Supporting Information

Total Synthesis of Teixobactin.

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General Methods and Materials

All reactions were carried out under an argon atmosphere and at room temperature (22 ^{o}C) unless the reaction was performed under aqueous conditions or unless otherwise specified. Reactions undertaken at -78 ^{o}C utilized a bath of dry ice and acetone. Reactions carried out at 0 ^{o}C employed a bath of water and ice. Anhydrous THF, CH_2Cl_2 and MeOH were obtained using a PureSolv® solvent purification system with water detectable only in low ppm levels. Reactions were monitored by thin layer chromatography (TLC) on aluminium backed silica plates (Merck Silica Gel 60 F254). Visualisation of TLC plates was undertaken with an ultraviolet (UV) light at λ = 254 nm and staining with solutions of vanillin, ninhydrin, phosphomolybdic acid (PMA), potassium permanganate or sulfuric acid, followed by exposure of the stained plates to heat. Silica flash column chromatography (Merck Silica Gel 60 40 – 63 μm) was undertaken to purify crude reaction mixtures using solvents as specified.

All commercially available reagents were used as obtained from Sigma-Aldrich, Merck or Acros Organics. Amino acids, coupling reagents and HMPB-ChemMatrix® resin were obtained from NovaBiochem or GL Biochem and peptide synthesis grade DMF was obtained from Merck or Labscan. All non-commercially available reagents were synthesized according to literature procedures as referenced.

^{1}H NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at frequencies of 400 MHz or 500 MHz respectively in $CDCl_3$, acetone-d_6 or DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). The residual solvent peaks were used as internal standards without the use of tetramethylsilane (TMS). ^{1}H NMR data is reported as follows: chemical shift values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) and relative integral. ^{13}C NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at 100 MHz or 125 MHz in $CDCl_3$, MeOD, acetone-d_6 or DMSO-d_6 unless otherwise specified. ^{13}C NMR data is reported as chemical shift values (ppm). Low resolution mass spectra for novel compounds were recorded on a Bruker amaZon SL mass spectrometer (ESI) operating in positive mode or on a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode for previously reported compounds. High resolution mass spectra were recorded on a Bruker-Daltronics Apex Ultra 7.0T Fourier transform (FTICR) mass spectrometer.

LC-MS was performed either on a Shimadzu 2020 LC-MS instrument with an LC-M20A pump, SPD-20A UV/Vis detector and a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode or on a Shimadzu UPLC-MS equipped with the same modules as above but with an SPD-M30A diode array detector. Separations on the LC-MS system were performed on a Waters Sunfire 5 μm, 2.1 x 150 mm (C18) column. On the UPLC-MS system, separations were performed on a Waters Acquity 1.7 μm, 2.1 x 50 mm (C18) column. These separations were performed using a mobile phase of 0.1 vol% trifluoroacetic acid (TFA) in water (Solvent A) and 0.1 vol% TFA in MeCN (Solvent B) using linear gradients. Preparative reverse-phase HPLC was performed using a Waters 500 pump with a 2996 photodiode array detector and a Waters 600 Multisolvent Delivery System. Compounds were purified using a Waters XBridge Prep OBD 5 μm 19 x 150 mm (C18) column using a 0-50 vol% MeCN focussed gradient (0-30vol% MeCN over 2 min, 30-50 vol% over 20 min) at a flow rate flow rate of 15 mL min^{-1}.

Fmoc Strategy Solid-Phase Peptide Synthesis (Fmoc-SPPS)

Fmoc-strategy solid-phase peptide synthesis (Fmoc-SPPS) procedures were employed on acid-labile 4-hydroxymethyl-3-methoxyphenoxybutyric acid (HMPB) functionalized polyethylene glycol resin (HMPB-NovaPEG) within fritted syringes (purchased from Torviq). All reagent equivalents are in regard to the amount of amino acid loaded to resin.

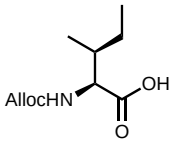
PyBOP Coupling Conditions

Loaded resin was washed with CH_2Cl_2 (x 5) and DMF (x 5) before being treated with a solution of 10 vol% piperidine in DMF (2 x 3 min). The resin was again washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). The resin was shaken for 2 h at room temperature with a solution of the desired Fmoc-protected amino acid (4 equiv.), (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (4 equiv.) and 4-methylmorpholine (NMM) (8 equiv.) in DMF (0.1 M in regard to loaded amino acid). The coupling solution was discharged and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5).

Experimental and Analytical Data

Synthesis of Alloc-L-Ile-OH

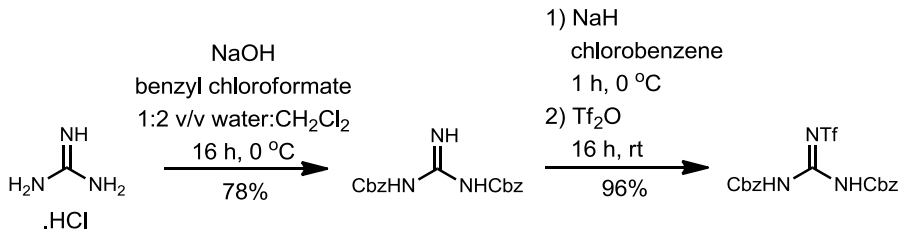
Alloc-L-Ile-OH



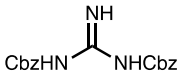
L-Isoleucine (1.00 g, 7.62 mmol) was suspended in saturated aqueous Na_2CO_3 (13 mL) and cooled to 0 ^oC. A mixture of allyl chloroformate (891 μL, 8.38 mmol) and 1,4-dioxane (28 mL) was added dropwise. The resulting reaction mixture was stirred at 0 ^oC for 2 h, then diluted with water (150 mL), washed with Et_2O (3 x 150 mL) and acidified to pH 2 *via* addition of 1 M aqueous HCl. The acidified mixture was extracted with ethyl acetate (3 x 200 mL) and the combined organic extracts were washed with brine (200 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford Alloc-L-Ile-OH as a colorless oil (1.57 g, 7.27 mmol, 95%) which was used without purification. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.70 (bs, 1H), 5.96-5.83 (m, 1H), 5.40-5.25 (m, 2H), 5.21 (dq, *J* = 1.2, 10.5 Hz, 1H), 4.63-4.52 (m, 2H), 4.35 (dd, *J* = 4.5, 9.0 Hz, 1H) 2.00-1.83 (m, 1H), 1.54-1.40 (m, 1H), 1.28-1.13 (m, 1H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz**)** δ (ppm); 176.6, 156.4, 132.63, 118.1, 66.1, 58.4, 37.8, 24.9, 15.6, 11.7; **LRMS:** (+ESI) *m/z* 216 $[M+H]^+$; **IR (ATR):** ν_{max} = 3326, 2964, 2928, 2879, 1710, 1528, 1461, 1409, 1331, 1242 cm^{-1}; **$[\alpha]_D$:** +9.6^o (c 0.3, CH_2Cl_2). These data are in agreement with those previously reported by Jad *et al.*[1]

*Synthesis of N,N'-di-Cbz-N''-Tf-guanidine (**5**)*

The synthesis of *N,N'*-di-Cbz-*N''*-Tf-guanidine (**5**) was adapted from previous work by Feichtinger *et al.*[2] All data are consistent with those previously reported.

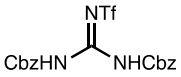


N,N'-di-Cbz-guanidine



Guanidine hydrochloride (3.82 g, 40.0 mmol) and sodium hydroxide (8.00 g, 200 mmol) were dissolved in a 1:2 v/v mixture of water:CH_2Cl_2 (120 mL) and cooled to 0 ^oC. Benzyl chloroformate (17.1 mL, 120 mmol) was added dropwise to the mixture and the resulting reaction was stirred at 0 ^oC for 16 h. Upon completion, the reaction mixture was poured over CH_2Cl_2 (100 mL) and separated with the aqueous phase being collected and re-extracted with CH_2Cl_2 (3 x 50 mL). All organic phases were combined and concentrated *in vacuo* to afford the crude product as a beige solid which was recrystallized from methanol to afford the title compound (10.2 g, 31.1 mmol, 78%) as a white solid. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm); 8.79 (bs, 2H), 7.37-7.26 (m, 10H), 5.04 (bs, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz**)** δ (ppm); 159.2, 135.8, 128.6, 128.4, 128.1, 67.4 (Guanidine signal missing due to overlap); **LRMS:** (+ESI) *m/z* 328 $[M+H]^+$; **IR (ATR):** ν_{max} = 3404, 3257, 3064, 3034, 2960, 1738, 1681, 1652, 1619, 1558, 1495, 1453, 1386, 1287, 1219 cm^{-1}; **m.p.:** 139-148 ^oC. These data are in agreement with those previously reported by Feichtinger *et al.*[2]

*N,N'-di-Cbz-N''-Tf-guanidine (**5**)*



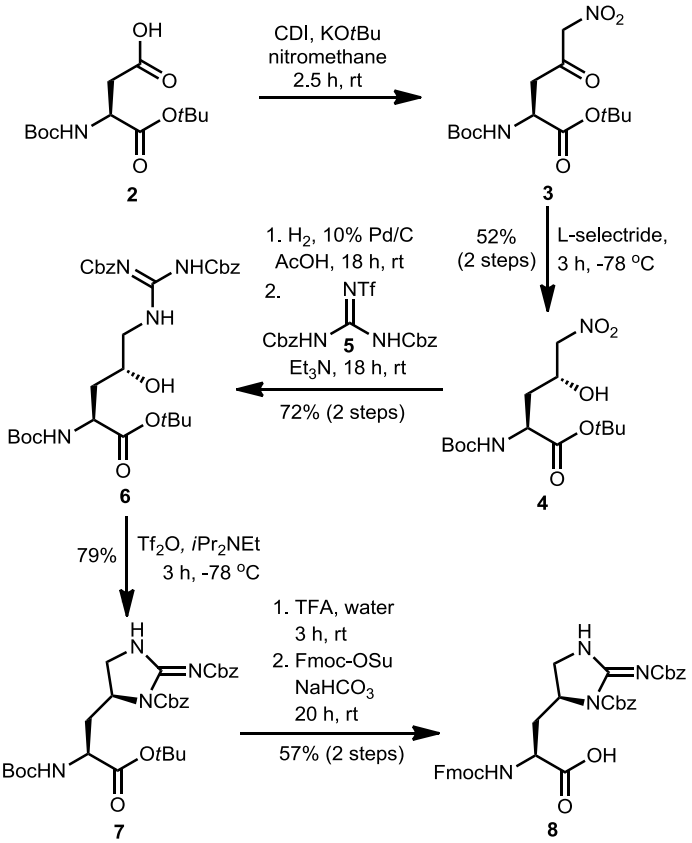
5

N,N'-di-Cbz-guanidine (1.5 g, 4.6 mmol) was dissolved in chlorobenzene (45 mL) and cooled to 0 ^oC prior to addition of sodium hydride (60% dispersion in oil) (370 mg, 9.2 mmol). The reaction mixture was stirred for 1 h at 0 ^oC, then cooled to -45 ^oC. Trifluoromethanesulfonic anhydride (770 μL, 4.6 mmol) was added dropwise to the solution which was then warmed to room temperature and stirred for 16 h. The reaction mixture was quenched with water and concentrated *in vacuo*. The resulting crude solid was redissolved in ethyl acetate (100 mL) and washed with 2 M

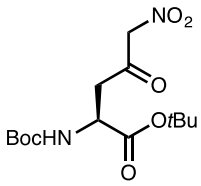
$NaHSO_4$ (2 x 100 mL), water (100 mL) and brine (100 mL). The washed organic phase was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a crude oil which was purified by flash chromatography (95:5 v/v CH_2Cl_2:Et_2O), affording the title compound **5** (2.0 g, 4.4 mmol, 96%) as a colorless oil. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm); 10.31 (bs, 2H), 7.40 (bs, 10H), 5.27 (bs, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz**)** δ (ppm); 151.0, 150.2, 133.7, 129.4, 129.0, 128.9, 119.3 (q, $J_{C\text{-}F}$ = 318 Hz), 69.9; **^{19}F-NMR** ($CDCl_3$, 470 MHz) δ (ppm); -78.64; **LRMS:** (+ESI) *m/z* 460 $[M+H]^+$; **IR (ATR):** ν_{max} = 3283, 1791, 1742, 1620, 1555, 1498, 1456, 1376, 1340, 1259 cm^{-1}. These data are in agreement with those previously reported by Feichtinger *et al*.[2]

*Synthesis of Fmoc-L-allo-enduracididine(Cbz)$_2$-OH (**8**)*

The synthesis of Fmoc-L-*allo*-enduracididine(Cbz)$_2$-OH took inspiration from previous work by Rudolph *et al.*[3] and Peoples *et al*.[4] All data for literature compounds are consistent with those previously reported.



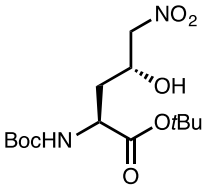
*tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-5-nitro-4-oxopentanoate (**3**)*



3

Boc-L-Asp-O*t*Bu **2** (3.00 g, 10.4 mmol) and 1,1'-carbonyldiimidazole (1.68 g, 10.4 mmol) were dried *in vacuo* for 1 h then dissolved in nitromethane (53 mL). The reaction mixture was stirred at room temperature for 45 min, at which point potassium *tert*-butoxide (2.24 g, 20.8 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for an additional 2.5 h, then quenched with 50 vol% glacial acetic acid in water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic phases were washed with water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL), water (100 mL) and brine (100 mL). The washed organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was azeotroped with toluene (x 3) and concentrated *in vacuo* to afford nitroketone **3** which was used without purification.

*tert-butyl (2S,4R)-2-((tert-butoxycarbonyl)amino)-4-hydroxy-5-nitropentanoate (**4**)*

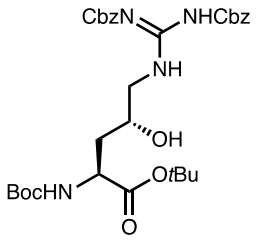


4

Crude nitroketone **3** was dissolved in anhydrous THF (150 mL) and cooled to -78 ^{o}C. To this solution was slowly added a 1 M solution of L-Selectride® in THF (6 mL, 6.00 mmol), the resulting reaction mixture was stirred at -78 ^{o}C for 3 h. The reaction mixture was then poured onto saturated aqueous NH_4Cl solution (150 mL) and diluted with water (50 mL). The resulting mixture was extracted with ethyl acetate (3 x 150 mL) and the combined organic phases were washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a crude yellow oil which was a 5:1 mixture of diastereomers that was purified by flash chromatography (95:5 v/v CH_2Cl_2:Et_2O), affording nitro-alcohol **4** as a single diastereomer (1.80 g, 5.38 mmol, 52% over 2 steps) as a white solid. **^{1}H NMR:** ($CDCl_3$, 500 MHz) δ (ppm); 5.46 (bd, 1H, *J* = 6.4 Hz), 4.57-4.37 (m, 3H), 4.30-4.19 (m, 1H), 3.55 (bs, 1H), 2.11-1.81 (m, 2H), 1.46 (s, 9H), 1.43 (s, 9H); **^{13}C NMR**

($CDCl_3$, 125 MHz**)** δ (ppm); 171.1, 155.9, 83.0, 80.6, 80.3, 66.2, 51.5, 37.0, 28.4, 28.0; **LRMS:** (+ESI) *m/z* 335 $[M+H]^+$; **IR (ATR):** ν_{max} = 3378, 2979, 2933, 1696, 1556, 1508, 1456, 1392, 1368, 1253 cm^{-1}; **$[\alpha]_D$:** +21.6° (*c* 0.3, CH_2Cl_2); **m.p.:** 106-120 °C. These data are in agreement with those previously reported by Rudolph *et al*.[3]

*tert-butyl (2S,4R)-5-((E)-2,3-bis((benzyloxy)carbonyl)guanidino)-2-((tert-butoxycarbonyl)amino)-4-hydroxypentanoate (**6**)*

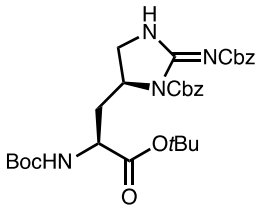


6

Nitro-alcohol **4** (1.80 g, 5.38 mmol) was dissolved in anhydrous methanol (54 mL) and to this solution was added 10% w/w palladium on activated carbon (575 mg, 540 μmol palladium), and glacial acetic acid (308 μL, 5.38 mmol). The reaction vessel was evacuated and flushed with nitrogen (x 3) then filled with an atmosphere of hydrogen (1 atm). The reaction was stirred at room temperature for 18 h, then evacuated and flushed with nitrogen (x 3), and filtered through celite. The filtrate was concentrated *in vacuo* to afford a crude beige foam which was azeotroped with toluene (x 3) and redissolved in CH_2Cl_2 (15 mL). To this solution was added a solution of guanidinylating reagent **5** (2.48 g, 5.40 mmol) in MeCN (15 mL) *via* canula. Et_3N (313 μL, 1.85 mmol) was added and the reaction mixture was stirred at 40 °C for 18 h, then poured onto a saturated aqueous NH_4Cl solution (30 mL). The mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude oil which was purified by flash chromatography (35 - 40% v/v ethyl acetate in *n*-hexanes), affording compound **6** (2.39 g, 3.89 mmol, 72%) as a white solid. **^{1}H NMR:** ($CDCl_3$, 400 MHz) δ (ppm); 11.69 (bs, 1H), 8.69 (t, 1H, *J* = 5.41 Hz), 7.42 – 7.24 (m, 10H), 5.49-5.37 (m, 1H), 5.18 (s, 2H), 5.10 (s, 2H), 4.27-4.16 (m, 1H), 3.99-3.90 (m, 1H), 3.63 (ddd, 1H, *J* = 2.5, 5.7, 14.0 Hz), 3.40 (ddd, 1H, *J* = 5.0, 7.5, 14.0 Hz), 2.01-1.90 (m, 1H), 1.82 (ddd, 1H, *J* = 6.6, 9.0, 14.3 Hz), 1.43 (s, 18H); **^{13}C NMR** ($CDCl_3$, 100 MHz**)** δ (ppm); 171.5, 163.2, 157.0, 155.7, 153.6, 136.5, 134.5, 128.8, 128.7, 128.5, 128.4, 128.1, 128.0, 82.2, 80.0, 68.6, 68.3, 67.1, 52.1, 47.2, 37.8, 28.3, 27.9; **LRMS:** (+ESI) *m/z* 637 $[M+Na]^+$; **HRMS:** (+ESI) Calc. for $C_{31}H_{42}N_4O_9$: 615.3025 $[M+H]^+$, Found: 615.3032 $[M+H]^+$; **IR**

(ATR): ν_{max} = 3335, 3306, 2958, 2926, 2854, 1732, 1643, 1625, 1571, 1499, 1455, 1382, 1368, 1352, 1326, 1257, 1214 cm^{-1}; **$[\alpha]_D$:** +6.0° (*c* 0.3, CH_2Cl_2); **m.p.:** 118-130 °C.

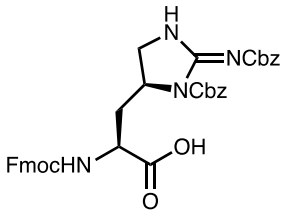
Boc-L-allo-End(Cbz)$_2$-OtBu (7)



7

Guanidinylated compound **6** (2.39 g, 3.89 mmol) was dissolved in anhydrous CH_2Cl_2 (140 mL) and cooled to -78 °C. To this solution was added Hünig's base (3.24 mL, 18.6 mmol) followed by dropwise addition of trifluoromethanesulfonic anhydride (669 μL, 4.66 mmol). The resulting reaction mixture was stirred at -78 °C for 3 h, then warmed to room temperature for 15 min and quenched with a saturated aqueous NH_4Cl solution (140 mL). This mixture was extracted with CH_2Cl_2 (2 x 100 mL) and the combined organic phases were washed with a saturated aqueous $NaHCO_3$ solution (100 mL). The washed organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a brown oil which was purified by flash chromatography (30 – 50% v/v ethyl acetate in *n*-hexanes), affording Boc-L-*allo*-End(Cbz)$_2$-O*t*Bu (**7**) (1.83 g, 3.07 mmol, 79%) as a white foam. **^{1}H NMR:** (acetone-d_6, 400 MHz) δ (ppm); 7.63-7.57 (m, 2H) 7.44-7.25 (m, 8H), 6.24 (d, *J* = 4 Hz, 1H), 5.33-5.24 (m, 2H), 5.16-5.06 (m, 2H), 4.62-4.53 (m, 1H), 4.15 (app. q, *J* = 7.2 Hz, 1H), 3.90 (dd, *J* = 9.1, 10.6 Hz, 1H), 3.65 (dd, *J* = 3.0, 7.6 Hz, 1H), 2.37 (ddd, *J* = 3.3, 6.9, 13.5 Hz, 1H), 2.08-1.99 (m, 1H, obscured by residual solvent), 1.46 (s, 9H), 1.41 (s, 9H); **^{13}C NMR** (acetone-d_6, 100 MHz**)** δ (ppm); 171.7, 164.1, 159.5, 156.2, 152.1, 138.6, 137.0, 129.2, 129.1, 128.7, 128.6, 128.4, 128.4, 82.1, 79.5, 68.4, 67.4, 54.9, 52.9, 47.0, 36.5, 28.6, 28.1; **LRMS:** (+ESI) *m/z* 619 $[M+Na]^+$; **HRMS:** (+ESI) Calc. for $C_{31}H_{40}N_4O_8$: 597.2919 $[M+H]^+$, Found: 597.2923 $[M+H]^+$; **IR (ATR):** ν_{max} = 3349, 2976, 2923, 2854, 2162, 1713, 1654, 1617, 1498, 1440, 1393, 1368, 1306, 1258 cm^{-1}; **$[\alpha]_D$:** +17.3° (*c* 0.3, CH_2Cl_2).

