Total Synthesis of Darobactin A

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Supporting Information

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General Procedures

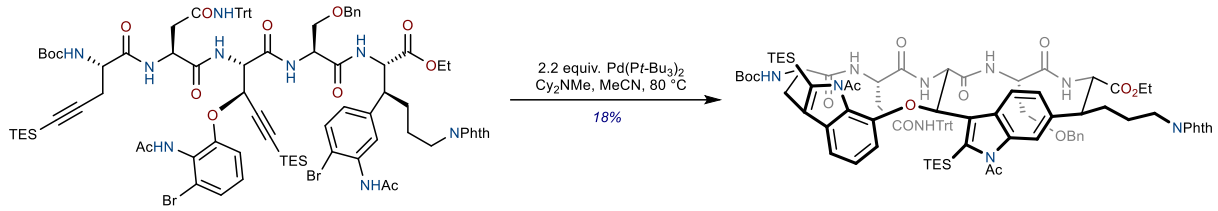
All chemicals were purchased from commercial suppliers and used as received, unless otherwise noted. *N*-bromosuccinimide was recrystallized from hot water prior to use. Diethyl ether (ACS grade), dichloromethane (ACS grade), tetrahydrofuran (HPLC grade), acetonitrile (HPLC grade), and toluene (ACS grade) were dried for reactions using the MB-SPS solvent purification system containing activated alumina manufactured by MBRAUN. Reaction temperatures correspond to the external temperature of the reaction vessel unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminum sheets. Visualization was accomplished with UV light and/or potassium permanganate ($KMnO_4$). Retention factor (R_f) values reported were measured using a 10 × 2 cm TLC plate in a developing chamber containing the solvent system described. Silicycle SiliaFlash® P60 (SiO_2, 40–63 μm particle size, 230–400 mesh) was used for flash column chromatography. Some compounds were purified using Biotage® Isolera™ One (AQ C18 column Spherical; 20 – 35μm; 100A) or Shimadzu Prominence reverse phase preparative HPLC with SPD-20A UV/Vis Photodiode array detector. ^{1}H NMR spectra were obtained at 500 MHz, 600 MHz, or 800 MHz. ^{13}C NMR were obtained at 126 MHz, 151 MHz, or 201 MHz. NMR spectra were recorded using a Bruker Avance III 500 MHz spectrometer equipped with BB CryoProbe, Bruker NEO NMR 600 MHz equipped with BBO prodigy probe, or Bruker 800 MHz Avance NEO NMR spectrometer equipped with 5mm TCI CryoProbe and were referenced to residual chloroform (7.26 ppm, 1 H), residual DMSO (2.50, 6 H), or DCM (5.32, 2 H). High temperature NMR experiments were recorded using Varian UNITY INOVA 600 MHz spectrometer equipped with 3mm BB probe. Chemical shifts are reported in parts per million (ppm) and multiplicities are indicated as: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), and br (broad). Coupling constants, J, are reported in Hertz. Mass spectrometry (MS) was performed by the University of Illinois Mass Spectrometry Laboratory. Electron Impact (EI+) spectra were performed at 70 eV using methane as the carrier gas, with time-of-flight (TOF) mass analyzer. Electrospray ionization (ESI+) spectra were performed using a time-of-flight (TOF) mass analyzer. Data are reported in the form of m/z. Infrared (IR) spectra were measured neat on a Perkin-Elmer Spectrum Two FT-IR ATR spectrometer. Peaks are reported in cm^{-1} with indicated relative intensities: s (strong, 0 – 33% T); m (medium, 34 – 66% T), w (weak, 67 – 100% T), and br (broad). Melting points were measured on a Buchi B-540 melting point apparatus and are uncorrected.

Abbreviations

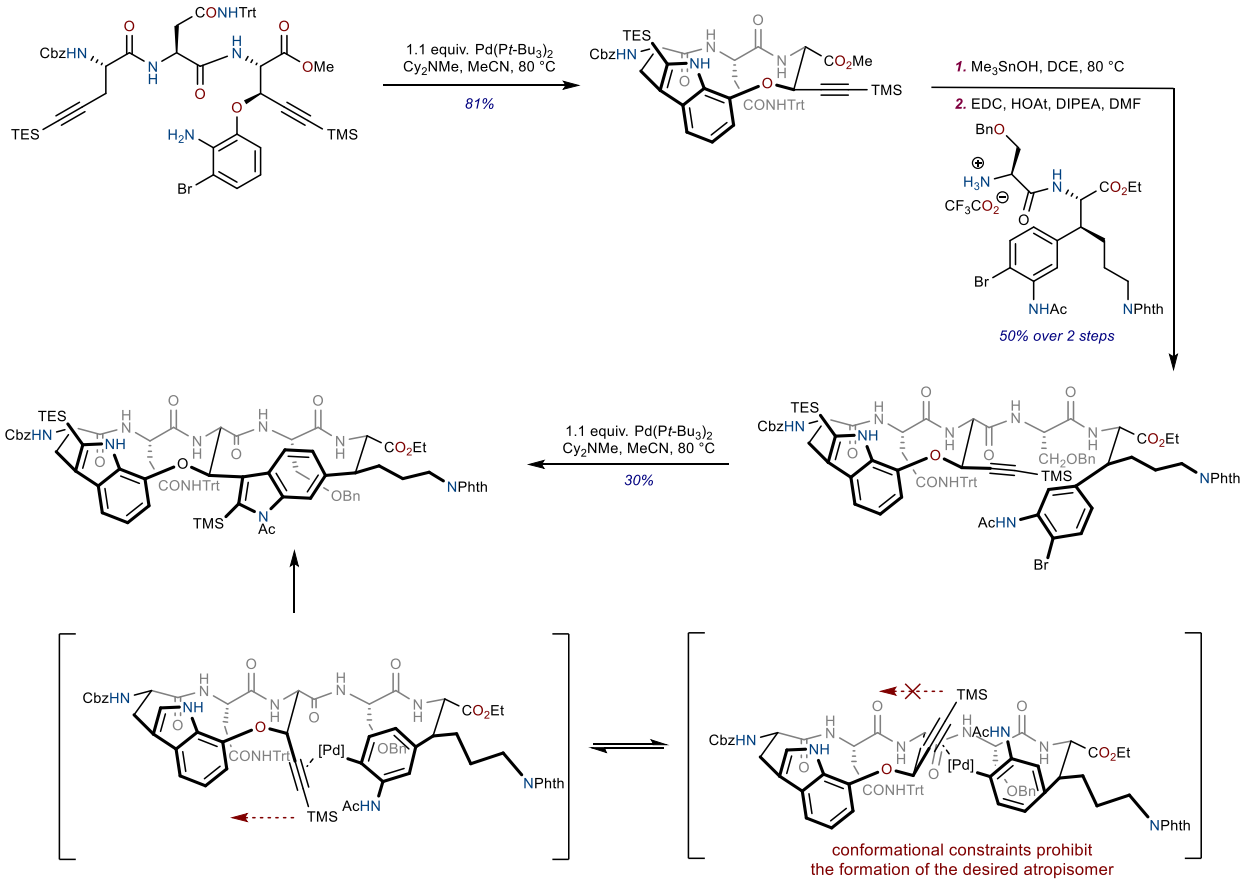
THF = tetrahydrofuran, DCE = 1,2-dichloroethane, MeCN = acetonitrile, PhMe = toluene, HOAt = 1-hydroxy-7-azabenzotriazole, EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, DIPEA/DIEA = *N*,*N*-Diisopropylethylamine, IPA = isopropyl alcohol, TFA = trifluoroacetic acid, NBS = *N*-Bromosuccinimide, NIS = *N*-iodosuccinimide, TEMPO = (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl, PIDA = (Diacetoxyiodo)benzene, DME = 1,2-dimethoxyethane, NIS = *N*-iodosuccinimide, DMS = dimethyl sulfide, DCM = dichloromethane, DMF = dimethylformamide EtOAc = ethyl acetate, Hex = hexanes, TMS = trimethylsilyl, TES = triethylsilyl.

Preliminary Macrocyclization studies

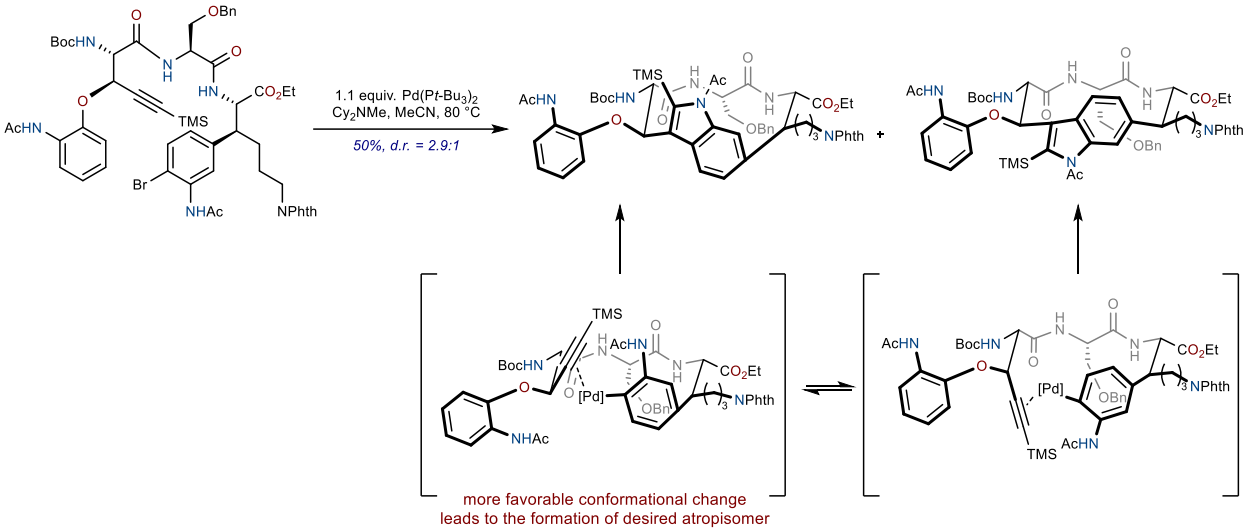
Attempted one-pot double Larock macrocyclization led to the formation of the undesired atropisomer of the eastern macrocycle:



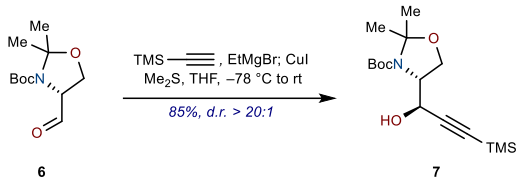
West-to-east sequential macrocyclization approach resulted in the formation of the undesired atropisomer of the eastern atropisomer as well, due to steric/conformational constraints imposed by the western macrocycle:



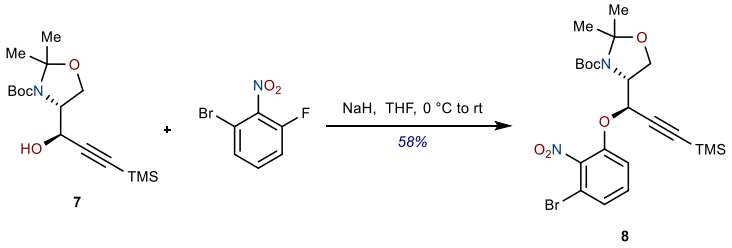
An east-to-west strategy successfully furnished the desired atropisomer of the eastern macrocycle:



Experimental Procedures



Alcohol 7 was prepared according to a known procedure[1]:
TMS-acetylene (13.0 mL, 91.6 mmol, 1.75 equiv.) was dissolved in THF (200 mL). The obtained solution was cooled to 0 ˚C, followed by dropwise addition of EtMgBr (26.2 mL, 3.0 M in ether, 28.3 mmol, 1.5 equiv.) at the same temperature. The mixture was heated to reflux and stirred for 1 hour. The solution was then cooled down to room temperature and cannulated into a solution of CuI (21.9 g, 115 mmol, 2.2 equiv.) in THF/DMS = 5:1 (300 mL total volume) at –78 ˚C. The obtained mixture was warmed to –30 ˚C, stirred for 30 min at this temperature then cooled back to –78 ˚C. To this solution was added dropwise D-Garner's aldehyde (**6**) (12.0 g, 52.3 mmol, 1.0 equiv.) as a solution in THF (50 mL). The reaction mixture was left to stir overnight, slowly warming up to room temperature. Saturated aqueous NH_4Cl (300 mL) was added to quench the reaction. After stirring for 30 min, the reaction mixture was transferred to a separatory funnel and extracted with Et_2O (3 x 200 mL). The organic extracts were combined, washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (SiO_2, Hex/EtOAc = 5:1 to 4:1) afforded alcohol **7** as a yellow oil (14.5 g, 52.3 mmol, 85%, >20:1 d.r.). Characterization data matched previously reported values.[1]

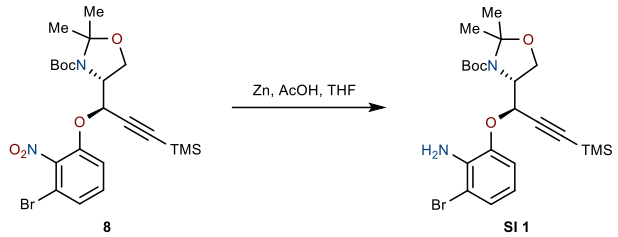


Ether 8
Alcohol **7** (14.25 g, 43.5 mmol, 1.0 equiv.) was dissolved in THF (870 mL, 0.05 M). The obtained solution was cooled to 0 ˚C, followed by portionwise addition of NaH (2.20 g, 54.4 mmol, 1.25 equiv., 60% in mineral oil). After 15 minutes of stirring at 0 ˚C, 1-bromo-3-fluoro-2-nitrobenzene (11.5 g, 52.2 mmol, 1.2 equiv.) was added in one portion. The reaction was allowed to slowly warm to room temperature and stir overnight. Upon completion (monitored by HPLC), the reaction mixture was cooled to 0 ˚C and quenched carefully with saturated aqueous $NaHCO_3$ (400 mL). The obtained solution was transferred to a separatory funnel and extracted with Et_2O (3 x 400 mL). The organic extracts were combined, washed with brine (400 mL), dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (Biotage Isolera, C_{18}-SiO_2, $MeCN/H_2O$ = 60% to 90%) afforded the ether (**8**) as a clear oil (13.0 g, 24.6 mmol, 58%).

R_f	0.3 (SiO_2, Hex/EtOAc = 8:1)
^{1}H NMR	(600 MHz, DMSO-d_6, 80 ˚C) δ 7.56 – 7.46 (m, 3H), 5.47 (s, 1H), 4.17 (s, 1H), 4.09 – 3.97 (m, 2H), 1.57 (s, 3H), 1.46 (s, 12H), 0.12 (s, 9H).

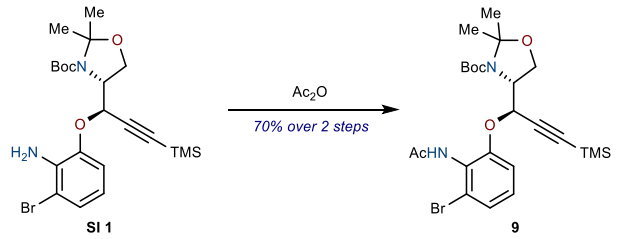
$^{13}C\{^{1}H\}$ NMR	(151 MHz, DMSO-d_6) δ 148.4, 142.5, 132.0, 126.1, 116.4, 111.9, 98.9, 94.7, 93.8, 79.9, 70.3, 63.4, 58.0, 27.6, 25.7, −1.1.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{22}H_{33}N_2O_6{}^{79}BrSi$ 527.1213; found 527.1196
IR	(ATR, neat, cm^{-1}) 2975 (w), 2936 (w), 1688 (m), 1545 (m), 1390 (m), 1366 (s), 1251 (m), 1169 (m), 1007 (w), 846 (s), 762 (m)
$[\alpha]_D^{23}$	(c = 0.19, $CHCl_3$) + 75.1˚

Due to rotamerism at room temperature, NMR spectra used for assignment were taken at 80 ˚C in DMSO-d_6.



Aniline SI 1

To a solution of ether **8** (3.85 g, 7.30 mmol 1.0 equiv.) in THF (73 mL) was added Zn (9.54 g, 146 mmol, 20 equiv., non-activated) and glacial AcOH (8.0 mL) at room temperature. After 1 hour of stirring (monitored by HPLC), the reaction mixture was filtered through celite. Water (200 mL) was added to the filtrate which was followed by neutralization with solid Na_2CO_3 until effervescence ceased. The obtained mixture was transferred to a separatory funnel and extracted with EtOAc (3 x 150 mL). The organic extracts were combined, washed with brine (150 mL), dried over $MgSO_4$, filtered, and concentrated to afford **SI 1** as an off–white foam that was taken forward without purification (quantitative yield was assumed, 3.63 g, 7.30 mmol).



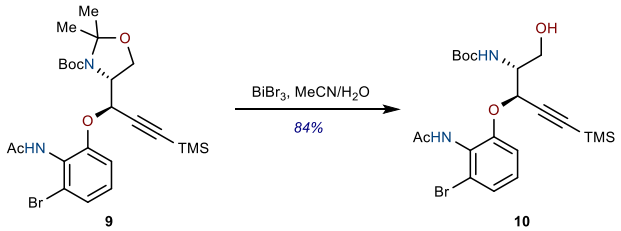
Acetanilide 9

SI 1 (3.63 g, 7.30 mmol, 1.0 equiv.) was dissolved in acetic anhydride (40 mL) and left to stir overnight. After full consumption of the starting material, acetic anhydride was removed at room temperature under high vacuum (heating the reaction mixture during concentration on the rotovap led to decomposition). The crude material was purified by flash column chromatography (SiO_2 Hex/EtOAc = 2:1) to afford acetanilide **9** (2.74 g, 5.08 mmol, 70% over 2 steps) as a white foam.

R_f	0.3 (SiO_2, Hex/EtOAc = 2:1)
^{1}H NMR	(600 MHz, DMSO-d_6, 80 °C) δ 9.05 (s, 1H), 7.31 (dd, J = 7.8, 1.5 Hz, 1H), 7.21 (t, J = 8.1 Hz, 1H), 7.18 (dd, J = 8.3, 1.5 Hz, 1H), 5.30 (s, 1H), 4.17 (dd,

	J = 9.0, 3.3 Hz, 1H), 4.15 – 4.12 (m, 1H), 4.08 (dd, J = 9.0, 6.5 Hz, 1H), 2.02 – 1.97 (s, 3H), 1.59 (s, 3H), 1.47 (s, 3H), 1.43 (s, 9H), 0.14 (s, 9H).
$^{13}C\{^{1}H\}$ NMR	(151 MHz, DMSO-*d*$_6$, 80 °C) δ 167.7, 154.0, 128.2, 127.3, 125.3, 123.3, 114.5, 100.4, 93.8, 93.1, 79.7, 68.9, 63.6, 58.1, 27.6, 25.4, 21.8, −0.9.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{24}H_{36}N_2O_5{}^{79}BrSi$ 539.1577; found 539.1583
IR	(ATR, neat, cm^{-1}): 3255 (br), 2976 (s), 1689 (s), 1473 (m), 1446 (m), 1392 (m), 1366 (m), 1251 (m), 1167 (m), 1062 (m), 1018 (m), 843 (m), 763 (m).
$[\alpha]_D^{23}$	(*c* = 0.49, $CHCl_3$) + 38.8˚

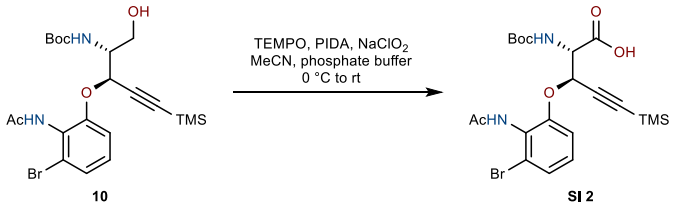
Due to rotamerism at room temperature, NMR spectra used for assignment were taken at 80 ˚C in DMSO-d$_6$*. The carbons at 68.9 ppm and 167.7 ppm didn't resolve at this temperature.*



Alcohol 10

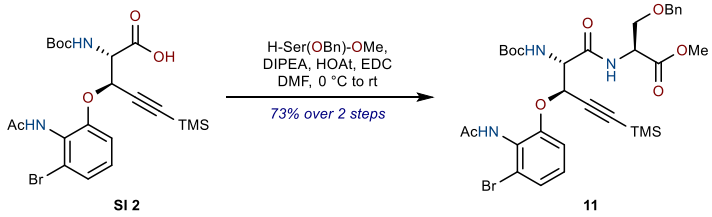
To a solution of acetanilide **9** (2.74 g, 5.08 mmol, 1.0 equiv.) in MeCN (50 mL, 0.10 M) was added bismuth (III) bromide (456 mg, 1.02 mmol, 0.20 equiv.) at room temperature. Subsequently, 0.50 mL of water were added, and the reaction mixture was left to stir for 3 hours (monitored by HPLC until complete). The reaction mixture was then quenched by addition of saturated aqueous $NaHCO_3$ (150 mL) and filtered through celite (celite was washed with 150 mL of EtOAc). The filtrate was transferred to a separatory funnel, the layers were separated, and the organic layer was further extracted with EtOAc (2 x 100 mL). The combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (SiO_2, Hex/EtOAc = 1:2) delivered alcohol **10** (2.14 g, 4.28 mmol, 84%) as a white foam.

R_f	0.3 (SiO_2, Hex/EtOAc = 1:2)
^{1}H NMR	(500 MHz, $CDCl_3$) δ 9.37 (s, 1H), 7.26 (dd, J = 7.5, 2.0 Hz, 1H), 7.20 – 7.12 (m, 2H), 6.61 (d, J = 9.2 Hz, 1H), 5.02 (d, J = 3.4 Hz, 1H), 4.84 (s, 1H), 3.97 – 3.85 (m, 1H), 3.55 – 3.40 (m, 2H), 2.03 (s, 3H), 1.42 (s, 9H), 0.10 (s, 9H).
$^{13}C\{^{1}H\}$ NMR	(126 MHz, DMSO–*d6*) δ 168.03, 155.37, 153.23, 128.02, 126.76, 124.95, 123.07, 113.44, 101.74, 92.27, 78.10, 67.76, 59.79, 55.80, 28.24, 22.73, -0.41.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{21}H_{32}N_2O_5Si^{79}Br$ 499.1264; found 499.1269
IR	(ATR, neat, cm^{-1}): 3280 (br), 2966 (s), 2177 (s), 1693 (m), 1669 (m), 1580 (s), 1510 (m), 1472 (m), 1446 (m), 1250 (m), 1168 (m), 843 (m), 762 (m)
$[\alpha]_D^{23}$	(*c* = 0.27, $CHCl_3$) + 7.2˚



Acid SI 2

To a solution of alcohol **10** (2.14 g, 4.28 mmol, 1.0 equiv.) in MeCN (55 mL) and phosphate buffer (30 mL, pH = 6.4, 0.10 M) were added PIDA (276 mg, 0.857 mmol, 0.2 equiv.) and TEMPO (268 mg, 1.71 mmol, 0.4 equiv.) at room temperature. The obtained mixture was cooled to 0 ˚C, followed by the addition of $NaClO_2$ (1.28 g, 14.1 mmol, 3.3 equiv.) in one portion. The resulting solution was warmed to room temperature and left to stir overnight. Saturated aqueous NH_4Cl (120 mL) was added, and the obtained mixture was transferred to a separatory funnel and extracted with EtOAc (4 x 100 mL). The organic extracts were combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The obtained crude acid **SI 2** was taken into the next step without further purification (assumed quantitative yield, 2.20 g, 4.28 mmol).

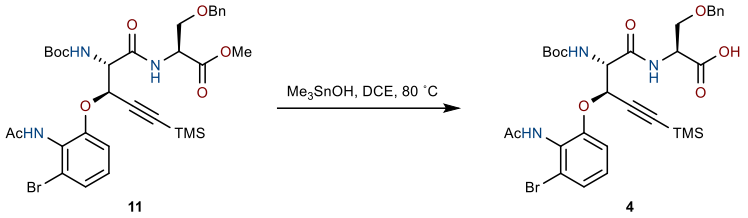


Dipeptide 11

Crude acid **SI 2** (2.20 g, 4.28 mmol, 1.0 equiv.) and *O*–benzyl serine methyl ester (1.66 g, 5.14 mmol, 1.2 equiv.) were dissolved in DMF (43 mL, 0.1 M). The solution was cooled to 0 ˚C before DIPEA (2.24 mL, 12.9 mmol, 3 equiv.) and HATU (1.96 g, 5.14 mmol, 1.2 equiv.) were added. The reaction was allowed to slowly warm to room temperature and stir at this temperature until complete (5 hours in total). The reaction was quenched with 1 M aqueous HCl (80 mL) and diluted with EtOAc (100 mL). The mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (60 mL), water (60 mL) and brine (60 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (SiO_2, Hex/EtOAc = 1:1) to yield dipeptide **11** (2.21 g, 3.14 mmol, 73% over two steps) as a white foam.

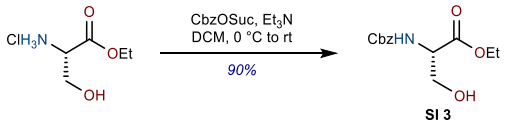
R_f	0.4 (SiO_2, Hex/EtOAc = 1:1)
^{1}H NMR	(600 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.39 (d, *J* = 7.9 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.30 – 7.23 (m, 4H), 7.16 (t, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 7.06 (d, *J* = 10.0 Hz, 1H), 5.29 (d, *J* = 3.0 Hz, 1H), 4.69 (dd, *J* = 9.9, 3.0 Hz, 1H), 4.52 (dt, *J* = 8.5, 4.5 Hz, 1H), 4.50 – 4.40 (m, 2H), 3.71 (dd, *J* = 9.8, 4.9 Hz, 1H), 3.59 (dd, *J* = 9.9, 4.2 Hz, 1H), 3.42 (s, 3H), 2.00 (s, 3H), 1.44 (s, 9H), 0.09 (s, 9H).

$^{13}C\{^{1}H\}$ NMR	(151 MHz, DMSO-d_6) δ 170.1, 168.0, 167.7, 155.3, 152.6, 137.8, 128.2, 127.7, 127.5, 127.5, 127.3, 125.4, 122.8, 113.5, 100.4, 92.8, 78.8, 72.1, 70.3, 69.1, 57.4, 52.4, 52.0, 28.2, 22.9, -0.5.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{32}H_{43}N_3O_8Si^{79}Br$ 704.2003; found 704.2008
IR	(ATR, neat, cm^{-1}): 3299 (w), 2958 (w), 2180 (w), 1672 (s), 1497 (s), 1366 (m), 1162 (s)
$[\alpha]_D^{23}$	(c = 4.0, $CHCl_3$) + 62.3˚



Dipeptide 4

Dipeptide **11** (1.81 g, 2.57 mmol, 1.0 equiv.) was dissolved in DCE (26 mL, 0.1 M). To the obtained solution was added Me_3SnOH (1.39 g, 6.77 mmol, 3.0 equiv.). The reaction was heated to 80 ˚C and left to stir overnight at this temperature. After cooling to room temperature, the solvent was removed *in vacuo*, and the residue was redissolved in 1:1 EtOAc/1 M aqueous HCl (20 mL) and stirred vigourously for 5 minutes. The mixture was transferred to a separatory funnel, the layers were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The organic layers were combined, washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated. Crude acid **4** was taken forward without further purification (quantitative yield was assumed, 1.77 g, 2.57 mmol).

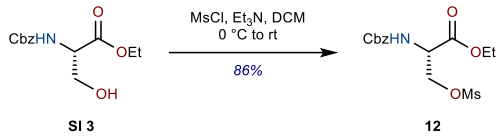


Ethyl ((benzyloxy)carbonyl)-*L*-serinate SI 3

A 1-neck 1 L round bottom flask containing a large magnetic stir bar was charged with ethyl *L*-serinate hydrochloride (38.4 g, 226 mmol, 1.0 equiv.) which was taken up in DCM (400 mL). NEt_3 (110 mL, 792 mmol, 3.5 equiv.) was added and the resulting nearly homogeneous solution was cooled to 0 °C before adding a DCM solution (250 mL) N-(Benzyloxycarbonyloxy)succinimide (59.2 g, 238 mmol, 1.05 equiv.) fast dropwise. After the addition was complete, the reaction mixture was warmed to room temperature where it was stirred for an addition 1 hour. At this time, the reaction mixture was transferred to a 2 L separatory funnel where it was washed with 1 M aq. $KHSO_4$ (2 x 500 mL), saturated aq. $NaHCO_3$ (500 mL), water (500 mL) and brine (250 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by SiO_2 flash column chromatography using 100% hexanes → 100% EtOAc as the mobile phase. The product **SI 3** was obtained as a colorless oil (54.5 g, 204 mmol, 90% yield).

R_f	0.3 (SiO_2, Hex/EtOAc = 3:2)

^{1}H NMR	(500 MHz, DMSO-d_6, major rotamer) δ 7.48 (d, J = 7.9 Hz, 1H), 7.34 (d, J = 21.8 Hz, 5H), 5.05 (s, 2H), 4.92 (t, J = 5.9 Hz, 1H), 4.10 (dq, J = 14.1, 7.1, 6.3 Hz, 3H), 3.65 (t, J = 5.5 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H).
^{13}C{^{1}H} NMR	(126 MHz, DMSO-d_6, major rotamer) δ 170.7, 156.0, 137.0, 128.40, 127.8, 127.7, 65.5, 61.3, 60.5, 56.8, 14.1.
HRMS	(ES+) *m/z*: [M+H$^+$] calcd. for $C_{13}H_{18}NO_5$ 268.1179; found 268.1184.
IR	(TE-MCT, cm^{-1}) 2980, 2930, 1721, 1506, 1343, 1204, 1052.
$[\alpha]_D^{23}$	(c = 1.0, $CHCl_3$) + 28.8˚

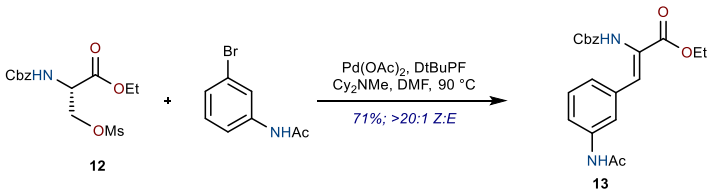


SI 3 12

Mesylate 12

A 200 mL round bottom flask containing a magnetic stir bar was charged with ethyl ((benzyloxy)carbonyl)-*L*-serinate (**SI 3**) (35.0 g, 131 mmol, 1.0 equiv.) which was taken up in DCM (400 mL before adding triethylamine (19.2 mL, 137 mmol, 1.05 equiv.). The solution was cooled 0 ˚C before methanesulfonyl chloride (10.6 mL, 137 mmol, 1.05 equiv.) was added fast dropwise. After 10 minutes at this temperature, the mixture was allowed to warm to room temperature where it was allowed to stir for an addition 5 minutes. At this time, the mixture was transferred to a separatory funnel where it was washed with 1:1 water:brine (2 x 500 mL), and brine (500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by SiO_2 flash column chromatography using 0% → 50% EtOAc in hexanes as the mobile phase. The product **12** was obtained as a colorless solid (38.9 g, 112 mmol, 86% yield).

R_f	0.3 (SiO_2, Hex/EtOAc = 3:2)
^{1}H NMR	(500 MHz, CD_2Cl_2) δ 7.48 – 7.14 (m, 5H), 5.90 (d, J = 7.1 Hz, 1H), 5.14 (s, 2H), 4.72 – 4.64 (m, 1H), 4.58 (d, J = 10.4 Hz, 1H), 4.51 (d, J = 10.0 Hz, 1H), 4.25 (q, J = 6.9 Hz, 2H), 2.96 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H).
^{13}C{^{1}H} NMR	(126 MHz, CD_2Cl_2) δ 168.7, 156.0, 136.8, 128.9, 128.6, 128.4, 69.3, 67.5, 62.9, 54.0, 37.7, 14.3.
HRMS	(ES+) *m/z*: [M+H$^+$] calcd. for $C_{14}H_{20}NO_7S$ 346.0955; found 346.0960.
IR	(TE-MCT, cm^{-1}) 1727, 1520, 1361, 1336, 1207, 1170, 1058, 1007.
$[\alpha]_D^{23}$	(c = 1.0, $CHCl_3$) + 60.0˚



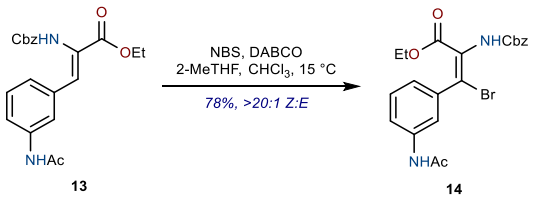
12 13

***Z*–enamide 13**

A 1000 mL round bottom flask was charged with *N*-(3-bromophenyl)acetamide (21.3 g, 99.0 mmol, 1.1 equiv.), ethyl *N*-((benzyloxy)carbonyl)-*O*-(methylsulfonyl)-*L*-serinate **12** (31.2 g, 90 mmol, 1.0 equiv.), $Pd(OAc)_2$ (1.01 g, 4.52 mmol, 5 mol%) and bis(2-(di-tert-butylphosphaneyl)cyclopenta-2,4-dien-1-yl)iron (D^tBPF, 4.29 g, 9.03 mmol, 10 mol%), which were taken up in degassed anhydrous DMF (550 mL, 60 minute N_2 sparge). To this solution, was

added distilled Cy_2NMe (48.4 mL, 226 mmol, 2.5 equiv., from KOH). The resulting solution was heated such that the internal temperature reached 90 °C where it was stirred for 19 hours. At this time, LC indicated full conversion of mesylated substrate and the dehydro-alanine intermediate resulting from *β*-mesylate elimination. This analysis also indicated that the product was present as a 96:4 *Z*:*E* ratio (210 nm UV detector). The reaction mixture was poured into a solution of distilled water (1000 mL) and 10% citric acid (200 mL), and was further diluted with EtOAc (700 mL). After separation of the layers, the aqueous layer was extracted with EtOAc (2 x 700 mL), and the combined organic layers were washed with 10% LiCl (5 x 500 mL) and brine (3 x 500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography using 0% → 100% EtOAc in hexanes as the mobile phase. The resulting residue was triturated by taking it up in a minimal amount of EtOAc (200 mL) and precipitating using hexanes (500 mL) while rapidly stirring. The solid formed in this step was separated by decantation, at which time the wet residue was taken up in Et_2O (250 mL). After stirring the heterogeneous mixture rapidly for 30 minutes, the solid product was separated from the orange liquor using vacuum filtration. The off-white solid **13** was dried to a constant weight under high vacuum (24.5 g, 64.1 mmol, 71% yield, 99:1 *Z*:*E* ratio [210 nm UV detector]).

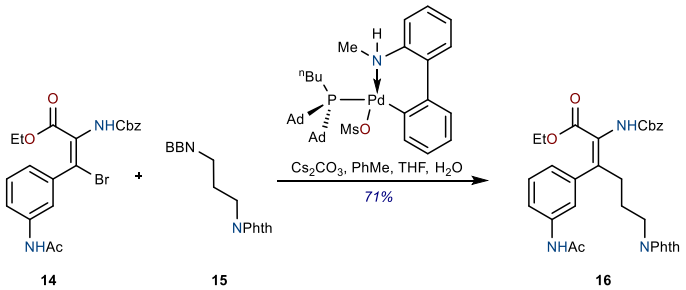
R_f	0.3 (SiO_2, Hex/EtOAc = 1:4)
^{1}H NMR	(500 MHz, DMSO-d_6) δ 10.02 (s, 1H), 9.14 (s, 1H), 7.85 (s, 1H), 7.55 (s, 1H), 7.46 – 7.25 (m, 7H), 7.13 (s, 1H), 5.11 (s, 2H), 4.16 (s(br), 2H), 2.05 (s, 3H), 1.20 (s(br), 3H).
^{13}C{^{1}H} NMR	(126 MHz, DMSO-d_6) δ 168.4, 165.2, 154.7, 139.5, 136.8, 133.7, 131.7, 128.9, 128.3, 127.9, 127.7, 126.4, 124.5, 120.6, 120.2, 65.9, 61.0, 24.0, 14.0.
HRMS	(ES+) *m/z*: [M+H$^+$] calcd. for $C_{21}H_{23}N_2O_5$ 383.1601; found 383.1601.
IR	(TE-MCT, cm^{-1}) 1738, 1702, 1530, 1489, 1370, 1306, 1273, 1230, 1218.



***Z*–Bromoenamide 14**

A 1000 mL recovery flask containing a large magnetic stir bar was charged with ethyl (Z)-3-(3-acetamidophenyl)-2-(((benzyloxy)carbonyl)amino)acrylate **13** (23.8 g, 62.3 mmol, 1.0 equiv) which was taken up in 2-MeTHF:$CHCl_3$ (312 mL) to generate a suspension. The solution was cooled to ~15 °C before adding freshly recrystallized 1-bromopyrrolidine-2,5-dione (13.9 g, 78.0 mmol, 1.25 equiv) portion-wise. The resulting mixture was allowed to stir for 20 minutes at this temperature before 1,4-diazabicyclo[2.2.2]octane (7.69 g, 68.5 mmol, 1.1 equiv) was added portion-wise. The resulting mixture was allowed to stir at this temperature for an additional 15 mins before warming to room temperature where it was stirred for an additional 30 minutes at which time LC indicated full conversion and a >20:1 *Z*:*E* ratio [210 nm UV detector]. At this time, the reaction mixture was filtered through Celite and concentrated *in vacuo*. The residue was purified via SiO_2 flash column chromatography using 30% → 100% EtOAc in hexanes as the mobile phase. The resulting material was triturated with hexanes:Et_2O (1:1, 500 mL), and the resulting solid was collected via vacuum filtration and dried *in vacuo*. The product **14** was obtained as a colorless solid (22.4 g, 48.6 mmol, 78% yield, >20:1 *Z*:*E* ratio [210 nm UV detector]).

R_f	0.5 (SiO_2, Hex/EtOAc = 3:2)
^{1}H NMR	(500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 1H), 7.52 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 6H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.75 (s, 1H), 5.17 (s, 2H), 4.01 (s(br), 2H), 2.14 (s, 3H), 0.91 (s(br), 3H).
^{13}C{^{1}H} NMR	(126 MHz, $CDCl_3$) δ 169.0, 162.9, 153.1, 138.4, 137.9, 135.2, 129.3, 128.9, 128.7, 128.6, 128.4, 124.5, 120.7, 120.2, 115.8, 68.1, 62.1, 24.5, 13.4.
HRMS	(ES+) *m/z*: [M+H$^+$] calcd. for $C_{21}H_{22}{}^{79}BrN_2O_5$ 461.0707; found 461.0706.
IR	(TE-MCT, cm^{-1}) 1736, 1695, 1532, 1475, 1321, 1212, 1060.



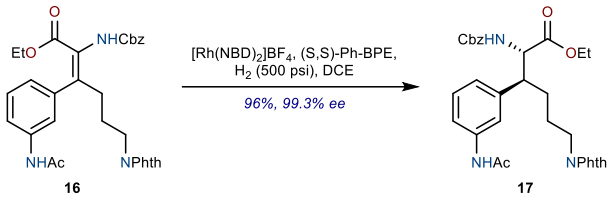
Tetrasubstituted enamide 16

A dry 500 mL round bottom flask containing a magnetic stir bar was charged with 9-BBN (267 mL, 134 mmol, 1.0 equiv., 0.5 M in THF). The solution was cooled to 0 °C before a THF solution (85 mL) of the 2-allylisoindoline-1,3-dione (25.0 g, 134 mmol, 1 equiv.) was added fast dropwise. The mixture was allowed to warm to room temperature where it was allowed to stir for 18 hours. This yellow solution of alkyl borane **15** was directly utilized in the coupling step.

