Supplementary Data for

Synthesis and biological evaluation of novel 2,3-dihydro-1*H*-1,5-benzodiazepin-2-ones; potential imaging agents of the metabotropic glutamate 2 receptor

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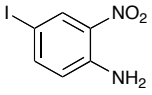
1. General Experimental

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. Brine refers to a saturated solution of sodium chloride in distilled water. 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 were visualised by staining with potassium permanganate. ^{1}H 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), residual chloroform (δ_H 7.26 and δ_C 77.16), dimethylsulfoxide (δ_H 2.50 and δ_C 39.52), or methanol (δ_H 3.31 and δ_C 49.00) as standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Infrared spectra were obtained neat using a SHIMADZU spectrometer. Mass spectra were obtained using a JEOL JMS-700 or Bruker Microtof-q spectrometer. Melting points were determined on a Gallenkamp melting point apparatus.

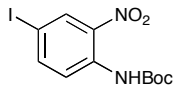
2. Experimental Procedures and Spectroscopic Data For All Compounds

4-Iodo-2-nitroaniline (8)[1]



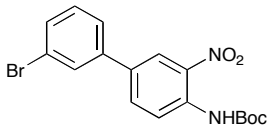
Iodine monochloride (1.0 M in dichloromethane) (19.0 mL, 19.0 mmol) was added to a solution of 2-nitroaniline (**7**) (2.50 g, 18.1 mmol) and sodium acetate (1.56 g, 19.0 mmol) in acetic acid (20 mL). The reaction mixture was stirred at 90 °C for 0.5 h before being allowed to cool to room temperature and poured on to ice water. A precipitate formed that was collected by filtration to give 4-iodo-2-nitroaniline (**8**) as an orange solid (4.20 g, 88%). Mp 117−119 °C (lit.,[1] 118−119 °C); δ_H (400 MHz, $CDCl_3$) 6.11 (1H, br s, NH_2), 6.61 (1H, d, *J* 8.8 Hz, 6-H), 7.57 (1H, dd, *J* 8.8, 1.9 Hz, 5-H), 8.44 (1H, d, *J* 1.9 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 75.9 (C), 120.6 (CH), 133.2 (C), 134.4 (CH), 143.8 (CH), 144.0 (C); *m/z* (CI) 265 (MH^+, 56%), 235 (24), 186 (10), 158 (24), 139 (32), 79 (98).

1-(*tert*-Butoxycarbonyl)amino-4-iodo-2-nitrobenzene (9)[2]



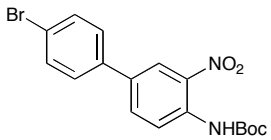
To a solution of 4-iodo-2-nitroaniline (**8**) (3.54 g, 13.4 mmol) in dichloromethane (150 mL) was added di-*tert*-butyl dicarbonate (6.44 g, 29.5 mmol), 4-dimethylaminopyridine (0.330 g, 2.68 mmol) and triethylamine (4.11 mL, 29.5 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (100 mL) and extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then purified by dry flash chromatography eluting with 30% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene as a white solid (4.35 g, 70%). Mp 121−123 °C; ν_{max}/cm^{-1} (neat) 2984 (CH), 1763 (CO), 1742 (CO), 1525, 1346, 1273, 1150, 1001, 827; δ_H (400 MHz, $CDCl_3$) 1.41 (18H, s, 6 × CH_3), 7.06 (1H, d, *J* 8.3 Hz, 6-H), 7.95 (1H, dd, *J* 8.3, 2.0 Hz, 3-H), 8.37 (1H, d, *J* 2.0 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 27.9 (6 × CH_3), 84.2 (C), 92.3 (C), 132.8 (CH), 133.2 (C), 133.8 (CH), 142.8 (CH), 146.0 (C), 150.0 (C); *m/z* (EI) 464.0443 (M^+. $C_{16}H_{21}IN_2O_6$ requires 464.0444), 364 (30%), 308 (100), 264 (74), 216 (37). To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (1.17 g, 2.52 mmol) in dichloromethane (30.0 mL) at 0 ºC was added trifluoroacetic acid (0.39 mL, 5.04 mmol). The mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with a saturated solution of sodium hydrogen carbonate (20 mL), extracted with dichloromethane (3 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**9**) as a yellow solid (0.90 g, 98%). Mp 96−98 °C (lit.,[2] 92−94 °C); ν_{max}/cm^{-1} (neat) 3364 (NH), 2970 (CH), 1728 (CO), 1566, 1497, 1335, 1242, 1142; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 7.85 (1H, dd, *J* 9.0, 1.9 Hz, 5-H), 8.37 (1H, d, *J* 9.0 Hz, 6-H), 8.50 (1H, d, *J* 1.9 Hz, 3-H), 9.61 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 82.5 (C), 83.1 (C), 122.5 (CH), 134.2 (CH), 135.9 (C), 136.4 (C), 144.3 (CH), 152.0 (C); *m/z* (EI) 363.9921 (M^+. $C_{11}H_{13}IN_2O_4$ requires 363.9920), 308 (16%), 264 (23), 84 (81), 57 (100).

4-(3'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (10)



To a solution of 1-(*tert*-butoxycarbonylamino)-2-nitro-4-iodobenzene (**9**) (1.50 g, 3.81 mmol) in DMF and water (100 mL, 9:1) was added 3-bromophenylboronic acid (0.820 g, 4.12 mmol), potassium carbonate (1.42 g, 10.3 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.092 g, 0.08 mmol). The reaction mixture was heated to 110 ºC and stirred for 1.5 h. After cooling to room temperature, the solution was concentrated *in vacuo*, redissolved in chloroform (100 mL), filtered through Celite® and concentrated *in vacuo*. The resulting solid was dissolved in diethyl ether (100 mL), washed with water (6 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 10% ethyl acetate in petroleum ether (40–60) to give 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**10**) as a yellow solid (1.16 g, 72%). Mp 107−109 °C; ν_{max}/cm^{-1} 3345 (NH), 2986, (CH), 1732 (CO), 1577 (C=C), 1522, 1340, 1248, 1152, 892; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 7.34 (1H, t, *J* 7.9 Hz, 5'-H), 7.50−7.54 (2H, m, 4'-H and 6'-H), 7.73 (1H, t, *J* 1.8 Hz, 2'-H), 7.81 (1H, dd, *J* 8.9, 2.3 Hz, 5-H), 8.39 (1H, d, *J* 2.3 Hz, 3-H), 8.66 (1H, d, *J* 8.9 Hz, 6-H), 9.69 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 82.3 (C), 121.4 (CH), 123.4 (C), 124.0 (CH), 125.5 (CH), 129.9 (CH), 130.8 (CH), 131.2 (CH), 133.6 (C), 134.2 (CH), 135.6 (C), 136.2 (C), 140.4 (C), 152.3 (C); *m/z* (EI) 392.0363 (M^+. $C_{17}H_{17}{}^{79}BrN_2O_4$ requires 392.0372), 336 (32%), 292 (78), 246 (19), 167 (43), 57 (99).

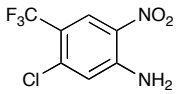
4-(4'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (11)



The reaction was carried out as described above using 1-(*tert*-butoxycarbonylamino)-2-nitro-4-iodobenzene (**9**) (3.00 g, 8.24 mmol), 4-bromophenylboronic acid (1.74 g, 8.65 mmol), potassium carbonate (2.85 g, 20.6 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.18 g, 0.16 mmol) in DMF:water (125 mL, 9:1) to give 4-(4'-bromophenyl)-1-(*tert*-

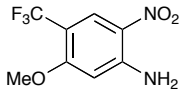
butoxycarbonylamino)-2-nitrobenzene (**11**) as a yellow solid (2.51 g, 78%). Mp 134−136 °C; ν_{max}/cm^{-1} (neat) 3366 (NH), 2970 (CH), 1730 (CO), 1489, 1341, 1244, 1143, 812; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 7.46 (2H, d, *J* 8.4 Hz, 2'-H and 6'-H), 7.60 (2H, d, *J* 8.4 Hz, 3'-H and 5'-H), 7.81 (1H, dd, *J* 8.9, 2.1 Hz, 5-H), 8.39 (1H, d, *J* 2.1 Hz, 3-H), 8.65 (1H, d, *J* 8.9 Hz, 6-H), 9.69 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.2 (3 × CH_3), 82.1 (C), 121.3 (CH), 122.4 (C), 123.6 (CH), 128.2 (2 × CH), 132.2 (2 × CH), 133.8 (C), 133.9 (CH), 135.2 (C), 136.1 (C), 137.0 (C), 152.1 (C); *m/z* (EI) 392.0367 (M^+. $C_{17}H_{17}{}^{79}BrN_2O_4$ requires 392.0372), 336 (40%), 292 (59), 246 (22), 167 (21), 139 (24), 57 (100).

5-Chloro-2-nitro-4-(trifluoromethyl)aniline (13)[3]



A solution of 1,5-dichloro-2-nitro-4-(trifluoromethyl)benzene (**12**) (4.80 g, 18.5 mmol) in 1,4-dioxane (10 mL) was saturated with ammonia gas. The reaction vessel was sealed and heated to 100 °C for 4 days. The solution was again saturated with ammonia gas, the reaction vessel sealed and heated to 100 °C for a further 2 days. The reaction mixture was allowed to cool to room temperature, then poured on to water (100 mL) and stirred until a yellow precipitate formed. The precipitate was collected by filtration to give 5-chloro-2-nitro-4-(trifluoromethyl)aniline (**13**) as a yellow solid (3.34 g, 76%). Mp 111–112 °C (lit.,[3] 113–114 °C); δ_H (400 MHz, $CDCl_3$) 6.39 (2H, br s, NH_2), 6.97 (1H, s, 6-H), 8.50 (1H, s, 3-H); δ_C (126 MHz, $CDCl_3$) 117.3 (q, $J_{C\text{-}C\text{-}F}$ 33.4 Hz, C), 120.6 (CH), 122.3 (q, $J_{C\text{-}F}$ 271.8 Hz, C), 127.0 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz, CH), 129.6 (C), 139.1 (C), 146.5 (C); *m/z* (EI) 240.0 (M^+, 62%), 210 (12), 194 (35), 182 (16), 132 (13), 83 (100).

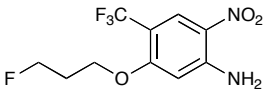
5-Methoxy-2-nitro-4-(trifluoromethyl)aniline (14)



5-Chloro-2-nitro-4-(trifluoromethyl)aniline (**13**) (2.30 g, 9.56 mmol) was added to a solution of potassium hydroxide (1.18 g, 21.0 mmol) in DMSO (10 mL) and methanol (10 mL). The reaction mixture was heated to 60 °C and stirred for 5 h. After cooling to room temperature, the reaction was poured on to 1 M hydrochloric acid (40 mL) and extracted with ethyl acetate

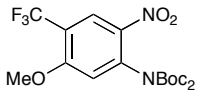
(3 × 30 mL). The combined organic extracts were then washed with water (3 × 30 mL) and brine (2 × 30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 5-methoxy-2-nitro-4-(trifluoromethyl)aniline (**14**) as a yellow solid (2.19 g, 97%). Mp 136–138 °C; ν_{max}/cm^{-1} (neat) 3341 (NH), 1636 (C=C), 1566, 1420, 1327, 1234, 1111, 918, 841; δ_H (400 MHz, $CDCl_3$) 3.92 (3H, s, OCH_3), 6.21 (1H, s, 6-H), 6.46 (2H, br s, NH_2), 8.41 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 56.5 (CH_3), 99.0 (CH), 109.8 (q, $J_{C\text{-}F}$ 32.9 Hz, C), 125.2 (C), 122.8 (q, $J_{C\text{-}F}$ 271.2 Hz, C), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$, 5.7 Hz, CH), 149.0 (C), 162.4 (C); *m/z* (EI) 236.0406 (M^+. $C_8H_7F_3N_2O_3$ requires 236.0409), 217 (22%), 206 (82), 190 (38), 178 (24), 147 (30), 83 (68).

5-(3'-Fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (15)



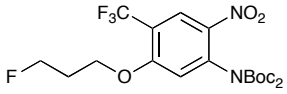
To a solution of 5-chloro-2-nitro-4-(trifluoromethyl)aniline (**13**) (1.00 g, 4.16 mmol) in DMSO (2.00 mL) was added 3-fluoropropan-1-ol (3.13 mL, 41.6 mmol) and potassium carbonate (1.15 g, 8.32 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h. Potassium carbonate (1.15 g, 8.32 mmol) was added and the mixture stirred for a further 48 h at 90 °C. The reaction mixture was then diluted with 1 M hydrochloric acid (80 mL) and extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified using dry flash chromatography on silica eluting with 30% ethyl acetate in petroleum ether (40–60) to give 5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (**15**) as a yellow solid (0.88 g, 71%). Mp 148–149 °C; (Found: C, 42.49; H, 3.47; N, 9.81. $C_{10}H_{10}F_4N_2O_3$ requires C, 42.56; H, 3.58; N, 9.93%); ν_{max}/cm^{-1} (neat) 3480 (NH), 3354 (NH), 1641 (C=C), 1571, 1306, 1245, 1115, 1034, 914, 851; δ_H (400 MHz, $CDCl_3$) 2.22 (2H, dquin, *J* 26.4, 5.7 Hz, 2'-H_2), 4.18 (2H, t, *J* 5.7 Hz, 1'-H_2), 4.65 (2H, dt, *J* 46.9, 5.7 Hz, 3'-H_2), 6.22 (1H, s, 6-H), 6.45 (2H, br s, NH_2), 8.42 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 30.1 (d, $J_{C\text{-}C\text{-}F}$ 20.2 Hz, CH_2), 64.9 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.9 Hz, CH_2), 80.1 (d, $J_{C\text{-}F}$ 164.9 Hz, CH_2), 99.6 (CH), 109.9 (q, $J_{C\text{-}C\text{-}F}$ 33.2 Hz, C), 122.9 (q, $J_{C\text{-}F}$ 269.5 Hz, C), 125.4 (C), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH), 148.8 (C), 161.5 (C); *m/z* (CI) 283 (MH^+, 100%), 253 (18), 233 (8), 113 (16), 85 (34).

1-[Bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl)benzene



To a solution of 5-methoxy-2-nitro-4-(trifluoromethyl)aniline (**14**) (3.27 g, 13.8 mmol) in dichloromethane (150 mL) was added di-*tert*-butyl dicarbonate (6.66 g, 30.5 mmol), 4-dimethylaminopyridine (0.340 g, 2.76 mmol) and triethylamine (4.25 mL, 30.5 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (100 mL) and extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then purified by dry flash chromatography eluting with 30% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl)benzene as a white solid (5.70 g, 95%). Mp 123–125 °C; ν_{max}/cm^{-1} (neat) 2986 (CH), 1805 (CO), 1620 (C=C), 1589, 1528, 1319, 1242, 1150, 1096, 849; δ_H (400 MHz, $CDCl_3$) 1.44 (18H, s, 6 × CH_3), 4.01 (3H, s, OCH_3), 6.91 (1H, s, 6-H), 8.43 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 28.0 (6 × CH_3), 57.2 (CH_3), 84.6 (C), 114.8 (CH), 118.9 (q, $J_{C\text{-}C\text{-}F}$ 33.1 Hz, C), 122.1 (q, $J_{C\text{-}F}$ 273.1 Hz, C), 125.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 138.0 (C), 139.0 (C), 150.2 (C), 161.1 (C); *m/z* (EI) 336.0925 ($M^+ - C_5H_8O_2$. $C_{13}H_{15}F_3N_2O_5$ requires 336.0933), 280 (8%), 236 (12), 83 (90), 57 (100).

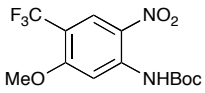
1-[Bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene



The reaction was performed according to the above procedure using 5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (**14**) (1.33 g, 4.71 mmol), di-*tert*-butyl dicarbonate (2.26 g, 10.4 mmol), 4-dimethylaminopyridine (0.12 g, 0.94 mmol) and triethylamine (1.44 mL, 10.4 mmol) in dichloromethane (65 mL). The material was then purified by dry flash chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene as a white solid (1.86 g, 82%). Mp 84–86 °C; (Found: C, 49.84; H, 5.43; N, 5.87. $C_{20}H_{26}F_4N_2O_7$ requires C, 49.79; H, 5.43; N, 5.81%); ν_{max}/cm^{-1} (neat) 2982 (CH), 1794 (CO), 1620 (C=C), 1586, 1523, 1327, 1254, 1138, 1099, 918, 849; δ_H (400 MHz, $CDCl_3$) 1.44 (18H, s, 6 × CH_3), 2.26 (2H, dquin, *J* 26.3, 5.8 Hz, 2'-H_2), 4.27 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.66 (2H, dt, *J* 46.9, 5.8 Hz,

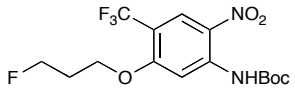
3'-H_2), 6.91 (1H, s, 6-H), 8.43 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 28.0 (6 × CH_3), 30.1 (d, $J_{C\text{-}C\text{-}F}$ 20.1 Hz, CH_2), 65.6 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.7 Hz, CH_2), 79.9 (d, $J_{C\text{-}F}$ 165.2 Hz, CH_2), 84.7 (C), 115.4 (CH), 119.0 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.2 (q, $J_{C\text{-}F}$ 272.9 Hz, C), 125.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 138.2 (C), 139.0 (C), 150.2 (C), 160.2 (C); *m/z* (CI) 383 (MH^+–$C_5H_8O_2$, 37%), 353 (24), 327 (98), 283 (61), 253 (30).

1-(*tert*-Butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (16)



To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (1.30 g, 2.98 mmol) in dichloromethane (40 mL) at 0 ºC was added trifluoroacetic acid (0.46 mL, 5.96 mmol). The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with a saturated solution of sodium hydrogen carbonate (30 mL) and extracted with dichloromethane (3 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (**16**) as a yellow solid (1.00 g, 100%). Mp 108–109 °C; ν_{max}/cm^{-1} (neat) 3395 (NH), 1636 (CO), 1481, 1335, 1258, 1134, 964, 856; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, 3 × CH_3), 4.03 (3H, s, CH_3), 8.38 (1H, s, 6-H), 8.50 (1H, s, 3-H), 10.20 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.0 (3 × CH_3), 56.7 (CH_3), 82.7 (C), 101.7 (CH), 112.5 (q, $J_{C\text{-}C\text{-}F}$ 33.1 Hz, C), 122.3 (q, $J_{C\text{-}F}$ 271.9 Hz, C), 126.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.5 Hz, CH), 127.4 (C), 141.5 (C), 151.8 (C), 162.6 (C); *m/z* 336.0929 (M^+. $C_{13}H_{15}F_3N_2O_5$ requires 336.0933), 280 (8%), 236 (24), 206 (9), 83 (100).

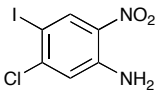
1-(*tert*-Butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (17)



The reaction was carried out according to the above procedure using 1-[bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (1.55 g, 3.21 mmol) and trifluoroacetic acid (0.49 mL, 6.42 mmol) in dichloromethane (40 mL) to give 1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**17**) as a yellow solid (1.20 g, 98%). Mp 75–76 °C; (Found: C, 47.13; H, 4.71; N, 7.21.

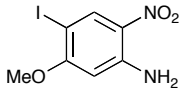
$C_{15}H_{18}F_4N_2O_5$ requires C, 47.12; H, 4.75; N, 7.33%); ν_{max}/cm^{-1} (neat) 3356 (NH), 2988 (CH), 1743 (CO), 1631 (C=C), 1580, 1440, 1343, 1233, 1133, 1049, 924, 849; δ_H (400 MHz, $CDCl_3$) 1.55 (9H, s, 3 × CH_3), 2.24 (2H, dquin, *J* 25.8, 5.8 Hz, 2'-H_2), 4.33 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.66 (2H, dt, *J* 46.9, 5.8 Hz, 3'-H_2), 8.39 (1H, s, 6-H), 8.50 (1H, s, 3-H), 10.19 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 30.0 (d, $J_{C\text{-}C\text{-}F}$ 20.4 Hz, CH_2), 65.4 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.0 Hz, CH_2), 80.0 (d, $J_{C\text{-}F}$ 165.2 Hz, CH_2), 82.9 (C), 102.5 (CH), 112.9 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.5 (q, $J_{C\text{-}F}$ 271.8 Hz, C), 126.6 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 127.8 (C), 141.6 (C), 152.0 (C), 162.0 (C); *m/z* (EI) 382 (M^+, 3%), 282 (23), 222 (17), 131 (6), 57 (100).

5-Chloro-4-iodo-2-nitroaniline (19)[4]



Iodine monochloride (1.0 M in dichloromethane) (45.7 mL, 45.7 mmol) was added to a solution of 5-chloro-2-nitroaniline (**18**) (7.00 g, 40.6 mmol) and sodium acetate (3.75 g, 45.7 mmol) in acetic acid (35 mL). The reaction mixture was heated to 80 °C and stirred for 3 h. The mixture was then allowed to cool to room temperature and the remaining dichloromethane removed *in vacuo*. The resulting residue was poured onto ice water with stirring and the precipitate formed was collected by filtration to give 5-chloro-4-iodo-2-nitroaniline (**19**) as an orange solid (11.1 g, 92%). Mp 200–201 °C (lit.,[4] 202−203 °C); ν_{max}/cm^{-1} (neat) 3350 (NH), 1609 (C=C), 1543, 1478, 1309, 1234, 1124, 892; δ_H (400 MHz, DMSO-d_6) 7.26 (1H, s, 6-H), 7.59 (2H, br s, NH_2), 8.36 (1H, s, 3-H); δ_C (101 MHz, DMSO-d_6) 79.0 (C), 118.5 (CH), 130.3 (C), 136.0 (CH), 143.8 (C) 146.0 (C); *m/z* (EI) 297.8999 (M^+. $C_6H_4{}^{35}ClIN_2O_2$ requires 297.9006), 268 (10%), 252 (32), 225 (9), 172 (4), 125 (37).

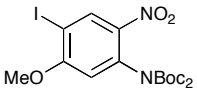
4-Iodo-5-methoxy-2-nitroaniline (20)[4]



5-Chloro-4-iodo-2-nitroaniline (**19**) (5.00 g, 16.8 mmol) was added to a solution of potassium hydroxide (2.08 g, 37.0 mmol) in DMSO (17 mL) and methanol (17 mL). The mixture was heated to 60 °C and stirred for 6 h. After cooling to room temperature, the solution was poured onto 1 M hydrochloric acid (100 mL) and extracted with ethyl acetate (3 × 70 mL). The organic extracts were combined, washed with 1 M hydrochloric acid (6 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 4-iodo-5-methoxy-2-

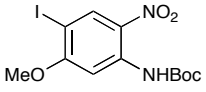
nitroaniline (**20**) as an orange solid (4.66 g, 94%). Mp 182–183 °C (lit.,[4] 189 °C); ν_{max}/cm^{-1} (neat) 3340 (NH), 1639 (C=C), 1571, 1332, 1233, 1107, 921, 840; δ_H (400 MHz, $CDCl_3$) 3.90 (3H, s, OCH_3), 6.11 (1H, s, 6-H), 6.25 (2H, br s, NH_2), 8.55 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 56.8 (CH_3), 71.2, (C), 97.7 (CH), 127.9 (C), 137.1 (CH), 146.8 (C), 163.1 (C); *m/z* (EI) 293.9504 (M^+. $C_7H_7IN_2O_3$ requires 293.9501), 264 (47%), 248 (53), 121 (48), 106 (50).

1-[Bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene



To a solution of 4-iodo-5-methoxy-2-nitroaniline (**20**) (2.95 g, 10.0 mmol) in dichloromethane (135 mL) was added di-*tert*-butyl dicarbonate (4.80 g, 22.0 mmol), 4-dimethylaminopyridine (0.240 g, 2.00 mmol) and triethylamine (3.07 mL, 22.0 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (100 mL), extracted with dichloromethane (3 × 70 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then purified by dry flash chromatography eluting with 0–50% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene as a white solid (4.76 g, 96%). Mp 186–188 °C; ν_{max}/cm^{-1} (neat) 2977 (CH), 1793 (CO), 1570 (C=C), 1515, 1339, 1282, 1229, 1153, 1098, 1009, 847; δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s, 6 × CH_3), 3.97 (3H, s, OCH_3), 6.66 (1H, s, 6-H), 8.60 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 27.9 (6 × CH_3), 57.4 (CH_3), 84.2 (C), 84.3 (C), 112.4 (CH), 135.8 (C), 136.3 (CH), 139.1 (C), 150.2 (C), 162.4 (C); *m/z* (EI) 494.0552 (M^+. $C_{17}H_{23}IN_2O_7$ requires 494.0550), 394 (12%), 338 (93), 321 (10), 294 (58), 246 (10), 61 (28), 43 (100).

