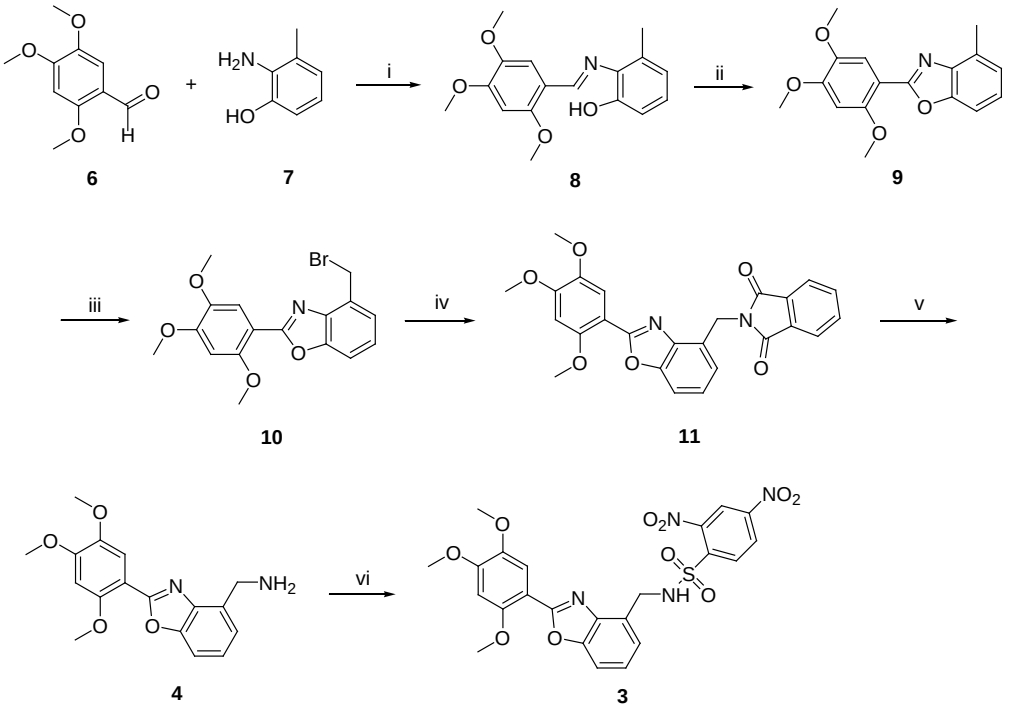
Supporting Information

Rational design of a highly selective and sensitive fluorescent PET probe for discrimination of thiophenols and aliphatic thiols

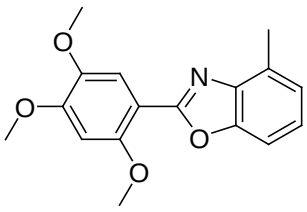
Wei Jiang, Yanting Cao, Yuan Liu, and Wei Wang[*]

Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM 87131-0001, USA

General Information: Commercial reagents were used as received, unless otherwise stated. Merck 60 silica gel was used for chromatography, and Whatman silica gel plates with fluorescence F_{254} were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 500, and tetramethylsilane (TMS) was used as a reference. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm. Mass Spectra were obtained from University of New Mexico mass spectral facility.



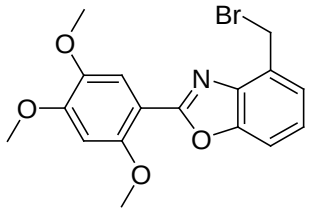
Scheme S1. Synthesis of probe **3**. (i) Benzene, reflux, 24 h; (ii) $BaMnO_4$, benzene, reflux, 8 h; (iii) NBS, AIBN, CCl_4, reflux, 6 h; (iv) Potasium Succinimide, DMF, 50 °C, 8 h; (v) NH_2NH_2, THF, reflux, 2 h; (vi) 2,4-Dinitrobenzensulfonyl chloride, pyridine, CH_2Cl_2, 0 °C to r.t



4-Methyl-2-(2,4,5-trimethoxyphenyl)benzo[d]oxazole (9).

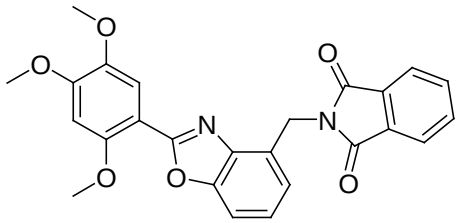
A mixture of 2,4,5-trimethoxybenzaldehyde (1.96 g, 10 mmol) and 2-amino-*m*-cresol (1.23 g, 10 mmol) in 100 mL of benzene was refluxed for 24 h under a nitrogen atmosphere with an Dean-stark apparatus to remove water. The reaction mixture was cooled down to room temperature and the solvent was removed in *vacuo* to afford the Schiff base compound **8** as a dark orange solid, which was used for the next reaction without further purification.

To a solution of unpurified **8** in 100 mL of dry benzene was added $BaMnO_4$ (10.3 g, 40 mmol) and the solution was refluxed for 6 h under a nitrogen atmosphere. After the reaction mixture was cooled down to room temperature, $BaMnO_4$ was removed through Celite and the filtrate was concentrated in *vacuo*. The black residue was purified by silica gel chromatography using ethyl acetate/hexane (1:2) as elute to afford a pale yellow solid in 66 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (s, 1H), 7.39 (d, 1H, *J* = 8.0 Hz), 7.20 (t, 1H, *J* = 8.0 Hz), 7.11 (d, 1H, *J* = 7.0 Hz), 6.63 (s, 1H), 3.98 (s, 3H), 3.96 (s, 6H), 2.69 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ161.3, 154.0, 152.6, 150.3, 143.4, 141.4, 130.2, 124.9, 124.3, 113.5, 108.1, 107.8, 98.1, 57.3, 56.6, 56.2, 16.8.



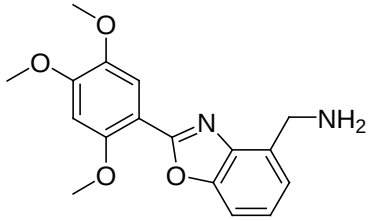
4-(Bromomethyl)-2-(2,4,5-trimethoxyphenyl)benzo[d]oxazole (10).