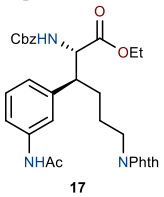
A 3-neck 1000 mL round bottom flask was fitted with an overhead stirrer and dried under a constant flow of N_2 for 10 minutes. The flask was then charged with enamide **14** (16.5 g, 35.8 mmol, 1.0 equiv.) which was taken up in PhMe (200 mL, 60 minute N_2 sparge) before adding cataCXium®-Pd-G4 (0.665 g, 0.896 mmol). Alkyl borane **15** (169 mL, 62.7 mmol, 1.79 equiv., 0.38 M in THF) and a degassed aqueous solution of Cs_2CO_3 (71.7 mL, 107 mmol, 1.5 equiv., 3.0 M in H_2O) were added in that order, and the resulting biphasic mixture was rapidly stirred (500 rpm) for 1 hour and 40 minutes. After LCMS confirmed full consumption of the starting vinyl bromide, the reaction was cooled before diluting with water (500 mL) and EtOAc (500 mL) and quenching with 10% aqueous citric acid (200 mL). After separation of the layers, the aqueous layer was extracted with EtOAc (2 x 250 mL), and the combined organic layers were washed with brine (250 mL), dried over Na_2SO_4, filtered through a Celite-impregnated filter frit, and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography using 30% → 90% EtOAc in DCM as the mobile phase. The fractions containing the product were collected and treated with 2 g of active charcoal. After stirring for 10 minutes, the solution was filtered through a Celite-impregnated filter frit, and the filtrate was concentrated *in vacuo* to yield an off-white solid which was suspended in Et_2O (250 mL). After vigorously stirring for 5 minutes, the solid was separated from the yellow mother liquor by vacuum filtration. The cake was washed with Et_2O (50 mL) and dried under a constant N_2 flow with the aid of house vacuum. The product **16** was obtained as a colorless solid (14.5 g, 25.4 mmol, 71% yield).

R_f	0.4 (SiO_2, Hex/EtOAc = 3:2)

^{1}H NMR	(500 MHz, DMSO-d_6, major rotamer) δ 9.89 (s, 1H), 9.40 (s, 1H), 7.82 (d, J = 2.6 Hz, 4H), 7.50 (d, J = 8.0 Hz, 1H), 7.44 – 7.23 (m, 6H), 7.20 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 7.5 Hz, 1H), 5.05 (s, 2H), 3.75 (q, J = 6.9 Hz, 2H), 3.53 (t, J = 6.9 Hz, 2H), 2.49 – 2.44 (m, 2H), 2.00 (s, 3H), 1.55 – 1.46 (m, 2H), 0.73 (t, J = 7.0 Hz, 3H).
^{13}C{^{1}H} NMR	(126 MHz, DMSO-d_6, major rotamer) δ 168.2, 167.9, 165.0, 154.5, 140.0, 139.2, 138.3, 136.6, 134.5, 131.7, 128.4, 128.3, 128.0, 127.9, 125.5, 122.9, 122.0, 117.9, 117.8, 65.9, 59.8, 37.4, 30.8, 26.1, 24.0, 13.2.
HRMS	(ES+) *m/z*: [M+H$^+$] calcd. for $C_{32}H_{32}N_3O_7$ 570.2235; found 570.2245.
IR	(TE-MCT, cm^{-1}) 1734, 1712, 1530, 1479, 1399, 1312, 1226, 1199, 1031.



HTE optimization for the synthesis of 17

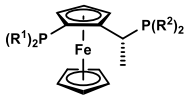


Microscale high-throughput experimentation was conducted in 8 × 30 mm glass vial inserts in 96-well SBS-format microplates inside pressure vessels. Yields are reported as SFC area%, and % ee was determined by chiral SFC. Chiral analysis was performed on a Waters UPC^2, OJ3 4.6x150 mm, 3 µm, 3 mL / min, 5.0 -17.5% MeOH w/ 25 mM iBuNH$_2$ / CO$_2$ in 5 min, 200 bar, 40 °C, 210 nm. **16**: 4.55 min, (*S*,*S*)-**17**: 3.32 min, (*R*,*R*)-**17**: 3.51 min.

Evaluation of chiral ligands for the asymmetric hydrogenation of **16**:
In a glovebox with O_2 < 5 ppm, to 8 × 30 mm vials containing 0.42 µmol (26.25 mol %) of 192 different chiral bidentate phosphine ligands, 100 µL of a 4 mM stock solution of $(NBD)_2RhBF_4$ in DCE (0.4 µmol, 25 mol %) was added, and the mixture was stirred using magnetic tumble stirring for 15 min at room temperature. The volatiles were removed on a vacuum centrifuge, and 100 µL of a 16 mM stock solution of **16** in MeOH (4 µmol) was added. The plates were sealed in pressure vessels and removed from the glovebox. The plates were purged with 3 × N_2 / vent cycles, 3 × H_2 / vent cycles, pressurized to 500 psi with H_2, and heated to 50 °C with 500 rpm shaking overnight. Ligands giving > 90% ee of product **17** are presented below:

Ligand	**17**	**16**	%ee **17**	**B**	**A**	%ee **B**
SL-T027-2	97.2	0.2	97.5	2.5	0.1	
(S,S)-Ph-BPE	78.4	0.2	96.8	21.5	0.0	92.4
SL-J014-1	99.7	0.1	96.7	0.2	0.0	
SL-J011-1	75.0	0.0	96.5	25.0	0.0	94.5
SL-J002-1	99.7	0.0	95.9	0.3	0.0	
SL-T025-2	47.7	1.3	95.3	50.2	0.7	85.4
SL-J013-1	99.7	0.0	95.2	0.3	0.0	
SL-J203-2	97.2	0.1	-93.5	2.4	0.3	

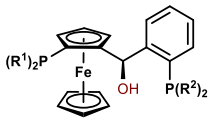
(+)-Cy-Segphos	98.1	0.8	91.4	1.1	0.0	
SL-J012-1	99.5	0.0	91.4	0.5	0.0	
SL-T021-2	37.4	20.1	90.0	27.4	15.0	78.2



Josiphos

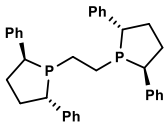
Ligand	R^1	R^2
SL-J014-1	4-FPh	tBu
SL-J011-1	4-CF_3Ph	tBu
SL-J002-1	Ph	tBu
SL-J013-1	3,5-Me_2-4-MeOPh	tBu
SL-J203-2*	3,5-tBu_2-4-MeOPh	tBu
SL-J012-1	4-MePh	tBu

*enantiomer of structure drawn

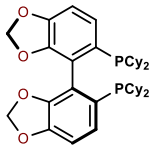


HO-Taniaphos

Ligand	R^1	R^2
SL-T027-2	3,5-Me_2Ph	3,5-$(CF_3)_2$Ph
SL-T025-2	3,4-Me_2Ph	3,5-Me_2Ph
SL-T021-2	Ph	Ph

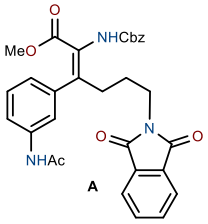


(*S*,*S*)-Ph-BPE

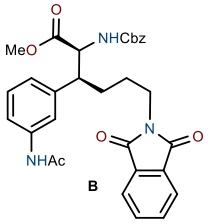


(+)-Cy-Segphos

Under the experimental conditions, significant amounts of transesterification with the MeOH reaction solvent to yield alkene **A** and product **B** was also observed.



A



B

Evaluation of solvents for the asymmetric hydrogenation of **16**:
In a glovebox with O_2 < 5 ppm, solutions of 20 µmol of $(NBD)_2RhBF_4$ and 21 µmol of chiral bidentate phosphine ligands in 4 mL DCE were stirred for 15 min at room temperature. 40 µL of the catalyst stock solutions (0.2 µmol, 4 mol %) were added to 8 × 30 mm vials, followed by 100 µL of a 50 mM stock solution of **16** in DCE (5 µmol). The volatiles were removed on a vacuum centrifuge, and 100 µL of 16 different reaction solvents were added. The plates were sealed in pressure vessels and removed from the glovebox. The plates were purged with 3 × N_2 / vent cycles, 3 × H_2 / vent cycles, pressurized to 500 psi with H_2, and heated to 50 °C with 500 rpm shaking overnight.

17	(*S*,*S*)-Ph-BPE	SL-J011-1	SL-J002-1	SL-J013-1
MeOH	99.3	99.5	99.6	99.6
EtOH	100.0	99.8	99.7	99.7
iPrOH	99.6	98.7	98.8	99.3
TFE	67.4	100.0	100.0	100.0
DCE	99.9	100.0	99.8	99.3
$PhCF_3$	17.9	94.5	88.6	99.1
PhCl	27.0	100.0	99.6	99.4
PhMe	0.4	99.6	99.6	100.0
2-Me-THF	98.9	100.0	100.0	100.0
CPME	1.7	99.9	99.9	99.9
DME	99.9	100.0	100.0	100.0
EtOAc	82.5	100.0	100.0	100.0
iPrOAc	14.1	100.0	100.0	99.9
MEK	100.0	100.0	100.0	99.9
MIBK	99.7	99.5	99.2	98.8
sulfolane	1.0	18.7	15.0	11.0

%ee 17	(*S*,*S*)-Ph-BPE	SL-J011-1	SL-J002-1	SL-J013-1
MeOH	97.8	91.3	95.6	95.1
EtOH	97.7	94.3	95.5	94.6
iPrOH	97.7	92.8	94.5	94.3
TFE	97.5	93.3	93.9	95.0
DCE	99.1	95.0	95.7	94.9
$PhCF_3$	98.2	94.1	93.3	92.8
PhCl	98.2	95.0	95.4	94.3
PhMe		94.1	94.3	93.7
2-Me-THF	97.0	93.7	95.6	95.2
CPME		93.0	93.8	94.2
DME	98.1	93.2	96.2	95.2
EtOAc	97.1	94.1	95.2	95.2
iPrOAc	93.1	93.5	94.3	94.7
MEK	98.1	93.9	92.4	93.6
MIBK	97.8	94.1	94.4	94.5
sulfolane		89.1	93.3	93.7

16	(*S*,*S*)-Ph-BPE	SL-J011-1	SL-J002-1	SL-J013-1
MeOH	0.1	0.2	0.1	0.1
EtOH	0.0	0.2	0.3	0.3
iPrOH	0.4	1.3	1.2	0.7
TFE	32.6	0.0	0.0	0.0
DCE	0.1	0.0	0.2	0.7
$PhCF_3$	82.1	5.5	11.4	0.9
PhCl	73.0	0.0	0.4	0.6
PhMe	99.6	0.4	0.4	0.0
2-Me-THF	1.1	0.0	0.0	0.0
CPME	98.3	0.1	0.1	0.1
DME	0.1	0.0	0.0	0.0
EtOAc	17.5	0.0	0.0	0.0
iPrOAc	85.9	0.0	0.0	0.1
MEK	0.0	0.0	0.0	0.1
MIBK	0.3	0.5	0.8	1.2
sulfolane	99.0	81.3	85.0	89.0

Optimization of catalyst loading for the asymmetric hydrogenation of **16**:

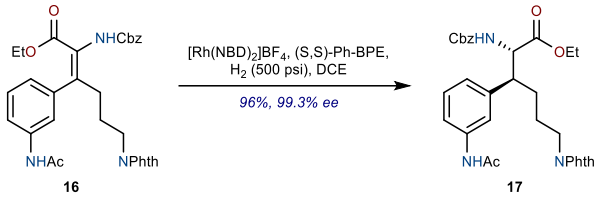
In a glovebox with O_2 < 5 ppm, solutions of 20 µmol of $(NBD)_2RhBF_4$ and 21 µmol of chiral bidentate phosphine ligands in 4 mL DCE were stirred for 15 min at room temperature. 12.5 - 100 µL of the catalyst stock solutions (0.05 - 0.4 µmol, 0.5 - 4 mol %) were added to 8 × 30 mm vials. The volatiles were removed on a vacuum centrifuge, and 100 µL of 100 mM stock solutions of **16** in MeOH, DCE, DME, or MEK (10 µmol) were added. The plates were sealed in pressure vessels and removed from the glovebox. The plates were purged with 3 × N_2 / vent cycles, 3 × H_2 / vent cycles, pressurized to 500 psi with H_2, and heated to 50 °C with 500 rpm shaking overnight.

17	4%	2%	1%	0.5%	4%	2%	1%	0.5%
MeOH	99.1	99.4	93.3	47.3	99.6	99.5	99.1	63.2
DCE	97.6	97.5	57.1	17.1	97.5	93.6	93.1	84.3
DME	97.7	98.9	91.0	9.3	100.0	99.9	96.9	23.0
MEK	98.0	99.9	97.8	78.6	99.9	99.9	95.6	88.9
	(*S*,*S*)-Ph-BPE				SL-J002-1			

%ee 17	4%	2%	1%	0.5%	4%	2%	1%	0.5%
MeOH	97.5	97.6	97.7	97.1	95.2	94.9	94.3	93.5
DCE	98.9	98.9	99.3	94.8	95.6	95.8	95.6	95.4
DME	97.8	98.1	98.1	88.8	95.7	95.7	94.6	93.7
MEK	98.0	97.9	98.0	97.9	91.8	93.2	93.6	93.4
	(*S*,*S*)-Ph-BPE				SL-J002-1			

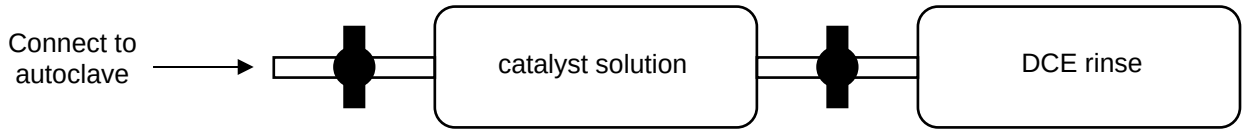
16	4%	2%	1%	0.5%	4%	2%	1%	0.5%
MeOH	0.1	0.0	6.2	52.2	0.1	0.2	0.6	36.4
DCE	2.4	2.5	42.9	82.9	2.5	6.4	6.9	15.7
DME	2.3	1.1	9.0	90.7	0.0	0.1	3.1	77.0
MEK	2.0	0.1	2.2	21.4	0.1	0.1	4.4	11.1
	(*S*,*S*)-Ph-BPE				SL-J002-1			

Scale-Up of the Optimized Hydrogenation Conditions Obtained from HTE



β–Aryl Lysine 17

In a glovebox with O_2 < 5 ppm, 194 mg $(NBD)_2RhBF_4$ (2.5 mol %), 276 mg (*S*,*S*)-Ph-BPE (2.63 mol %), and 10 mL DCE were stirred at room temperature for 25 minutes. The catalyst solution was transferred to a charge bomb assembly with the aid of 2 × 2.5 mL DCE rinses. 15 mL DCE was transferred to the rinse bomb, and the assembly was sealed and removed from the glovebox.

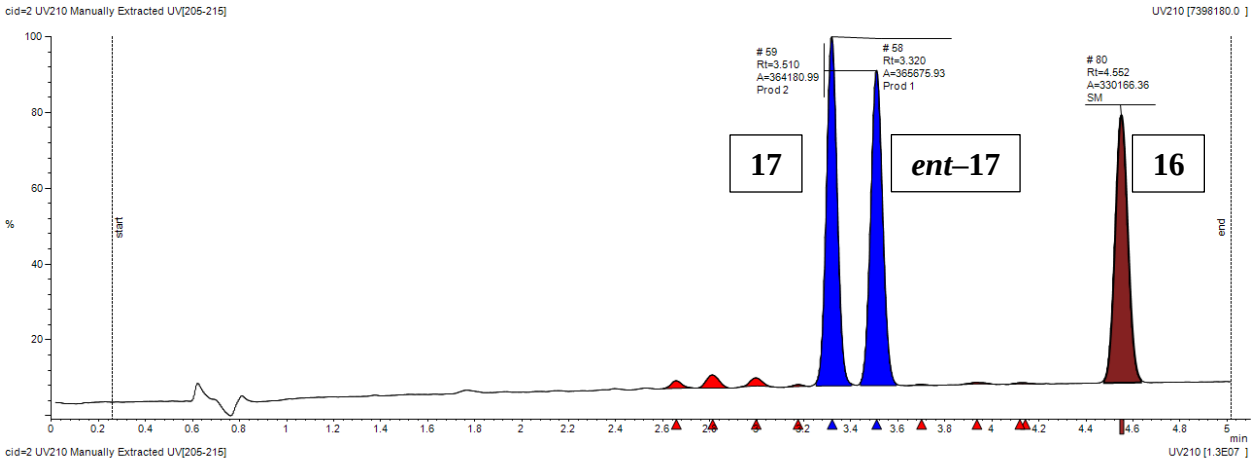


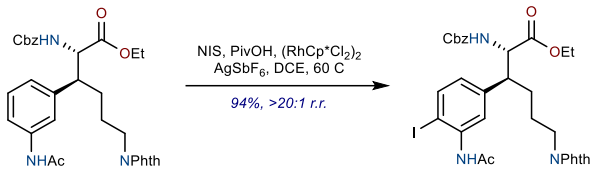
To a 1 L Autoclave Engineers Zipperclave, tetrasubstituted enamide **16** (11.8 g, 20.72 mmol, 1.0 equiv.) and 210 mL DCE were added under air. The autoclave was sealed, and the catalyst charge bomb assembly was connected via flexible tubing. The vessel and transfer line were inerted twice with vacuum and then refilled with nitrogen. Then reactor and transfer line were degassed by pressurizing with nitrogen, agitating briefly, and then evacuated with partial vacuum. This degassing was repeated a total of three times. The autoclave was placed under partial vacuum, then the catalyst solution was drawn into the autoclave by opening the valve on the catalyst charge bomb assembly. Then the charge bomb assembly valve was closed and was rinsed by opening the rinse valve. The rinse was then drawn into the autoclave. The autoclave was purged three times with hydrogen followed by venting to ambient pressure and then pressurized with hydrogen to 500 psig. The reaction was heated to 50 °C with 1000 rpm stirring for 20 h. The vessel was cooled to room temperature, vented to atmospheric pressure, and the reaction mixture was removed with the aid of a 250 mL CH_2Cl_2 rinse. SFC analysis showed complete conversion and 99.3% ee. The combined reaction mixture and rinse was concentrated on a rotary evaporator. The residue was purified by SiO_2 flash column chromatography using 80% → 90% EtOAc in hexanes as the mobile phase. The product **17** was obtained as a colorless solid (11.4 g, 19.94 mmol, 96% yield).

R_f	0.2 (SiO_2, 1:2 Hex/EtOAc)
^{1}H NMR	(600 MHz, DMSO-*d*$_6$) δ 9.85 (s, 1H), 7.86 – 7.80 (m, 4H), 7.53 – 7.49 (m, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.37 (t, *J* = 1.9 Hz, 1H), 7.33 – 7.25 (m, 3H), 7.21 – 7.14 (m, 3H), 6.88 (d, *J* = 7.6 Hz, 1H), 4.90 (d, *J* = 2.0 Hz, 2H), 4.24 (t, *J* = 8.7 Hz, 1H), 4.04 (q, *J* = 7.1 Hz, 2H), 3.48 (t, *J* = 6.9 Hz, 2H), 2.92 (q, *J* = 8.0 Hz, 1H), 2.00 (s, 3H), 1.55 (p, *J* = 7.1 Hz, 2H), 1.35 (m, 2H), 1.13 (t, *J* = 7.1 Hz, 3H).
^{13}C{^{1}H} NMR	(151 MHz, DMSO-*d*$_6$) δ 171.5, 168.2, 167.9, 155.8, 140.7, 139.4, 136.8, 134.3, 131.6, 128.5, 128.3, 127.7, 127.5, 123.2, 123.0, 118.3, 117.4, 65.4, 60.6, 58.8, 46.4, 37.2, 28.9, 25.9, 24.0, 13.9.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{32}H_{34}N_3O_7$, 572.2397; found 572.2402
IR	(ATR, neat, cm^{-1}): 3340 (br), 1709 (s), 1547 (w), 1189 (w)

$[\alpha]_D^{23}$ (c = 0.55, $CHCl_3$) 51.65˚

SFC (Waters UPC^2, OJ3 4.6x150 mm, 3 µm, 3 mL / min, 5.0 -17.5% MeOH w/ 25 mM $iBuNH_2$ / CO_2 in 5 min, 200 bar, 40 °C, 210 nm): **17**: 4.55 min, (*S*,*S*)-**18**: 3.32 min, (*R*,*R*)-**18**: 3.51 min. 99.3% ee.





17 **18**

Iodide 18

β–aryl lysine **17** (2.47 g, 4.32 mmol, 1.0 equiv.) was dissolved in DCE (43 mL, 0.1 M). To this was added pivalic acid (485 mg, 4.75 mmol, 1.1 equiv.), silver hexafluoroantimonate (371 mg, 1.08 mmol, 0.25 equiv.), and $[RhCp^*Cl_2]_2$ (267 mg, 432 µmol, 0.1 equiv.). Lastly, NIS (1.02 g, 4.54 mmol, 1.05 equiv.) was added and the reaction was heated to 60 ˚C for 6 hours. Upon completion, the reaction was cooled to room temperature and filtered through celite. The filter pad was washed with DCM (20 mL) and the solvent was removed on the rotary evaporator. The crude material was purified by column chromatography (SiO_2, Hex/EtOAc 3:1 to 1:1) to produce iodide **18** (2.82 g, 4.04 mmol, 94%) as a light-brown foam.

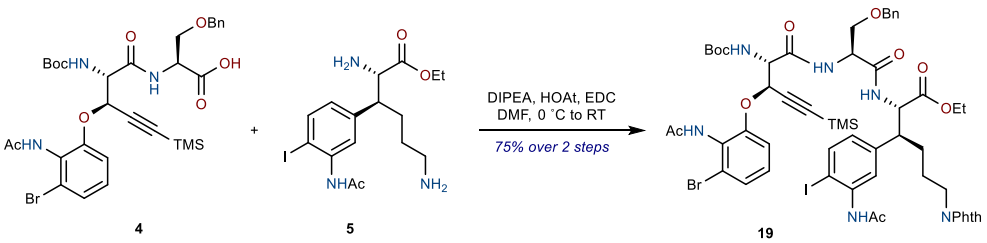
R_f 0.4 (SiO_2, 1:2 Hex/EtOAc)

^{1}H NMR	(600 MHz, DMSO-d_6) δ 9.35 (s, 1H), 7.88 – 7.77 (m, 4H), 7.73 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.35 – 7.26 (m, 4H), 7.22 (d, J = 7.4 Hz, 2H), 6.86 (d, J = 8.2 Hz, 1H), 4.97 – 4.84 (m, 2H), 4.23 (t, J = 8.6 Hz, 1H), 4.03 (q, J = 7.1 Hz, 2H), 3.48 (t, J = 6.8 Hz, 2H), 2.95 (td, J = 9.2, 4.8 Hz, 1H), 2.02 (s, 3H), 1.66 – 1.48 (m, 2H), 1.35 (dh, J = 17.7, 6.8 Hz, 2H), 1.12 (t, J = 7.1 Hz, 3H).
^{13}C{^{1}H} NMR	(151 MHz, DMSO-d_6) δ 179.4, 171.3, 168.1, 167.9, 155.9, 141.0, 139.5, 138.6, 136.8, 134.4, 131.6, 128.3, 127.8, 127.6, 127.2, 123.0, 94.4, 65.5, 60.6, 58.6, 45.7, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 37.2, 28.5, 25.8, 13.9.
HRMS	(ES+) m/z: $[M+H]^+$ calcd. for $C_{32}H_{33}N_3O_7I$, 698.1363; found 698.1367
IR	(ATR, neat, cm^{-1}) 3333 (w), 2940 (w), 1771 (w), 1708 (s), 1518 (w), 1397 (w), 1184 (w)
$[\alpha]_D^{23}$	(c = 0.71, $CHCl_3$) + 56.7˚

Satellite peaks can be observed in the 1H and ^{13}C NMR due to rotamerism at room temperature. Only the peaks for the major rotamer are reported. HMBC is included to prove that the desired regioisomer is formed.

Amine 5

Iodide **18** (1.78 g, 2.55 mmol, 1.0 equiv.) was dissolved in DCM (32 mL, 0.08 M) and cooled to 0 ˚C. To this was added 1 M BBr_3 in DCM (2.81 mL, 2.81 mmol, 1.1 equiv.) dropwise. The ice bath was removed and the reaction was left to stir until complete (about 1 hour). The mixture was cooled to 0 ˚C and quenched by dropwise addition of methanol (3 mL). The obtained solution was concentrated and the residue was triturated with hexanes and dried under vacuum to yield amine **5** as a light orange foam that was taken forward without further purification (quantitative yield was assumed, 1.44 g, 2.55 mmol).

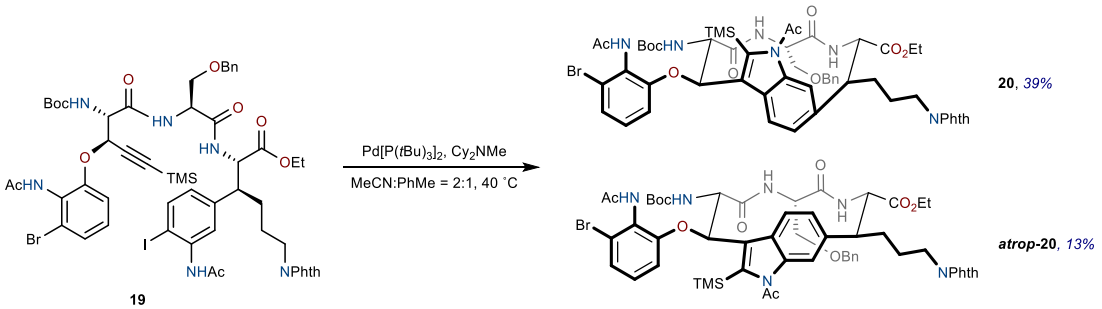


Tripeptide 19

Acid **4** (1.77 g, 2.56 mmol, 1.0 equiv.) and amine **5** (1.44 g, 2.56 mmol, 1.0 equiv.) were dissolved in DMF (26 mL, 0.1 M). To the obtained solution was added DIPEA (1.34 mL, 7.67 mmol, 3 equiv.). The solution was cooled to 0 ˚C before HOAt (417 mg, 3.07 mmol, 1.2 equiv.) was added. Subsequently EDC (588 mg, 3.07 mmol, 1.2 equiv.) was added. The mixture was allowed to warm to room temperature and stir overnight. The reaction was quenched with 1 M aqueous HCl (60 mL) and diluted with EtOAc (80 mL). The mixture was transferred to a separatory funnel and the

layers were separated. The aqueous layer was extracted with EtOAc (2 x 80 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (60 mL), water (60 mL) and brine (60 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (SiO_2, Hex/EtOAc = 1:1 to 1:2) to yield tripeptide **19** (2.35 g, 1.92 mmol, 75% yield over 2 steps) as a white foam.

R_f	0.3 (SiO_2, 1:2 Hex/EtOAc)
^{1}H NMR	(600 MHz, DMSO-d_6) δ 9.34 (s, 1H), 9.23 (s, 1H), 7.99 (s, 1H), 7.89 (d, *J* = 7.1 Hz, 1H), 7.82 (dtt, *J* = 9.0, 6.5, 3.3 Hz, 4H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.20 (m, 7H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.12 – 7.02 (m, 2H), 6.77 (d, *J* = 8.2 Hz, 1H), 5.31 (d, *J* = 3.0 Hz, 1H), 4.57 (ddd, *J* = 17.8, 12.3, 7.0 Hz, 3H), 4.38 (s, 2H), 3.94 (qt, *J* = 10.9, 5.0 Hz, 2H), 3.54 – 3.49 (m, 1H), 3.46 (dt, *J* = 14.1, 6.5 Hz, 3H), 3.08 (dd, *J* = 9.4, 5.6 Hz, 1H), 2.04 (s, 3H), 1.99 (s, 3H), 1.60 (dq, *J* = 19.3, 8.7 Hz, 2H), 1.43 (s, 9H), 1.37 (s, 2H), 1.05 (t, *J* = 7.2 Hz, 3H), 0.07 (s, 9H).
^{13}C{^{1}H} NMR	(151 MHz, DMSO-d_6) δ 170.1, 169.3, 168.3, 168.0, 167.9, 167.6, 155.4, 152.5, 140.0, 139.4, 138.7, 137.9, 134.4, 131.6, 128.2, 127.7, 127.44, 127.41, 127.37, 127.30, 127.05, 125.6, 123.0, 122.8, 114.3, 100.4, 94.2, 92.8, 78.9, 72.2, 69.8, 60.7, 57.9, 56.1, 52.4, 45.7, 37.0, 28.2, 27.8, 25.7, 23.3, 22.9, 13.8, −0.5.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{55}H_{65}N_5O_{12}Si^{79}BrI$, 1235.2658; found 1235.2629
IR	(ATR, neat, cm^{-1}) 3261 (w), 2176 (w), 1771 (w), 1710 (s), 1520 (m), 1367 (m), 1026 (m)
$[\alpha]_D^{23}$	(*c* = 2.0, $CHCl_3$) + 26.7°



Macrocycles 20 and *atrop*-20

The following reaction was set up under argon atmosphere

To a flask containing tripeptide **19** (200 mg, 162 µmol, 1.0 equiv.) was added $Pd(t\text{-}Bu_3P)_2$ (91 mg, 178 µmol, 1.1 equiv.). Dry and degassed MeCN (100 mL) was cannulated into the flask. Subsequent addition of Cy_2NMe (45 µL, 210 µmol, 1.3 equiv.) was followed by cannulation of dry and degassed PhMe (50 mL) into the flask. The resulting solution was sonicated for 1 minute to dissolve $Pd(t\text{-}Bu_3P)_2$ and Cy_2NMe and obtain a homogeneous solution. The reaction mixture was heated to 40 ˚C and left to stir for 5 hours at this temperature. Upon completion (determined by HPLC analysis, atropisomeric ratio 3.3:1), the crude reaction mixture was frozen using a liquid nitrogen bath and the solvent was removed on the lyopholizer (solvent removal on a rotary evaporator led to some product decomposition as the crude mixture was concentrated). The residue

was redissolved in DCM (20 mL) and the resulting solution was washed with saturated aqueous sodium thiosulfate (10 mL) and 1 M aqueous HCl (10 mL). The organic layer was transferred to a flask and stirred with an aqueous solution of *N*-Ac-Cys-OH (264 mg, 1.62 mmol, 10 equiv. in 10 mL of water) for 1 hour. The mixture was transferred to a separation funnel and the organic layer was separated and washed with brine (10 mL). Upon drying with $MgSO_4$ and removal of the solvent *in vacuo*, the obtained residue was purified by flash column chromatography (SiO_2, PhMe/acetone/MeOH = 7:1:0.1, this fraction contains the undesired atropisomer, then 6:1:0.1, this fraction contains the desired atropisomer).

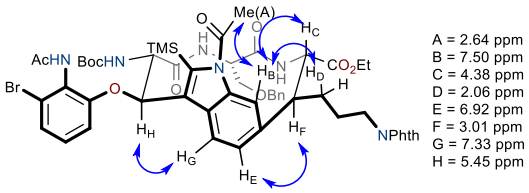
The fraction containing the desired atropisomer was further purified with preparative TLC (SiO_2, PhMe/acetone/MeOH = 3:1:0.1) to afford a white solid (**20**) (70 mg, 63 µmol, 39% yield).

The fraction containing the undesired atropisomer was further purified with preparative TLC (SiO_2, Hex/EtOAc = 1:1) to afford a white solid (***atrop*-20**) (23 mg, 21 µmol, 13% yield).
The total yield is 52%, *d.r.* = 3:1.

Desired atropisomer:

R_f	0.4 (SiO_2, 3:1:0.1 PhMe/Acetone/MeOH)
^{1}H NMR	(600 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.80 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.71 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.50 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.24 – 7.19 (m, 3H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.11 – 7.09 (m, 2H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.81 (t, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 6.51 (s, 1H), 5.45 (d, *J* = 9.9 Hz, 1H), 5.27 (d, *J* = 8.6 Hz, 1H), 4.57 – 4.51 (m, 1H), 4.43 (m, 1H), 4.38 (t, *J* = 10.6 Hz, 1H), 4.35 (d, *J* = 12.3 Hz, 1H), 4.30 (d, *J* = 12.3 Hz, 1H), 4.28 – 4.18 (m, 2H), 3.72 (t, *J* = 6.9 Hz, 2H), 3.19 (d, *J* = 7.2 Hz, 2H), 3.00 (td, *J* = 11.3, 3.8 Hz, 1H), 2.64 (s, 3H), 2.27 (s, 3H), 2.11 – 2.02 (m, 1H), 1.97 (ddt, *J* = 18.1, 12.5, 6.5 Hz, 1H), 1.83 (ddt, *J* = 12.8, 9.8, 5.0 Hz, 1H), 1.72 (ddd, *J* = 13.2, 10.6, 6.2 Hz, 1H), 1.46 (s, 9H), 1.27 (t, *J* = 7.1 Hz, 3H), 0.31 (s, 9H).
^{13}C{^{1}H} NMR	(151 MHz, $CDCl_3$) δ 171.0, 169.8, 168.4, 168.2, 167.3, 156.5, 153.7, 142.8, 139.5, 137.6, 135.9, 134.2, 132.0, 130.2, 129.7, 129.1, 128.4, 127.8, 127.7, 127.5, 126.5, 126.0, 123.4, 122.1, 117.2, 113.8, 112.2, 80.3, 73.2, 70.1, 62.0, 61.0, 59.3, 52.9, 50.2, 37.6, 28.7, 27.0, 26.7, 26.2, 23.7, 14.2, 2.3.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{55}H_{64}N_6O_{12}Si^{79}Br$, 1107.3535; found 1107.3540
IR	(ATR, neat, cm^{-1}) 3319 (br), 2978 (w), 2935 (w), 1771 (w), 1708 (s), 1663 (s), 1499 (m), 1255 (m), 1172 (m), 851 (m)
$[\alpha]_D^{23}$	(*c* = 1.40, $CHCl_3$) – 35.8˚

Key NOE correlations in the desired atropisomer:

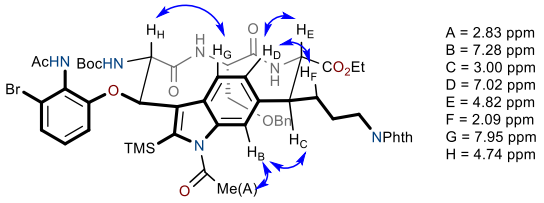


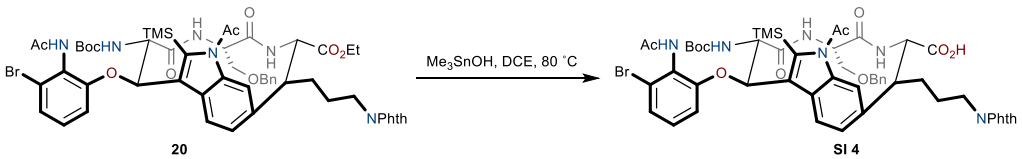
Undesired atropisomer:

R_f	0.3 (SiO_2, 5:1:0.1 PhMe/Acetone/MeOH)

^{1}H NMR	(800 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.4 Hz, 1H), 7.85 (s, 1H), 7.82 (td, *J* = 5.4, 2.9 Hz, 1H), 7.79 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.70 (ddt, *J* = 7.9, 5.3, 2.6 Hz, 1H), 7.67 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.29 – 7.26 (m, 2H), 7.25 – 7.20 (m, 3H), 7.15 – 7.13 (m, 2H), 7.09 (t, *J* = 8.2 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.64 (s, 1H), 5.84 (s, 1H), 5.50 (s, 1H), 4.83 (t, *J* = 10.2 Hz, 1H), 4.74 (s, 1H), 4.36 (d, *J* = 11.9 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H), 4.22 – 4.16 (m, 2H), 3.72 (t, *J* = 7.3 Hz, 2H), 3.51– 3.48 (m, 1H), 3.42 (t, *J* = 9.1 Hz, 1H), 3.36 (dd, *J* = 9.1, 5.6 Hz, 1H), 3.01 (td, *J* = 10.0, 4.9 Hz, 1H), 2.84 (s, 3H), 2.46 (s, 3H), 2.11 – 2.08 (m, 1H), 1.86 (dt, *J* = 14.0, 6.9 Hz, 1H), 1.76 (d, *J* = 18.9 Hz, 1H), 1.45 (d, *J* = 8.0 Hz, 9H), 1.22 (t, *J* = 7.2 Hz, 3H), 0.41 (s, 9H).
^{13}C{^{1}H} NMR	(151 MHz, $CDCl_3$) δ 171.2, 167.0, 169.3, 168.4, 168.3, 167.4, 155.2, 154.5, 137.9, 137.5, 136.9, 134.2, 132.1, 132.1, 130.5, 128.5, 128.4, 127.8, 127.6, 126.8, 126.3, 123.5, 123.3, 122.7, 122.6, 122.0, 116.8, 114.7, 81.4, 80.8, 73.4, 69.5, 62.0, 57.1, 53.8, 50.9, 37.5, 29.7, 28.5, 28.4, 27.1, 26.4, 23.7, 14.2, 3.0.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{55}H_{64}N_6O_{12}Si^{79}Br$, 1107.3535; found 1107.3527
IR	(ATR, neat, cm^{-1}) 3345 (br), 2977 (w), 2932 (w), 1771 (w), 1707 (s), 1665 (s), 1444 (m), 1396 (m), 1248 (m), 847 (m)
$[\alpha]_D^{23}$	(*c* = 0.61, $CHCl_3$) + 16.0˚

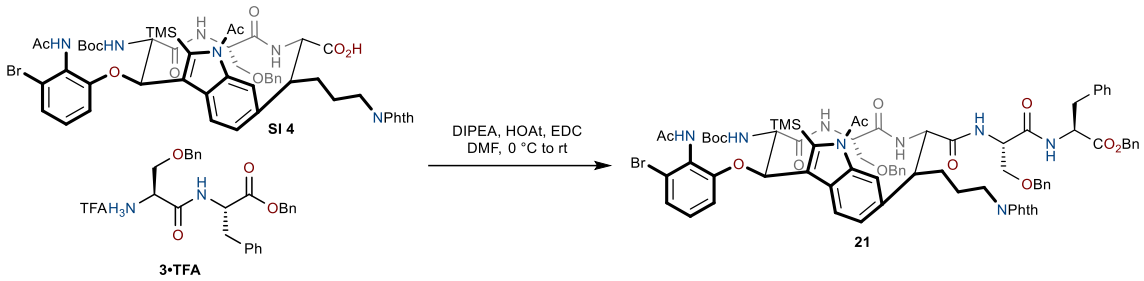
Key NOE correlations in the undesired atropisomer:





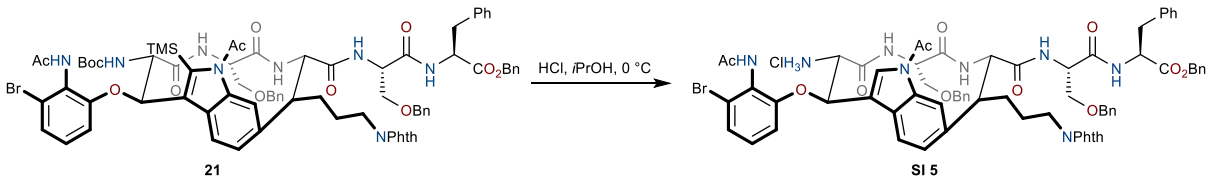
Acid SI 4

Macrocycle **20** (70 mg, 63 µmol, 1.0 equiv.) was dissolved in DCE (0.63 mL, 0.1 M). To the obtained solution was added trimethyl tin hydroxide (57 mg, 0.32 mmol, 5.0 equiv.) and the mixture was heated to 80 ˚C. After stirring for 12 hours at this temperature, the reaction was completed (monitored by HPLC) and the solvent was removed *in vacuo*. The obtained residue was redissolved in EtOAc (0.40 mL) and 1 M aqueous HCl (0.40 mL) was added. After vigorous stirring for 5 minutes, the layers were separated, and the aqueous layer was extracted with EtOAc (2 x 0.40 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to provide the crude carboxylic acid. Quantitative yield was assumed (68 mg, 63 µmol).



