Supporting Information

Apoptosis Induced by 2-Aryl Benzothiazoles-Mediated Photodynamic Therapy in Melanomas via Mitochondrial Dysfunction

Yin-Kai Chen,[‡, b] *Gopal Chandru Senadi,*[‡,a] *Yi-Min Tsai,*[c] *Yan-Ren Chen,*[a] *Wan-ping Hu,*[*, b] *Yu-Wei Chou,*[b] *and Jeh-Jeng Wang* *[a]

[a]Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung, Taiwan.
[b]Department of Biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan.
[c]School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

jjwang@kmu.edu.tw, wphu@kmu.edu.tw

Table of Contents

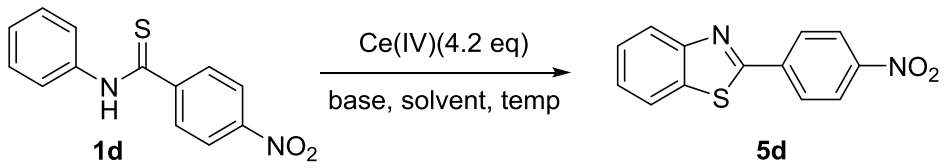
1. Optimization studies for intramolecular oxidative cyclization reaction

Initial cyclization studies of **1d**, obtained from the reaction of benzamides with Lawesson's reagent in refluxing chlorobenzene, using 4.2 equivalents of CAN and 6 equivalents of $NaHCO_3$ as a base in the mixture of CH_3CN and H_2O (10:1) under nitrogen at 0 ^{0}C for 10 min provided the desired benzothiazole **5d** in a good yield of 81% (Table S1, entry 1). Compound **1d** was used as a model substrate to optimize the reaction conditions. The presence of the base proved to be important for the reaction, as the yield was reduced to 67% without the base and only 72% with 2 equivalents of the base (entries 2-4). The use of mixture of CH_3CN and H_2O as the solvent gave the best results. Other solvents such as CH_2Cl_2, CH_3OH, and THF or at different temperature gave much lower yields (entries 5-8). Hitherto, to our knowledge, no example of CAN-mediated intramolecular carbon-sulfur bond-forming reaction has been reported.

Since oxidations utilizing cerium(IV)-based reagents have been use to carry out a number of bond-forming reactions, we then examined other cerium(IV) based reagents. Cerium(IV) tetrabutylammonium nitrate (CTAN) is a very mild reagent that is soluble in common organic solvents like CH_2Cl_2. However, the reaction resulted in lower yield (entry 9). Other solvents such as CH_3CN and CH_3OH were less effective (entries 10-11). Cerium(IV) sulfate showed no reaction at all in CH_3CN because of the solubility problem, while it proceeded in a CH_3CN-H_2O (10:1) mixture in 43% yield (entries 12-13). Similarly, cerium(IV) fluoride gave the desired benzothiazole **2a** in fair yield (48%, entry 14).

From the above results, it is clear that use of 4.2 equivalents of CAN and 6 equivalents of $NaHCO_3$ as a base in the mixture of CH_3CN and H_2O (10:1) at 0^{o}C (entry 1) serves as an optimum condition to achieve the desired compound in maximum yield.

Table S1. Optimization studies for intramolecular oxidative cyclization reaction[a]



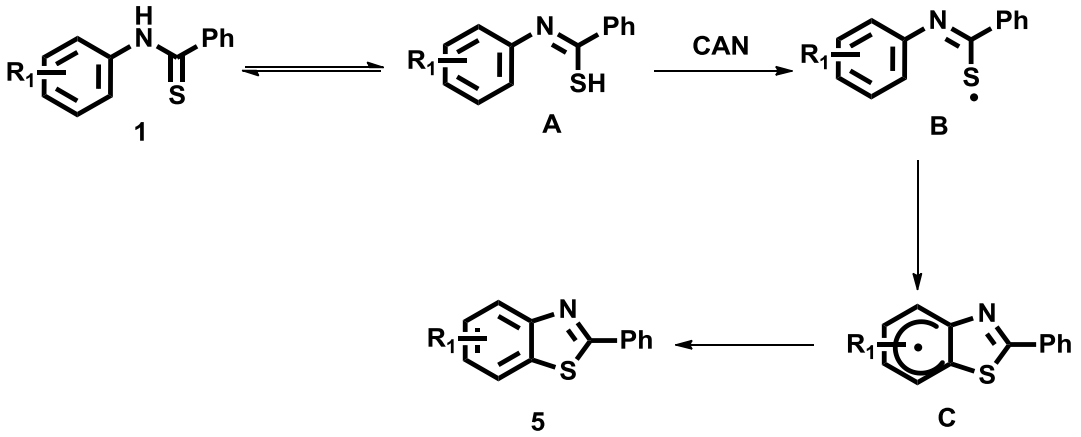
1d 5d

entry	Ce source	solvent	$NaHCO_3$ (equiv)	temp (°C)	time(min)	Yield (%)
1	**CAN**	**CH_3CN+H_2O**	**6**	**0**	**10**	**81**
2	CAN	CH_3CN+H_2O	4	0	10	76
3	CAN	CH_3CN+H_2O	2	0	10	72
4	CAN	CH_3CN+H_2O	0	0	10	67
5	CAN	CH_2Cl_2	6	RT	60	21
6	CAN	CH_2Cl_2	6	reflux	24(hr)	24
7	CAN	CH_3OH	6	RT	10	10
8	CAN	THF	6	0	10	35
9	CTAN	CH_2Cl_2	6	0	10	55
10	CTAN	CH_3CN	6	0	10	41
11	CTAN	CH_3OH	6	RT	10	8
12	$Ce(SO_4)_2$	CH_3CN	6	0	10	NR
13	$Ce(SO_4)_2$	CH_3CN+H_2O	6	0	10	43
14	CeF_4	CH_3CN+H_2O	6	0	10	48

[a]All reactions were performed with (**1d**, 1mmol) and solvent (2.0 mL).

2. Proposed reaction mechanism

A plausible mechanistic interpretation of the CAN-mediated intramolecular cyclization of **1** is illustrated in Scheme S1. *N*-phenyl-thiobenzamide (**1**) can exist as tautomeric thioimidol **A**; the latter reacting with CAN to form the thiyl radical **B** while cerium(IV) is reduced to cerium(III) at the same time. Then, 1,5-homolytic radical cyclization of **B** followed by aromatization of radical **C**, forms 2-arylbenzothiazole (**5**).



Scheme S1. A plausible reaction mechanism

3. X- ray structure of compound 5o:

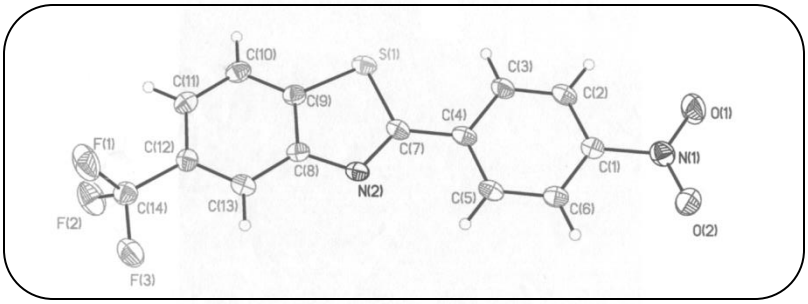


Figure S1. X-ray structure of compound **5o**

4. Spectra Characterization of compounds 5d-f,h,m,q-s,w and **6d,h,m,q,r,w**

2-(4-Nitrophenyl)benzothiazole (5d):[3] White solid; yield: 81%; mp 228-230 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.27 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.56 (dt, *J* = 8.0 and 1.2 Hz, 1H), 7.47 (dt, *J* = 8.0 and 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.8 (s), 154.1 (s), 149.0 (s), 139.2 (s), 135.5 (s), 128.2 (d), 126.9 (d), 126.2 (d), 124.3 (d), 123.9 (d), 121.8 (d); HRMS (EI, m/z) for $C_{13}H_9N_2O_2S$, calcd 256.0308, found 256.0306; Anal. Calcd for $C_{13}H_8N_2O_2S$: C, 60.93; H, 3.15; N, 10.93. Found: C, 60.89; H, 3.34; N, 10.75.

6-Ethyl-2-(4-nitrophenyl)benzothiazole (5e):[3] Yellow solid; yield: 93%; mp 150-152 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.34 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.24 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 0.8 Hz, 1H), 7.39 (dd, *J* = 8.4 and 1.6 Hz, 1H), 2.82 (q, *J* = 7.6 Hz, 2H), 1.33 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8 (s), 152.4 (s), 148.8 (s), 143.0 (s), 139.3 (s), 135.7 (s), 128.0 (d), 127.6 (d), 124.3 (d), 123.5 (d), 120.3 (d), 29.0 (t), 15.7 (q); HRMS (ESI, m/z) for $C_{15}H_{13}N_2O_2S$, calcd 285.0698, found 285.0695; Anal. Calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found; C, 62.89; H, 4.45; N, 9.55.

6-Methoxyl-2-(4-nitrophenyl)benzothiazole (5f):[3] Yellow solid; yield: 89%; mp 214-216 ^{o}C; 1H NMR (CDCl3, 400 MHz) δ 8.33 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.20 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.38 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 9.2 and 2.4 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2 (s), 158.6 (s), 148.68 (s), 148.65 (s), 139.3 (s), 137.0 (s), 127.8 (d), 124.5 (d), 124.3 (d), 116.6 (d), 104.0 (d), 55.9 (q); HRMS (ESI, m/z) for $C_{14}H_{11}N_2O_3S$, calcd 287.0490, found 287.0492; Anal. Calcd for $C_{14}H_{10}N_2O_3S$: C, 58.73; H, 3.52; N, 9.78. Found; C, 58.68; H, 3.47; N, 9.75.

5-Methoxyl-2-(4-nitrophenyl)benzothiazole (5g):[3] Yellow solid; yield: 72%; mp: 195‑196 ^{o}C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.33 (d, *J* = 8.8 Hz, 2H), 8.21 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 9.0 and 2.4 Hz, 1H), 3.91 (s, 3H).

7-Methoxyl-2-(4-nitrophenyl)benzothiazole (5h):[3] Yellow solid; yield: 8%; mp = 228-229 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, *J* = 8.8 Hz, 2H), 7.75-7.73 (m, 1H), 7.49 (t, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.38, 155.62, 154.32, 148.93, 139.20, 128.16, 127.82, 124.29, 116.35, 105.93, 56.02 HRMS (ESI, m/z) for $C_{14}H_{10}N_2O_3SNa$, calcd 309.0310, found 309.0308; Anal. Calcd for $C_{14}H_{10}N_2O_3S$: C, 58.73; H, 3.52; N, 9.78. Found; C, 58.94; H, 3.79; N, 9.65.

6-Trifluoromethyl-2-(4-nitrophenyl)benzothiazole (5m):[3] Yellow solid; yield: 282 mg (87%); mp 149-151 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (dt, *J* = 9.2 and 2.0 Hz, 2H), 8.27-8.23 (m, 3H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.77 (dd, *J* = 8.4 and 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.8 (s), 155.8 (s), 149.4 (s), 138.3 (s), 135.4 (s), 128.4 (d), 128.2 (q), 124.3 (d), 124.2 (d), 123.9 (q), 123.8 (q), 119.5 (q); HRMS (EI, *m/z*) for $C_{14}H_7F_3N_2O_2S$, calcd 324.0175, found 324.0178; Anal. Calcd for $C_{14}H_7F_3N_2O_2S$: C, 51.85; H, 2.18; N, 8.64. Found: C, 52.24; H, 2.57; N, 8.86.

2-(3-Methyl-4-nitrophenyl)benzothiazole (5q):[3] White solid; yield: 235 mg (87%); mp 163-165 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13-8.08 (m, 3H), 8.02 (dd, *J* = 8.0 and 2.0 Hz, 1H), 7.95 (dd, *J* = 8.0 and 1,2 Hz, 1H), 7.55 (dt, *J* = 8.0 and 1.2 Hz, 1H), 7.45 (dt, *J* = 8.0 and 1.2 Hz, 1H), 2.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1 (s), 154.0 (s), 150.1 (s), 137.5 (s), 135.4 (s), 134.7 (s), 131.4 (d), 126.8 (d), 126.1 (d), 125.8 (d), 125.5 (d), 123.8 (d), 121.8 (d), 20.6 (s); HRMS (ESI, *m/z*) for $C_{14}H_{11}N_2O_2S$, calcd 271.0541, found 271.0541; Anal. calcd for $C_{14}H_{11}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36. Found: C, 62.07; H, 3.88; N, 10.26.

6-Methoxy-2-(3-methyl-4-nitrophenyl)benzothiazole (5r):[3] Yellow solid; yield: 273 g (91%); mp 195-197 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 0.8 Hz, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.95 (dd, *J* = 8.4 and 2.0 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.13 (dd, *J* = 9.2 and 2.4 Hz, 1H), 3.91 (s, 3H), 2.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4 (s), 158.4 (s), 149.7 (s), 148.6 (s), 137.7 (s), 136.9 (s), 134.6 (s), 131.0 (d), 125.5 (d), 125.4 (d), 124.3 (d), 116.4 (d), 104.0 (s), 55.8 (q), 20.6 (q); HRMS (ESI, *m/z*) for $C_{15}H_{14}N_2O_3S$, calcd 301.0647, found 301.0648; Anal. calcd for $C_{15}H_{14}N_2O_3S$: C, 59.93; H, 4.03; N, 9.33. Found: C, 60.37 H, 4.13; N, 9.36.

6-Ethyl-2-(3-methyl-4-nitrophenyl)benzothiazole (5s):[3] Yellow solid; yield: 268 mg (90%); mp 118-120 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10 (d, 8.8 Hz, 1H), 8.08 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 8.01 (dd, *J* = 8.4 and 2.0 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.39 (dd, *J* = 8.4 and 2.0 Hz, 1H), 2.82 (q, 7.6 Hz, 2H), 2.72 (s, 3H), 1.33 (t, 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.1 (s), 152.3 (s), 149.9 (s), 142.8 (s), 137.7 (s), 135.6 (s), 134.6 (s), 131.3 (d), 127.5 (d), 125.7 (d), 125.6 (d), 123.4 (d), 120.3 (d), 29.0 (t), 20.6(q), 15.70 (q); HRMS (ESI, *m/z*) for $C_{16}H_{15}N_2O_2S$, calcd 299.0854, found 299.0856; Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 64.41; H, 4.73; N, 9.38. Found; C, 64.15; H, 4.80; N, 9.36.

2-(3-Methyl-4-nitrophenyl)-6-trifluoromethyl-benzothiazole (5w):[3] Yellow solid; yield: 68%; mp 99-100 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21−8.15 (m, 2H), 8.08-8.06 (m, 2H), 8.00 (dd, *J* =8.8 Hz and 1.6 Hz, 1H), 7.76 (dd, *J* = 8.8 Hz and 1.6 Hz, 1H), 2.69 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.0, 155.7, 150.4, 136.6, 135.2, 134.6, 131.60, 127.9 (q, *J* = 32.6 Hz, 1C), 125.9 (d, *J* = 43.2 Hz, 1C), 125.49, 124.08, 123.8, 122.2, 119.4 (q, *J* = 4.5 Hz, 1C), 20.4; HRMS (ESI, *m/z*) for $C_{15}H_{10}N_2O_2F_3S$, calcd 339.0415, found 339.0414; Anal. Calcd for $C_{15}H_9N_2O_2F_3S$: C, 53.25; H, 2.68; N, 8.28. Found; C, 53.56; H, 3.18; N, 7.95.

2-(4-Aminophenyl)benzothiazole (6d):[3] Yellow solid; 94% yield; mp 130–132 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (m, 1H), 7.89 (m, 2H), 7.84 (m, 1H), 7.44 (m, 1H), 7.32 (m, 1H), 6.72 (dt, *J* = 4.2 and 2.0 Hz, 2H), 4.00 (br s, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.5, 154.2, 149.2, 134.6, 129.1, 126.0, 124.4, 123.9, 122.5, 121.4, 114.8; HRMS (ESI, m/z) for $C_{13}H_{10}N_2S$ calcd 226.0565, found 226.0567; Anal. Calcd for $C_{13}H_{10}N_2S$: C, 69.00; H, 4.45; N, 12.38. Found: C, 69.01; H, 4.69; N, 12.29.

5-Methoxy-2-(4-aminophenyl)-1,3-benzothiazole (6g):[4] Yellow solid; 75% yield; mp 108–109 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.50 (d, *J* = 2.6 Hz, 1H), 6.97 (dd, *J* = 8.9 Hz and 2.6 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 2H), 3.88 (s, 3H); HRMS (ESI, m/z) for $C_{14}H_{13}N_2OS$ calcd 257.0749, found 257.0748. Anal. Calcd for $C_{14}H_{12}N_2O_S$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.33; H, 4.92; N, 10.65.

2-(4-Aminophenyl)-7-methoxybenzothiazole (6h):[3] Yellow solid; 95% yield; mp 142–144 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.0Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 2H), 3.99 (s, 5H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 169.1, 15.9, 154.2, 149.2, 129.1, 126.9, 124.0, 123.0, 115.3, 114.8, 104.7, 55.9; HRMS (ESI, m/z) for $C_{14}H_{13}N_2OS$ calcd 257.0749, found 257.0748; Anal. Calcd for $C_{14}H_{12}N_2OS$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.66; H, 4.86; N, 10.92.

2-(4-Aminophenyl)-6-trifluoromethylbenzothiazole (6m):[3] Yellow solid; 91% yield; mp 181–183 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.90 (dt, *J* = 4.8 and 2.4 Hz, 2H), 7.67 (dd, *J* = 6.8 and 1.6 Hz, 1H), 6.73 (dt, *J* = 4.8 and 2.4 Hz, 2H), 4.08 (br s, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 158.1, 149.9, 132.3, 129.4, 123.1, 123.0, 122.5, 119.0, 119.0, 114.7; HRMS (ESI, m/z) for $C_{14}H_9F_3N_2S$ calcd 294.0439, found 294.0438; Anal. Calcd for $C_{14}H_9F_3N_2S$: C, 57.14; H, 3.08; N, 9.52. Found: C, 57.20; H, 3.18; N, 9.49.

2-(4-Amino-3-methylphenyl)benzothiazole (6q):[3] Yellow solid; 94% yield; mp 147–149 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, *J* = 8.0 Hz, 1H), 7.85–7.83 (m, 2H), 7.75 (dd, *J* = 8.0 and 2.0 Hz, 1H), 7.44 (td, *J* = 8.0 and 1.2 Hz, 1H), 7.31 (td, *J* = 8.0 and 1.2 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 3.94 (br s, NH_2), 2.23 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.7, 154.2, 147.5, 134.5, 129.7, 126.9, 126.0, 124.3, 123.8, 122.4, 122.1, 121.3, 114.5, 17.1; HRMS (ESI, m/z) for $C_{14}H_{12}N_2OSNa$ calcd 263.0619, found 263.0618. Anal. Calcd for $C_{14}H_{12}N_2OS$: C, 69.97; H, 5.03; N, 11.66. Found: C, 69.84; H, 5.01; N, 11.70.

2-(4-Amino-3-methylphenyl)-6-methoxybenzothiazole (6r):[3] Yellow solid; 95% yield; mp 151–153 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 2.0 Hz, 1H), 7.69 (dd, *J* = 6.0 and 2.4 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 7.03 (dd, *J* = 6.0 and 2.4 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 3.86 (s, 5H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.4, 157.2, 148.7, 147.2, 135.8, 129.4, 126.6, 124.0, 122.9, 122.2, 115.0, 114.6, 104.3, 55.8, 17.2; HRMS (ESI, m/z) for $C_{15}H_{15}N_2OS$ calcd 271.0905, found 271.0906; Anal. Calcd for $C_{15}H_{14}N_2OS$: C, 66.64; H, 5.22; N, 10.36. Found: C, 66.68; H, 5.38; N, 10.48.

2-(4-Amino-3-methylphenyl)-6-trifluoromethylbenzothiazole (6w):[3] Yellow solid; 91% yield; mp 152–154 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 1.2 Hz, 1H), 7.77 (dd, *J* = 6.0 and 2.4 Hz, 1H), 7.66 (dd, *J* = 6.8 and 1.6 Hz, 1H), 6.72 (d, *J* = 8 Hz, 1H), 4.20 (br s, NH_2), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 156.3, 148.3, 134.6, 130.0, 127.3, 126.4 (q, *J* = 65.5 Hz, 1C), 123.0 (q, *J* = 5.6 Hz, 1C), 122.4, 122.1, 118.9 (q, *J* = 5.6 Hz, 1C), 114.5, 17.2; HRMS (ESI, m/ z) for $C_{15}H_{12}F_3N_2S$ calcd 308.0595, found 308.0597. Anal. Calcd for $C_{15}H_{11}F_3N_2S$: C, 58.43; H, 3.60; N, 9.09. Found: C, 58.50; H, 3.60; N, 9.09.

5. Experimental protocol for biological studies

Cell culture. [1] Two melanoma cell lines A375 (human) and B16F10 (murine), purchased from American Type Culture Collection (Manassas, VA), were maintained in Dulbecco's minimal essential medium (DMEM) supplemented with10% FCS and 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate (Gibco, BRL). Cells were passaged at the confluence after treatment with 5 mM EDTA (Gibco, BRL) and incubated at 37 ^{o}C in a humidified atmosphere containing 5% CO_2.

UVA irradiation.[2] For UVA irradiation, a specific UVA lamp emitting a peak wavelength of 365 nm (UVP, Upland, CA, USA) was used. The cultured cells were pretreated with different agents at 5 μM for 4 h before UVA irradiation. The cultured cells were rinsed with phosphate-buffered saline (PBS) and then irradiated with 1 J/cm^2 UVA in PBS to avoid the formation of medium-derived toxic photoproducts induced by UV exposure. The doses of irradiation were measured by a UVX digital radiometer (UVP, Upland, CA, USA) and the incident irradiance at the surface of the cells was found to be 4.762 mW/cm^2 at the target distance of 17 cm. The calculation formula of designated time for UVA treatment is energy (J/cm^2) = power (W/cm^2) $\times$ exposure time (s). Immediately after photo treatment, PBS was removed and media were added to the cells. All the following experiments were performed three times in triplicate.

Cell viability.[2] Cell viability was assessed by the MTT assay, a mitochondrial function assay based on the ability of viable cells to reduce the redox indicator MTT to insoluble formazan crystals by mitochondrial dehydrogenase. Briefly, cells were seeded in a 96-well plate at the cell density of 10000 cells/well. After an overnight incubation, the cells were treated with compounds at 5 μM for 4 h followed by 1J/cm^2 UVA irradiation. Forty-eight hours after exposure, the medium was discarded and replaced with 10 μL of MTT dye. Plates were

incubated at 37 °C for 2 h. The resulting formazan crystals were solubilized in 100 μL DMSO, and the optical density was read at 540 nm with a microplate reader (MRX-II, Dynex technology, Chantilly, VA).

Fluorescence measurement of uptake of 6l. Cultured A375 cells were seeded on glass coverslips with a density of 2×10^4 cells/well in 24-well plate for 24 hours until cell attachment. Then the cells were exposed to **6l** at 5 μM for indicated time in the dark. The cells were washed twice with PBS and were then fixed with 4 % paraformaldehyde at 4℃ for 30 min. The qualitative expression of cell fluorescence was determined using a Leica inverted microscope (Leica DMI6000, Wetzlar Germany).

Morphological observation. A375 cells ($5x10^5$ cells/well) seeded in 6 well plate. Cells were treated with 5 μM compounds for 4 h followed by 1 J/cm^2 UVA irradiation. Twenty four hours after exposure, pictures were taken by using a microscope at 200x phase.

