Synthesis and in Vitro Cytotoxicity Profile of the *R*-Enantiomer of 3,4-Dihydroxymethamphetamine (*R*-(–)-HHMA) : Comparison with Related Catecholamines.

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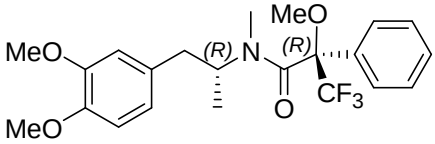
I.Experimental procedures for the determination of enantiomeric excess

1-Enantiomeric purity of (*R*)-3,4-dimethoxymethamphetamine (*R*)-MMMA 6. The enantiomeric excess of (*R*)-MMMA **6** was determined by HPLC after derivatization with (*R*)-α–methoxy-α-trifluoromethylphenyl acetic chloride [(*R*)-MTPA-Cl] (*1*). In order to set up reaction conditions, the racemic compound (±)-MMMA was used as a reference. After derivatization with (*R*)-MTPA-Cl, two diastereoisomers were isolated after purification by semipreparative HPLC, and characterized separately. Their mixture was analyzed by HPLC to establish optimal conditions of separation. Then, the same process of synthesis and analysis was applied to (*R*)-MMMA.

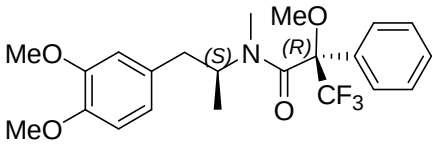
(±)-MMMA Derivatization. Synthesis of (*R*,*R*) and (*R*,*S*)-*N*-methyl-ethyl-[2-(3,4-dimethoxy-phenyl)]-3,3,3-trifluoro-2-methoxy-*N*-methyl-2-phenyl-propionamide 10 and 11. A solution of (±)-MMMA (519 mg, 1.98 mmol) and triethylamine (0.27 mL, 1.98 mmol, 1eq.) in dry CH_2Cl_2 (20 mL), was added dropwise, under inert atmosphere, to a solution of (*R*)-MTPA-Cl (1.98 mmol, 1 eq.) in dry CH_2Cl_2 (20 mL). After stirring 3h at room temperature, 20 mL HCl 0.5 N solution were added. The organic layer was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, filtered off and the solvent was evaporated to dryness. The resulting colorless oil was purified by reversed-phase HPLC, using a mixture of [H_2O + 1 ‰TFA (further noted solvent A)]/MeCN 60/40 as the eluent (flow rate: 16 mL.min^{-1}). Fractions containing diastereoisomer **10** and those containing diastereoisomer **11** were separately collected and freeze-dried. Compound **10** was recrystallized in diethylether (266 mg, 0.63 mmol, 29%), while compound **11** was recrystallized with an hexane/diethylether mixture (285 mg, 0.67 mmol, 34%). Their degrees of purity (100%) were determined by analytical HPLC (eluent, solvent A/MeCN 53/47; flow rate: 0.9 mL.min^{-1}). Spectroscopic data show the existence of two rotamers for each compound.



10

Compound **10**. ***Rotamer A***: ^{1}H NMR ($CDCl_3$) δ 1.18 (d, J = 6.5 Hz, 3H), 2.46 (s, 3H), 2.70 (m, 2H), 3.64 (s, 3H), 3.84 (s, 3H), 3.92 (s, 3H), 5.27 (m, 1H), 6.75 (m, 3H), 7.25 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 17.4, 28.1, 39.0, 50.0, 53.5, 55.9 × 2, 85.0, 111.1, 112.1, 121.0, 126.4 × 2, 127.2, 128.1 × 2, 128.9, 130.3, 133.7, 147.6, 148.7, 165.1. ***Rotamer B***: ^{1}H NMR ($CDCl_3$) δ

0.25 (d, *J* = 6.5 Hz, 3H), 2.30 (m, 2H), 2,94 (s, 3H), 3.78 (s, 3H), 3.86 (s, 3H), 3.92 (s, 3H), 4.22 (m, 1H), 6.75 (m, 3H), 7.25 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 14.9, 27.8, 41.1, 50.0, 54.9, 55.8 × 2, 85.0, 111.1, 112.1, 121.0, 126.4 × 2, 127.2, 128.1 × 2, 128.3, 128.9, 130.6, 147.6, 148.7, 165.1.



11

Compound **11**. ***Rotamer A***: ^{1}H NMR ($CDCl_3$) δ 1.19 (d, *J* = 6.5 Hz, 3H), 2.41 (s, 3H), 2.67 (dd, *J* = 14.6 Hz, 1H), 2.85 (dd, *J* = 14.6 Hz, 1H), 3.07 (s, 3H), 3.82 (s, 3H), 3.89 (s, 3H), 5.30 (m, 1H), 6.80 (m, 3H), 7.50 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 16.6, 27.8, 39.2, 49.5, 54.5, 55.9, 56.0, 85.0, 111.2, 111.8, 120.8, 123.7, 127.1× 2, 128.5 × 2, 129.1, 130.6, 134.0, 147.7, 149.0, 165.3. ***Rotamer B***: ^{1}H NMR ($CDCl_3$) δ 0.99 (d, *J* = 6.5 Hz, 3H), 2.02 (dd, *J* = 12.7 Hz, 1H), 2.24 (dd, *J* = 12.7 Hz, 1H), 3.07 (s, 3H), 3.69 (s, 3H), 3.76 (s, 3H), 3.80 (s, 3H), 4.24 (m, 1H), 6.80 (m, 3H), 7.50 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 16.6, 27.9, 39.4, 49.5, 52.9, 55.8, 56.3, 85.0, 111.0, 112.2, 121.3, 123.7, 126.5 × 2, 128.8 × 2, 129.6, 129.9, 134.6, 147.6, 148.7, 165.3.

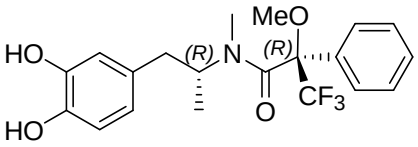
Conditions of separation of an equimolar mixture of compounds **10** and **11** were determined by analytical HPLC (eluent: solvent A/MeCN 53/47; flow rate: 0.9 mL.min^{-1}).

(*R*)-MMMA Derivatization. Previous method of derivatization, replacing (±)-MMMA by (*R*)-MMMA **6** afforded a colorless oil, whose spectroscopic data were identical with those of previous (*R*,*R*)-compound **10**. Its degree of purity (99.5%) was directly determined by analytical HPLC (eluent, solvent A/MeCN 53/47; flow rate: 0.9 mL.min^{-1}).

2-Enantiomeric purity of (*R*)-3,4-dihydroxymethamphetamine *R*-(–)-HHMA 8. Similarly, the enantiomeric excess of (*R*)-HHMA **8** was determined by HPLC after derivatization with (*R*)-MTPA-Cl. In order to set up reaction conditions, the racemic compound (±)-HHMA was used as a reference. After derivatization with (*R*)-MTPA-Cl, two diastereoisomeric Mosher amides, along with the corresponding diastereoisomeric Mosher esters resulting from the reaction with the phenol groups, were isolated after purification by flash column chromatography. The mixture of diastereoisomeric Mosher amides was analyzed by HPLC to establish optimal conditions of separation. Then, the same process of synthesis, and analysis, was applied to (*R*)-HHMA **8**.

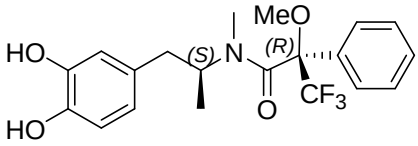
(±)-HHMA Derivatization. Synthesis of (*R*,*R*) and (*R*,*S*)-*N*-methyl-ethyl-[2-(3,4-dihydroxy-phenyl)]-3,3,3-trifluoro-2-methoxy-*N*-methyl-2-phenyl-propionamide

12 and 13. A solution of (±)-HHMA,HBr (131 mg, 0.5 mmol) and triethylamine (0.35 mL, 5 eq.) in a mixture of dry CH_2Cl_2 (6 mL) and DMSO (1 mL), was added dropwise, under nitrogen, to a solution of (*R*)-MTPA-Cl (139 mg, 0.55 mmoles, 1.1 éq.) in dry CH_2Cl_2 (5.5 mL). After stirring 2h30 at room temperature, 50 mL of dichloromethane and 10 mL HCl 0.5 N solution were added. The organic layer was dried over $MgSO_4$, filtered off and evaporated to dryness. Flash column chromatography (toluene/acetone 90:10 v/v) afforded a mixture of the Mosher amides **12** and **13** as a pale yellow oil (31mg, 0.08 mmol, 15.5%), along with a fraction containing the four diastereoisomeric Mosher esters (65 mg, 0.16 mmol, 33%).



12

Compound **12**. ^{1}H NMR ($CDCl_3$) δ 1.25 (d, J = 6.5 Hz, 3H), 2.46 (s, 3H), 2.55 (dd, J = 12.5 Hz, 1H), 2.74 (dd, J = 12.5 Hz, 1H), 3.65 (s, 3H), 5.29 (m, 1H), 6.75 (m, 3H), 7.25 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 17.6, 28.0, 38.7, 50.0, 55.0, 85.0, 115.3, 115.9, 121.4, 126.3 × 2, 128.2 × 2, 128.6, 129.1, 129.7, 133.3, 143.2, 143.7, 165.8.



13

Compound **13**. ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.5 Hz, 3H), 2.44 (s, 3H), 2.60 (m, 1H), 2.90 (m, 1H), 3.65 (s, 3H), 5.26 (m, 1H), 6.75 (m, 3H), 7.25 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 16.8, 27.8, 38.9, 50.2, 54.6, 85.0, 115.1, 115.6, 121.1, 126.4 × 2, 128.3 × 2, 128.6, 129.2, 130.0, 133.8, 143.1, 144.0, 166.1.

Conditions of separation of compounds **12** and **13** were determined by analytical HPLC (eluent: solvent A/MeCN 70/30; flow rate: 0.58 mL.min^{-1}).

Derivatization of (*R*)-3,4-dihydroxymethamphetamine *R*-(–)-HHMA 8. The previous method, replacing (±)-HHMA,HBr by *R*-(−)-HHMA,HBr **8** afforded the corresponding Mosher amide as a pale yellow oil (31mg, 0.08 mmol, 15.5%), whose degree of purity (99.5%) was determined by analytical HPLC (eluent: solvent A/MeCN 70/30; flow rate: 0.58 mL.min^{-1}) (Figure 1).

