

Supporting Information

**Development of tumor-targeting aza-vesamicol derivatives with high affinity for sigma
receptors for cancer theranostics**

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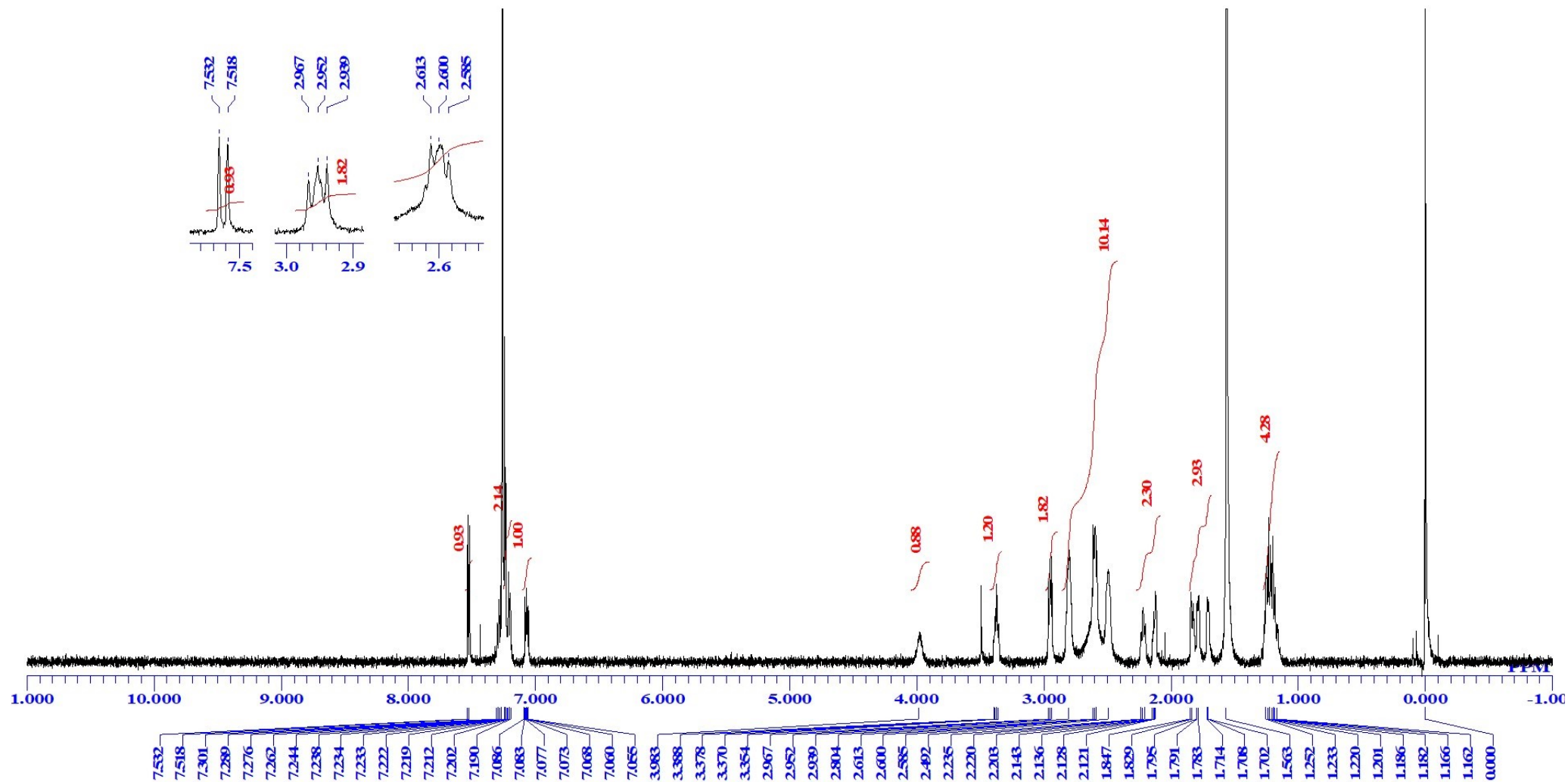
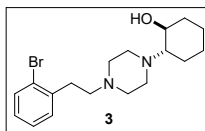
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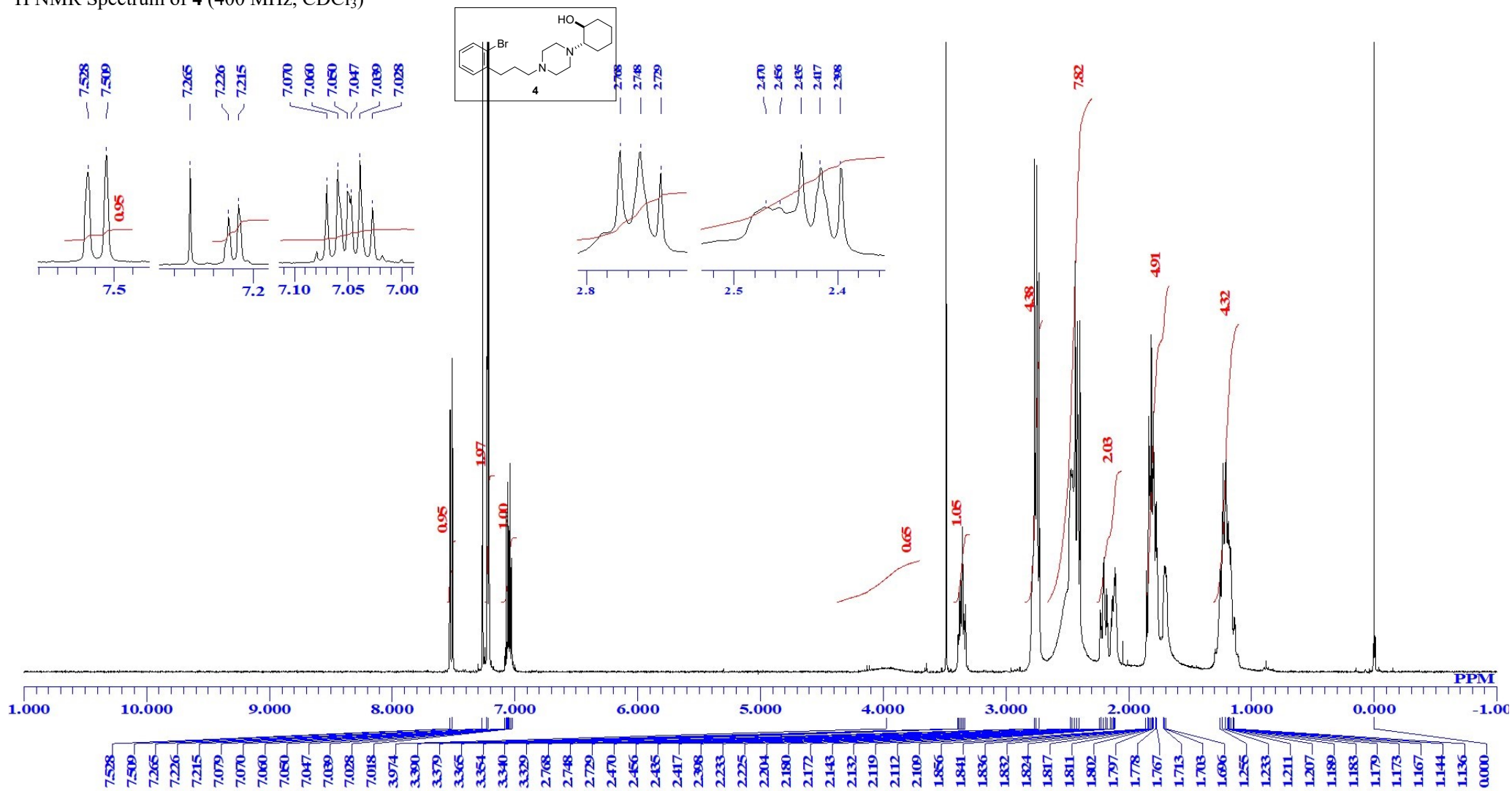
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1. NMR spectra

