Supporting Information

Bulky Dehydroamino Acids Enhance Proteolytic Stability and Folding in β-Hairpin Peptides

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General Experimental Details

N,*N*-Dimethylformamide and tetrahydrofuran were dried by passage through a solvent drying system containing cylinders of activated alumina.[1] Other solvents and reagents were purchased from commercial vendors and used without purification. Flash chromatography was carried out using 60–230 mesh silica gel. ^{1}H NMR spectra were acquired on a 500 MHz spectrometer with chloroform (7.27 ppm) or methanol (3.31 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ^{13}C NMR spectra were acquired on a spectrometer operating at 125 MHz with chloroform (77.23 ppm) or methanol (49.00 ppm) as internal reference. NMR samples of peptides were at concentrations of 1–3.5 mM in 10% v/v D_2O/H_2O buffered to pD 3.9 with 5000 mM NaOAc-d_3. DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) was used as the internal standard. Samples were transferred to a Varian 500 MHz magnet and data collection was controlled using vnmrJ software. Water suppression was achieved using an excitation sculpting sequence. 2D TOCSY experiments were collected with 32 scans, 400 t1 increments, and 150 ms mixing time. 2D adiabatic ROESY experiments were collected with 64 scans, 256 t1 increments, and 200 ms mixing time. Data were processed using vnmrJ. Peak assignments were made with the assistance of the ccpNMR software package. Infrared spectra were obtained on an FT-IR spectrometer. Mass spectral data were obtained using ESI techniques. Circular dichroism measurements were made with an Aviv 420 Circular Dichroism Spectropolarimeter, using quartz cuvettes with a path length of 0.1 cm.

[1] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.

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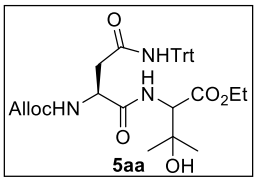
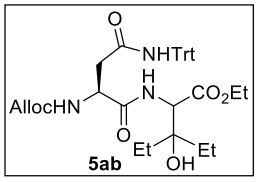
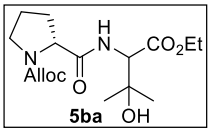


Ethyl 2-((*S*)-2-(((Allyloxy)carbonyl)amino)-4-oxo-4-(tritylamino) butanamido)-3-hydroxy-3-methylbutanoate (5aa). A solution of Alloc-Asn(Trt)-OH (**3a**, 695.2 mg, 1.52 mmol, 1.5 equiv) in DMF (30 mL) at 0 °C was treated with EDC·HCl (290.8 mg, 1.52 mmol, 1.5 equiv) and HOBt·H_2O (256.9 mg, 1.52 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C for 15 min, then treated with a solution of *β*-hydroxyamino ester **4a**[2] (163.6 mg, 1.01 mmol) in DMF (5 mL) and stirred at 0 °C to rt for 12 h. The reaction was then quenched with sat aq $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (3 × 15 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (75 mL of SiO_2, 0–6% MeOH in CH_2Cl_2 gradient elution), afforded **5aa** (472.7 mg, 0.786 mmol, 77%) as a white film that was a 1:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 (d, *J* = 8.4 Hz, 1H), 7.33–7.23 (m, 9H), 7.18 (d, *J* = 7.8 Hz, 6H), 6.86 (br s; 1H), 6.43 (d, *J* = 8.1 Hz, 1H), 5.97–5.85 (m, 1H), 5.31 (dd, *J* = 17.2, 1.3 Hz, 1H), 5.23 (dd, *J* = 10.4, 1.2 Hz, 1H), 4.63–4.56 (m, 2H), 4.56–4.51 (m, 1H), 4.47 (d, *J* = 8.8 Hz, 1H), 4.29–4.16 (m, 2H), 3.11 (dd, *J* = 15.6, 3.7 Hz, 1H), 2.72–2.63 (m, 1H), 2.69 (br s, 1H), 1.30 (t, *J* = 7.0 Hz, 3H), 1.21 (s, 3H), 1.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 170.8, 170.2, 156.4, 144.2 (3C), 132.3, 128.7 (6C), 128.0 (6C), 127.1 (3C), 118.0, 71.9, 70.9, 66.1, 61.6, 60.3, 51.7, 37.6, 26.7, 26.4, 14.1; IR (film) ν_{max} 3330, 2923, 1659, 1524 cm^{-1}; HRMS (ESI) *m/z* 602.2831 (MH^+, $C_{34}H_{39}N_3O_7H^+$ requires 602.2822).

