.2
130.1
126.5
126.4
117.9
117.7
115.7
115.5
104.9
80.8
53.1
52.9
29.5
28.4
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

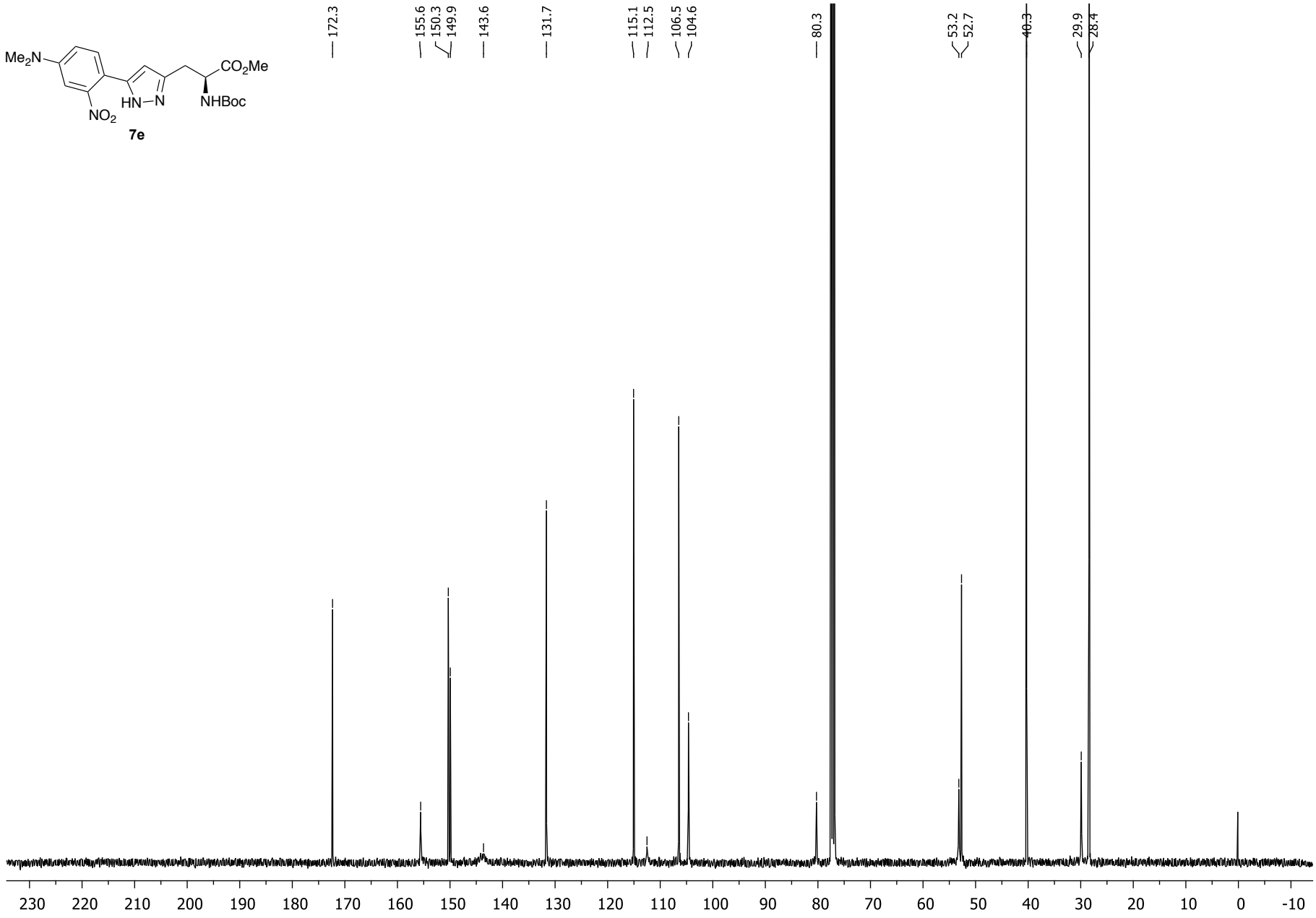

MeO
MeO
NO2
HN–N
CO2Me
NHBoc
7d
7.44
7.04
6.18
5.52
5.51
4.65
3.96
3.95
3.76
3.23
3.22
1.42
0.99
1.00
0.97
0.99
0.80
6.13
3.00
1.99
9.47
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

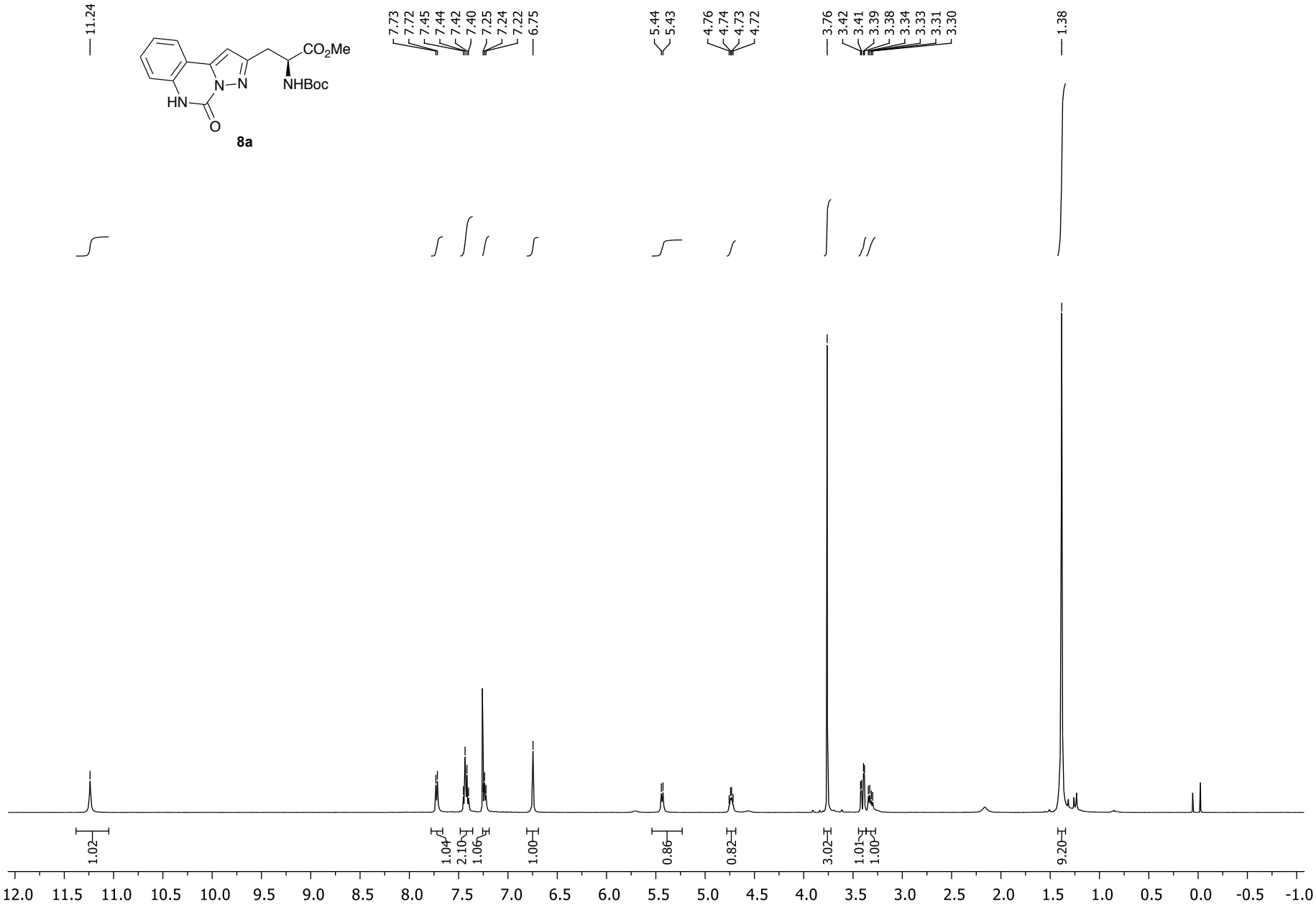

MeO
MeO
NO2
HN–N
CO2Me
NHBoc
7d
172.1
155.5
152.2
148.6
145.5
142.1
141.3
121.3
113.0
107.7
105.4
80.4
56.5
56.4
53.1
52.7
29.7
28.3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

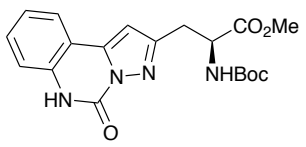

Me2N
CO2Me
HN–N
NHBoc
NO2

7e

7.44
7.42
6.95
6.95
6.83
6.83
6.81
6.81
6.11
5.51
5.49
4.61
3.73
3.18
3.17
3.02
1.42
0.97
0.97
1.01
1.00
1.09
0.89
3.14
1.93
6.09
9.50
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

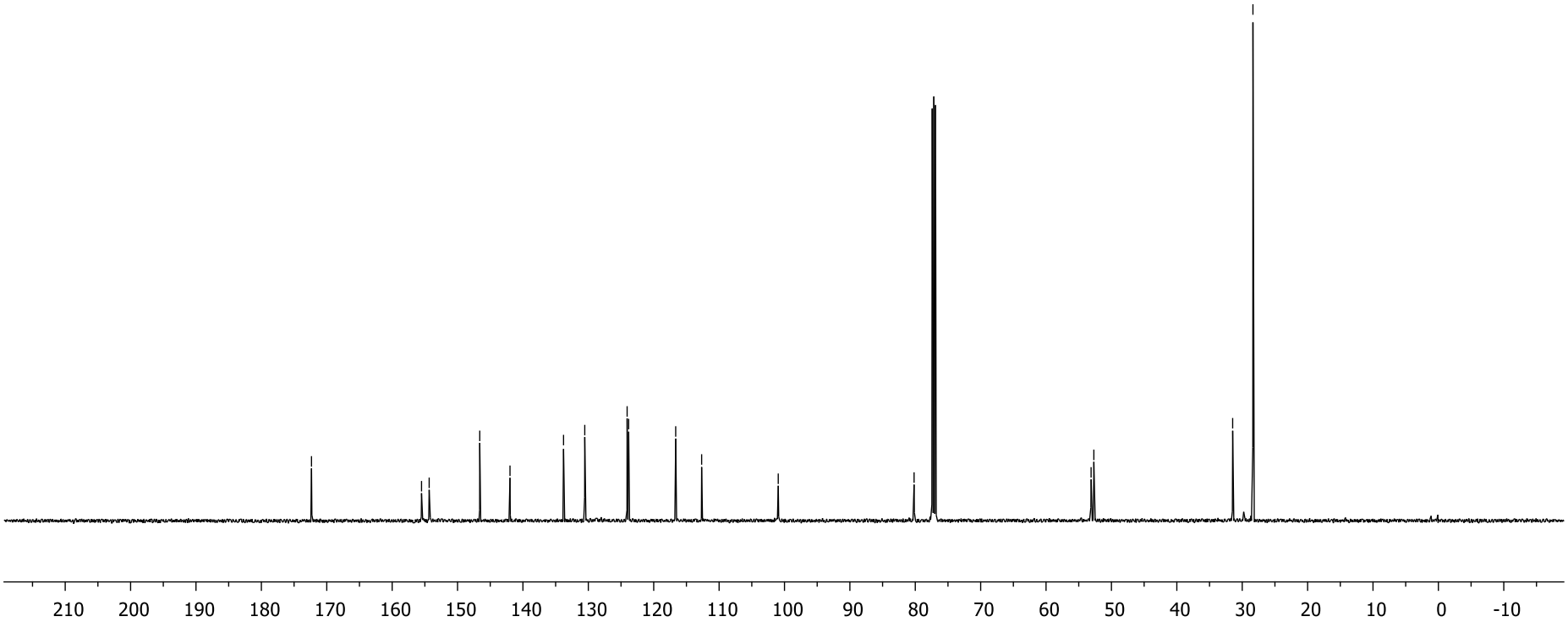

Me2N
NO2
HN–N
CO2Me
NHBoc
7e
172.3
155.6
150.3
149.9
143.6
131.7
115.1
112.5
106.5
104.6
80.3
53.2
52.7
40.3
29.9
28.4
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

