Advanced
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Supporting Information

Supporting Information

Dehydrative Cyclization Catalyzed by the Combination of Molybdenum(VI) Oxides and Benzoic Acids: First Synthesis of the Antitumor Substance BE-70016

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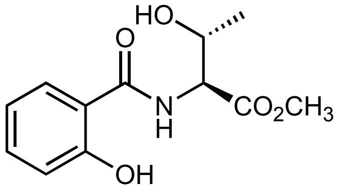
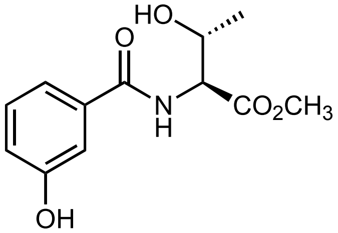
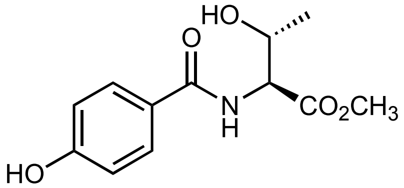
General Remarks: IR spectra were recorded on a JASCO FT/IR-460 plus spectrometer. ^{1}H spectra were measured on a Varian Gemini-2000 spectrometer (300 MHz) at ambient temperature. Data were recorded as follows: chemical shift in ppm from internal tetramethysilane on the d scale, multiplicity (s = singlet; d = doublet; t = triplet; m = multiplet), coupling constant (Hz), and integration. ^{13}C NMR spectra were measured on a Varian Gemini-2000 spectrometer (75 MHz) or INOVA spectrometer (125 MHz) at ambient temperature. Chemical shifts were recorded in ppm from the solvent resonance employed as the internal standard ($CDCl_3$ at 77.0 ppm). All experiments were carried out under an atmosphere of dry nitrogen. For TLC analysis, Merck precoated TLC plates (silica gel 60 F_{254} 0.25 mm) were used. For preparative column chromatography, Merck silica gel 60 (0.040–0.063 mm) was used. High resolution mass spectral analysis (HRMS) was performed at Chemical Instrument Center, Nagoya University. Dry toluene was purchased from Wako as the “anhydrous” and stored under nitrogen. Dichloromethane and triethylamine were freshly distilled from calcium hydride. $(NH_4)_2MoO_4$ (Aldrich), $MoO_2(acac)_2$ (Wako), $(CF_3)_2C_6H_3CO_2H$ (TCI), 4-$(NO_2)C_6H_4CO_2H$ (Kishida) and other materials were obtained from commercial supplies and used without further purification.



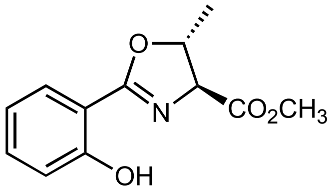
***N*-(*o*-Hydroxybenzoyl)-L-threonine methyl ester (1a).** To a solution of L-threonine methylester (1.70 g, 10 mmol), salicylic acid (1.38 g, 10 mmol), HOBt (135 mg, 1.0 mmol) and Et_3N (1.39 mL, 10 mmol) in CH_2Cl_2 (30 mL) was added a solution of WSCI•HCl (2.11 g, 11 mmol) in CH_2Cl_2 (30 mL) at 0 °C. After stirring at 6 °C for 12 h, CH_2Cl_2 was removed in vacuo and dissolved in EtOAc (80 mL). The resulting solution was washed with 1 M HCl (80 mL), saturated aqueous $NaHCO_3$ (2 × 80 mL) and brine (80 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using a mixture of hexane–EtOAc (2:1 → 3:2) as an eluent to give **1a** (2.25 g, 89%): colorless oil; IR (neat) 3373, 1745, 1644, 1600, 1538, 1493, 1439, 1361, 1308, 1217, 1175 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (d, J = 6.3 Hz, 3H), 3.17 (br s, 1H), 3.78 (s, 3H), 4.48 (dq, J = 2.1, 6.3 Hz, 1H), 4.78 (d, J = 2.4 Hz, 0.5H), 4.81 (d, J = 2.4 Hz, 0.5H), 6.85 (ddd, J = 1.2, 7.2, 7.8 Hz, 1H), 6.97 (dd, J = 1.2, 8.4 Hz, 1H), 7.39 (ddd, J = 1.5, 7.2, 8.4 Hz, 1H), 7.59 (dd, J = 1.5, 7.8 Hz, 1H), ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.9, 52.7, 57.3, 67.7, 114.0, 117.9, 119.0, 126.7, 134.4, 160.5, 170.1, 171.4; HRMS (FAB) calcd for $C_{12}H_{16}NO_5$ $[M+H]^+$ 254.1028, found 254.1022.



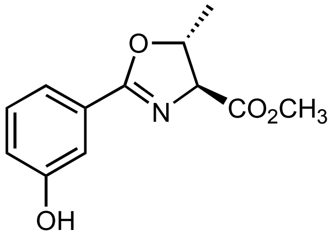
***N*-(*m*-Hydroxybenzoyl)-L-threonine methyl ester (1b):** colorless oil; IR (neat) 3366, 1739, 1644, 1585, 1530, 1486, 1439, 1316, 1217, 1158, 1085 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.23 (d, J = 6.6 Hz, 3H), 3.77 (s, 3H), 4.38 (dq, J = 3.3, 6.6 Hz, 1H), 4.66 (d, J = 3.3 Hz, 1H), 6.97 (td, J = 2.4, 6.9 Hz, 1H), 7.25–7.35 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.4, 52.9, 59.8, 68.5, 115.3, 119.2, 120.0, 130.8, 136.4, 158.9, 170.6, 172.6; HRMS (FAB) calcd for $C_{12}H_{16}NO_5$ $[M+H]^+$ 254.1028, found 254.1022.



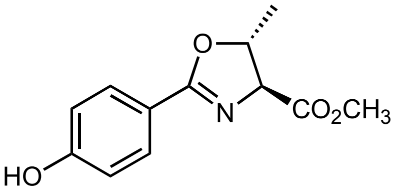
***N*-(*p*-Hydroxybenzoyl)-L-threonine methyl ester (1c):** colorless oil; IR (neat) 3358, 1740, 1640, 1609, 1588, 1541, 1507, 1439, 1281, 1239, 1177, 1111, 1084, 1022 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.22 (d, J = 6.6 Hz, 3H), 3.75 (s, 3H), 4.37 (dq, J = 3.3, 6.6 Hz, 1H), 4.66 (d, J = 3.3 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.4, 52.9, 59.7, 68.6, 116.2, 125.6, 130.5, 162.4, 170.2, 172.8; HRMS (FAB) calcd for $C_{12}H_{16}NO_5$ $[M+H]^+$ 254.1028, found 254.1018.



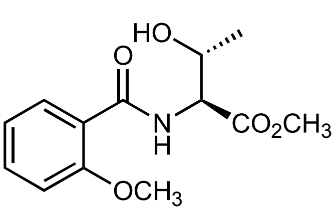
Methyl (4*S*,5*R*)-2-(*o*-hydroxyphenyl)-5-methyl-4-oxazolinecarboxylate (2a). A solution of **1a** (253 mg, 1 mmol), $(NH_4)_2MoO_4$ (20 mg, 0.10 mmol) and $C_6F_5CO_2H$ (21 mg, 0.10 mmol) in toluene (10 mL) was heated at azeotropic reflux with the removal of water using a Dean-Stark apparatus. After 12 hours, the reaction mixture was cooled to ambient temperature, diluted with EtOAc (10 mL) and washed with 1 M citric acid in saturated aqueous NaCl (15 mL), saturated aqueous $NaHCO_3$ and NaCl (15 mL), and brine (15 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a crude product. Yields were determined by ^{1}H NMR analysis. The crude product was purified by column chromatography on silica gel using a mixture of hexane–EtOAc (15:1 → 13:1 → 10:1) as an eluent to give **2a**: colorless oil; IR (neat) 1743, 1638, 1614, 1491, 1438, 1355, 1310, 1259, 1229, 1207, 1157, 1134, 1072, 1038 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (d, J = 6.3 Hz, 3H), 3.80 (s, 3H), 4.50 (d, J = 6.9 Hz, 1H), 4.98 (qd, J = 6.3, 6.9 Hz, 1H), 6.87 (ddd, J = 0.9, 7.2, 7.8 Hz, 1H), 7.01 (dd, J = 0.9, 8.4 Hz, 1H), 7.39 (ddd, J = 1.5, 7.2, 8.4 Hz, 1H), 7.66 (dd, J = 1.5, 7.8 Hz, 1H), 11.8 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9, 52.8, 73.7, 78.5, 110.4, 117.0, 118.8, 128.4, 134.0, 160.1, 167.0, 171.0; HRMS (FAB) calcd for $C_{12}H_{14}NO_4$ $[M+H]^+$ 236.0923, found 236.0925.



