Synthesis of nitrogenated lignin-derived compounds and reactivity with laccases. Study of their application in mild chemoenzymatic oxidative processes

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SUPPORTING INFORMATION (page 1 of 82 pages)

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1. Structures of compounds employed in this study

The different derivatives synthesized from vanillin, syringaldehyde or 3,4-dihydroxybenzaldehyde are shown in Figure S1, as well as the 3,4-dihydroxybenzaldehyde (**3c**) and the 5-hydrazynyl-1*H*-tetrazole (**13**), which were also chemically prepared.

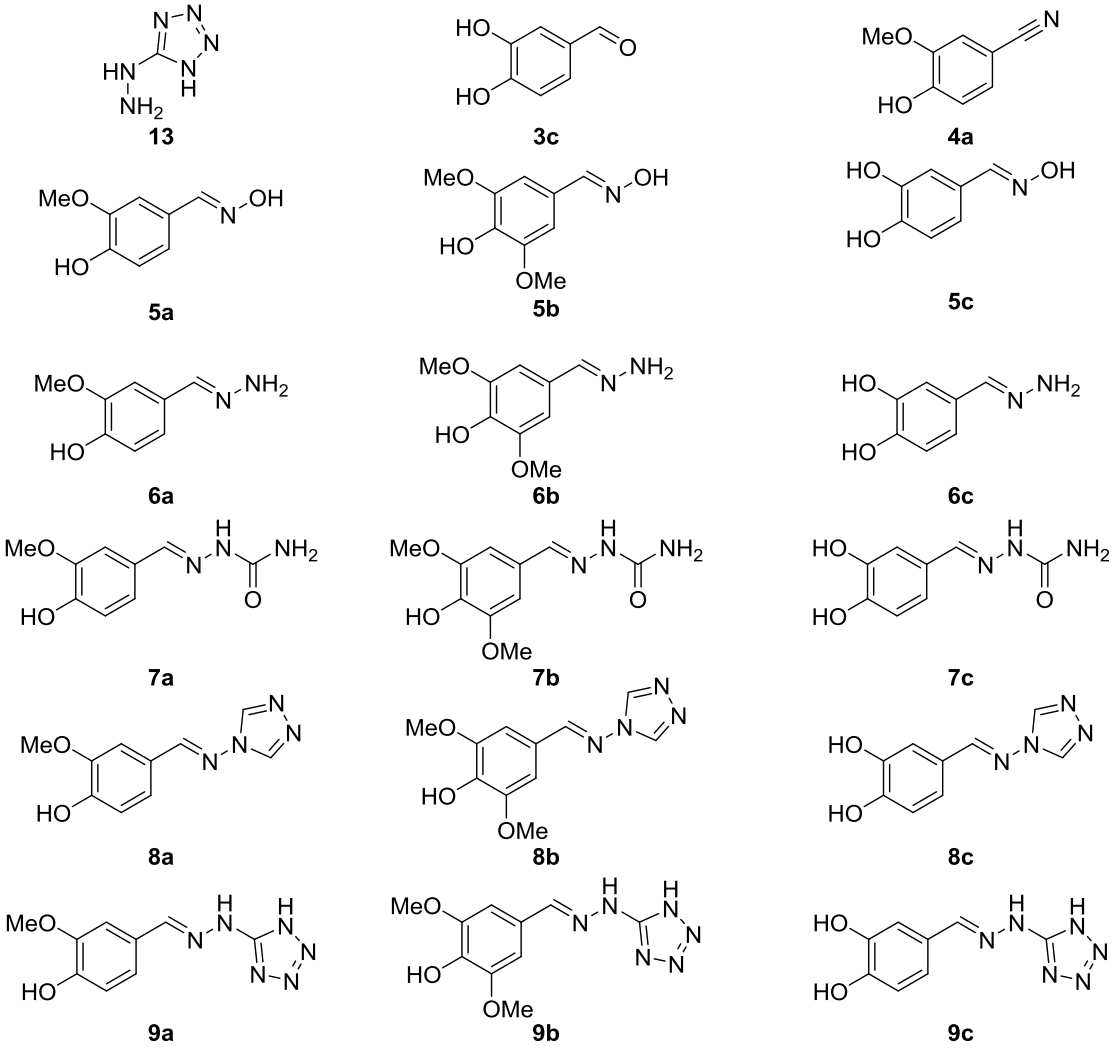
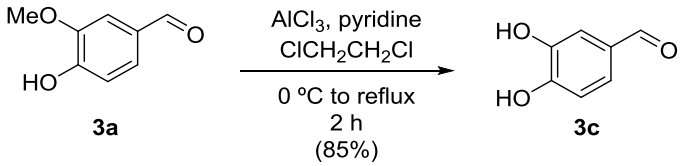


Fig. S1. Structures of compounds employed in this study.

2. Chemical synthesis of 3,4-dihydroxybenzaldehyde (3c)

3,4-Dihydroxybenzaldehyde (**3c**) was synthesized *via* demethylation of vanillin (Scheme S1) as previously described in J. Ravindran, G. V. Subbaraju, M. V. Ramani, B. Sung and B. B. Aggarwal, *Biochem. Pharmacol.*, 2010, **79**, 1658-1666.



Scheme S1. Synthesis of 3,4-dihydroxybenzaldehyde (**3c**) from vanillin (**3a**).

3,4-dihydroxybenzaldehyde (3c). Aluminium chloride (1.53 g, 11.5 mmol) was added to a solution of **3a** (700 mg, 4.60 mmol) in ethylene dichloride (14 mL) at 0-5 ºC. To the resulting suspension, pyridine (3.5 mL, 43.2 mmol) was added dropwise for 15 min, and the formed solution was stirred under reflux for 2 h. Then, a concentrated aqueous HCl solution was added (25 mL) at 0 ºC, followed by addition of H_2O (25 mL). The mixture was extracted with EtOAc (4 x 30 mL), the organic layers were combined, washed with H_2O (3 x 20 mL), and dried over Na_2SO_4. The solvent was removed by distillation under reduced pressure and the reaction crude purified by column chromatography on silica gel (35% EtOAc/hexane) isolating the aldehyde **3c** in 85% yield.

^{1}H NMR (300.13 MHz, MeOD-d_4): δ 4.91 (br s, 1H), 6.91 (d, $^3J_{HH}$ = 8.2 Hz, 1H), 7.29 (s, 1H), overlapped with 7.28-7.33 (m, 1H), 9.69 (s, 1H) ppm. ^{13}C NMR (75.5 MHz, MeOD-d_4): δ 115.2 (CH), 116.2 (CH), 126.5 (CH), 130.7 (C), 147.0 (C), 153.6 (C), 193.1 (CH) ppm.

3. Procedure for the control experiment of 6a with the denaturized protein

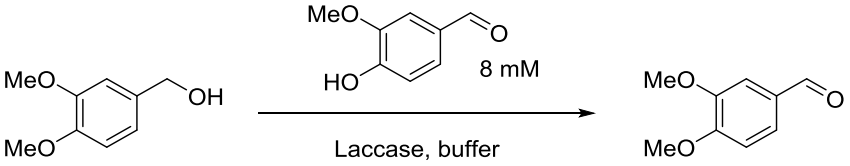
In a test tube, a suspension of the LTv (25 U) in acetate buffer 100 mM pH 4.7 (2.7 mL) was shaken at 100 ºC for 2 h. After this time the reaction was allowed to cool at 30 ºC and the hydrazone **6a** dissolved in DMSO (300 μL, 10% v/v) was added. A balloon that was filled with oxygen was attached to the test tube and the mixture was stirred at 30 ºC for 16 h. Then, the reaction was finished by extraction with EtOAc (3 x 2 mL), and the organic layers were combined and dried with Na_2SO_4. After solvent evaporation under reduced pressure, the reaction crude was analyzed by ^{1}H-NMR.

4. Optimization study of veratryl alcohol (VA) oxidation

The optimization study for vanillin, syringaldehyde and all the new synthesized compounds employed as potential mediators in the laccase-catalyzed veratryl alcohol (**1**) oxidation are detailed in the Tables S1 to S18.

4.1. Optimization study of veratryl alcohol oxidation with natural compounds 3a and 3b

Table S1. Laccase-catalyzed VA oxidation using vanillin (**3a**) as mediator.[a]

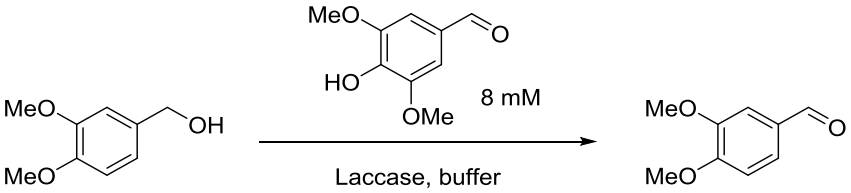


Entry	Laccase	T (ºC)	[VA] (mM)	t (h)	pH	Conversion (%)[b]
1	LTv	30	3	48	4.7	2
2	LTv	30	3	48	5	4
3	LTv	30	3	72	5	4
4	LTv	30	10	72	5	1
5	LTv	30	15	72	5	1
6	LTv	30	20	72	5	1
7	LTv	30	3	48	6	1
8	Novozym	50	3	48	5	4
9	Novozym	50	3	48	6	6
10	Novozym	50	3	72	6	6
11	Novozym	50	10	72	6	3
12	Novozym	50	15	72	6	3
13	Novozym	50	20	72	6	3
14	Novozym	50	3	48	7	4

[a] Reaction conditions: **3a** (2.4 mg, 16 μmol), buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U). For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S2. Laccase-catalyzed VA oxidation using syringaldehyde (**3b**) as mediator.[a]



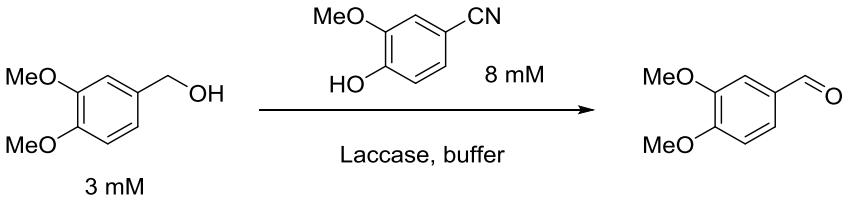
Entry	Laccase	T (ºC)	[VA] (mM)	t(h)	pH	Conversion (%)[b]
1	LTv	30	3	48	4.7	4
2	LTv	30	3	48	5	4
3	LTv	30	3	48	6	6
4	LTv	30	3	72	6	5
5	LTv	30	10	72	6	4
6	LTv	30	15	72	6	5
7	LTv	30	20	72	6	5
8	Novozym	50	3	48	5	7
9	Novozym	50	3	48	6	7
10	Novozym	50	3	72	6	7
11	Novozym	50	10	72	6	7
12	Novozym	50	15	72	6	7
13	Novozym	50	20	72	6	6
14	Novozym	50	3	48	7	4

[a] Reaction conditions: **3b** (2.9 mg, 16 μmol), buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U). For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

4.2. Optimization study of veratryl alcohol oxidation with vanillin-derived compounds 4a-9a

Table S3. Laccase-catalyzed VA oxidation using 4-hydroxy-3-methoxybenzonitrile (**4a**) as potential mediator.[a]

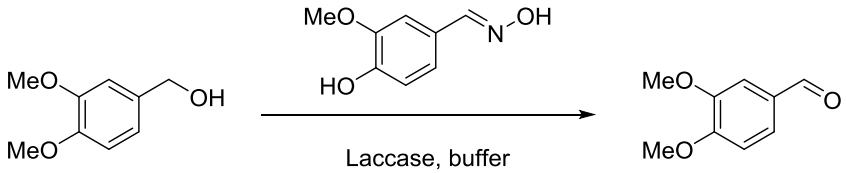


Entry	Laccase	T (ºC)	*t* (h)	pH	Conversion (%)[b]
1	LTv	30	48	4.7	3
2	LTv	30	72	4.7	2
3	LTv	30	48	5	<1
4	LTv	30	48	6	1
5	Novozym	50	48	5	3
6	Novozym	50	48	6	6
7	Novozym	50	72	6	5
8	Novozym	50	48	7	5

[a] Reaction conditions: **4a** (2.4 mg, 16 mmol), buffer (2 mL), veratryl alcohol (0.9 µL, 6 µmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U). For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S4. Laccase-catalyzed VA oxidation using 4-hydroxy-3-methoxybenzaldehyde oxime (**5a**) as potential mediator.[a]

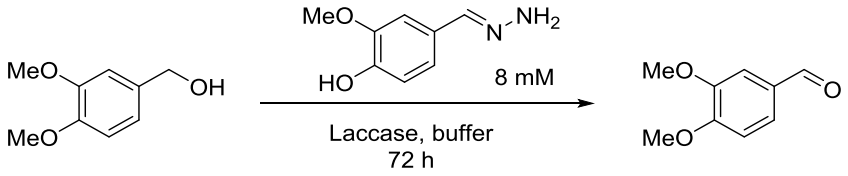


Entry	Laccase	T (ºC)	[Med] (mM)	[VA] (mM)	Cosolvent	*t* (h)	pH	Conversion (%)[b]
1	LTv	30	4	3	--	24	4.7	9
2	LTv	30	8	3	--	24	4.7	10
3	LTv	30	8	3	--	48	4.7	13
4	LTv	50	8	3	--	48	4.7	13
5	LTv	30	8	3	EtOH	48	4.7	9
6	LTv	30	8	3	1,4-Dioxane	48	4.7	9
7	LTv	30	8	3	--	72	4.7	27
8	LTv	30	8	3	--	48	5	20
9	LTv	30	8	3	--	48	6	24
10	LTv	30	8	3	MTBE	48	6	19
11	LTv	30	8	3	*t*-BuOH	48	6	14
12	LTv	30	8	3	--	72	6	37
13	LTv	30	8	10	--	72	6	17
14	LTv	30	8	15	--	72	6	12
15	LTv	30	8	20	--	72	6	10
16	LTv	30	8	3	--	96	6	3
17	Novozym	50	8	3	--	48	5.5	26
18	Novozym	50	8	3	--	48	6	28
19	Novozym	50	8	3	--	48	7	24
20	Novozym	50	8	3	--	72	7	26
21	Novozym	50	8	3	--	72	6	34
22	Novozym	50	8	3	--	96	6	34
23	Novozym	50	4	3	--	96	6	23
24	Novozym	50	8	10	--	72	6	26
25	Novozym	50	8	10	--	96	6	27
26	Novozym	50	8	15	--	72	6	18
27	Novozym	50	8	20	--	72	6	14

[a] Reaction conditions: **5a**, cosolvent (10% v/v), buffer (for a total volume of 2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U). For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S5. Laccase-catalyzed VA oxidation using 4-(hydrazonomethyl)-2-methoxyphenol (**6a**) as potential mediator.[a]



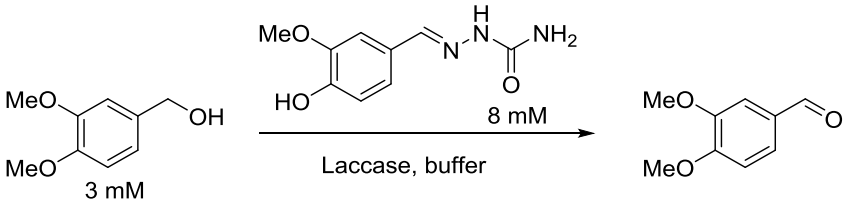
Entry	Laccase	T (ºC)	[VA] (mM)	Cosolvent	pH	Conversion (%)[b]
1	LTv	30	3	DMSO	4.7	14
2	LTv	30	3	DMSO	5	22
3	LTv	30	3	DMSO	6	17
4	LTv	30	3	--	4.7	39
5	LTv	30	10	--	4.7	21
6	LTv	30	15	--	4.7	12
7	LTv	30	20	--	4.7	10
8	LTv	30	3	--	5	3
9	LTv	30	3	--	6	4
10	Novozym	50	3	DMSO	5	22
11	Novozym	50	3	DMSO	6	28
12	Novozym	50	3	DMSO	7	13
13	Novozym	50	3	--	5	32
14	Novozym	50	3	--	6	54
15	Novozym	50	3	--	7	48
16	Novozym	50	10	--	6	41
17	Novozym	50	15	--	6	26[c]
18	Novozym	50	20	--	6	18

[a] Reaction conditions: **6a** (2.7 mg, 16 μmol), DMSO (10% v/v), buffer (for a total volume of 2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

[c] Conversion value corresponds to the average of duplicated reactions (±5%).

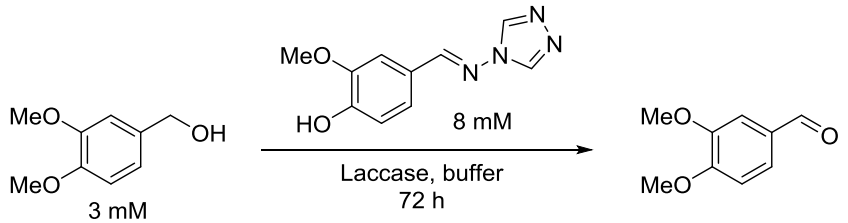
Table S6. Laccase-catalyzed VA oxidation using 2-(4-hydroxy-3-methoxybenzylidene)hydrazine-1-carboxamide (**7a**) as potential mediator.[a]



Entry	Laccase	T (ºC)	Cosolvent	*t* (h)	pH	Conversion (%)[b]
1	LTv	30	DMSO	72	4.7	5
2	LTv	30	DMSO	72	5	6
3	LTv	30	DMSO	72	6	8
4	LTv	30	--	48	4.7	9
5	LTv	30	--	72	4.7	14
6	LTv	30	--	48	5	5
7	LTv	30	--	48	6	9
8	LTv	30	--	72	6	14
9	Novozym	50	DMSO	72	5	6
10	Novozym	50	DMSO	72	6	7
11	Novozym	50	DMSO	72	7	4
12	Novozym	50	--	72	5	11
13	Novozym	50	--	72	6	16
14	Novozym	50	--	72	7	13

[a] Reaction conditions: **7a** (3.3 mg, 16 μmol), DMSO (10% v/v), buffer (for a total volume of 2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U). For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

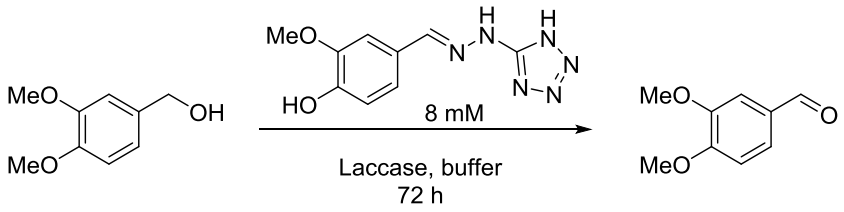
Table S7. Laccase-catalyzed VA oxidation using 4-(((4*H*-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol (**8a**) as potential mediator.[a]



Entry	Laccase	T (ºC)	Cosolvent	pH	Conversion (%)[b]
1	LTv	30	--	4.7	8
2	LTv	30	DMSO	4.7	3
3	LTv	30	--	5	<1
4	LTv	30	--	6	<1
5	Novozym	50	--	5	4
6	Novozym	50	--	6	9
7	Novozym	50	DMSO	6	4
8	Novozym	50	--	7	4

[a] Reaction conditions: **8a** (3.5 mg, 16 μmol), DMSO (10% v/v), buffer (for a total volume of 2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S8. Laccase-catalyzed VA oxidation using 4-((2-(1*H*-tetrazol-5-yl)hydrazono)methyl)-2-methoxyphenol (**9a**) as potential mediator.[a]



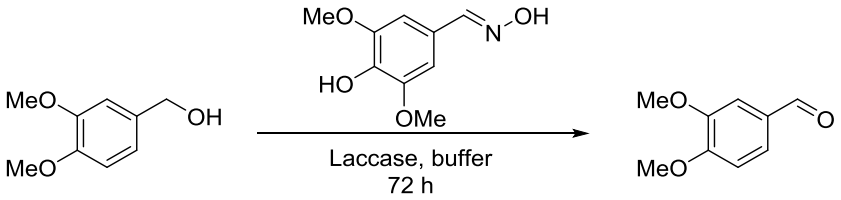
Entry	Laccase	[VA] (mM)	T (ºC)	pH	Conversion (%)[b]
1	LTv	3	30	4.7	7
2	LTv	3	30	5	15
3	LTv	3	30	6	39
4	LTv	3	30	7	25
5	LTv	10	30	6	20
6	LTv	15	30	6	13
7	LTv	20	30	6	13
8	Novozym	3	50	5	5
9	Novozym	3	50	6	24
10	Novozym	3	50	7	13
11	Novozym	10	50	6	10
12	Novozym	15	50	6	16
13	Novozym	20	50	6	15

[a] Reaction conditions: **9a** (3.7 mg, 16 μmol), buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

4.3. Optimization study of veratryl alcohol oxidation with syringyl-derived compounds 5b-9b

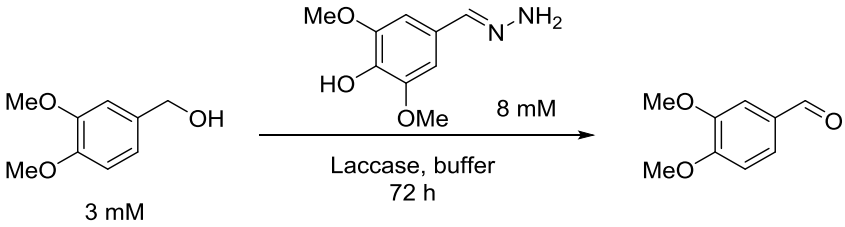
Table S9. Laccase-catalyzed VA oxidation using 4-hydroxy-3,5-dimethoxybenzaldehyde oxime (**5b**) as potential mediator.[a]



Entry	Laccase	T (ºC)	[Med] (mM)	[VA] (mM)	pH	Conversion (%)[b]
1	LTv	30	4	3	4.7	11
2	LTv	30	8	3	5	<1
3	LTv	30	8	3	6	1
4	Novozym	50	8	3	5	19
5	Novozym	50	8	3	6	34
6	Novozym	50	8	3	7	35
7	Novozym	50	8	10	7	37
8	Novozym	50	12	3	7	36
9	Novozym	50	3	3	7	23
10	Novozym	50	8	15	7	32[c]
11	Novozym	50	8	20	7	31[c]

[a] Reaction conditions: **5b**, buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.
[c] Conversion value corresponds to the average of duplicated reactions (±4%).

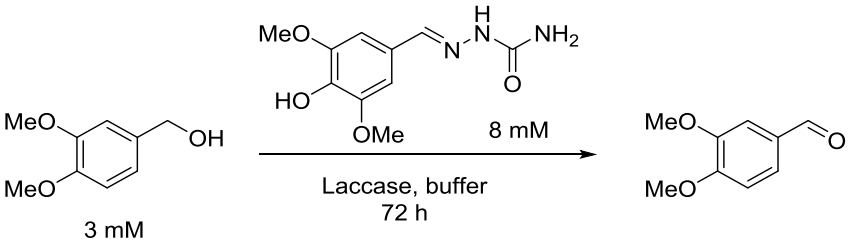
Table S10. Laccase-catalyzed VA oxidation using 4-(hydrazonomethyl)-2,6-dimethoxyphenol (**6b**) as potential mediator.[a]



Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	<1
2	LTv	30	5	<1
3	LTv	30	6	<1
4	Novozym	50	5	3
5	Novozym	50	6	3
6	Novozym	50	7	3

[a] Reaction conditions: **6b** (3.1 mg, 16 µmol), buffer (2 mL), veratryl alcohol (0.9 µL, 6 µmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

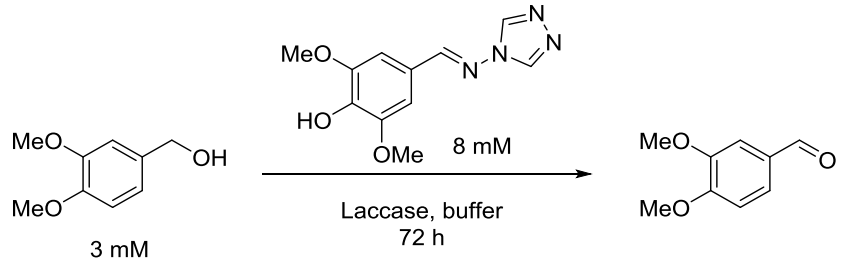
Table S11. Laccase-catalyzed VA oxidation using 2-(4-hydroxy-3,5-dimethoxybenzylidene)hydrazine-1-carboxamide (**7b**) as potential mediator.[a]



Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	23
2	LTv	30	5	<1
3	LTv	30	6	<1
4	Novozym	50	5	13
5	Novozym	50	6	22
6	Novozym	50	7	23

[a] Reaction conditions: **7b** (3.8 mg, 16 µmol), buffer (2 mL), veratryl alcohol (0.9 µL, 6 µmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

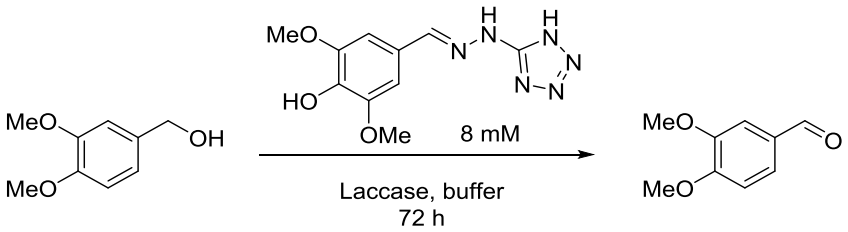
Table S12. Laccase-catalyzed VA oxidation using 4-(((4*H*-1,2,4-triazol-4-yl)imino)methyl)-2,6-dimethoxyphenol (**8b**) as potential mediator.[a]



Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	5
2	LTv	30	5	<1
3	LTv	30	6	<1
4	Novozym	50	5	13
5	Novozym	50	6	16
6	Novozym	50	7	14

[a] Reaction conditions: **8b** (4.0 mg, 16 µmol), buffer (2 mL), veratryl alcohol (0.9 µL, 6 µmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S13. Laccase-catalyzed VA oxidation using 4-((2-(1*H*-tetrazol-5-yl)hydrazono)methyl)-2,6-dimethoxyphenol (**9b**) as potential mediator.[a]



Entry	Laccase	[VA] (mM)	T (ºC)	pH	Conversion (%)[b]
1	LTv	3	30	4.7	33
2	LTv	3	30	5	36
3	LTv	3	30	6	32
4	LTv	10	30	5	18
5	LTv	15	30	5	17
6	LTv	20	30	5	14
7	Novozym	3	50	5	11
8	Novozym	3	50	6	32
9	Novozym	3	50	7	14
10	Novozym	10	50	6	28
11	Novozym	15	50	6	24
12	Novozym	20	50	6	25[c]

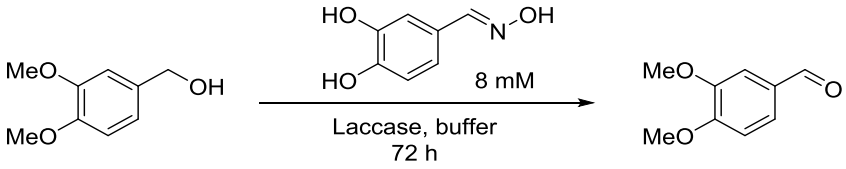
[a] Reaction conditions: **9b** (4.2 mg, 16 μmol), buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

[c] Conversion value corresponds to the average of duplicated reactions (±1%).

4.4. Optimization study of veratryl alcohol oxidation with 3,4-dihydroxybenzaldehyde-derived compounds 5c-9c

Table S14. Laccase-catalyzed VA oxidation using 3,4-dihydroxybenzaldehyde oxime (**5c**) as potential mediator.[a]



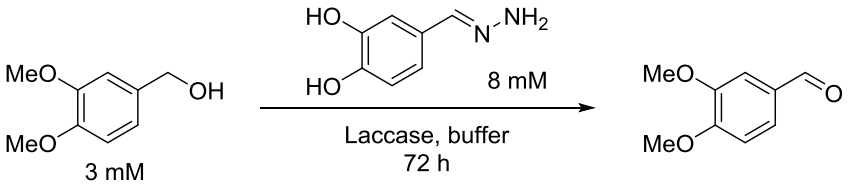
Entry	Laccase	T (ºC)	[VA] (mM)	pH	Conversion (%)[b]
1	LTv	30	3	4.7	48
2	LTv	30	3	5	70
3	LTv	30	3	6	67
4	LTv	30	10	5	52[c]
5	LTv	30	15	5	40[c]
6	LTv	30	20	5	30[c]
7	Novozym	50	3	5	9
8	Novozym	50	3	6	21
9	Novozym	50	3	7	10
10	Novozym	50	10	6	11
11	Novozym	50	15	6	10
12	Novozym	50	20	6	9

[a] Reaction conditions: **5c** (2.5 mg, 16 μmol), buffer (2 mL), veratryl alcohol, laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.

[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

[c] Conversion value corresponds to the average of duplicated reactions (±5%).

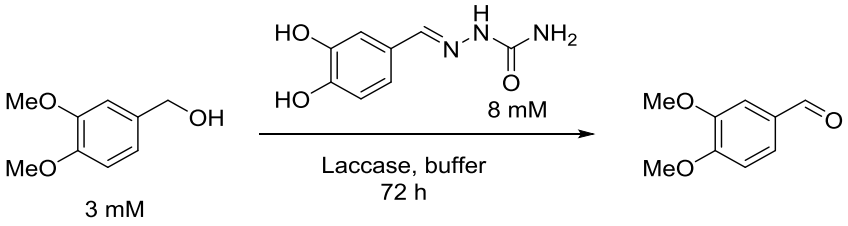
Table S15. Laccase-catalyzed VA oxidation using 4-(hydrazonomethyl)benzene-1,2-diol (**6c**) as potential mediator.[a]



Entry	Laccase	T (ºC)	Cosolvent	pH	Conversion (%)[b]
1	LTv	30	--	4.7	7
2	LTv	30	--	5	7
3	LTv	30	DMSO	5	6
4	LTv	30	--	5.5	8
5	LTv	30	--	6	7
6	Novozym	50	--	5	10
7	Novozym	50	DMSO	5	4
8	Novozym	50	--	6	8
9	Novozym	50	--	7	3

[a] Reaction conditions: **6c** (2.4 mg, 16 μmol), DMSO (10% v/v), buffer (for a total volume of 2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

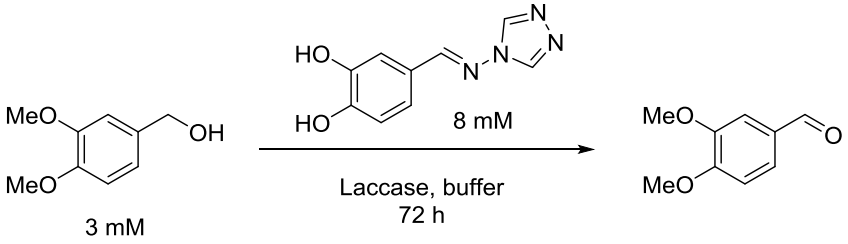
Table S16. Laccase-catalyzed VA oxidation using 2-(3,4-dihydroxybenzylidene)hydrazine-1-carboxamide (**7c**) as potential mediator.[a]



Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	8
2	LTv	30	5	<1
3	LTv	30	6	6
4	Novozym	50	5	5
5	Novozym	50	6	4
6	Novozym	50	7	<1

[a] Reaction conditions: **7c** (3.1 mg, 16 μmol), buffer (2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

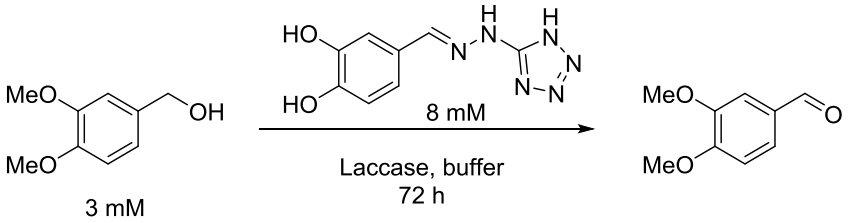
Table S17. Laccase-catalyzed VA oxidation using 4-(((4*H*-1,2,4-triazol-4-yl)imino)methyl)benzene-1,2-diol (**8c**) as potential mediator.[a]



Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	4
2	LTv	30	5	<1
3	LTv	30	6	<1
4	Novozym	50	5	2
5	Novozym	50	6	2
6	Novozym	50	7	1

[a] Reaction conditions: **8c** (3.3 mg, 16 μmol), buffer (2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

Table S18. Laccase-catalyzed VA oxidation using 4-((2-(1*H*-tetrazol-5-yl)hydrazono)methyl)benzene-1,2-diol (**9c**) as potential mediator.[a]



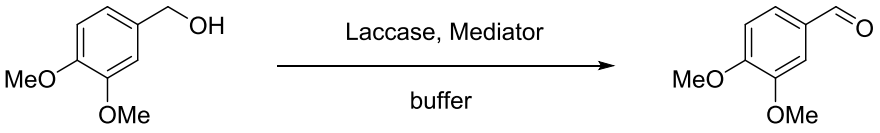
Entry	Laccase	T (ºC)	pH	Conversion (%)[b]
1	LTv	30	4.7	11
2	LTv	30	5	11
3	LTv	30	6	9
4	Novozym	50	5	2
5	Novozym	50	6	3
6	Novozym	50	7	1

[a] Reaction conditions: **9c** (3.5 mg, 16 μmol), buffer (2 mL), veratryl alcohol (0.9 μL, 6 μmol), laccase from *Trametes versicolor* (LTv, 25 U) or Novozym 51003 (31.4 U), 72 h. For the buffers used: buffer acetate 100 mM pH 4.7; buffer citrate 50 mM pH 5; buffer citrate 50 mM pH 5.5; buffer citrate 50 mM pH 6; buffer phosphate 100 mM pH 7.
[b] Conversion values were determined by GC analysis after isolation of products by extraction under acidic media.

5. Analytics

Gas chromatography analyses (GC) were carried out for monitoring the laccase-catalyzed oxidation of veratryl alcohol (**1**). An Agilent 7890A GC-apparatus and a chiral stationary phase HP-1 column (30 m x 0.320 mm x 0.25 µm) were used for the measurement of reaction conversion values. The conditions are shown in Table S19.

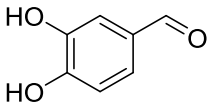
Table S19. Analytical conditions for the GC measurement of conversions values in the enzymatic oxidation of **1**.

