Electronic Supplementary Information for:

Kinetics of Curcumin Oxidation by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH$^{\bullet}$): an Interesting Case of Separated Coupled Proton-Electron Transfer

Mario C. Foti,[*,a] Adriana Slavova-Kazakova,[b] Concetta Rocco[a] and Vessela D. Kancheva[b]

[a] Istituto di Chimica Biomolecolare del CNR, Via P. Gaifami 18 – I 95126 Catania (Italy);

[b] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str., Bl.9 - 1113 Sofia (Bulgaria)

Email: mario.foti@cnr.it

The NMR spectra are in CDCl3 at room temperature.

Curcumin 1	13C	1H
C-3	183.16	
C-4′	147.75	
C-3′	146.69	
C-1	140.3	7.60
C-1′	127.60	
C-6′	122.76	7.20
C-2	121.69	6.48

C-5’	114.73	6.94
C-2’	109.55	7.09
C-4	101.0	5.81
-OCH3	55.87	3.96
Phenol OH		5.89
Enol OH		16.05

Compound 2	13C	1H
C-3	183.14	
C-3’	151.1	
C-4’	149.19	
C-1	140.26	7.60
C-1’	128.01	
C-6’	122.48	7.14
C-2	121.96	6.50
C-5’	111.11	6.88
C-2’	109.80	7.08
C-4	101.10	5.82
-OCH3	55.88/55.82	3.94/3.92

Compound 4	13C	1H
C-3	198.4	
C-4′	148.56	
C-3’	146.76	
C-1	144.47	7.66

C-1’	126.66	
C-6’	124.02	7.07
C-2	118.9	6.62
C-5’	114-74	6.87
C-2’	109.76	6.97
C-4	60.69	
-OCH3	55.96	3.88
-CH3	21.11	1.46
Phenolic OH	6.2	

Compound 5	13C	1H
C-3/5	183.32/183.01	
C-4”	150.93	
C-3’’	149.12	
C-4’	147.81	
C-3’	146.72	
C-1	140.50	7.61
C-7	140.25	7.78
C-1”	127.96	
C-1’	127.55	
C6’/C6’’	122.82	7.12 H-6’
C6’/C6’’	122.56	7.09 H-6’’
C2/C6	121.93	6.50 H-2
C2/C6	121.68	4.46 H-6
C-5’	114.75	6.92

C5″	111.03	6.86
C-2’	109.67	7.03
C-2″	109.56	7.07
C-4	101.14	5.80
-OCH3	55.86/55.83/55.79	3.93/3.91
Phenolic OH		6.01
Enol OH		16.05

compound 3 enol form	13C δ	1H δ
C-3	182.39	
C-3’	147.65	
C-4’	146.65	
C-1	141.30	7.69
C-1’	128.08	
C-6’	122.51	7.19
C-2	118.33	6.97
C-5’	114.75	6.95
C-2’	109.57	7.05
C-4	105.54	
-OCH3	55.91	3.96
-CH3	11.99	2.17
Phenolic OH		5.89
Enol OH		17.4

Compound 3 keto form	13C δ	1H δ
C-3	196.06	
C-3’	148.53	
C-4’	146.70	

C-1	144.76	7.63
C1’	126.66	
C-6’	124.14	7.11
C-2	121.07	6.69
C-5’	114.75	6.92
C-2’	109.57	7.04
C-4	58.8	4.16
-OCH3	55.91	3.93
OH		5.95
-CH3	13.07	1.49

Acetylated Curcumin	13C	1H
C-3	183.00	
CO (Ac)	168.68	
C-3’	151.32	
C-4’	141.22	
C-1	139.84	7.61
C-1’	133.87	
C-2	124.17	6.56
C-5’	123.19	7.06
C-6’	120.96	7.15
C-2’	111.38	7.12
C-4	101.69	5.85
-OCH3	55.81	3.88
Ac	20.70	2.33
Enol OH		15.89

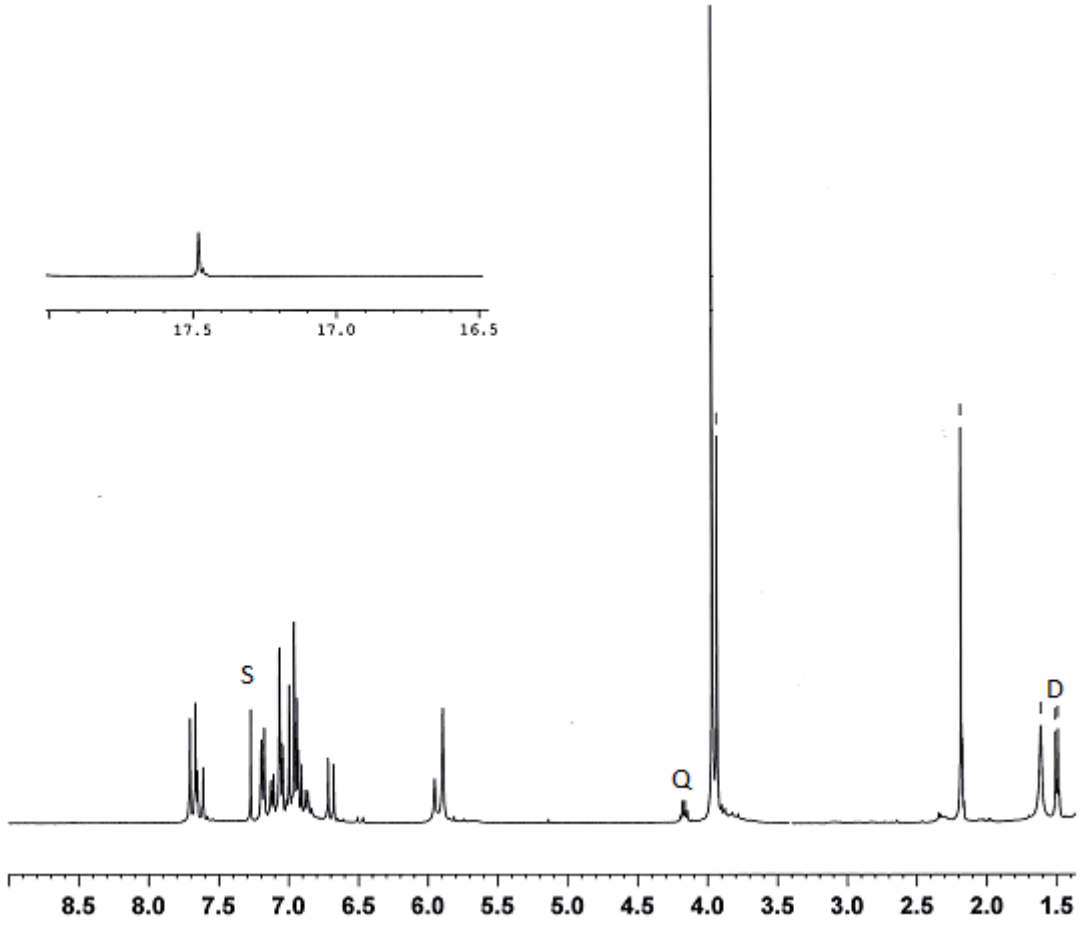
Acetylated 4	13C	1H
C-3	197.79	

C=O Ac	168.54	
C-3’	151.34	
C1	143.68	7.70
C-4’	141.84	
C-1’	133.03	
C-5’	123.19	7.02
C-6’	121.77	7.13
C-2	121.40	6.70
C-2’	111.74	7.06
C-4	60.86	
-OCH3	56.00	3.89
-CH3	20.92	1.49
Ac	20.51	2.32

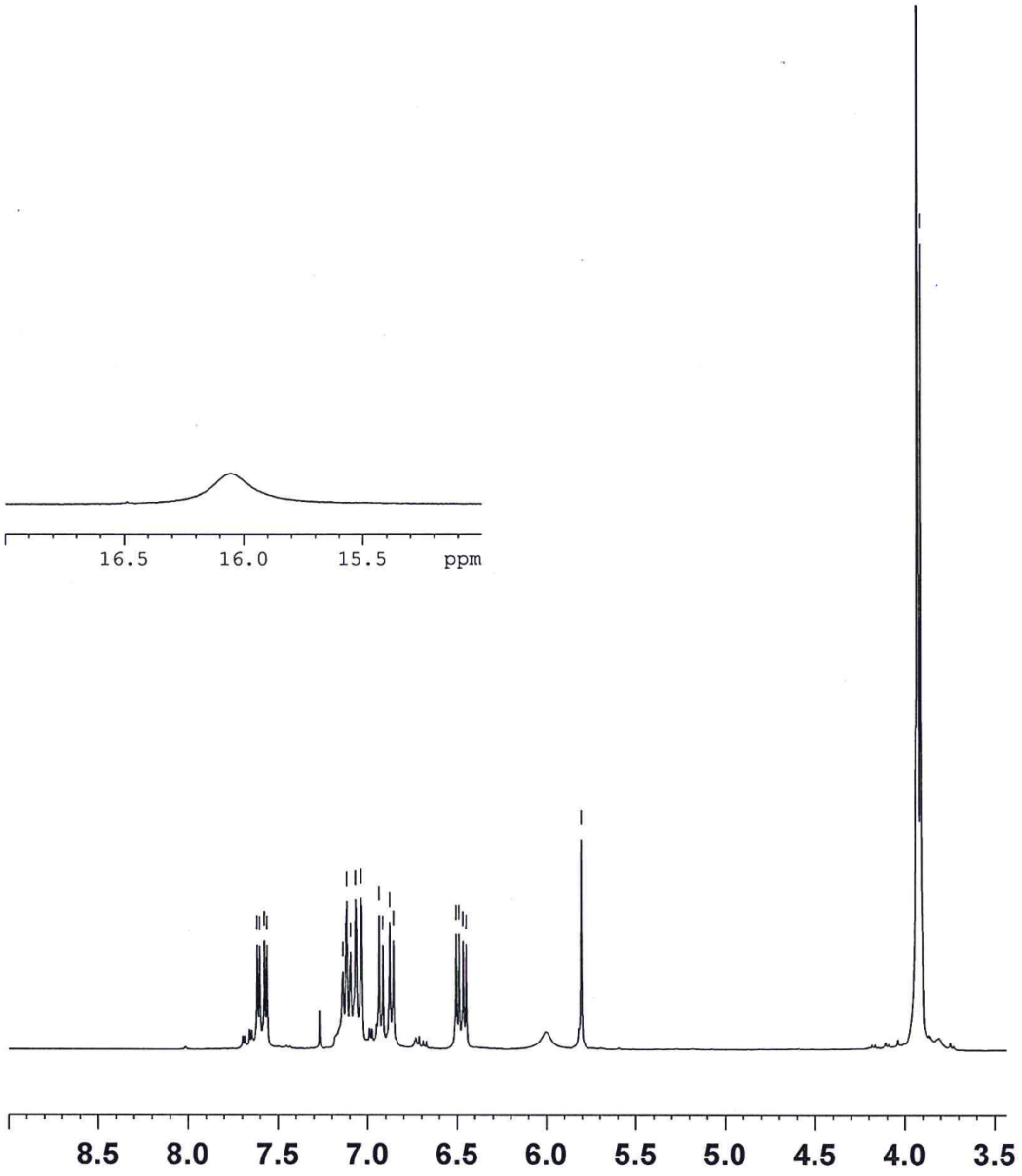
Acetylated 3 as enol	13C δ	1H δ
C-3	182.27	
CO Ac	168.6	
C-3’	151.31	
C-4’	141.1	
C-1	140.69	7.70
C-1’	134.37	
C-6’	120.74	7.21
C-2	123.16	7.06
C-5’	120.89	7.08
C-2’	111.79	7.14