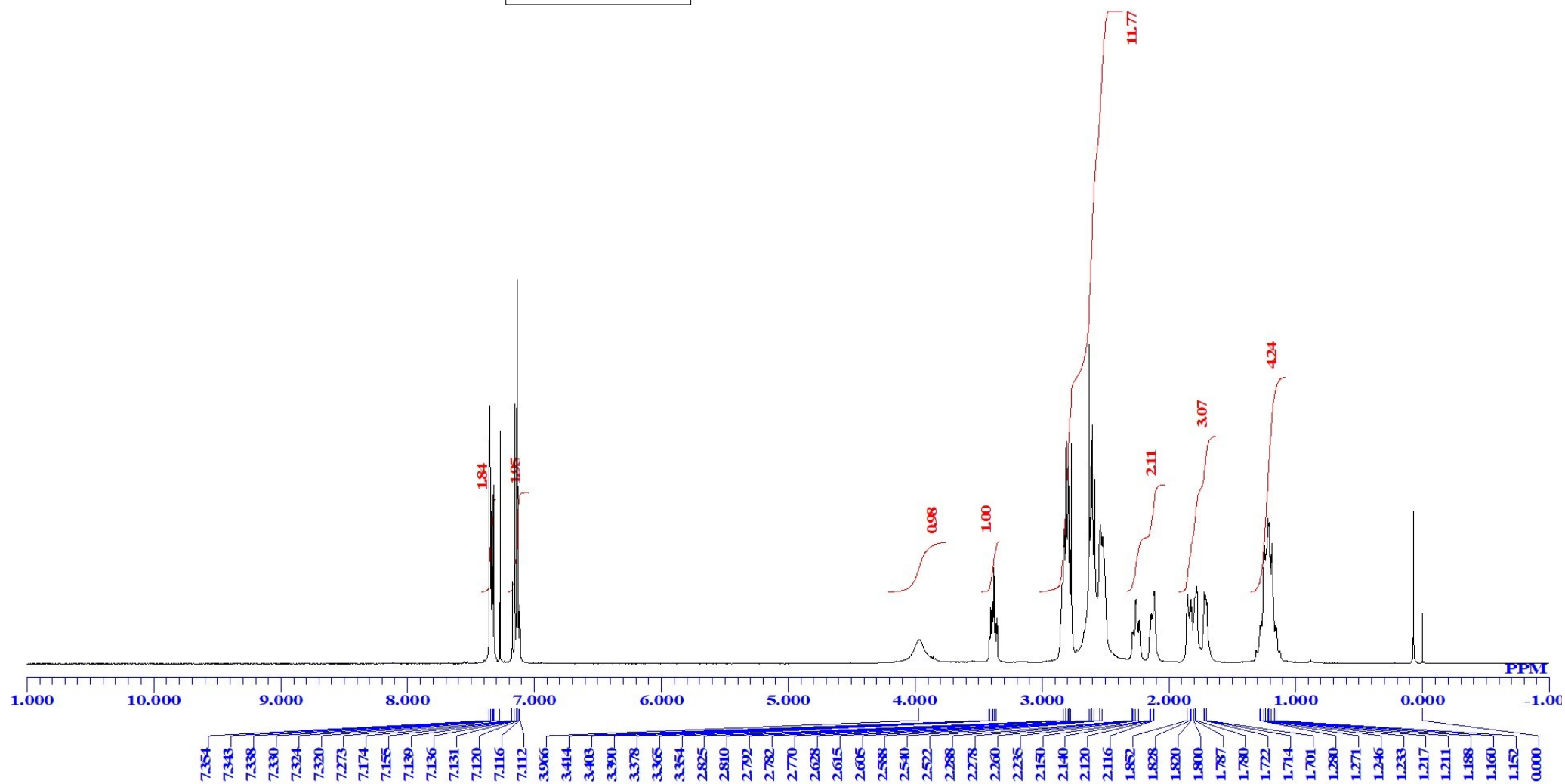
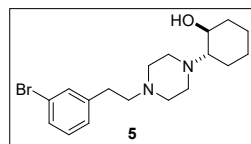
^1H NMR Spectrum of **3** (600 MHz, CDCl_3)



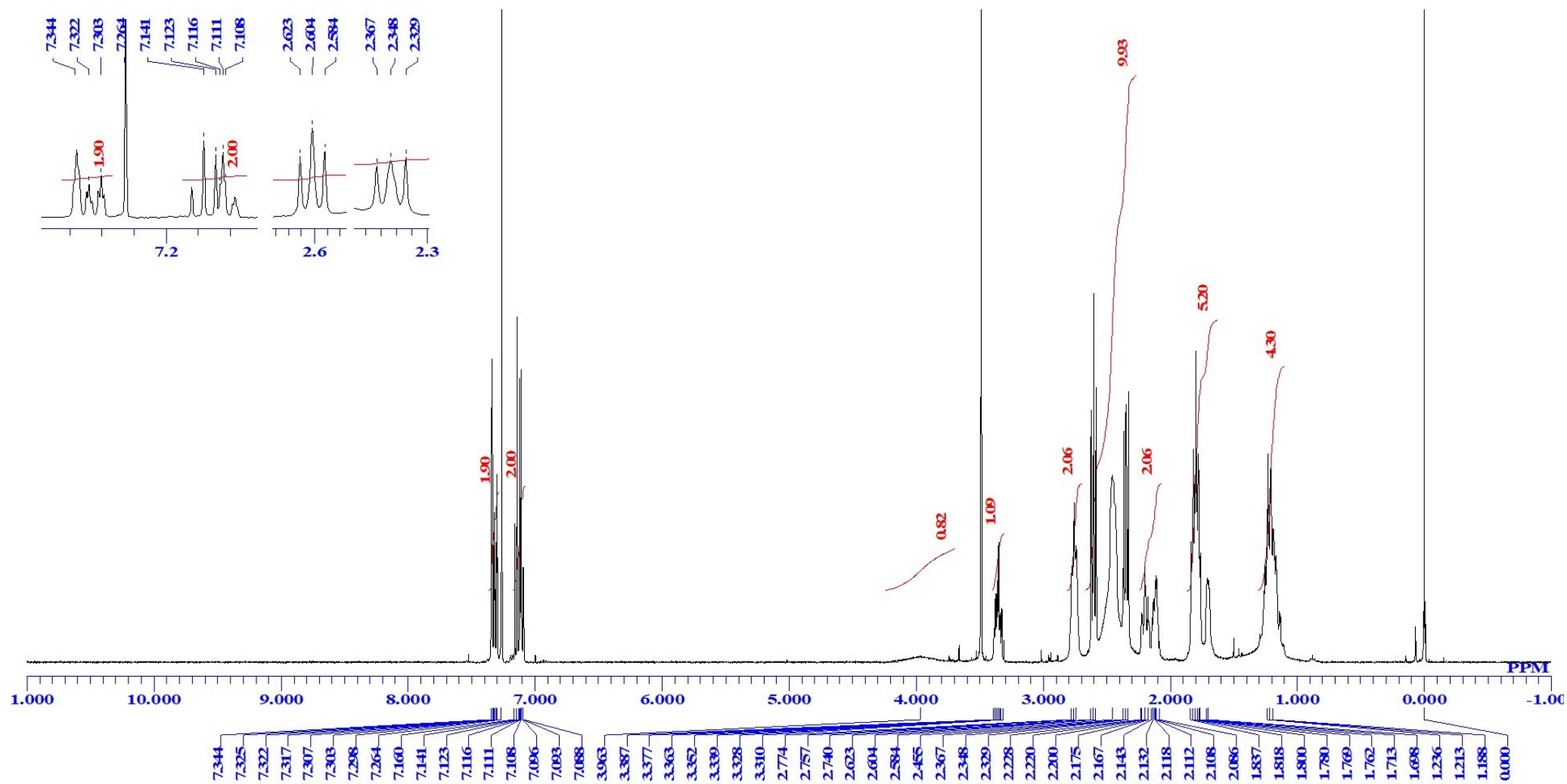
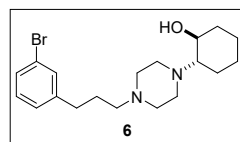
¹H NMR Spectrum of 4 (400 MHz, CDCl₃)



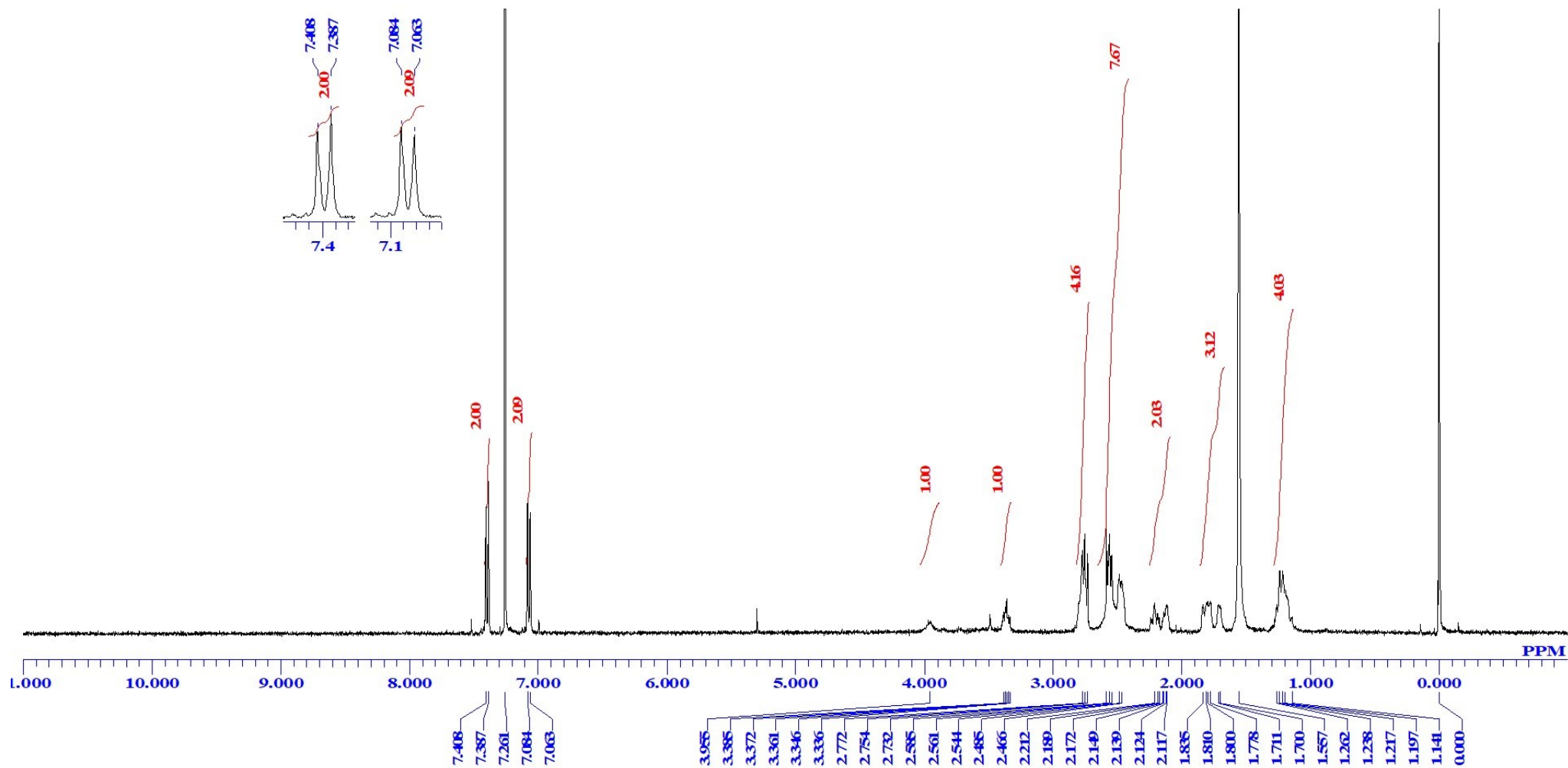
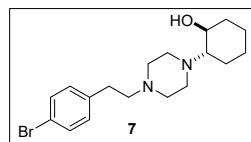
¹H NMR Spectrum of **5** (400 MHz, CDCl₃)