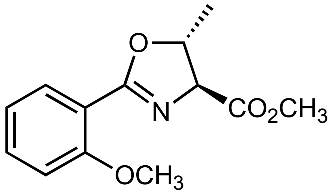
Methyl (4*S*,5*R*)-2-(*m*-hydroxyphenyl)-5-methyl-4-oxazolinecarboxylate (2b): colorless oil; IR (neat) 1740, 1670, 1586, 1453, 1438, 1388, 1216, 1094 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 1.49 (d, J = 6.3 Hz, 3H), 3.78 (s, 3H), 4.48 (d, J = 7.2 Hz, 1H), 4.99 (qd, J = 6.3, 7.2 Hz, 1H), 6.97 (ddd, J = 1.2, 2.7, 8.1 Hz, 1H), 7.26 (dd, J = 7.8, 8.1 Hz, 1H), 7.34 (dd, J = 1.5, 2.7 Hz, 1H), 7.40 (ddd, J = 1.2, 1.5, 7.8 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 21.0, 53.0, 75.4, 80.7, 116.0, 120.4, 120.6, 129.2, 130.8, 158.8, 167.6, 172.8; HRMS (FAB) calcd for $C_{12}H_{14}NO_4$ $[M+H]^+$ 236.0923, found 236.0919.



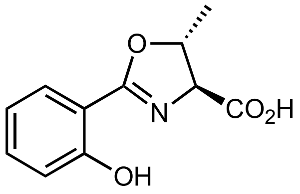
Methyl (4*S*,5*R*)-2-(*p*-hydroxyphenyl)-5-methyl-4-oxazolinecarboxylate (2c): colorless crystal (cryst. from EtOAc); mp 156–157 °C; IR (KBr) 1730, 1601, 1514, 1446, 1372, 1349, 1245, 1165, 1089, 1016 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 1.49 (d, J = 6.3 Hz, 3H), 3.78 (s, 3H), 4.44 (d, J = 6.9 Hz, 1H), 4.96 (qd, J = 6.3, 6.9 Hz, 1H), 6.82 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 21.0, 53.0, 75.3, 80.4, 116.3, 118.9, 131.5, 162.7, 167.8, 173.0; HRMS (FAB) calcd for $C_{12}H_{14}NO_4$ $[M+H]^+$ 236.0923, found 236.0924.



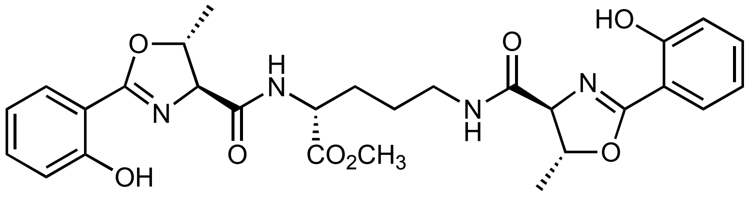
***N*-(*o*-Methoxybenzoyl)-L-threonine methyl ester (3).** colorless oil; IR (neat) 3381, 1739, 1642, 1601, 1531, 1484, 1299, 1242, 1211, 1162, 1047, 1020 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (d, J = 6.3 Hz, 3H), 2.83 (br s, 1H), 3.79 (s, 3H), 4.00 (s, 3H), 4.41 (m, 1H), 4.82 (d, J = 2.7 Hz, 0.5H), 4.84 (d, J = 2.4 Hz, 0.5H), 7.00 (d, J = 8.1 Hz, 1H), 7.07 (dd, J = 6.9, 7.5 Hz, 1H), 7.47 (ddd, *J* = 1.8, 6.9, 8.4 Hz, 1H), 8.18 (dd, *J* = 1.8, 7.5 Hz, 1H), 8.74 (br d, *J* = 7.8 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.0, 52.4, 56.0, 58.1, 67.8, 114.4, 120.6, 121.1, 132.1, 133.2, 157.8, 165.8, 171.7.



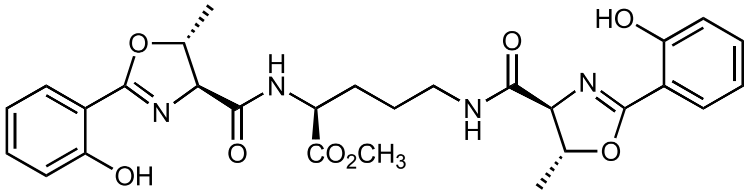
Methyl (4*S*,5*R*)-2-(*o*-methoxyphenyl)-5-methyl-4-oxazolinecarboxylate (4). colorless oil; IR (KBr) 1739, 1631, 1601, 1493, 1468, 1437, 1344, 1271, 1212, 1126, 1084, 1043, 1021 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, *J* = 6.3 Hz, 3H), 3.80 (s, 3H), 3.91 (s, 3H), 4.51 (d, *J* = 7.5 Hz, 1H), 4.94 (qd, *J* = 6.3, 7.5 Hz, 1H), 6.92–7.02 (m, 2H), 7.44 (ddd, *J* = 1.8, 7.5, 8.4 Hz, 1H), 7.79 (dd, *J* = 1.8, 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 52.7, 56.2, 75.5, 78.2, 111.8, 116.6, 120.3, 131.7, 132.8, 158.7, 164.7, 171.9.