*Fmoc-L-allo-End(Cbz)$_2$-OH (**8**)*



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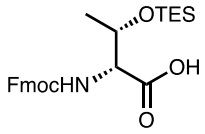
Boc-L-*allo*-End(Cbz)$_2$-O*t*Bu (**7)** (263 mg, 441 μmol) was dissolved in a mixture of TFA (4.5 mL) and water (0.45 mL). The mixture was stirred at room temperature for 3 h, then concentrated under a stream of nitrogen. The resulting crude oil was azeotroped with toluene (3 x 10 mL) and concentrated *in vacuo* to remove residual TFA. The concentrated crude material was then dissolved in a mixture of THF (4 mL) and saturated aqueous $NaHCO_3$ solution (2.5 mL). Fmoc-succinamide (156 mg, 459 μmol) was added to this mixture and the reaction was stirred at room temperature for 20 h. The reaction mixture was acidified to pH 2 with a 1 M aqueous HCl solution, then extracted with ethyl acetate (3 x 20 mL). The combined ethyl acetate phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude white foam that was purified by flash chromatography (9:1 ethyl acetate:MeOH) to afford Fmoc-L-*allo*-End(Cbz)$_2$-OH (**8**) (168 mg, 253 μmol, 57%) as a white foam. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm); 7.89 (d, *J* = 7.9 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.71 (dd, *J* = 2.4, 7.7 Hz, 2H), 7.52-7.24 (m, 14H), 5.26-5.14 (m, 2H), 5.09-4.99 (m, 2H), 4.46-4.16 (m, 4H), 4.14-4.04 (m, 1H), 3.68 (dd, *J* = 9.4, 10.4 Hz, 1H), 3.39 (dd, *J* = 2.4, 10.7 Hz, 1H), 2.29-2.19 (m, 1H), 1.97-1.85 (m, 1H) **^{13}C NMR** (DMSO-d_6, 100 MHz**)** δ (ppm); 173.1, 162.4, 157.7, 156.0, 150.6, 143.7, 140.7, 137.2, 135.8, 128.2, 128.0, 127.7, 127.6, 127.6, 127.4, 127.1, 125.3, 125.2, 120.1, 67.2, 66.1, 65.7, 53.9, 51.5, 46.6, 45.8, 34.9; **LRMS:** (+ESI) *m/z* 685 $[M+Na]^+$; **HRMS:** (+ESI) Calc. for $C_{37}H_{34}N_4O_8$: 663.2449 $[M+H]^+$, Found: 663.2460 $[M+H]^+$; **IR (ATR):** ν_{max} = 2950, 2925, 2855, 2163, 1706, 1218 cm^{-1}; **$[\alpha]_D$:** +4.0^o (*c* 0.1, CH_2Cl_2).

*Synthesis of Fmoc-D-Thr(OTES)-OH (**9**)*

*Fmoc-D-Thr(OTES)-OH (**9**)*

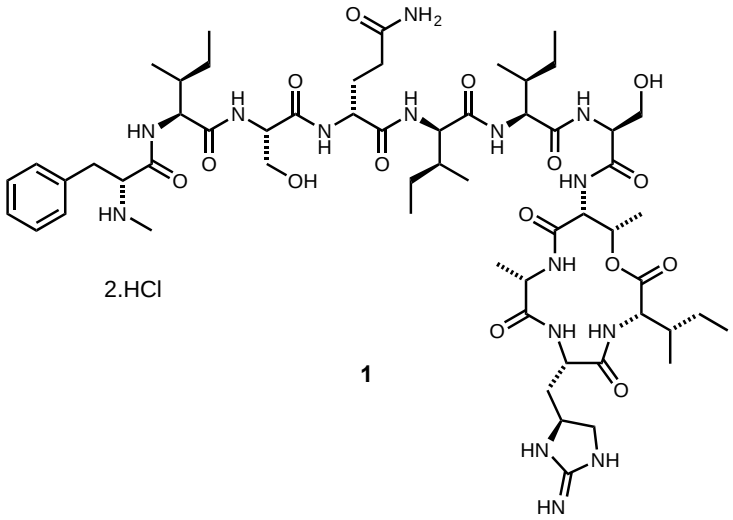


9

D-Threonine (5.00 g, 42.0 mmol) and Fmoc-succinamide (14.9 g, 44.1 mmol) were dissolved in a 2:1 v/v mixture of THF:saturated aqueous $NaHCO_3$ (100 mL). The reaction mixture was stirred at room temperature for 16 h. The reaction was then diluted with water (50 mL) and the pH of the mixture was adjusted to pH 9 *via* addition of saturated aqueous $NaHCO_3$. The mixture was extracted with diethyl ether (3 x 50 mL) and the aqueous layer was acidified to pH 1 *via* addition of 1 M HCl. The acidic aqueous mixture was extracted with ethyl acetate (3 x 100 mL) and the combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford crude Fmoc-D-Thr-OH (14.3 g) as a white foam which was deemed to be sufficiently pure and used without further purification. A portion of the crude Fmoc-D-Thr-OH (3.00 g, 8.79 mmol) was dissolved in anhydrous DMF (20 mL) and cooled to 0 ^oC. To this cooled solution was added Hünig's base (4.90 mL, 28.1 mmol) followed by chlorotriethylsilane (1.48 mL, 17.6 mmol) dropwise. The reaction mixture was stirred at 0 ^oC for 20 min then warmed to room temperature and stirred for an additional 16 h. The reaction mixture was then cooled to 0 ^oC, diluted with water (20 mL) and poured onto saturated aqueous NH_4Cl (20 mL). The mixture was washed with ethyl acetate (2 x 50 mL), acidified to pH 2 *via* addition of 1 M HCl and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (100 mL), water (100 mL) and brine (100 mL) before being dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a crude colourless oil which was purified by flash chromatography (20% - 50% ethyl acetate in *n*-hexanes) to afford Fmoc-D-Thr(OTES)-OH (**9**) (1.47 g, 3.23 mmol, 37% over two steps) as a colourless oil. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm); 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (ap. t, *J* = 6.8 Hz, 2H), 7.40 (ap. t, *J* = 7.5 Hz, 2H), 7.32 (ap. t, *J* = 7.5 Hz, 2H), 5.62 (d, *J* = 8.5 Hz, 1H), 4.55-4.37 (m, 3H), 4.35 (dd, *J* = 2.2, 8.2 Hz, 1H), 4.27 (t, *J* = 7.2 Hz, 1H), 1.23 (d, *J* = 6.3 Hz, 3H), 0.98 (t, *J* = 8.1 Hz, 9H), 0.64 (q, *J* = 8.2 Hz, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz**)** δ (ppm); 174.5, 156.6, 144.0, 143.8, 141.5, 127.9, 127.2, 125.3, 120.1, 68.4, 67.4, 59.4, 47.3, 20.0, 6.8, 4.8 (1 extra signal due to restricted rotation about the Fmoc group); **LRMS:** (+ESI) *m/z* 478 $[M+Na]^+$; **HRMS:** (+ESI) Calc.

for $C_{25}H_{33}NO_5Si$: 478.2020 $[M+Na]^+$, Found: 478.2024 $[M+Na]^+$; **IR (ATR):** ν_{max} = 3437, 3067, 2955, 2911, 2876, 1719, 1510, 1478, 1450, 1413, 1378, 1341, 1310, 1209 cm^{-1}; **$[\alpha]_D$:** -1.5° (*c* 10.0, CH_2Cl_2).

*Solid-Phase Synthesis of teixobactin (**1**)*



1

Fmoc-D-Thr(OTES)-OH (**9**) (549 mg, 1.2 mmol, 10 equiv.) was dissolved in anhydrous CH_2Cl_2 (6 mL) and cooled to 0 °C. *N,N'*-diisopropylcarbodiimide (DIC) (94 μL, 0.60 mmol, 5 equiv.) was then added to the cooled solution, which was then warmed to room temperature and stirred under an atmosphere of argon for 30 min. The reaction mixture was concentrated under a stream of nitrogen and the resulting crude solid was redissolved in a 1:1 v/v mixture of CH_2Cl_2:DMF (1.2 mL). This mixture, along with a solution of 4-dimethylaminopyridine (DMAP) (cat.) in DMF (0.1 mL), was shaken for 16 h at room temperature with HMPB-NovaPEG resin (234 mg, 0.64 mmol g^{-1}) in a fritted syringe, which had been swollen in CH_2Cl_2 for 30 min and washed with CH_2Cl_2 (x 5), affording the resin-bound Fmoc-D-Thr(OTES) **10**.

The loading mixture was discharged from the fritted syringe and the resin was washed with CH_2Cl_2 (x 5) and DMF (x 5). The loaded resin was then capped *via* treatment with 10 vol% acetic anhydride in pyridine (3 mL) with dissolved DMAP (cat.) for 45 min at room temperature. The resin was again washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). Resin loading was determined after Fmoc-deprotection of the loaded amino acid, in which the resin was treated with a solution of 10 vol% piperidine in DMF (2 x 3 min) then washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). Combined deprotection solutions were made up to 10 mL with 10 vol% piperidine in DMF and diluted 1:100 with 10 vol% piperidine in DMF. Resin loading was determined to be 115 μmol by

measurement of the UV absorbance at λ = 301 nm of the diluted deprotection solution. The resin was then subjected to a mixture of 1 M tetrabutylammonium fluoride (TBAF) in THF (2.30 mL, 2.30 mmol, 20 equiv.), glacial acetic acid (131 μL, 2.30 mmol, 20 equiv.) and CH_2Cl_2 (2.3 mL) at room temperature for 2 h (x 2), effecting OTES deprotection and affording the the resin-bound dipeptide **11** as judged by HPLC-MS analysis.

The deprotection solution was discharged and the resin was washed with CH_2Cl_2 (x 5), DMF (x 5) and CH_2Cl_2 (x 5). Fmoc-Ser(*t*Bu)-OH was coupled to the α-amine of the resin-bound D-Thr according to standard PyBOP coupling conditions (see *PyBOP Coupling Conditions*). Alloc-L-Ile-OH (247 mg, 1.15 mmol, 10 equiv.) was dissolved in anhydrous CH_2Cl_2 (5.75 mL) and cooled to 0 ^oC. *N,N'*-diisopropylcarbodiimide (90 μL, 575 μmol, 5 equiv.) was added to this solution which was then warmed to room temperature and stirred for 30 min. The reaction mixture was concentrated under a stream of nitrogen and subsequently redissolved in a 1:1 v/v mixture of CH_2Cl_2:DMF (1.2 mL). This solution, along with a solution of DMAP (cat.) in DMF (0.1 mL), was shaken with the OTES-deprotected resin-bound dipeptide **11** for 16 h at room temperature to afford the resin-bound depsitripeptide **12**.

The esterification solution was discharged and the resin was washed with CH_2Cl_2 (x 5), DMF (x 5) and CH_2Cl_2 (x 5). Completion of the on-resin esterification reaction was judged by HPLC-MS analysis. The linear peptide was elongated using standard PyBOP coupling conditions (see *PyBOP Coupling Conditions*), incorporating the commercially available amino acids Fmoc-L-Ile-OH, Fmoc-D-*allo*-Ile-OH, Fmoc-D-Gln(Trt)-OH, Fmoc-L-Ser(*t*Bu)-OH, Fmoc-L-Ile-OH and *N*-methyl-Boc-D-Phe-OH, to afford resin-bound depsipeptide **13**.

Alloc deprotection of the esterified Alloc-L-Ile was then effected *via* treatment with a solution of $Pd(PPh_3)_4$ (27 mg, 23 μmol, 0.2 equiv.) and $PhSiH_3$ (283 μL, 2.3 mmol, 20 equiv.) in CH_2Cl_2 (1.2 mL) for 20 min (x 2) at room temperature. The deprotection solution was discharged and the resin was washed with CH_2Cl_2 (x 5) and DMF (x 5) before coupling of Fmoc-L-*allo*-End$(Cbz)_2$-OH (**8**) (114 mg, 174 mmol, 1.5 equiv.) with 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (66 mg, 174 mmol, 1.5 equiv.), 1-Hydroxy-7-azabenzotriazole (HOAt) (47 mg,348 mmol, 3 equiv.) and Hünig's base (60 μL, 348 mmol, 3 equiv.) in DMF (1.7 mL) for 16 h at room temperature. The resin was washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5), and the final amino acid, Fmoc-L-Ala-OH was coupled under standard PyBOP coupling conditions (see *PyBOP Coupling Conditions*), however, with shortened 10 vol% piperidine in DMF treatment of 30 sec (to prevent diketopiperazine formation). The branched N-

terminus of the resin-bound undecadepsipeptide **15** was Fmoc-deprotected *via* treatment with 10 vol% piperidine in DMF (2 x 3 min). The resin was then thoroughly washed with DMF (x 5) and CH_2Cl_2 (x 20) prior to selective cleavage of the undecadepsipeptide from the resin *via* treatment with 1 vol% TFA in CH_2Cl_2 (4 x 20 min), affording the protected undecadepsipeptide **16** as a crude solid after concentration of the deprotection solutions under a stream of nitrogen, azeotroping with toluene (x 3) and concentration *in vacuo*.

Crude **16** (116 mg, 115 μmol) was used without purification and dissolved in DMF (11.5 mL) to a concentration of 10 mM. To this solution was added 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM.BF_4) (56.0 mg, 173 μmol) and Hünig's base (60.0 μL, 345 μmol). The reaction was stirred at room temperature for 16 h and monitored by HPLC-MS. Upon completion, the reaction mixture was concentrated under a stream of nitrogen and re-dissolved in a mixture of 70:10:12:8 v/v/v/v TFA:thioanisole:TfOH:*m*-cresol (1 mL) at 0 ^oC. The reaction was stirred at 0 ^oC for 1 h to afford teixobactin (**1**) as a crude solid after concentration under a stream of nitrogen. The crude teixobactin (**1**) was purified by RP-HPLC (see *General Methods and Materials*) and lyophilized to give pure teixobactin (**1**) as a TFA salt. Re-lyophilisation in the presense of 5 mM HCl (x 3) afforded afforded teixobactin (**1**) (4.99 mg, 3.80 μmol) as its *bis*-HCl salt in 3.3% yield over 24 linear steps. **LRMS:** (+ESI) *m/z* 1243 $[M+H]^+$; **HRMS:** (+ESI) Calc. for $C_{58}H_{95}N_{15}O_{15}$: 1242.7205 $[M+H]^+$, Found: 1242.7201 $[M+H]^+$; **IR (ATR):** ν_{max} = 3281, 2964, 2932, 2877, 1742, 1662, 1631, 1526, 1458, 1384, 1301, 1260 cm^{-1}; (see table S1 and table S2 for NMR characterization).

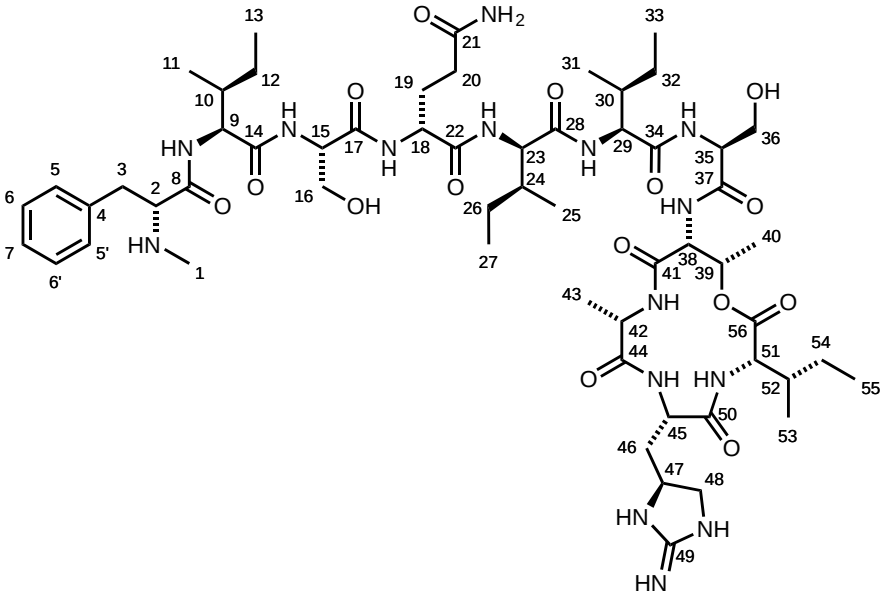


Figure S1. Structure of teixobactin (**1**) with numbered carbon centres for use in NMR analysis.

Table S1. ^{1}H-NMR comparison of natural and synthetic Teixobactin (**1**) in DMSO-d_6 referenced at 2.50 ppm. All assignments were made based on COSY, TOCSY, HSQC and HMBC data in comparison with the isolated material (see Figure S1 for positions of carbon centres).

Position	Natural δ ^{1}H /ppm (no. H, mult, *J* Hz)	Synthetic δ ^{1}H /ppm (no. H, mult, *J* Hz)	Δδ /ppm		Position	Natural δ ^{1}H /ppm (no. H, mult, *J* Hz)	Synthetic δ ^{1}H /ppm (no. H, mult, *J* Hz)	Δδ /ppm
1	2.5 (3H, brs)	2.47 (3H, br t, 4.4)	0.03		29	4.29 (1H, m)	4.39 (1H, m)	-0.1
2	4.21 (1H,dd, 9.4, 5.3)	4.27 (1H, m)	-0.06		29-NH	7.78 (1H, d, 8.8)	7.89 (1H, d, 8.1)	-0.11
2-NH	9.3, 9.0 (2H, v br s)	10.09, 9.08 (v br s)	na		30	1.83 (1H, m)	1.82 (1H, m)	0.01
3	3.00 (1H, dd, 13.2, 9.4)	2.95 (1H, dd, 12.7, 10.8)	0.05		31	0.84 (3H, m)	0.88 (3H, d, 6.7)	-0.04
	3.15 (1H, 13.2, 5.3)	3.29 (1H, dd, 12.8, 4.5)	-0.14		32	1.11 (1H, m)	1.08 (1H, m)	0.03
4						1.42 (1H, m)	1.44 (1H, m)	-0.02
5,5’	7.24 (2H,m)	7.21 (m, 2H)	0.03		33	0.85 (3H, m)	0.77 (3H, m)	-0.07
6,6’	7.31 (2H,m)	7.28 (m, 2H)	0.03		34			
7	7.27 (1H,m)	7.22 (m, 1H)	0.05		35	4.47 (1H, dt, 5.0, 5.2)	4.64* (1H, m)	-0.19
8					35-NH	8.37 (1H, d, 5.2)	8.99 (1H, d, 8.7)	-0.62
9	4.12 (1H, dd, 7.8, 7.2)	4.07 (1H, ap t, 7.3)	0.05		36	3.64 (1H, m)	3.56 (1H, m)	0.08
9-NH	8.43 (1H, d, 7.2)	8.64 (1H, d, 8.3)	-0.21			3.80 (1H, dd, 10.8, 5.0)	3.87 (1H, m)	-0.07
10	1.56 (1H, m)	1.56 (1H, m)	0		36-OH	exchanged	exchanged	
11	0.62 (3H, d, 6.7)	0.53 (3H, d, 6.6)	0.09		37			
12	0.76 (1H, m)	0.72 (1H, m)	0.04		38	4.64 (1H, dd, 9.5, 2.2)	4.69 (1H, ap. d, 11.0)	-0.05
					38-NH	Not reported	8.93 (1H, d, 9.9)	na
	1.07 (1H, m)	1.02 (1H, m)	0.05		39	5.36 (1H, dq, 2.2, 6.4)	5.37 (1H, dq, 2.0, 6.2)	-0.01
13	0.66 (3H, t, 7.1)	0.61 (3H, t, 7.1)	0.05		40	1.13 (3H, d, 6.4)	1.05 (3H, 6.4)	0.08
14					41			
15	4.34 (1H, m)	4.30 (1H, m)	0.04		42	3.97 (1H, dq, 5.1, 7.5)	3.89 (1H, m)	0.08
15-NH	7.88 (1H, d, 7.9)	8.09 (1H, d, 7.6)	-0.21		42-NH	8.05 (1H, d, 5.1)	8.16 (1H, d, 5.2)	-0.11
16	3.57 (1H, dd, 10.8, 5.6)	3.54 (1H, m)	0.03		43	1.34 (3H, d, 7.5)	1.26 (3H, d, 7.3)	0.08
	3.63 (1H, m)	3.62 (1H, m)	0.01		44			
16-OH	exchanged				45	4.38 (1H, m)	4.35 (1H, m)	0.03
17					45-NH	8.32 (1H, d, 9.1)	8.85 (1H, d, 10)	-0.53
18	4.33 (1H, m)	4.30 (1H, m)	0.03		46	2.03 (2H, m)	2.13 (2H, m)[§]	-0.1
18-NH	7.85 (1H, d, 7.9)	8.02 (1H, d 8.0)	-0.17		47	3.90 (1H, m)	3.82 (1H, m)	0.08
19[#]	1.74 (1H, m)	1.71 (1H, m)	0.03		47-NH	7.95 (1H, br s)	8.00 (1H, br s)	-0.05
	1.92 (1H, m)	1.87 (1H, m)	0.05		48	3.36 (1H, dd, 9.4, 7.7)	3.44 (1H, ap t, 8.0)	-0.08
20[#]	2.10 (2H, m)	2.08 (2H, m)	0.02			3.66 (1H, t, 9.4)	3.60 (1H, m)	0.06
21					48-NH	8.1 (1H, br s)	8.05 (1H, br s)	0.05
21-NH2	6.63 (1H, br s)	6.76 (1H, br s)	-0.13		49			
	7.11 (1H, br s)	7.26 (1H, br s)	-0.15		49-NH	7.76 (2H, br s)	7.80 (2H, br s)	-0.04
22					50			
23	4.36 (1H, m)	4.37 (1H, m)	-0.01		51	4.03 (1H, t, 9.4)	4.01 (1H, t, 9.8)	0.02
23-NH	7.70 (1H, d, 8.8)	7.75 (1H, d, 9)	-0.05		51-NH	8.01 (1H, d 9.4)	8.75 (1H, d, 9.8)	-0.74
24	1.8 (2H, m)	1.80 (1H, m)	0.0		52	1.77 (1H, m)	1.88 (1H, m)	-0.11
25	0.82 (3H, m)	0.77 (3H, m)	0.05		53	0.81 (3H, m)	0.78 (3H, m)	0.03
26	1.09 (1H, m)	1.06 (1H, m)	0.03		54	0.77 (1H, m)	1.13 (1H, m)	-0.36
	1.32 (1H, m)	1.28 (1H, m)	0.04			1.07 (1H, m)	1.41 (1H, m)	-0.34
27	0.82 (3H, m)	0.82 (3H, m)	0.0		55	0.82 (3H, m)	0.80 (3H, m)	0.02
28					56			

Note: discrepencies in NH chemical shifts are attributed to differences in pH and concentration. [#]methylene protons at C-19 and C-20 were misassigned in the isolation paper by Ling et al.[5] These assignments have been corrected in our data. *the data in the isolation paper appears to be quoted with the incorrect chemical shift. [§]methyelene protons appear as two separate signals in our data at 2.04 and 2.22 ppm each 1H, with the average (2.13) presented in the table

Table S2. ^{13}C-NMR comparison of natural and synthetic Teixobactin (**1**) in DMSO-d_6 referenced to 39.52 ppm. ^{13}C were extracted from the HSQC and HMBC spectra. All assignments were made based on COSY, TOCSY, HSQC and HMBC data in comparison with the isolated material (see Figure S1 for positions of carbon centres).

