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Design, Synthesis and Structure–Activity Relationship Studies on Erianin Analogues as Pyruvate Carboxylase (PC) Inhibitors Against Hepatocellular Carcinoma Cells

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content

Synthesis of 2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)propyl)phenol 4[1]

The obtained ketone **3** (150.0 mg, 0.43 mmol) was dissolver in acetic acid (2 mL), 10% palladium on carbon (25.2 mg) was added to the solution. The reaction vessel was evacuated and backfilled with hydrogenation (H_2) for 3 times. The mixture was stirred at 25 °C for 12 h and then filtration through celite gave a filtrate. This filtrate was evaporated to oil, which was purified by chromatography on silica gel (ethyl acetate: hexane = 1: 10) to give 15 (130.6 mg, 91.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 2.24 Hz, 1H), 6.76 (d, J = 8.36 Hz, 1H), 6.67 (dd, J = 2.00, 8.20 Hz, 1H), 6.39 (s, 2 H), 3.87 (s, 3H), 3.85 (s, 3 H), 3.83 (s, 3H), 2.61 (t, J = 7.76 Hz, 4H), 1.89 - 1.95 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.07, 145.50, 144.78, 138.17, 136.05, 135.54, 119.75, 114.71, 110.63, 105.33, 60.86, 56.07, 35.76, 34.82, 33.02.

Synthesis of compound 5[2] and 14[3]

The synthesis of compound **5** and **7** followed the synthesis procedure of compound **15** in scheme 3.

2-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethan-1-one (**5**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 2H), 6.84 (d, J = 2.00 Hz, 1H), 6.81 (d, J= 8.20 Hz, 1H), 6.75 (dd, J = 2.00 Hz, 8.20 Hz, 1H), 5.64 (s, 1H), 4.14 (s, 2H), 3.40 (s, 3H), 3.88 (s, 6H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.61, 153.01, 145.82, 145.62, 142.57, 131.70, 127.97, 120.73, 115.54, 110.94, 106.32, 60.91, 56.26, 55.95, 45.03.

1-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethan-1-one (**14**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, J = 1.92 Hz, 8.28 Hz, 1H), 7.57 (d, J = 1.92 Hz, 1H), 6.96 (d, J = 8.28 Hz, 1H), 6.48 (s, 2H), 6.13 (br s, 1H), 4.17 (s, 2H), 3.94 (s, 3H), 3.83 (s, 3 H), (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.19, 153.32, 150.60, 146.77, 136.91, 130.57, 129.48, 124.07, 113.86, 110.33, 106.45, 60.82, 56.11, 56.03, 45.26, 29.69.

Synthesis of compound 6 and 7[4]

The synthesis of compound **6** and **7** followed the synthesis procedure of compound **8** and **9** in scheme 4. 2-methoxy-5-((methyl(3,4,5- trimethoxyphenyl)amino)methyl)-

phenol (**6**). ^{1}H NMR (400 MHZ, $CDCl_3$) δ 6.84 (s, 1H), 6.81 (d, *J* = 10.52, 1H), 6.76 (d, *J* = 8.04, 1H), 6.00 (s, 2H), 5.64 (s, 1H)， 4.38 (s, 2H), 3.88 (s, 3H) 3.80 (s, 6H) 3.77 (s, 3H), 2.97 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.68, 145.79, 145.64, 118.41, 113.25, 110.70, 90.90, 61.09, 57.04, 56.01, 55.99, 38.97.

2-methoxy-5-(methyl(3,4,5-trimethoxybenzyl)amino)phenol (**7**). ^{1}H NMR (400 MHZ, $CDCl_3$), *δ* 6.77 (d, *J* = 8.8Hz, 1H), 6.48 (d, *J* = 2.92Hz, 1H), 6.47 (s, 2H), 6.26 (dd, *J* = 2.92Hz, 8.8Hz, 1H), 5.63 (s, 1H) 4.34 (s, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.80 (s, 6H), 2.90 (s, 3H), ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 153.40, 146.42, 145.92, 138.94, 136.76, 135.06, 112.27, 104.45, 103.70, 101.25, 60.85, 58.11, 56.78, 56.09, 38.96.

Figure S1. IC_{50} values of the erianin analogues 1, 2, 3, 4 and 6 on cell growth inhibition in HCCLM3

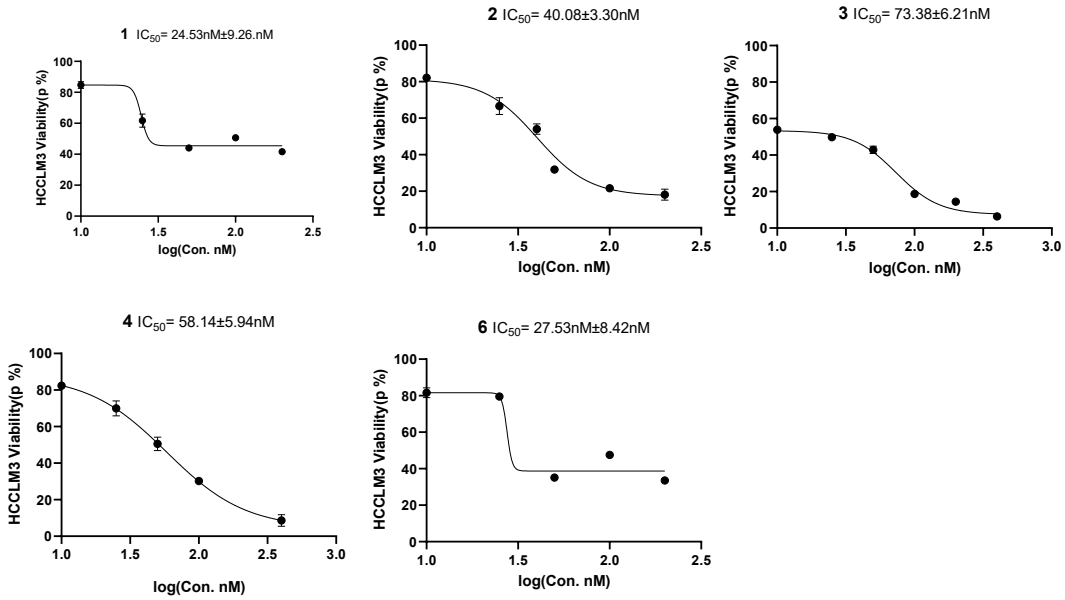


Figure S2 Compound 34 and human PC (PDB ID:3BG3) binding modes

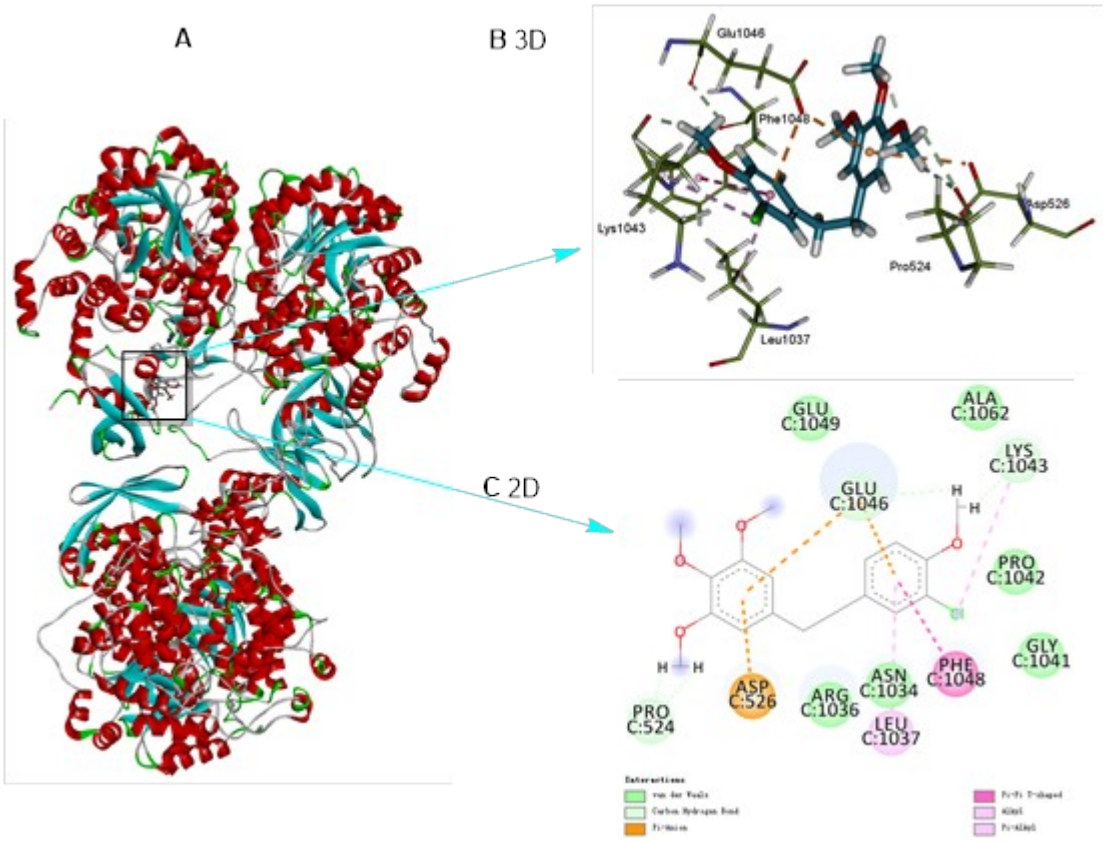


Fig. S2 Binding conformations of **34** with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound **34** in the 3BG3; panel B: binding 3D modes of compound **34** in the active site of 3BG; panel C, binding 2D modes of compound **34** the active site of 3BG3.

Figure S3 Compound 35 and human PC (PDB ID:3BG3) binding modes

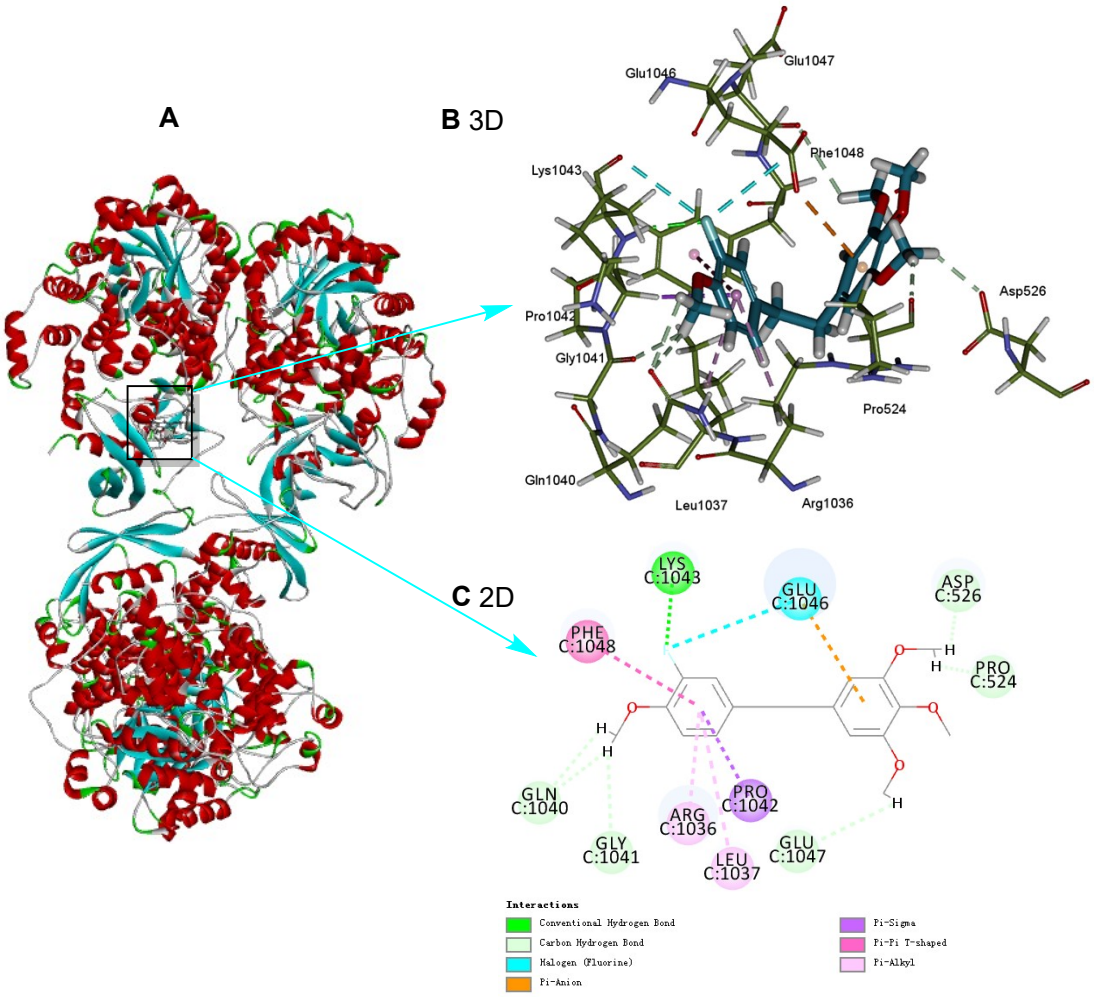


Fig. S3 Binding conformations of **35** with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound **35** in the 3BG3; panel B: binding 3D modes of compound **35** in the active site of 3BG; panel C, binding 2D modes of compound **35** the active site of 3BG3.

Figure S4 Erianin and human PC (PDB ID:3BG3) binding modes

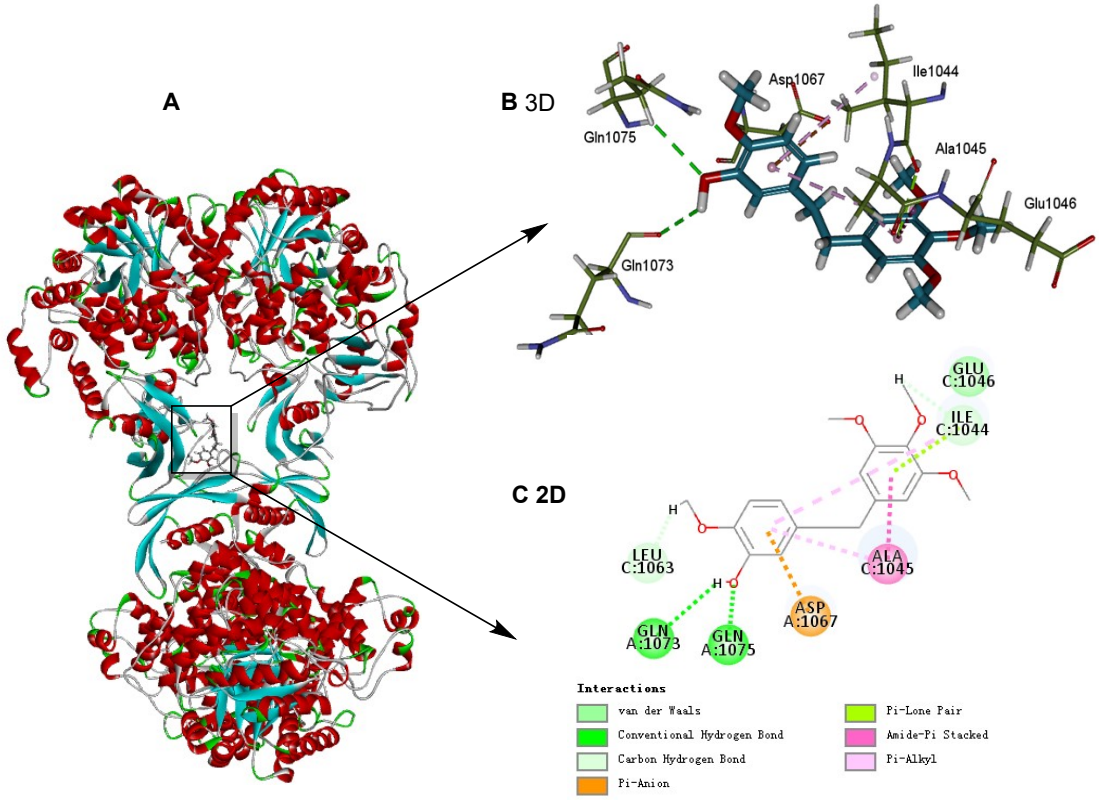


Fig. S4 Binding conformations of erianin with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound erianin in the 3BG3; panel B: binding 3D modes of compound erianin in the active site of 3BG; panel C, binding 2D modes of compound erianin the active site of 3BG3.

