Basavarajappa HD, Lee B, Fei X, Lim D, Callaghan B, Mund JA, Case J, Rajashekhar G, Seo S-Y, Corson TW. Synthesis and mechanistic studies of a novel homoisoflavanone inhibitor of endothelial cell growth.

SUPPLEMENTAL METHODS S1

Experimental Section

General

All starting materials and reagents were obtained commercially and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane and acetonitrile were freshly distilled from calcium hydride. All solvents used for routine product isolation and chromatography were of reagent grade and glass distilled. Reaction flasks were dried at 100°C before use, and air- and moisture-sensitive reactions were performed under argon. Flash column chromatography was performed using silica gel 60 (230−400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Mass spectra were obtained using a Waters Auto Purification instrument, and high resolution mass spectra were obtained using a JEOL JMS-AX 505WA unit. ^{1}H and ^{13}C spectra were recorded on either a Bruker AVANCE III 400MHz, or a Bruker AVANCE III 600MHz spectrometer as solutions in deuteriochloroform ($CDCl_3$) and methanol-d4. ^{1}H NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (*J*) in hertz (Hz).

*1-(6-hydroxy-2,3,4-trimethoxyphenyl)ethanone (**4**)*

To an acetic anhydride (2 mL) solution of 3,4,5-trimethoxyphenol (**3**) (1.2 g, 6.6 mmol), BF_3-Et_2O (0.07 mL) was added at 0°C. After stirring at 60°C for 3 h, the reaction mixture was diluted with ethyl acetate and the reaction mixture was cooled to ca. 0°C for 2 h and the crystallized cake filtered with ethyl acetate. H_2O (10 mL) and Et_3N (1 mL) were added. After stirring for 1 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (Ethyl acetate / *n*-hexanes = 1 : 1) to afford the methyl ketone (**4**) (1.4 g, 95%). The compound **4** was reported. See Supplemental Ref [1].

*(E)-3-(3'-benzyloxy-4'-methoxyphenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (**6**)*

To a solution of methyl ketone (**4**) (1.5 g, 6.5 mmol) in MeOH (10 mL) was added 3-benzyloxy-4-methoxybenzaldehyde (**5**) (2.0 g, 8.0 mmol) and KOH (1.5 g, 25 mmol) at 0°C, then warmed to rt. To the reaction mixture was stirred at 35°C for 72 h followed by the addition of water and dilution with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (Ethyl acetate / *n*-hexane = 1 : 3) to afford 3'-benzyloxy-4'-methoxychalcone (**6**) (1.6 g, 56%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 13.7 (s, 1H), 7.74 (s, 2H), 7.47 (d, 2H, *J* = 7.2 Hz); 7.40 (t, 2H, *J* = 7.2 Hz); 7.33 (d, 1H, *J* = 7.2 Hz); 7.24 (dd, 1H, *J* = 8.4 and 2.4 Hz); 7.17 (d, 1H, *J* = 1.2 Hz); 6.92 (d, 1H, *J* = 8.4 Hz); 6.28 (s, 1H), 5.22 (s, 2H), 3.94 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H); ^{13}C-NMR

(150 MHz, $CDCl_3$) δ 192.7, 162.6, 159.9, 154.9, 151.9, 148.3, 143.5, 136.7, 135.2, 128.7, 128.2, 128.0, 127.2, 124.2, 123.5, 113.0, 111.5, 108.7, 96.6, 71.12, 61.9, 61.32, 56.12, 29.7; LRMS (ESI) *m/z* 315 (M+H).

*1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(3'-hydroxy-4'-methoxyphenyl)propan-1-one (**7**)*

3'-(benzyloxy)-4'-methoxychalcone (**6**) (850 mg, 1.9 mmol) in isopropanol (10 mL) was added HCO_2Na (513 mg, 7.5 mmol), Pd/C (195 mg, 1.8 mmol) and HCO_2H (1 mL) at 0°C. The reaction mixture was stirred at 60°C for 6 h. The mixture was filtered through a short pad of silica gel. After the filtrate was concentrated in vacuo, purification of the residue via flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 3) afforded dihydrochalcone (**7**) (517 mg, 79%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 13.38 (s, 1H), 6.82 (d, 1H, *J* = 8.28 Hz); 6.73 (s, 2H), 6.21 (s, 1H), 5.53 (s, 1H), 3.93 (s, 3H), 3.85 (d, 6H, *J* = 1.96 Hz); 3.74 (s, 3H), 3.31 (m, 2H), 2.94 (d, 2H, *J* = 7.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.8, 161.7, 159.8, 155.0, 146.3, 143.7, 134.6, 133.3, 120.7, 114.2, 111.1, 108.1, 96.1, 61.0, 60.9, 55.9, 55.7, 45.1, 30.2; LRMS (ESI) *m/z* 317 (M+H).

*3-(3'-hydroxy-4'-methoxybenzyl)-3-(hydroxymethyl)-5,6,7-trimethoxychroman-4-one (**8**) and 1-(6-hydroxy-2,3,4-trimethoxyphenyl)-2-(3'-hydroxy-4'-methoxybenzyl)prop-2-en-1-one (**10**)*

The dihydrochalcone (**7**) (700 mg, 1.9 mmol) was dissolved in 50% aqueous NaOH (0.96 mL), H_2O (3.8 mL) and stirred with formalin (0.16 mL, 5.8 mmol) at 60°C for 3h. After stirring for 3 h, the reaction mixture was diluted with ethyl acetate and washed with NH_4Cl and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 2) to afford a mixture of compound **8** (383 mg, 54%), **9** (71 mg, 10%) and **10** (106 mg, 15%), respectively. For compound **8**, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.83-6.80 (m, 2H), 6.75-6.73 (m, 1H), 6.28 (s, 1H), 5.78 (bs, 1H), 4.04-4.03 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.79 (s, 2H), 3.58-3.50 (m, 2H), 3.21 (bs, 1H), 2.98 (d, 1H, *J* = 13 Hz); 2.85 (d, 1H, *J* = 14 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 196.1, 159.7, 159.4, 154.5, 146.3, 144.5, 137.5, 126.7, 123.3, 114.1, 113.0, 107.8, 95.9, 69.6, 62.2, 61.4, 61.2, 56.0, 55.8, 49.9, 34.8. LRMS (ESI) *m/z* 405 (M+H); For compound **10**, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.7 (s, 1H), 7.29 (s, 1H), 7.18 (s, 1H), 6.79 (d, 1H, *J* = 7.8 Hz); 6.67-6.65 (m, 2H), 6.19 (s, 1H), 5.44 (s, 1H), 5.10 (s, 1H), 4.96 (s, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.71 (s, 3H), 3.66 (s, 3H), 3.56 (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 201.7, 160.6, 160.2, 151.8, 146.4, 144.1, 134.9, 129.7, 128.3, 122.2, 115.3, 114.2, 112.0, 108.3, 95.9, 61.4, 61.0, 56.1, 55.8, 38.6; LRMS (ESI) *m/z* 375 (M+H).

*3-(3'-hydroxy-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**9**)*

The compound **8** (100 mg, 0.25 mmol) was dissolved in ethanol (2 mL), and stirred with K_2CO_3 (54 mg, 0.49 mmol) at 90°C for 3 h. After stirring for 3 h, the reaction mixture was diluted with ethyl acetate and washed with 1 N HCl and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 2) affording 5,6,7-trimethoxychromanone (**9**) (45 mg, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.24 (s, 1H), 6.83 (d, 1H, *J* = 7.8 Hz); 6.71 (d, 2H, *J* = 1.9 Hz); 6.23 (s, 1H), 5.53 (s, 1H), 4.23 (m, 1H), 4.10 (m, 1H), 3.91 (s, 3H), 3.85 (d, 6H, *J* = 1.9 Hz); 3.79 (s, 3H), 3.16 (m, 1H), 2.70 (m, 1H), 2.63 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.3, 159.6, 159.2, 154.4, 146.5,

144.2, 137.4, 130.2, 121.8, 114.3, 111.4, 108.6, 95.9, 69.0, 61.5, 61.2, 56.0, 55.9, 48.5, 32.5; LRMS (ESI) *m/z* 375 (M+H). From the compound **10** (100 mg, 0.27 mmol), the same reaction condition afforded 5,6,7-trimethoxychromanone (**9**) (72 mg, 72%).