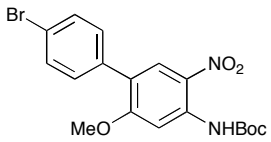
1-(*tert*-Butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (21)



To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene (1.14 g, 2.31 mmol) in dichloromethane (30 mL) at 0 ºC was added trifluoroacetic acid (0.35 mL, 4.62 mmol). The reaction mixture was then warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with a saturated solution of sodium hydrogen carbonate (30 mL)

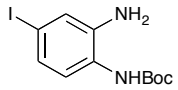
and extracted with dichloromethane (3 × 40 mL). The organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (**21**) as a yellow solid (0.91 g, 100%). Mp 193–194 °C; ν_{max}/cm^{-1} (neat) 3347 (NH), 2989 (CH), 1719 (CO), 1572 (C=C), 1430, 1330, 1229, 1042, 845; δ_H (400 MHz, $CDCl_3$) 1.55 (9H, s, 3 × CH_3), 4.00 (3H, s, OCH_3), 8.20 (1H, s, 6-H), 8.65 (1H, s, 3-H), 10.06 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 57.4 (CH_3), 76.1 (C), 82.4 (C), 100.6 (CH), 130.0 (C), 136.9 (CH), 139.1 (C), 152.3 (C), 163.9 (C); *m/z* (EI) 394.0029 (M^+. $C_{12}H_{15}IN_2O_5$ requires 394.0026), 338 (45%), 294 (50), 248 (10), 168 (9), 83 (58), 44 (100).

4-(4'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (22)



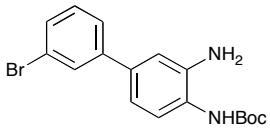
The reaction was carried out as described for the synthesis of **10** and **11** using 1-(*tert*-butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (**21**) (2.00 g, 5.07 mmol), 4-bromophenylboronic acid (1.02 g, 5.07 mmol), potassium carbonate (1.40 g, 10.1 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.116 g, 0.101 mmol) in DMF:water (100 mL, 9:1) to give 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**22**) as a yellow solid (1.41 g, 66%). Mp 159–160 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2986 (CH), 1735 (CO), 1620 (C=C), 1574, 1443, 1273, 1142, 1018, 849; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 3.96 (3H, s, OCH_3), 7.37 (1H, d, *J* 8.5 Hz, 2'-H and 6'-H), 7.54 (1H, d, *J* 8.5 Hz, 3'-H and 5'-H), 8.20 (1H, s, 3-H), 8.30 (1H, s, 6-H), 10.15 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 56.6 (CH_3), 82.2 (C), 101.4 (CH), 122.1 (C), 124.0 (C), 128.1 (CH), 129.0 (C), 131.1 (2 × CH), 131.6 (2 × CH), 134.8 (C), 138.5 (C), 152.4 (C), 162.5 (C); *m/z* (EI) 422.0482 (M^+. $C_{18}H_{19}{}^{79}BrN_2O_5$ requires 422.0477), 366 (33%), 322 (36), 292 (6), 197 (13), 154 (8), 126 (8), 105 (8), 57 (100).

2-Amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (23)[2]



To a solution of 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**9**) (0.20 g, 0.55 mmol) in ethanol (17 mL) was added tin(II) chloride dihydrate (0.62 g, 2.75 mmol). The reaction mixture was heated to 70 °C and stirred for 3 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The resulting solid was dissolved in ethyl acetate (20 mL) and a saturated solution of sodium hydrogen carbonate (10 mL) was added. The mixture was then extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 2-amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (**23**) as an off-white solid (0.17 g, 91%). Mp 140−141 °C (lit.,[2] 127−130 °C); ν_{max}/cm^{-1} (neat) 3349 (NH), 2922 (CH), 1678 (CO), 1587 (C=C), 1506, 1489, 1410, 1248, 1157, 1051, 853; δ_H (400 MHz, $CDCl_3$) 1.50 (9H, s, 3 × CH_3), 3.74 (2H, br s, NH_2), 6.18 (1H, br s, NH), 7.01 (1H, d, *J* 8.2 Hz, 6-H), 7.05–7.11 (2H, m, 3-H and 5-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 80.9 (C), 89.8 (C), 124.7 (C), 126.0 (CH), 126.1 (CH), 128.5 (CH), 141.3 (C), 153.6 (C); *m/z* (CI) 335.0265 (MH^+. $C_{11}H_{16}IN_2O_2$ requires 335.0257), 311 (10%), 279 (100), 209 (22), 153 (43), 113 (17), 69 (64).

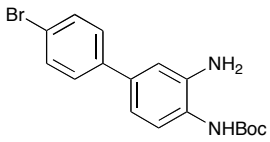
2-Amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (24)



To a solution of 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**10**) (0.680 g, 1.73 mmol) in ethyl acetate and pyridine (70 mL, 6:1) was added tin(II) chloride dihydrate (1.95 g, 8.65 mmol), and the mixture stirred at room temperature for 6 h. The reaction mixture was then filtered through Celite® and concentrated *in vacuo*. The resulting solid was redissolved in ethyl acetate (100 mL), washed with water (4 × 50 mL) and brine (2 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–50% ethyl acetate in petroleum ether (40−60) to give 2-amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**24**) as a white solid (0.452 g, 72%). Mp 146–149 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2925 (CH), 1685 (CO), 1503, 1247, 1158, 1058, 862; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.87 (2H br s, NH_2),

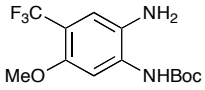
6.25 (1H, br s, NH), 6.96 (1H, d, *J* 1.8 Hz, 3-H), 6.99 (1H, dd, *J* 8.1, 1.8 Hz, 5-H), 7.27 (1H, t, *J* 7.9 Hz, 5'-H), 7.37 (1H, d, *J* 8.1 Hz, 6-H), 7.42–7.47 (2H, m, 4'-H and 6'-H), 7.67 (1H, t, *J* 1.7 Hz, 2'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 80.9 (C), 116.3 (CH), 118.7 (CH), 122.9 (C), 124.9 (CH), 124.9 (C), 125.7 (CH), 130.1 (2 × CH), 130.3 (CH), 137.6 (C), 140.1 (C), 143.1 (C), 153.9 (C); *m/z* (EI) 362.0630 (M^+. $C_{17}H_{19}{}^{79}BrN_2O_2$ requires 362.0630), 306 (14%), 289 (20), 262 (99), 234 (13), 181 (15), 154 (19), 139 (16), 127 (11), 91 (10), 57 (66).

2-Amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (25)



The reaction was carried out according to the above procedure using 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**11**) (1.82 g, 4.63 mmol) and tin(II) chloride dihydrate (5.22 g, 23.1 mmol) in ethyl acetate and pyridine (175 mL, 6:1) to give 2-amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**25**) as a white solid (1.06 g, 63%). Mp 194–196 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2978 (CH), 1686 (CO), 1502, 1248, 1163, 1057, 805; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.84 (2H, br s, NH_2), 6.25 (1H, br s, NH), 6.95 (1H, d, *J* 2.0 Hz, 3-H), 6.98 (1H, dd, *J* 8.1, 2.0 Hz, 5-H), 7.36 (1H, d, *J* 8.1 Hz, 6-H), 7.39 (2H, d, *J* 8.5 Hz, 2'-H and 6'-H), 7.52 (2H, d, *J* 8.5 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 80.9 (C), 116.2 (CH), 118.5 (CH), 121.4 (C), 124.7 (C), 125.0 (CH), 128.7 (2 × CH), 131.9 (2 × CH), 138.0 (C), 139.9 (C), 140.1 (C), 153.9 (C); *m/z* (EI) 362.0626 (M^+. $C_{17}H_{19}{}^{79}BrN_2O_2$ requires 362.0630), 306 (15%), 262 (95), 234 (16), 181 (12), 154 (19), 83 (58).

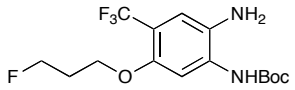
2-Amino-1-(*tert*-butoxycarbonylamino)-5-methoxy-4-(trifluoromethyl)benzene (26)



The reaction was carried out as described above using 1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (**16**) (0.88g, 2.62 mmol) and tin(II) chloride dihydrate (2.96 g, 13.1 mmol) and stirred at room temperature for 18 h. This gave 2-amino-1-(*tert*-butoxycarbonylamino)-5-methoxy-4-(trifluoromethyl)benzene (**26**) as a white solid (0.68

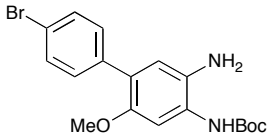
g, 85%). Mp 151–152 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 2986 (CH), 1690 (CO), 1528, 1497, 1427, 1296, 1111, 1057, 887; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.16 (2H, br s, NH_2), 3.87 (3H, s, OCH_3), 6.92 (1H, br s, NH), 7.04 (1H, s, 3-H), 7.58 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 56.6 (CH_3), 81.5 (C), 105.4 (CH), 113.5 (q, $J_{C\text{-}C\text{-}F}$ 31.0 Hz, C), 119.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.7 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 126.0 (C), 133.9 (C), 153.0 (C), 153.5 (C); *m/z* (EI) 306.1192 (M^+. $C_{13}H_{17}F_3N_2O_3$ requires 306.1191), 250 (52%), 232 (14), 206 (70), 191 (42), 163 (15), 134 (13), 83 (100), 57 (98).

2-Amino-1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-4-(trifluoromethyl)benzene (27)



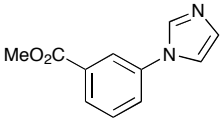
The reaction was carried out according to the above procedure using 1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**17**) (1.13 g, 2.96 mmol) and tin(II) chloride dihydrate (3.34 g, 14.8 mmol) in ethyl acetate and pyridine (12 mL, 6:1). The crude material was purified using flash column chromatography eluting with 0–30% ethyl acetate in petroleum ether (40–60) to give 2-amino-1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-4-(trifluoromethyl)aniline (**27**) as a white solid (0.89 g, 83%). Mp 121–122 °C; (Found: C, 51.02; H, 5.69; N, 7.81. $C_{15}H_{20}F_4N_2O_3$ requires C, 51.13; H, 5.72; N, 7.95%); ν_{max}/cm^{-1} (neat) 3358 (NH), 2994 (CH), 1690 (CO), 1526, 1499, 1441, 1296, 1219, 1111, 1059, 957, 880; δ_H (400 MHz, $CDCl_3$) 1.52 (9H, s, 3 × CH_3), 2.16 (2H, dquin, *J* 25.5, 5.8 Hz, 2'-H_2), 3.18 (2H, br s, NH_2), 4.13 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.64 (2H, dt, *J* 47.0, 5.8 Hz, 3'-H_2), 6.92 (1H, br s, NH), 7.03 (1H, s, 3-H), 7.57 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 30.4 (d, $J_{C\text{-}C\text{-}F}$ 20.0 Hz, CH_2), 64.7 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH_2), 80.7 (d, $J_{C\text{-}F}$ 163.9 Hz, CH_2), 81.3 (C), 105.9 (CH), 113.5 (q, $J_{C\text{-}C\text{-}F}$ 31.2 Hz, C), 118.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.8 Hz, CH), 123.7 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 127.5 (C), 133.3 (C), 152.0 (C), 152.9 (C); *m/z* (CI) 353 (MH^+, 24%), 297 (100), 279 (10), 253 (38), 81 (42).

2-Amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (28)



The reaction was carried out as described above using 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**22**) (1.00 g, 2.36 mmol) and tin(II) chloride dihydrate (2.66 g, 11.8 mmol) and the reaction mixture stirred at room temperature for 17 h. The gave 2-amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (**28**) as a white solid (0.697 g, 75%). Mp 188–189 °C; ν_{max}/cm^{-1} (neat) 3364 (NH), 2978 (CH), 1682 (CO), 1597 (C=C), 1505, 1250, 1165, 1057, 833; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 3.32 (2H, br s, NH_2), 3.75 (3H, s, OCH_3), 6.69 (1H, br s, NH), 6.80 (1H, s, 3-H), 7.30 (1H, s, 6-H), 7.35 (2H, d, *J* 8.4 Hz, 2'-H and 6'-H), 7.49 (2H, *J* 8.4 Hz, 3'-H and 5-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 56.3 (CH_3), 81.1 (C), 106.7 (CH), 121.1 (C), 122.1 (CH), 125.9 (C), 128.4 (C), 128.6 (C), 131.1 (2 × CH), 131.2 (2 × CH), 136.9 (C), 151.8 (C), 153.6 (C); *m/z* (CI) 393.0819 (MH^+. $C_{18}H_{22}{}^{79}BrN_2O_3$ requires 393.0814), 337 (51%), 315 (100), 293 (28), 259 (65), 215 (27), 113 (70), 73 (80).

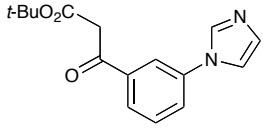
Methyl 3-(1'*H*-imidazol-1'-yl)benzoate[5]



Methyl 3-aminobenzoate (**29**) (10.0 g, 66.2 mmol) was dissolved in methanol (45 mL) and 40% glyoxyl solution (7.61 mL, 66.2 mmol) was added. The mixture was stirred at room temperature for 18 h before the addition of ammonium chloride (7.08 g, 132.4 mmol), 37% formaldehyde (10.7 mL, 132.4 mmol), and methanol (240 mL). The reaction mixture was heated under reflux and stirred for 1 h before adding 85% hydrochloric acid (9.26 mL). The mixture was heated under reflux for a further 5 h before concentrating *in vacuo*. The resulting residue was dissolved in water (300 mL), basified to pH 9 using 30% potassium hydroxide solution, and extracted with diethyl ether (4 × 300 mL). The organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 50−100% ethyl acetate in petroleum ether (40−60) to give methyl 3-(1'*H*-imidazol-1'-yl)benzoate as a brown oil (7.48 g, 58%). Spectroscopic data in accordance with literature.[5] δ_H (400 MHz, $CDCl_3$) 3.96 (3H, s, CH_3), 7.23 (1H, br s, 4'-H),

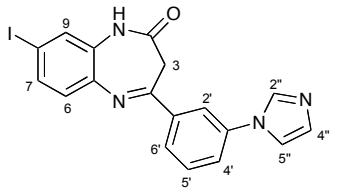
7.35 (1H, t, *J* 1.3 Hz, 5'-H), 7.54−7.62 (2H, m, 4-H and 5-H), 7.93 (1H, br s, 2'-H), 8.03 (1H, dt, *J* 7.3, 1.6 Hz, 6-H), 8.05−8.07 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 52.6 (CH_3), 118.2 (CH), 122.3 (CH), 125.5 (CH), 128.5 (CH), 130.1 (CH), 130.5 (CH), 132.1 (C), 135.5 (CH), 137.5 (C), 165.9 (C); *m/z* (EI) 202 (M^+, 100%), 171 (93), 143 (47), 116 (50), 84 (31).

tert-Butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (30)



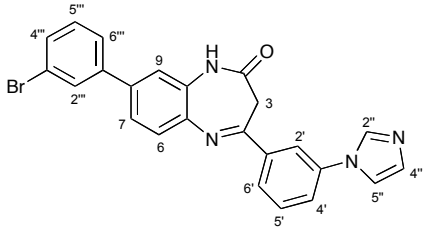
A solution of lithium hexamethyldisilazide (1.0 M in tetrahydrofuran) (41.5 mL, 41.5 mmol) in dry THF (100 mL) was cooled to –78 ºC under argon and *tert*-butyl acetate (5.57 mL, 41.5 mmol) was added. After stirring for 1 h, methyl 3-(1'*H*-imidazol-1'-yl)benzoate (3.50 g, 17.3 mmol) was added and stirring was continued at –78 °C for a further 2 h. The reaction mixture was allowed to warm to room temperature before the addition of a saturated solution of ammonium chloride (200 mL). The solution was extracted with ethyl acetate (4 × 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was purified by flash column chromatography eluting with 0−5% methanol in dichloromethane. Further purification was carried out by flash column chromatography eluting with 100% ethyl acetate to give *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**30**) as a brown oil (3.94 g, 80%). ν_{max}/cm^{-1} (neat) 3117, 2978 (CH), 1728 (CO), 1690 (CO), 1589 (C=C), 1505, 1312, 1250, 1142, 1057, 795; NMR spectra are a mixture of keto-enol tautomers (1:0.13). Signals are given for the major tautomer: δ_H (400 MHz, DMSO-d_6) 1.39 (9H, s, 3 × CH_3), 4.18 (2H, s, 2-H_2), 7.15 (1H, br s, 4"-H), 7.69 (1H, t, *J* 7.9 Hz, 5'-H), 7.84−8.01 (3H, m, 4'-H, 6'H and 5"-H), 8.15 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H); δ_C (101 MHz, DMSO-d_6) 27.6 (3 × CH_3), 47.0 (CH_2), 80.9 (C), 118.1 (CH), 120.0 (CH), 125.1 (CH), 126.4 (CH), 130.1 (CH), 130.4 (CH), 135.7 (CH), 137.2 (C), 137.4 (C), 166.7 (C), 193.2 (C); *m/z* (FAB) 287.1395 (MH^+. $C_{16}H_{19}N_2O_3$ requires 287.1396), 231 (17%), 213 (22), 187 (30), 147 (12), 73 (30).

4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-iodo-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (31)



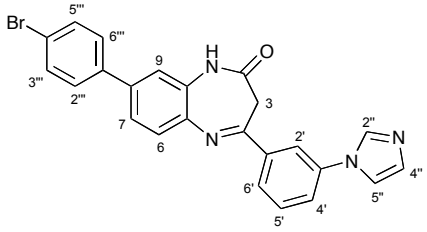
To a solution of 2-amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (**23**) (0.145 g, 0.434 mmol) in toluene (1 mL) was added *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**30**) (0.149 g, 0.520 mmol). The solution was heated under reflux for 4 h, cooled to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–4% ethanol in dichloromethane to give 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-iodobenzene as a white foam (0.158 g, 67%). To a solution of 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-iodobenzene (0.124 g, 0.227 mmol) in dichloromethane (1 mL) at 0 °C was added trifluoroacetic acid (0.3 mL). The mixture was warmed to room temperature and stirred for 0.5 h. A saturated solution of sodium hydrogen carbonate (10 mL) was added, the solution extracted with dichloromethane (2 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was then triturated with toluene to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-iodo-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**31**) as a white solid (0.056 g, 58%). Mp 238−240 °C (decomposition); ν_{max}/cm^{-1} (neat) 3074 (NH), 2082 (CH), 1679 (CO), 1587, 1502, 1311, 1229, 1054, 909; δ_H (400 MHz, DMSO-d_6) 3.65 (2H, s, 3-CH_2), 7.16 (1H, br s, 4"-H), 7.23 (1H, d, *J* 8.8 Hz, 6-H), 7.53–7.59 (2H, m, 7-H and 9-H), 7.68 (1H, t, *J* 7.9 Hz, 5'-H), 7.82–7.89 (2H, m, 4'-H and 5-"H), 8.03 (1H, br d, *J* 7.9 Hz, 6'-H), 8.22 (1H, br s, 2'-H), 8.37 (1H, br s, 2"-H), 10.64 (1H, br s NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 91.0 (C), 118.3 (CH), 119.5 (CH), 123.2 (CH), 126.1 (CH), 129.8 (CH), 130.0 (2 × CH), 130.4 (CH), 131.6 (C), 132.6 (CH), 135.8 (CH), 137.4 (C), 138.7 (C), 138.8 (C), 158.2 (C), 166.0 (C); *m/z* (EI) 428.0128 (M^+. $C_{18}H_{13}IN_4O$ requires 428.0134), 386 (84%), 359 (13), 260 (18), 193 (10), 170 (10), 78 (11).

8-(3”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (32)



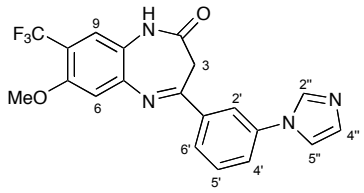
The reaction was carried out according to the above procedure, using 2-amino-4-(3’-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**24**) (0.347 g, 0.955 mmol) and *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**30**) (0.275 g, 0.960 mmol) in toluene (10 mL) to give 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene as a white foam (0.344 g, 63%). Cyclisation was performed as described above, using 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (0.080 g, 0.139 mmol) and trifluoroacetic acid (5 mL) in dichloromethane (0.5 mL) to give 8-(3”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**32**) as a white solid (0.056 g, 88%). Mp 204–205 °C (decomposition); ν_{max}/cm^{-1} (neat) 3055 (NH), 2854 (CH), 1683 (CO), 1503, 1256, 1112, 1061, 913, 863, 776; δ_H (500 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4”-H), 7.47 (1H, t, *J* 7.9 Hz, 5”’-H), 7.53 (1H, d, *J* 1.9 Hz, 9-H), 7.55 (1H, d, *J* 8.4 Hz, 6-H), 7.58–7.62 (2H, m, 7-H and 6”’-H), 7.67–7.72 (2H, m, 5’-H and 4”’-H), 7.79–7.88 (3H, m, 4’-H, 5”-H and 2”’-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.24 (1H, br s, 2’-H), 8.32 (1H, br s, 2”-H), 10.53 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 118.3 (CH), 119.6 (CH), 120.1 (CH), 122.5 (C), 122.8 (CH), 123.2 (CH), 125.7 (CH), 126.2 (CH), 128.8 (CH), 129.2 (CH), 130.1 (CH), 130.4 (CH), 130.6 (CH), 130.7 (C), 131.3 (CH), 135.9 (CH), 136.5 (C), 137.4 (C), 138.8 (C), 139.0 (C), 141.5 (C), 157.9 (C), 166.1 (C); *m/z* (ESI) 457.0644 (MH^+. $C_{24}H_{18}{}^{79}BrN_4O$ requires 457.0659).

8-(4”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (33)



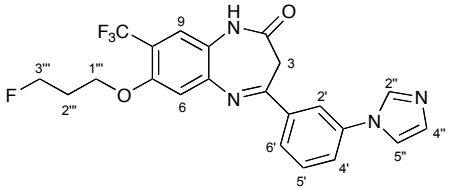
The reaction was carried out according to the procedure described above, using 2-amino-4-(4’-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**25**) (0.178 g, 0.490 mmol) and *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**30**) (0.109 g, 0.381 mmol) in toluene (10 mL) to give 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene as a white foam (0.160 g, 73%). Cyclisation was performed as described above, using 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (0.230 g, 0.400 mmol) and trifluoroacetic acid (1 mL) in dichloromethane (8 mL) to give 8-(4”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**33**) as a white solid (0.166 g, 91%). Mp 200–202 °C (decomposition); ν_{max}/cm^{-1} (neat) 3079 (NH), 2923 (CH), 1682 (CO), 1579, 1504, 1474, 1315, 1216, 1059, 812; δ_H (400 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4”-H), 7.49 (1H, d, *J* 1.9 Hz, 9-H), 7.54 (1H, d, *J* 8.4 Hz, 6-H), 7.59 (1H, dd, *J* 8.4, 1.9 Hz, 7-H), 7.62–7.73 (5H, m, 5’-H, 2”’-H, 3”’-H, 5”’-H and 6”’-H), 7.83–7.89 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.25 (1H, br s, 2’-H), 8.37 (1H, br s, 2”-H), 10.69 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 118.3 (CH), 119.5 (CH), 119.7 (CH), 121.2 (C), 122.4 (CH), 123.1 (CH), 126.1 (CH), 128.6 (2 × CH), 128.7 (CH), 130.0 (CH), 130.3 (CH), 130.6 (C), 132.0 (2 × CH), 135.8 (CH), 136.8 (C), 137.4 (C), 138.2 (C), 138.8 (2 × C), 157.7 (C), 166.0 (C); *m/z* (FAB) 457.0668 (MH^+. $C_{24}H_{18}{}^{79}BrN_4O$ requires 457.0664), 441 (34%), 219 (60), 203 (22), 169 (81), 147 (34), 84 (100).

