SUPPLEMENTARY INFORMATION

for

Novel *O*-methyl goniofufurone and 7-*epi*-goniofufurone derivatives: Synthesis, in vitro cytotoxicity and SAR analysis

Jovana Francuz [a], Mirjana Popsavin [a], Sanja Djokić [a], Vesna Kojić [b], Tatjana Srdić-Rajić [c], Marko V. Rodić [a], Dimitar Jakimov [b] and Velimir Popsavin [a,d,*]

[a] *Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*

[b] *Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia*

[c] *Institute for Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia*

[d] *Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia*

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* Corresponding author. E-mail address: velimir.popsavin@dh.uns.ac.rs (V. Popsavin).

I. X-RAY CRYSTAL STRUCTURE DETERMINATION

Diffraction experiments were performed on a Gemini S diffractometer (Oxford Diffraction) equipped with a Sapphire 3 CCD detector. A sealed tube with the Mo-target anode and graphite monochromator was used as the source of X-radiation (Mo $K\alpha$, $\lambda = 0.71073$ Å). Data were collected with the ω-scan technique at room temperature. The reflection intensities were integrated and corrected for background, Lorentz-polarization effect and absorption with the *CrysAlisPRO* [1]. Structures were solved by *SIR92* [2] or *SHELXT* [3]. All structures were refined with the *SHELXL* [4] by full-matrix least-squares procedure based on F^2. The *SHELXLE* [5] was used as a graphical user interface. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms connected to carbon atoms were introduced in idealized positions and refined using riding model. Hydrogen atoms bonded to oxygen atoms were found in difference Fourier maps and refined isotropically, while is some cases distance restraints d(O–H) = 0.82(2) Å and ADP constraints U_{iso}(H) = 1.2U_{eq}(O) were applied (see CIF data for details). Absolute structures of all compounds were determined by reference to the stereocenters of know chirality (unchanged during synthetic procedure). The Flack parameters were meaningless, due to weak anomalous scattering power of the compounds. Pertinent crystallographic and refinement data are listed in Table S1.

Crystallographic data reported in this paper have been deposited at the Cambridge Crystallographic Data Centre. CCDC numbers: 1843444 (**5**), 1843445 (**23**), 1843446 (**3**), 1843447 (**21**), 1843448 (**9**) and 1843449 (**8**). Copies of the data can be obtained, free of charge, on the following Internet address: https://www.ccdc.cam.ac.uk/structures/

[1] CrysAlisPRO Software system, Rigaku Oxford Diffraction, Oxford, UK.
[2] G. M. Sheldrick *Acta Crystallogr. Sect. A 71* (2015) 3-8.
[3] A. Altomare, G. Cascarano, C. Giacovazzo, A. Gualardi *J. Appl. Cryst. 26* (1993) 343-350.
[4] G. M. Sheldrick, *Acta Crystallogr. Sect. C 71* (2015) 3-8.
[5] C. B. Hübschle, G. M. Sheldrick, B. Dittrich, *J. Appl. Cryst. 44* (2011) 1281-1284.

Table S1. Crystal data and structure refinement details for **3**, **5**, **8**, **9**, **21** and **23**.

	3	**5**	**8**	**9**	**21**	**23**
Chemical formula	$C_{14}H_{16}O_5$	$C_{15}H_{18}O_5$	$C_{15}H_{18}O_5$	C14 H14 O5	$C_{15}H_{20}O_5$	$C_{15}H_{20}O_5$
M_r	264.27	278.29	278.29	262.25	280.31	280.31
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
T / K	294	294	294	294	294	294
a/ Å	7.6832 (2)	8.2861 (10)	8.1993 (2)	4.6740(2)	11.0585 (4)	8.8306 (3)
b / Å	7.5202 (3)	9.8316 (10)	9.5498 (2)	12.9175(7)	11.4256 (3)	10.0761 (3)
c/ Å	11.6492 (4)	18.092 (2)	18.6176 (4)	21.3349(7)	11.8876 (4)	17.1204 (6)
β/ °	91.533 (3)	90	90	90	90	90
V/ Å^3	672.84 (4)	1473.9 (3)	1457.79 (6)	1288.12(10)	1502.00 (8)	1523.34 (9)
Z	2	4	4	4	4	4
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
μ/ mm^{-1}	0.10	0.09	0.10	0.10	0.09	0.09
Crystal size / mm	0.6 × 0.33 × 0.28	0.65 × 0.36 × 0.03	0.70 × 0.52 × 0.37	0.51 × 0.30 × 0.18	0.8 × 0.62 × 0.6	1.0 × 0.3 × 0.28
Absorption correction	Multi-scan	Analytical	Analytical	Analytical	Multi-scan	Multi-scan
T_{min}, T_{max}	0.937, 1	0.938, 0.995	0.952, 0.975	0.966, 0.984	0.933, 1	0.900, 1
Measured reflections	14574	4375	19102	9417	12380	13354
Independent reflections	3236	2428	3548	2988	3542	3626
Observed reflections[I> 2σ(I)]	3035	1631	2797	2246	3338	3218
R_{int}	0.018	0.124	0.089	0.028	0.016	0.017
(sin $\theta/\lambda)_{max}$/ Å^{-1}	0.681	0.581	0.684	0.684	0.684	0.679
R[F^2> 2σ(F^2)]	0.039	0.119	0.097	0.042	0.039	0.036
wR(F^2)	0.098	0.268	0.201	0.011	0.098	0.089
S	1.06	1.09	1.17	1.03	1.09	1.05
Parameters	176	183	184	173	188	189
Restraints	2	11	0	0	0	0
H-atom treatment	Mixed	Constrained	Constrained	Constrained	Mixed	Mixed
$\Delta\rho_{max}$/ e Å^{-3}	0.16	0.36	0.37	0.12	0.23	0.17
$\Delta\rho_{min}$/ e Å^{-3}	−0.18	−0.59	−0.63	−0.14	−0.21	−0.13
Absolute structure parameter	Meaningless	Meaningless	Meaningless	Meaningless	Meaningless	Meaningless

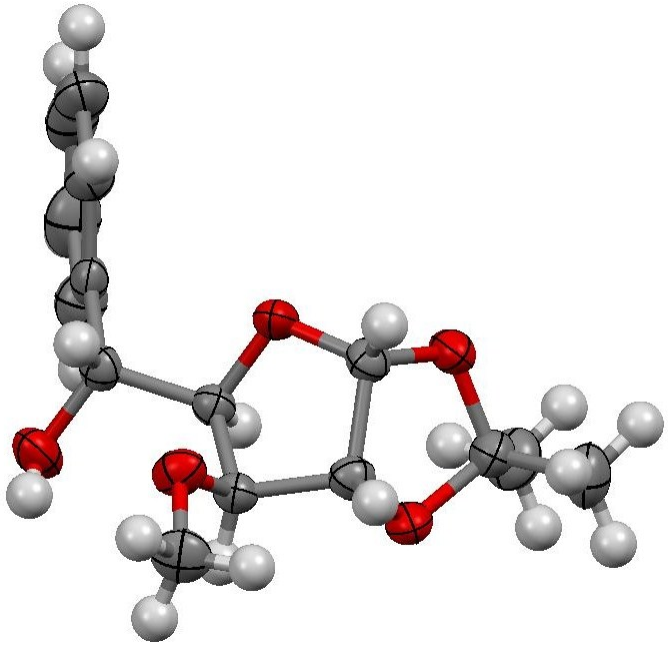


Figure S1. ORTEP presentation of compound **21**.

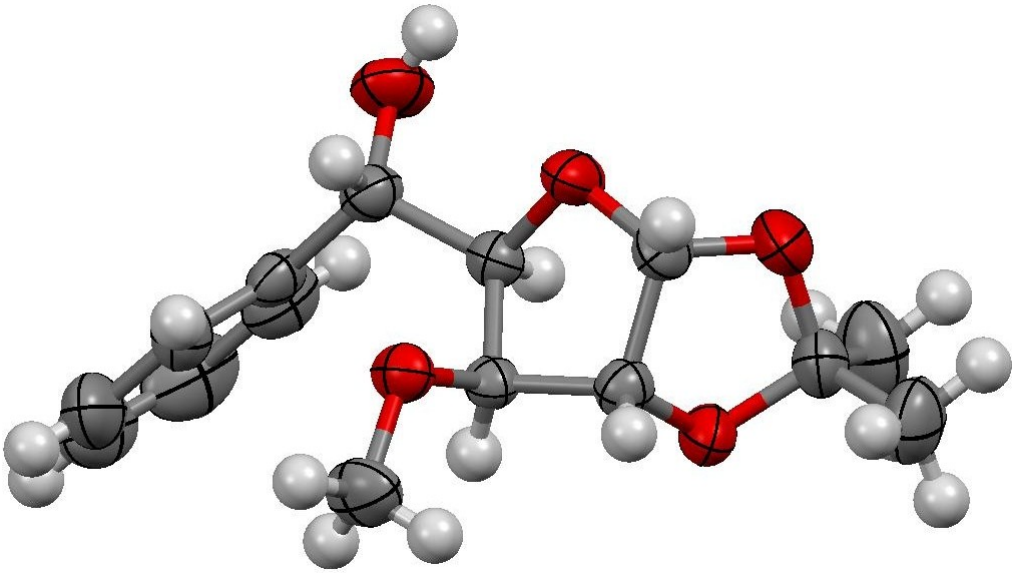


Figure S2. ORTEP presentation of compound **23**.

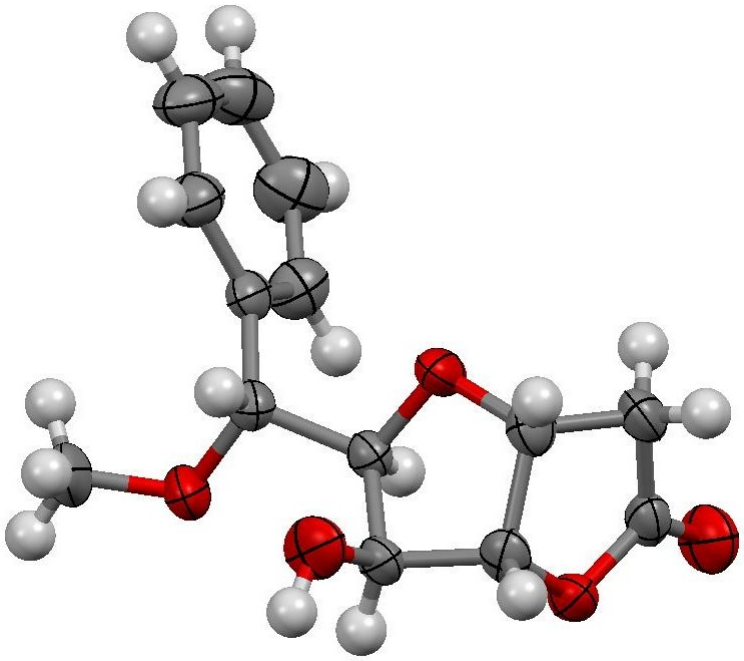


Figure S3. ORTEP presentation of compound **3**.

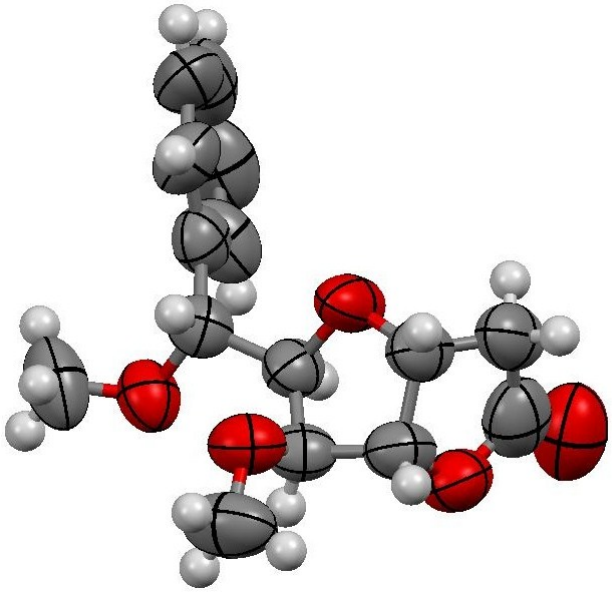


Figure S4. ORTEP presentation of compound **5**.

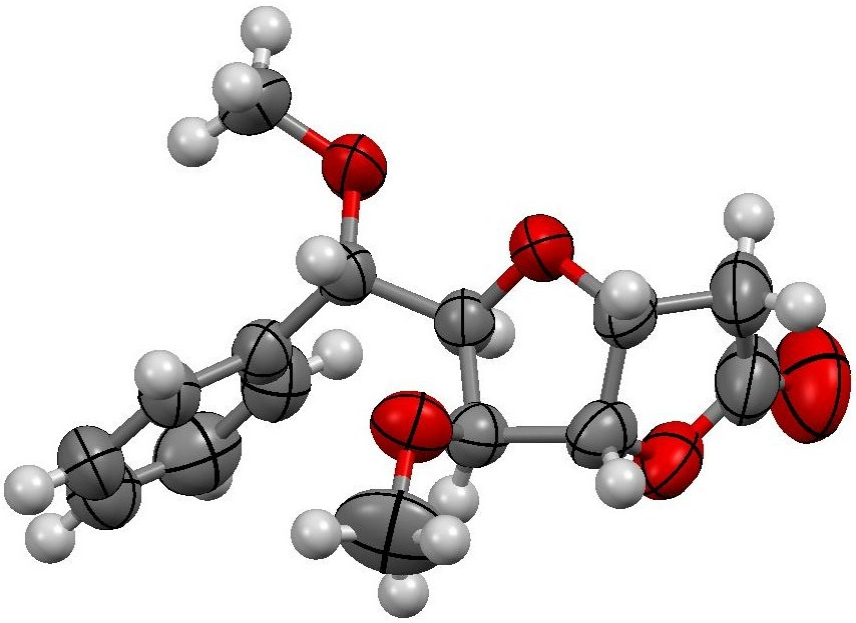


Figure S5. ORTEP presentation of compound **8**.

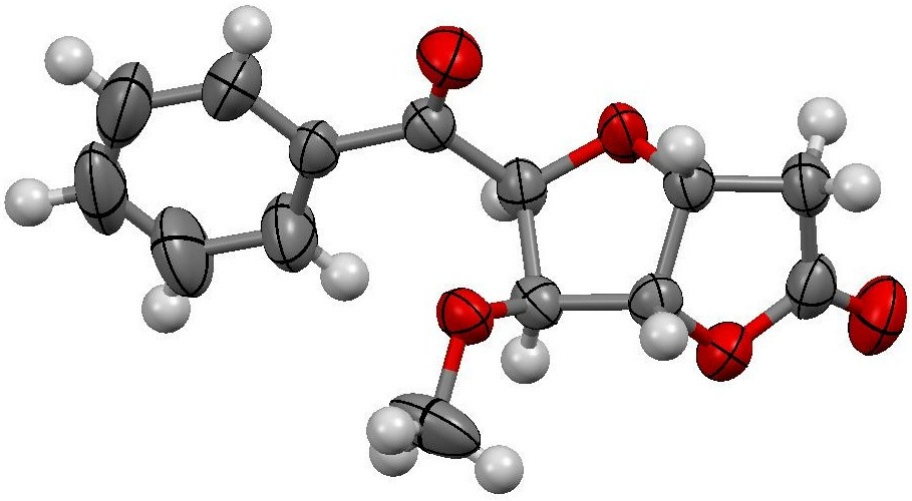


Figure S6. ORTEP presentation of compound **9**.

II. SAR ANALYSIS

Table S2. Cytotoxicity data for SAR analysis.

Compounds	IC_{50} (μM), 72 h							
	K562	HL-60	Jurkat	Raji	MCF-7	MDA-MB 231	HeLa	A549
1	0.41	201.32	32.45	18.45	16.59	75.34	8.32	35.21
3	7.95	2.96	15.64	15.64	935.56	0.064	5.64	2.64
4	5.36	25.01	24.85	8.69	4.04	28.64	8.63	23.55
5	14.65	2.21	2.36	12.36	121.23	1.36	56.37	26.34
2	0.028	22.02	18.64	1.25	9.24	58.70	0.89	21.02
6	11.54	1.63	12.64	21.08	472.73	0.13	8.46	5.31
7	8.45	18.95	15.46	12.22	12.65	35.22	11.01	38.25
8	9.24	4.36	1.69	10.47	129.34	2.65	89.34	25.05
9	22.47	11.21	8.97	25.61	3.44	41.02	6.39	2.58
10	28.69	2.36	9.79	18.95	11.36	51.02	12.64	15.08

The structure-activity relationships were accessed as follows: the IC_{50} values of two compounds were compared, and the Δ log IC_{50} was calculated (Δ log IC_{50} is a difference between the log IC_{50} values of an analogue and the corresponding control compound). Positive Δ log IC_{50} values show a decrease of antiproliferative activity, whereas negative values indicate an increase in the activity upon the structural modification being considered. The results are presented in Figure S7.

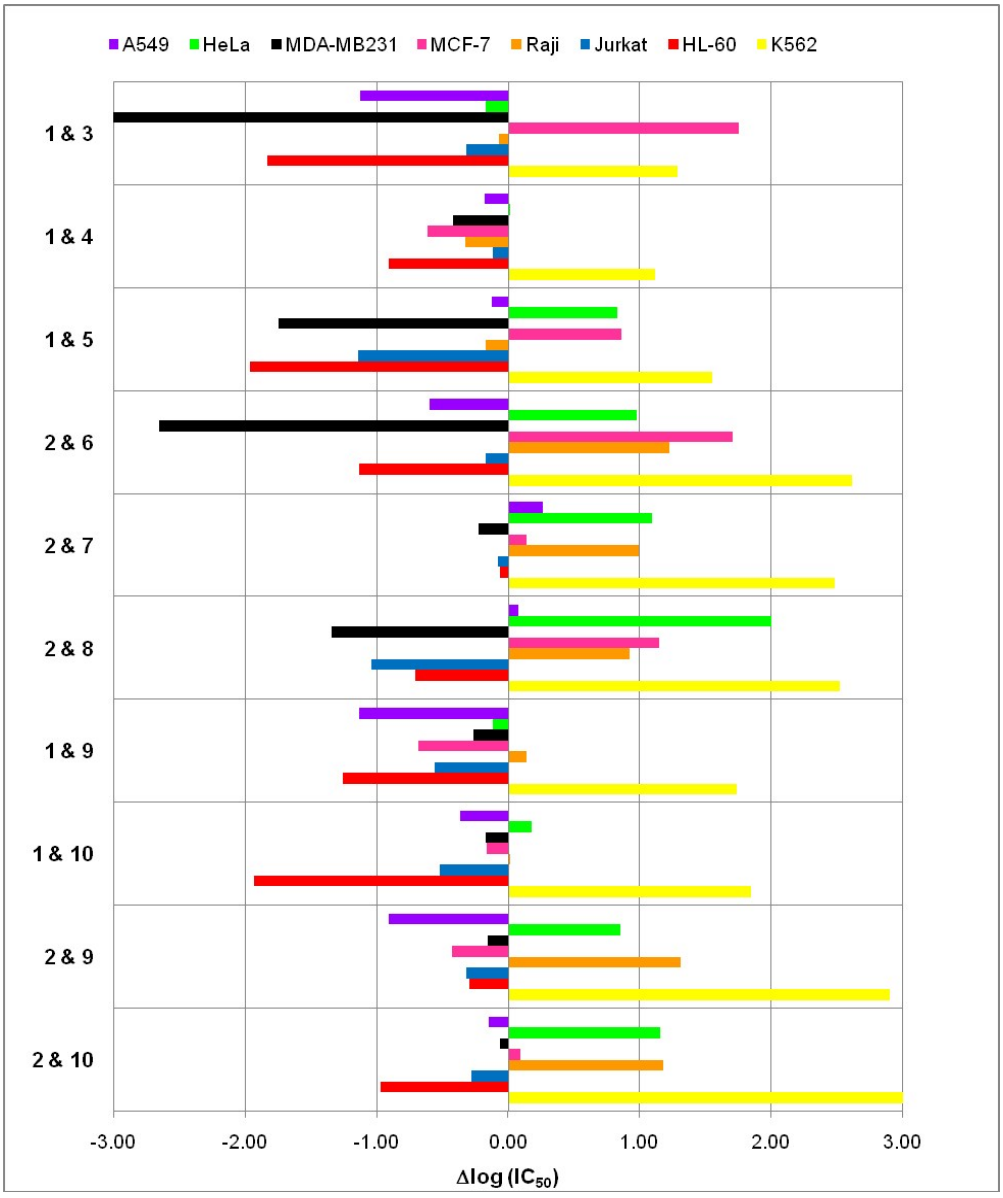


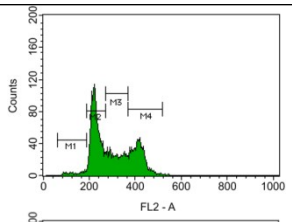
Figure S7. Contributions of selected structural features to the antiproliferative activities. The influence of OH → OMe replacement.

