Supporting information

Synthesis of α-Amino Squaric Acid-containing Peptide on Solid Phase

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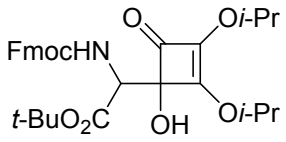
General Information:

All reagents and solvents were purchased from either Aldrich Chemical Company, Inc., Merck & Co., Inc., Nacalai Tesque Company, Ltd., Peptide Institute, Tokyo Kasei Kogyo Co., Ltd., Wako Pure Chemical Industries, Ltd., or Watanabe Chemical Industries, Ltd. and used without further purification unless otherwise indicated. Dichloromethane (CH_2Cl_2) was distilled from phosphoric pentaoxide (P_2O_5). Tetrahydrofuran (THF), Acetonitrile (CH_3CN) and dimethylformamide (DMF) of anhydrous grade were used.

Optical rotations were taken on a JASCO P-1030 polarimeter with a sodium lamp (D line). FTIR spectra was measured on a JASCO FT/IR-6200 infrared spectrophotometer. ^1H NMR spectra were reported on an either Bruker AVANCE-300 or JEOL JNM-LA 400 (400 MHz) spectrometer. Chemical shifts of ^1H NMR were reported as δ values in ppm relative to $CHCl_3$ (δ = 7.26) in $CDCl_3$, CD_2HOD (δ = 3.31) in CD_3OD, HDO (δ = 4.79) in D_2O. Chemical shifts of ^{13}C NMR spectra were reported as δ values in ppm relative to $CHCl_3$ (δ = 77.0) in $CDCl_3$, CH_3OH (δ = 49.0) in CD_3OD. Low resolution mass spectra (LRMS) and High resolution mass spectra (HRMS) were obtained on an JEOL JMS-AX500 for fast atom bombardment ionization (FAB) or Bruker solariX XR (9.4T) for electrospray ionization (ESI). Mass spectra of peptide library were obtained on a KRATOS AXIMA-CFRplus (SHIMAZU) for matrix assisted laser desorption/ionization (MALDI).

All reactions were monitored by thin layer chromatography (TLC), which was performed with precoated plates (silica gel 60 F-254, 0.25 mm layer thickness, manufactured by Merck). TLC visualization was accompanied using UV lamp (254 nm), ninhydrin solution (TCI N-094) or phosphomolybdic acid solution (10 g dissolved in 150 mL of EtOH). Daisogel IR-60 1002W(40/63 mm) was used for flash column chromatography on silica gel. Reversed phase chromatography was performed on Cosmosil® 140C_{18}-PREP.

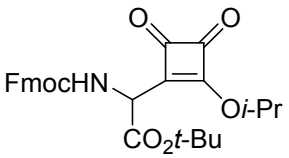
***tert*-Butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(1-hydroxy-2,3-diisopropoxy-4-oxocyclobut-2-en-1-yl)acetate (7)**



7

To a solution of **5** (6.40 g, 13.8 mmol) in MeOH (28 mL) was added 20% Pd-C (1.28 g) at room temperature. The mixture was stirred under H_2 for 3 h at room temperature, filtrated through and a celite pad. The filtrate was concentrated *in vacuo* to give amine **6** (4.59 g). The amine was subjected to the next step without purification. FmocOSu (4.65 g, 13.8 mmol) was added to a solution of **6** (4.59 g) in CH_3CN (16 ml) at room temperature. The mixture was stirred for 4 h and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/1) to give **7** (6.19 g, 81% from **5**), a 1:2 inseparable mixture as yellow oil:
FTIR (neat) 3409, 2979, 2935, 1770, 1729, 1621, 1515, 1452, 1386, 1375, 1321, 1218, 1157, 1099, 1056 cm^{-1};
^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.2 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.2 Hz, 2H), 5.83 (br d, 2/3H), 5.68 (br d, 1/3H), 4.94-4.81 (m, 2H), 4.72 (m, 1H), 4.37 (d, J = 6.9 Hz, 2H), 4.22 (d, J = 7.2 Hz, 1H), 1.51 (s, 3H), 1.49 (s, 6H), 1.39-1.23 (m, 12H);
^{13}C NMR (75 MHz, $CDCl_3$) δ 182.1, 181.5, 168.2, 163.6, 163.4, 156.4, 143.7, 141.2, 133.1, 132.7, 127.7, 127.0, 125.2, 119.9, 86.7, 85.7, 84.0, 83.4, 73.9, 67.6, 57.8, 47.0, 27.8, 22.7, 22.4, 22.2;
HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{31}H_{37}NO_8+H]^+$ 552.2592, found 552.2598.

***tert*-Butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(2-isopropoxy-3,4-dioxocyclobut-1-en-1-yl) acetate (8)**



8

To a solution of **7** (702 mg, 1.27 mmol) in CH_2Cl_2 (10 mL) was added 12*N* HCl (0.083 mL) at room temperature. The mixture was stirred for 3 h, quenched with $NaHCO_3$, and filtrated through a celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/1) to give **8** (499 mg, 80%) as yellow oil:

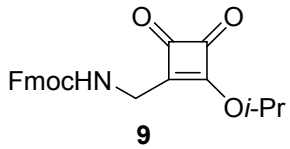
FTIR (neat) 3345, 2983, 2937, 1799, 1727, 1596, 1511, 1450, 1390, 1322, 1249, 1151, 1095, 1052 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.61 (d, *J* = 7.2 Hz , 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 6.06 (d, *J* = 7.2 Hz, 1H) , 5.52-4.40 (m, 2H), 4.37-4.31 (m, 2H), 4.25 (t, *J* = 7.2 Hz, 1H), 1.48 (s, 9H), 1.46-1.42 (m, 6H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 196.5, 193.5, 191.4, 174.5, 165.1, 155.4, 143.6, 141.2, 127.7, 127.1, 125.1, 119.9, 84.7, 80.3, 67.6, 51.4, 46.9, 27.8, 22.7, 22.6;

HRMS (FAB) *m/z* $(M+H)^+$calcd for $[C_{28}H_{29}NO_7+H]^+$ 492.2017, found 492.2014.

(9H-Fluoren-9-yl)methyl ((2-isopropoxy-3,4-dioxocyclobut-1-en-1-yl)methyl)carbamate (9)
[FmocHN-[Sq-Gly]-O*i*-Pr]



9

To a solution of **8** (290 mg, 0.589 mmol) in CH_2Cl_2 (6 mL) was added TFA (1.4 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C, warmed to room temperature, and stirred for 12 h. The mixture was quenched with sat. $NaHCO_3$ (10 mL) and extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/1) to give **9** (175 mg, 76%) as yellow oil:

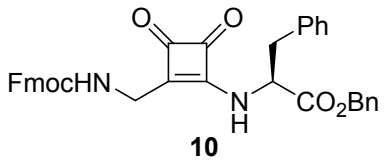
FTIR (neat) 3347, 3066, 3018, 2985, 2938, 1795, 1751, 1714, 1590, 1517, 1450, 1400, 1324, 1243, 1143, 1093, 1049, 1004, cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz , 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.46 (br s, 1H), 5.39 (sept, *J* = 6.3 Hz, 1H), 4.52-4.39 (m, 4H), 4.23 (t, *J* = 6.6 Hz, 1H), 1.43 (d, *J* = 6.3 Hz, 6H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7, 193.3, 193.0, 177.7, 156.2, 143.6, 141.2, 127.7, 127.0, 125.0, 119.9, 80.0, 67.2, 47.0, 36.5, 22.6;

HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{23}H_{21}NO_5+H]^+$ 392.1492, found 392.1488.

FmocHN-[Sq-Gly]-(L)-Phe-OBn (10)



10

To a solution of **9** (25.0 mg, 0.064 mmol) in THF (0.5 mL) was added a solution of H_2N-(L)-Phe-OBn (49.0 mg, 0.192 mmol) in THF (0.5 mL) at room temperature. The mixture was stirred for 3 h and washed with 4*N* HCl (3 mL). The organic layer was washed with sat.$NaHCO_3$ (5 mL) and brine (5 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/1) to give **10** (27.0 mg, 71%) as pale yellow amorphous solid:

$[\alpha]^{24.8}_D$ -4.0° (*c* 0.95, $CHCl_3$);

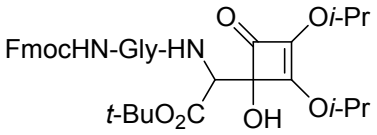
FTIR (neat) 3318, 3029, 2952, 1787, 1739, 1700, 1606, 1517, 1450, 1334, 1251, 1216, 1178, 1106, 1079, 1052, 1002 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.57 (t, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.35-7.00 (m, 12H), 5.89 (br s, 1H), 5.22- 5.12 (m, 3H), 4.42 (dd, *J* = 9.6, 6.0 Hz, 1H), 4.28-4.15 (m, 2H), 4.12 (d, *J* = 6.0 Hz, 2H), 3.23 (dd, *J* = 13.8, 5.4 Hz, 1H), 3.05 (dd, *J* = 13.8, 78.1 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 192.7, 190.0, 183.8, 169.9, 166.3, 158.3, 143.6, 143.4, 141.2, 134.6, 134.4, 129.3, 128.6, 128.4, 127.8, 127.3, 127.0, 125.1, 124.9, 120.0, 67.7, 67.6, 57.5, 46.8, 39.7, 33.3;

HRMS (FAB) *m/z* $(M+H)^+$calcd for $[C_{36}H_{30}N_2O_6+H]^+$ 587.2177, found 587.2177.

***tert*-Butyl 2-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-oxoethyl)hydrazinyl)- 2-(1-hydroxy-2,3-diisopropoxy-4-oxocyclobut-2-en-1-yl)acetate (14)**



14

To a solution of **5** (12.4 g, 26.8 mmol) in MeOH (55 mL) was added 10% Pd-C (2.48 g) at room temperature. The mixture was stirred under H_2 for 3 h at room temperature and filtrated through a celite pad. The filtrate was concentrated *in vacuo* to give **6** (9.0 g). The amine **6** was subjected to the next step without purification. FmocHN-Gly-OH (8.1 g, 27.3 mmol), EDCI (5.26 g, 27.4 mmol) was successively added to a solution of **6** (9.0 g) in THF (78 ml) at 0 ^oC. The mixture was stirred for 10 min at 0 ^oC, warmed to room temperature, and stirred for 3 h. The mixture was quenched with sat. NH_4Cl and extracted with EtOAc (20 mL x 3). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 5/1-1/3) to give **14** (16.0 g, 98% (2 steps from **5**), an inseparable 2:1 mixture of diastereomers) as yellow amorphous solid:

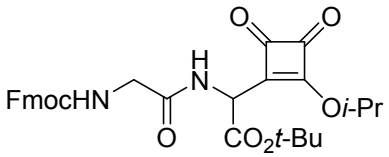
FTIR (neat) 3338, 2981, 2937, 1769, 1727, 1615, 1520, 1450, 1386, 1373, 1320, 1249, 1157, 1097, 1048, 997, 960, 938, 909, 843, 759, 735 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 7.4 Hz, 2H), 7.59 (br d, 2H, J = 7.4 Hz), 7.36 (t, J = 7.4 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 7.19 (br s, 1H), 6.10 (br s, 2/3H), 6.00 (br s, 1/3H), 5.02 (d, J = 8.7 Hz, 2/3H), 4.95 (d, J = 8.1 Hz, 1/3H), 4.91-4.75 (m, 2H), 4.35 (d, J = 7.2 Hz, 2H), 4.22 (t, J = 7.2 Hz, 1H), 4.12-3.91 (m, 2H), 1.51-1.15 (m, 21H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 182.8, 182.3, 170.0, 169.7, 167.6, 167.5, 164.7, 164.6, 156.7, 156.5, 143.8, 143.7, 141.1, 132.2, 127.5, 127.0, 125.1, 119.7, 85.5, 85.4, 83.2, 83.1, 77.5, 67.3, 56.9, 56.4, 46.9, 44.3, 27.7, 22.6, 22.4, 22.1;

HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{33}H_{40}N_2O_9+H]^+$ 609.2807, found 609.2823.

***tert*-Butyl 2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-(2-isopropoxy-3,4-dioxocyclobut-1- en-1-yl)acetate (15)**



15

To a solution of **14** (1.40 g, 2.30 mmol) in CH_2Cl_2 (22 mL) was added 12 *N* HCl (0.18 mL) at room temperature. The mixture was stirred for 3 h, quenched with $NaHCO_3$, and filtrated through a celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 5/1-1/3) to give **15** (876 mg, 70%) as yellow amorphous solid:

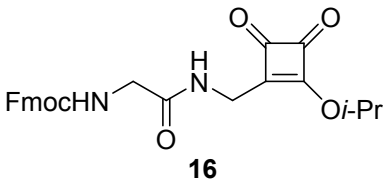
FTIR (neat) 3342, 2981, 1797, 1733, 1682, 1594, 1519, 1450, 1392, 1326, 1251, 1150, 1093, 1047, 910, 761, 734 cm^{-1};

^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.22 (br s, 1H) , 5.58 (d, *J* = 6.9 Hz, 1H), 5.53 (br s, 1H), 5.46 (sept, *J* = 6.0 Hz, 1H), 4.43 (d, *J* = 6.6 Hz, 2H), 4.24 (t, *J* = 6.6 Hz, 1H), 4.00 (br s, 1H), 1.49-1.44 (m, 15H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 196.5, 193.3, 191.6, 173.7, 169.2, 164.7, 156.5, 143.7, 141.1, 127.5, 126.9, 125.0, 119.8, 84.6, 80.4, 67.2, 49.9, 46.9, 44.0, 27.7, 22.6, 22.5;

HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{30}H_{32}N_2O_8+H]^+$ 549.2231, found 549.2234.

FmocHN-Gly-[Sq-Gly]-O*i*-Pr (16)



16

To a solution of **15** (1.40 g, 2.55 mmol) in CH_2Cl_2 (26 mL) was added TFA (6 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C, warmed to room temperature, and stirred for 12 h. The mixture was quenched with *sat*. $NaHCO_3$ and extracted with EtOAc (15 mL x 3). The combined organic layers were washed with brine, and dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 3/1-1/6) to give **16** (780 mg, 69%) as yellow amorphous solid:

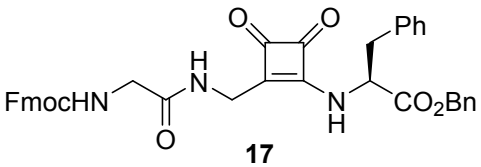
FTIR (neat) 3628, 3338, 2987, 1796, 1748, 1670, 1587, 1449, 1404, 1322, 1251, 1201, 1136, 1093, 1049, 990, 899, 800 cm^{-1};

^1H NMR (300 MHz, CD_3OD) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 7.5 Hz , 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 5.33 (sept, *J* = 6.0 Hz, 1H), 4.40-4.25 (m, 4H), 4.20 (t, *J* = 6.9 Hz, 1H), 3.82 (s, 2H), 1.41 (d, *J* = 6.0 Hz, 6H);

^{13}C NMR (100 MHz, CD_3OD) δ 198.4, 195.5, 194.3, 178.9, 172.8, 158.9, 145.2, 142.6, 128.9, 128.3, 126.3, 121.1, 81.4, 68.2, 48.3, 44.9, 35.5, 23.0;

HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{25}H_{24}N_2O_6+H]^+$ 449.1707, found 449.1709

FmocHN-Gly-[Sq-Gly]-(L)-Phe-OBn (17)



17

To a solution of **16** (50.7 mg, 0.113 mmol) in THF (0.5 mL) was added a solution of H_2N-(L)-Phe-OBn (86 mg, 0.339 mmol) in THF (0.5 mL) at room temperature. The mixture was stirred for 3 h and quenched with with 4*N* HCl (3 mL). The organic layer was washed with sat.$NaHCO_3$ and brine (15 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/9) to give **17** (47.0 mg, 64%) as yellow amorphous solid:

$[\alpha]^{30.0}_D$ -23.8 ^{o} (*c* 1.3, MeOH);

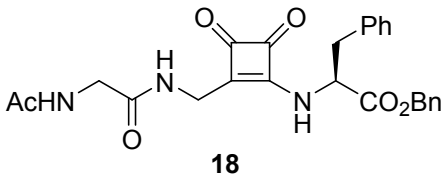
FTIR (neat) 3734, 3709, 3628, 3566, 3289, 1786, 1733, 1607, 1540, 1455, 1244, 1166 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.41-7.00 (m, 15H), 5.68 (br s, 1H), 5.19-5.10 (m, 3H), 4.45 (d, *J* = 6.3 Hz, 2H), 4.19 (t, *J* = 6.3 Hz, 1H), 4.12 (br s, 2H), 3.78 (br d, 2H), 3.25 (dd, *J* = 13.8, 5.1 Hz, 1H), 3.07 (dd, *J* = 13.8, 8.4 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 190.1, 183.7, 171.8, 170.0, 166.0, 156.7, 143.7, 143.6, 141.3, 134.8, 134.7, 129.4, 128.6, 128.5, 127.7, 127.3, 127.0, 124.9, 120.0, 67.7, 67.0, 57.8, 47.1, 44.3, 39.4, 32.5;

HRMS (FAB) *m/z* $(M+H)^+$ calcd for $[C_{38}H_{33}N_3O_7+H]^+$, 644.2391, found 644.2398.

AcHN-Gly-[Sq-Gly]-(L)-Phe-OBn (18)



18

To a solution of **17** (34.0 mg, 0.053 mmol) was added 20% Et_2NH/THF (1 mL) at room temperature. The mixture was stirred for 30 min and concentrated *in vacuo*. To the residue was added acetic anhydride (1 mL) at room temperature and stirred for 1 h and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 1/1 and EtOAc/MeOH = 40/1) to give **18** (17.5 mg, 72%) as yellow oil:

$[\alpha]^{30.0}_{D}$ -31.9 ^{o} (*c* 1.36, MeOH);

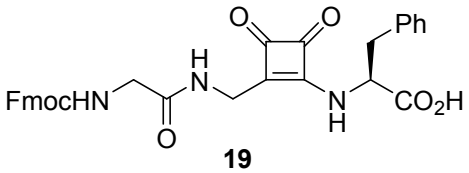
FTIR (neat) 3733, 3628, 3595, 3301, 2980, 2934, 1741, 1653, 1616, 1455, 1271, 1213, 1105, 1050, 753 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 9.0 Hz, 1H), 7.66 (t, *J* = 5.7 Hz, 1H), 7.37-7.08 (m, 10H), 6.57 (t, *J* = 5.4 Hz, 1H), 5.24-5.08 (m, 3H), 4.19 (dd, *J* = 15.9, 5.7 Hz, 1H), 4.19 (dd, *J* = 15.9, 5.7 Hz, 1H), 3.91 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.82 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.28 (dd, *J* = 14.1, 5.1 Hz, 1H), 3.09 (dd, *J* = 14.1, 9.0 Hz, 1H), 2.00 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 190.1, 183.7, 171.6, 171.3, 170.2, 166.1, 134.9, 134.7, 129.5, 128.7, 128.6, 128.5, 127.4, 67.8, 57.8, 43.2, 39.5, 32.6, 22.9;

HRMS (FAB) *m/z* $(M-H)^-$ calcd for $[C_{25}H_{25}N_3O_6-H]^-$, 462.1671, found 462.1660.

FmocHN-Gly-[Sq-Gly]-(L)-Phe-OH (19)



19

To a suspension of FmocHN-Phe-Wang resin (Watanabe Chemical Industries, Ltd., 0.67 mmol/resin(g), 68 mg, 0.046 mmol) in a small reaction tube with a membrane filter (LibraTube, HiPep Lab. Inc.) was added a solution of piperidine/DMF 1:4 (2 mL). The mixture was shaken with a vortex mixer for 1 min. The tube was equipped with a rotary shaker and mixing was continued for 15 min. The solution was removed by filtration and the resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). To a suspension of the residual resin in AcOEt (2 mL), FmocHN-Gly-[Sq-Gly]-O*i*-Pr **16** (41 mg, 0.091 mmol) was added. The mixture was shaken with a vortex mixer for 1 min and subjected to mixing with a rotary shaker for 24 h. The mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). This coupling procedure was repeated once again. The residual resin was moved to a test tube and treated with TFA (3 mL) for 3 h. The mixture was filtrated and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc (1% AcOH) = 2/1-0/1) to give **19** (20 mg, 80%, a 4:1 mixture of two rotamers) as white amorphous solid:

$[\alpha]^{27.5}_{D}$ -13.0 ^{o} (*c* 1.32, MeOH);

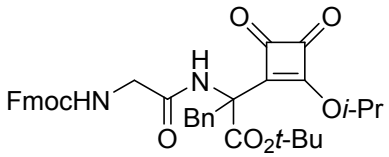
FTIR (neat) 3322, 3019, 2935, 1787, 1722, 1671, 1602, 1531, 1448, 1342, 1247, 1176, 1105, 1045, 993, 755, 701 cm^{-1};

^{1}H NMR (300 MHz, CD_3OD) δ 7.78 (d, *J* = 7.2 Hz, 2H), 7.65 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.2 Hz, 2H), 7.34-7.12 (m, 7H), 5.05 (dd, *J* = 9.9, 4.2 Hz, 4/5H), 4.62 (dd, *J* = 9.6, 4.2 Hz, 1/5H), 4.40 (d, *J* = 6.9 Hz, 8/5H), 4.36 (d, *J* = 6.9 Hz, 2/5H), 4.27-3.95 (m, 3H), 3.90-3.64 (m, 2H), 3.36 (m, 1H), 3.08 (m, 1H);

^{13}C NMR (75 MHz, CD_3OD) δ 193.4, 192.9, 192.3, 184.7, 173.3, 173.2, 172.4, 166.7, 164.3, 159.3, 145.2, 145.2, 142.6, 137.8, 137.6, 130.7, 130.5, 129.7, 129.6, 128.8, 128.2, 128.1, 126.2, 120.9, 68.2, 61.0, 59.6, 48.3, 45.0, 44.8, 39.9, 39.2, 34.6, 34.3;

HRMS (FAB) *m/z* $(M-H)^-$ calcd for $[C_{31}H_{27}N_3O_7-H]^-$ 552.1776, found 552.1777.