4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-7-methoxy-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (4)



The reaction was carried out according to the procedure described above, using 2-amino-1-(*tert*-butoxycarbonylamino)-5-methoxy-4-(trifluoromethyl)benzene (**26**) (0.654 g, 2.14 mmol) and *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**30**) (0.733 g, 2.56 mmol) in toluene (4 mL) to give 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-5-methoxy-4-(trifluoromethyl)benzene as a white solid (0.723 g, 69%). Cyclisation was performed as described above, using 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-5-methoxy-4-(trifluoromethyl)benzene (0.490, 0.945 mmol) and trifluoroacetic acid (1 mL) in dichloromethane (5 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**4**) as a white solid (0.266 g, 70%). Mp 222–223 °C (decomposition); ν_{max}/cm^{-1} (neat) 2842 (CH), 1685 (CO), 1497, 1403, 1300, 1223, 1101, 1044, 919, 827; δ_H (400 MHz, DMSO-d_6) 3.69 (2H, s, 3-H_2), 3.93 (3H, s, OCH_3), 7.16 (1H, br s, 4"-H), 7.24 (1H, s, 6-H), 7.48 (1H, s, 9-H), 7.70 (1H, t, *J* 7.9 Hz, 5'-H), 7.83–7.90 (2H, m, 4'-H and 5"-H), 8.06 (1H, d, *J* 7.9 Hz, 6'-H), 8.27 (1H, br s, 2'-H), 8.36 (1H, br s, 2"-H), 10.56 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 40.0 (CH_2), 56.3 (CH_3), 110.6 (CH), 114.9 (q, $J_{C\text{-}C\text{-}F}$ 30.8 Hz, C), 118.1 (CH), 119.6 (CH), 120.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 123.0 (C), 123.1 (q, $J_{C\text{-}F}$ 271.9, C), 123.4 (CH), 126.1 (CH), 129.9 (CH), 130.2 (CH), 135.6 (CH), 137.3 (C), 138.4 (C), 143.1 (C), 152.8 (C), 159.8 (C), 165.7 (C); *m/z* (FAB) 401.1224 (MH^+. $C_{20}H_{16}F_3N_4O_2$ requires 401.1225), 238 (15%), 169 (26), 136, (13), 107 (16), 86 (100).

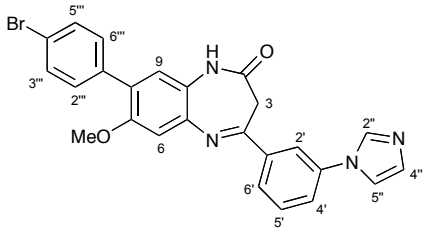
7-(3"'-Fluoropropoxy)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (5)



The reaction was carried out as described above, using 2-amino-1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-4-(trifluoromethyl)benzene (**27**) (0.400 g, 1.14 mmol) and *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**30**) (0.389 g, 1.36

mmol) in toluene (3 mL) to give 1-(*tert*-butoxycarbonylamino)-5-(3''''fluoropropoxy)-2-{3'-[3''-(1'''*H*-imidazol-1'''-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene as a white solid (0.507 g, 79%). Cyclisation was carried out as described above, using 1-(*tert*-butoxycarbonylamino)-5-(3''''fluoropropoxy)-2-{3'-[3''-(1'''*H*-imidazol-1'''-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene (0.070 g, 0.124 mmol) and trifluoroacetic acid (0.35 mL) in dichloromethane (0.7 mL) to give 7-(3'''-fluoropropoxy)-4-[3'-(1''*H*-imidazol-1''-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**5**) as a white solid (0.052 g, 91%). Mp 225–227 °C (decomposition); ν_{max}/cm^{-1} (neat) 2854 (CH), 1683 (CO), 1584, 1495, 1396, 1218, 1180, 1104, 1048, 954; δ_H (500 MHz, DMSO-d_6) 2.14 (1H, dquin, *J* 25.2, 5.9 Hz, 2'''-H_2), 3.69 (2H, s, 3-H_2), 4.25 (2H, t, *J* 5.9 Hz, 1'''-H_2), 4.62 (2H, dt, *J* 47.2, 5.9 Hz, 3'''-H_2), 7.16 (1H, br s, 4''-H), 7.27 (1H, s, 6-H), 7.48 (1H, s, 9-H), 7.70 (1H, t, *J* 7.9 Hz, 5'-H), 7.84–7.90 (2H, m, 4'-H and 5'''-H), 8.06 (1H, br d, *J* 7.9 Hz, 6'-H), 8.27 (1H, br s, 2'-H), 8.37 (1H, br s, 2''-H), 10.60 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 29.6 (d, $J_{C\text{-}C\text{-}F}$ 19.9 Hz, CH_2), 40.0 (CH_2), 64.6 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.8 Hz, CH_2), 80.5 (d, $J_{C\text{-}F}$, 161.9 Hz, CH_2), 111.4 (CH), 115.2 (q, $J_{C\text{-}C\text{-}F}$ 30.9 Hz, C), 118.3 (CH), 119.7 (CH), 120.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH), 123.3 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 123.3 (C), 123.6 (CH), 126.3 (CH), 130.1 (CH), 130.5 (CH), 135.8 (CH), 137.4 (C), 138.4 (C), 143.3 (C), 152.0 (C), 160.0 (C), 166.0 (C); *m/z* (FAB) 447.1448 (MH^+. $C_{22}H_{19}F_4N_4O_2$ requires 447.1444), 307 (21%), 289 (15), 155 (11), 89 (12).

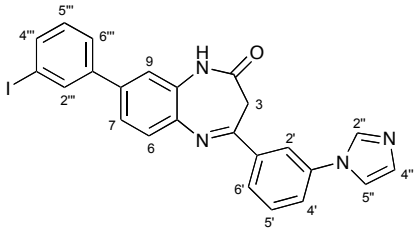
8-(4'''-Bromophenyl)-4-[3'-(1''*H*-imidazol-1''-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (34)



The reaction was carried out as described above, using 2-amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (0.200 g, 0.509 mmol) (**28**) and *tert*-butyl 3-[3'-(1''*H*-imidazol-1''-yl)phenyl]-3-oxopropanoate (**30**) (0.175 g, 0.611 mmol) in toluene (1 mL) to give 4-(4''''-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3'-[3''-(1'''*H*-imidazol-1'''-yl)phenyl]-3'-oxopropanamido}-5-methoxybenzene as a white solid (0.226, 73%). Cyclisation was carried out as described above, using 4-(4''''-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3'-[3''-(1'''*H*-imidazol-1'''-yl)phenyl]-3'-oxopropanamido}-5-methoxybenzene (0.420 g, 0.694 mmol) and trifluoroacetic acid (0.5 mL) in dichloromethane (4 mL) to give 8-(4'''-bromophenyl)-4-[3'-(1''*H*-imidazol-1''-yl)phenyl]-7-methoxy-2,3-

dihydro-1*H*-1,5-benzodiazepin-2-one (**34**) as a white solid (0.317 g, 94%). Mp 187–188 °C (decomposition); ν_{max}/cm^{-1} (neat) 3121 (NH), 2839 (CH), 1688 (CO), 1480, 1375, 1236, 1046, 817; δ_H (400 MHz, DMSO-d_6) 3.66 (2H, s, 3-H_2), 3.83 (3H, s, OCH_3), 7.11–7.19 (3H, m, 6-H, 9-H, 4”-H), 7.47 (2H, d, *J* 8.4 Hz, 2”’-H and 6”’-H), 7.65 (2H, d, *J* 8.4 Hz, 3”’-H and 5”’-H), 7.70 (1H, t, *J* 7.9 Hz, 5’-H), 7.83–7.89 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.26 (1H, br s, 2’-H), 8.37 (1H, s, 2”-H), 10.52 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 55.9 (CH_3), 109.7 (CH), 118.3 (CH), 119.6 (CH), 120.7 (C), 123.2 (CH), 123.4 (CH), 123.9 (C), 126.1 (CH), 127.4 (C), 130.1 (CH), 130.4 (CH), 131.2 (2 × CH), 131.3 (2 × CH), 135.9 (CH), 136.3 (C), 137.4 (C), 138.9 (C), 139.7 (C), 152.4 (C), 157.7 (C), 165.7 (C); *m/z* (ESI) 487.0746 (MH^+. $C_{25}H_{20}{}^{79}BrN_4O_2$ requires 487.0764).

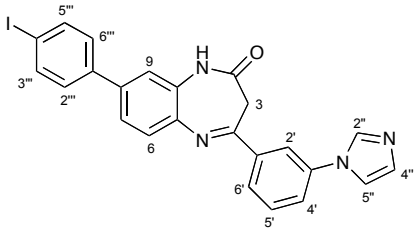
4-[3’-(1”*H*-Imidazol-1”-yl)phenyl]-8-(3”’-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (2)



A solution of 8-(3”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-(3”’-bromophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**32**) (0.030 g, 0.066 mmol) in 1,4-dioxane (1 mL) was degassed for 0.25 h before the addition of hexamethylditin (54 μL, 0.260 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.008 g, 0.007 mmol). The reaction mixture was heated to 90 ºC and stirred under argon for 48 h. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography on silica eluting with 0–4% ethanol in dichloromethane to give 4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-[3”’-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.29, 81%). To a solution of 4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-[3”’-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.029 g, 0.054 mmol) in ethanol (50 mL) was added sodium iodide (0.011 g, 0.073 mmol) in 0.01 M sodium hydroxide (11 mL). The solution was then acidified to pH 4–5 using 0.05 M hydrochloric acid. A solution of chloramine-T (0.032 g, 0.141 mmol) in water (30 mL) was added and the mixture stirred at room temperature for 0.5 h. The reaction was then quenched by the addition of sodium metabisulfite (0.400 g) in water (20 mL). The solution was then diluted with a saturated solution of sodium hydrogen carbonate (40 mL), extracted with dichloromethane (4 × 80 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid

was then triturated with diethyl ether to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**2**) as a white solid (0.023 g, 85%). Mp 208–209 °C (decomposition); ν_{max}/cm^{-1} (neat) 3190 (NH), 3090 (CH), 1676 (CO), 1551, 1505, 1312, 1269, 1055, 874, 777; δ_H (400 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4"-H), 7.31 (1H, t, *J* 7.7 Hz, 5"'-H), 7.49 (1H, br s, 9-H), 7.53 (1H, d, *J* 8.3 Hz, 6-H), 7.59 (1H, d, *J* 8.3 Hz, 7-H), 7.65–7.80 (3H, m, 5'-H, 4"'-H and 6"'-H), 7.82–7.90 (2H, m, 4'-H and 5"-H), 8.00–8.10 (2H, m, 6'-H and 2"'-H), 8.25 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H), 10.66 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 95.7 (C), 118.3 (CH), 119.5 (CH), 120.0 (CH), 122.7 (CH), 123.2 (CH), 126.0 (CH), 126.1 (CH), 128.7 (CH), 130.1 (CH), 130.4 (CH), 130.6 (C), 131.2 (CH), 135.0 (CH), 135.8 (CH), 136.4 (CH), 136.5 (C), 137.4 (C), 138.8 (C), 138.9 (C), 141.4 (C), 157.8 (C), 166.0 (C); *m/z* (ESI) 505.0508 (MH^+. $C_{24}H_{18}IN_4O$ requires 505.0520).

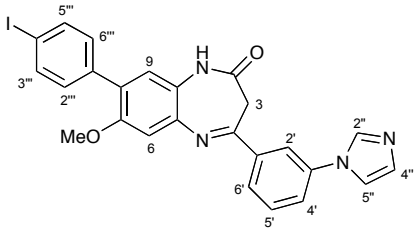
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (3)



The reaction was carried out as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-bromophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**33**) (0.200 g, 0.437 mmol), hexamethylditin (0.36 mL, 1.76 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.051 g, 0.044 mmol) in 1,4-dioxane (4.8 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.136 g, 57%). Iododestannylation was performed as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.035 g, 0.065 mmol), sodium iodide (0.010 g, 0.065 mmol) and chloramine-T (0.030 g, 0.132 mmol) in ethanol (25 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**3**) as a white solid (0.024 g, 73%). Mp 217–218 °C (decomposition); ν_{max}/cm^{-1} (neat) 2919 (CH), 1672 (CO), 1500, 1314, 1242, 1102, 1057, 802; δ_H (500 MHz, DMSO-d_6) 3.67 (2H, s, 3-H_2), 7.17 (1H, br s, 4"-H), 7.44–7.60 (5H, m, 6-H, 7-H, 9-H, 2"'H and 6"'-H), 7.69 (1H, t, *J* 7.6 Hz, 5'-H), 7.77–7.91 (4H, m, 4'-H, 5"-H, 3"'-H and 5"'-H), 8.06 (1H, br d, *J* 7.6 Hz, 6'-H), 8.25 (1H, br s, 2'-H), 8.33 (1H, br s, 2"-H), 10.58 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 94.2 (C), 118.3 (CH), 119.5 (CH),

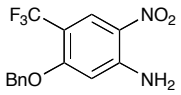
119.6 (CH), 122.4 (CH), 123.2 (CH), 126.1 (CH), 128.7 (3 × CH), 130.1 (CH), 130.4 (CH), 130.7 (C), 135.8 (CH), 137.0 (C), 137.4 (C), 137.9 (2 × CH), 138.5 (C), 138.8 (2 × C), 157.7 (C), 166.0 (C); *m/z* (ESI) 505.0507 (MH^+. $C_{24}H_{18}IN_4O$ requires 505.0520).

4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (6)



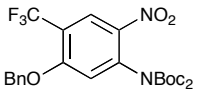
The reaction was carried out as described above, using 8-(4"'-bromophenyl)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**34**) (0.080 g, 0.164 mmol), hexamethylditin (136 μL, 0.660 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.019 g, 0.016 mmol) in 1,4-dioxane (2 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.084 g, 89%). Iododestannylation was then performed as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (0.084 g, 0.147 mmol), sodium iodide (0.023 g, 0.153 mmol) and chloramine-T (0.068g, 0.299 mmol) in ethanol (100 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**6**) as a yellow solid (0.042 g, 53%). Mp 203–204 °C (decomposition); ν_{max}/cm^{-1} (neat) 3109 (NH), 2932 (CH), 1678 (CO), 1582 (C=C), 1500, 1477, 1312, 1230, 1038, 1003, 817; δ_H (400 MHz, DMSO-d_6) 3.65 (2H, s, 3-H_2), 3.82 (3H, s, OCH_3), 7.08–7.23 (3H, m, 6-H, 9-H and 4"-H), 7.32 (2H, d, *J* 8.2 Hz, 2"'-H and 6"'-H), 7.70 (1H, t, *J* 7.8 Hz, 5'-H), 7.81 (2H, d, *J* 8.2 Hz, 3"'-H and 5"'-H), 7.83–7.91 (2H, m, 4'-H and 5"-H), 8.06 (1H, br d, *J* 7.8 Hz, 6'-H), 8.26 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H), 10.52 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 39.9 (CH_2), 55.9 (CH_3), 93.6 (C), 109.7 (CH), 118.4 (CH), 119.6 (CH), 123.2 (CH), 123.3 (CH), 123.9 (C), 126.1 (CH), 127.5 (C), 130.1 (CH), 130.4 (CH), 131.4 (2 × CH), 136.0 (CH), 136.6 (C), 137.0 (2 × CH), 137.4 (C), 138.9 (C), 139.7 (C), 152.4 (C), 157.7 (C), 165.7 (C); *m/z* (FAB) 535.0638 (MH^+. $C_{25}H_{20}IN_4O_2$ requires 535.0631), 409 (9), 304 (7), 282 (29), 119 (21), 96 (41), 85 (50), 56 (100).

5-Benzyloxy-2-nitro-4-(trifluoromethyl)aniline (36)



To a solution of 5-chloro-2-nitro-4-(trifluoromethyl)aniline **13** (1.30 g, 5.40 mmol) in benzyl alcohol (17 mL) was added tetra-*n*-butylammonium bromide (0.087 g, 0.027 mmol) and potassium hydroxide (0.727 g, 13.0 mmol). The reaction mixture was heated to 60 °C and stirred for 48 h. After cooling to room temperature, the reaction was quenched with 1 M hydrochloric acid (100 mL), extracted with ethyl acetate (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The addition of diethyl ether (50 mL) and petroleum ether (40–60) (50 mL) resulted in the formation of a precipitate that was collected by filtration to give 5-benzyloxy-2-nitro-4-(trifluoromethyl)aniline (**36**) as a yellow solid (1.01 g, 60%). Mp 146–147 °C; (Found: C, 53.84; H, 3.48; N, 8.86. $C_{14}H_{11}F_3N_2O_3$ requires C, 53.85; H, 3.55; N, 8.97%); ν_{max}/cm^{-1} (neat) 3350 (NH), 1640 (C=C), 1576, 1451, 1335, 1236, 1103, 907, 833; δ_H (400 MHz, $CDCl_3$) 5.19 (2H, s, CH_2), 6.25 (1H, s, 6-H), 6.43 (2H, br s, NH_2), 7.32–7.44 (5H, m, Ph), 8.45 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 70.8 (CH_2), 100.1 (CH), 110.1 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.7 (q, $J_{C\text{-}F}$ 271.2 Hz, C), 125.3 (C), 126.7 (2 × CH), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz, CH), 128.4 (CH), 128.8 (2 × CH), 134.9 (C), 148.6 (C), 161.1 (C); *m/z* (CI) 313 (MH^+, 100%), 283 (80), 223 (100), 147 (54), 119 (63), 89 (90).

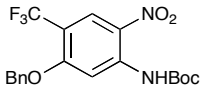
5-Benzyloxy-1-bis[*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene



To a solution of 5-benzyloxy-2-nitro-4-(trifluoromethyl)aniline (**36**) (1.70 g, 5.44 mmol) in dichloromethane (60 mL) was added di-*tert*-butyl dicarbonate (2.61 g, 12.0 mmol), 4-dimethylaminopyridine (0.13 g, 1.09 mmol) and triethylamine (1.67 mL, 12.0 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (60 mL), extracted with dichloromethane (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then purified by dry flash chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 5-benzyloxy-1-bis[*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene as a white solid (2.73 g, 98%). Mp 166–168 °C; (Found: C, 56.17; H, 5.29; N, 5.48. $C_{24}H_{27}F_3N_2O_7$ requires C, 56.25; H,

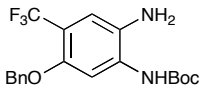
5.31; N, 5.47%); ν_{max}/cm^{-1} (neat) 2992 (CH), 1790 (CO), 1624 (C=C), 1530, 1343, 1233, 1150, 1098, 912, 847; δ_H (500 Hz, $CDCl_3$) 1.37 (18H, s, 6 × CH_3), 5.30 (2H, s, CH_2), 6.90 (1H, s, 6-H), 7.33–7.41 (5H, m, Ph), 8.45 (1H, s, 3-H); δ_C (126 MHz, $CDCl_3$) 27.7 (6 × CH_3), 71.3 (CH_2), 84.4 (2 × C), 115.8 (CH), 119.2 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.0 (q, $J_{C\text{-}F}$ 272.9 Hz, C), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 126.8 (2 × CH), 128.6 (CH), 129.0 (2 × CH), 134.4 (C), 137.9 (C), 138.7 (C), 149.8 (C), 159.8 (2 × C); *m/z* (CI) 413 ($MH^+ - CO_2{}^tBu$, 59%), 401 (26), 375 (39), 357 (100), 327 (33), 313 (15), 267 (23), 237 (14), 113 (13).

5-Benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (37)



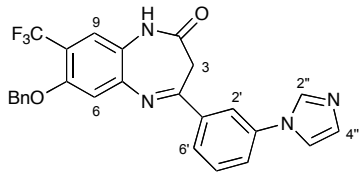
To a solution of 5-benzyloxy-1-[bis(*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene (2.71g, 5.29 mmol) in dichloromethane (73 mL) at 0 ºC was added trifluoroacetic acid (0.81 mL, 10.6 mmol). The reaction mixture was then warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with a saturated solution of sodium hydrogen carbonate (50 mL), extracted with dichloromethane (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (**37**) as a yellow solid (2.12 g, 97%). Mp 159–160 °C; (Found: C, 55.14; H, 4.59; N, 6.74. $C_{19}H_{19}F_3N_2O_5$ requires C, 55.34; H, 4.65; N, 6,79%); ν_{max}/cm^{-1} (neat) 3343 (NH), 2992 (CH), 1732 (CO), 1632 (C=C), 1580, 1439, 1341, 1236, 1140, 978, 843; δ_H (400 MHz, $CDCl_3$) 1.57 (9H, s, 3 × CH_3), 5.30 (2H, s, CH_2), 7.32–7.50 (5H, m, Ph), 8.50 (1H, s, 6-H), 8.52 (1H, s, 3-H), 10.18 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.1 (3 × CH_3), 71.2 (CH_2), 82.7 (C), 102.8 (CH), 113.1 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.4 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 126.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.5 Hz, CH), 127.4 (2 × CH), 127.7 (C), 128.5 (CH), 128.7 (2 × CH), 134.7 (C), 141.4 (C), 151.9 (C), 161.6 (C); *m/z* (CI) 413 (MH^+, 37%), 383 (11), 357 (100), 327 (13), 313 (13), 267 (23), 223 (12).

2-Amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (38)



To a solution of 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (**37**) (2.10 g, 5.09 mmol) in ethanol (145 mL) was added tin(II) chloride dihydrate (5.75 g, 25.5 mmol). The reaction mixture was heated to 70 °C and stirred for 6 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The resulting solid was dissolved in ethyl acetate (100 mL) and a saturated solution of sodium hydrogen carbonate (100 mL) was added. The mixture was then extracted with ethyl acetate (3 × 70 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 2-amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (**38**) as an off-white solid (1.98 g, 98%). Mp 129–130 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2992 (CH), 1688 (CO), 1597 (C=C), 1497, 1437, 1296, 1223, 1123, 1063, 883; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 3.12 (2H, br s, NH_2), 5.13 (2H, s, CH_2), 6.87 (1H, br s, NH), 7.07 (1H, s, 3-H), 7.28−7.49 (5H, m, Ph), 7.66 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 70.9 (CH_2), 81.2 (C), 106.4 (CH), 113.9 (q, $J_{C\text{-}C\text{-}F}$ 31.9 Hz, C), 118.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.6 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 127.1 (2 × CH), 127.1 (C), 127.8 (CH), 128.5 (2 × CH), 133.3 (C), 136.6 (C), 152.0 (C), 152.8 (C); *m/z* (CI) 383.1590 (MH^+. $C_{19}H_{22}F_3N_2O_3$ requires 383.1583), 327 (33%), 283 (18), 113 (26), 71 (100).

