Supplementary Information

Divergent Response of Homologous ATP Sites to Stereospecific Ligand Fluorination for Selectivity Enhancement

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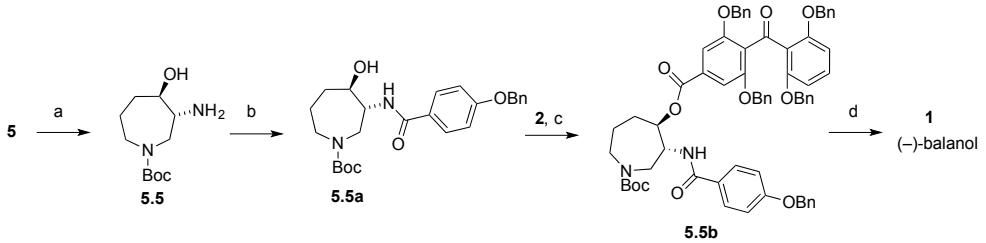
I. Synthesis of (–)-balanol 1 and balanoids 1a–1e.

A. General information.

All reactions were conducted under N_2 atmosphere. Unless otherwise specified, all reagents were purchased from Sigma–Aldrich and used without further purification. CH_2Cl_2 was obtained from a solvent purification system (Innovative Technology SPS400) and stored over MS 4Å beads. Acetone, EtOAc and petroleum ether were distilled before use. Petroleum ether refers to the fraction collected between 60 ºC and 80 ºC. THF was distilled from Na–benzophenone and stored over MS 4Å beads. ^{1}H NMR spectra were recorded at 25 °C on either a Bruker DRX600K or DPX400 NMR spectrometer and are reported in ppm using the specified solvent as the internal standard ($CDCl_3$ at 7.26 ppm, $(CD_3)_2SO$ at 2.50 ppm, CD_3CN at 1.94 ppm). ^{13}C NMR spectra are reported in ppm using the specified solvent as the internal standard ($CDCl_3$ at 77.16 ppm, $(CD_3)_2SO$ at 39.52 ppm, CD_3CN at 1.32 and 118.26 ppm).

B. Synthesis and characterization of the final balanol **1** and balanoids **1a–1e**.



Scheme S1.1. Synthesis of balanol **1**. *Reagents and conditions*: (a) H_2, Pd/C, triflic acid, MeOH, 14 h, 25 °C; (b) Et_3N, 4–benzyloxybenzoyl chloride, 2 h, 25 °C, 52% over two steps; (c) 2–chloro–1–methylpyridinium iodide, DMAP, NEt_3, DCM, 8 h, 25 °C, 75%; (d) i) H_2, Pd/C, THF, H_2O, AcOH, 25 °C, 10 h; ii) TFA, neat, 5 min., 25 °C, 70%.

The benzophenone (**2**) and azepane fragment **5** were synthesized as described previously).[1] The coupling of benzophenone (**2**) and azepane (**5.5a**) fragments was successfully accomplished via esterification using the Mukaiyama procedure to afford the fully protected balanol progenitor **5.5b** in 75% yield (Scheme S1.1). The universal benzyl deprotection was achieved by mild palladium–catalyzed hydrogenolysis of **5.5b** in acidic media to obtain the Boc protected balanol as a yellow solid, which was subjected to purification by reverse phase HPLC. The Boc deprotection was readily achieved by treatment with neat TFA to furnish balanol (**1**) in 70% yield, which exhibited characterization data consistent with that from previous reports.

2–(2,6–dihydroxy–4–(((3*R*,4*R*)–3–(4–hydroxybenzamido)azepan–4–yloxy)carbonyl) benzoyl)–3–hydroxybenzoic acid (1)

$[\alpha]^{20}_{D}$ = −109.3 (*c* 0.12, MeOH); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 11.67 (s, 2H), 9.88 (brs, 1H), 9.06 (brs, 1H), 8.98 (brs, 1H), 8.51 (d, *J* = 7.91 Hz, 1H), 7.64 (d, *J* = 8.87 Hz, 2H), 7.37 (dd, *J* = 7.78, 1.01 Hz, 1H), 7.27 (t, *J* = 7.90 Hz, 1H), 7.05 (dd, *J* = 8.00, 1.01 Hz, 1H), 6.78 (d, *J* = 8.66 Hz, 2H), 6.78 (s, 2H), 5.29–5.23 (m, 1H), 4.53–4.45 (m, 1H), 3.39–3.25 (m, 2H), 3.20–3.11 (brs, 2H), 2.17–2.08 (m, 1H), 2.03–1.79 (m, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 201.6, 166.9, 166.2, 164.2, 161.5, 160.5, 153.3, 135.5, 132.5, 129.3, 129.0, 128.9, 124.4, 120.0, 119.7, 114.9, 113.5, 107.3, 75.9, 50.8, 46.0, 45.9, 28.2; MS (ESI): *m/z*, 551.17 [M^+ + H].

(3*R*,4*R*)–3–(4–(benzyloxy)benzamido)–4–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (5.5a)

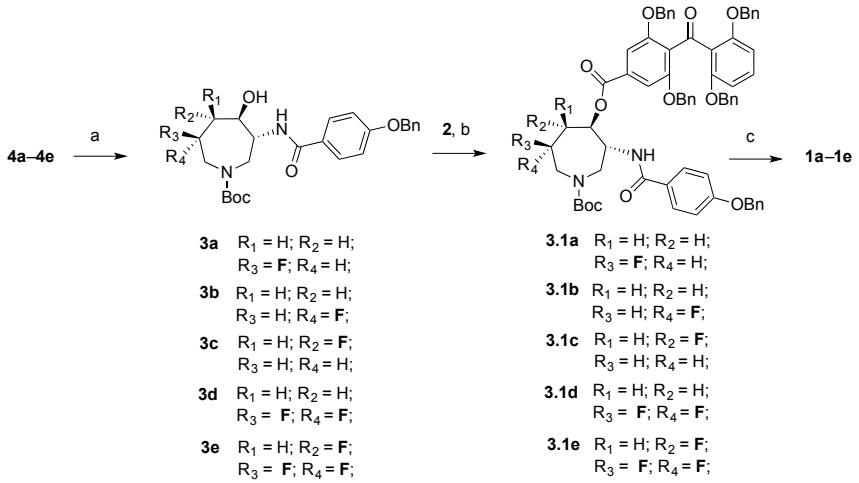
A solution of compound **5** (20 mg, 58.1 μmol) and trifluoromethanesulfonic acid (4 μL, 58.1 μmol) in MeOH (1.21 mL) was treated with Pd/C (5% w/w). The resulting suspension was stirred under H_2 (1 atm) for 14 h. The reaction mixture was then filtered through a pad of Celite which was rinsed with MeOH (1 mL × 5). The solvent was evaporated under reduced pressure to yield the desired amine **5.5** which was used in the next step without further purification. 4–benzyloxybenzoyl chloride (15.6 mg, 68.1 μmol) was added to the solution of amine **5.5** and Et_3N (64 μL, 681 μmol) in CH_2Cl_2 (0.81 mL) under N_2 atmosphere. The reaction mixture was stirred for 2 h at 25 °C before it was quenched by the addition of MeOH (0.15 mL) and pyridine (0.15 mL). The volatiles were evaporated under vacuum and the residue was dissolved in EtOAc. The organic phase was successively washed with aqueous 2 *N* HCl, water, aqueous saturated $NaHCO_3$, and brine. The organic layer was then dried (Na_2SO_4) before vacuum evaporation to obtain the crude mixture, which was purified by flash chromatography (petroleum ether/ethyl acetate, 1/1) to afford azepane **5.5a** (15.6 mg, 52% over two steps) as a colourless oil with NMR, optical rotation and mass spectra matching to those reported previously.[1] R_f (petroleum ether/EtOAc, 1/1) 0.33; $[\alpha]^{20}_{D}$ = −4.1 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.96 (d, *J* = 4.98 Hz, 1H), 7.90 (d, *J* = 8.71 Hz, 2H), 7.51–7.36 (m, 5H), 7.08 (d, *J* = 8.71 Hz, 2H), 5.17 (s, 2H), 4.11–4.02 (m, 3H), 3.82–3.75 (m, 1H), 3.33 (dd, *J* = 15.46, 4.90 Hz, 1H), 2.79 (ddd, *J* = 13.88, 12.30, 3.50 Hz, 1H), 1.95–1.63 (m, 4H), 1.54 (s, 9H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 168.5, 162.0, 157.7, 137.0, 129.4, 129.0, 128.5, 128.0, 126.5, 115.0, 81.0, 78.0, 70.5, 61.2, 50.7, 50.1, 33.1, 28.5, 27.5; MS (ESI): *m/z*, 441.23 [M^+ + H].

(3*R*,4*R*)–*tert*–butyl–3–(4–(benzyloxy)benzamido)–4–(3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–(benzyloxycarbonyl)benzoyl)benzoyloxy)azepane–1–carboxylate (5.5b)

A suspension of hydroxyazepane benzamide **5.5a** (15.6 mg, 35.4 μmol), benzophenone **2** (24.0 mg, 35.4 μmol) and 2–chloro–1–methylpyridinium iodide (11.8 mg, 46.0 μmol) in CH_2Cl_2 (0.35 mL) was treated with Et_3N (9.9 μL, 70.8 μmol) and allowed to stir for 30 min at 25 °C. The reaction

mixture was then reacted with 4–dimethylaminopyridine (2.2 mg, 17.7 μmol) and was allowed to stir for 8 h at 25 °C before vacuum evaporation to obtain the crude mixture for purification by flash chromatography (petroleum ether/ethyl acetate, 3/2) to afford the fully protected balanol **5.5b** (29.2 mg, 75%) as a colourless oil with NMR, optical rotation and mass spectra matching to those reported previously.[2] R_f (petroleum ether/EtOAc, 3/2) 0.31; $[\alpha]^{20}_D = -61.1$ (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.60 Hz, 1H), 7.77 (d, *J* = 8.56 Hz, 2H), 7.42–7.03 (m, 27H), 6.93 (brd, *J* = 8.82 Hz, 3H), 6.82 (d, *J* = 7.92 Hz, 2H), 5.11 (s, 2H), 5.05 (s, 2H), 4.84 (s, 4H), 4.67 (s, 2H), 4.13–3.94 (m, 2H), 3.35 (dd, *J* = 15.66, 5.77 Hz, 1H), 2.89–2.79 (m, 1H), 2.09–1.69 (m, 6H), 1.57 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 167.5, 166.4, 165.8, 161.4, 158.1, 156.4, 136.5, 135.9, 135.8, 132.9, 130.6, 129.0, 128.8, 128.4, 128.3, 128.0, 127.9, 127.6, 127.5, 127.4, 122.1, 115.5, 114.7, 107.1, 81.0, 77.9, 70.6, 70.1, 67.2, 53.7, 50.3, 49.8, 28.6; MS (ESI): *m/z*, 1101.24 [M^+ + H].

A solution of fully protected balanol **5.5b** (20.0 mg, 18.2 μmol) in THF/AcOH/Water (16/4/1) at 25 °C was treated with Pd/C (10% w/w) and stirred under H_2 atmosphere (1 atm) for 10 h at 25 °C before it was filtered through a pad of celite and vacuum evaporated to obtain the crude mixture. The crude was then chromatographed on a Phenomenex Gemini C18 column (150 × 21.20 mm) eluting with MeCN/water/TFA (from 35:65:0.1 to 39:61:0.1 over 25 min; flow rate: 8.80 mL/min; retention time 19.5 min) to provide boc–protected balanol as a pale yellow solid powder. The compound was treated with TFA (1 mL) at 25 °C for 5 min before TFA was vacuum evaporated. The reaction flask was kept under high vacuum (0.005 Torr, 25 °C) for 3 h to remove traces of TFA, and the pale yellow film obtained was characterized as balanol (**1**) (7.0 mg, 70%) with NMR, optical rotation and mass spectra matching to those reported previously.[3] $[\alpha]^{20}_D = -109.3$ (*c* 0.12, MeOH); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 11.67 (s, 2H), 9.88 (brs, 1H), 9.06 (brs, 1H), 8.98 (brs, 1H), 8.51 (d, *J* = 7.91 Hz, 1H), 7.64 (d, *J* = 8.87 Hz, 2H), 7.37 (dd, *J* = 7.78, 1.01 Hz, 1H), 7.27 (t, *J* = 7.90 Hz, 1H), 7.05 (dd, *J* = 8.00, 1.01 Hz, 1H), 6.78 (d, *J* = 8.66 Hz, 2H), 6.78 (s, 2H), 5.29–5.23 (m, 1H), 4.53–4.45 (m, 1H), 3.39–3.25 (m, 2H), 3.20–3.11 (brs, 2H), 2.17–2.08 (m, 1H), 2.03–1.79 (m, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 201.6, 166.9, 166.2, 164.2, 161.5, 160.5, 153.3, 135.5, 132.5, 129.3, 129.0, 128.9, 124.4, 120.0, 119.7, 114.9, 113.5, 107.3, 75.9, 50.8, 46.0, 45.9, 28.2; MS (ESI): *m/z*, 551.17 [M^+ + H].



Scheme S1.2. Synthesis of balanoids **1a–1e**. *Reagents and conditions*: (a) i) H_2, Pd/C, triflic acid, MeOH, 14 h, 25 °C; ii) Et_3N, 4–benzyloxybenzoyl chloride, 2 h, 25 °C; (b) 2–chloro–1–methylpyridinium iodide, DMAP, NEt_3, DCM, 8 h, 25 °C; (c) i) H_2, Pd/C, THF, H_2O, AcOH, 25 °C, 10 h; ii) TFA, neat, 5 min., 25 °C.

The azepanes **4a–4e** were prepared as described previously[4] and subjected to the same coupling protocol that used in the total synthesis of (−)–balanol (**1**). The palladium catalyzed hydrogenation in acidic conditions followed by amidation to afforded the azepane benzamides (**3a–3e**) which were then coupled with benzophenone (**2**) to furnish the fluorinated, fully protected balanoids (**3.1a–3.1e**). Finally, removal of both the benzyl and Boc groups furnished the fluorinated balanol analogues (**1a–1e**) in fair yields (Scheme S1.2).

(3*R*,4*R*,6*S*)–3–(4–(benzyloxy)benzamido)–6–fluoro–4–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (3a)

The procedure for the synthesis of **5.5a** was followed: yield 50% over two steps; colourless oil; R_f (petroleum ether/EtOAc, 1/1) 0.34; $[\alpha]^{20}_D = +19.7$ (*c* 1.4, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3500–3100 (br), 1673, 1634, 1607, 1508, 1243, 1200, 1178, 1134, 1049, 849, 799, 721; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, J = 5.34 Hz, 1H), 7.84 (d, J = 8.78 Hz, 2H), 7.45–7.31 (m, 5H), 7.02 (d, J = 8.76 Hz, 2H), 5.52 (brs,1H), 5.12 (s, 2H), 4.70–4.50 (m, $^1J_{HF}$ = 45.93 Hz, 1H), 4.37 (dd, J = 13.54, 5.55 Hz, 1H), 4.18–4.09 (m, 2H), 3.79 (dd, J = 11.03, 6.67 Hz, 1H), 3.33 (dd, J = 15.74, 5.47 Hz, 1H), 2.88–2.78 (m, 1H), 2.37 (dd, J = 15.24, 15.15 Hz, 1H), 2.23–2.12 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 161.9, 157.2, 136.5, 129.3, 128.8, 128.3, 127.6, 125.6, 114.9, 87.6 (d, $^1J_{CF}$ = 176.82 Hz), 82.0, 73.7 (d, $^3J_{CF}$ = 14.86 Hz), 70.3, 60.2, 53.3 (d, $^2J_{CF}$ = 34.19 Hz), 50.4, 39.5 (d, $^2J_{CF}$ = 19.96 Hz), 28.4; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{25}H_{32}FN_2O_5$ 459.2295, found 459.2299.

(3*R*,4*R*,6*R*)–3–(4–(benzyloxy)benzamido)–6–fluoro–4–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (3b)

The procedure for the synthesis of **5.5a** was followed: yield 48% over two steps; colourless oil; R_f (petroleum ether/EtOAc, 1/1) 0.35; $[\alpha]^{20}_D = -38.8$ (*c* 0.9, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3500–3100 (br), 1673, 1634, 1607, 1508, 1243, 1200, 1178, 1134, 1049, 849, 799, 721; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, J = 4.88 Hz, 1H), 7.85 (d, J = 8.79 Hz, 2H), 7.44–7.30 (m, 5H), 7.01 (d, J = 8.83 Hz, 2H), 5.11 (s, 2H), 4.87–4.72 (m, $^1J_{HF}$ = 45.06 Hz, 1H), 4.43 (dd, J = 15.94, 15.28 Hz, 1H), 4.27–4.06 (m, 3H), 3.21 (dd, J = 15.48, 5.38 Hz, 1H), 2.98 (dd, J = 15.81, 2.02 Hz, 1H), 2.39–1.97 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 161.9, 157.8, 135.8, 129.3, 128.8, 128.3, 127.6, 125.8, 114.8, 88.7 (d, $^1J_{CF}$ = 178.54 Hz), 81.7, 72.0 (d, $^3J_{CF}$ = 6.67 Hz), 70.2, 60.2, 54.4 (d, $^2J_{CF}$ = 22.99 Hz), 50.1, 37.4 (d, $^2J_{CF}$ = 20.06 Hz), 28.4; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{25}H_{32}FN_2O_5$ 459.2295, found 459.2300.

(3*R*,4*S*,5*S*)–3–(4–(benzyloxy)benzamido)–5–fluoro–4–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (3c)

The procedure for the synthesis of **5.5a** was followed: yield 48% over two steps; colourless oil; R_f (petroleum ether/EtOAc, 1/1) 0.34; $[\alpha]^{20}_D = +44.5$ (*c* 1.2, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3500–3100 (br), 1673, 1634, 1607, 1508, 1243, 1200, 1178, 1134, 1049, 849, 799, 721; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 4.83 Hz, 1H), 7.79 (d, J = 8.28 Hz, 2H), 7.43–7.30 (m, 5H), 6.99 (d, J = 8.93 Hz, 2H), 5.10 (s, 2H), 4.81–4.62 (dt, J = 45.88 ($^1J_{HF}$), 9.43, 9.0 Hz, 1H), 4.20–3.89 (m, 3H), 3.28–3.20 (m, 1H), 3.06–2.95 (m, 2H), 2.28–2.14 (m, 1H), 2.07–1.93 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 162.2, 157.3, 136.7, 129.6, 129.1, 128.6, 127.9, 125.8, 115.1, 92.2 (d, $^1J_{CF}$ = 172.0 Hz), 81.8, 78.6 (d, $^2J_{CF}$ = 20.9 Hz), 70.5, 57.7 (d, $^3J_{CF}$ = 7.41 Hz), 47.8, 44.7 (d, $^3J_{CF}$ = 14.77 Hz), 33.1 (d, $^2J_{CF}$ = 21.8 Hz), 28.7; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{25}H_{32}FN_2O_5$ 459.2295, found 459.2297.

(5*R*,6*R*)–6–(4–(benzyloxy)benzamido)–3,3–difluoro–5–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (3d)

The procedure for the synthesis of **5.5a** was followed: yield 55% over two steps; colourless oil; R_f (petroleum ether/EtOAc, 1/1) 0.34; $[\alpha]^{20}_D = -27.4$ (*c* 1.0, $CHCl_3$); IR (film) ν_{max} (cm^{-1}): 3500–3100 (br), 2360, 1716, 1683, 1652, 1630, 1613, 1518, 1249, 1210, 1171, 1139, 1049, 840, 793, 726, 608; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (d, J = 5.25 Hz, 1H), 7.85 (d, J = 8.70 Hz, 2H), 7.45–7.31 (m, 5H), 7.03 (d, J = 8.58 Hz, 2H), 5.38 (brs,1H), 5.13 (s, 2H), 4.43 (dd, J = 14.99, 13.34 Hz, 1H), 4.21–4.09 (m, 2H), 4.03 (dd, J = 9.97, 7.34 Hz, 1H), 3.32 (dd, J = 15.78, 5.26 Hz, 1H), 3.09 (dd, J = 31.99, 14.94 Hz, 1H), 2.49–2.24 (m, 2H), 1.51 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8,

161.9, 157.2, 136.5, 129.3, 128.8, 128.3, 127.6, 125.6, 121.7 (d, $^1J_{CF}$ = 238.33 Hz), 114.9, 82.5, 72.1 (dd, J = 11.18 ($^2J_{CF}$), 3.82 ($^3J_{CF}$) Hz), 70.3, 60.0, 55.7 (dd, J = 41.22 ($^2J_{CF}$), 28.80 ($^3J_{CF}$) Hz), 50.1, 41.2 (t, $^2J_{CF}$ = 23.80 Hz), 28.3; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{25}H_{31}F_2N_2O_5$ 477.2201, found 477.2203.

(4*R*,5*S*,6*R*)–6–(4–(benzyloxy)benzamido)–3,3,4–trifluoro–5–hydroxyazepane–1–carboxylic acid–*tert*–butyl ester (3e)

The procedure for the synthesis of **5.5a** was followed: yield 58% over two steps; white crystals; R_f (petroleum ether/EtOAc, 1/1) 0.37; $[\alpha]^{20}_D = -11.4$ (*c* 1.0, $CHCl_3$); IR (film) ν_{max} (cm^{-1}): 3500–3100 (br), 2360, 1716, 1683, 1652, 1630, 1613, 1518, 1249, 1210, 1171, 1139, 1049, 840, 793, 726, 608; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (d, J = 4.87 Hz, 1H), 7.82 (d, J = 8.66 Hz, 2H), 7.45–7.32 (m, 5H), 7.03 (d, J = 8.59 Hz, 2H), 5.45 (s,1H), 5.12 (s, 2H), 4.68 (dddd, J = 46.57 ($^1J_{HF}$), 18.31, 9.42, 1.57 Hz, 1H), 4.47–4.39 (m, 1H), 4.30–4.24 (m, 1H), 4.21–4.10 (m, 2H), 4.34–4.23 (m, 2H), 1.51 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 162.0, 156.7, 136.4, 129.3, 128.8, 128.4, 127.6, 125.4, 121.7 (dd, J = 252.11 ($^1J_{CF}$), 18.99 ($^3J_{CF}$) Hz), 114.9, 90.3 (dt, J = 193.17 ($^1J_{CF}$), 22.29 ($^2J_{CF}$) Hz), 82.9, 73.2 (d, $^2J_{CF}$ = 16.55 Hz), 70.3, 57.6 (d, $^3J_{CF}$ = 5.71 Hz), 52.4 (dd, J = 37.68 ($^2J_{CF}$), 27.76 ($^2J_{CF}$) Hz), 48.9, 28.3; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{25}H_{30}F_3N_2O_5$ 495.2107, found 495.2104.

(3*R*,4*R*,6*S*)–3–(4–(benzyloxy)benzamido)–4–(3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–(benzyloxycarbonyl)benzoyl)benzoyloxy)–6–fluoroazepane–1–carboxylic acid–*tert*–butyl ester (3.1a)

The procedure for the synthesis of **5.5b** was followed: yield 70%; colourless oil; R_f (petroleum ether/EtOAc, 3/2) 0.33; $[\alpha]^{20}_D = +34.3$ (*c* 0.9, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3365, 2390, 2324, 2288, 1715, 1688, 1666, 1652, 1638, 1585, 1493, 1129, 978, 919, 613; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 7.08 Hz, 1H), 7.75 (d, J = 8.56 Hz, 2H), 7.43–7.30 (m, 7H), 7.26–7.14 (m, 15H), 7.09–7.02 (m, 5H), 6.98–6.89 (m, 3H), 6.81 (d, J = 7.55 Hz, 2H), 5.09 (s, 2H), 5.05 (s, 2H), 4.95–4.77 (m, 1H), 4.81 (s, 2H), 4.80 (s, 2H), 4.67 (s, 2H), 4.32 (dd, J = 14.63, 6.70 Hz, 1H), 4.15 (d, J = 15.24 Hz, 1H), 3.54–3.44 (m, 1H), 3.39 (dd, J = 15.67, 4.98 Hz, 1H), 2.89 (dd, J = 24.21, 11.39 Hz, 1H), 2.46–2.30 (m, 2H), 1.55 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 167.8, 166.5, 165.8, 161.9, 158.4, 157.4, 156.7, 136.7, 136.6, 136.2, 136.1, 133.2, 132.9, 132.7, 130.9, 129.3, 129.1, 128.8, 128.7, 128.6, 128.3, 128.2, 127.9, 127.8, 127.6, 127.5, 126.8, 122.4, 115.7, 115.1, 107.4, 104.3, 87.8 (d, $^1J_{CF}$ = 177.72 Hz), 82.2, 72.6 (d, $^3J_{CF}$ = 7.78 Hz), 70.9, 70.5, 67.5, 67.3, 53.5, 53.1 (d, $^2J_{CF}$ = 34.37 Hz), 51.6, 34.4 (d, $^2J_{CF}$ = 20.91 Hz), 28.7; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{68}H_{64}FN_2O_{12}$ 1119.4443, found 1119.4413.

(3*R*,4*R*,6*R*)–3–(4–(benzyloxy)benzamido)–4–(3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–(benzyloxycarbonyl)benzoyl)benzoyloxy)–6–fluoroazepane–1–carboxylic acid–*tert*–butyl ester (3.1b)

The procedure for the synthesis of **5.5b** was followed: yield 72%; colourless oil; R_f (petroleum ether/EtOAc, 3/2) 0.31; $[\alpha]^{20}_D = -67.5$ (*c* 0.6, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3365, 2390, 2324, 2288, 1715, 1688, 1666, 1652, 1638, 1585, 1493, 1129, 978, 919, 613; ^1H NMR (400 MHz, $CDCl_3$) δ 8.21–7.62 (m, 3H), 7.57–7.67 (m, 32H), 5.24–2.76 (m, 16H), 2.70–1.93 (m, 3H), 1.51 (s, 9H); ^{13}C NMR (150 MHz, $(CD_3)_2CO$) δ 190.8, 168.2, 167.0, 166.0, 162.6, 162.3, 162.2, 157.6, 155.6, 138.0, 137.9, 136.8, 132.6, 132.5, 130.0, 130.0, 129.8, 129.3, 129.1, 129.0, 128.9, 128.8, 128.5, 122.6, 116.2, 115.3, 115.2, 92.2 (d, $^1J_{CF}$ = 169.37 Hz), 81.6, 80.6, 76.3, 71.8, 71.5, 70.7, 70.6, 67.5, 60.0, 54.0, 53.2, 50.5, 46.7, 46.1, 39.7 (d, $^2J_{CF}$ = 21.27 Hz), 28.6, 28.4; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{68}H_{64}FN_2O_{12}$ 1119.4443, found 1119.4447.

(3*R*,4*S*,5*S*)–3–(4–(benzyloxy)benzamido)–4–(3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–(benzyloxycarbonyl)benzoyl)benzoyloxy)–5–fluoroazepane–1–carboxylic acid–*tert*–butyl ester (3.1c)

The procedure for the synthesis of **5.5b** was followed: yield 67%; colourless oil; R_f (petroleum ether/EtOAc, 3/2) 0.34; $[\alpha]^{20}_D = +54.9$ (*c* 1.0, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3365, 2390, 2324, 2288, 1715, 1688, 1666, 1652, 1638, 1585, 1493, 1129, 978, 919, 613; ^1H NMR (600 MHz, $CDCl_3$) δ 7.76–7.66 (m, 2H), 7.42–6.79 (m, 32H), 5.17–4.87 (m, 5H), 4.81–4.56 (m, 6H), 4.00–3.12 (m, 6H), 2.32–2.16 (m, 2H), 1.56 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.3, 167.5, 166.2, 165.7, 161.9, 158.1, 156.6, 155.1, 136.4, 135.9, 135.7, 132.2, 131.1, 130.7, 129.0, 128.8, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 122.2, 115.4, 115.2, 114.9, 114.8, 107.2, 103.9, 91.1 (d, $^1J_{CF}$ = 175.6 Hz), 81.8, 81.3 (d, $^2J_{CF}$ = 16.36 Hz), 76.1, 70.9, 70.7, 70.2, 51.2, 47.8, 46.3, 41.5, 37.3, 32.1, 28.5; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{68}H_{64}FN_2O_{12}$ 1119.4443, found 1119.4425.

(5*R*,6*R*)–6–(4–(benzyloxy)benzamido)–5–((3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–((benzyloxy) carbonyl)benzoyl)benzoyl)oxy)–3,3–difluoroazepane–1–carboxylic acid–*tert*–butyl ester (3.1d)

The procedure for the synthesis of **5.5b** was followed: yield 70%; colourless oil; R_f (petroleum ether/EtOAc, 3/2) 0.33; $[\alpha]^{20}_D = -74.9$ (*c* 0.6, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3370, 2383, 2322, 2282, 1711, 1687, 1652, 1649, 1631, 1581, 1484, 1122, 999, 921, 627; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03–7.94 (m, 1H), 7.84–7.76 (m, 2H), 7.46–6.82 (m, 32 H), 5.50 (t, *J* = 7.42 Hz, 1H), 5.13 (s,

2H), 5.09 (s, 4H), 4.81 (s, 4H), 4.68–4.58 (m, 1H), 4.50–4.34 (m, 1H), 4.19 (d, J = 14.98 Hz, 1H), 3.44 (dd, J = 15.13, 4.88 Hz, 1H), 3.30 (dd, J = 15.12, 4.22 Hz, 1H), 2.76–2.47 (m, 2H), 1.56 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 167.5, 166.5, 161.7, 158.1, 157.1, 136.5, 135.9, 135.8, 132.3, 130.7, 129.1, 128.8, 128.6, 128.4, 127.8, 127.6, 127.2, 126.3, 122.2, 115.4, 114.9, 107.2, 82.4, 76.1, 70.7, 70.6, 70.2, 67.2, 55.7 (d, $^2J_{CF}$ = 27.60 Hz), 53.4, 50.6, 36.3 (d, $^2J_{CF}$ = 27.70 Hz), 28.3; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{68}H_{63}F_2N_2O_{12}$ 1137.4349, found 1137.4318.

(4*R*,5*S*,6*R*)–6–(4–(benzyloxy)benzamido)–5–((3,5–bis(benzyloxy)–4–(2–(benzyloxy)–6–((benzyloxy)carbonyl)benzoyl)benzoyl)oxy)–3,3,4–trifluoroazepane–1–carboxylic acid–*tert*–butyl ester (3.1e)

The procedure for the synthesis of **5.5b** was followed: yield 74%; colourless oil; R_f (petroleum ether/EtOAc, 3/2) 0.36; $[\alpha]^{20}_D$ = −57.1 (*c* 0.9, CH_2Cl_2); IR (film) ν_{max} (cm^{-1}): 3378, 2388, 2343, 2267, 1721, 1689, 1659, 1641, 1639, 1587, 1489, 1129, 987, 926, 607; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.64 (m, 2H), 7.43–6.76 (m, 32H), 5.74–5.60 (m, 1H), 5.11 (s, 2H), 5.06 (s, 2H), 4.97–4.75 (m, 1H), 4.76 (s, 4H), 4.74 (s, 2H), 4.45–4.30 (m, 1H), 4.06 (brd, J = 14.54 Hz, 1H), 4.00–3.82 (m, 1H), 3.58–3.34 (m, 2H), 1.56 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.8, 167.6, 166.3, 161.8, 158.1, 156.2, 136.4, 135.9, 129.0, 128.8, 128.6, 128.4, 128.1, 127.9, 127.8, 127.6, 127.3, 126.4, 122.2, 115.2, 114.9, 107.3, 89.4 (d, $^1J_{CF}$ = 217.71 Hz), 82.6 (d, $^2J_{CF}$ = 24.57 Hz), 78.3, 70.9, 70.6, 70.2, 67.4, 67.3, 51.8 (d, $^3J_{CF}$ = 9.44 Hz), 30.4, 28.3; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{68}H_{62}F_3N_2O_{12}$ 1155.4255, found 1155.4226.