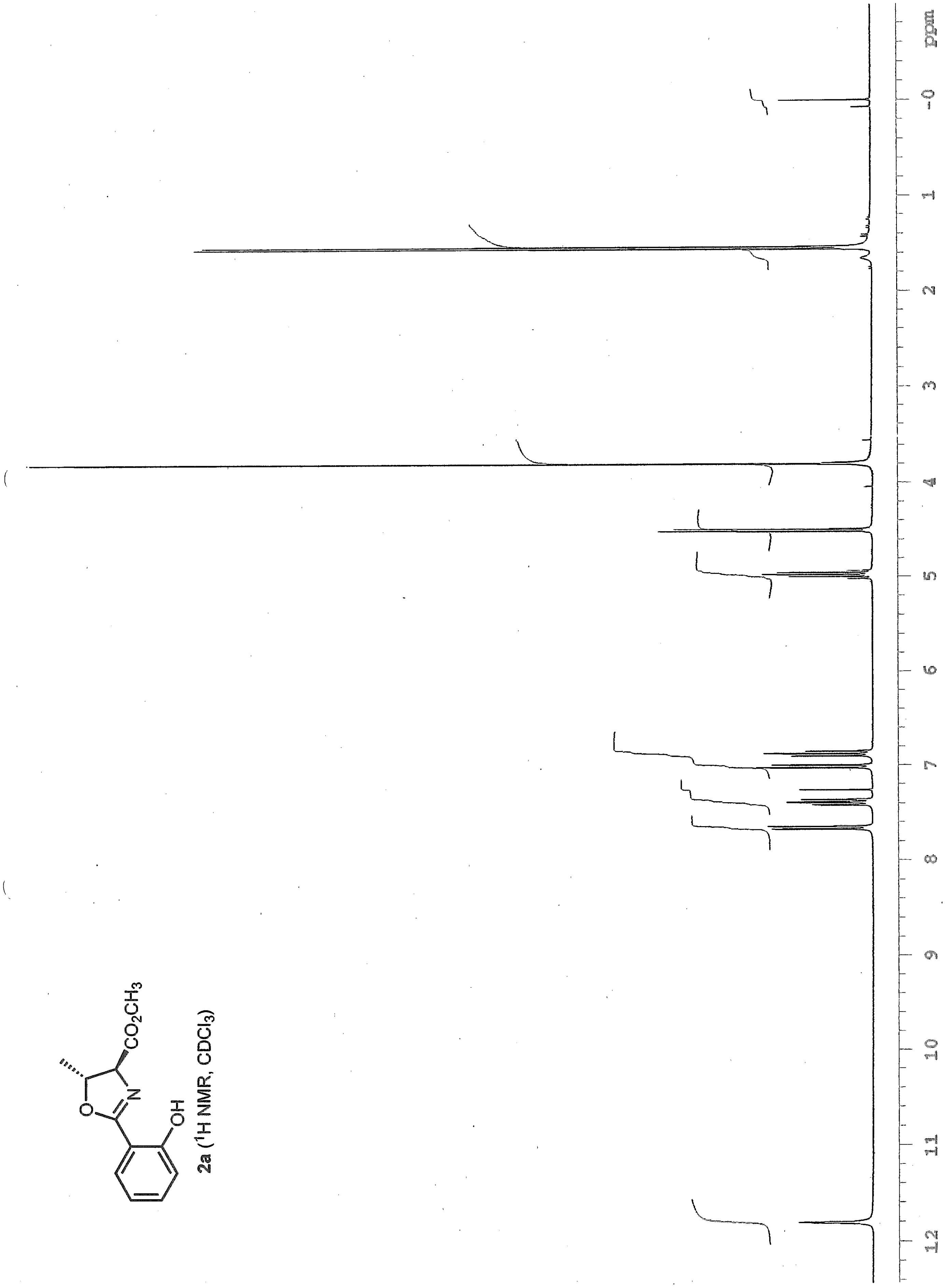
(4*S*,5*R*)-2-(*o*-Hydroxyphenyl)-5-methyl-4-oxazolinecarboxylic acid (5). To a solution of **2a** (588 mg, 2.5 mmol) in methanol (10 mL) was added 1.0 M aqueous solution of LiOH (10 mL, 10 mmol) at ambient temperature and stirred for 2.5 h. The reaction mixture was cooled to 0 °C, and acidified (pH 2) with conc. aqueous HCl. After removing MeOH *in vacuo*, the resulting aqueous layer was extracted with EtOAc (3 × 15 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated, to give **5** (550 mg, 99%): colorless amorphous powder; IR (KBr) 1732, 1637, 1491, 1446, 1372, 1308, 1259, 1158, 1133, 1073, 1037 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, *J* = 6.3 Hz, 3H), 4.39 (d, *J* = 7.5 Hz, 1H), 4.93 (qd, *J* = 6.3, 7.5 Hz, 1H), 6.78 (dd, *J* = 7.8, 8.4 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.31 (dd, *J* = 8.4, 8.4 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 11.8 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7, 73.5, 78.4, 110.3, 116.8, 118.4, 128.2, 133.6, 160.1, 166.6, 171.6; HRMS (FAB) calcd for $C_{11}H_{12}NO_4$ $[M+H]^+$ 222.0766, found 222.0772.



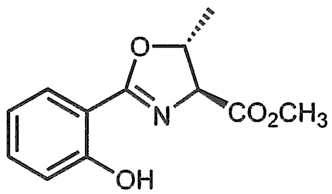
(*R*)-Ornithine derivative (BE-70016) (7). To a solution of **2a** (465 mg, 2.1 mmol), H–L-Orn–OCH_3•2HCl (153 mg, 0.70 mmol), HOBt (189 mg, 1.4 mmol) and Et_3N (195 μL, 1.4 mmol) in CH_2Cl_2 (10 mL) was added a solution of WSCI•HCl (403 mg, 2.1 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After stirring at 6 °C for 15 h then at ambient temperature for 2 h, CH_2Cl_2 was removed in vacuo and dissolved in EtOAc (50 mL). The resulting solution was washed with 1 M HCl (40 mL), saturated aqueous $NaHCO_3$ (2 × 40 mL) and brine (40 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using a mixture of hexane–acetone (3:1 → 2:1→ 1:1) as a eluent to give **7** (340 mg, 88%): colorless amorphous powder; $[\alpha]^{23}_{D}$ +10.6 (CH_3OH, *c* 0.25); IR (KBr) 3352, 1743, 1668, 1637, 1613, 1523, 1490, 1445, 1372, 1350, 1310, 1258, 1227, 1157, 1134, 1074, 1039 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (d, *J* = 6.5 Hz, 3H), 1.62 (d, *J* = 6.0 Hz, 3H), 1.6–1.7 (m, 2H), 1.76 (m, 1H), 1.93 (dddd, *J* = 5.5, 8.5, 9.0, 14.0 Hz, 1H), 3.33 (ddd, *J* = 6.5, 7.0, 13.5 Hz, 1H), 3.38 (ddd, *J* = 6.5, 7.0, 13.5 Hz, 1H), 3.69 (s, 3H), 4.39 (d, *J* = 7.5 Hz, 1H) 4.43 (d, *J* = 8.0 Hz, 1H), 4.58 (dt, *J* = 5.0, 7.5 Hz, 1H), 4.84–4.93 (m, 2H), 6.67 (br s, 1H), 6.90 (ddd, *J* = 1.0, 7.5, 8.0 Hz, 2H), 6.91 (ddd, *J* = 1.0, 7.5, 8.0 Hz, 2H), 7.02 (m, 2H), 7.08 (br d, *J* = 8.0 Hz, 1H), 7.41 (ddd, J = 1.0, 7.5, 8.5 Hz, 1H), 7.42 (ddd, J = 1.0, 7.5, 8.5 Hz, 1H), 7.69 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.69 (dd, *J* = 1.5, 8.0 Hz, 1H), 11.5 (br s, 1H), 11.5 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.7, 21.8, 25.8, 29.7, 38.7, 51.8, 52.7, 74.3, 74.3, 79.4, 79.7, 110.4, 117.0, 117.1, 119.1, 119.2, 128.7, 128.7, 134.3, 134.3, 159.9, 160.0, 167.2, 167.5 170.7, 170.8, 172.0; HRMS (FAB) calcd for $C_{28}H_{33}N_4O_8$ $[M+H]^+$ 553.2298, found 553.2294.



(*S*)-Ornithine derivative (6). **6** was prepared according to the same manner with **7** using (*S*)-L-ornithine methylester instead of (*R*)-D-ornithine methyl ester: 85% yield; colorless amorphous powder; $[\alpha]^{23}_{D}$ +1.6 (CH_3OH, *c* 0.25); IR (KBr) 1740, 1655, 1610, 1534, 1490, 1444, 1374, 1354, 1309, 1260, 1228, 1212, 1158, 1134, 1072, 1046 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (d, *J* = 6.3 Hz, 3H), 1.60 (d, *J* = 6.3 Hz, 3H), 1.72 (m, 1H), 1.83–1.97 (m, 1H), 3.20 (m, 1H), 3.38 (m, 1H), 3.76 (s, 3H), 4.36 (d, *J* = 7.5 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.59 (ddd, *J* = 5.4, 7.8, 7.8 Hz, 1H), 4.88 (qd, *J* = 6.3, 7.5 Hz, 1H), 4.91 (qd, *J* = 6.3, 7.8 Hz, 1H), 6.75 (br t, *J* = 6.3 Hz, 1H), 6.90 (ddd, *J* = 1.2, 7.2, 7.8 Hz, 1H), 6.91 (ddd, *J* = 1.2, 7.2, 7.8 Hz, 1H), 7.02 (dd, *J* = 1.2, 8.1 Hz, 2H), 7.15 (br d, *J* = 7.8 Hz, 1H), 7.41 (ddd, *J* = 1.8, 7.2, 8.1 Hz, 1H), 7.43 (ddd, *J* = 1.8, 7.2, 8.1 Hz, 1H), 7.69 (dd, *J* = 1.8, 7.8 Hz, 2H), 11.49 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 21.8, 25.9, 29.5, 38.7, 52.1, 52.7, 74.3, 74.4, 79.4, 79.7 110.3, 110.3, 117.0, 117.1, 119.1, 119.2, 128.7, 128.7, 134.3, 134.3, 159.9, 160.0, 167.2, 167.2, 170.6, 170.8, 172.1; HRMS (FAB) calcd for $C_{28}H_{33}N_4O_8$ $[M+H]^+$ 553.2298, found 553.2316.