***tert*-Butyl 2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-(2-isopropoxy-3,4-dioxocyclobut-1-en-1-yl)-3-phenylpropanoate (20)**



20

To a solution of **15** (1.70 g, 3.10 mmol) in CH_2Cl_2 (30 mL) was added BnBr (2.95 mL, 24.8 mmol) and TBAI (9.15 g, 24.8 mmol) at room temperature. Et_3N (0.517 mL, 3.72 mmol) was added to the mixture. The mixture was stirred for 20 min and filtrated through a celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc = 10/1-1/1) to give **23** (1.75 g, 90%) as pale yellow amorphous solid:

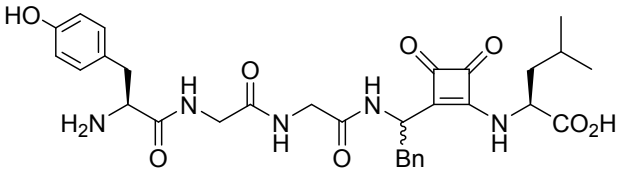
FTIR (neat) 3387, 3010, 2982, 2936, 1794, 1758, 1734, 1680, 1591, 1496, 1450, 1389, 1371, 1343, 1330, 1250, 1220, 1150, 1095, 1047, 1006 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$), δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.59 (dd, *J* = 7.6, 4.1 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.28-7.18 (m, 3H), 7.09 (d, *J* = 7.3 Hz, 2H), 6.98 (s, 1H), 5.50 (sept, *J* = 6.4 Hz, 1H), 5.40 (br s, 1H), 4.39 (m, 2H), 4.22 (t, *J* = 7.3 Hz, 1H), 3.93 (m, 2H), 3.90 (d, *J* = 13.9 Hz, 1H), 3.63 (d, *J* = 13.9 Hz, 1H), 1.50-1.44 (m, 12H), 1.43 (d, *J* = 6.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 195.7, 193.6, 191.1, 178.0, 168.3, 166.3, 156.3, 143.7, 141.2, 133.9, 130.0, 128.4, 127.7, 127.6, 127.0, 125.1, 119.9, 85.3, 80.3, 67.3, 63.3, 47.0, 44.3, 37.8, 27.8, 22.8, 22.7;

HRMS (FAB) *m/z* $(M-H)^-$calcd for $[C_{37}H_{38}N_2O_8-H]^-$ 637.2555, found 637.2565.

Solid phase synthesis of [Sq-Phe^4] Enkephalin 21



21

To a suspension of FmocHN-Leu-Wang resin (Watanabe Chemical Industries, Ltd., 0.60 mmol/resin(g), 167 mg, 0.10 mmol) in a reaction tube with a membrane filter (LibraTube, HiPep Lab. Inc.) was added a solution of piperidine/DMF 1:4 (2 mL). The reaction tube was vigorously shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 15 min, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). FmocHN-Gly-[Sq-Phe(CO_2*t*-Bu)]-O*i*-Pr (**20**) (255 mg, 0.40 mmol) and DIEA (35 μL, 0.20 mmol) was added to a suspension of the residual resin in AcOEt (2 mL). The reaction tube was vigorously shaken with a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 60 h, the mixture was filtrated and washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). The subsequent removal of the Fmoc group was carried out using Et_2NH/DMF 1:4 (2 mL) (In the case of using peperidine/DMF, a byproduct which peperidine added to carbonyl group of α-Asq in desired peptide observed by MALDI-TOF MS analysis after cleavage reaction from the resin.). FmocHN-Gly-OH, HBTU, HOBt, and DIEA (0.4 mmol, 0.36 mmol, 0.4 mmol, and 0.8 mmol) were added to a suspension of the residual resin in DMF (2 mL). The reaction tube was shaken with a vortex mixer for 2 min and equipped with a rotary shaker. After mixing for 1.5 h, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). According to the methods described above, the subsequent removal of the Fmoc group using Et_2NH/DMF 1:4 (2 mL) and coupling reaction of Fmoc-Tyr(*t*-Bu)-OH were carried out. After the removal of the Fmoc group, the residual resin was moved to a test tube and treated with TFA (3 mL) for 3 h. The mixture was filtrated and concentrated *in vacuo*. The crude mixture (36 mg) was purified by flash column chromatography on Cosmosil^{R} (H_2O/MeOH = 2/1-1/1) and reverse-phase HPLC (Column : Nacalai Cosmosil 5C_{18}-MS-Ⅱ, Column Size : 20 x 250 mm, solvent 33% MeCN (0.1% TFA)/H_2O (0.1% TFA), flow rate 6 mL/min, temperature : 25 ^{o}C, UV detection at 254 nm, sample conc : 36 mg/mL, injection volume 90 μL) to give **21a** (7.5 mg ; retention time at 17.5 min) and **21b** (10 mg ; retention time at 20.9 min) as pale yellow sticky oil, respectively: HPLC profile is shown in the next page. NMR signals are multiple and broaden because of the presence of rotamers arising from the N-Sq bond (see attached spectral data of ^{1}H- and ^{13}C-NMR (300 MHz, CD_3OD) of **21a** and **21b** in SI).

21a

$[\alpha]^{21.9}_{D}$ +9.7 ^{o} (*c* 0.65, MeOH);

FTIR (neat) 2959, 1785, 1733, 1678, 1604, 1519, 1430, 1253, 1202, 1142, 1027 cm^{-1};

HRMS (ESI) *m/z* $(M+H)^+$calcd for $[C_{31}H_{37}N_5O_8+H]^+$ 608.2715, found 608.2714.

HRMS (ESI) *m/z* $(M+Na)^+$calcd for $[C_{31}H_{37}N_5O_8+Na]^+$ 630.2534, found 630.2534.

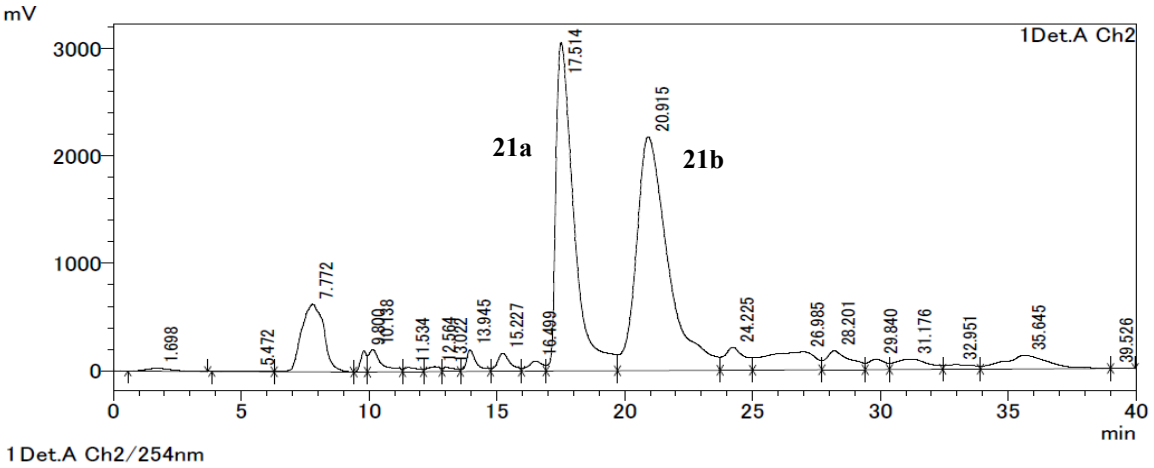
21b

$[\alpha]^{22.4}_{D}$ +0.35 ^{o} (*c* 0.69, MeOH);

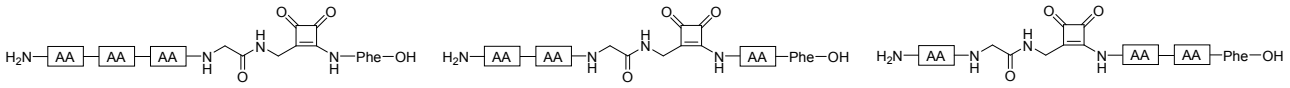
FTIR (neat) 2969, 1785, 1734, 1675, 1604, 1517, 1457, 1364, 1231, 1203, 1142, 1029 cm^{-1};

HRMS (ESI) *m/z* $(M+H)^{+}$calcd for $[C_{31}H_{37}N_5O_8+H]^{+}$ 608.2715, found 608.2715.

HRMS (ESI) *m/z* $(M+Na)^{+}$calcd for $[C_{31}H_{37}N_5O_8+Na]^{+}$ 630.2534, found 630.2533.



Solid phase synthesis of peptide libraries 22-24



22 **23** **24**

Coupling reactions were performed by a series of sequential transformations.

22: (a), (b), (c), (d), (c), (d), (c), (d), (c), (e)

23: (a), (d), (a), (b), (c), (d), (c), (d), (c), (e)

24: (a), (d), (a), (d), (a), (b), (c), (d), (c), (e)

The solid phase synthesis of libraries **22-24** was performed in a small reaction tube with a membrane filter (LibraTube, HiPep Lab. Inc.) using FmocHN-Phe-Wang resin (Watanabe Chemical Industries, Ltd., 0.67 mmol/resin(g), 68 mg, 0.046 mmol).

(a) Removal of the Fmoc group from peptides not containing the α-Asq

The resin was treated with piperidine/DMF 1:4 (2 mL). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 15 min. the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

(b) Linkage of FmocHN-Gly-[Sq-Gly]-O*i*-Pr (**16**)

To a suspension of the NH_2 group-free peptide linked to the resin in AcOEt (2 mL) were added **16** (41 mg, 0.091 mmol). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 24 h, the mixture was filtered. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2). This procedure was repeated once again.

(c) Removal of the Fmoc group from peptides containing the α-Asq

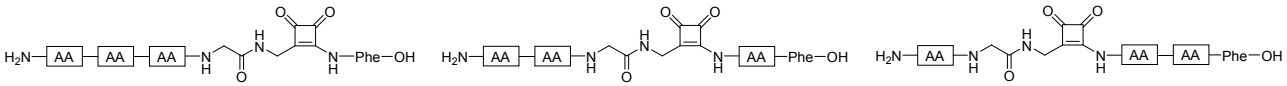
The resin was treated with Et_2NH/DMF 1:4 (2 mL). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 15 min, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

(d) Coupling reaction of Fmoc-amino acids

To a suspension of the residual resin in DMF (2 mL) were added a mixture of amino acids (FmocHN-Phe-OH/FmocHN-Tyr(*t*-Bu)-OH/FmocHN-His(Trt)-OH: 0.60 mmol each), HBTU (1.64 mmol), HOBt (1.82 mmol), and DIEA (3.64 mmol). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 1.5 h, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

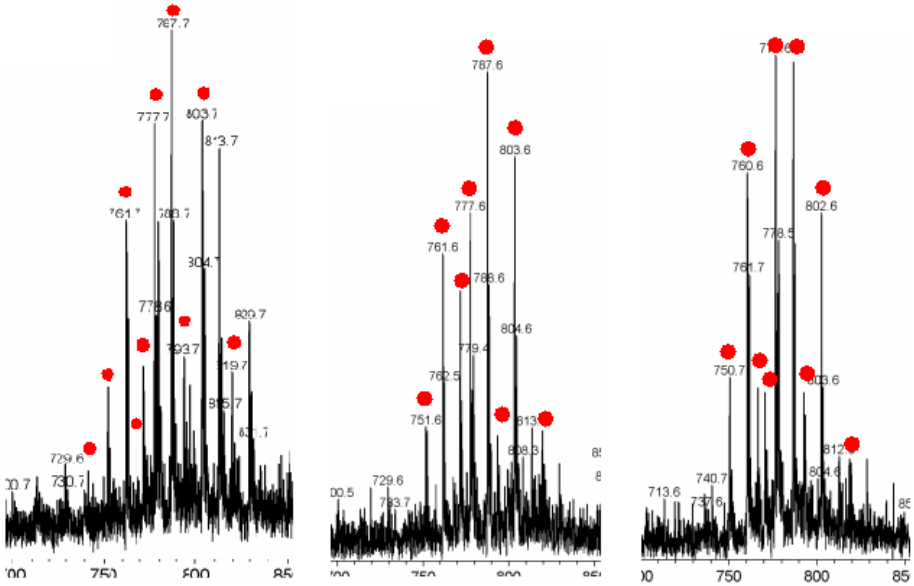
(e) Cleavage of the peptide from resin

The residual resin was moved to a test tube and treated with TFA/H_2O 19:1 (3 mL) for 3 h. The mixture was filtrated. The filtrate was concentrated *in vacuo* to give a crude mixture (38 mg). MALDI-TOF MS data of the crude mixtures were depicted in SI-Figure 1.



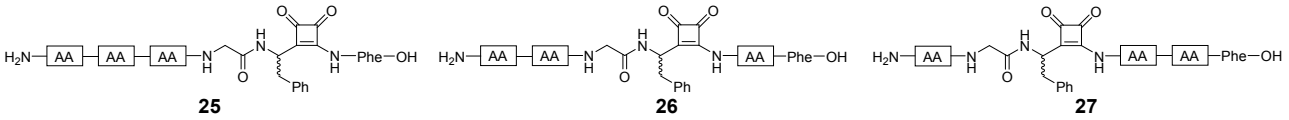
22 **23** **24**

Matrix: α-CHCA, Negative mode



SI-Figure 1. MALDI-TOF MS Analysis of Peptide Library **22-24**.

Solid phase synthesis of peptide libraries 25-27



25 **26** **27**

Coupling reactions were performed by a series of sequential transformations.

25: (a), (b), (c), (d), (c), (d), (c), (d), (c), (e)

26: (a), (d), (a), (b), (c), (d), (c), (d), (c), (e)

27: (a), (d), (a), (d), (a), (b), (c), (d), (c), (e)

The solid phase synthesis of libraries **25-27** was performed in a small reaction tube with a membrane filter (LibraTube, HiPep Lab. Inc.) using FmocHN-Phe-Wang resin (Watanabe Chemical Industries, Ltd., 0.67 mmol/resin(g), 150 mg, 0.10 mmol).

(a) Removal of the Fmoc group from peptides not containing the α-Asq

The resin was treated with piperidine/DMF 1:4 (2 mL). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 15 min. the mixture was filtrated. The residual resin was washed with DMF (2 mL x 5) and CH_2Cl_2 (2 mL x 2).

(b) Linkage of dipeptide unit **20**

To a suspension of the NH_2 group-free peptide linked to the resin in AcOEt (2 mL) were added **20** (116 mg, 0.40 mmol) and DIEA (35 μL, 0.20 mmol). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 60 h, the mixture was filtered. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

(c) Removal of the Fmoc group from peptides containing the α-Asq

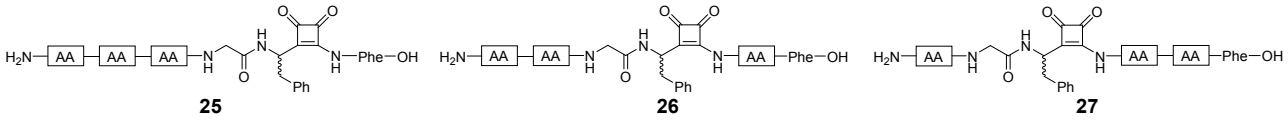
The resin was treated with Et_2NH/DMF 1:4 (2 mL). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 15 min, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

(d) Coupling reaction of Fmoc-amino acids

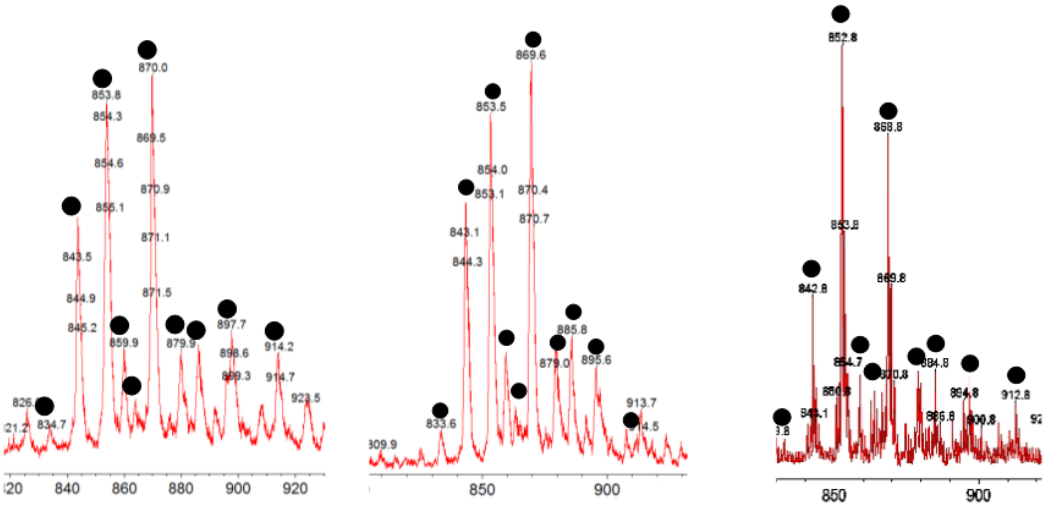
To a suspension of the residual resin in DMF (2 mL) were added a mixture of amino acids [FmocHN-Phe-OH/FmocHN-Tyr(*t*-Bu)-OH/FmocHN-His(Trt)-OH (1.33 mmol each)], HBTU (3.6 mmol), HOBt (4 mmol), and DIEA (8 mmol). The reaction tube was shaken by a vortex mixer for 1 min and equipped with a rotary shaker. After mixing for 1.5 h, the mixture was filtrated. The residual resin was washed with DMF (2 mL x 3) and CH_2Cl_2 (2 mL x 2).

(e) Cleavage of the peptide from resin

The residual resin was moved to a test tube and treated with TFA/H_2O 19:1 (3 mL) for 3 h. The filtrate was concentrated *in vacuo* to give a crude mixture (ca. 105 mg). MALDI-TOF MS data of the crude mixtures were depicted in SI-Figure 2.