Determination of intracellular ROS level.[3] To evaluate intracellular reactive oxygen species (ROS) levels, 2',7'-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes) fluorescent dye was used to clarify this issue. The nonpolar DCFH-DA is converted to the polar derivative DCFH by esterases when it is taken up by the cell. DCFH is non-fluorescent but is rapidly oxidized to the highly fluorescent DCF by intracellular H_2O_2 or nitric oxide. After indicated irradiation, DCFH-DA (10 μM) was immediately added into cultured cells for 30 min at 37 ^oC. The fluorescence of the samples was measured with a flow cytometer. The 2',7'-dichlorofluorescein (DCF) data were recorded using FL-1 photomultiplier.

Assessment of mitochondrial membrane potential ($\Delta\Psi_{mt}$).[3] A375 cells were cultured in 35-mm dishes and allowed to reach exponential growth for 24 h before treatment. Cells were pretreated with various concentrations (0-5 μM) of **6l** for 4 h before irradiation. The medium was

removed and the adherent cells trypsinized. The cells were pelleted by centrifugation at 400*g* for 5 min and stained in a 100 nM/ml $DiOC_6$ dye (Molecular Probes, Eugene, OR) for 30 min at room temperature and washed with PBS twice and resuspended in PBS. The samples were analyzed immediately for fluorescence (FL-1 detector, filter 530/30 nm band pass) on a FACScan flow cytometer (Elite ESP, Beckman Coulter, Brea, CA). Histograms were analyzed using Windows Multiple Document interface software (WinMDI).

ATP content bioluminescence assay.[3] The amount of intracellular ATP was determined by bioluminescent assay based on the measurement of the light output of the luciferin-luciferase reaction. After treatment with various concentrations of **6l** plus UVA, total cell extracts from cultured A375 cells were obtained immediately by lysing solution. After centrifugation to remove cell debris, we collected supernatants for ATP measurement. The total amount of intracellular ATP was determined according to the protocol provided with the ATPLite assay kit (Perkin Elmer, Boston, MA).

Quantitative RT-PCR.[2] Total RNA was extracted from A375 cells with or without **6l** plus UVA treatment using the Trizol reagent (Invitrogen). Two micrograms of total RNA were used for reverse transcription using RevertAidTM H Minus First Strand cDNA Synthesis Kit (Fermentas, MA, USA) following the manufacturer's instructions. For real-time qPCR, the ABI PRISM 7900 Sequence Detection System (ABI) was used. Nine microlitres of master-mix (2X Maxima® SYBR Green/ROXqPCR Master Mix, 0.3 μM forward primer, 0.3 μM reverseb primer) and 1 microlitre of 100 nanograms cDNA were added to the 96-well plates and amplified using a suitable program. At the completion of cycling, melting curve analysis was performed to establish the specificity of the amplicon production. Data were analyzed according to the comparative Ct method and were normalized by GAPDH expression.

The primers used were:

mtDNA 4977-bp deletion: forward, 5'- ACTACGGTCAATGCTCTG - 3'; reverse, 5' - GGAGGTTGAAGTGAGAGGTATG - 3'

GAPDH: forward, 5' - CCTCAACTACATGGTTTACATGTTCC - 3'; reverse, 5' - ATGGGATTTCCATTGATGACAAG - 3'

Transmission Electron Microscopy (TEM). Cell after **6l** plus UVA treatment were cultured at 37 ^{o}C incubator for 24h, harvested and fixed with 2% paraformadehyde and 2.5% glutaraldehyde for 2 h at 4 ^{o}C. Then, cells were washed with PBS, and postfixed cells in 2% OsO_4 for 1h at 4 ^{o}C. Cells were dehydrated with different concentration of ethanol and propylene oxide, and embedded in Epikote. Ultrathin sections were counterstained with uranyl acetate and lead citrate before observation with JEM－2000EXII (JEOL Ltd.).

Caspase-3 colorimetric assay.[3] Twenty-four hours after irradiation, cells were collected by centrifugation, washed once with PBS, and cell pellets were counted and resuspended in 25 μL/1 x 10^6 cells of cold lysis buffer and homogenized. Homogenates were centrifuged at 12,000 rpm for 10 min at 4^{o}C, supernatants were used for measuring caspase-3 activity using an ELISA-based assay, according to the manufacturer's instructions. (R&D Systems, Minneapolis, MN). The results were presented as mean ± SD.

Protein extraction and western blot analysis.[2] Total cell extracts from cultured A375 cells were obtained by lysing the cells in ice-cold RIPA buffer (1 X PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing 100 μg/mL PMSF, 2 μg/mL aprotinin, 2 μg/mL leupeptin and 100 μg/mL NaF. After centrifugation at 14,000*g* for 30 min, protein in the supernatants was quantified by Bradford method (Bio-Rad). Forty micrograms of protein per lane was applied in 10% SDS-poly-acrylamide gel. After electrophoresis, protein was transferred from the gel to

polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membranes were blocked at room temperature for 1 h in PBS + 0.1% Tween 20 (PBS-T) containing 5% skim milk. After being briefly rinsed with PBS-T, the blots were probed with respective primary antibodies at room temperature for 2 h or at 4^{o}C overnight. Rabbit polyclonal antibodie against poly(ADP-ribose)polymerase (PARP; H-250) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal antibody against NDUFS3 and actin were purchased from Novus Biologicals (Littleton, CO, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Mouse polyclonal antibody against UQCRC2 was purchased from Novus Biologicals (Littleton, CO, USA). Rabbit monoclonal antibodie against COX II was purchased from Abcam (Burlingame, CA, USA) The membrane was incubated with the corresponding horseradish peroxidase-labeled secondary antibody (Santa Cruz Biotechnology) at room temperature for 1 h. Membranes were washed with PBS-T four times for 15 min, and the protein blots were visualized with Western Lightning Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences, Boston, MA, USA). The relative amounts of specific proteins were quantified by densitometry scanning of X-ray films and analyzed by Eagle Eye Image System (Stratagene, La Jolla, CA, USA).

Immunocytochemical staining. To clarify the role of the tumor apoptosis marker M30 in A375 cells, the mitochondrial marker COX IV and cytochrome *c* in B16 cells treated with **6l**-PDT and its expression were correlated with apoptotic. Cells were seeded on glass coverslips with a density of 1 x 10^4 cells and were incubated overnight. The cells were treated with different concentration of compound **6l** for 4 h followed by 1J/cm^2 UVA irradiation. Immunocytochemistry was performed on all test samples. Cells were washed several times by using PBS and fix by 4% formaldehyde for 5 min at 4 ^{o}C, then permeabilized cell with 0.5%

Triton X-100 for 5 min. Non-specific binding was blocked by 5% bovine serum albumin at 37 ^{o}C for 45 min. Cells were incubated 1 h with primary antibodies (mouse anti- M30 at 1:250, rabbit anti-COX IV and mouse anti-cytochrome c at 1:1000) at RT. Subsequently, slides were incubated with secondary antibodies for 30 min at RT. Finally, added about 40 μl mounting medium contains DAPI to slide and cover with glass coverslips then sealed with nail polish.

Animal experiment. Seven-week-old female ICR strain mice were obtained from BioLASCO Taiwan. The animals were housed under controlled temperature (24± 2°C), humidity (50 ± 10%) and light (12 h light / 12 h dark cycles, without any UV emission). The animals were acclimatized for at least one week prior to the start of the experiments. A total of 5×10^6 B16 cells were inoculated into female ICR mice (about 19-21g / 7week). The subcutaneous inoculation of tumor cells resulted in a tumor generation at the injection site. When tumors reached about 4×4 mm in diameter, mice were separated into groups. Each group had four mice in each experiment and injected 4mg/kg compound **6l** into the tumor site then exposed tumor to different dose of UVA on the day after injection. Tumor volume was measured by calipers each five days after agent injection, and tumor volume was calculated by following formula: Tumor volume = 1/2 X Length X Width^2.

Statistical analysis.[1] All results were expressed as means values ± standard deviation (SD) and analyzed by using the statistical analysis system (SPSS, SPSS Inc., Chicago, IL). Differences among groups were analyzed by Student's *t*-test. *P* values <0.05 were considered as significant for all statistical tests.

6. Photo irradiation of compound 6l with longer wavelengths

We have also confirmed the photo irradiation of compound **6l** at longer wavelength such as red, yellow and blue for their inhibitory activity as shown in Table 5 and results shown that UVA plus compound **6l** shown a maximum inhibitory activity for A375 cells. This confirms that compound **6l** have maximum absorption at UVA region.

Table S2. Photo irradiation of compound **6l** with other wavelengths.[a]

S.No	6l+photoirradiation	**Survival (%)**
1	**6l**+Red light	91.08±0.01
2	**6l**+Yellow light	97.62±0.03
3	**6l**+Blue light	104.29±0.02
4	**6l**+UVA	60.30±0.01

[a]Cells were cultured with the agents at 5 μM for 4 h before 1 J/cm^2 UVA irradiation. twenty-four hours after irradiation, cell survival was assessed using the MTT assay. The data are expressed as the mean ± SD. Red light (630nm±5nm), yellow light(590nm±5nm), blue light (415nm±5nm), UVA 365nm.

7. References:

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^{1}H and ^{13}C NMR of 5a-5y

