Supporting information

(7*S*)-Kaitocephalin: A Highly NMDA Receptor Selective Ligand

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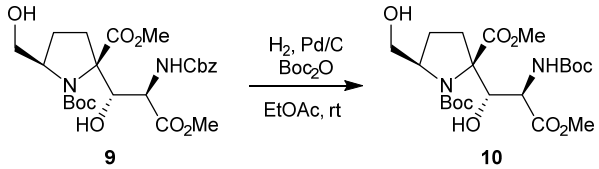
Contents:

General.

All reagents and solvents were purchased from either Aldrich Chemical Company, Inc., Kanto Kagaku Co., Inc., Merck KGaA, Inc., Nacalai Tesque Company, Ltd., Peptide Institute, Tokyo Kasei Kogyo Co., Ltd., or Wako Pure Chemical Industries, Ltd., and used without further purification unless otherwise indicated. Dichloromethane (CH_2Cl_2) was distilled from phosphorus pentaoxide (P_2O_5). Tetrahydrofuran (THF) and toluene of anhydrous grade were used. [^{3}H]CGP 39653, [^{3}H]AMPA, and [^{3}H]KA were purchased from PerkinElmer, Inc.

Optical rotations were taken on a JASCO P-1030 polarimeter with a sodium lamp (D line). Melting points were determined with a Yanaco MP-21 melting point apparatus and were uncorrected. FTIR spectra were measured on a JASCO FT/IR-6200 infrared spectrophotometer. ^{1}H NMR spectra were recorded on an either Bruker AVANCE 300 (300 MHz) or JEOL JNM-LA 400 (400 MHz) spectrometer. Chemical shifts of ^{1}H NMR were reported in parts per million (ppm, δ) relative to $CHCl_3$ (δ = 7.26) in $CDCl_3$ or HDO (δ = 4.79) in D_2O. ^{13}C NMR spectra were recorded on an either Bruker AVANCE 300 (75 MHz) or JEOL JNM-LA 400 (100 MHz) spectrometer. Chemical shifts of ^{13}C NMR were reported in ppm (δ) relative to $CHCl_3$ (δ = 77.0) in $CDCl_3$ or CD_2HOD (δ = 49.0) in D_2O. Low resolution mass spectra (LRMS) and high resolution mass spectra (HRMS) were obtained on a JEOL JMS-AX500 for fast atom bombardment ionization (FAB). All reactions were monitored by thin layer chromatography (TLC), which was performed with precoated plates (silica gel 60 F-254, 0.25 mm thickness, manufactured by Merck). TLC visualization was accompanied using UV lamp (254 nm) or a charring solution (ethanoic phosphomolybdic acid, aqueous potassium permanganate and butanoic ninhydrin). Daisogel IR-60 1002W (40/63 μm) was used for flash column chromatography on silica gel.

(2*R*,5*R*)-1-*tert*-Butyl 2-methyl 2-((1*S*,2*R*)-2-(*tert*-butoxycarbonylamino)-1-hydroxy-3-methoxy-3-oxopropyl)-5-(hydroxymethyl)pyrrolidine-1,2-dicarboxylate (10)



To a solution of **9** (315 mg, 617 μmol) in EtOAc (3.1 mL) were added Boc_2O (0.43 mL, 1.85 mmol) and 10% Pd/C (31.5 mg, 10 wt%). The mixture was stirred for 21 h under hydrogen at room temperature and filtered through a thin Celite® pad. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5:1 to 2:1) to give **10** (264 mg, 90%);

Viscous oil;

$[\alpha]_D^{25}$ = +1.4 (*c* 1.34, $CHCl_3$);

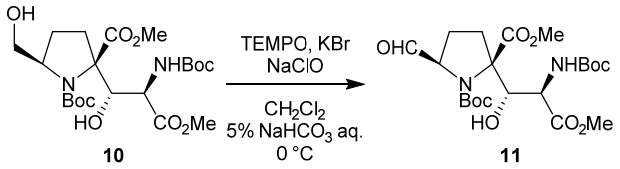
FTIR (neat) 3336, 2979, 1747, 1716, 1676, 1506, 1394, 1369, 1255, 1165 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (brd, *J* = 9.3 Hz, 1 H), 5.59 (brd, *J* = 8.1 Hz, 1 H), 4.39 (m, 1 H), 4.30 (m, 1 H), 4.09–3.98 (m, 2 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.51 (m, 1 H), 2.69 (brs, 1 H), 2.45 (m, 1 H), 2.36–2.20 (m, 2 H), 1.91 (m, 1 H), 1.51 (s, 9 H), 1.43 (s, 9 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 170.7, 156.5, 155.1, 82.4, 80.3, 77.9, 73.8, 64.5, 63.5, 54.8, 53.3, 52.3, 36.1, 28.4, 28.3, 26.3;

HRMS (FAB) calcd for $C_{21}H_{37}N_2O_{10}$ *m*/*z* 477.2448 $[M+H]^+$, found 477.2458.

(2*R*,5*R*)-1-*tert*-Butyl 2-methyl 2-((1*S*,2*R*)-2-(*tert*-butoxycarbonylamino)-1-hydroxy-3-methoxy-3-oxopropyl)-5-formylpyrrolidine-1,2-dicarboxylate (11)



To a mixture of **10** (102 mg, 214 μmol) and TEMPO (1.0 mg, 6.43 μmol) in CH_2Cl_2 (2.0 mL) and KBr in H_2O (43 μL, 21.4 μmol, 0.5 M solution) was added the mixture of aq. NaOCl solution/5% $NaHCO_3$ aq. (1:1) at 0 °C with vigorous stirring until the starting material was consumed (monitored by TLC). The organic layer was separated and the aqueous layer was extracted with EtOAc (5 mL × 2). The combined organic layers were washed with brine (10 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5:1 to 3:1) to give **11** (89.9 mg, 89%);

Colorless viscous oil;

$[\alpha]_D^{23}$ = +1.0 (*c* 2.58, $CHCl_3$);

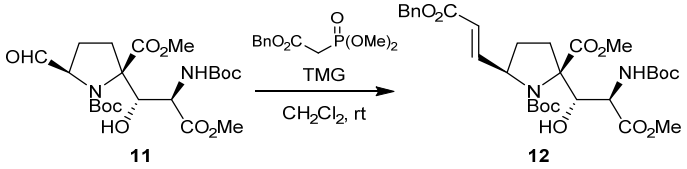
FTIR (neat) 3359, 2979, 1738, 1714, 1687, 1504, 1456, 1437, 1390, 1369, 1257, 1163 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 9.60 (d, *J* = 3.6 Hz, 1 H), 6.23 (brd, *J* = 11.1 Hz, 1 H), 5.49 (brd, *J* = 8.4 Hz, 1 H), 4.49 (brdd, *J* = 8.4, 6.0 Hz, 1 H), 4.31 (dd, *J* = 11.1, 6.0 Hz, 1 H), 4.21 (dt, *J* = 7.2, 3.6 Hz, 1 H), 3.81 (s, 3 H), 3.74 (s, 3 H), 2.40 (m, 1 H), 2.39 (m, 1 H), 2.14 (m, 1 H), 1.76 (m, 1 H), 1.49 (s, 9 H), 1.43 (s, 9 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 199.8, 171.7, 170.9, 155.7, 154.7, 83.8, 80.4, 75.0, 74.9, 68.1, 55.1, 53.2, 52.3, 34.1, 28.3, 28.1, 24.1;

HRMS (FAB) calcd for $C_{21}H_{35}N_2O_{10}$ *m*/*z* 475.2292 $[M+H]^+$, found 475.2293.

(2*R*,5*R*)-1-*tert*-Butyl 2-methyl 5-((*E*)-3-(benzyloxy)-3-oxoprop-1-enyl)-2-((1*S*,2*R*)-2-(*tert*-butoxycarbonylamino)-1-hydroxy-3-methoxy-3-oxopropyl)pyrrolidine-1,2-dicarboxylate (12)



To a solution of benzyl dimethylphosphonoacetate (300 mg, 1.16 mmol) in CH_2Cl_2 (2.0 mL) was added TMG (146 mL, 1.16 mmol) at 0 °C under argon and stirred for 10 min. To the mixture was added a solution of **11** (184 mg, 388 μmol) in CH_2Cl_2 (1.9 mL) at 0 °C. The mixture was stirred for 23 h at room temperature and quenched with sat. NH_4Cl (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (5 mL × 2). The combined organic layers were washed with brine (10 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 3:1) to give **12** (209 mg, 89%);

Colorless oil;

$[\alpha]_D^{25} = -16.8$ (*c* 3.4, $CHCl_3$);

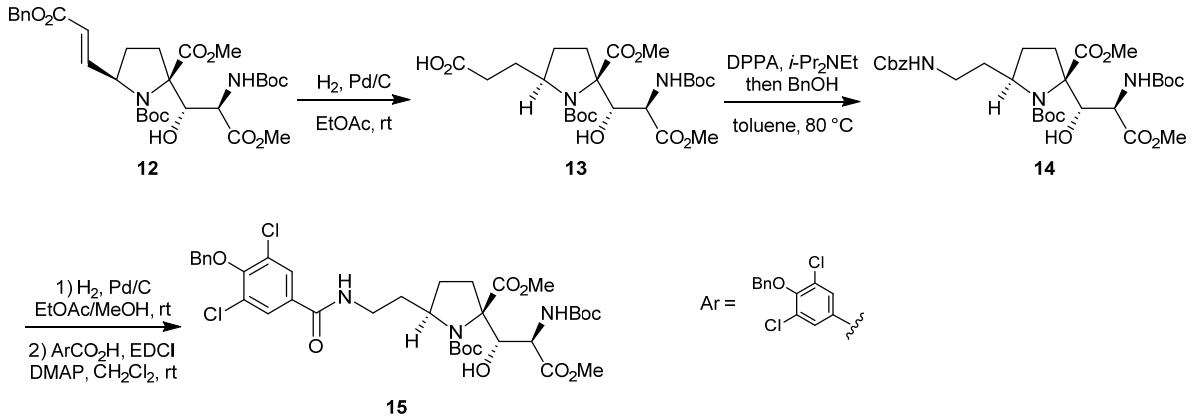
FTIR (neat) 3342, 2979, 1747, 1716, 1678, 1498, 1456, 1389, 1369, 1257, 1163 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.33 (m, 5 H), 6.97 (dd, *J* = 15.8, 7.7 Hz, 1 H), 6.26 (brs, 1 H), 6.00 (dd, *J* = 15.8, 0.8 Hz, 1 H), 5.57 (brd, *J* = 8.4 Hz, 1 H), 5.23 (d, *J* = 12.5 Hz, 1 H), 5.18 (d, *J* = 12.5 Hz, 1 H), 4.51–4.41 (m, 2 H), 4.30 (dd, *J* = 10.2, 4.5 Hz, 1 H), 3.78 (s, 3 H), 3.72 (s, 3 H), 2.36–2.27 (m, 3 H), 1.68 (m, 1 H), 1.43 (s, 9 H), 1.42 (s, 9 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.7, 166.0, 156.2, 155.0, 148.3, 136.0, 128.5, 128.2, 128.1, 121.0, 82.5, 80.2, 77.2, 73.5, 66.1, 62.2, 55.2, 52.9, 52.2, 34.0, 28.9, 28.2, 28.1;

HRMS (FAB) calcd for $C_{30}H_{43}N_2O_{11}$ *m/z* 607.2867 $[M+H]^+$, found 607.2867.

(2*R*,5*R*)-1-*tert*-Butyl 2-methyl 5-(2-(4-(benzyloxy)-3,5-dichlorobenzamido)ethyl)-2-((1*S*,2*R*)-2-(*tert*-butoxycarbonylamino)-1-hydroxy-3-methoxy-3-oxopropyl)pyrrolidine-1,2-dicarboxylate (15)



To a solution of **12** (90.5 mg, 149 μmol) in EtOAc (1.5 mL) was added 10% Pd/C (9.1 mg, 10 wt%). The mixture was stirred for 24 h under hydrogen at room temperature and filtered through a thin

Celite® pad. The filtrate was concentrated under reduced pressure to give corresponding carboxylic acid **13** and the product was used without further purification. To a solution of the crude **13** in toluene (1.5 mL) were added *i*-Pr_2NEt (25.6 mL, 149 μmol) and DPPA (37.2 mL, 164 μmol, 95% purity) under argon. The mixture was stirred for 30 min at room temperature, then for 30 min at 80 °C. To the mixture was added BnOH (17.0 μL, 164 μmol) and additionary stirred for 16 h at 80 °C. After cooling to room temperature, the mixture was quenched with sat. NH_4Cl (5 mL) and extracted with EtOAc (5 mL × 3). The combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5:1 to 2:1) to give crude **14** (67 mg, R_f value = 0.3, hexane/AcOEt = 1:1). The crude **14** contained a byproduct that showed the same R_f value with **14**. Since these were inseparable by further silica gel column chromatography, the mixture was subjected to the next step without further purification. To a solution of the crude **14** (67.0 mg) in EtOAc/MeOH (1:1, 2.0 mL) was added 10% Pd/C (6.7 mg, 10 wt%). The mixture was stirred for 14 h under hydrogen at room temperature and filtered. The filtrate was concentrated under reduced pressure to give corresponding amine and the product was used without further purification. To a solution of the crude amine in CH_2Cl_2 (1.0 mL) were added 3,5-dichloro-4-benzyloxybenzoic acid (31.8 mg, 107 μmol), EDCI (20.5 mg, 107 μmol) and DMAP (13.1 mg, 107 μmol) under argon. The mixture was stirred for 5 h at room temperature and quenched with sat. NH_4Cl (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (5 mL × 2). The combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by PLC (silica gel 60 F-254, 0.5 mm thickness, manufactured by Merck)(hexane/EtOAc = 1:1) to give **15** (43.9 mg, 38% over 4 steps) as a 1:1 mixture of rotamers;

Colorless amorphous solid;

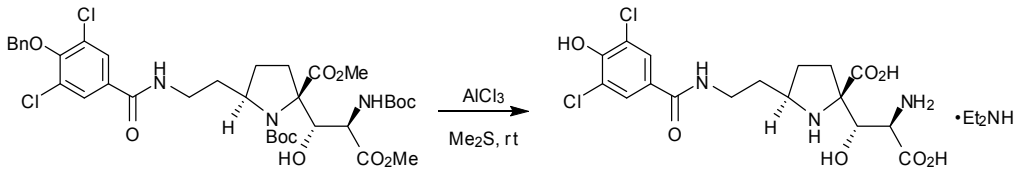
$[\alpha]_D^{24} = -27.3$ (*c* 1.3, $CHCl_3$);

FTIR (neat) 3336, 2978, 1745, 1716, 1668, 1550, 1456, 1392, 1369, 1259, 1167 cm^{-1};

The NMR signals of **15** are broaden and complicated because of observation of rotamers. Therefore, NMR data assignments are not given for **15**. The actual spectra are shown in P23–24.

HRMS (FAB) calcd for $C_{36}H_{48}Cl_2N_3O_{11}$ *m/z* 768.2666 $[M+H]^+$, found 768.2653.

(2*R*,5*R*)-2-((1*S*,2*R*)-2-Amino-2-carboxy-1-hydroxyethyl)-5-(2-(3,5-dichloro-4-hydroxybenzamido)ethyl) pyrrolidine-2-carboxylic acid (5)



15 **5**

To a solution of **15** (15.8 mg, 20.6 μmol) in Me_2S (1.0 mL) was added $AlCl_3$ (54.9 mg, 412 μmol) at 0 °C under argon. The mixture was stirred for 15 h at room temperature and quenched with water (1 mL) at 0 °C. The mixture was stirred for 1 h at room temperature and concentrated under reduced pressure. The residue was purified by Dowex® 50WX4 (elution with 1*N* NH_4OH) and reversed-phase column chromatography (elution with water) to give roughly purified product. The product was purified by HPLC (COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ20 × 250 mm, elution with 10% MeOH/20 mM Et_2NH-CO_2 buffer pH 7, 6.0 mL/min) to give **5** (7.4 mg, 69%) as a diethylamine salt;

White solid;

$[\alpha]_D^{25}$ = –24.7 (*c* 0.11, H_2O);

FTIR (neat) 3057, 1628, 1468, 1298, 1095 cm^{-1};

^1H NMR (300 MHz, D_2O) δ 7.68 (s, 2 H), 4.51 (s, 1 H), 4.24 (s, 1 H), 3.73 (m, 1 H), 3.49 (m, 2 H), 3.06 (t, *J* = 7.3 Hz, 4 H, Et_2NH), 2.41 (m, 1 H), 2.26–1.99 (m, 4 H), 1.70 (m, 1 H), 1.27 (t, *J* = 7.3 Hz, 6 H, Et_2NH);

^{13}C NMR (75 MHz, D_2O) δ 174.4, 170.9, 169.2, 162.2, 127.4, 124.2, 117.7, 76.5, 70.8, 59.1, 55.6, 42.4 (Et_2NH), 37.0, 32.1, 31.9, 29.4, 10.6 (Et_2NH);

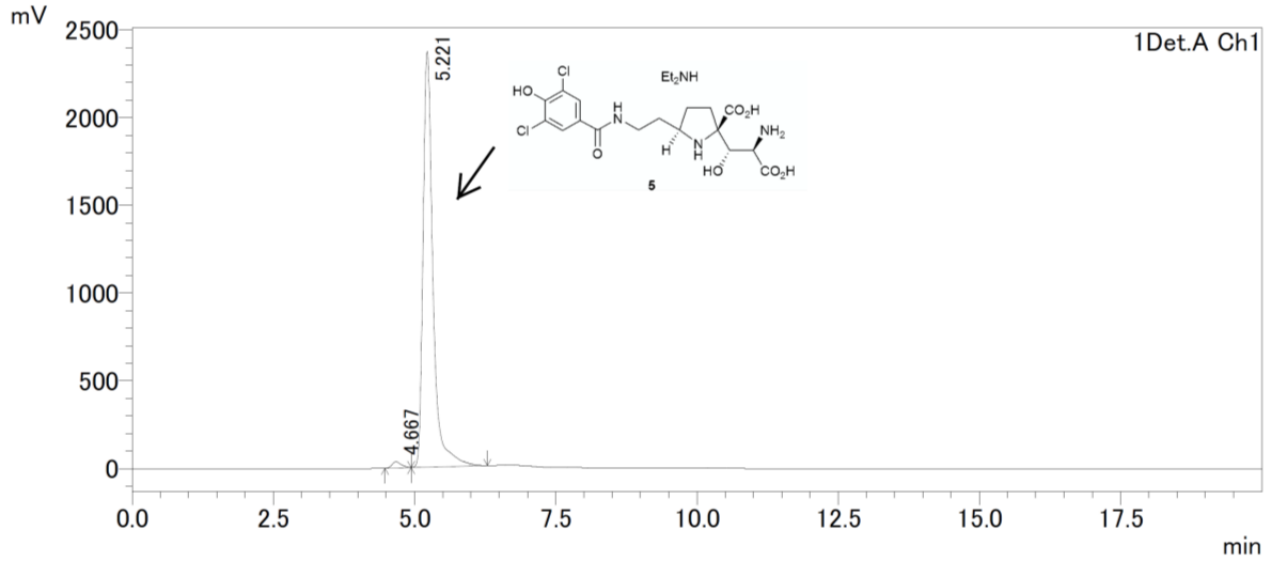
HRMS (FAB) calcd for $C_{17}H_{22}Cl_2N_3O_7$ *m*/*z* 450.0835 $[M+H]^+$, found 450.0840.

HPLC data of **5** after purification:

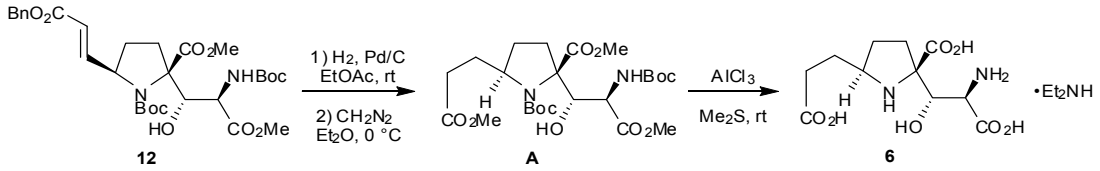
column: COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ4.6 × 250 mm

elution: 10% MeOH/20 mM Et_2NH-CO_2 buffer pH 7

flow rate: 1 mL/min, detect: 300 nm



(2*R*,5*R*)-2-((1*S*,2*R*)-2-Amino-2-carboxy-1-hydroxyethyl)-5-(2-carboxyethyl)pyrrolidine-2-carboxylic acid (6)



To a solution of **12** (38.2 mg, 62.9 μmol) in EtOAc (1.0 mL) was added 10% Pd/C (3.8 mg, 10 wt%). The mixture was stirred for 24 h under hydrogen at room temperature and filtered through a thin Celite® pad. The filtrate was concentrated under reduced pressure to give the crude carboxylic acid and the product was used without further purification. To a solution of the crude in Et_2O (1.0 mL) was added excess amount of the solution of CH_2N_2 in Et_2O at 0 °C with stirring until the starting material was consumed (monitored by TLC). The mixture was warm to 40 °C to remove residual CH_2N_2 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5:1 to 3:1) to give **A** (29.5 mg, 88%). The data of **A** are as follows;

Colorless oil;

$[\alpha]_D^{27}$ = –22.7 (*c* 1.45, $CHCl_3$);

FTIR (neat) 3317, 2978, 1743, 1714, 1672, 1506, 1436, 1392, 1369, 1255, 1163 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 6.47 (brs, 1 H), 5.53 (brd, *J* = 5.4 Hz, 1 H), 4.38 (m, 1 H), 4.26 (dd, *J* = 10.4, 4.4 Hz, 1 H), 3.87 (m, 1 H), 3.78 (s, 3 H), 3.72 (s, 3 H), 3.67 (s, 3 H), 2.34–2.13 (m, 6 H), 1.65–1.55 (m, 2 H), 1.51 (s, 9 H), 1.43 (s, 9 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 172.5, 170.8, 156.4, 155.2, 82.1, 80.3, 77.3, 72.7, 61.6, 55.1, 52.9, 52.3, 51.7, 34.5, 31.8, 28.43, 28.36, 28.0, 26.8;

HRMS (FAB) calcd for $C_{24}H_{41}N_2O_{11}$ *m*/*z* 533.2710 $[M+H]^+$, found 533.2711.