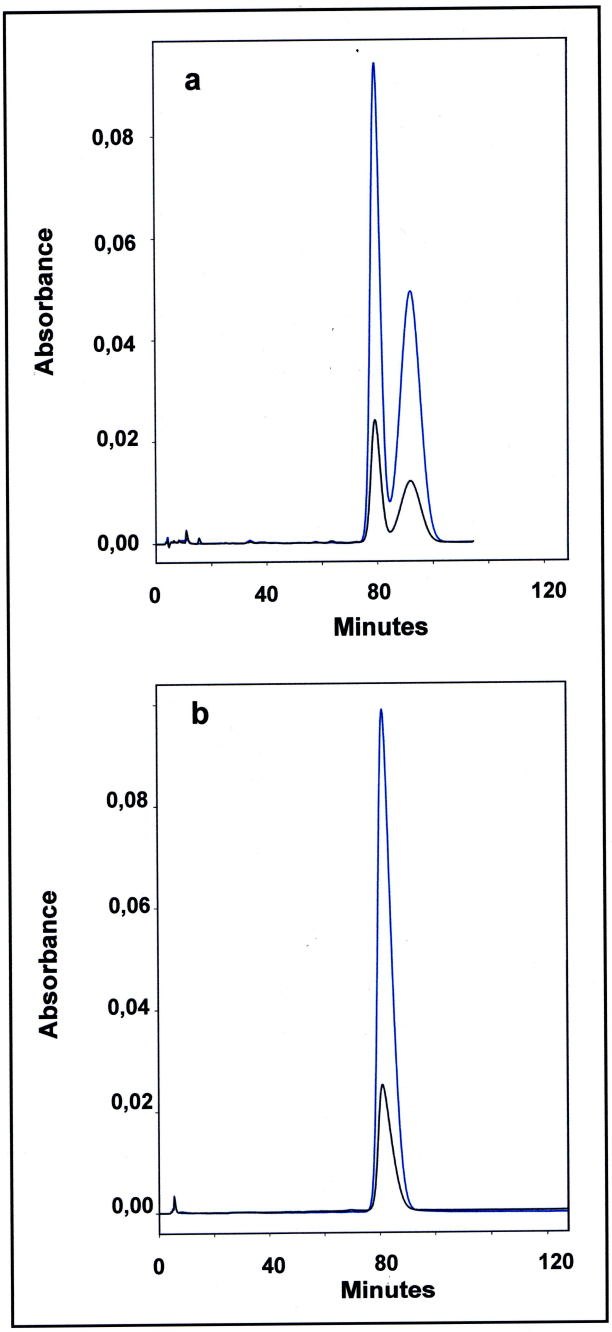
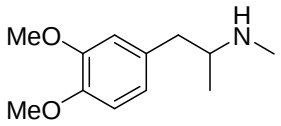
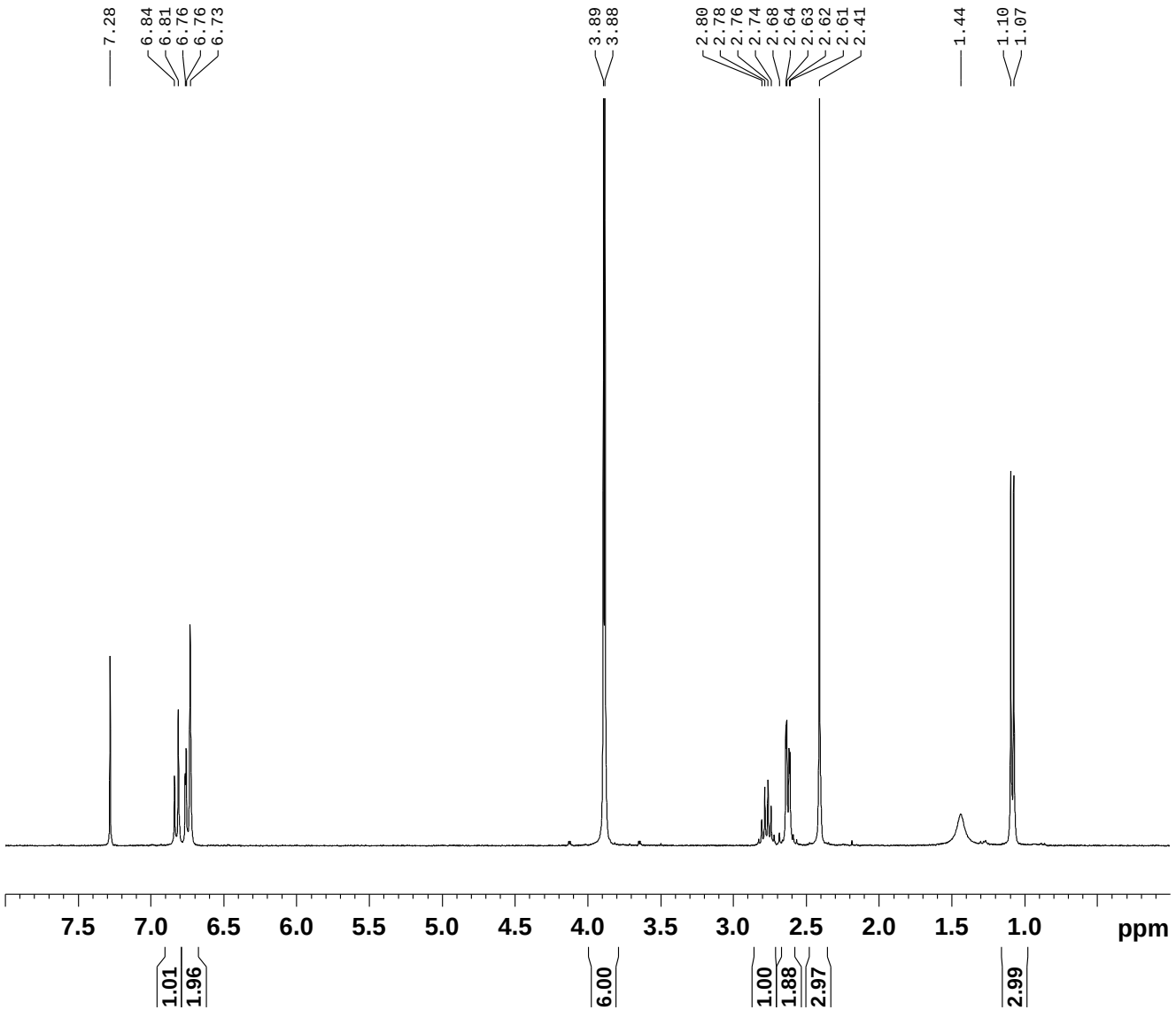
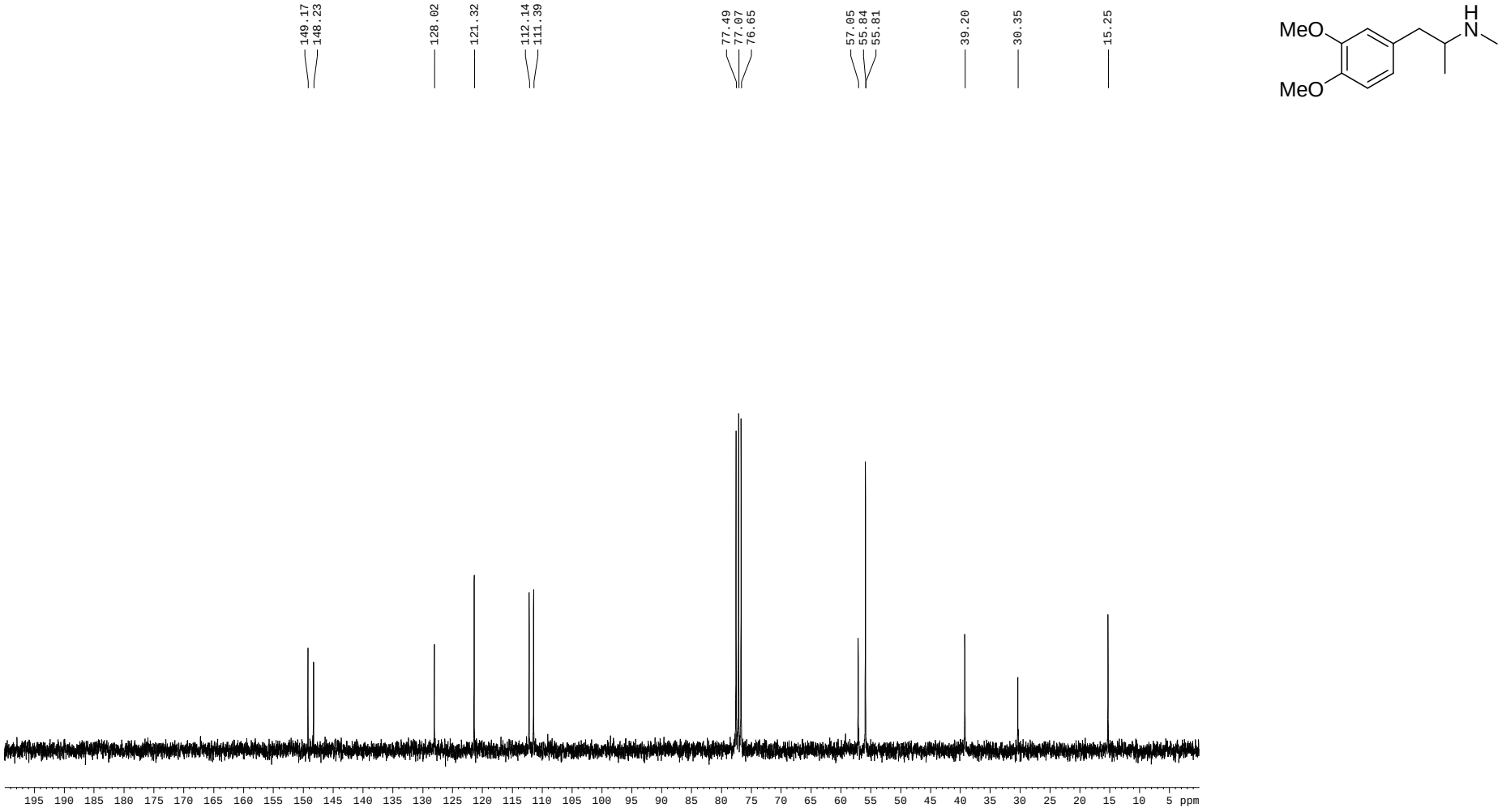


Figure 1. HPLC chromatograms for the analytical determination of the enantiomeric excess of *R*-(−)-HHMA **8** (solvent A/MeCN 53/47; flow rate: 0.9 mL.min^{-1}) : a) mixture of the Mosher amides obtained from (±)-HHMA racemate (compounds **12** and **13**); b) Mosher amide prepared from enantiomerically pure *R*-(−)-HHMA **8**.