2a (^{1}H NMR, CDCl$_3$)



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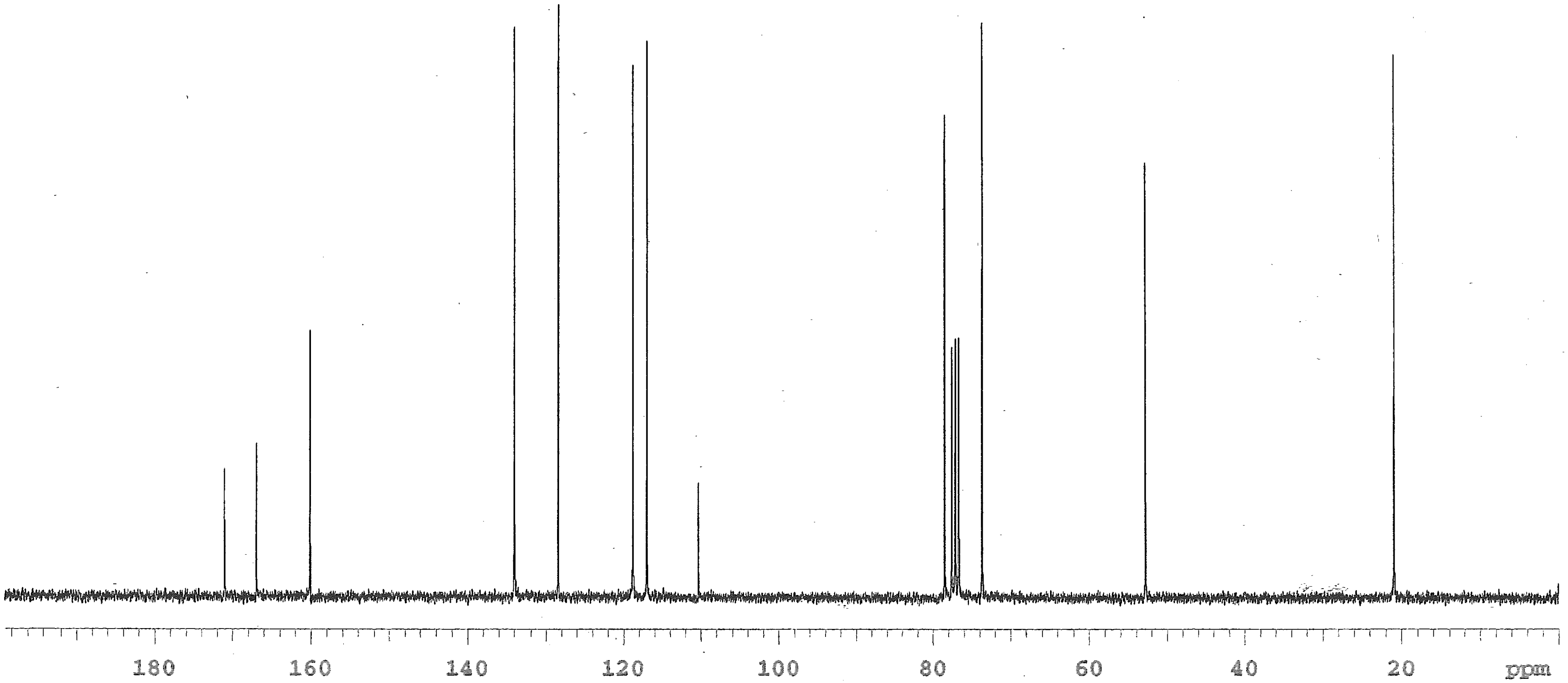
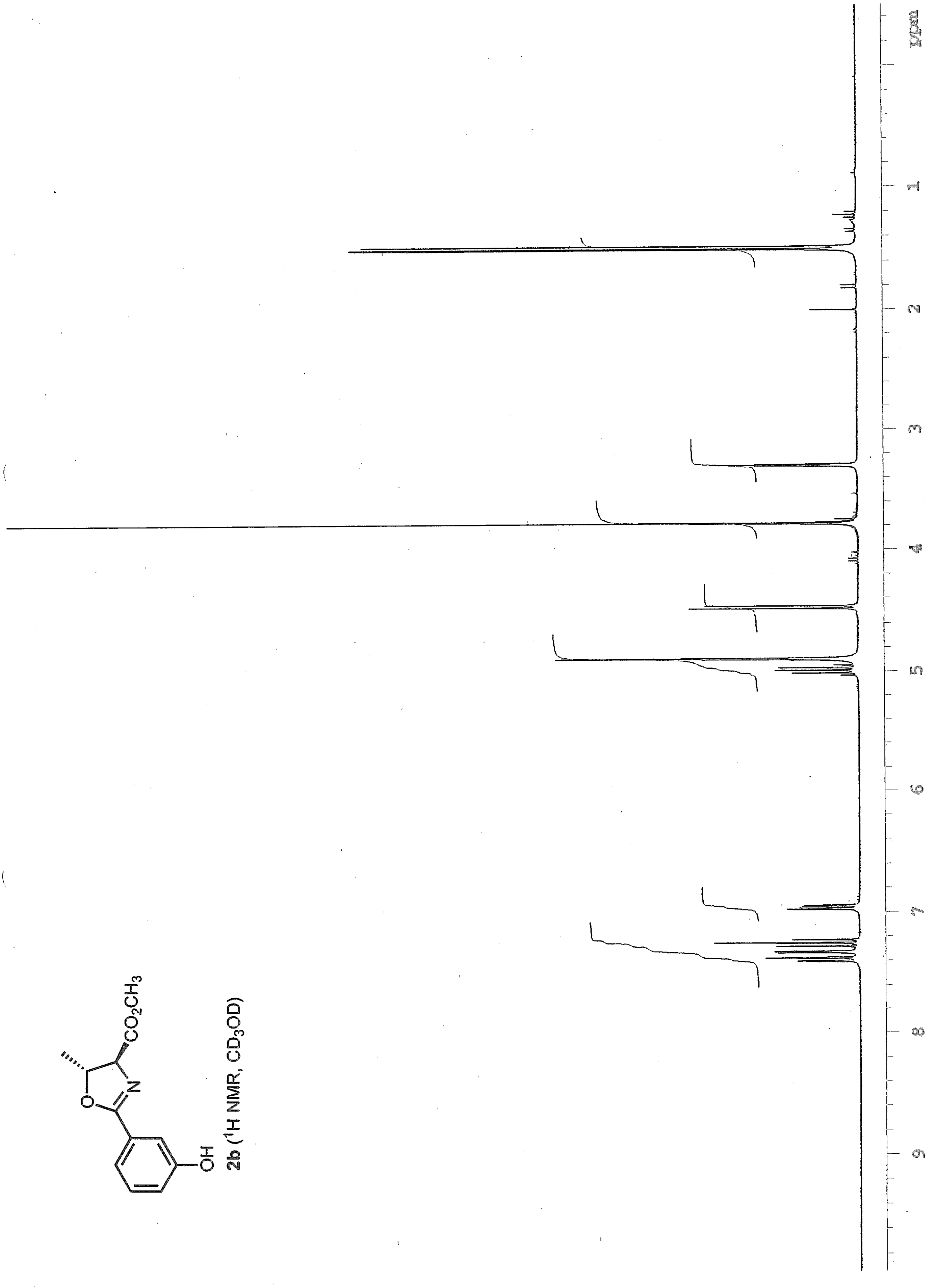
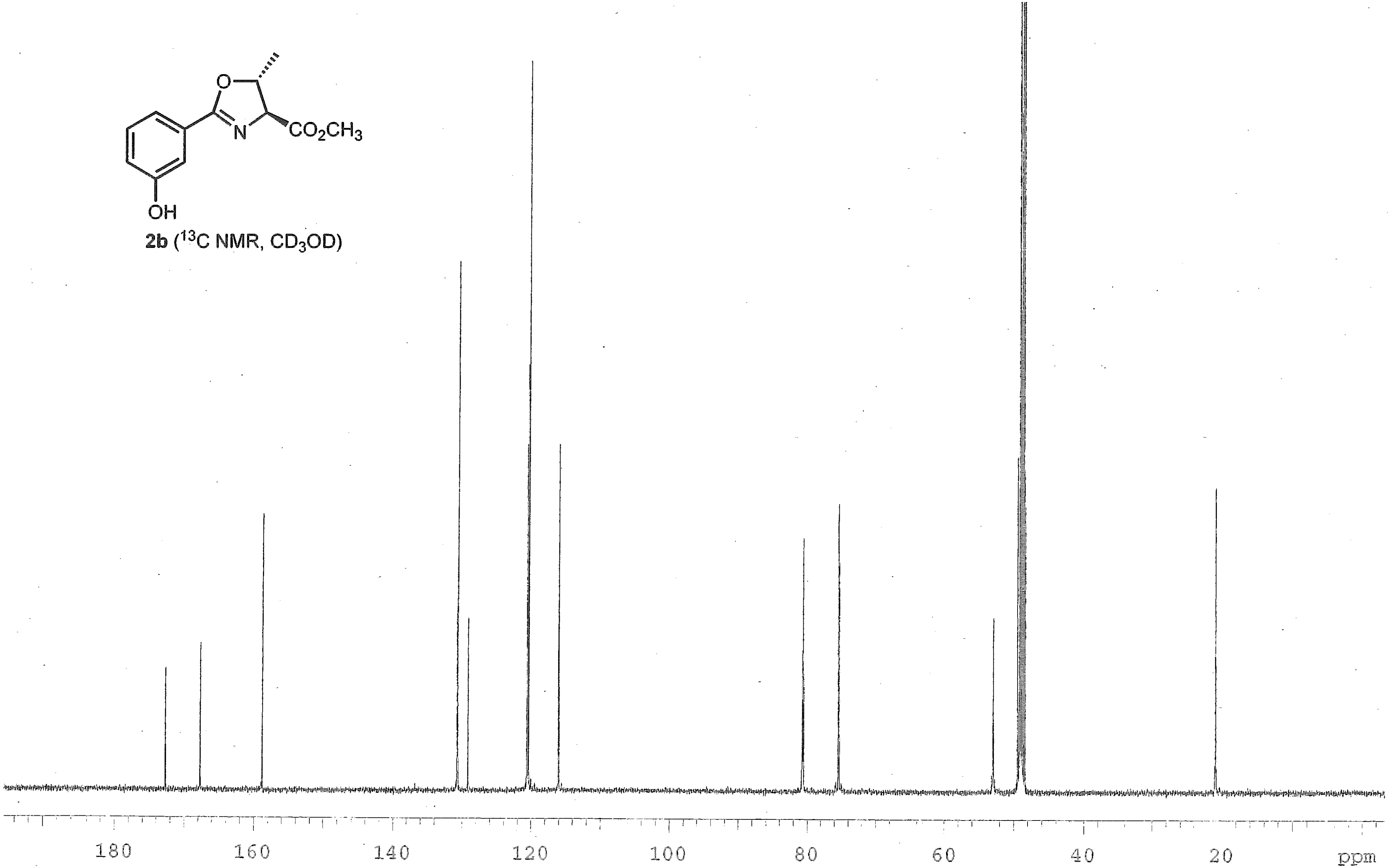
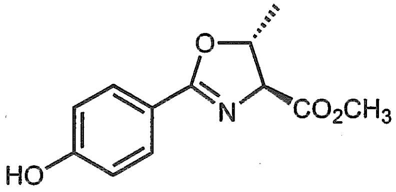