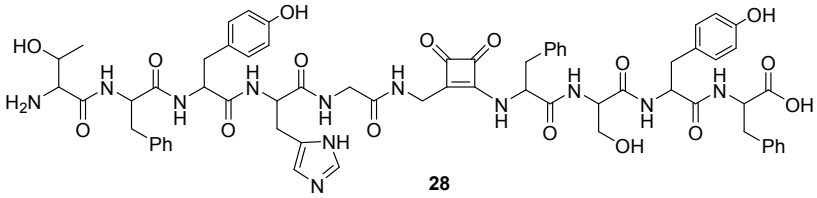


Matrix: α-CHCA, Positive mode



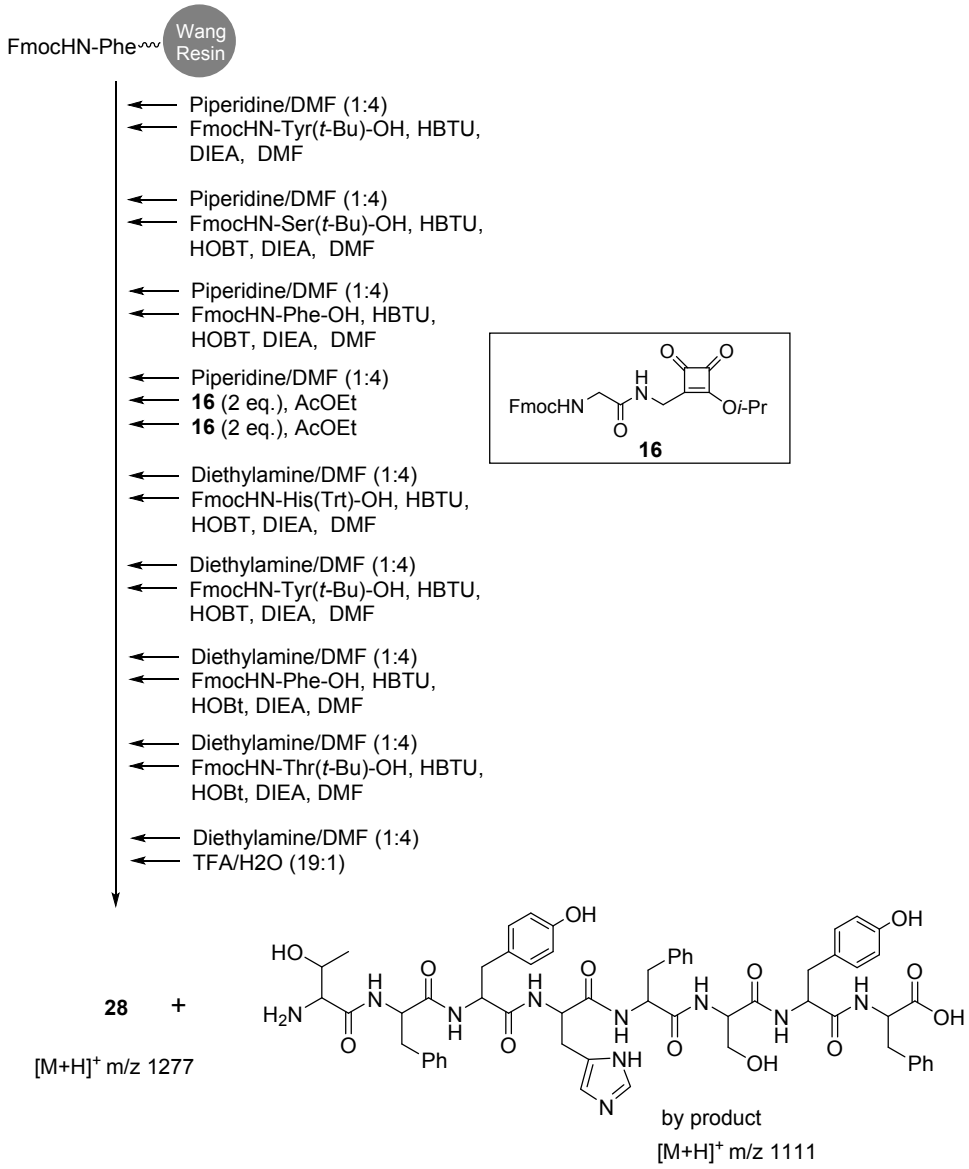
SI-Figure 2. MALDI-TOF MS data of Peptide library **25**-**27**. Matrix: α-CHCA, Positive mode.

Solid phase synthesis of 28



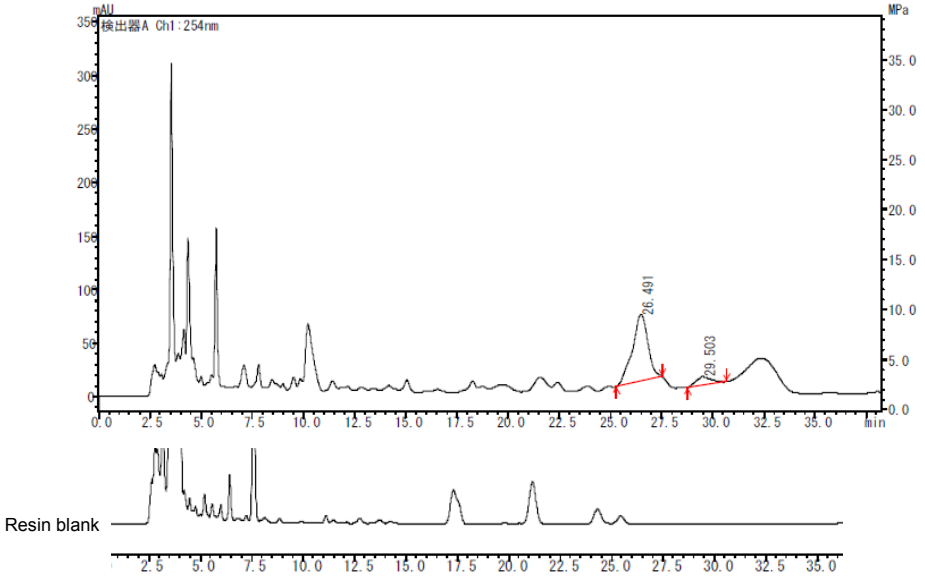
28

In according to the method for the solid phase synthesis of peptide libraries **20**-**22**, the model peptide **28** was prepared starting from the resin (0.05 mmol) by the following coupling sequence.



HPLC purification of the resulting peptide mixture

10 µL of a solution of the crude peptide in H_2O (24 mg/mL) was subjected to reverse phase HPLC several times [column: Nacalai Cosmosil Packed Column Cholester, Column Size : 4.6 x 250 mm, solvent: 25% MeCN (0.1% TFA)/H_2O (0.1% TFA), flow rate: 1 mL/min, UV detection at 254 nm, retention time: **28** for 26.5 min, by product for 29.5 min). After lyophilization, peptide analog **28** (< 0.1 mg) was obtained. The purity was confirmed by the MALDI-TOF MS analysis (Figure 2 (a) in the text).

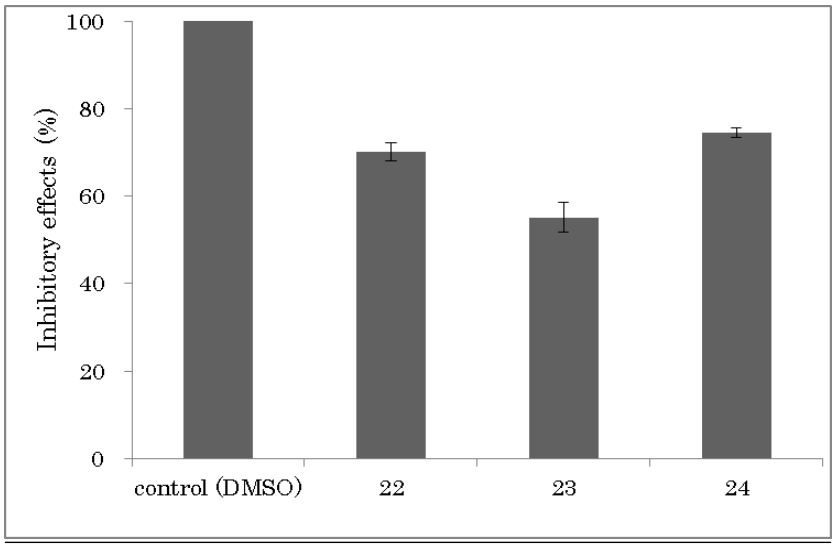


Reaction of 28 with carboxypeptidase Y

Peptide **28** purified by the HPLC purification described above was dissolved in water (15 µL). A buffer solution of carboxypeptidase Y (5 µL, 2.5 mM / 50 mM ammonium bicarbonate) was added to a solution 5 µL of peptide solution in an eppendorf-tube. The reaction mixture was incubated at 30 °C for 30 min. The time course of the reaction was monitored by by MALDI-TOF/MS (see Figure 4. in the text)

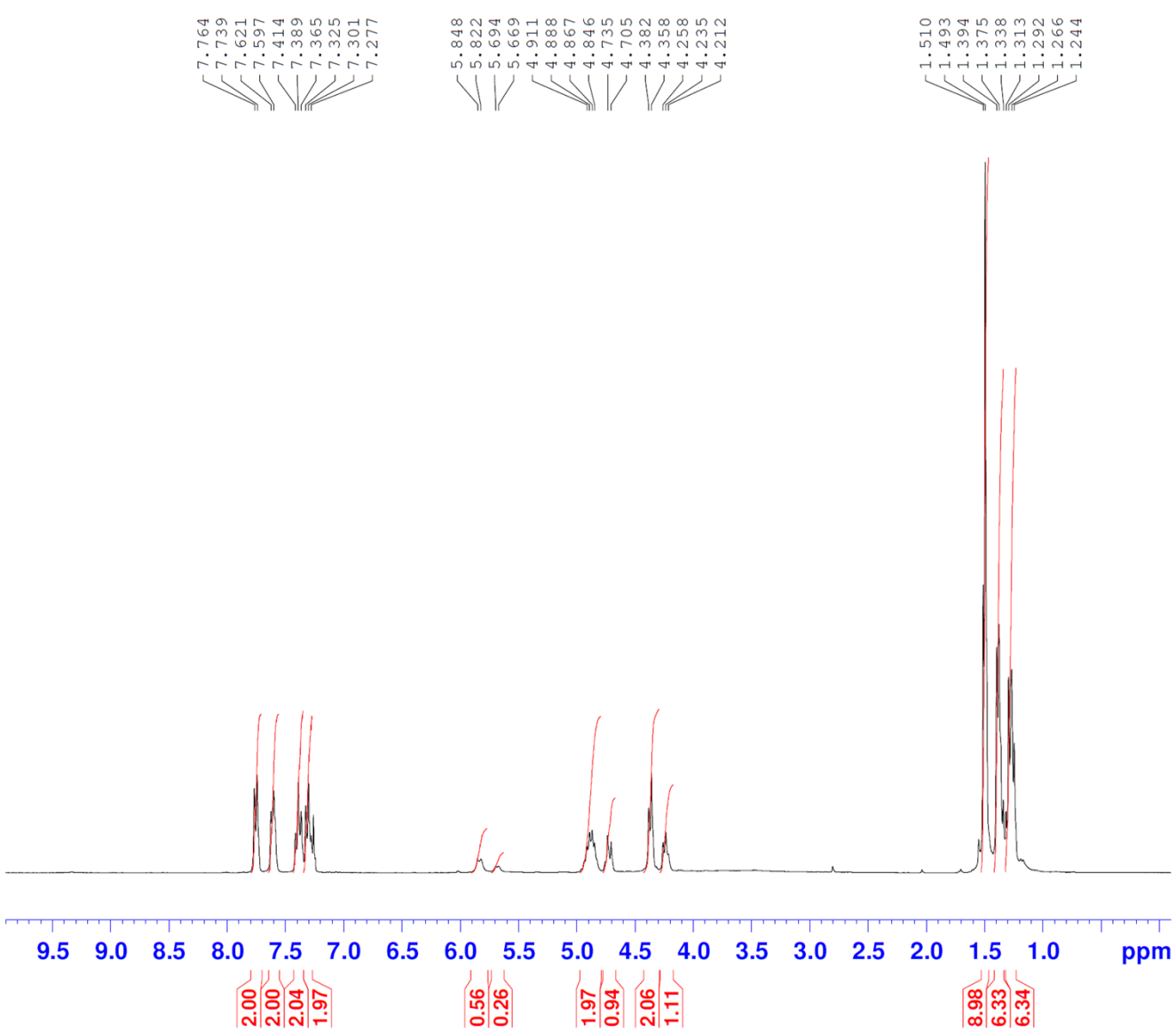
Growth inhibitory effects of HepG2 Cell

According to the modified method of a previous paper (Yang et al., 2007), the cultures of rat hepatoma cells (dRLh84) were maintained at 37 °C in humidified atmosphere containing 5% CO_2. Exponentially growing cells were trypsinized, seeded at an appropriate density and incubated for 24 h to allow adhesion and growth. Trypsinized cells were suspended in the culture medium at a density of 5 x 104 cells/mL and 100 μL of the suspension was dispended into each well of a 96-well microtiter plate. In a 24 h pre-incubation , DMSO used as a control or peptides for assays were added to culture medium of cells. After subsequent 48 h incubation, cells were trypsinized and determined by means of counting in Burker-Turk hemacytometer. Results were expressed as means±S.E. of three independent experiments.



P. Yang, S. Abe, Y. Sato, T. Yamashita, F. Mtsuda, T. Hamayasu, K. Imai and K. Suzuki (2007) A palmitonyl conjugate of an insect pentapeptide causes growth arrest in mammalian cells and mimics the action of diapause hormone. *J. Insect Biotech. Sericol.* **76**, 63-69.

^{1}H- and ^{13}C-NMR data of new compounds.



BRUKER

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PROCNO                   1

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DS                     2
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RG                    32
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DE                 6.50 usec
TE                295.4 K
D1             1.00000000 sec

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P1                 15.00 usec
PLW1          7.50000000 W
SFO1         300.1318534 MHz

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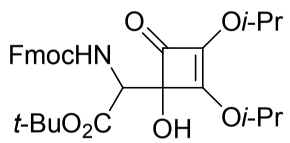
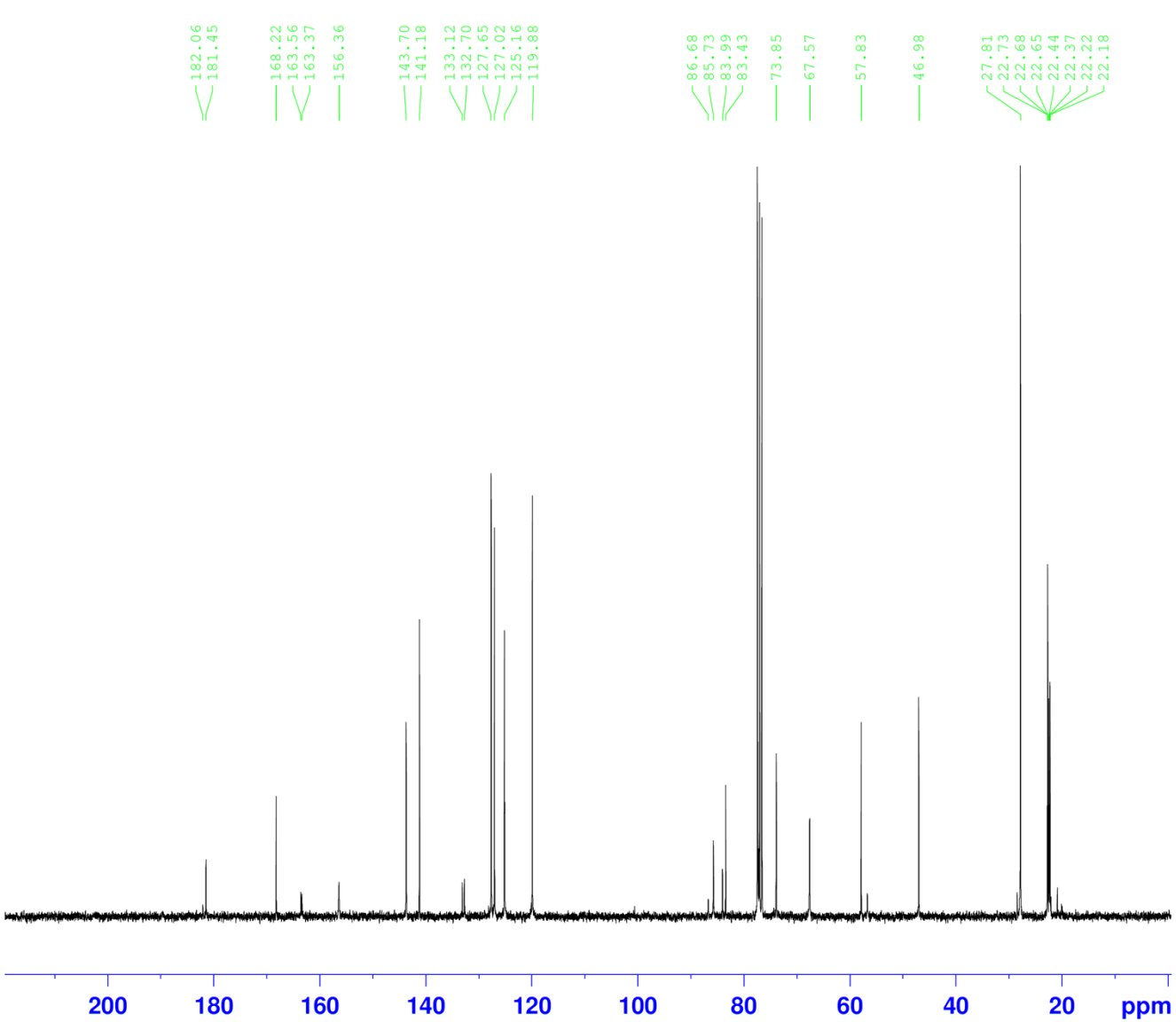


7



BRUKER

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PLW1         32.00000000 W
SFO1          75.4752953 MHz

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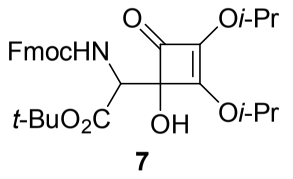
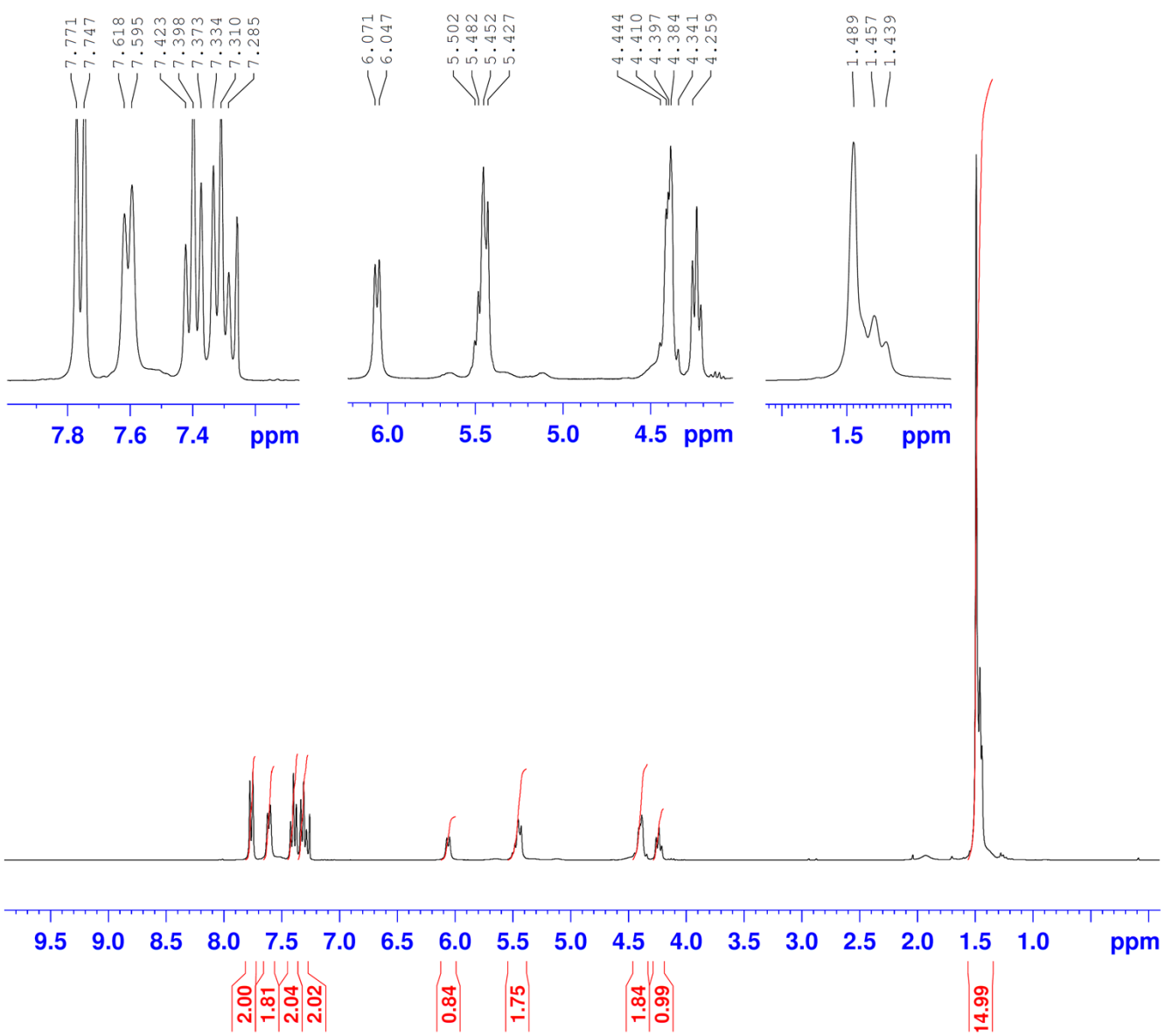