A mixture of **9 (**345 mg, 1.15 mmol), *N*-bromosuccinimide (218 mg, 1.21 mmol), and AIBN (20 mg, 0.12 mmol) in 30 mL of anhydrous CCl_4 was refluxed for 10 h under a nitrogen atmosphere. The reaction mixture was cooled to 0 °C and the precipitate was removed by filtration while maintaining the temperature at 0 °C. After the solvent was evaporated, the residue was directly purified by silica gel chromatography using ethyl acetate/hexane (1:2) as elute to afford **10** in 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.51 (d, 1H, *J* = 7.5 Hz), 7.39 (d, 1H, *J* = 7.5 Hz), 7.28 (q, 1H, *J* = 8.0 Hz), 6.63 (s, 1H), 4.97 (s, 2H), 4.00 (s, 3H), 3.99 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.5, 154.3, 153.1, 150.7, 143.6, 140.9, 129.3, 125.4, 124.7, 113.6, 110.6, 107.8, 98.3, 57.5, 56.7, 56.3, 28.3.



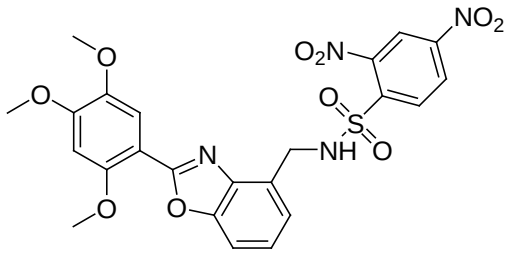
2-((2-(2,4,5-Trimethoxyphenyl)benzo[d]oxazol-4-yl)methyl)isoindoline-1,3-dione (11).

A mixture of **11** (320 mg, 0.85 mmol) and potassium phthalimide (176 mg, 0.93 mmol) in 30 mL of DMF was heated at 50 °C for 8 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and the solvent was removed in *vacuo*. The residue was dissolved in water and extracted with dichloromethane (3 × 20 mL). The combined organic layer was washed by brine, dried with magnesium sulfate, filtered and evaporated in *vacuo*. The residue was purified by silica gel chromatography using ethyl acetate/hexane (2:3) as elute to afford **4** in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (s, 2H), 7.71(s, 2H), 7.58 (s, 1H), 7.47 (s, 1H), 7.27-7.19 (m, 2H), 6.61 (s, 1H), 3.96 (s, 6H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.3, 162.1, 154.3, 152.9, 150.8, 143.5, 140.4, 134.1, 132.6, 127.7, 124.5, 123.5, 122.5, 113.7, 109.8, 108.1, 98.4, 57.4, 56.7, 56.3, 37.9.



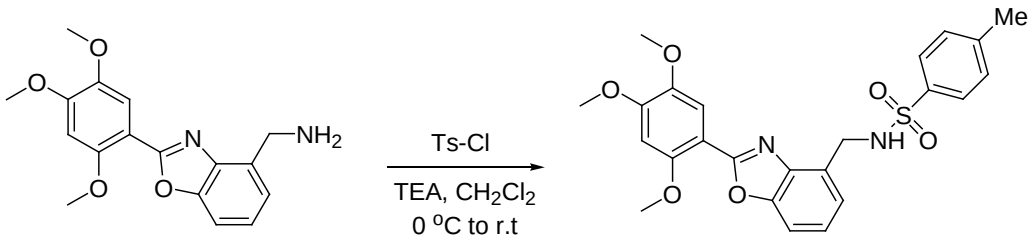
(2-(2,4,5-Trimethoxyphenyl)benzo[d]oxazol-4-yl)methanamine (4).

A mixture of **11 (**280 mg, 0.63 mmol) and hydrazine (0.8 mL, 25 mmol) in 20 mL THF was refluxed for 2 h under a nitrogen atmosphere, during which a large amount of white solid appeared. The reaction mixture was cooled to room temperature and the solvent was removed in *vacuo*. The residue was dissolved in 10 mL of ethyl acetate, 10 mL HCl (1N) was added and the mixture solution for 30 minutes. Aqueous layer was separated and the organic layer was exacted with 1N HCl (2×10 mL). Combined the aqueous layers and concentrated to 10 mL. Sodium hydroxide was added to just the pH of solution to 13, which was then extracted with dichloromethane (3×20 mL). The combined organic layer was washed by brine, dried with magnesium sulfate, filtered and evaporated in *vacuo* to afford **4** in 75% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.454 (d, 2H, *J* = 7.5 Hz), 7.25-7.21 (m, 2H), 6.62 (s, 1H), 3.98 (s, 3H), 3.95 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 154.0, 152.7, 150.5, 143.3, 140.3, 135.1, 124.4, 122.4, 113.4, 108.9, 107.8, 98.1, 57.2, 56.5, 56.1, 42.9.



2,4-Dinitro-N-((2-(2,4,5-trimethoxyphenyl)benzo[d]oxazol-4-yl)methyl)benzenesulfonamide (3).

To a solution of **4** (150 mg, 0.478 mmol) and pyridine (116 μL, 1.433 mmol) in 30 mL of dichloromethane was added 2.4-dinitrobenzenesulfonyl chloride (158 mg, 0.573 mmol) in 2 mL of dichloromethane dropwise at 0 °C. The reaction solution was stirred overnight under a nitrogen atmosphere and let it warm up to room temperature gradually, during which yellow solid appeared. Evaporated the solvents in *vacuo*. The residue was dissolved in 50 mL of chloroform and washed with 1N HCl and brine, dried with magnesium sulfate, filtered and evaporated in *vacuo.* The residue was recrystallized in dichloromethane to give **3** as bright yellow solid in 50% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.72 (s, 1H), 8.36 (d, 1H, *J* = 8.0 Hz), 8.04 (d, 1H, *J* = 8.5 Hz), 7.59 (d, 1H, *J* = 4.0 Hz), 7.51 (s, 1H), 7.29 (s, 2H), 6.87 (s, 1H), 4.61 (d, 2H, *J* = 5.0 Hz), 3.92 (s, 6H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.6, 153.8, 153.2, 149.6, 149.2, 147.4, 142.7, 139.5, 138.1, 131.2, 127.7, 126.6, 124.5, 123.5, 119.7, 113.4, 109.7, 105.8, 98.6, 56.7, 56.2, 55.9, 41.9.