To a solution of methyl ester **A** (15.7 mg, 29.5 μmol) in Me_2S (1.0 mL) was added $AlCl_3$ (78.7 mg, 590 μmol) at 0 °C under argon. The mixture was stirred for 20 h at room temperature, quenched with water (1 mL) at 0 °C. The mixture was stirred for 1 h and concentrated under reduced pressure. The residue was purified by Dowex® 50WX4 (elution with 1*N* NH_4OH) and reversed-phase column chromatography (elution with water) to give roughly purified product. The product was purified by HPLC (COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ20 × 250 mm, elution with 0.5% MeOH/20 mM Et_2NH-CO_2 buffer pH 7, 6.0 mL/min) to give **6** (5.0 mg, 47%) as a diethylamine salt;

White solid;

$[\alpha]_D^{25}$ = –35.7 (*c* 0.41, H_2O);

FTIR (neat) 2989, 1631, 1552, 1452, 1358, 1308, 1090, 1065 cm^{-1};

^1H NMR (300 MHz, D_2O) δ 4.50 (s, 1 H), 4.26 (s, 1 H), 3.65 (m, 1 H), 3.06 (t, *J* = 7.3 Hz, 4 H, Et_2NH), 2.43–2.30 (m, 3 H), 2.28–2.05 (m, 3 H), 1.96 (m, 1 H), 1.64 (m, 1 H), 1.26 (t, *J* = 7.3 Hz, 6 H, Et_2NH);

^{13}C NMR (75 MHz, D_2O) δ 181.4, 174.5, 171.4, 76.6, 71.0, 61.3, 55.7, 42.4 (Et_2NH), 34.4, 32.2, 29.3, 28.9, 10.7 (Et_2NH);

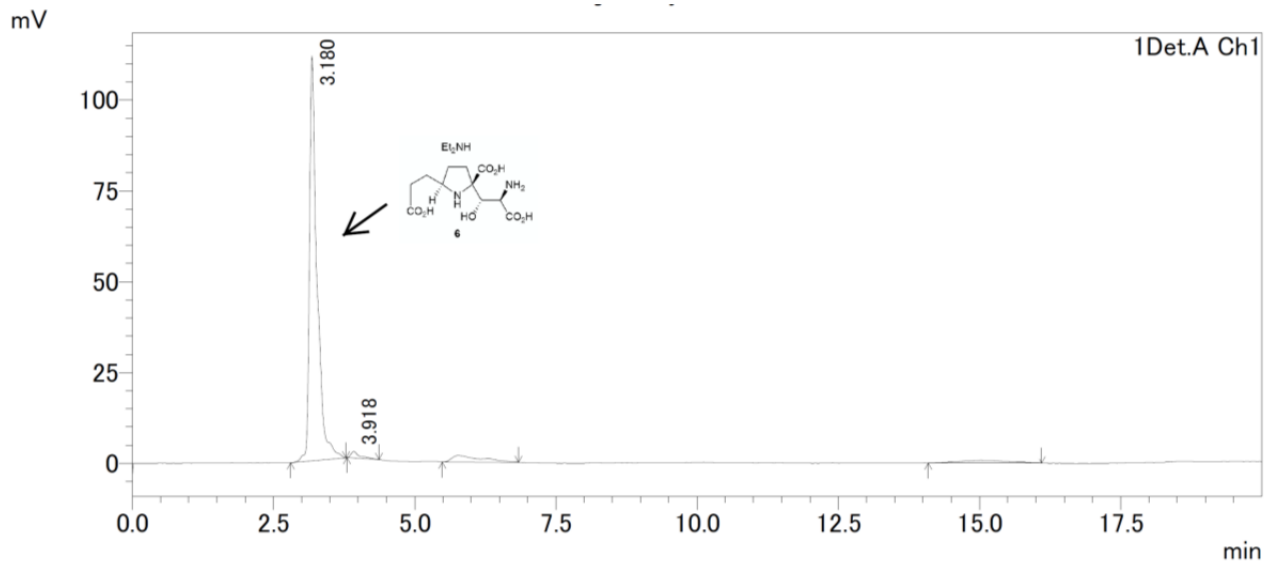
HRMS (FAB) calcd for $C_{11}H_{17}N_2O_7$ *m*/*z* 289.1036 $[M–H]^-$, found 289.1052.

HPLC data of **6** after purification:

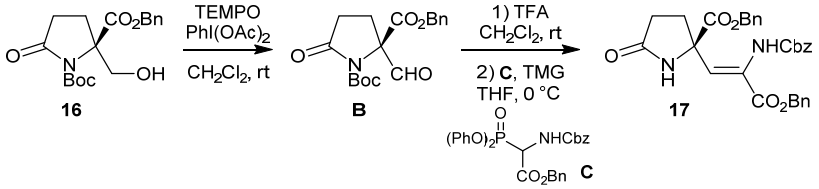
column: COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ4.6 × 250 mm

elution: 0.5% MeOH/20 mM Et_2NH-CO_2 buffer pH 7

flow rate: 1 mL/min, detect: 210 nm



Synthesis of 17 from 16



To a solution of **16**[1] (972 mg, 2.78 mmol) in CH_2Cl_2 (5.6 mL) were added TEMPO (43.4 mg, 0.278 mmol) and $PhI(OAc)_2$ (1.08 g, 3.34 mmol) at 0 °C. The mixture was stirred for 19 h at room temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5:1 to 2:1) to give **B** (896 mg, 94%). The data of **B** are as follows;

Colorless oil;

$[\alpha]_D^{25}$ = +23.1 (*c* 1.4, $CHCl_3$);

FTIR (neat) 3469, 2979, 2931, 2354, 1795, 1731, 1455, 1371, 1288, 1259, 1151, 1072, 1025 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ 10.2 (s, 1 H), 7.36 (m, 5 H), 2.58–2.53 (m, 2 H), 2.26 (dt, *J* = 13.5, 8.1 Hz, 1 H), 2.13 (m, 1 H), 1.38 (s, 9 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 193.4, 171.9, 168.6, 148.2, 134.1, 128.5, 128.4, 128.3, 84.3, 71.8, 67.7, 29.9, 27.2, 24.5;

HRMS (FAB) calcd for $C_{18}H_{22}NO_6$ *m/z* 348.1447 $[M+H]^+$, found 348.1447.

To a solution of **B** (152 mg, 442 μmol) in CH_2Cl_2 (4.4 mL) was added TFA (0.34 mL, 4.42 mmol) at 0 °C. The mixture was stirred for 1 h under argon at room temperature and concentrated under reduced pressure. The residual TFA was removed by azeotropic distillation with toluene (3 mL × 3). The resulting aldehyde was subjected to the next olefination without further purification. To a solution of **C** (707 mg, 1.33 mmol) (Synthetic procedure of **C** was described in the next column) in THF (4.4 mL) was added TMG (180 μL, 1.46 mmol) at 0 °C. The mixture was stirred for 15 min under argon, then the crude aldehyde in THF (2.2 mL) was added to the mixture. The mixture was stirred for 30 min at 0 °C, quenched by sat. NH_4Cl (10 mL), and extracted with EtOAc (10 mL × 3). The combined organic layers were washed brine (30 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 7:1 to 1:2) to give **17** (142 mg, 61%);

Colorless amorphous solid;

$[\alpha]_D^{29}$ = –35.0 (*c* 1.0, $CHCl_3$);

FTIR (neat) 3262, 3032, 1718, 1498, 1455, 1379, 1215, 1182, 1163, 1127, 1064 cm^{-1};

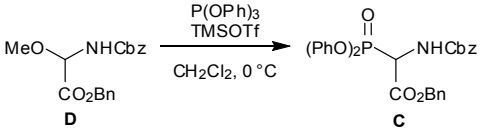
^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 15 H), 6.92 (brs, 1 H), 6.65 (brs, 1 H), 6.57 (s, 1 H), 5.18–5.05 (m, 6 H), 2.54 (m, 1 H), 2.39–2.28 (m, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 171.4, 163.8, 154.5, 135.4, 135.0, 134.8, 131.7, 128.63, 128.60,

128.57, 128.5, 128.40, 128.36, 128.30, 128.28, 67.9, 64.2, 33.4, 29.1;

HRMS (FAB) calcd for $C_{30}H_{29}N_2O_7$ *m*/*z* 529.1975 $[M+H]^+$, found 529.1972.

Benzyl 2-(benzyloxycarbonylamino)-2-(diphenoxyphosphoryl)acetate (C)



To a solution of **D**[2] (1.90 g, 5.75 mmol) in CH_2Cl_2 (12 mL) was added triphenyl phosphite (2.3 mL, 8.63 mmol) and TMSOTf (1.2 mL, 6.90 mmol) at 0 °C under argon. The mixture was stirred for 3 h, and quenched with sat. $NaHCO_3$ (15 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (15 mL × 2). The combined organic layers were washed with brine (45 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 10:1 to 4:1), and recrystallized from EtOAc/hexane to give **C** (2.23 g, 73%);

White solid;

mp 102–105 °C;

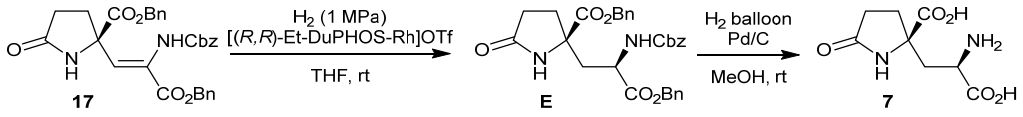
FTIR (neat) 3295, 3064, 2952, 1723, 1591, 1490, 1456, 1379, 1286, 1207, 1185, 1163, 1049, 1027, 1006 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ 7.34–7.23 (m, 14 H), 7.18–7.14 (m, 2 H), 7.07 (d, *J* = 7.8 Hz, 4 H), 5.76 (brd, *J* = 9.5 Hz, 1 H), 5.32 (dd, *J* = 23.2, 9.5 Hz, 1 H), 5.27 (d, *J* = 12.2 Hz, 1 H), 5.23 (d, *J* = 12.2 Hz, 1 H), 5.15 (d, *J* = 12.1 Hz, 1 H), 5.11 (d, *J* = 12.1 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.9, 155.53, 155.46, 150.0, 149.9, 135.7, 134.5, 129.8, 128.6, 128.53, 128.52, 128.3, 128.2, 125.6, 120.39, 120.35, 120.29, 120.25, 68.5, 67.8, 53.8, 52.3;

HRMS (FAB) calcd for $C_{29}H_{27}NO_7P$ *m*/*z* 532.1525 $[M+H]^+$, found 532.1526.

(*R*)-2-((*R*)-2-Amino-2-carboxyethyl)-5-oxopyrrolidine-2-carboxylic acid (7)



In a glove box, [Rh(I)(COD)-(*R*,*R*)-Et-DuPHOS]OTf (28.4 mg, 39.3 μmol) and **17** (208 mg, 394 μmol) was dissolved in THF (2.6 mL). The mixture was placed in a high-pressure hydrogen tube under argon. The tube was sealed cooled to −78 °C. The tube was vacuumed and then hydrogen was introduced into the tube. After repeating of the gas-exchange process for 3 times, the mixture was stirred for 72 h at room temperature under 1.0 MPa of hydrogen atmosphere. The hydrogenation was carefully leaked from the

tube, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 3:1 to 1:2) to give **E** (161 mg, 77%, dr = >25:1). The data of **E** are as follows;

Colorless amorphous solid;

$[\alpha]_D^{21}$ = –21.2 (*c* 1.11, $CHCl_3$);

FTIR (neat) 3355, 3018, 1721, 1498, 1456, 1378, 1340, 1261, 1215, 1167, 1081, 1048, 1028 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.29 (m, 15 H), 6.33 (brs, 1 H), 5.43 (brs, 1 H), 5.17–5.04 (m, 6 H), 4.48 (brs, 1 H), 2.61 (brd, *J* = 11.0 Hz, 1 H), 2.40–2.30 (m, 3 H), 2.14–2.04 (m, 2 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 177.1, 172.7, 171.5, 156.1, 136.1, 135.0, 128.8, 128.7, 128.6, 128.5, 128.4, 67.9, 67.4, 64.1, 51.5, 40.8, 32.1, 29.2;

HRMS (FAB) calcd for $C_{30}H_{31}N_2O_7$ *m*/*z* 531.2131 $[M+H]^+$, found 531.2156.

To a solution of **E** (139 mg, 0.262 mmol, dr = >25:1) in MeOH (2.6 mL) was added 10% Pd/C (13.9 mg, 10 wt%). The mixture was stirred under hydrogen for 3.5 h at room temperature and filtration through a thin Celite® pad. The filtrate was concentrated under reduced pressure. The residue was purified by Dowex® 50WX4 (elution with 1*N* NH_4OH) to give **7** (51.5 mg, 84%, dr = >25:1) as an ammonium salt;

Brown solid;

mp 170–173 °C;

$[\alpha]_D^{29}$ = –3.6 (*c* 1.21, H_2O);

FTIR (H_2O) 2927, 1655, 1597, 1458, 1396 cm^{-1};

^1H NMR (300MHz, D_2O) δ 3.77 (dd, *J* = 8.9, 3.5 Hz, 1 H), 2.48–2.42 (m, 2 H), 2.40–2.16 (m, 4 H);

^{13}C NMR (100 MHz, D_2O) δ 181.5, 181.0, 175.2, 67.4, 52.7, 39.9, 31.6, 30.7;

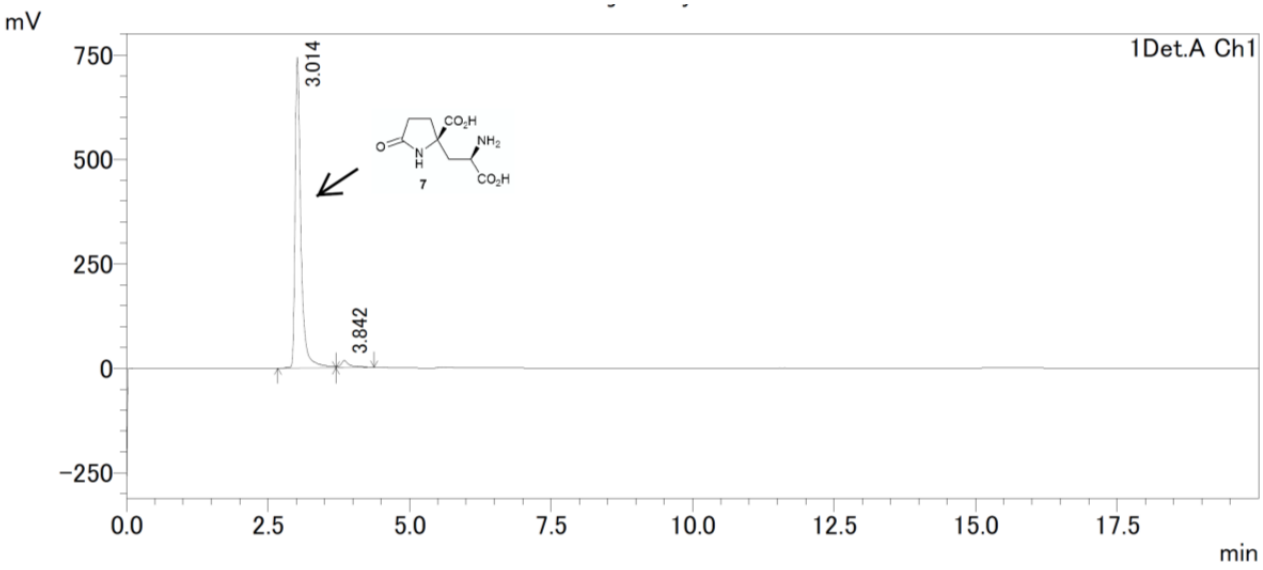
HRMS (FAB) calcd for $C_8H_{13}N_2O_5$ *m*/*z* 217.0824 $[M+H]^+$, found 217.0816.

HPLC data of **7** after purification:

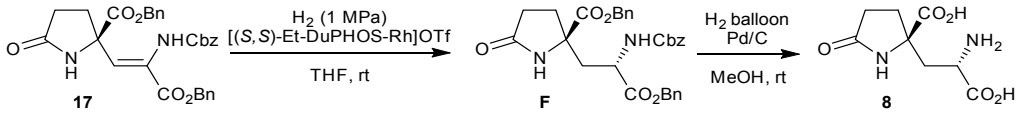
column: COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ4.6 × 250 mm

elution: 0.5% MeOH/20 mM Et_2NH-CO_2 buffer pH 7

flow rate: 1 mL/min, detect: 210 nm



(*R*)-2-((*S*)-2-Amino-2-carboxyethyl)-5-oxopyrrolidine-2-carboxylic acid (8)



According to the experimental procedure of **7** from **17**, A mixture of **17** (211 mg, 399 μmol) and [Rh(I)(COD)-(*S*,*S*)-Et-DuPHOS]OTf (29 mg, 40.0 μmol) in THF (2.7 mL) was placed in a hydrogenation tube and pressurized to 1.0 MPa with hydrogen at room temperature for 72 h. The mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 3:1 to 1:2), followed by preparative TLC (hexane/EtOAc = 1:2) to give **F** (168 mg, 79%, dr = 25:1). The data of **F** are as follows;

Colorless amorphous solid;

$[\alpha]_D^{21}$ = –3.4 (*c* 1.22, $CHCl_3$);

FTIR (neat) 3320, 3033, 2952, 1737, 1700, 1518, 1499, 1456, 1378, 1218, 1178, 1072 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (m, 15 H), 6.98 (s, 1 H), 5.19–4.89 (m, 7 H), 4.59 (m, 1 H), 2.53–2.06 (m, 6 H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 173.6, 171.2, 156.4, 135.8, 135.1, 134.9, 128.8, 128.7, 128.63, 128.55, 128.51, 128.47, 128.1, 67.9, 67.8, 67.5, 63.4, 51.0, 41.3, 33.0, 29.2;

HRMS (FAB) calcd for $C_{30}H_{31}N_2O_7$ *m*/*z* 531.2131 $[M+H]^+$, found 531.2126.

To a solution of **F** (142 mg, 0.268 mmol, dr = 25:1) in MeOH (2.7 mL) was added 10% Pd/C (14.2 mg, 10 wt%), the mixture was stirred under hydrogen for 3 h at room temperature and filtration through a thin Celite® pad. The filtrate was concentrated under reduced pressure. The residue was purified by Dowex® 50WX4 (elution with 1*N* NH_4OH) to give **8** (40.7 mg, 65%, dr = 25:1) as an ammonium salt;

Brown solid;

mp 171–176 °C;

$[\alpha]_D^{21}$ = +10.4 (*c* 1.04, H_2O);

FTIR (H_2O) 3031, 1652, 1591, 1459, 1444, 1395 cm^{-1};

^1H NMR (300MHz, D_2O) δ 3.70 (brt, *J* = 6.2 Hz, 1 H), 2.49–2.31 (m, 5 H), 2.15 (m, 1 H);

^{13}C NMR (75 MHz, D_2O) δ 181.9, 180.7, 175.3, 67.8, 53.1, 40.7, 33.7, 30.5;

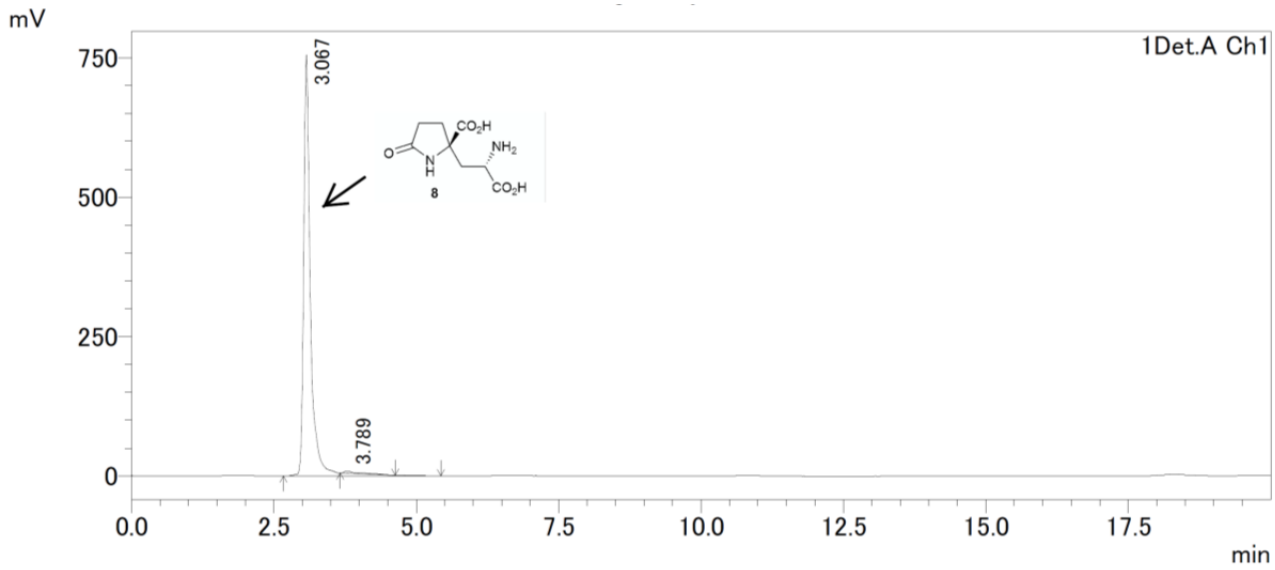
HRMS (FAB) calcd for $C_8H_{11}N_2O_5$ *m*/*z* 215.0668 $[M–H]^-$, found 215.0663.