7



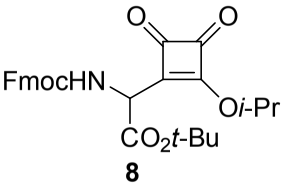
BRUKER

```
Current Data Parameters
NAME      MK-Fmoc-SqGly(CO2Bu)-OPr-H1
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140719
Time              22.39
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   2
SWH           6188.119 Hz
FIDRES       0.094423 Hz
AQ           5.2953587 sec
RG                  36
DW               80.800 usec
DE                6.50 usec
TE              295.4 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                15.00 usec
PLW1         7.50000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                32768
SF          300.1300064 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```



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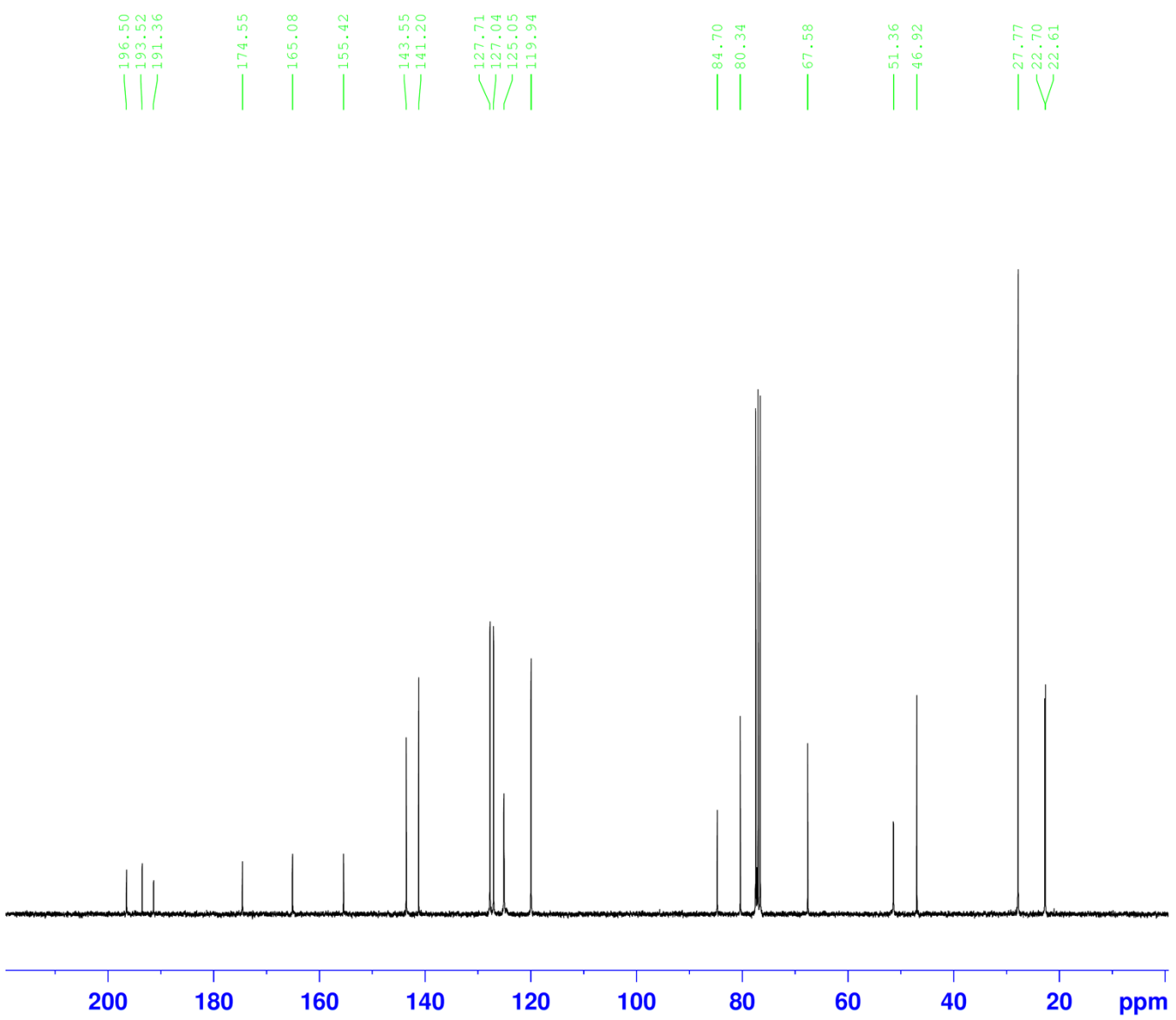


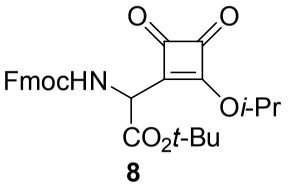
Current Data Parameters
NAME MK-Fmoc-SqGly(CO2Bu)-OPr-C13
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140719
Time 23.57
INSTRUM av300
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1200
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 203
DW 27.733 usec
DE 6.50 usec
TE 296.1 K
D1 2.00000000 sec
D11 0.03000000 sec

======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PLW1 32.00000000 W
SFO1 75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PLW2 7.80000019 W
PLW12 0.21667001 W
PLW13 0.17550001 W
SFO2 300.1312005 MHz

F2 - Processing parameters
SI 32768
SF 75.4677560 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



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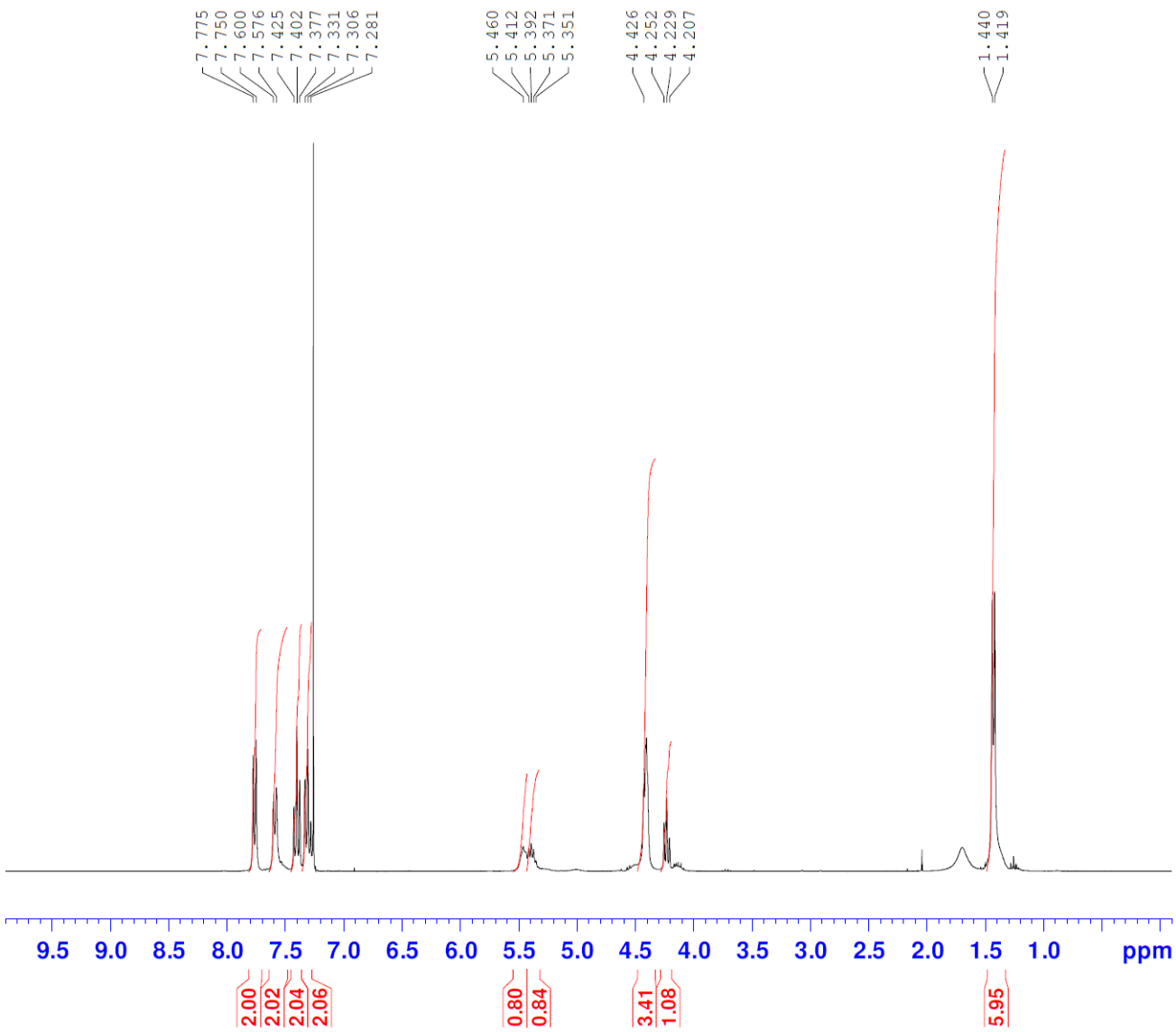


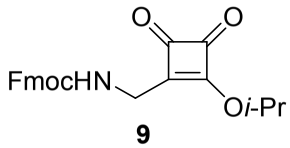
BRUKER

```
Current Data Parameters
NAME      MK-Fmoc-SqGly-OPr-H1
EXPNO                   40
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140724
Time              10.28
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG           zg30
TD                65536
SOLVENT           CDCl3
NS                  48
DS                   2
SWH            6188.119 Hz
FIDRES         0.094423 Hz
AQ             5.2953587 sec
RG                 203
DW               80.800 usec
DE                6.50 usec
TE               295.5 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                15.00 usec
PLW1         7.50000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                32768
SF          300.1300067 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



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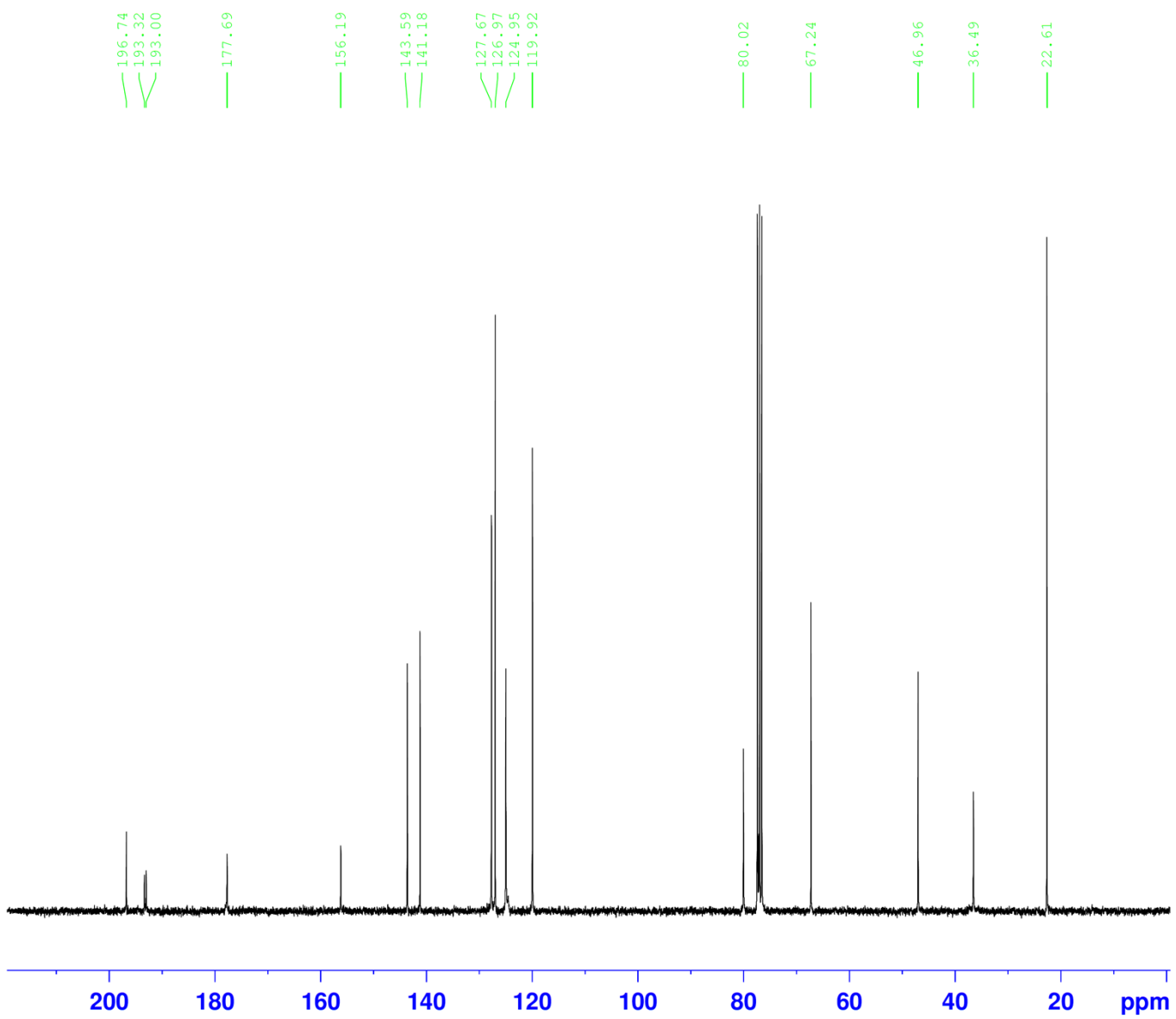
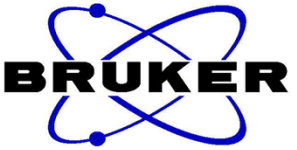




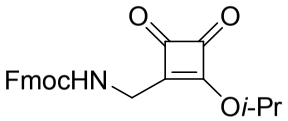

```
Current Data Parameters
NAME      MK-Fmoc-SqGly-OPr-C13
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_             20140721
Time                  5.16
INSTRUM             av300
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                    2400
DS                       2
SWH              18028.846 Hz
FIDRES            0.275098 Hz
AQ                1.8175818 sec
RG                     203
DW                  27.733 usec
DE                   6.50 usec
TE                  296.1 K
D1              2.00000000 sec
D11             0.03000000 sec

======== CHANNEL f1 ========
NUC1                   13C
P1                   10.00 usec
PLW1          32.00000000 W
SFO1           75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2                90.00 usec
PLW2           7.80000019 W
PLW12          0.21667001 W
PLW13          0.17550001 W
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                   32768
SF              75.4677586 MHz
WDW                    EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```



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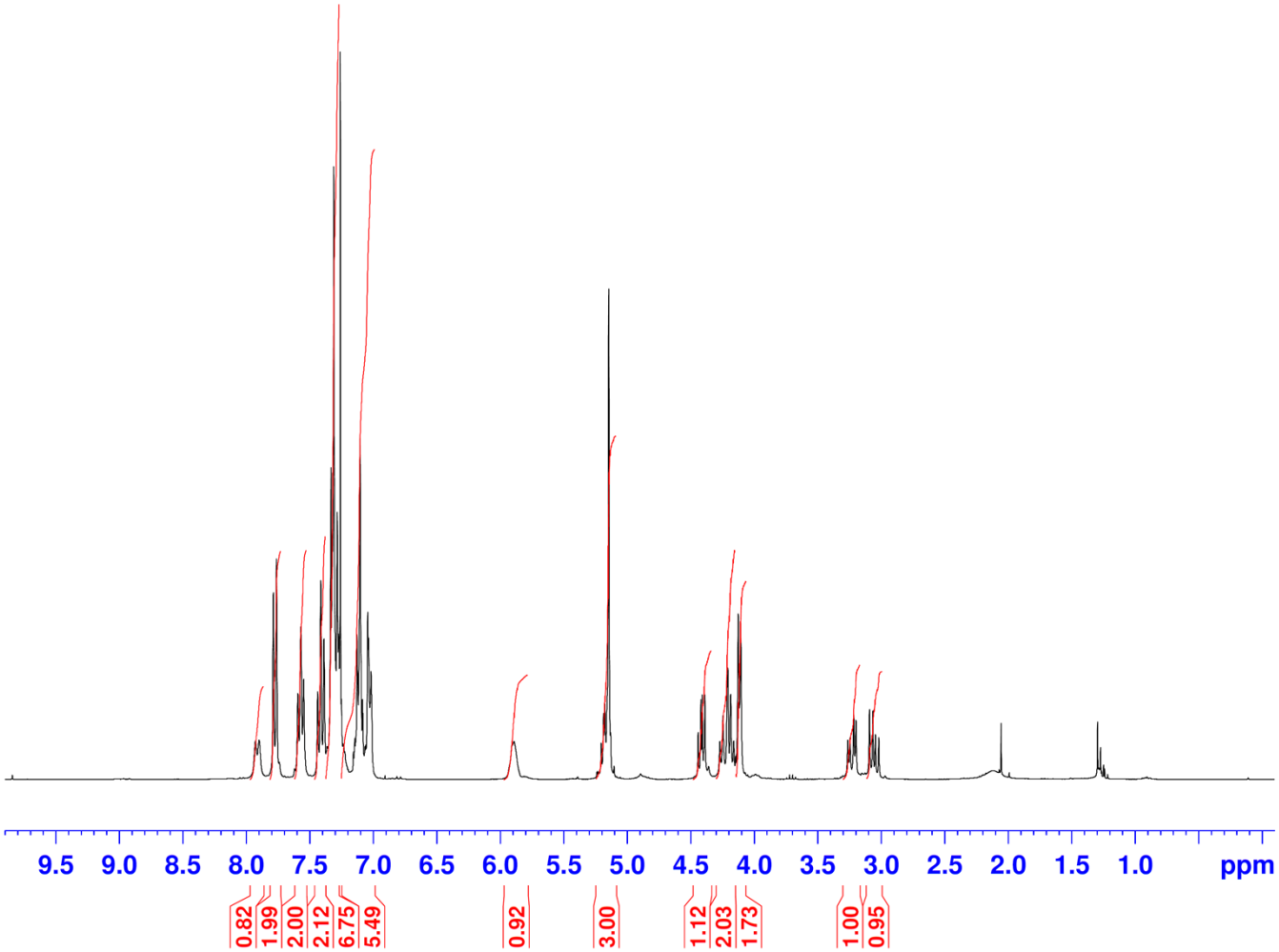
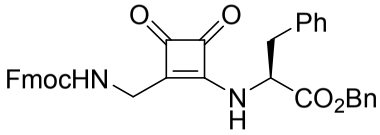



```
Current Data Parameters
NAME      MK-Fmoc-SqGly-Phe-OBn-H1
EXPNO                   20
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140722
Time                6.39
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6188.119 Hz
FIDRES          0.094423 Hz
AQ             5.2953587 sec
RG                    36
DW               80.800 usec
DE                6.50 usec
TE               295.6 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                15.00 usec
PLW1          7.50000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300063 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



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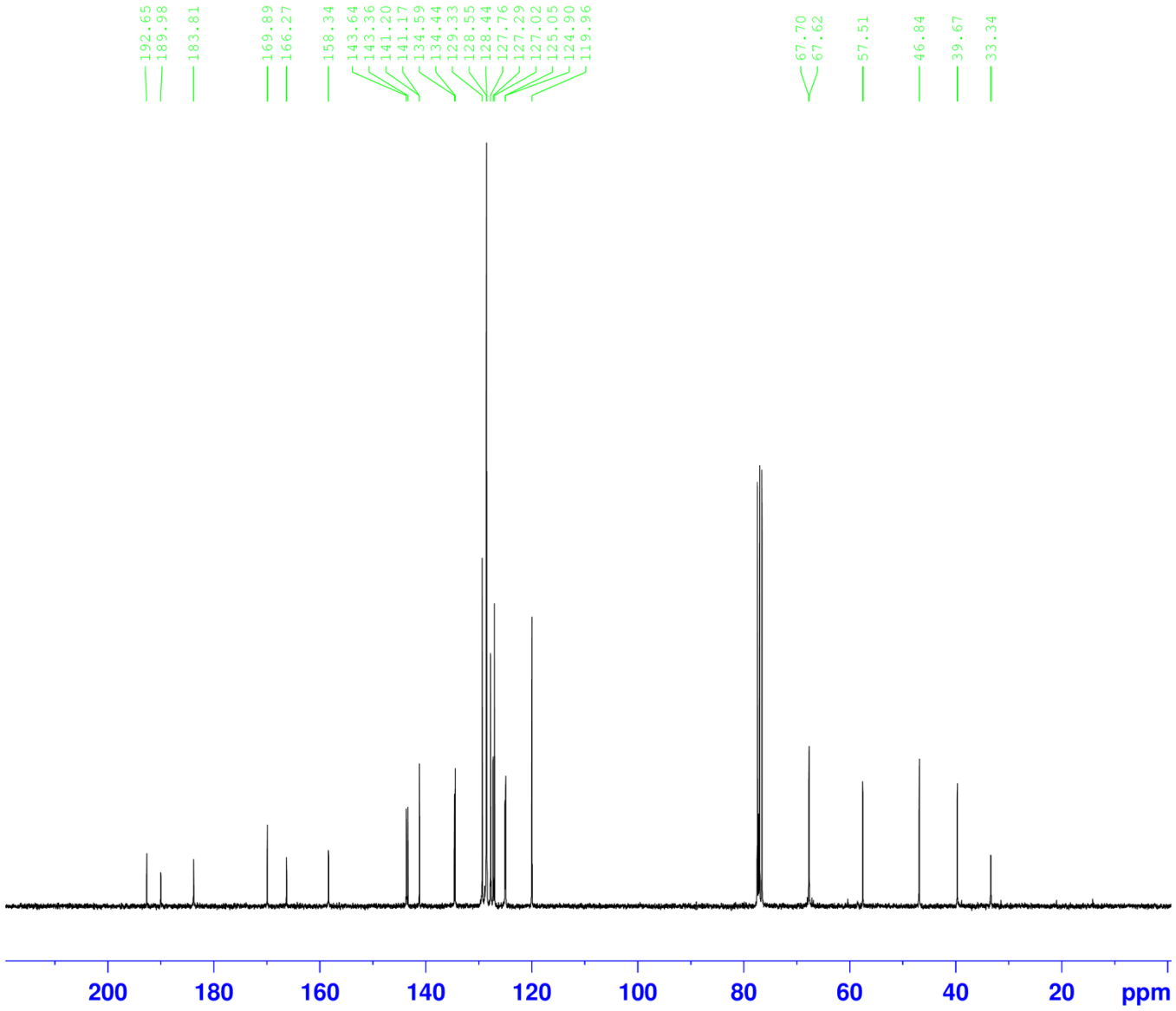
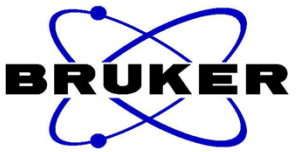