4 **5**

4-Methyl-N-((2-(2,4,5-trimethoxyphenyl)benzo[d]oxazol-4-yl)methyl)benzenesulfonamide (5).

To a solution of **4** (32 mg, 0.10 mmol) and triethylamine (40 μL, 0.3 mmol) in 3 mL of dichloromethane was added *p*-toluenesulfonyl chloride (29 mg, 0.15 mmol) at 0 °C. The reaction solution was stirred 3 h under a nitrogen atmosphere and let the solution warmed up to room temperature gradually. The residue was washed with water, and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layer was washed by brine, dried with magnesium sulfate, filtered and evaporated in *vacuo*. The residue was purified by silica gel chromatography to afford **5** in 92% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.64 (d, 2H, *J* = 8.0 Hz), 7.61 (s, 1H), 7.38 (d, 1H, *J* = 8 Hz), 7.15 (t, 1H, *J* = 8.0 Hz), 7.05 (d, 3H, *J* = 7.5 Hz), 6.66 (s, 1H), 6.50 (t, 1H, *J* = 5.5 Hz), 4.57 (d, 2H, *J* = 5.5 Hz), 4.04 (s, 3H), 4.00 (s, 3H), 3.98 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 154.6, 153.3, 150.2, 143.5, 140.2, 137.1, 129.3, 127.4, 127.2, 124.5, 123.3, 113.2, 109.6, 107.1, 98.0, 57.2, 56.8, 56.3, 45.0, 21.6.

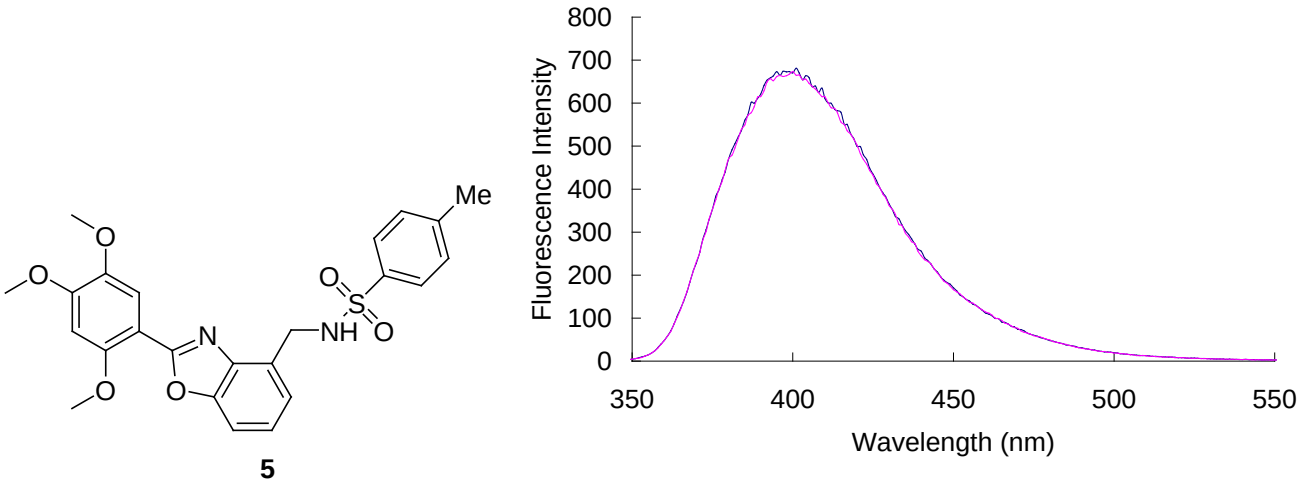


Figure S1. Fluorescence response of the benzoxazole derivative **5** towards thiophenol. Probe **5** (1×10^{-6}M), prepared from a stock solution (1 mM) in DMF, was sampled for fluorescence measurement in a phosphate buffer (pH 7.3, 0.01M) containing 0.5% of DMF at room temperature before and 20 min after the addition of thiophenol (2.0 equivalents) with λ_{ex} = 335 nm.