C-4	106.3	
-OCH3	55.91	3.89
-CH3	12.07	2.18
Ac	20.9	2.33
-enol OH		17.26

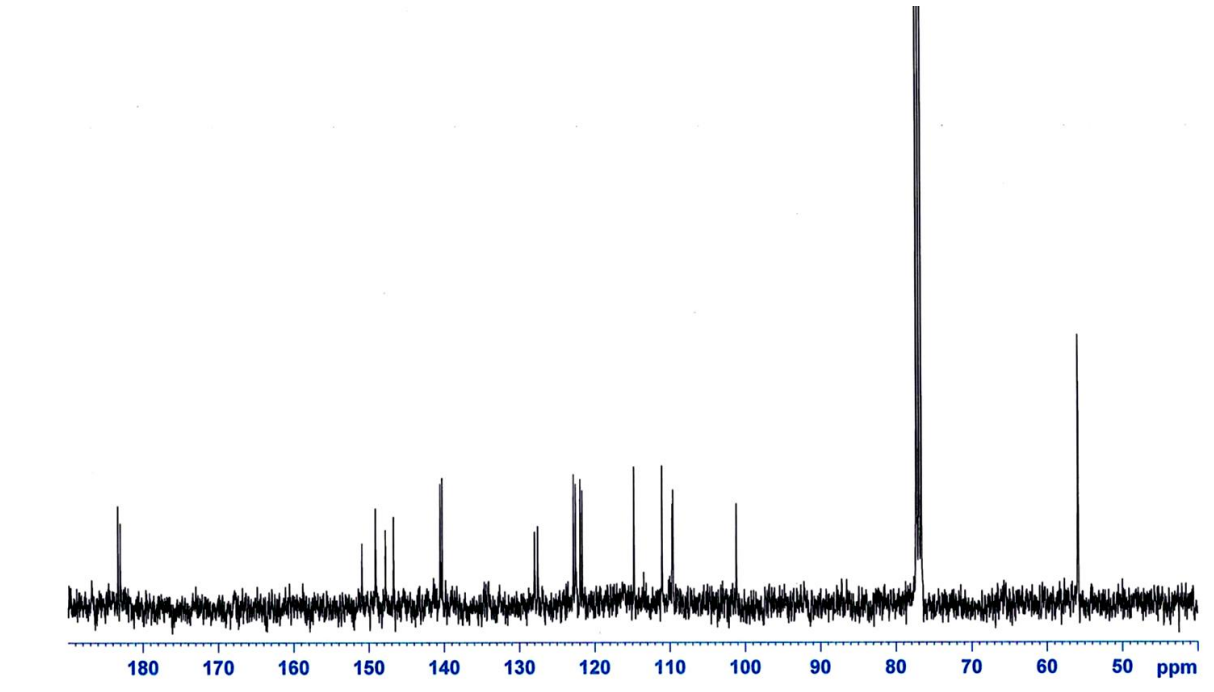
Acetylated Compound 3 as keto form	13C δ	1H δ
C-3	196.06	
CO Ac	168.4	
C-3’	151.31	
C-4’	141.1	
C-1	144.0	7.71
C1’	132.76	
C-6’	121.93	6.77
C-2	123.16	6.77
C-5’	122.0	6.92
C-2’	111.8	7.15
C-4	58.9	4.20
-OCH3	55.91	3.87
Ac	20.9	2.37
-CH3	12.07	1.49



Compound 3. 400 MHz ^{1}H NMR spectrum of compound **3** in $CDCl_3$ at room temperature. The two tautomeric forms **3a** and **3b** are present in solution in a 2.45:1 ratio. The signal of the CH_3 group at position 4 of the 1,6-heptadiene moiety is split in the *β*-diketo form into a doublet at about 1.5 ppm (peak D) by the methine proton at C-4, which in turn appears as a quartet (peak Q) at ca. 4.15 ppm. The CH_3 group of the enolic form appears as a singlet at 2.18 ppm. The methoxy groups of the two tautomeric forms are distinguishable since they resonate as singlets at 3.93 (*β*-diketo form) and 3.97 ppm (enol form). The peaks at ca. 5.9 ppm are due to the phenolic OHs of the two forms while the enolic OH gives a downfield signal at 17.49 ppm. The signals in the range 6.65 – 7.65 ppm (except for peak S) are due to the aromatic and diene moieties. The peaks at 1.61 and at 7.28 ppm (peak S) are due to water and chloroform, respectively, contained in the deuterated solvent.



Compound 5. 400 MHz ^{1}H NMR of **5** in $CDCl_3$ at room temperature. The spectrum shows the asymmetry of the two phenyl rings and diene bonds in the signals at 6.4 – 7.7 ppm. The singlet at 5.8 ppm is due to the C-H of the keto-enol moiety while the broad signal at 6.0 ppm is due to the phenolic O-H. The two singlets (one has a shoulder) at 3.9 ppm are due to the three methoxy groups. The broad downfield signal at 16.05 ppm is due to the enol O-H. The presence of *small* quantities of diketo form cannot be excluded because the impurities present in the sample prevent any possible assignment.



Compound 5. 100.62 MHz ^{13}C NMR of **5** in $CDCl_3$ at room temperature. The spectrum shows many split signals due to the asymmetry of the molecule, including the two signals at 183.36 and 183.05 ppm attributed to the keto-enol carbonyls of **5**.

Experimental Section: syntheses and characterization of compounds 2 – 5

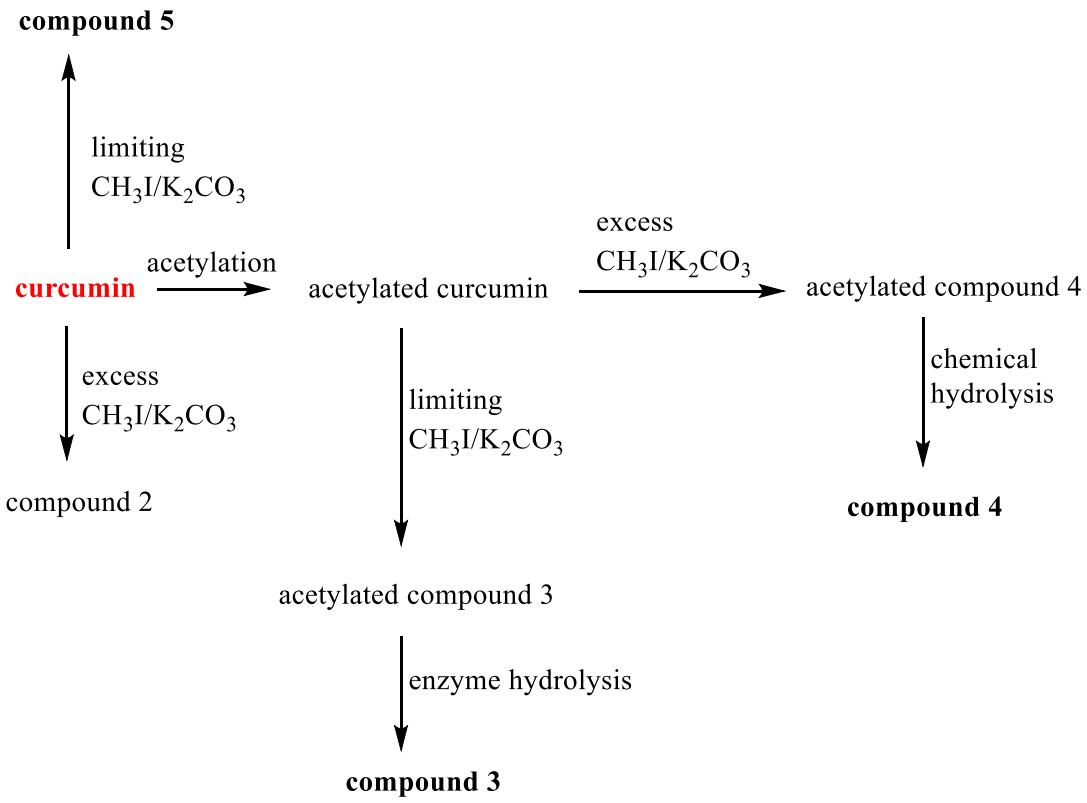
^{1}H NMR and ^{13}C NMR spectra. The spectra were recorded in $CDCl_3$ (room temperature) on a Bruker Avance^{TM} spectrometer at 400.13 (^{1}H) and 100.62 (^{13}C) MHz. The adopted numbering system for **1**, **2**, **3**, **4** is reported in Scheme 1 while that for **5** is reported in Scheme 2, see the paper.

Purified Curcumin. ^{1}H NMR spectrum in $CDCl_3$: δ = 3.96 (s, 6H, OCH_3), 5.81 (s, 1H, H_4), 5.85 (broad phenolic OH), 6.49 (d, J = 15.6 Hz, 2H, H_2), 6.94 (d, J = 8.4 Hz, 2H, $H_{5'}$), 7.06 (s, 2H, $H_{2'}$), 7.20 (d, J = 8.0 Hz, 2H, $H_{6'}$), 7.60 (d, J = 16.0 Hz, 2H, H_1), 16.05 (broad enolic OH).

For compounds **2 – 5** various syntheses are known, see 1) A. Sundaryono et al. *Photochem. Photobiol. Sci.*, 2003, **2**, 914–920; 2) B.M. Liu et al. *Chem. Pharm. Bull*. 2013, **61**, 757; 3) C. Changtam et al. *Eur. J. Med. Chem*. 2010, **45**, 4446-4457.

Compound **4** can also be purchased from Sigma-Aldrich (commercial name FLLL31).

We have adopted the syntheses described below because they are simple and the yields are good. See the following scheme:



Synthesis of compound 2. 150 mg of purified curcumin (0.41 mmol) were solubilized in 30 ml of acetone containing 2 ml of DMF. Then, 450 mg of K_2CO_3 (3.26 mmol) and 400 µL of CH_3I were added. The mixture was left under stirring at room temperature for 3 hours and then was filtered and neutralized with 0.1 N HCl. Extractive workup with dichloromethane gave a residue which was roughly purified over silica gel 25-40 µm using a gradient of ethyl acetate (20 to 30%) in n-hexane. The fractions containing compound **2** were mixed and were purified again over silica gel 25-40 µm using a gradient of dichloromethane (60 – 100 %) in n-hexane. The final yield was 20%. ^{1}H NMR spectrum in $CDCl_3$: δ = 3.93 (s, 6H, OCH_3), 3.94 (s, 6H, OCH_3), 5.83 (s, 1H, H_4), 6.50 (d, J = 15.6 Hz, 2H, H_2), 6.88 (d, J = 8.4 Hz, 2H, $H_{5'}$), 7.09 (s, 2H, $H_{2'}$), 7.15 (d, J = 8.0 Hz, 2H, $H_{6'}$), 7.61 (d, J = 16.0 Hz, 2H, H_1), 16.05 (broad enolic OH).

