SUPPORTING INFORMATIONS

α-Ketothioamide derivatives: a promising tool to interrogate phosphoglycerate dehydrogenase (PHGDH)

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Content

I. Synthetic procedure of compounds 20-35

1-(2-Fluorophenyl)-2-morpholino-2-thioxoethanone (**20**). This compound was synthesized according to the general procedure describe above. 1-(2-Fluorophenyl)ethanone (1.50 g, 11.10 mmol) and dibromine (0.65 mL, 13.00 mmol) were mixed in chloroform (15 mL) to obtain the 2-bromo-1-(2-fluorophenyl)-ethanone and this intermediate was reacted in a second time with morpholine (2.84 mL, 32.50 mmol) and sulfur (0.52 g, 16.20 mmol) in DMF (10 mL). Methanol was used for recrystallization to afford the title compound as a yellow solid (1.09 g, 39%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 83-85°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.48-3.50 (m, 2H), 3.71-3.73 (m, 2H), 3.90-3.92 (t, 2H, J = 4.8 Hz), 4.26-4.28 (t, 2H, J = 4.8 Hz), 7.10-7.16 (Ddd, 1 ArH, J_{HF} = 10.9 Hz J_{HH} = 8.3 and 1.0 Hz), 7.29-7.33 (DDd, 1 ArH, J_{HH} = 7.8 and 1.0 Hz), 7.59-7.61 (m, 1 ArH), 8.00-8.05 (Ddd, 1 ArH, J_{HF} = 15.2 Hz J_{HH} = 7.6 and 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 44.97, 49.65, 63.86, 63.87, 114.30 (d, J_{CF} = 22 Hz), 120.91 (d, J_{CF} = 23 Hz), 122.74 (d, J_{CF} = 23 Hz), 129.49 (d, J_{CF} = 23 Hz), 133.76 (d, J_{CF} = 23 Hz), 158.06 (D, J_{CF} = 255 Hz), 181.35 (C=O), 194.23 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}FNO_2S$ $(M + H)^+$ 254.0645, found 254.0644.

1-(3-Fluorophenyl)-2-morpholino-2-thioxoethanone (**21**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(3-fluorophenyl)ethanone (0.50 g, 2.30 mmol), morpholine (0.6 mL, 6.90 mmol) and sulfur (0.11 g, 3.45 mmol) in DMF (10 mL). Methanol was used for recrystallization to afford the title compound as a yellow solid (0.13 g, 23%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 95-97°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.59-3.62 (t, 2H, J = 4.8 Hz), 3.70-3.73 (t, 2H, J = 4.8 Hz), 3.90-3.93 (t, 2H, J = 4.8 Hz), 4.32-4.34 (t, 2H, J = 4.8 Hz), 7.30-7.35 (DDdd, 1 ArH, J_{HF} = 16.4 Hz J_{HH} = 8.1 and 2.5 Hz), 7.46-7.51 (DDd, 1 ArH, J_{HH} = 8.1 Hz J_{HF} = 5.4 Hz), 7.68-7.72 (Ddd, 1 ArH, J_{HF} = 9.0 Hz J_{HH} = 1.6 Hz), 7.76-7.78 (Ddd, 1 ArH, J_{HH} = 7.7 Hz and 1.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.07, 51.87, 66.25, 66.38, 116.03 (d, J_{CF} = 23 Hz), 121.31 (d, J_{CF} = 23 Hz), 125.59 (d, J_{CF} = 23 Hz), 130.58 (d, J_{CF} = 23 Hz), 135.34 (d, J_{CF} = 23 Hz), 161.45 (D, J_{CF} = 247 Hz), 186.05 (C=O), 194.59 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}FNO_2S$ $(M + H)^+$ 254.0645, found 254.0644.

1-(4-Fluorophenyl)-2-morpholino-2-thioxoethanone (**22**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(4-fluorophenyl)ethanone (0.50 g, 2.30 mmol), morpholine (0.6 mL, 6.90 mmol) and sulfur (0.11 g, 3.45 mmol) in DMF

(10 mL). Methanol was used for recrystallization to afford the title compound as a beige solid (0.23 g, 41%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 131-133°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.59-3.71 (m, 4H), 3.89-3.91 (t, 2H, *J* = 4.8 Hz), 4.31-4.34 (t, 2H, *J* = 4.8 Hz), 7.15-7.19 (m, 2 ArH), 8.02-8.05 (m, 2 ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.17, 51.97, 66.39, 66.53, 116.19 (2C, d, *J* = 22 Hz), 129.74, 132.63 (2C, d, *J* = 9 Hz), 165.17 (D, *J* = 257 Hz), 186.38 (C=O), 195.16 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}FNO_2S$ $(M + H)^+$ 254.0645, found 254.0642.

1-(2-Chlorophenyl)-2-morpholino-2-thioxoethanone (**23**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(2-chlorophenyl)ethanone (0.50 g, 2.14 mmol), morpholine (0.56 mL, 6.42 mmol) and sulfur (0.10 g, 3.21 mmol) in DMF (10 mL). Acetonitrile was used for recrystallization to afford the title compound as a yellow solid (0.20 g, 36%). R_f 0.3 (cyclohexane/EtOAc 8:2). Mp: 78-80°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.82-3.92 (m, 6H), 4.26-4.28 (m, 2H), 7.40-7.60 (m, 3 ArH), 7.92-7.94 (m, 1 ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.70, 52.15, 66.01, 66.06, 127.29, 130.73, 132.41, 132.69, 133.80, 134.58, 185.07 (C=O), 195.72 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{13}ClNO_2S$ $(M + H)^+$ 270.0350, found 270.0350.

1-(3-Chlorophenyl)-2-morpholino-2-thioxoethanone (**24**). This compound was synthesized according to the general procedure describe above. 1-(3-Chlorophenyl)ethanone (2.00 g, 12.90 mmol) and dibromine (0.78 mL, 15.50 mmol) were mixed in chloroform (15 mL) to obtain the 2-bromo-1-(3-chlorophenyl)-ethanone and this intermediate was reacted in a second time with morpholine (3.38 mL, 38.80 mmol) and sulfur (0.62 g, 19.40 mmol) in DMF (10 mL). The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 8:2) and the obtained oil was collected by filtration with diethyl ether to give the title compound as a yellow solid (1.50 g, 43%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 92-94°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.59-3.61 (t, 2H, *J* = 4.8 Hz), 3.70-3.72 (t, 2H, *J* = 4.8 Hz), 3.90-3.92 (t, 2H, *J* = 4.8 Hz), 4.31-4.33 (t, 2H, *J* = 4.8 Hz), 7.42-7.46 (DD, 1 ArH, *J* = 7.8 Hz), 7.57-7.59 (Ddd, 1 ArH, *J* = 8.0 and 1.0 Hz), 7.85-7.87 (Ddd, 1 ArH, *J* = 7.8 and 1.4 Hz), 7.96-7.97 (dd, 1 ArH, *J* = 1.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.21, 52.00, 66.39, 66.52, 127.98, 129.61, 130.28, 134.33, 135.05, 135.30, 186.07 (C=O), 194.60 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}ClNO_2S$ $(M + H)^+$ 270.0277, found 270.0278.

1-(4-Chlorophenyl)-2-morpholino-2-thioxoethanone (**25**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(2-chlorophenyl)ethanone (0.50 g, 2.14 mmol), morpholine (0.56 mL, 6.42 mmol) and sulfur (0.10 g, 3.21 mmol) in DMF (10 mL). Acetonitrile was used for recrystallization to afford the title compound as a yellow solid (0.22 g, 38%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 135-137°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.58-3.71 (m, 4H), 3.89-3.92 (t, 2H, *J* = 4.8 Hz), 4.31-4.33 (t, 2H, *J* = 4.8 Hz), 7.46-7.48 (D, 2 ArH, *J* = 8.8 Hz), 7.93-7.95 (D, 2 ArH, *J* = 8.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.18, 51.97, 66.39, 66.53, 129.36 (2C), 131.22 (2C), 131.74, 141.06, 186.45 (C=O), 194.94 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{13}ClNO_2S$ $(M + H)^+$ 270.0350, found 270.0350.

1-(2-Bromophenyl)-2-morpholino-2-thioxoethanone (**26**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(2-bromophenyl)-ethanone (0.50 g, 1.81 mmol), morpholine (0.48 mL, 5.43 mmol) and sulfur (0.08 g, 2.72 mmol) in DMF (10 mL). Ethanol was used for recrystallization to afford the title compound as a colorless solid (0.28 g, 52%). R_f 0.3 (cyclohexane/EtOAc 8:2). Mp: 81-83°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.48-3.85 (m, 4H), 3.90-3.92 (t, 2H, *J* = 4.8 Hz), 4.25-4.28 (t, 2H, *J* = 4.8 Hz), 7.27-7.40 (ddd, 1 ArH, *J* = 1.8 and 7.8 Hz), 7.40-7.46 (ddd, 1 ArH, *J* = 1.2 and 7.5 Hz), 7.59-7.62 (dd, 1 ArH, *J* = 1.1 and 7.9 Hz), 7.84-7.87 (dd, 1 ArH, *J* = 1.8 and 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.86, 52.28, 66.02, 66.05, 120.59, 127.69, 132.96, 133.64, 134.02, 136.45, 185.56 (C=O), 194.99 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}BrNO_2S$ $(M + H)^+$ 313.9844, found 313.9845.

1-(3-Bromophenyl)-2-morpholino-2-thioxoethanone (**27**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(3-bromophenyl)-ethanone (0.50 g, 1.81 mmol), morpholine (0.48 mL, 5.43 mmol) and sulfur (0.08 g, 2.72 mmol) in DMF (10 mL). Methanol was used for recrystallization to afford the title compound as a colorless solid (0.14 g, 26%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 104-106°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.61-3.64 (t, 2H, *J* = 4.8 Hz), 3.72-3.74 (t, 2H, *J* = 4.8 Hz), 3.90-3.93 (t, 2H, *J* = 4.8 Hz), 4.33-4.35 (t, 2H, *J* = 4.8 Hz), 7.36-7.40 (m, 1 ArH), 7.73-7.75 (m, 1 ArH), 7.90-7.92 (m, 1 ArH), 8.13-8.15 (m, 1 ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 44.79, 49.58, 63.97, 64.11, 120.80, 126.03, 128.08, 130.10, 132.81, 134.81, 183.54 (C=O), 192.09 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}BrNO_2S$ $(M + H)^+$ 313.9844, found 313.9844.

1-(4-Bromophenyl)-2-morpholino-2-thioxoethanone (**28**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(4-bromophenyl)-ethanone (0.50 g, 1.81 mmol), morpholine (0.47 mL, 5.44 mmol) and sulfur (0.08 g, 2.71 mmol) in DMF (10 mL). Cyclohexane was used for recrystallization to afford the title compound as a colorless solid (0.11 g, 19%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 157-159°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.58-3.61 (t, 2H, *J* = 4.8 Hz), 3.69-3.72 (t, 2H, *J* = 4.8 Hz), 3.89-3.92 (t, 2H, *J* = 4.8 Hz), 4.31-4.34 (t, 2H, *J* = 4.8 Hz), 7.63-7.65 (D, 2 ArH, *J* = 8.6 Hz), 7.85-7.87 (D, 2 ArH, *J* = 8.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.18, 51.97, 66.39, 66.54, 129.91, 131.26 (2C), 132.17, 132.35 (2C), 186.59 (C=O), 194.89 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}BrNO_2S$ $(M + H)^+$ 313.9844, found 313.9841.

1-(2-Iodophenyl)-2-morpholino-2-thioxoethanone (**29**). This compound was synthesized according to the general procedure describe above. 1-(2-Iodophenyl)ethanone (1.00 g, 4.00 mmol) and dibromine (0.24 mL, 4.87 mmol) were mixed in chloroform (15 mL) to obtain the 2-bromo-1-(2-iodophenyl)-ethanone and this intermediate was reacted in a second time with morpholine (1.06 mL, 12.20 mmol) and sulfur (0.19 g, 6.10 mmol) in DMF (10 mL). The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 8:2) to give the title compound as a yellow oil (0.70 g, 47%). R_f 0.2 (cyclohexane/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.80-3.85 (m, 4H), 3.91-3.93 (t, 2H, *J* = 4.8 Hz), 4.27-4.30 (t, 2H, *J* = 4.8 Hz), 7.17-7.21 (DDd, 1 ArH, *J* = 7.5 and 1.5 Hz), 7.43-7.47 (Ddd, 1 ArH, *J* = 7.5 and 1.1 Hz), 7.74-7.77 (Dd, 1 ArH, *J* = 7.8 and 1.5 Hz), 7.97-7.99 (Dd, 1 ArH, *J* = 7.9 and 1.1 Hz). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{13}INO_2S$ $(M + H)^+$ 361.9706, found 361.9706.

1-(3-Iodophenyl)-2-morpholino-2-thioxoethanone (**30**). This compound was synthesized according to the general procedure describe above. 1-(3-Iodophenyl)ethanone (1.00 g, 4.06 mmol) and dibromine (0.24 mL, 4.87 mmol) were mixed in chloroform (15 mL) to obtain the 2-bromo-1-(3-iodophenyl)-ethanone and this intermediate was reacted in a second time with morpholine (1.07 mL, 12.18 mmol) and sulfur (0.19 g, 6.09 mmol) in DMF (10 mL). The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 8:2) to give the title compound as a yellow solid (0.75 g, 51%). R_f 0.3 (cyclohexane/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.60-3.625 (t, 2H, *J* = 4.8 Hz), 3.70-3.72 (t, 2H, *J* = 4.8 Hz), 3.91-3.93 (t, 2H, *J* = 4.8 Hz), 4.31-4.33 (t, 2H, *J* = 4.8 Hz), 7.21-7.25 (DD, 1 ArH, *J* = 7.8 Hz),

7.92-7.94 (Dd, 2 ArH, J = 7.1 and 0.7 Hz), 8.32-8.33 (dd, 1 ArH, J = 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 46.60, 51.40, 65.79, 65.92, 93.94, 128.43, 129.94, 134.59, 137.76, 142.47, 185.29 (C=O), 193.88 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{13}INO_2S$ $(M + H)^+$ 361.9706, found 361.9704.

1-(4-Iodophenyl)-2-morpholino-2-thioxoethanone (**31**). This compound was synthesized according to the general procedure describe above. 1-(4-Iodophenyl)ethanone (1.00 g, 4.06 mmol) and dibromine (0.24 mL, 4.87 mmol) were mixed in chloroform (15 mL) to obtain the 2-bromo-1-(3-iodophenyl)-ethanone and this intermediate was reacted in a second time with morpholine (1.07 mL, 12.18 mmol) and sulfur (0.19 g, 6.09 mmol) in DMF (10 mL). The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 8:2) to give the title compound as a yellow solid (0.92 g, 63%). R_f 0.3 (cyclohexane/EtOAc 8:2). Mp: 175-177°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.59 (m, 4H), 3.82 (t, 2H, J = 4.8 Hz), 4.21 (t, 2H, J = 4.8 Hz), 7.67 (D, 2 ArH, J = 8.1 Hz), 7.98 (D, 2ArH, J = 8.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 46,92, 51.83, 65.54, 65.88, 103.86, 130.92 (2C), 132.27, 138.13 (2C), 186.51 (C=O), 192.99 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{13}INO_2S$ $(M + H)^+$ 361.9706, found 361.9705.

1-(2-Nitrophenyl)-2-morpholino-2-thioxoethanone (**32**). This compound was synthesized according to the general procedure describe above using 2-bromo-1-(2-nitrophenyl)-ethanone (0.50 g, 2.05 mmol), morpholine (0.54 mL, 6.17 mmol), and sulfur (0.09 g, 3.07 mmol) in DMF (10 mL). A mixture of cyclohexane/EtOAc (8:2) was used for recrystallization to afford the title compound as a yellow solid (0.15 g, 26%). R_f 0.3 (cyclohexane/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.86-3.88 (t, 2H, J = 4.8 Hz), 3.94-3.96 (t, 2H, J = 4.8 Hz), 4.11-4.13 (t, 2H, J = 4.8 Hz), 4.22-4.24 (t, 2H, J = 4.8 Hz), 7.62-7.66 (m, 1 ArH), 7.74-7.78 (m, 1 ArH), 7.84-7.86 (m, 1 ArH), 8.04-8.06 (m, 1 ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 49.06, 52.33, 66.36, 66.71, 123.84, 131.70, 132.57, 134.03, 134.92, 145.78, 182.95 (C=O), 191.17 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}N_2O_4S$ $(M + H)^+$ 281.0590, found 281.0586.