II. ^{1}H and ^{13}C NMR spectra



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N
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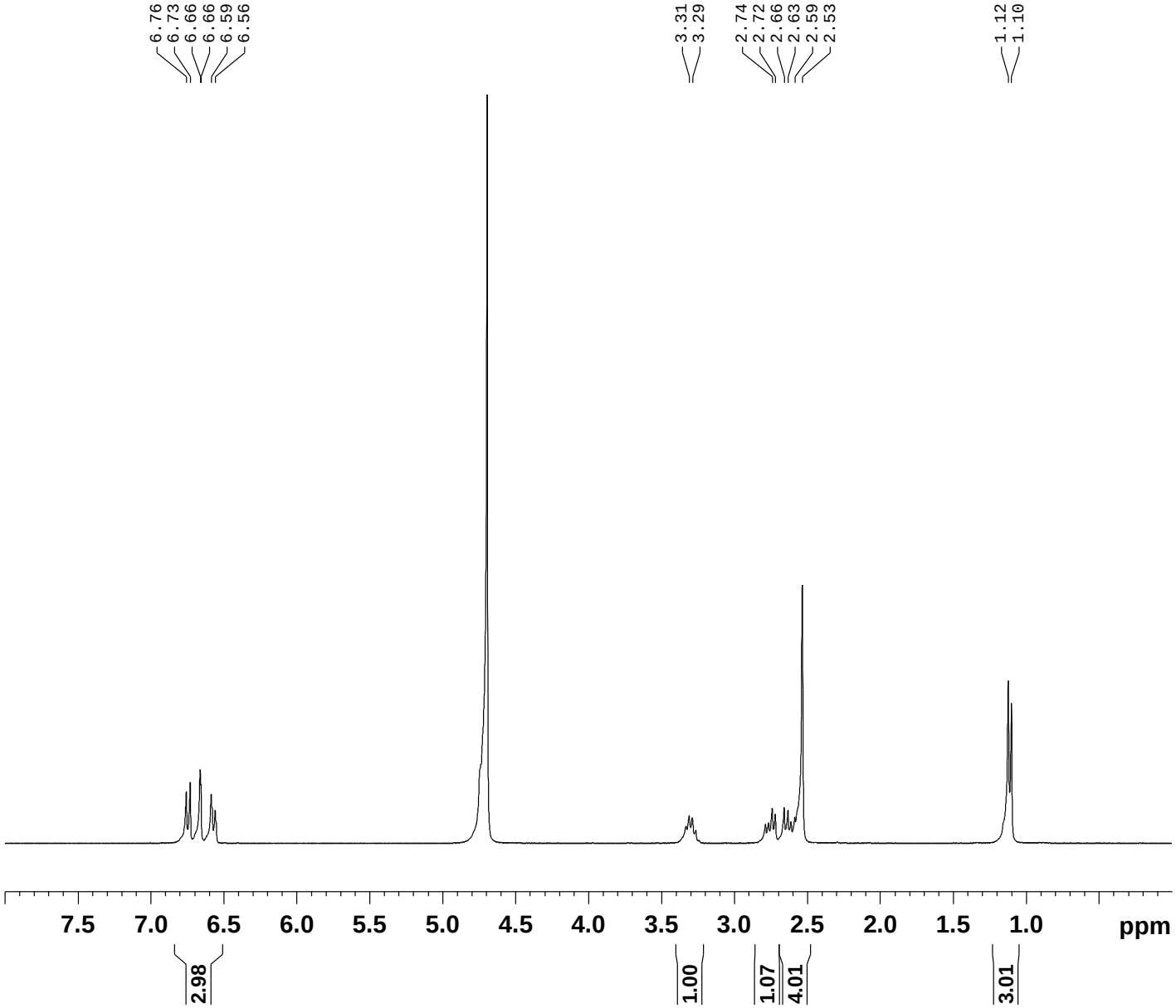
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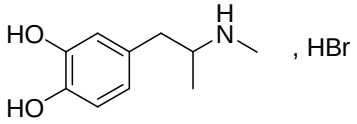
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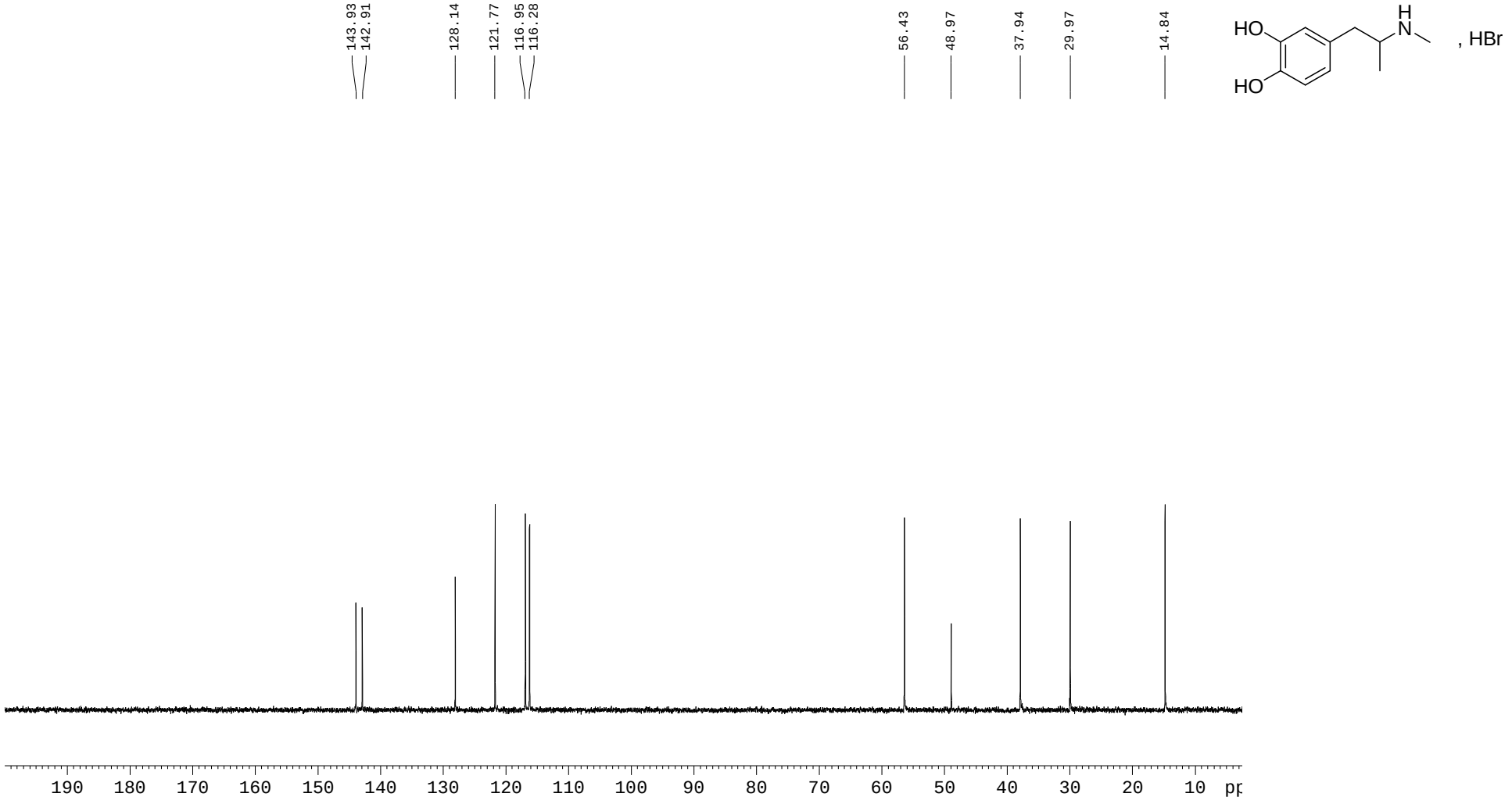
N
, HBr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, HBr
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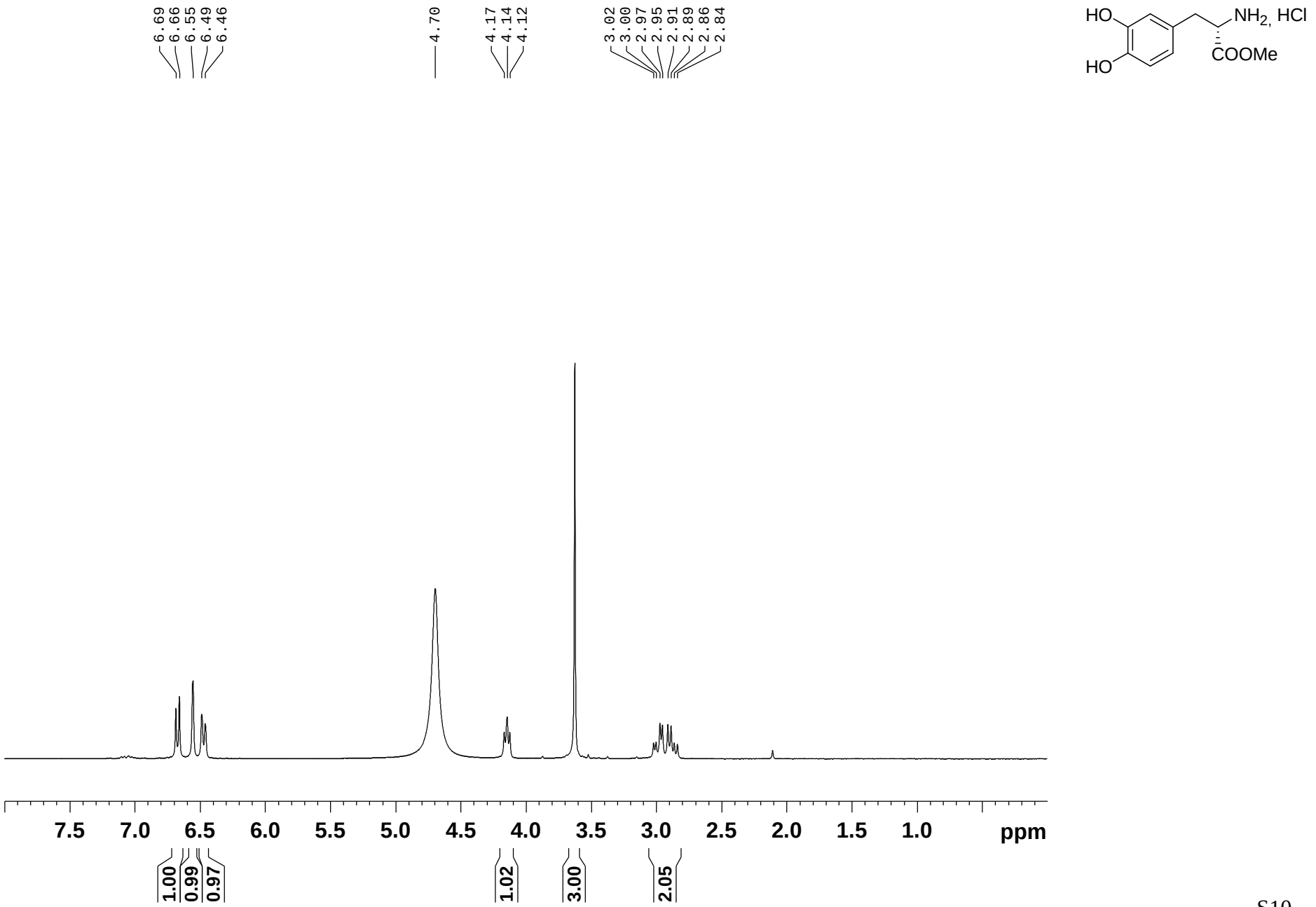
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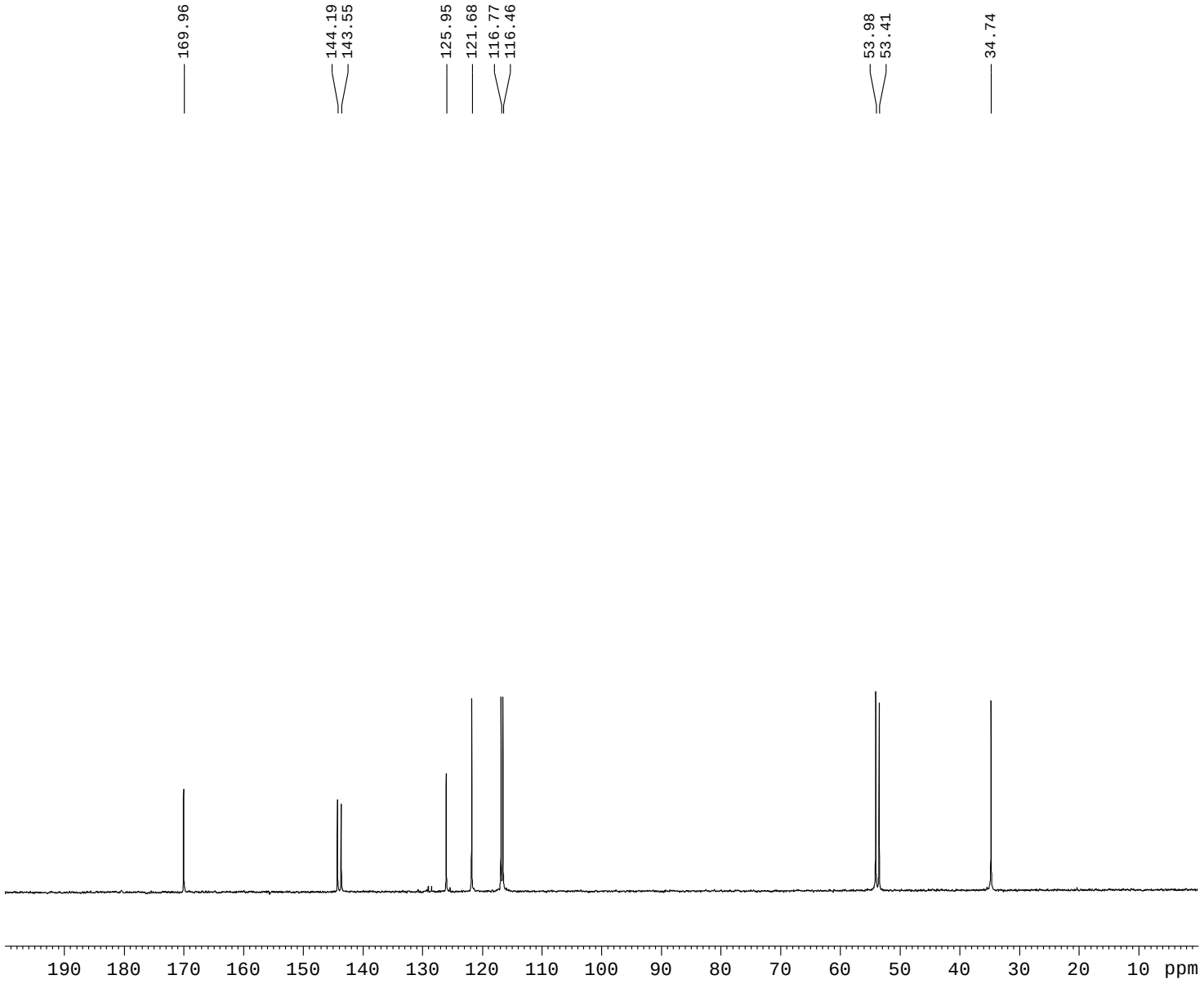
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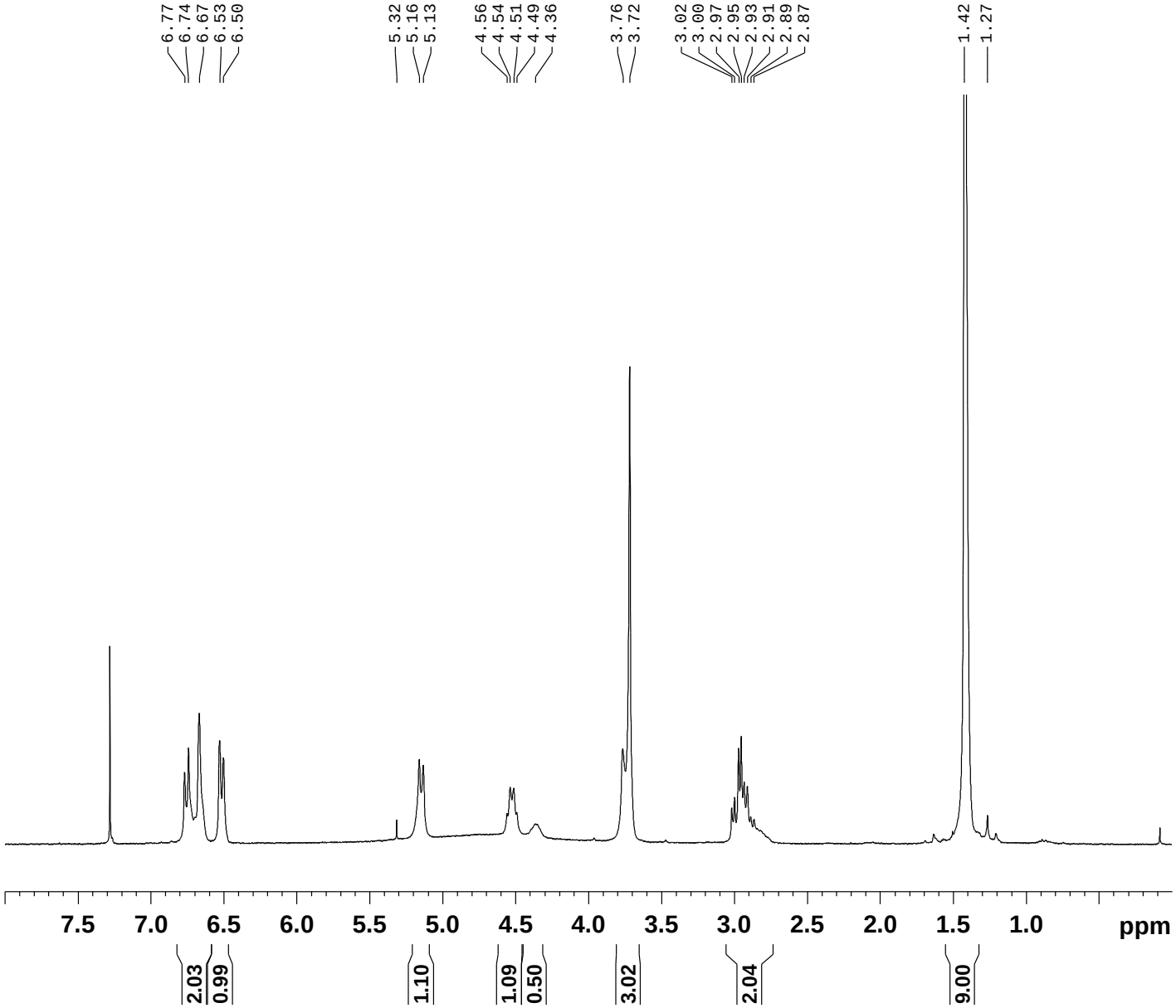
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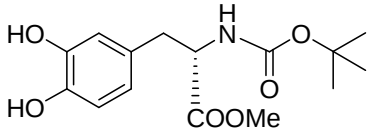
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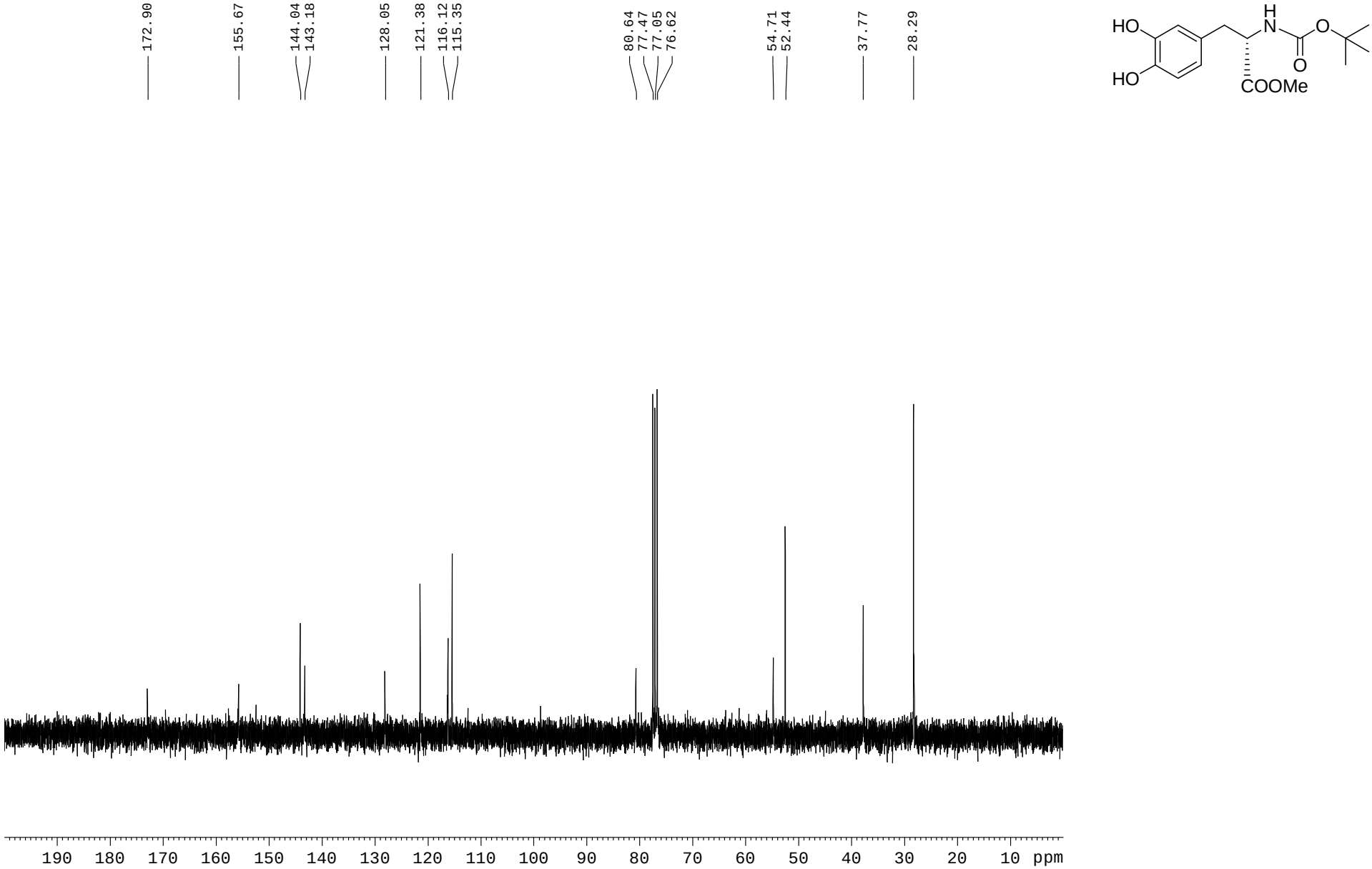
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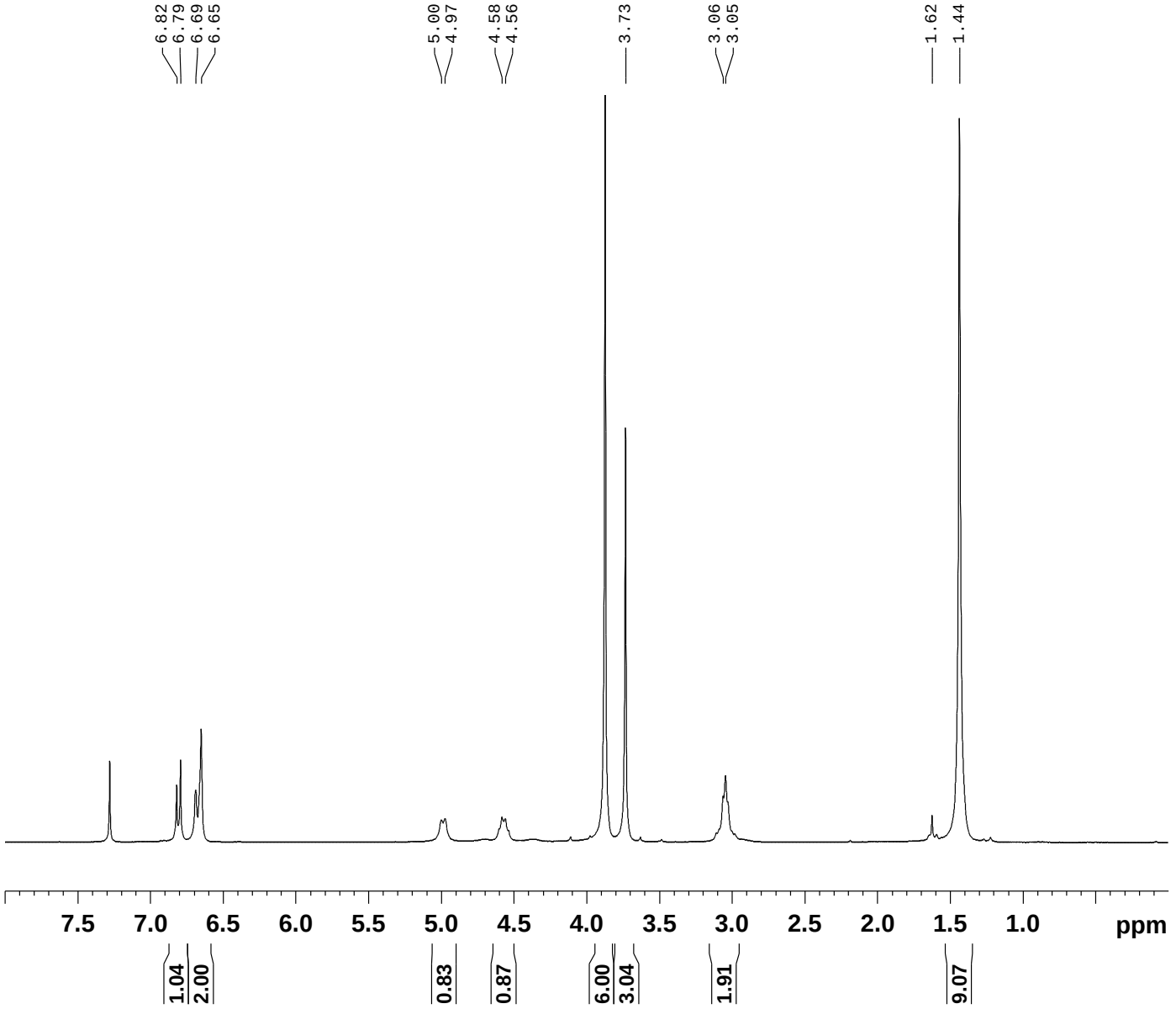
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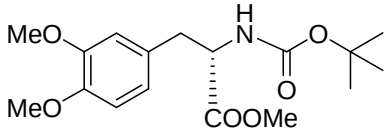
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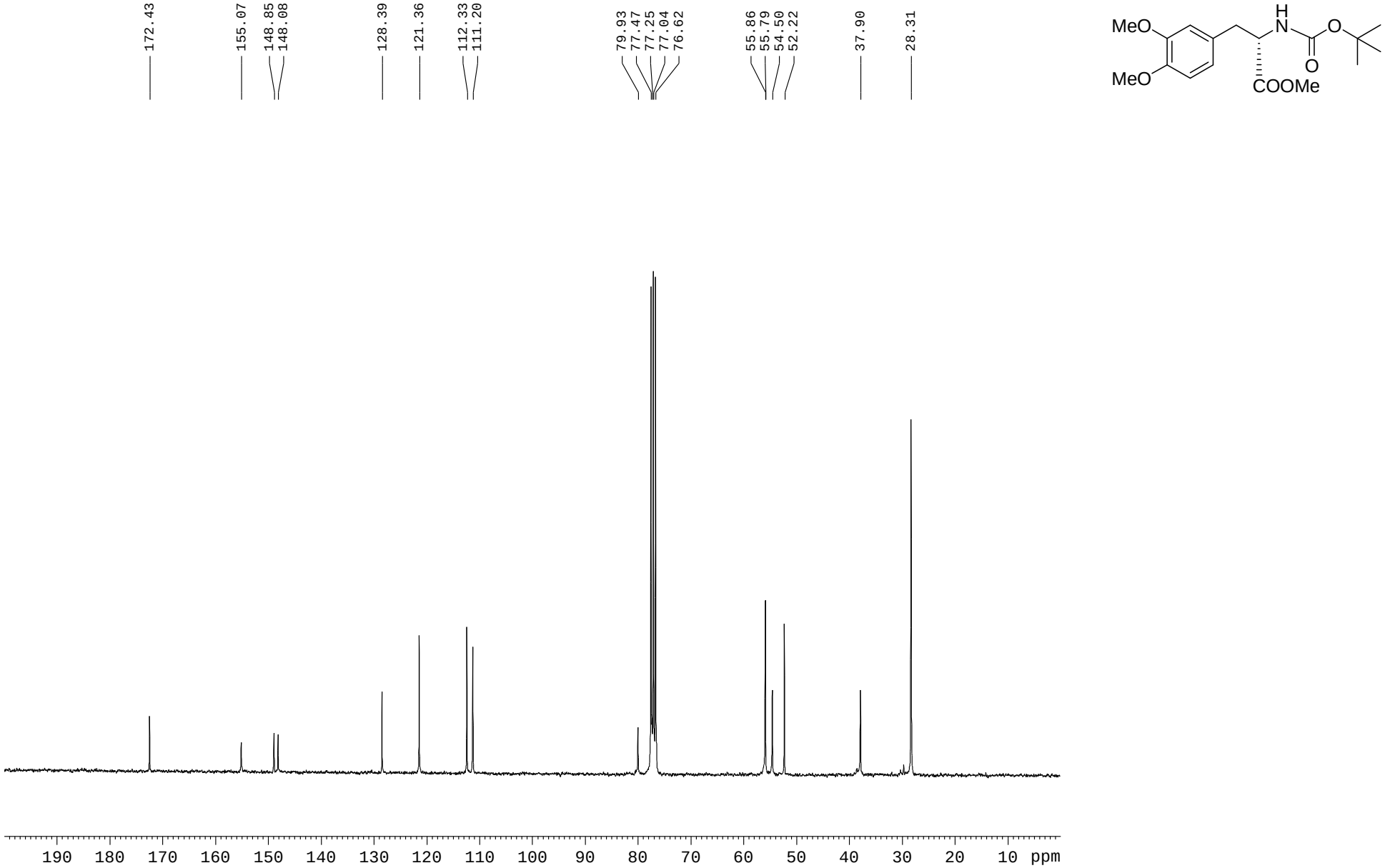
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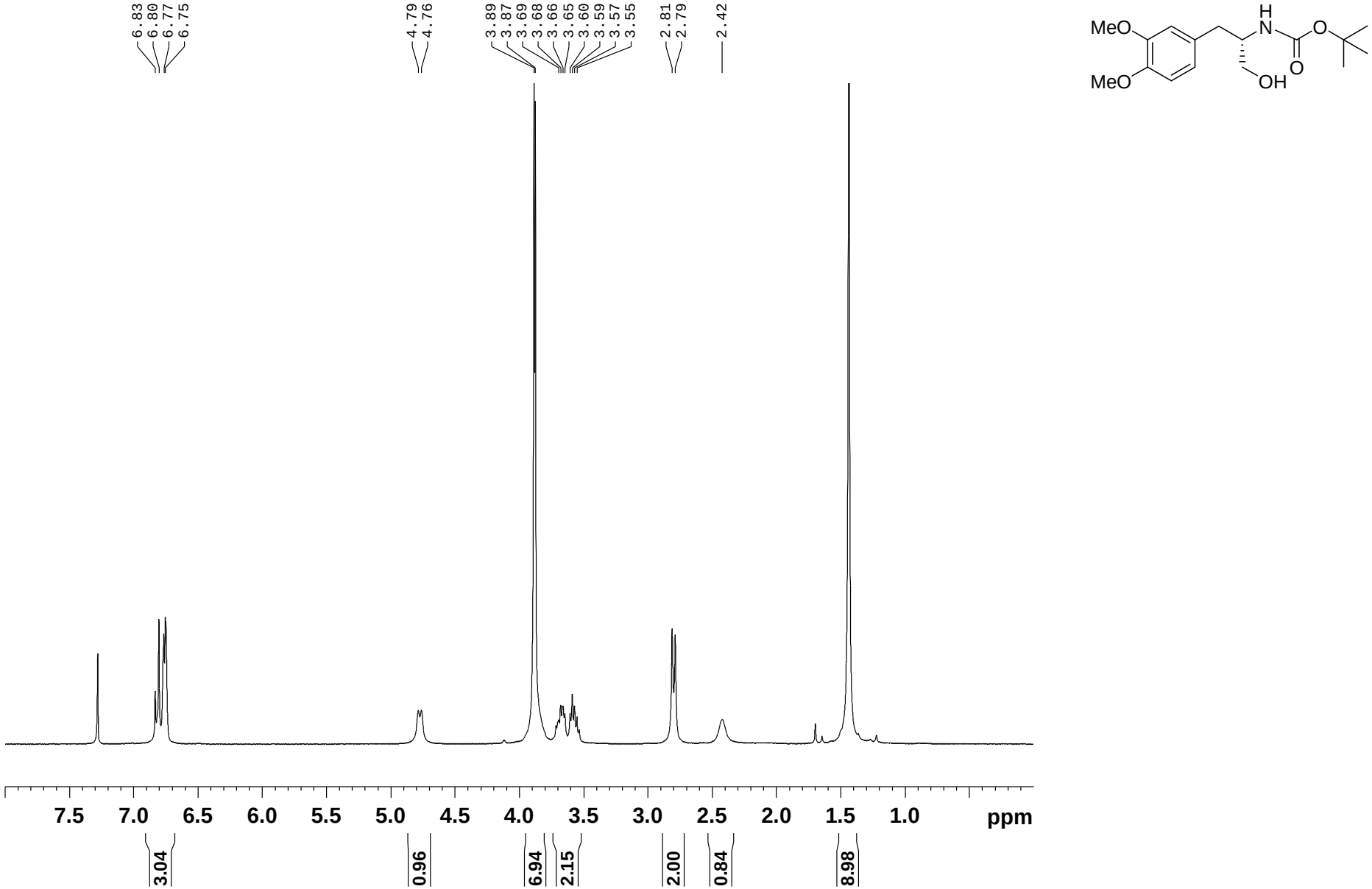
