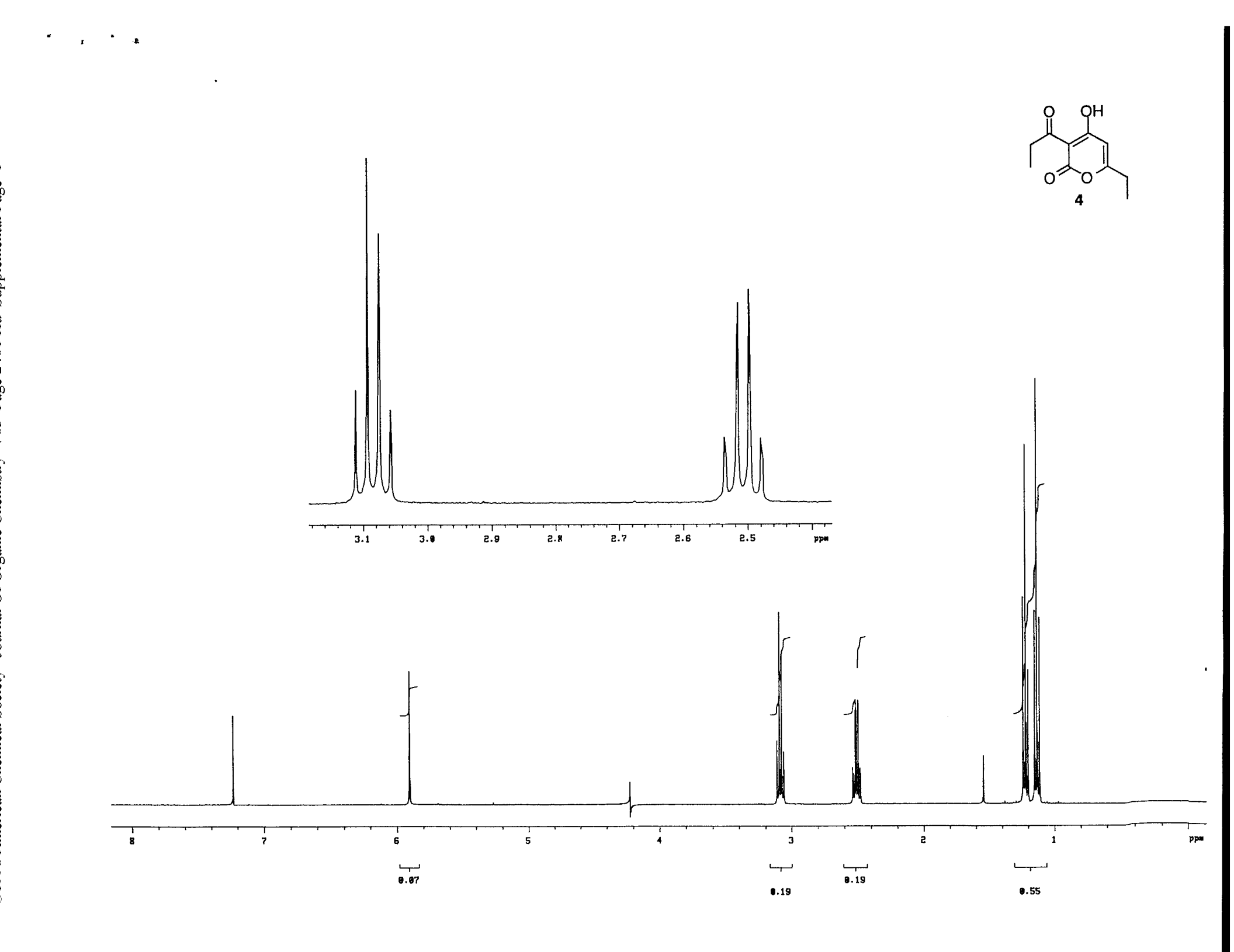
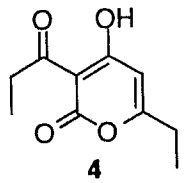
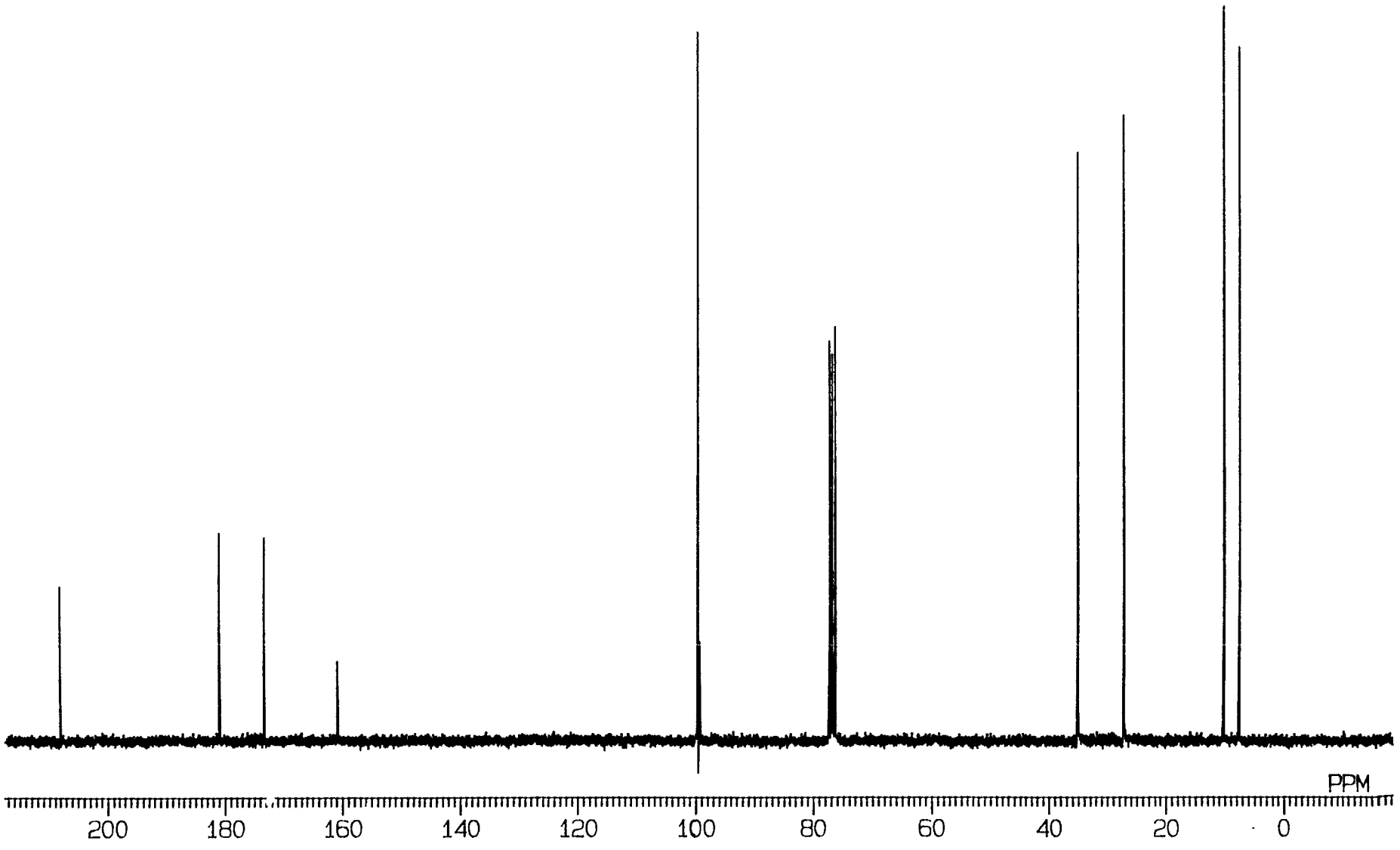
JOC The Journal of Organic Chemistry

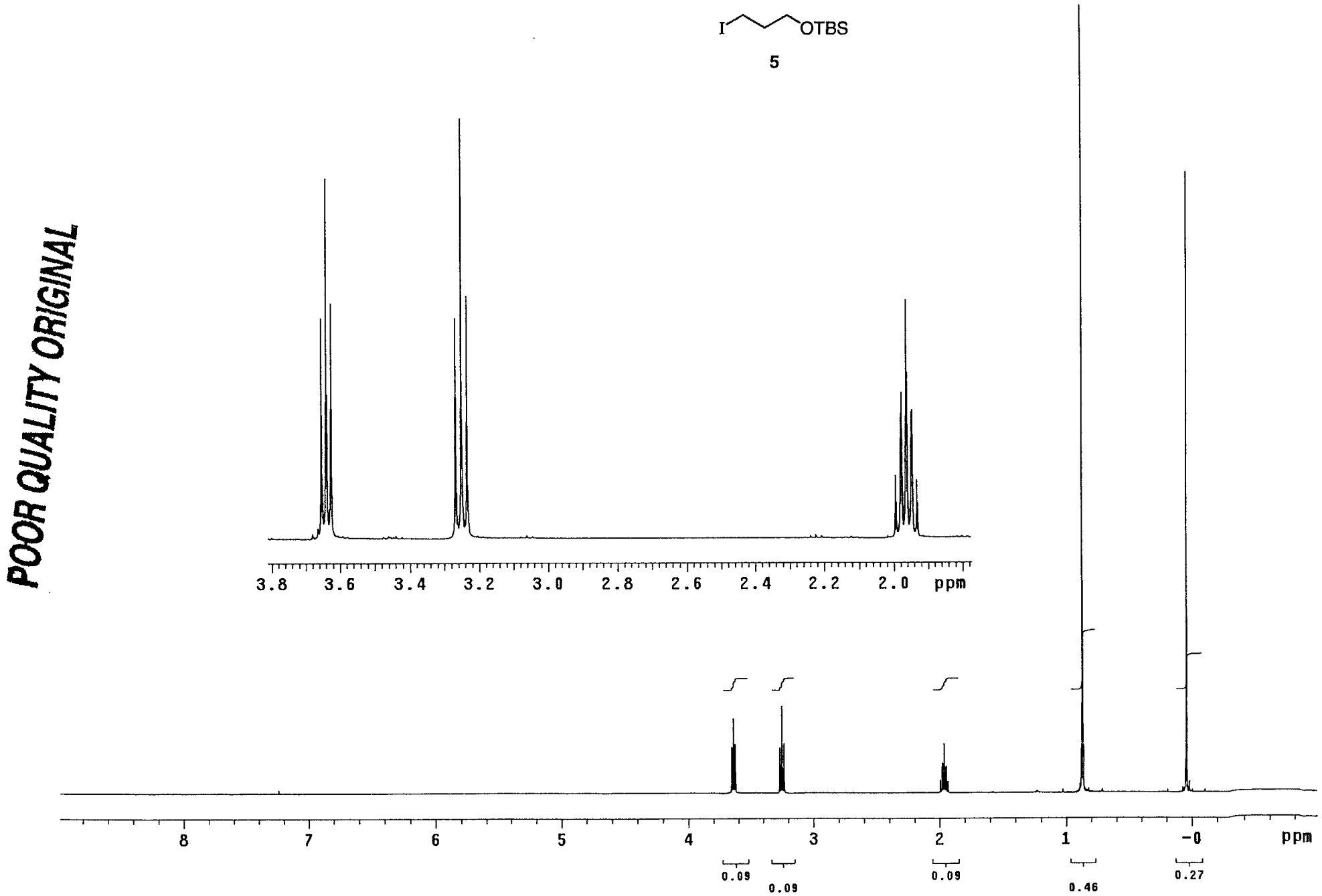
J. Org. Chem., 1998, 63(7), 2401-2406, DOI:10.1021/jo9721610

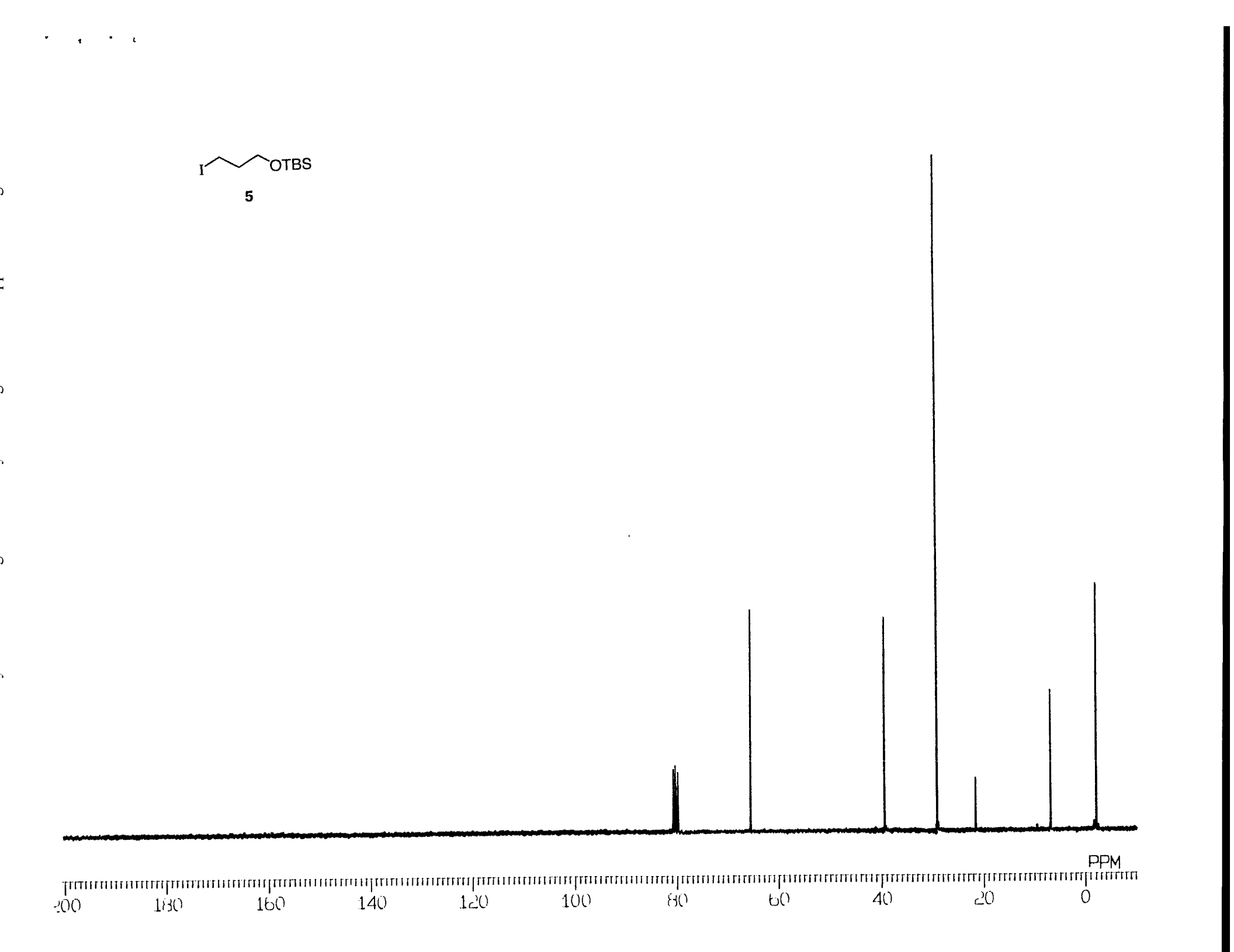

O
OH
O
O
4
3.1
3.0
2.9
2.8
2.7
2.6
2.5
ppm
8
7
6
5
4
3
2
1
ppm
0.07
0.19
0.19
0.55