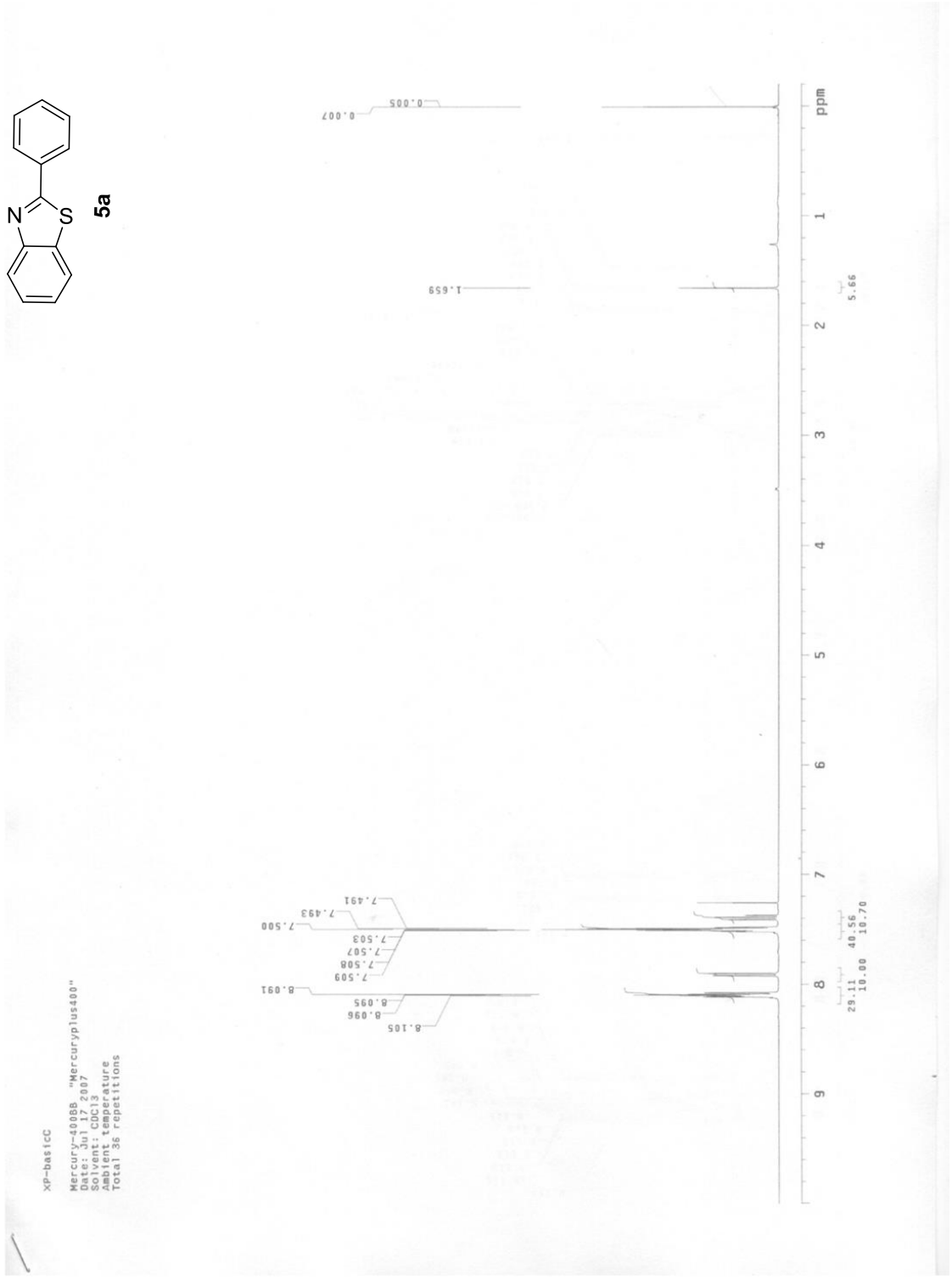


Figure S2. ^{1}H NMR spectrum of compound **5a**

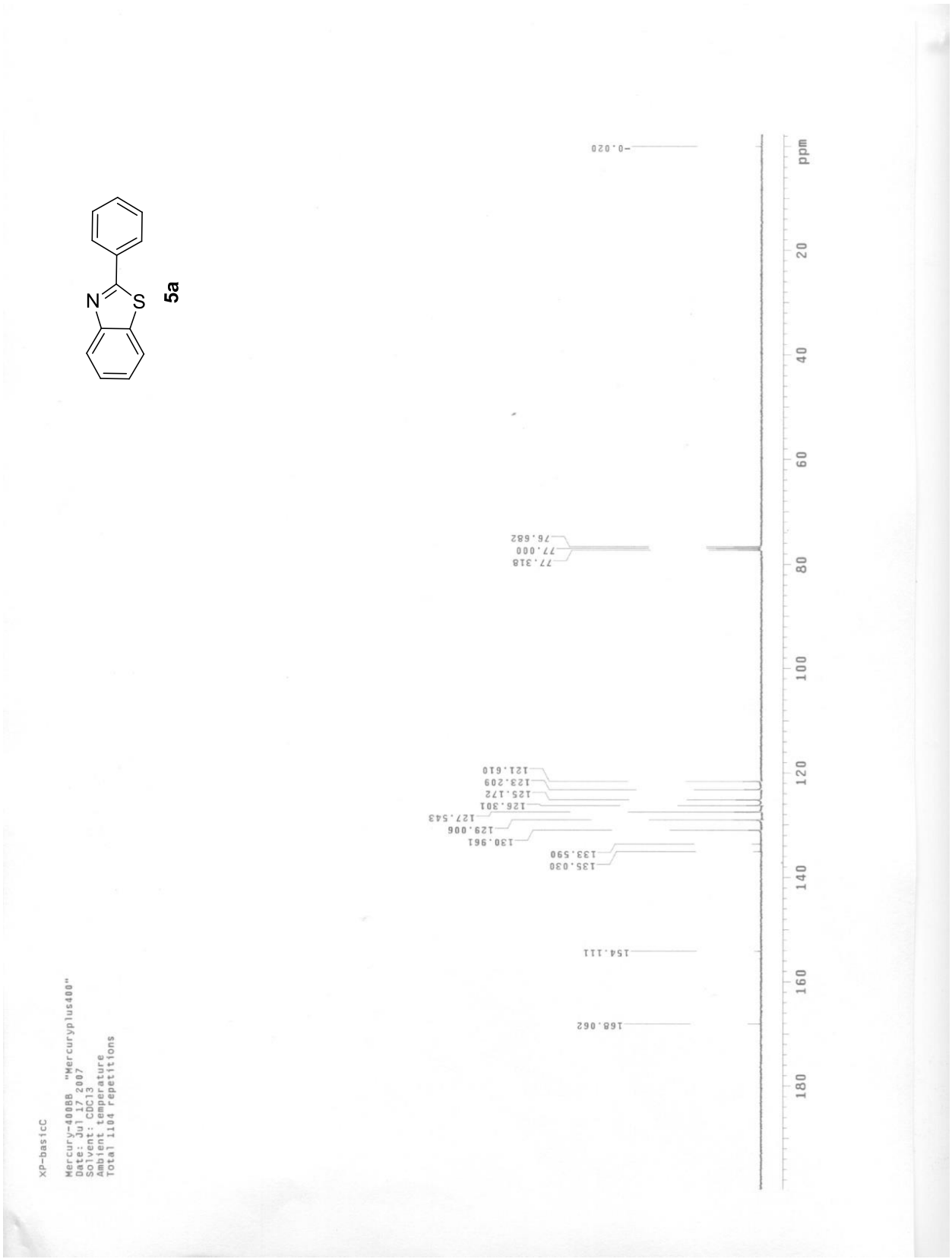


Figure S3. ^{13}C spectrum of compound **5a**

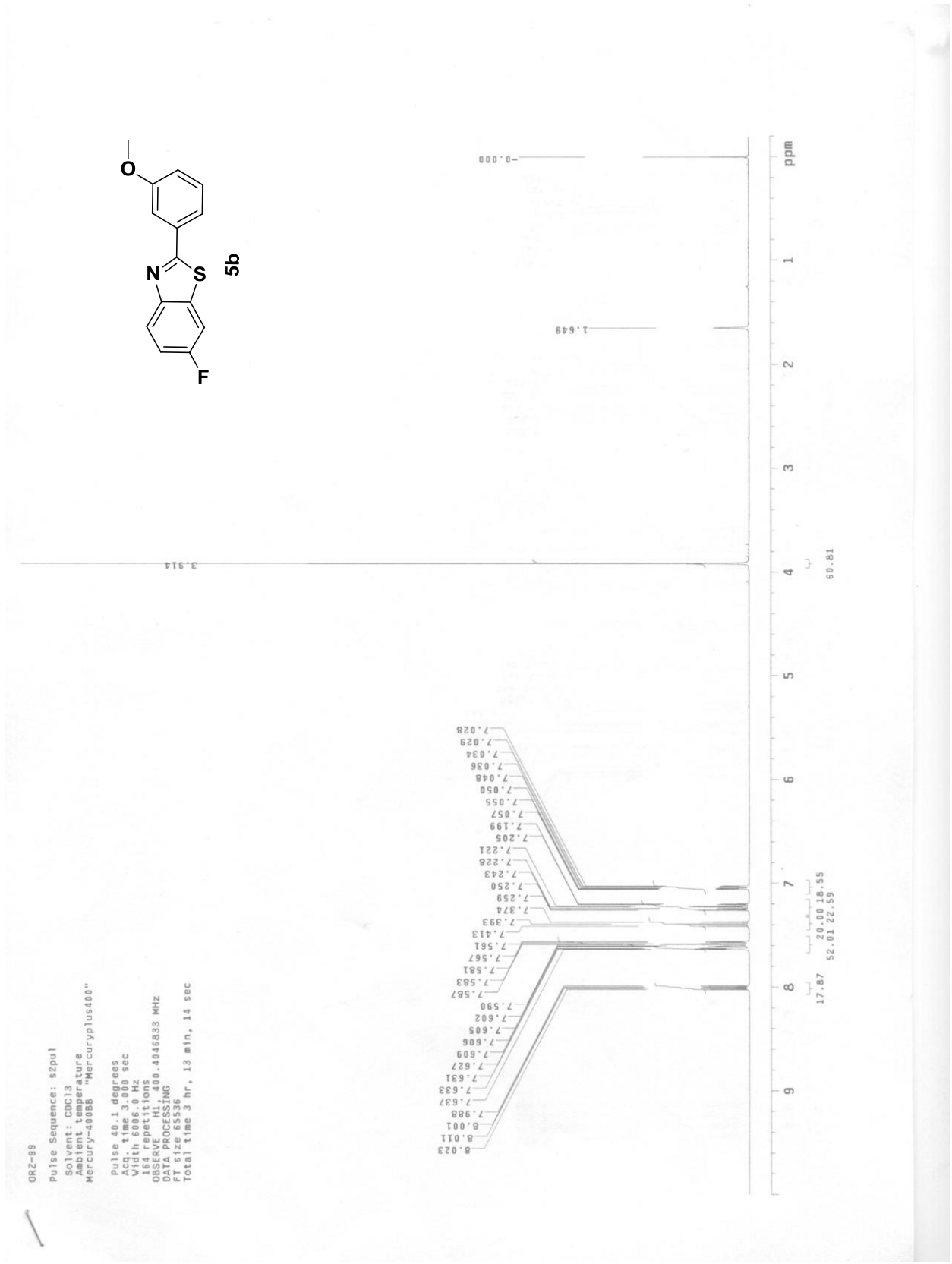


Figure S4. ^{1}H NMR spectrum of compound **5b**

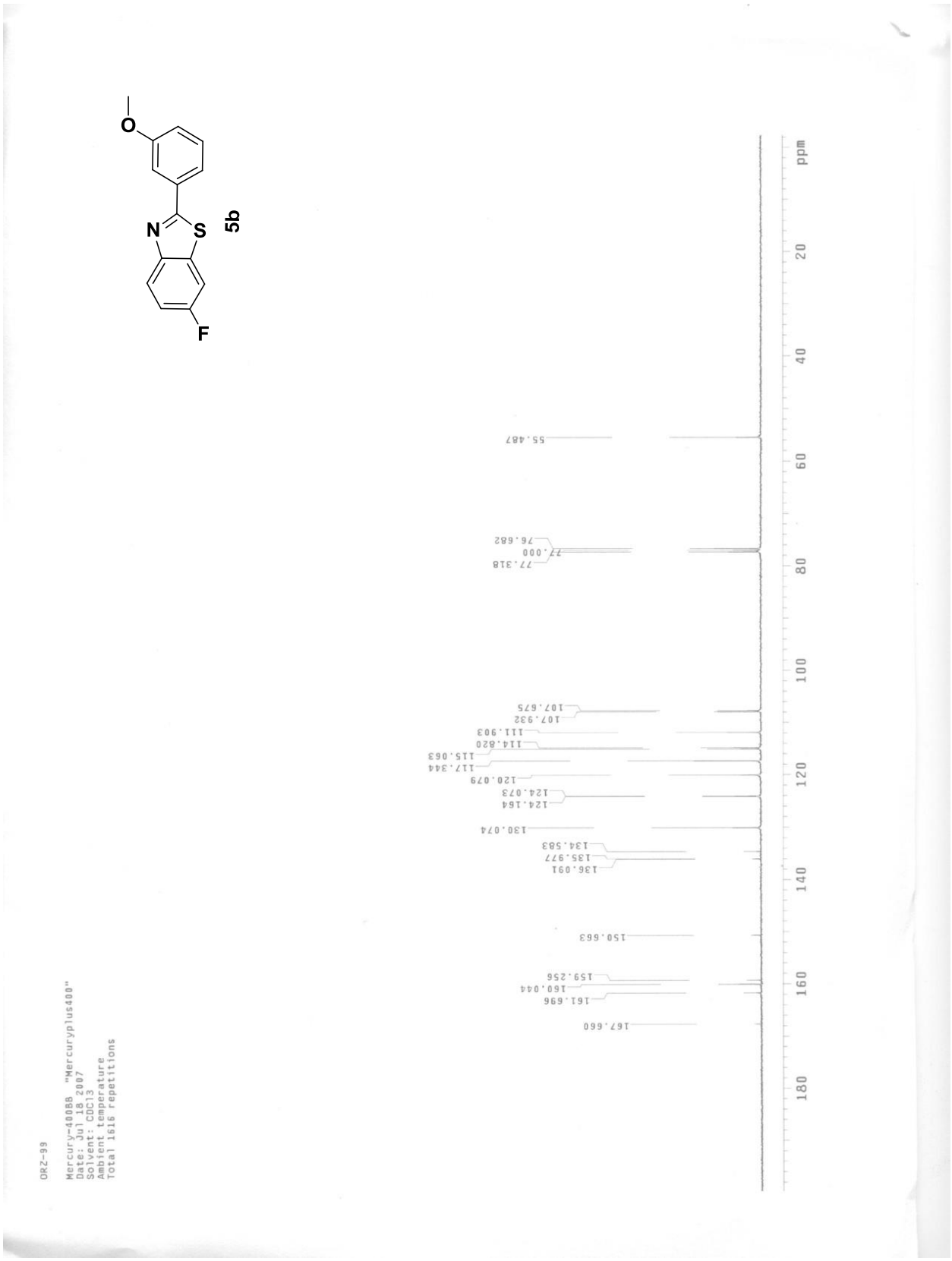


Figure S5. ^{13}C NMR spectrum of compound **5b**

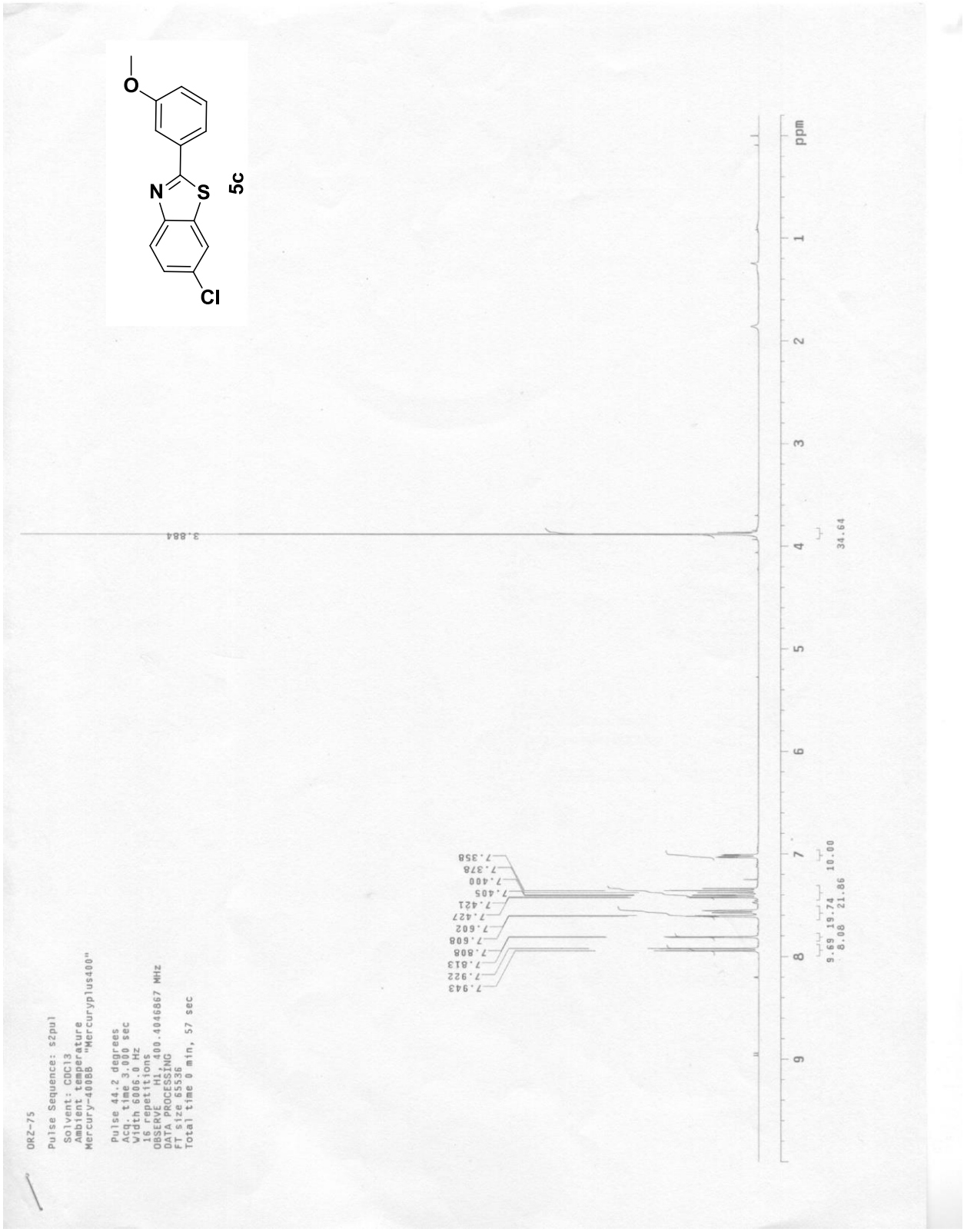


Figure S6. ^{1}H NMR spectrum of compound **5c**

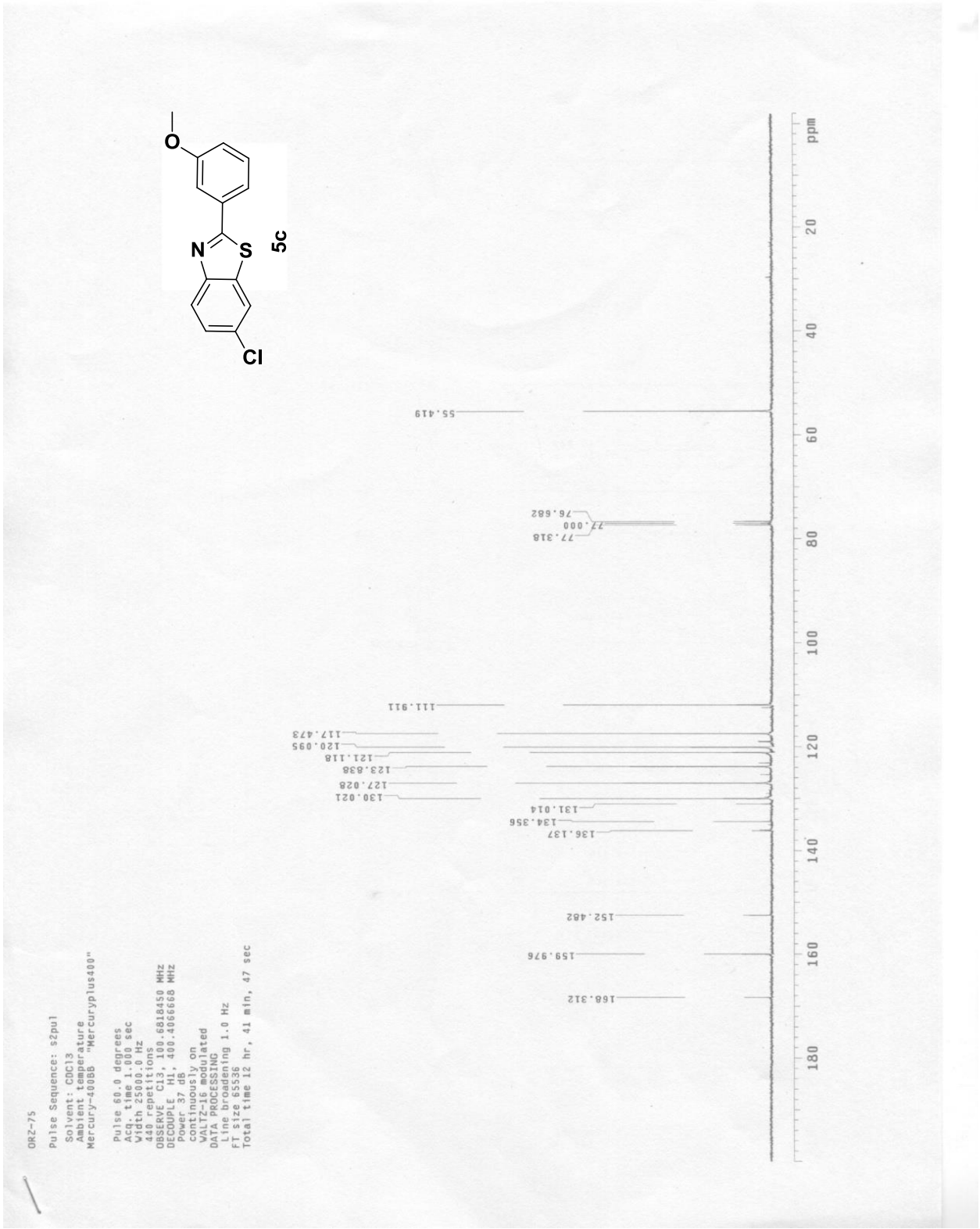


Figure S7. 13C NMR spectrum of compound **5c**