Position	Natural δ ^{13}C /ppm	Synthetic δ ^{13}C /ppm	Δδ /ppm		Position	Natural δ ^{13}C /ppm	Synthetic δ ^{13}C /ppm	Δδ /ppm
1	31.9	30.9	1		29	57.3	56.6	0.7
2	61.9	61.0	0.9		29-NH			
2-NH					30	36.9	36.7^	0.2
3	36.4	35.5	0.9		31	15.4	15.4[b]	0.0
					32	25.3	24.1	1.2
4	135.0	134.7	0.3					
5,5’	129.7	129.0	0.7		33	11.2	11.4[c]	-0.2
6,6’	128.9	128.3	0.6		34	171.6	170.7[a]	0.9
7	127.5	126.8	0.7		35	56.5	55.0	1.5
8	167.1	166.6	0.5		35-NH			
9	57.9	57.4	0.5		36	62.7	63.5	-0.8
9-NH								
10	36.5	35.8	0.7		36-OH			
11	15.5	14.9	0.6		37	171.7	171.4a	0.3
12	24.4	23.8	0.6		38	56.2	55.3	0.9
					38-NH			
					39	71.2	70.0	1.2
13	11.3	10.8	0.5		40	15.9	15.3	0.6
14	170.6	170.1	0.5		41	168.9	167.9	1.0
15	55.6	55.2	0.4		42	52.2	51.6	0.6
15-NH					42-NH			
16	62.4	61.7	0.7		43	17.1	16.5	0.6
					44	173.1	172.5	0.6
16-OH					45	52.2	51.9	0.3
17	170.2	169.7	0.5		45-NH			
18	52.7	52.0	0.7		46	37.2	36.2	1
18-NH					47	53.5	53.2	0.3
19[#]	28.4	27.9	0.5		47-NH			
20[#]	31.9	31.4	0.5		48	48.3	47.7	0.6
21	174.4	173.9	0.5		48-NH			
21-NH2					49	160.0	159.0	1
					49-NH			
22	170.9	170.9[a]	0.0		50	171.8	172.5[a]	-0.7
23	56.8	55.5	1.3		51	57.8	57.0	0.8
23-NH					51-NH			
24	37.4	36.7^	0.7		52	36.3	35.2	1.1
25	14.7	14.3[b]	0.4		53	16.0	15.0	1.0
26	26.2	25.6	0.8		54	24.5	24.8	-0.3
27	10.6	11.2[c]	-0.6		55	11.8	10.1[c]	1.7
28	171.4	170.8[a]	0.6		56	169.3	169.3	0.0

Note: [#]methylene carbons at C-19 and C-20 were misassigned in the isolation paper by Ling et al.[5] These assignments have been corrected in our data. ^assignment difficult due to signal overlap [a,b,c]correspond to signals in the isolation paper in which the ‘assignments may be switched due to overlap’ and were similarly difficult to assign in this case.

Antimicrobial Screening

Resazurin Assay for Mtb[6]

The compounds were originally stored as 10 mM stock solutions in 100% DMSO. Two fold serial dilutions of the compounds were made in a 96 well plate using Middlebrook 7H9 medium supplemented with ADC (0.5% v/v glycerol and 0.05% v/v Tween-80). *M. tuberculosis* H37Rv was grown to mid-exponential phase to an OD_{600} of 0.6-0.8 in 7H9 media at 37 °C. On the day of the assay, culture was diluted to an OD_{600} of 0.002 and 100 μl of bacterial suspension was added to the 96 well plate containing 100 μl of the diluted compounds. The plate was incubated for 5 days at 37 °C in a humidified incubator and 30 μl of Resazurin (0.02% w/v) and 12.5 μl of Tween-80 was added to each well and incubated for further 24 h. On day 6, the fluorescence was read using a BMG Labtech Polarstar plate reader (excitation 530 nm and emission 590 nm). The results are presented as *M. tuberculosis* survival as a percentage of negative control (no drug controls).

High-Throughput Antibacterial Inhibition Assay

Bacterial test strains were grown on fresh agar plates and individual colonies used to to inoculate 3 mL of sterile media. All staphylococcal strains were grown in tryptic soy broth (17 g tryptone, 3 g soytone, 2.5 g dextrose, 5 g NaCl and 2.5 g dipotassium phosphate in 1 L distilled water; pH 7.5). *P. alcalifaciens, O. anthropi, E. aerogenes and A. baumanii* were grown in nutrient broth (Difco, USA) while *B. subtilis, E. coli, V. cholerae, S. typhimurium, P. aeruginosa* and *Y. pseudotuberculosis* cultures were grown in Luria Broth (10 g tryptone, 5 g yeast extract and 10 g NaCl in 1 L distilled water; pH 7.5). All three media were autoclaved at 121 °C for 30 min. Inoculated cultures were grown overnight with shaking (200 rpm; 30 °C). Saturated overnight cultures were diluted 1:1000 or 1:100 according to turbidity and dispensed into sterile clear polypropylene 384 well plates (30 μL screening volume). Optical density (OD_{600}) of cultures at a 1:100 dilution were recorded (Shimadzu UV-Visible Spectrophotometer) and further diluted on agar plates to calculate colony forming units (CFU) per milliliter of culture. DMSO solutions of test compounds (200 nL) were pinned into each well at t_0 using a high-throughput pinning robot (Perkin Elmer Janus MDT). In the 384 well plate lanes 1 and 2 were reserved for DMSO vehicle negative controls, while lanes 23 and 24 contained only culture medium and test organisms. After compound addition, screening plates were stacked in an automated plate reader/shaker (Perkin Elmer EnVision)

and a OD_{600} reading was collected every 1 h for 16 - 20 h. The resulting growth curves for each dilution series were used to determine MIC values for all test compounds following standard procedures.[7]

Bacterial Strains

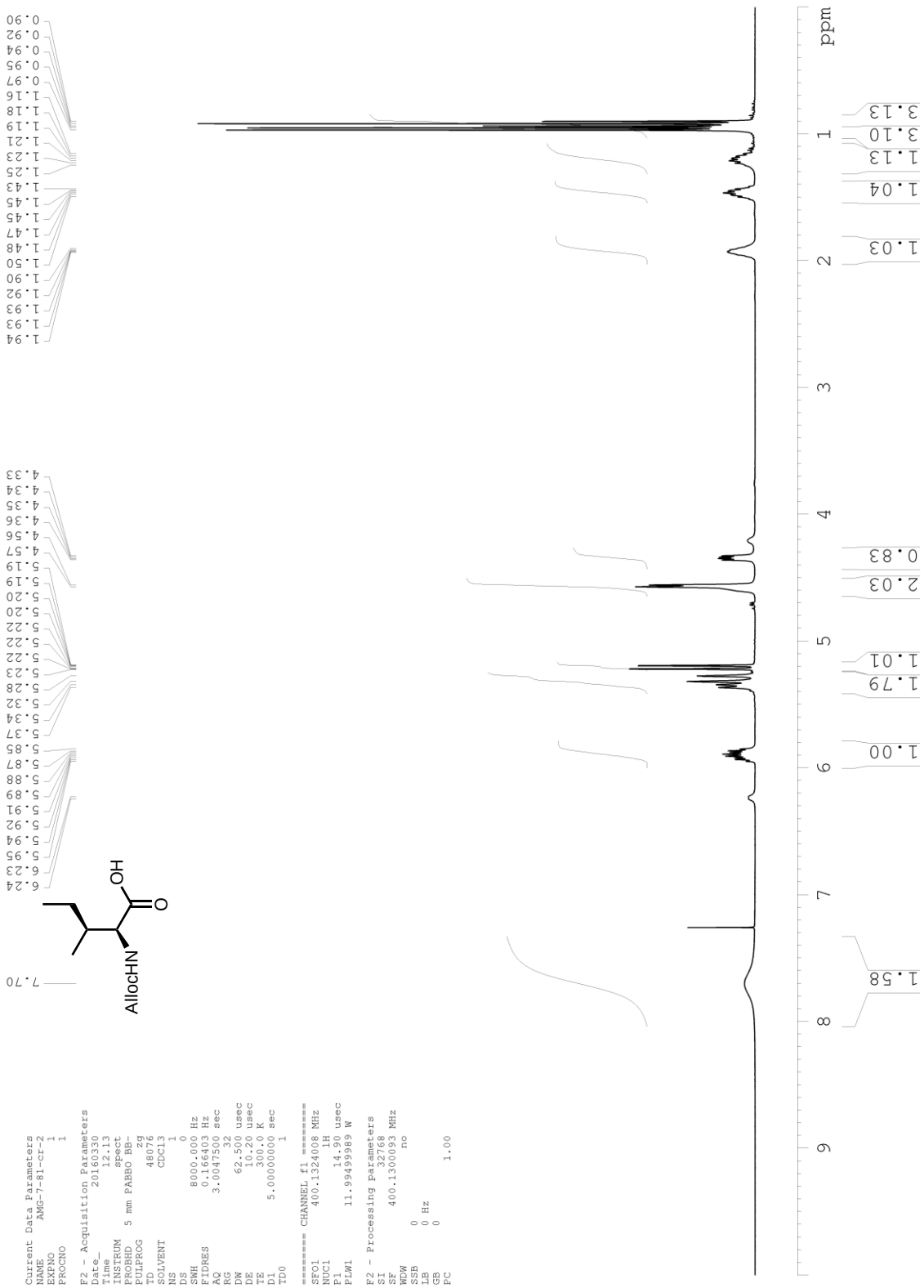
Gram-positive: *Bacillus subtilis* 168, Methicillin susceptible *Staphylococcus aureus* (MSSA) (ATCC 29213), Methicillin resistant *S. aureus* (MRSA) (BAA-44).
Gram-negative: *Escherichia coli* K12 (BW 25113), *Providencia alcalifaciens* (ATCC 9886), *Ochrobactrum anthropi* (ATCC 49687), *Enterobacter aerogenes* (ATCC 35029), *Acinetobacter baumanii* (NCIMB 12457, *Vibrio cholerae* O1 (biotype El Tor A1552), *Salmonella typhimurium* LT2, *Pseudomonas aeruginosa* (ATCC 27853), *Yersinia pseudotuberculosis* (IP2666 pIBI).

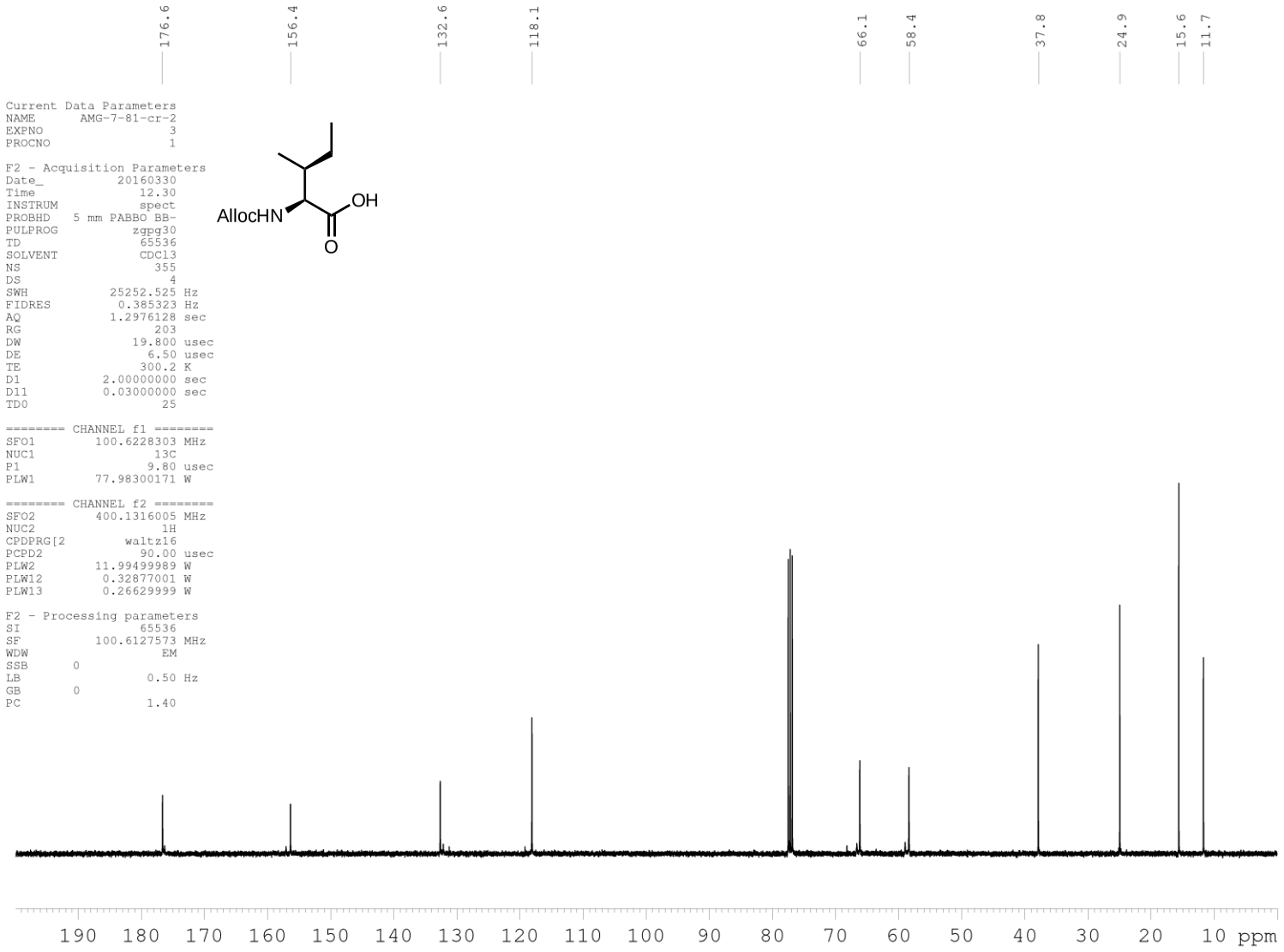
High-Throughput Antibacterial Inhibition Assay Results

Table S3. Average MIC values (μM) for teixobactin (**1**) and clinically relevant antiobiotics derived from high-throughput antibacterial screening (see above) for select Gram-negative and Gram-positive bacterial strains.

	Screening Dilution	Average OD	Average CFU	Teixobactin MIC (μM)	Vancomycin MIC (μM)	Linezolid MIC (μM)	Ciprofloxacin MIC (μM)
S. aureus (MSSA)	1000	0.55	3.3E+09	1.1	0.69	1.4	0.69
S. aureus (MRSA)	1000	0.47	4.9E+09	1.1	0.87	1.2	>66
E. coli	1000	0.58	3.2E+09	>27	>66	>66	0.013
B. subtilis	1000	0.43	3.0E+08	0.21	0.17	0.22	0.13
P. alcalifaciens	100	0.11	1.9E+10	>27	>66	>66	0.027
O. anthropi	100	0.17	7.0E+07	>27	>66	>66	0.85
E. aerogenes	100	0.2	3.8E+10	>27	>66	>66	0.022
A. baumanii	100	0.19	1.8E+10	>27	>66	>66	2.4
V. cholerae	1000	0.43	1.4E+11	>27	>66	>66	0.016
S. typhimurium	1000	0.4	5.0E+09	>27	>66	>66	0.027
P. aeruginosa	1000	0.48	2.0E+07	>27	>66	>66	1.4
Y. pseudotuberculosis	1000	0.42	2.0E+07	>27	>66	>66	0.0081

^{1}H and ^{13}C NMR Spectra



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TD0                 25

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PLW1        77.98300171 W

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NUC2                1H
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PCPD2            90.00 usec
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PLW13        0.26629999 W