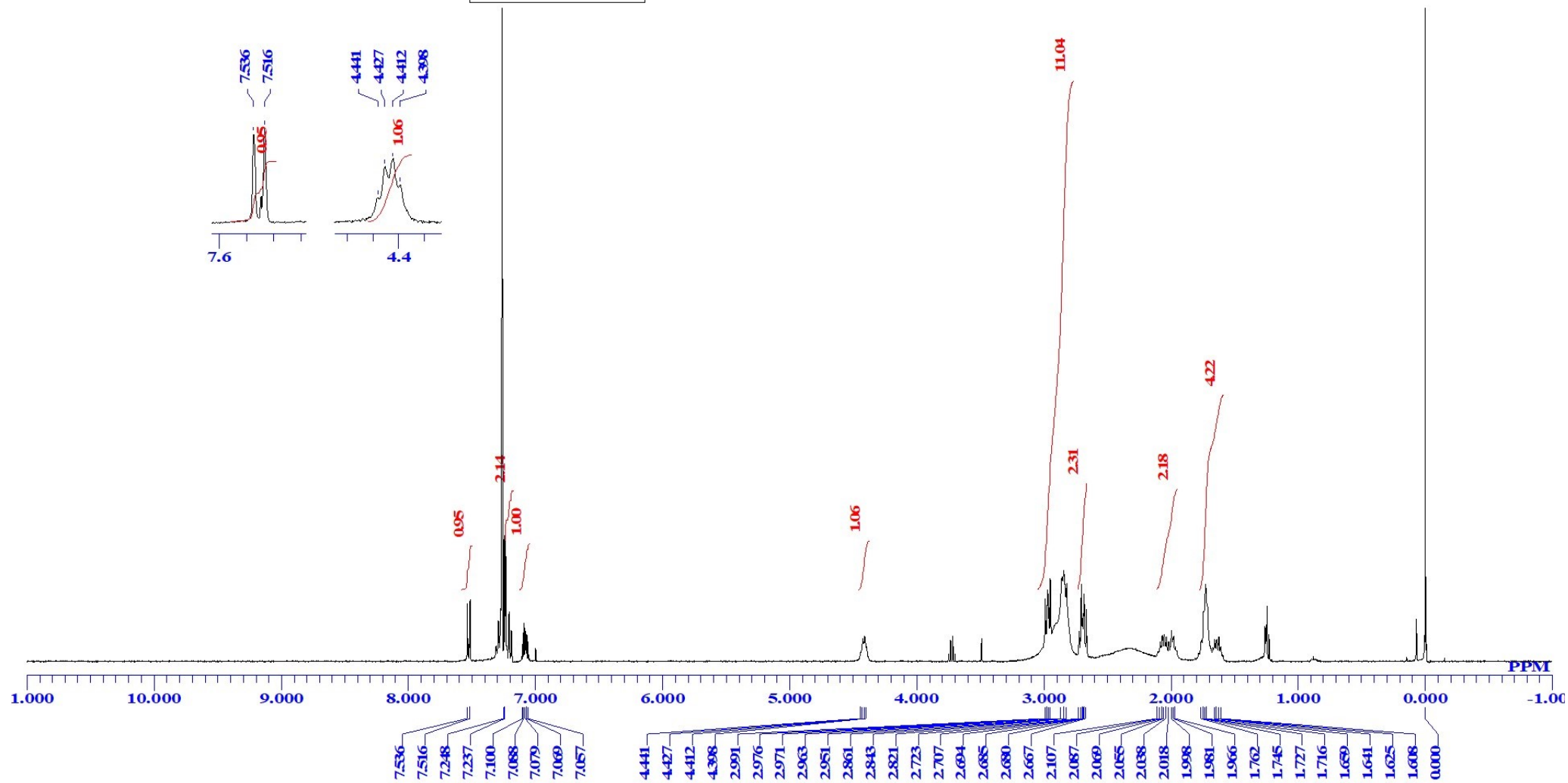
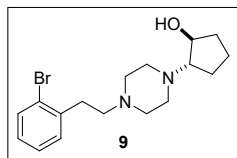
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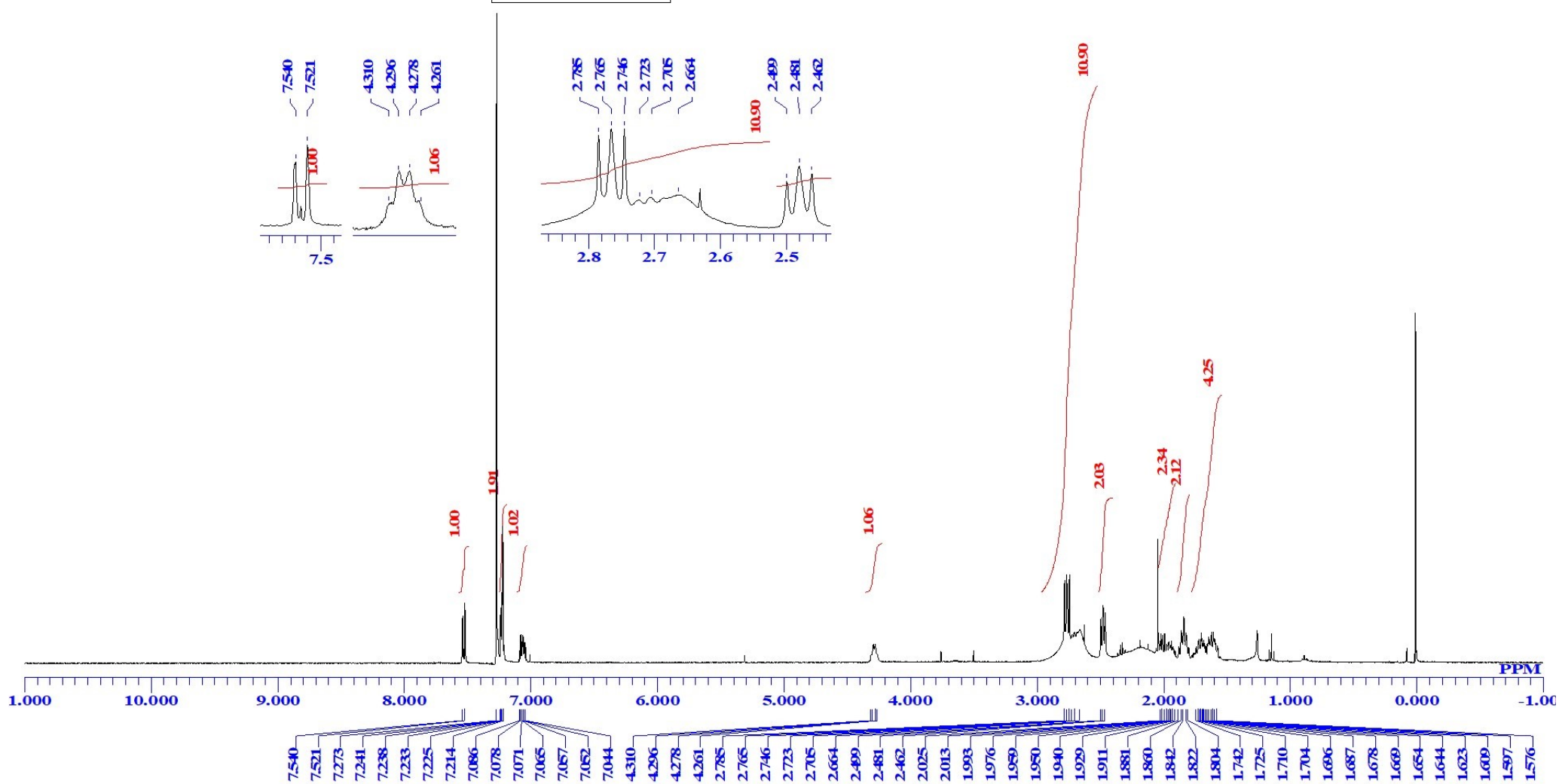
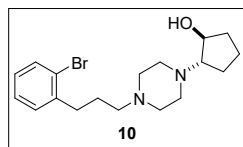
^1H NMR Spectrum of 7 (400 MHz, CDCl_3)