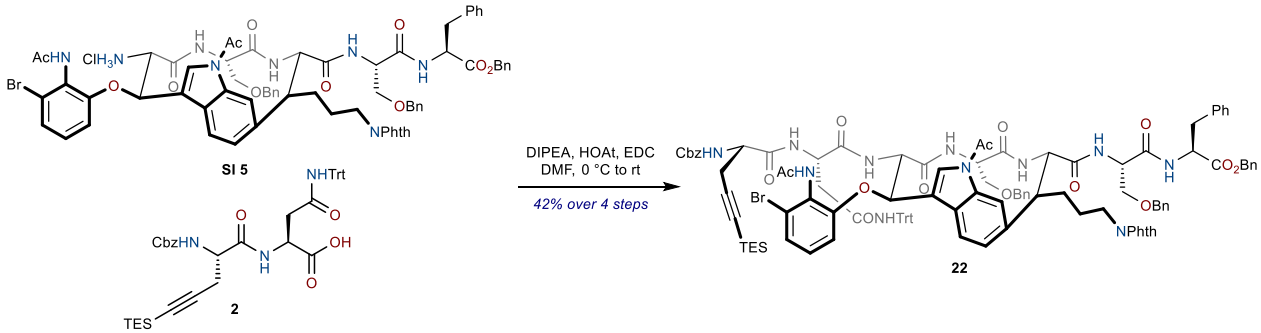
Pentapeptide 21

Acid **SI 4** (68 mg, 63 µmol, 1.0 equiv.) and ammonium salt **3•TFA** (41 mg, 76 µmol, 1.2 equiv.) were dissolved in DMF (0.63 mL, 0.10 M). To this was added DIPEA (26 µL, 0.15 mmol, 2.4 equiv.). The solution was cooled to 0 ˚C and HOAt (10 mg, 76 µmol, 1.2 equiv.) and EDC (14 mg, 76 µmol, 1.2 equiv.) were added successively. The mixture was allowed to slowly warm to room temperature and stir for 5 hours in total. Upon completion, the solution was transferred to a separation funnel and diluted with EtOAc (40 mL). This was washed with 1 M aqueous HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), water (5 mL) and brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Due to poor solubility, the crude product **21** was carried into the next step without additional purification (quantitative yield was assumed, 94 mg, 63 µmol).



Hydrochloride Salt SI 5

Pentapeptide **21** (94 mg, 63 µmol, 1.0 equiv.) was suspended in DCM (3 mL), which was followed by the addition of HCl in IPA (0.5 mL, 5.5-6 M) at 0 ˚C. After stirring for 90 minutes at 0 ˚C, the solvents were removed *in vacuo*. The residue was suspended in toluene (3 mL) and concentrated again. The obtained hydrochloride salt **SI 5** was used in the next step without additional purification (assumed quantitative yield, 85 mg, 63 µmol).

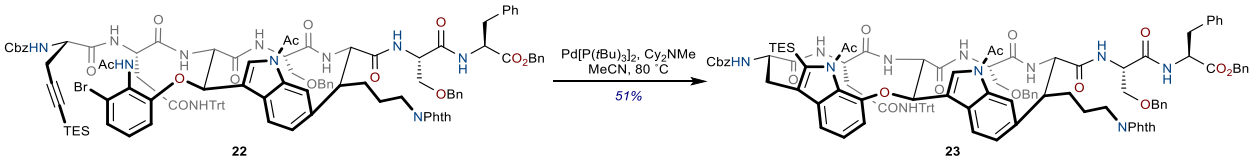


Heptapeptide 22

Ammonium salt **SI 5** (85 mg, 63 µmol, 1.0 equiv.) and acid **2** (54 mg, 75 µmol, 1.2 equiv.) were dissolved in DMF (0.63 mL, 0.10 M). To the obtained solution was added DIPEA (33 µL, 0.19 mmol, 3.0 equiv.). The solution was cooled to 0 ˚C and HOAt (10 mg, 75 µmol, 1.2 equiv.) and EDC (14 mg, 75 µmol, 1.2 equiv.) were added successively. The mixture was allowed to slowly warm to room temperature and stir for 5 hours in total. Upon completion, the reaction was diluted with EtOAc (1.0 mL) and quenched with 1 M aqueous HCl (1.0 mL). The layers were separated,

and the aqueous phase was extracted with EtOAc (2 x 1.0 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (1.0 mL), water (1.0 mL) and brine (1.0 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The obtained residue was purified by flash column chromatography (SiO_2, PhMe/acetone/MeOH = 7/1/0.1 to 3/1/0.1) to provide heptapeptide **22** (53 mg, 26 µmol, 42% over 4 steps) as a white solid.

R_f	0.3 (SiO_2, PhMe/acetone/MeOH = 3:1:0.1)
^{1}H NMR	(600 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.57 (d, *J* = 7.8 Hz, 1H), 8.45 (s, 1H), 8.41 (d, *J* = 7.4 Hz, 1H), 8.32 (s, 1H), 8.17 (s, 1H), 7.81 – 7.74 (m, 5H), 7.72 (s, 1H), 7.54 (t, *J* = 8.7 Hz, 2H), 7.37 – 7.08 (m, 45H), 7.02 (t, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 5.36 (d, *J* = 9.3 Hz, 1H), 5.06 (d, *J* = 5.5 Hz, 2H), 5.02 (d, *J* = 3.1 Hz, 2H), 4.71 – 4.65 (m, 1H), 4.60 (dq, *J* = 12.0, 7.0, 6.2 Hz, 1H), 4.55 (q, *J* = 7.3 Hz, 1H), 4.42 – 4.34 (m, 2H), 4.33 – 4.22 (m, 5H), 3.54 – 3.46 (m, 5H), 3.08 – 2.98 (m, 4H), 2.93 – 2.82 (m, 1H), 2.49 (s, 3H), 2.15 (s, 3H), 2.03 – 1.94 (m, 1H), 1.92 – 1.85 (m, 1H), 1.77 – 1.69 (m, 1H), 1.57 – 1.50 (m, 1H), 0.92 (t, *J* = 7.9 Hz, 9H), 0.50 (q, *J* = 7.9 Hz, 6H).
^{13}C{^{1}H} NMR	(151 MHz, DMSO-d_6) δ 171.0, 170.7, 169.9, 169.7, 169.5, 169.2, 168.9, 168.7, 168.0, 167.8, 166.9, 166.8, 155.8, 154.2, 144.7, 138.1, 138.0, 137.5, 136.9, 136.7, 136.1, 135.6, 134.4, 134.2, 131.7, 131.6, 129.2, 129.0, 128.7, 128.6, 128.4, 128.31, 128.27, 128.2, 128.1, 128.07, 128.01, 127.93, 127.90, 127.8, 127.63, 127.61, 127.53, 127.47, 127.37, 127.34, 127.30, 127.26, 127.18, 127.11, 126.6, 126.4, 126.3, 126.2, 124.8, 124.1, 123.5, 123.0, 122.9, 118.0, 116.7, 114.5, 112.4, 105.0, 82.3, 79.2, 73.7, 72.0, 71.5, 70.2, 69.8, 69.3, 66.1, 65.5, 59.7, 53.6, 53.4, 53.0, 51.5, 50.0, 49.2, 40.1, 37.4, 36.8, 26.9, 25.6, 23.5, 23.4, 22.9, 7.3, 4.0.
HRMS	(ES+) *m/z*: $[M+2H]^{2+}$ calcd. for ($C_{113}H_{116}{}^{79}BrN_{11}O_{18}Si$)/2, 1010.8726; found 1010.8707.
IR	(ATR, neat, cm^{-1}) 3299 (br), 3061 (w), 3031 (w), 2953 (w), 2177 (w), 1718 (s), 1679 (m), 1643 (s), 1516 (m), 698 (m).
$[\alpha]_D^{23}$	(*c* = 1.05, $CHCl_3$) + 10.5˚



Protected darobactin 23

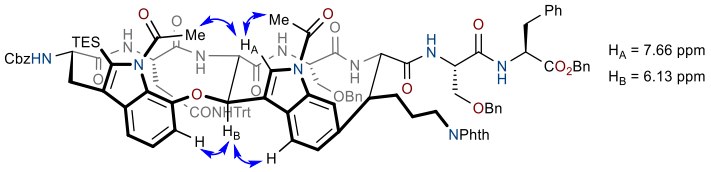
The following reaction was set up under argon atmosphere

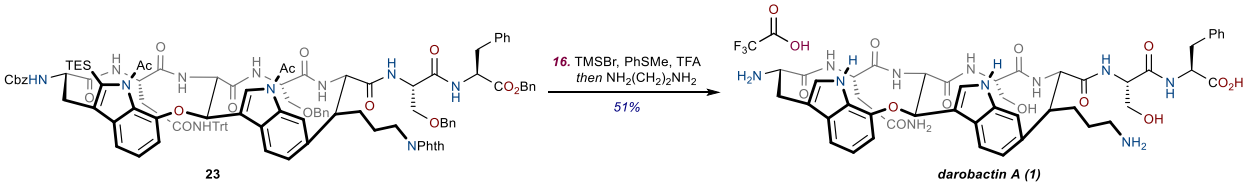
To a flask containing heptapeptide **22** (37 mg, 18 µmol, 1.0 equiv.) was added Pd(*t*-$Bu_3P)_2$ (10 mg, 20 µmol, 1.1 equiv.). Dry and degassed MeCN (18 mL, 1.0 mM) was cannulated into the flask. Cy_2NMe (12 µL, 55 µmol, 3.0 equiv.) was added and the resulting solution was heated to 80 ˚C and left to stir for 2 hours at this temperature. Upon completion (determined by TLC analysis), MeCN was removed *in vacuo* and the obtained residue was redissolved in DCM (10 mL) and transferred to a separation funnel. The DCM solution was washed with 1 M aqueous HCl (3 mL). The organic layer was transferred to a flask and stirred with an aqueous solution of *N*-Ac-Cys-OH (42 mg, 0.26 mmol, 10 equiv. in 5 mL of water) for 1 hour. The mixture was transferred to a separation funnel and the organic layer was separated and washed with brine (5 mL). Upon drying

with $MgSO_4$ and removal of the solvent *in vacuo*, the obtained residue was purified by flash column chromatography (SiO_2, PhMe/acetone/MeOH = 6/1/0.1, then 5/1/0.1) to afford protected darobactin **23** (18 mg, 9.3 µmol, 51%) as a white foamy solid.

R_f	0.3 (SiO_2, PhMe/Acetone/MeOH = 5:1:0.1)
^{1}H NMR	(600 MHz, DMSO-d_6) δ 8.57 (d, *J* = 7.8 Hz, 1H), 8.44 – 8.39 (m, 2H), 8.22 (s, 1H), 7.82 (d, *J* = 7.6 Hz, 0H), 7.81 – 7.76 (m, 4H), 7.66 (s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.41 – 7.36 (m, 5H), 7.34 – 7.30 (m, 4H), 7.28 – 7.18 (m, 22H), 7.16 – 7.10 (m, 8H), 7.09 – 7.07 (m, 1H), 7.06 (d, *J* = 7.5 Hz, 1H), 7.03 (d, *J* = 7.1 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.95 – 6.92 (m, 2H), 6.13 (d, *J* = 8.6 Hz, 1H), 5.57 (d, *J* = 5.6 Hz, 1H), 5.10 – 5.04 (m, 3H), 4.99 (d, *J* = 12.6 Hz, 1H), 4.76 (t, *J* = 9.3 Hz, 1H), 4.60 (q, *J* = 6.8 Hz, 1H), 4.55 (q, *J* = 7.3 Hz, 1H), 4.38 (d, *J* = 1.8 Hz, 2H), 4.35 – 4.25 (m, 2H), 4.08 (s, 2H), 3.88 (q, *J* = 8.2 Hz, 1H), 3.55 – 3.48 (m, 4H), 3.15 – 3.09 (m, 2H), 3.04 –3.01 (m, 2H), 2.95 (dd, *J* = 10.0, 6.6 Hz, 1H), 2.88 (dd, *J* = 10.1, 6.8 Hz, 1H), 2.77 (s, 3H), 2.73 (s, 3H), 2.03 – 1.98 (m, 1H), 1.91 – 1.85 (m, 1H), 1.80 – 1.71 (m, 1H), 1.57 (dd, *J* = 20.2, 11.7 Hz, 2H), 1.17 (m, 9H), 1.02 (m, 6H).
^{13}C{^{1}H} NMR	(151 MHz, DMSO) δ 173.0, 171.0, 170.03, 169.95, 169.3, 168.6, 168.5, 168.4, 168.0, 167.8, 166.7, 165.8, 155.35, 145.6, 145.0, 144.8, 138.0, 137.9, 137.7, 136.9, 136.7, 135.9, 135.6, 134.4, 134.2, 133.5, 133.3, 131.7, 131.6, 129.2, 129.1, 128.6, 128.4, 128.3, 128.1, 128.1, 128.03, 127.93, 127.9, 127.8, 127.53, 127.46, 127.37, 127.34, 127.2, 127.13, 127.09, 127.03, 126.95, 126.6, 126.2, 124.4, 123.0, 122.9, 122.6, 118.6, 117.5, 114.6, 112.4, 110.9, 79.2, 76.0, 72.0, 71.4, 70.4, 69.8, 69.6, 66.1, 65.6, 61.1, 59.6, 59.0, 53.6, 52.9, 51.1, 49.3, 48.8, 40.1, 38.6, 38.3, 37.5, 36.8, 28.6, 28.3, 27.0, 26.0, 23.9, 8.0.
HRMS	(ES+) *m/z*: $[M+2H]^{2+}$ calcd. for ($C_{113}H_{115}N_{11}O_{18}Si$)/2, 970.9095; found 970.9082.
IR	(ATR, neat, cm^{-1}) 3295 (br), 2931 (w), 2872 (w), 1714 (s), 1640 (s), 1496 (m), 1718 (s), 1224 (m), 698 (m).
$[\alpha]_D^{23}$	(*c* = 0.35, $CHCl_3$), – 27.4˚

Key NOE correlations in the bismacrocycle:





Darobactin A (1)

To protected darobactin **23** (20 mg, 10 μmol, 1.0 equiv.) and thioanisole (61 μL, 0.52 mmol, 50 equiv.) were added TFA (1.0 mL) and TMSBr (68 μL, 0.52 mmol, 50 equiv.) dropwise in succession at 0 °C. After stirring for 2 hours at this temperature, the mixture was concentrated under a stream of nitrogen. The obtained residue was concentrated three more times from PhMe (3 x 0.4 mL), then triturated with hexanes (3 x 0.4 mL). The crude was redissolved in MeOH (1.0 mL) and treated with ethylenediamine (69 μL, 1.0 mmol, 100 equiv.) at room temperature. After stirring for 2 hours, the mixture was concentrated under a stream of nitrogen. The crude mixture (20 mg scale) was taken up in 1.5 mL of 1:1 MeCN:H_2O + 3 drops of DMSO + 5 drops TFA to homogenize the resulting mixture, then purified by semi-preparative reverse-phase HPLC (Gilson GX-281 liquid handler w/ 333,334 pumps and UV/VIS-155 detector Gilson equipped with a Waters SunFire C_{18} OBD Prep Column, 100 Å, 5 μm, 30 mm X 150 mm). H_2O (A; +0.1 % TFA) and MeCN (B; +0.1% TFA) were used as the mobile phase with a gradient of 0-30% B over 17 minutes, holding at 26% for 3.5 minutes, 20 mL/min, t_R = 14.45-15.13 min) to afford **1•TFA** (5.5 mg, 5.1 μmol, 51%) as a fluffy white solid.

^{1}H NMR (1•TFA)	(800 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2) δ 10.63 (d, *J* = 2.6 Hz, 1H), 10.44 (d, *J* = 2.7 Hz, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 8.32 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 10.6 Hz, 1H), 7.86 – 7.81 (m, 2H), 7.50 (s, 2H), 7.47 (s, 1H), 7.46 – 7.40 (m, 3H), 7.39 – 7.30 (m, 5H), 7.23 (t, *J* = 8.5 Hz, 2H), 7.18 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 9.1 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.65 (s, 1H), 6.18 (d, *J* = 9.0 Hz, 1H), 4.47 (d, *J* = 6.8 Hz, 1H), 4.25 (t, *J* = 10.9 Hz, 1H), 4.03 (dd, *J* = 11.5, 7.7 Hz, 1H), 3.96 (q, *J* = 7.5 Hz, 1H), 3.80 (h, *J* = 6.5 Hz, 2H), 3.55 (dd, *J* = 14.3, 7.6 Hz, 1H), 3.35 – 3.27 (m, 2H), 3.23 (td, *J* = 12.5, 6.2 Hz, 2H), 3.18 – 3.09 (m, 2H), 3.01 (ddd, *J* = 23.8, 12.2, 5.4 Hz, 3H), 2.22 – 2.03 (m, 4H), 1.88 (tdd, *J* = 16.5, 11.4, 6.9 Hz, 1H), 1.74 (qdd, *J* = 13.8, 9.9, 6.4 Hz, 1H). *Note: due to water suppression in the ^{1}H NMR, signals corresponding to H-16 (4.68 ppm), H-36 (4.46 ppm), and H-39 (4.72 ppm) are hidden.*
^{13}C{^{1}H} NMR (1•TFA)	(200 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2) δ 177.5, 176.6, 174.5, 173.7, 171.2, 171.1, 170.8, 170.7, 147.9, 139.9, 139.3, 135.7, 132.2, 131.8, 131.7, 131.5, 130.0, 127.8, 127.7, 127.6, 127.3, 122.9, 120.9, 116.4, 114.4, 113.3, 111.6, 110.9, 79.5, 66.1, 64.7, 64.0, 63.0, 58.4, 57.6, 57.2, 56.9, 53.6, 51.0, 42.4, 41.8, 39.5, 29.1, 28.42, 28.38.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{47}H_{56}N_{11}O_{12}$, 966.4104; found 966.4105.
$[\alpha]_D^{23}$	(*c* = 0.055, 0.1% aqueous formic acid), + 7.20˚

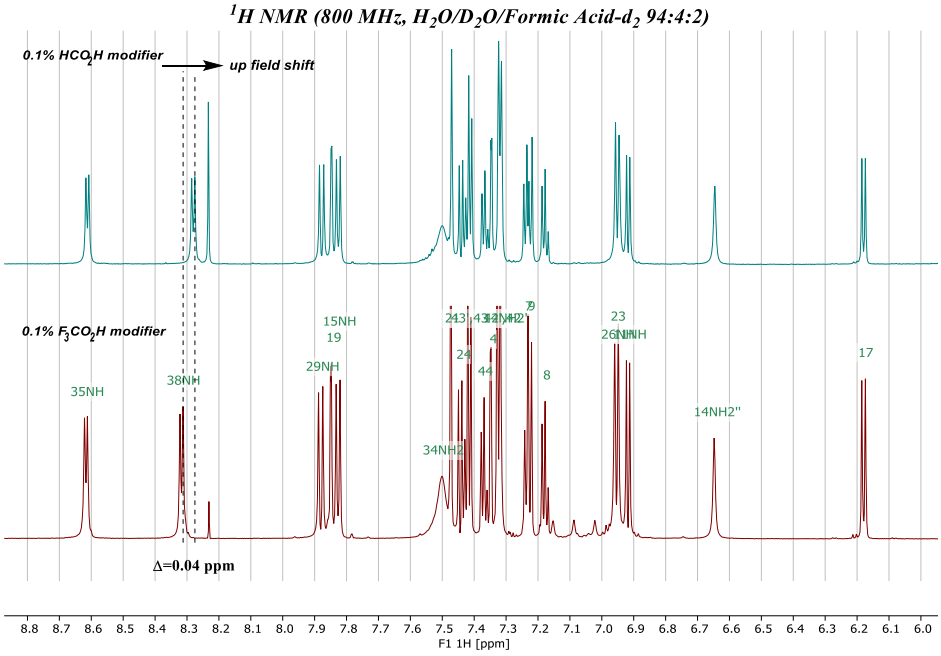
*Using the above protocol, synthetic darobactin A (**1**) was isolated as a TFA salt. The original isolation is as a formic acid salt. As a result, slight pH-dependent differences in the ^{1}H and ^{13}C NMR spectra can be seen at the C–terminus when compared to the isolated compound.[2] As such, the above purification was repeated using the following conditions:*

The crude mixture was taken up in 1.5 mL of 1:1 MeCN:H_2O + 5 drops of DMSO to homogenize the resulting mixture, then purified by semi-preparative reverse-phase HPLC (Gilson GX-281 liquid handler w/ 333,334 pumps and UV/VIS-155 detector. Gilson equipped with a Waters SunFire C18 OBD Prep Column, 100 Å, 5 μm, 30 mm X 150 mm). H_2O (A; +0.1 % HCO_2H) and

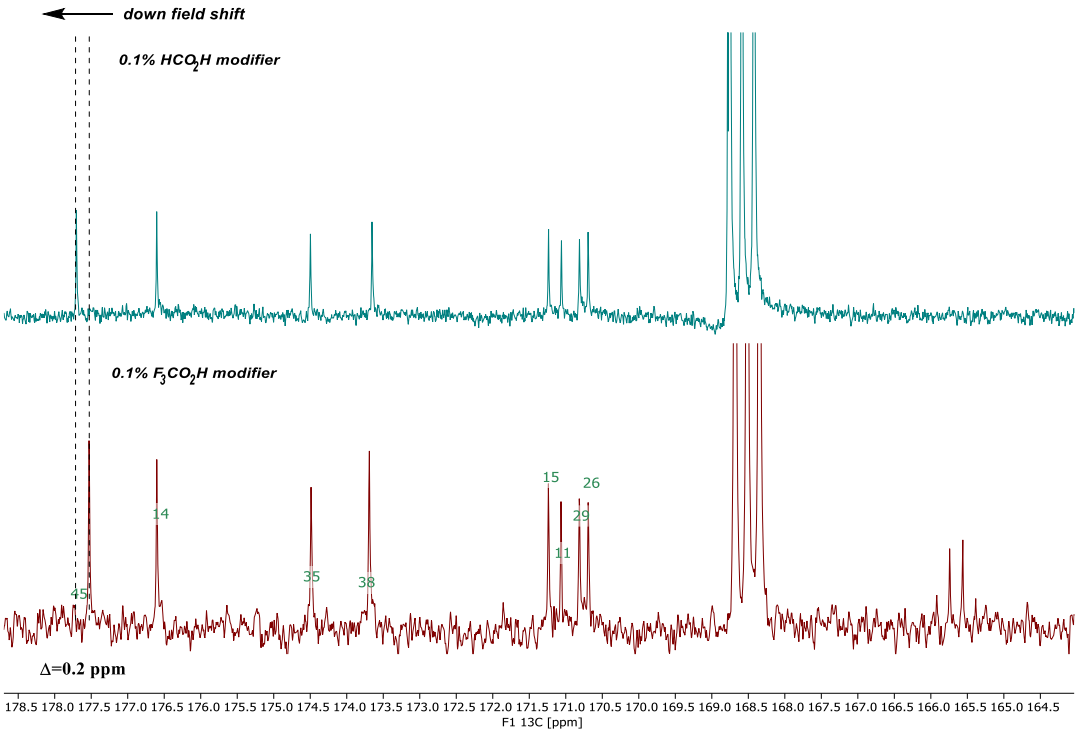
MeCN (B; +0.1% HCO_2H) were used as the mobile phase with a gradient of 0-30% B over 15 minutes, holding at 25% for 3.5 minutes, 20 mL/min, t_R = 12.25-13.50 min). The relevant fractions were concentrated via lyophilization to afford a colorless solid.

^{1}H NMR (1•HCOOH)	(800 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2) δ 10.62 (d, *J* = 2.7 Hz, 1H), 10.43 (d, *J* = 2.7 Hz, 1H), 8.61 (d, *J* = 7.2 Hz, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 10.6 Hz, 1H), 7.84 – 7.82 (m, 1H), 7.50 (s, 2H), 7.47 (s, 1H), 7.45 – 7.40 (m, 3H), 7.38 – 7.33 (m, 2H), 7.33 – 7.30 (m, 3H), 7.23 (m, 2H), 7.17 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.64 (s, 1H), 6.18 (d, *J* = 8.9 Hz, 1H), 4.45 (m, 0H), 4.24 (t, *J* = 10.9 Hz, 1H), 4.03 (dd, *J* = 11.5, 7.7 Hz, 1H), 3.94 (q, *J* = 7.2 Hz, 1H), 3.81 – 3.76 (m, 2H), 3.55 (dd, *J* = 14.4, 7.7 Hz, 1H), 3.31 (m, 2H), 3.22 (td, *J* = 9.5, 6.0 Hz, 2H), 3.15 – 3.11 (m, 2H), 3.05 – 2.94 (m, 3H), 2.20 – 2.04 (m, 4H), 1.90 – 1.85 (m, 1H), 1.75 – 1.72 (m, 1H).
^{13}C{^{1}H} NMR (1•HCOOH)	(200 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2) δ 177.7, 176.6, 174.5, 173.7, 171.2, 171.1, 170.8, 170.7, 147.9, 139.9, 139.3, 135.7, 132.2, 131.8, 131.7, 131.5, 129.9, 127.8, 127.7, 127.5, 127.3, 122.9, 120.0, 116.4, 114.4, 113.3, 111.6, 110.9, 79.5, 66.1, 64.7, 63.9, 63.0, 58.4, 57.6, 57.3, 56.9, 53.6, 51.0, 42.4, 41.8, 39.5, 29.1, 28.42, 28.38.

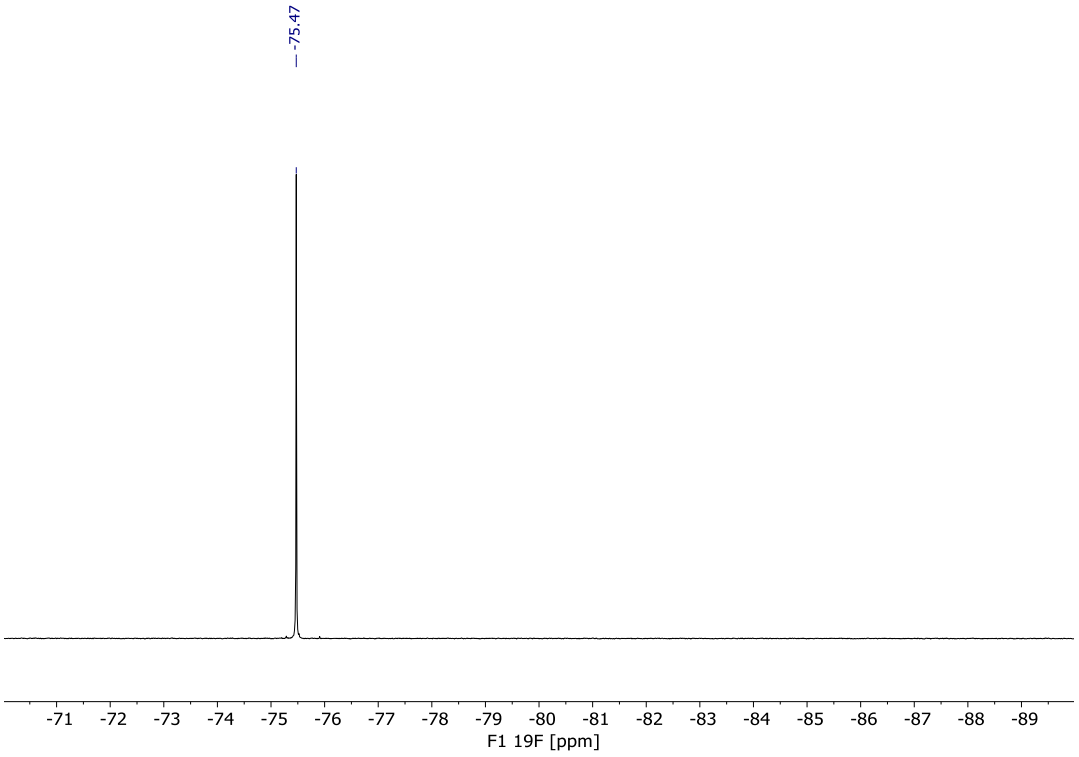
Comparison of the ^{1}H and ^{13}C NMR spectra (H_2O/D_2O/Formic Acid-d_2 94:4:2) of samples obtained from the above two purification protocols indicated shifts in two main regions, namely *NH38* (lit. 8.14 ppm)[2] and *C45* (lit. 178.4 ppm)[2]. Therein, the *NH38* shifted up field from 8.32 ppm (F_3CO_2H modifier) to 8.28 ppm (HCO_2H modifier), while *C45* shifted downfield from 177.5 ppm (F_3CO_2H modifier) to 177.7 ppm (HCO_2H modifier). Both shifts trended in the direction of the literature values, however, the remaining discrepancies can be rationalized by the presence of a partial TFA salt. This species is assumed to originate during the final deprotection steps (TFA, TMSBr and PhSMe then ethylene diamine), and is not fully converted to the formate salt during semi-prep HPLC.



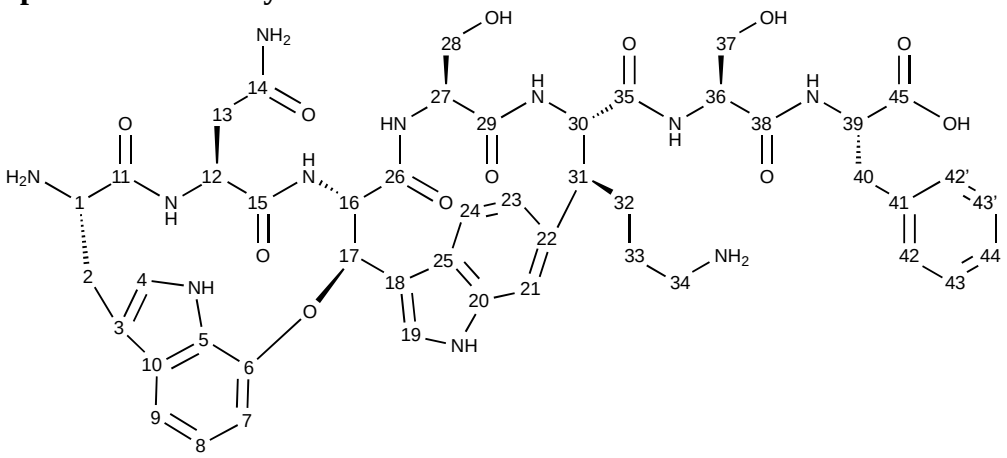
^{13}C NMR (200 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2)



^{19}F NMR (471 MHz, H_2O/D_2O/Formic Acid-d_2 94:4:2)

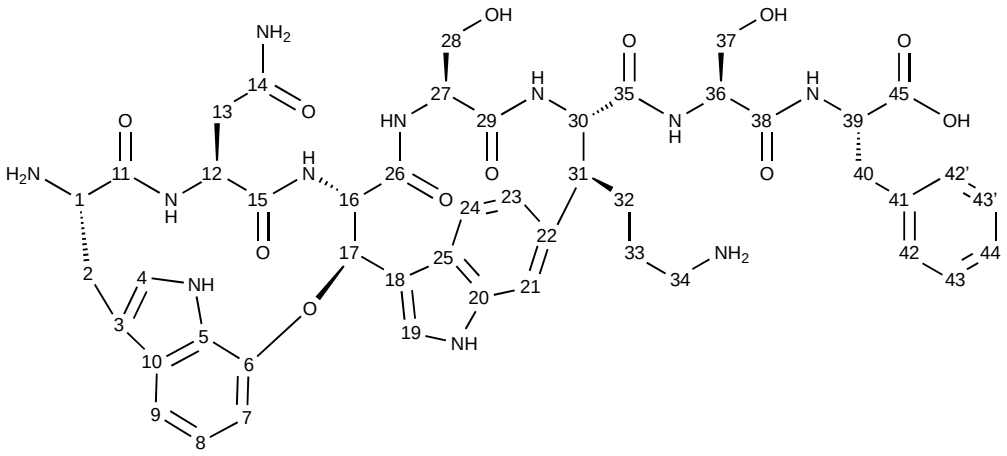


NMR comparison between synthetic and natural darobactin A



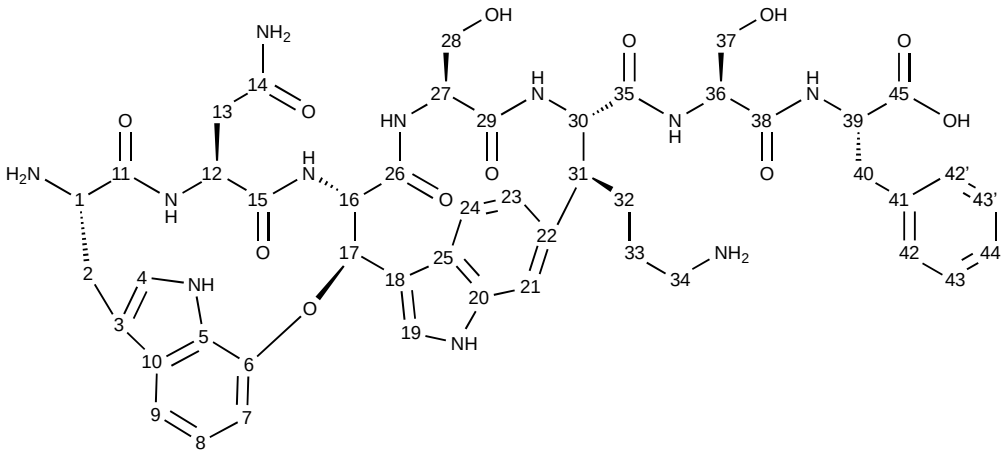
*^{1}H NMR Comparison of TFA Salt (**1•TFA**) (Taken in H_2O/D_2O/Formic acid-d_2, 94:4:2)*

#	***Synthetic***	***Isolated*[2]**	*Δ^{1}H*	*24*	7.44	7.45	-0.01
1	4.03	4.04	-0.01	*26-NH*	6.96	6.95	0.01
1-NH2				*27*	3.96	3.95	0.01
2'	3.55	3.55	0	*28'*	3.22	3.22	0
2''	3.30	3.3	0	*28''*	3.13	3.14	-0.01
4	7.35	7.35	0	*29-NH*	7.88	7.88	0
4-NH	10.63	10.63	0	*30*	4.24	4.25	-0.01
7	7.24	7.24	0	*31*	3.02	3.03	-0.01
8	7.18	7.18	0	*32*	2.08	2.08	0
9	7.23	7.22	0.01	*33'*	1.88	1.88	0
11-NH	6.92	6.92	0	*33''*	1.74	1.74	0
12	3.32	3.33	-0.01	*34*	2.99	2.99	0
13'	2.17	2.19	-0.02	*34-NH2*	7.5	7.51	-0.01
13''	2.12	2.13	-0.01	*35-NH*	8.62	8.62	0
14-NH2'	7.31	7.31	0	*36*	4.46	4.46	0
14-NH2''	6.65	6.64	0.01	*37*	3.8	3.8	0
15-NH	7.83	7.83	0	*38-NH*	8.32	8.14	0.18
16	4.68	4.69	-0.01	*39*	4.72	4.64	0.08
17	6.18	6.18	0	*40'*	3.14	3.11	0.03
19	7.85	7.85	0	*40''*	3.24	3.22	0.02
20-NH	10.44	10.44	0	*42, 42'*	7.32	7.32	0
21	7.48	7.48	0	*43, 43'*	7.42	7.42	0
23	6.96	6.96	0	*44*	7.37	7.37	0



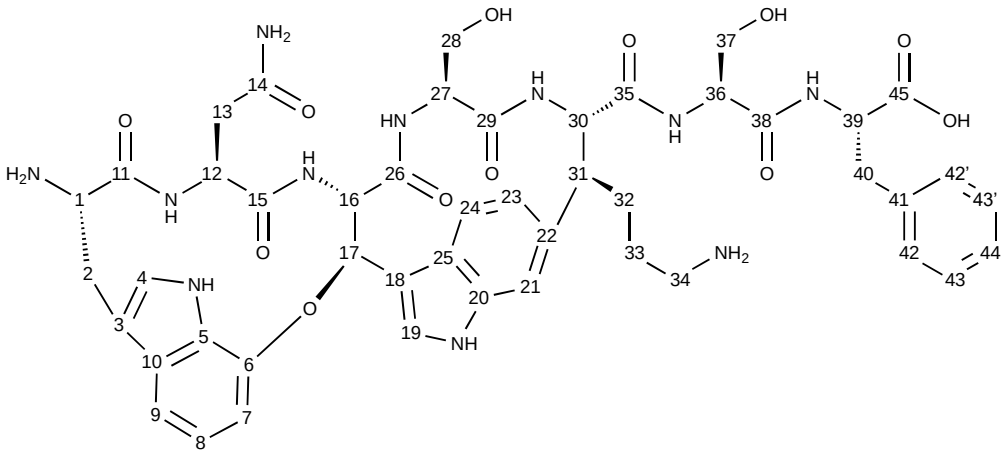
*$^{13}C\{^{1}H\}$ NMR Comparison of TFA salt (**1•TFA**) (Taken in H_2O/D_2O/Formic acid-d_2, 94:4:2)*

#	Synthetic	Isolated[2]	$\Delta^{13}C$	#	Synthetic	Isolated[2]	$\Delta^{13}C$
1	57.6	57.6	0.0	*23*	127.7	127.7	0.0
2'	29.1	29.2	-0.1	*24*	120.0	120.0	0.0
3	110.9	111	-0.1	*25*	127.8	127.8	0.0
4	127.5	127.6	-0.1	*26*	170.7	170.7	0.0
5	131.7	131.8	-0.1	*27*	56.9	56.9	0.0
6	148.0	147.9	0.1	*28'*	64.7	64.8	-0.1
7	111.6	111.6	0.0	*29*	170.8	170.9	-0.1
8	122.9	123	-0.1	*30*	63.0	63	0.0
9	116.4	116.5	-0.1	*31*	51.0	51	0.0
10	131.8	131.8	0.0	*32*	28.4	28.5	-0.1
11	171.1	171.1	0.0	*33'*	28.4	28.5	-0.1
12	53.6	53.7	-0.1	*34*	42.4	42.4	0.0
13'	41.8	41.9	-0.1	*35*	174.5	174.6	-0.1
14	176.6	176.6	0.0	*36*	58.6	58.5	0.0
15	171.2	171.3	-0.1	*37*	64.0	64	0.0
16	66.1	66.1	0.0	*38*	173.8	173.5	0.3
17	79.5	79.5	0.0	*39*	57.2	57.9	-0.7
18	114.4	114.5	-0.1	*40'*	39.5	39.8	-0.3
19	127.3	127.4	-0.1	*41*	139.3	139.6	-0.3
20	139.9	139.9	0.0	*42, 42'*	132.2	132.3	-0.1
21	113.3	113.3	0.0	*43, 43'*	131.5	131.5	0.0
22	135.7	135.7	0.0	*44*	129.9	129.9	0.0
				45	177.5	178.4	-0.9



*^{1}H NMR Comparison of formic acid salt (**1•HCOOH**) (Taken in H_2O/D_2O/Formic acid-d_2, 94:4:2)*

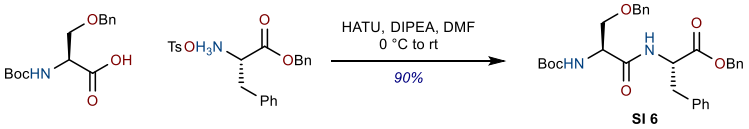
#	***Synthetic***	***Isolated*[2]**	*Δ^{1}H*
1	4.03	4.04	-0.01
1-NH2			
2'	3.55	3.55	0
2''	3.3	3.3	0
4	7.35	7.35	0
4-NH	10.63	10.63	0
7	7.24	7.24	0
8	7.18	7.18	0
9	7.22	7.22	0
11-NH	6.91	6.92	-0.01
12	3.32	3.33	-0.01
13'	2.17	2.19	-0.02
13''	2.12	2.13	-0.01
14-NH2'	7.31	7.31	0
14-NH2''	6.64	6.64	0
15-NH	7.83	7.83	0
16	4.68	4.69	-0.01
17	6.18	6.18	0
19	7.85	7.85	0
20-NH	10.43	10.44	-0.01
21	7.47	7.48	-0.01
23	6.95	6.96	-0.01
24	7.44	7.45	-0.01
26-NH	6.95	6.95	0
27	3.95	3.95	0
28'	3.22	3.22	0
28''	3.13	3.14	-0.01
29-NH	7.88	7.88	0
30	4.24	4.25	-0.01
31	3.02	3.03	-0.01
32	2.08	2.08	0
33'	1.88	1.88	0
33''	1.74	1.74	0
34	2.99	2.99	0
34-NH2	7.50	7.51	-0.01
35-NH	8.61	8.62	-0.01
36	4.45	4.46	-0.01
37	3.79	3.8	-0.01
38-NH	8.28	8.14	0.14
39	4.70	4.64	0.06
40'	3.12	3.11	0.01
40''	3.23	3.22	0.01
42, 42'	7.32	7.32	0
43, 43'	7.42	7.42	0
44	7.37	7.37	0



*$^{13}C\{^{1}H\}$ NMR Comparison of formic acid salt (**1•HCOOH**) (Taken in H_2O/D_2O/Formic acid-d_2, 94:4:2)*

#	***Synthetic***	***Isolated*[2]**	*$\Delta^{13}C$*	*23*	127.7	127.7	0.0
1	57.6	57.6	0.0	*24*	120.0	120.0	0.0
2'	29.1	29.2	-0.1	*25*	127.7	127.8	-0.1
3	110.9	111	-0.1	*26*	170.7	170.7	0.0
4	127.5	127.6	-0.1	*27*	56.9	56.9	0.0
5	131.7	131.8	-0.1	*28'*	64.7	64.8	-0.1
6	147.9	147.9	0	*29*	170.8	170.9	-0.1
7	111.6	111.6	0.0	*30*	63.0	63	0.0
8	122.9	123	-0.1	*31*	51.0	51	0.0
9	116.4	116.5	-0.1	*32*	28.4	28.5	-0.1
10	131.8	131.8	0.0	*33'*	28.4	28.5	-0.1
11	171.1	171.1	0.0	*34*	42.4	42.4	0.0
12	53.6	53.7	-0.1	*35*	174.5	174.6	-0.1
13'	41.8	41.9	-0.1	*36*	58.4	58.5	-0.1
14	176.6	176.6	0.0	*37*	63.9	64	-0.1
15	171.2	171.3	-0.1	*38*	173.7	173.5	0.2
16	66.1	66.1	0.0	*39*	57.3	57.9	-0.6
17	79.5	79.5	0.0	*40'*	39.5	39.8	-0.3
18	114.4	114.5	-0.1	*41*	139.3	139.6	-0.3
19	127.3	127.4	-0.1	*42, 42'*	132.2	132.3	-0.1
20	139.9	139.9	0.0	*43, 43'*	131.5	131.5	0.0
21	113.3	113.3	0.0	*44*	129.9	129.9	0.0
22	135.7	135.7	0.0	*45*	177.7	178.4	-0.7

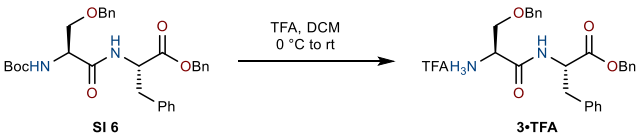
Sequence for the Synthesis of Sidechain 3•TFA



Protected sidechain SI 6

Boc-*L*-Ser(OBn)-OH (2.00 g, 6.77 mmol, 1.0 equiv.) and H-Phe-OBn•TsOH (2.90 g, 6.77 mmol, 1.0 equiv.) were dissolved in DMF (34 mL). The solution was cooled to 0 ˚C, followed by sequential addition of DIPEA (1.77 mL, 10 mmol, 1.5 equiv.) and HATU (2.57 g, 6.77 mmol, 1.0 equiv.). The mixture was left to slowly warm up to room temperature and stir for 5 hours. The reaction was quenched by the addition of 1 M aqueous HCl (150 mL). The obtained mixture was transferred to a separation funnel and extracted with EtOAc (3 x 150 mL). The organic layers were combined, washed with saturated aqueous $NaHCO_3$ (150 mL), H_2O (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (SiO_2, Hex/EtOAc = 3:1) to afford the dipeptide **SI 6** (3.25 g, 6.77 mmol, 90%) as a clear oil that solidified upon standing.