[2] Ma, Z.; Naylor, B. C.; Loertscher, B. M.; Hafen, D. D.; Li, J. M.; Castle, S. L. *J. Org. Chem.* **2012**, *77*, 1208.



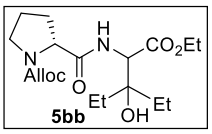
Ethyl 2-((*S*)-2-(((Allyloxy)carbonyl)amino)-4-oxo-4-(tritylamino) butanamido)-3-ethyl-3-hydroxypentanoate (5ab). Subjection of β-hydroxyamino ester **4b**[3] (135.2 mg, 0.714 mmol) to the procedure described above for the synthesis of **5aa** with stirring for 16 h and purification by flash chromatography (60 mL of SiO_2, 0–5% MeOH in CH_2Cl_2 gradient elution), afforded **5ab** (320.4 mg, 0.509 mmol, 71%) as a white film that was a 1:1 mixture of diastereomers: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.30–7.19 (m, 15H), 6.00–5.91 (m, 1H), 5.34 (d, *J* = 17.2 Hz, 1H), 5.21 (d, *J* = 10.3 Hz, 1H), 4.60–4.56 (m, 2H), 4.55–4.51 (m, 1H), 4.50 (s, 1H), 4.23–4.17 and 4.16–4.09 (2m, 2H), 2.86–2.66 (m, 2H), 1.65–1.45 (m, 4H), 1.27 and 1.26 (2t, *J* = 7.3 and 7.3 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.84 and 0.83 (2t, *J* = 7.5 and 7.4 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.1, 170.4 and 170.3, 170.1, 156.7 and 156.6, 144.4 (3C), 132.7, 128.6 (6C), 127.3 (6C), 126.4 (3C), 116.5, 75.6 and 75.5, 70.4, 65.6 and 65.5, 60.9, 57.8 and 57.7, 52.1, 38.2, 27.4, 27.0 and 26.9, 13.0, 6.7, 6.6; IR (film) ν_{max} 3328, 2970, 1735, 1663, 1522, 1275, 1268 cm^{-1}; HRMS (ESI) *m/z* 630.3110 (MH^+, $C_{36}H_{43}N_3O_7H^+$ requires 630.3135).



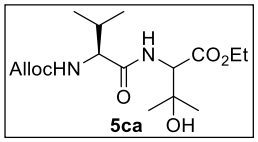
Allyl (2*R*)-2-((1-Ethoxy-3-hydroxy-3-methyl-1-oxobutan-2-yl)carbamoyl) pyrrolidine-1-carboxylate (5ba). Subjection of *β*-hydroxyamino ester **4a** (220.7 mg, 1.37 mmol) to the procedure described above for the synthesis of **5aa** with Alloc-D-Pro-OH (**3b**, 406.3 mg, 2.04 mmol, 1.5 equiv) as coupling partner and purification by flash chromatography (60 mL of SiO_2, 0–6.5% MeOH in CH_2Cl_2 gradient elution) afforded **5ba** (378.8 mg, 1.11 mmol, 81%) as a

[3] Jiang, J.; Luo, S.; Castle, S. L. *Tetrahedron Lett.* **2015**, *56*, 3311.

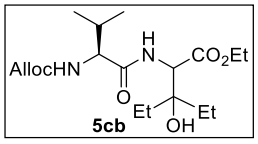
colorless oil that was a 1:1 mixture of diastereomers: ^{1}H NMR (CD_3OD, 500 MHz, mixture of rotamers and diastereomers) δ 6.03–5.83 (m, 1H), 5.40–5.09 (m, 2H), 4.64–4.52 (m, 2H), 4.46–4.35 (m, 2H), 4.27–4.14 (m, 2H), 3.64–3.56 (m, 1H), 3.55–3.46 (m, 1H), 2.34–2.16 (m, 1H), 2.07–1.85 (m, 3H), 1.33–1.27 (m, 6H); 1.24 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz, mixture of rotamers and diastereomers) δ 175.2/175.0, 171.7/171.6, 156.7/156.3, 134.2/134.1/134.0, 117.9/117.7/117.6, 72.5/72.4, 67.2, 62.3/62.2, 61.9, 61.6/61.5/61.2, 48.1, 32.6/32.5/31.3/31.2, 27.7/27.4/27.3, 27.2/27.1, 25.4/25.3/24.6/24.5, 14.5; IR (film) ν_{max} 3417, 2917, 1678, 1540, 1408 cm^{-1}; HRMS (ESI) *m/z* 343.1824 (MH^+, $C_{16}H_{26}N_2O_6H^+$ requires 343.1824).