*SH-11052 (**2**)*

To a solution of 5,6,7-trimethoxychromanone (37 mg, 0.10 mmol) in $CHCl_3$ (1 mL) was added TMSI (113 μL, 0.80 mmol) at 0°C and the reaction mixture was heated at 60°C for 4 h. The mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 1) to afford SH-11052 (**2**) (17 mg, 49%). ^{1}H-NMR (600 MHz, CD_3OD) δ 6.85 (d, 1H, J = 8.4 Hz), 6.70 (d, 1H, J = 2.4 Hz), 6.67 (dd, 1H, J = 2.4 and 8.4 Hz), 6.13 (s, 1H), 4.25 (dd, 1H, J = 4.2 and 11.4 Hz), 4.09 (dd, 1H, J = 7.8 and 11.4 Hz), 3.87 (s, 3H), 3.82 (s, 3H), 3.08 (dd, 1H, J = 4.8 and 13.8 Hz), 2.84-2.79 (m, 1H), 2.64 (dd, 1H, J = 10.2 and 13.8 Hz); ^{1}H-NMR (600 MHz, $CDCl_3$) δ 11.7 (s, 1H), 6.78 (d, 1H, J = 2.4 Hz), 6.78 (d, 1H, J = 9.6 Hz), 6.67 (dd, 1H, J = 2.4 and 8.4 Hz), 6.02 (s, 1H), 5.60 (s, 1H), 5.02 (s, 1H), 4.25 (dd, 1H, J = 4.2 and 11.4 Hz), 4.10 (dd, 1H, J = 7.8 and 11.4 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 3.15 (dd, 1H, J = 4.8 and 13.8 Hz), 2.82-2.78 (m, 1H), 2.64 (dd, 1H, J = 10.8 and 14.4 Hz); ^{13}C-NMR (150 MHz, CD_3OD) δ 200.7, 157.6, 157.6, 150.4, 148.0, 147.8, 132.4, 128.7, 121.4, 117.1, 113.0, 103.5, 92.25, 70.63, 56.82, 56.58, 33.26; ^{13}C-NMR (150 MHz, $CDCl_3$) δ 200.7, 158.0, 156.6, 150.1, 147.7, 147.4, 133.0, 129.2, 122.6, 117.1, 112.8, 104.4, 93.0, 71.4, 58.3, 58.0, 48.8, 34.1; LRMS (EI) *m/z* 346 (M^+); HRMS (EI) *m/z* 346.1057 (M^+) [calc. $C_{18}H_{18}O_7$ 346.1053].

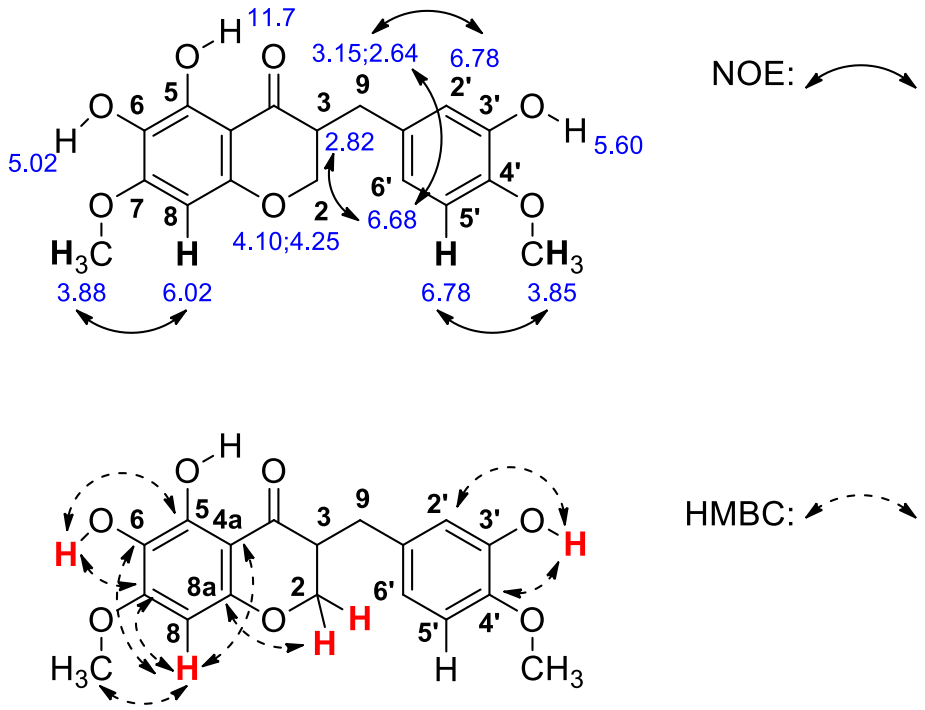

Figure S1. Structural assignment of SH-11052 (**2**) by NOESY and HMBC. (Blue color: chemical shift of ^{1}H-NMR)

Supplemental Reference

1. Chen DZ, Yang J, Yang B, Wu YS, Wu T (2010) Total synthesis of baicalein. J Asian Nat Prod Res 12: 124-128.

#6

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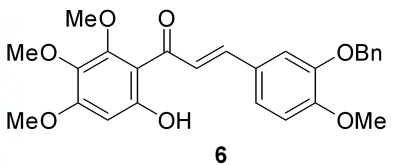

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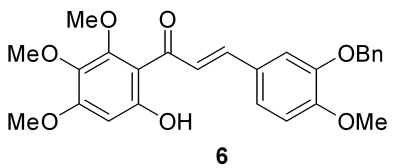