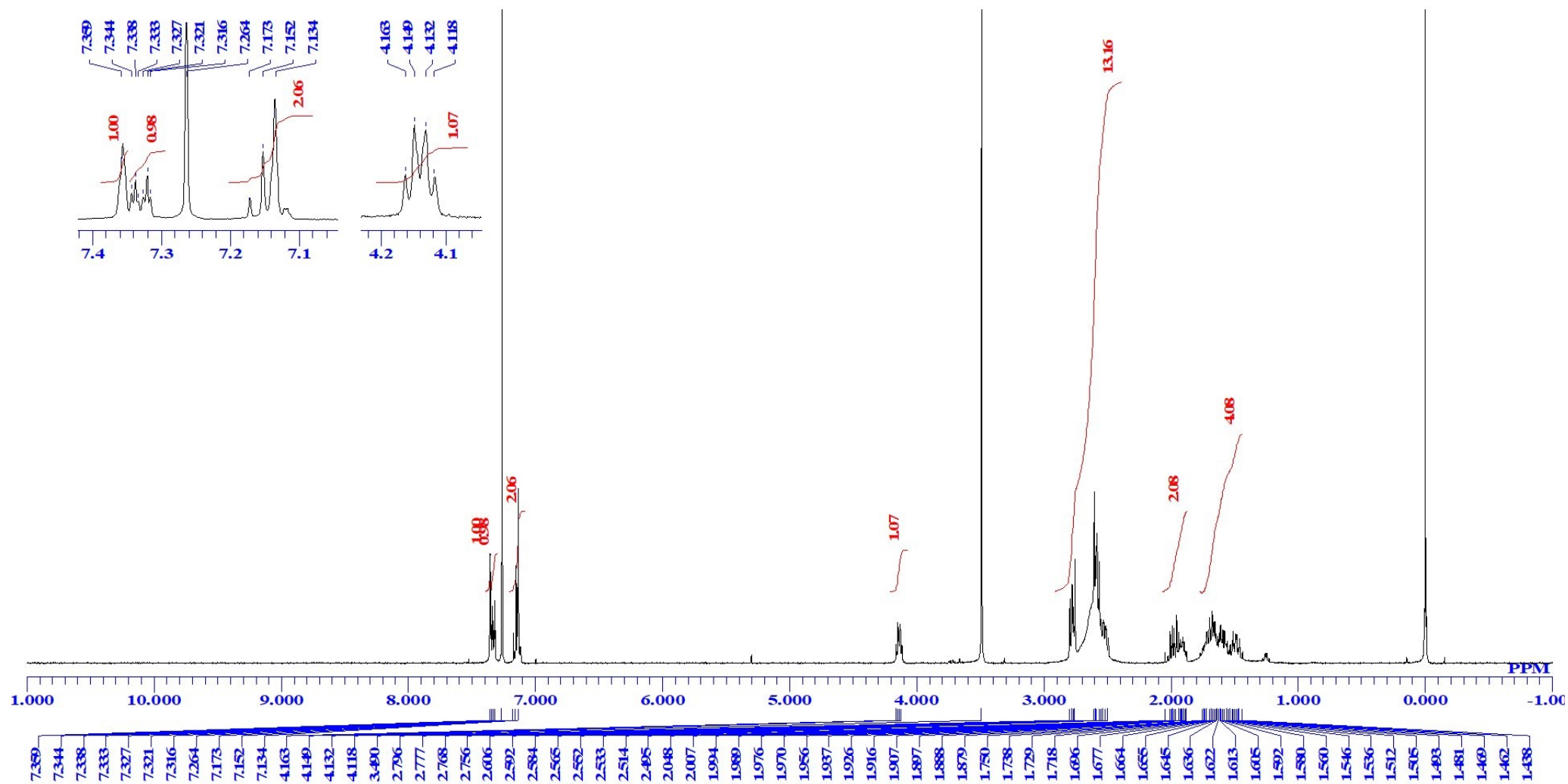
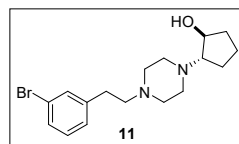
^1H NMR Spectrum of **9** (400 MHz, CDCl_3)



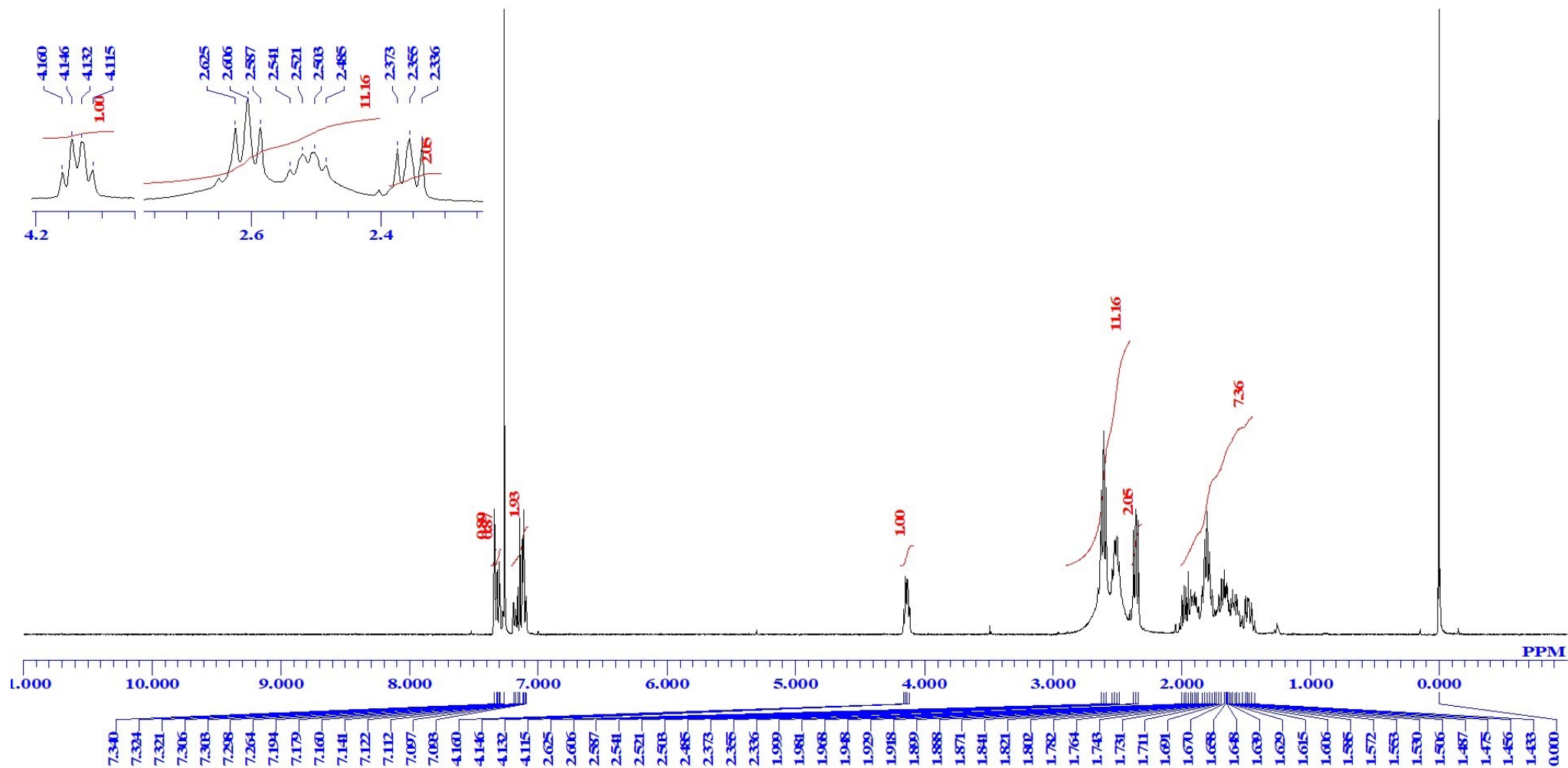
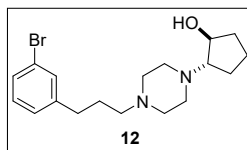
¹H NMR Spectrum of **10** (400 MHz, CDCl₃)