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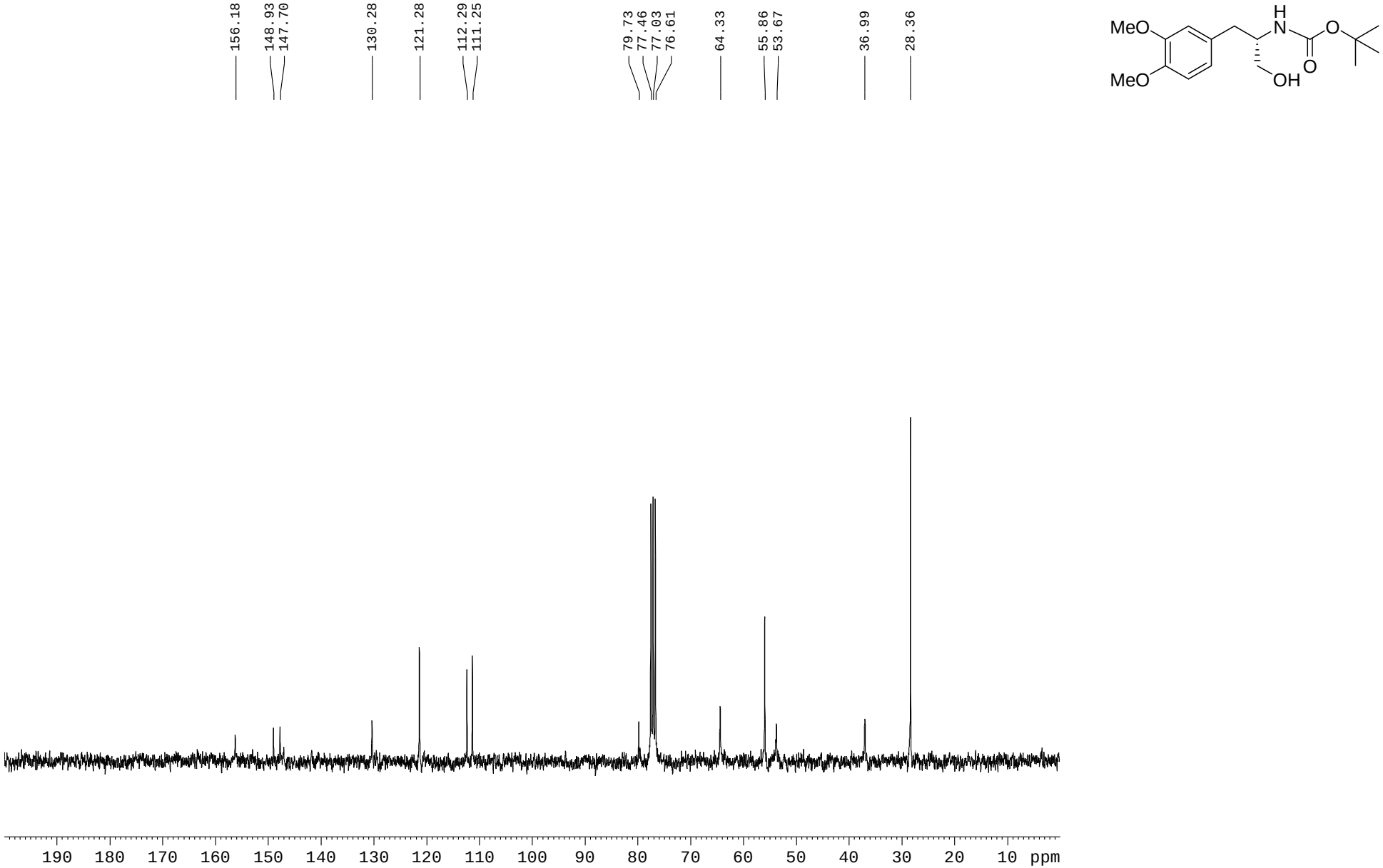
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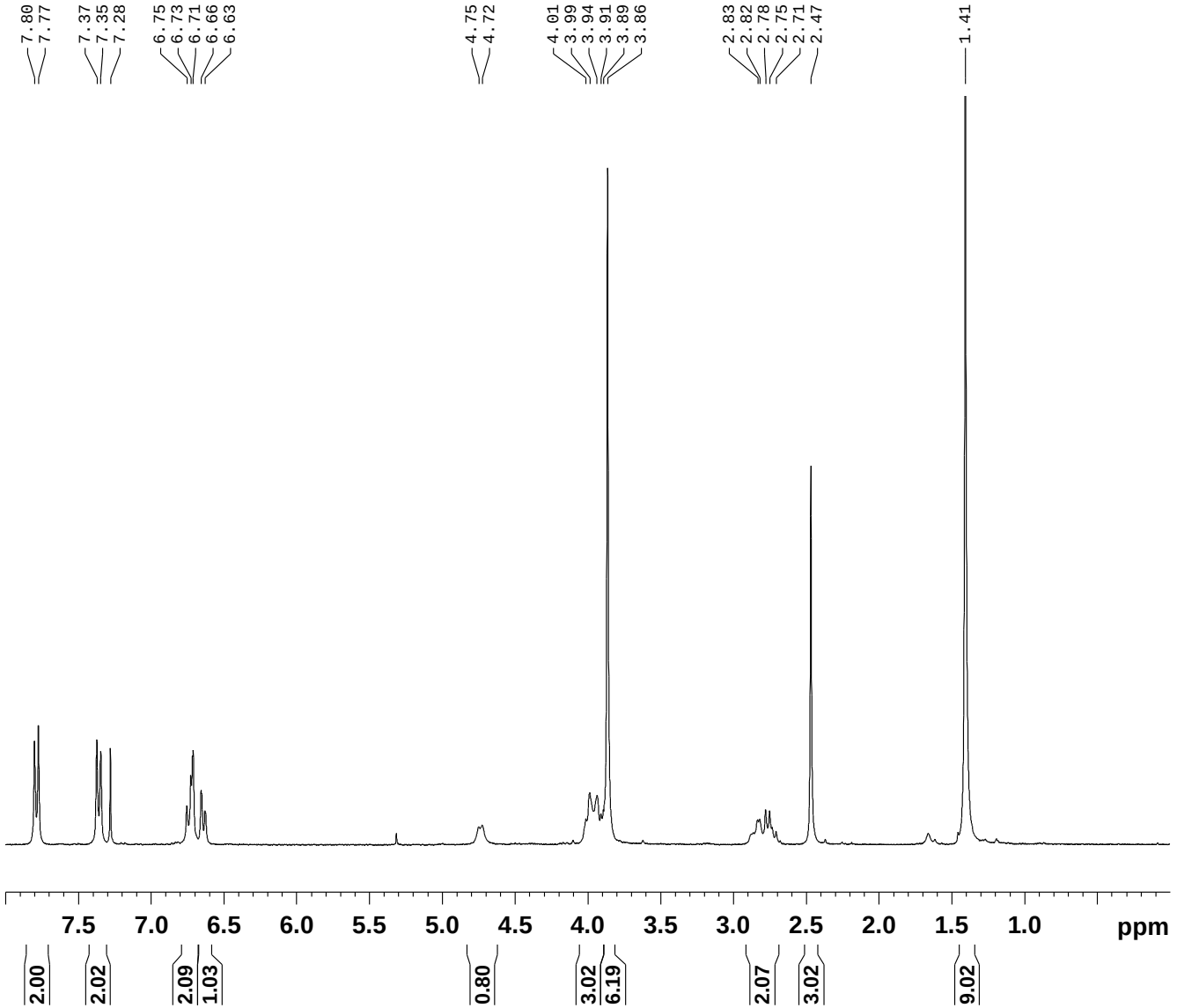
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3.86
2.83
2.82
2.78
2.75
2.71
2.47
1.41
7.5
7.0
6.5
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5.0
4.5
4.0
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2.5
2.0
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1.03
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3.02
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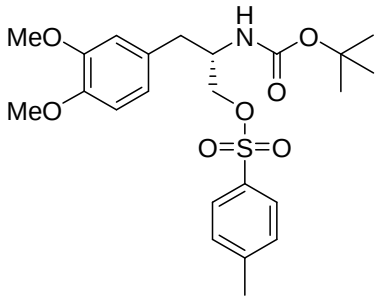
3.02
9.02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MeO
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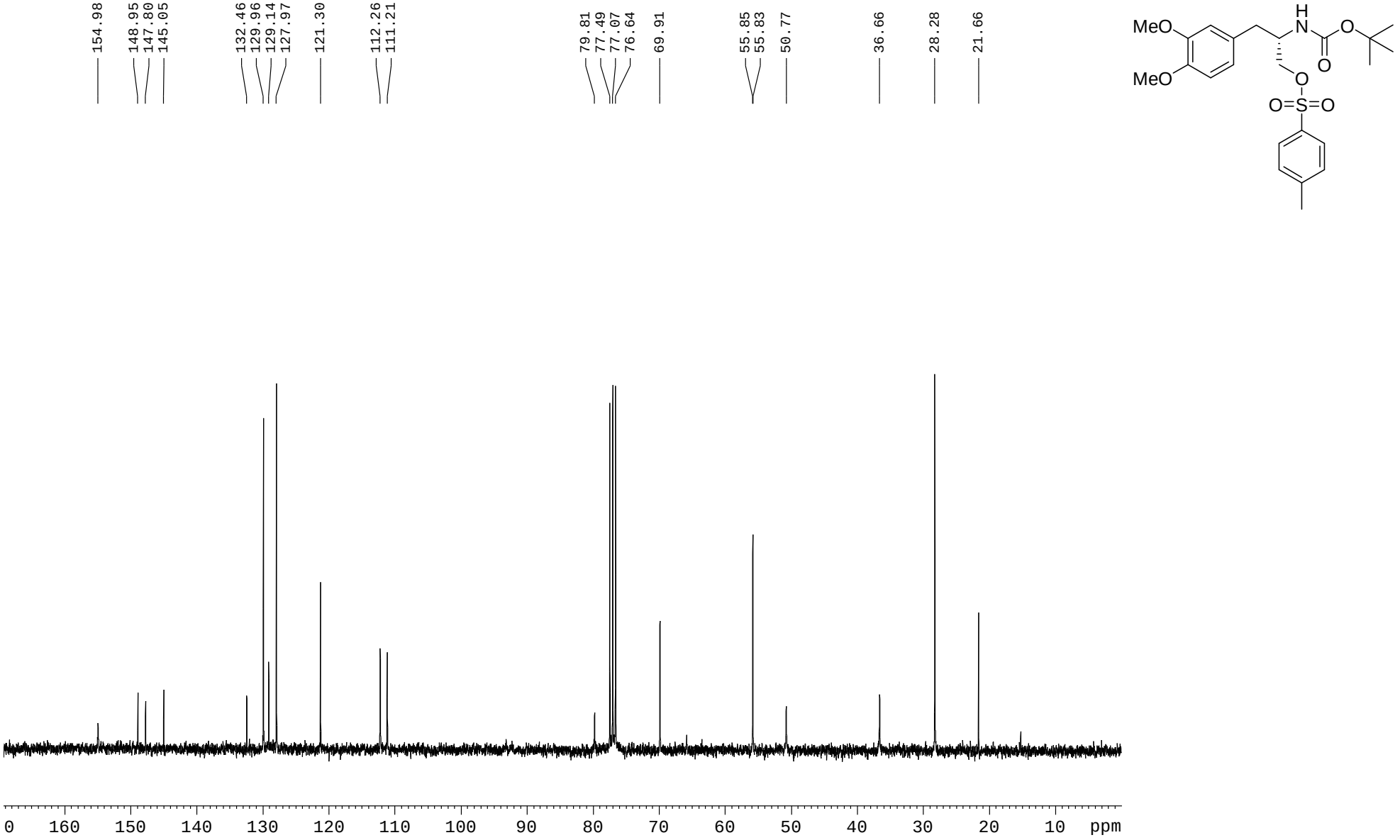
N
O
O
O
O=S=O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127.97
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111.21
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77.49
77.07
76.64
69.91
55.85
55.83
50.77
36.66
28.28
21.66
MeO
MeO
H
N
O
O
O
O=S=O
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140
130
120
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70
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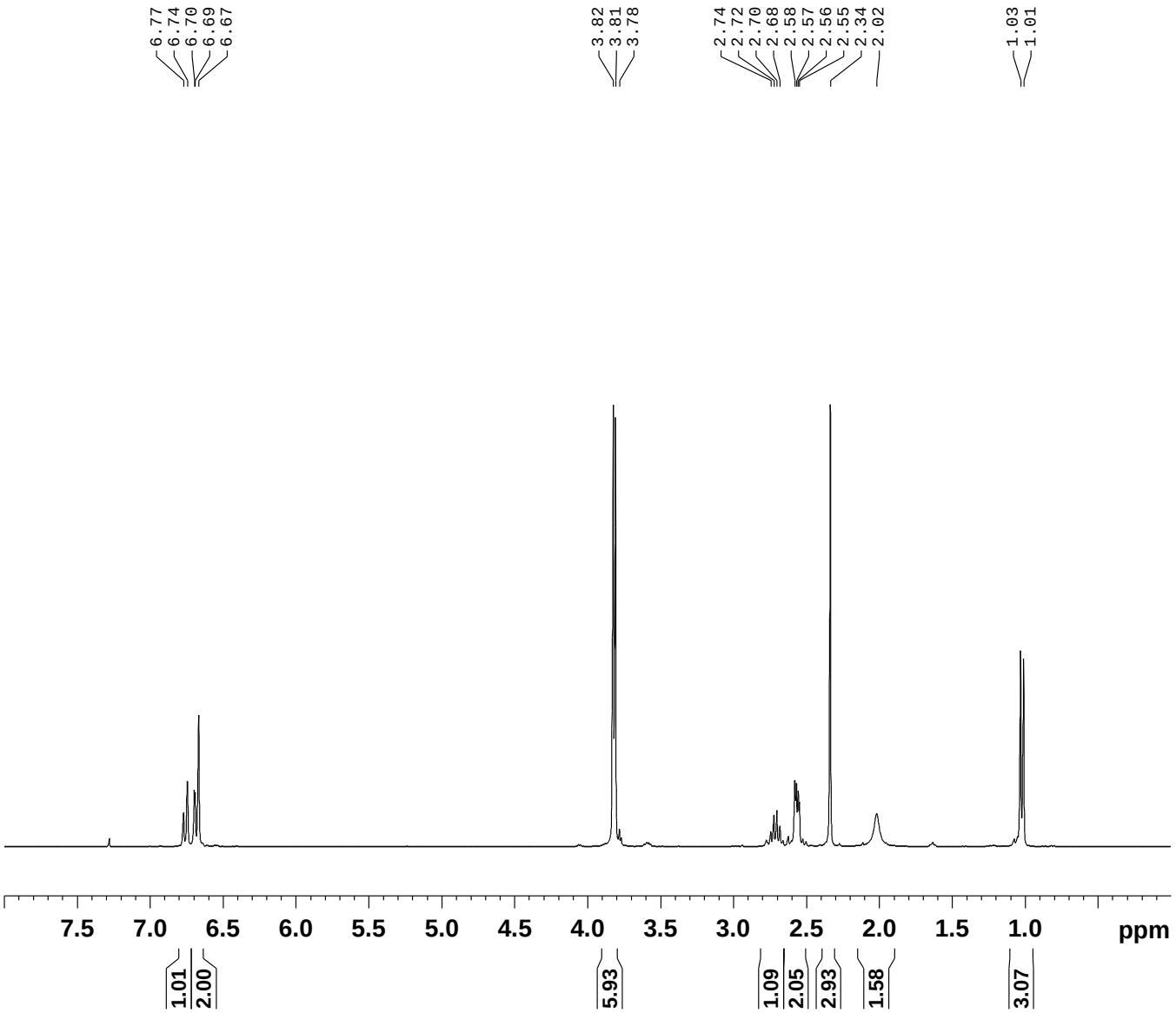
40
30
20
10
ppm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6.67
3.82
3.81
3.78
2.74