Acetylation of curcumin 1. 400 mg of purified curcumin (1.09 mmol) were dissolved in 100 ml of CH_2Cl_2 containing 1 ml of pyridine and 1 ml of acetic anhydride. The solution was kept

at reflux under stirring for 7 hours and then was neutralized with 0.1 N HCl and was extracted with CH_2Cl_2. The residue obtained after solvent removal was constituted by di-acetylated curcumin which was used in the syntheses of **3** and **4**. ^{1}H NMR spectrum in $CDCl_3$: δ = 2.33 (s, 6H, CH_3COO-), 3.88 (s, 6H, CH_3O), 5.86 (s, 1H, H_4), 6.56 (d, J = 15.6 Hz, 2H, H_2), 7.06 (d, J = 8.4 Hz, 2H, $H_{5'}$), 7.12 (s, 2H, $H_{2'}$), 7.15 (d, J = 8.4 Hz, 2H, $H_{6'}$), 7.61 (d, J = 16.0 Hz, 2H, H_1), 15.87 (broad enolic OH).

Synthesis of acetylated compound 3. 380 mg of acetylated curcumin (0.81mmol) were solubilized in 65 ml of acetone. Afterwards, 300 mg of K_2CO_3 (2.17 mmol) and 400 μl of CH_3I were added and the reaction at reflux (80 °C; *shielded light*) was followed by ^{1}H NMR spectroscopy until the signal at 17.26 ppm due to the enolic OH of the acetylated compound **3** was observed to increase. The NMR spectra were done in a coaxial NMR tube containing a deuterated solvent. Therefore, the reaction was stopped after about two hours and 15 minutes by cooling the flask down to room temperature. The mixture was then neutralized with 0.1 N HCl and was extracted with CH_2Cl_2. After solvent removal, the residue was purified over silica gel 40-63 μm with a gradient of acetone (20 – 25 %) in cyclohexane *under shielded light*. The yield was ca. 50%. It is worthy of being noted that this compound appeared on TLC as two separated spots due to the keto-enol and diketo tautomeric forms because the proton-shift equilibrium is slow. The ratio keto-enol:di-keto (KE:KK) was 1:0.33. ^{1}H NMR spectrum in $CDCl_3$: δ = 1.50 (d, J = 6.9 Hz, CH_3-4 KK), 2.19 (s, CH_3-4, KE), 2.33 (s, CH_3COO- KK), 2.37 (s, CH_3COO- KE), 3.87 (s, CH_3O KK), 3.90 (s, CH_3O KE), 4.20 (q, J = 6.9 Hz, H_4 KK), 6.76 (d, J = 14.7 Hz, H_2 KK), 7.04 – 7.20 (H_2 of KE and aromatic signals of KE + KK), 7.6 – 7.8 (H_1 of KE and KK), 17.26 (sharp enolic OH).

Deacetylation of the above compound. Deacetylation of the above compound was done enzymatically (Lipozyme® 42 U/g, immobilized from *Mucor miehei*) because chemical

hydrolysis with K_2CO_3 gave a complex mixture of compounds. To this end, 15 mg of acetylated compound **3** (0.03 mmol) were put in an Erlenmeyer flask with a plug seal cup and were solubilized in 15 ml of *tert*-butyl methyl ether. Then, 15 μL of n-butanol and 30 mg of Lipozyme from *Mucor miehei* were added. The Erlenmeyer flask was therefore put in a shaker at 25 °C and 300 rounds per minute for 20 hours e 30 minutes. Successively, the mixture was filtered and the solvent removed to give compound **3** as a mixture of the two tautomeric forms in a ratio keto-enol:diketo (KE:KK) of *ca*. 1:0.44. ^{1}H NMR spectrum in $CDCl_3$: δ = 1.50 (d, J = 6.8 Hz, CH_3-4 KK), 2.18 (s, CH_3-4 KE), 3.93 (s, CH_3O KK), 3.97 (s, CH_3O KE), 4.15 (q, J = 6.8 Hz, H_4 KK), 5.85 (s, phenolic OH KE), 5.95 (s, phenolic OH KK), 6.70 (d, J = 16.7 Hz, H_2 KK), 6.97 – 7.2 (H_2 of KE and aromatic signals of KE + KK), 7.63 – 7.70 (H_1 of KK and KE), 17.47 (sharp enolic OH).

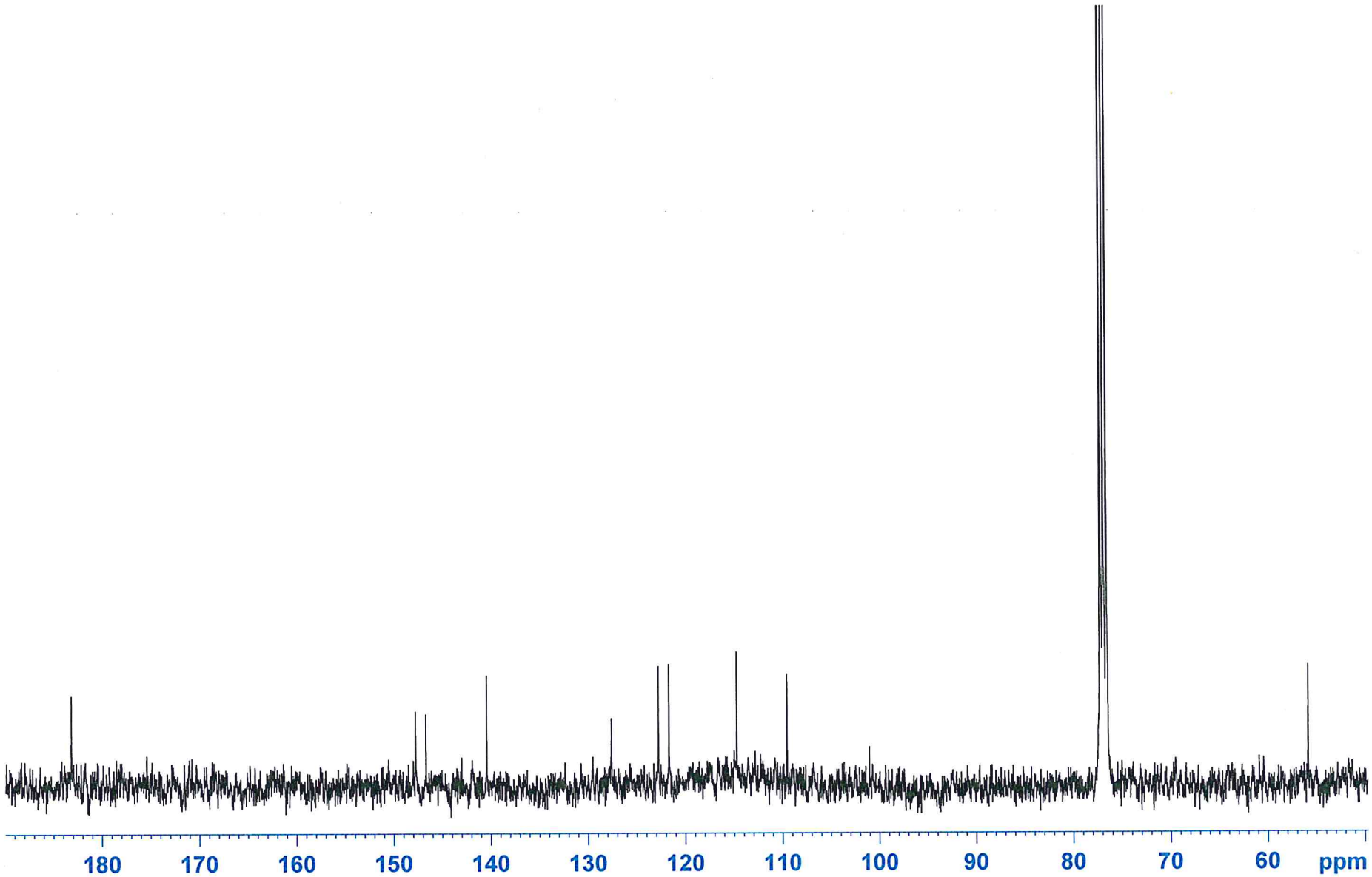
Synthesis of acetylated compound 4. 200 mg of acetylated curcumin (0.44 mmol) were dissolved in 50 ml of acetone. Then, 500 mg of K_2CO_3 and 900 μL of CH_3I were added and the mixture was kept at reflux under stirring for about 2 hours. Subsequently, the mixture was cooled down to room temperature, filtrated, concentrated and finally neutralized with 0.1 N HCl. The mixture was extracted with aliquots of CH_2Cl_2 and the organic layers were recombined and then were washed with a solution of $NaHCO_3$. After solvent removal, the residue constituted of acetylated compound **4** plus a small quantity of acetylated compound **3** was purified over silica gel 40-63 μm with 25% acetone in n-hexane. Final yield ca. 50%. ^{1}H NMR spectrum in $CDCl_3$: δ = 1.49 (s, 6H, CH_3-4), 2.31 (s, 6H, CH_3COO-), 3.86 (s, 6H, CH_3O), 6.71 (d, J = 15.6 Hz, 2H, H_2), 7.03 (d, J = 8.0 Hz, 2H, $H_{5'}$), 7.07 (s, 2H, $H_{2'}$), 7.13 (d, J = 8.0 Hz, 2H, $H_{6'}$), 7.69 (d, J = 15.6 Hz, 2H, H_1).

Deacetylation of the above compound. 45 mg of acetylated **4** were dissolved in a solvent mixture made of 10 ml of acetone and 2 ml of methanol. Subsequently, 100 mg of Cs_2CO_3

were added to the solution and the mixture was kept at reflux for about 10 minutes. Then, the solvent was removed and the residue was first neutralized with 0.1 N HCl and after was extracted with CH_2Cl_2. The organic layer was washed with a solution of $NaHCO_3$ and then was dried over Na_2SO_4. Solvent removal afforded a residue constituted of pure **4**. ^{1}H NMR spectrum in $CDCl_3$: δ = 1.48 (s, 6H, CH_3-4), 3.88 (s, 6H, CH_3O), 6.03 (s, phenolic OH), 6.63 (d, *J* = 15.2 Hz, 2H, H_2), 6.89 (d, *J* = 8.0 Hz, 2H, $H_{5'}$), 6.99 (s, 2H, $H_{2'}$), 7.09 (d, *J* = 8.4 Hz, 2H, $H_{6'}$), 7.67 (d, *J* = 15.6 Hz, 2H, H_1).