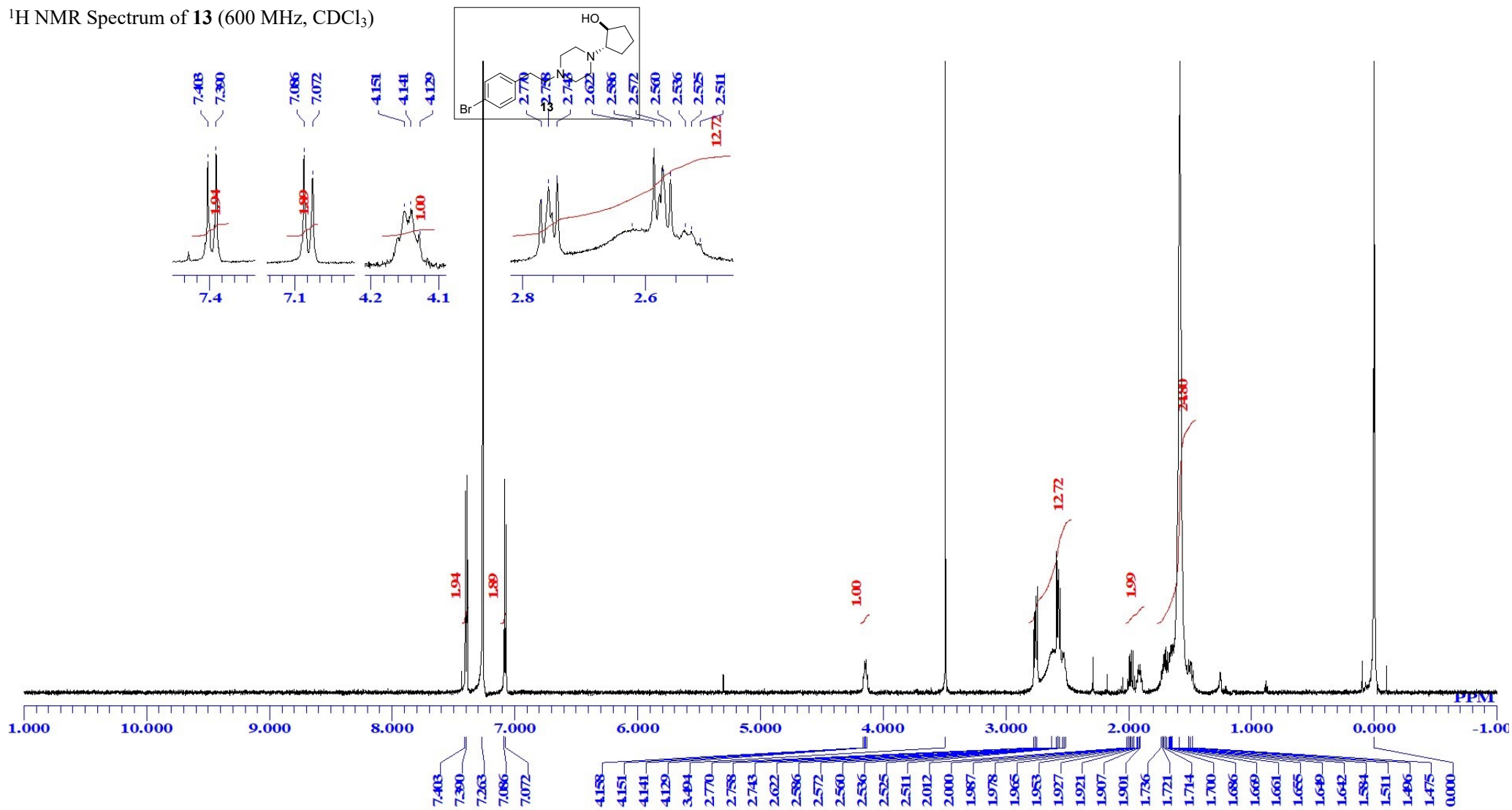
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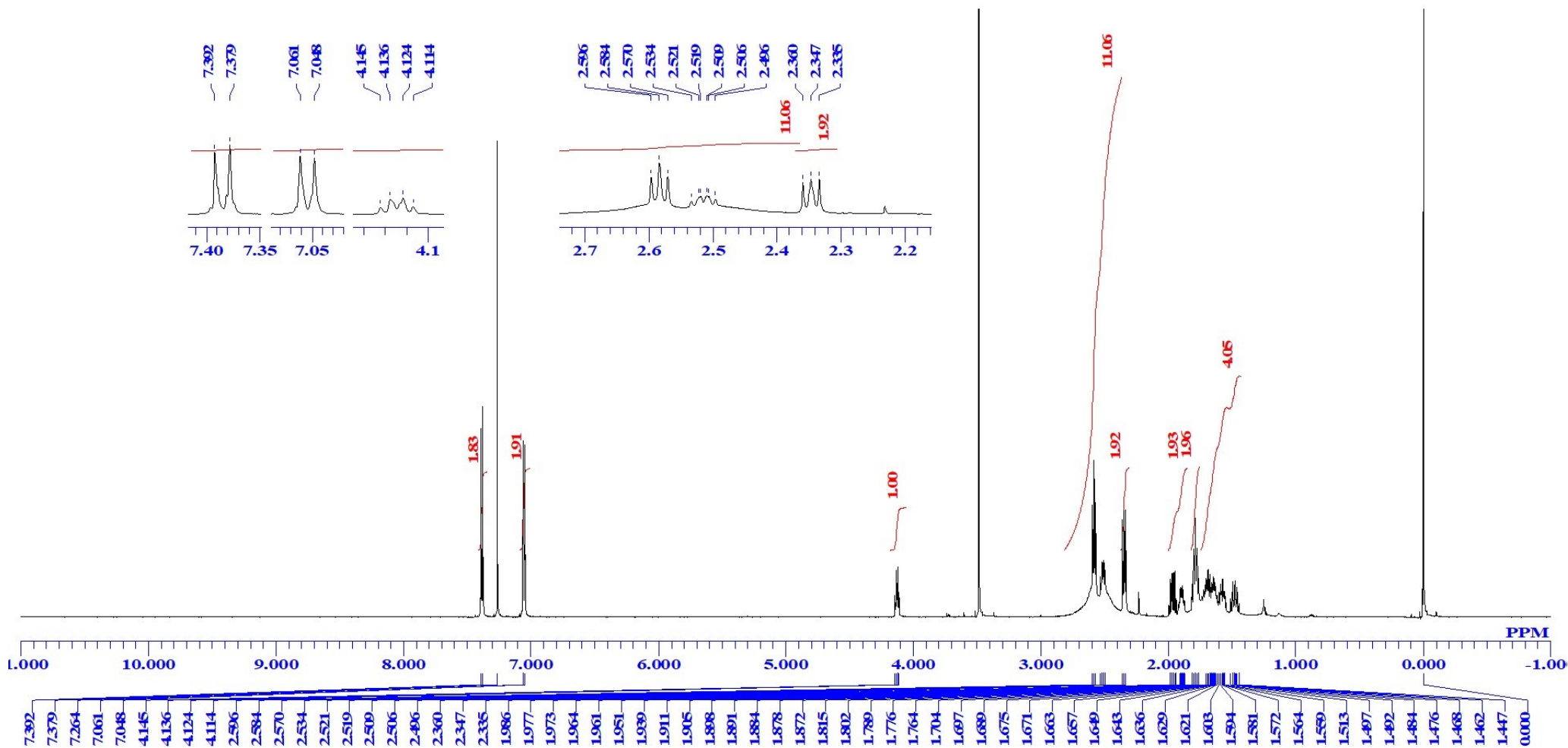
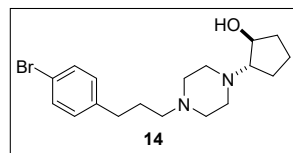
¹H NMR Spectrum of **12** (400 MHz, CDCl₃)



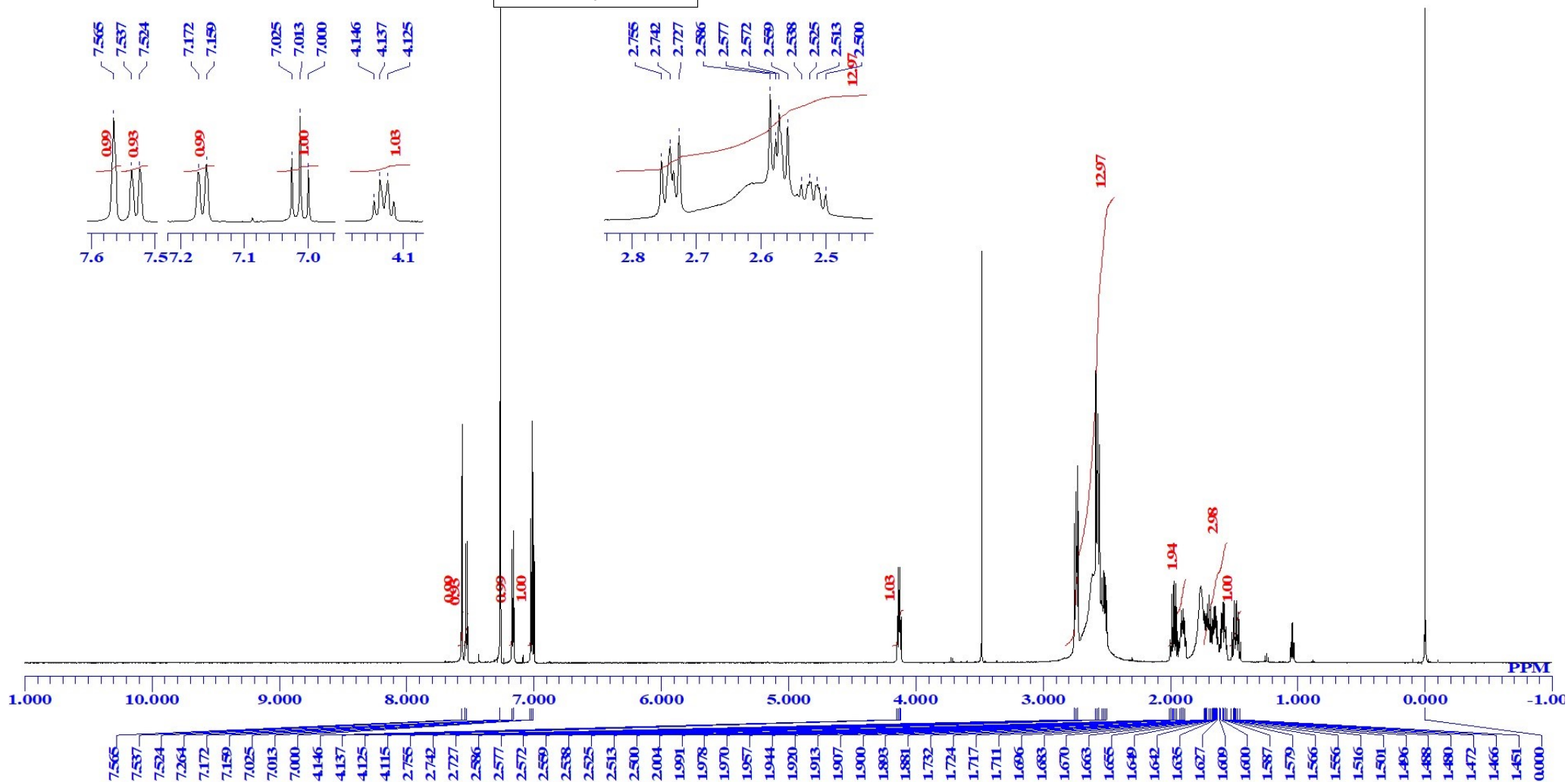
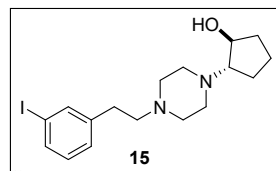
¹H NMR Spectrum of **13** (600 MHz, CDCl₃)