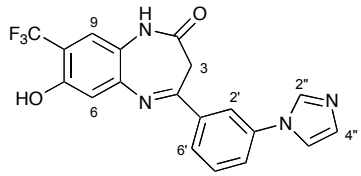
7-Benzyloxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (39)



To a solution of 2-amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (**38**) (0.250 g, 0.654 mmol) in toluene (1.5 mL) was added *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl]phenyl)-3-oxopropanoate (**30**) (0.223 g, 0.779 mmol). The solution was heated under reflux for 4 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–5% ethanol in dichloromethane to give 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-

(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene as a white solid (0.286 g, 74%). To a solution of 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene (0.085 g, 0.143 mmol) in dichloromethane (0.75 mL) at 0 °C was added trifluoroacetic acid (0.25 mL). The reaction mixture was warmed to room temperature and stirred for 2 h. The solution was diluted with dichloromethane (5 mL). A saturated solution of sodium hydrogen carbonate (5 mL) was added, the solution extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 7-benzyloxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**39**) as a white solid (0.075 g, 88%). Mp 209−211 °C (decomposition); ν_{max}/cm^{-1} (neat) 3100 (NH), 2870 (CH), 1674 (CO), 1493, 1404, 1306, 1223, 1121, 1055, 914, 895; δ_H (400 MHz, DMSO-d_6) 3.70 (2H, s, 3-H_2), 5.31 (2H, s, OCH_2Ph), 7.16 (1H, br s, 4"-H), 7.32−7.52 (7H, m, 6-H, 9-H and Ph), 7.71 (1H, t, *J* 7.9 Hz, 5'-H), 7.84−7.90 (2H, m, 4'-H and 5"-H), 8.06 (1H, br d, *J* 7.9 Hz, 6'-H), 8.26 (1H, t, *J* 1.8 Hz, 2'-H), 8.36 (1H, br s, 2"-H), 10.60 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 40.1 (CH_2), 70.1 (CH_2), 111.8 (CH), 115.5 (q, $J_{C\text{-}C\text{-}F}$ 30.9 Hz, C), 118.3 (CH), 119.8 (CH), 120.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.4 (q, $J_{C\text{-}F}$ 271.9 Hz, C), 123.4 (C), 123.6 (CH), 126.3 (CH), 127.1 (2 × CH), 128.0 (CH), 128.6 (2 × CH), 130.1 (CH), 130.5 (CH), 135.9 (CH), 136.4 (C), 137.4 (C), 138.4 (C), 143.2 (C), 151.8 (C), 160.1 (C), 166.0 (C); *m/z* (ESI) 477.1517 (MH^+. $C_{26}H_{20}F_3N_4O_2$ requires 477.1533).

7-Hydroxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (40)



To a suspension of 7-benzyloxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**39**) (0.150 g, 0.315 mmol) in dichloromethane (4 mL) was added boron tribromide (1.0 M in dichloromethane) (1.55 mL, 1.55 mmol). After stirring for 8 h, the solution was diluted with a saturated solution of sodium hydrogen carbonate (5 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid was then triturated with dichloromethane to give 7-hydroxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5- benzodiazepin-2-one (**40**) as a pale yellow solid (0.099 g, 81%). Mp 232−234 °C (decomposition); ν_{max}/cm^{-1} (neat) 3071

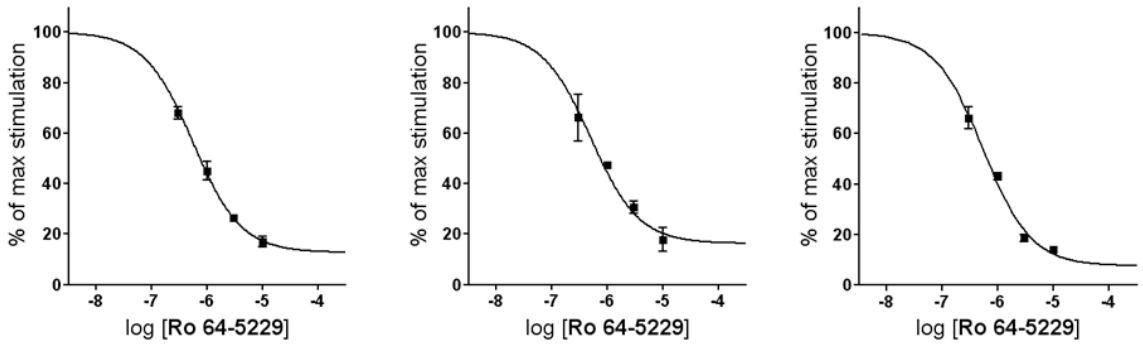
(NH), 2922 (CH), 2743, 1651 (CO), 1582, 1491, 1412, 1317, 1221, 1105, 1059, 889; δ_H (500 MHz, DMSO-d_6) 3.65 (2H, s, 3-H_2), 7.03 (1H, s, 6-H), 7.15 (1H, s, 4"-H), 7.40 (1H, s, 9-H), 7.69 (1H, t, *J* 7.9 Hz, 5'-H), 7.79−7.87 (2H, m, 4'-H and 5"-H), 8.04 (1H, br d, *J* 7.9 Hz, 6'-H), 8.21 (1H, br s, 2'-H), 8.32 (1H, s, 2"-H), 10.37 (1H, s, NH), 10.51 (1H, br s, OH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 113.8 (CH), 114.0 (q, $J_{C\text{-}C\text{-}F}$ 30.4 Hz, C), 118.1 (CH), 119.6 (CH), 120.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.3 Hz, CH), 122.1 (C), 123.4 (CH), 123.4 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 126.1 (CH), 129.9 (CH), 130.2 (CH), 135.7 (CH), 137.3 (C), 138.5 (C), 143.0 (C), 151.4 (C), 159.8 (C), 165.8 (C); *m/z* (EI) 386.0984 (M^+. $C_{19}H_{13}F_3N_4O_2$ requires 386.0991), 344 (50%), 324 (46), 296 (12), 169 (8), 84 (14).

3. Experimental for [^{35}S]GTPγS Binding Assay[6]

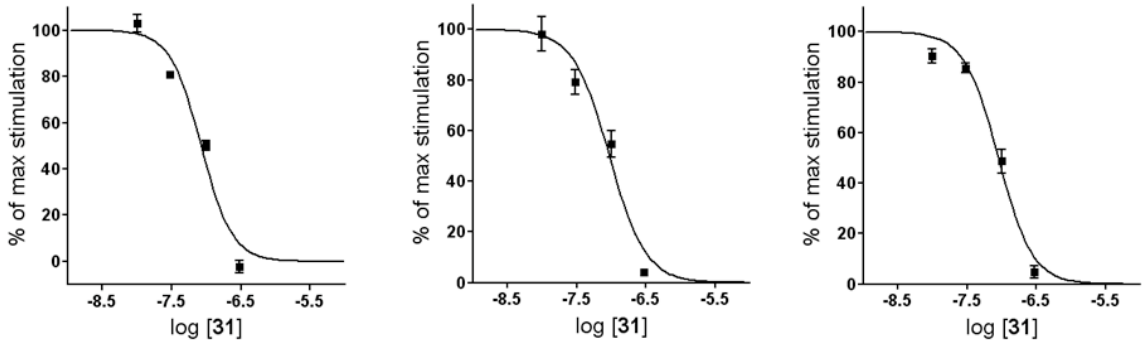
ChemiSCREEN™ membrane preparation (recombinant human mGluR2 metabotropic glutamate receptor) was obtained from Millipore. Membranes were permeabilised by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]GTPγS (0.1 nM), L-glutamate (10 μM) and various concentrations of test compound, in HEPES (20 mM), sodium chloride (100 mM), magnesium chloride (10 mM), GDP (0.5 μM), pH 7.4 (final volume 100 μL). Incubation was carried out for 30 min at 30 ºC. Basal binding was determined without L-glutamate or test compound present, and stimulated binding without test compound present. Reactions were terminated by rapid filtration through Whatman GF/B glass fibre filters pre-soaked with water using a 24-well Brandel cell harvester. The filters were washed 3 times (1 mL per well per wash) with ice-cold sodium phosphate (10 mM), pH 7.4. Disintegrations per minute were determined by liquid scintillation analysis and IC_{50} values derived from nonlinear regression analysis using GraphPad Prism Version 4 (GraphPad Software Inc).

4. IC_{50} Binding Curves

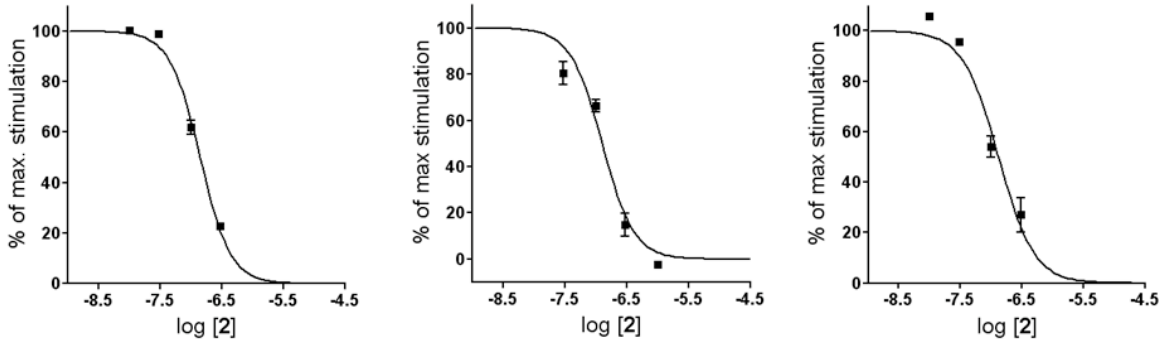
Ro 64-5229 35:



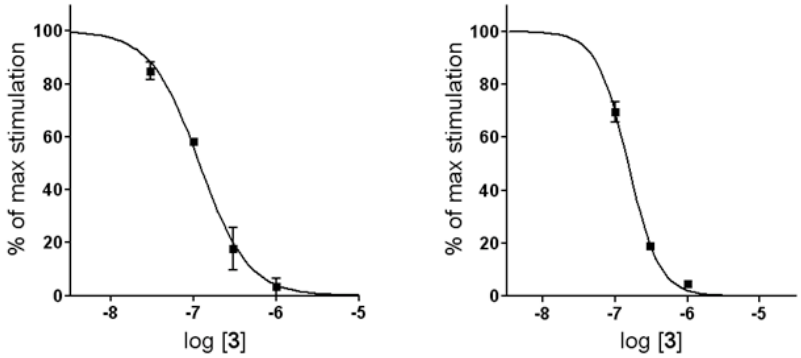
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-iodo-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (31)



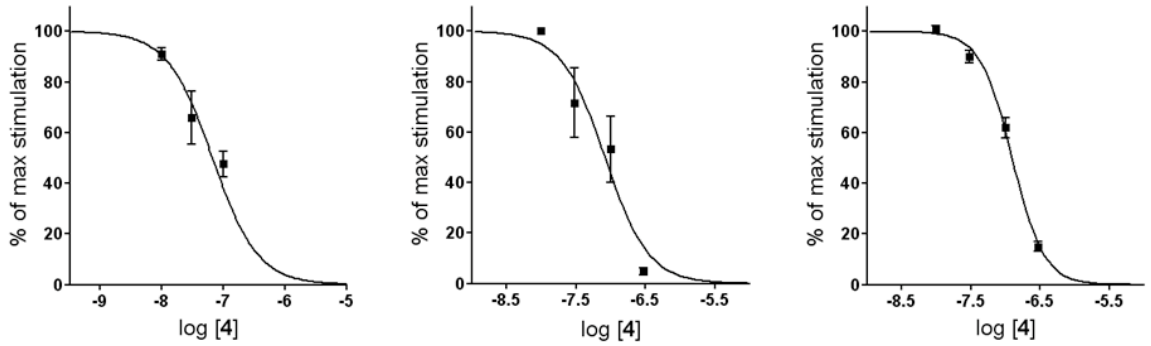
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (2):



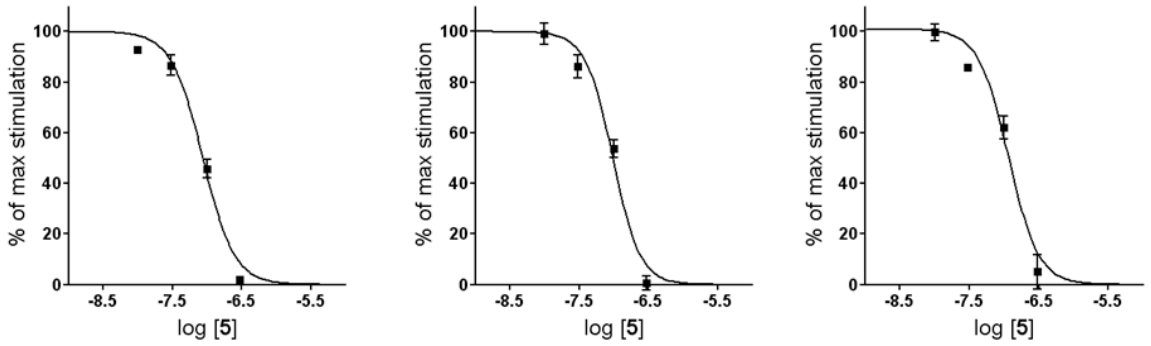
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (3):



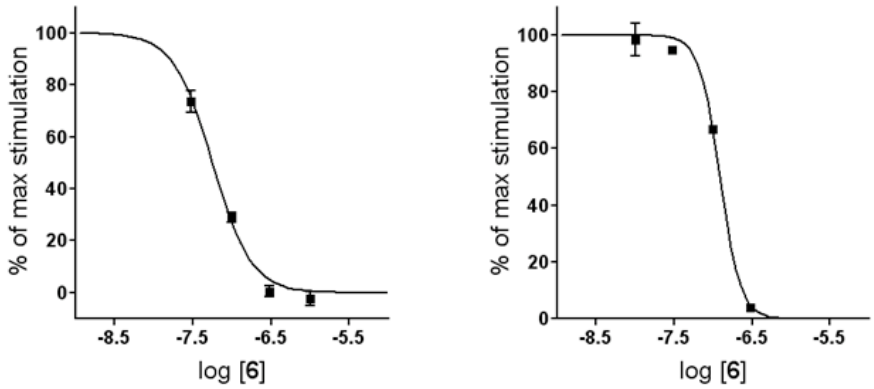
4-[3'-(1"H-Imidazol-1"-yl)phenyl]-7-methoxy-8-trifluoromethyl-2,3-dihydro-1H-1,5-benzodiazepin-2-one (4):



7-(3"'-Fluoropropoxy)-4-[3'-(1"H-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1H-1,5-benzodiazepin-2-one (5):



4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (6):



5. HPLC Methodology[7]

All physicochemical analyses were performed using a Dionex Ultimate 3000 series, and data acquisition and processing performed using Chromeleon 6.8 Chromatography software. Standard and test compounds were dissolved in 1:1 organic/aqueous phases, and prepared to a concentration of 0.5 mg/mL. The HPLC system was set to 25 °C, and UV detection achieved using a diode array detector (190 – 800 nm). Analysis was performed using 5 μL sample injections.

C_{18} chromatography for determination of lipophilicity (Log*P*)

Log*P* values were determined using a Phenomenex Luna 5 micron C_{18} 100 A (50 × 3 mm) column. The retention time for each compound of interest was measured using filtered acetonitrile and 0.01 mM phosphate buffered saline as the mobile phase at pH 7.4, pH 2.5 and pH 10.0, and pH was adjusted by the addition of concentrated hydrochloric acid and 0.05 M sodium hydroxide solution, respectively. The mobile phase flow rate was set at 1.0 mL/min. The CHI value for all compounds of interest was determined by measuring the retention time of each compound under the following mobile phase conditions: 0–10.5 min, 0–100% acetonitrile; 10.5–11.5 min, 100% acetonitrile; 11.5–12.0 min, 100–0% acetonitrile; 12.0–15.0 min, 0% acetonitrile. System calibration was achieved using the following compounds and plotting their mean CHI values against the measured retention time under all three pH conditions: theophylline (CHI = 15.76), phenyltetrazole (CHI = 20.18), benzimidazole (CHI = 30.71), colchicine (CHI = 41.37), acetophenone (CHI = 64.90), indole (CHI = 69.15), propiophenone (CHI = 78.41), butyrophenone (CHI = 88.49) and valerophenone (CHI = 97.67). Using the calibration curves obtained and the following equations from a validated study, the Log*P* of the compounds were calculated using Excel 2008 Software.

CHI Log *D* = 0.054 CHI – 1.467

where CHI Log *D* is the CHI value projected to the logarithmic scale

Log *P* = 0.054 CHIN + 1.32 A – 1.88

where CHIN = CHI values of the compound in a neutral form, and A = Abraham H-bond acidity parameter, which was determined using ADME Suite 5.0 software.

Immobilised Artificial Membrane (IAM) chromatography for determination of membrane permeability (P_m) and membrane partition coefficient (K_m)

P_m and K_m values were determined using previously developed methodology on a Registech IAM.PC.DD2 (15 cm × 4.6 mm) column. Acetonitrile and 0.01 mM phosphate buffered saline at pH 7.4 was used as the mobile phase, with a flow rate of 1.0 mL/min. The retention time of each compound was measured under an isocratic mobile phase with the percentage of acetonitrile ranging from 30–40%. The retention time of citric acid, as an unretained compound, under an isocratic mobile phase of 100% phosphate buffered saline was used for system corrections. The following equations were used to calculate P_m and K_m of the compounds of interest using Excel 2008 Software.

$$k_{IAM} = \frac{(t_r - t_0)}{t_0}$$

where k_{IAM} = solute capacity factor on the column, t_r = compound retention time and t_0 = unretained compound retention time

$$k_{IAM} = \left(\frac{V_s}{V_m}\right) \times K_m$$

where V_s = volume of the IAM interphase created by the immobilised phospholipids, V_m = total volume of the solvent within the IAM column and K_m = membrane partition coefficient

$$V_m = \frac{W_{PhC}}{\delta_{PhC}} + \frac{W_{C10}}{\delta_{C10}} + \frac{W_{C3}}{\delta_{C3}}$$

where the specific weight of PhC (δ_{PhC}) = 1.01779 g/mL and C_{10}/C_3 ($\delta_{C10/C3}$) = 0.86 g/mL; W_{PhC} = 133 mg, W_{C10} = 12.73 mg and W_{C3} = 2.28 mg

$$V_m = f_r \times t_0$$

where f_r = flow rate

$$P_m = \frac{K_m}{MW}$$

where P_m = permeability and MW = molecular weight

Human Serum Albumin (HSA) chromatography for determination of percentage of plasma protein binding (%PPB)

%PPB values were determined using previously developed methodology on a ChromTech HSA 5 μm (3.0 × 50 mm) column. Isopropanol and 0.01 mM phosphate buffered saline at pH 7.4 was used as the mobile phase, with a flow rate of 1.8 mL/min. The retention time of each compound was measured under the following mobile phase conditions: 0–3 min, 0–30% IPA; 3–10 min, 30% IPA; 10.5–11.0 min, 30–0% IPA; 11.0–15.0 min, 0% IPA. System calibration was achieved using the following compounds and plotting %PPB values against their mean retention times: warfarin (%PPB = 98.0), nizatidine (%PPB = 35.0), bromazepam (%PPB = 60.0), carbamazepine (%PPB = 75.0), budesonide (%PPB = 88.0), nicardipine (%PPB = 95.0), ketoprofen (%PPB = 98.7), indomethacin (%PPB = 99.0) and diclofenac (%PPB = 99.8). For each standard compound, the literature %PPB value was converted to its corresponding Log k value, which when plotted against t_r on the HSA column, afforded a line equation from which the Log k value of the unknown compounds could be extracted. The Log k values of the unknown compounds could then be converted to %PPB. Log k and subsequent %PPB calculation for the compounds of interest were performed using Excel 2008 Software.

$$Logk = Log\left[\frac{\%PPB}{(101 - \%PPB)}\right]$$

$$\%PPB = \left[\frac{(101 - 10^{\log k})}{(1 + 10^{\log k})}\right]$$

6. References

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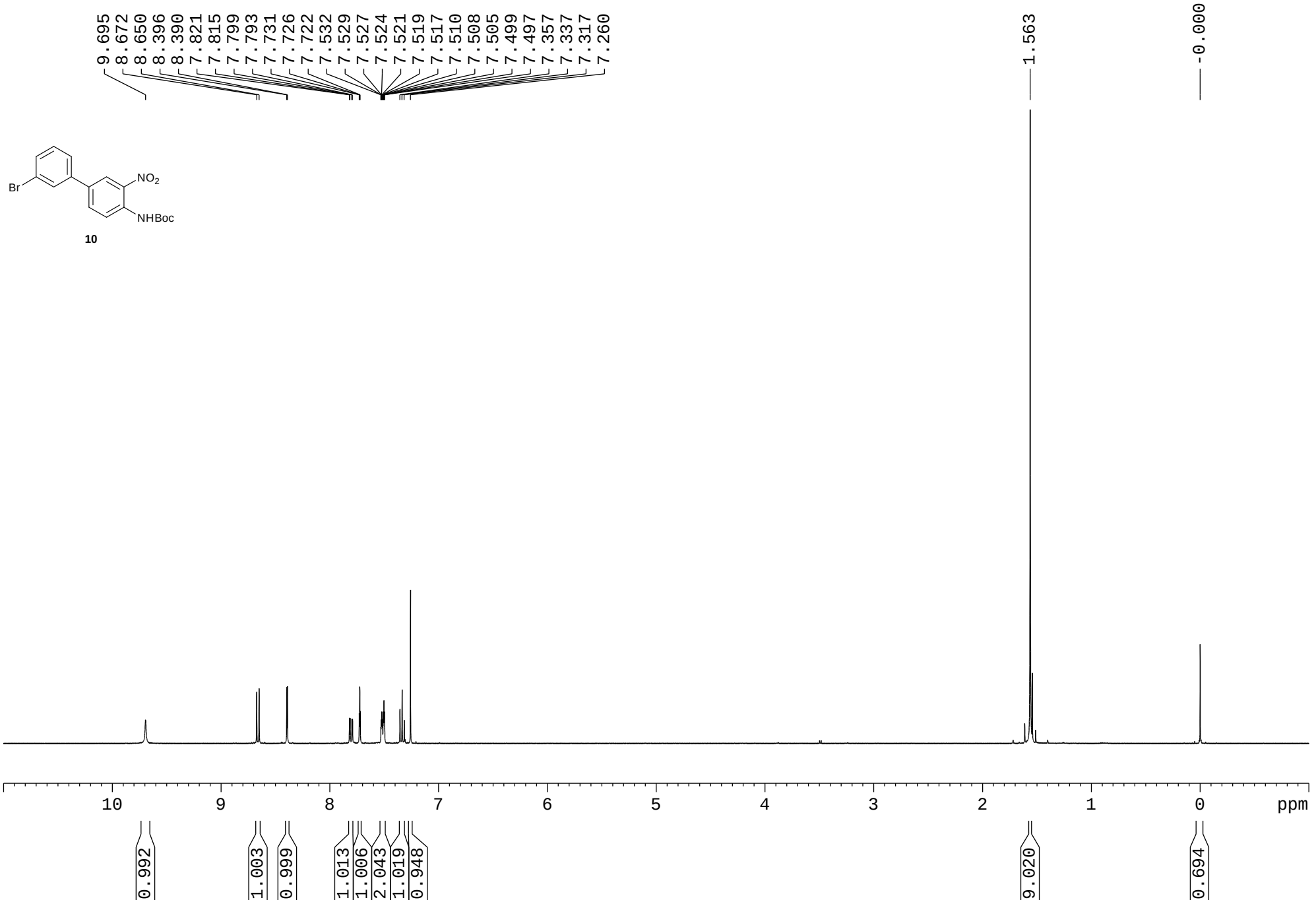