2.72
2.70
2.68
2.58
2.57
2.56
2.55
2.34
2.02
1.03
1.01
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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2.00
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1.09
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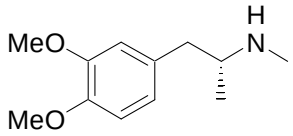
1.58
3.07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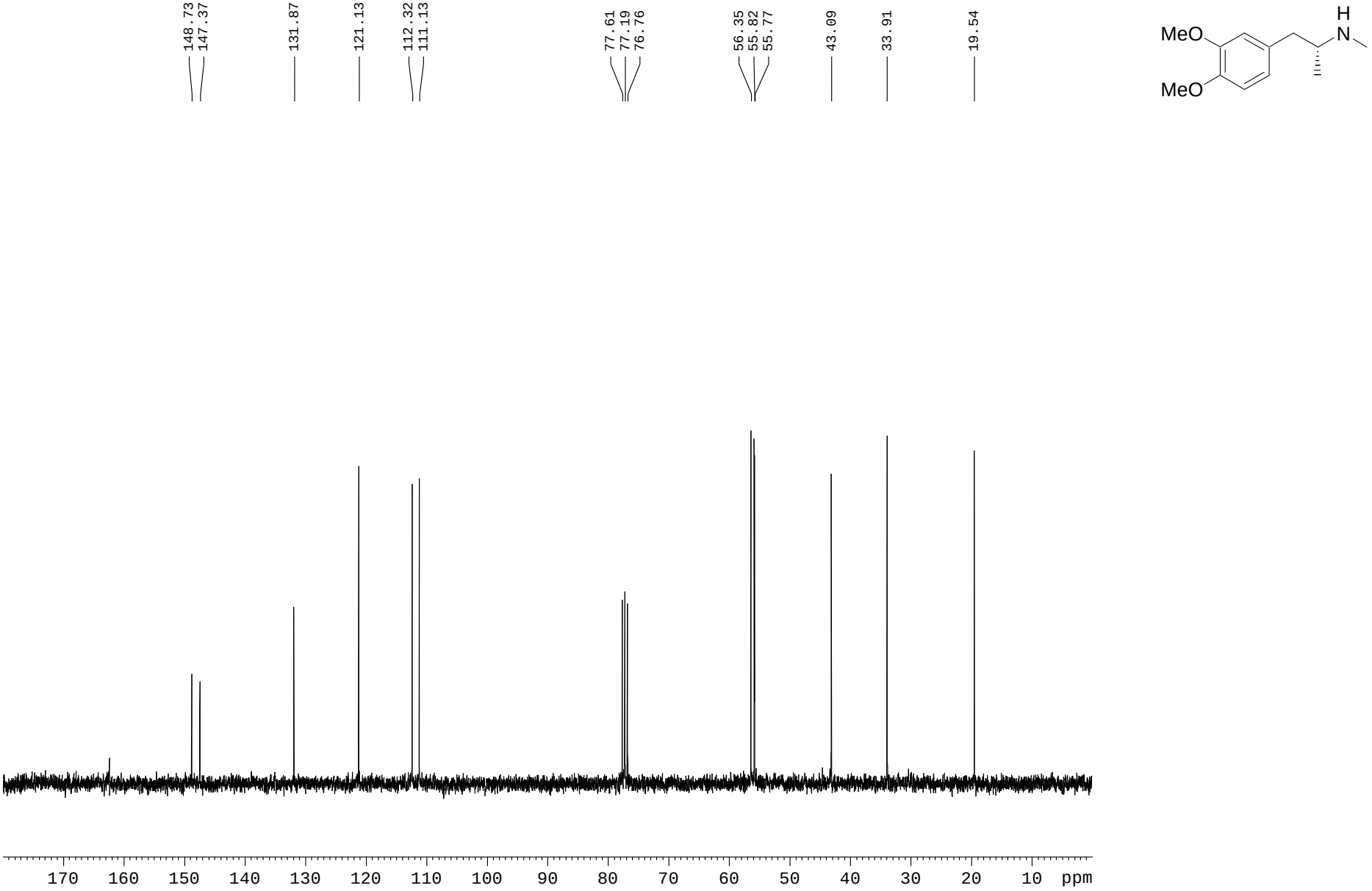
MeO
H
N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111.13
77.61
77.19
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56.35
55.82
55.77
43.09
33.91
19.54
MeO
MeO
H
N
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160
150
140
130
120
110
100
90
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70
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30
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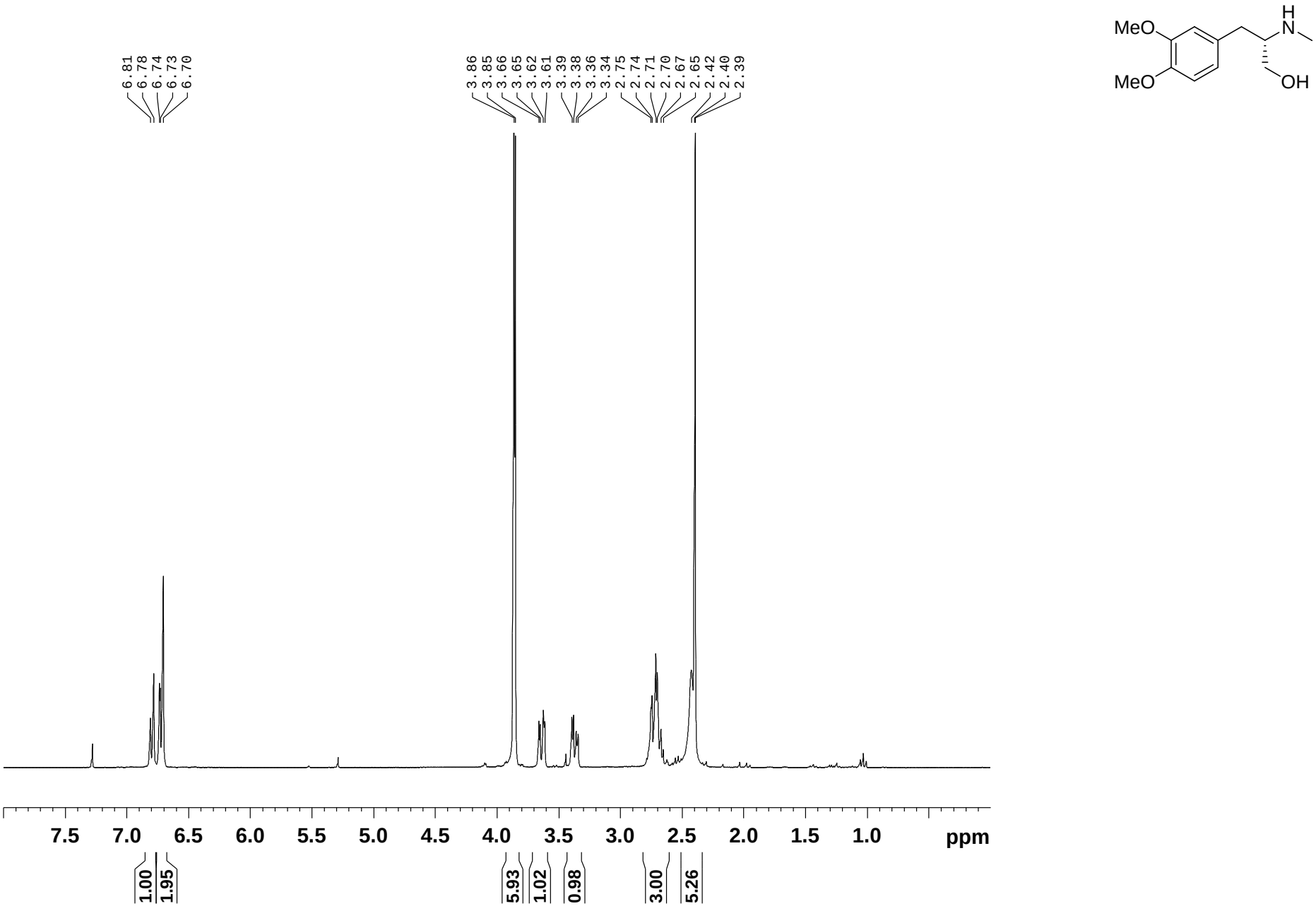
10
ppm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3.66
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3.39
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3.36
3.34
2.75
2.74
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2.70
2.67
2.65
2.42
2.40
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4.0
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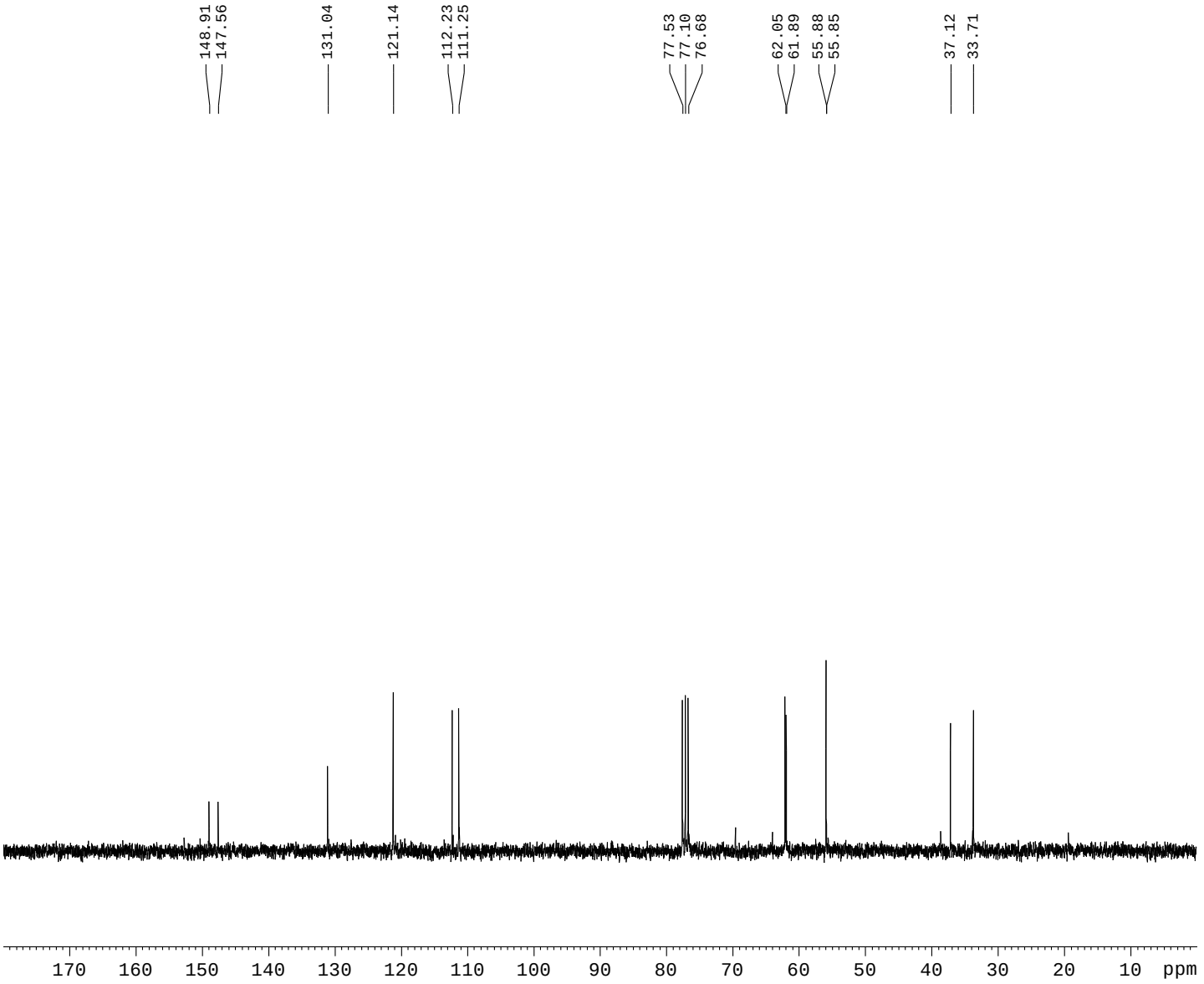
3.00
5.26