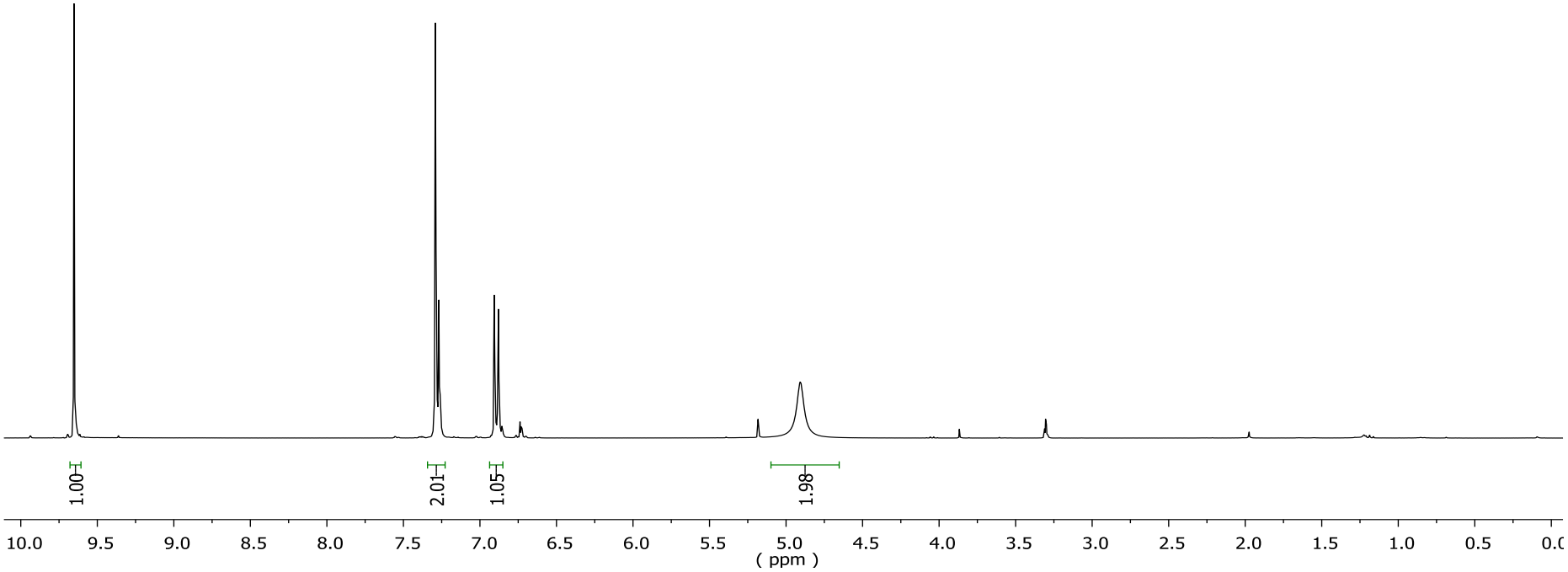


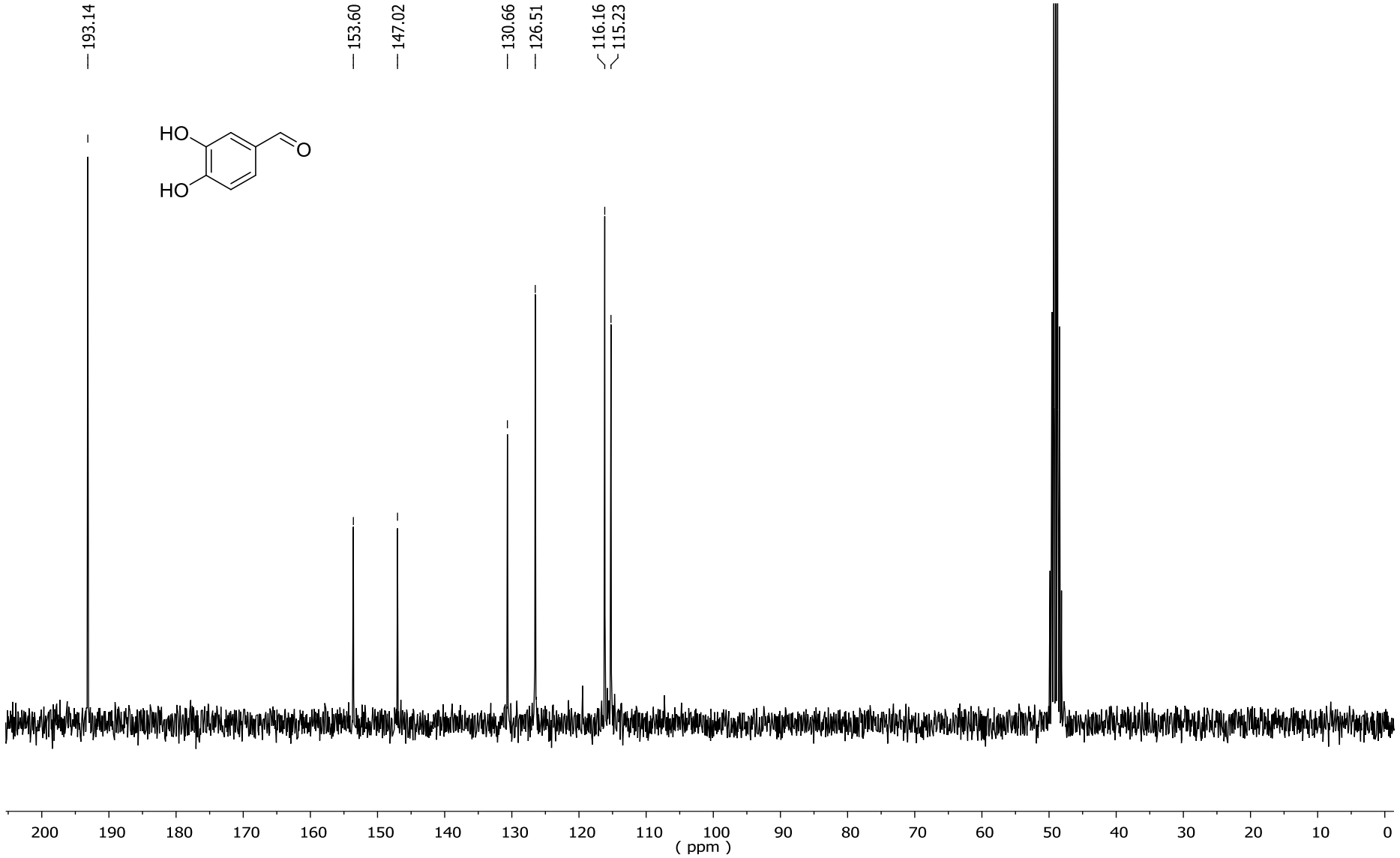
1 **18**

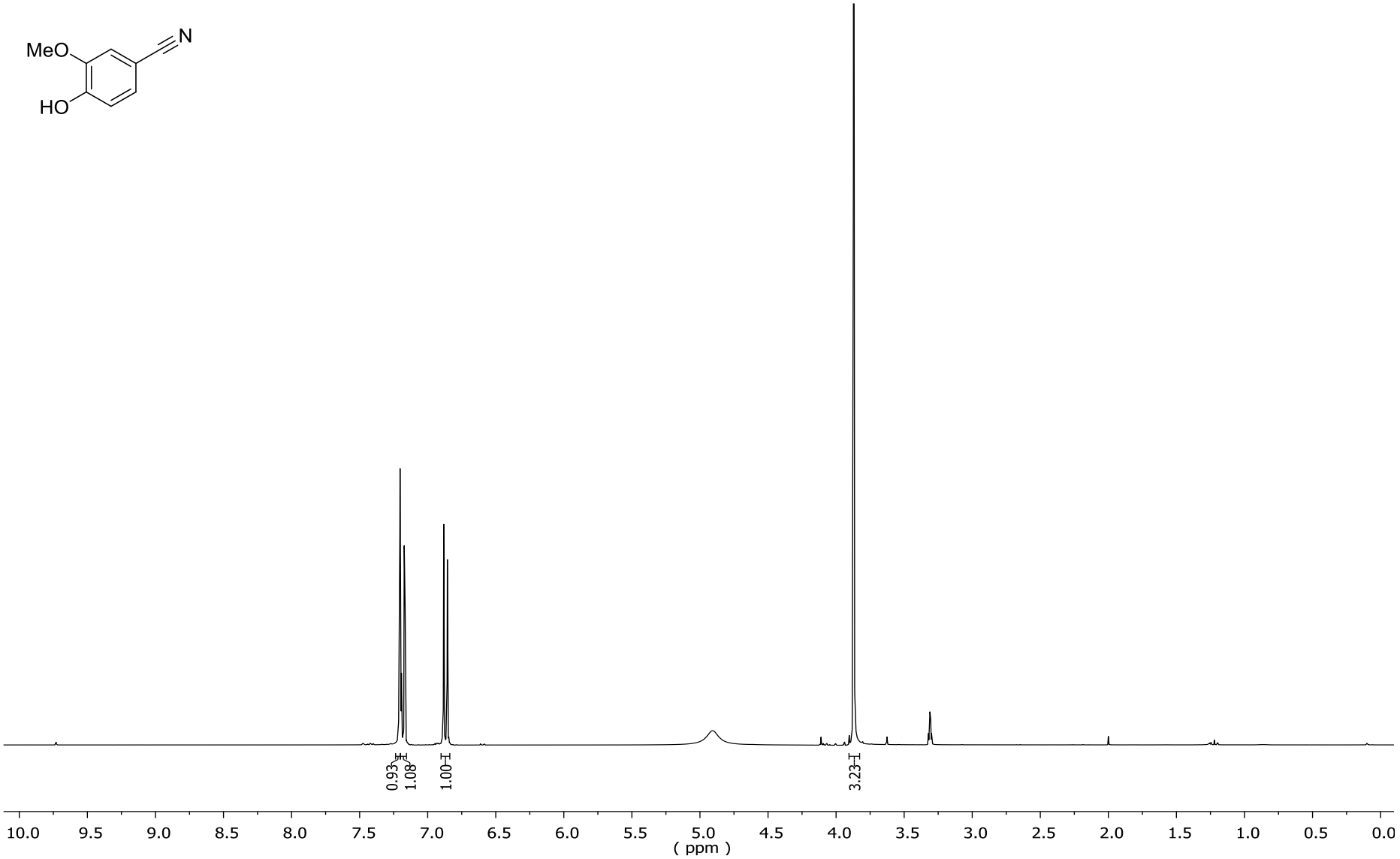
Compound	Temperature program	t_R (min)
1	90 ºC (0 min), 10 ºC/min until 200 ºC (0 min), 40 ºC until 300 ºC (0 min)	6.2
18	90 ºC (0 min), 10 ºC/min until 200 ºC (0 min), 40 ºC until 300 ºC (0 min)	6.6

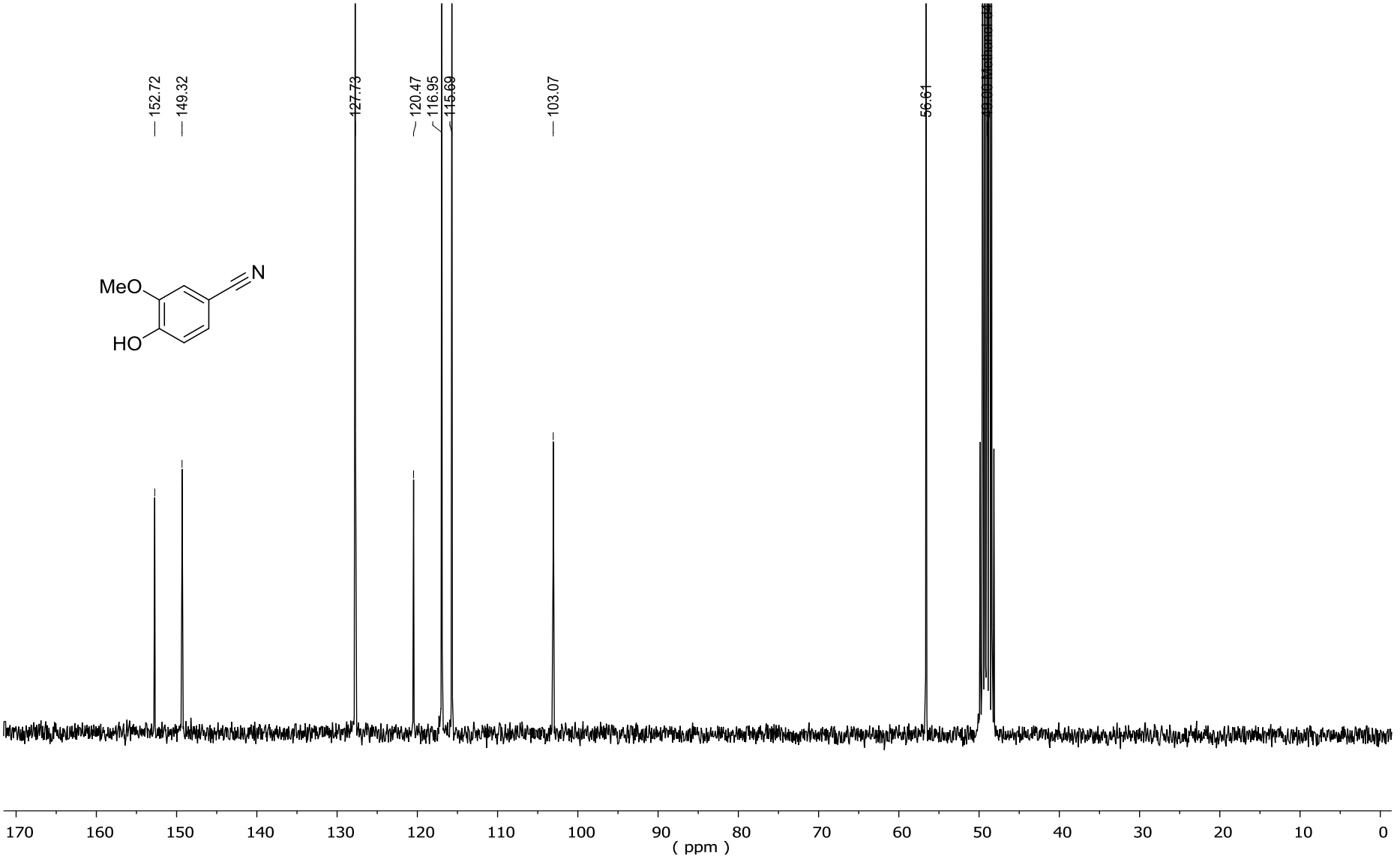
6. Copy of ^{1}H, ^{13}C and DEPT NMR spectra






193.14
153.60
147.02
130.66
126.51
116.16
115.23
HO
HO
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


MeO
HO
N
0.93
1.08
1.00
3.23
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)


152.72
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127.73
120.47
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115.69
103.07
56.61
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HO
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120
110
100
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30
20
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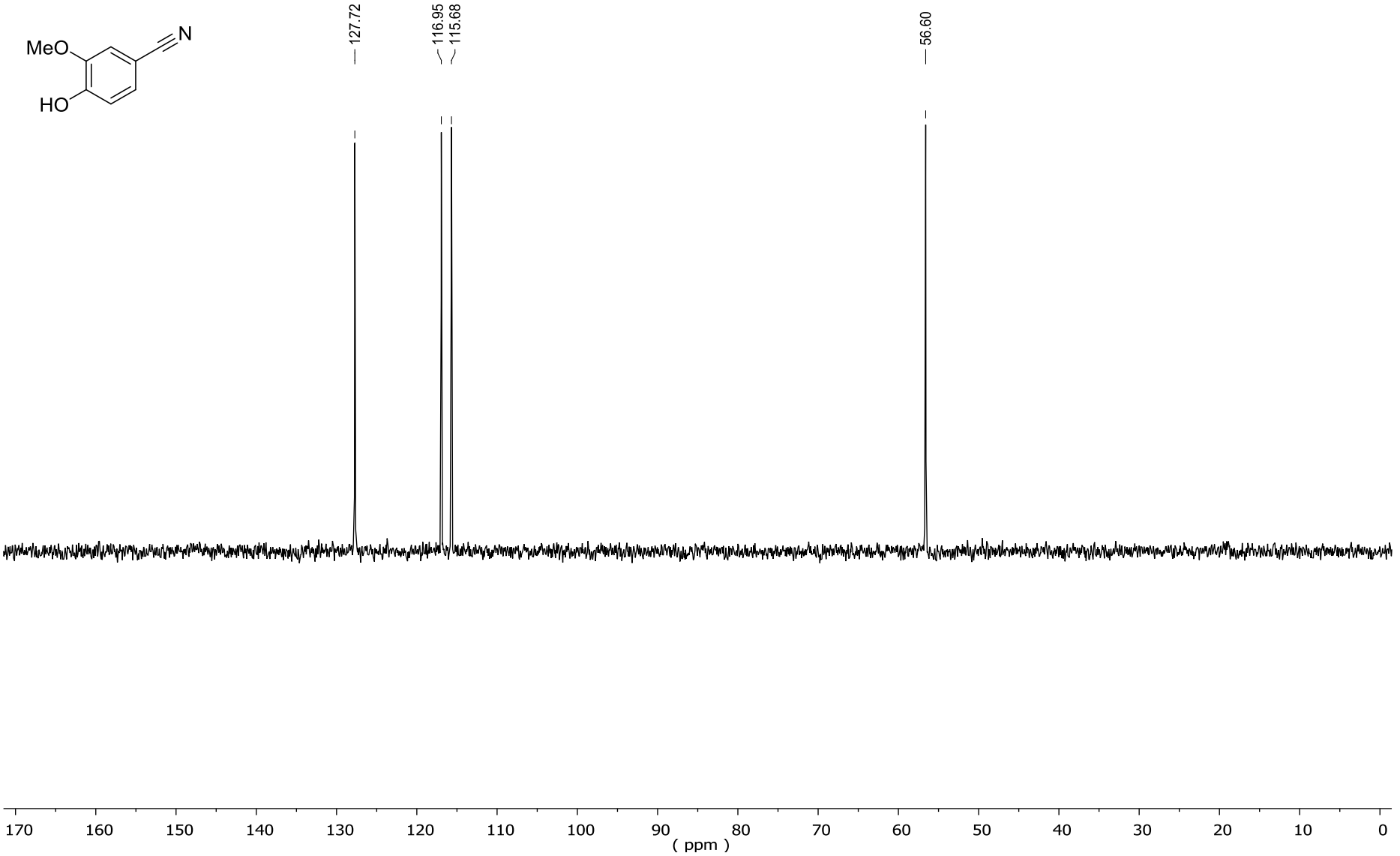
0
(ppm)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116.95
115.68
56.60
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120
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100
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80
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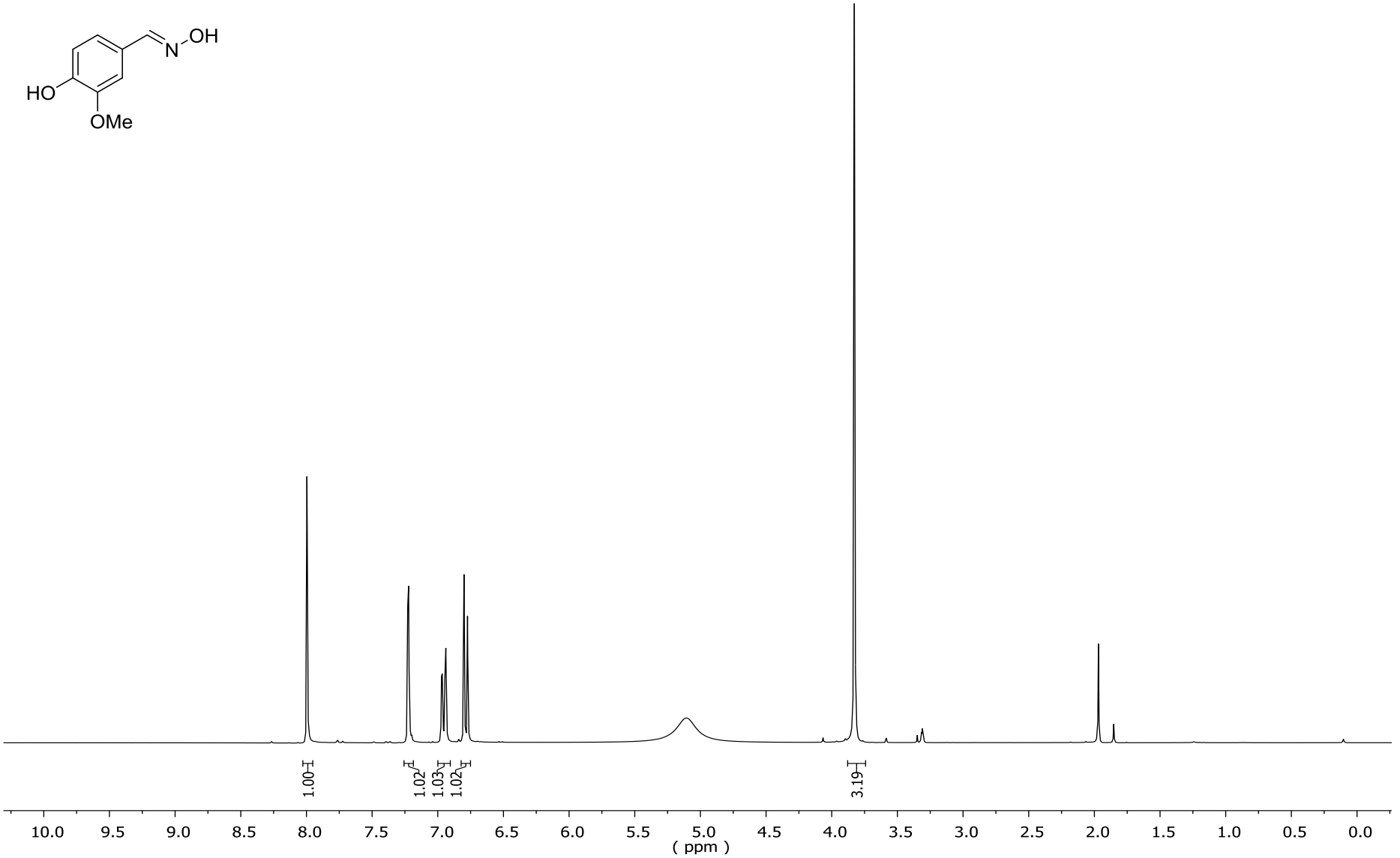
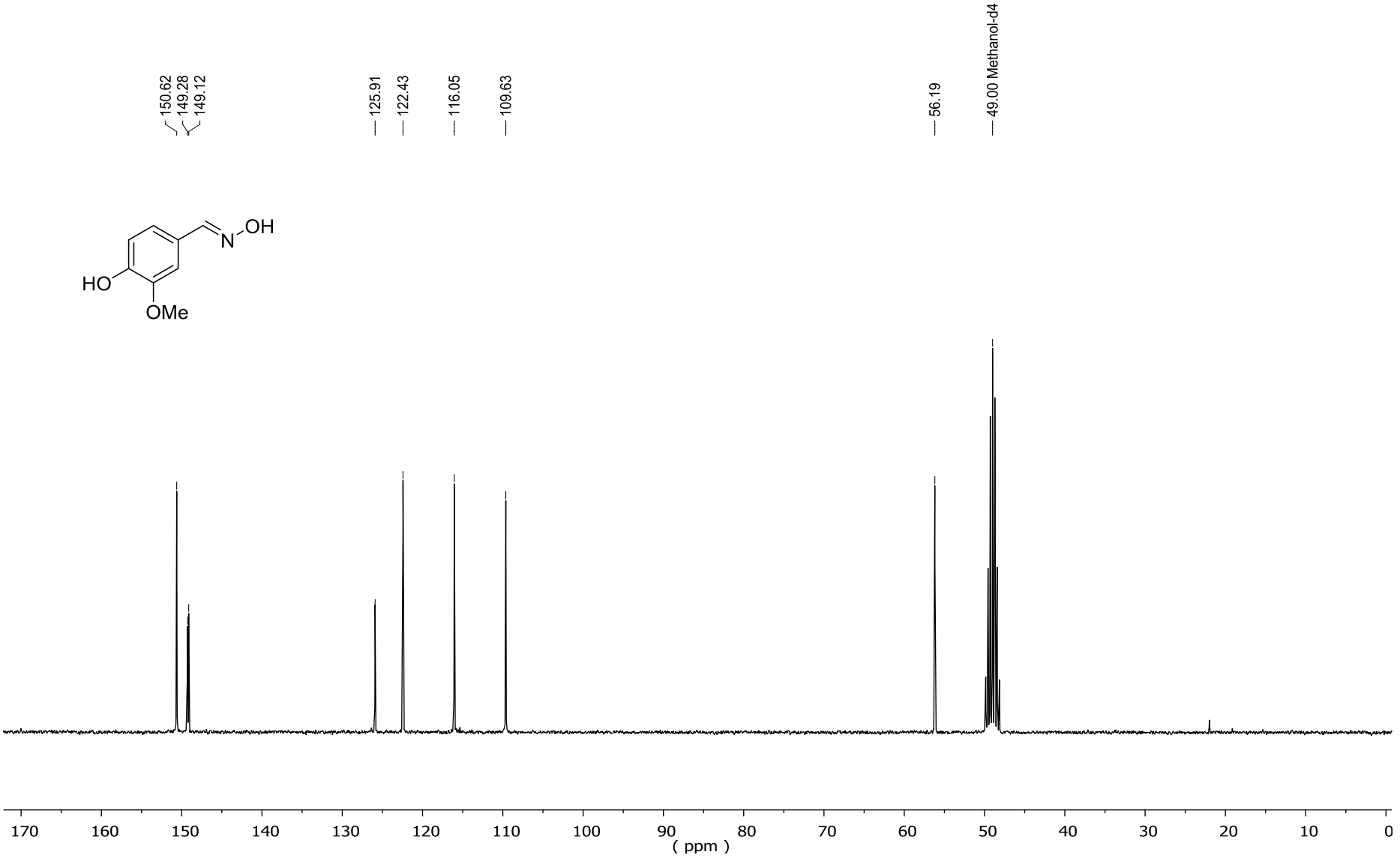
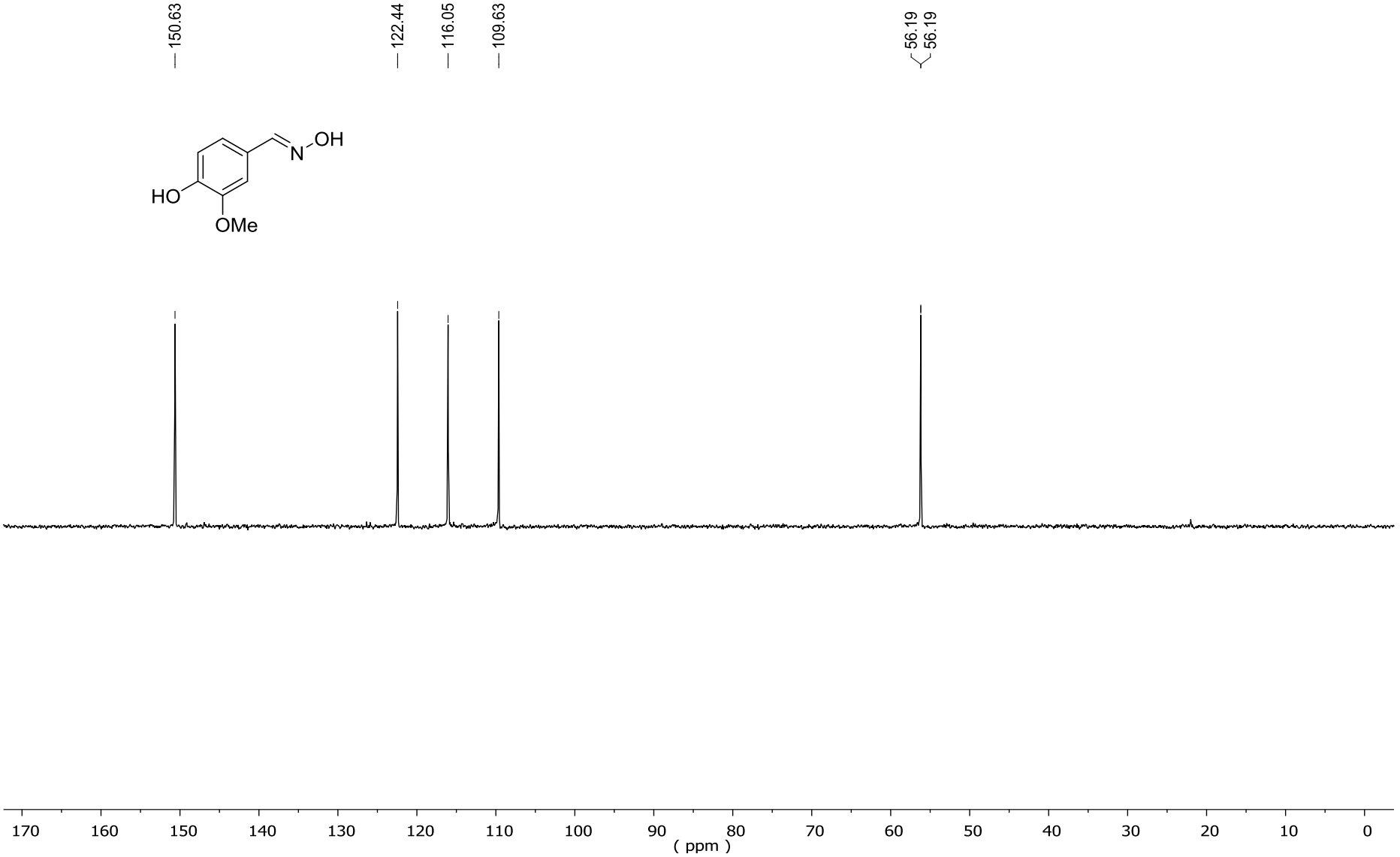
60
50
40
30
20
10
0
(ppm)


HO
OMe
N
OH
1.00
1.02
1.03
1.02
3.19
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)


150.62
149.28
149.12
125.91
122.43
116.05
109.63
56.19
49.00 Methanol-d4
HO
N
OH
OMe
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)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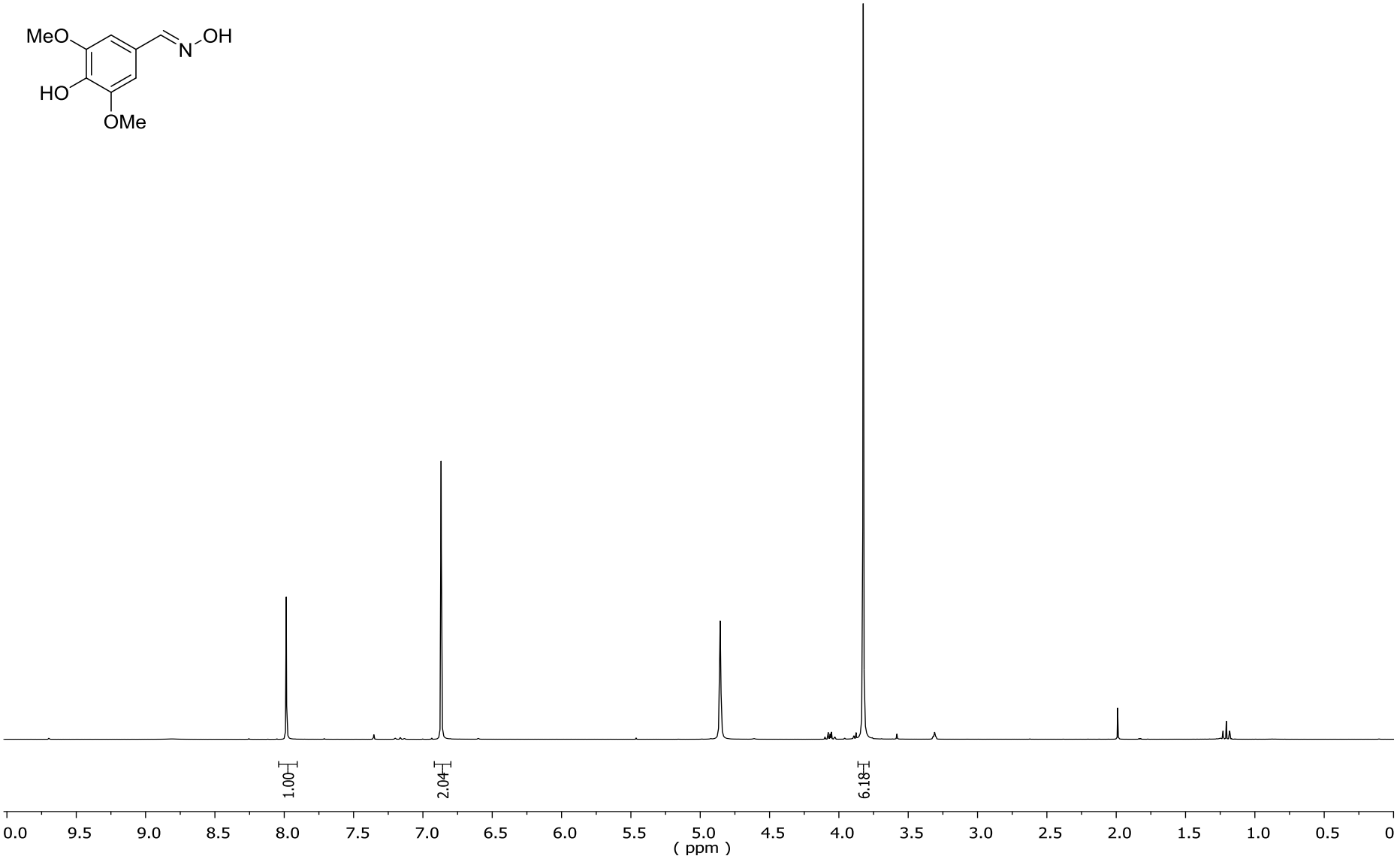
122.44
116.05
109.63
56.19
56.19
HO
N
OH
OMe
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