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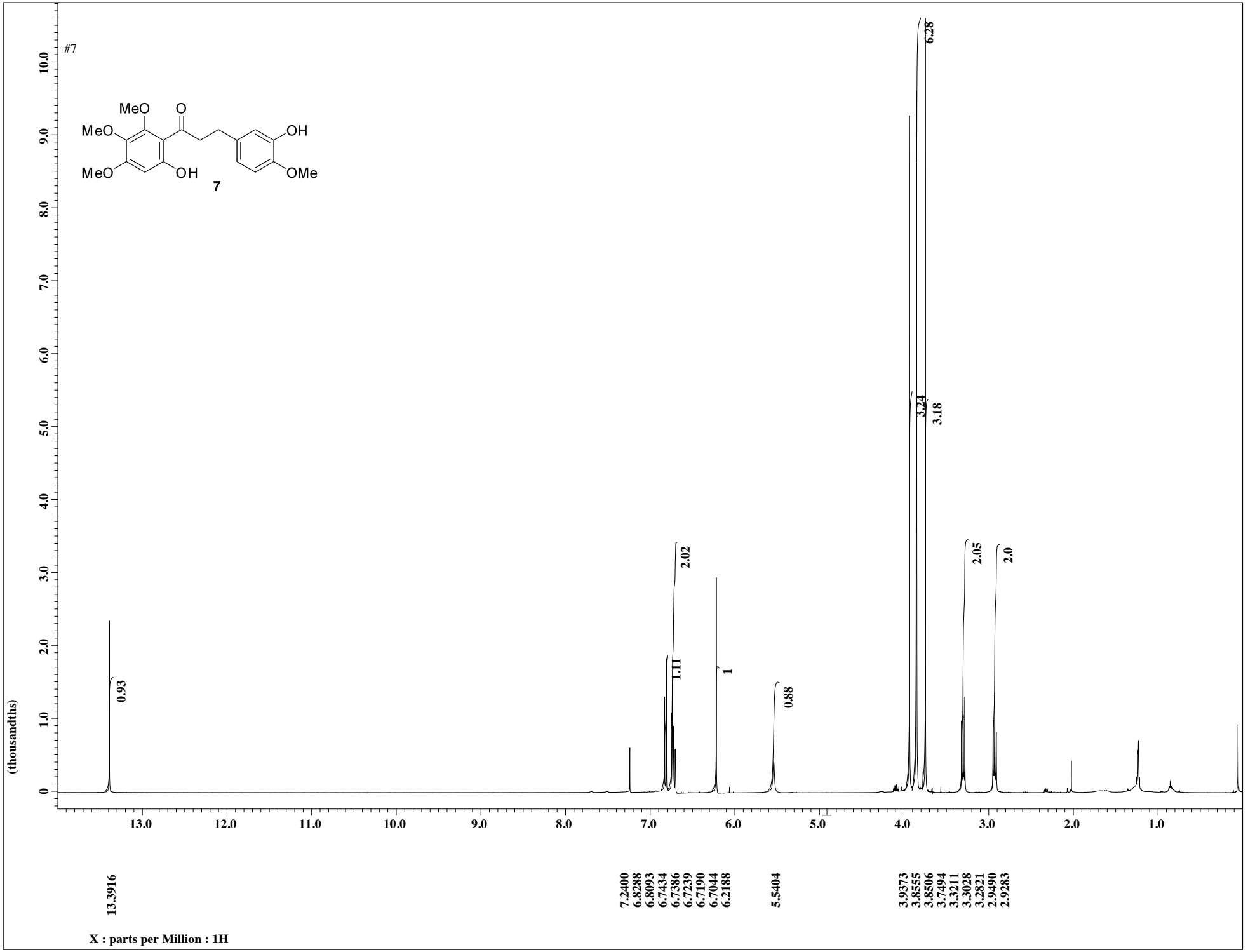
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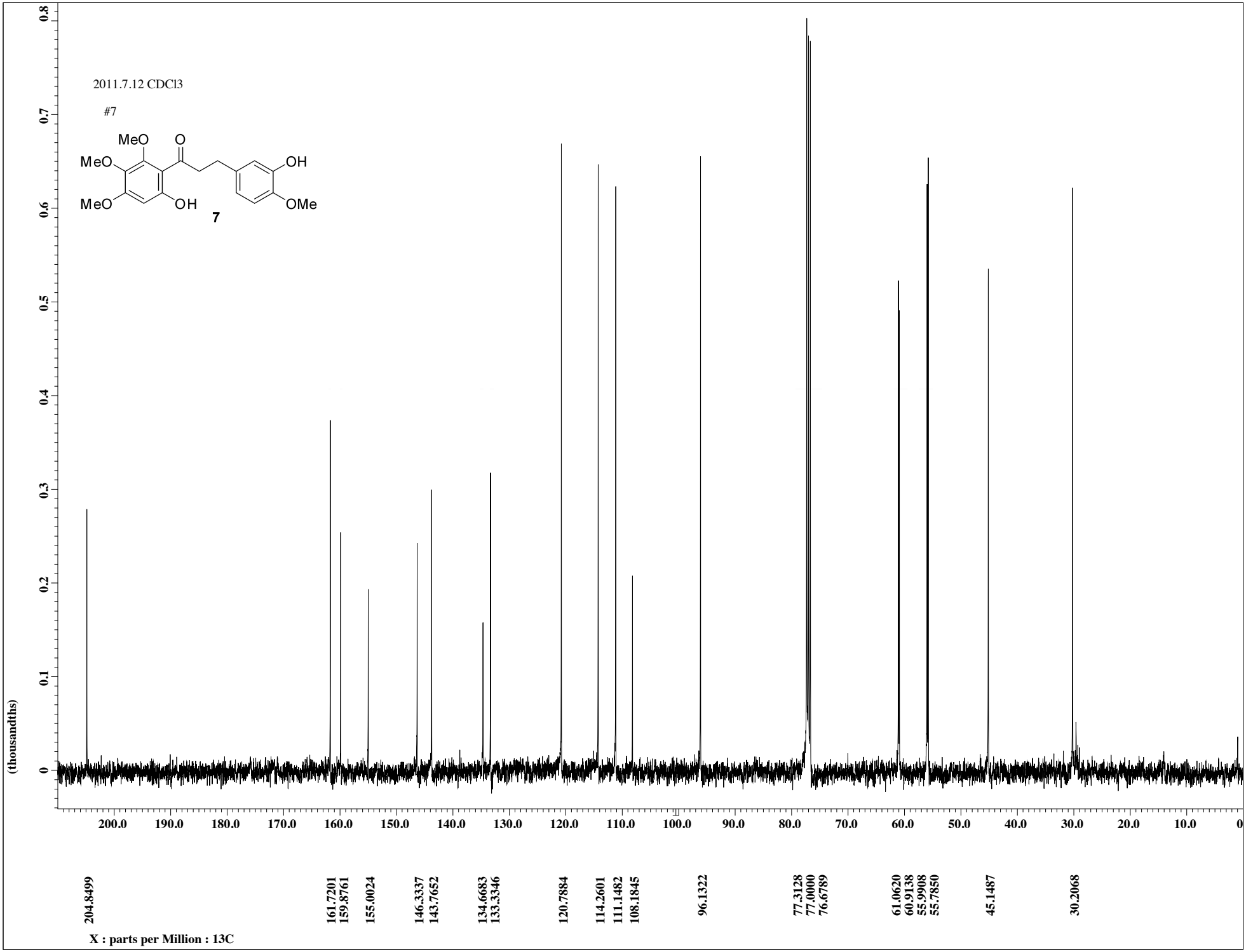
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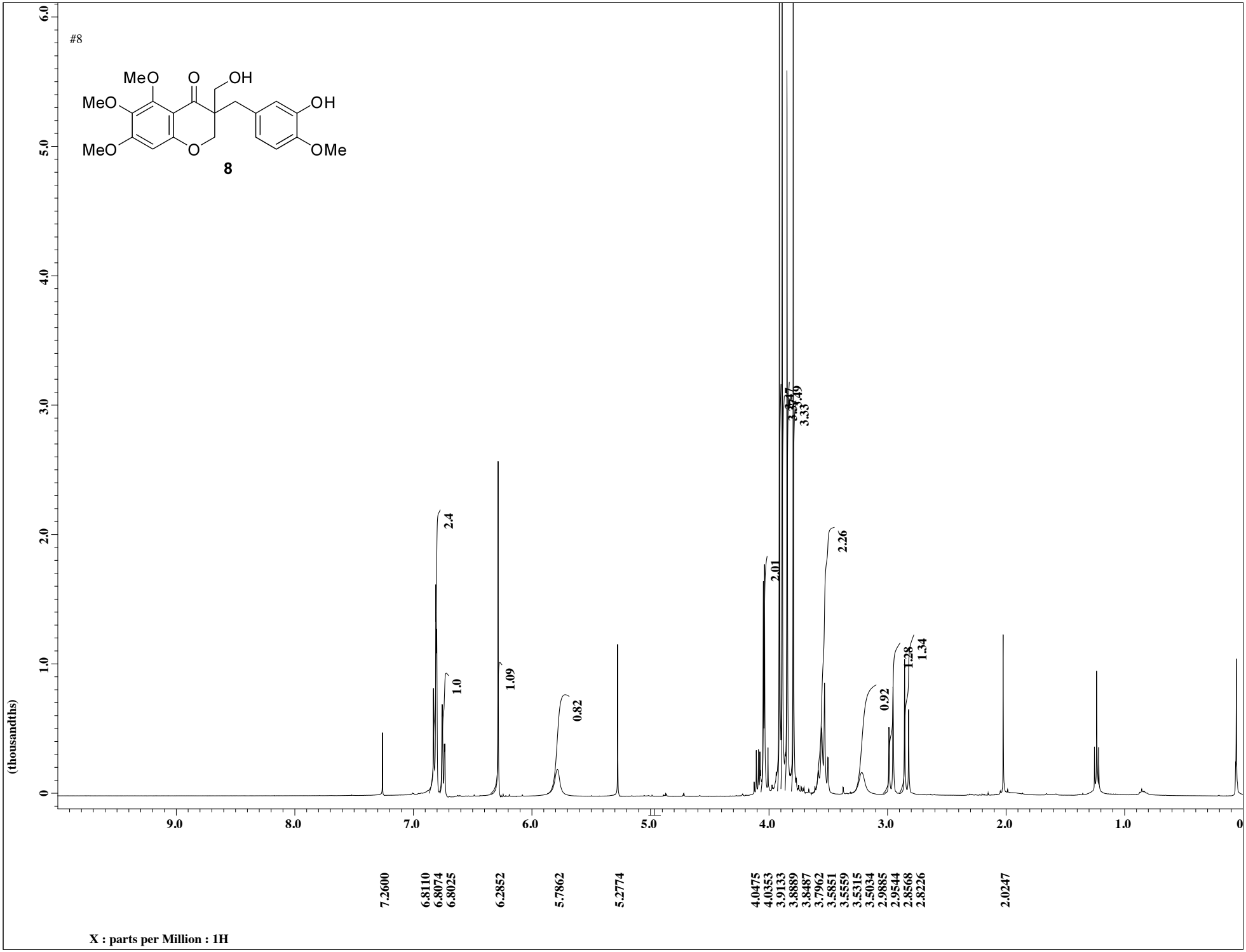