Figure S5. ^{1}H NMR of Compound 4

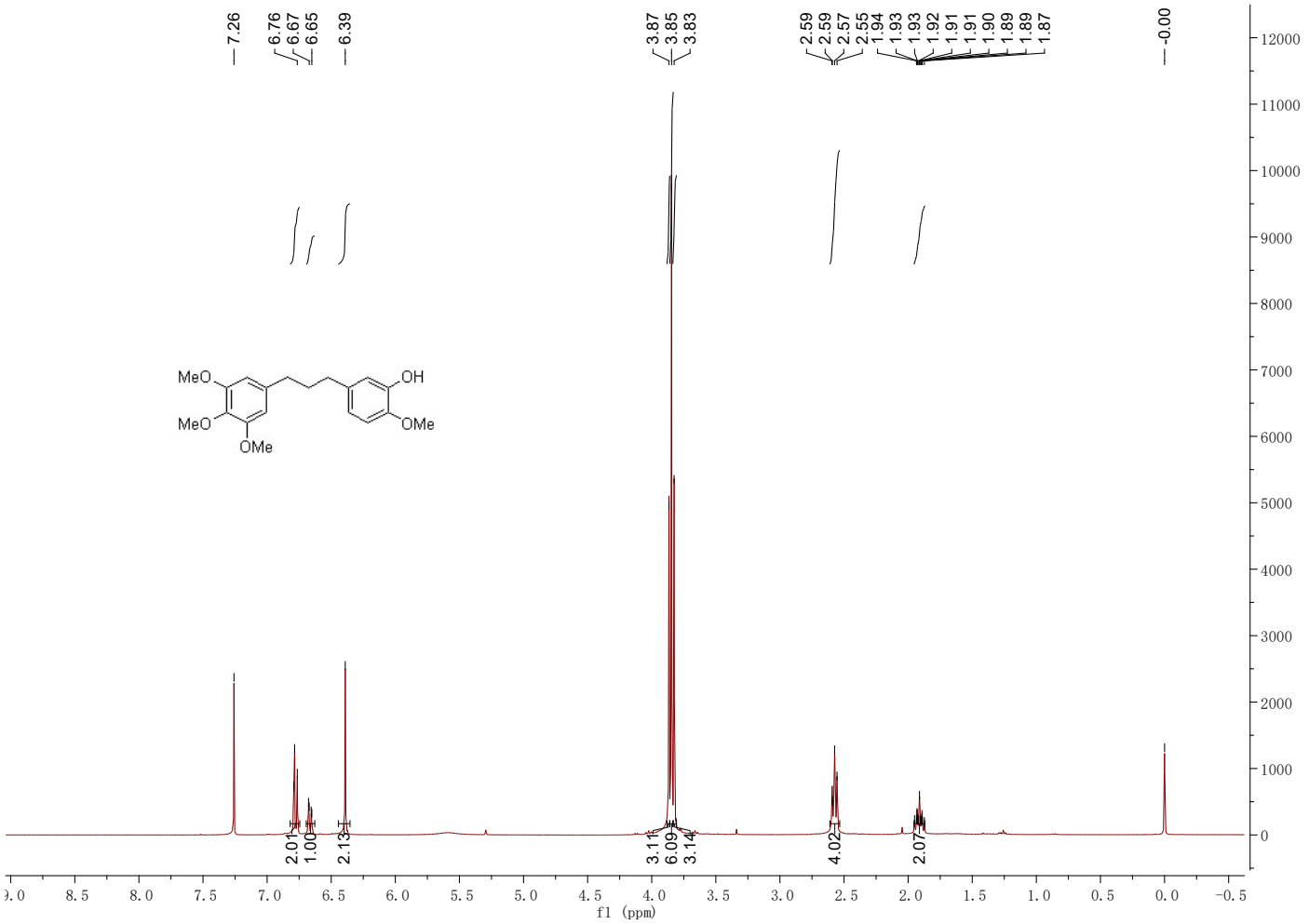


Figure S6. ^{13}C NMR of Compound 4

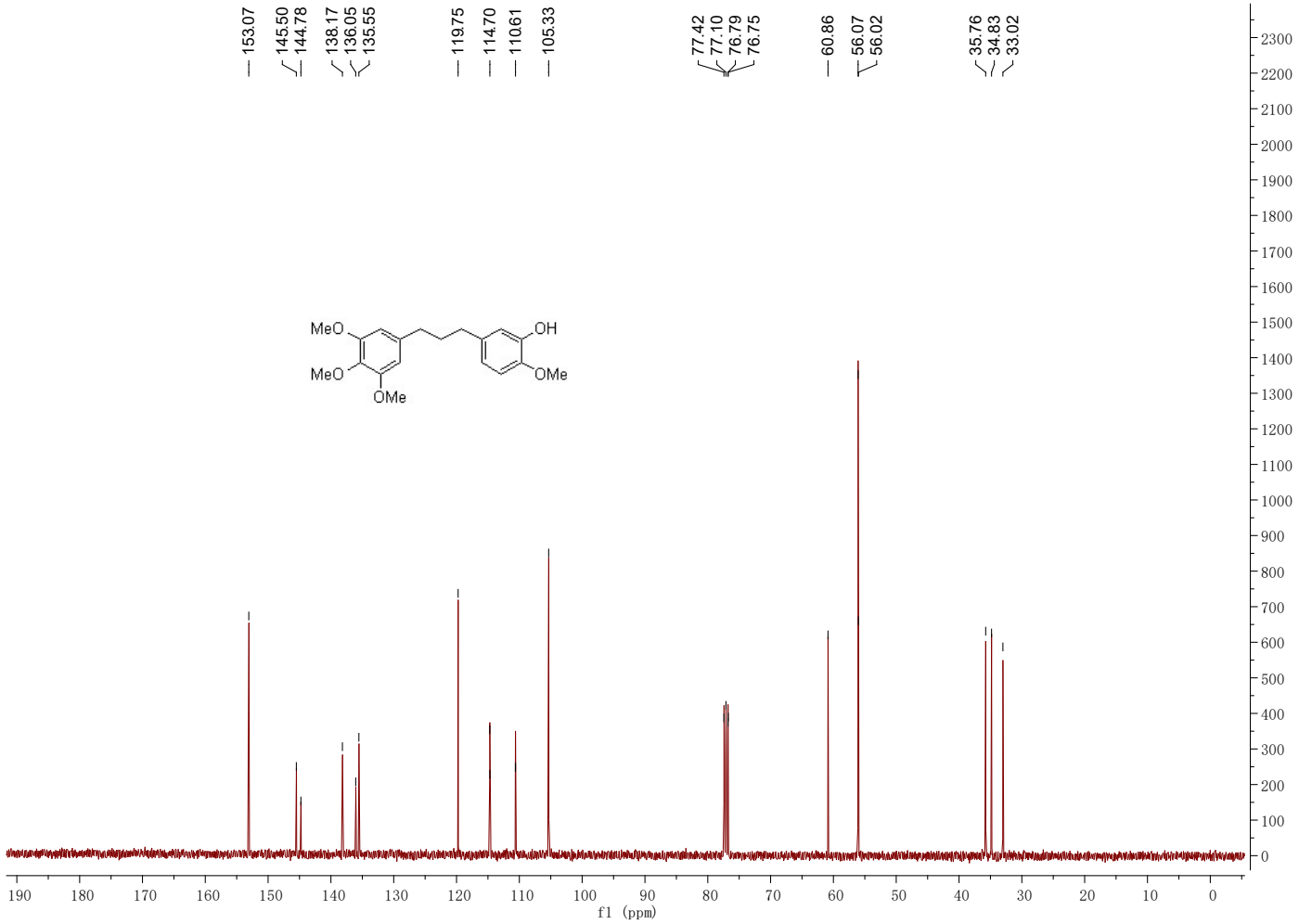


Figure S7. ^{1}H NMR of Compound 14

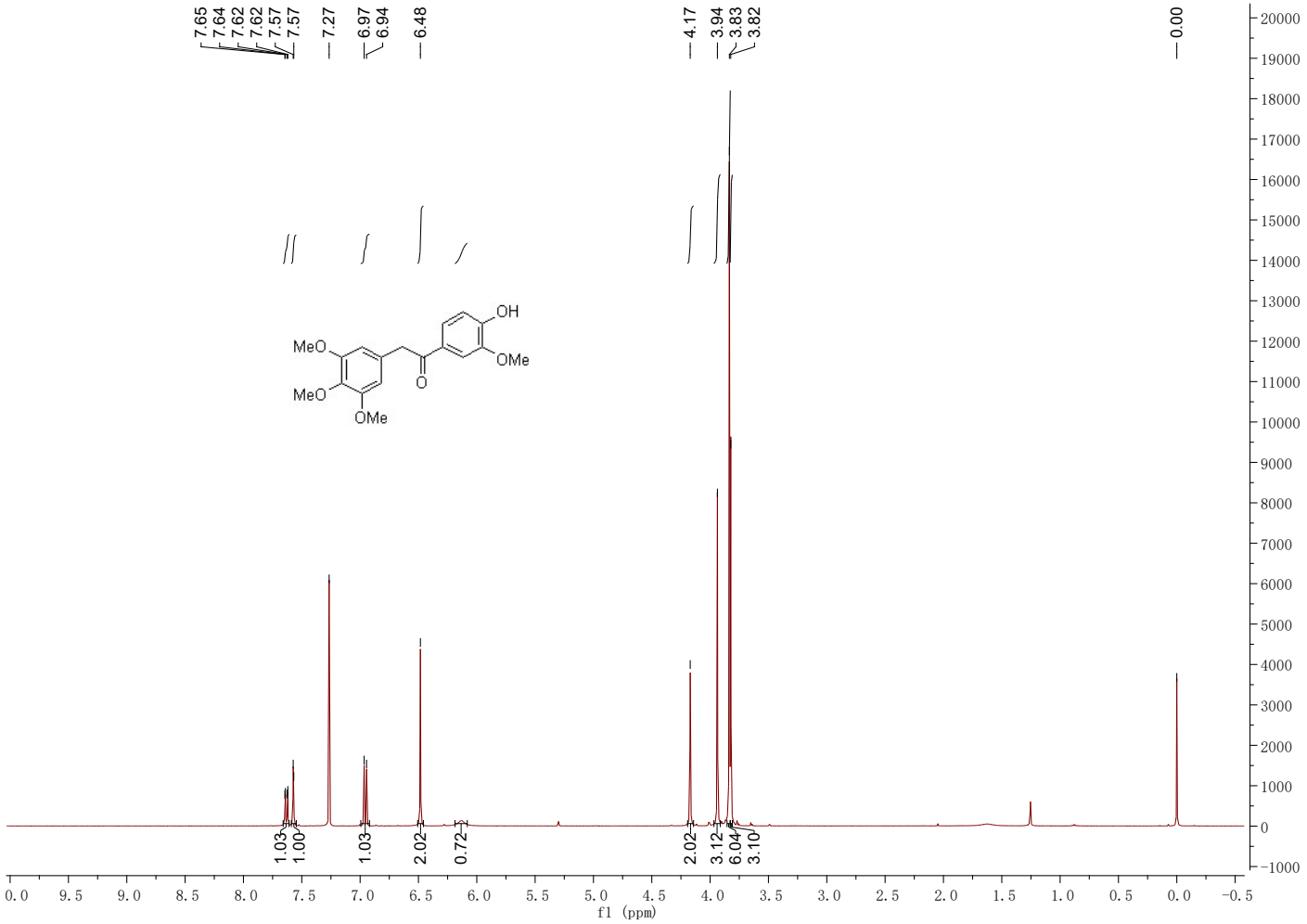


Figure S8. ^{13}C NMR of Compound **14**

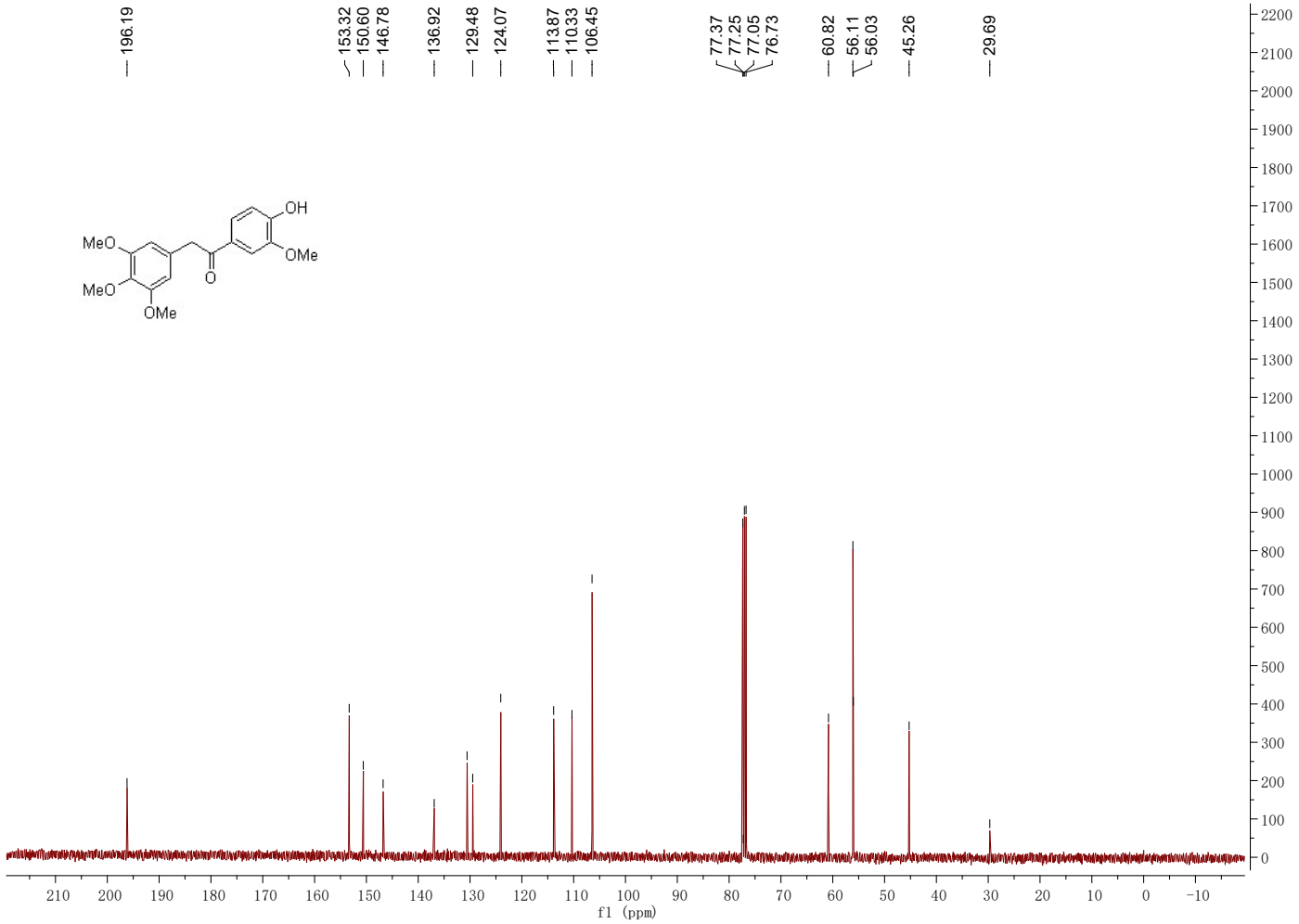


Figure S9. ^{13}C NMR of Compound 5

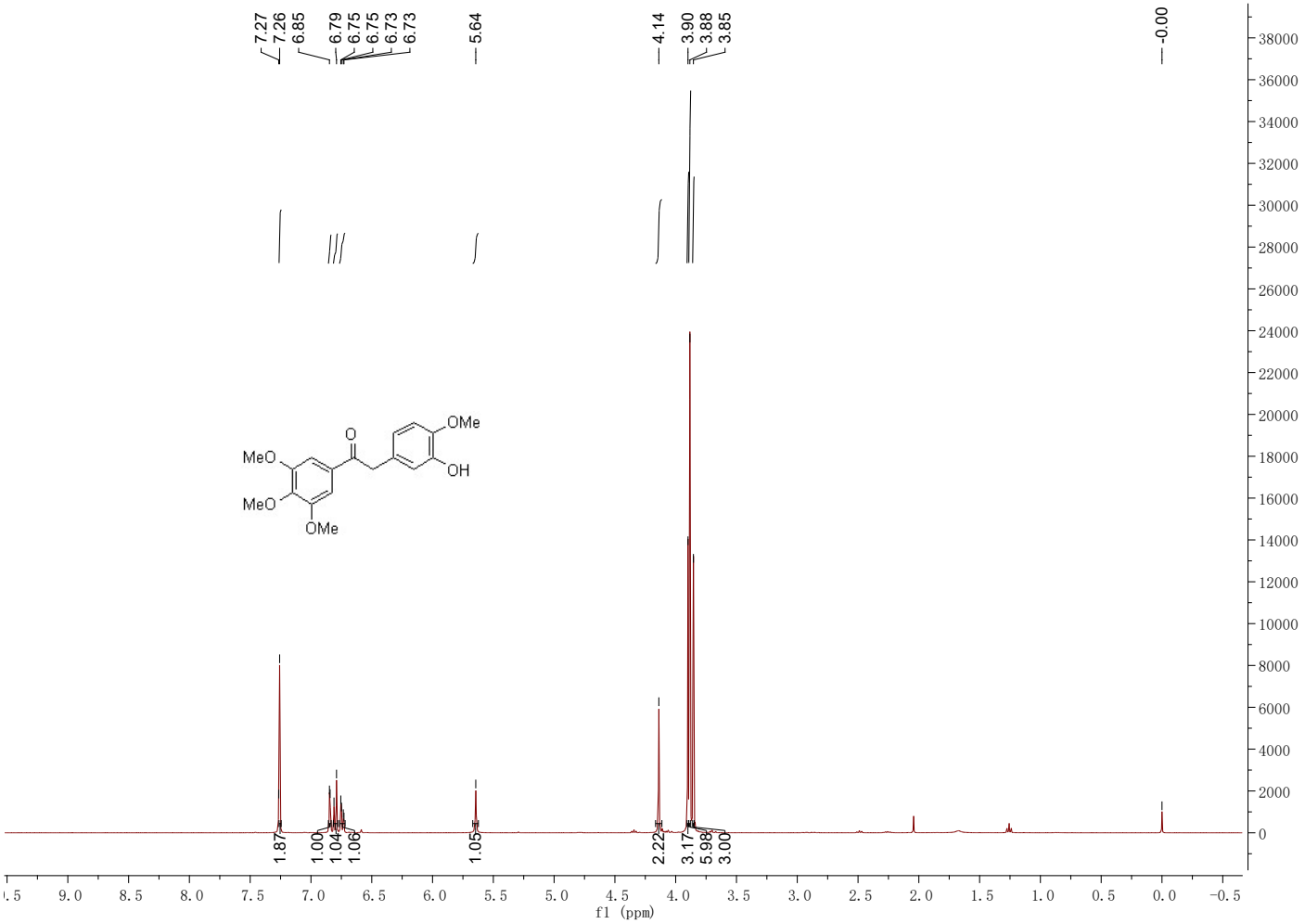


Figure S10. ^{13}C NMR of Compound 5

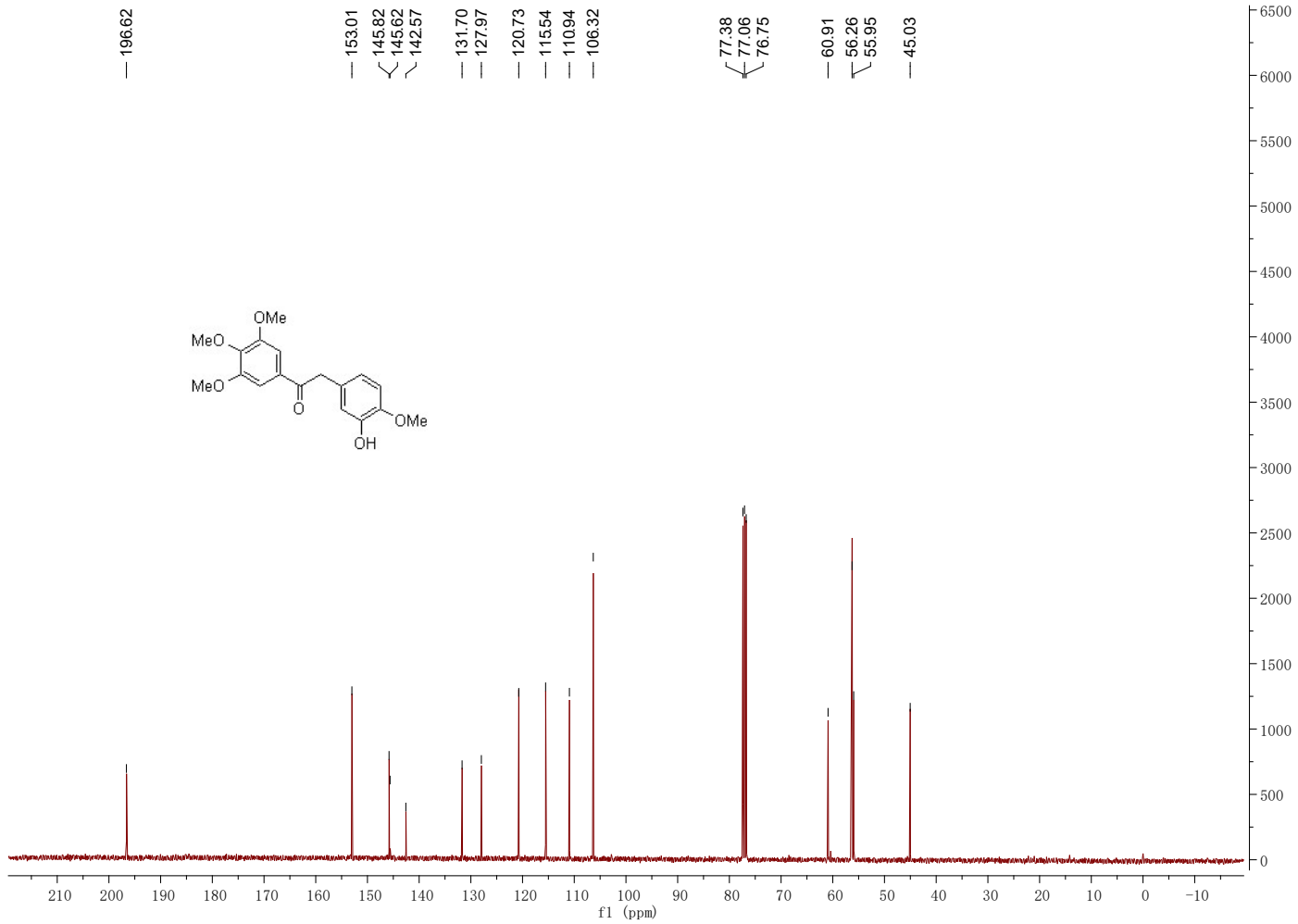


Figure S11. ^{1}H NMR of Compound 6

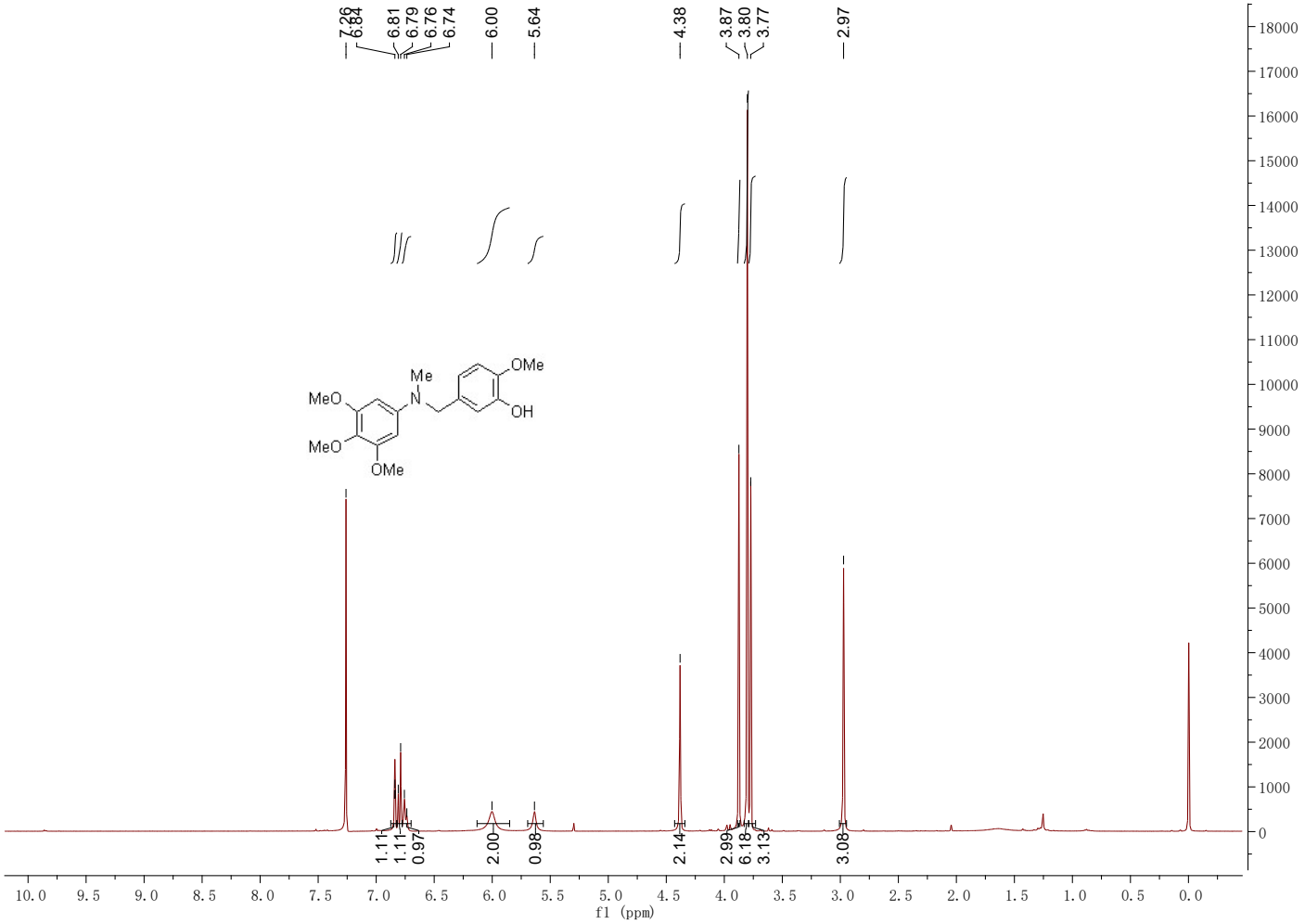


Figure S12. ^{13}C NMR of Compound 6

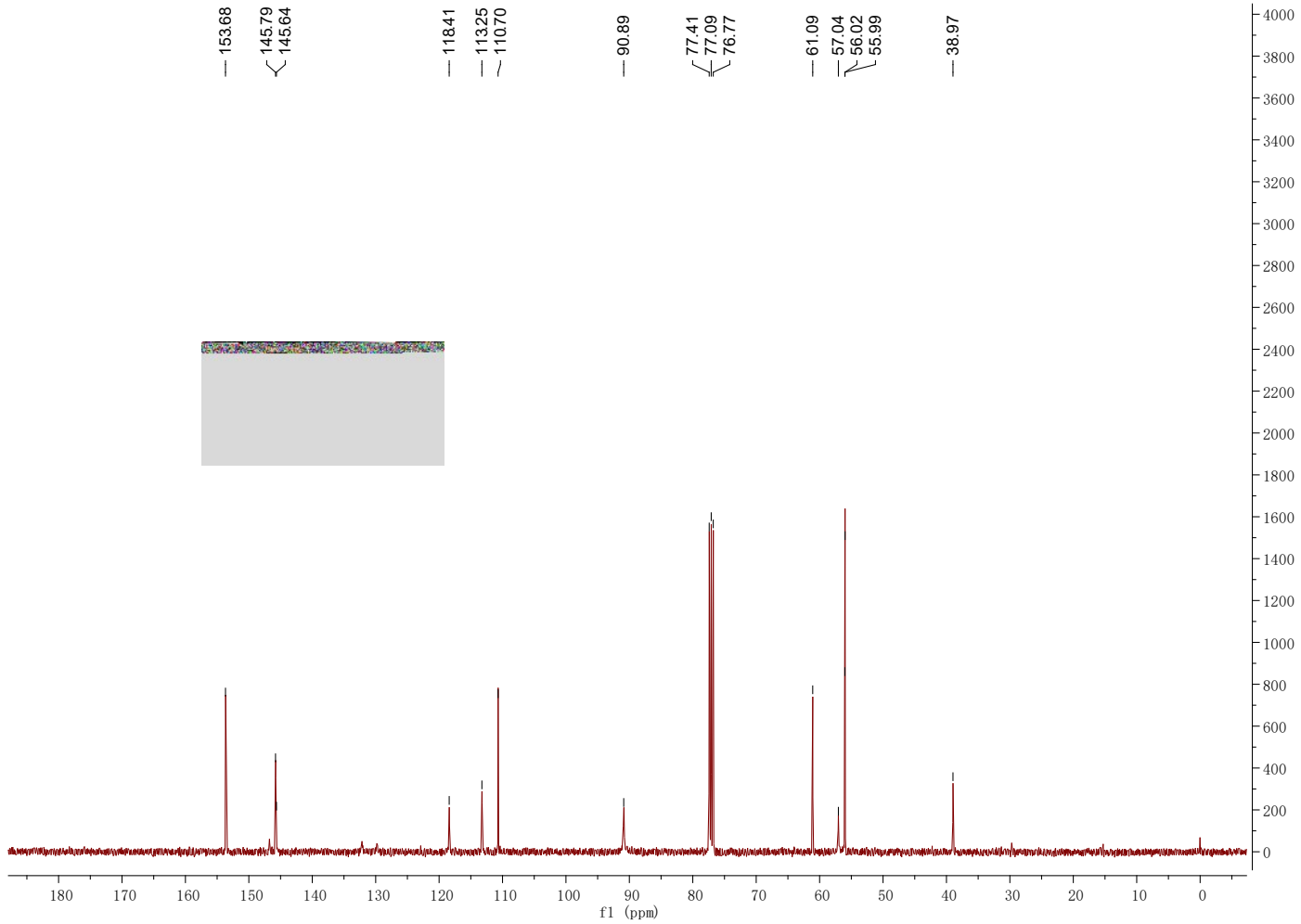


Figure S13. ^{1}H NMR of Compound 7

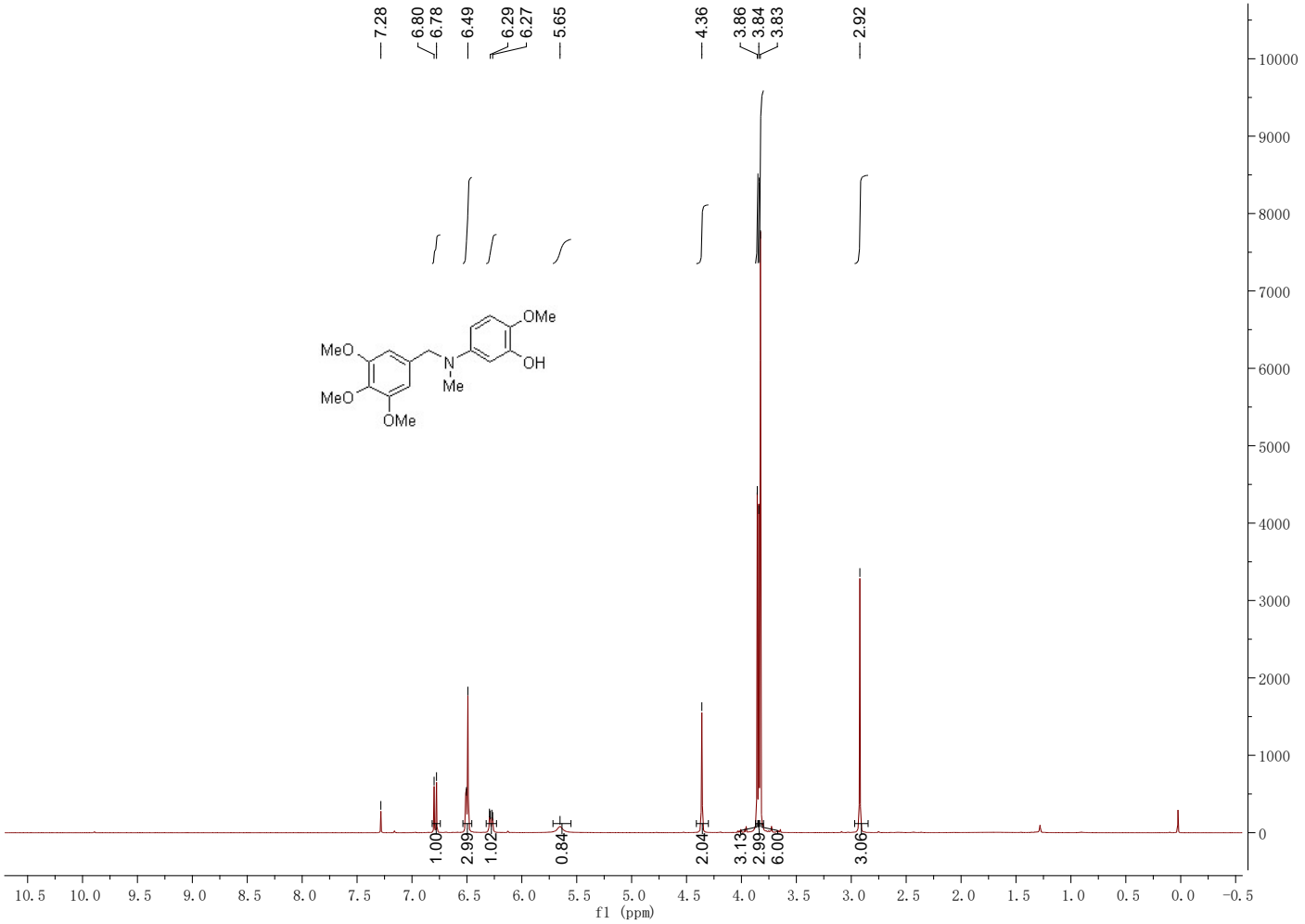


Figure S14. ^{13}C NMR of Compound 7

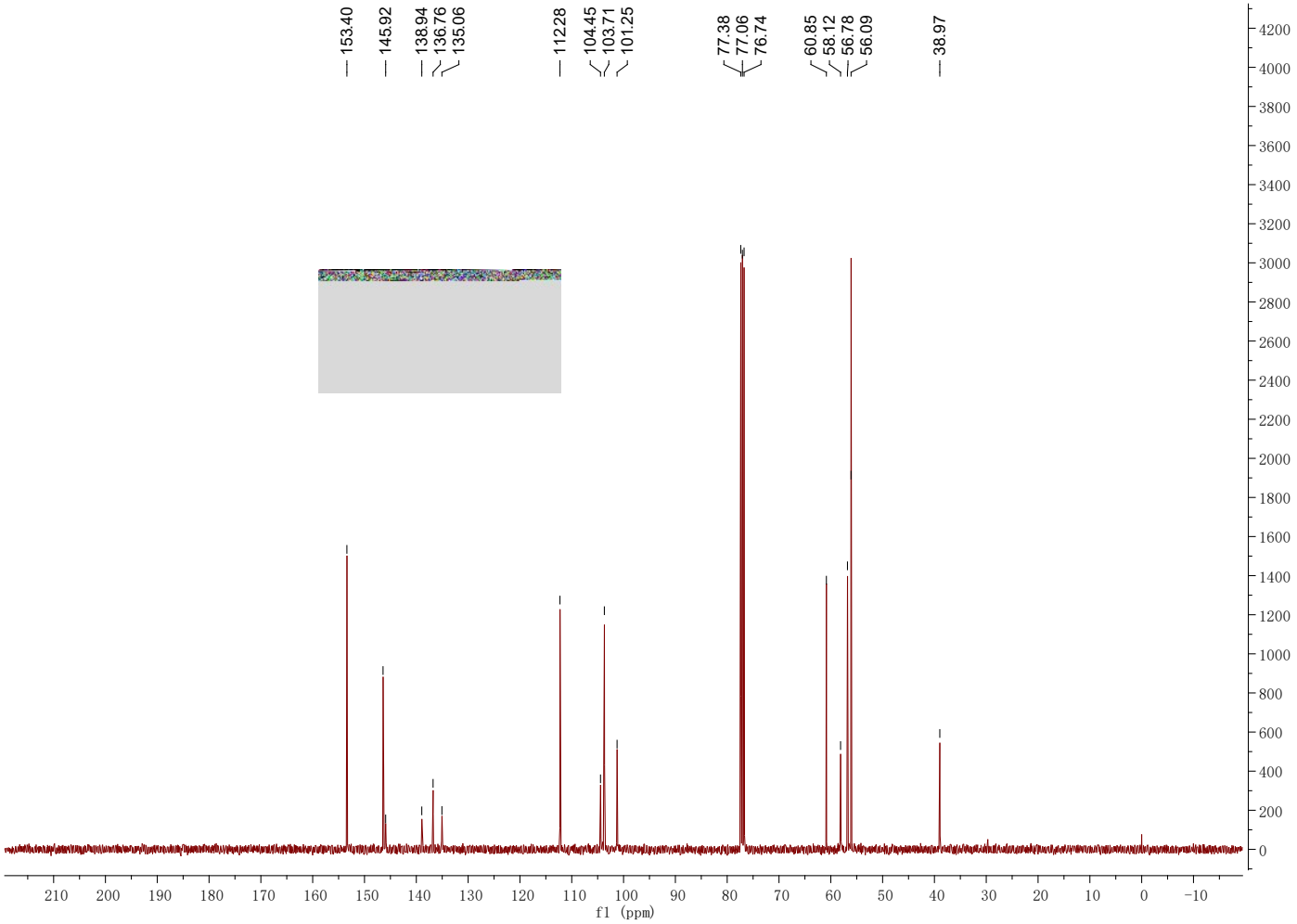


Figure S15. ^{1}H NMR of Compound 8

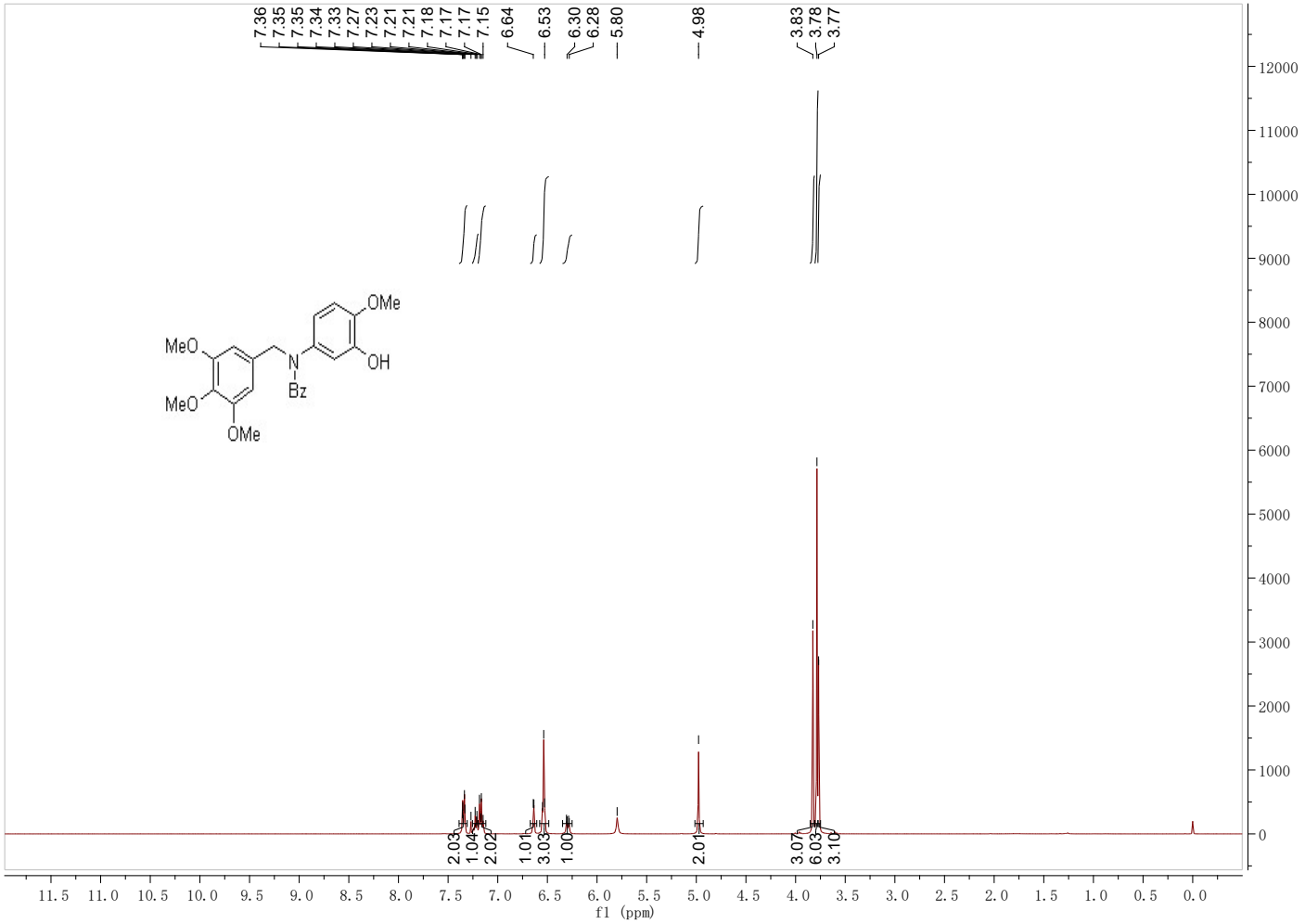


Figure S16. ^{13}C-NMR of Compound 8

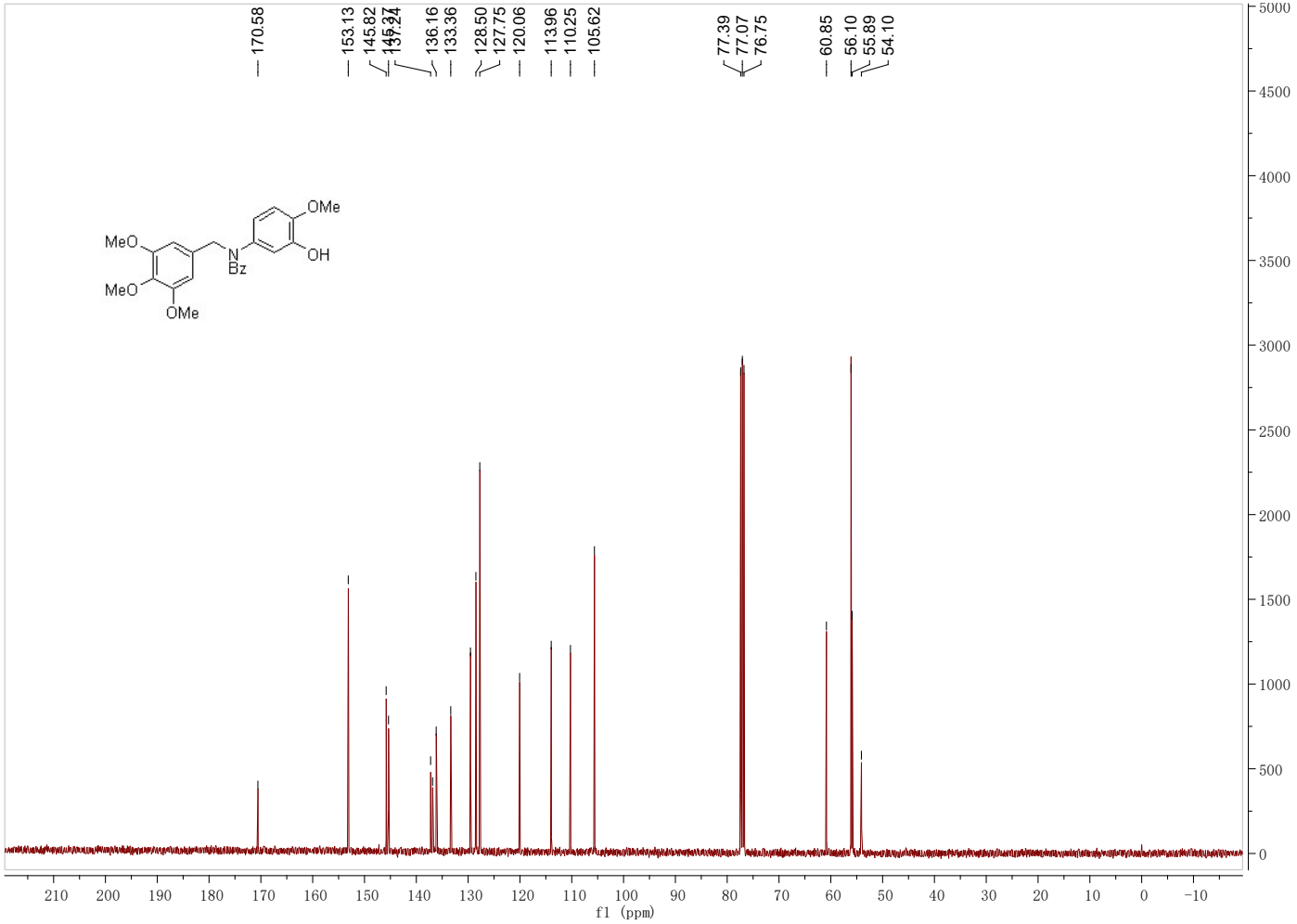


Figure S17. ^{1}H NMR of Compound 9