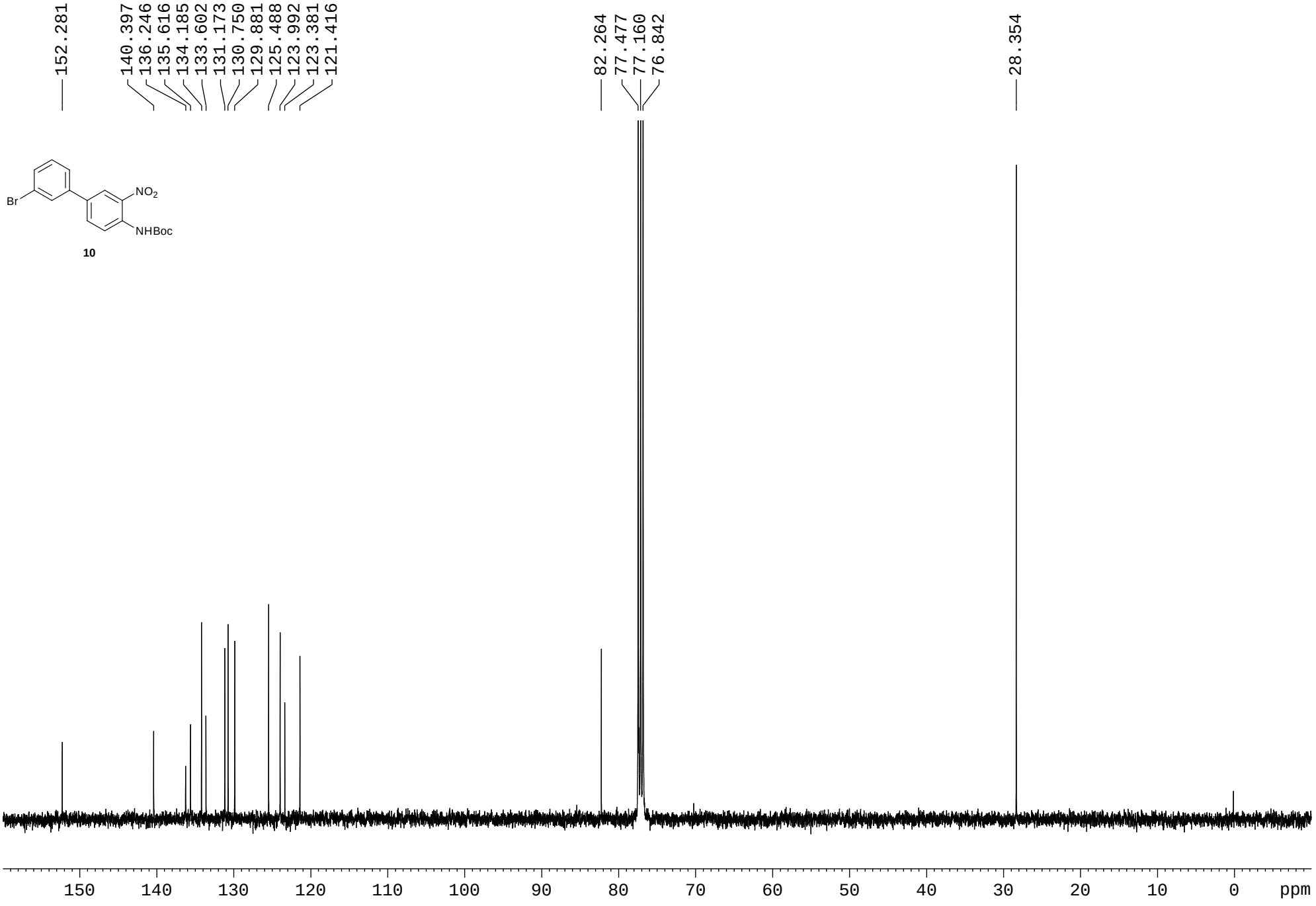

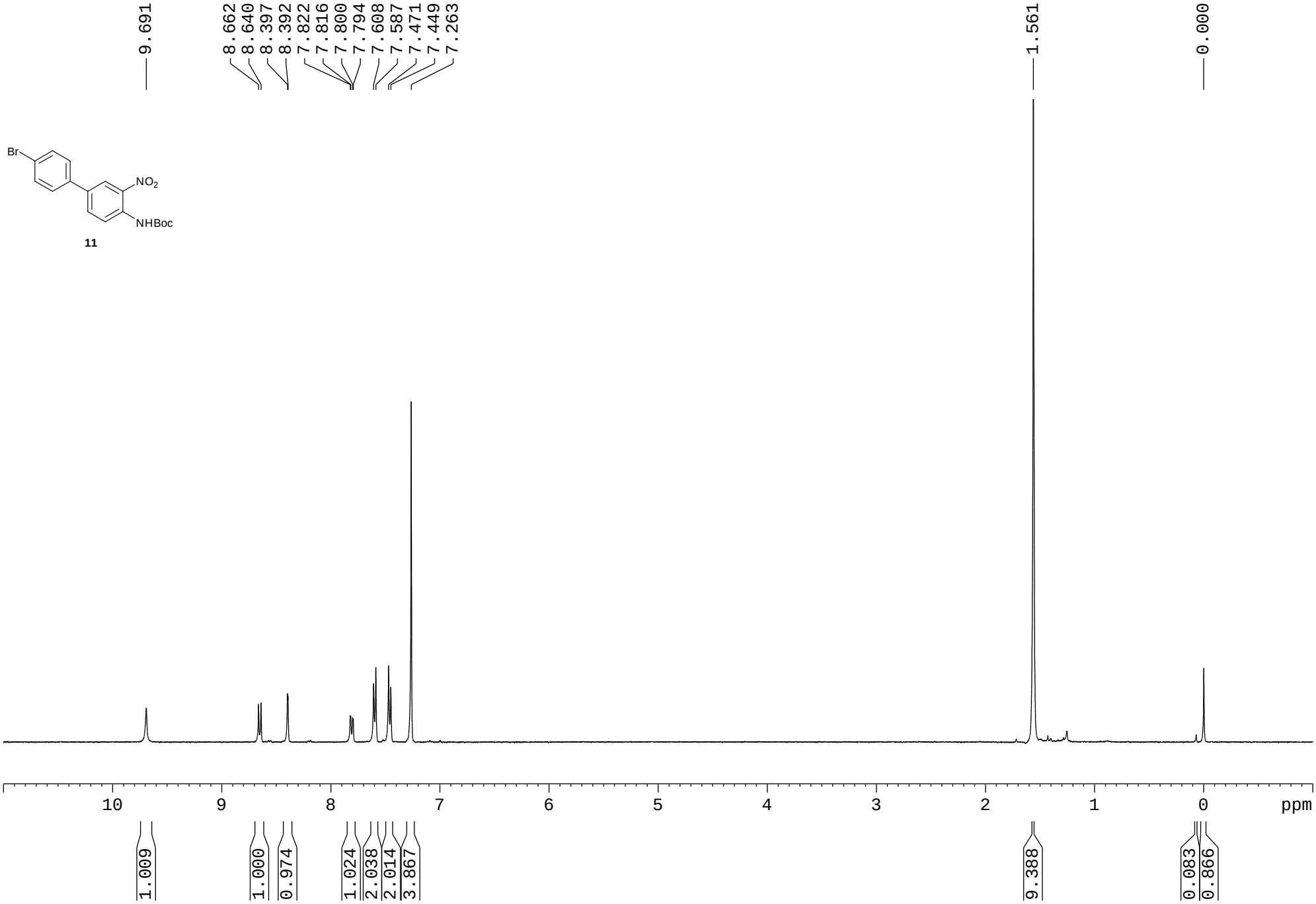

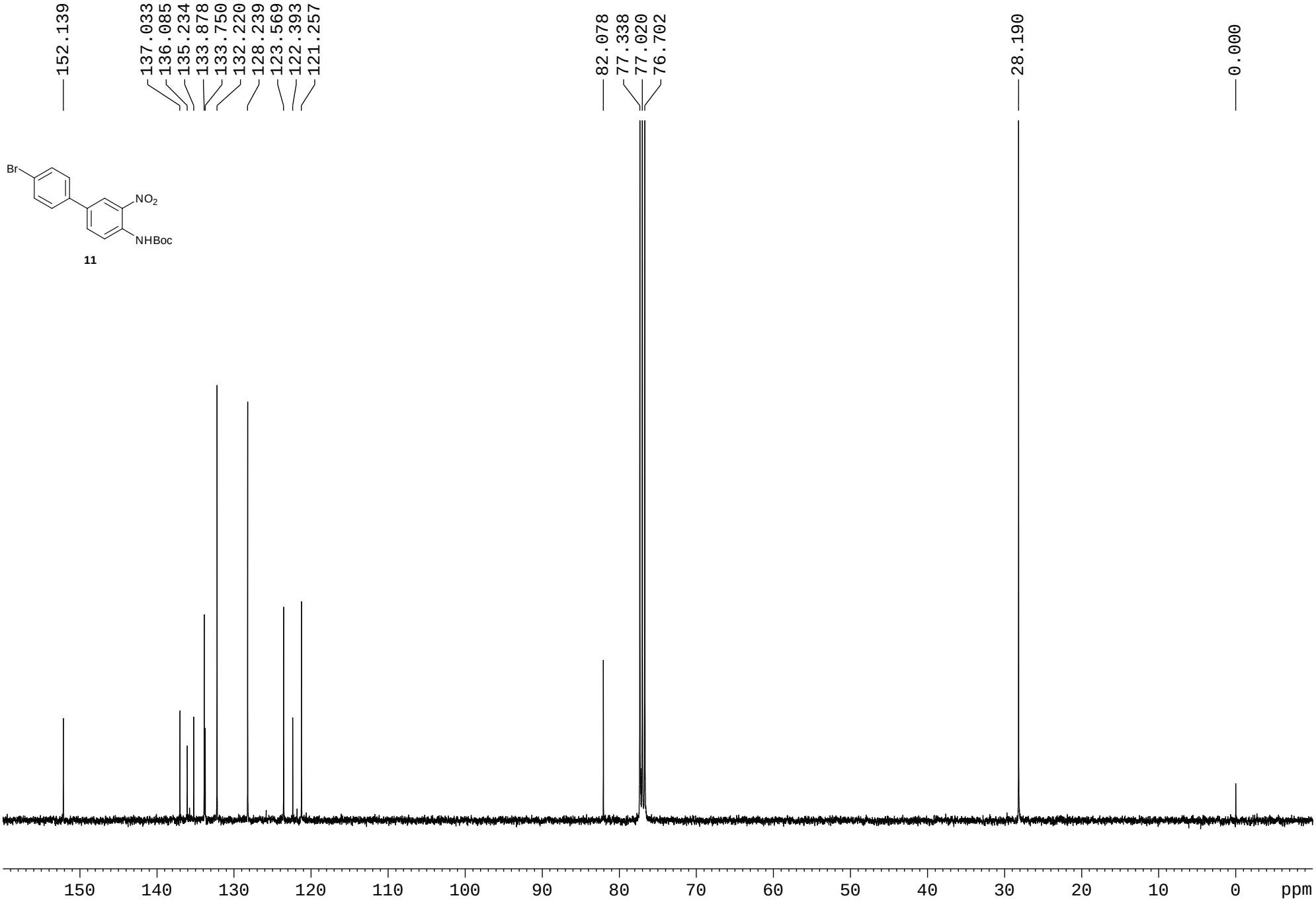

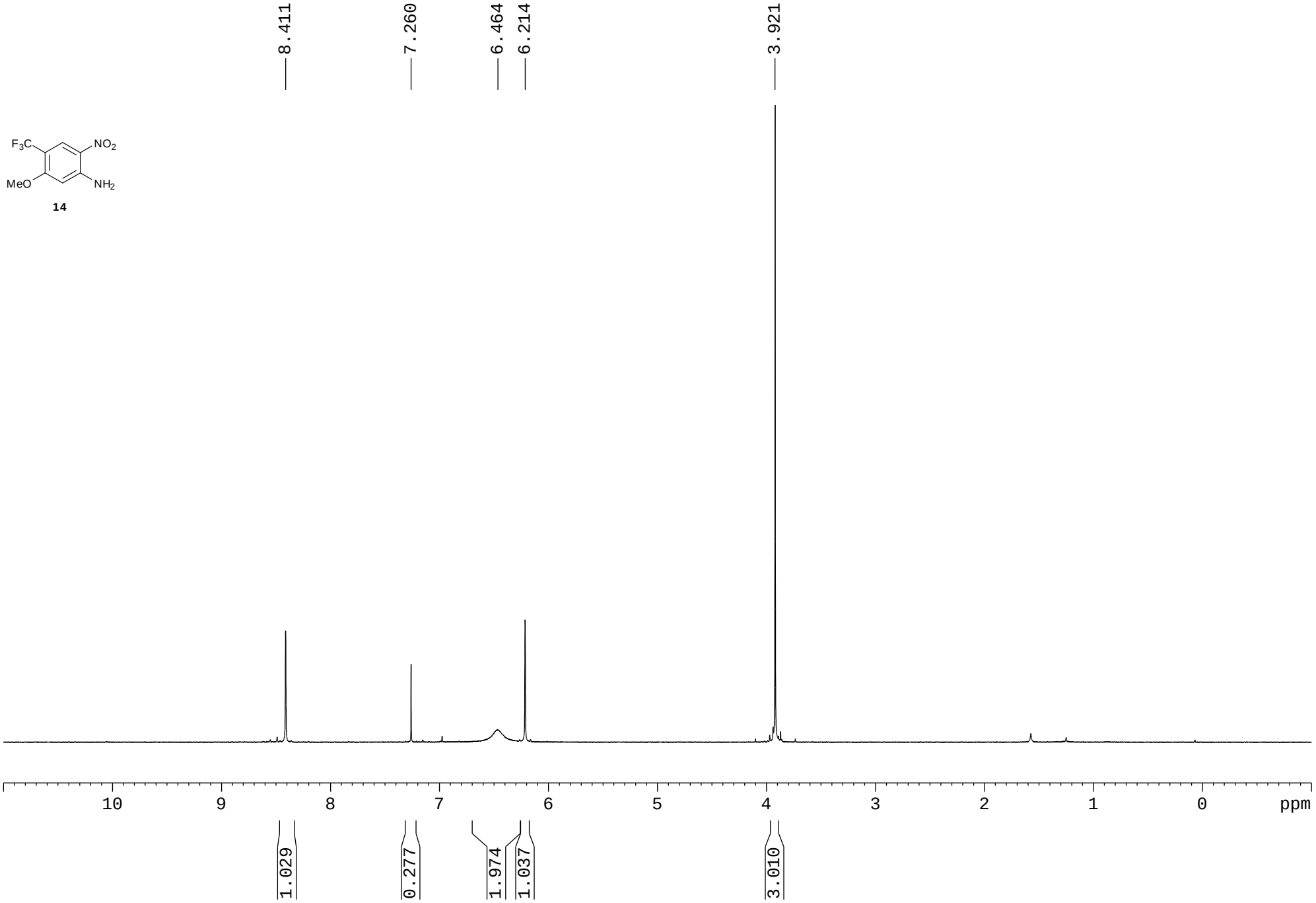

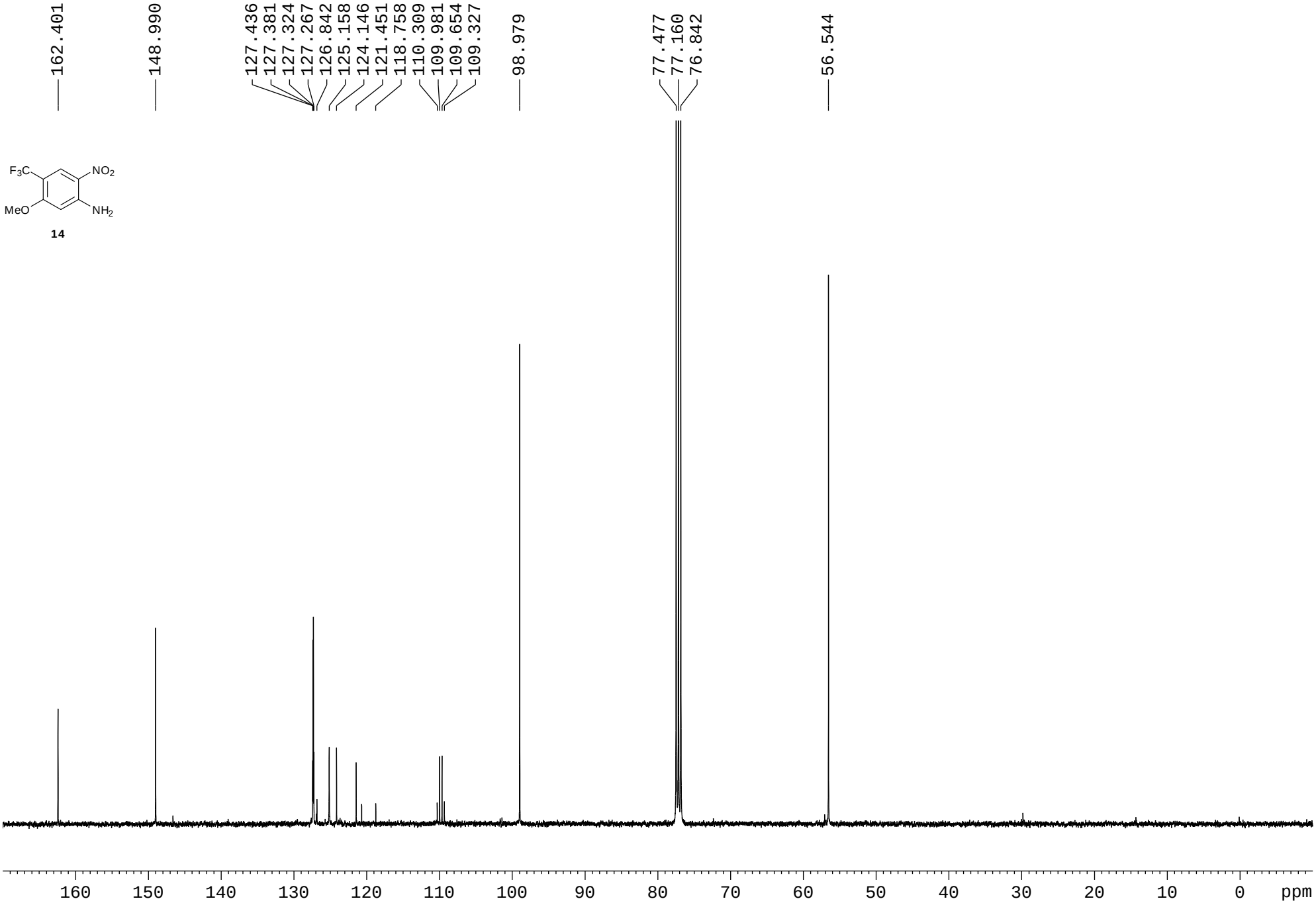

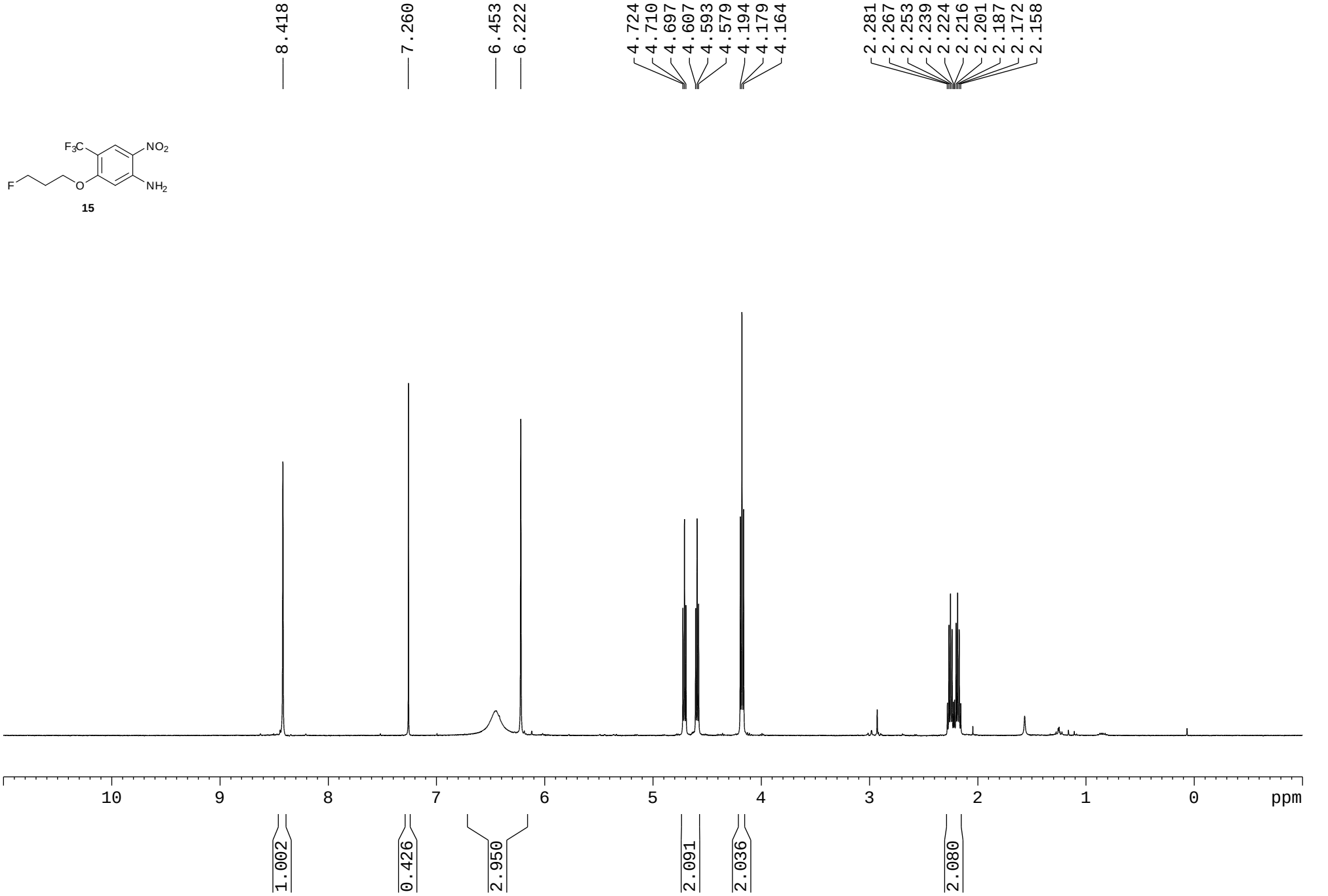

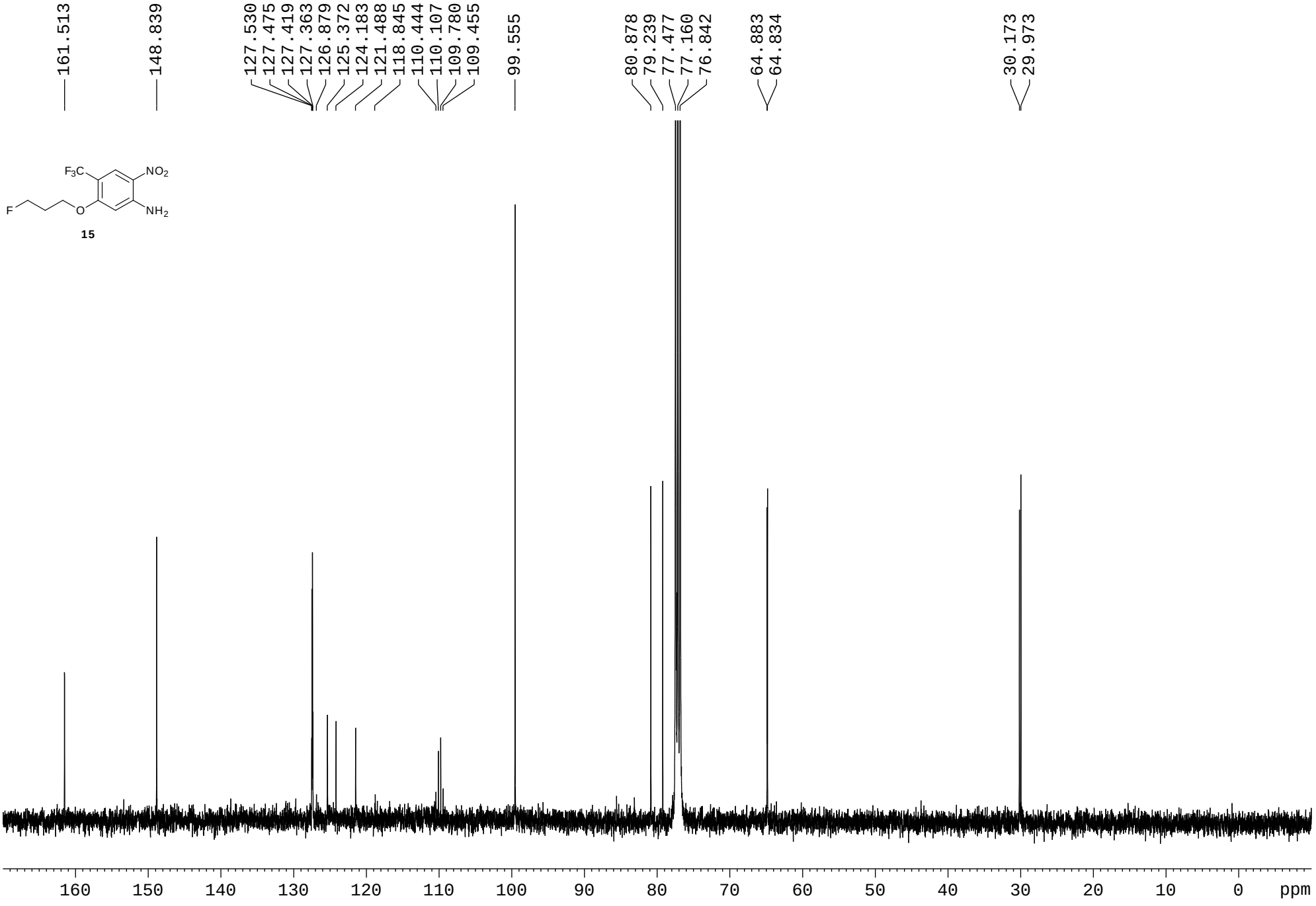

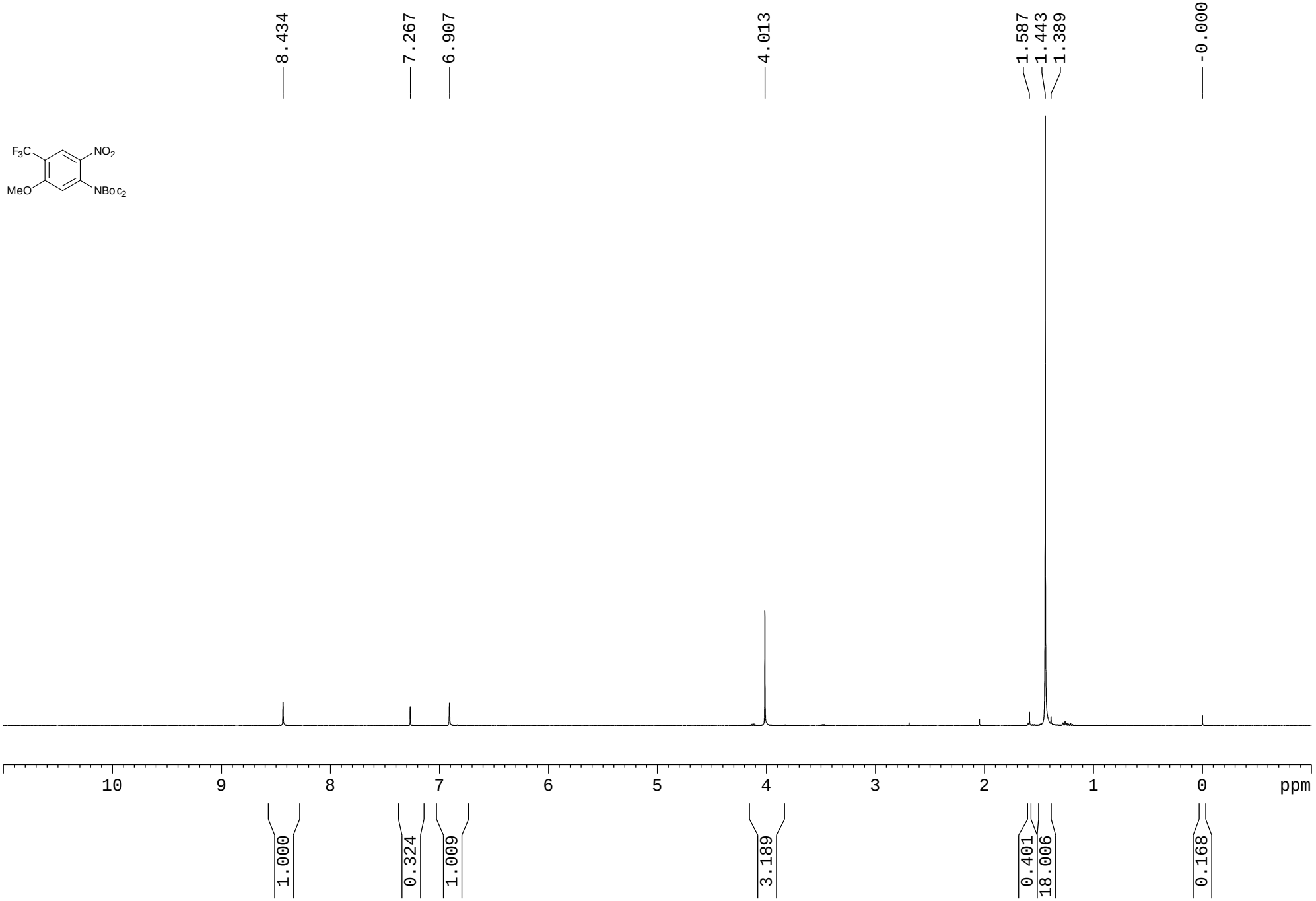