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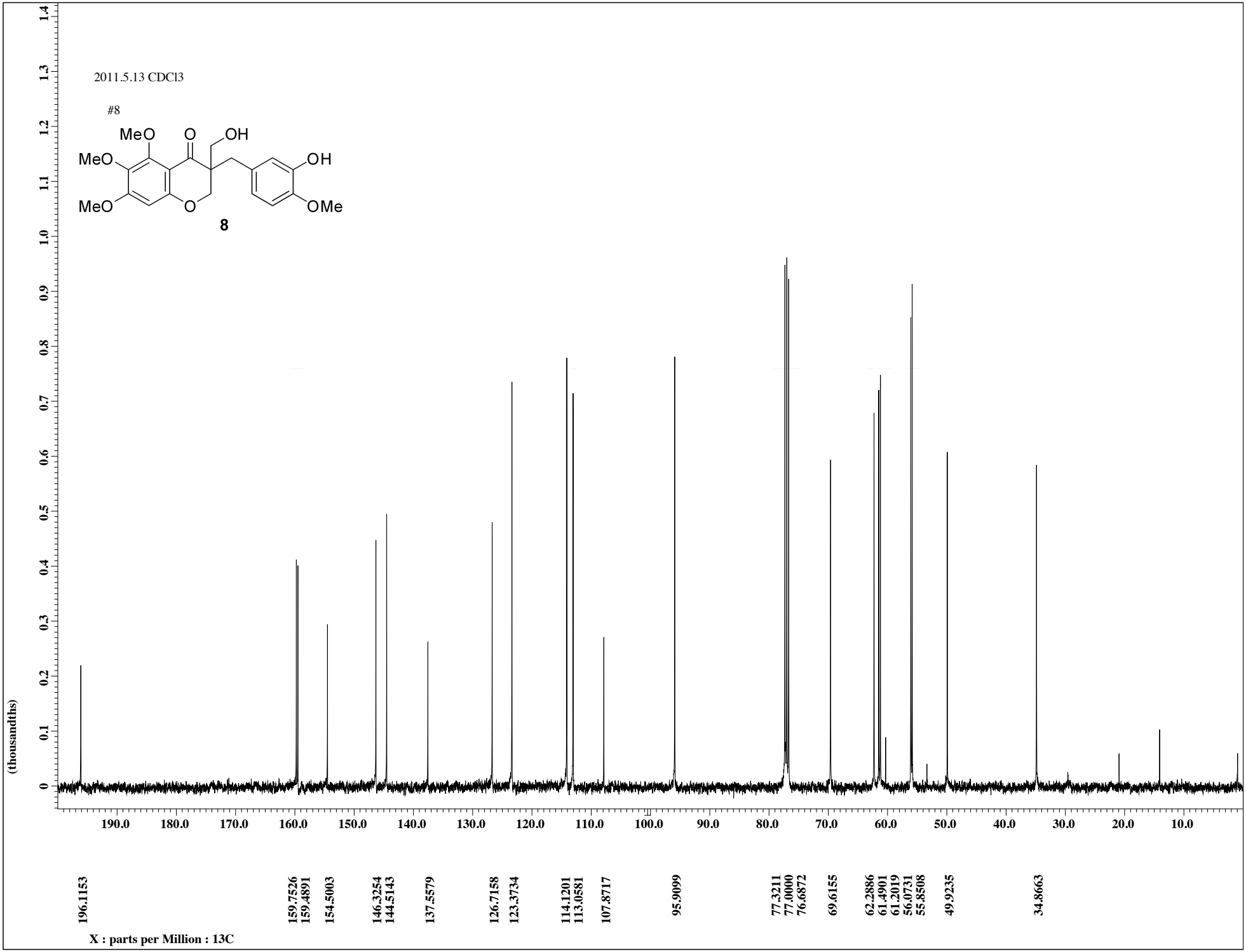
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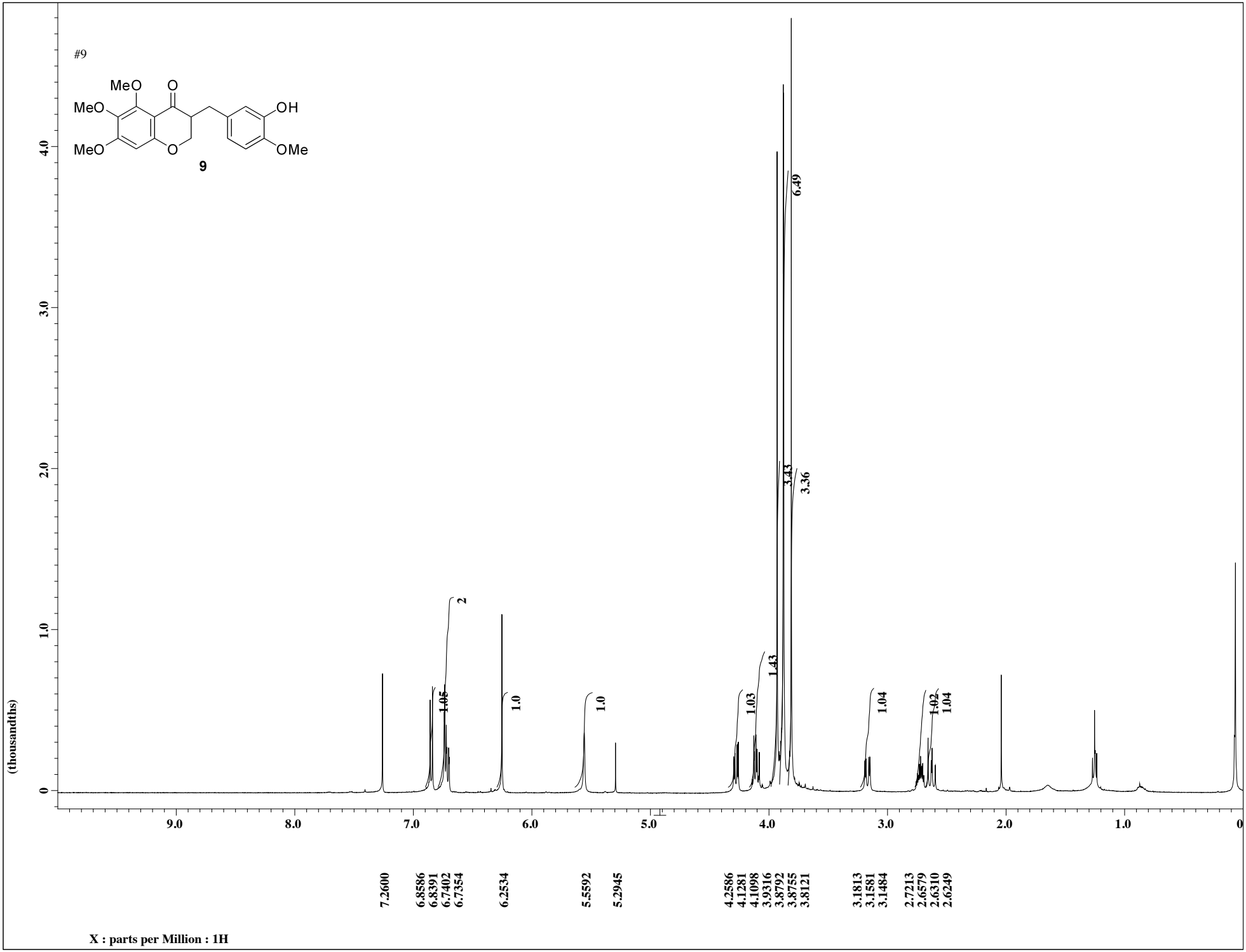
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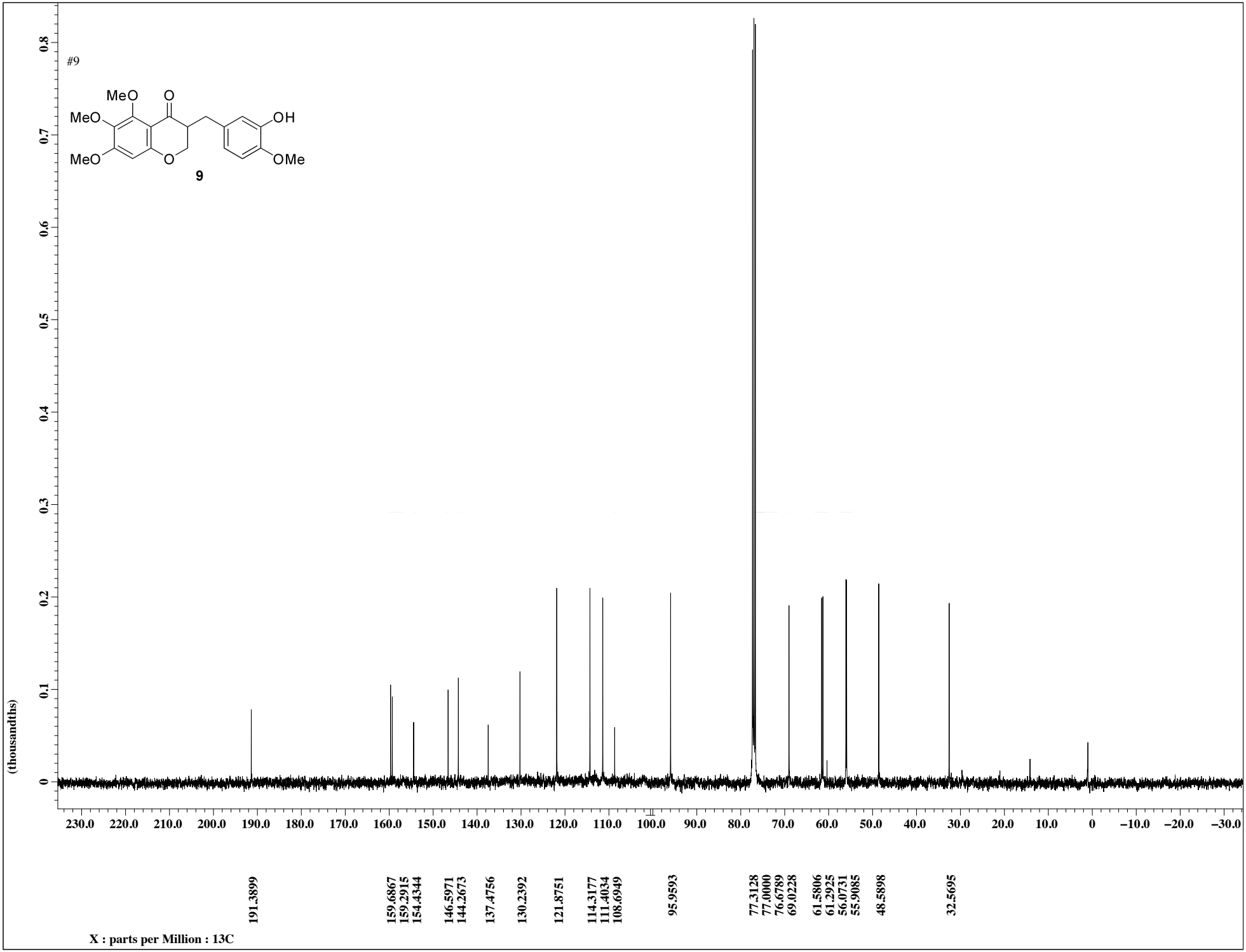