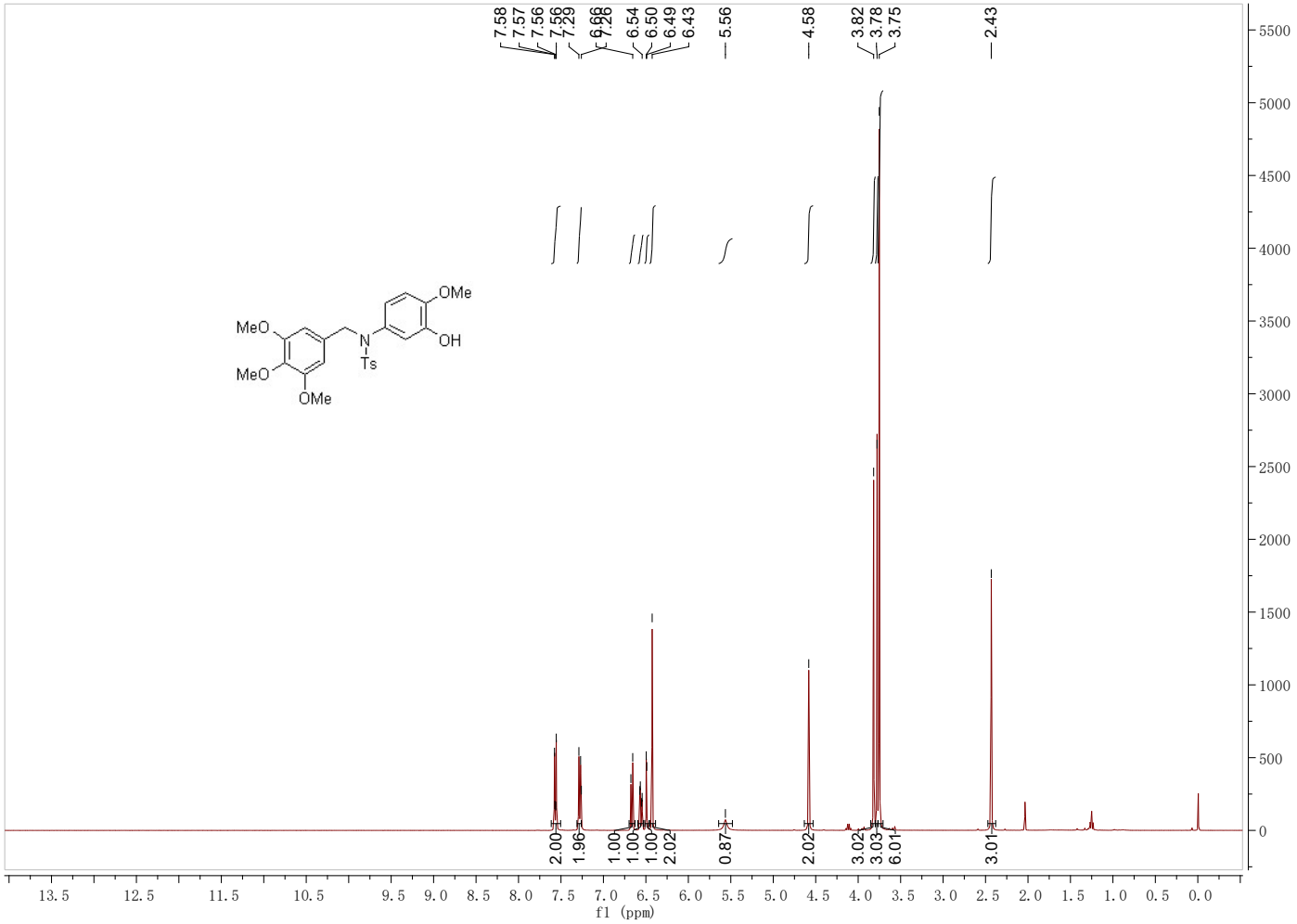


Figure S18. ^{13}C-NMR of Compound 9

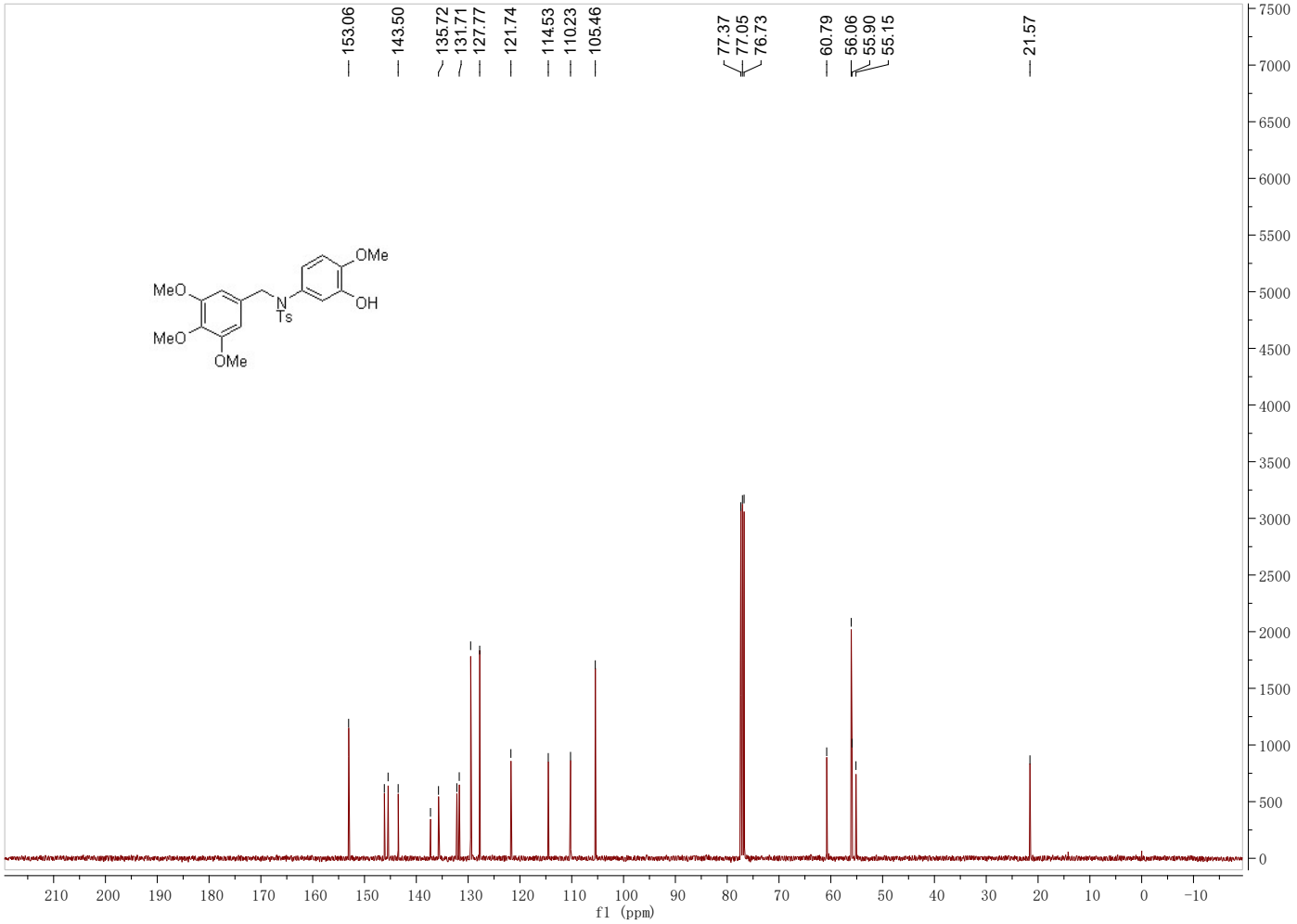


Figure S19. ^{1}H NMR of Compound 11

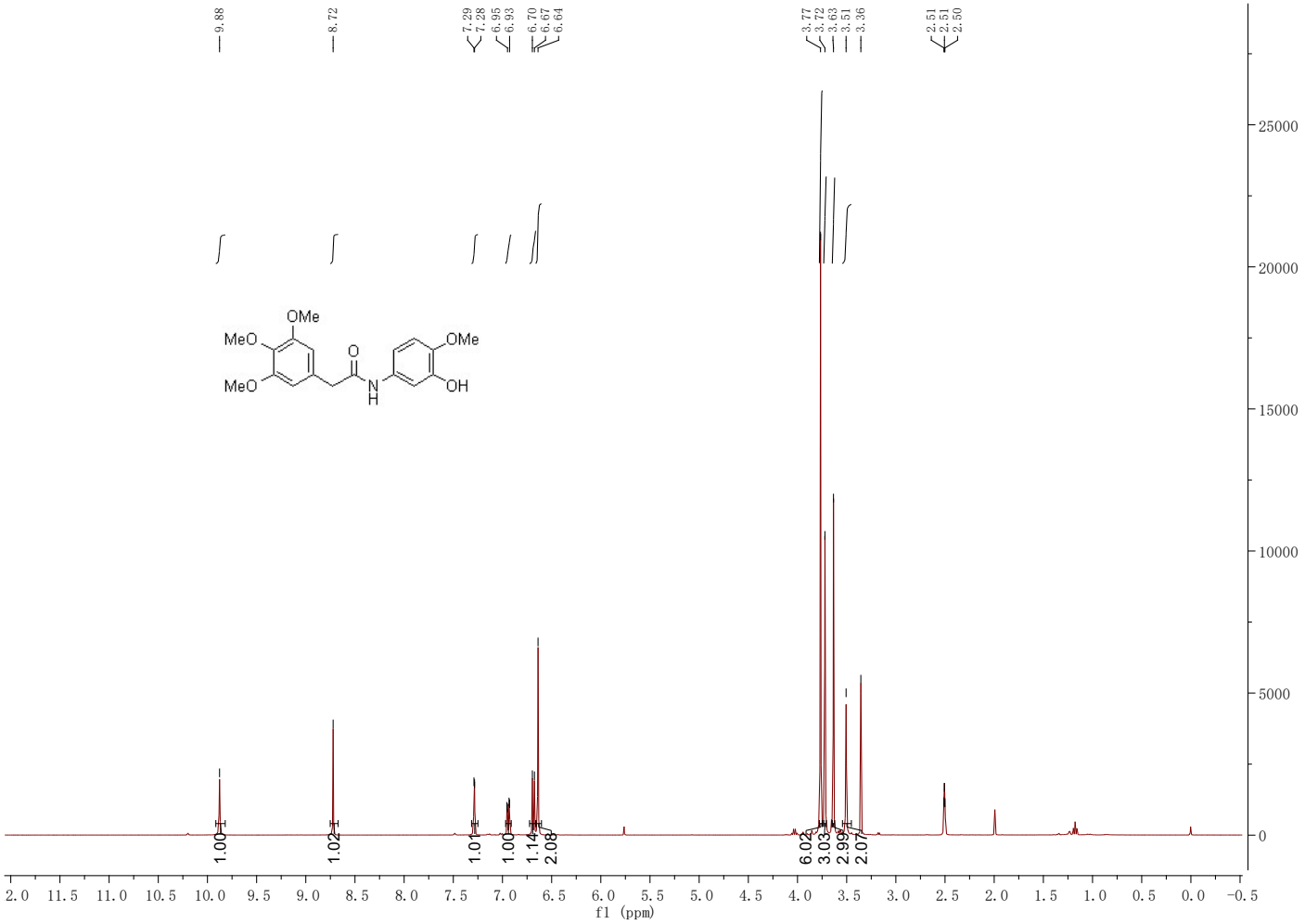


Figure S20. ^{13}C-NMR of Compound 11

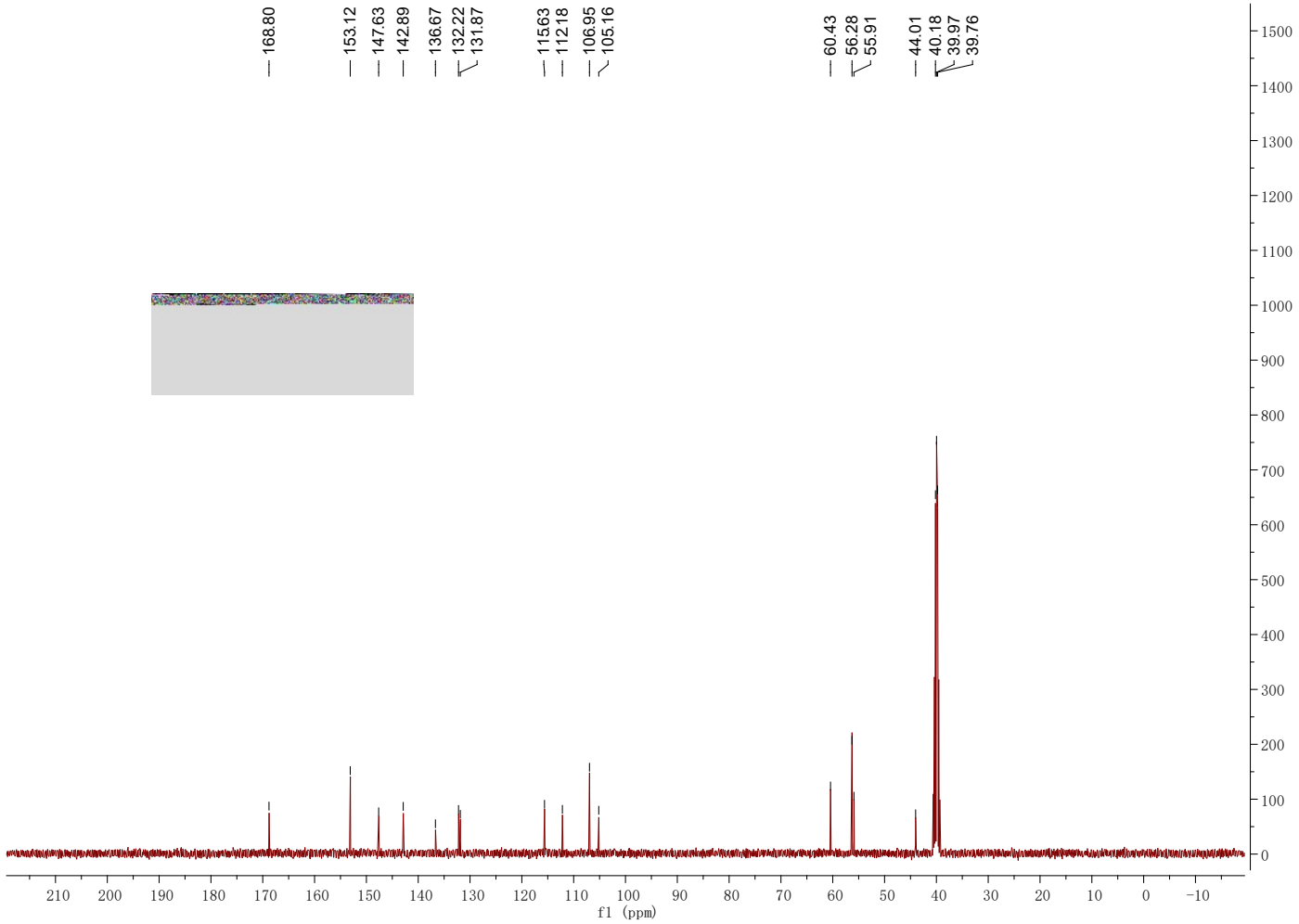


Figure S21. ^{1}H NMR of Compound 12

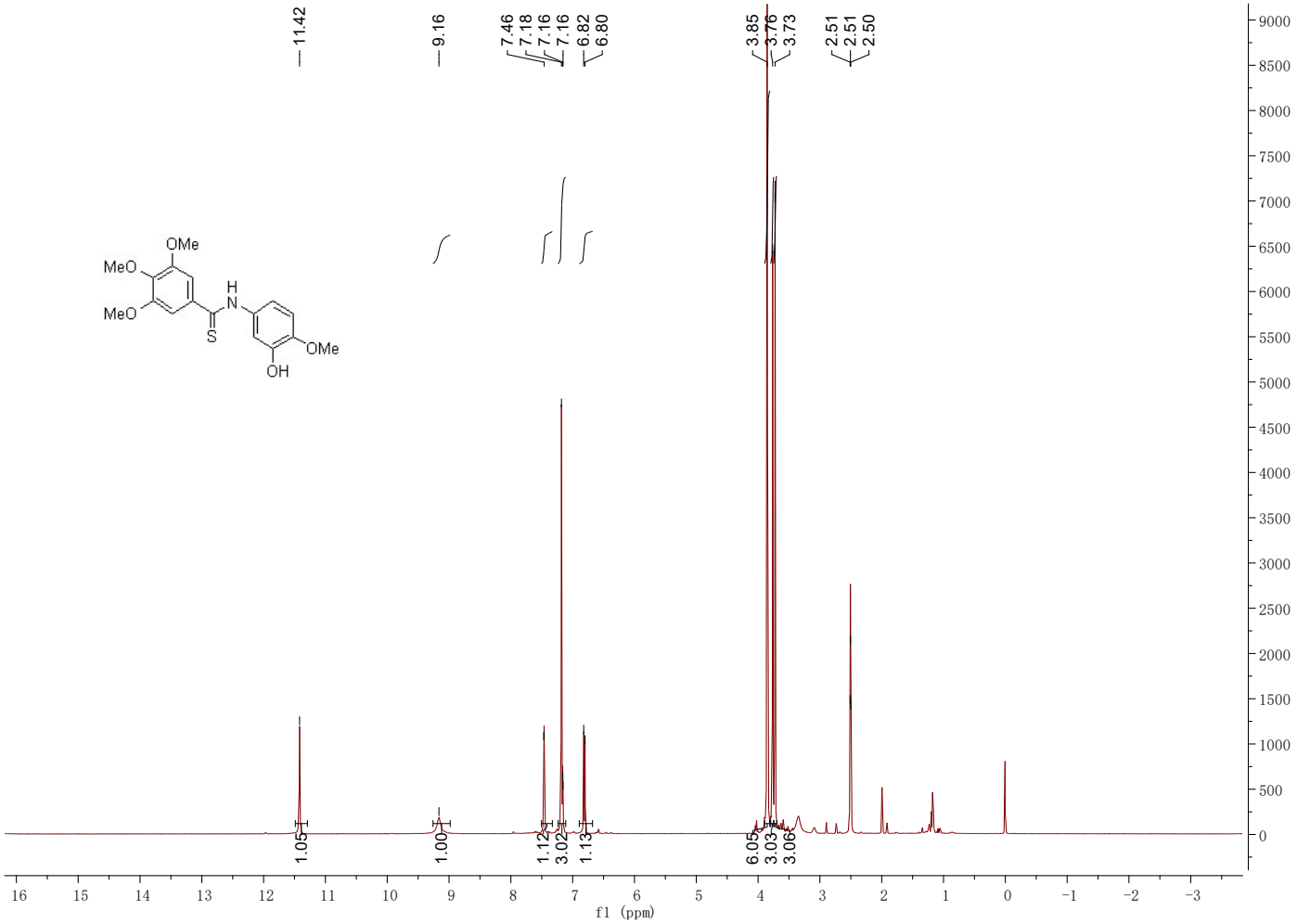


Figure S22. ^{13}C-NMR of Compound 12

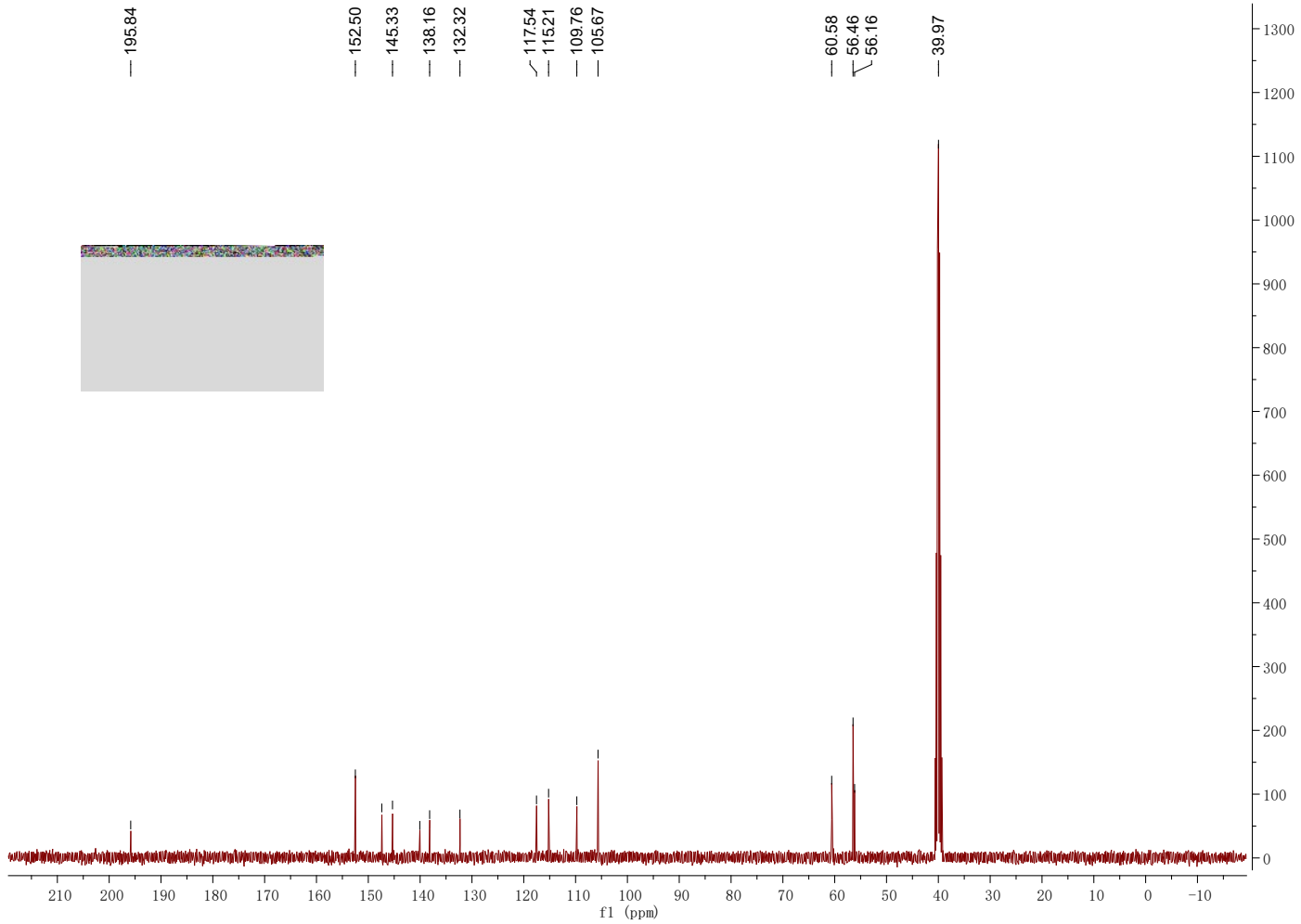


Figure S23. ^{1}H NMR of Compound 13

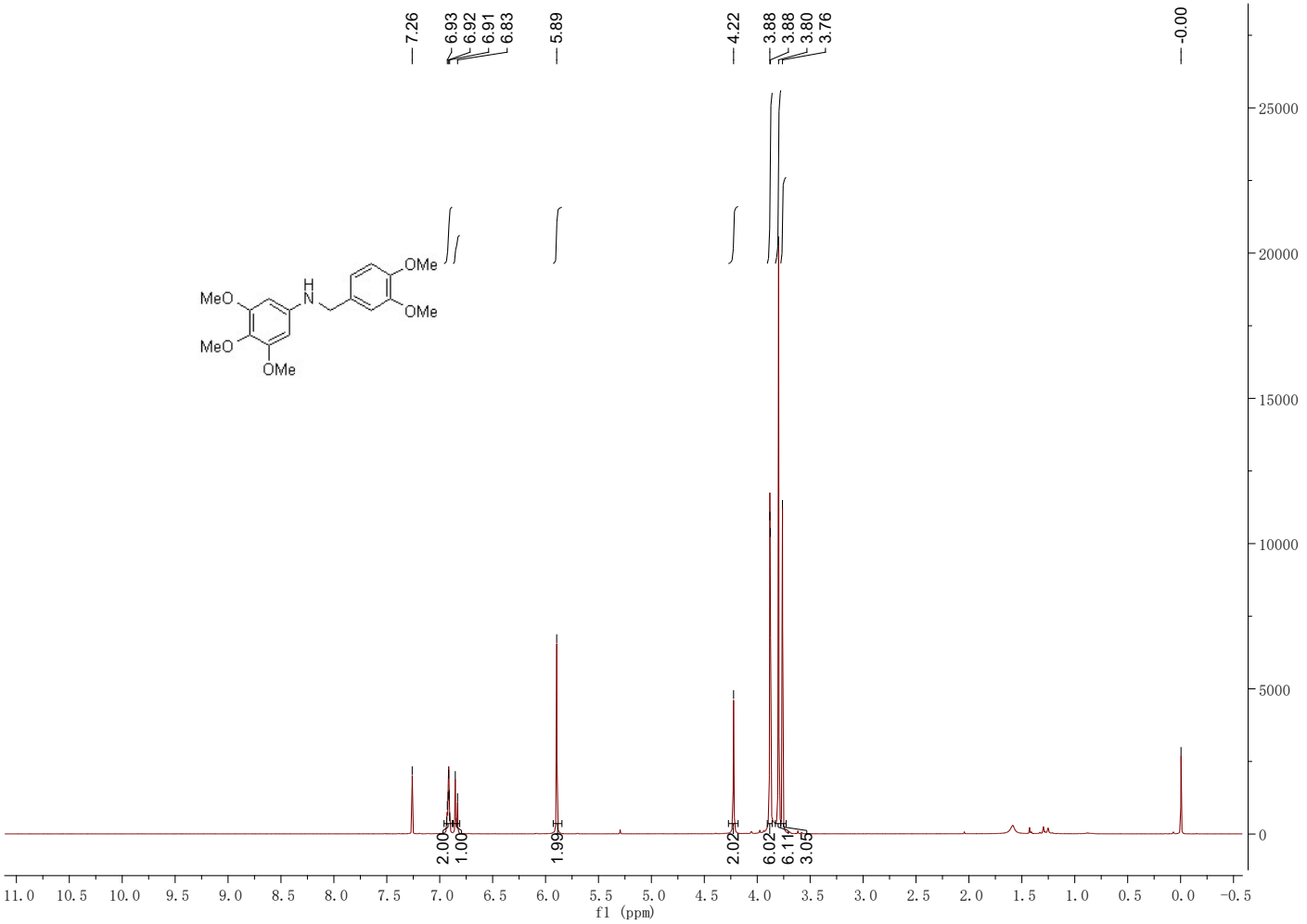


Figure S24. ^{13}C-NMR of Compound 13

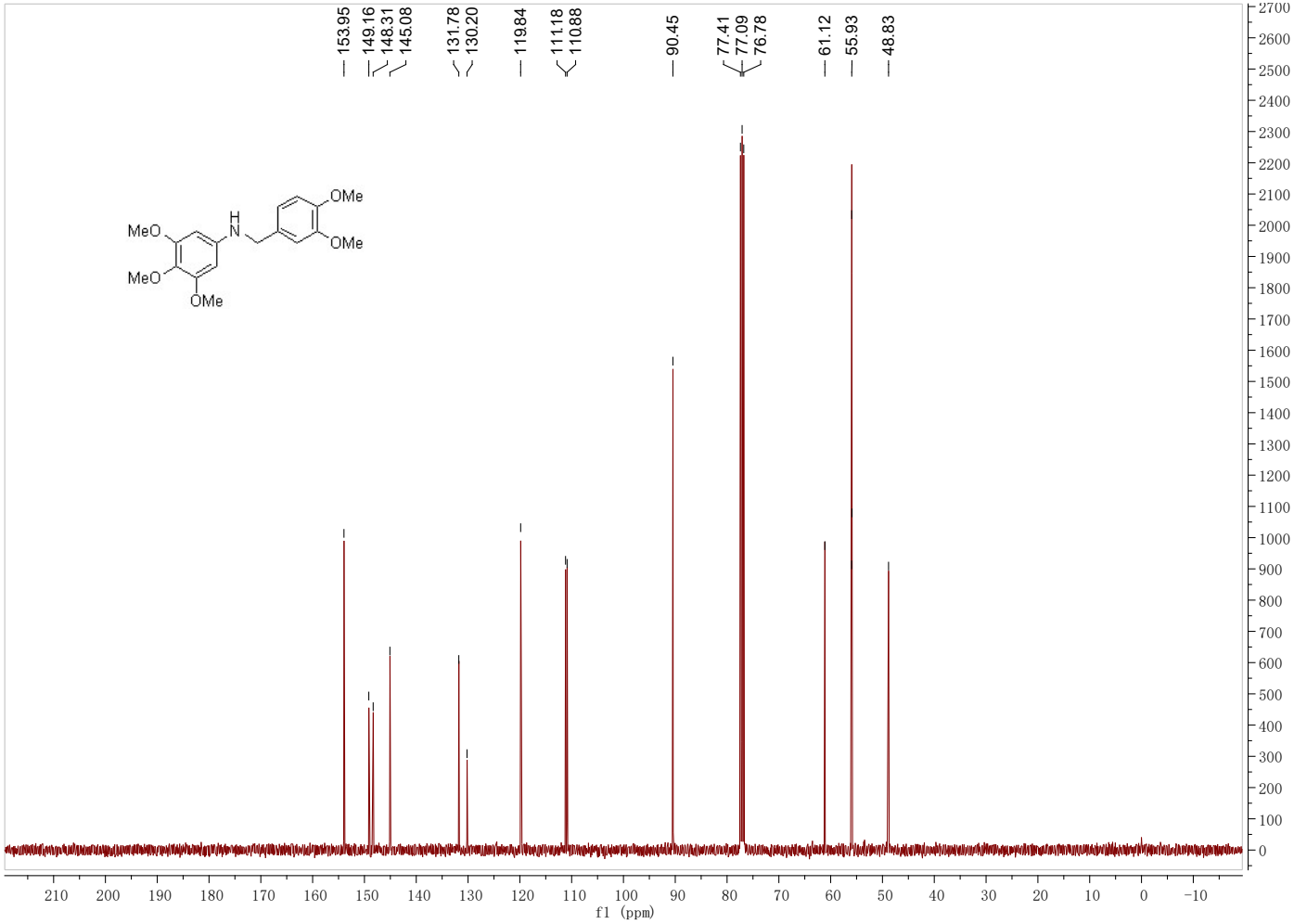


Figure S25. ^{1}H NMR of Compound 15

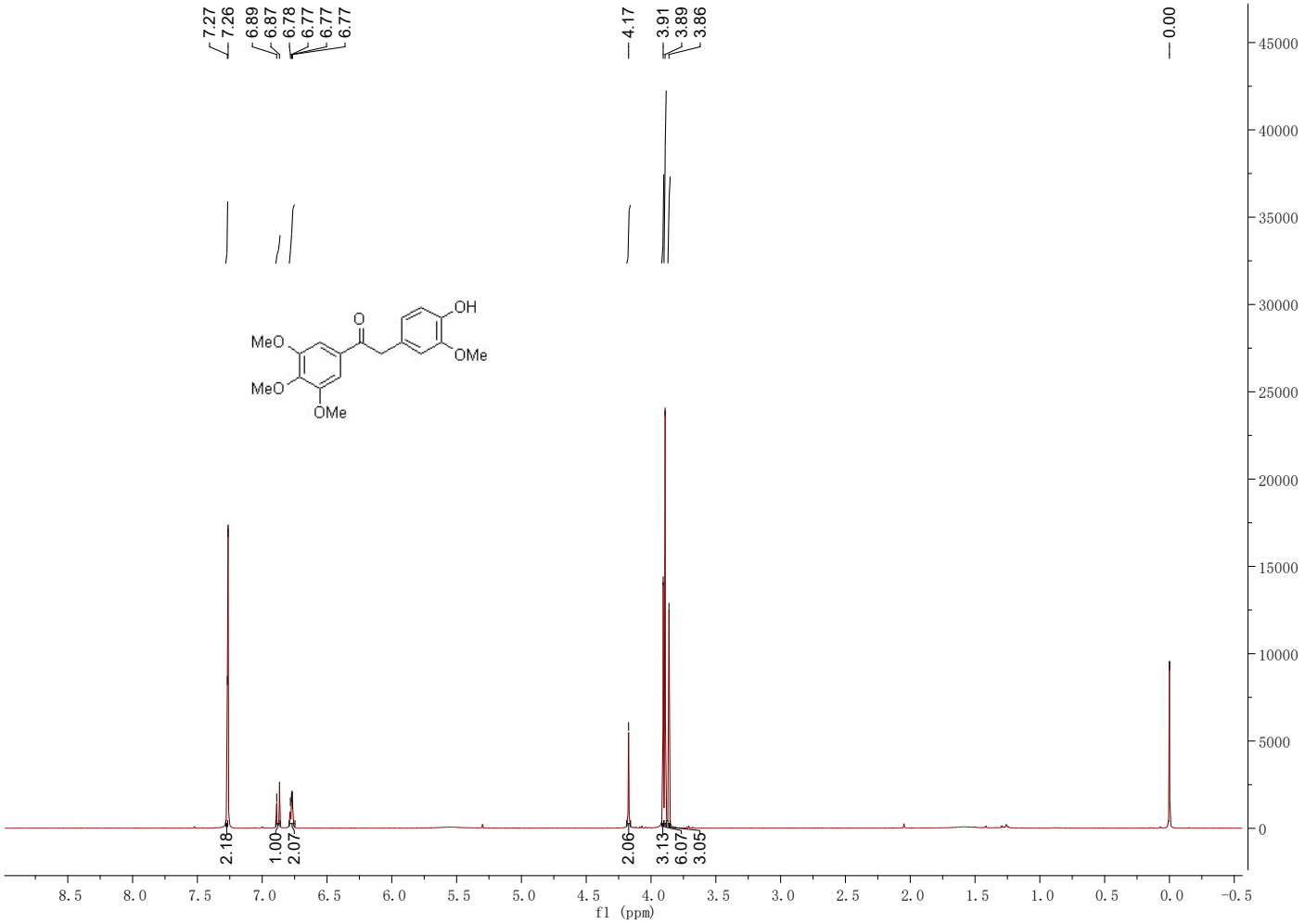


Figure S26. ^{13}C-NMR of Compound 15

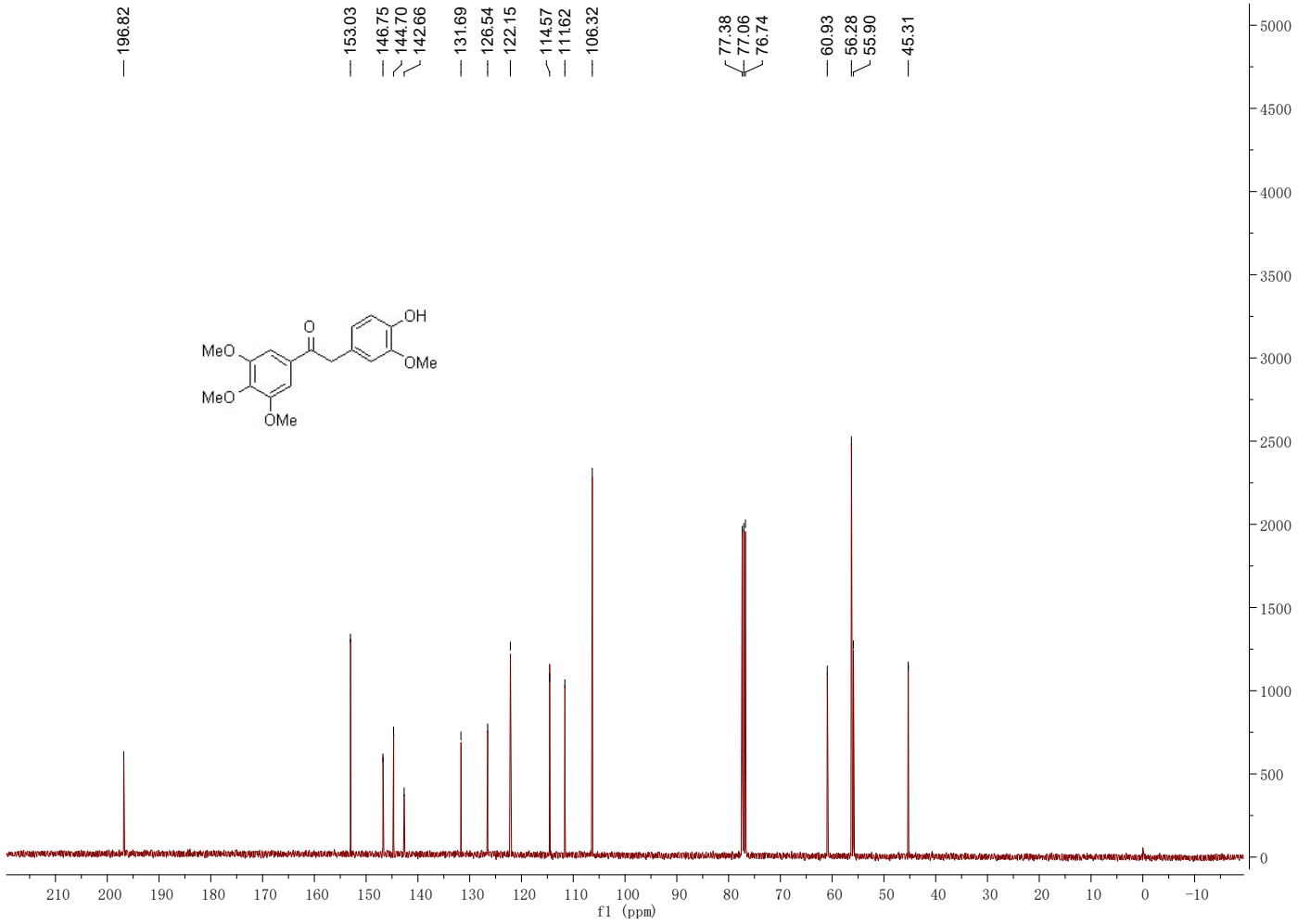


Figure S27. ^{1}H NMR of Compound 16

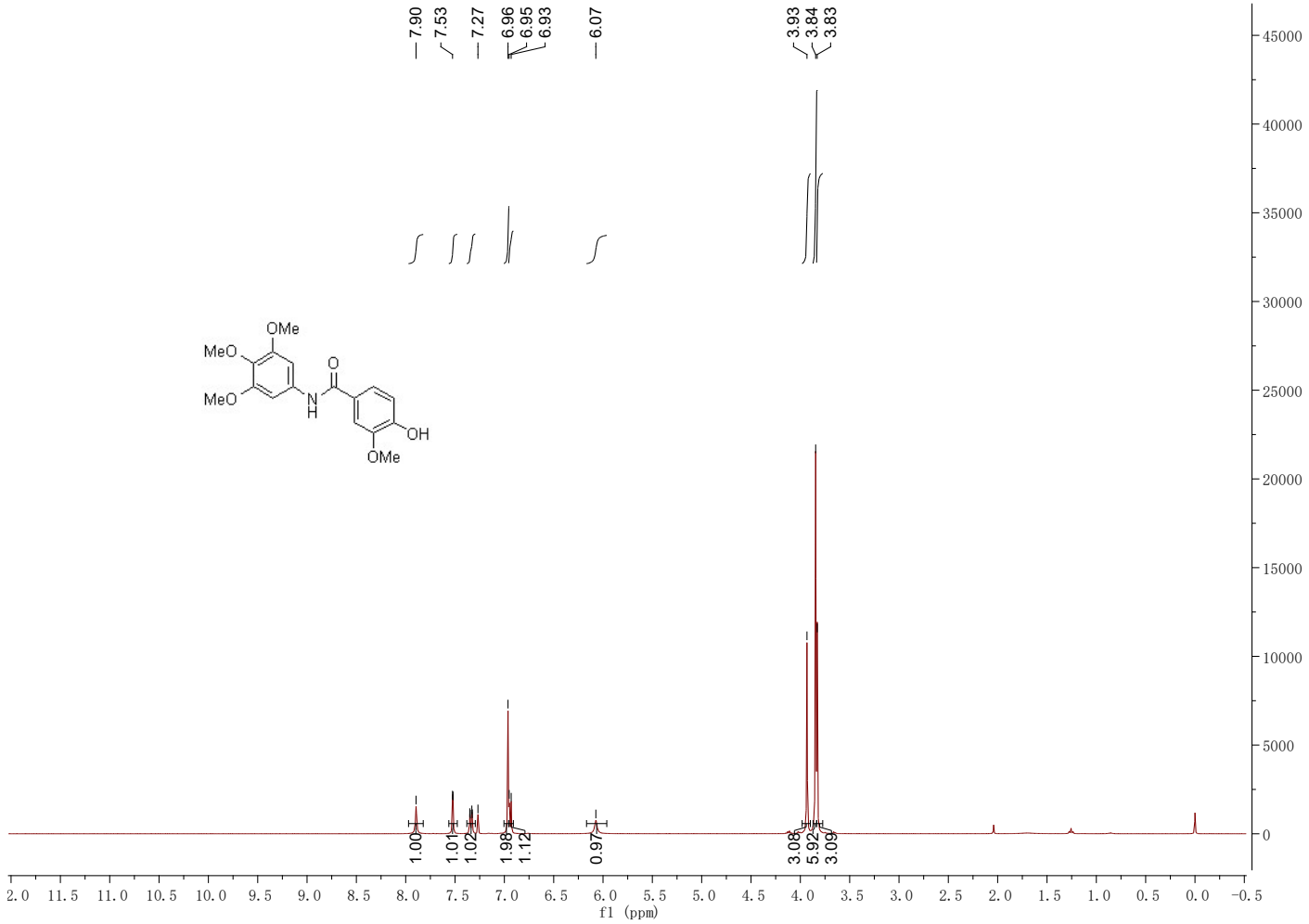


Figure S28. ^{13}C-NMR of Compound 16

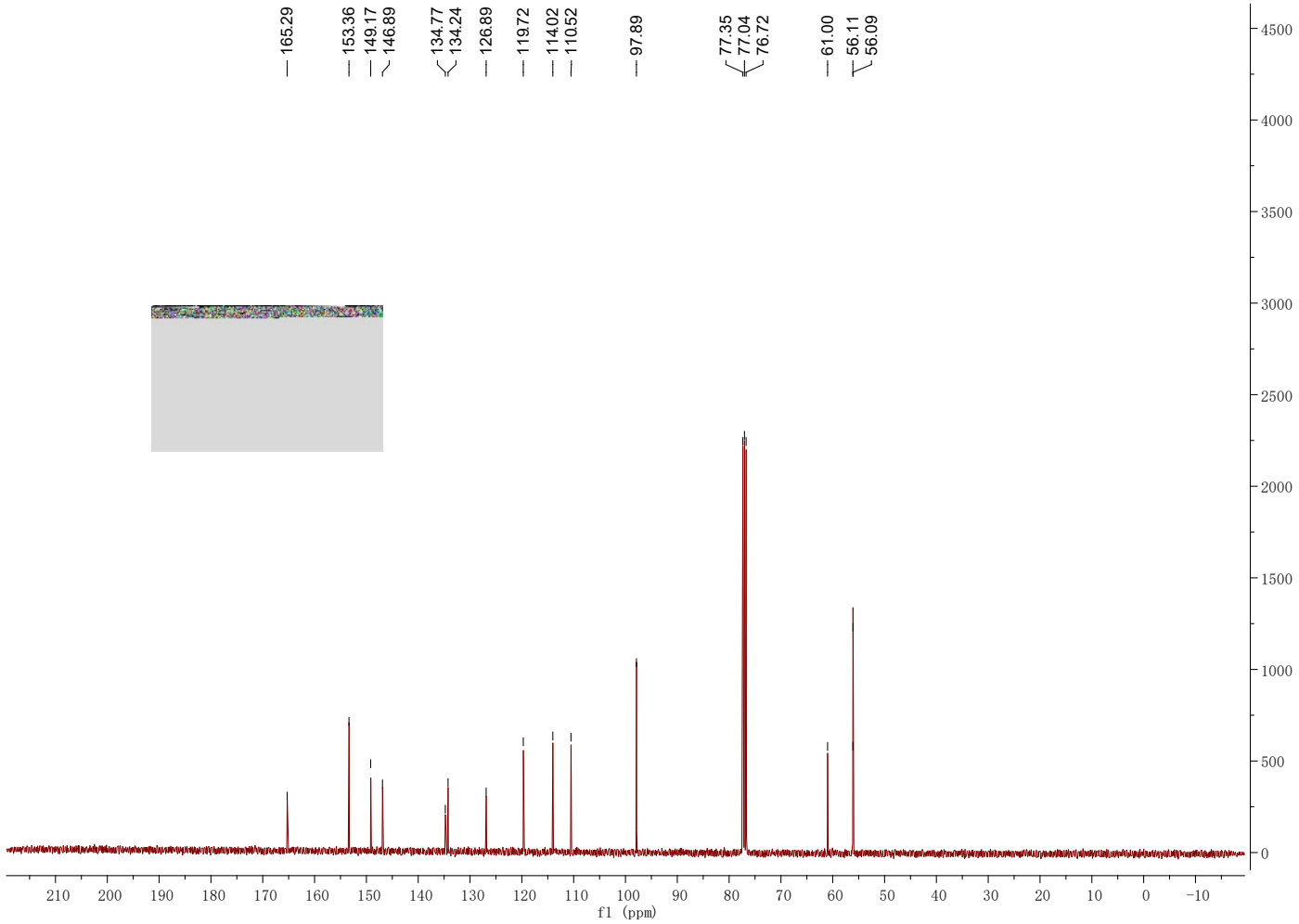


Figure S29. ^{1}H NMR of Compound 17

