Electronic Supplementary Information

Synthesis and structure confirmation of Fuscachelins A and B, structurally unique natural product siderophores from *Thermobifida fusca*

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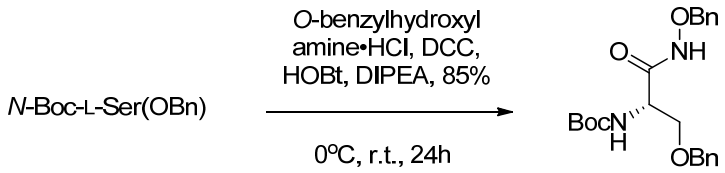
General Experimental Methods. Unless stated otherwise, reactions were performed under an atmosphere of dry nitrogen in flame-dried glassware and stirred with teflon coated magnetic stir bars. Dry acetone and methanol were prepared via distillation over $CaSO_4$. All other organic solvents were obtained from a solvent purification system or were purchased anhydrous from commercial suppliers. Flash chromatography was performed with 32-63 μm, 230-400 mesh active silica gel. Thin layer chromatography (TLC) was performed on Silica Gel 60 F_{254}, 250 μm, glass-backed normal phase plates. TLC plates were visualized under short wave UV illumination and/or were stained by dipping in iodine coated silica or 1% w/v ninhydrin in 3% v/v acetic acid/ethanol and subsequently heated with a heat gun. NMR spectra were acquired on either a Varian Unity 400 MHz, INOVA 500 MHz, or Agilent 600 MHz NMR spectrometer and were analyzed using MestReNova v5.3.1-4825 and v7.1.1-9649 NMR processing software. NMR solvents were purchased from Cambridge Isotope Laboratories. Spectra were referenced to internal tetramethylsilane standard or residual protio-solvent. All NMR chemical shifts are reported in δ (ppm downfield from tetramethylsilane) and all coupling constants (*J*) are reported in Hertz (Hz). ^{1}H NMR peak multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), b (broad), m (multiplet). The following abbreviations are used: **Boc**: *t*-butoxycarbonyl; **DCC**: dicyclohexyl-carbodiimide; **DHB**: dihydroxybenzoyl; **DIPEA**: diisopropylethylamine; **DMAP**: 4-(dimethylamino)pyridine; **EDC**: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; **Fmoc**: 9-fluorenylmethoxycarbonyl; **HOBt**: 1-hydroxybenzotriazole; **NMM**: *N*-methylmor-pholine; **TFA**: trifluoroacetic acid



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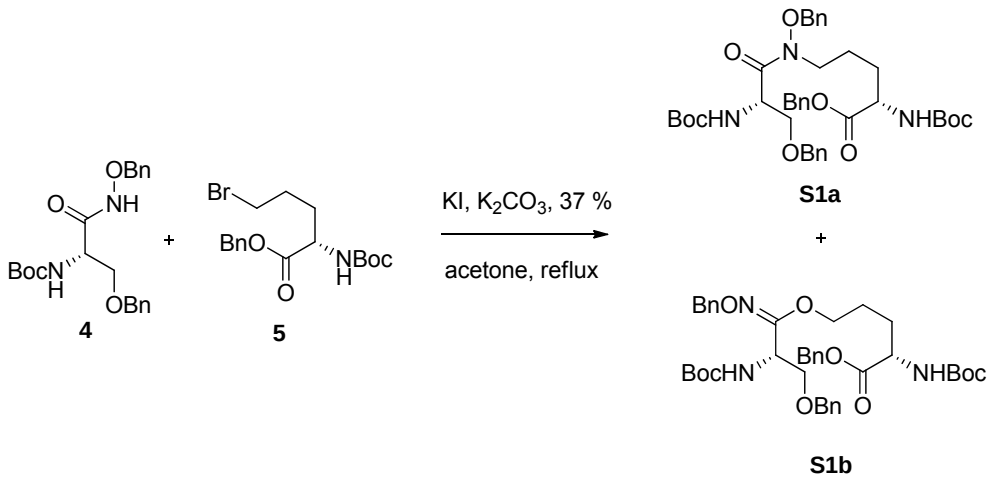
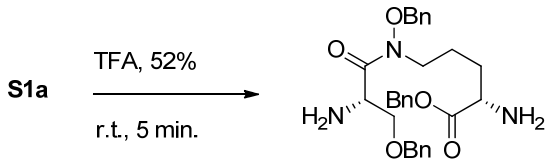
(L)-*N*-α-Boc-serinyl-3-(*O*-benzyl)-1-*O*-benzylhydroxamate (4). (L)-*N*-α-Boc-3-(*O*-benzyl)serine (5.00 g, 16.9 mmol) and HOBt (2.74 g, 20.3 mmol) were taken up in 42 mL THF and the mixture was cooled to 0 ºC. DCC (4.19 g, 20.3 mmol) was then added to the cooled mixture and the OBt ester was allowed to form. Simultaneously, *O*-benzylhydroxylamine (3.24 g, 20.3 mmol) was taken up in 120 mL 1:1 DCM:THF and then DIPEA (7.07 mL, 40.6 mmol) was added to the stirring mixture. After 10 min, the *O*-benzylhydroxylamine/DIPEA mixture was added dropwise to the freshly prepared (L)-*N*-α-Boc-3-(*O*-benzyl)serine-OBt ester. The reaction was run for 24 h and allowed to slowly warm to room temperature. After 24 h, the reaction was concentrated *in vacuo*. Diethyl ether was added to the resulting solid and the slurry was filtered through a plug of celite. The filtrate was washed twice with 0.2 M citric acid, once with 5% $NaHCO_3$ and three times with 0.4 M NaOH. The combined NaOH layers were washed once with diethyl ether, acidified to pH 6.7 with 0.2 M citric acid and extracted twice with DCM. The combined organic layers were dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to afford the product as a white powder (5.75 g, 85%). R_f = 0.7 (10% IPA/$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.45 – 7.18 (m, 10H), 5.38 (bs, 1H), 4.88 (s, 2H), 4.47 (2d, *J* = 11.7 Hz, 2H), 4.34 – 4.17 (bs, 1H), 3.78 (dd, *J* = 9.2, 4.4 Hz, 1H), 3.52 (bdd, *J* = 9.2 Hz, 1H), 1.48 – 1.34 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 155.4, 137.2, 135.1, 129.2, 128.7, 128.5 (2C), 128.3 (2C) 128.2, 127.9, 127.8, 127.7, 80.4, 78.3, 73.4, 69.5, 52.2, 28.3. ESI$^+$ MS calc for $C_{22}H_{29}N_2O_5$ $[M+H]^+$ 401.2077, found 401.2072.



(L,L)-*N*-α-Boc-*N*-δ-benzyloxy-*N*-δ-[*N*-α-Boc-3-(*O*-benzyl)serinyl]-ornithine benzyl ester (S1a).[1, 2] (L)-*N*-α-Boc-serinyl-3-(*O*-benzyl)-1-*O*-benzylhydroxamate (**4**,1.22 g, 3.0 mmol), K_2CO_3 (1.38 g, 10 mmol), and KI (0.168 g, 1.0 mmol) were taken up in 20 mL of anhydrous acetone, stirred and heated to 70 ºC. After 10 min, (L)-*N*-Boc-5-bromonorvaline benzyl ester (**5**)[3] (0.977 g, 2.5 mmol) was dissolved in 30 mL of anhydrous acetone and added dropwise to the refluxing solution. The reaction was run for 24 h at 70 ºC. The suspension was then filtered and the acetone removed *in vacuo*. The residue was dissolved in Et_2O, which was extracted twice with 0.5 N NaOH, once with brine, dried over $MgSO_4$, filtered and concentrated to dryness *in vacuo*. A silica column was run using a stepwise gradient of 20%, 30% and 40% EtOAc in hexanes to afford the *N*-alkylated product as a clear, colorless oil (0.67 g, 37%). R_f = 0.2 (30% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.14 (m, 15H), 5.51-5.37 (d, *J* = 7.34 Hz, 1H), 5.09 (2d, *J* = 12.3 Hz, 2H, benzylic; Boc-NH, 1H), 4.95 (bs, 1H), 4.86 (overlapping d, *J* = 10.2 Hz, 2H), 4.42 (2d, *J* = 12.2 Hz, 2H), 4.33-4.26 (m, 1H), 3.99-3.82 (m, 1H), 3.63 (2dd, *J* = 9.6, 4.8 Hz, 2H), 3.49-3.34 (m, 1H), 1.90-1.73 (m, 1H), 1.73-1.51 (m, 2H), 1.62-1.52 (m, 1H), 1.44 (s, 9H), 1.41 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 171.1, 155.5, 137.8, 135.4, 134.1, 129.4, 129.3, 129.1, 128.8, 128.7, 128.4, 128.3, 127.9, 127.7, 127.7, 79.9, 79.8, 77.0, 73.0, 69.8, 67.1, 53.4, 51.3, 45.1, 29.6, 28.5, 23.1. DART$^+$ MS calc for $C_{39}H_{52}N_3O_9$ $[M+H]^+$ 706.3704, found 706.3684.

The undesired *O*-alkylated regioisomer of (L,L)-*N*-α-Boc-*N*-δ-benzyloxy-*N*-δ-[*N*-α-Boc-3-(*O*-benzyl)serinyl]-ornithine benzyl ester (**S1b**)[2, 3] was also isolated as a white solid (0.530 g, 30%). R_f = 0.5 (30% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$, δ

7.39-7.22 (m, 15H), 5.21-5.06 (m, 3H), 5.03 (bd, *J* = 8.0 Hz, 1H), 4.95 (2d, *J* = 12.5 Hz, 2H), 4.48 (2d, *J* = 12.0 Hz, 2H), 4.42-4.35 (m, 1H), 4.35-4.24 (m, 2H), 4.23-4.15 (m, 1H), 3.64-3.53 (m, 2H), 2.27-2.13 (m, 1H) 2.02-1.89 (m, 1H), 1.89-1.77 (m, 1H), 1.67-1.54 (m, 2H), 1.43(bs, 18H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 172.5, 155.5, 155.3, 153.9, 152.8, 138.0, 137.7, 135.8, 135.5, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.8, 127.7, 80.0, 79.8, 76.6, 73.2, 71.5, 70.2, 67.1, 66.7, 59.3, 59.0, 53.4, 51.4, 46.7, 46.5, 31.0, 30.0, 29.0, 28.5, 28.4, 26.2, 24.4, 23.7. DART$^+$ MS calc for $C_{39}H_{52}N_3O_9$ $[M+H]^+$ 706.3704, found 706.3729.



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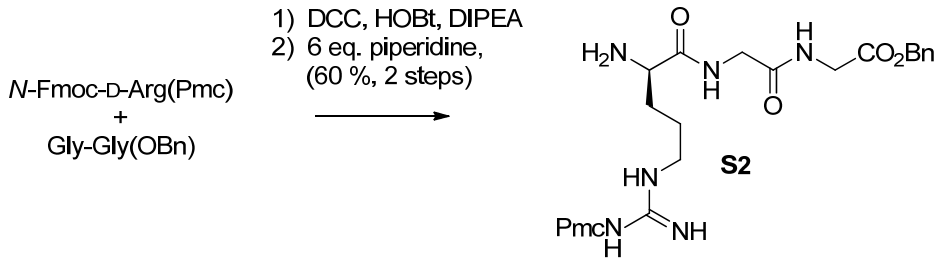
(L,L)-*N*-δ-benzyloxy-*N*-δ-[3-(*O*-benzyl)serinyl]-ornithine benzyl ester (6). (L,L)-*N*-α-Boc-*N*-δ-benzyloxy-*N*-δ-[*N*-α-Boc-3-(*O*-benzyl)serinyl]-ornithine benzyl ester (0.67 g, 0.95 mmol) was dissolved in 5 mL TFA and stirred for 5 min. The TFA was then removed *in vacuo*. The dry residue was then dissolved in DCM and extracted twice with 5% $NaHCO_3$. The combined acqueous layers were washed once with DCM and the pooled organic layers dried, first with brine and then with anhydrous $MgSO_4$. The dried organic extract was then filtered and concentrated *in vacuo*. The residue was then taken up in a small amount of DCM and applied to a silica plug. The plug was rinsed with 3 volumes of DCM and the deprotected peptide was then eluted in 20% IPA/DCM as a clear, colorless oil (0.48 g, 52%). R_f = 0.4 (20% IPA/DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.22 (m, 15H), 5.12 (overlapping d, *J* = 12.3 Hz, 2H), 4.81 (2d, *J* = 10.4 Hz, 2H), 4.47 (2d, J = 12.1 Hz, 2H), 4.03 (bt, *J* = 5.1 Hz, 1H), 3.81-3.73 (m, 1H), 3.59 (dd, *J* = 9.0, 5.6 Hz, 1H), 3.60-3.53 (m, 1H), 3.48 (dd, *J* = 9.1, 6.5 Hz, 1H), 3.45 (dd, *J* = 8.0, 4.7 Hz, 1H), 1.79-1.66 (m, 3H), 1.64-1.48 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.6, 174.5, 138.0, 135.7, 134.2, 129.3, 129.1, 128.8, 128.6, 128.4, 128.4, 128.3,

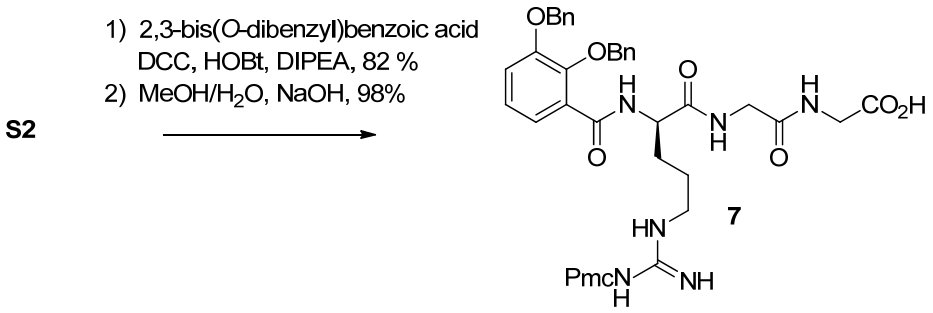
127.7, 127.7, 76.6, 73.2, 72.5, 66.7, 54.1, 51.6, 44.9, 31.7, 23.1. ESI^+ MS calc for $C_{29}H_{36}N_3O_5$ $[M+H]^+$ 506.2649, found 506.2636.



(D)-(*N*-η-Pmc-argininyl)glycinylglycine benzyl ester (S2). Glycinylglycine benzyl ester *p*-toluenesulfonate (2.38 g, 6.0 mmol) was taken up in 20 mL of THF and then DIPEA (3.14 mL, 18 mmol) was added to the slurry and the solid was allowed to dissolve at room temperature. Meanwhile, (D)-*N*-α-Fmoc-*N*-η-Pmc-arginine (2.00 g, 3.0 mmol) and HOBt (0.489 g, 3.6 mmol) were taken up in 40 mL THF and cooled to 0 ºC. DCC (0.747 g, 3.6 mmol) was then added to the mixture and the OBt ester was allowed to form for 10 min. The glycinylglycine benzyl ester *p*-toluenesulfonate/DIPEA mixture was then added dropwise and the reaction was allowed to slowly warm to room temperature over 2 h. Solid was removed via filtration through a plug of celite and a silica column was run (20% IPA/$CHCl_3$) to afford the tripeptide product as a white solid (2.71 g, theoretical yield: 2.62 g). R_f = 0.7 (20%IPA/$CHCl_3$).

The product (2.71 g, 3.1 mmol) was then dissolved in 45 mL of THF. Piperidine (1.85 mL, 18.7 mmol) was added to the solution and the reaction was allowed to proceed at room temperature for 6 h. Solvent was removed *in vacuo* and a silica column was run using a stepwise gradient of 5%, 10%, 20% and 25% MeOH in $CHCl_3$ to afford pure deprotected tripeptide as a white solid (1.17 g, 60% over two steps). R_f = 0.3 (25% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (bs, 1H), 7.62 (m, 1H), 7.32 – 7.23 (m, 5H), 6.44 (bs, 3H), 5.08 (s, 2H), 4.06 – 3.90 (m, 4H), 3.51 (t, *J* = 6.8 Hz, 1H), 3.19 (s, 2H), 2.58 (dd, *J* = 6.6, 5.9 Hz, 2H), 2.55 – 2.50 (m, 6H), 2.07 (s, 3H), 1.77 (dd, *J* = 8.9, 4.8 Hz, 3H), 1.58 (s, 3H), 1.28 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 170.2, 169.9, 156.5, 153.6, 135.4, 135.2, 134.7, 133.3, 128.6, 128.4, 128.2, 124.1, 118.0, 73.6,

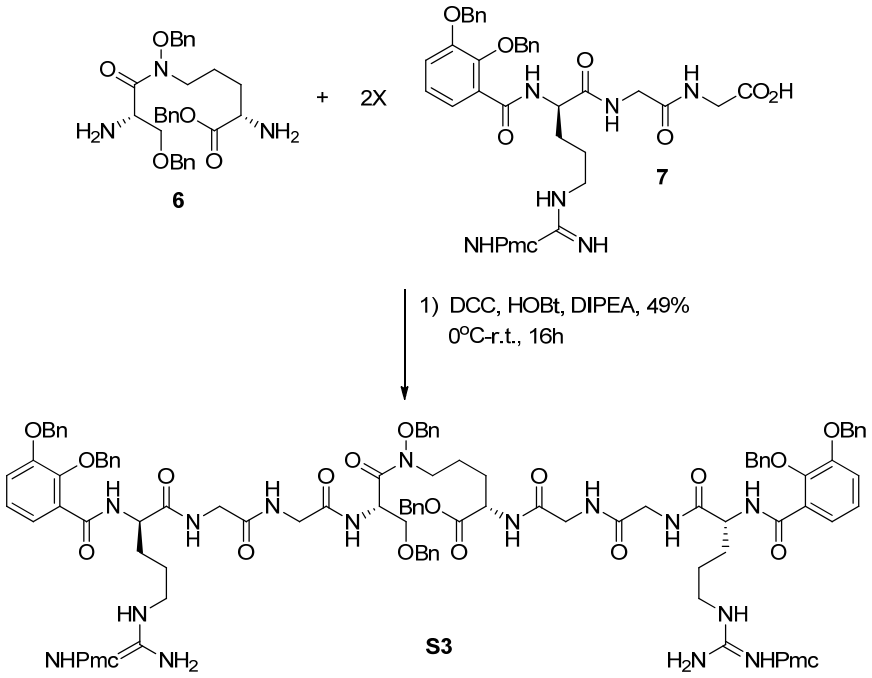
67.1, 54.4, 42.7, 41.3, 32.8, 31.8, 26.8, 25.4, 21.4, 18.5, 17.5, 12.2. ESI^+ MS calc for $C_{31}H_{45}N_6O_7S$ $[M+H]^+$ 645.3065, found 645.3089.