2–(4–((((3*R*,4*R*,6*S*)–6–fluoro–3–(4–hydroxybenzamido)azepan–4–yl)oxy)carbonyl)–2,6–dihydroxybenzoyl)–3–hydroxybenzoic acid (1a)

The procedure for the synthesis of **1** was followed: yield 64%; yellow film; retention time 20.0 min (Phenomenex Gemini C18 column (150 × 21.20 mm); MeCN/water/TFA (from 35:65:0.1 to 44:56:0.1 over 30 min; flow rate: 8.80 mL/min); $[\alpha]^{20}_D$ = +82.1 (*c* 0.5, MeOH); IR (film) ν_{max} (cm^{-1}): 3400–2800 (br), 2360, 2340, 1717, 1683, 1652, 1646, 1635, 1489, 1102, 991, 922, 632; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 11.63 (s, 2H), 10.00 (s, 1H), 9.83 (s, 1H), 9.24 (brs, 1H), 8.46 (d, J = 8.00 Hz, 1H), 7.62 (d, J = 8.74 Hz, 2H), 7.35 (d, J = 7.80 Hz, 1H), 7.26 (t, J = 7.90 Hz, 1H), 7.04 (d, J = 8.13 Hz, 1H), 6.77 (d, J = 8.60 Hz, 2H), 6.76 (s, 2H), 5.36–5.31 (m, 1H), 5.29–5.17 (m, $^1J_{HF}$ = 44.09 Hz, 1H), 4.57–4.51 (m, 1H), 3.50–3.31 (m, 4H), 2.57–2.42 (m, 2H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 201.5, 166.9, 166.1, 164.1, 161.4, 160.4, 153.2, 135.3, 132.4, 129.2, 128.9, 128.8, 124.5, 120.0, 119.6, 114.8, 113.5, 107.3, 86.2 (d, $^1J_{CF}$ = 172.92 Hz), 71.5, 50.8, 49.2 (d, $^2J_{CF}$ = 25.48 Hz), 47.7, 33.9 (d, $^2J_{CF}$ = 22.28 Hz); HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{28}H_{26}FN_2O_{10}$ 569.1571, found 569.1575.

2–(4–((((3*R*,4*R*,6*R*)–6–fluoro–3–(4–hydroxybenzamido)azepan–4–yl)oxy)carbonyl)–2,6–dihydroxybenzoyl)–3–hydroxybenzoic acid (1b)

The procedure for the synthesis of **1** was followed: yield 69%; yellow film; retention time 27.4 min (Phenomenex Gemini C18 column (150 × 21.20 mm); MeCN/water/TFA (from 5:95:0.1 to 55:45:0.1 over 30 min; flow rate: 8.80 mL/min); $[\alpha]^{20}_{D} = -77.9$ (*c* 0.4, MeOH); IR (film) ν_{max} (cm^{-1}): 3400–2800 (br), 2360, 2340, 1717, 1683, 1652, 1646, 1635, 1489, 1102, 991, 922, 632; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 11.67 (s, 2H), 10.07 (brs, 1H), 9.87 (s, 1H), 9.34 (brs, 2H), 8.55 (d, J = 8.47 Hz, 1H), 7.61 (d, J = 8.69 Hz, 2H), 7.36 (d, J = 7.61 Hz, 1H), 7.27 (t, J = 8.01 Hz, 1H), 7.04 (d, J = 8.01 Hz, 1H), 6.78 (d, J = 8.66 Hz, 2H), 6.76 (s, 2H), 5.55 (ddd, J = 10.33, 9.93, 2.02 Hz, 1H), 5.33–5.21 (m, $^{1}J_{HF}$ = 47.37 Hz, 1H), 4.59–4.53 (m, 1H), 3.58 (dd, J = 17.42, 15.96 Hz, 1H), 3.52–3.40 (m, 2H), 3.30 (dd, J = 14.14, 7.39 Hz, 1H), 2.57–2.52 (m, 1H), 2.39–2.27 (m, 1H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 201.6, 166.9, 166.3, 164.0, 161.5, 160.6, 153.2, 135.3, 132.4, 129.2, 128.9, 128.8, 124.2, 120.0, 119.6, 114.9, 113.5, 107.4, 86.0 (d, $^{1}J_{CF}$ = 169.84 Hz), 70.1, 50.5, 50.3 (d, $^{2}J_{CF}$ = 24.68 Hz), 47.1, 32.3 (d, $^{2}J_{CF}$ = 19.15 Hz); HRMS (ESI): $[M + H]^{+}$, *m/z* calcd for $C_{28}H_{26}FN_2O_{10}$ 569.1571, found 569.1577.

2–(4–(((3*R*,4*S*,5*S*)–5–fluoro–3–(4–hydroxybenzamido)azepan–4–yloxy)carbonyl)–2,6–dihydroxybenzoyl)–3–hydroxybenzoic acid (1c)

The procedure for the synthesis of **1** was followed: yield 72%; yellow film; retention time 21.2 min (Phenomenex Gemini C18 column (150 × 21.20 mm); MeCN/water/TFA (from 35:65:0.1 to 44:56:0.1 over 30 min; flow rate: 8.80 mL/min); $[\alpha]^{20}_{D} = +58.7$ (*c* 0.2, MeOH); IR (film) ν_{max} (cm^{-1}): 3400–2800 (br), 2360, 2340, 1717, 1683, 1652, 1646, 1635, 1489, 1102, 991, 922, 632; ^{1}H NMR (600 MHz, (CD_3CN) δ 10.69 (brs, 1H), 8.00 (d, J = 6.91 Hz, 1H), 7.68 (d, J = 8.06 Hz, 2H), 7.51 (d, J = 7.62 Hz, 1H), 7.33 (t, J = 7.95 Hz, 1H), 7.11 (d, J = 7.62 Hz, 1H), 7.02 (s, 2H), 6.83 (d, J = 8.06 Hz, 2H), 5.49 (t, J = 7.98 Hz, 1H), 5.29–5.16 (m, $^{1}J_{HF}$ = 43.56 Hz, 1H), 4.41 (brs, 1H), 3.71–3.64 (m, 2H), 3.59–3.54 (m, 1H), 3.42–3.34 (m, 1H), 2.63–2.46 (m, 2H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 202.5, 169.5, 167.5, 162.5, 161.6, 160.5, 153.7, 137.1, 130.6, 130.6, 130.4, 125.5, 122.3, 121.2, 116.1, 109.2, 103.7, 86.9 (d, $^{1}J_{CF}$ = 167.92 Hz), 71.2 (d, $^{2}J_{CF}$ = 19.71 Hz), 53.0, 50.6, 50.3, 35.3 (d, $^{2}J_{CF}$ = 24.12 Hz); HRMS (ESI): $[M + H]^{+}$, *m/z* calcd for $C_{28}H_{26}FN_2O_{10}$ 569.1571, found 569.1567.

2–(4–((((3*R*,4*R*)–6,6–difluoro–3–(4–hydroxybenzamido)azepan–4–yl)oxy)carbonyl)–2,6–dihydroxybenzoyl)–3–hydroxybenzoic acid (1d)

The procedure for the synthesis of **1** was followed: yield 75%; yellow film; retention time 27.5 min (Phenomenex Gemini C18 column (150 × 21.20 mm); MeCN/water/TFA (from 30:70:0.1 to 70:30:0.1 over 50 min; flow rate: 8.80 mL/min); $[\alpha]^{20}_{D} = -98.4$ (*c* 0.7, MeOH); IR (film) ν_{max} (cm^{-1}): 3400–2800 (br), 2363, 2341, 1711, 1687, 1652, 1649, 1631, 1484, 1122, 999, 921, 627; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 11.68 (s, 2H), 10.09 (brs, 1H), 9.89 (s, 1H), 8.54 (d, J = 8.13 Hz, 1H), 7.63 (d, J = 8.58 Hz, 2H), 7.37 (d, J = 7.63 Hz, 1H), 7.28 (t, J = 7.99 Hz, 1H), 7.05 (d, J = 8.16 Hz, 1H), 6.78 (d, J = 8.64 Hz, 2H), 6.78 (s, 2H), 5.45–5.41 (m, 1H), 4.59–4.54 (m, 1H), 3.77–3.68 (m, 2H), 3.52–3.47 (m, 1H), 3.39–3.33 (m, 1H), 2.83–2.74 (m, 2H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 201.6, 167.0, 166.3, 164.0, 161.5, 160.6, 153.3, 135.1, 132.5, 129.3, 129.0, 128.9, 124.3, 121.8 (d, $^{1}J_{CF}$ = 243.01 Hz), 120.0, 119.7, 115.0, 113.6, 107.5, 69.4, 51.7 (t, $^{2}J_{CF}$ = 38.37 Hz), 50.6, 48.1, 37.1 (t, $^{2}J_{CF}$ = 25.41 Hz); HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{28}H_{25}F_2N_2O_{10}$ 587.1477, found 587.1467.

2–(2,6–dihydroxy–4–((((3*R*,4*S*,5*R*)–5,6,6–trifluoro–3–(4–hydroxybenzamido)azepan–4–yl)oxy)carbonyl)benzoyl)–3–hydroxybenzoic acid (1e)

The procedure for the synthesis of **1** was followed: yield 68%; yellow film; retention time 27.6 min (Phenomenex Gemini C18 column (150 × 21.20 mm); MeCN/water/TFA (from 30:70:0.1 to 70:30:0.1 over 50 min; flow rate: 8.80 mL/min); $[\alpha]^{20}_{D} = -64.6$ (*c* 0.4, MeOH); IR (film) ν_{max} (cm^{-1}): 3400–2800 (br), 2360, 2346, 1719, 1667, 1659, 1640, 1639, 1480, 1129, 987, 946, 607; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 11.68 (s, 2H), 10.01 (brs, 1H), 9.86 (s, 1H), 8.48 (d, J = 8.05 Hz, 1H), 7.57 (d, J = 8.46 Hz, 2H), 7.36 (d, J = 7.80 Hz, 1H), 7.28 (t, J = 7.97 Hz, 1H), 7.05 (d, J = 7.95 Hz, 1H), 6.76 (d, J = 8.59 Hz, 2H), 6.76 (s, 2H), 5.67–5.60 (m, 1H), 5.48–5.35 (m, $^{1}J_{HF}$ = 45.30 Hz, 1H), 4.58–4.52 (m, 1H), 3.38–3.34 (m, 1H), 3.30–3.20 (m, 2H), 3.19–3.10 (m, 1H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 201.5, 166.9, 165.9, 164.1, 161.5, 160.4, 153.3, 134.7, 132.4, 129.1, 129.0, 128.9, 124.5, 121.8 (d, $^{1}J_{CF}$ = 251.98 Hz), 120.0, 119.7, 114.9, 113.7, 107.4, 89.4 (dd, J = 186.95 ($^{1}J_{CF}$), 25.49 ($^{2}J_{CF}$) Hz), 72.0 (d, $^{2}J_{CF}$ = 22.64 Hz), 63.0 (d, $^{2}J_{CF}$ = 34.43 Hz), 49.5, 48.4; HRMS (ESI): $[M + H]^+$, *m/z* calcd for $C_{28}H_{24}F_3N_2O_{10}$ 605.1383, found 605.1389.

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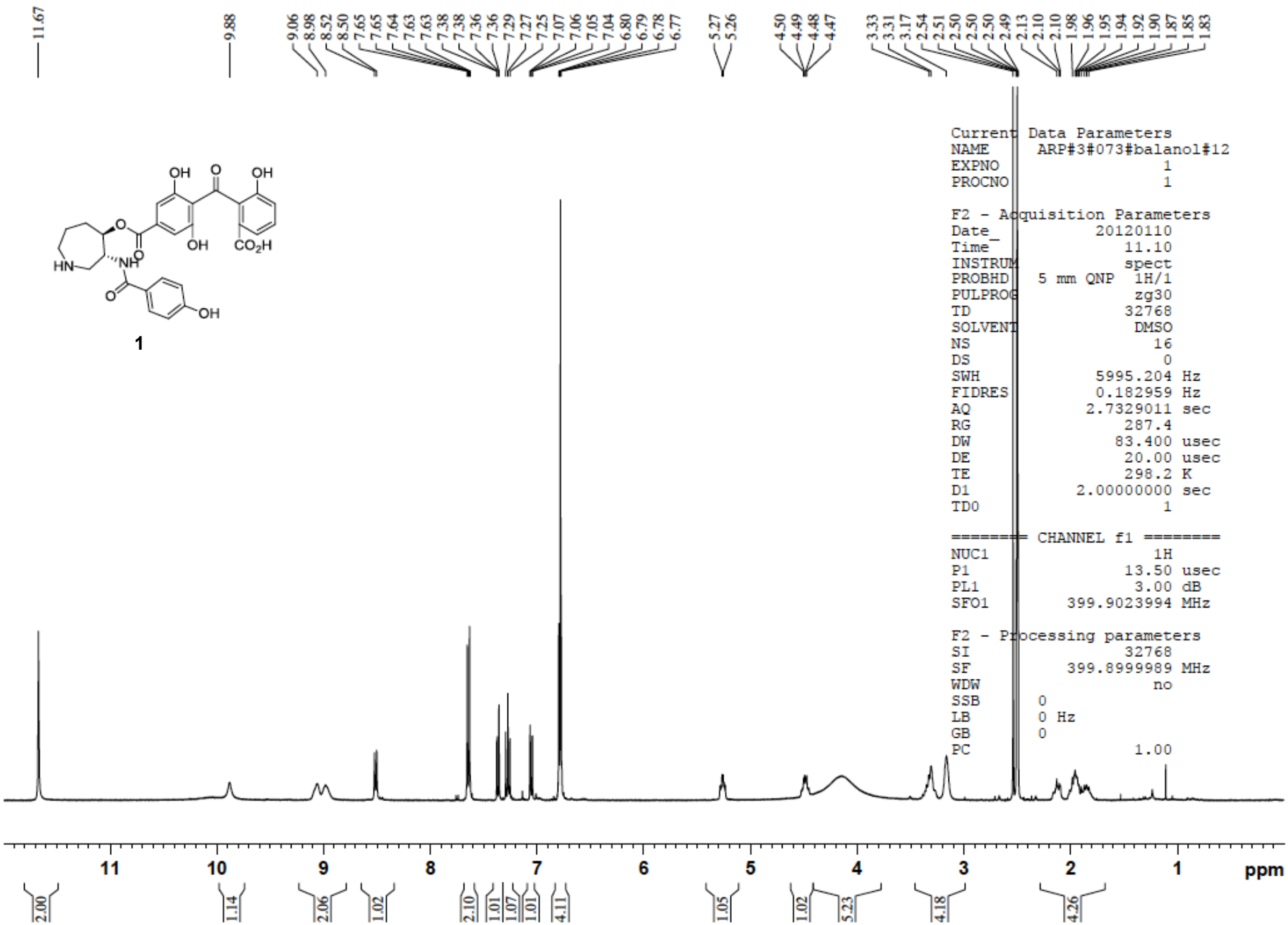
D. NMR spectra of **1**, **3a–3e, 3.1a–3.1e**, and **1a–1e**.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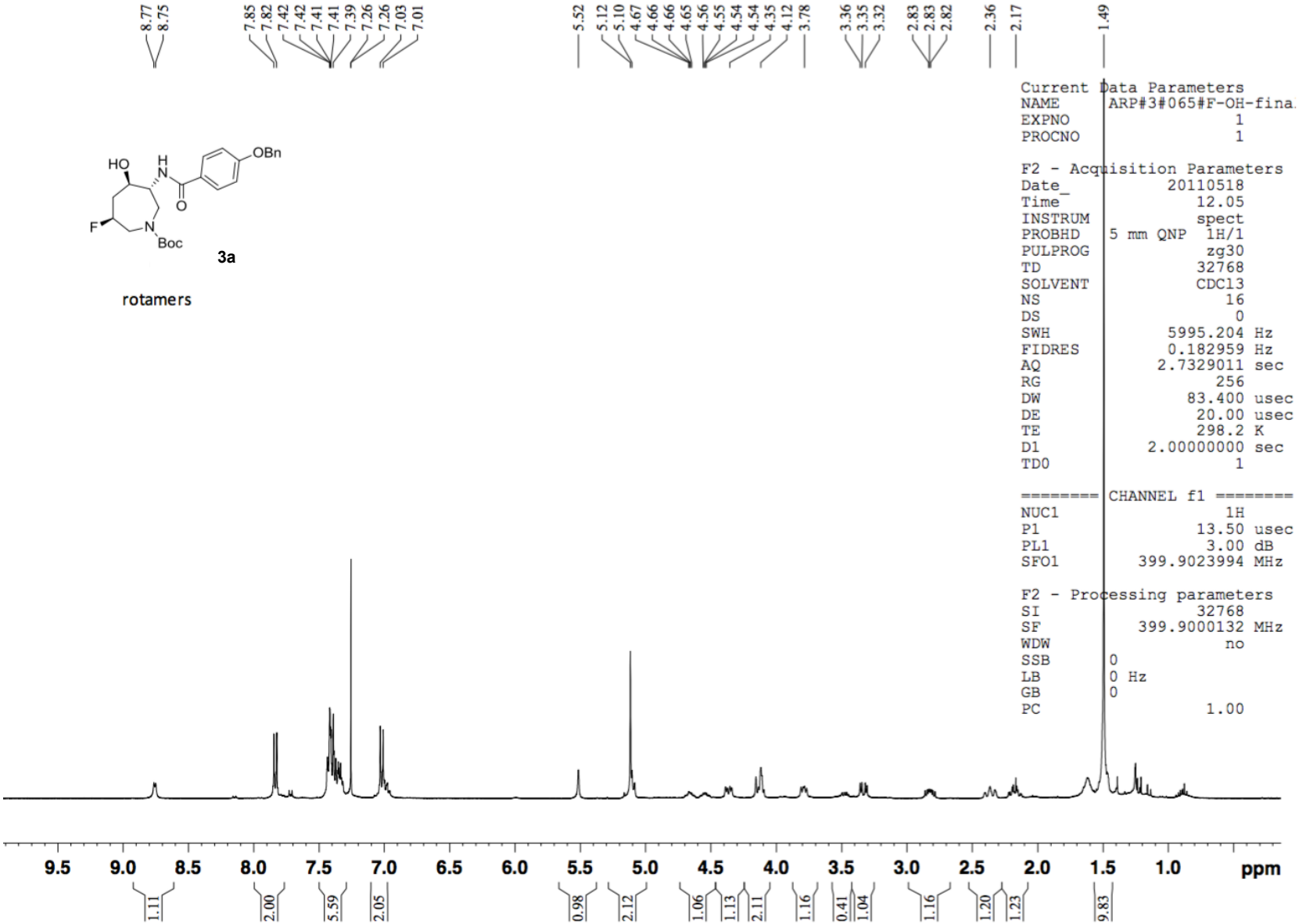
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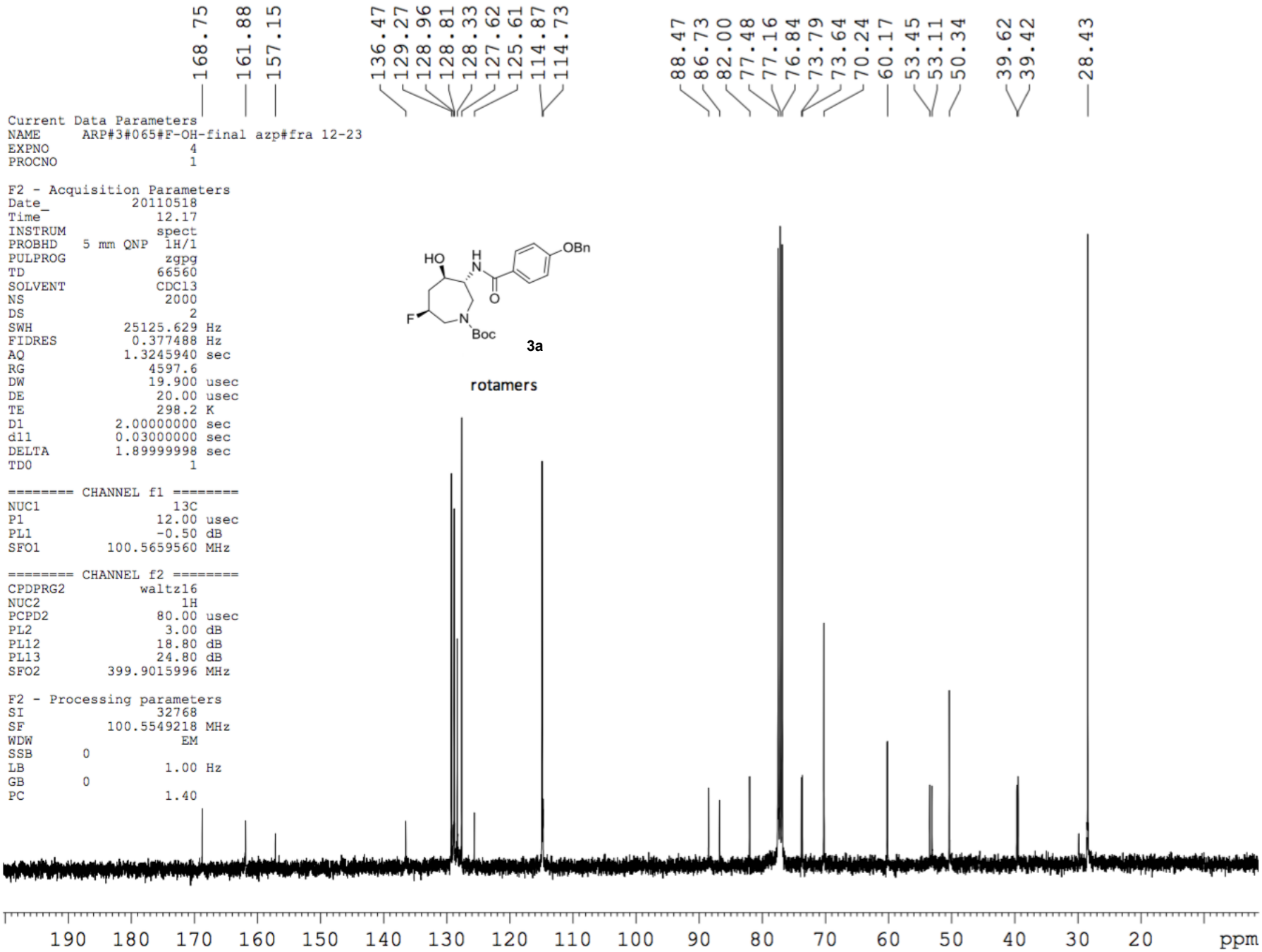
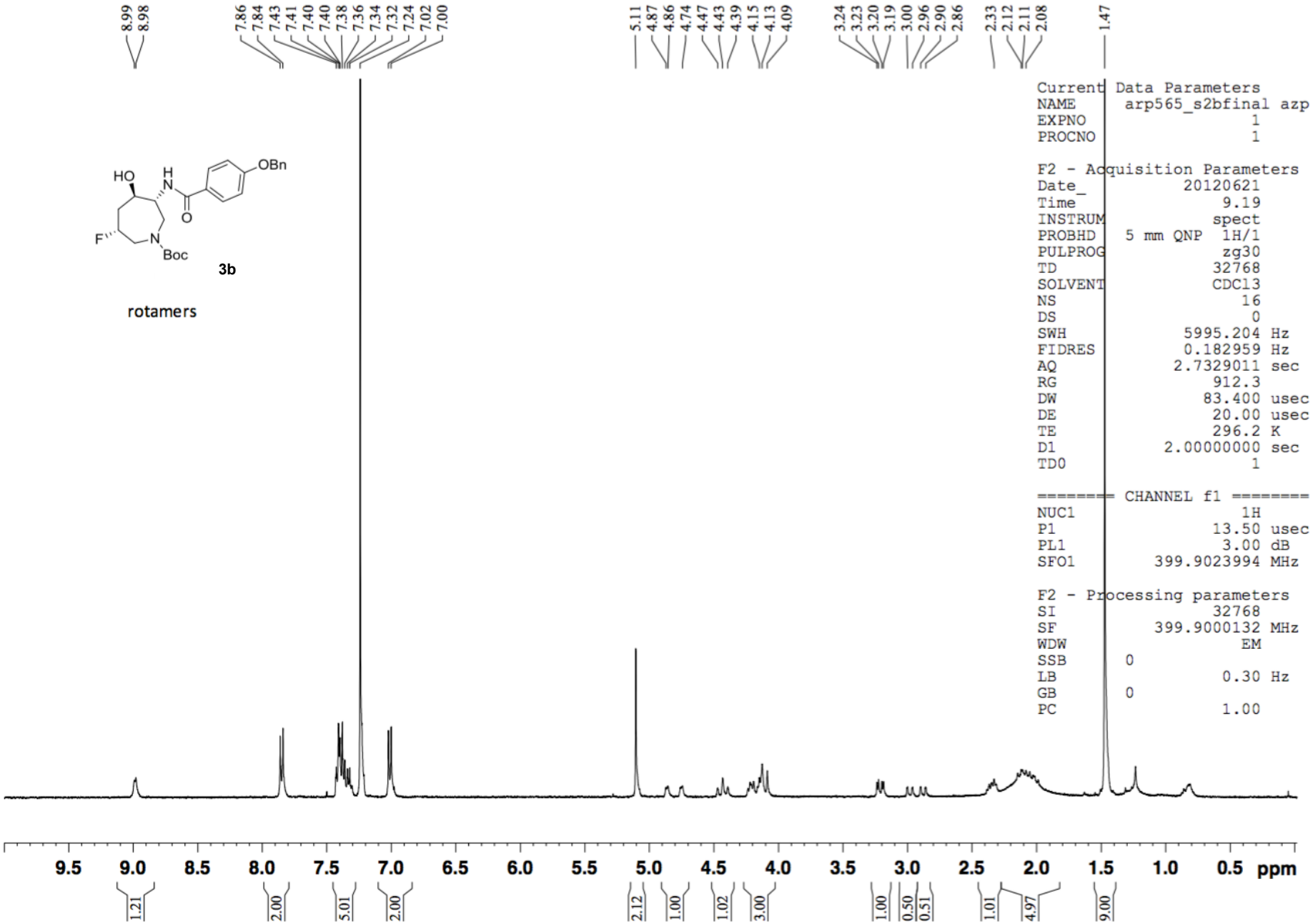
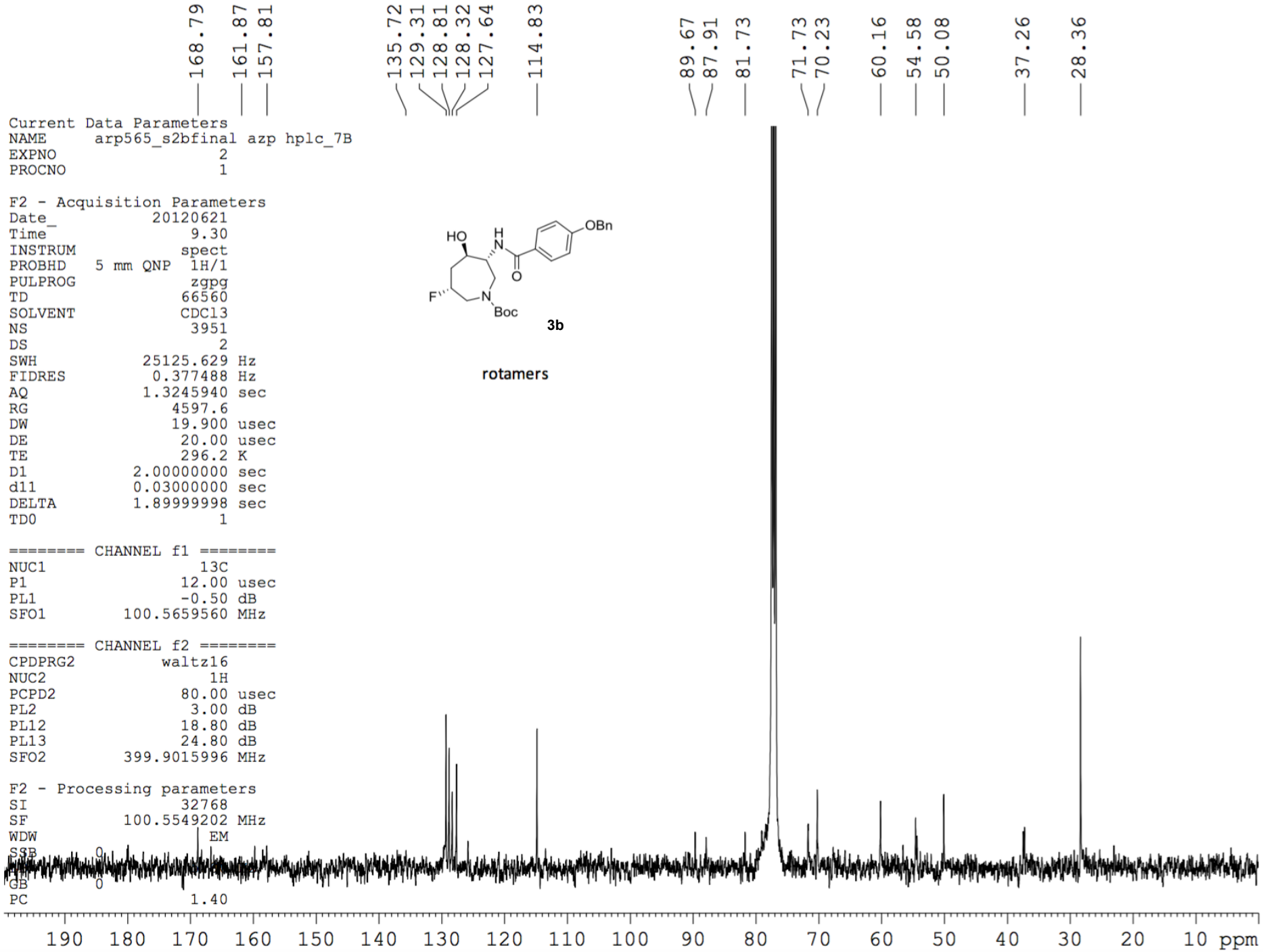
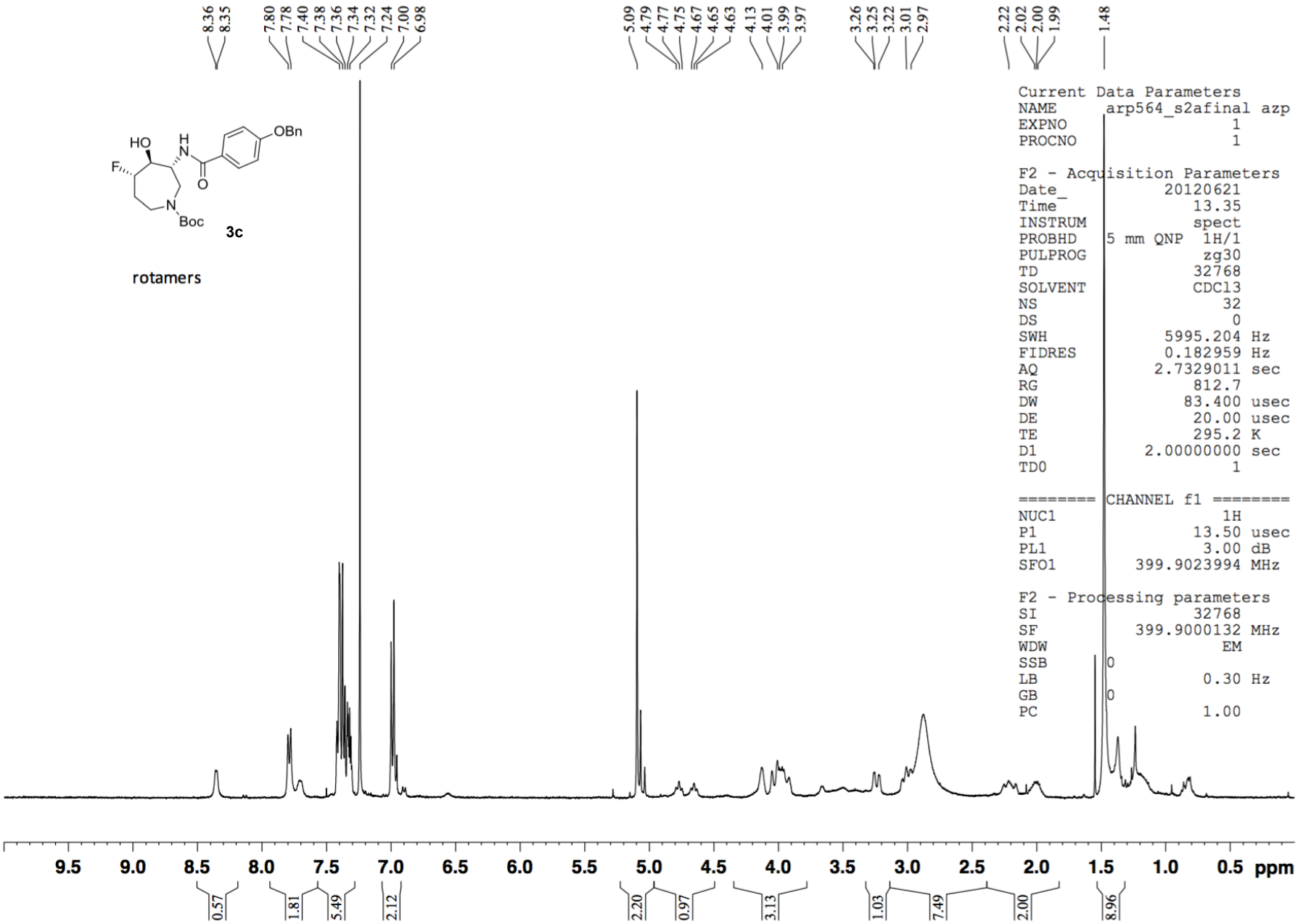
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 256
DW 83.400 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.50 usec
PL1 3.00 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000132 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.11
2.00
5.59
2.05
0.98
2.12
1.06
1.13
2.11
1.16
0.41
1.04
1.16
1.20
1.23
9.83