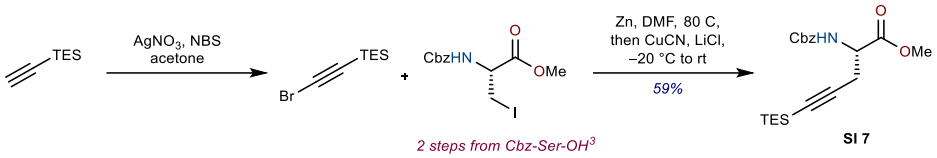
R_f	0.5 (SiO_2, Hex/EtOAc, 3:1)
^{1}H NMR	(600 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 6H), 7.30 – 7.25 (m, 4H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.13 (dd, *J* = 8.2, 6.7 Hz, 2H), 7.08 (s, 1H), 6.99 – 6.94 (m, 2H), 5.36 (d, *J* = 6.8 Hz, 1H), 5.14 (d, *J* = 12.1 Hz, 1H), 5.10 (d, *J* = 12.1 Hz, 1H), 4.89 (q, *J* = 6.4 Hz, 1H), 4.51 (d, *J* = 11.9 Hz, 1H), 4.46 (d, *J* = 11.6 Hz, 1H), 4.29 (s, 1H), 3.93 – 3.88 (m, 1H), 3.52 (dd, *J* = 9.2, 6.7 Hz, 1H), 3.13 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.06 (dd, *J* = 13.9, 5.8 Hz, 1H), 1.43 (s, 9H).
^{13}C{^{1}H} NMR	(151 MHz, $CDCl_3$) δ 171.0, 170.1, 155.5, 137.4, 135.7, 135.2, 129.4, 128.7, 128.6, 128.6, 128.0, 128.0, 127.1, 80.3, 73.5, 69.9, 67.3, 53.8, 53.6, 37.8, 28.4.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{31}H_{37}N_2O_6$, 533.2652; found 533.2646
IR	(ATR, neat, cm^{-1}) 3320 (w), 2977 (w), 2176 (w), 1674 (s), 1497 (s), 1167 (s), 1111 (m)
$[\alpha]_D^{23}$	(*c* = 3.0, $CHCl_3$) + 15.0˚



Ammonium salt 3•TFA

Boc protected dipeptide **SI 6** (3.25 g, 6.77 mmol, 1.0 equiv.) was dissolved in DCM (30 mL, 0.2 M) and cooled to 0 ˚C. To this was added TFA (9.40 mL, 122 mmol, 20 equiv.). The reaction was allowed to warm to room temperature and stirred until complete (about 4 hours). The solvent was removed on a rotary evaporator and the residue was redissolved in toluene. The toluene was removed, and this process was repeated two more times to remove residual TFA. The ammonium salt **3•TFA** was isolated as a white solid (assumed quantitative yield, 3.24 g, 6.10 mmol) that was used without further purification.

Sequence for the Synthesis of Dipeptide 2

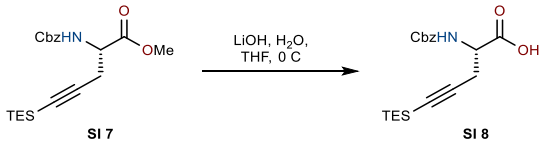


Alkyne SI 7

The following procedure was adapted from a previous report[3]

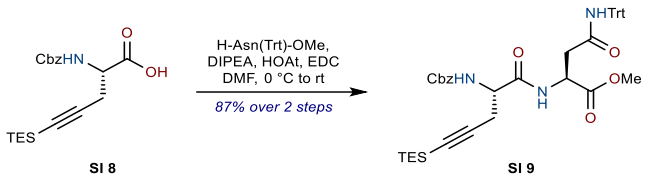
Triethyl(ethynyl)silane (1.17 g, 8.33 mmol, 1.4 equiv.) was dissolved in acetone (28 mL, 0.3 M). Silver nitrate (141 mg, 833 µmol, 0.14 equiv.) and recrystallized NBS (1.59 g, 8.92 mmol, 1.5 equiv.) were added successively, each in a single portion. After 2 hours, the reaction mixture was quenched by its addition to ice water (20 mL), extracted with pentane (2 × 20 mL), washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. The crude material was used directly in the coupling reaction.

To a flask containing the zinc dust (1.40 g, 21.4 mmol, 3.6 equiv.) was added DMF (6 mL), followed by 1,2-dibromoethane (103 µL, 1.19 mmol, 0.2 equiv.). The suspension was heated at 80 ˚C for 30 minutes. After cooling to room temperature, distilled TMSCl (75.5 µL, 595 µmol, 0.1 equiv.) was added and the suspension was stirred an additional 30 minutes at room temperature. To this suspension was added Cbz-iodoserine methyl ester[4] (2.16 g, 5.95 mmol, 1.0 equiv.) in DMF (4 mL) over 2 minutes, which resulted in an exotherm. After returning to room temperature, stirring was ceased and the alkyl zinc reagent was transferred dropwise via cannula to a cooled (–20 ˚C) solution of CuCN (479 mg, 5.35 mmol, 0.9 equiv.) and LiCl (454 mg, 10.7 mmol, 1.8 equiv.) in DMF (10 mL; 0.25 M total concentration relative to alkyl iodide). After a period of 15 minutes, neat 1–bromo-2–triethylsilylacetylene was added dropwise to the reaction mixture at the same temperature. The reaction mixture was then allowed to slowly warm to room temperature over a 3 hour period and stirring was continued at that temperature overnight. At this time, the reaction was quenched by the addition of water (100 mL) and extracted with Et_2O (4 × 25 mL). The organic extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (SiO_2, Hex/EtOAc = 5:1) to yield alkyne **SI 7** (1.32 g, 3.51 mmol, 59% based on iodoserine). Characterization data matched that of a previous report.[5]



Acid SI 8

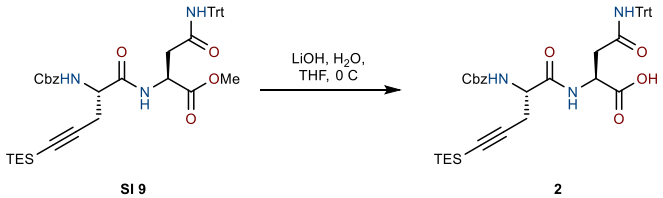
Alkyne **SI 7** (1.60 g, 4.26 mmol, 1.0 equiv.) was dissolved in THF (21 mL) and water (10 mL) and cooled to 0 ˚C. A 1 M aqueous solution of LiOH (153 mg, 6.39 mmol ,6.39 mL, 1.5 equiv.) was added and the reaction was allowed to warm to room temperature. After 1 hour, the reaction was complete (by TLC analysis), and the reaction was quenched by the slow addition of 1 M aqueous HCl until the pH was around 4. The reaction mixture was extracted with EtOAc (3 x 70 mL), the organic layers were combined, washed with brine (50 mL), dried with $MgSO_4$, and concentrated. The crude acid **SI 8** was used without further purification (assumed quantitative yield, 1.54 g, 4.26 mmol).



Methyl Ester SI 9

Acid **SI 8** (1.54 g, 4.26 mmol, 1.0 equiv.) and H-Asn(Trt)-OMe (1.82 g, 4.69 mmol, 1.1 equiv.) were dissolved in DMF (45 mL) (0.1 M) and cooled to 0 ˚C. To this solution was added DIPEA (1.78 mL, 10.2 mmol, 2.4 equiv.), HOAt, (696 mg, 5.11 mmol, 1.2 equiv.), and EDC (980 mg, 5.11 mmol, 1.2 equiv.) in that order. The mixture was allowed to warm to room temperature and left to stir overnight. The reaction was quenched by addition of aqueous 1 M HCl (60 mL) and the mixture was transferred to a separatory funnel. The layers were separated and the aquesous layer was extracted with ethyl acetate (3 x 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (40 mL), water (40 mL), and brine (40 mL), followed by drying over $MgSO_4$, filtration and removal of the solvent *in vacuo*. The crude product was purified by flash column chromatography (SiO_2, Hex/EtOAc = 2:1 to 1:1) to yield the methyl ester (**SI 9**) (2.72 g, 3.72 mmol, 87% yield over 2 steps) as a white solid.

R_f	0.2 (SiO_2, 2:1 Hex/EtOAc)
^{1}H NMR	(600 MHz, $CDCl_3$) δ 7.28 (tt, *J* = 27.2, 5.9 Hz, 14H), 7.16 (d, *J* = 7.8 Hz, 7H), 6.70 (s, 1H), 5.44 (d, *J* = 7.8 Hz, 1H), 5.08 (q, *J* = 12.2 Hz, 2H), 4.79 (dt, *J* = 8.8, 4.4 Hz, 1H), 4.29 (q, *J* = 6.7 Hz, 1H), 3.65 (s, 3H), 3.06 (dd, *J* = 15.9, 4.2 Hz, 1H), 2.76 (td, *J* = 16.8, 5.0 Hz, 2H), 2.65 (dd, *J* = 17.2, 7.0 Hz, 1H), 0.94 (t, *J* = 7.9 Hz, 9H), 0.54 (q, *J* = 7.9 Hz, 6H).
^{13}C{^{1}H} NMR	(151 MHz, $CDCl_3$) δ 171.04, 169.87, 169.30, 155.93, 144.38, 136.28, 128.75, 128.62, 128.24, 128.17, 127.33, 102.05, 85.84, 71.06, 67.24, 53.54, 52.86, 49.24, 38.32, 24.26, 7.57, 4.43.
HRMS	(ES+) *m/z*: $[M+H]^+$ calcd. for $C_{55}H_{64}N_6O_{12}Si^{79}Br$, 1107.3535; found 1107.3540
IR	(ATR, neat, cm^{-1}) 3317 (w), 2953 (w), 2176 (w), 1732 (m), 1665 (s), 1494 (s), 1216 (m), 1045 (w)
$[\alpha]_D^{23}$	(*c* = 4.0, $CHCl_3$) + 62.3˚



Dipeptide 2

Methyl ester **SI 9** (2.72 g, 3.72 mmol, 1.0 equiv.) was dissolved in THF/water (3:1, 40 mL total, 0.1 M) and cooled to 0 ˚C. To this was added a 1 M aqueous solution of LiOH (222 mg, 9.29 mmol, 9.29 mL, 2.5 equiv.) dropwise. The reaction was allowed to stir at 0 ˚C until complete (about 1 hour, monitored by TLC). The reaction was quenched by addition of 1 M aqueous HCl (until pH ~ 4) and diluted with EtOAc (80 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 80 mL). The combined organic layers were combined, washed with

brine, dried over $MgSO_4$, filtered and concentrated. The crude acid **2** was used without further purification.

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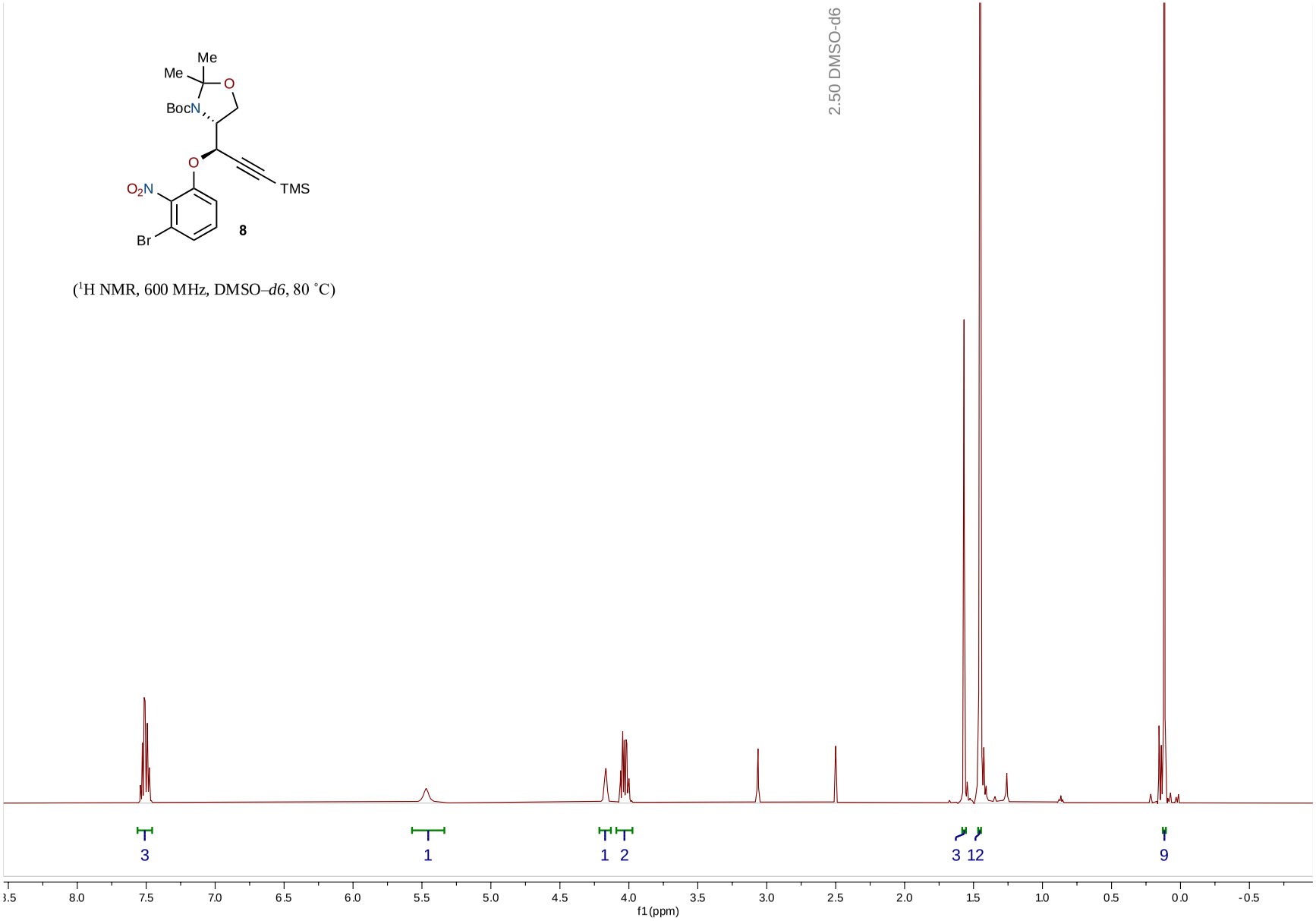
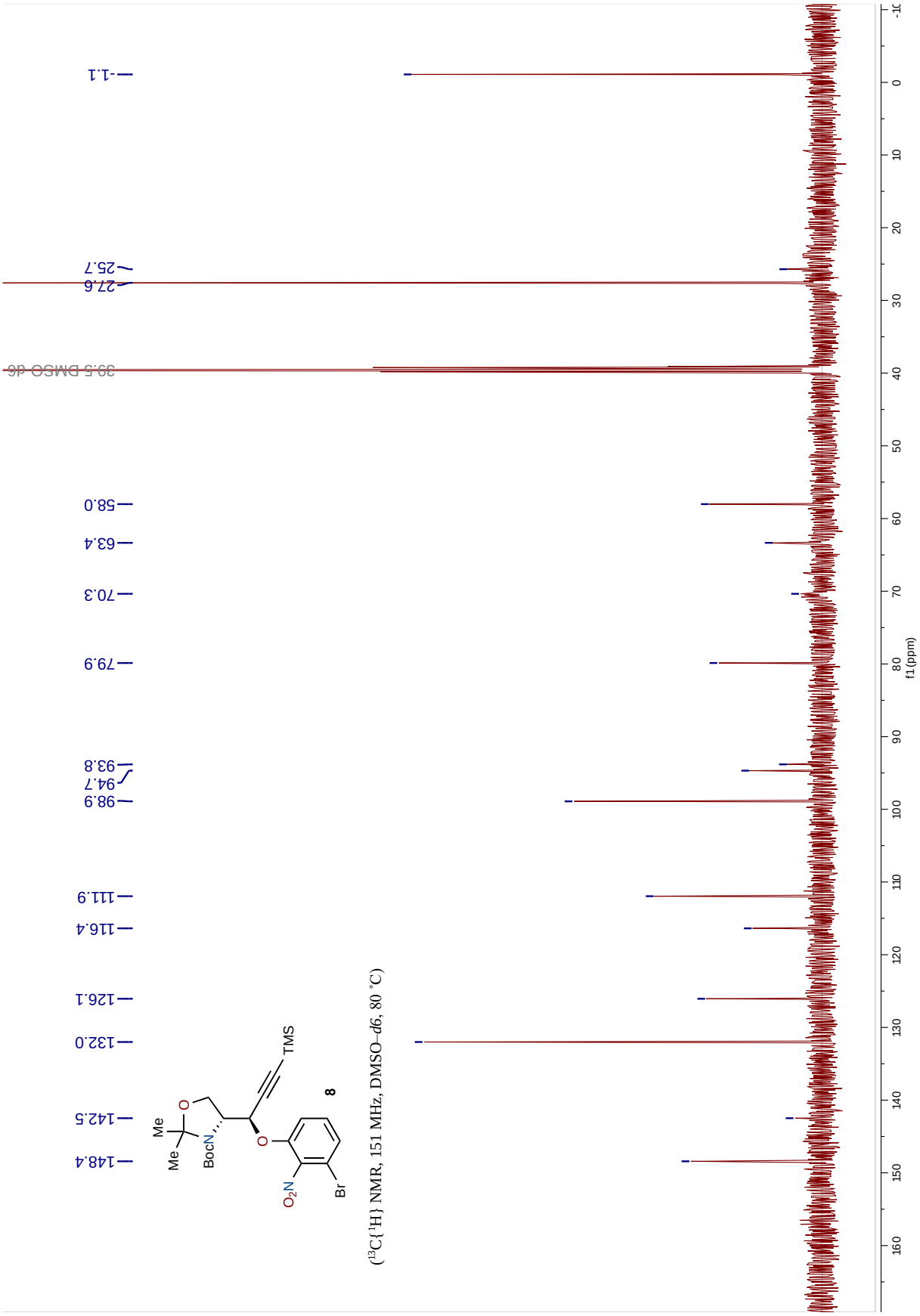
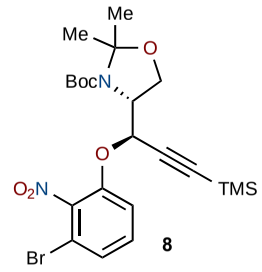
Spectroscopic Data



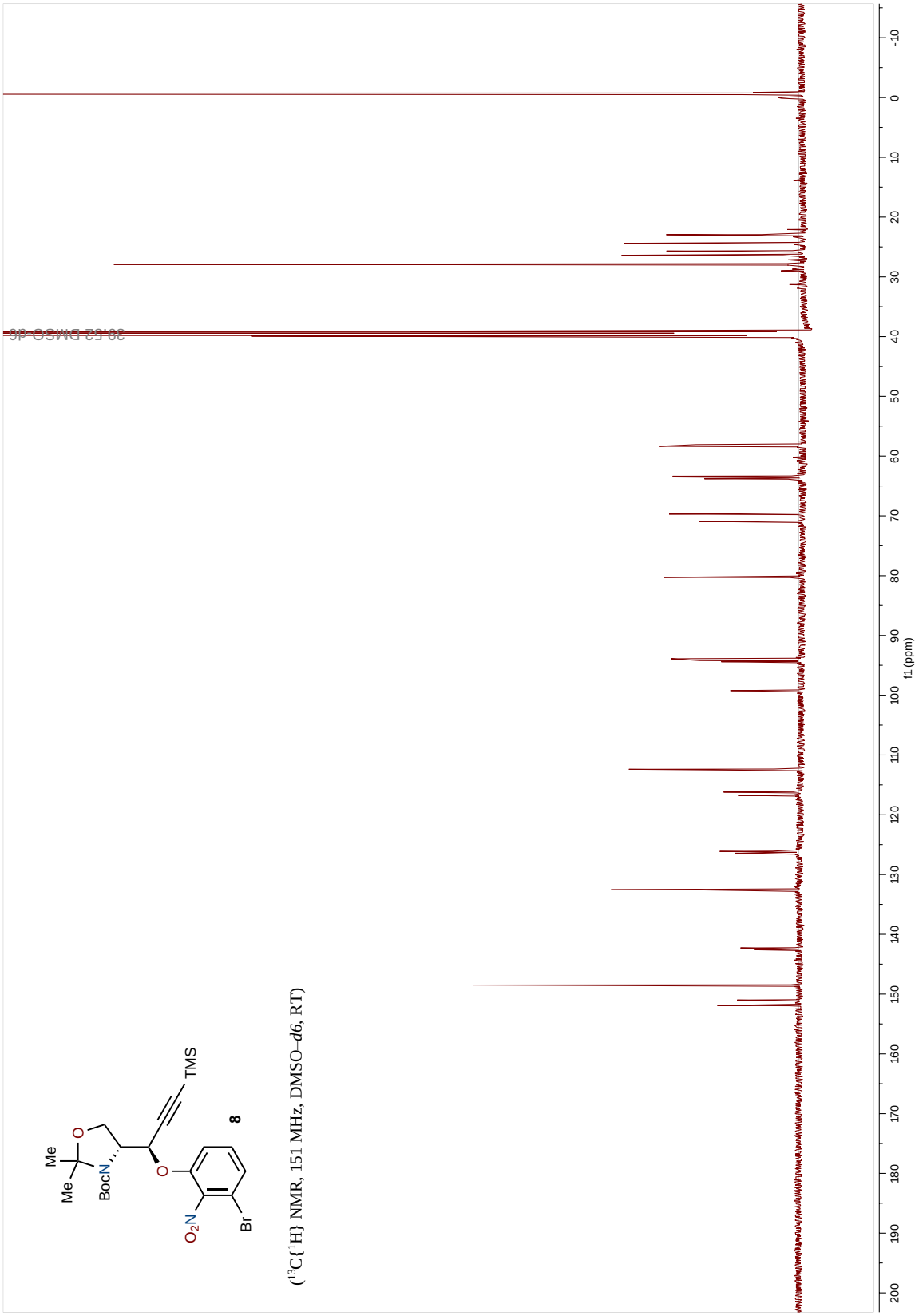
(^{1}H NMR, 600 MHz, DMSO–*d6*, 80 ˚C)

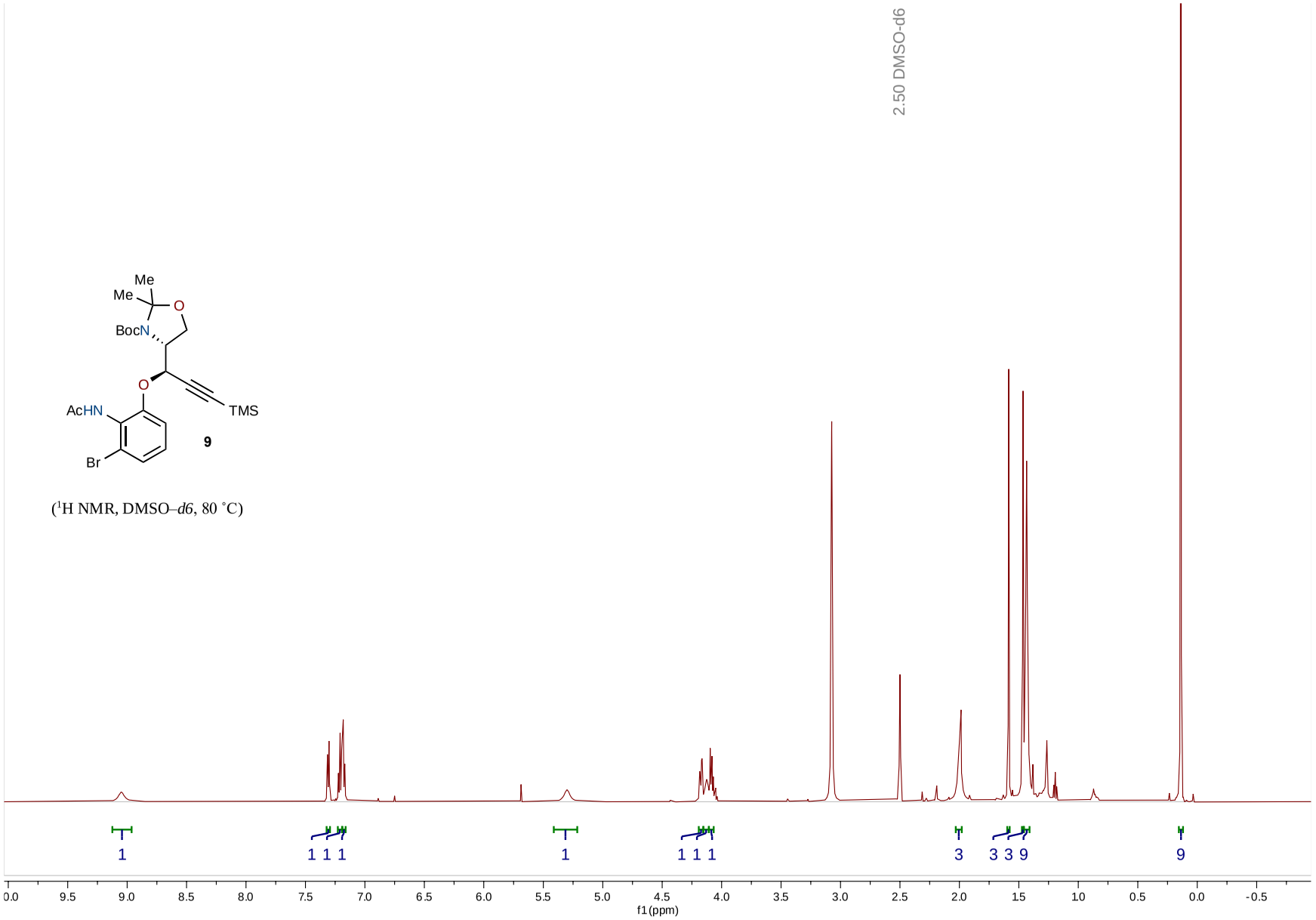

-1.1
25.7
27.6
39.5 DMSO-d6
58.0
63.4
70.3
79.9
93.8
94.7
98.9
111.9
116.4
126.1
132.0
142.5
148.4
f1 (ppm)
(13C{1H} NMR, 151 MHz, DMSO–d6, 80 °C)
8
TMS
Me
Me
BocN
O
O
O2N
Br



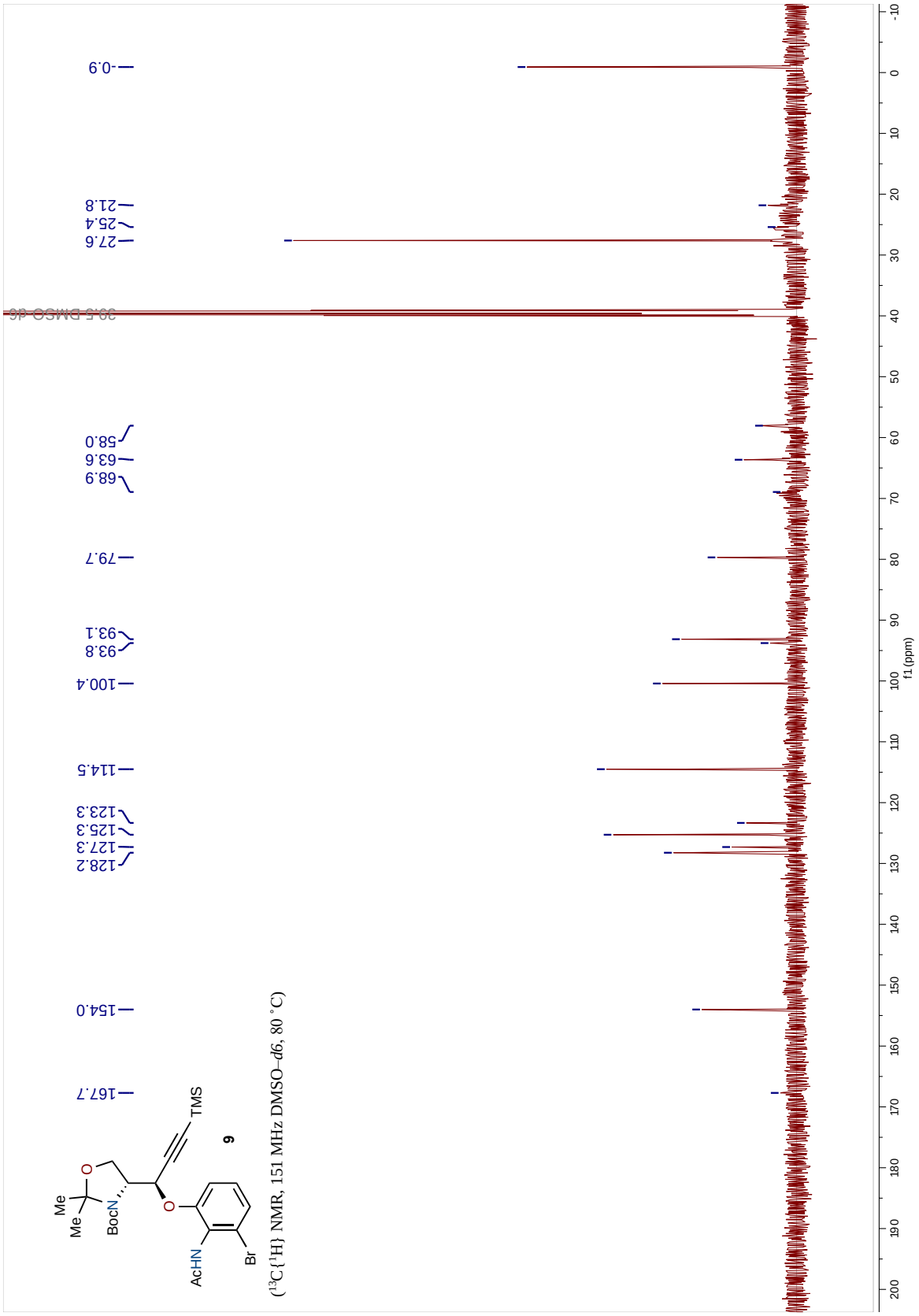
8

(^{1}H NMR, 600 MHz, DMSO–*d6*, RT)


Me
Me
O
BocN
O
TMS
O2N
Br
8
(^{13}C{^{1}H} NMR, 151 MHz, DMSO–*d6*, RT)
39.52 DMSO -d6
f1 (ppm)



(^{1}H NMR, DMSO–*d6*, 80 ˚C)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21.8
25.4
27.6
39.5 DMSO-d6
58.0
63.6
68.9
79.7
93.1
93.8
100.4
114.5
123.3
125.3
127.3
128.2
154.0
167.7
f1 (ppm)
Me
Me
BocN
O
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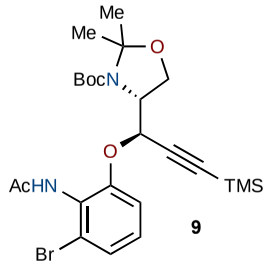
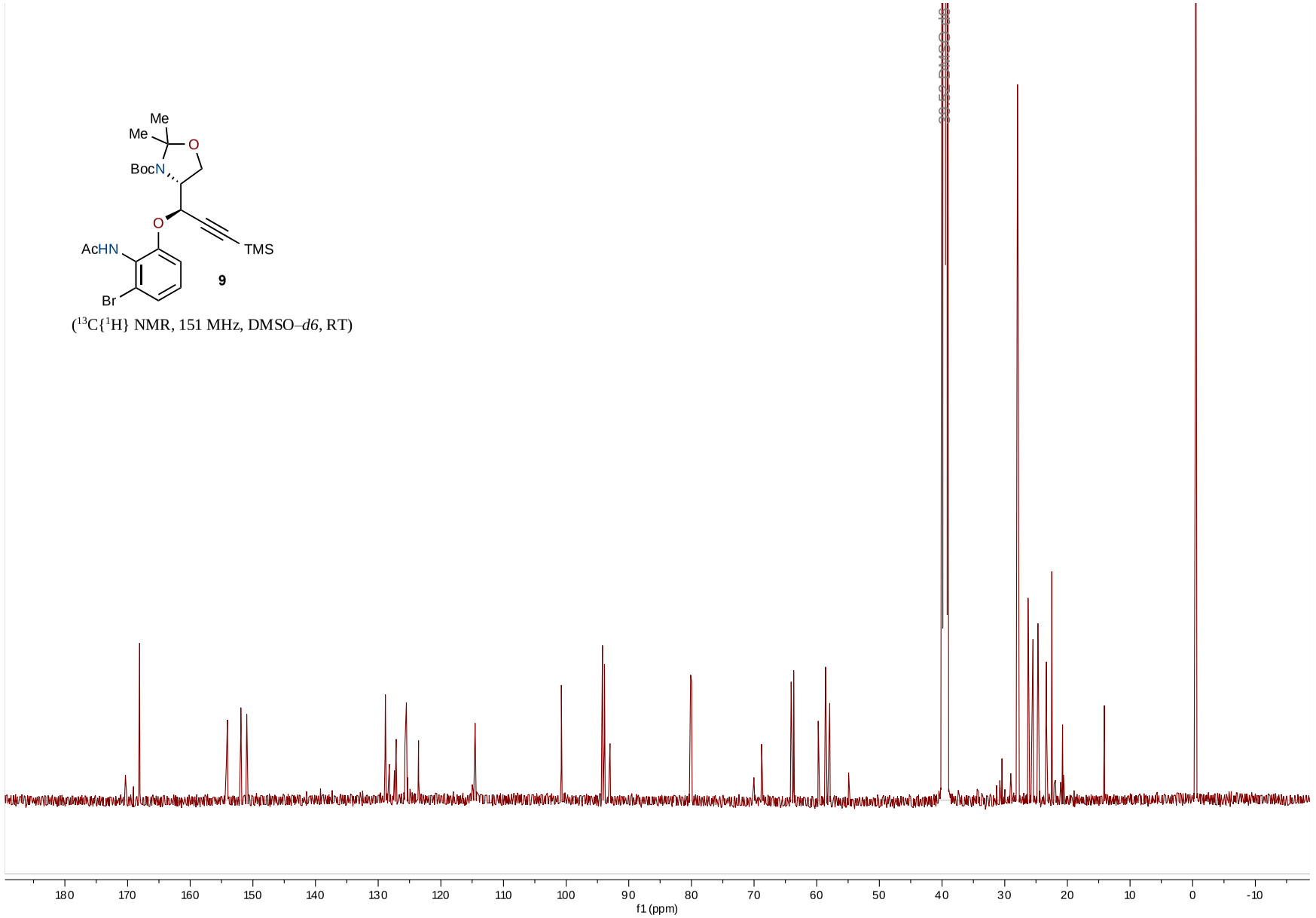
AcHN
Br
TMS
9
($^{13}C\{^{1}H\}$ NMR, 151 MHz DMSO-*d6*, 80 °C)