(D)-2,3-bis(dibenzyloxy)benzoyl(*N*-η-Pmc-argininyl))glycinylglycine (7). 2,3-bis(dibenzyloxy)benzoic acid[4] (0.143 g, 0.43 mmol), DCC (0.104 g, 0.5 mmol) and HOBt (0.068 g, 0.5 mmol) were taken up in 2.3 mL DCM and the OBt ester was allowed to form over 30 min. At the same time, the (D)-(*N*-η-Pmc-argininyl)glycinylglycine benzyl ester (**S2**, 0.250 g, 0.39 mmol) was taken up in 2.3 mL DCM to which 406 μL of DIPEA was added and allowed to stir. After 30 min, the tripeptide/DIPEA mixture was transferred to the flask containing 2,3-bis(dibenzyloxy)benzoyl-OBt ester and the reaction was allowed to proceed for 2 h at room temperature. The mixture was then dried *in vacuo* and a silica column run using a stepwise gradient of 0%, 5% and 10% IPA in DCM to give the tetrapeptide product as a white solid (0.305 g, 82%).

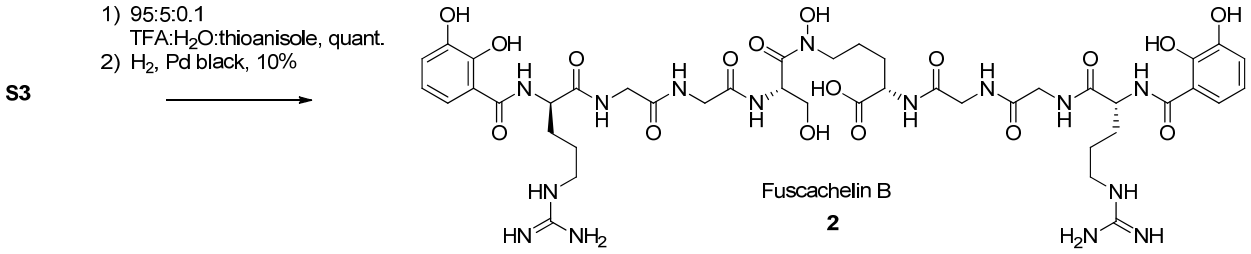
The product (2.19 g, 2.3 mmol) was next dissolved in 122 mL of MeOH. 30 mL of 5 M NaOH was then added to the solution and the reaction was allowed to proceed for 5 h at room temperature. Organic solvent was then removed *in vacuo*. The aqueous residue was then diluted with DI H_2O and the solution was acidified to pH 2 with 3 M HCl, at which point the protonated acid precipitated from solution, affording the saponified tetrapeptide as a white solid (1.95 g, 98%). R_f = 0.7 (25% MeOH/DCM). ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.97 (bs, 1H), 7.79 (bs, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.43 – 7.28 (m, 7H), 7.17 (bs, 3H), 7.08 (d, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H), 6.32 (bs, 2H), 6.21 (bs, 1H), 5.09 (d, *J* = 10.4 Hz, 1H), 5.05 (2, 2H), 5.00 (d, *J* = 10.4 Hz, 1H), 4.44 (bs, 1H), 4.06 – 3.72 (m, 4H), 3.02 (bs, 1H), 2.93 (bs, 1H), 2.53 (t, *J* = 6.1

Hz, 2H), 2.49 (s, 3H), 2.48 (s, 3H), 2.04 (s, 3H), 1.95 – 1.83 (m, 1H), 1.72 (t, *J* = 6.5 Hz, 2H), 1.65 – 1.55 (m, 2H), 1.25 (s, 3H), 1.24 (s. 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 170.9, 166.0, 156.3, 153.5, 151.7, 147.0, 136.3, 136.2, 135.5, 134.8, 128.9, 128.7, 128.6, 128.2, 127.7, 126.1, 124.4, 124.0, 122.9, 117.9, 117.6, 104.2, 76.2, 73.6, 71.3, 63.8, 53.8, 49.2, 42.8, 41.7, 40.1, 34.0, 32.8, 28.8, 26.8, 25.6, 24.9, 21.4, 18.5, 17.9, 17.5, 15.4, 12.1. ESI$^+$ MS calc for $C_{45}H_{54}N_6O_{10}SNa$ $[M+Na]^+$ 893.3514, found 893.3486.



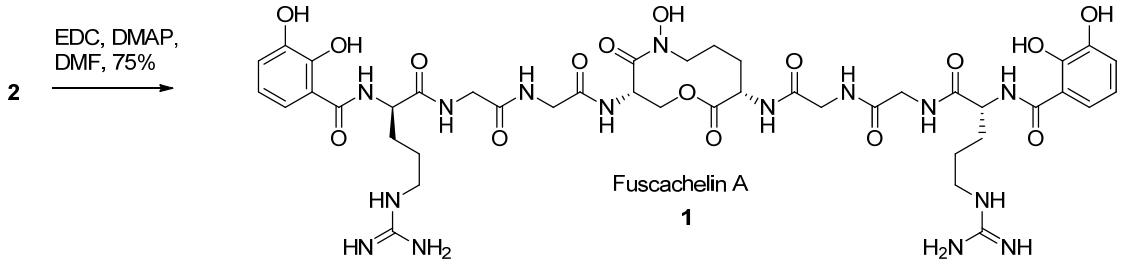
Fuscachelin B-(Bn)$_7$(Pmc)$_2$ (S3). (D)-2,3-bis(dibenzyloxy)benzoyl(*N*-η-Pmc-arginin-yl))glycinylglycine (**7**, 0.296 g, 0.34 mmol) and HOBt (0.051 g, 0.37 mmol) were dissolved in 3.4 mL THF and cooled in a bath of ice and water. DCC (0.077 g, 0.37 mmol) was then added to the stirring solution and the OBt ester was allowed to form over 10 min. DIPEA (0.355 mL, 2.0 mmol) and (L,L)-*N*-δ-benzyloxy-*N*-δ-[3-(*O*-benzyl)serinyl]-ornithine benzyl ester (0.086 g, 0.17 mmol, dissolved in 3.4 mL THF) were added in succession. The reaction was allowed to warm to room temperature. After 2 h, an additional equivalent of DCC (0.035 g, 0.17 mmol) and HOBt (0.023 g, 0.17 mmol) were added. The reaction was then run for 12 h, at which point, one more

equivalent of both DCC and HOBt were added. The reaction was run for an additional 2 h. The insoluble dicyclohexylurea byproduct was removed via filtration through fine pore filter paper. THF was removed *in vacuo* and a silica column was run using a stepwise gradient of 0%, 5%, 10% 15%, 20% and 25% IPA in DCM to afford the fully protected decapeptide as a white solid (0.185 g, 49%). R_f = 0.8 (20% IPA/DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56-8.38 (m, 2H), 7.61-7.52 (m, 2H), 7.48-7.14 (m, 38H), 7.13-7.03 (m, 4H), 6.48-6.01 (m, 5H), 5.23-4.95 (m, 12H), 4.90-4.78 (m, 3H), 4.57-4.50 (m, 1H), 4.50-4.34 (m, 4H), 4.34-4.29 (m, 1H), 4.28-4.18 (m, 1H), 4.07-3.32 (m, 16H), 3.20-2.85 (m, 4H), 2.62-2.49 (m, 14H), 2.07 (s, 6H), 1.79-1.49 (m, 12H), 1.26 (s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 175.1, 173.3, 173.0, 171.8, 171.3, 170.5, 170.0, 169.7, 169.0, 165.9, 165.8, 156.3, 153.5, 151.7, 147.0, 137.4, 136.2, 135.4, 134.7, 134.0, 133.4, 129.3, 129.0, 128.9, 128.9, 128.9, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.2, 128.2, 128.0, 127.6, 126.1, 124.4, 123.9, 122.9, 122.9, 117.8, 117.5, 117.5, 76.2, 73.5, 73.0, 71.3, 69.4, 68.7, 66.9, 66.6, 64.3, 54.1, 53.8, 49.9, 49.5, 49.3, 49.1, 45.0, 44.7, 44.6, 43.1, 43.0, 39.9, 32.7, 26.7, 25.3, 21.4, 18.5, 17.4, 12.1. ESI$^+$ MS calc for $C_{119}H_{139}N_{15}O_{23}S_2Na$ $[M+Na]^+$ 2232.9507, found 2232.9511.



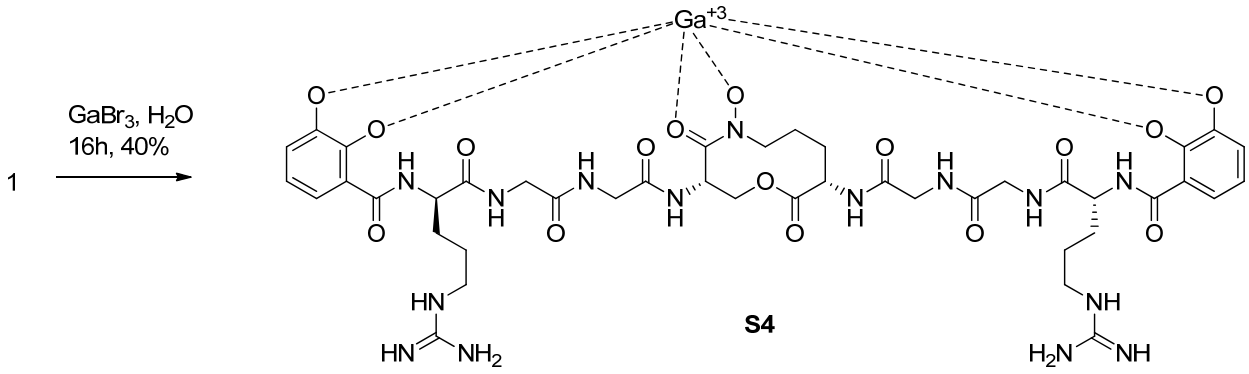
Fuscachelin B (2). Fuscachelin B-$(Bn)_7(Pmc)_2$ (**S3**, 0.155 g, 70 μmol) was taken up in 5 mL of a solution of 95:5:0.1 TFA:H_2O:thioanisole and stirred at room temperature for 2.5 h. The liquid was then removed *in vacuo* and the residue dissolved in water and lyophilized. Then, 10 mg of the freeze dried material was dissolved in 1.5 mL of 25 % MeOH/glacial acetic acid (v/v), 15 mg of Pd black was added and the vessel was purged with dry nitrogen for 10 min. The slurry was then purged with hydrogen gas (balloon conditions, ambient temperature and pressure) for 16 h. The Pd black was pelleted at 13,000 rpm for 1 min using an Eppendorf centrifuge 5417C equipped with an

F45-30-11 rotor. The supernatant was collected and evaporated *in vacuo*, the residue dissolved in water and the material lyophilized. The freeze dried solid was then taken up in 1 mL of 23% MeOH/ 0.1 % TFA (v/v) water and applied to a Vydac 218TP1022 Protein and Peptide C18 column (250 x 22 mm, 10 μm) on a Shimadzu LC-6AD liquid chromatography system equipped with a Shimadzu SPD-10A UV-Vis detector. A detection wavelength of 320 nm was chosen to monitor elution of the 2,3-dihydroxybenzoyl moiety. Two, 500 μL portions were injected onto the column and purified using a linear gradient of 23-28% methanol in water/0.1% TFA over 30 min at 8 mL min^{-1}. The material was collected at 17 min. Methanol was removed by rotary evaporation and the aqueous solution was dried by lyophilization to afford the peptide as a white solid (530 μg, 10%). MALDI$^+$ MS calc for $C_{42}H_{62}N_{15}O_{17}$ $[M+H]^+$ 1048.4448, found 1048.4431. Note: extra signals present in the proton NMR (p. 33/34) likely arise from impurities corresponding to chelation with metal contaminants.



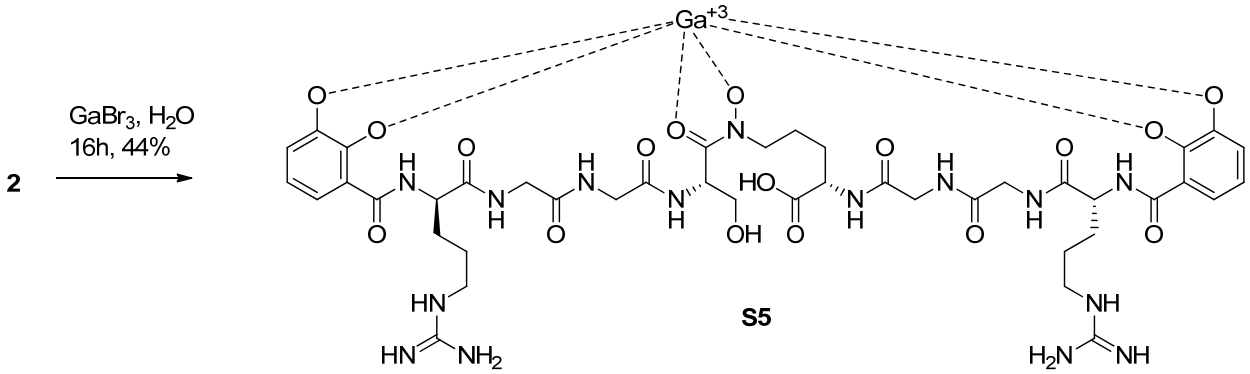
Fuscachelin A (1).[5] 2 mg of Fuscachelin B (1.9 μmol) was taken up in 1.178 mL of dry DMF. To this solution was added EDC (3.8 μmol, 38 μL from a freshly prepared 100 mM solution in dry DMF) and DMAP (5.7 μmol, 57 μL from a freshly prepared 100 mM solutionin dry DMF). The mixture was gently agitated at room temperature for 36 h. At 12 h and 24 h, an additional equivalent of EDC (19 μL, 100 mM stock) and DMAP (19 μL, 100 mM stock) was added to the mixture. The reaction was terminated by flash freezing in liquid nitrogen. The solvent was then removed via lyophilization. The product was purified by preparative HPLC using a Vydac 218TP1022 Protein and Peptide C18 column (250 x 22 mm, 10 μm) on a Shimadzu LC-6AD liquid chromatography system equipped with a Shimadzu SPD-10A UV-Vis detector. A

detection wavelength of 320 nm was chosen to monitor elution of the 2,3-dihydroxybenzoyl moiety. The lyophilized material was dissolved in 500 μL of 23% methanol/ 0.1% TFA (v/v) water. Two, 250 μL portions were injected onto the column and purified using a linear gradient of 23-28% methanol in 0.1% TFA (v/v) water over 30 min at 8 mL min^{-1}. The material was collected at 22 min. Methanol was removed by rotary evaporation and the aqueous solution was dried by lyophilization to afford the peptide as a white solid (1.47 mg, 75%). LCQ ESI^+ MS calc for $C_{42}H_{60}N_{15}O_{16}$ $[M+H]^+$ 1030.4, found 1030.5. HR LC ESI^+ calc for $C_{42}H_{60}N_{15}O_{16}$ $[M+H]^+$ 1030.4342, found 1030.4373. HR LC ESI^+ calc for $C_{42}H_{60}N_{15}O_{16}$ $[M+2H]^{2+}$ 515.7205, found 515.7230.

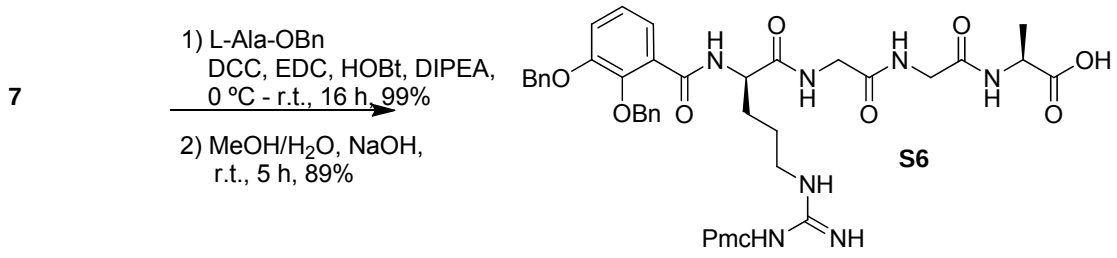


Fuscachelin A – Ga^{3+} complex (S4). This reaction was performed in a flask that had been washed with a 1:1 solution of HNO_3 and dd H_2O. To 8.6 mL of dd H_2O was added 97.1 μL of a 0.01 M fuscachelin A stock solution (1.0 mg, 0.97 μmol) and 971 μL of a 0.01 M $GaBr_3$ solution (3.00 mg, 9.7 μmol). After 16 h, the reaction was flash frozen and lyophilized. The complex was purified via HPLC (same equipment as stated above for **1** and **2**) using a linear gradient of 2-80% B in A (where buffer A is 5 mM ammonium acetate pH 6.5 and buffer B is methanol) over 30 min following an initial 3 min wash with 2% B. A flow rate of 10 mL min^{-1} and detection wavelengths of 220 and 320 nm were used. The product eluted at 18 min. Organic solvent was removed via rotary evaporation and water was removed via lyophilization to give the complex as a white

solid (400 μg, 40%). MALDI$^+$ MS calc for $C_{42}H_{57}N_{15}O_{16}Ga$ $[M+Ga]^+$ 1096.3, found 1096.2.