168.75
161.88
157.15
136.47
129.27
128.96
128.81
128.33
127.62
125.61
114.87
114.73
88.47
86.73
82.00
77.48
77.16
76.84
73.79
73.64
70.24
60.17
53.45
53.11
50.34
39.62
39.42
28.43
Current Data Parameters
NAME ARP#3#065#F-OH-final azp#fra 12-23
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110518
Time 12.17
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 2000
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 4597.6
DW 19.900 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 18.80 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5549218 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
HO
H
N
OBn
O
F
N
Boc
3a
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


8.99
8.98
7.86
7.84
7.43
7.41
7.40
7.40
7.38
7.36
7.34
7.32
7.24
7.02
7.00
5.11
4.87
4.86
4.74
4.47
4.43
4.39
4.15
4.13
4.09
3.24
3.23
3.20
3.19
3.00
2.96
2.90
2.86
2.33
2.12
2.11
2.08
1.47
HO
H
N
OBn
O
F
N
Boc
3b
rotamers
Current Data Parameters
NAME arp565_s2bfinal azp
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120621
Time 9.19
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 912.3
DW 83.400 usec
DE 20.00 usec
TE 296.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.50 usec
PL1 3.00 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.21
2.00
5.01
2.00
2.12
1.00
1.02
3.00
1.00
0.50
0.51
1.01
4.97
9.00

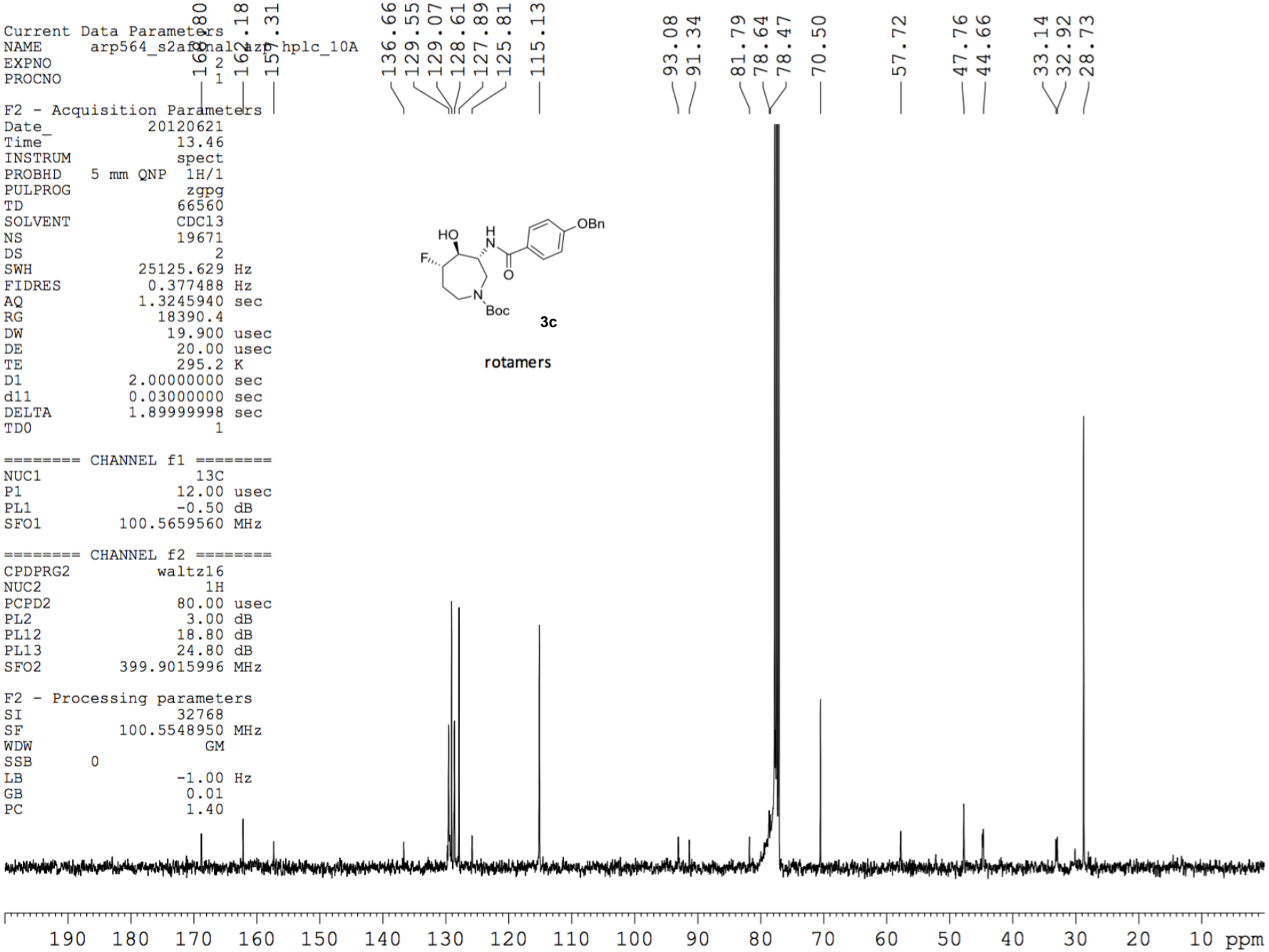

168.79
161.87
157.81
135.72
129.31
128.81
128.32
127.64
114.83
89.67
87.91
81.73
71.73
70.23
60.16
54.58
50.08
37.26
28.36
Current Data Parameters
NAME arp565_s2bfinal azp hplc_7B
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120621
Time 9.30
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 3951
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 4597.6
DW 19.900 usec
DE 20.00 usec
TE 296.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 18.80 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5549202 MHz
WDW EM
SSB 0
LB 0
GB 0
PC 1.40
HO
H
N
OBn
O
F
N
Boc
3b
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


8.36
8.35
7.80
7.78
7.40
7.38
7.36
7.34
7.32
7.24
7.00
6.98
5.09
4.79
4.77
4.75
4.67
4.65
4.63
4.13
4.01
3.99
3.97
3.26
3.25
3.22
3.01
2.97
2.22
2.02
2.00
1.99
1.48
HO
H
N
OBn
F
O
N
Boc
3c
rotamers
Current Data Parameters
NAME arp564_s2afinal azp
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120621
Time 13.35
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 32
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 812.7
DW 83.400 usec
DE 20.00 usec
TE 295.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.50 usec
PL1 3.00 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.57
1.81
5.49
2.12
2.20
0.97
3.13
1.03
7.49
2.00
8.96

```
Current Data Parameters
NAME      arp564_s2afonalc4azp_hplc_10A
EXPNO                               2
PROCNO                              1

F2 - Acquisition Parameters
Date_                        20120621
Time                            13.46
INSTRUM                         spect
PROBHD    5 mm QNP  1H/1
PULPROG                          zgpg
TD                              66560
SOLVENT                         CDCl3
NS                              19671
DS                                  2
SWH                         25125.629 Hz
FIDRES                       0.377488 Hz
AQ                          1.3245940 sec
RG                            18390.4
DW                             19.900 usec
DE                             20.00 usec
TE                             295.2 K
D1                         2.00000000 sec
d11                        0.03000000 sec
DELTA                      1.89999998 sec
TD0                                 1

======== CHANNEL f1 ========
NUC1                              13C
P1                             12.00 usec
PL1                            -0.50 dB
SFO1                     100.5659560 MHz

======== CHANNEL f2 ========
CPDPRG2                      waltz16
NUC2                              1H
PCPD2                          80.00 usec
PL2                             3.00 dB
PL12                           18.80 dB
PL13                           24.80 dB
SFO2                     399.9015996 MHz

F2 - Processing parameters
SI                              32768
SF                       100.5548950 MHz
WDW                                GM
SSB       0
LB                             -1.00 Hz
GB                              0.01
PC                              1.40
```

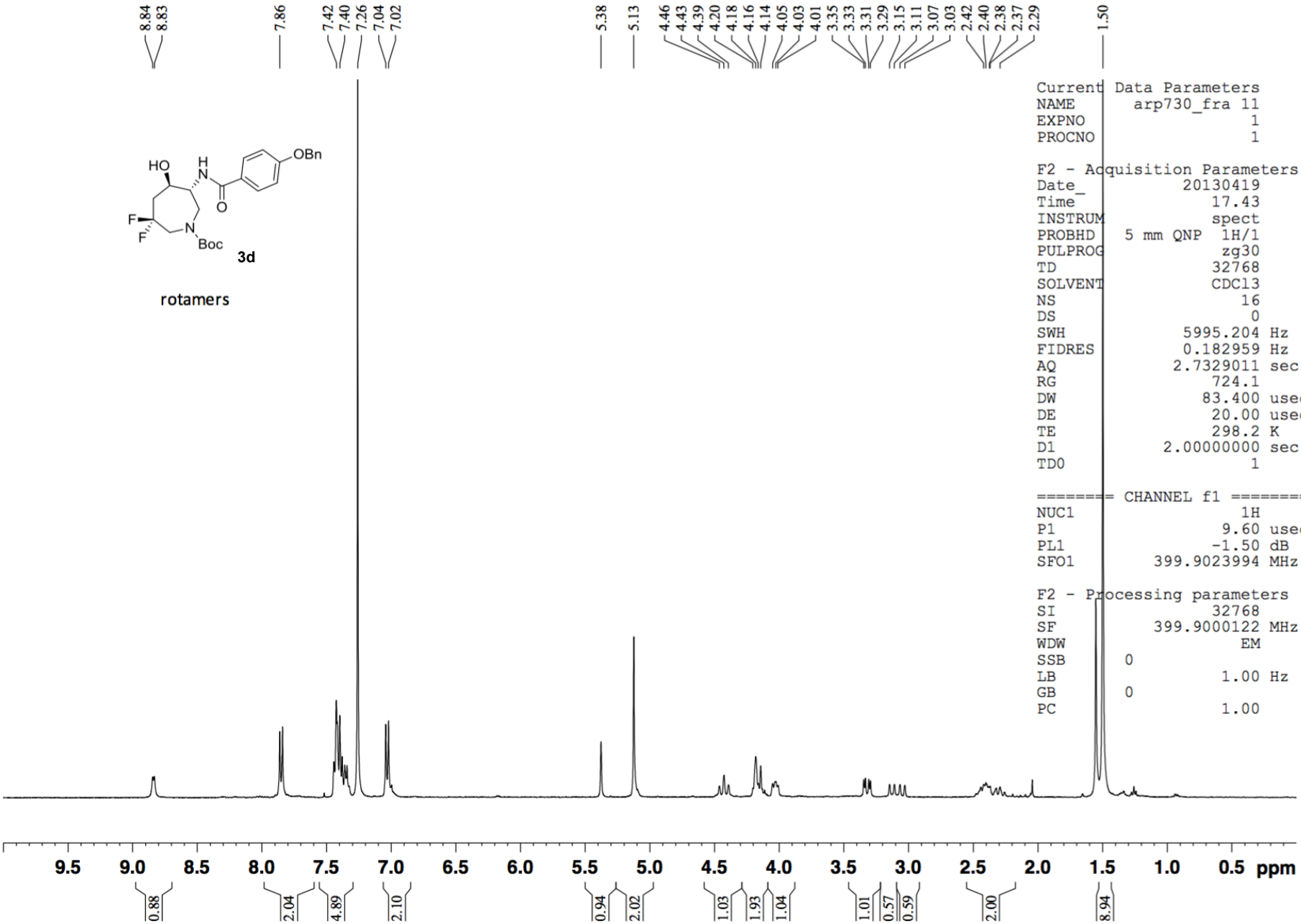

3c

rotamers

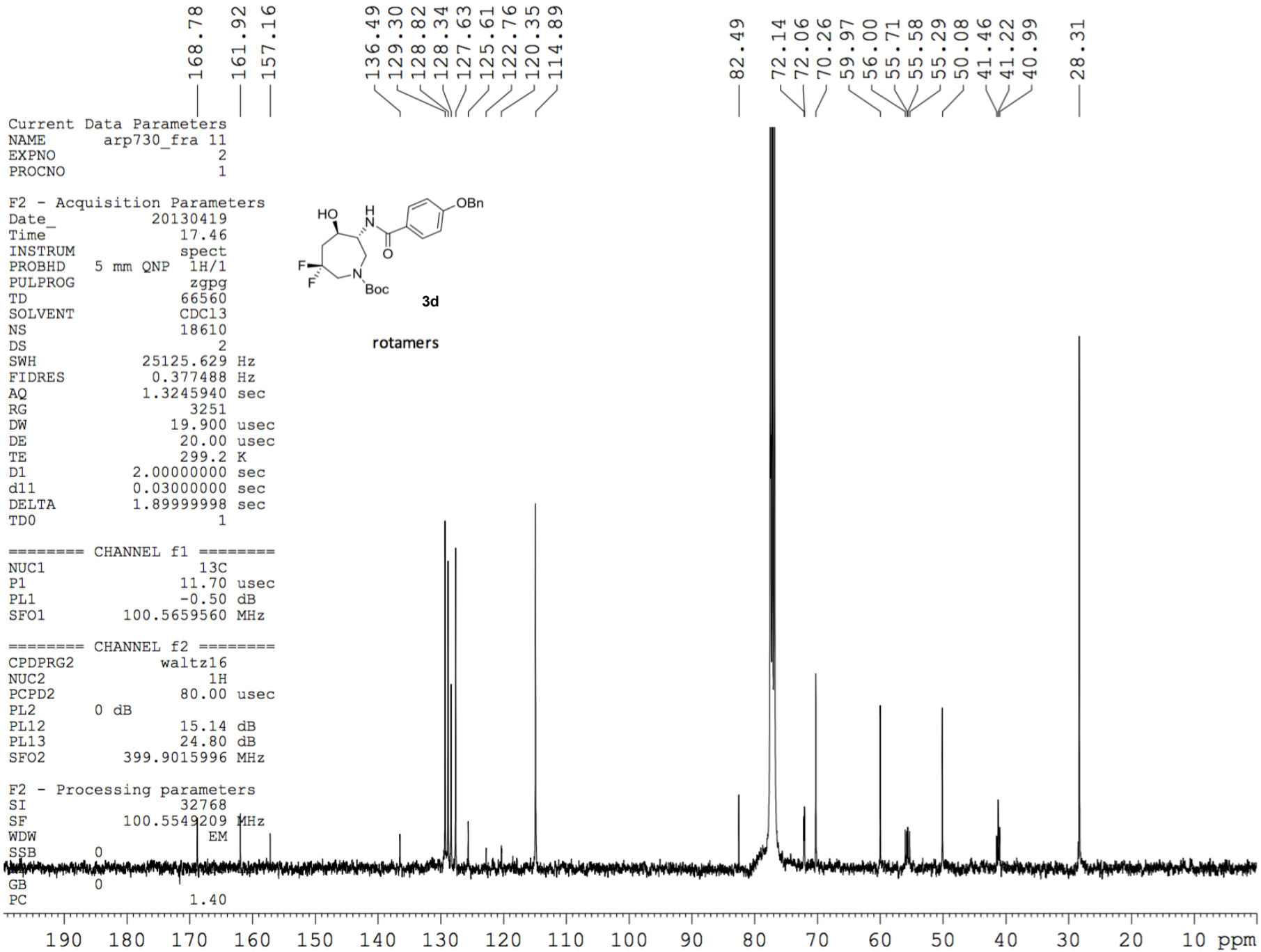


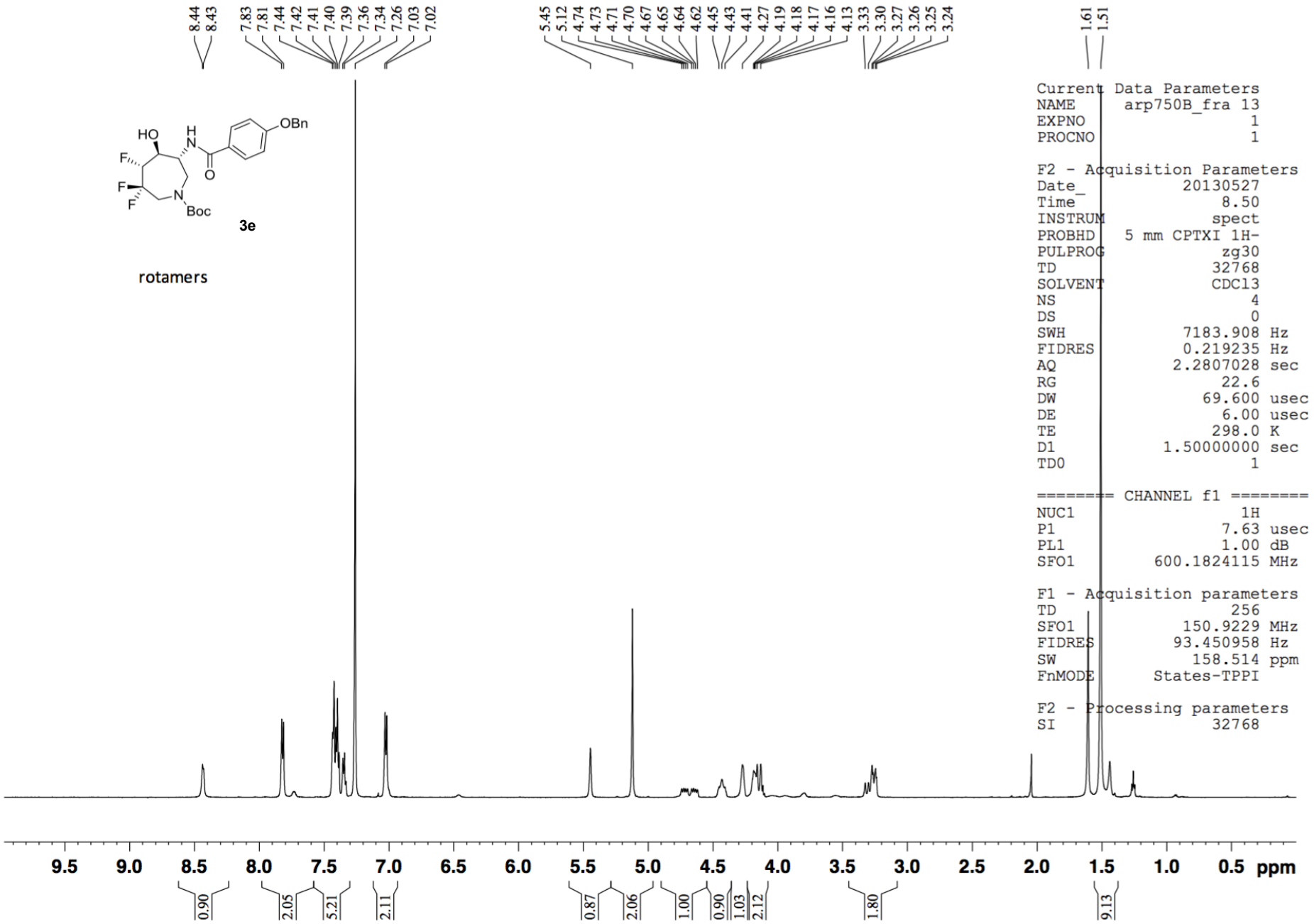
3d

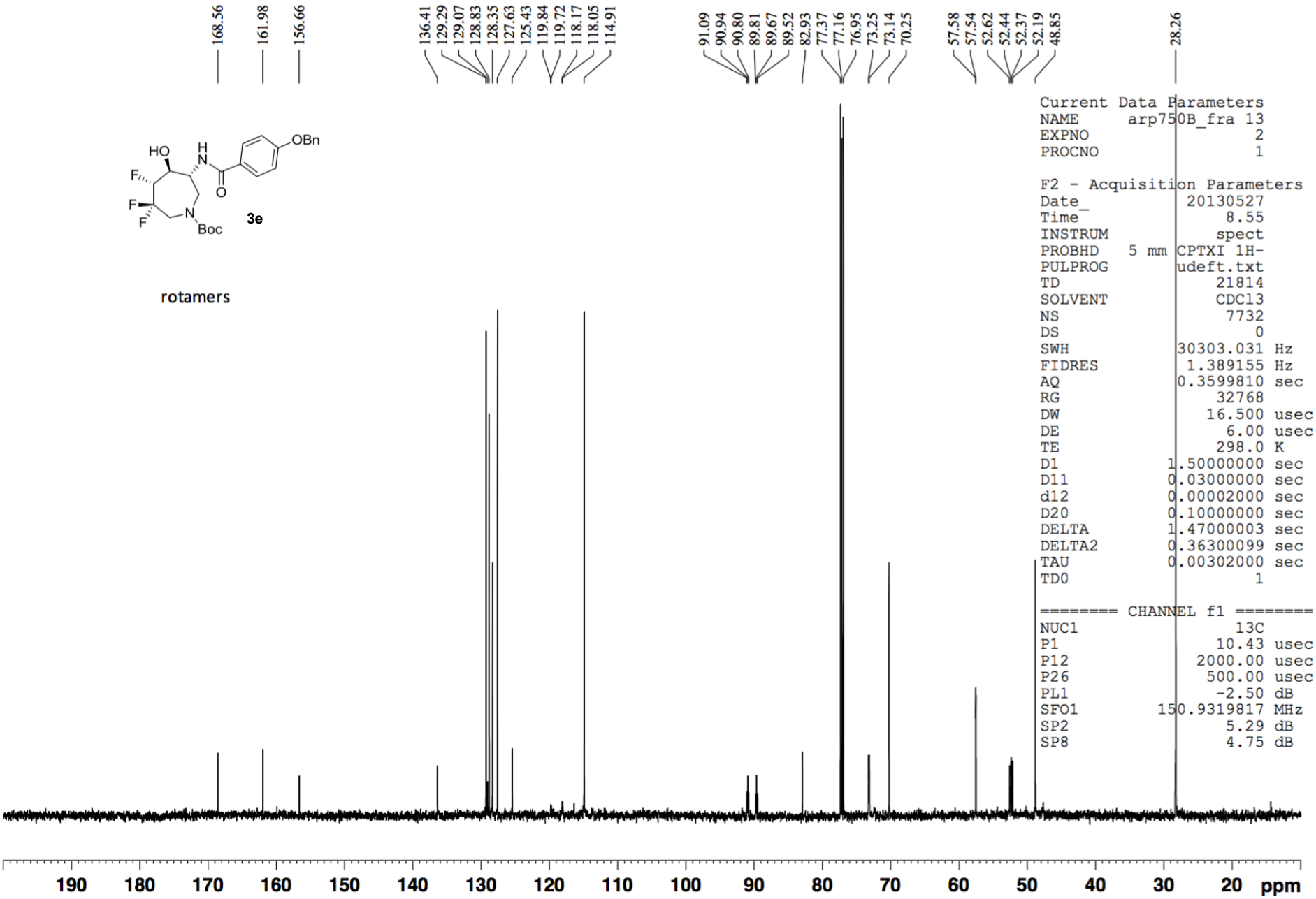
rotamers

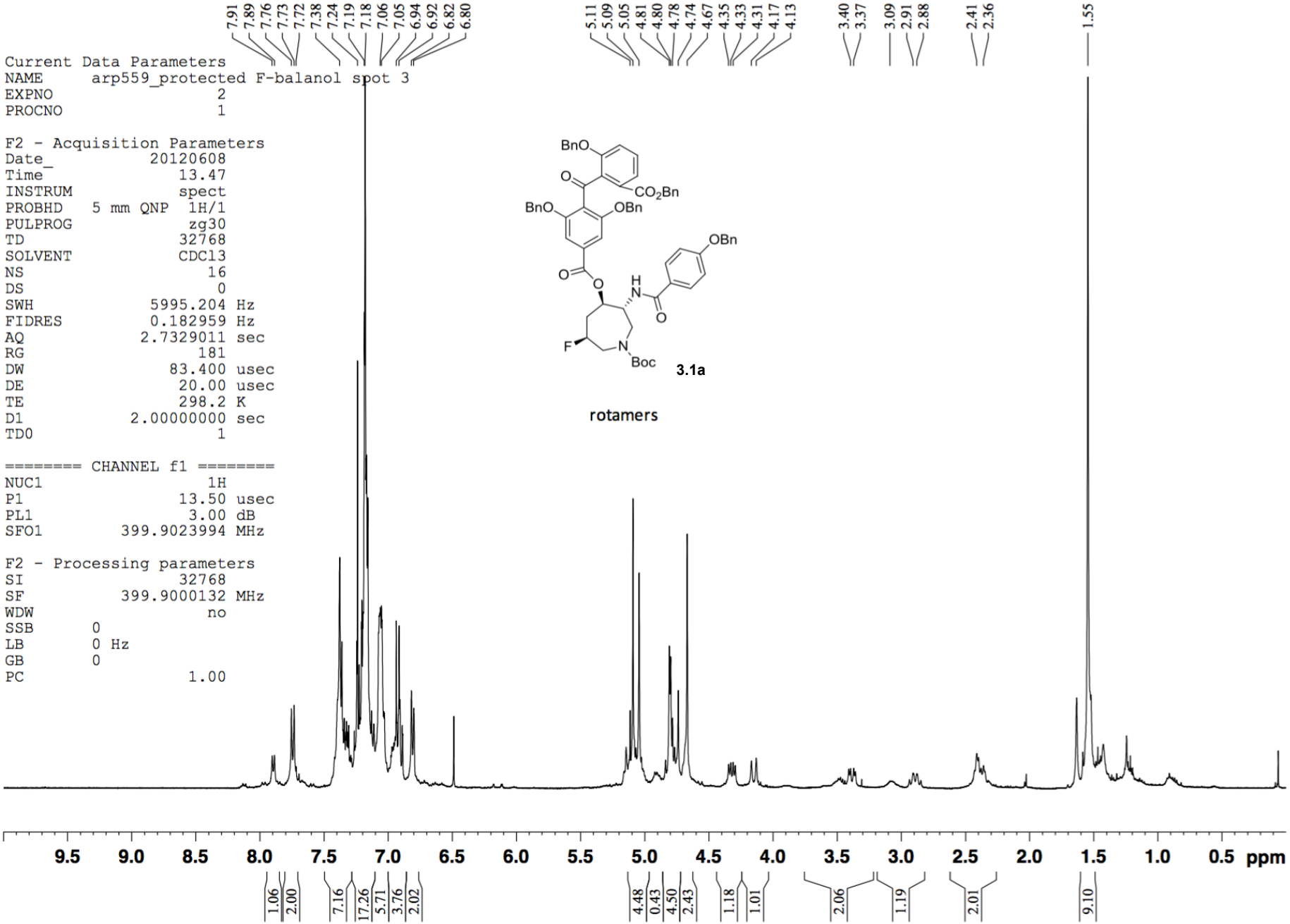


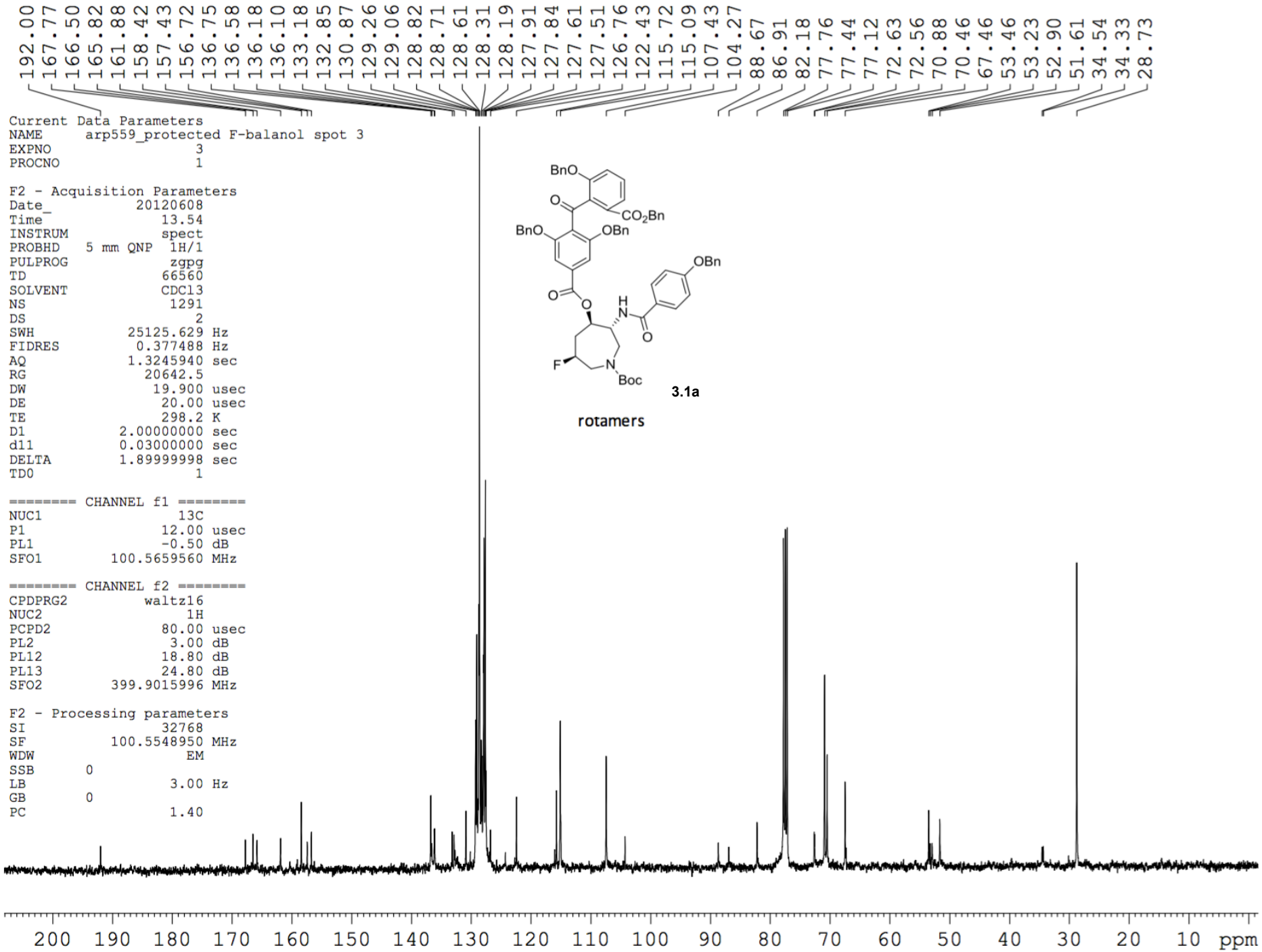

```
Current Data Parameters
NAME        arp730_fra 11
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_            20130419
Time                17.43
INSTRUM             spect
PROBHD    5 mm QNP  1H/1
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     16
DS                      0
SWH             5995.204 Hz
FIDRES          0.182959 Hz
AQ             2.7329011 sec
RG                  724.1
DW                 83.400 usec
DE                 20.00 usec
TE                 298.2 K
D1            2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                  9.60 usec
PL1                -1.50 dB
SFO1         399.9023994 MHz

F2 - Processing parameters
SI                  32768
SF           399.9000122 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.00
```