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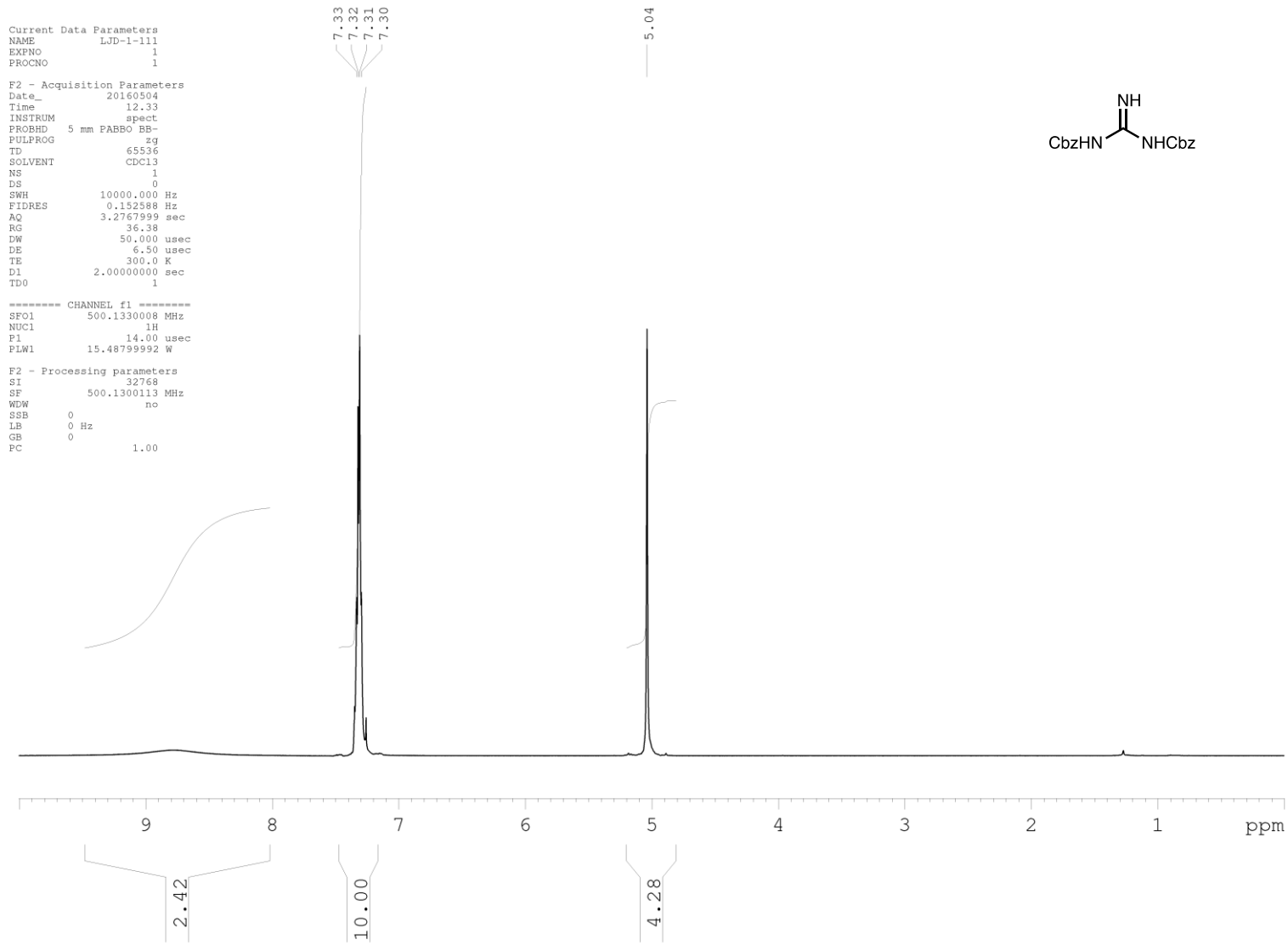
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SOLVENT CDCl3
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7.32
7.31
7.30
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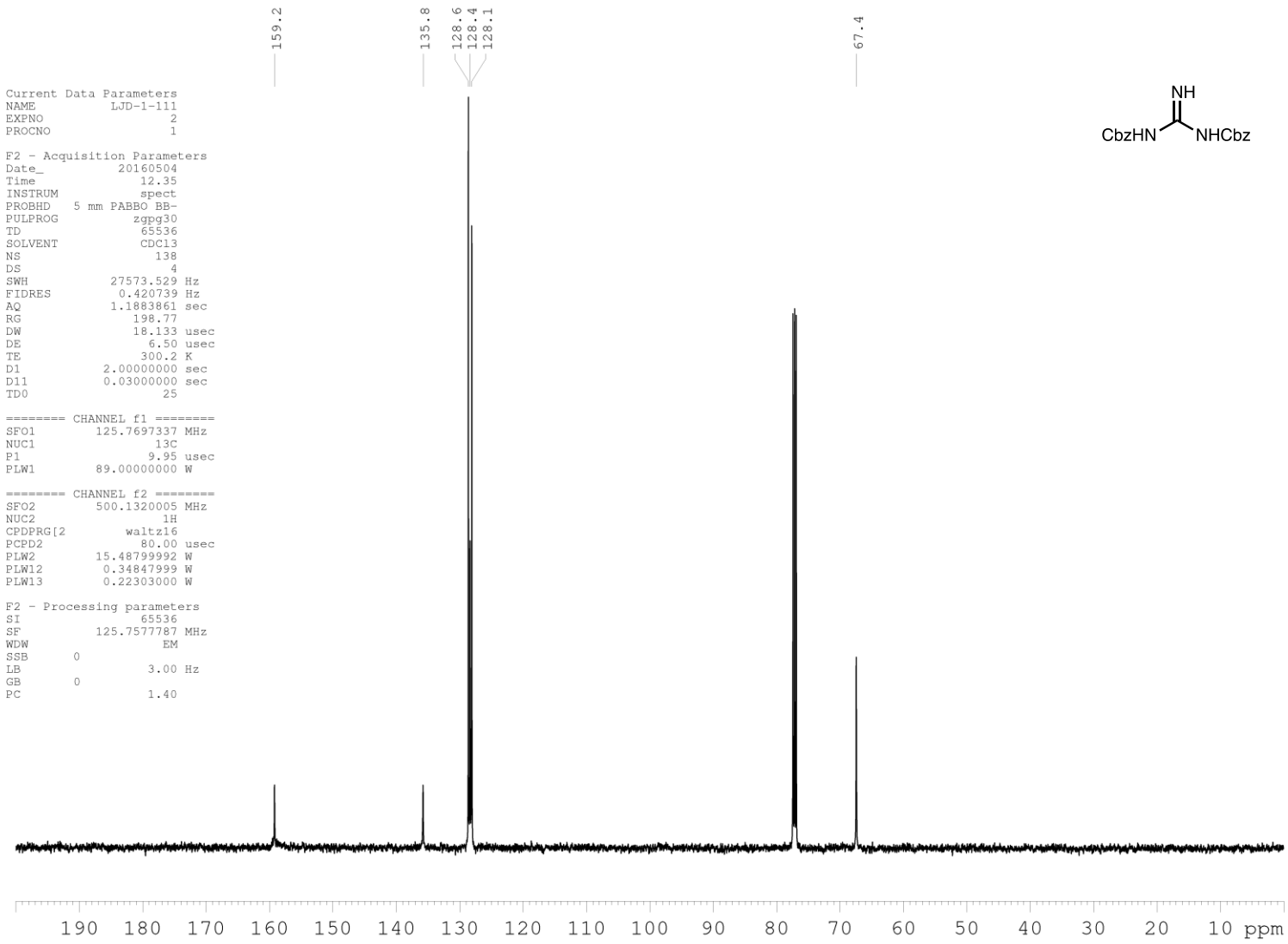
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PLW1 89.00000000 W
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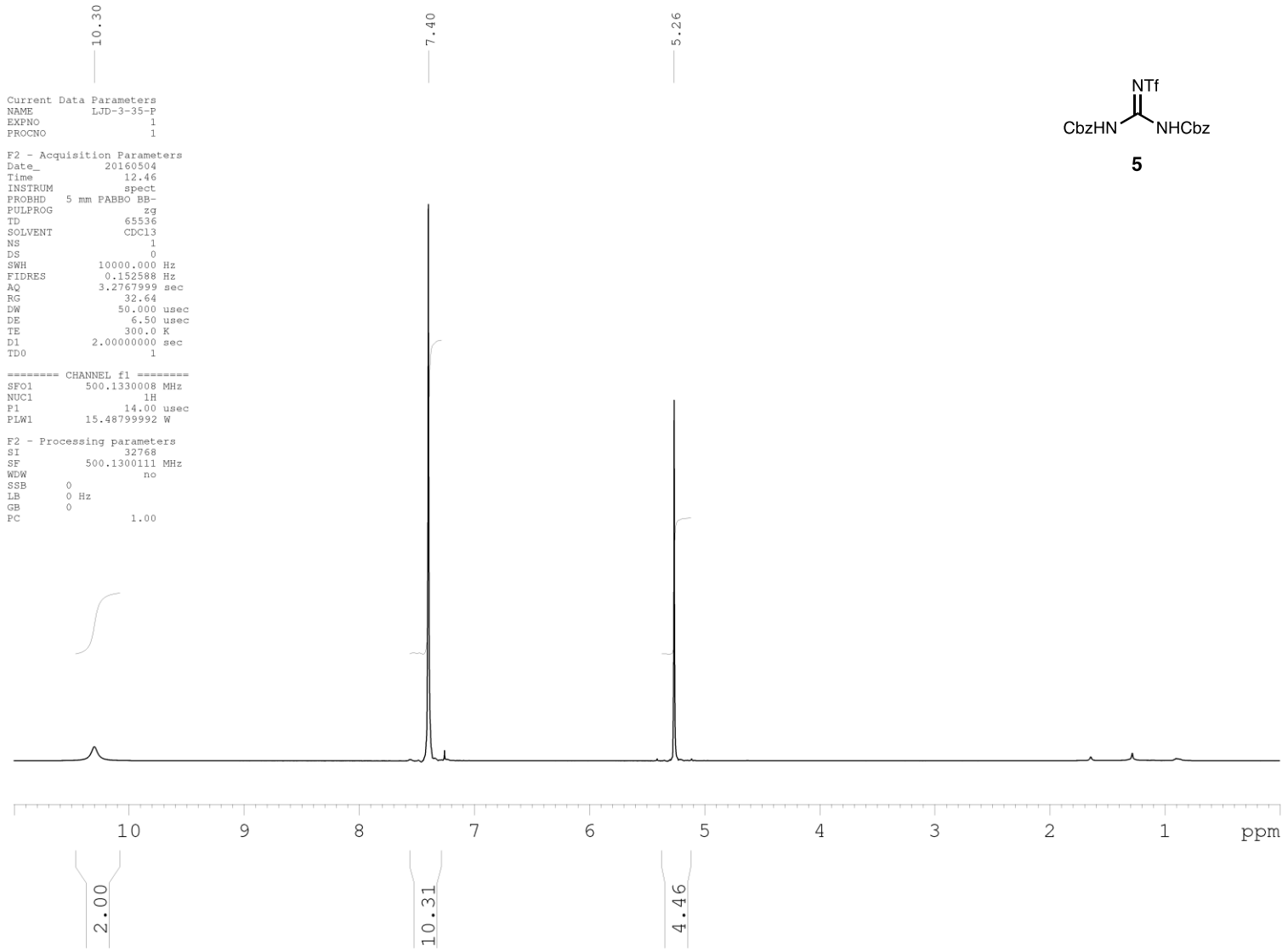
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SOLVENT CDCl3
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SSB 0
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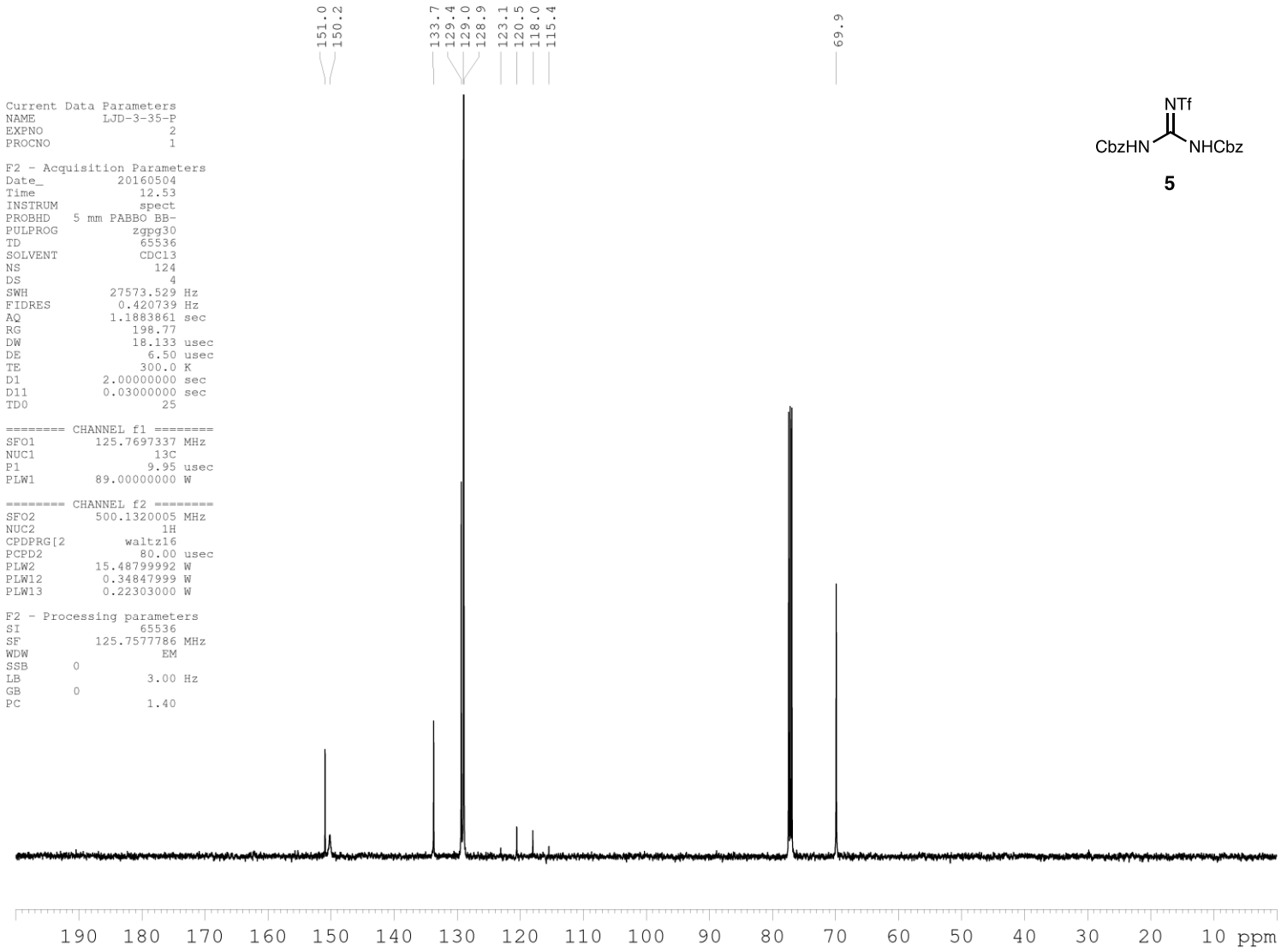
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EXPNO 2
PROCNO 1
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D1 2.00000000 sec
D11 0.03000000 sec
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NUC1 13C
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PLW1 89.00000000 W
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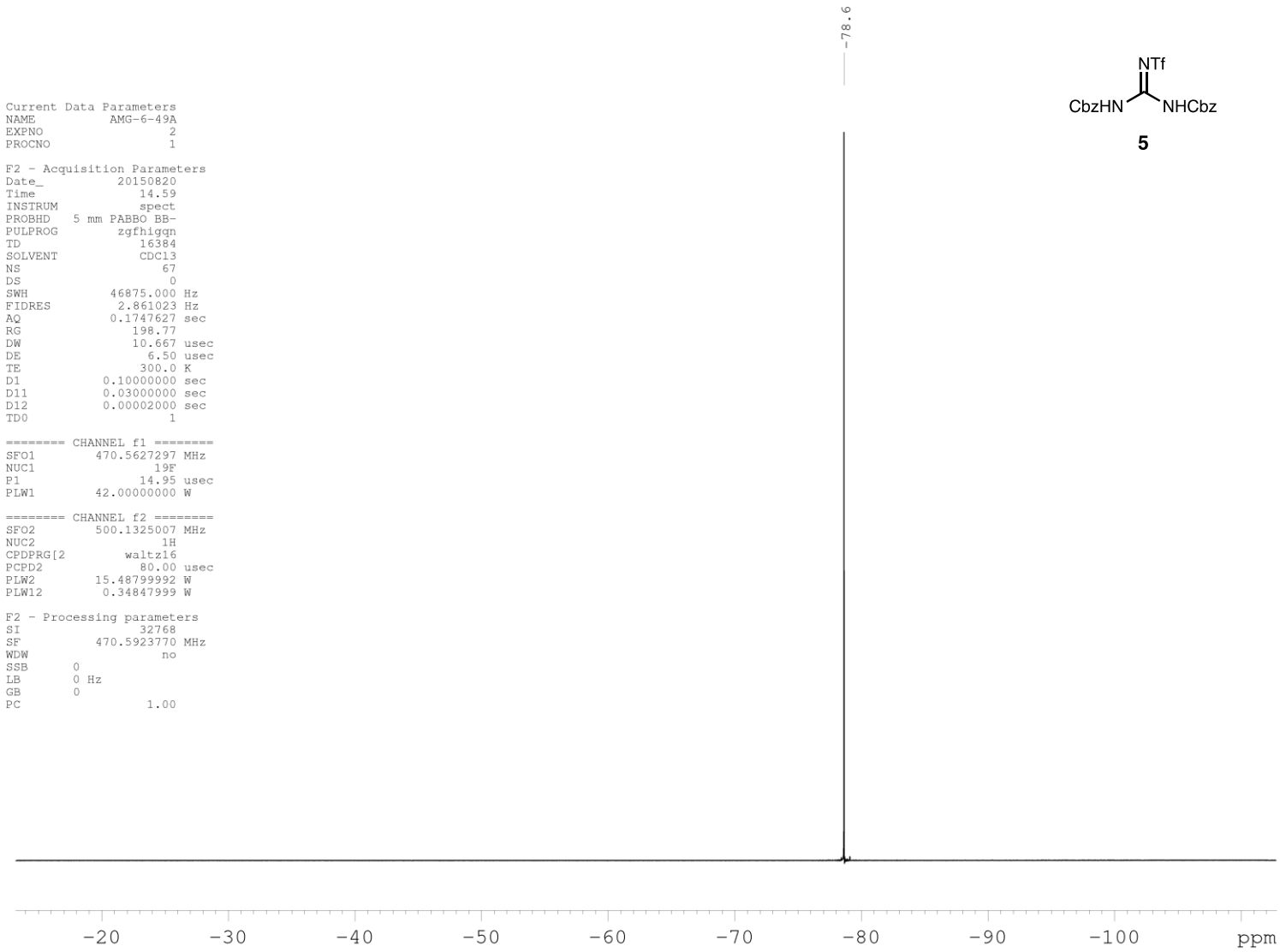