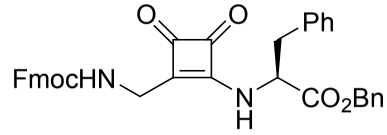
```
Current Data Parameters
NAME      MK-Fmoc-SqGly-Phe-OBn-C13
EXPNO                20
PROCNO                1

F2 - Acquisition Parameters
Date_          20140722
Time               8.23
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1600
DS                    2
SWH           18028.846 Hz
FIDRES         0.275098 Hz
AQ            1.8175818 sec
RG                  203
DW               27.733 usec
DE                6.50 usec
TE                296.3 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1               10.00 usec
PLW1        32.00000000 W
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2            90.00 usec
PLW2         7.80000019 W
PLW12        0.21667001 W
PLW13        0.17550001 W
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF           75.4677591 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



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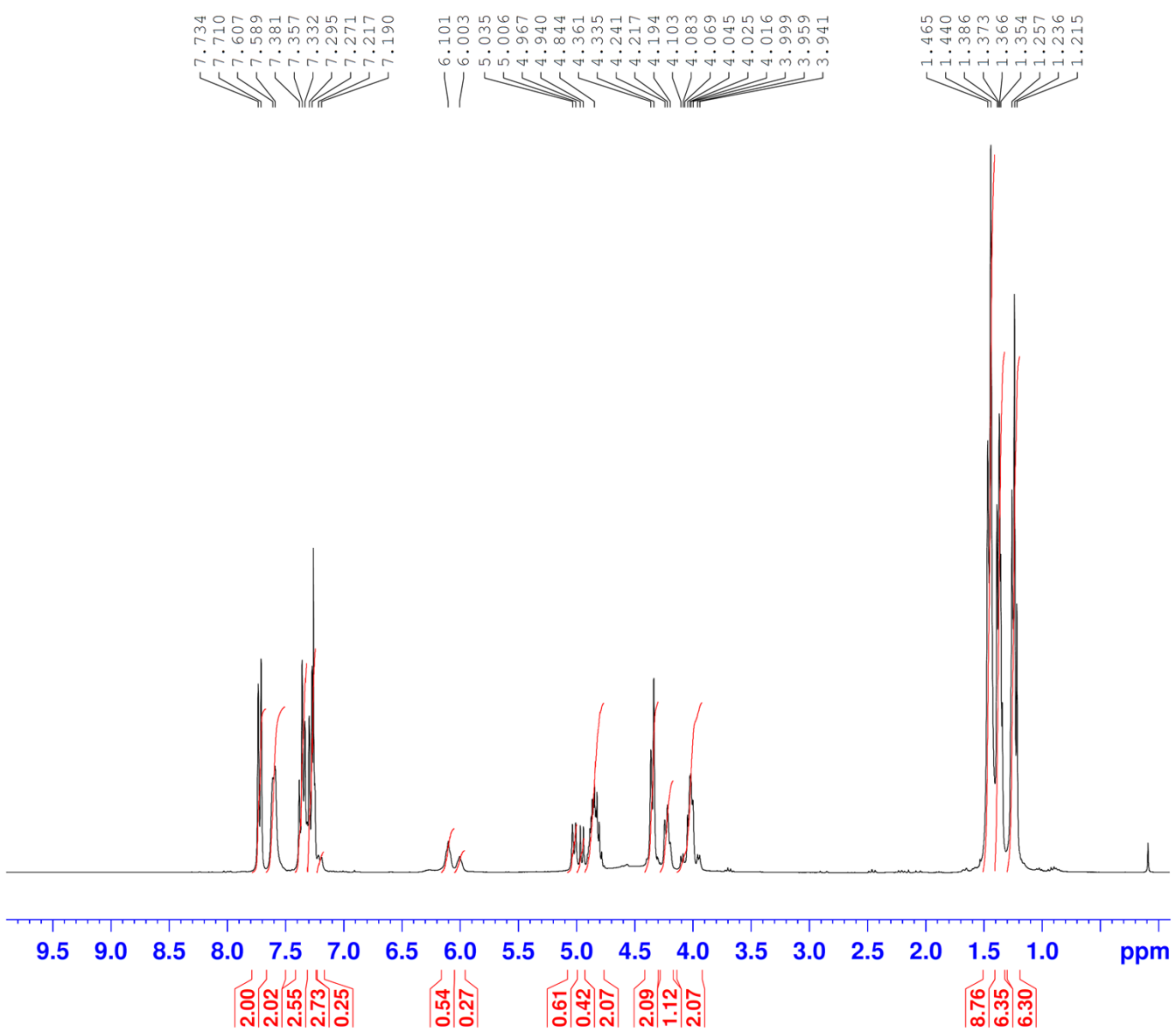

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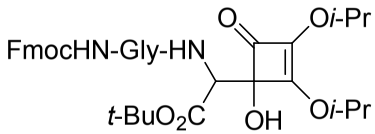
```
Current Data Parameters
NAME      MK-FmocGly-Gly-HCB-H1
EXPNO                   10
PROCNO                   1

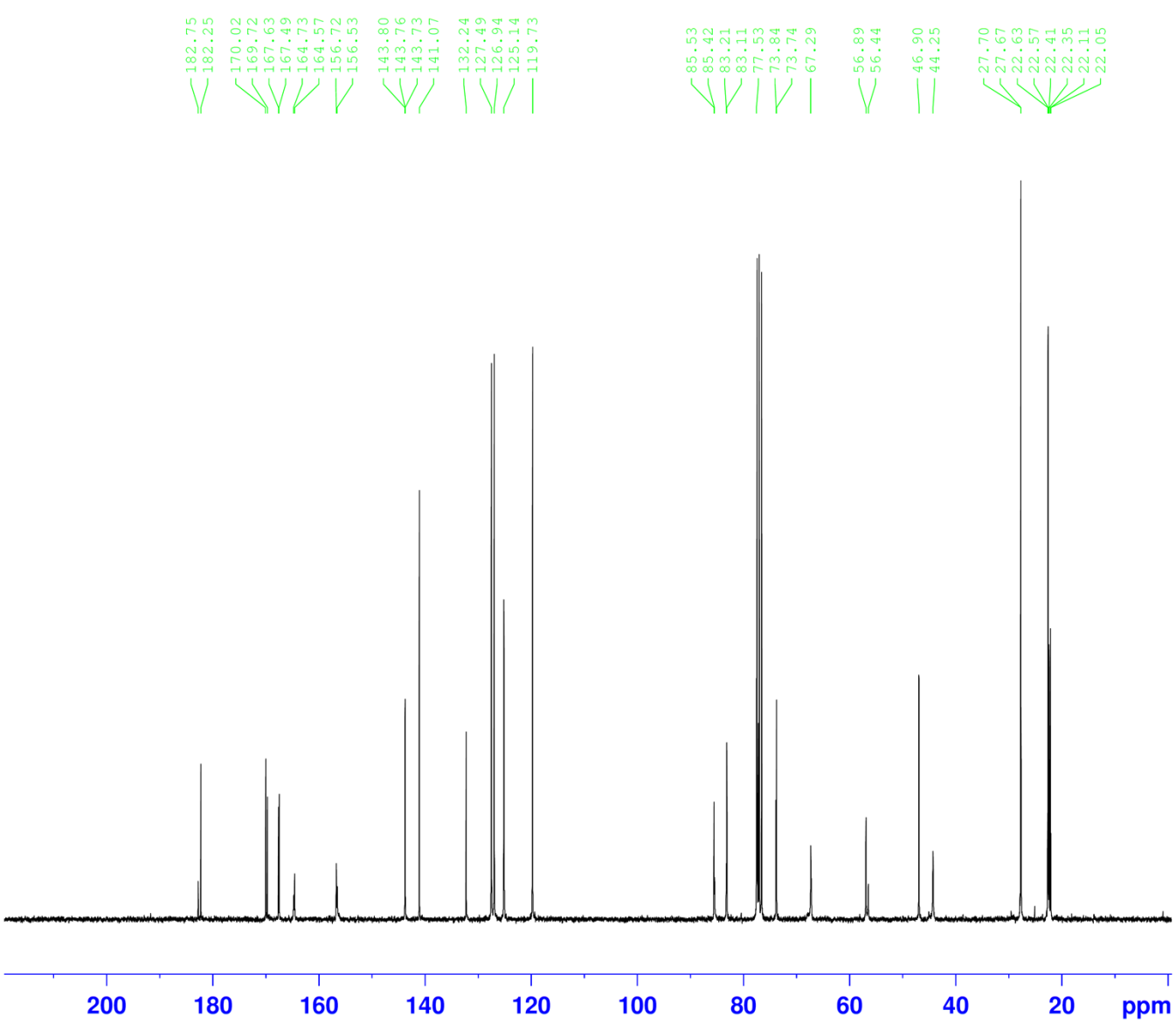
F2 - Acquisition Parameters
Date_           20140720
Time                2.28
INSTRUM           av300
PROBHD    5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6188.119 Hz
FIDRES          0.094423 Hz
AQ              5.2953587 sec
RG                  22.6
DW                80.800 usec
DE                 6.50 usec
TE                295.4 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  15.00 usec
PLW1          7.50000000 W
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                  32768
SF           300.1300064 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



14



BRUKER

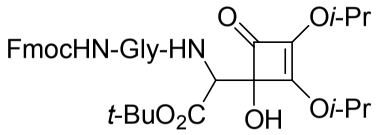
```
Current Data Parameters
NAME      MK-FmocGly-Gly-HCB-C13
EXPNO                         10
PROCNO                         1

F2 - Acquisition Parameters
Date_               20140720
Time                    5.04
INSTRUM                av300
PROBHD    5 mm PABBO BB-
PULPROG                zgpg30
TD                      65536
SOLVENT                 CDC13
NS                       2400
DS                          2
SWH                 18028.846 Hz
FIDRES                0.275098 Hz
AQ                    1.8175818 sec
RG                          203
DW                     27.733 usec
DE                      6.50 usec
TE                     296.0 K
D1                  2.00000000 sec
D11                 0.03000000 sec

======== CHANNEL f1 ========
NUC1                       13C
P1                      10.00 usec
PLW1               32.00000000 W
SFO1                75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                   90.00 usec
PLW2                7.80000019 W
PLW12               0.21667001 W
PLW13               0.17550001 W
SFO2                300.1312005 MHz

F2 - Processing parameters
SI                        32768
SF                  75.4677605 MHz
WDW                          EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
```



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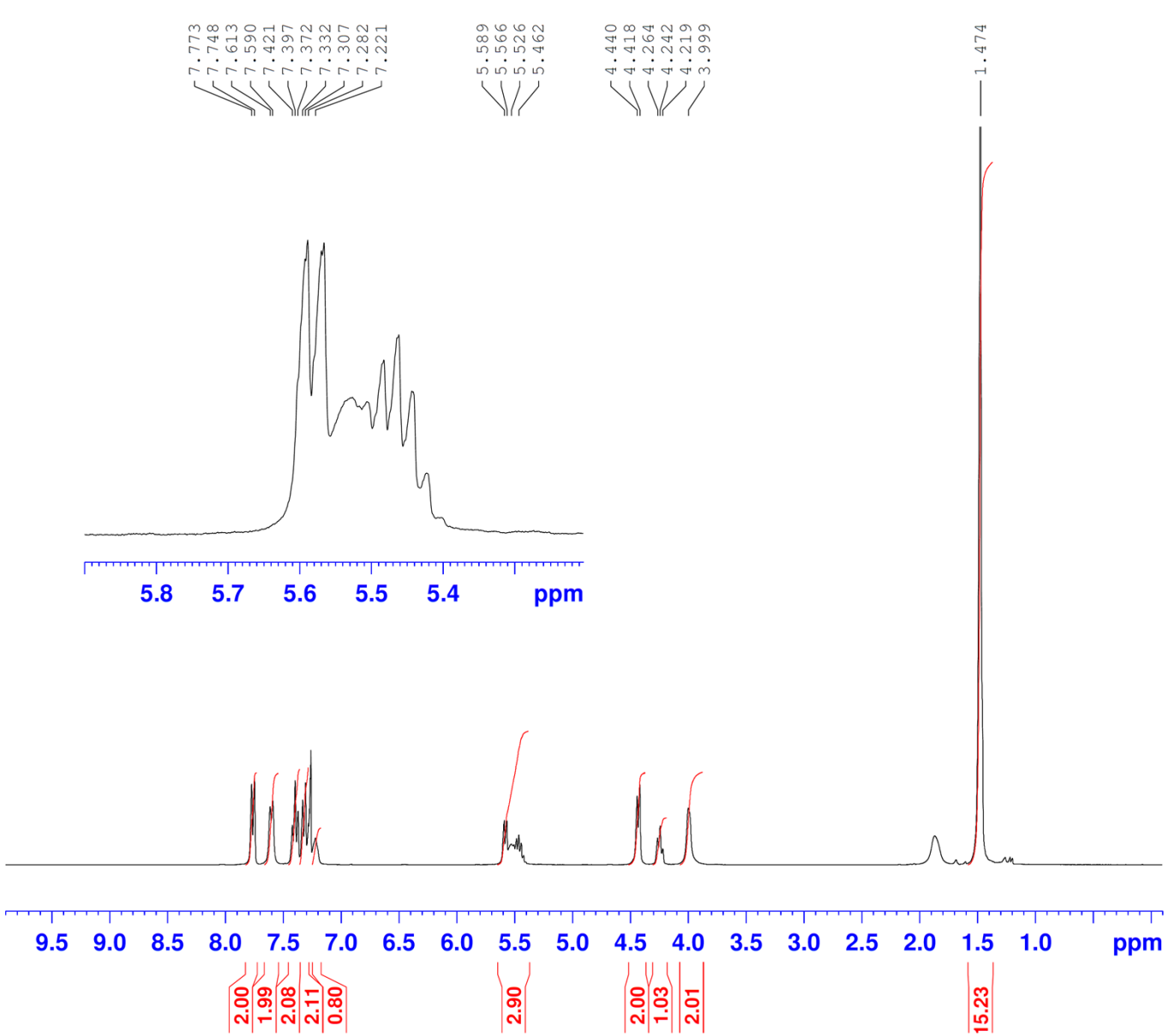


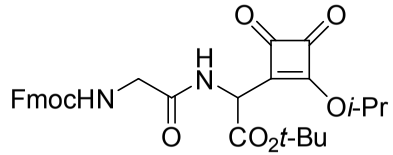
BRUKER

```
Current Data Parameters
NAME      MK-Fmoc-Gly-SqGly(CO2Bu)-OPr-H1
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140720
Time              13.31
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT           CDC13
NS                   48
DS                    2
SWH            6188.119 Hz
FIDRES        0.094423 Hz
AQ            5.2953587 sec
RG                  203
DW               80.800 usec
DE                6.50 usec
TE                295.8 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1               15.00 usec
PLW1         7.50000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                32768
SF          300.1300050 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```



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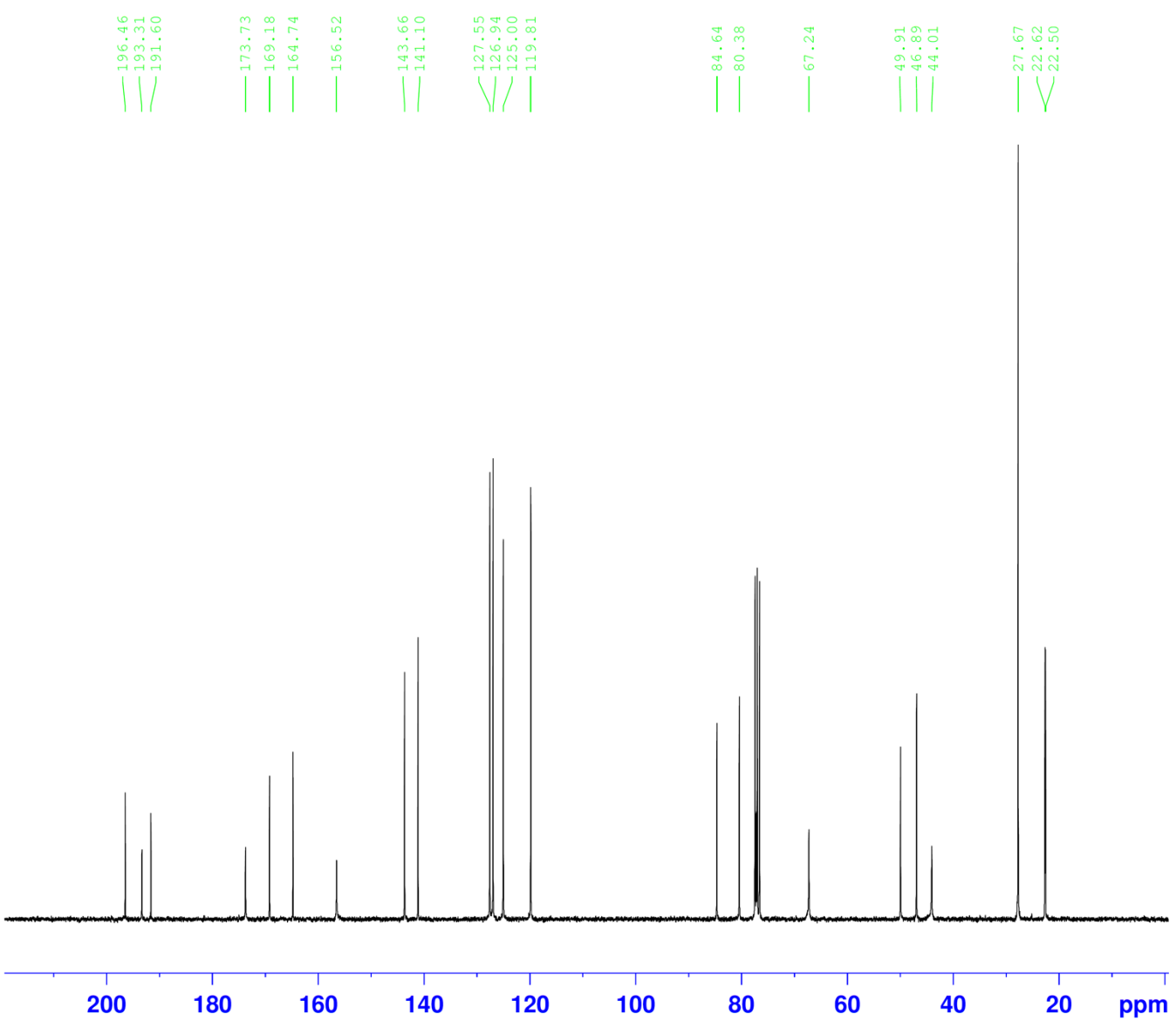
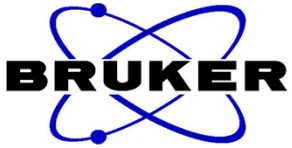




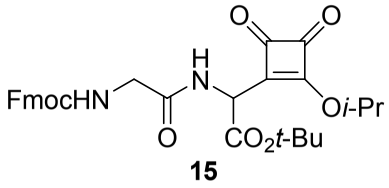

```
Current Data Parameters
NAME      MK-FmocGly-SqGly(CO2Bu)-OPr-C13
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140720
Time                7.43
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2400
DS                    2
SWH           18028.846 Hz
FIDRES         0.275098 Hz
AQ             1.8175818 sec
RG                  203
DW               27.733 usec
DE                6.50 usec
TE                296.0 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PLW1         32.00000000 W
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            90.00 usec
PLW2          7.80000019 W
PLW12         0.21667001 W
PLW13         0.17550001 W
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4677611 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