2b (^{1}H NMR, CD_3OD)



2b (^{13}C NMR, CD_3OD)



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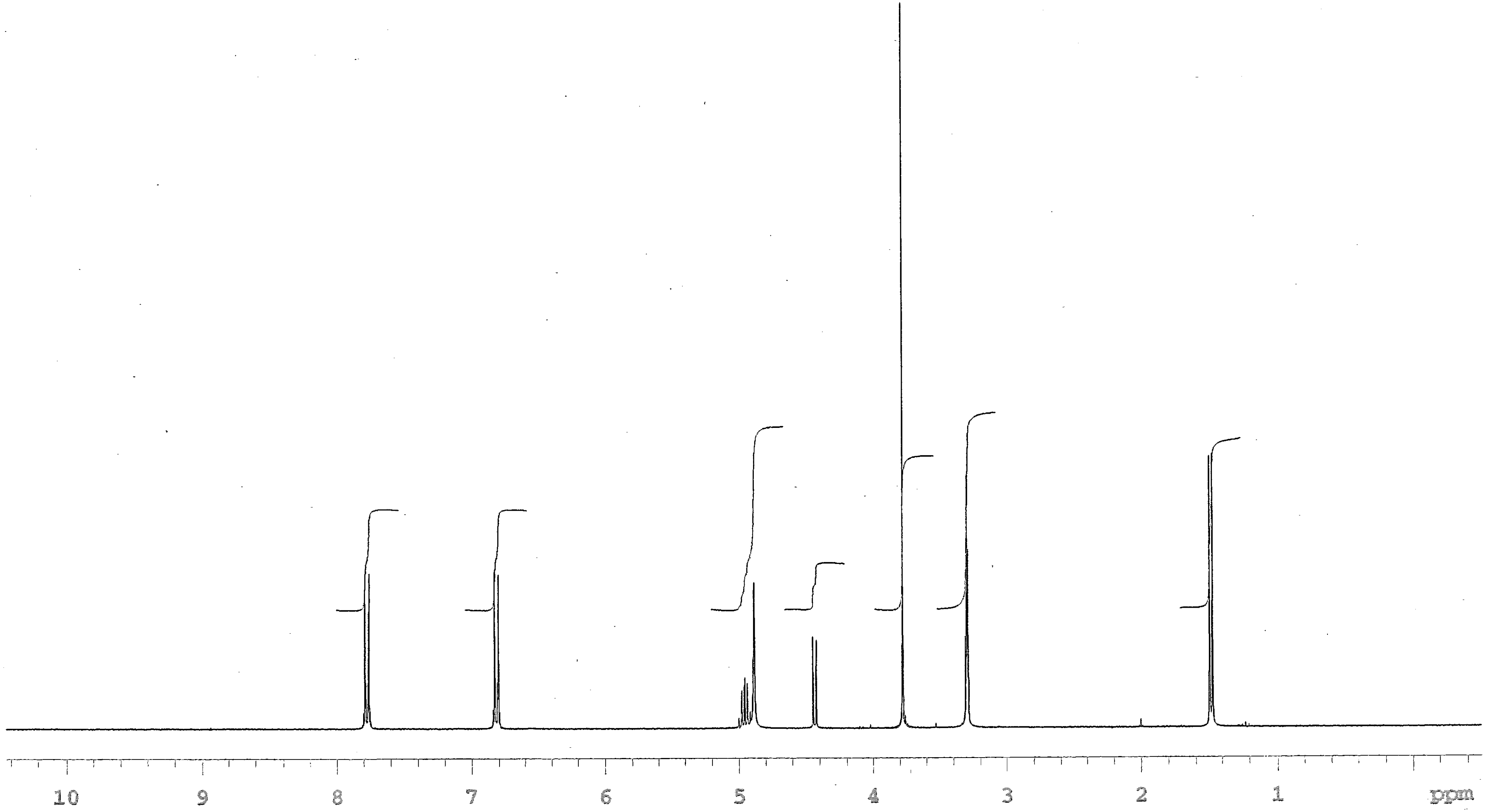