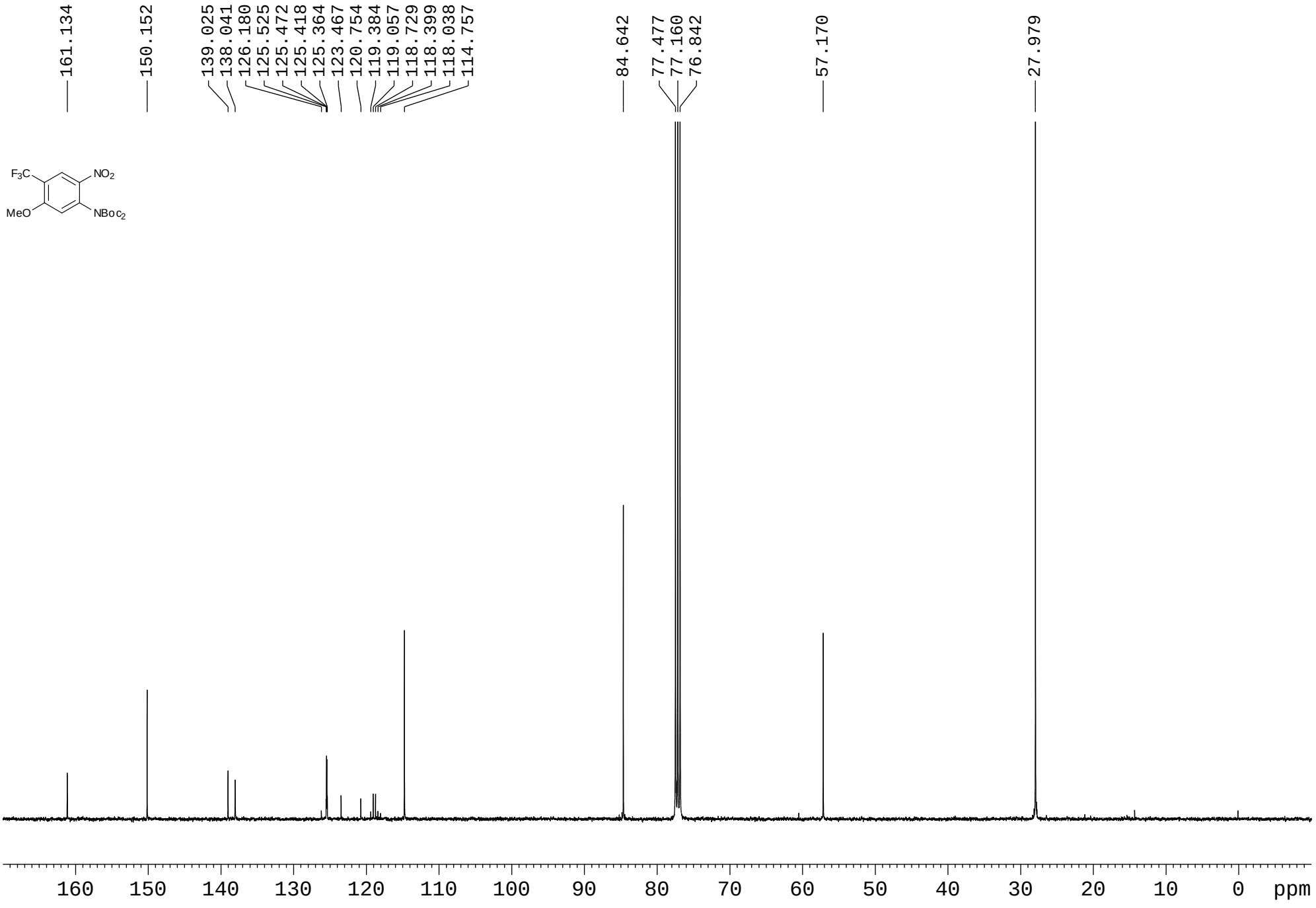

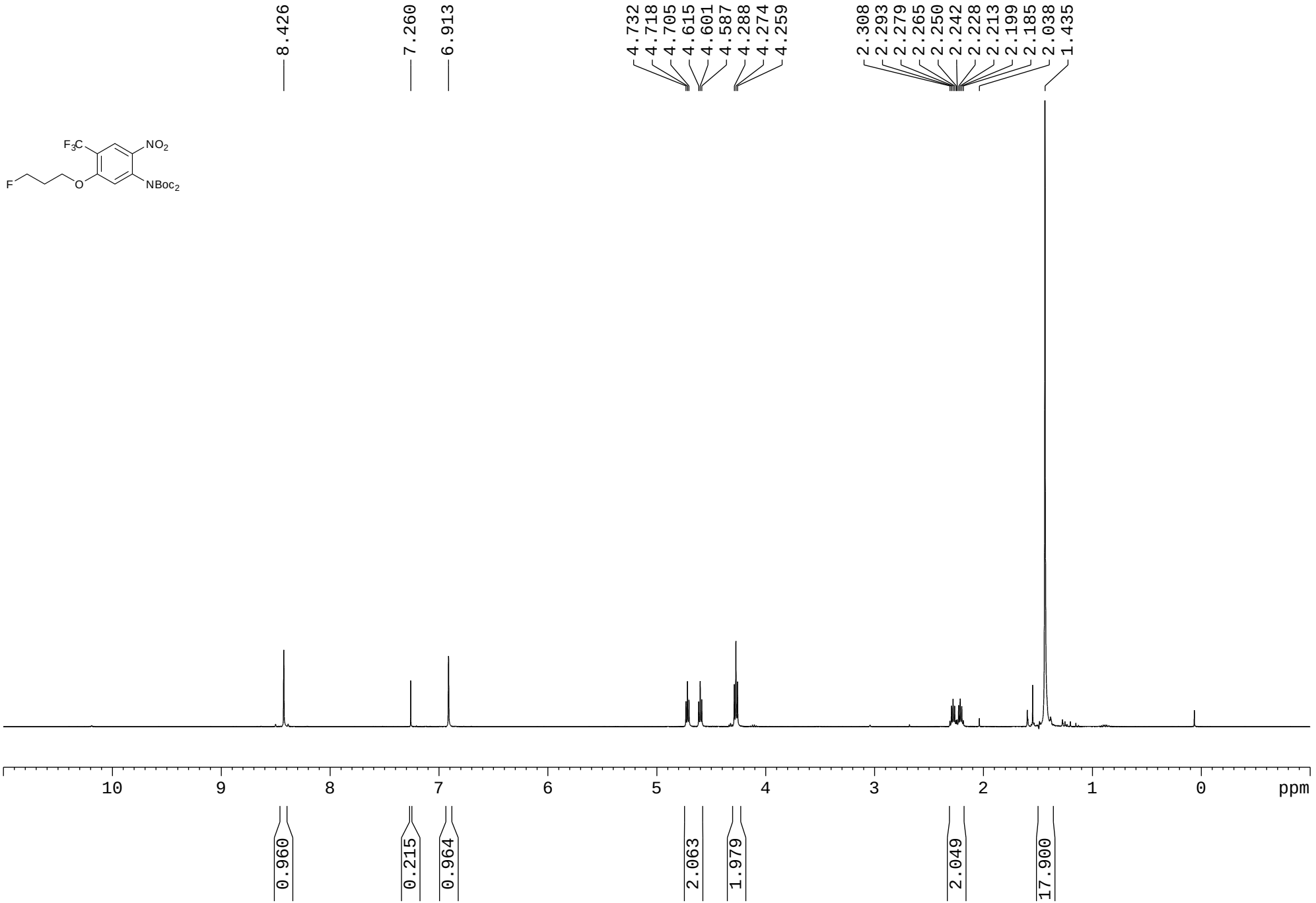

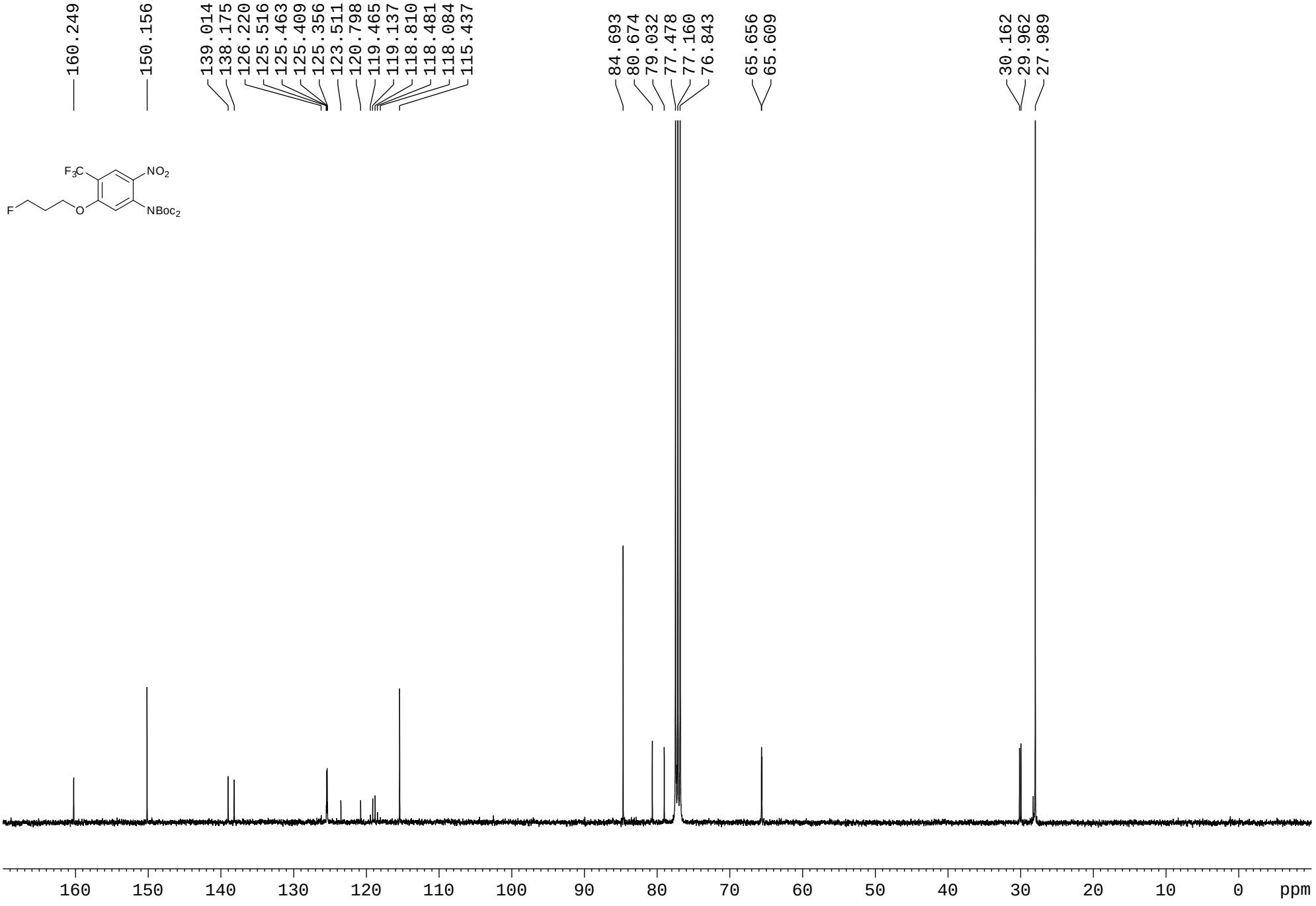

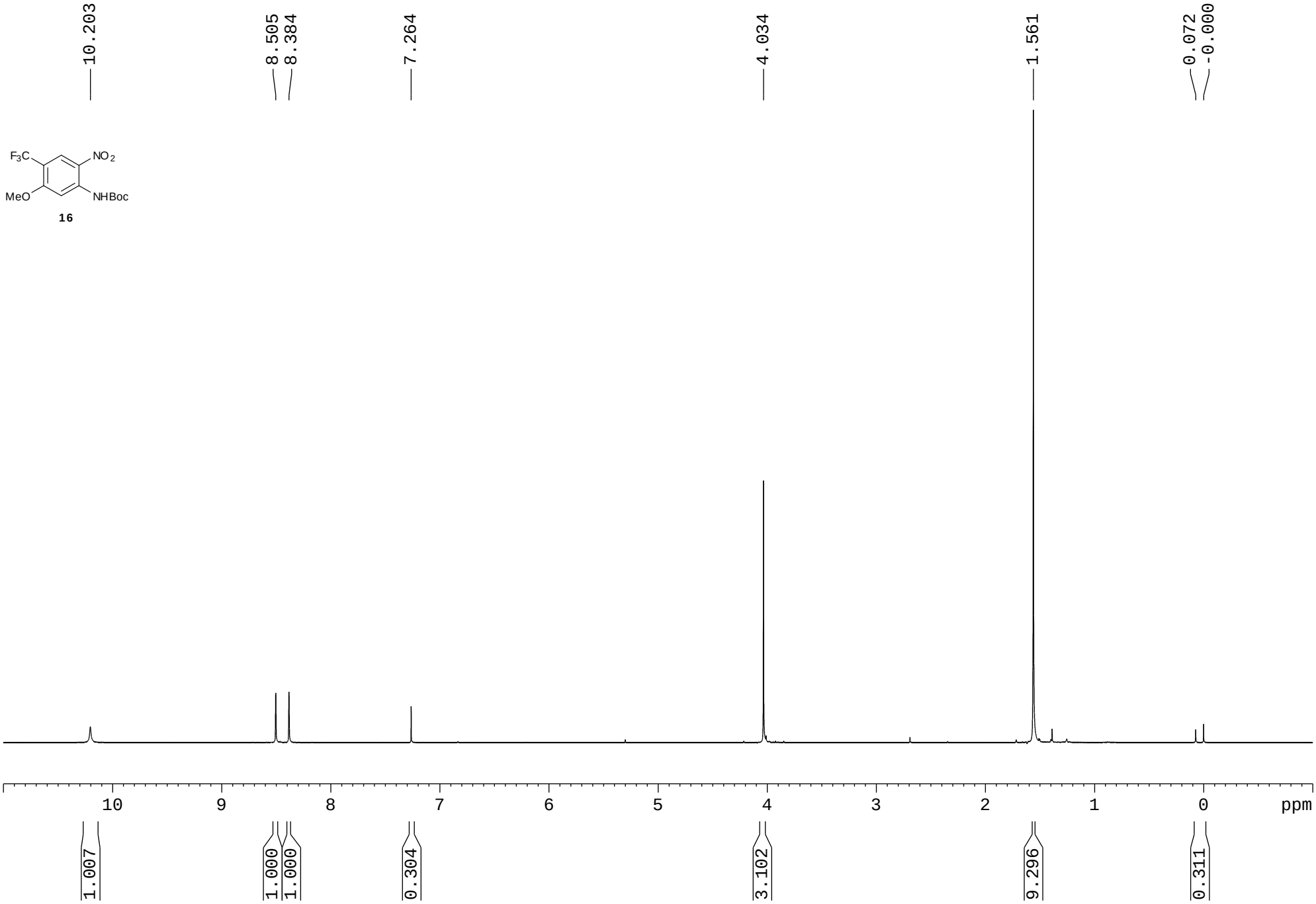

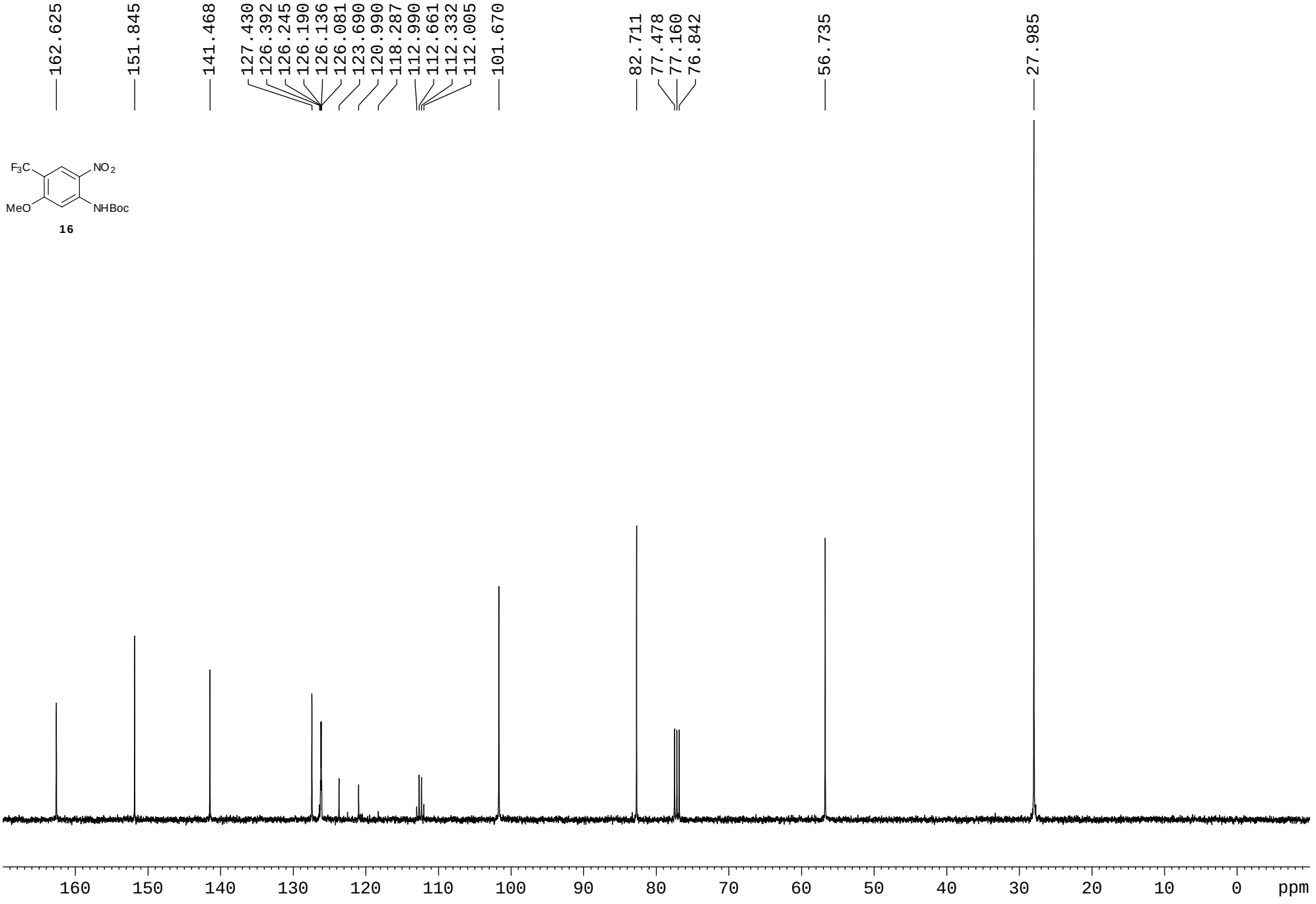

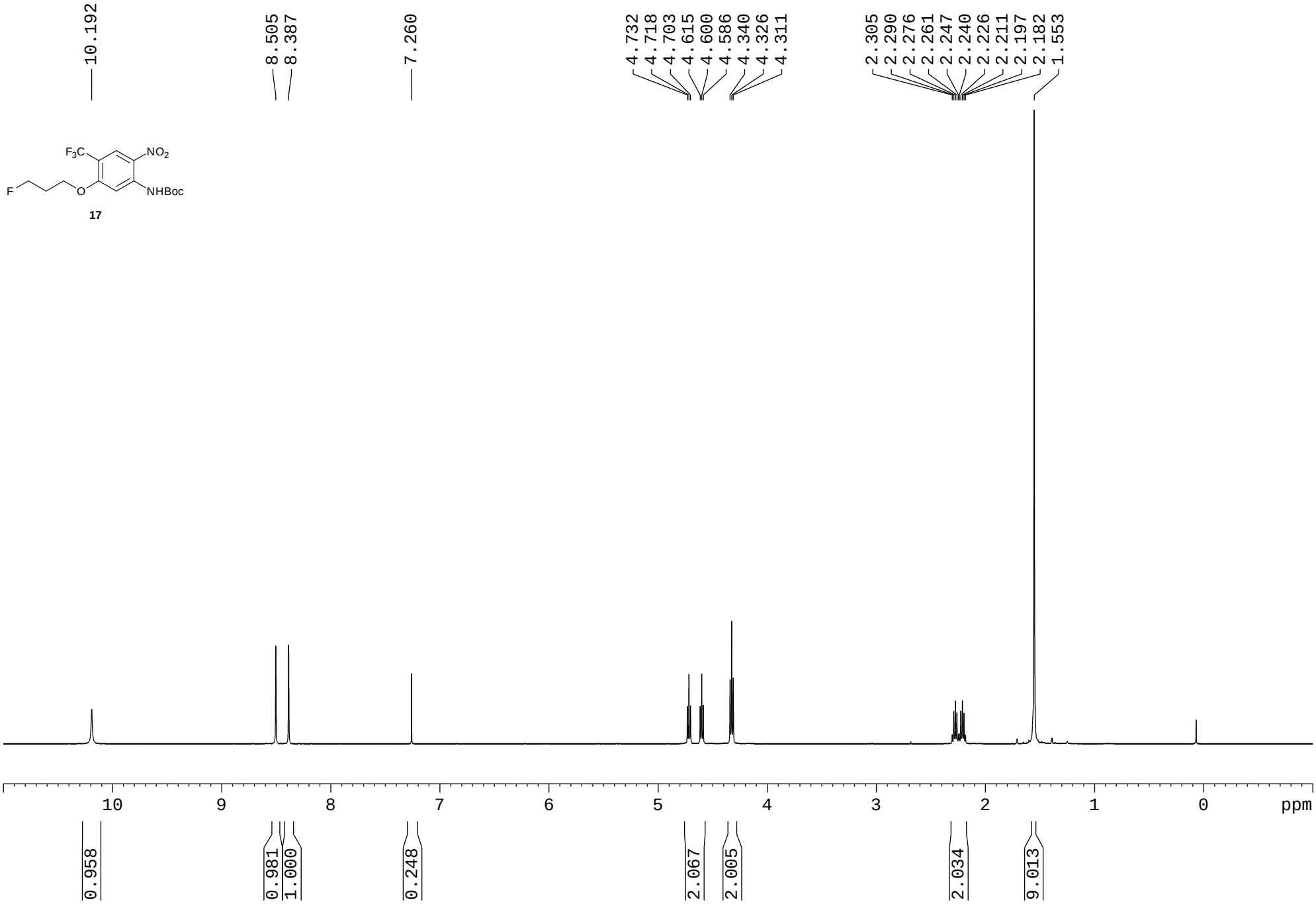

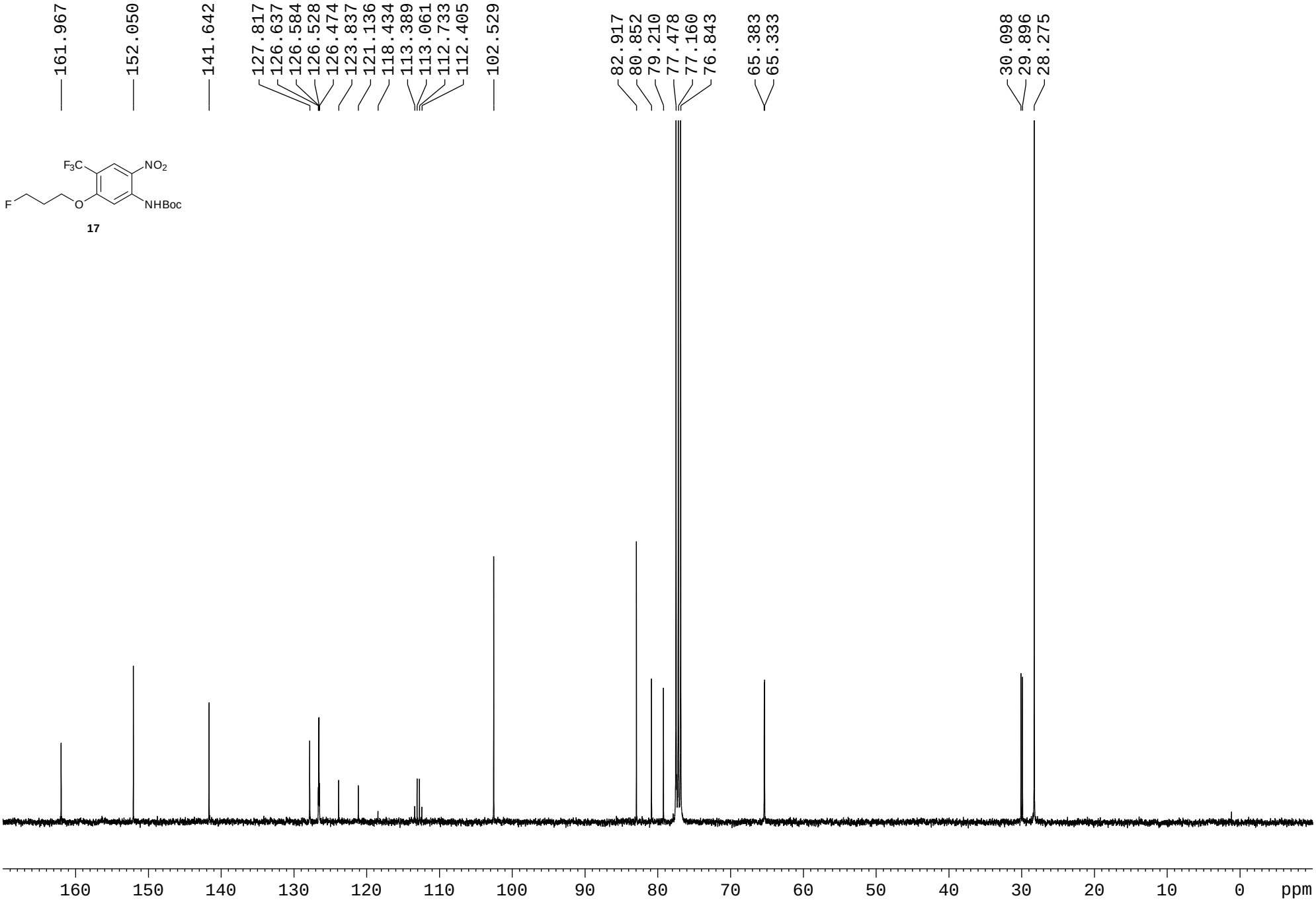