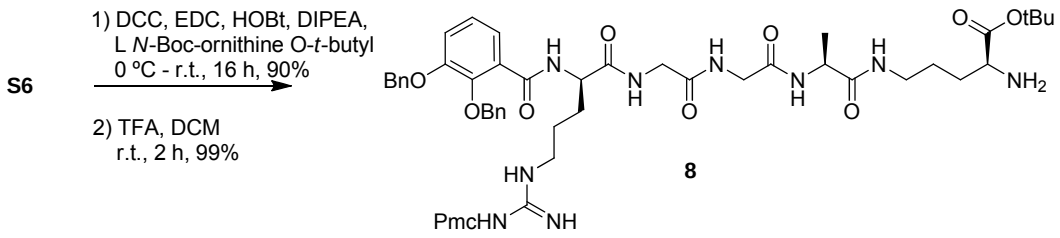


Fuscachelin B – Ga^{3+} complex (S5). This reaction was performed in a flask that had been washed with a 1:1 solution of HNO_3 and dd H_2O. To 8.6 mL of dd H_2O was added 9.5 μL of a 0.1 M fuscachelin B stock solution (1.0 mg, 0.95 μmol) and 950 μL of a 0.01 M $GaBr_3$ solution (2.96 mg, 9.5 μmol). After 16 h, the reaction was flash frozen and lyophilized. The complex was purified via HPLC (same equipment as stated above for **1** and **2**) using a linear gradient of 2-80% B in A (where buffer A is 5 mM ammonium acetate pH 6.5 and buffer B is methanol) over 30 min following an initial 3 min wash with 2% B. A flow rate of 10 mL min^{-1} and detection wavelengths of 220 and 320 nm were used. The product eluted at 15 min. Organic solvent was removed via rotary evaporation and water was removed via lyophilization to give the complex as a white solid (450 μg, 44%). MALDI$^+$ MS calc for $C_{42}H_{59}N_{15}O_{17}Ga$ $[M+Ga]^+$ 1114.3464, found 1114.3471.



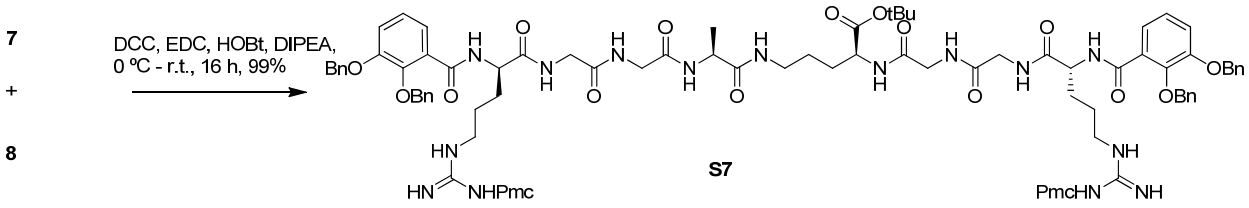
(D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-argininyl))glycinylglycinylalanine (S6). (D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-argininyl))glycinylglycine (**7**, 0.348 g, 0.40 mmol), DCC (0.165 g, 0.8 mmol), EDC (0.153g, 0.8 mmol) and HOBt (0.245g, 1.6

mmol) were taken up in 5 mL DCM/5 mL THF on ice and the OBt ester was allowed to form over 30 min. At the same time, the (L)-alanine benzyl ester (0.093 g, 0.52 mmol) was taken up in 5 mL DCM/5 mL THF to which 697 μL of DIPEA was added and allowed to stir. After 30 min, the alanine/DIPEA mixture was transferred to the flask containing the tetrapeptide-OBt ester and the reaction was allowed to proceed for 16 h at room temperature. The mixture was then dried *in vacuo* and a silica column run using a stepwise gradient of 0%, 5%, 10%, and 20% MeOH in $CHCl_3$ to give the pentapeptide product as a white solid. The product (0.425g) was taken up in 14.4 mL MeOH to which 3.6 mL 5.0 M NaOH was added. After 5 hr stirring at room temperature, the mixture was dried *in vacuo*, diluted with 20 mL ddH_2O, acidified to pH 2 with 6 N HCl, and extracted with 3x20 mL EtOAc. The organic fractions were combined, dried over $MgSO_4$, and concentrated *in vacuo* to yield the pure product as a white solid (0.344g, 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.51 (d, 1H), 7.42 (m, 1H), 7.38 - 7.33 (m, 3H), 7.12 (d, 1H), 7.08 (m, 1H), 6.98 (s, 1H), 5.11 - 5.01 (m, 4H), 4.28 (m, 2H), 3.88 (m, 4H), 3.04 - 2.94 (m, 2H), 2.57 (t, 2H), 2.50 (m, 3H), 2.27 (s, 3H), 2.07 (s, 3H), 1.76 (m, 2H), 1.43 (s, 10H), 1.27 (s, 3H). ESI$^+$ MS calc for $C_{48}H_{59}N_7NaO_{11}S$ $[M + Na]^+$ 964.3885, found 964.3882.



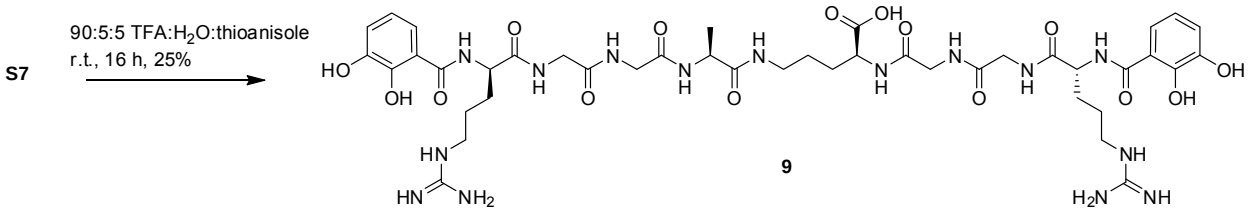
(D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-argininyl))glycinylglycinylalaninyl-ornithine t-butyl ester (8). (D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-argininyl))glycinylglycinylalanine (**S6**, 0.344g, 0.37 mmol), DCC (0.150 g, 0.73 mmol), EDC (0.140g, 0.73 mmol) and HOBt (0.224g, 1.5 mmol) were taken up in 5 mL DCM/5 mL THF, cooled on ice and the OBt ester was allowed to form over 30 min. At the same time, the (L)-*N*-Boc-ornithine t-butyl ester (0.154 g, 0.47 mmol) was taken up in 5 mL DCM/5 mL THF (on ice) to which 636 μL of DIPEA was added and allowed to stir. After

30 min, the ornithine/DIPEA mixture was transferred to the flask containing the pentapeptide-OBt ester and the reaction was allowed to proceed for 16 h at room temperature. The mixture was then dried *in vacuo* and a silica column run using a stepwise gradient of 0%, 5%, 10%, and 20% MeOH in $CHCl_3$ to give the protected hexapeptide product as a white solid (0.370 g, 90%). The product (95 mg, 102 mmol) was taken up in 4.25 mL DCM and placed in an ice bath with N_2 atmosphere. To this mixture was added 0.75 mL TFA dropwise. After 2hr stirring on ice, the reaction was diluted with chelexed ddH_2O and extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo* to yield the free hexapeptide as the TFA salt , (91 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.47 (m, 1H), 7.40 (m, 3H), 7.39 (m, 2H), 7.20 - 6.98 (m, 4H), 5.20 - 5.01 (m, 4H), 4.40 - 4.31 (m, 2H), 3.95 (m, 4H), 3.19 - 3.06 (m, 4H), 2.60 - 2.54 (m, 6H), 2.27 (s, 3H), 2.09 (s, 3H), 1.79 (t, 3H), 1.43 (m, 10H), 1.29 (m, 3H). ESI$^+$ MS calc for $C_{57}H_{77}N_9O_{12}S$ $[M + H]^+$ 1112.5485, found 1112.5493.

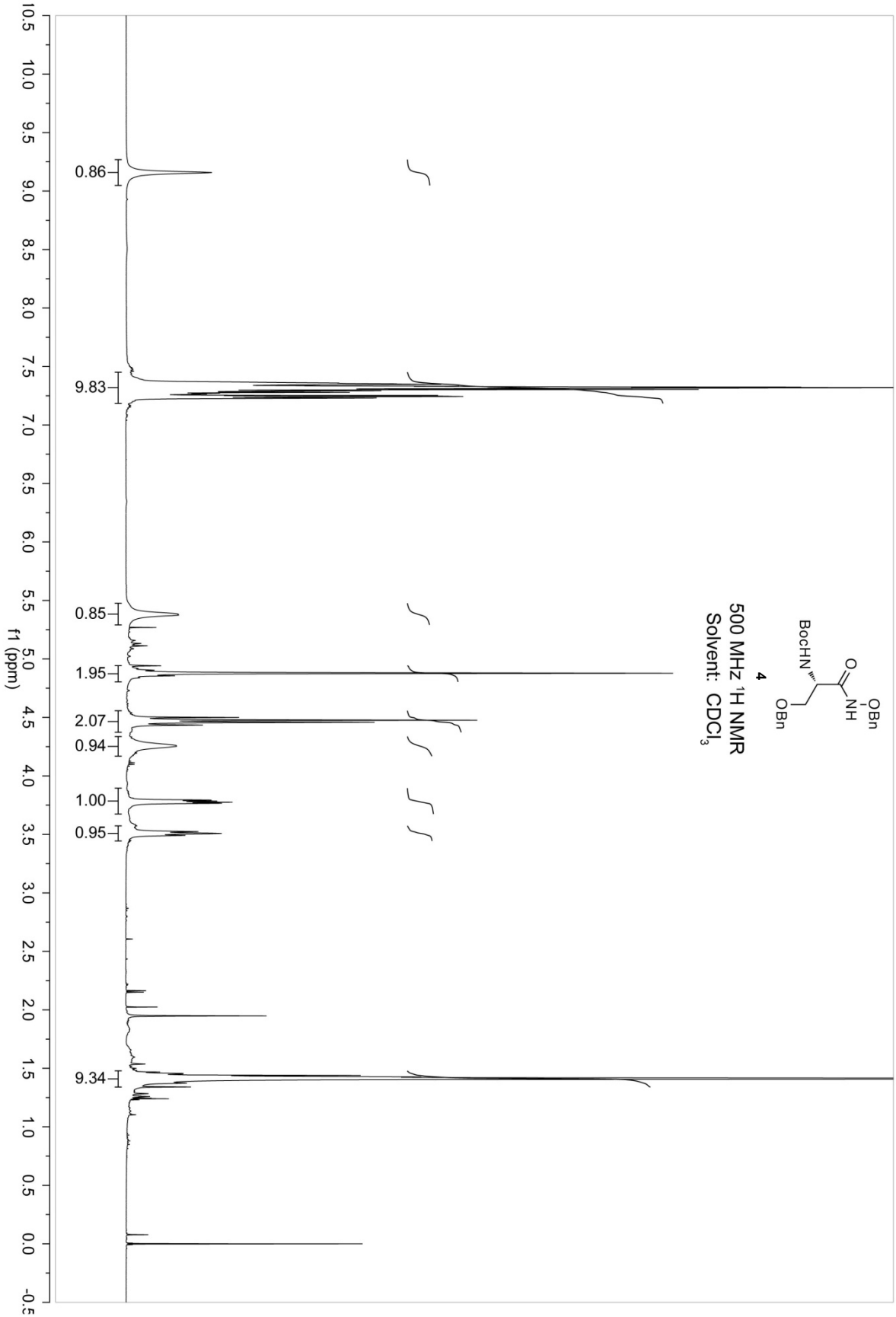


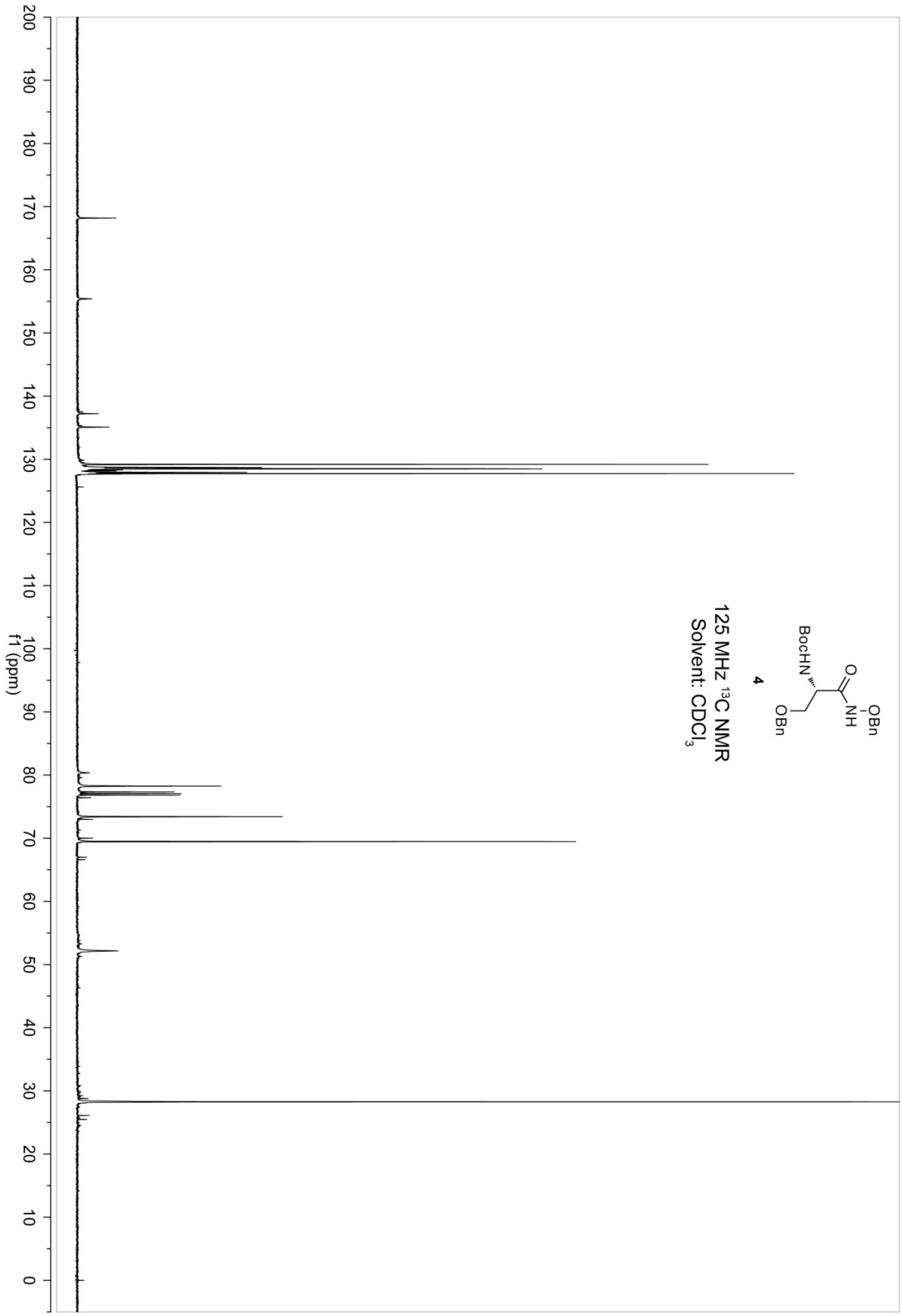
Fuscachelin analog(tBu)(Pmc)$_2$(Bn)$_4$ (S7). (D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-arginin-yl))glycinylglycine (**7**, 0.124 g, 0.11 mmol), DCC (0.069 g, 0.33 mmol), and HOBt (0.051g, 0.33 mmol) were taken up in 1.5 mL DCM/1.5 mL THF in an ice bath and the OBt ester was allowed to form over 30 min. At the same time, the (D)-2,3-bis(dibenzyloxy)benzoyl(*N*-h-Pmc-argininyl))glycinylglycinylalaninyl(*N*-Boc-ornithine) (**8,** 0.117 g, 0.13 mmol) was taken up in 1.5 mL DCM plus 1.5 mL THF to which 233 μL of DIPEA was added and allowed to stir. After 30 min, the hexapeptide/DIPEA mixture was transferred to the flask containing the tetrapeptide-OBt ester and the reaction was allowed to proceed for 16 h at room temperature. The mixture was then dried *in vacuo* and a silica column run using a stepwise gradient of 0%, 5%, 10%, and 20% MeOH in $CHCl_3$ to give the pentapeptide product as a white solid (yield 0.221 g, theoretical yield 0.219 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (s, 2H), 7.53 (b, 2H), 7.43