MeO
MeO
H
N
OH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77.10
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55.85
37.12
33.71
MeO
MeO
H
N
OH
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150
140
130
120
110
100
90
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70
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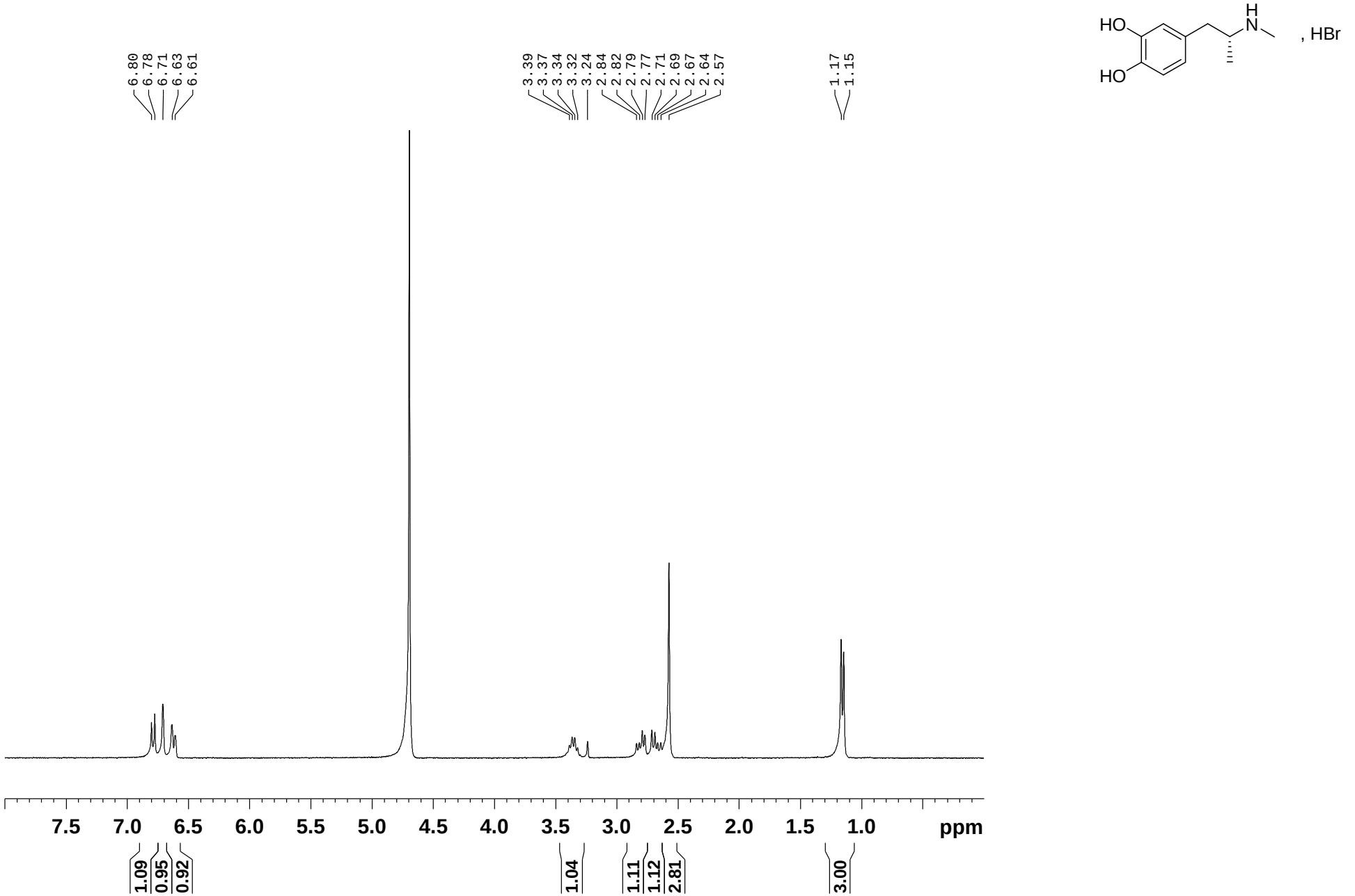
40
30
20
10
ppm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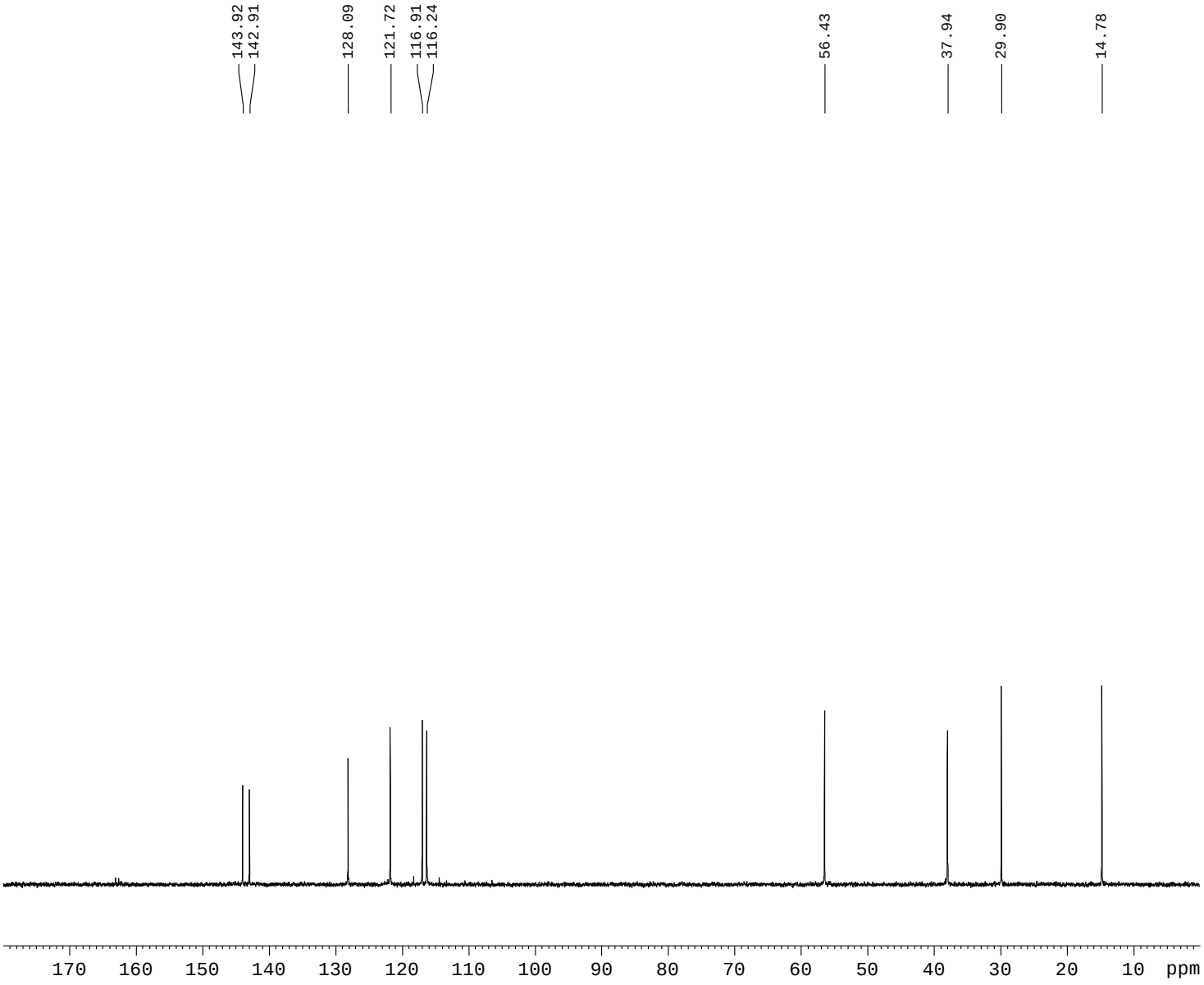
6.71
6.63
6.61
3.39
3.37
3.34
3.32
3.24
2.84
2.82
2.79
2.77
2.71
2.69
2.67
2.64
2.57
1.17
1.15
HO
HO
H
N
, HBr
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.09
0.95
0.92
1.04
1.11
1.12
2.81
3.00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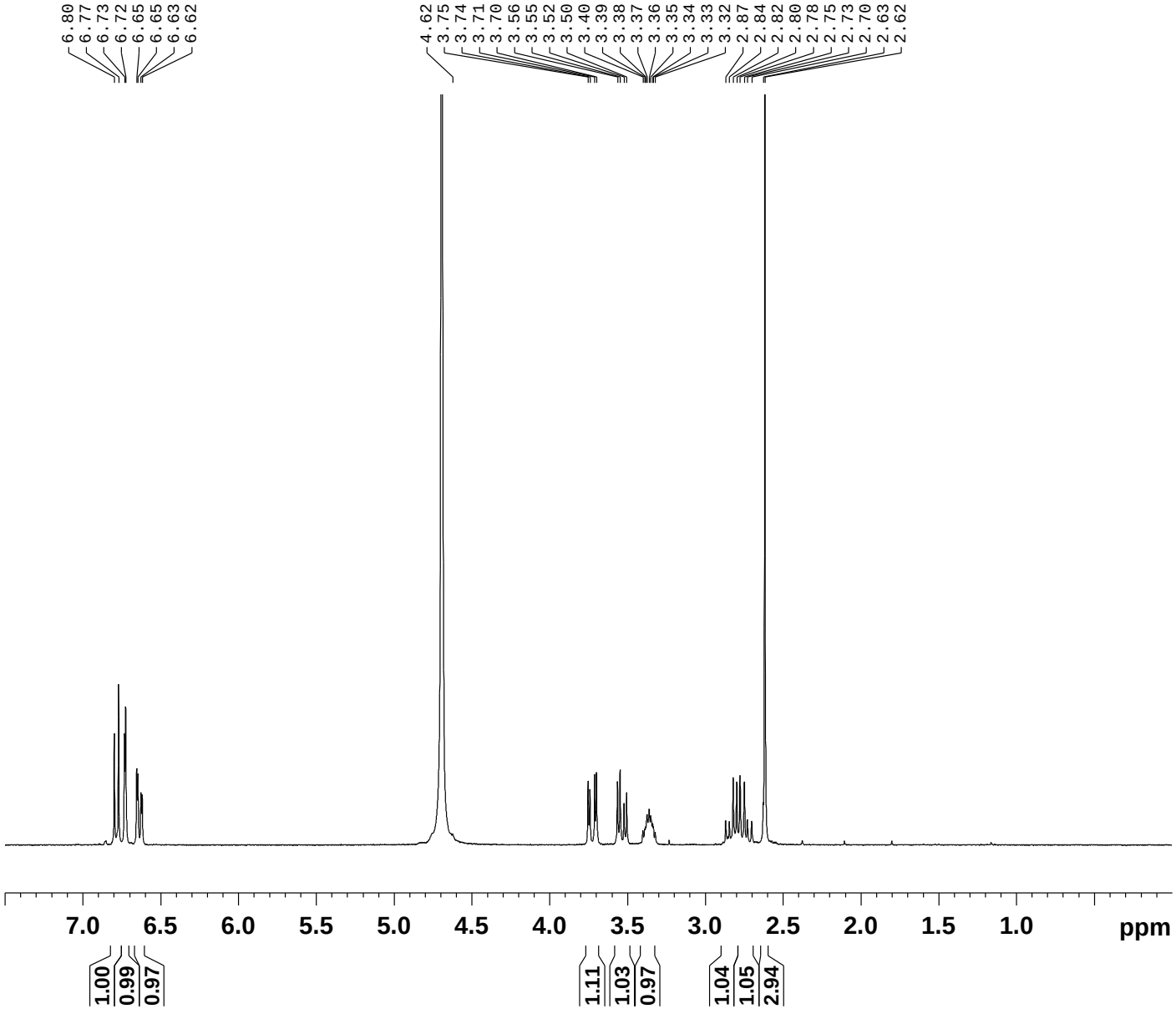
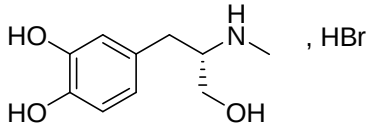
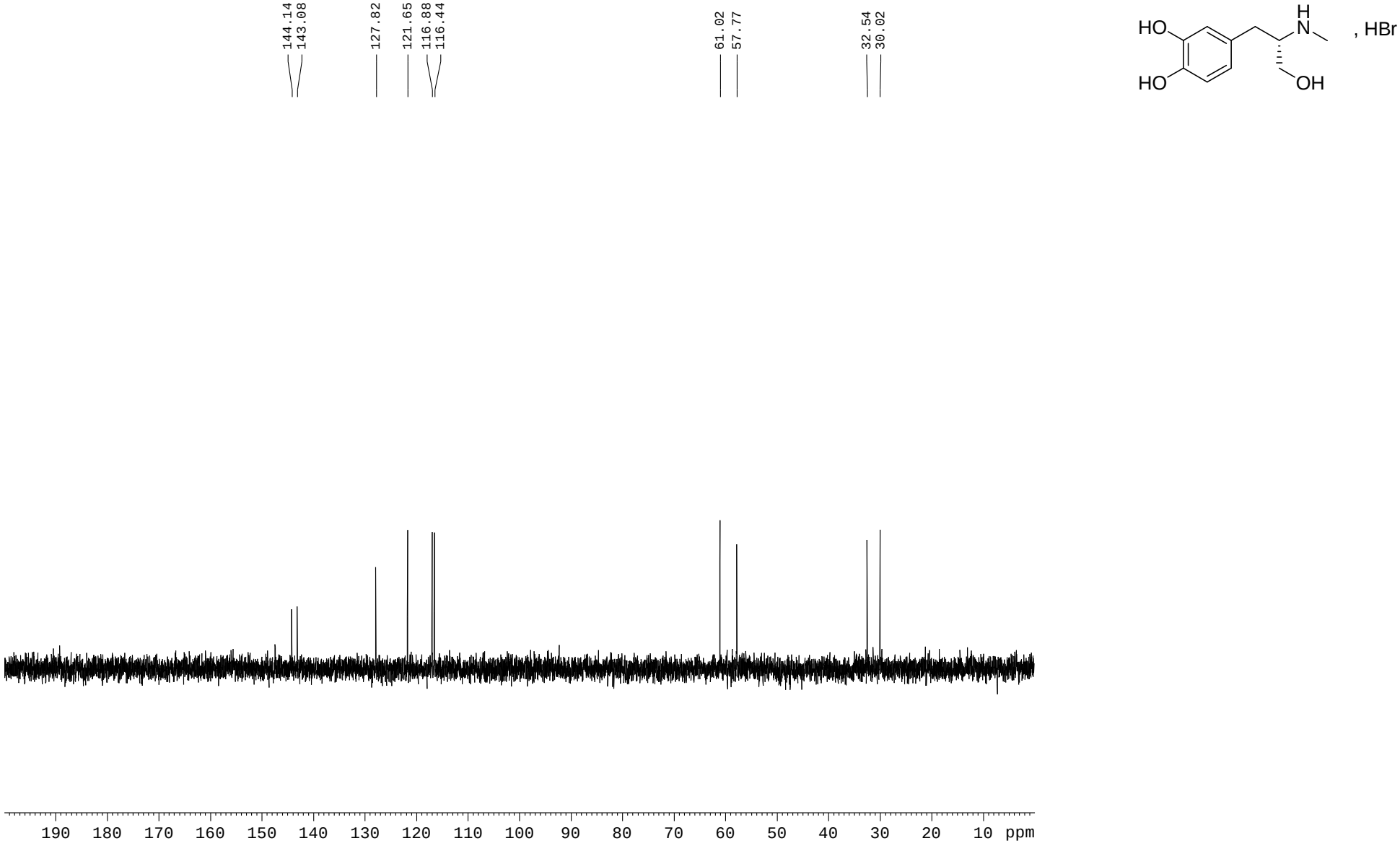
142.91
128.09
121.72
116.91
116.24
56.43
37.94
29.90
14.78
HO
HO
H
N
, HBr
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