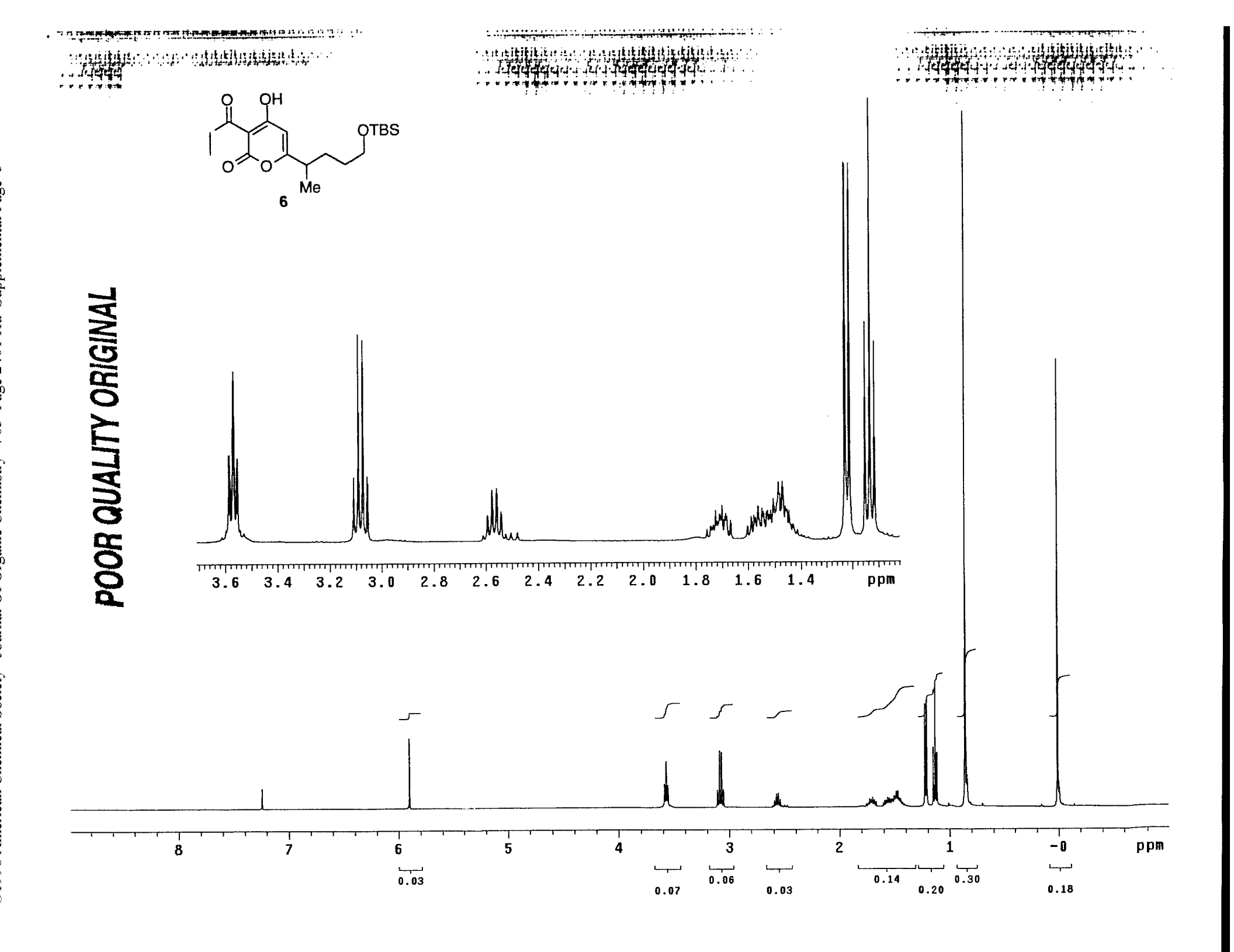


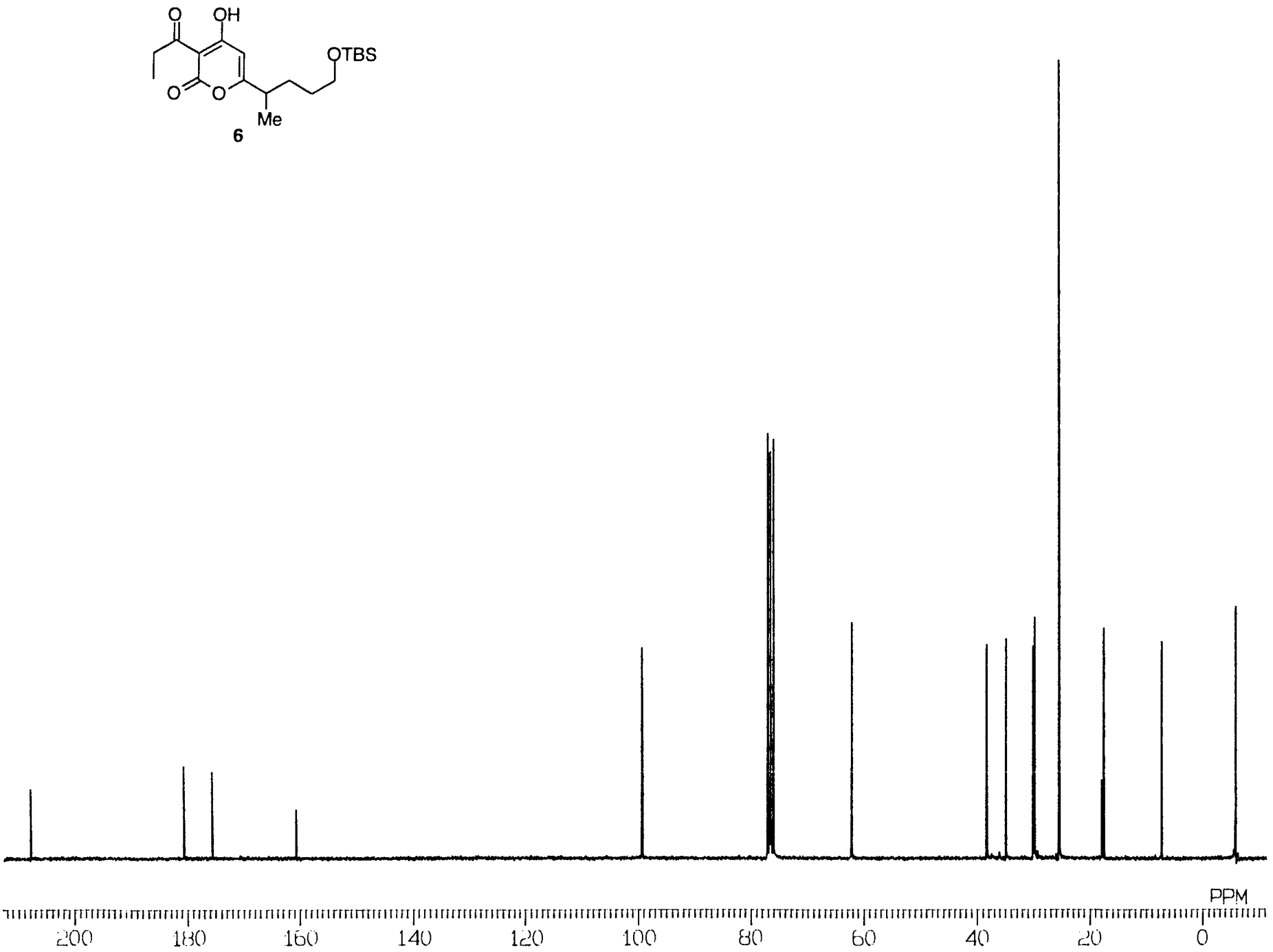
4

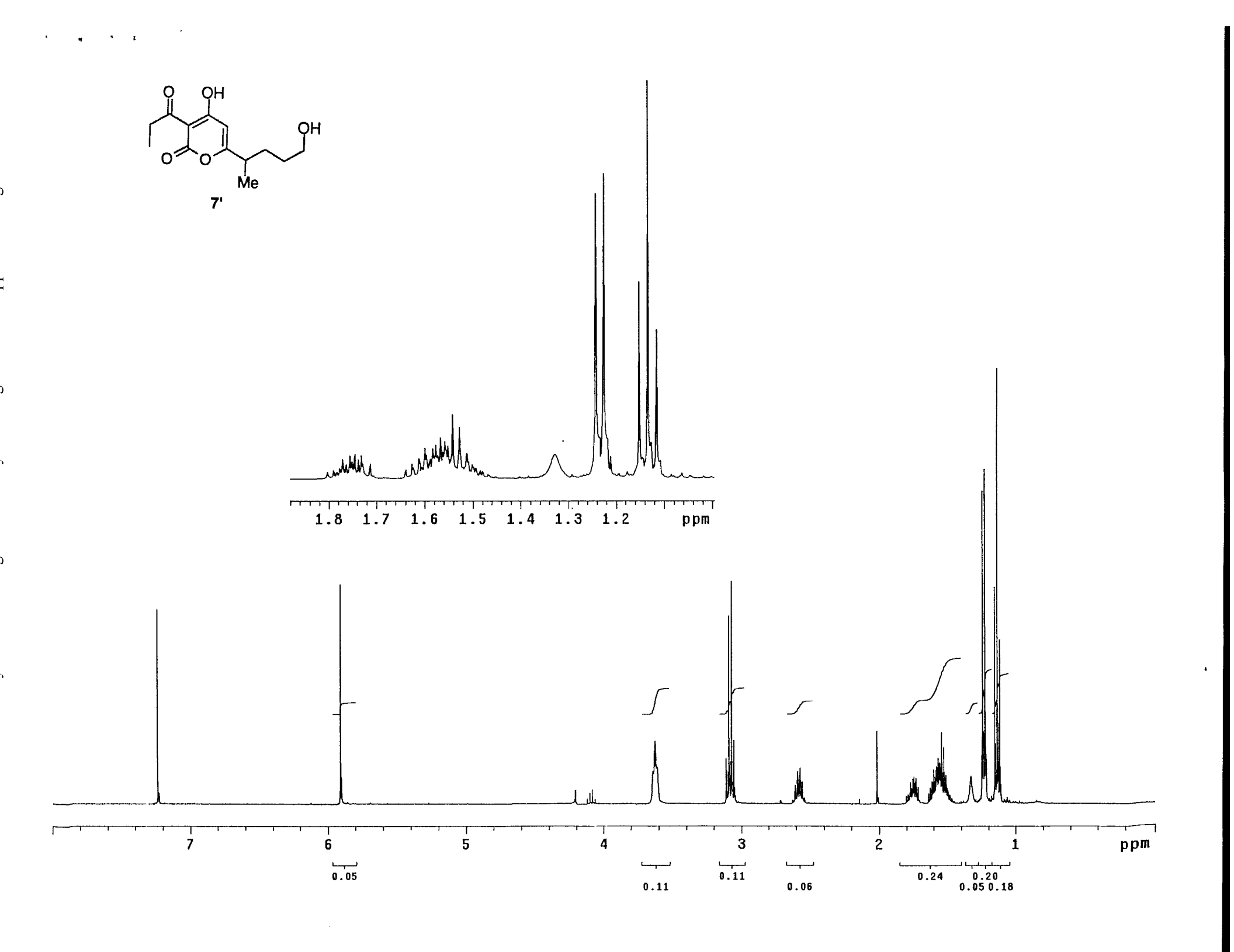


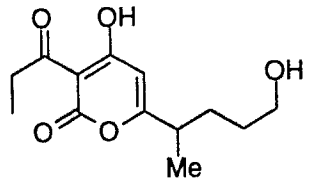



I
OTBS
5
PPM
200
180
160
140
120
100
80
60
40
20
0

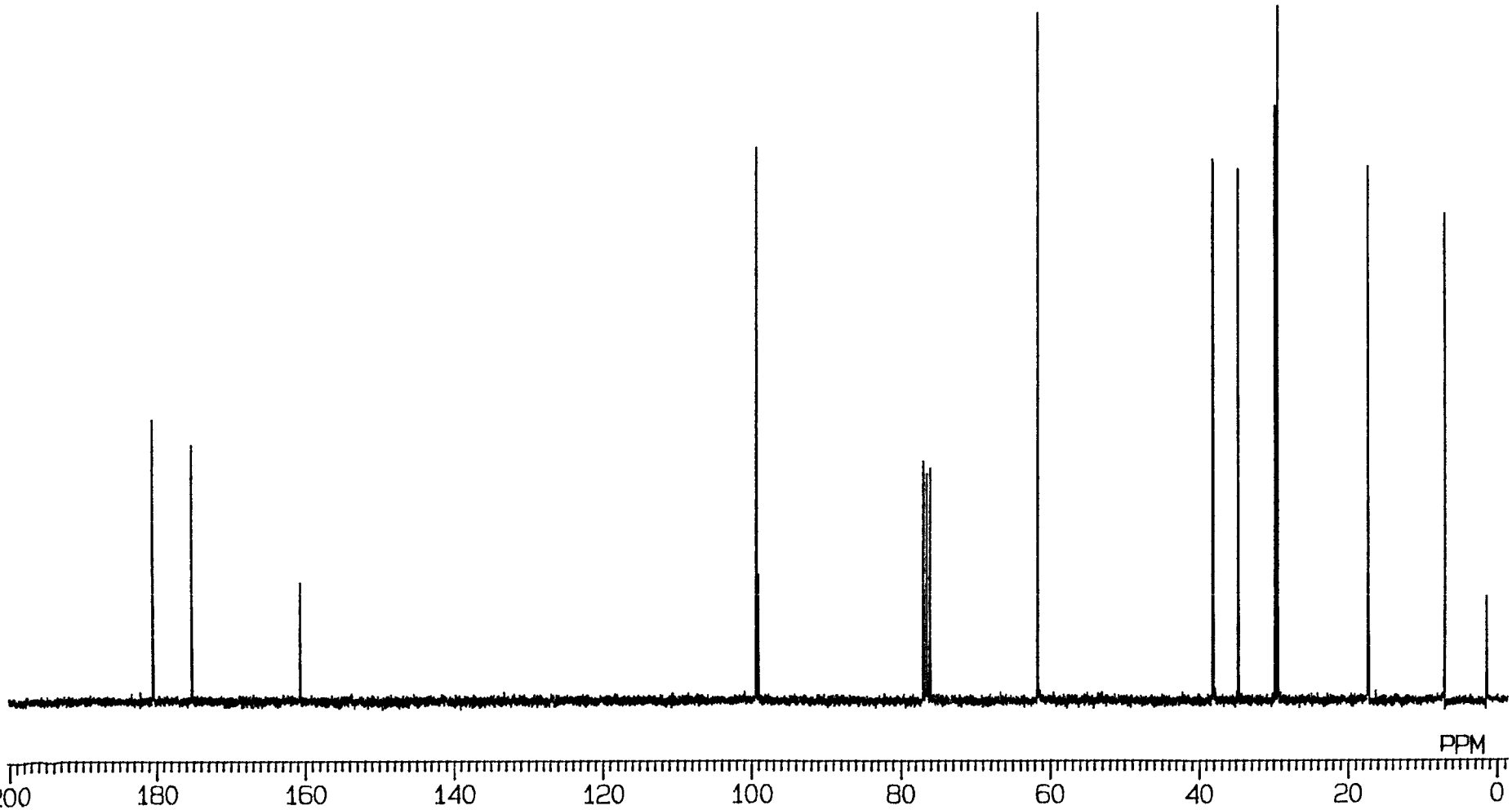


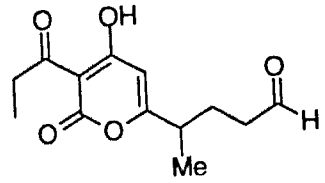

O
OH
OTBS
O
O
Me
6
PPM
200
180
160
140
120
100
80
60
40
20
0


O
OH
OH
O
O
Me
7'
1.8 1.7 1.6 1.5 1.4 1.3 1.2 ppm
7 6 5 4 3 2 1 ppm
0.05
0.11
0.11
0.06
0.24
0.20
0.05 0.18



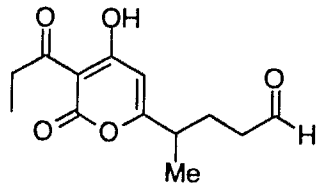
7'



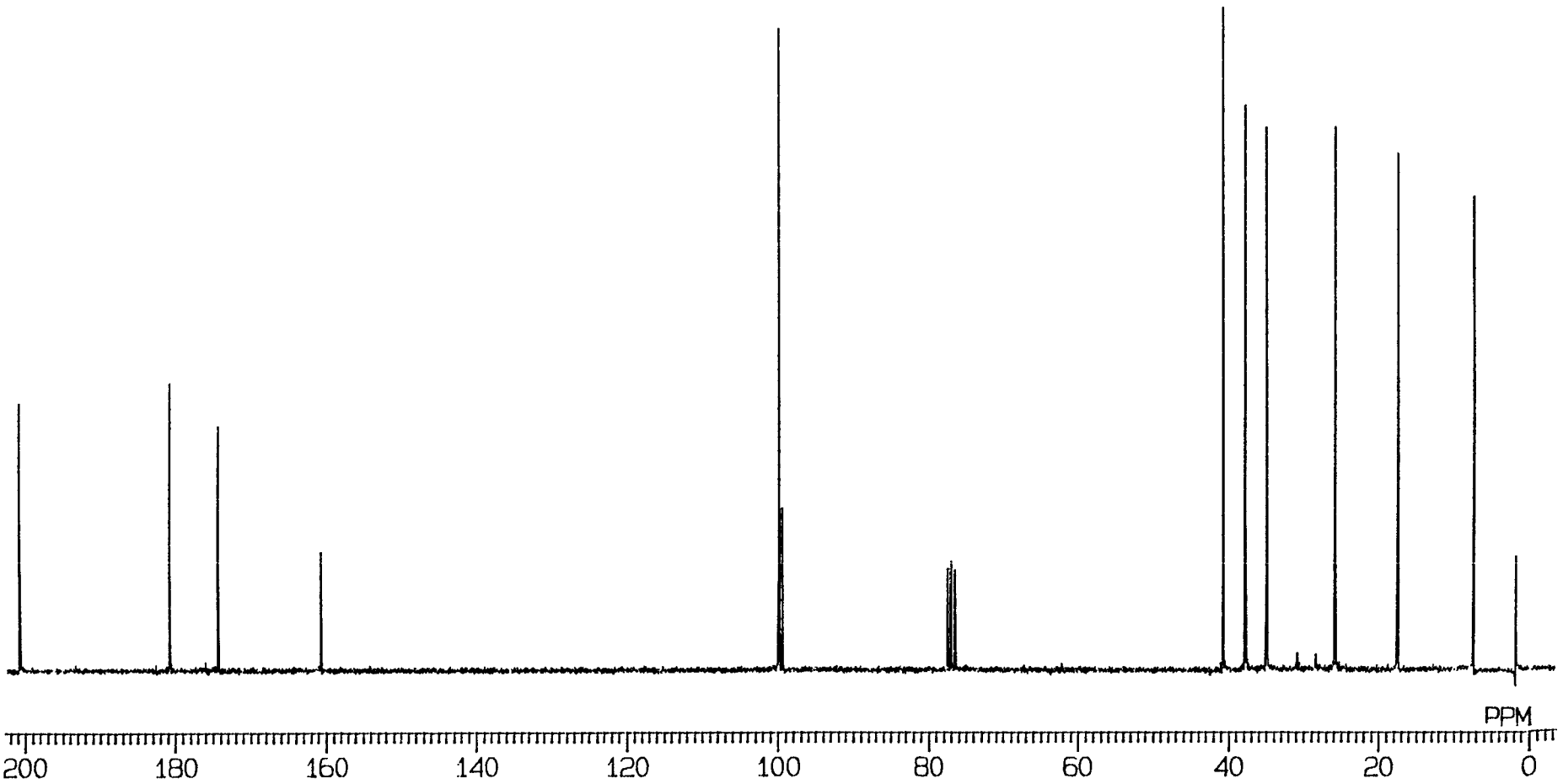

O
OH
O
O
O
H
Me

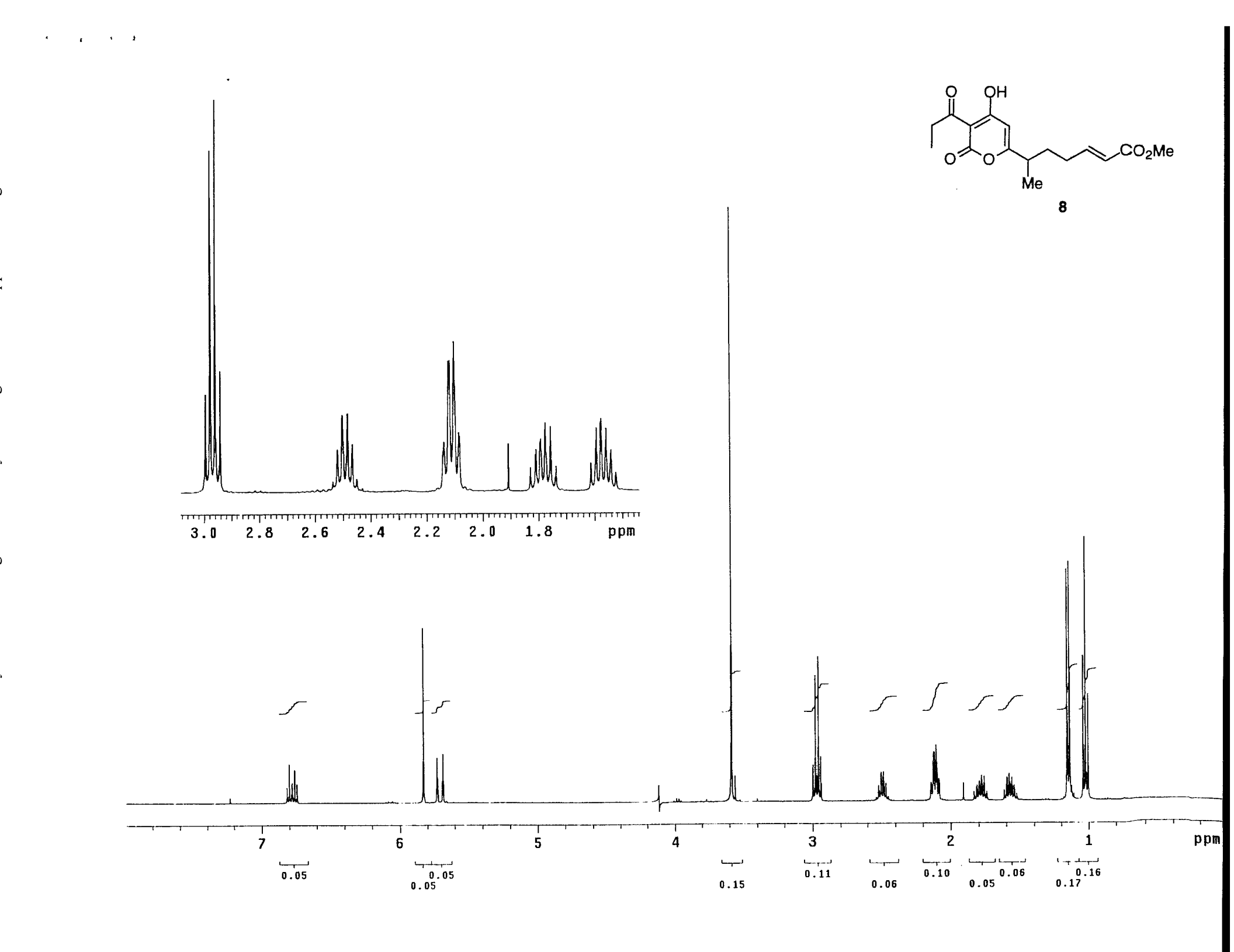
7

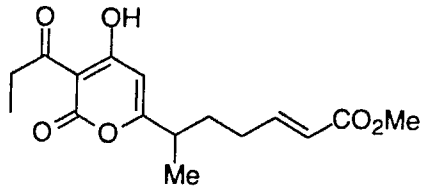
3.2
3.0
2.8
2.6
2.4
2.2
2.0
ppm
10
9
8
7
6
5
4
3
2
1
ppm
0.04
0.05
0.12
0.06
0.12
0.14
0.05
0.41



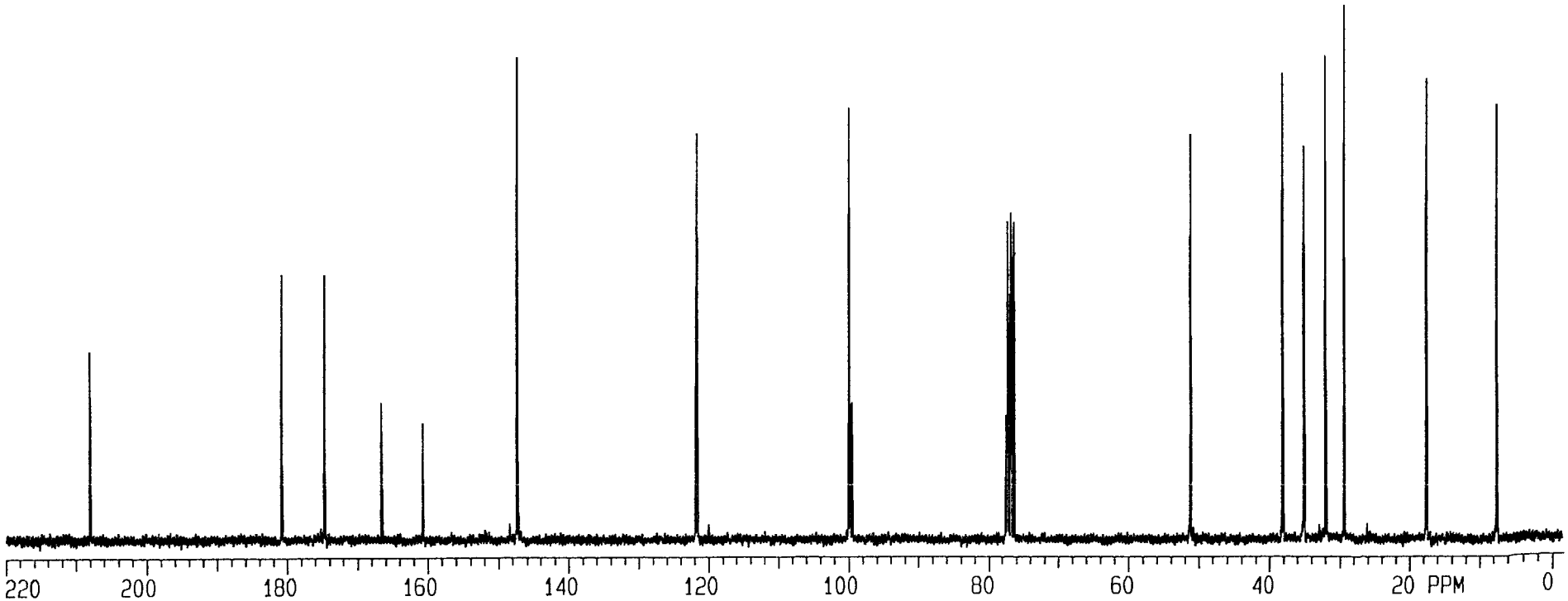
7

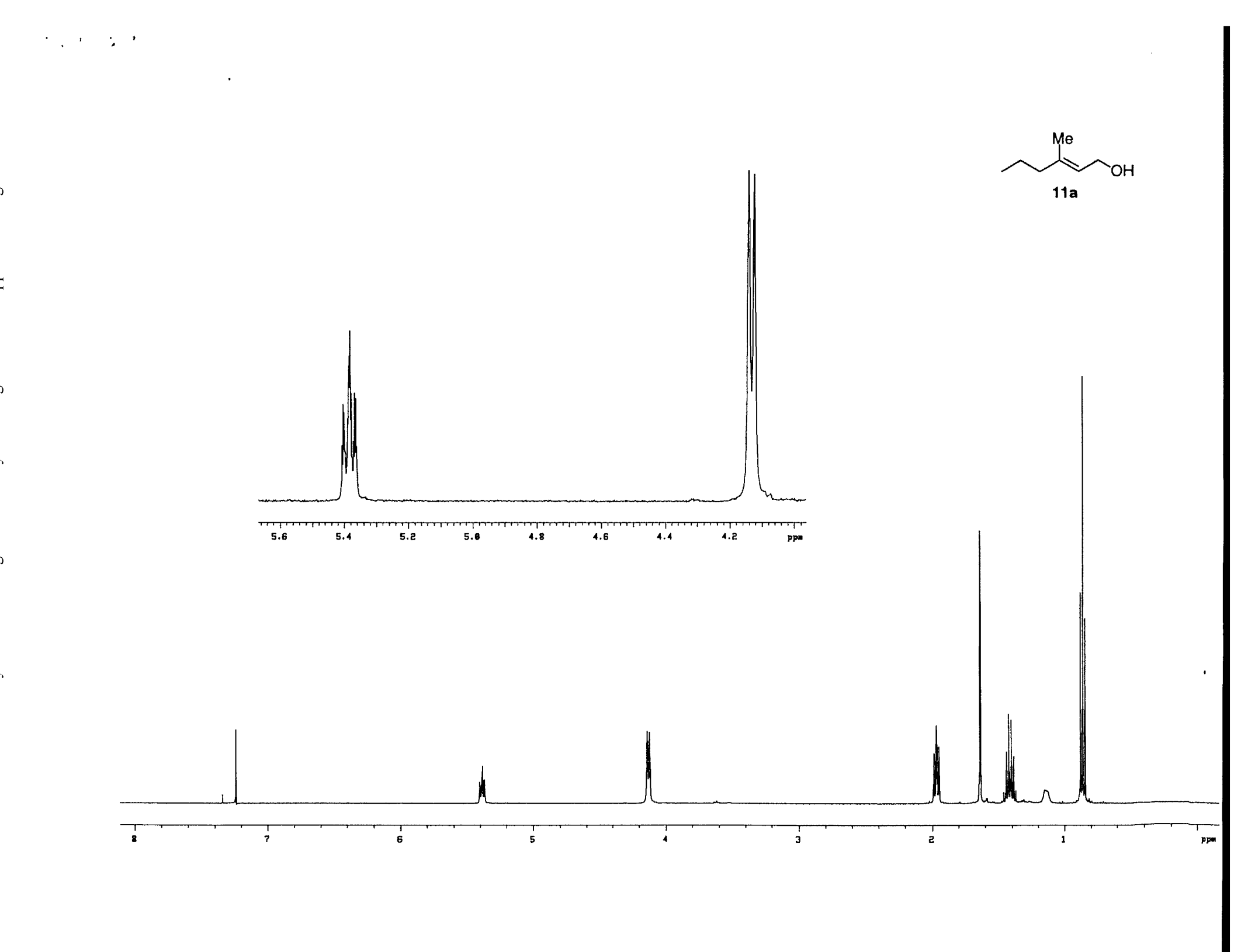


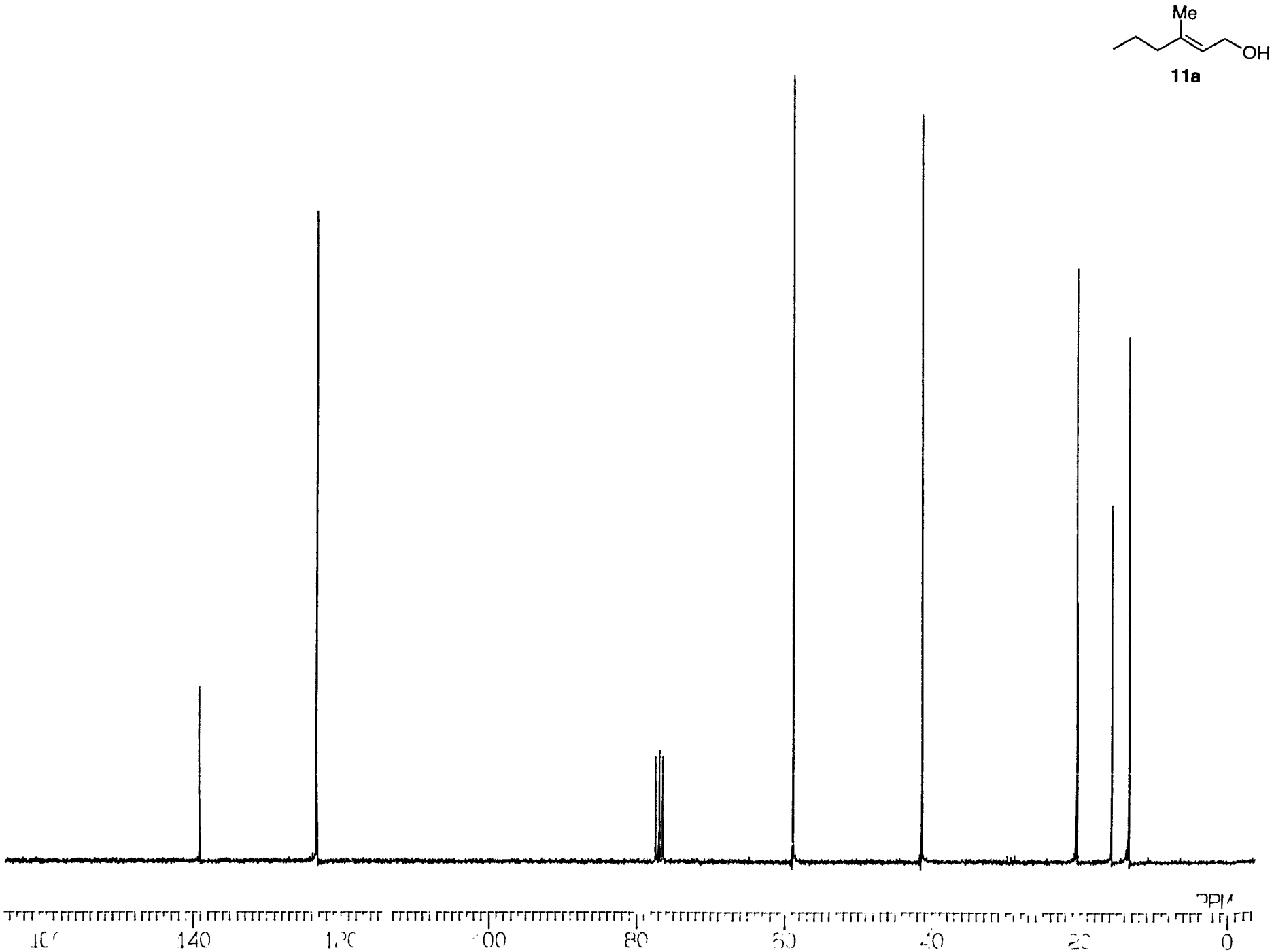

O
OH
O
O
CO2Me
Me
8
3.0
2.8
2.6
2.4
2.2
2.0
1.8
ppm
7
6
5
4
3
2
1
ppm
0.05
0.05
0.05
0.15
0.11
0.06
0.10
0.05
0.06
0.17
0.16

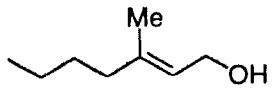


8

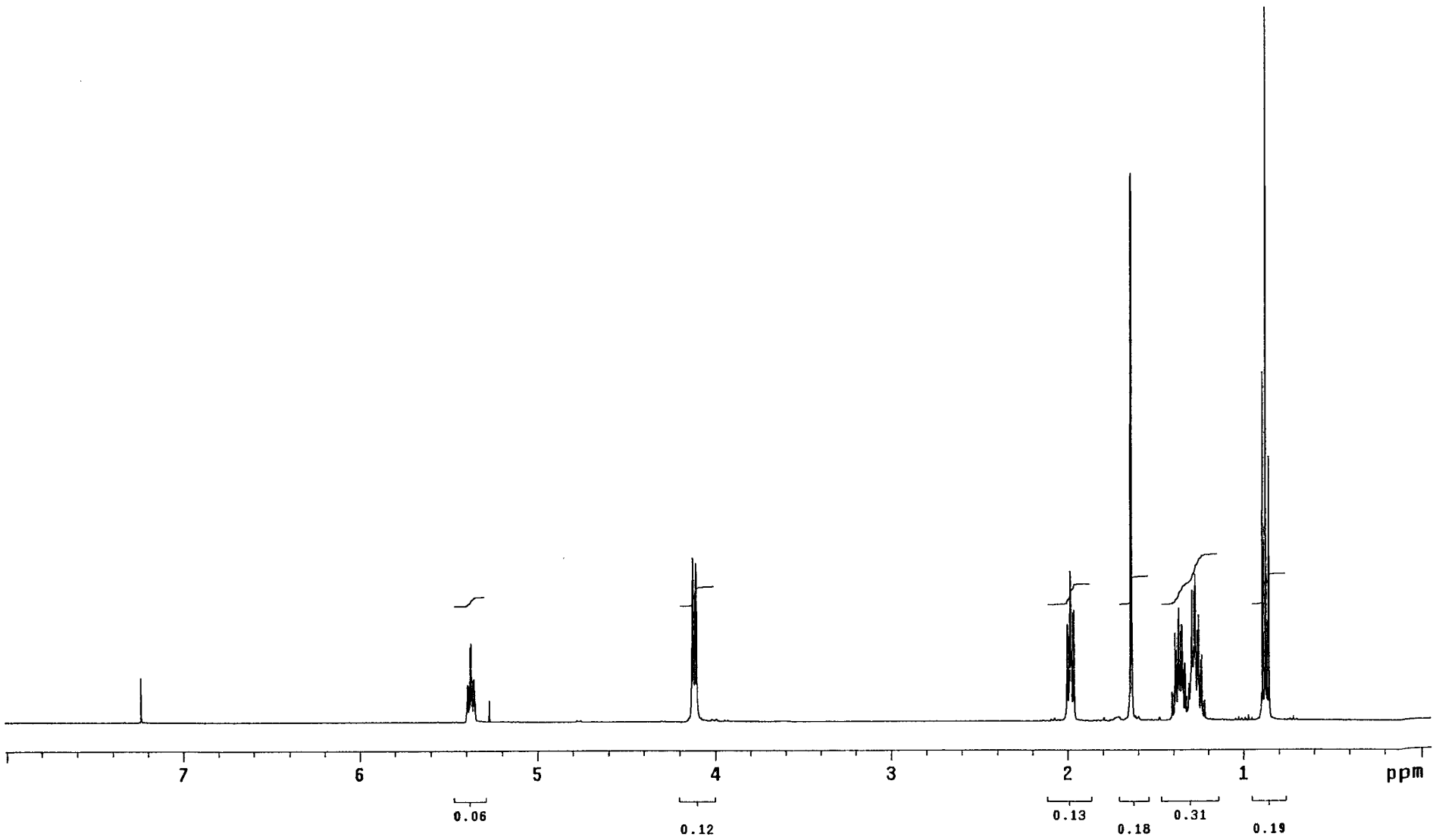


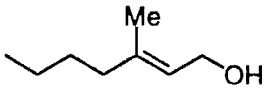

Me
OH
11a
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
ppm
8
7
6
5
4
3
2
1
ppm


Me
OH
11a
PPM
140
100
80
60
40
20
0

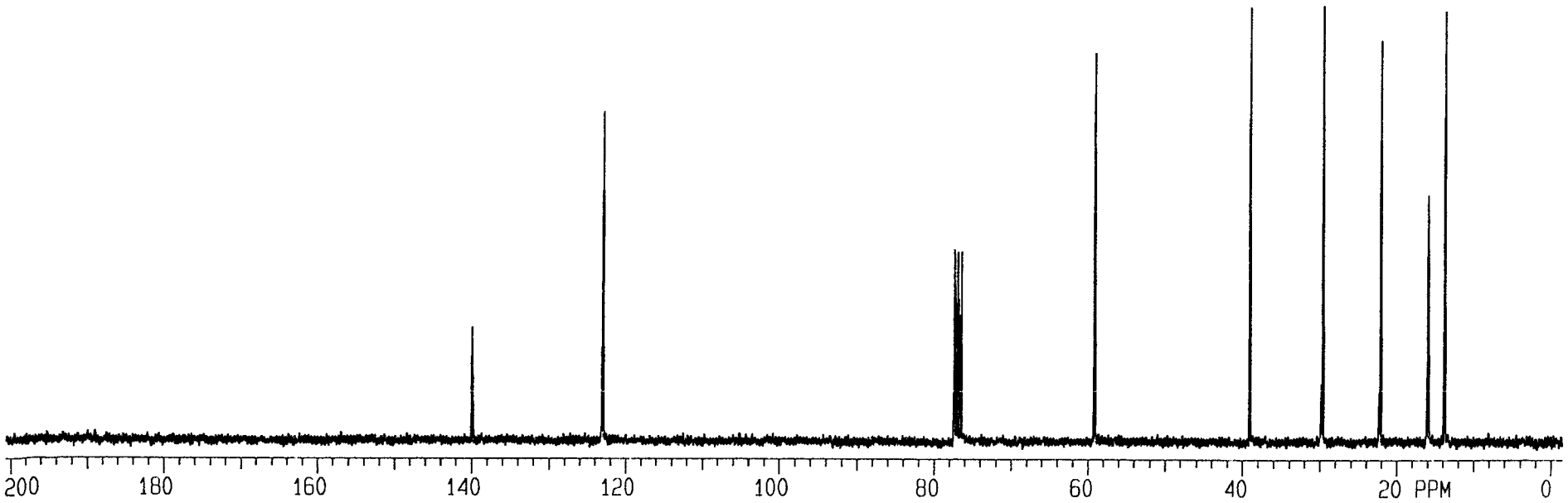


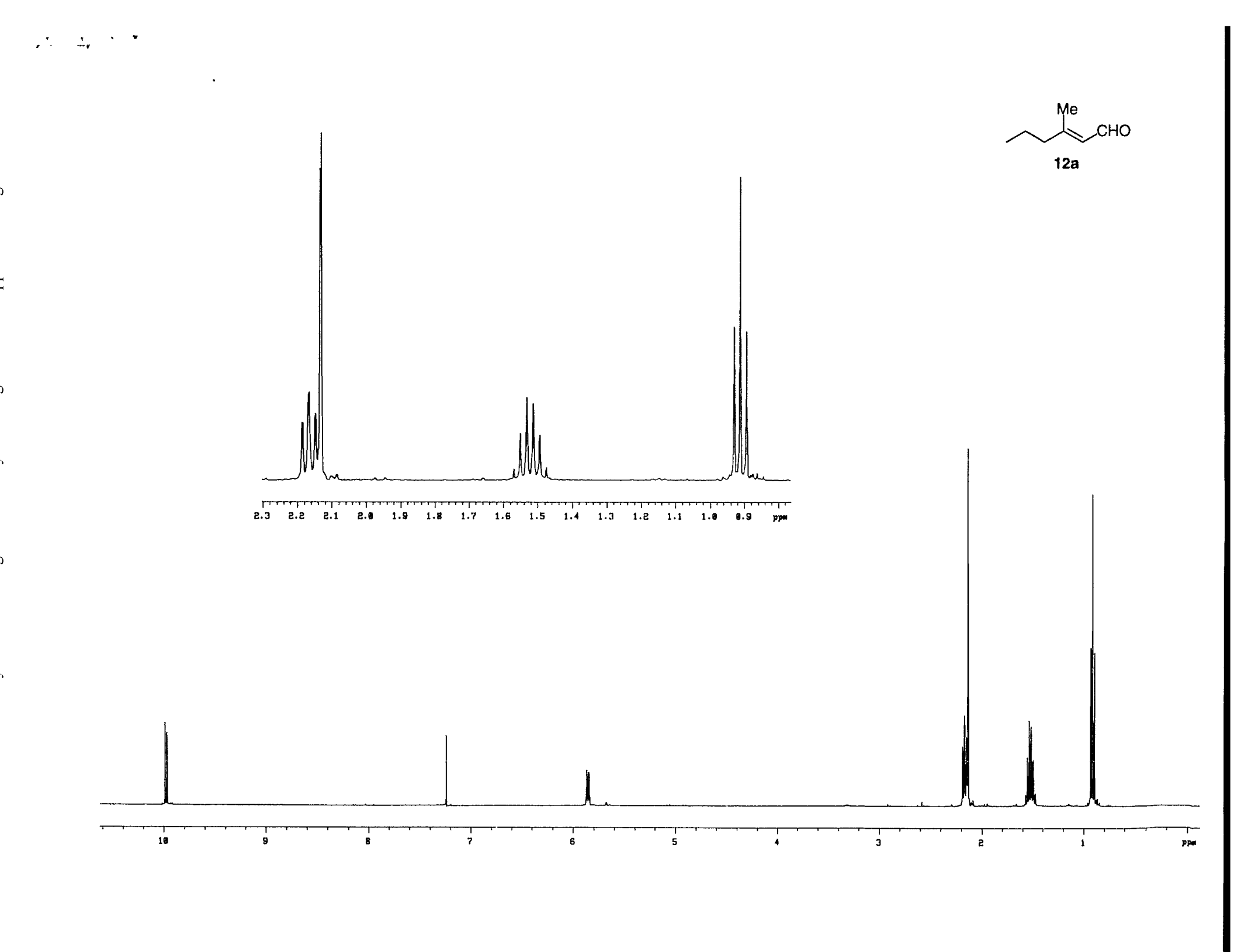
11b

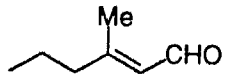




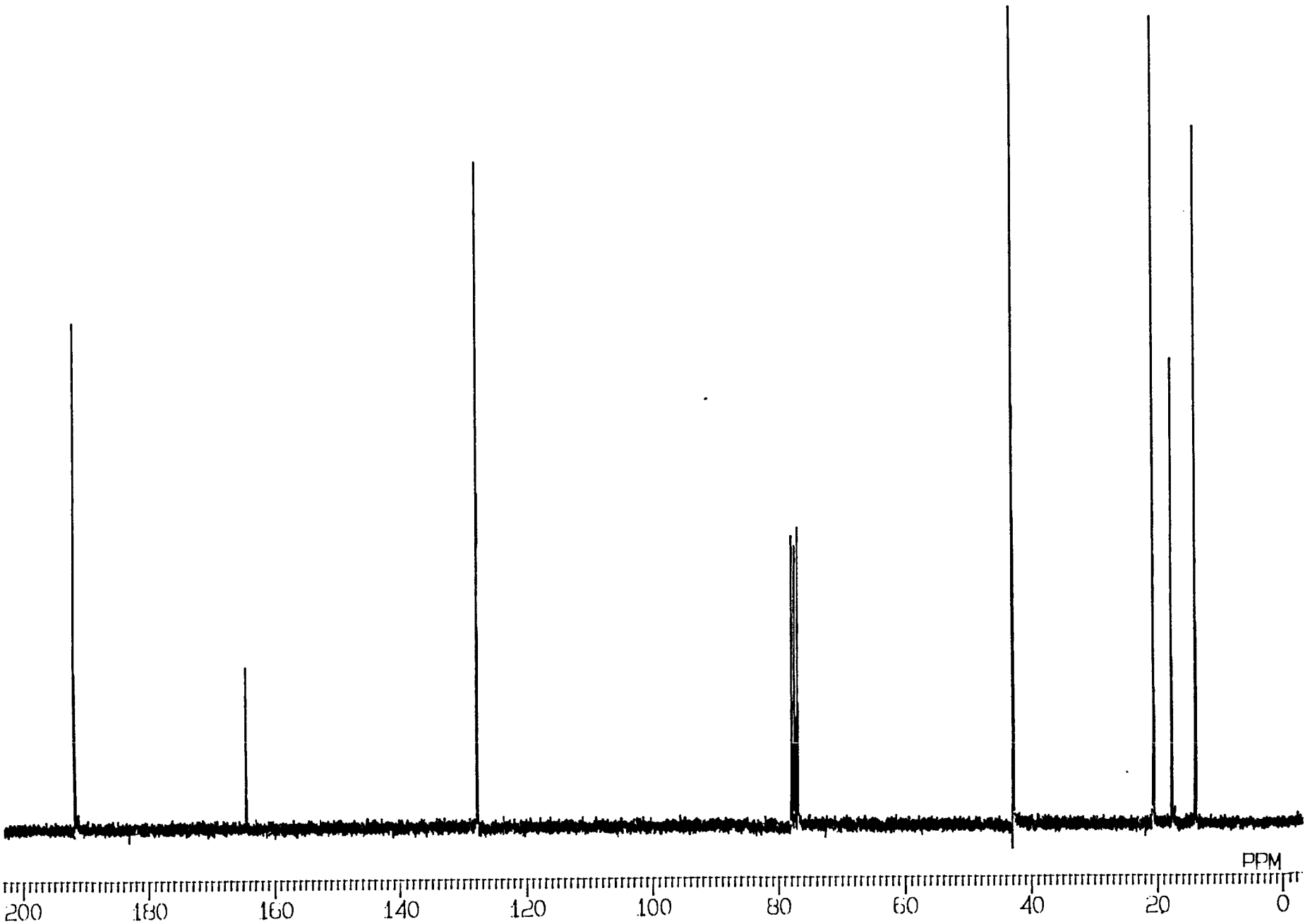
11b

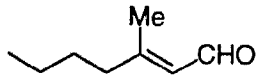



Me
CHO
12a
2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 ppm
10 9 8 7 6 5 4 3 2 1 ppm

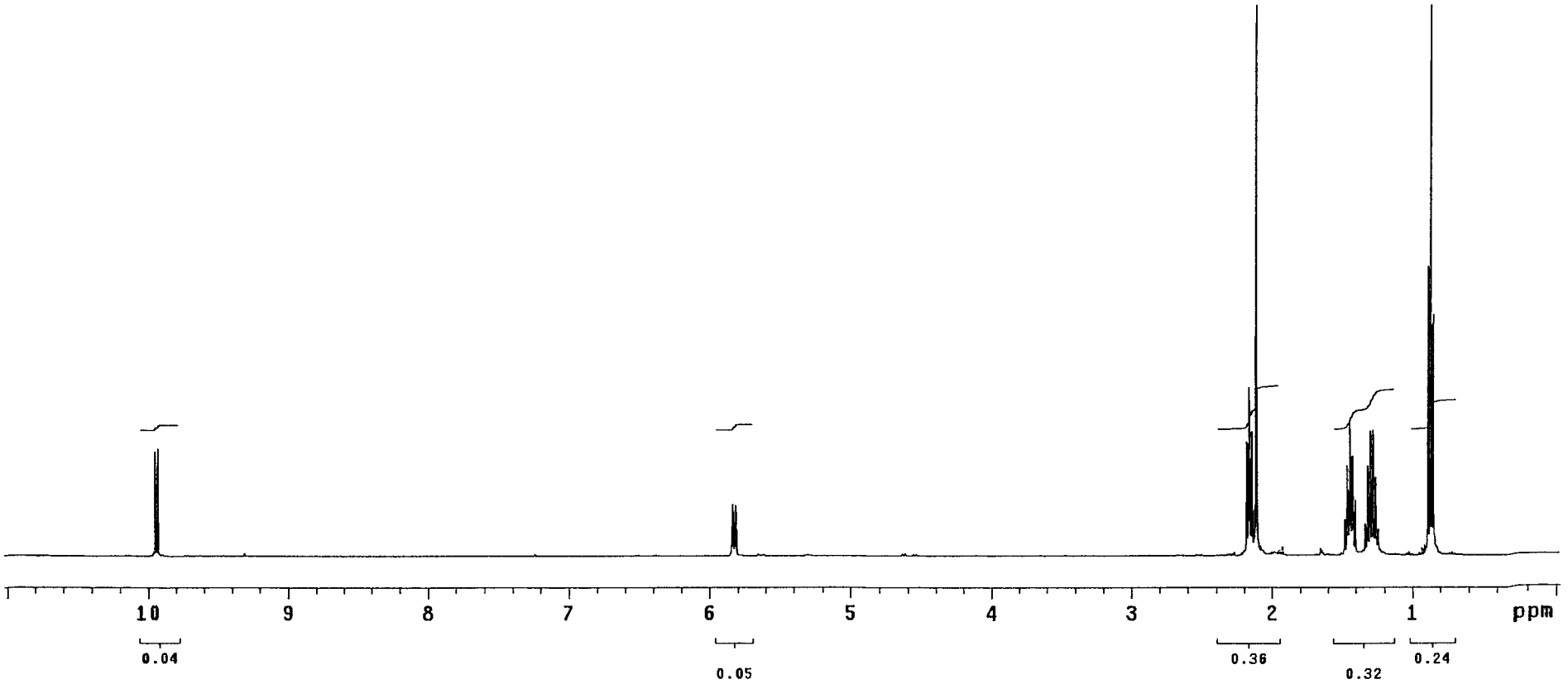


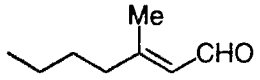
12a



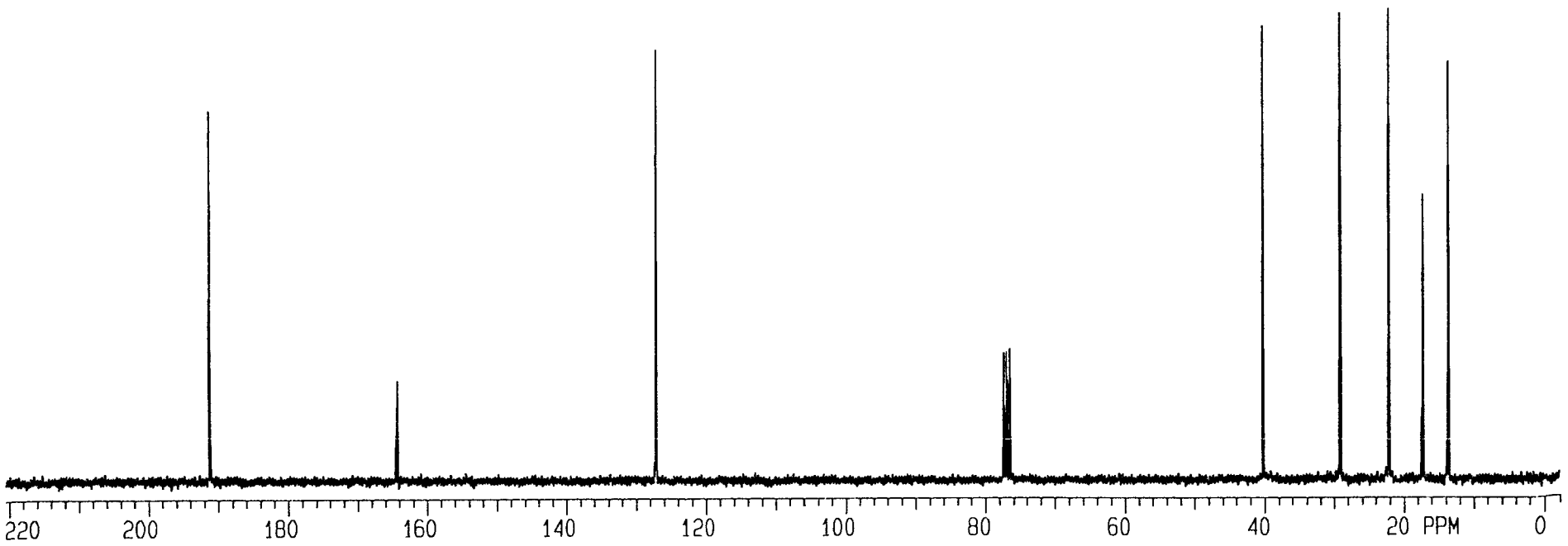


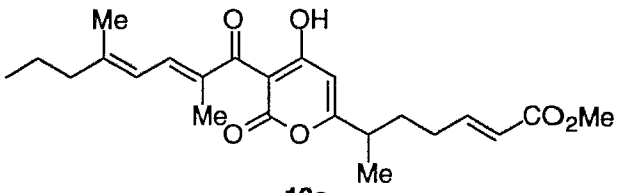
12b



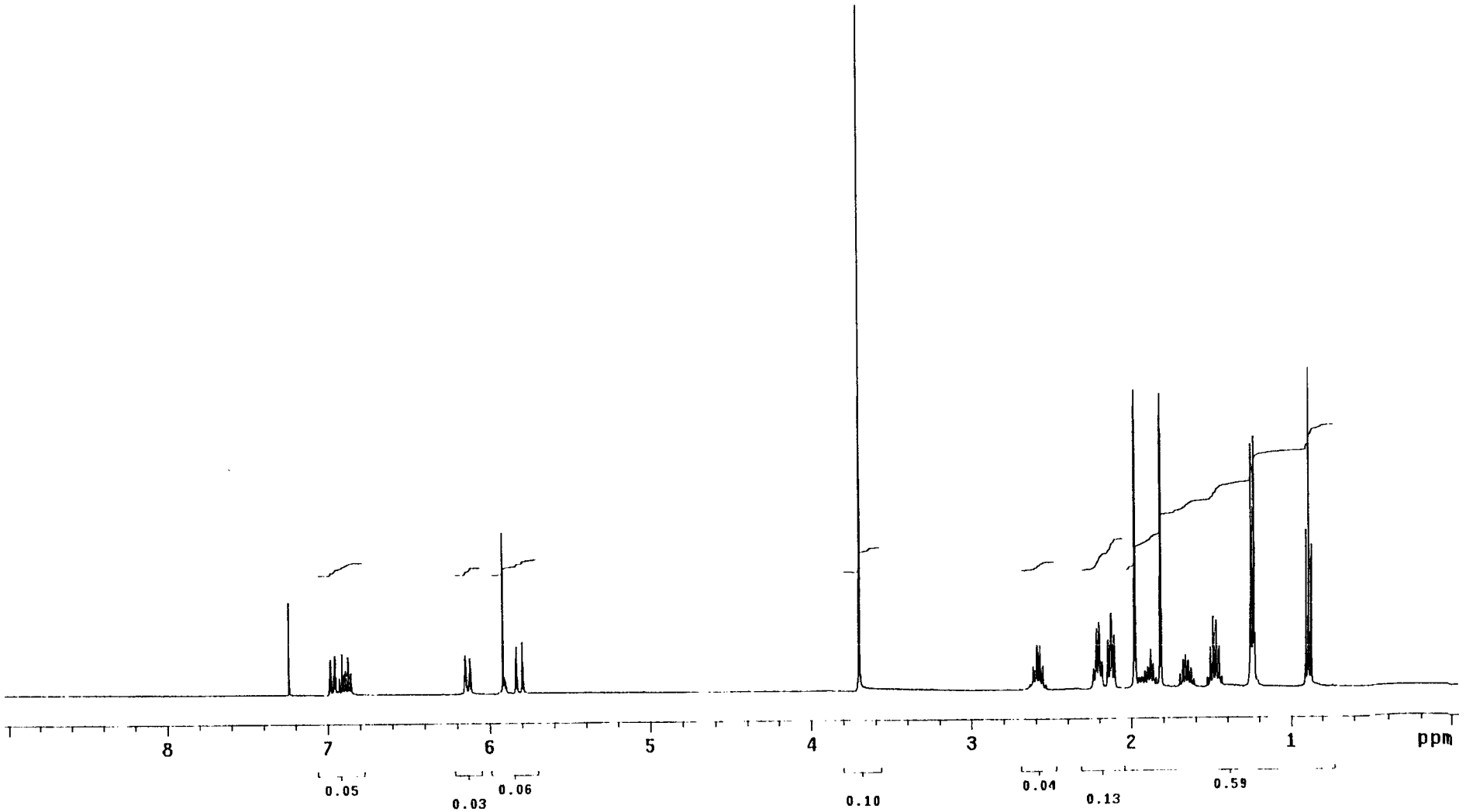


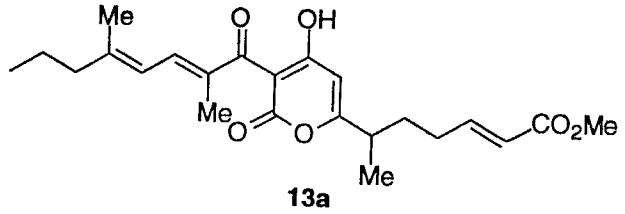
12b



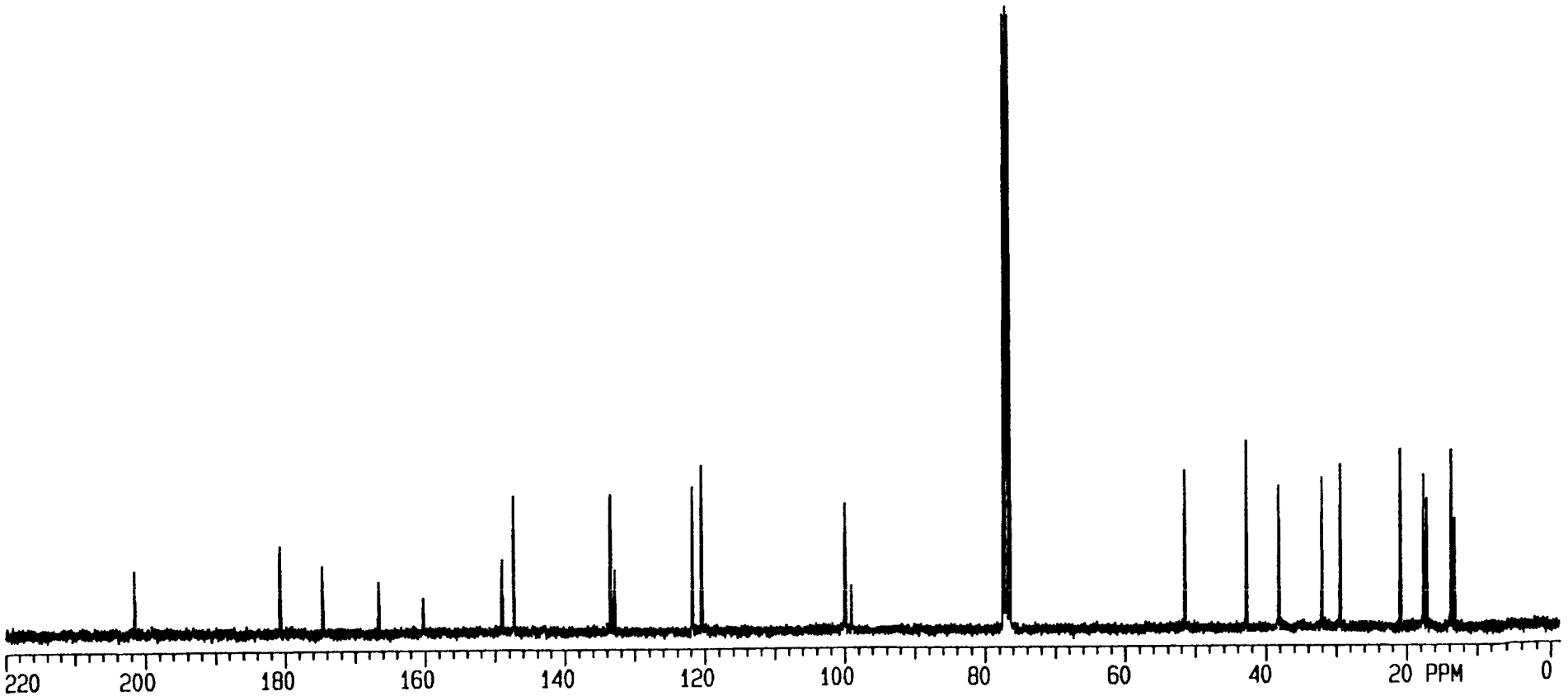


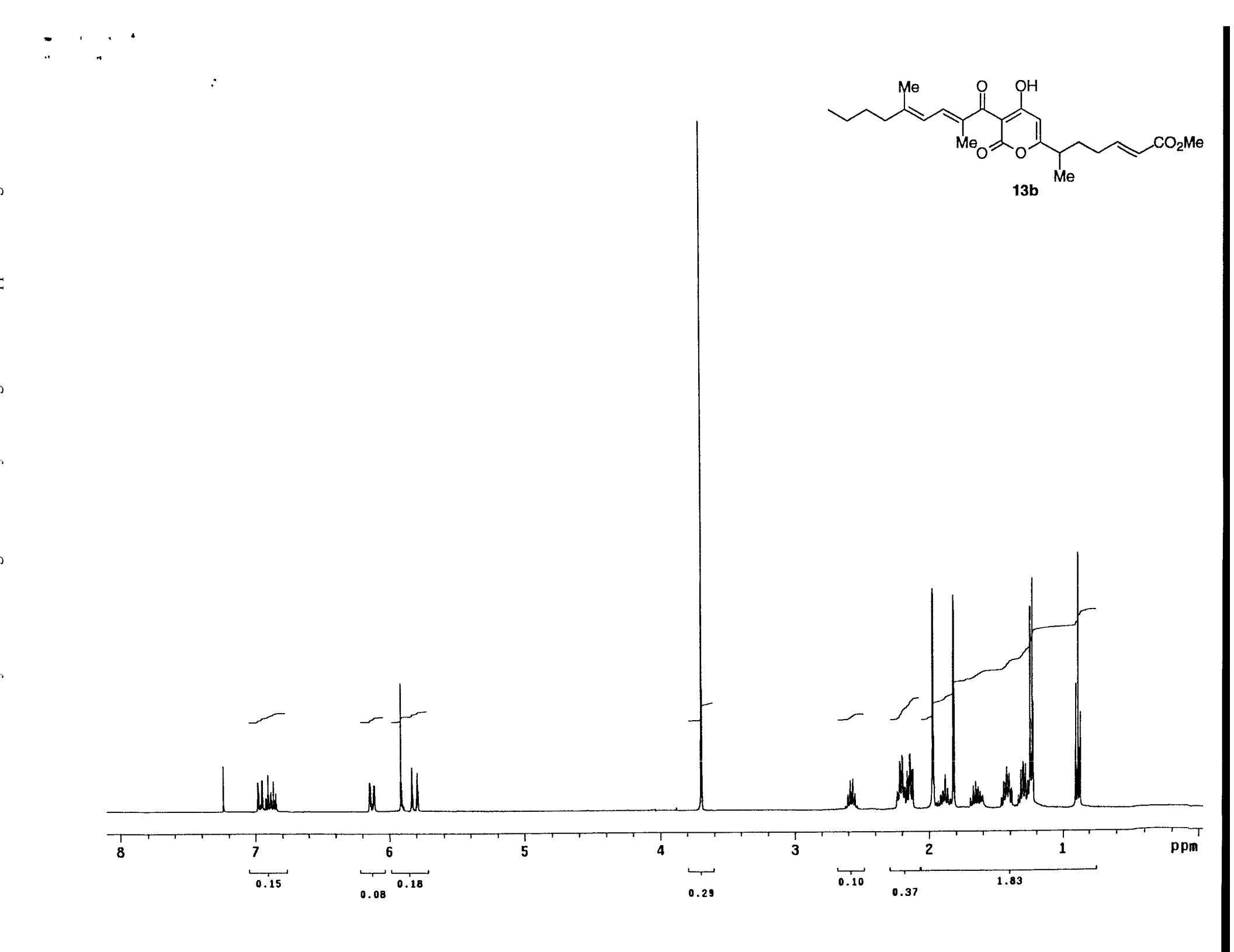
13a

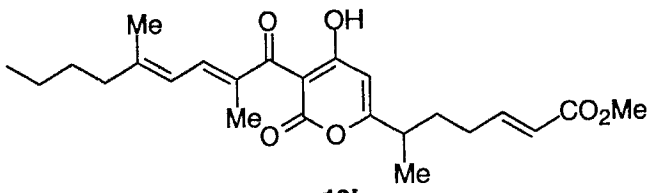




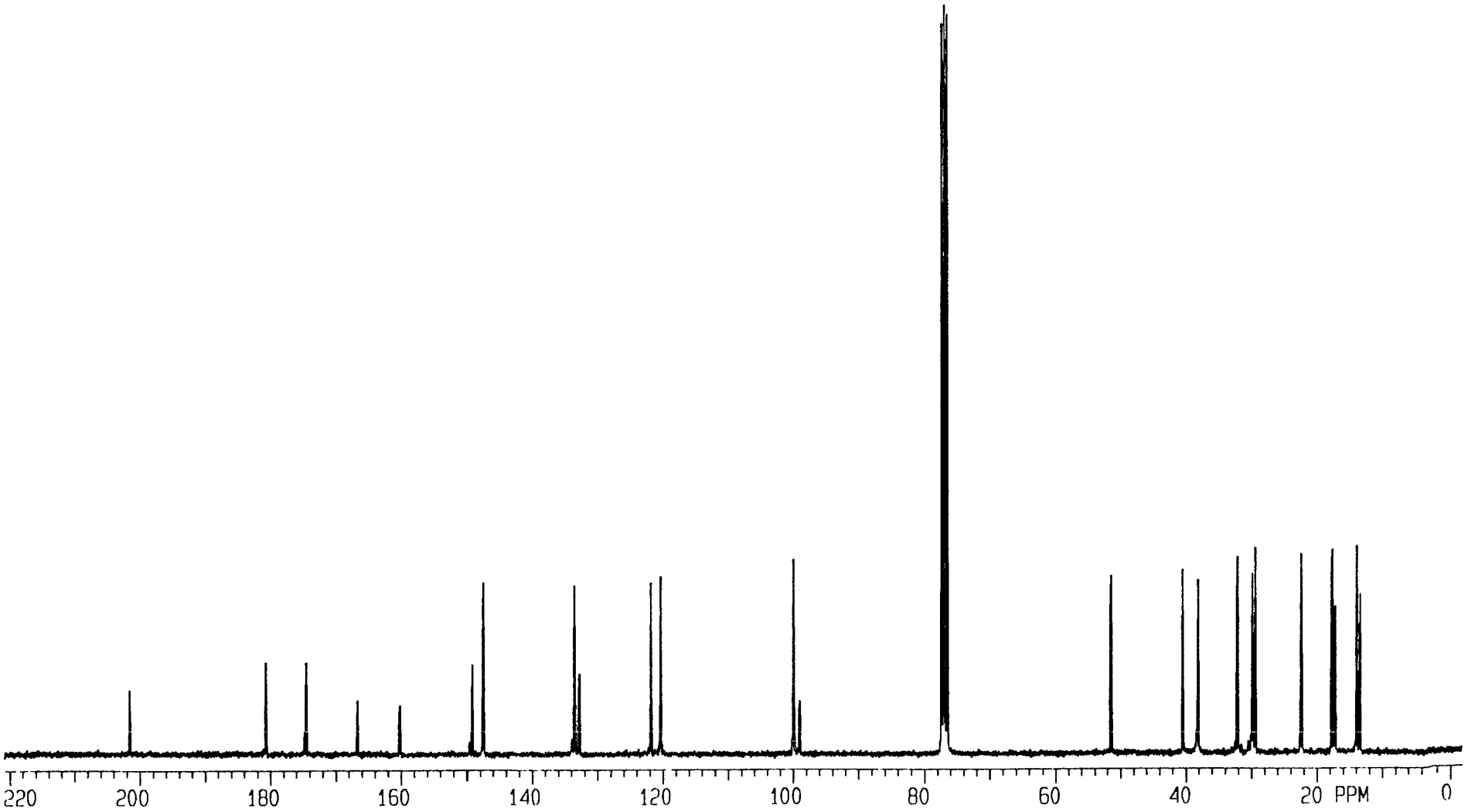
13a

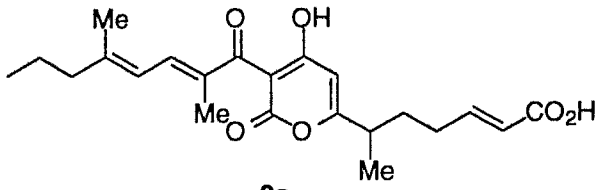



Me
O
OH
Me
O
O
CO2Me
Me
13b
8
7
6
5
4
3
2
1
ppm
0.15
0.08
0.18
0.29
0.10
0.37
1.83

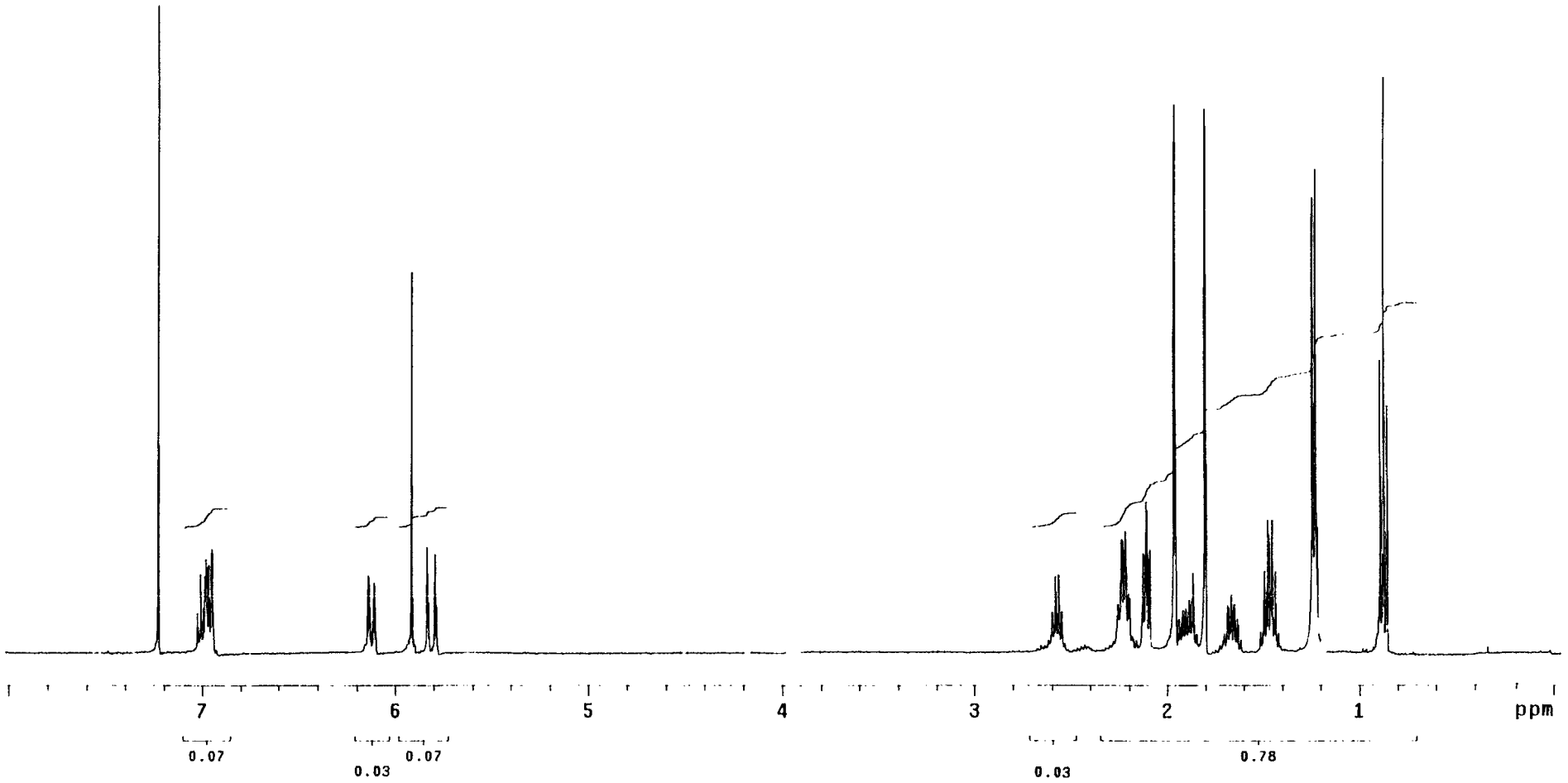


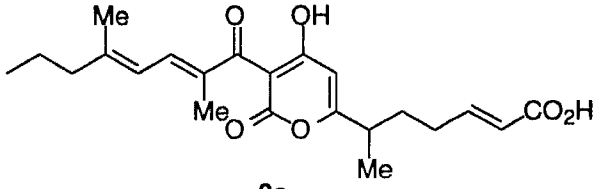
13b



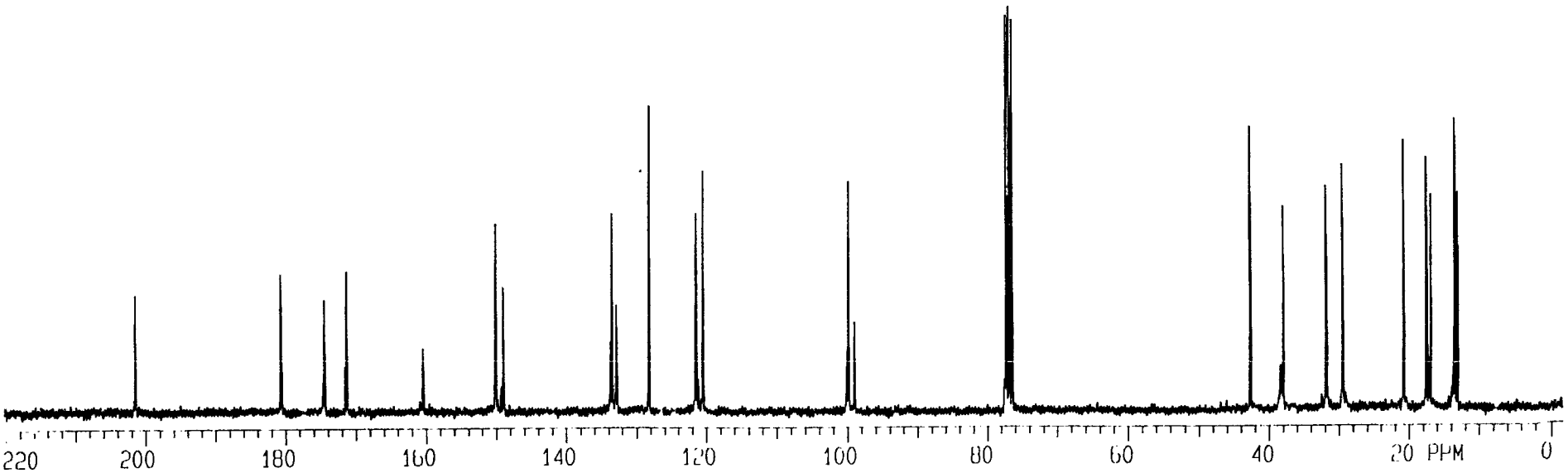


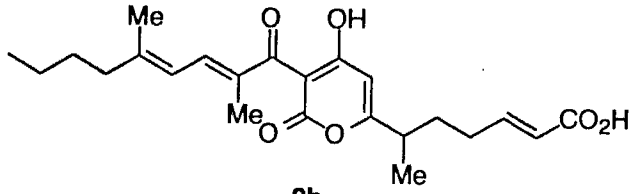
2a



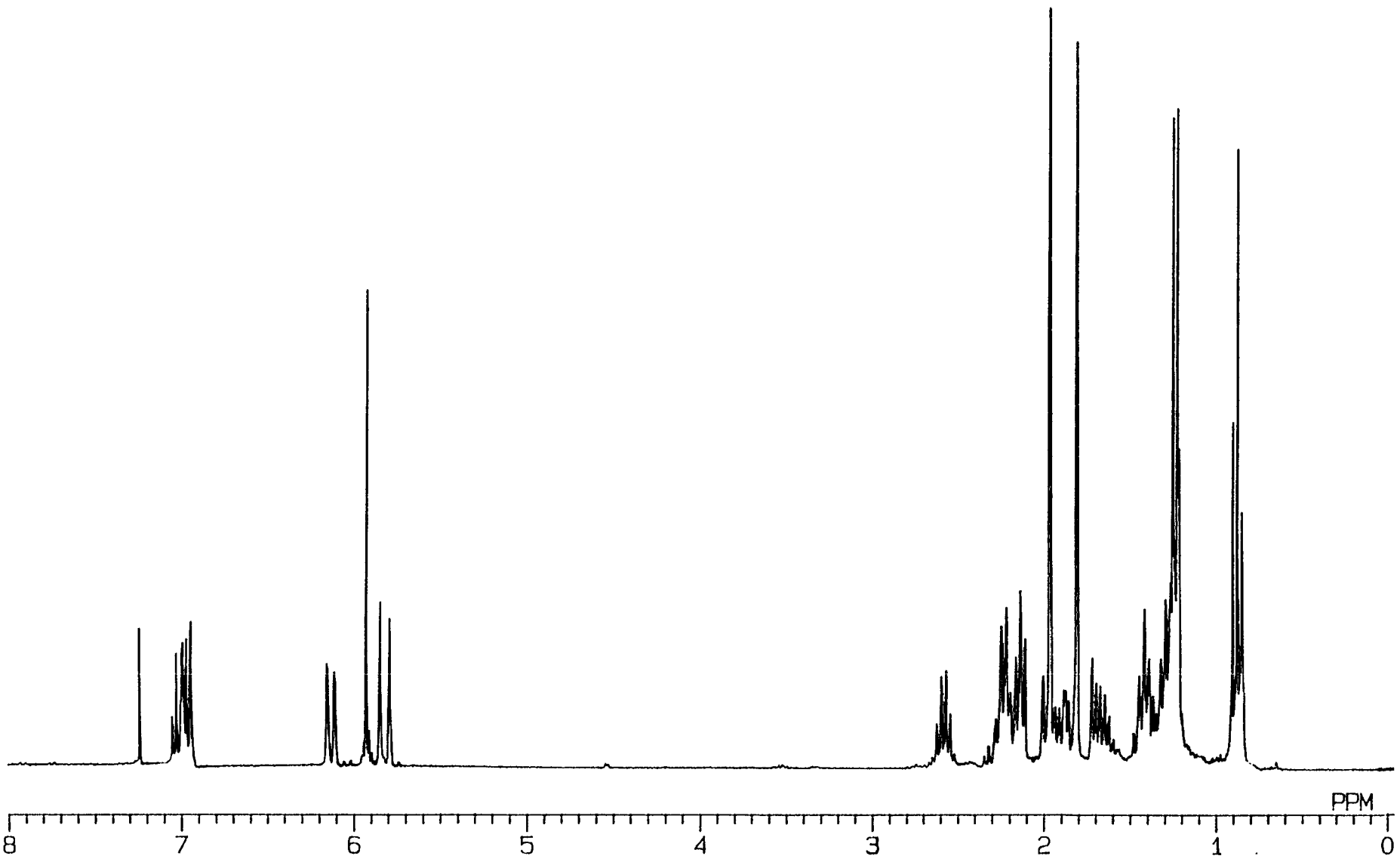


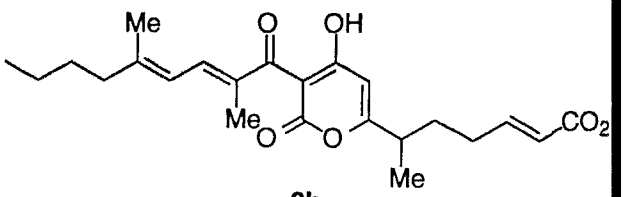
2a



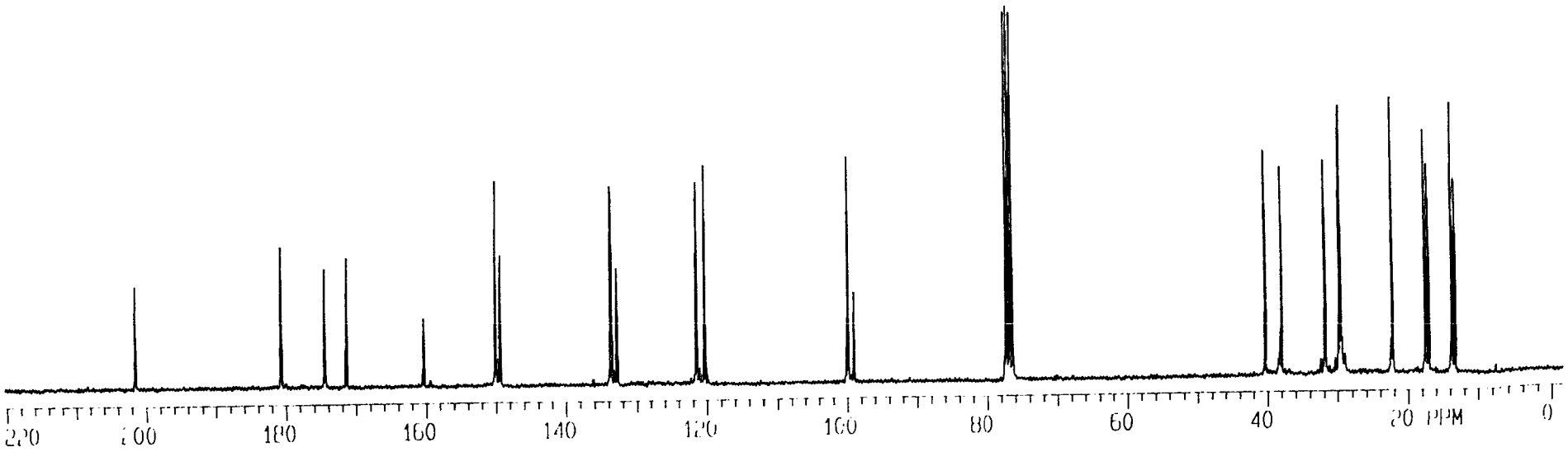


2b

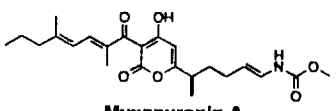




2b



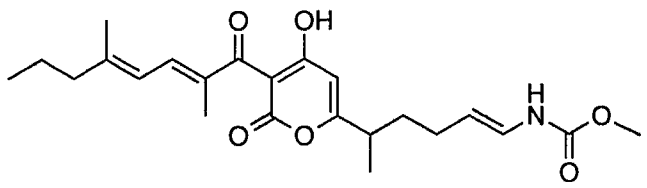
G. Höfle *et al.*, ^{1}H NMR (400 MHz, CD_3OD)



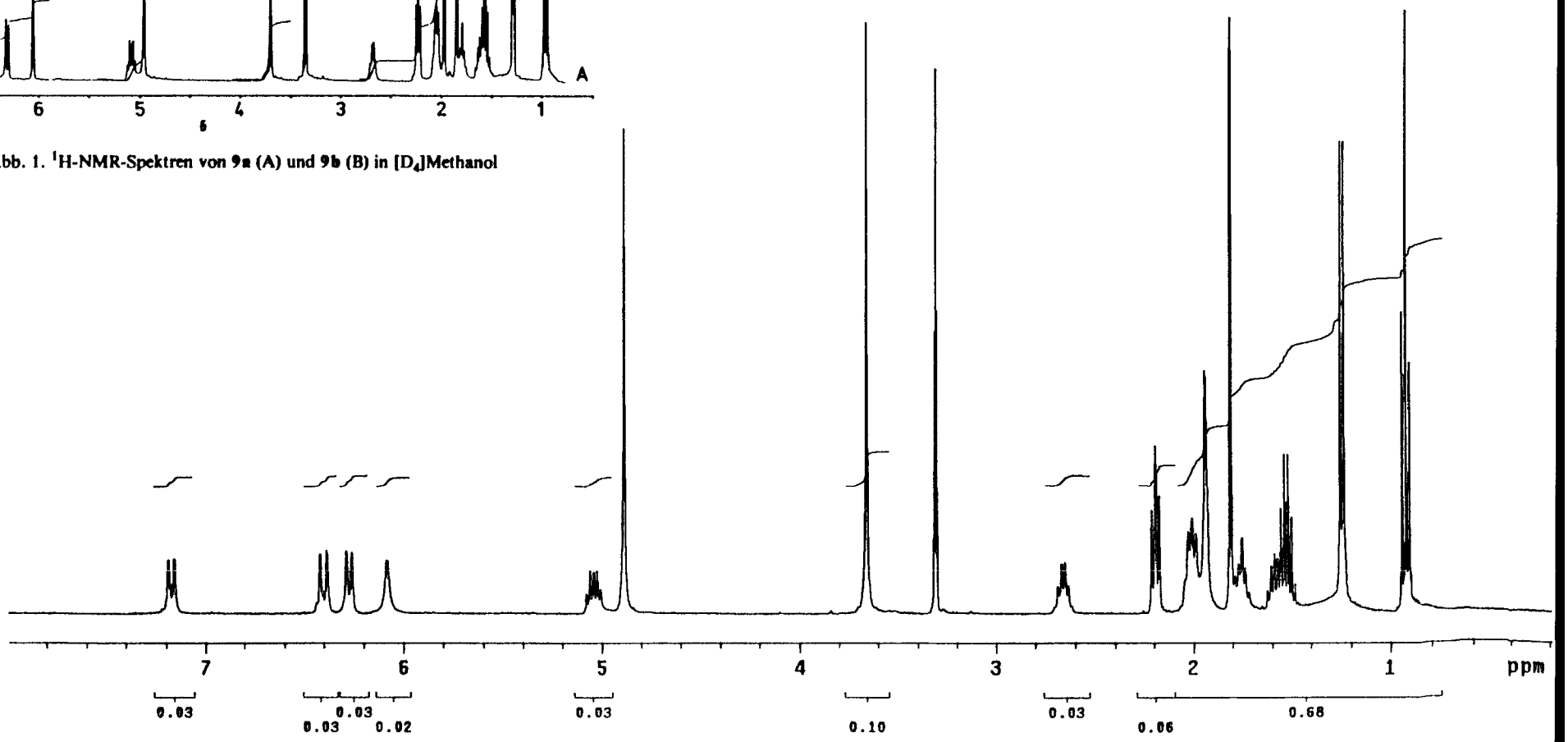
Myxopyronin A

Abb. 1. ^{1}H-NMR-Spektren von **9a** (A) und **9b** (B) in [D_4]Methanol

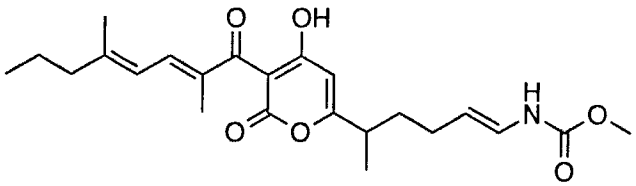
T. Hu, ^{1}H NMR (400 MHz, CD_3OD)



1a; (±) Myxopyronin A

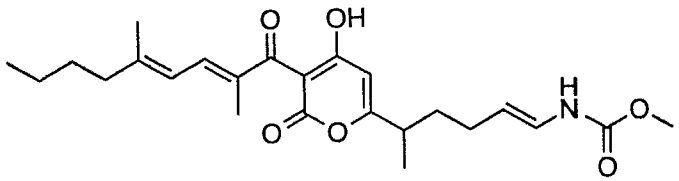


T. Hu, ^{13}C NMR (75 MHz, CD_3OD)



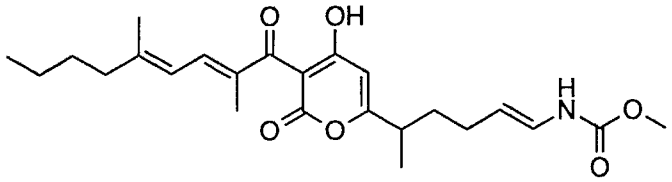
1a; (±) Myxopyronin A

T. Hu, ^{1}H NMR (400 MHz, CD_3OD)



1b; (±) Myxopyronin B

T. Hu, ^{13}C NMR (75 MHz, CD_3OD)



1b; (±) Myxopyronin B

Supporting Information

Total Synthesis and Preliminary Antibacterial Evaluation of the RNA Polymerase Inhibitors (±)-Myxopyronin A & B

Tao Hu[†], Jennifer V. Schaus[†], Kelvin Lam[§], Michael G. Palfreyman[§], Mark Wuonola[§], Gary Gustafson[§] and James S. Panek[†*]

Department of Chemistry, Metcalf Center for Science and Engineering
590 Commonwealth Avenue, Boston University, Boston, Massachusetts 02215 and
Scriptgen Pharmaceuticals Inc., Department of Biology, 200 Boston Avenue, Medford,
Massachusetts 02155

Experimental Section: ^{1}H and ^{13}C NMR spectra were taken in $CDCl_3$ at 400 MHz and 75 MHz respectively unless specified otherwise. Chemical shifts are reported in parts per million using the solvent resonance internal standard (chloroform, 7.24 and 77.0 ppm respectively, unless specified otherwise). NMR Data are reported as follows: chemical shift, multiplicity (app = apparent, par obsc = partially obscured, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, abq = ab quartet), coupling constant, and integration. Infrared Resonance (IR) spectra were recorded on a FTIR spectrophotometer. High resolution mass spectra were obstained on a Finnegan spectrometer in the Boston University Mass Spectrometry Laboratory. Reversed phase preparative HPLC was conducted with Diode Array detector, using 22 x 250 mm C18 column (218TP1022). Methylene chloride (CH_2Cl_2), methanol (MeOH), benzene (C_6H_6), toluene, and hexane were distilled from calcium hydride, and tetrahydrofuran (THF) and hexamethyl phosphoramide (HMPA) were distilled from sodium and benzophenone prior to use. Titanium tetrachloride ($TiCl_4$) was freshly distilled from copper powder under reduced presure before each use. Anhydrous 1, 2-dichloro ethane ($ClCH_2CH_2Cl$), trimethyl aluminum ($AlMe_3$, 2.0 **M** solution in hexanes) and zirconocene dichloride (Cp_2ZrCl_2) was purchased from Aldrich Chemical Company Inc. and used without further purification. All other reagents were used as supplied. All reactions were carried out in oven-dried glassware under argon atmosphere unless otherwise noted. Analytical thin layer chromatography was performed 0.25 mm silica gel 60-A plates. Flash chromatography was performed on silica gel 230-400 mesh.

(*E*)-3-Methyl-hex-2-en-1-ol (11a). To a white slurry of zirconocene dichloride (11.7 g, 40 mmol) in 100 mL of $(CH_2)_2Cl_2$ was added $AlMe_3$ (40 mL, 2.0 **M** in hexanes, 80 mmol) at 0 °C, stirred for 45 min, and then warmed to RT for 1.5 h. To this lemon-yellow solution was added 1-pentyne **9a** (2.72 g, 40 mmol, dissolved in 20 mL $(CH_2)_2Cl_2$ at RT. The reaction was allowed to stir for 3.0 h. The volatile components were evaporated under reduced pressure (maximum 50 °C, 0.3 mm Hg, 2.5h). The remaining orange-yellow organic residue was extracted with dry hexanes (4 x 30 mL), and the yellow extract was transferred to a 500 mL round-bottom flask via a cannula. To this was added ^{n}BuLi (16 mL, 2.5 **M** in hexanes, 40 mmol) at 0 °C. This orange-yellow slurry was stirred from 0 °C to RT for 1.5 h, and then THF (70 mL) was added to dissolve the precipitate. The resulting solution (homogeneous, brown-yellow color) was cannulated to a suspension of paraformaldehyde (6.0 g, 200 mmol) in THF (100mL) under a N_2 atmosphere. This orange-yellow mixture was allowed to stir at RT for 20 h before it was cooled to 0 °C (ice water bath). Ice was added to dilute the reaction, and then saturated NH_4Cl was added. The ice bath was removed and the reaction was further acidified with 3 **M** HCl until the reaction turned to a clear yellow (homogeneous) solution. At this time, the reaction pH was measured 2~3. The organic layer was separated, and the aqueous layer was extracted with ether. The organic extracts were combined and washed with a saturated $NaHCO_3$ solution, then dried with Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude allylic alcohol **11a**. This material was purified by flash chromatography (20% EtOAc in hexanes as the eluant) to afford alcohol **11a** (3.37 g, 74% yield) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (t, J = 7.0 Hz,1H); 4.13 (d, J = 7.0 Hz, 2H); 1.97 (t, J = 7.3 Hz, 2H); 1.64 (s, 3H); 1.4 2(m, 2H), 1.15(br, 1H), 0.85 (t, J = 7.3 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 139.2, 123.3, 59.0, 59.0, 41.5, 20.6, 15.9, 13.6; IR (neat) ν max: 3353, 2959, 1457 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_7H_{14}O_1$ (M^+) 114.1045, found: 114.1035.

(*E*)-3-Methyl-hex-2-en-1-al (12a). To a suspension solution of alcohol **11a** (892 mg, 7.82 mmol) , 4 Å molecular sieves (4.0 g, activated), and NMO (1.83 g, 15.64 mmol) in CH_2CL_2 (12 mL) at 0 °C, was added TPAP (165 mg,0.47 mmol) in one-portion. The resulting dark reaction mixture was allowed to stir at 0 °C for 30 min, before it was diluted with CH_2Cl_2, and then

filtrated through a short pad of silica gel. The eluent was concentrated *in vacuo* to afford aldehyde **12a** (823 mg, 94% yield) as a viscous, colorless oil. This unstable aldehyde was sufficiently pure, and used immediately without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (d, J = 8.2 Hz, 1H), 5.85 (d, J = 8.2 Hz, 1H), 2.17 (t, J = 7.3 Hz, 2H), 2,14 (s, 3H), 1.52 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 191.7, 164.5, 127.8, 43.0, 20.7, 17.8, 14.0; IR (neat) *v*max: 2961, 1678 cm^{-1}.

3-[(*E,E*)-2,5-Dimethyl-2,4-octadienoyl]-4-hydroxy-6-(methyl 6-methyl-hex-2-enoate)-2-pyrone (13a). Compound **8** (58 mg, 0.188 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and stirred at -78 °C under argon atmosphere. To this yellow solution was added freshly distilled $TiCl_4$ (82 µl, 0.75 mmol), the reaction turned to an orange-yellow slurry mixture immediately. After stirring for 45 min at -78 °C, DIPEA (144 µL, 0.83 mmol) was added, and the reaction became a red-dark reaction mixture. This reaction mixture was allowed to stir at -78 °C for 4.0 h, then aldehyde **12a** (84 mg, 0.75 mmol) dissolved in 1.0 mL CH_2Cl_2 was added via a cannula. This red-dark reaction mixture was stirred at -78 °C for 50 h and then 0 °C for 5-10 min, before it was quenched with distilled water. The reaction was extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was flash chromatographed (20% EtOAc in hexanes eluant) to provide diene **13a** (44 mg, 58% yield) as a sticky yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (d, J = 11.4 Hz, 1H), 6.89 (dt, J = 6.5, 15.3 Hz, 1H), 6.13 (d, J = 11.4 Hz, 1H), 5.92 (s, 1H); 5.83 (d, J = 15.3 Hz, 1H), 3.70 (s, 3H), 2.58 (m, 1H), 2.21 (m, 2H), 2.13 (t, J = 7.3 Hz, 2H), 1.98 (s, 3H), 1.90 (m, 1H), 1.82 (s, 3H), 1.66 (m, 1H), 1.48 (m, 2H), 1.24 (d, J = 7.3 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.6, 180.7, 174.7, 166.7, 160.3, 149.1, 147.5, 133.6, 132.9, 121.9, 120.6, 100.0, 99.1,51.5, 42.8, 38.2, 32.2, 29.6, 21.0, 17.7, 17.2, 13.8, 13.5; IR (neat) *v* max: 2958, 1726, 1637, 1436 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{23}H_{31}O_6$ ($M + H^+$) 403.2120, found: 403.2108.

3-[(*E,E*)-2,5-Dimethyl-2,4-octadienoyl]-4-hydroxy-6-(6-methyl-hex-2-enoic acid)-2-pyrone (2a). To a stirred solution of **13a** (60 mg, 0.15 mmol) in THF (8.0 mL) was added LiOH aqueous solution (2.0 mL, 1.0 **M** aq., 2.0 mmol) at RT, the resulting reaction mixture (THF/H_2O = 4:1) was allowed to stir at RT for 20 h before it was diluted with EtOAc, and then

quenched by saturated NH_4Cl solution. The reaction was further acidified to pH = 2 by slow addition of 5% HCL solution. The solution was extracted with EtOAc, dried (Na_2SO_4), filtered through a short pad of silica gel, and concentrated *in vacuo* to afford the crude acid **2a** (58 mg, 100% yield) as a yellow, sticky oil. This material was sufficiently pure and used in the Curtius reaction withought further purification: ^1H NMR (400 MHz, $CDCl_3$): δ 7.03–6.96 (m, 2H), 6.14 (d, J = 11.6 Hz, 1H), 5.92 (s, 1H), 5.82 (d, J = 15.9 Hz, 1H), 2.59 (m, 1H), 2.24 (m, 2H), 2.13 (t, J = 7.6 Hz, 2H), 1.98 (s, 3H), 1.89 (m, 1H), 1.82 (s, 3H), 1.68 (m, 1H), 1.48 (m, 2H), 1.30 (m 2H), 1.24 (d, J = 6.7 Hz, 3 H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 180.7, 174.5, 171.4, 160.4, 150.2, 149.1, 133.6, 132.9, 128.3, 121.6, 120.6, 100.1, 99.1, 42.8, 38.2, 32.0, 29.7, 20.9, 17.7, 17.2, 13.8, 13.4; IR (neat) ν max: 2960, 1724, 1636, 1561 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{22}H_{29}O_6$ ($M + H^+$) 389.1964, found: 389.1968.

(±) **Myxopyronin A (1a).** To a stirred solution of acid **2a** (34 mg, 0.0876 mmol) in dry acetone (1.5 mL) was added DIPEA (37 μL, 0.21 mmol) and then ethyl chloroformate (18 μL, 0.193 mmol) at 0 °C. The reaction was stirred at 0 °C for 1.5 h, then NaN_3 (17 mg, 0.263 mmol, dissolved in 300 μL distilled H_2O) was added via a syringe. The resulting reaction mixture was stirred at 0 °C for 45 min before being diluted with ice water. The reaction mixture was extracted with distilled toluene, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The organic residue was taken up in dry toluene (6 mL) and refluxed for 2.5 h before fresh distilled MeOH (3.0 mL) was added to trap the isocyanate imtermediate. The resulting solution was refluxed for an additional 8.0 h and then concentrated *in vacuo* to provide crude (±)myxopyronin A (**1a**) as a yellow oil. The crude material was purified by preparative reverse phase HPLC (70:30:4 MeOH:H_2O:AcOH) to provide pure (±)myxopyronin A (**1a**, 26 mg, 71%) as a sticky yellow oil: ^1H NMR (400 MHz, CD_3OD, 3.31 ppm) δ 7.17 (d, J = 11.6 Hz, 1H), 6.40 (d, J = 14.0 Hz, 1H), 6.27 (d, J = 11.6 Hz, 1H), 6.08 (s, 1H), 5.04 (dt, J = 7.3, 14.0 Hz, 1H), 3.66 (s, 3H), 2.66 (m, 1H), 2.20 (t, J = 7.3 Hz, 2H), 2.01 (m, 2H), 1.94 (s, 3H), 1.81 (s, 3H), 1.76 (m, 1H), 1.59 (m, 1H), 1.53 (m, 2H), 1.25 (d, J = 6.7 Hz, 3H), 0.93 (t, J = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD, 49.15 ppm) δ 199.0, 174.9, 173.5, 164.8, 156.9, 151.4, 138.3, 135.2, 126.2, 122.4, 110.7, 102.5, 101.4, 52.8, 44.0, 39.3, 35.9, 28.7, 22.2, 18.6, 17.4, 14.2, 12.0; IR (neat) ν max: 3313, 2931,

1717, 1681 cm^{-1}; UV (methanol): λ_{max} (log ε) = 213, 298 nm; CIHRMS (NH_3 gas) calcd for $C_{23}H_{32}N_1O_6$ ($M + H^+$) 418.2230, found: 418.2198.

Microdilution Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays. The minimal inhibitory concentration (MIC) is defined as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the microtiter plate. The MIC is reported as a range between the concentration at which no growth is observed and the concentration of the dilution which immediately followed. Selected inhibitors from the RNA polymerase screen described above were tested for their ability to inhibit bacterial growth in a broth microdilution assay as follows. Mueller-Hinton broth containing 20-25 mg/L Ca2+ and 10-12.5 mg/L Mg2+ (Difco #0757-07-8) is recommended as the medium (pH 7.2 and 7.4 at room temperature) of choice by the NCCLS for rapidly growing or faculative organisms and it demonstrates good batch-to-batch reproducibility for susceptibility testing; is low in sulfonamide, trimethoprim, and tetracycline inhibitors; and yields satisfactory growth of most pathogens. Dilution of antimicrobial agents is performed in a sterile, covered 96-well microtiter plate with flat bottom wells (Costar #9017), and each well contains 100 µL of broth +/- antimicrobial agent. The final concentrations of the small molecule antimicrobial agents are 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.20, 0.10, and 0.05 µg/mL, respectively. Different dilutions are performed for natural product extracts. They are first diluted 100-fold with Mueller-Hinton broth. The final dilutions of the natural product extracts are 200, 400, 800, 1600, 3200, 6400, 12800, 25600, 51200, 1 x 105, 2 x 105, and 4 x 105 -fold. A 1% DMSO (no-drug) row is prepared in Mueller-Hinton broth as a control for 100% growth on each plate. A Mueller-Hinton broth only with no bacteria growth is also included as a negative control for each plate. Ampicillin and rifampin are used as positive controls against all bacterial strains in every experiment.

The overnight culture of a single colony is diluted in sterile Mueller-Hinton broth so that, after inoculation, each well contains approximately 5 x 105 CFU/mL. Within 15 minutes of preparation, 50 mL of the adjusted inoculum suspension is added to the microtiter plate. Each well

is diluted with an equal volume of the antimicrobial agent/control substance diluted with sterile Mueller-Hinton broth. The inoculated microtiter plate is incubated at 35 °C for 16-20 hours. The turbidity of each well is determined by measuring the absorbance at 595 nm on the BioRad Model 3550-UV microplate reader. The rows containing broth only (no cells) serve as a control, and the rows containing the titration of 1% DMSO serve as a control for 100% growth. The average of the broth only controls is subtracted from the average of each duplicate. This value is subsequently normalized to the average of the DMSO controls.

The minimum bactericidal concentration (MBC) is defined as the concentration of antimicrobial agent from which no colonies grow on petri plates or in the medium. In practice, the MBC is arbitrarily defined as the concentration at which a 1000-fold reduction in colony forming units is observed with respect to the original inoculum (survival of 0.1%). The broth dilution method consists of inoculating the wells from an MIC microtiter plate using a 96-well inoculation grid into a fresh microtiter plate containing 100 μL Mueller-Hinton broth per well. The MBC plates are incubated at 37 °C for 16-20 hrs and the MBC values are determined.

The MIC data suggest that these compounds, like rifampicin, do not penetrate *E. coli* efficiently since it does inhibit the growth of a permeabilized *E. coli*. An attractive feature of this series is the activity against strains that are resistant to rifampicin. The MIC for rifampicin is 10 μM against susceptible strains, but inhibitory activity is greatly reduced against rifampicin resistant strains (> 100 μM), illustrating the limitation of rifamipcin and the need for discovery of new agents. Myxopyronins, however, are equiactive against rifampicin susceptible and rifampicin resistant *S. aureus* (Figure 2a,b).

Additional References

1. Burgess, R. R. *J. Biol. Chem.* **1969**, *244*, 6168-6176.

2. McClure, W. R. *Ann. Rev. Biochem* **1985,** *54*, 171-204.

3. Jansen, R.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1985**, 822-836.

4. Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1983**, 1656-1667.

5. Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1984**, 1088-1093.

6. Sampson, B. A.; Misra, R.; Benson, S. *Genetics* **1989**, *122*, 491-501.

7. Shorenstein, R. G.; Losick, R. *J. Biol Chem.* **1973,** *248*, 6170-6173.