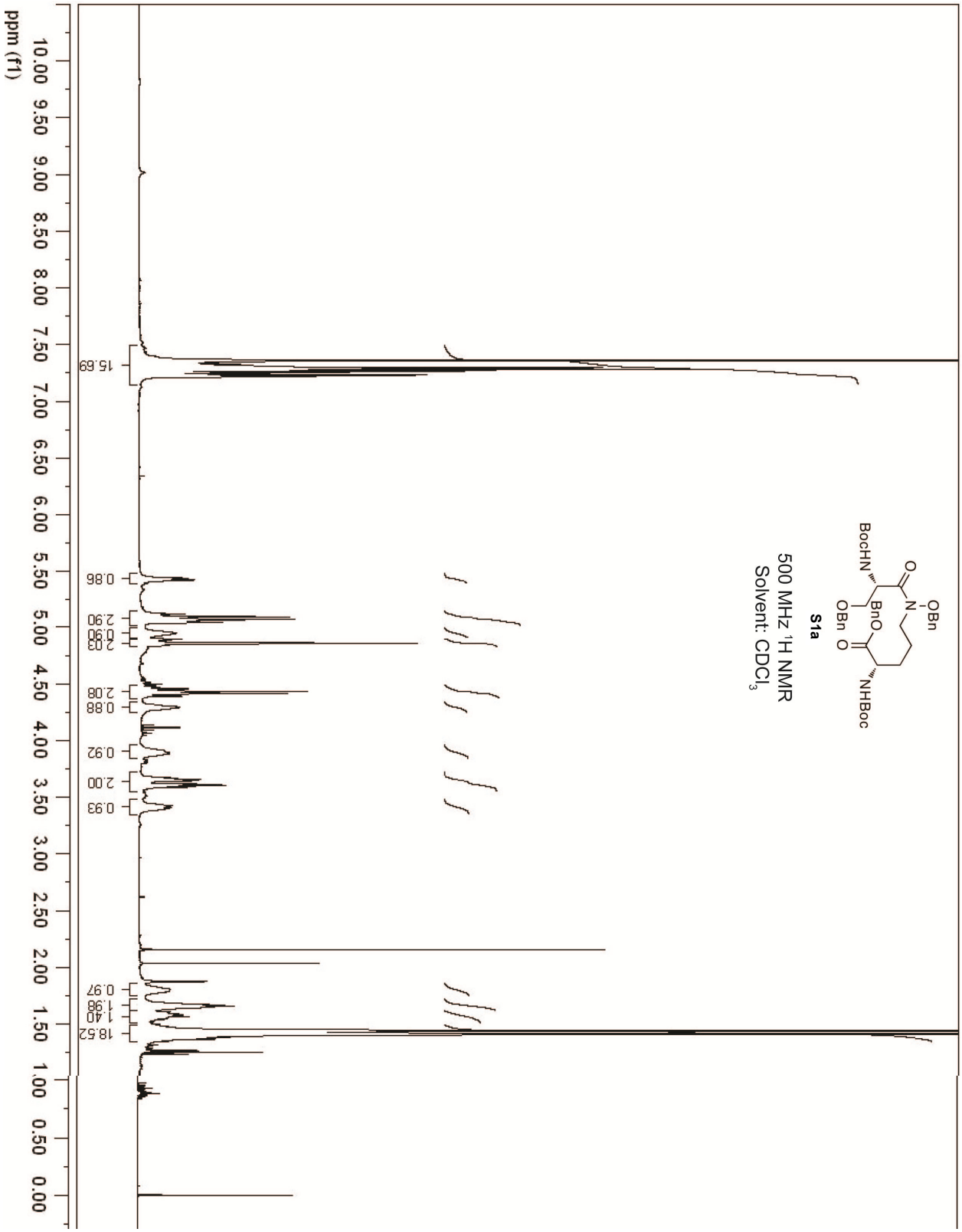
(d, 2H), 7.38 (m, 5H), 7.14 (d, 2H), 7.08 (m, 2H), 6.98 (s, 2H), 5.13 - 5.01 (m, 8H), 4.26 (m, 4H), 4.00 - 3.78 (m, 8H), 3.09 (b, 6H), 2.59 (b, 2H), 2.56 (m, 2H), 2.49 (b, 6H), 2.27 (s, 6H), 2.07 (s, 6H), 1.77 (t, 2H), 1.53 (m, 4H), 1.43 (s, 6H), 1.39 (s, 6H), 1.29 (s, 6H). ESI$^+$ MS calc for $C_{102}H_{129}N_{15}Na_2O_{21}S_2$ $[M + 2Na]^{2+}$ 1004.9357, found 1004.9330.

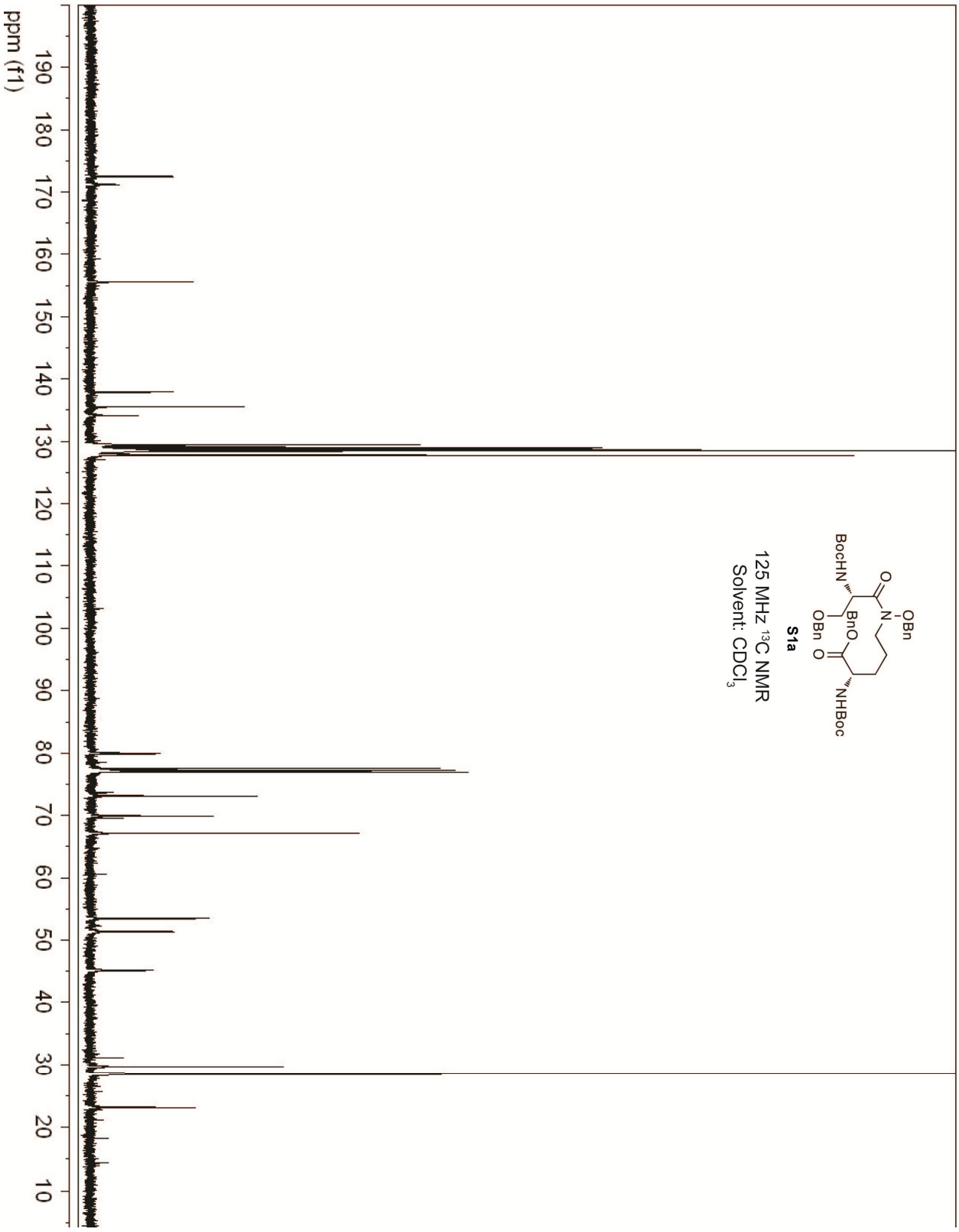


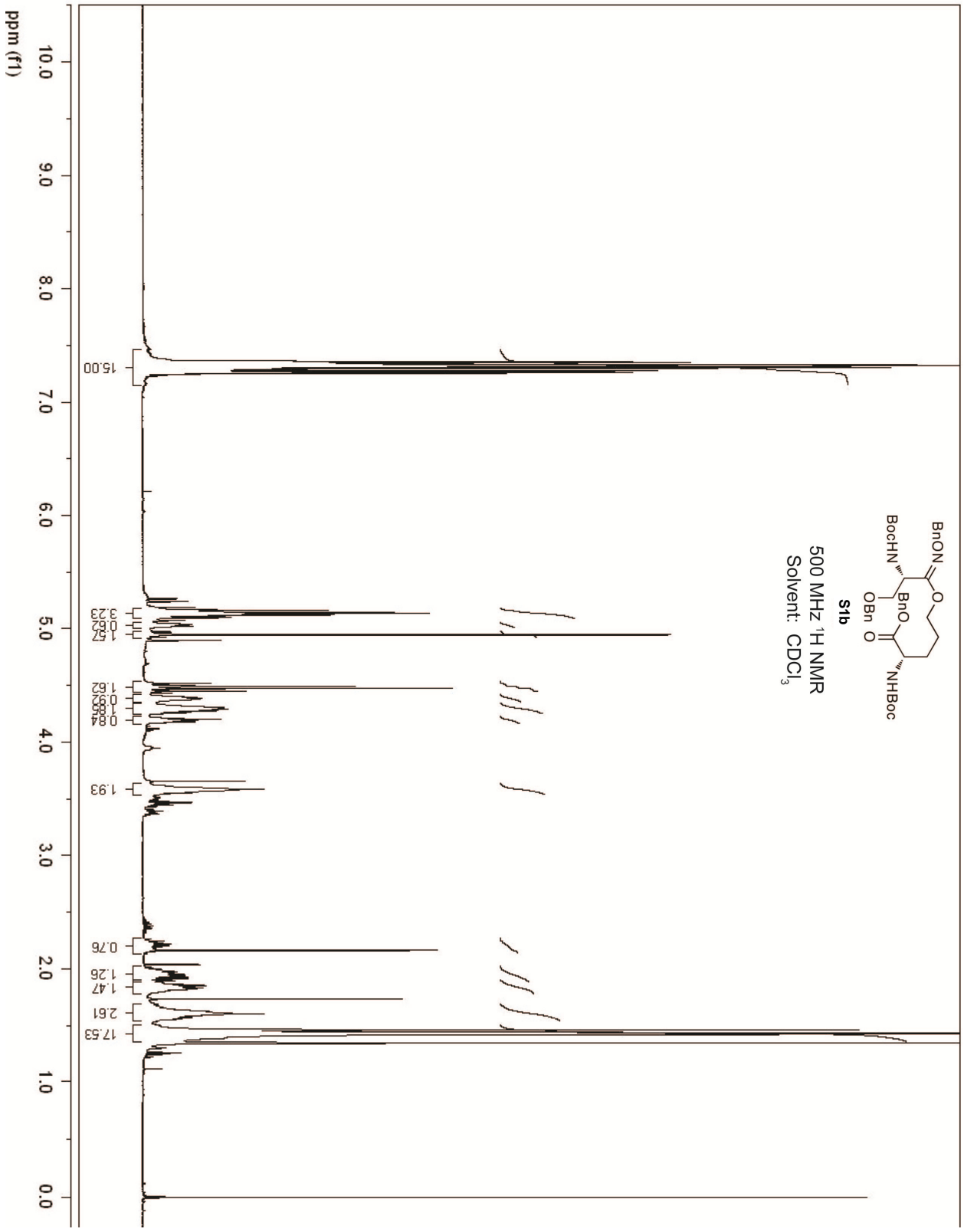
Fuscachelin analog (9) Fuscachelin analog(tBu)(Pmc)$_2$(Bn)$_4$ (**S7**, 11.9 mg, 6.0 µmol) was dissolved in 2.0 mL 90:5:5 TFA:thioanisole:chelexed ddH_2O and allowed to stir at room temp. under N_2 for 16 hr. The reaction was then diluted with chelexed ddH_2O, flash frozen, and lyophilized. The dry material was taken up in 25% MeOH/chelexed ddH_2O and purified by RP-HPLC using a linear gradient of 10-100% acetonitrile in water/0.1% TFA over 40 minutes. The product eluted at 18.5 min. Rotary evaporation of the acetonitrile followed by lyophilization gave the product as a white solid (2.5 mg, 25%). ^{1}H NMR (600 MHz, D_2O) δ 7.26 (d, J = 7.7 Hz, 2H), 7.03 (m, 2H), 6.84 (t, J = 8.0 Hz, 2H), 4.54 (m, 2H), 4.17 (m, 2H), 3.95 (m, 8H), 3.21 (m, 4H), 3.03 (m, 2H), 1.97 (m, 2H), 1.88 (m, 2H), 1.72 (m, 4H), 1.57 (m, 2H), 1.45 (m, 2H) 1.22 (d, J = 7.2 Hz, 3H). MALDI-TOF-MS calc for $C_{42}H_{61}N_{15}O_{15}$ $[M + H]^+$ 1016.4543, found 1016.4572.