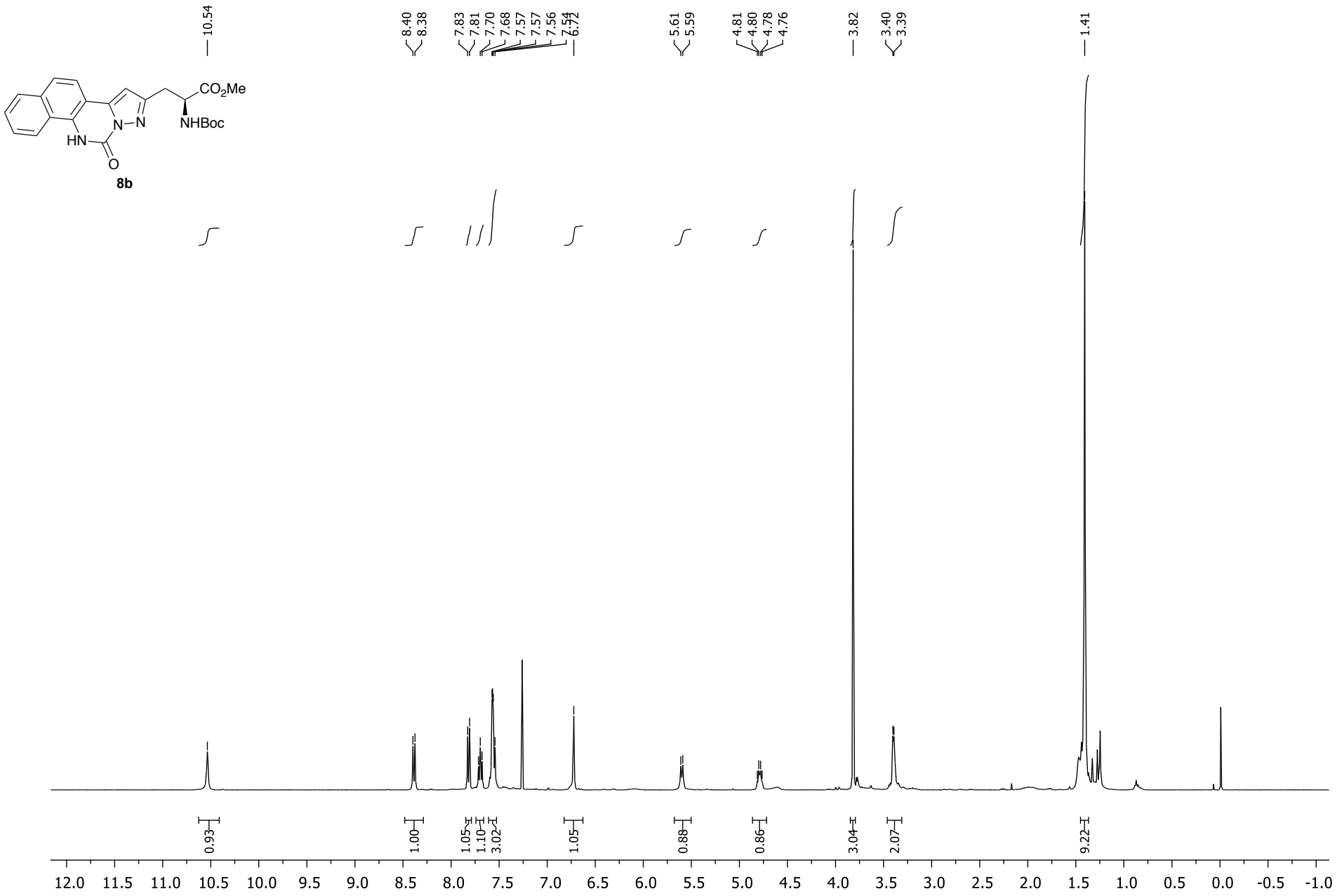

11.24
7.73
7.72
7.45
7.44
7.42
7.40
7.25
7.24
7.22
6.75
5.44
5.43
4.76
4.74
4.73
4.72
3.76
3.42
3.41
3.39
3.38
3.34
3.33
3.31
3.30
1.38
CO2Me
NHBoc
HN
N–N
O
8a
1.02
1.04
2.10
1.06
1.00
0.86
0.82
3.02
1.01
1.00
9.20
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0



8a

172.3
155.5
154.3
146.6
142.0
133.8
130.5
124.1
123.8
116.6
112.7
101.0
80.2
53.1
52.7
31.5
28.4

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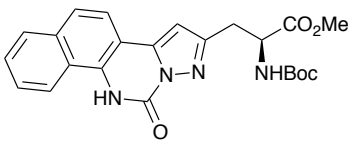
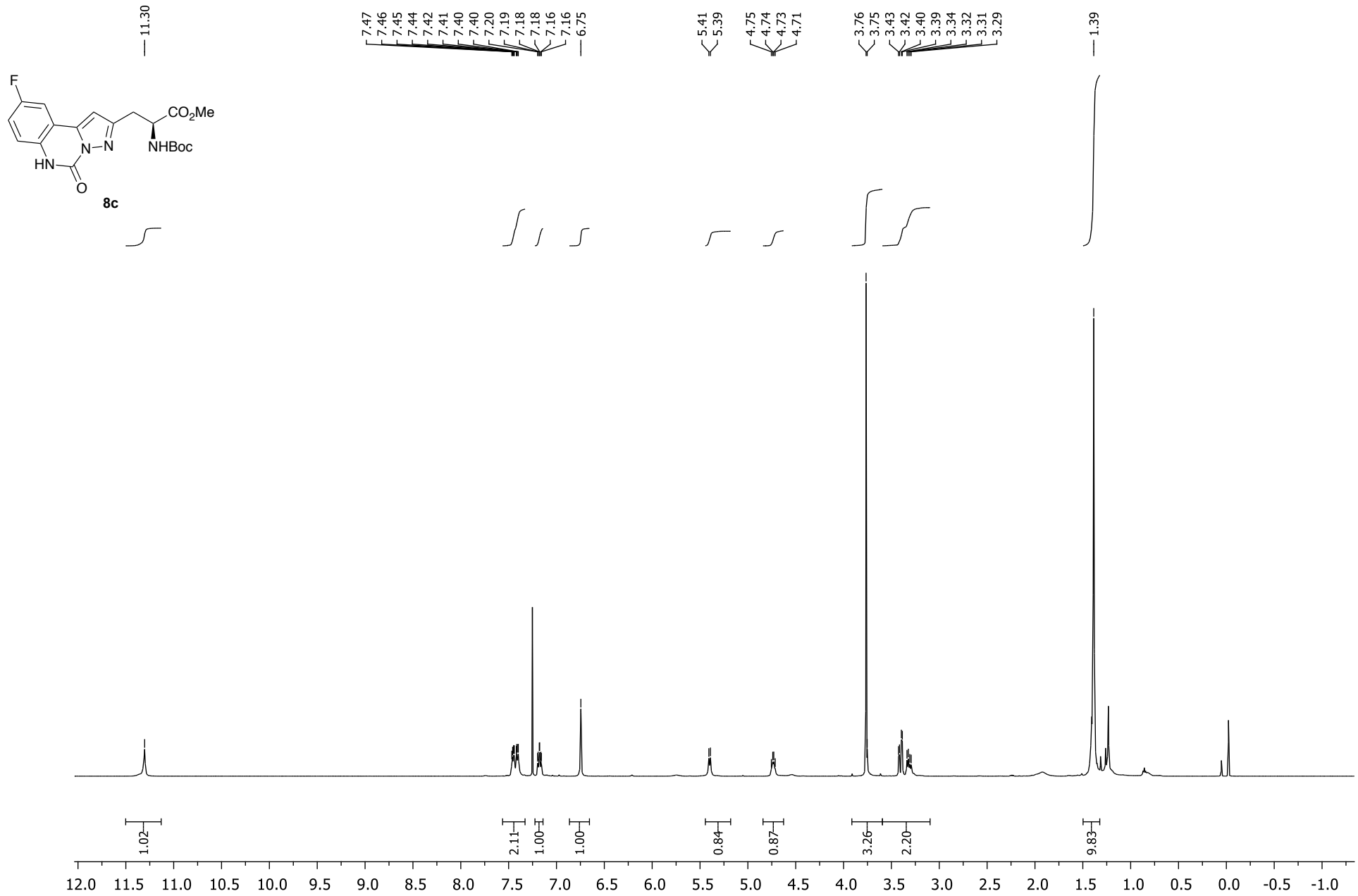
8.40
8.38
7.83
7.81
7.70
7.68
7.57
7.57
7.56
7.54
6.72
5.61
5.59
4.81
4.80
4.78
4.76
3.82
3.40
3.39
1.41
CO2Me
NHBoc
HN
N
N
O
8b
0.93
1.00
1.05
1.10
3.02
1.05
0.88
0.86
3.04
2.07
9.22
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0