1-(3-Nitrophenyl)-2-morpholino-2-thioxoethanone (**33**). This compound was synthesized according to the general procedure describe above using commercial 2-bromo-1-(3-nitrophenyl)-ethanone (1.50 g, 6.17 mmol), morpholine (1.61 mL, 18.52 mmol) and sulfur (0.29 g, 9.26 mmol) in DMF (10 mL). Methanol was used for recrystallization to afford the

title compound as a yellow solid (1.26 g, 73%). R_f 0.1 (cyclohexane/EtOAc 8:2). Mp: 179-181°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.58-3.60 (t, 2H, *J* = 4.8 Hz), 3.67-3.69 (t, 2H, *J* = 4.8 Hz), 3.86-3.89 (t, 2H, *J* = 4.8 Hz), 4.28-4.30 (t, 2H, *J* = 4.8 Hz), 7.63-7.67 (DD, 1 ArH, *J* = 8.6 Hz), 8.26-8.29 (m, 1 ArH), 8.38-8.40 (m, 1 ArH), 8.73-8.74 (dd, 1 ArH, *J* = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 47.42, 52.12, 66.41, 66.57, 124.56, 128.36, 130.20, 135.18, 148.51, 184.29 (C=O), 193.48 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}N_2O_4S$ $(M + H)^+$ 281.0590, found 281.0588.

1-(4-Nitrophenyl)-2-morpholino-2-thioxoethanone (**34**). This compound was synthesized according to the general procedure describe above using commercial 2-bromo-1-(4-nitrophenyl)-ethanone (1.50 g, 6.17 mmol), morpholine (1.61 mL, 18.52 mmol) and sulfur (0.29 g, 9.26 mmol) in DMF (10 mL). Methanol was used for recrystallization to afford the title compound as a yellow solid (0.81 g, 47%). R_f 0.1 (cyclohexane/EtOAc 8:2). Mp: 171-173°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm)) 3.59 (t, 2H, *J* = 4.8 Hz), 3,73 (t, 2H, *J* = 4.8 Hz), 3.92 (t, 2H, *J* = 4.8 Hz), 4.33 (t, 2H, *J* = 4.8 Hz), 8.16 (D, 2 ArH, *J* = 8.2 Hz), 8.32 (D, 2 ArH, *J* = 8.2 Hz). HRMS (ESI^+): *m/z* calcd for $C_{12}H_{12}N_2O_4S$ $(M + H)^+$ 281.0590, found 281.0588.

1-([1,1'-biphenyl]-4-yl)-2-morpholino-2-thioxoethanone (**35**). This compound was synthesized according to the general procedure describe above using commercial 1-([1,1'-biphenyl]-4-yl)-2-bromoethanone (1.00 g, 3.64 mmol), morpholine (0.94 mL, 10.92 mmol) and sulfur (0.17 g, 5,46 mmol) in DMF (10 mL). The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 8:2) to give the title compound as a yellow solid (0.69 g, 61%). R_f 0.2 (cyclohexane/EtOAc 8:2). Mp: 132-134°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 3.55-3.58 (t, 2H, *J* = 4.8 Hz), 3.68-3.71 (t, 2H, *J* = 4.8 Hz), 3.82-3.85 (t, 2H, *J* = 4.8 Hz), 4.28-4.30 (t, 2H, *J* = 4.8 Hz), 7.32-7.45 (m, 3 ArH), 7.52-7.55 (m, 2 ArH), 7.61-7.63 (D, 2 ArH, *J* = 8.5 Hz), 7.99-8.01 (D, 2 ArH, *J* = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 45.93, 50.75, 65.19, 65.34, 126.12 (2C), 126.40 (2C), 127.38, 127.84 (2C), 129.23 (2C), 130.72, 138.32, 146.01, 186.33 (C=O), 194.52 (C=S). HRMS (ESI^+): *m/z* calcd for $C_{18}H_{17}NO_2S$ $(M + H)^+$ 312.1052, found 312.1052.

II. Enzymatic assay optimization

PHGDH oxidizes 3-PG to 3-PPyr with NAD^+ as the electron acceptor to yield NADH. The formation of 3-PPyr is directly correlated with the NADH formation (Ex 340 nm / Em 460 nm). Thus, the enzymatic activity of PHGDH can be monitored by following the fluorescence intensity at an excitation wavelength of 340 nm and emission wavelength of 460 nm. Prior to developing a robust quantitative assay, we initially set out to optimize the activity of the PHGDH. Oxidoreductase activity was screened at varying enzyme, cofactor and substrate concentrations by monitoring the oxidation of NADH spectrophotometrically. The tolerance of the enzymatic assay to DMSO was studied at a DMSO concentration ranging from 5% to 20% (**Fig. S1**). For this present study, the concentrations of Tris HCl pH 8.8, NaCl and DTT were set at 100 mM, 400 mM and 0.2 mM, respectively, and the temperature was kept at 25°C.

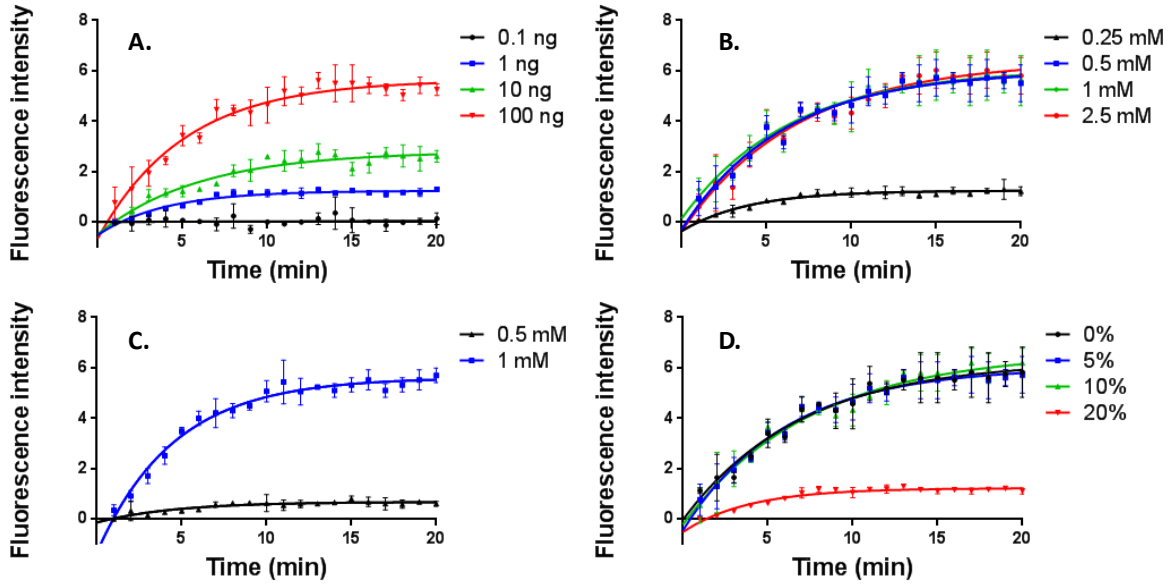


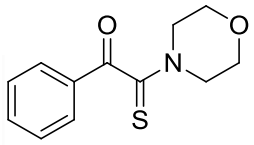
Figure S1. Optimization of the enzymatic assay for drug screening. (**A**) Variation of PHGDH concentration (2.5 mM 3-PG, 1 mM NAD). (**B**) Variation of 3-PG concentration (100 ng PHGDH, 1 mM NAD). (**C**) Variation of NAD concentration (100 ng PHGDH, 2.5 mM 3-PG). (**D**) Influence of DMSO (100 ng PHGDH, 2.5 mM 3-PG, 1 mM NAD). Data were analyzed using GraphPad software.

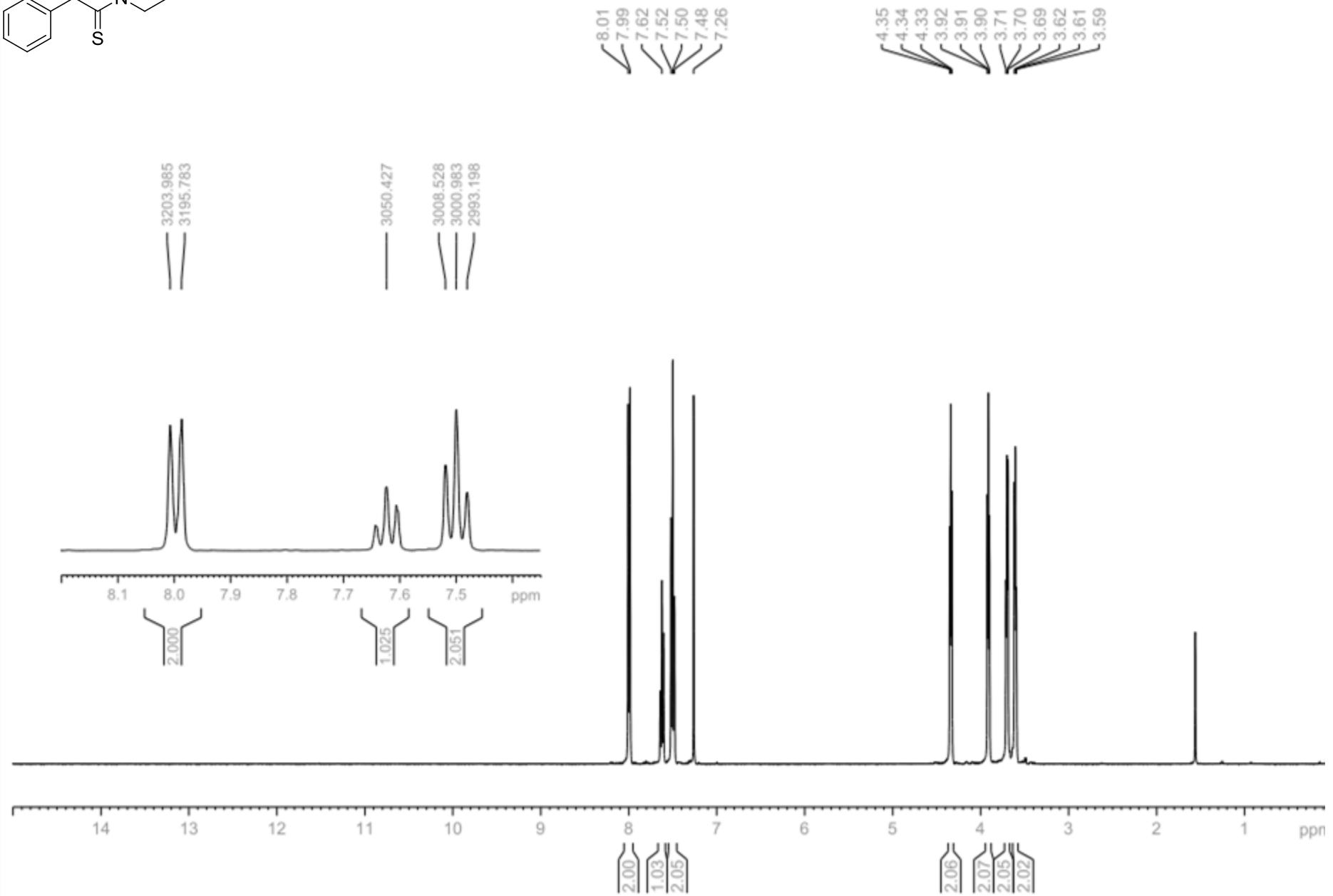
Minimizing the amount of enzyme was an important consideration for controlling the cost of assays. Thus, we sought to determine enzyme concentrations that would generate a sufficient fluorescent signal. Representative curves obtained with PHGDH quantity ranging from 1 ng to 100 ng, are shown in **Fig. S1A**. As depicted, a quantity of 100 ng of PHGDH was sufficient to achieve a robust assay window. The concentration of 3-PG and NAD^+ in an assay is also an important consideration. Concentration of 3- PG was varied between 0.25 and 2.5 mM and two concentrations of NAD^+ were evaluated (**Fig. S1B** and **Fig. S1C**). Optimal concentrations of 3-PG and NAD were 0.5 mM and 1 mM respectively to detect a correct fluorescent signal. Compounds from the database were stored at 10 mM in DMSO and the final concentration of DMSO in assay solutions was 10%. As depicted in

Fig. S1D, this percentage of DMSO was tolerated by PHGDH. Thus, the optimized conditions consisted of PHGDH (100 ng), 3-PG (0.5 mM), and NAD (1 mM). In these conditions, the rate of substrate conversion was found to be linear during the five first minutes. The K_m value of 3-PG was then determined by fitting the data to the Michaelis–Menten equation. The calculated K_m value (0.19 ± 0.03 mM) is consistent with the reported K_m value of 0.26 ± 0.03 mM.

III. NMR Spectral Data

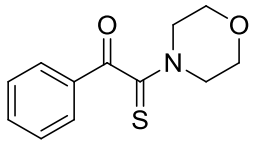
1-Phenyl-2-morpholino-2-thioxoethanone (**19**) **^{1}H NMR**

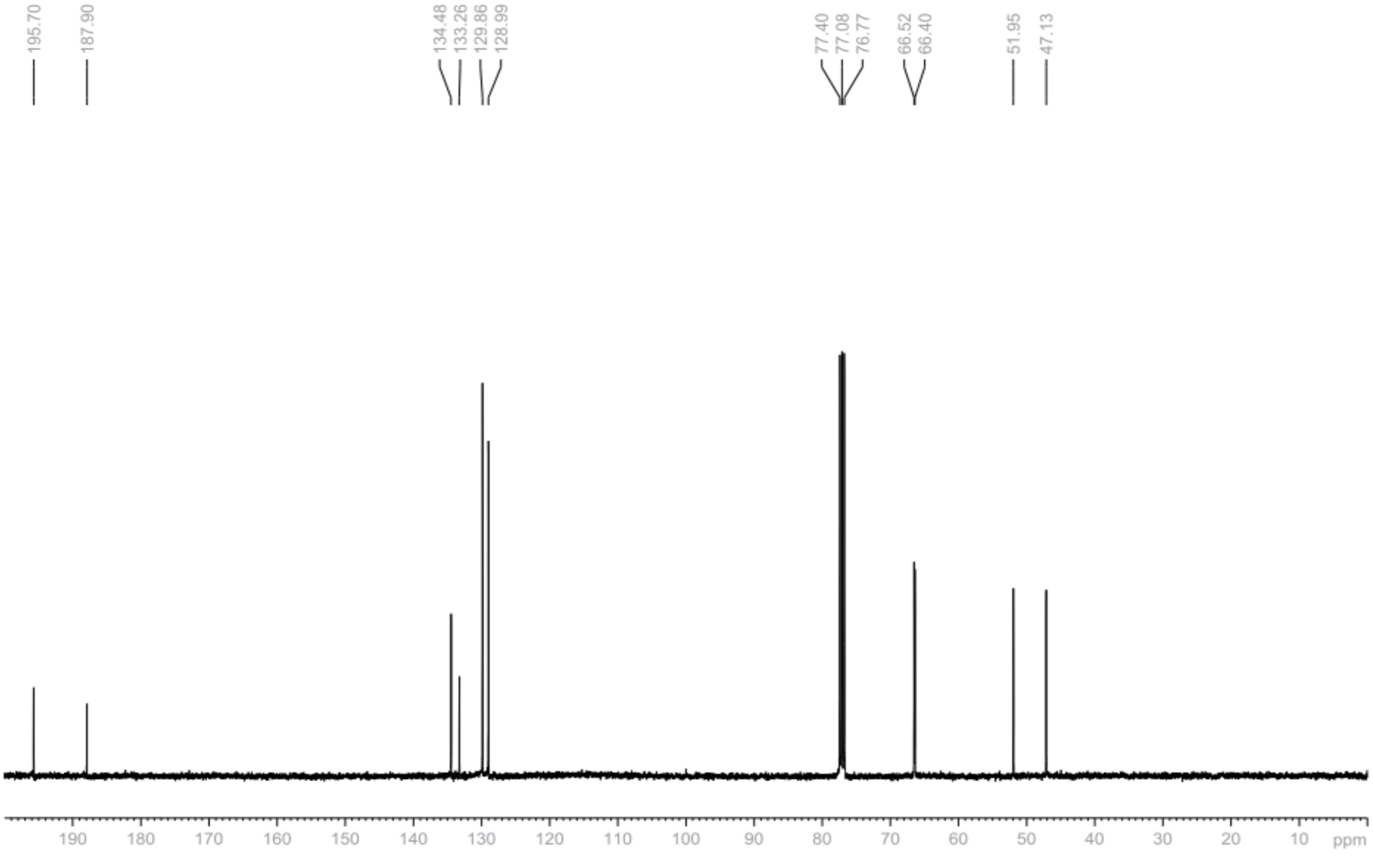




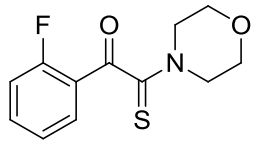
1-Phenyl-2-morpholino-2-thioxoethanone (**19**)