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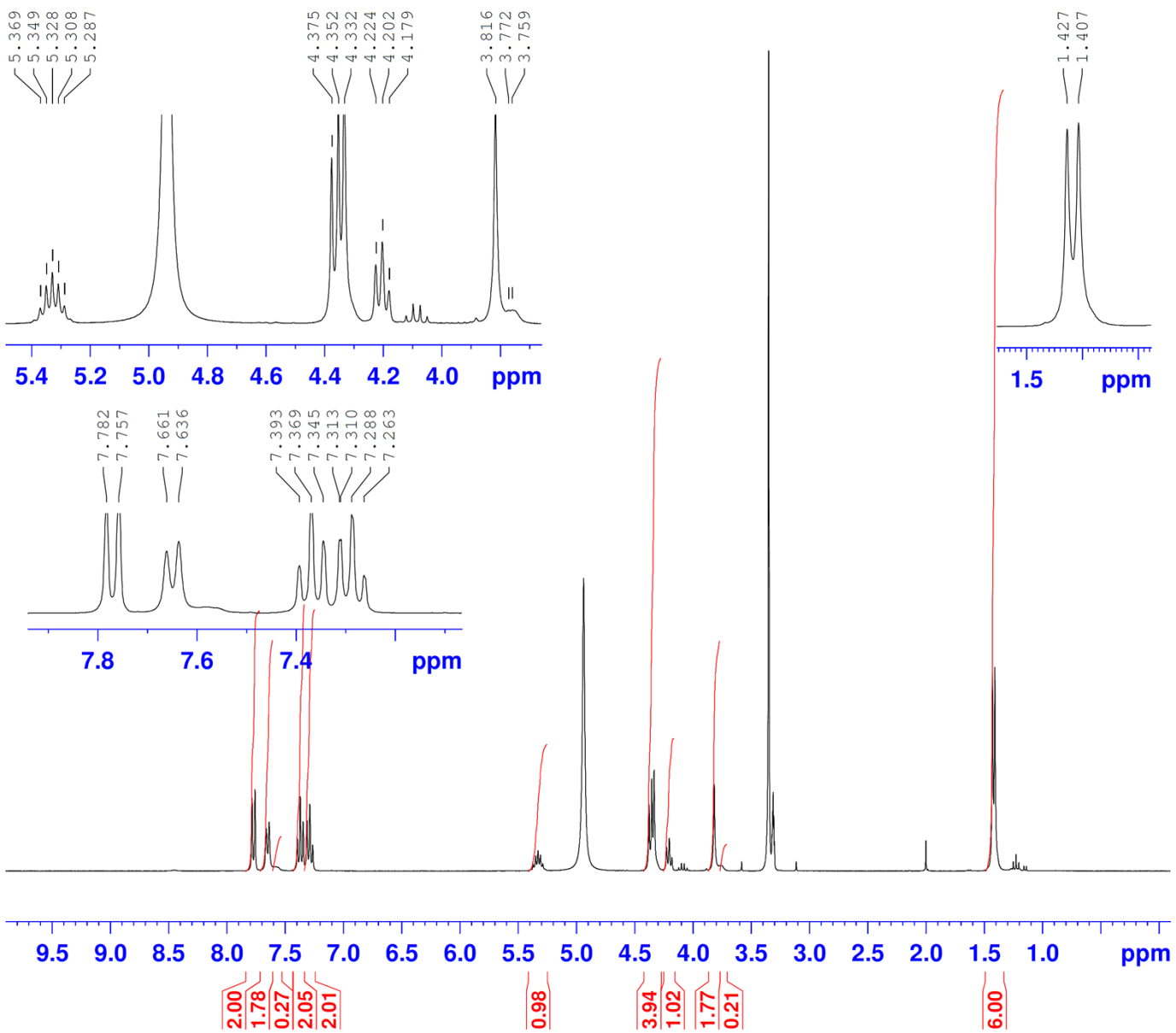

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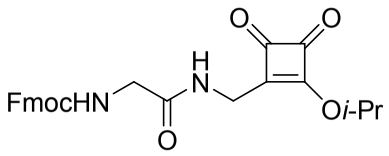
```
Current Data Parameters
NAME      MK-Fmoc-Gly-Sqgly-OiPr
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140626
Time                3.36
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH            6188.119 Hz
FIDRES        0.094423 Hz
AQ            5.2953587 sec
RG                   128
DW                80.800 usec
DE                 6.50 usec
TE                295.2 K
D1           1.00000000 sec

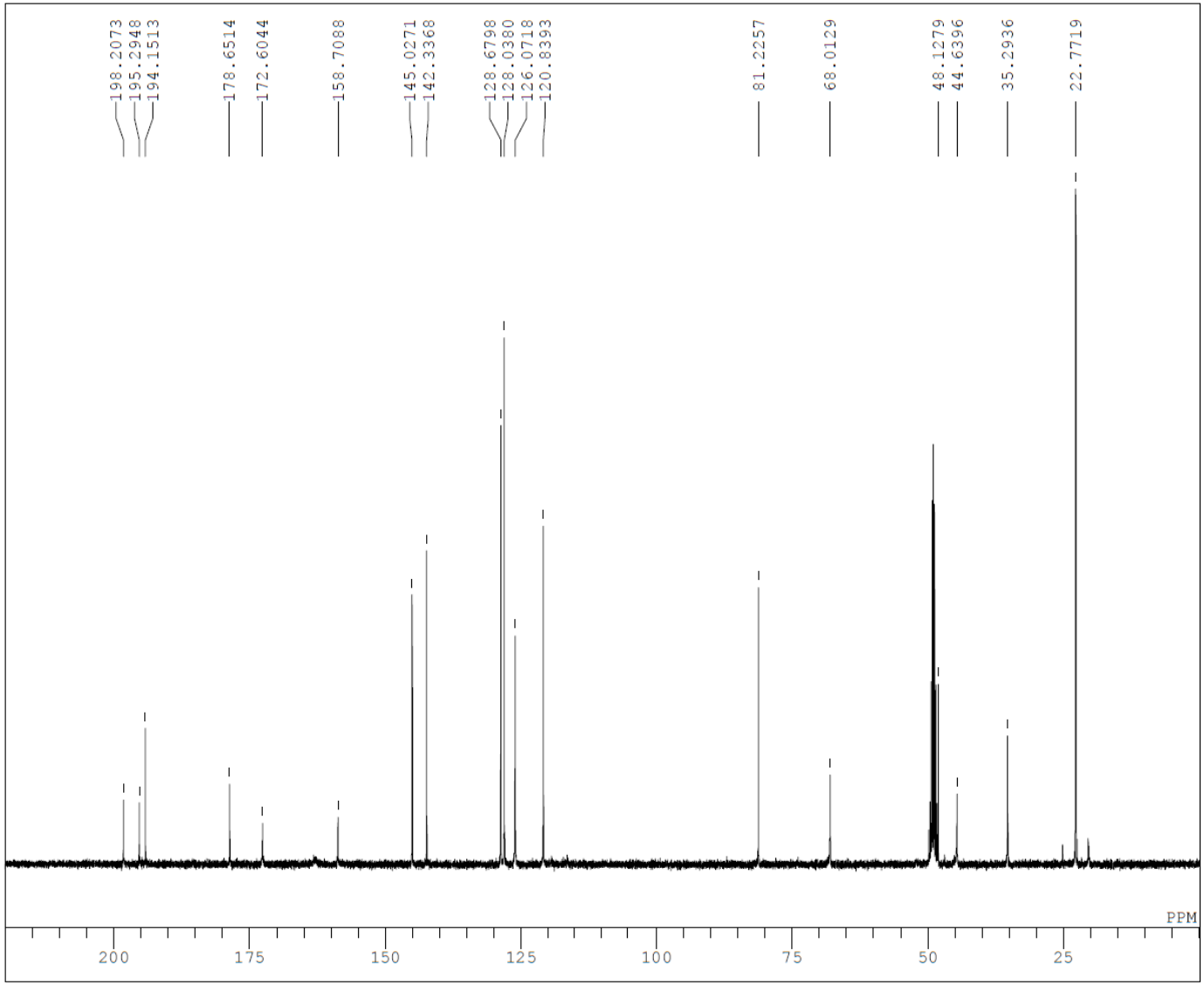
======== CHANNEL f1 ========
NUC1                1H
P1                15.00 usec
PLW1        15.00000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF          300.1300047 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



16

F:\ČZmNMRdata\FmocGlySQGlyOiPrCNMR.als




```
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COMNT
DATIM   Fri Aug 06 16:00:20 2010
OBNUC   13C
EXMOD   bcm
OBFRQ         100.40 MHz
OBSET           0.00 KHz
OBFIN      135500.0 Hz
POINT          32768
FREQU       27100.3 Hz
SCANS           1024
ACQTM          1.209 sec
PD             1.791 sec
PW1             5.0 us
IRNUC   1H
CTEMP           27.5 c
SLVNT   CD3OD
EXREF          49.00 ppm
BF              0.62 Hz
RGAIN             19
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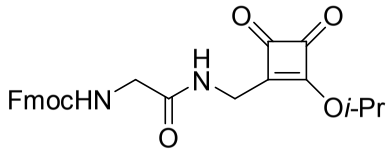

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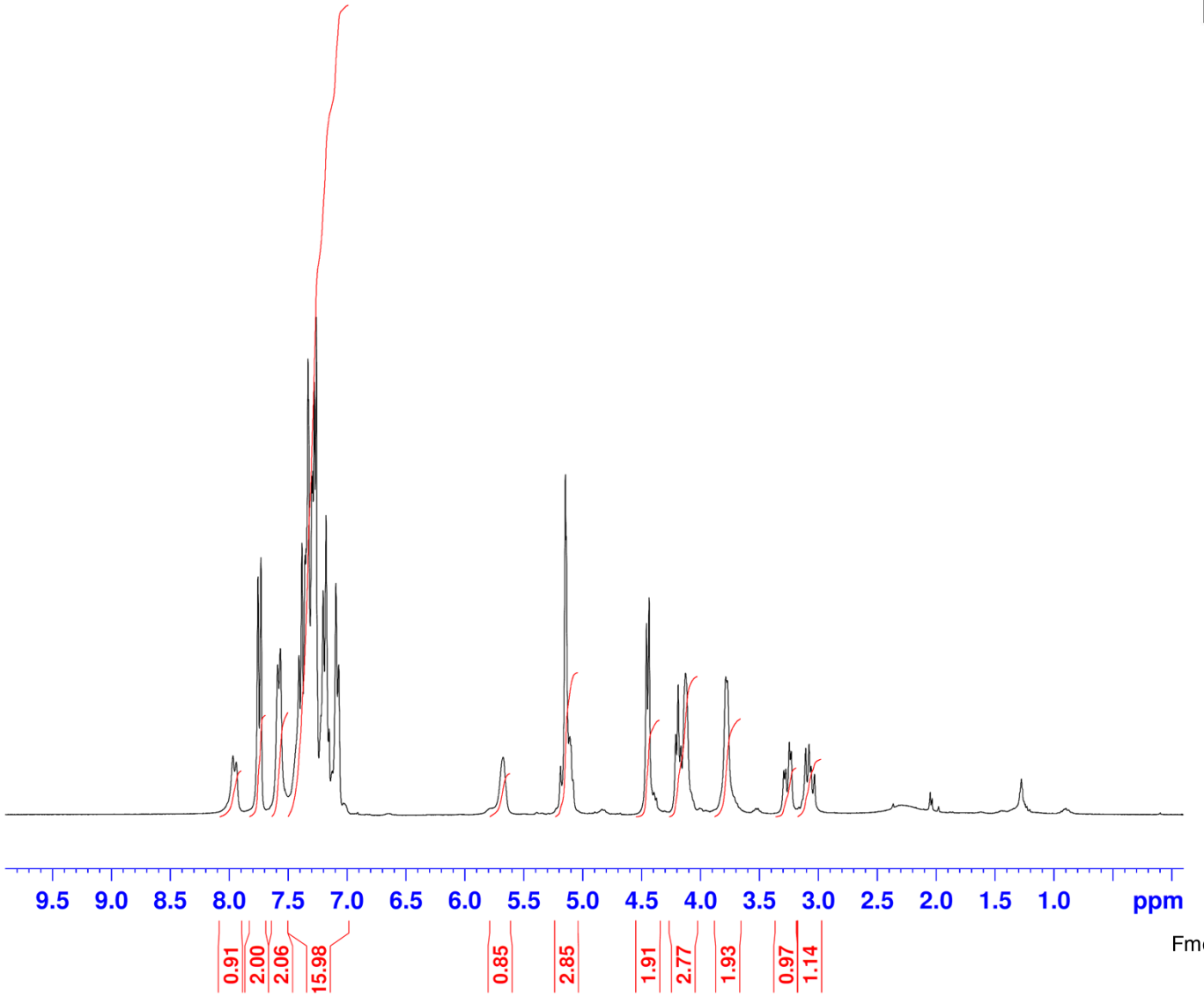
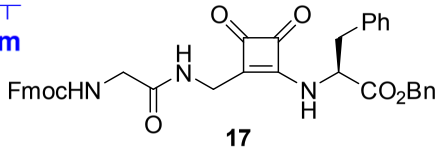



```
Current Data Parameters
NAME      MK-Fmoc-Gly-SqGly-Phe-OBn-H1
EXPNO                 10
PROCNO                 1

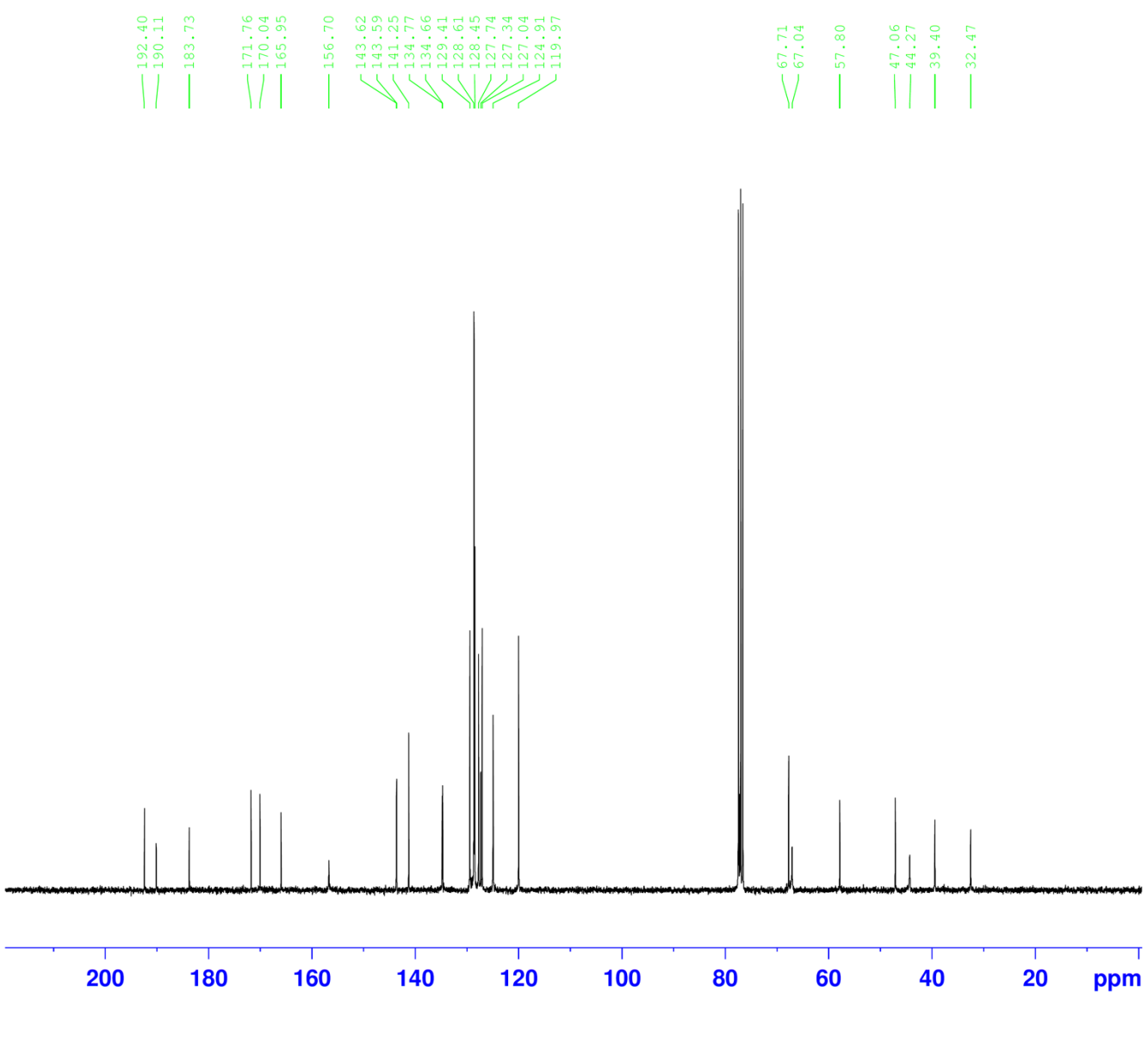
F2 - Acquisition Parameters
Date_           20140721
Time                0.25
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDC13
NS                    16
DS                     2
SWH             6188.119 Hz
FIDRES          0.094423 Hz
AQ             5.2953587 sec
RG                    64
DW                80.800 usec
DE                 6.50 usec
TE                295.5 K
D1           1.00000000 sec

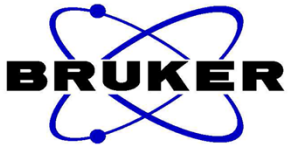
======== CHANNEL f1 ========
NUC1                  1H
P1                 15.00 usec
PLW1          7.50000000 W
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300055 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



17





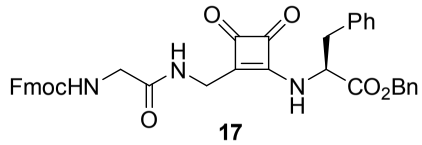

```
Current Data Parameters
NAME      MK-Fmoc-Gly-SqGly-Phe-OBn-C13
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20140721
Time               2.35
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2000
DS                    2
SWH           18028.846 Hz
FIDRES         0.275098 Hz
AQ             1.8175818 sec
RG                  203
DW               27.733 usec
DE                6.50 usec
TE               296.1 K
D1            2.00000000 sec
D11           0.03000000 sec

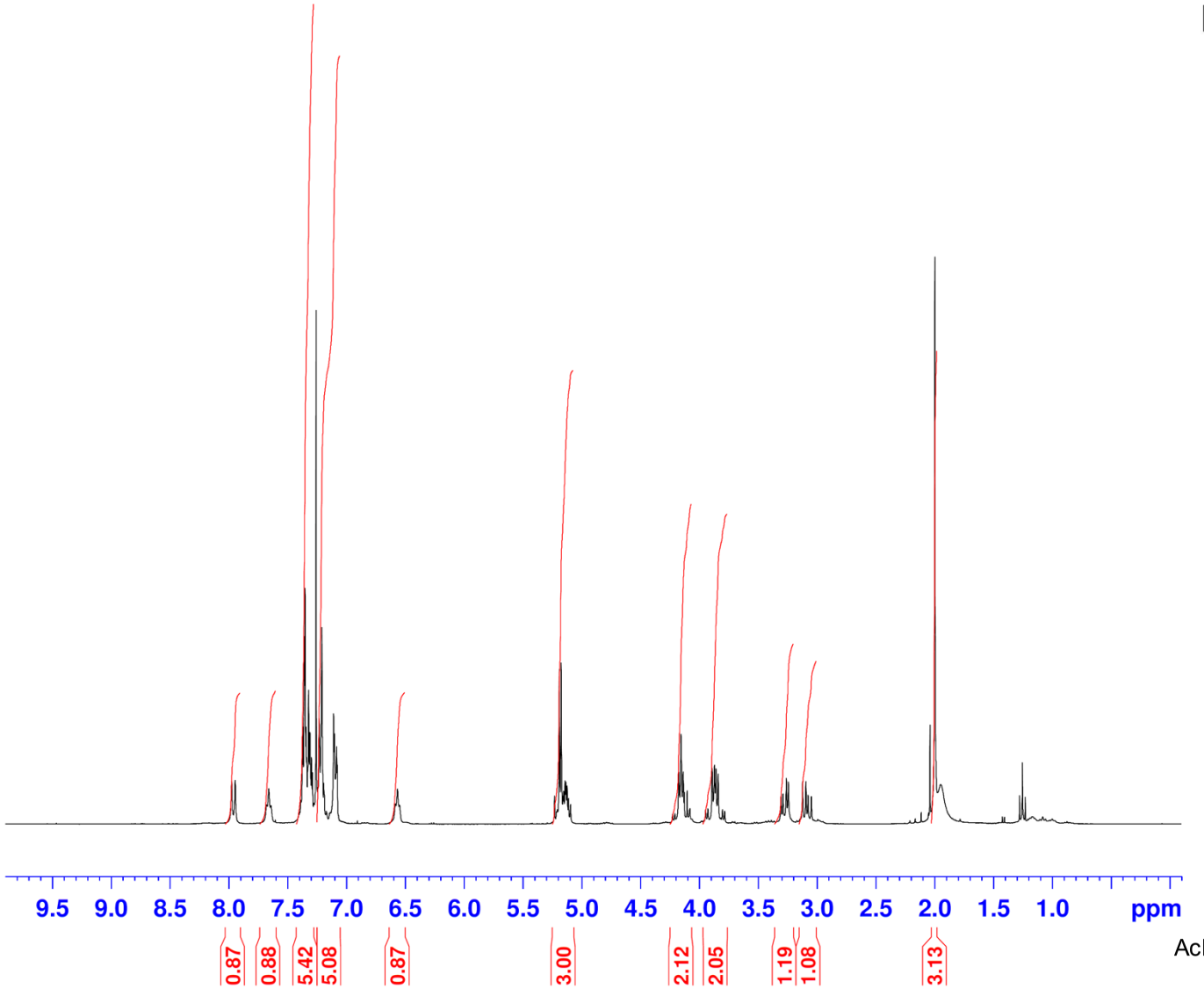
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PLW1         32.00000000 W
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            90.00 usec
PLW2          7.80000019 W
PLW12         0.21667001 W
PLW13         0.17550001 W
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                32768
SF           75.4677553 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