CO2Me
NHBoc
N
N
HN
O

8b

172.5
155.6
154.7
146.0
142.4
133.9
129.7
128.9
127.9
127.8
124.6
121.7
120.9
120.4
108.5
100.9
80.2
53.1
52.8
31.4
28.4
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10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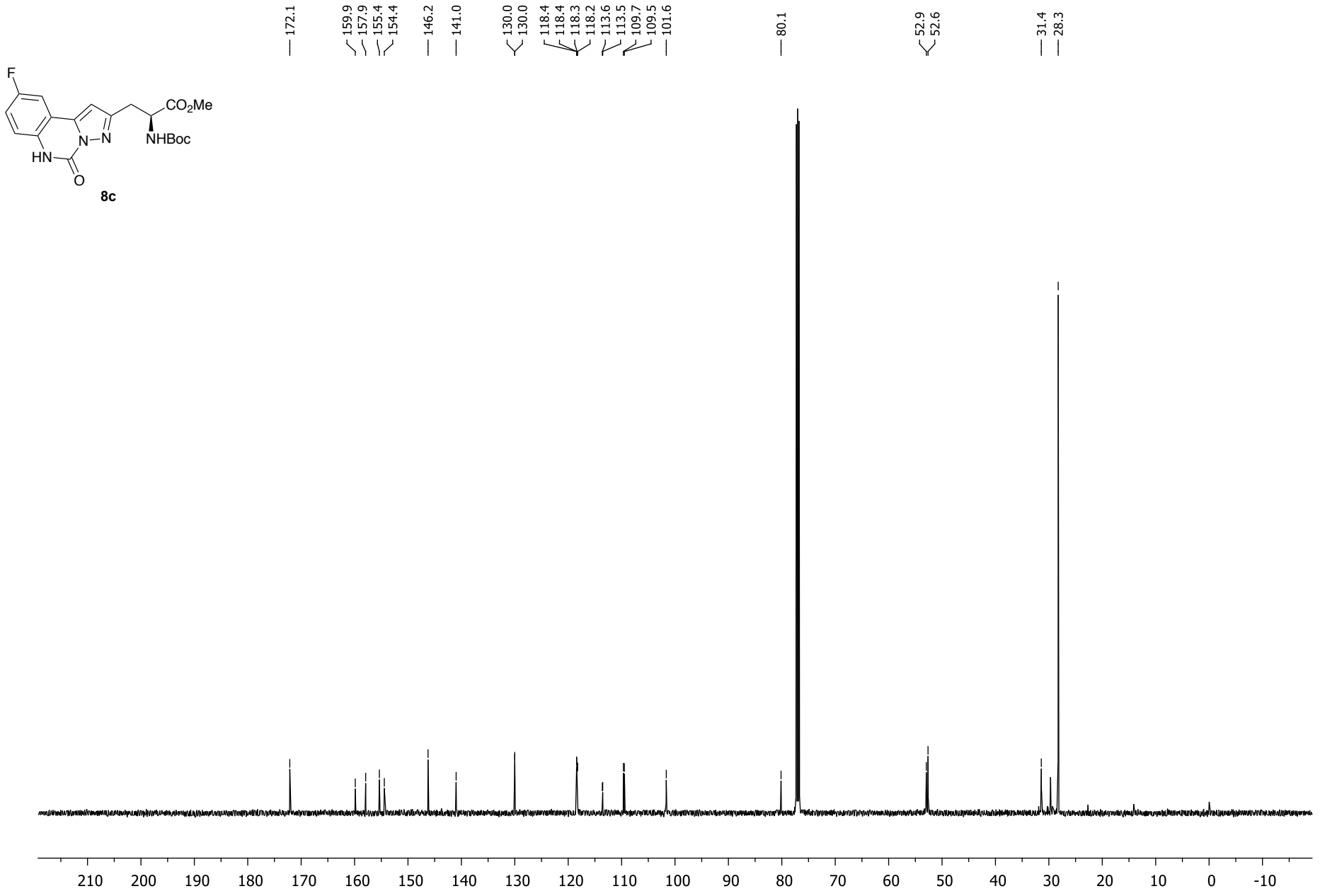
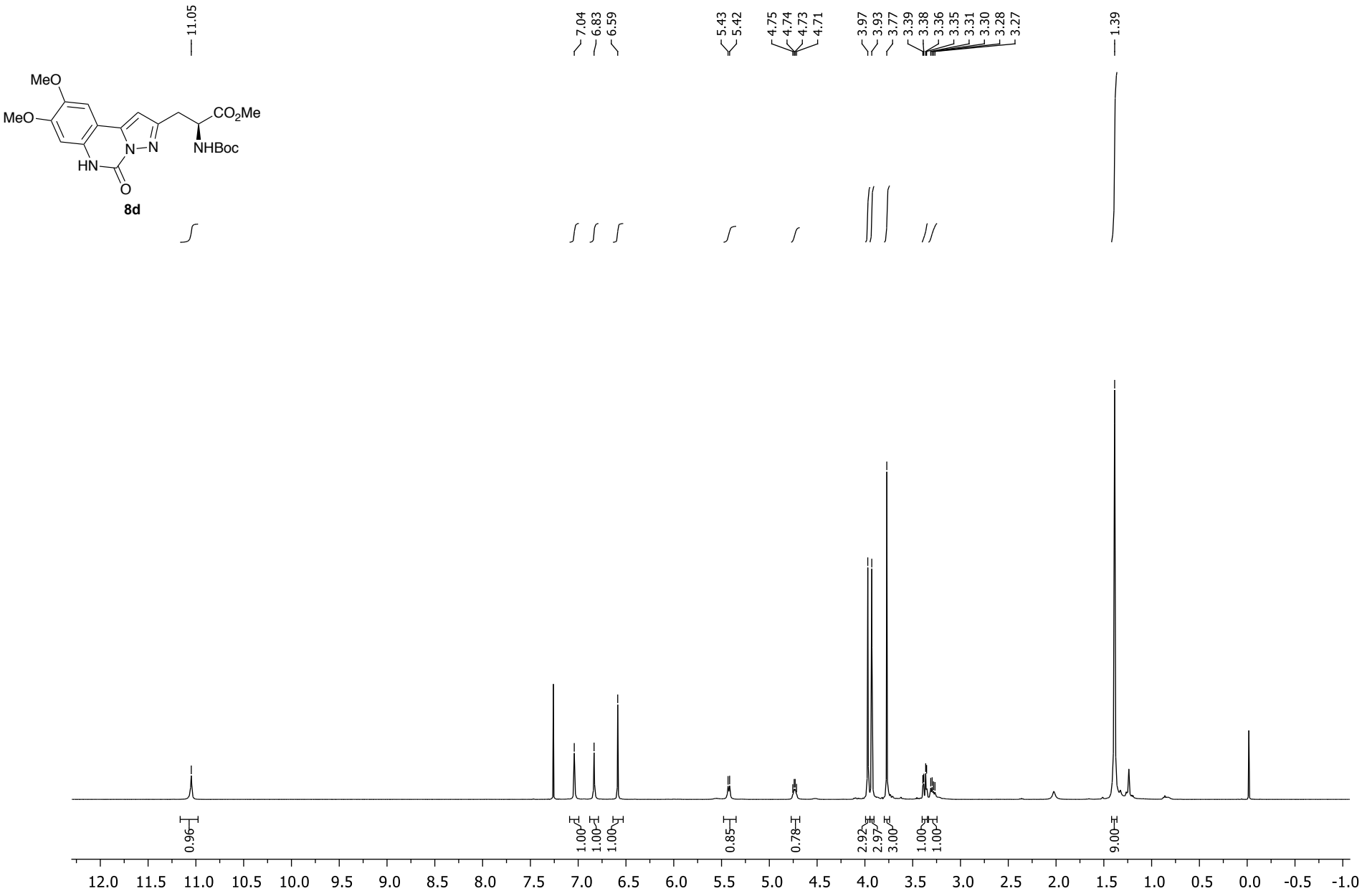
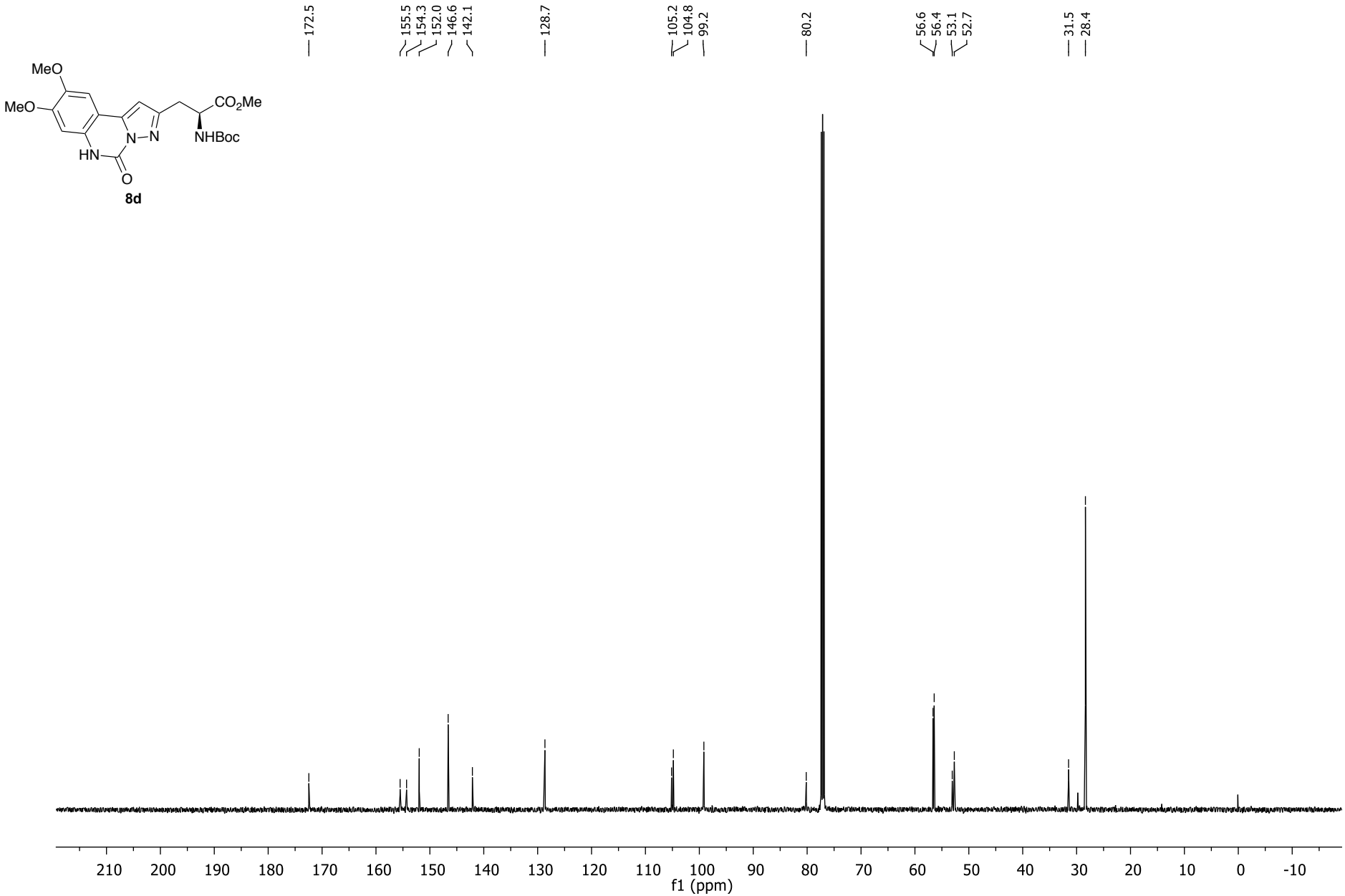
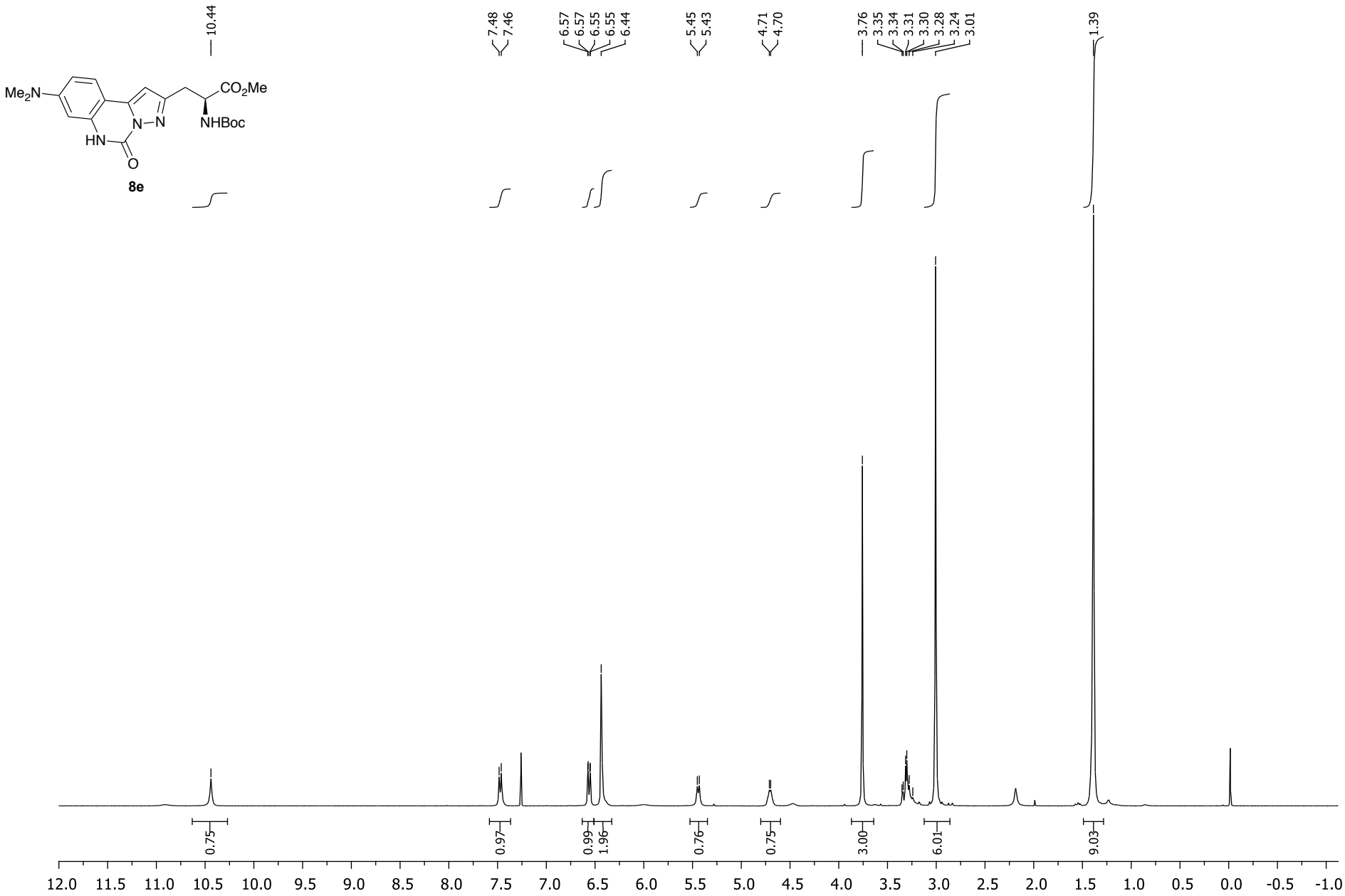
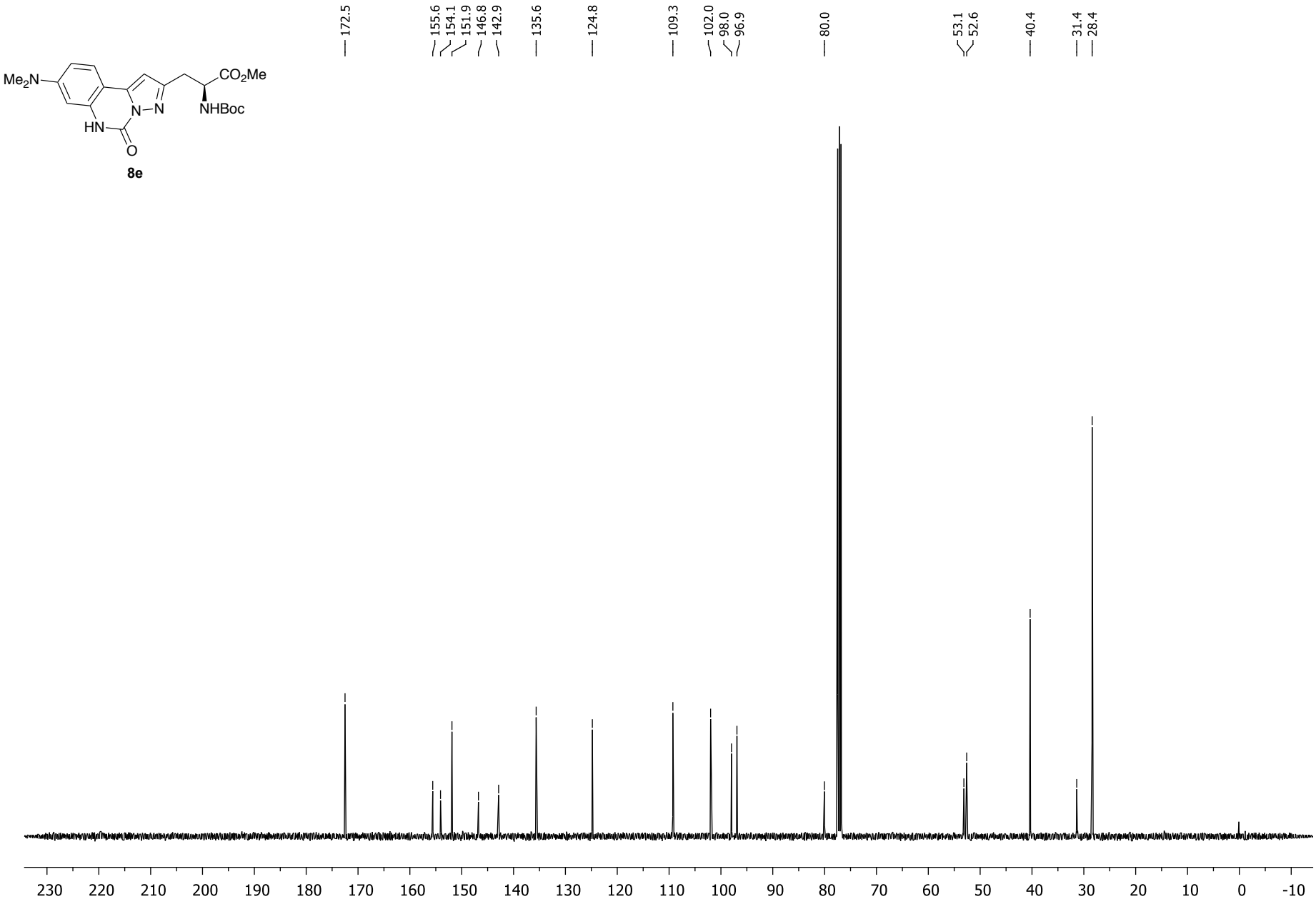
7.46
7.45
7.44
7.42
7.41
7.40
7.40
7.20
7.19
7.18
7.18
7.16
7.16
6.75
5.41
5.39
4.75
4.74
4.73
4.71
3.76
3.75
3.43
3.42
3.40
3.39
3.34
3.32
3.31
3.29
1.39
F
CO2Me
NHBoc
HN
N
N
O
8c
1.02
2.11
1.00
1.00
0.84
0.87
3.26
2.20
9.83
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0