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TD                16384
SOLVENT           CDC13
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TE                300.0 K
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PCPD2             80.00 usec
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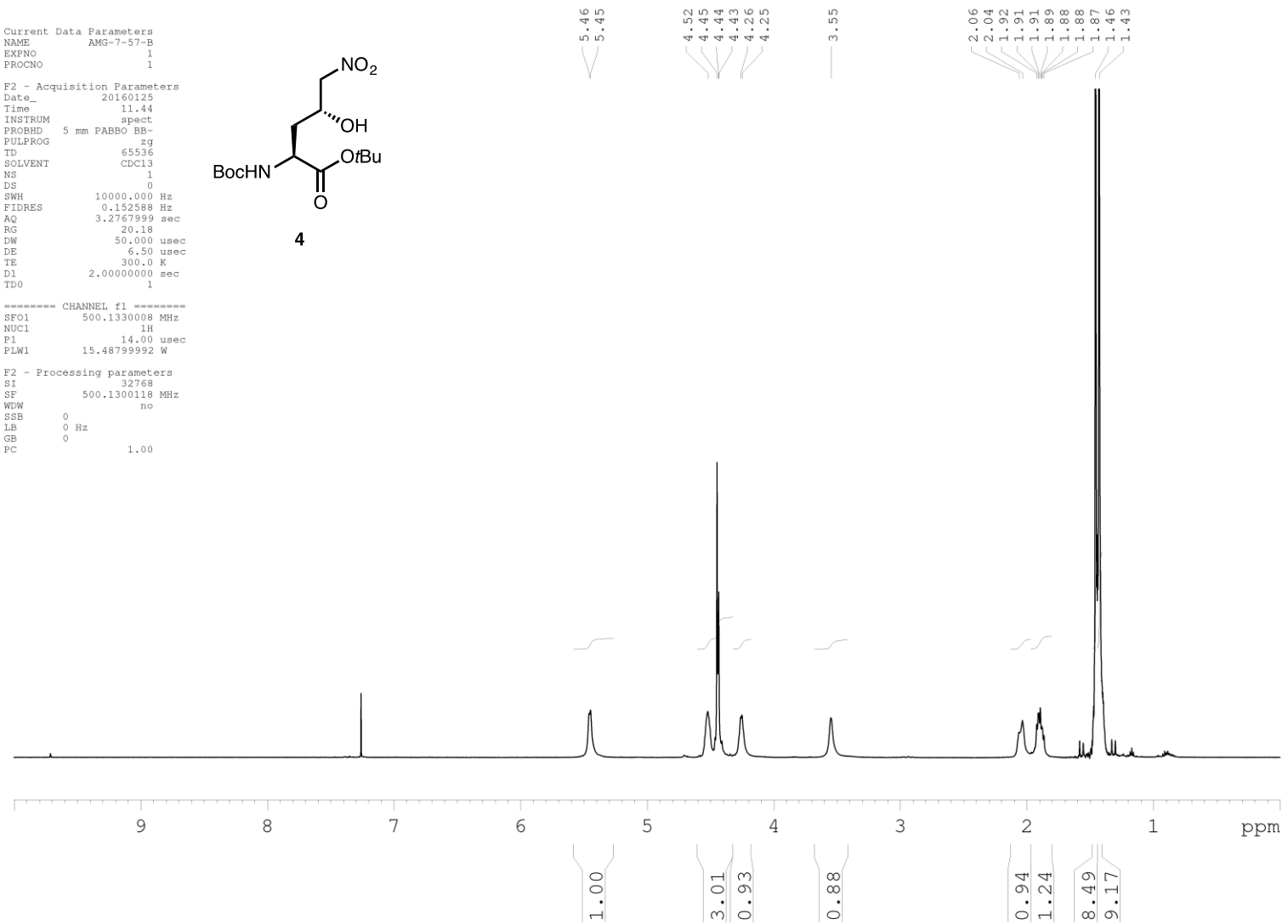
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SOLVENT CDCl3
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DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
TD0 1
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NUC1 1H
P1 14.00 usec
PLW1 15.48799992 W
F2 - Processing parameters
SI 32768
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WDW no
SSB 0
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NO2
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4.43
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4.25
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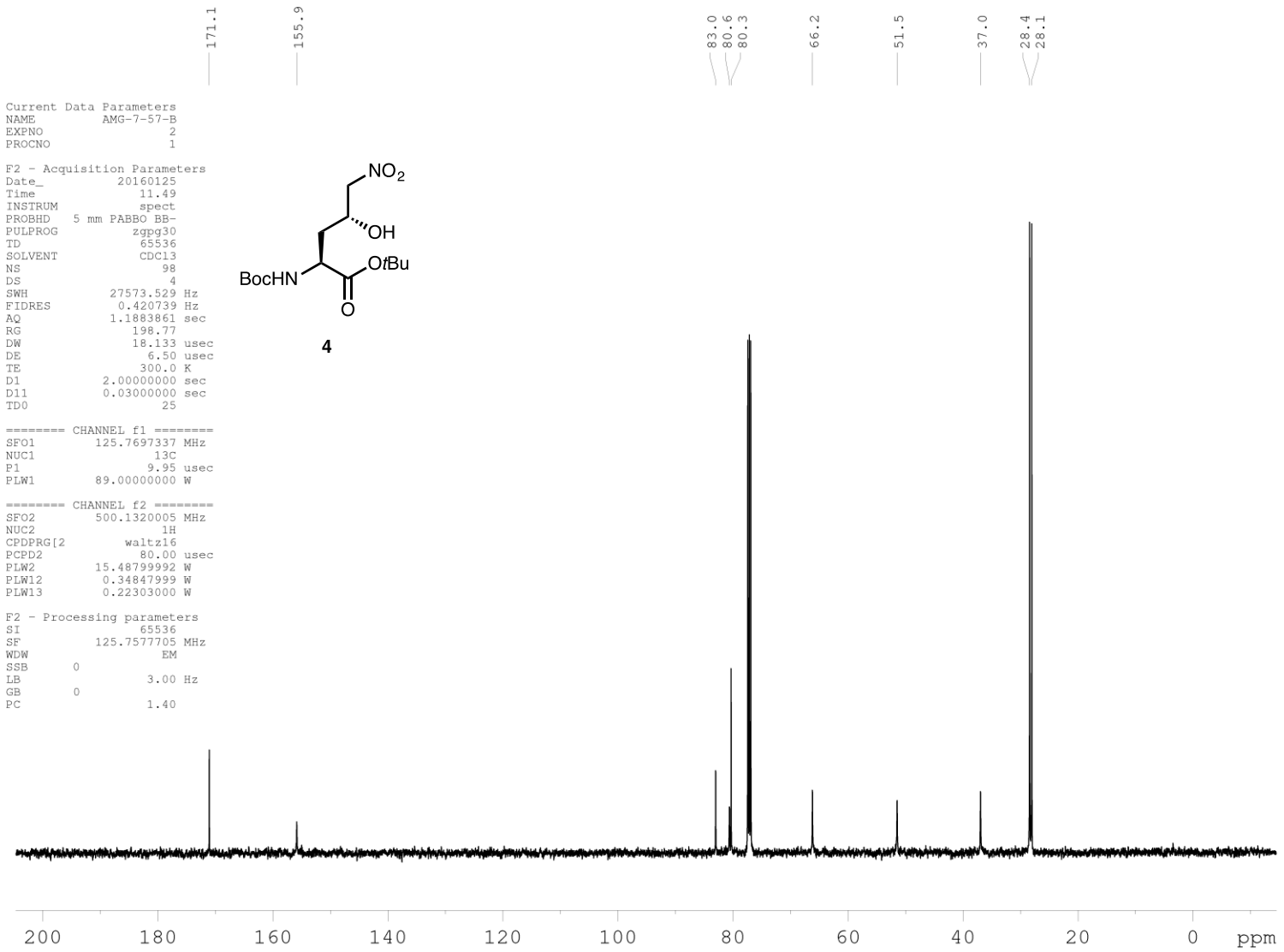
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28.1
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EXPNO 2
PROCNO 1
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Date_ 20160125
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SOLVENT CDC13
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FIDRES 0.420739 Hz
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RG 198.77
DW 18.133 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 25
======== CHANNEL f1 ========
SFO1 125.7697337 MHz
NUC1 13C
P1 9.95 usec
PLW1 89.00000000 W
======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.48799992 W
PLW12 0.34847999 W
PLW13 0.22303000 W
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WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40
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120
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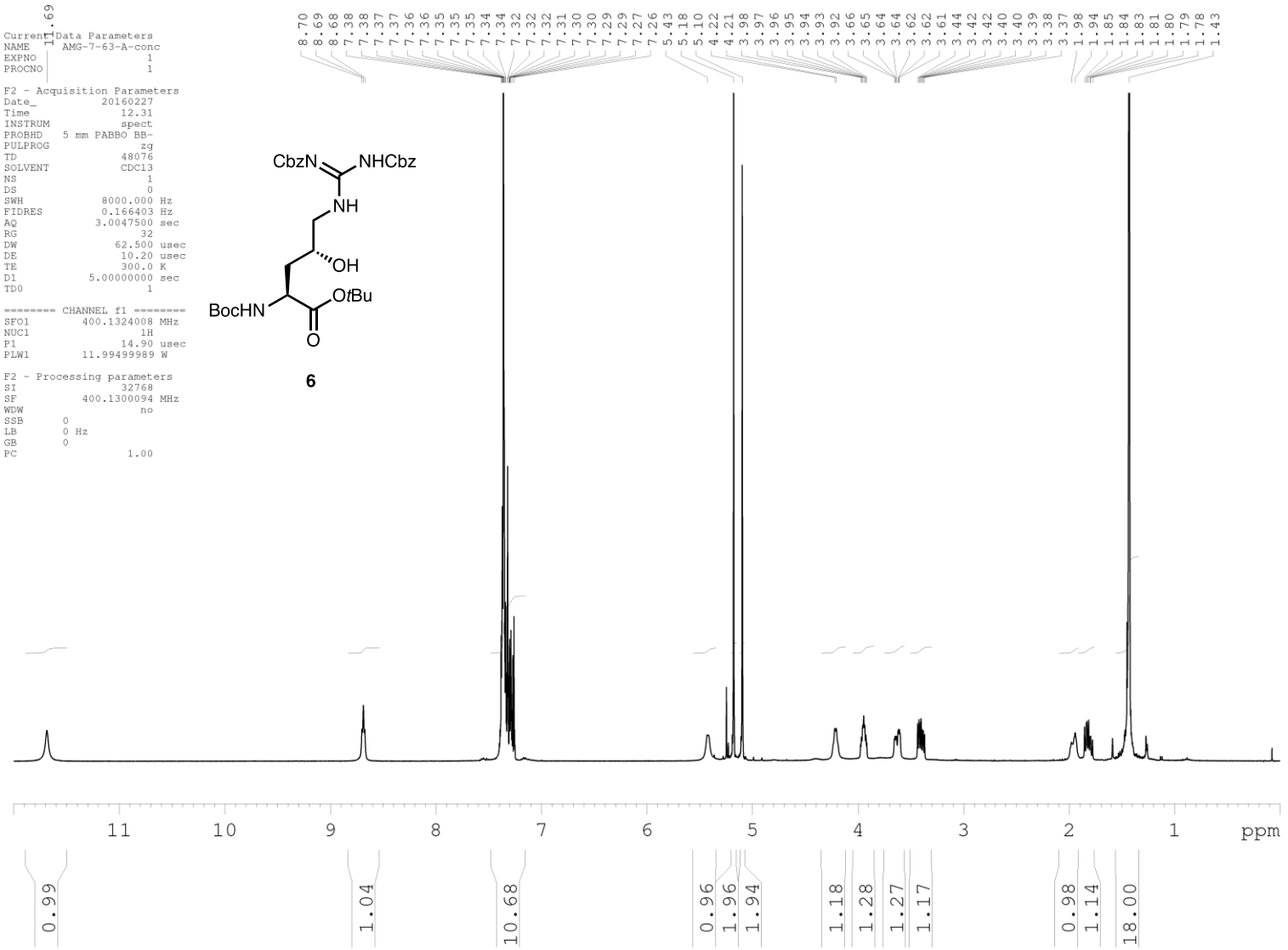
ppm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D1 5.00000000 sec
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PLW1 11.99499989 W
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WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
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NHCbz
NH
OH
OtBu
BocHN
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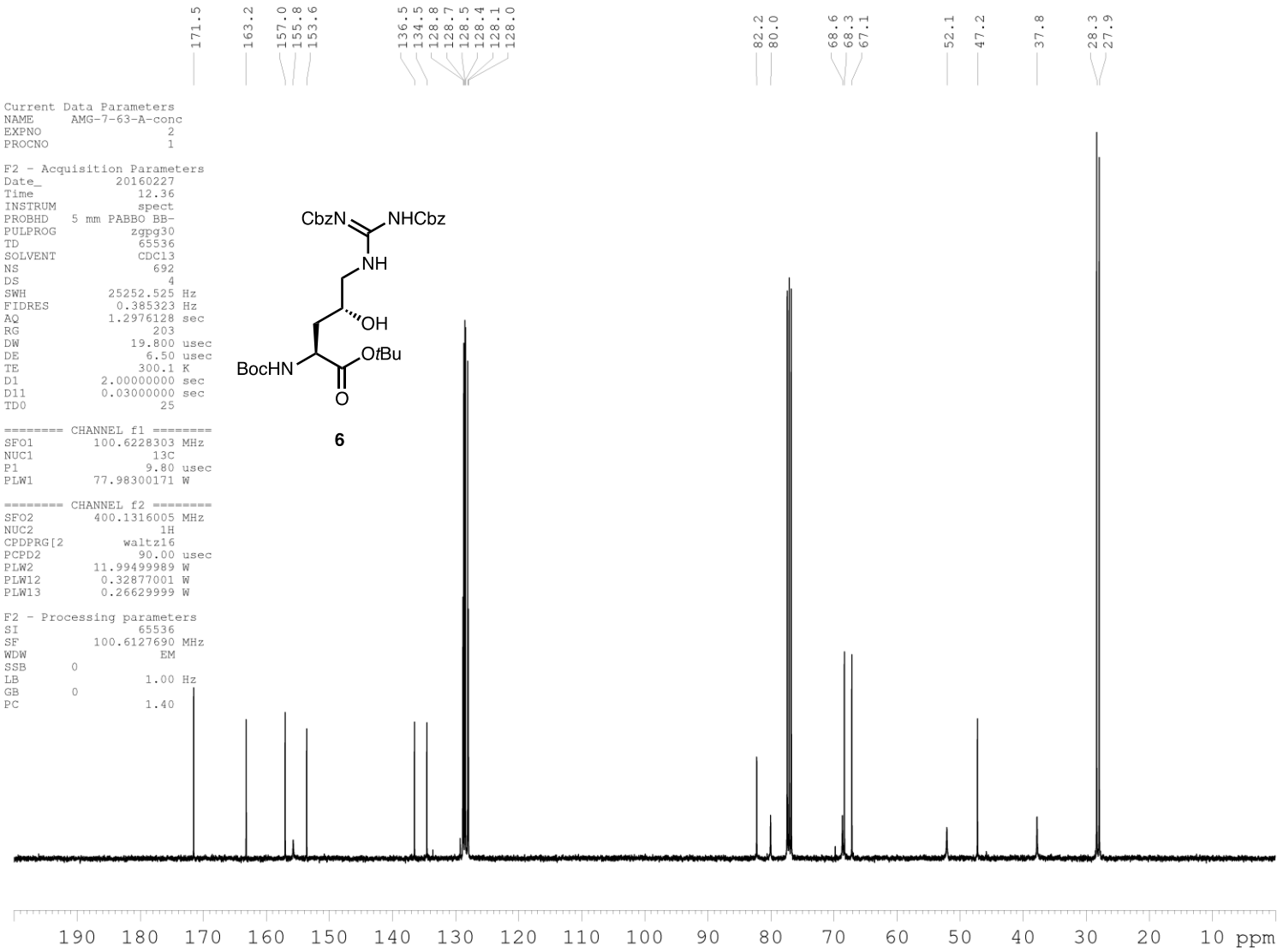
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128.0
82.2
80.0
68.6
68.3
67.1
52.1
47.2
37.8
28.3
27.9
Current Data Parameters
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EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
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FIDRES 0.385323 Hz
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DW 19.800 usec
DE 6.50 usec
TE 300.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 25
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SFO1 100.6228303 MHz
NUC1 13C
P1 9.80 usec
PLW1 77.98300171 W
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SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 11.99499989 W
PLW12 0.32877001 W
PLW13 0.26629999 W
F2 - Processing parameters
SI 65536
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
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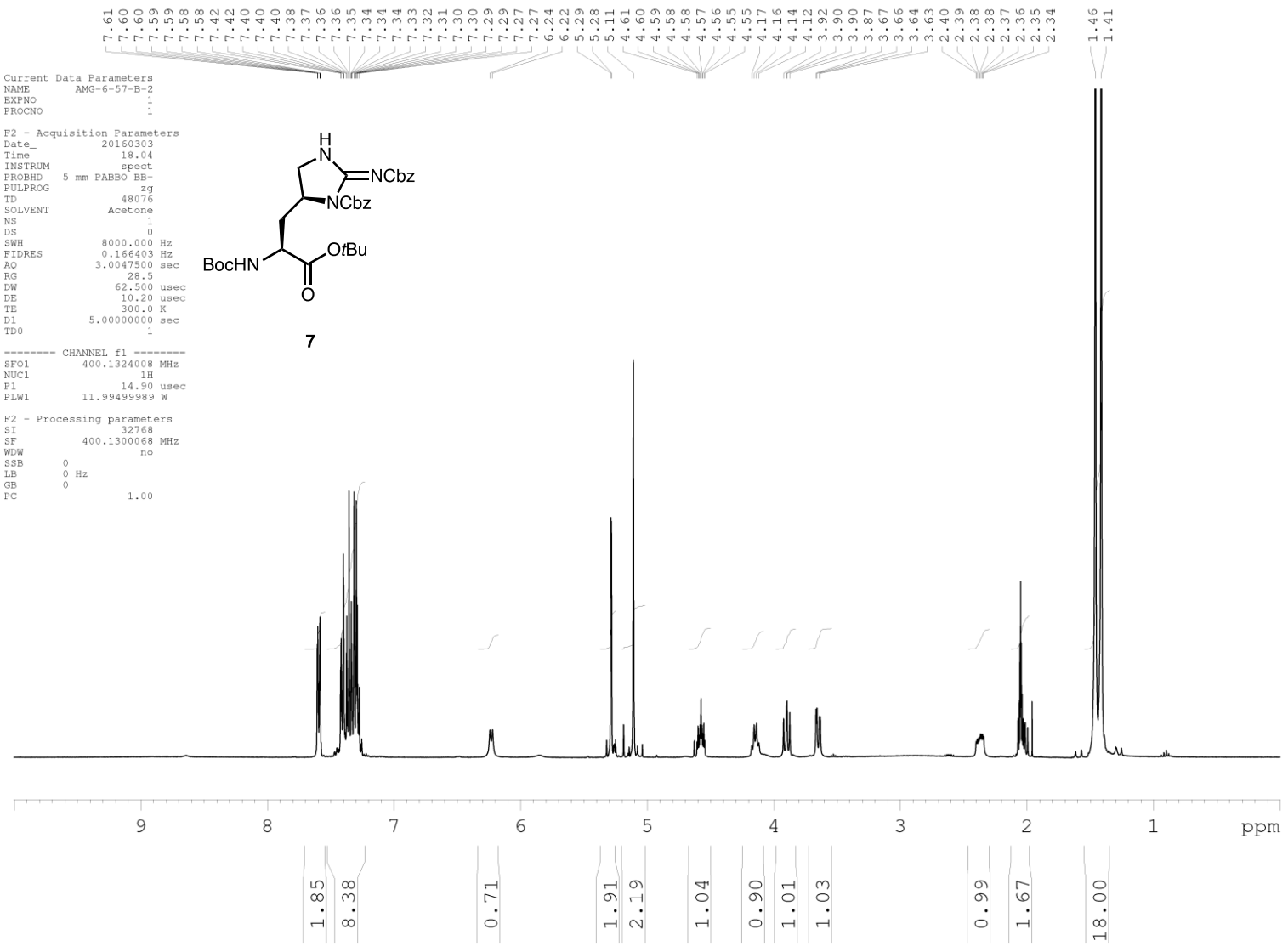
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EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160303
Time 18.04
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PULPROG zg
TD 48076
SOLVENT Acetone
NS 1
DS 0
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FIDRES 0.166403 Hz
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DW 62.500 usec
DE 10.20 usec
TE 300.0 K
D1 5.00000000 sec
TD0 1
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PLW1 11.99499989 W
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SSB 0
LB 0 Hz
GB 0
PC 1.00
H
N
NCbz
NCbz
BocHN
OtBu
O
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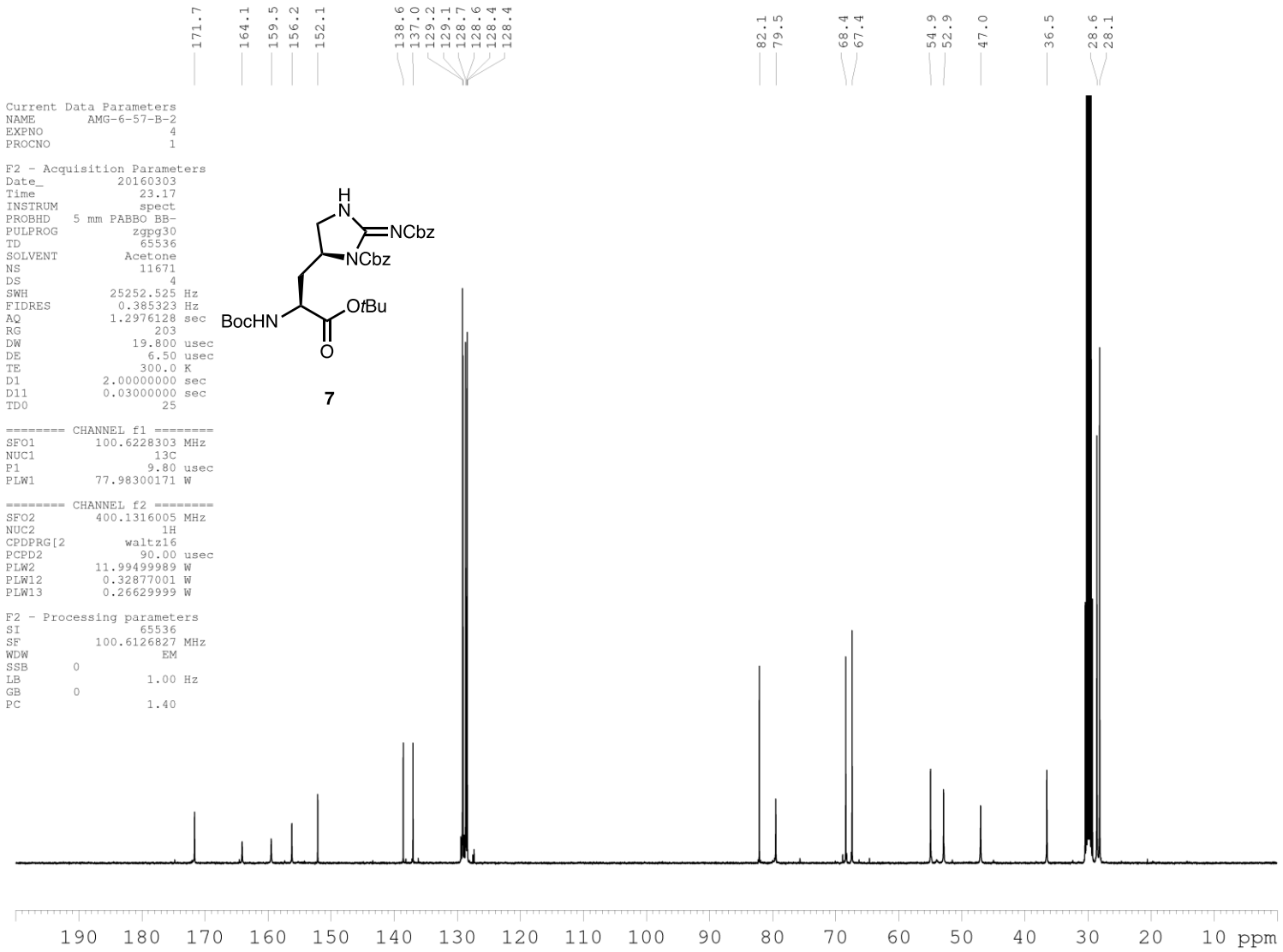
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128.6
128.4
128.4
82.1
79.5
68.4
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54.9
52.9
47.0
36.5
28.6
28.1
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PROCNO 1
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PULPROG zgpg30
TD 65536
SOLVENT Acetone
NS 11671
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 25
======== CHANNEL f1 ========
SFO1 100.6228303 MHz
NUC1 13C
P1 9.80 usec
PLW1 77.98300171 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 11.99499989 W
PLW12 0.32877001 W
PLW13 0.26629999 W
F2 - Processing parameters
SI 65536
SF 100.6126827 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
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NCbz
NCbz
BocHN
OtBu
O
7
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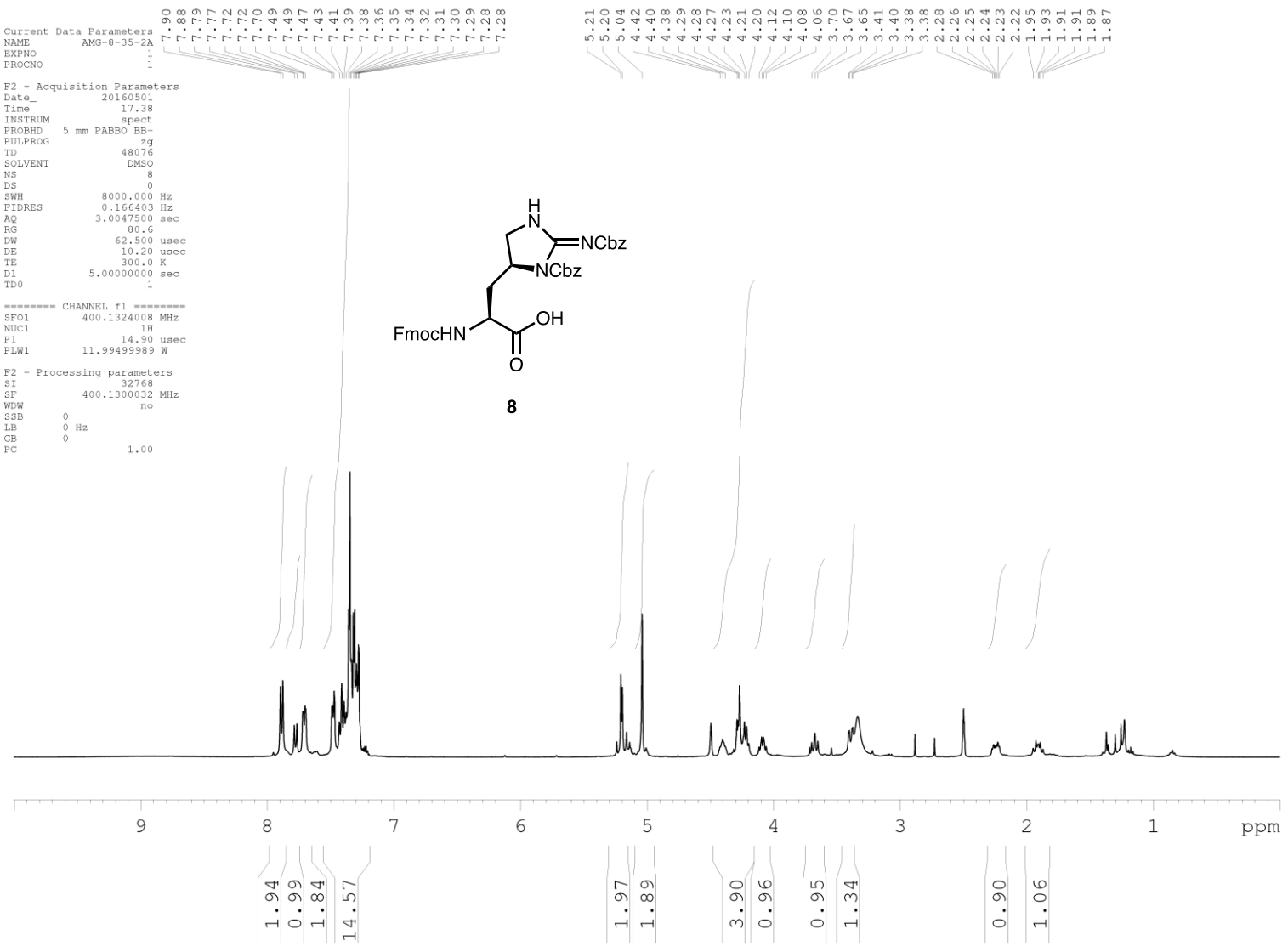
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PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 0
SWH 8000.000 Hz
FIDRES 0.166403 Hz
AQ 3.0047500 sec
RG 80.6
DW 62.500 usec
DE 10.20 usec
TE 300.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1324008 MHz
NUC1 1H
P1 14.90 usec
PLW1 11.99499989 W
F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
7.90 7.88 7.79 7.77 7.72 7.72 7.70 7.49 7.49 7.47 7.43 7.41 7.39 7.38 7.36 7.35 7.34 7.32 7.31 7.30 7.29 7.28 7.28
5.21 5.20 5.04 4.42 4.40 4.38 4.29 4.28 4.27 4.23 4.21 4.20 4.12 4.10 4.08 4.06 3.70 3.67 3.65 3.41 3.40 3.38 3.38 2.28 2.26 2.25 2.24 2.23 2.22 1.95 1.93 1.91 1.91 1.89 1.87
H
N
NCbz
NCbz
FmocHN
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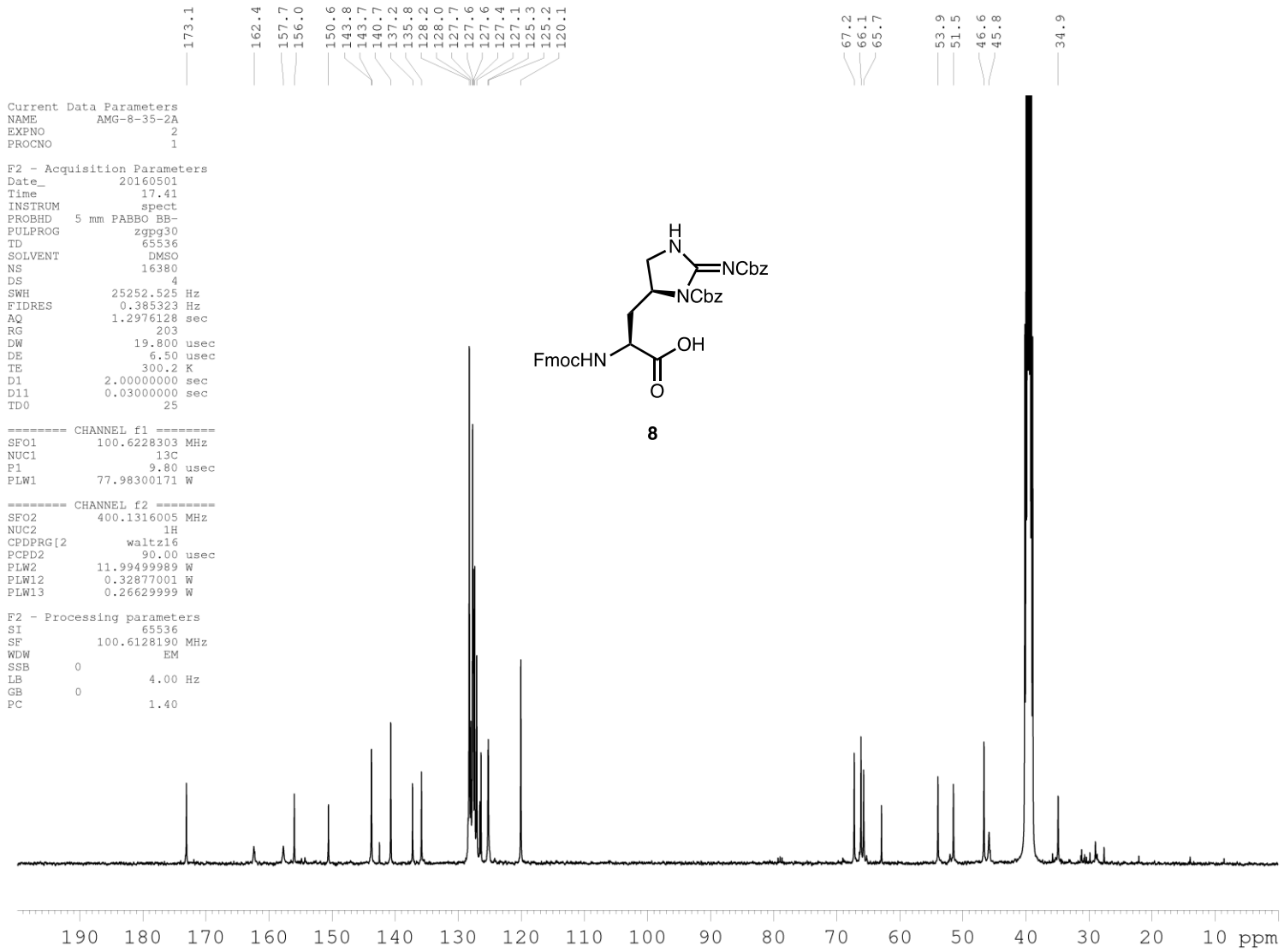
173.1
162.4
157.7
156.0
150.6
143.8
143.7
140.7
137.2
135.8
128.2
128.0
127.7
127.6
127.6
127.4
127.1
125.3
125.2
120.1
67.2
66.1
65.7
53.9
51.5
46.6
45.8
34.9
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EXPNO 2
PROCNO 1
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Date_ 20160501
Time 17.41
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TD 65536
SOLVENT DMSO
NS 16380
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 6.50 usec
TE 300.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 25
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NUC1 13C
P1 9.80 usec
PLW1 77.98300171 W
======== CHANNEL f2 ========
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NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 11.99499989 W
PLW12 0.32877001 W
PLW13 0.26629999 W
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WDW EM
SSB 0
LB 4.00 Hz
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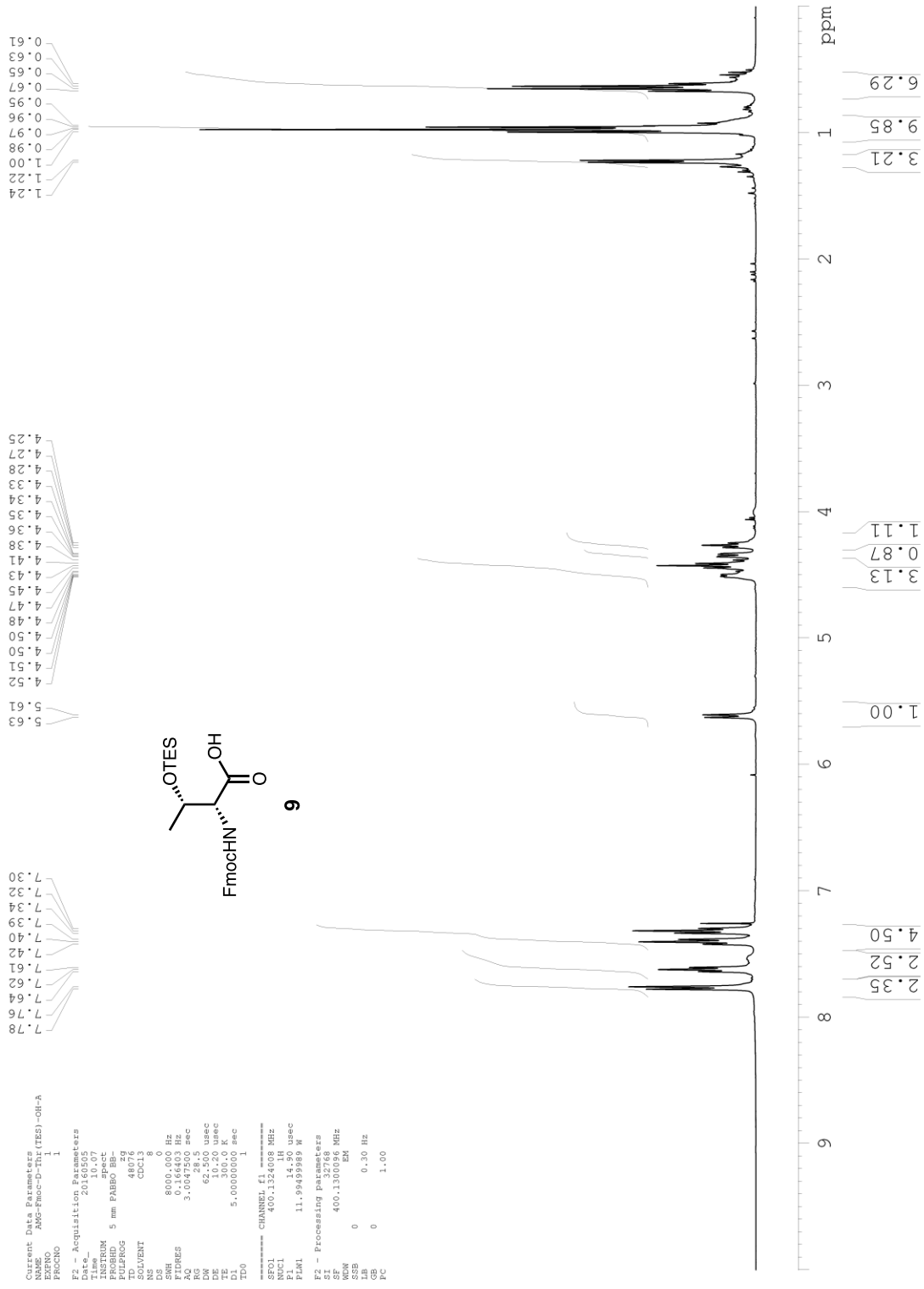
H
N
NCbz
NCbz
OH
FmocHN
O
8
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