HPLC data of **8** after purification:

column: COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ4.6 × 250 mm

elution: 0.5% MeOH/20 mM Et_2NH-CO_2 buffer pH 7

flow rate: 1 mL/min, detect: 210 nm

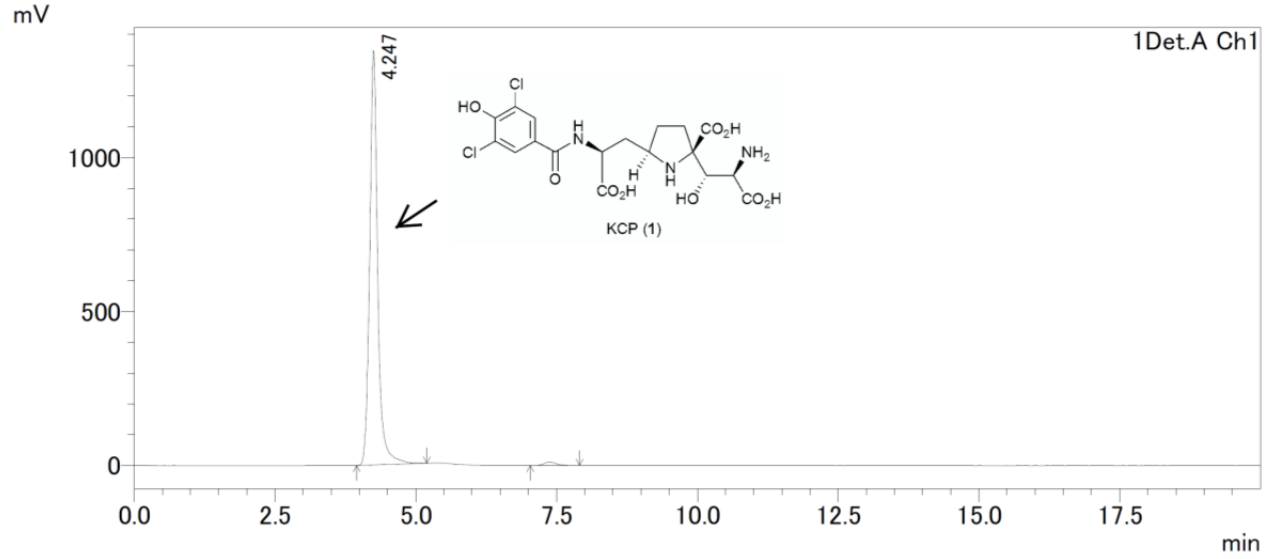


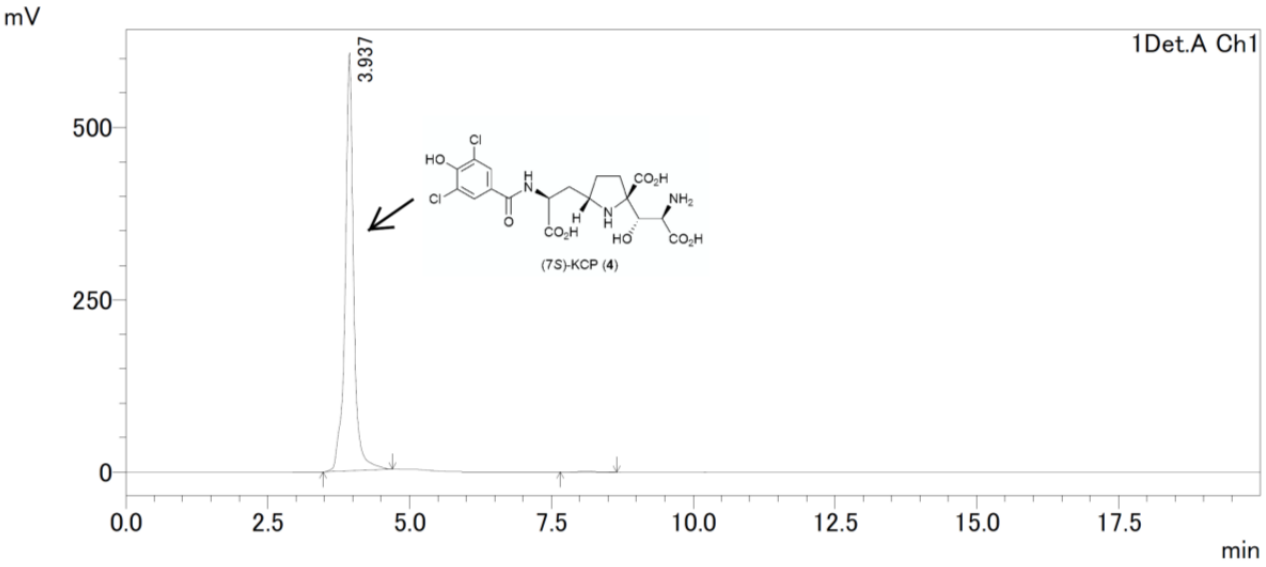
HPLC data of KCP (1) and (7*S*)-KCP (4)

column: COSMOSIL® $5C_{18}$-PAQ Packed Column, ϕ4.6 × 250 mm

elution: 5% MeOH/20 mM Et_2NH-CO_2 buffer pH 7

flow rate: 1 mL/min, detect: 300 nm





Receptor binding assay.

Rat brain synaptic membranes were prepared[3] and modified[4] as described previously and stored at −78 °C until use. On the day of the assay, the membrane suspension was incubated in a buffer containing 0.04% Triton X-100 at 37 °C for 15 min. Triton X-100 was removed by centrifugation, and the pallet was washed three times with an assay buffer. Binding assays were performed according to published methods.[5] Incubation conditions were as follows (ligand, ligand concentration, temperature, time, buffers);
for NMDA receptors: [^{3}H]CGP 39653, 2 nM, 4 °C, 1 h, 50 mM Tris-HCl buffer pH 7.6;
for AMPA receptors: [^{3}H]AMPA, 5 nM, 4 °C, 1 h, 50 mM Tris-HCl buffer pH 7.6 containing 100 mM KSCN;
for KA receptors: [^{3}H]KA, 1 nM, 4 °C, 1 h, 50 mM Tris-HCl buffer pH 7.6.

Calculation of K_i values.

K_i values were calculated from the equation $K_i = IC_{50}/(1 + [^3\text{H-labelled ligand}]/K_d)$. The K_d values were used 19 nM for [^{3}H]CGP 39653, 10 nM for [^{3}H]AMPA, and 3.8 nM for [^{3}H]KA. Each IC_{50} value for **1** or **4**–**8** was determined based on the concentration-inhibition curve (Fig. 4 and Fig. S1) using GraphPad Prism 3.03. K_i values are indicated as the mean ± SE for three determinations.

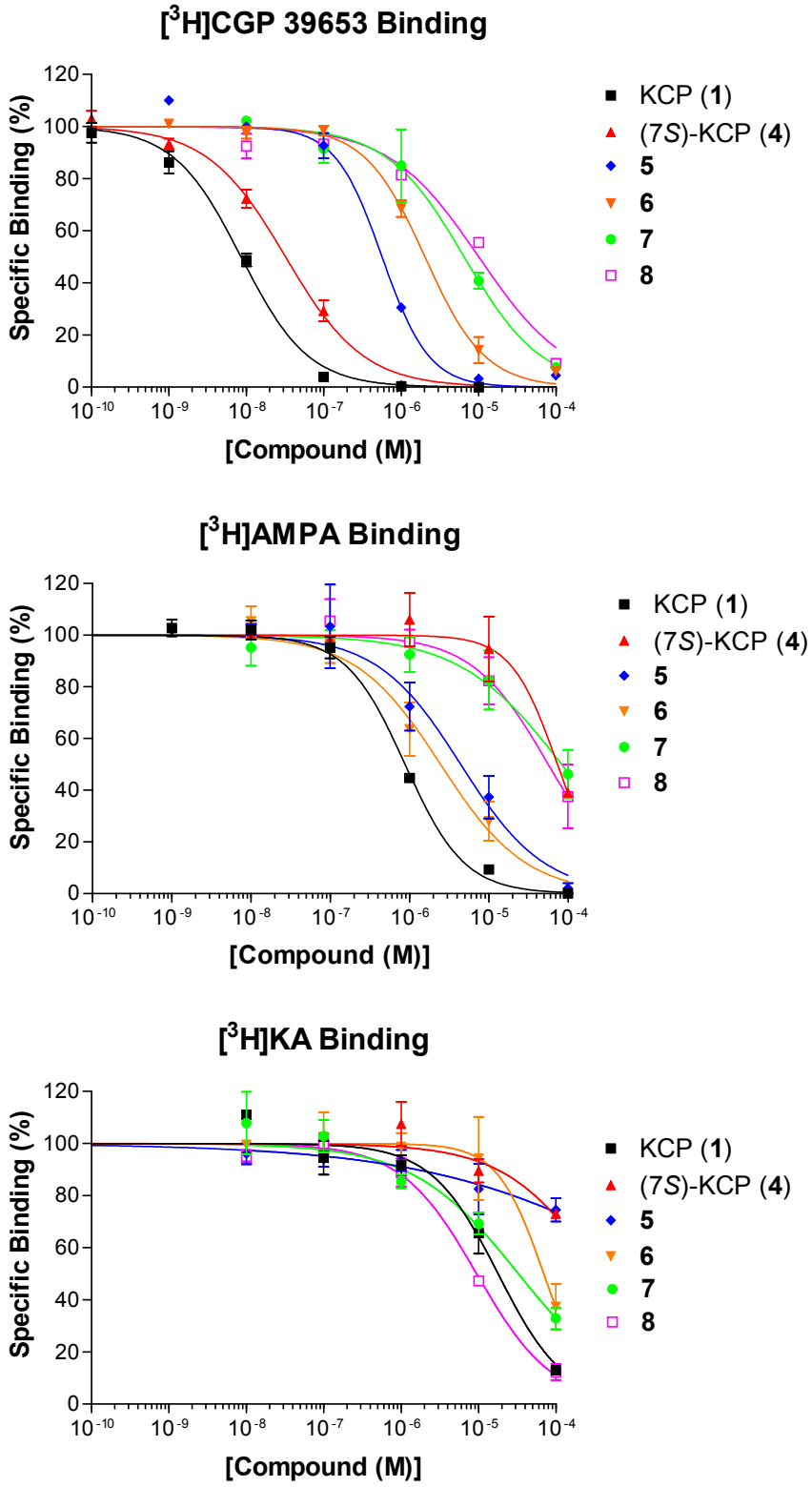


Fig. S1 Displacement of the specific ^{3}H-labelled ligand binding (NMDA, AMPA, and KA receptors) to rat synaptic membranes by increasing concentrations of **1** and **4**–**8**. Each point is the mean of triplicate determinations.

References

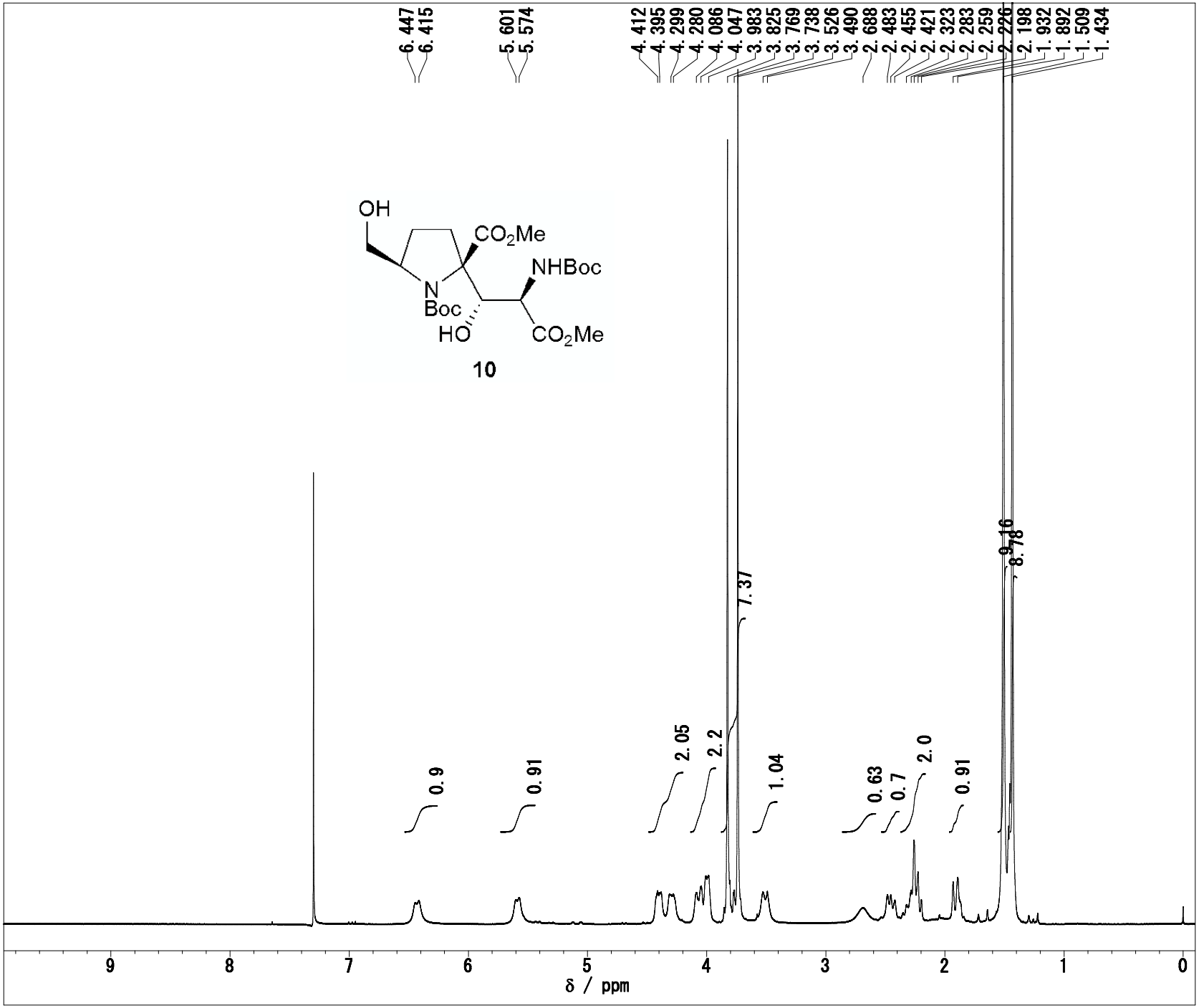
1. T. Shinada, H. Yoshida, Y. Ohfune, *Synthesis* **2009**, 3751.

2. Y. Harayama, M. Yoshida, D. Kamimura, Y. Wada, Y. Kita, *Chem. Eur. J*. **2006**, *12*, 4893.

3. S. J. Enna, S. H. Snyder, *Mol. Pharmacol*. **1977**, *13*, 422.

4. D. E. Murphy, A. J. Hutchison, S. D. Hurt, M. Williams, M. A. Sills, *Br. J. Pharmacol*. **1988**, *95*, 932.

5. (a) M. Kawai, Y. Horikawa, T. Ishihara, K.Shimamoto, Y. Ohfune, *Eur. J. Pharmacol*. **1992**, *211*, 195. (b) D. E. Murphy, E. W. Snowhill, M. Williams, *Neurochem. Res*. **1987**, *12*, 775. (c) E. D. London, J. T. Coyle, *Mol. Pharmacol*. **1979**, *15*, 492.



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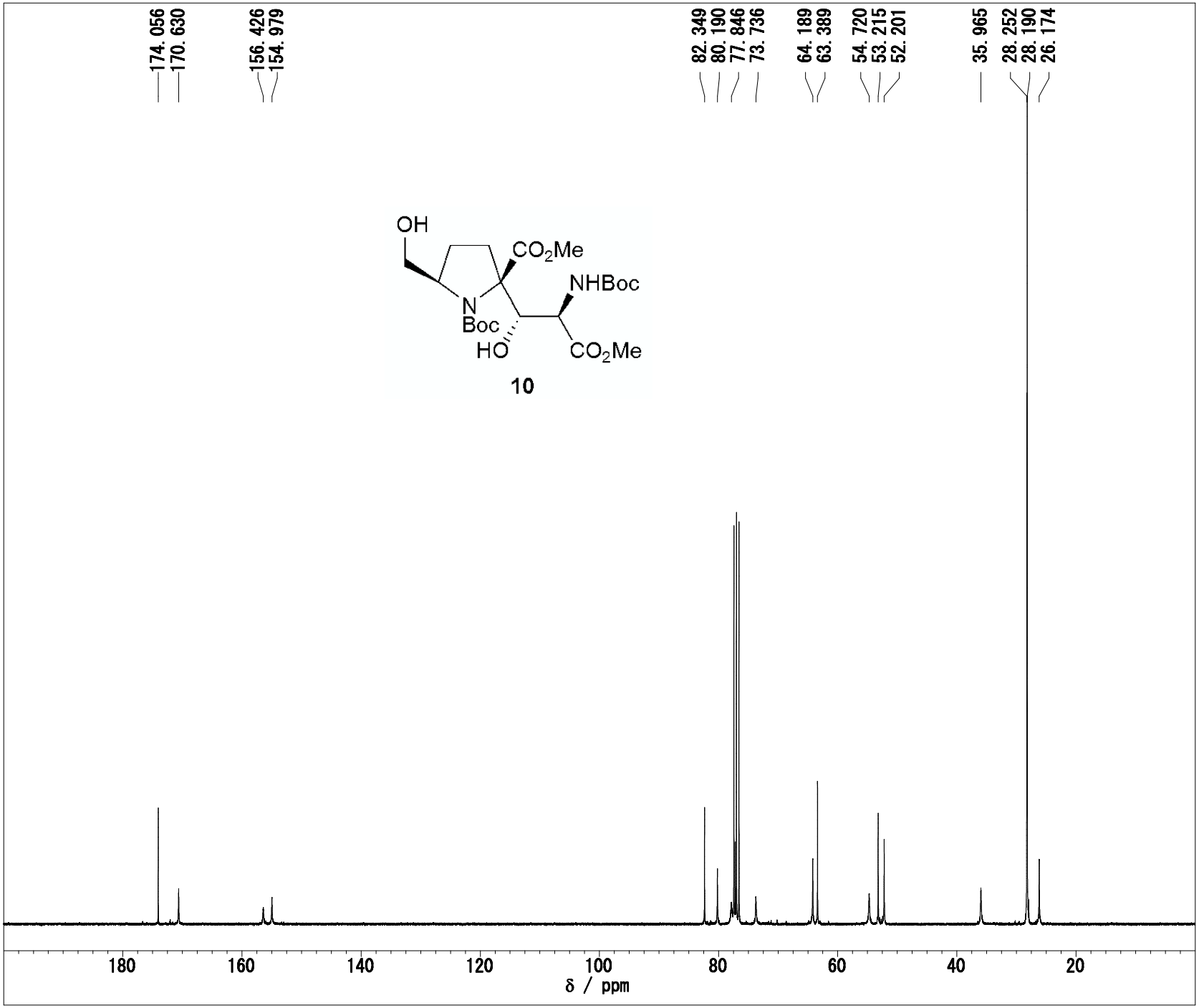

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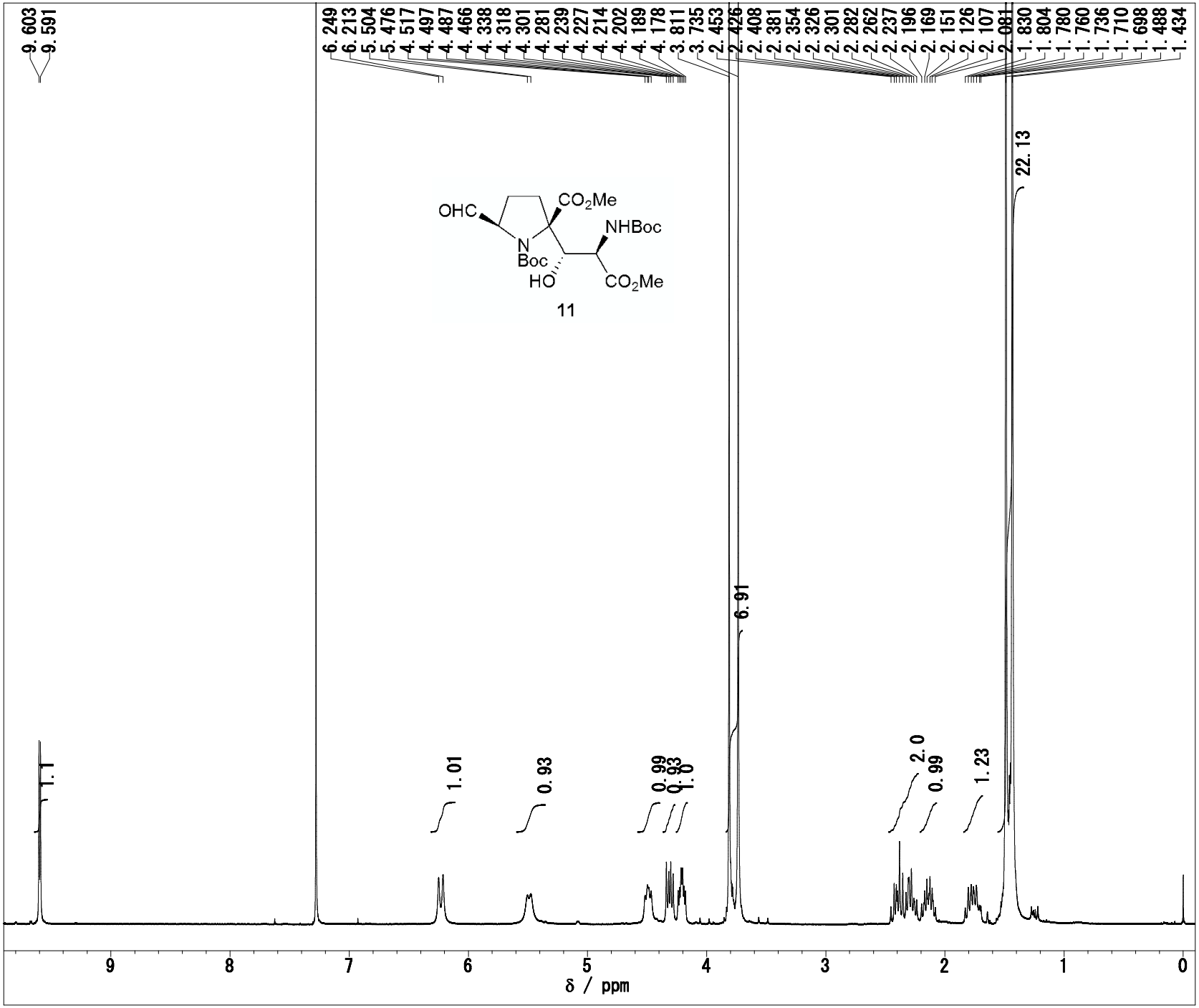