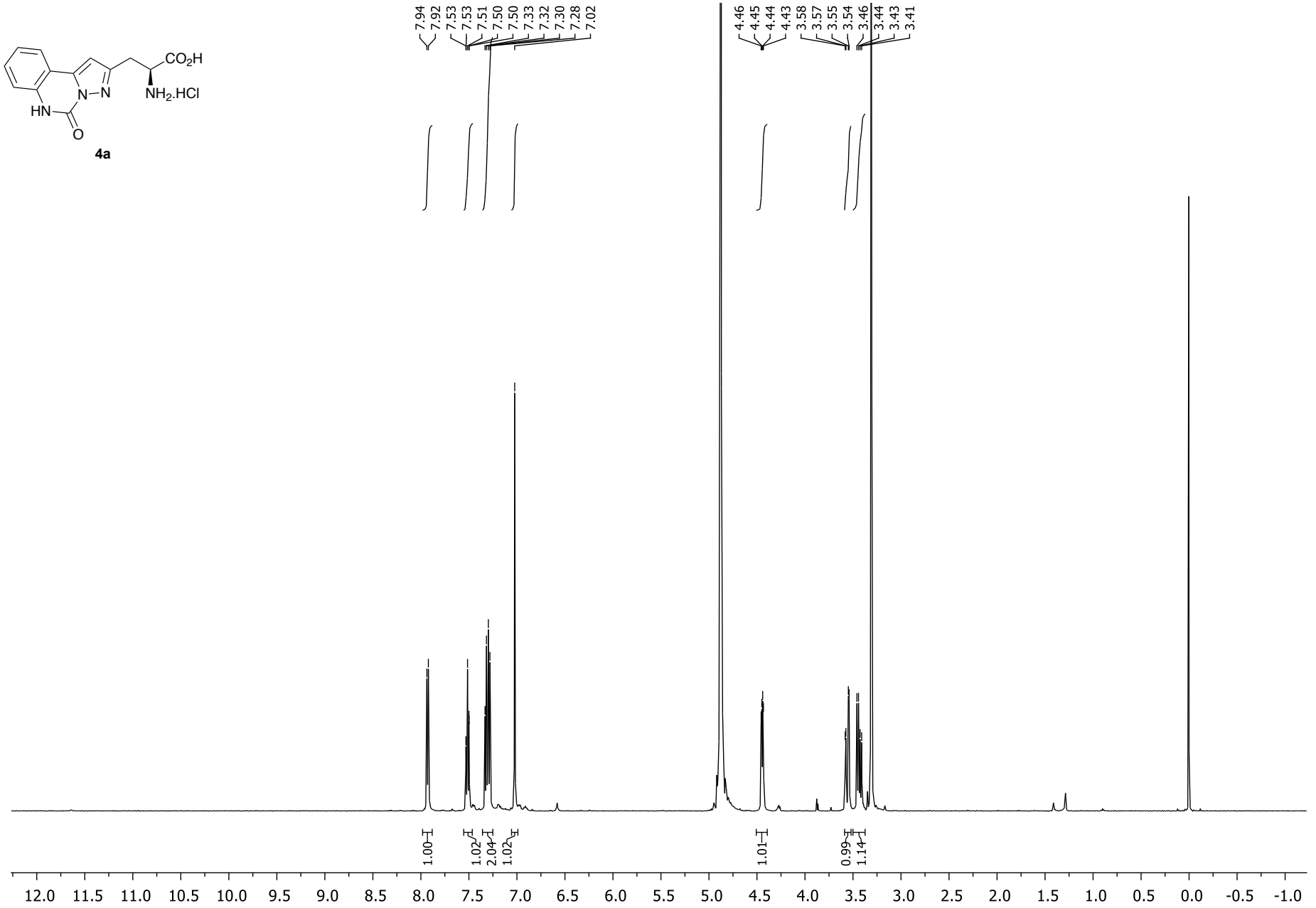

172.1
159.9
157.9
155.4
154.4
146.2
141.0
130.0
130.0
118.4
118.4
118.3
118.2
113.6
113.5
109.7
109.5
101.6
80.1
52.9
52.6
31.4
28.3
F
CO2Me
NHBoc
HN
O
N
N
8c
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