MeO
N
OH
HO
OMe
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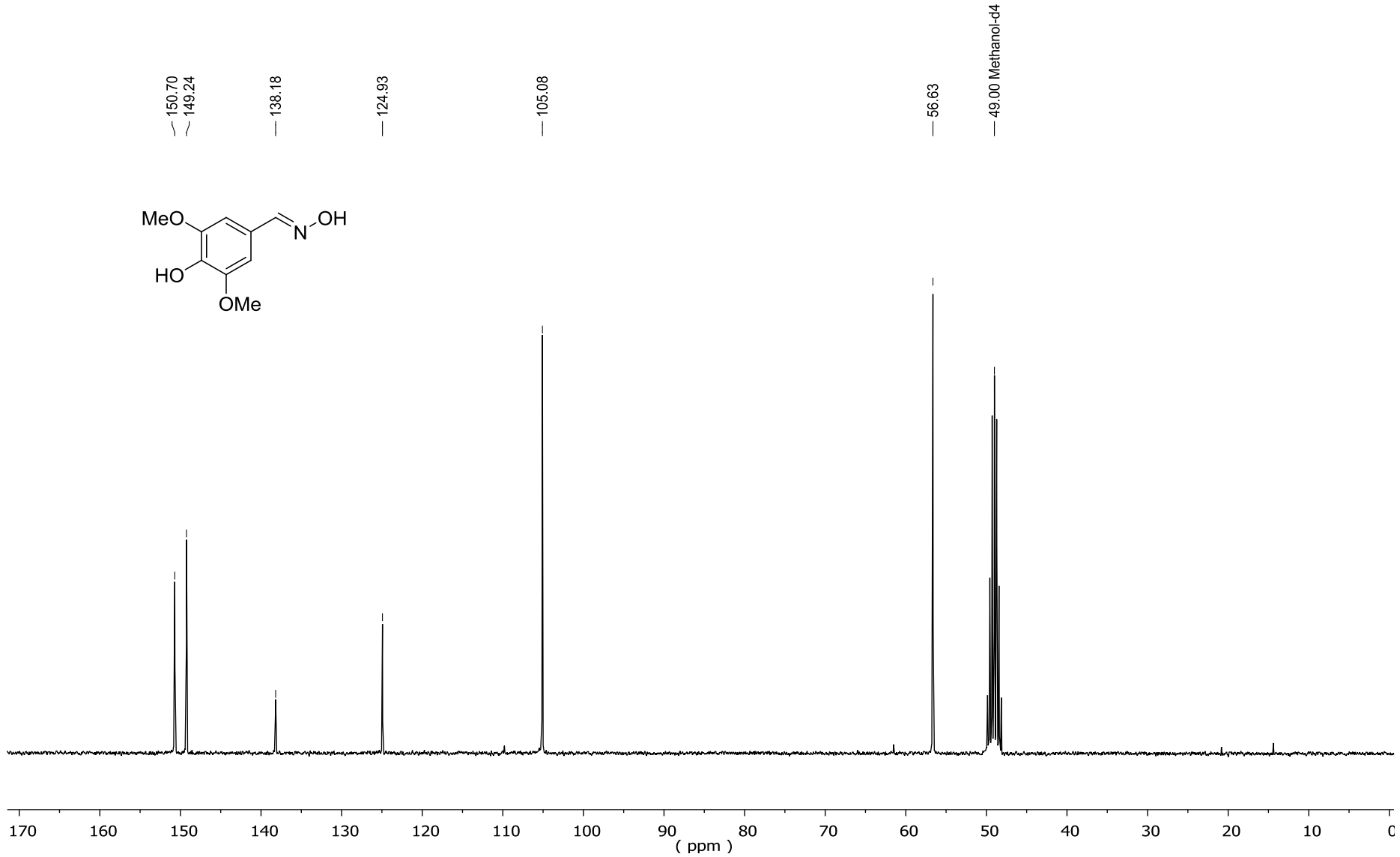
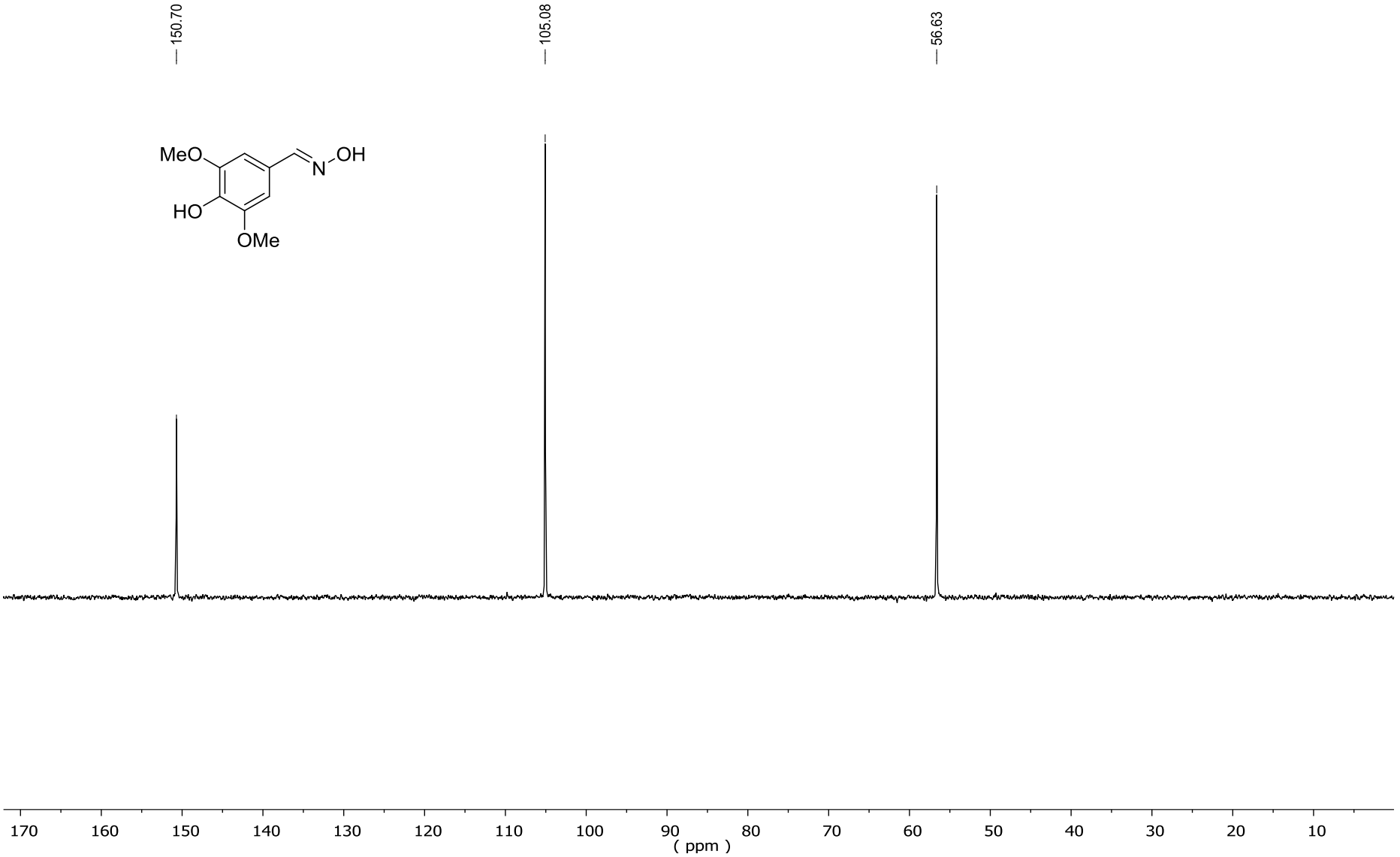
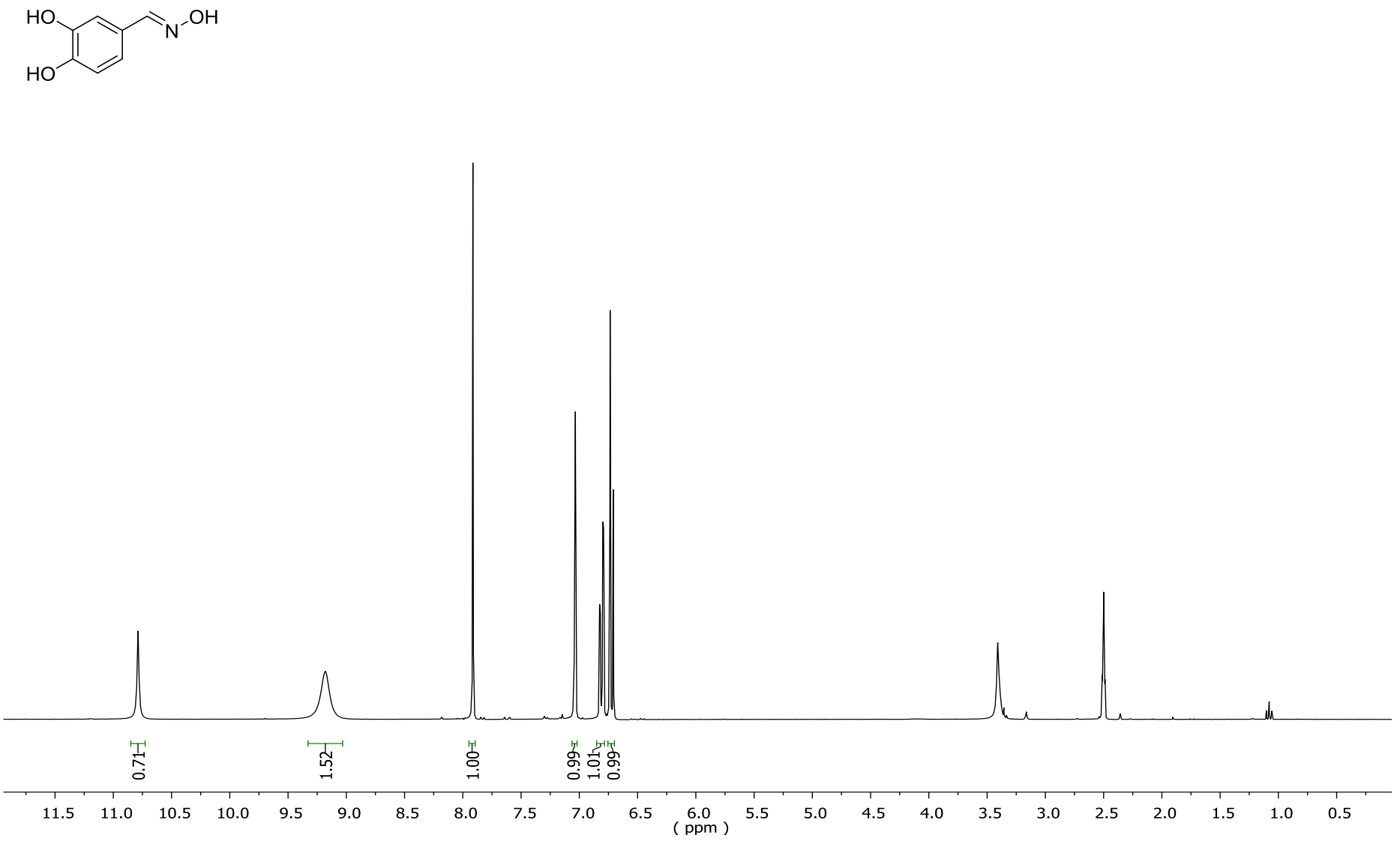
2.04
6.18
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
(ppm)


150.70
149.24
138.18
124.93
105.08
56.63
49.00 Methanol-d4
MeO
HO
OMe
N
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


150.70
105.08
56.63
MeO
HO
OMe
N
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


HO
HO
N
OH
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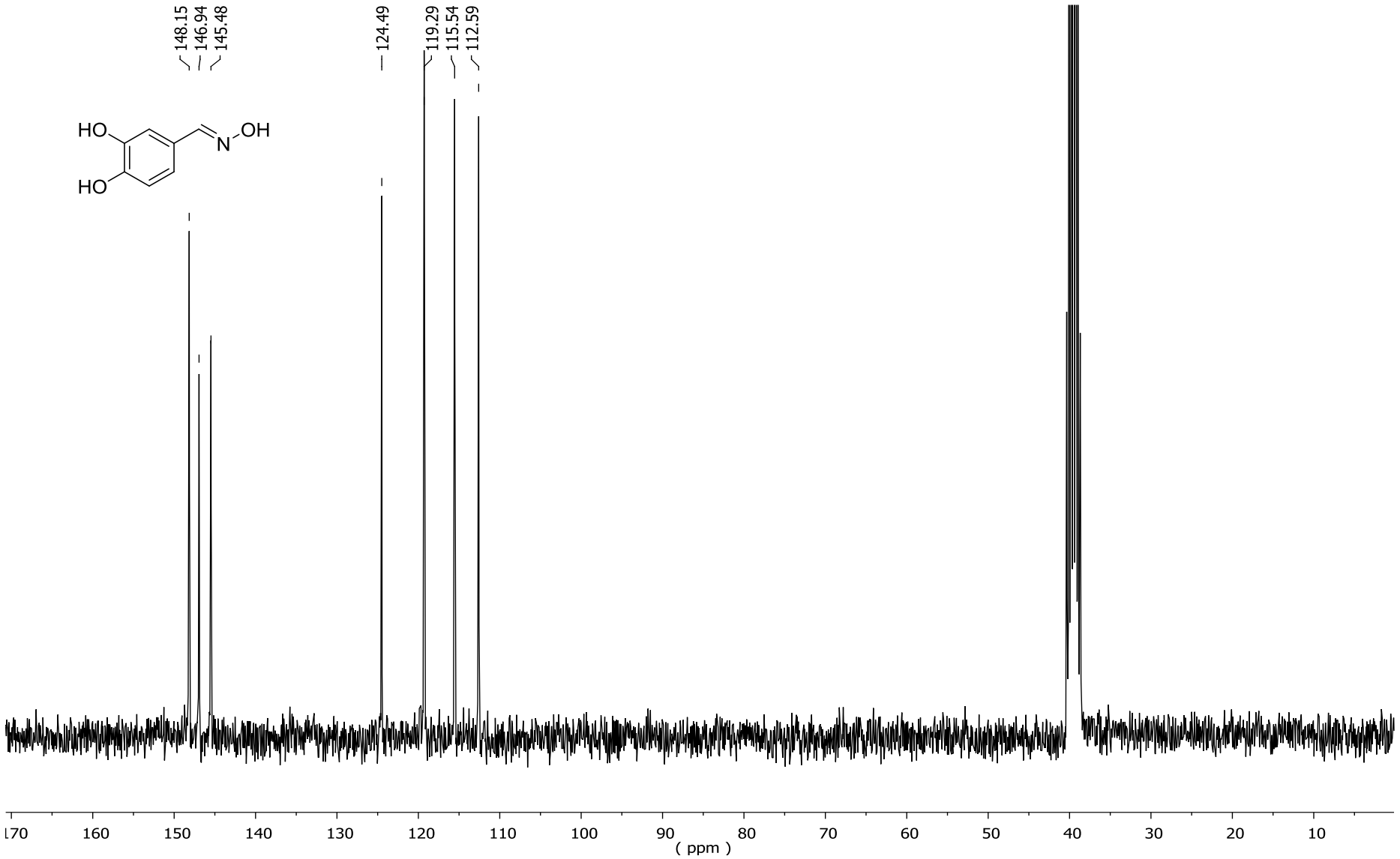
1.52
1.00
0.99
1.01
0.99
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)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146.94
145.48
124.49
119.29
115.54
112.59
HO
HO
N
OH
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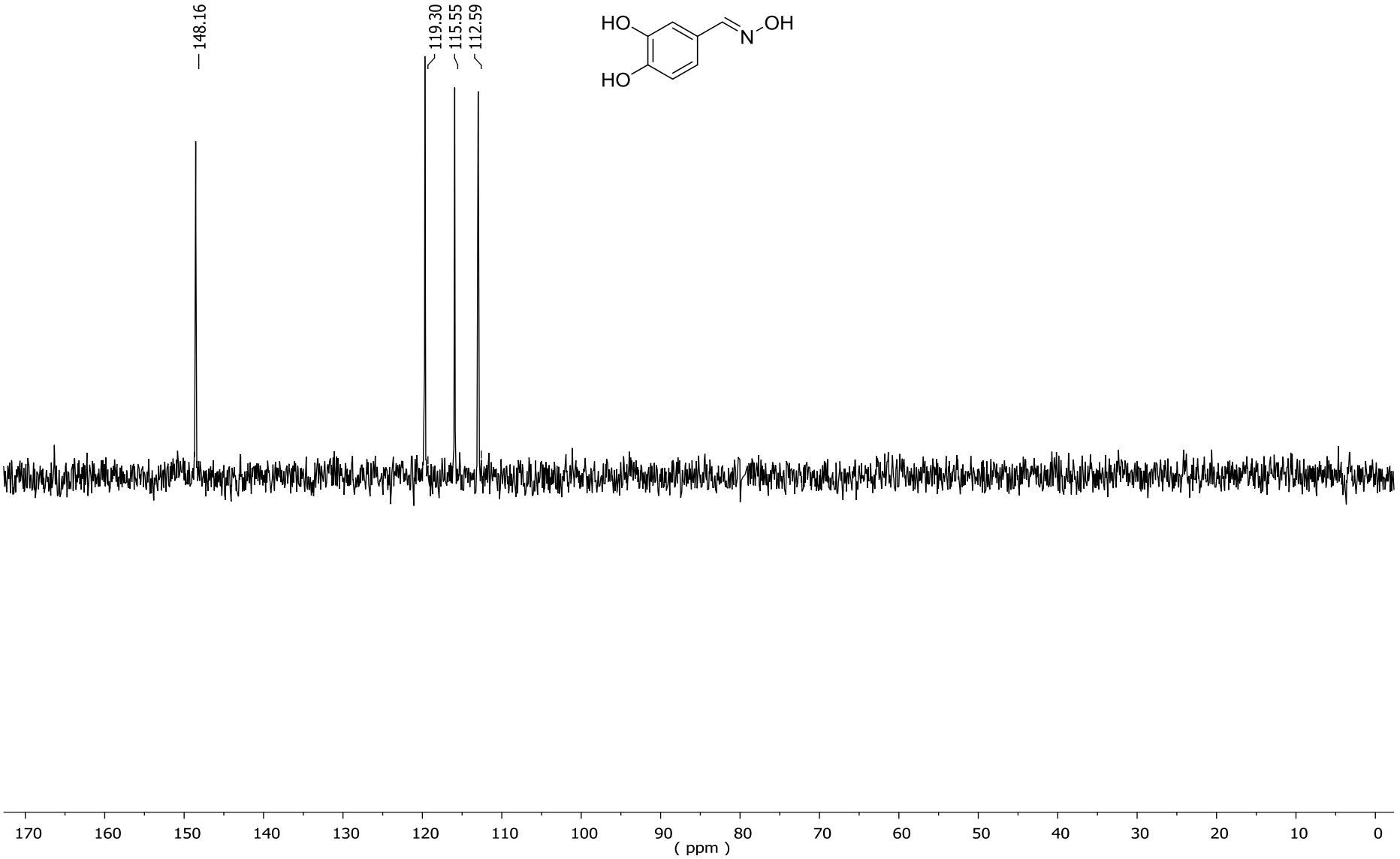
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)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115.55
112.59
HO
HO
N
OH
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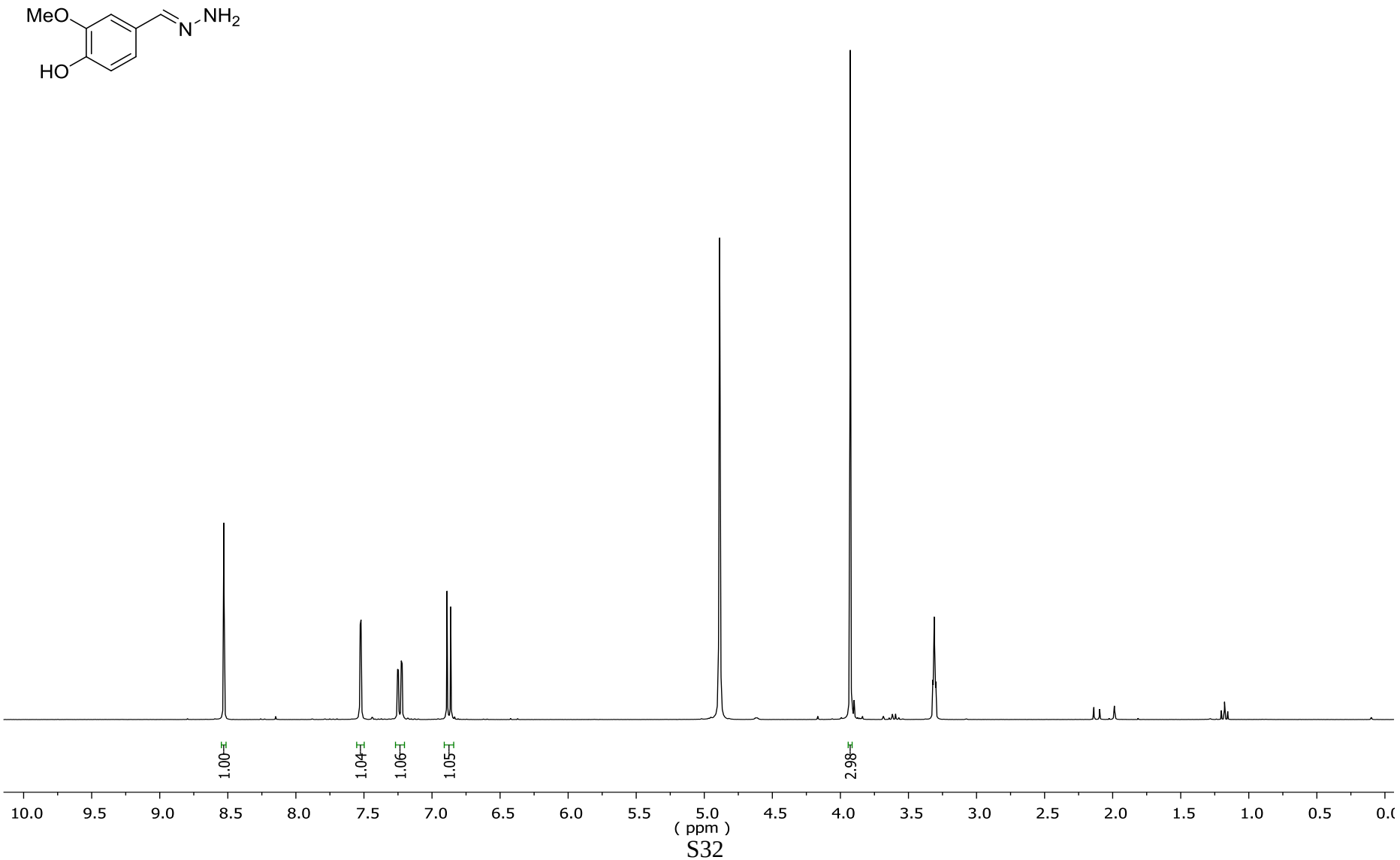
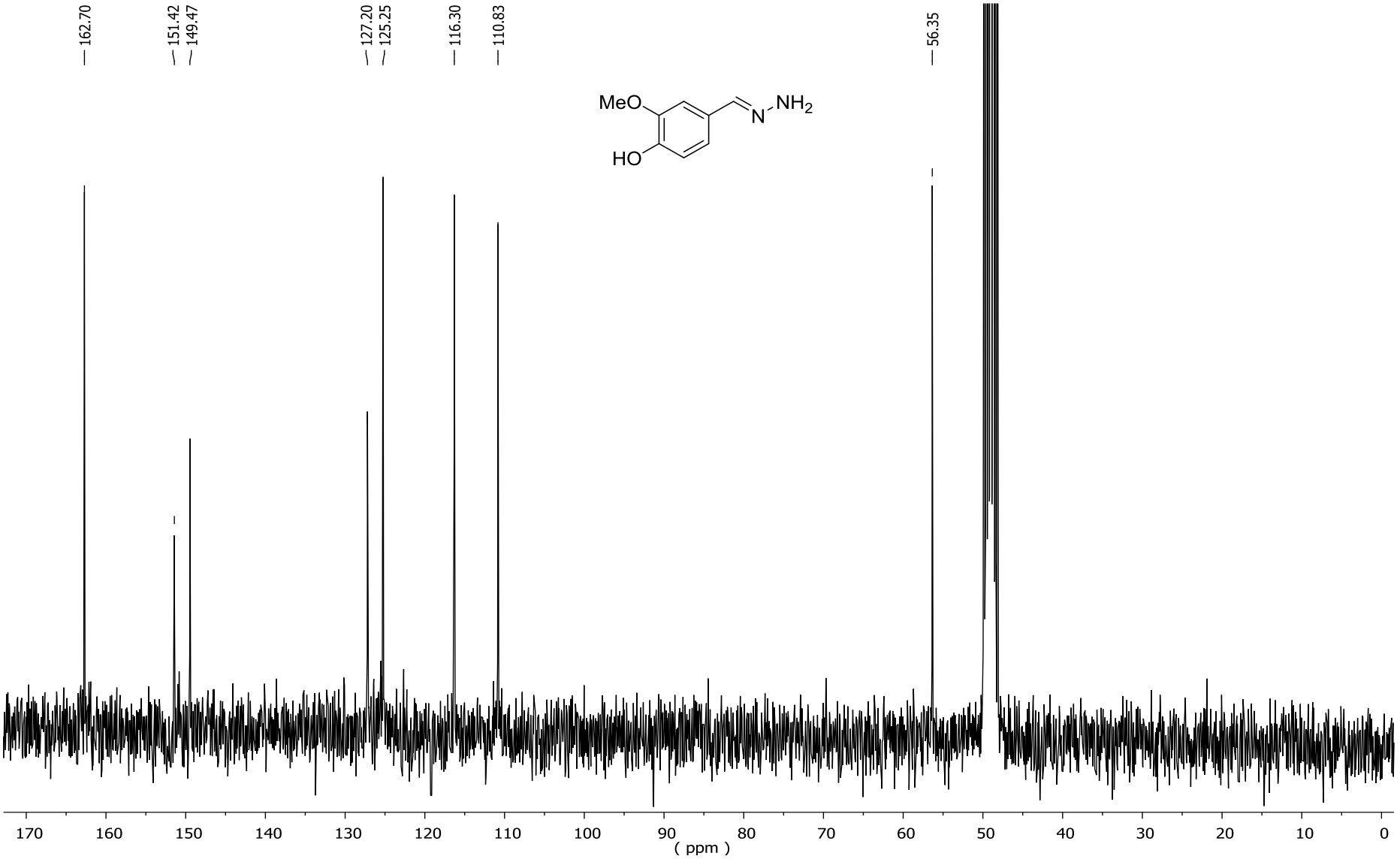
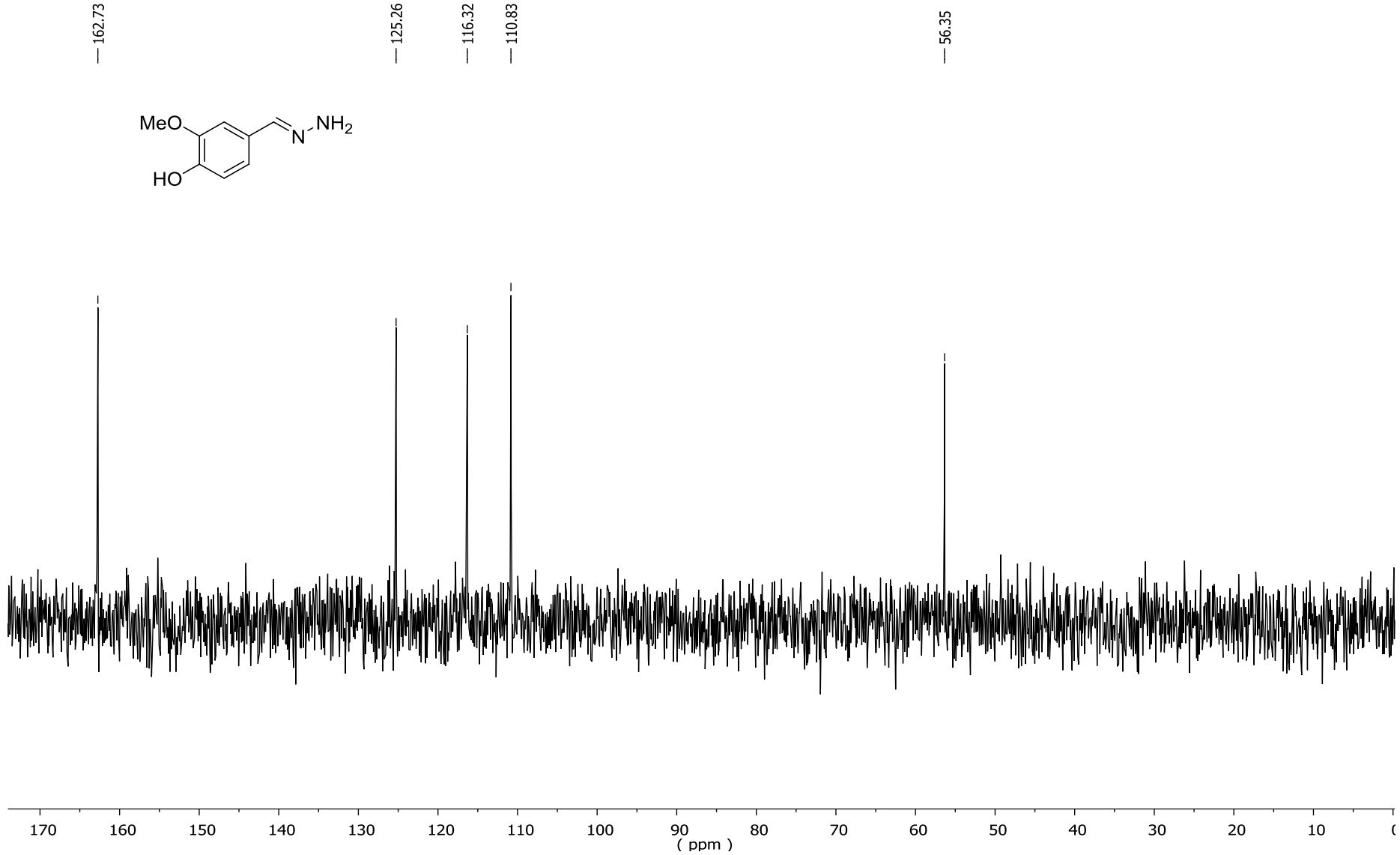
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


MeO
HO
N
NH2
1.00
1.04
1.06
1.05
2.98
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)


162.70
151.42
149.47
127.20
125.25
116.30
110.83
56.35
MeO
HO
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