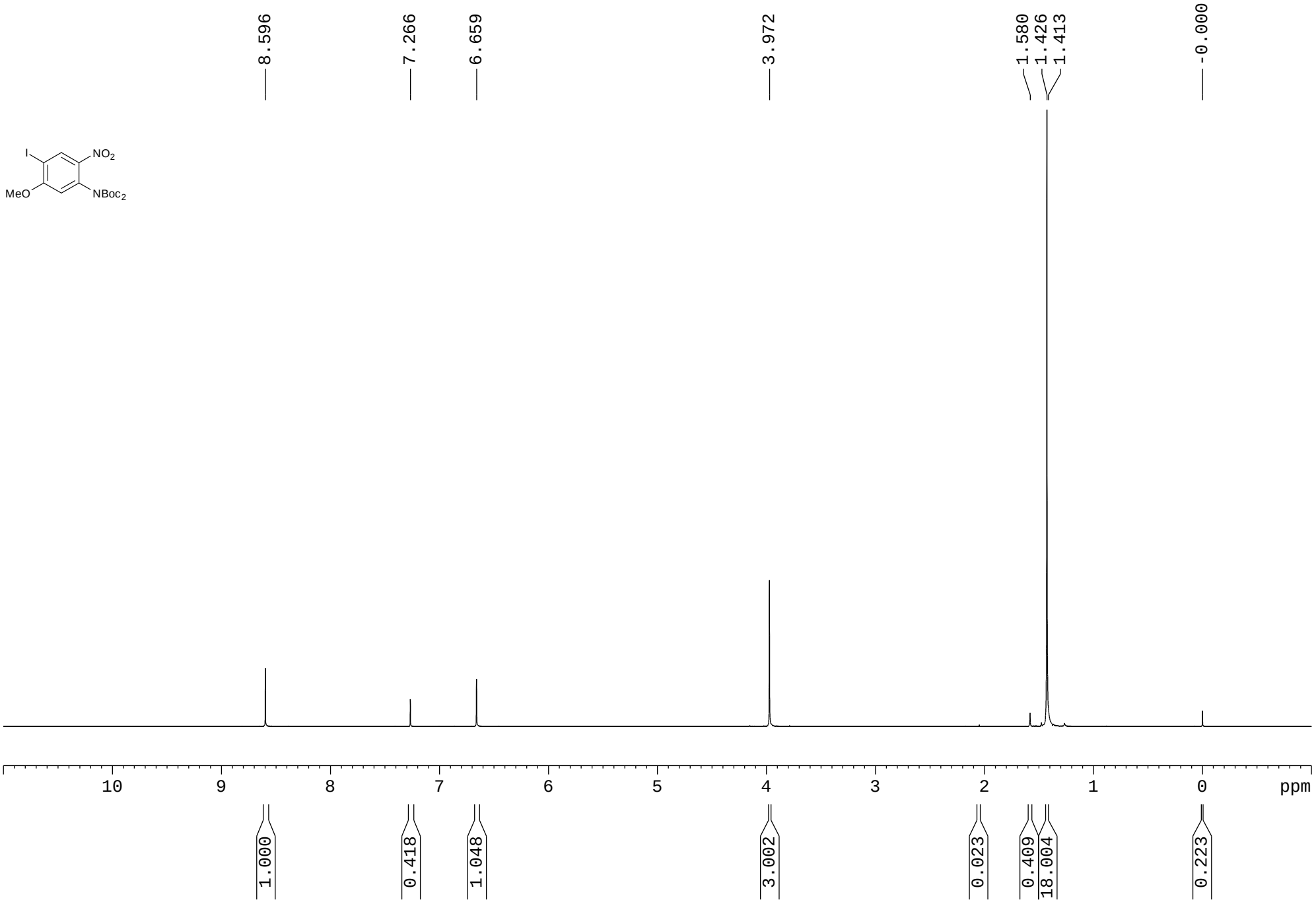

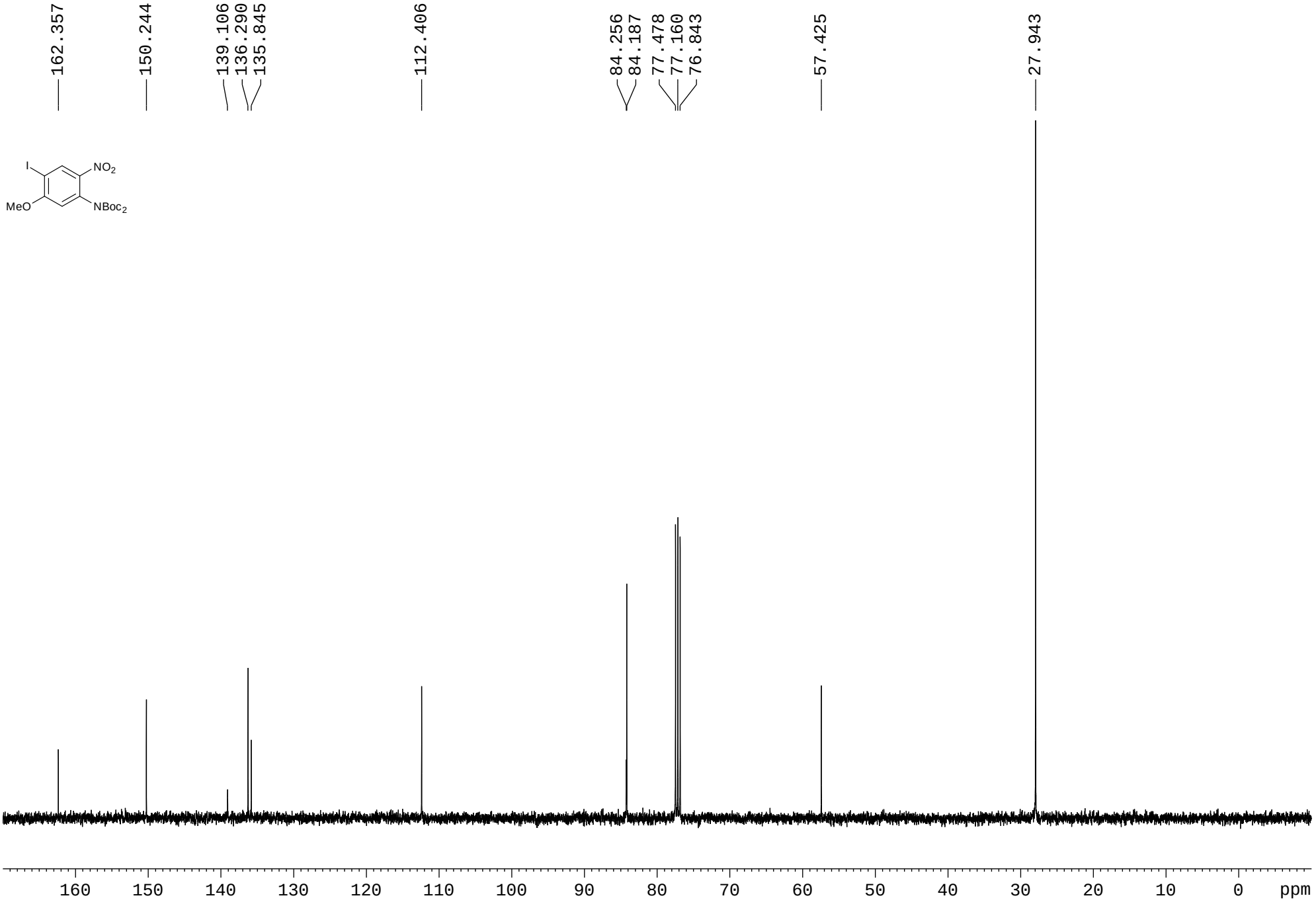

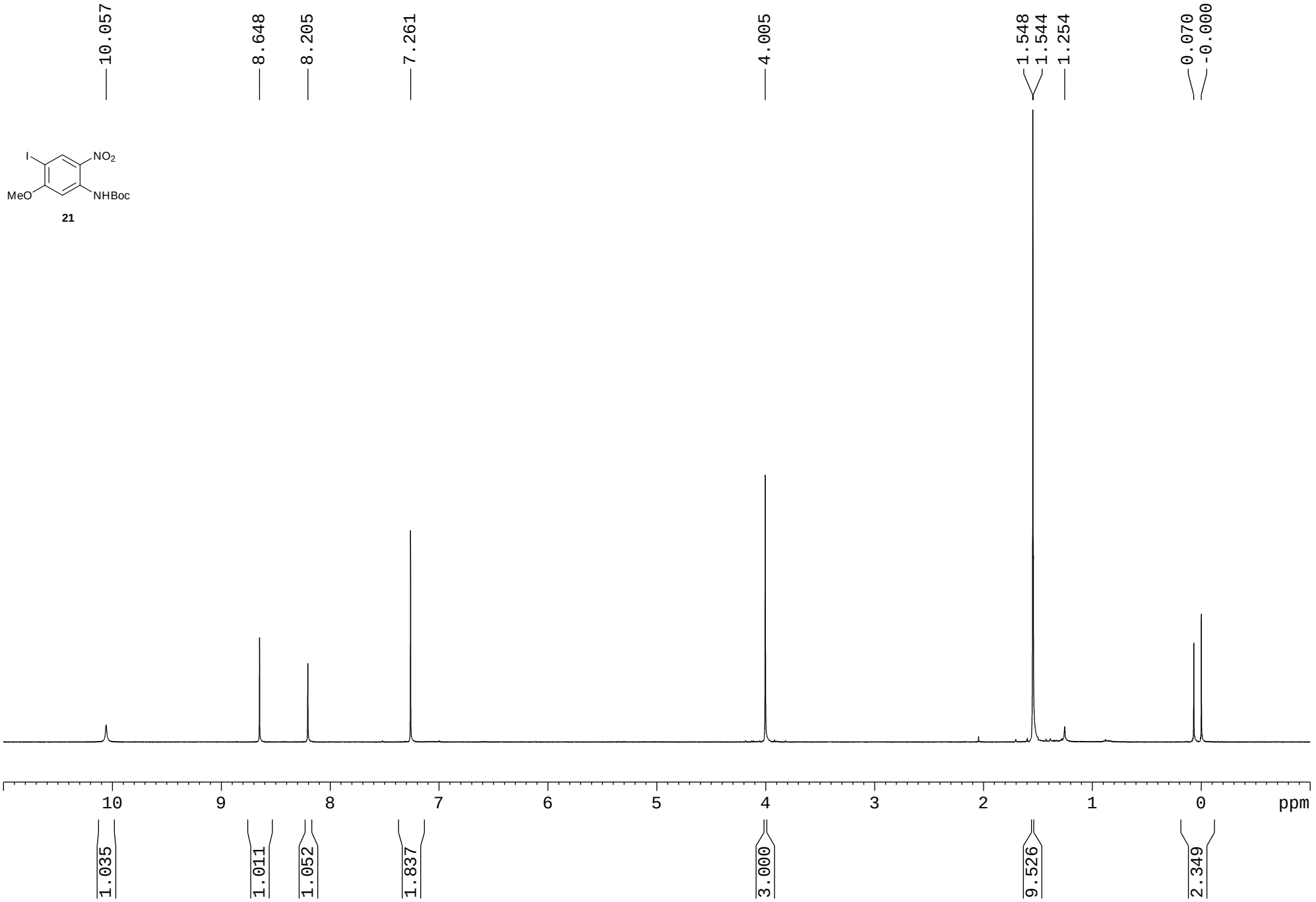

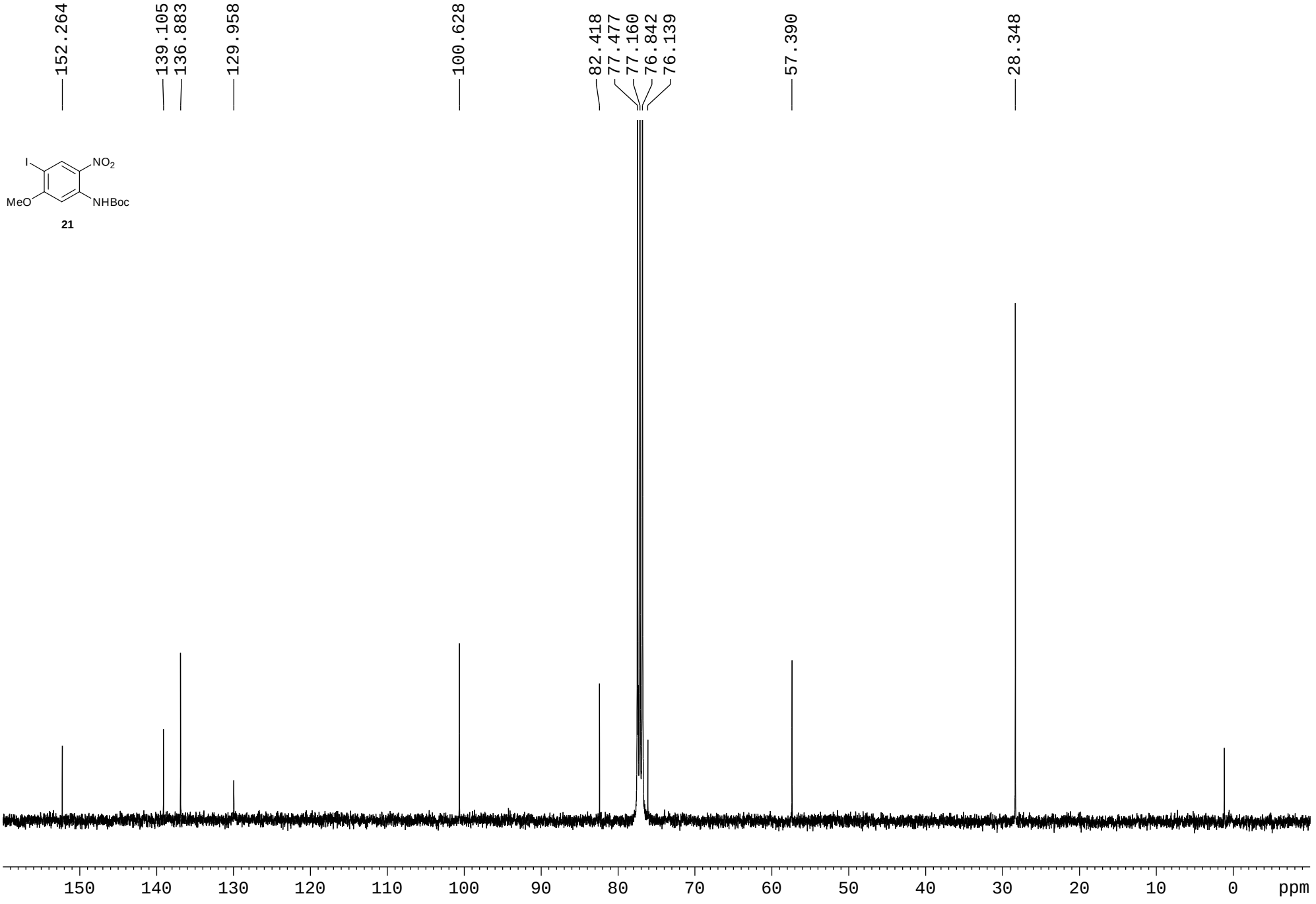

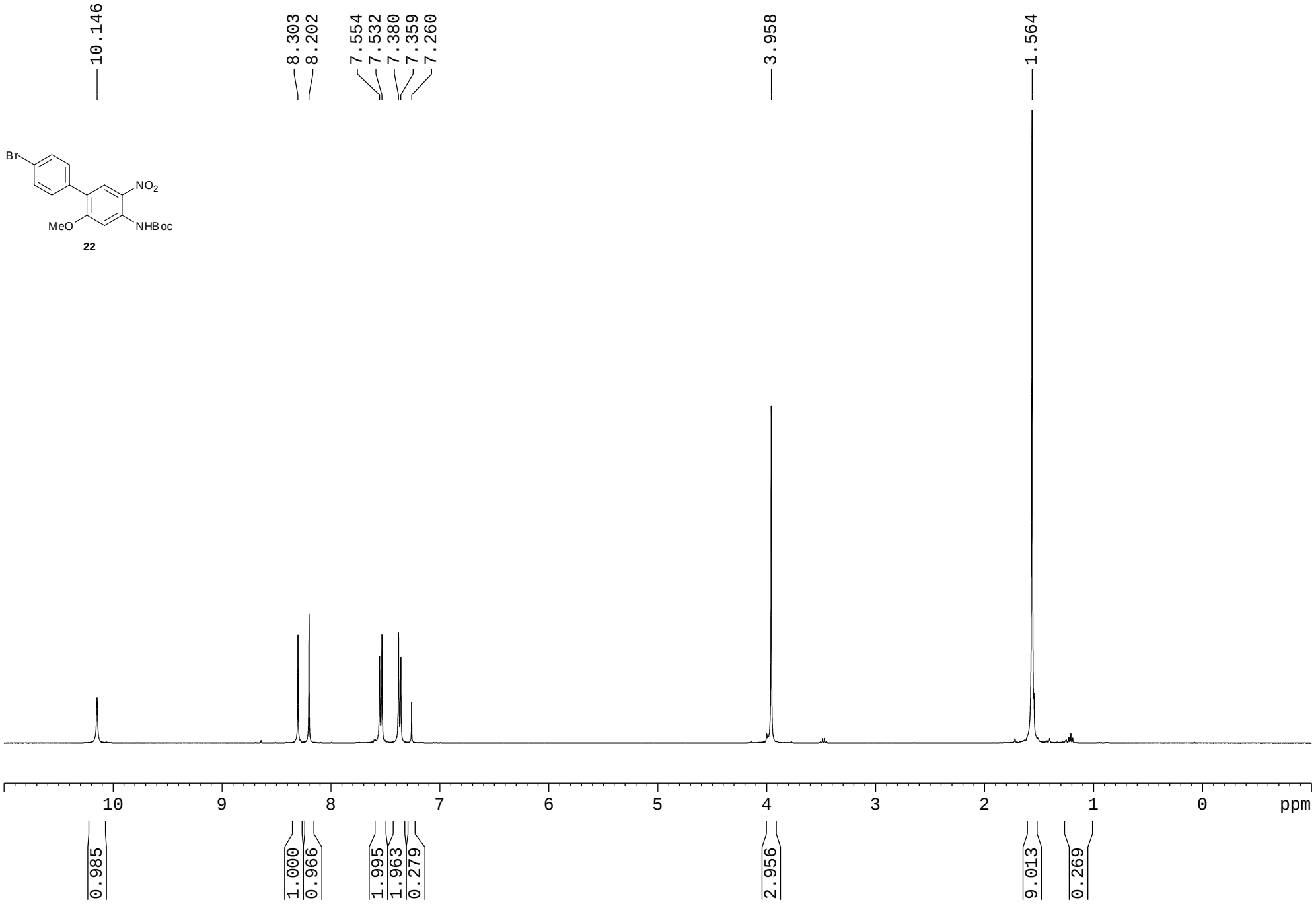

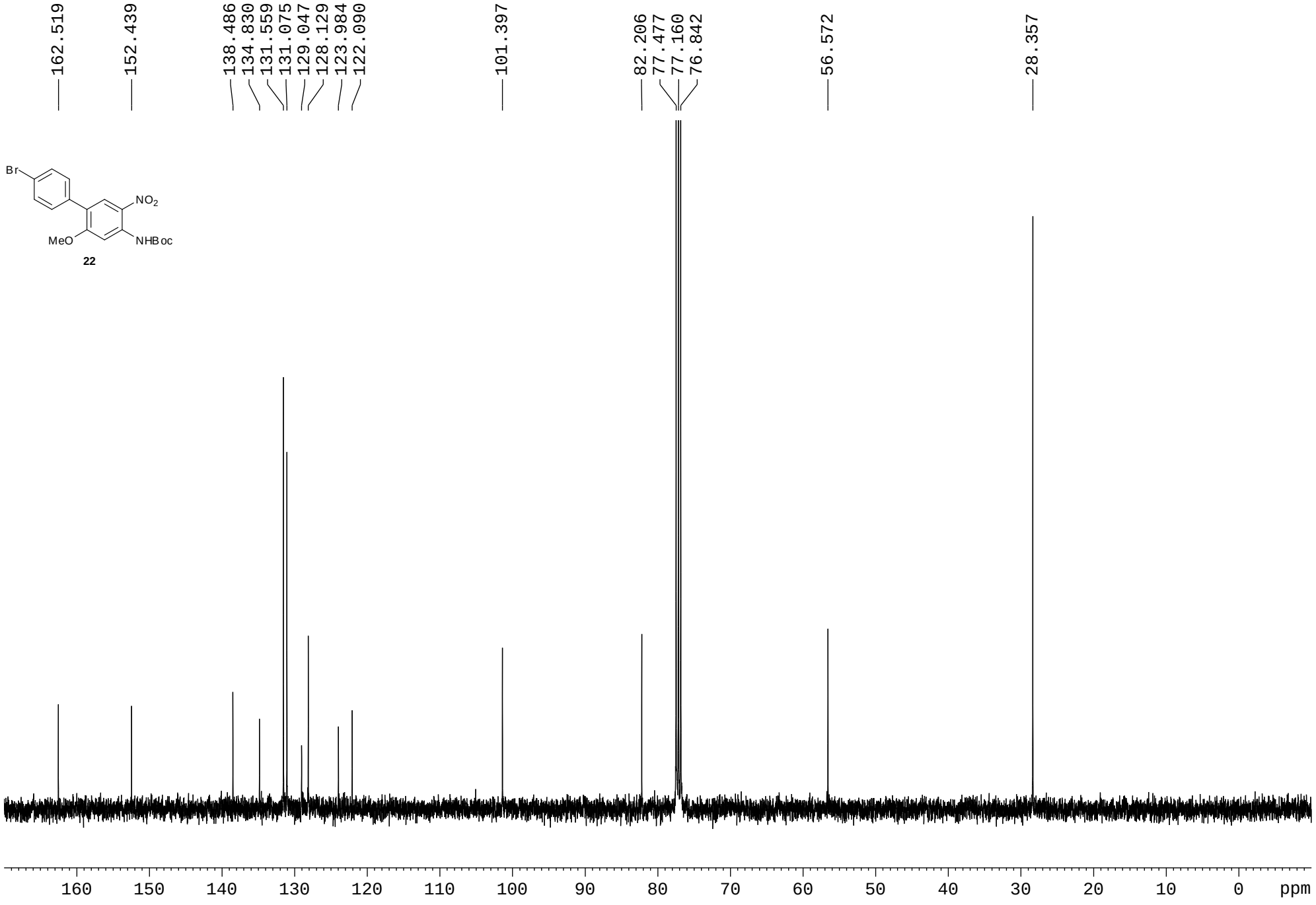

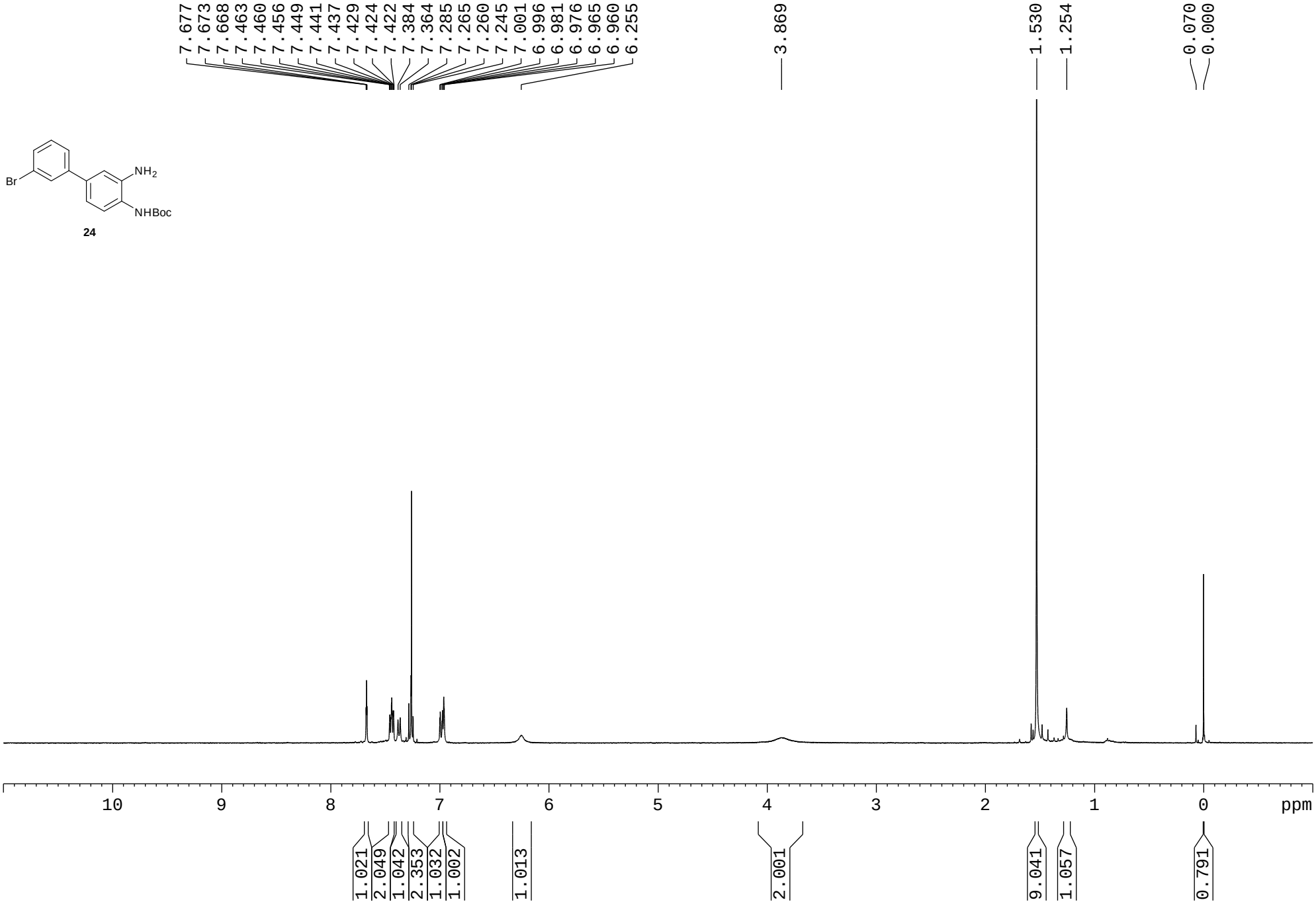

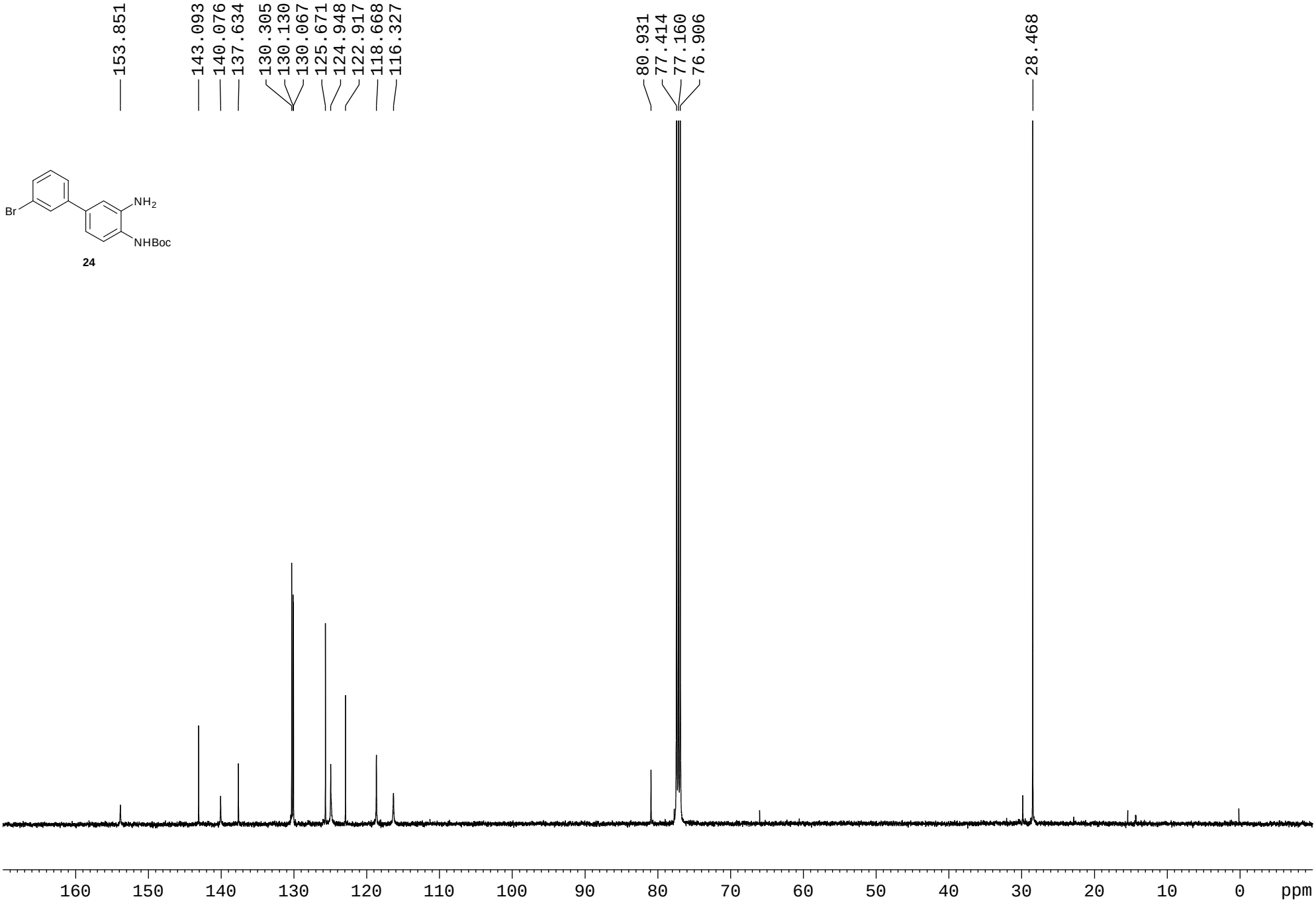