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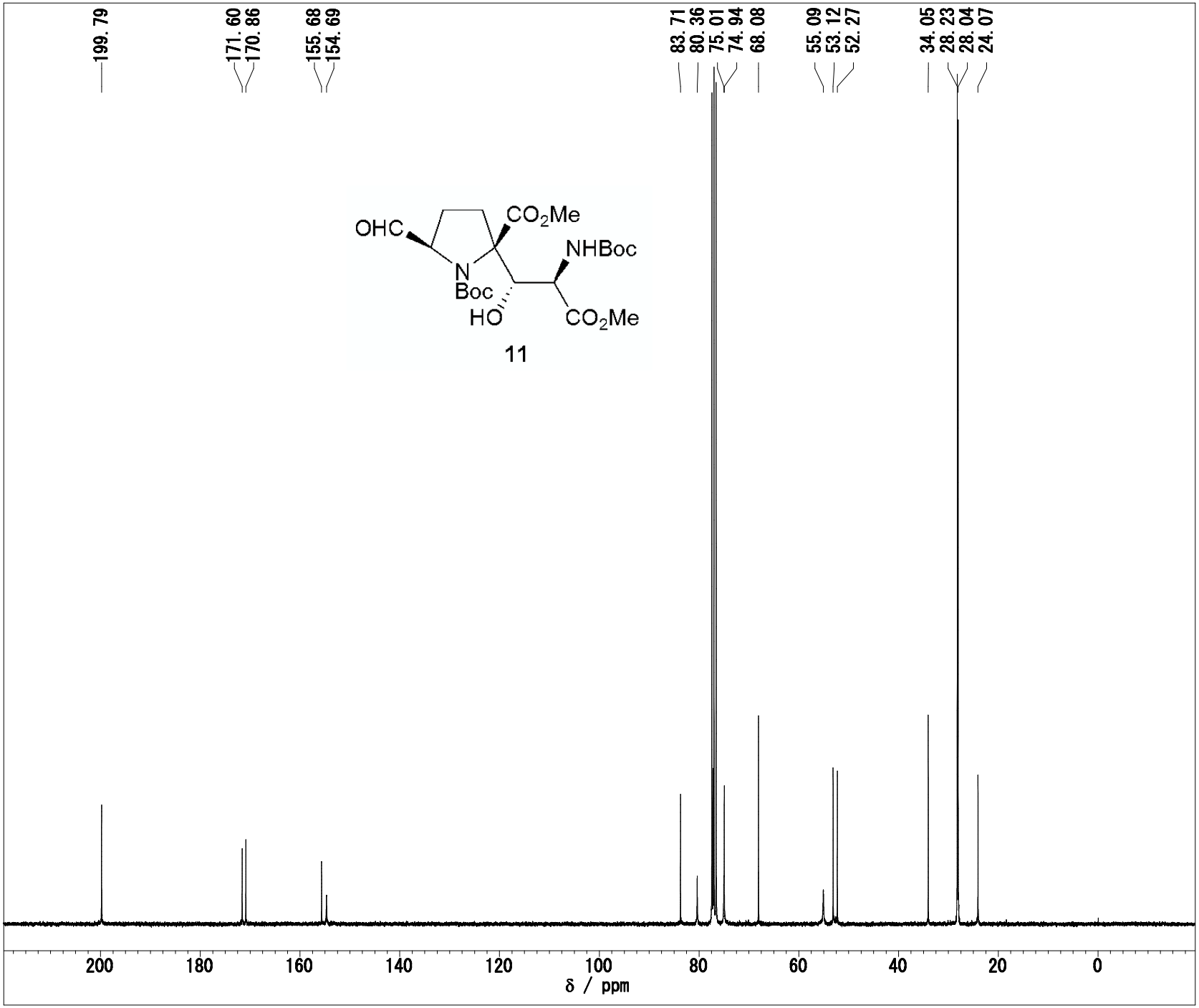

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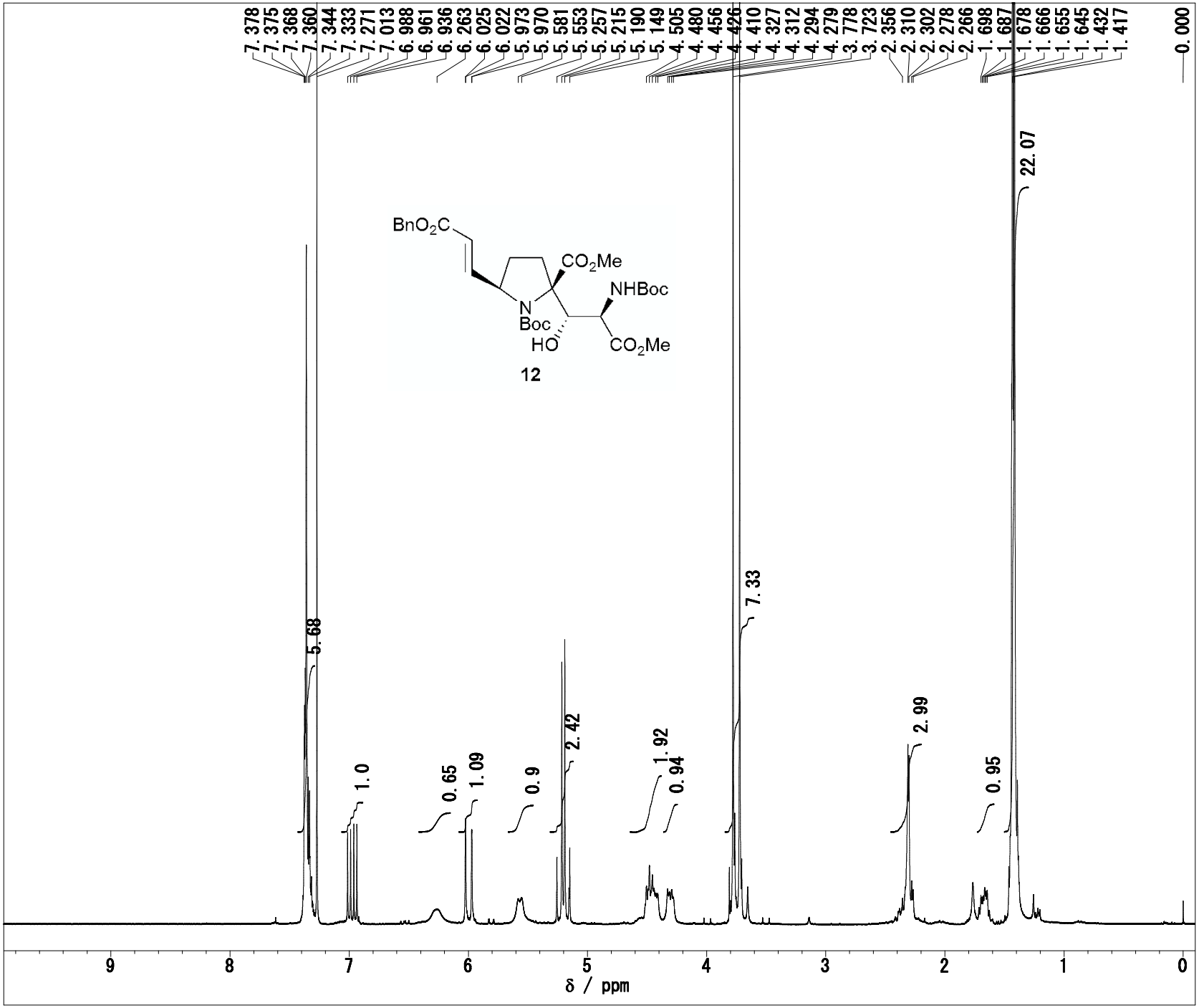

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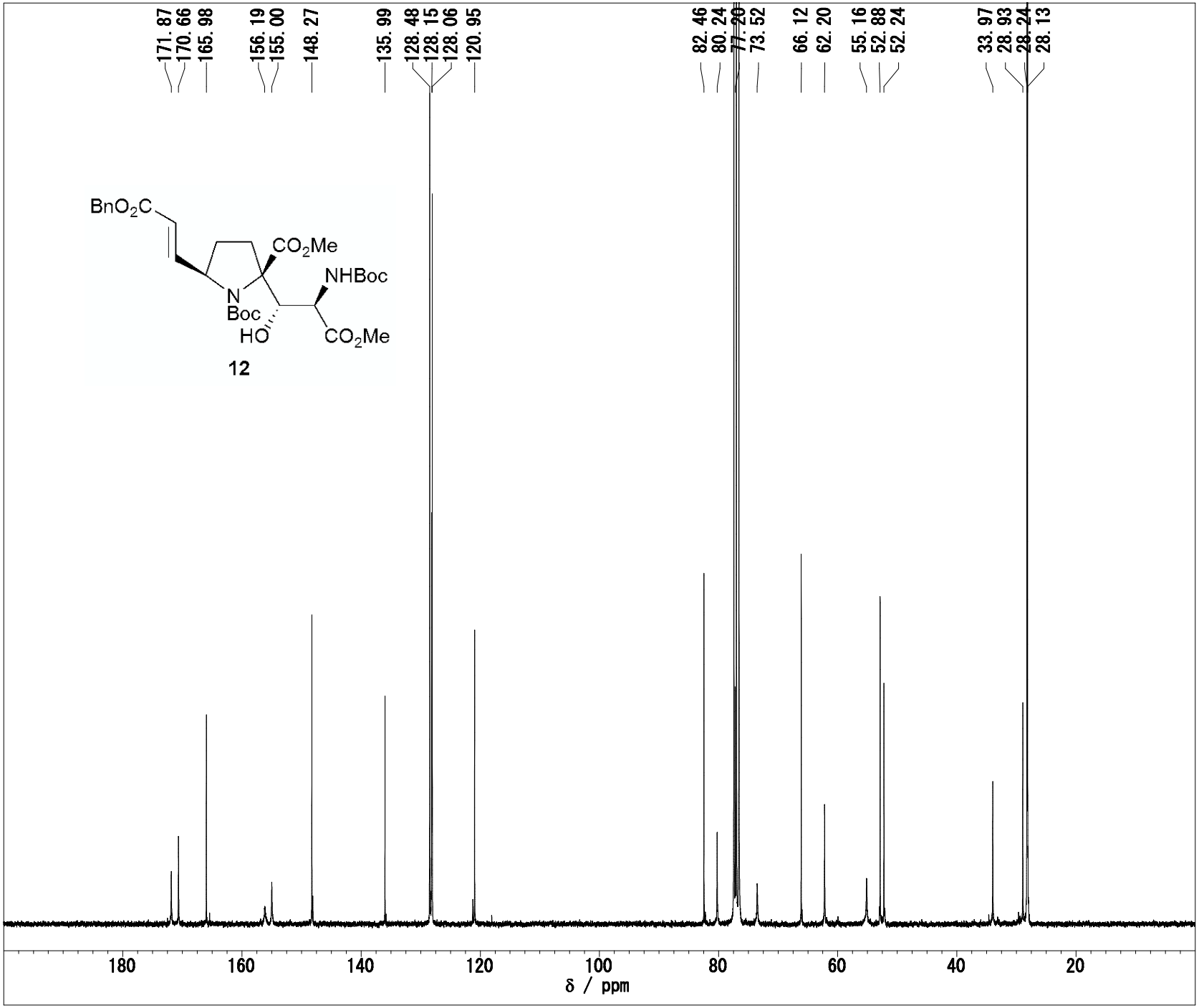

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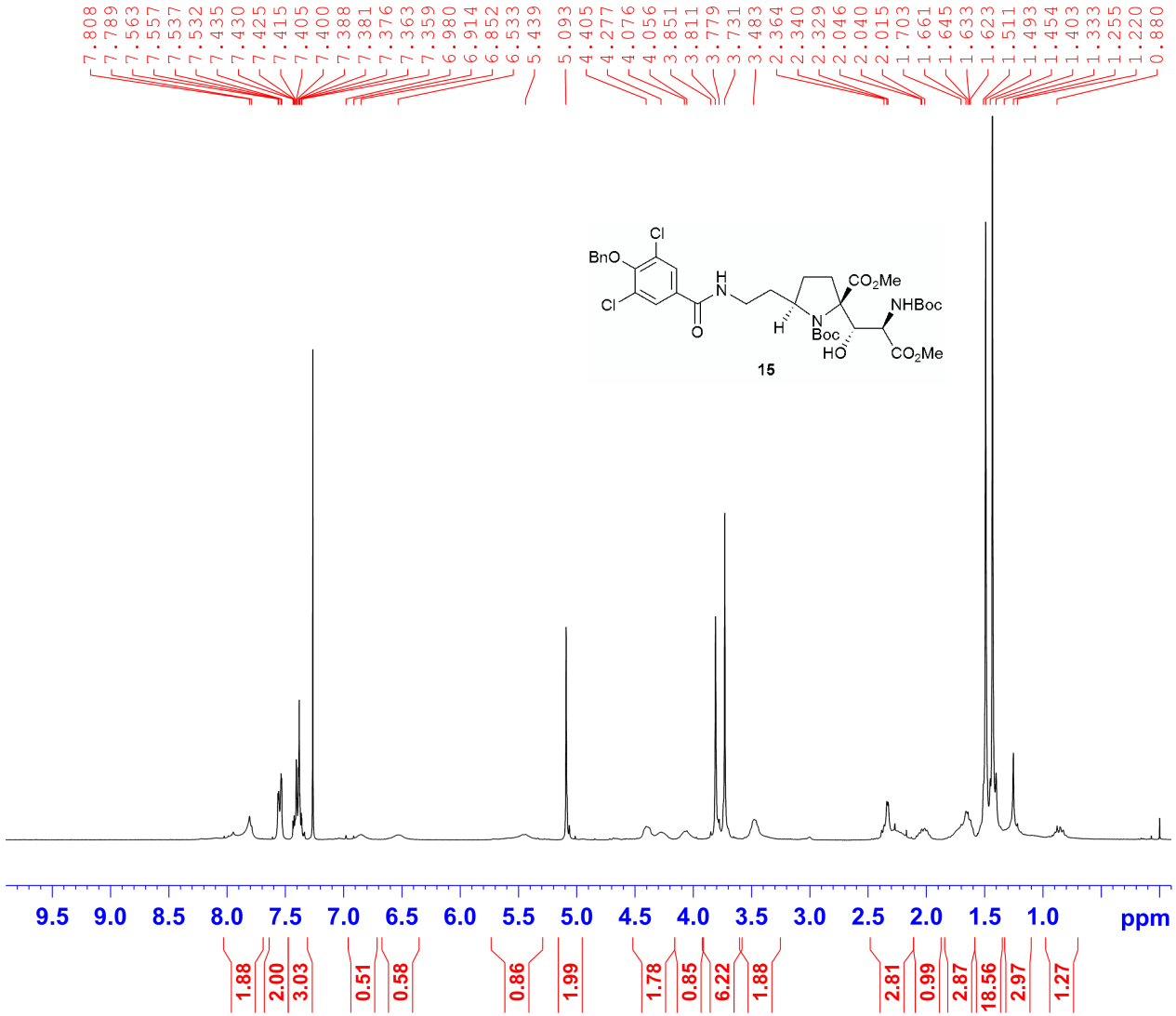

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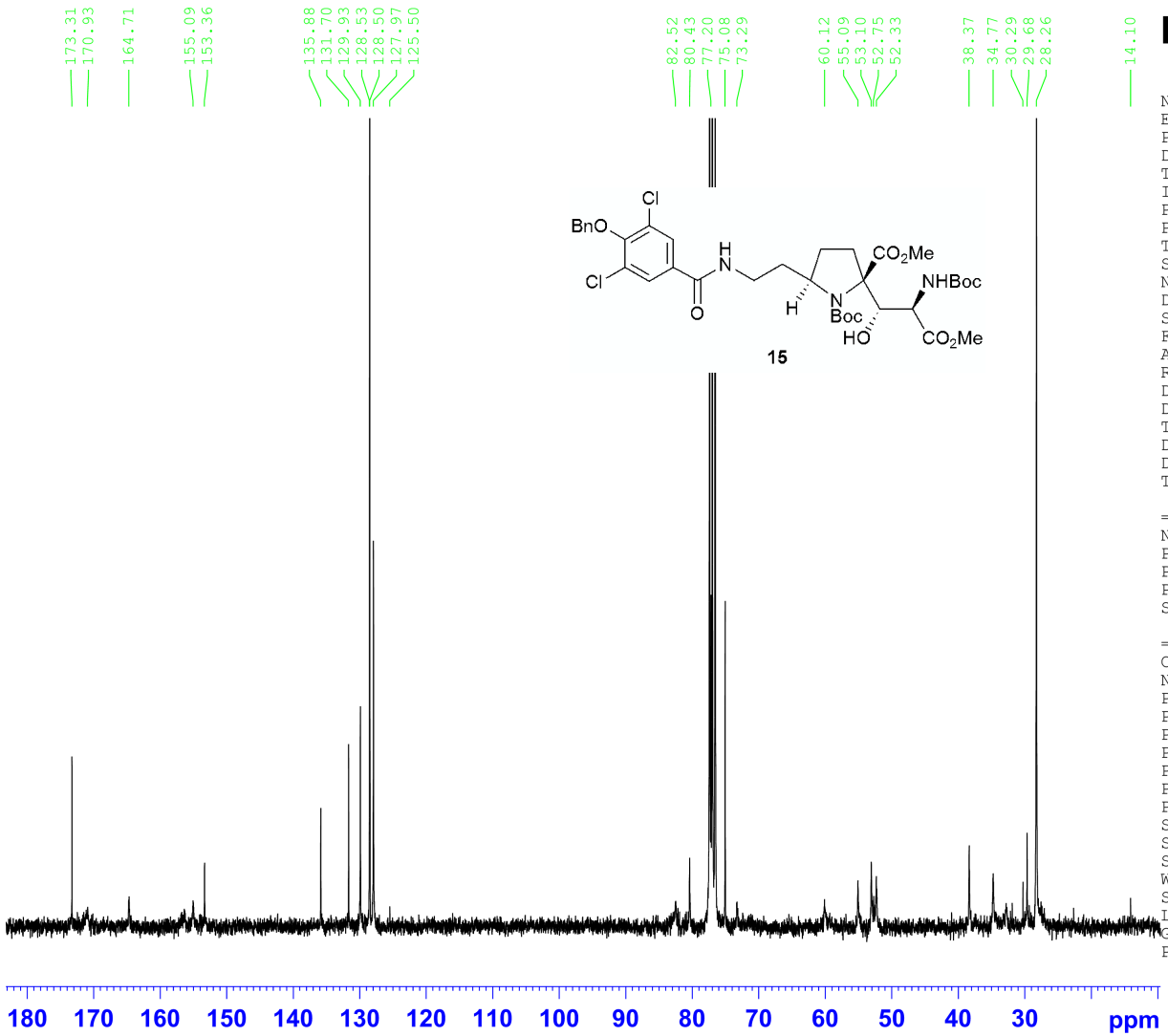
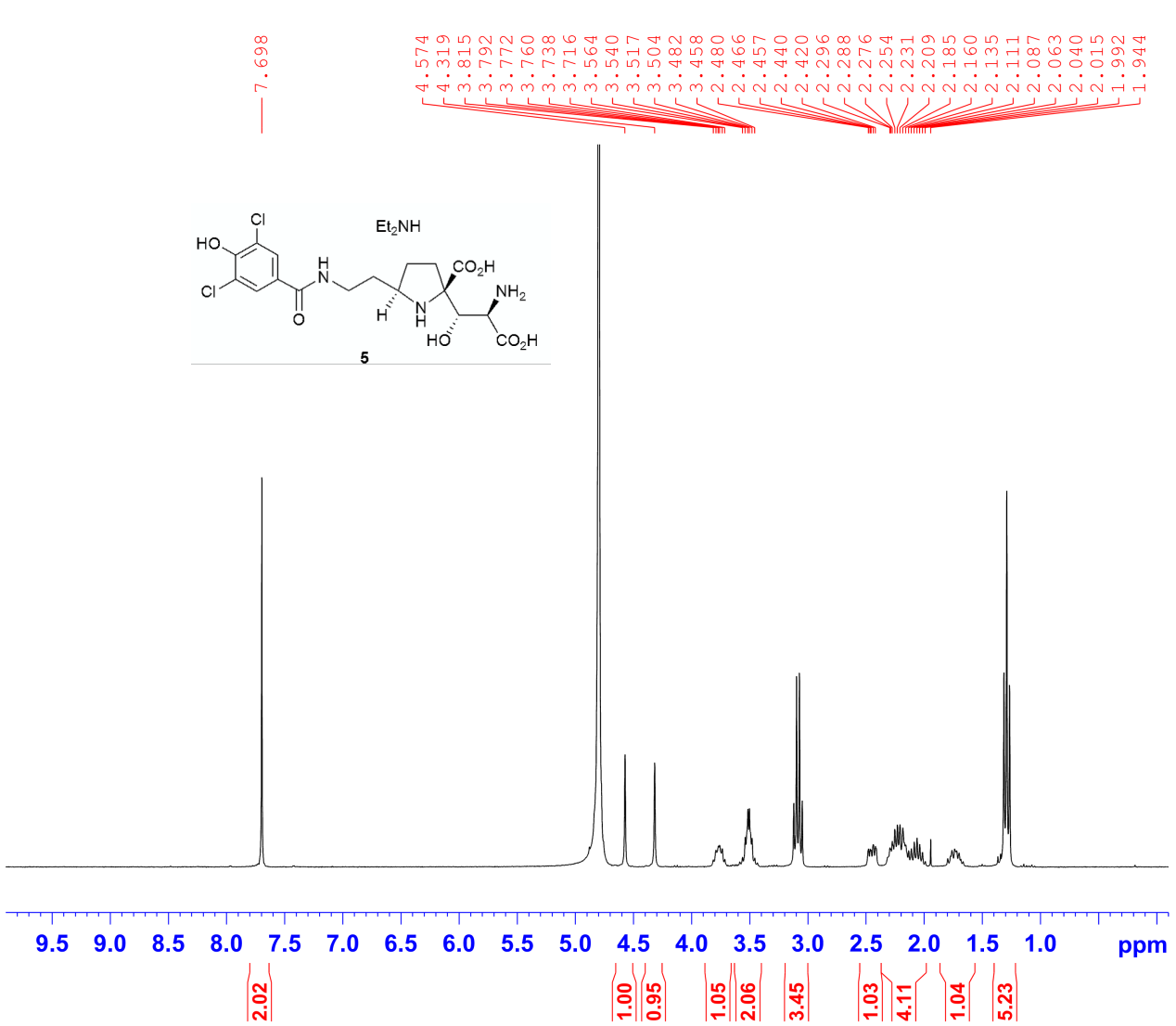
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	6188.119 Hz
FIDRES	0.094423 Hz
AQ	5.2953587 sec
RG	101
DW	80.800 usec
DE	6.50 usec
TE	293.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	15.00 usec
PL1	2.00 dB
PL1W	7.25881100 W
SFO1	300.1318534 MHz
SI	32768
SF	300.1300008 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