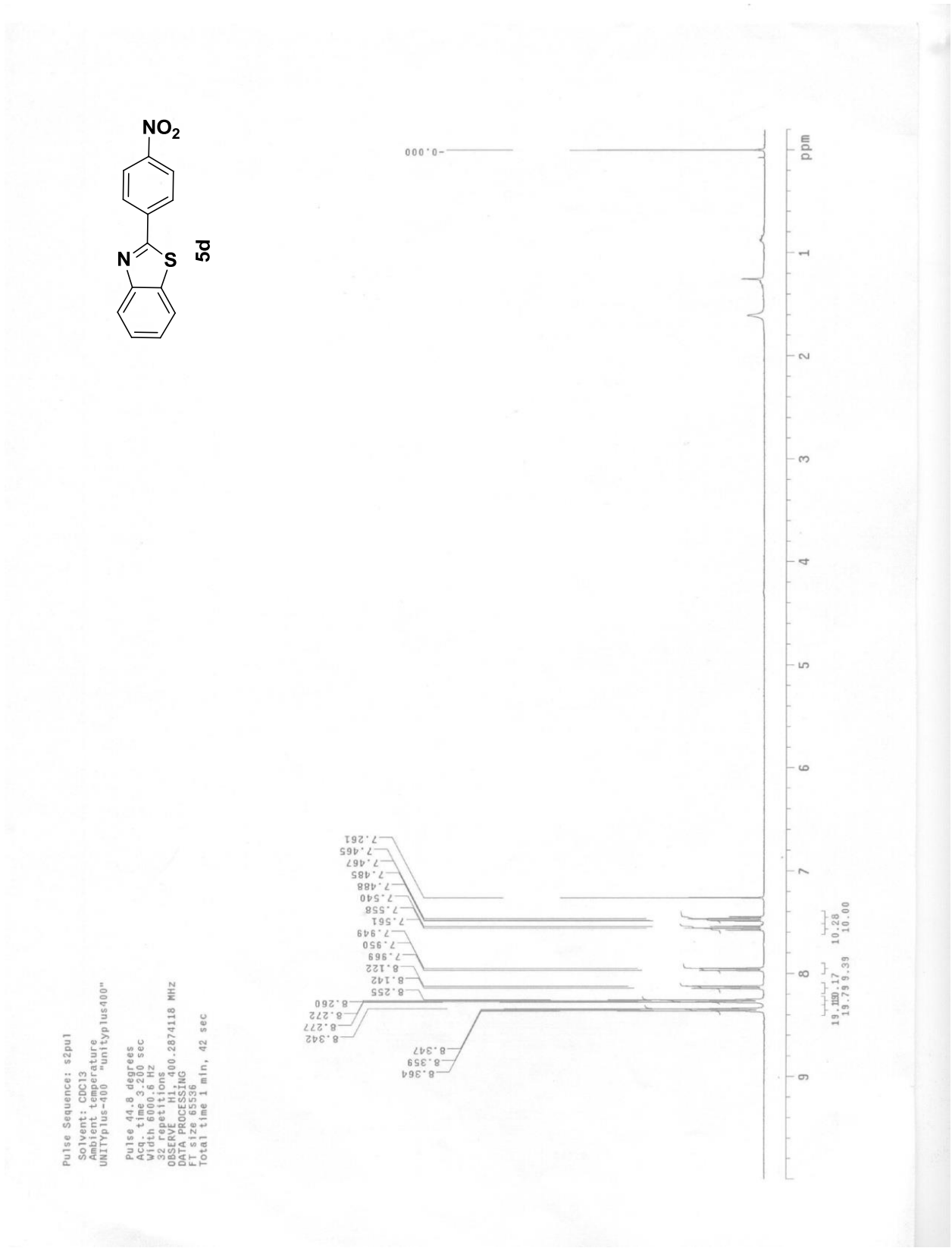


Figure S8. ^{1}H NMR spectrum of compound **5d**

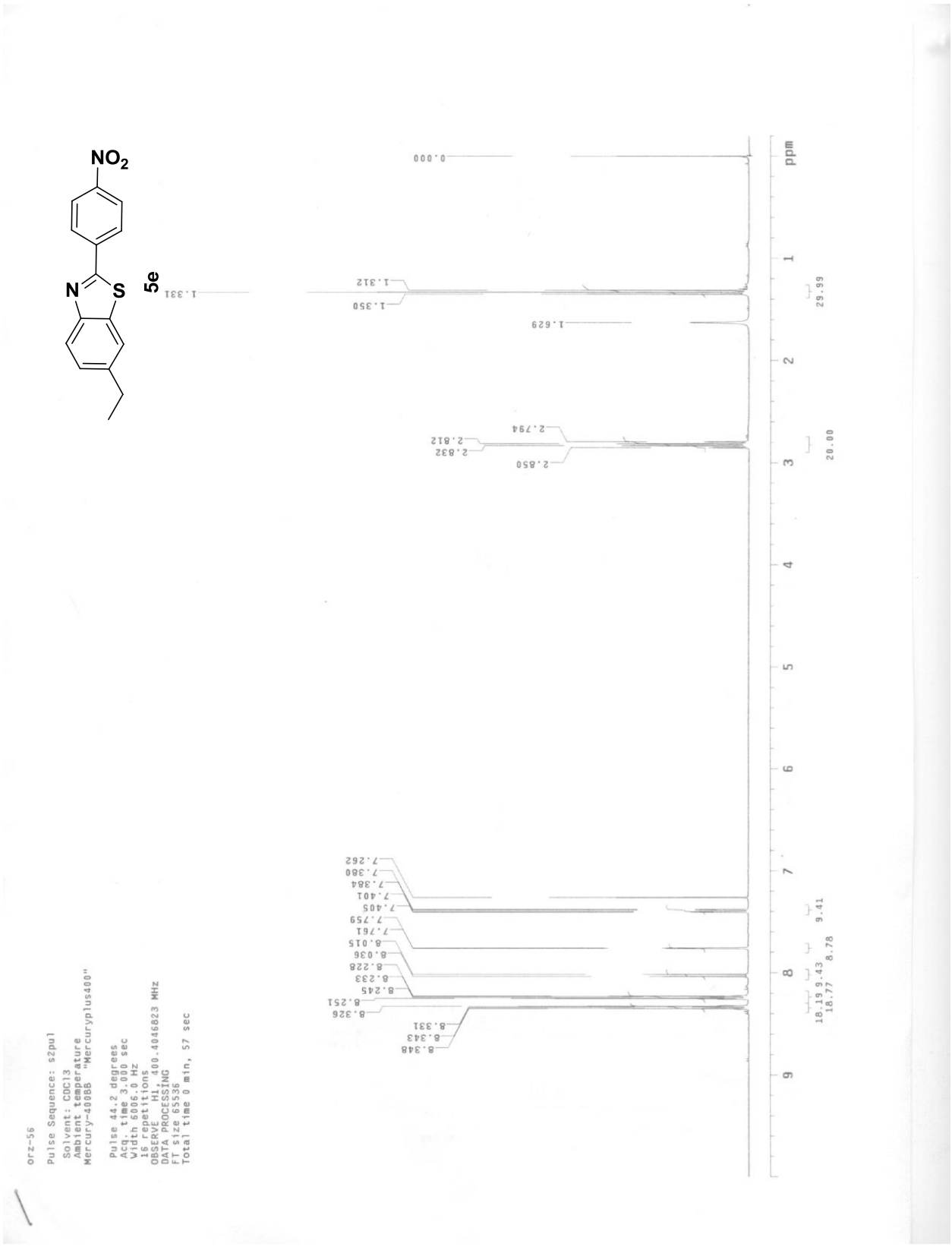


Figure S9. ^{1}H NMR spectrum of compound **5e**

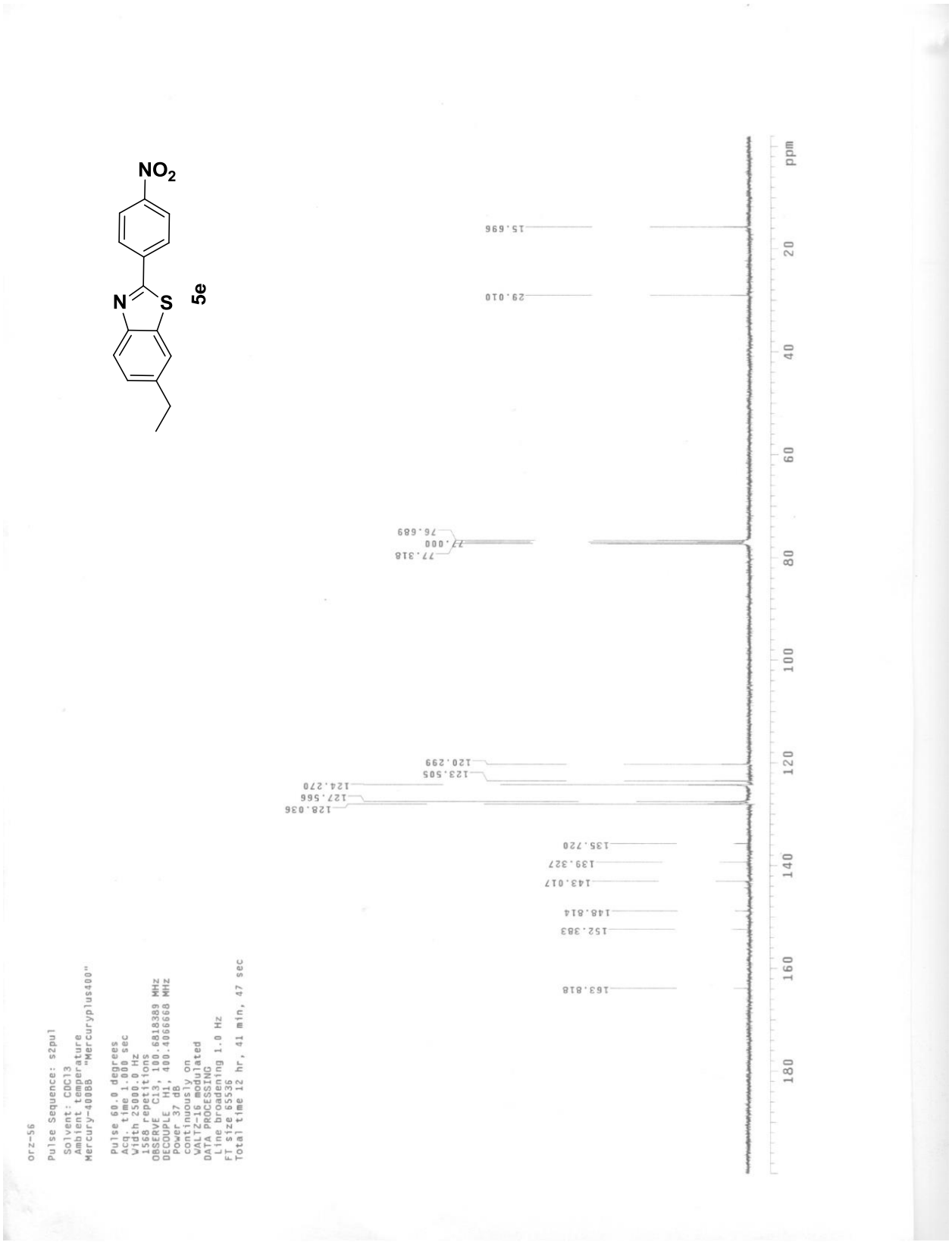


Figure S10. ^{13}C NMR spectrum of compound **5e**

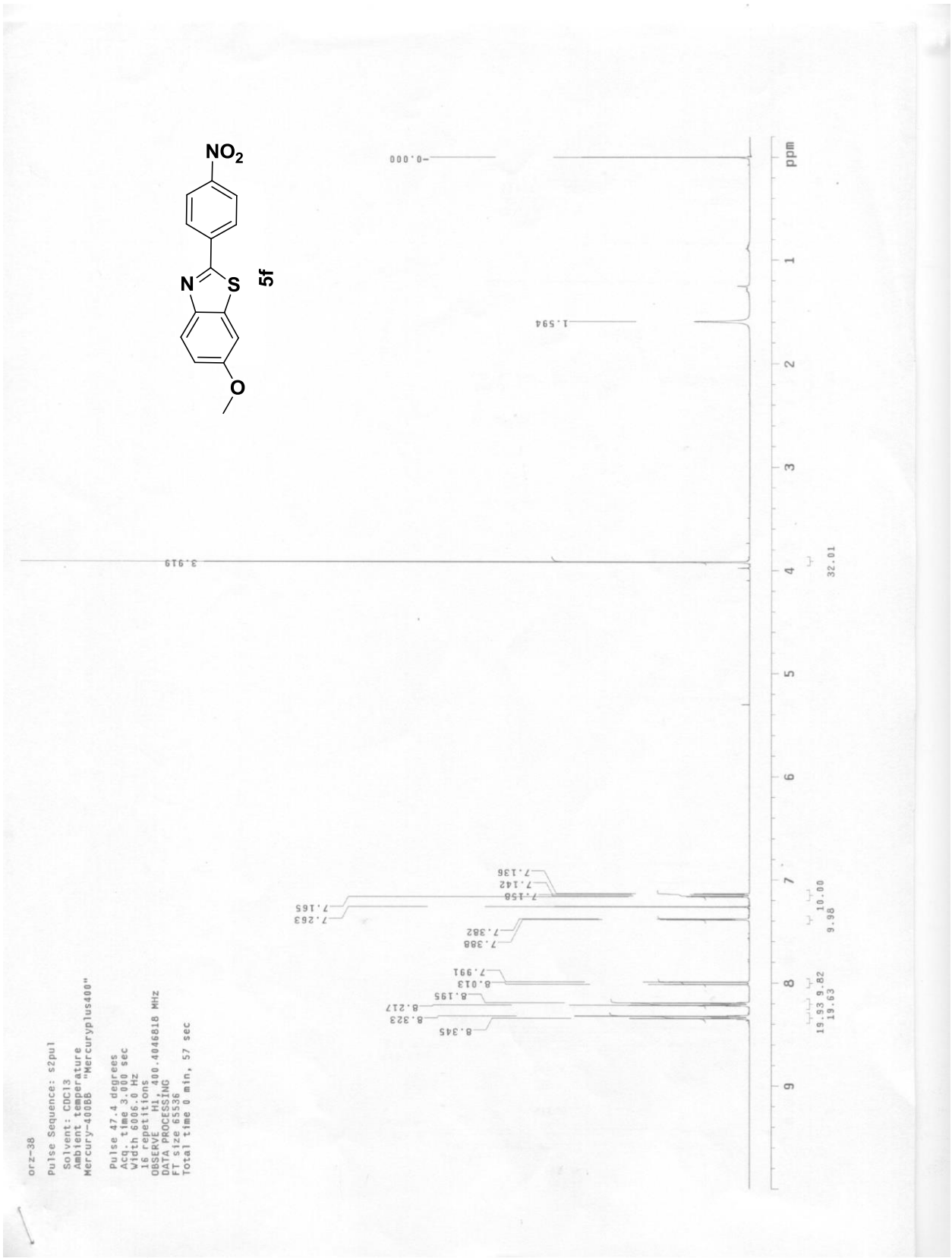


Figure S11. ^{1}H NMR spectrum of compound **5f**

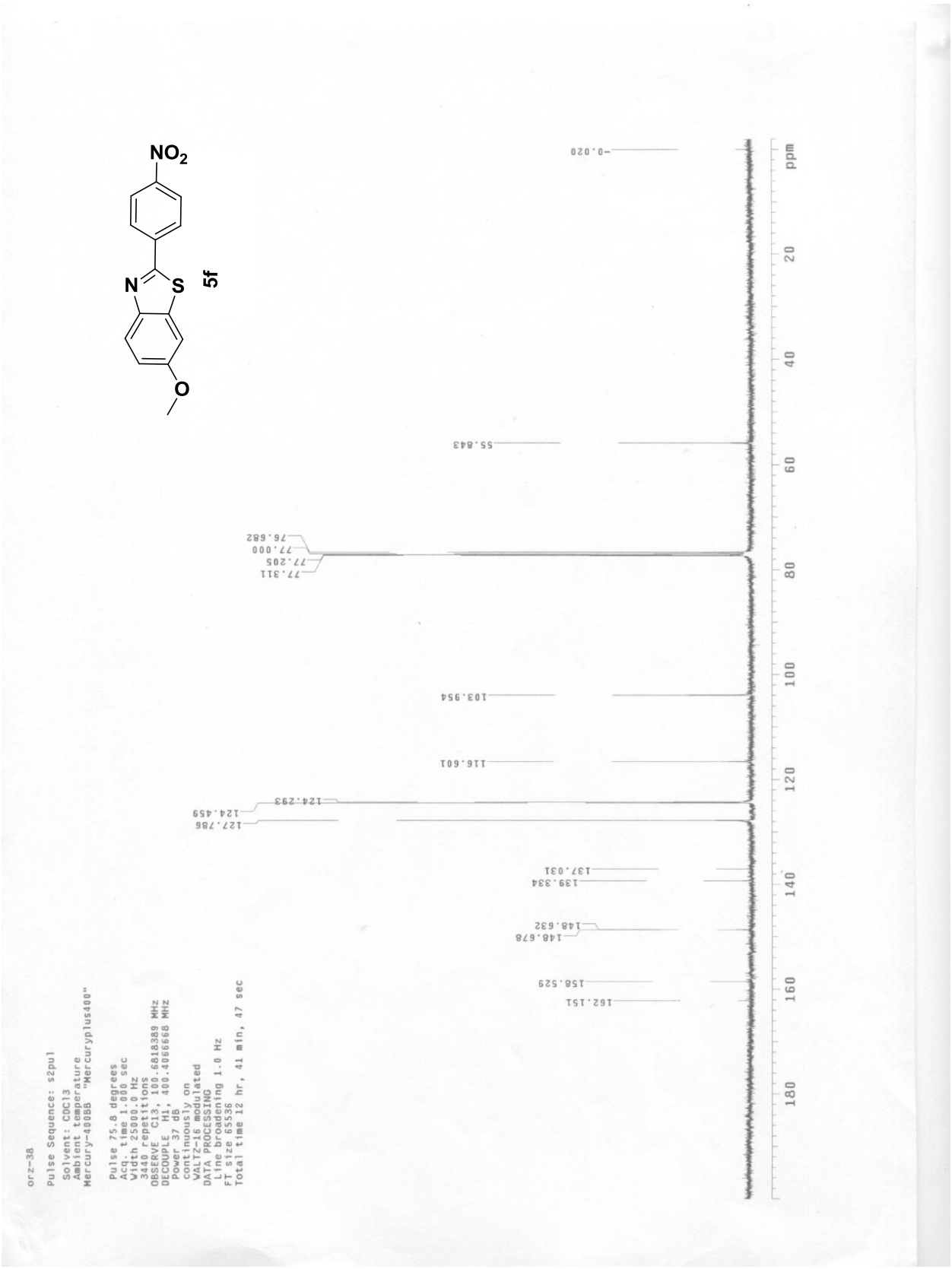


Figure S12. ^{13}C NMR Spectrum of compound **5f**

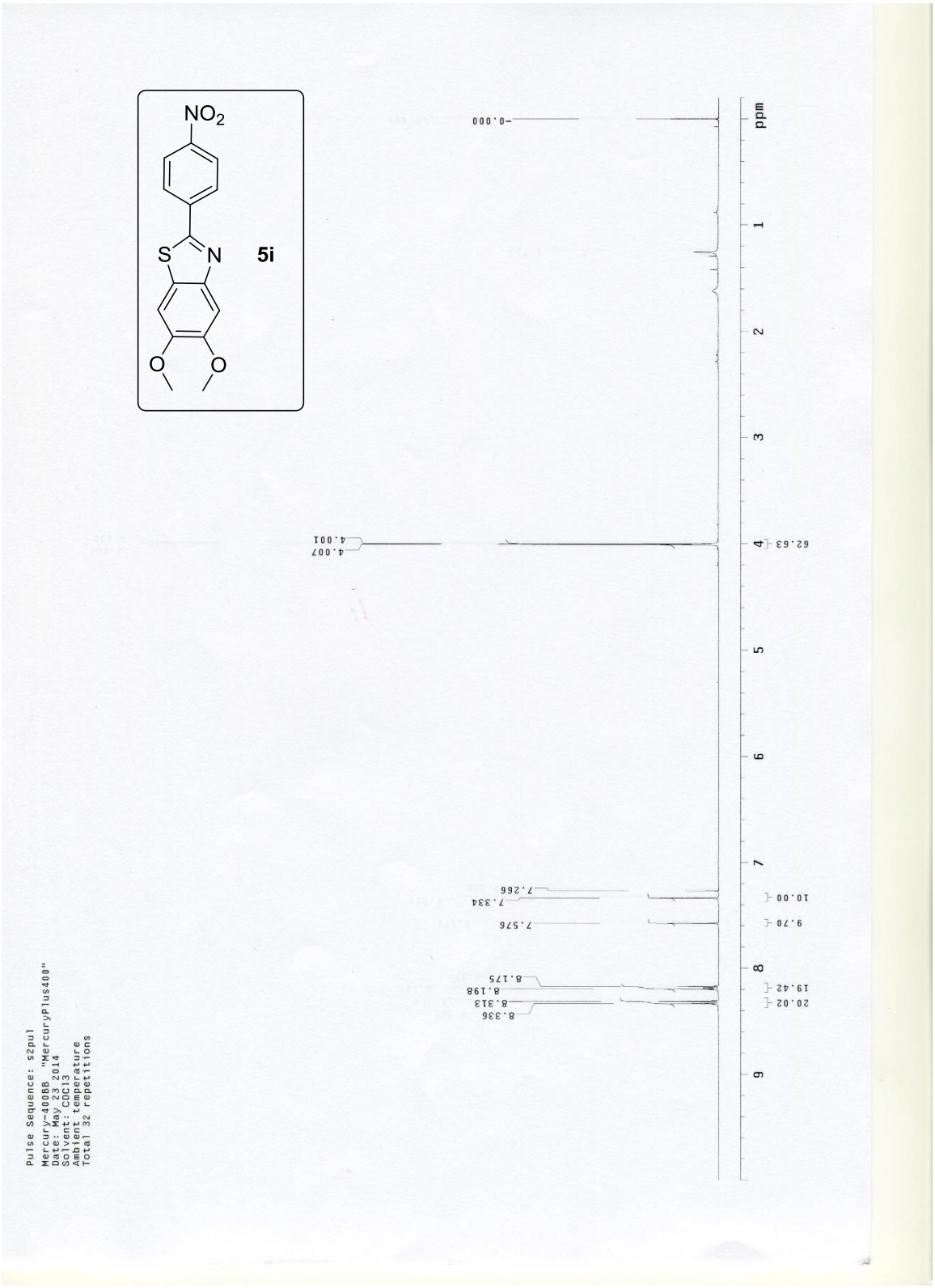
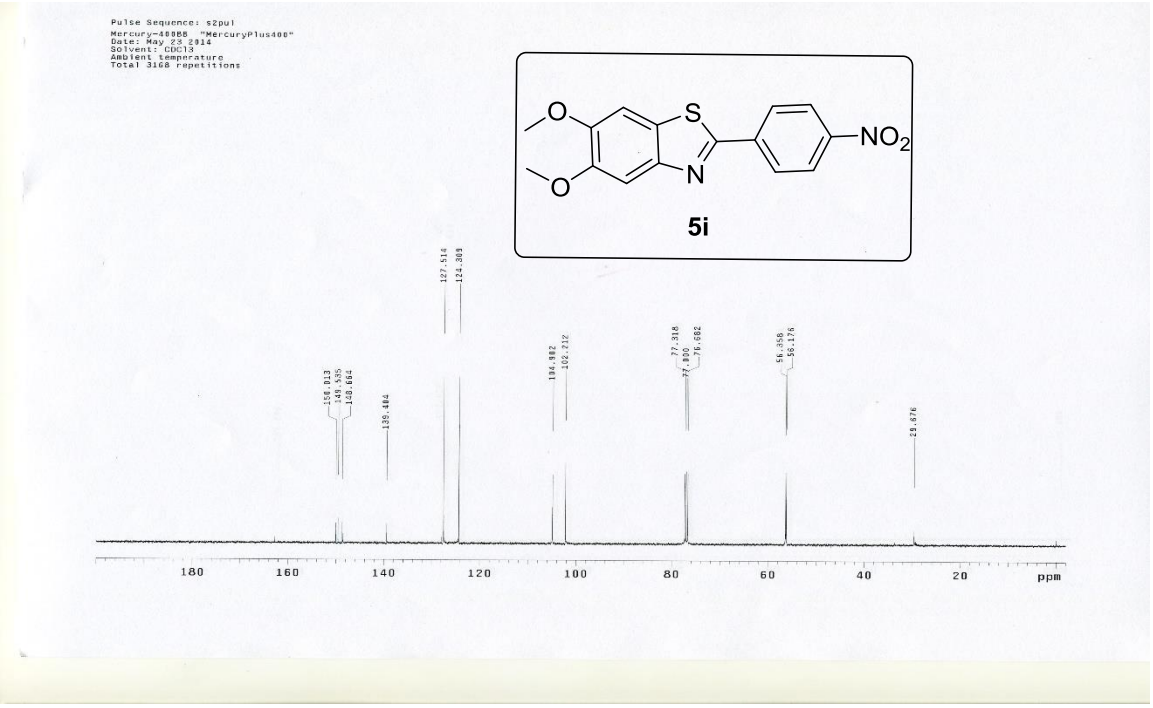