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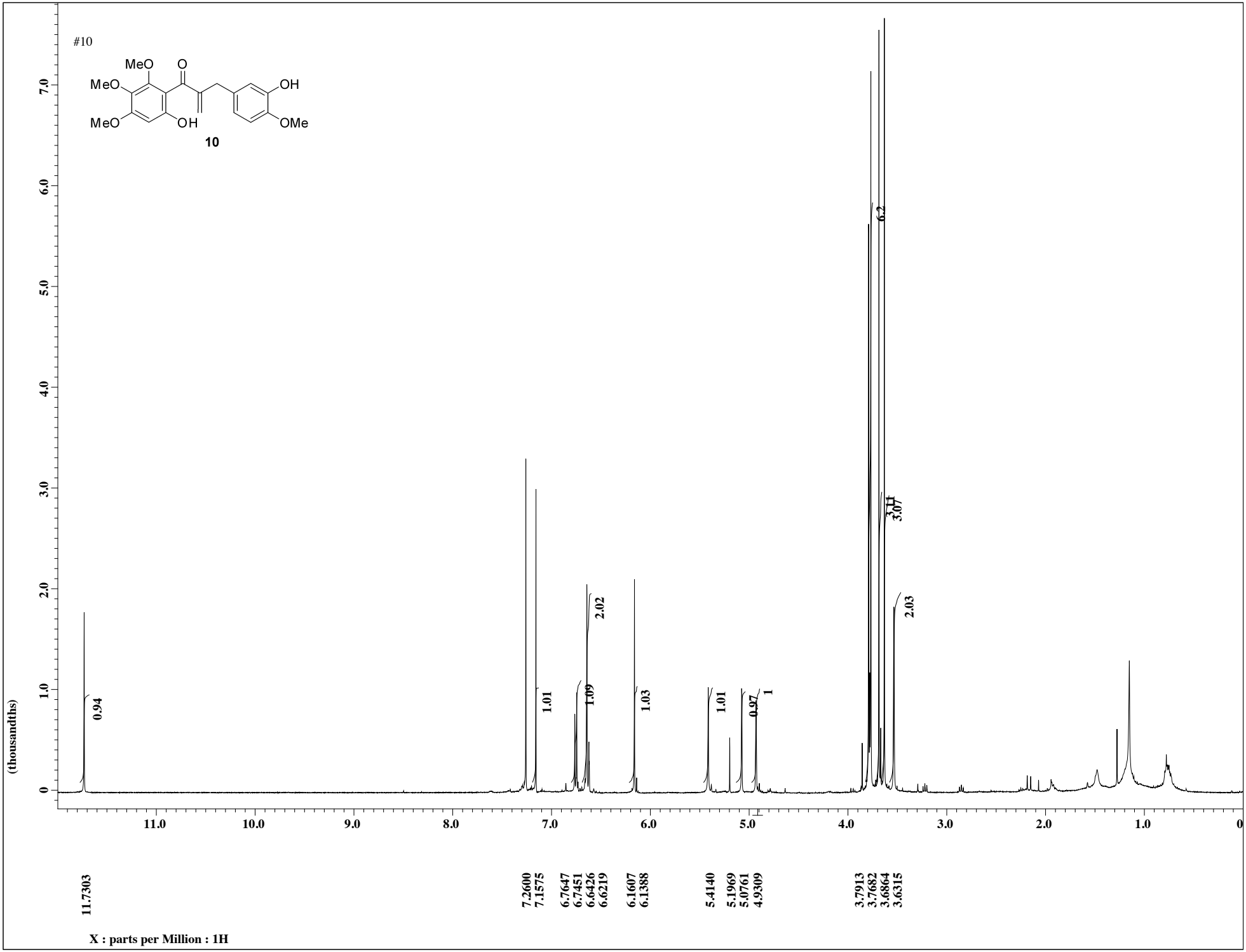
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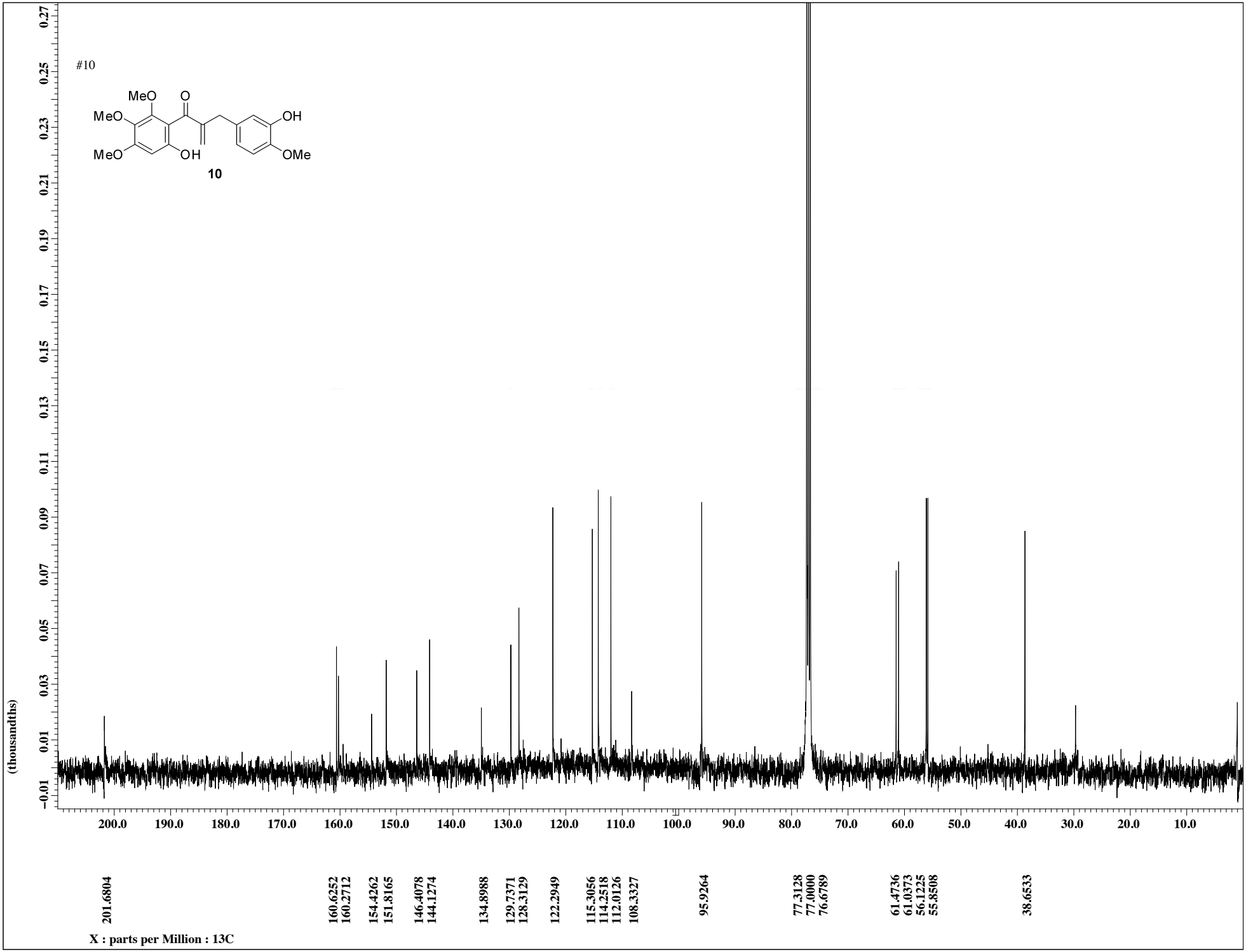
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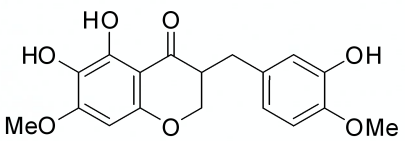
SH-11052