^{13}C NMR

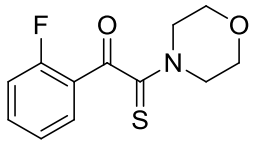


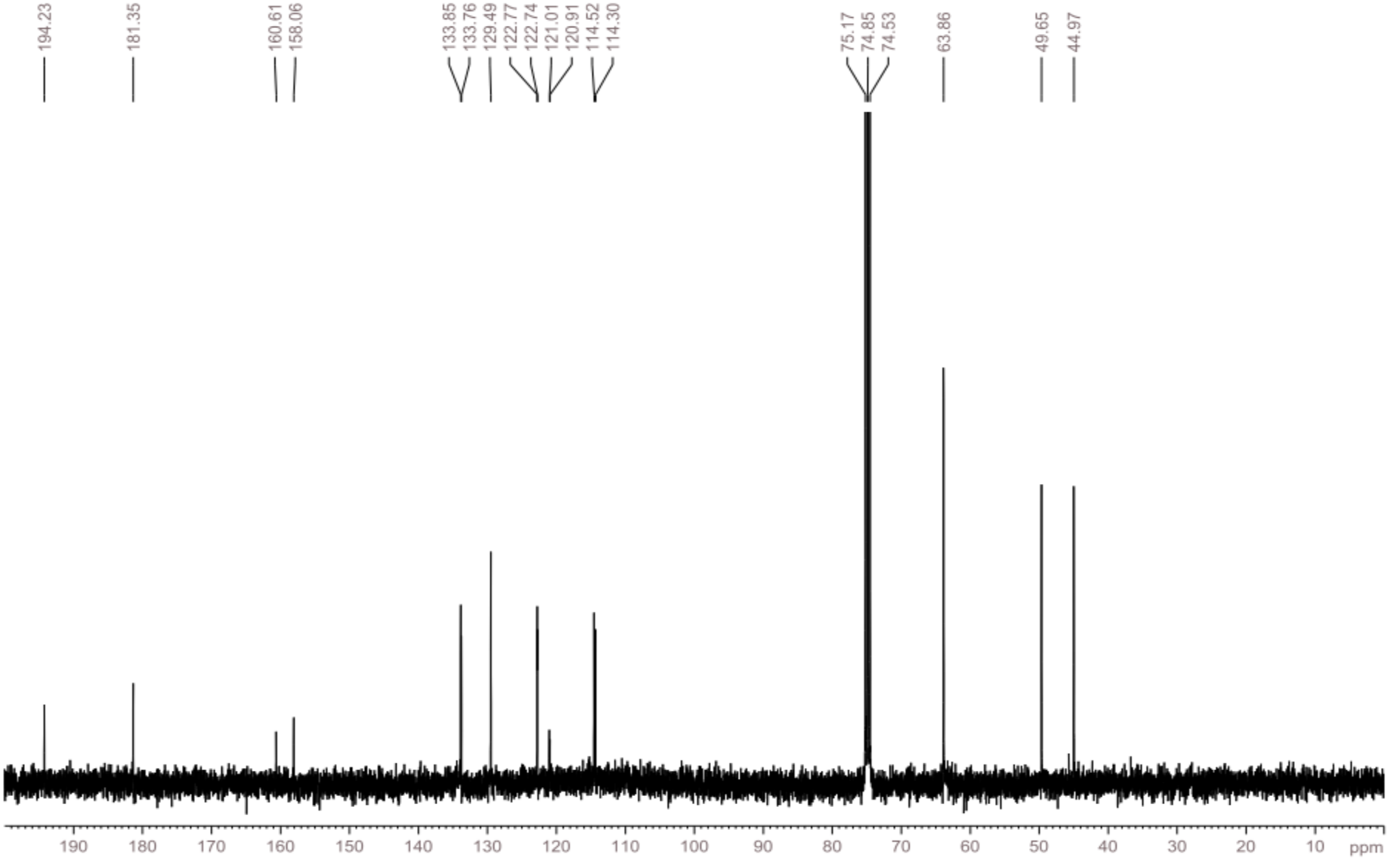


1-(2-Fluorophenyl)-2-morpholino-2-thioxoethanone (**20**) **^{1}H NMR**

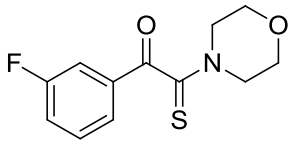


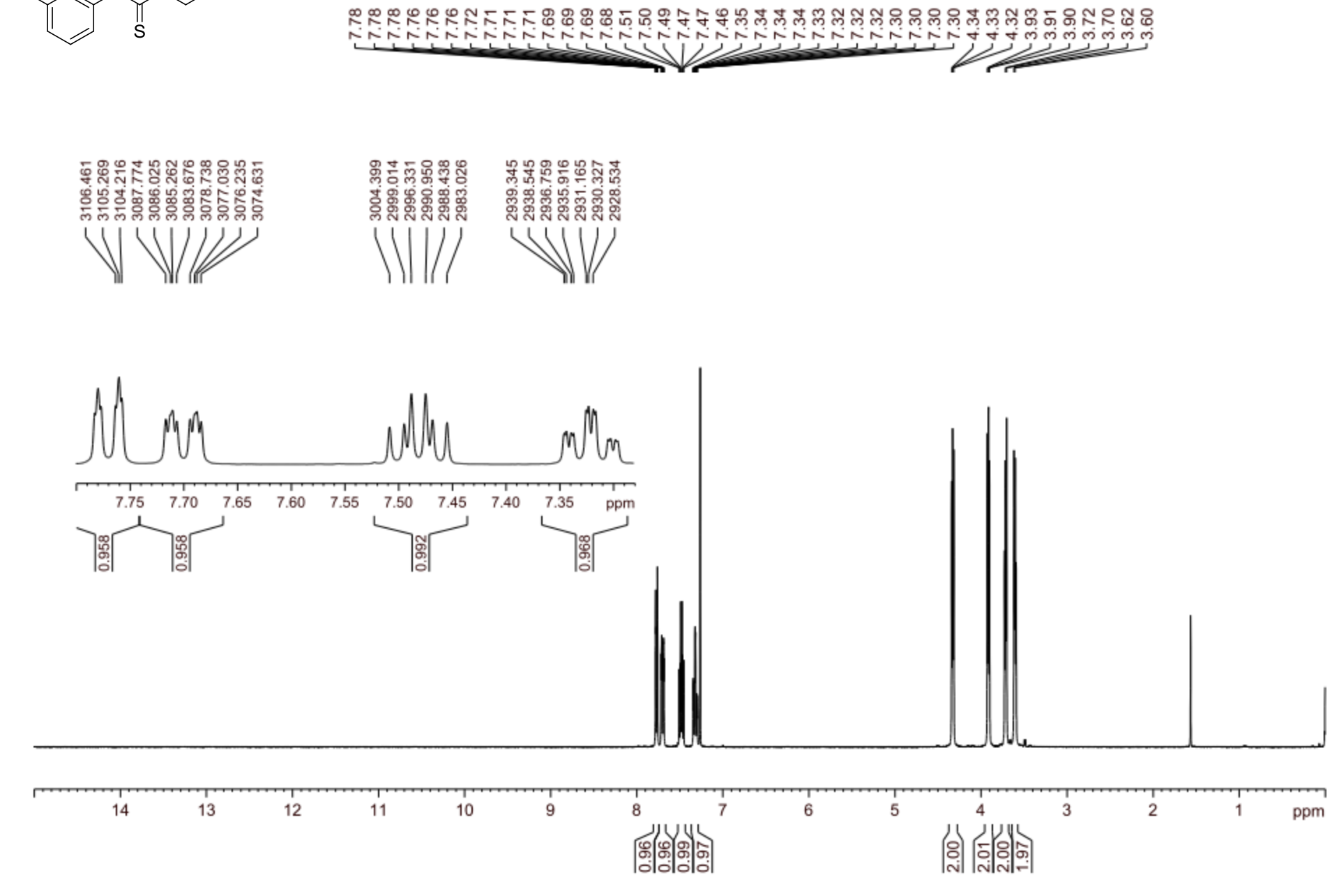
1-(2-Fluorophenyl)-2-morpholino-2-thioxoethanone (**20**) **^{13}C NMR**



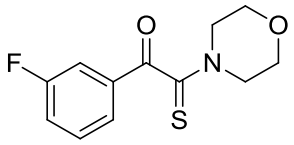


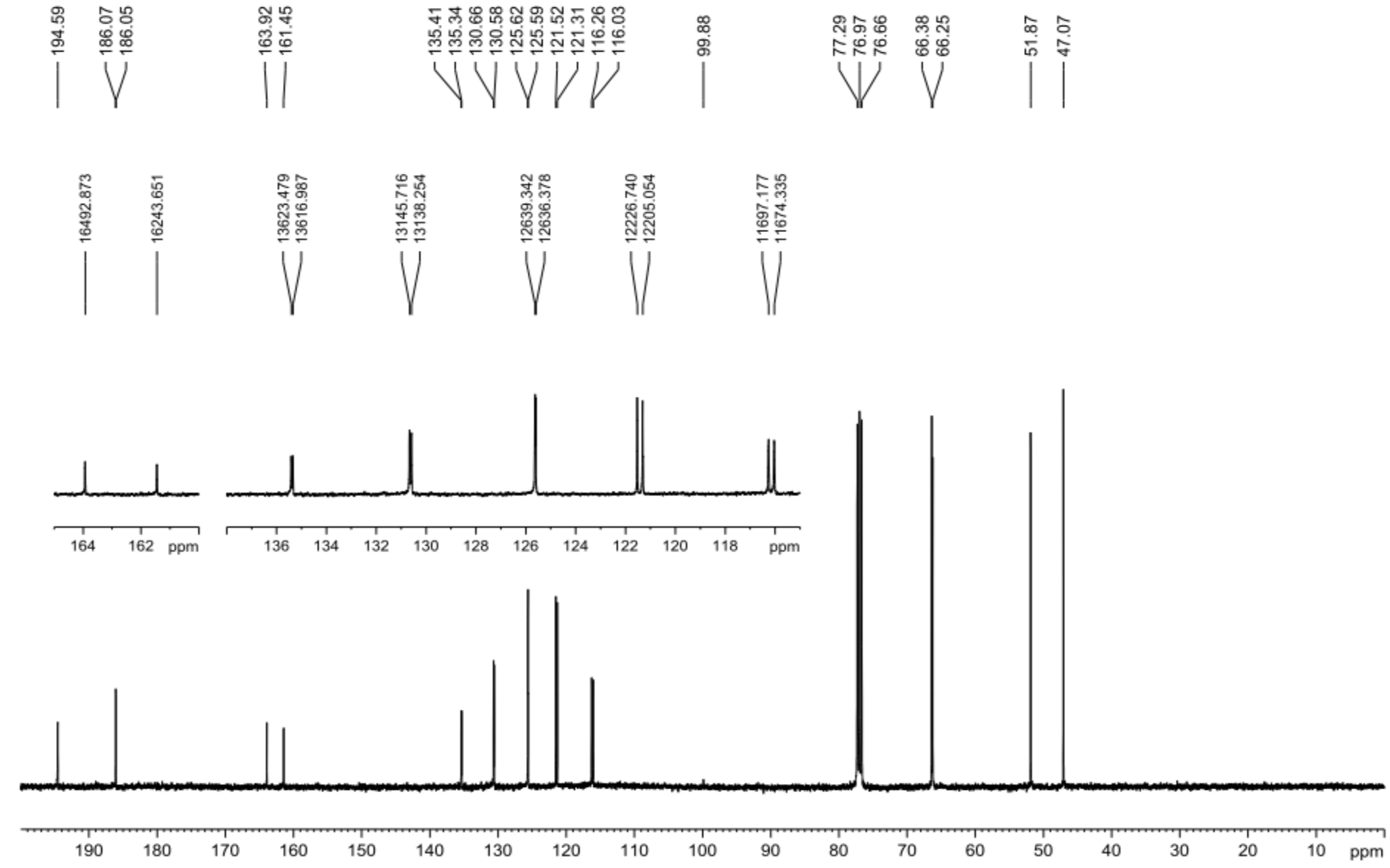
1-(3-Fluorophenyl)-2-morpholino-2-thioxoethanone (**21**) **^{1}H NMR**



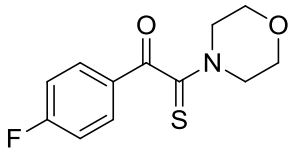


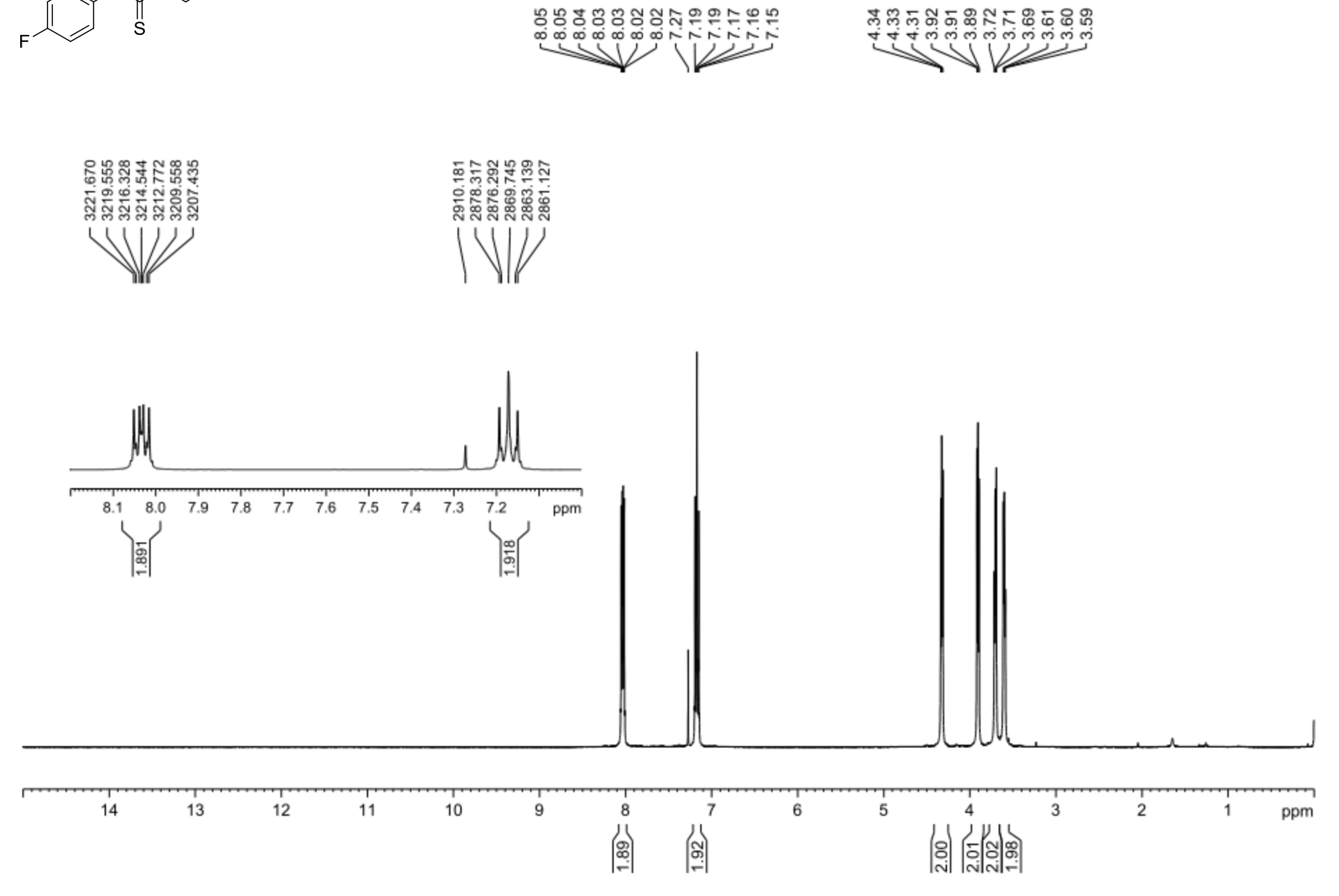
1-(3-Fluorophenyl)-2-morpholino-2-thioxoethanone (**21**) **^{13}C NMR**