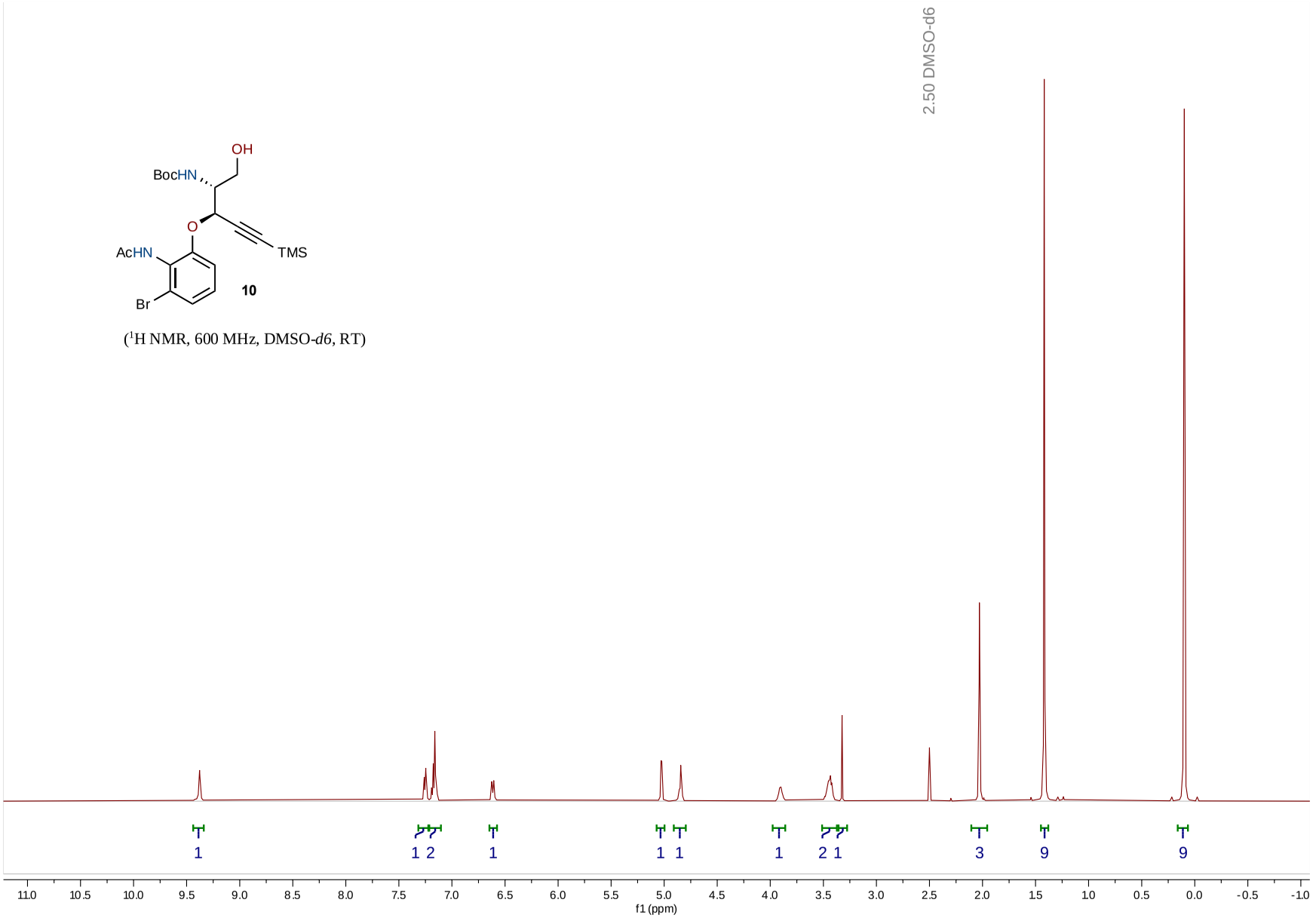


9

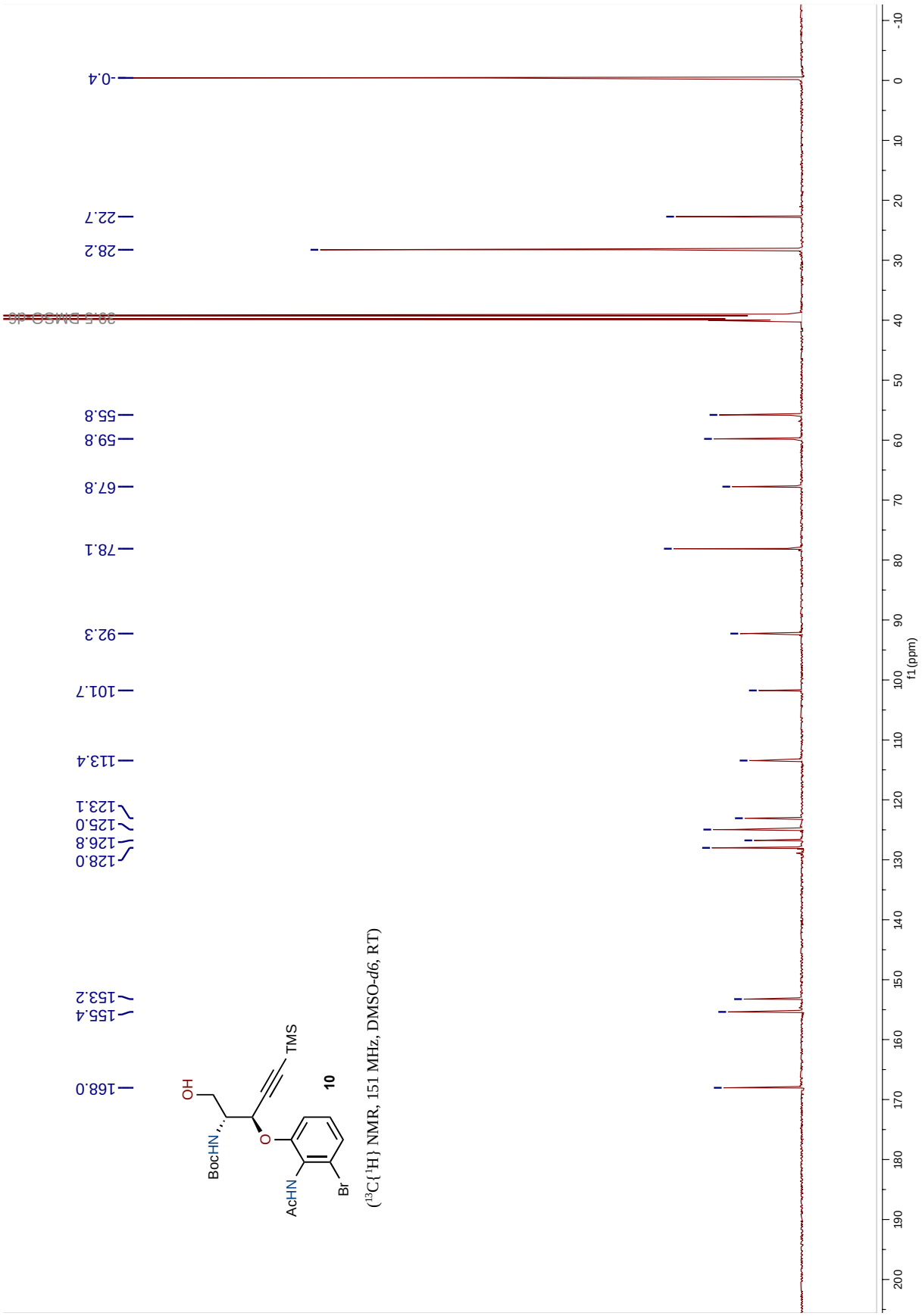
(^{1}H NMR, 600 MHz, DMSO–*d6*, RT)



($^{13}C\{^{1}H\}$ NMR, 151 MHz, DMSO–*d6*, RT)



(^{1}H NMR, 600 MHz, DMSO-*d6*, RT)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28.2
39.5 DMSO-d6
55.8
59.8
67.8
78.1
92.3
101.7
113.4
123.1
125.0
126.8
128.0
153.2
155.4
168.0
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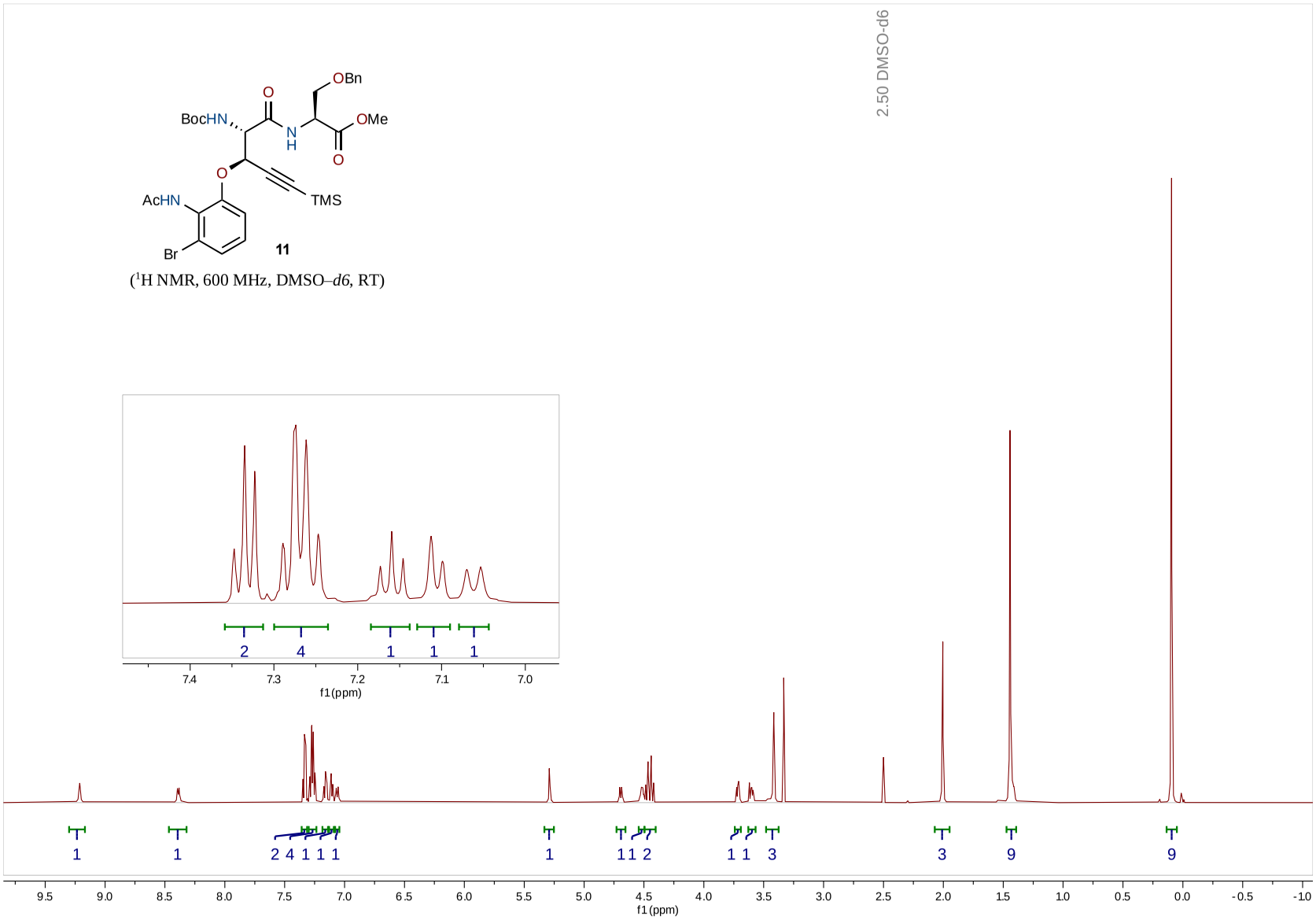
BocHN
O
TMS
AcHN
Br
10
(13C{1H} NMR, 151 MHz, DMSO-d6, RT)
f1 (ppm)


OBn
O
BocHN
N
H
OMe
O
O
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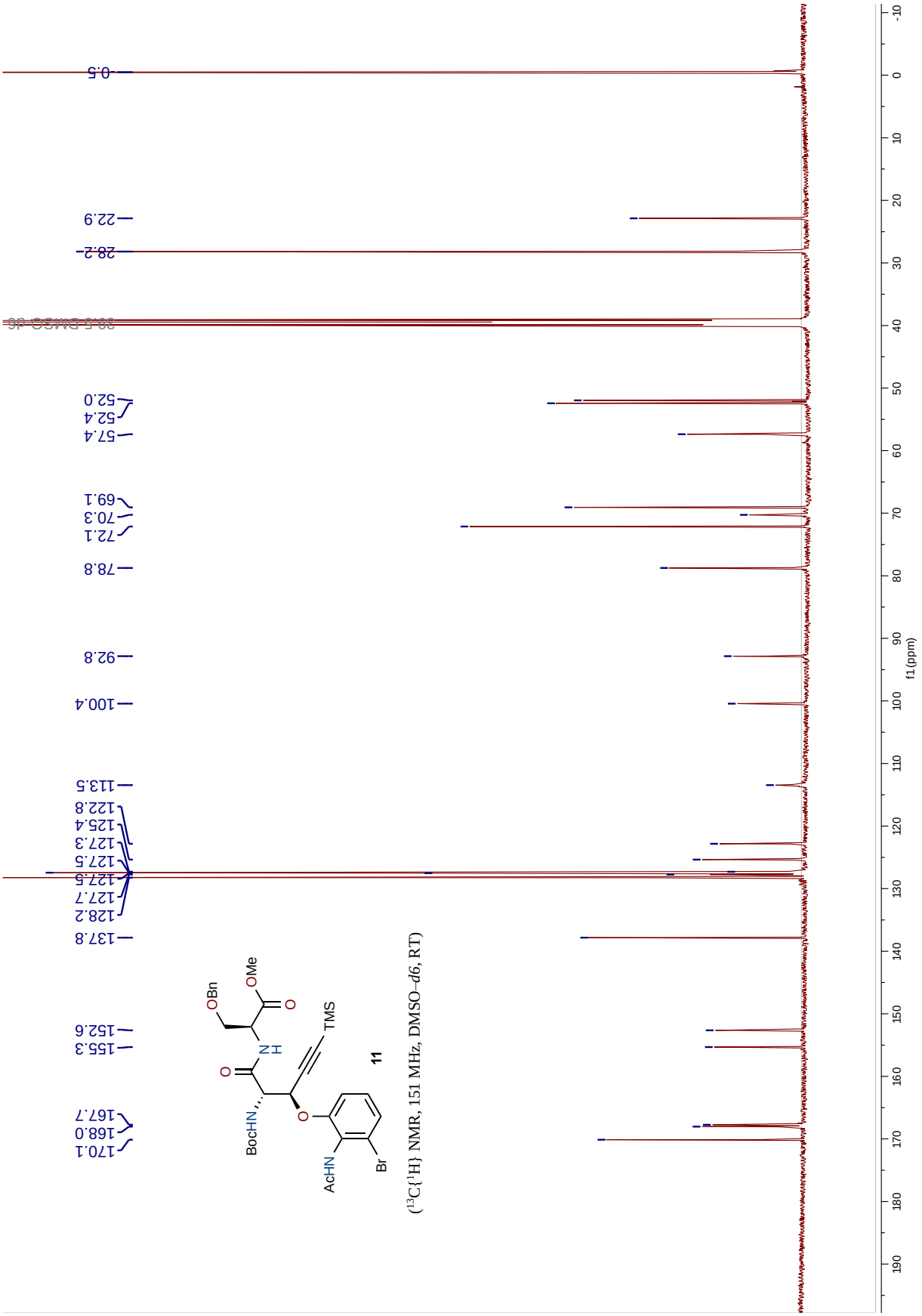
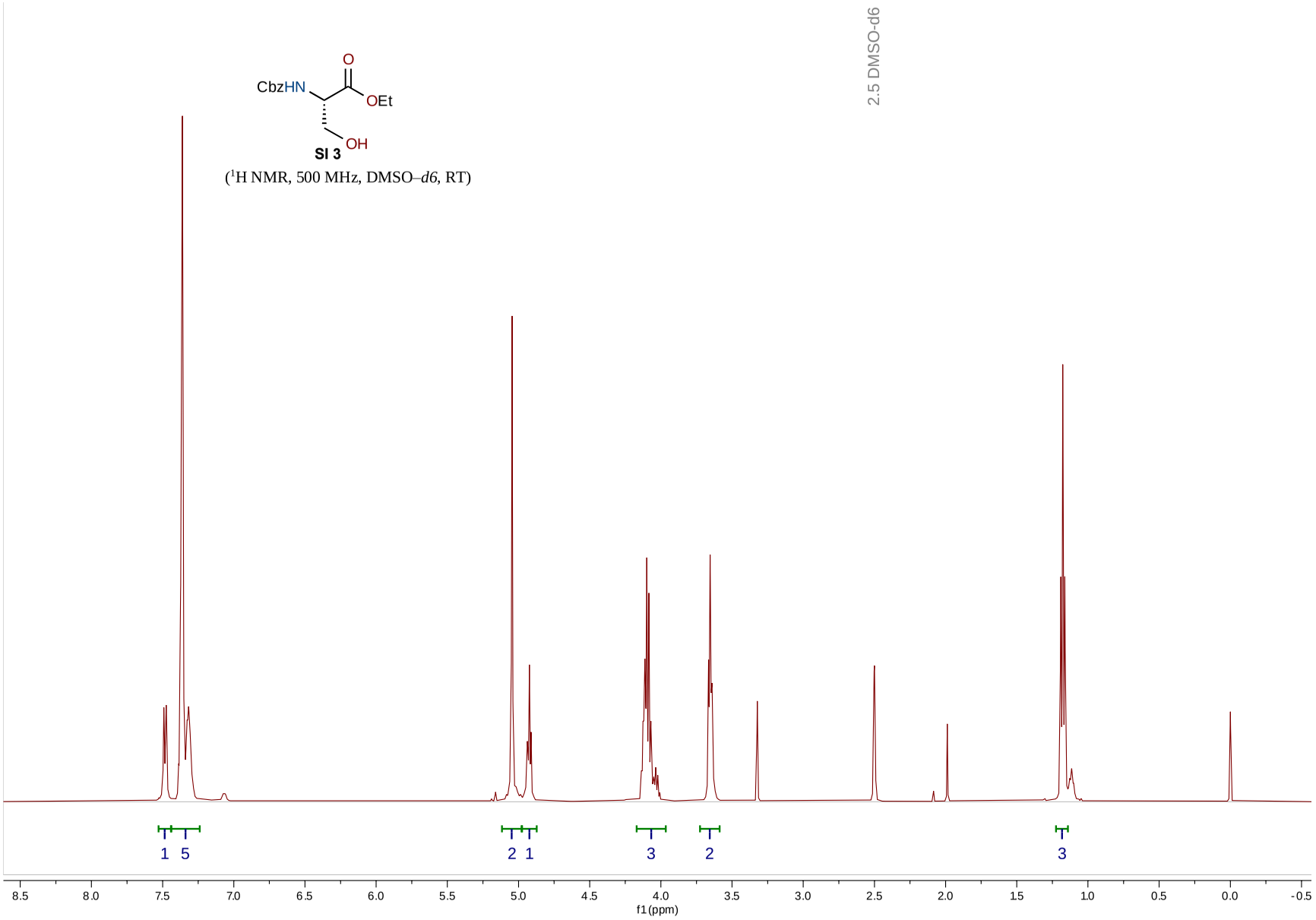
TMS
Br
11
(1H NMR, 600 MHz, DMSO–d6, RT)
2.50 DMSO-d6
2 4 1 1 1
7.4 7.3 7.2 7.1 7.0
f1(ppm)
1 1 2 4 1 1 1 1 1 1 1 2 1 1 3 3 9 9
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1(ppm)

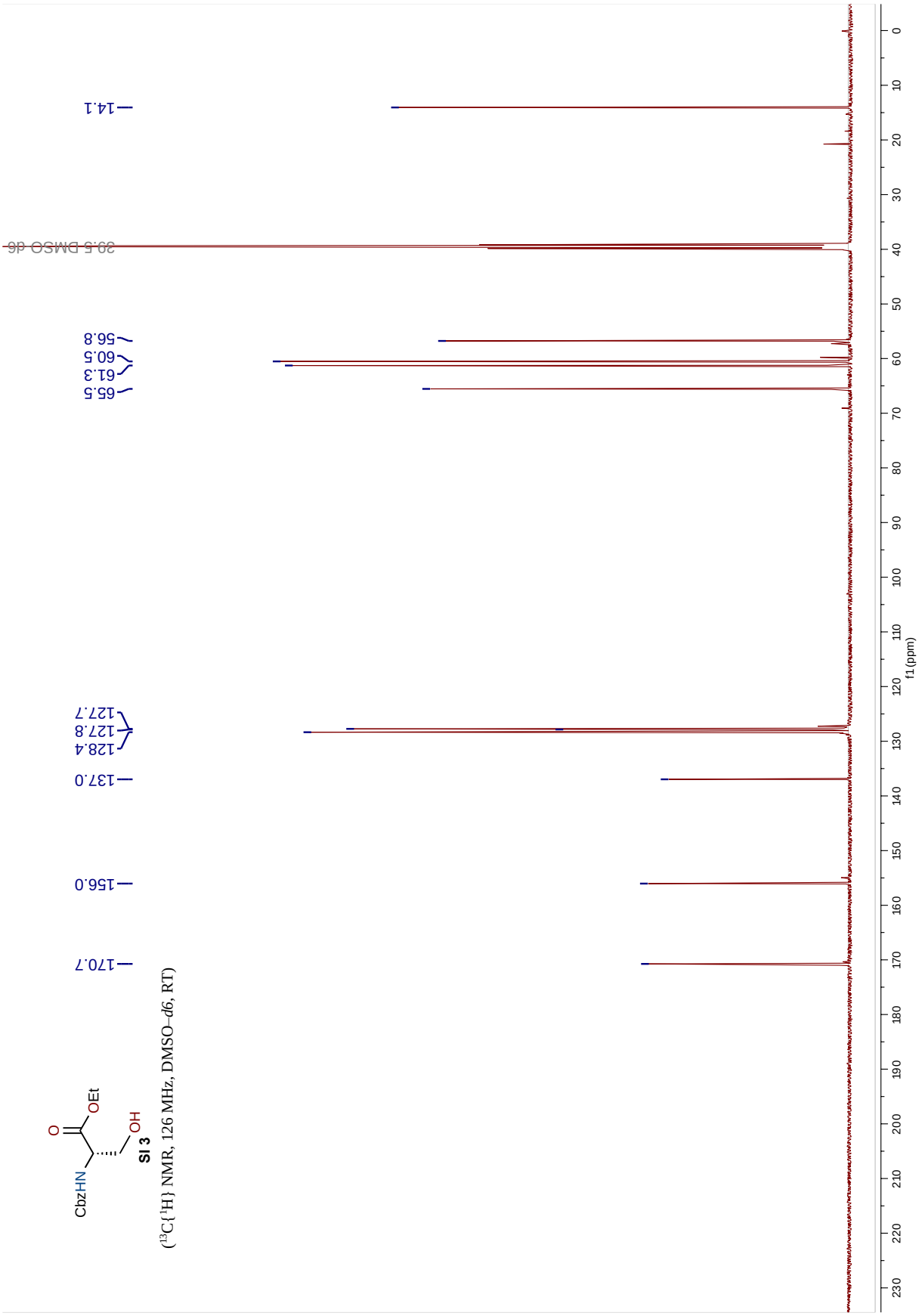


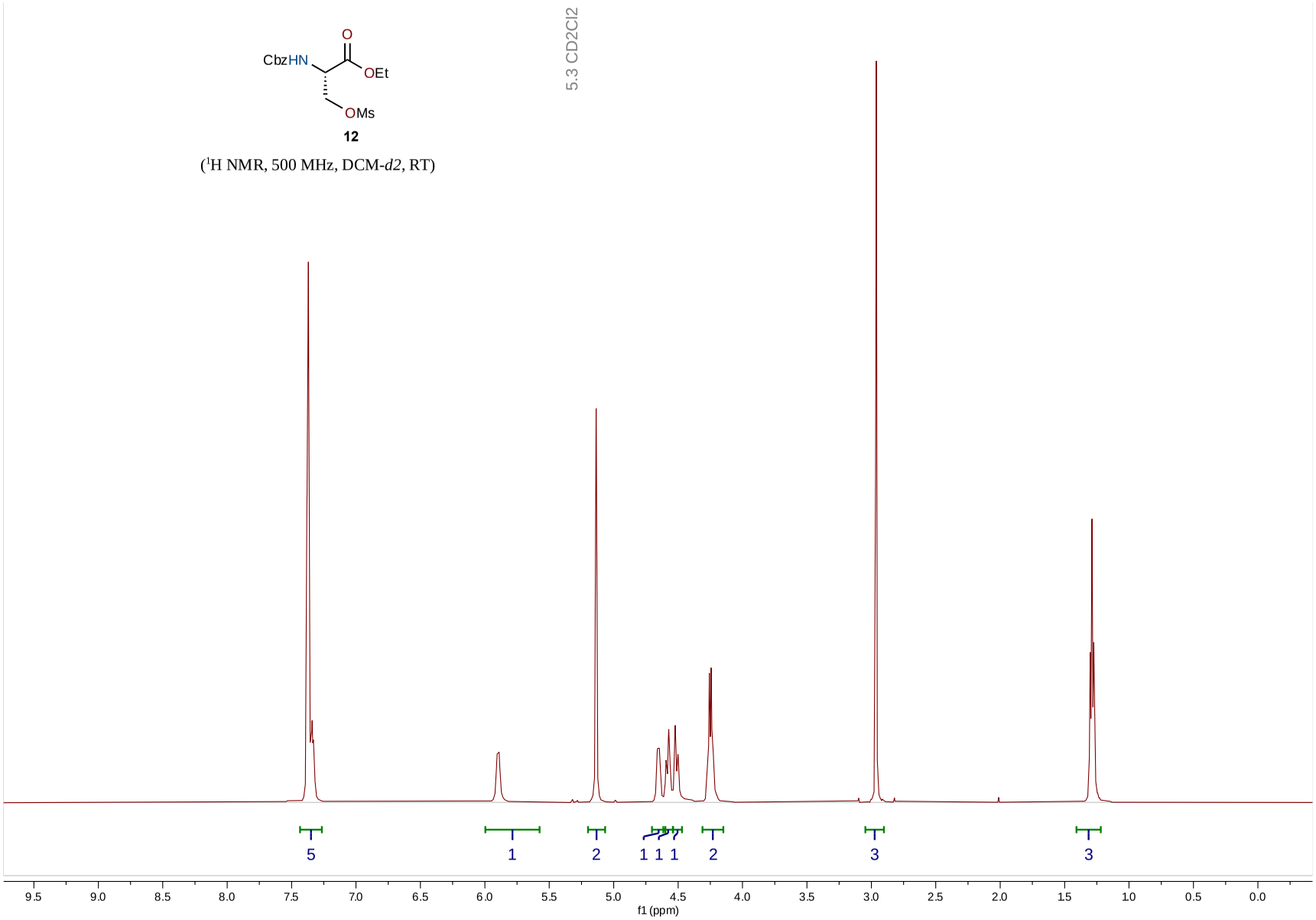
($^{13}C\{^{1}H\}$ NMR, 151 MHz, DMSO–*d6*, RT)

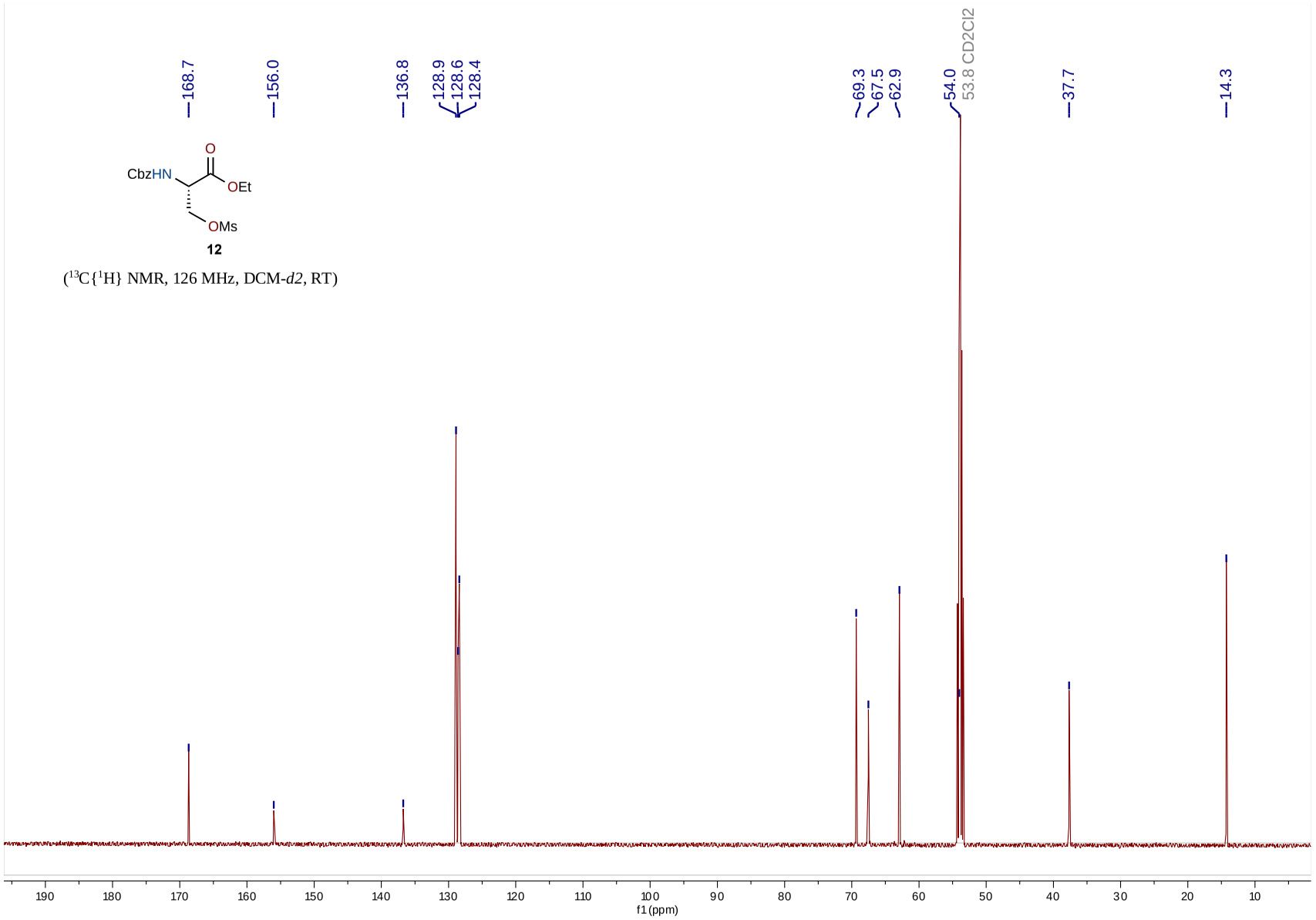


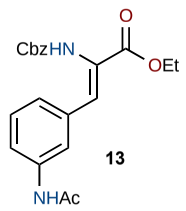
SI 3

(^{1}H NMR, 500 MHz, DMSO–*d6*, RT)


CbzHN
O
OEt
OH
SI 3
(13C{1H} NMR, 126 MHz, DMSO–d6, RT)
170.7
156.0
137.0
128.4
127.8
127.7
65.5
61.3
60.5
56.8
39.5 DMSO d6
14.1
f1 (ppm)

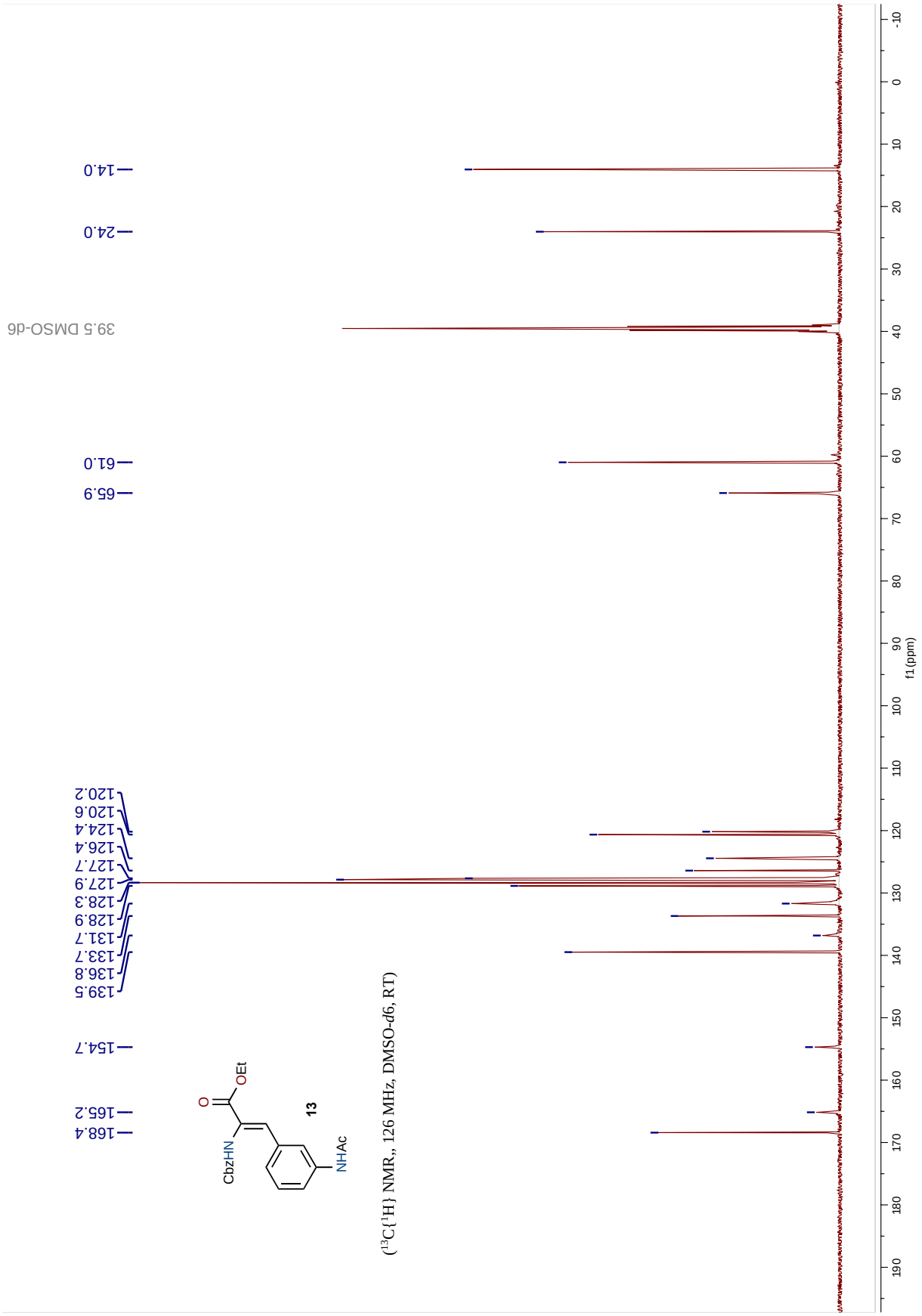

CbzHN
O
OEt
OMs
12
(1H NMR, 500 MHz, DCM-d2, RT)
5.3 CD2Cl2
5
1
2
1 1 1
2
3
3
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

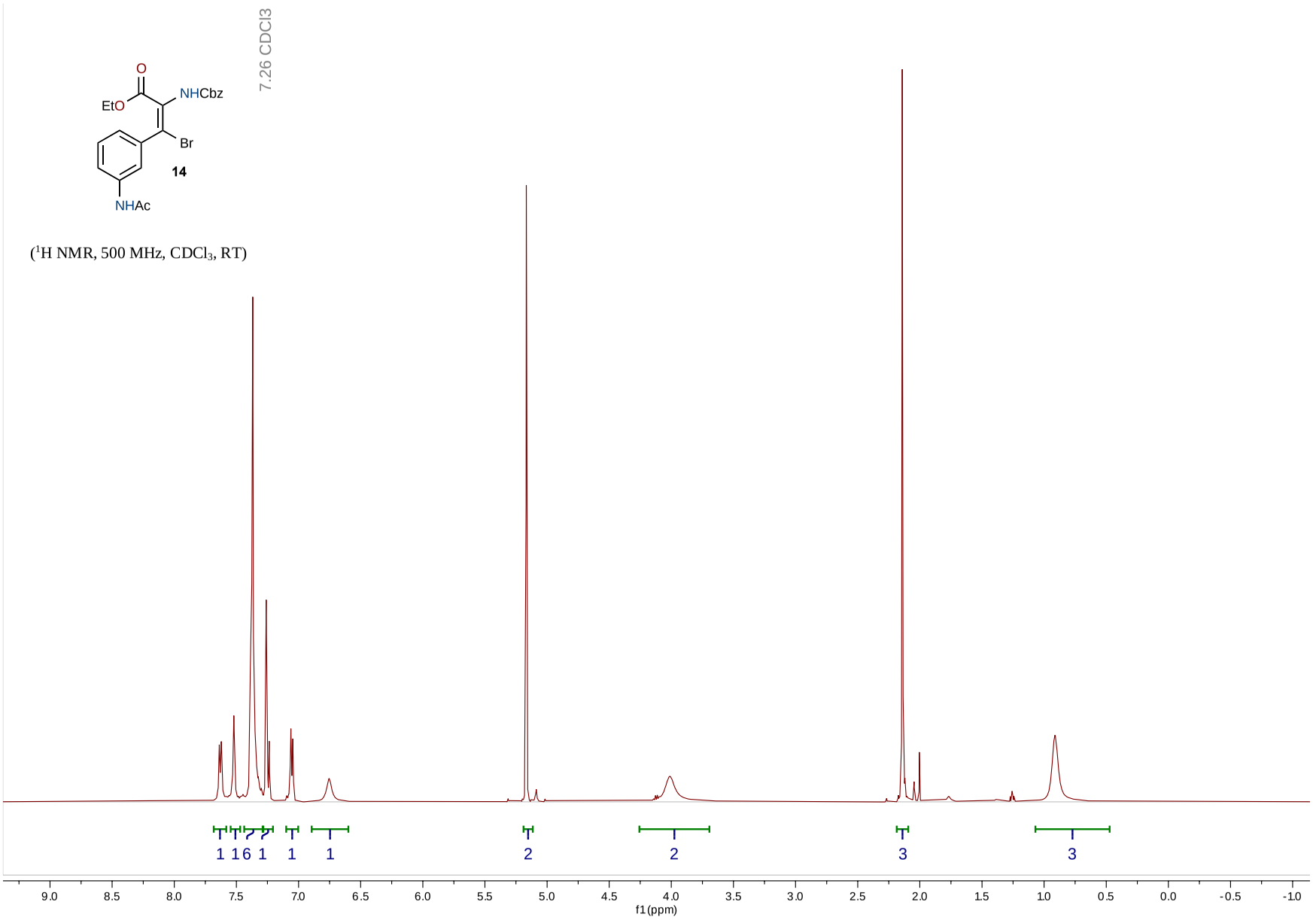

168.7
156.0
136.8
128.9
128.6
128.4
69.3
67.5
62.9
54.0
53.8 CD2Cl2
37.7
14.3
CbzHN
O
OEt
OMs
12
(13C{1H} NMR, 126 MHz, DCM-d2, RT)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