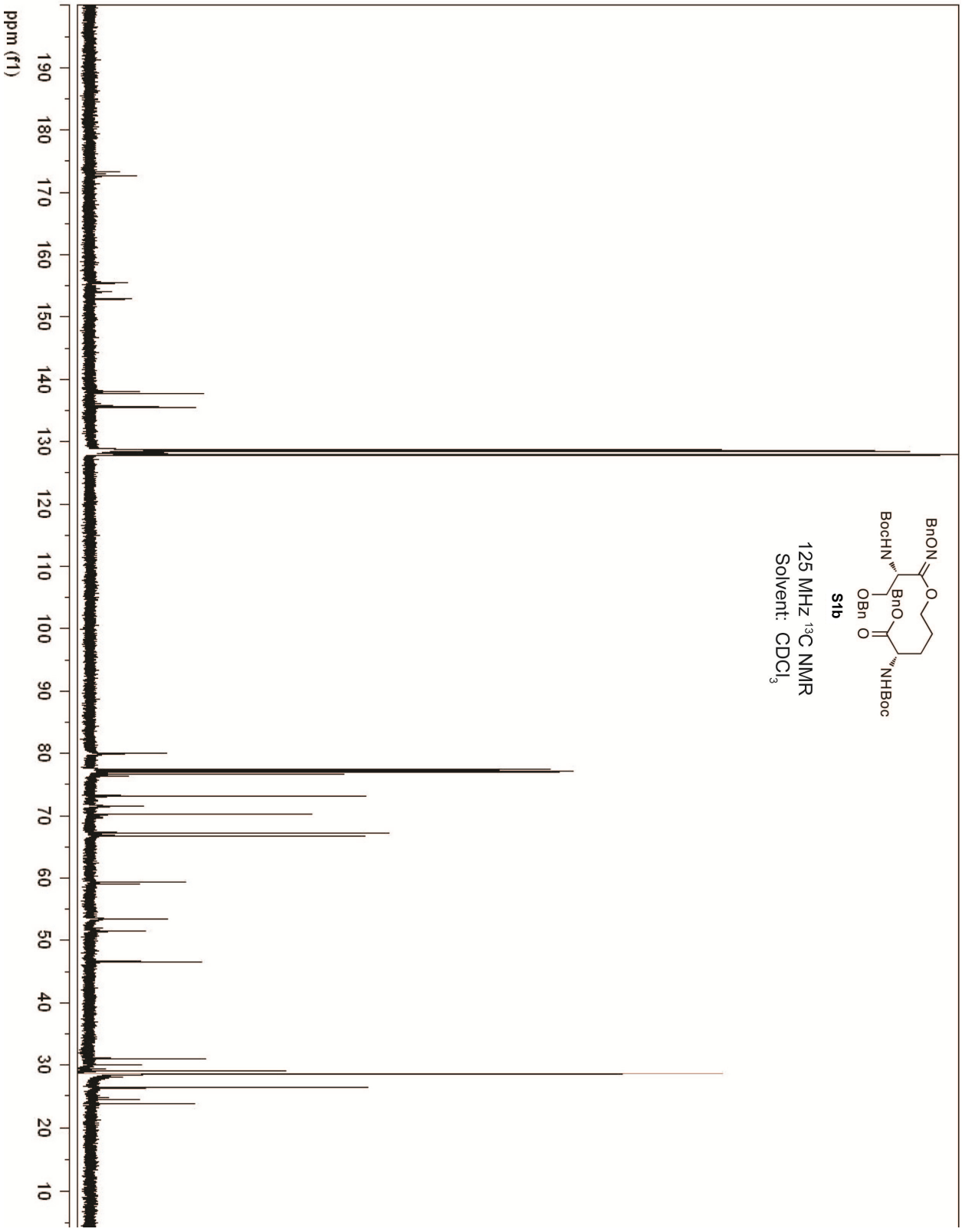


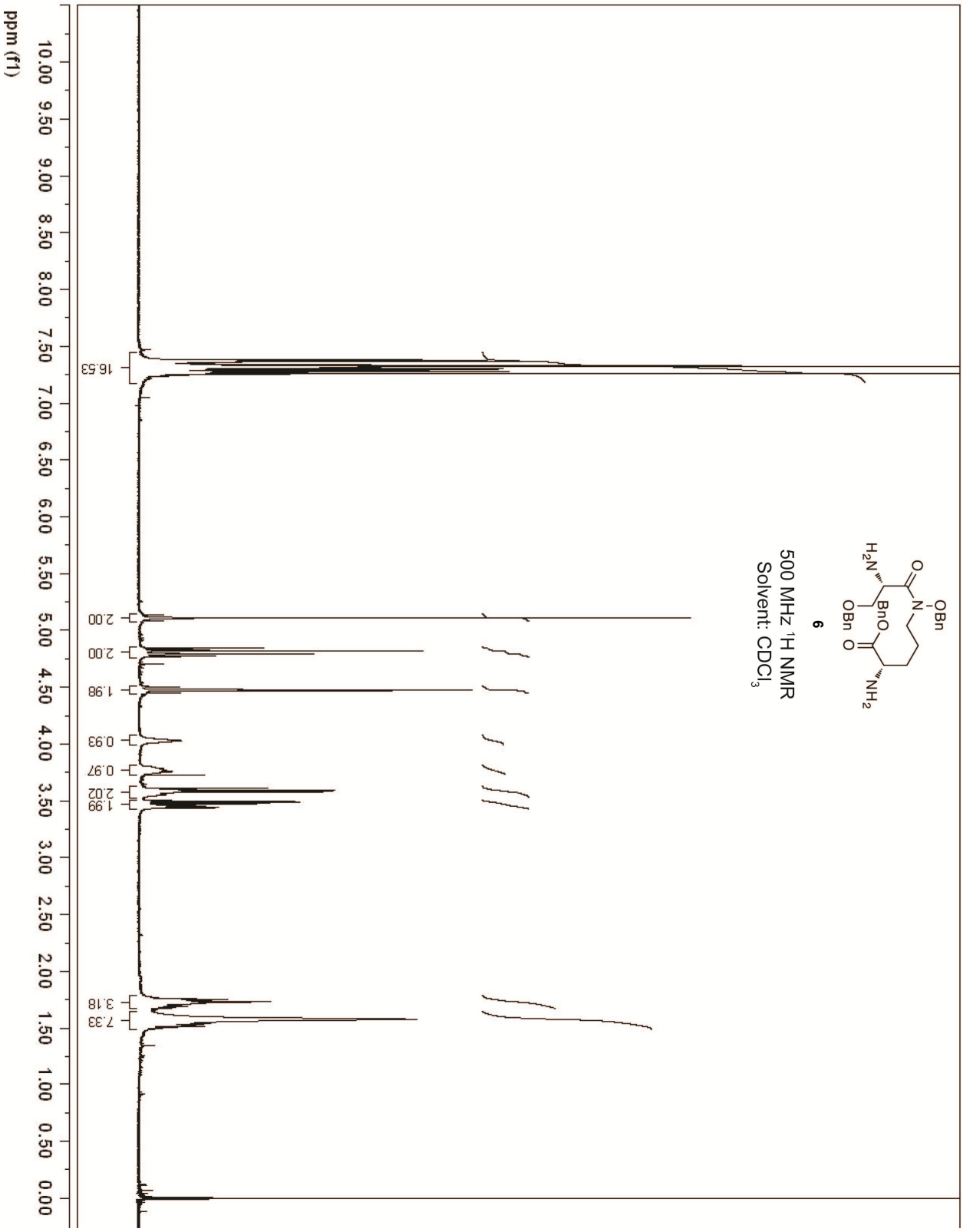


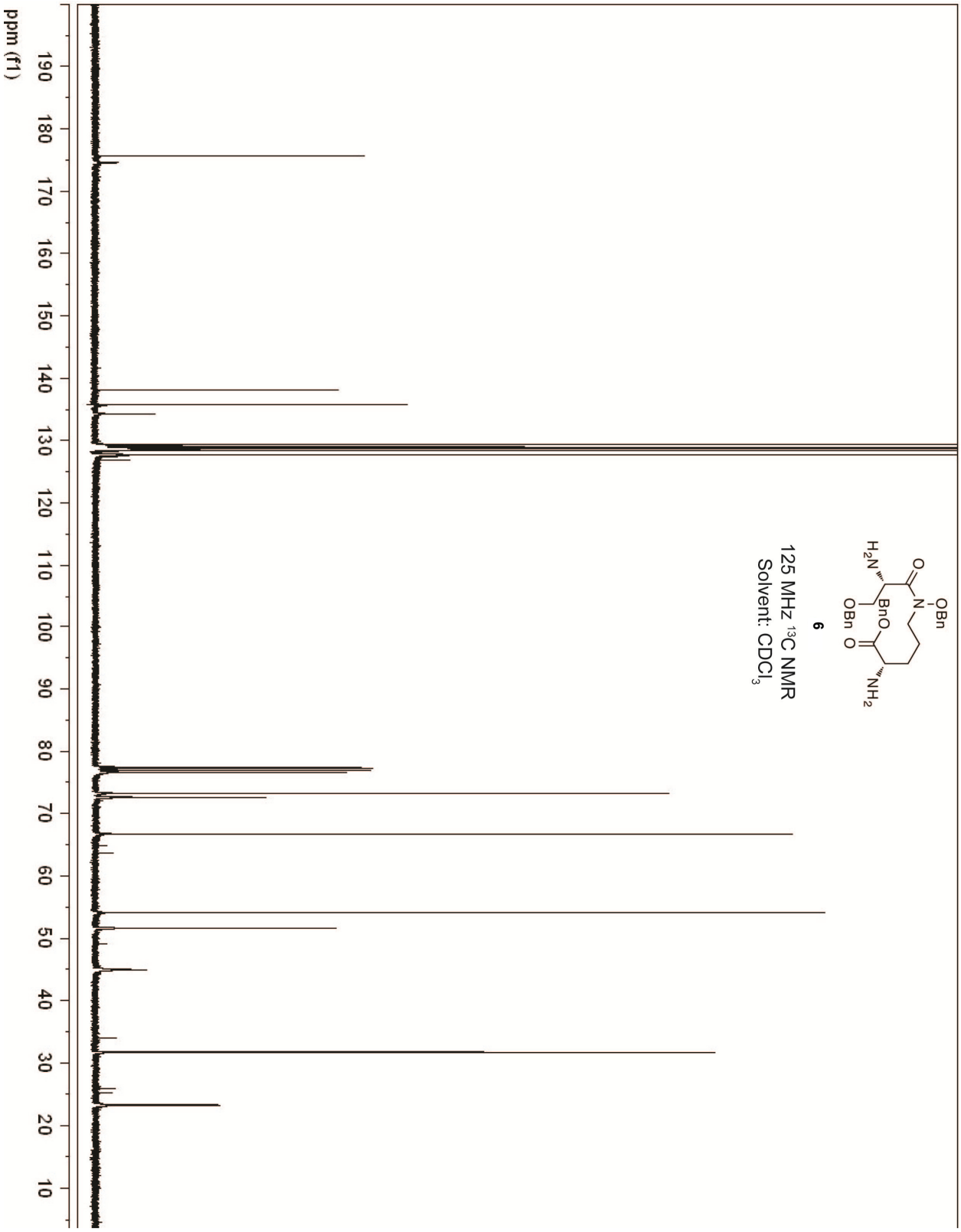


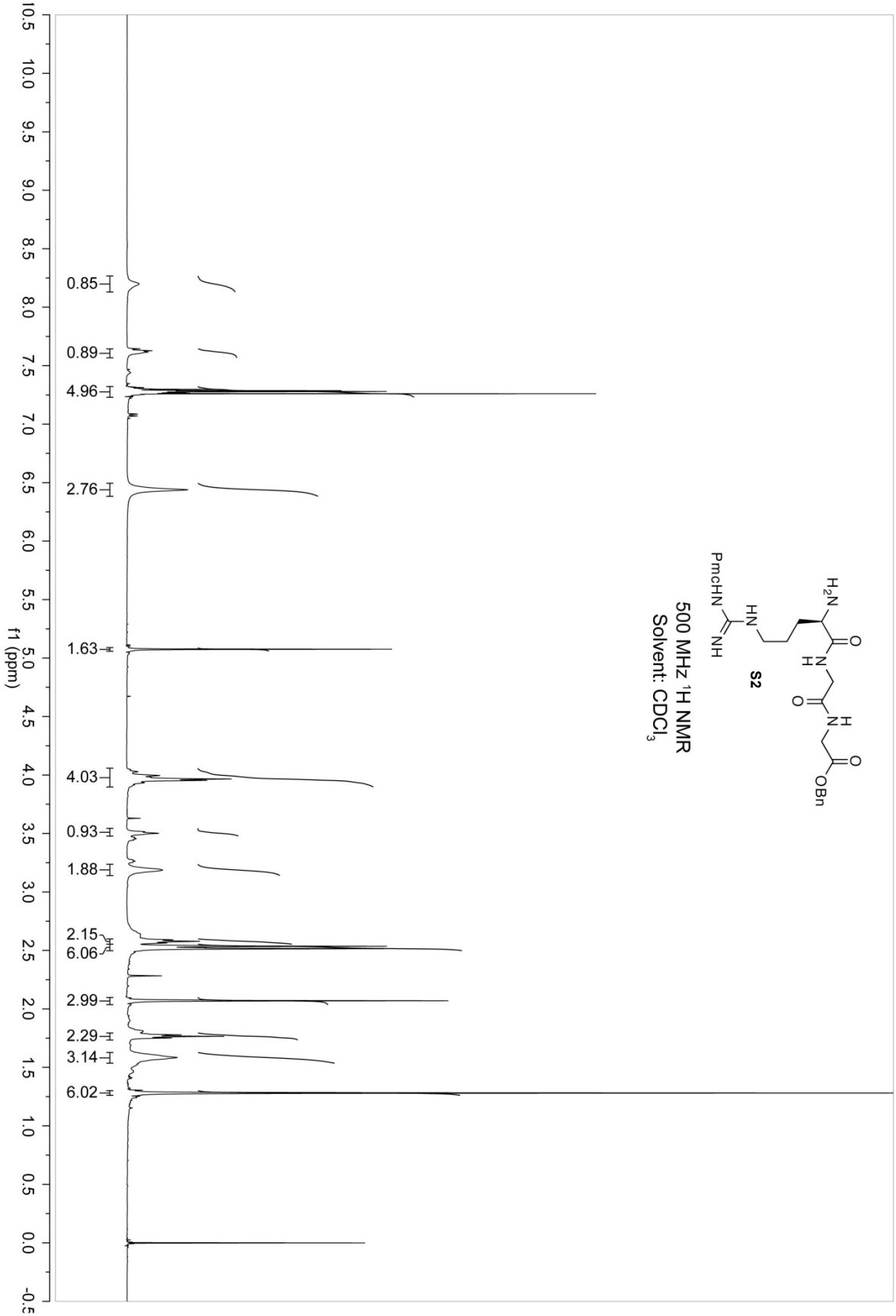


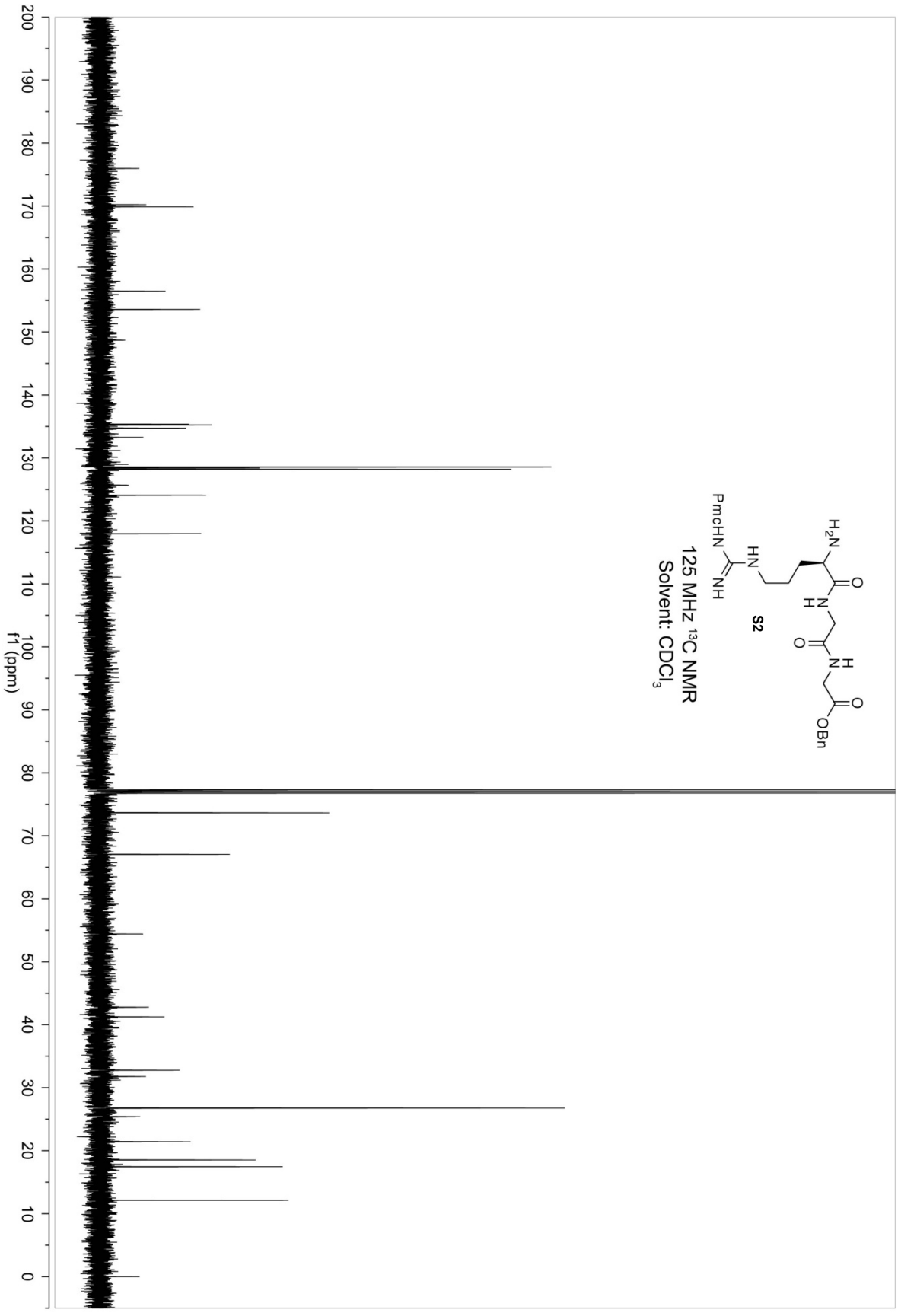


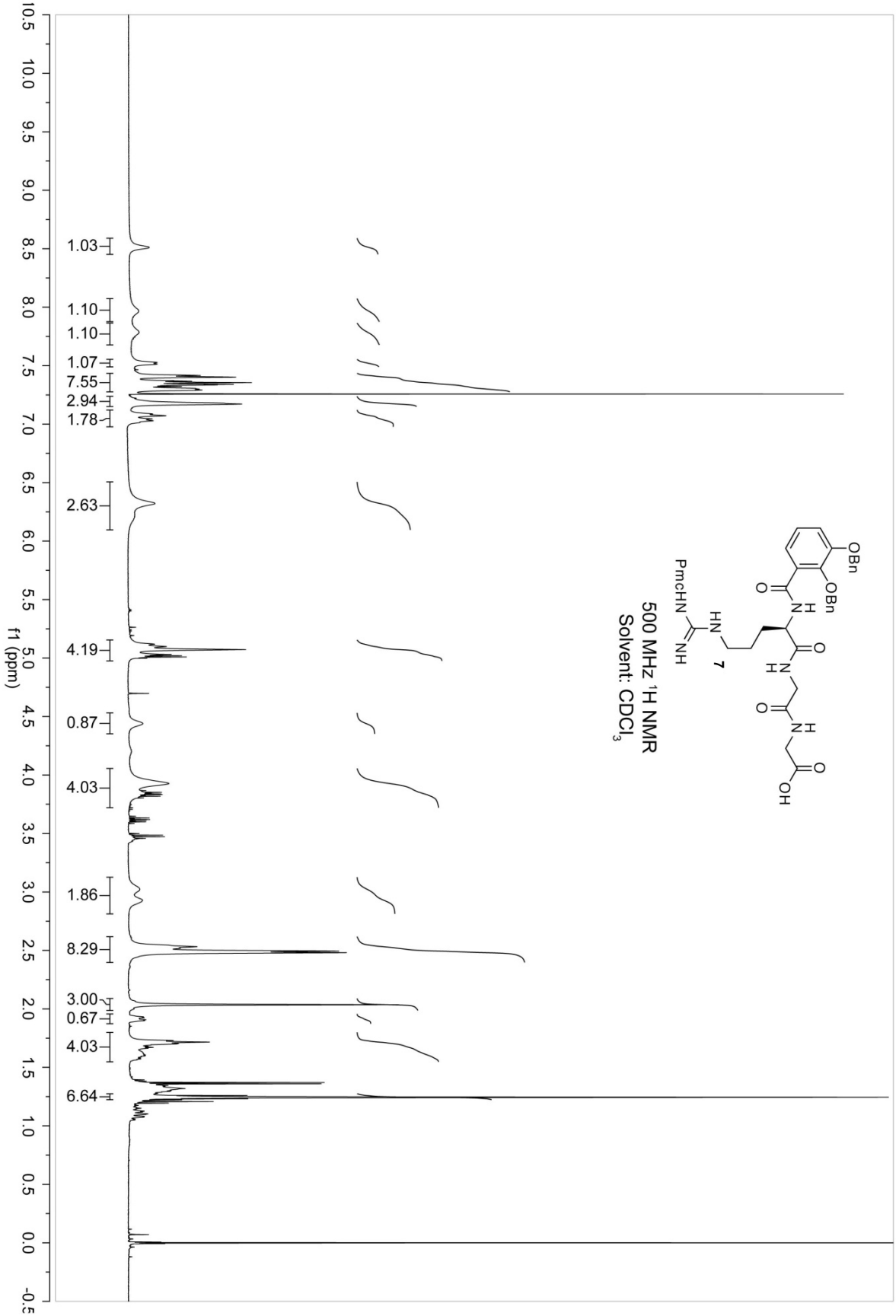


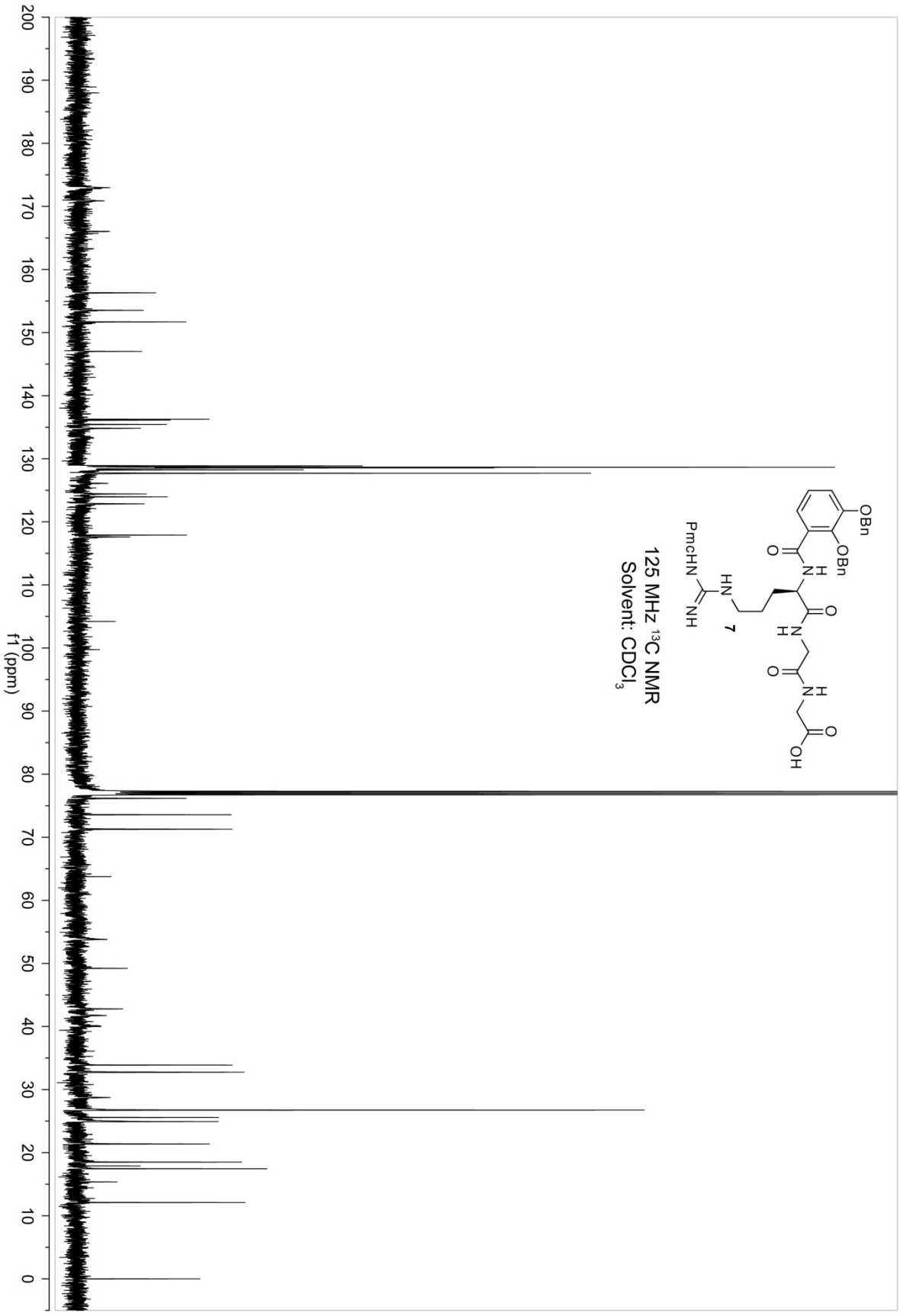


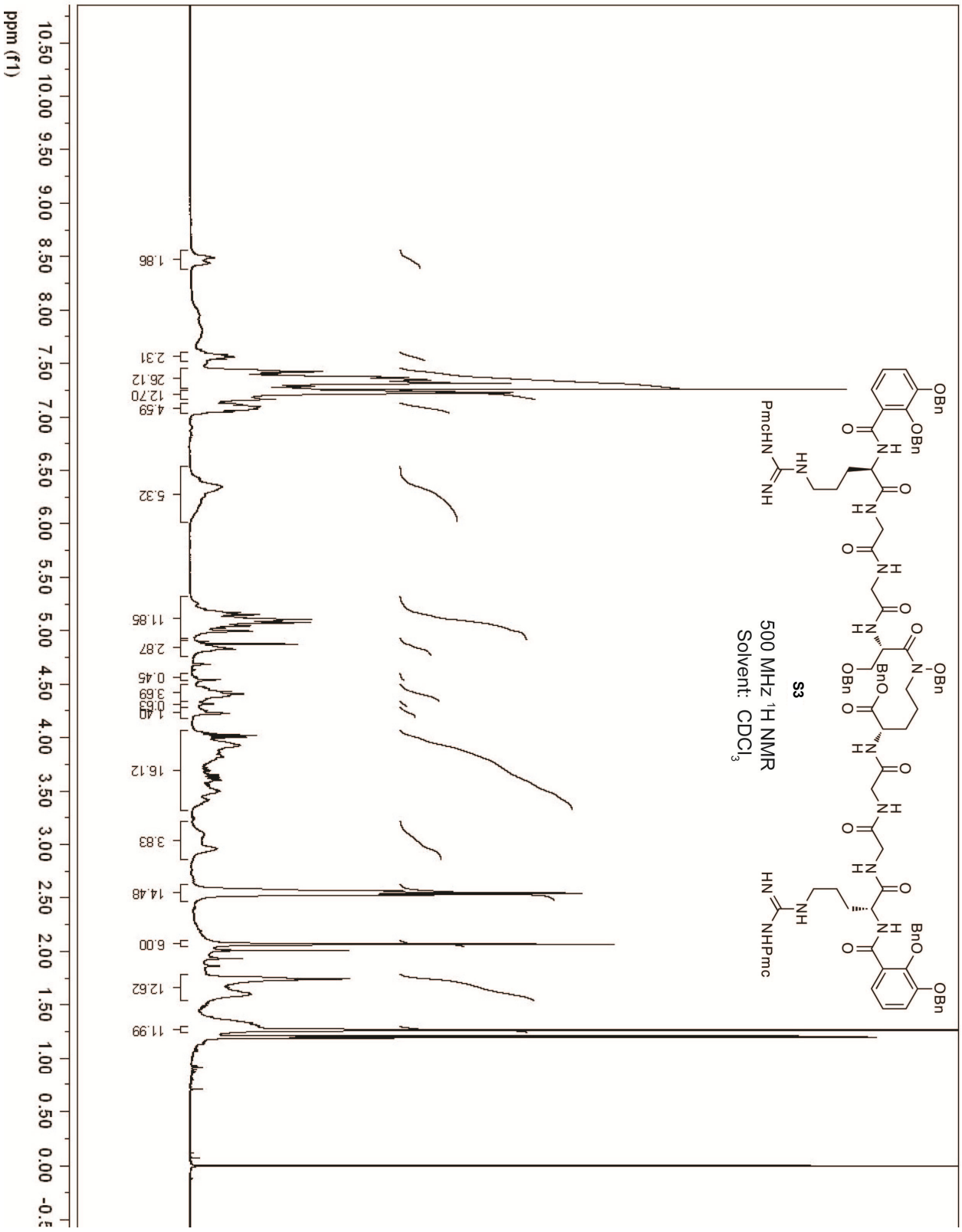