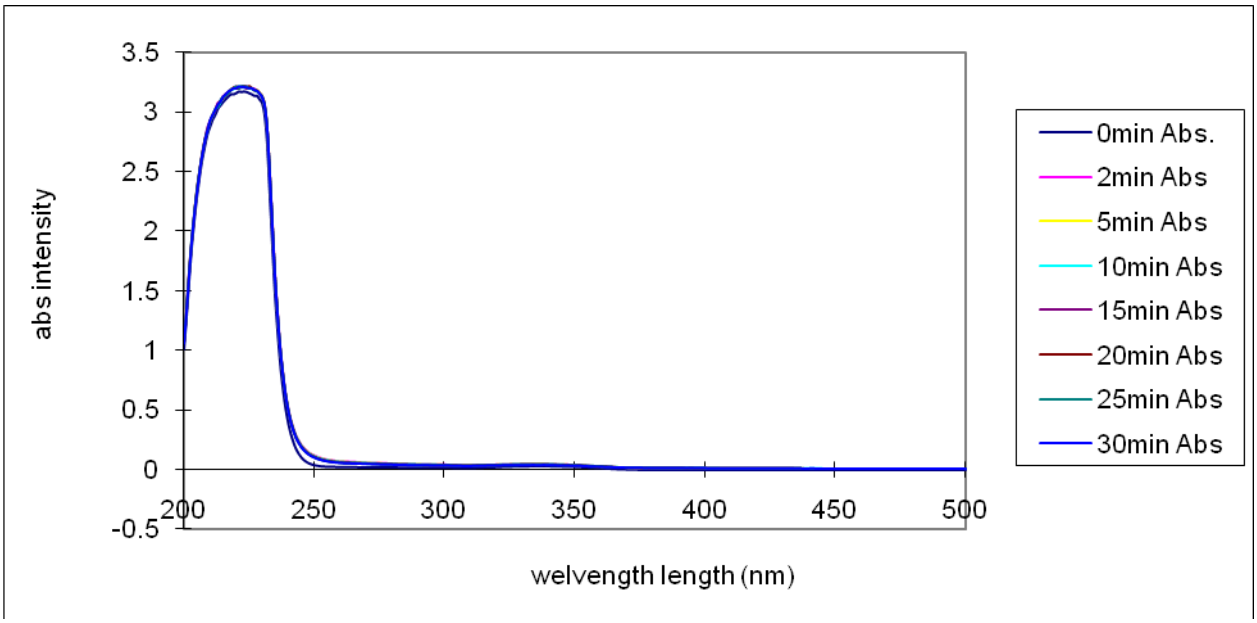
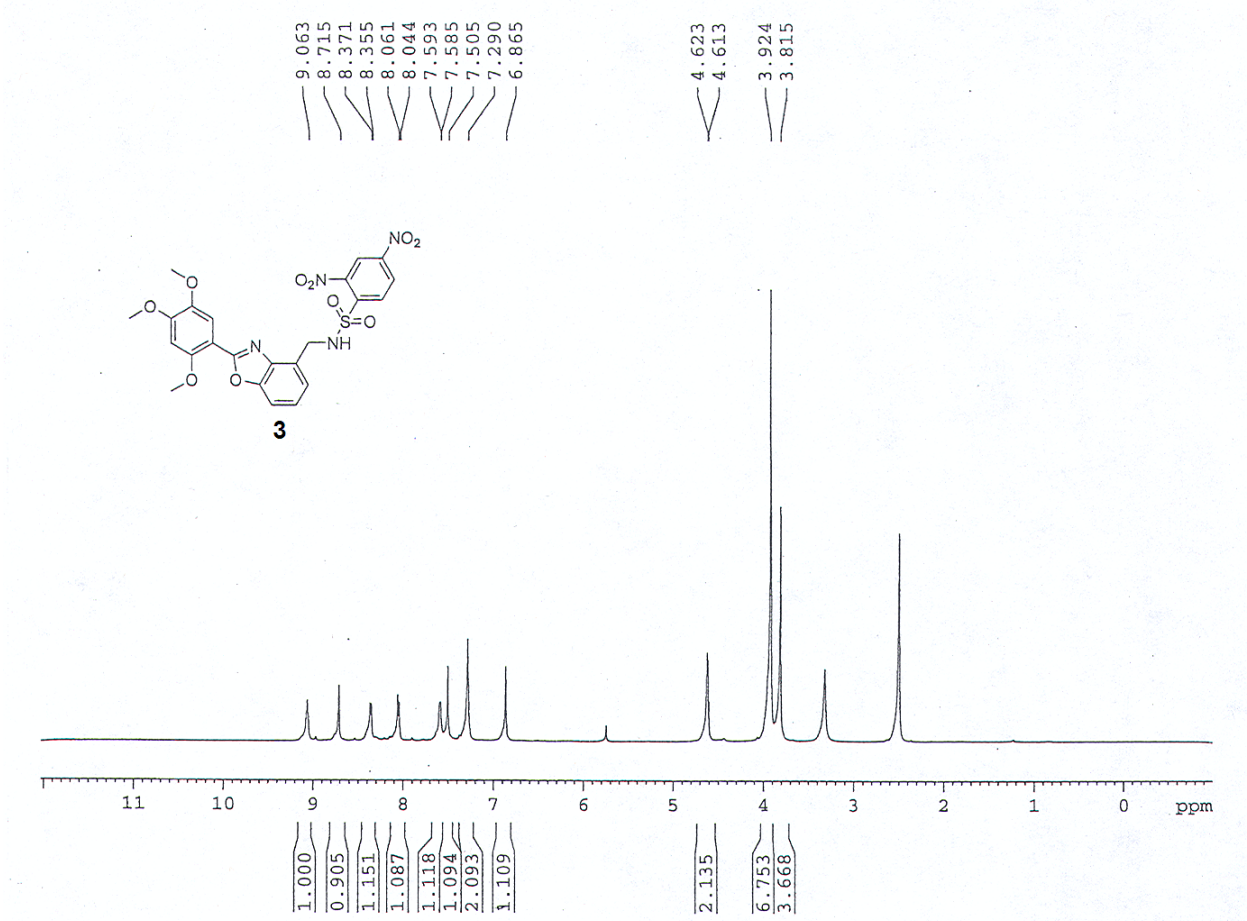