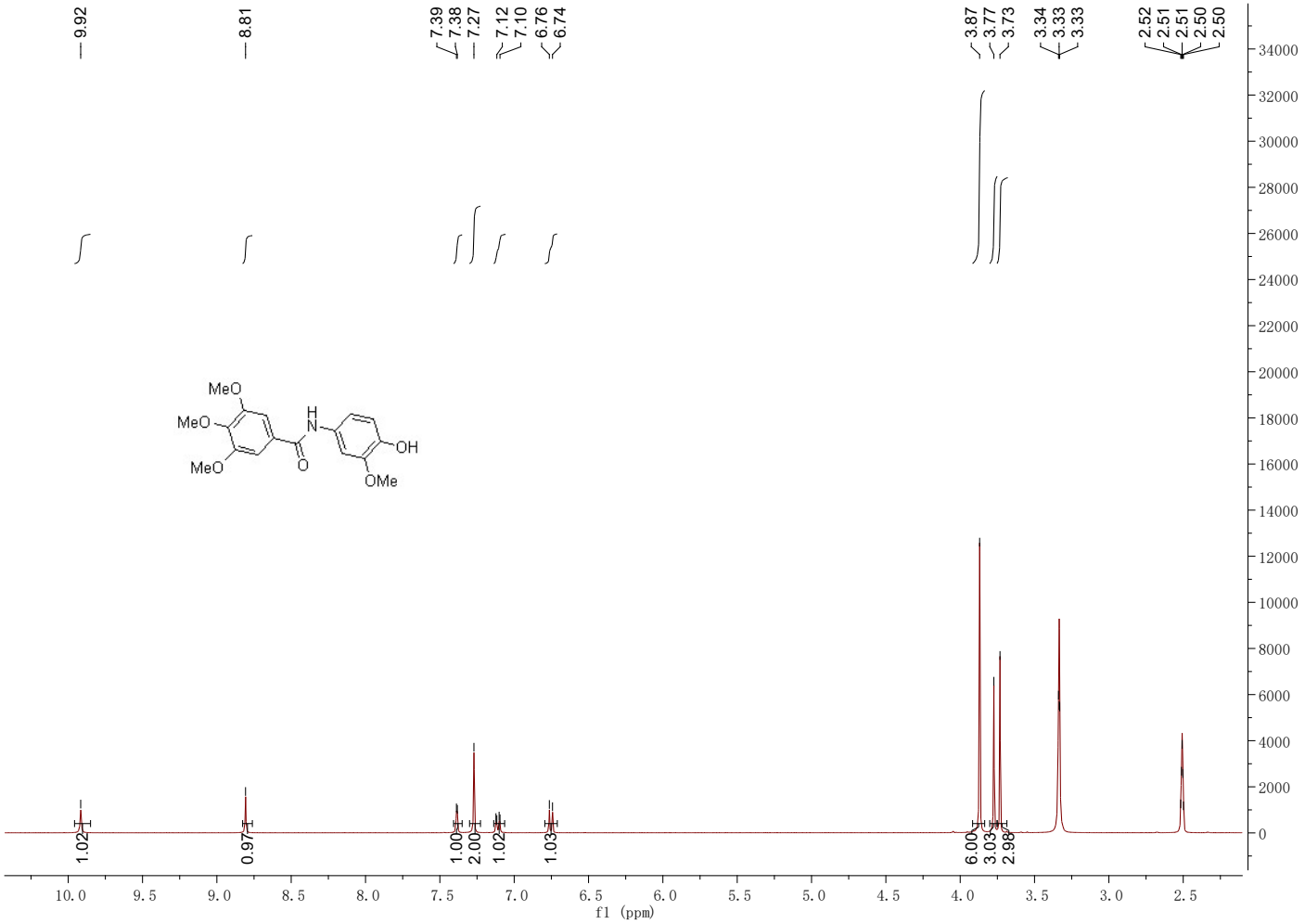


Figure S30. ^{13}C-NMR of Compound 17

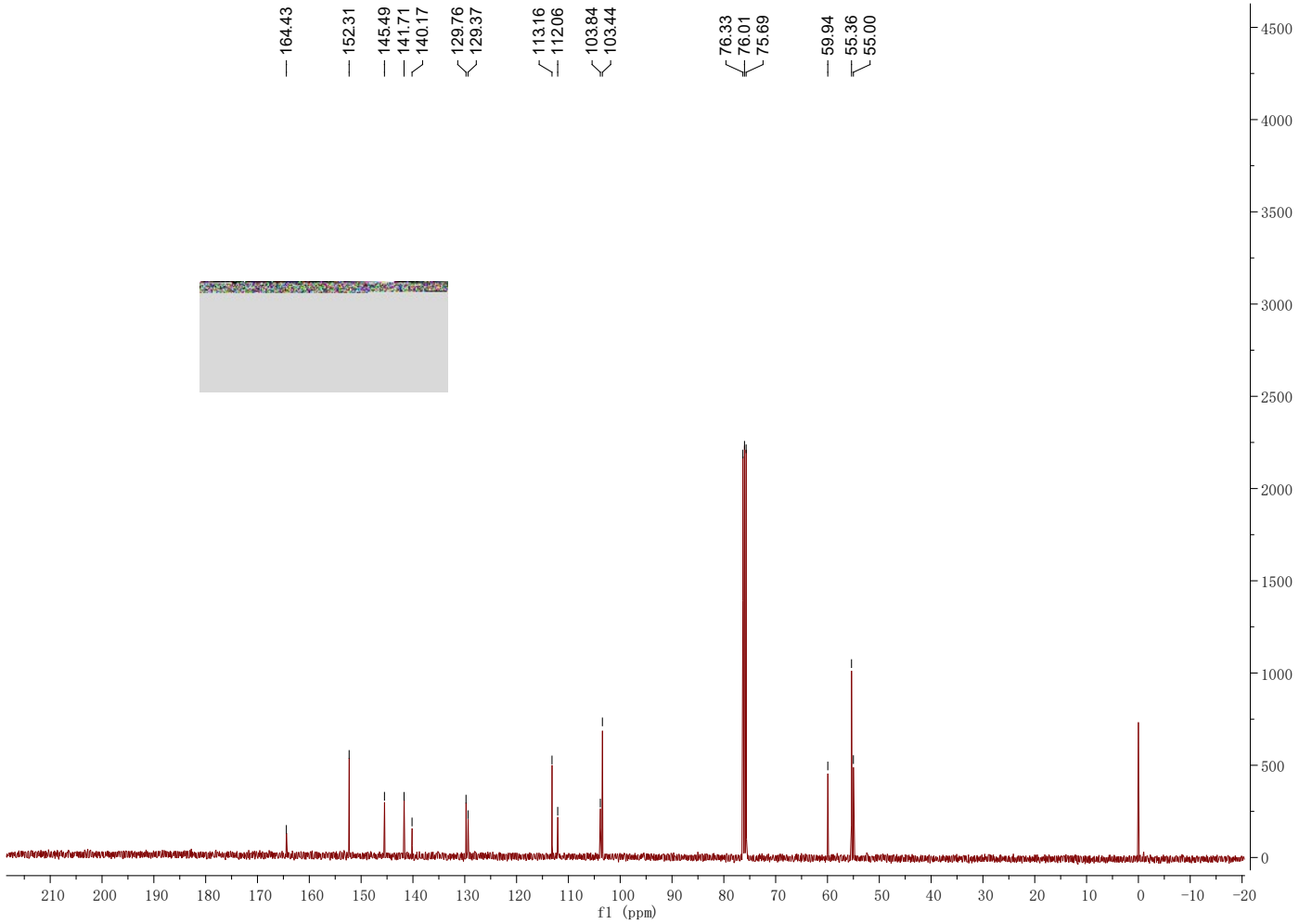


Figure S31. ^{1}H NMR of Compound 18

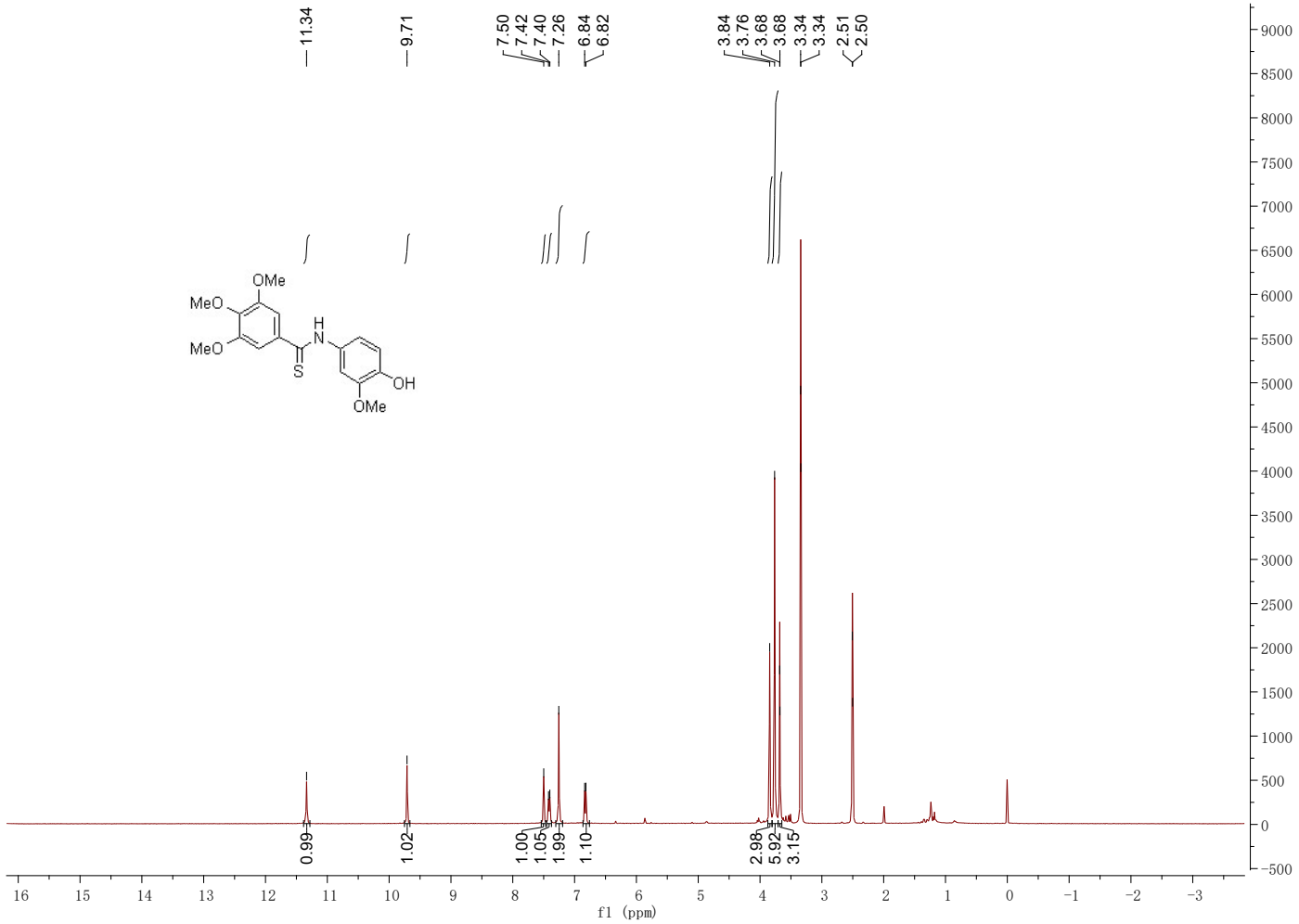


Figure S32. ^{13}C-NMR of Compound 18

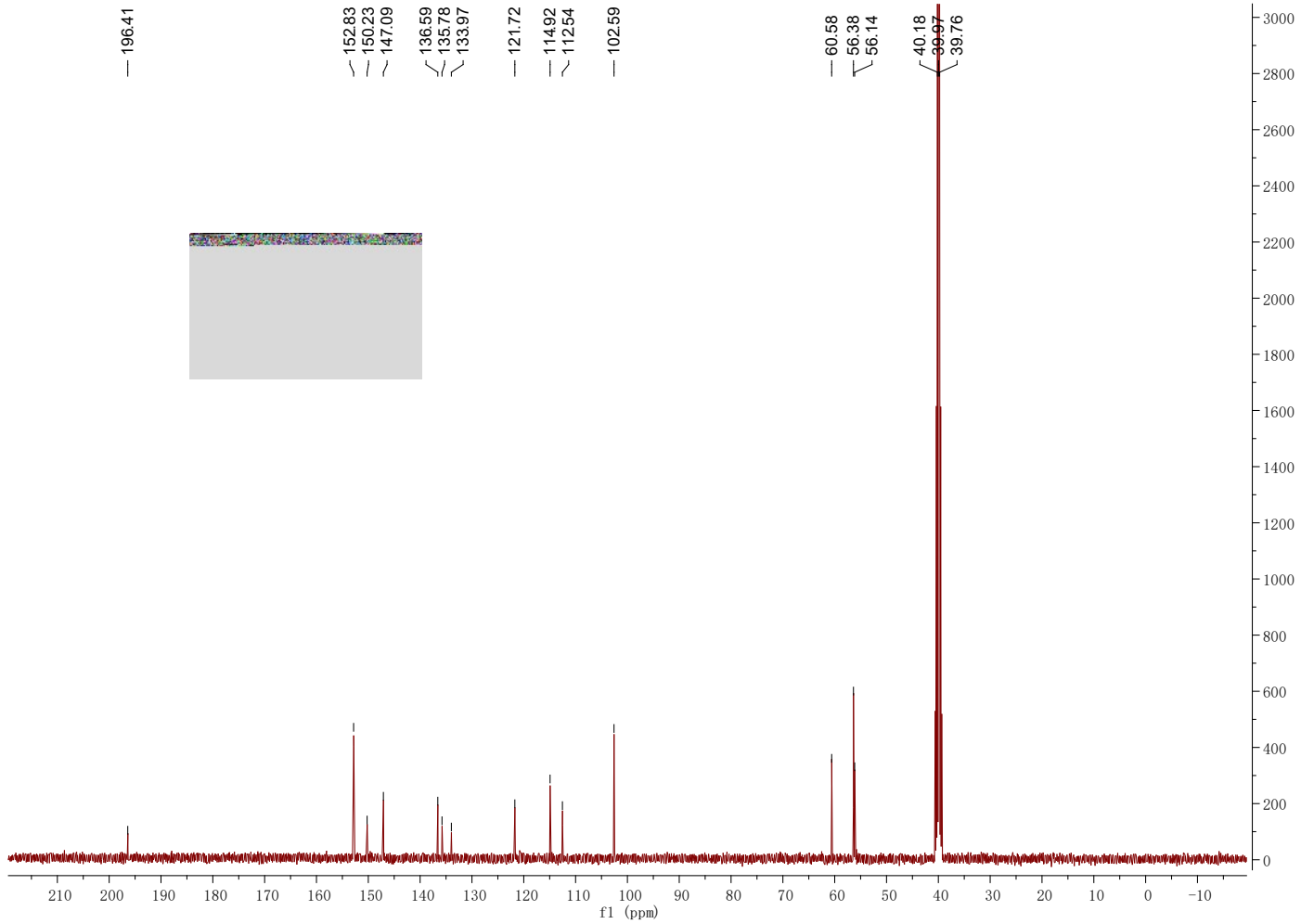


Figure S33. ^{1}H NMR of Compound 19

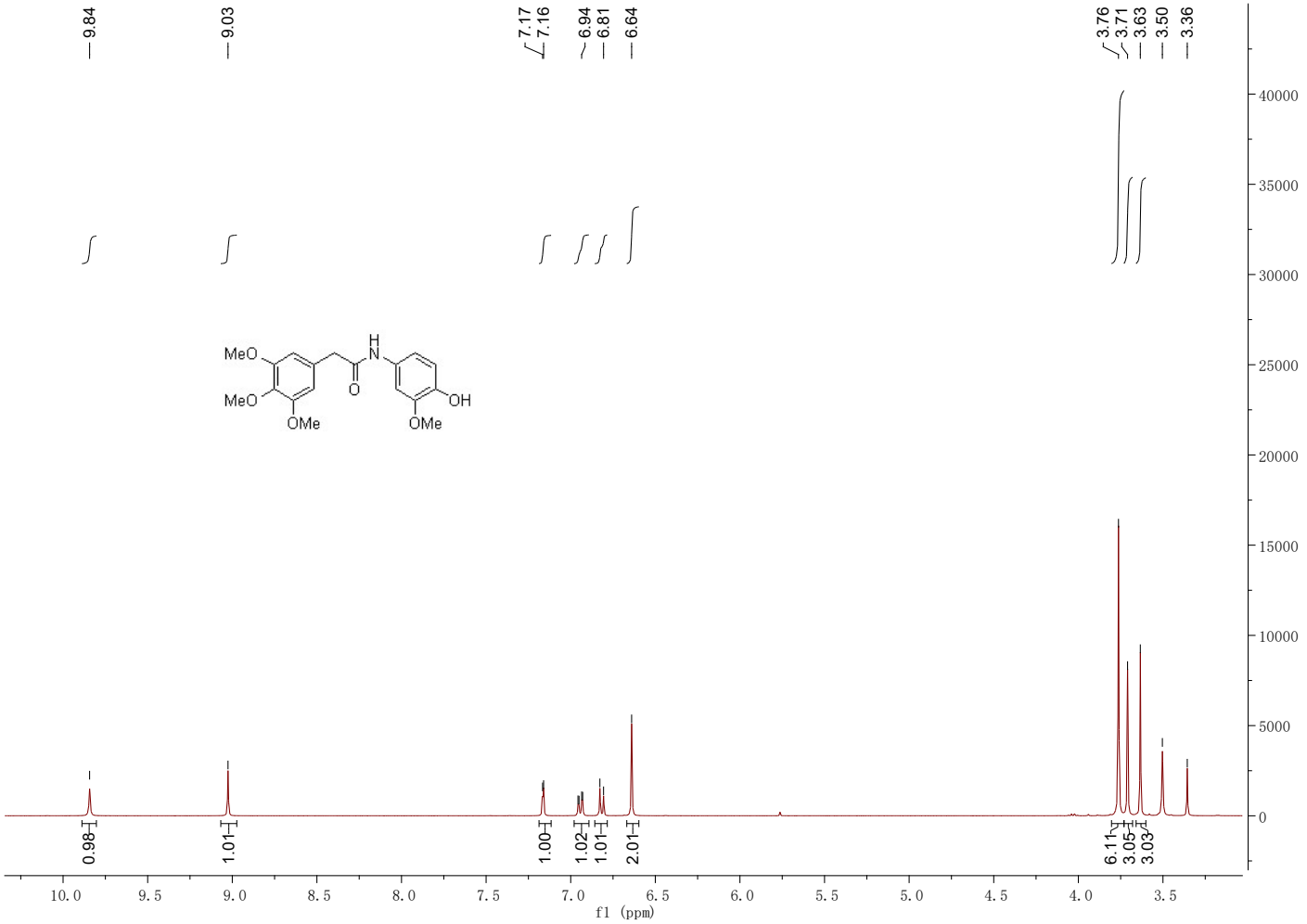


Figure S34. ^{13}C-NMR of Compound 19

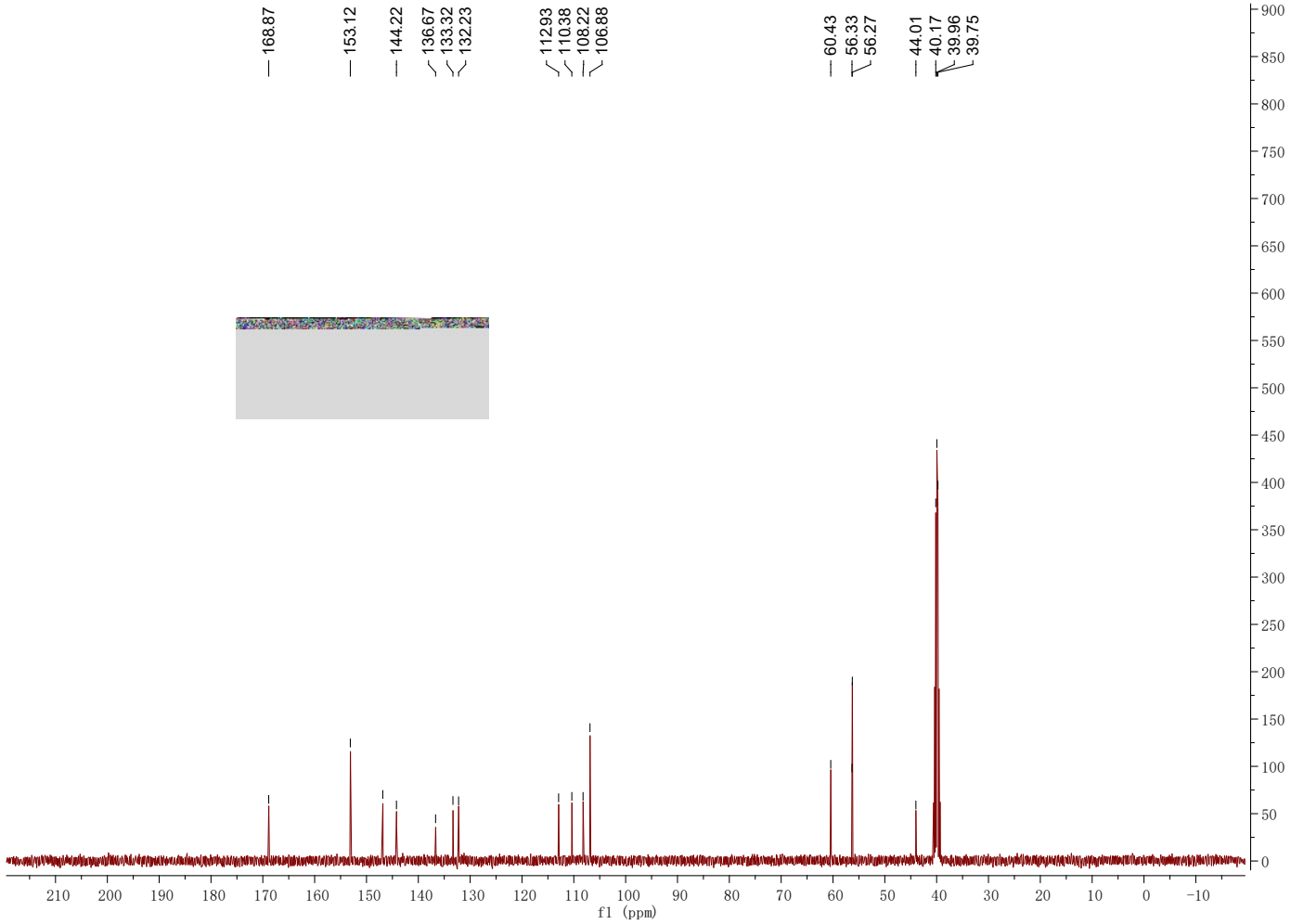


Figure S35. ^{1}H NMR of Compound 20

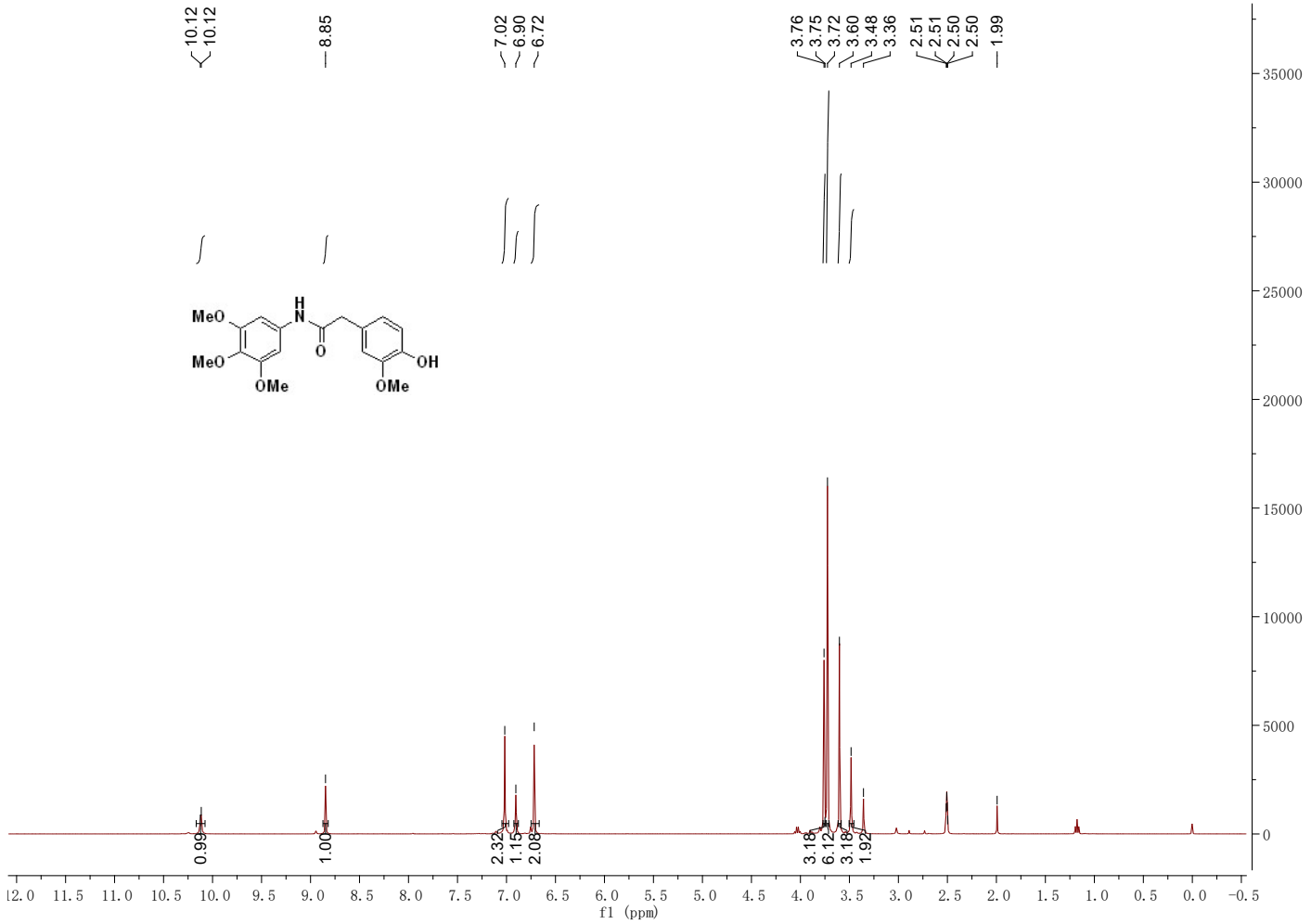


Figure S36. ^{13}C-NMR of Compound 20

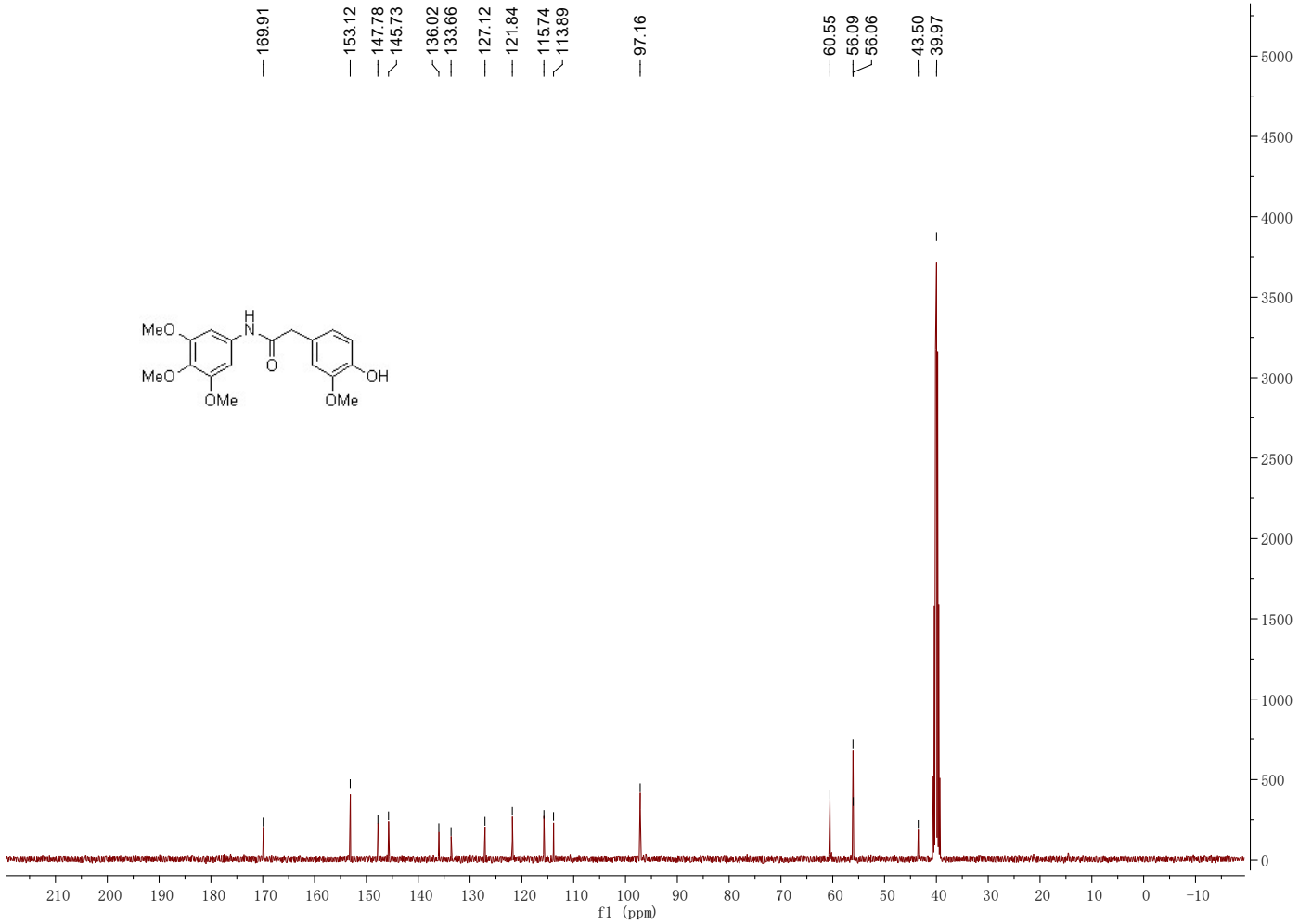


Figure S37. ^{1}H NMR of Compound 22

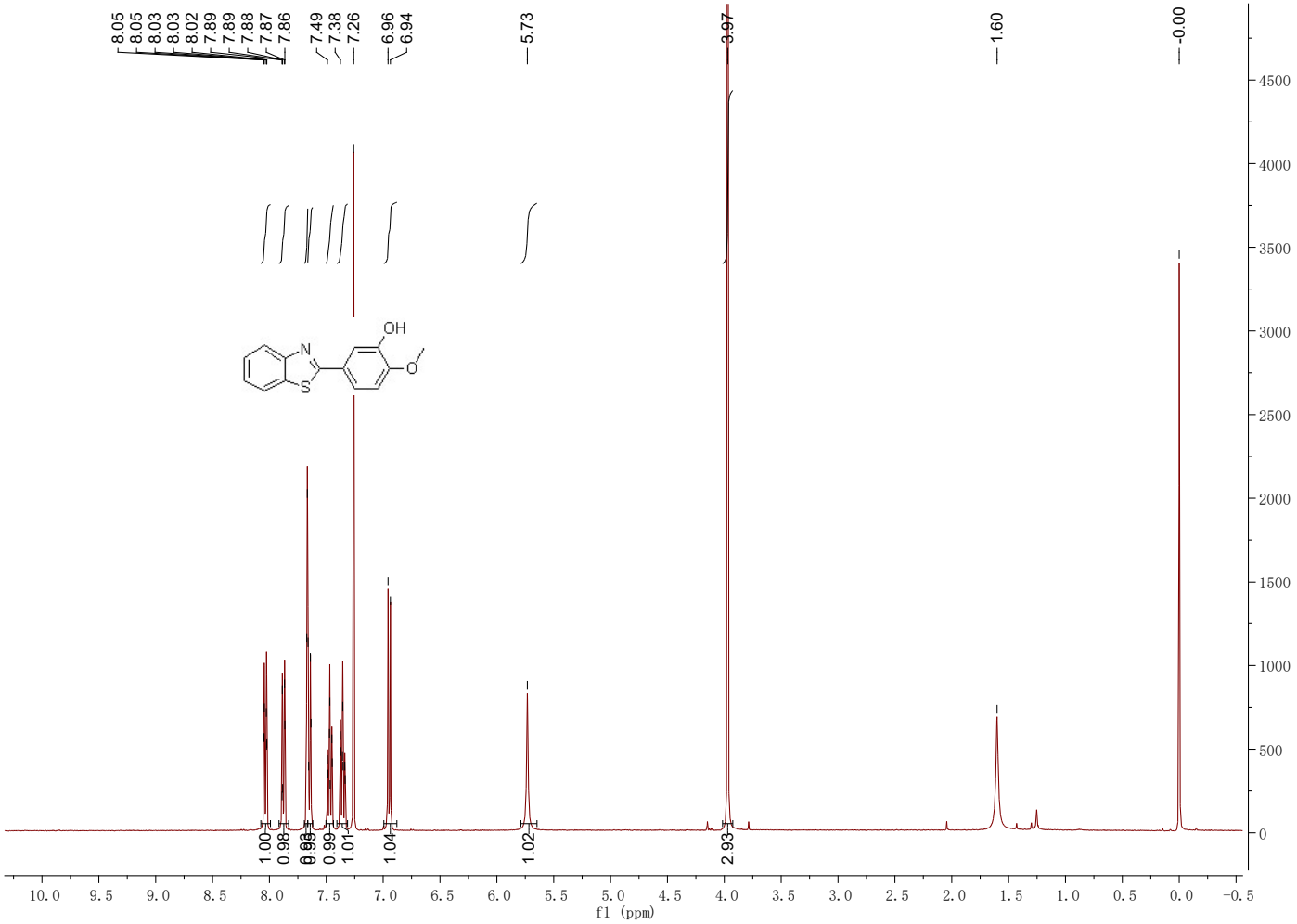


Figure S38. ^{13}C-NMR of Compound 22

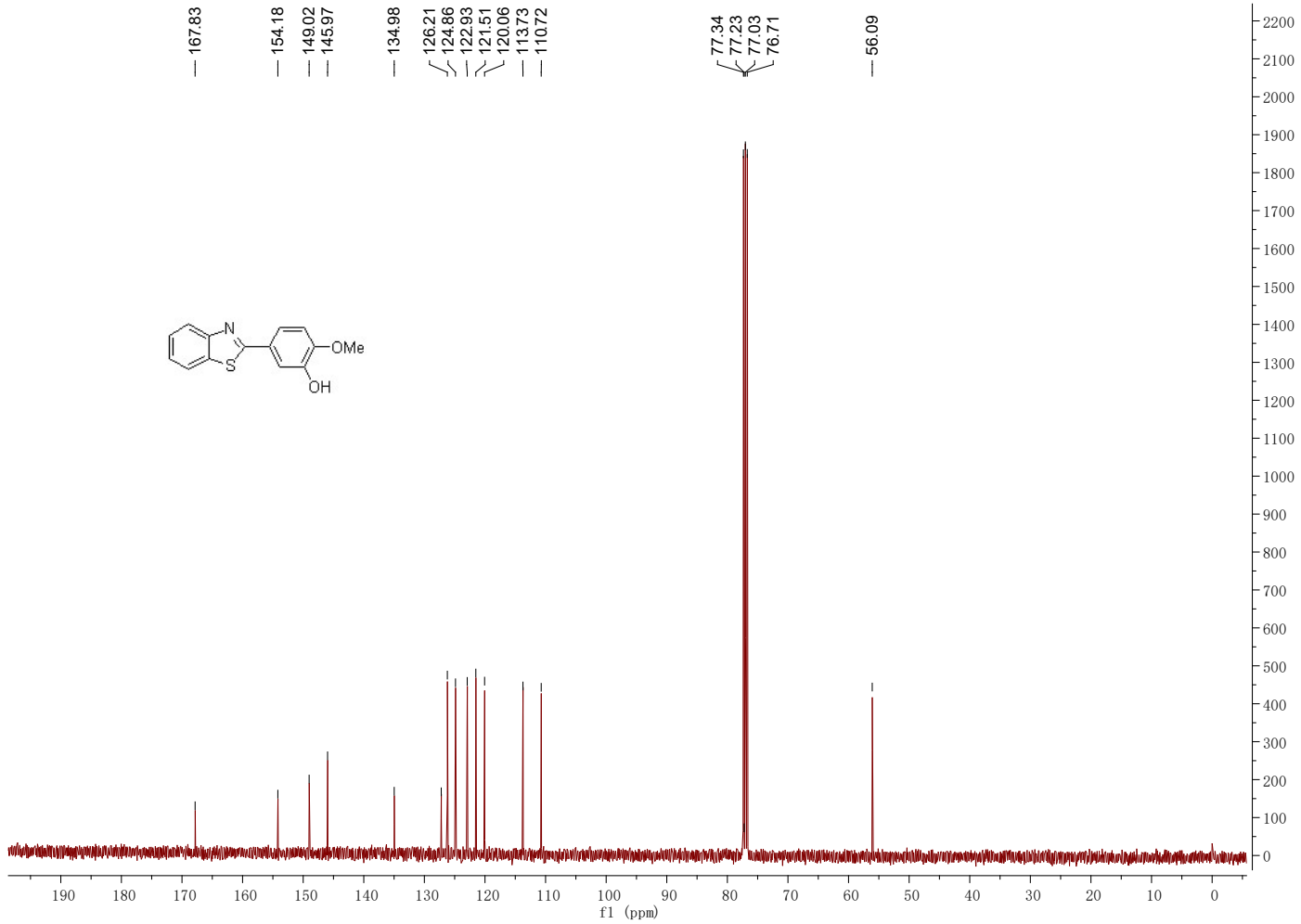


Figure S39. ^{1}H NMR of Compound 23

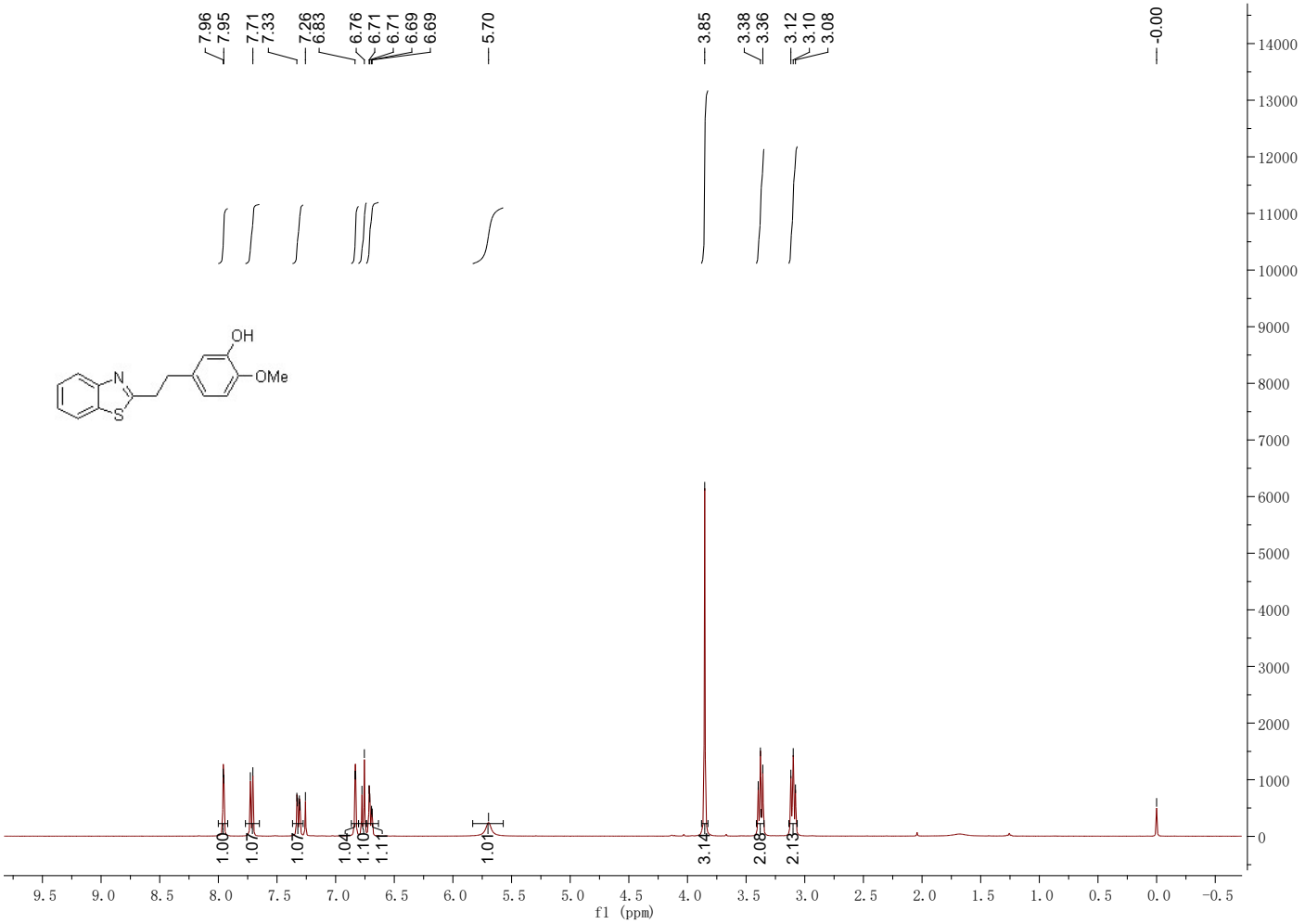


Figure S40. ^{13}C-NMR of Compound 23

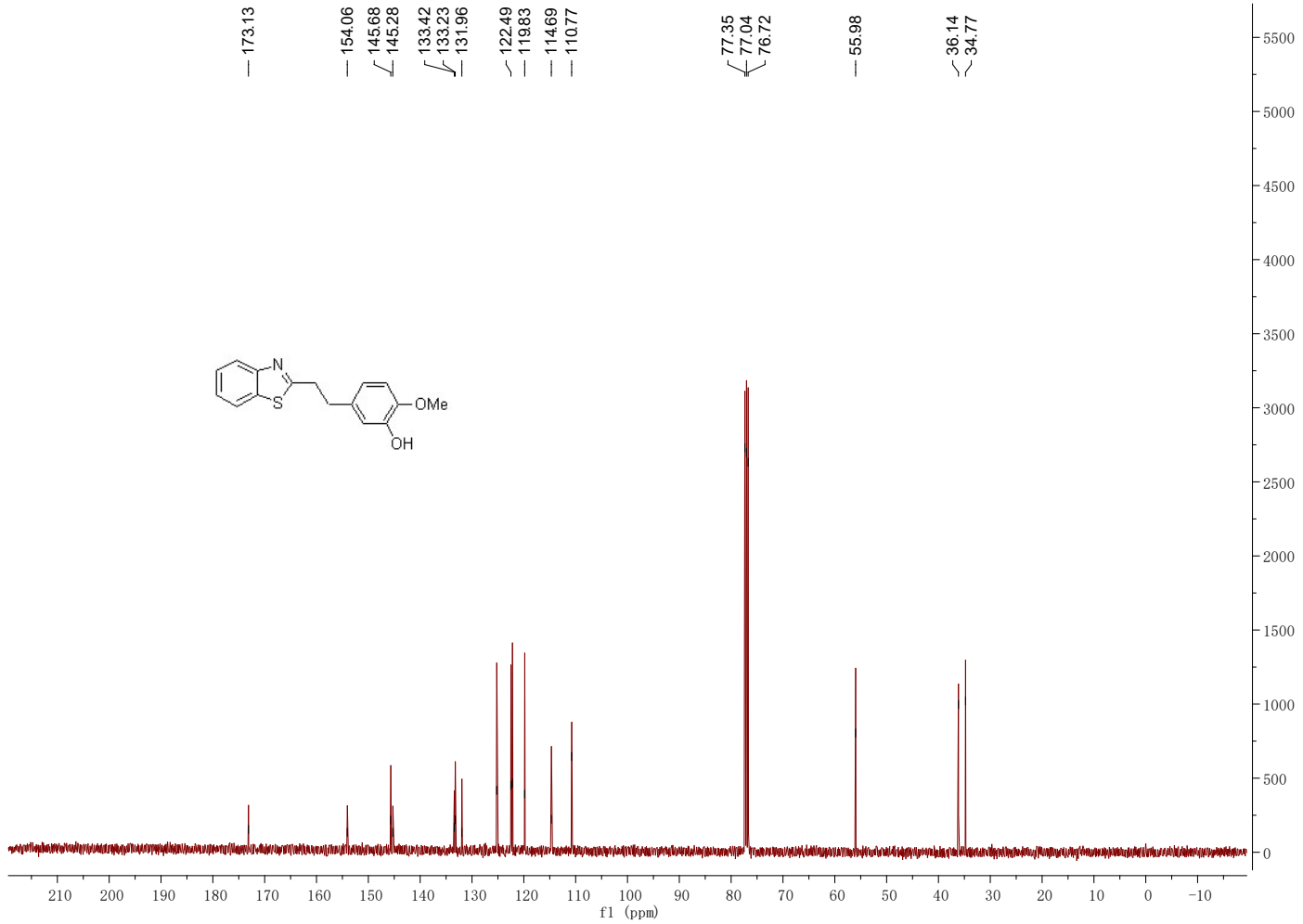


Figure S41. ^{1}H NMR of Compound 24