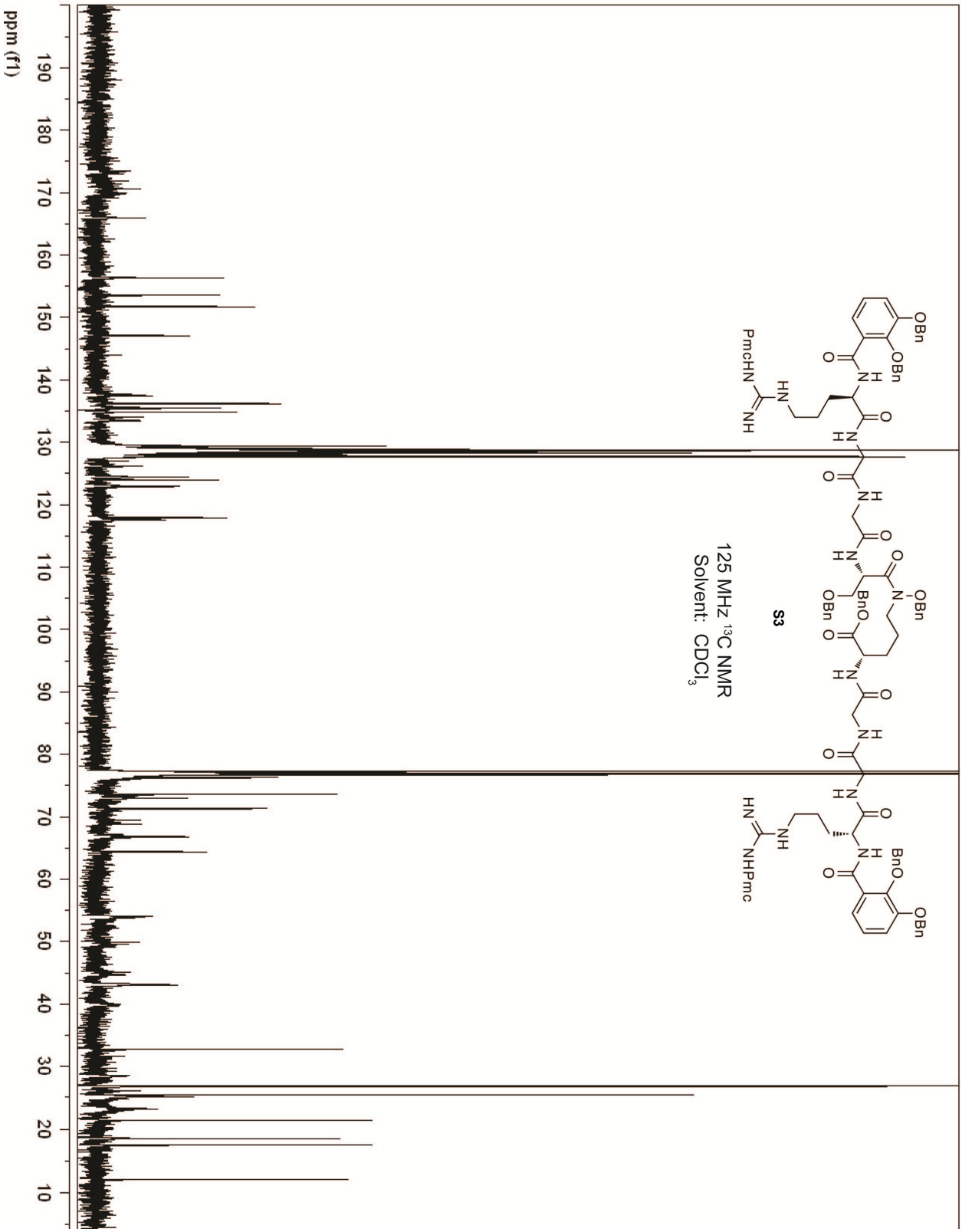


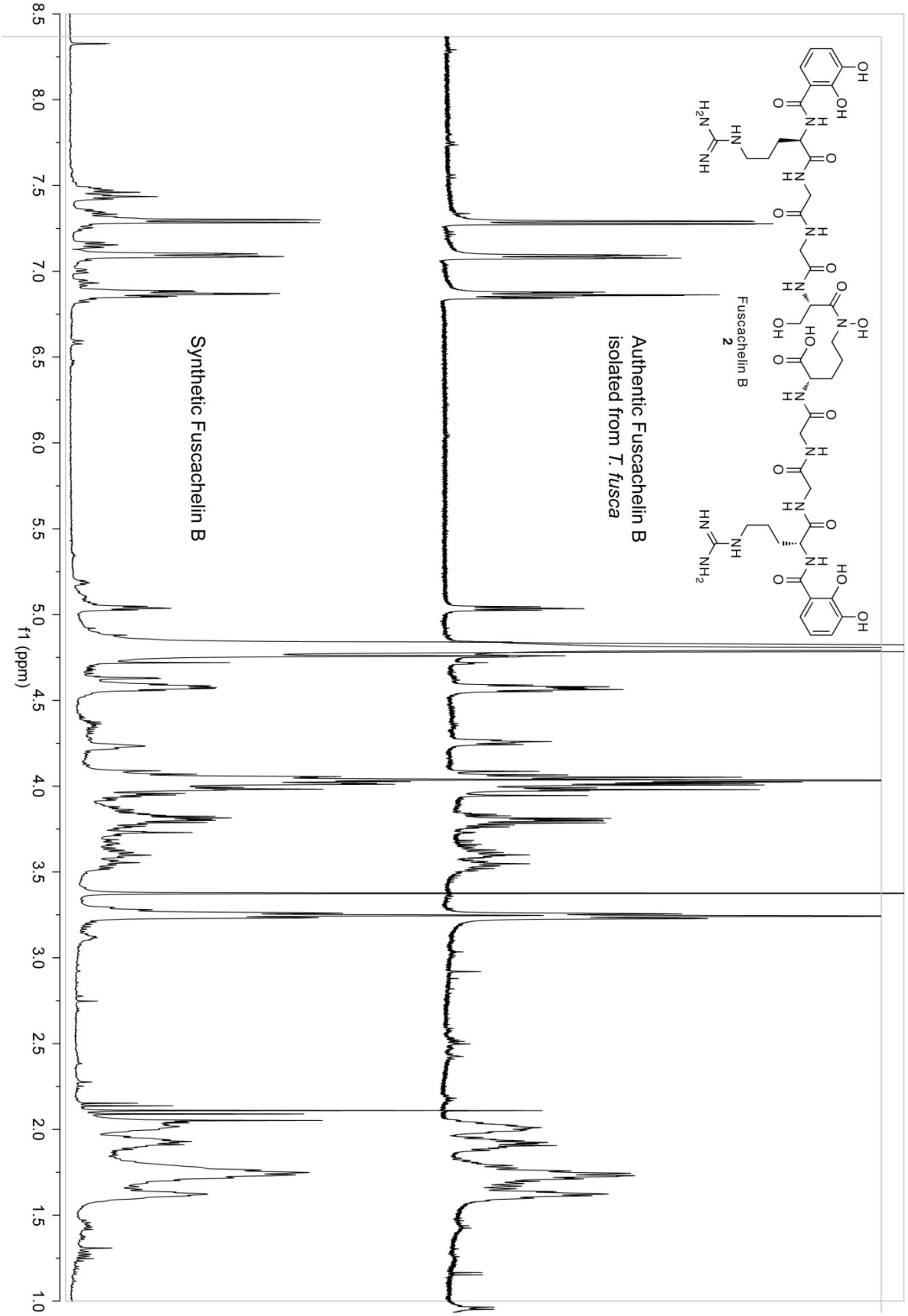


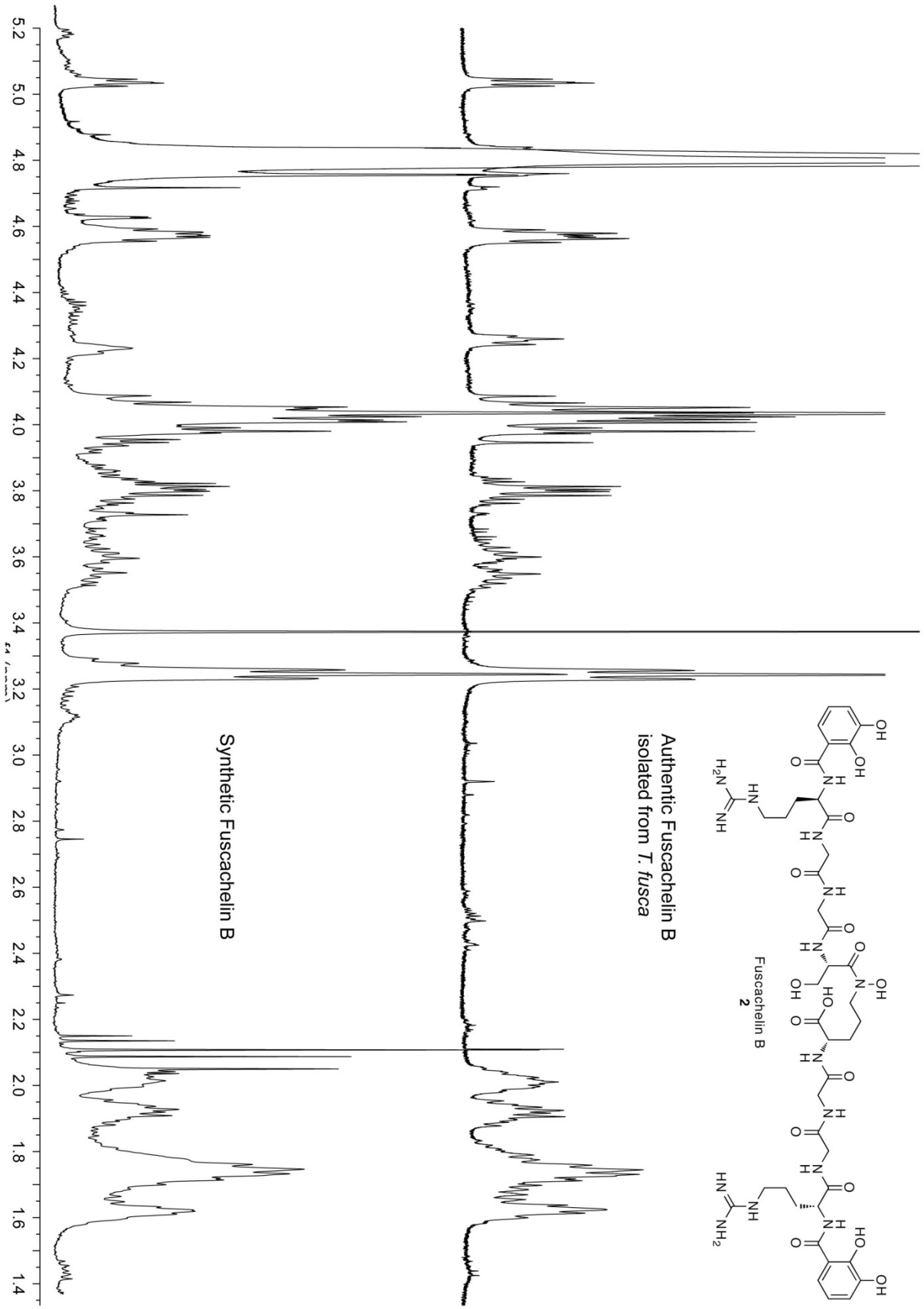


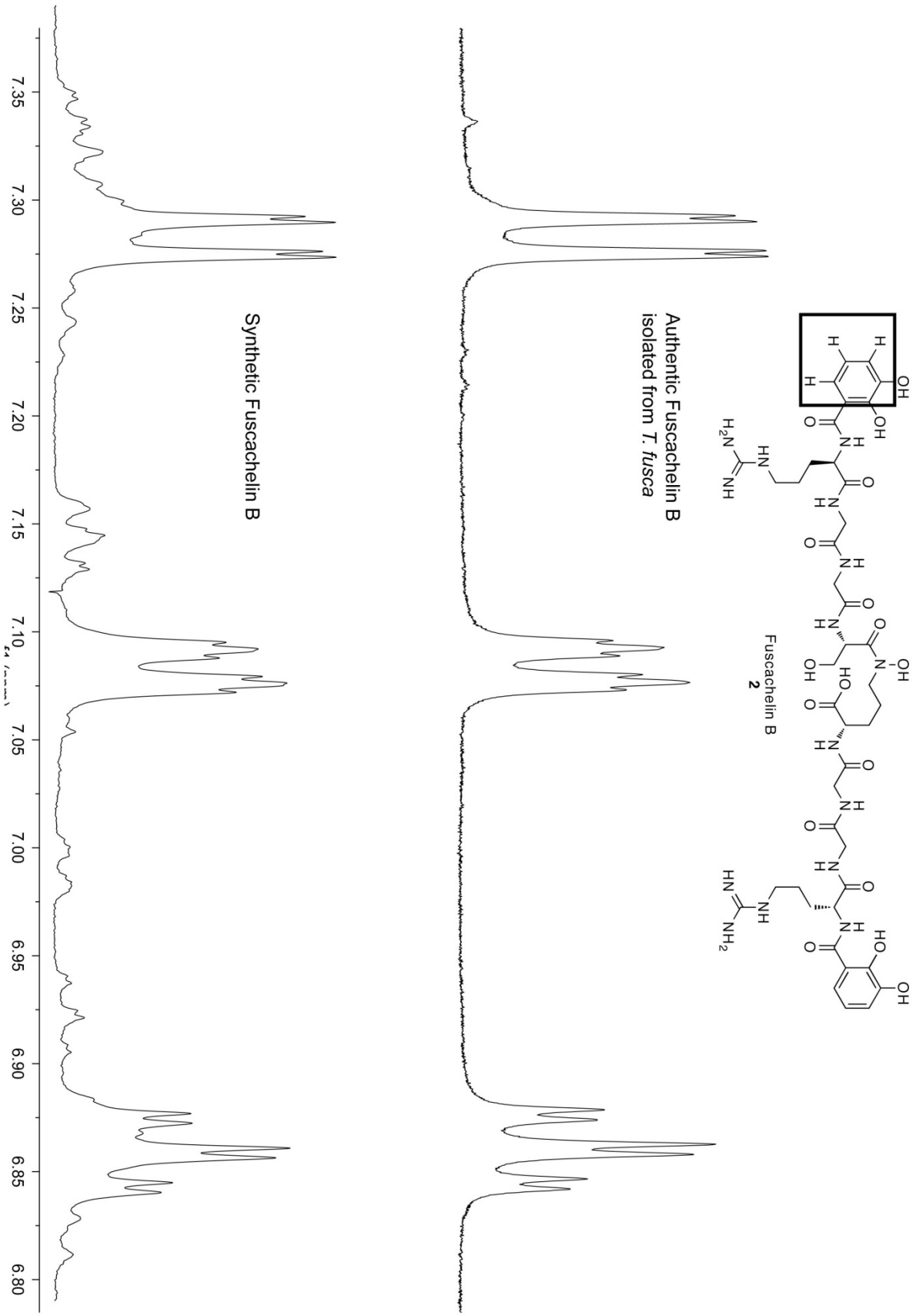


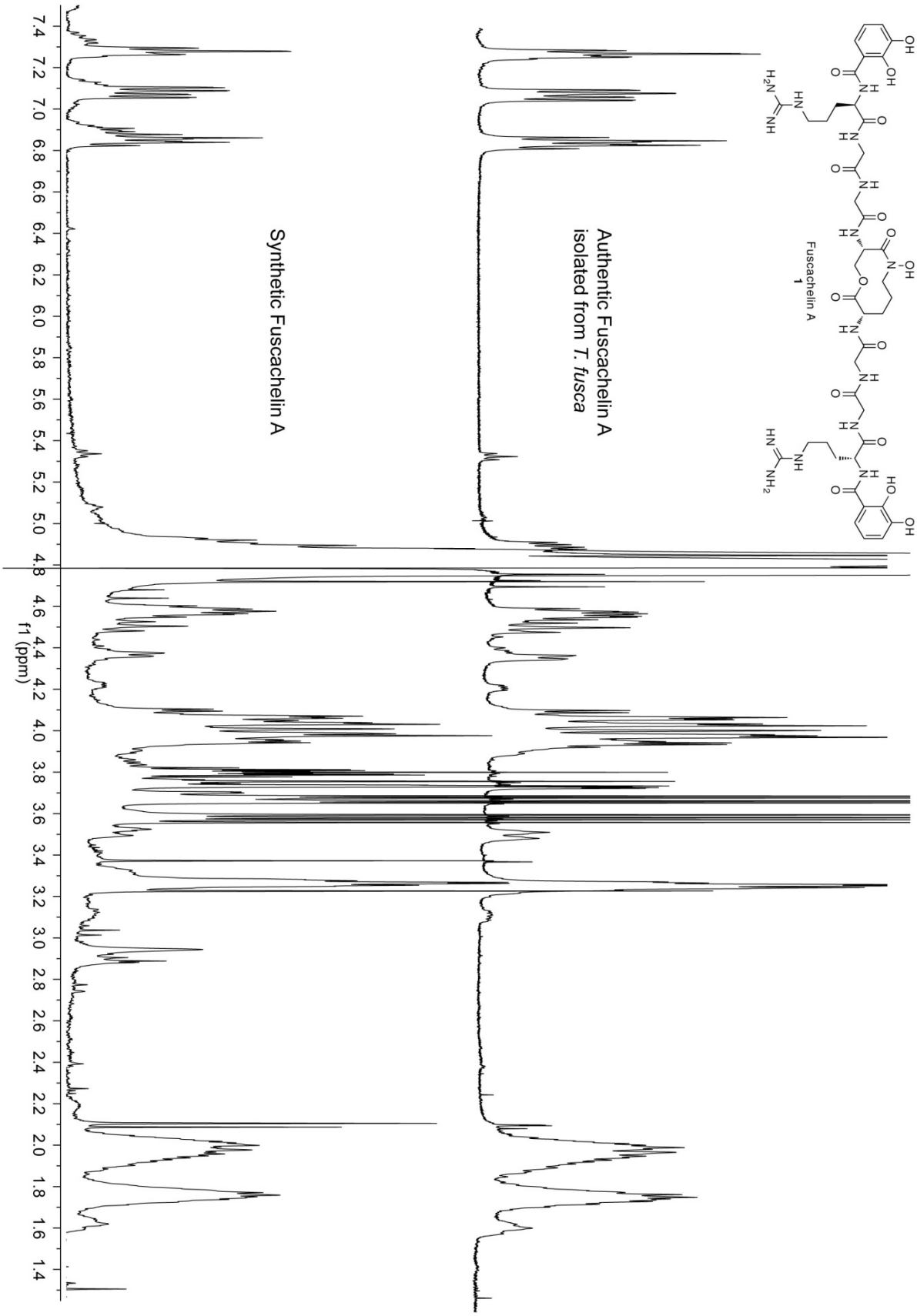


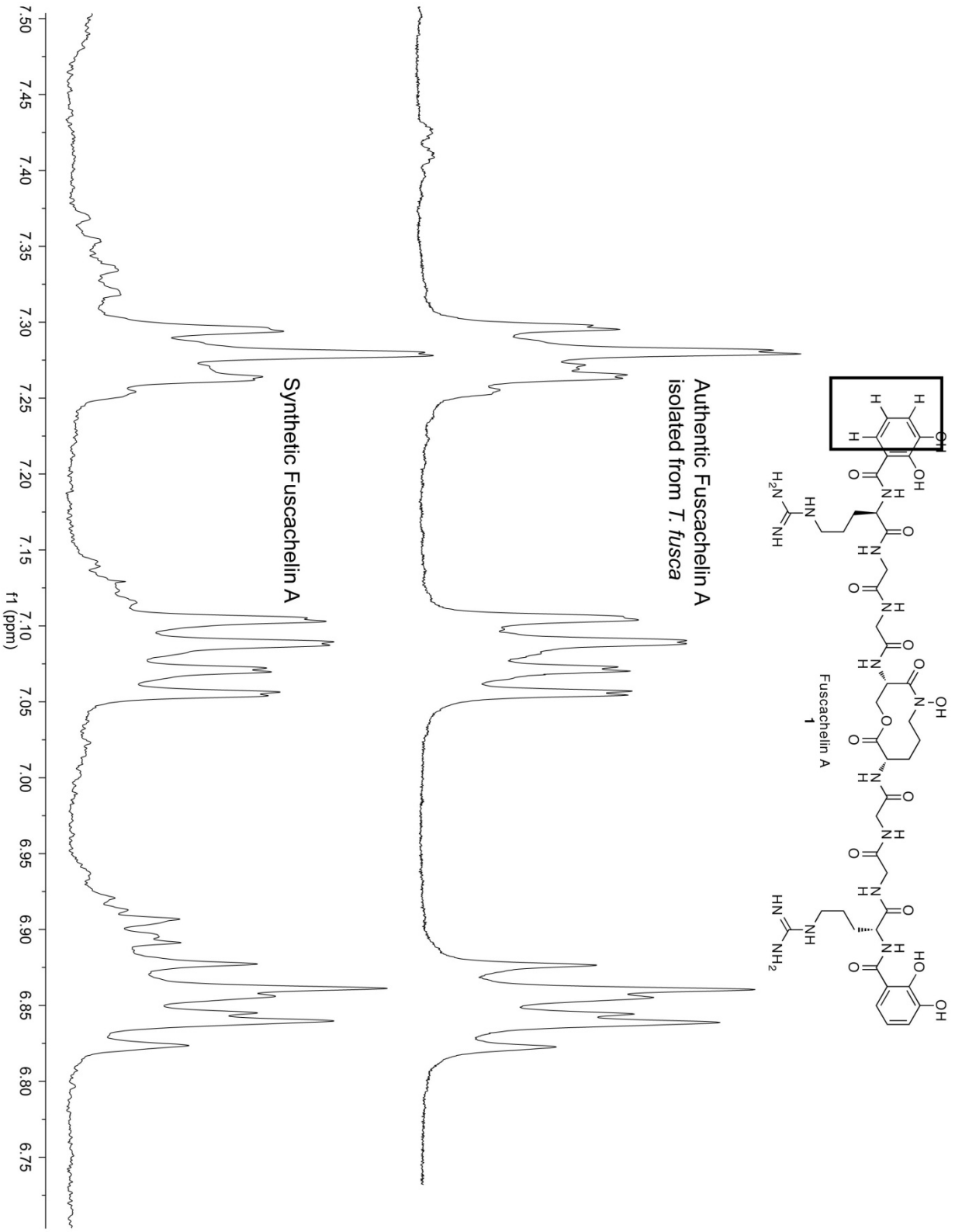


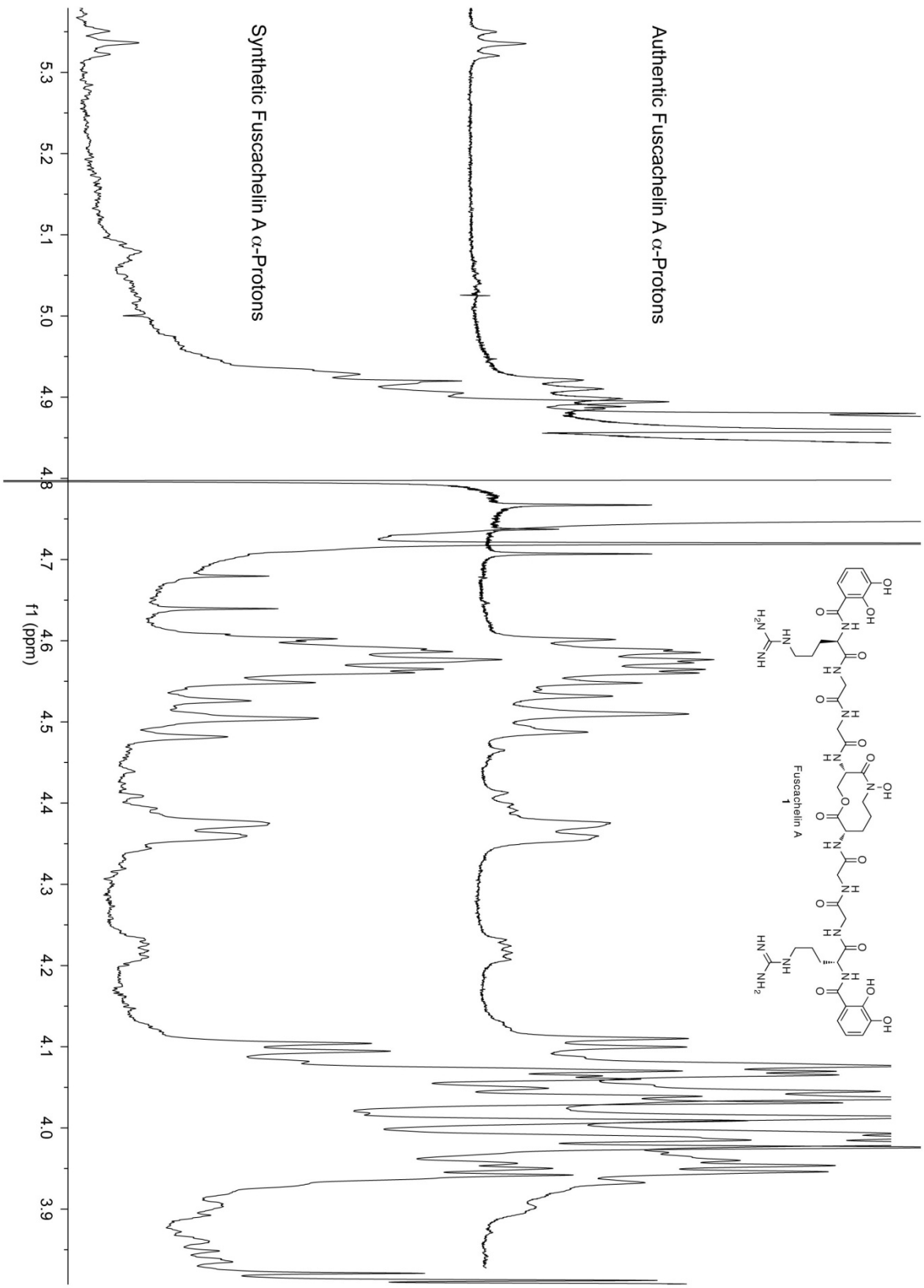


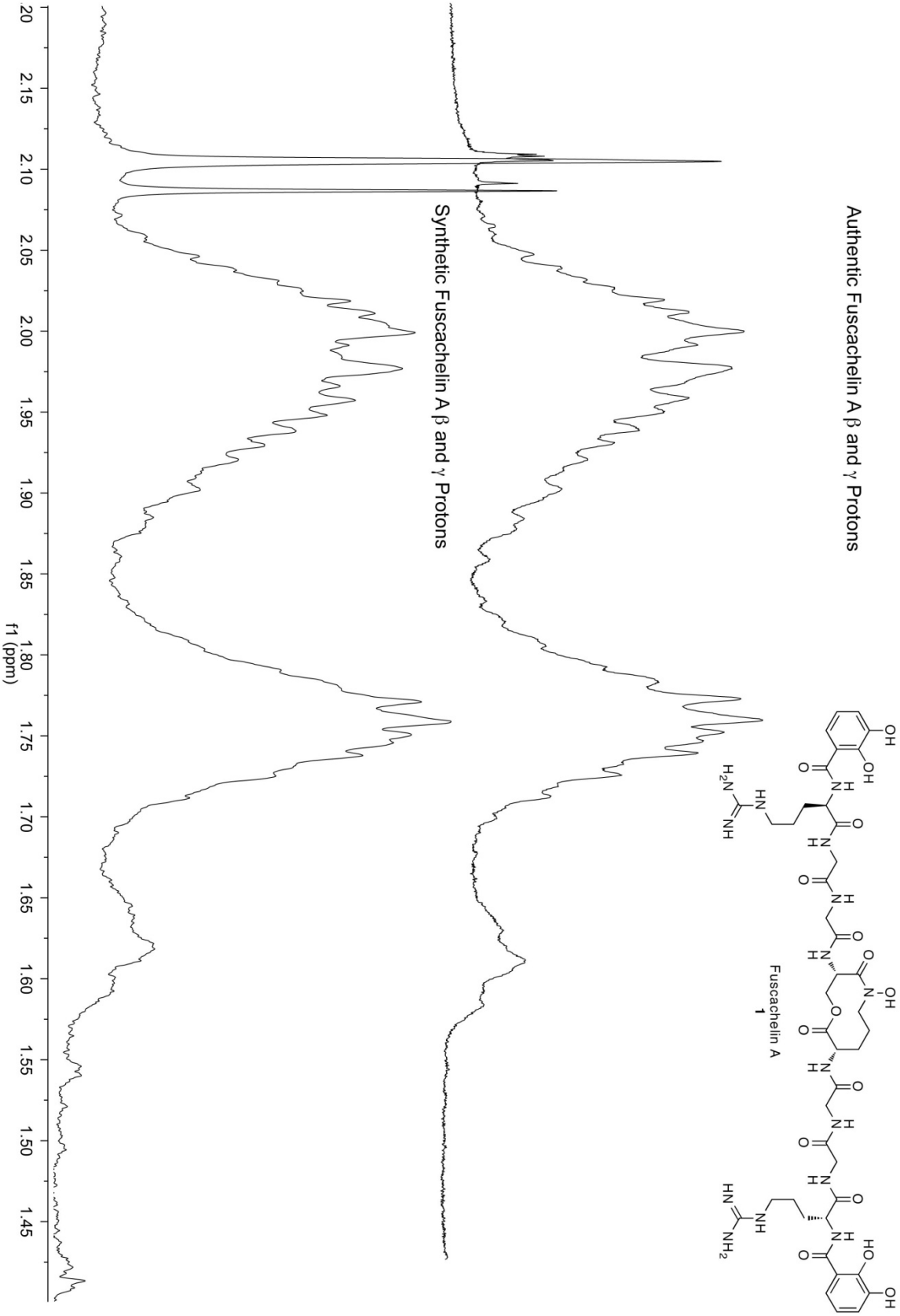