Figure S13. ^{1}H NMR spectrum of compound **5i**



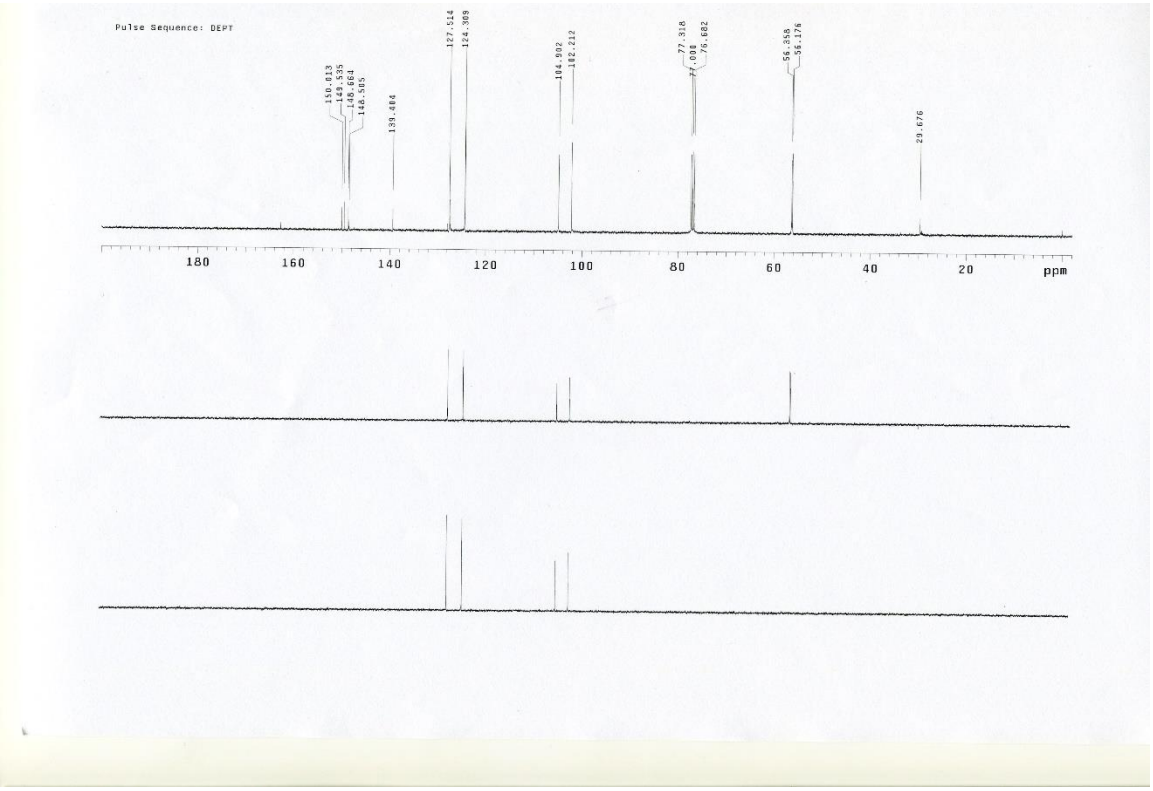


Figure S14. ^{13}C and DEPT NMR spectra of compound **5i**

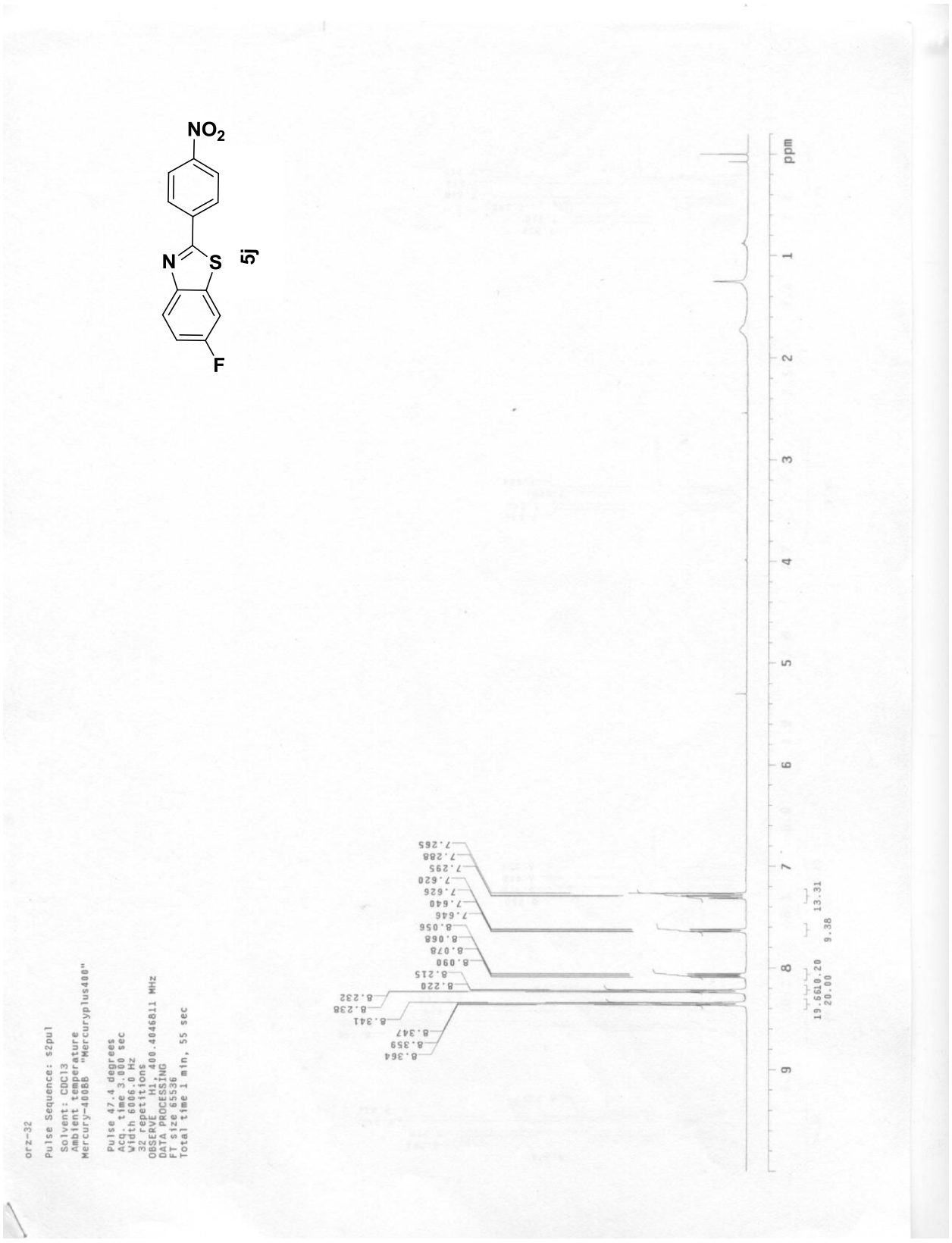


Figure S15. ^{1}H NMR spectrum of compound **5j**

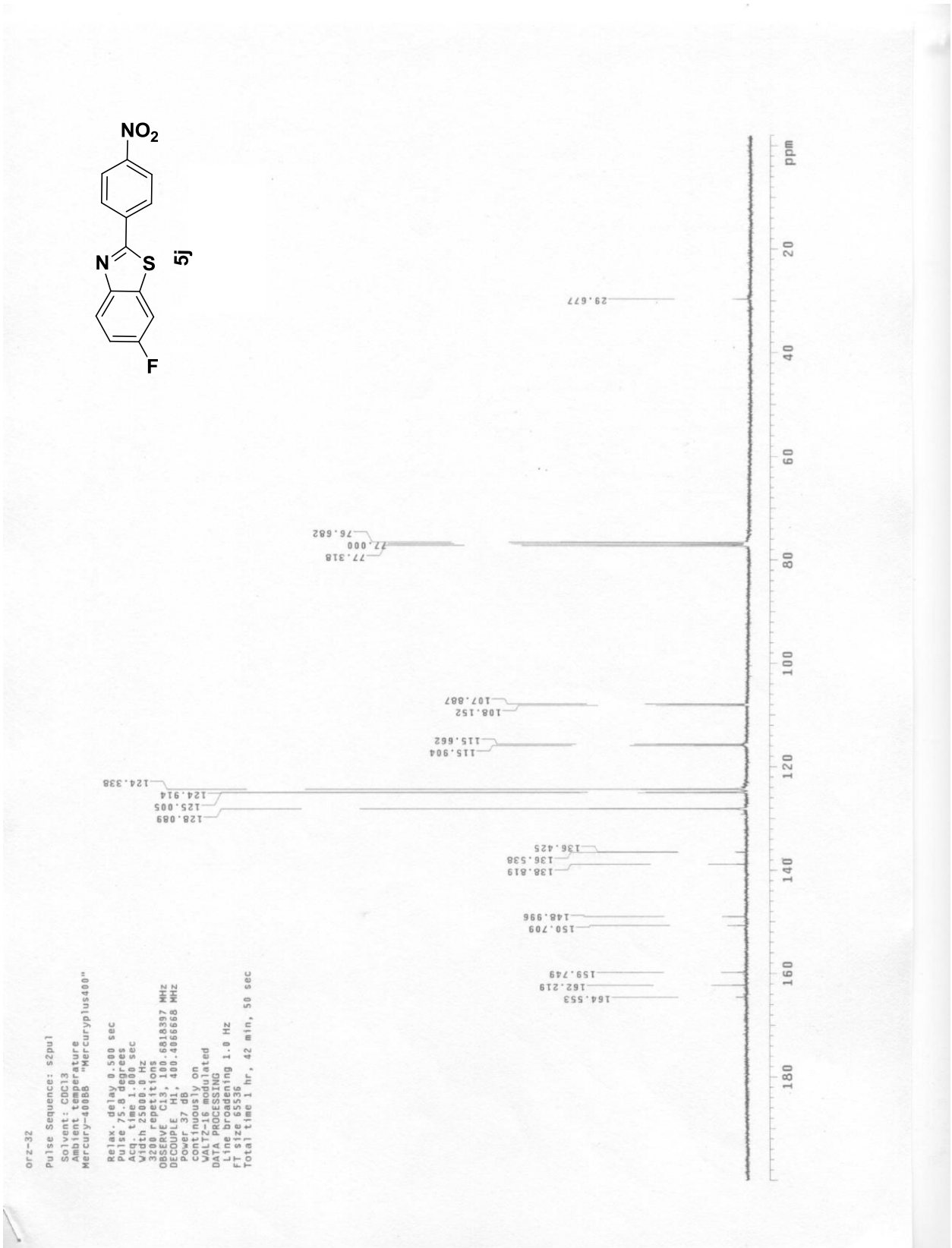


Figure S16. ^{13}C NMR spectrum of compound **5j**

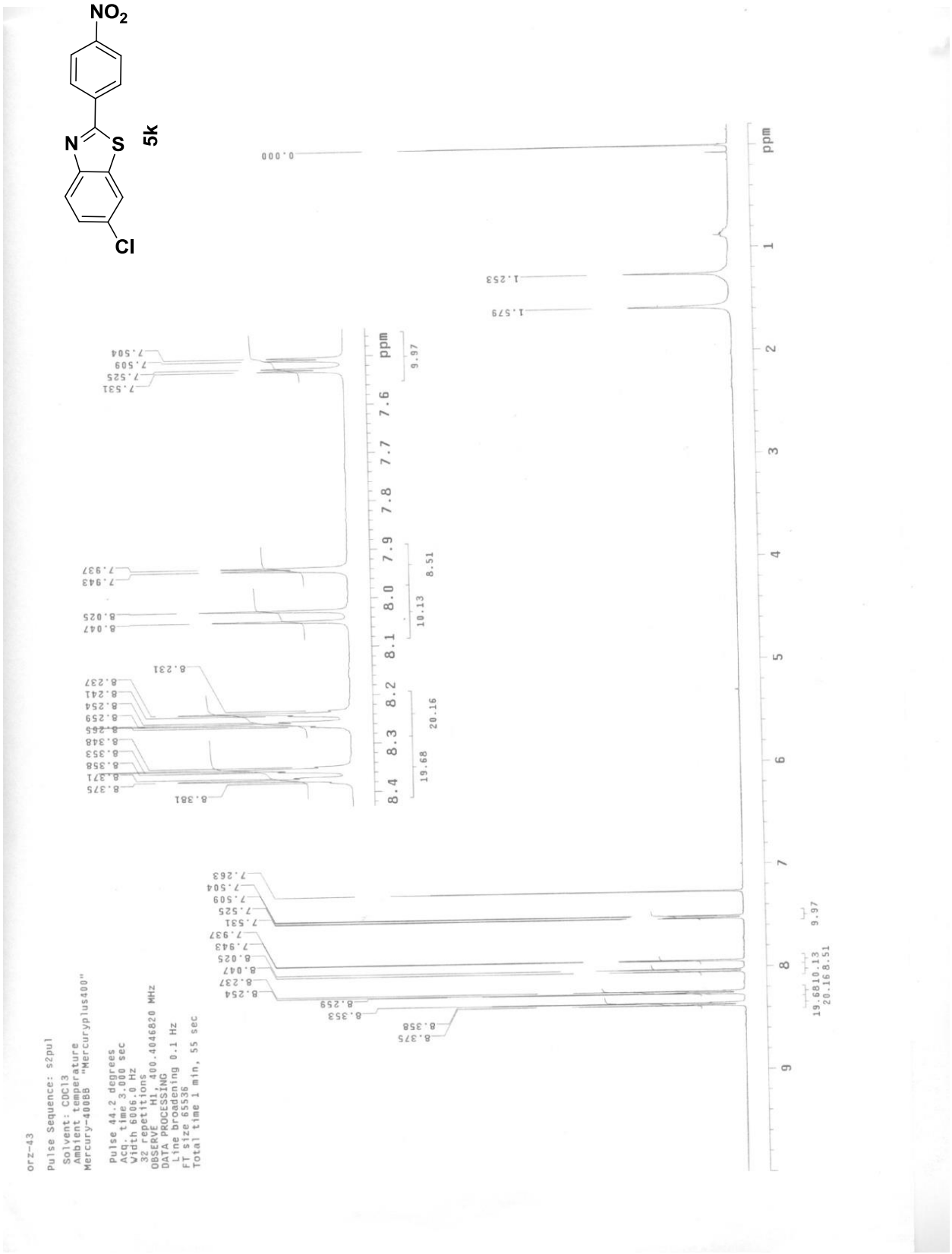


Figure S17. ^{1}H NMR spectrum of compound **5K**

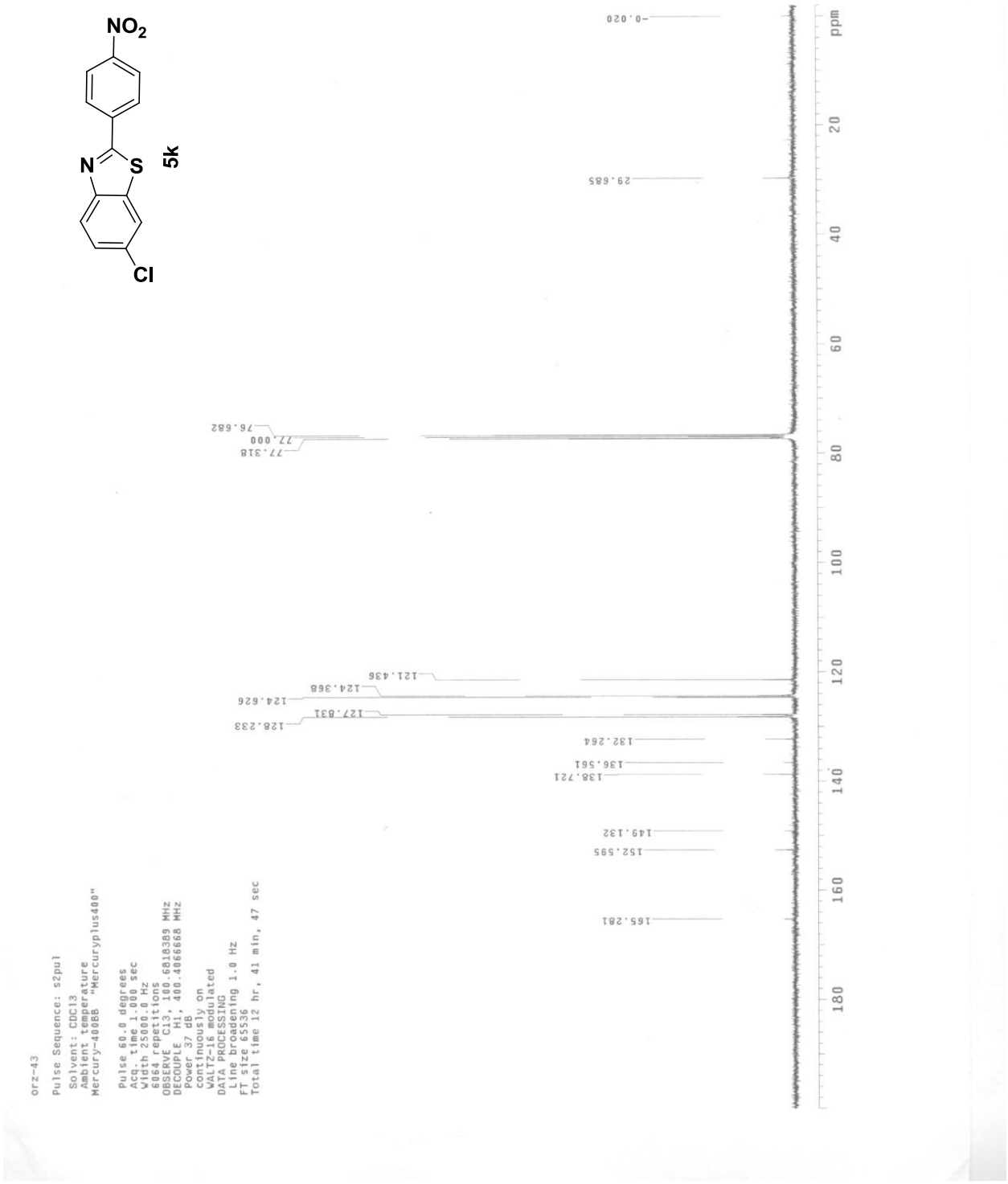


Figure S18. ^{13}C NMR spectrum of compound **5k**

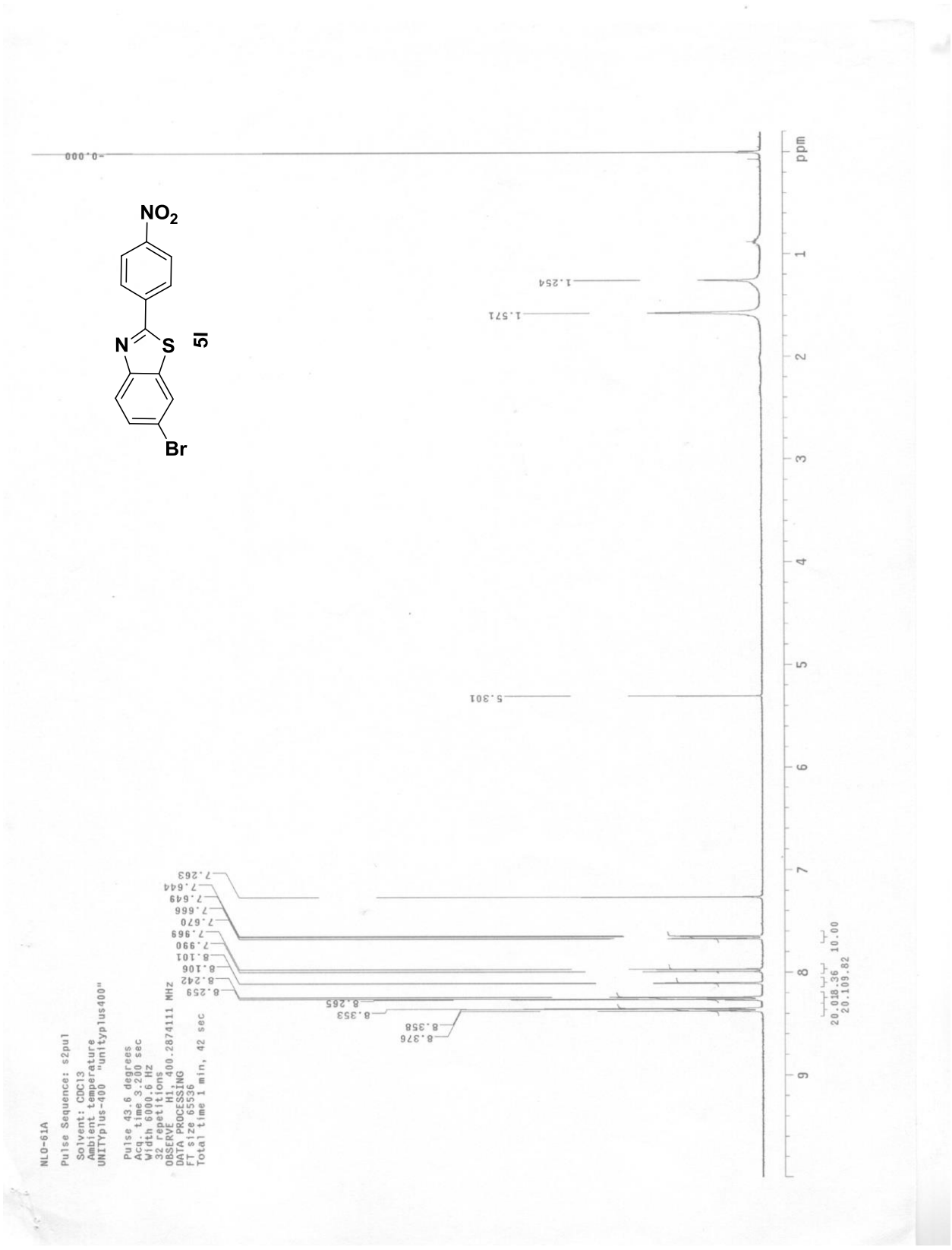


Figure S19. ^{1}H NMR spectrum of compound **5l**

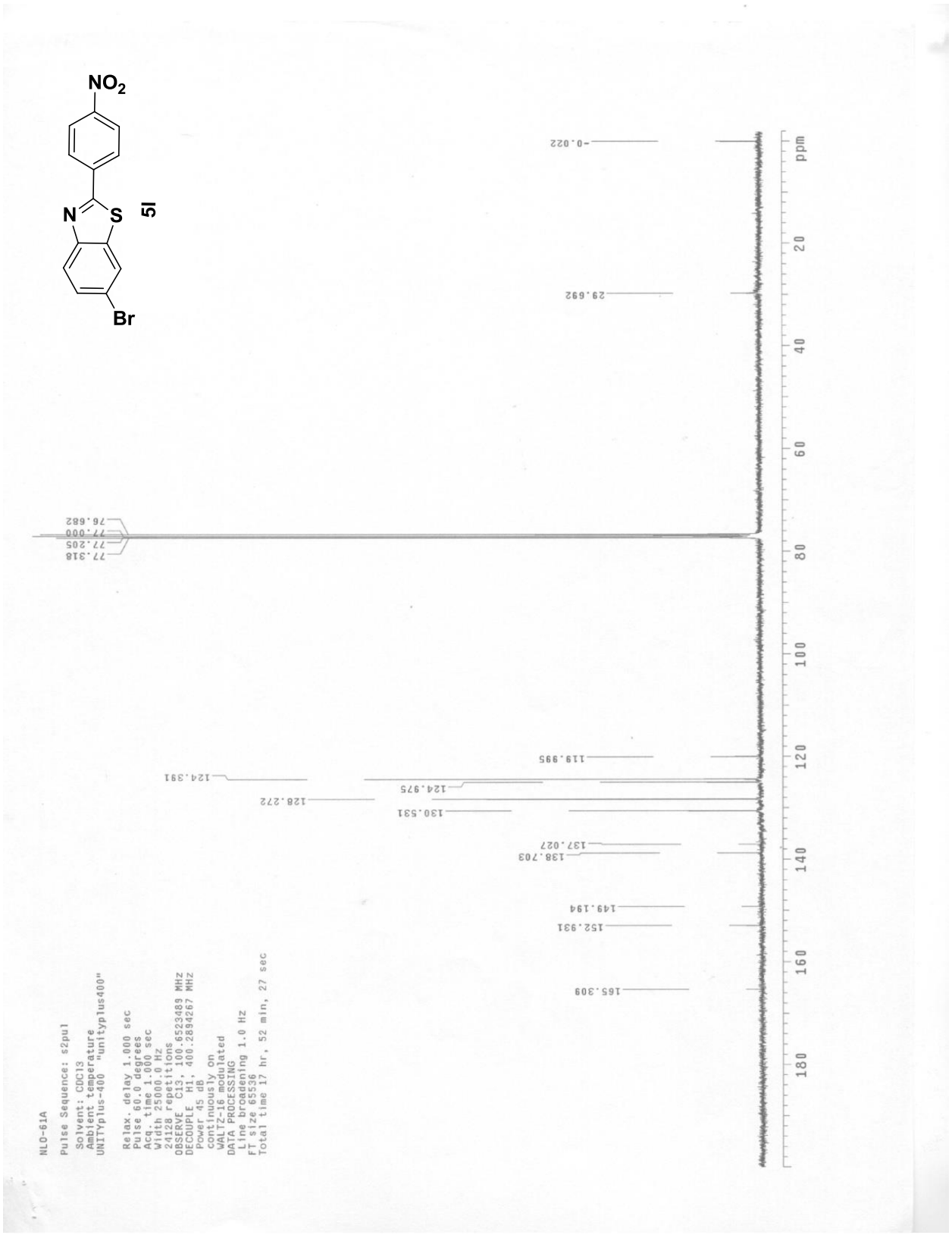


Figure S20. ^{13}C NMR spectrum of compound **5l**