168.78
161.92
157.16
136.49
129.30
128.82
128.34
127.63
125.61
122.76
120.35
114.89
82.49
72.14
72.06
70.26
59.97
56.00
55.71
55.58
55.29
50.08
41.46
41.22
40.99
28.31
Current Data Parameters
NAME arp730_fra 11
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130419
Time 17.46
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 18610
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 3251
DW 19.900 usec
DE 20.00 usec
TE 299.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 11.70 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0 dB
PL12 15.14 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5549209 MHz
WDW EM
SSB 0
LB 0
GB 0
PC 1.40
HO
H
N
OBn
O
F
F
N
Boc
3d
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


8.44
8.43
7.83
7.81
7.44
7.42
7.41
7.40
7.39
7.36
7.34
7.26
7.03
7.02
5.45
5.12
4.74
4.73
4.71
4.70
4.67
4.65
4.64
4.62
4.45
4.43
4.41
4.27
4.19
4.18
4.17
4.16
4.13
3.33
3.30
3.27
3.26
3.25
3.24
1.61
1.51
HO
H
N
OBn
F
F
F
N
Boc
O
3e
rotamers
Current Data Parameters
NAME arp750B_fra 13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130527
Time 8.50
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 4
DS 0
SWH 7183.908 Hz
FIDRES 0.219235 Hz
AQ 2.2807028 sec
RG 22.6
DW 69.600 usec
DE 6.00 usec
TE 298.0 K
D1 1.50000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 7.63 usec
PL1 1.00 dB
SFO1 600.1824115 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9229 MHz
FIDRES 93.450958 Hz
SW 158.514 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 32768
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.90
2.05
5.21
2.11
0.87
2.06
1.00
0.90
1.03
2.12
1.80
9.13


168.56
161.98
156.66
136.41
129.29
129.07
128.83
128.35
127.63
125.43
119.84
119.72
118.17
118.05
114.91
91.09
90.94
90.80
89.81
89.67
89.52
82.93
77.37
77.16
76.95
73.25
73.14
70.25
57.58
57.54
52.62
52.44
52.37
52.19
48.85
28.26
HO
H
N
OBn
O
F
F
F
N
Boc
3e
rotamers
Current Data Parameters
NAME arp750B_fra 13
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130527
Time 8.55
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG udeft.txt
TD 21814
SOLVENT CDCl3
NS 7732
DS 0
SWH 30303.031 Hz
FIDRES 1.389155 Hz
AQ 0.3599810 sec
RG 32768
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 1.50000000 sec
D11 0.03000000 sec
d12 0.00002000 sec
D20 0.10000000 sec
DELTA 1.47000003 sec
DELTA2 0.36300099 sec
TAU 0.00302000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.43 usec
P12 2000.00 usec
P26 500.00 usec
PL1 -2.50 dB
SFO1 150.9319817 MHz
SP2 5.29 dB
SP8 4.75 dB
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


7.91 7.89 7.76 7.73 7.72 7.38 7.24 7.19 7.18 7.06 7.05 6.94 6.92 6.82 6.80
5.11 5.09 5.05 4.81 4.80 4.78 4.74 4.67 4.35 4.33 4.31 4.17 4.13
3.40 3.37
3.09 2.91 2.88
2.41 2.36
1.55
Current Data Parameters
NAME arp559_protected F-balanol spot 3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120608
Time 13.47
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 181
DW 83.400 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.50 usec
PL1 3.00 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000132 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
N
Boc
3.1a
rotamers
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.06 2.00 7.16 17.26 5.71 3.76 2.02 4.48 0.43 4.50 2.43 1.18 1.01 2.06 1.19 2.01 9.10


192.00 167.77 166.50 165.82 161.88 158.42 157.43 156.72 136.75 136.58 136.18 136.10 133.18 132.85 130.87 129.26 129.06 128.82 128.71 128.61 128.31 128.19 127.91 127.84 127.61 127.51 126.76 122.43 115.72 115.09 107.43 104.27 88.67 86.91 82.18 77.76 77.44 77.12 72.63 72.56 70.88 70.46 67.46 53.46 53.23 52.90 51.61 34.54 34.33 28.73
Current Data Parameters
NAME arp559_protected F-balanol spot 3
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120608
Time 13.54
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 1291
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 20642.5
DW 19.900 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 18.80 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5548950 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
N
Boc
3.1a
rotamers
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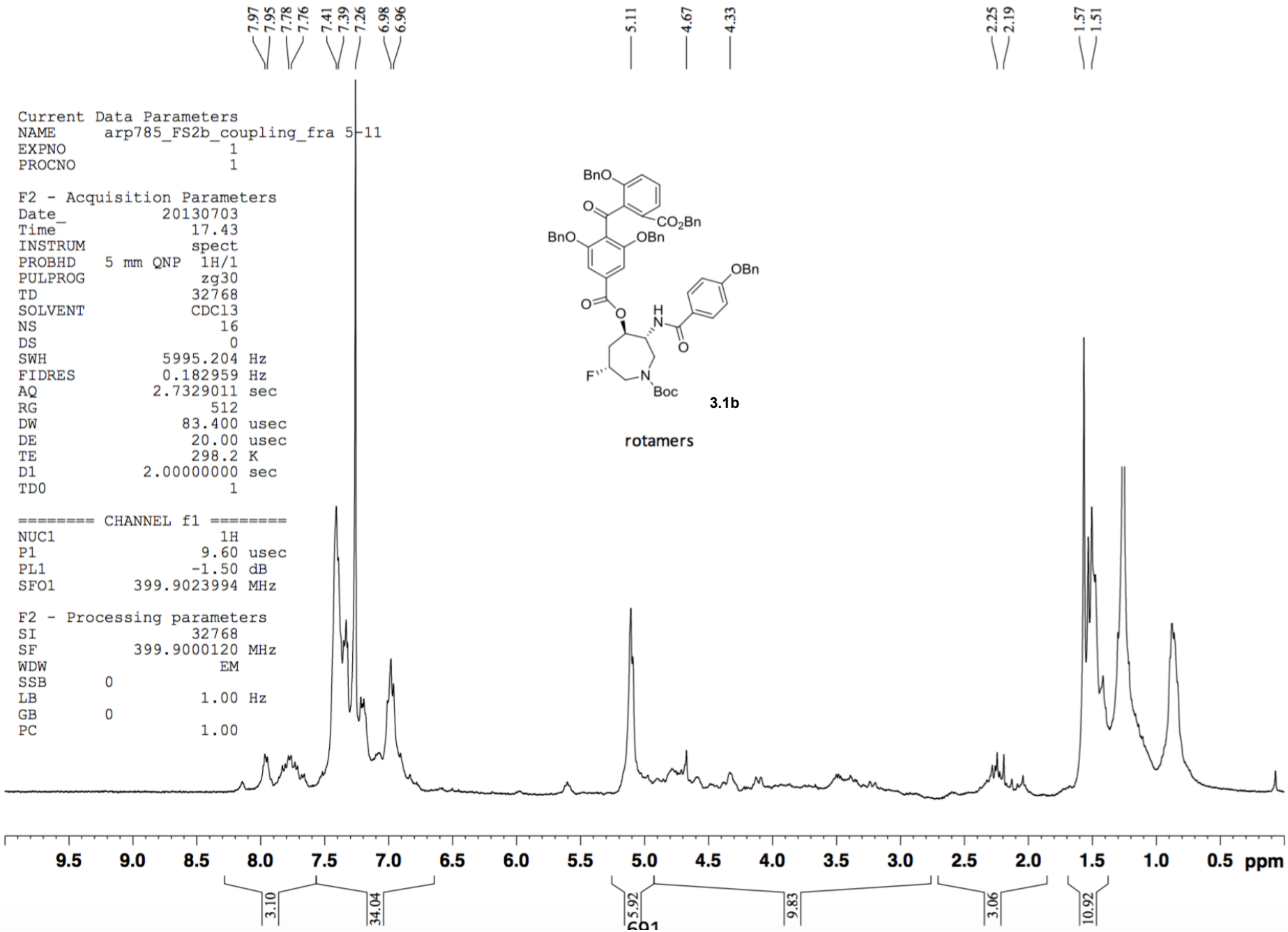
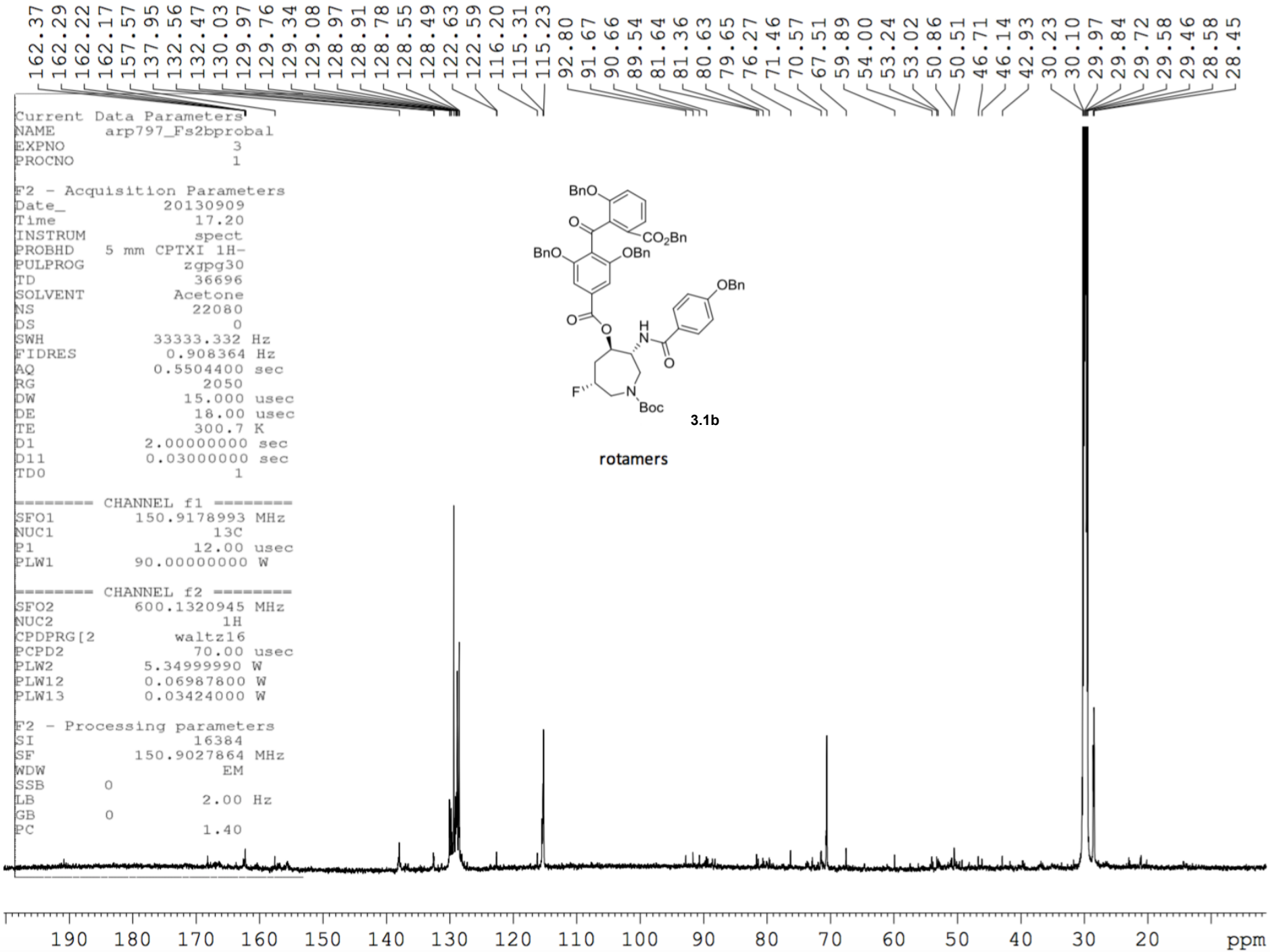
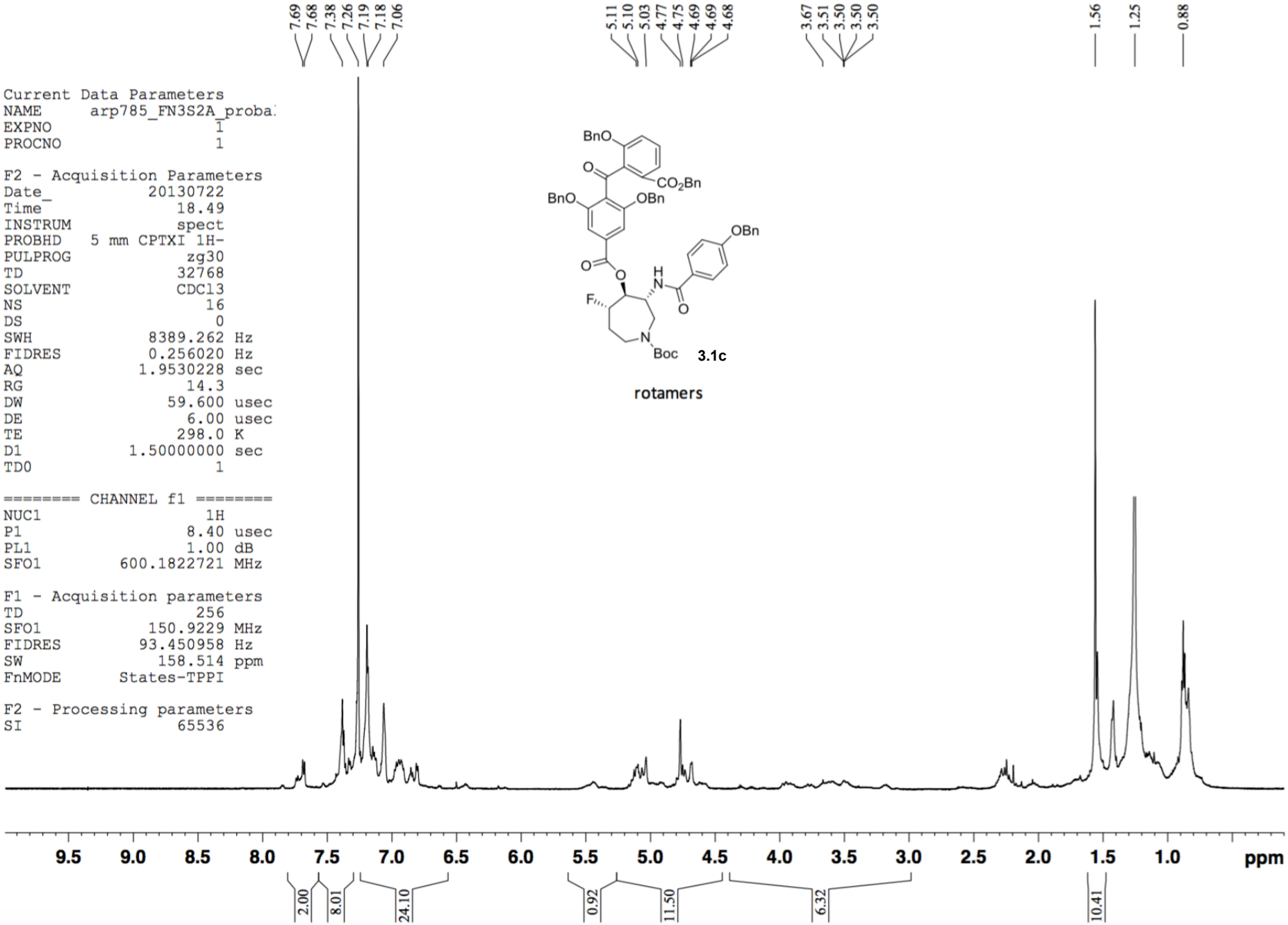
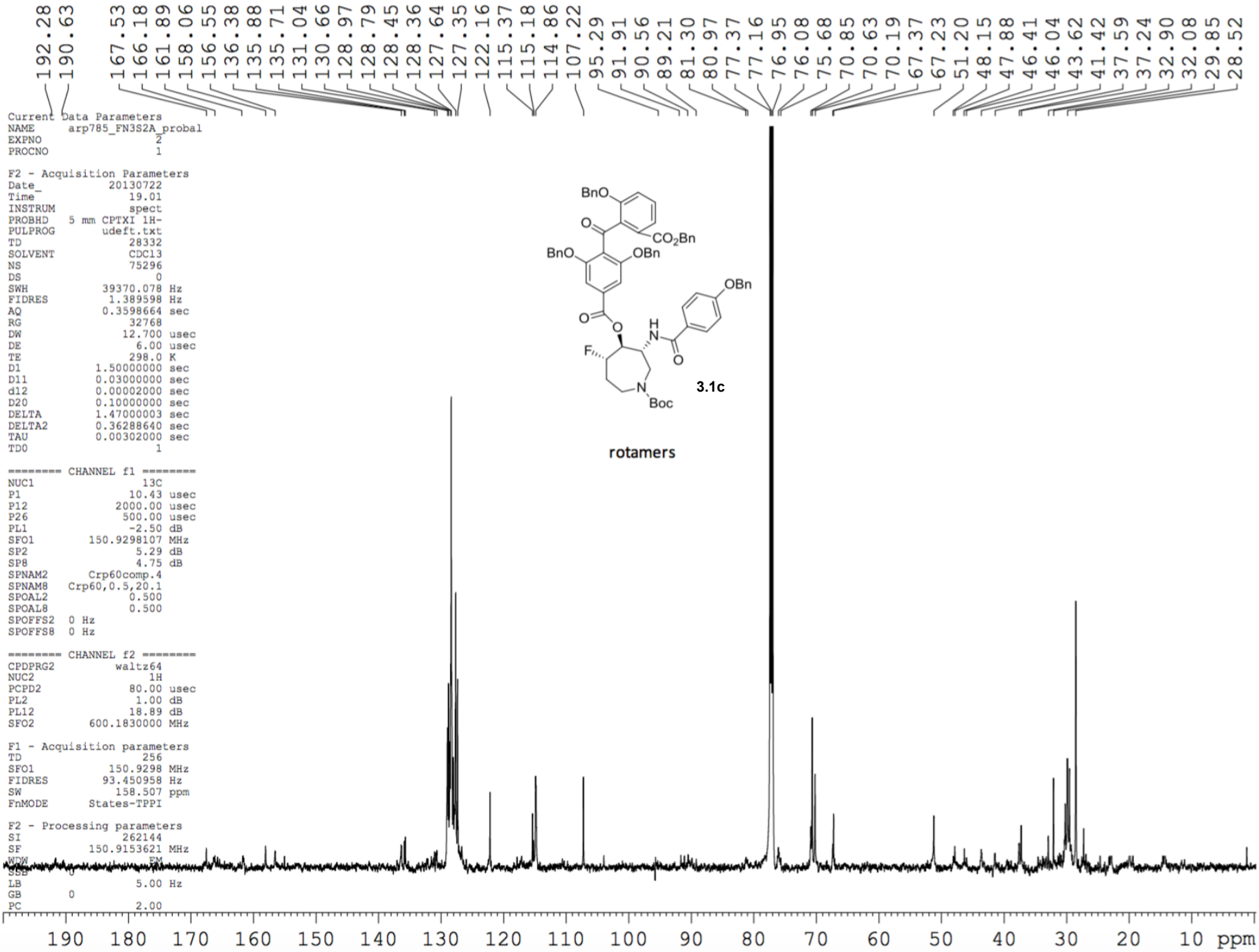
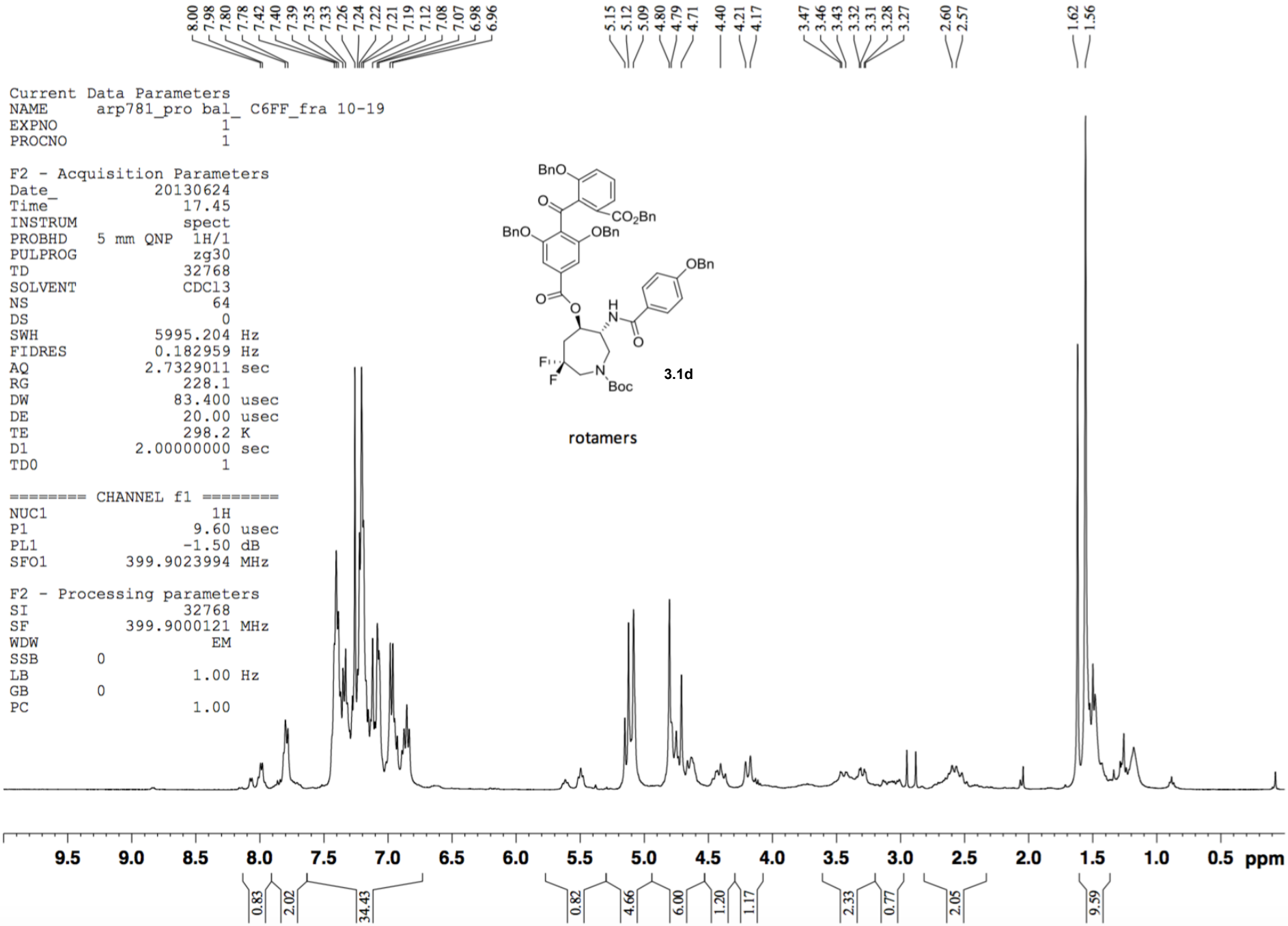
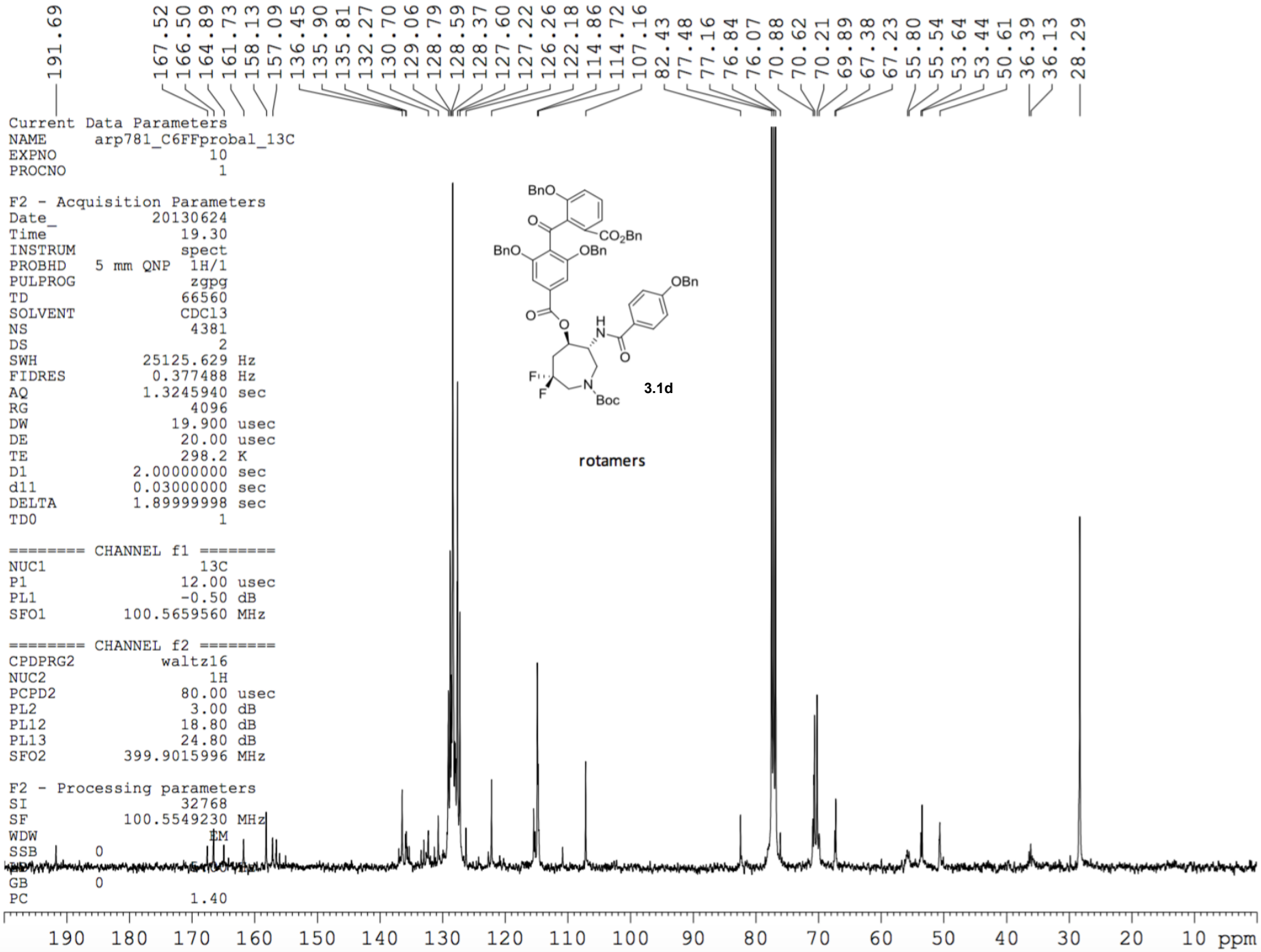

7.97
7.95
7.78
7.76
7.41
7.39
7.26
6.98
6.96
5.11
4.67
4.33
2.25
2.19
1.57
1.51
Current Data Parameters
NAME arp785_FS2b_coupling_fra 5-11
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130703
Time 17.43
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 512
DW 83.400 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.60 usec
PL1 -1.50 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000120 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
N
Boc
3.1b
rotamers
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
3.10
34.04
5.92
9.83
3.06
10.92

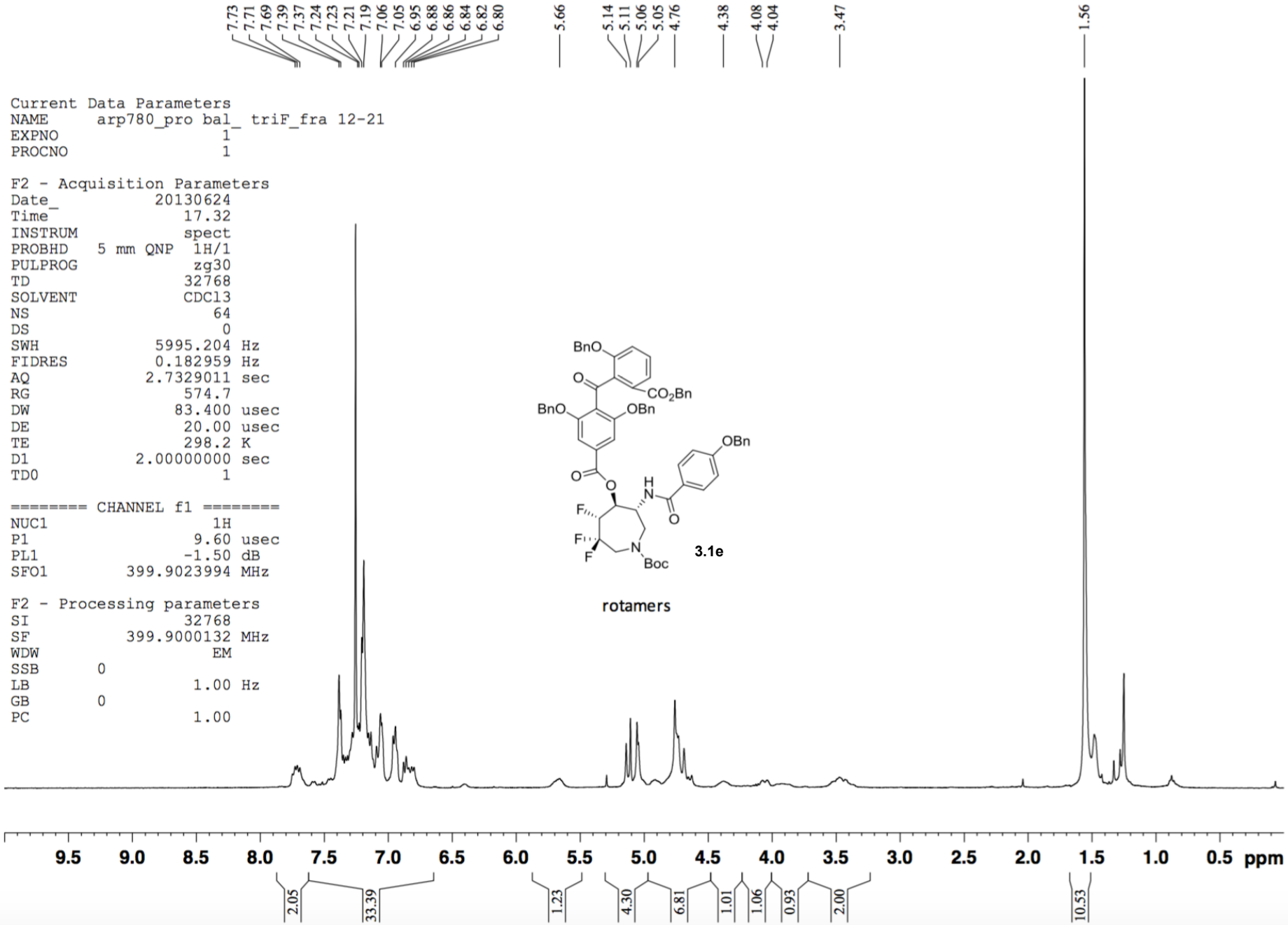

162.37 162.29 162.22 162.17 157.57 137.95 132.56 132.47 130.03 129.97 129.76 129.34 129.08 128.97 128.91 128.78 128.55 128.49 122.63 122.59 116.20 115.31 115.23 92.80 91.67 90.66 89.54 81.64 81.36 80.63 79.65 76.27 71.46 70.57 67.51 59.89 54.00 53.24 53.02 50.86 50.51 46.71 46.14 42.93 30.23 30.10 29.97 29.84 29.72 29.58 29.46 28.58 28.45
Current Data Parameters
NAME arp797_Fs2bprobal
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130909
Time 17.20
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG zgpg30
TD 36696
SOLVENT Acetone
NS 22080
DS 0
SWH 33333.332 Hz
FIDRES 0.908364 Hz
AQ 0.5504400 sec
RG 2050
DW 15.000 usec
DE 18.00 usec
TE 300.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 150.9178993 MHz
NUC1 13C
P1 12.00 usec
PLW1 90.00000000 W
======== CHANNEL f2 ========
SFO2 600.1320945 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 70.00 usec
PLW2 5.34999990 W
PLW12 0.06987800 W
PLW13 0.03424000 W
F2 - Processing parameters
SI 16384
SF 150.9027864 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
N
Boc
3.1b
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