OTES
OH
O
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1.11
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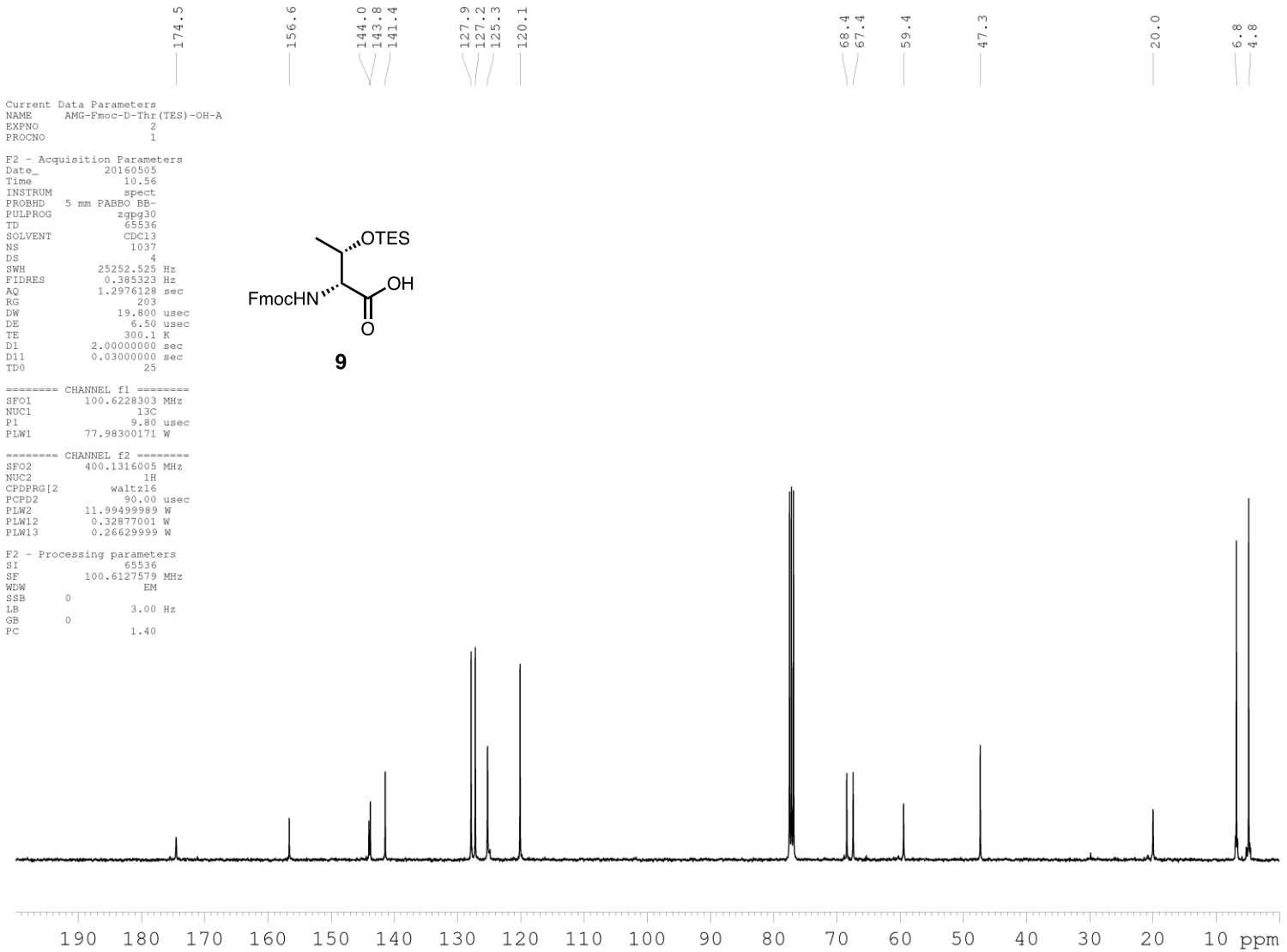
6.29
9.85
3.21


174.5
156.6
144.0
143.8
141.4
127.9
127.2
125.3
120.1
68.4
67.4
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47.3
20.0
6.8
4.8
Current Data Parameters
NAME AMG-Fmoc-D-Thr(TES)-OH-A
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160505
Time 10.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1037
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
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DW 19.800 usec
DE 6.50 usec
TE 300.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 25
======== CHANNEL f1 ========
SFO1 100.6228303 MHz
NUC1 13C
P1 9.80 usec
PLW1 77.98300171 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 11.99499989 W
PLW12 0.32877001 W
PLW13 0.26629999 W
F2 - Processing parameters
SI 65536
SF 100.6127579 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40
OTES
OH
FmocHN
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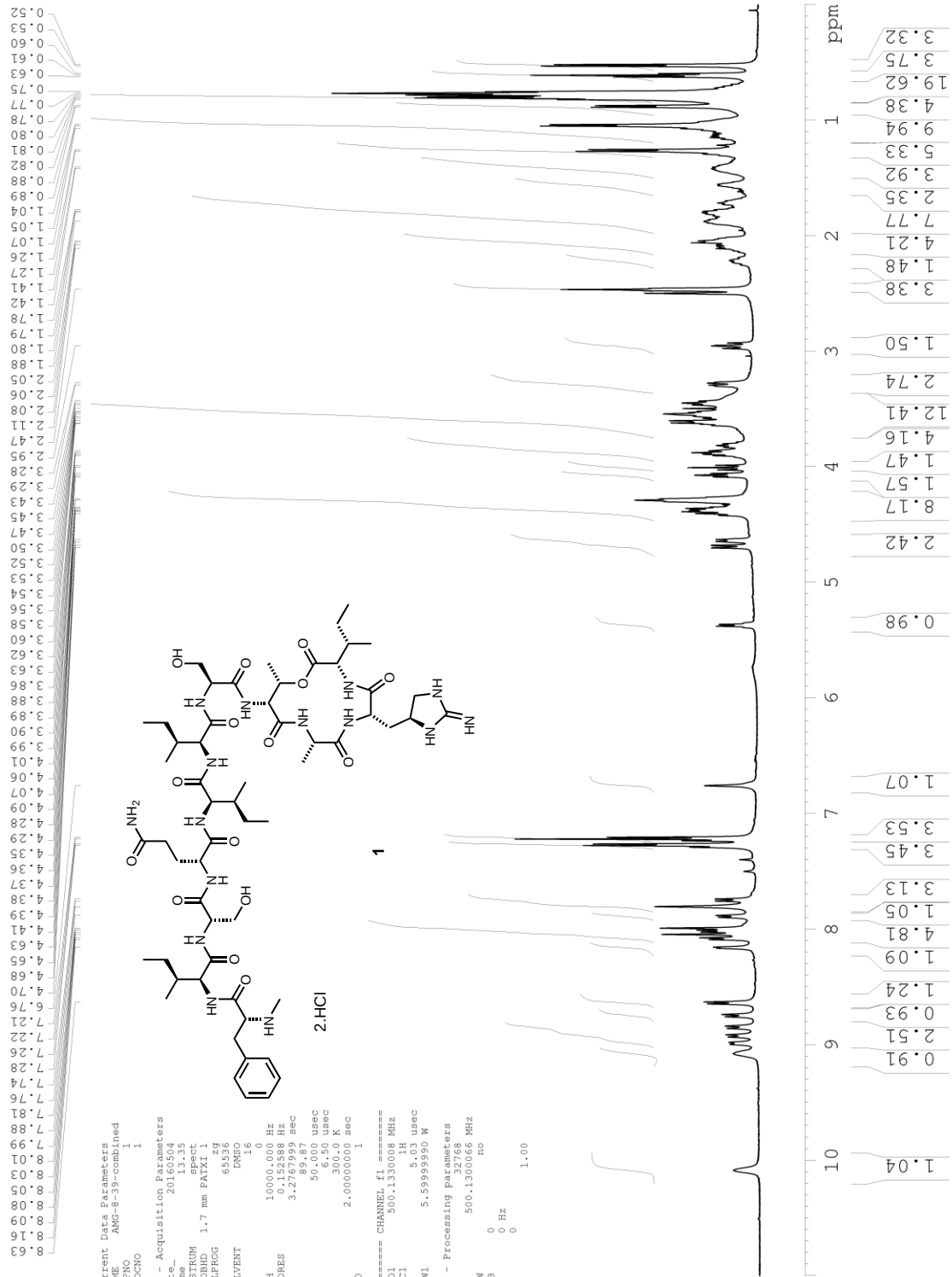
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EXPNO 1
PROCNO 1
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Date_ 20160504
Time 13.35
INSTRUM spect
PROBHD 1.7 mm PATXI 1
PULPROG zg
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 89.87
DW 50.000 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.1330008 MHz
NUC1 1H
P1 5.00 usec
PLW1 5.59999990 W
F2 - Processing parameters
SI 32768
SF 500.1300066 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
2.HCl
1
ppm
1.04
0.91
2.51
0.93
1.24
1.09
4.81
1.05
3.13
3.45
3.53
1.07
0.98
2.42
8.17
1.57
1.47
4.16
12.41
2.74
1.50
3.38
1.48
4.21
7.77
2.35
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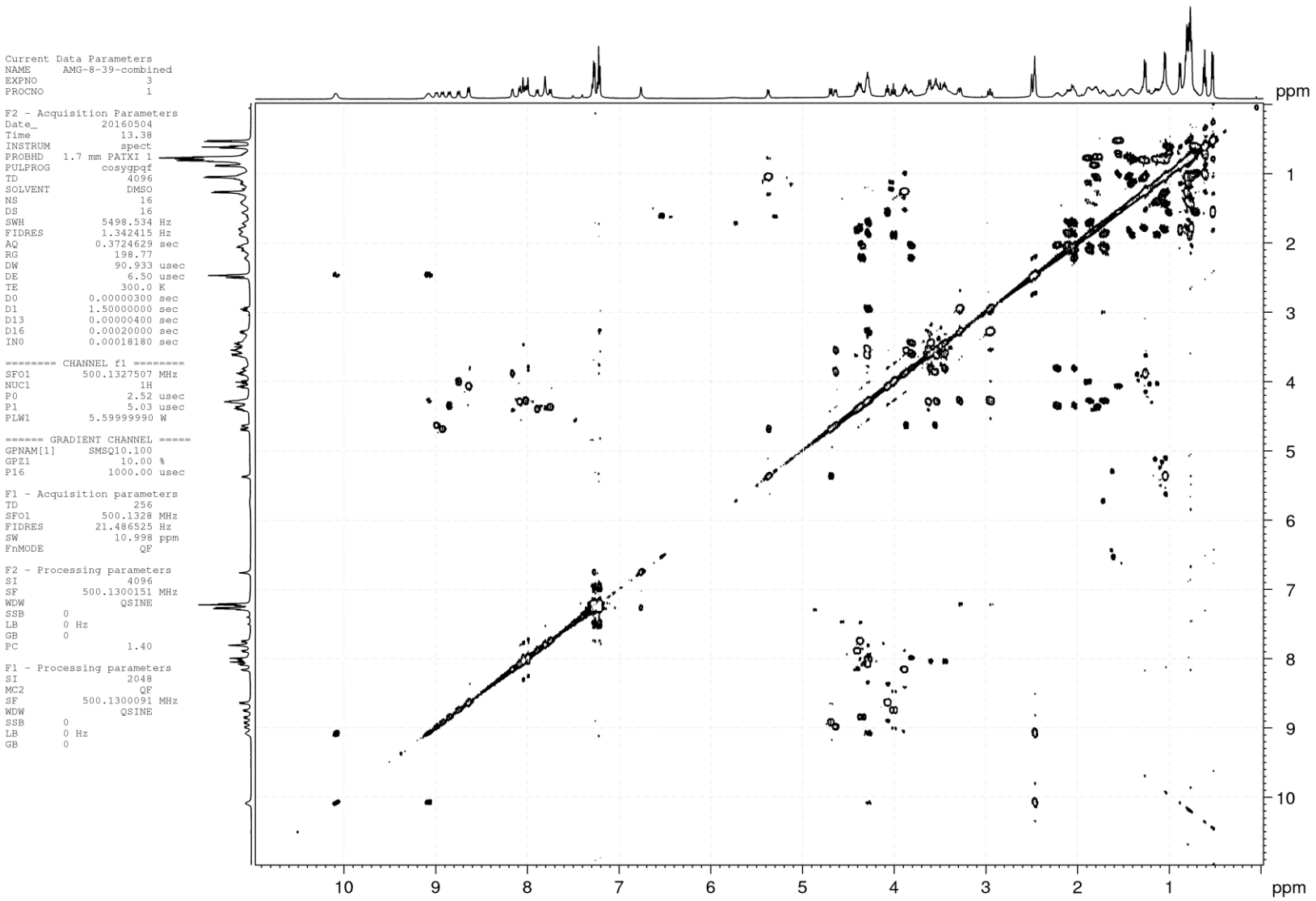
9.94
4.38
19.62
3.75
3.32