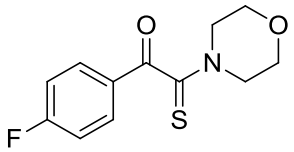


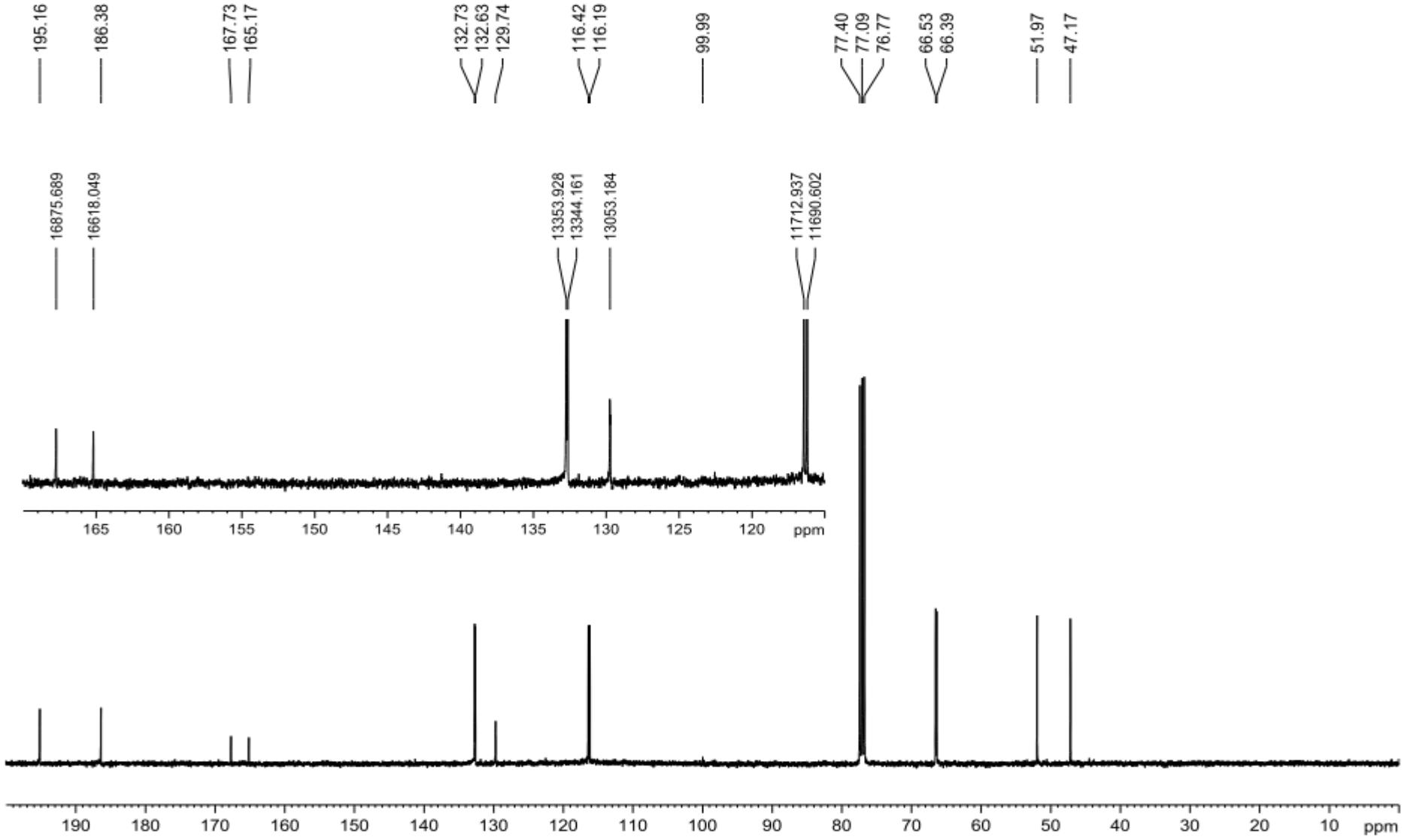
1-(4-Fluorophenyl)-2-morpholino-2-thioxoethanone (**22**) **^{1}H NMR**



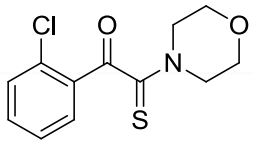


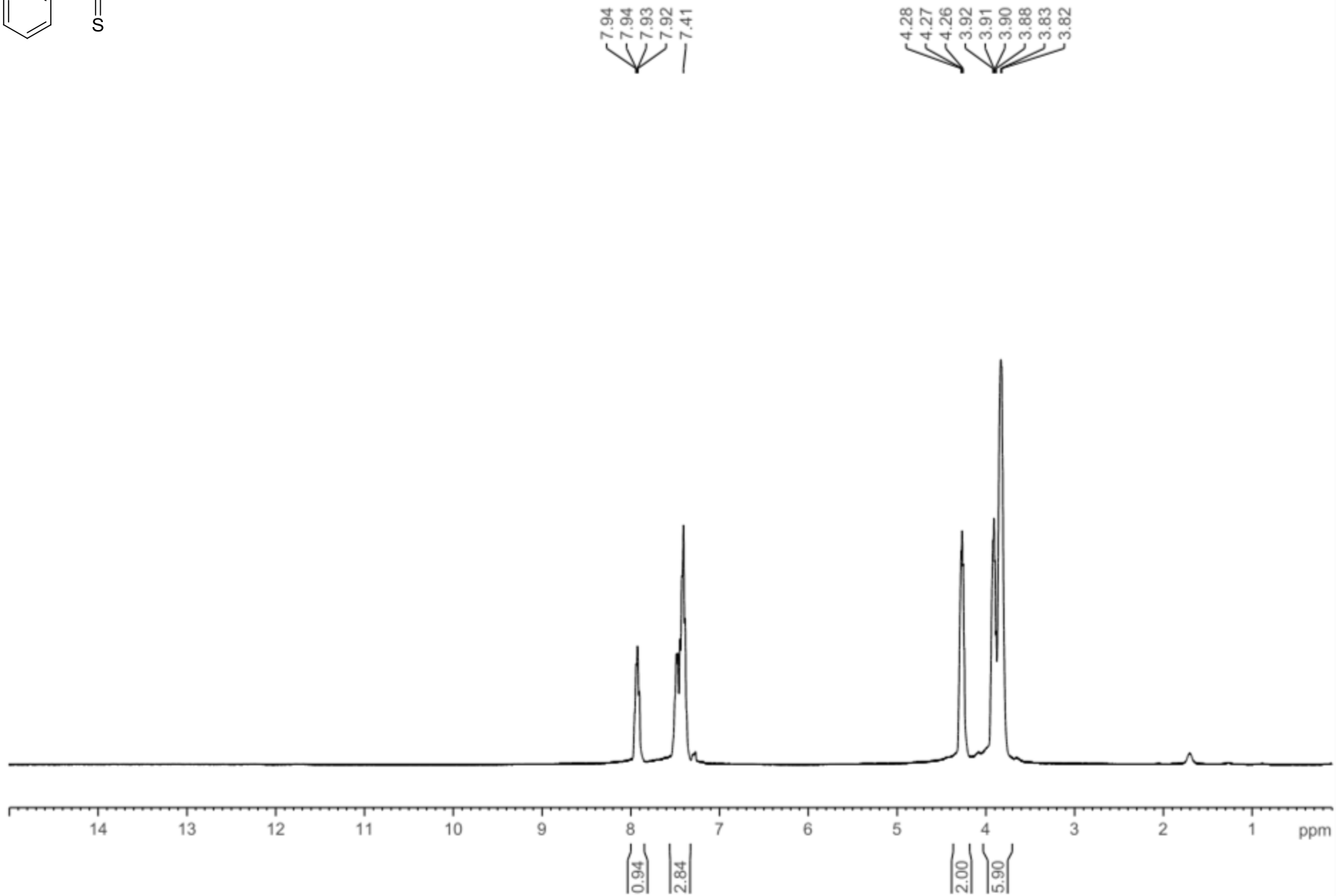
1-(4-Fluorophenyl)-2-morpholino-2-thioxoethanone (**22**) **^{13}C NMR**



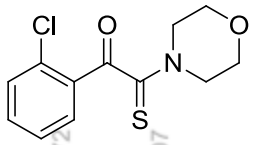


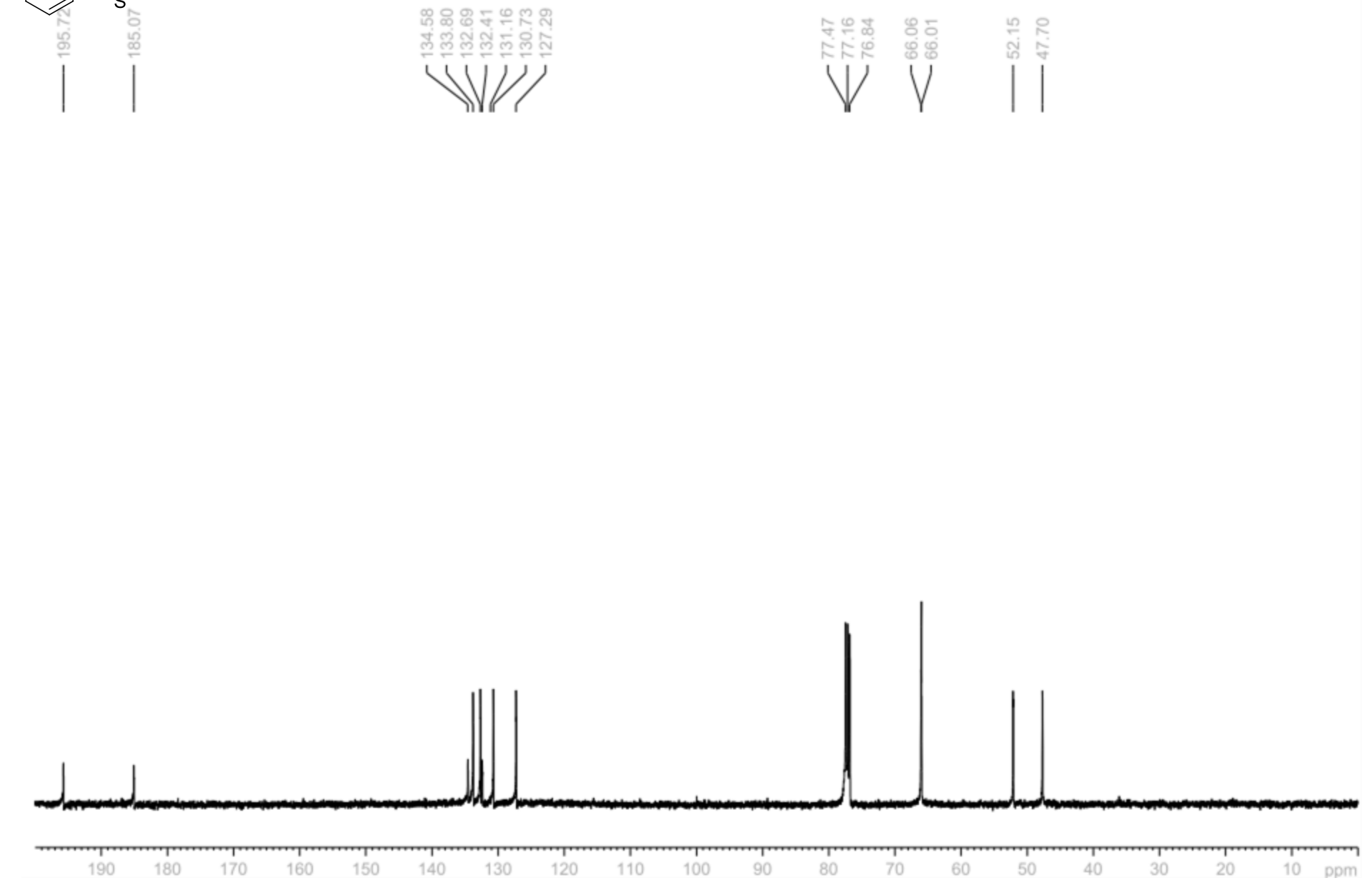
1-(2-Chlorophenyl)-2-morpholino-2-thioxoethanone (**23**) **^{1}H NMR**