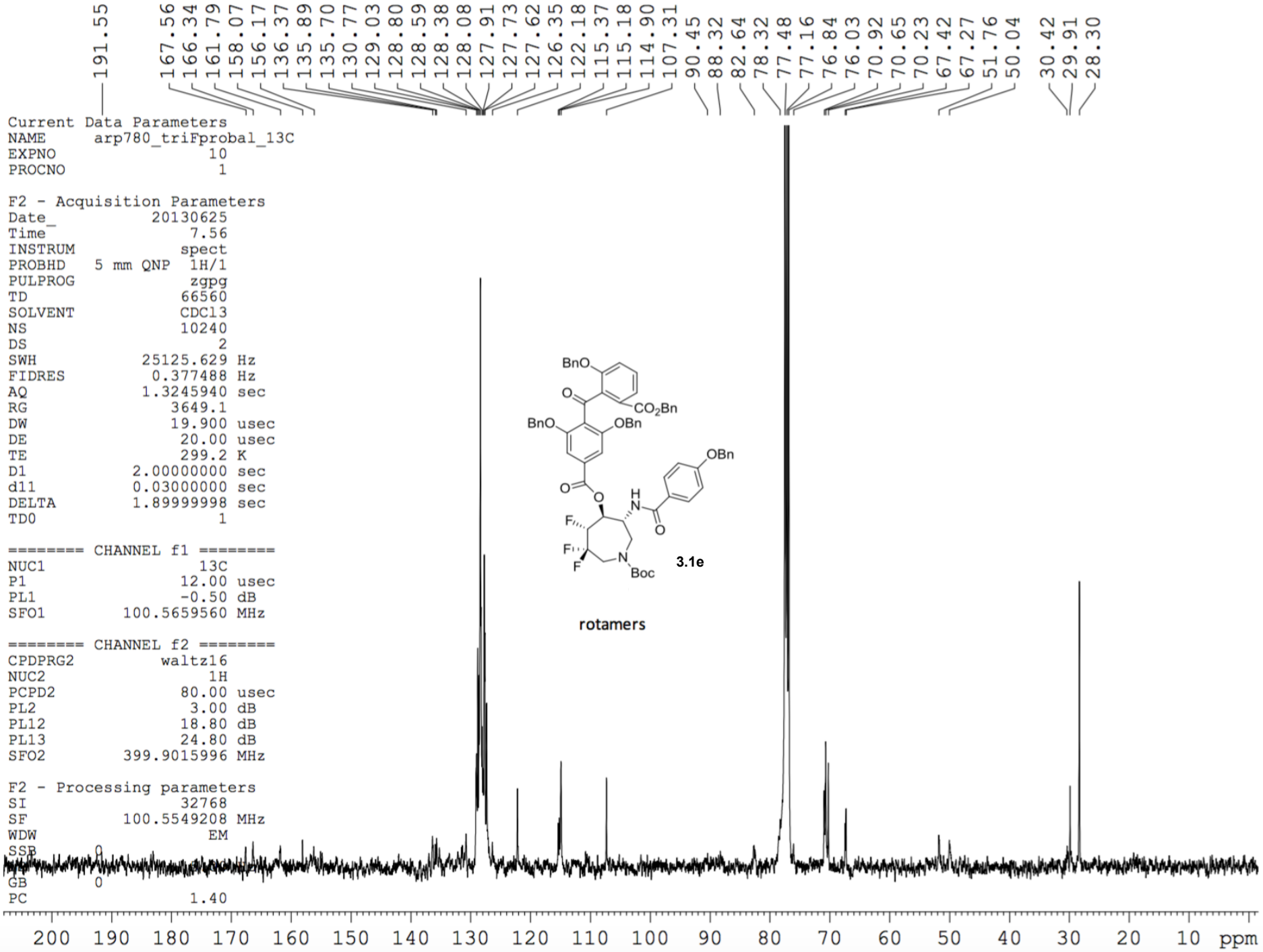

7.69
7.68
7.38
7.26
7.19
7.18
7.06
5.11
5.10
5.03
4.77
4.75
4.69
4.69
4.68
3.67
3.51
3.50
3.50
3.50
1.56
1.25
0.88
Current Data Parameters
NAME arp785_FN3S2A_proba
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130722
Time 18.49
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 8389.262 Hz
FIDRES 0.256020 Hz
AQ 1.9530228 sec
RG 14.3
DW 59.600 usec
DE 6.00 usec
TE 298.0 K
D1 1.50000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.40 usec
PL1 1.00 dB
SFO1 600.1822721 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9229 MHz
FIDRES 93.450958 Hz
SW 158.514 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 65536
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
F
O
N
Boc
3.1c
rotamers
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
2.00
8.01
24.10
0.92
11.50
6.32
10.41

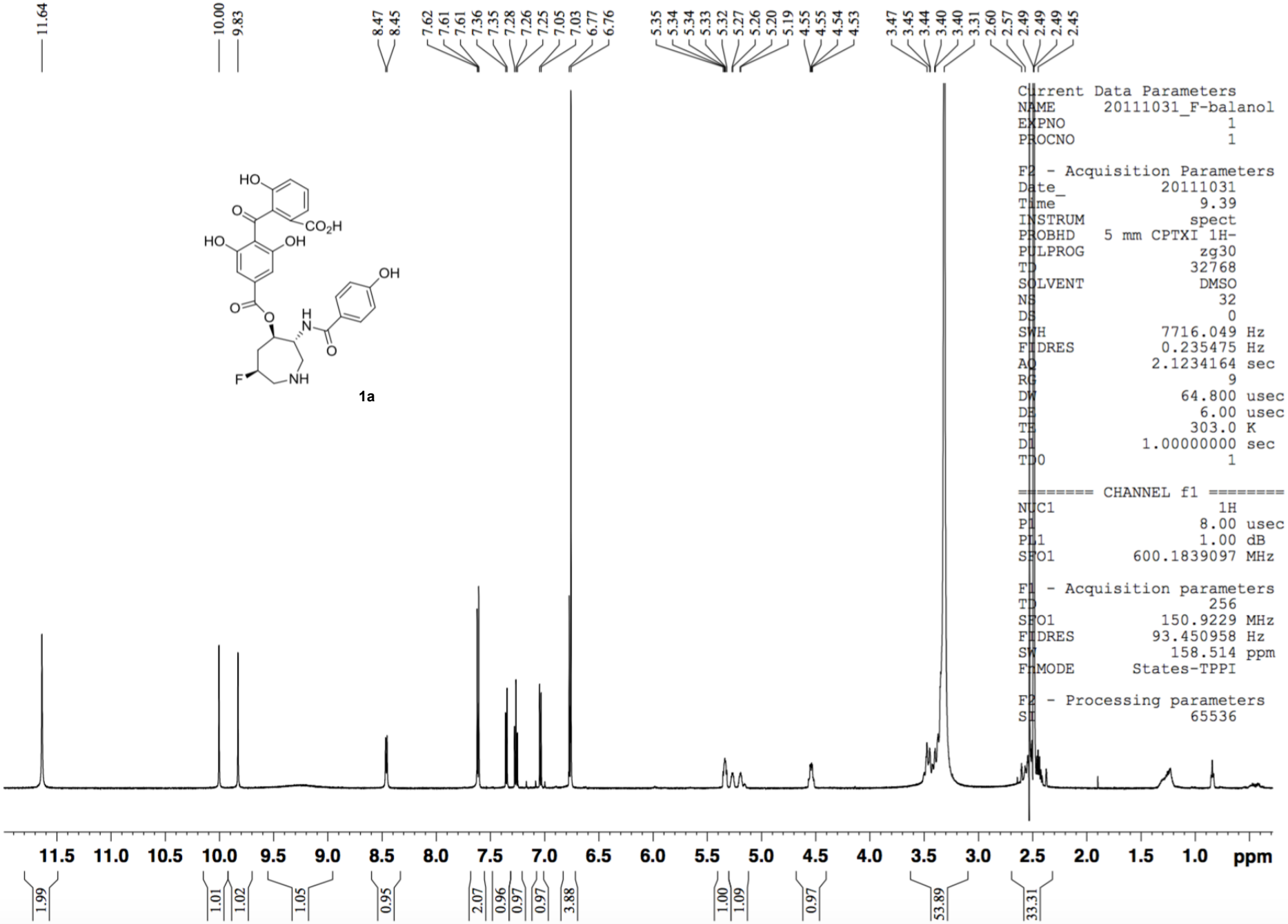

192.28
190.63
167.53
166.18
161.89
158.06
156.55
136.38
135.88
135.71
131.04
130.66
128.97
128.79
128.45
128.36
127.64
127.35
122.16
115.37
115.18
114.86
107.22
95.29
91.91
90.56
89.21
81.30
80.97
77.37
77.16
76.95
76.08
75.68
70.85
70.63
70.19
67.37
67.23
51.20
48.15
47.88
46.41
46.04
43.62
41.42
37.59
37.24
32.90
32.08
29.85
28.52
Current Data Parameters
NAME arp785_FN3S2A_probal
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130722
Time 19.01
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG udeft.txt
TD 28332
SOLVENT CDCl3
NS 75296
DS 0
SWH 39370.078 Hz
FIDRES 1.389598 Hz
AQ 0.3598664 sec
RG 32768
DW 12.700 usec
DE 6.00 usec
TE 298.0 K
D1 1.50000000 sec
D11 0.03000000 sec
d12 0.00002000 sec
D20 0.10000000 sec
DELTA 1.47000003 sec
DELTA2 0.36288640 sec
TAU 0.00302000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.43 usec
P12 2000.00 usec
P26 500.00 usec
PL1 -2.50 dB
SFO1 150.9298107 MHz
SP2 5.29 dB
SP8 4.75 dB
SPNAM2 Crp60comp.4
SPNAM8 Crp60,0.5,20.1
SPOAL2 0.500
SPOAL8 0.500
SPOFFS2 0 Hz
SPOFFS8 0 Hz
======== CHANNEL f2 ========
CPDPRG2 waltz64
NUC2 1H
PCPD2 80.00 usec
PL2 1.00 dB
PL12 18.89 dB
SFO2 600.1830000 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9298 MHz
FIDRES 93.450958 Hz
SW 158.507 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 262144
SF 150.9153621 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 2.00
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
F
O
N
Boc
3.1c
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

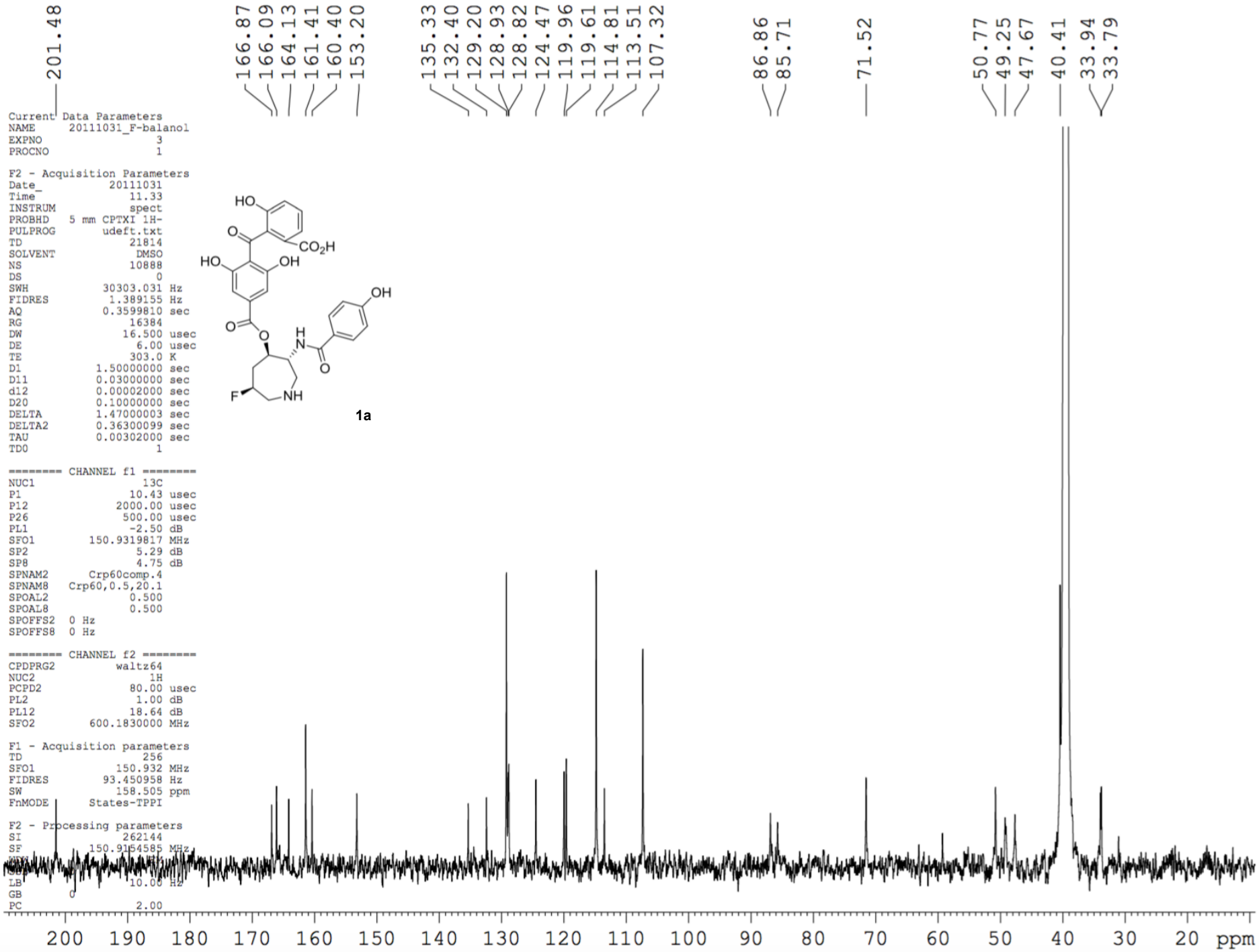

8.00 7.98 7.80 7.78 7.42 7.40 7.39 7.35 7.33 7.26 7.24 7.22 7.21 7.19 7.12 7.08 7.07 6.98 6.96
5.15 5.12 5.09 4.80 4.79 4.71 4.40 4.21 4.17
3.47 3.46 3.43 3.32 3.31 3.28 3.27
2.60 2.57
1.62 1.56
Current Data Parameters
NAME arp781_pro bal_ C6FF_fra 10-19
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130624
Time 17.45
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 228.1
DW 83.400 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.60 usec
PL1 -1.50 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000121 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
F
N
Boc
3.1d
rotamers
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.83 2.02 34.43 0.82 4.66 6.00 1.20 1.17 2.33 0.77 2.05 9.59

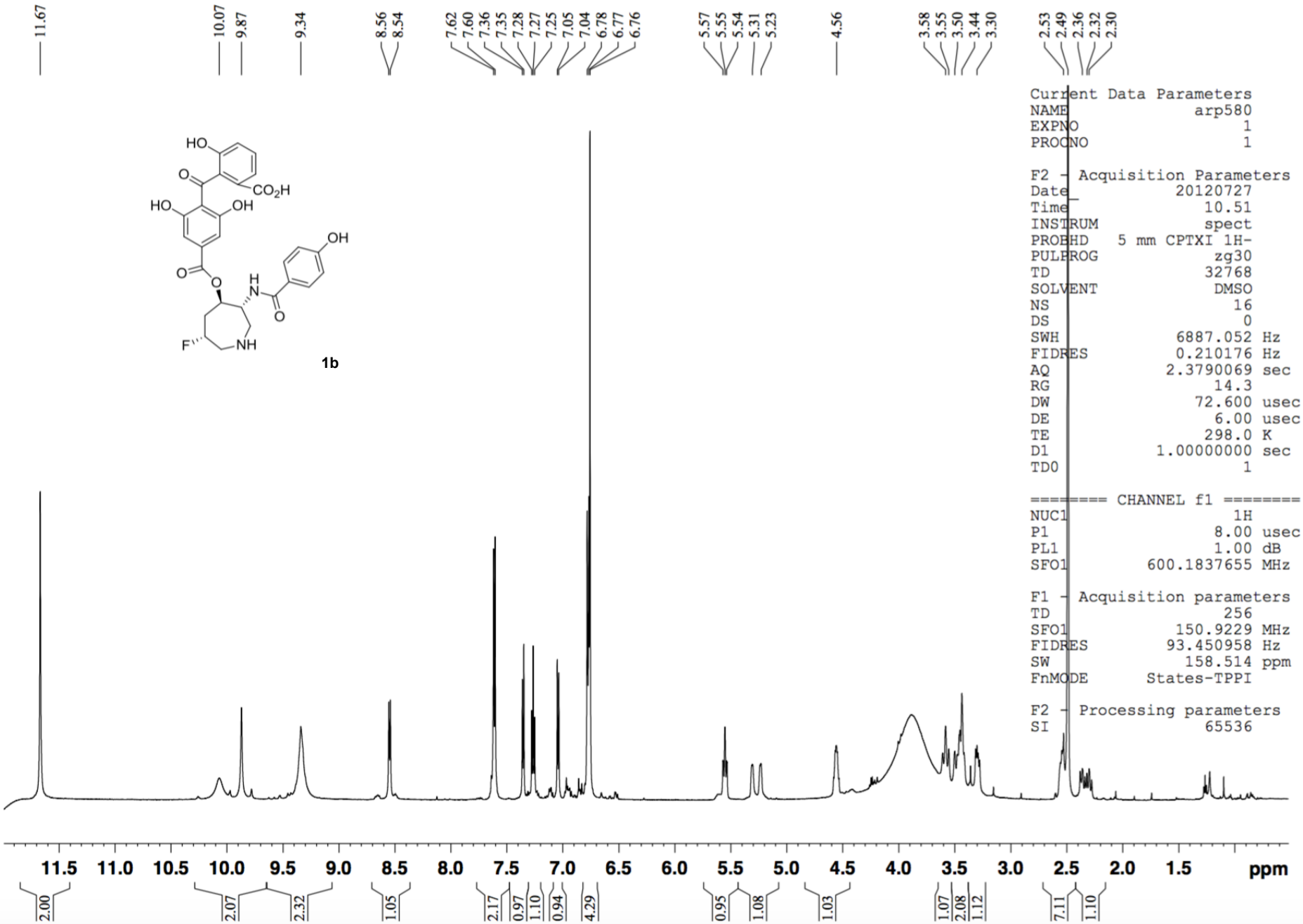

191.69
167.52
166.50
164.89
161.73
158.13
157.09
136.45
135.90
135.81
132.27
130.70
129.06
128.79
128.59
128.37
127.60
127.22
126.26
122.18
114.86
114.72
107.16
82.43
77.48
77.16
76.84
76.07
70.88
70.62
70.21
69.89
67.38
67.23
55.80
55.54
53.64
53.44
50.61
36.39
36.13
28.29
Current Data Parameters
NAME arp781_C6FFprobal_13C
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130624
Time 19.30
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 4381
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 4096
DW 19.900 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 18.80 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5549230 MHz
WDW EM
SSB 0
GB 0
PC 1.40
BnO
CO2Bn
BnO
OBn
OBn
H
N
O
O
O
O
F
F
N
Boc
3.1d
rotamers
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

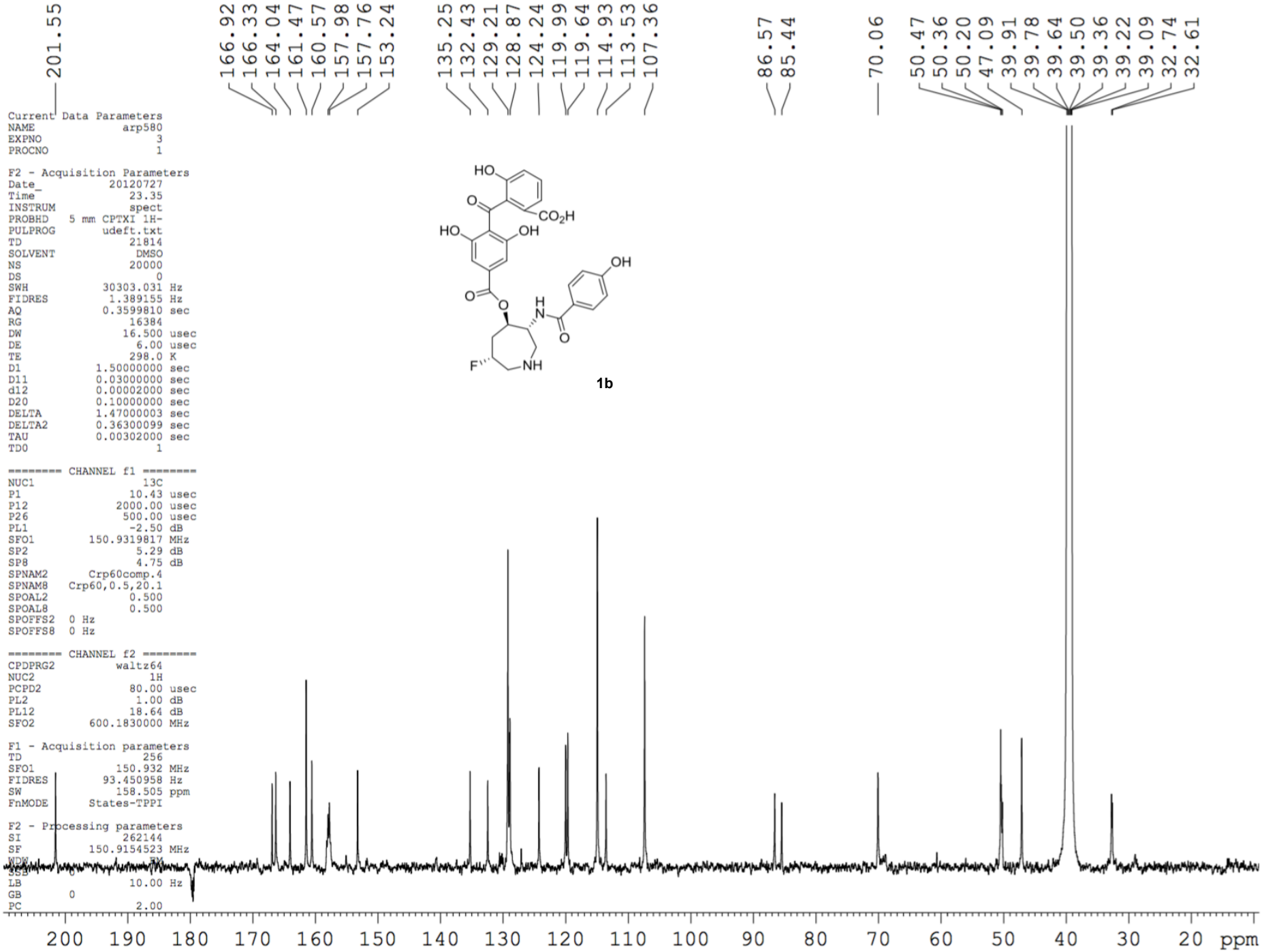

7.73
7.71
7.69
7.39
7.37
7.24
7.23
7.21
7.19
7.06
7.05
6.95
6.88
6.86
6.84
6.82
6.80
5.66
5.14
5.11
5.06
5.05
4.76
4.38
4.08
4.04
3.47
1.56
Current Data Parameters
NAME arp780_pro bal_ triF_fra 12-21
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130624
Time 17.32
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 574.7
DW 83.400 usec
DE 20.00 usec
TE 298.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.60 usec
PL1 -1.50 dB
SFO1 399.9023994 MHz
F2 - Processing parameters
SI 32768
SF 399.9000132 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00
BnO
O
CO2Bn
BnO
OBn
OBn
O
O
H
N
O
F
F
F
N
Boc
3.1e
rotamers
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.05
33.39
1.23
4.30
6.81
1.01
1.06
0.93
2.00
10.53


191.55
167.56
166.34
161.79
158.07
156.17
136.37
135.89
135.70
130.77
129.03
128.80
128.59
128.38
128.08
127.91
127.73
127.62
126.35
122.18
115.37
115.18
114.90
107.31
90.45
88.32
82.64
78.32
77.48
77.16
76.84
76.03
70.92
70.65
70.23
67.42
67.27
51.76
50.04
30.42
29.91
28.30
Current Data Parameters
NAME arp780_triFprobal_13C
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130625
Time 7.56
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 66560
SOLVENT CDCl3
NS 10240
DS 2
SWH 25125.629 Hz
FIDRES 0.377488 Hz
AQ 1.3245940 sec
RG 3649.1
DW 19.900 usec
DE 20.00 usec
TE 299.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -0.50 dB
SFO1 100.5659560 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 18.80 dB
PL13 24.80 dB
SFO2 399.9015996 MHz
F2 - Processing parameters
SI 32768
SF 100.5549208 MHz
WDW EM
SSB 0
LB 0
GB 0
PC 1.40
BnO
CO2Bn
BnO
OBn
OBn
H
N
O
F
F
F
N
Boc
3.1e
rotamers
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


11.64
10.00
9.83
8.47
8.45
7.62
7.61
7.61
7.36
7.35
7.28
7.26
7.25
7.05
7.03
6.77
6.76
5.35
5.34
5.34
5.33
5.32
5.27
5.26
5.20
5.19
4.55
4.55
4.54
4.53
3.47
3.45
3.44
3.40
3.40
3.31
2.60
2.57
2.49
2.49
2.49
2.45
HO
O
CO2H
HO
OH
OH
O
O
H
N
O
F
NH
1a
Current Data Parameters
NAME 20111031_F-balanol
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20111031
Time 9.39
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 0
SWH 7716.049 Hz
FIDRES 0.235475 Hz
AQ 2.1234164 sec
RG 9
DW 64.800 usec
DE 6.00 usec
TE 303.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.00 usec
PL1 1.00 dB
SFO1 600.1839097 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9229 MHz
FIDRES 93.450958 Hz
SW 158.514 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 65536
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.99
1.01
1.02
1.05
0.95
2.07
0.96
0.97
0.97
3.88
1.00
1.09
0.97
53.89
33.31


201.48
166.87
166.09
164.13
161.41
160.40
153.20
135.33
132.40
129.20
128.93
128.82
124.47
119.96
119.61
114.81
113.51
107.32
86.86
85.71
71.52
50.77
49.25
47.67
40.41
33.94
33.79
Current Data Parameters
NAME 20111031_F-balanol
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20111031
Time 11.33
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG udeft.txt
TD 21814
SOLVENT DMSO
NS 10888
DS 0
SWH 30303.031 Hz
FIDRES 1.389155 Hz
AQ 0.3599810 sec
RG 16384
DW 16.500 usec
DE 6.00 usec
TE 303.0 K
D1 1.50000000 sec
D11 0.03000000 sec
d12 0.00002000 sec
D20 0.10000000 sec
DELTA 1.47000003 sec
DELTA2 0.36300099 sec
TAU 0.00302000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.43 usec
P12 2000.00 usec
P26 500.00 usec
PL1 -2.50 dB
SFO1 150.9319817 MHz
SP2 5.29 dB
SP8 4.75 dB
SPNAM2 Crp60comp.4
SPNAM8 Crp60,0.5,20.1
SPOAL2 0.500
SPOAL8 0.500
SPOFFS2 0 Hz
SPOFFS8 0 Hz
======== CHANNEL f2 ========
CPDPRG2 waltz64
NUC2 1H
PCPD2 80.00 usec
PL2 1.00 dB
PL12 18.64 dB
SFO2 600.1830000 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.932 MHz
FIDRES 93.450958 Hz
SW 158.505 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 262144
SF 150.9154585 MHz
LB 10.00 Hz
GB 0
PC 2.00
HO
O
CO2H
HO
OH
OH
O
O
H
N
O
F
NH
1a
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