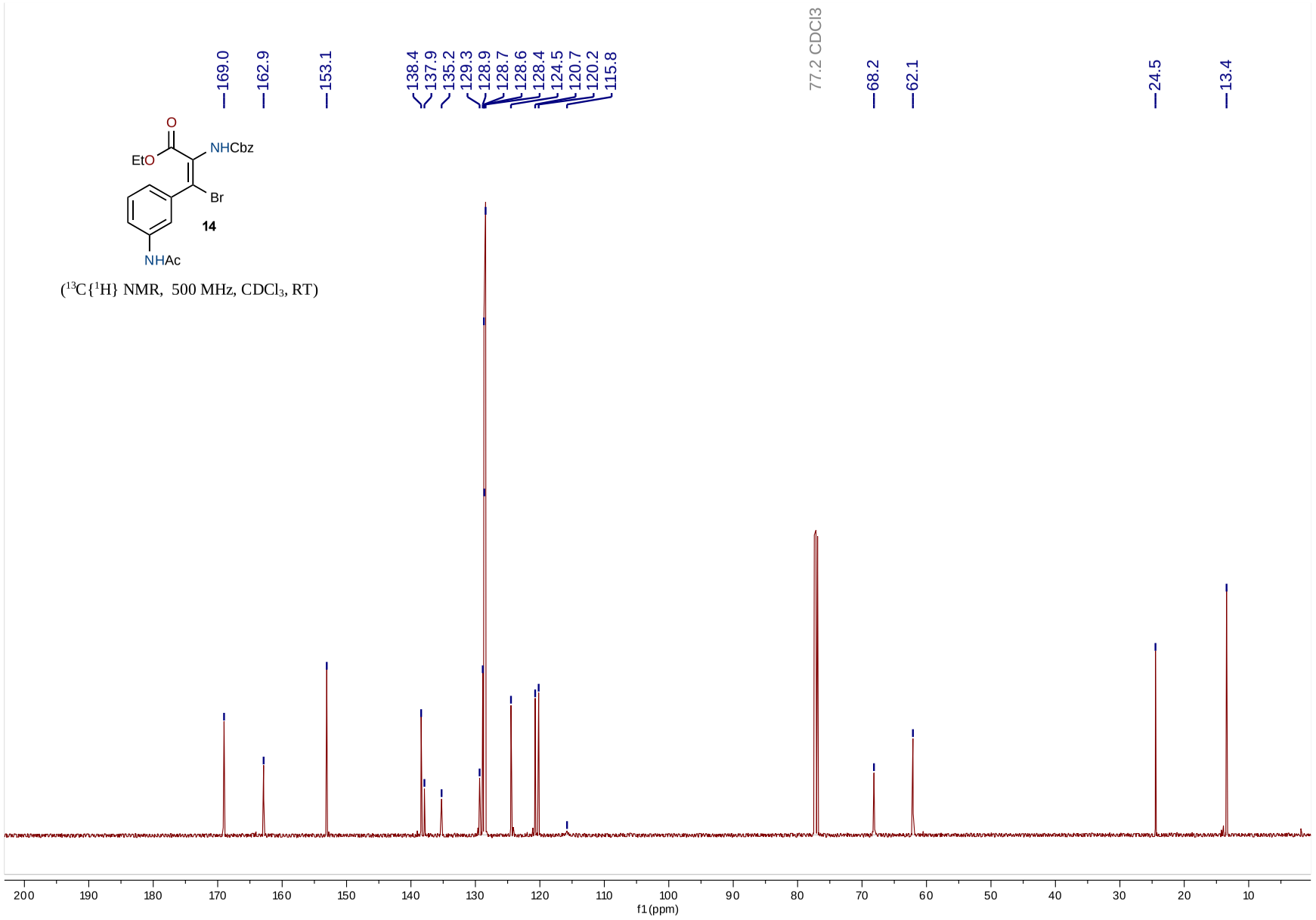


13

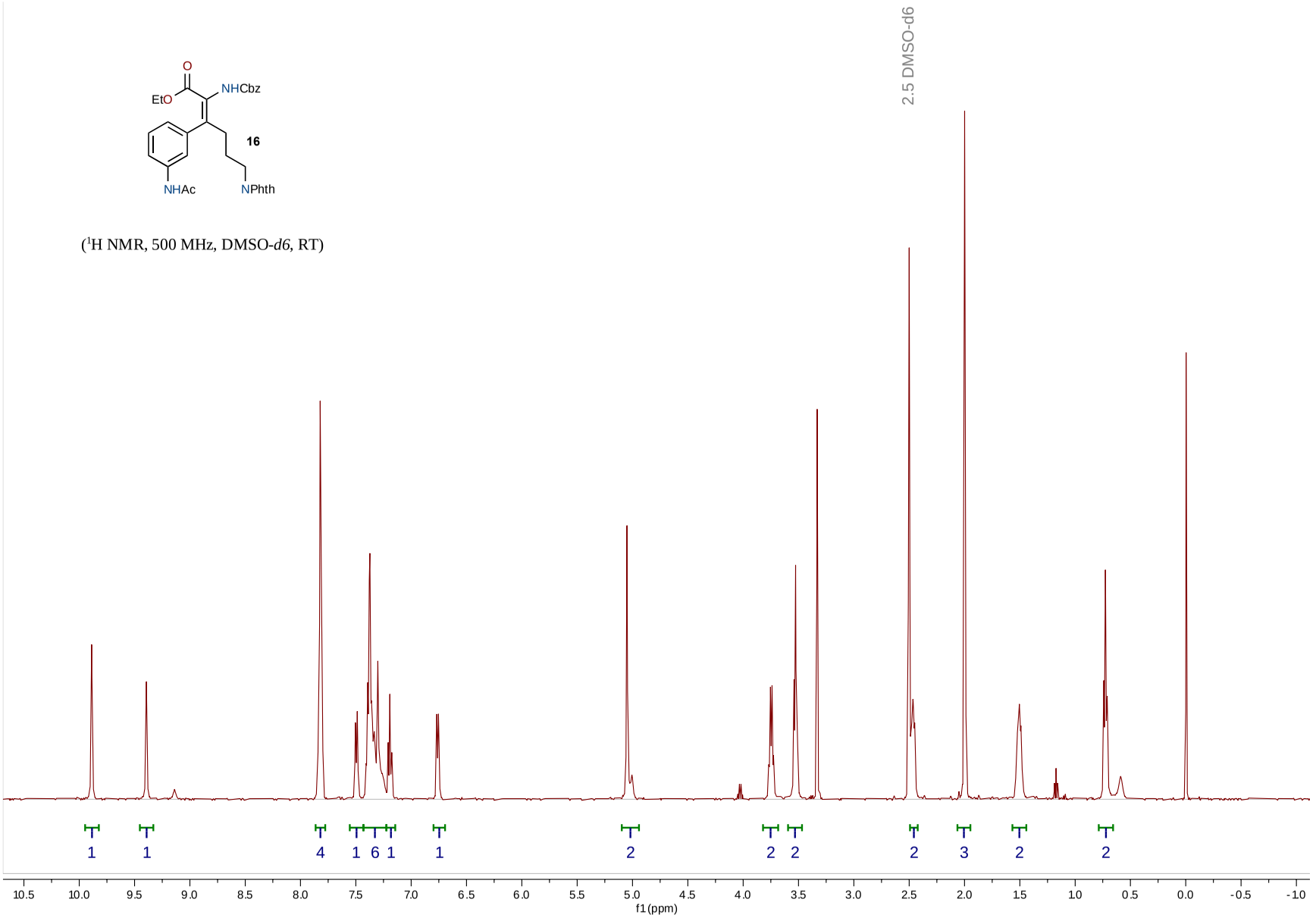
(^{1}H NMR, 500 MHz, DMSO-*d6*, RT)

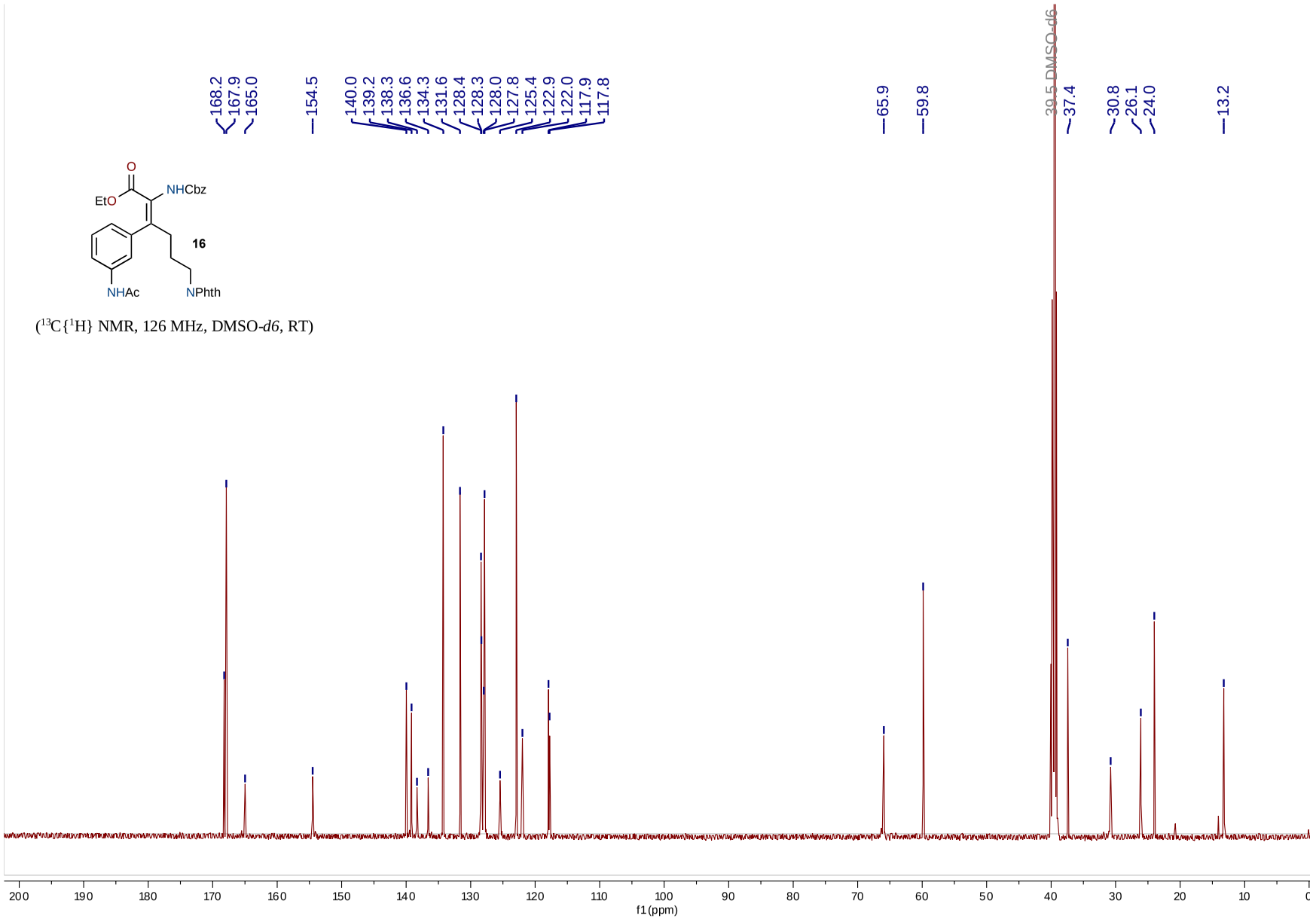

($^{13}C\{^1H\}$ NMR,, 126 MHz, DMSO-*d6*, RT)
13
CbzHN
NHAc
OEt
O
168.4
165.2
154.7
139.5
136.8
133.7
131.7
128.9
128.3
127.9
127.7
126.4
124.4
120.6
120.2
65.9
61.0
39.5 DMSO-d6
24.0
14.0
f1 (ppm)


7.26 CDCl3
O
EtO
NHCbz
Br
14
NHAc
(1H NMR, 500 MHz, CDCl3, RT)
1 1 6 1 1 1 2 2 3 3
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 0.0 -0.5 -1.0
f1 (ppm)

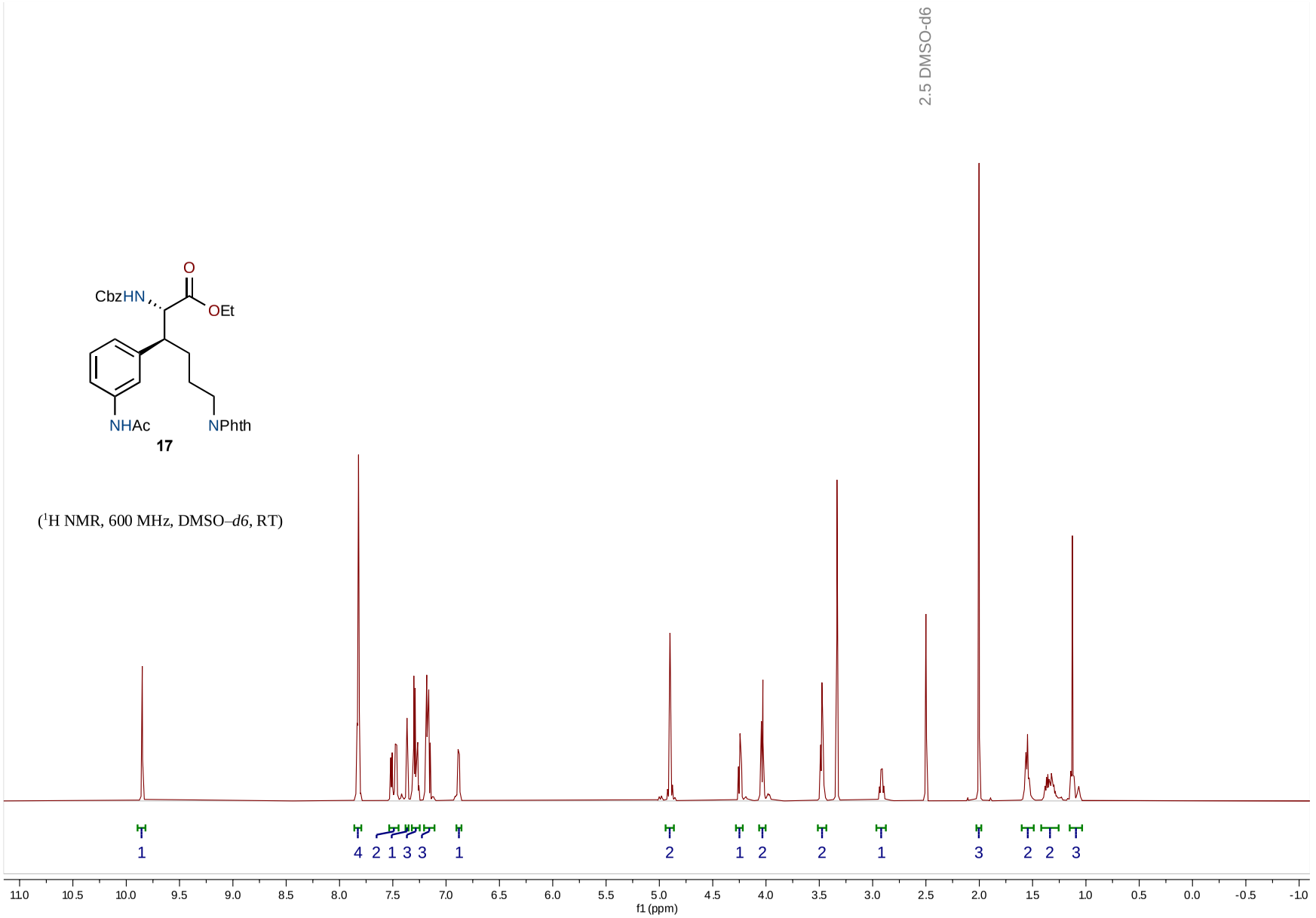


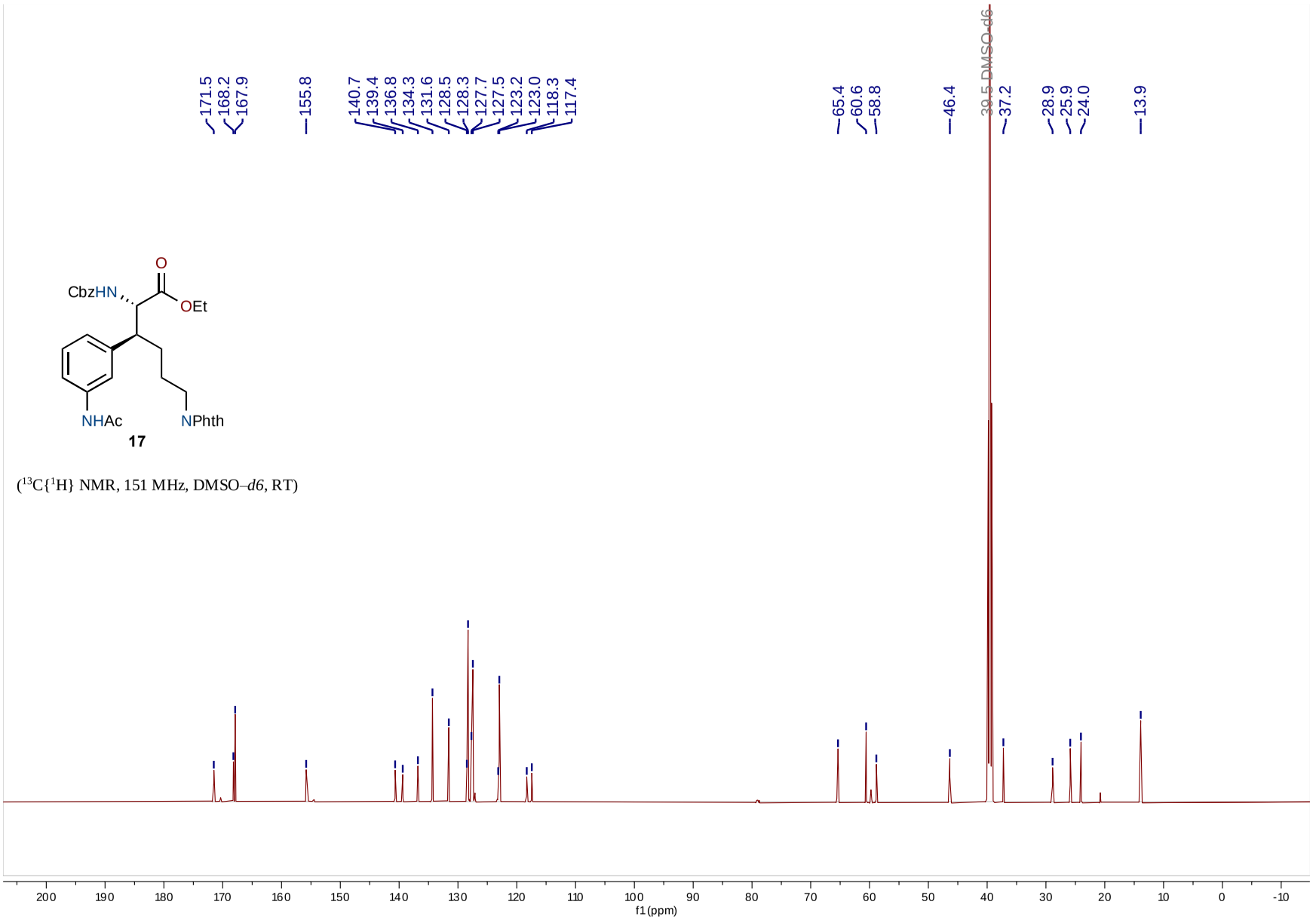
($^{13}C\{^{1}H\}$ NMR, 500 MHz, $CDCl_3$, RT)


O
EtO
NHCbz
16
NHAc
NPhth
(1H NMR, 500 MHz, DMSO-d6, RT)
2.5 DMSO-d6
1 1 4 1 6 1 1 2 2 2 2 3 2 2
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 0.5 0.0 -0.5 -1.0
f1 (ppm)

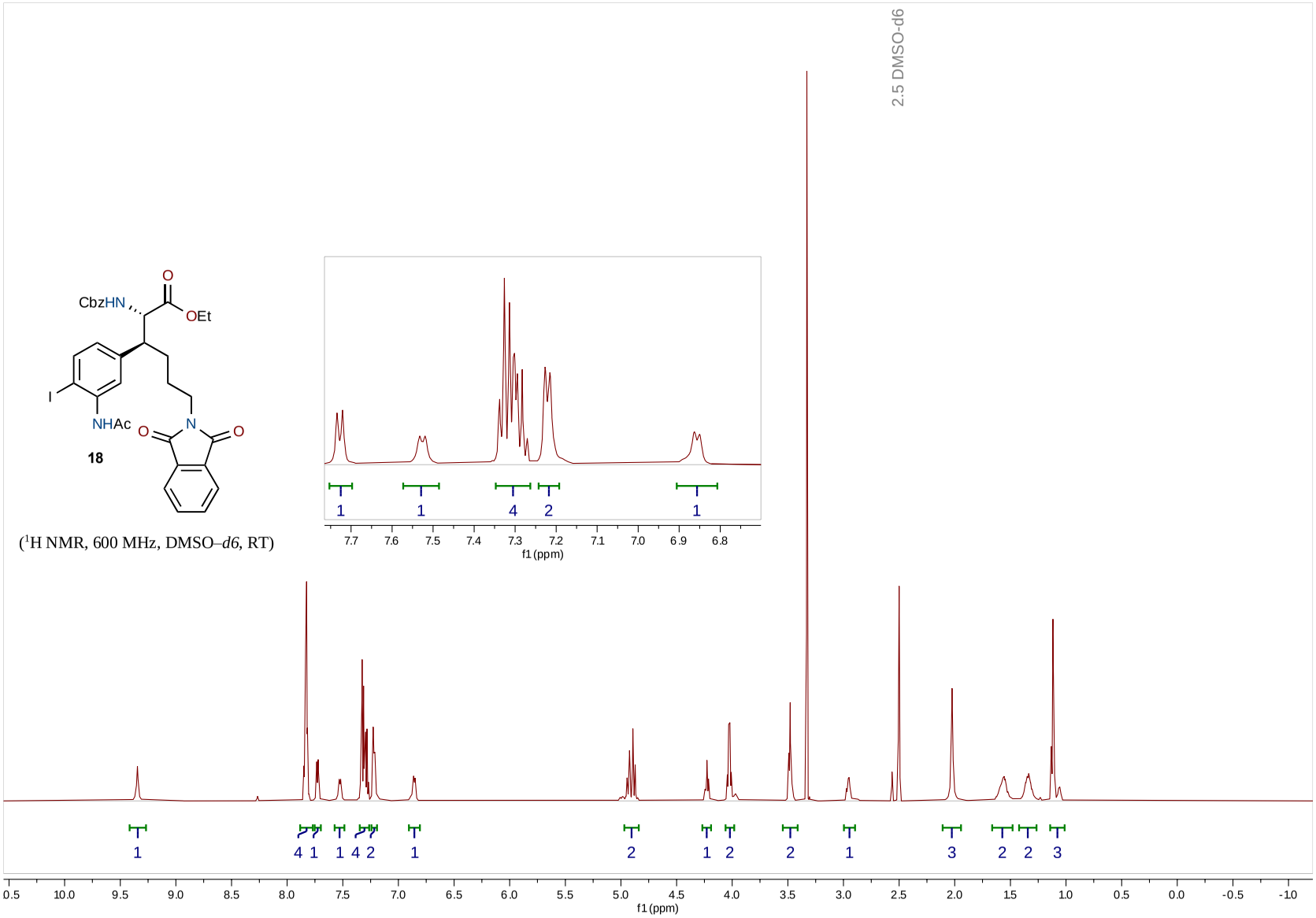


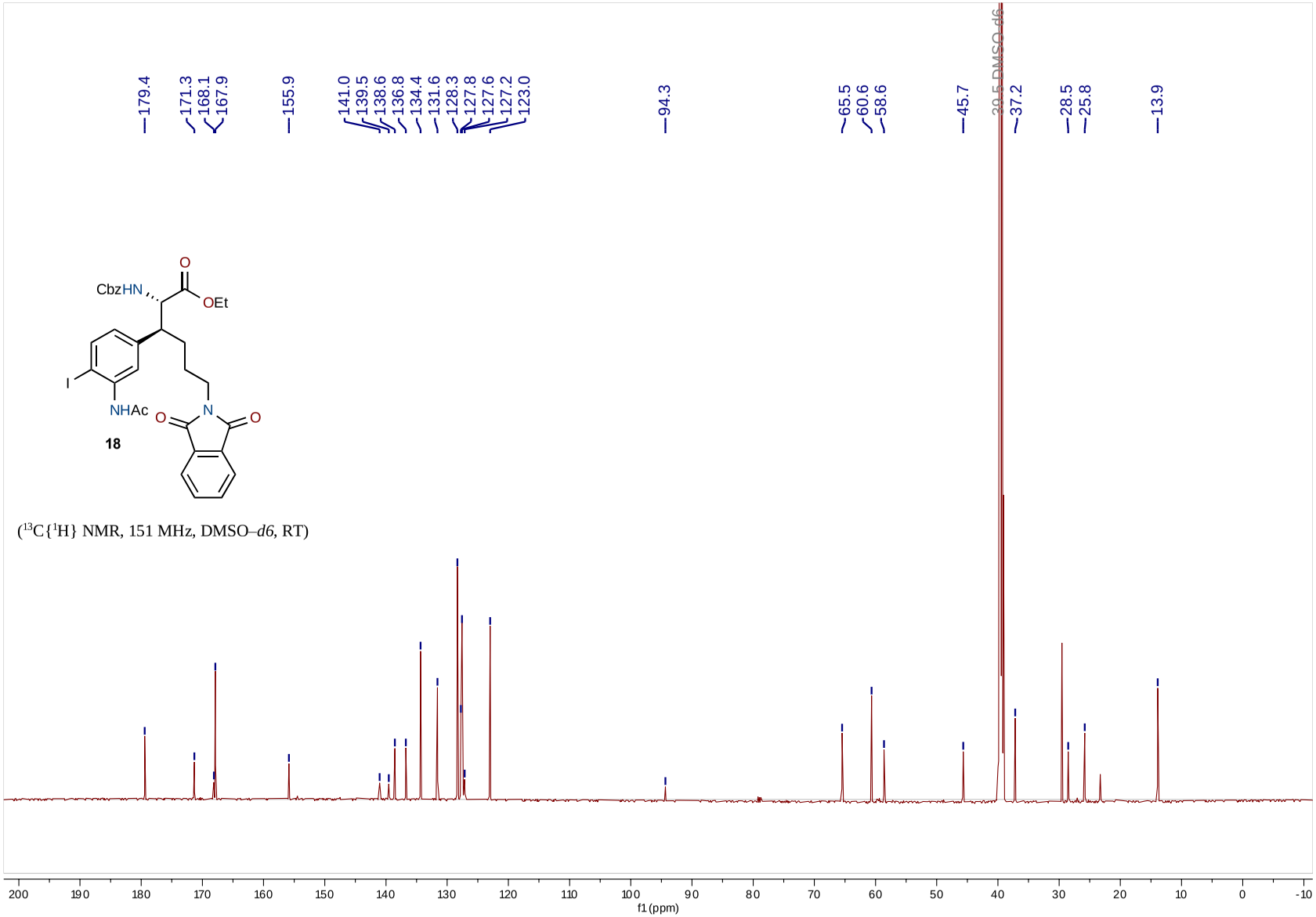
($^{13}C\{^{1}H\}$ NMR, 126 MHz, DMSO-*d6*, RT)


2.5 DMSO-d6
O
CbzHN
OEt
NHAc
NPhth
17
(1H NMR, 600 MHz, DMSO–d6, RT)
1
4 2 1 3 3
1
2
1 2
2
1
3
2 2 3
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 0.5 0.0 -0.5 -1.0
f1 (ppm)

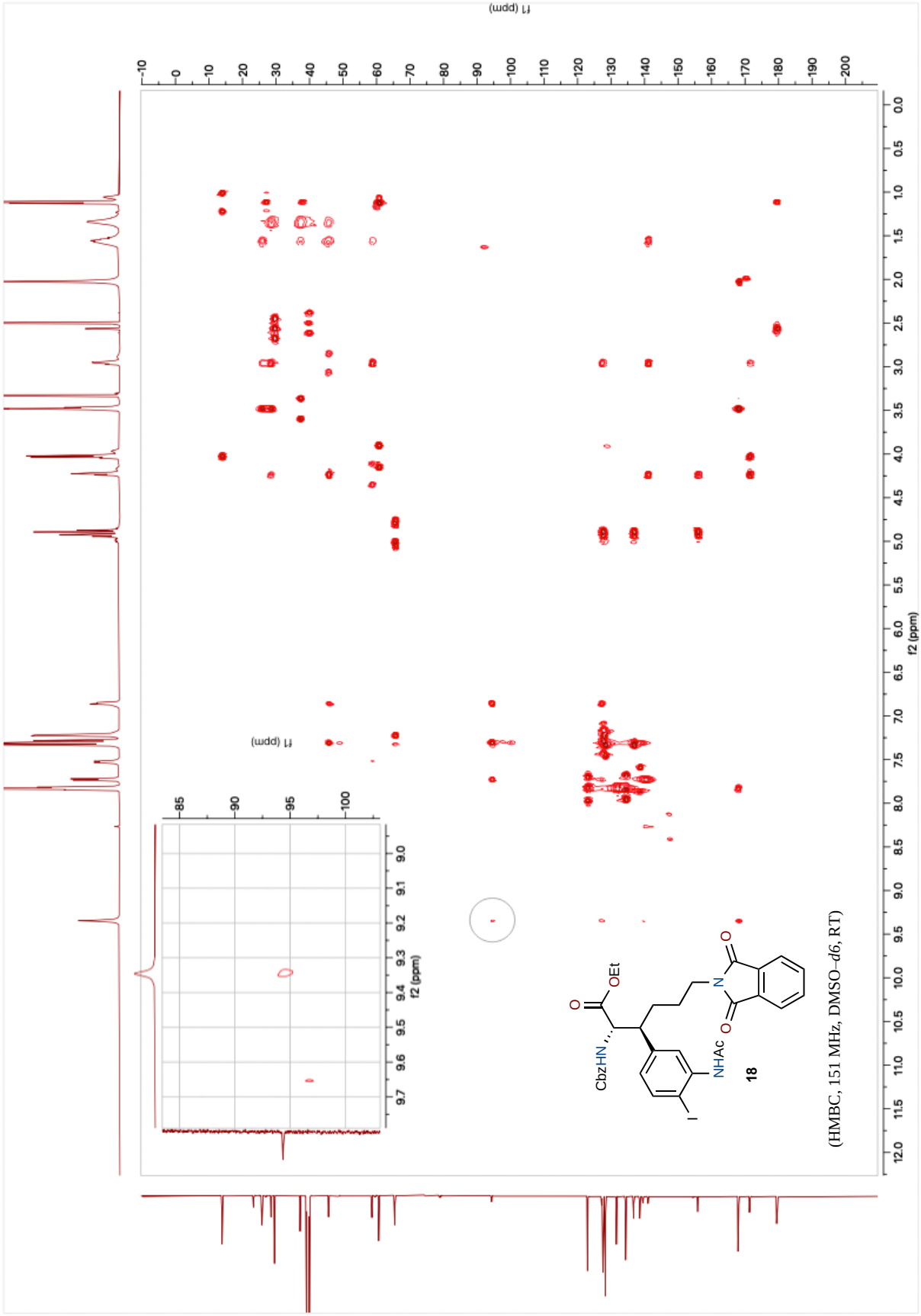


($^{13}C\{^{1}H\}$ NMR, 151 MHz, DMSO–*d6*, RT)

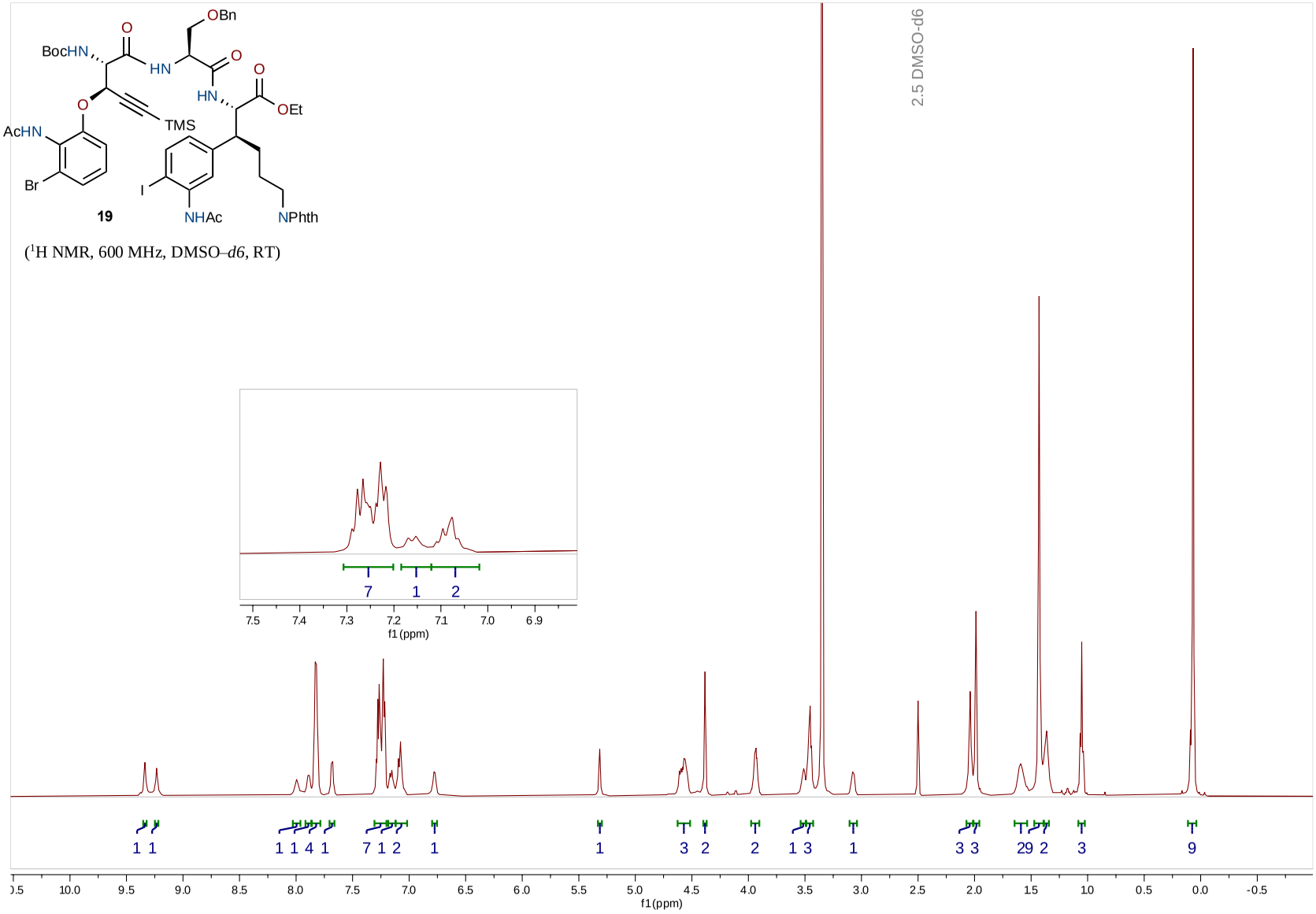

2.5 DMSO-d6
CbzHN
OEt
O
I
NHAc
N
O
O
18
(1H NMR, 600 MHz, DMSO–d6, RT)
1 1 4 2 1
7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8
f1 (ppm)
1 4 1 1 4 2 1 2 1 2 2 1 3 2 2 3
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -10
f1 (ppm)



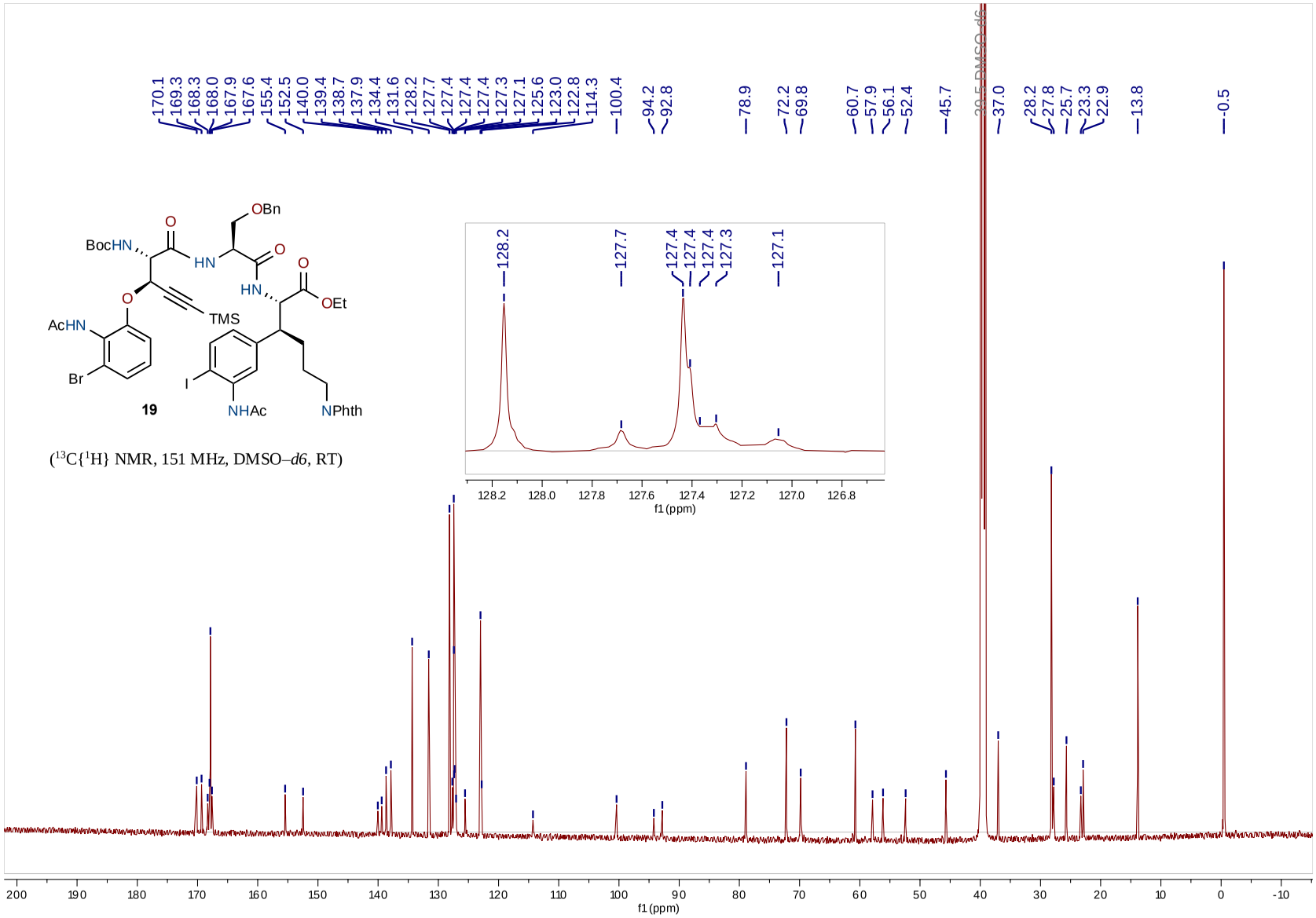
($^{13}C\{^{1}H\}$ NMR, 151 MHz, DMSO–*d6*, RT)



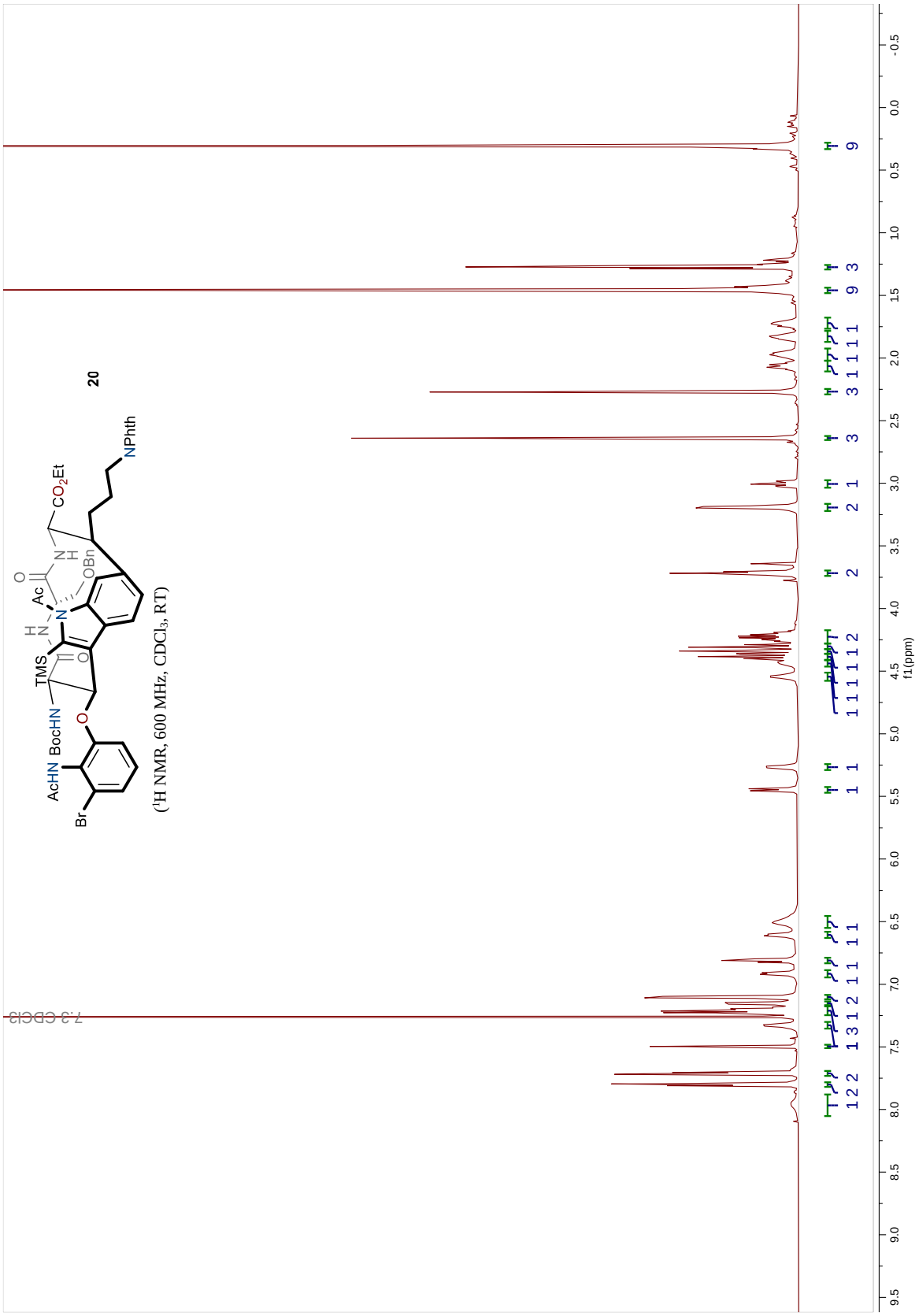
(HMBC, 151 MHz, DMSO-*d6*, RT)