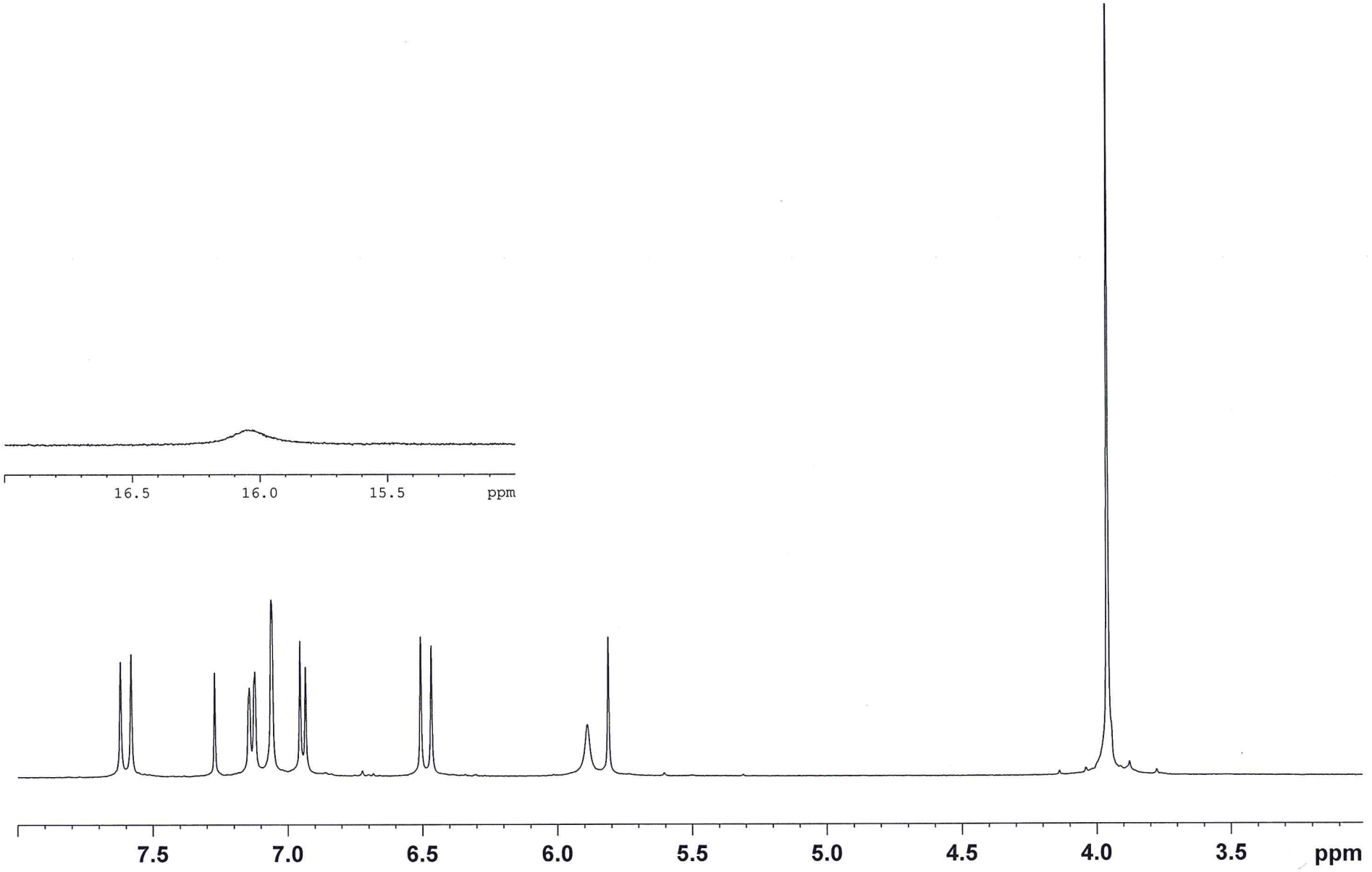
Synthesis of compound 5. 150 mg of purified curcumin were dissolved in 30 mL of acetone. After the addition of 350 mg of K_2CO_3, a solution of CH_3I (400 μL of CH_3I in 20 ml of acetone) was added drop by drop under stirring at room temperature in about 20 minutes. Then, the solution was filtrated and the solvent partially removed. The final solution was neutralized with 0.1 N HCl and then was extracted with CH_2Cl_2. The organic layers were combined and the solvent removed. The residue was purified over silica gel 40 – 63 μm with 25% acetone in *n*-hexane. Final yield ca. 40%. ^{1}H NMR spectrum in $CDCl_3$: δ = 3.91 (s, 3H, OCH_3), 3.93 (s with a shoulder, 6H, OCH_3), 5.81 (s, 1H, H_4), 6.0 (broad phenolic OH), 6.47 (d, *J* = 16.0 Hz, 1H, H_6), 6.49 (d, *J* = 15.6 Hz, 1H, H_2), 6.87 (d, J = 8.4 Hz, 1H, $H_{5''}$), 6.93 (d, J = 8.4Hz, 1H, $H_{5'}$), 7.04 (s, 1H, $H_{2'}$), 7.07 (s, 1H, $H_{2''}$), 7.11 (d, *J* = 8.4 Hz, 1H, $H_{6''}$), 7.13 (dd, *J* = 8.4 Hz, 1H, $H_{6'}$), 7.58 (d, *J* = 15.6 Hz, 1H, H_7), 7.60 (d, *J* = 16.0 Hz, 1H, H_1), 16.05 (broad enolic OH).