11.67
10.07
9.87
9.34
8.56
8.54
7.62
7.60
7.36
7.35
7.28
7.27
7.25
7.05
7.04
6.78
6.77
6.76
5.57
5.55
5.54
5.31
5.23
4.56
3.58
3.55
3.50
3.44
3.30
2.53
2.49
2.36
2.32
2.30
HO
O
CO2H
HO
OH
OH
O
O
H
N
O
F
NH
1b
Current Data Parameters
NAME arp580
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date 20120727
Time 10.51
INSTRUM spect
PROBHD 5 mm CPTXI 1H-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 16
DS 0
SWH 6887.052 Hz
FIDRES 0.210176 Hz
AQ 2.3790069 sec
RG 14.3
DW 72.600 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.00 usec
PL1 1.00 dB
SFO1 600.1837655 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9229 MHz
FIDRES 93.450958 Hz
SW 158.514 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 65536
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
2.00
2.07
2.32
1.05
2.17
0.97
1.10
0.94
4.29
0.95
1.08
1.03
1.07
2.08
1.12
7.11
1.10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113.53
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TAU 0.00302000 sec
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P26 500.00 usec
PL1 -2.50 dB
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SP2 5.29 dB
SP8 4.75 dB
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SPNAM8 Crp60,0.5,20.1
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SPOFFS8 0 Hz
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NUC2 1H
PCPD2 80.00 usec
PL2 1.00 dB
PL12 18.64 dB
SFO2 600.1830000 MHz
F1 - Acquisition parameters
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SFO1 150.932 MHz
FIDRES 93.450958 Hz
SW 158.505 ppm
FnMODE States-TPPI
F2 - Processing parameters
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SF 150.9154523 MHz
WDW EM
SSB 0
LB 10.00 Hz
GB 0
PC 2.00
HO
O
CO2H
HO
OH
OH
O
O
H
N
O
F
NH
1b
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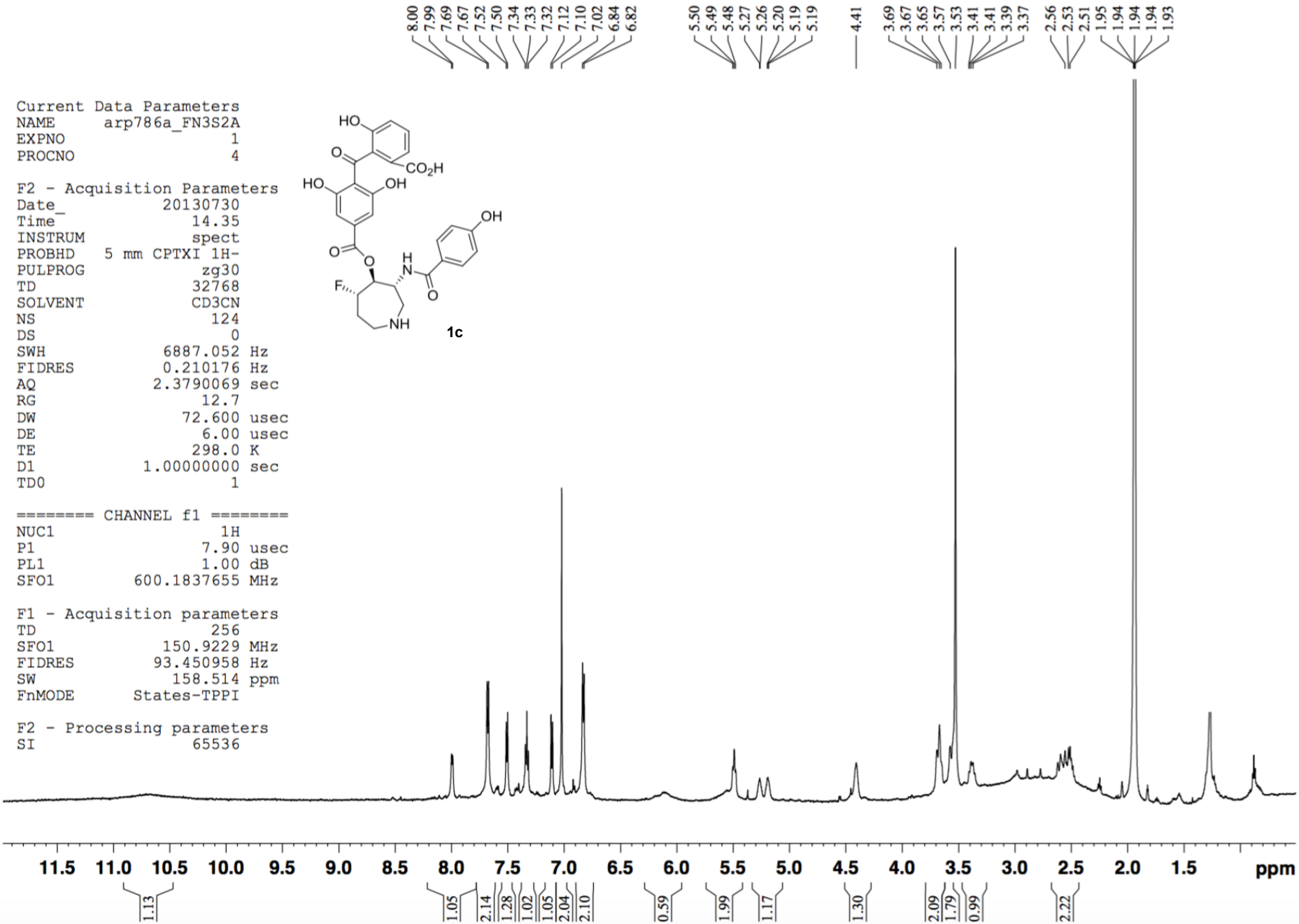
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4.41
3.69 3.67 3.65 3.57 3.53 3.41 3.41 3.39 3.37
2.56 2.53 2.51 1.95 1.94 1.94 1.94 1.93
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CO2H
HO
OH
OH
O
O
H
N
O
F
NH
1c
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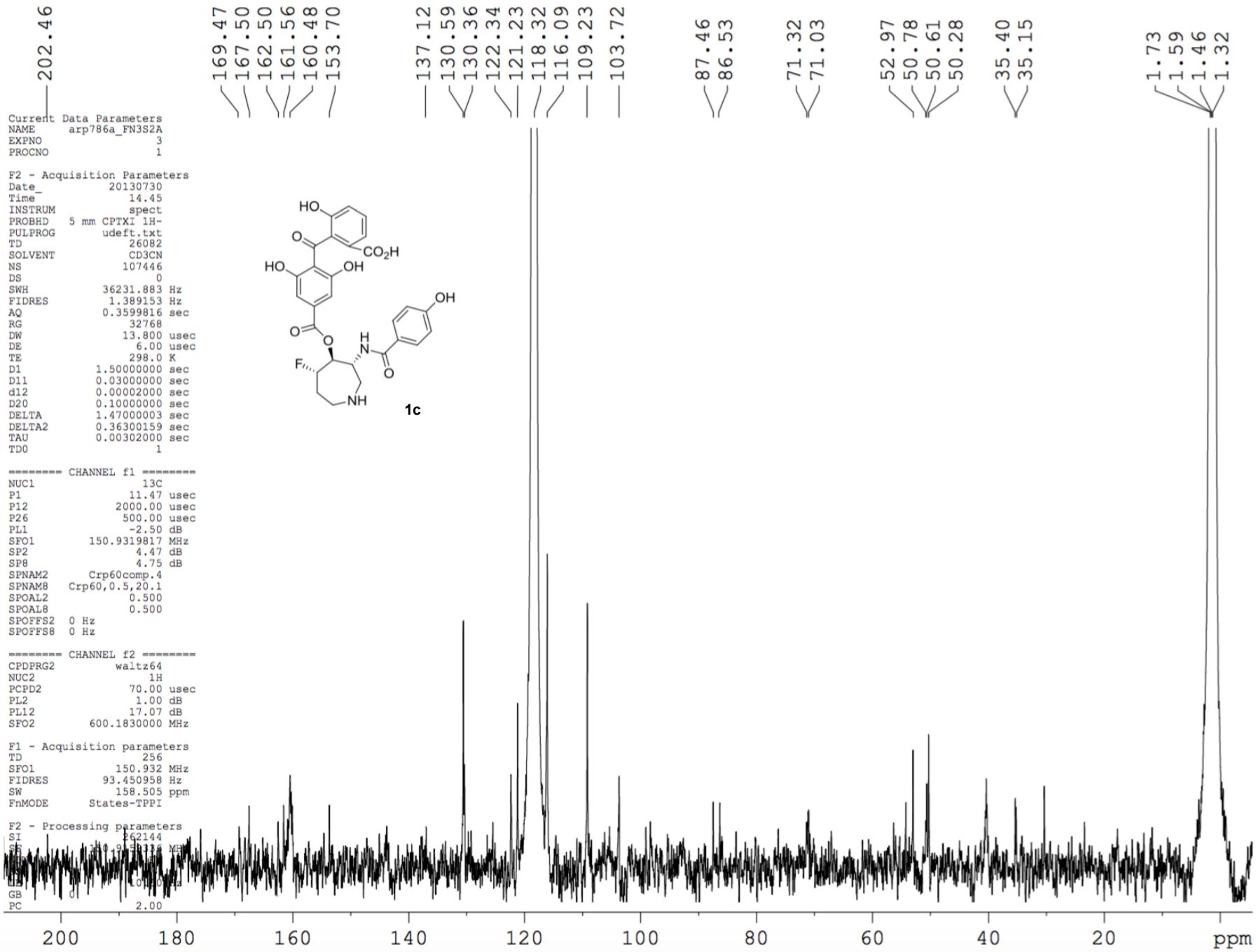


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D20           0.10000000 sec
DELTA         1.47000003 sec
DELTA2        0.36300159 sec
TAU           0.00302000 sec
TD0                     1

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SP8                  4.75 dB
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SPNAM8    Crp60,0.5,20.1
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SPOFFS2  0 Hz
SPOFFS8  0 Hz

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PCPD2               70.00 usec
PL2                  1.00 dB
PL12                17.07 dB
SFO2         600.1830000 MHz

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SFO1              150.932 MHz
FIDRES          93.450958 Hz
SW               158.505 ppm
FnMODE       States-TPPI

F2 - Processing parameters
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WDW                     EM
SSB                     0
LB                   [illegible] Hz
GB                      0
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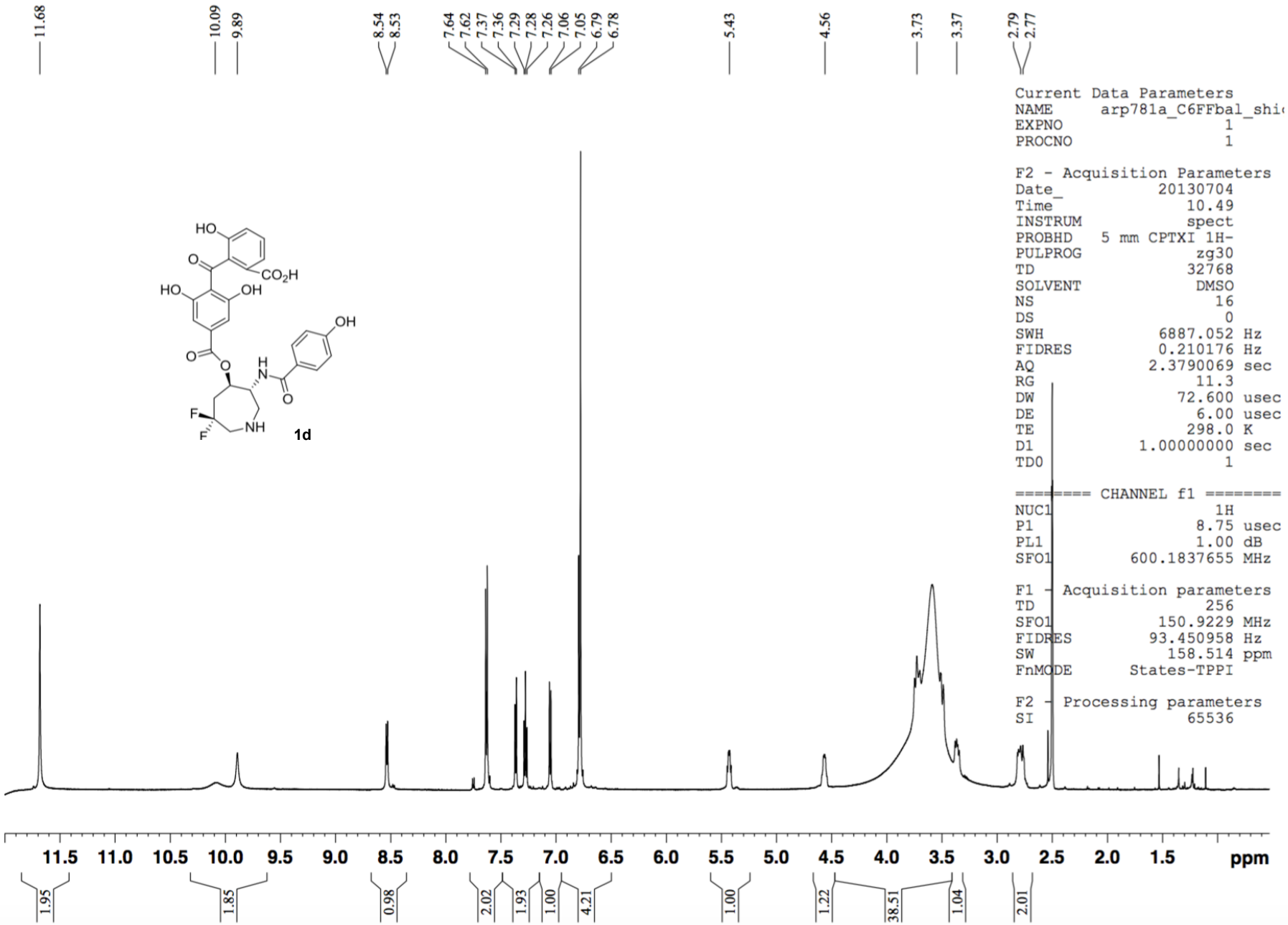
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CO2H
HO
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OH
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F
F
NH
1d
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DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
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PL1 1.00 dB
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F1 - Acquisition parameters
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SFO1 150.9229 MHz
FIDRES 93.450958 Hz
SW 158.514 ppm
FnMODE States-TPPI
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10.0
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9.0
8.5
8.0
7.5
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
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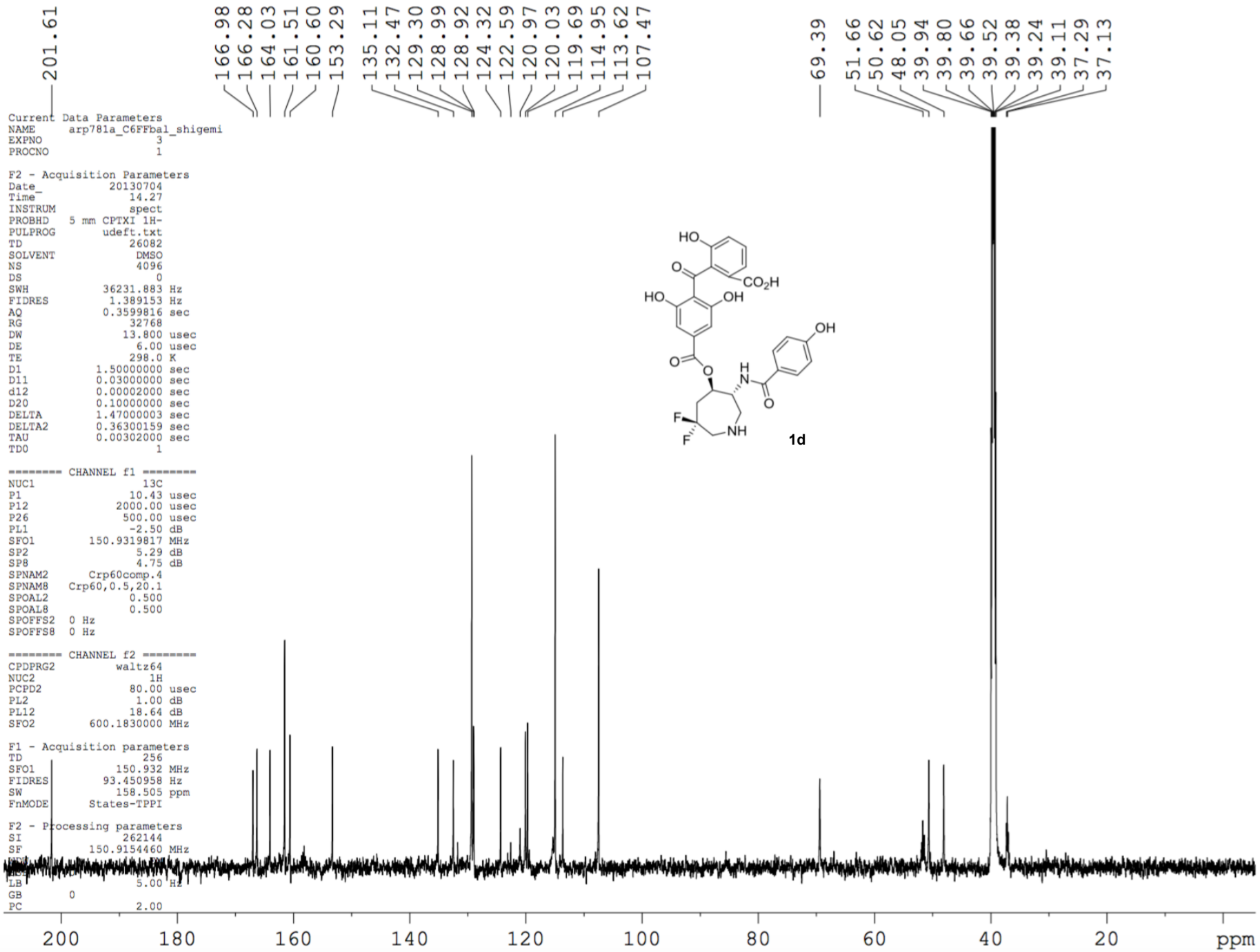
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69.39
51.66
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39.94
39.80
39.66
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39.38
39.24
39.11
37.29
37.13
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RG 32768
DW 13.800 usec
DE 6.00 usec
TE 298.0 K
D1 1.50000000 sec
D11 0.03000000 sec
d12 0.00002000 sec
D20 0.10000000 sec
DELTA 1.47000003 sec
DELTA2 0.36300159 sec
TAU 0.00302000 sec
TD0 1
======== CHANNEL f1 ========
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P1 10.43 usec
P12 2000.00 usec
P26 500.00 usec
PL1 -2.50 dB
SFO1 150.9319817 MHz
SP2 5.29 dB
SP8 4.75 dB
SPNAM2 Crp60comp.4
SPNAM8 Crp60,0.5,20.1
SPOAL2 0.500
SPOAL8 0.500
SPOFFS2 0 Hz
SPOFFS8 0 Hz
======== CHANNEL f2 ========
CPDPRG2 waltz64
NUC2 1H
PCPD2 80.00 usec
PL2 1.00 dB
PL12 18.64 dB
SFO2 600.1830000 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.932 MHz
FIDRES 93.450958 Hz
SW 158.505 ppm
FnMODE States-TPPI
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LB 5.00 Hz
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PC 2.00
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CO2H
HO
OH
OH
O
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H
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O
F
F
NH
1d
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140
120
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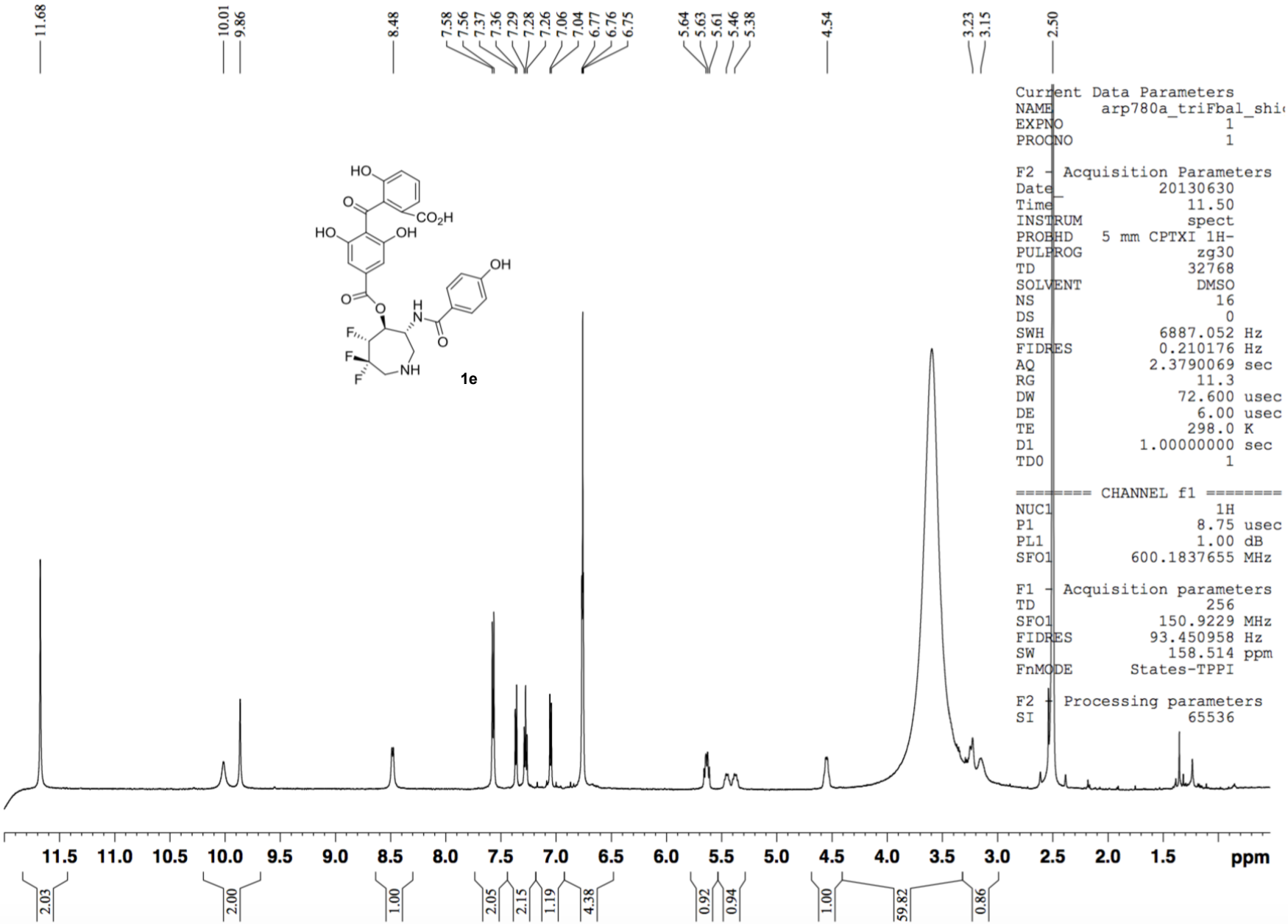
80
60
40
20
ppm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6.77
6.76
6.75
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5.63
5.61
5.46
5.38
4.54
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3.15
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CO2H
HO
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O
O
H
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F
F
F
NH
1e
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SOLVENT DMSO
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FIDRES 0.210176 Hz
AQ 2.3790069 sec
RG 11.3
DW 72.600 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.75 usec
PL1 1.00 dB
SFO1 600.1837655 MHz
F1 - Acquisition parameters
TD 256
SFO1 150.9229 MHz
FIDRES 93.450958 Hz
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FnMODE States-TPPI
F2 - Processing parameters
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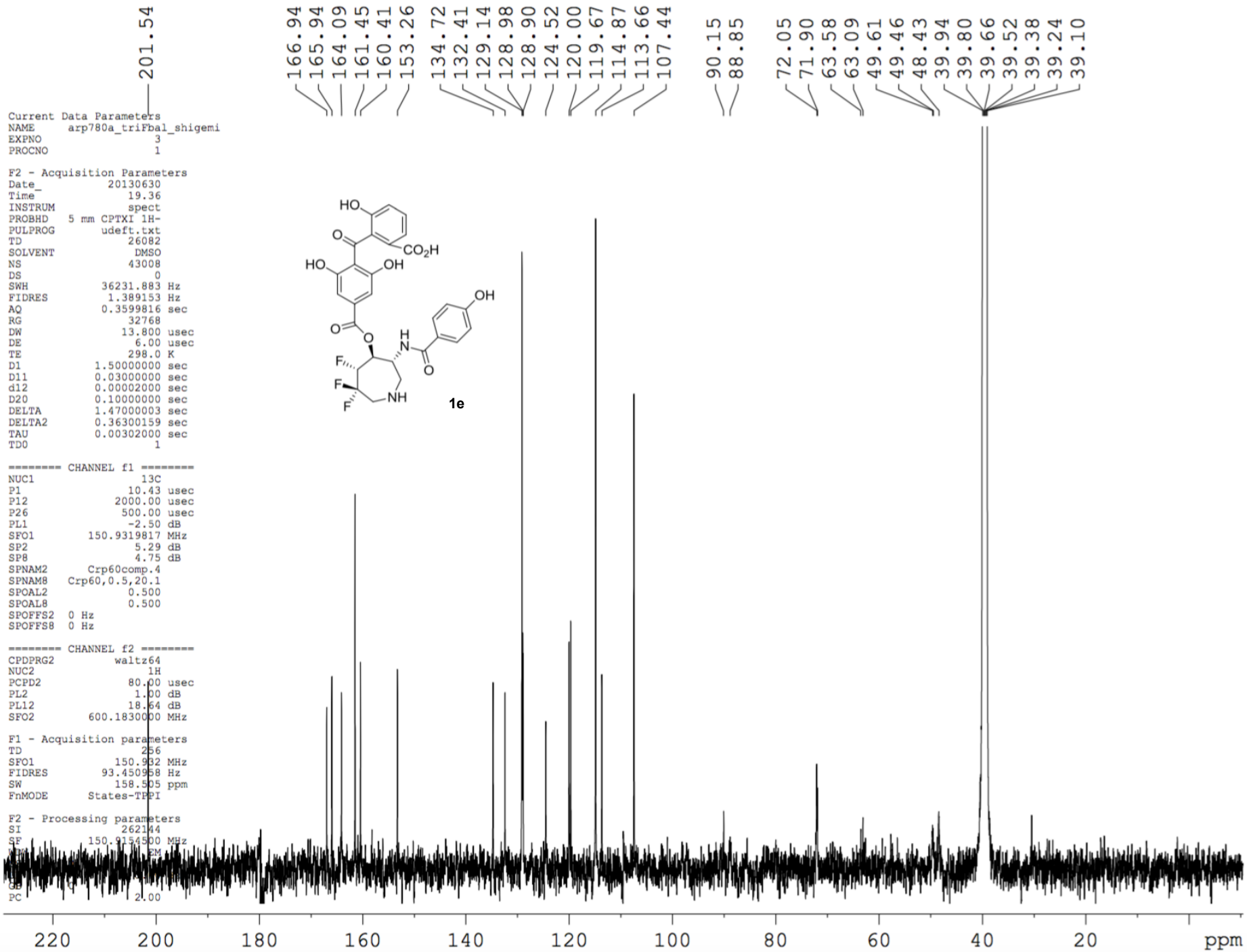
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72.05
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DE 6.00 usec
TE 298.0 K
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D11 0.03000000 sec
d12 0.00002000 sec
D20 0.10000000 sec
DELTA 1.47000003 sec
DELTA2 0.36300159 sec
TAU 0.00302000 sec
TD0 1
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NUC1 13C
P1 10.43 usec
P12 2000.00 usec
P26 500.00 usec
PL1 -2.50 dB
SFO1 150.9319817 MHz
SP2 5.29 dB
SP8 4.75 dB
SPNAM2 Crp60comp.4
SPNAM8 Crp60,0.5,20.1
SPOAL2 0.500
SPOAL8 0.500
SPOFFS2 0 Hz
SPOFFS8 0 Hz
======== CHANNEL f2 ========
CPDPRG2 waltz64
NUC2 1H
PCPD2 80.00 usec
PL2 1.00 dB
PL12 18.64 dB
SFO2 600.1830000 MHz
F1 - Acquisition parameters
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FIDRES 93.450958 Hz
SW 158.505 ppm
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CO2H
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F
NH
1e
220
200
180
160
140
120
100
80
60
40
20
ppm

II. Binding affinity measurements.

A. Binding assay for balanol and balanoids.

Compounds were tested by by DiscoverRx, USA using the assay KINOME*scan*™.[1] KINOME*scan*™ is a competition–binding assay with quantitative measurement of the ability of a compound to compete with an immobilized, active–site directed ligand. The assay utilizes three components: a DNA–tagged kinase; an immobilized ligand; and a test compound. The ability of the test compound to compete with the immobilized ligand is measured via quantitative PCR of the DNA tag.

KINOME*scan*™ Assay Principle

Compounds that bind the kinase active site and directly (sterically) or indirectly (allosterically) prevent kinase binding to the immobilized ligand and consequently reduce the amount of kinase captured on the solid support (A & B). Conversely, test molecules that do not bind the kinase have no effect on the amount of kinase captured on the solid support (C) (Figure 6.2). The dissociation constants (K_d's) for test compound–kinase interactions are calculated by measuring the amount of kinase captured on the solid support as a function of the test compound concentration by using a quantitative, precise and ultra–sensitive qPCR method that detects the associated DNA label (D).

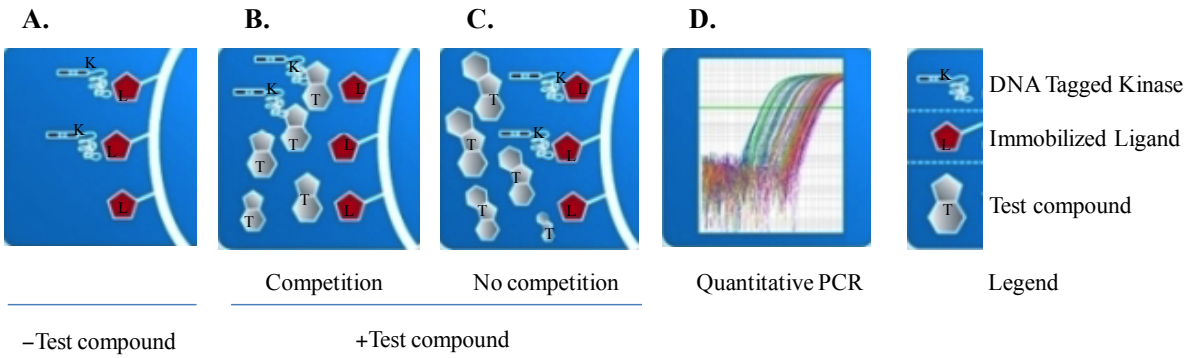


Figure S2.1. KINOME*scan*™ assay schematic representation as provided by DiscoverX.[1]

KINOME*scan*™ Assay Protocol (as provided by DiscoverRx)

For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2µm) to remove

cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 % SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05 %Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

Binding Affinities (K_d's)

The binding constants (K_d's) were calculated with a standard dose–response curve using the Hill equation:

$$\text{Response} = \frac{\text{Signal - Background}}{1 + (K_d^{\text{Hill Slope}}/\text{Dose}^{\text{Hill Slope}})}$$

The Hill Slope was set to −1. Curves were fitted using a non–linear least square fit with the Levenberg–Marquardt algorithm.

References

[1] M. A. Fabian, W. H. Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J.-M. Lelias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar, D. J. Lockhart, *Nat Biotech* **2005**, *23*, 329-336.

B. Full set of binding constants and curves measured for balanol **1** and balanoids **1a**–**1e** against PKA/C isozymes.

The compounds were tested in duplicate experiments (Figure S2.2). The binding constants are tabulated in Table S2.1. The cross-references of compound/protein names and the assay codes are listed in Table S2.2.

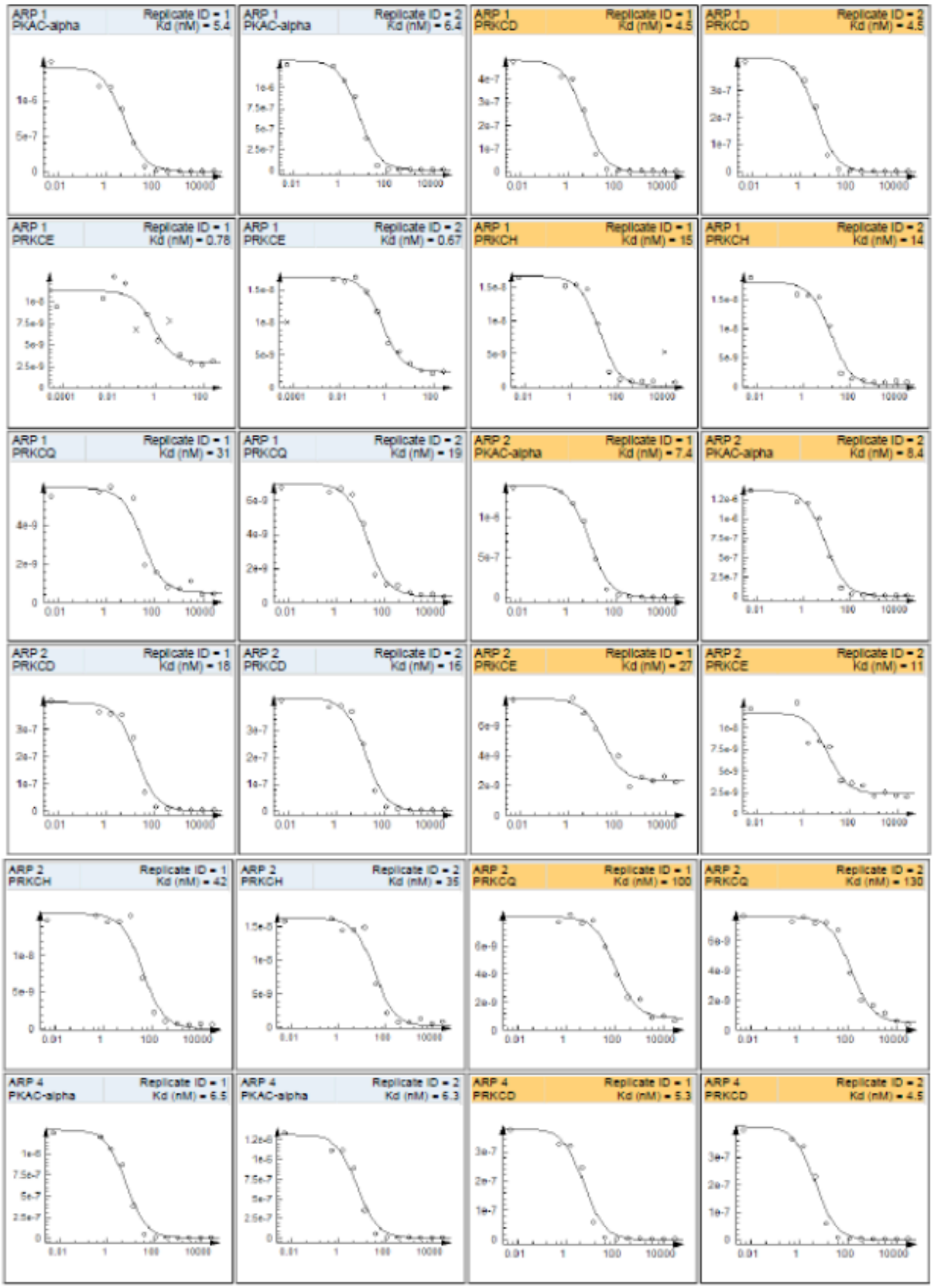
Table S2.1. K_d (nM) values of **1** and **1a**–**1e** against PKA and PKC isozymes in duplicate experiments.

Compound	K_d (nM)				
	PKA	**PKCδ**	**PKCε**	**PKCη**	**PKCθ**
1	5.4	4.5	0.78	15	31
1	6.4	4.5	0.78	14	19
1a	7.4	18	27	42	100
1a	8.4	16	11	35	130
1b	7.3	13	28	13	150
1b	6.5	16	21	18	130
1c	6.5	5.3	0.42	13	25
1c	6.3	4.5	0.39	13	24
1d	8.4	19	130	19	640
1d	10	20	93	20	520
1e	40	19	49	10	770
1e	47	18	26	14	930

Table S2.2. Cross-references of compound/protein names and assay codes.