COSY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PROCNO 1
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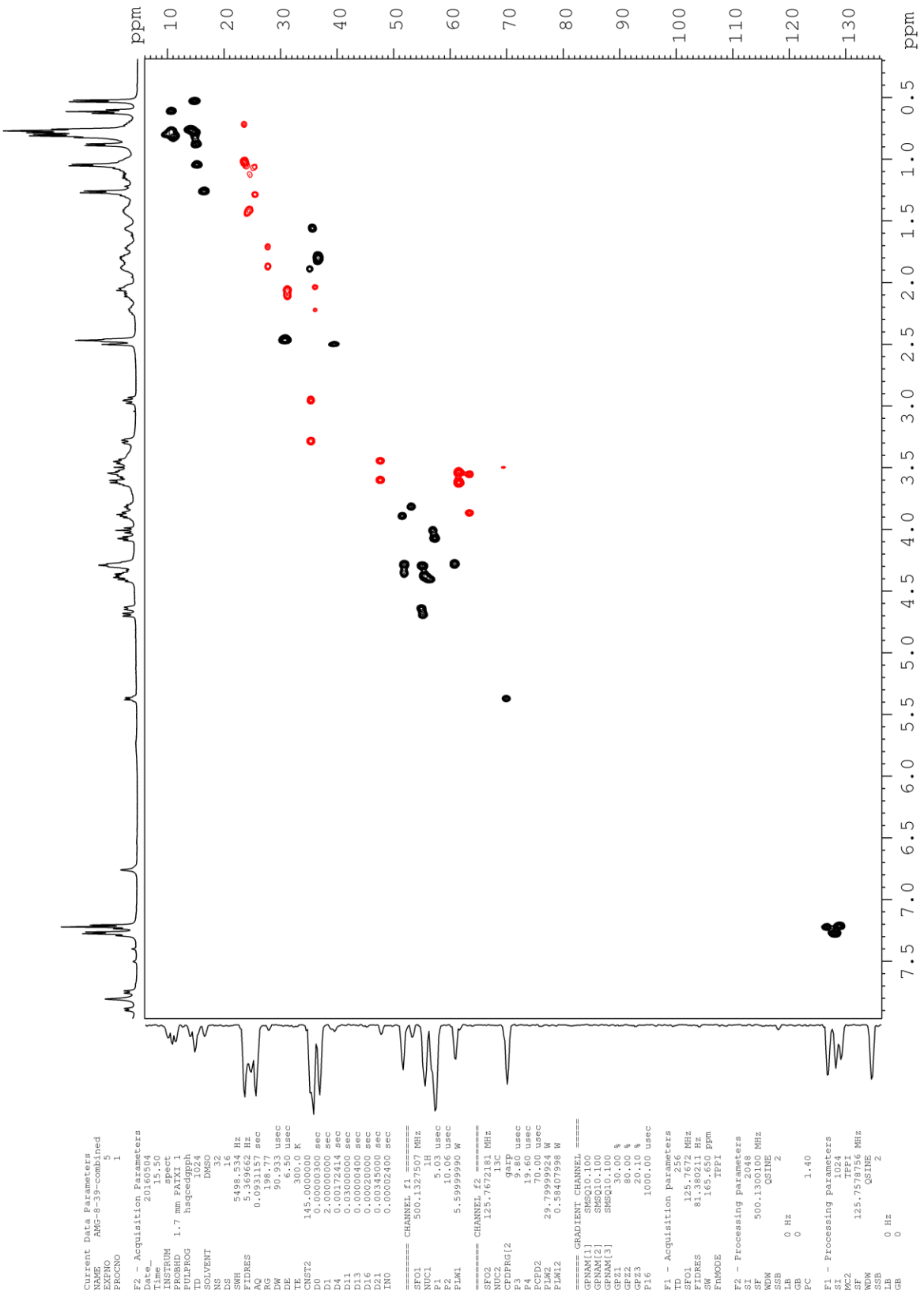
Date_ 20160504
Time 13.38
INSTRUM spect
PROBHD 1.7 mm PATXI 1
PULPROG cosygpqf
TD 4096
SOLVENT DMSO
NS 16
DS 16
SWH 5498.534 Hz
FIDRES 1.342415 Hz
AQ 0.3724629 sec
RG 198.77
DW 90.933 usec
DE 6.50 usec
TE 300.0 K
D0 0.00000300 sec
D1 1.50000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00018180 sec
======== CHANNEL f1 ========
SFO1 500.1327507 MHz
NUC1 1H
P0 2.52 usec
P1 5.03 usec
PLW1 5.59999990 W
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPZ1 10.00 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 256
SFO1 500.1328 MHz
FIDRES 21.486525 Hz
SW 10.998 ppm
FnMODE QF
F2 - Processing parameters
SI 4096
SF 500.1300151 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 2048
MC2 QF
SF 500.1300091 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
ppm
1
2
3
4
5
6
7
8
9
10
ppm
10
9
8
7
6
5
4
3
2
1

HSQC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EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
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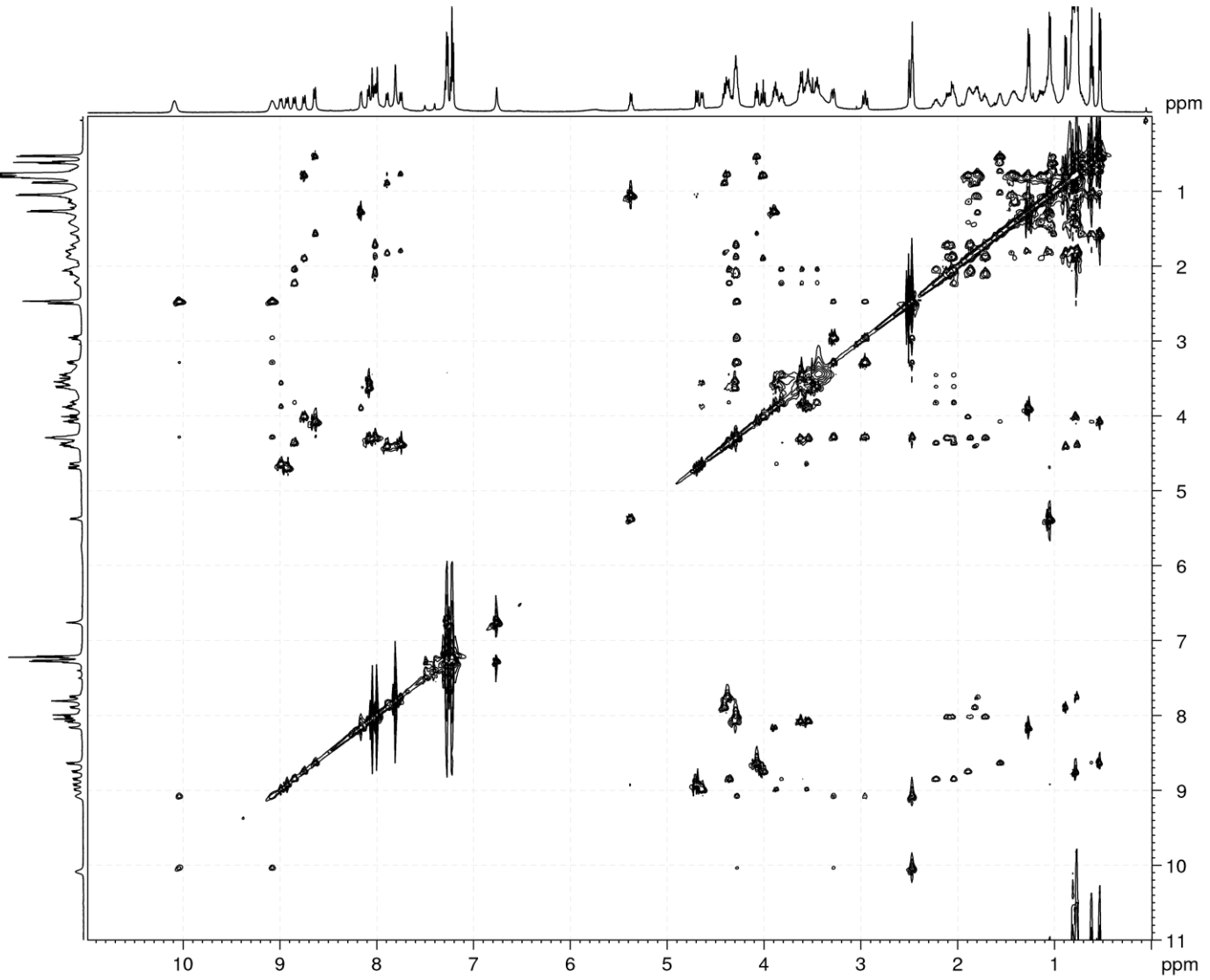
Time 15.50
INSTRUM spect
PROBHD 1.7 mm PAXYI 1
PULPROG hsqcedqpph
TD 1024
SOLVENT DMSO
NS 32
DS 16
SWH 5498.534 Hz
FIDRES 5.369662 Hz
AQ 0.0931157 sec
RG 196.77
DW 90.933 usec
DE 6.50 usec
TE 300.0 K
CNST2 145.0000000
D0 0.00000300 sec
D1 2.00000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
D21 0.00345100 sec
IN0 0.00002400 sec
======== CHANNEL f1 ========
SFO1 500.1327507 MHz
NUC1 1H
P1 5.03 usec
P2 10.06 usec
PLW1 5.59999990 W
======== CHANNEL f2 ========
SFO2 125.7672181 MHz
NUC2 13C
CPDPRG[2 garp
P3 9.80 usec
P4 19.60 usec
PCPD2 70.00 usec
PLW2 29.79999924 W
PLW12 0.58407998 W
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 30.00 %
GPZ2 80.00 %
GPZ3 20.10 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 256
SFO1 125.7672 MHz
FIDRES 81.437071 Hz
SW 165.650 ppm
FnMODE TPPI
F2 - Processing parameters
SI 2048
SF 500.1300100 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 1024
MC2 TPPI
SF 125.7578756 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
ppm
ppm

TOCSY

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Current Data Parameters
NAME      AMG-8-39-combined
EXPNO                    9
PROCNO                   1

F2 - Acquisition Parameters
Date_              20160505
Time                 10.05
INSTRUM              spect
PROBHD   1.7 mm PATXI 1
PULPROG    dipsi2etgpsi
TD                   4096
SOLVENT              DMSO
NS                     32
DS                     16
SWH             5498.534 Hz
FIDRES          1.342415 Hz
AQ              0.3724629 sec
RG                 178.57
DW                  90.933 usec
DE                    6.50 usec
TE                   300.0 K
D0             0.00000300 sec
D1             2.04529405 sec
D9             0.06000000 sec
D11            0.03000000 sec
D16            0.00020000 sec
D20            0.00001000 sec
D21            0.00001000 sec
IN0            0.00018180 sec
L1                     20

======== CHANNEL f1 ========
SFO1          500.1327507 MHz
NUC1                  1H
P1                   5.03 usec
P2                  10.06 usec
P6                  26.00 usec
PLW1          5.59999990 W
PLW10         0.19890000 W

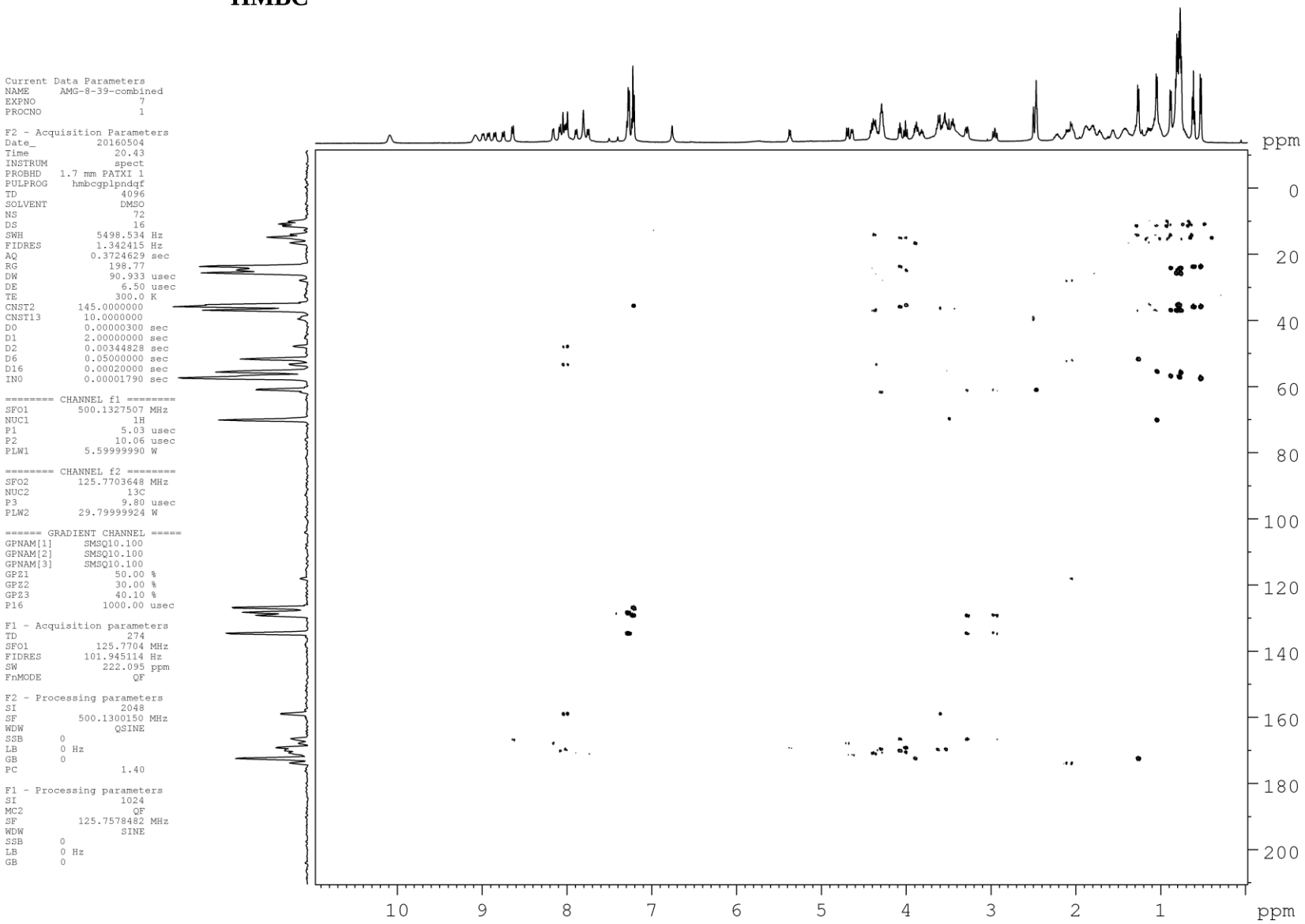
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPZ1                30.00 %
GPZ2                30.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1            500.1328 MHz
FIDRES         21.486525 Hz
SW                10.998 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                   1024
SF            500.1300057 MHz
WDW                  QSINE
SSB                      2
LB        0 Hz
GB        0
PC                    1.40