162.73
125.26
116.32
110.83
56.35
MeO
HO
N
NH2
170
160
150
140
130
120
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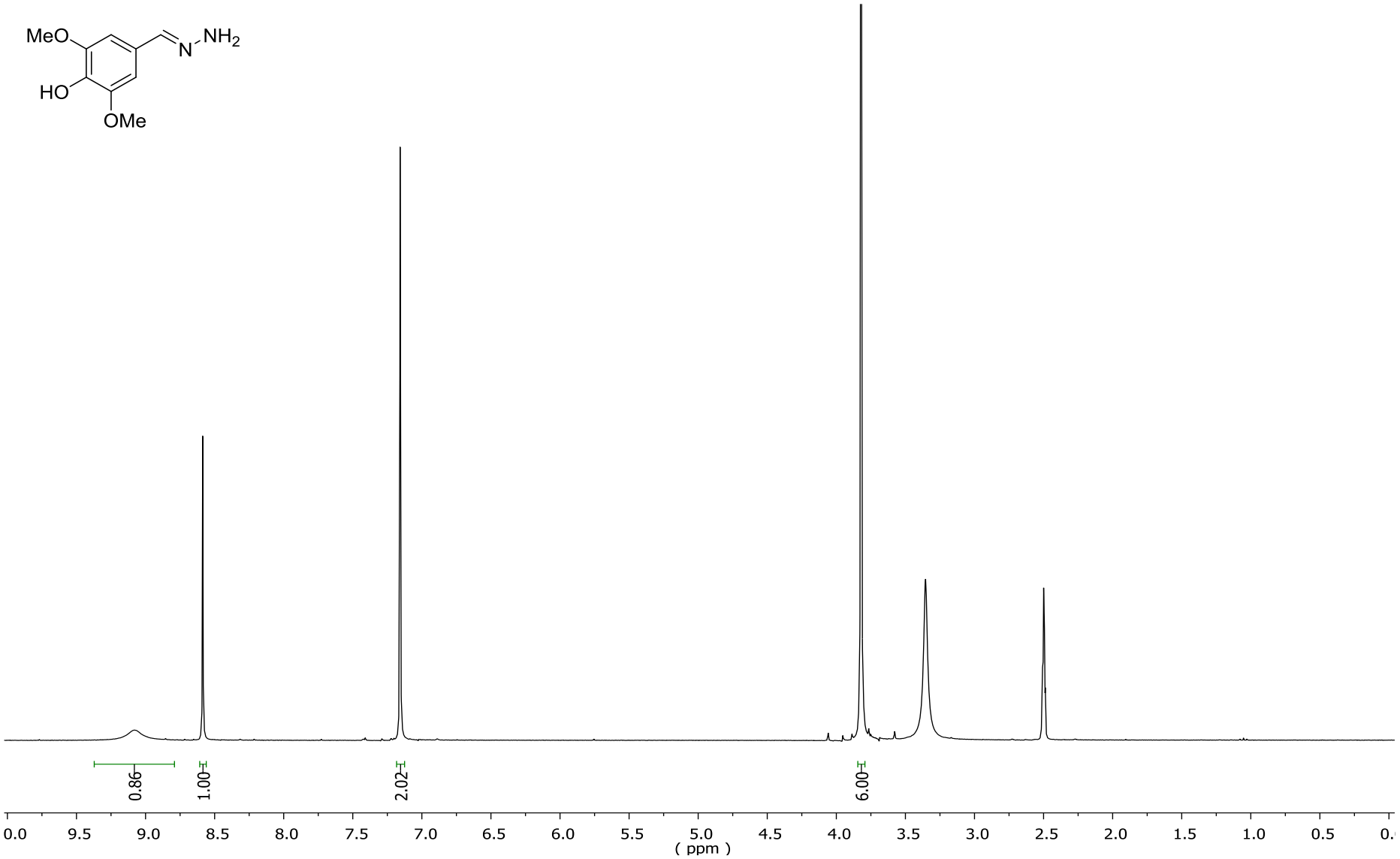
100
90
80
70
60
50
40
30
20
10
(ppm)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HO
OMe
N
NH2
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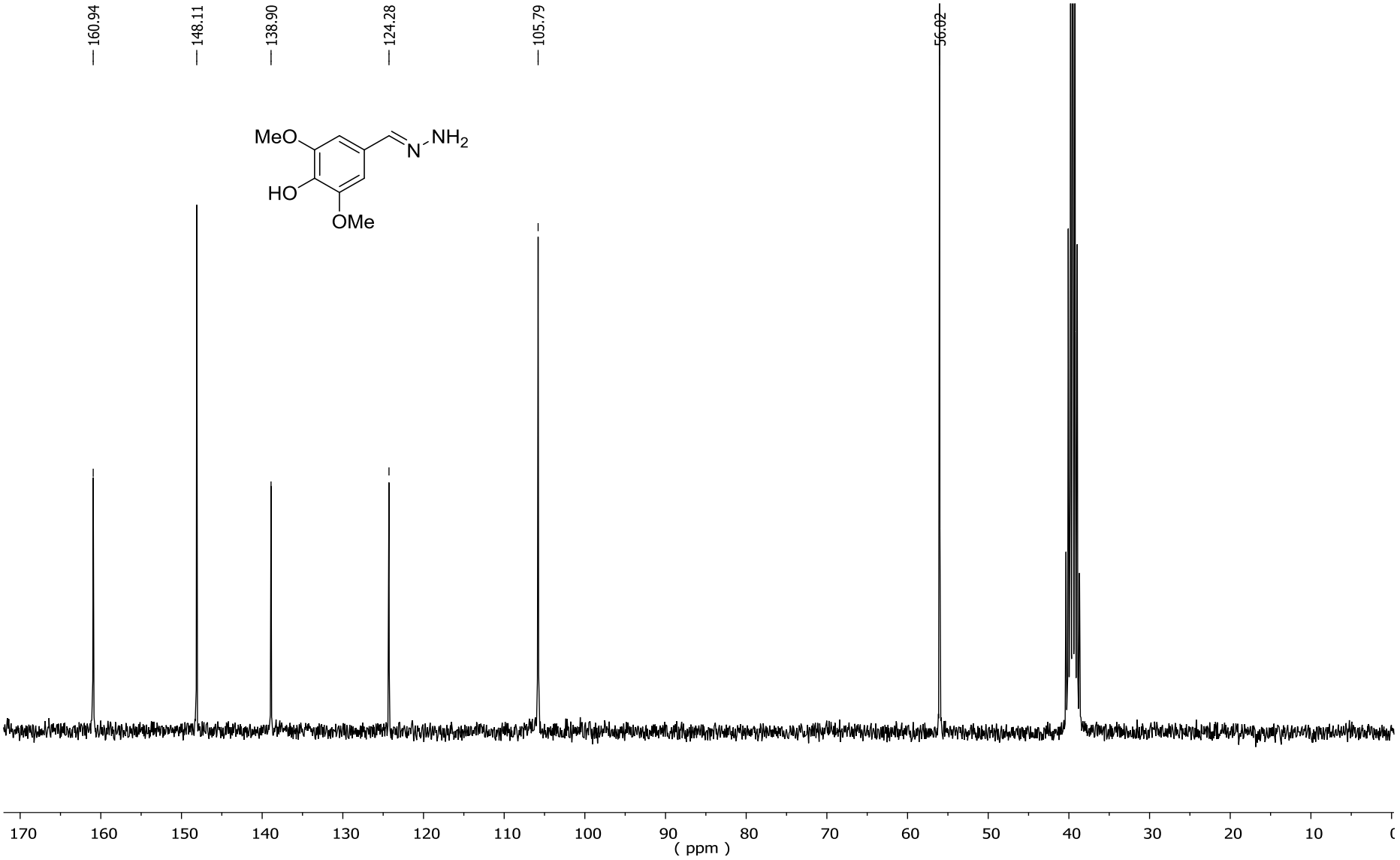
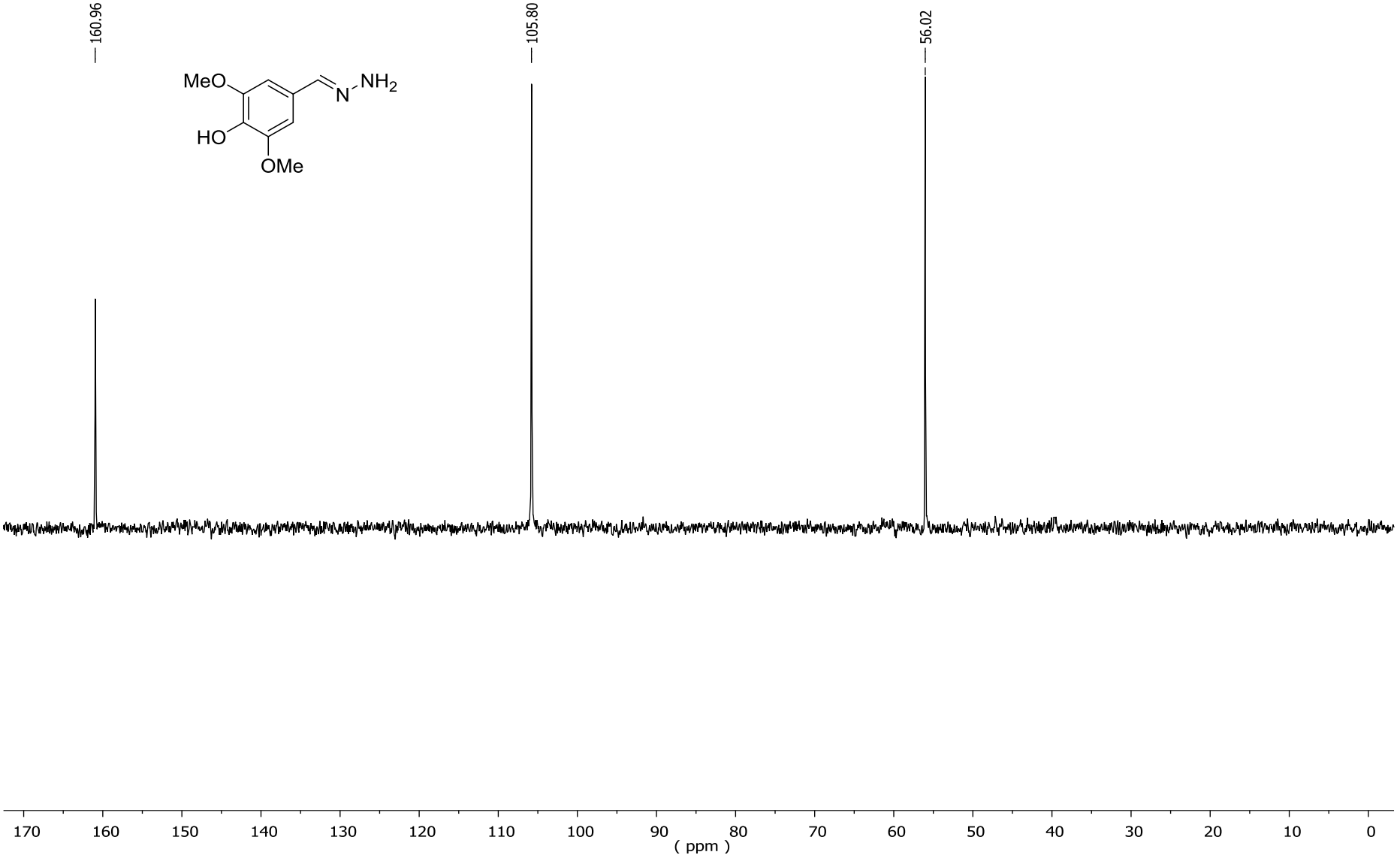
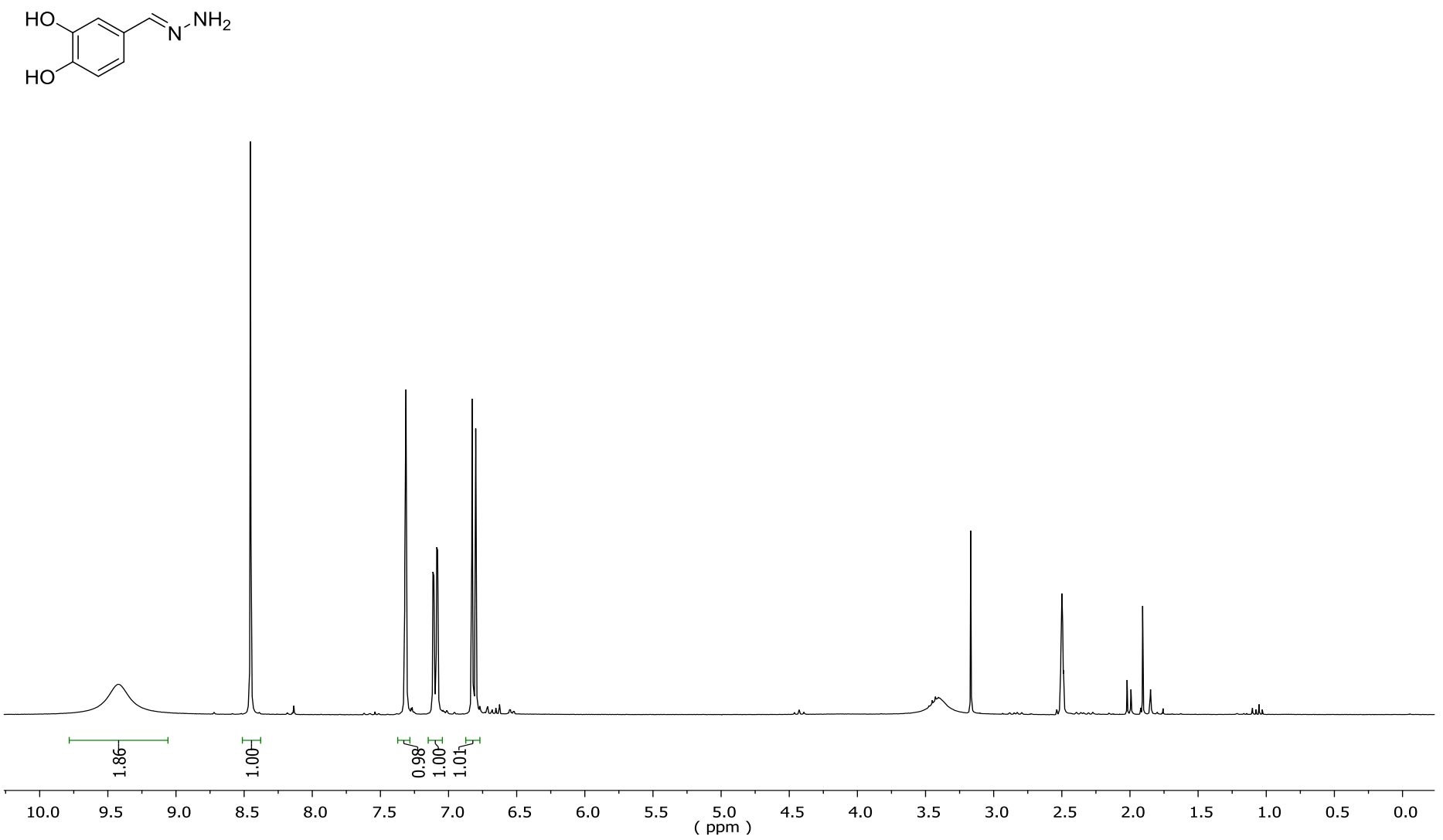
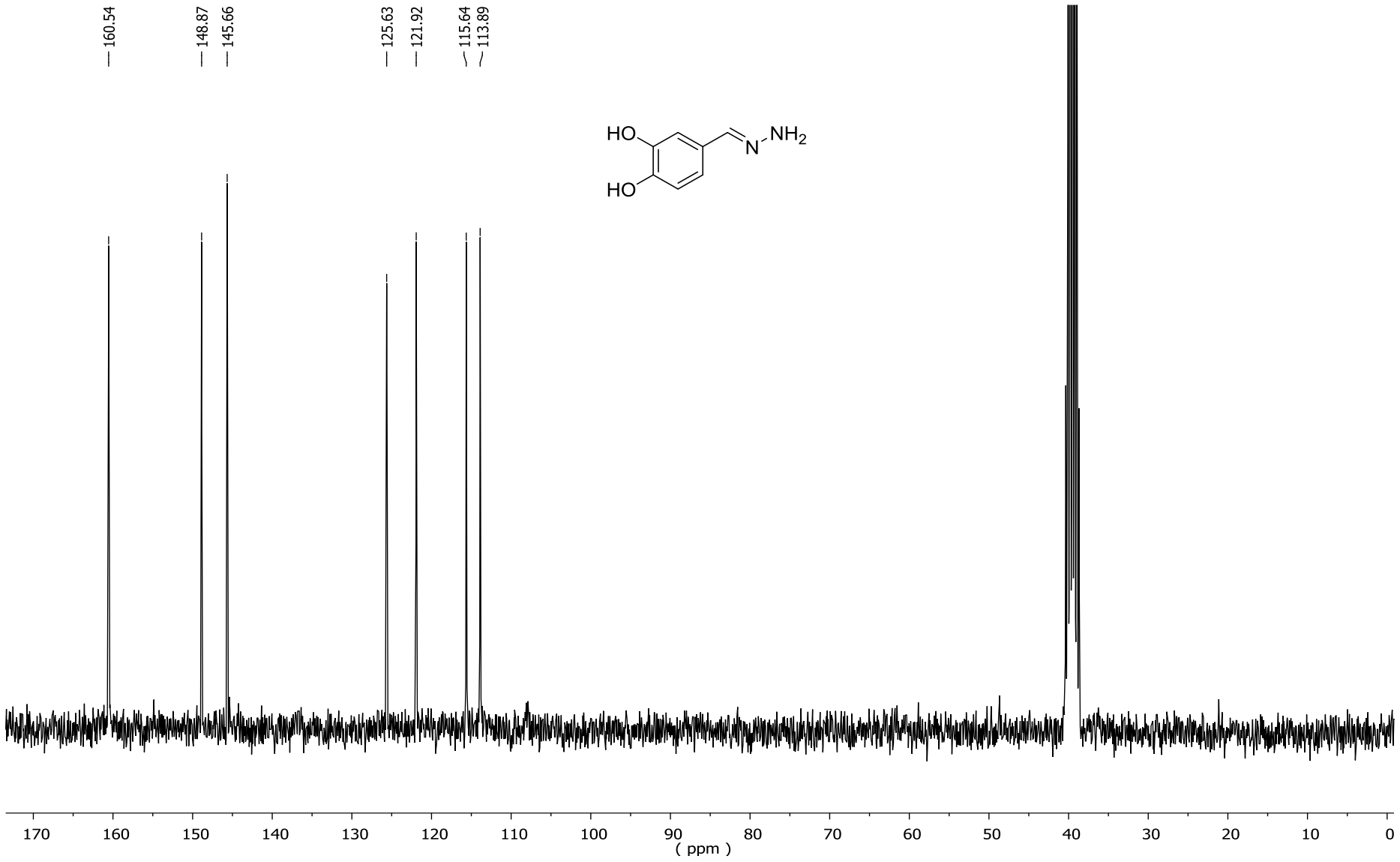
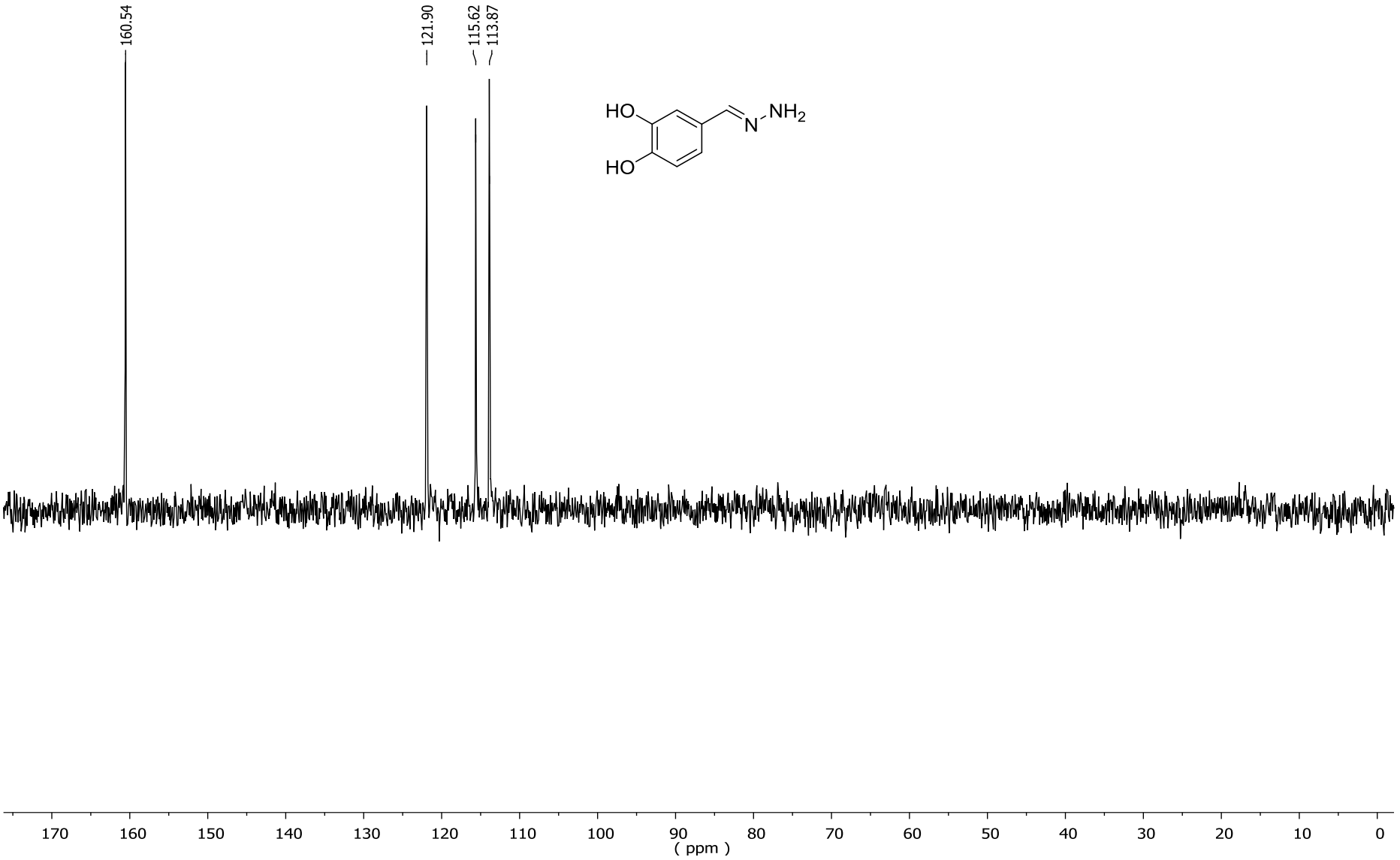
2.02
6.00
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

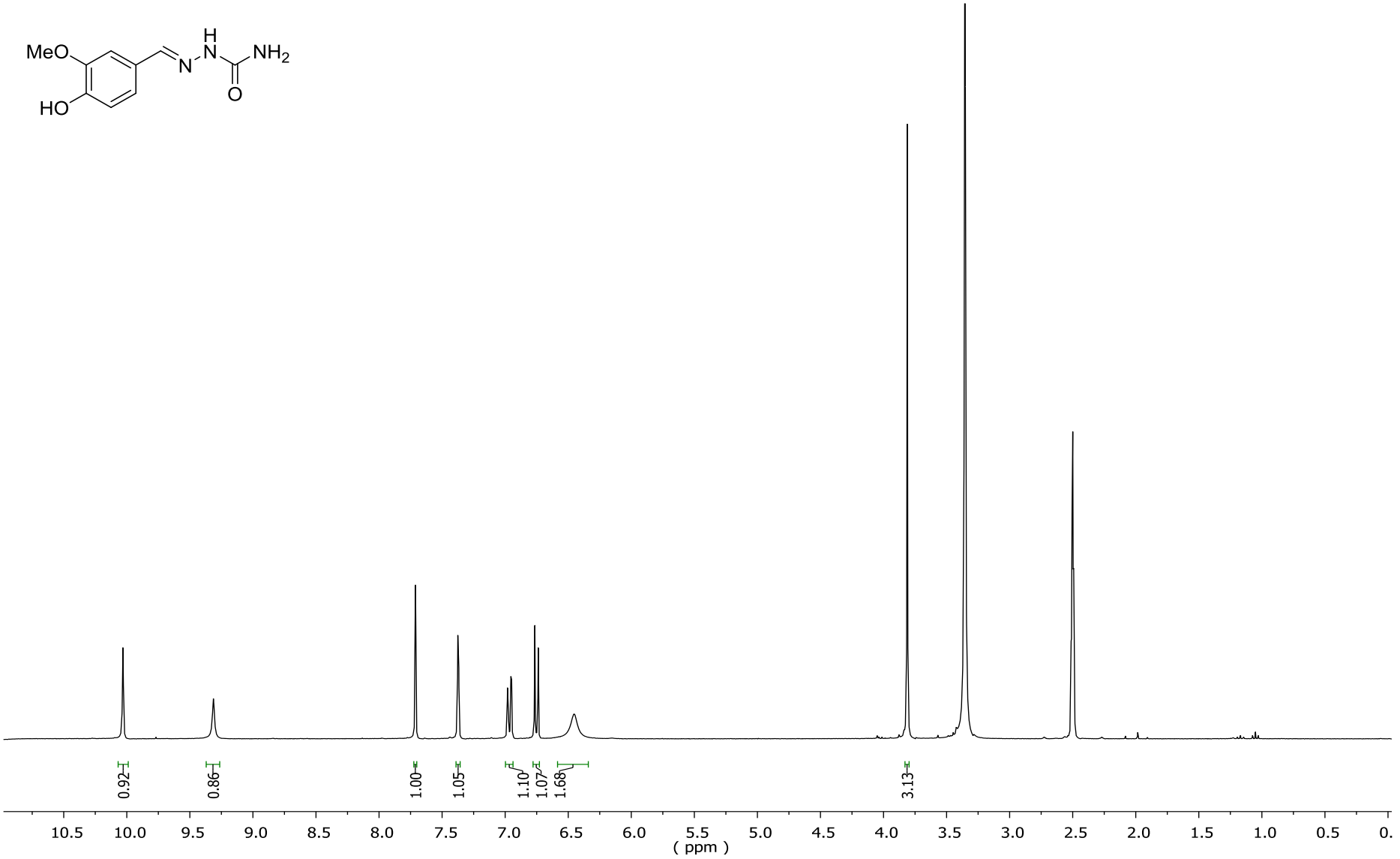

160.94
148.11
138.90
124.28
105.79
56.02
MeO
HO
OMe
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

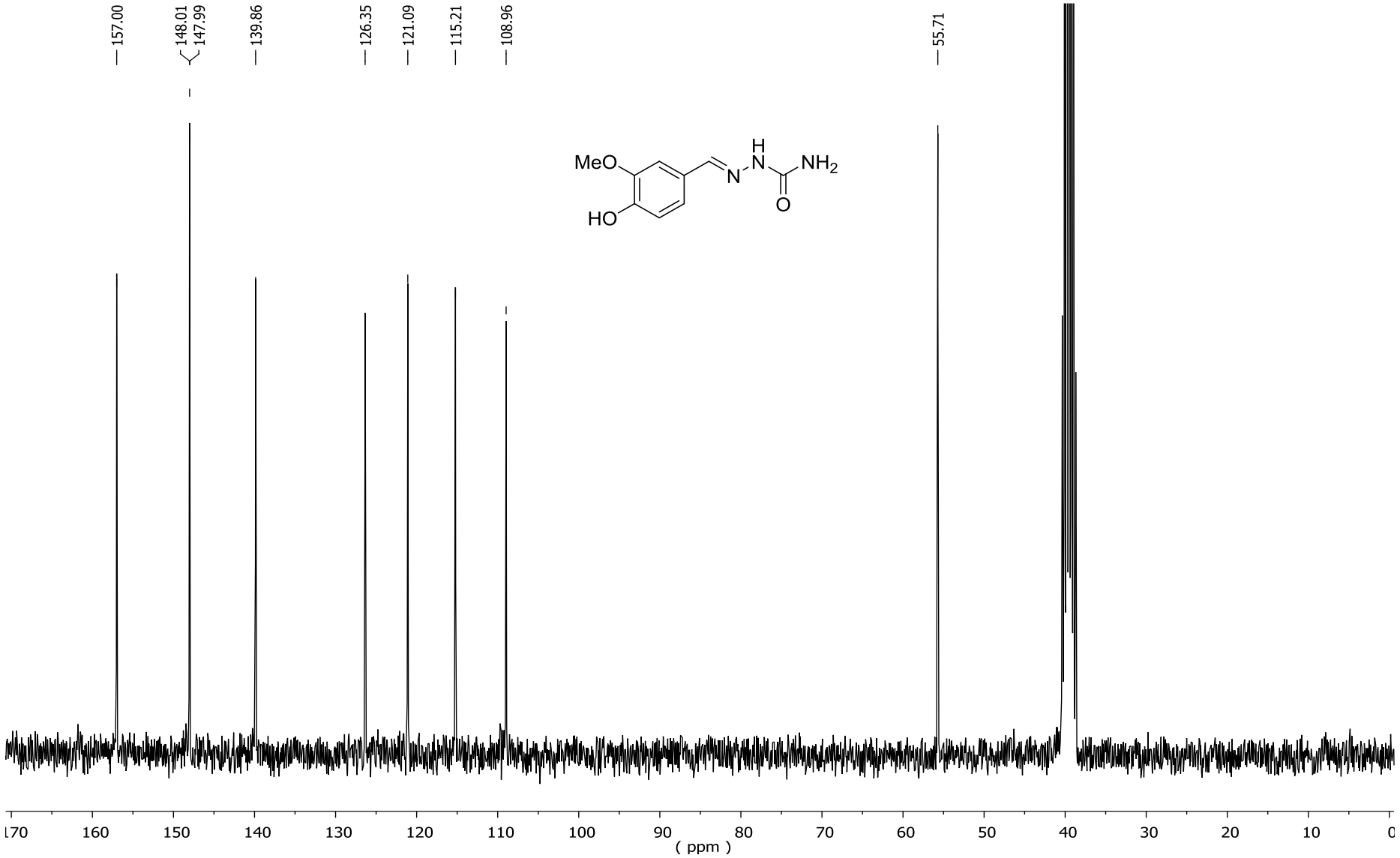

160.96
105.80
56.02
MeO
HO
OMe
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

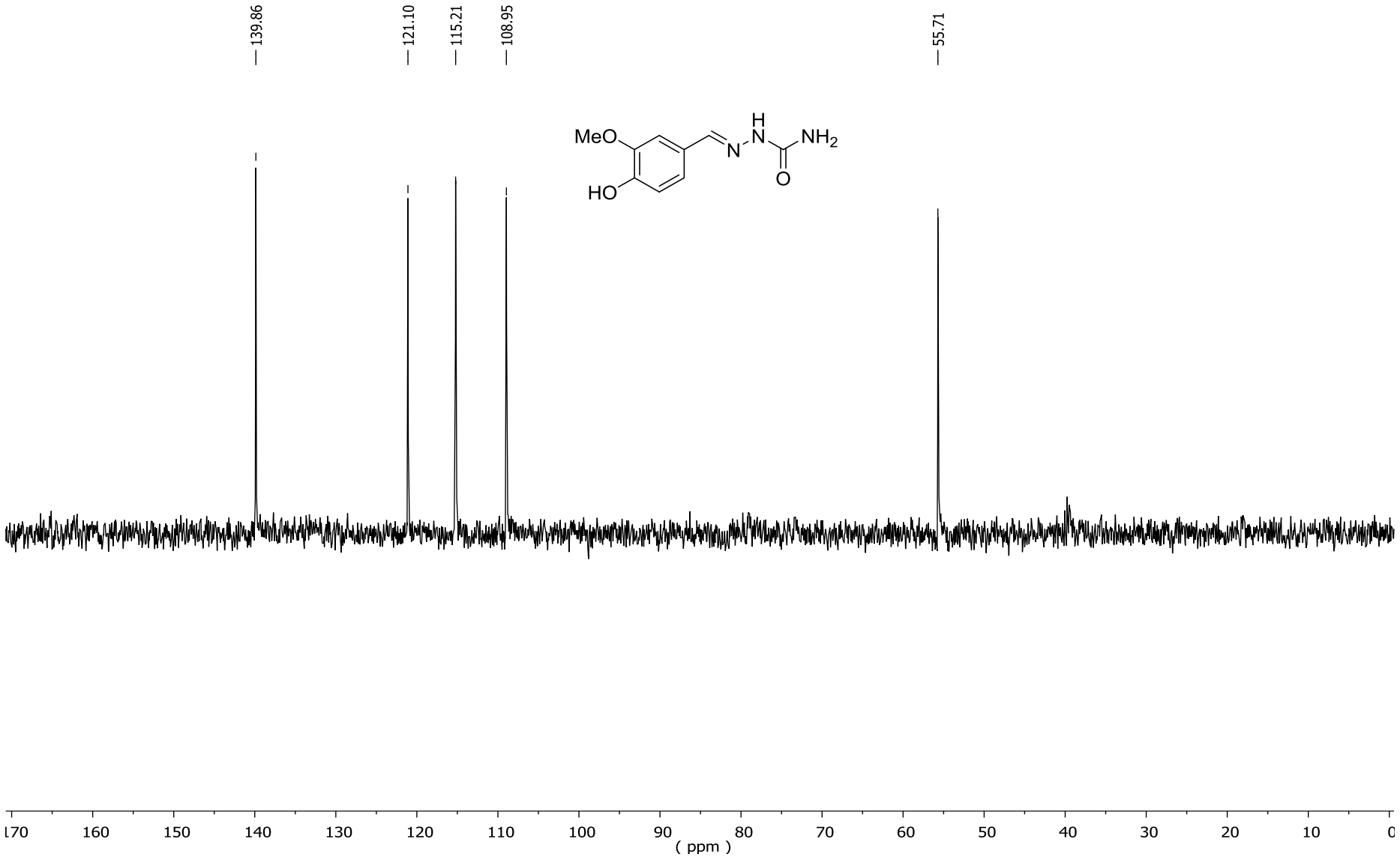

HO
HO
N
NH2
1.86
1.00
0.98
1.00
1.01
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

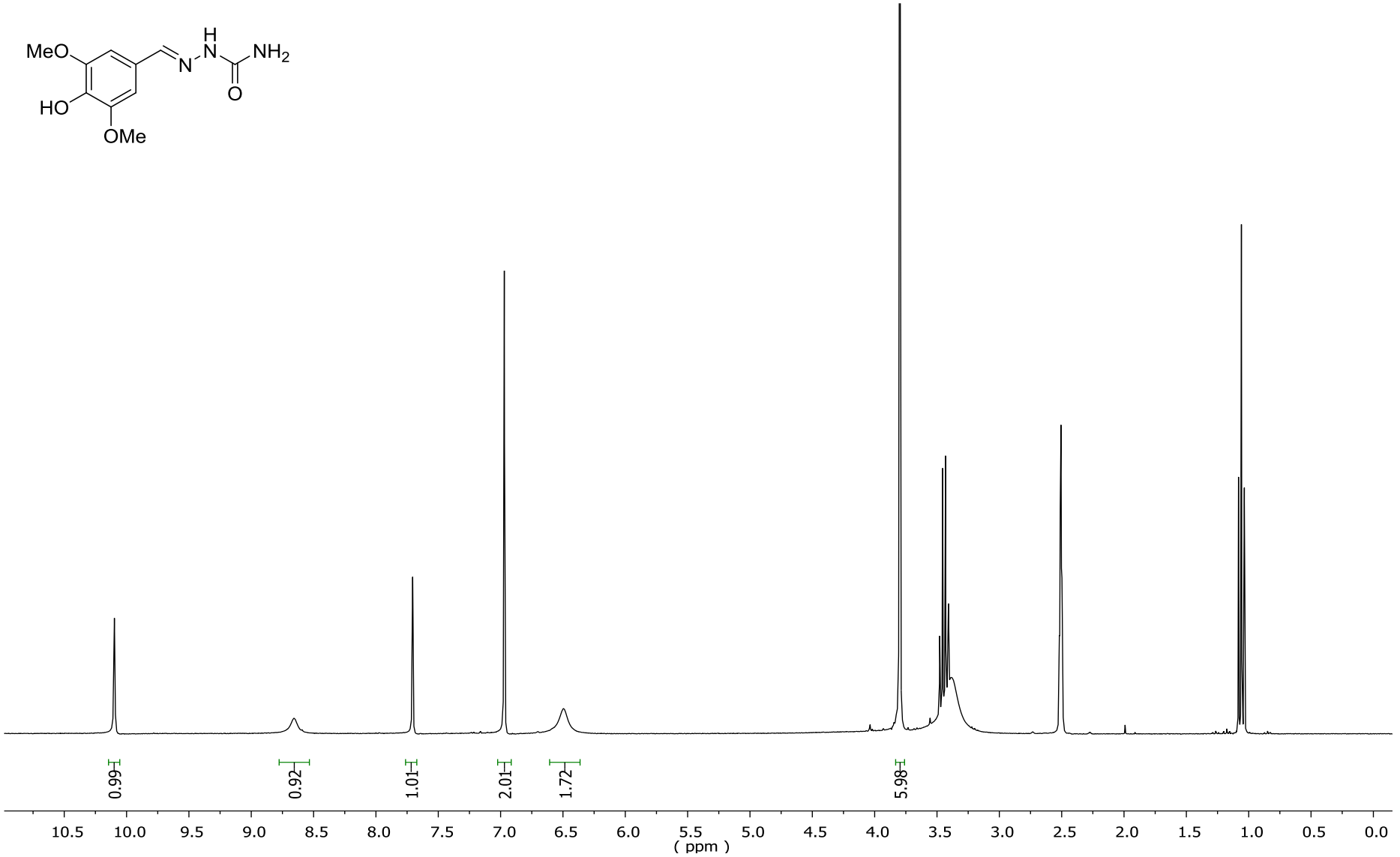

160.54
148.87
145.66
125.63
121.92
115.64
113.89
HO
HO
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

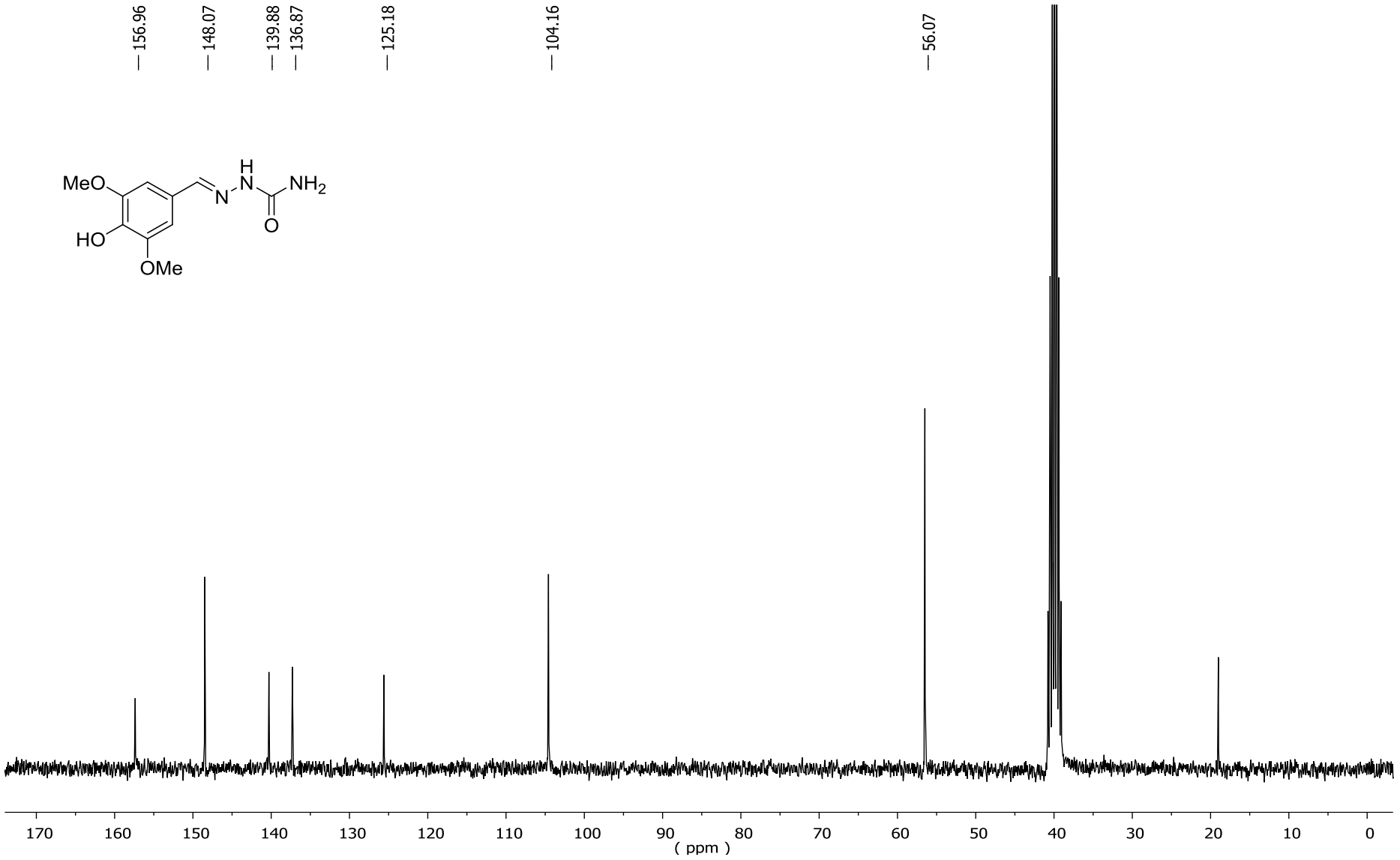

160.54
121.90
115.62
113.87
HO
HO
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