CURCUMIN



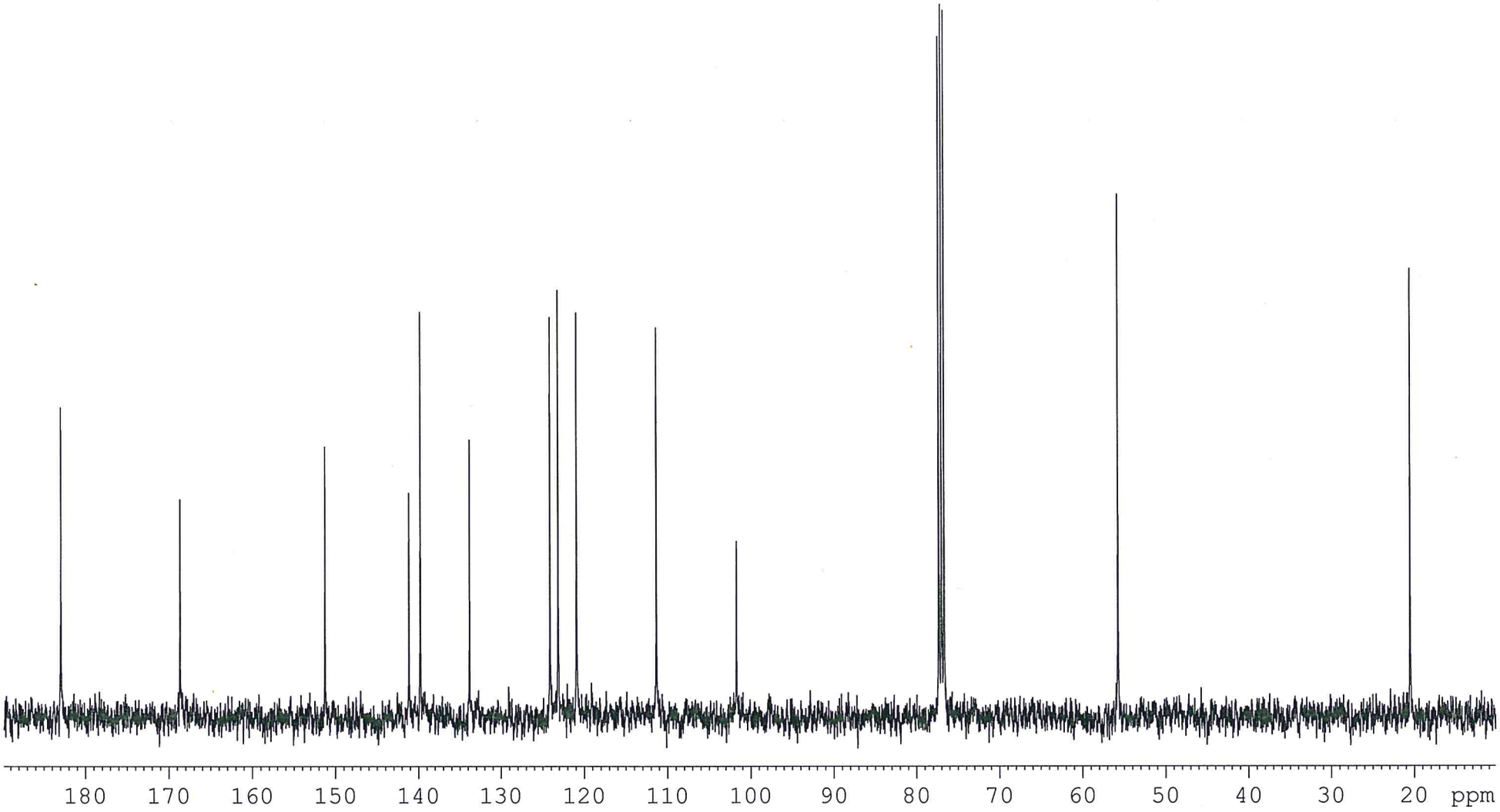
curcumin

CURCUMIN



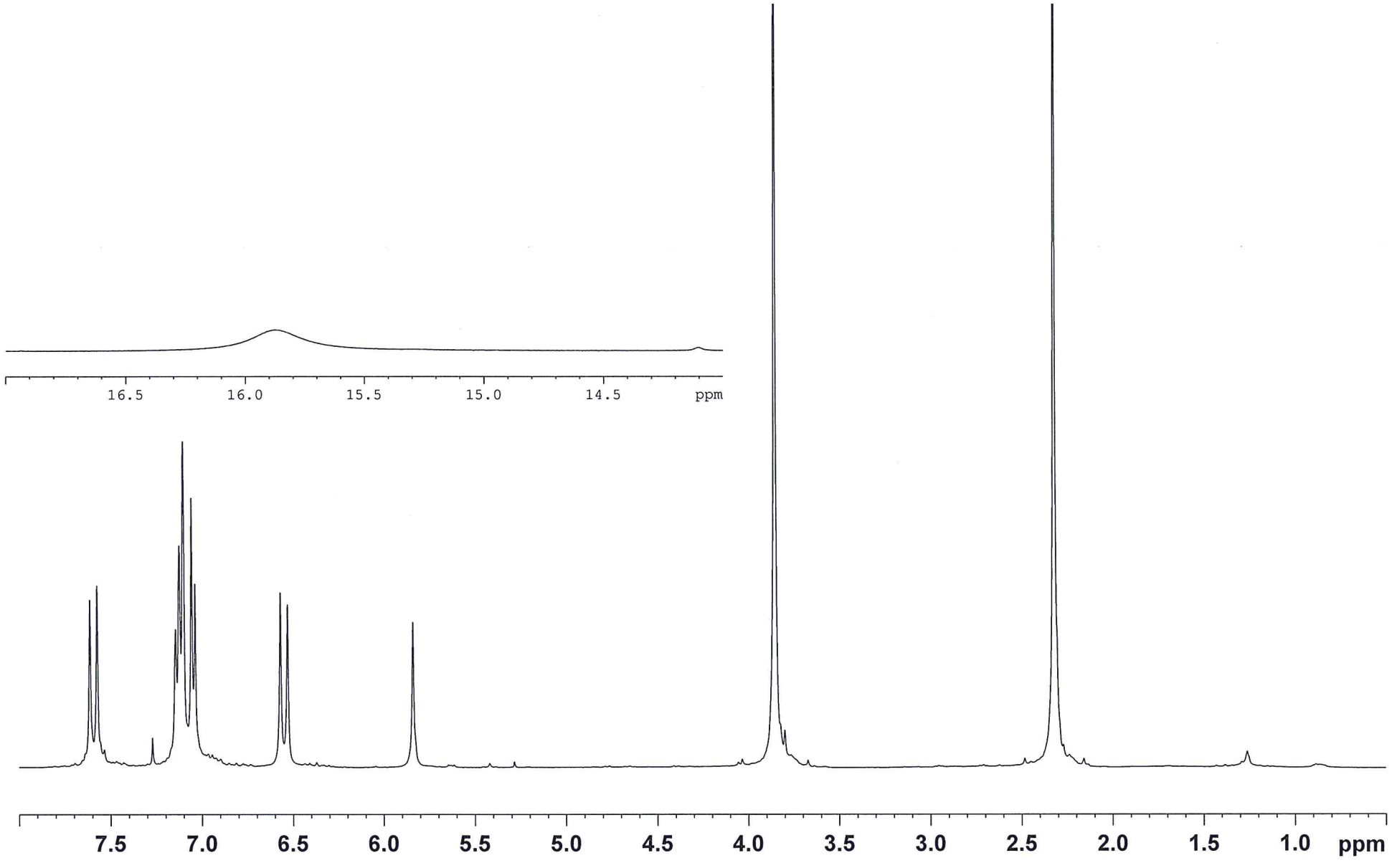
Curcumin Ac

ACETYLATED CURCUMIN



Curcumin Ac

ACETYLATED CURCUMIN



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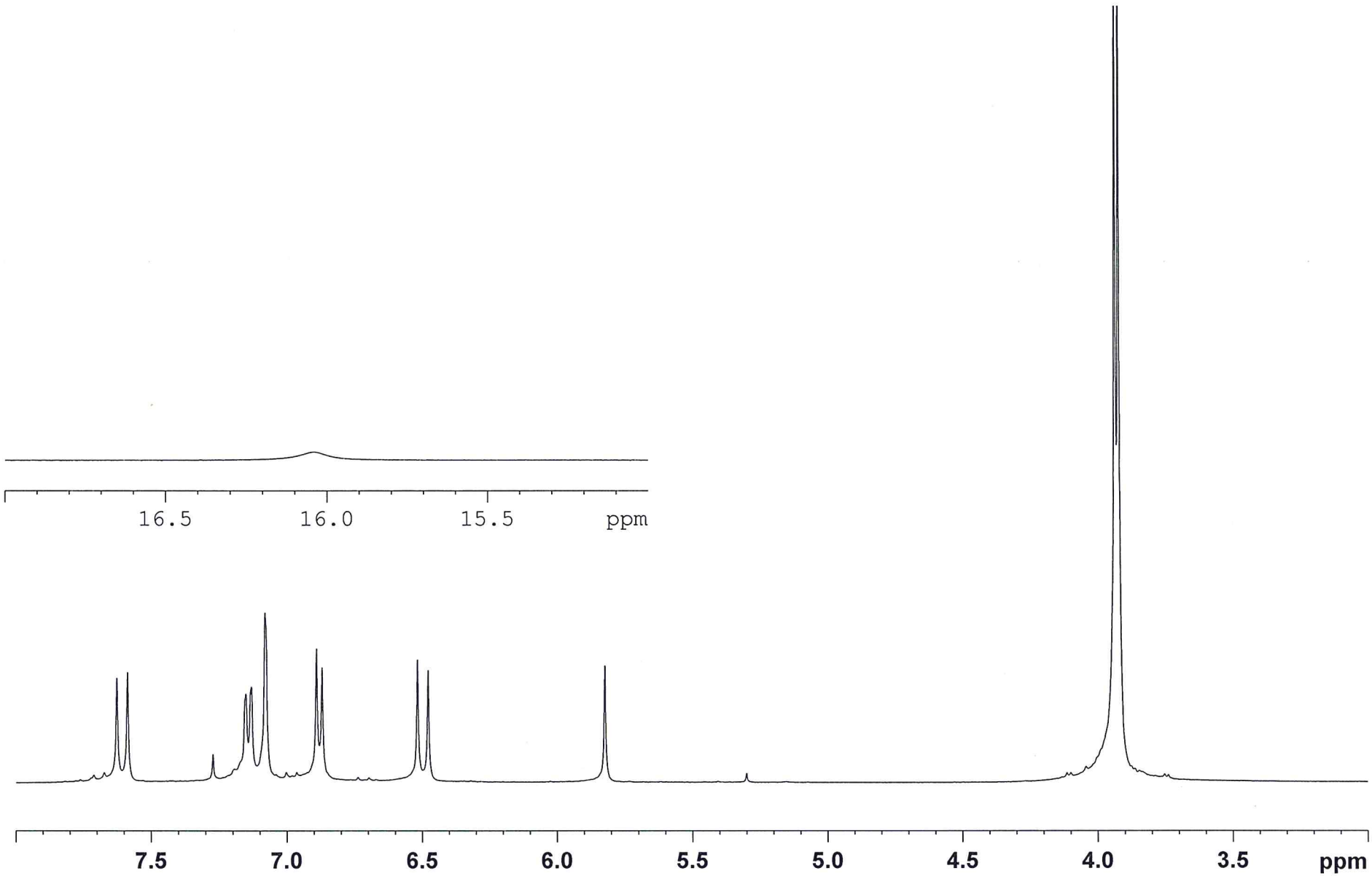
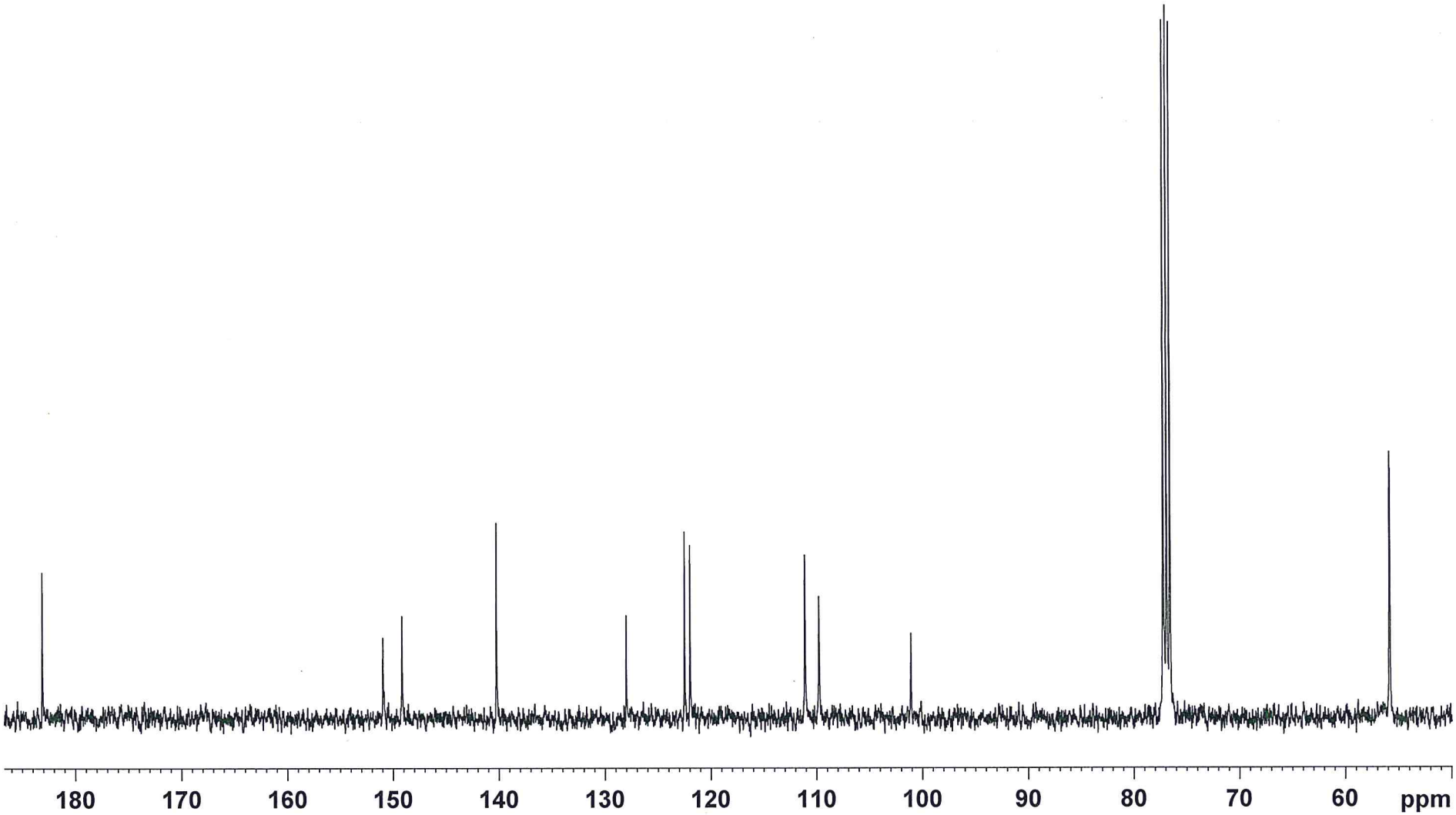


Comp 2

COMPOUND 2



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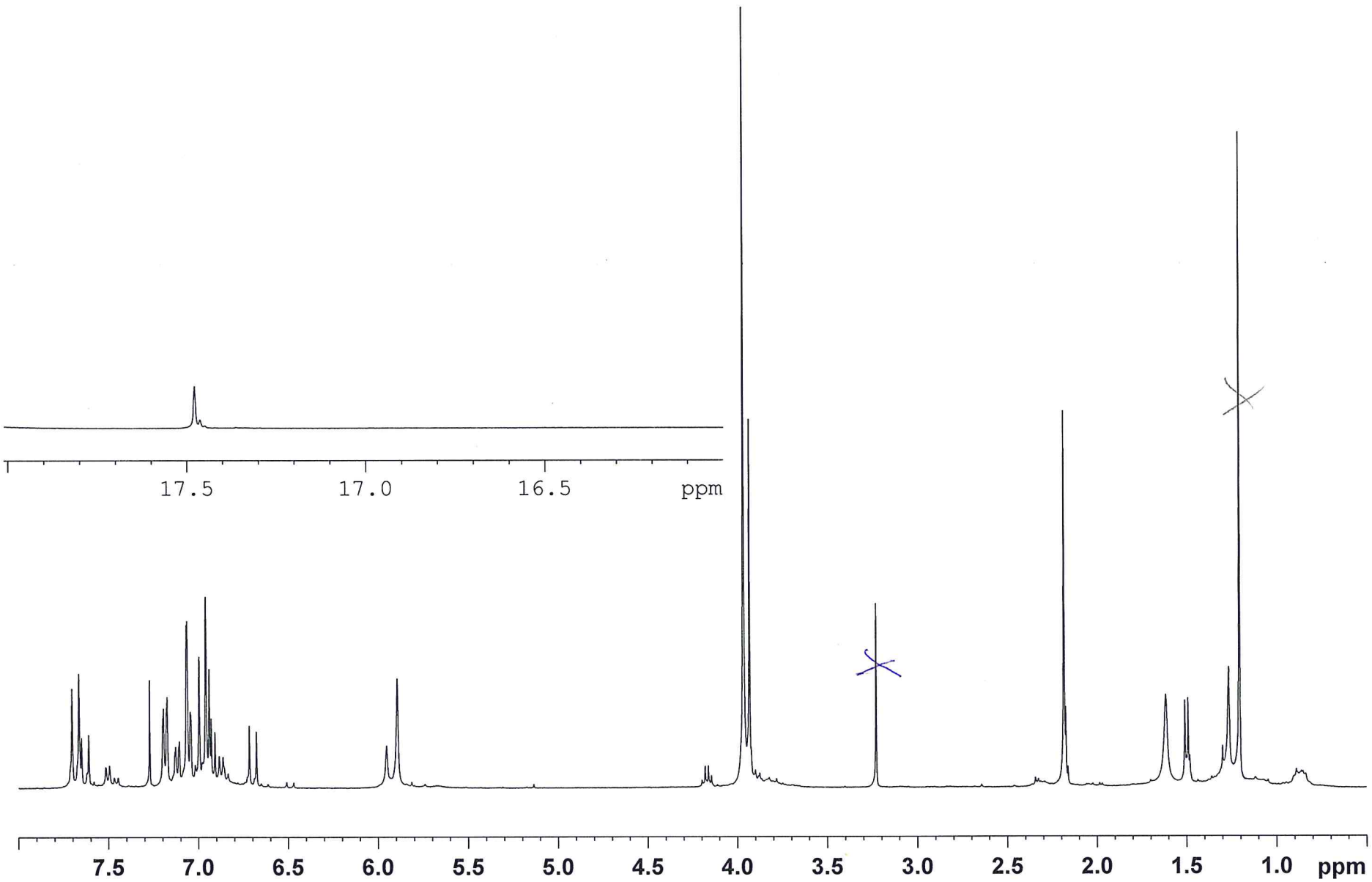