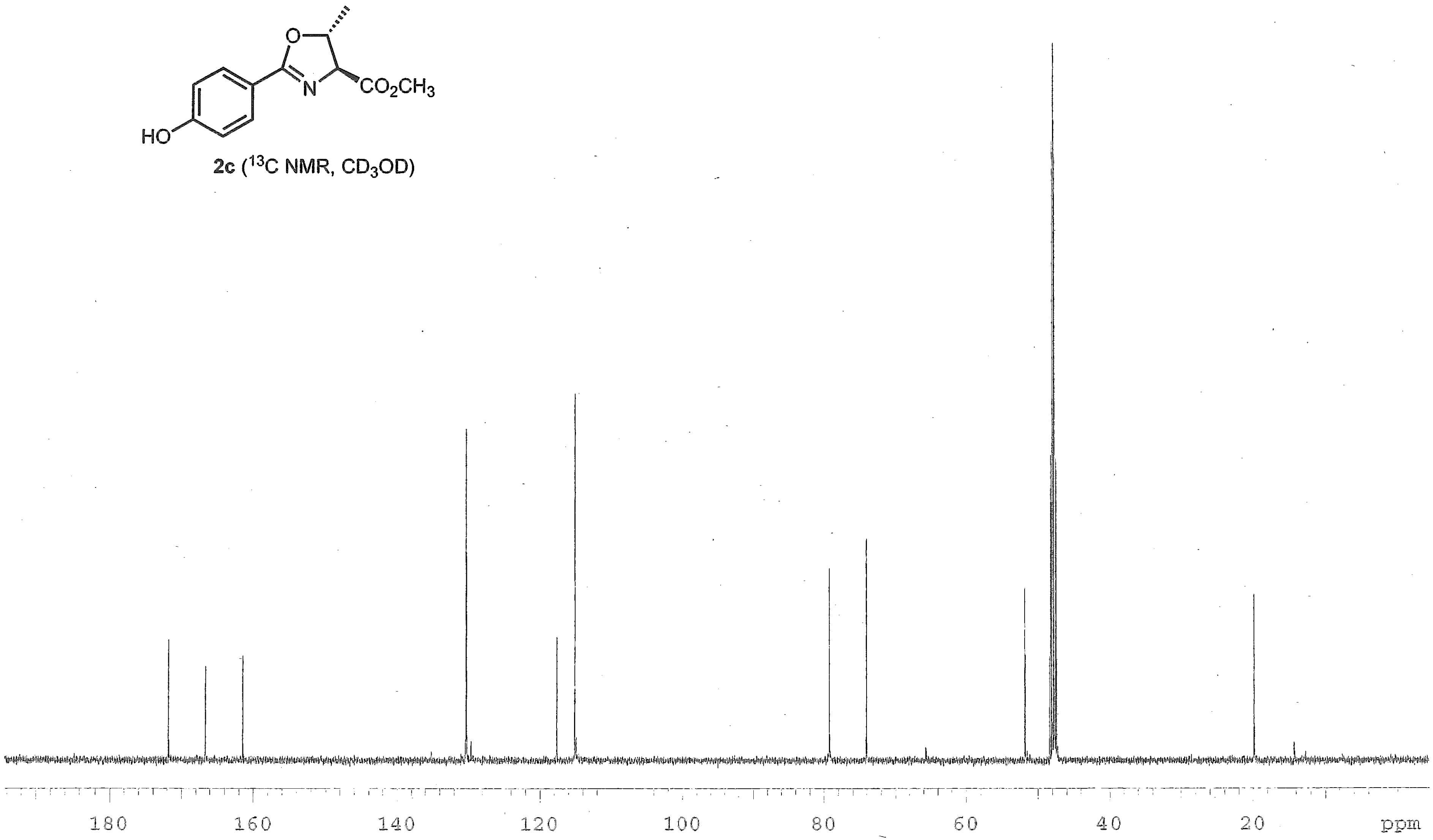
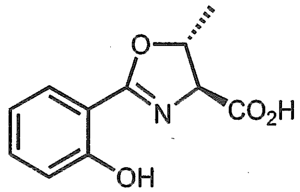
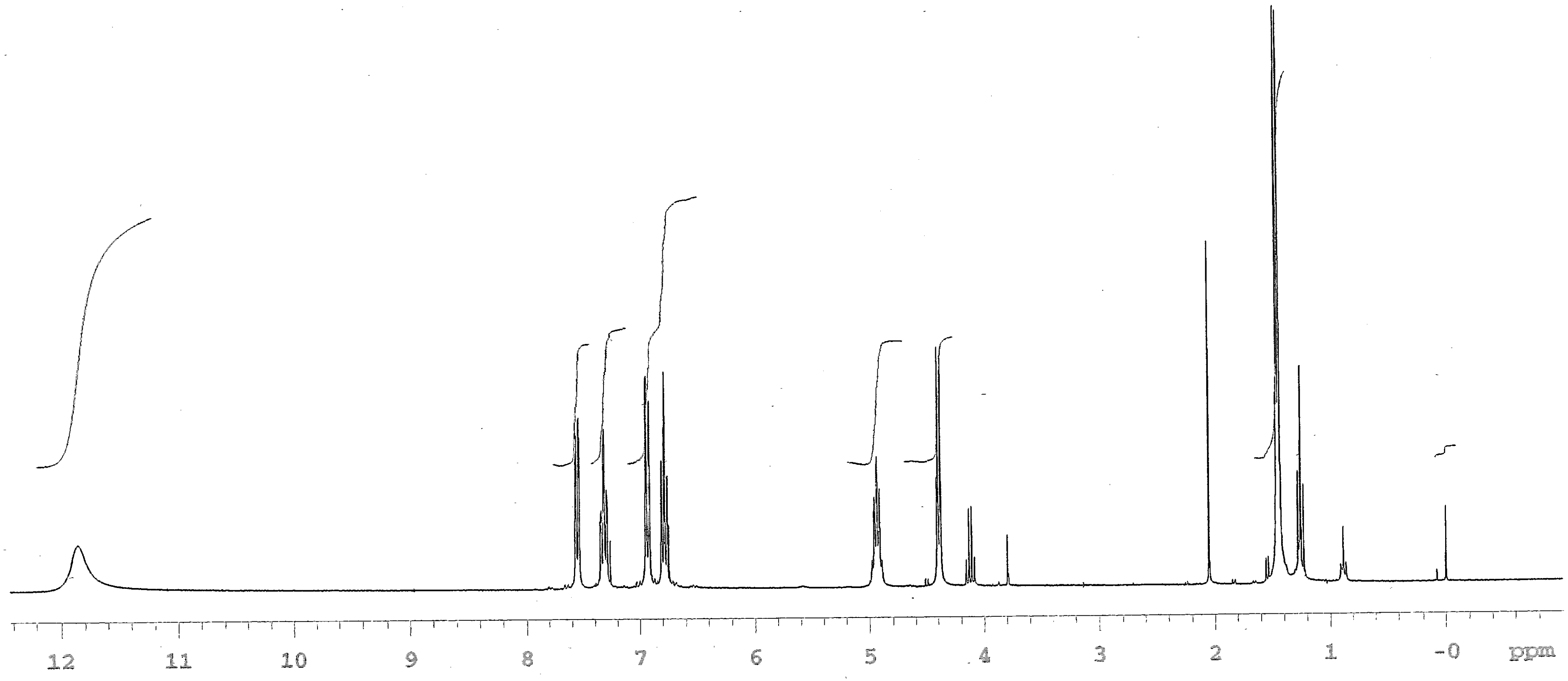