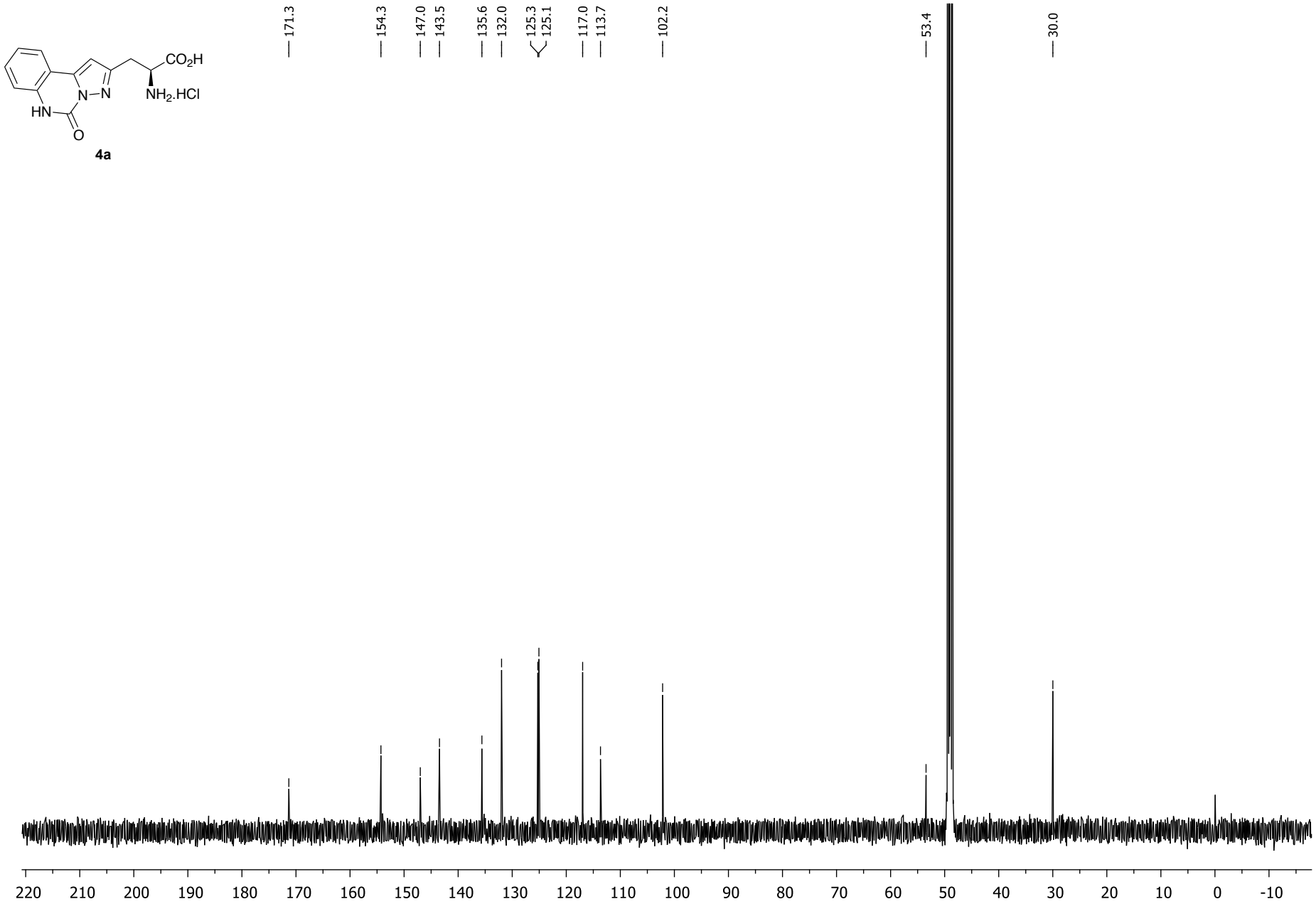

11.05
7.04
6.83
6.59
5.43
5.42
4.75
4.74
4.73
4.71
3.97
3.93
3.77
3.39
3.38
3.36
3.35
3.31
3.30
3.28
3.27
1.39
MeO
MeO
CO2Me
NHBoc
HN
N–N
O
8d
0.96
1.00
1.00
1.00
0.85
0.78
2.92
2.97
3.00
1.00
1.00
9.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

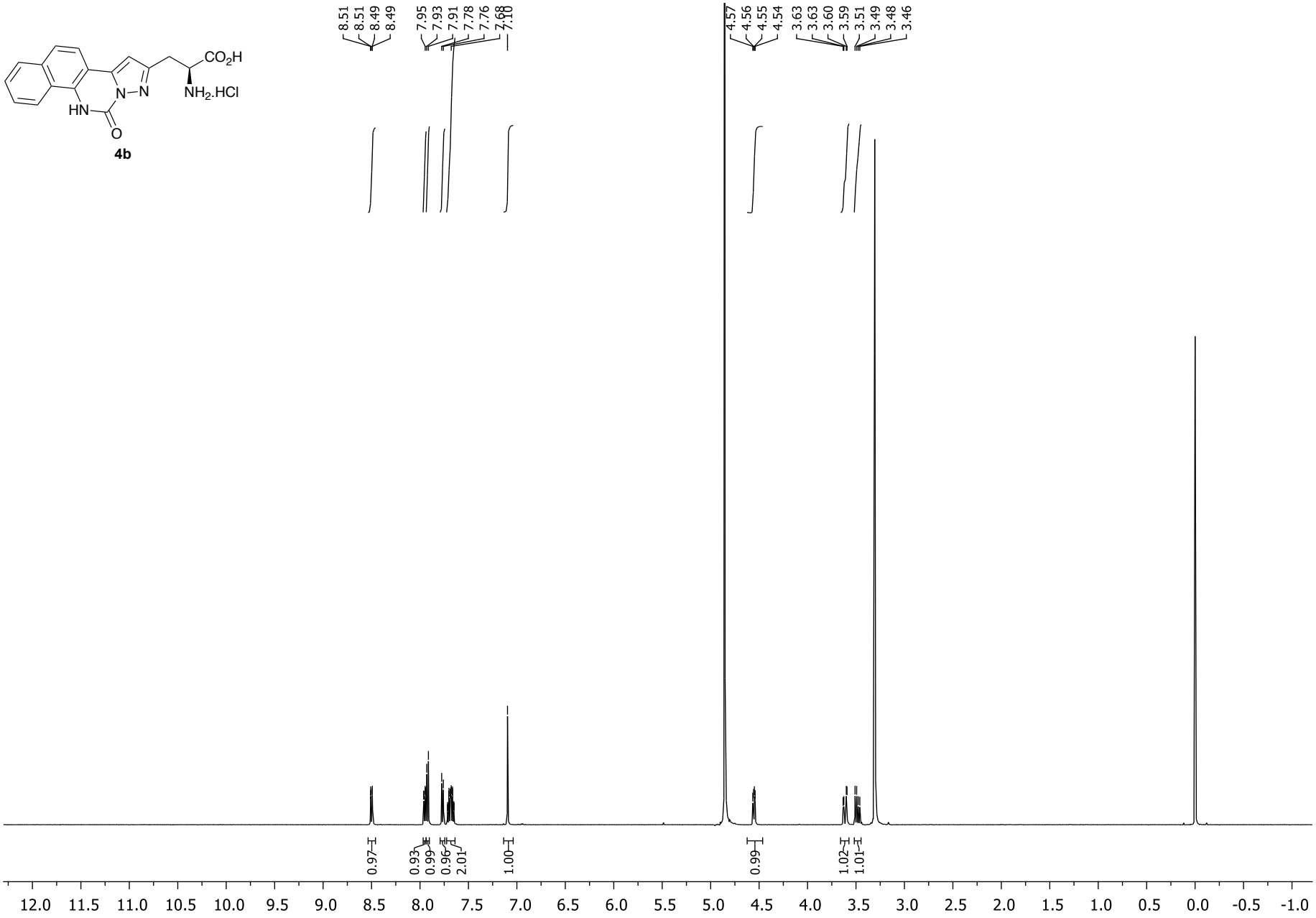

172.5
155.5
154.3
152.0
146.6
142.1
128.7
105.2
104.8
99.2
80.2
56.6
56.4
53.1
52.7
31.5
28.4
MeO
MeO
CO2Me
NHBoc
N–N
HN
O
8d
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

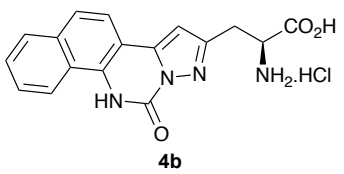

10.44
7.48
7.46
6.57
6.57
6.55
6.55
6.44
5.45
5.43
4.71
4.70
3.76
3.35
3.34
3.31
3.30
3.28
3.24
3.01
1.39
Me2N
CO2Me
NHBoc
N–N
HN
O
8e
0.75
0.97
0.99
1.96
0.76
0.75
3.00
6.01
9.03
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0


172.5
155.6
154.1
151.9
146.8
142.9
135.6
124.8
109.3
102.0
98.0
96.9
80.0
53.1
52.6
40.4
31.4
28.4
Me2N
CO2Me
NHBoc
HN
O
N
N
8e
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


CO2H
NH2.HCl
N–N
HN
O
4a
7.94
7.92
7.53
7.53
7.51
7.50
7.50
7.33
7.32
7.30
7.28
7.02
4.46
4.45
4.44
4.43
3.58
3.57
3.55
3.54
3.46
3.44
3.43
3.41
1.00
1.02
2.04
1.02
1.01
0.99
1.14
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0

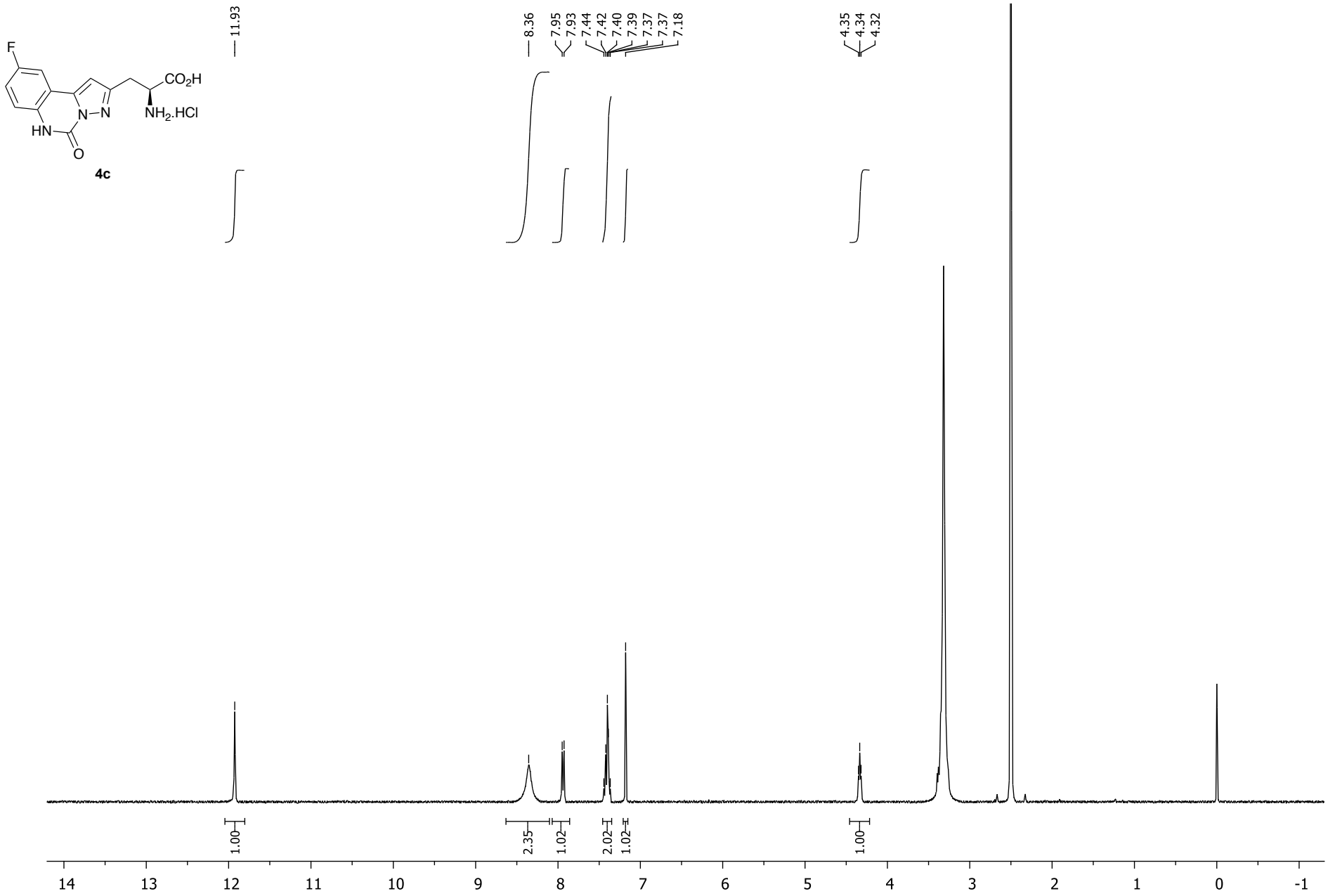

171.3
154.3
147.0
143.5
135.6
132.0
125.3
125.1
117.0
113.7
102.2
53.4
30.0
CO2H
NH2.HCl
N–N
HN
O
4a
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