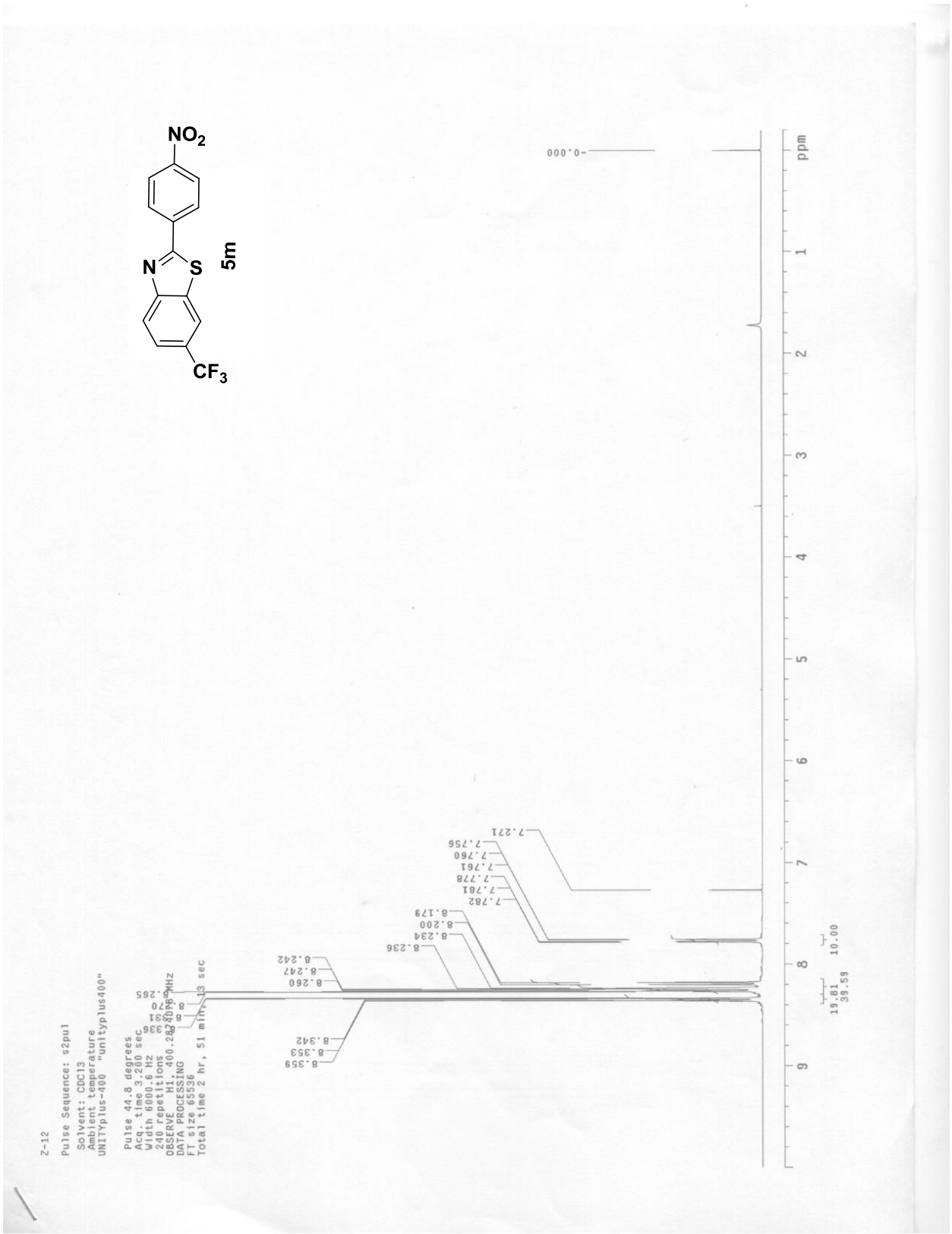


Figure S21. ^{1}H NMR spectrum of compound **5m**

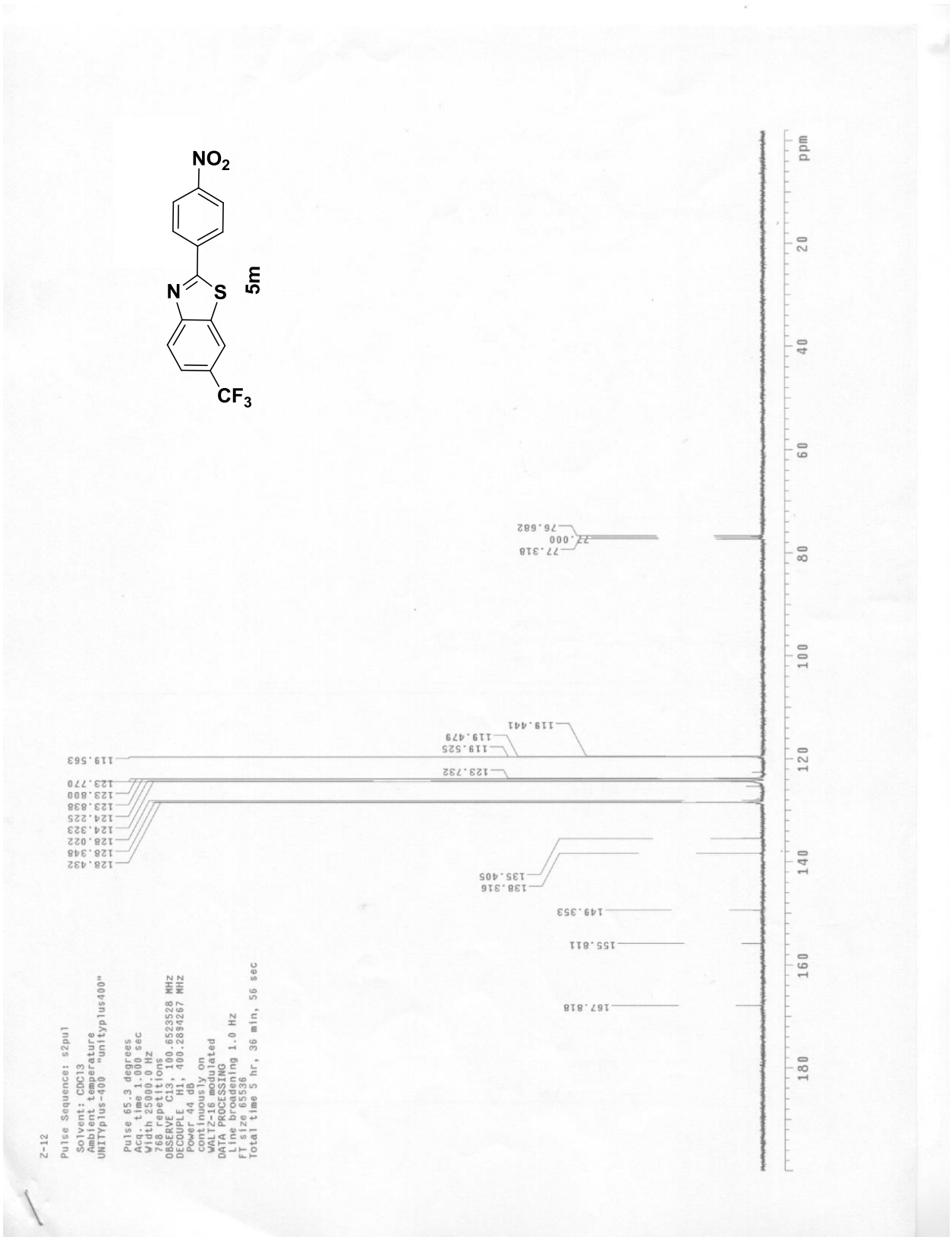


Figure S22. ^{13}C NMR spectrum of compound **5m**

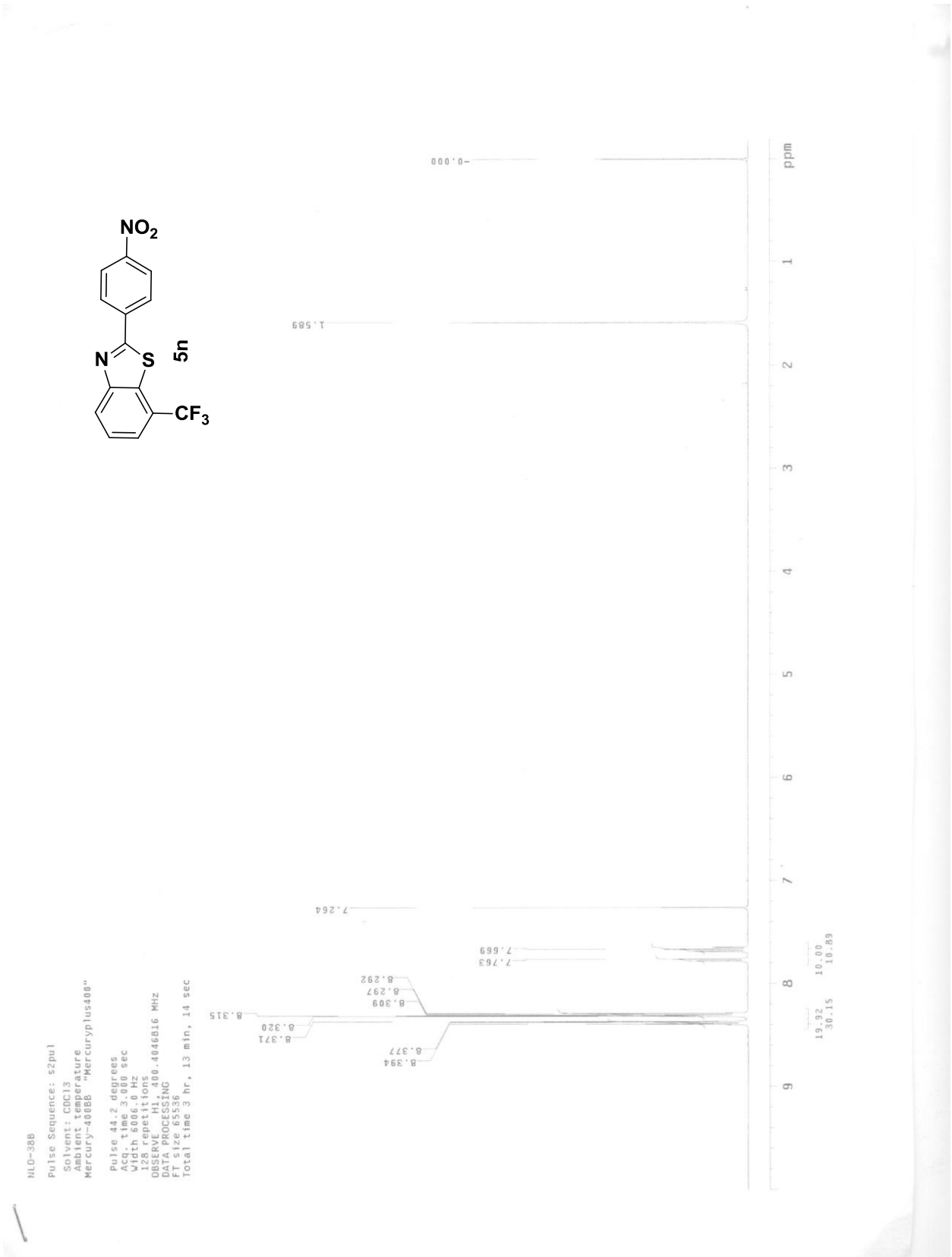


Figure S23. ^{1}H NMR spectrum of compound **5n**

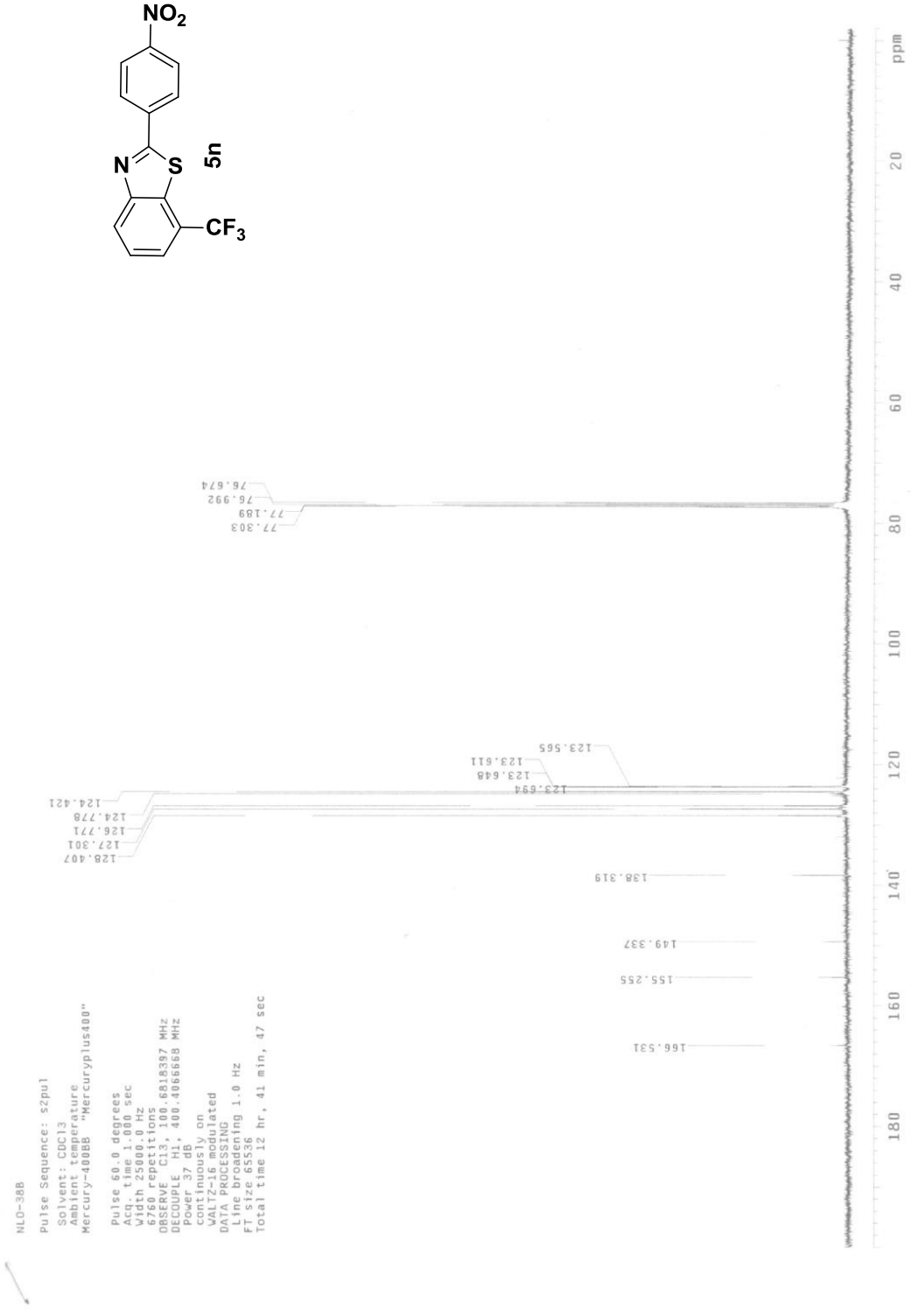


Figure S 24. ^{13}C NMR spectrum of compound **5n**

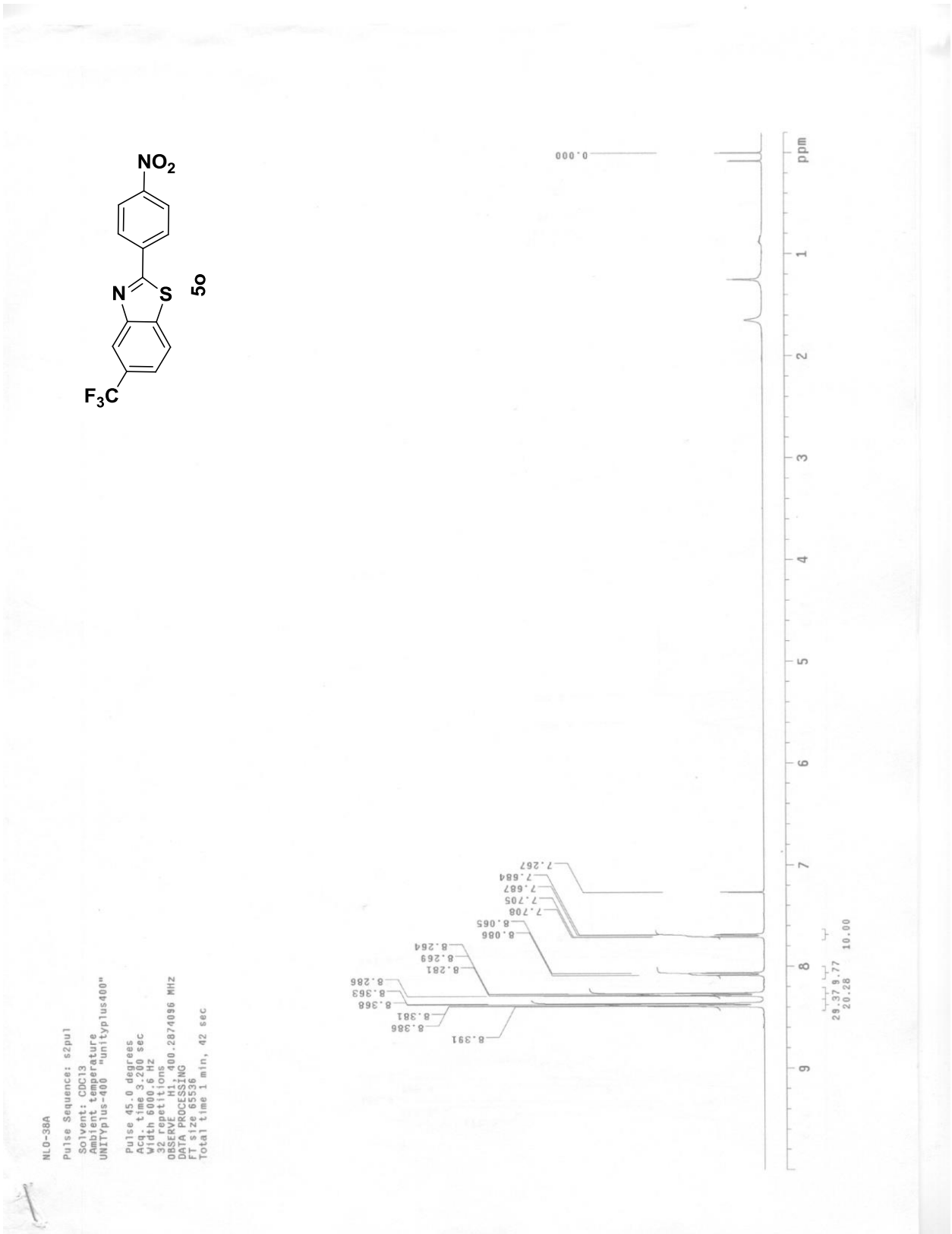


Figure S25. ^{1}H NMR spectrum of compound **5o**

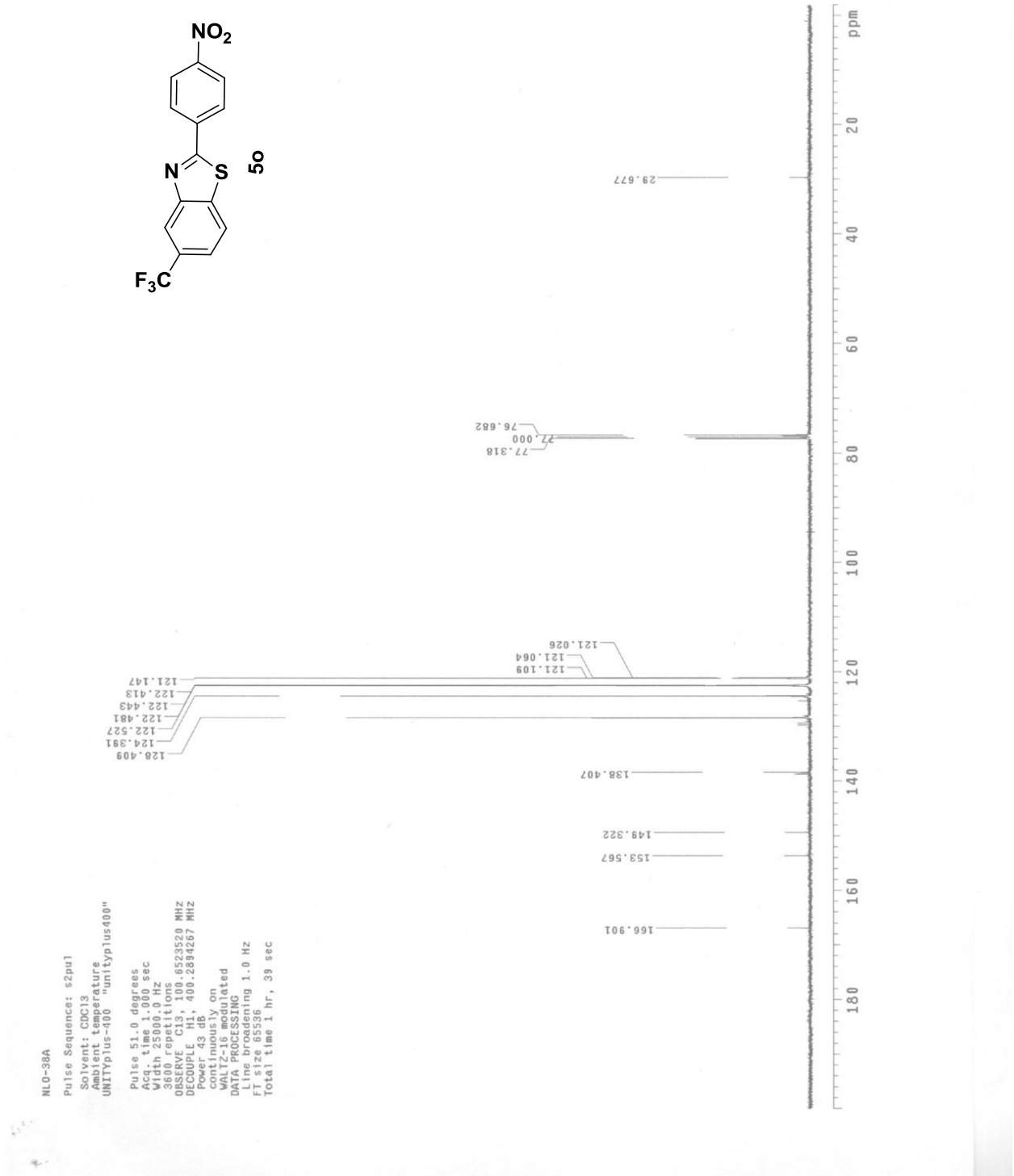


Figure S26. ^{13}C NMR spectrum of compound **5o**

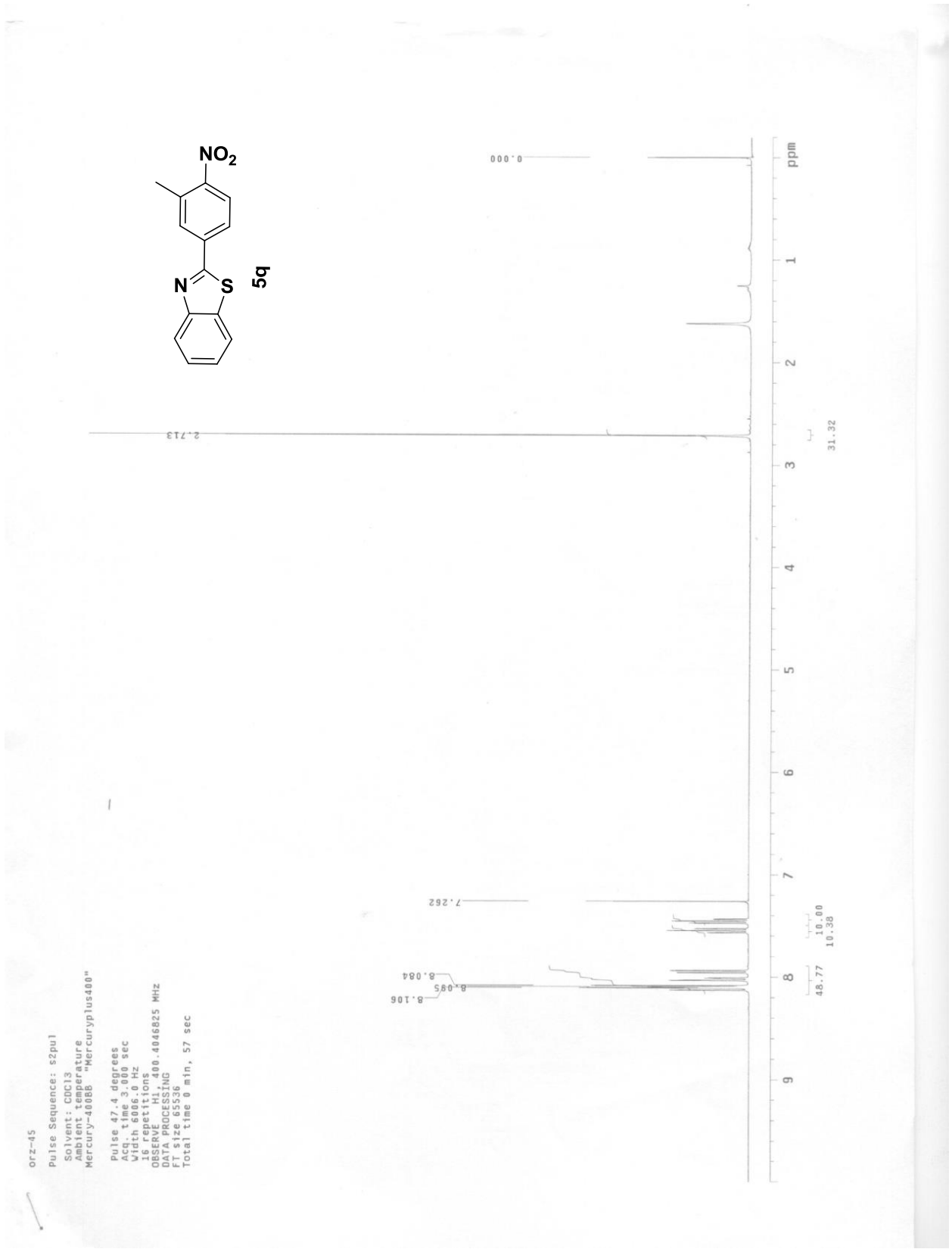


Figure S27. ^{1}H NMR spectrum of compound **5q**

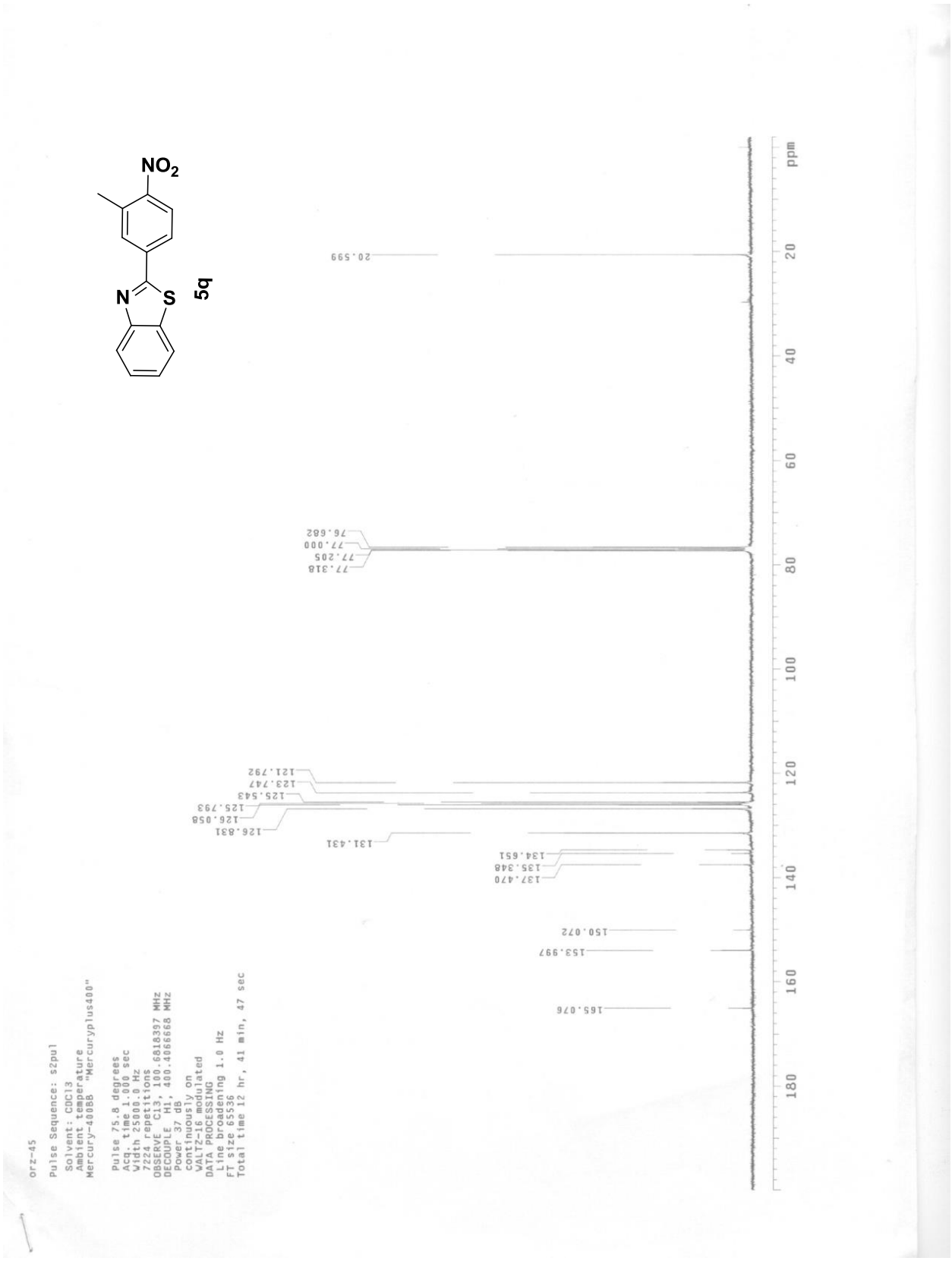


Figure S28. ^{13}C NMR of compound **5q**