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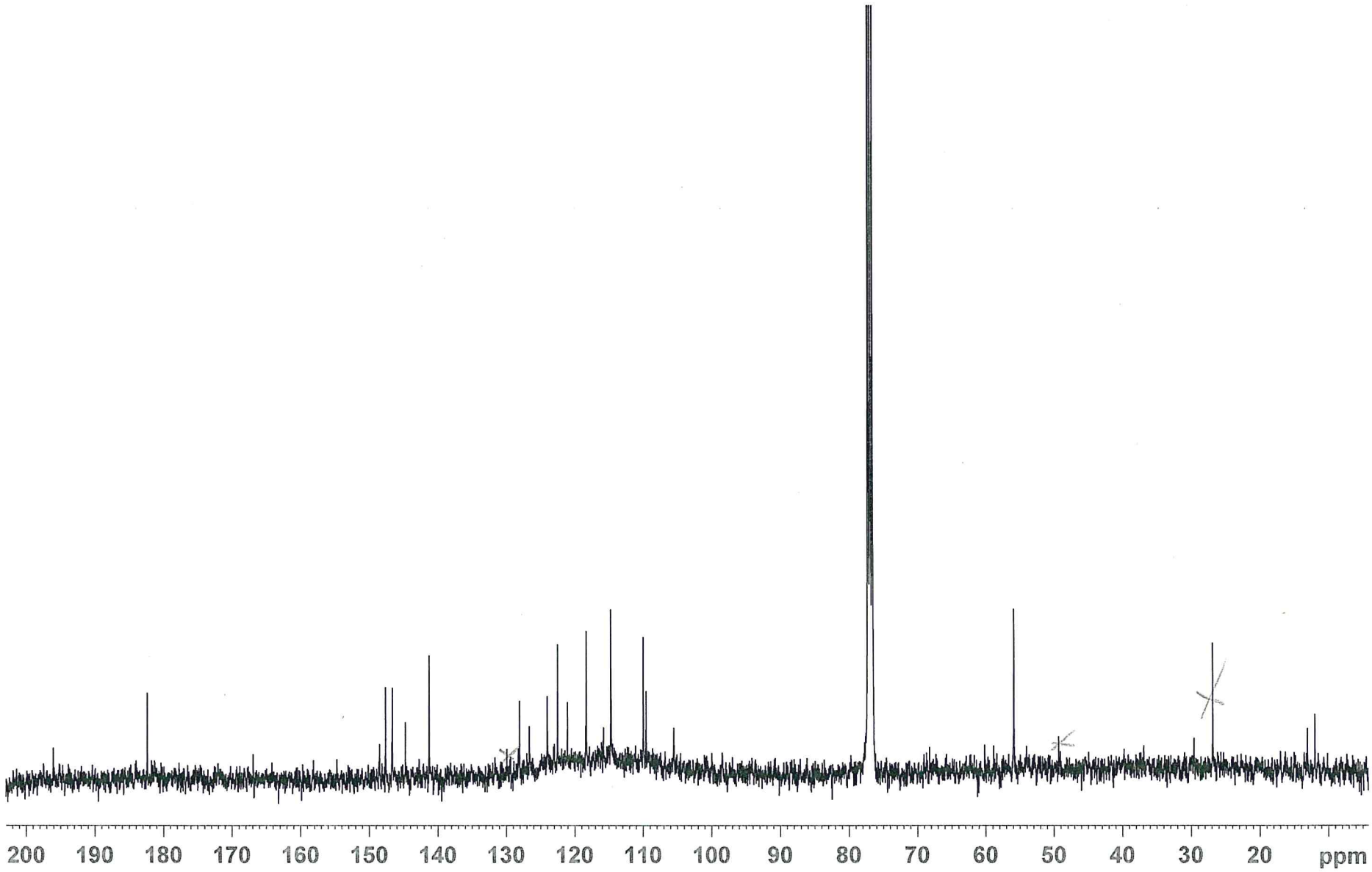
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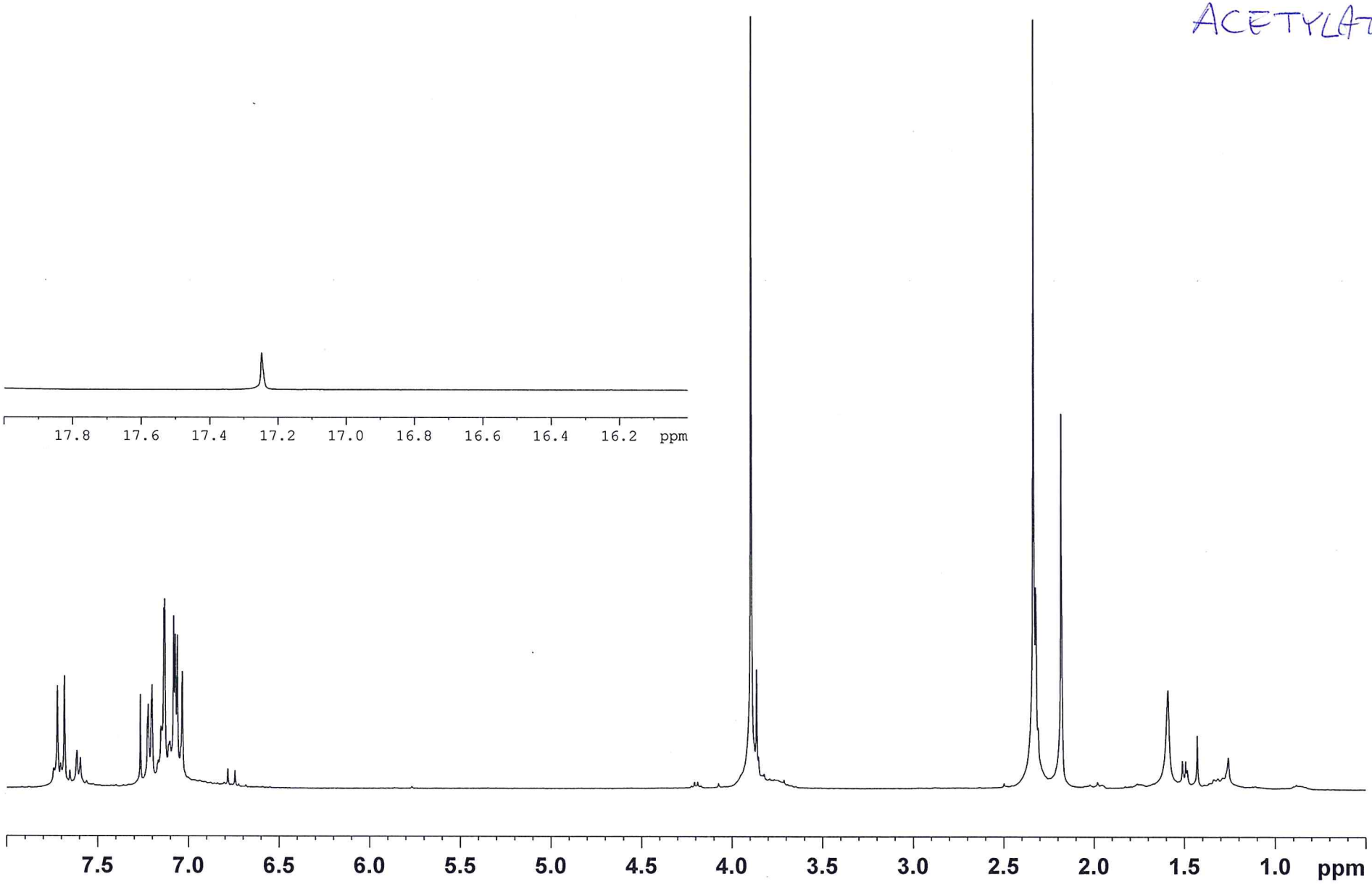
3Ac