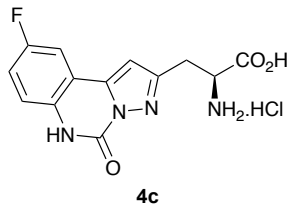

8.51
8.51
8.49
8.49
7.95
7.93
7.91
7.78
7.76
7.68
7.10
4.57
4.56
4.55
4.54
3.63
3.63
3.60
3.59
3.51
3.49
3.48
3.46
CO2H
NH2.HCl
N–N
HN
O
4b
0.97
0.93
0.99
0.96
2.01
1.00
0.99
1.02
1.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0


CO2H
NH2.HCl
N
N
HN
O

4b

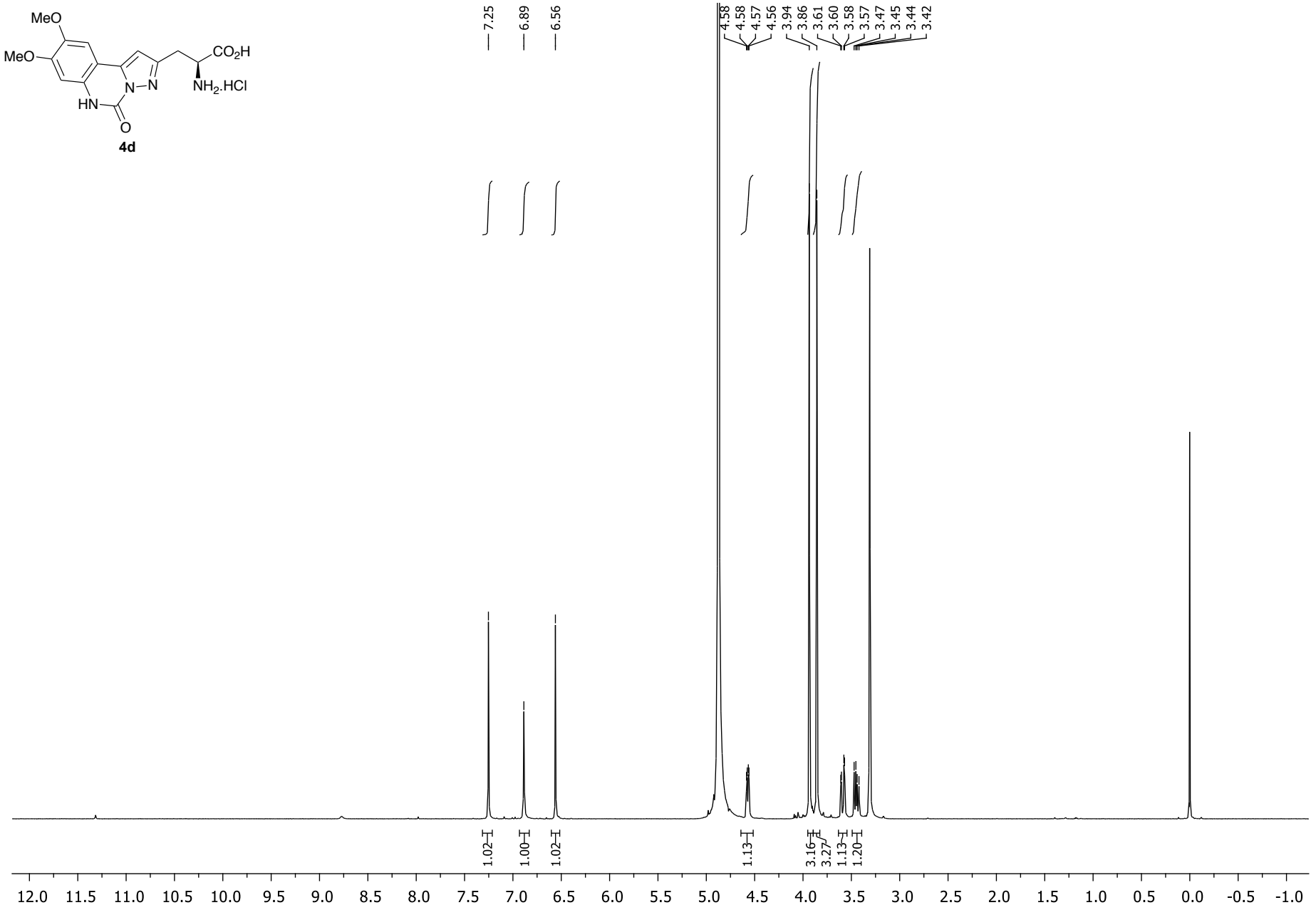
170.9
154.6
147.4
144.0
135.7
131.7
130.1
129.1
128.6
125.9
123.3
122.5
121.7
109.6
102.1
53.1
29.9
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

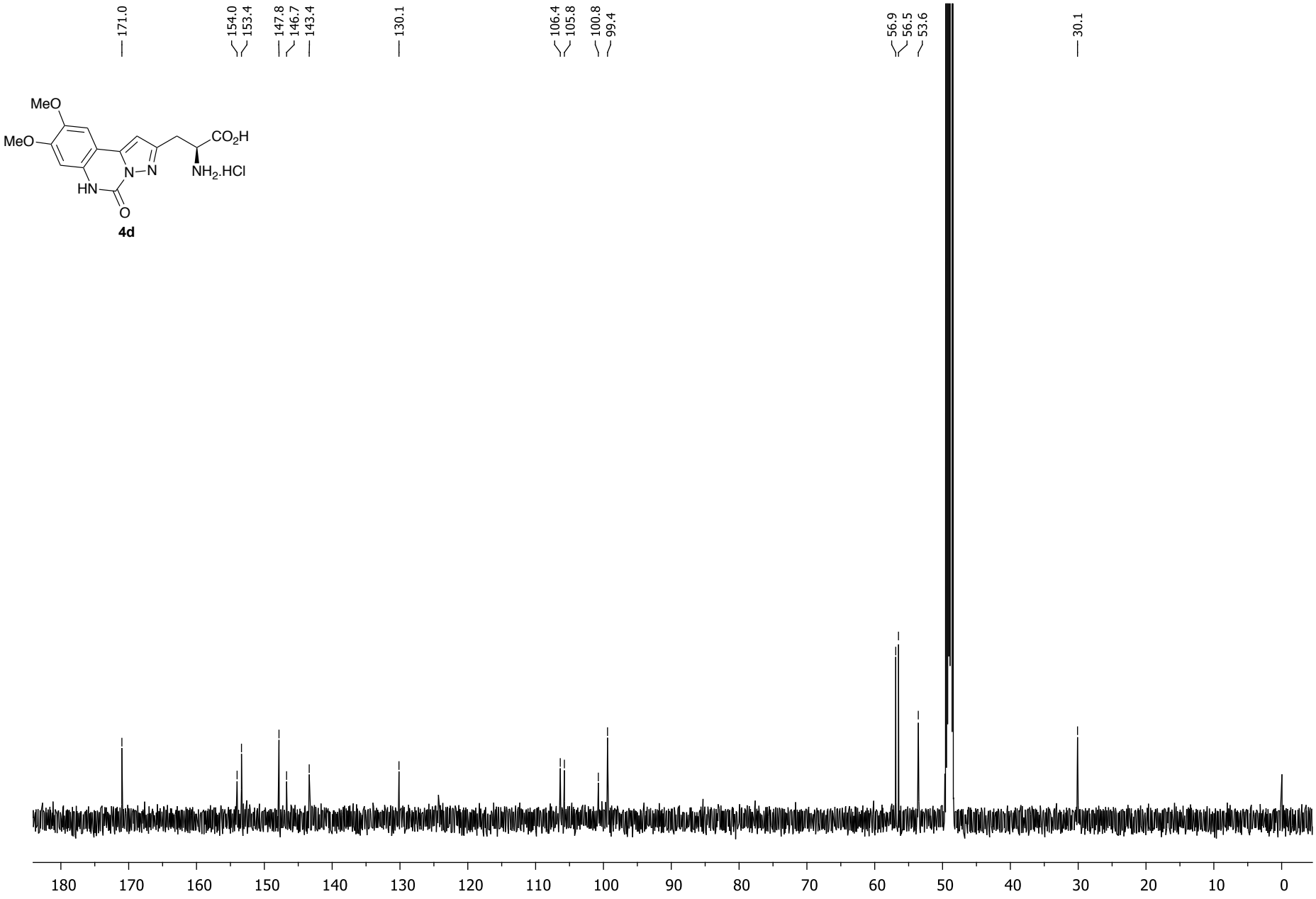

F
CO2H
NH2.HCl
N–N
HN
O
4c
11.93
8.36
7.95
7.93
7.44
7.42
7.40
7.39
7.37
7.37
7.18
4.35
4.34
4.32
1.00
2.35
1.02
2.02
1.02
1.00
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1