III. FLOW CYTOMETRY

Sample — Cell Cycle — Apoptosis

Control

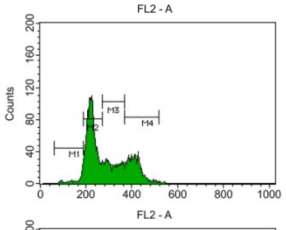
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	88.77	235.71
M1	49	176	1.84	1.63	126.31
M2	177	262	47.59	42.25	218.00
M3	263	373	26.56	23.58	316.82
M4	374	524	24.17	21.46	411.46



Quad Label	Events	% Gated	% Total
UL	69	0.72	0.69
UR	64	0.67	0.64
LL	9021	94.13	90.21
LR	430	4.49	4.3

1 (Goniofufurone)

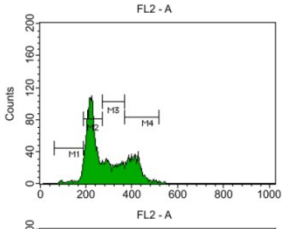
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	87.00	273.02
M1	40	167	2.16	1.88	131.18
M2	168	253	52.48	45.66	209.83
M3	254	352	21.03	18.30	300.54
M4	352	502	24.34	21.18	397.07



Quad Label	Events	% Gated	% Total
UL	39	0.41	0.39
UR	65	0.68	0.65
LL	9096	94.78	90.96
LR	397	4.14	3.97

2 (7-*epi*-goniofufurone)

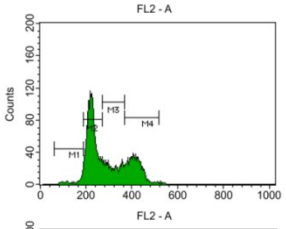
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	87.69	278.56
M1	49	176	2.73	2.39	148.11
M2	177	262	51.51	45.17	213.73
M3	263	361	22.13	19.41	310.56
M4	361	511	23.73	20.81	403.74



Quad Label	Events	% Gated	% Total
UL	37	0.39	0.37
UR	71	0.74	0.71
LL	9046	94.66	90.46
LR	402	4.21	4.02

3

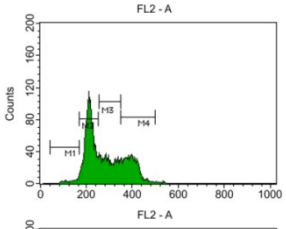
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	86.71	280.71
M1	49	176	2.40	2.08	142.86
M2	177	262	52.08	45.16	215.20
M3	263	361	20.61	17.87	310.81
M4	361	511	24.89	21.58	404.63



Quad Label	Events	% Gated	% Total
UL	21	0.22	0.21
UR	54	0.56	0.54
LL	9094	94.88	90.94
LR	416	4.34	4.16

4

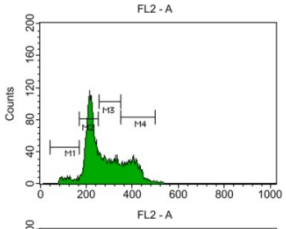
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	90.04	270.99
M1	31	158	1.82	1.64	127.26
M2	159	244	47.39	42.67	204.57
M3	245	343	26.55	23.91	292.23
M4	343	493	24.34	21.92	386.56



Quad Label	Events	% Gated	% Total
UL	17	0.18	0.17
UR	78	0.81	0.78
LL	8919	92.12	89.19
LR	668	6.90	6.68

5

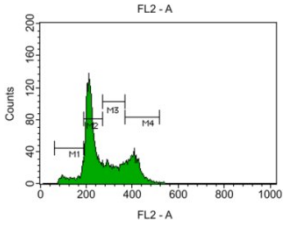
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	88.76	286.19
M1	49	176	6.40	2.13	140.55
M2	177	262	45.16	43.19	216.67
M3	263	361	20.62	20.14	311.41
M4	361	511	28.49	23.42	405.51



Quad Label	Events	% Gated	% Total
UL	78	0.83	0.78
UR	109	1.16	1.09
LL	8454	89.91	84.54
LR	762	8.10	7.62

6

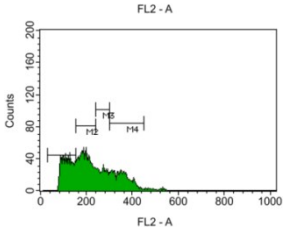
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	92.43	265.12
M1	49	176	6.04	5.58	130.46
M2	177	262	53.47	49.42	210.21
M3	263	361	19.71	18.22	311.30
M4	361	511	20.93	19.35	400.80



Quad Label	Events	% Gated	% Total
UL	119	1.27	1.19
UR	176	1.89	1.76
LL	7747	82.98	77.47
LR	1294	13.86	12.94

7

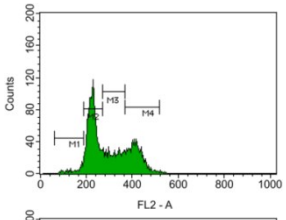
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	82.74	209.71
M1	18	145	28.51	23.59	106.99
M2	146	231	34.84	28.83	185.23
M3	232	293	15.00	12.41	261.49
M4	293	443	21.09	17.45	343.51



Quad Label	Events	% Gated	% Total
UL	132	3.27	2.6
UR	458	11.36	9.03
LL	1570	38.95	30.97
LR	1871	46.42	36.9

8

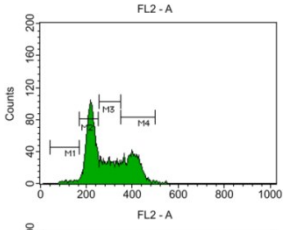
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	88.76	286.19
M1	49	176	2.40	2.13	140.55
M2	177	262	48.66	43.19	216.67
M3	263	361	22.69	20.14	311.41
M4	361	511	26.39	23.42	405.51



Quad Label	Events	% Gated	% Total
UL	27	0.28	0.27
UR	102	1.07	1.02
LL	8896	92.95	88.96
LR	546	5.70	5.46

9

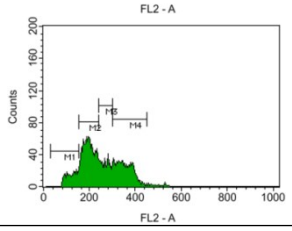
Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	89.76	283.34
M1	31	158	1.33	1.19	117.68
M2	159	244	45.43	40.78	209.53
M3	245	343	23.66	21.24	293.37
M4	343	493	29.51	26.49	394.54



Quad Label	Events	% Gated	% Total
UL	13	0.13	0.13
UR	88	0.91	0.88
LL	8947	92.72	89.47
LR	602	6.24	6.02

10

Marker Label	Left	Right	% Gated	% Total	X Mean
All	0	1023	100.00	85.11	239.83
M1	18	145	10.07	8.57	110.95
M2	146	231	43.10	36.68	187.16
M3	232	293	17.67	15.04	261.48
M4	293	443	28.59	24.33	344.80



Quad Label	Events	% Gated	% Total
UL	671	9.39	7.95
UR	571	7.99	6.76
LL	3844	53.77	45.52
LR	2063	28.86	24.43

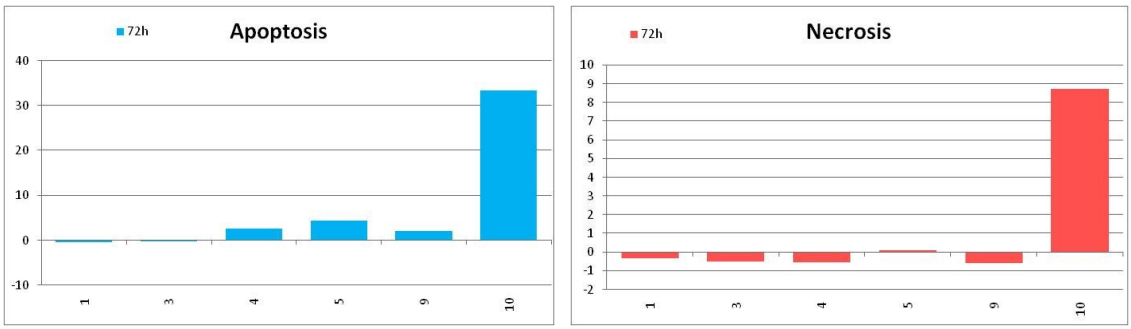


Figure S8. Percents of specific apoptosis and necrosis induced with compounds **1**, **3**, **4**, **5**, **9** and **10**.

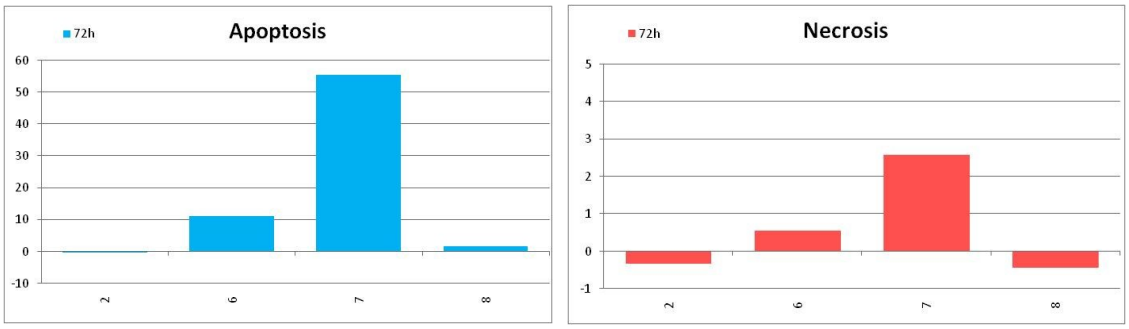


Figure S9. Percents of specific apoptosis and necrosis induced with compounds **2**, **6**, **7** and **8**.

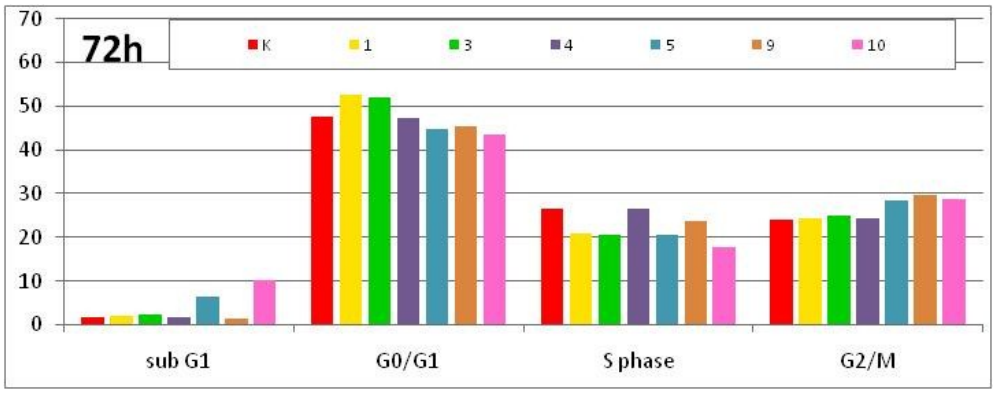


Figure S10. Influence of compounds **1**, **3**, **4**, **5**, **9** and **10** on the K562 cell cycle.

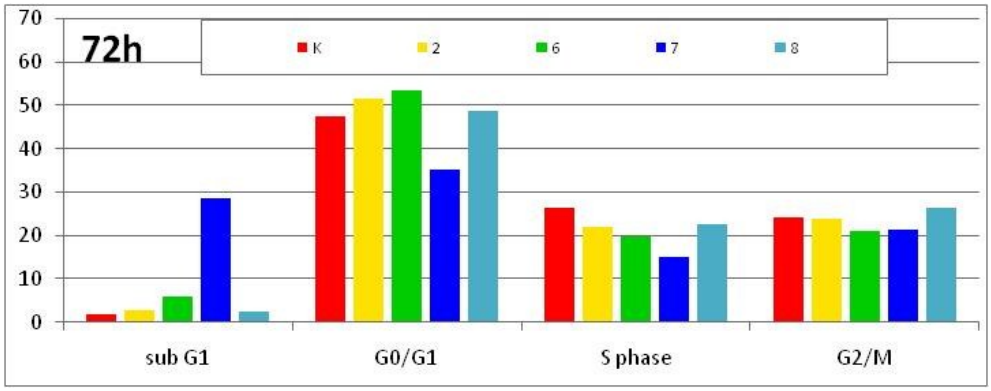


Figure S11. Influence of compounds **2**, **6**, **7** and **8** on the K562 cell cycle.

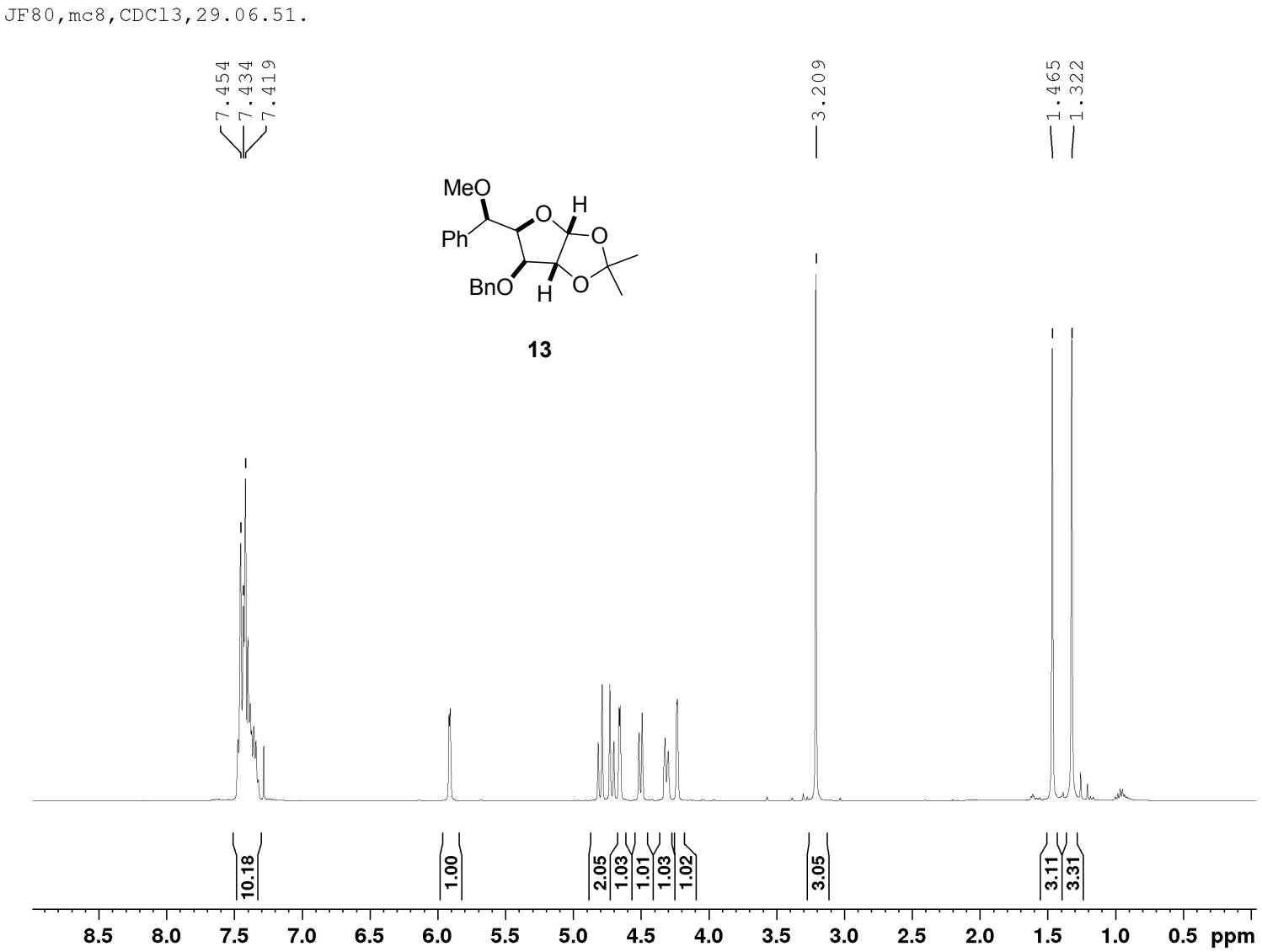
IV. WESTERN BLOT ANALYSIS

Table S3. Results of Western blot analysis

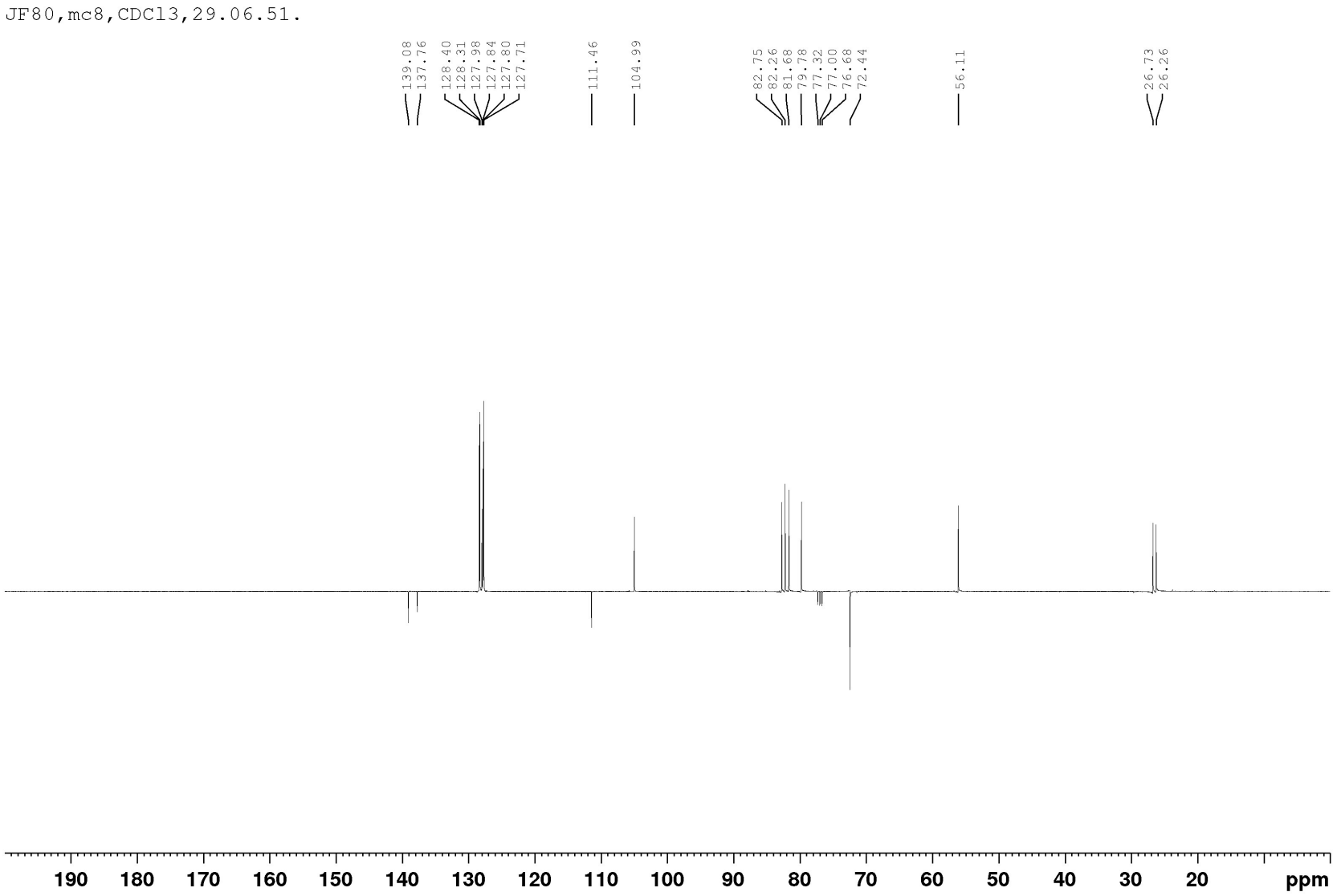
Compounds	% of control					
	Bcl-2	Bax	Caspase-3 precursor (32 kD)	Caspase-3 active subunit (18 kD)	PARP (112 kD)	PARP (85 kD)
1	136.54	114.33	78.86	61.60	78.66	204.22
2	115.57	96.74	115.06	36.92	84.85	136.85
3	41.56	47.89	8.96	37.00	27.78	145.51
4	91.92	104.97	108.40	78.14	29.82	163.37
5	124.89	114.85	187.06	127.34	58.49	148.84
6	96.37	74.73	25.07	44.25	40.43	124.65
7	88.47	91.39	49.26	86.61	73.28	267.34
8	115.43	128.29	39.98	149.43	60.61	201.87
9	164.02	173.72	72.23	119.35	68.52	139.02
10	164.45	200.10	163.30	251.76	55.93	159.55