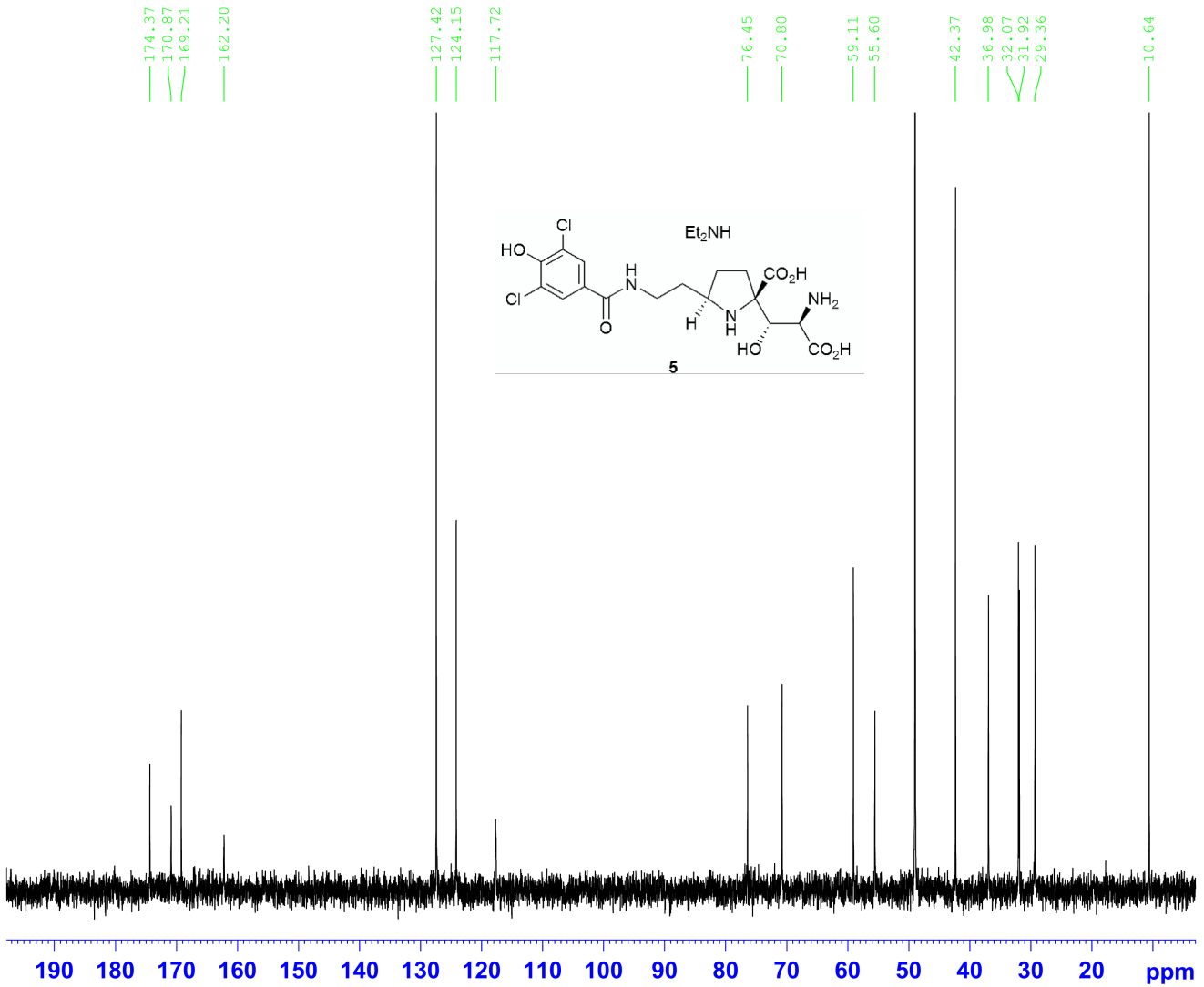


BRUKER

NAME	04302009-HM1440
EXPNO	11
PROCNO	1
Date_	20090501
Time	9.20
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	12200
DS	4
SWH	18028.846 Hz
FIDRES	0.275098 Hz
AQ	1.8175818 sec
RG	406
DW	27.733 usec
DE	6.50 usec
TE	293.9 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-0.80 dB
PL1W	38.05139160 W
SFO1	75.4752953 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	2.00 dB
PL12	16.50 dB
PL13	16.50 dB
PL2W	7.25881100 W
PL12W	0.25755233 W
PL13W	0.25755233 W
SFO2	300.1312005 MHz
SI	32768
SF	75.4677496 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

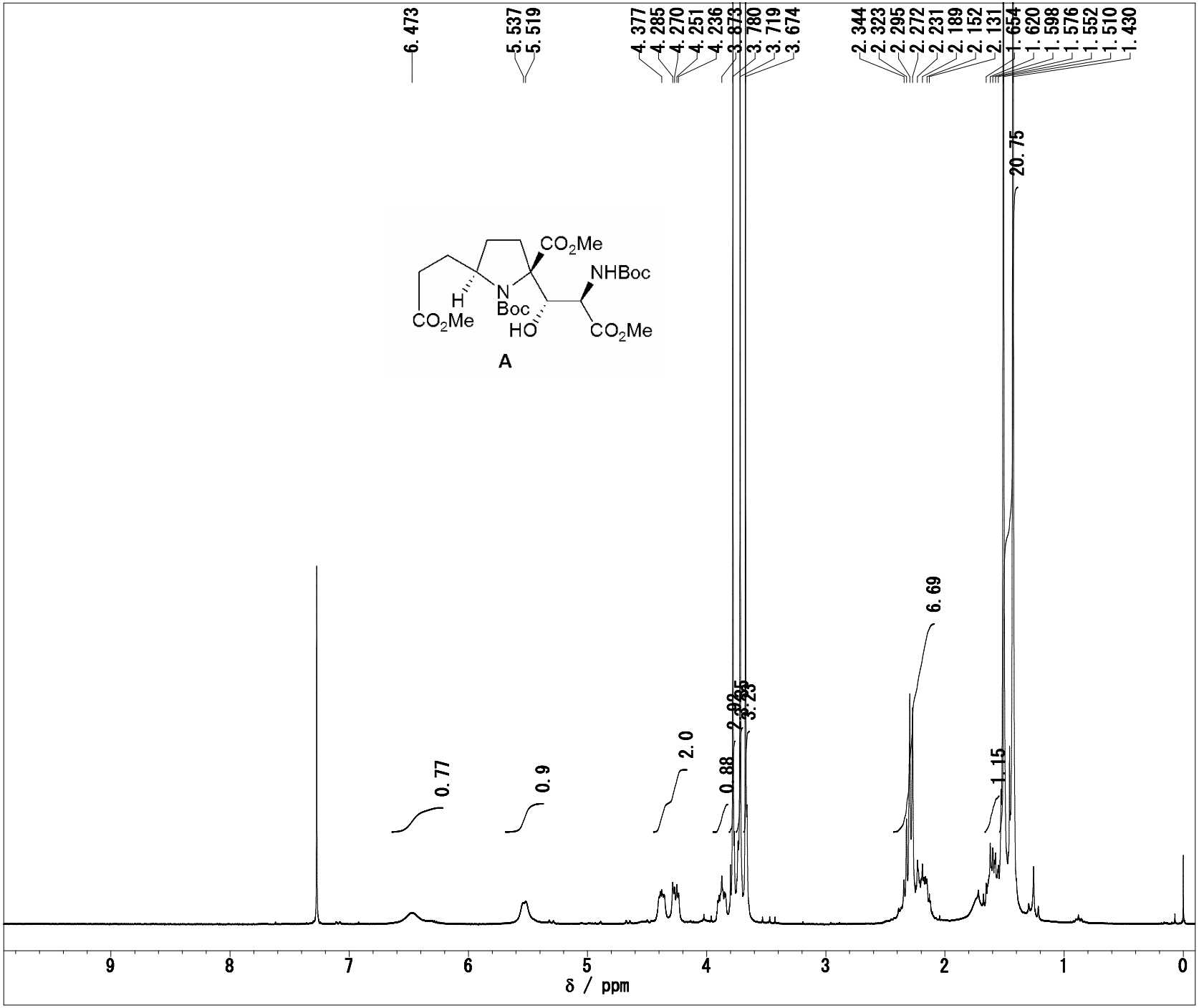


NAME	09182009-HM1495
EXPNO	10
PROCNO	1
Date_	20090918
Time	11.33
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	D2O
NS	8
DS	2
SWH	6188.119 Hz
FIDRES	0.094423 Hz
AQ	5.2953587 sec
RG	256
DW	80.800 usec
DE	6.50 usec
TE	296.2 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	15.00 usec
PL1	2.00 dB
PL1W	7.25881100 W
SFO1	300.1318534 MHz
SI	32768
SF	300.1299648 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



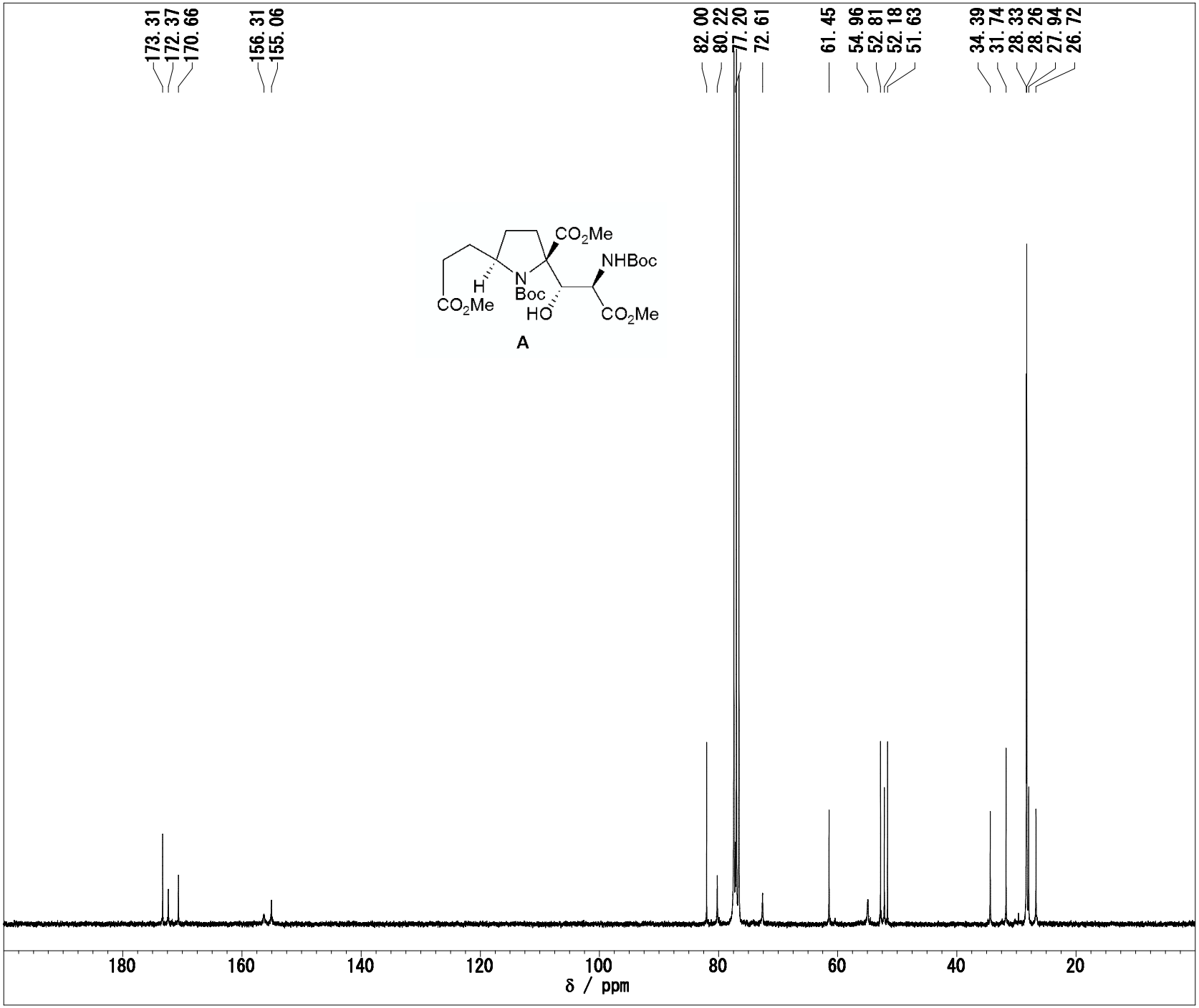
BRUKER

NAME	09182009-HM1495
EXPNO	20
PROCNO	1
Date_	20090919
Time	9.13
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	12200
DS	4
SWH	18028.846 Hz
FIDRES	0.275098 Hz
AQ	1.8175818 sec
RG	406
DW	27.733 usec
DE	6.50 usec
TE	296.9 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-0.80 dB
PL1W	38.05139160 W
SFO1	75.4752953 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	2.00 dB
PL12	16.50 dB
PL13	16.50 dB
PL2W	7.25881100 W
PL12W	0.25755233 W
PL13W	0.25755233 W
SFO2	300.1312005 MHz
SI	32768
SF	75.4677369 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40



File C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥08182009-PRODAKC¥10¥PDATA¥1¥1R
Original File: C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥08182009-PRODAKC¥10¥PDATA¥1¥1R
Date 18/Aug/2009 20:13:41
Comment:

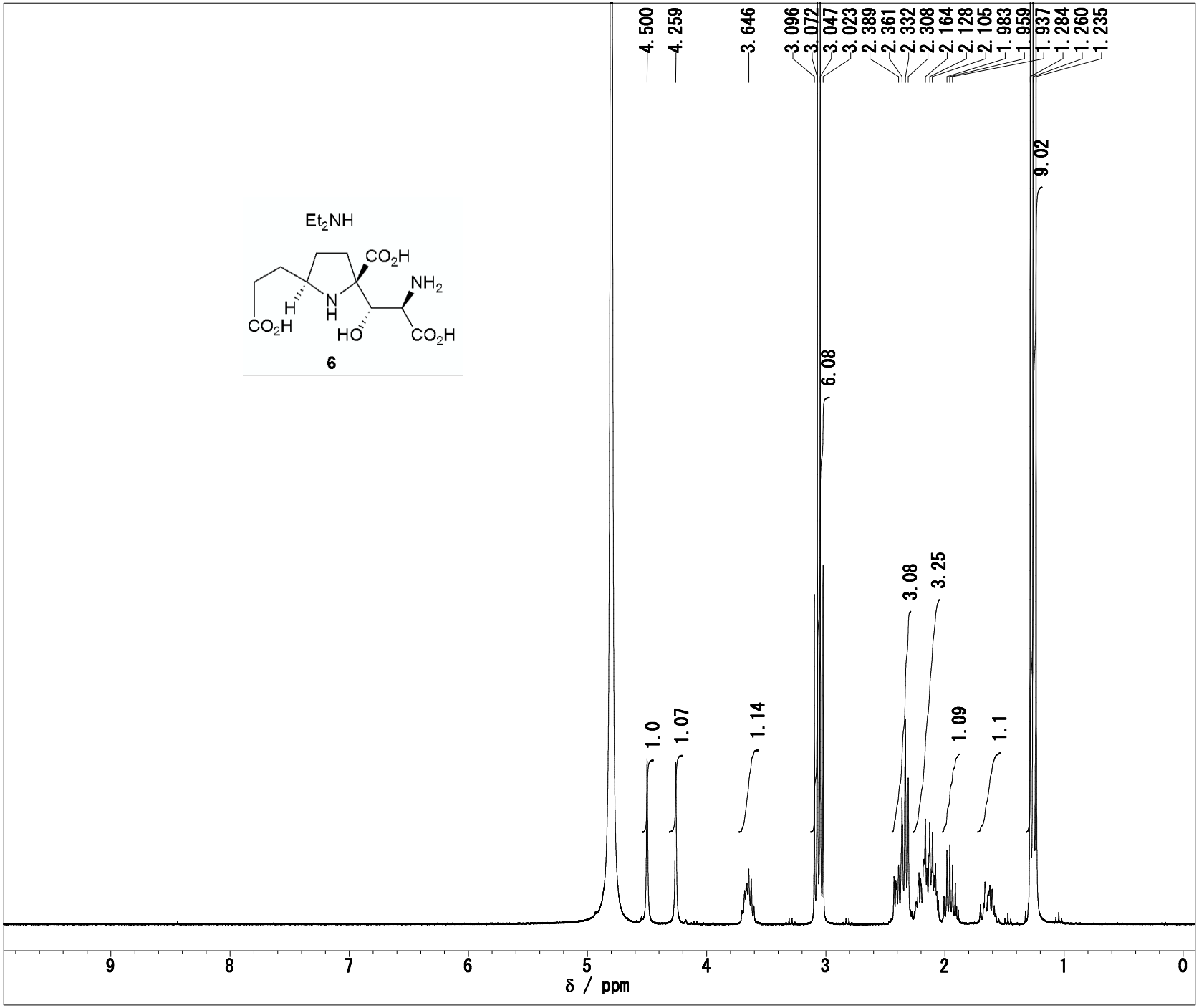
ObsNuc	^{1}H
ObsFreq	300.13 MHz
ObsSet	0.0 kHz
ObsFine	9996.736 Hz
Pulse1	15.0 µs
Pulse2	15.0 µs
Pulse3	30.0 µs
PI1	1.0 ms
PI2	0.0 ms
PI3	0.0 ms
Loop1	0
Point	32768
Scan	16
DummyScan	2
Frequecy(Span)	6188.119 Hz
AcqTime	5.2953 s
PD	1.0 s
RGain	72
Broad.Factor	0.25 Hz
ExMode	ZG30
IrrNuc	OFF
IrrFreq	0.0 MHz
IrrSet	0.0 kHz
IrrFine	0.0 Hz
IrrPulse	0 µs
IrrAttn	0
Spinning	8.0 Hz
Temperature	23.11 ℃
Printed	2015/Nov/23 16:10:06



File C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥08182009-PRODAKC¥11¥PDATA¥1¥1R
Original File: C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥08182009-PRODAKC¥11¥PDATA¥1¥1R
Date 19/Aug/2009 09:11:35
Comment:

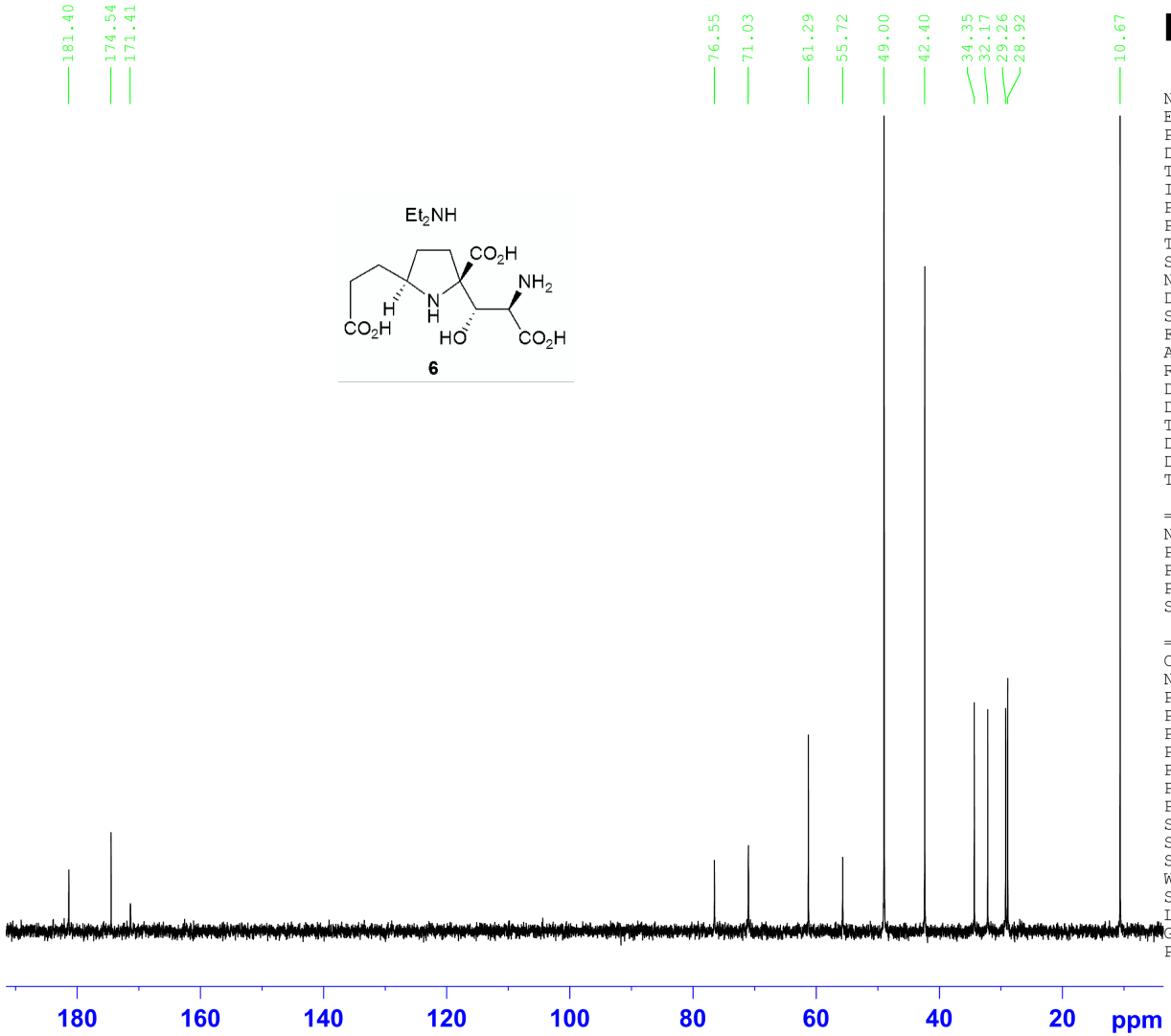
ObsNuc	^{13}C
ObsFreq	75.47 MHz
ObsSet	0.0 kHz
ObsFine	10001.42 Hz
Pulse1	10.0 µs
Pulse2	10.0 µs
Pulse3	20.0 µs
PI1	2.0 ms
PI2	0.0035 ms
PI3	0.0 ms
Loop1	0
Point	32768
Scan	12000
DummyScan	4
Frequecy(Span)	18028.85 Hz
AcqTime	1.8175 s
PD	2.0 s
RGain	322
Broad.Factor	0.25 Hz
ExMode	ZGPG30
IrrNuc	OFF
IrrFreq	0.0 MHz
IrrSet	0.0 kHz
IrrFine	0.0 Hz
IrrPulse	0 µs
IrrAttn	0
Spinning	6.0 Hz
Temperature	24.31 ℃

Printed 2015/Nov/23 16:13:10



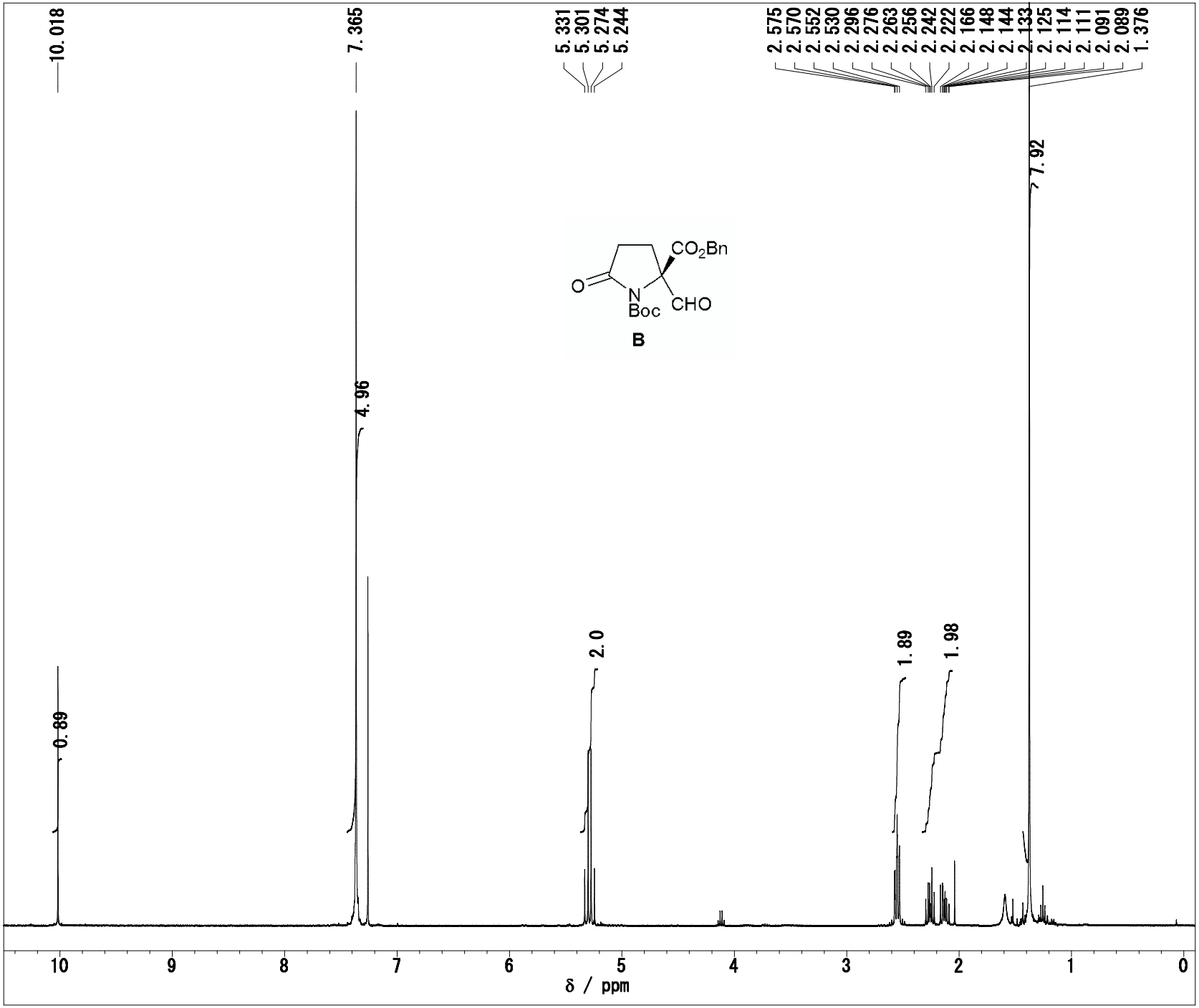
File C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥05012009-DEACYLKC¥10¥PDATA¥1¥1R
Original File: C:¥USERS¥OCU¥DOCUMENTS¥卒業生フォルダ¥2010卒業生¥HAMADA¥NMR¥BRUKER2¥BRUKER¥DATA¥MCM¥NMR¥05012009-DEACYLKC¥10¥PDATA¥1¥1R
Date 01/May/2009 20:21:19
Comment:

ObsNuc	^{1}H
ObsFreq	300.13 MHz
ObsSet	0.0 kHz
ObsFine	9996.736 Hz
Pulse1	15.0 µs
Pulse2	15.0 µs
Pulse3	30.0 µs
PI1	1.0 ms
PI2	0.0 ms
PI3	0.0 ms
Loop1	0
Point	32768
Scan	16
DummyScan	2
Frequecy(Span)	6188.119 Hz
AcqTime	5.2953 s
PD	1.0 s
RGain	114
Broad.Factor	0.25 Hz
ExMode	ZG30
IrrNuc	OFF
IrrFreq	0.0 MHz
IrrSet	0.0 kHz
IrrFine	0.0 Hz
IrrPulse	0 µs
IrrAttn	0
Spinning	20.0 Hz
Temperature	19.71 ℃
Printed	2015/Nov/21 20:25:42



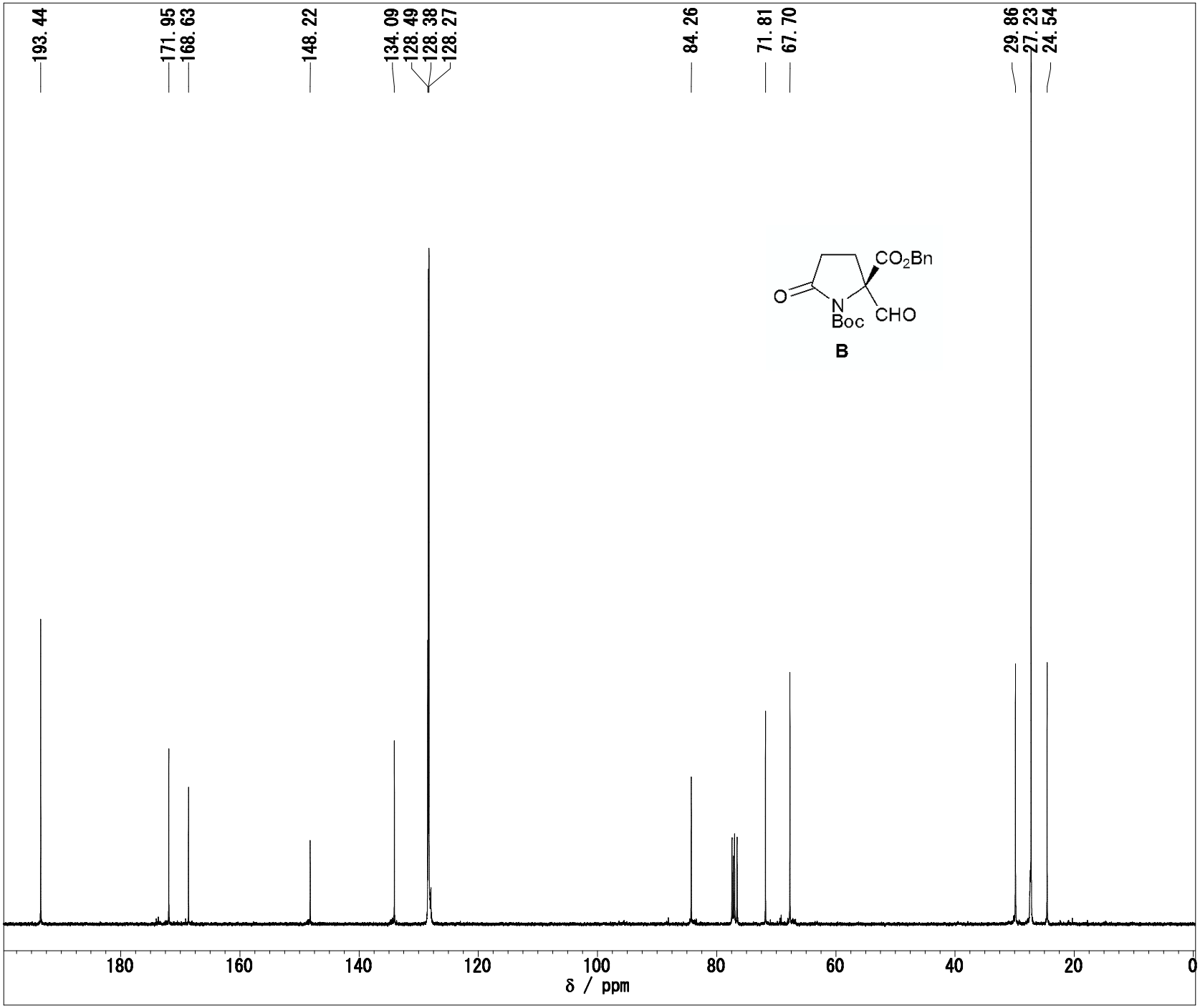
BRUKER

NAME	05012009-deacylKC
EXPNO	21
PROCNO	1
Date_	20090502
Time	9.20
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	12000
DS	4
SWH	18028.846 Hz
FIDRES	0.275098 Hz
AQ	1.8175818 sec
RG	456
DW	27.733 usec
DE	6.50 usec
TE	294.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-0.80 dB
PL1W	38.05139160 W
SFO1	75.4752953 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	2.00 dB
PL12	16.50 dB
PL13	16.50 dB
PL2W	7.25881100 W
PL12W	0.25755233 W
PL13W	0.25755233 W
SFO2	300.1312005 MHz
SI	32768
SF	75.4677326 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40



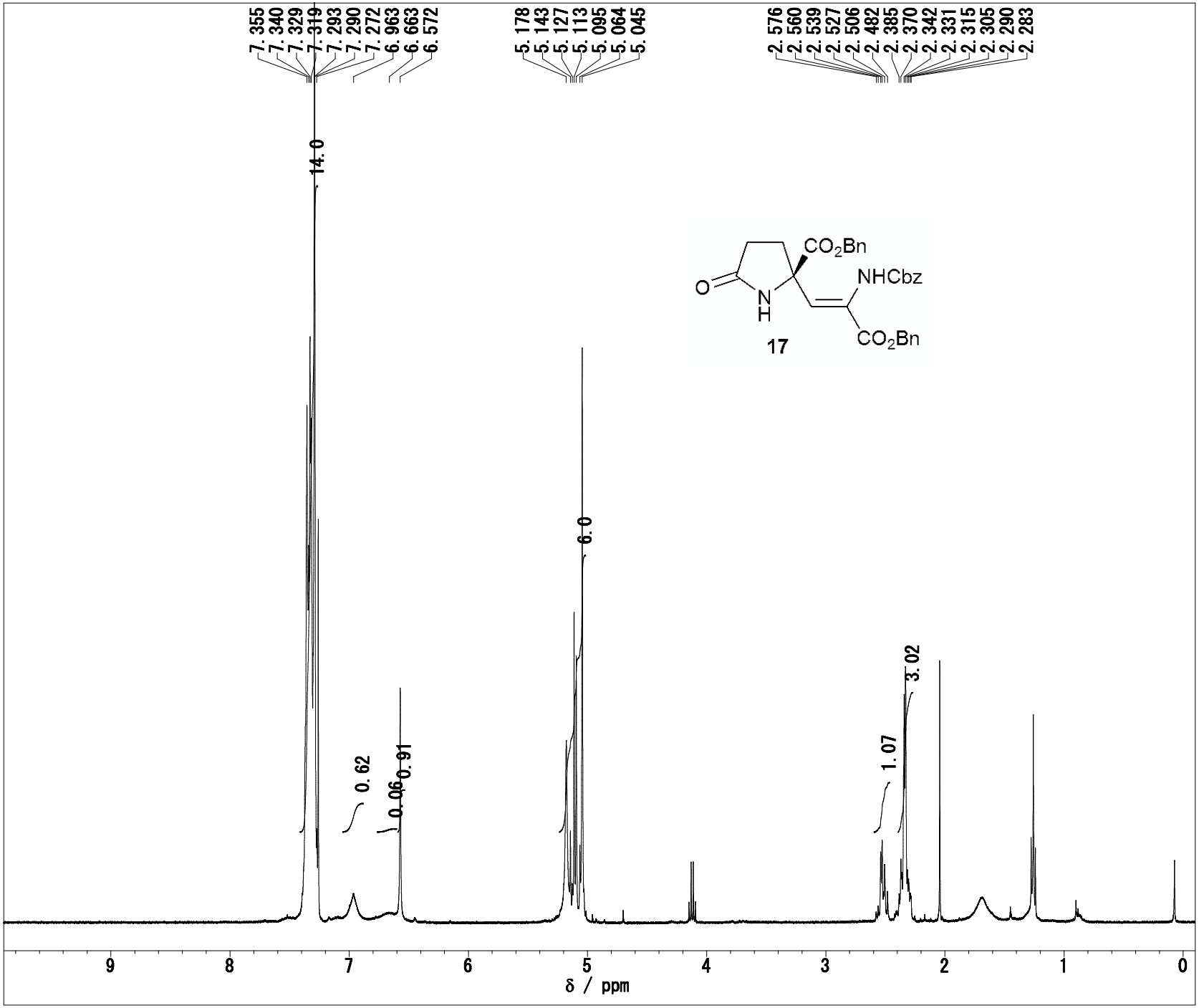
File F:¥vol.1¥090715 14.als
Original File: F:¥vol.1¥090715 14.als
Date 15/Jul/2009 17:07:50
Comment:

ObsNuc	^{1}H
ObsFreq	399.65 MHz
ObsSet	0.0 kHz
ObsFine	134300.0 Hz
Pulse1	6.25 µs
Pulse2	9.0 µs
Pulse3	10.0 µs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	131072(ZeroFi 11:×4)
Scan	4
DummyScan	1
Frequecy(Span)	7993.605 Hz
AcqTime	4.0993 s
PD	2.901 s
RGain	17
Broad.Factor	0.122 Hz
ExMode	non
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	45 µs
IrrAttn	511
Spinning	14.0 Hz
Temperature	26.7 ℃
Printed	2015/Nov/23 17:40:47



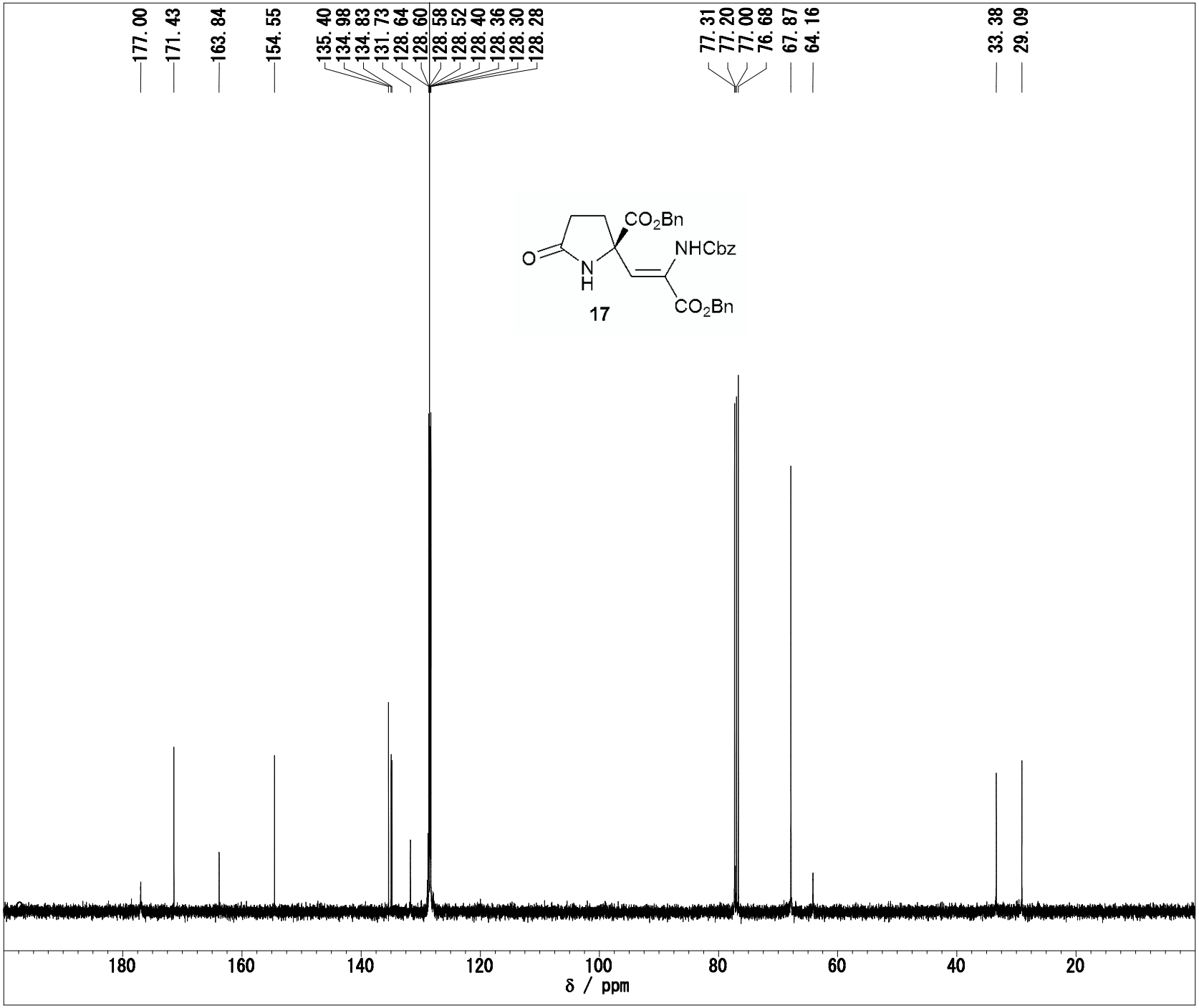
File F:¥DATA¥MCM¥NMR¥PYROGLU-ALDEHYDE 13C¥20¥PDATA¥1¥1R
Original File: F:¥DATA¥MCM¥NMR¥PYROGLU-ALDEHYDE 13C¥20¥PDATA¥1¥1R
Date 24/Nov/2015 12:37:51
Comment:

ObsNuc	^{13}C
ObsFreq	75.47 MHz
ObsSet	0.0 kHz
ObsFine	10001.42 Hz
Pulse1	10.0 µs
Pulse2	10.0 µs
Pulse3	20.0 µs
PI1	2.0 ms
PI2	0.0035 ms
PI3	0.0 ms
Loop1	0
Point	32768
Scan	800
DummyScan	2
Frequecy(Span)	18028.85 Hz
AcqTime	1.8175 s
PD	2.0 s
RGain	203
Broad.Factor	0.25 Hz
ExMode	ZGPG30
IrrNuc	OFF
IrrFreq	0.0 MHz
IrrSet	0.0 kHz
IrrFine	0.0 Hz
IrrPulse	0 µs
IrrAttn	0
Spinning	0.0 Hz
Temperature	22.31 ℃
Printed	2015/Nov/24 20:46:23



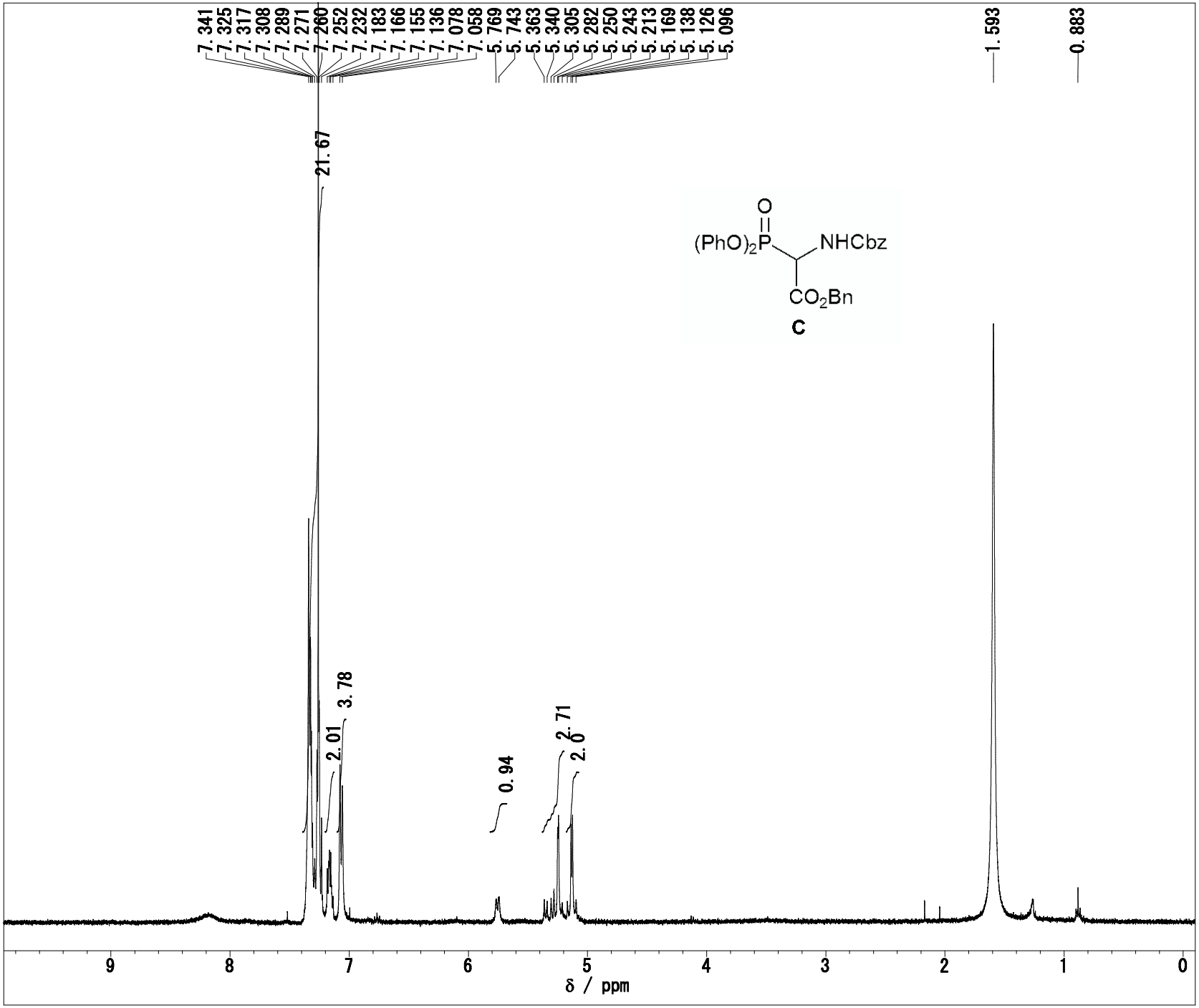
File F:¥vol.3¥51-100¥100510 60-1-1.als
Original File: :¥vol.3¥51-100¥100510 60-1-1.als
Date 10/May/2010 18:43:54
Comment:

ObsNuc	^{1}H
ObsFreq	399.65 MHz
ObsSet	0.0 kHz
ObsFine	134300.0 Hz
Pulse1	6.25 μs
Pulse2	9.0 μs
Pulse3	10.0 μs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	32768
Scan	4
DummyScan	1
Frequecy(Span)	7993.605 Hz
AcqTime	4.0993 s
PD	2.901 s
RGain	17
Broad.Factor	0.122 Hz
ExMode	non
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	45 μs
IrrAttn	511
Spinning	11.0 Hz
Temperature	25.3 ℃
Printed	2015/Nov/26 13:22:58



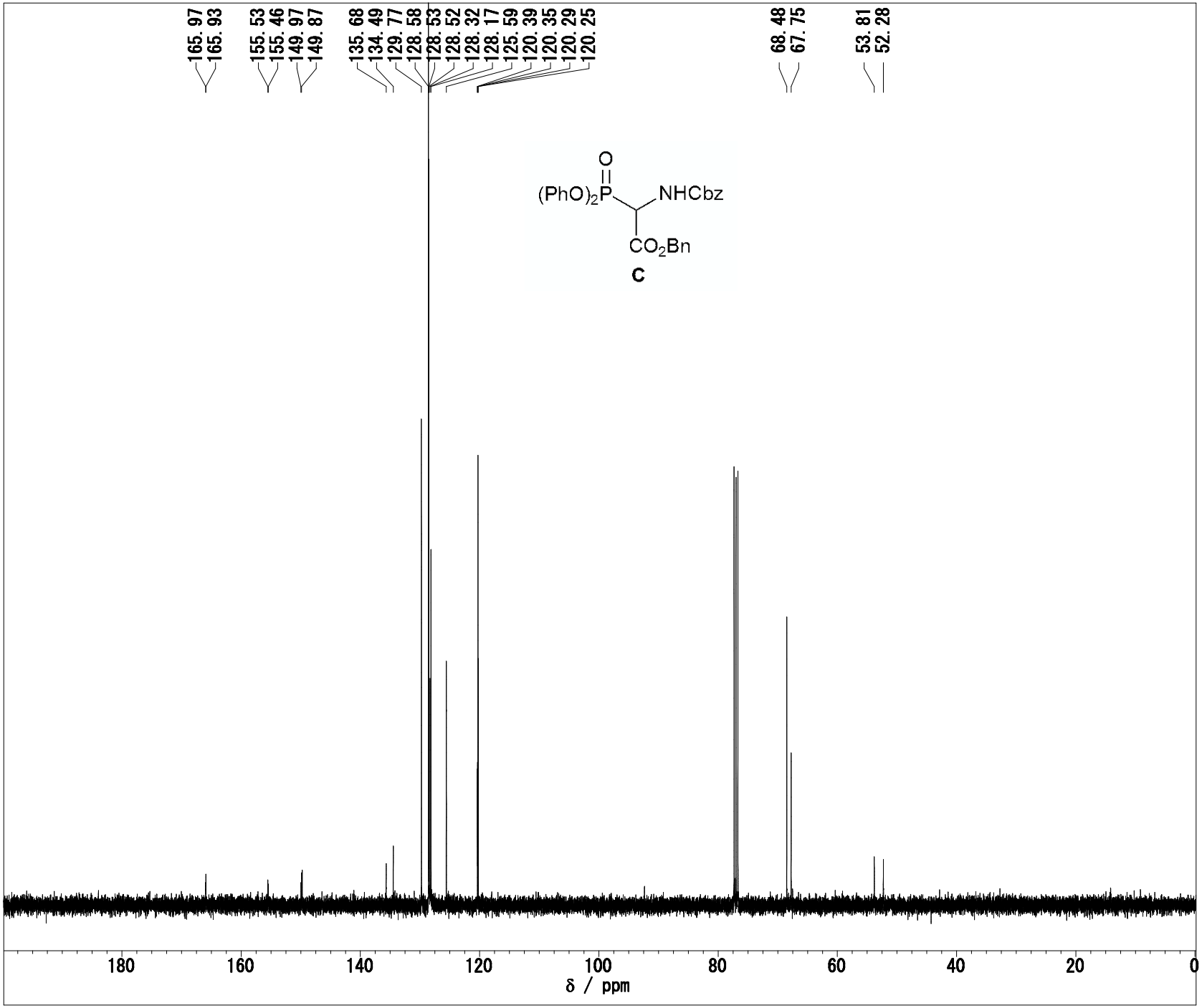
File F:¥Date¥(R)-isomer¥13C-NMR¥100818 Olefine-Bnester.als
Original File: Date¥(R)-isomer¥100818 Olefine-Bnester.als
Date 19/Aug/2010 20:29:43
Comment: 0

ObsNuc	^{13}C
ObsFreq	100.4 MHz
ObsSet	0.0 kHz
ObsFine	135500.0 Hz
Pulse1	5.0 µs
Pulse2	9.0 µs
Pulse3	10.0 µs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	32768
Scan	1024
DummyScan	1
Frequecy(Span)	27100.27 Hz
AcqTime	1.2091 s
PD	1.791 s
RGain	26
Broad.Factor	0.4135 Hz
ExMode	bcm
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	50 µs
IrrAttn	511
Spinning	14.0 Hz
Temperature	27.6 ℃
Printed	2015/Nov/26 13:10:46



File F:¥vol.4¥1-50¥100726 23.als
Original File: :¥vol.4¥1-50¥100726 23.als
Date 26/Jul/2010 17:21:37
Comment:

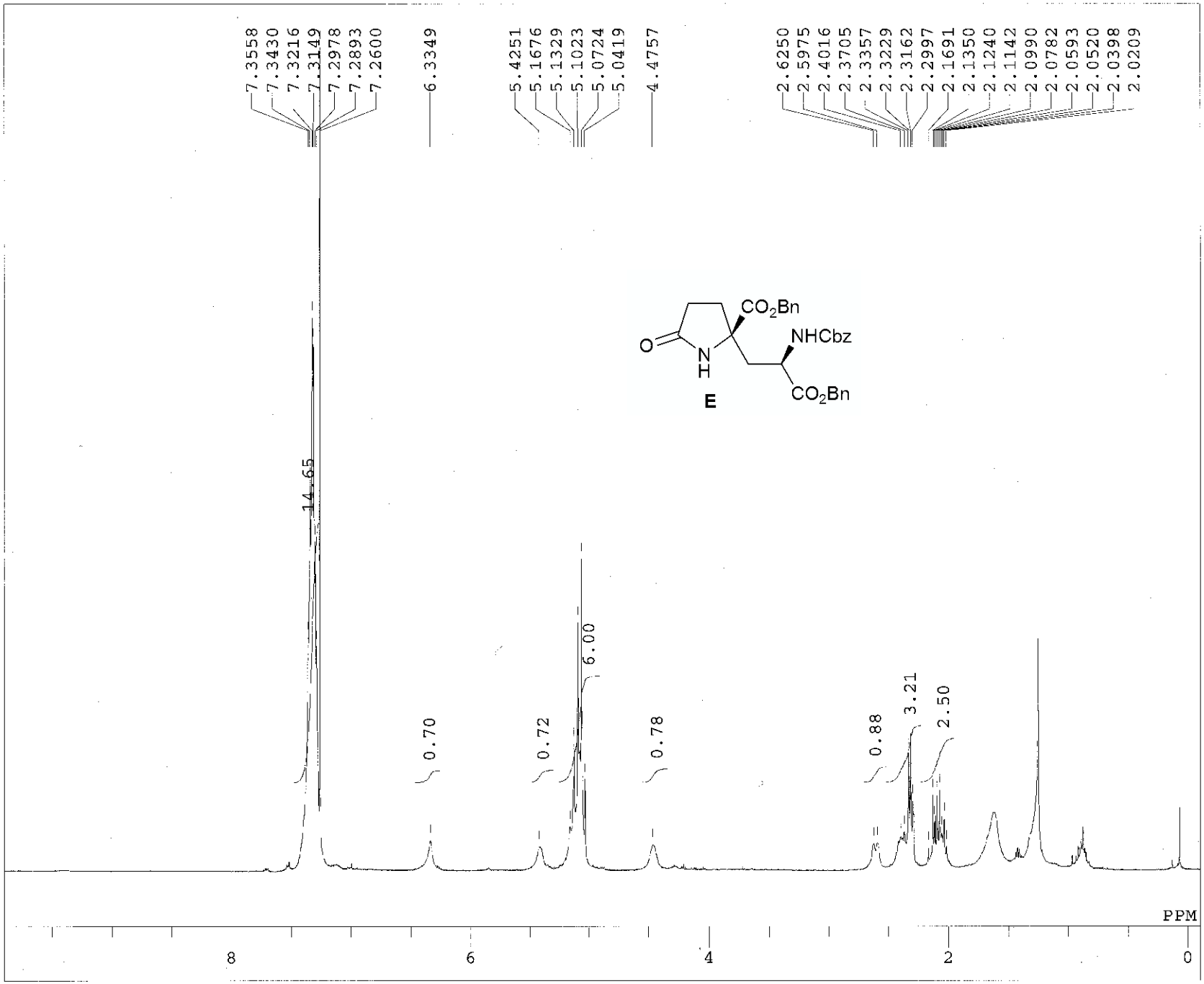
ObsNuc	^{1}H
ObsFreq	399.65 MHz
ObsSet	0.0 kHz
ObsFine	134300.0 Hz
Pulse1	6.25 μs
Pulse2	9.0 μs
Pulse3	10.0 μs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	32768
Scan	8
DummyScan	1
Frequecy(Span)	7993.605 Hz
AcqTime	4.0993 s
PD	2.901 s
RGain	24
Broad.Factor	0.122 Hz
ExMode	non
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	45 μs
IrrAttn	511
Spinning	13.0 Hz
Temperature	26.3 ℃
Printed	2015/Nov/23 18:41:48



File F:¥Date¥101008 phosphite-Bnester-2.als
Original File: :¥Date¥101008 phosphite-Bnester-2.als
Date 08/Oct/2010 15:59:27
Comment:

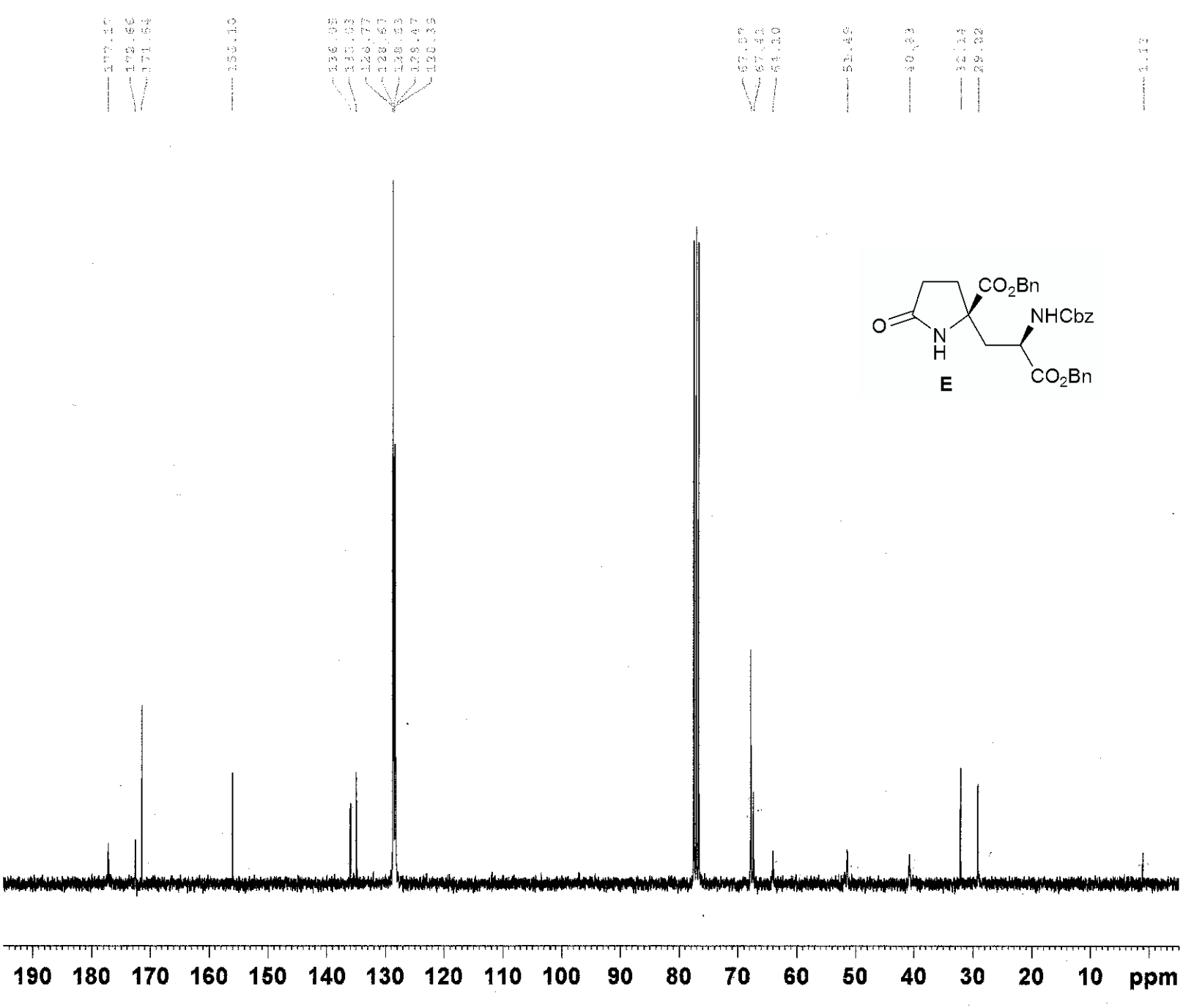
ObsNuc	^{13}C
ObsFreq	100.4 MHz
ObsSet	0.0 kHz
ObsFine	135500.0 Hz
Pulse1	5.0 µs
Pulse2	9.0 µs
Pulse3	10.0 µs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	32768
Scan	321
DummyScan	1
Frequecy(Span)	27100.27 Hz
AcqTime	1.2091 s
PD	1.791 s
RGain	26
Broad.Factor	0.4135 Hz
ExMode	bcm
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	50 µs
IrrAttn	511
Spinning	14.0 Hz
Temperature	27.3 ℃
Printed	2015/Nov/23 19:05:41

E:\5_51-100\100828 13-sai2.als



E

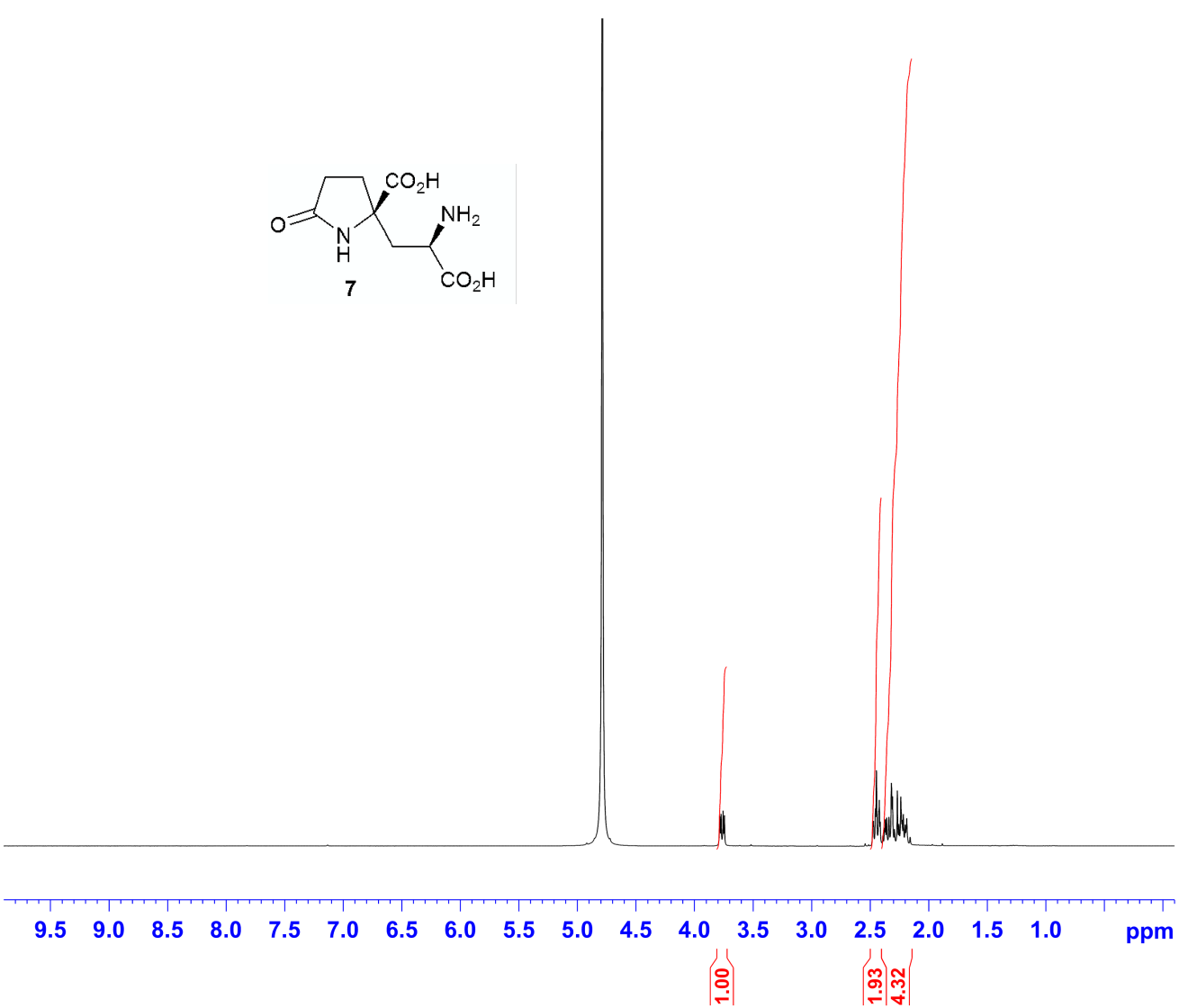
DFILE	E:\5_51-100\100828
COMNT	
DATIM	Sat Aug 28 15:51:10
OBNUC	1H
EXMOD	non
OBFRQ	399.65 MHz
OBSET	0.00 KHz
OBFIN	134300.0 Hz
POINT	32768
FREQU	7993.6 Hz
SCANS	36
ACQTM	4.099 sec
PD	2.901 sec
PW1	6.2 us
IRNUC	1H
CTEMP	25.7 c
SLVNT	CDCL3
EXREF	7.26 ppm
BF	0.12 Hz
RGAIN	20