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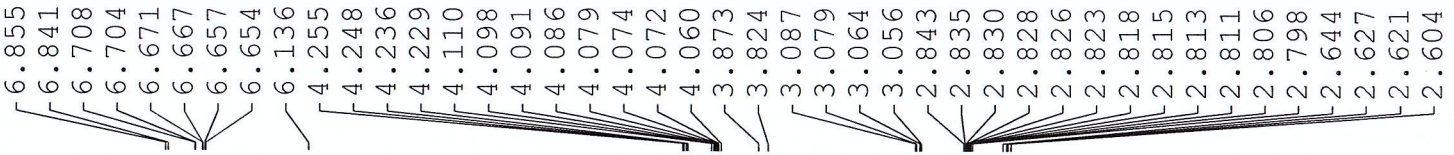
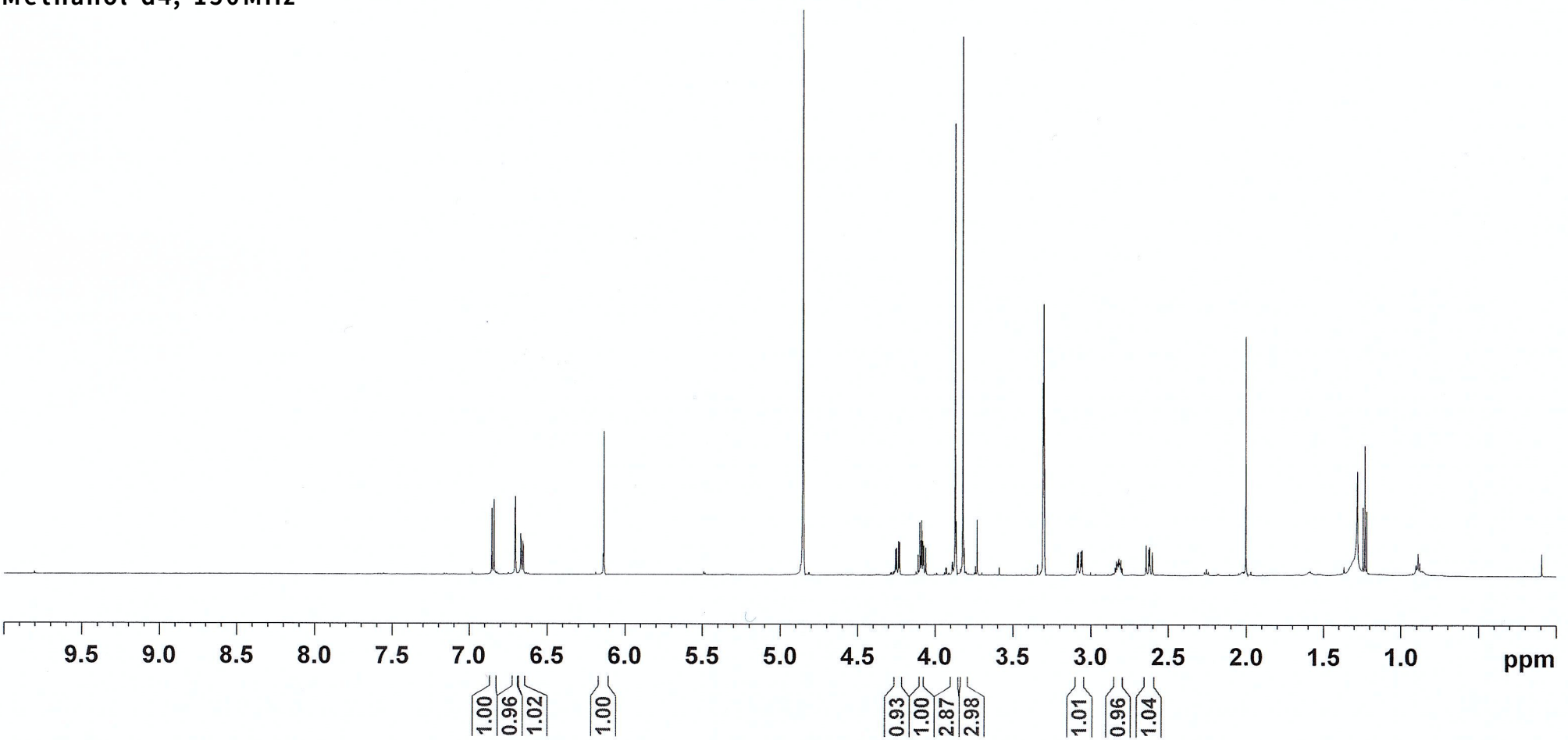
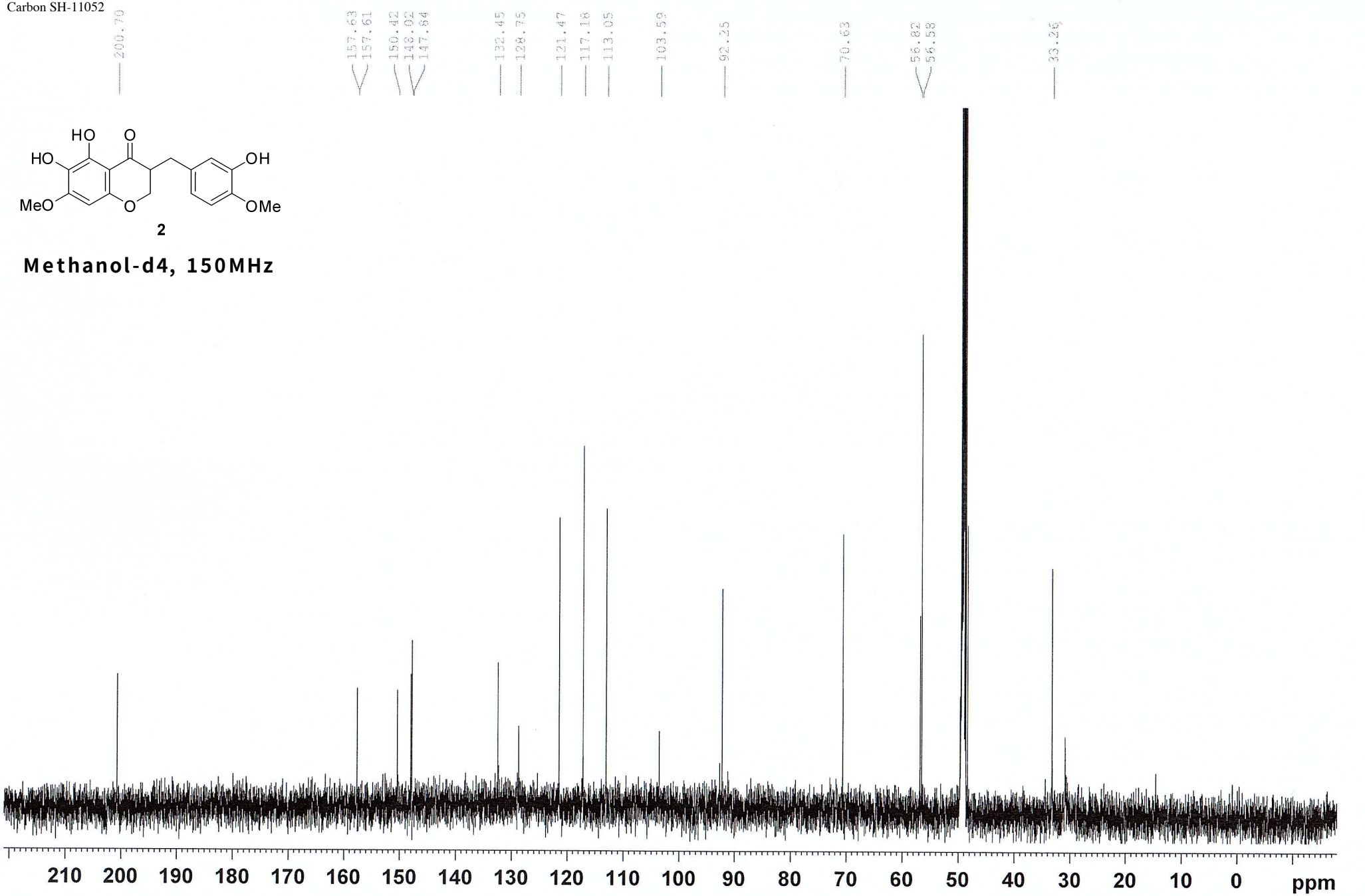