Figure S2. Absorption spectrum of **3** upon the addition of thiophenol. Probe **3** (2×10^{-6}M), prepared from a stock solution (1 mM) in DMF, was sampled for absorption measurement in a phosphate buffer (pH 7.3, 0.01M) containing 0.5% of DMF at room temperature at different times.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3.924
3.815
NO2
O2N
O
S=O
NH
N
O
3
11
10
9
8
7
6
5
4
3
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1
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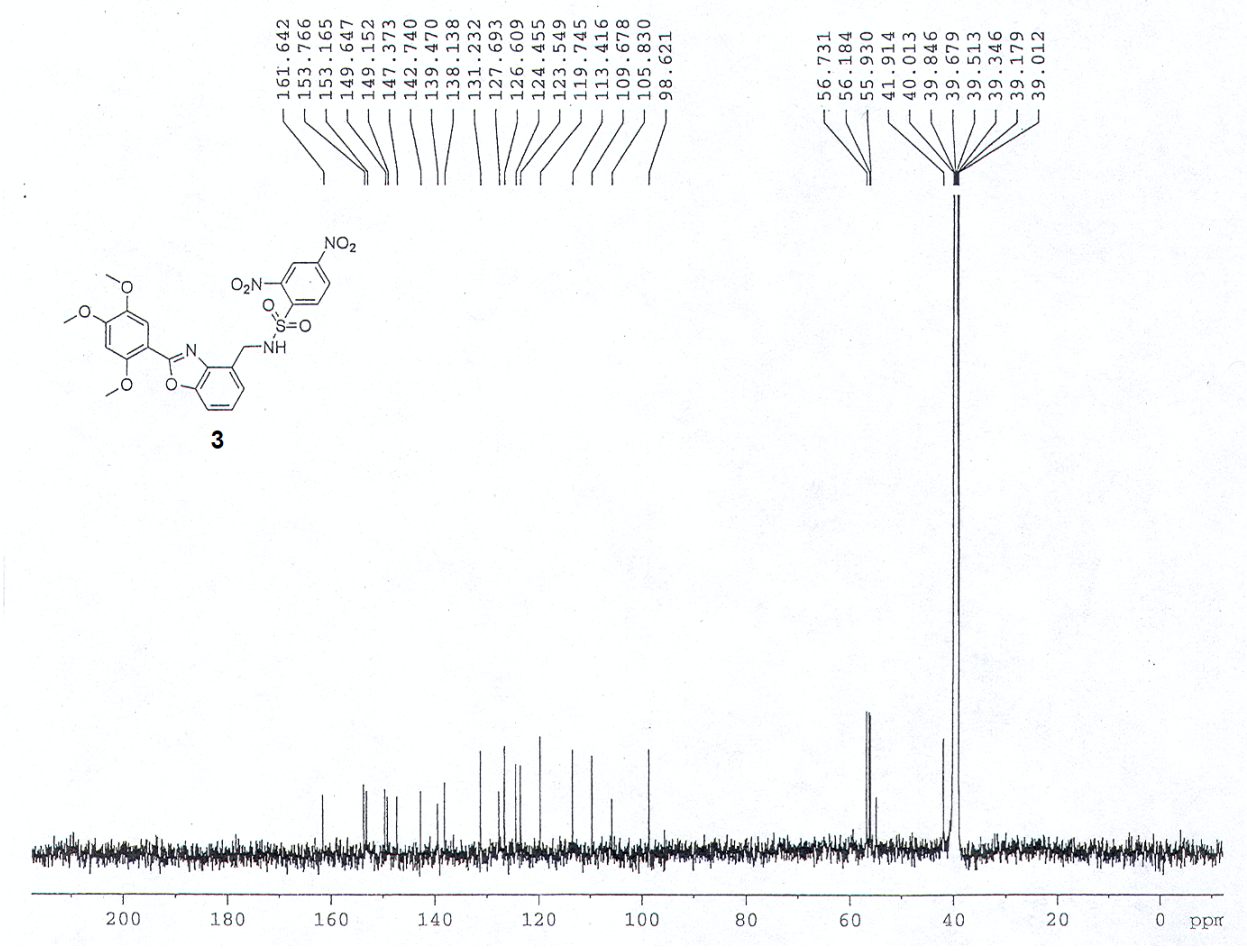
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123.549
119.745
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56.731
56.184
55.930
41.914
40.013
39.846
39.679
39.513
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39.179
39.012
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NH
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O
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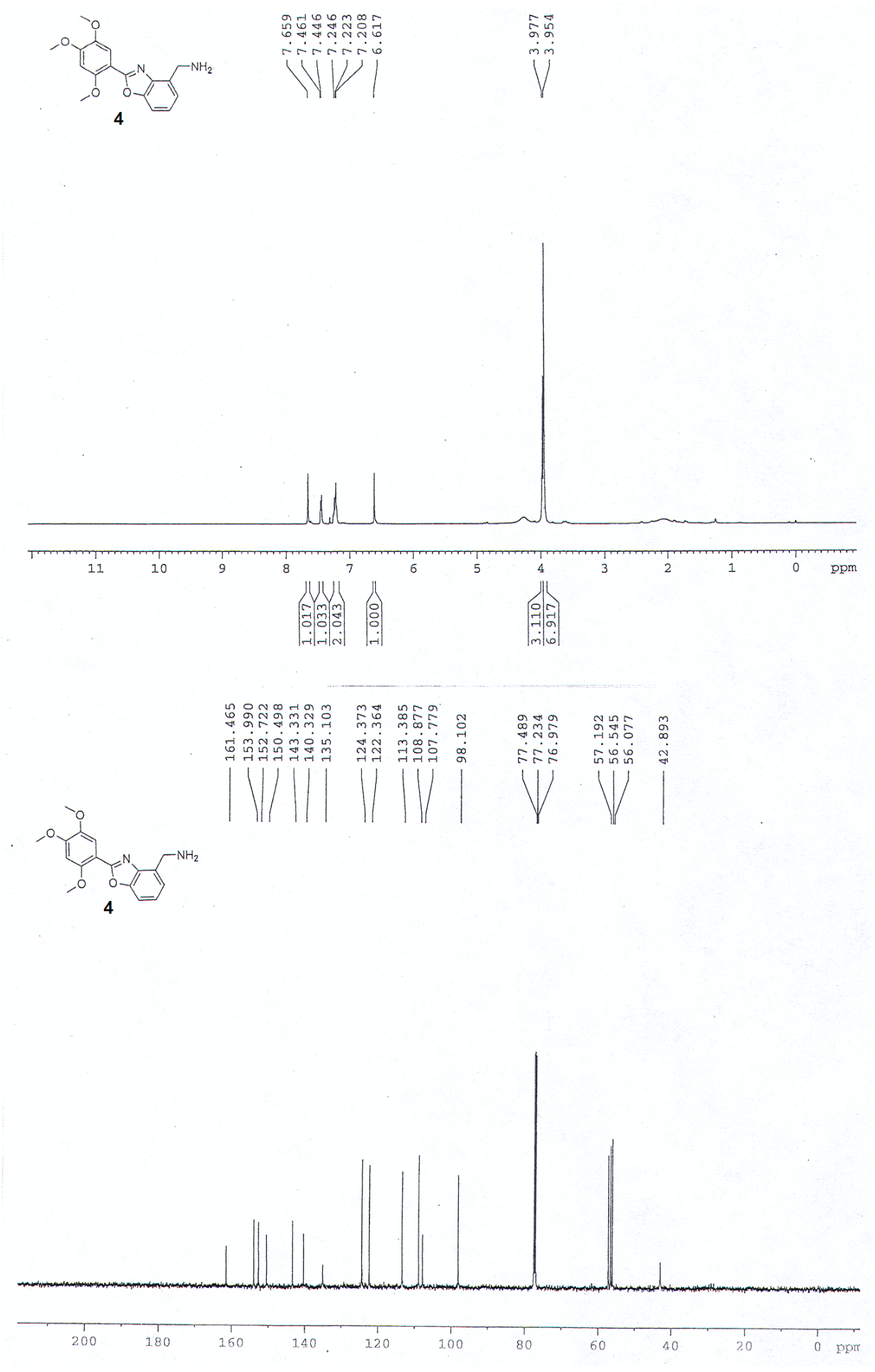
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6
5
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1.000
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56.077
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100
80
60
40
20
0
ppm