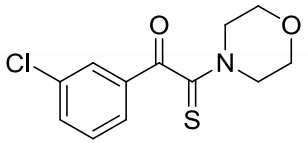


1-(2-Chlorophenyl)-2-morpholino-2-thioxoethanone (**23**) **^{13}C NMR**

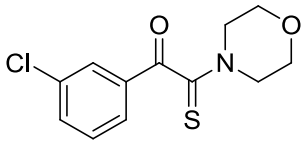


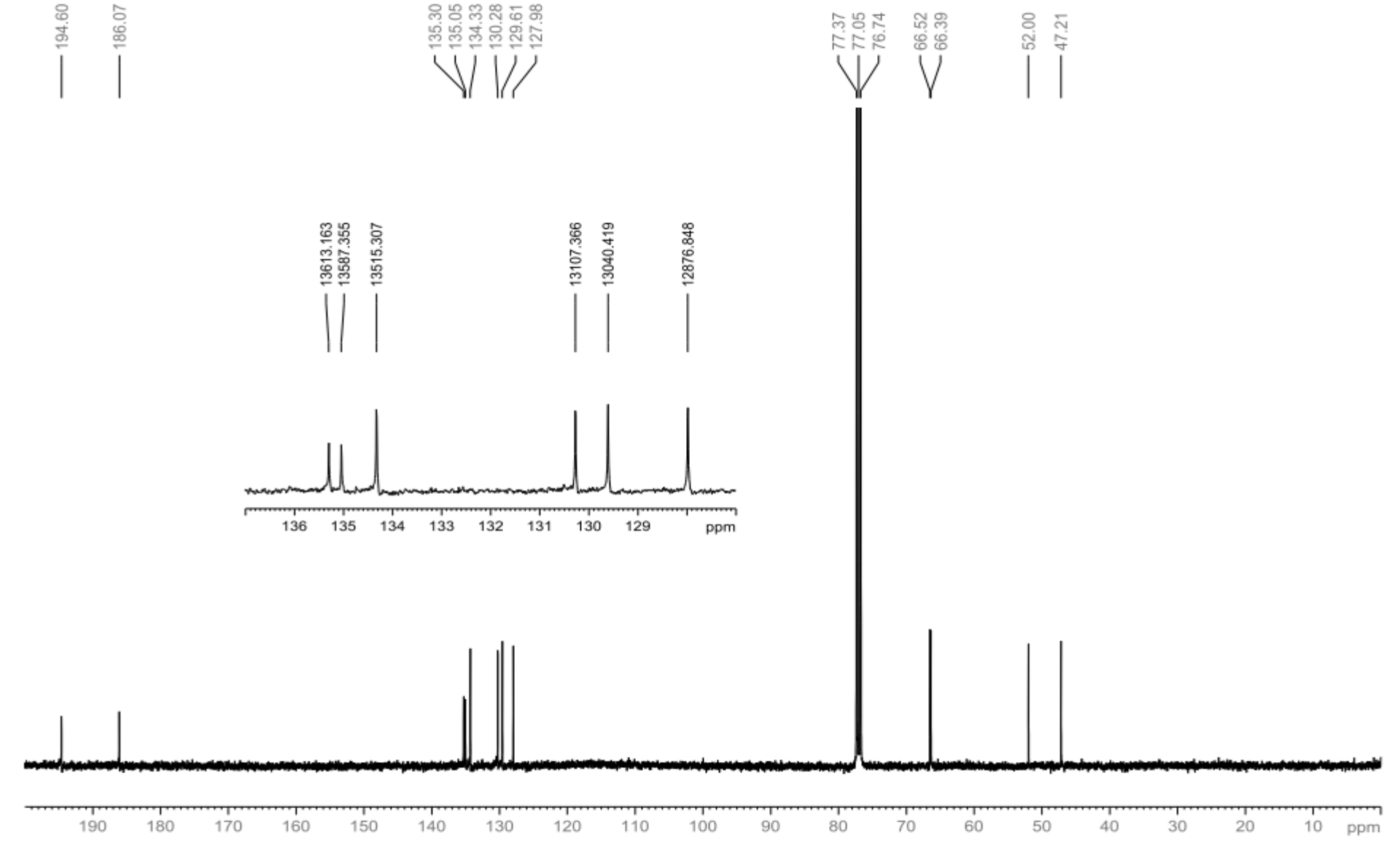


1-(3-Chlorophenyl)-2-morpholino-2-thioxoethanone (**24**) **^{1}H NMR**

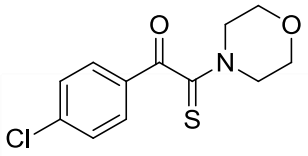


1-(3-Chlorophenyl)-2-morpholino-2-thioxoethanone (**24**) **^{13}C NMR**

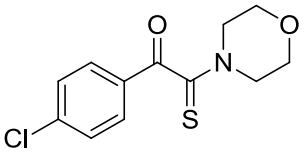


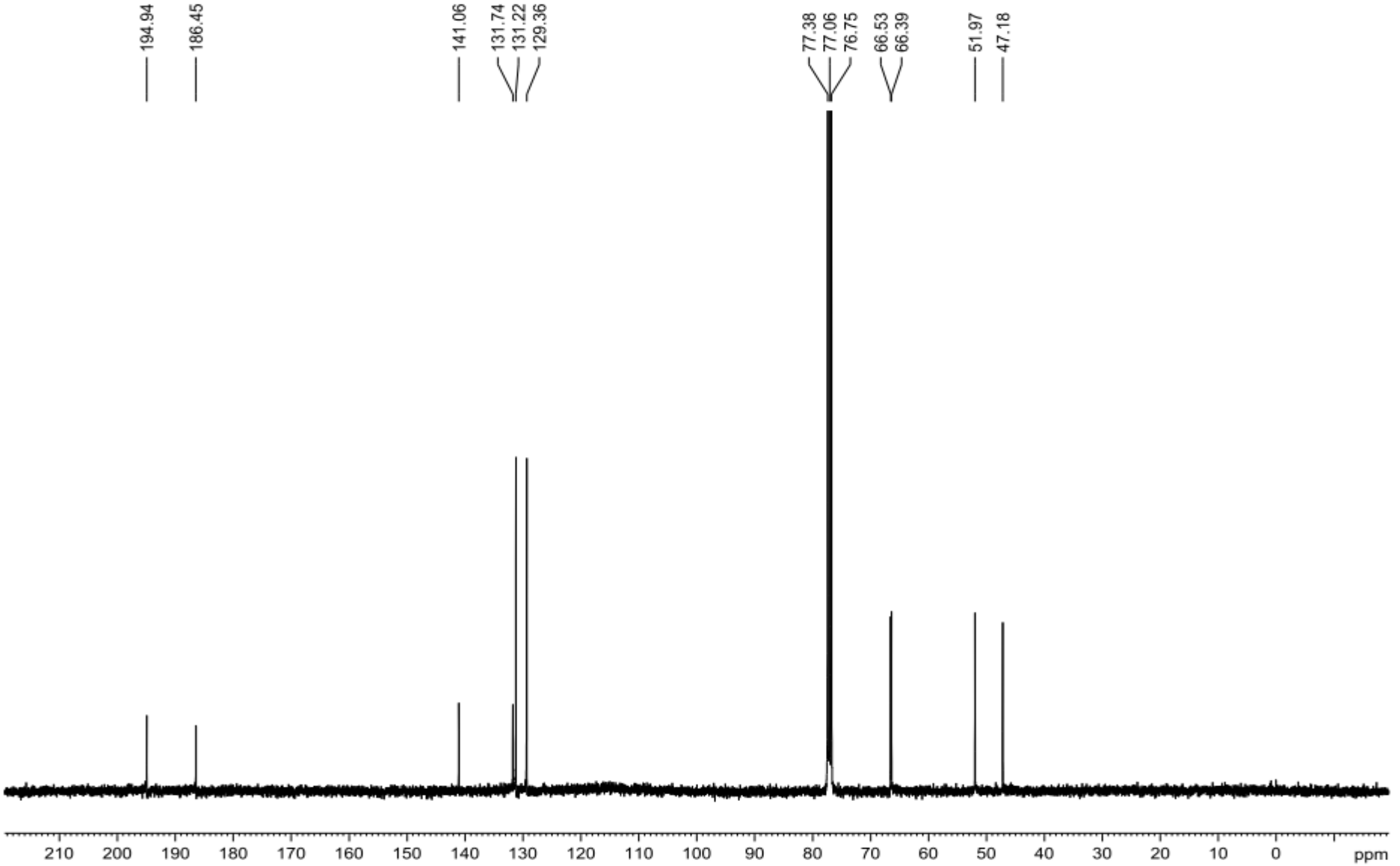


1-(4-Chlorophenyl)-2-morpholino-2-thioxoethanone (**25**) **^{1}H NMR**

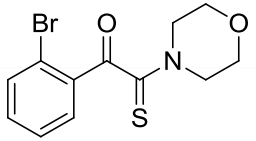


1-(4-Chlorophenyl)-2-morpholino-2-thioxoethanone (**25**) **^{13}C NMR**

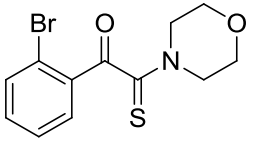


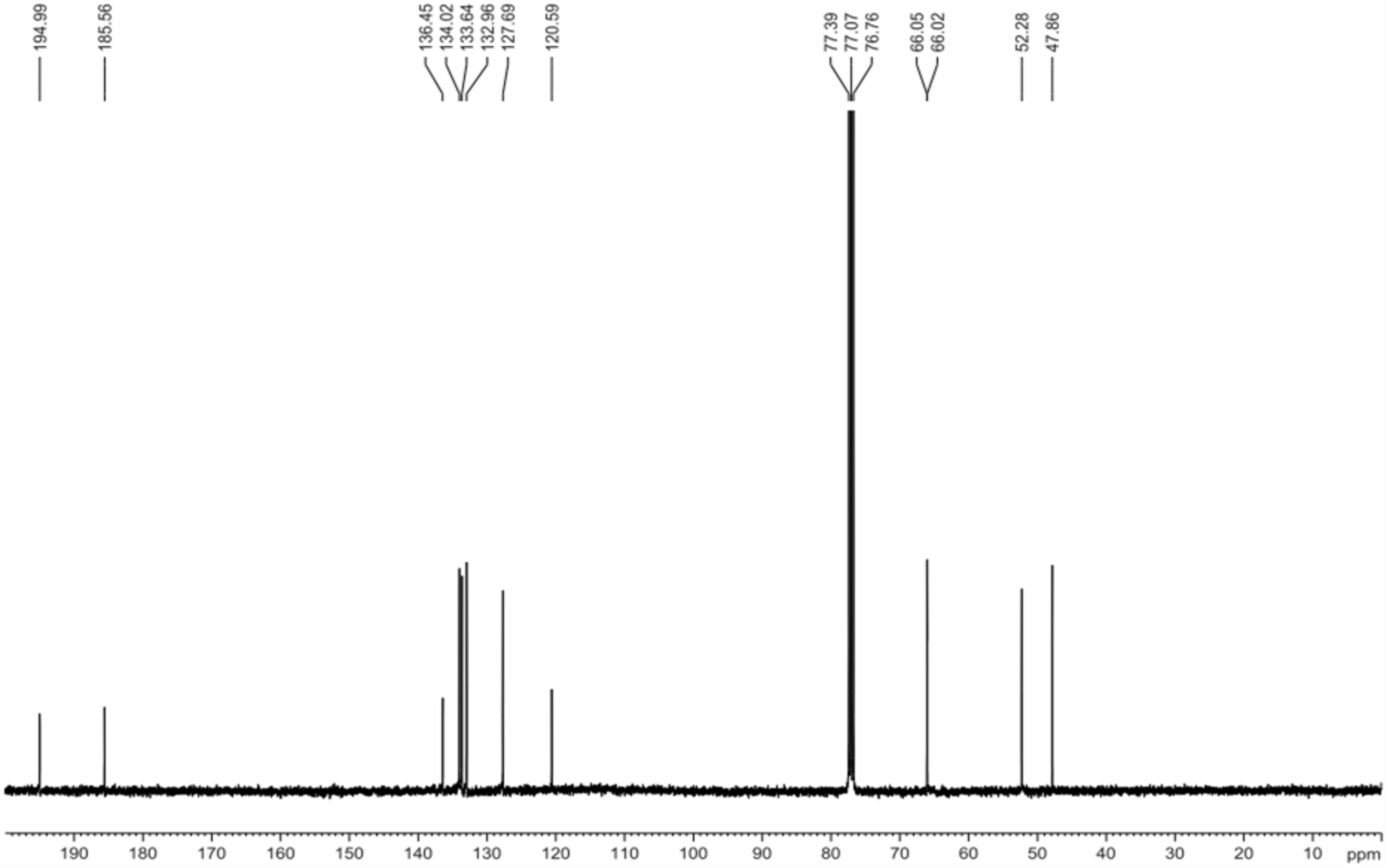


1-(2-Bromophenyl)-2-morpholino-2-thioxoethanone (**26**) **^{1}H NMR**

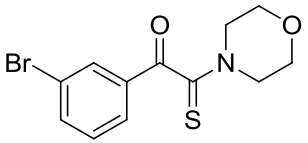


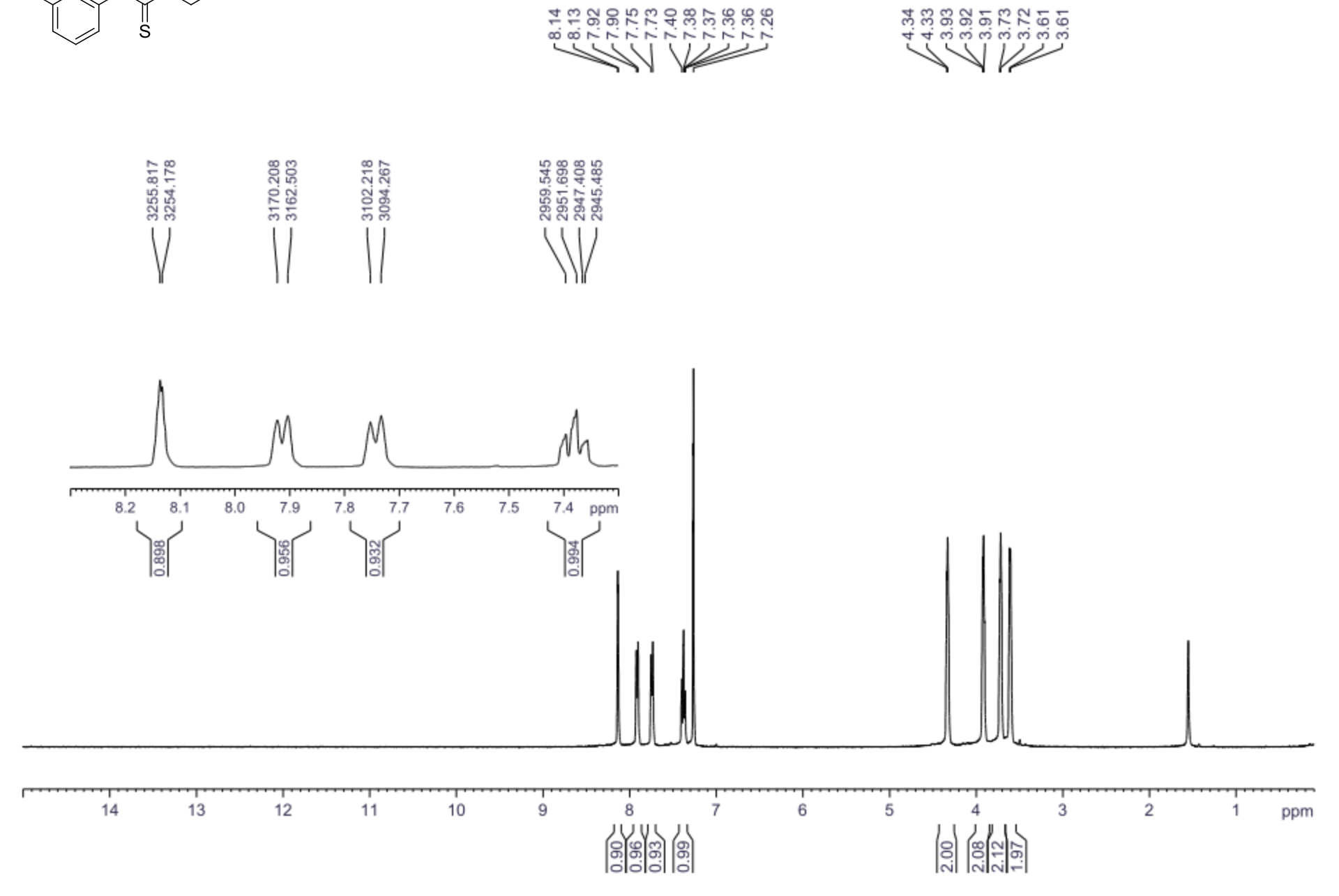
1-(2-Bromophenyl)-2-morpholino-2-thioxoethanone (**26**) **^{13}C NMR**



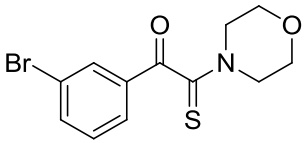


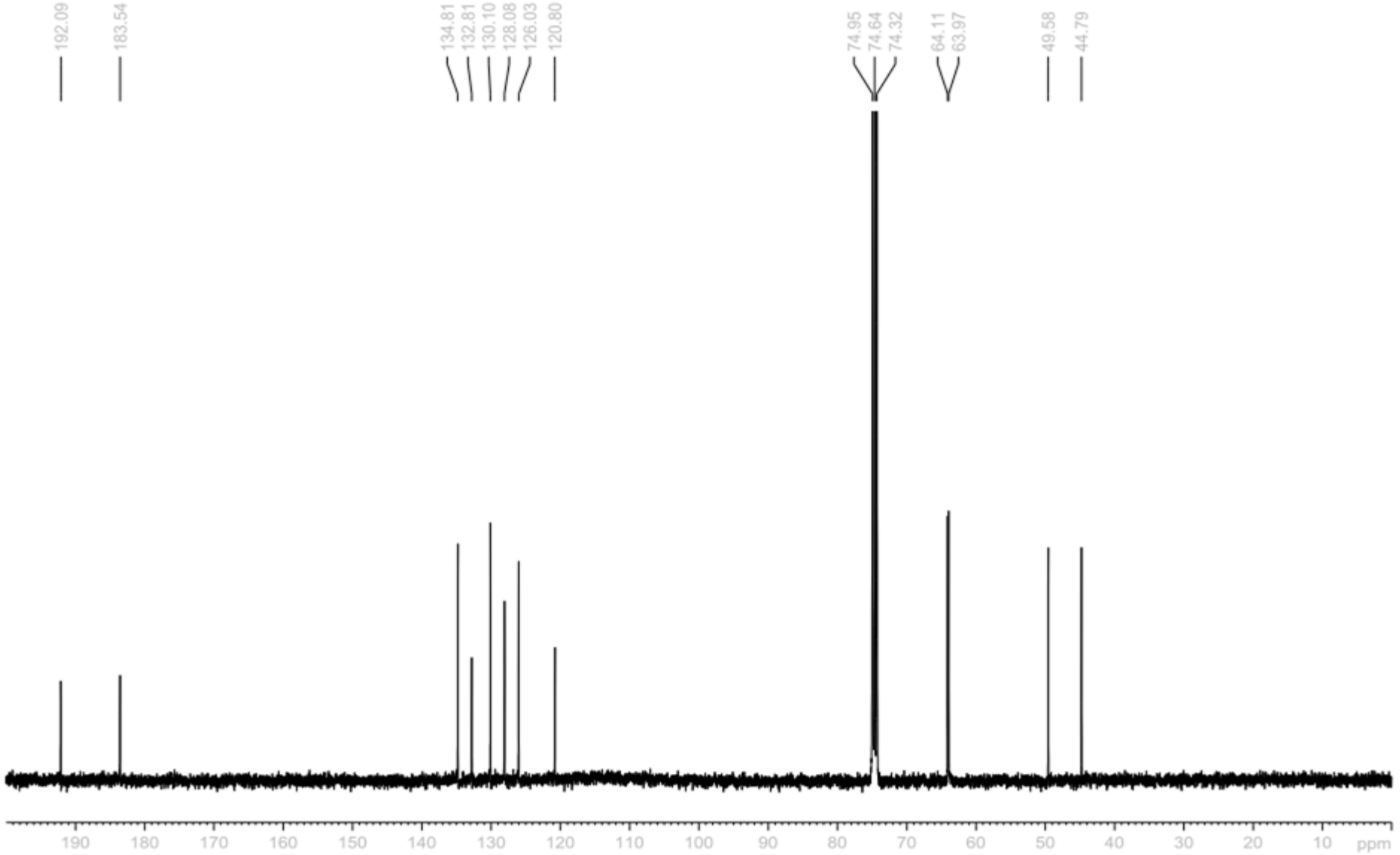
1-(3-Bromophenyl)-2-morpholino-2-thioxoethanone (**27**) **^{1}H NMR**