E

BRUKER

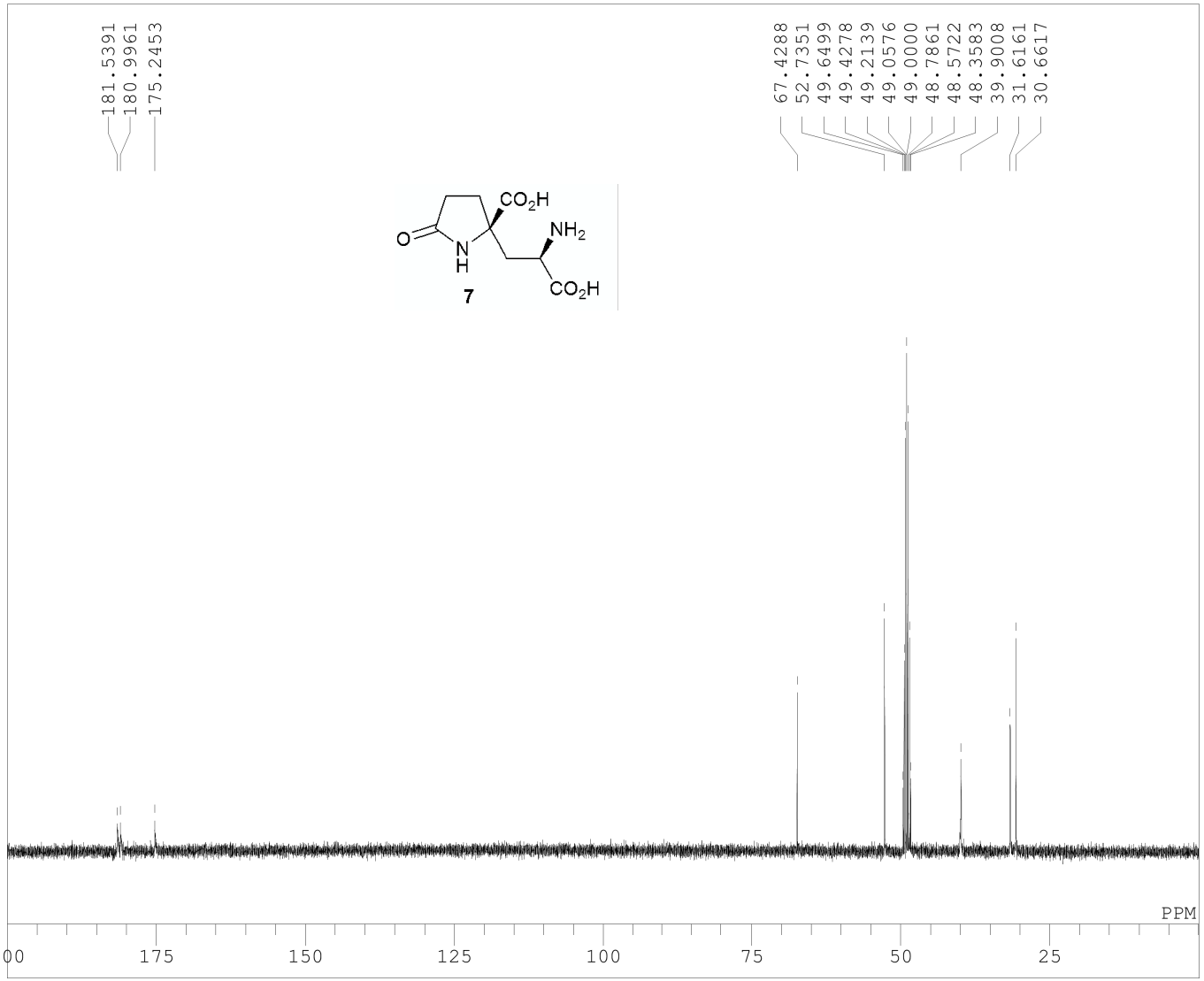
NAME	R-R-13C	
EXPNO	20	
PROCNO	1	
Date_	20101207	
Time	12.40	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	542	
DS	4	
SWH	18028.846	Hz
FIDRES	0.275098	Hz
AQ	1.8175818	sec
RG	287	
DW	27.733	usec
DE	6.50	usec
TE	294.6	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	13C	
P1	10.00	usec
PL1	-0.80	dB
PL1W	38.05139160	W
SFO1	75.4752953	MHz
======== CHANNEL f2 ========		
CPDPRG2	waltz16	
NUC2	1H	
PCPD2	80.00	usec
PL2	2.00	dB
PL12	16.50	dB
PL13	16.50	dB
PL2W	7.25881100	W
PL12W	0.25755233	W
PL13W	0.25755233	W
SFO2	300.1312005	MHz
SI	32768	
SF	75.4677410	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	



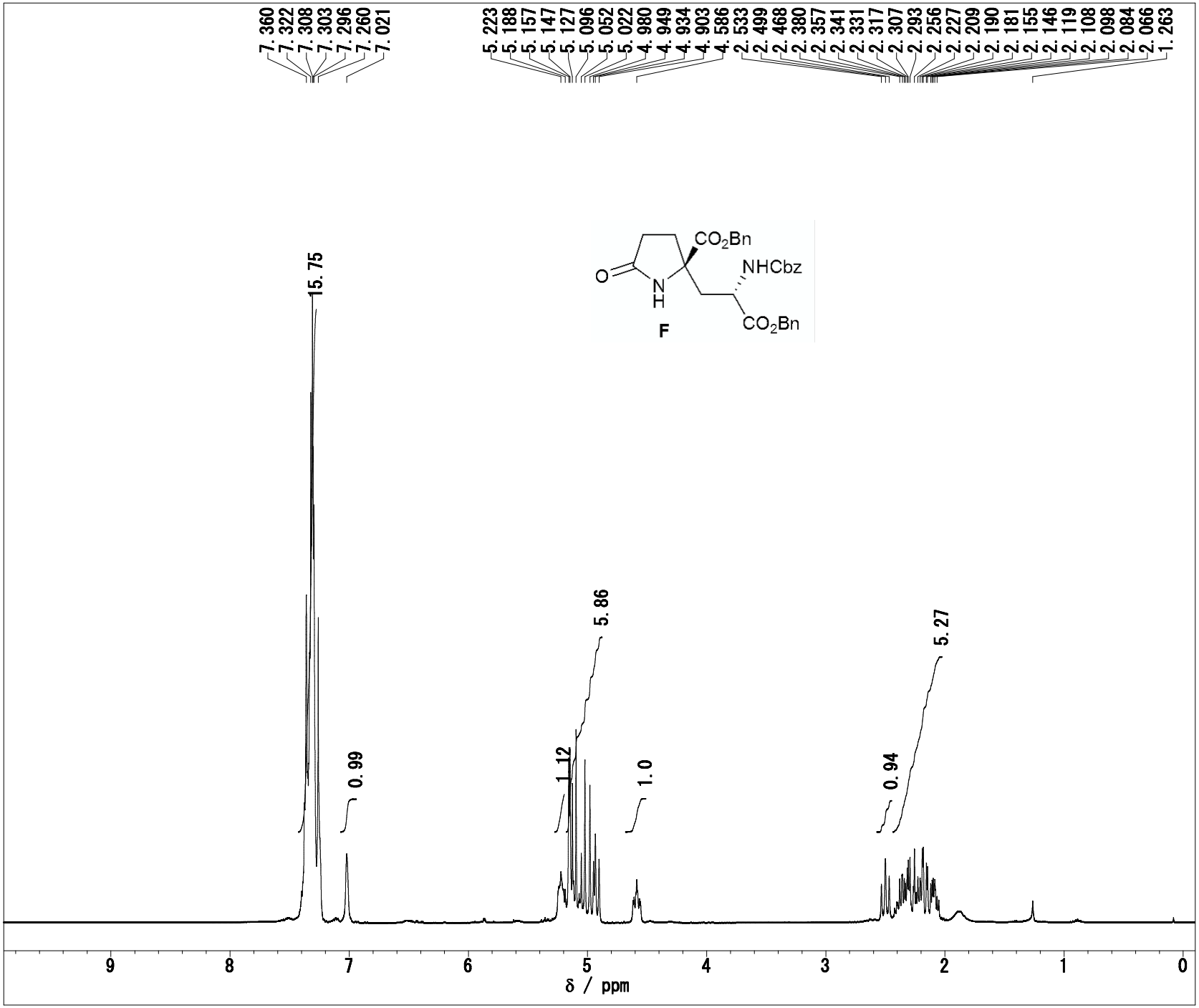
BRUKER

NAME	71
EXPNO	10
PROCNO	1
Date_	20110210
Time	16.09
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	D2O
NS	64
DS	2
SWH	6188.119 Hz
FIDRES	0.094423 Hz
AQ	5.2953587 sec
RG	80.6
DW	80.800 usec
DE	6.50 usec
TE	292.9 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	15.00 usec
PL1	2.00 dB
PL1W	7.25881100 W
SFO1	300.1318534 MHz
SI	32768
SF	300.1299677 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

C:\Documents and Settings\henkan\My Documents\NMR\yasuno\Data\(R)-isomer\13C\100922 2R-aminoacid+MeOH-d.als

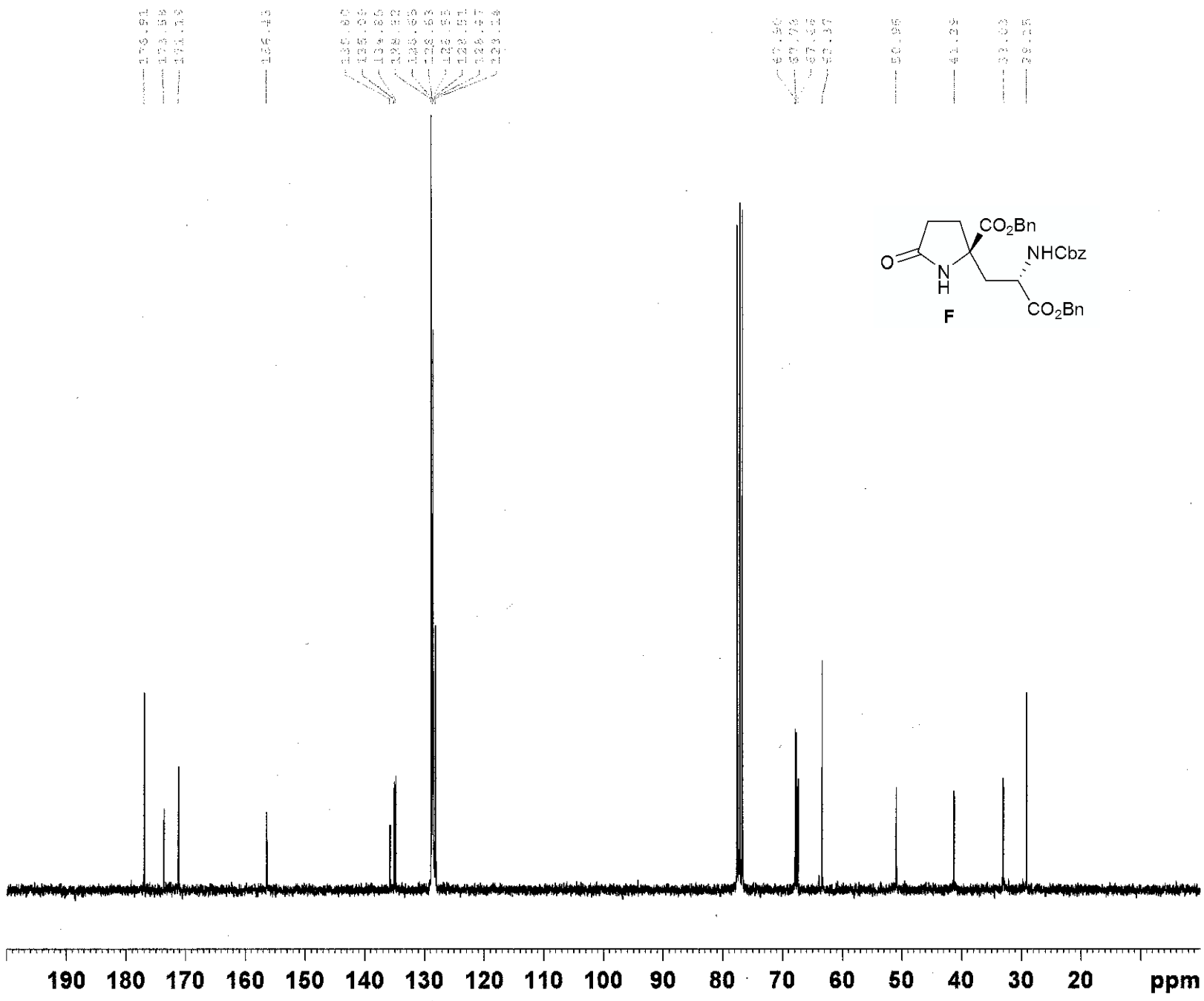


DFILE	C:\Documents and Settir
COMNT	
DATIM	Wed Sep 22 08:56:16 201
OBNUC	13C
EXMOD	bcm
OBFRQ	100.40 MHz
OBSET	0.00 KHz
OBFIN	135500.0 Hz
POINT	32768
FREQU	27100.3 Hz
SCANS	14861
ACQTM	1.209 sec
PD	1.791 sec
PW1	5.0 us
IRNUC	1H
CTEMP	27.3 c
SLVNT	D2O
EXREF	49.00 ppm
BF	0.12 Hz
RGAIN	27

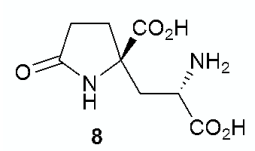


File F:¥vol.4¥51-100¥100909 51-2sai.als
Original File: :¥vol.4¥51-100¥100909 51-2sai.als
Date 09/Sep/2010 19:47:03
Comment:

ObsNuc	^{1}H
ObsFreq	399.65 MHz
ObsSet	0.0 kHz
ObsFine	134300.0 Hz
Pulse1	6.25 µs
Pulse2	9.0 µs
Pulse3	10.0 µs
PI1	0.01 ms
PI2	0.25 ms
PI3	1.0 ms
Loop1	1
Point	32768
Scan	32
DummyScan	1
Frequecy(Span)	7993.605 Hz
AcqTime	4.0993 s
PD	2.901 s
RGain	13
Broad.Factor	0.122 Hz
ExMode	non
IrrNuc	^{1}H
IrrFreq	399.65 MHz
IrrSet	0.0 kHz
IrrFine	134500.0 Hz
IrrPulse	45 µs
IrrAttn	511
Spinning	11.0 Hz
Temperature	25.9 ℃
Printed	2015/Nov/24 11:11:46



NAME	R-S-13C	
EXPNO	10	
PROCNO	1	
Date_	20101208	
Time	18.56	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	720	
DS	4	
SWH	18028.846	Hz
FIDRES	0.275098	Hz
AQ	1.8175818	sec
RG	256	
DW	27.733	usec
DE	6.50	usec
TE	293.9	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	13C	
P1	10.00	usec
PL1	-0.80	dB
PL1W	38.05139160	W
SFO1	75.4752953	MHz
======== CHANNEL f2 ========		
CPDPRG2	waltz16	
NUC2	1H	
PCPD2	80.00	usec
PL2	2.00	dB
PL12	16.50	dB
PL13	16.50	dB
PL2W	7.25881100	W
PL12W	0.25755233	W
PL13W	0.25755233	W
SFO2	300.1312005	MHz
SI	32768	
SF	75.4677415	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	



8

BRUKER

```
NAME          R-Samino acid
EXPNO                    10
PROCNO                    1
Date_              20110310
Time                  10.37
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                 D2O
NS                      128
DS                        2
SWH               6188.119 Hz
FIDRES            0.094423 Hz
AQ                5.2953587 sec
RG                       57
DW                  80.800 usec
DE                   6.50 usec
TE                   294.4 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                   15.00 usec
PL1                   2.00 dB
PL1W           7.25881100 W
SFO1          300.1318534 MHz
SI                    32768
SF            300.1299685 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
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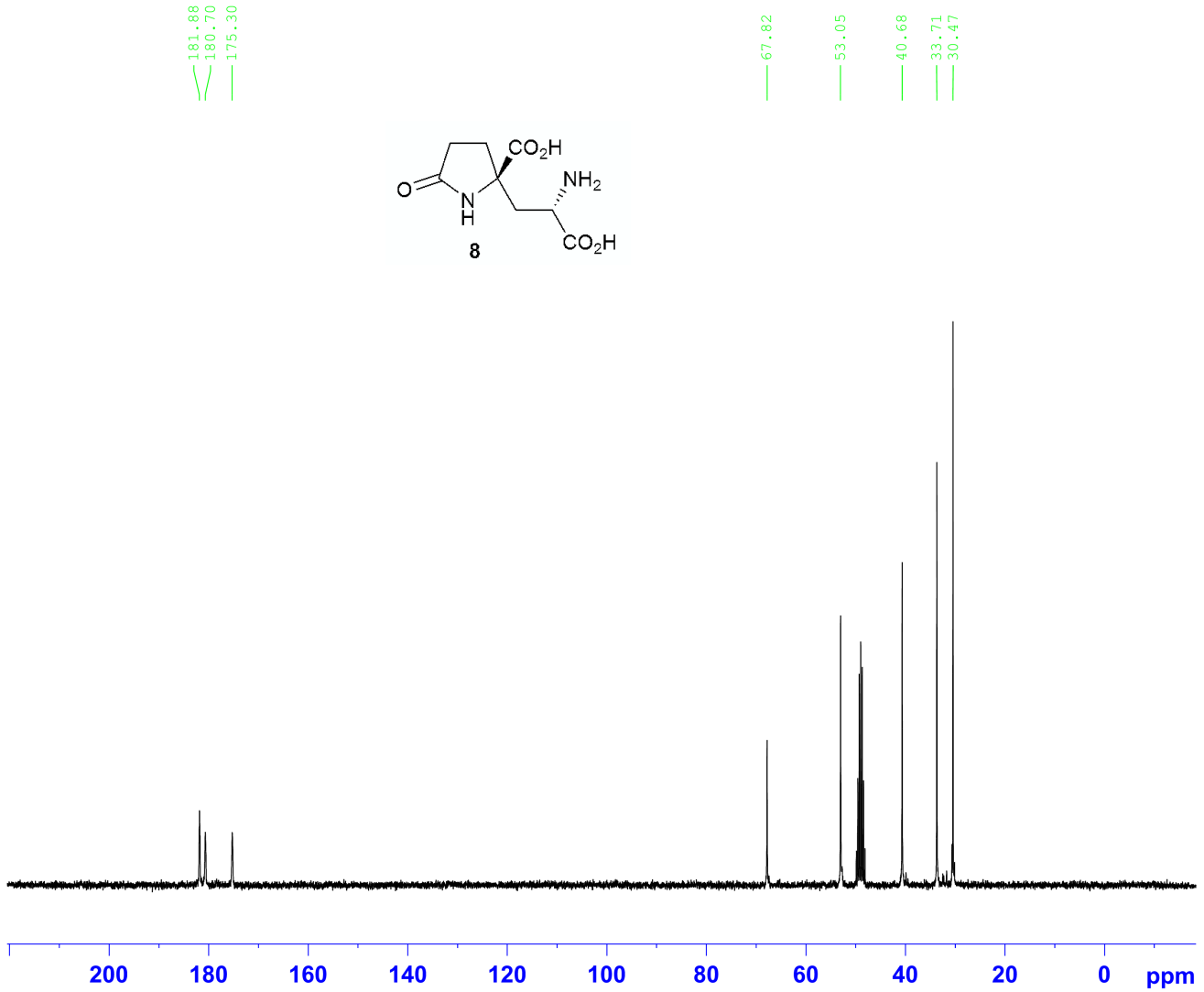

BRUKER

NAME	R-S-amino acid	
EXPNO	10	
PROCNO	1	
Date_	20110305	
Time	9.01	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zgpg30	
TD	65536	
SOLVENT	D2O	
NS	11200	
DS	4	
SWH	18028.846	Hz
FIDRES	0.275098	Hz
AQ	1.8175818	sec
RG	203	
DW	27.733	usec
DE	6.50	usec
TE	294.4	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	13C	
P1	10.00	usec
PL1	-0.80	dB
PL1W	38.05139160	W
SFO1	75.4752953	MHz
======== CHANNEL f2 ========		
CPDPRG2	waltz16	
NUC2	1H	
PCPD2	80.00	usec
PL2	2.00	dB
PL12	16.50	dB
PL13	16.50	dB
PL2W	7.25881100	W
PL12W	0.25755233	W
PL13W	0.25755233	W
SFO2	300.1312005	MHz
SI	32768	
SF	75.4676686	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	