V. COPIES OF NMR SPECTRA OF SYNTHESIZED PRODUCTS

400 MHz ^{1}H NMR Spectrum of Compound 13 ($CDCl_3$)

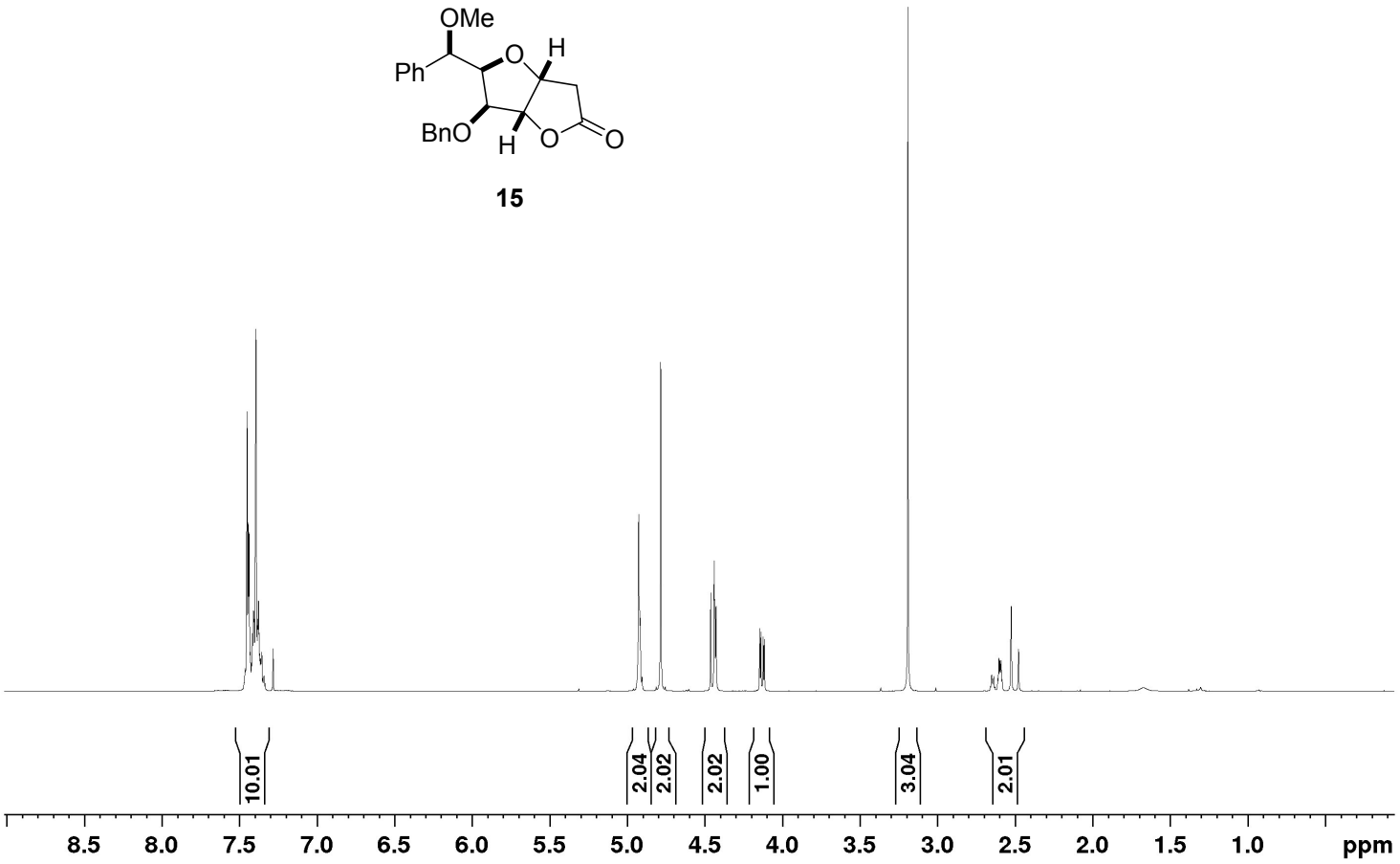


100 MHz ^{13}C NMR Spectrum of Compound 13 ($CDCl_3$)

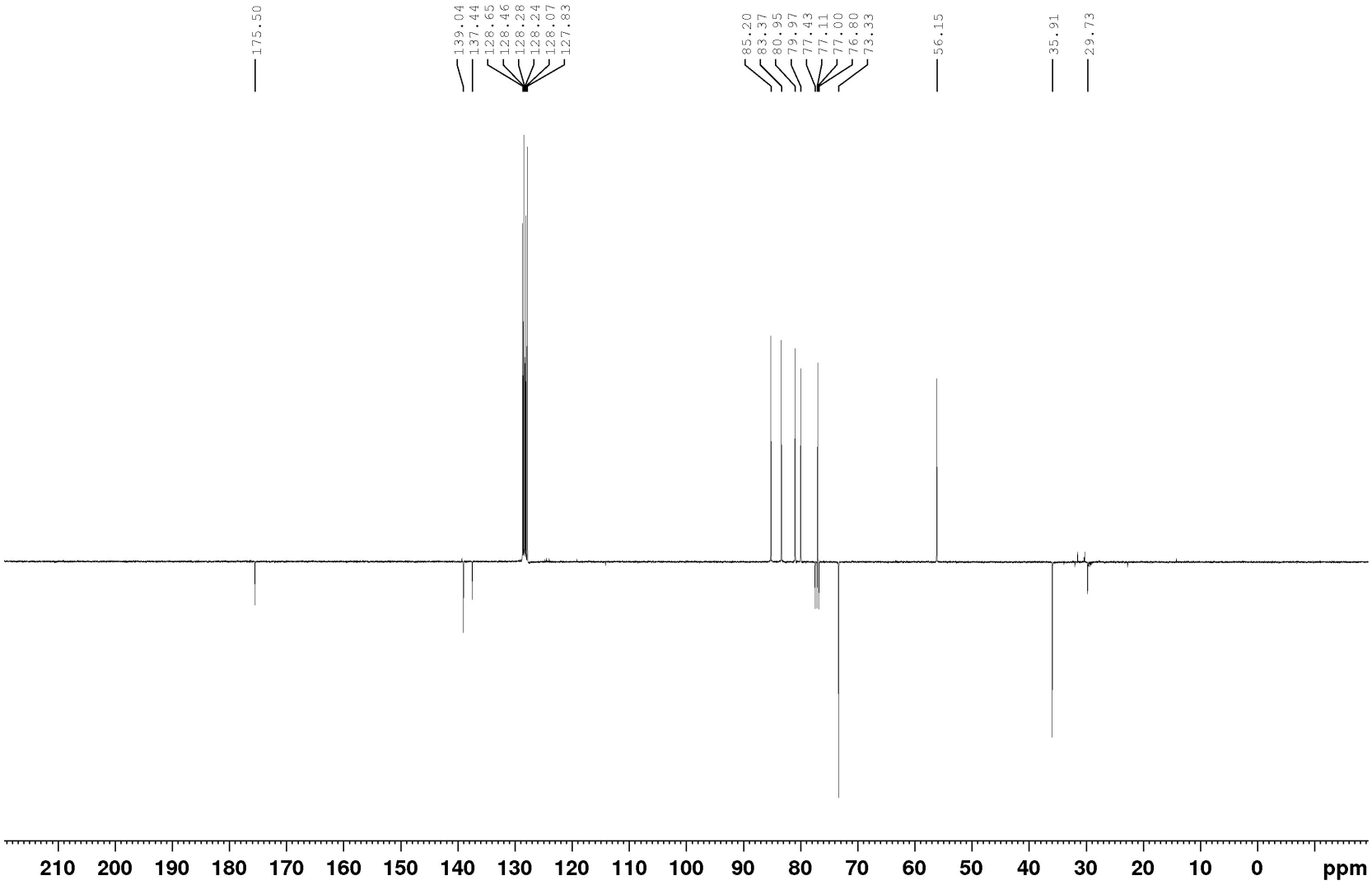


400 MHz ^{1}H NMR Spectrum of Compound 15 ($CDCl_3$)

JF82,MC11,CDCl3, 10.7.15

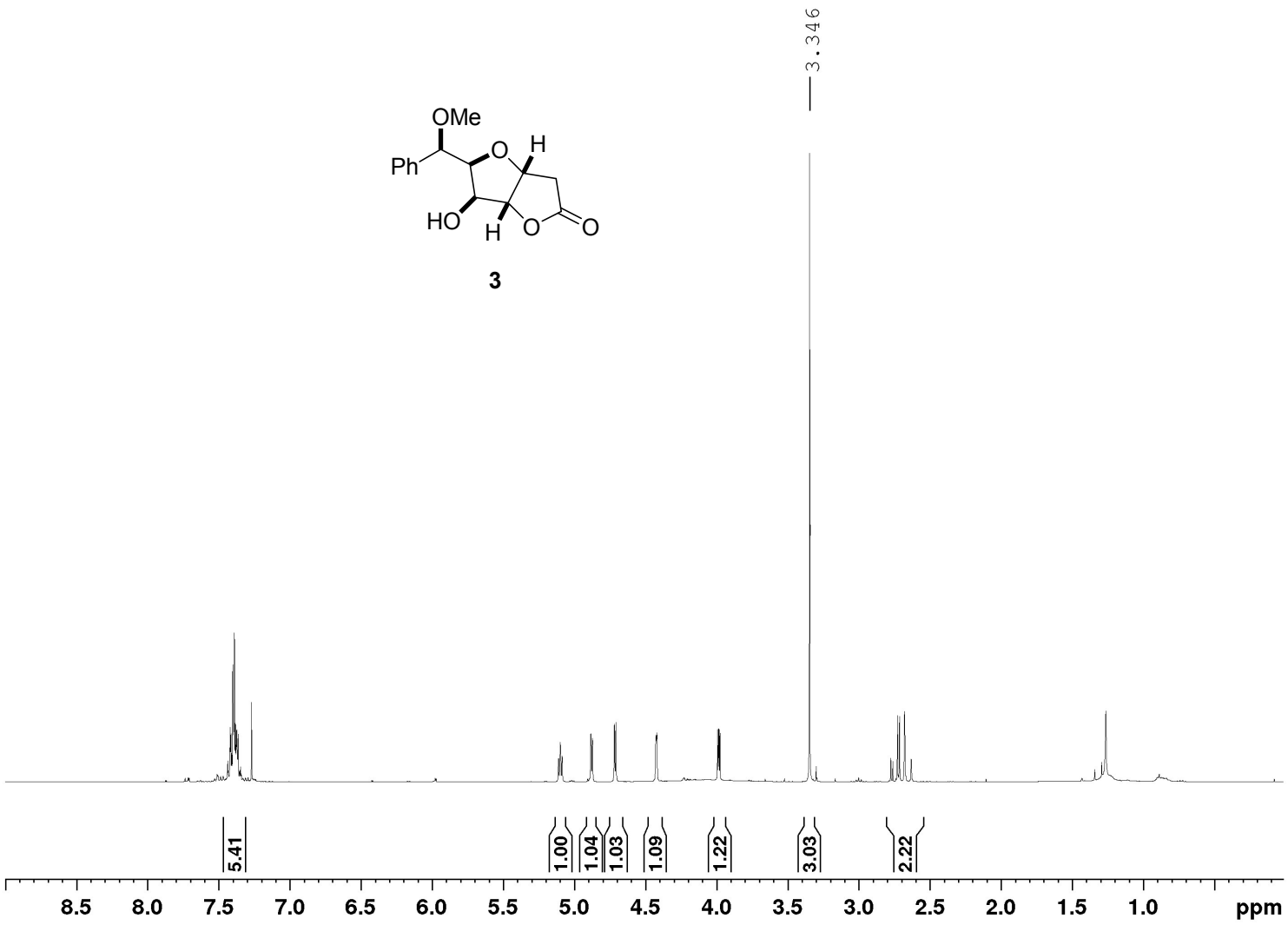


100 MHz ^{13}C NMR Spectrum of Compound 15 ($CDCl_3$)



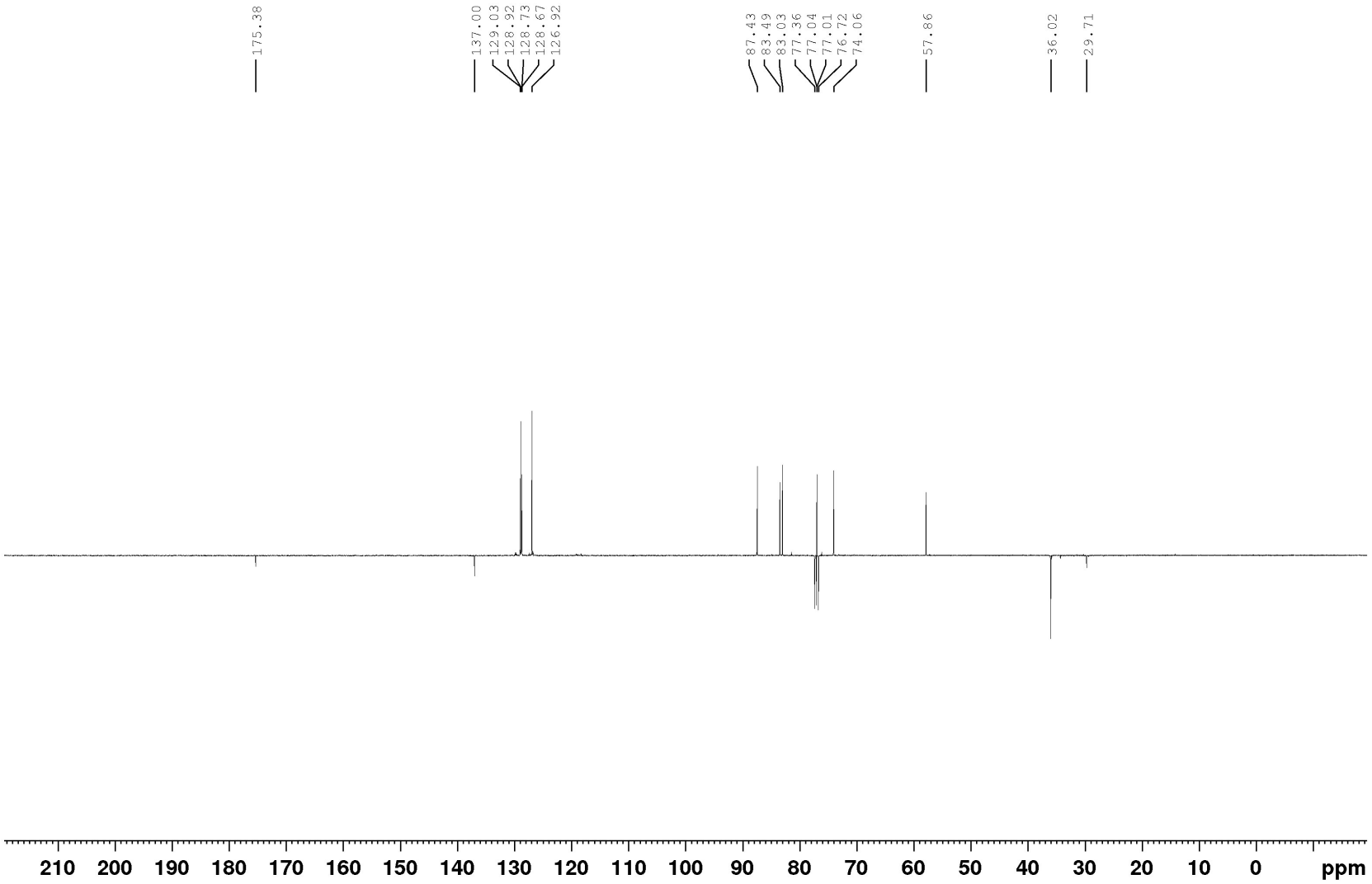
400 MHz ^{1}H NMR Spectrum of Compound 3 ($CDCl_3$)

JF83,MC17,CDCl3,08.9.15.rotacija



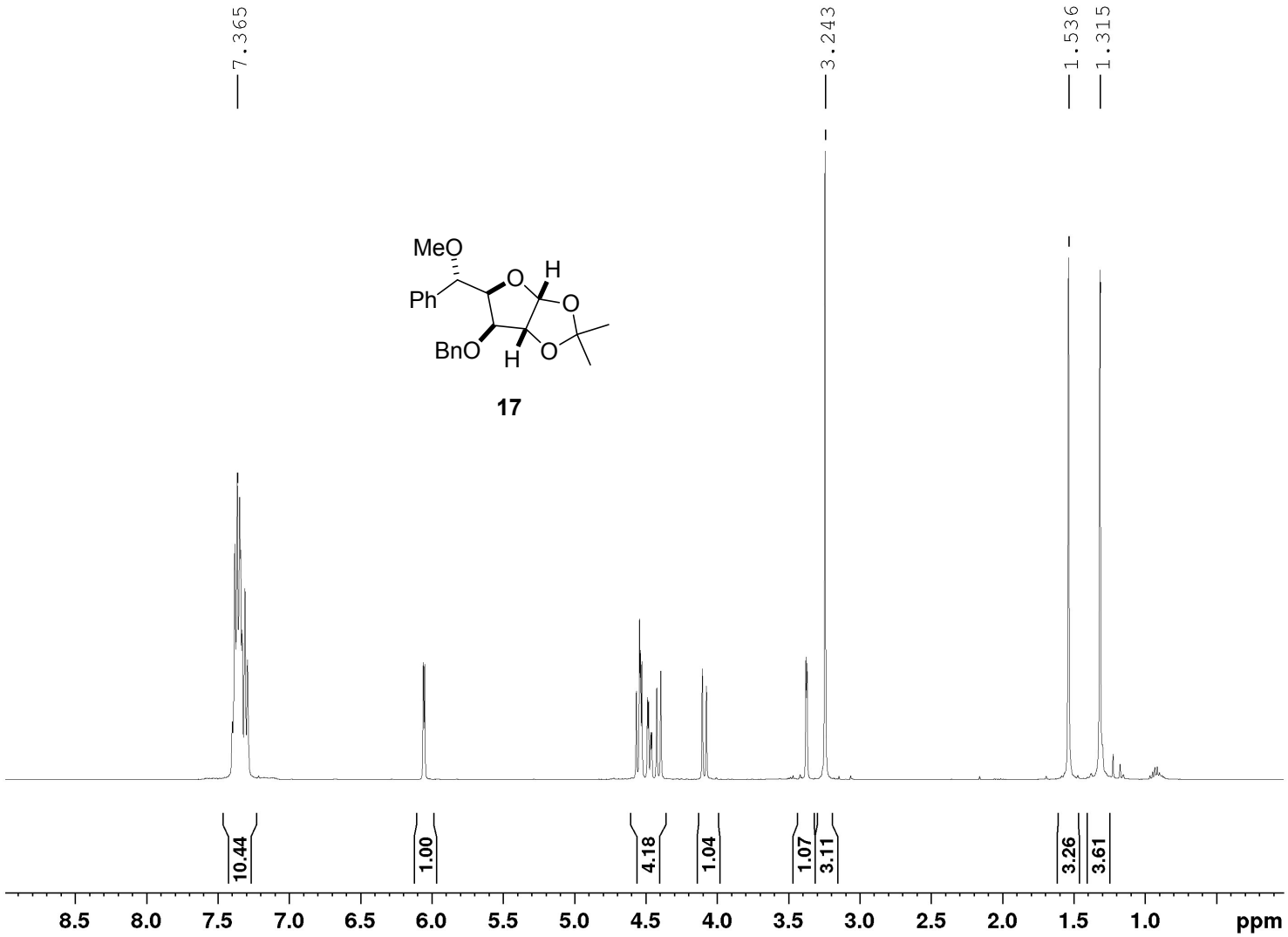
100 MHz ^{13}C NMR Spectrum of Compound 3 ($CDCl_3$)

JF83,MC17,CDCl3,08.9.15.