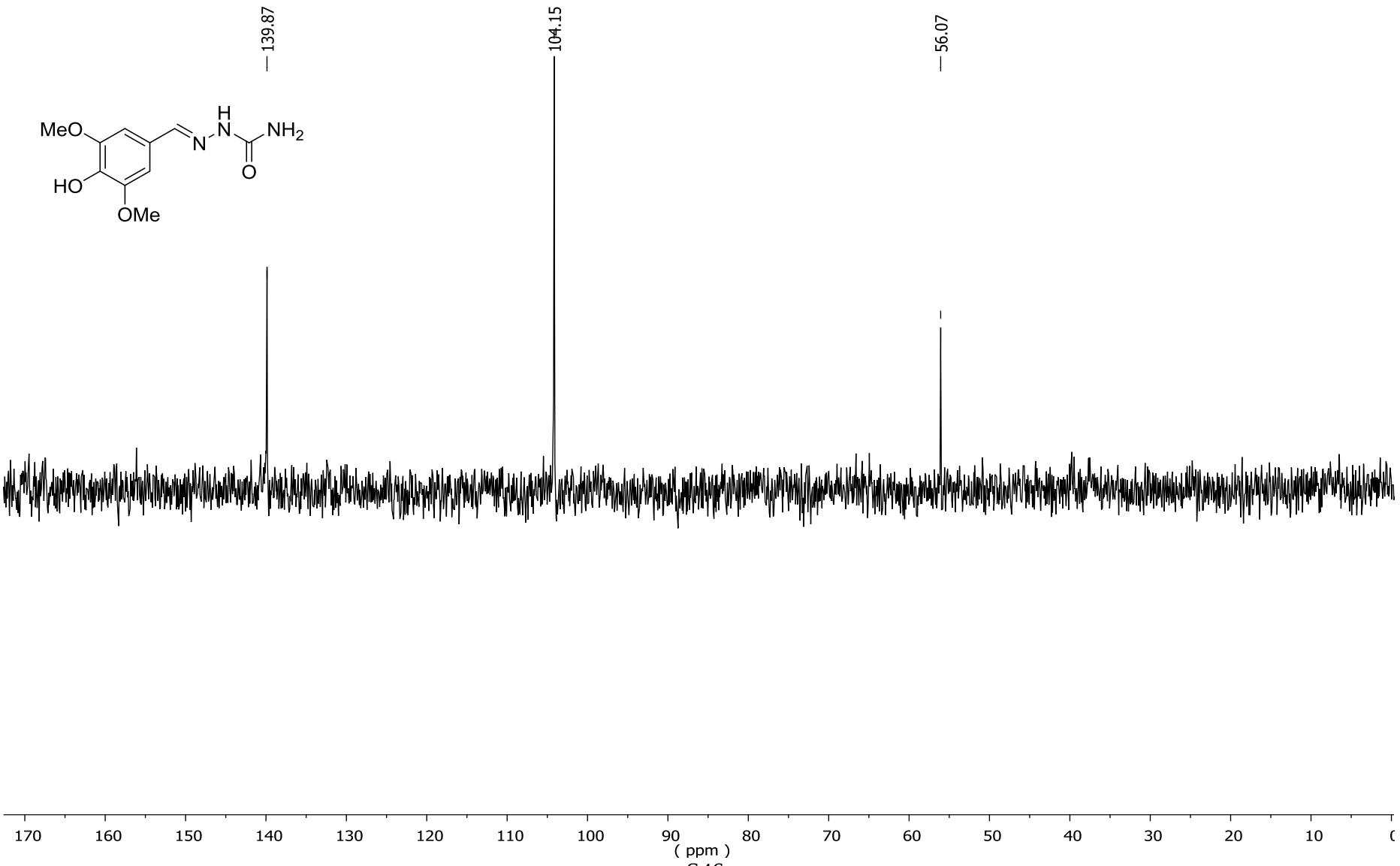

MeO
HO
H
N
N
NH2
O
0.92
0.86
1.00
1.05
1.10
1.07
1.68
3.13
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)

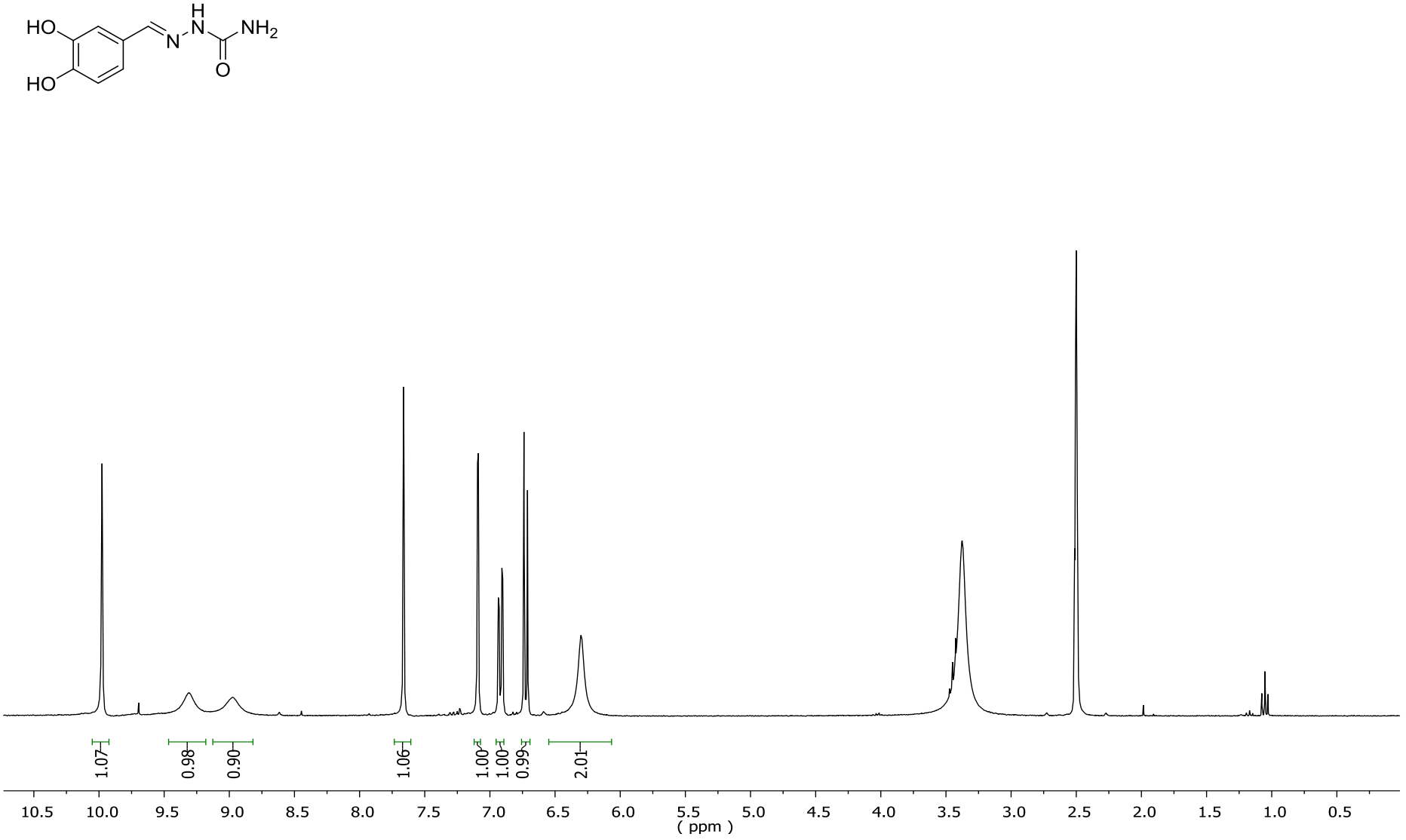

157.00
148.01
147.99
139.86
126.35
121.09
115.21
108.96
55.71
MeO
HO
H
N
N
NH2
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

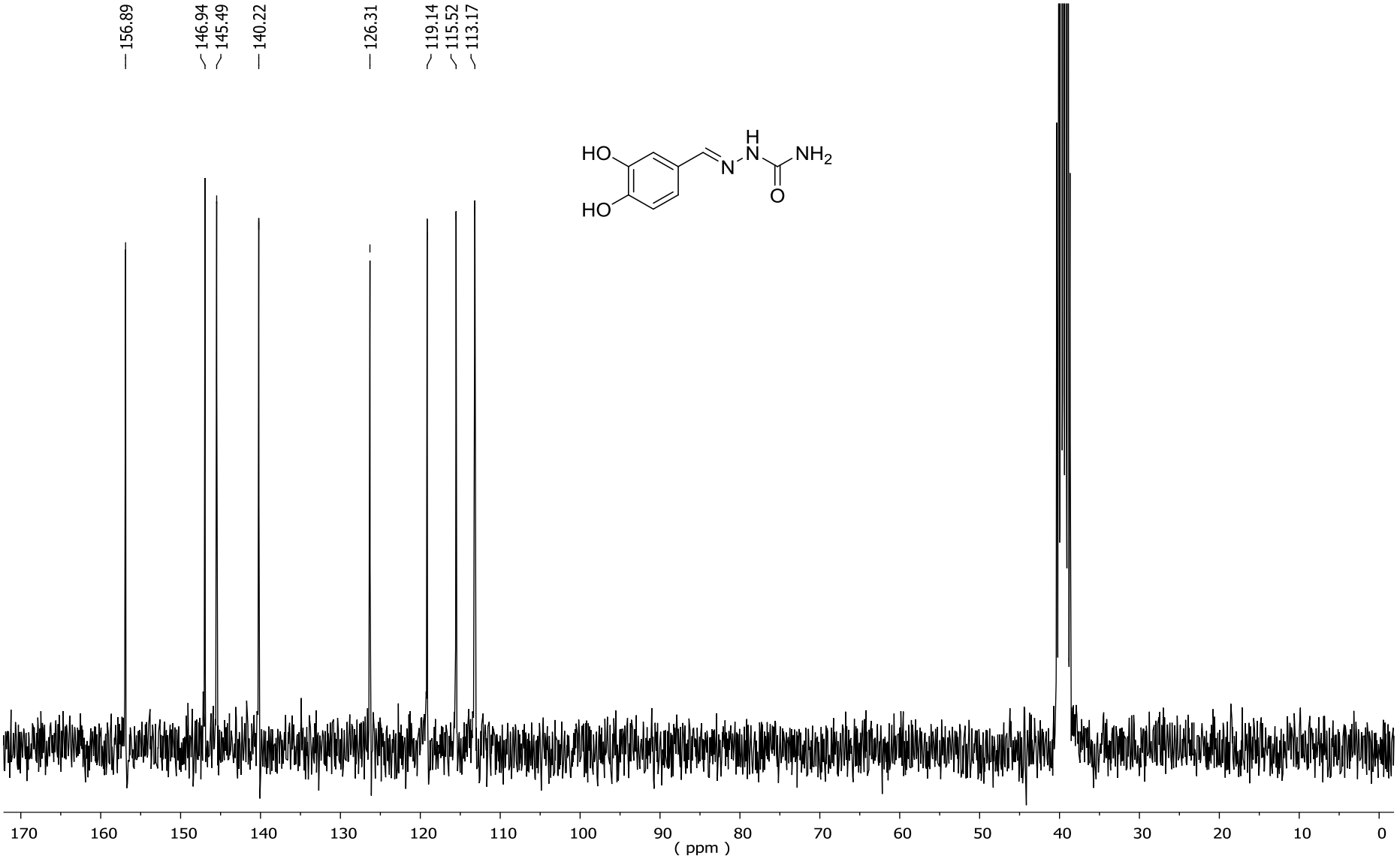

139.86
121.10
115.21
108.95
55.71
MeO
HO
H
N
N
NH2
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

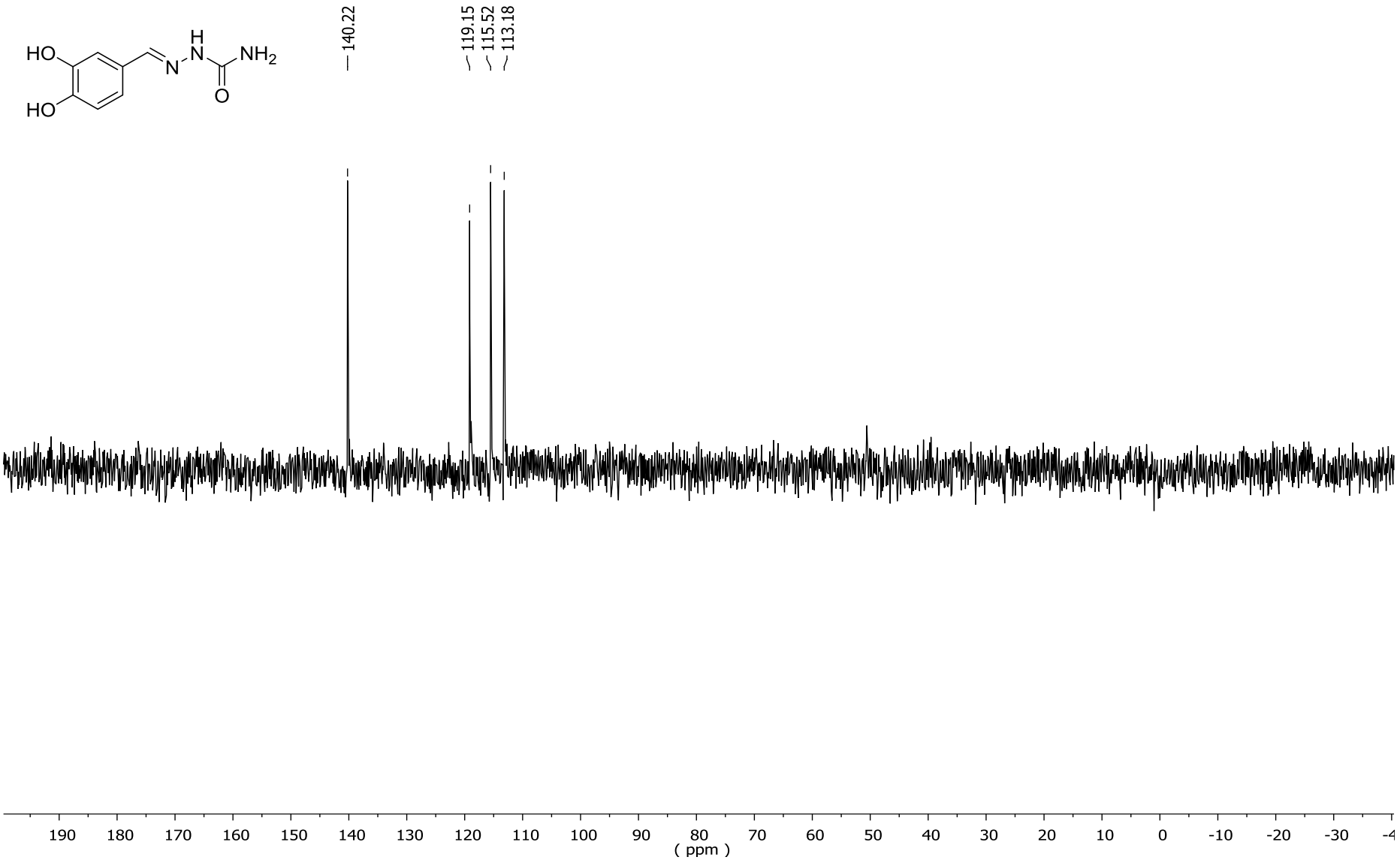

MeO
HO
OMe
H
N
N
NH2
O
0.99
0.92
1.01
2.01
1.72
5.98
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

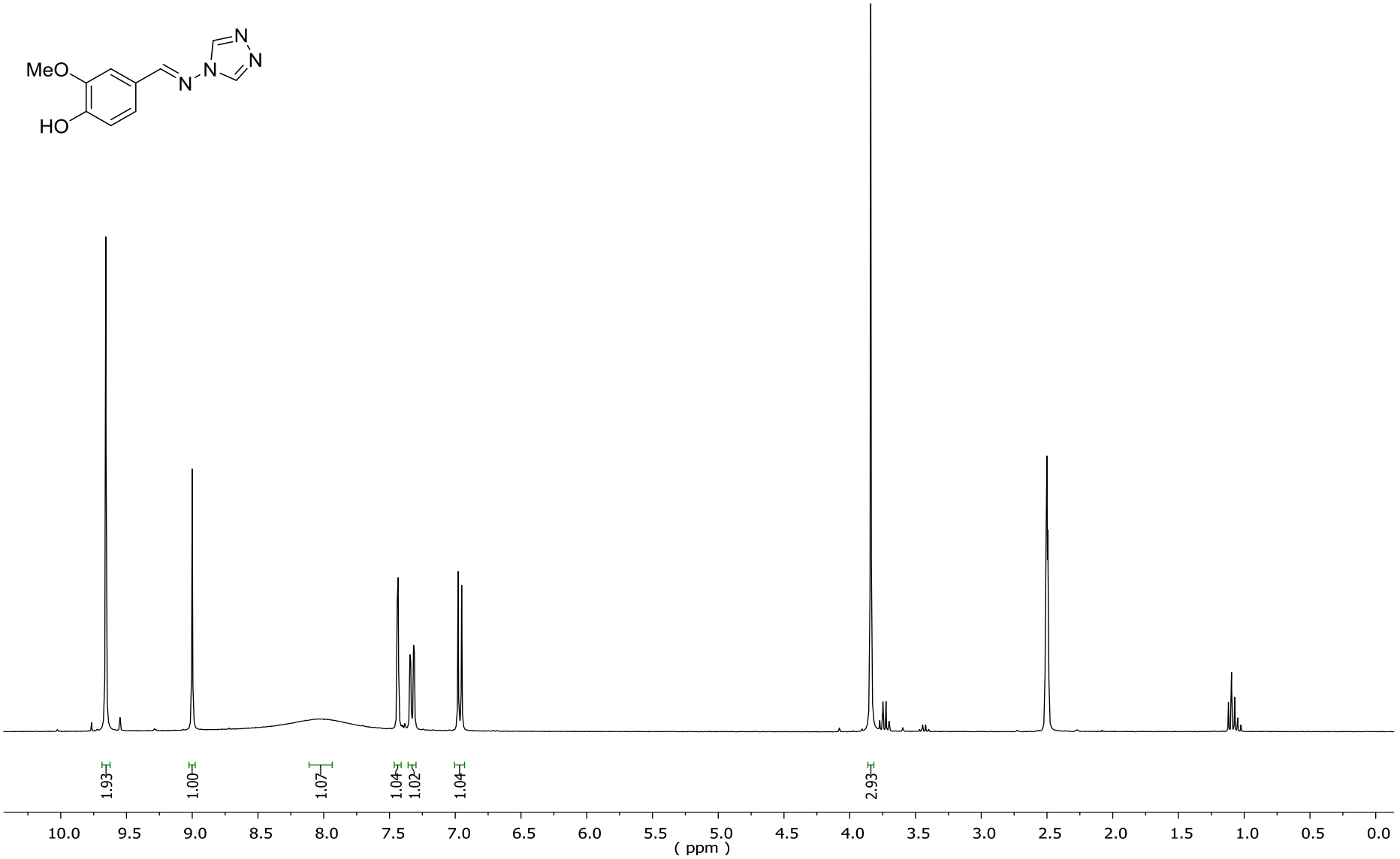

156.96
148.07
139.88
136.87
125.18
104.16
56.07
MeO
HO
OMe
H
N
N
NH2
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

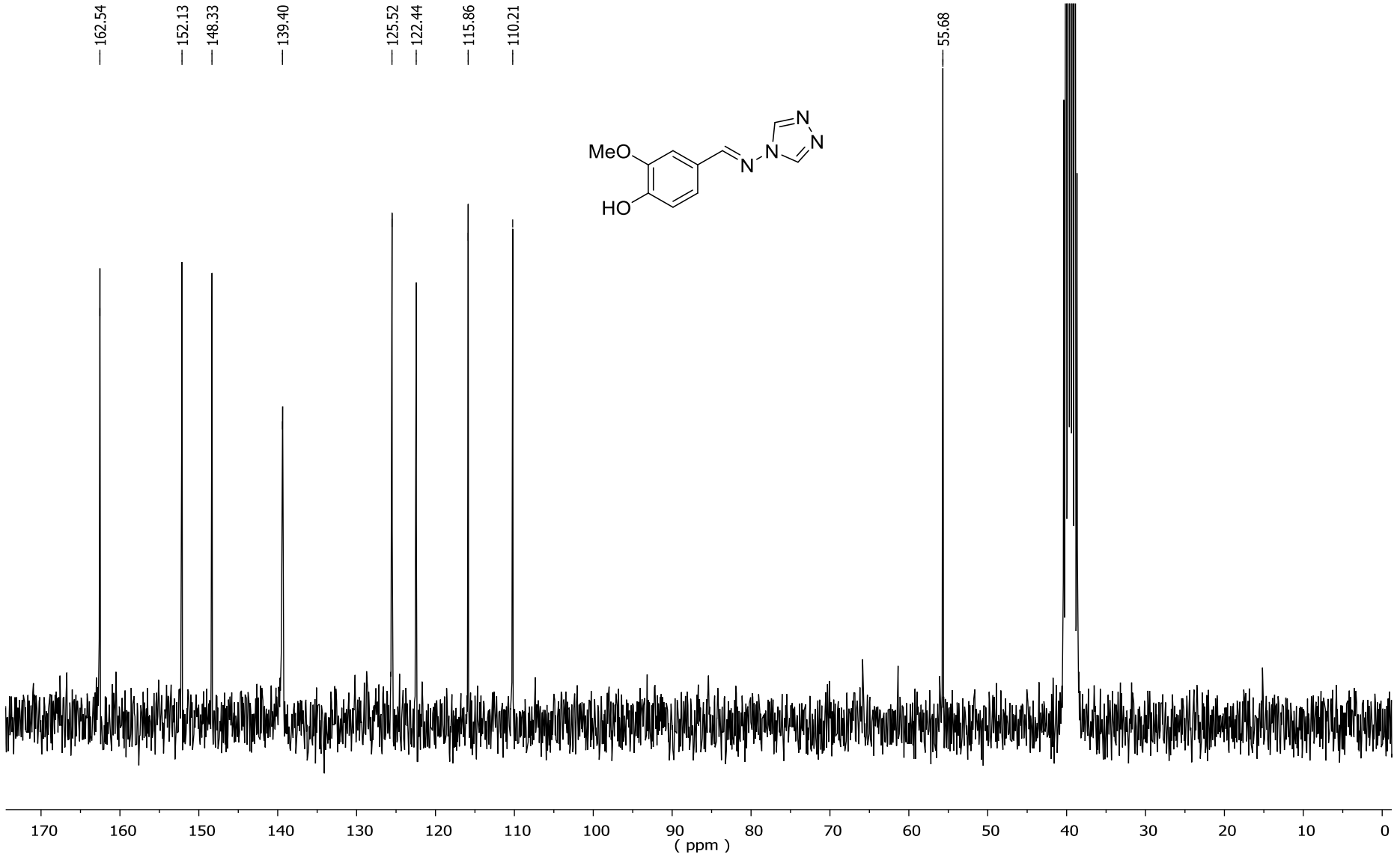

139.87
104.15
56.07
MeO
HO
OMe
H
N
N
NH2
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

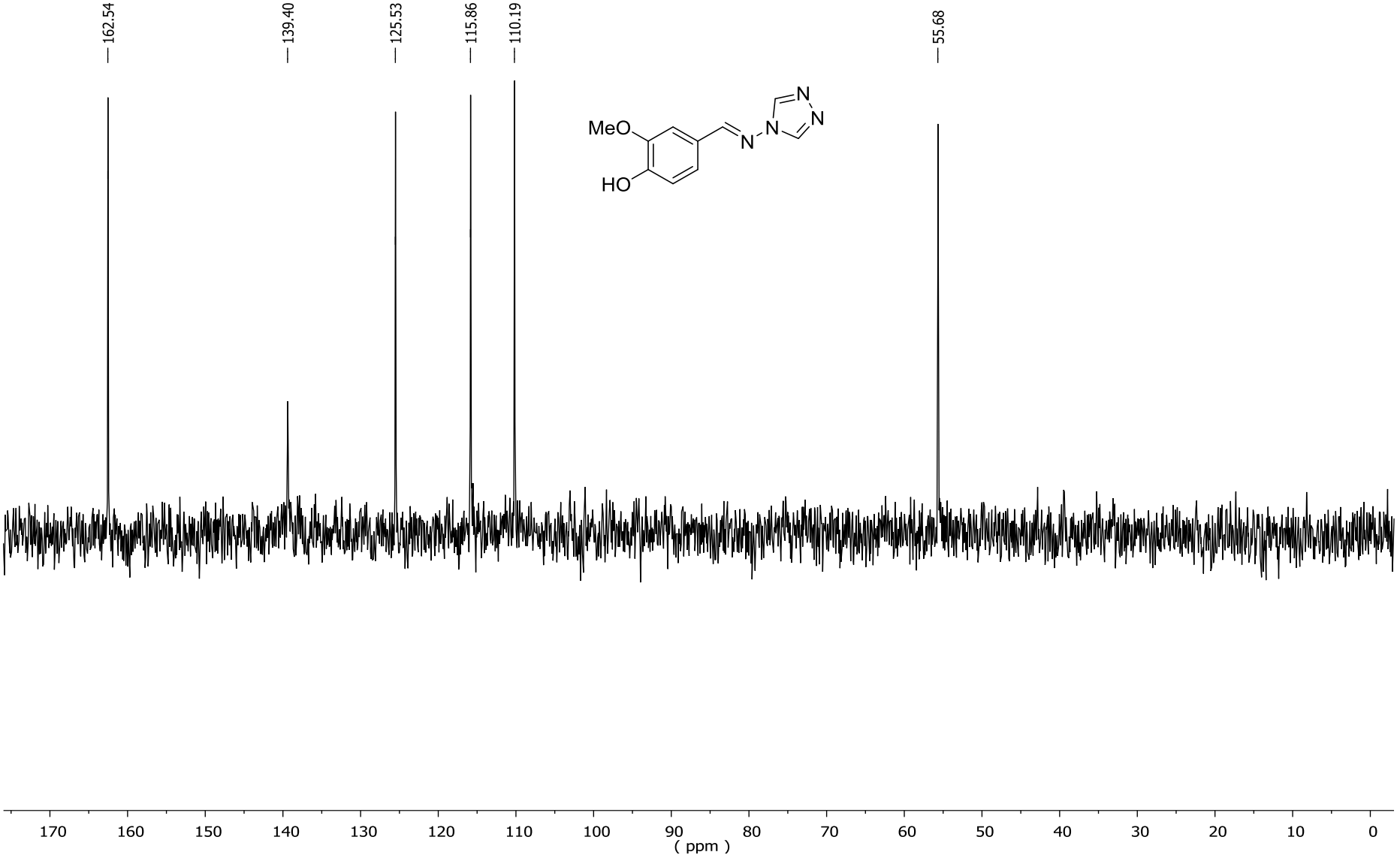

HO
HO
H
N
N
NH2
O
1.07
0.98
0.90
1.06
1.00
1.00
0.99
2.01
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

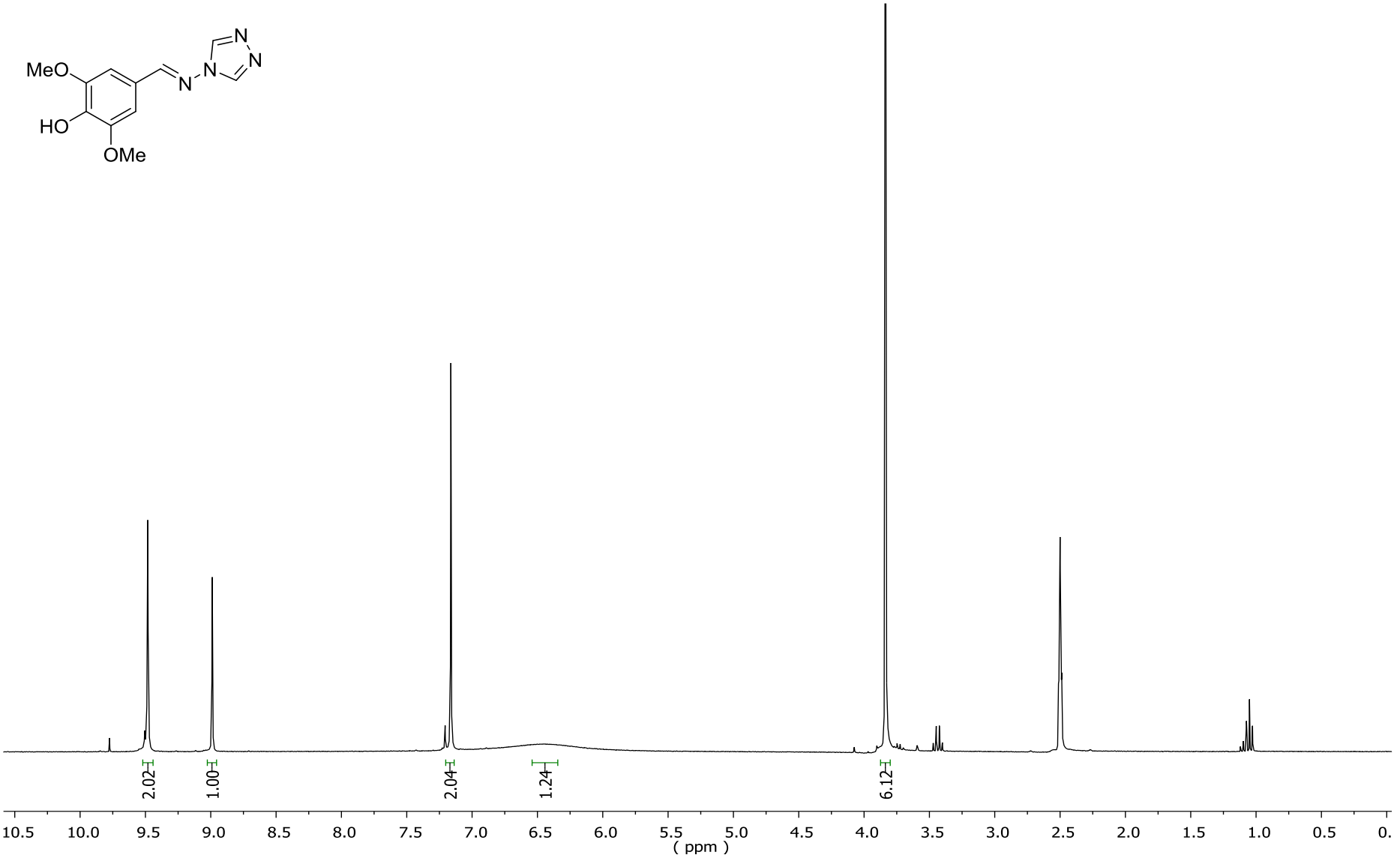

156.89
146.94
145.49
140.22
126.31
119.14
115.52
113.17
HO
HO
H
N
N
NH2
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

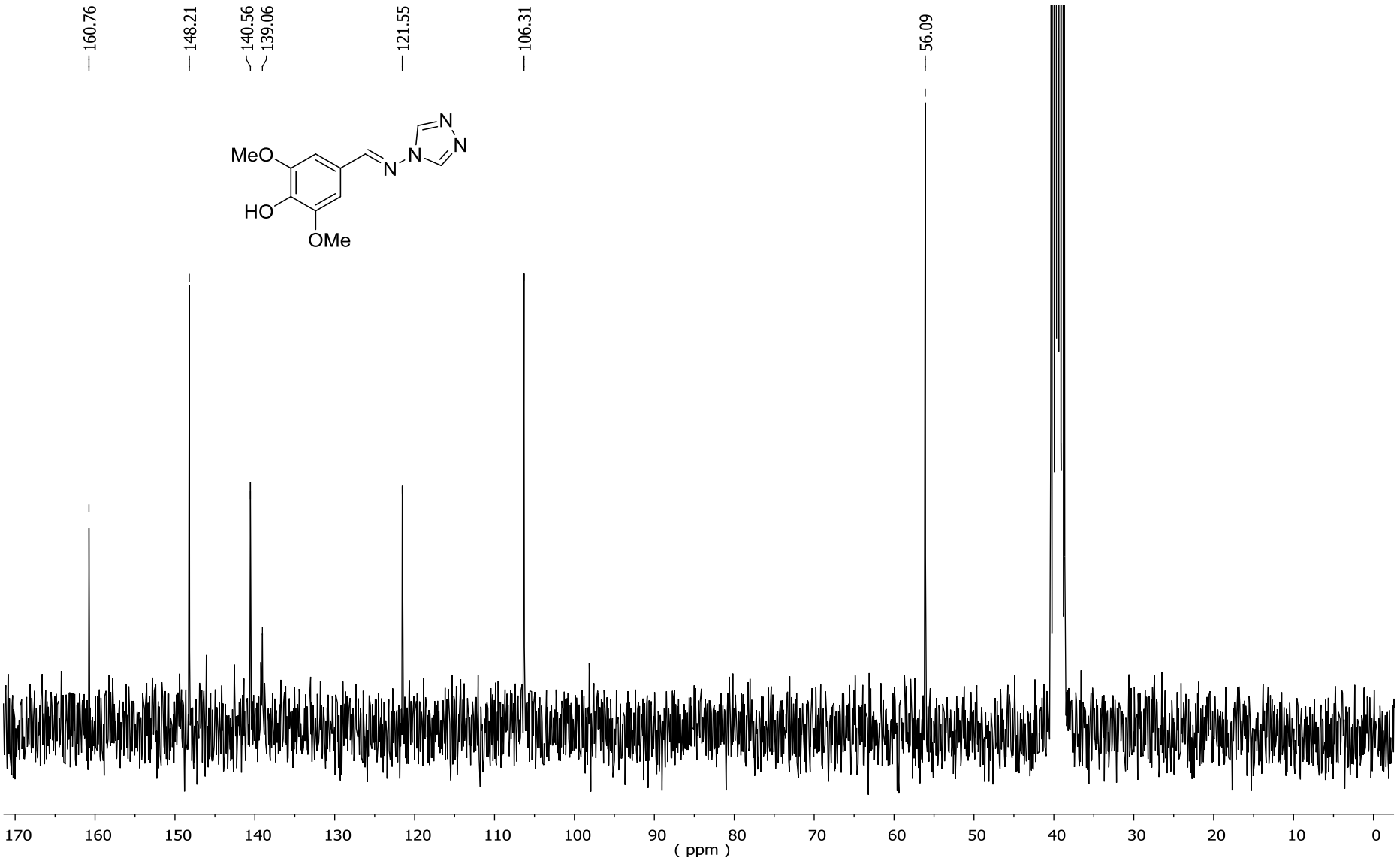

HO
HO
H
N
N
NH2
O
140.22
119.15
115.52
113.18
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
(ppm)