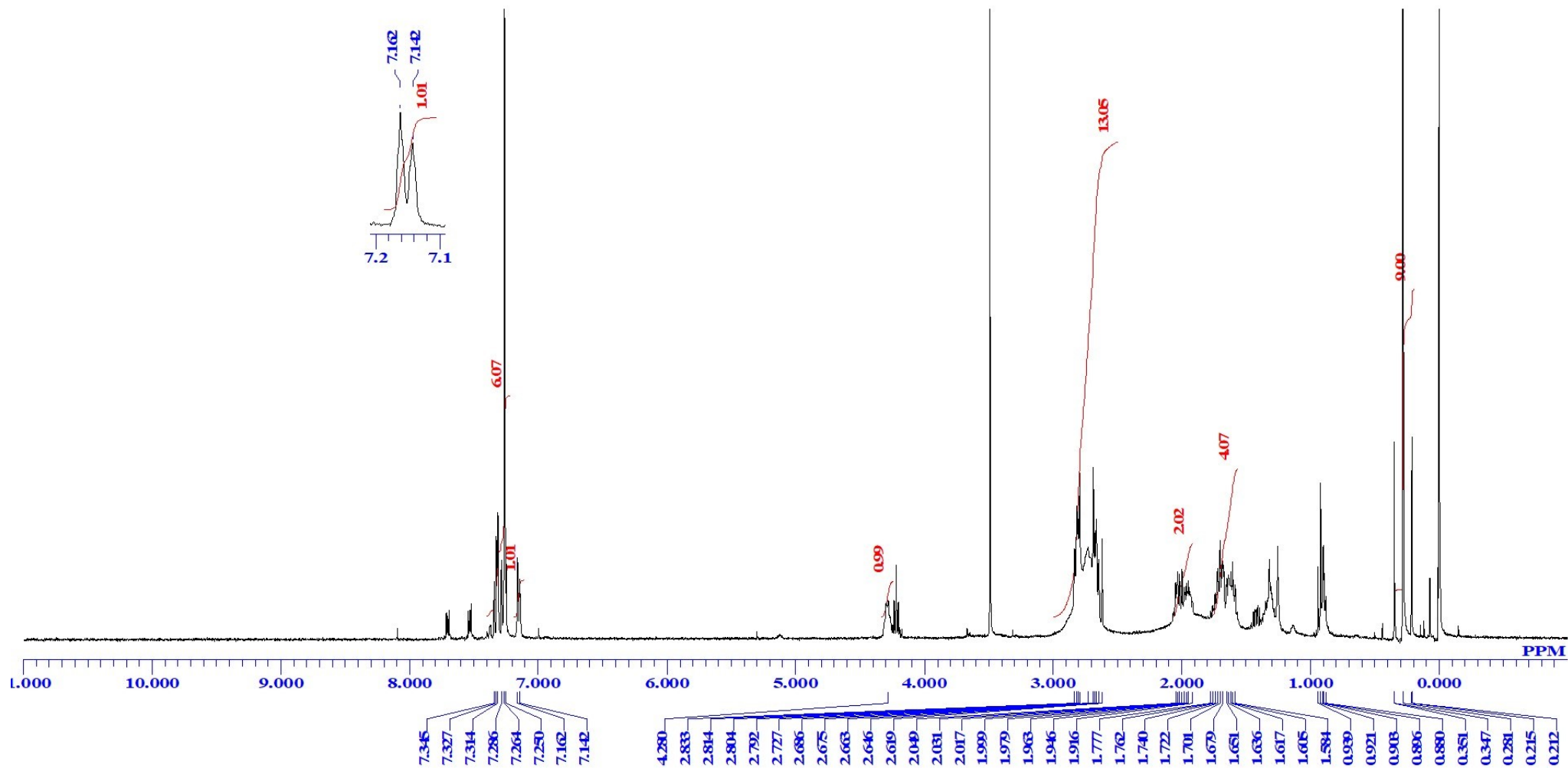
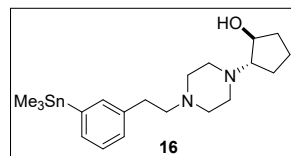
^1H NMR Spectrum of **14** (600 MHz, CDCl_3)



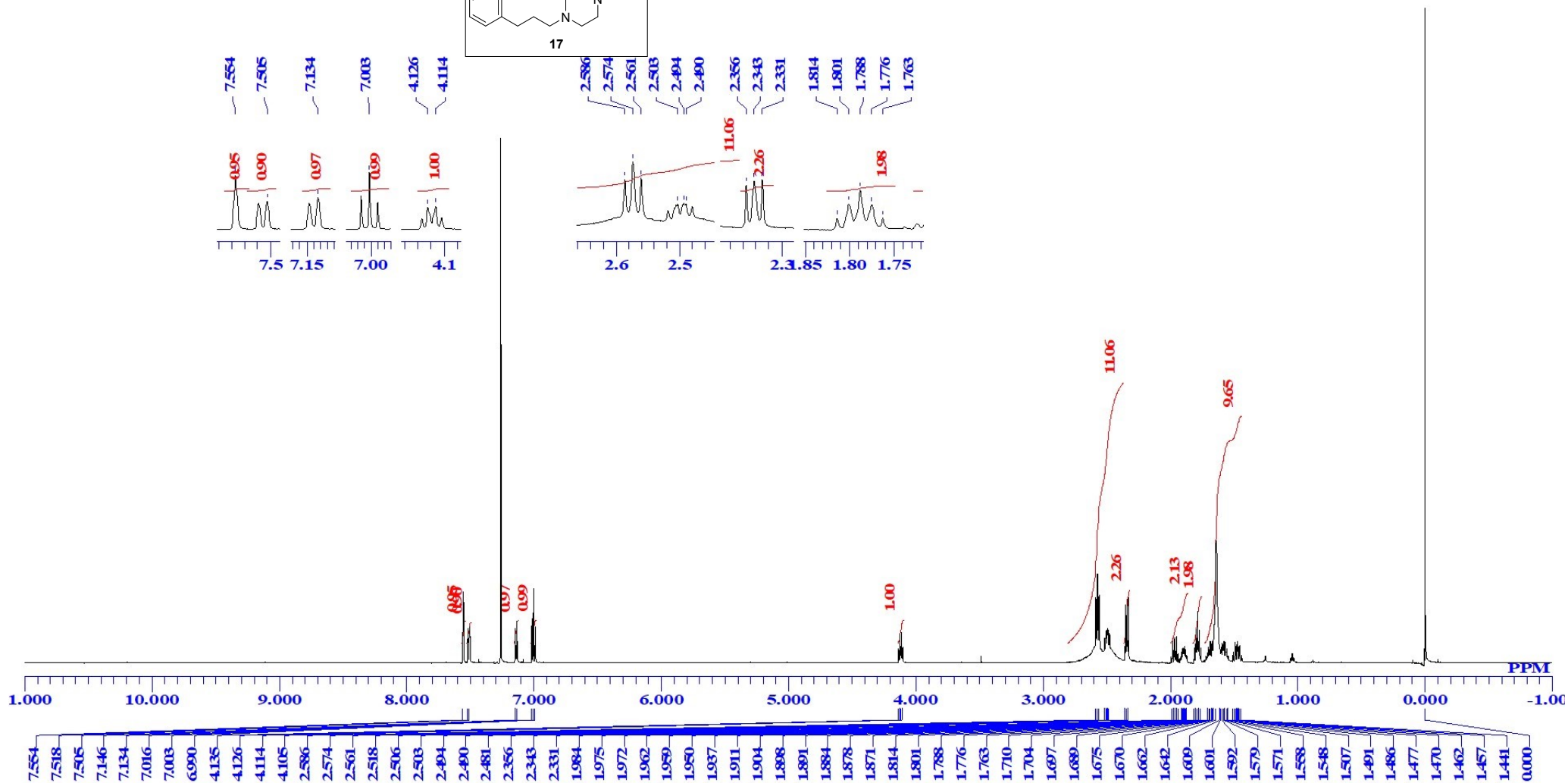
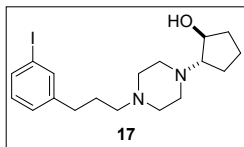
^1H NMR Spectrum of **15** (600 MHz, CDCl_3)