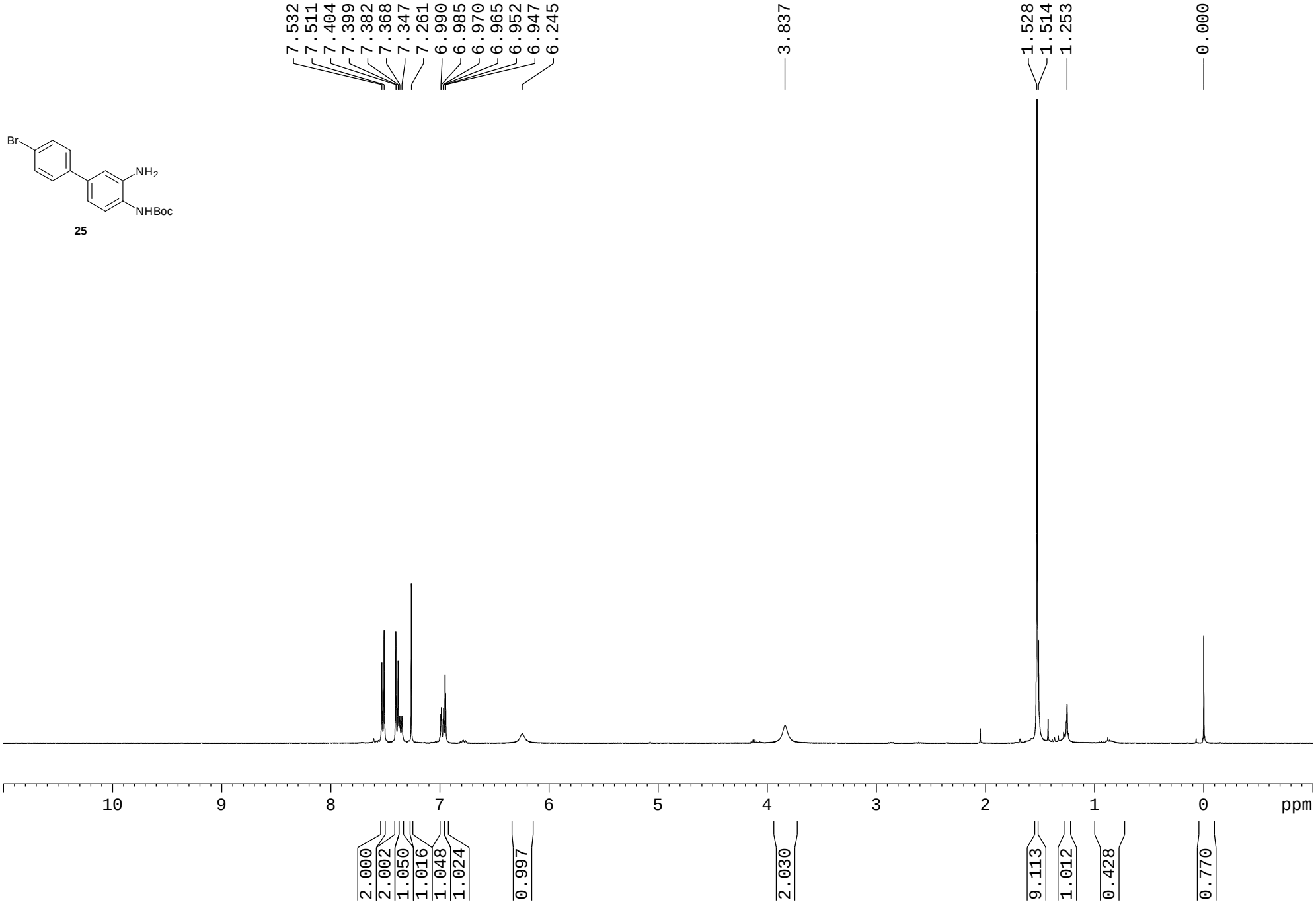

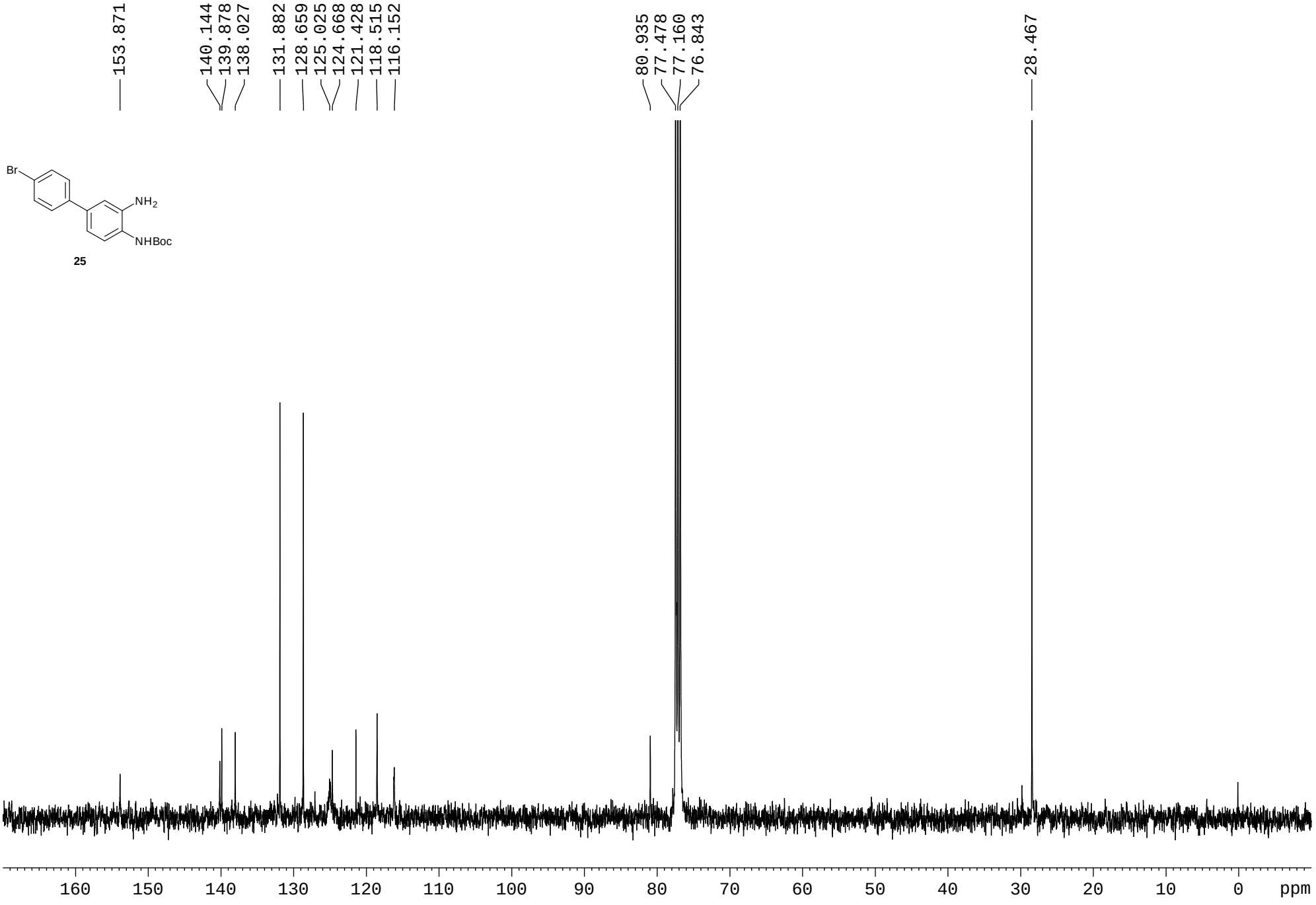

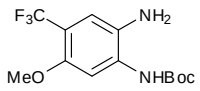

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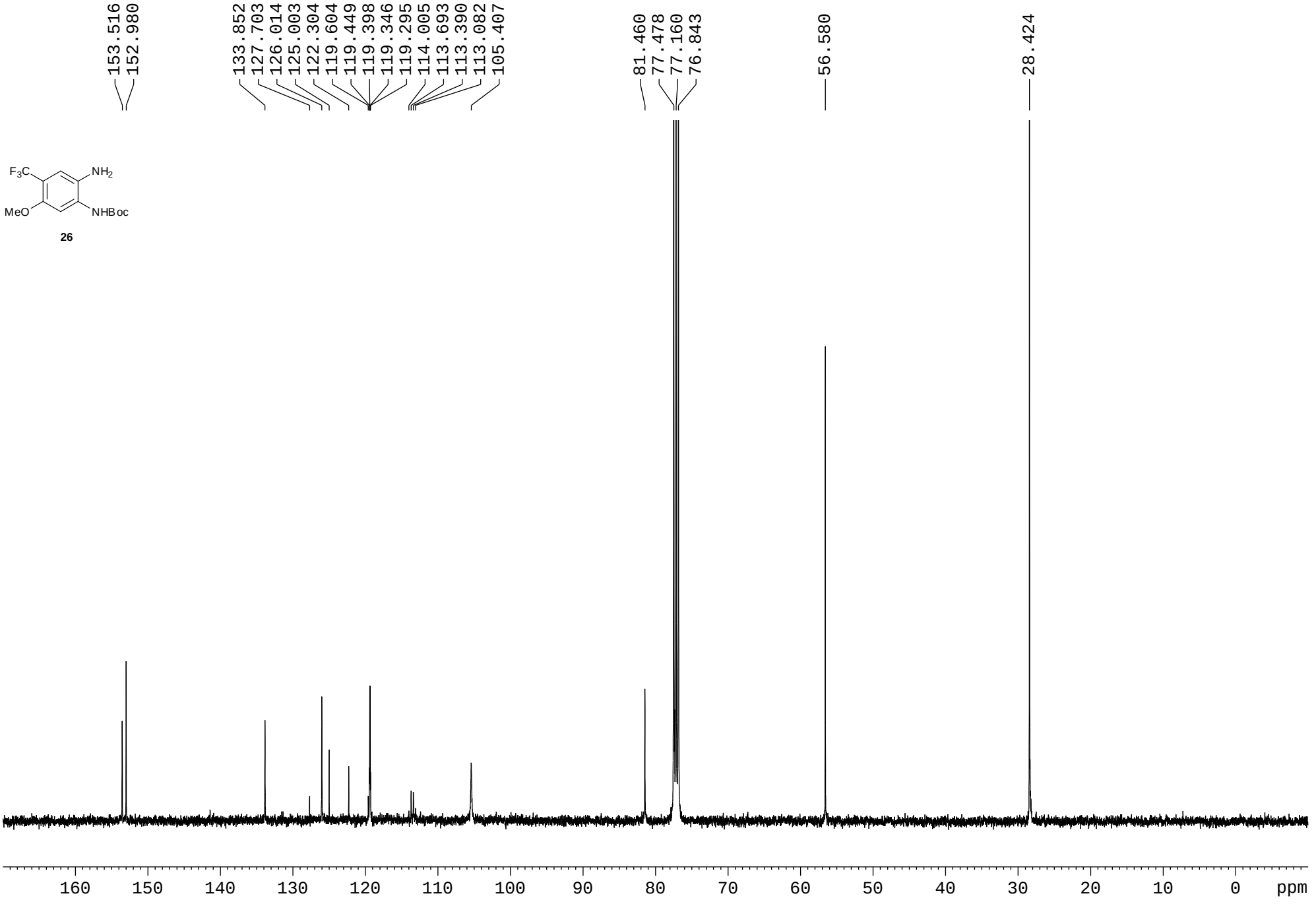

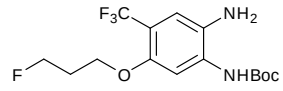

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