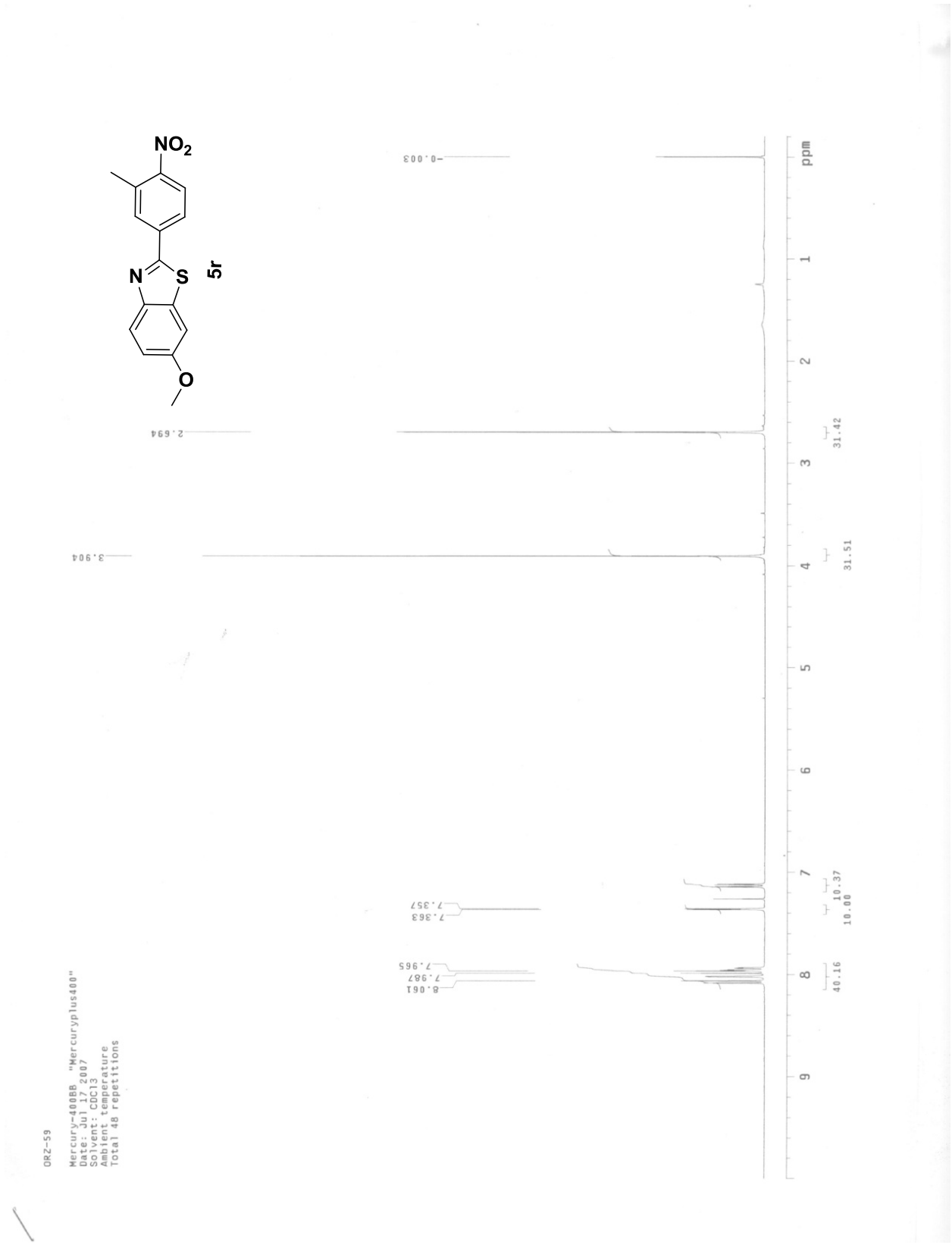


Figure S29. ^{1}H NMR spectrum of compound **5r**

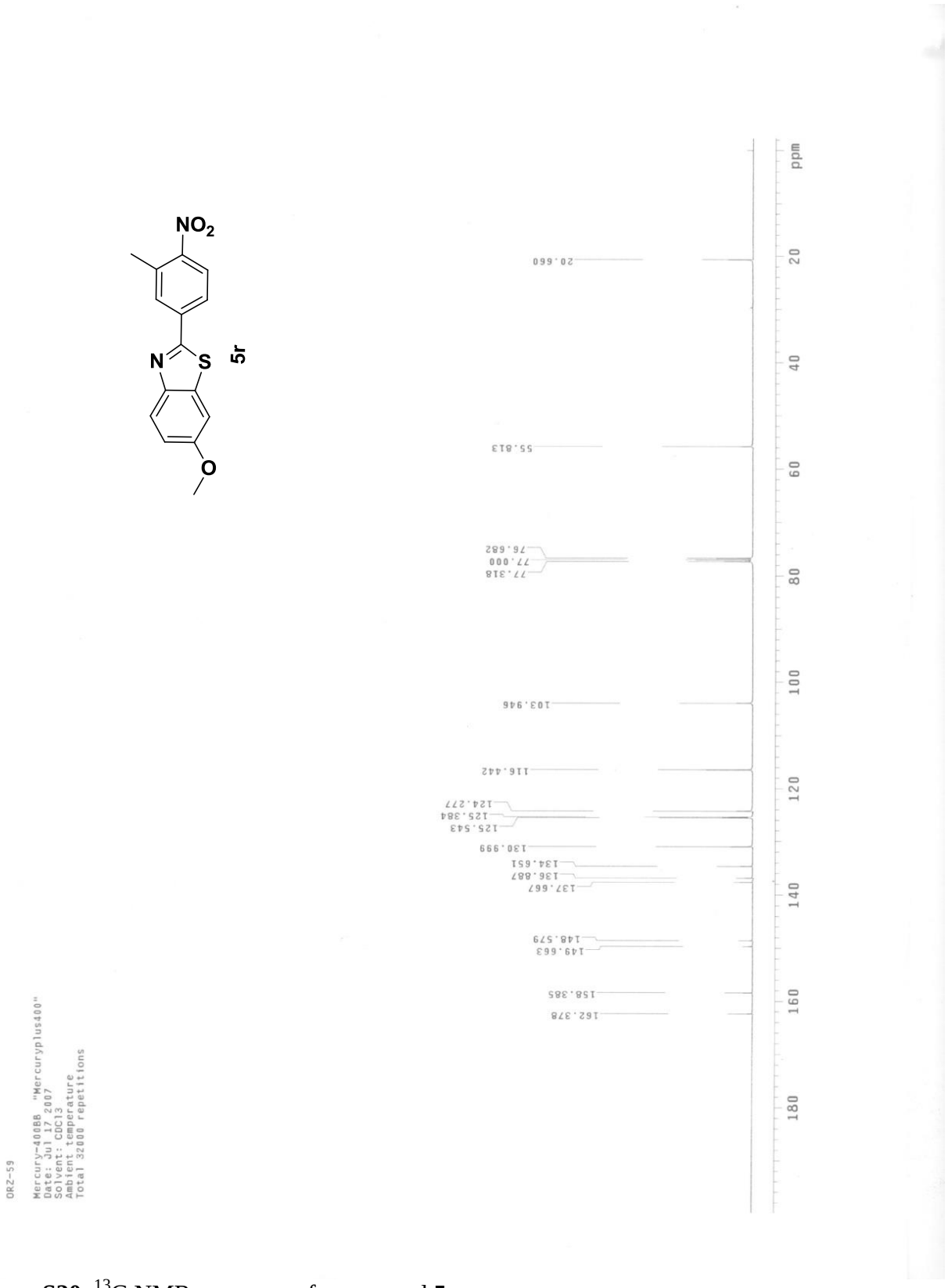


Figure S30. ^{13}C NMR spectrum of compound **5r**

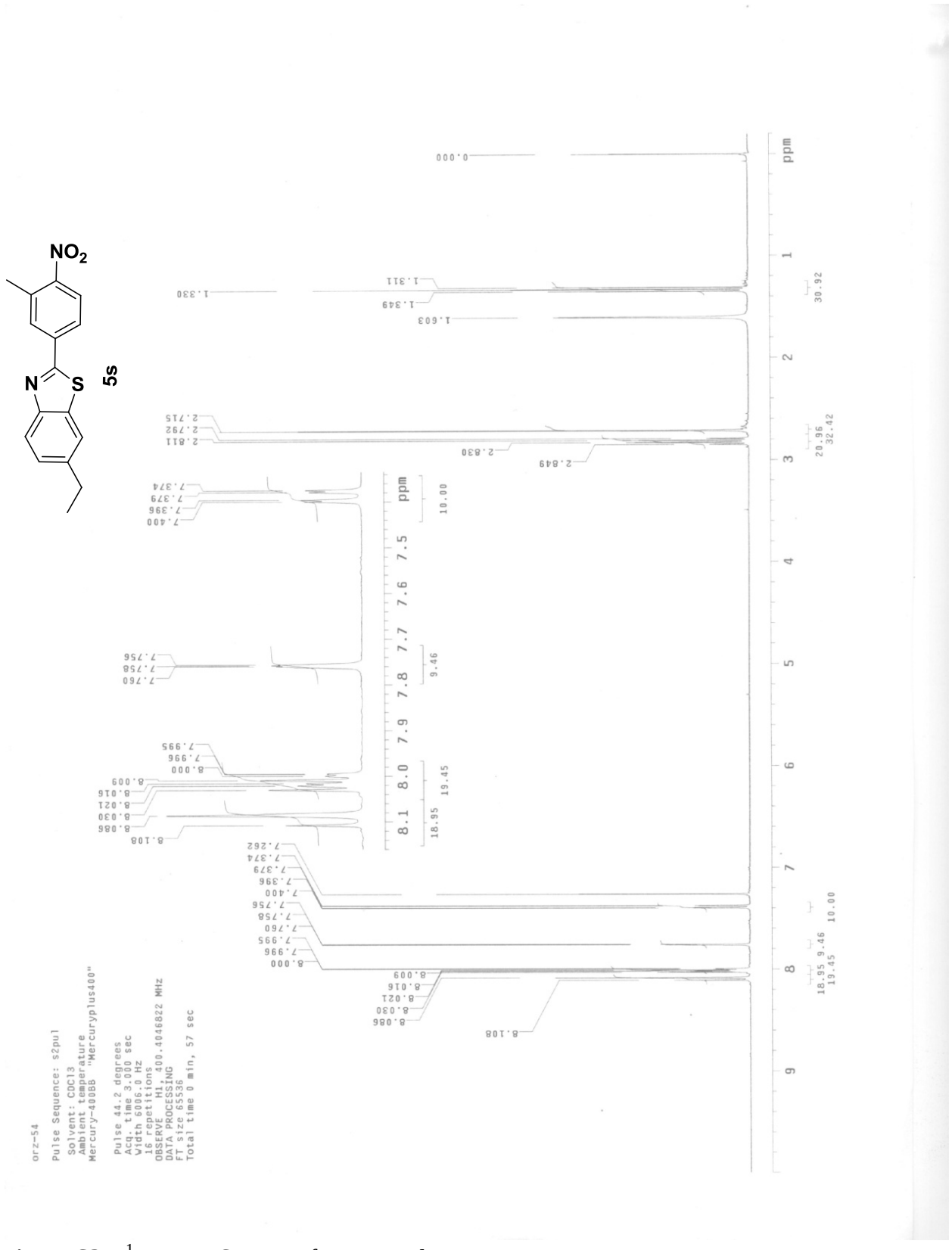


Figure S31. ^{1}H NMR Spectra of compound **5s**

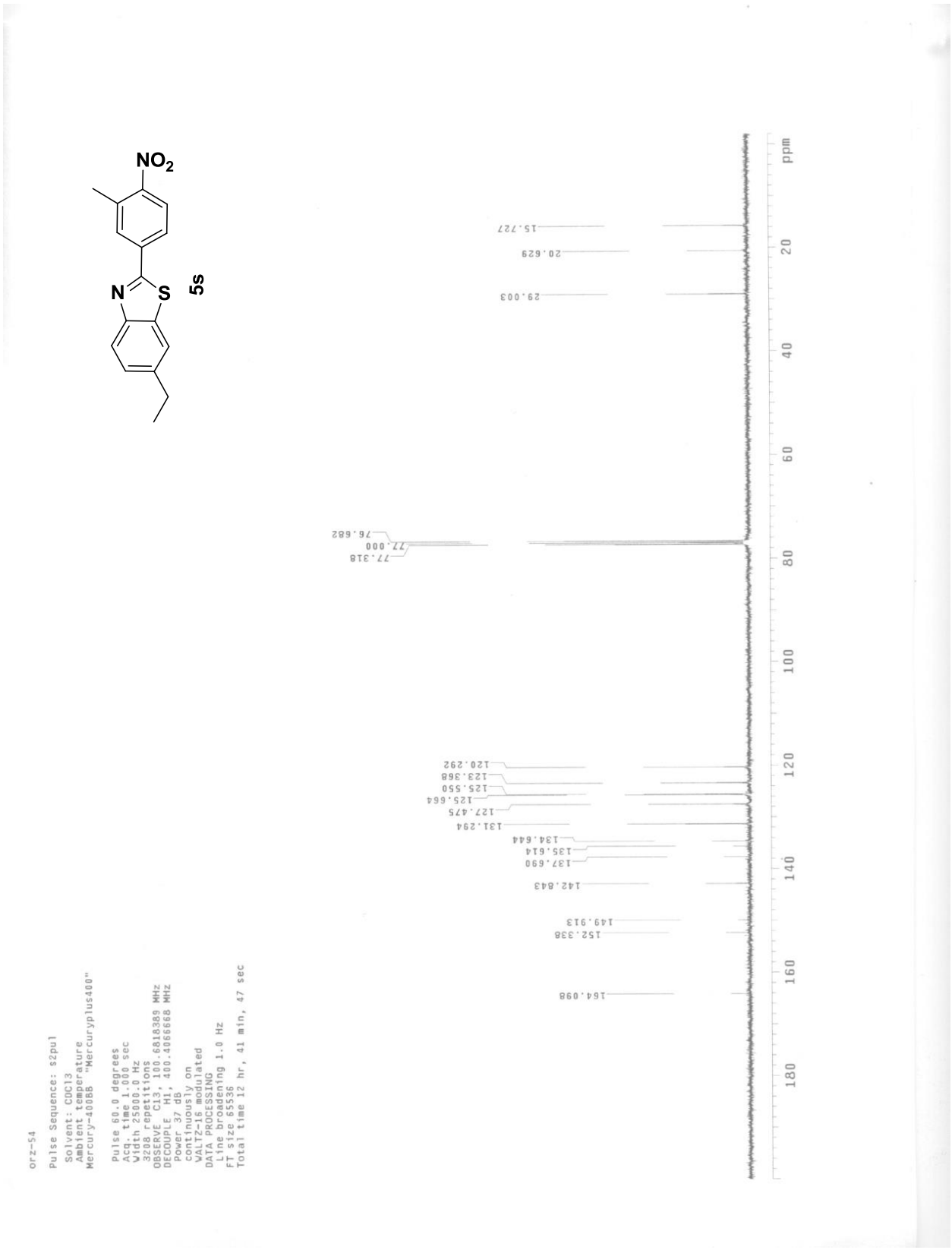


Figure S32. ^{13}C NMR spectrum of compound **5s**

Figure S33. ^{1}H NMR spectrum of compound **5t**

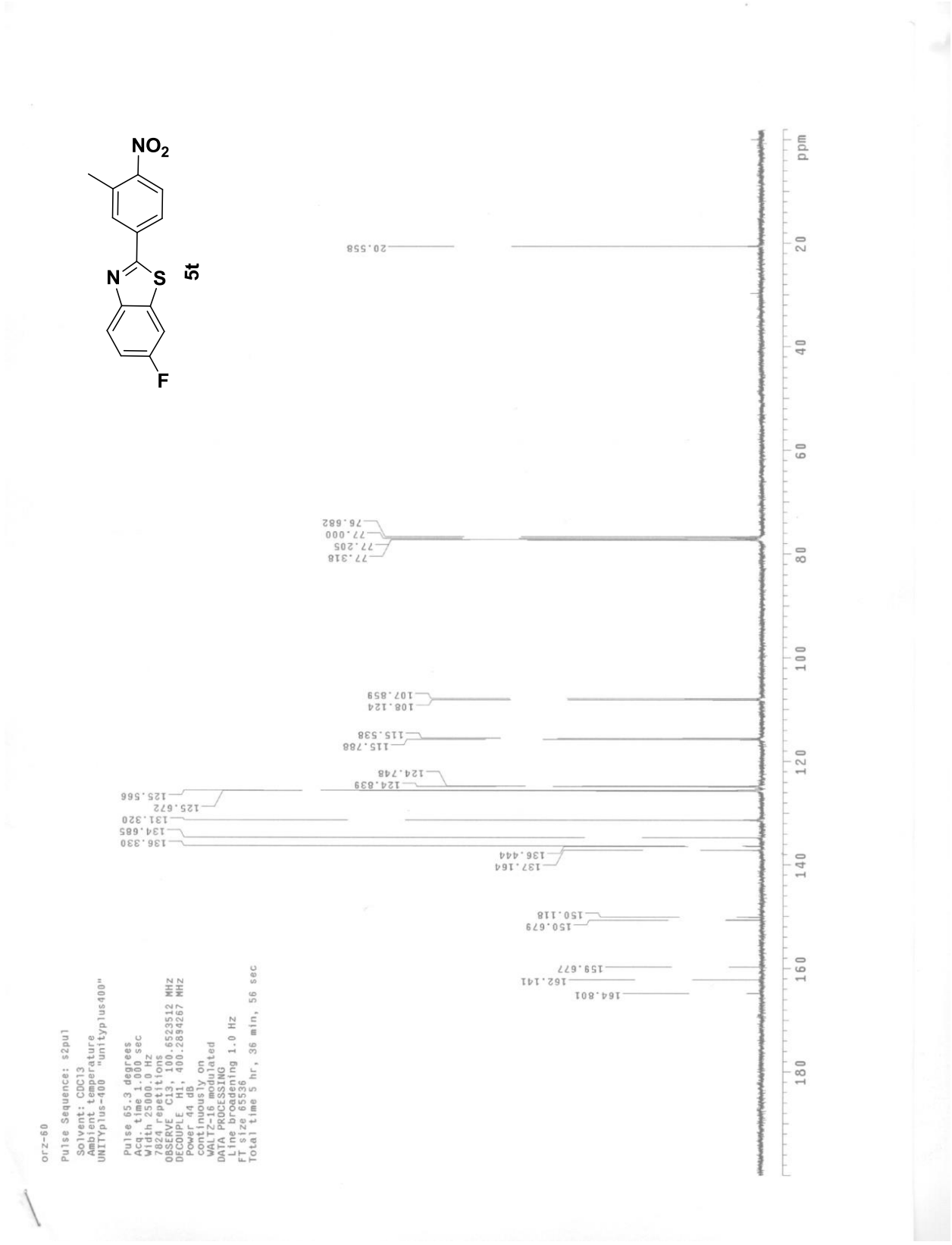


Figure S34. ^{13}C NMR spectrum of compound **5t**

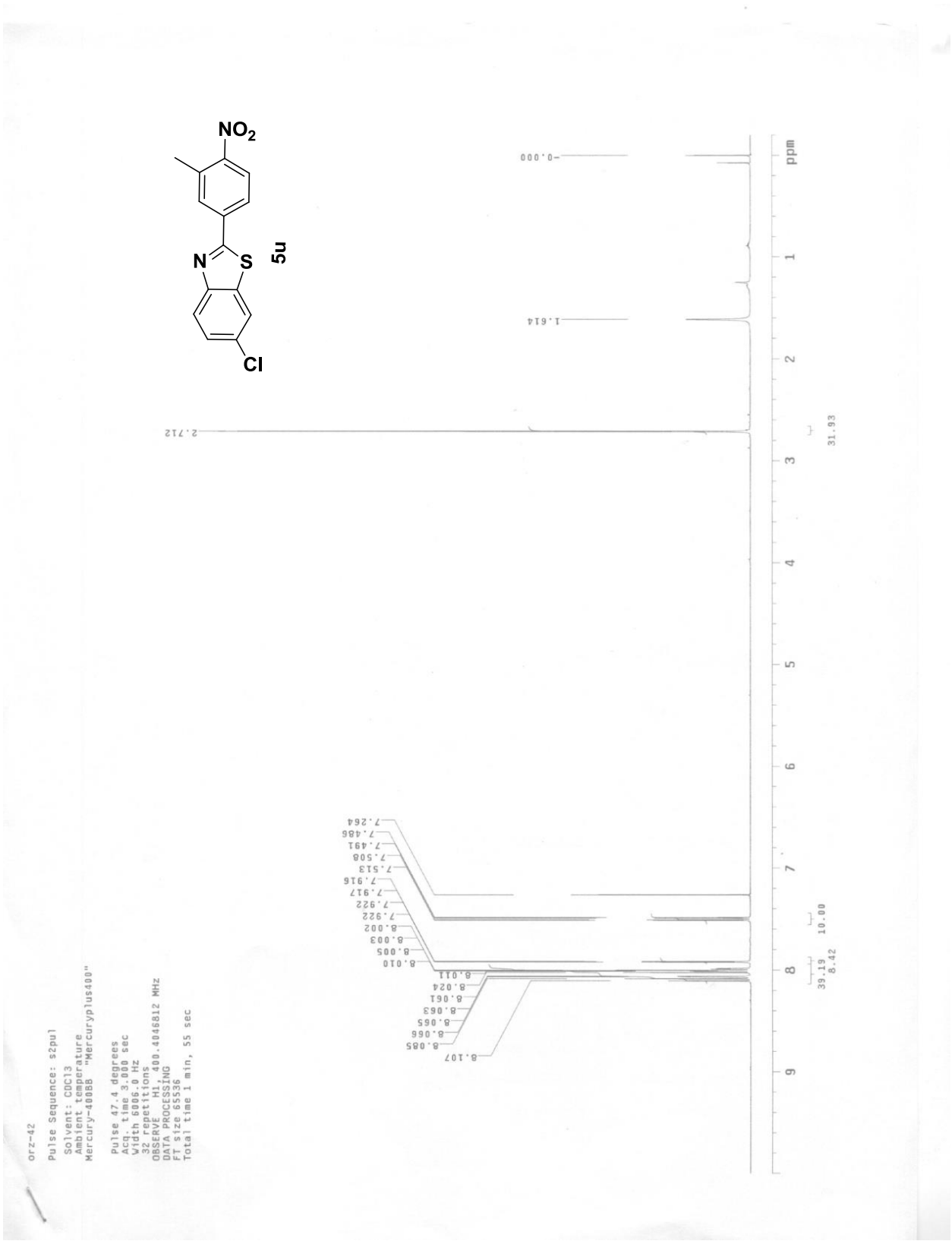


Figure S35. ^{1}H NMR spectrum of compound **5u**

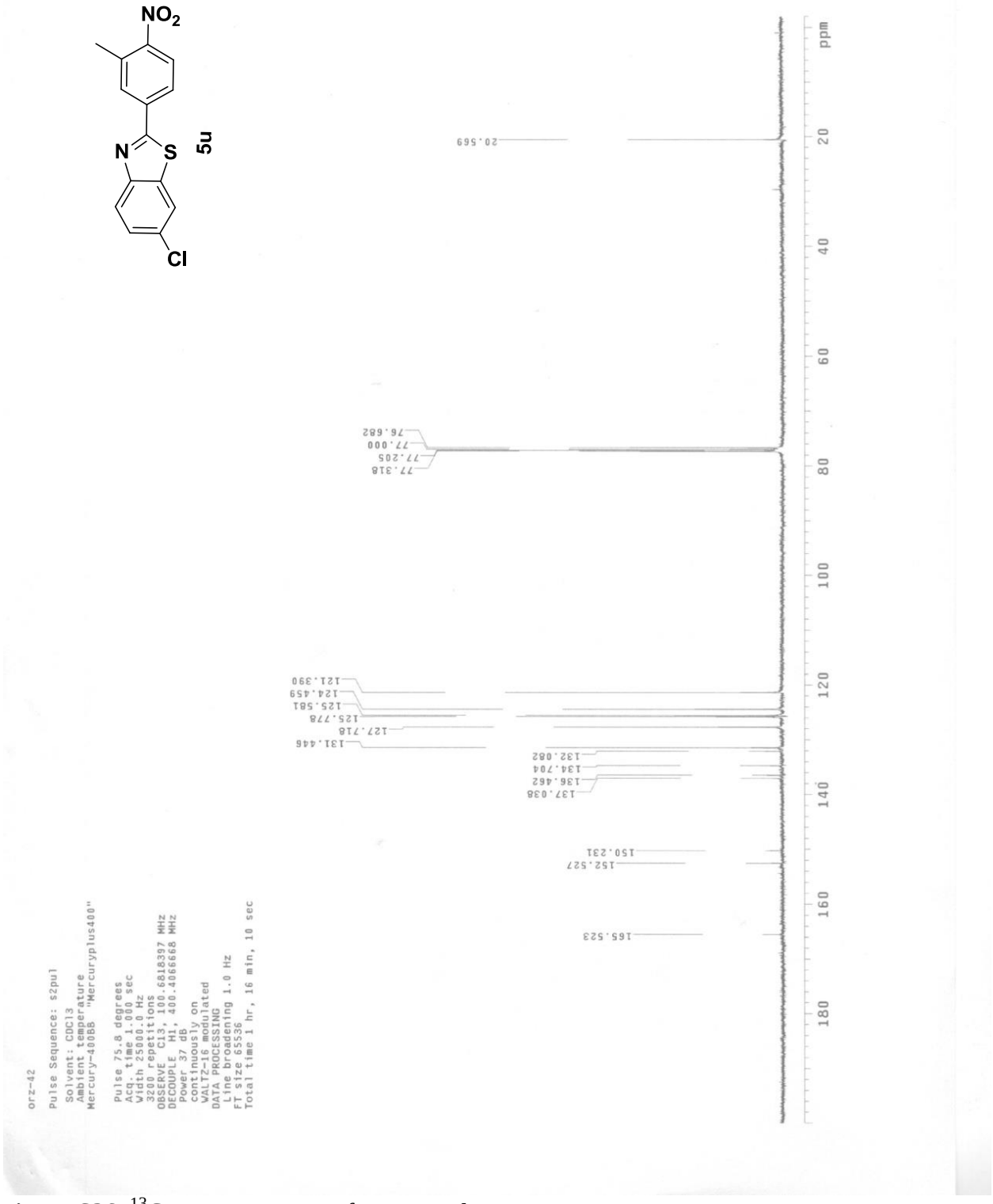


Figure S36. ^{13}C NMR spectrum of compound **5u**