Compound	Assay code	Proteins	Protein code
1	ARP1	**PKA**	PKAC-alpha
1a	ARP2	**PKCδ**	PRKCD
1b	ARP3	**PKCε**	PRKCE
1c	ARP4	**PKCη**	PRKCH
1d	ARP30	**PKCθ**	PRKCQ
1e	ARP31	**PKCi**	PRKCI

Figure S2.2. Binding curve images of compounds **1** and **1a**–**1e** provided by DiscoveRx.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ARP 1 PRKCD Replicate ID = 2 Kd (nM) = 4.5
ARP 1 PRKCE Replicate ID = 1 Kd (nM) = 0.78
ARP 1 PRKCE Replicate ID = 2 Kd (nM) = 0.67
ARP 1 PRKCH Replicate ID = 1 Kd (nM) = 15
ARP 1 PRKCH Replicate ID = 2 Kd (nM) = 14
ARP 1 PRKCQ Replicate ID = 1 Kd (nM) = 31
ARP 1 PRKCQ Replicate ID = 2 Kd (nM) = 19
ARP 2 PKAC-alpha Replicate ID = 1 Kd (nM) = 7.4
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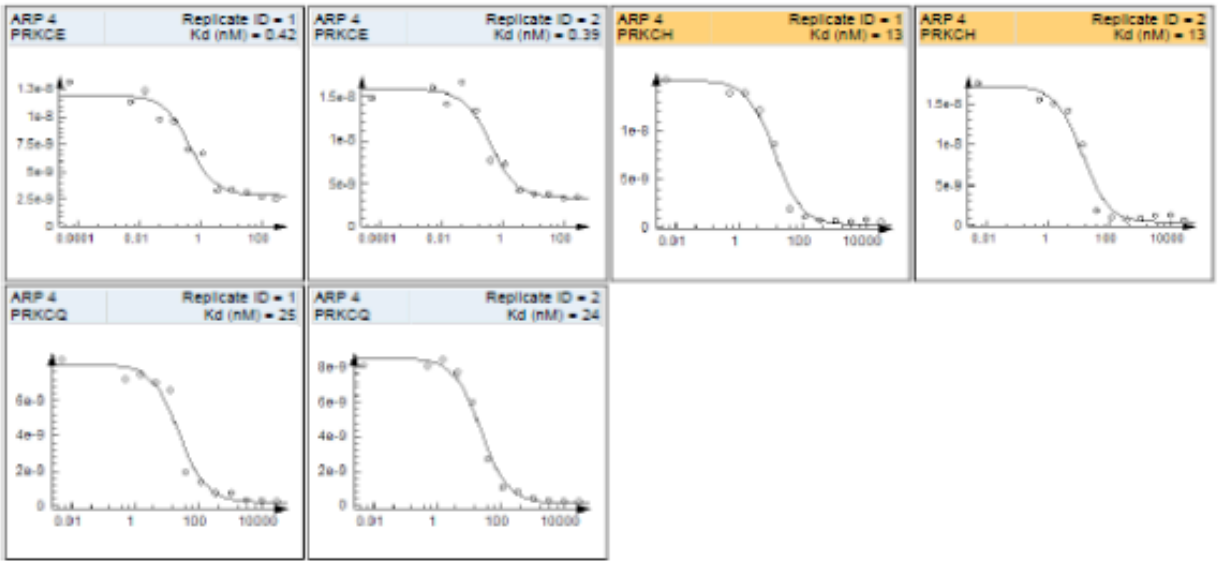
ARP 2 PRKCD Replicate ID = 1 Kd (nM) = 18
ARP 2 PRKCD Replicate ID = 2 Kd (nM) = 16
ARP 2 PRKCE Replicate ID = 1 Kd (nM) = 27
ARP 2 PRKCE Replicate ID = 2 Kd (nM) = 11
ARP 2 PRKCH Replicate ID = 1 Kd (nM) = 42
ARP 2 PRKCH Replicate ID = 2 Kd (nM) = 35
ARP 2 PRKCQ Replicate ID = 1 Kd (nM) = 100
ARP 2 PRKCQ Replicate ID = 2 Kd (nM) = 130
ARP 4 PKAC-alpha Replicate ID = 1 Kd (nM) = 6.5
ARP 4 PKAC-alpha Replicate ID = 2 Kd (nM) = 6.3
ARP 4 PRKCD Replicate ID = 1 Kd (nM) = 5.3
ARP 4 PRKCD Replicate ID = 2 Kd (nM) = 4.5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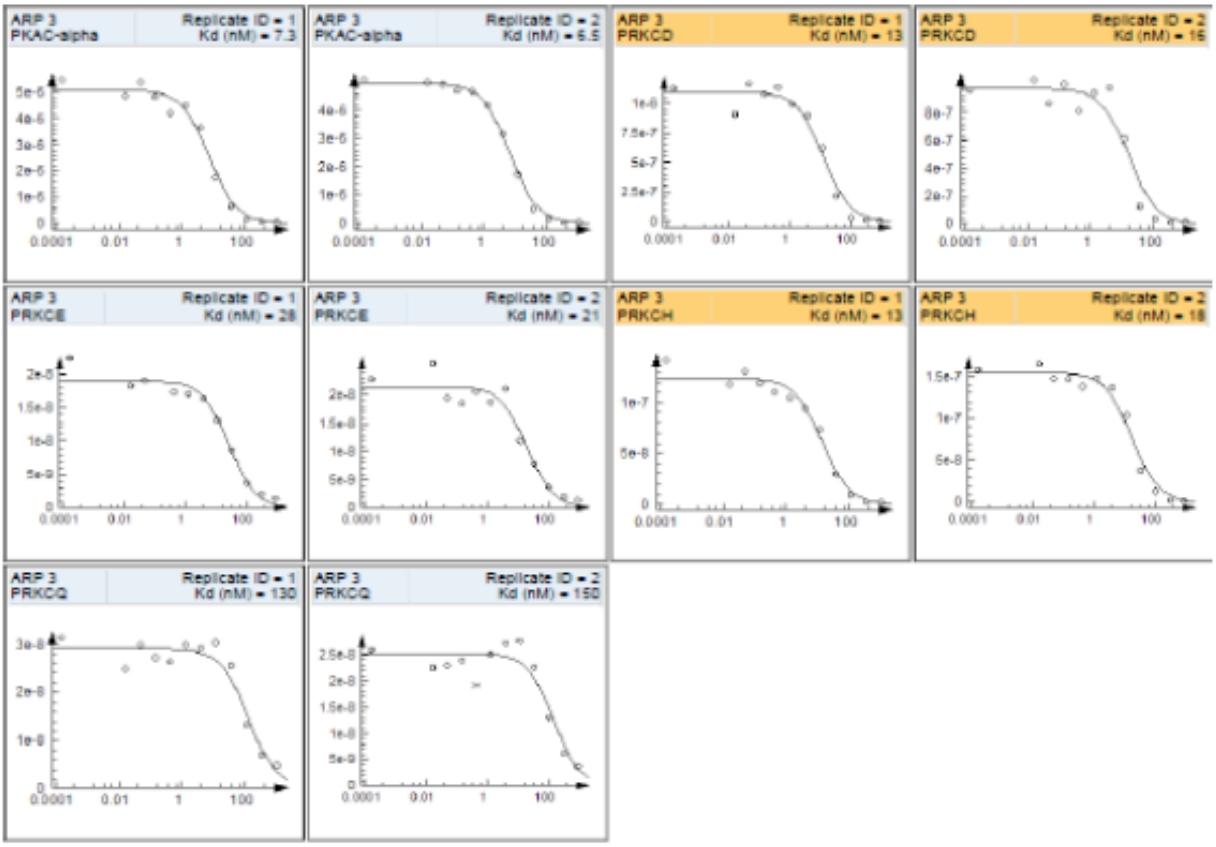
PRKCE
Replicate ID = 1
Kd (nM) = 0.42
ARP 4
PRKCE
Replicate ID = 2
Kd (nM) = 0.39
ARP 4
PRKCH
Replicate ID = 1
Kd (nM) = 13
ARP 4
PRKCH
Replicate ID = 2
Kd (nM) = 13
ARP 4
PRKCQ
Replicate ID = 1
Kd (nM) = 25
ARP 4
PRKCQ
Replicate ID = 2
Kd (nM) = 24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PKAC-alpha
Replicate ID = 2
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Replicate ID = 1
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ARP 3
PRKCD
Replicate ID = 2
Kd (nM) = 16
ARP 3
PRKCE
Replicate ID = 1
Kd (nM) = 28
ARP 3
PRKCE
Replicate ID = 2
Kd (nM) = 21
ARP 3
PRKCH
Replicate ID = 1
Kd (nM) = 13
ARP 3
PRKCH
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PRKCQ
Replicate ID = 1
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PRKCQ
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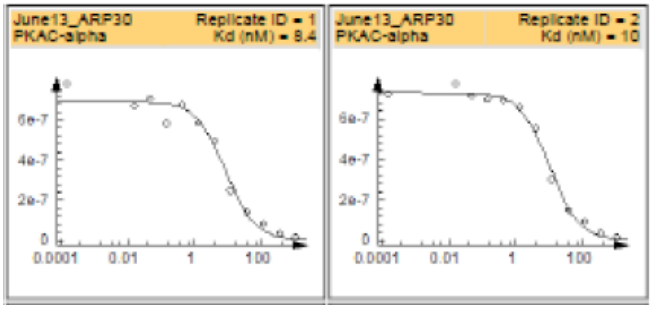
Kd (nM) = 150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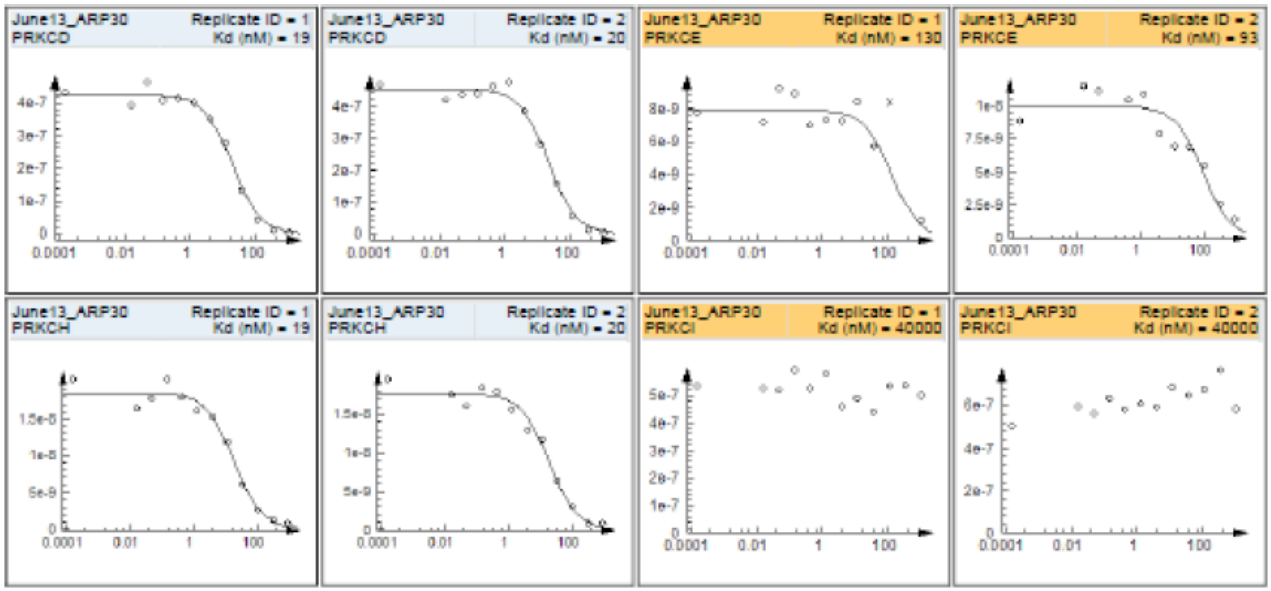
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June13_ARP30 Replicate ID = 1 PRKCE Kd (nM) = 130
June13_ARP30 Replicate ID = 2 PRKCE Kd (nM) = 93
June13_ARP30 Replicate ID = 1 PRKCH Kd (nM) = 19
June13_ARP30 Replicate ID = 2 PRKCH Kd (nM) = 20
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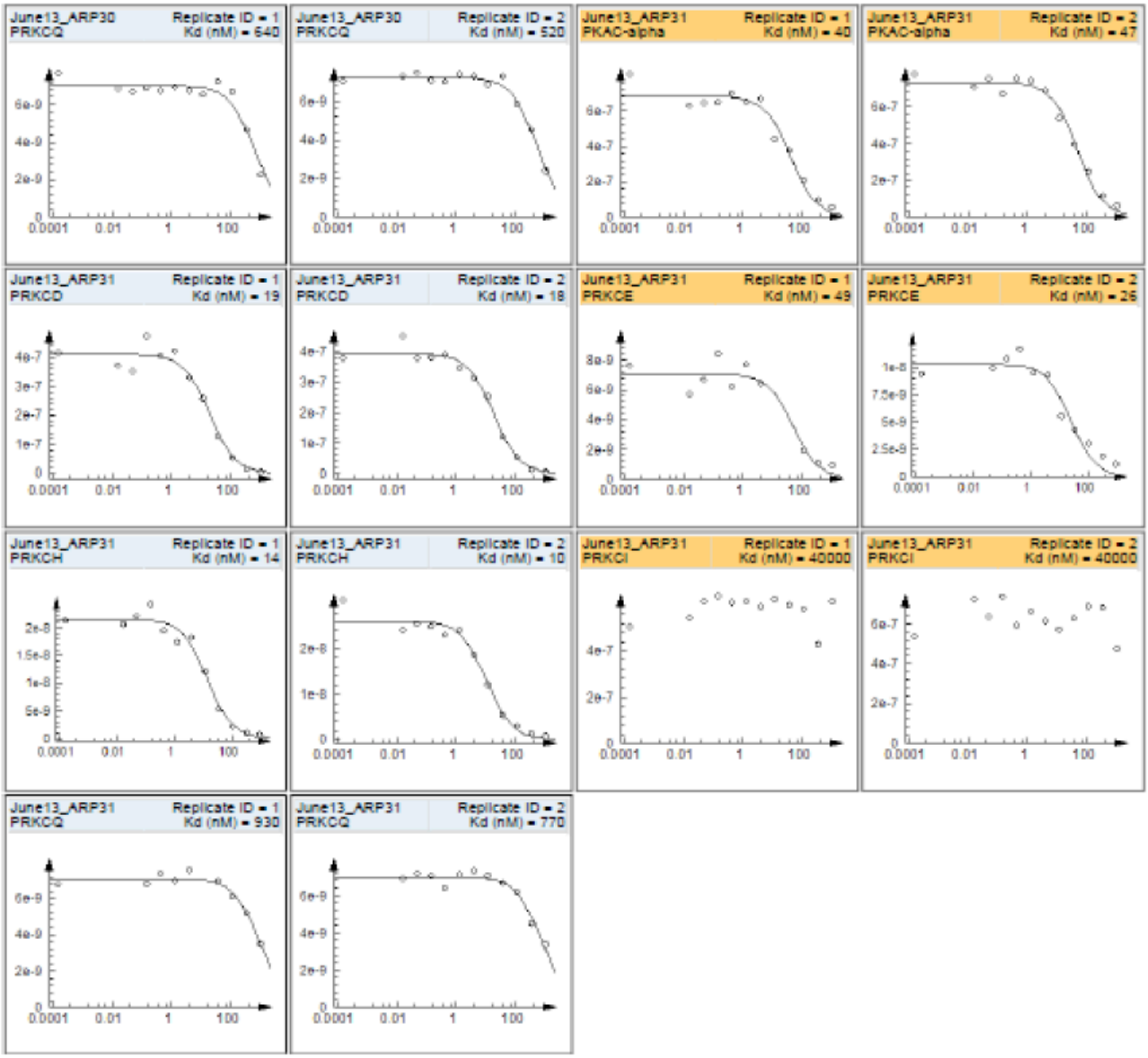
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June13_ARP31 Replicate ID = 2 PKAC-alpha Kd (nM) = 47
June13_ARP31 Replicate ID = 1 PRKCD Kd (nM) = 19
June13_ARP31 Replicate ID = 2 PRKCD Kd (nM) = 18
June13_ARP31 Replicate ID = 1 PRKCE Kd (nM) = 49
June13_ARP31 Replicate ID = 2 PRKCE Kd (nM) = 26
June13_ARP31 Replicate ID = 1 PRKCH Kd (nM) = 14
June13_ARP31 Replicate ID = 2 PRKCH Kd (nM) = 10
June13_ARP31 Replicate ID = 1 PRKCI Kd (nM) = 40000
June13_ARP31 Replicate ID = 2 PRKCI Kd (nM) = 40000
June13_ARP31 Replicate ID = 1 PRKCQ Kd (nM) = 930
June13_ARP31 Replicate ID = 2 PRKCQ Kd (nM) = 770

III. Docking analysis.

A. Development of docking protocols using the balanol-PKA X-ray crystal structure 1BX6.

In order to find the optimum docking procedure which would reproduce the observed conformation of the balanol docked to PKA (1BX6), a few re-docking procedures were performed using the Molsoft ICM 3.7-2a Package (Neves et al. 2012). ICM exploits a Biased Probability Monte Carlo (BPMC) simulation (Abagyan & Totrov 1994) to find docking solutions. This Monte Carlo simulation globally optimizes internal coordinates of ligand within grid potential maps of a receptor binding site. The simulation involves four stages: (1) a random move which is applied to either the rotational, translational or conformational variables of the ligand in the binding pocket; (2) a minimization of differentiable terms of energy function; (3) a calculation of desolvation energy; and (4) a Metropolis selection criterion to accept or reject final minimized conformation. The stages are iteratively run until reaching the maximal number of steps. For the scoring function, ICM uses a weighted sum of ligand-receptor van der Waals interactions (with coefficients α_1 to α_5) of H-bond interactions (ΔE_{Hbond}), hydrogen bond donor–acceptor desolvation energy ($\Delta E_{HBDesol}$), solvation electrostatic energy upon ligand binding (ΔE_{SolEl}), hydrophobic free energy gain (ΔE_{HPhob}) and a size correction term proportional to the number of ligand atoms (Q_{Size}) together with an internal force field energy of the ligand (ΔE_{IntEF}) and free energy changes due to conformational energy loss upon ligand binding ($T\Delta S_{Tor}$), (Neves et al. 2012). The scoring function is expressed as follows:

$$\Delta G = \Delta E_{\mathrm{IntFF}} + T\Delta S_{\mathrm{Tor}} + \alpha_1 \Delta E_{\mathrm{HBond}} + \alpha_2 \Delta E_{\mathrm{HBDesol}} + \alpha_3 \Delta E_{\mathrm{SolEl}} + \alpha_4 \Delta E_{\mathrm{HPhob}} + \alpha_5 Q_{\mathrm{Size}}$$

ICM also provides an option for semi-flexible docking through ligand and receptor complex refinement. The docking involves a two-step docking process. The first step is the docking of a flexible ligand and a rigid receptor and is followed by a refinement of the entire ligand-receptor complex. The procedure allows the side chains of the receptor to be fully flexible (Neves et al. 2012).

Before performing docking, molecules were assigned MMFF atom types for ligands and partial charges of protein atoms from library of ECEPP/3 residue templates. In addition, molecules were globally optimized to avoid steric clashes (Neves et al. 2012).

During the preliminary step of re-docking of balanol was carried out using two different approaches. In the first group of procedures (labelled group I), the approach of flexible ligand/rigid

receptor was used, while in the second (referred to as group II), the approach was to use flexible ligand/semi-flexible receptor docking simulation in which the side chain amino acid residues interacting with ligand were allowed to be flexible.

In group I docking, we investigated flexible ligand/rigid receptor docking simulations with or without water molecules as shown in the crystal structure, as well as running the simulation with different grid box size (25.09 x 26.63 x 24.07 Å or 32 x 32 x 32 Å). Docking simulations with and without water molecules were also conducted for the second group but without using the larger grid box size as the results from group I indicated that a smaller grid box size would be more reliable, which were then used exclusively for group II (flexible ligand and receptor side chain simulation). Seven replications were conducted for each simulation. The lowest root mean squared deviation (RMSD) for heavy atoms of the re-docked balanol conformation in each simulation were listed in Table S3.1.

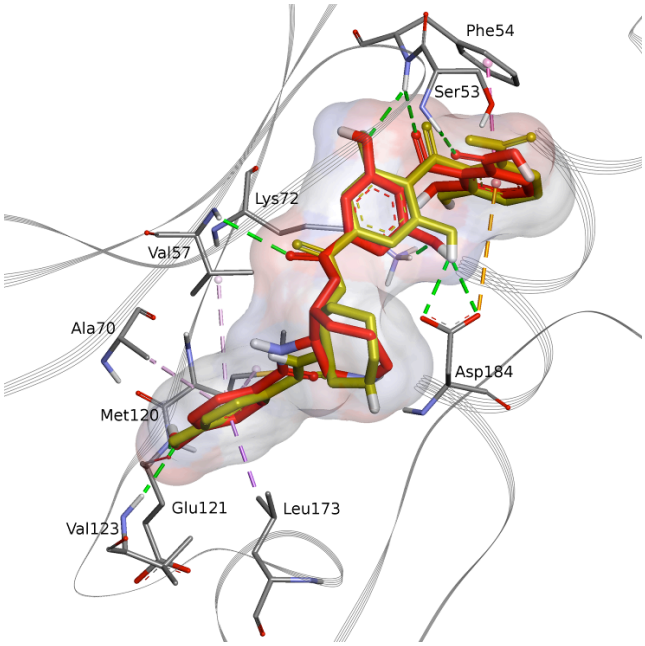
Table S3.1. The lowest RMSD for heavy atoms of balanol in 1BX6 obtained by various docking protocols.

Docking simulation procedures tested	**The lowest RMSD from heavy atoms of balanol (Å)**
Group I: Flexible ligand and rigid receptor	
In the presence of water molecules (25.09 x 26.63 x 24.07 Å)	1.01
In the absence of water molecules (25.09 x 26.63 x 24.07 Å)	1.03
Bigger size of grid box size (32 x 32 x 32 Å)	1.44
Group II: Flexible ligand and flexible side chain residues of the receptor interacting with ligand	
In the presence of water molecules (25.09 x 26.63 x 24.07 Å)	0.74
In the absence of water molecules (25.09 x 26.63 x 24.07 Å)	0.73

The lowest RMSD values are 0.74 and 0.73 Å for group II procedures with or without water molecules respectively, suggesting that flexible ligand/semi-flexible receptor docking procedure would be more appropriate for assessing balanol binding to PKA. This is comparable to existing methods that have also found that inclusion of flexibility is important for optimal docking (Wong et al. 2005; Rezácová et al. 2008). Docking simulation with or without water molecules appeared to give comparable results, which is consistent with the observation that no water molecule appear in the key interactions identified in the crystallographic structure of balanol and PKA complex.

The least RMSD value of 0.73 Å without water molecules was the best outcome for method validation. The overlay of the X-ray complex structure 1BX6 and this docking simulated structure of balanol-PKA is shown in Figure S3.1. The docking method reproduced the binding mode of balanol with very slight changes in the azepane and benzophenone conformations.

A



B

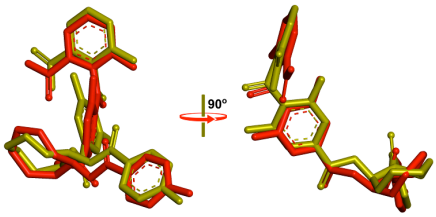


Figure S3.1. Structural comparison between 1BX6 and docking simulated structure of balanol bound to PKA. (A) The X-ray structure of balanol in the ATP site from 1BX6 (balanol in red) overlaid with the docking simulation structure for the same site with balanol (balanol in gold). The protein backbone is shown in grey ribbons and the amino acid sidechains are shown in sticks. (B) Overlay of the two balanol structures alone (balanol from 1BX6 in red and balanol from docking simulation in gold).

B. Construction of the PKCε structural homology model (from PDB 3TXO and 1BX6).

X-Ray crystal structure for the human PKCε (Q02156) is unavailable yet, although there is a crystal structure (PDB: 3TXO) for human PKCη with naphthyridine bound in the adenine subsite of the ATP pocket. BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated 83.2% primary sequence homology (69.8 % identity) between PKCε and PKCη. Likewise, mouse PKA (PDB: 1BX6) and human PKCε showed 54.1% primary sequence homology (36.4% identity by CLUSTAL 2.1, http://www.ebi.ac.uk/Tools/msa/clustalo/) (McWilliam et al. 2013). The alignment of three proteins is shown in Figure S3.2.

```
CLUSTAL 2.1 multiple sequence alignment

3txoA  353                                DNFEFIRVLGK    GSFGKVMLARVKETGD   379
1bx6A   12 QESVKEFLAKAKEDFLKKWETPSQNTAQLDQFDRIKTLGTGSFG    RVMLVKHKESGN    67
PKCε   406                                DEFNFIKVLGKGSFG    KVMLAELKGKDE   432
                                          *:*: *:.**.        :***.. * ..:

3txoA  380 LYAVKVLKKDVILQDDDVECTMTEKRILSLARNHPFLTQLFCCFQTPDRLFFVMEFVNGG   439
1bx6A   68 HYAMKILDKQKVVKLKQIEHTLNEKRILQAVN FPFLVKLEFSFKDNSNLYMVMEYVAGG   126
PKCε   433 VYAVKVLKKDVILQDDDVDCTMTEKRILALARKHPYLTQLYCCFQTKDRLFFVMEYVNGG   492
            **:*:*.*: ::: .::: *:.*****  .. .*:*.:*  .*:  ..*::***:* **

3txoA  440 DLMFHIQKSRRFDEARARFYAAEIISALMFLHDKGIIYRDLKLDNVLLDHEGHCKLADFG   499
1bx6A  127 EMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFG   186
PKCε   493 DLMFQIQRSRKFDEPRSRFYAAEVTSALMFLHQHGVIYRDLKLDNILLDAEGHCKLADFG   552
           ::: ::::  :*.*.::*****::  :: :**. .:****** :*:*:* :*: :::***

3txoA  500 MCKEGI         CGTPDYIAPEILQEMLYGPAVDWWAMGVLLYEMLCGHAPFEAENE   559
1bx6A  187 FAKRVKG   RTWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFADQP   243
PKCε   553 MCKEGILNGVTTTTFCGTPDYIAPEILQELEYGPSVDWWALGVLMYEMMAGQPPFEADNE   612
           :.*.           ****:*:****: .  *. :*****:***:*** .* .** *::

3txoA  560 DDLFEAILNDEVVYPTWLHEDATGILKSFMTKNPTMRLGSLT QGGEHAILRHPFFKEID   618
1bx6A  244 IQIYEKIVSGKVRFPSHFSSDLKDLLRNLLQVDLTKRFGNLK  NGVNDIKNHKWFATTD   301
PKCε   613 DDLFESILHDDVLYPVWLSKEAVSILKAFMTKNPHKRLGCVASQNGEDAIKQHPFFKEID   672
           :::* *: ..* :*  : .:  .:*: ::  :   *:* :   .* . * .* :*   *

3txoA  619 WAQLNHRQIEPPFRPRIKSREDVSNFDPDFIKEEPVLTPIDEGHLPMINQDEFRNFEYVS   678
1bx6A  302 WIAIYQRKVEAPFIPKFKGPGDTSNFDD  YEEEEIRVSINEKCG     KEFTEF       350
PKCε   673 WVLLEQKKIKPPFKPRIKTKRDVNNFDQDFTREEPVLTLVDEAIVKQINQEEFKGFSYFG   732
           *  : ::::::.** *::*   *..***    .** : . ::*        .**  *

3txoA  679 P
1bx6A
PKCε   733 E
```

Figure S3.2. Human PKCη (P24723), PKA mouse (P05132) and PKCε human (Q02156) alignment using CLUSTAL 2.1. "*" - identical residue; ":" - one of the following 'strong' groups is fully conserved residue; "." - one of the following 'weaker' groups is fully conserved.

The catalytic domain of protein kinases have two forms with either "open" or "closed" conformation, both of which can be bound to a ligand in the ATP site. The structure 3TXO shows the naphthyridine ligand bound in the "closed" conformation of the receptor, while in 1BX6, balanol is bound to the "open" conformation. PKCε is likely to bind to balanol in a conformation analogous to that of PKA with balanol bound (Narayana et al. 1999). Hence, 1BX6 was used as the

main structural template to model the open conformation of PKCε around the ligand and 3TXO as a template for the rest of the protein.

Firstly, 3TXO and 1BX6 were structurally aligned using jCE algoritm (Prlic et al. 2010) to examine the equivalency of both structures. The result showed that the RMSD of their structures is 1.65 Å and they share identity and similarity at 34.23% and 51.35%, respectively. Next, the sequence of human PKCε was aligned with the sequences of 3TXO and 1BX6 using CLUSTAL 2.1 and also edited manually to maintain the "open" conformation at the glycine-rich loop (GXGXXG) in 3TXO (Taylor et al. 2011).

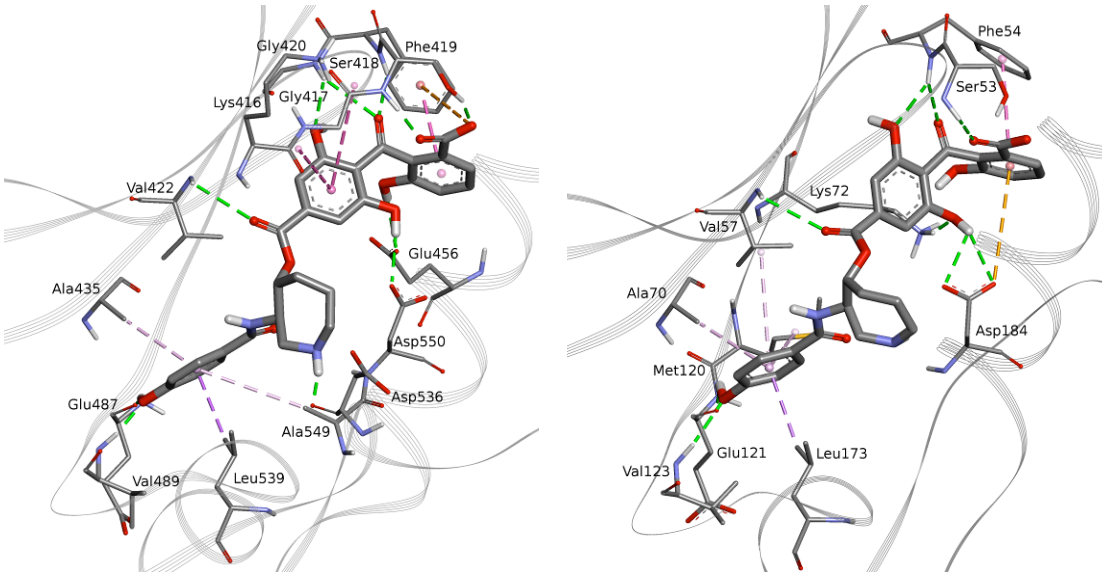
The three dimensional model of human PKCε with bound balanol was built using Modeller 9.14 (Eswar et al. 2008). The resulting models were ranked using the DOPE score (Shen & Sali 2006). The score represents the quality of the model and the lower the score, the more native-like the corresponding model. The model was further assessed (Table S3.2) using Ramachandran plot on PROCHECK webserver (Laskowski et al. 2001).

Table S3.2. Statistics of Ramachandran plot of the PKCε structure homology model with balanol bound.

	Number of residues	%
Residues in most favoured regions	271	**91.8**
Residues in additional allowed regions	16	5.5%
Residues in generously allowed regions	6	2.0%
Residues in disallowed regions	2	0.7%

PROCHECK has suggested that a good quality model would be expected to have over 90% in the most favoured regions, based on an analysis of 118 structures of at least 2.0 Å resolution and R-factor no greater than 20%, (Laskowski et al. 2001). Thereby, the selected PKCε model can be considered of good quality, with a score of 91.8% in the most favoured regions.

The docking simulated structure of balanol bound to PKCε is compared to the crystal structure of balanol bound to PKA in Figure S3.3. The close contact between the benzophenone and the glycine-rich loop is similar in these two structures, and the key backbone H-bonding between the *p*-hydroxybenzamide phenol to the conserved Glu amino acid residue (Glu 121 in PKA and Glu 487 in PKCε) is also maintained in both. However, the azepane conformation in these two structures is significantly different, with the azepane ring making more H-bonding interactions in the case of the PKCε model. The key ligand-protein interactions in both structures are tabulated in Table S3.3.



PKCε-Balanol PKA-balanol

Figure S3.3. The docking simulated structure of balanol bound to PKCε is compared to the crystal structure of balanol bound to PKA.

Table S3.3. Key amino acid residue contact comparison for balanol binding to mouse PKA (1BX6) and human PKCε (from docking simulation). These amino acid residues are important in balanol binding in the ATP site according to 1BX6.