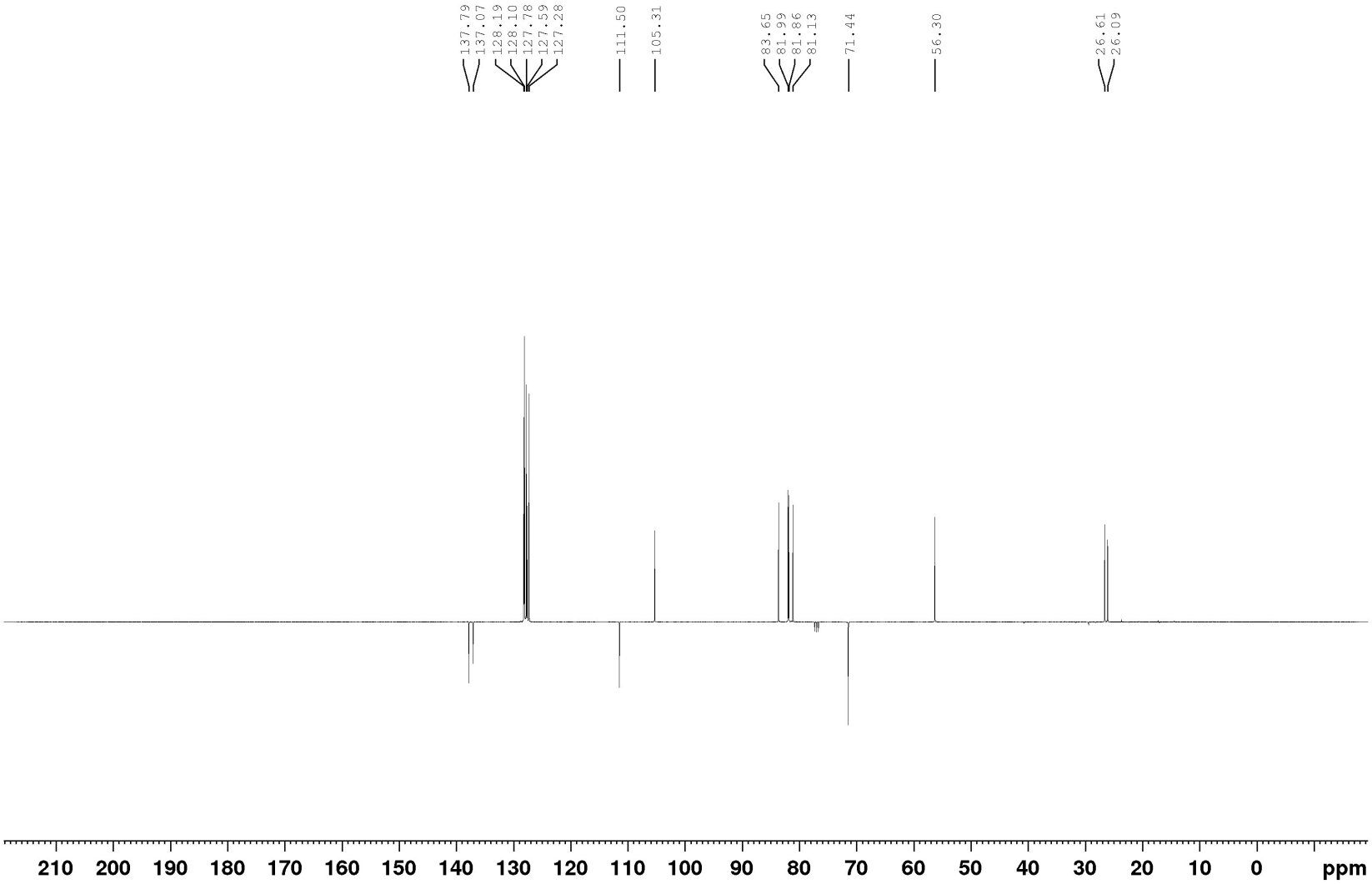
400 MHz ^{1}H NMR Spectrum of Compound 17 ($CDCl_3$)

JF84,mc9CDCl3,2.7.15.



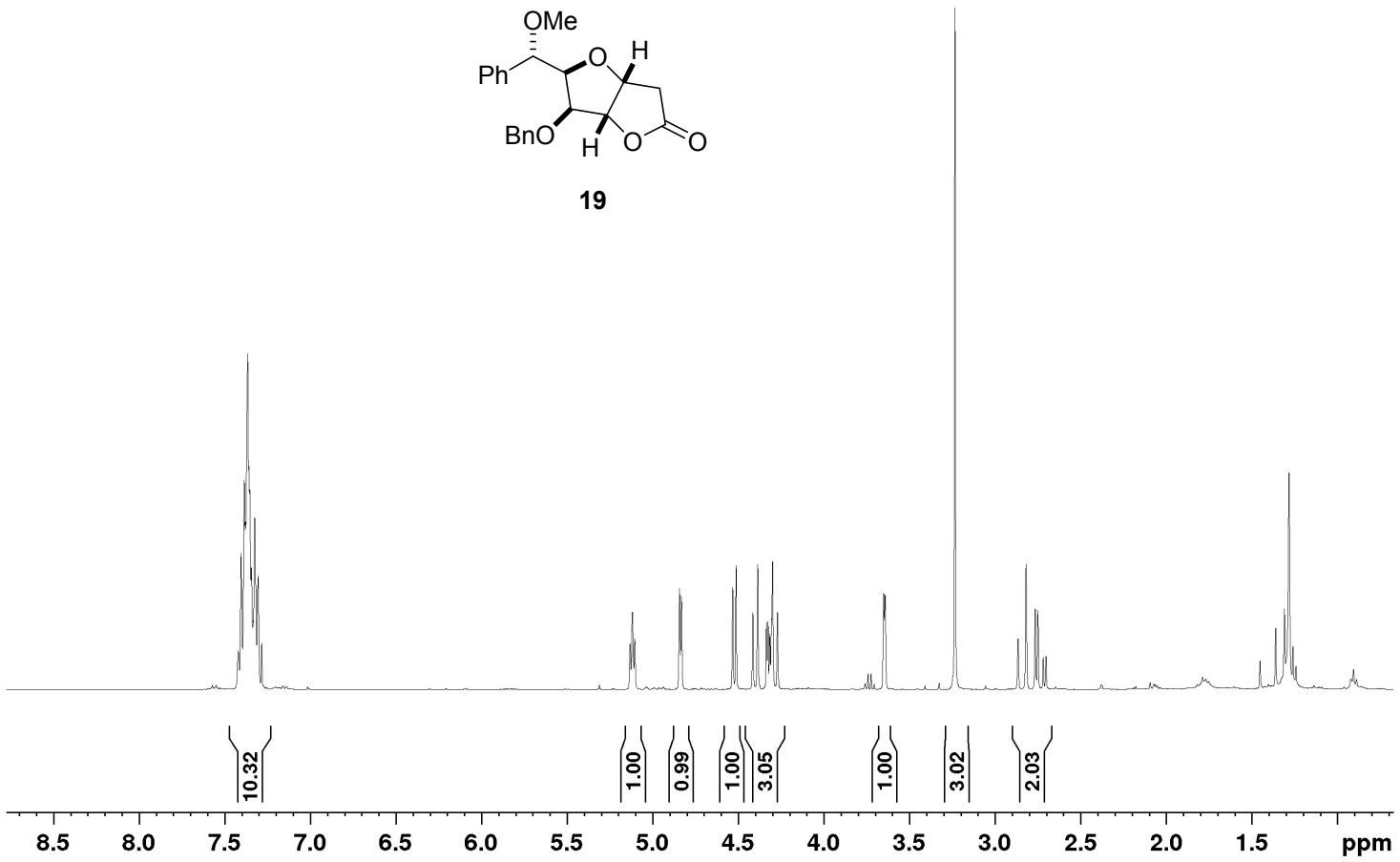
100 MHz ^{13}C NMR Spectrum of Compound 17 ($CDCl_3$)

JF84,mc9CDCl3,2.7.15.

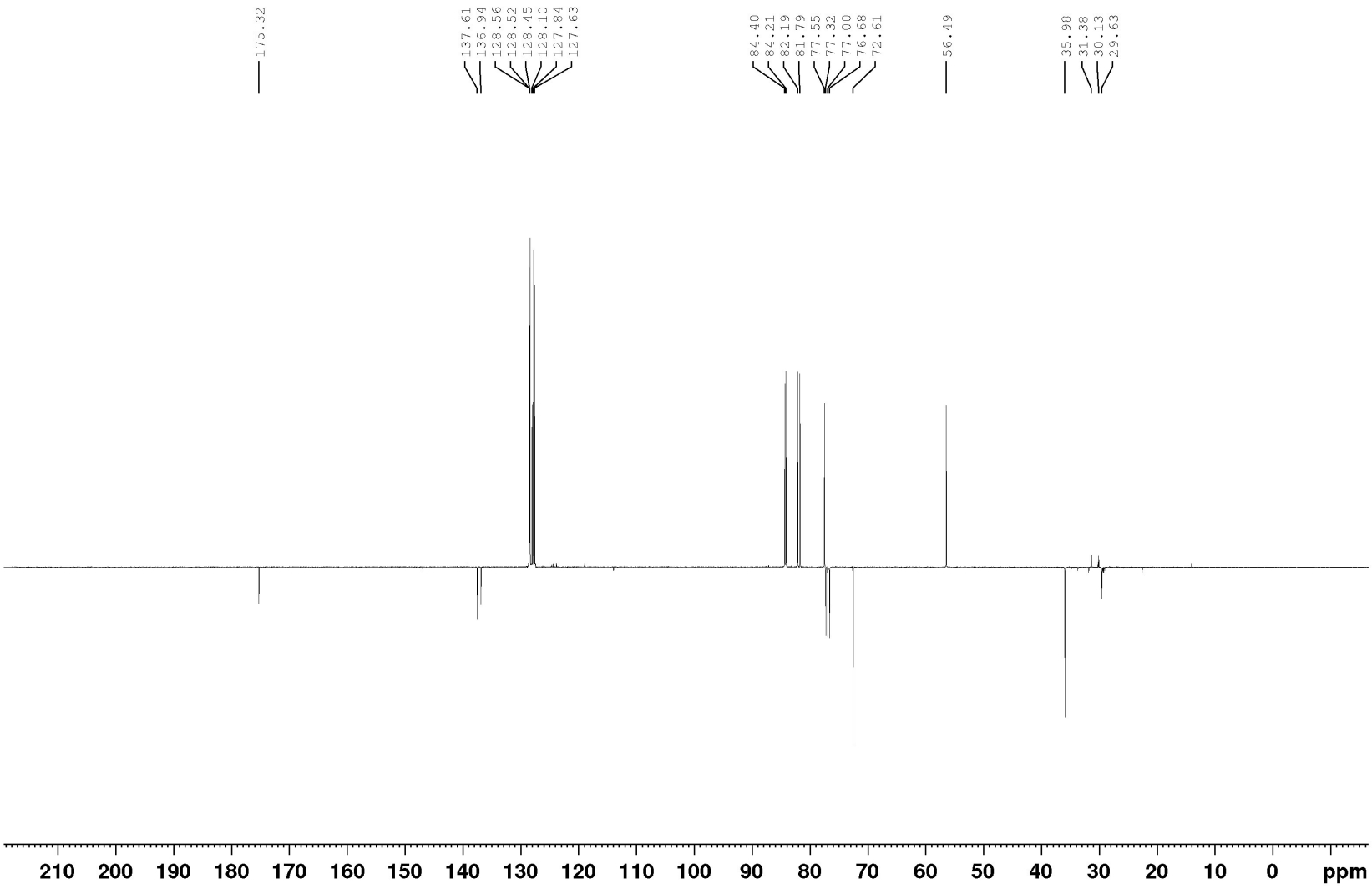


400 MHz ^{1}H NMR Spectrum of Compound 19 ($CDCl_3$)

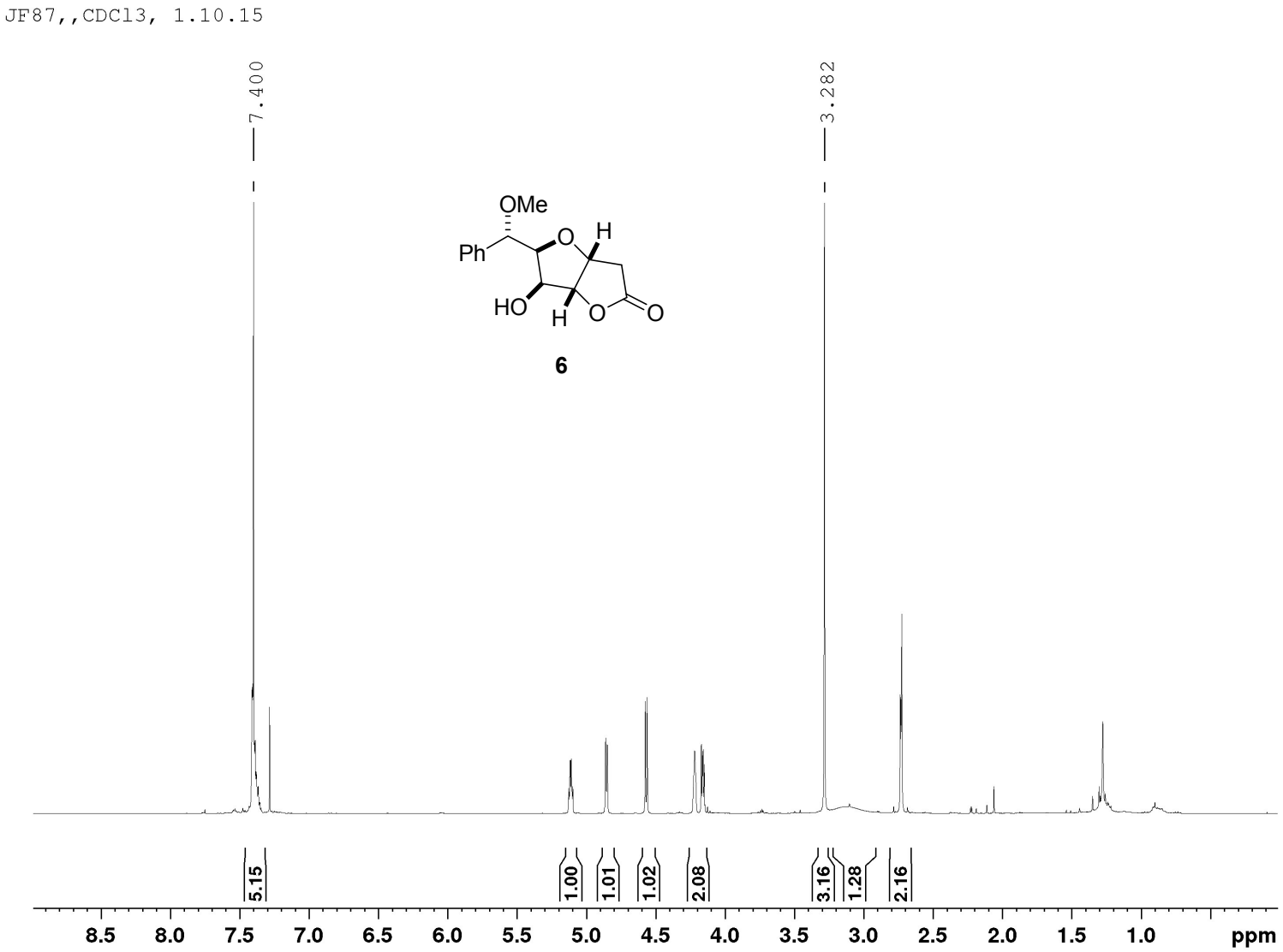
JF86,MC13,CDCl3, 14.7.15



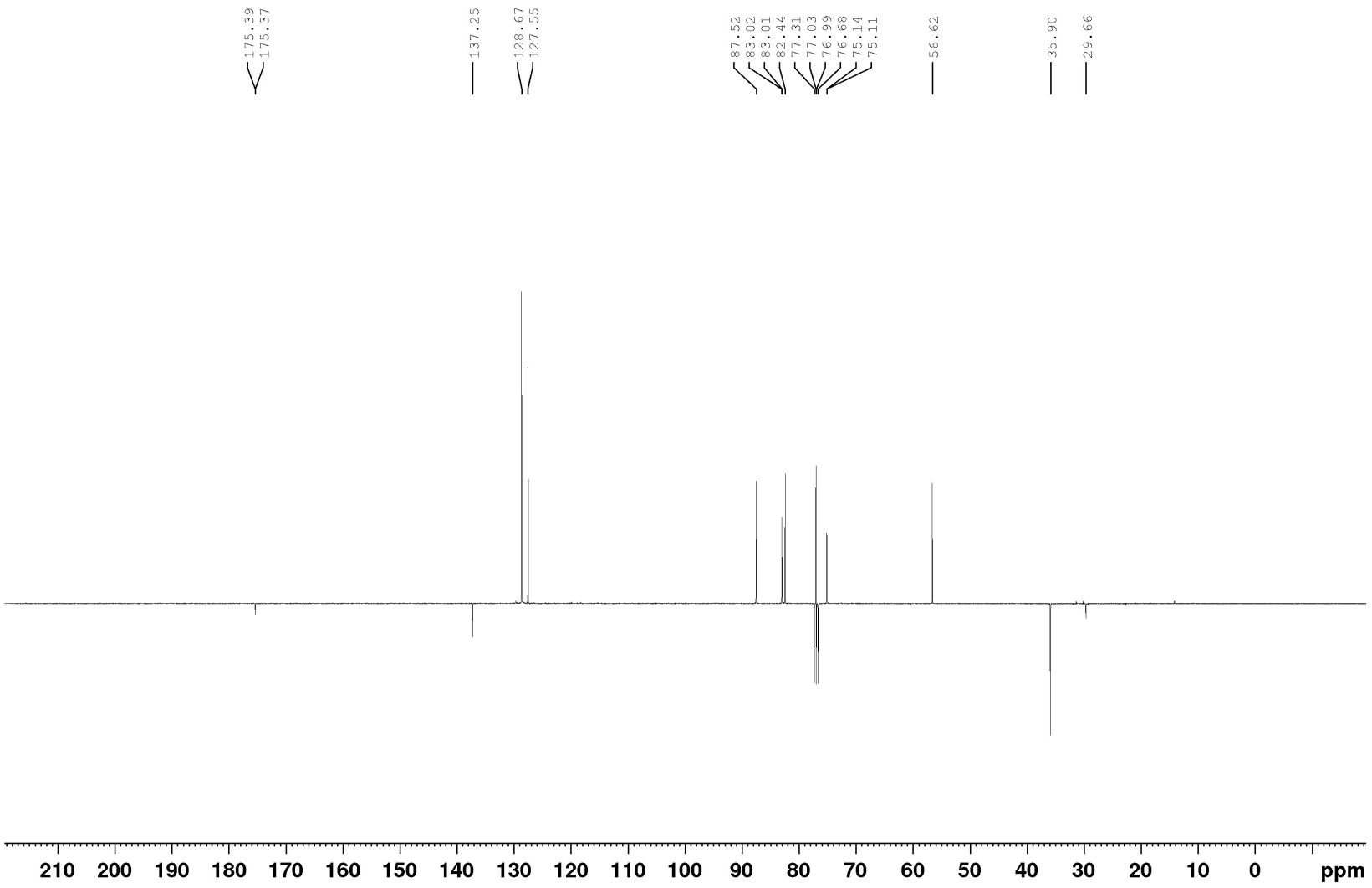
100 MHz ^{13}C NMR Spectrum of Compound 19 ($CDCl_3$)