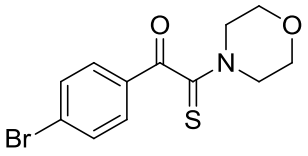


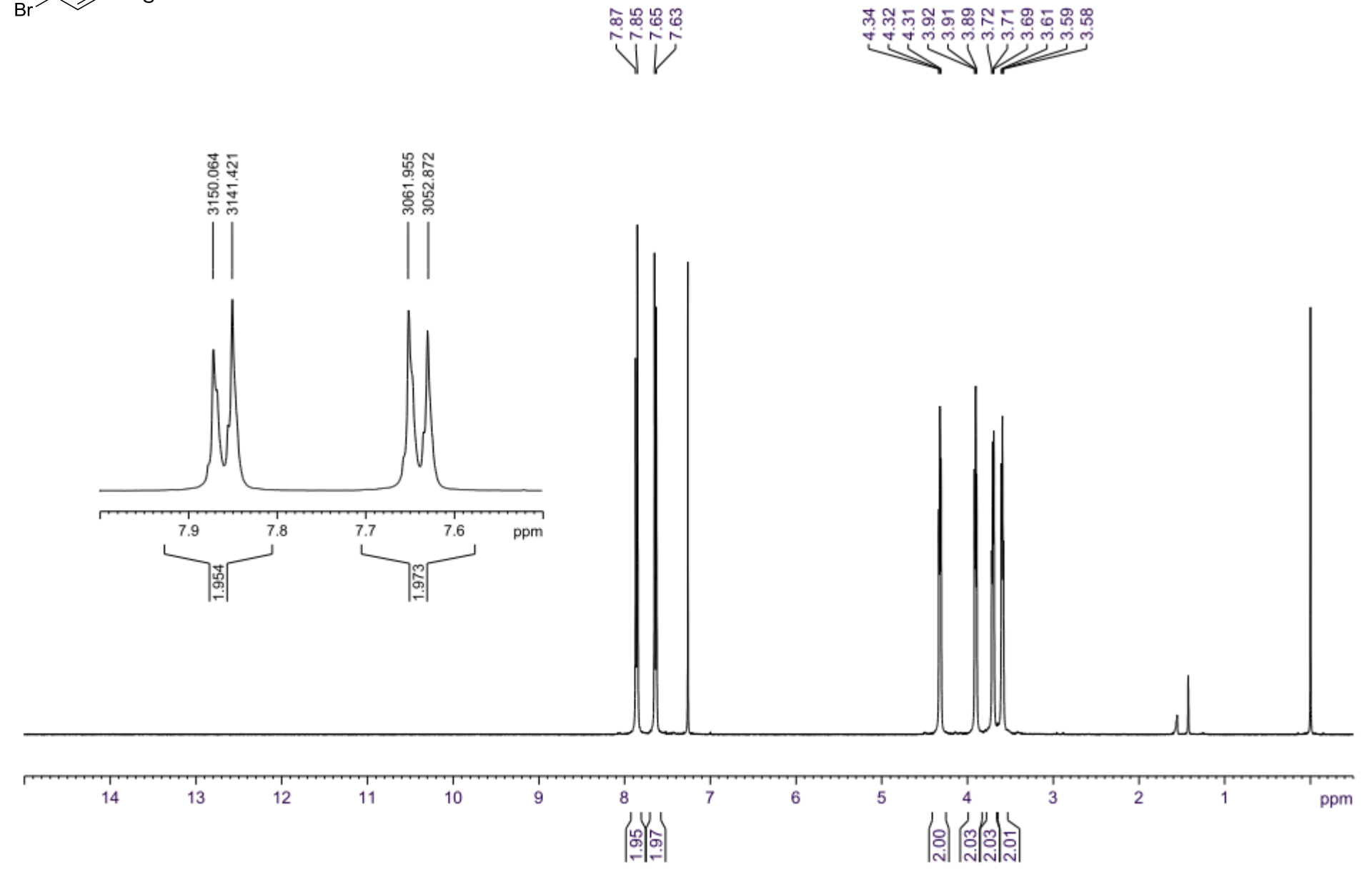
1-(3-Bromophenyl)-2-morpholino-2-thioxoethanone (**27**) **^{13}C NMR**



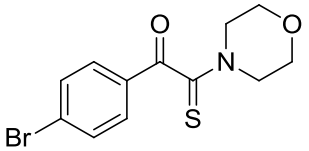


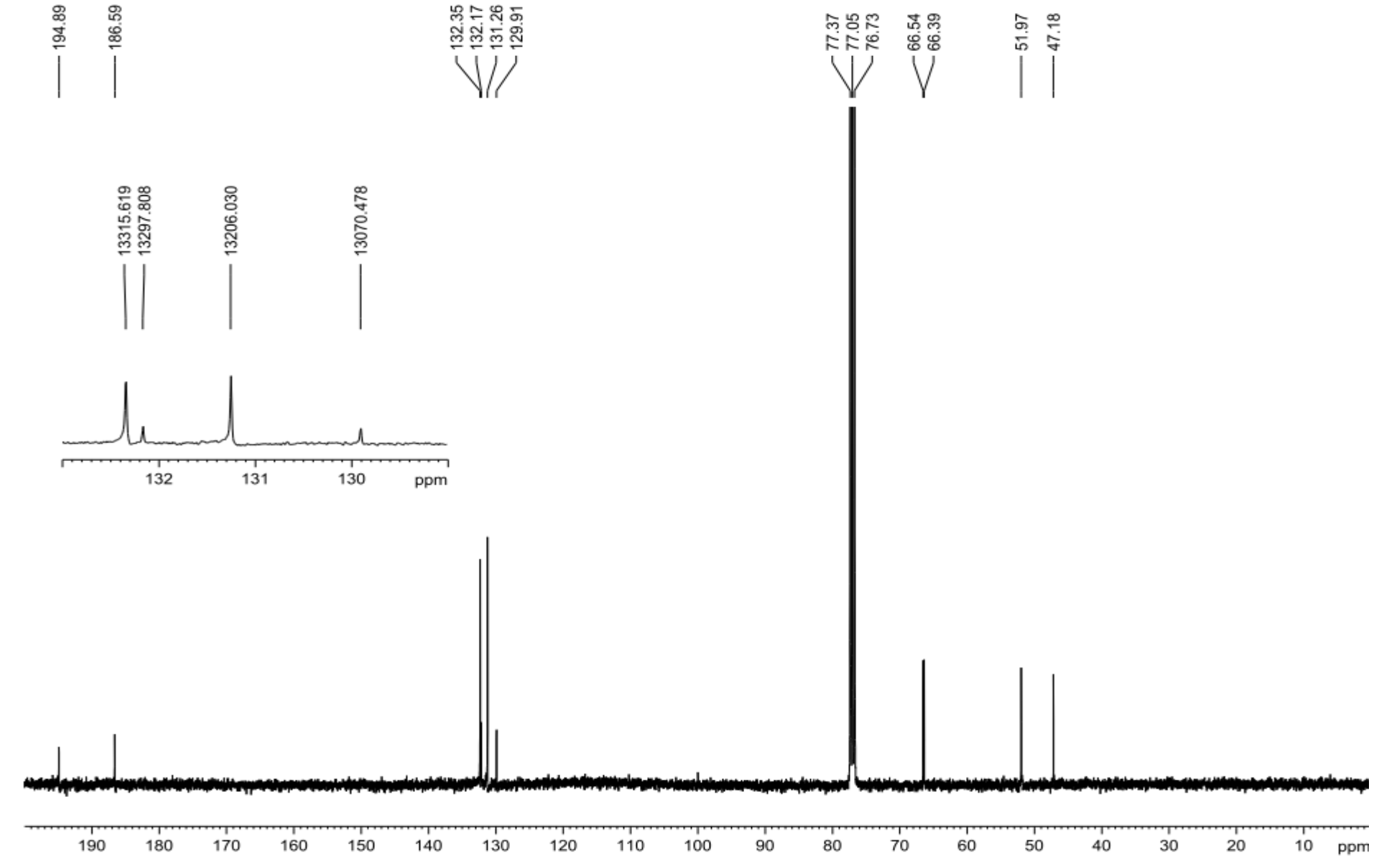
1-(4-Bromophenyl)-2-morpholino-2-thioxoethanone (**28**) **^{1}H NMR**



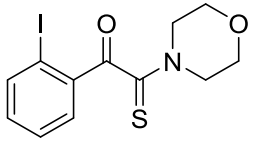


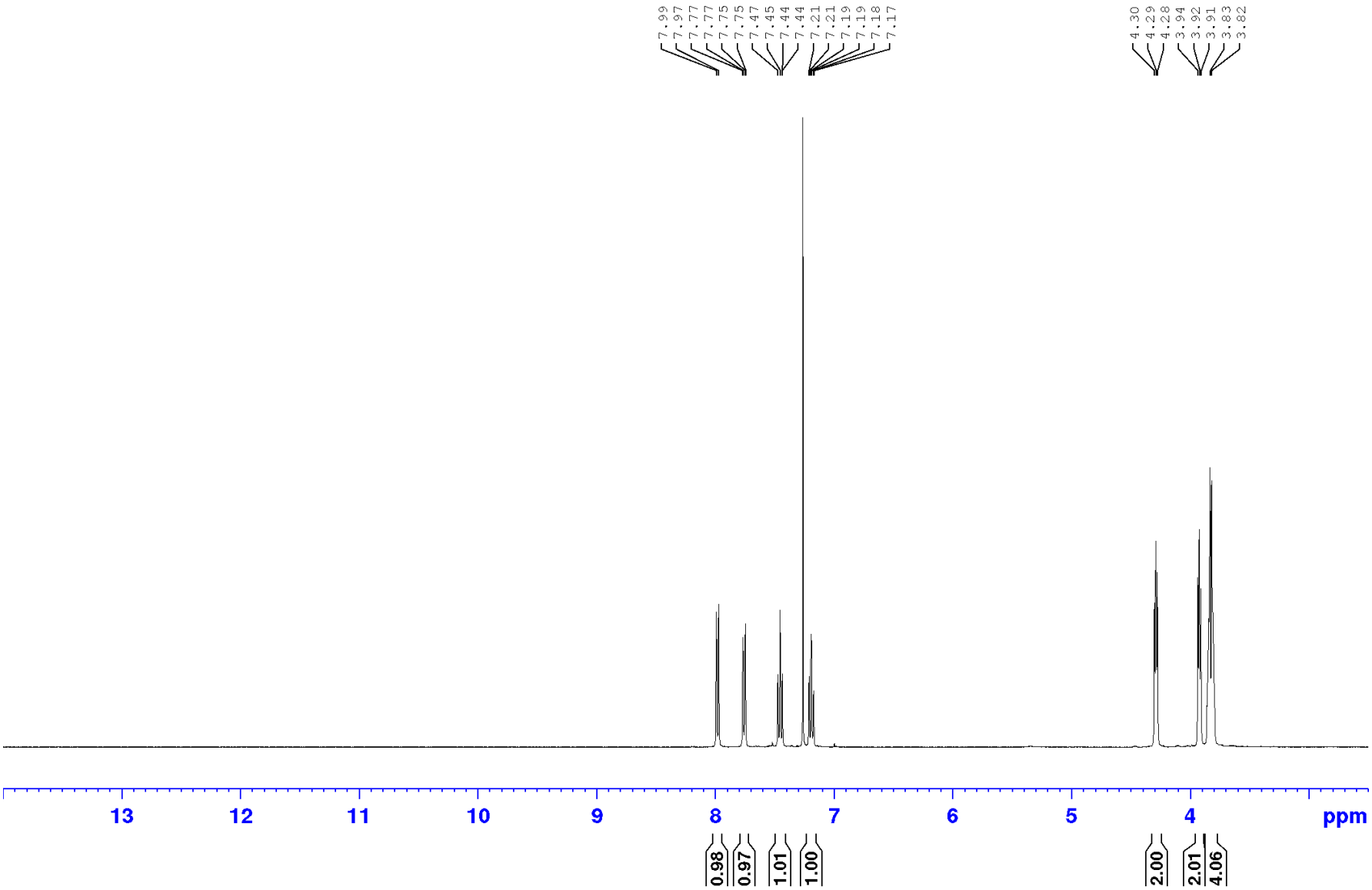
1-(4-Bromophenyl)-2-morpholino-2-thioxoethanone (**28**) **^{13}C NMR**



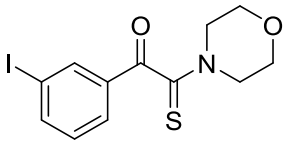


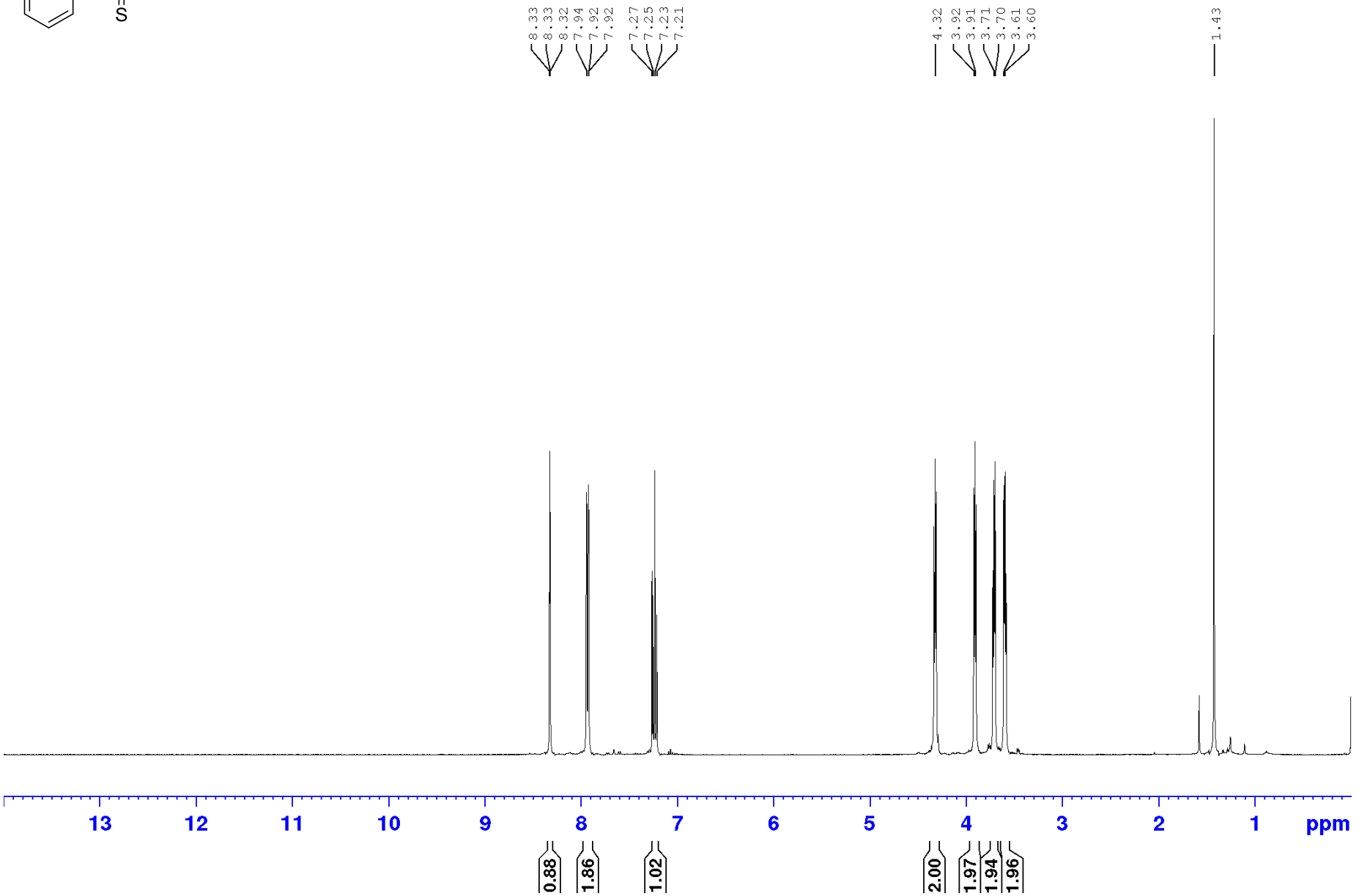
1-(2-Iodophenyl)-2-morpholino-2-thioxoethanone (**29**) **^{1}H NMR**