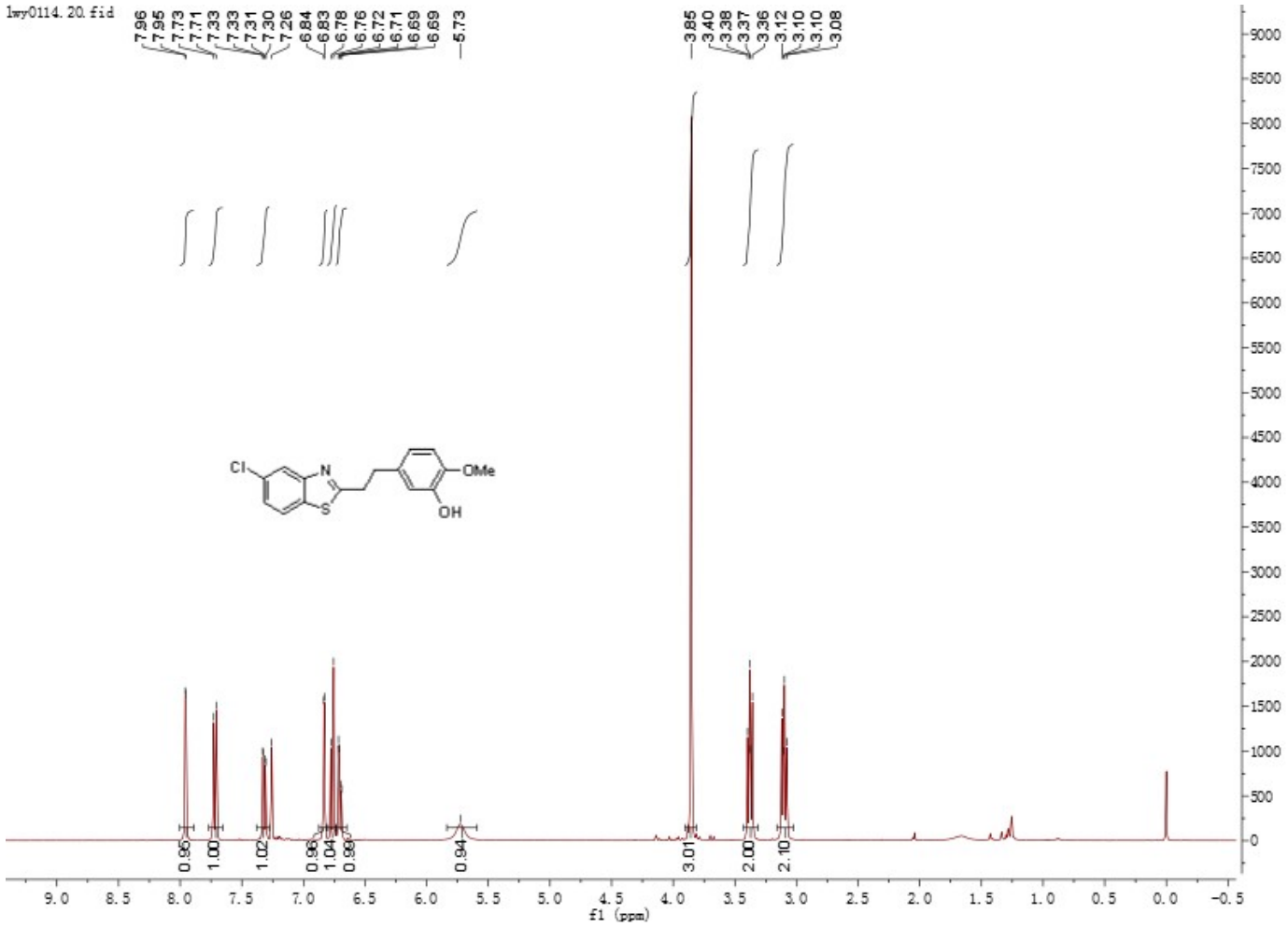


Figure S42. ^{13}C-NMR of Compound 24

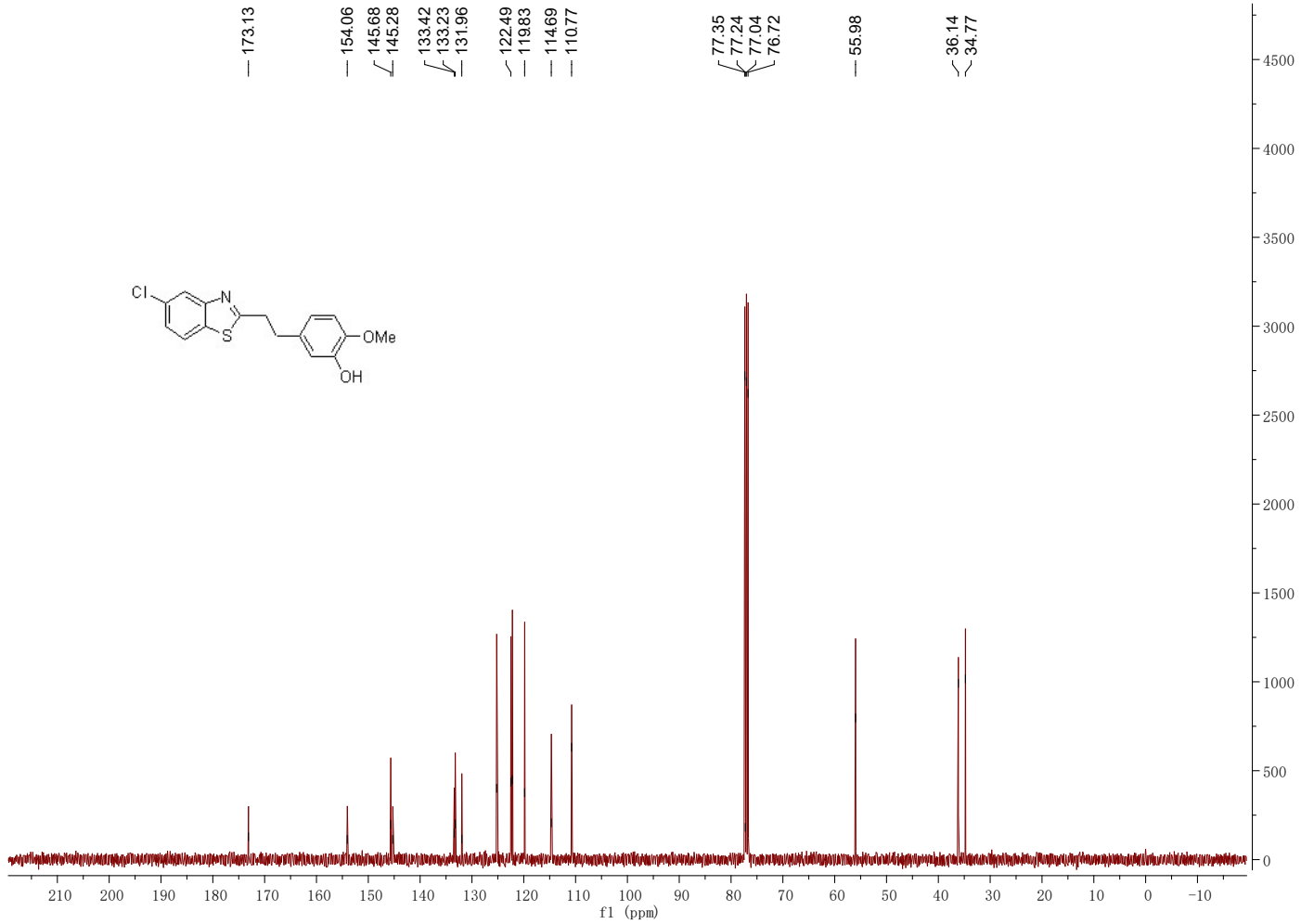


Figure S43. ^{1}H NMR of Compound 25

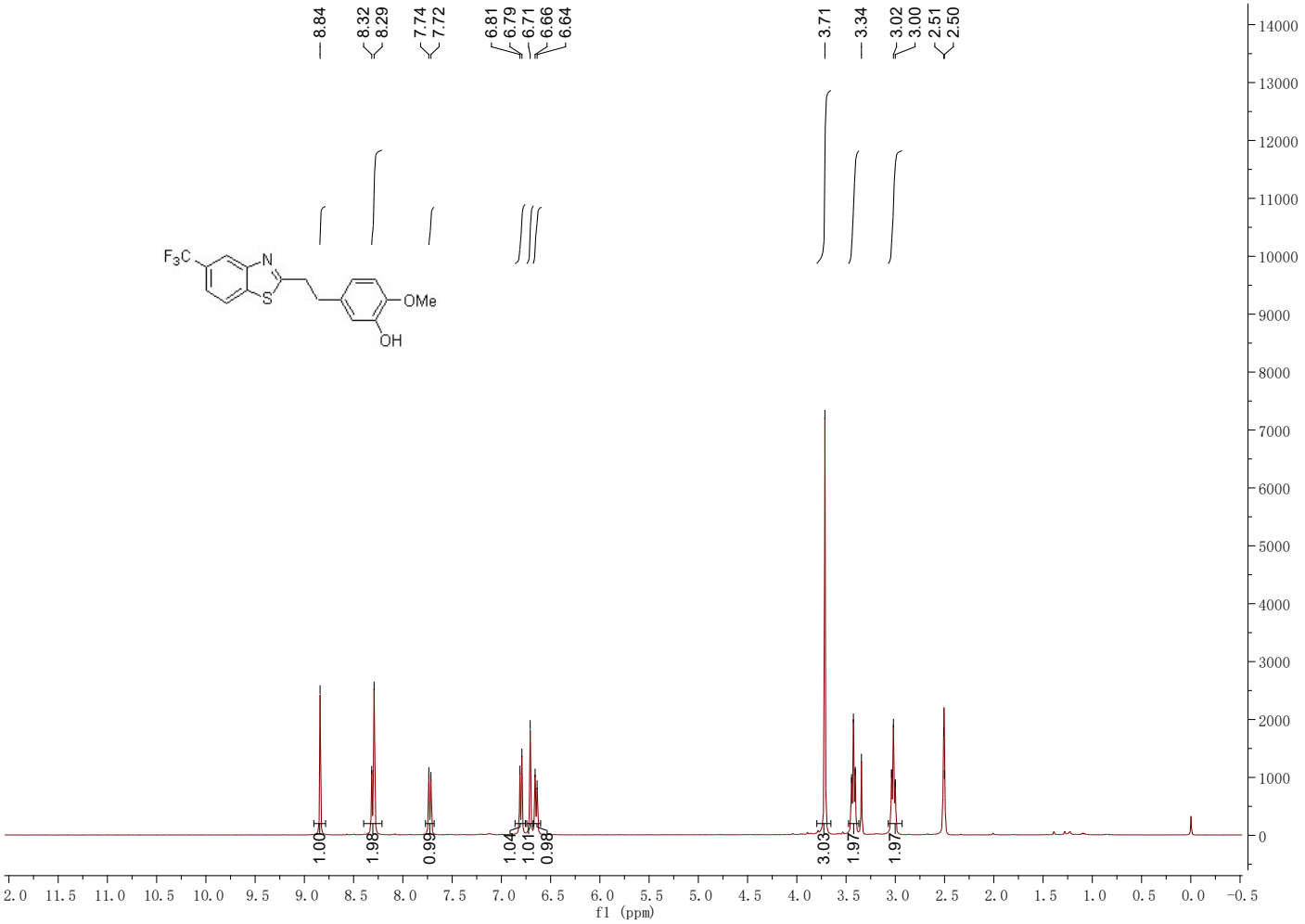


Figure S44. ^{13}C-NMR of Compound 25

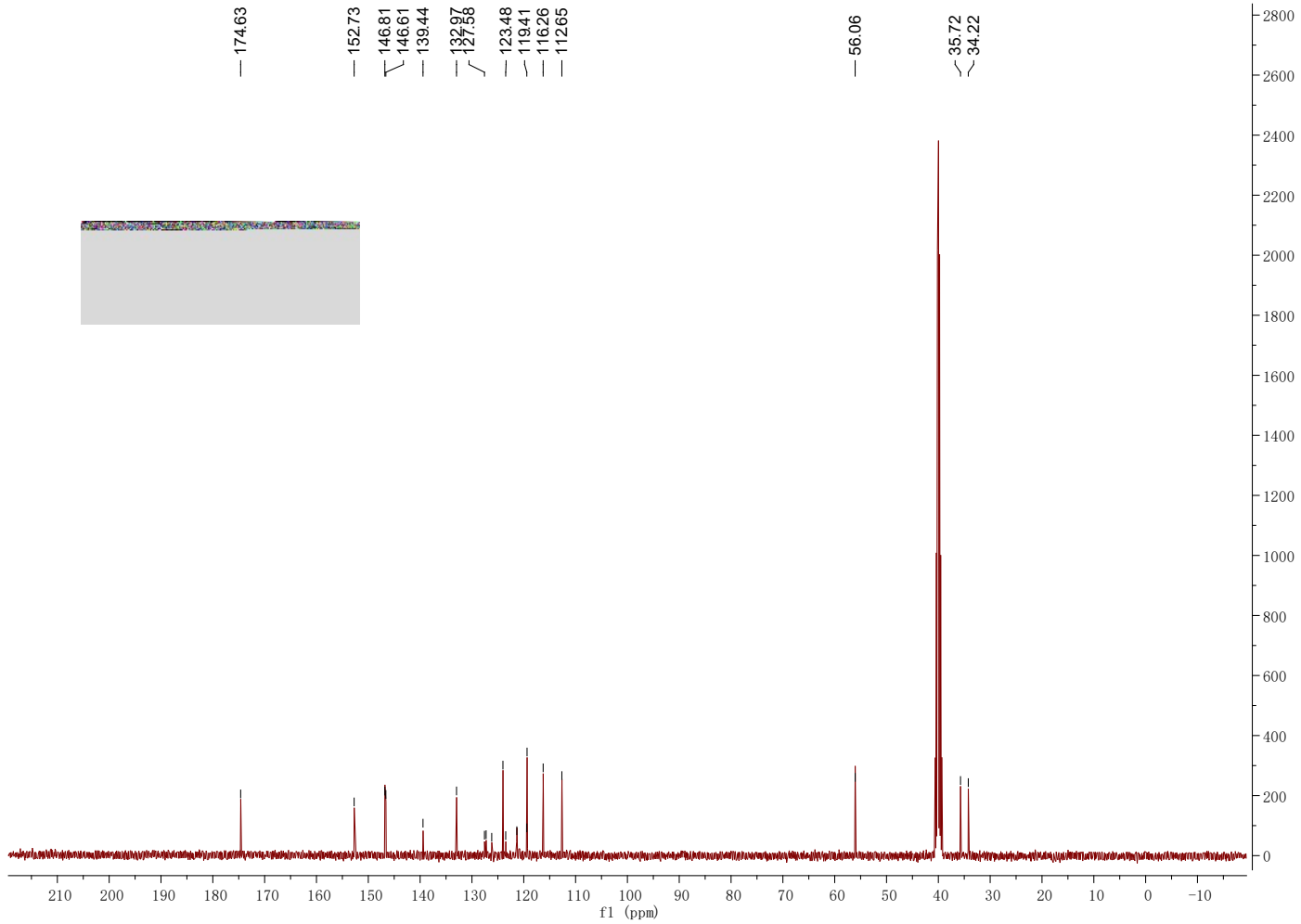


Figure S45 ^{1}H NMR of Compound 26

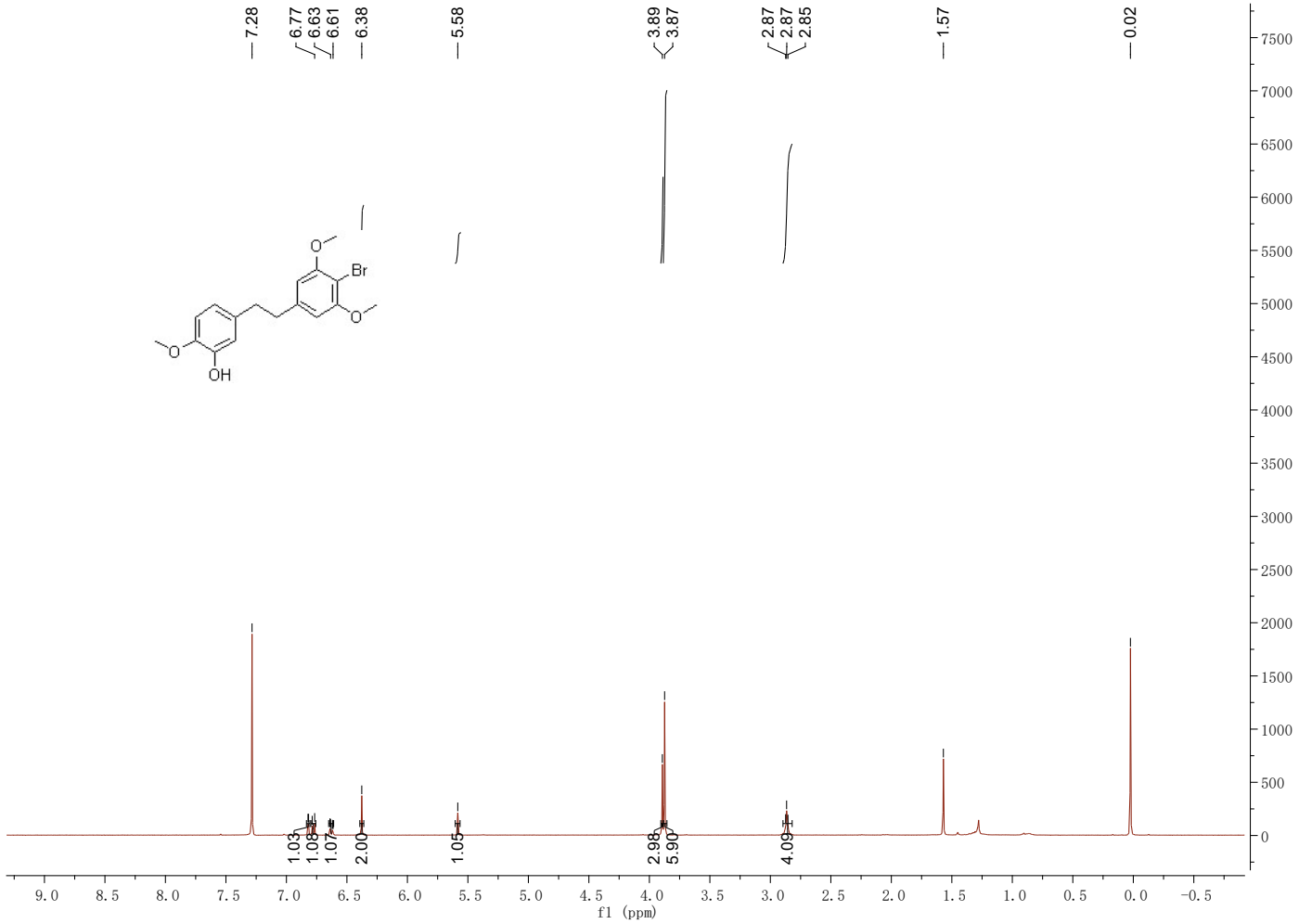


Figure S46. ^{13}C-NMR of Compound 26

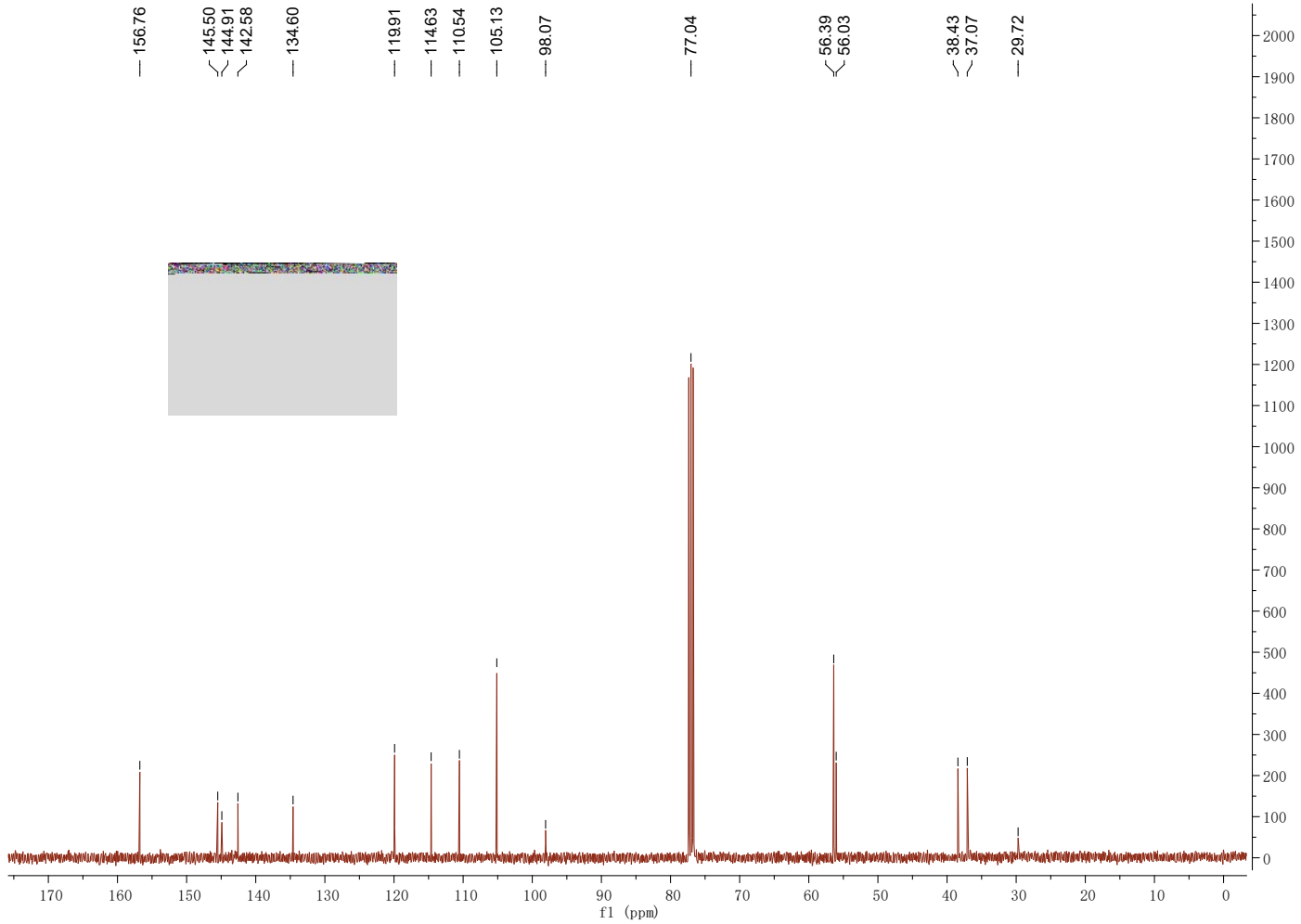


Figure S47. ^{1}H NMR of Compound 27

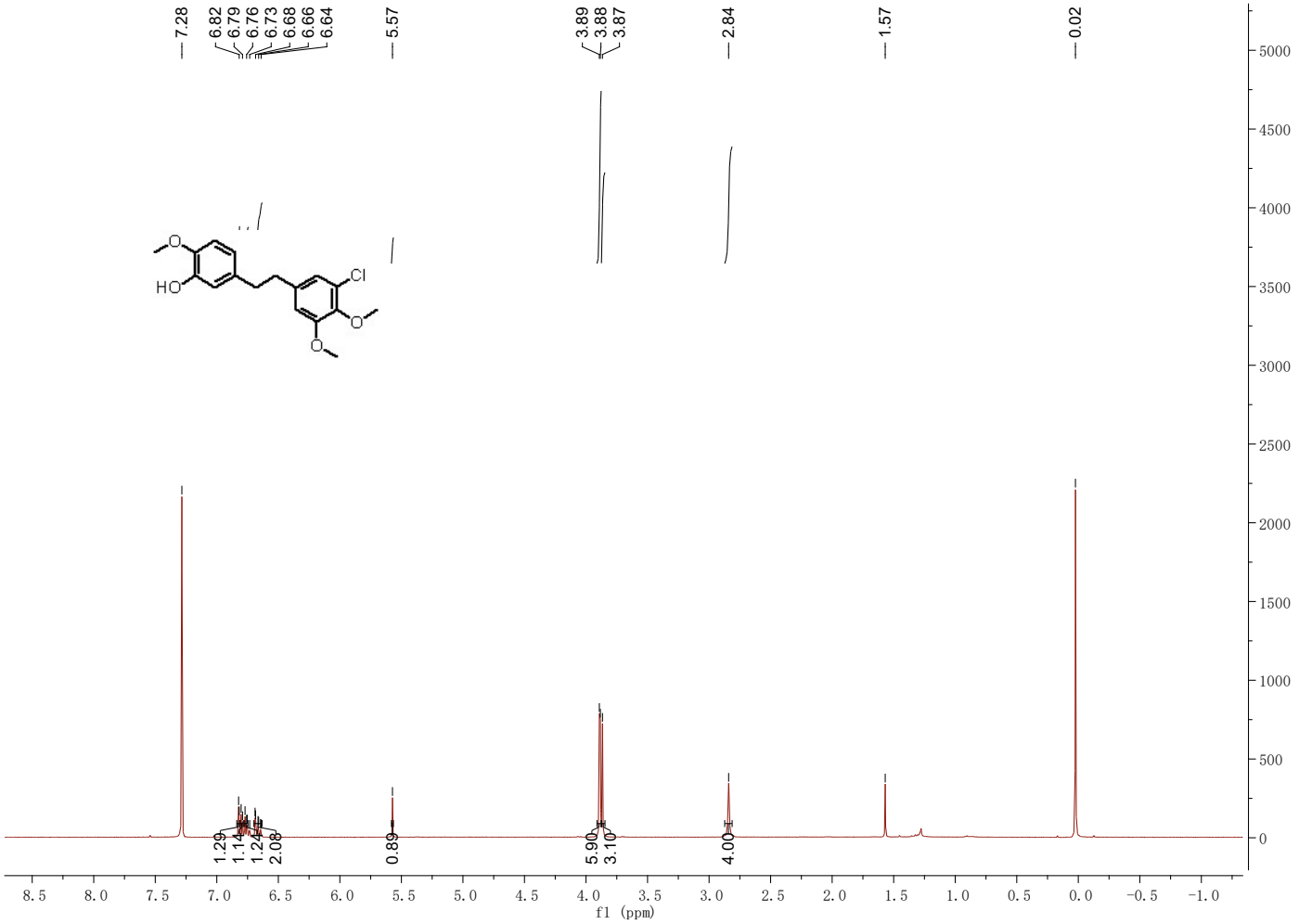


Figure S48. ^{13}C-NMR of Compound 27

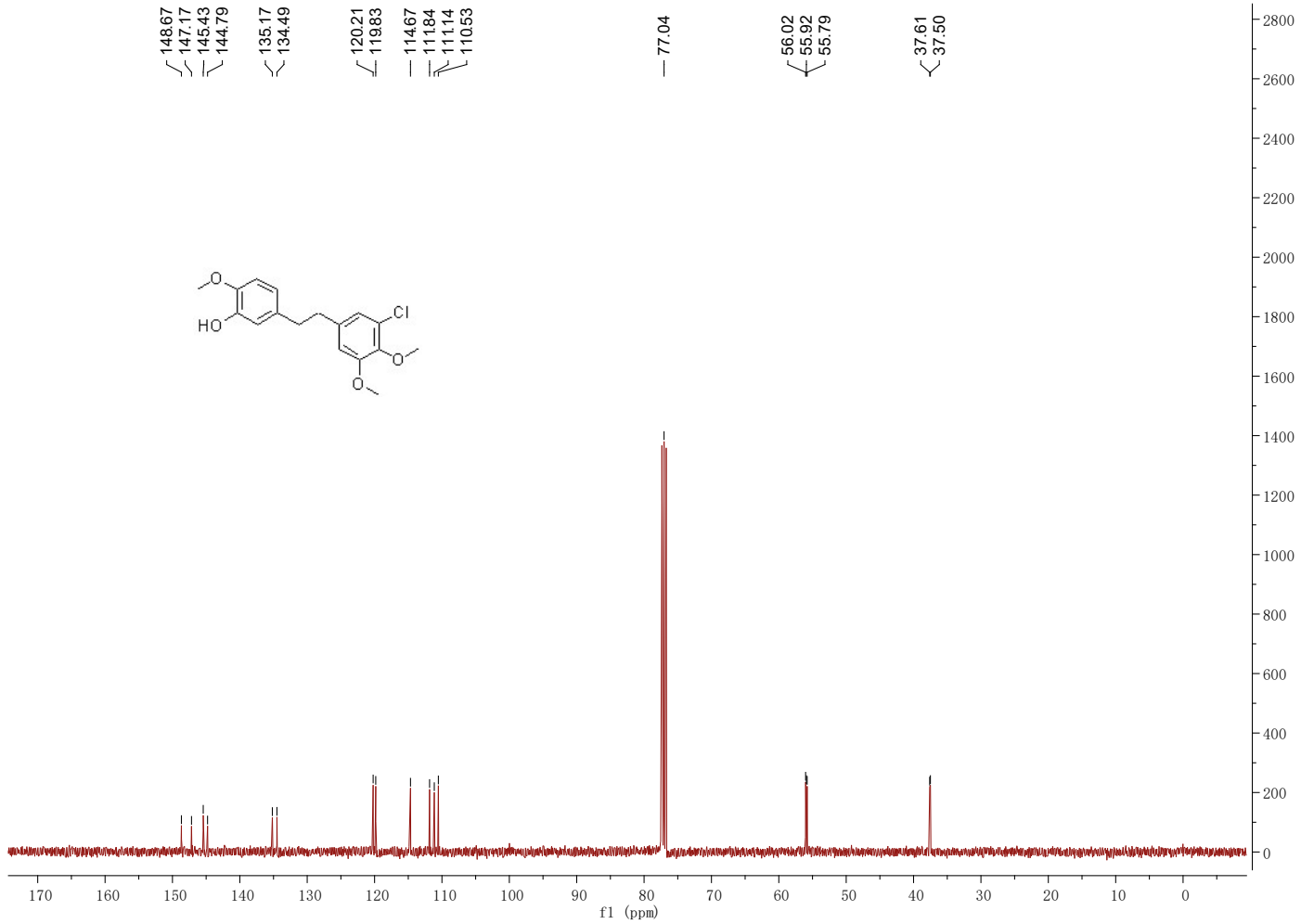


Figure S49. ^{1}H NMR of Compound 28

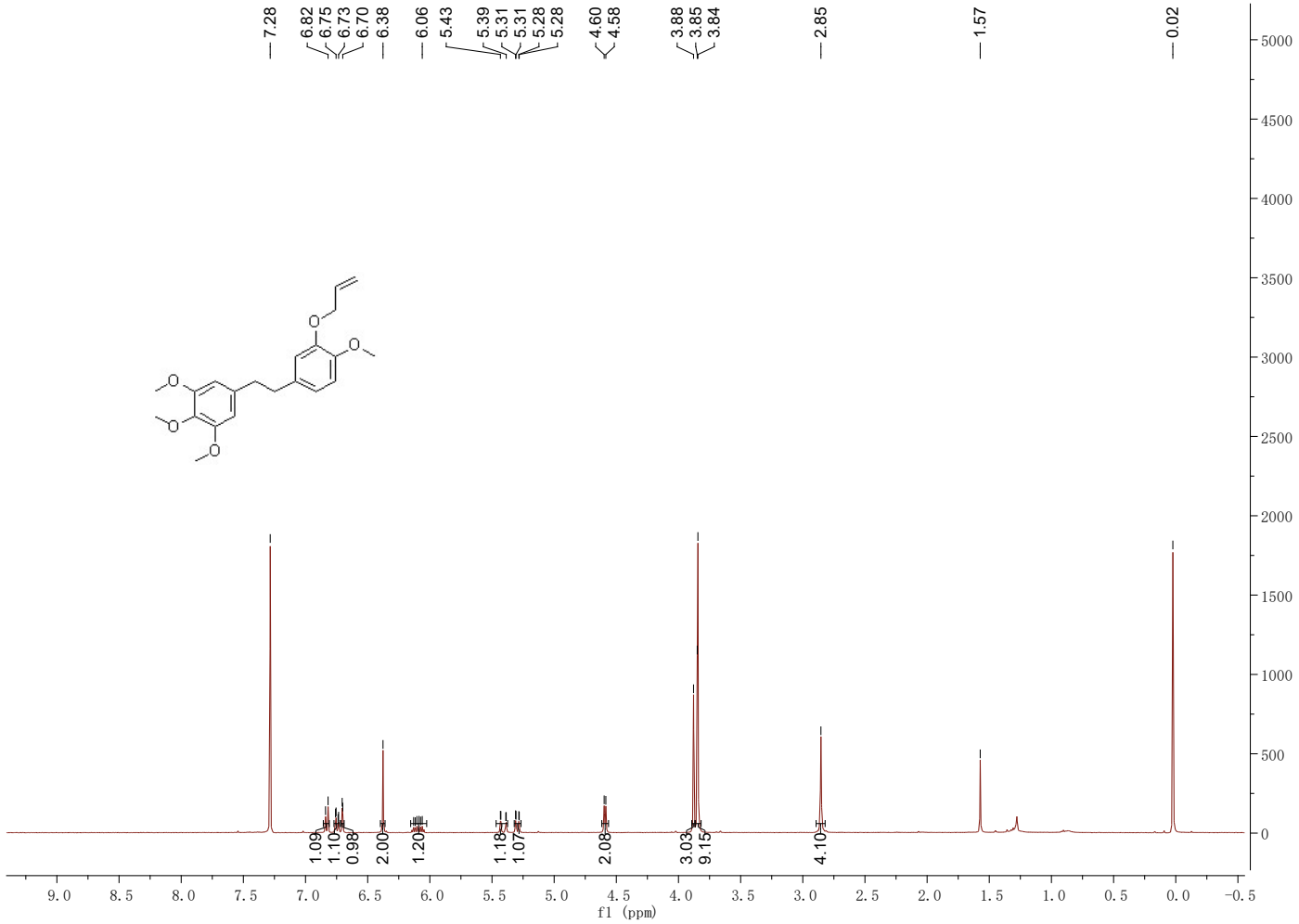


Figure S50 ^{13}C-NMR of Compound 28

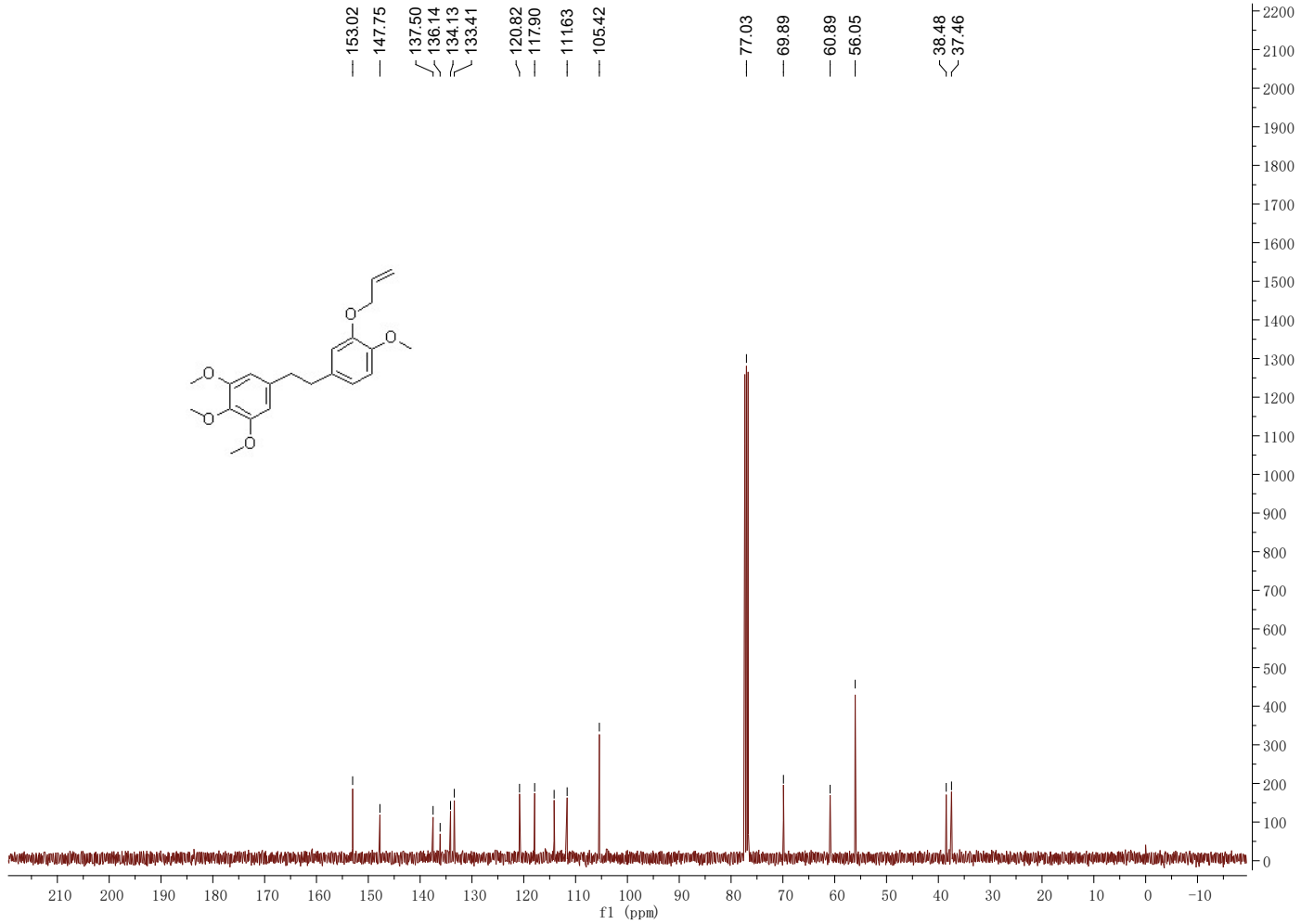


Figure S51. ^{1}H NMR of Compound 29

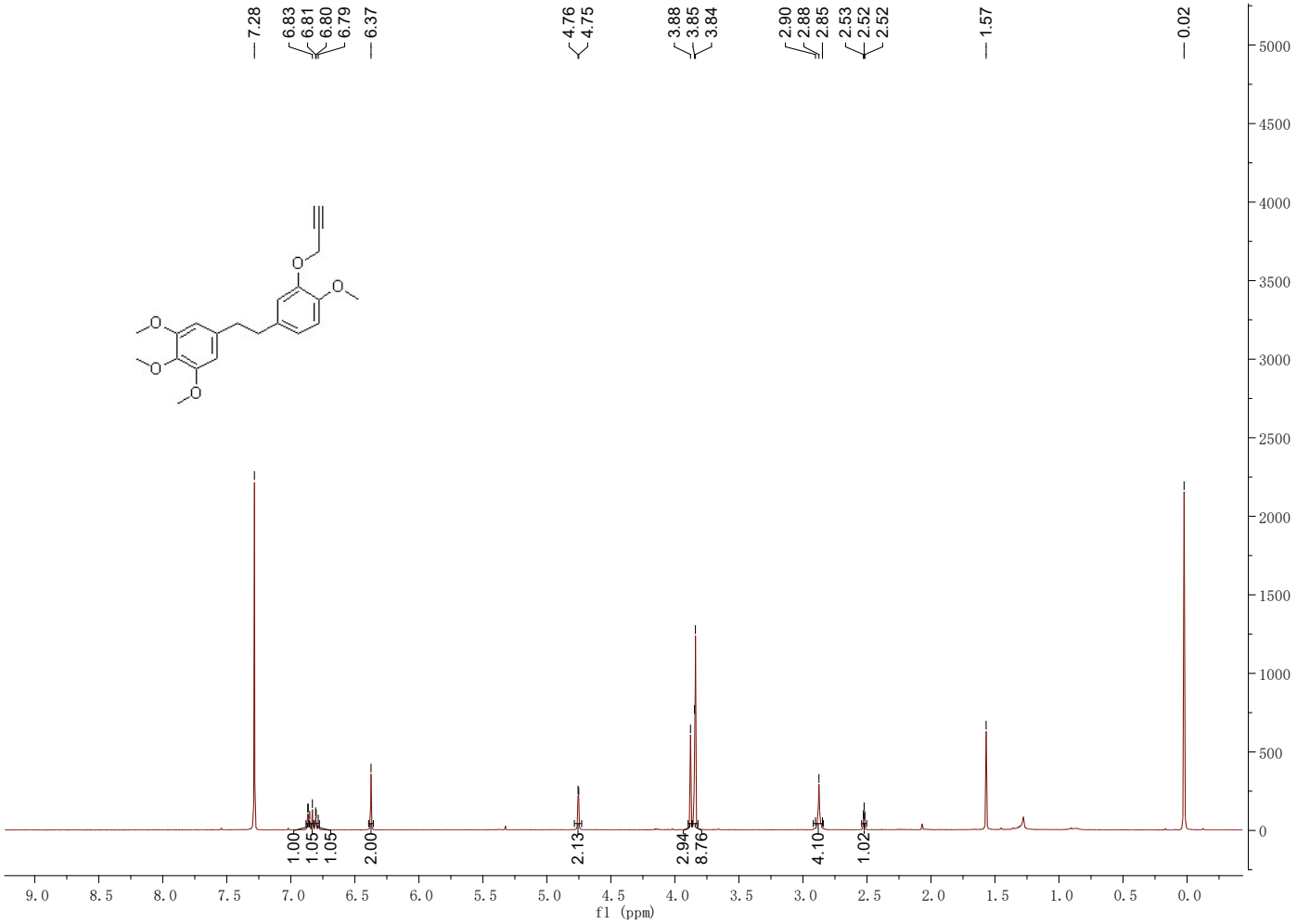


Figure S52. ^{13}C-NMR of Compound 29

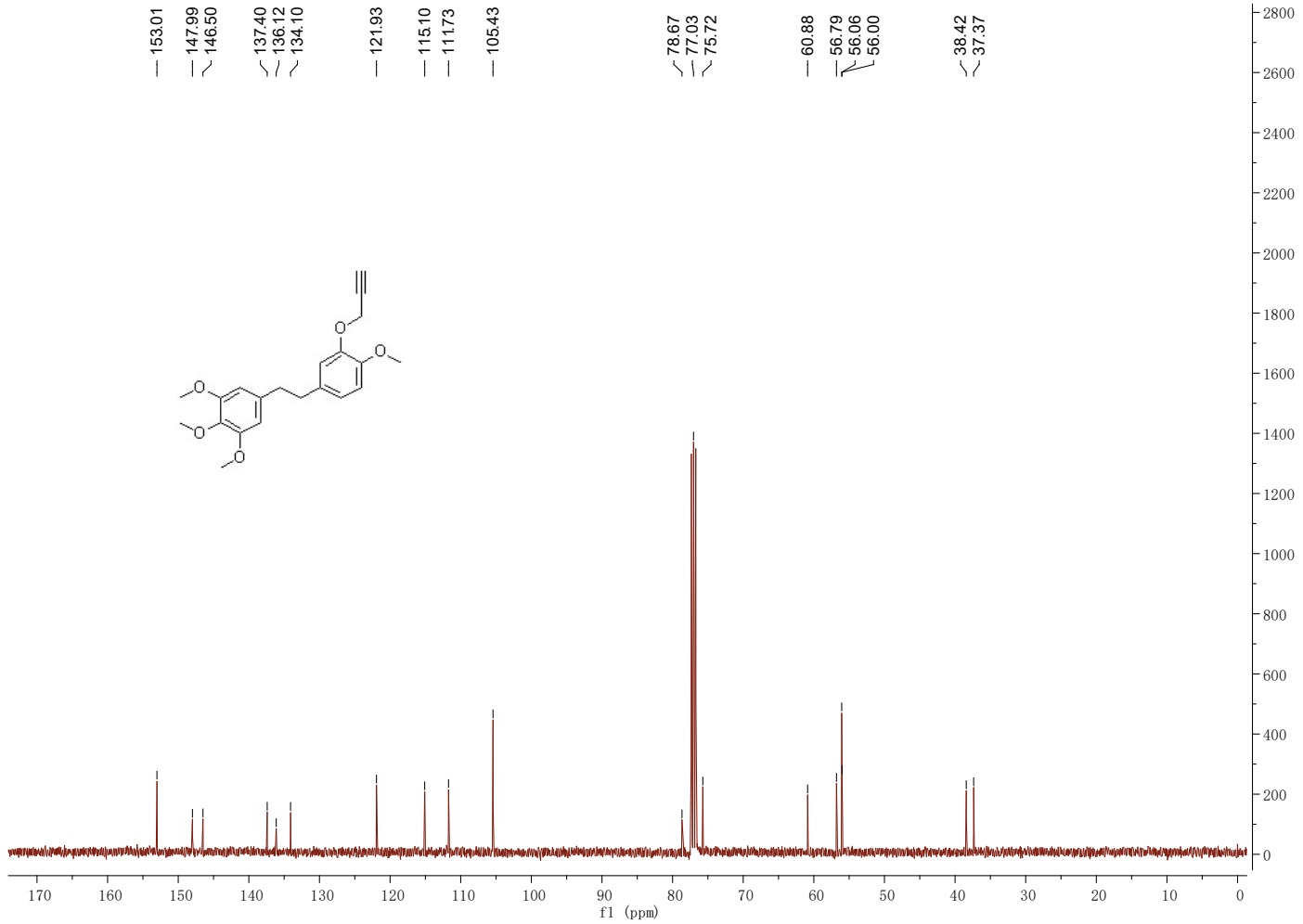


Figure S53. ^{1}H NMR of Compound 30

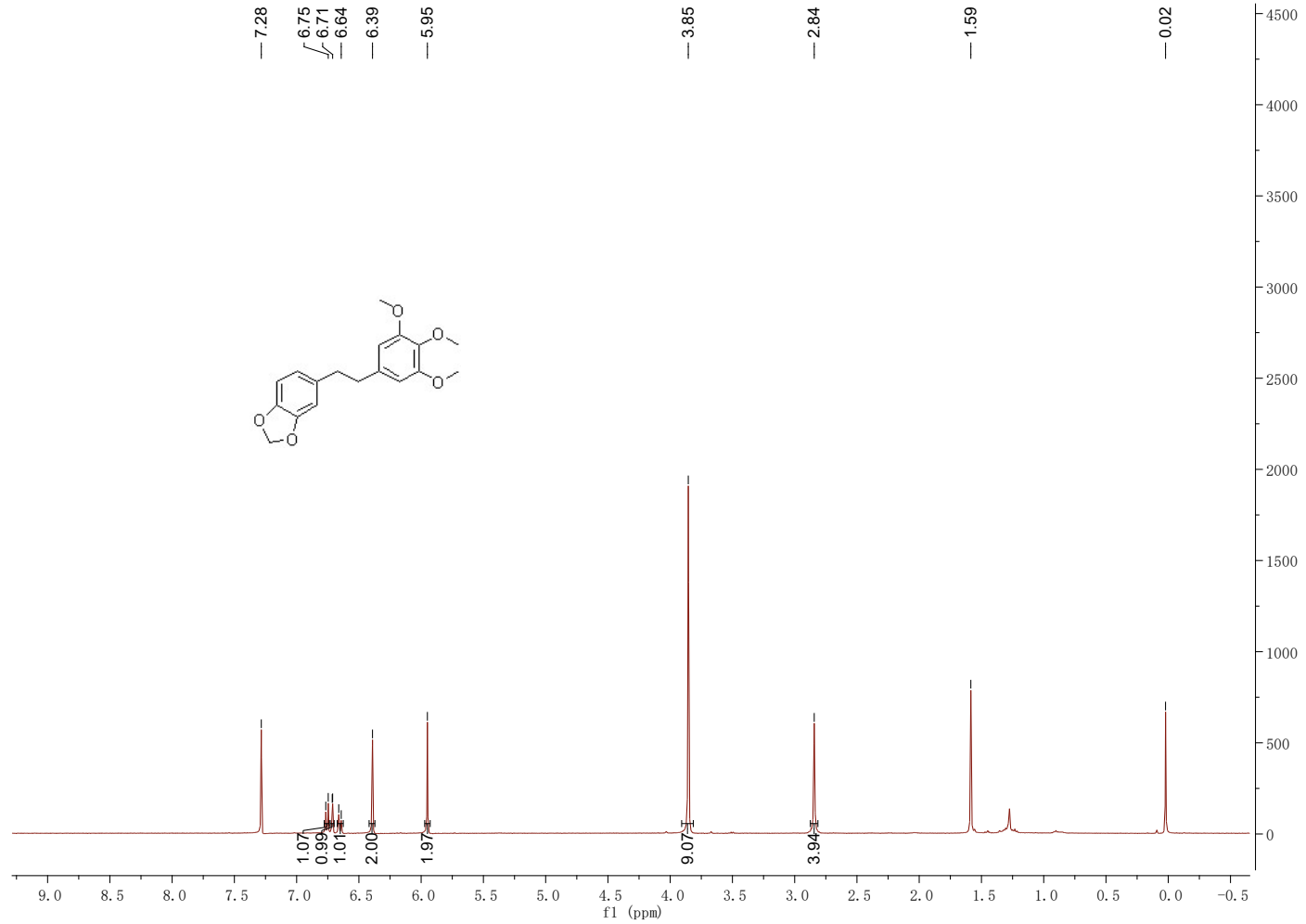