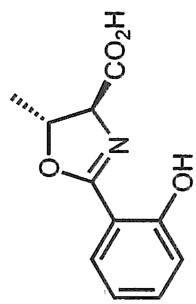


2c (^{13}C NMR, CD_3OD)

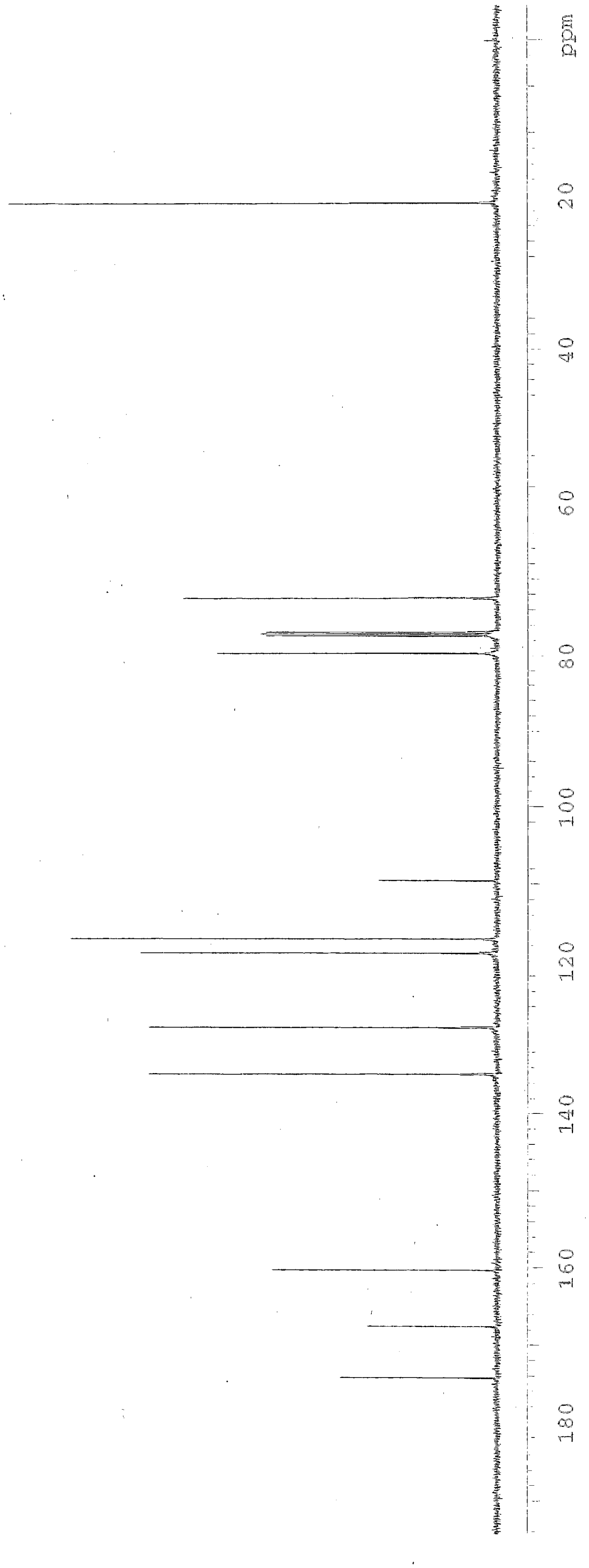


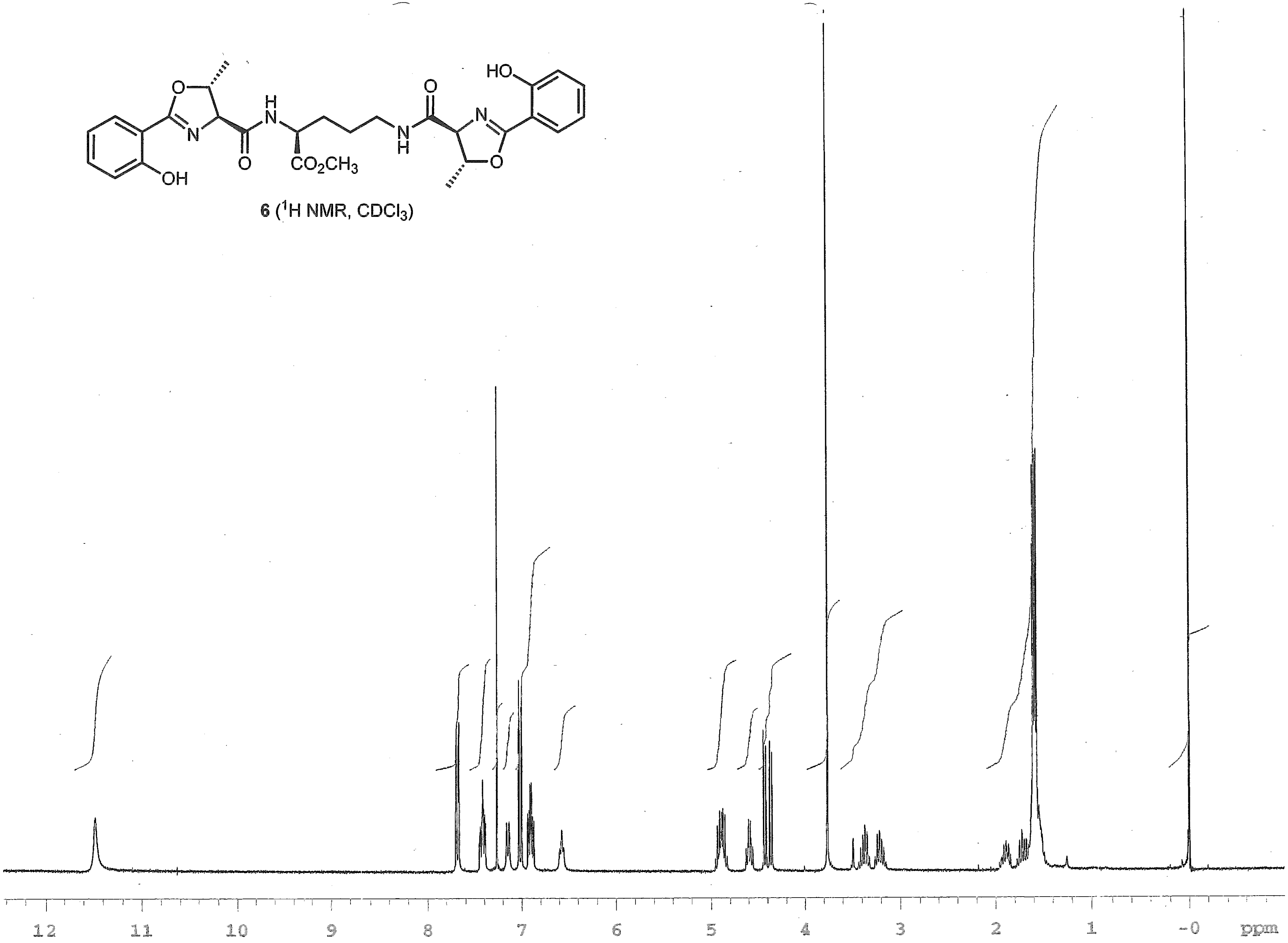
5 (¹H NMR, CDCl$_3$)