COMPOUND 3

ACETYLATED



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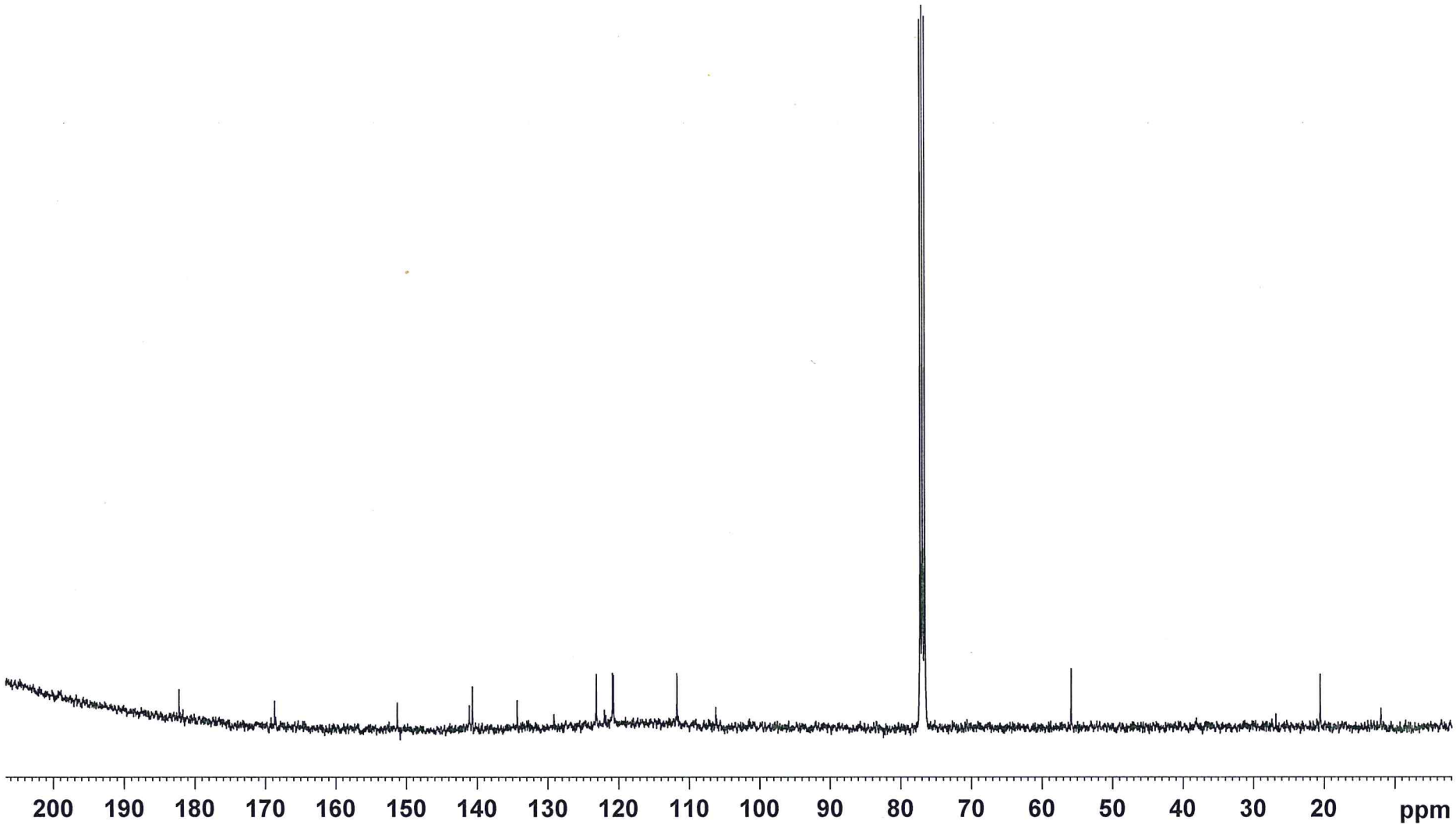
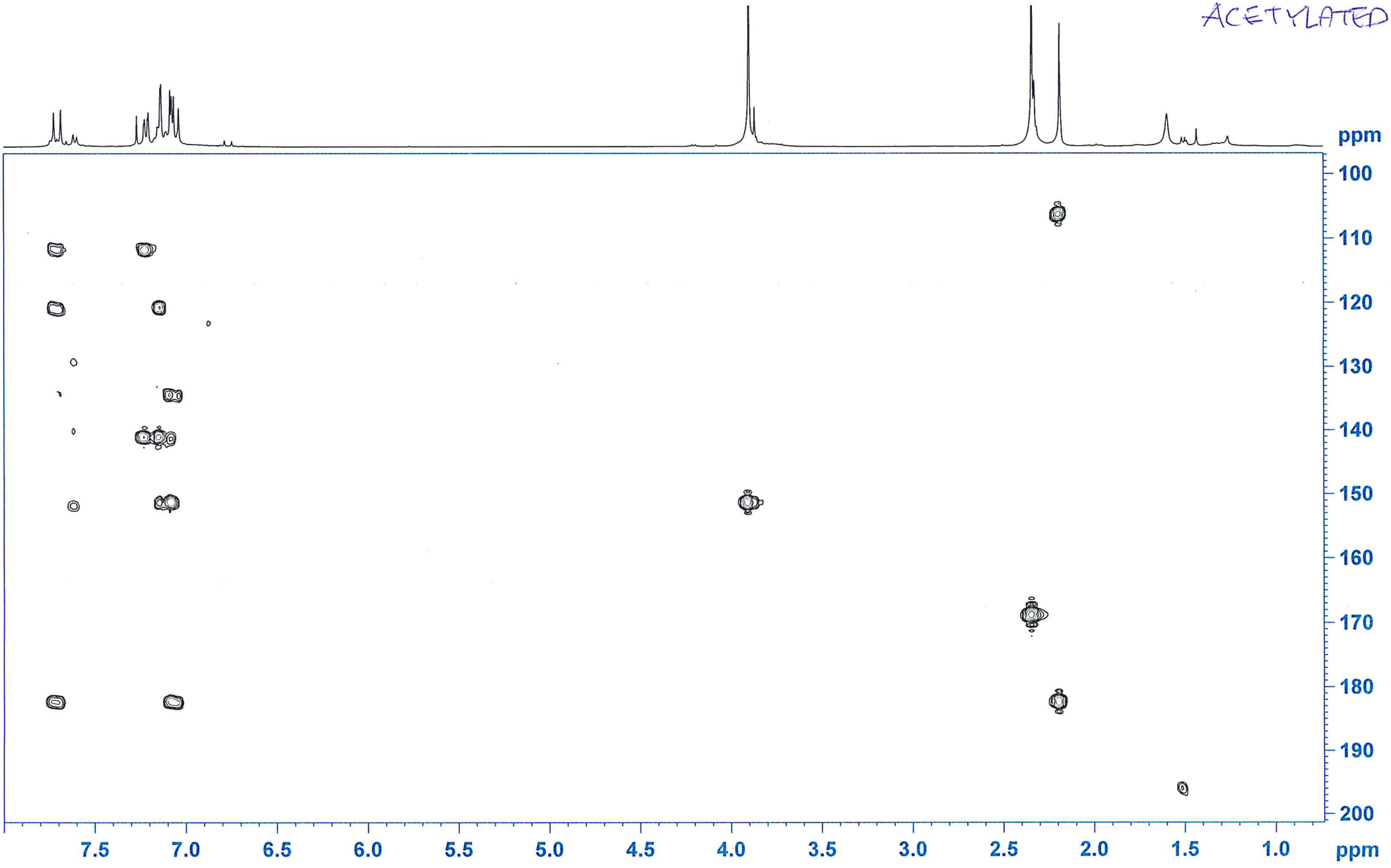


34e

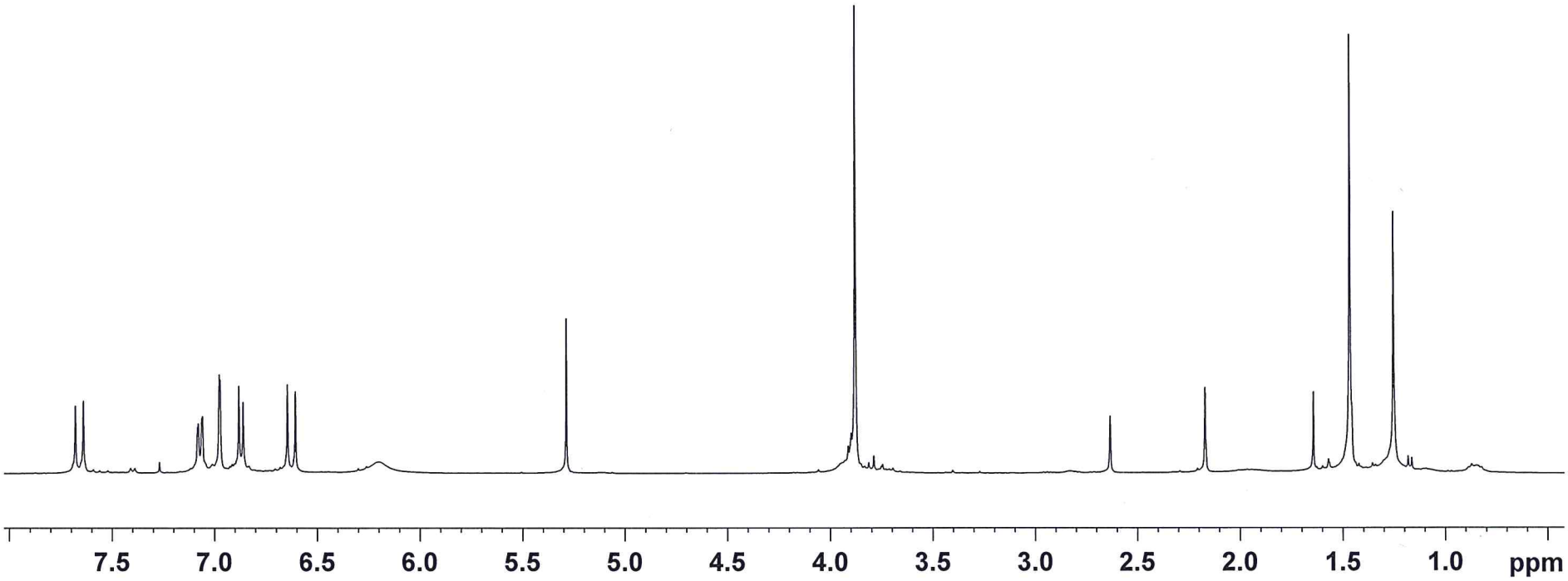
COMPOUND 3

ACETYLATED



(4)