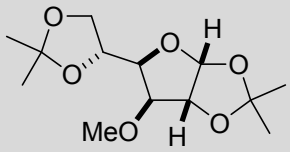
400 MHz ^{1}H NMR Spectrum of Compound 6 ($CDCl_3$)



100 MHz ^{13}C NMR Spectrum of Compound 6 ($CDCl_3$)

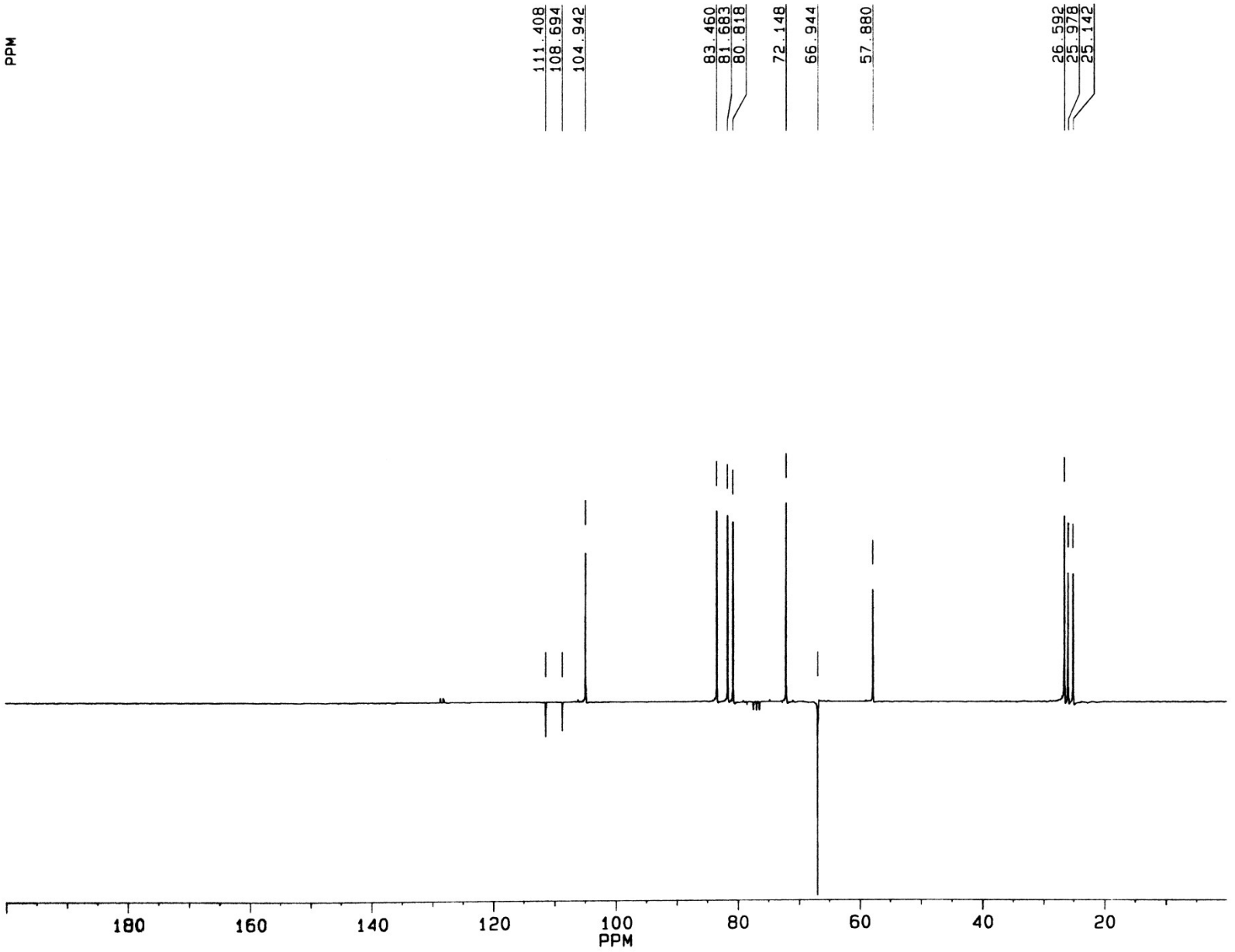


250 MHz ^{1}H NMR Spectrum of Compound 20 ($CDCl_3$)

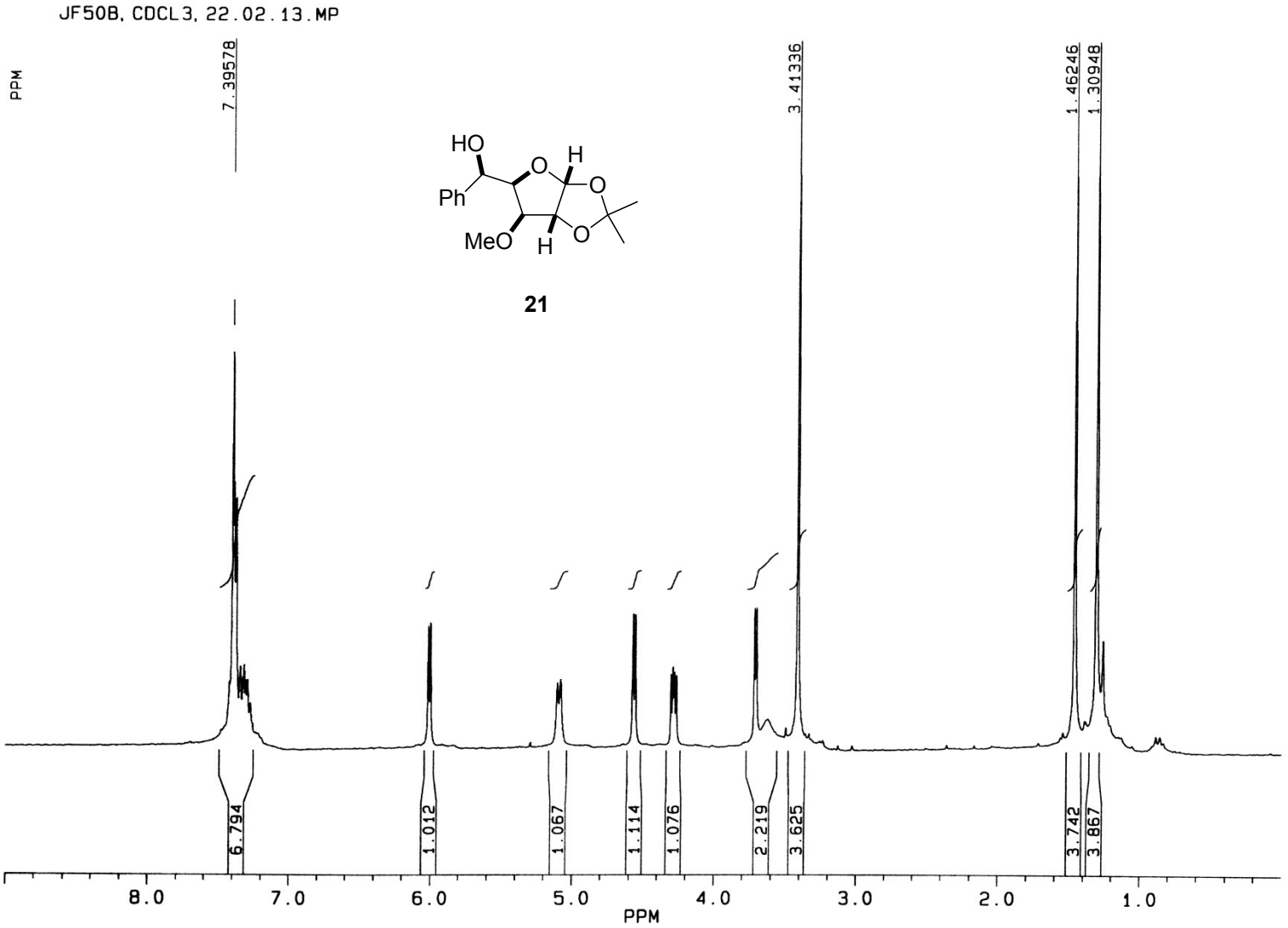


20

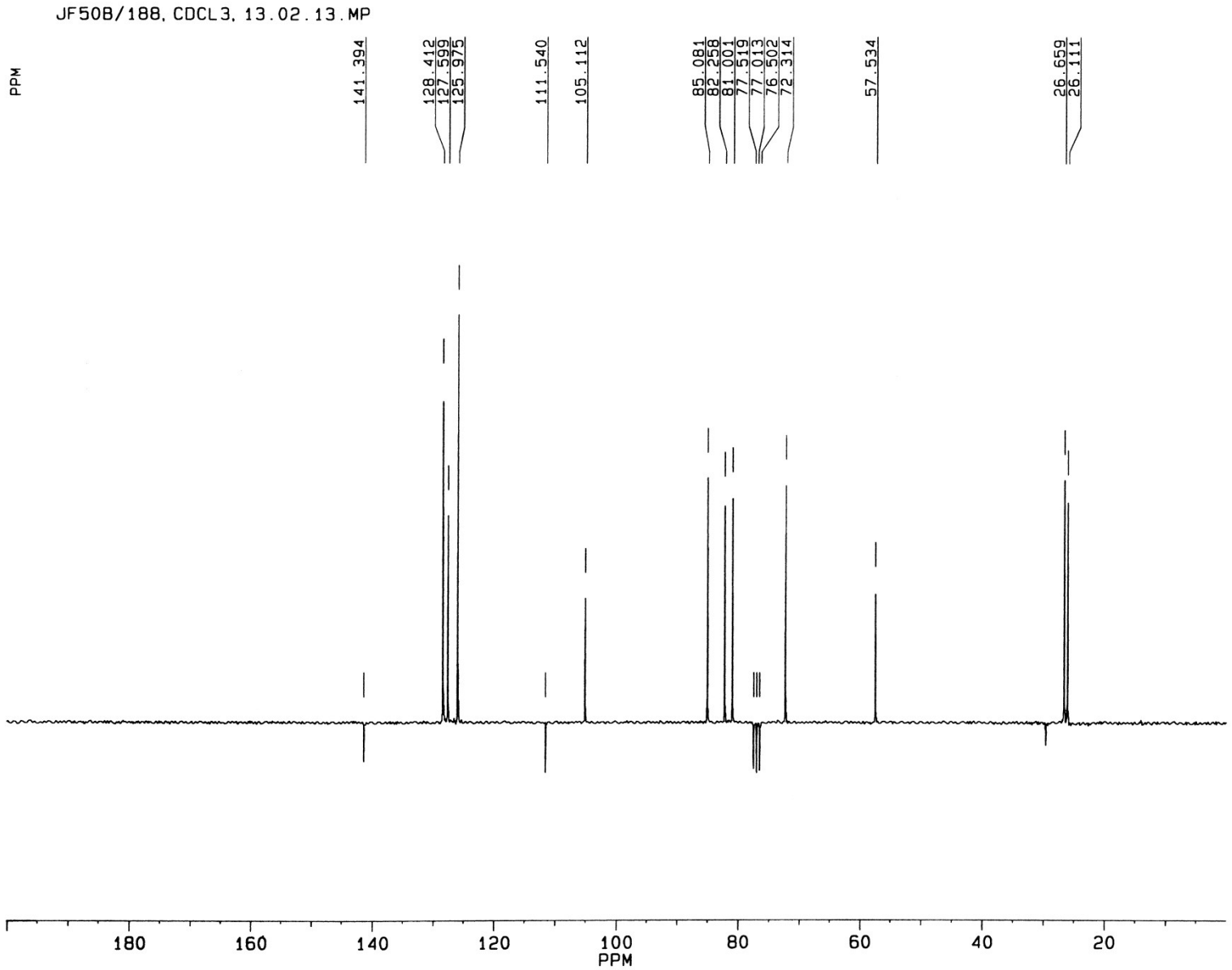
62.9 MHz ^{13}C NMR Spectrum of Compound 20 ($CDCl_3$)



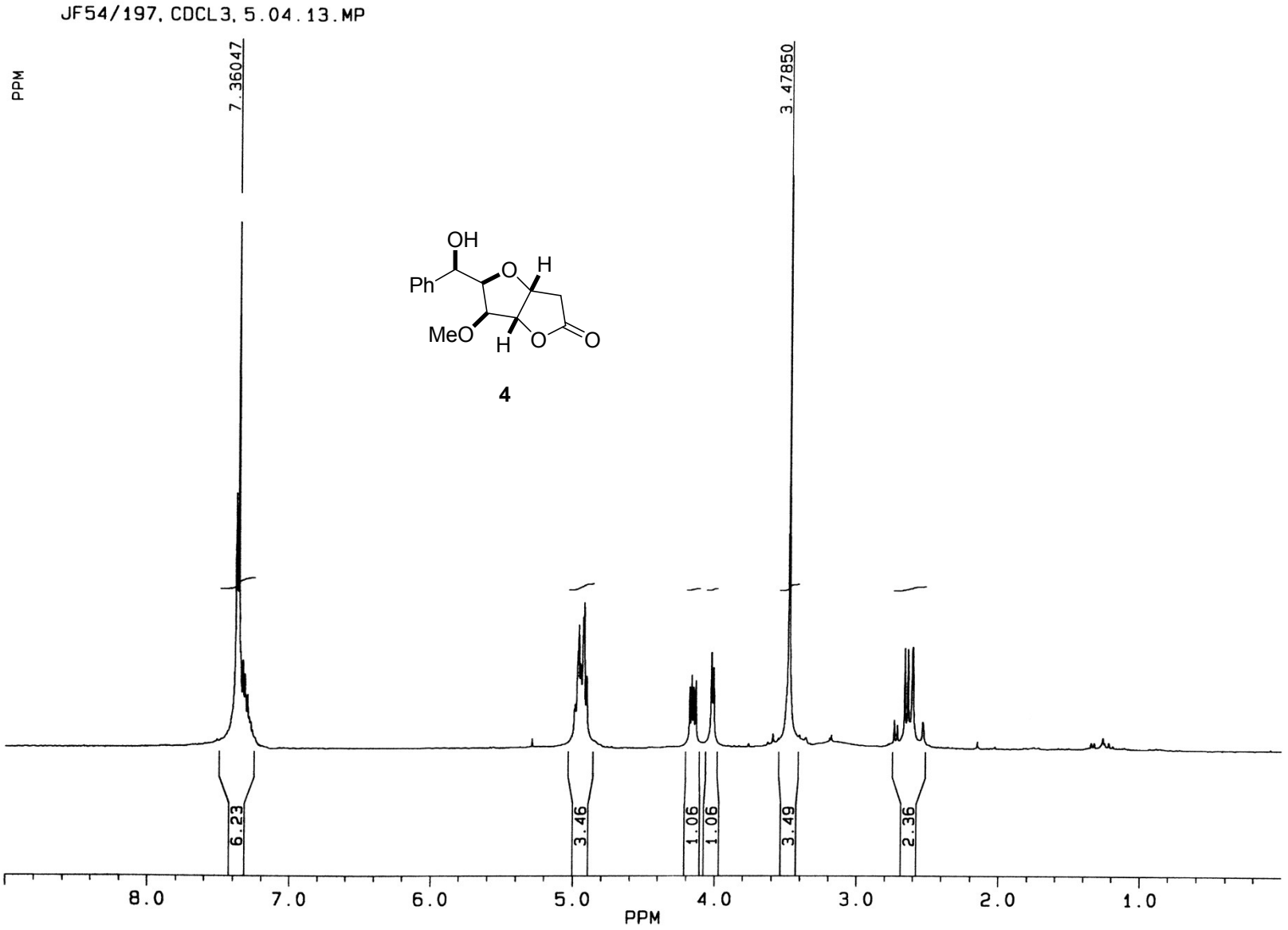
250 MHz ^{1}H NMR Spectrum of Compound 21 ($CDCl_3$)