17

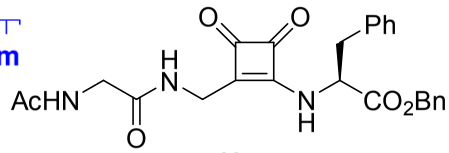



```
Current Data Parameters
NAME      MK-Ac-Gly-SqGly-Phe-OBn-H1
EXPNO                 10
PROCNO                 1

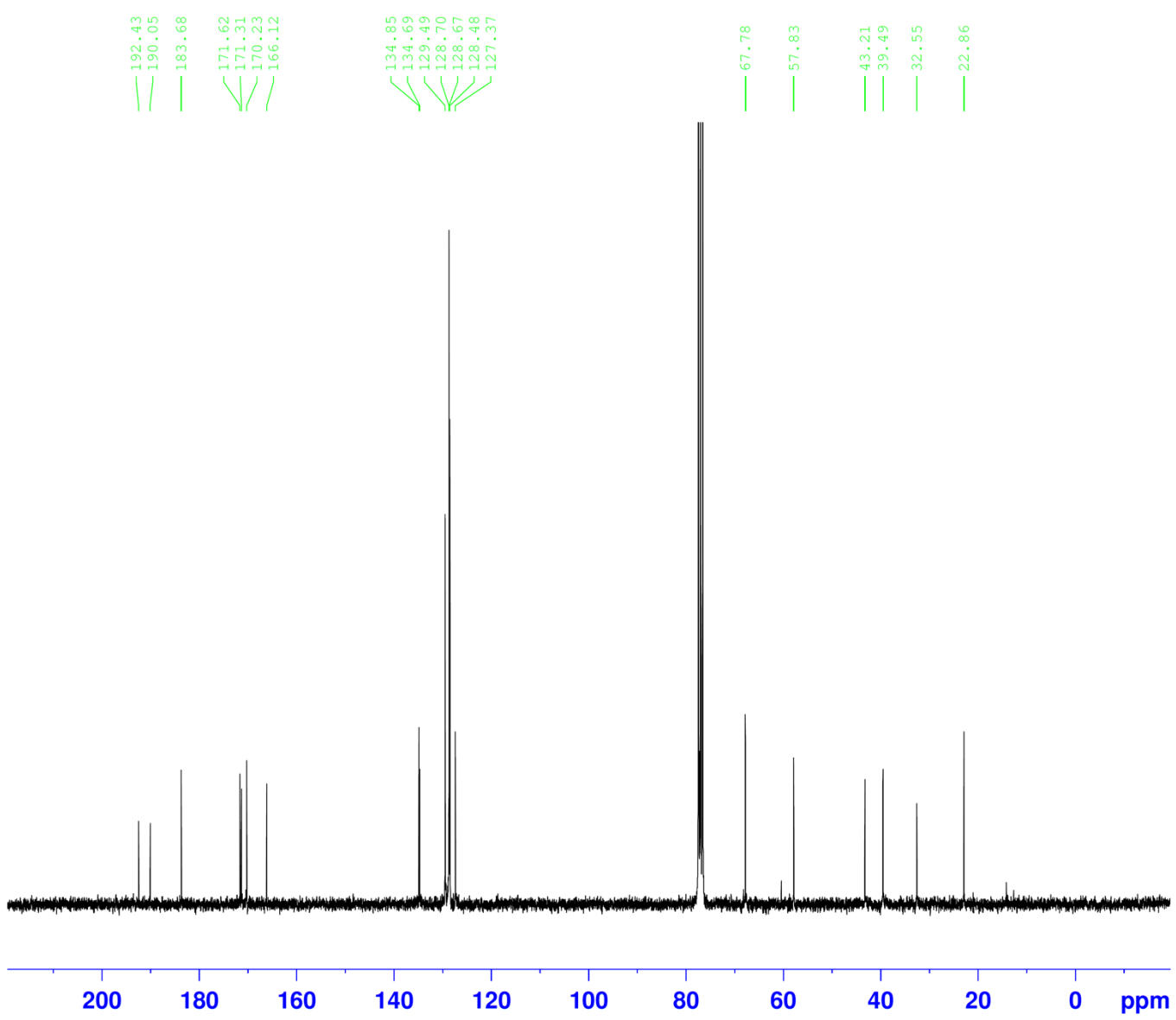
F2 - Acquisition Parameters
Date_           20140721
Time              23.55
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  48
DS                   2
SWH            6188.119 Hz
FIDRES        0.094423 Hz
AQ            5.2953587 sec
RG                 203
DW               80.800 usec
DE                6.50 usec
TE               295.4 K
D1            1.00000000 sec

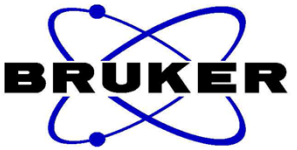
======== CHANNEL f1 ========
NUC1                1H
P1                15.00 usec
PLW1         7.50000000 W
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                32768
SF          300.1300068 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



18





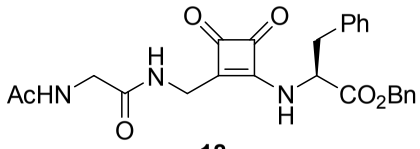
```
Current Data Parameters
NAME      MK-Ac-Gly-SqGly-Phe-OBn-C13
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140722
Time               6.29
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD               65536
SOLVENT          CDCl3
NS                6000
DS                   2
SWH          18028.846 Hz
FIDRES        0.275098 Hz
AQ            1.8175818 sec
RG                 203
DW              27.733 usec
DE               6.50 usec
TE              295.7 K
D1           2.00000000 sec
D11          0.03000000 sec

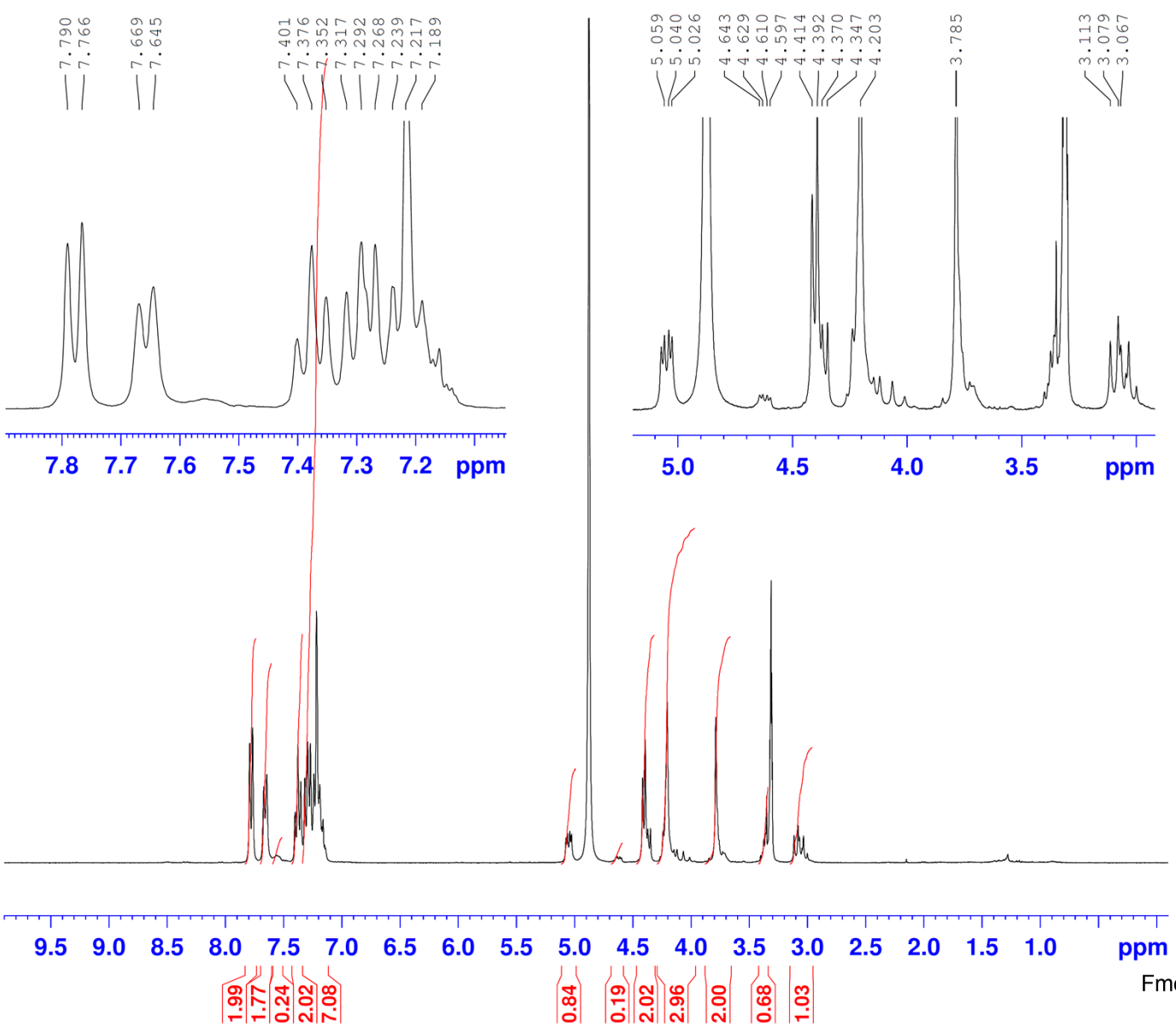
======== CHANNEL f1 ========
NUC1               13C
P1               10.00 usec
PLW1        32.00000000 W
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2               1H
PCPD2            90.00 usec
PLW2         7.80000019 W
PLW12        0.21667001 W
PLW13        0.17550001 W
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                32768
SF           75.4677519 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



18

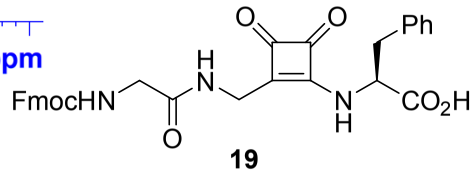



```
Current Data Parameters
NAME          MK-10-42-2
EXPNO                 20
PROCNO                 1

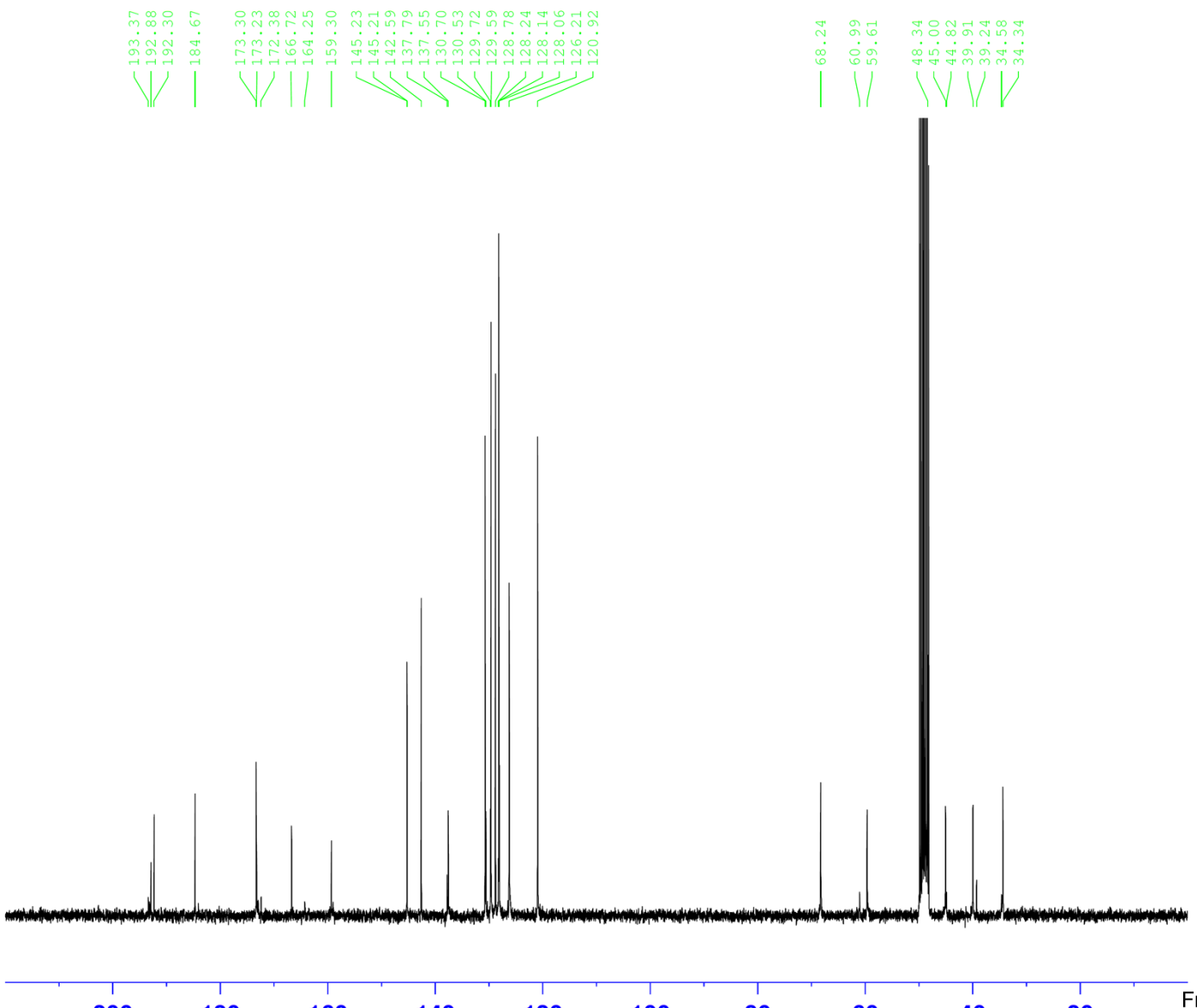
F2 - Acquisition Parameters
Date_           20140607
Time               21.23
INSTRUM            av300
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           6188.119 Hz
FIDRES        0.094423 Hz
AQ            5.2953587 sec
RG                   203
DW              80.800 usec
DE               6.50 usec
TE               294.9 K
D1           1.00000000 sec

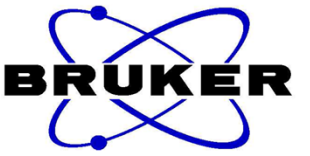
======== CHANNEL f1 ========
NUC1                  1H
P1              15.00 usec
PLW1        15.00000000 W
SFO1       300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF         300.1300046 MHz
WDW                   EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```



19





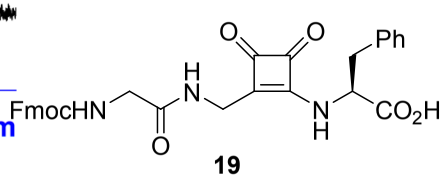

```
Current Data Parameters
NAME      MK-Fmoc-Gly-SqGly-Phe-OH-C13
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140724
Time               2.12
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD               65536
SOLVENT            MeOD
NS                 3200
DS                    2
SWH           18028.846 Hz
FIDRES         0.275098 Hz
AQ             1.8175818 sec
RG                  203
DW               27.733 usec
DE                6.50 usec
TE               296.3 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PLW1         32.00000000 W
SFO1          75.4752953 MHz

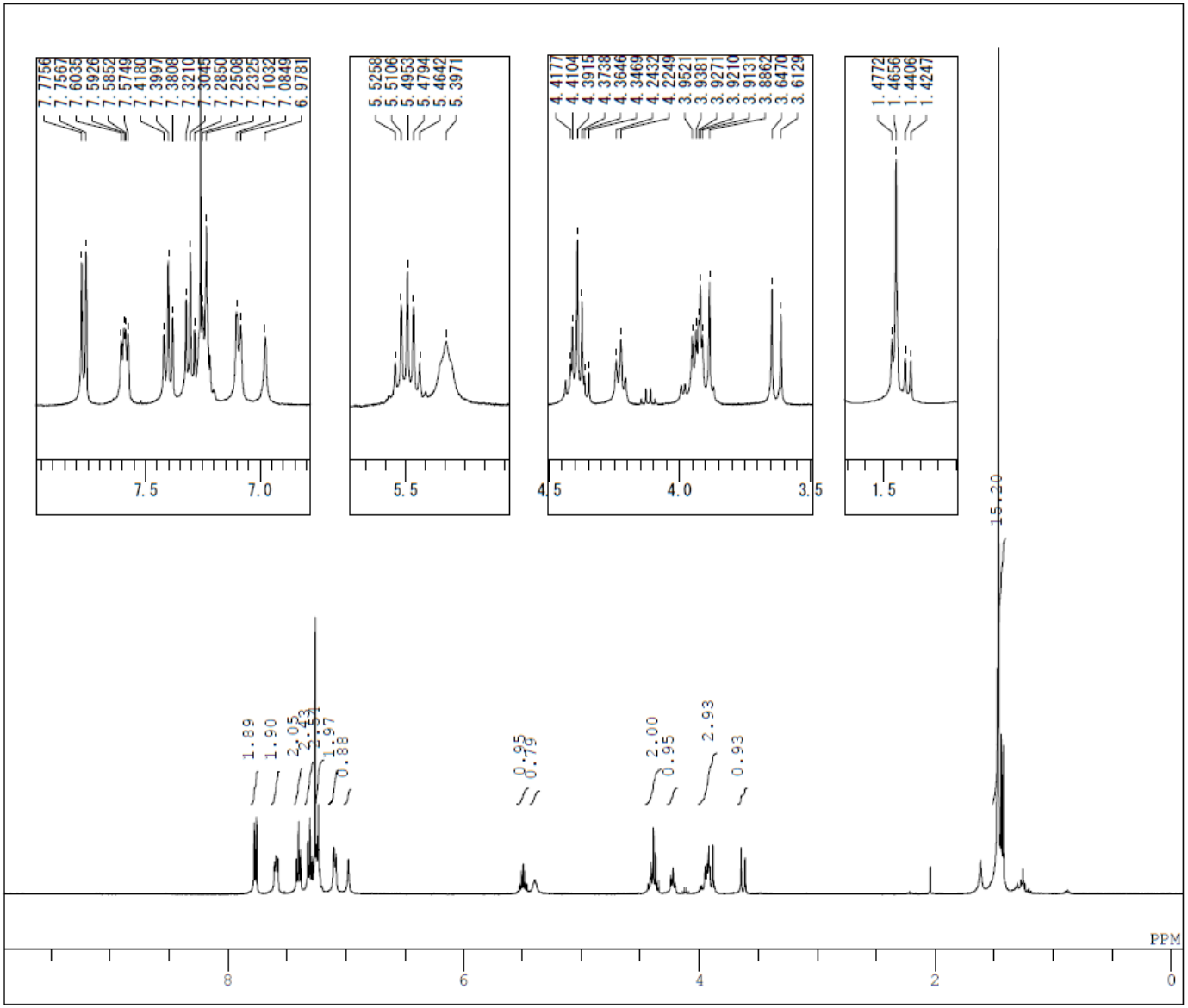
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             90.00 usec
PLW2          7.80000019 W
PLW12         0.21667001 W
PLW13         0.17550001 W
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4676448 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