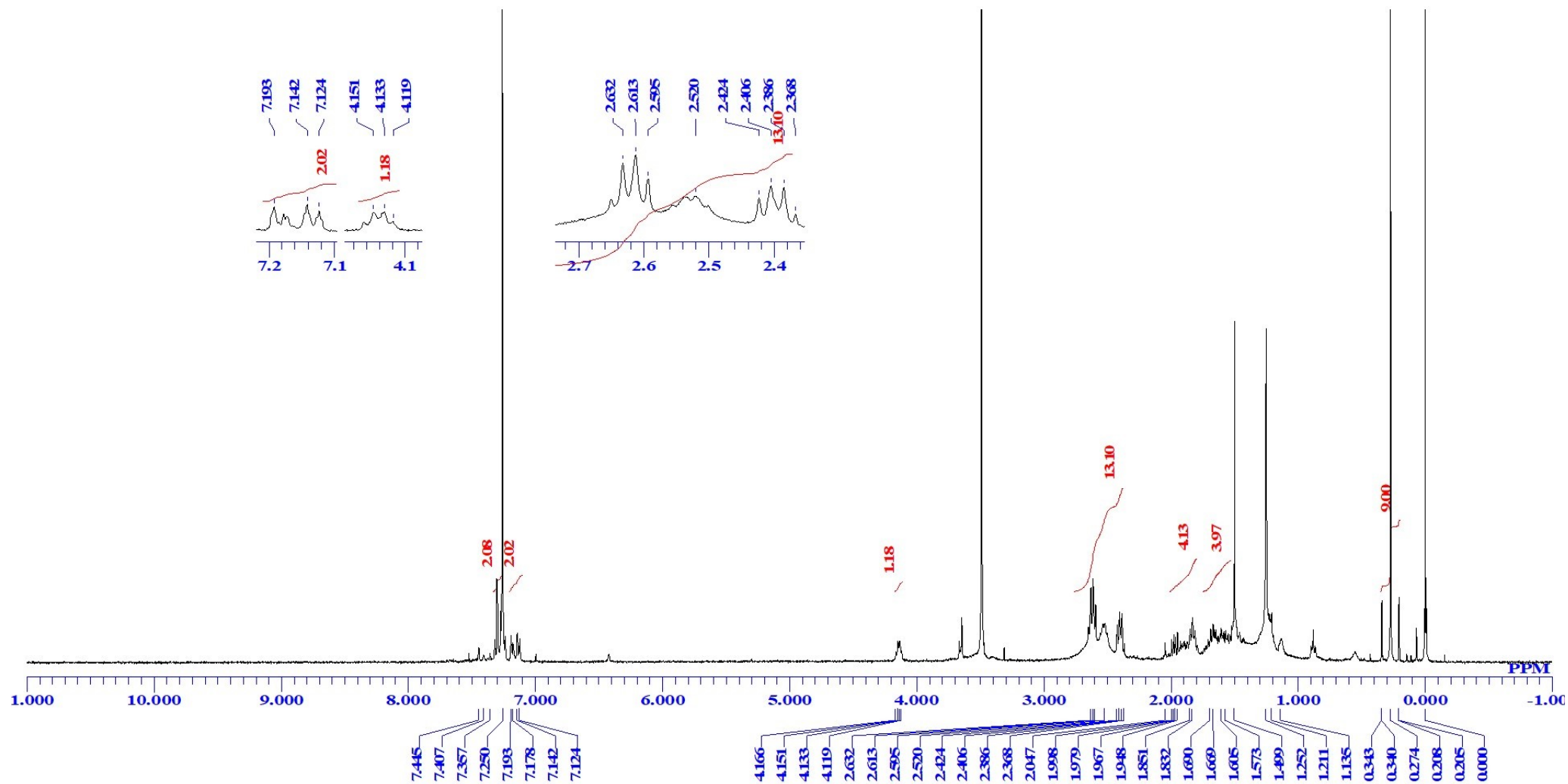
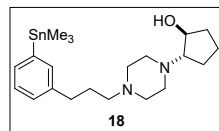
^1H NMR Spectrum of **16** (400 MHz, CDCl_3)



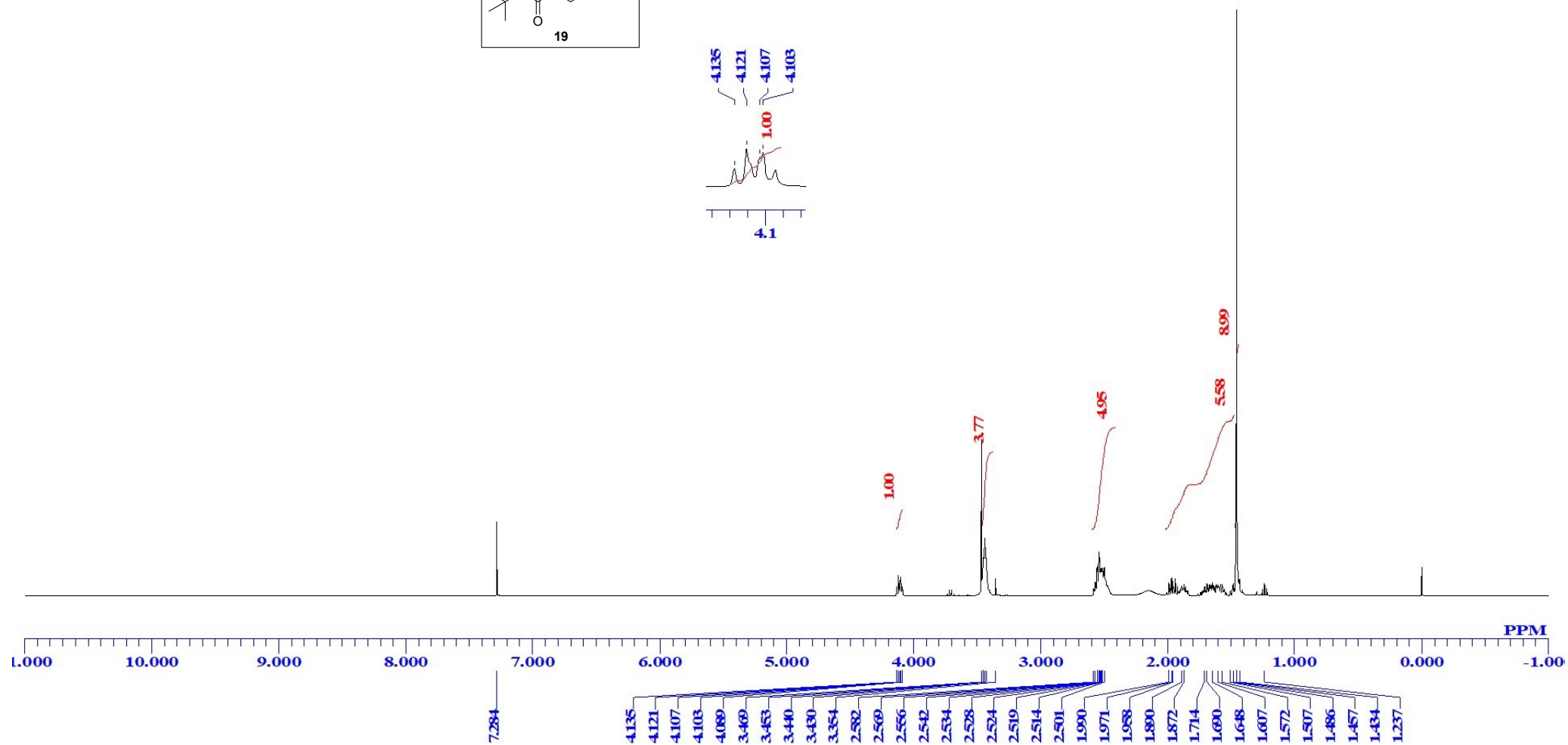
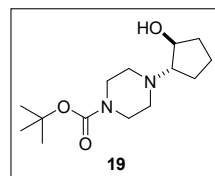
¹H NMR Spectrum of 17 (600 MHz, CDCl₃)



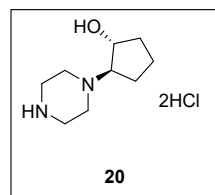
¹H NMR Spectrum of **18** (400 MHz, CDCl₃)



^1H NMR Spectrum of **19** (400 MHz, CDCl_3)



^1H NMR Spectrum of **20** (400 MHz, D_2O)



2. HPLC analysis

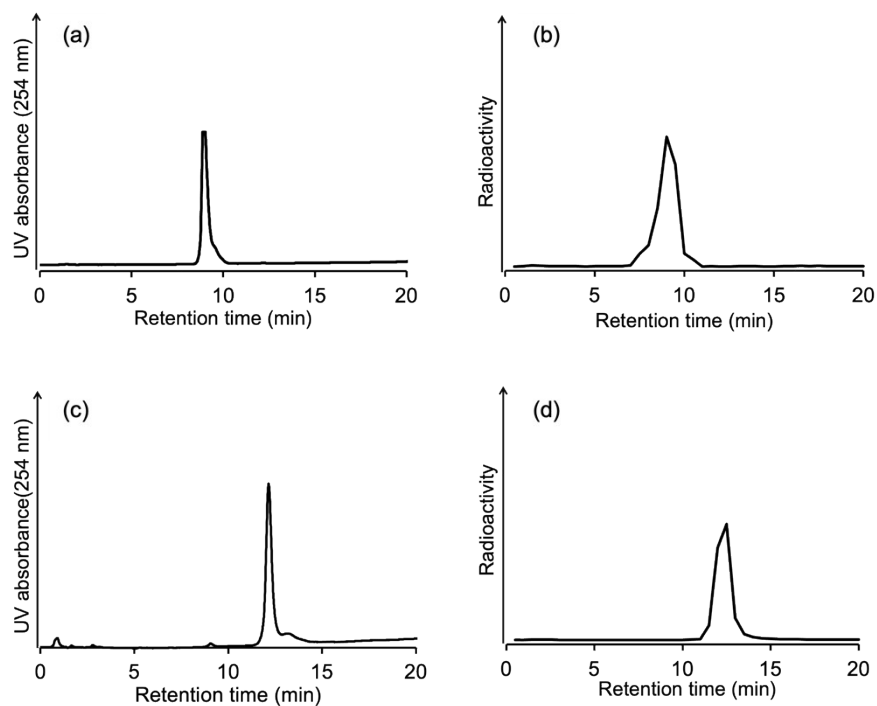


Figure S1. RP-HPLC chromatograms of (a) nonradioactive **15**, (b) [¹²⁵I]**15**, (c) nonradioactive **17**, (d) [¹²⁵I]**17**. Conditions: A flow rate of 1 mL/min with a gradient mobile phase of 60% methanol in water containing 0.05% TEA to 90% methanol in water containing 0.05% TEA for 20 min.

3. Detailed biodistribution data

Table S1. Biodistribution of radioactivity at 1 and 24 h after intravenous injection of [¹²⁵I]**15** in DU145 tumor-bearing mice.