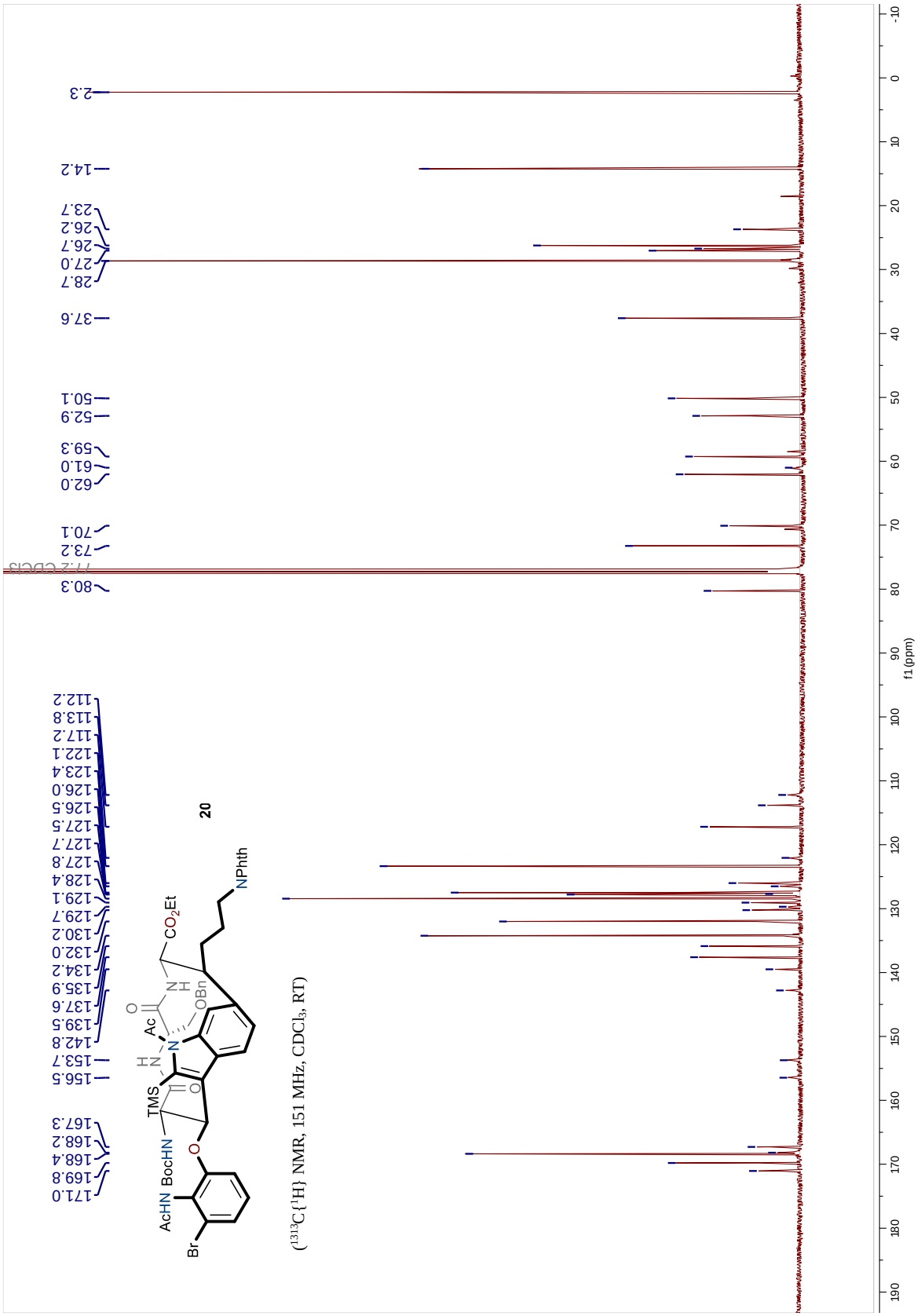


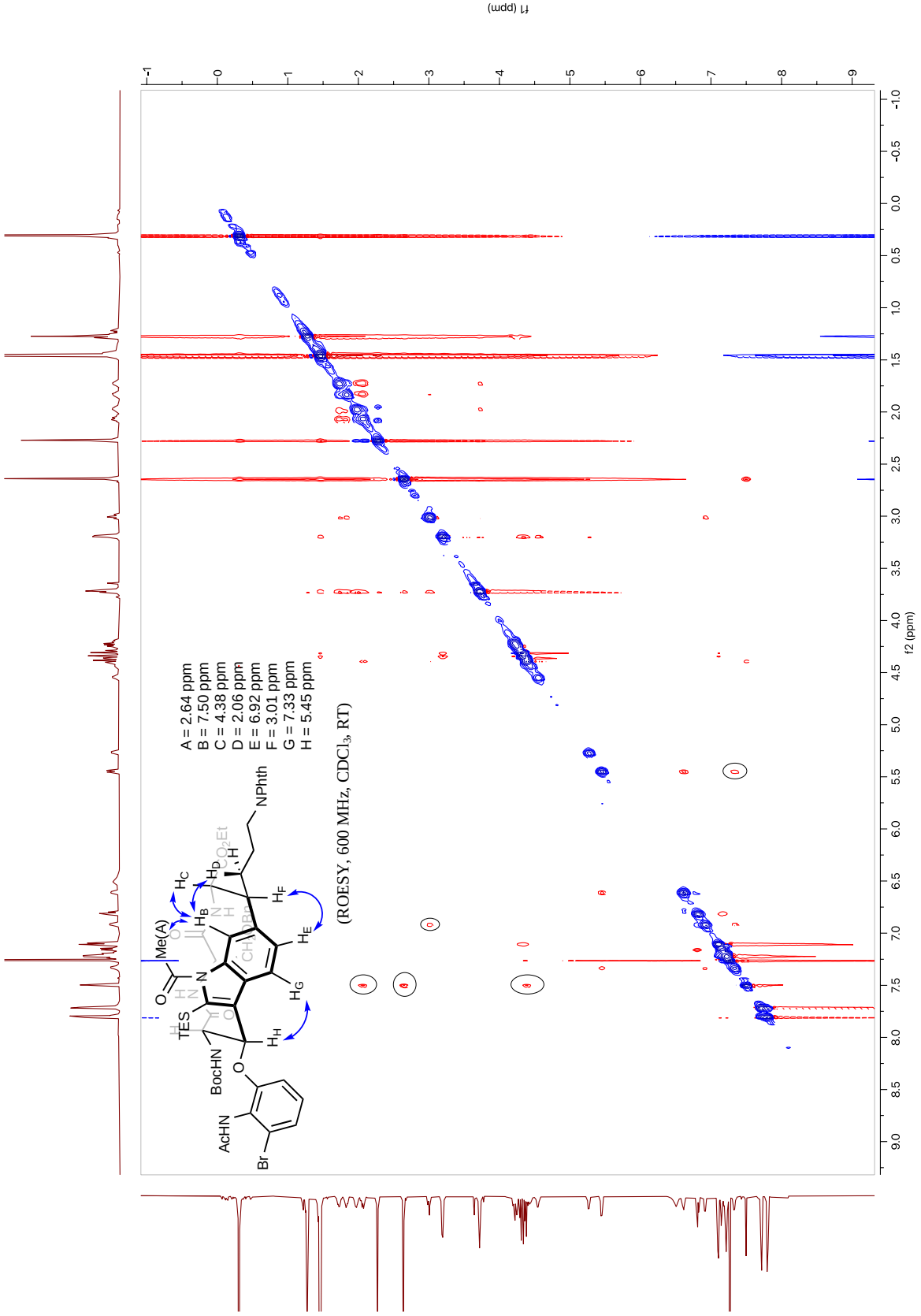
(^{1}H NMR, 600 MHz, DMSO–*d6*, RT)

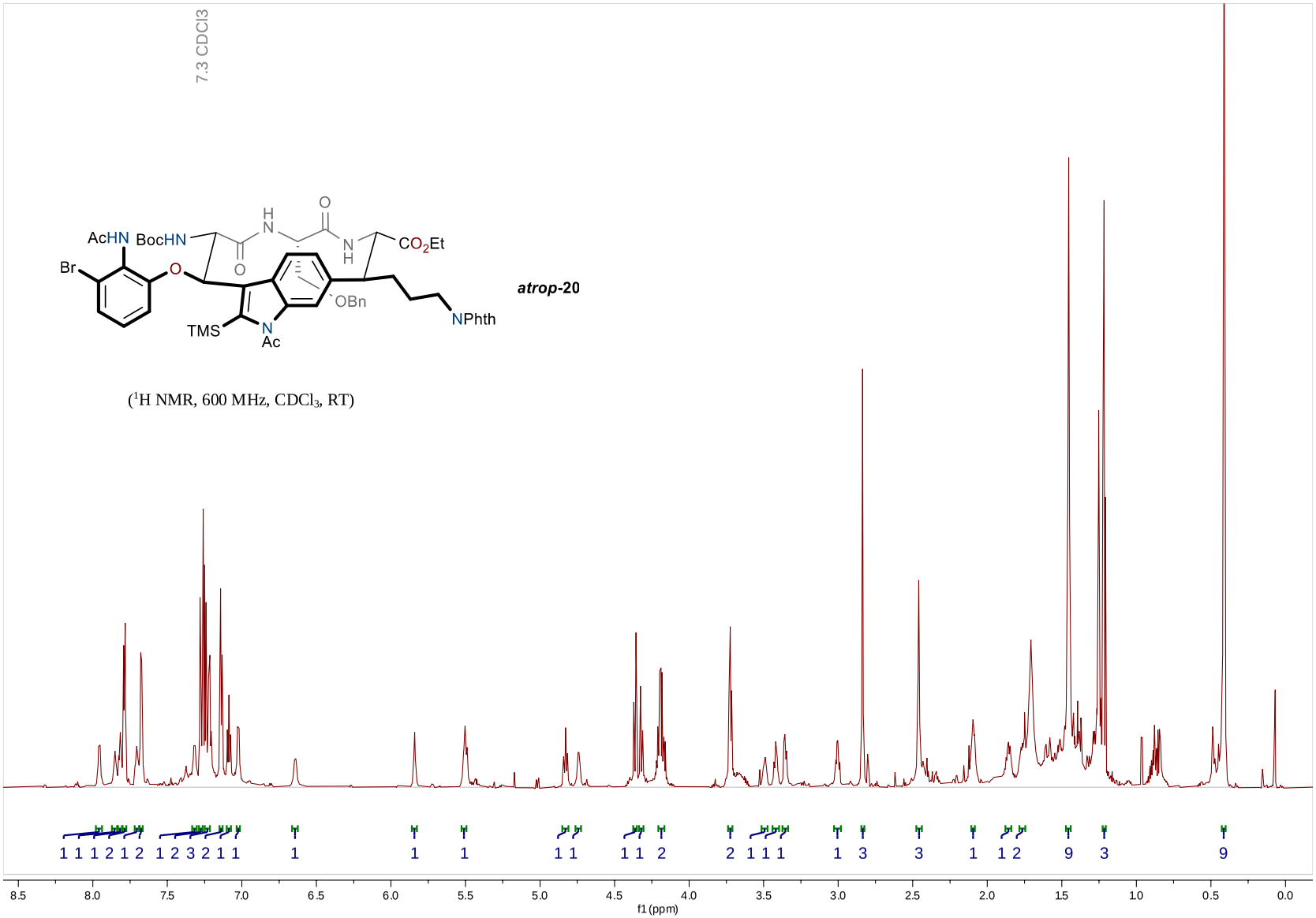


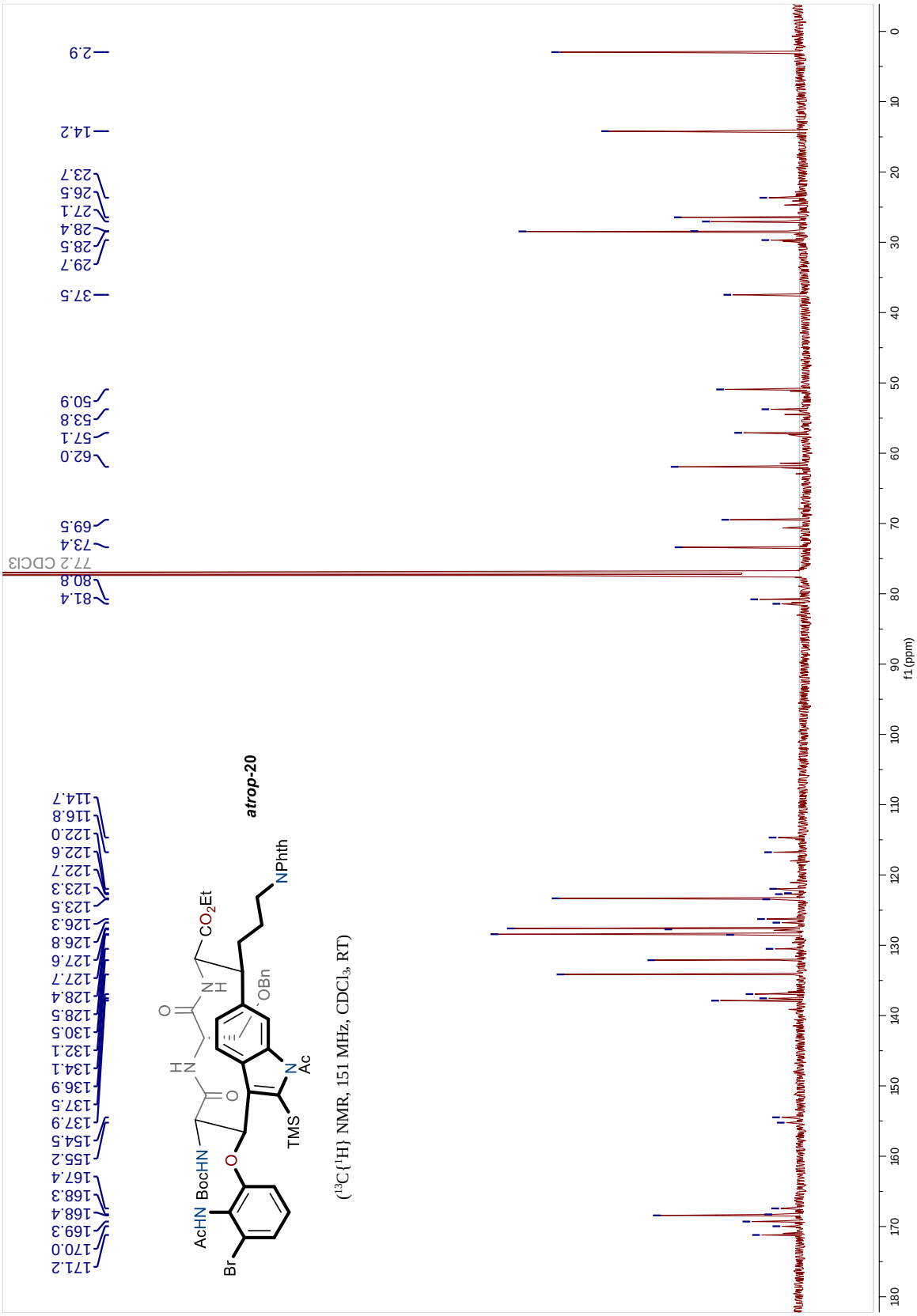
($^{13}C\{^{1}H\}$ NMR, 151 MHz, DMSO–*d6*, RT)

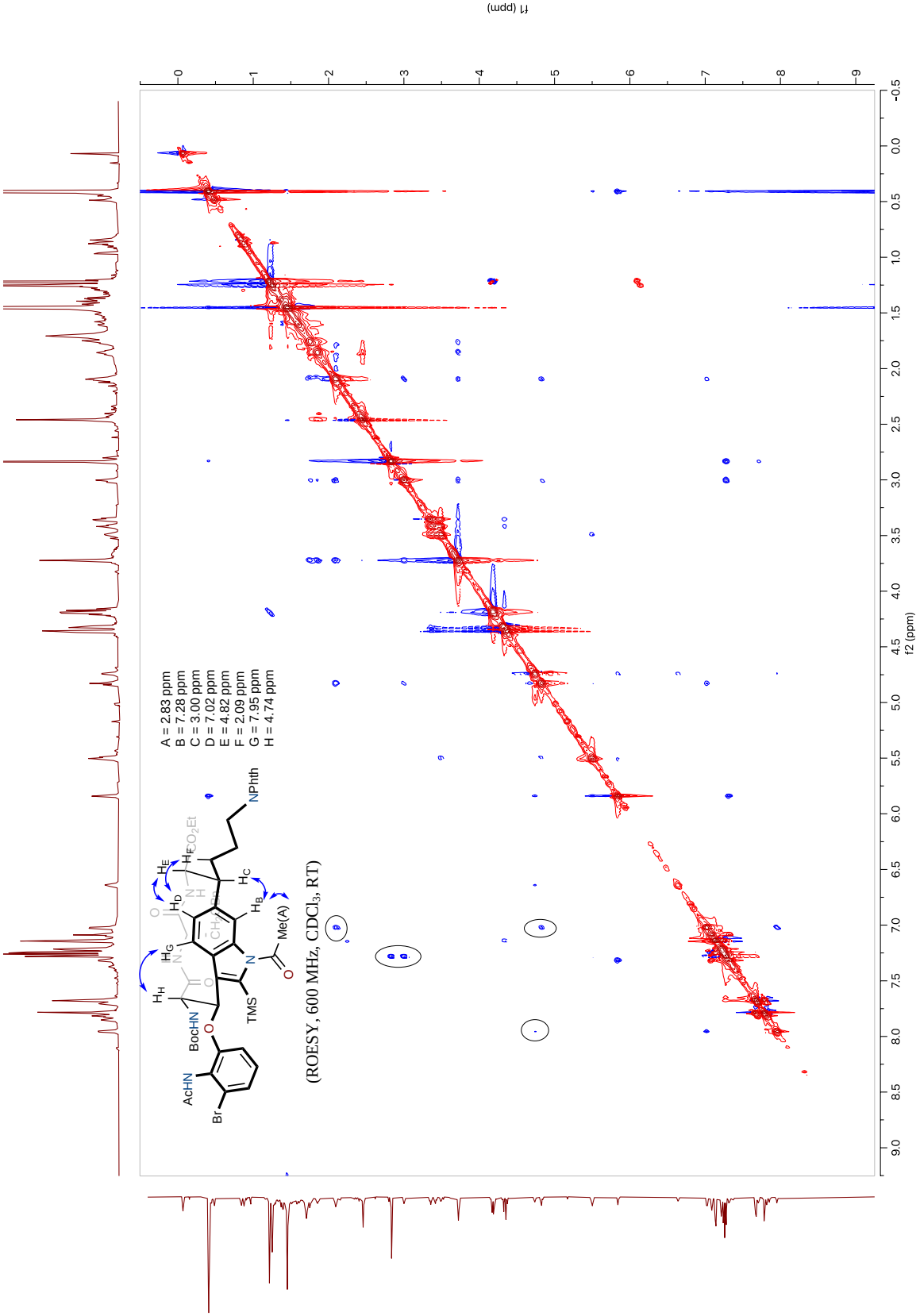

20
(^{1}H NMR, 600 MHz, $CDCl_3$, RT)
7.3 CDCl3
f1(ppm)

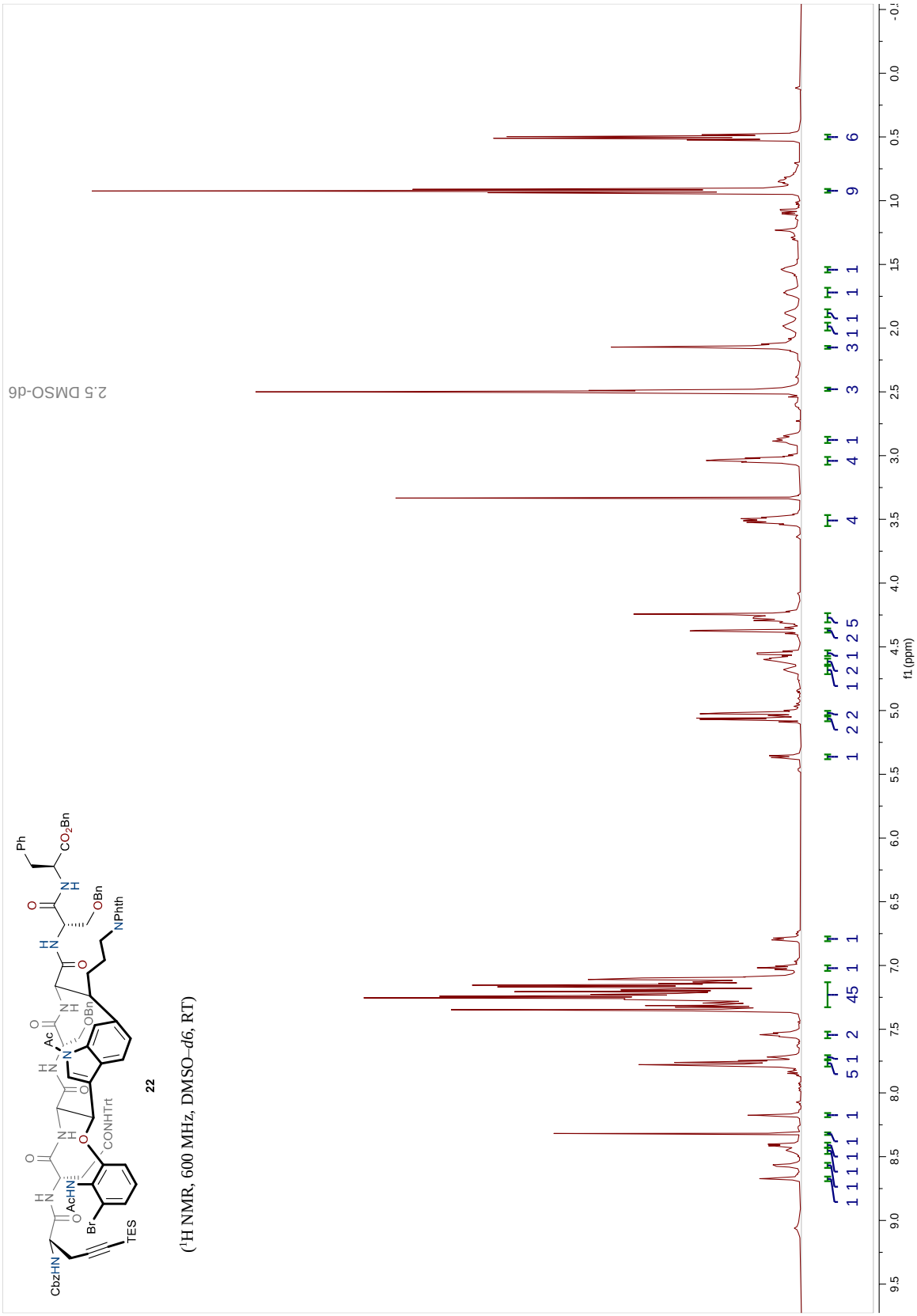

2.3
14.2
23.7
26.2
26.7
27.0
28.7
37.6
50.1
52.9
59.3
61.0
62.0
70.1
73.2
77.2 CDCl3
80.3
112.2
113.8
117.2
122.1
123.4
126.0
126.5
127.5
127.7
127.8
128.4
129.1
129.7
130.2
132.0
134.2
135.9
137.6
139.5
142.8
153.7
156.5
167.3
168.2
168.4
169.8
171.0
AcHN
BocHN
Br
TMS
Ac
OBn
CO2Et
NPhth
20
(1313C{1H} NMR, 151 MHz, CDCl3, RT)
f1 (ppm)


A = 2.64 ppm
B = 7.50 ppm
C = 4.38 ppm
D = 2.06 ppm
E = 6.92 ppm
F = 3.01 ppm
G = 7.33 ppm
H = 5.45 ppm
(ROESY, 600 MHz, CDCl3, RT)
f2 (ppm)
f1 (ppm)


7.3 CDCl3
AcHN
BocHN
Br
O
TMS
N
Ac
OBn
NPhth
CO2Et
atrop-20
(1H NMR, 600 MHz, CDCl3, RT)
1 1 1 2 1 2 1 2 3 2 1 1 1 1 1 1 1 2 2 1 1 1 1 3 3 1 1 2 9 3 9
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 0.0
f1 (ppm)

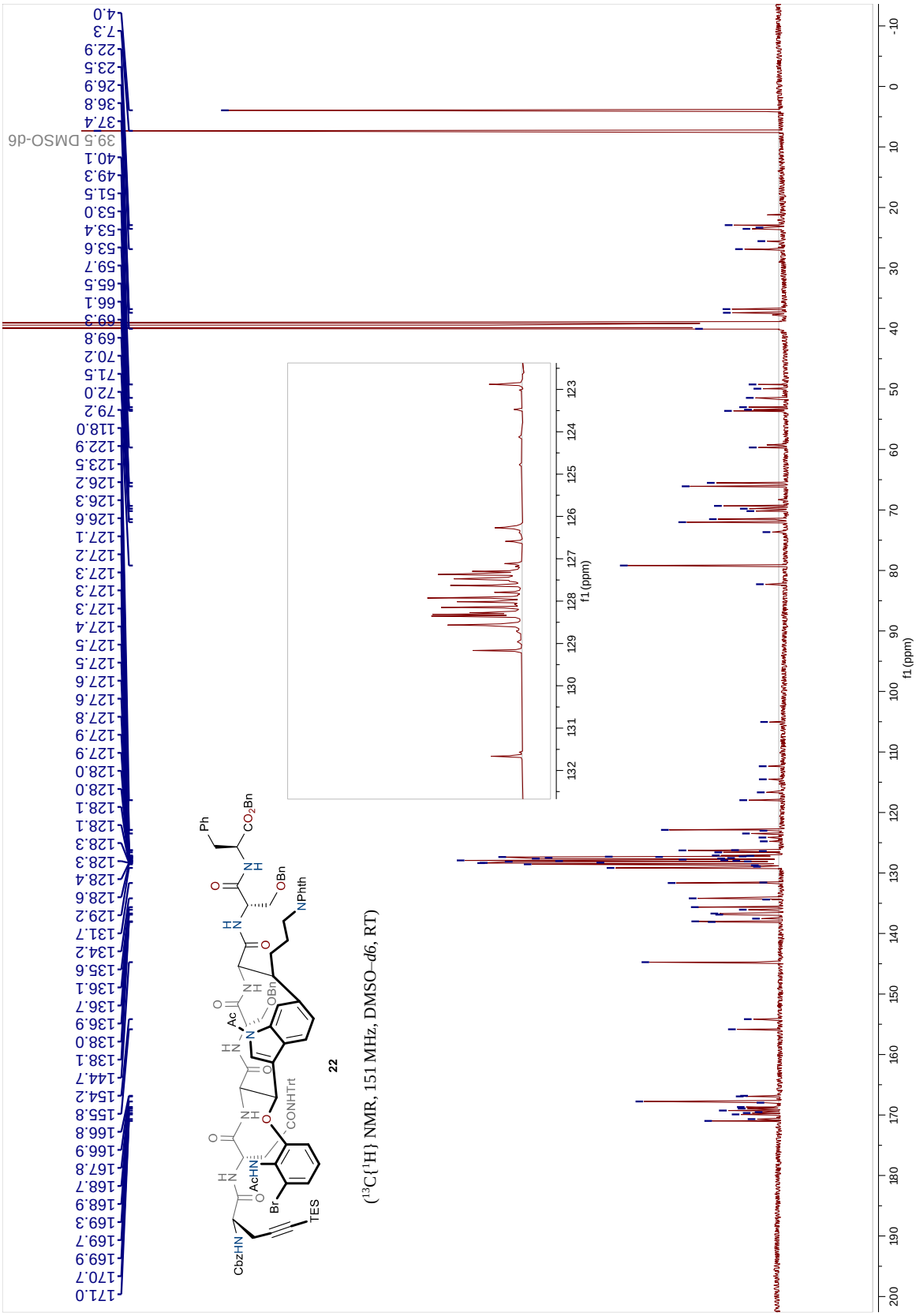

atrop-20
($^{13}C\{^1H\}$ NMR, 151 MHz, $CDCl_3$, RT)
AcHN
BocHN
Br
TMS
Ac
OBn
NPhth
CO_2Et
77.2 CDCl3
f1 (ppm)

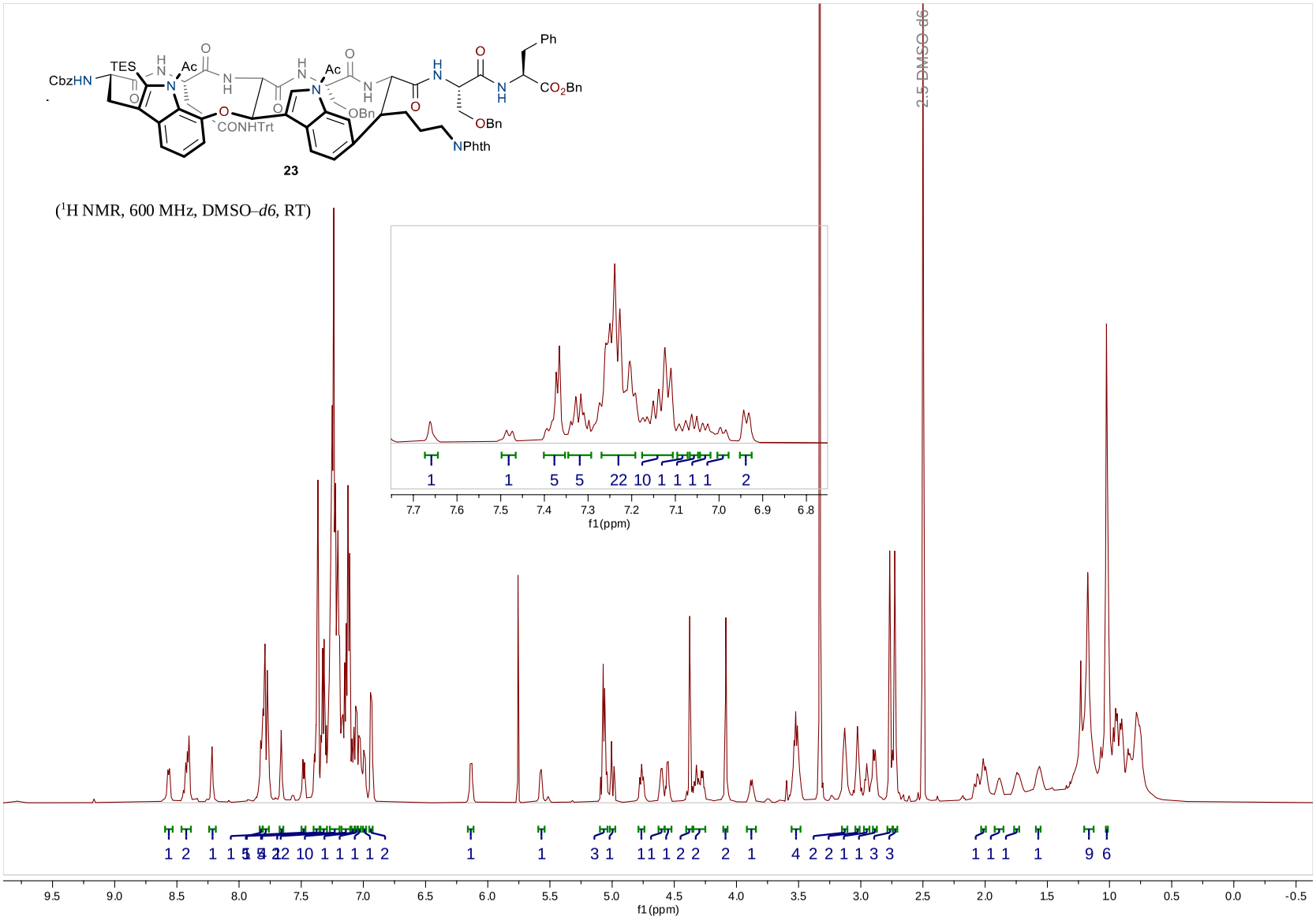

A = 2.83 ppm
B = 7.28 ppm
C = 3.00 ppm
D = 7.02 ppm
E = 4.82 ppm
F = 2.09 ppm
G = 7.95 ppm
H = 4.74 ppm
(ROESY, 600 MHz, CDCl3, RT)
f2 (ppm)
f1 (ppm)

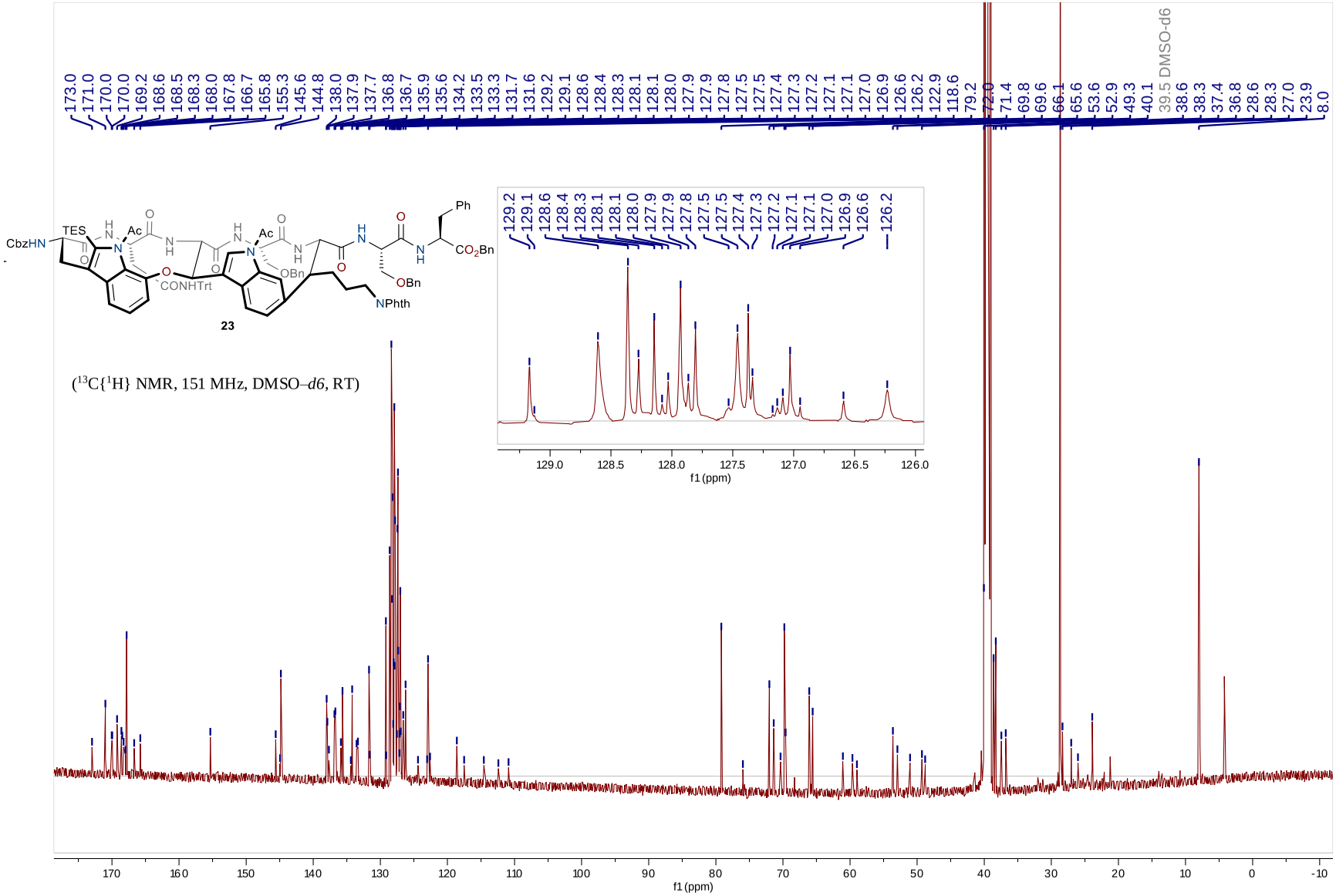


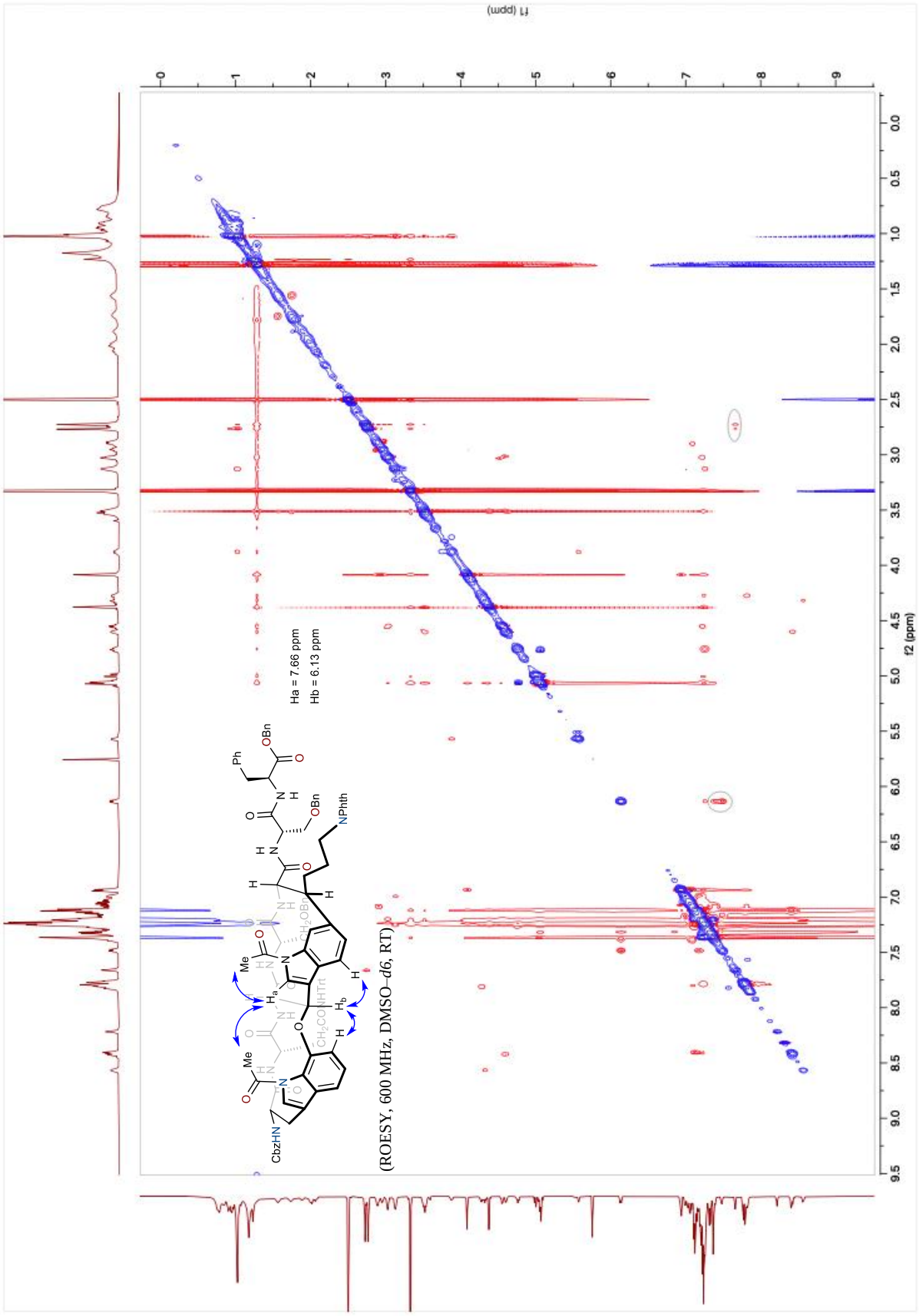
22

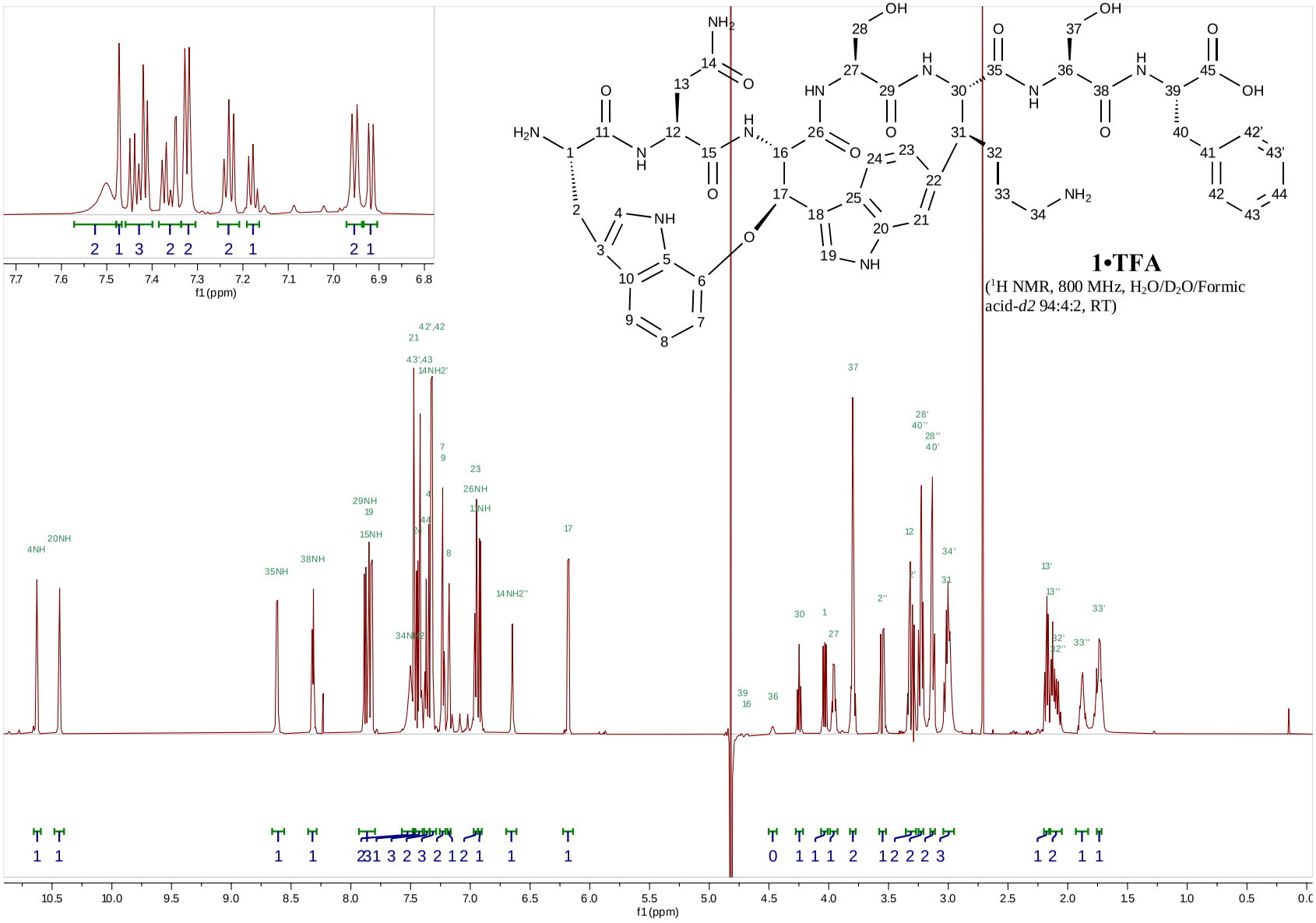
(^{1}H NMR, 600 MHz, DMSO-*d6*, RT)