62.9 MHz ^{13}C NMR Spectrum of Compound 21 ($CDCl_3$)

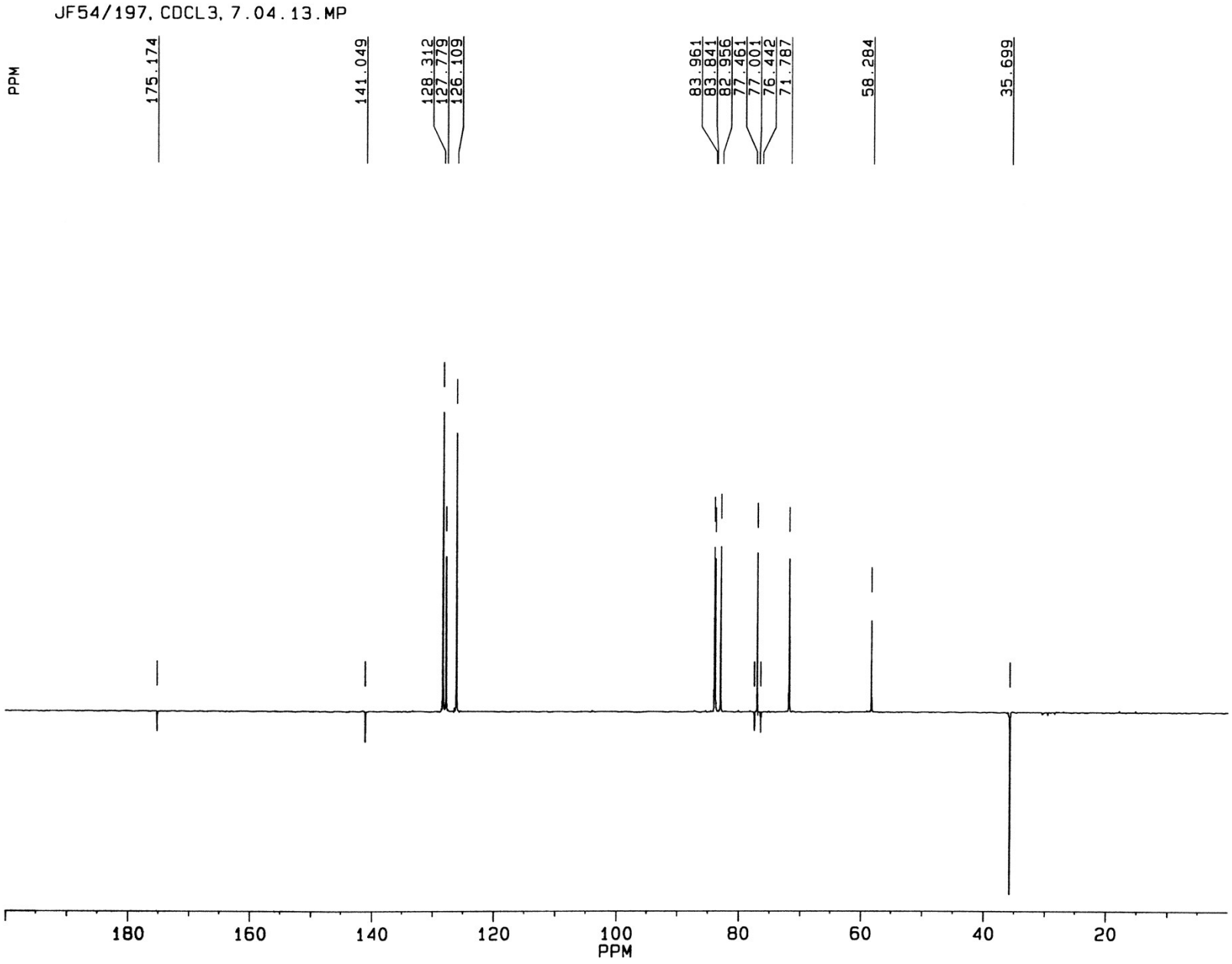


250 MHz ^{1}H NMR Spectrum of Compound 4 ($CDCl_3$)

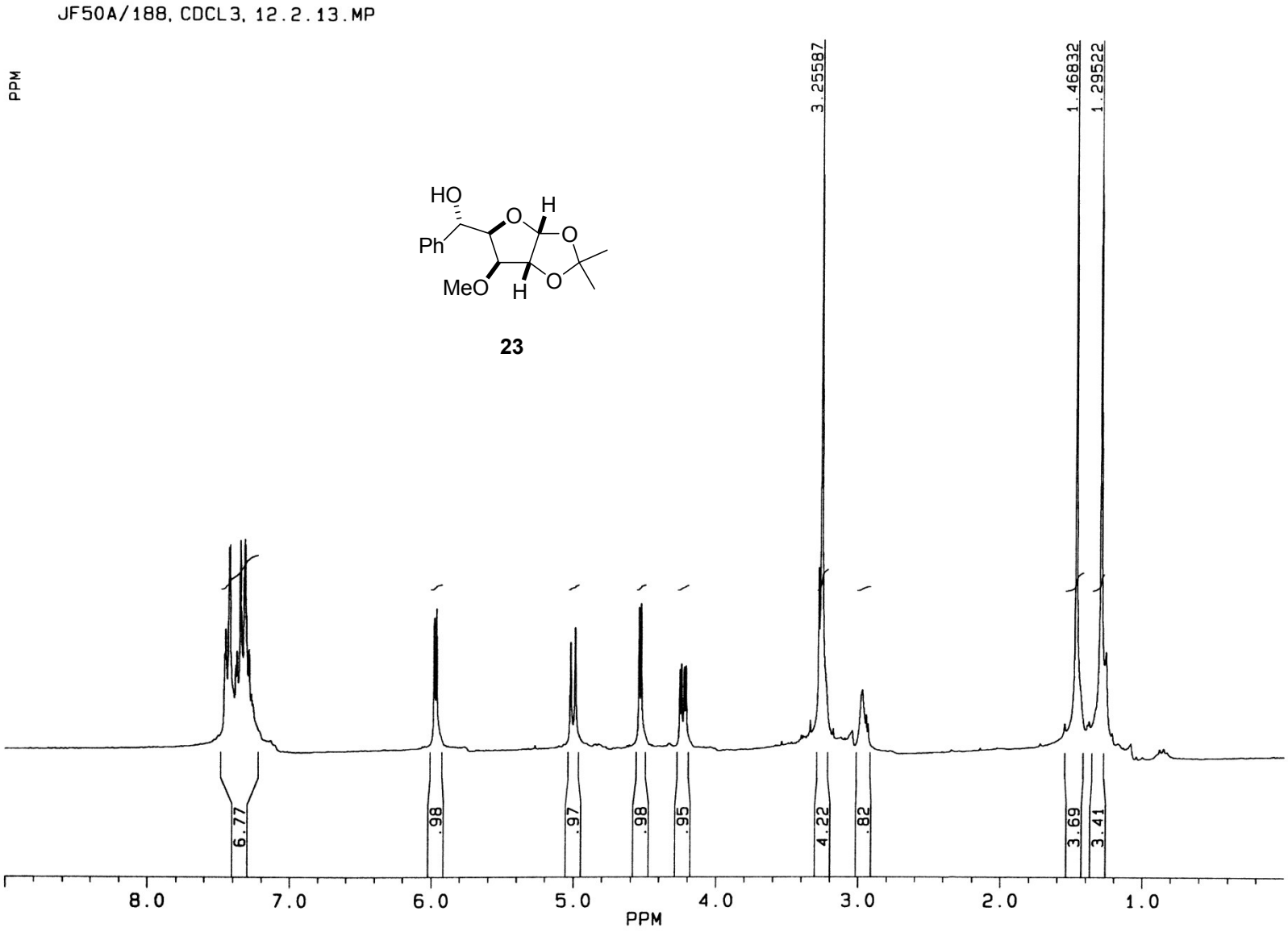


4

62.9 MHz ^{13}C NMR Spectrum of Compound 4 ($CDCl_3$)

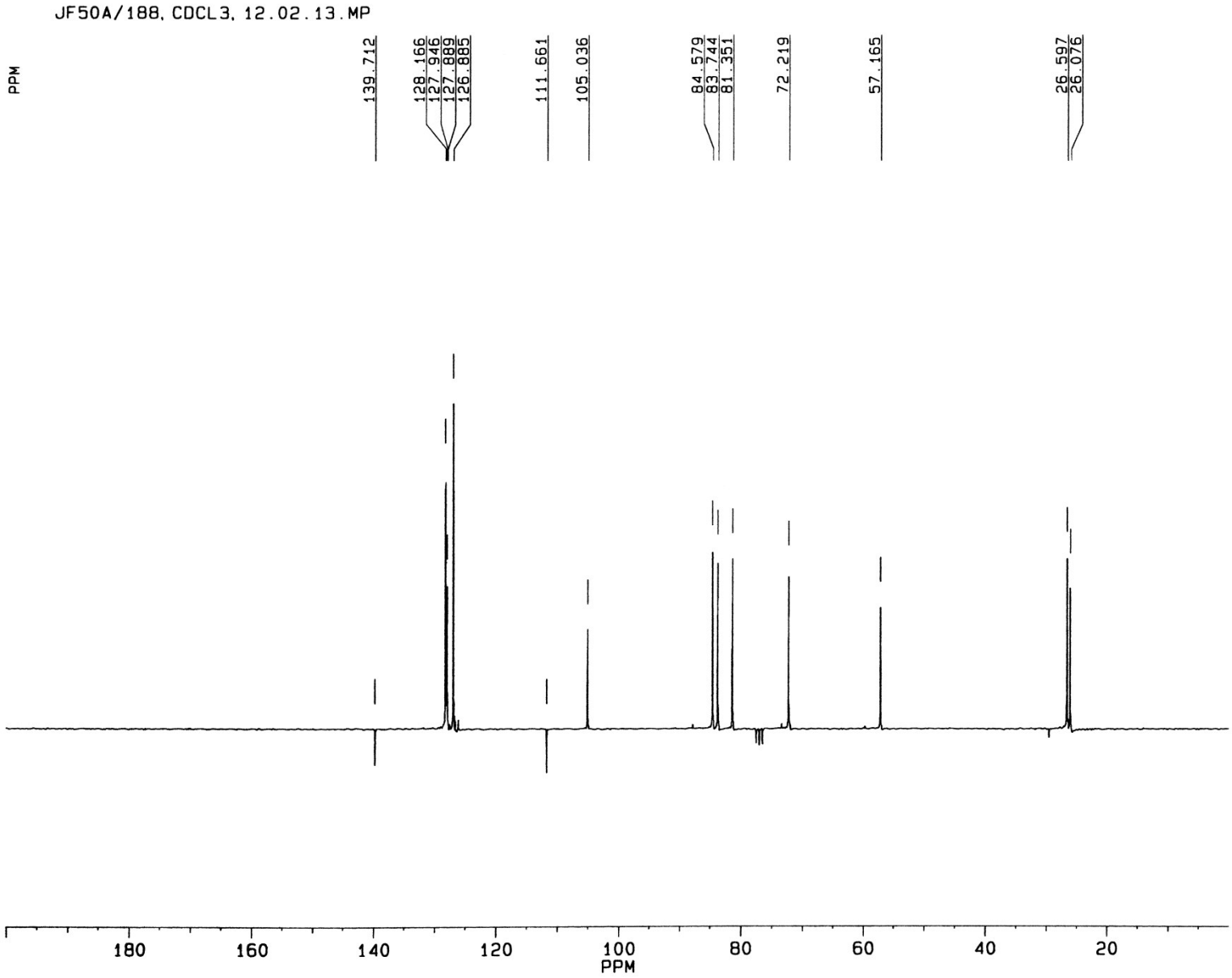


250 MHz ^{1}H NMR Spectrum of Compound 23 ($CDCl_3$)

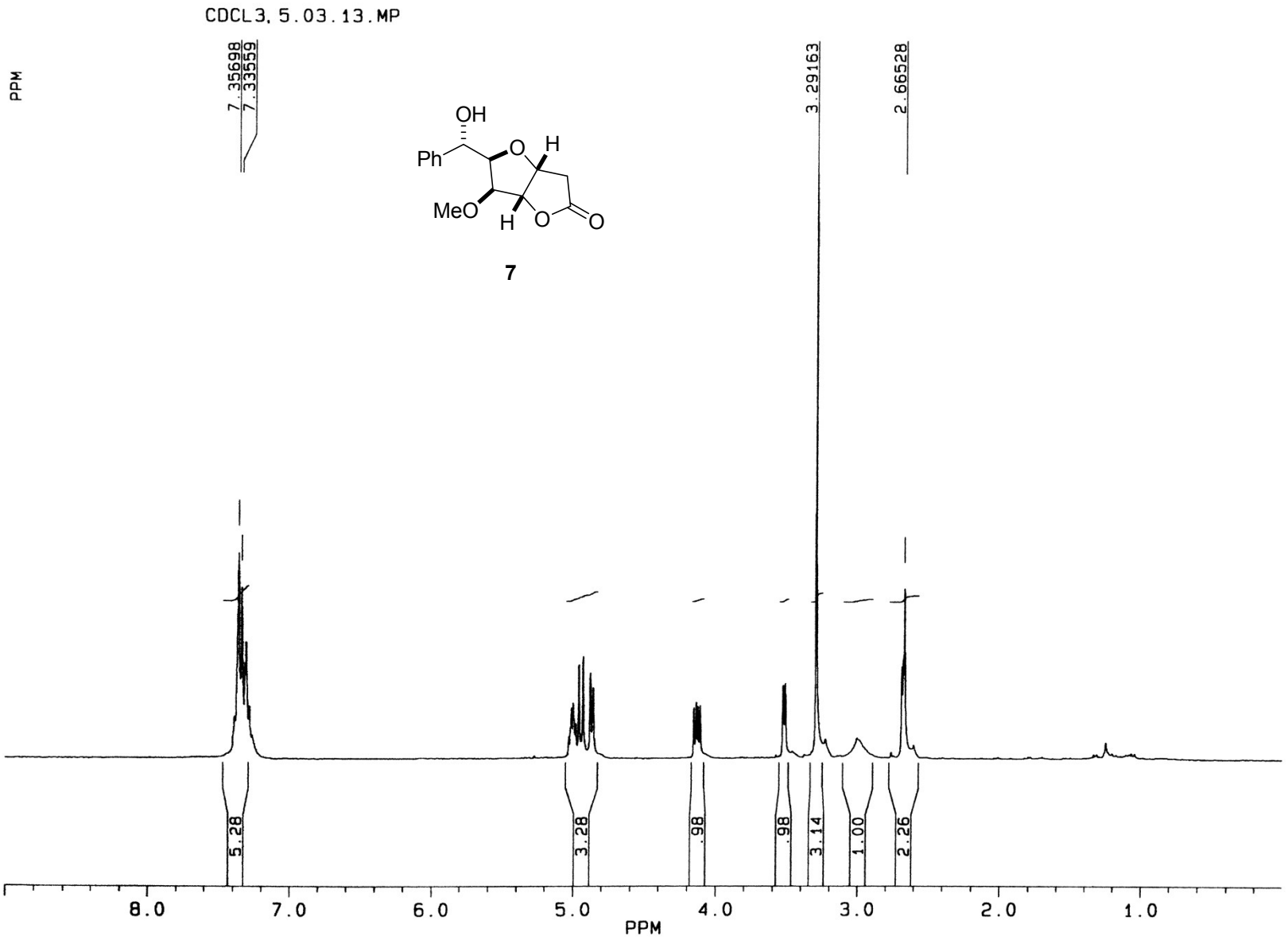


23

62.9 MHz ^{13}C NMR Spectrum of Compound 23 ($CDCl_3$)

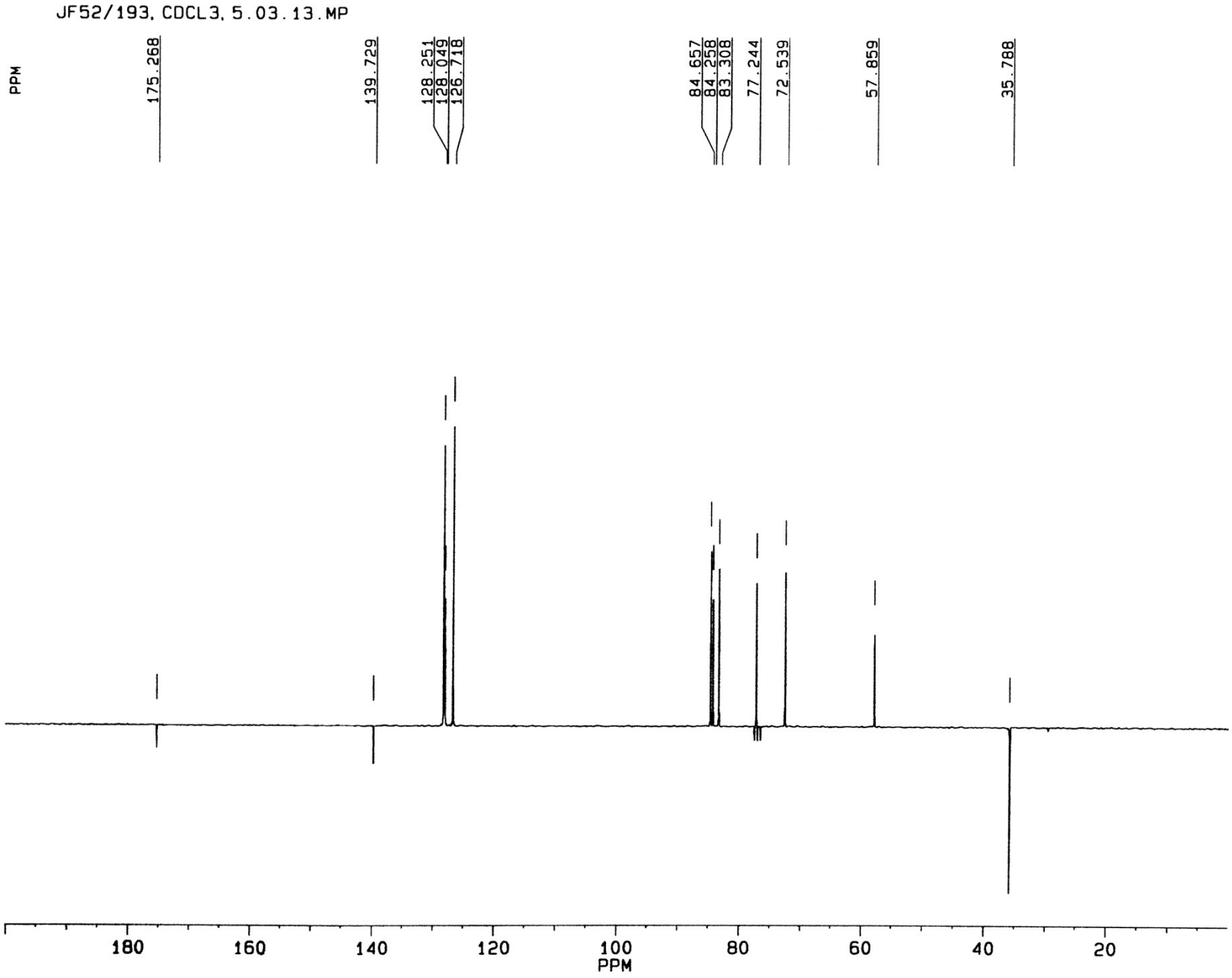


250 MHz ^{1}H NMR Spectrum of Compound 7 ($CDCl_3$)



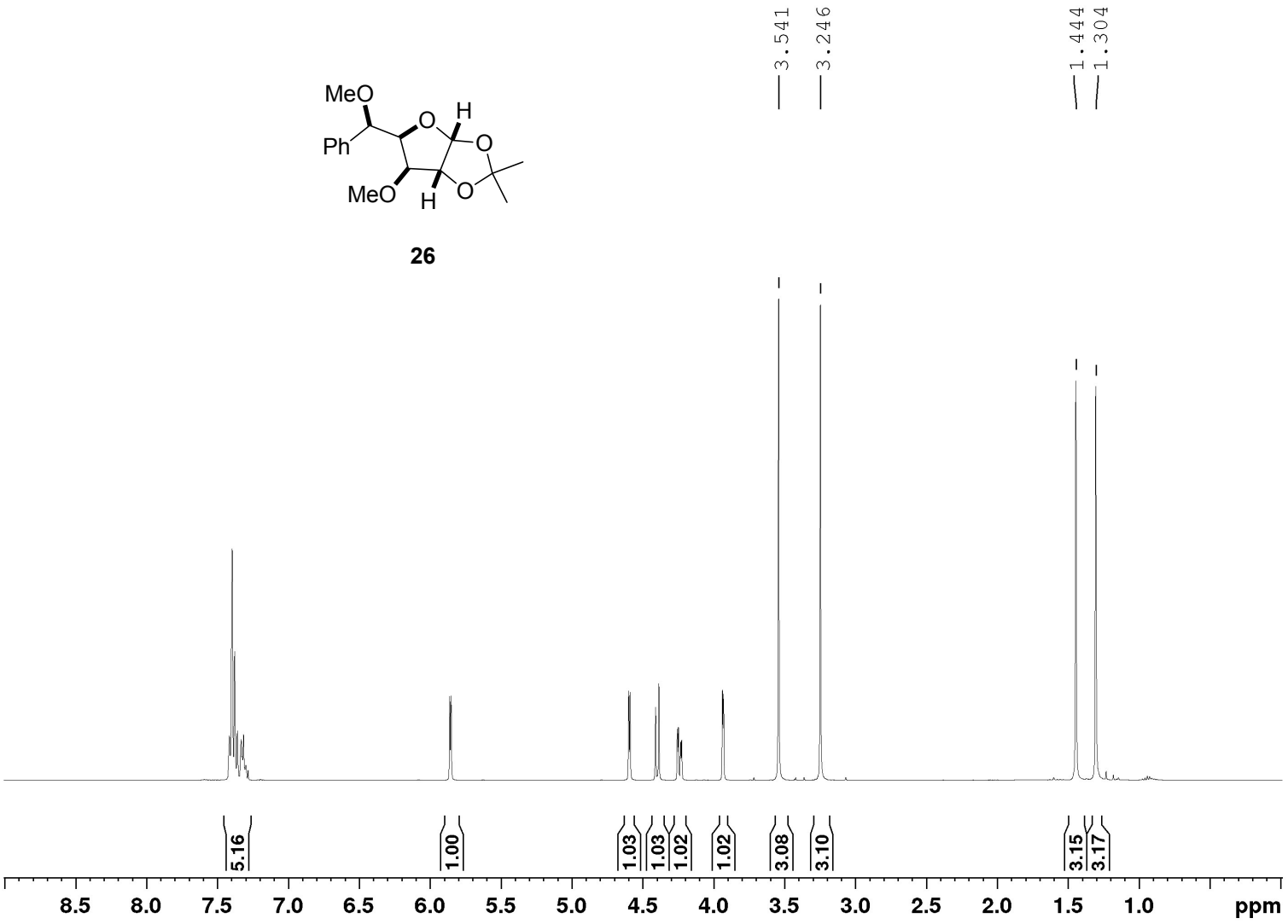