Figure S54. ^{13}C-NMR of Compound 30

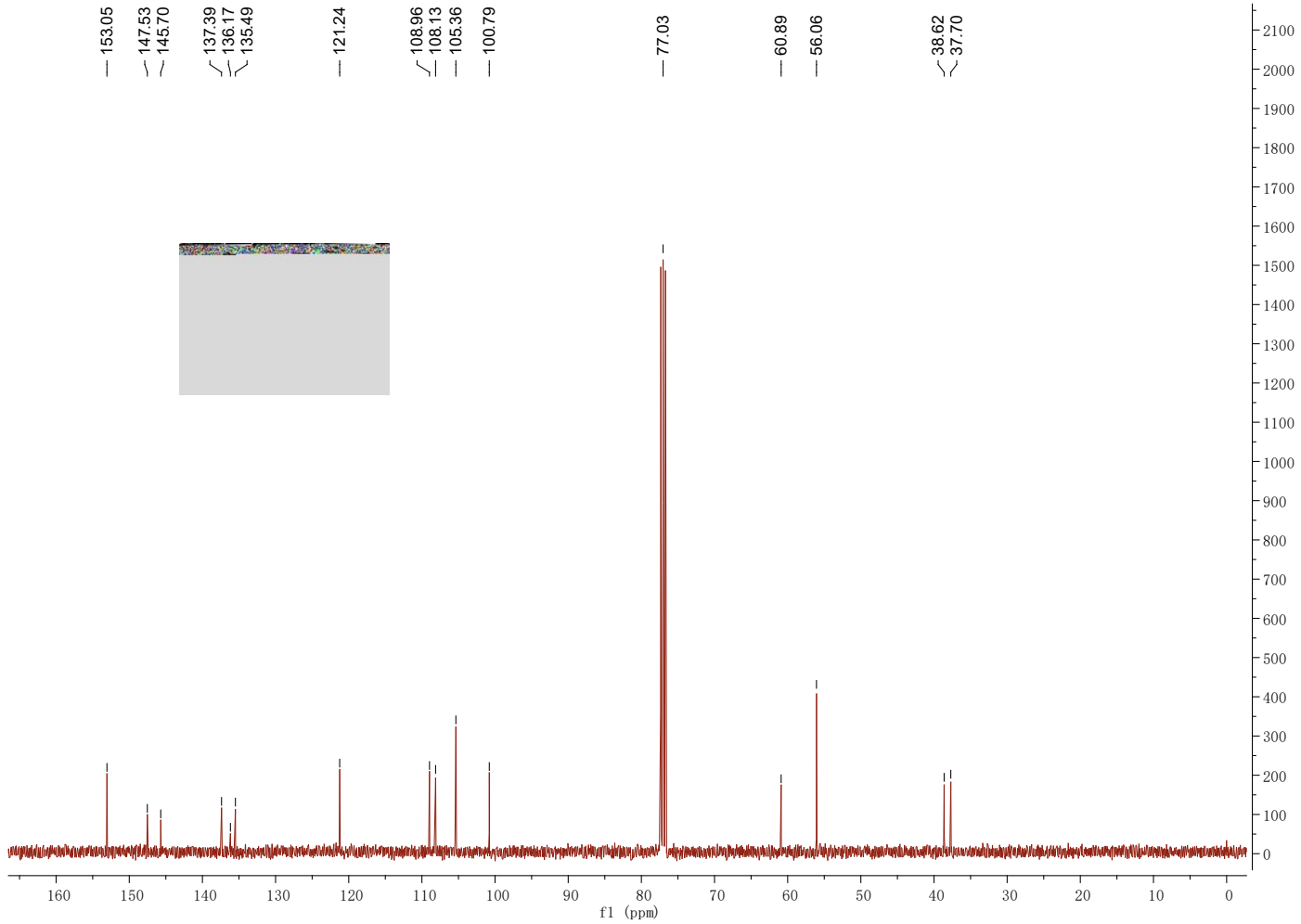


Figure S55. ^{1}H NMR of Compound 31

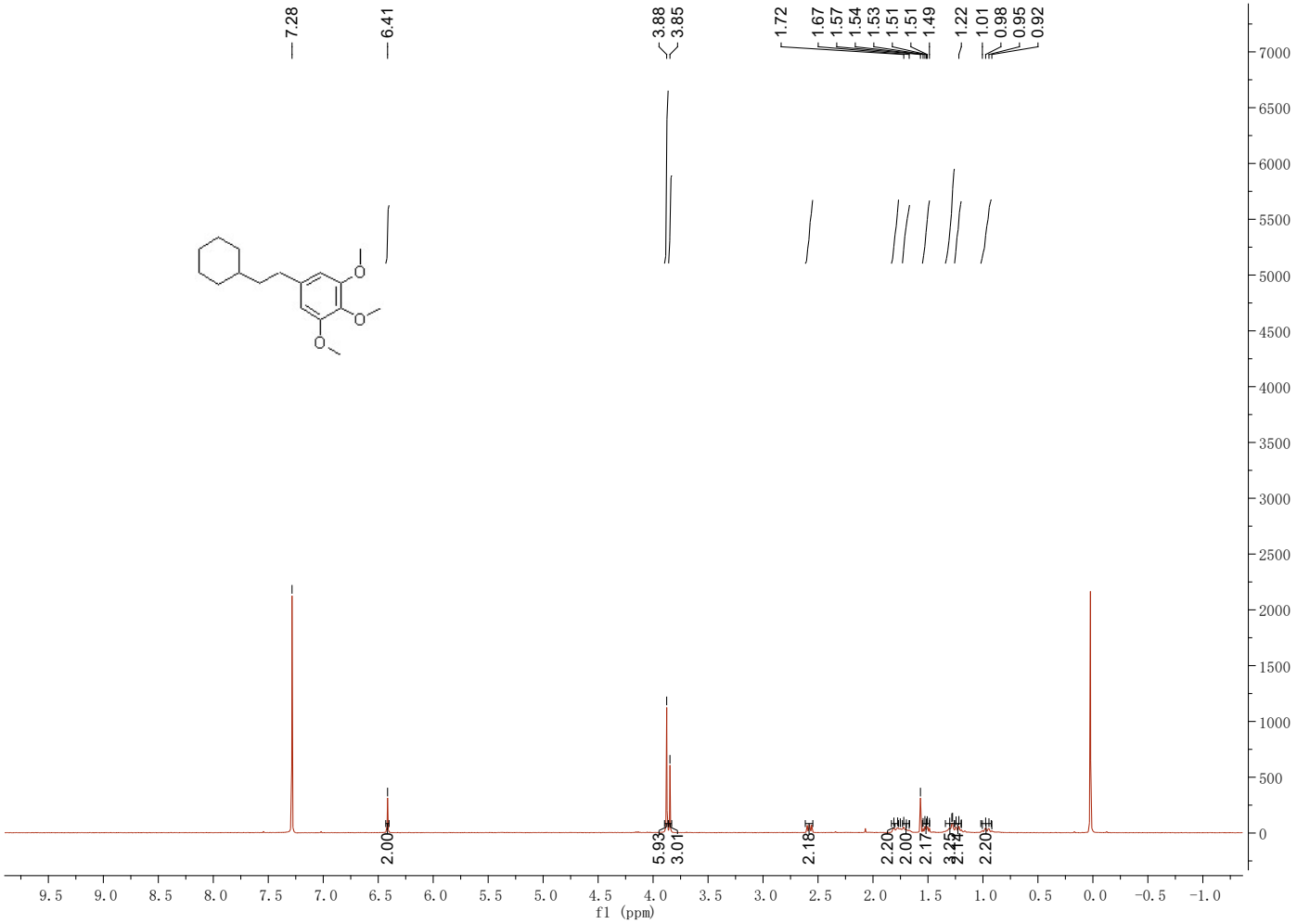


Figure S56. ^{13}C-NMR of Compound 31

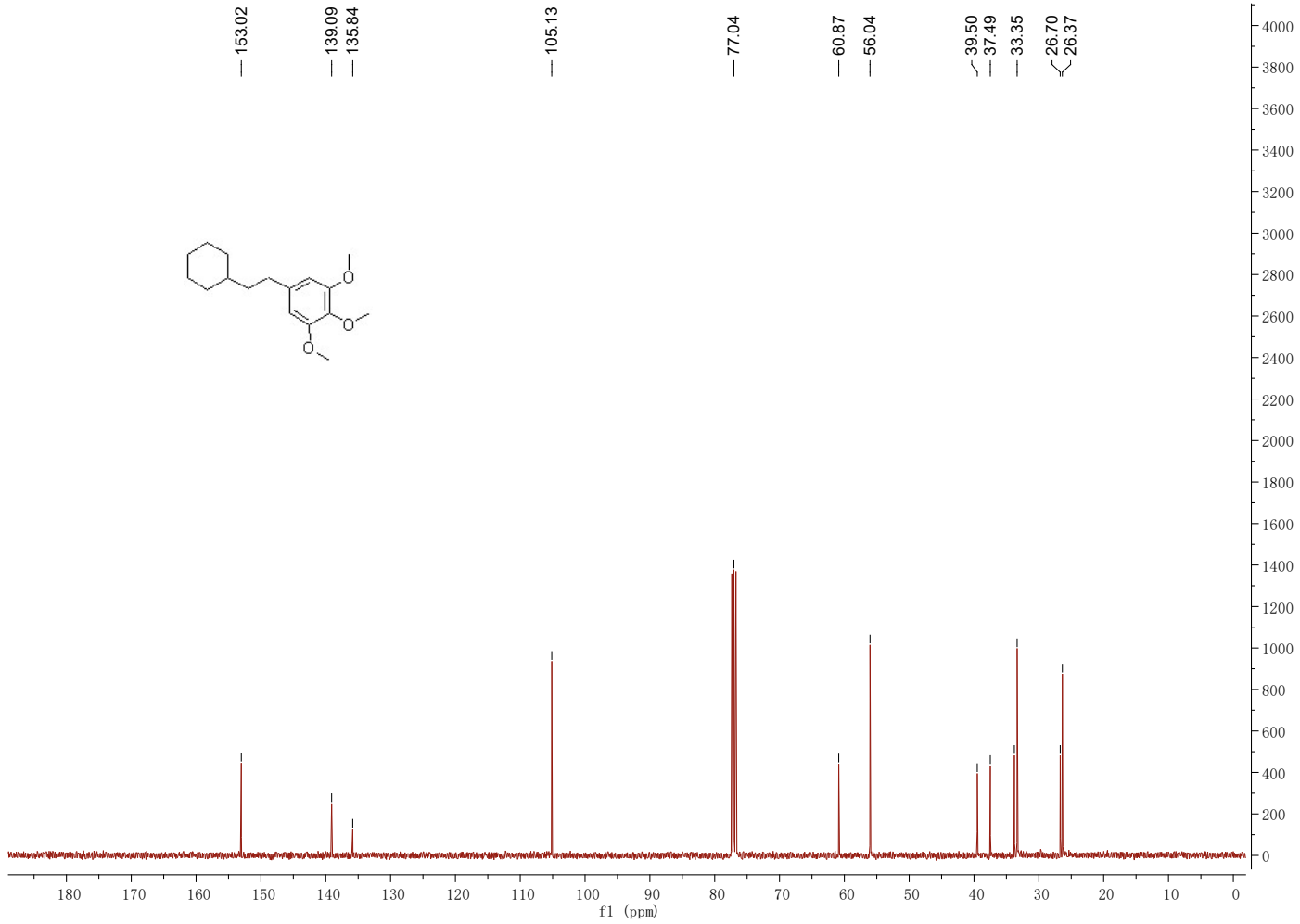


Figure S57. ^{1}H NMR of Compound 32

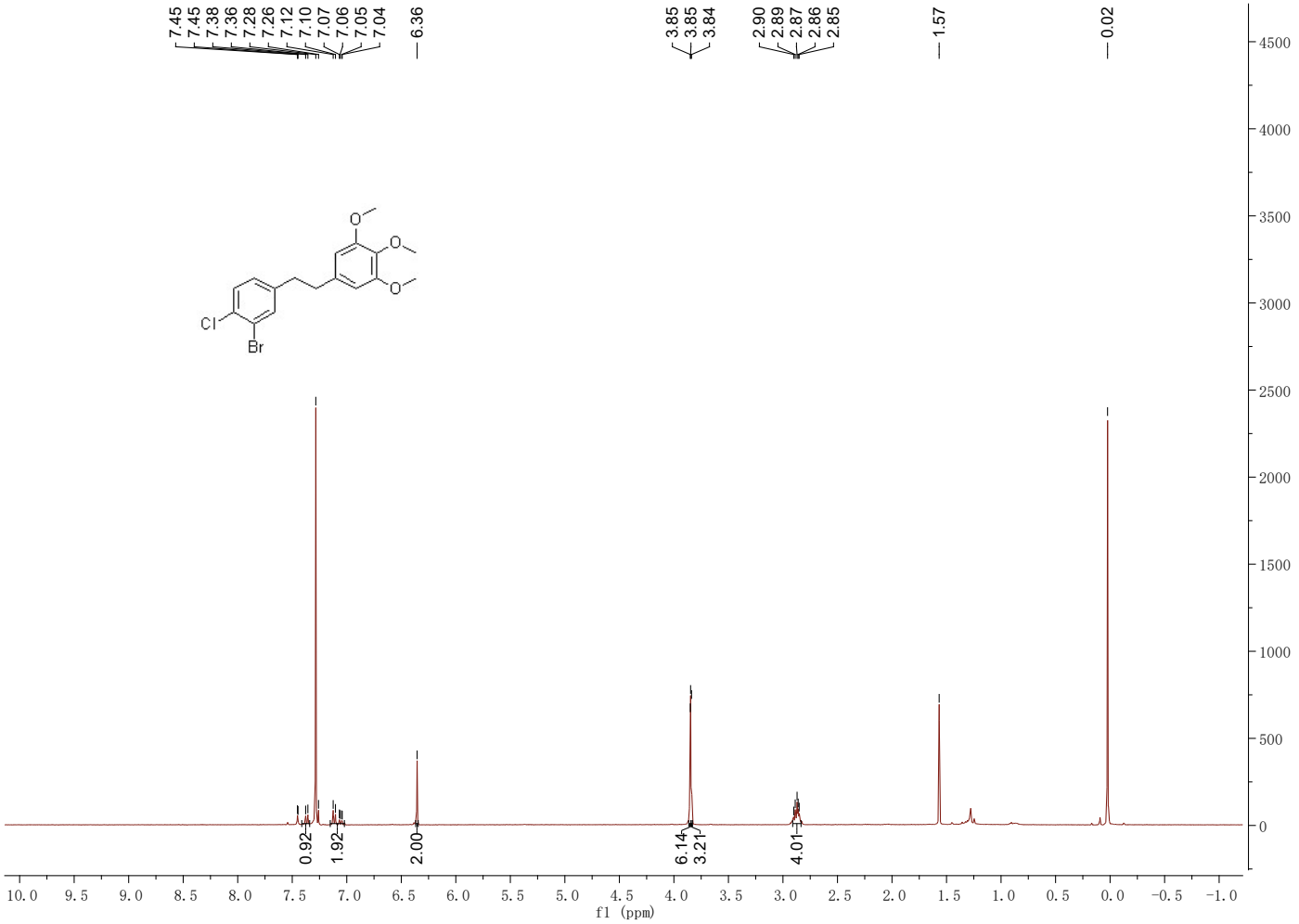


Figure S58. ^{13}C-NMR of Compound 32

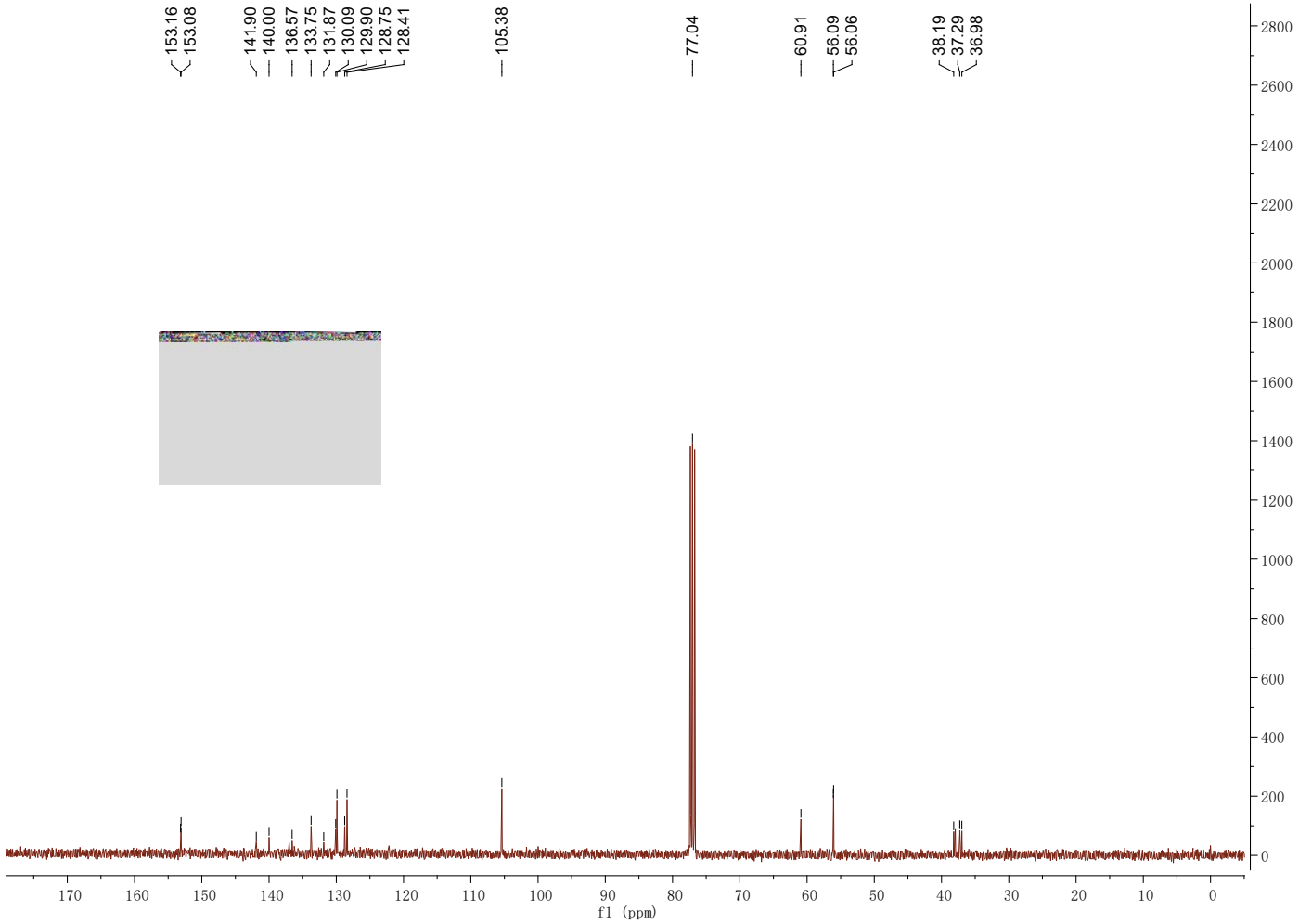


Figure S59. ^{1}H NMR of Compound 33

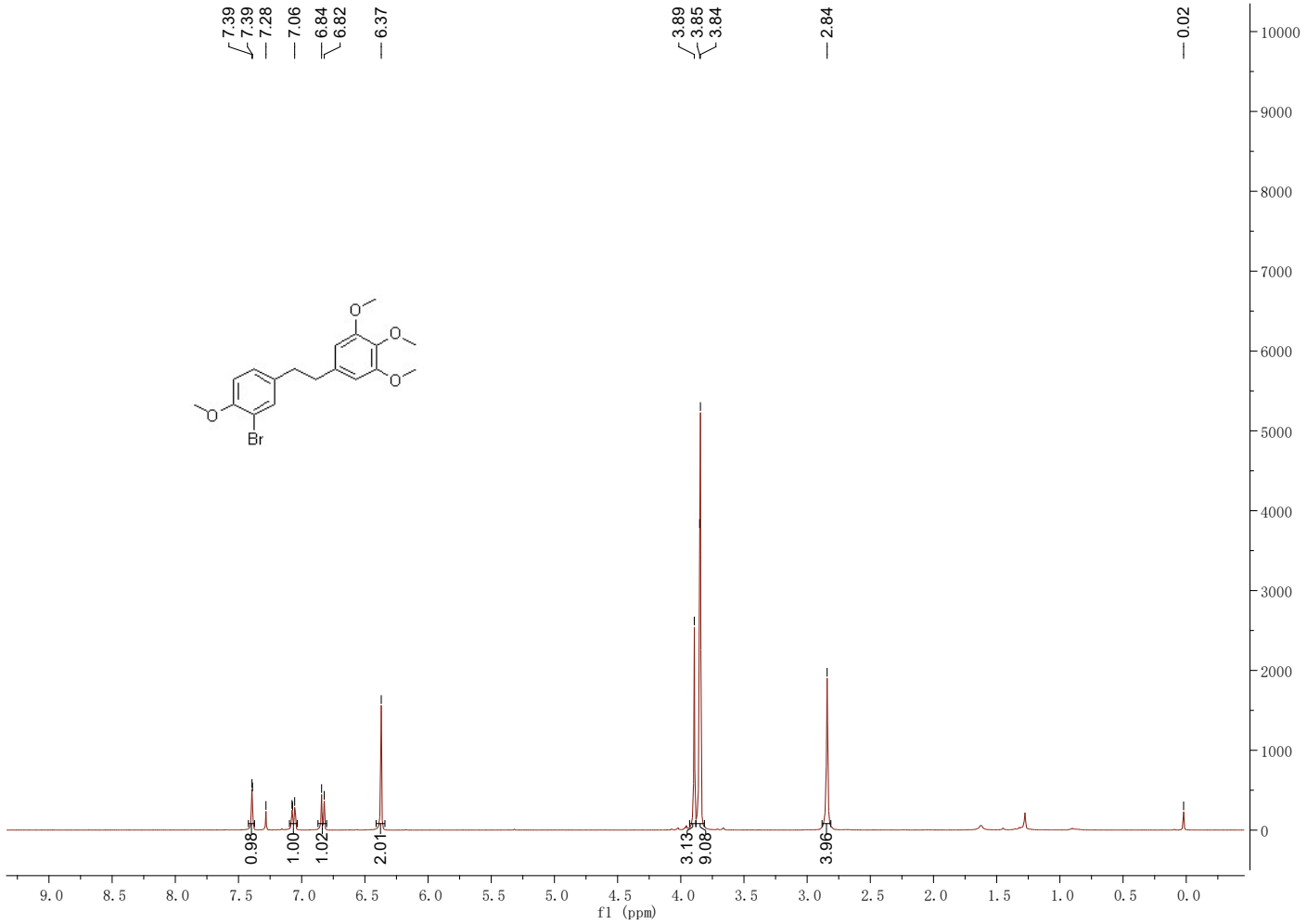


Figure S60. ^{13}C-NMR of Compound 33

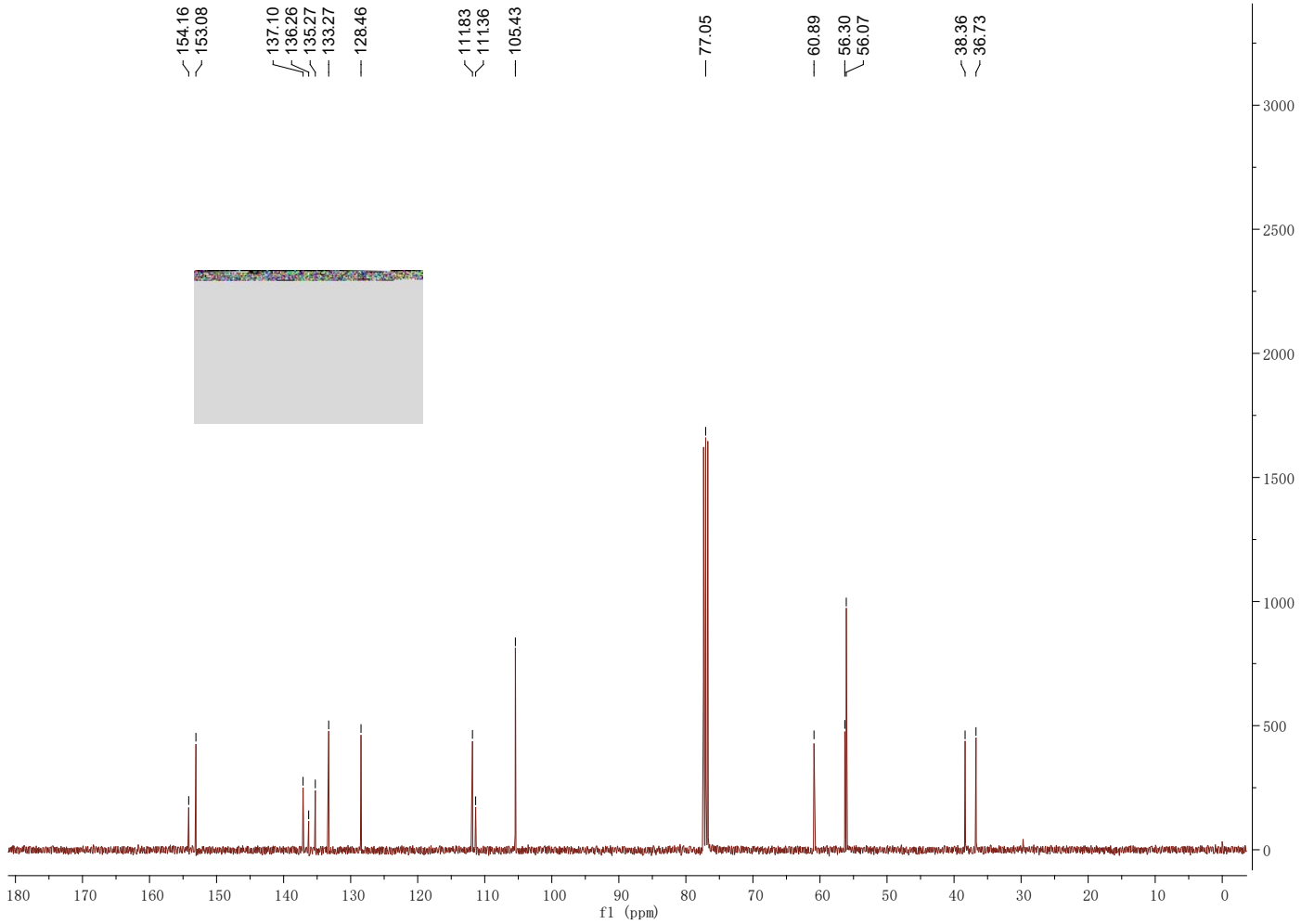


Figure S61. ^{1}H NMR of Compound 34

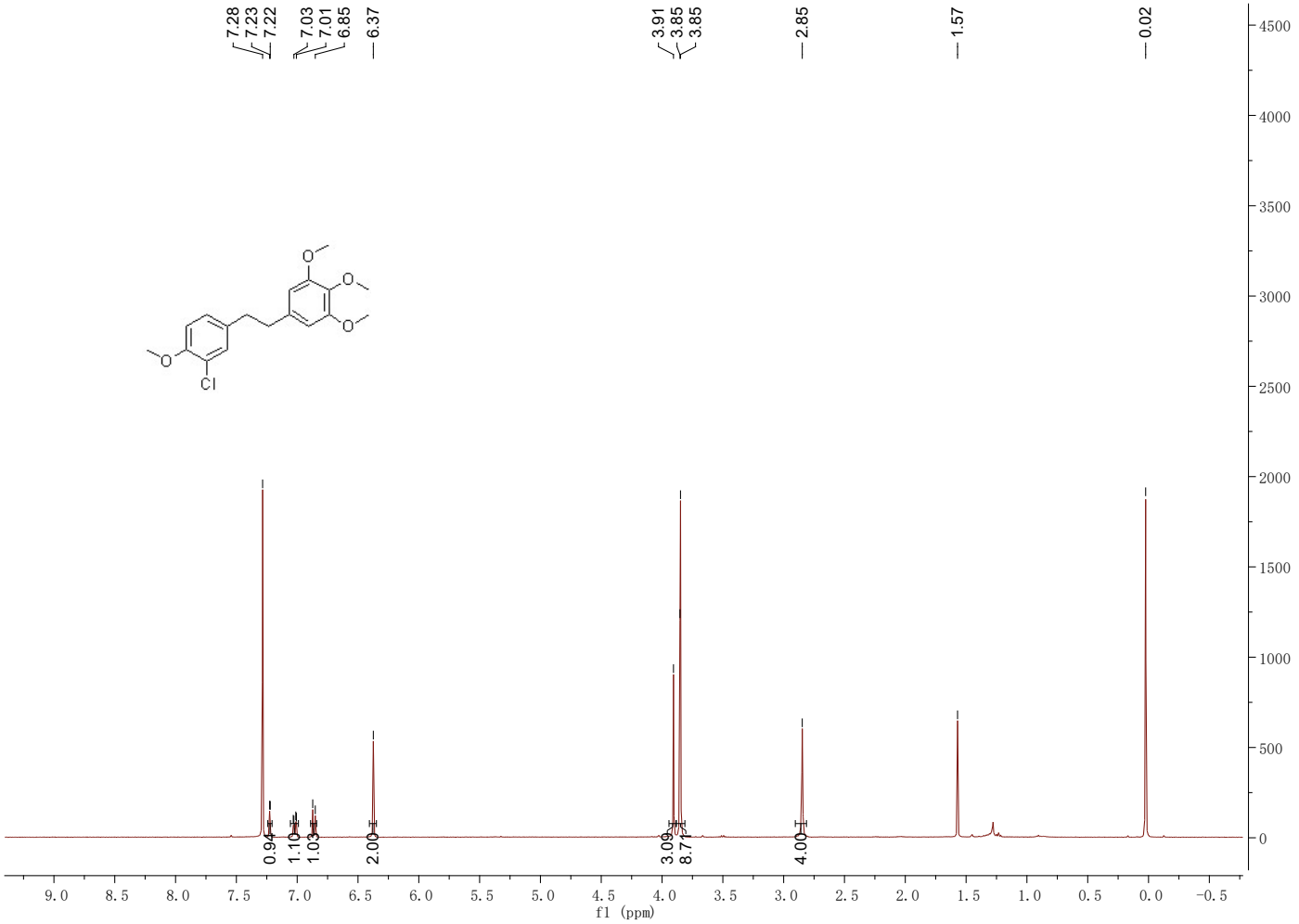


Figure S62. ^{13}C-NMR of Compound 34

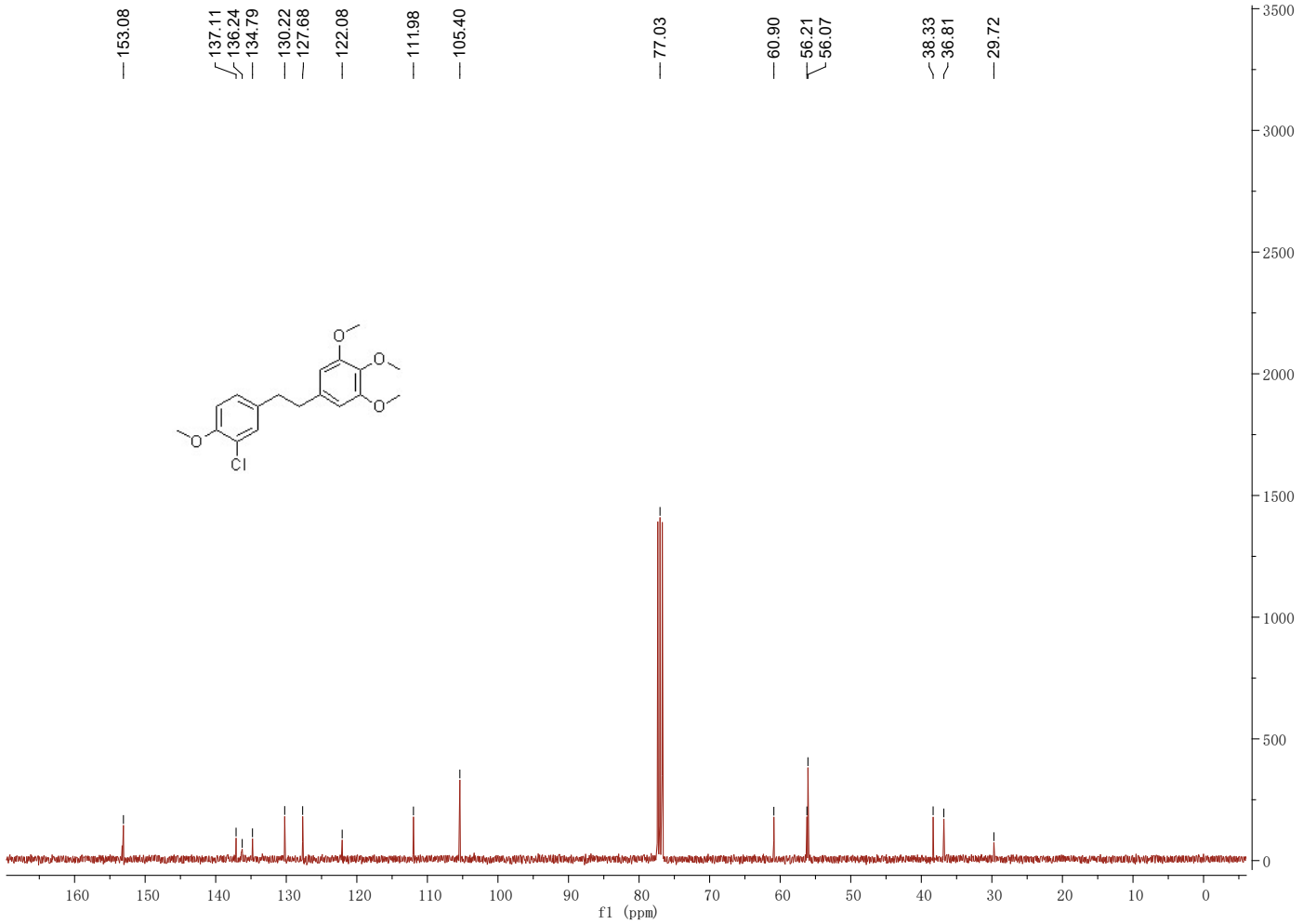


Figure S63. ^{1}H NMR of Compound 35

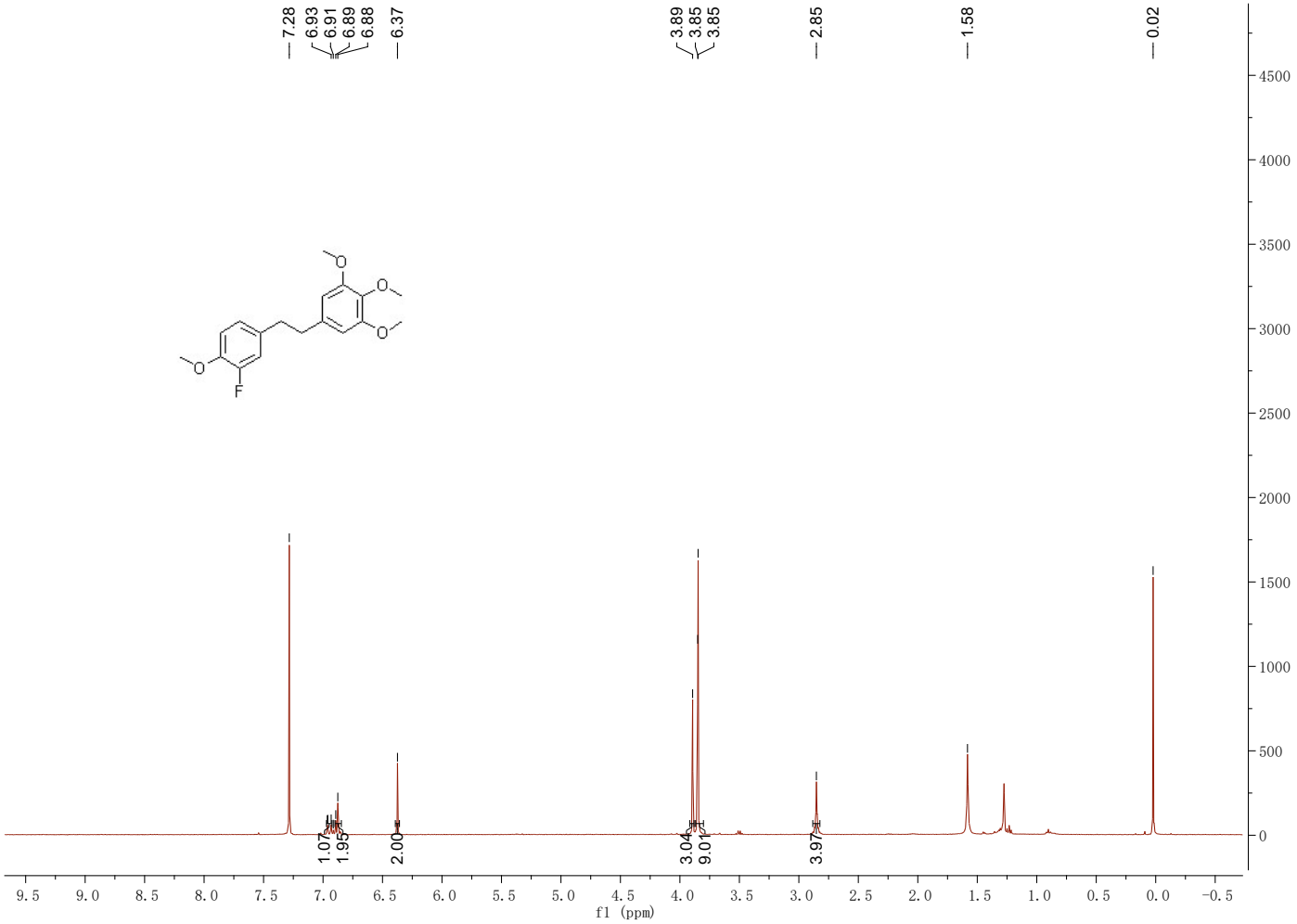


Figure S64. ^{13}C-NMR of Compound 35

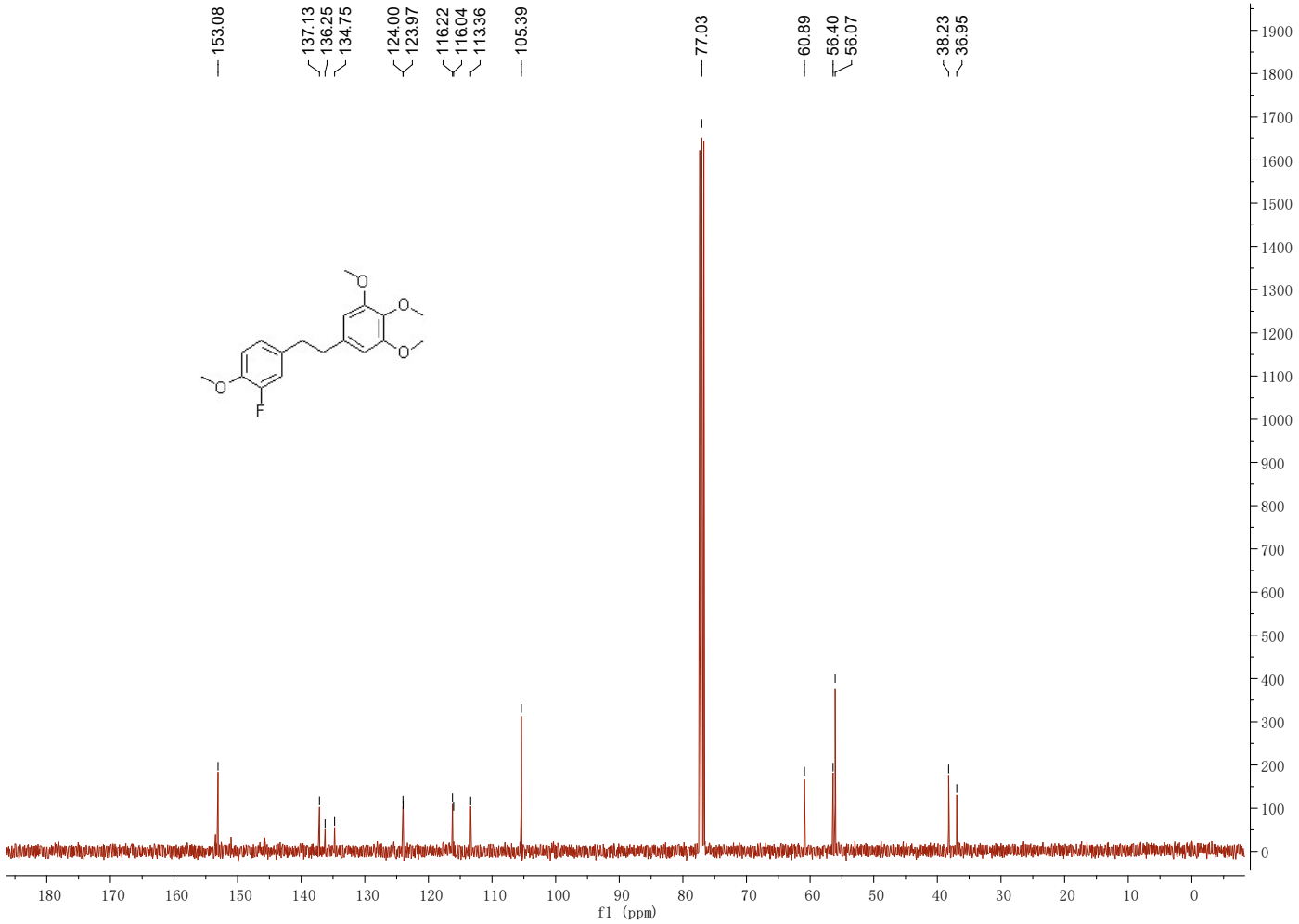


Figure S65. ^{1}H NMR of Compound 36

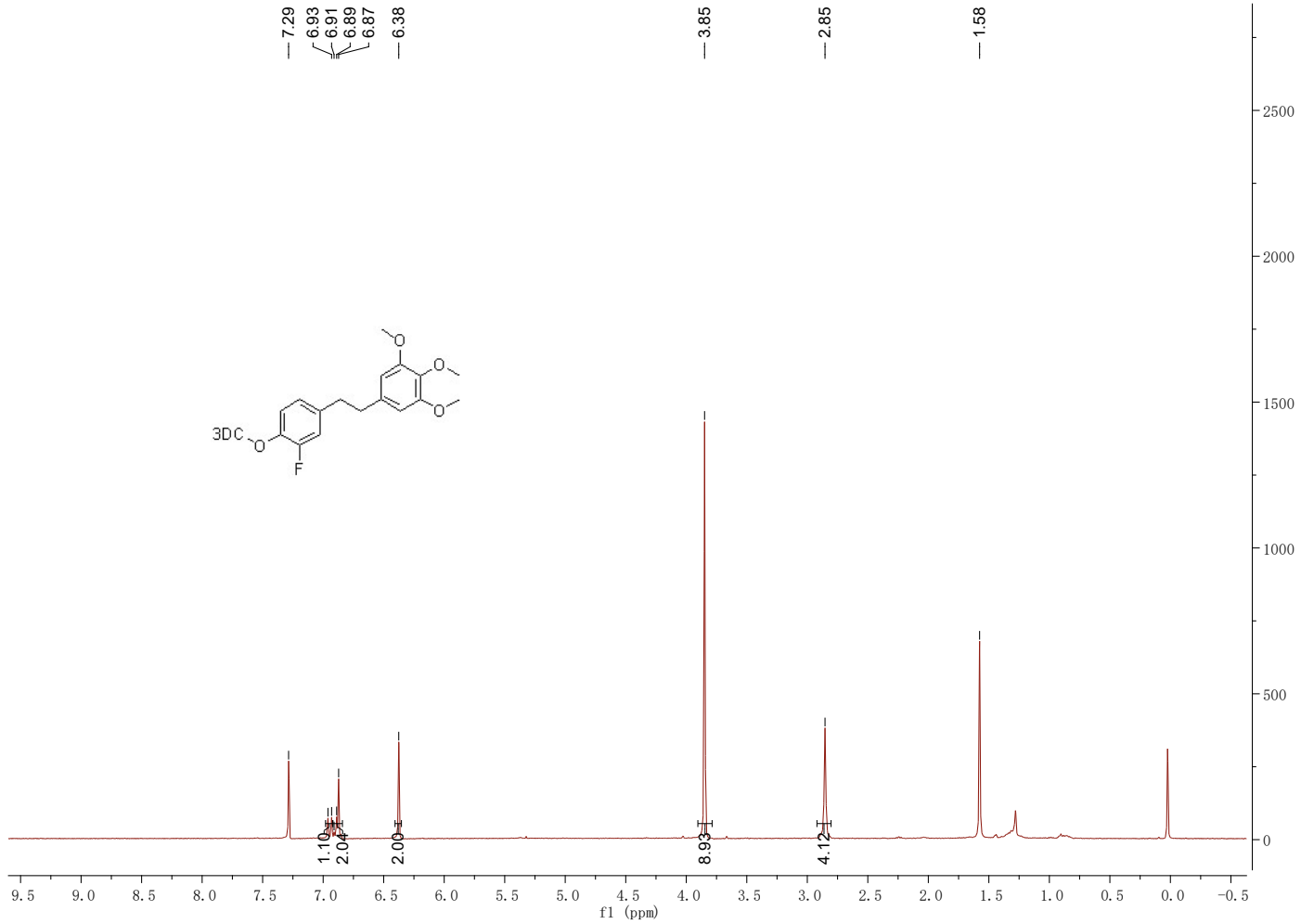