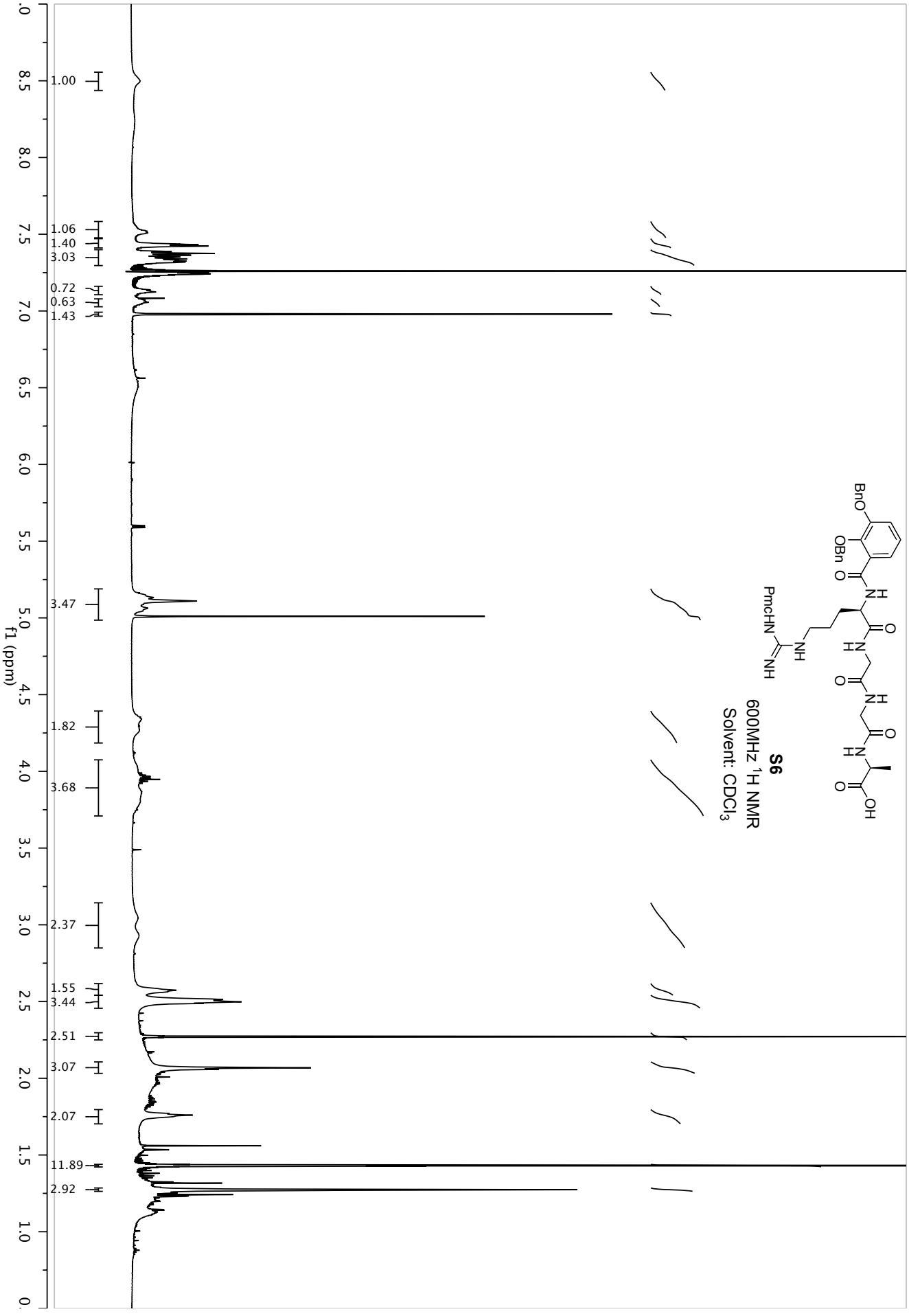


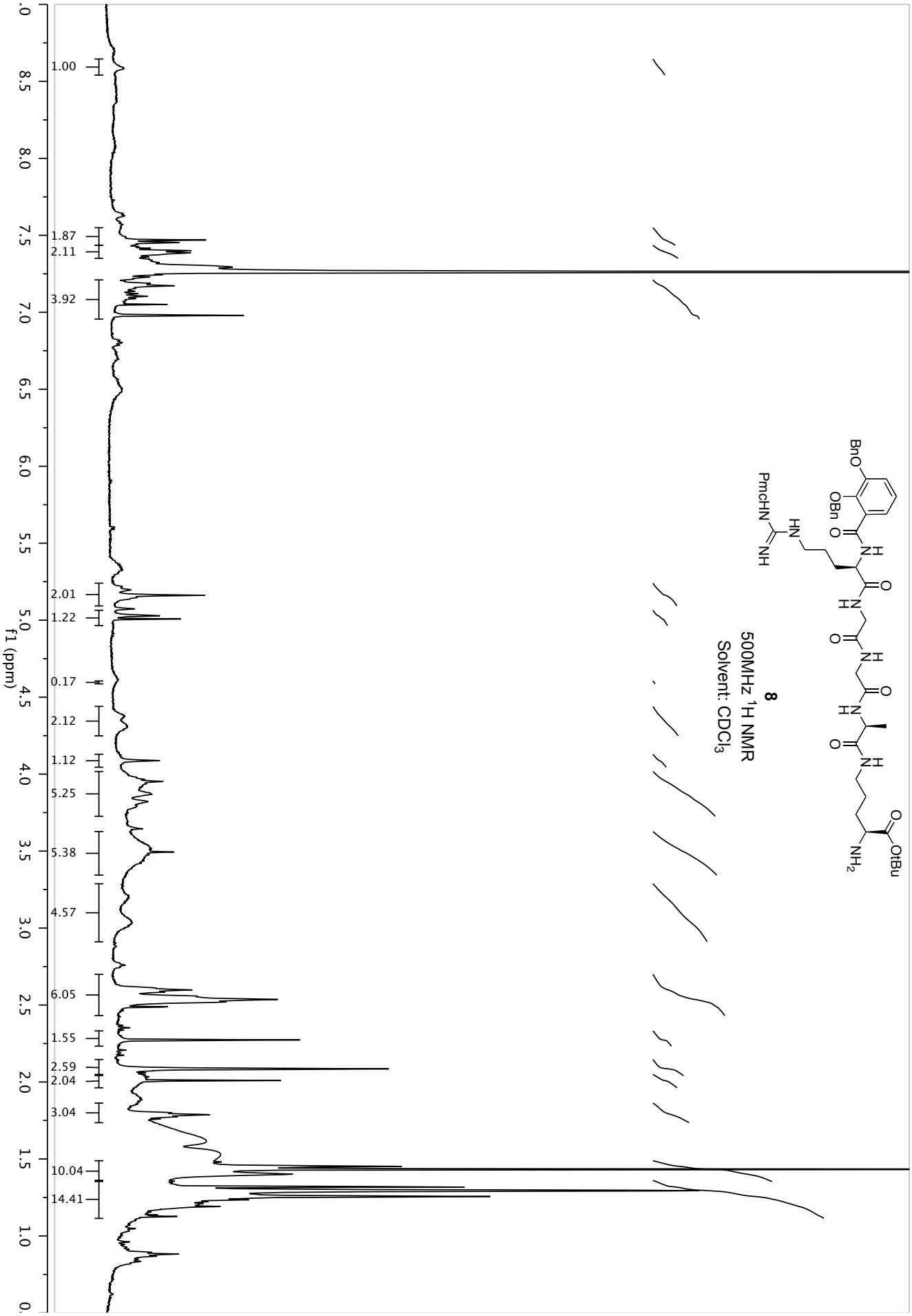


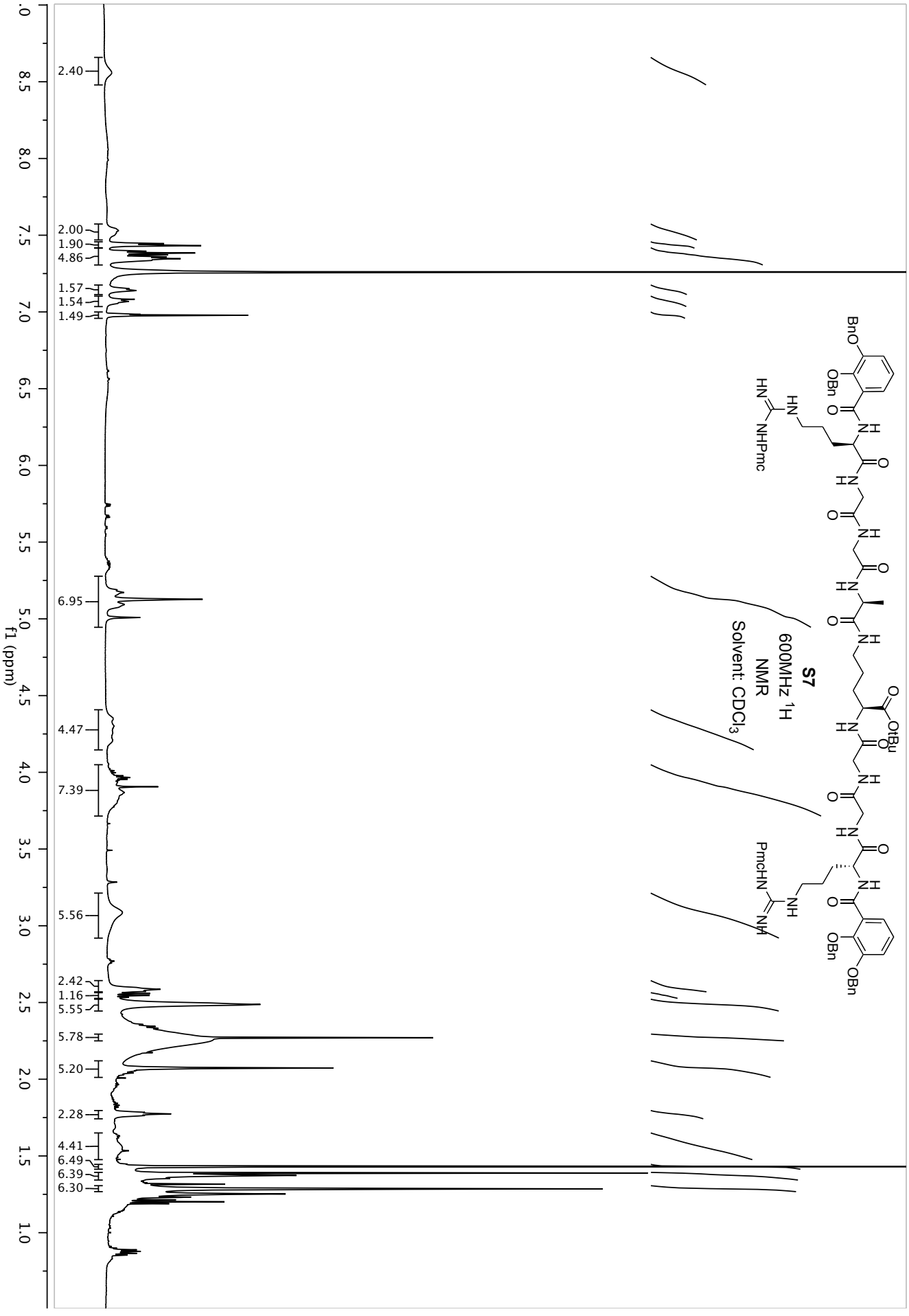


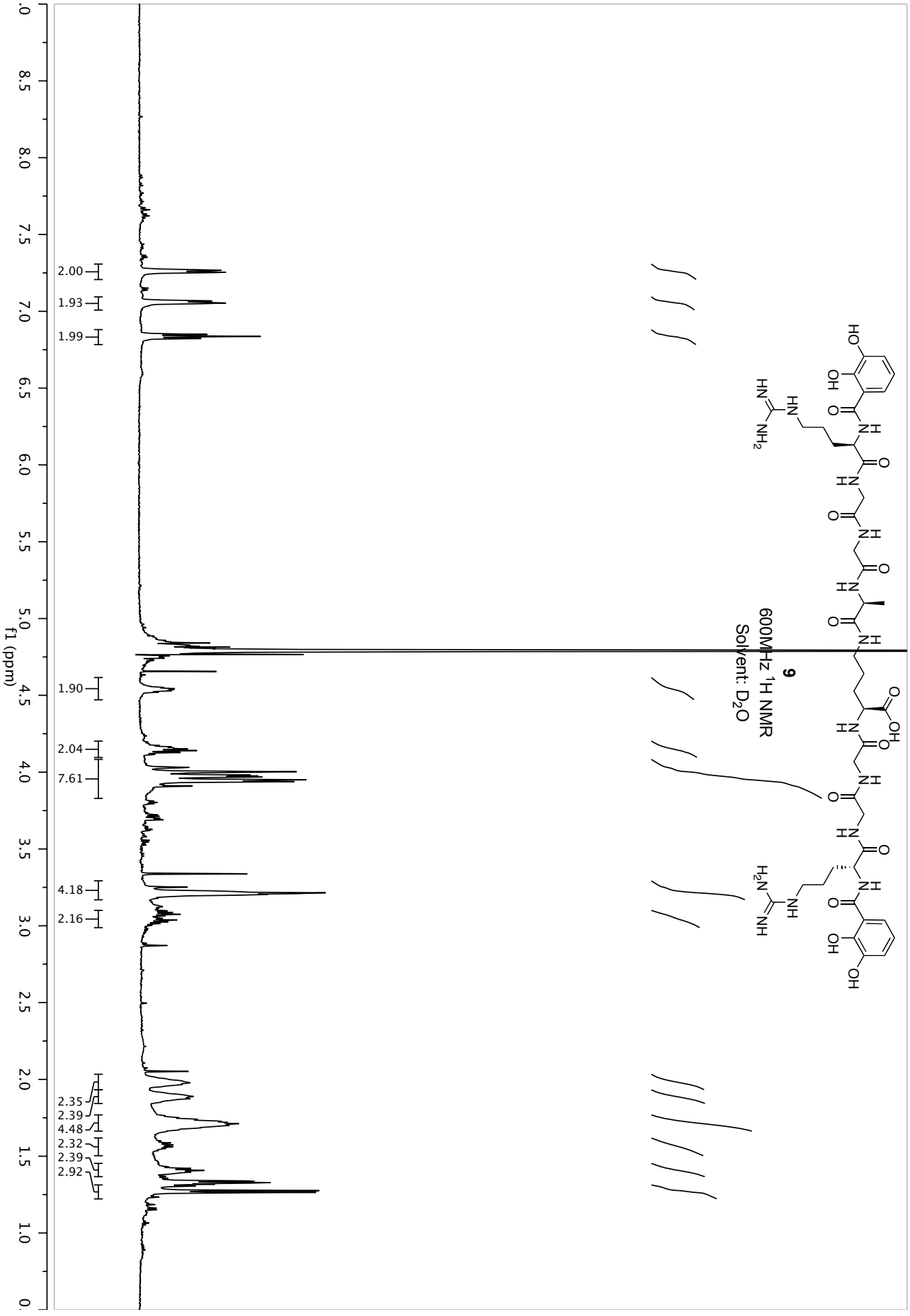












Notes and References

1. K. A. Fennell, U. Mollmann, M. J. Miller, *J. Org. Chem.*, 2008, **73**, 1018.
2. P. J. Maurer, M. J. Miller, *J. Am. Chem. Soc.*, 1982, **104**, 3096.
3. U. Diederichsen, D. Weicherding, N. Diezemann, *Org. Biomol. Chem.*, 2005, **3**, 1058.
4. R. A. Gardner, R. Kinkade, C. Wang, O. Phanstiel, *J. Org. Chem.*, 2004, **69**, 3530.
5. Upon LC MS analysis, both the ferric and desferrifuscachelin A species were detected, resulting from the binding of trace Fe^{3+} in the LC MS instrument and LC solvent.