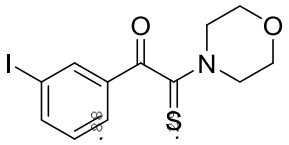


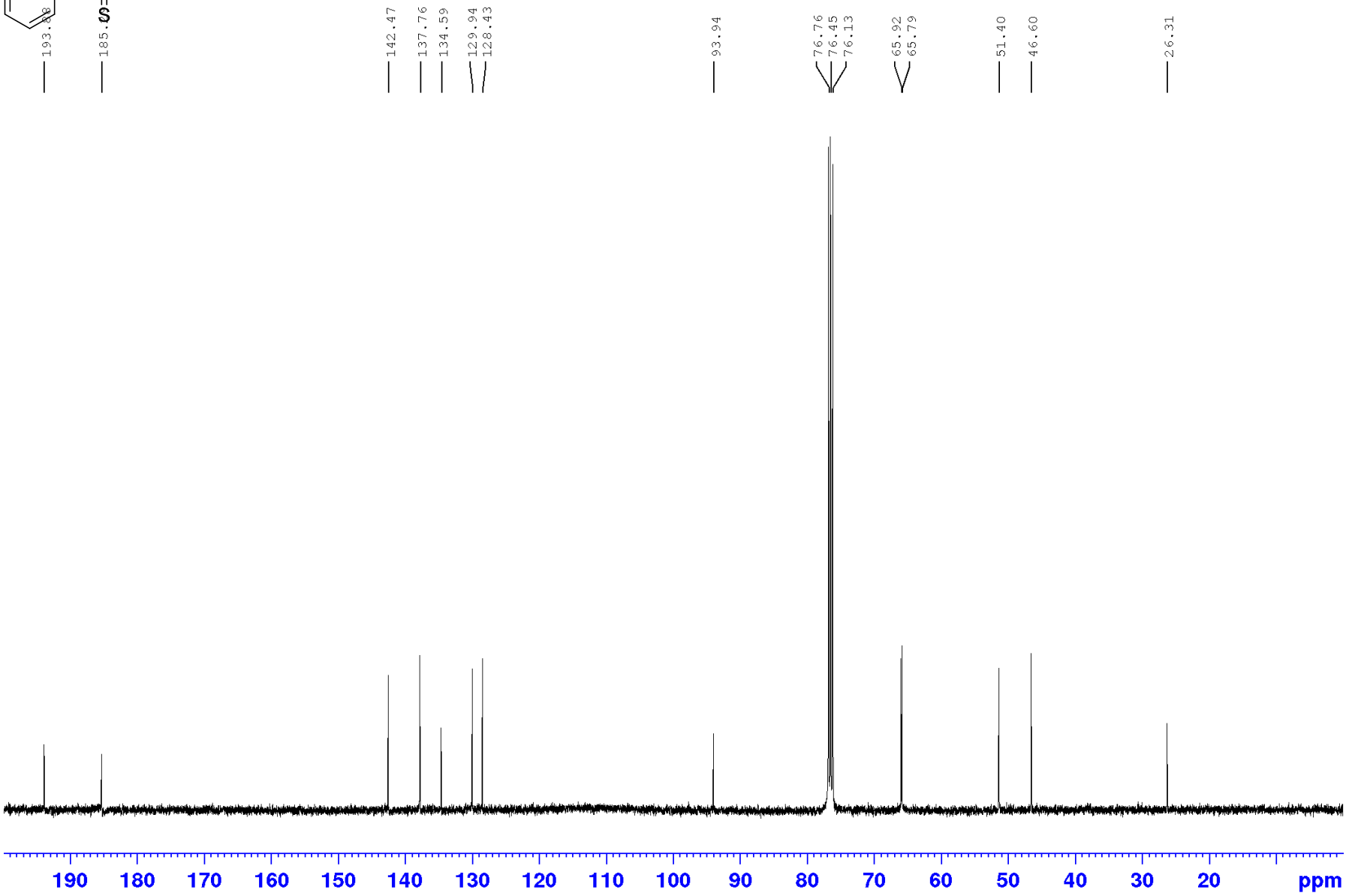
1-(3-Iodophenyl)-2-morpholino-2-thioxoethanone (**30**) **^{1}H NMR**



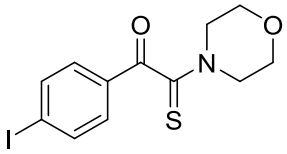


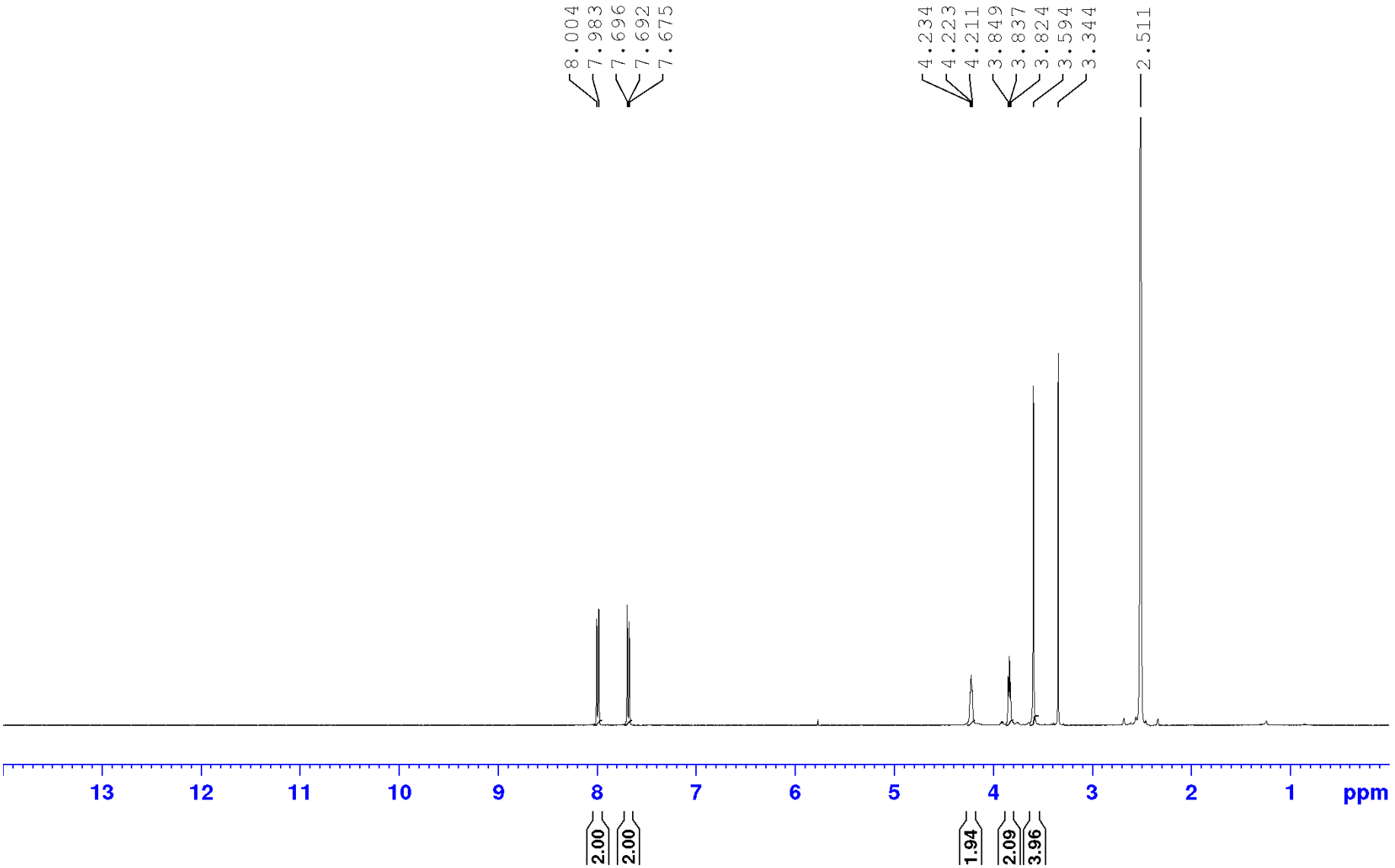
1-(3-Iodophenyl)-2-morpholino-2-thioxoethanone (**30**) **^{13}C NMR**





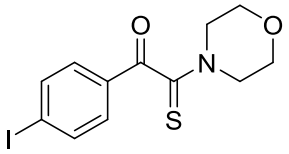
1-(4-Iodophenyl)-2-morpholino-2-thioxoethanone (**31**) **^{1}H NMR**

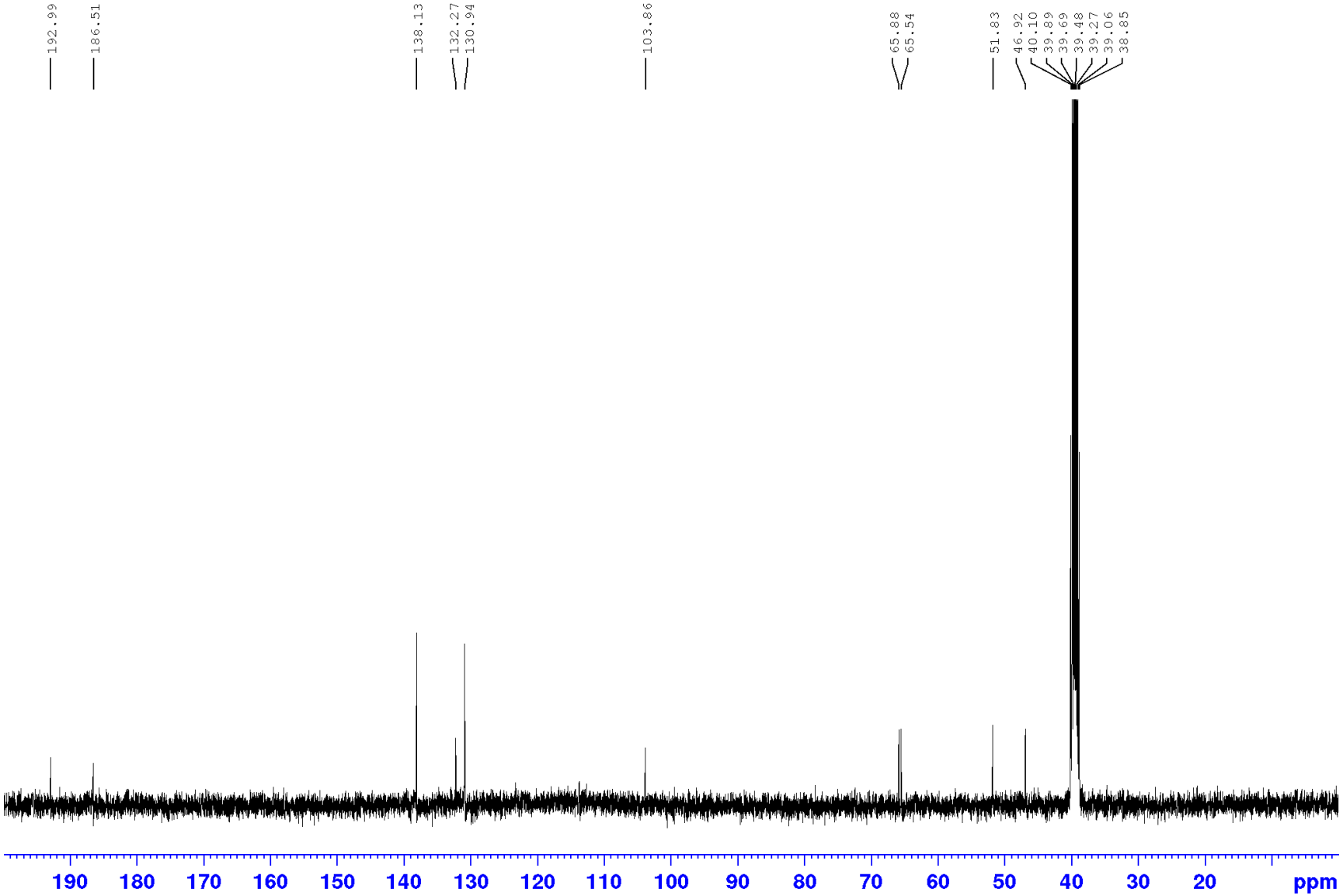




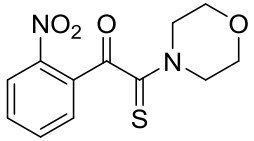
1-(4-Iodophenyl)-2-morpholino-2-thioxoethanone (**31**)