Key ATP binding contact

Glycine rich loop *Catalytic lysine* *Activation loop*

PKA ^{47}KTLGTGSFGRVMLVKHKESGNHYAMK^{72}...^{120}MEYVAGGEMF^{129} ...^{184}DFG

PKC$_{\varepsilon}$ ^{412}KVLGKGSFGKVMLAELKGKDEVYAVK^{437}...^{486}MEYVNGGDLMF^{496}...^{550}DFG

PKCε residues	Balanol motifs	Type[a]	Subtype[a]	Matching PKA residues	Balanol motifs	Type	Subtype
Lys416^	C2′-C7′ ring	π- π	BB				
Gly417^	C2′-C7′ ring	π- π	BB				
Ser418#	C15″- CO_2H	H-bond	BB	Ser53	C15″- CO_2H	H-bond	BB
Phe419#	C15″- CO_2H	π-Anion	SC	Phe54	C4″-OH	H-bond	BB
Phe419#	C9″- C14″ ring	π-π Stacking	SC	Phe54	C9″- C14″ ring	π-π Stacking	SC
Phe419^	C8′=O	H-bond	BB	Phe54	C8″=O	H-bond	BB
Gly420^	C8′=O	H-bond	BB				
Gly420#	C4″-OH	H-bond	BB				
Val422#	C1″=O	H-bond	BB	Val57	C1″=O	H-bond	BB
				Val57	C2′-C7′ ring	π-Alkyl	SC
Ala435^	C2′-C7′ ring	π-Alkyl	SC	Ala70	C2′-C7′ ring	π-Alkyl	SC
				Lys72	C6″-OH	H-bond	SC
Glu456#	C10″-OH	H-bond	SC				
				Met120	C2′-C7′ ring	π-Alkyl	SC
Glu487^	C5′-OH	H-bond	BB	Glu121	C5′-OH	H-bond	BB
Val489^	C5′-OH	H-bond	BB	Val123	C5′-OH	H-bond	BB
Leu539^	C2′-C7′ ring	π-σ	SC	Leu173	C2′-C7′ ring	π-σ	SC
Asp550#	C6″-OH	H-bond	SC	Asp184	C6″-OH	H-bond	SC
				Asp184	C9″- C14″ ring	π-Anion	SC
Asp536*	N1	H-bond	BB				
Ala549^	C2′-C7′ ring	π-σ	SC				

[a] H-bond: hydrogen bond; BB : backbone; SC : side chain
^ p-Hydroxybenzamide moiety binding interactions
* Azepan moiety binding interactions
# Benzophenone moiety binding interactions

C. Docking simulation of balanoids to PKA/C using optimized docking simulation procedure. Balanoids (**1a**, **1c**, and **1d**) were used for docking analysis using the mouse PKA crystal structure (1BX6) and the PKCε structural homology model (Figure S3.4). These balanoids were built by adding fluorine atoms at particular positions of the seven-membered azepane ring of the natural

balanol. Each balanoid was subjected to the optimised docking procedure and docked into PKA (see section A) with seven replication each. The simulation indicates that all balanoids have highly similar conformations and occupy the ATP site of PKA in similar positions and binding orientations (Figure S3.5).

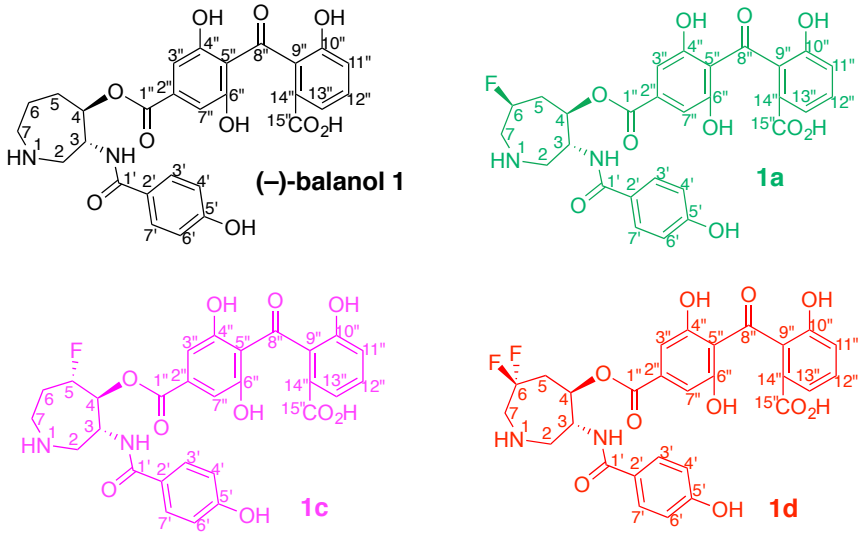
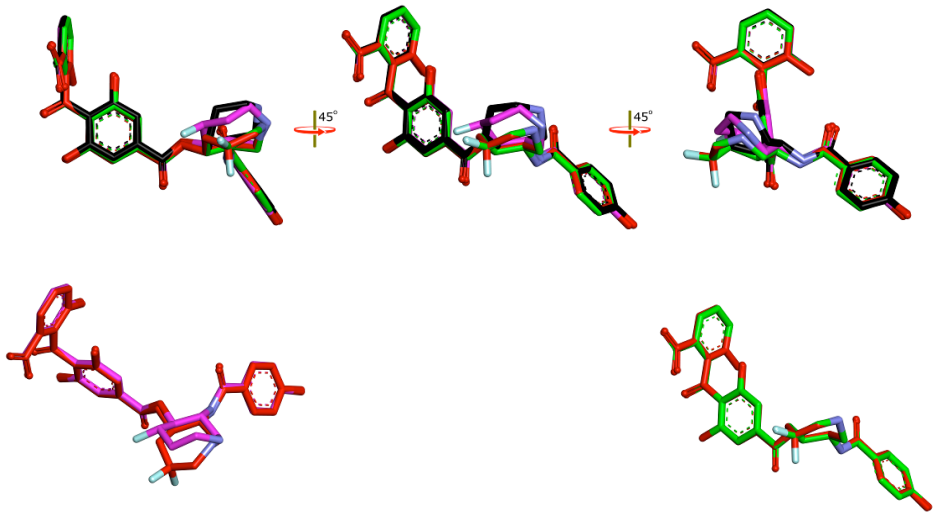


Figure S3.4. Structures of balanol (**1**) and balanoids (**1a**, **1c**, and **1d**) used for docking analysis. Balanol **1**: black; **1a**: green; **1c**: pink; and **1d**: red. The colour codes are used throughout the section.



1c and **1d** **1a** and **1d**

Figure S3.5. An overlay of the bound form of balanol with balanoids **1a**, **1c**, and **1d** in PKA (according to the same color codes as in Figure S3., Balanol **1**: black; **1a**: green; **1c**: pink; and **1d**: red) viewed from multiple angles. Pairwise overlay of the bound conformations of balanoids are also shown to highlight the similarities and differences in their conformations.

In particular, the benzophenone binding conformations are similar across balanol and the balanoids, which is consistent with the experimental observations that these compounds have similar binding affinities to PKA. In addition, the *p*-hydroxybenzamide binding conformations of these compounds

are also very similar, and the azepane moiety is the only part of that undergoes noticeable conformational changes. These results again suggest good correlation between the measure binding affinities and interaction profiles identified by the docking analysis.

Likewise, each balanoid was subjected to the optimised docking procedure (section B) with seven replicates each. The grid maps derived from the PKCε structure homology model was used for the docking simulation. The calculated binding energies ($\Delta G°$) were listed along with the experimental K_d in Table S3.4.

Table S3.4. Experimental K_d values of the balanoids binding to PKCε and their calculated ΔG° obtained from docking simulation.

Balanoids	K_d **(nM)**	**ln** K_d	**Calculated** $\Delta G°$
1	0.73	-21.04	-26.11
7 **1a**	19	-17.78	-23.75
9 **1c**	0.4	-21.64	-26.36
12 **1d**	110	-16.02	-21.44

Graphical plotting between ln K_d of the balanoids and calculated ΔG° showed good linear correlation (Figure 3.6).

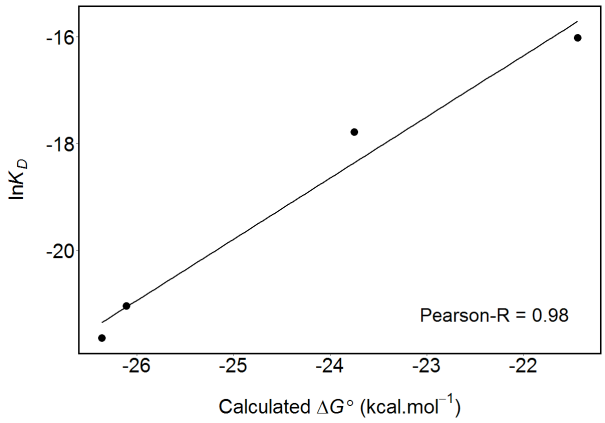


Figure S3.6. Correlation between ln K_d and calculated ΔG° of binding between the balanoids and PKCε.

As was the case with PKA, the bound ligand conformation to PKCε is similar across the panel. In particular, the binding interactions of the benzophenone moiety appeared to be very conserved across all members. Larger conformational differences of the azepane ring appeared in the ribose-binding subsite and the *p*-hydroxybenzamide motif in the adenine subsite, with **1**, **1a**, and **1c** being comparable, and **1d** different from the other three (Figure 3.7).

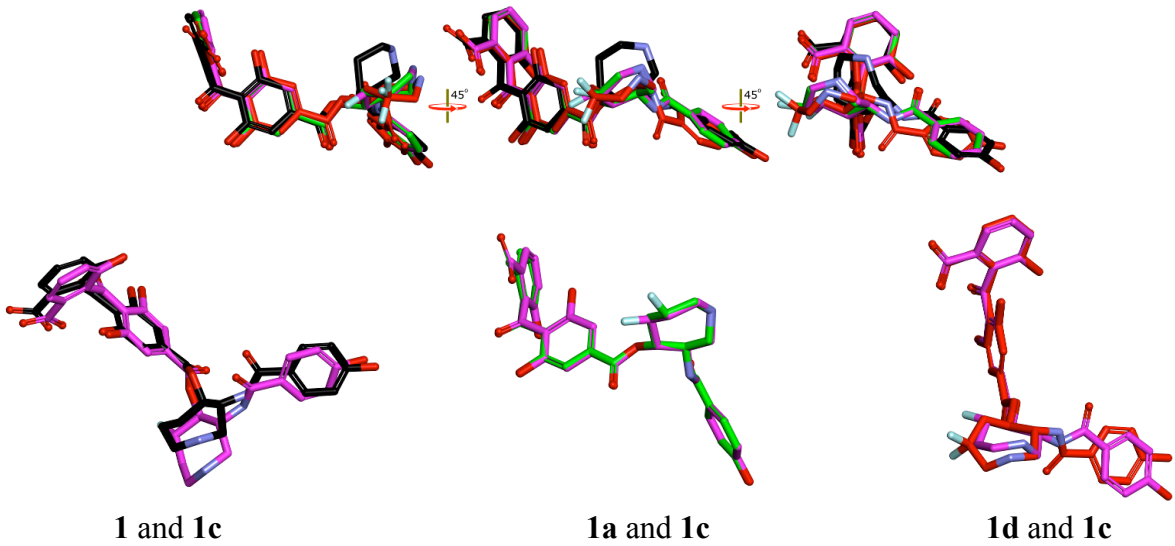


Figure S3.7. An overlay of bound balanol/balanoids conformations in PKCε (according to the same color codes as in Figure S3., Balanol **1**: black; **1a**: green; **1c**: pink; and **1d**: red). Pairwise overlay of the bound conformations of balanoids are also shown to highlight the similarities and differences in their conformations.

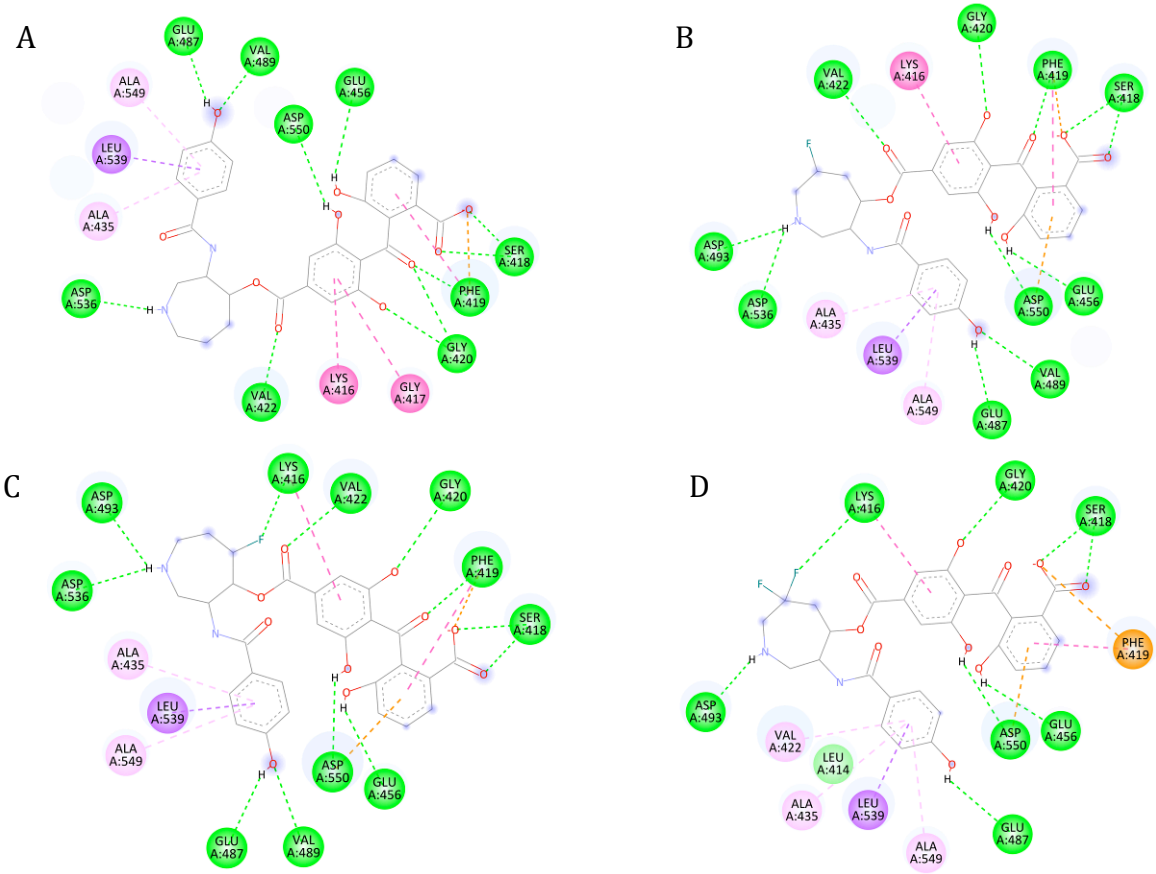


Figure S3.8. Two-dimensional graphical representations of ligand-receptor interactions of balanoids (A) **1**, (B) **1a**, (C) **1c**, and (D) **1d** with PKCε. The interaction visualisation was generated using Discovery Studio Visualizer (Systèmes 2016).

In general, the key interactions, as identified earlier in section I between the benzophenone motif of balanol and PKA, are preserved here in the case of balanoids **1**, **1a**, **1c** and **1d** with PKCε (Table S3.5, Figure S3.8, and Figure S3.9). These include: hydrogen bonds with hydroxyl of Ser418 and carboxyl of Glu456. The first ring also interacted with Phe419 via π- π interaction. Meanwhile, the Gly420 backbone and the side chain of Asp550 made hydrogen bonds with the second ring of benzophenone moiety. Those interactions suggest stabilisation of the benzophenone moiety of balanol analogues at the ATP binding site of PKCε.

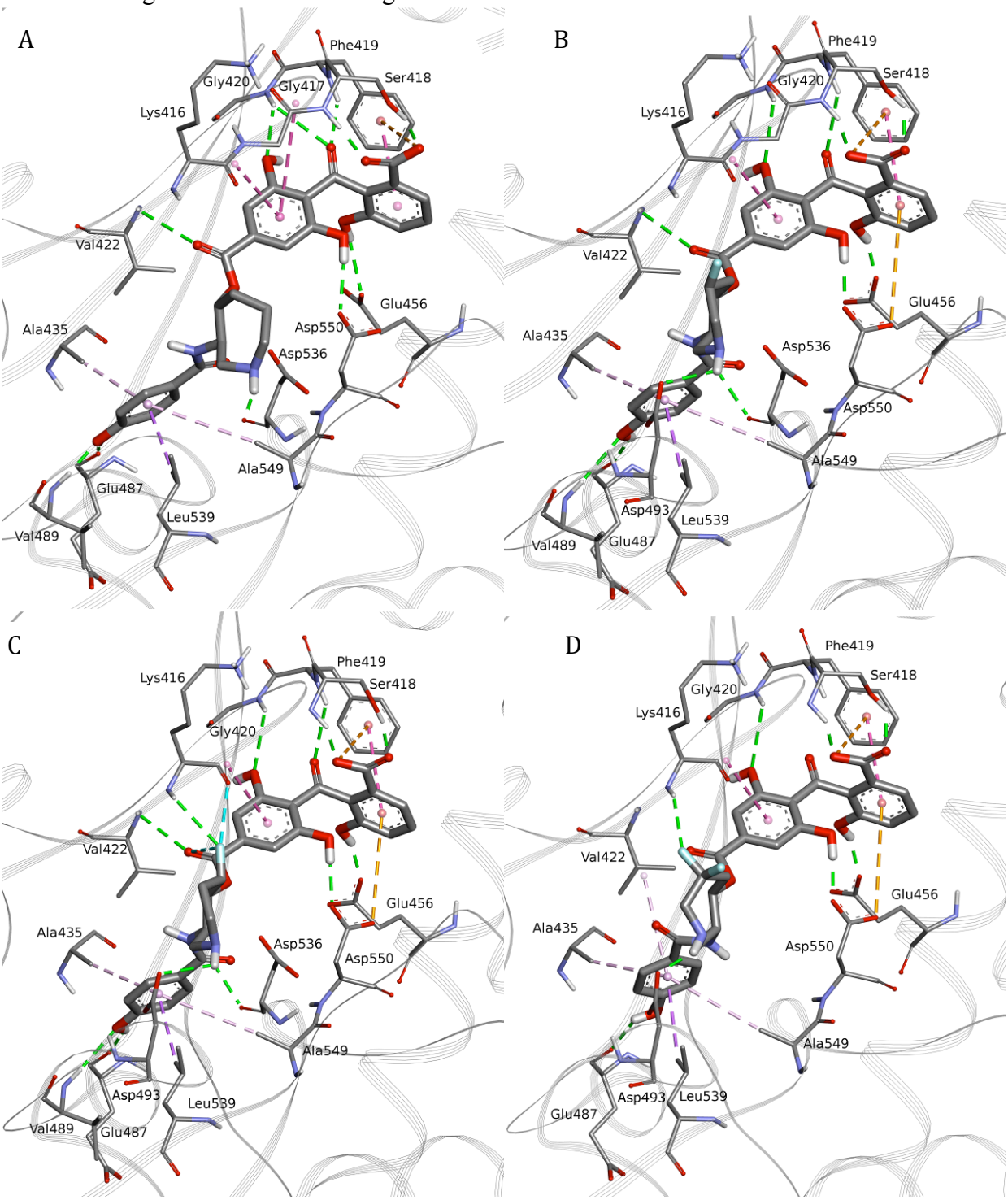


Figure S3.9. Ligand-receptor interactions of balanoids (A) **1**, (B) **1a**, (C) **1c**, and (D) **1d** with PKCε.

Table S3.5. Comparison of ligand-receptor interactions of balanoids **1**, **1a**, **1c**, and **1d** with PKCε.

Residues	Balanol1 (**1**)			Balanol7 (**1a**)			Balanol9 (**1c**)			Balanol12 (**1d**)		
	Balanol motifs	Type[a]	Subtype[a]	Balanol motifs	Type	Subtype	Balanol motifs	Type	Subtype	Balanol motifs	Type	Subtype
Lys416^	C2′-C7′ ring	π- π	BB	C2′-C7′ ring	π- π	BB	C2′-C7′ ring	π- π	BB	C2′-C7′ ring	π- π	BB
Lys416*							C5-F	H-bond	BB	C6-F	H-bond	BB
Gly417^	C2′-C7′ ring	π- π	BB									
Ser418#	C15″- CO_2H	H-bond	BB	C15″- CO_2H	H-bond	BB	C15″- CO_2H	H-bond	BB	C15″- CO_2H	H-bond	BB
Ser418#	C15″- CO_2H	H-bond	SC	C15″- CO_2H	H-bond	SC	C15″- CO_2H	H-bond	SC	C15″- CO_2H	H-bond	SC
Phe419#	C15″- CO_2H	π-Anion	SC	C15″- CO_2H	π-Anion	SC	C15″- CO_2H	π-Anion	SC	C15″- CO_2H	π-Anion	SC
Phe419#	C9″- C14″ ring	π-π Stacking	SC	C9″- C14″ ring	π-π Stacking	SC	C9″- C14″ ring	π-π Stacking	SC	C9″- C14″ ring	π-π Stacking	SC
Phe419^	C8′=O	H-bond	BB	C8′=O	H-bond	BB	C8′=O	H-bond	BB			
Gly420^	C8′=O	H-bond	BB									
Gly420#	C4″-OH	H-bond	BB	C4″-OH	H-bond	BB	C4″-OH	H-bond	BB	C4″-OH	H-bond	BB
Val422#	C1″=O	H-bond	BB	C1″=O	H-bond	BB	C1″=O	H-bond				
Ala435^	C2′-C7′ ring	π-Alkyl	SC	C2′-C7′ ring	π-Alkyl	SC	C2′-C7′ ring	π-Alkyl	SC	C2′-C7′ ring	π-Alkyl	SC
Glu456#	C10″-OH	H-bond	SC	C10″-OH	H-bond	SC	C10″-OH	H-bond	SC	C10″-OH	H-bond	SC
Glu487^	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB
Val489^	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB			
Asp493*				N1	H-bond	SC	N1	H-bond	SC	N1	H-bond	SC
Asp536*	N1	H-bond	BB	N1	H-bond	BB	N1	H-bond	BB			
Leu539^	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC
Ala549^	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC
Asp550#	C6″-OH	H-bond	SC	C6″-OH	H-bond	SC	C6″-OH	H-bond	SC	C6″-OH	H-bond	SC
				C9″- C14″ ring	π-Anion	SC	C9″- C14″ ring	π-Anion	SC	C9″- C14″ ring	π-Anion	SC

[a] H-bond: hydrogen bond; BB: backbone; SC: side chain. ^ Benzamide moiety binding interactions. * Azepan moiety binding interactions. # Benzophenone moiety binding interactions

The details of H-bonds are presented in Table S3.6. The balanoid **1c** has the maxi H-bonds (12), followed closely by **1a** (with 11) and **1** (10), while **1d** has the leas bonds (8). These are consistent with the lnK_d values shown in Table S3.4, wher higher affinity than **1**, **1a**, and **1d**. The interaction with Gly420 is specific to **1**, balanoids interact with Asp493, which is not seen with **1**.

Table S3.6. H-bond distances among balanoids in PKCε binding site.

	H-bond distances (Å)			
	Balanol1 (**1**)	Balanol7 (**1a**)	Balanol9 (**1c**)	Balanol12 (**1d**)
Lys416			2.90	2.73
Ser418	1.97	1.98	1.92	1.99
Ser418	1.86	1.86	1.75	1.84
Phe419	2.07	2.88	2.81	
Gly420	2.36			
Gly420	2.93	2.85	2.86	3.00
Val422	3.06	2.97	2.96	
Glu456	2.47	1.80	1.83	1.84
Glu487	1.85	2.13	2.07	2.48
Val489	1.77	2.50	2.41	
Asp493		2.37	2.50	1.78
Asp536	1.83	2.34	2.16	
Asp550	2.24	1.99	2.02	1.94

A comparison of **1c** bound to PKA and to PKCε is informative in understand interactions that are unique to each complex (Figure S3.10). We note that the H-bor of **1c** and Asp493 of PKCε (which is conserved among all balanoids; Table S3.6) i the case of PKA, where this residue is replaced by Glu127, which is beyond H-b from N1H. Furthermore, the interaction between F (on C5) of **1c** and the backbone Lys416 of PKCε is replaced by a single interaction in PKA with the CO of equivalent residue, Thr51. A comparison of **1c** and **1d** bound to PKA is shown in F Table S3.7. The interactions in the azepane, benzophenone and the *p*-hydroxyber regions are comparable for these two compounds.

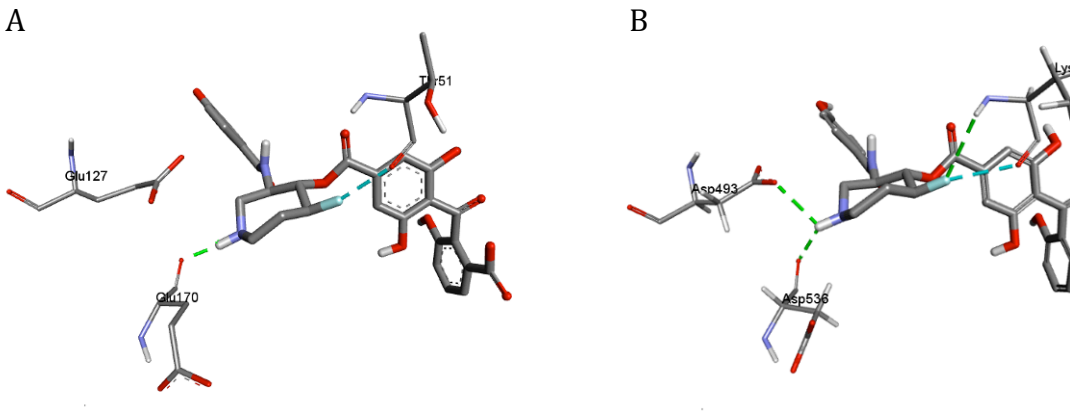


Figure S3.10. A comparison of **1c** docked on (A) PKA and (B) PKCε. Two H-bond ring are missing in the complex of 1c and PKA.

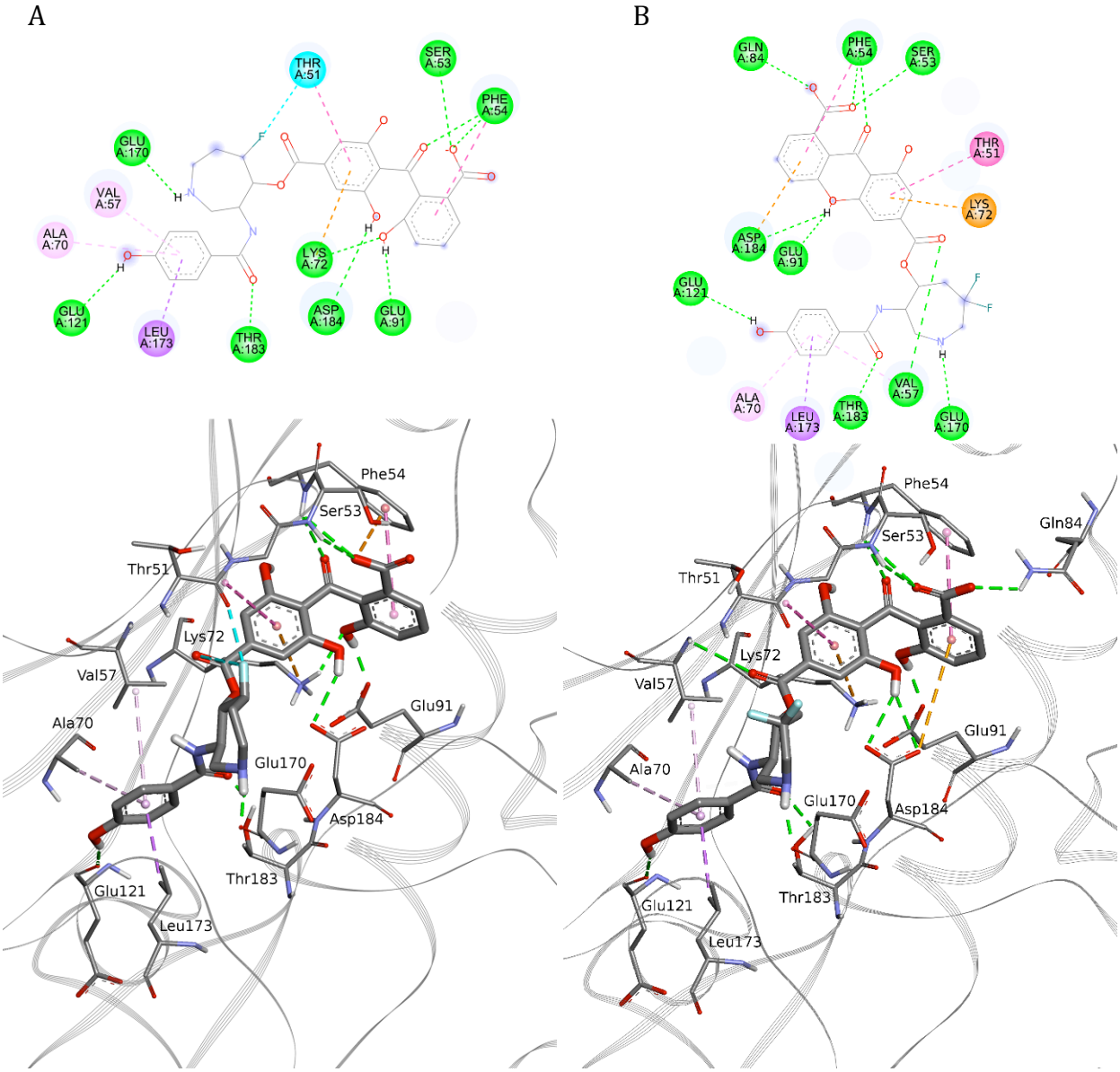


Figure S3.11. Ligand-receptor interactions of balanoids (A) **1c**, and (B) **1d** with PKA.

Table S3.7. Comparison of Non-covalent interaction of the balanoid **1c** and **1d** in PKA.

	1c			**1d**		
Residues	Balanol motifs	Type[a]	Subtype[a]	Balanol motifs	Type	Subtype
Thr51[#]	C2″-C7″ ring	π-Alkyl	BB	C2″-C7″ ring	π-Alkyl	BB
Ser53[#]	C15″- CO_2H	H-bond	BB	C15″- CO_2H	H-bond	BB
Phe54[#]	C15″- CO_2H	H-bond	BB	C15″- CO_2H	H-bond	BB
Phe54[#]	C15″- CO_2H	π-Anion	side chain			
Phe54[#]	C9″- C14″ ring	π-π Stacking	SC	C9″- C14″ ring	π-π Stacking	SC
Phe54[#]	C8″=O	H-bond	BB	C8″=O	H-bond	BB
Val57^	C2′-C7′ ring	π-Alkyl	SC	C2′-C7′ ring	π-Alkyl	SC
Val57[#]				C1″=O	H-bond	BB
Ala70^	C2′-C7′ ring	π-Alkyl	SC	C2′-C7′ ring	π-Alkyl	SC
Lys72[#]	C10″-OH	H-bond	SC			
Lys72[#]	C2″-C7″ ring	π-Cation	SC	C2″-C7″ ring	π- Cation	SC
Gln84[#]				C15″- CO_2H	H-bond	SC
Glu91[#]	C10″-OH	H-bond	SC	C10″-OH	H-bond	SC
Glu121^	C5′-OH	H-bond	BB	C5′-OH	H-bond	BB
Glu170*	N1	H-bond	BB	N1	H-bond	BB
Leu173^	C2′-C7′ ring	π-σ	SC	C2′-C7′ ring	π-σ	SC
Thr183^	C1′=O	H-bond	SC	C1′=O	H-bond	SC
Asp184[#]	C6″-OH	H-bond	SC	C6″-OH	H-bond	SC
Asp184[#]				C9″- C14″ ring	π-Anion	SC

[a] H-bond: hydrogen bond; BB: backbone; SC: side chain.
^ Benzamide moiety
* Azepan moiety
[#] Benzophenone moiety

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