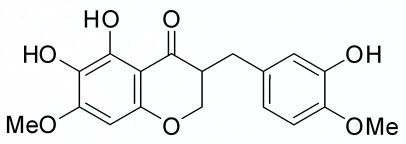
Methanol-d4, 150MHz



Carbon SH-11052



1H 1D BL-360

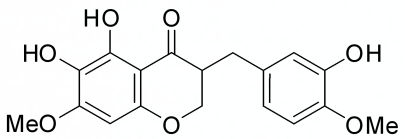


2

Chloroform-d, 600MHz

Carbon 1DBL-360

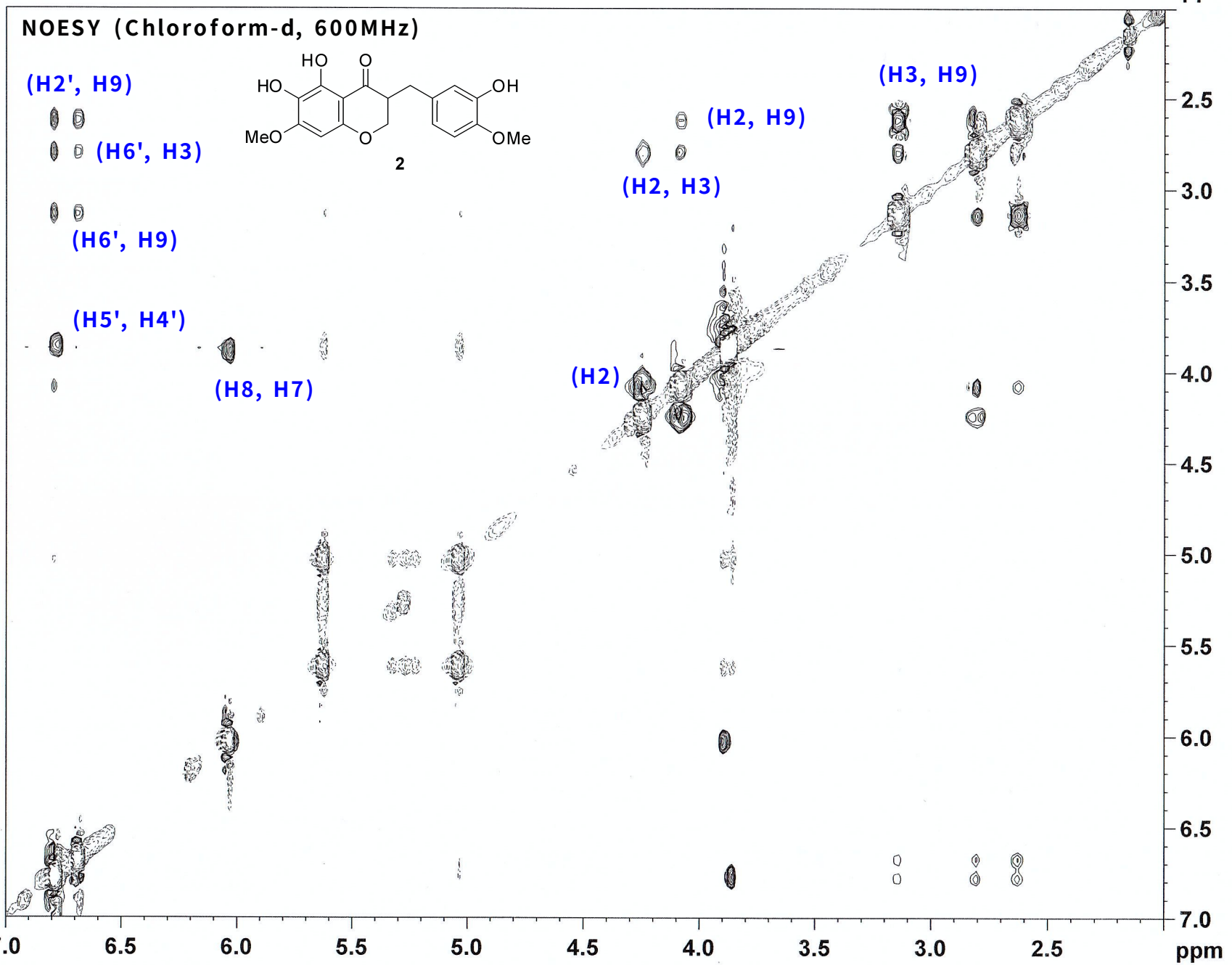
200.70
158.01
156.65
150.12
147.74
147.46
133.03
129.25
122.62
117.19
112.83
104.44
93.04
71.39
58.32
58.03
48.88
34.15