Tissue	Time after injection	
	1 h	24 h
Blood	0.69 ± 0.10	0.08 ± 0.02
Tumor	5.39 ± 1.44	2.13 ± 0.18
Liver	3.53 ± 0.10	0.50 ± 0.04
Kidney	9.75 ± 0.75	1.13 ± 0.16
Small Intestine	10.40 ± 1.42	1.29 ± 0.21
Large Intestine	2.97 ± 0.45	0.88 ± 0.12
Spleen	6.80 ± 0.91	0.91 ± 0.15
Pancreas	14.43 ± 0.42	3.58 ± 0.95
Lung	6.15 ± 1.48	0.50 ± 0.10
Heart	2.96 ± 0.41	0.18 ± 0.04
Stomach [†]	1.32 ± 0.23	0.26 ± 0.05
Bone	2.00 ± 0.25	0.36 ± 0.19
Brain	3.47 ± 0.13	0.38 ± 0.04
Muscle	0.93 ± 0.13	0.17 ± 0.07

Data are expressed as % injected dose per gram tissue. Each value represents the mean ± SD for four animals.

[†]Data are expressed as % injected dose.

Table S2. Biodistribution of radioactivity at 1 and 24 h after intravenous injection of [¹²⁵I]**17** in DU145 tumor-bearing mice.

Tissue	Time after injection	
	1 h	24 h
Blood	0.85 ± 0.05	0.08 ± 0.02
Tumor	4.79 ± 0.77	4.08 ± 0.91
Liver	7.45 ± 0.35	0.62 ± 0.10
Kidney	14.34 ± 0.83	1.35 ± 0.27
Small Intestine	14.57 ± 0.41	1.00 ± 0.17
Large Intestine	3.73 ± 0.64	1.17 ± 0.35
Spleen	11.69 ± 0.75	1.50 ± 0.15
Pancreas	31.41 ± 2.26	11.63 ± 2.85
Lung	15.07 ± 2.44	1.80 ± 0.80
Heart	4.08 ± 0.28	0.31 ± 0.06
Stomach [†]	1.49 ± 0.19	0.23 ± 0.07
Bone	3.18 ± 0.86	0.45 ± 0.10
Brain	5.73 ± 0.47	0.52 ± 0.22
Muscle	1.63 ± 0.35	0.37 ± 0.19

Data are expressed as % injected dose per gram tissue. Each value represents the mean ± SD for four animals.

[†]Data are expressed as % injected dose.

Table S3. Biodistribution of radioactivity at 1 and 24 h after intravenous injection of [¹²⁵I]2 in DU145 tumor-bearing mice.

Tissue	Time after injection	
	1 h	24 h
Blood	0.78 ± 0.09	0.20 ± 0.03
Tumor	6.23 ± 0.55	6.27 ± 2.36
Liver	26.02 ± 1.99	7.42 ± 0.48
Kidney	26.15 ± 2.08	3.41 ± 0.58
Small Intestine	12.19 ± 0.70	3.00 ± 0.63
Large Intestine	4.11 ± 0.38	2.02 ± 0.31
Spleen	14.87 ± 0.99	2.15 ± 0.31
Lung	18.00 ± 3.18	1.89 ± 0.51
Heart	3.66 ± 0.32	0.40 ± 0.08
Stomach [†]	1.73 ± 0.41	0.52 ± 0.33
Brain	4.77 ± 0.60	0.66 ± 0.13
Muscle	1.84 ± 0.36	0.26 ± 0.09

Data are expressed as % injected dose per gram tissue. Each value represents the mean ± SD for four animals.

[†]Data are expressed as % injected dose.

This biodistribution data from a reference.¹

Table S4. Biodistribution of radioactivity at 10 min, 30 min, 1 h, 4 h, 8 h, and 24 h after intravenous injection of [¹²⁵I]**15** in normal mice.

Tissue	Time after injection					
	10 min	30 min	1 h	4 h	8 h	24 h
Blood	0.85 ± 0.03	0.65 ± 0.06	0.58 ± 0.09	0.58 ± 0.10	0.40 ± 0.01	0.07 ± 0.01
Liver	4.74 ± 0.36	4.09 ± 0.82	4.21 ± 0.81	3.40 ± 0.47	3.97 ± 0.36	0.39 ± 0.04
Kidney	18.01 ± 1.32	13.43 ± 1.02	13.38 ± 2.15	8.66 ± 1.30	9.18 ± 0.95	0.97 ± 0.25
S-Intestine	5.19 ± 0.29	7.56 ± 2.20	9.35 ± 1.55	6.62 ± 0.79	4.98 ± 0.36	0.65 ± 0.16
L-Intestine	2.30 ± 0.14	2.35 ± 0.25	2.60 ± 0.47	9.64 ± 2.32	6.58 ± 0.89	0.75 ± 0.07
Spleen	7.65 ± 1.40	7.15 ± 0.77	6.86 ± 1.04	3.11 ± 1.16	2.57 ± 0.59	0.58 ± 0.08
Pancreas	13.47 ± 2.30	13.35 ± 1.51	16.02 ± 1.84	12.70 ± 1.23	8.05 ± 1.26	2.28 ± 0.55
Lung	14.44 ± 4.55	12.63 ± 2.05	9.82 ± 1.51	4.49 ± 0.68	5.69 ± 1.27	0.61 ± 0.09
Heart	4.94 ± 0.57	3.81 ± 0.30	3.16 ± 0.40	1.68 ± 0.15	0.92 ± 0.04	0.17 ± 0.02
Stomach [†]	3.55 ± 0.19	3.45 ± 0.46	3.78 ± 0.67	2.94 ± 0.97	2.65 ± 0.88	0.52 ± 0.09
Bone	2.85 ± 0.33	2.60 ± 0.16	2.76 ± 0.22	1.56 ± 0.34	1.13 ± 0.07	0.38 ± 0.09
Brain	4.89 ± 0.34	4.28 ± 0.27	3.89 ± 0.37	2.18 ± 0.11	1.24 ± 0.02	0.43 ± 0.04
Muscle	2.38 ± 0.33	2.01 ± 0.24	1.93 ± 0.12	1.23 ± 0.19	0.79 ± 0.08	0.14 ± 0.02
Neck [†]	0.19 ± 0.03	0.31 ± 0.07	0.63 ± 0.11	1.78 ± 0.50	3.01 ± 0.49	4.23 ± 0.62

Data are expressed as % injected dose per gram tissue. Each value represents the mean ± SD for four animals.

S-Intestine and L-Intestine mean small intestine and large intestine, respectively.

[†]Data are expressed as % injected dose.

Table S5. Biodistribution of radioactivity at 10 min, 30 min, 1 h, 4 h, 8 h, and 24 h after intravenous injection of [¹²⁵I]17 in normal mice.

Tissue	Time after injection					
	10 min	30 min	1 h	4 h	8 h	24 h
Blood	0.66 ± 0.08	0.56 ± 0.08	0.40 ± 0.04	0.44 ± 0.06	0.42 ± 0.14	0.14 ± 0.02
Liver	5.96 ± 1.70	6.24 ± 1.03	5.23 ± 1.11	4.90 ± 0.86	3.16 ± 0.40	1.10 ± 0.20
Kidney	16.58 ± 2.94	12.31 ± 0.92	10.28 ± 0.88	6.94 ± 0.09	4.27 ± 0.49	1.26 ± 0.30
S-Intestine	5.17 ± 1.48	7.02 ± 1.17	7.66 ± 0.77	6.49 ± 0.27	2.99 ± 0.37	1.10 ± 0.17
L-Intestine	2.28 ± 0.65	2.02 ± 0.13	2.01 ± 0.38	8.43 ± 0.93	5.80 ± 0.52	1.73 ± 0.16
Spleen	7.89 ± 2.45	7.75 ± 1.75	6.14 ± 0.85	5.10 ± 0.36	3.03 ± 0.36	0.91 ± 0.12
Pancreas	12.65 ± 4.20	12.50 ± 0.34	12.37 ± 2.38	15.37 ± 3.24	18.37 ± 0.55	11.42 ± 2.08
Lung	18.66 ± 2.57	12.02 ± 1.57	8.93 ± 0.64	4.28 ± 0.97	2.67 ± 0.51	0.71 ± 0.15
Heart	4.72 ± 0.53	3.44 ± 0.41	2.66 ± 0.28	1.58 ± 0.10	0.91 ± 0.07	0.21 ± 0.04
Stomach [†]	2.90 ± 0.83	2.89 ± 0.91	2.71 ± 0.95	2.74 ± 0.81	2.70 ± 0.76	1.31 ± 0.30
Bone	2.77 ± 0.53	2.38 ± 0.42	2.39 ± 0.18	1.98 ± 0.24	1.52 ± 0.11	0.48 ± 0.07
Brain	5.58 ± 0.68	4.69 ± 0.45	4.29 ± 0.63	3.41 ± 0.28	2.28 ± 0.17	0.61 ± 0.06
Muscle	2.69 ± 0.43	2.05 ± 0.12	1.76 ± 0.22	1.30 ± 0.12	0.82 ± 0.09	0.20 ± 0.02
Neck [†]	0.19 ± 0.05	0.27 ± 0.05	0.37 ± 0.03	1.30 ± 0.11	2.67 ± 0.26	3.78 ± 0.53

Data are expressed as % injected dose per gram tissue. Each value represents the mean ± SD for four animals.

S-Intestine and L-Intestine mean small intestine and large intestine, respectively.

[†]Data are expressed as % injected dose.

Reference

- (1) Ogawa, K.; Masuda, R.; Mishiro, K.; Wang, M.; Kozaka, T.; Shiba, K.; Kinuya, S.; Odani, A. Syntheses and evaluation of a homologous series of aza-vesamicol as improved radioiodine-labeled probes for sigma-1 receptor imaging. *Bioorg. Med. Chem.* **2019**, *27* (10), 1990-1996.