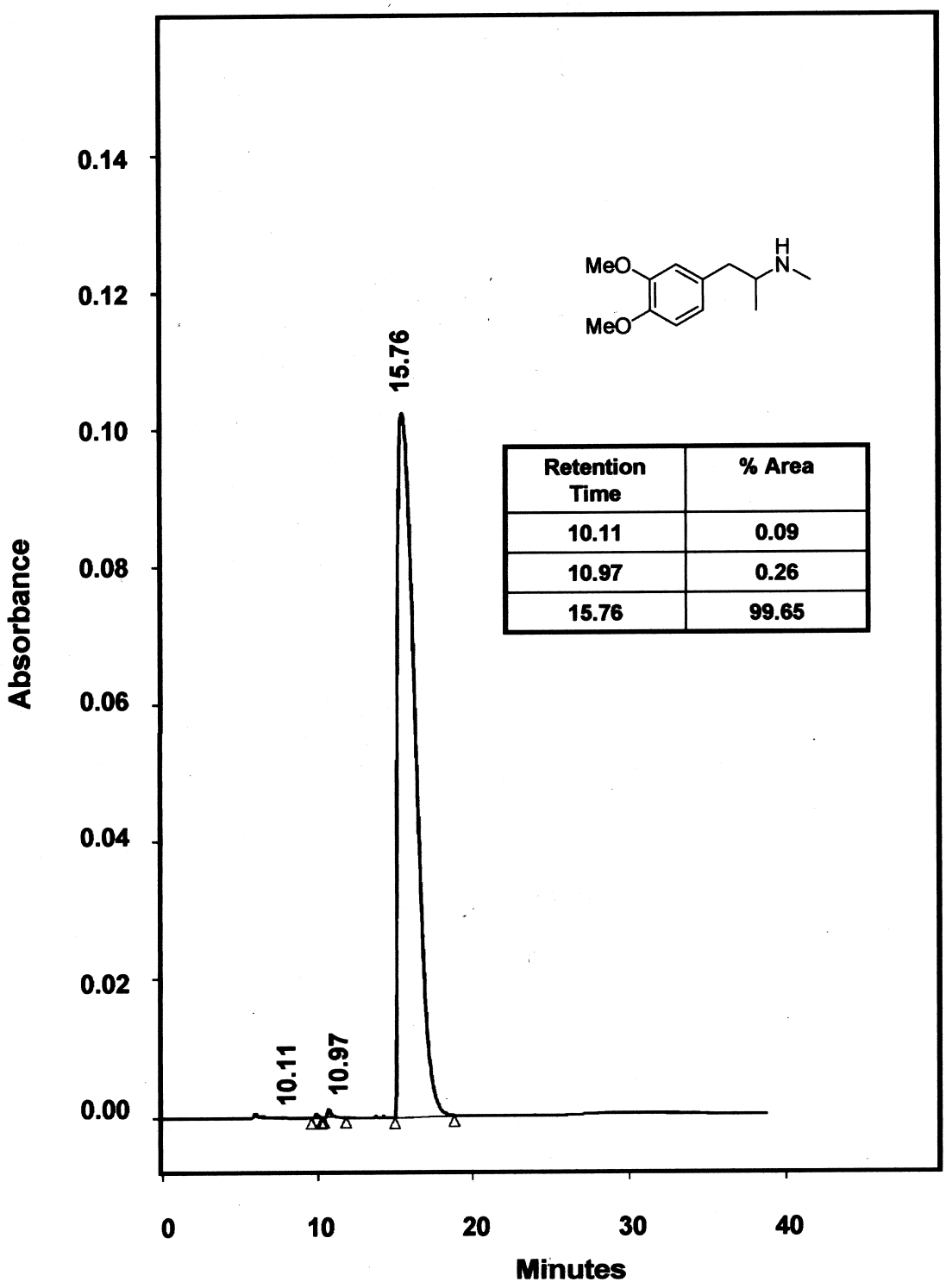

6.80
6.77
6.73
6.72
6.65
6.63
6.62
4.62
3.75
3.74
3.71
3.70
3.56
3.55
3.52
3.50
3.40
3.39
3.38
3.37
3.36
3.35
3.34
3.33
3.32
2.87
2.84
2.82
2.80
2.78
2.75
2.73
2.70
2.63
2.62
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
0.99
0.97
1.11
1.03
0.97
1.04
1.05
2.94