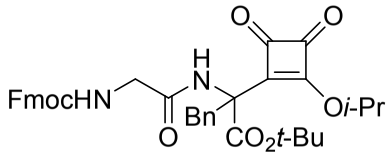


19

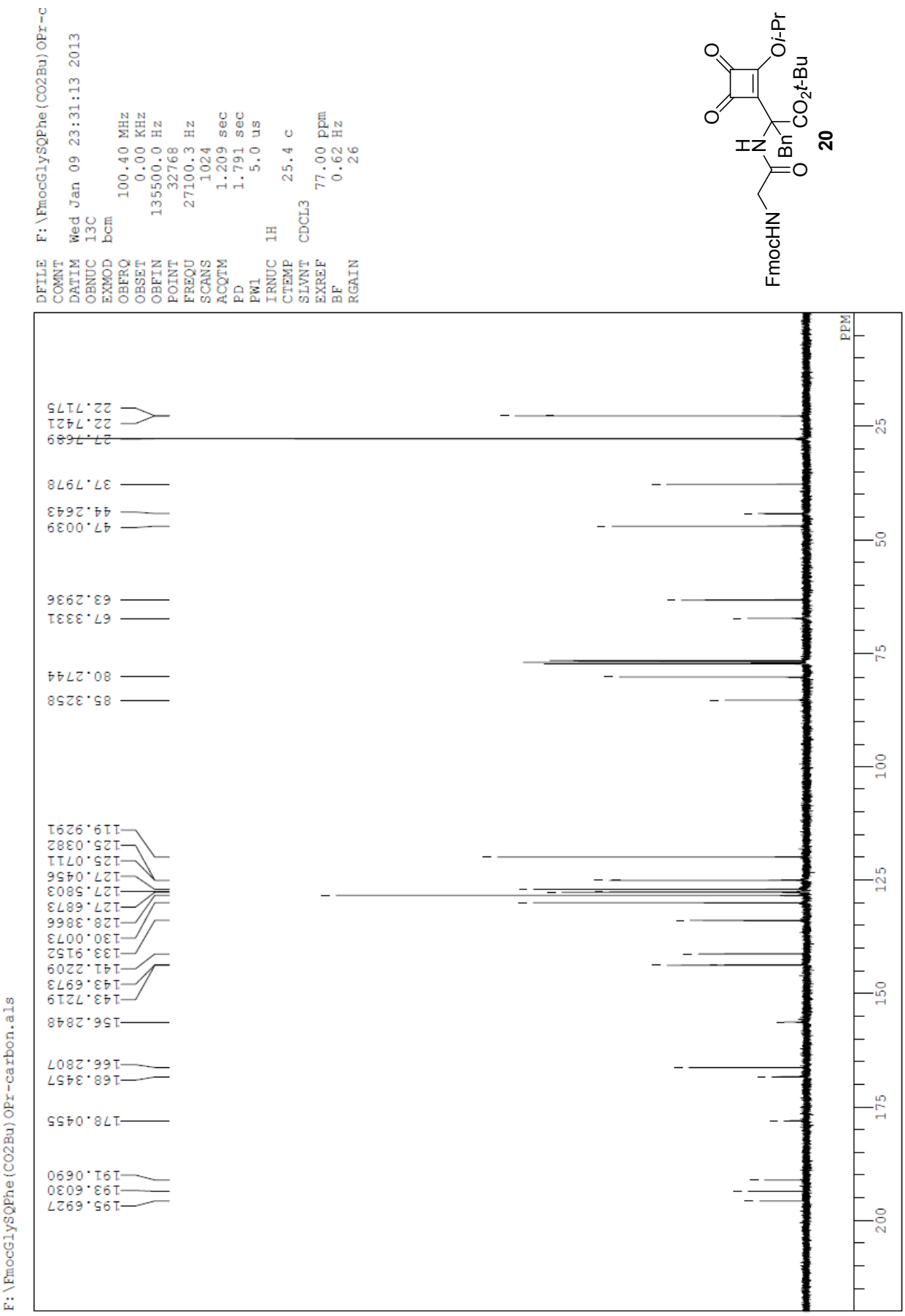
F:\FmocGlySQPhe(CO2Bu)OPr.als

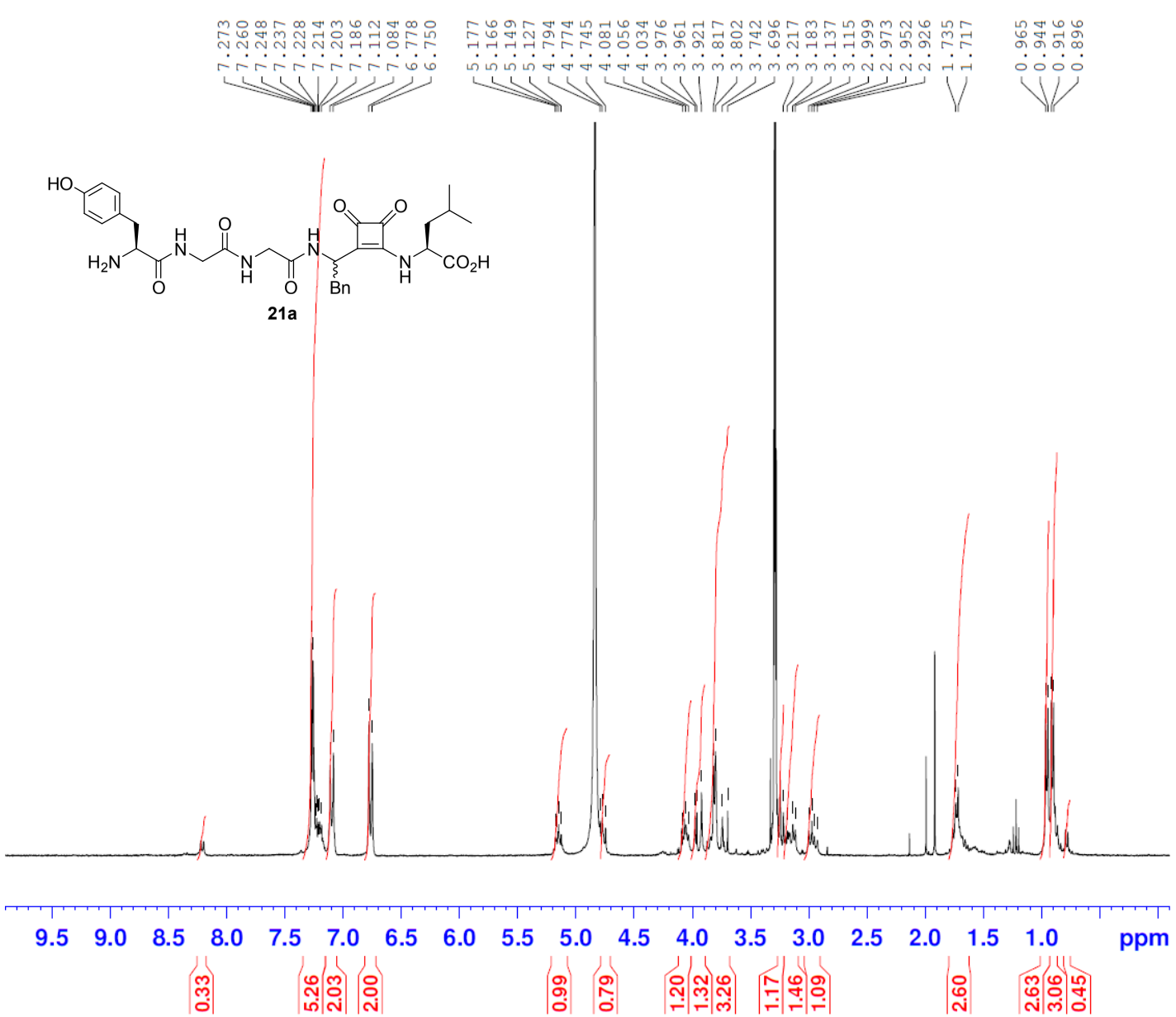


DFILE F:\FmocGlySQPhe(CO2Bu)OPr.a
COMNT
DATIM Tue Jan 08 20:06:26 2013
OBNUC 1H
EXMOD non
OBFRQ 399.65 MHz
OBSET 0.00 KHz
OBFIN 134300.0 Hz
POINT 32768
FREQU 7993.6 Hz
SCANS 8
ACQTM 4.099 sec
PD 2.901 sec
PW1 6.2 us
IRNUC 1H
CTEMP 23.4 c
SLVNT CDCL3
EXREF 7.26 ppm
BF 0.62 Hz
RGAIN 18



20


F:\FmocGlySQPhe(CO2Bu)OPr-carbon.als
DFILE F:\FmocGlySQPhe(CO2Bu)OPr-c
COMNT
DATIM Wed Jan 09 23:31:13 2013
OBNUC 13C
EXMOD bcm
OBFRQ 100.40 MHz
OBSET 0.00 KHz
OBFIN 135500.0 Hz
POINT 32768
FREQU 27100.3 Hz
SCANS 1024
ACQTM 1.209 sec
PD 1.791 sec
PW1 5.0 us
IRNUC 1H
CTEMP 25.4 c
SLVNT CDCL3
EXREF 77.00 ppm
BF 0.62 Hz
RGAIN 26
22.7175
22.7421
27.7689
37.7978
44.2643
47.0039
63.2936
67.3331
80.2744
85.3258
119.9291
125.0382
125.0711
127.0456
127.5803
127.6873
128.3866
130.0073
133.9152
141.2209
143.6973
143.7219
156.2848
166.2807
168.3457
178.0455
191.0690
193.6030
195.6927
PPM
25
50
75
100
125
150
175
200
FmocHN
H
N
O
Bn
CO2t-Bu
O
O
Oi-Pr
20

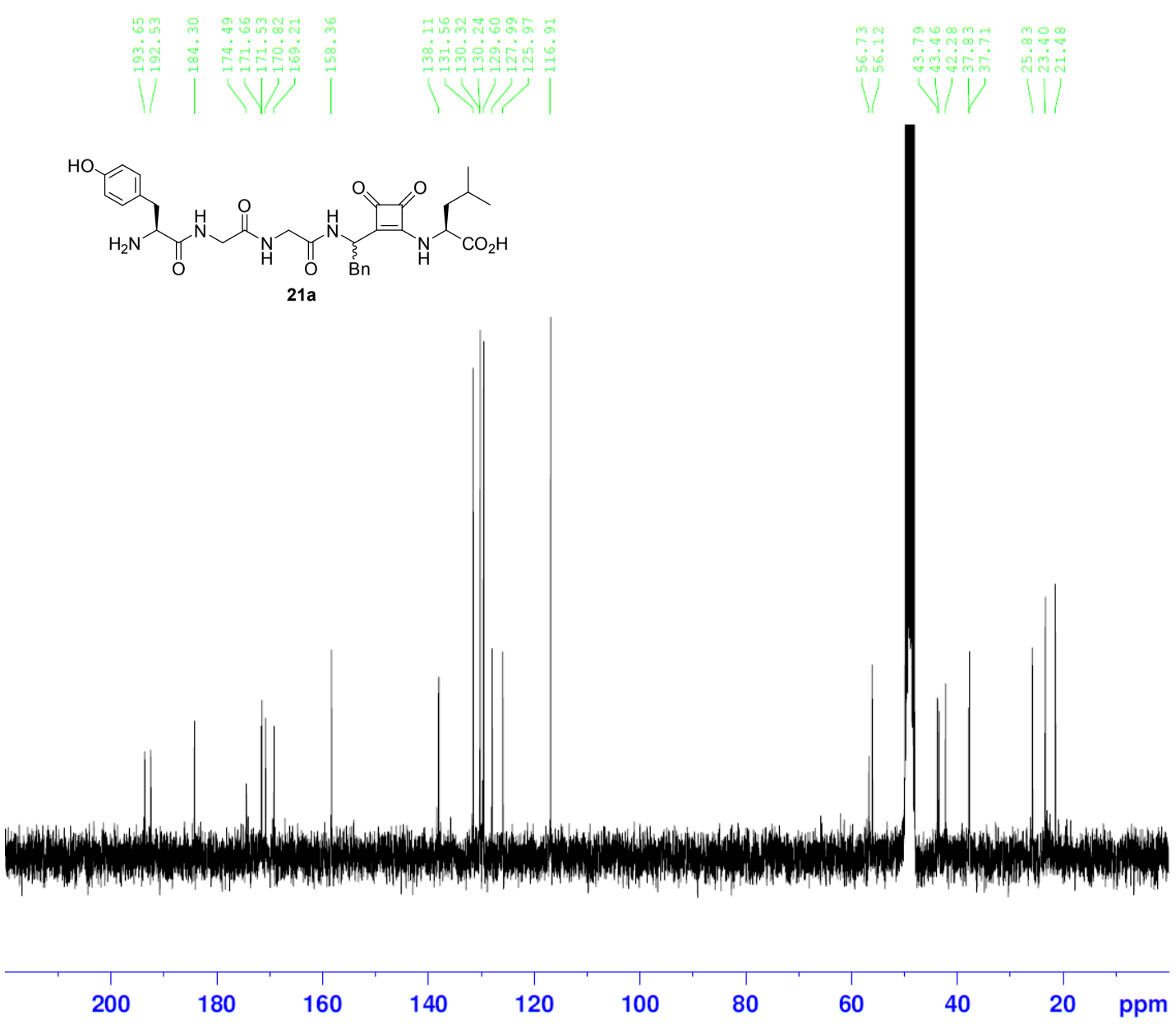



```
Current Data Parameters
NAME                  7-20-1
EXPNO                     10
PROCNO                     1

F2 - Acquisition Parameters
Date_              20140416
Time                  20.09
INSTRUM               av300
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                MeOD
NS                        8
DS                        2
SWH               6188.119 Hz
FIDRES             0.094423 Hz
AQ                5.2953587 sec
RG                      203
DW                 80.800 usec
DE                  6.50 usec
TE                  295.8 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                    15.00 usec
PLW1           7.50000000 W
SFO1          300.1318534 MHz

F2 - Processing parameters
SI                    32768
SF            300.1300107 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```



BRUKER

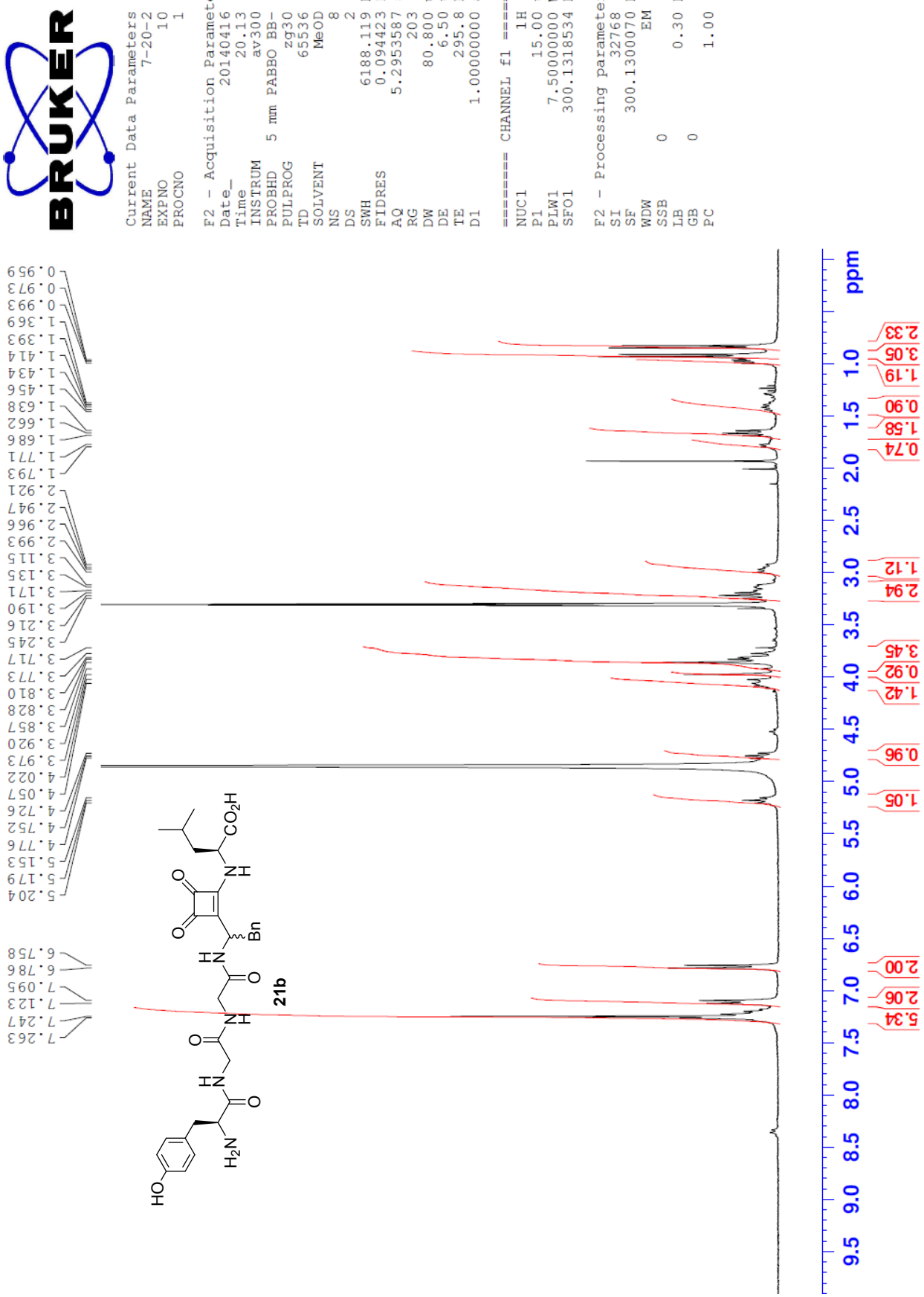
```
Current Data Parameters
NAME                 7-20-1-C13
EXPNO                        10
PROCNO                        1

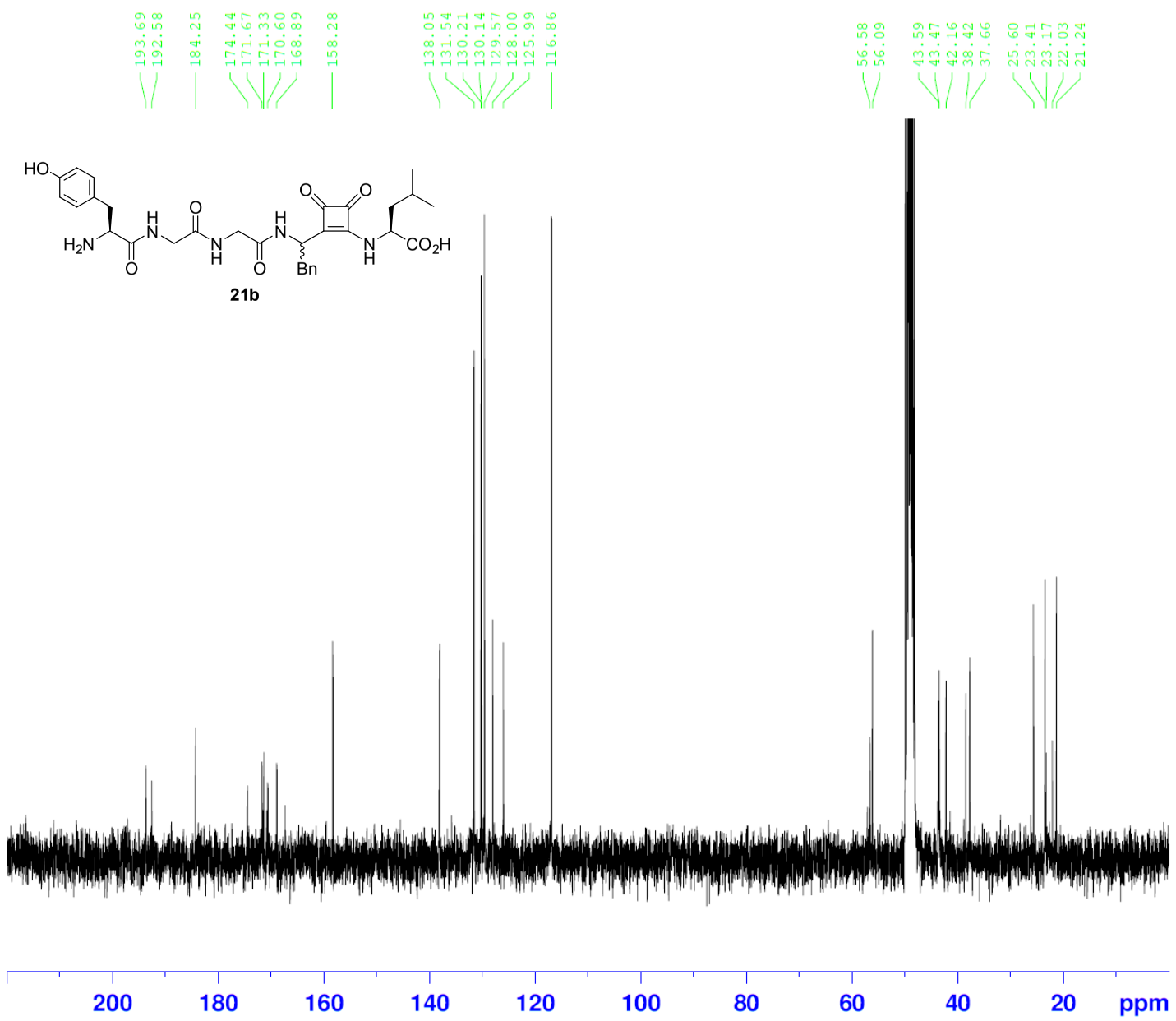
F2 - Acquisition Parameters
Date_                 20140417
Time                      1.50
INSTRUM                 av300
PROBHD     5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                   MeOD
NS                        5000
DS                           2
SWH                  18028.846 Hz
FIDRES                0.275098 Hz
AQ                    1.8175818 sec
RG                         203
DW                       27.733 usec
DE                        6.50 usec
TE                       296.4 K
D1                    2.00000000 sec
D11                   0.03000000 sec

======== CHANNEL f1 ========
NUC1                        13C
P1                        10.00 usec
PLW1                32.00000000 W
SFO1                 75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2                 waltz16
NUC2                        1H
PCPD2                     90.00 usec
PLW2                 7.80000019 W
PLW12                0.21667001 W
PLW13                0.17550001 W
SFO2                300.1312005 MHz

F2 - Processing parameters
SI                        32768
SF                   75.4676464 MHz
WDW                         EM
SSB                  0
LB                        1.00 Hz
GB                   0
PC                        1.40
```


Current Data Parameters
NAME 7-20-2
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140416
Time 20.13
INSTRUM av300
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 203
DW 80.800 usec
DE 6.50 usec
TE 295.8 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 7.50000000 W
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300070 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
21b
ppm



BRUKER

Current Data Parameters
NAME 7-20-2-C13
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140417
Time 7.16
INSTRUM av300
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 5000
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 203
DW 27.733 usec
DE 6.50 usec
TE 296.3 K
D1 2.00000000 sec
D11 0.03000000 sec

======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PLW1 32.00000000 W
SFO1 75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PLW2 7.80000019 W
PLW12 0.21667001 W
PLW13 0.17550001 W
SFO2 300.1312005 MHz

F2 - Processing parameters
SI 32768
SF 75.4676446 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40