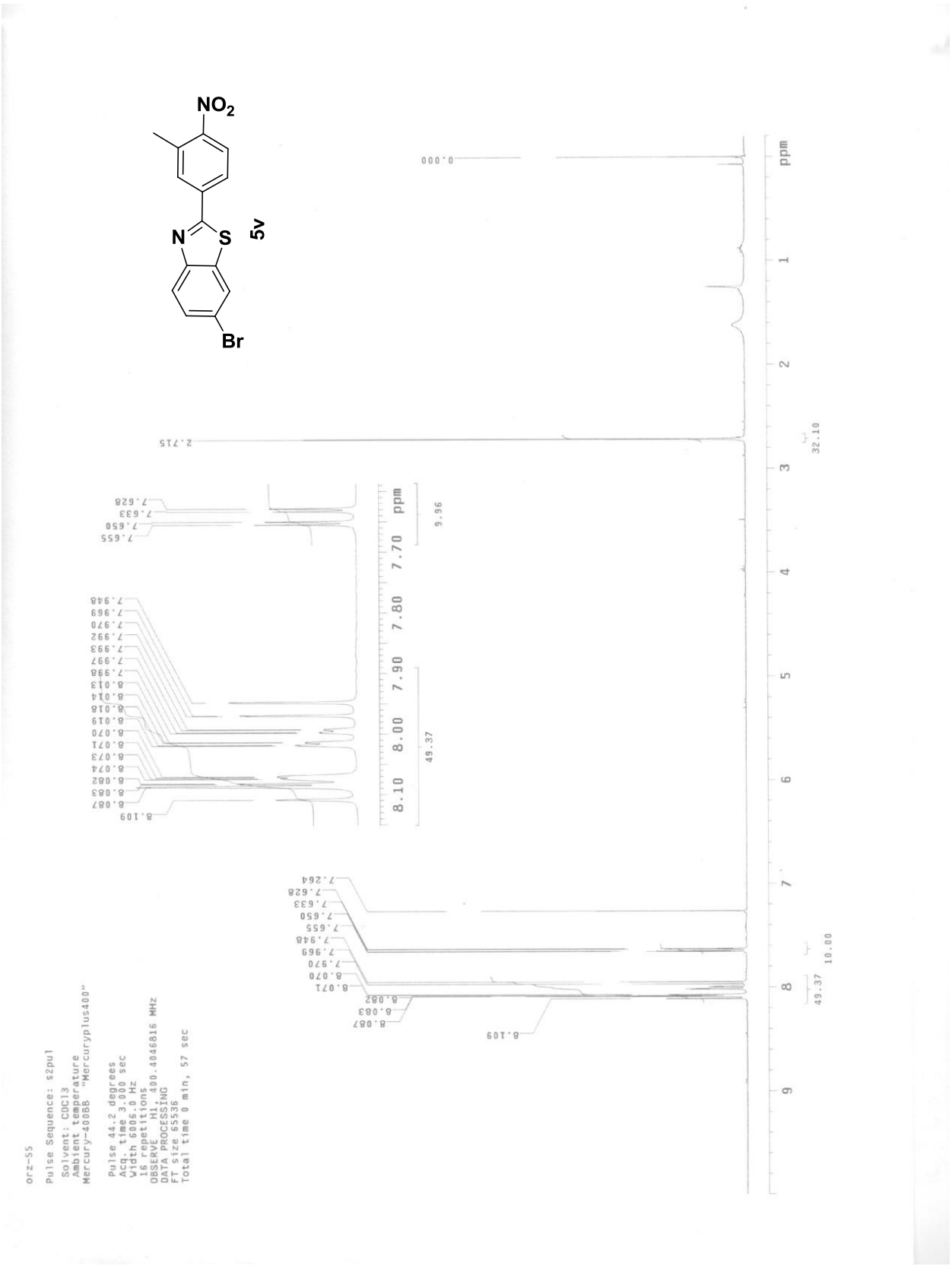


Figure S37. ^{1}H NMR spectrum of compound **5v**

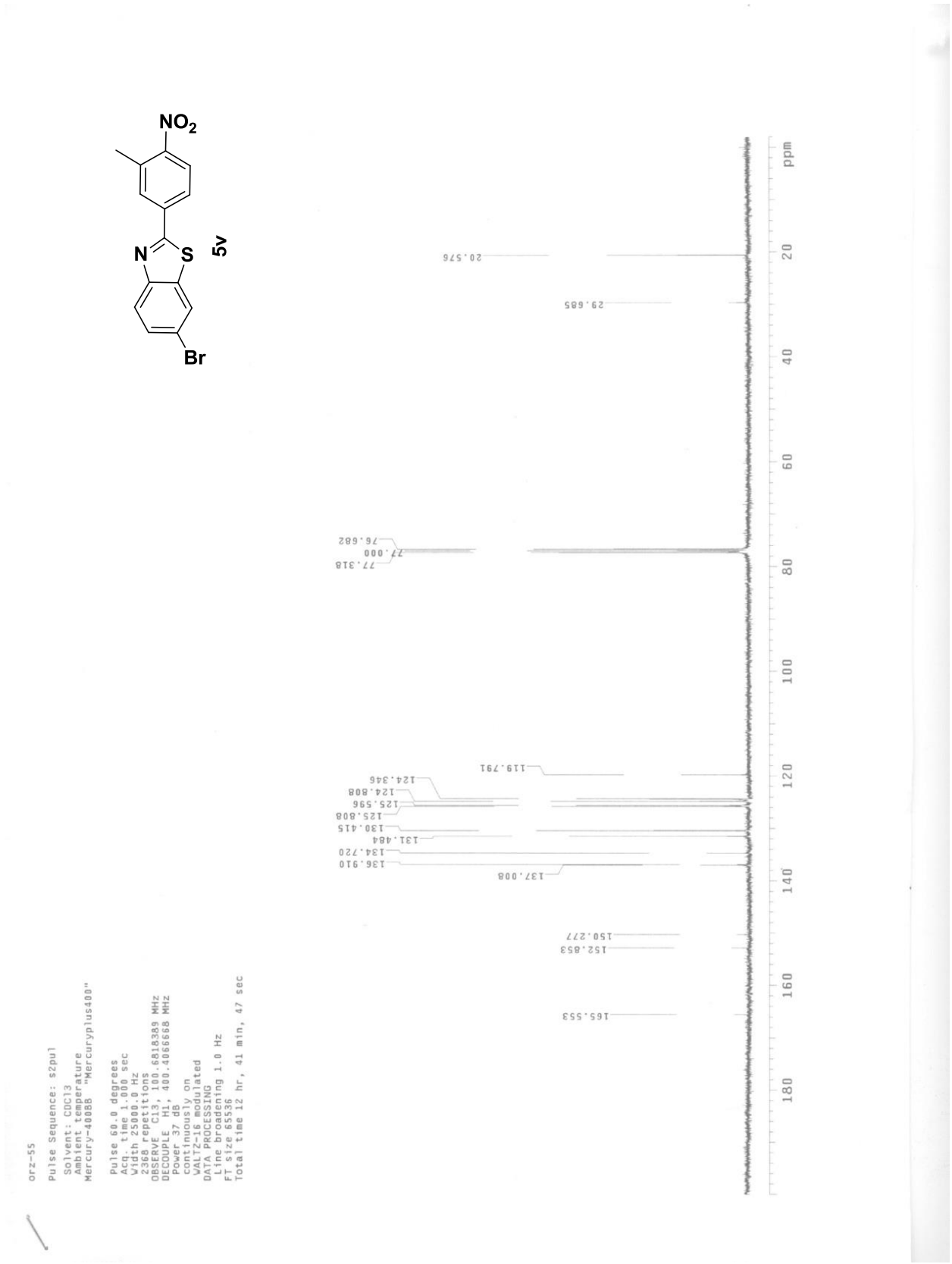


Figure S38. ^{13}C NMR spectrum of compound **5v**

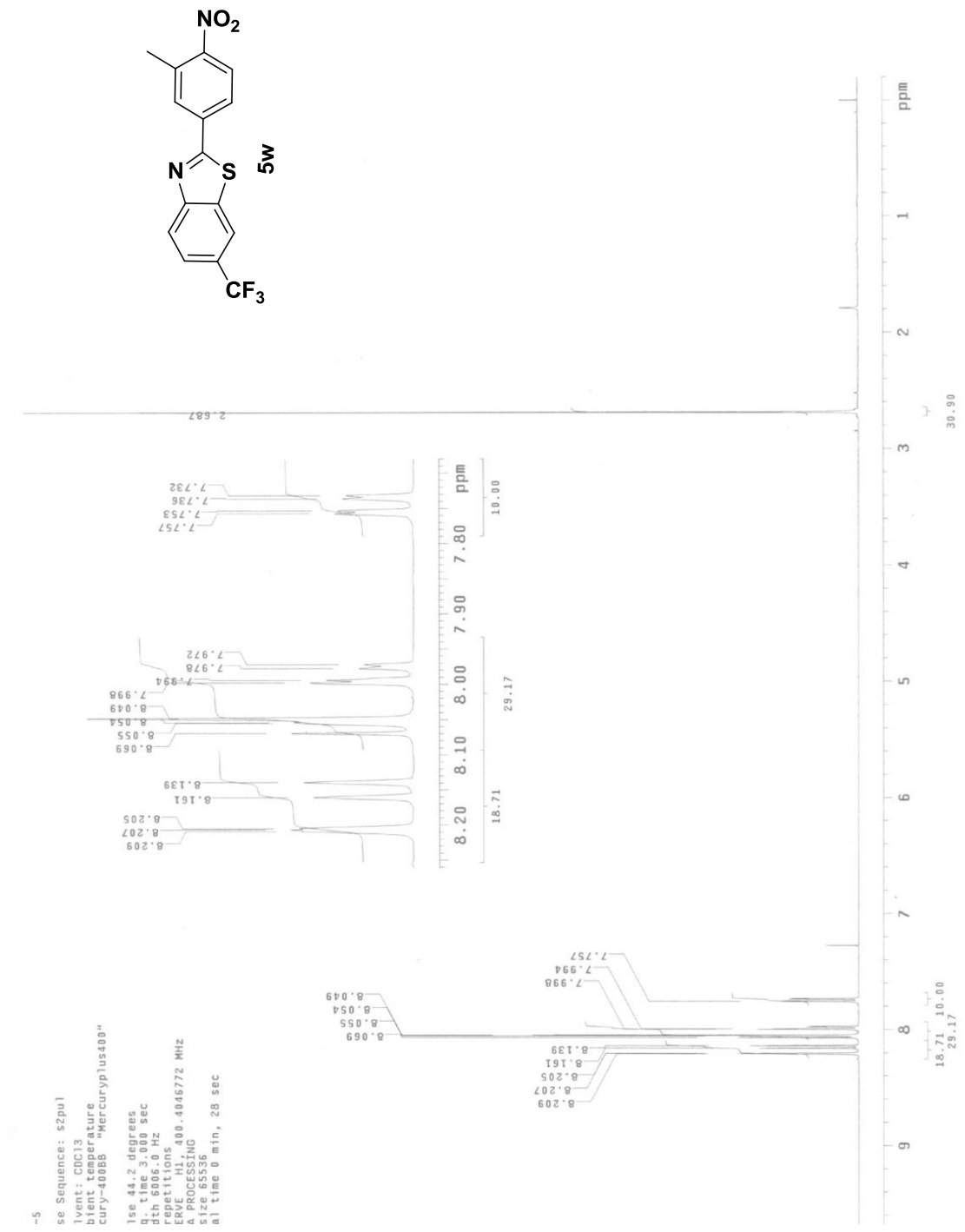


Figure S39. ^{1}H NMR spectrum of compound **5w**

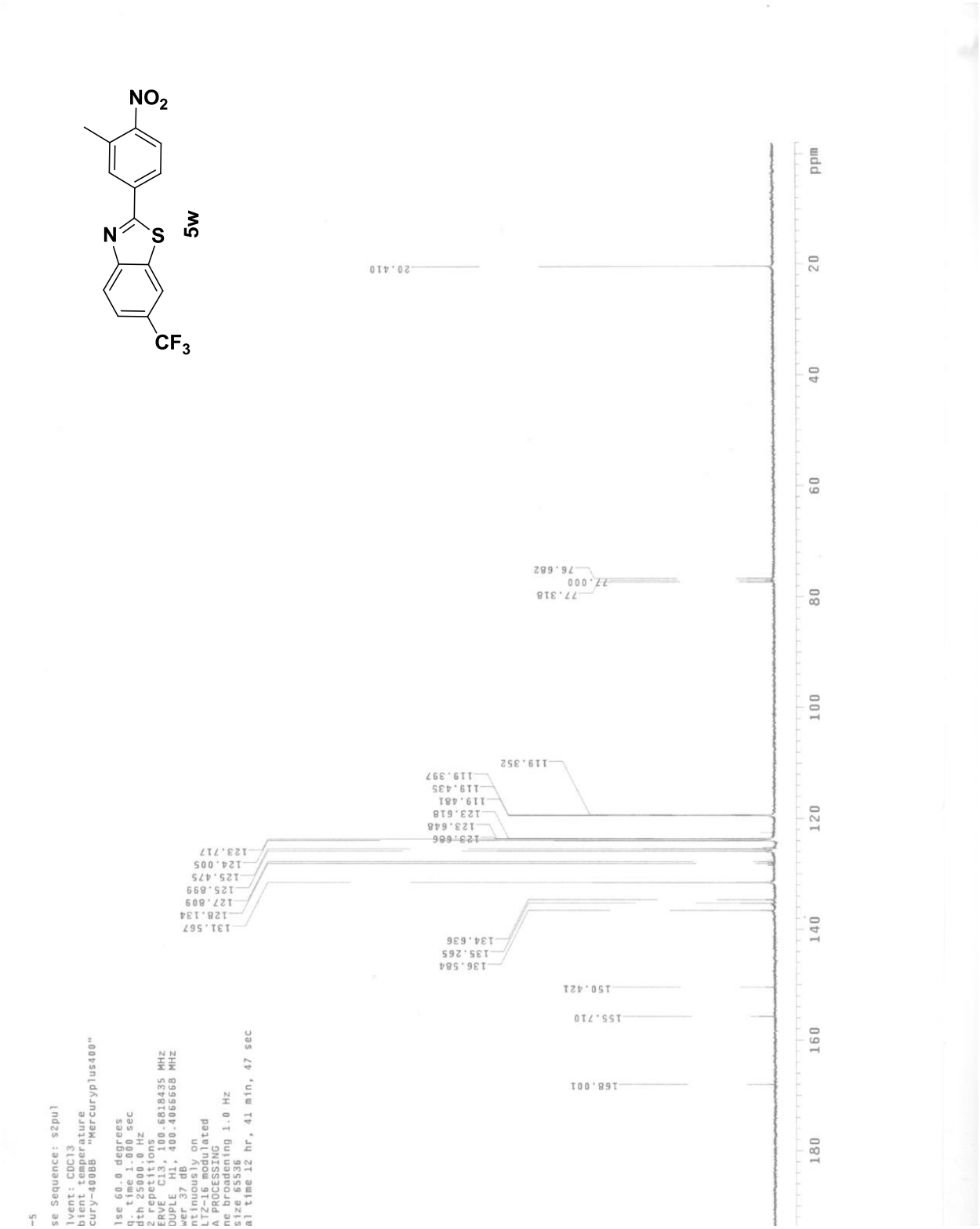


Figure S40. ^{13}C NMR spectrum of compound **5w**

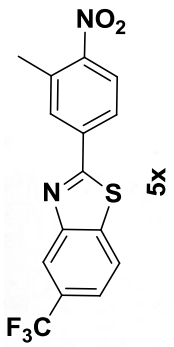


Figure S41. ^{1}H NMR spectrum of compound **5x**

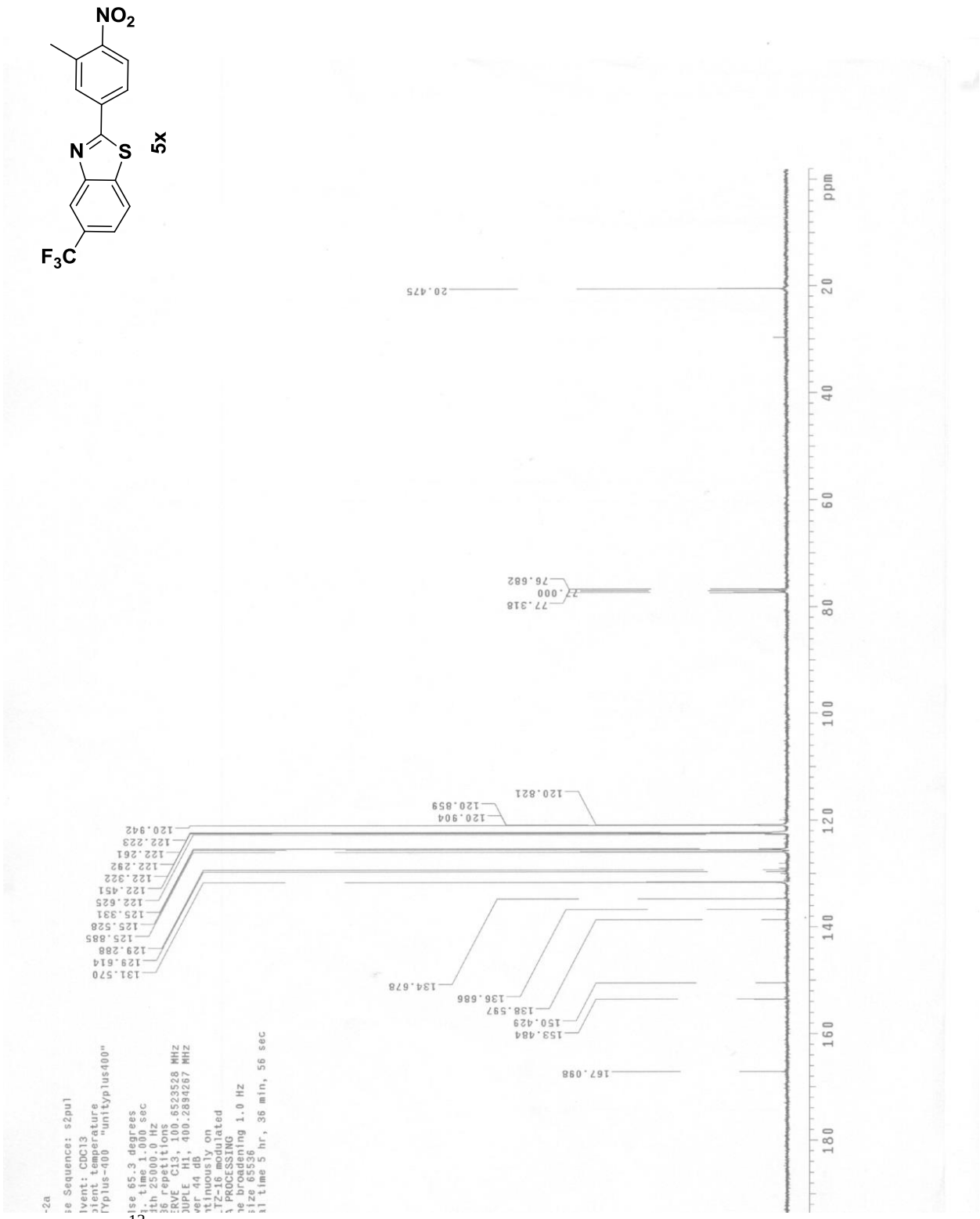


Figure S42. ^{13}C NMR spectrum of compound **5x**

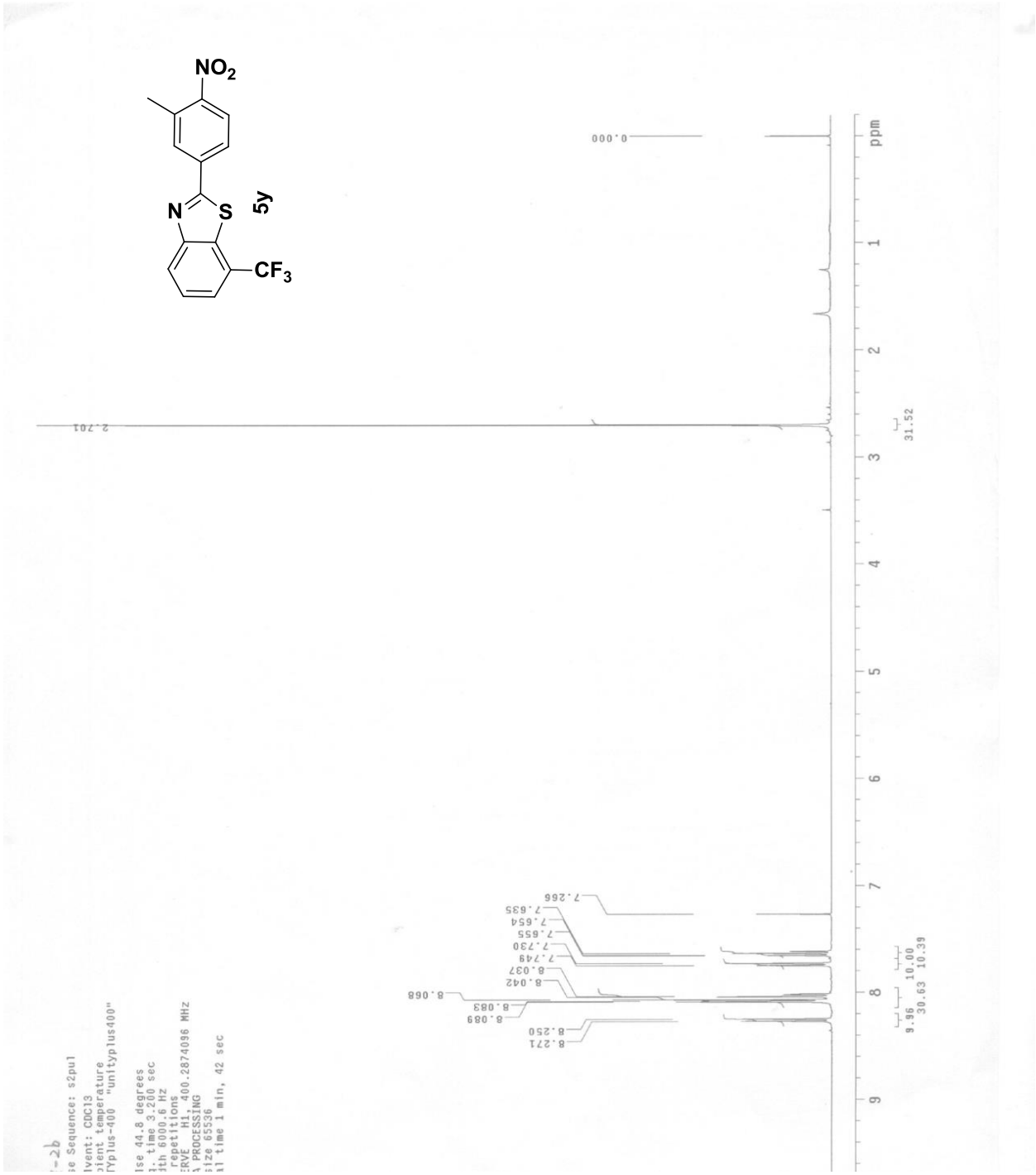


Figure S 43. ^{1}H NMR spectrum of compound **5y**

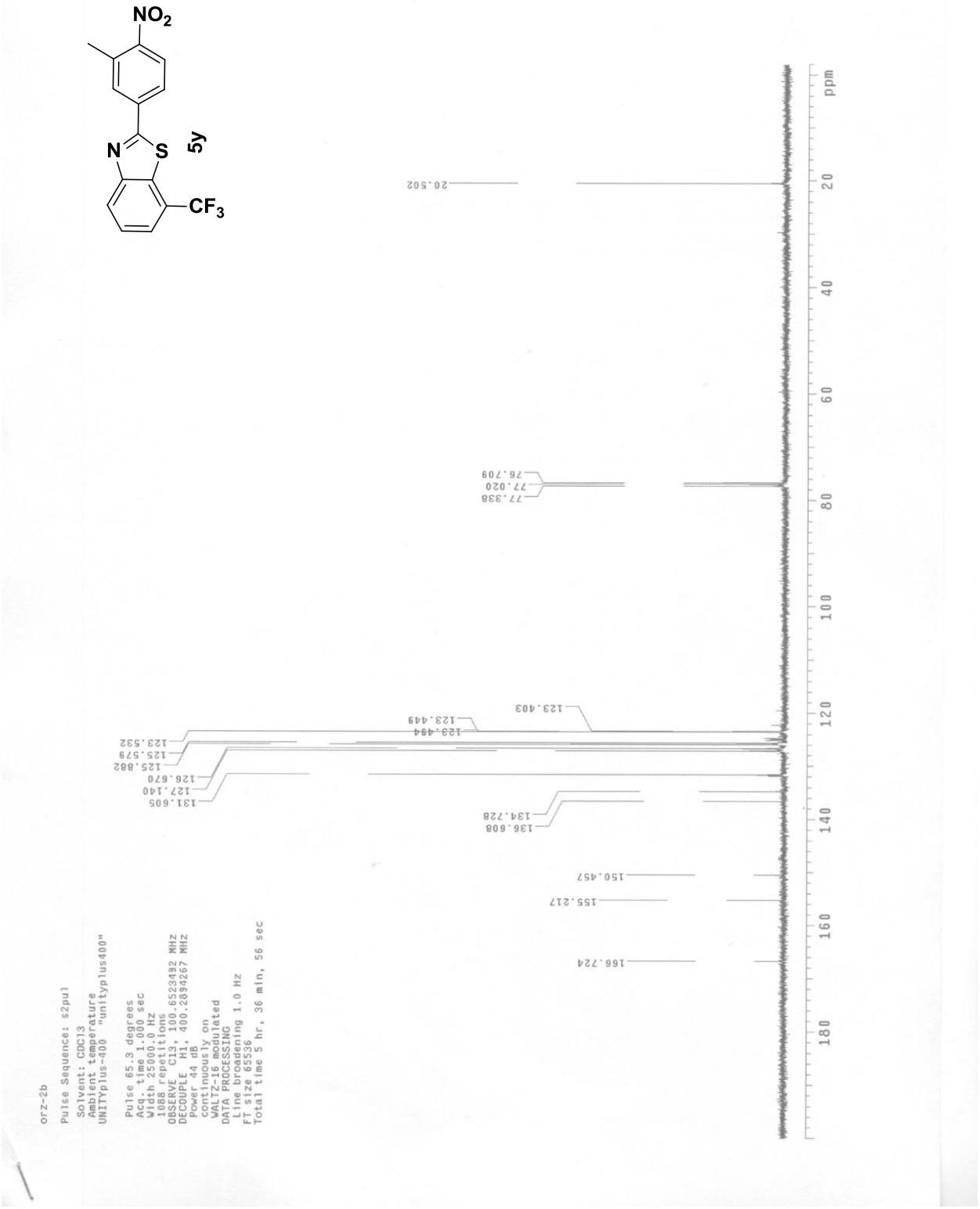


Figure S44. ^{13}C NMR spectrum of compound **5y**