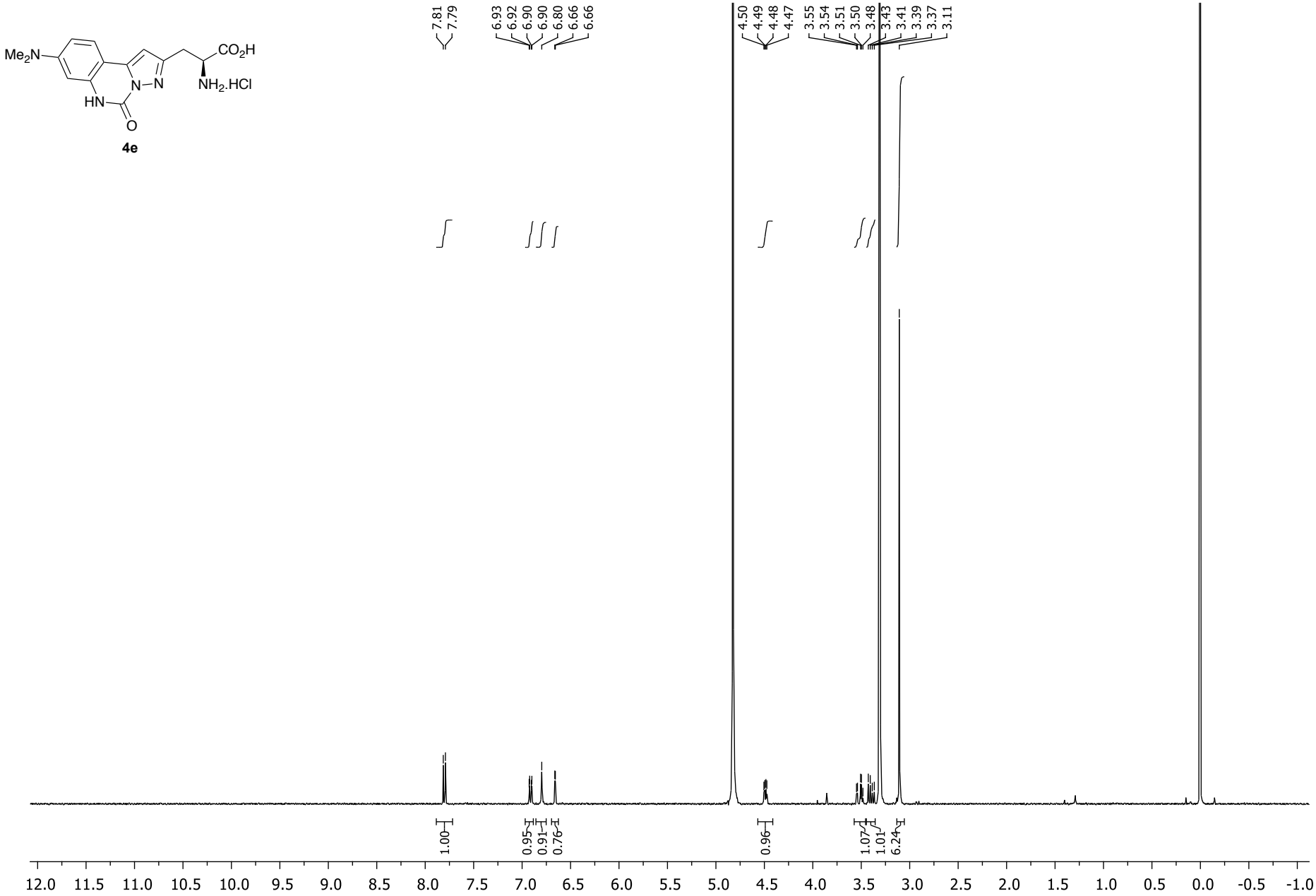

F
CO2H
N–N
NH2.HCl
HN
O

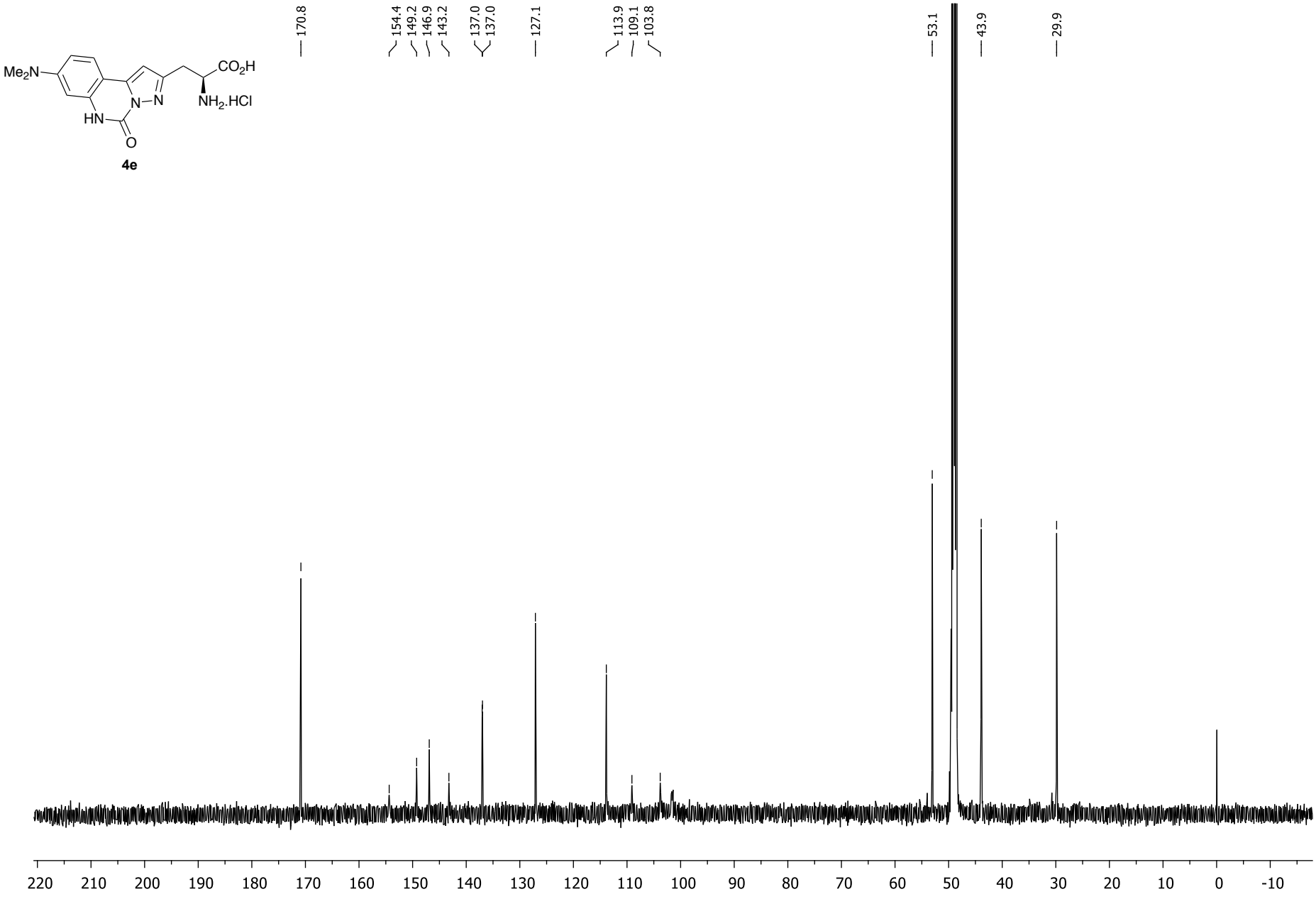
4c

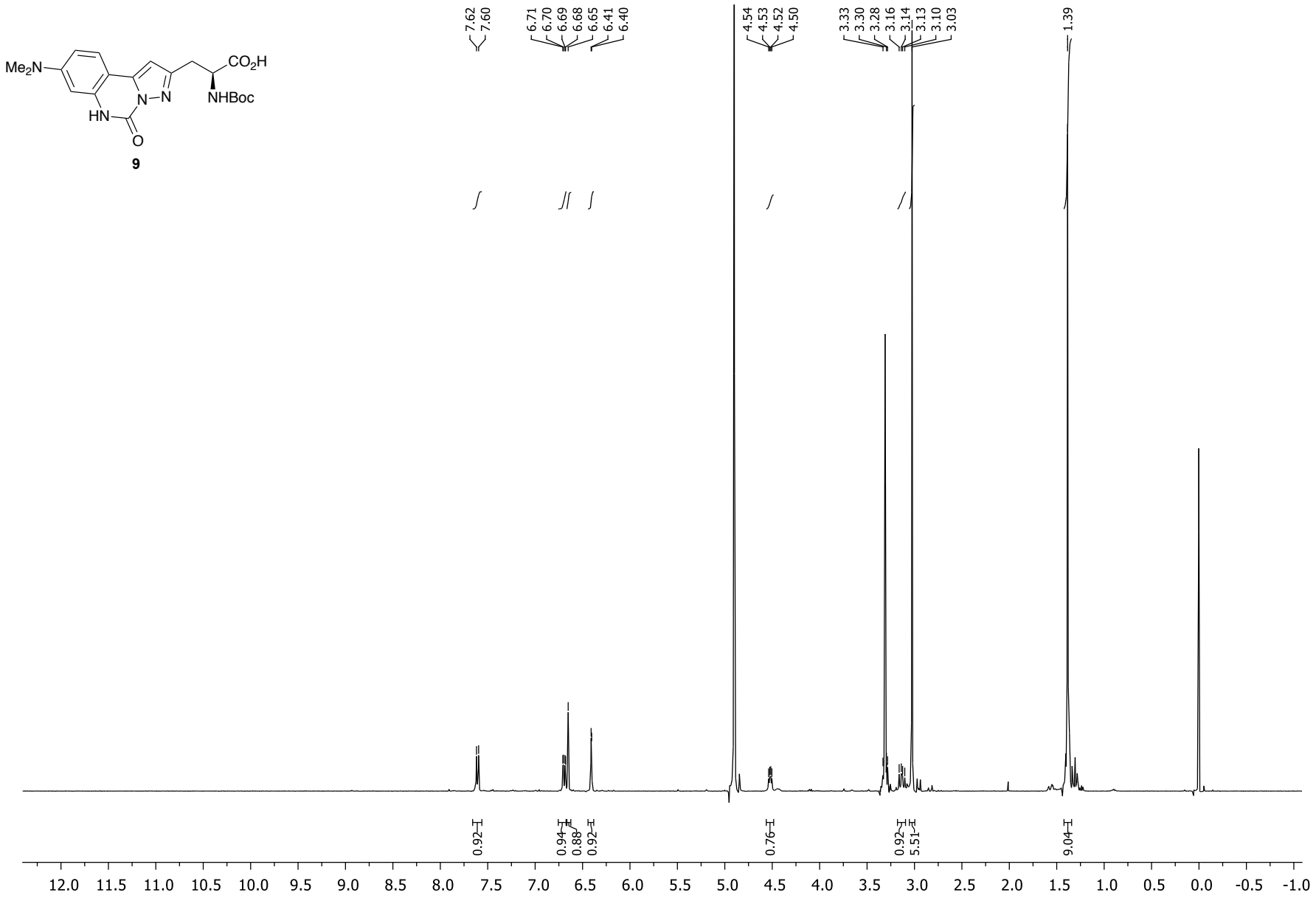
169.9
159.0
156.6
151.6
143.9
140.7
140.6
131.0
131.0
118.2
117.9
117.8
117.7
113.1
113.0
109.9
109.6
102.4
51.6
29.0
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

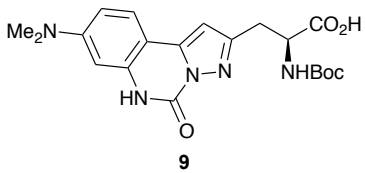

MeO
MeO
CO2H
NH2.HCl
HN
O
N
N
4d
7.25
6.89
6.56
4.58
4.58
4.57
4.56
3.94
3.86
3.61
3.60
3.58
3.57
3.47
3.45
3.44
3.42
1.02
1.00
1.02
1.13
3.16
3.27
1.13
1.20
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0


171.0
154.0
153.4
147.8
146.7
143.4
130.1
106.4
105.8
100.8
99.4
56.9
56.5
53.6
30.1
MeO
MeO
CO2H
NH2.HCl
HN
N
N
O
4d
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


Me2N
CO2H
NH2.HCl
HN
O
N–N
4e
7.81
7.79
6.93
6.92
6.90
6.90
6.80
6.66
6.66
4.50
4.49
4.48
4.47
3.55
3.54
3.51
3.50
3.48
3.43
3.41
3.39
3.37
3.11
1.00
0.95
0.91
0.76
0.96
1.07
1.01
6.24
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0


Me2N
CO2H
NH2.HCl
HN
O
N
N
4e
170.8
154.4
149.2
146.9
143.2
137.0
137.0
127.1
113.9
109.1
103.8
53.1
43.9
29.9
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


Me2N
CO2H
NHBoc
N–N
HN
O
9
7.62
7.60
6.71
6.70
6.69
6.68
6.65
6.41
6.40
4.54
4.53
4.52
4.50
3.33
3.30
3.28
3.16
3.14
3.13
3.10
3.03
1.39
0.92
0.94
0.88
0.92
0.76
0.92
5.51
9.04
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0


Me2N
CO2H
N–N
NHBoc
HN
O

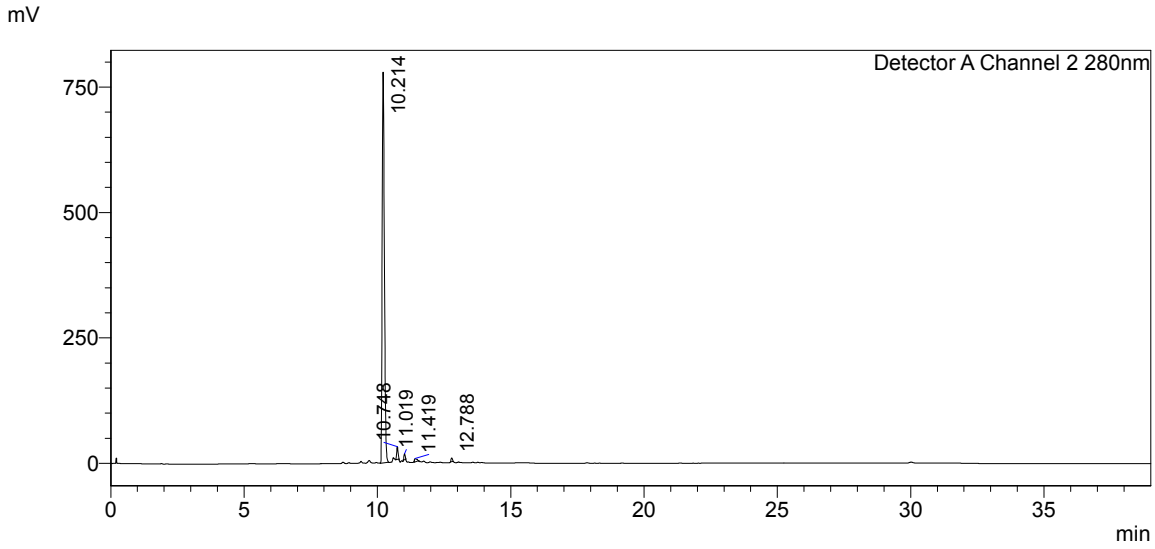
9

175.2
157.9
156.0
153.5
147.2
143.9
137.3
125.9
110.3
103.0
99.1
97.4
80.6
54.7
40.3
31.9
28.7
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

8. HPLC Traces for Amino Acids 4a–e

Amino acids **4a–d** were analysed on a reverse-phase HPLC system using a C18 (150 × 4.6 mm) analytical column, a gradient solution of 0.1% TFA in water/acetonitrile (10% to 90%) over 40 minutes and with a flow rate of 1 mL per minute. Amino acid **4e** was analysed on a reverse-phase HPLC system using a C18 (250 × 4.6 mm) analytical column, a gradient solution of 0.1% TFA in water/acetonitrile (10% to 90%) over 30 minutes and with a flow rate of 1 mL per minute.