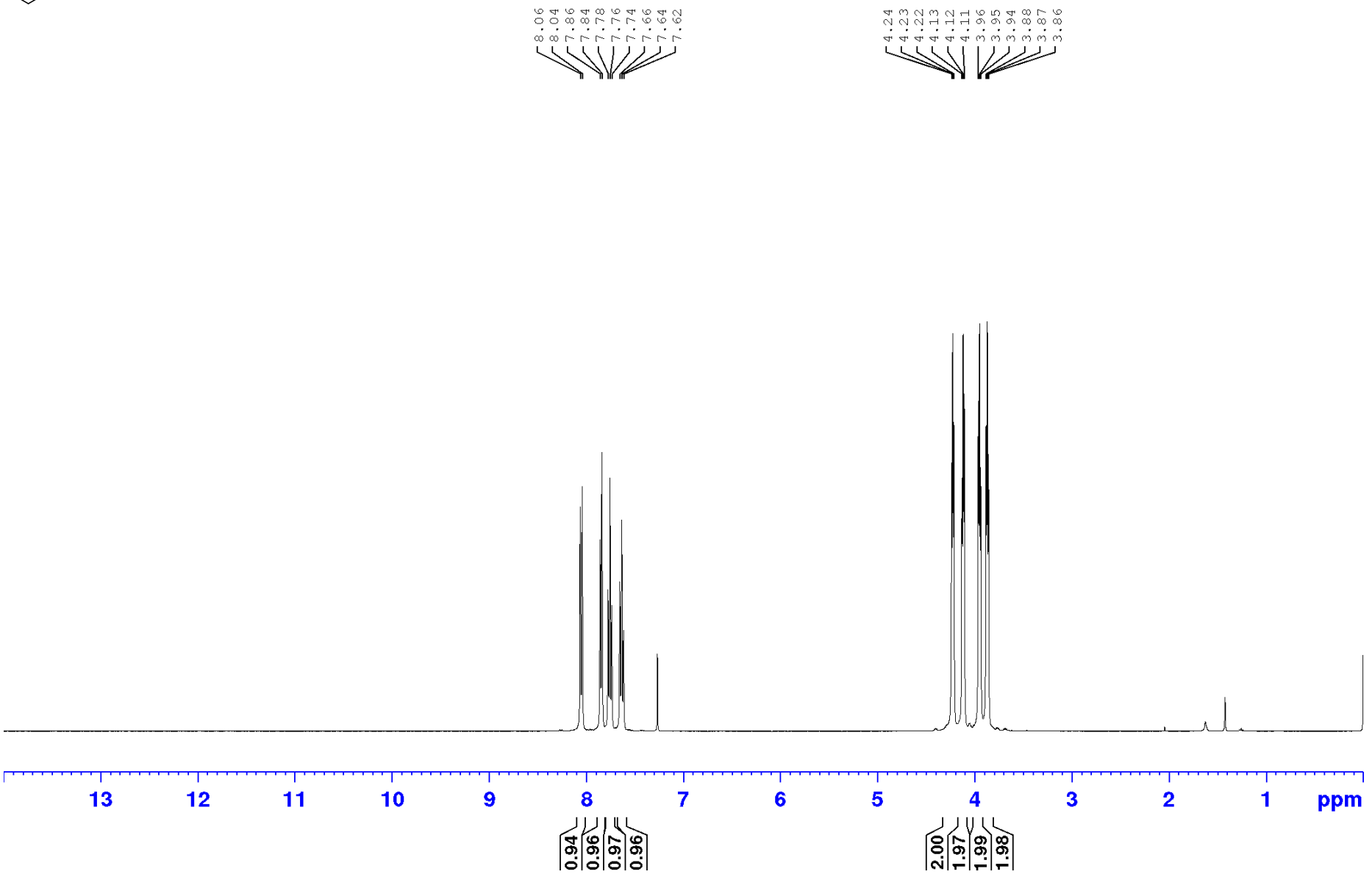
^{13}C NMR



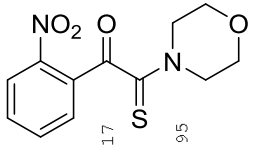


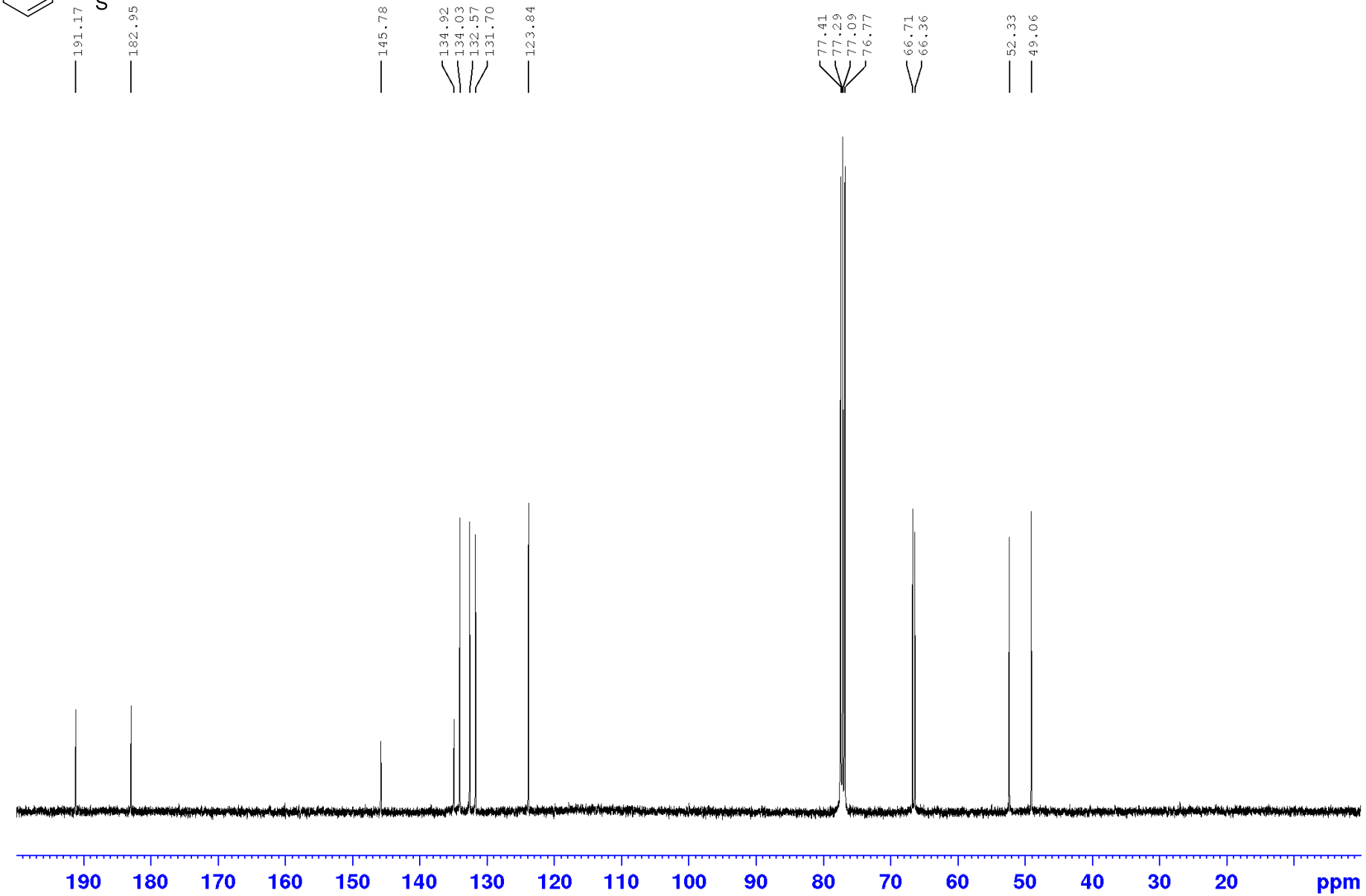
1-(2-Nitrophenyl)-2-morpholino-2-thioxoethanone (**32**) **^{1}H NMR**



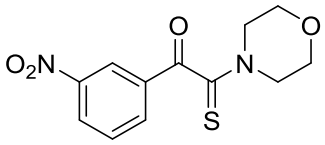


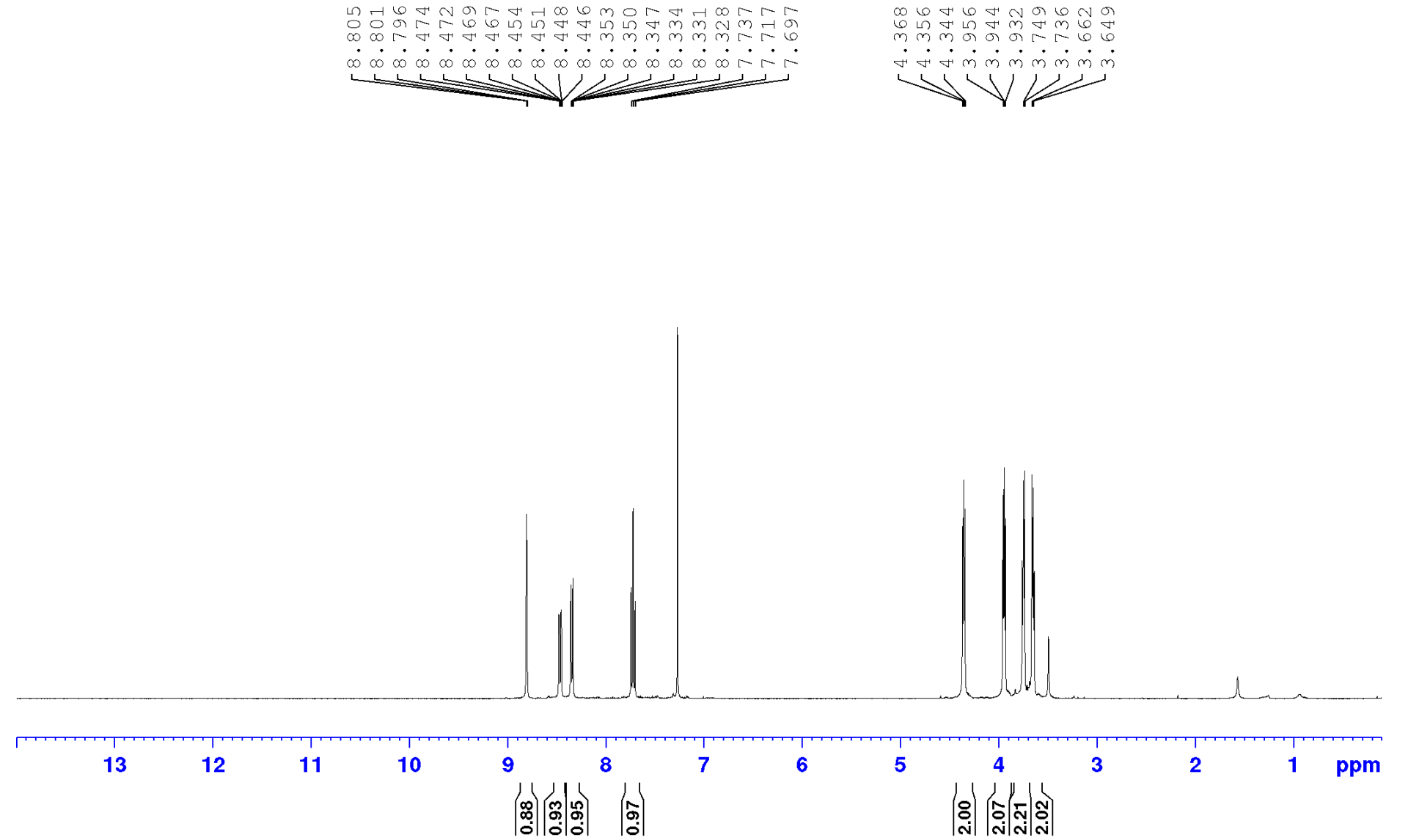
1-(2-Nitrophenyl)-2-morpholino-2-thioxoethanone (**32**) **^{13}C NMR**





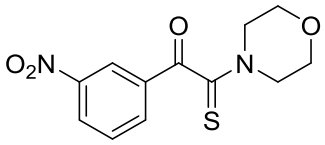
1-(3-Nitrophenyl)-2-morpholino-2-thioxoethanone (**33**) **^{1}H NMR**

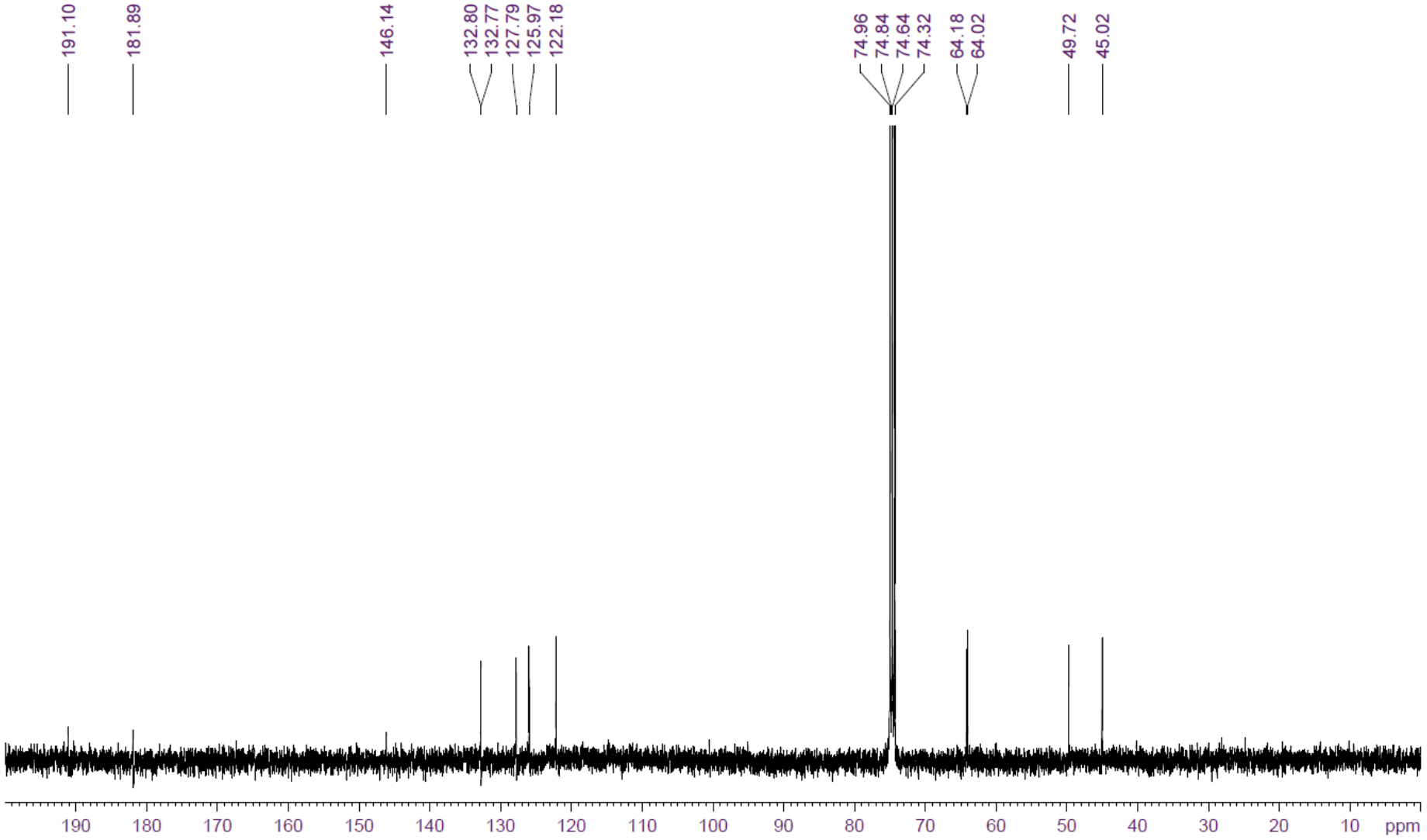




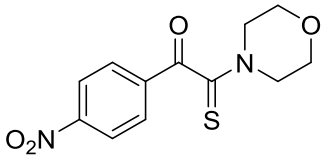
1-(3-Nitrophenyl)-2-morpholino-2-thioxoethanone (**33**)

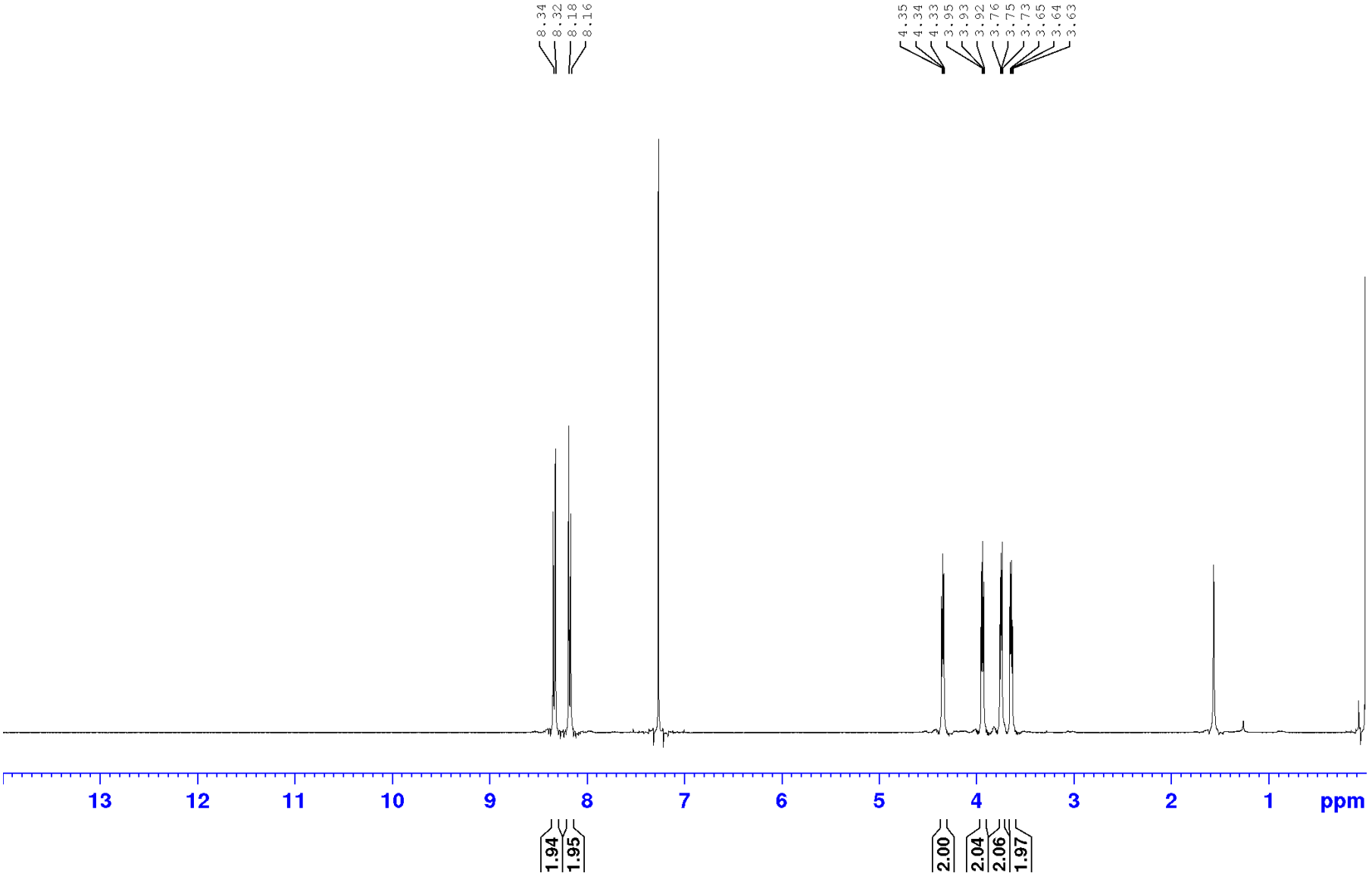
^{13}C NMR



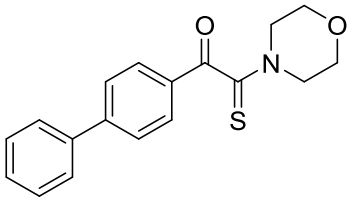


1-(4-Nitrophenyl)-2-morpholino-2-thioxoethanone (**34**) **^{1}H NMR**





1-([1,1'-biphenyl]-4-yl)-2-morpholino-2-thioxoethanone (**35**) **^{1}H NMR**



1-([1,1'-biphenyl]-4-yl)-2-morpholino-2-thioxoethanone (**35**) **^{13}C NMR**