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7.135
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O
S
O
N
NH
O
O
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10
9
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NH
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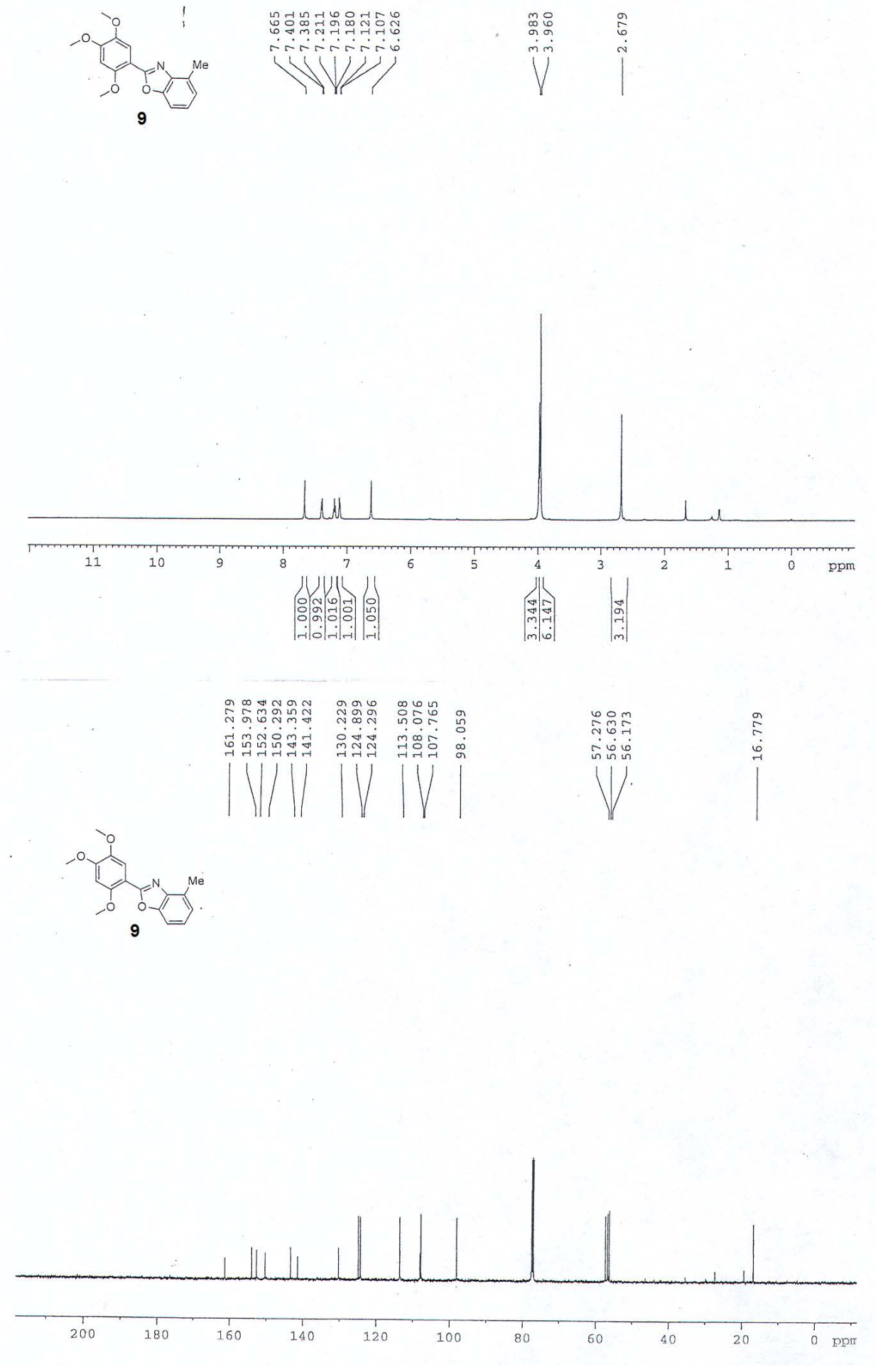