HO
HO
H
N
OH

, HBr

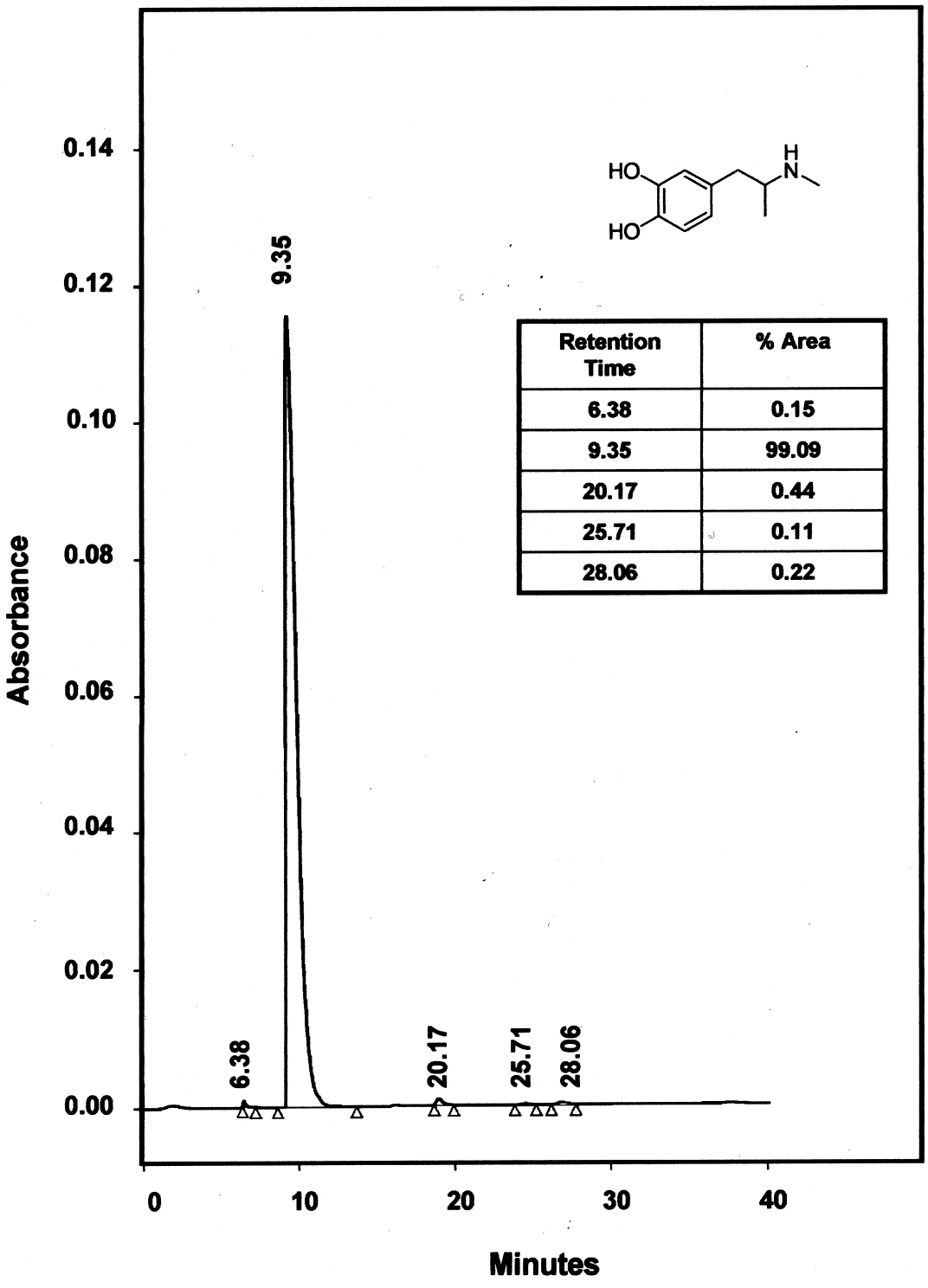

144.14
143.08
127.82
121.65
116.88
116.44
61.02
57.77
32.54
30.02
HO
HO
H
N
OH
, HBr
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

III. Analytical HPLC chromatograms



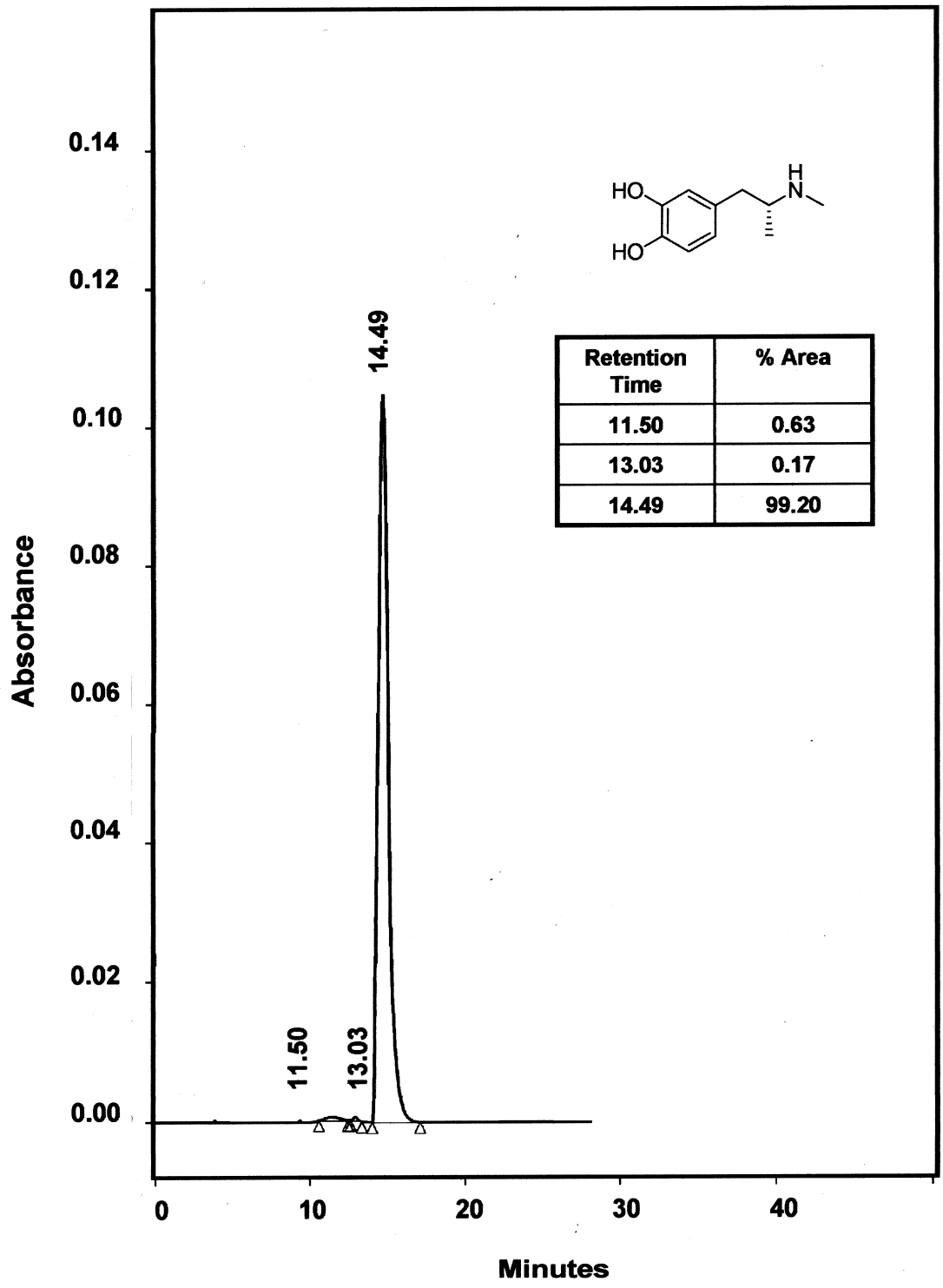
Retention Time	% Area
10.11	0.09
10.97	0.26
15.76	99.65

Conditions of HPLC analysis :
Column Kromasil C18, 250 x 4.6 mm, - 5 μm;
Mobile phase: (H_2O + 1‰ TFA)/ MeCN 88/12;
Flow rate: 0.6 mL min^{-1};
UV detector wavelength: 278 nm.



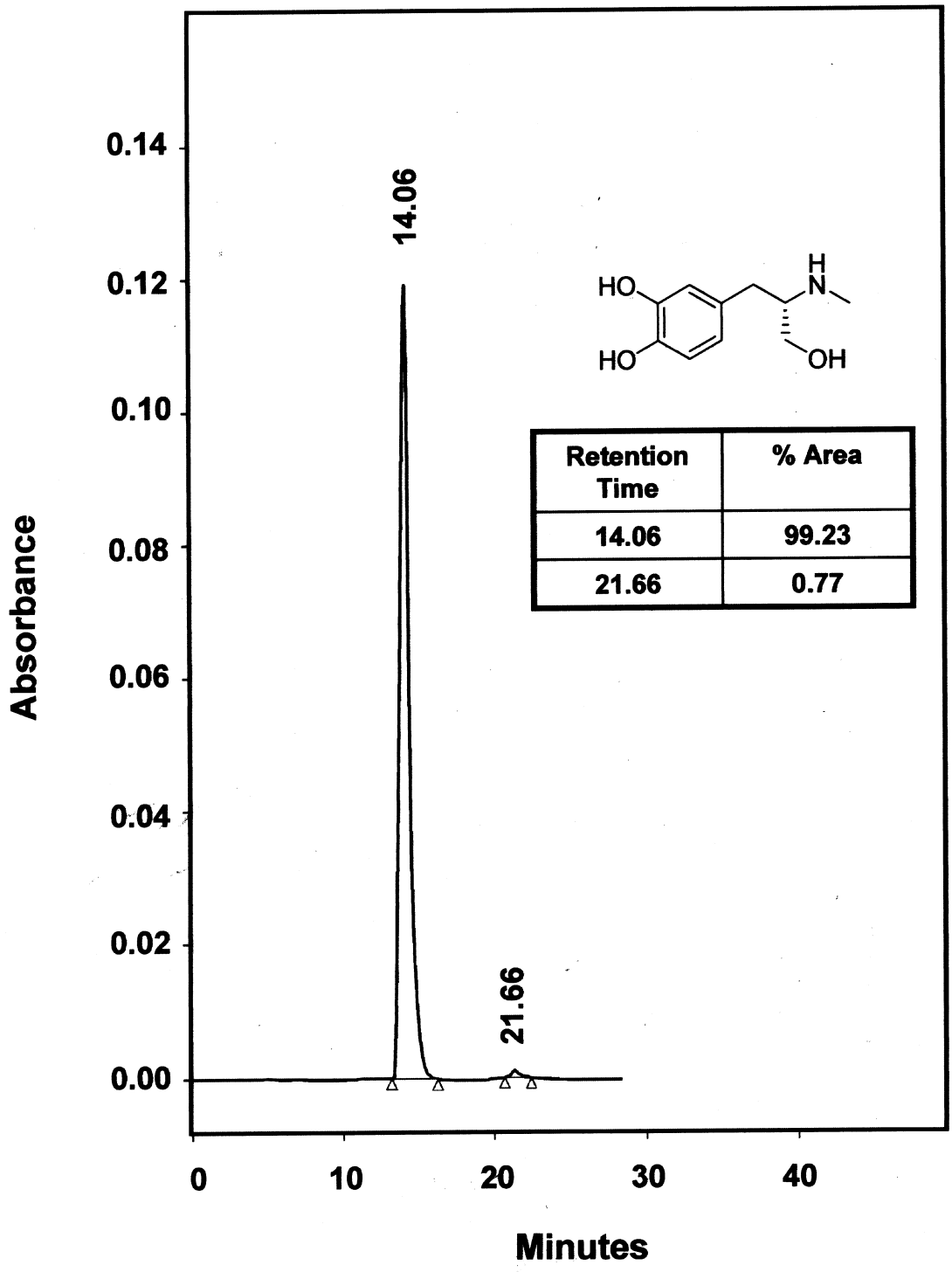
Retention Time	% Area
6.38	0.15
9.35	99.09
20.17	0.44
25.71	0.11
28.06	0.22

Conditions of HPLC analysis :
Column Kromasil C18, 250 x 4.6 mm, - 5 μm;
Mobile phase: (H_2O + 1‰ TFA)/ MeCN 94/06.
Flow rate: 0.7 mL min^{-1};
UV detector wavelength: 278 nm.



Retention Time	% Area
11.50	0.63
13.03	0.17
14.49	99.20

Conditions of HPLC analysis :
Column Kromasil C18, 250 x 4.6 mm, - 5 μm;
Mobile phase: (H_2O + 1‰ TFA)/ MeCN 94/6;
Flow rate: 0.6 mL min^{-1};
UV detector wavelength: 278 nm.



Retention Time	% Area
14.06	99.23
21.66	0.77

Conditions of HPLC analysis :
Column Kromasil C18, 250 x 4.6 mm, - 5 μm;
Mobile phase: (H_2O + 1‰ TFA)/ MeCN 94/6;
Flow rate: 0.6 mL min^{-1};
UV detector wavelength: 278 nm.