(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4a)

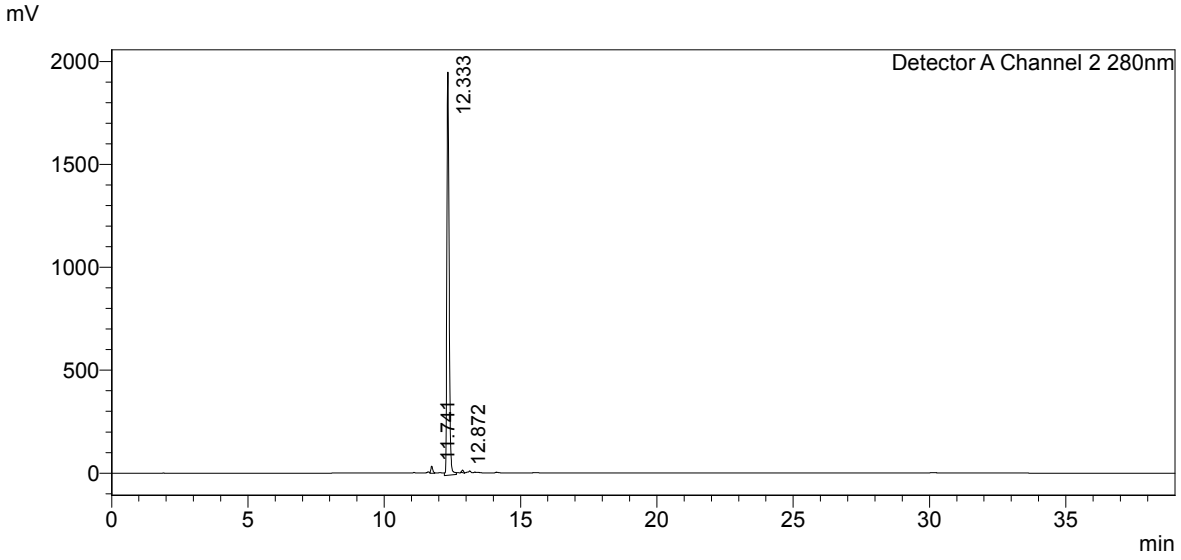


<Peak Table>

Detector A Channel 2 280nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	10.214	4447336	778828	95.791		M	
2	10.748	91606	25503	1.973		M	
3	11.019	37928	12029	0.817		M	
4	11.419	39050	6764	0.841		M	
5	12.788	26853	7709	0.578		M	
Total		4642772	830832				

(2*S*)-2-Amino-3-(5′-oxo-5′,6′-dihydrobenzo[h]pyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4b)

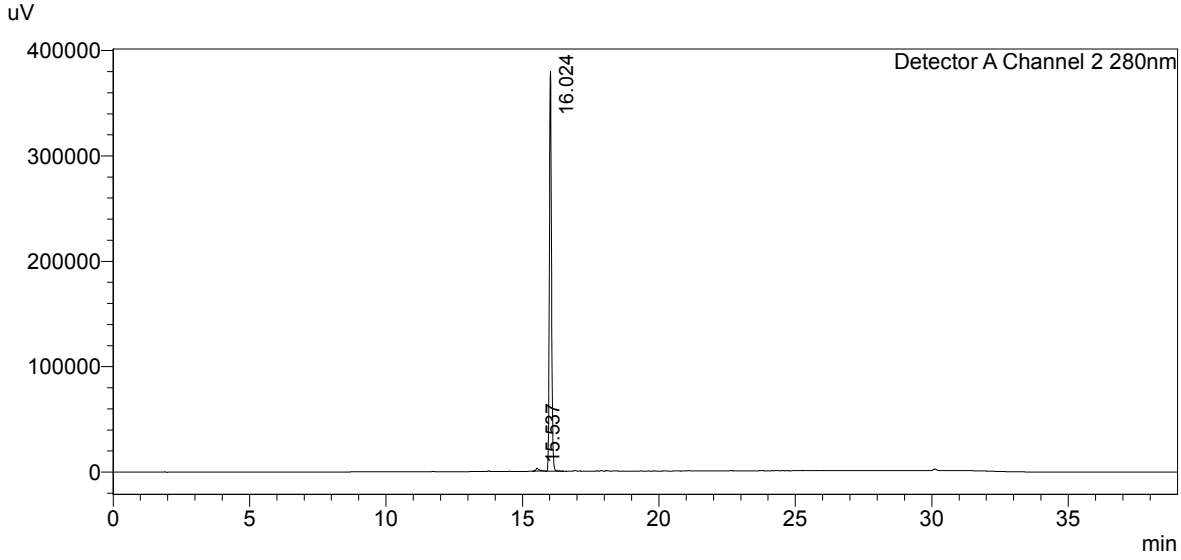


<Peak Table>

Detector A Channel 2 280nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	11.741	163388	34264	1.409		M	
2	12.333	11385163	1958353	98.158		M	
3	12.872	50321	12001	0.434		M	
Total		11598872	2004618				

(2*S*)-2-Amino-3-(9′-fluoro-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4c)

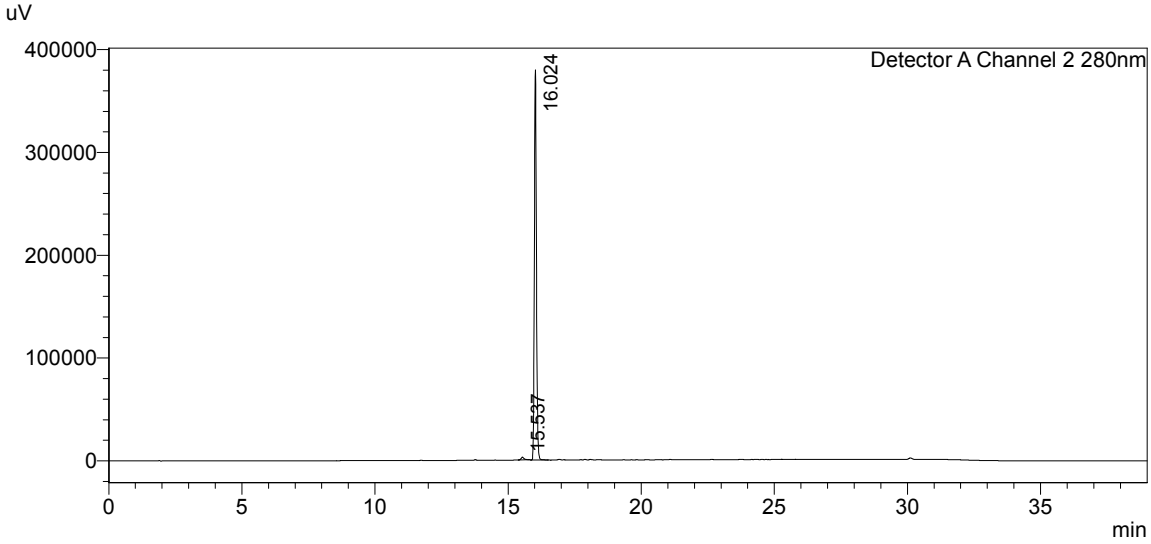


<Peak Table>

Detector A Channel 2 280nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	15.537	21190	2846	1.027		M	
2	16.024	2041210	379340	98.973		M	
Total		2062401	382186				

(2*S*)-2-Amino-3-(8′,9′-dimethoxy-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4d)

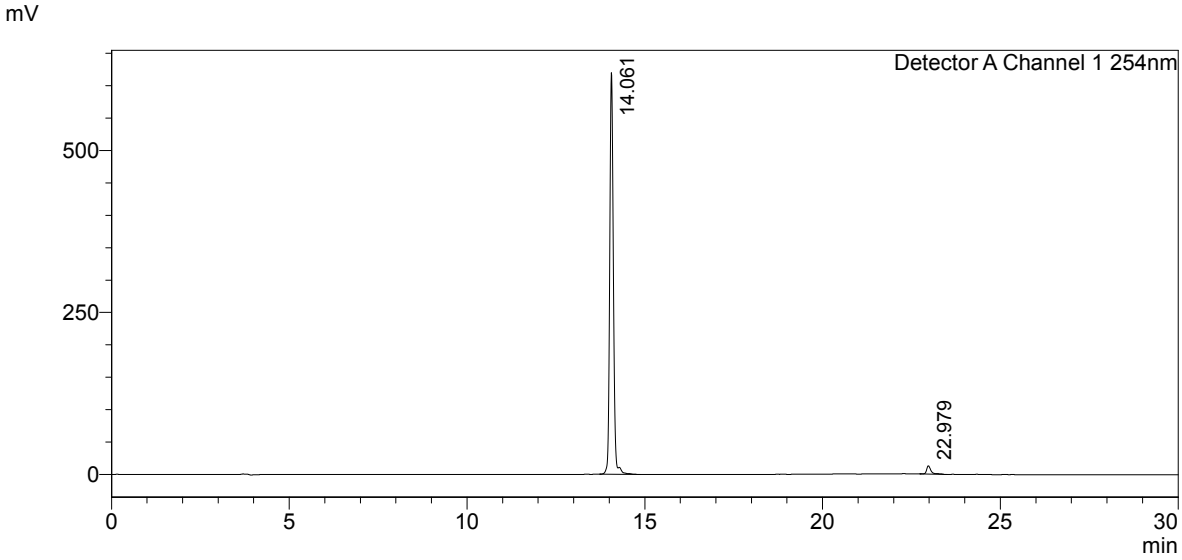


<Peak Table>

Detector A Channel 2 280nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	15.537	21190	2846	1.027		M	
2	16.024	2041210	379340	98.973		M	
Total		2062401	382186				

(2*S*)-2-Amino-3-(8′-dimethylamino-5′-oxo-5′,6′-dihydropyrazolo[1′,5′-c]quinazolin-2′-yl)propanoic acid hydrochloride (4e)



<Peak Table>

Detector A Channel 1 254 nm

Peak#	Ret. Time	Area	Height	Conc.
1	14.061	4444601	619320	97.552
2	22.979	111524	12680	2.448
Total		4556125	632000	