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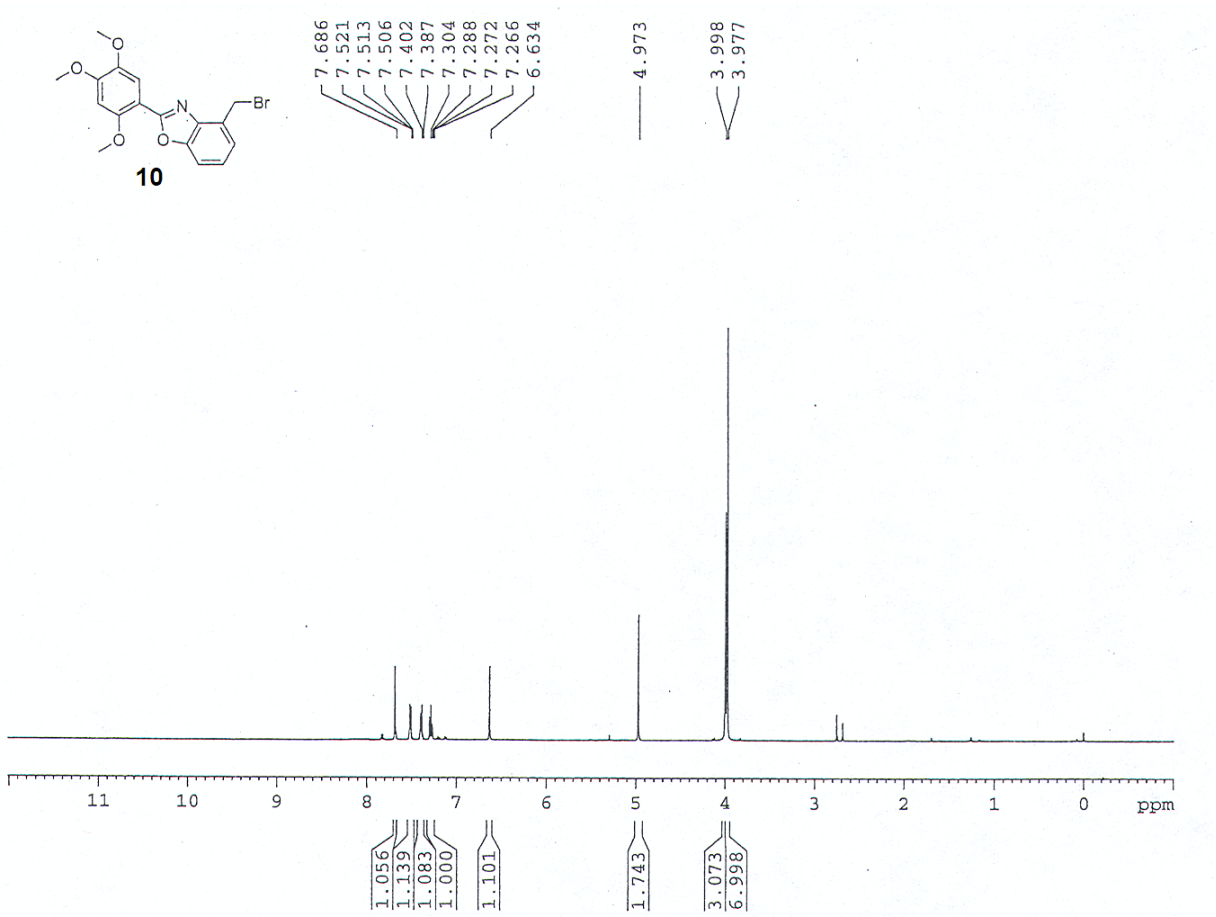
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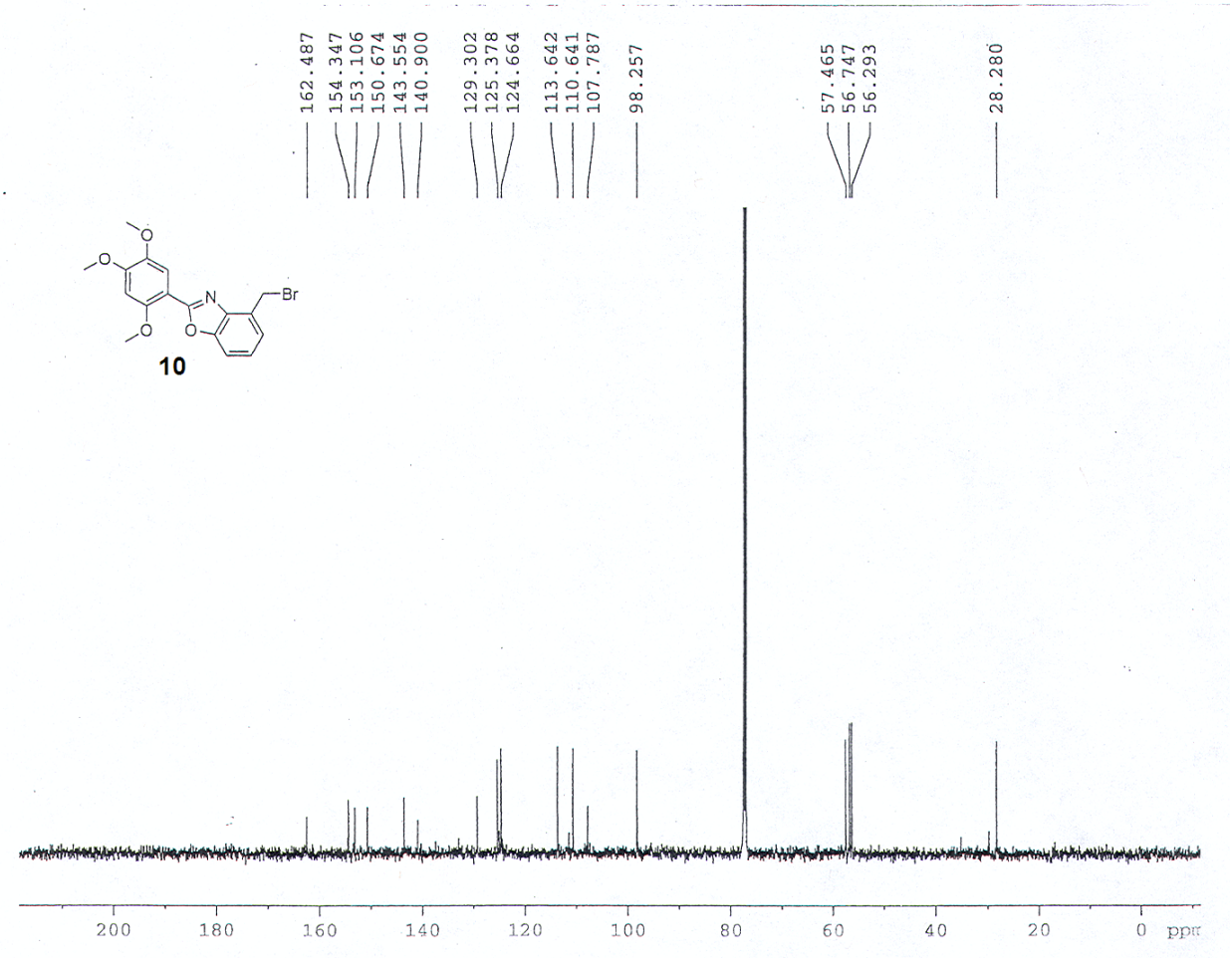
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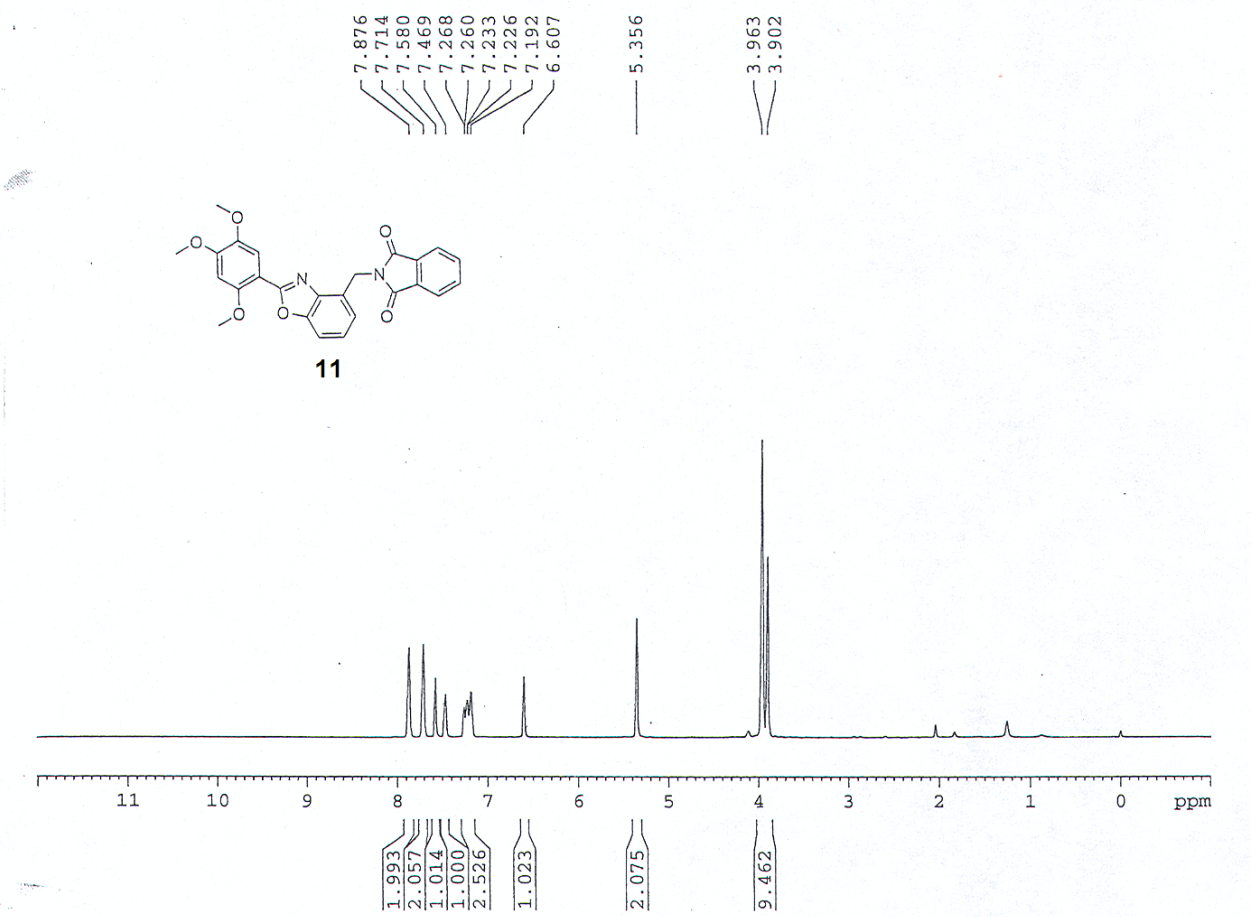