Figure S66. ^{13}C-NMR of Compound 36

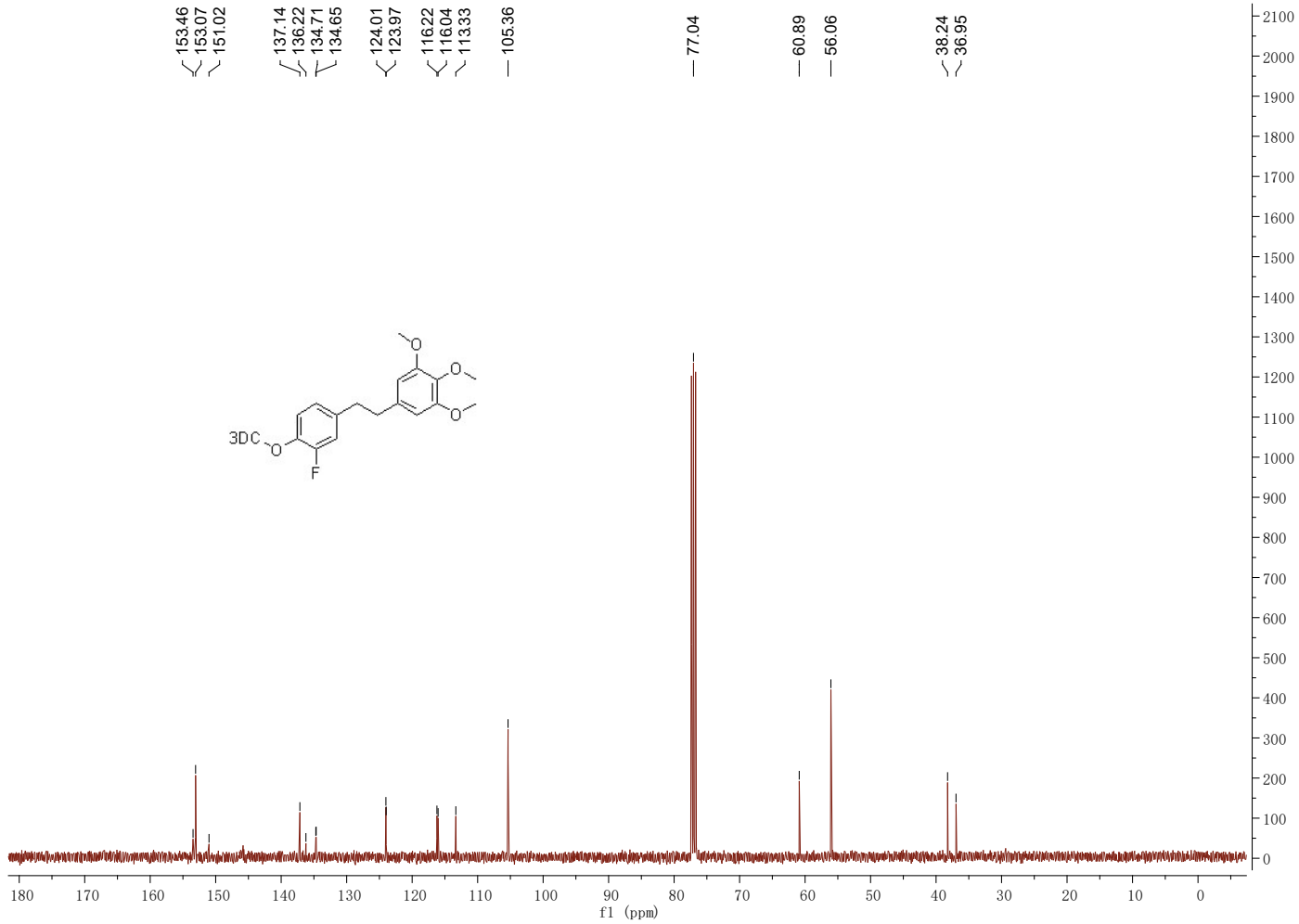


Figure S67. ^{1}H NMR of Compound 37

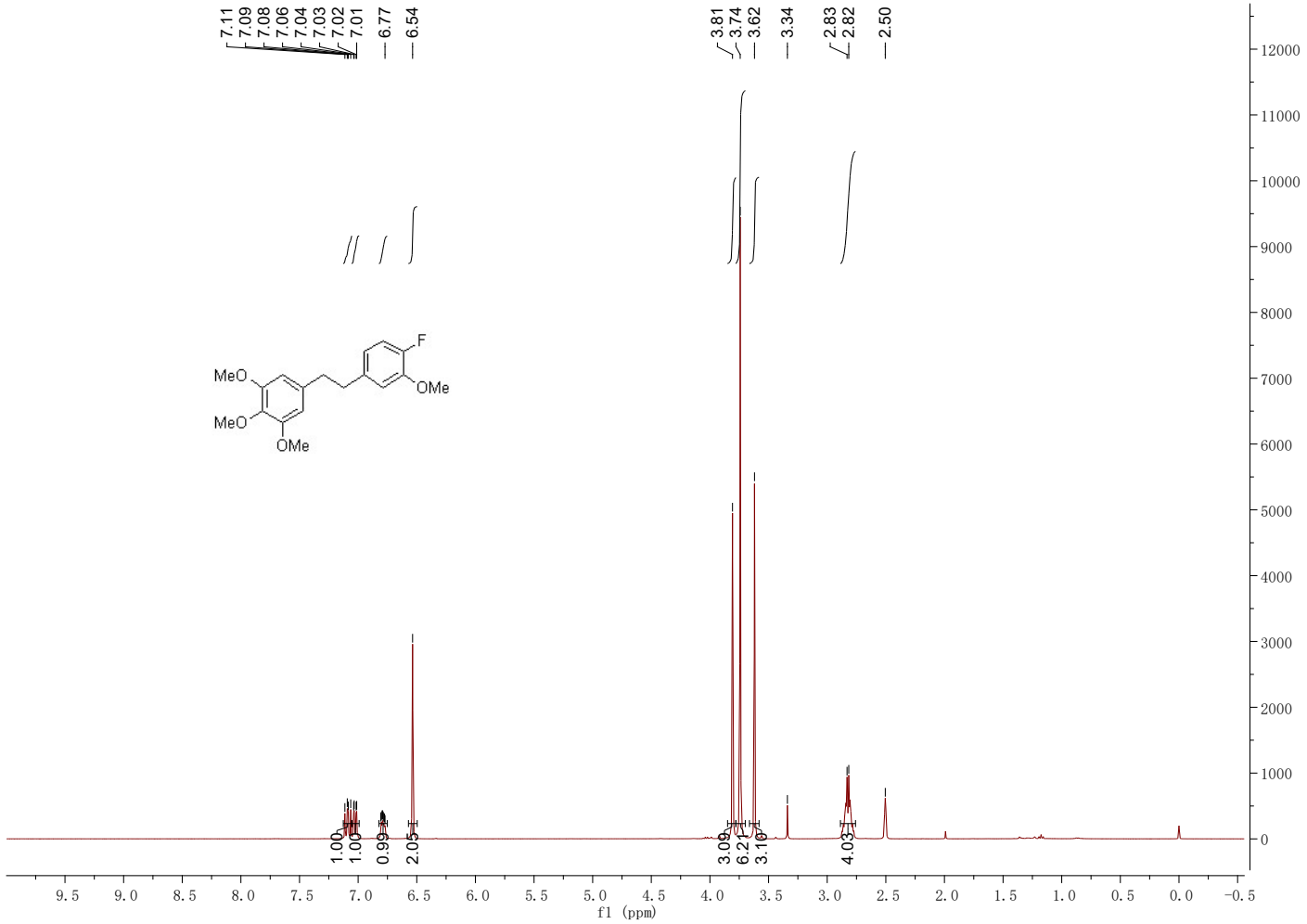


Figure S68. ^{13}C-NMR of Compound 37

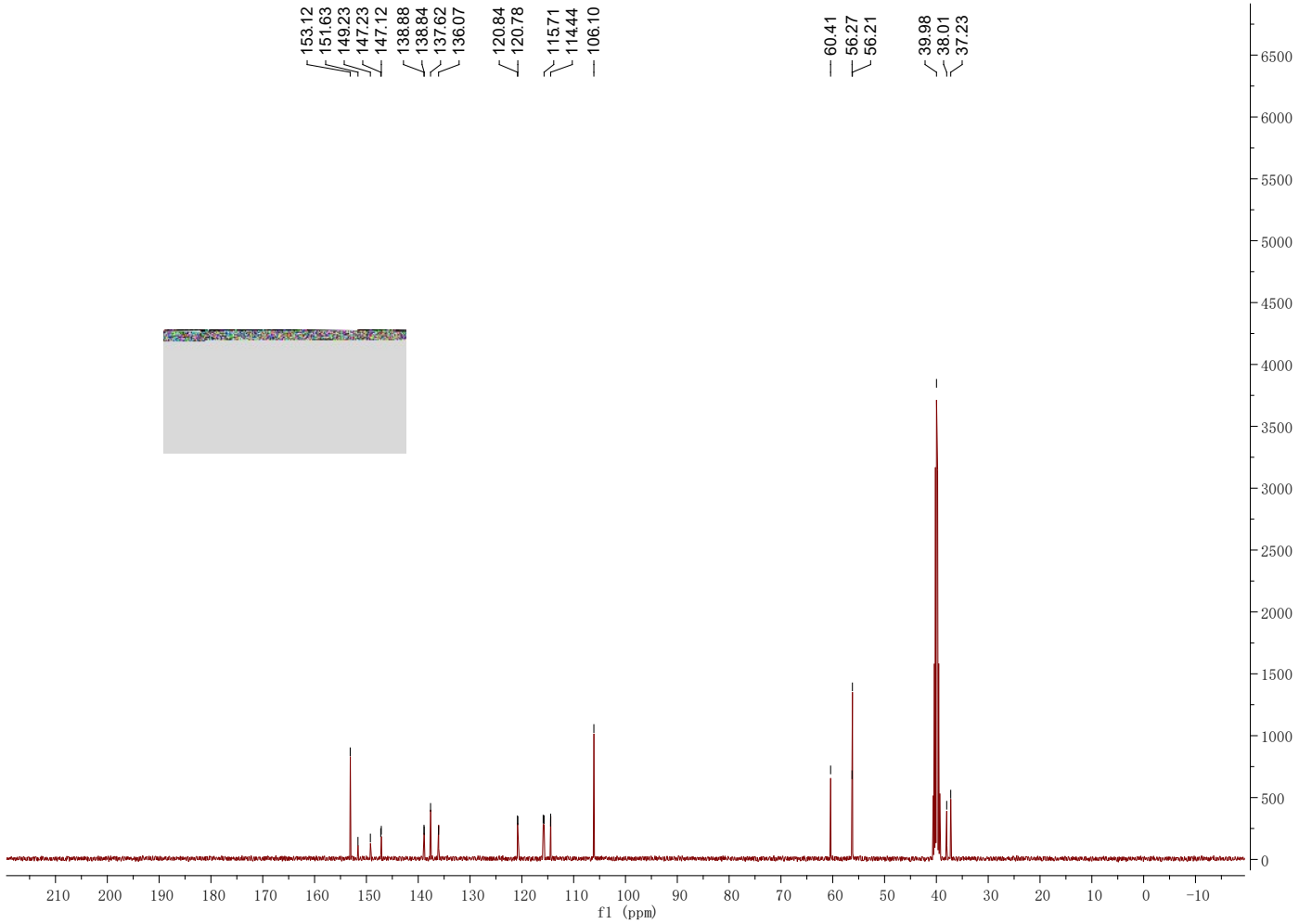


Figure S69. ^{1}H NMR of Compound 38

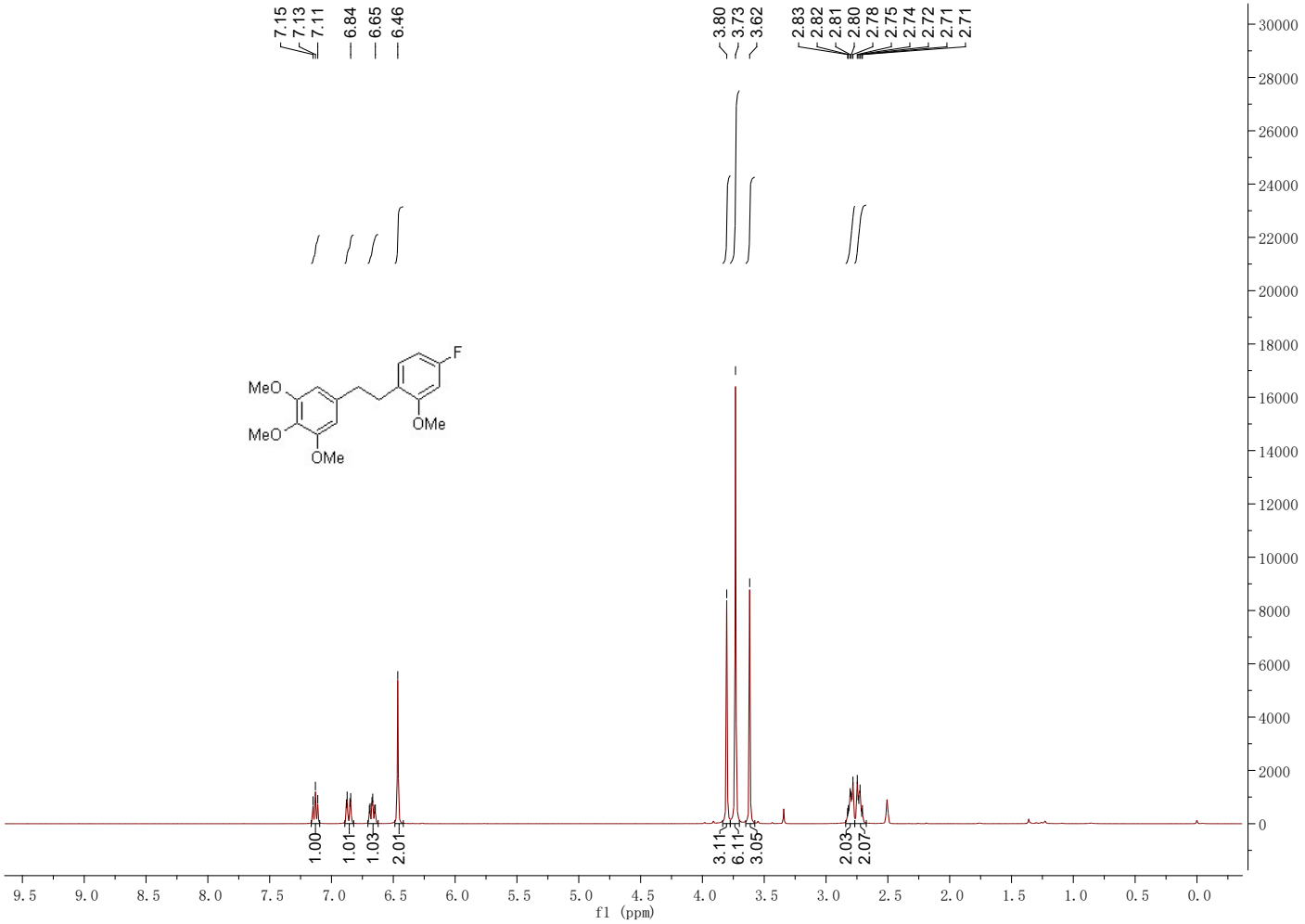


Figure S70. ^{13}C-NMR of Compound 38

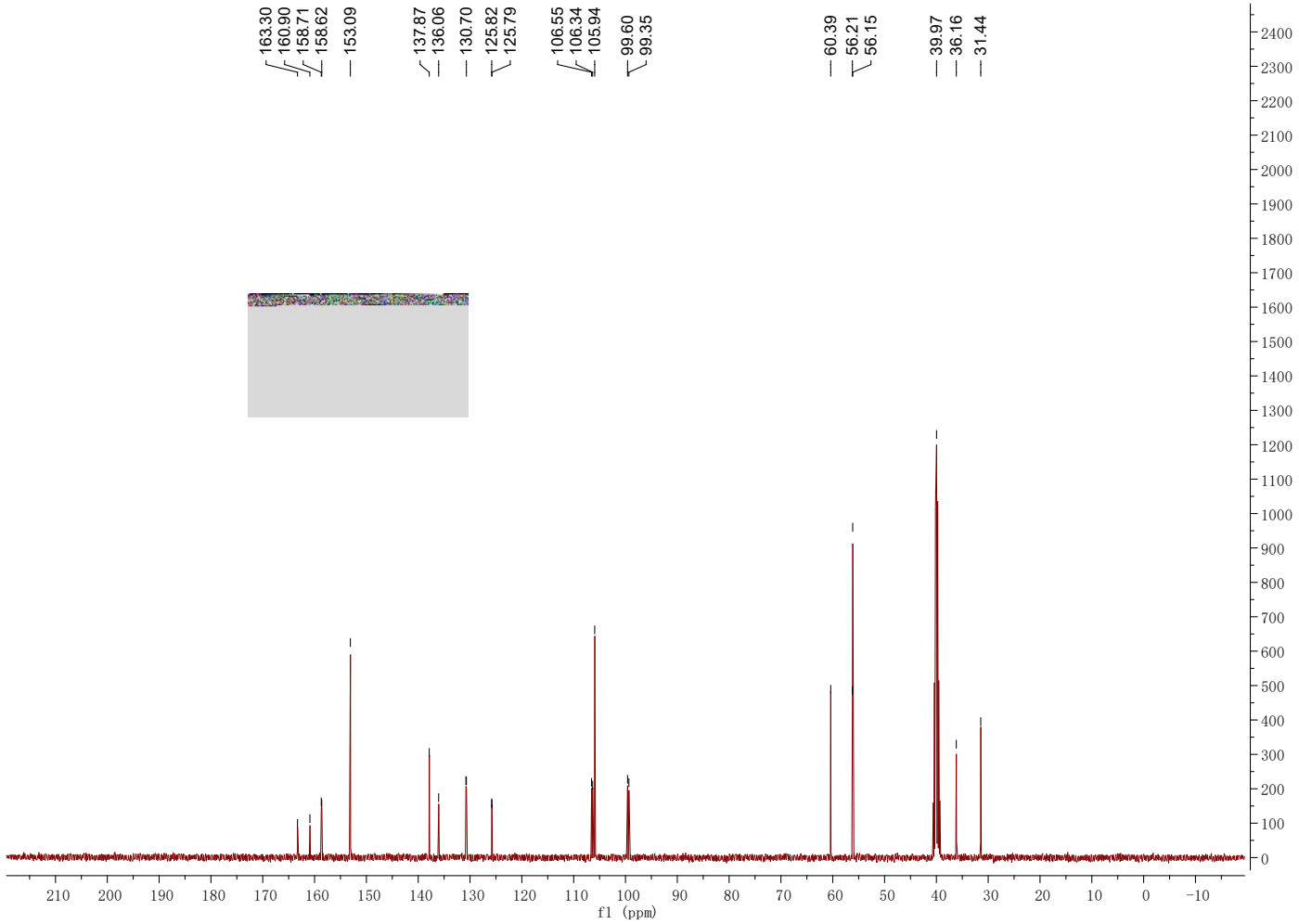


Figure S71. ^{1}H NMR of Compound 39

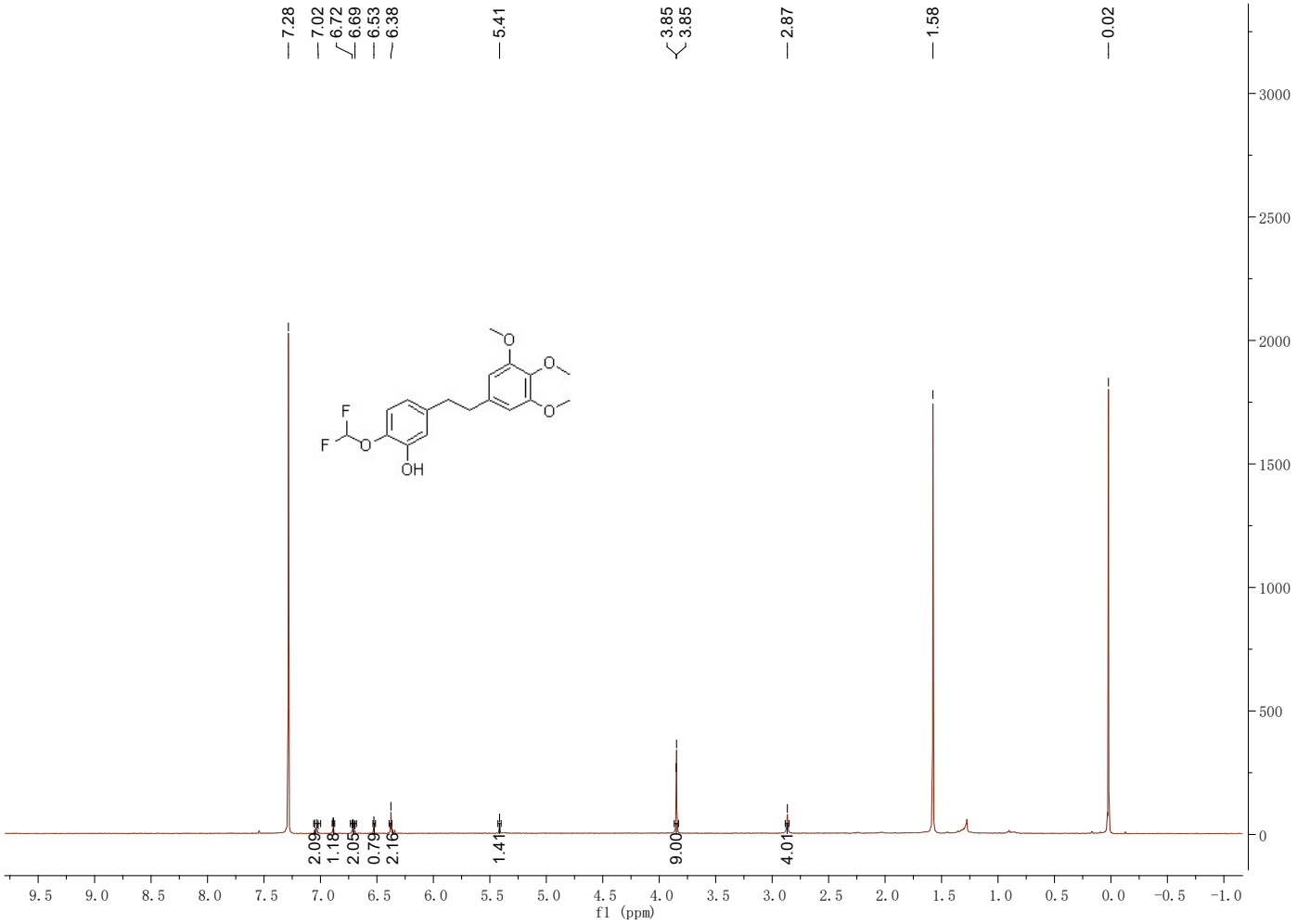


Figure S72. ^{13}C-NMR of Compound 39

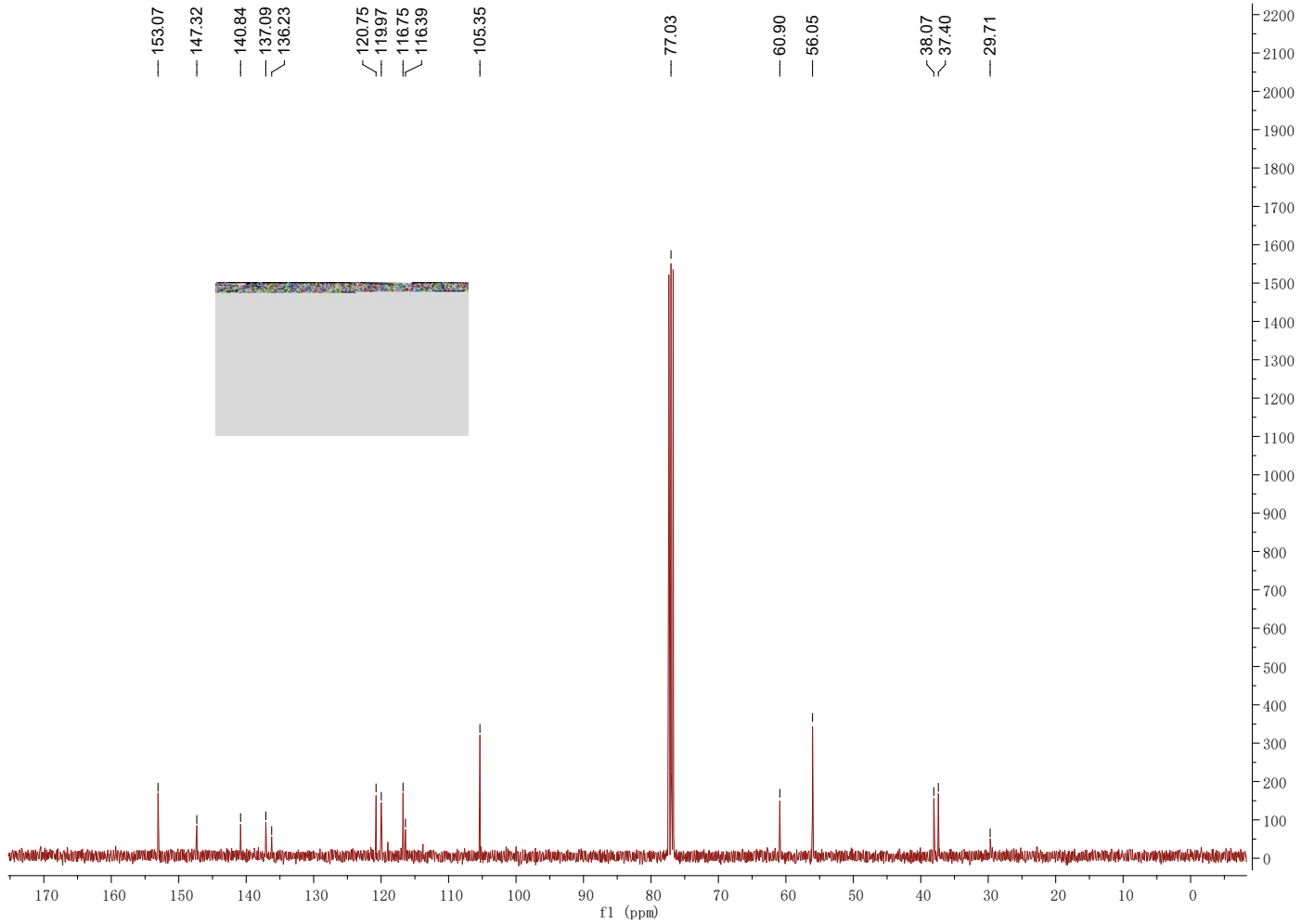


Figure S73. ^{1}H NMR of Compound 41

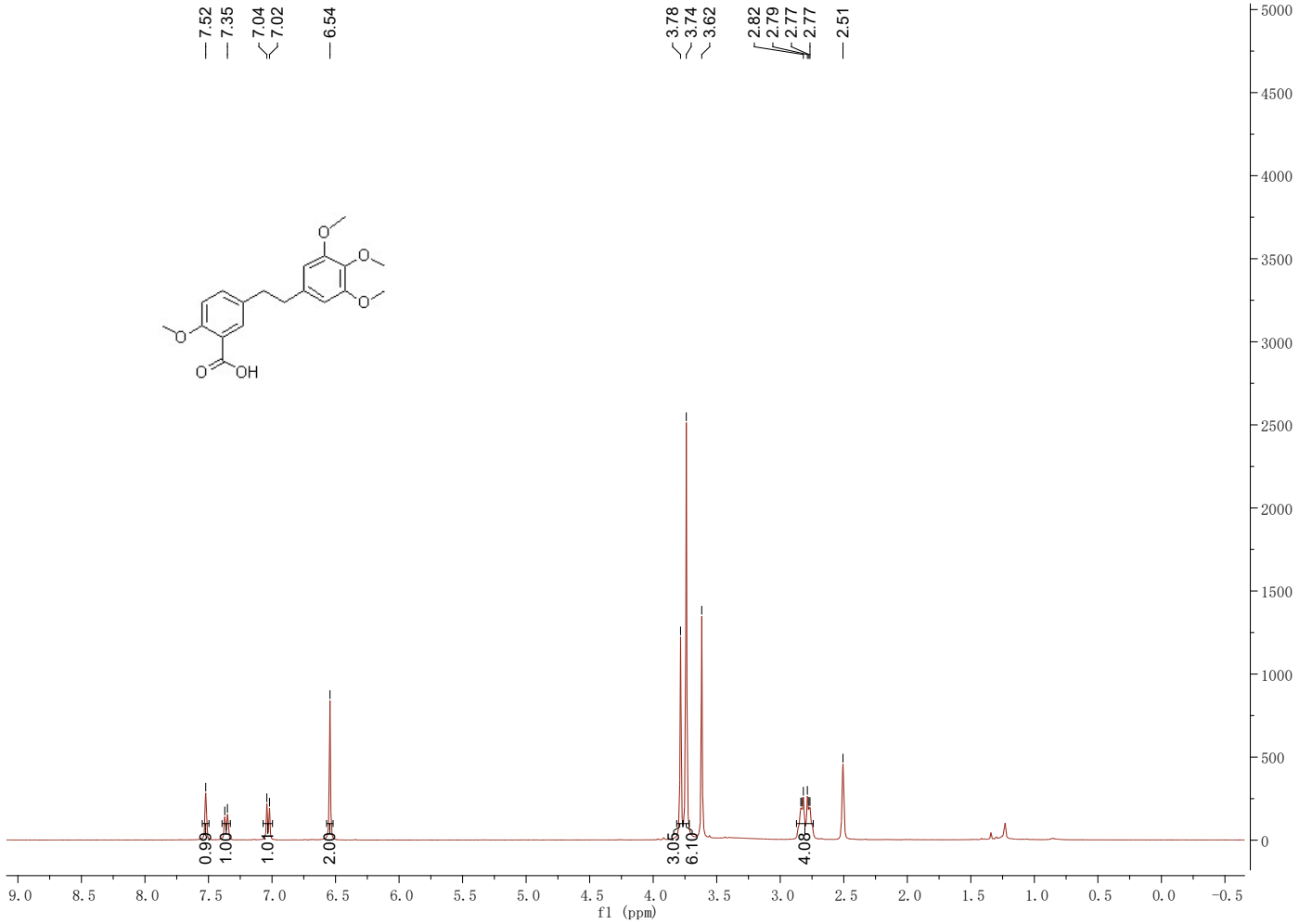


Figure S74. ^{13}C-NMR of Compound 41

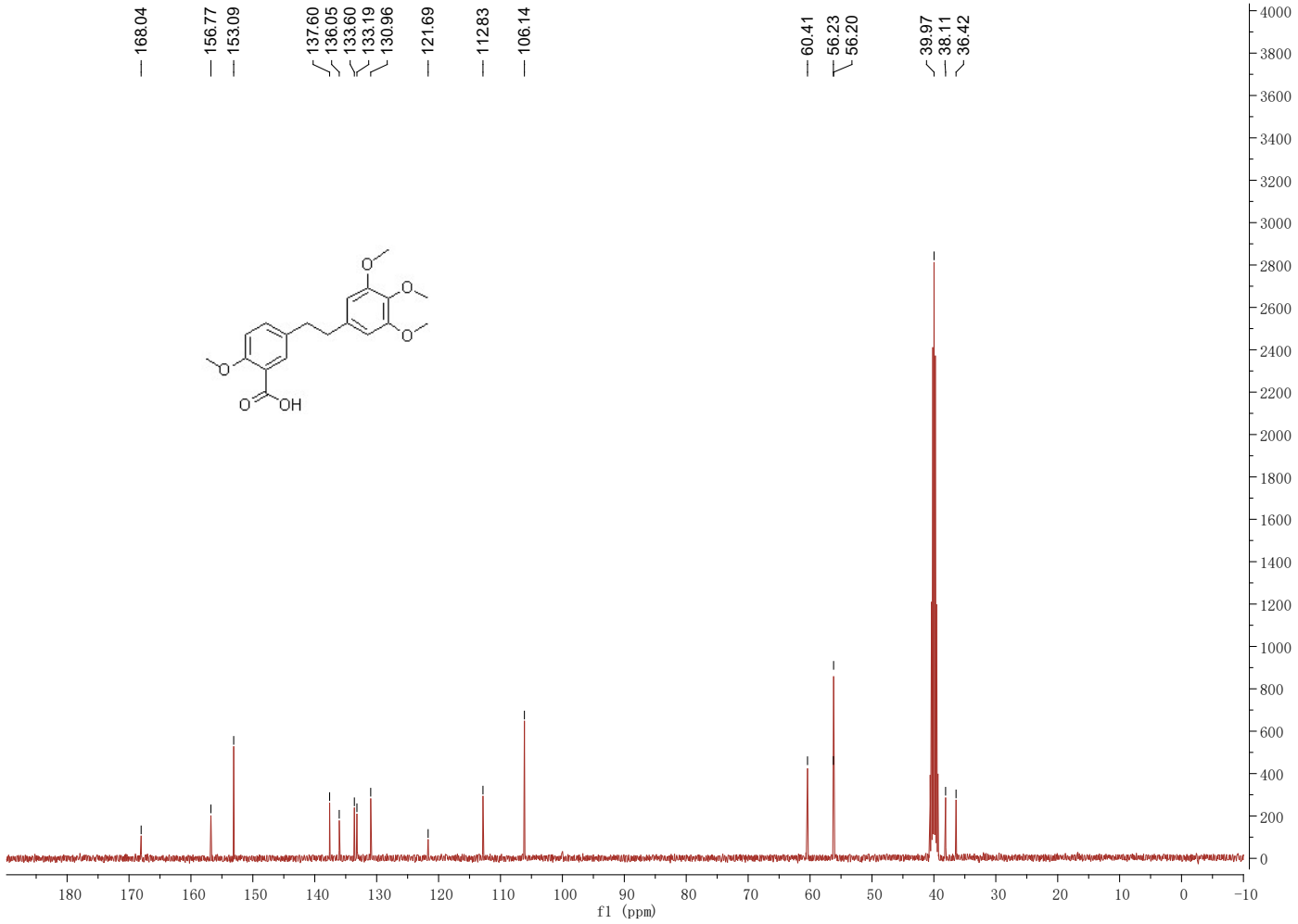


Figure S75. ^{1}H NMR of Compound 42

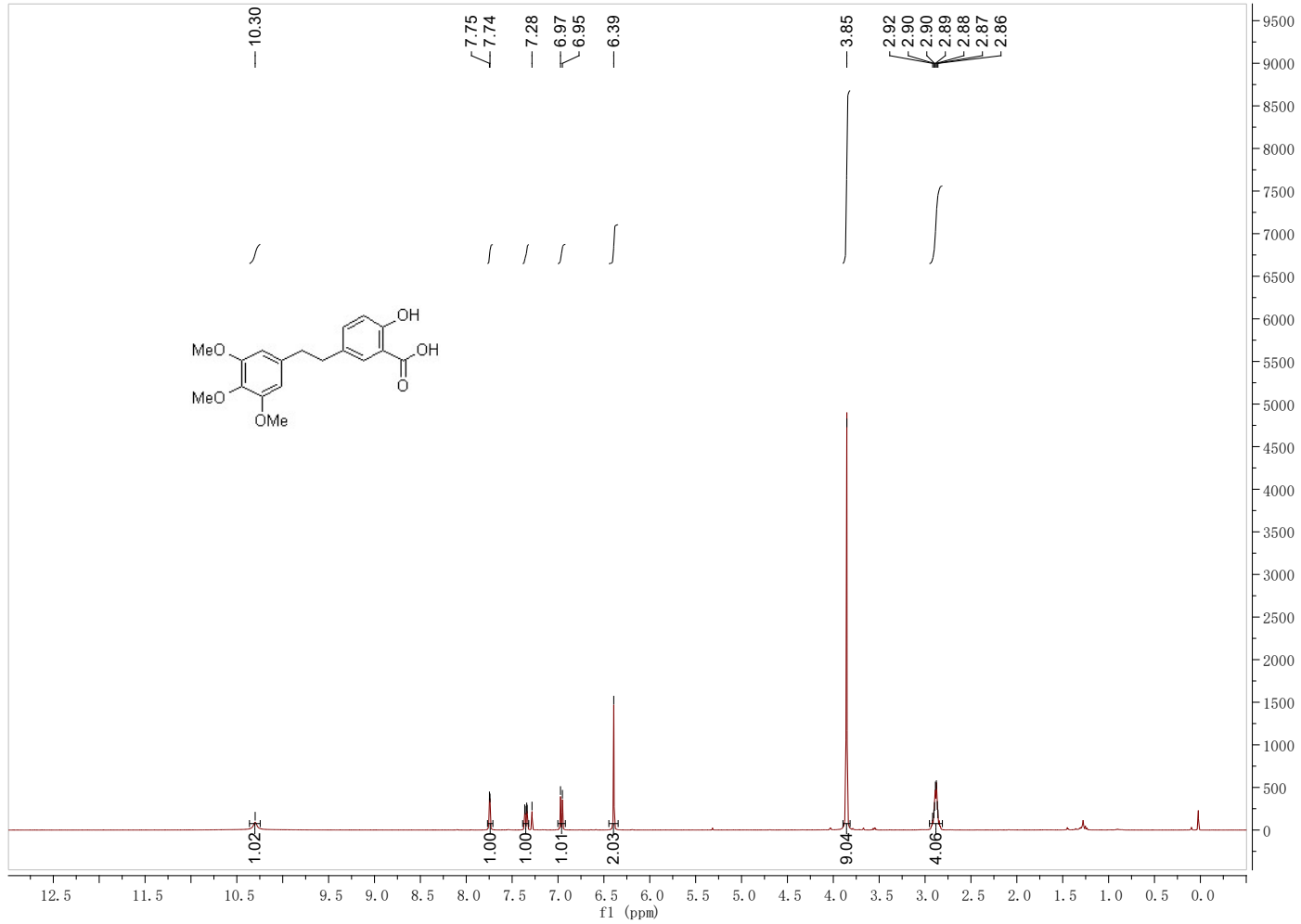


Figure S76. ^{13}C-NMR of Compound 42

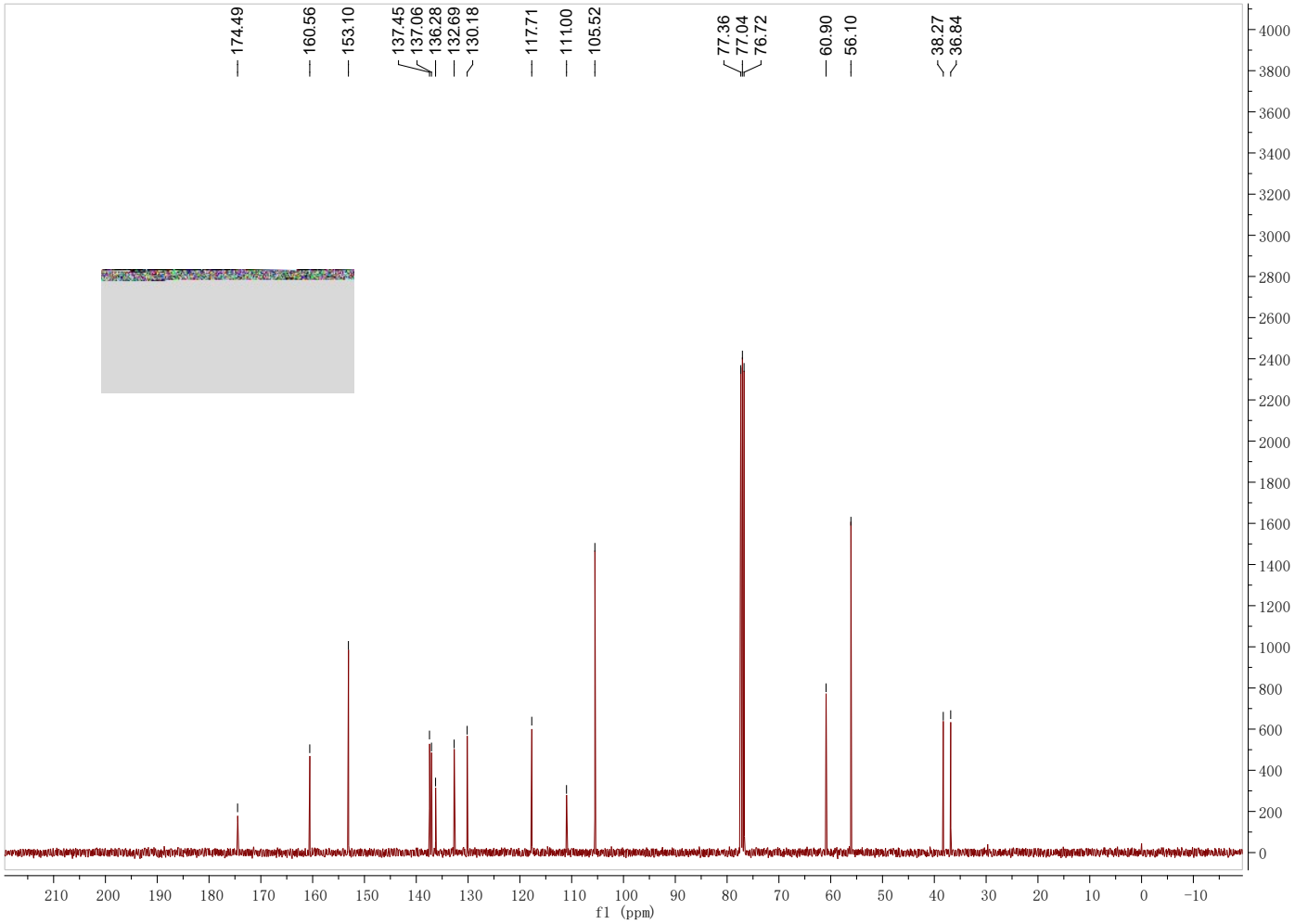


Figure S77. ^{1}H NMR of Compound 43

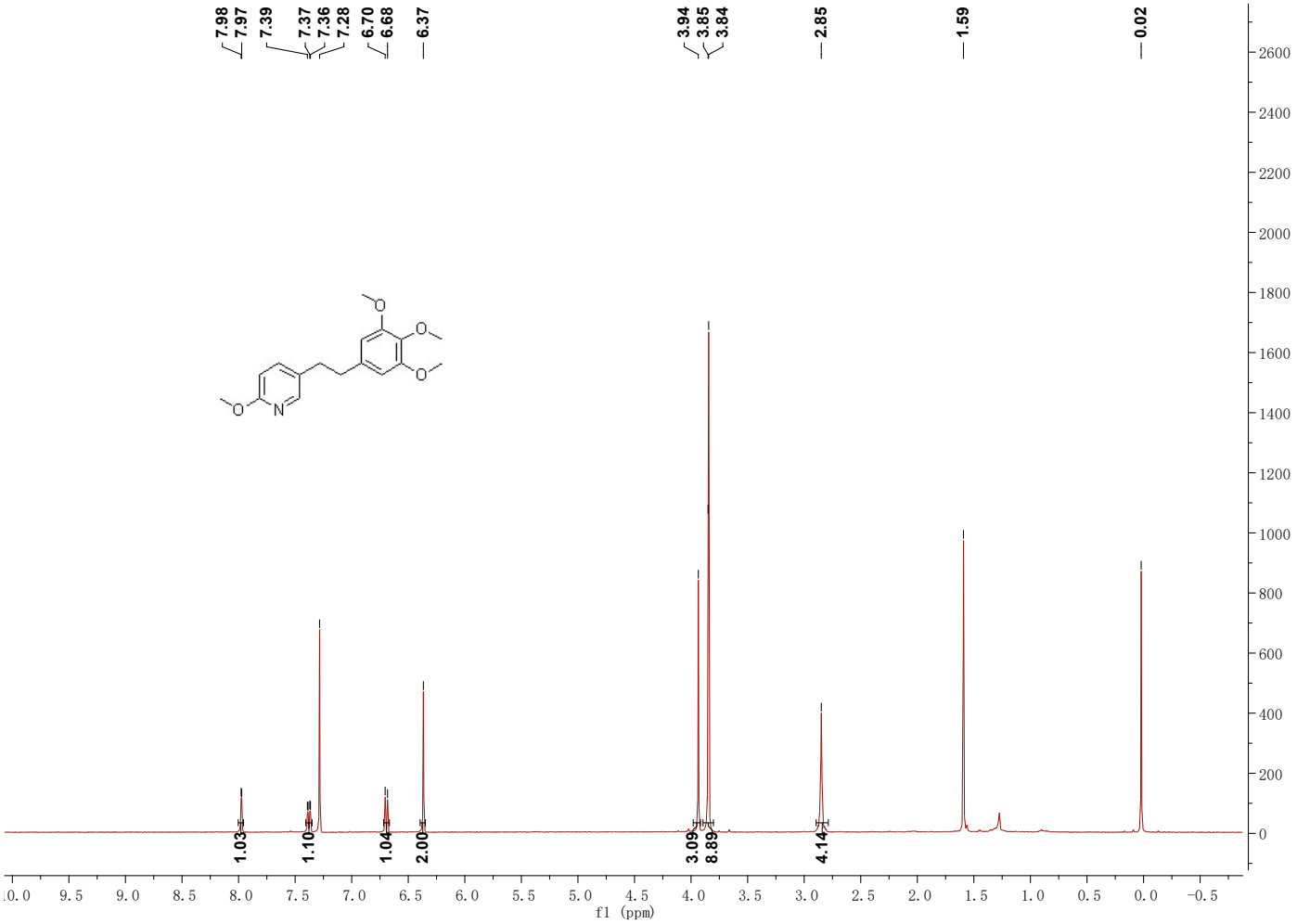