F1 - Processing parameters
SI                   1024
MC2         echo-antiecho
SF            500.1299991 MHz
WDW                  QSINE
SSB                      2
LB        0 Hz
GB        0
```

HMBC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EXPNO 7
PROCNO 1
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PULPROG hmbcgplpndqf
TD 4096
SOLVENT DMSO
NS 72
DS 16
SWH 5498.534 Hz
FIDRES 1.342415 Hz
AQ 0.3724629 sec
RG 198.77
DW 90.933 usec
DE 6.50 usec
TE 300.0 K
CNST2 145.0000000
CNST13 10.0000000
D0 0.00000300 sec
D1 2.00000000 sec
D2 0.00344828 sec
D6 0.05000000 sec
D16 0.00020000 sec
IN0 0.00001790 sec
======== CHANNEL f1 ========
SFO1 500.1327507 MHz
NUC1 1H
P1 5.03 usec
P2 10.06 usec
PLW1 5.59999990 W
======== CHANNEL f2 ========
SFO2 125.7703648 MHz
NUC2 13C
P3 9.80 usec
PLW2 29.79999924 W
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 274
SFO1 125.7704 MHz
FIDRES 101.945114 Hz
SW 222.095 ppm
FnMODE QF
F2 - Processing parameters
SI 2048
SF 500.1300150 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 1024
MC2 QF
SF 125.7578482 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
ppm
0
20
40
60
80
100
120
140
160
180
200
10
9
8
7
6
5
4
3
2
1
ppm

High Resolution Mass Spectra (+ESI)

Generic Display Report

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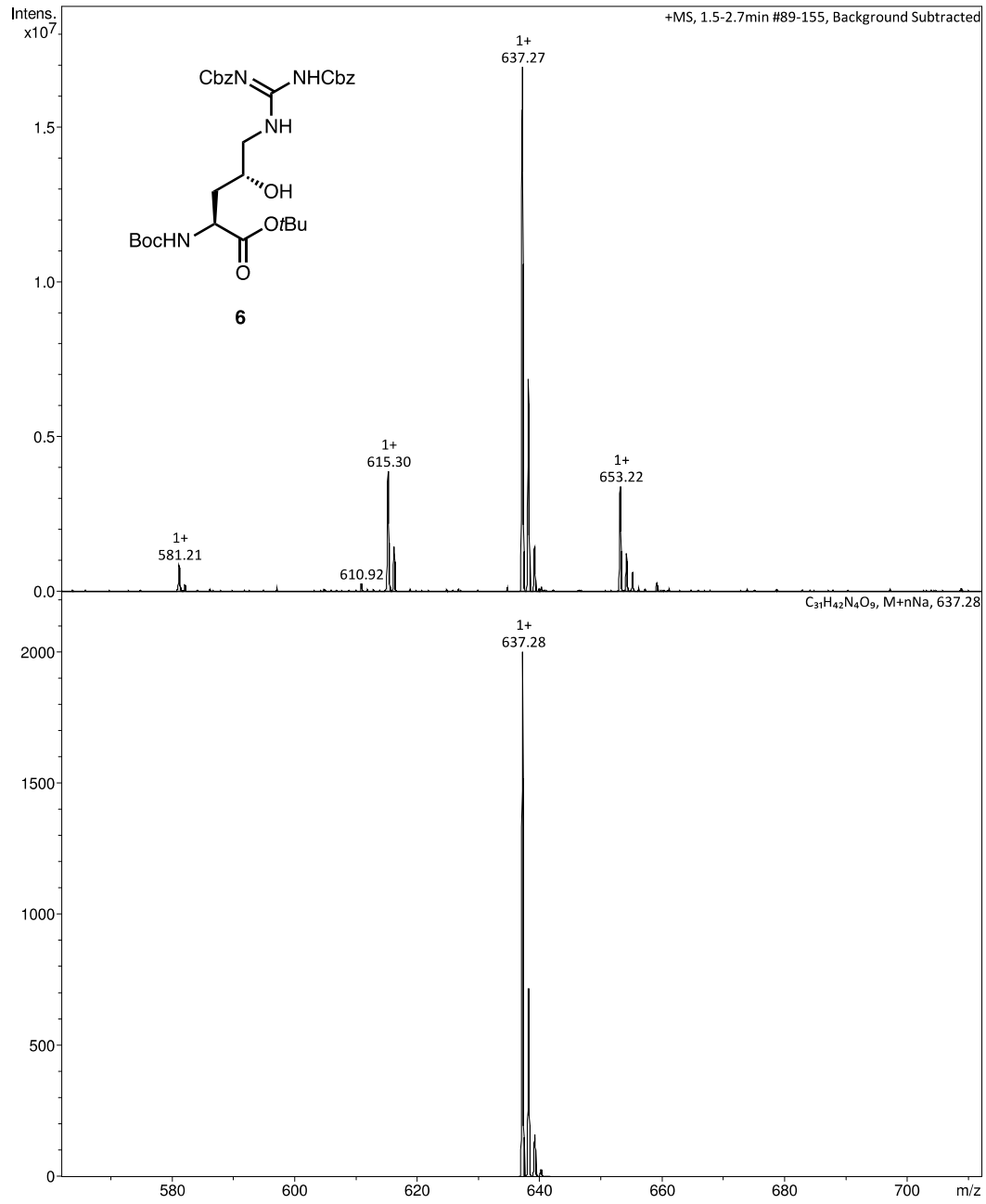
Sample Name	LJD-2-76	Instrument	amaZon SL
Comment	MeOH		



Generic Display Report

Analysis Info

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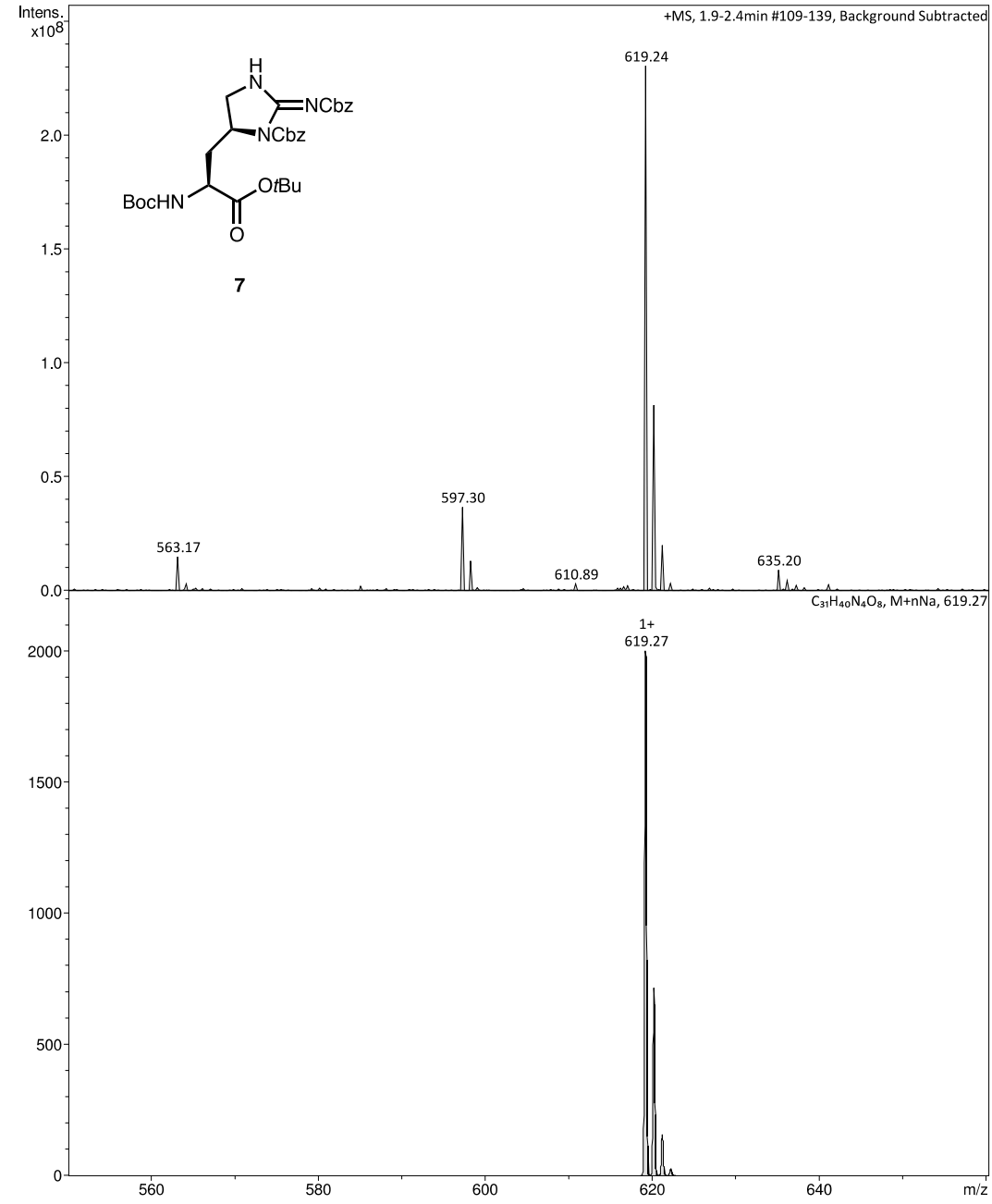
Comment	MeOH		



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Generic Display Report

Analysis Info

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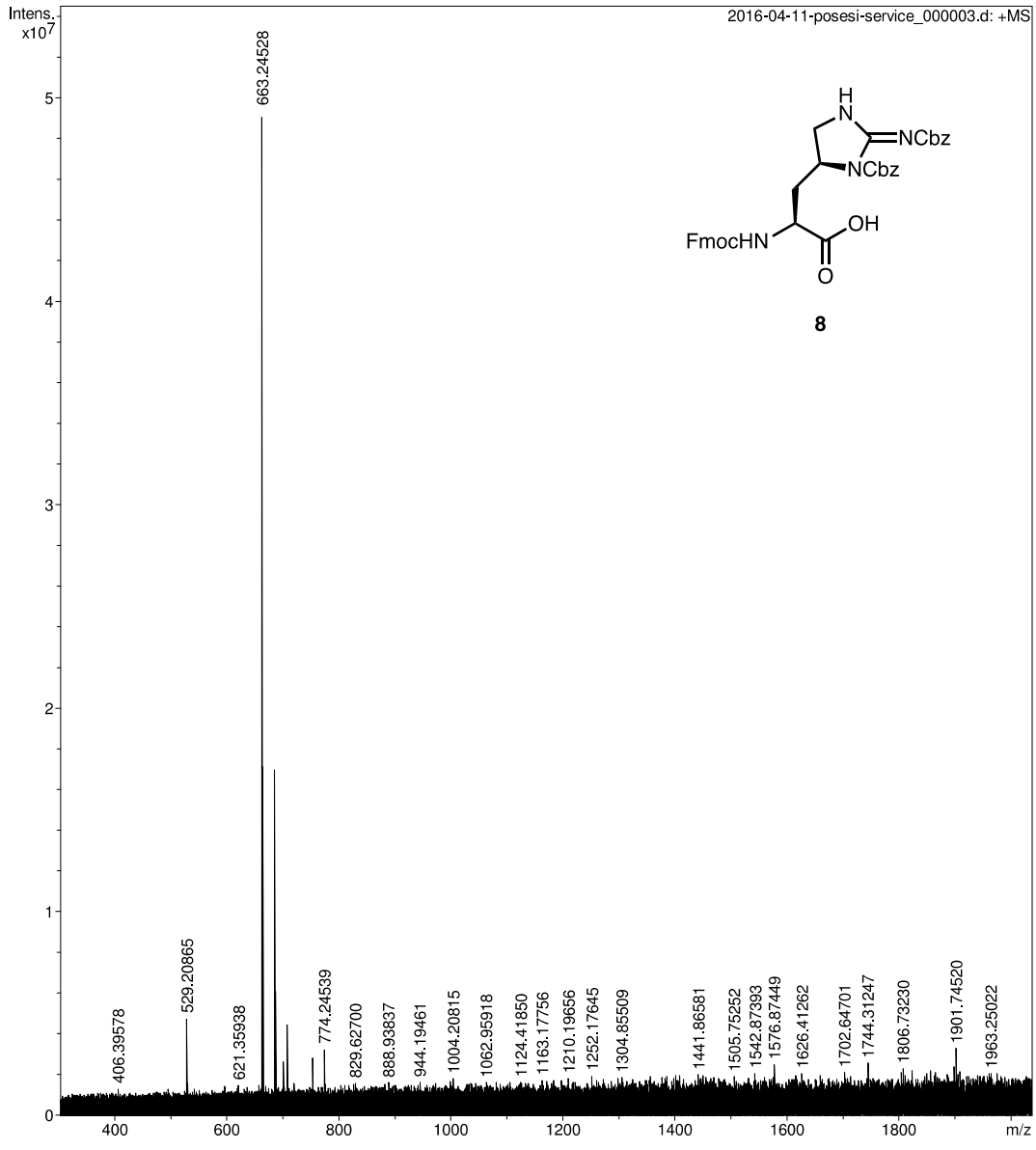
Comment	MeOH 1M TOF delay 0.0007s, Q1 300 m/z		



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Generic Display Report

Analysis Info

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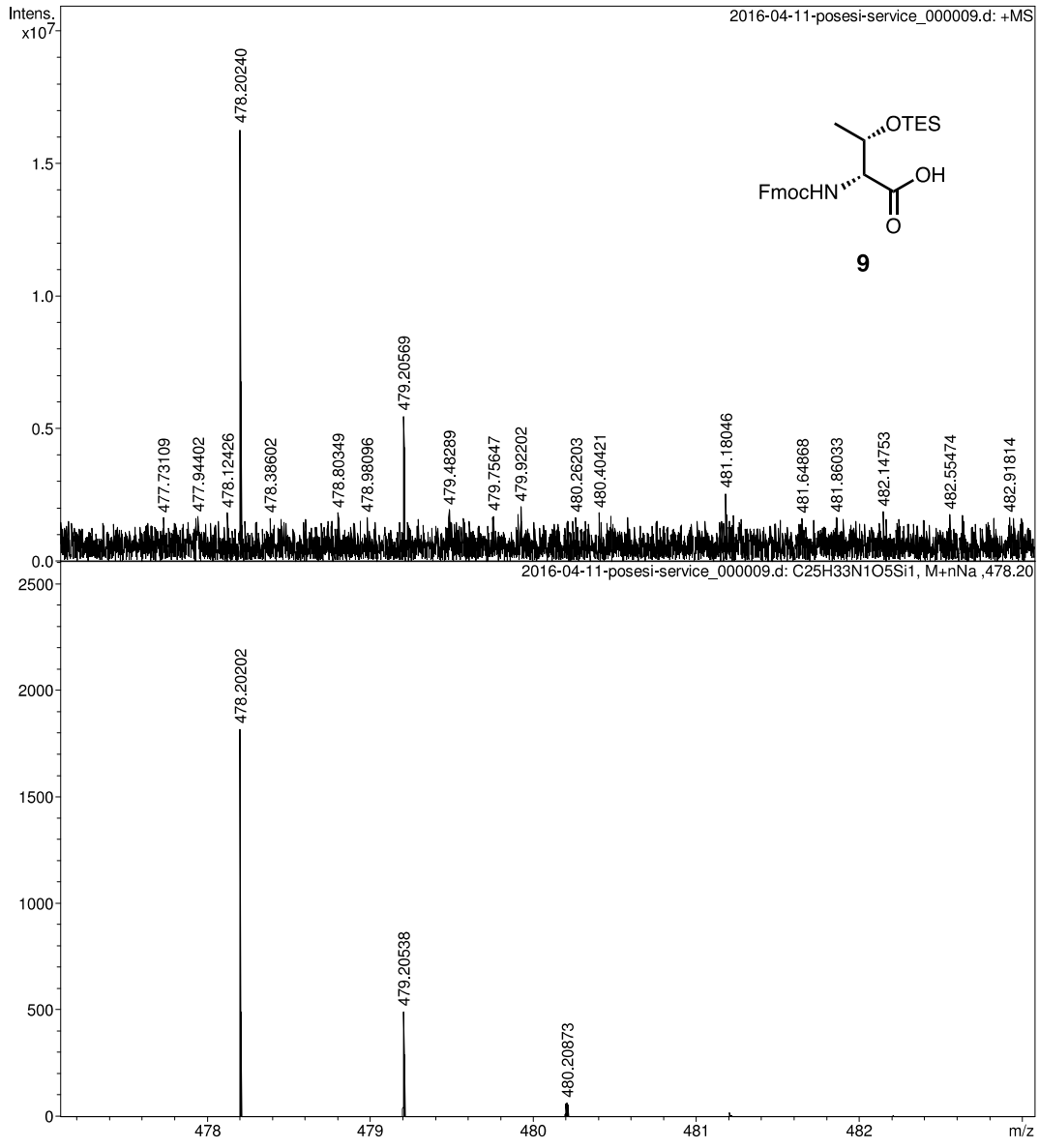
Method	1MW Positive ESI	Operator	
Sample Name	AMG-8-9-2A	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0007s, Q1 300 m/z		



Generic Display Report

Analysis Info

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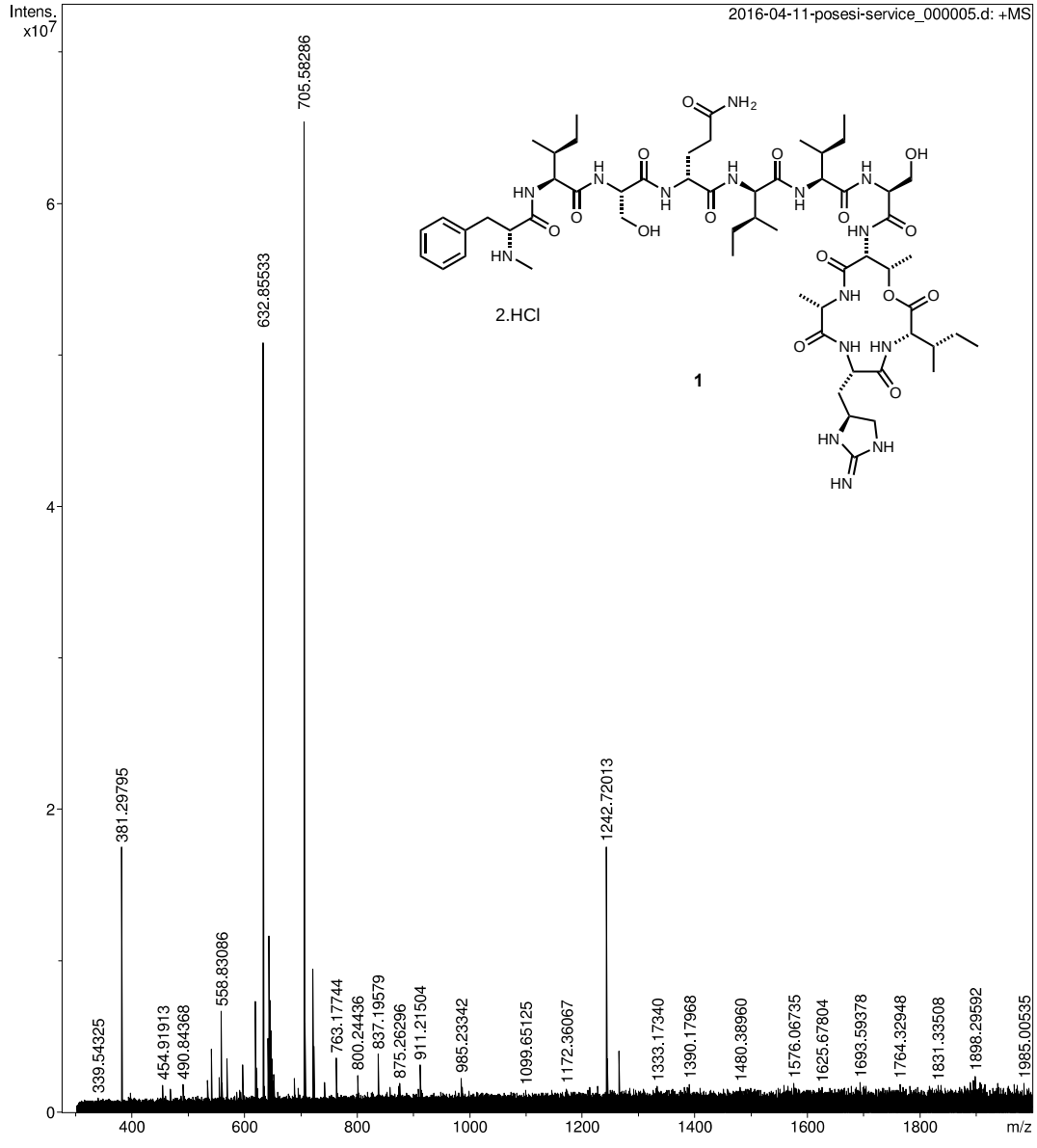
Sample Name	AMG-8-3-A	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0007s, Q1 300 m/z		



Bruker Compass DataAnalysis 4.0 printed: 11/04/2016 10:22:20 AM Page 1 of 1

Generic Display Report

Analysis Info

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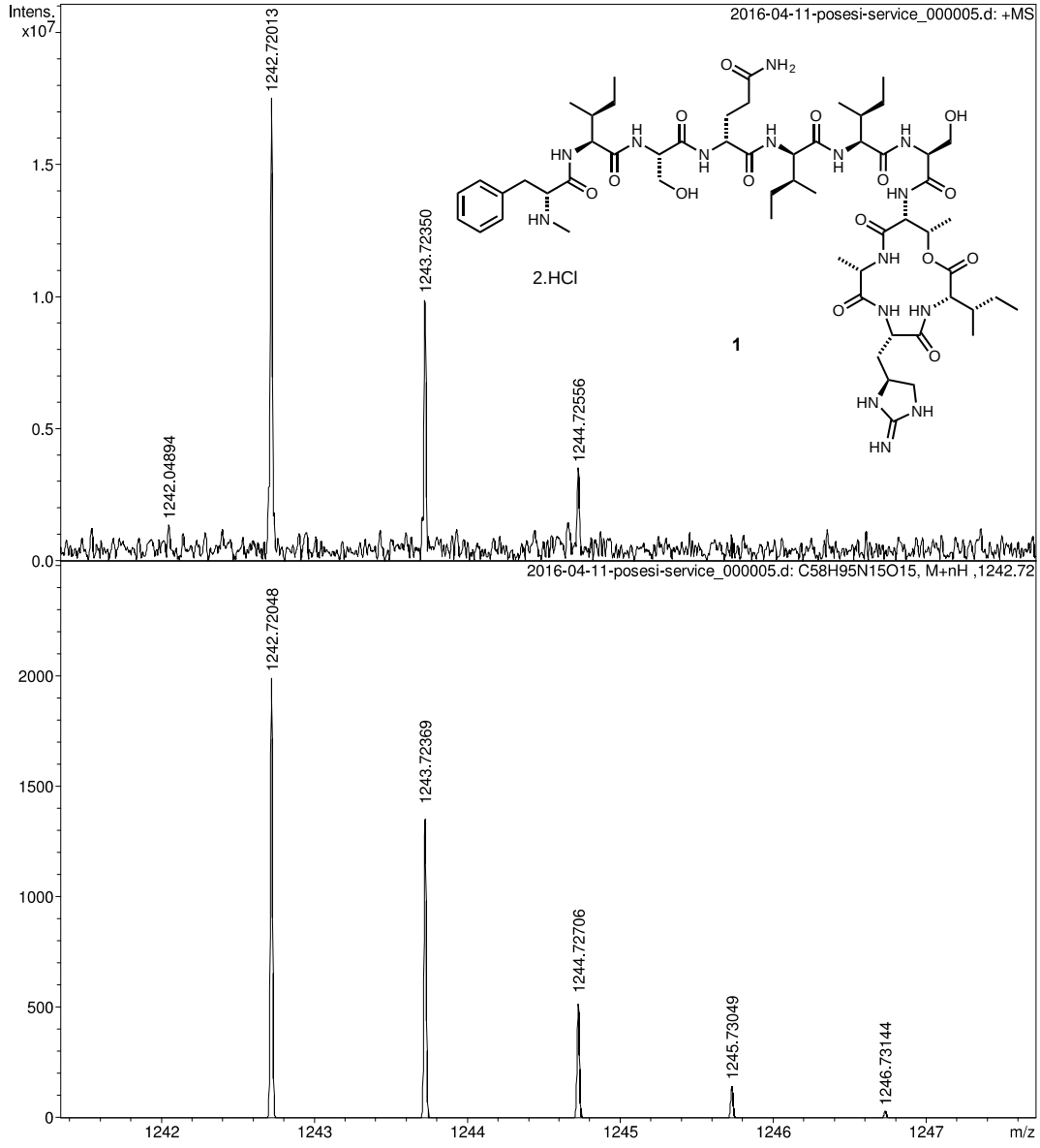
Sample Name	AMG-8-3-A	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0007s, Q1 300 m/z		



Analytical HPLC Trace of Teixobactin (1)

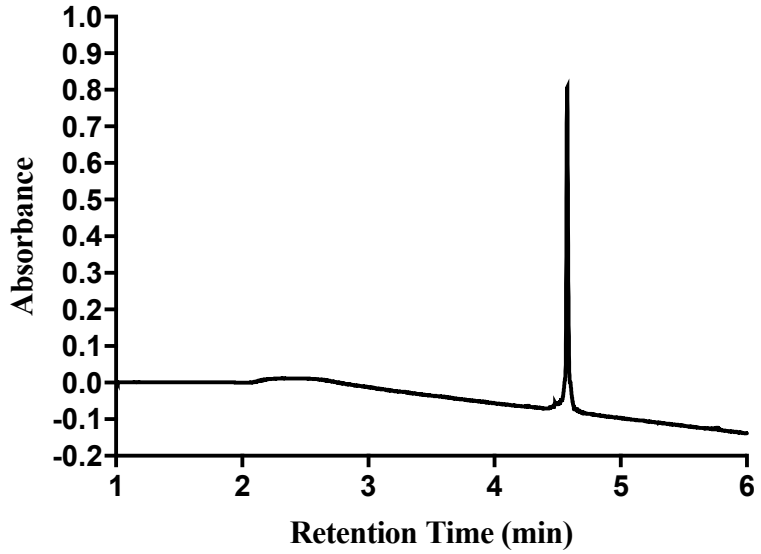


Figure S2. RP-HPLC trace of teixobactin (**1**). Gradient: 0 – 70 vol% MeCN:water (0.1% formic acid) linear gradient over 5 min (gradient starts at t = 1 min). λ = 214 nm.

References

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