COMPOUND 4



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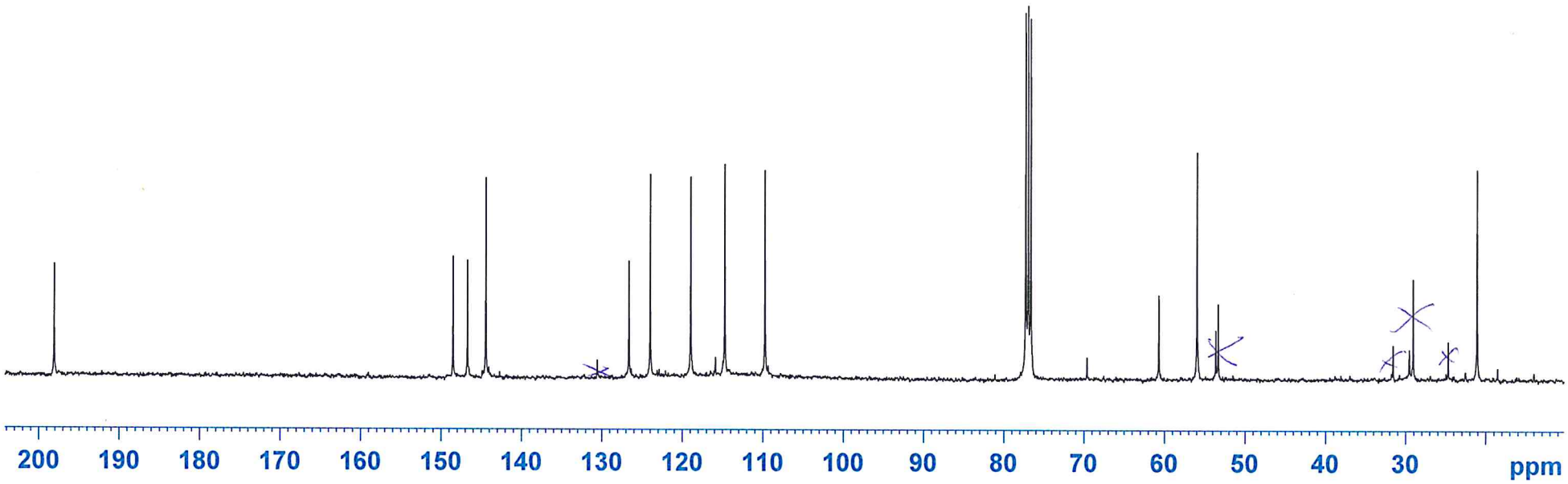
200
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160
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140
130
120
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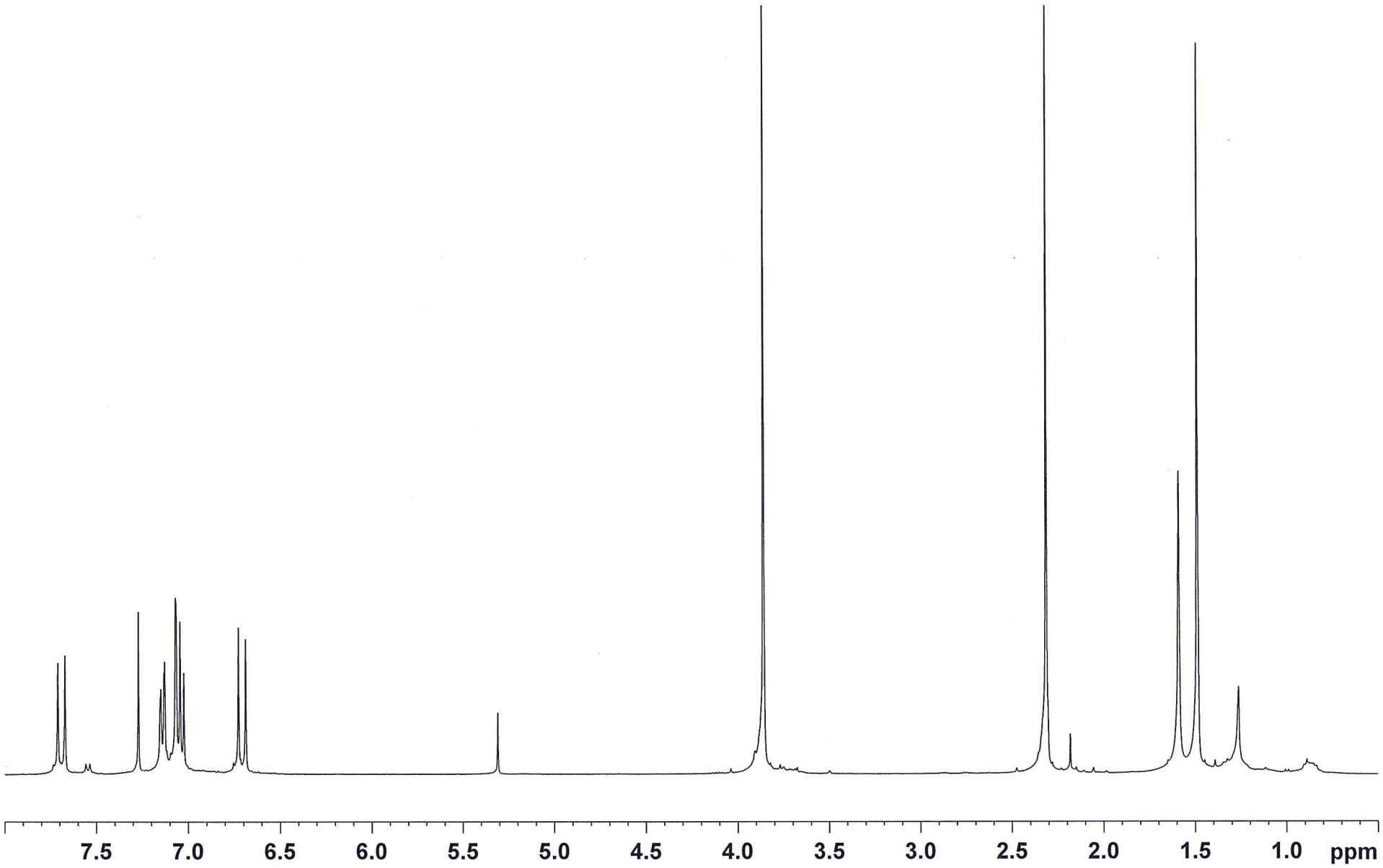
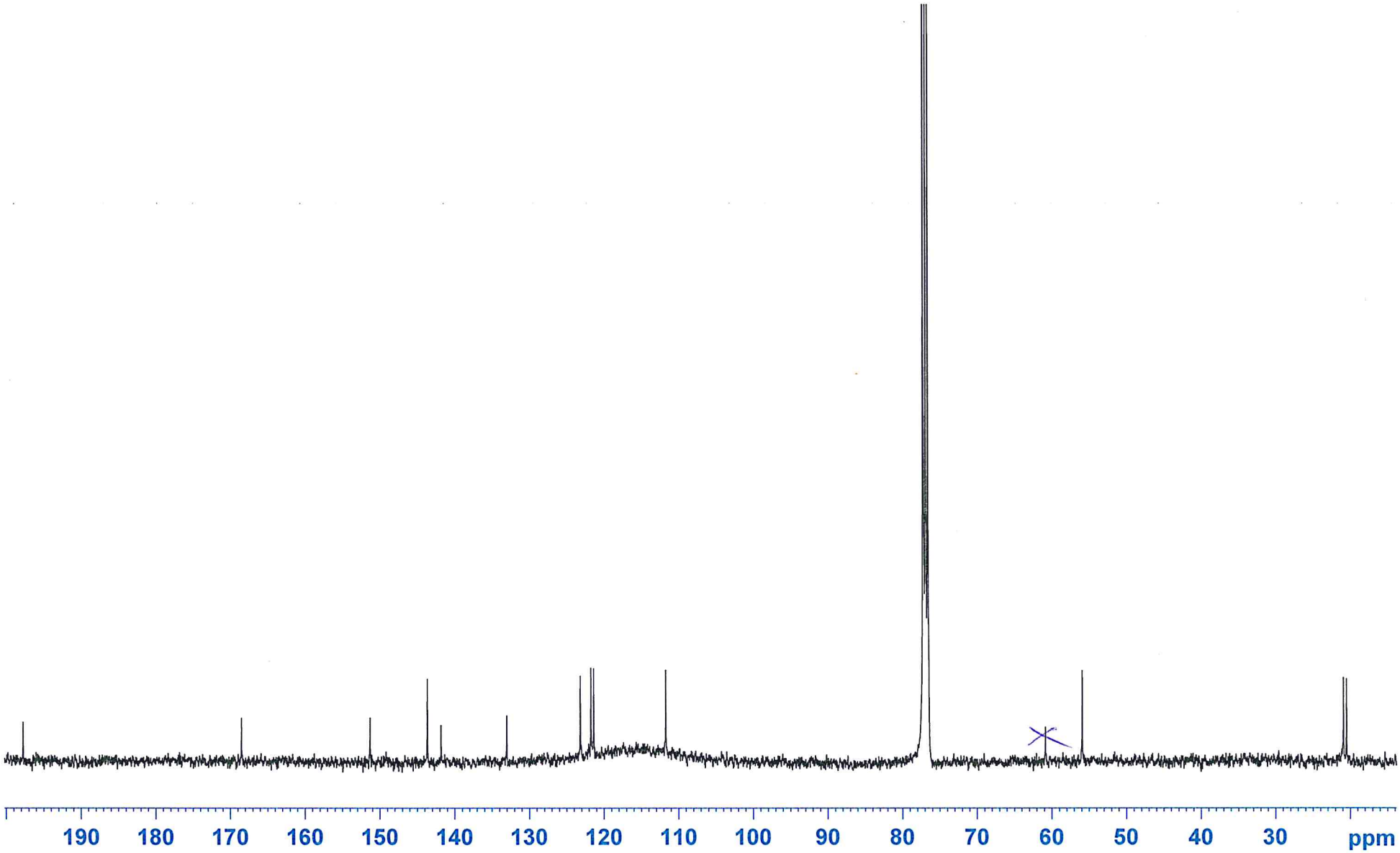
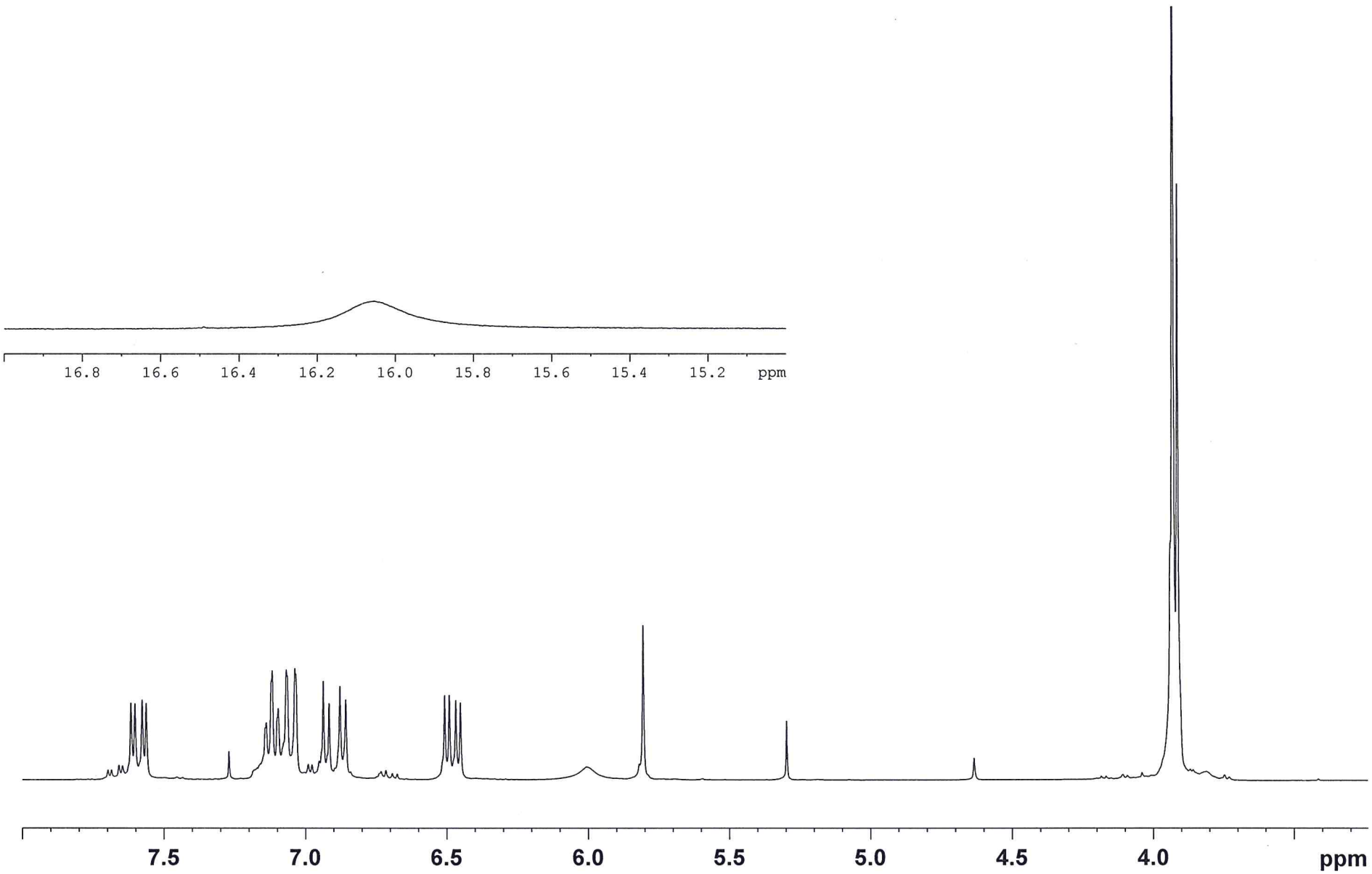
30
ppm


hNMR
ACETYLATED
4
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

ACETYLATED 4



comp 5



Comp 5