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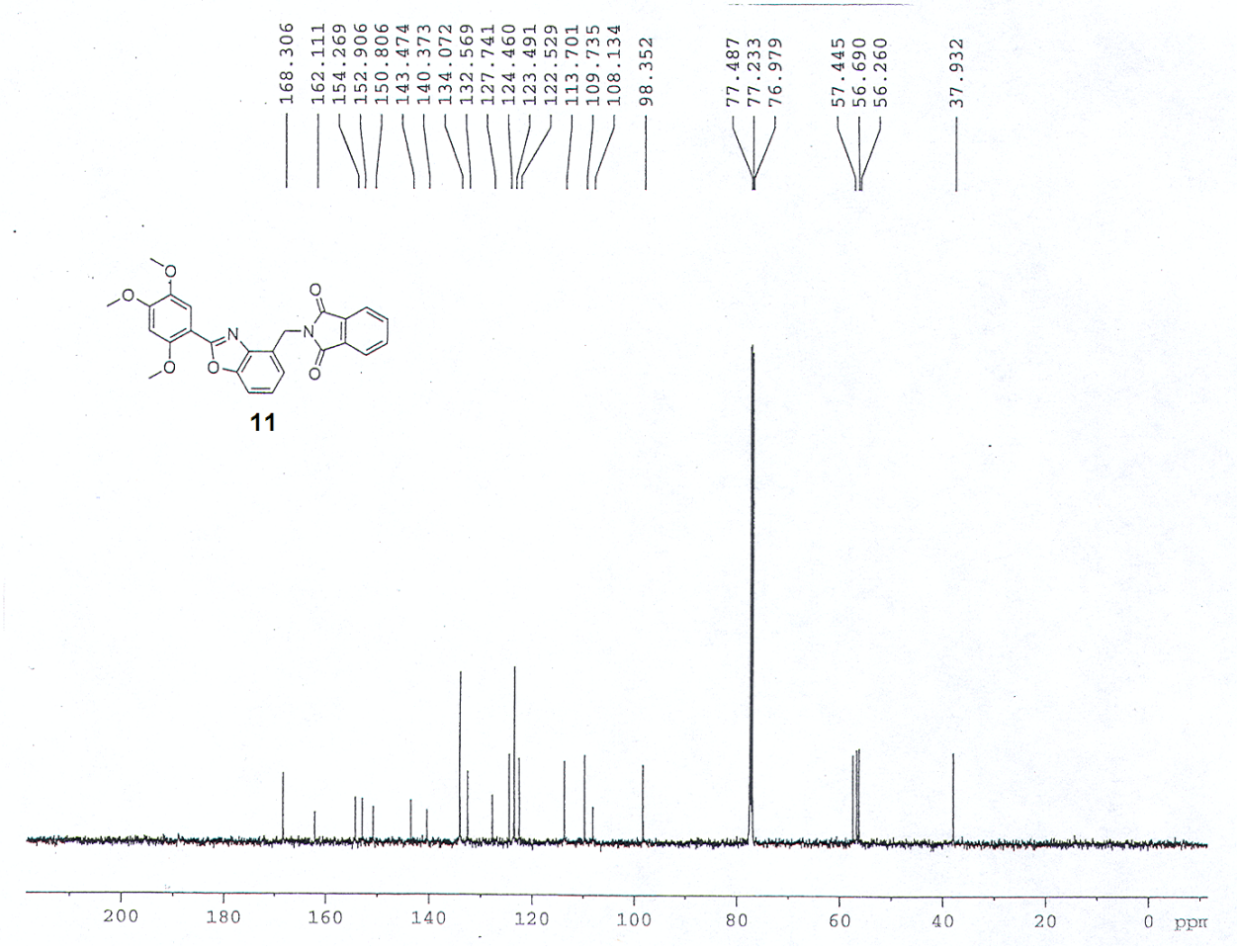
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109.735
108.134
98.352
77.487
77.233
76.979
57.445
56.690
56.260
37.932
11
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