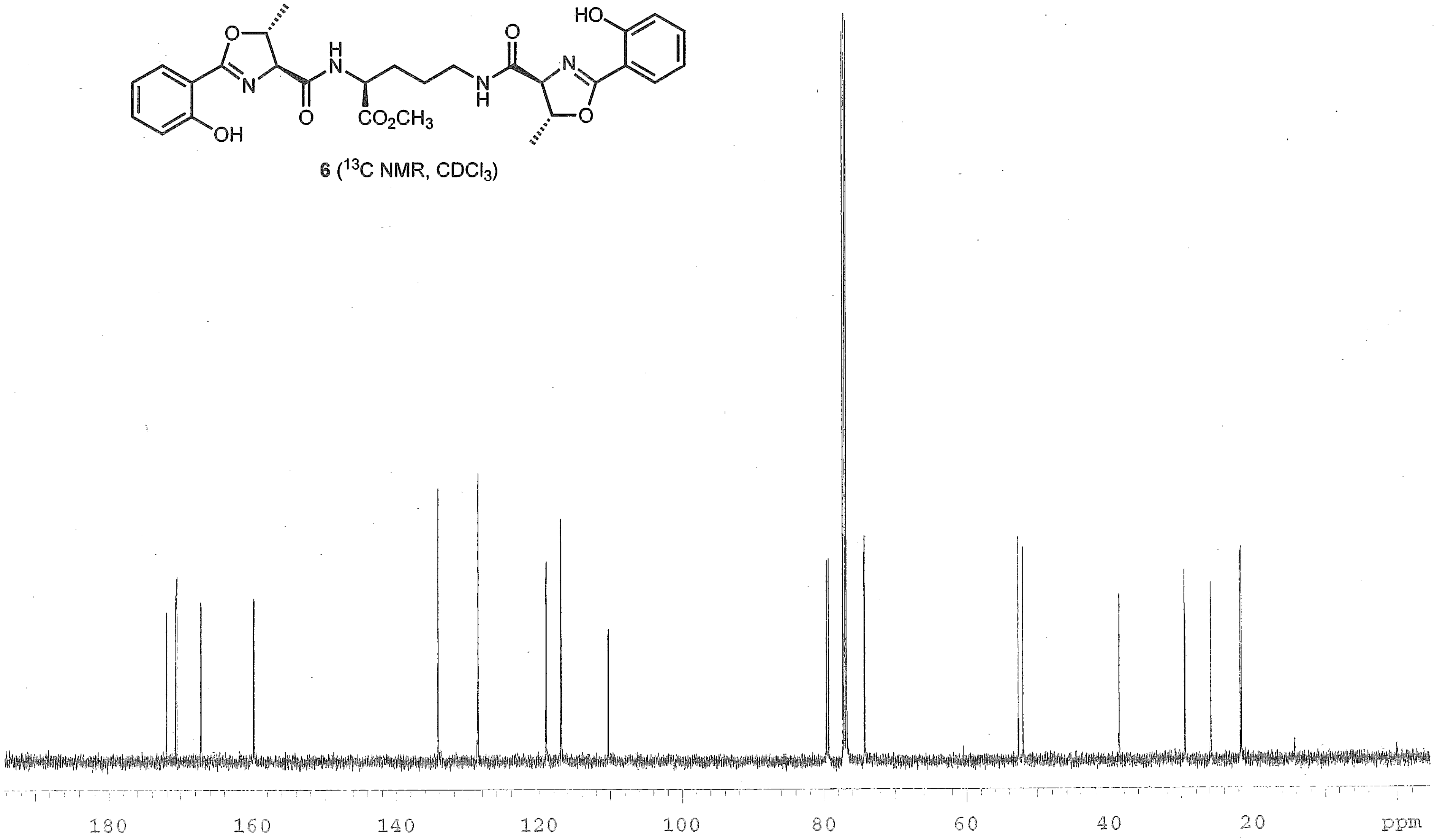


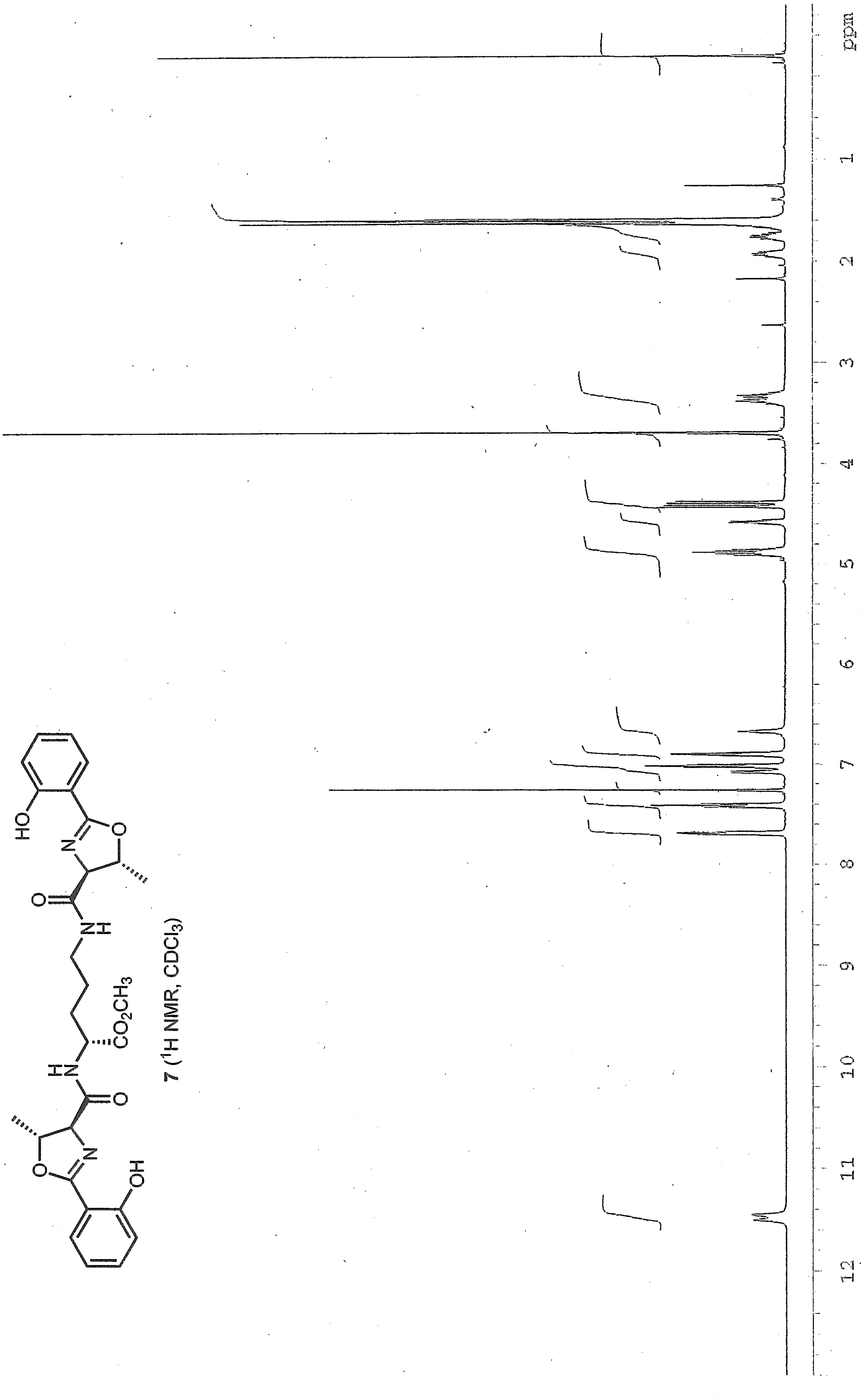
5 (^{13}C NMR, CDCl$_3$)



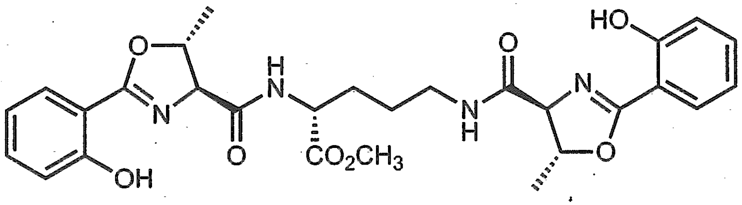


6 (^{1}H NMR, CDCl$_3$)


6 (^{13}C NMR, $CDCl_3$)



7 (^{1}H NMR, $CDCl_3$)



7 (^{13}C NMR, $CDCl_3$)