7

62.9 MHz ^{13}C NMR Spectrum of Compound 7 ($CDCl_3$)



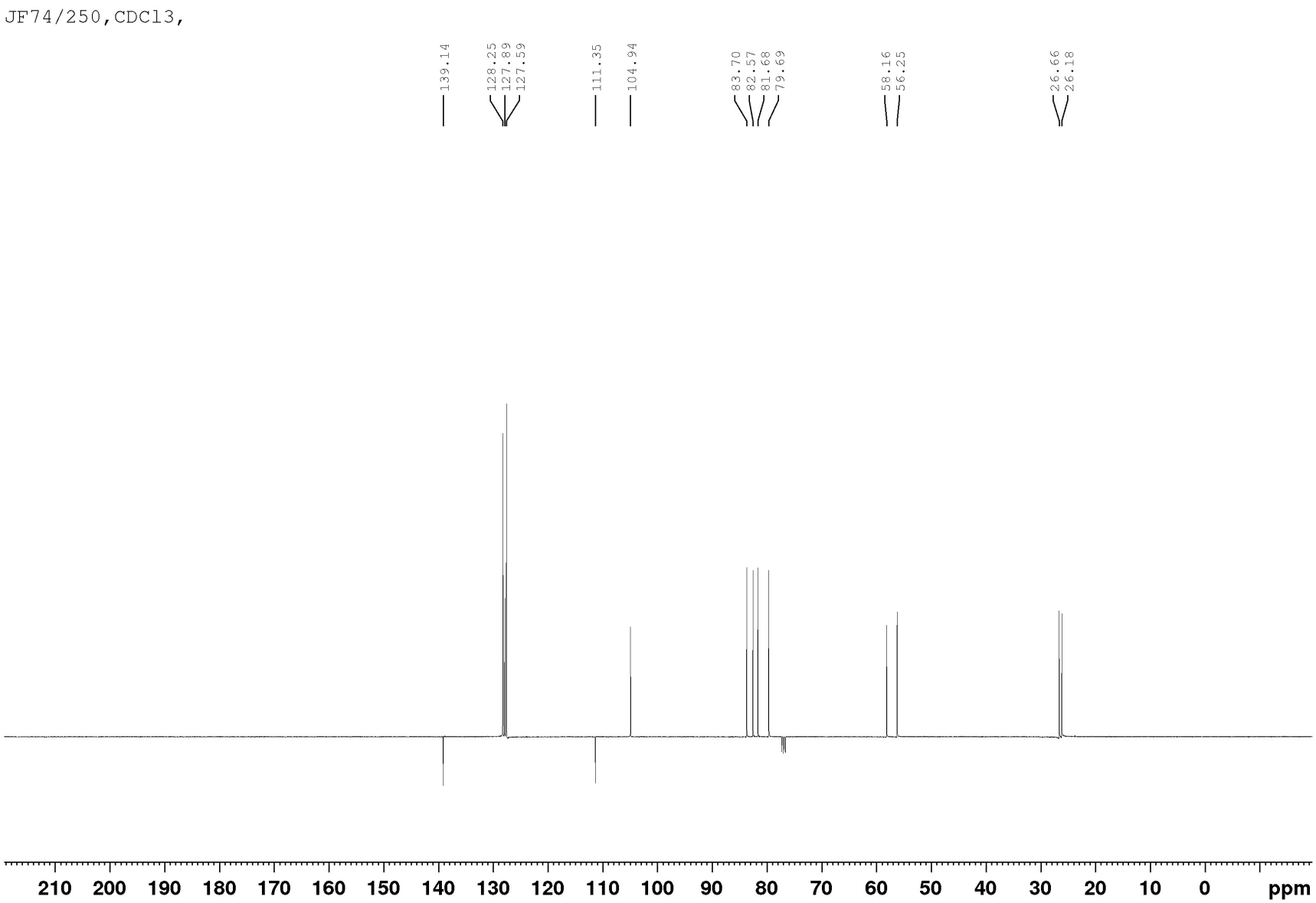
400 MHz ^{1}H NMR Spectrum of Compound 26 ($CDCl_3$)

JF74/250,CDCl3,



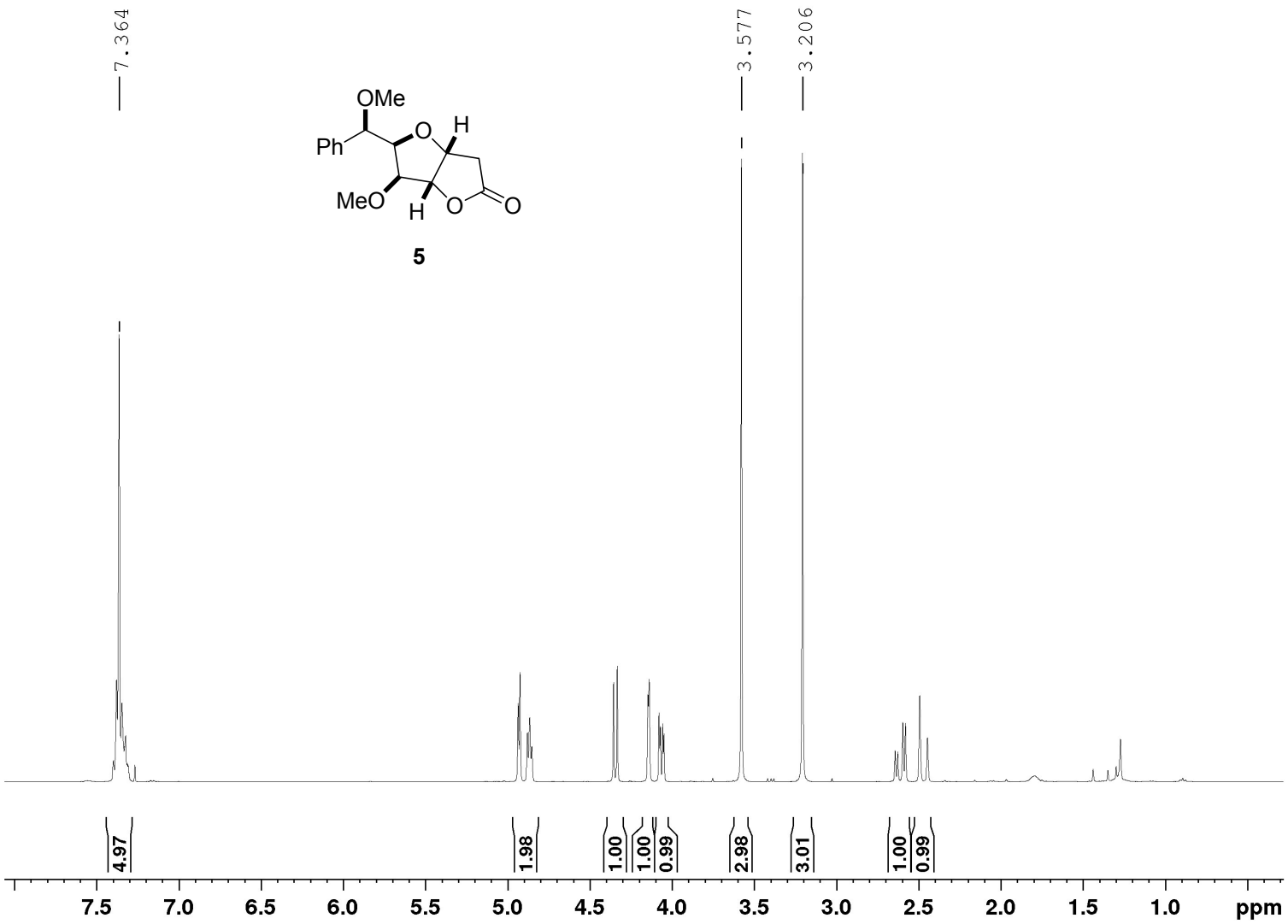
26

100 MHz ^{13}C NMR Spectrum of Compound 26 ($CDCl_3$)

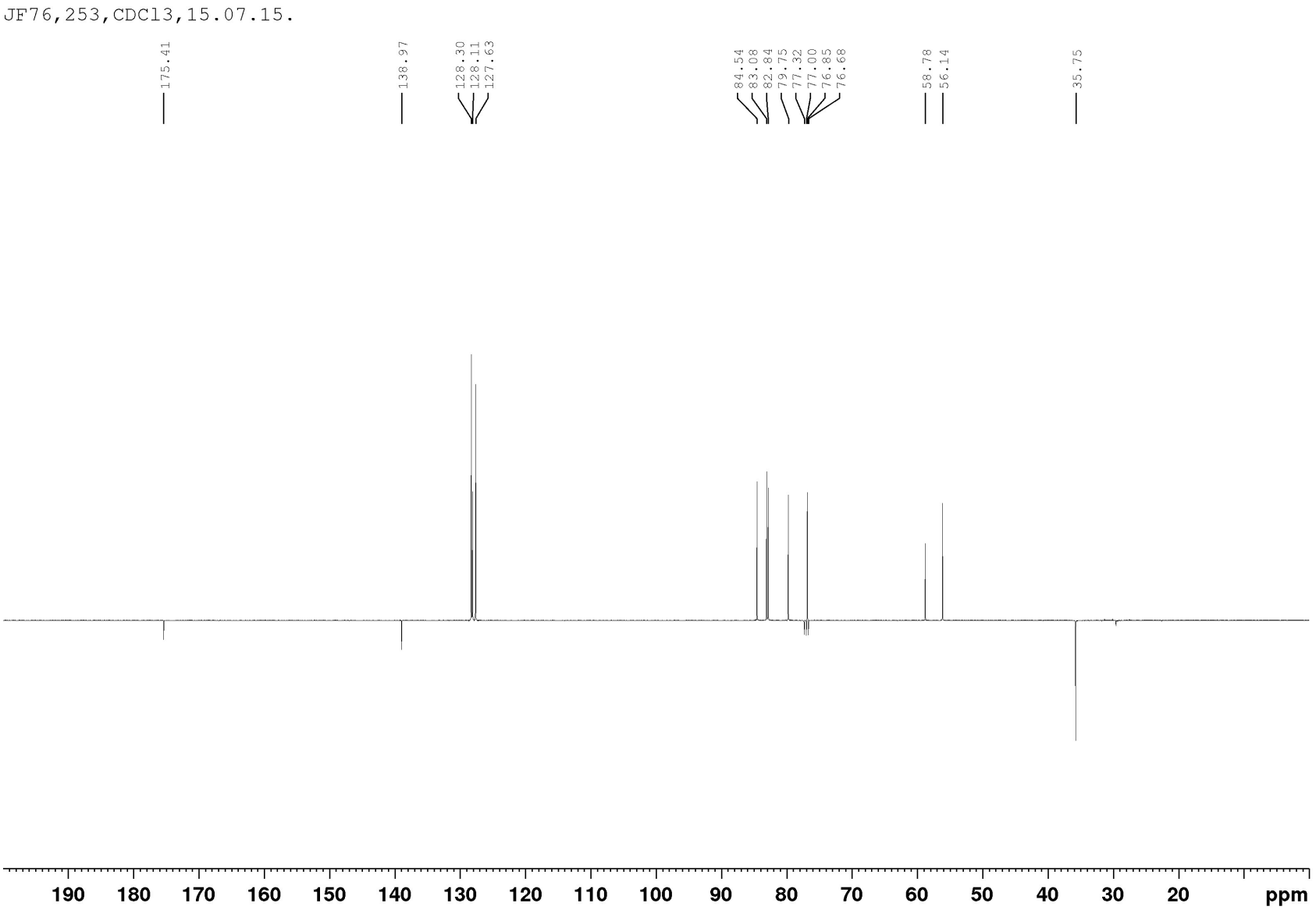


400 MHz ^{1}H NMR Spectrum of Compound 5 ($CDCl_3$)

JF76,253,CDCl3,15.07.15.

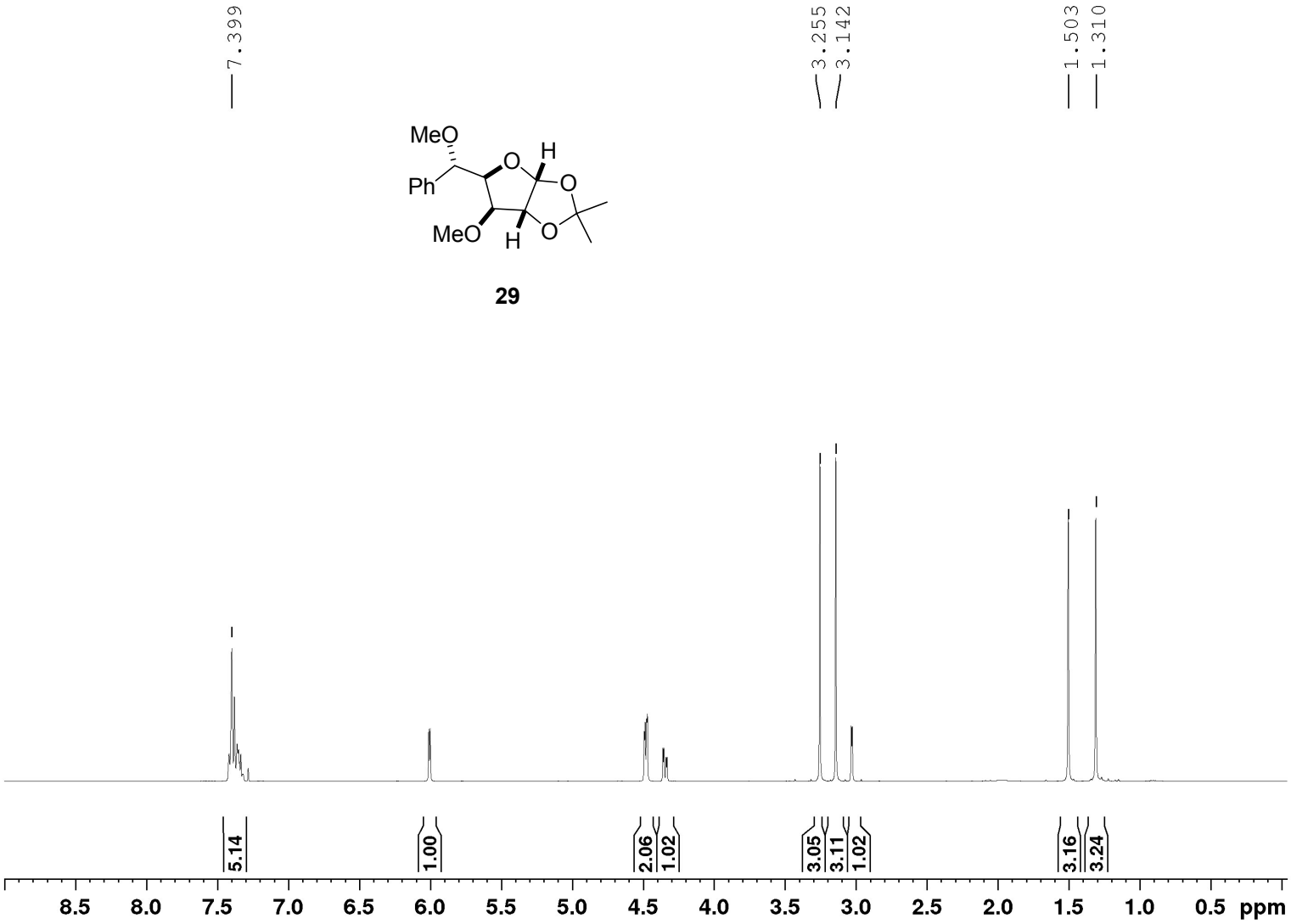


100 MHz ^{13}C NMR Spectrum of Compound 5 ($CDCl_3$)



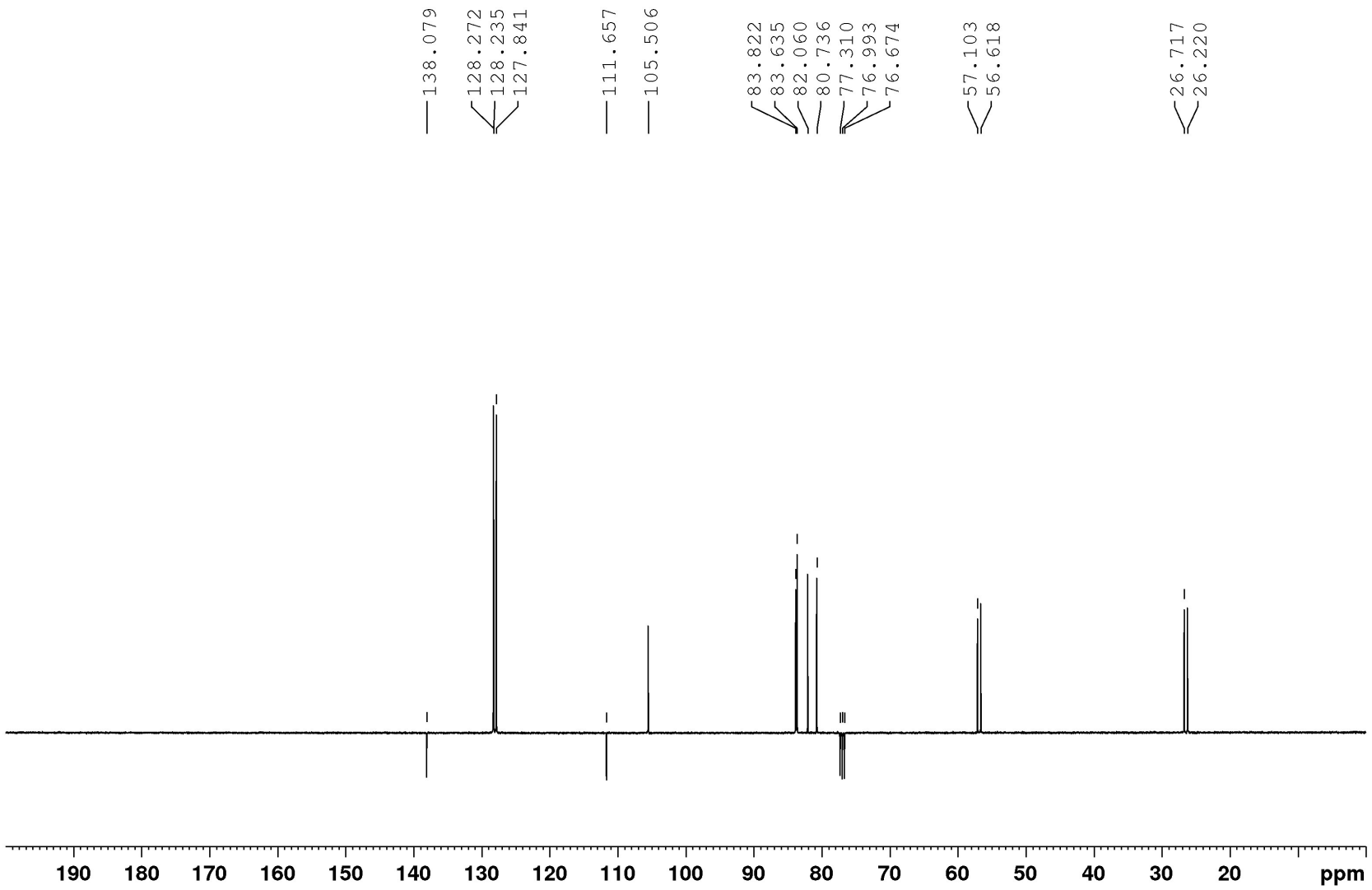
400 MHz ^{1}H NMR Spectrum of Compound 29 ($CDCl_3$)