Figure S78. ^{13}C-NMR of Compound 43

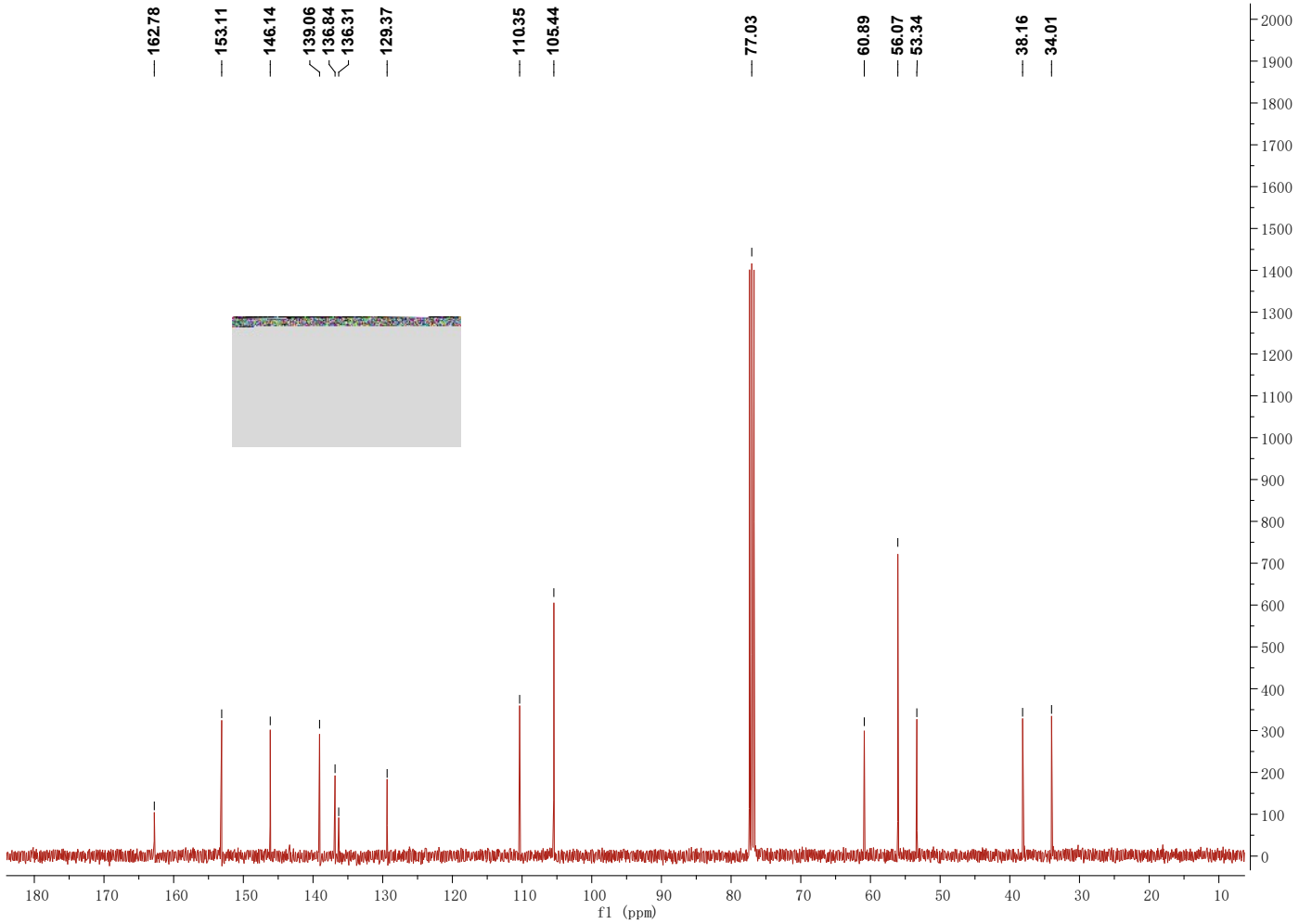


Figure S79. ^{1}H NMR of Compound 44

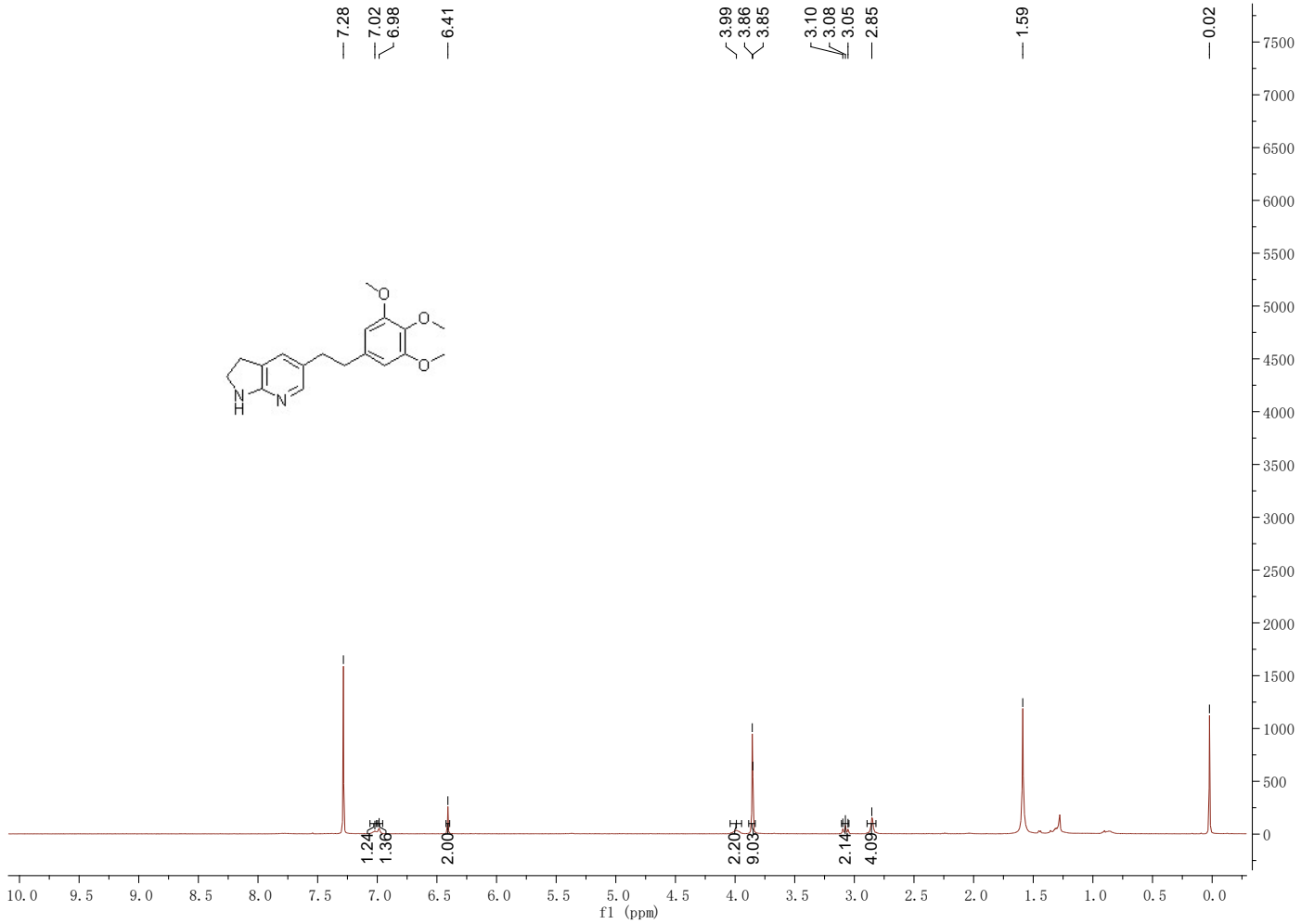


Figure S80. ^{13}C-NMR of Compound 44

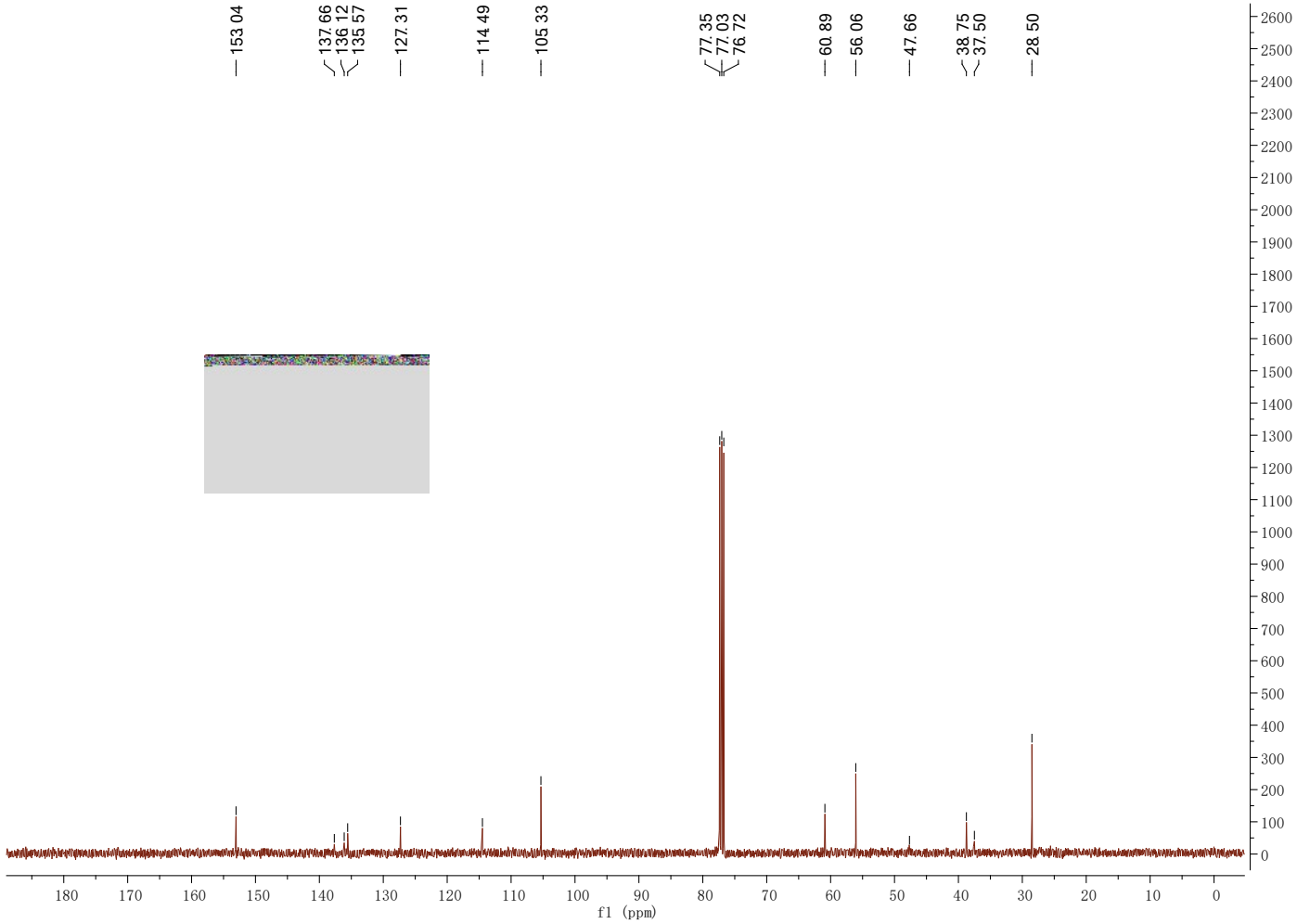


Figure S81. ^{1}H NMR of Compound 45

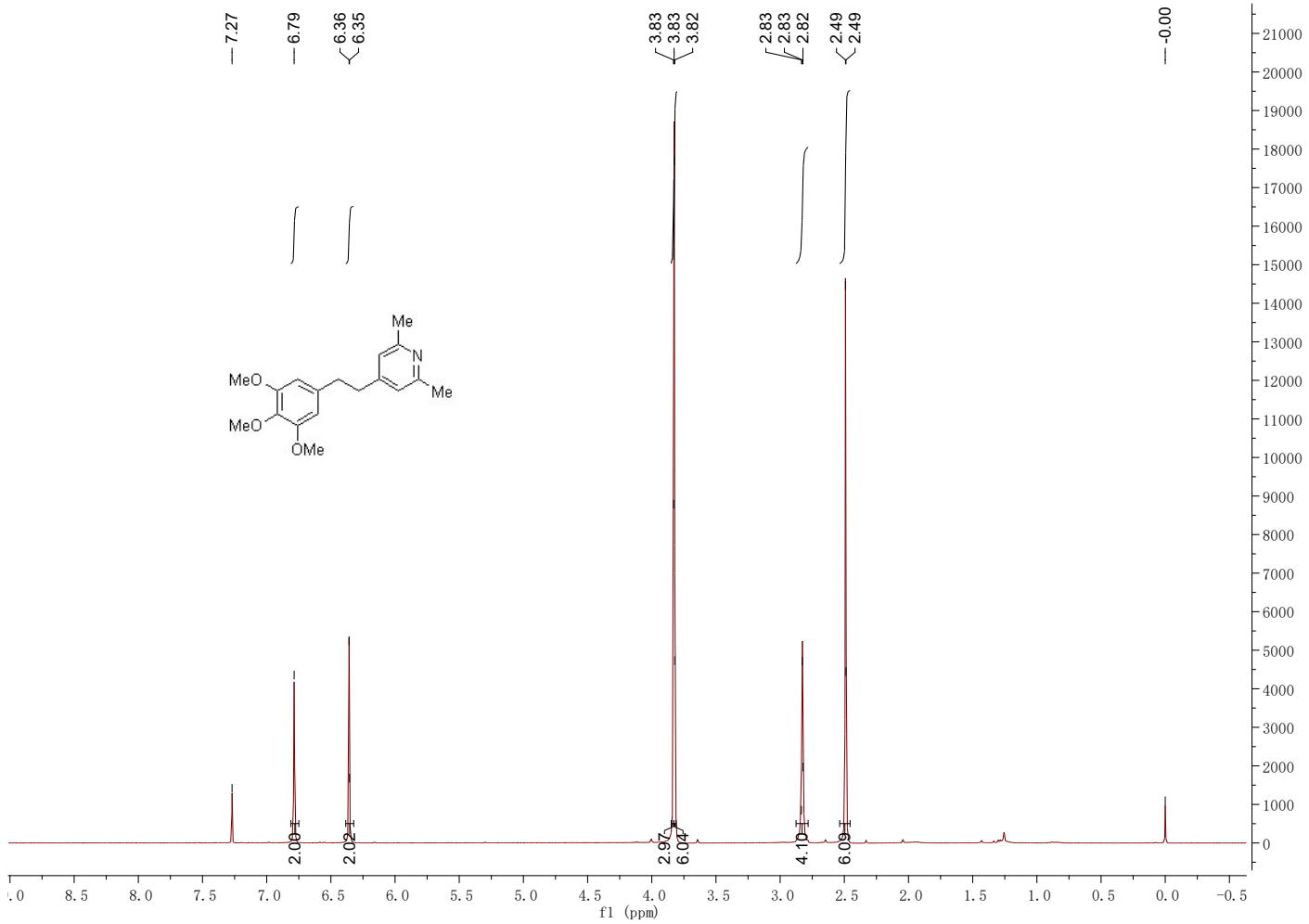


Figure S82. ^{13}C-NMR of Compound 45

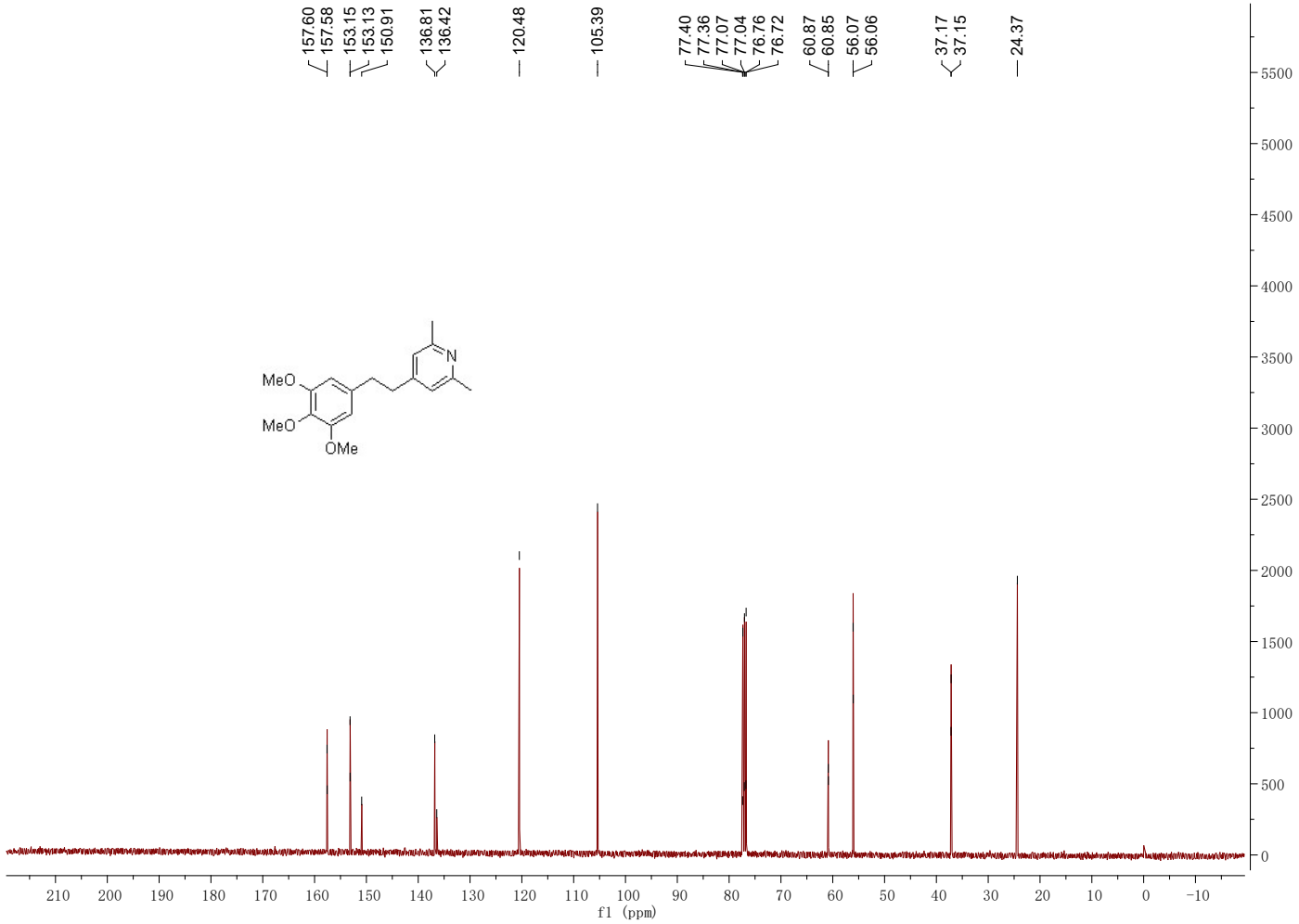


Figure S83. ^{1}H NMR of Compound 46

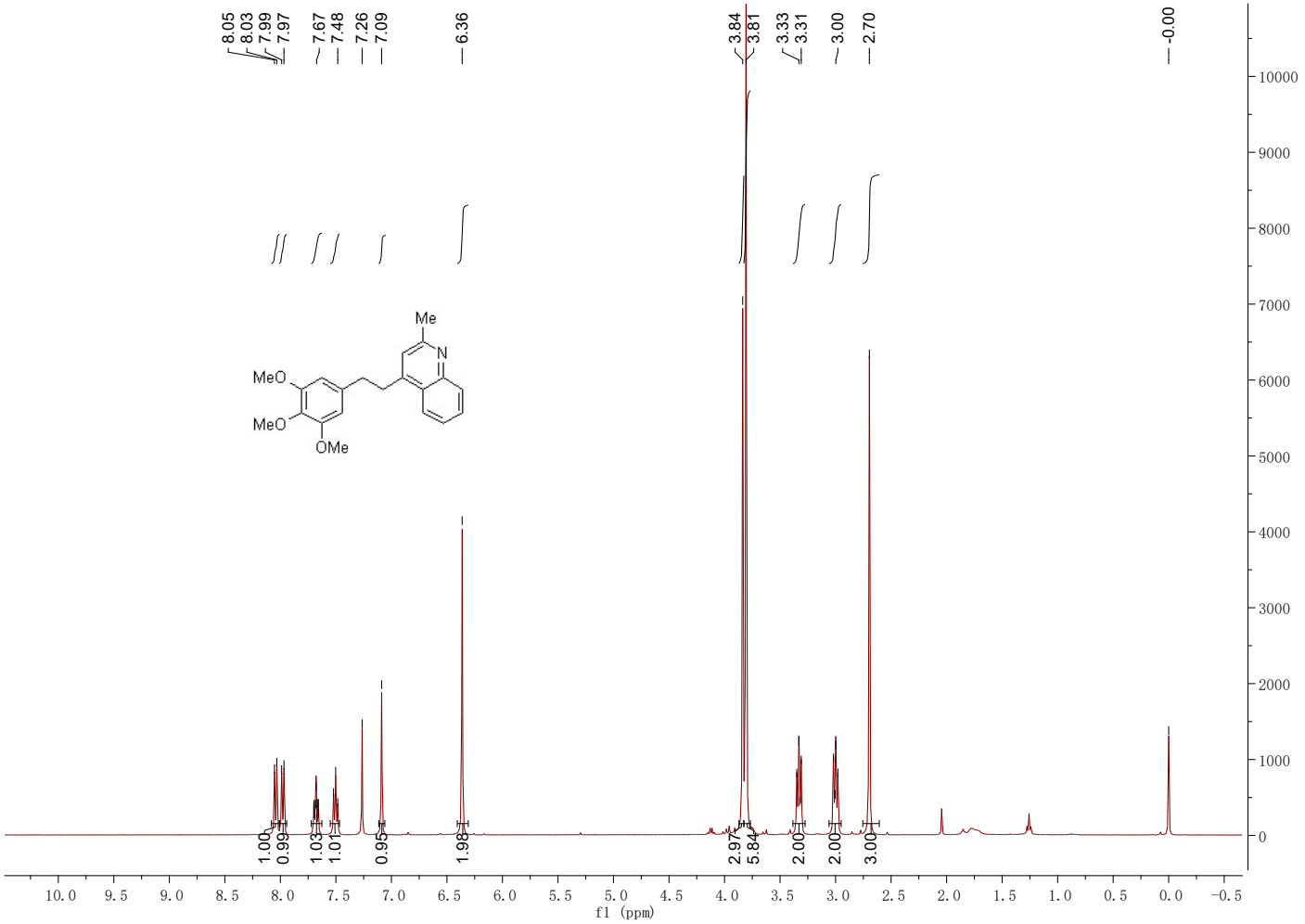


Figure S84. ^{13}C-NMR of Compound 46

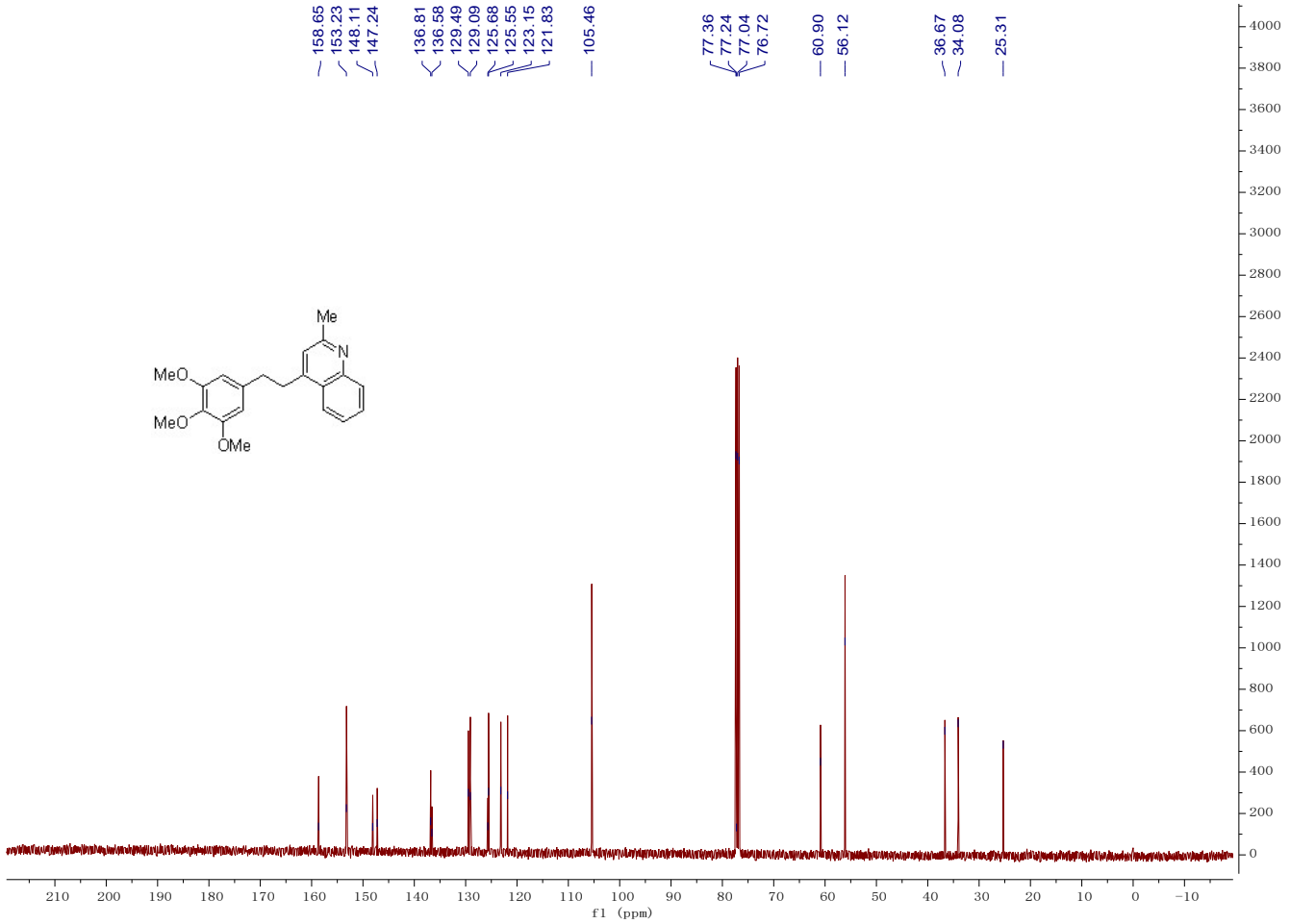


Figure S85. ^{1}H NMR of Compound 47

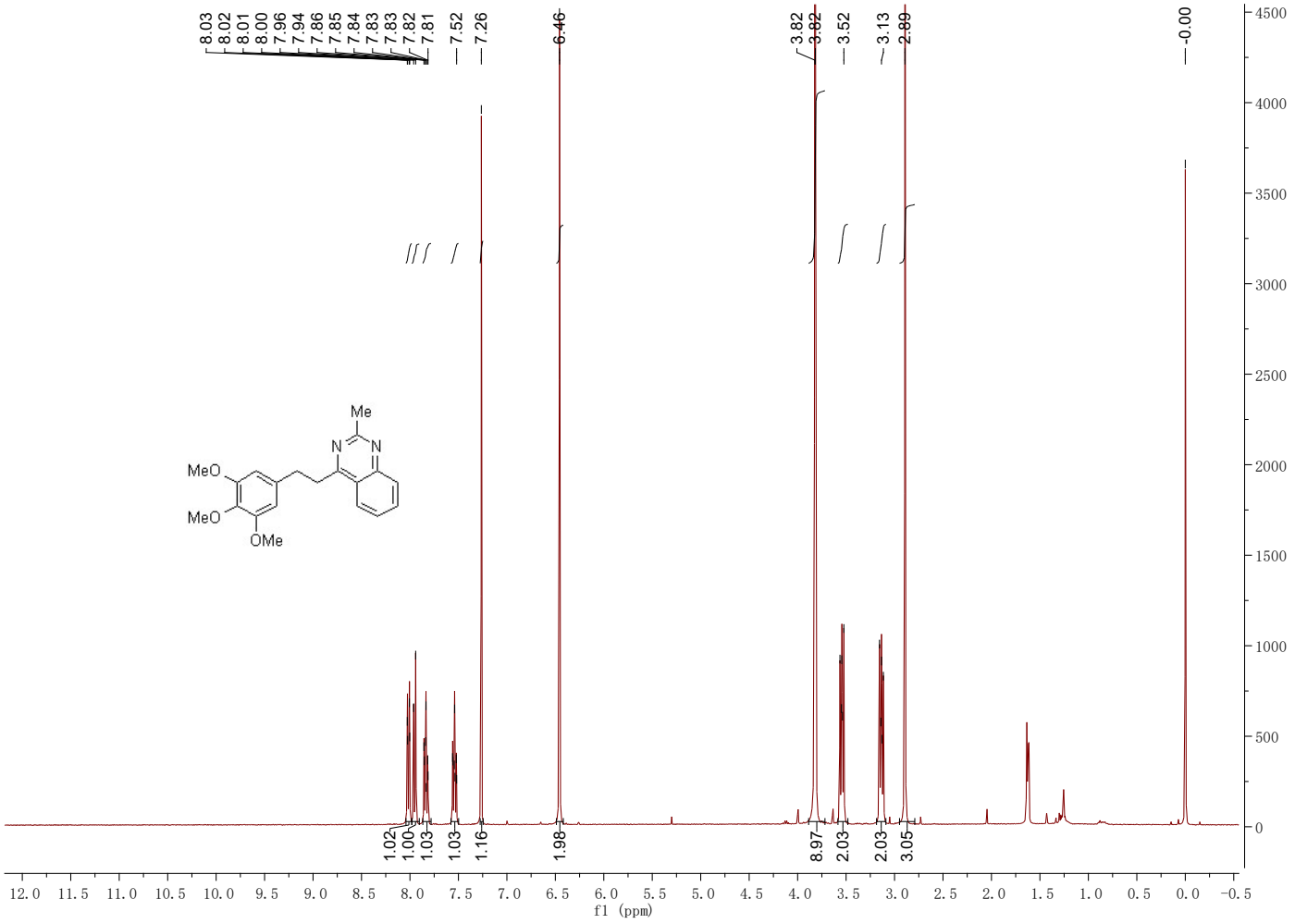
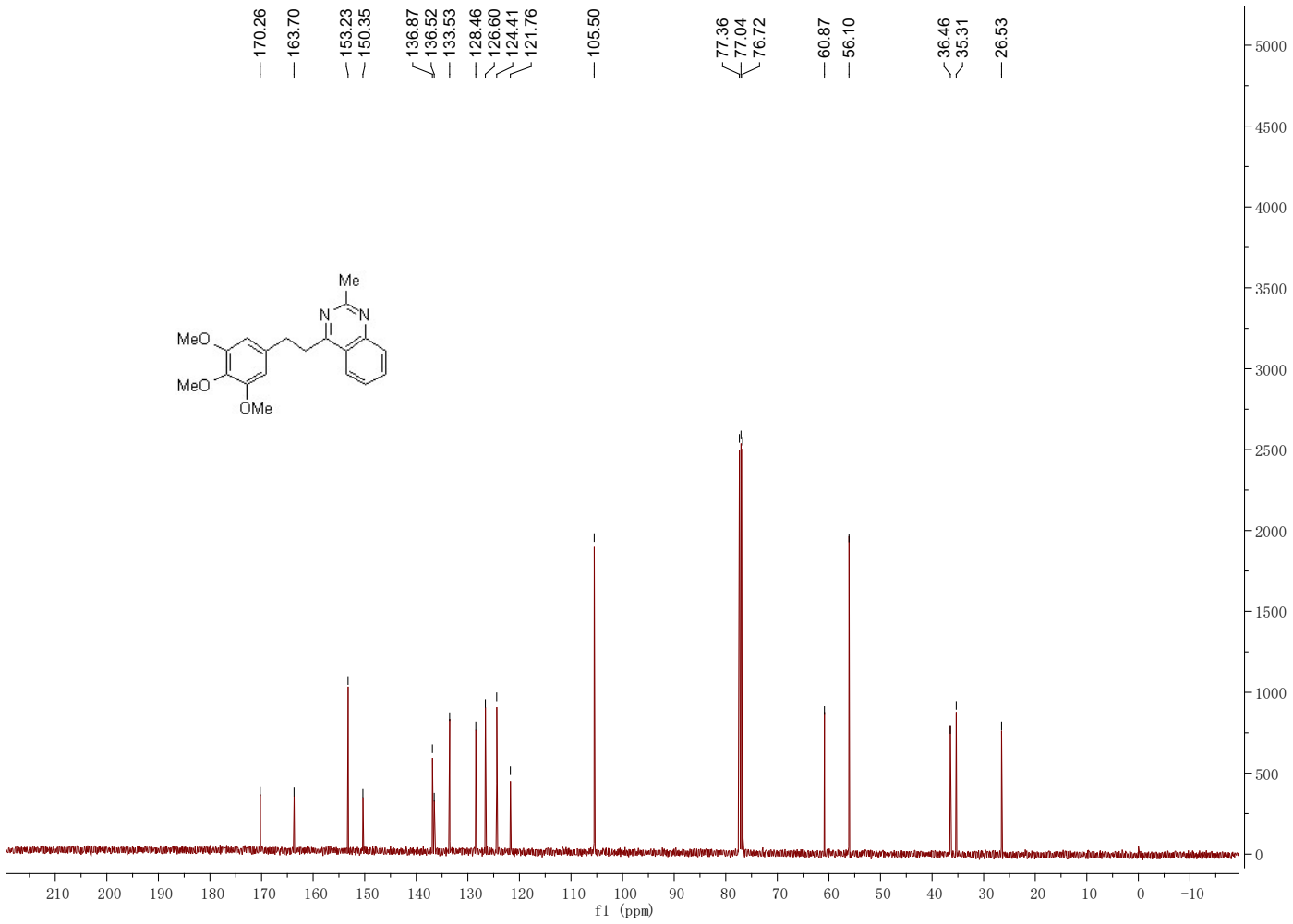


Figure S86. ^{13}C-NMR of Compound 47


170.26
163.70
153.23
150.35
136.87
136.52
133.53
128.46
126.60
124.41
121.76
105.50
77.36
77.04
76.72
60.87
56.10
36.46
35.31
26.53
Me
N
N
MeO
MeO
OMe
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

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