2

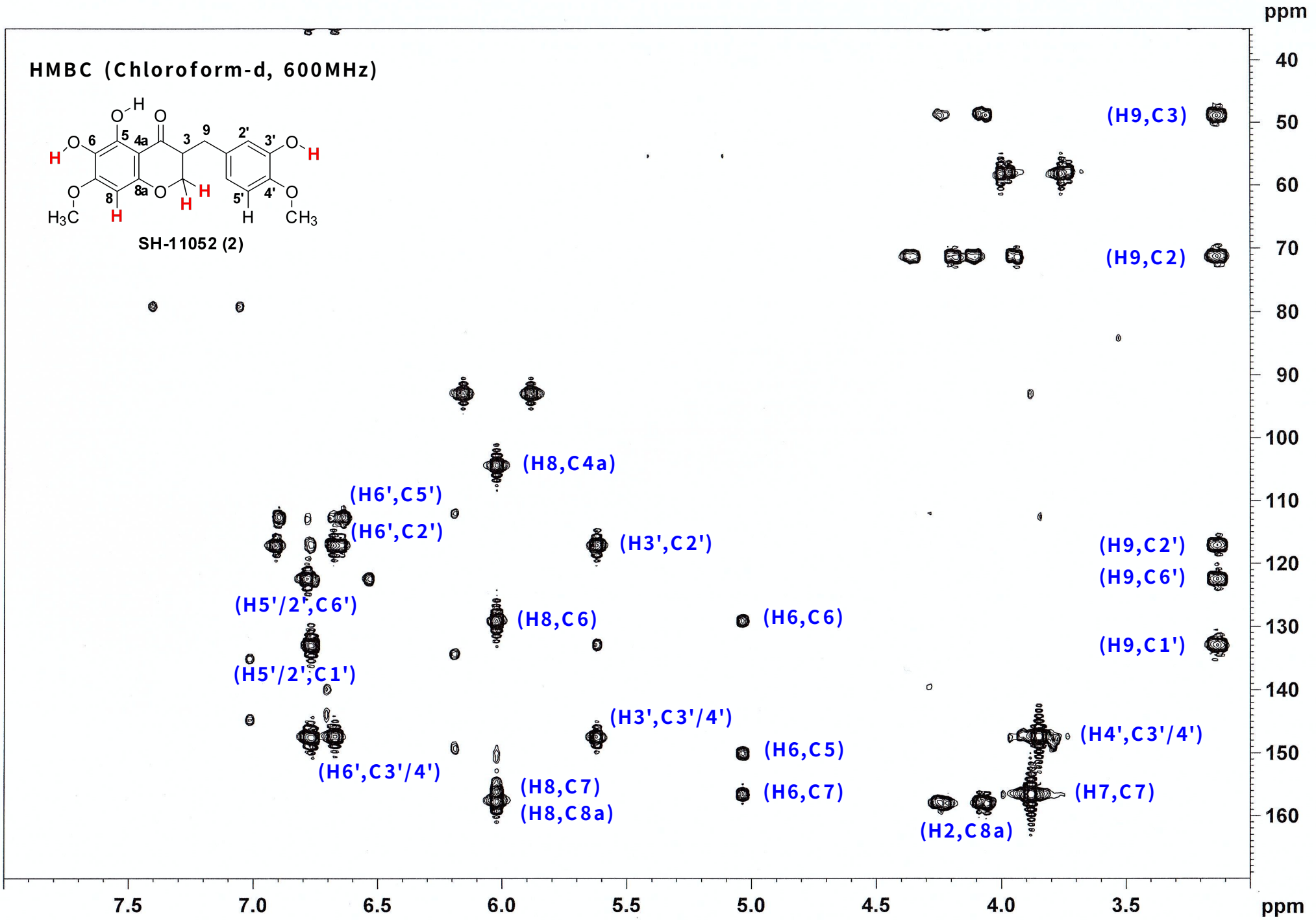
Chloroform-d, 150MHz

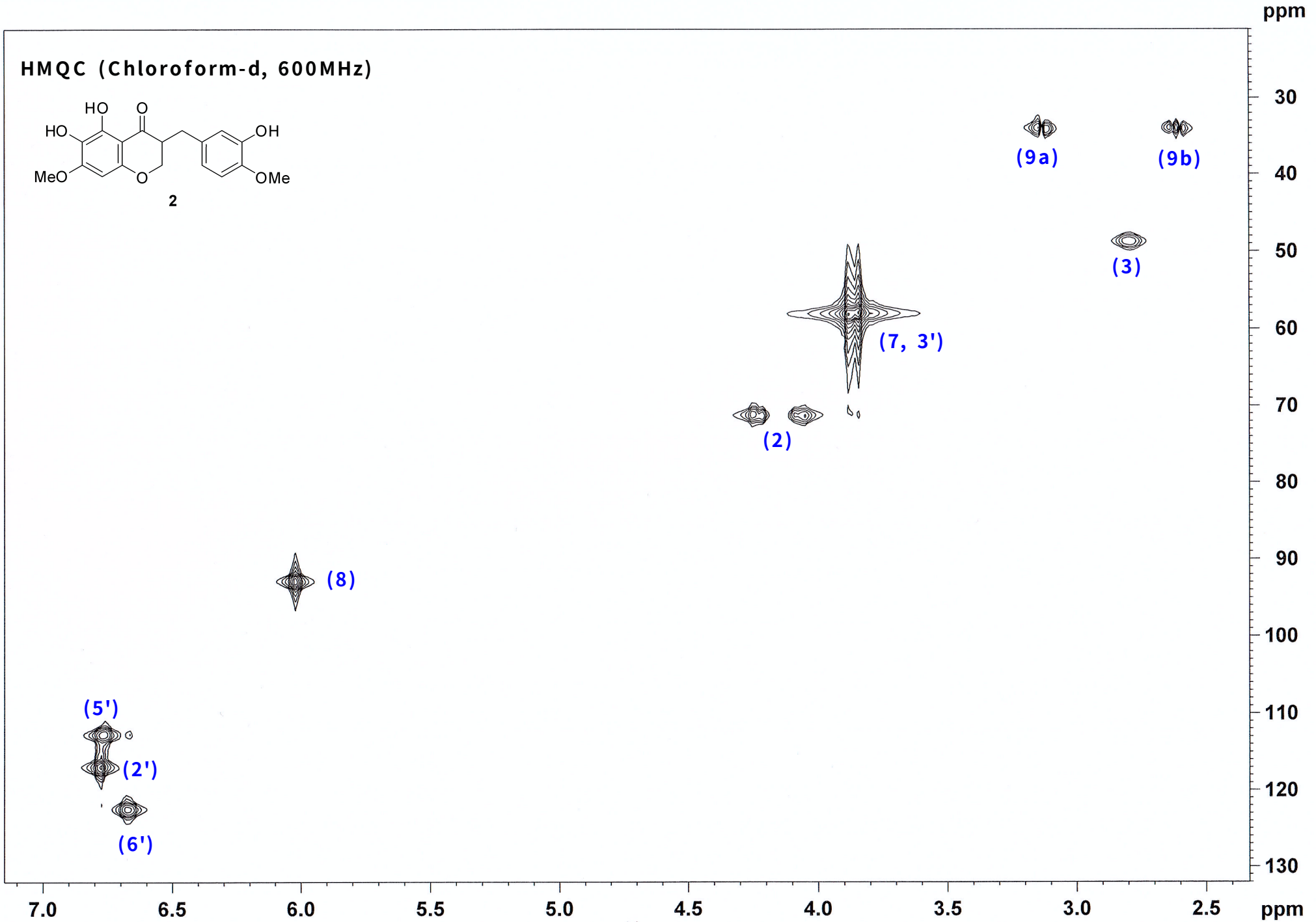
H noesy



BRUKER

```
Current Data Parameters
NAME                   BL
EXPNO                 604
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140102
Time                14.17
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG     noesygpphpp
TD                  2048
SOLVENT             CDCl3
NS                    48
DS                    32
SWH           12019.230 Hz
FIDRES         5.868765 Hz
AQ             0.0851968 sec
RG                 35.72
DW                41.600 usec
DE                10.00 usec
TE                298.0 K
D0            0.00003154 sec
D1            2.00000000 sec
D8            0.50000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D16           0.00020000 sec
IN0           0.00008320 sec

======== CHANNEL f1 ========
SFO1         600.2436014 MHz
NUC1                1H
P1                 7.90 usec
P2                15.80 usec
P17             2500.00 usec
PLW1          7.00000000 W
PLW10         0.64626002 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPZ1              40.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1          600.2436 MHz
FIDRES       23.475060 Hz
SW             20.024 ppm
FnMODE      States-TPPI

F2 - Processing parameters
SI                 1024
SF          600.2400233 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2          States-TPPI
SF          600.2400284 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```


HMBC (Chloroform-d, 600MHz)
SH-11052 (2)
(H9,C3)
(H9,C2)
(H8,C4a)
(H6',C5')
(H6',C2')
(H3',C2')
(H9,C2')
(H9,C6')
(H5'/2',C6')
(H8,C6)
(H6,C6)
(H9,C1')
(H5'/2',C1')
(H3',C3'/4')
(H4',C3'/4')
(H6,C5')
(H6',C3'/4')
(H8,C7)
(H6,C7)
(H7,C7)
(H8,C8a)
(H2,C8a)
ppm
40
50
60
70
80
90
100
110
120
130
140
150
160
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm


HMQC (Chloroform-d, 600MHz)
HO
O
HO
OH
MeO
O
OMe
2
(9a)
(9b)
(3)
(7, 3')
(2)
(8)
(5')
(2')
(6')
ppm
30
40
50
60
70
80
90
100
110
120
130
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm