Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Synthesis and biological activity profile of novel 2-cinnamylidene-1,3-diones related to coruscanone A. Promising new antileishmanial agents

Martín J. Riveira,[a] Babu L. Tekwani,[b] Guillermo R. Labadie*[a] and Mirta P. Mischne*[a]

[a] *Instituto de Química Rosario (IQUIR-CONICET-UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Suipacha 531, S2002LRK, Rosario, Argentina. Tel-Fax: +54-341-4370477 E-mail: mischne@iquir-conicet.gov.ar; labadie@iquir-conicet.gov.ar*

[b] *National Center for Natural Products Research & Department of Pharmacology, School of Pharmacy, University of Mississippi, University MS 38677, USA.*

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GENERAL INFORMATION

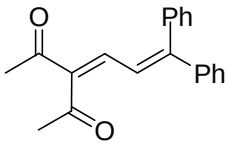
Chemical reagents were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents were analytical grade or were purified by standard procedures prior to use. Yields were calculated for material judged homogeneous by thin layer chromatography (TLC) and nuclear magnetic resonance (^{1}H NMR). All reactions were monitored by thin layer chromatography performed on silica gel 60 F_{254} pre-coated aluminum sheets, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 4-anisaldehyde. Column flash chromatography was performed using silica gel 60 (230 – 400 mesh). Melting points (m.p.) were taken on an electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were acquired at 300 MHz for ^{1}H and 75 MHz for ^{13}C using $CDCl_3$ as solvent. Chemical shifts for proton nuclear magnetic resonance spectra are reported in parts per million relative to the signal of tetramethylsilane (TMS) at 0 ppm (internal standard) and coupling constants (*J*) are reported in hertz (Hz). Chemical shifts for carbon nuclear magnetic resonance (^{13}C NMR) spectra are reported in parts per million relative to the center line of the $CDCl_3$ triplet at 76.9 ppm. The following abbreviations are used to indicate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad signal. IR spectra were obtained using an FT-IR spectrometer and only partial spectral data are listed. High resolution mass spectrometry (ESI-HRMS) was performed in a Bruker microTOF-QII instrument by direct infusion using an electrospray ion source.

EXPERIMENTAL SECTION AND SPECTROSCOPIC DATA

General procedure for the preparation of dienones

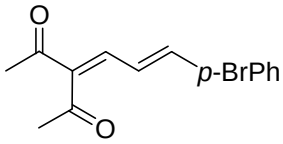
A mixture of 1,3-dicarbonyl substrate (1 mmol), unsaturated aldehyde (1 mmol) and either EDDA (36.0 mg, 0.2 mmol) or MTFA (40.2 mg, 0.2 mmol) in dichloromethane (5.0 ml) was heated at reflux for 4 hours. Solvent was then evaporated under reduced pressure and the residue was purified by flash column chromatography on silica gel (hexanes:ethyl acetate) to afford the following products **6b-d** and **6g-s**.

3-(3,3-Diphenyl-allylidene)-pentane-2,4-dione (6b).



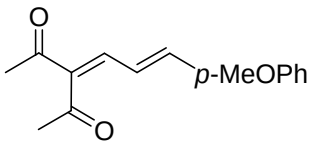
Yellow crystals. Mp.: 101.0-102.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3056, 3029, 3000, 2927, 1689, 1652, 1588, 1444, 1383, 1269, 1167. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.49-7.43 (m, 3H), 7.37-7.29 (m, 5H), 7.28-7.22 (m, 2H), 7.16 (d, J = 11.9 Hz, 1H), 7.03 (d, J = 11.9 Hz, 1H), 2.43 (s, 3H), 2.16 ppm (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 203.3 (s), 197.2 (s), 155.3 (s), 141.6 (s), 140.5 (s), 140.0 (d), 137.9 (s), 130.4 (2 × d), 129.3 (d), 128.8 (d), 128.4 (2 × d), 128.3 (2 × d), 128.2 (2 × d), 121.9 (d), 31.7 (q), 26.0 ppm (q). ESI-HRMS: *m/z* calcd. for $C_{20}H_{19}O_2$ [M + H$^+$] 291.1380, found 291.1375.

3-[(2*E*)-3-(4-Bromo-phenyl)-allylidene]-pentane-2,4-dione (6c).



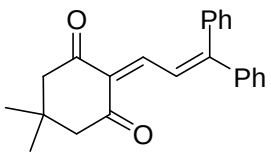
Pale yellow crystals. Mp.: 86.0-87.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3058, 3045, 3015, 2996, 1703, 1645, 1615, 1582, 1406, 1228, 1151, 1070. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.52-7.46 (m, 2H), 7.37-7.32 (m, 2H), 7.22-6.96 (m, 3H), 2.415 (s, 3H), 2.408 ppm (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 202.7 (s), 197.0 (s), 143.3 (d), 142.3 (d), 141.7 (s), 134.2 (s), 132.0 (2 × d), 129.0 (2 × d), 124.0 (s), 123.9 (d), 31.6 (q), 26.3 ppm (q). ESI-HRMS: *m/z* calcd. for $C_{14}H_{14}BrO_2$ ($M + H^+$) 293.0172, found 293.0170.

3-[(2*E*)-3-(4-Methoxy-phenyl)-allylidene]-pentane-2,4-dione (6d).



Pale yellow crystals. Mp.: 103.0-104.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3063, 3001, 2939, 1699, 1643, 1603, 1578, 1512, 1313, 1257, 1180. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.46-7.40 (m, 2H), 7.27-7.16 (m, 1H), 7.02-6.99 (m, 2H), 6.91-6.84 (m, 2H), 3.80 (s, 3H), 2.40 (s, 3H), 2.37 ppm (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 202.8 (s), 196.9 (s), 160.9 (s), 144.9 (d), 143.6 (d), 139.8 (s), 129.3 (2 × d), 128.0 (s), 120.9 (d), 114.1 (2 × d), 55.0 (q), 31.4 (q), 26.0 ppm (q). ESI-HRMS: *m/z* calcd. for $C_{15}H_{16}NaO_3$ ($M + Na^+$) 267.0992, found 267.0985.

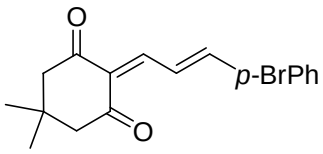
2-(3,3-Diphenyl-allylidene)-5,5-dimethylcyclohexane-1,3-dione (6g).



Yellow crystals. Mp.: 155.0-156.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3080, 3061, 2959, 2949, 2926, 1690, 1656, 1558, 1367, 1179. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.31

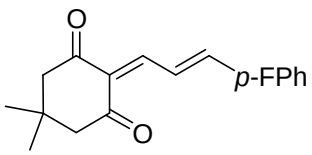
(d, *J* = 12.3 Hz, 1H), 7.75 (d, *J* = 12.3 Hz, 1H), 7.48-7.31 (m, 8H), 7.24-7.20 (m, 2H), 2.56 (bs, 2H), 2.50 (bs, 2H), 1.08 ppm (bs, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 199.0 (s), 197.8 (s), 163.3 (s), 148.3 (d), 140.9 (s), 137.7 (s), 130.9 (2 × d), 130.0 (d), 129.4 (s), 129.32 (d), 129.28 (2 × d), 128.3 (2 × d), 128.2 (2 × d), 124.4 (d), 54.1 (t), 52.3 (t), 30.0 (s), 28.4 ppm (2 × q). ESI-HRMS: *m/z* calcd. for $C_{23}H_{22}O_4K$ ($M + K^+$) 369.1251, found 369.1248.

2-[(2*E*)-3-(4-Bromo-phenyl)-allylidene]-5,5-dimethyl-cyclohexane-1,3-dione (6h).



Yellow crystals. Mp: 138.0-138.5 °C. IR (KBr): ν_{max}/cm^{-1} 3078, 3030, 2956, 2935, 1695, 1651, 1596, 1538, 1373, 1269, 1172, 1006. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.33 (dd, *J* = 15.4, 12.1 Hz, 1H), 7.74 (d, *J* = 12.3 Hz, 1H), 7.54-7.44 (m, 4H), 7.26 (d, *J* = 15.6 Hz, 1H), 2.56 (bs, 2H), 2.55 (bs, 2H), 1.09 ppm (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 198.8 (s), 197.6 (s), 151.1 (d), 150.3 (d), 134.4 (s), 132.1 (2 × d), 129.8 (2 × d), 129.1 (s), 125.9 (d), 125.1 (s), 53.9 (t), 52.2 (t), 29.9 (s), 28.4 ppm (2 × q). ESI-HRMS: *m/z* calcd. for $C_{17}H_{18}O_2Br$ ($M + H^+$) 333.0485, found 333.0488.

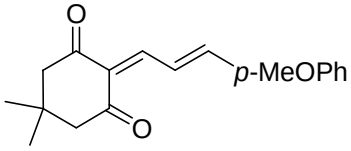
2-[(2*E*)-3-(4-Fluoro-phenyl)-allylidene]-5,5-dimethyl-cyclohexane-1,3-dione (6i).



Yellow to orange crystals. Mp: 111.0-112.0 °C. IR (KBr): ν_{max}/cm^{-1} 3063, 3050, 3005, 2962, 2931, 1686, 1651, 1596, 1582, 1543, 1366, 1236, 1161. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.29 (dd, *J* = 15.4, 12.1 Hz, 1H), 7.76 (d, *J* = 12.1 Hz, 1H),

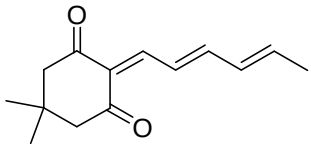
7.65-7.58 (m, 2H), 7.30 (d, *J* = 15.4 Hz, 1H), 7.13-7.05 (m, 2H), 2.56 (bs, 2H), 2.55 (bs, 2H), 1.09 ppm (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 198.8 (s), 197.6 (s), 164.1 (ds, *J* = 252.1 Hz), 151.5 (d), 150.8 (d), 131.8 (ds, *J* = 3.0 Hz), 130.5 (2 × dd, *J* = 9.0 Hz), 128.8 (s), 125.1 (dd, *J* = 2.2 Hz), 116.0 (2 × dd, *J* = 21.8 Hz), 53.9 (t), 52.2 (t), 29.9 (s), 28.4 ppm (2 × q). ESI-HRMS: *m/z* calcd. for $C_{17}H_{18}FO_2$ ($M + H^+$) 273.1285, found 273.1284.

2-[(2*E*)-3-(4-Methoxyphenyl)-allylidene]-5,5-dimethylcyclohexane-1,3-dione (6j).



Orange crystals. Mp.: 142.0-143.0 ºC. IR (KBr): ν_{max}/cm^{-1} 2956, 2935, 1684, 1641, 1586, 1526, 1326, 1271, 1168. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.30 (dd, *J* = 15.3, 12.1 Hz, 1H), 7.81 (d, *J* = 12.1 Hz, 1H), 7.62-7.57 (m, 2H), 7.33 (d, *J* = 15.3 Hz, 1H), 6.94-6.89 (m, 2H), 3.86 (s, 3H), 2.54 (bs, 2H), 2.53 (bs, 2H), 1.09 ppm (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 198.8 (s), 197.8 (s), 162.1 (s), 153.8 (d), 152.0 (d), 130.7 (2 × d), 128.5 (s), 127.5 (s), 123.5 (d), 114.4 (2 × d), 55.3 (q), 53.9 (t), 52.2 (t), 30.0 (s), 28.4 ppm (2 × q). ESI-HRMS: *m/z* calcd. for $C_{18}H_{20}O_3Na$ ($M + Na^+$) 307.1305, found 307.1290.

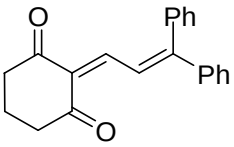
2-[(2*E*,4*E*)-Hexa-2,4-dien-1-ylidene]-5,5-dimethylcyclohexane-1,3-dione (6k).



Yellow to orange crystals. Mp.: 76.0-77.0 ºC. IR (KBr): ν_{max}/cm^{-1} 2954, 1645, 1575, 1530, 1242. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.75-7.62 (m, 2H), 7.07-6.93

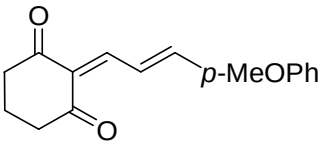
(m, 1H), 6.40 (ddq, *J* = 15.1, 10.5, 1.4 Hz, 1H), 6.24 (dq, *J* = 14.9, 6.8 Hz, 1H), 2.52 (bs, 4H), 1.91 (dd, *J* = 6.7, 1.2 Hz, 3H), 1.07 ppm (bs, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 198.6 (s), 197.6 (s), 154.1 (d), 151.4 (d), 141.5 (d), 132.1 (d), 127.9 (s), 127.4 (d), 53.8 (t), 52.1 (t), 29.8 (s), 28.3 (2 × q), 18.9 ppm (q). ESI-HRMS: *m/z* calcd. for $C_{14}H_{18}O_2Na$ ($M + Na^+$) 241.1199, found 241.1198.

2-(3,3-Diphenyl-allylidene)-cyclohexane-1,3-dione (6l).



Orange crystals. Mp.: 125.5-126.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3076, 3062, 2950, 1657, 1645, 1551, 1527, 1364, 1336, 1260, 1177. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.28 (d, *J* = 12.4 Hz, 1H), 7.76 (d, *J* = 12.4 Hz, 1H), 7.48-7.31 (m, 8H), 7.23-7.18 (m, 2H), 2.66 (t, *J* = 6.0 Hz, 2H), 2.59 (t, *J* = 6.2 Hz, 2H), 2.02 ppm (p, *J* = 6.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 199.2 (s), 198.0 (s), 163.2 (s), 148.9 (d), 140.9 (s), 137.7 (s), 130.9 (2 × d), 130.0 (d), 129.3 (d), 129.2 (2 × d), 128.3 (2 × d), 128.1 (2 × d), 124.5 (d), 40.2 (t), 38.6 (t), 18.0 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{21}H_{18}O_2Na$ ($M + Na^+$) 325.1199, found 325.1193.

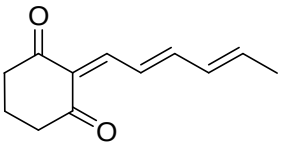
2-[(2*E*)-3-(4-Methoxyphenyl)-allylidene]cyclohexane-1,3-dione (6m).



Orange crystals. Mp.: 108.0-109.0 ºC. IR (film): ν_{max}/cm^{-1} 2954, 1681, 1644, 1590, 1529, 1367, 1263, 1165. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.27 (dd, *J* = 15.3, 12.1 Hz, 1H), 7.82 (d, *J* = 12.1 Hz, 1H), 7.61-7.57 (m, 2H), 7.32 (d, *J* = 15.3 Hz,

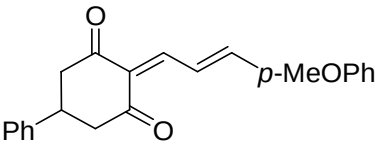
1H), 6.94-6.89 (m, 2H), 3.85 (s, 3H), 2.65 (t, *J* = 5.6 Hz, 2H), 2.63 (t, *J* = 6.1 Hz, 2H), 2.03 ppm (p, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 199.1 (s), 198.1 (s), 162.1 (s), 153.8 (d), 152.6 (d), 130.7 (2 × d), 129.0 (s), 128.4 (s), 123.5 (d), 114.4 (2 × d), 55.3 (q), 40.1 (t), 38.5 (t), 18.1 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{16}H_{16}O_3Na$ (M + Na^+) 279.0992, found 279.0979.

2-[(2*E*,4*E*)-Hexa-2,4-dien-1-ylidene]cyclohexane-1,3-dione (6n).



Yellow to orange crystals. Mp.: 96.0-97.0 ºC. IR (KBr): ν_{max}/cm^{-1} 2988, 2952, 1692, 1652, 1643, 1525, 1372, 1179, 1026. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.75-7.59 (m, 2H), 7.07-6.91 (m, 1H), 6.39 (dd, *J* = 15.3, 10.5 Hz, 1H), 6.31-6.17 (m, 1H), 2.61 (t, *J* = 6.0 Hz, 4H), 2.02 (p, *J* = 6.0 Hz, 2H), 1.91 ppm (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 199.0 (s), 198.0 (s), 154.2 (d), 152.2 (d), 141.7 (d), 132.2 (d), 129.5 (s), 127.5 (d), 40.1 (t), 38.5 (t), 18.9 (q), 18.0 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{12}H_{14}O_2Na$ (M + Na^+) 213.0886, found 213.0886.

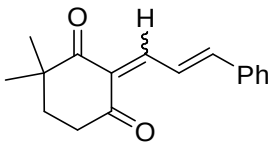
2-[(2*E*)-3-(4-Methoxyphenyl)-allylidene]-5-phenylcyclohexane-1,3-dione (6o).



Orange crystals. Mp.: 168.0-169.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3064, 3027, 3015, 2941, 2913, 1686, 1645, 1567, 1538, 1377, 1259, 1178. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.33 (dd, *J* = 15.4, 12.1 Hz, 1H), 7.86 (d, *J* = 12.1 Hz, 1H), 7.62-7.57 (m, 2H), 7.38-7.31 (m, 3H), 7.28-7.20 (m, 3H), 6.93-6.88 (m, 2H), 3.84 (s, 3H), 3.39 (tt, *J* =

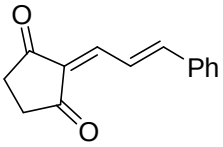
11.8, 4.1 Hz, 1H), 2.98-2.74 ppm (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 198.0 (s), 196.9 (s), 162.2 (s), 154.5 (d), 153.0 (d), 142.0 (s), 130.8 (2 × d), 128.7 (2 × d), 128.3 (s), 127.8 (s), 126.9 (d), 126.3 (2 × d), 123.4 (d), 114.4 (2 × d), 55.3 (q), 47.4 (t), 45.8 (t), 35.7 ppm (d). ESI-HRMS: *m/z* calcd. for $C_{22}H_{20}O_3Na$ (M + Na^+) 355.1305, found 355.1285.

(2*E*)-4,4-Dimethyl-2-[(2*E*)-3-phenyl-allylidene]-cyclohexane-1,3-dione and (2*Z*)-4,4-Dimethyl-2-[(2*E*)-3-phenyl-allylidene]-cyclohexane-1,3-dione (6p) (since all ^{1}H NMR signals of both diasteroisomers overlap we were unable to determine the diasteromeric ratio)**.**



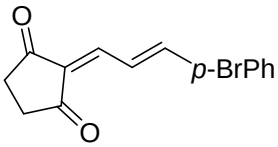
Yellow solid. Mp.: 86.5-87.5 ºC IR (KBr): ν_{max}/cm^{-1} 3078, 3069, 3059, 2955, 2922, 1684, 1645, 1533, 1447, 1362, 1273, 1171, 1072. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.39-8.22 (m, 2H), 7.82-7.72 (m, 2H), 7.65-7.55 (m, 4H), 7.40-7.34 (m, 6H), 7.30 (d, *J* = 15.6 Hz, 2H), 2.66 (t, *J* = 6.6 Hz, 4H), 1.87 (t, *J* = 6.8 Hz, 4H), 1.23 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 203.3 (s), 202.0 (s), 199.0 (s), 197.8 (s), 152.3 (d), 152.2 (d), 151.8 (d), 151.6 (d), 135.4 (2 × s), 130.5 (2 × d), 129.4 (2 × s), 128.6 (4 × d), 128.3 (4 × d), 125.4 (d), 125.3 (d), 42.7 (s), 41.5 (s), 35.9 (t), 34.5 (t), 31.6 (t), 31.5 (t), 24.5 (2 × q), 24.4 ppm (2 x q). ESI-HRMS: *m/z* calcd. for $C_{17}H_{19}O_2$ (M + H^+) 255.1380, found 255.1379.

2-[(2*E*)-3-Phenyl-allylidene]-cyclopentane-1,3-dione (6q).



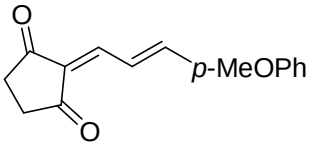
Orange crystals. Mp.: 135.0-136.0 ºC. IR (KBr): ν_{max}/cm^{-1} 3062, 3024, 3000, 2953, 2918, 1672, 1597, 1568, 1371, 1172. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.38 (dd, *J* = 15.5, 11.9 Hz, 1H), 7.69-7.65 (m, 2H), 7.53 (d, *J* = 11.9 Hz, 1H), 7.46-7.40 (m, 4H), 2.76-2.70 ppm (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 203.5 (s), 202.9 (s), 155.0 (d), 148.3 (d), 135.1 (s), 131.6 (d), 129.1 (2 × d), 129.0 (2 × d), 127.3 (s), 123.7 (d), 34.9 (t), 34.2 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{14}H_{12}O_2Na$ ($M + Na^+$) 235.0729, found 235.0723.

2-[(2*E*)-3-(4-Bromo-phenyl)-allylidene]-cyclopentane-1,3-dione (6r).



Orange crystals. Mp.: 230.0 ºC (dec.). IR (KBr): ν_{max}/cm^{-1} 3059, 3049, 2926, 2909, 1716, 1666, 1564, 1403, 1367, 1171, 1106. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.35 (dd, *J* = 15.3, 12.0 Hz, 1H), 7.58-7.46 (m, 5H), 7.33 (d, *J* = 15.6 Hz, 1H), 2.76-2.70 ppm (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 203.5 (s), 202.7 (s), 152.9 (d), 147.6 (d), 134.0 (s), 132.3 (2 × d), 130.2 (2 × d), 127.7 (s), 126.0 (s), 124.1 (d), 34.9 (t), 34.2 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{14}H_{12}BrO_2$ ($M + H^+$) 291.0015, found 291.0012.

2-[(2*E*)-3-(4-Methoxyphenyl)allylidene]-cyclopentane-1,3-dione (6s).



Orange to red crystals. Mp.: 150.0 ºC (dec.). IR (KBr): ν_{max}/cm^{-1} 2925, 1670, 1562, 1374, 1258, 1169, 1098. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.27 (dd, *J* = 15.3, 12.3 Hz, 1H), 7.67-7.61 (m, 2H), 7.51 (d, *J* = 12.1 Hz, 1H), 7.39 (d, *J* = 15.1 Hz, 1H), 6.97-6.92 (m, 2H), 3.88 (s, 3H), 2.75-2.64 ppm (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 203.7 (s), 203.1 (s), 162.8 (s), 155.5 (d), 149.1 (d), 131.3 (2 × d), 128.1 (s), 126.2 (s), 121.7 (d), 114.6 (2 × d), 55.4 (q), 34.8 (t), 34.1 ppm (t). ESI-HRMS: *m/z* calcd. for $C_{15}H_{14}O_3Na$ ($M + Na^+$) 265.0835, found 265.0827.

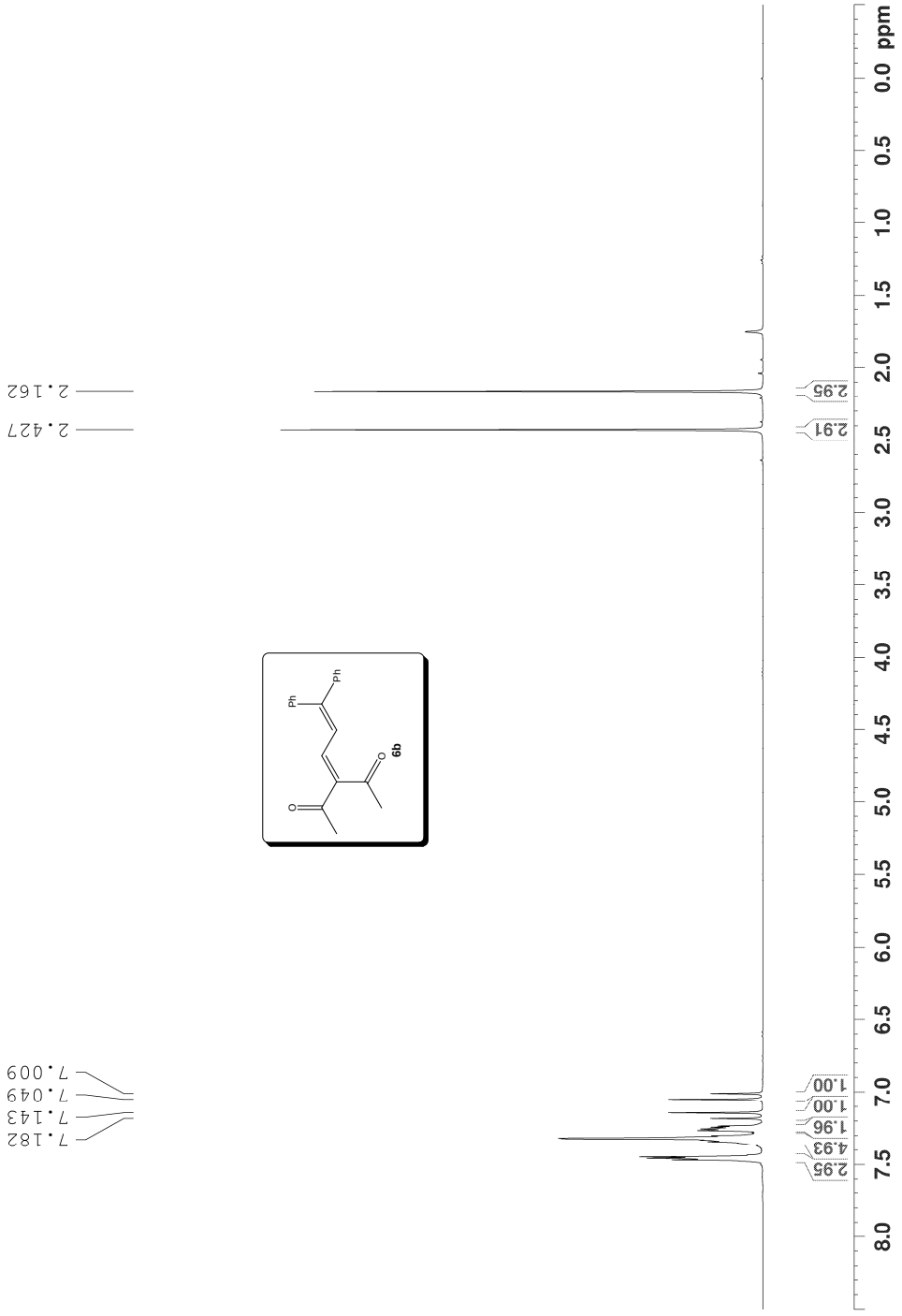


Fig. 1 ^{1}H NMR Spectra of Compound **6b**

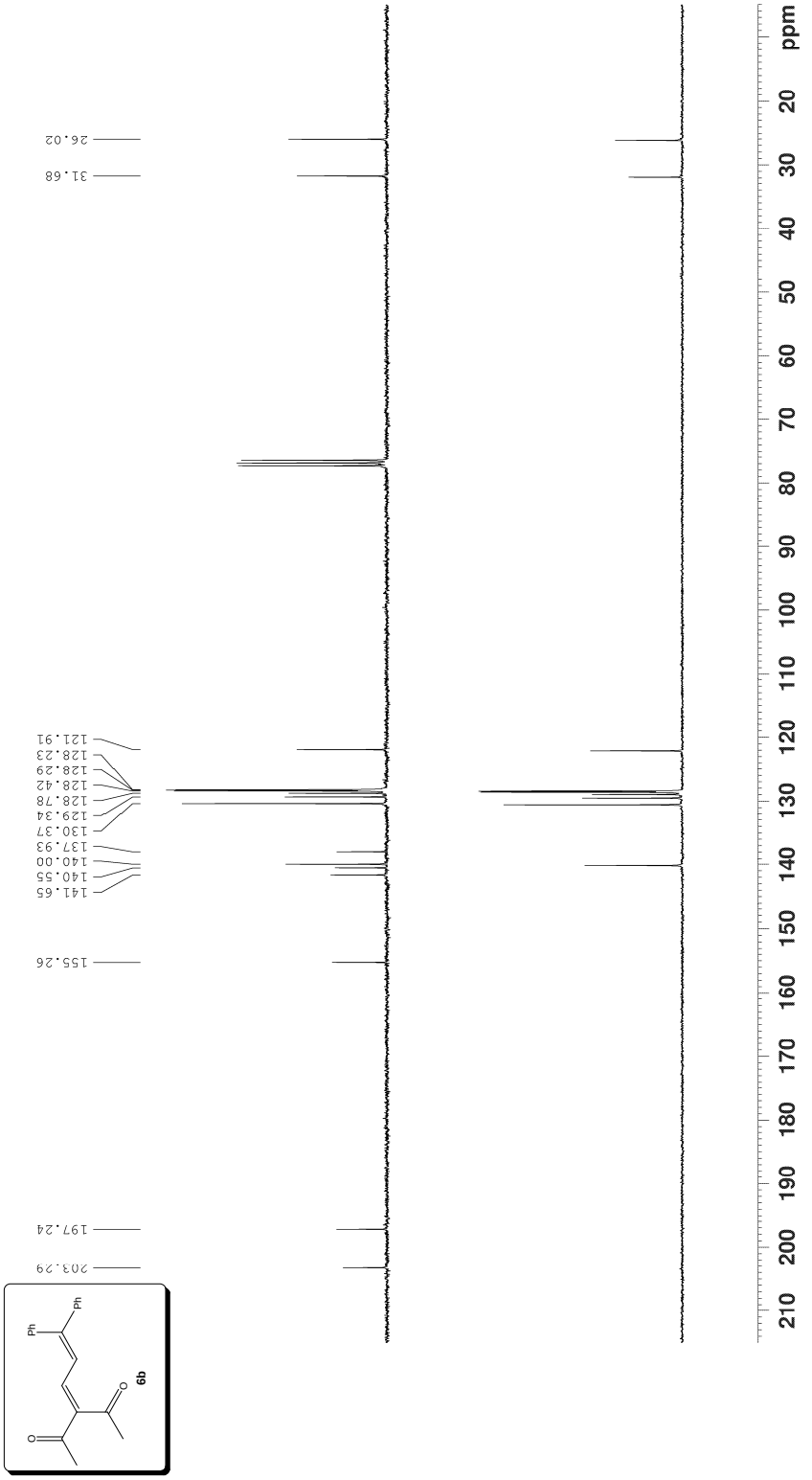


Fig. 2 ^{13}C NMR Spectra of Compound **6b**

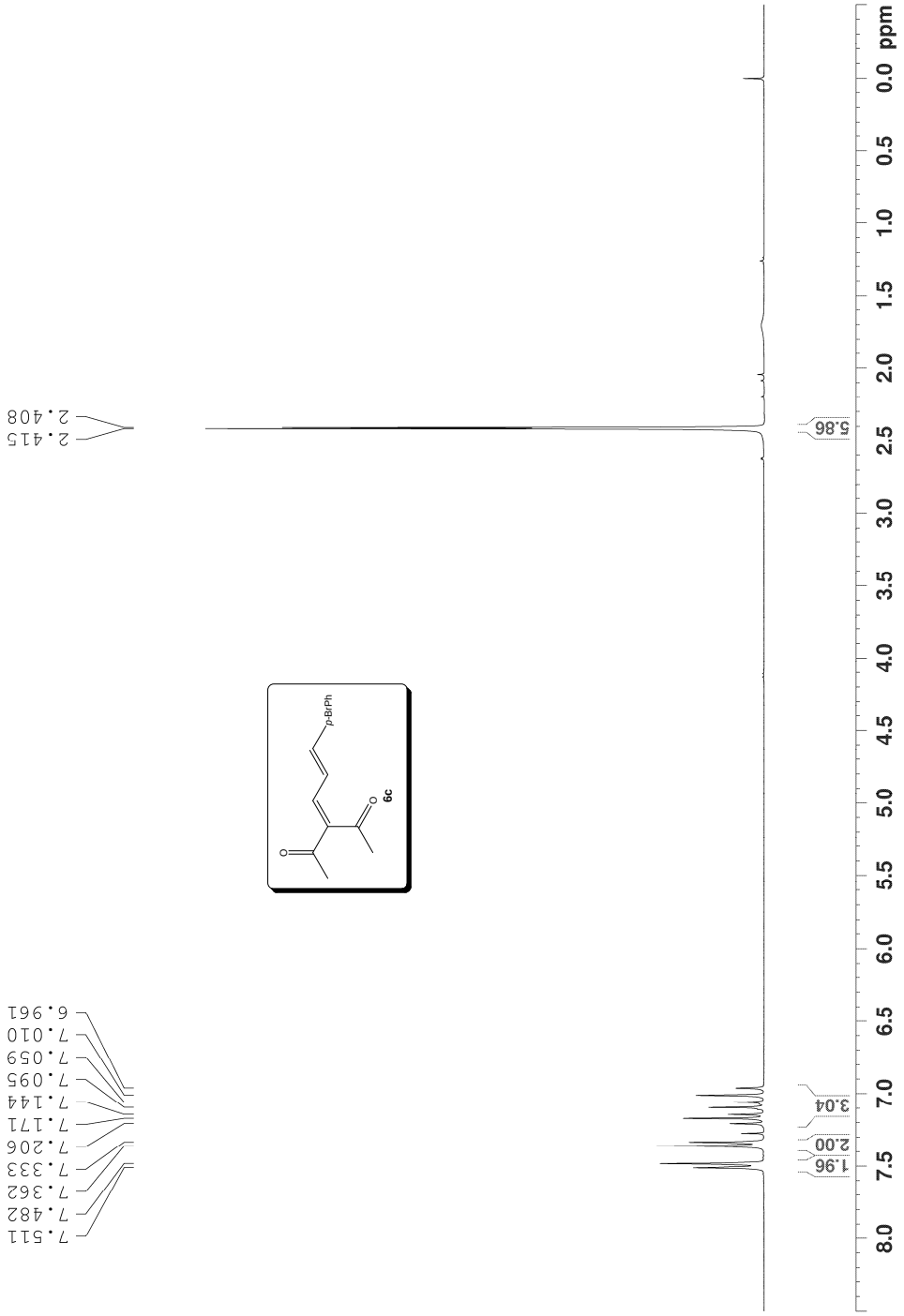


Fig. 3 ^{1}H NMR Spectra of Compound **6c**

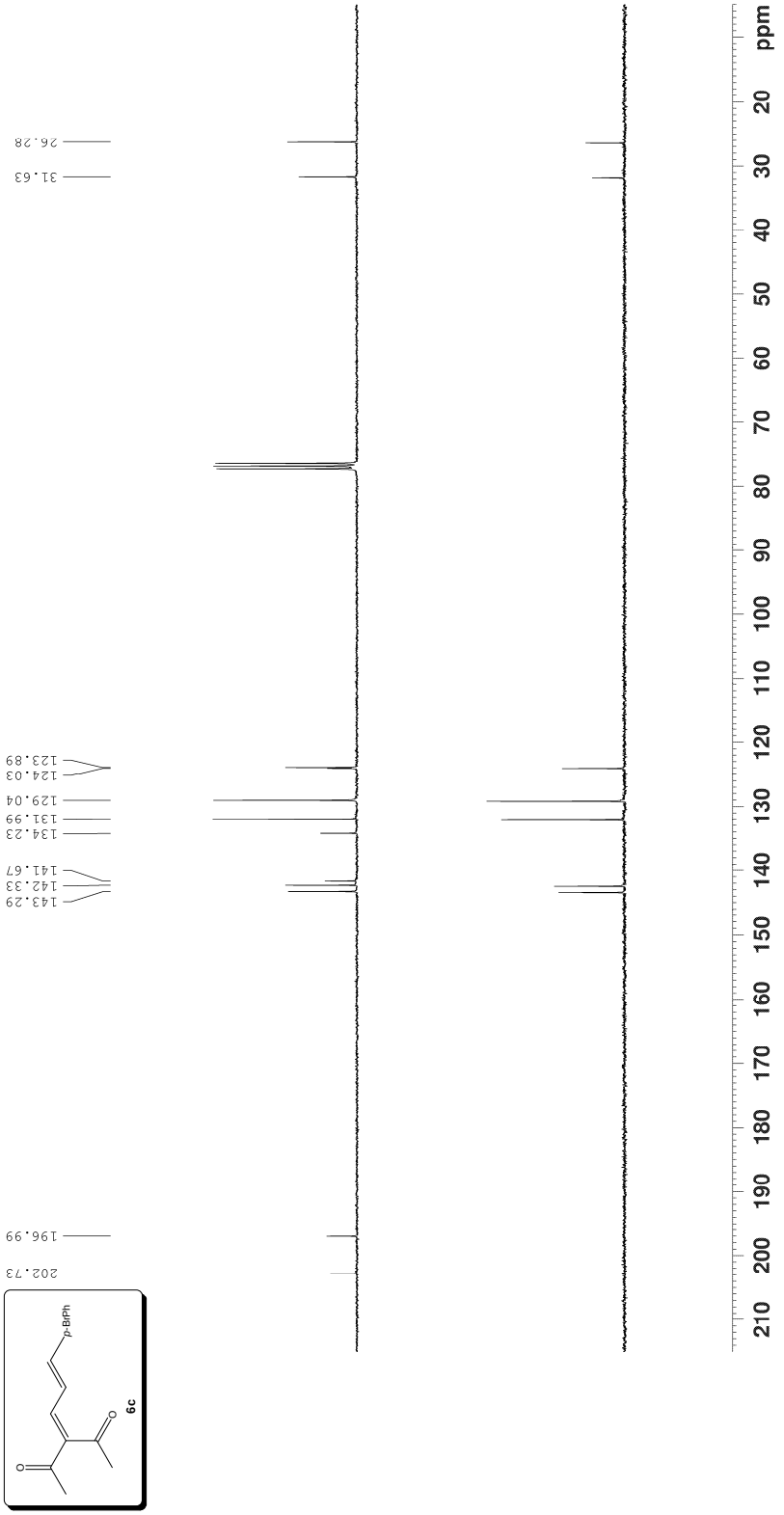


Fig. 4 ^{13}C NMR Spectra of Compound **6c**

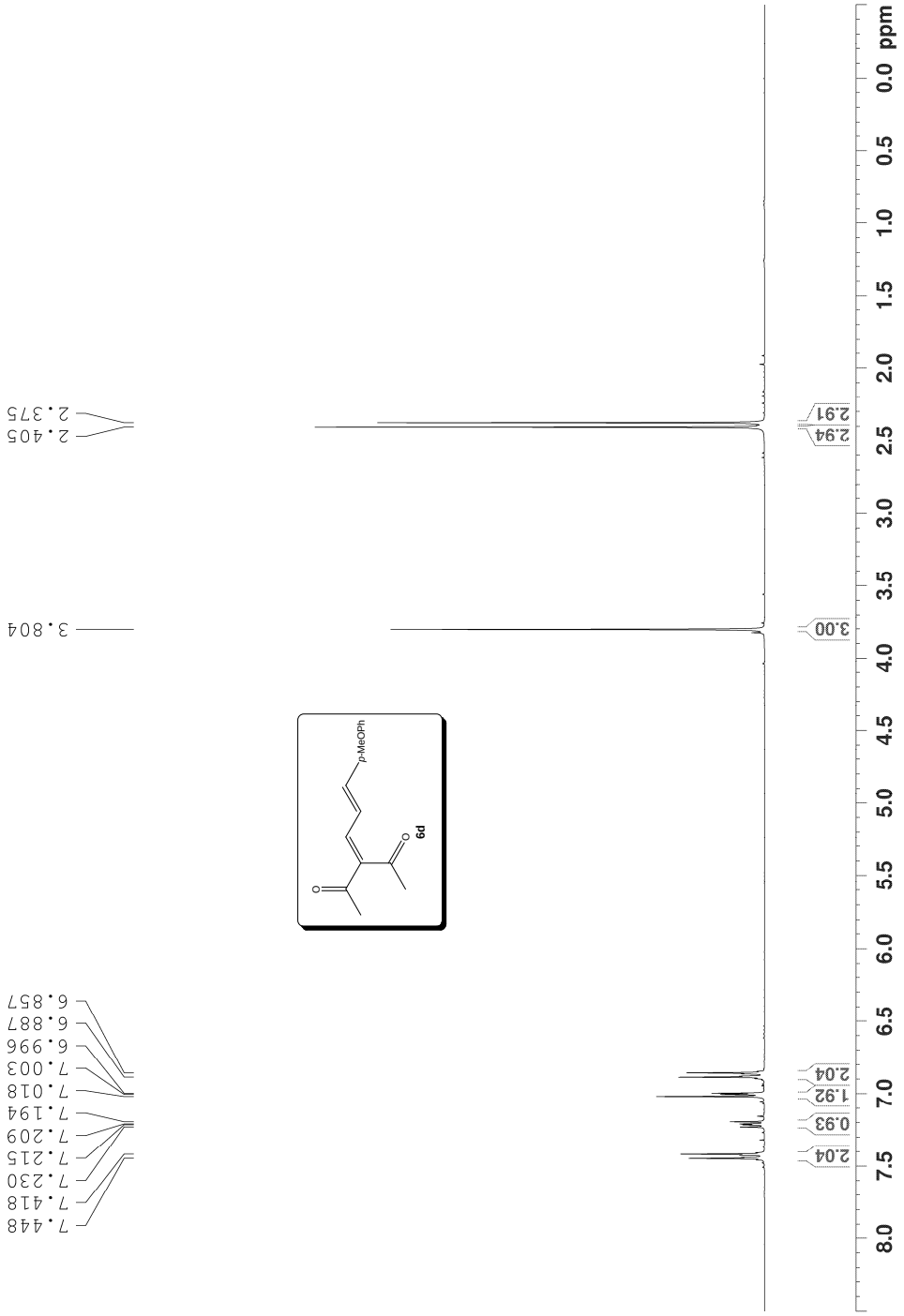


Fig. 5 ^{1}H NMR Spectra of Compound **6d**

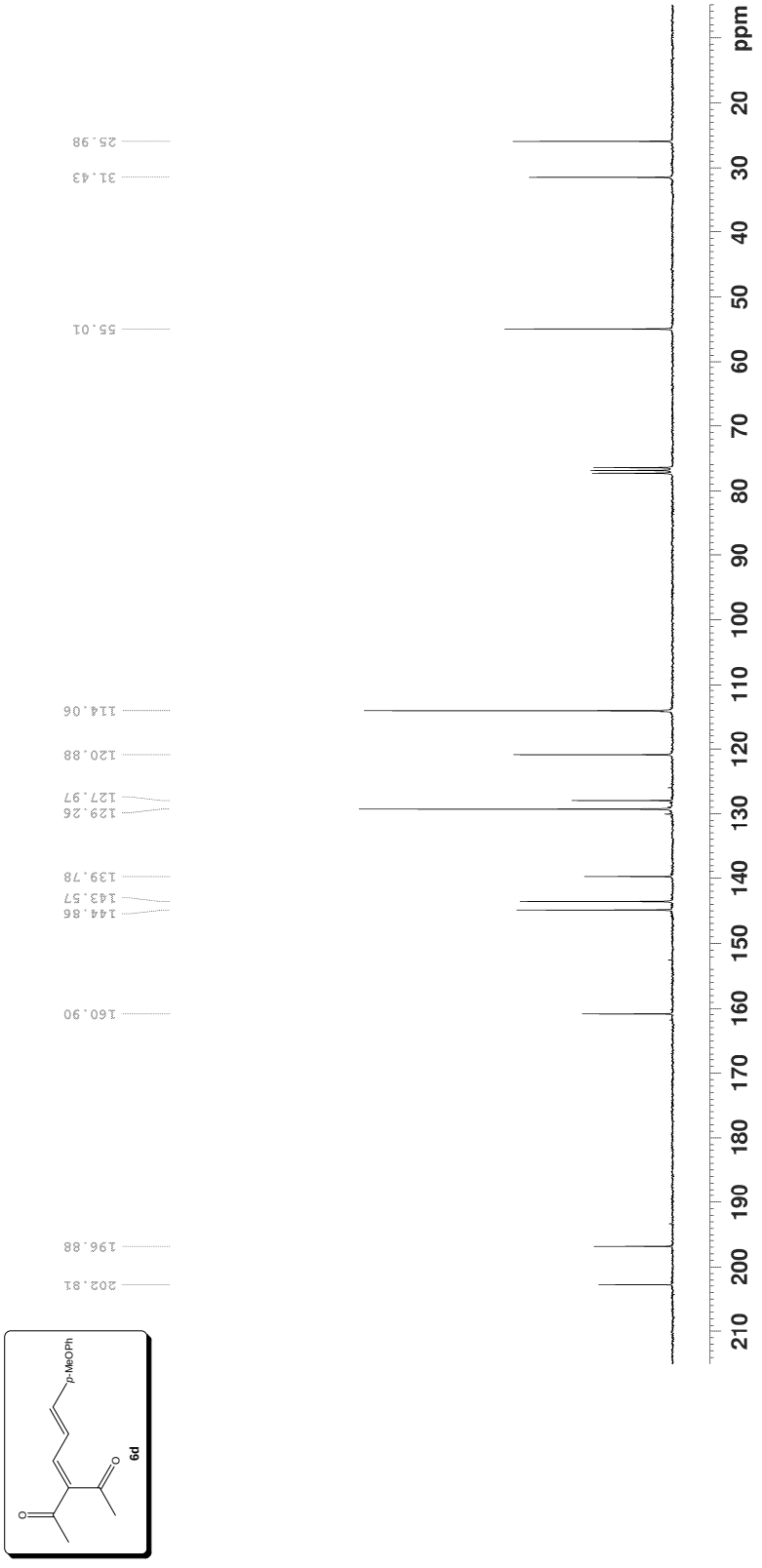


Fig. 6 ^{13}C NMR Spectra of Compound **6d**

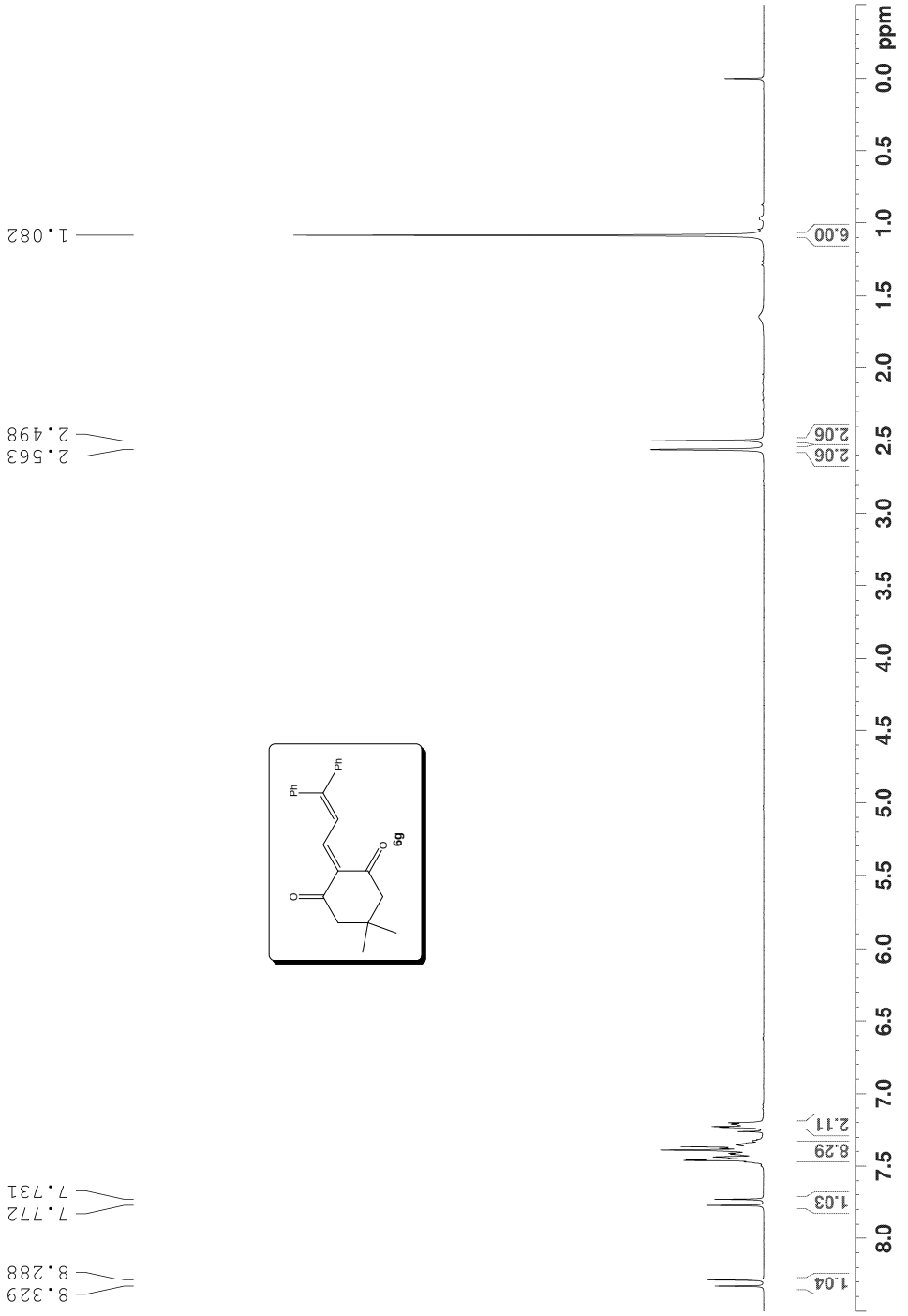


Fig. 7 ^{1}H NMR Spectra of Compound **6g**

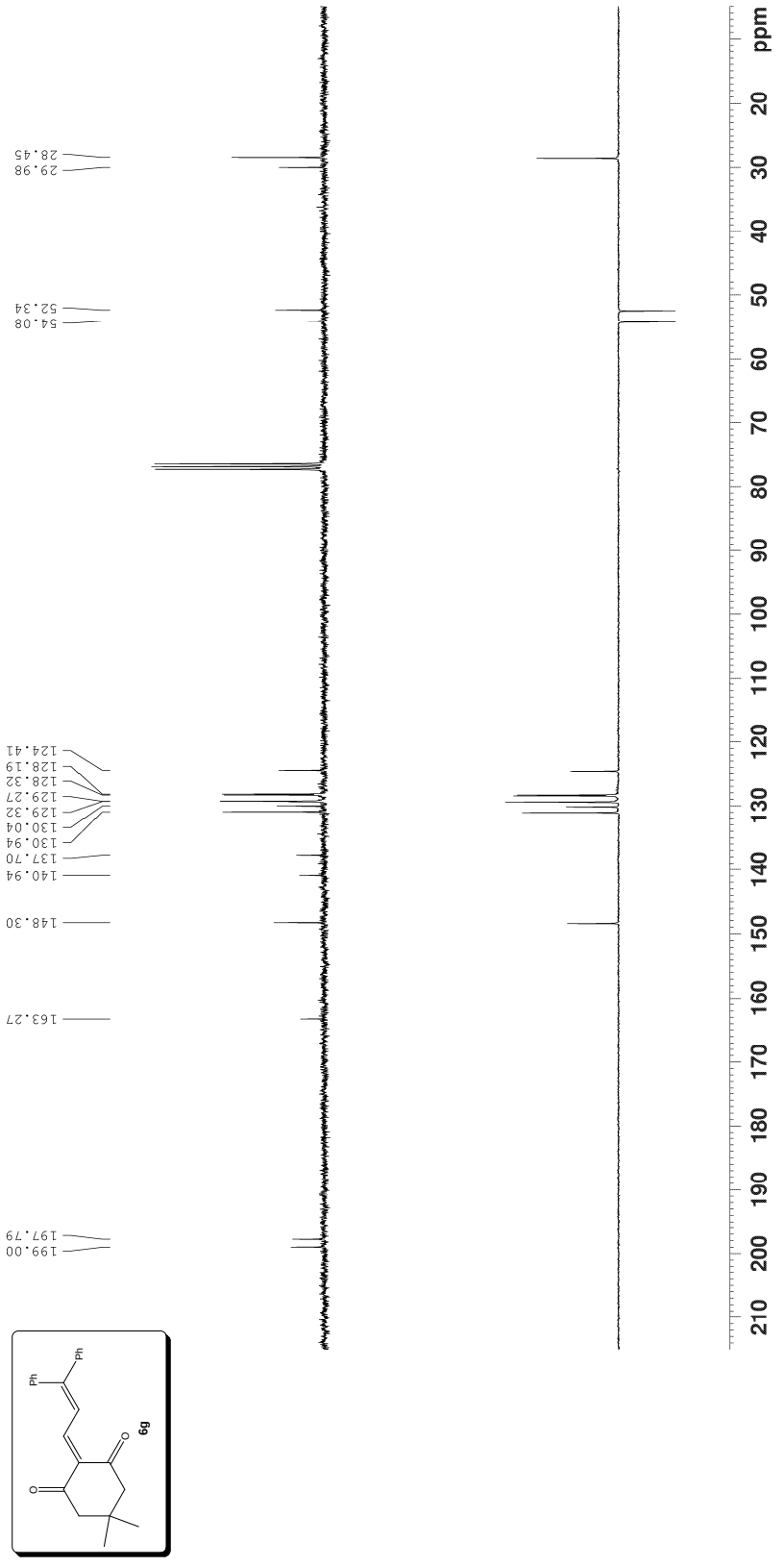


Fig. 8 ^{13}C NMR Spectra of Compound **6g**

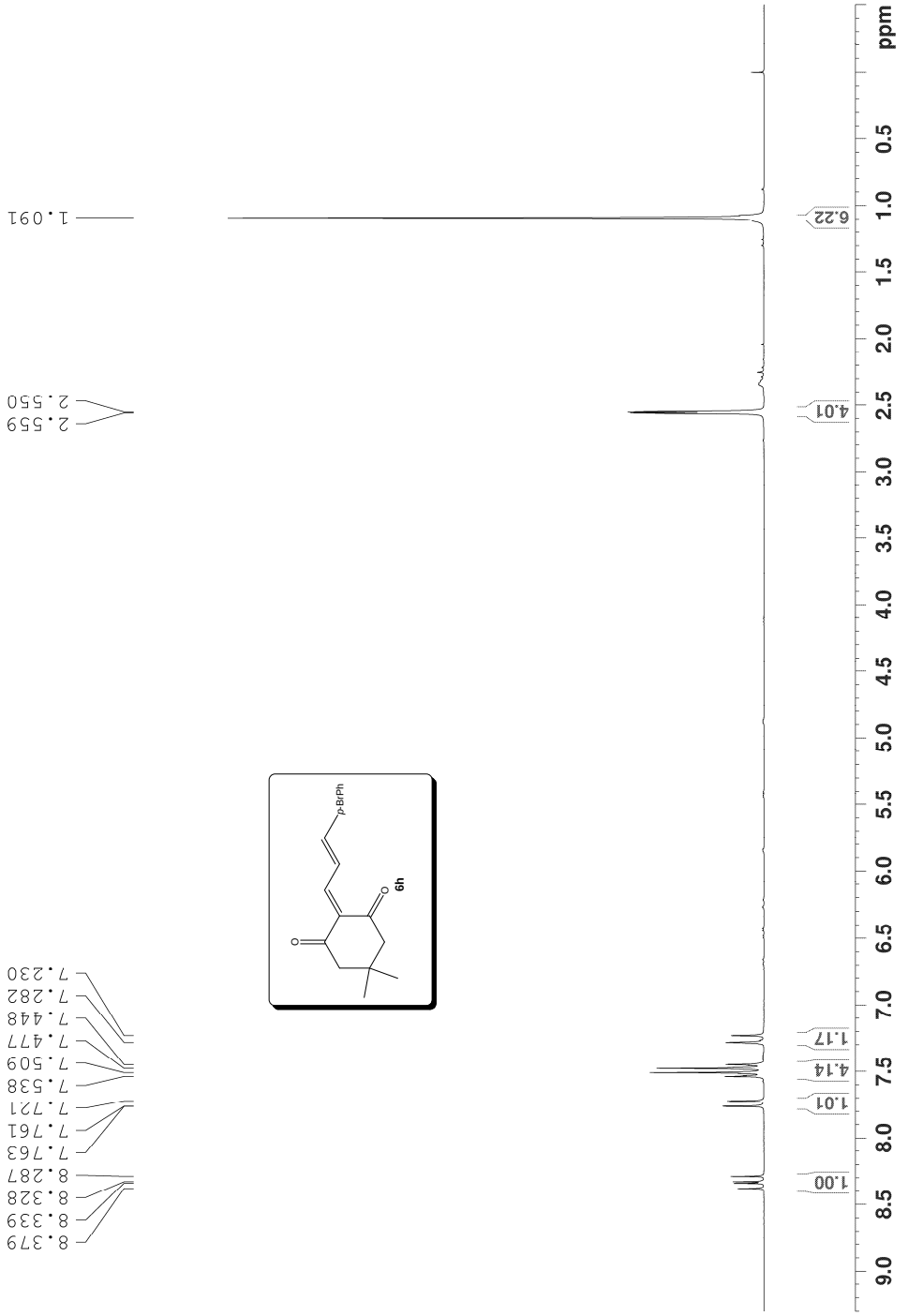


Fig. 9 ^{1}H NMR Spectra of Compound **6h**

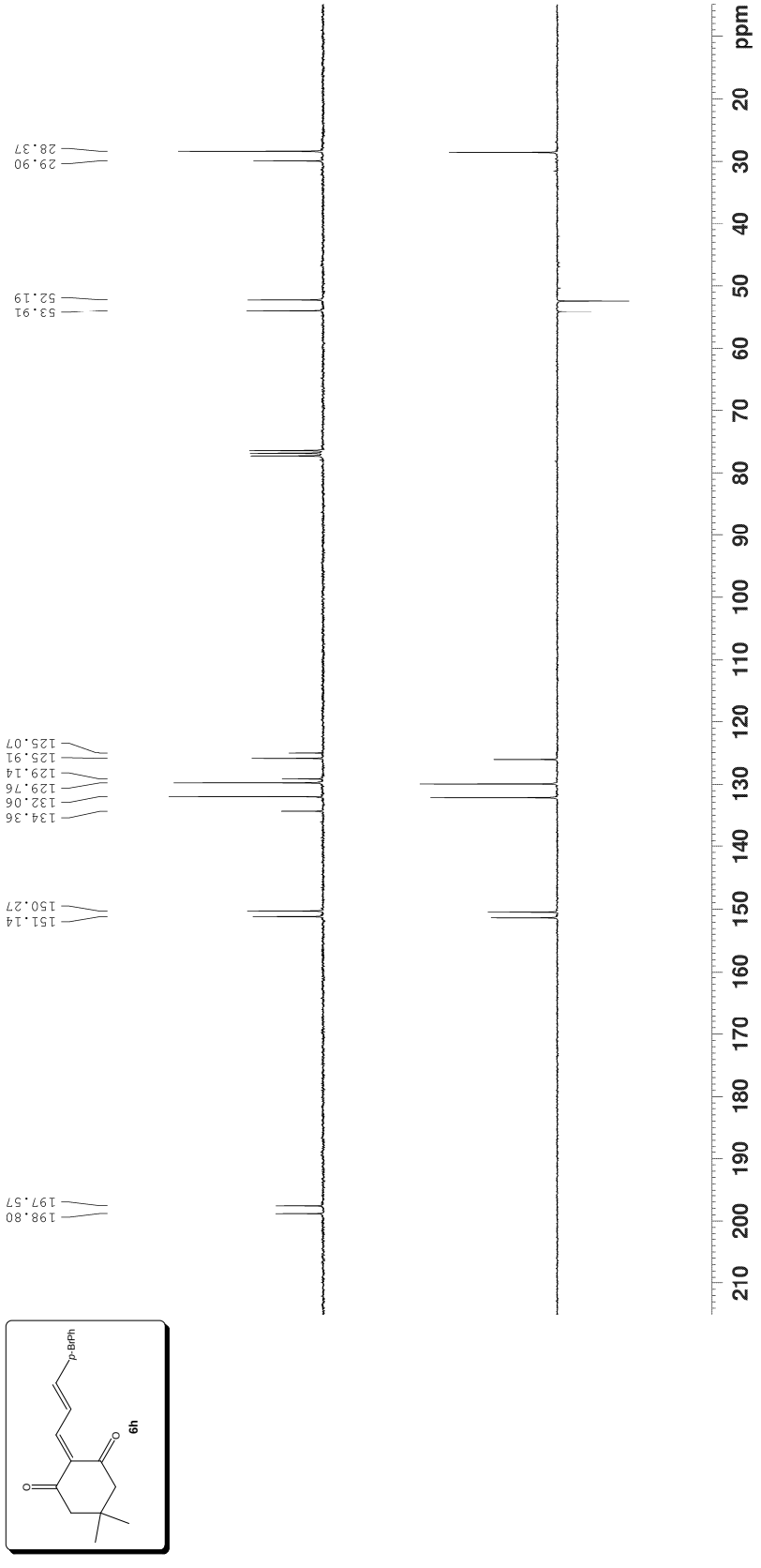


Fig. 10 ^{13}C NMR Spectra of Compound **6h**

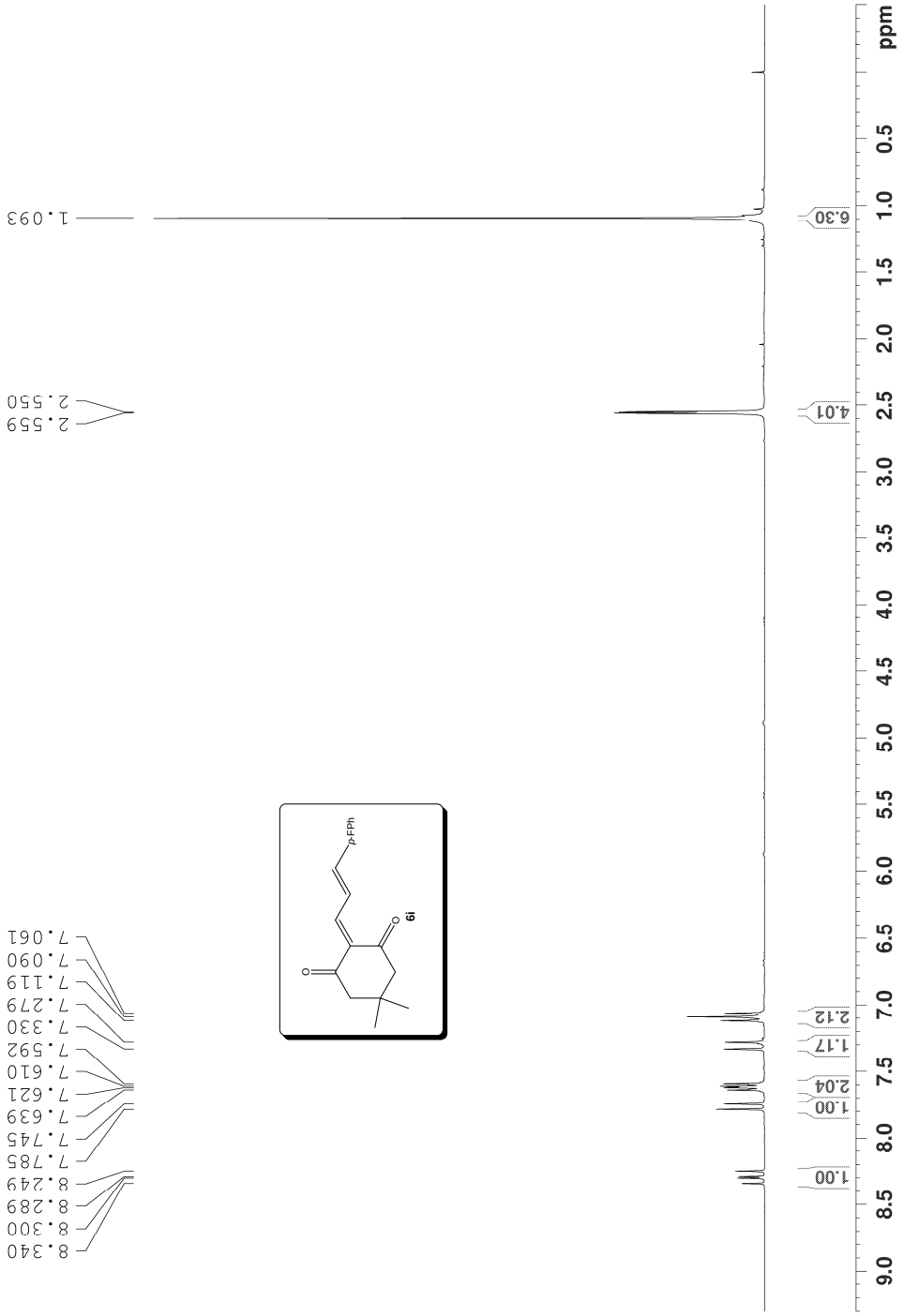


Fig. 11 ^{1}H NMR Spectra of Compound **6i**

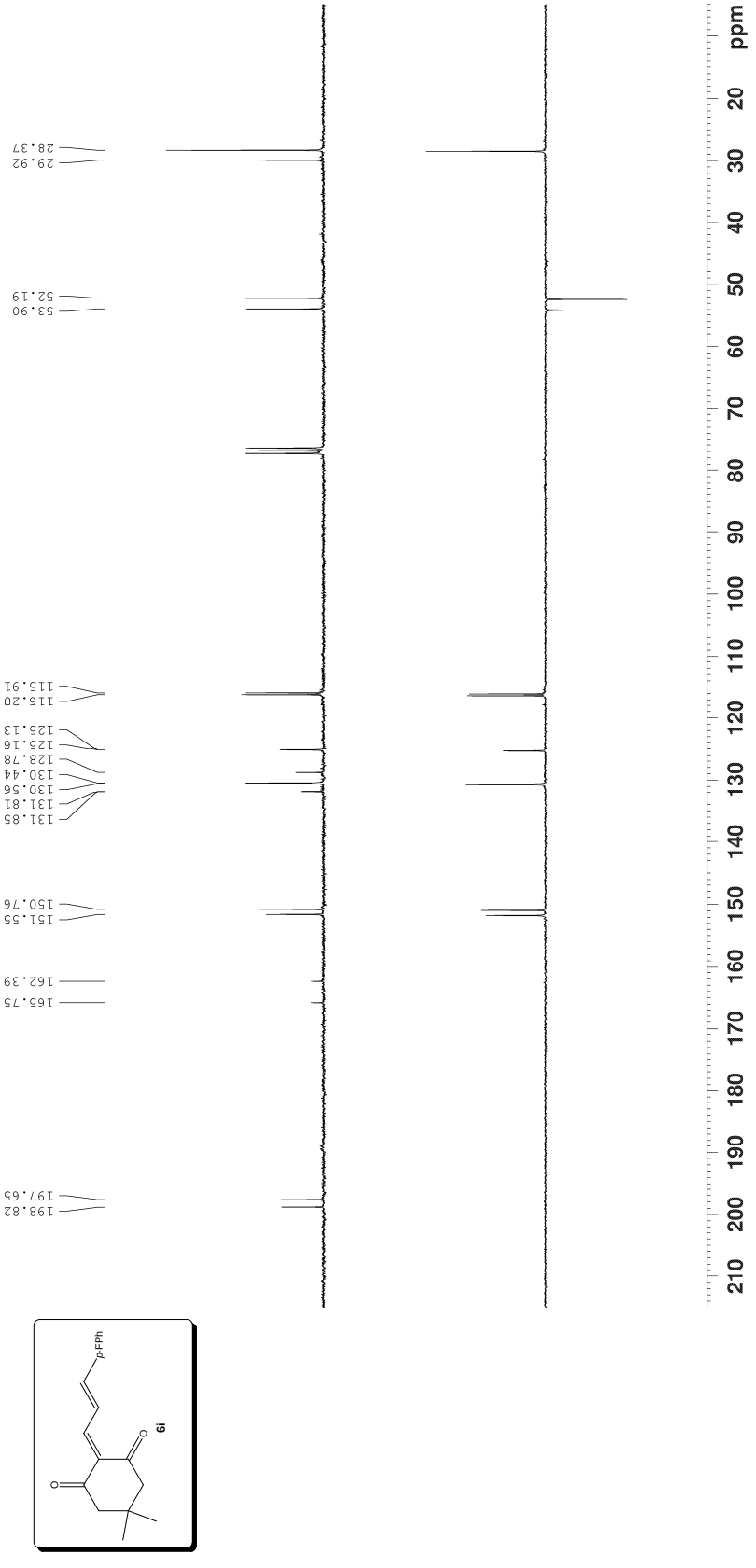


Fig. 12 ^{13}C NMR Spectra of Compound **6i**

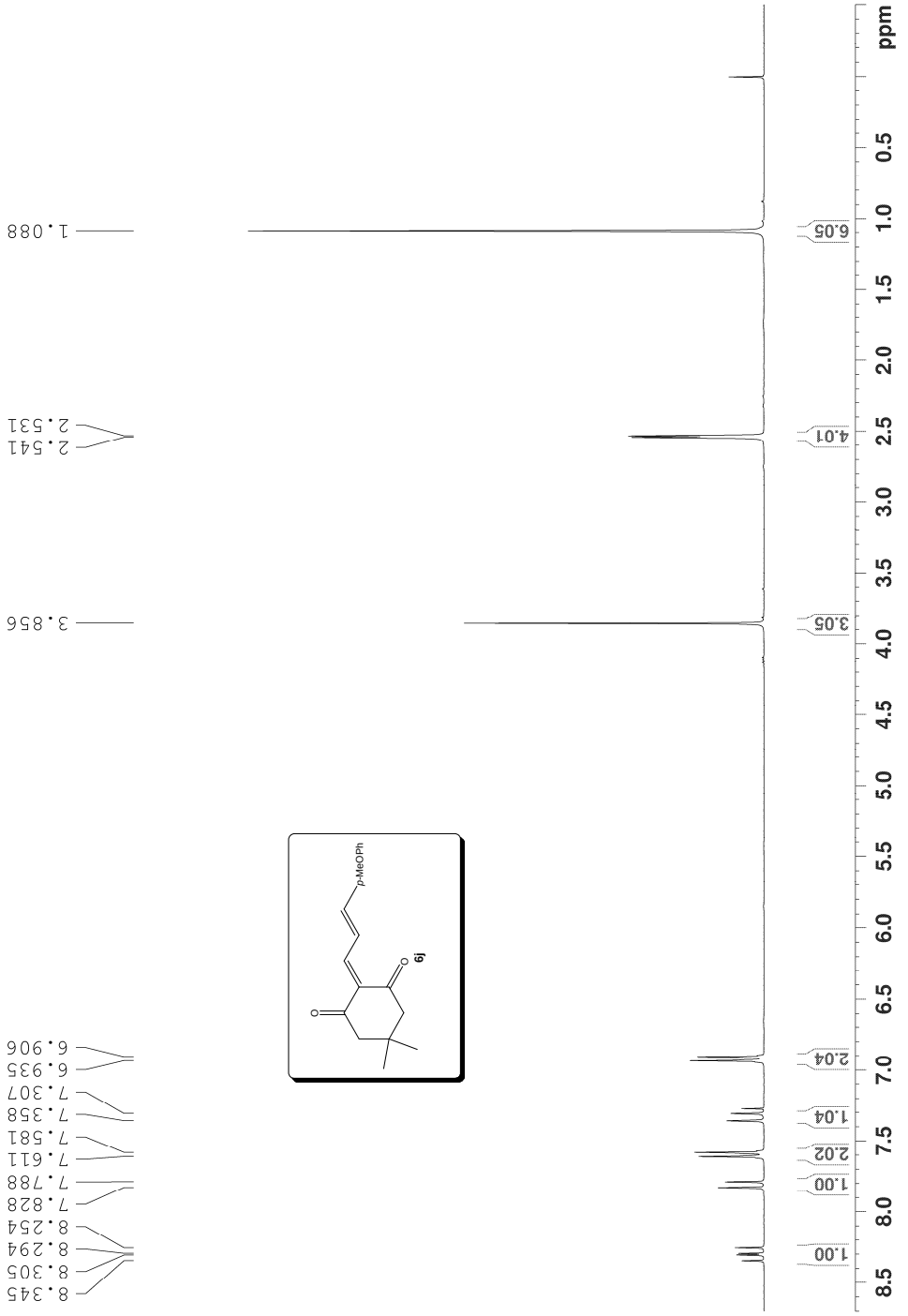


Fig. 13 ^{1}H NMR Spectra of Compound **6j**

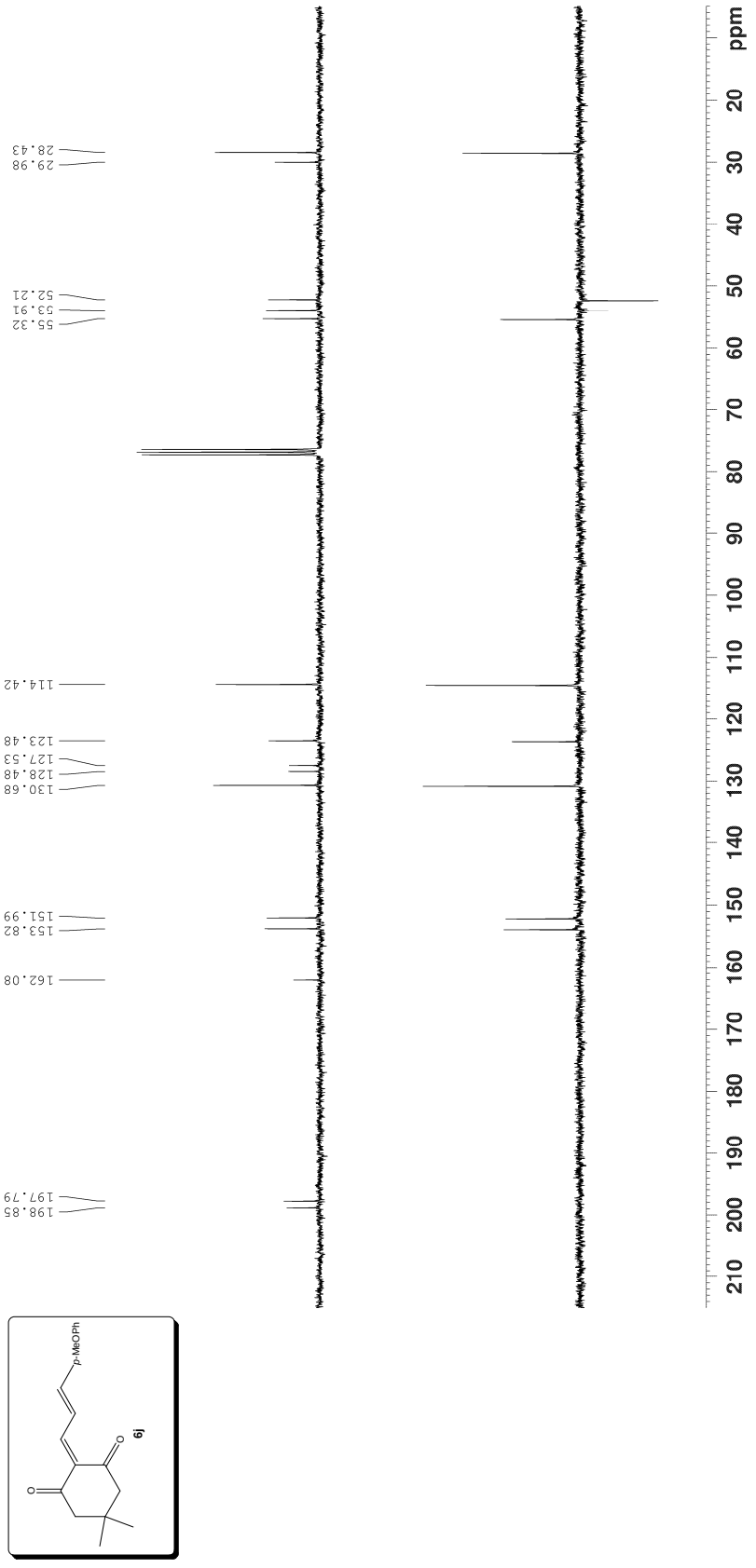


Fig. 14 ^{13}C NMR Spectra of Compound **6j**

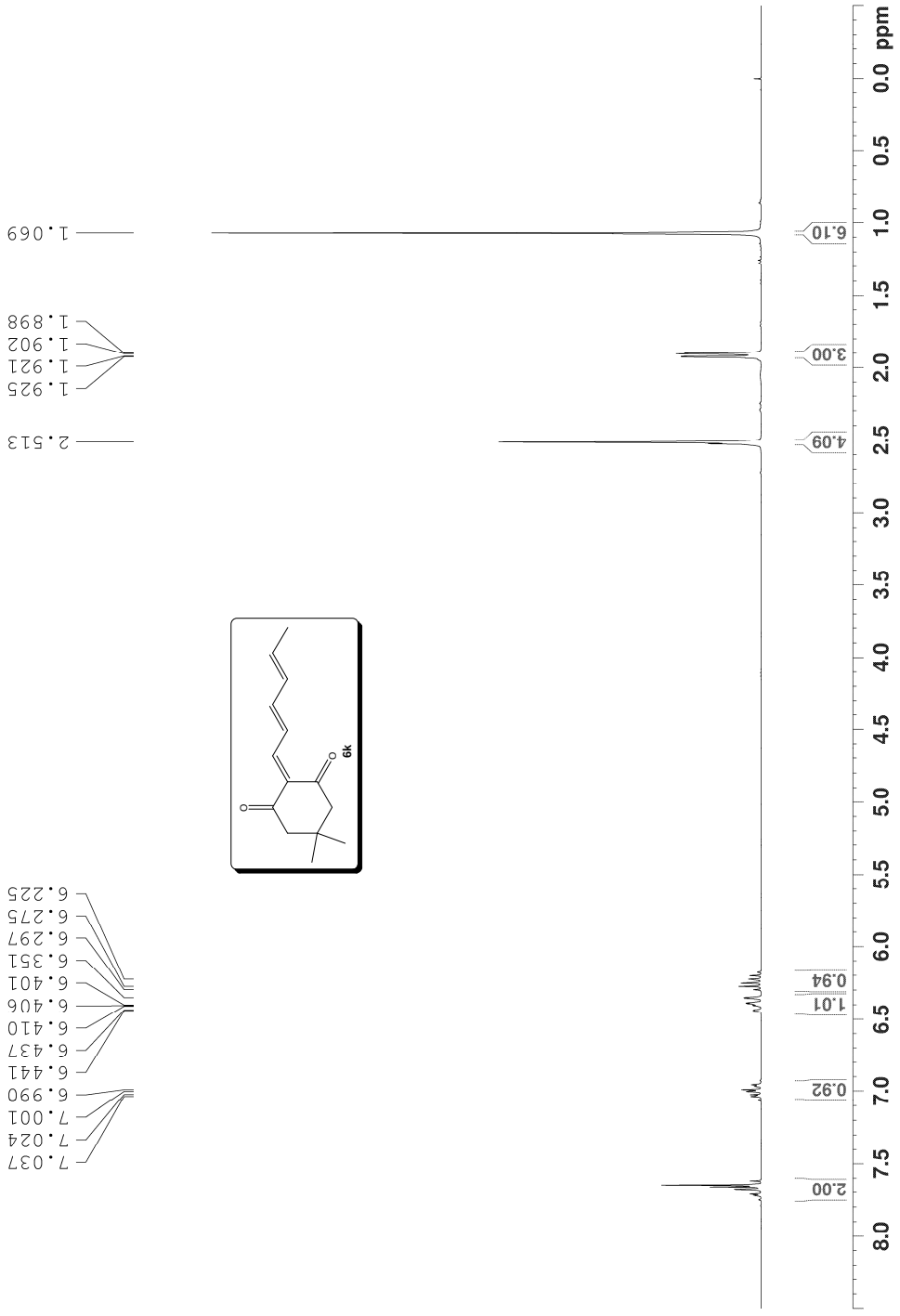


Fig. 15 ^{1}H NMR Spectra of Compound **6k**

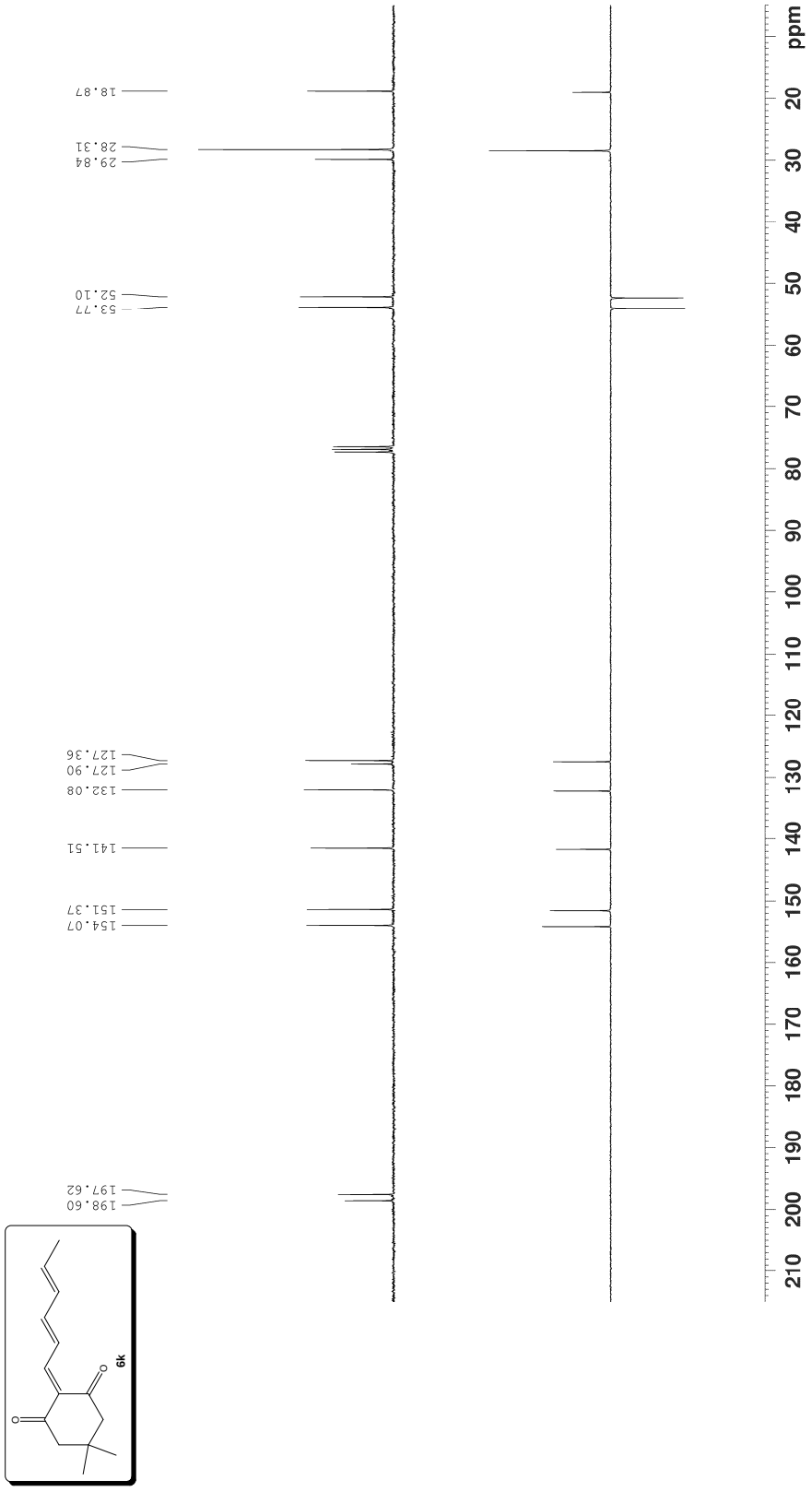


Fig. 16 ^{13}C NMR Spectra of Compound **6k**

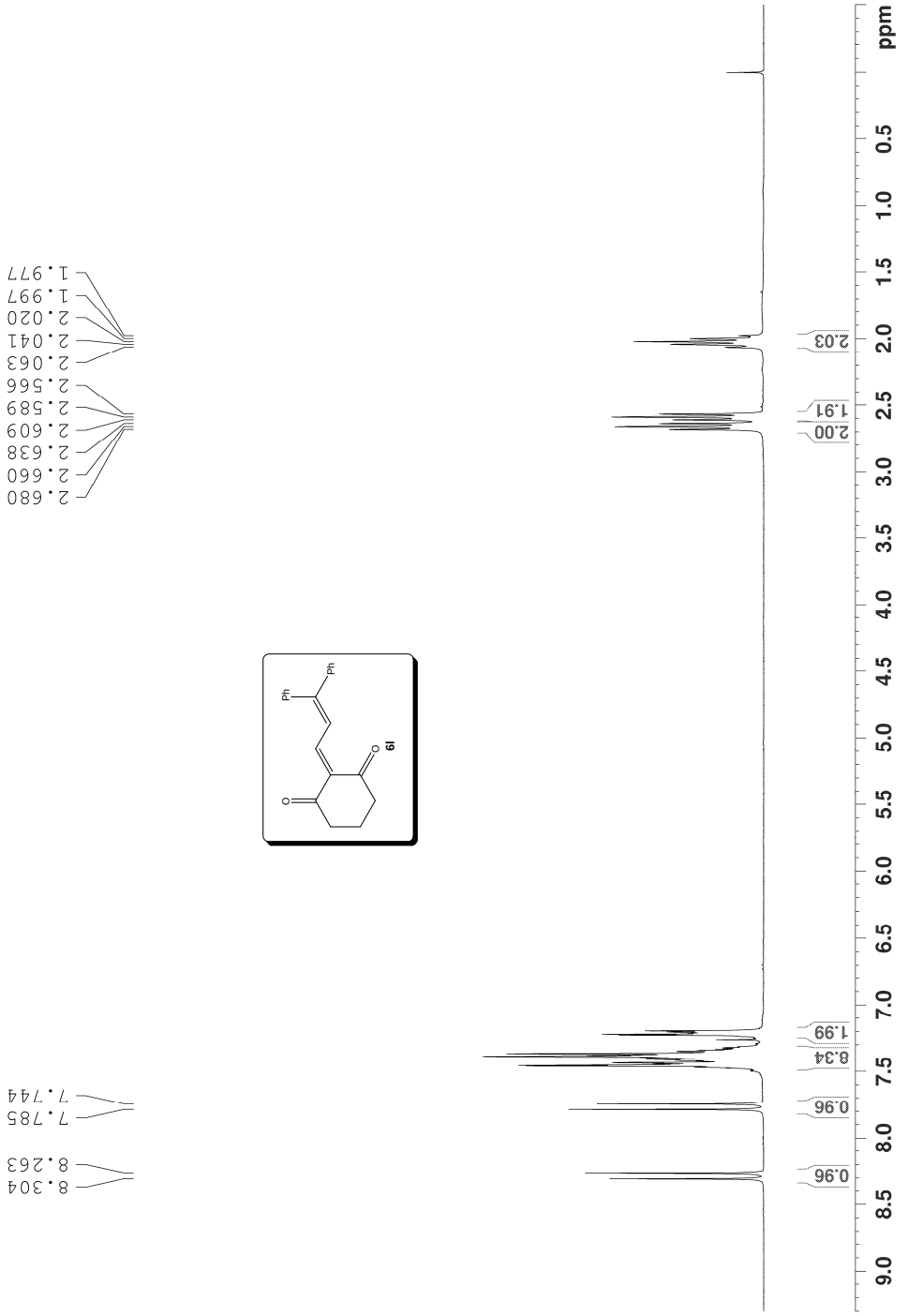


Fig. 17 ^{1}H NMR Spectra of Compound **6l**

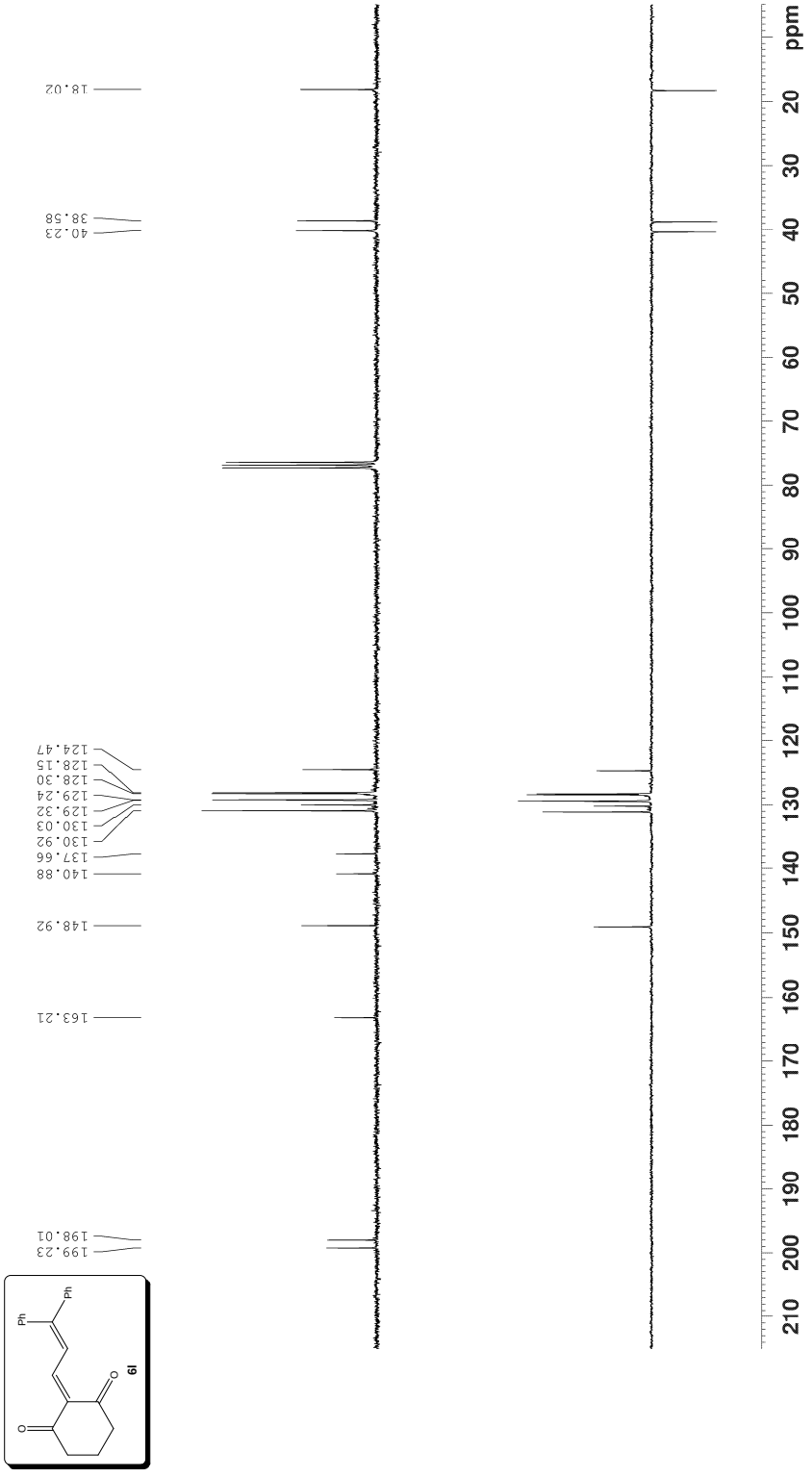


Fig. 18 ^{13}C NMR Spectra of Compound **6l**

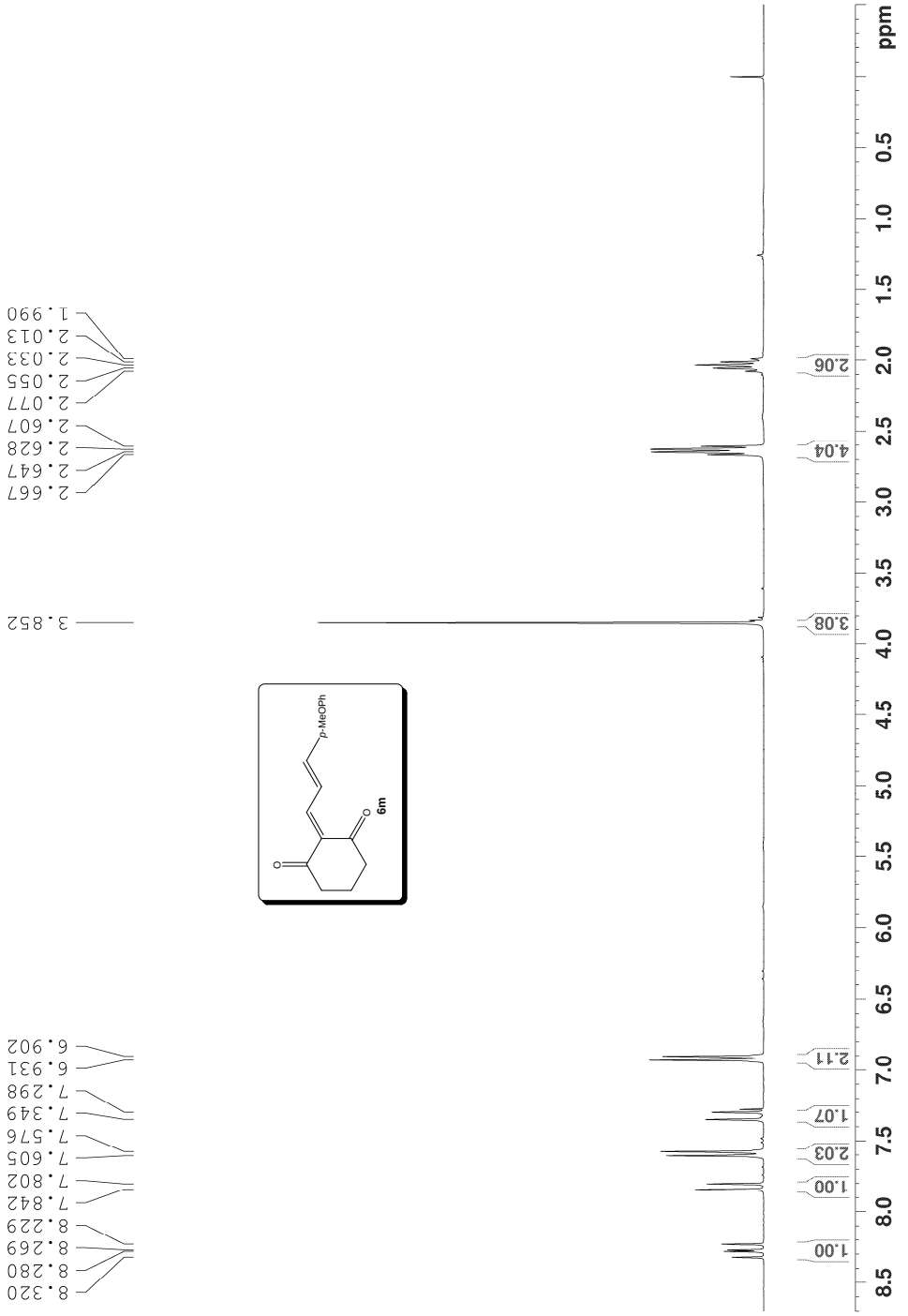


Fig. 19 ^{1}H NMR Spectra of Compound **6m**

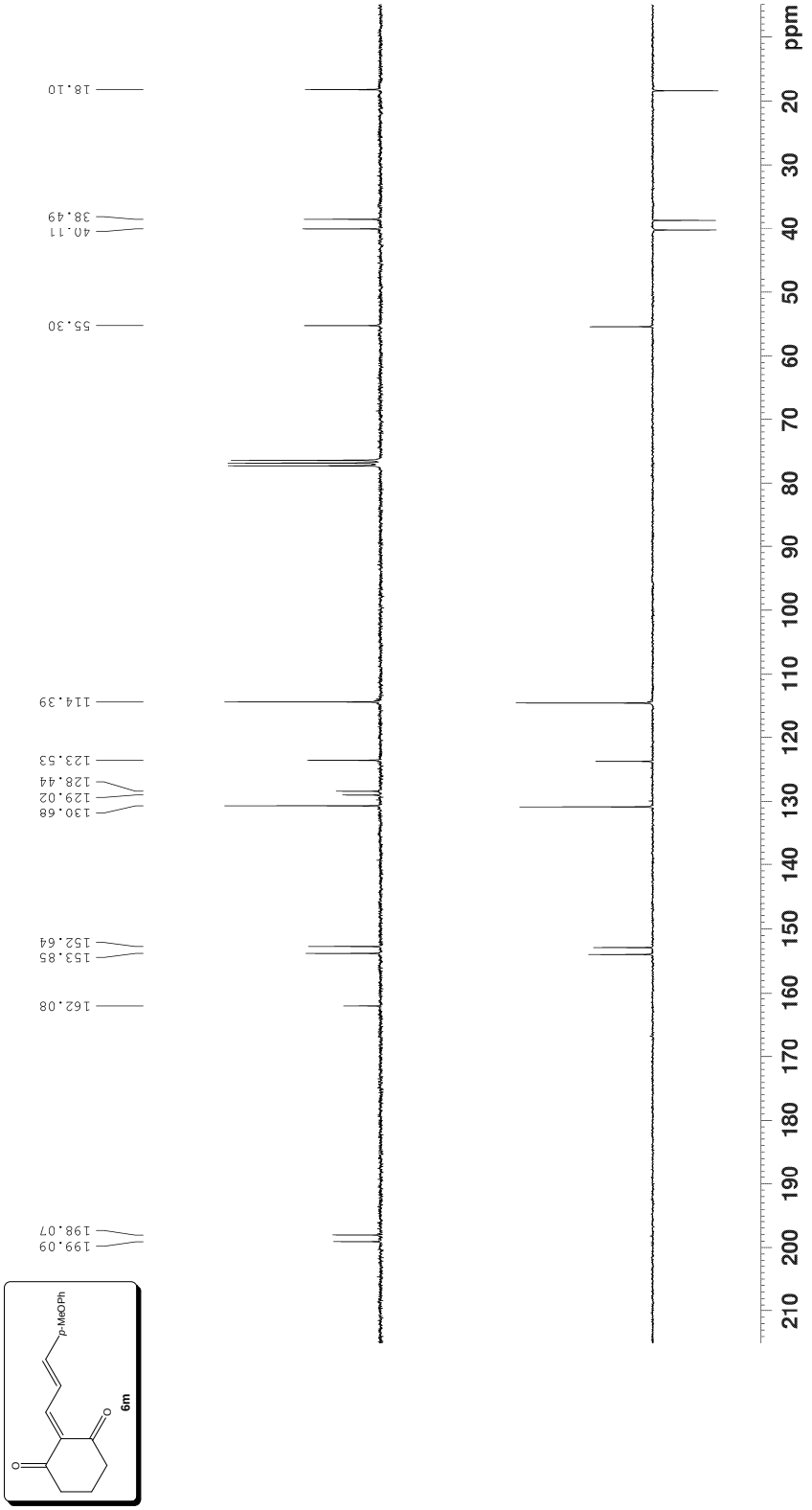


Fig. 20 ^{13}C NMR Spectra of Compound **6m**

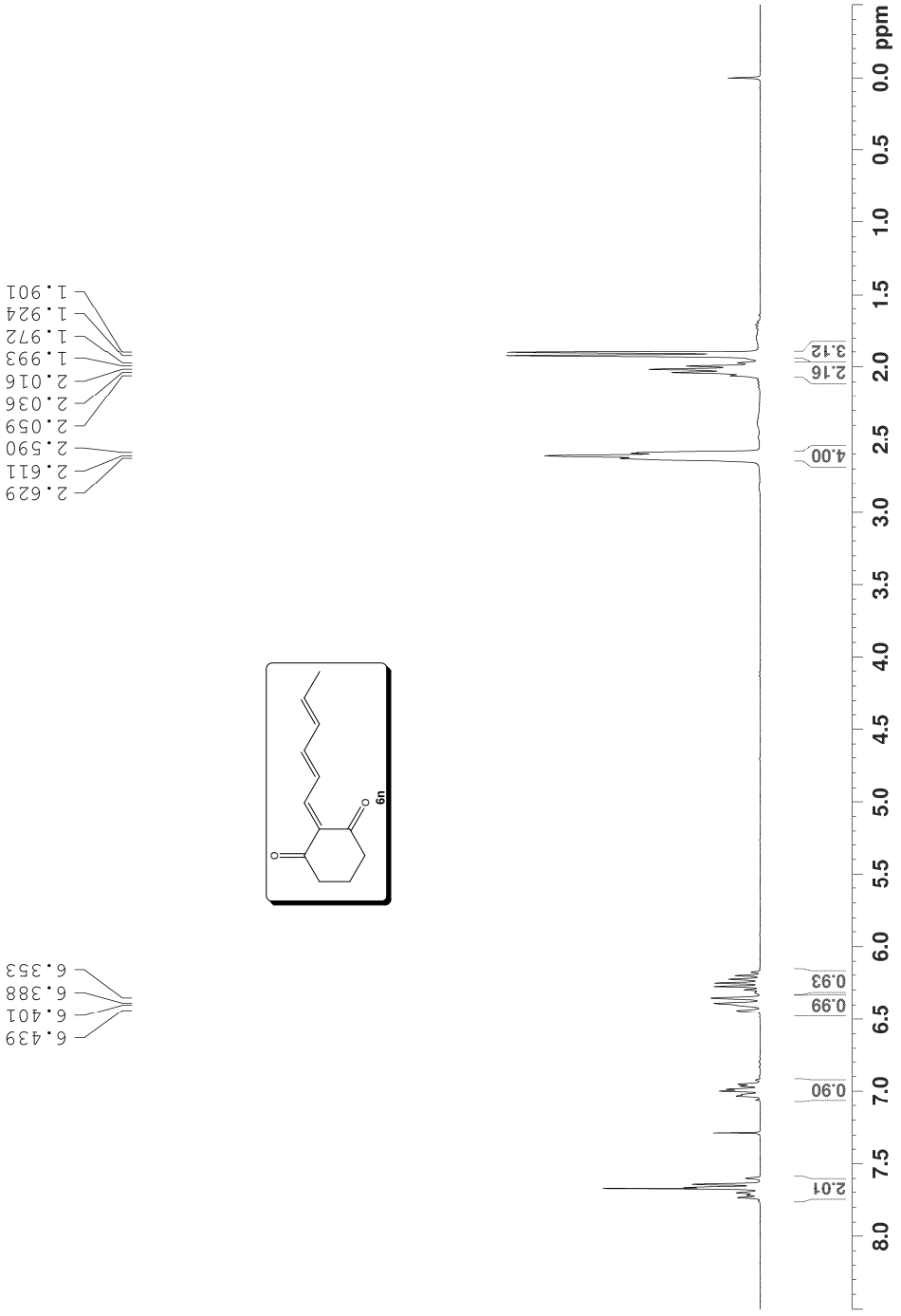


Fig. 21 ^{1}H NMR Spectra of Compound **6n**

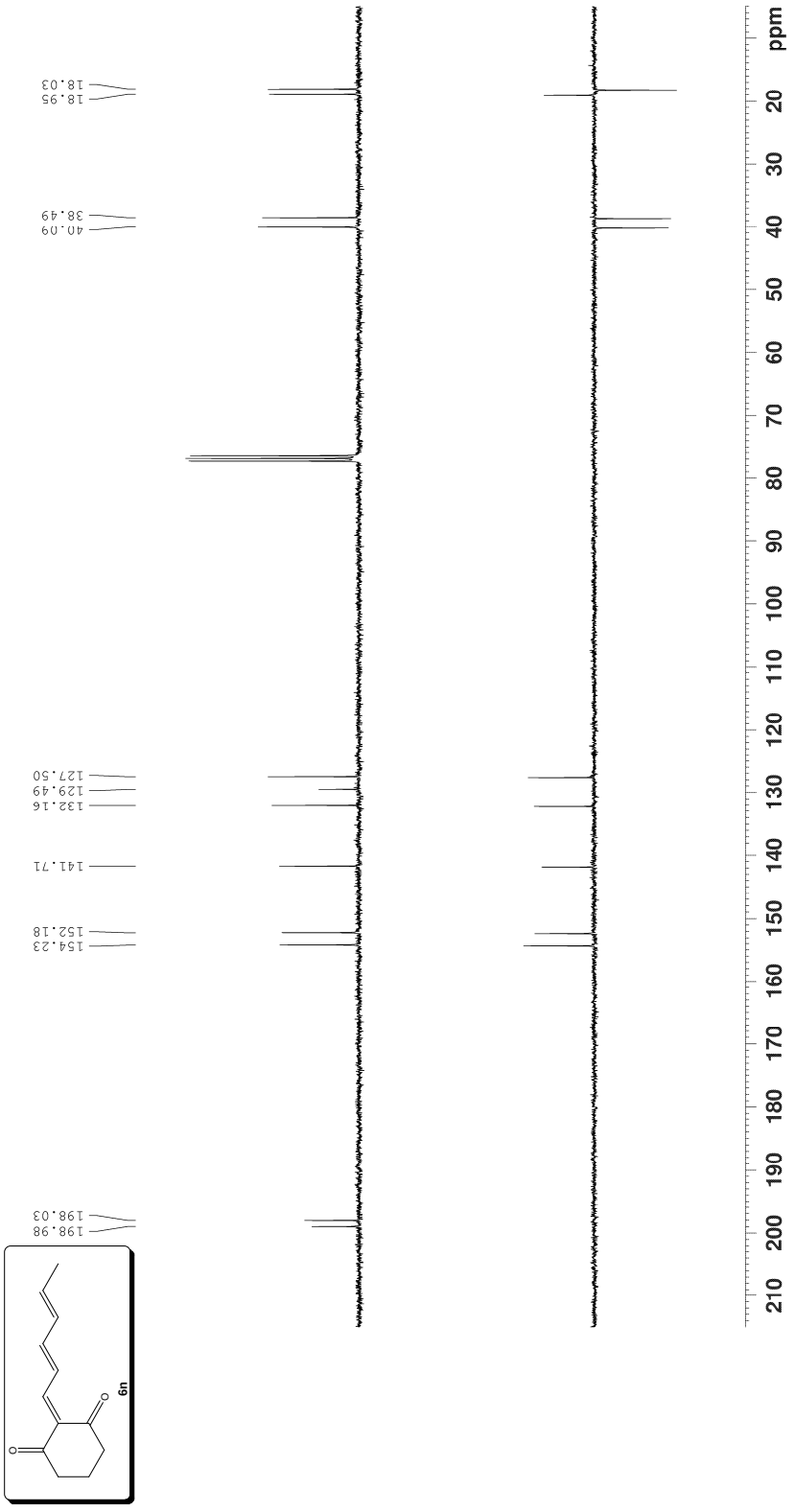


Fig. 22 ^{13}C NMR Spectra of Compound **6n**

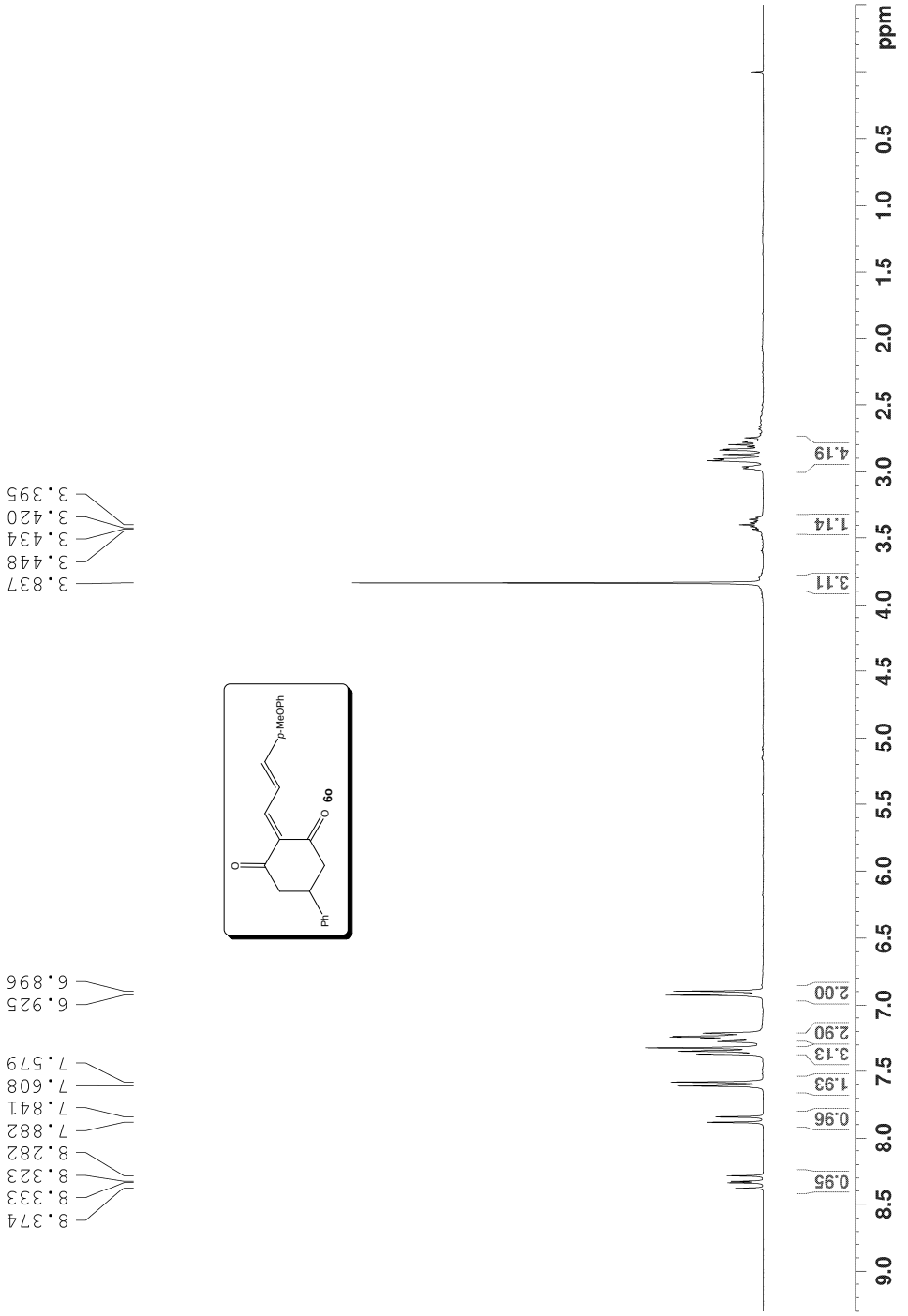


Fig. 23 ^{1}H NMR Spectra of Compound **6o**

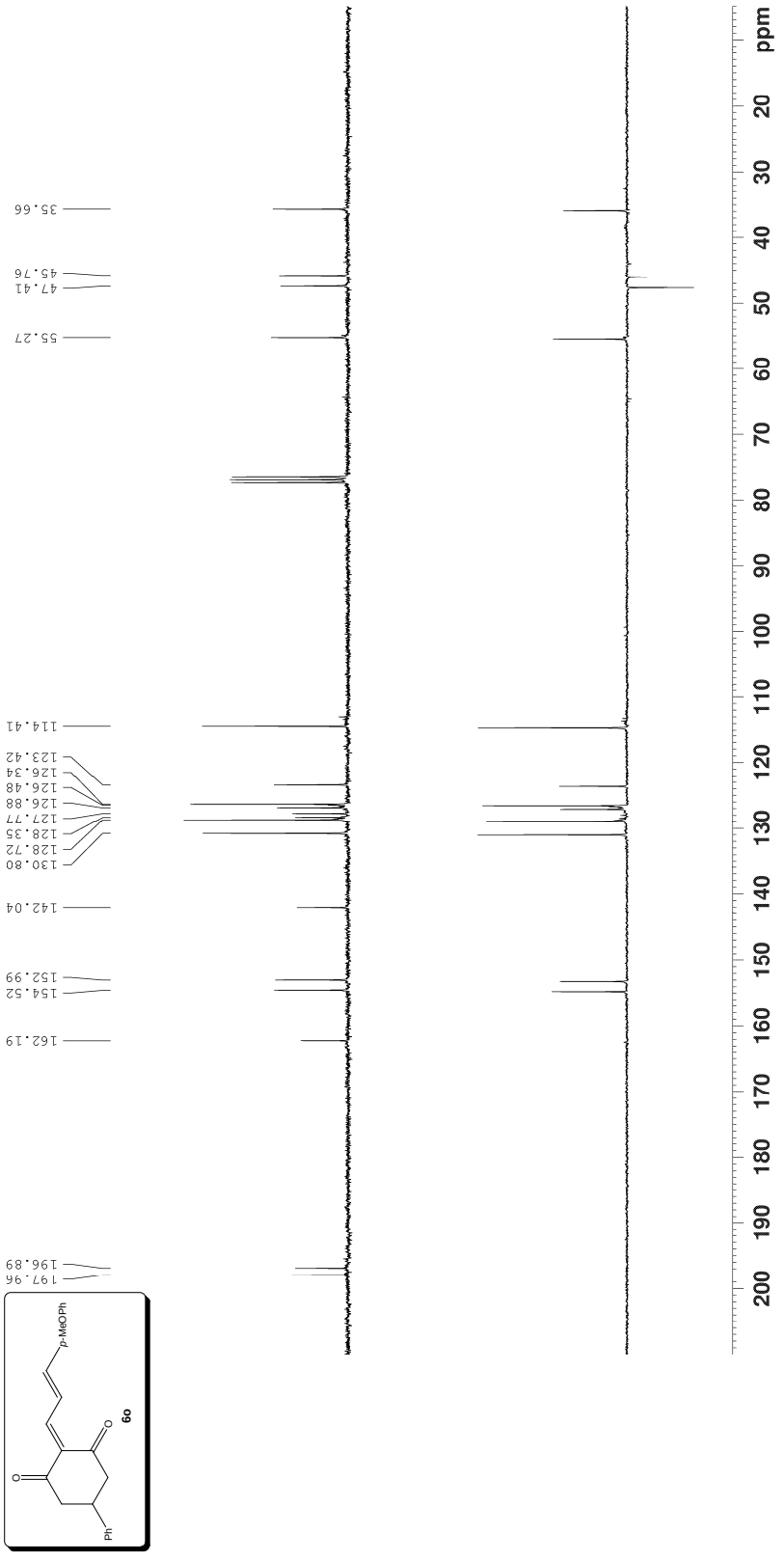


Fig. 24 ^{13}C NMR Spectra of Compound **6o**

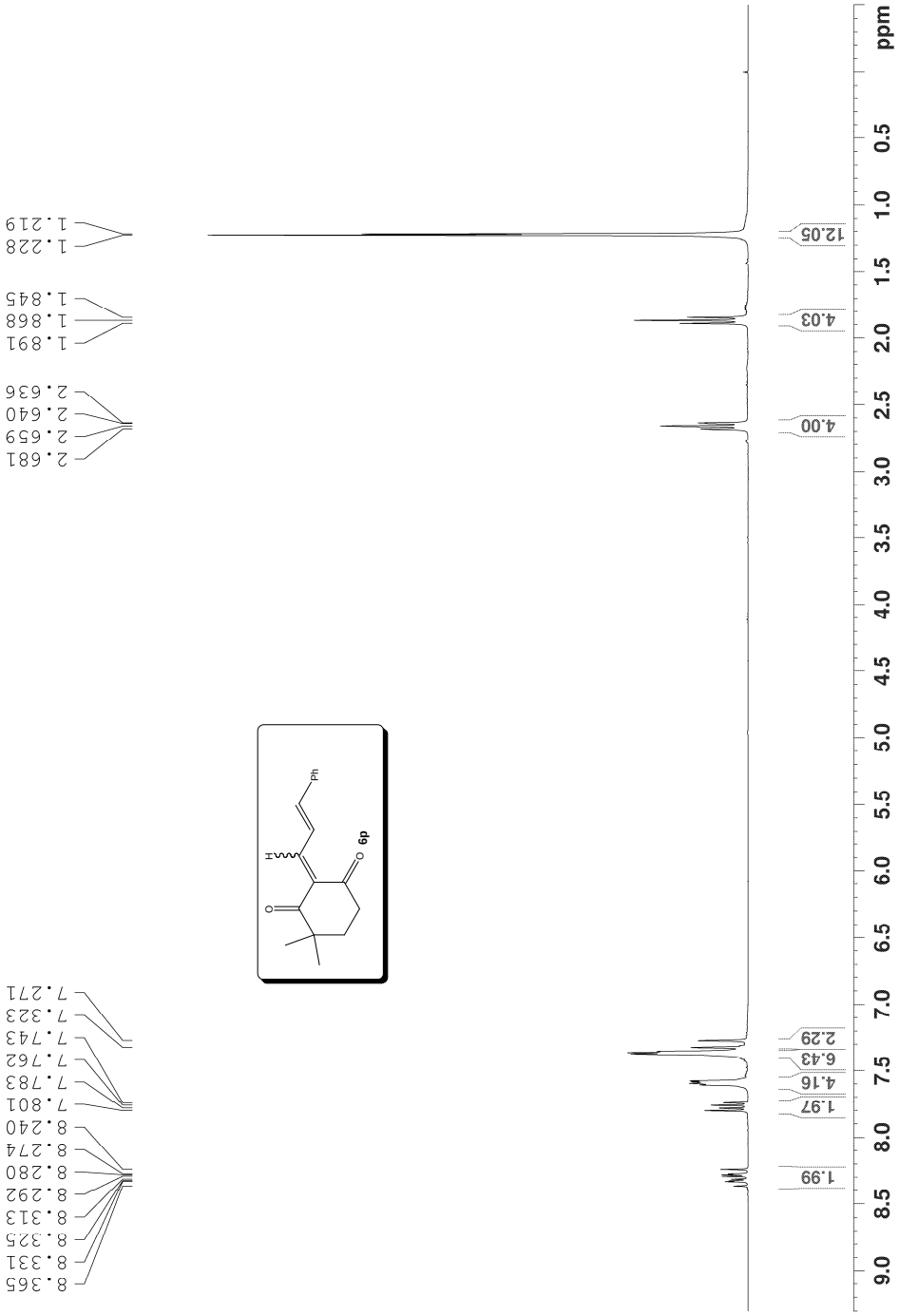


Fig. 25 ^{1}H NMR Spectra of Compounds **6p**

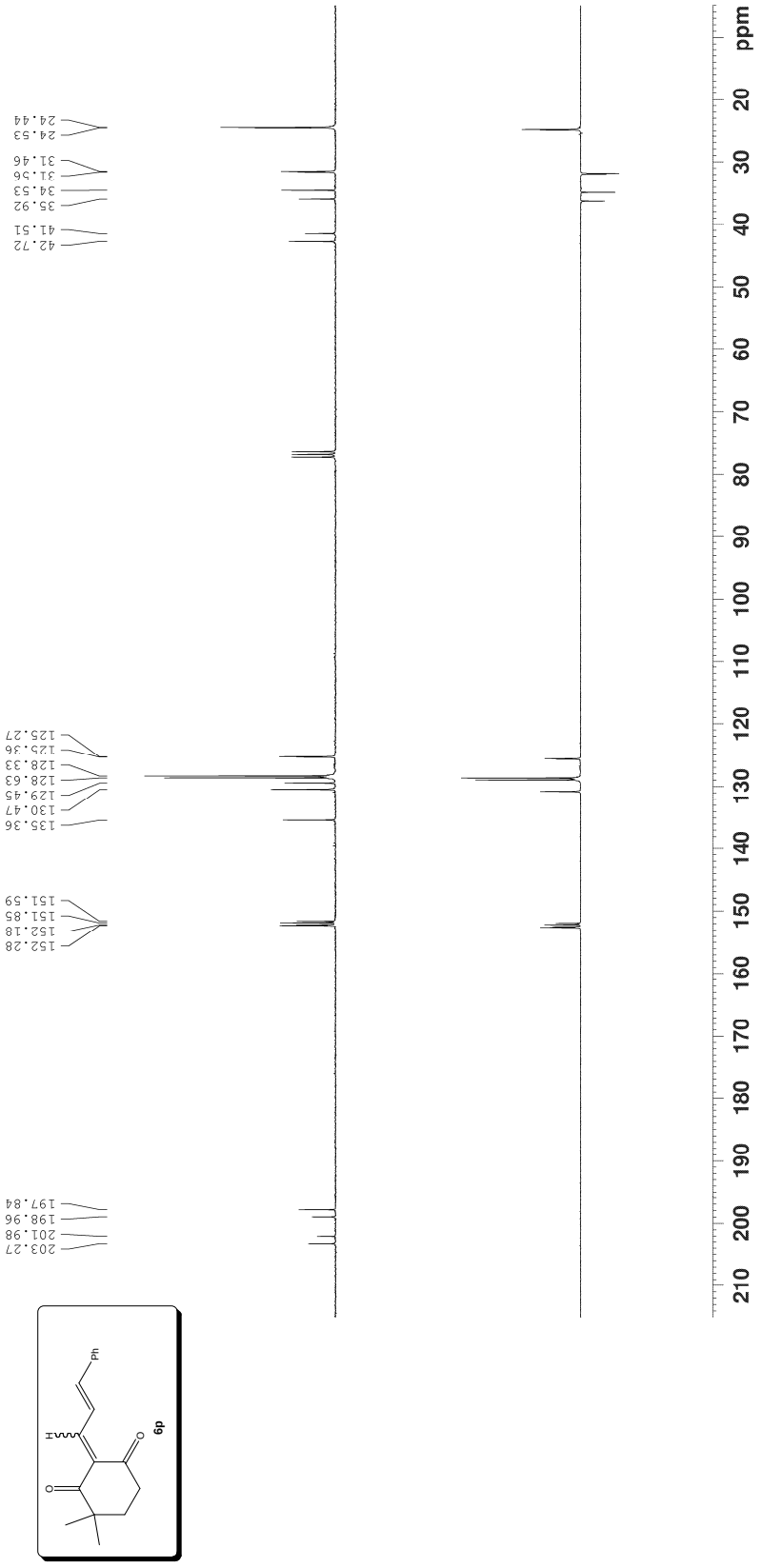


Fig. 26 ^{13}C NMR Spectra of Compounds **6p**

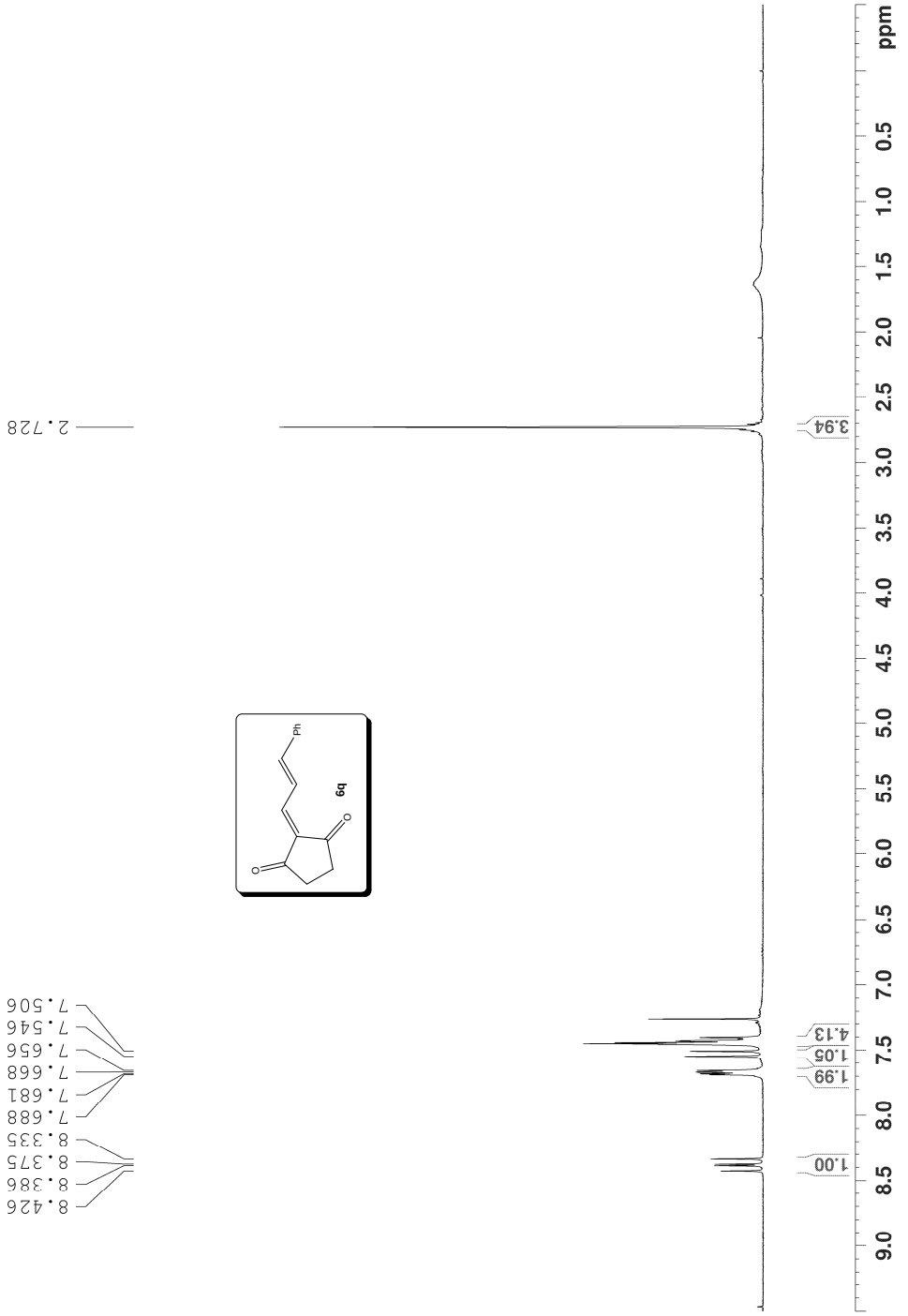


Fig. 27 ^{1}H NMR Spectra of Compound **6q**

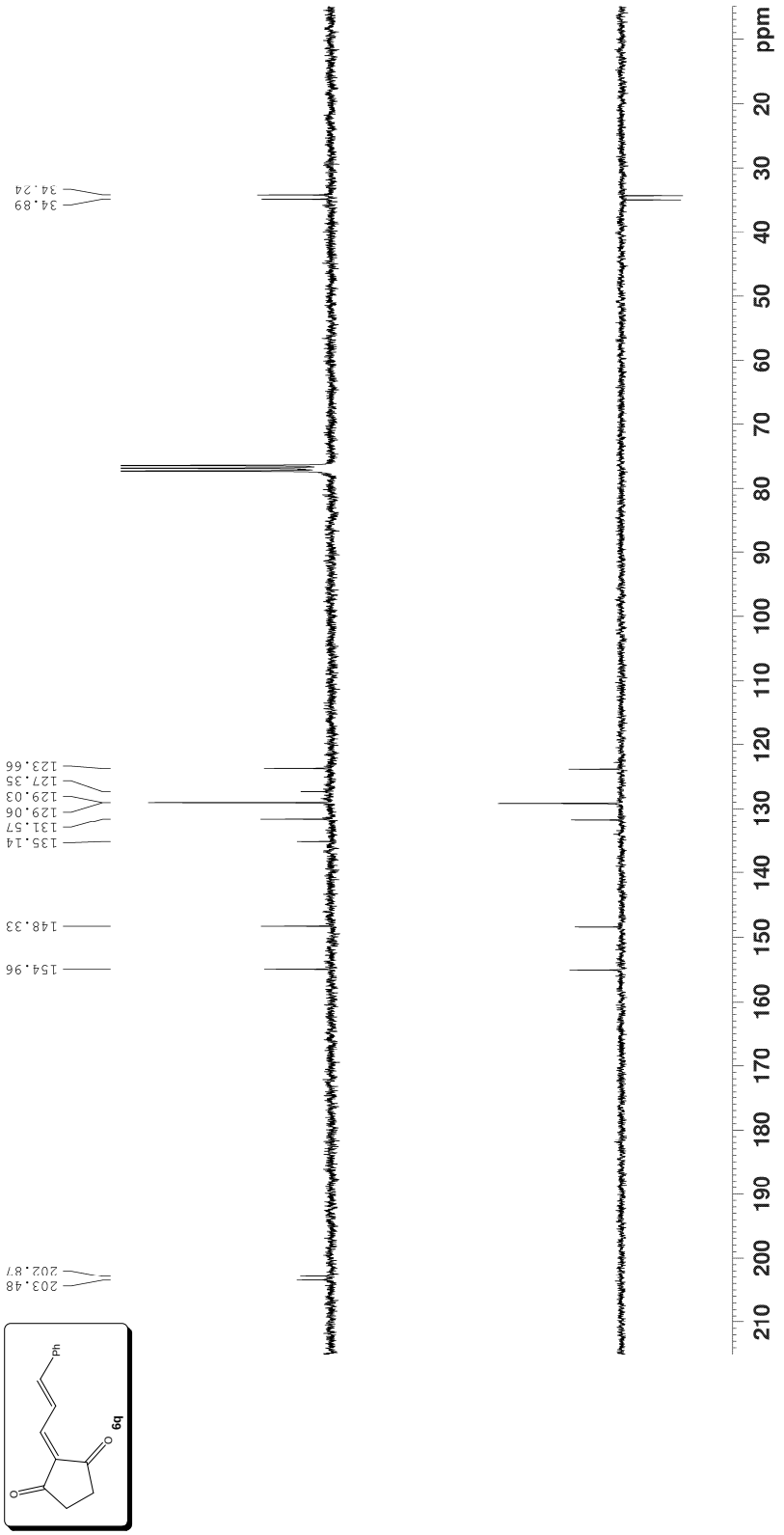


Fig. 28 ^{13}C NMR Spectra of Compound **6q**

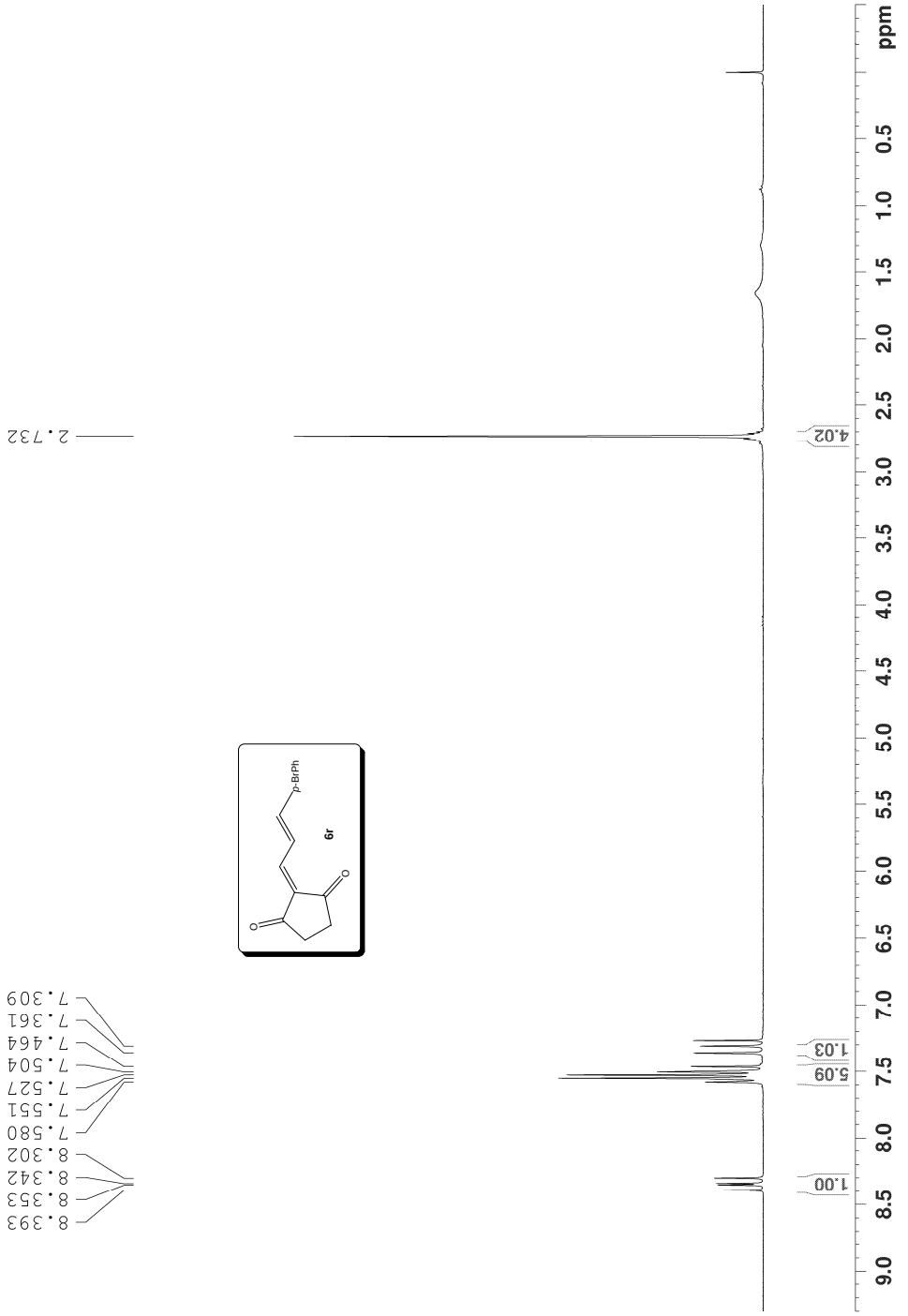


Fig. 29 ^{1}H NMR Spectra of Compound **6r**

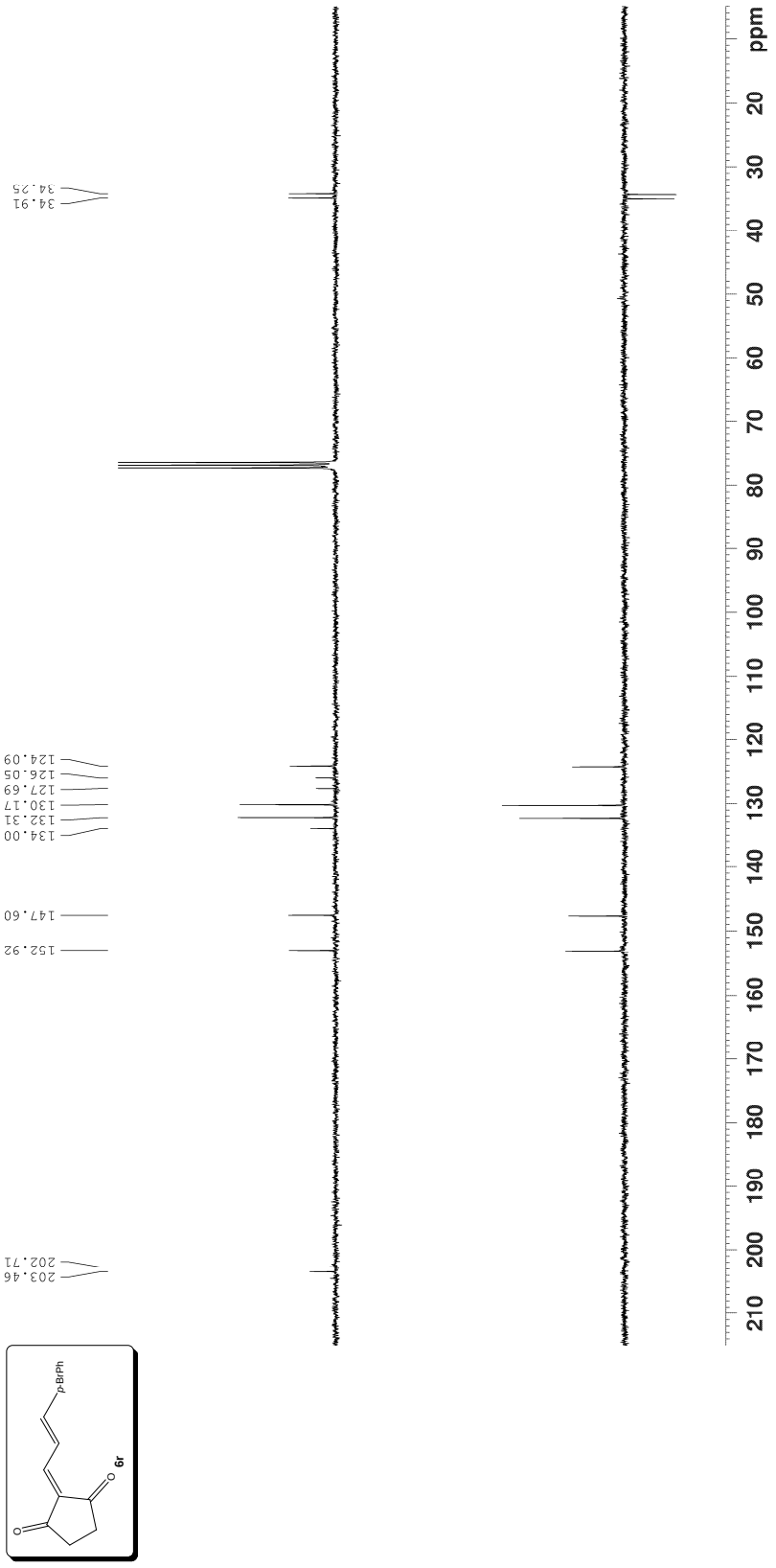


Fig. 30 ^{13}C NMR Spectra of Compound **6r**

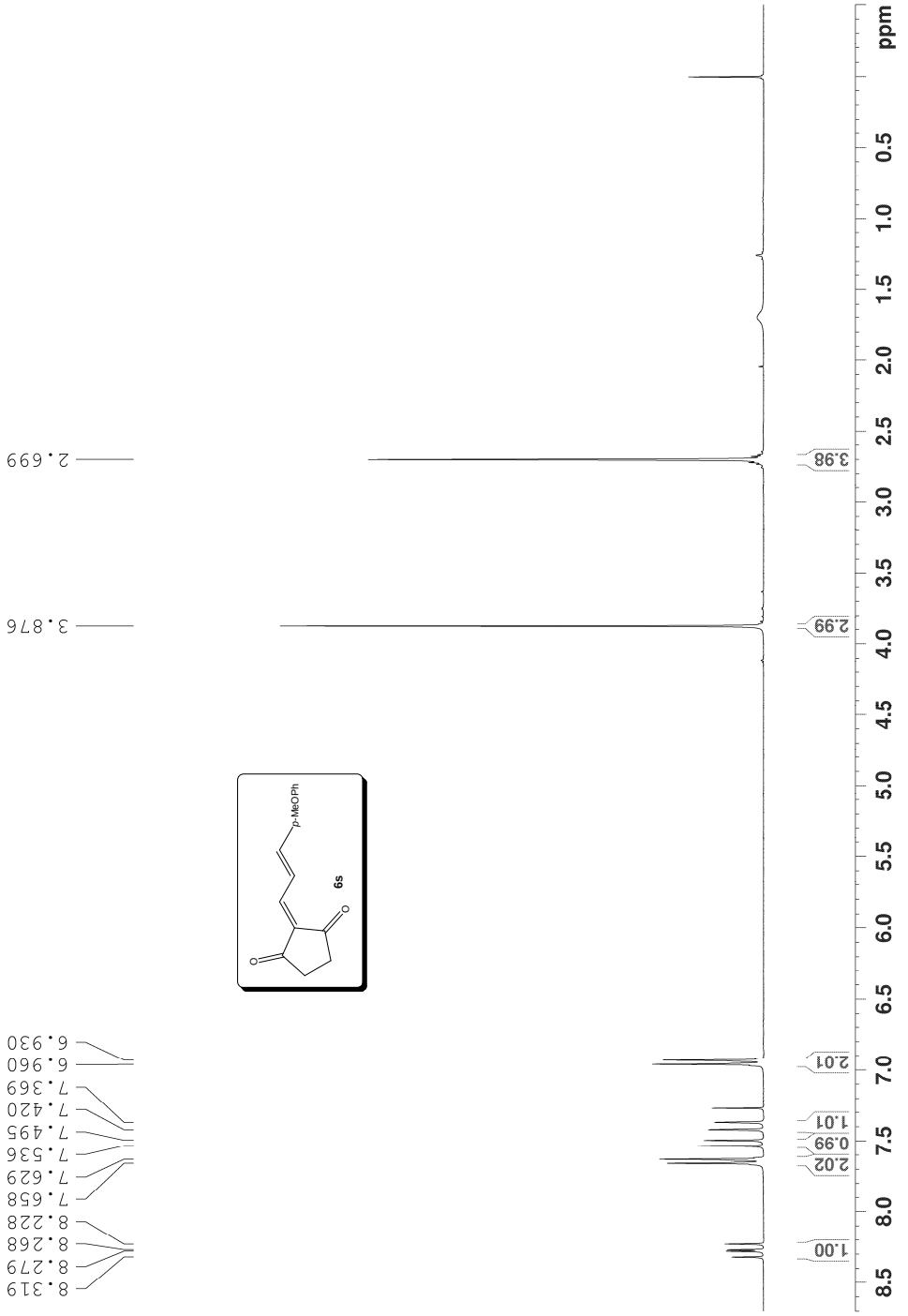


Fig. 31 ^{1}H NMR Spectra of Compound **6s**

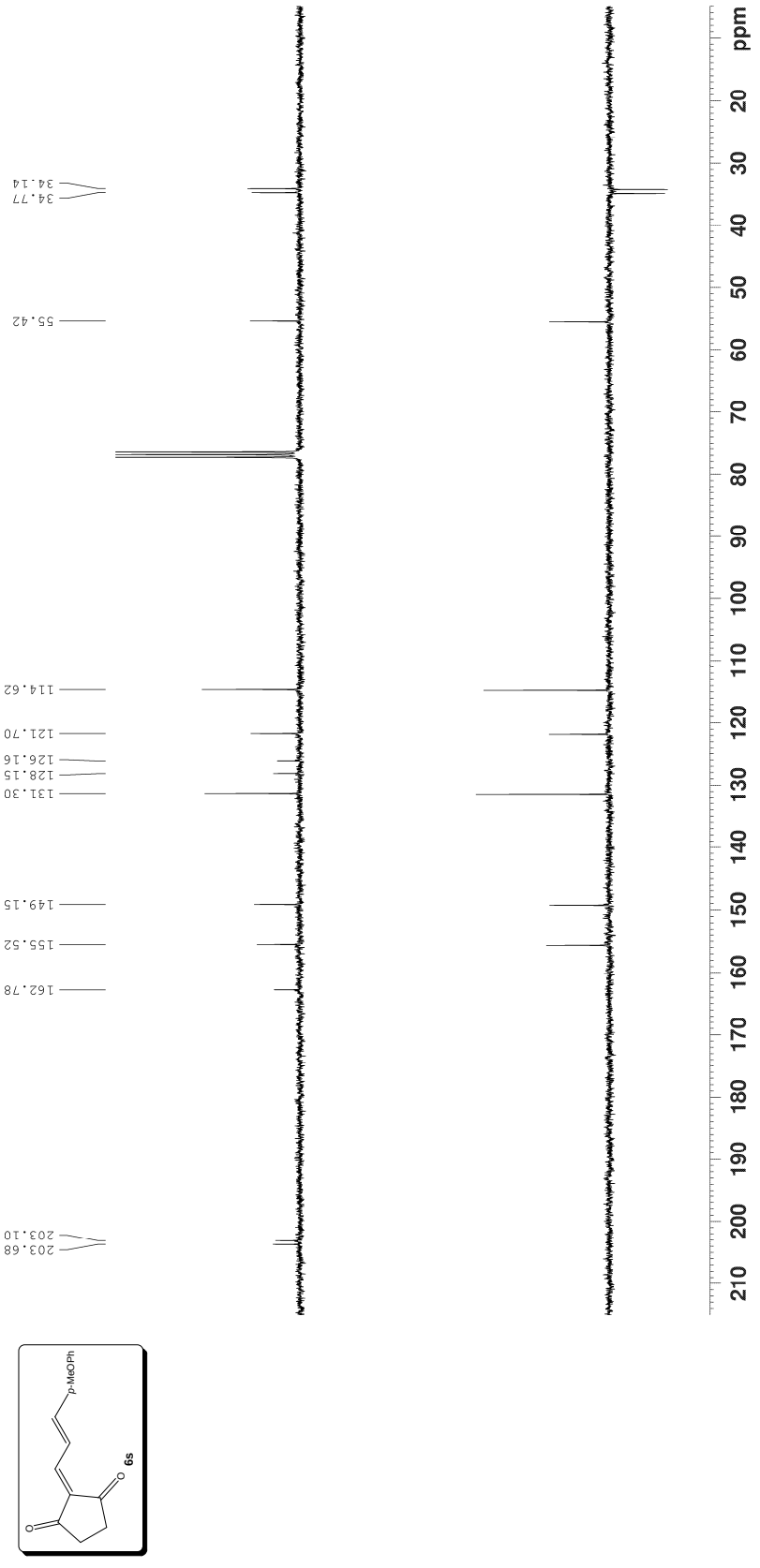


Fig. 32 ^{13}C NMR Spectra of Compound **6s**

Biological Assays

Antimicrobial Assays.

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods[1–2] as described by Samoylenko *et al*.[3] All organisms are tested using modified versions of the CLSI (formerly NCCLS) methods. For all organisms excluding *M. intracellulare* and *A. fumigatus*, optical density is used to monitor growth[1–2] Media supplemented with 5% Alamar Blue™ (BioSource International, Camarillo, CA) is utilized for growth detection of *M. intracellulare*,[4,5] and *A. fumigatus*.[6] Samples (dissolved in DMSO) are serially-diluted in 20% DMSO/saline and transferred (10µL) in duplicate to 96 well flat bottom microplates. Inocula are prepared by correcting the OD_{630} of microbe suspensions in incubation broth [RPMI 1640/0.2% dextrose/0.03% glutamine/MOPS @ pH 6.0 (Cellgro) for *Candida spp.*, Sabouraud Dextrose for *C. neoformans*, cation-adjusted Mueller-Hinton (Difco) @ pH 7.3 for *Staphylococcus spp.*, *E. coli*, and *P. aeruginosa*, 5% Alamar Blue™(BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment, pH = 7.0 for *M. intracellulare*, and 5% Alamar Blue™/RPMI 1640 broth (0.2% dextrose, 0.03% glutamine, buffered with 0.165M MOPS at pH 7.0) for *A. fumigatus* to afford an assay volume of 200µL and final target inocula of: *Candida spp.* and *C. neoformans*:

1.5 X 10^3, *M. intracellulare*: 2.0 X 10^6, *Staphylococcus spp.*, *E. coli*, *P. aeruginosa*: 5.0 X 10^5 CFU/ml, and *A. fumigatus*: 2.7 X 10^4 CFU/ml. Final sample test concentrations are 1/100th the DMSO stock concentration. Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for fungi] are included in each assay. All organisms are read at either 530nm using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or 544ex/590em, (*M. intracellulare, A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation: *Candida spp.* at 35°C for 46–50h, *Staphylococcus spp.*, *E. coli*, and *P. aeruginosa* at 35°C for 16–20h, *C. neoformans* at 35°C for 70–74h, *A. fumigatus* at 35°C for 46–50h, and *M. intracellulare* at 37°C and 10% CO_2 for 70–74h. IC_{50}s (concentrations that afford 50% inhibition relative to controls) are calculated using XL*fit* 4.2 software (IDBS, Alameda, CA) using fit model 201. The MIC is defined as the lowest test concentration that allows no detectable growth (for *M. intracellulare* and *A. fumigatus*, no color change from blue to pink). Minimum fungicidal or bactericidal concentrations are determined by removing 5µl from each clear (or blue) well, transferring to agar and incubating as previously mentioned. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar). Drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

In vitro Antileishmanial and Antimalarial Assays:

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes (Strain S1). In a 96 well microplate assay the compounds with appropriate dilution were added to the promastigotes culture (2 x 10^6 cell/mL) to get the final concentrations of 40, 8 and 1.6 µg/ml. The plates were

incubated at 26°C for 72 hours and growth was determined by Alamar blue assay.[7] Pentamidine and Amphotericin B were used as the standard antileishmanial agents. All the analogs were simultaneously tested for cytotoxicty on VERO (monkey kidney fibroblast) cells by Neutral Red assay.[8] IC_{50} value for each compound was computed from the growth inhibition curve. Antimalarial activity was determined *in vitro* on chloroquine sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) strains of *Plasmodium falciparum*. The 96 well microplate assay is based on evaluation of the effect of the compounds on growth of asynchronous cultures of *P. falciparum*, determined by the assay of parasite lactate dehydrogenase (pLDH) activity.[9] The appropriate dilutions of the compounds were prepared in DMSO or RPMI-1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set up in clear flat bottomed 96 well plates. The plates were placed into the humidified chamber and flushed with a gas mixture of 90% N_2, 5% CO_2 & 5% O_2. The cultures were incubated at 37°C for 48 hours. Growth of the parasite in each well was determined by pLDH assay using Malstat® reagent. The medium and RBC controls were also set-up in each plates. The standard antimalarial agents, chloroquine and artemisinin, were used as the positive controls while DMSO was tested as the negative control.

Table 1. Antimicrobial and antiparasitic activities for compounds **6a-6s.**[a]

	CA[b]	CG[c]	CK[d]	AF[e]	CN[f]	SA[g]	MRS[h]	EC[i]	PA[j]	MI[k]	LD[l]	LD[m]	PF[n]	PF2[o]	CV[p]
6a	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	19.13	52.26	>16	>16	>22.2
6b	>60	>60	>60	>60	35.30	>60	>60	>60	>60	>60	9.29	66.11	>16	>16	>16.4
6c	>60	>60	>60	>60	11.69	>60	>60	>60	>60	>60	9.55	24.55	>16	>16	>16.2
6d	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	38.47	>100	>16	>16	>19.5
6e	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	46.64	99.15	>16	>16	>17.5
6f	>60	35.27	>60	>60	19.03	>60	>60	>60	>60	>60	20.05	27.52	>16	>16	>18.7
6g	>60	>60	>60	>60	23.60	>60	>60	>60	>60	>60	9.38	26.63	>16	>16	>14.4
6h	>60	>60	>60	>60	24.73	>60	>60	>60	>60	>60	9.60	18.60	>16	>16	>14.3
6i	>60	35.55	>60	>60	16.63	>60	>60	>60	>60	>60	10.65	24.97	>16	>16	>17.5
6j	>60	>60	>60	>60	38.22	>60	>60	>60	>60	>60	30.59	68.93	>16	>16	>16.7
6k	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	45.80	>100	>16	>16	>21.8
6l	>60	>60	>60	>60	14.45	>60	>60	>60	>60	>60	10.25	26.12	>16	>16	>15.7
6m	>60	55.95	>60	>60	44.71	>60	>60	>60	>60	>60	19.51	91.69	>16	>16	>18.6
6n	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	56.78	>100	>16	>16	>25.0
6o	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	7.52	14.74	>16	>16	>14.3
6p	>60	>60	>60	>60	19.89	>60	>60	>60	>60	>60	11.79	20.05	>16	>16	>18.7
6q	>60	>60	42.60	>60	11.87	53.01	44.90	>60	>60	>60	2.16	3.58	>16	>16	>22.4
6r	>60	>60	>60	>60	26.86	>60	>60	>60	>60	>60	8.58	14.08	>16	>16	>16.3
6s	33.64	34.99	24.06	>60	13.37	54.90	33.68	>60	>60	>60	7.01	19.81	14.45	15.27	>19.6
CorA[q]	NT[r]	NT	NT	NT	8.02	NT	NT	NT	NT	NT	NT	NT	NT	NT	19.3
Amb[s]	0.63	0.28	0.78	1.12	0.35	NT	NT	NT	NT	NT	0.31	1.09	NT	NT	7.6
Pent[t]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	3.82	12.40	NT	NT	NT
Cip[u]	NT	NT	NT	NT	NT	0.36	0.30	0.015	0.29	0.75	NT	NT	NT	NT	NT
CQ[v]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.044	0.47	NT
ART[w]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.016	0.019	NT

[a] All the analogues were tested at least at six different concentrations, each in duplicate. The mean values were used to generate the growth inhibition curves and determination of IC_{50} values. [b] *Candida albicans* ATCC 90028, IC_{50} (μM). [c] *C. glabrata* ATCC 90030, IC_{50} (μM). [d] *C. krusei* ATCC 6258, IC_{50} (μM). [e] *Aspergillus fumigatus* ATCC 90906, IC_{50} (μM). [f] *Cryptococcus neoformans* ATCC 90113, IC_{50} (μM). [g] *Staphylococcus aureus* ATCC 29213, IC_{50} (μM). [h] Methicillin-resistant *S. aureus* ATCC 33591, IC_{50} (μM). [i] *Escherichia coli* ATCC 35218, IC_{50} (μM). [j] *Pseudomonas aeruginosa* ATCC 27853, IC_{50} (μM). [k] *Mycobacterium intracellulare* ATCC 23068, IC_{50} (μM). [l] *L. donovani*, IC_{50} (μM). [m] *L. donovani*, IC_{90} (μM). [n] *Plasmodium falciparum* (D6 Clone), IC_{50} (μM). [o] *P. falciparum* (W2 Clone), IC_{50} (μM). [p] Cytotoxicity Vero (African green monkey kidney fibroblast), TC_{50} (μM). [q] Coruscanone A. [r] Not tested. [s] Amphotericin B. [t] Pentamidine. [u] Ciprofloxacin. [v] Chloroquine. [w] Artemisinin.

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