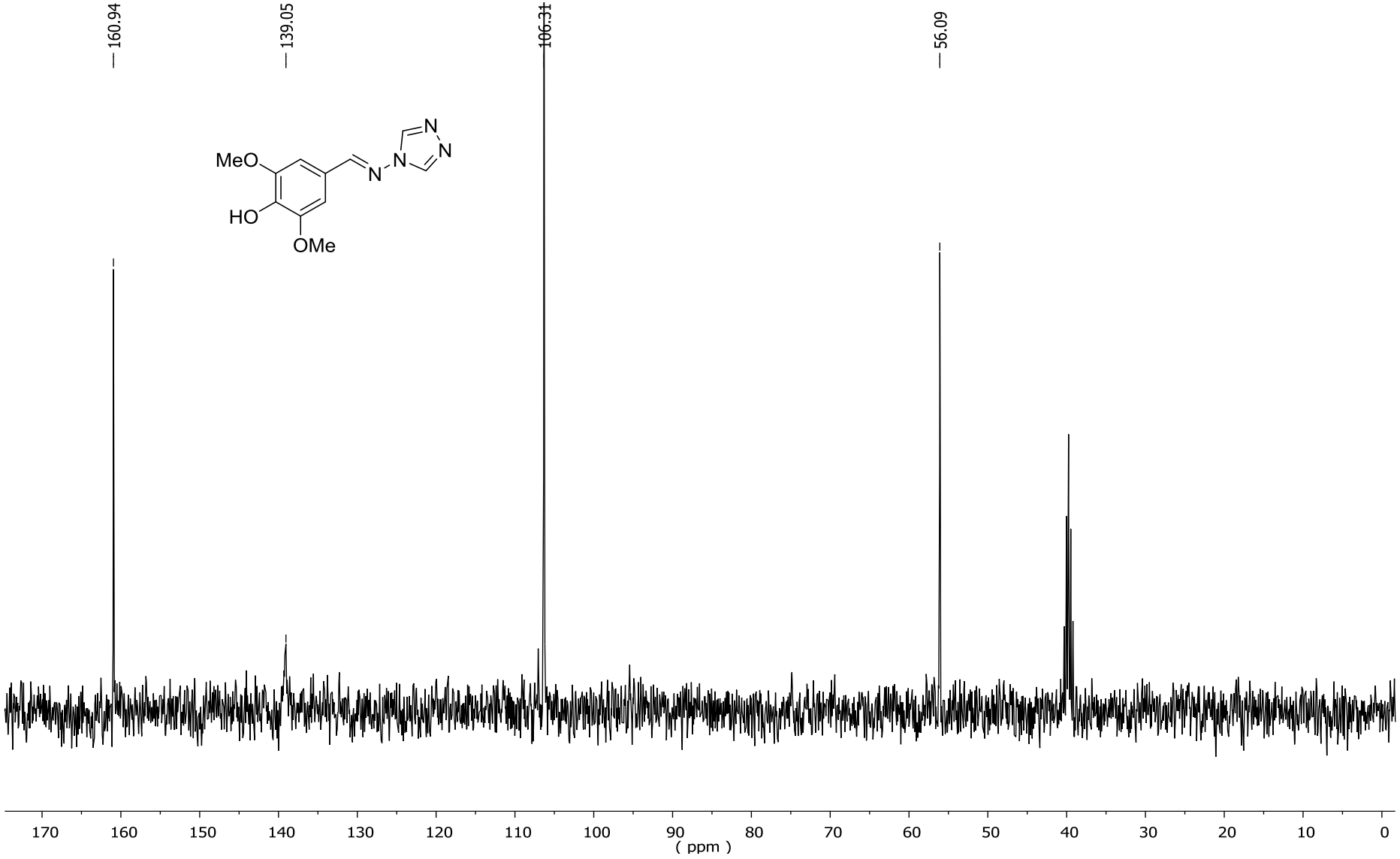

MeO
HO
N
N
N
N
1.93
1.00
1.07
1.04
1.02
1.04
2.93
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

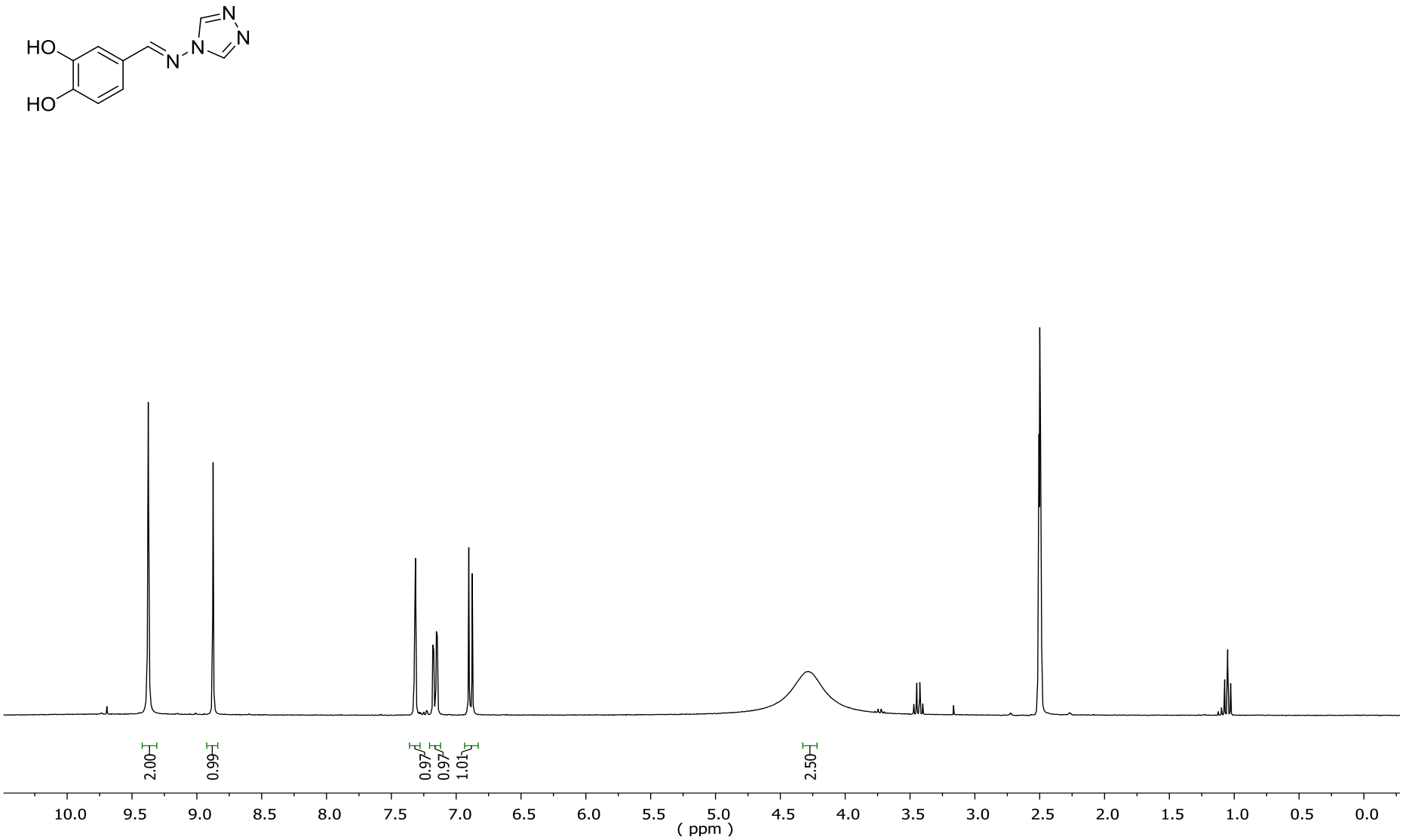

162.54
152.13
148.33
139.40
125.52
122.44
115.86
110.21
55.68
MeO
HO
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

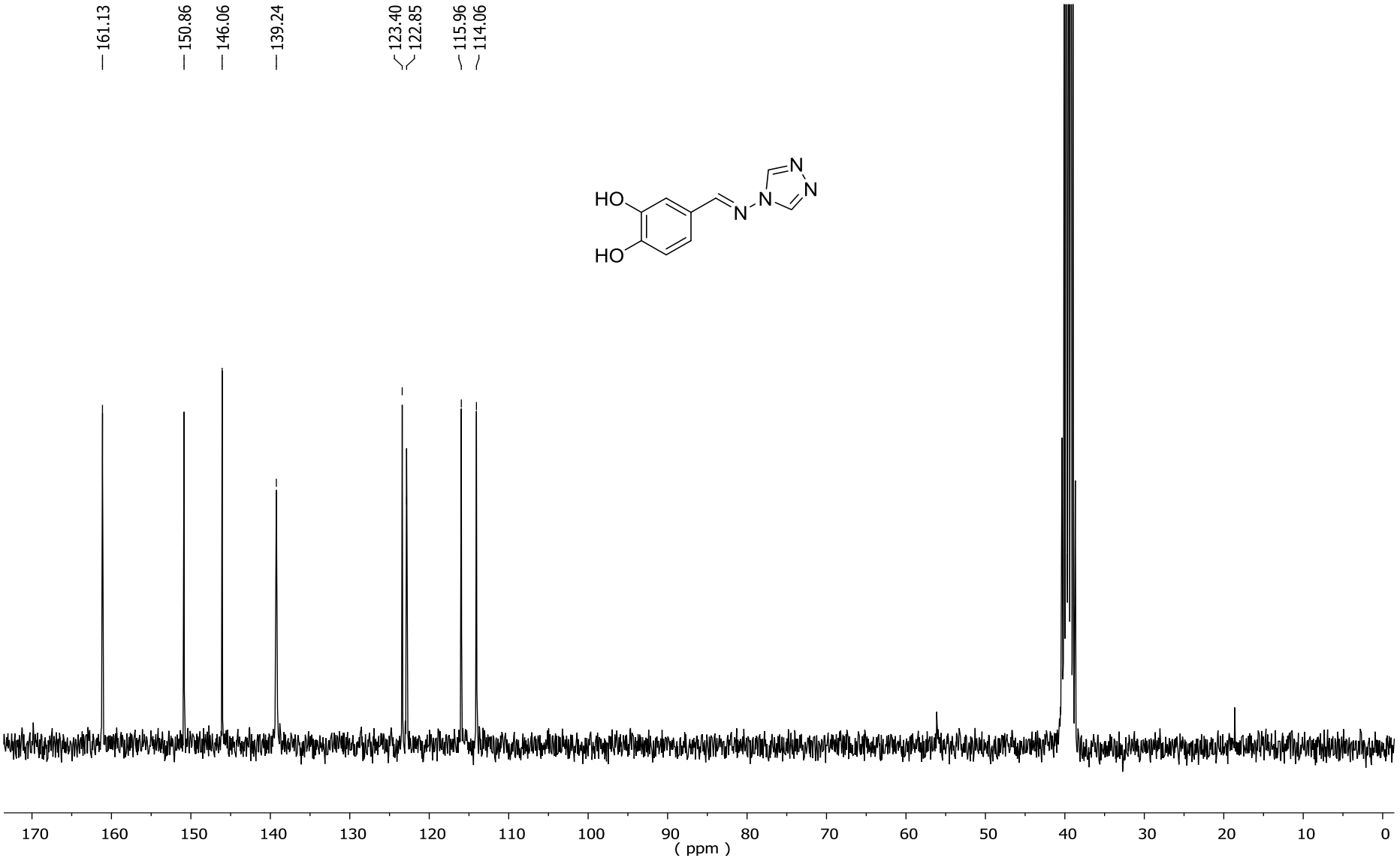

162.54
139.40
125.53
115.86
110.19
55.68
MeO
HO
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

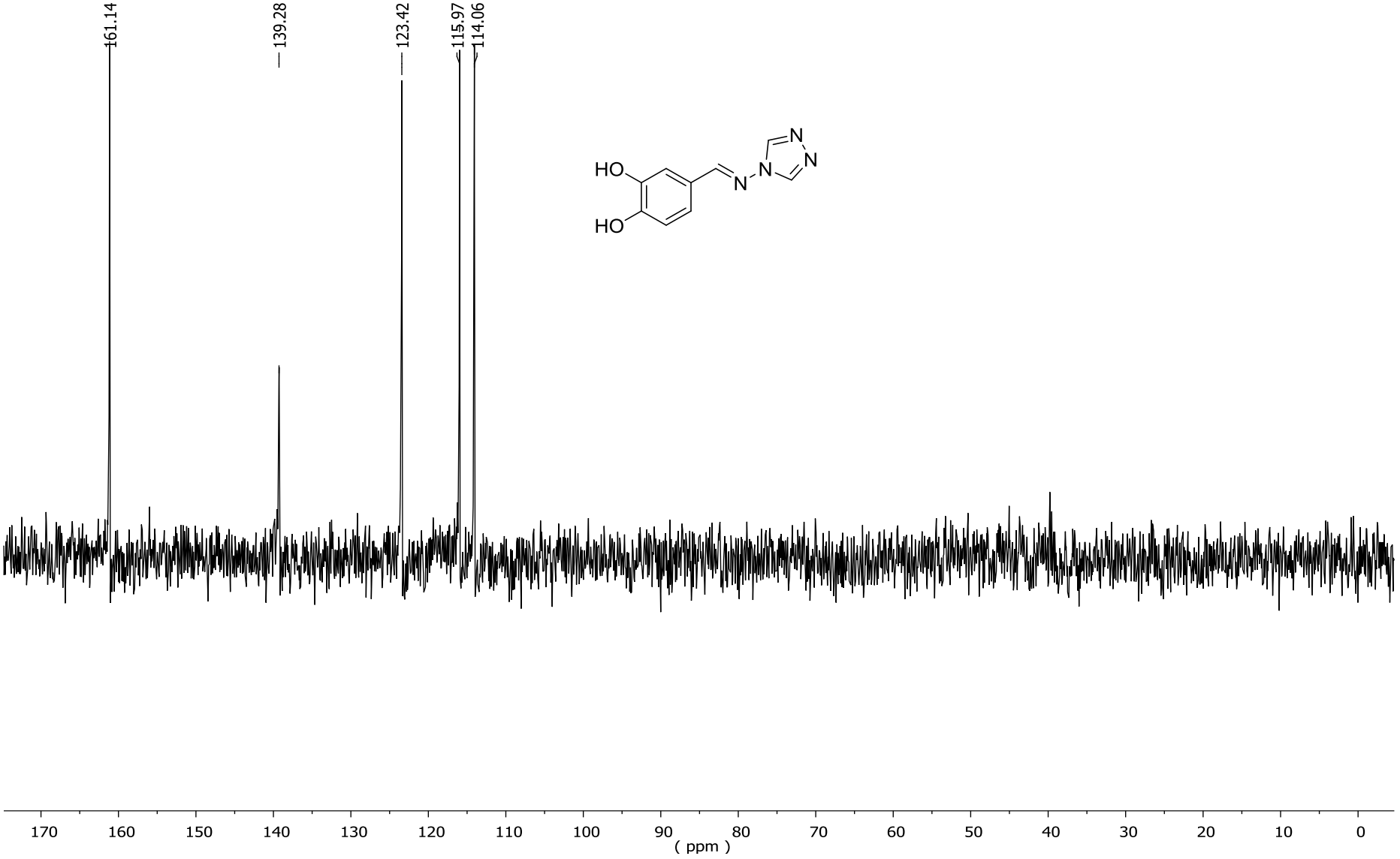

MeO
HO
OMe
N
N
N
N
2.02
1.00
2.04
1.24
6.12
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)

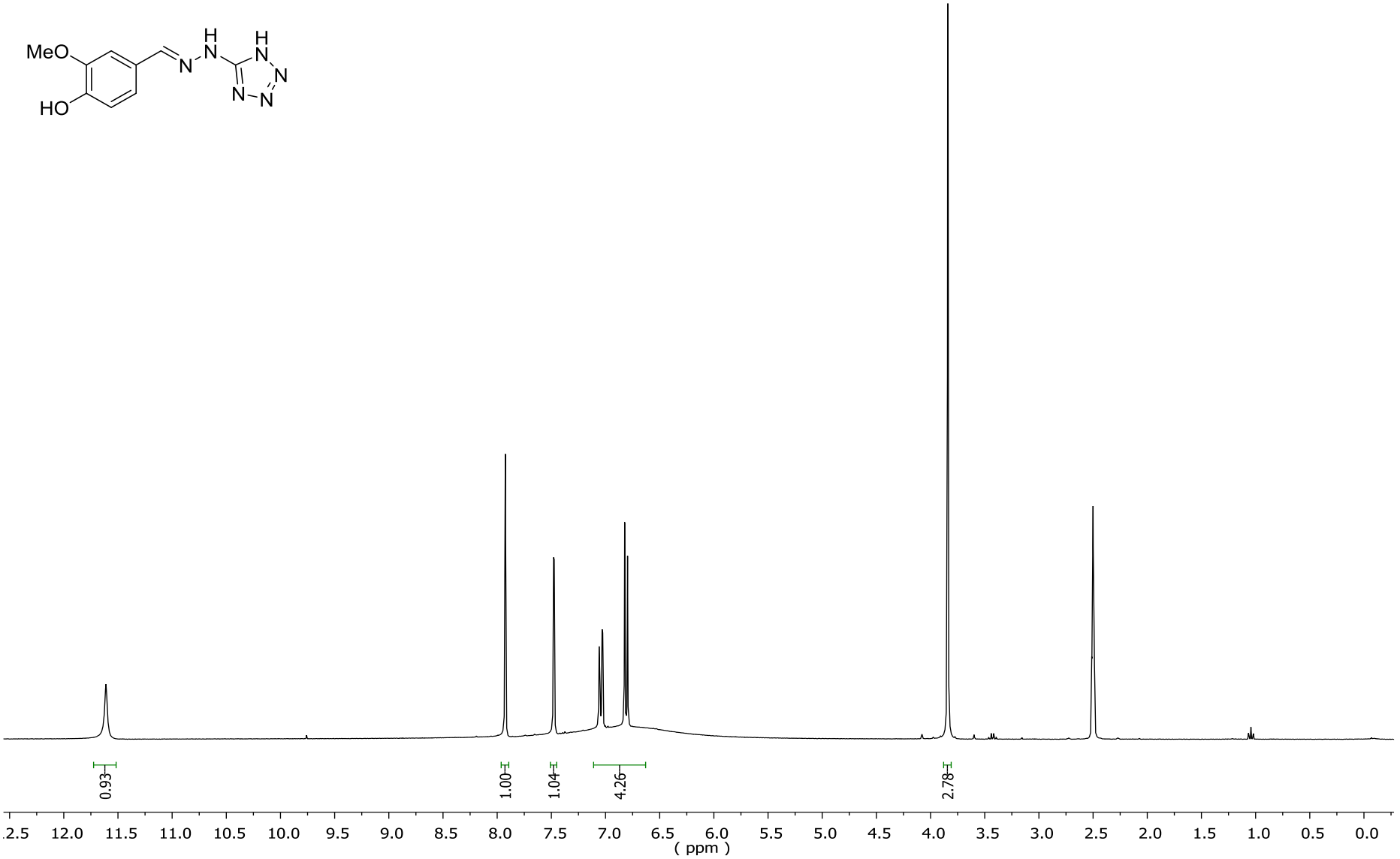

160.76
148.21
140.56
139.06
121.55
106.31
56.09
MeO
HO
OMe
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

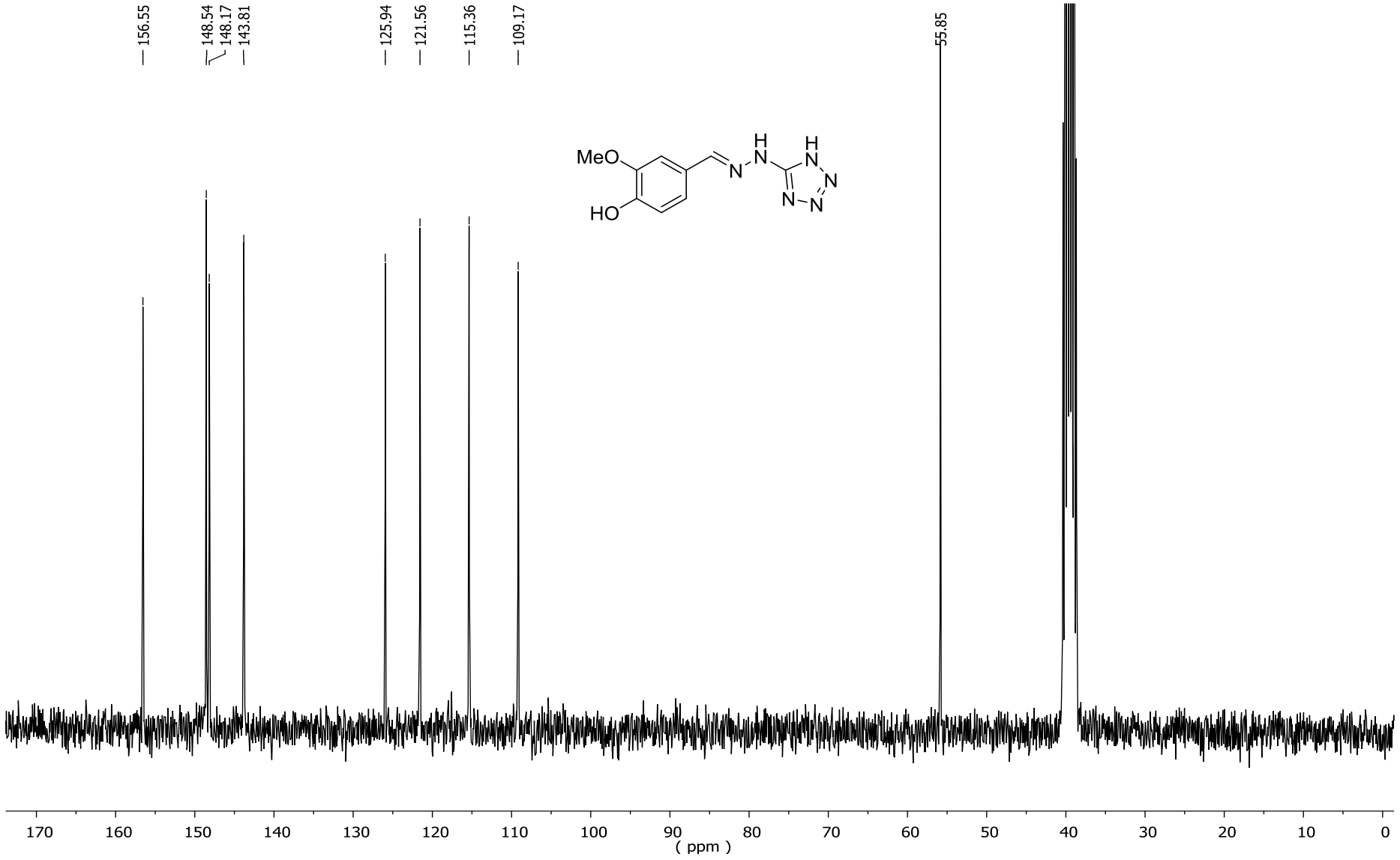

160.94
139.05
106.31
56.09
MeO
HO
OMe
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

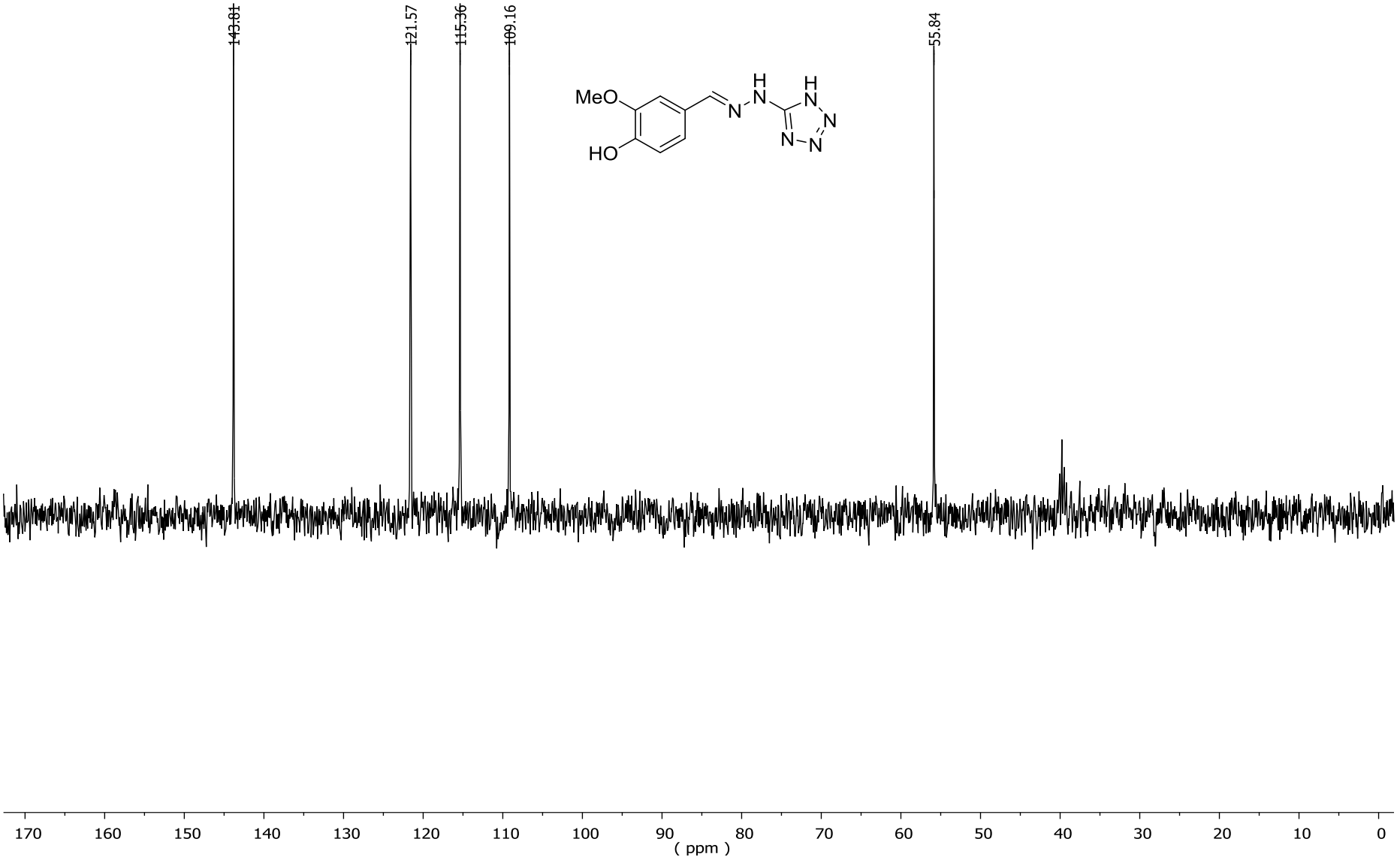

HO
HO
N
N
N
N
2.00
0.99
0.97
0.97
1.01
2.50
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

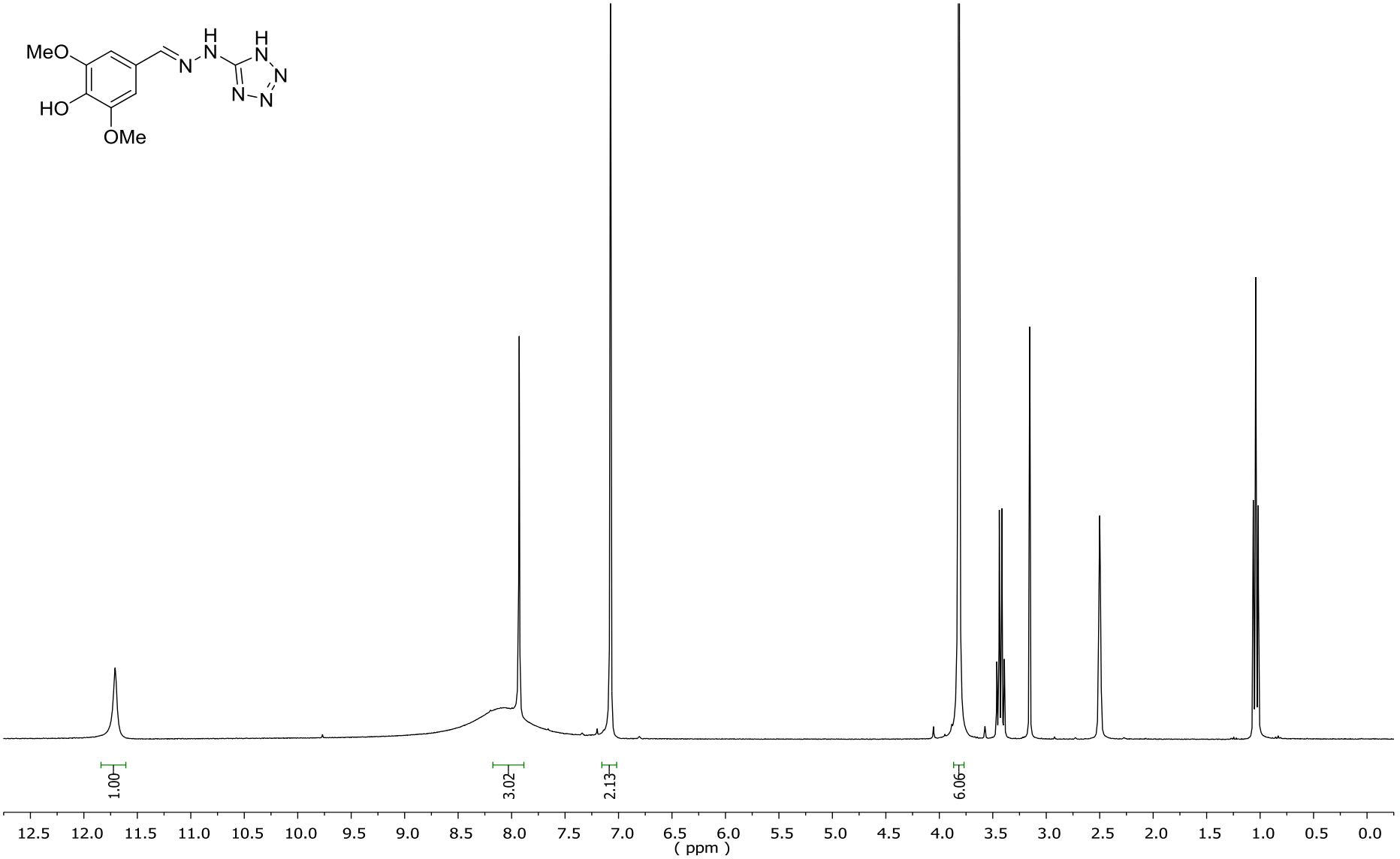

161.13
150.86
146.06
139.24
123.40
122.85
115.96
114.06
HO
HO
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

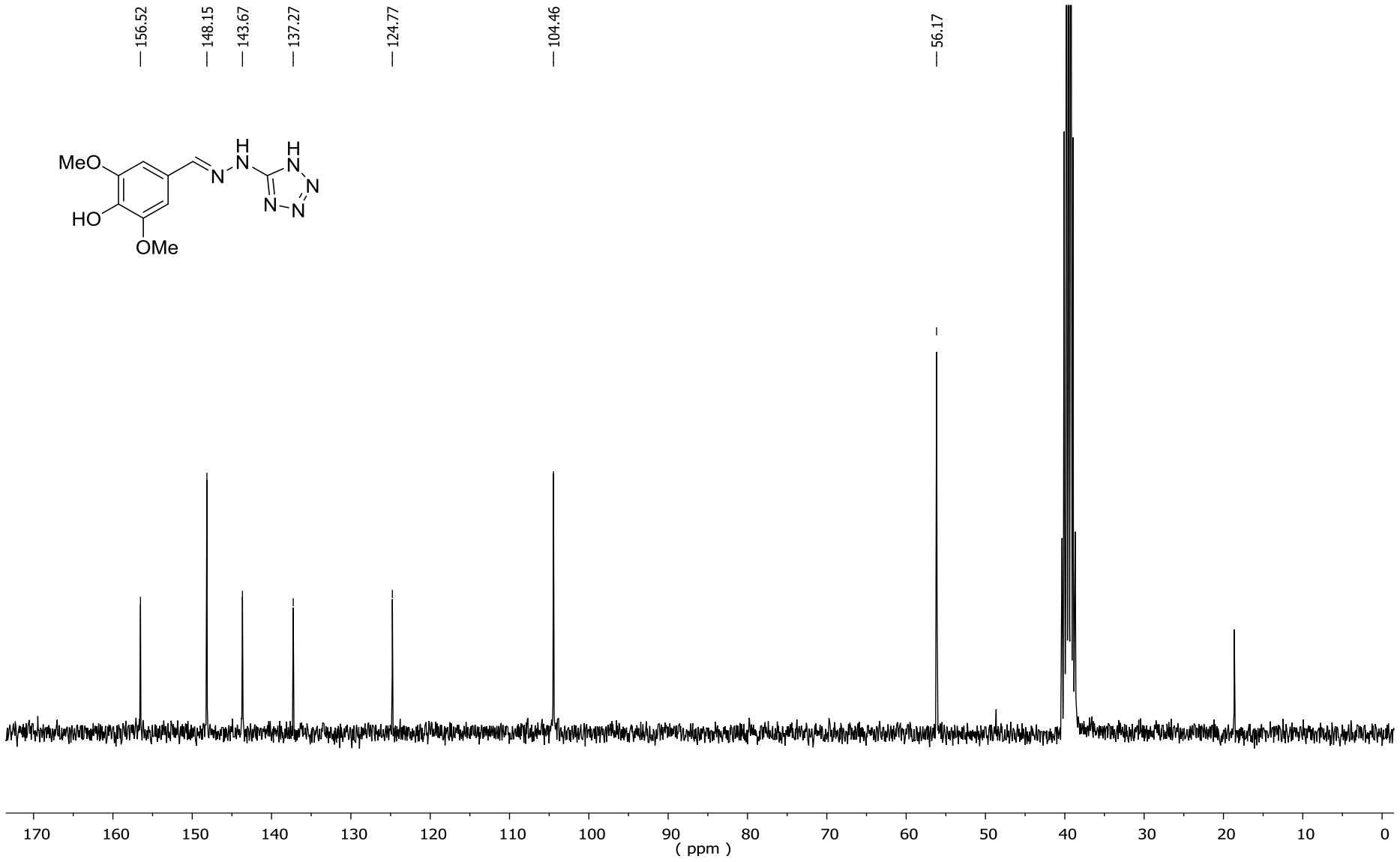

161.14
139.28
123.42
115.97
114.06
HO
HO
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

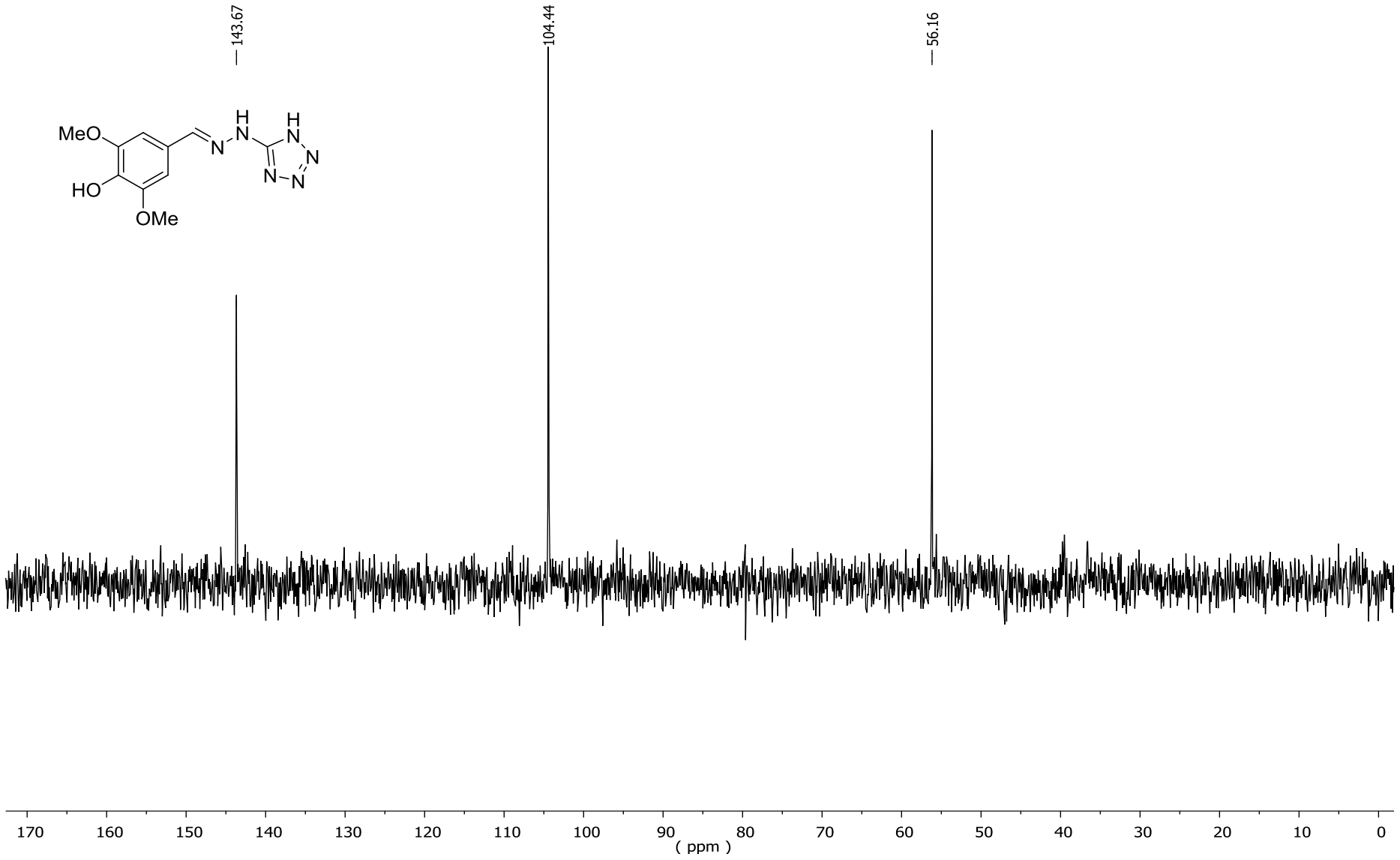

MeO
HO
H
N
N
H
N
N
N
N
0.93
1.00
1.04
4.26
2.78
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