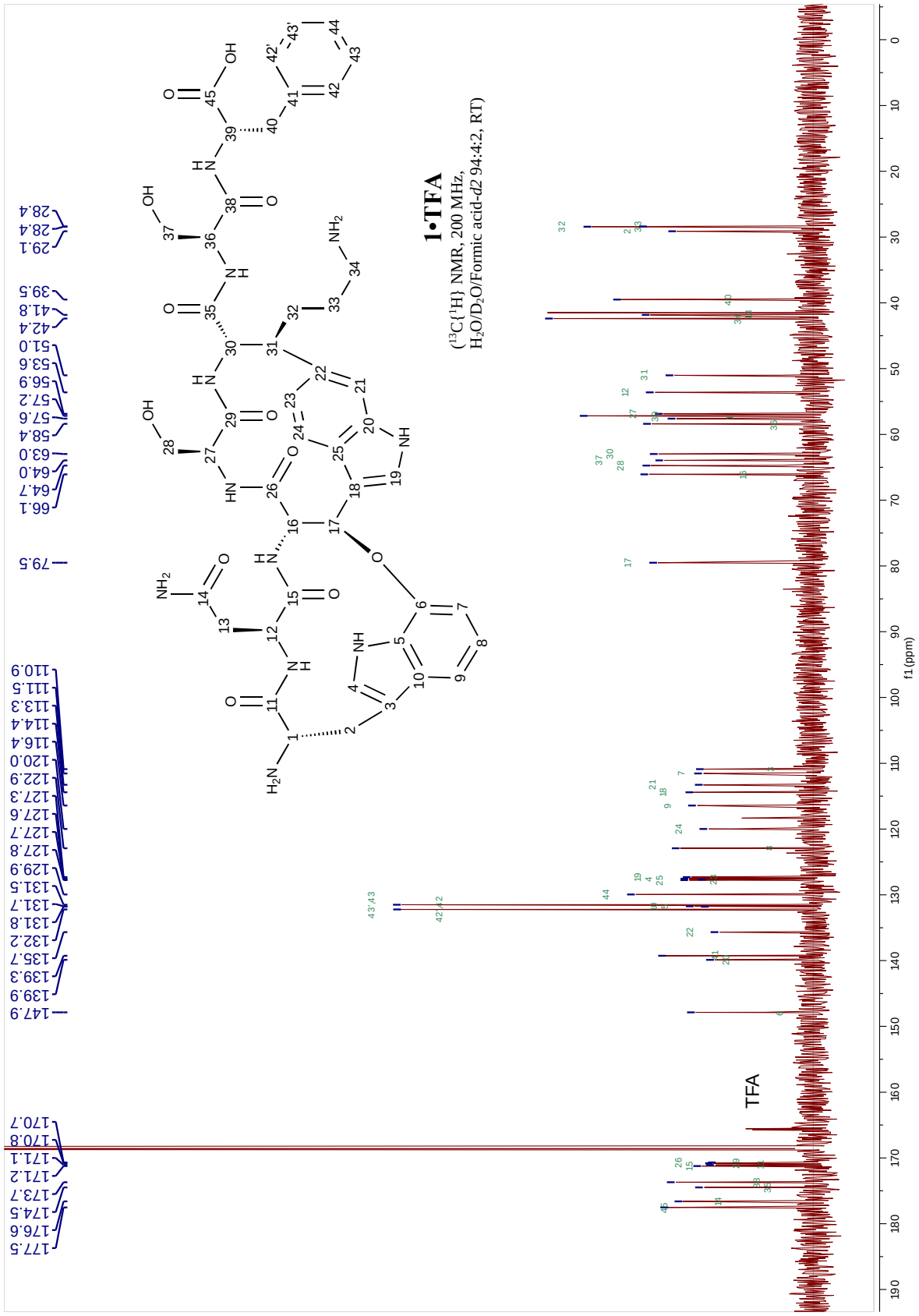

22
(^{13}C{^{1}H} NMR, 151 MHz, DMSO-*d6*, RT)
39.5 DMSO-d6
f1 (ppm)

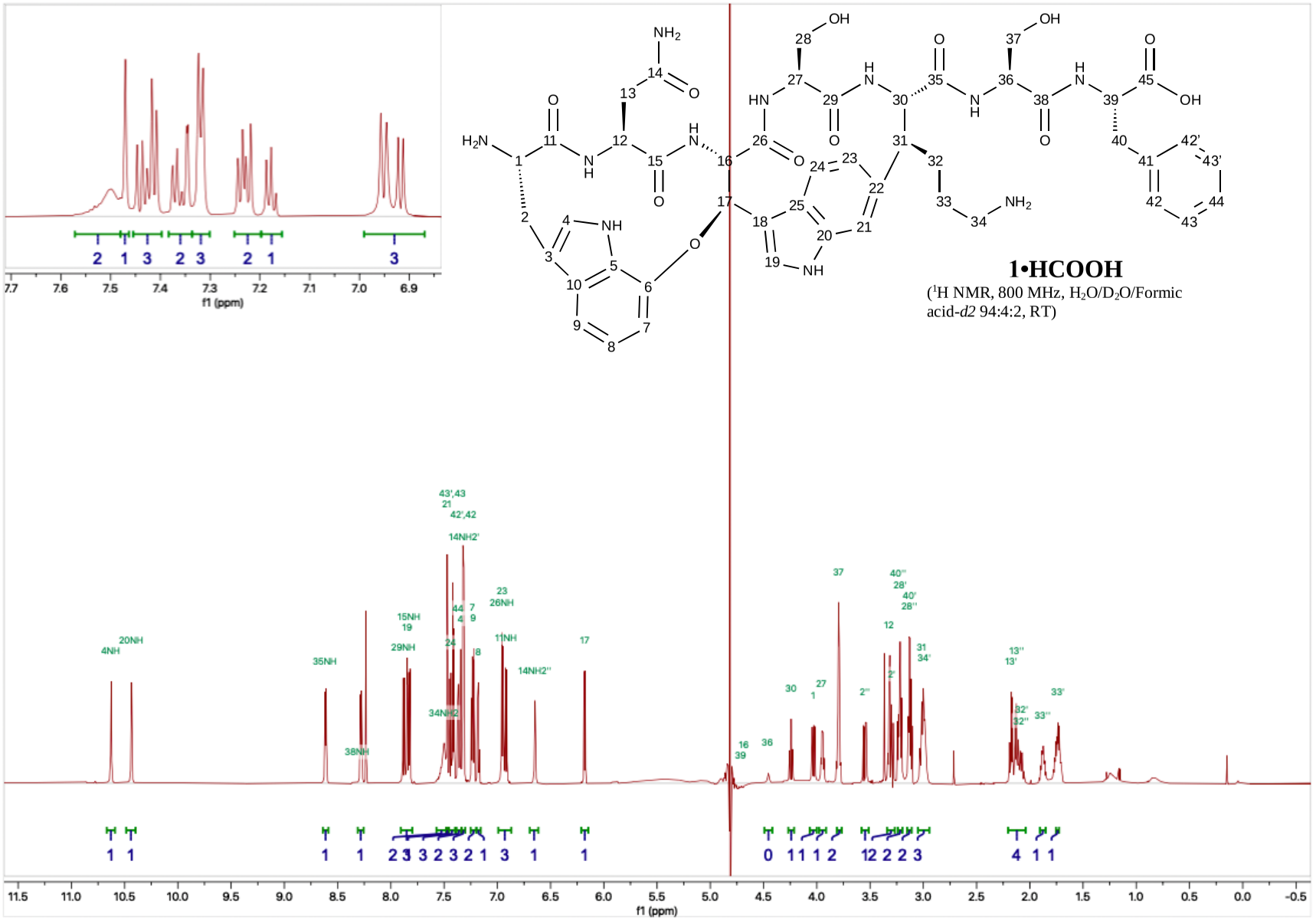

CbzHN
TES
Ac
Ac
CONHTrt
OBn
OBn
NPhth
Ph
CO2Bn
23
(1H NMR, 600 MHz, DMSO–d6, RT)
2.5 DMSO
f1 (ppm)

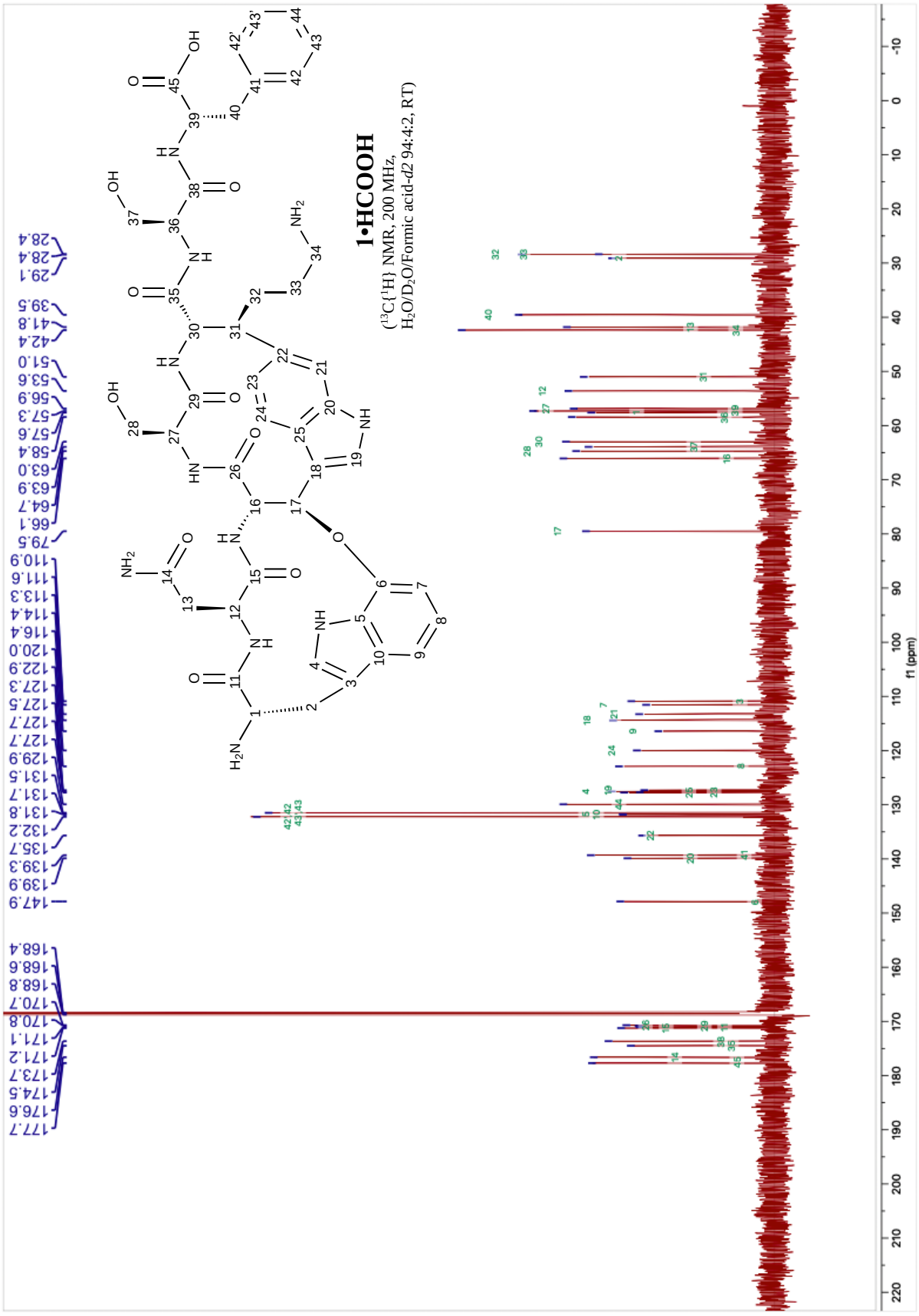

173.0
171.0
170.0
170.0
169.2
168.6
168.5
168.3
168.0
167.8
166.7
165.8
155.3
145.6
144.8
138.0
137.9
137.7
136.8
136.7
135.9
135.6
134.2
133.5
133.3
131.7
131.6
129.2
129.1
128.6
128.4
128.3
128.1
128.1
128.0
127.9
127.9
127.8
127.5
127.5
127.4
127.3
127.2
127.1
127.1
127.0
126.9
126.6
126.2
122.9
118.6
79.2
72.0
71.4
69.8
69.6
66.1
65.6
53.6
52.9
49.3
40.1
39.5 DMSO-d6
38.6
38.3
37.4
36.8
28.6
28.3
27.0
23.9
8.0
CbzHN
TES
Ac
Ac
CONHTrt
OBn
OBn
NPhth
Ph
CO2Bn
23
(13C{1H} NMR, 151 MHz, DMSO–d6, RT)
129.2
129.1
128.6
128.4
128.3
128.1
128.1
128.0
127.9
127.9
127.8
127.5
127.5
127.4
127.3
127.2
127.1
127.1
127.0
126.9
126.6
126.2
129.0
128.5
128.0
127.5
127.0
126.5
126.0
f1 (ppm)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

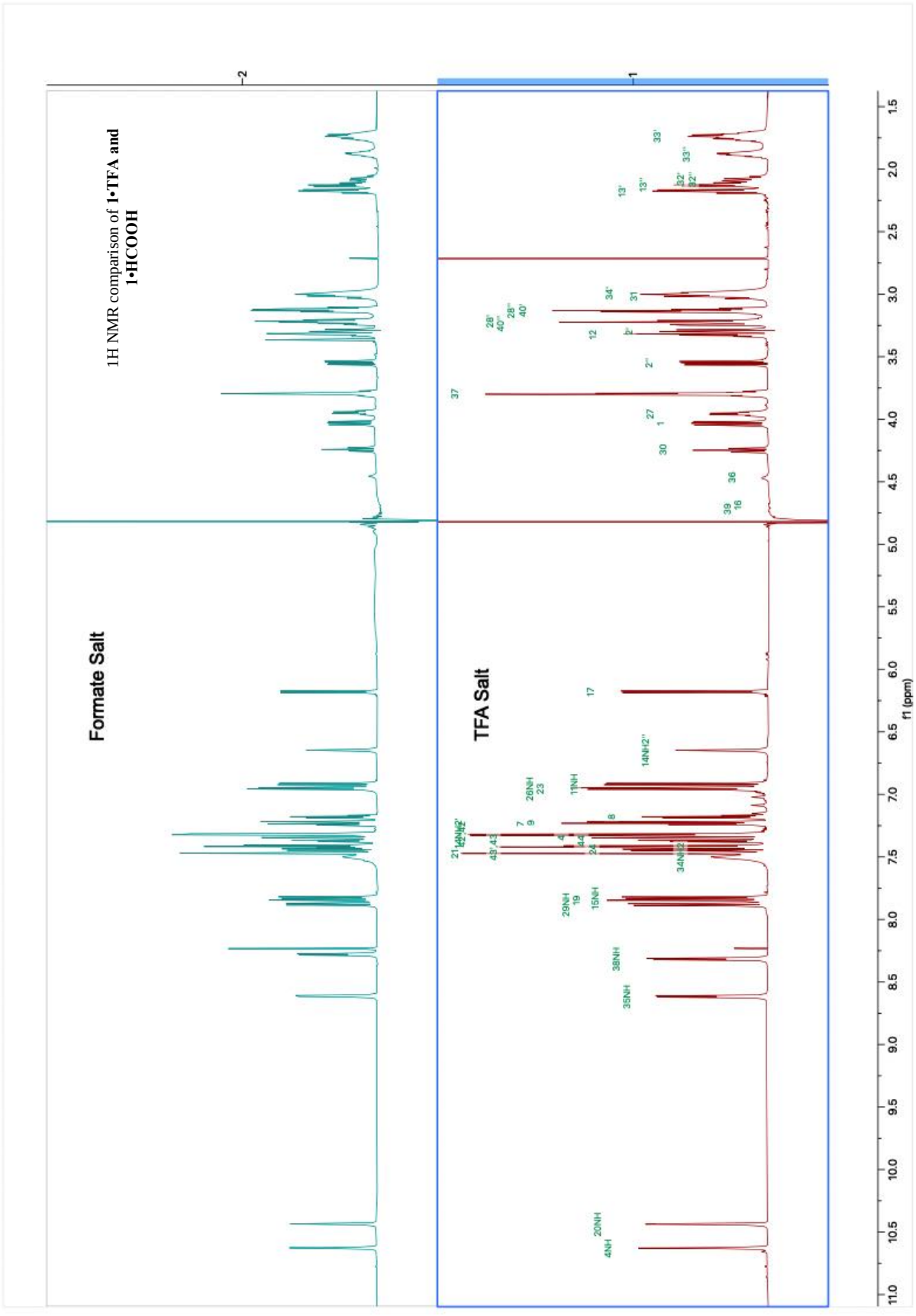

f1 (ppm)
f2 (ppm)
Ha = 7.66 ppm
Hb = 6.13 ppm
(ROESY, 600 MHz, DMSO–d6, RT)

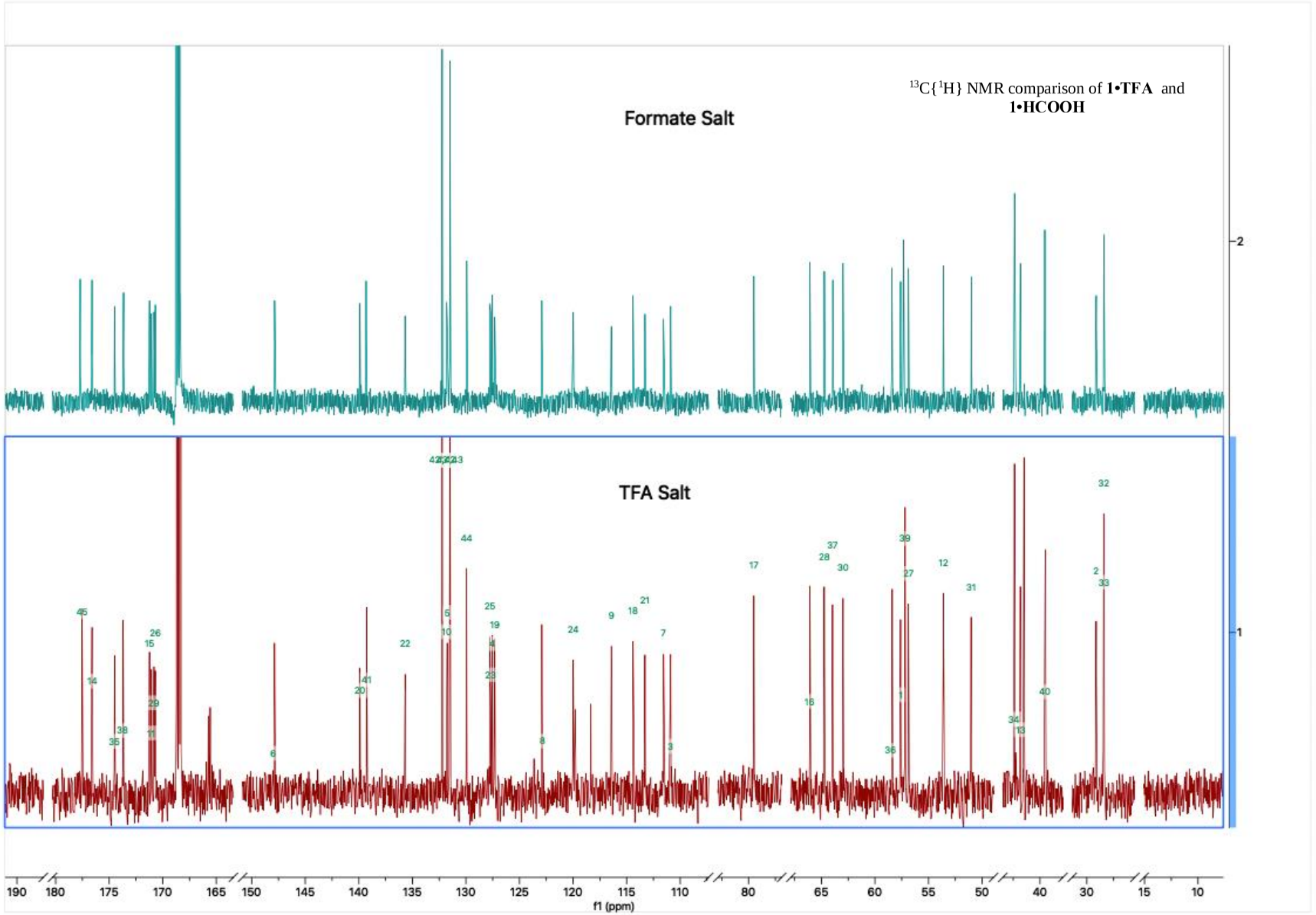

1•TFA
(1H NMR, 800 MHz, H2O/D2O/Formic acid-d2 94:4:2, RT)
f1 (ppm)

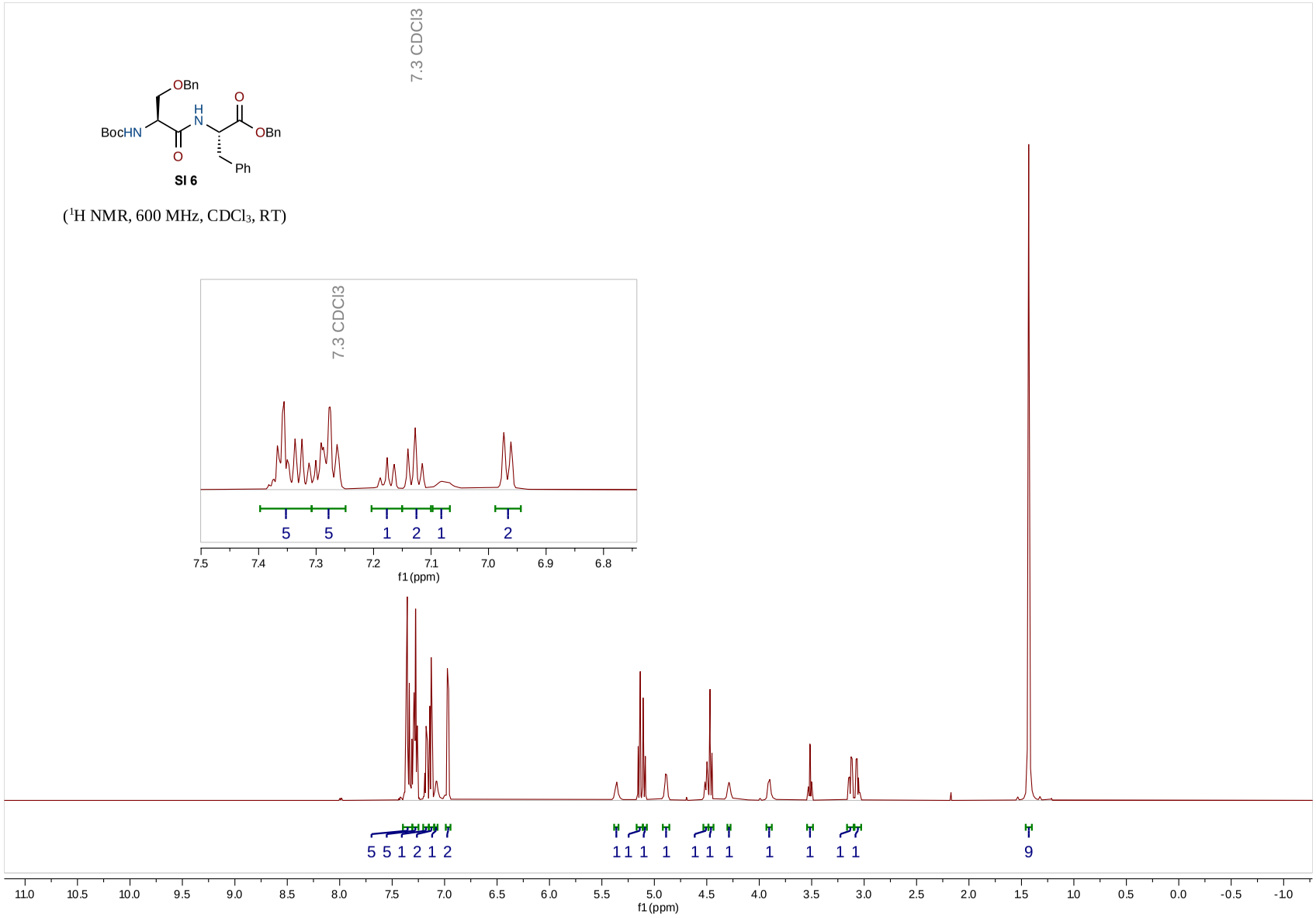

1•TFA
(^{13}C{^{1}H} NMR, 200 MHz,
H_2O/D_2O/Formic acid-*d2* 94:4:2, RT)
TFA
f1 (ppm)


1•HCOOH
(1H NMR, 800 MHz, H2O/D2O/Formic acid-d2 94:4:2, RT)
f1 (ppm)

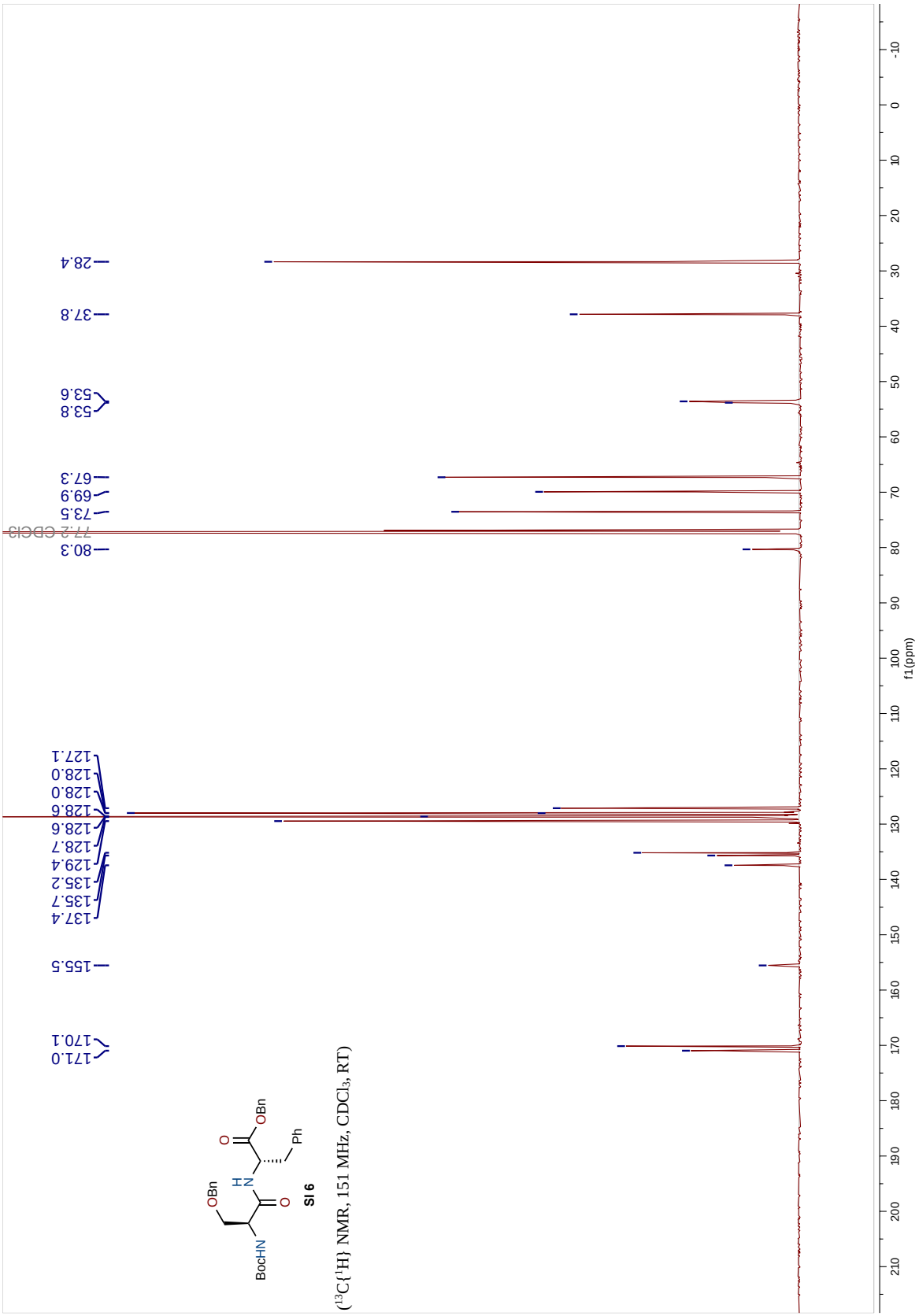

1•HCOOH
($^{13}C\{^1H\}$ NMR, 200 MHz,
H_2O/D_2O/Formic acid-*d2* 94:4:2, RT)
f1 (ppm)

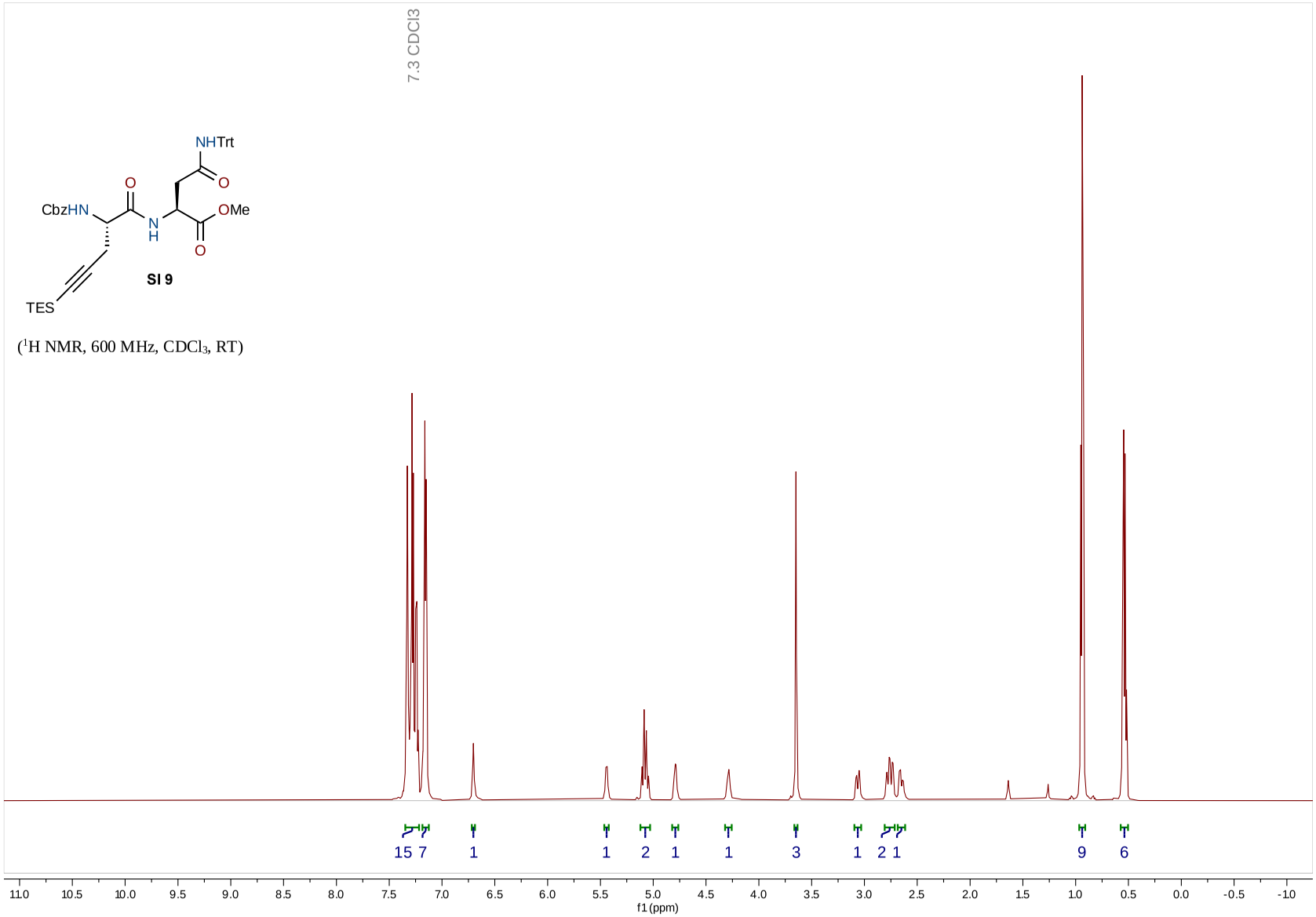

1H NMR comparison of 1•TFA and 1•HCOOH
Formate Salt
TFA Salt
f1 (ppm)


$^{13}C\{^{1}H\}$ NMR comparison of **1•TFA** and **1•HCOOH**
Formate Salt
TFA Salt
f1 (ppm)

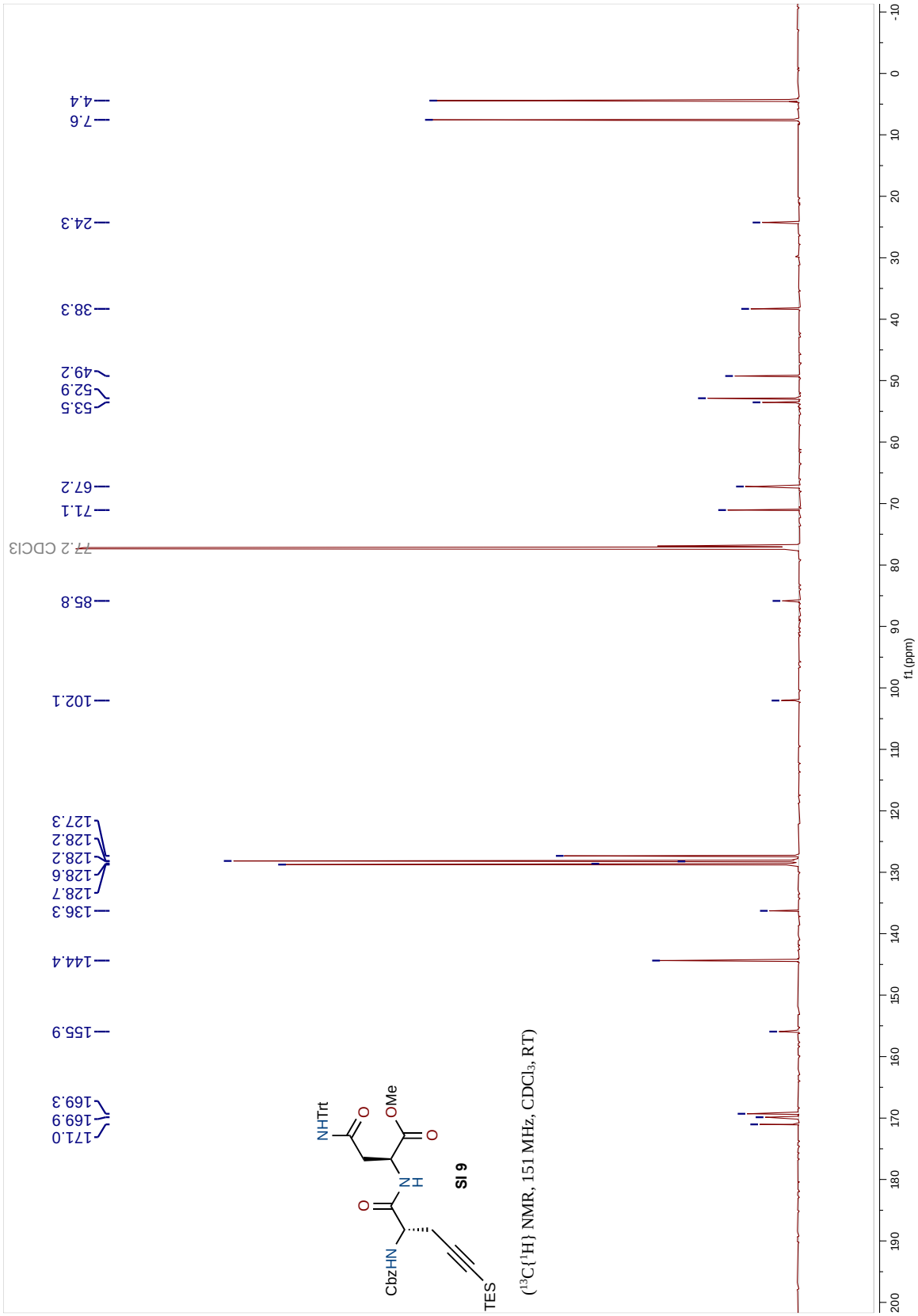


(^{1}H NMR, 600 MHz, $CDCl_3$, RT)


28.4
37.8
53.6
53.8
67.3
69.9
73.5
77.2 CDCl3
80.3
127.1
128.0
128.0
128.6
128.6
128.7
129.4
135.2
135.7
137.4
155.5
170.1
171.0
f1(ppm)
BocHN
OBn
NH
OBn
Ph
SI 6
(^{13}C{^{1}H} NMR, 151 MHz, CDCl$_3$, RT)



(^{1}H NMR, 600 MHz, $CDCl_3$, RT)


SI 9
(^{13}C{^{1}H} NMR, 151 MHz, $CDCl_3$, RT)
f1 (ppm)
77.2 CDCl3