JF77,254,CDCl3



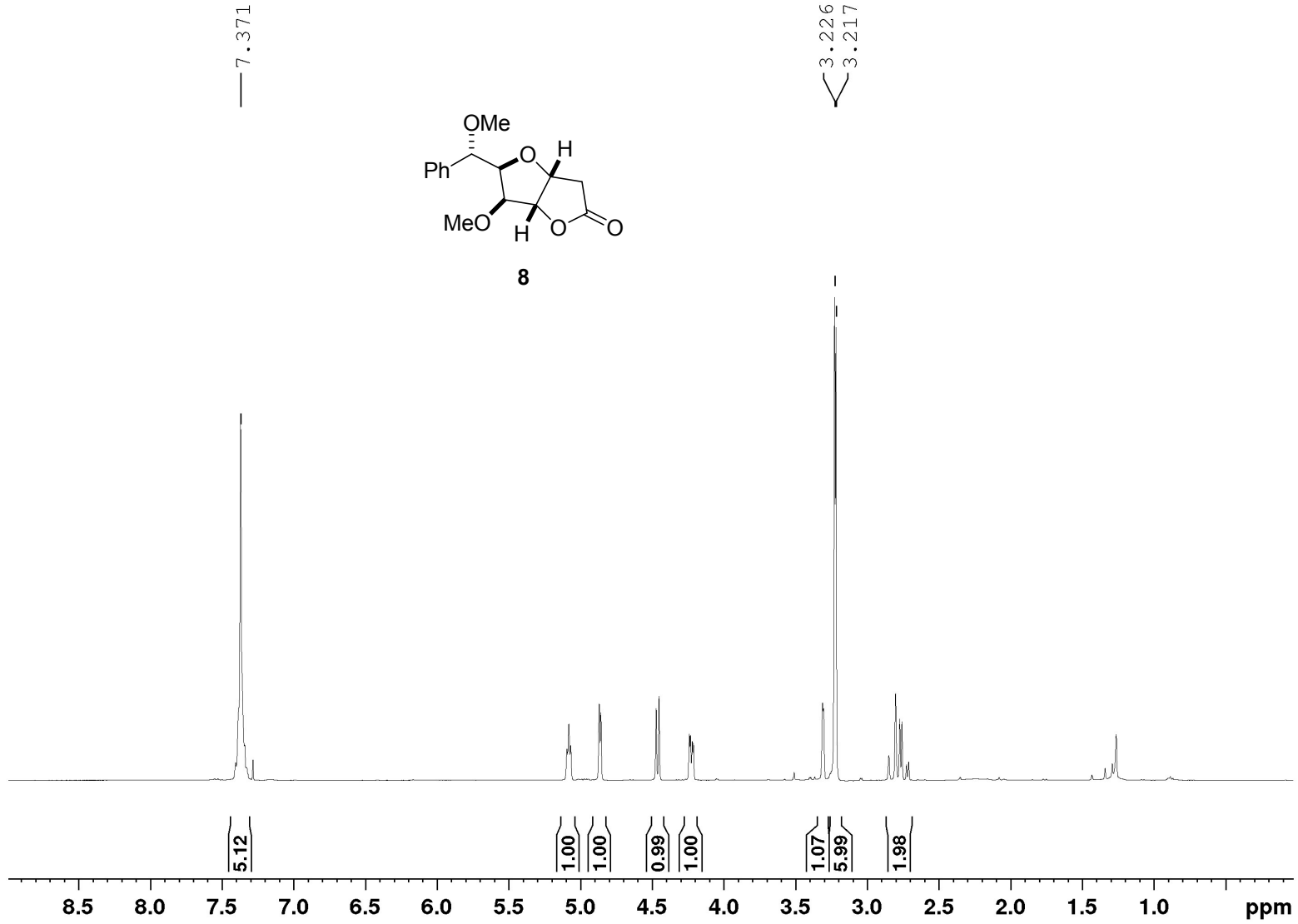
29

100 MHz ^{13}C NMR Spectrum of Compound 29 ($CDCl_3$)



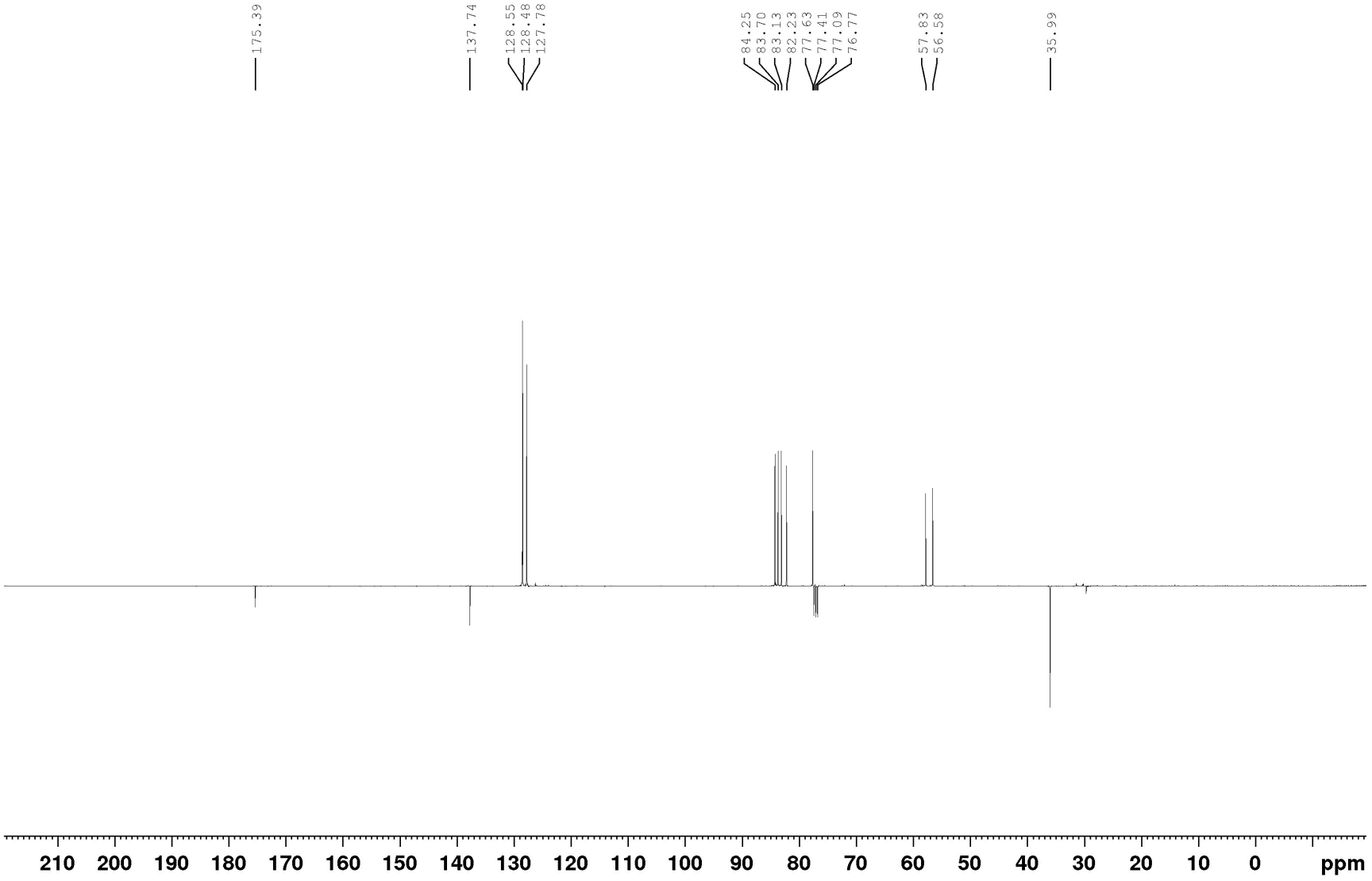
400 MHz ^{1}H NMR Spectrum of Compound 8 ($CDCl_3$)

JF79,257,CDCl3,2.7.15.

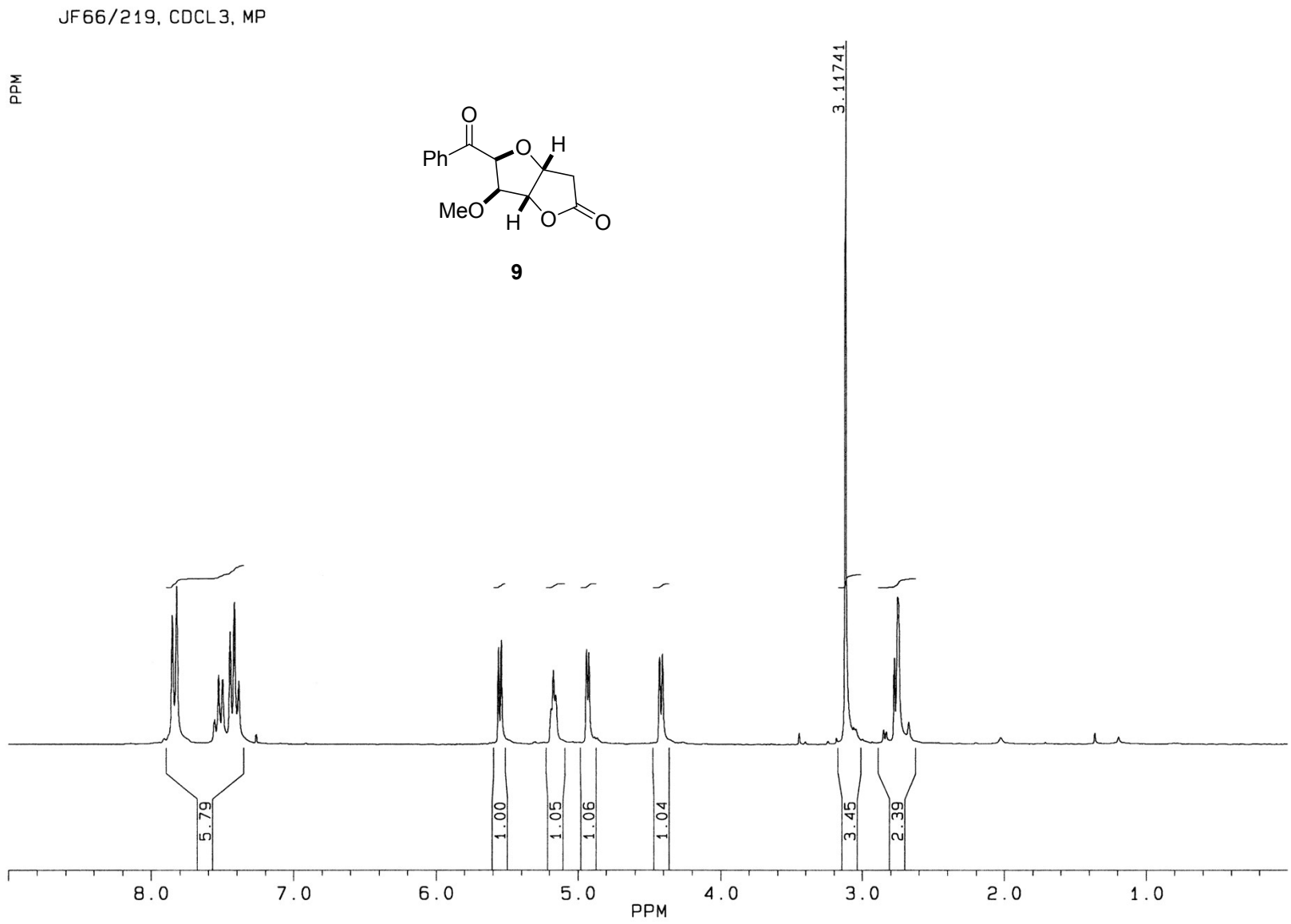


100 MHz ^{13}C NMR Spectrum of Compound 8 ($CDCl_3$)

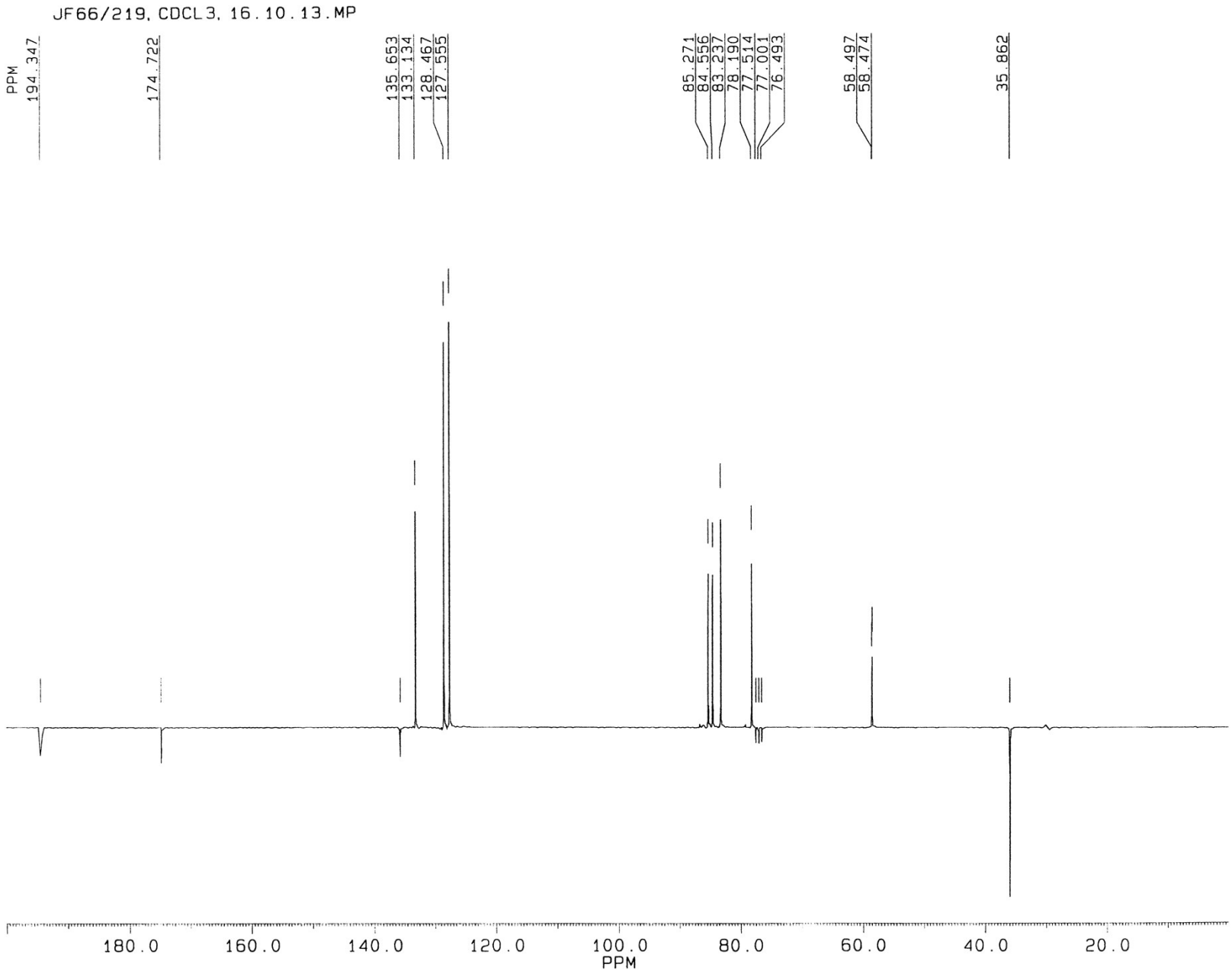
JF79,257,CDCl3,2.7.15.



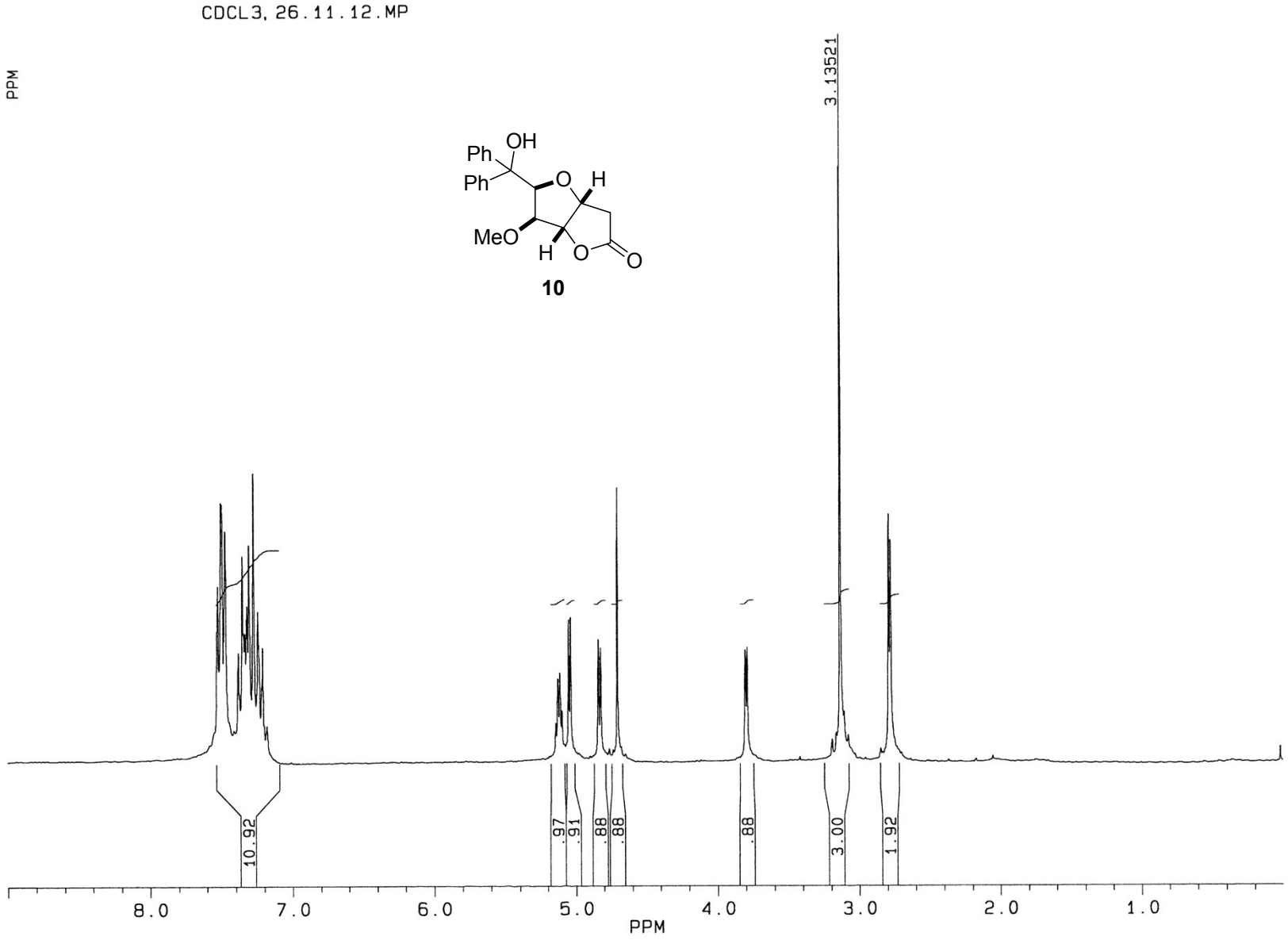
250 MHz ^{1}H NMR Spectrum of Compound 9 ($CDCl_3$)



62.9 MHz ^{13}C NMR Spectrum of Compound 9 ($CDCl_3$)



250 MHz ^{1}H NMR Spectrum of Compound 10 ($CDCl_3$)



62.9 MHz ^{13}C NMR Spectrum of Compound 10 ($CDCl_3$)