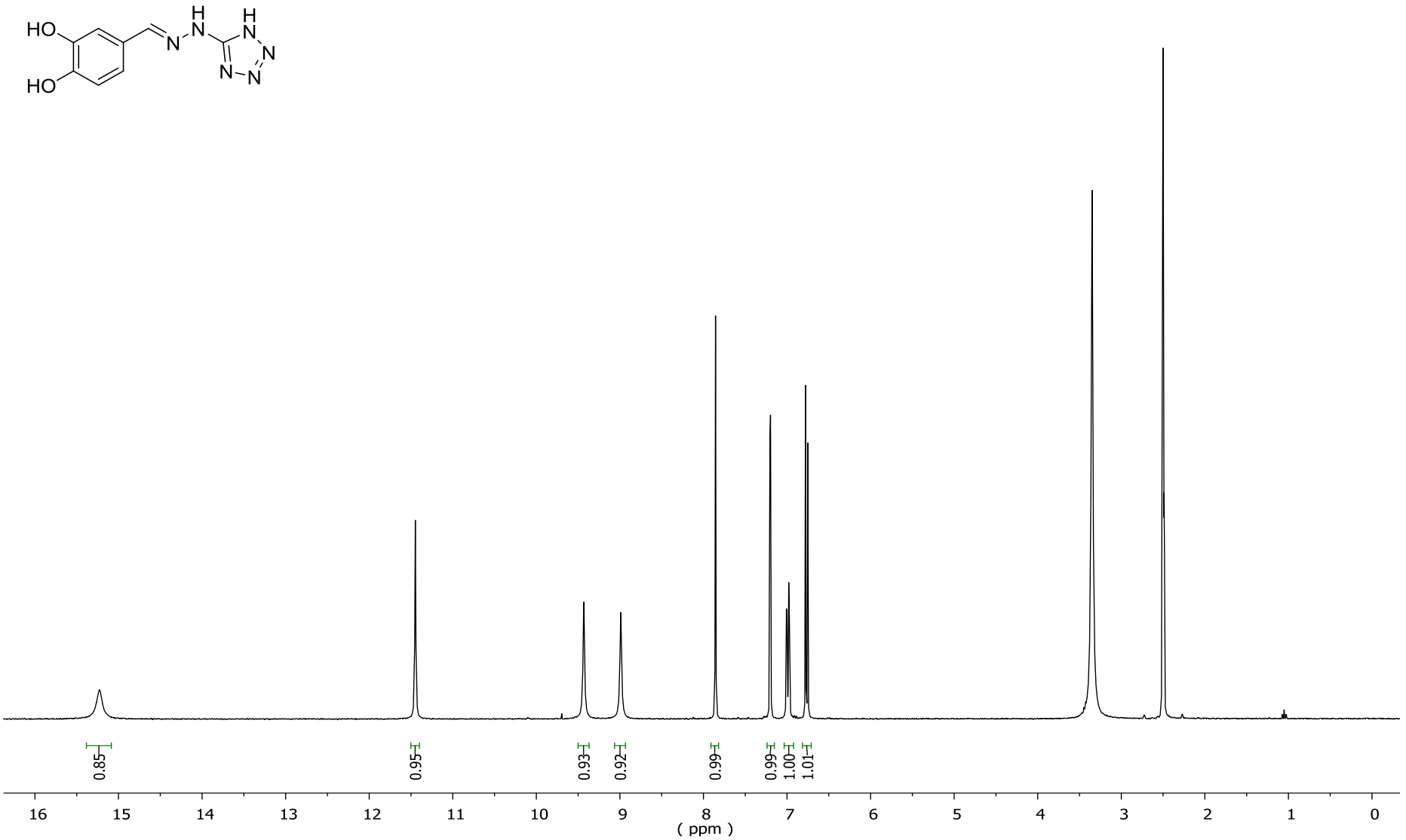

156.55
148.54
148.17
143.81
125.94
121.56
115.36
109.17
55.85
MeO
HO
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

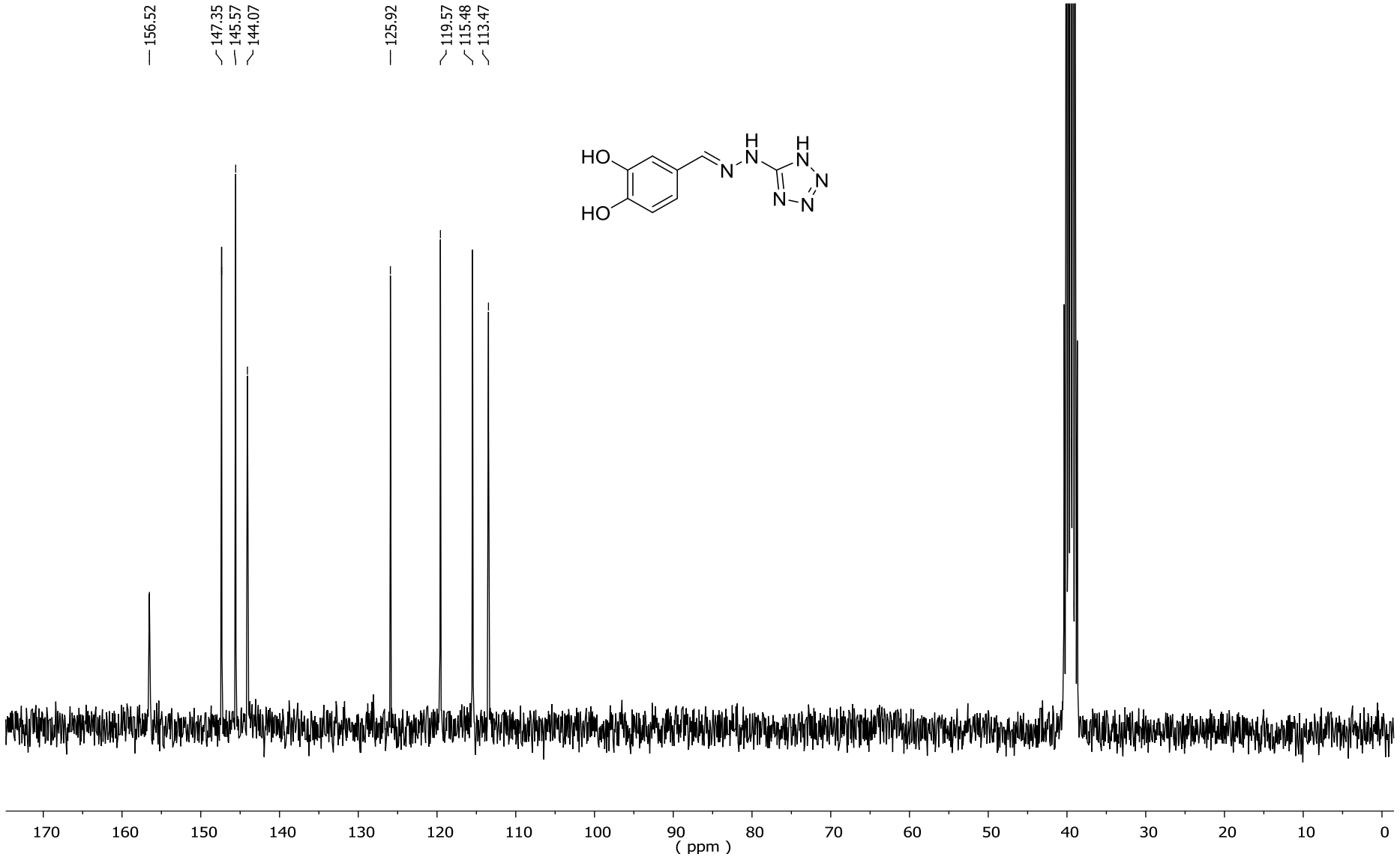

143.81
121.57
115.36
109.16
55.84
MeO
HO
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

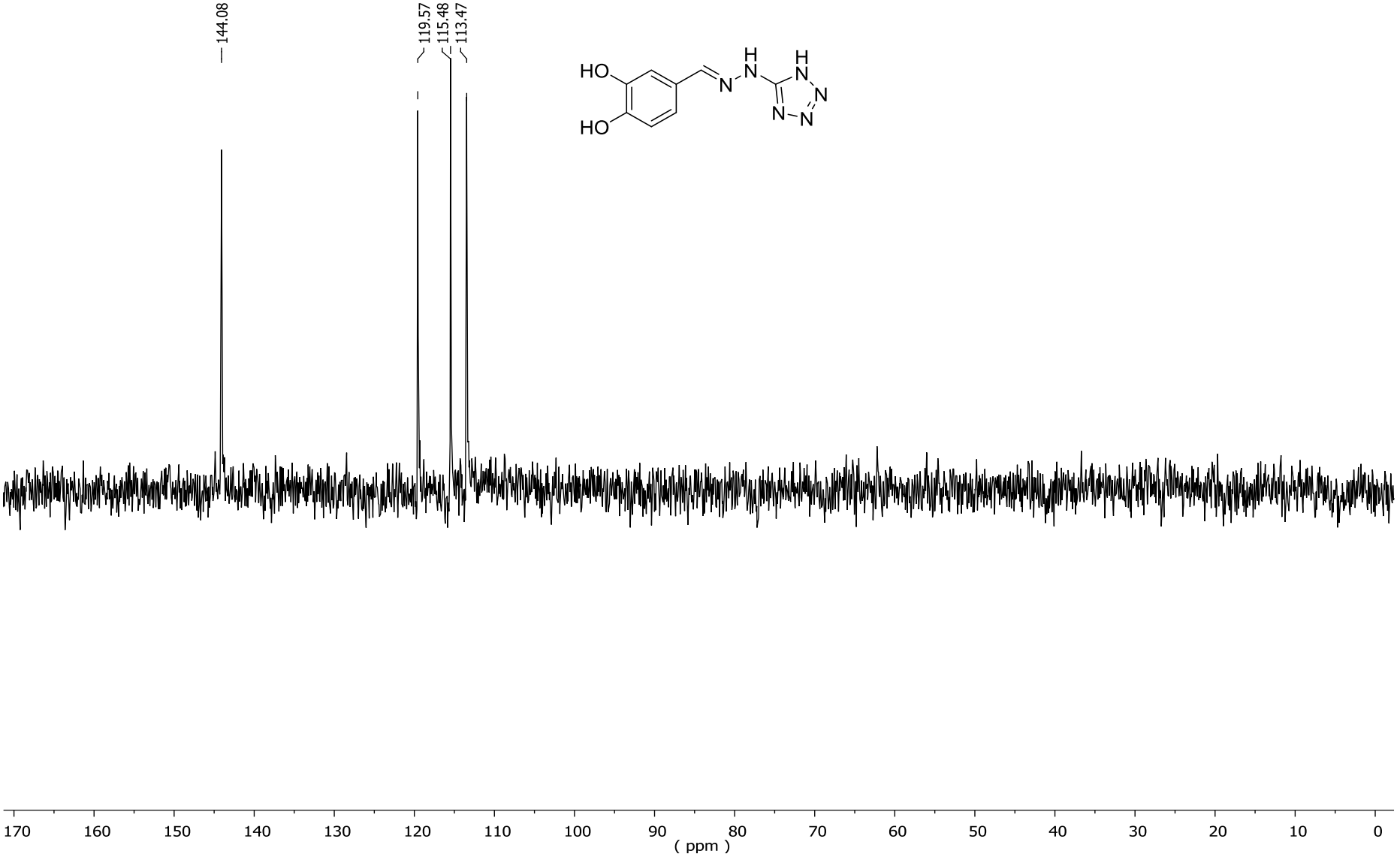

MeO
HO
OMe
H
N
N
H
N
N
N
N
1.00
3.02
2.13
6.06
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
(ppm)

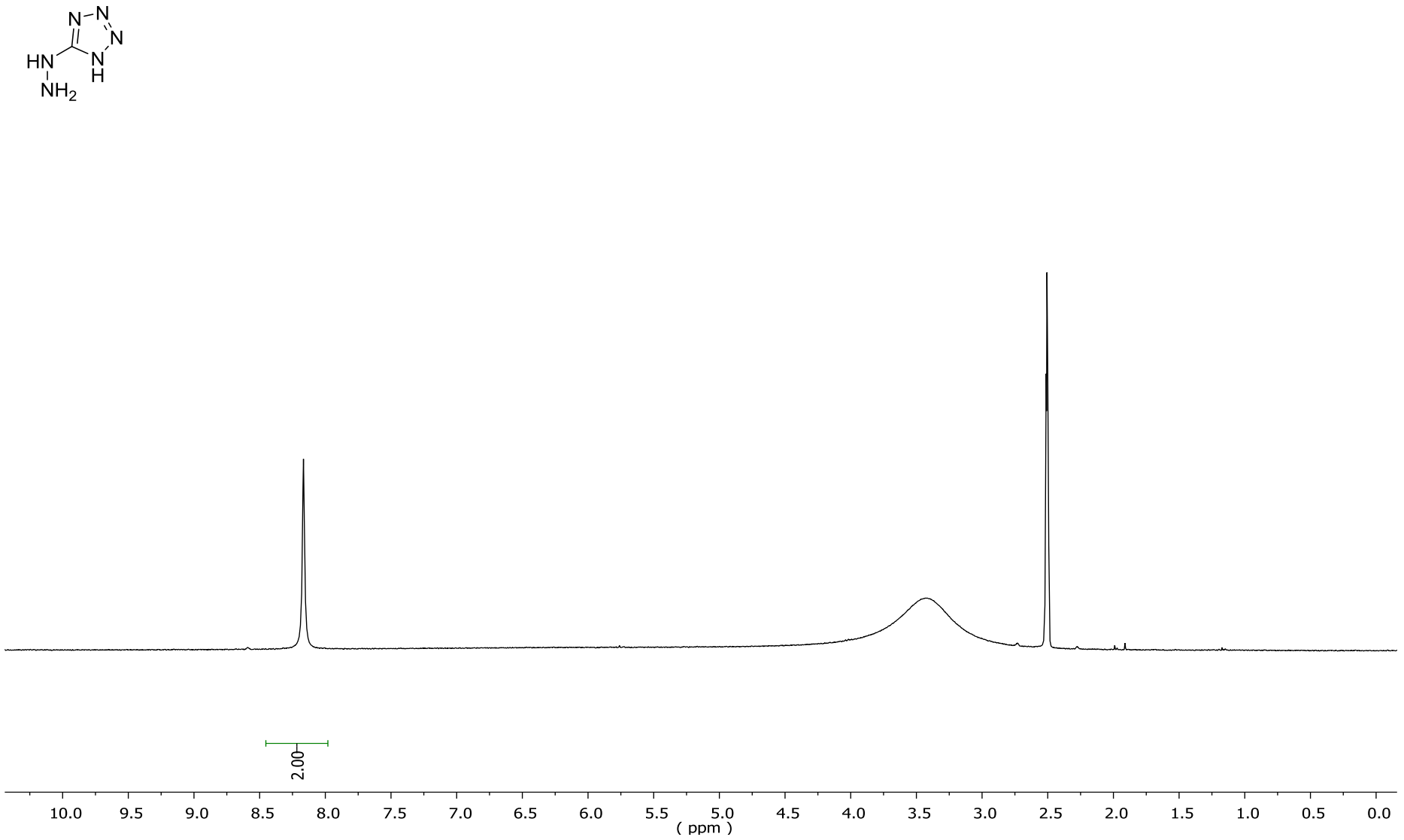

156.52
148.15
143.67
137.27
124.77
104.46
56.17
MeO
HO
OMe
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

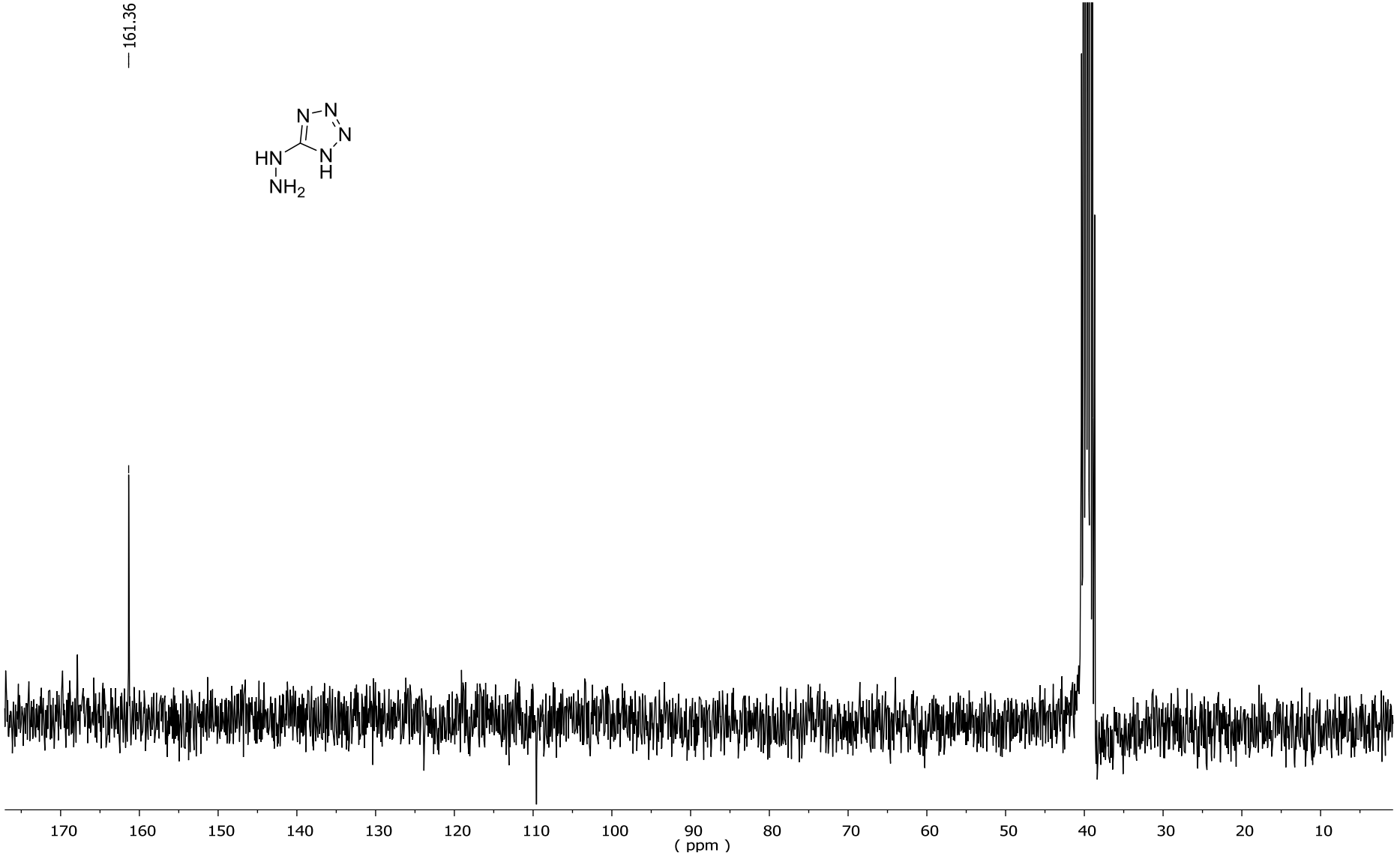

143.67
104.44
56.16
MeO
HO
OMe
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

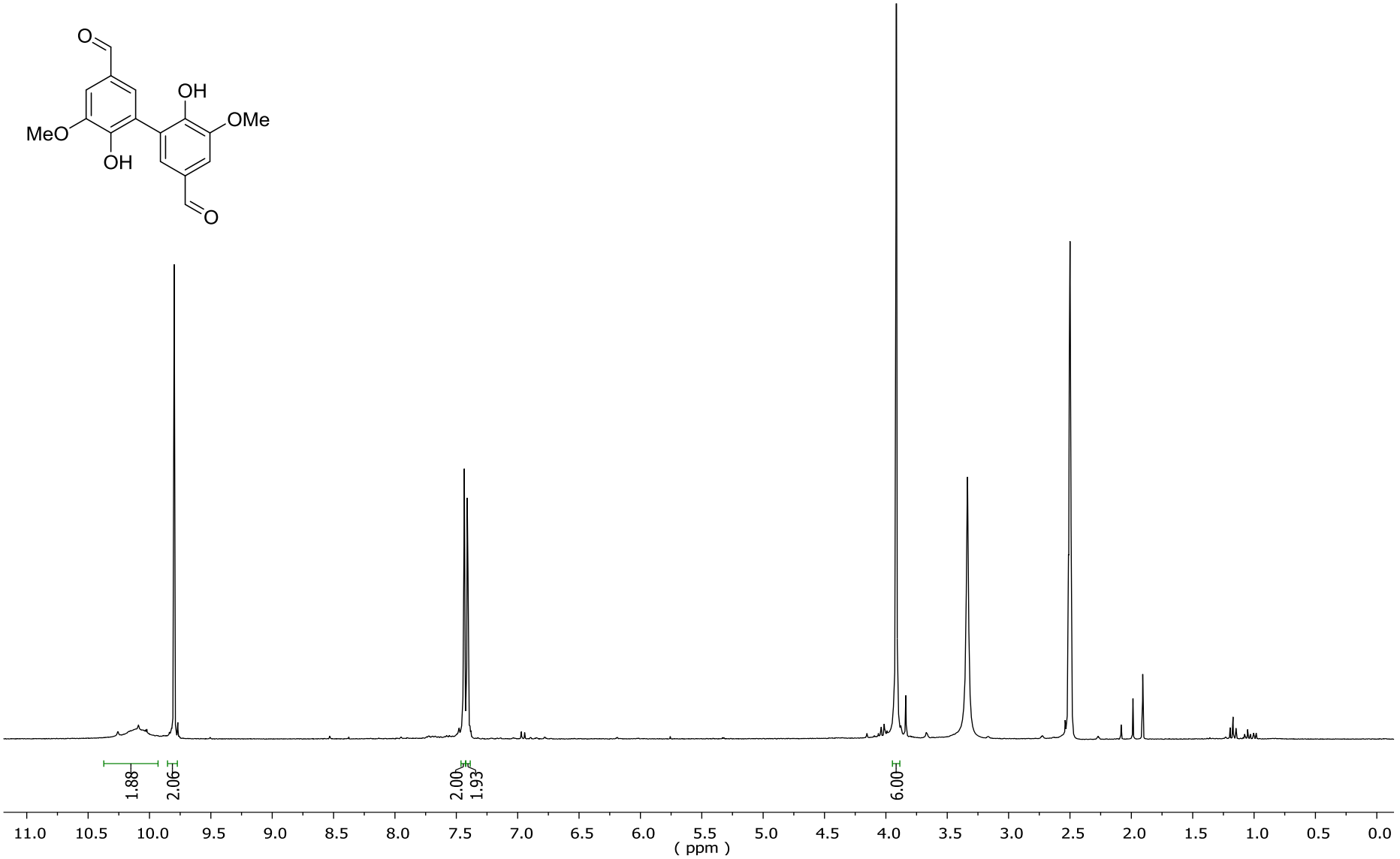

HO
HO
H
N
N
H
N
N
N
N
0.85
0.95
0.93
0.92
0.99
0.99
1.00
1.01
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

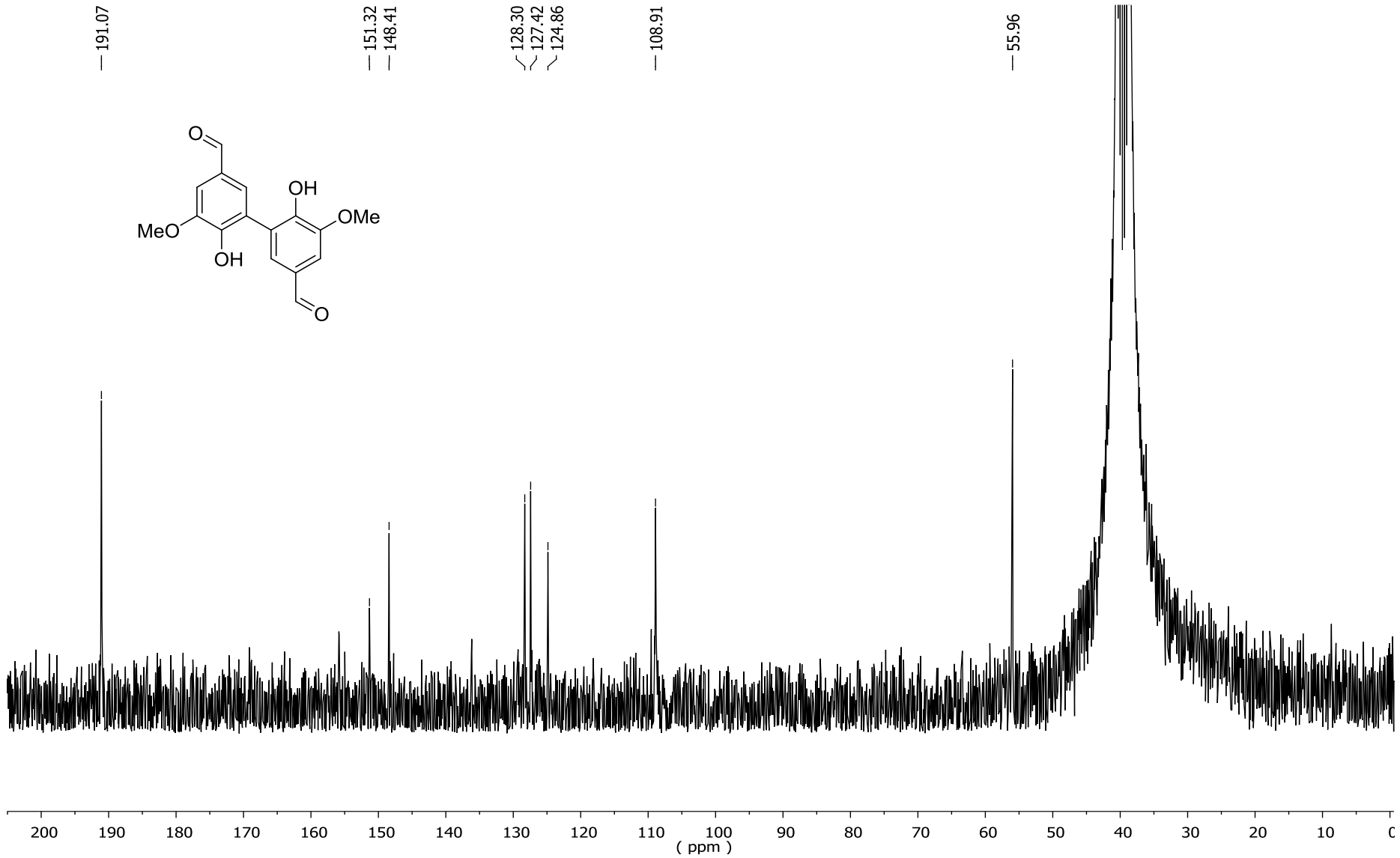

156.52
147.35
145.57
144.07
125.92
119.57
115.48
113.47
HO
HO
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

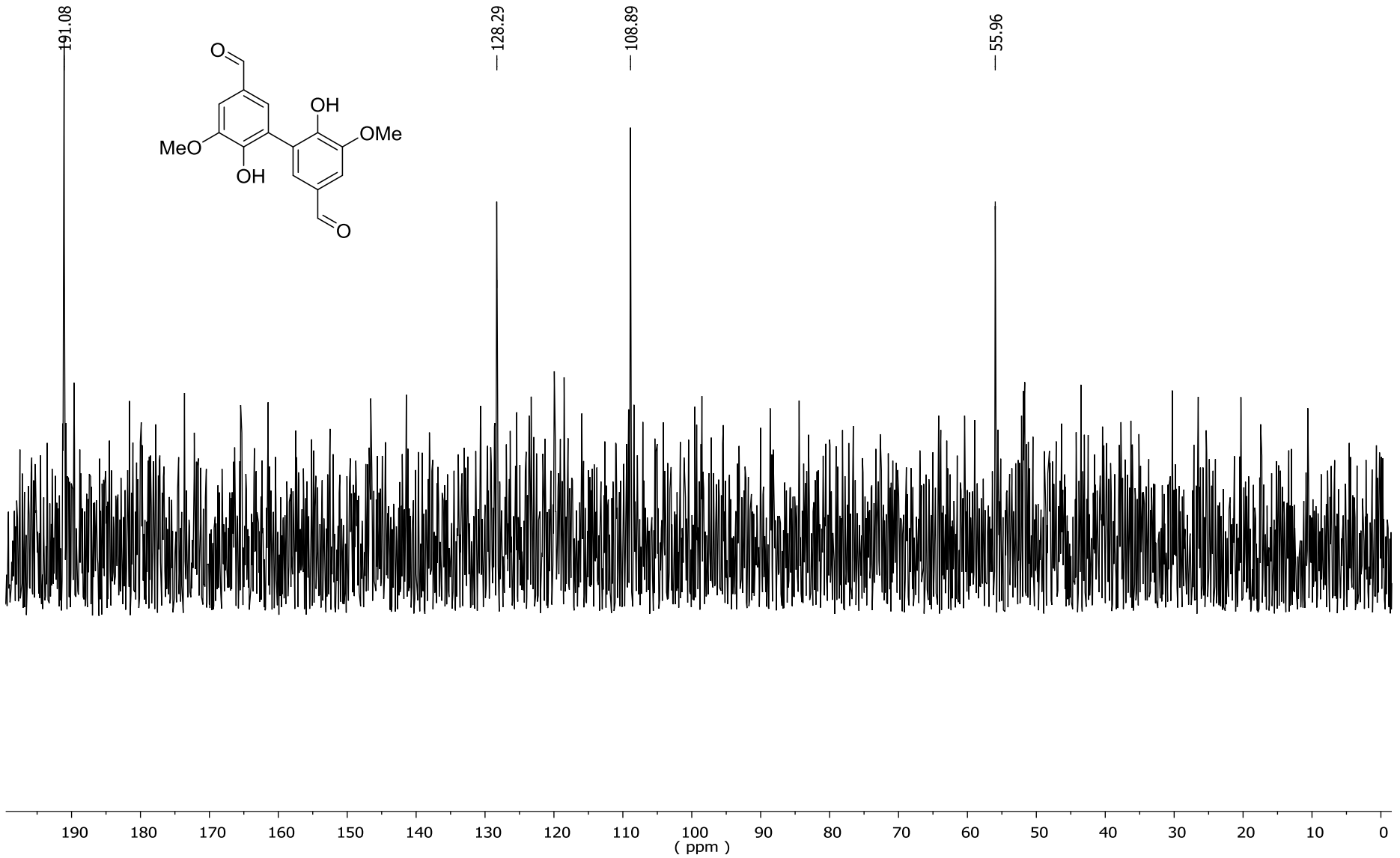

144.08
119.57
115.48
113.47
HO
HO
H
N
N
H
N
N
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

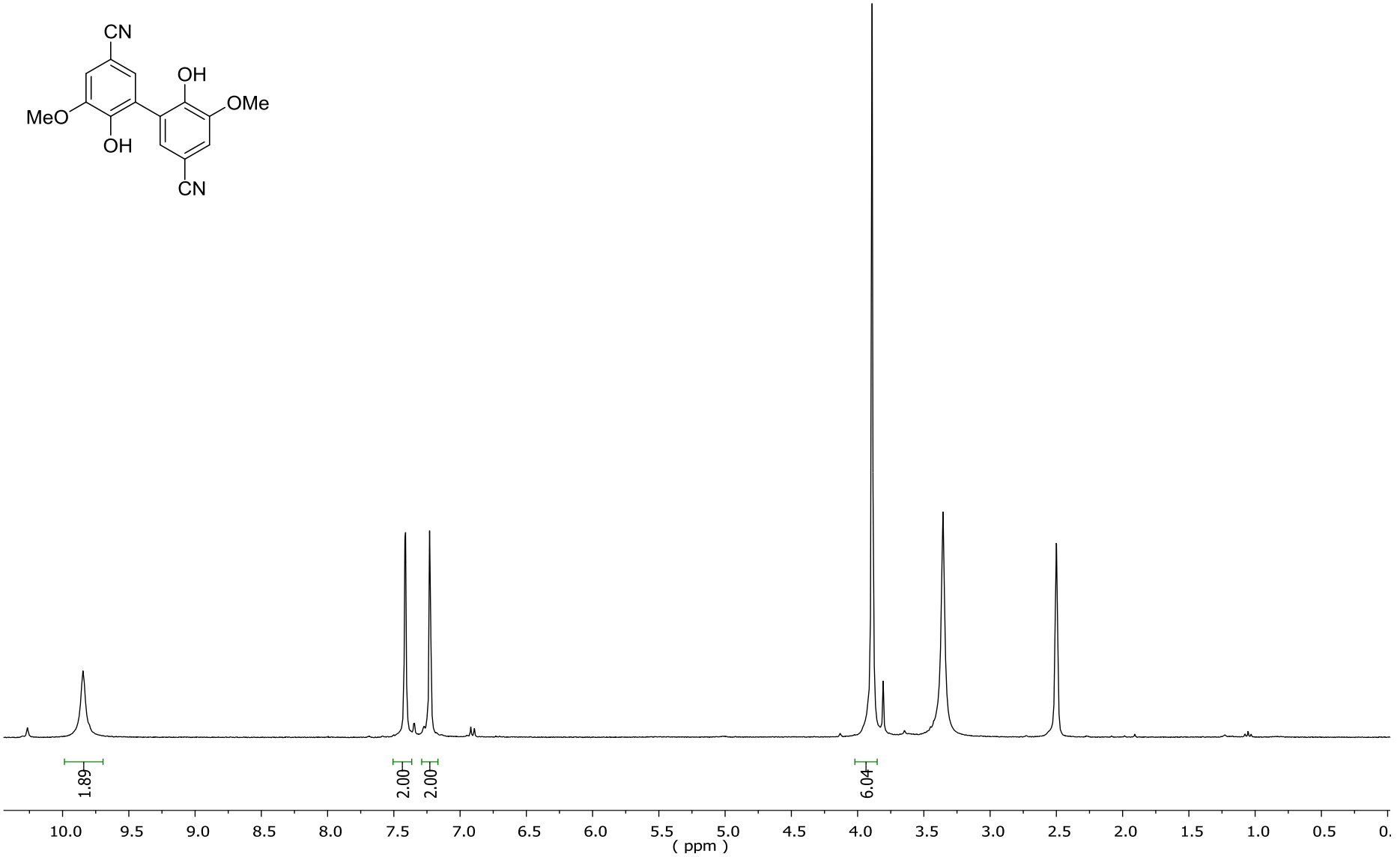

HN
NH2
N
N
N
N
H
2.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

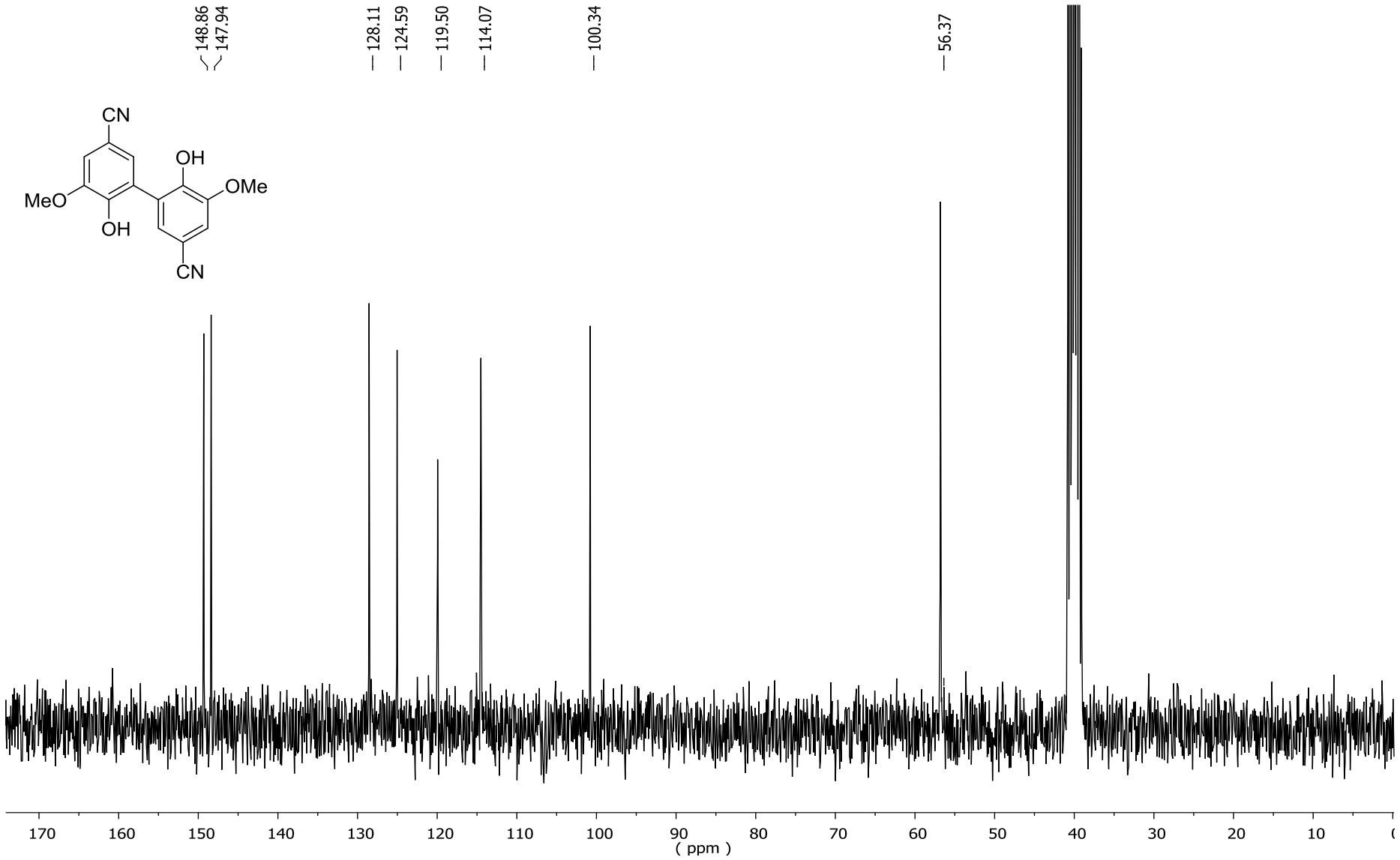

161.36
HN
NH2
N
N
N
N
H
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

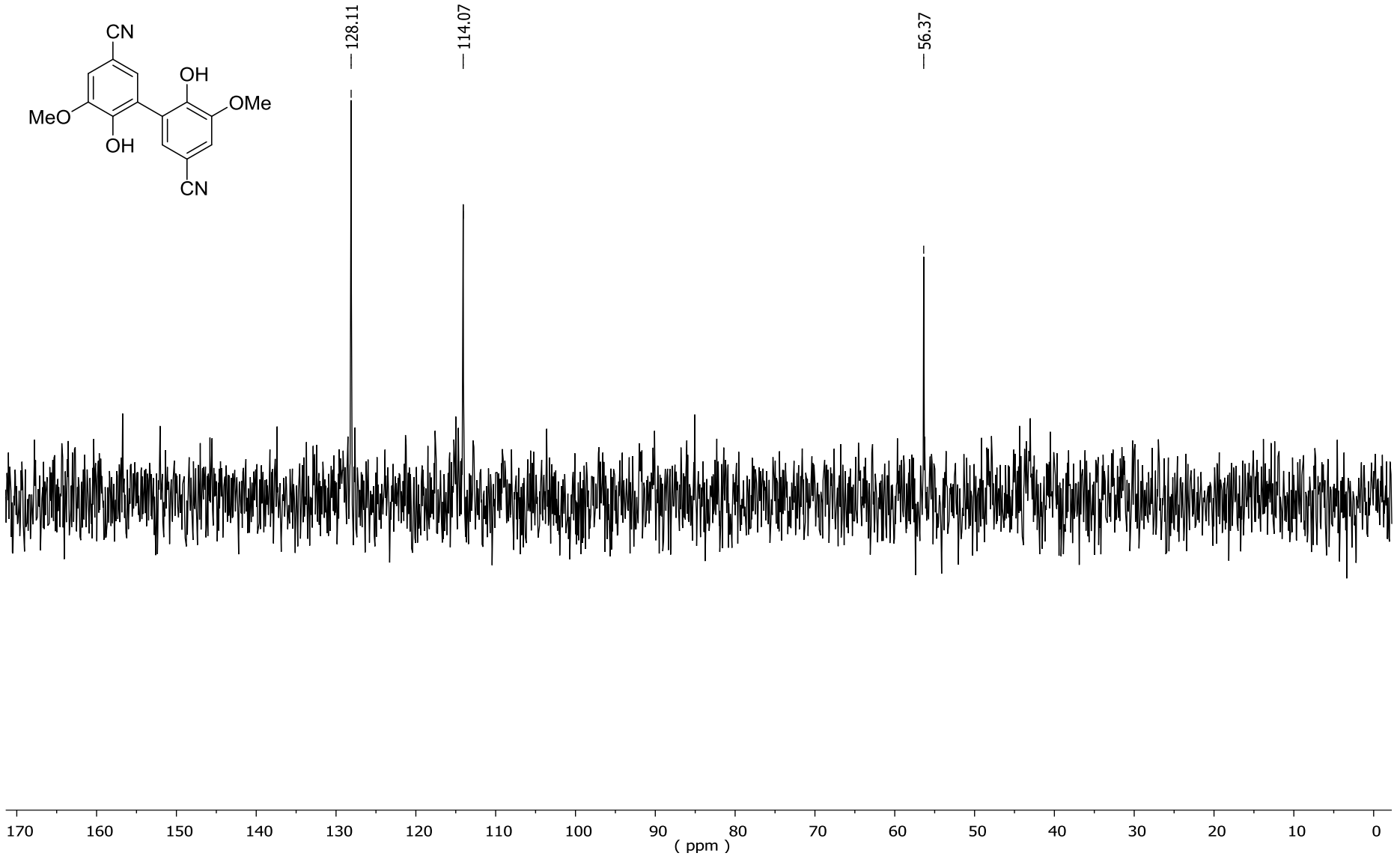

O
OH
OMe
MeO
OH
O
1.88
2.06
2.00
1.93
6.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

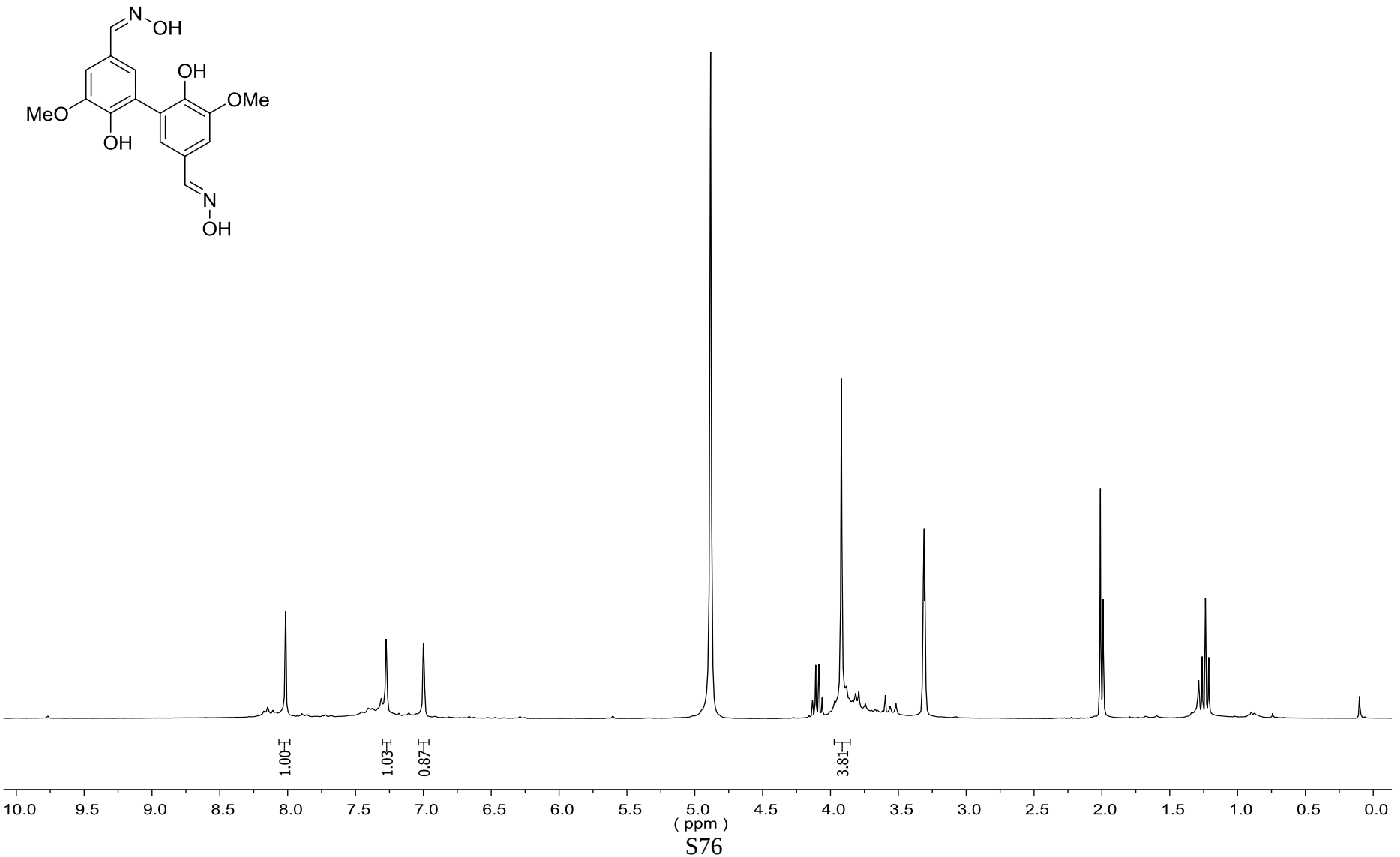

191.07
151.32
148.41
128.30
127.42
124.86
108.91
55.96
O
OH
OMe
MeO
OH
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

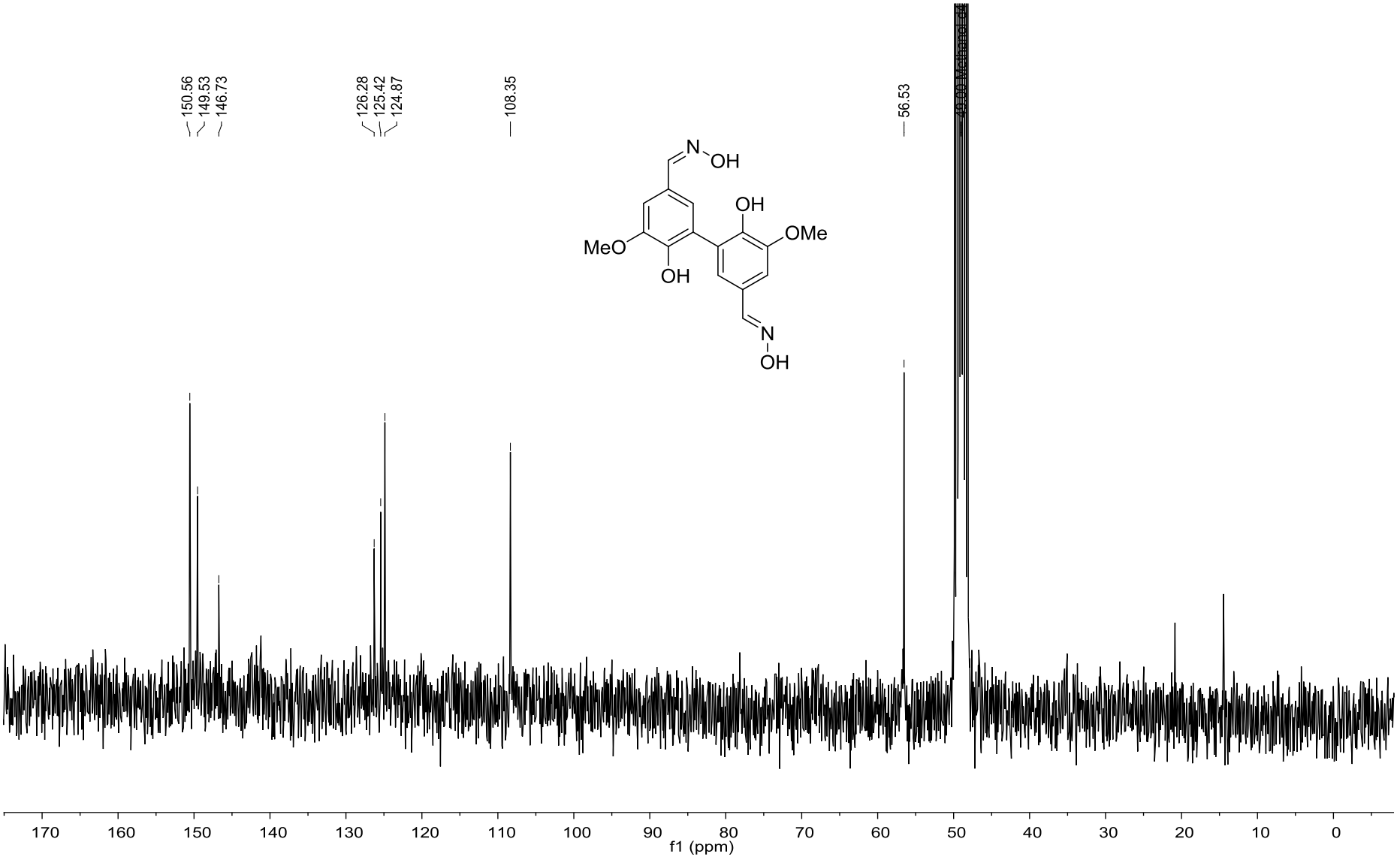

191.08
128.29
108.89
55.96
O
OH
OMe
MeO
OH
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

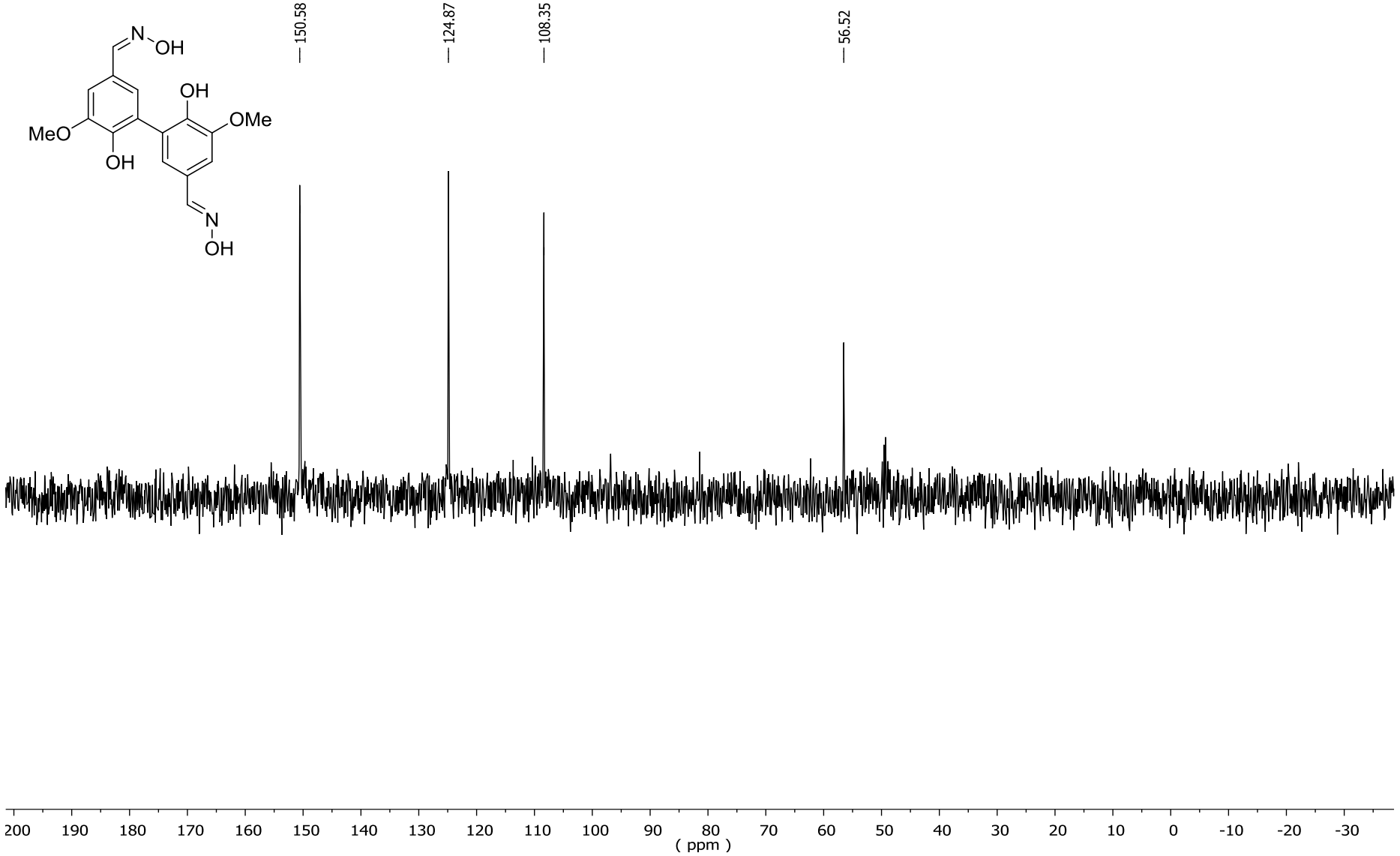

CN
OH
OMe
MeO
OH
CN
1.89
2.00
2.00
6.04
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

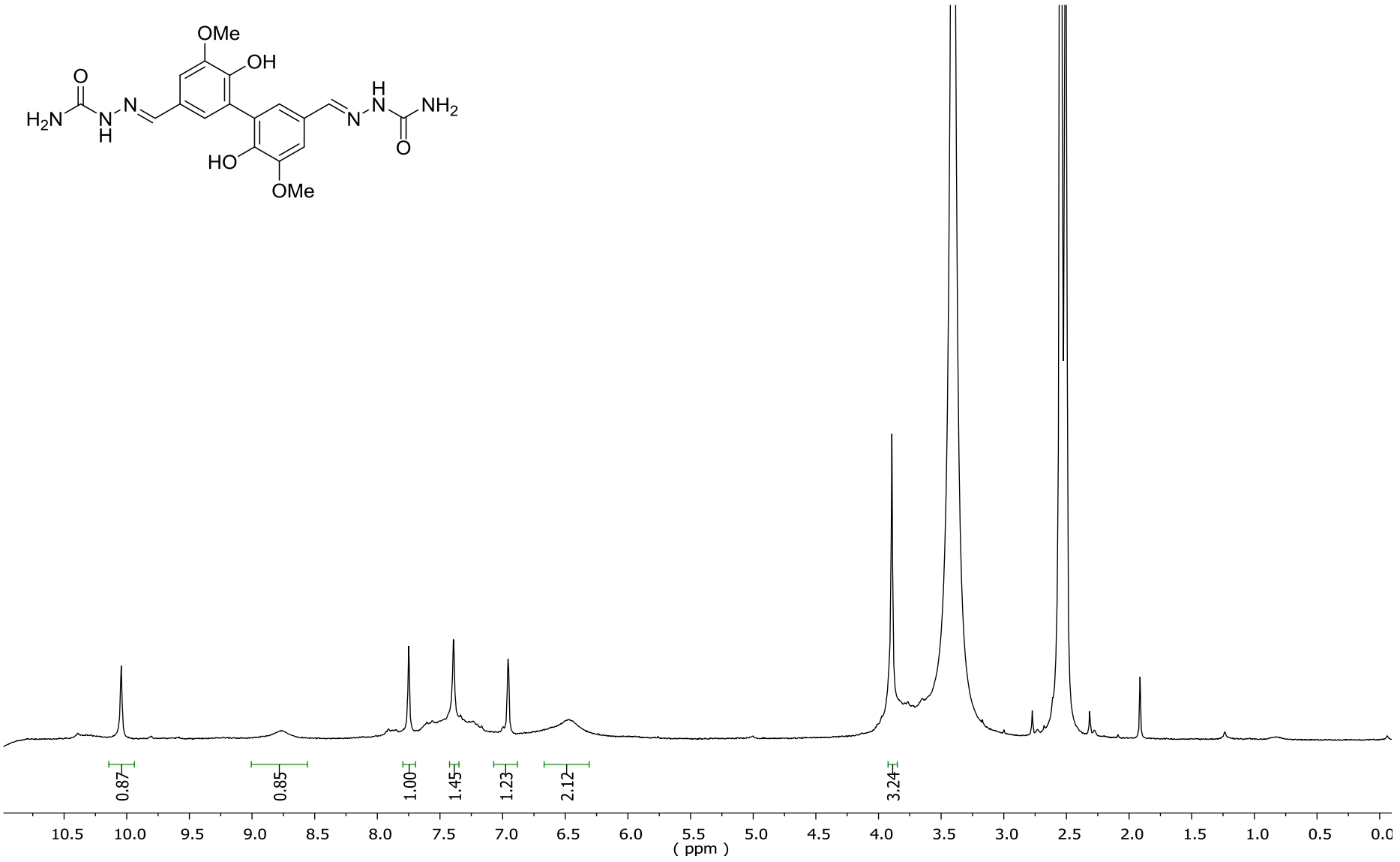

148.86
147.94
128.11
124.59
119.50
114.07
100.34
56.37
CN
OH
OMe
MeO
OH
CN
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

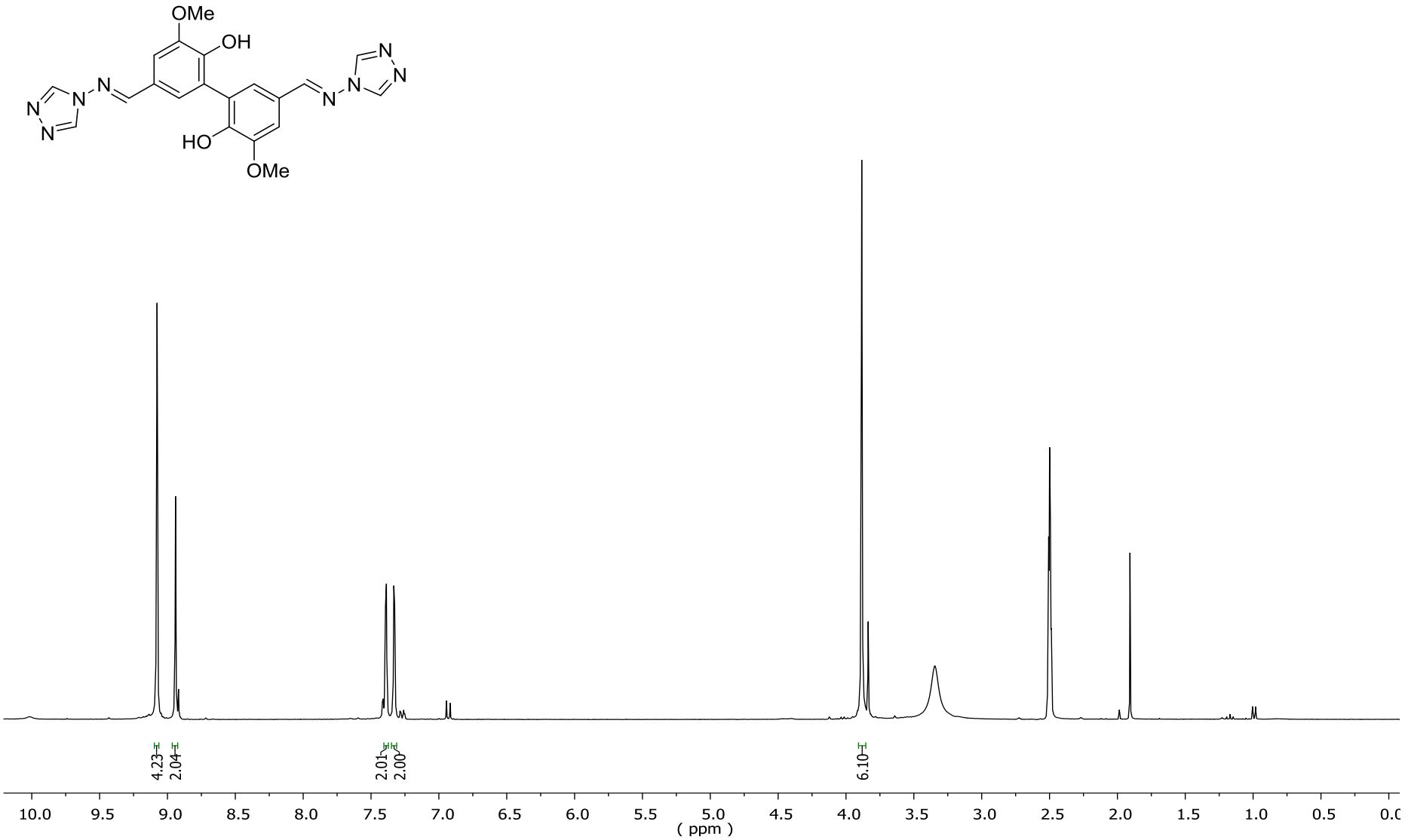

CN
OH
OMe
MeO
OH
CN
128.11
114.07
56.37
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

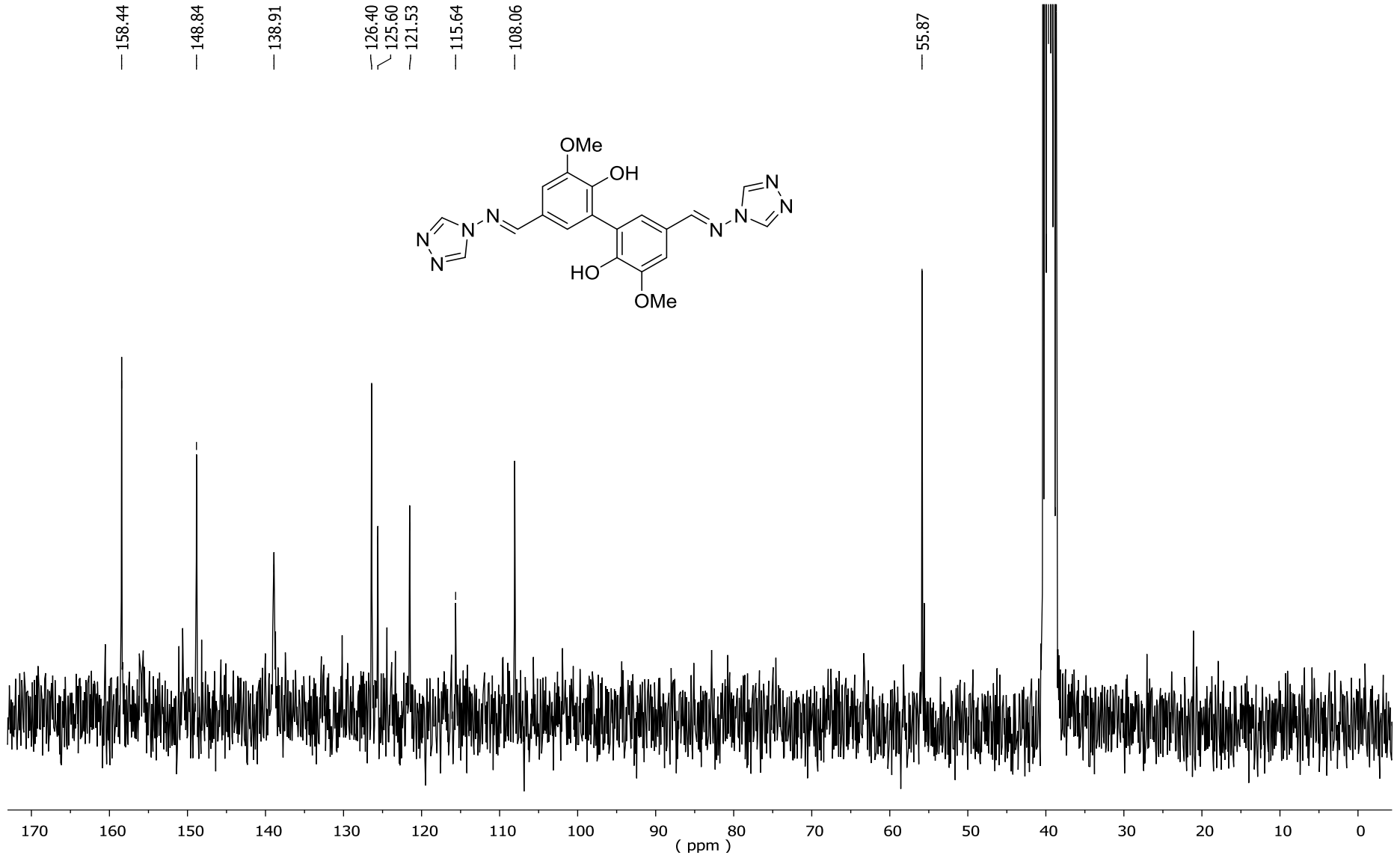

N
OH
OH
OMe
MeO
OH
N
OH
1.00
1.03
0.87
3.81
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

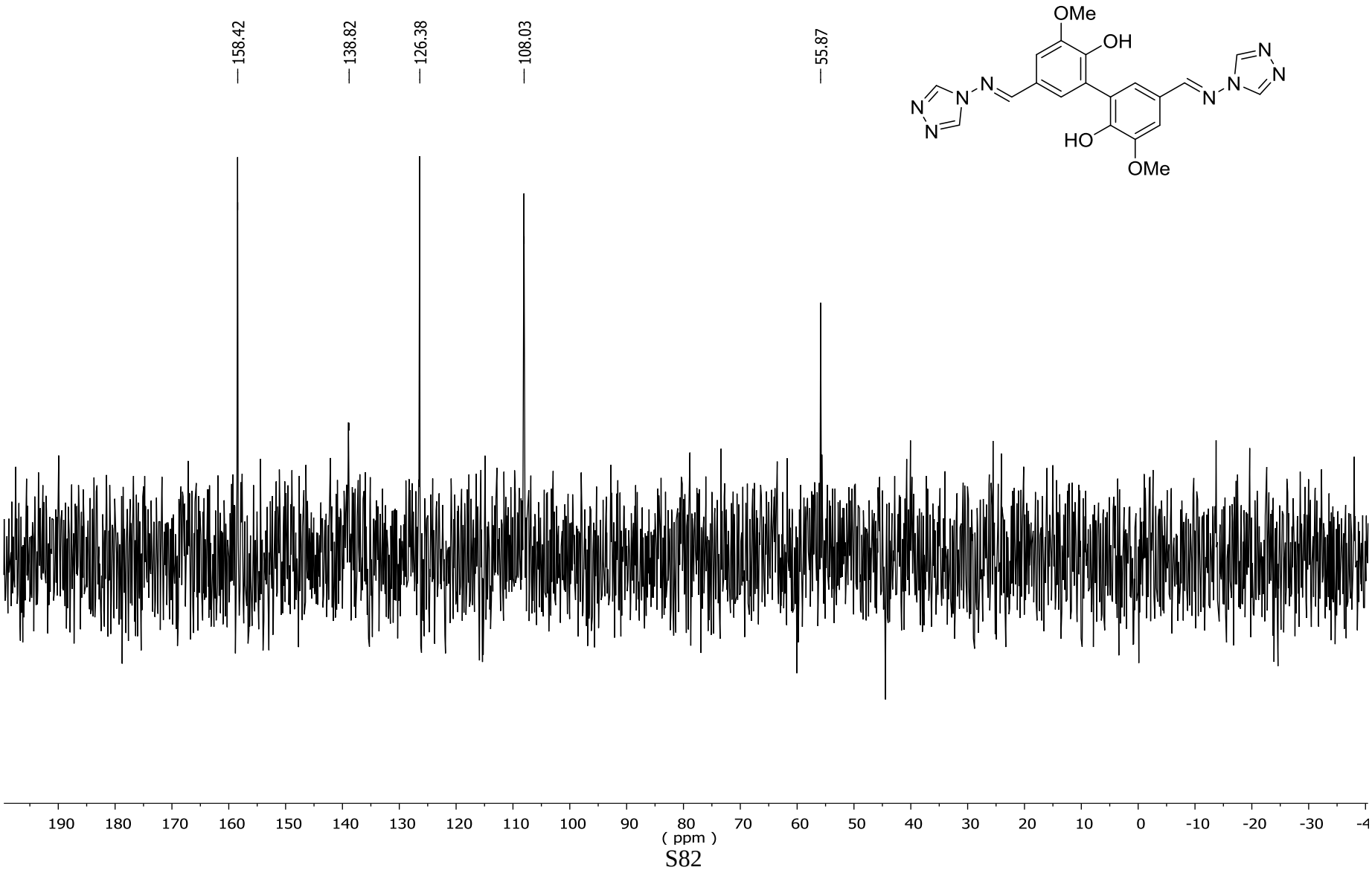

150.56
149.53
146.73
126.28
125.42
124.87
108.35
56.53
49.00
N
OH
OH
MeO
OMe
OH
N
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


150.58
124.87
108.35
56.52
N
OH
OH
MeO
OMe
OH
N
OH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
(ppm)


OMe
OH
O
H2N
N
H
N
HO
OMe
H
N
N
NH2
O
0.87
0.85
1.00
1.45
1.23
2.12
3.24
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)


OMe
OH
HO
OMe
4.23
2.04
2.01
2.00
6.10
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)


158.44
148.84
138.91
126.40
125.60
121.53
115.64
108.06
55.87
OMe
OH
HO
OMe
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


158.42
138.82
126.38
108.03
55.87
OMe
OH
HO
OMe
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -4
(ppm)