Allyl (2*R*)-2-((1-Ethoxy-3-ethyl-3-hydroxy-1-oxopentan-2-yl)carbamoyl) pyrrolidine-1-carboxylate (5bb). Subjection of β-hydroxyamino ester **4b** (278.1 mg, 1.47 mmol) to the procedure described above for the synthesis of **5aa** with Alloc-D-Pro-OH (**3b**, 437.9 mg, 2.20 mmol, 1.5 equiv) as coupling partner, stirring for 16 h, and purification by flash chromatography (75 mL of SiO_2, 0–6% MeOH in CH_2Cl_2 gradient elution) afforded **5bb** (431.7 mg, 1.17 mmol, 79%) as a colorless oil that was a 1:1 mixture of diastereomers: ^{1}H NMR (CD_3OD, 500 MHz, mixture of rotamers and diastereomers) δ 6.03–5.85 (m, 1H), 5.39–5.11 (m, 2H), 4.64–4.49 (m, 3H), 4.44–4.33 (m, 1H), 4.28–4.13 (m, 2H), 3.65–3.56 (m, 1H), 3.55–3.47 (m, 1H), 2.36–2.15 (m, 1H), 2.08–1.84 (m, 3H), 1.72–1.49 (m, 4H), 1.33–1.25 (m, 3H), 0.93 (t, *J* = 7.6 Hz, 3H), 0.90–0.82 (m, 3H); ^{13}C NMR (CD_3OD, 125 MHz, mixture of rotamers and diastereomers) δ 173.6/173.5, 170.5/170.4, 155.0/154.9, 132.7/132.6, 116.4/116.3/116.2/116.1, 75.6/75.5, 65.9/65.8, 60.8/60.7, 60.3/59.9, 57.7/57.6/57.4, 46.7, 31.1/31.0/29.9/29.8, 27.4, 27.0/26.9, 24.0/23.9/23.0, 13.0, 6.7, 6.6; IR (film) ν_{max} 3414, 2969, 1679, 1444, 1408 cm^{-1}; HRMS (ESI) *m/z* 371.2127 (MH^+, $C_{18}H_{30}N_2O_6H^+$ requires 371.2137).



Ethyl 2-((*S*)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)-3-hydroxy-3-methylbutanoate (5ca). Subjection of *β*-hydroxyamino ester **4a** (351.8 mg, 2.18 mmol) to the procedure described above for the synthesis of **5aa** with Alloc-Val-OH (**3c**, 658.3 mg, 3.27 mmol, 1.5 equiv) as coupling partner, stirring for 14 h, and purification by flash chromatography (100 mL of SiO_2, 0–2.5% MeOH in CH_2Cl_2 gradient elution) afforded **5ca** (632.7 mg, 1.84 mmol, 84%) as a colorless oil that was a 1:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.90 (d, *J* = 8.7 Hz, 1H), 5.96–5.86 (m, 1H), 5.50–5.43 (m, 1H), 5.34–5.27 (m, 1H), 5.24–5.19 (m, 1H), 4.61–4.55 (m, 2H), 4.51 (t, *J* = 8.5 Hz, 1H), 4.29–4.19 (m, 2H), 4.17–4.12 and 4.11–4.06 (2m, 1H), 3.12 and 2.92 (2 br s, 1H), 2.25–2.17 and 2.16–2.07 (2m, 1H), 1.32–1.28 (m, 3H), 1.30 (s, 3H), 1.26 (s, 3H), 1.00 and 0.97 (2d, *J* = 6.9 and 6.7 Hz, 3H), 0.95 and 0.92 (2d, *J* = 6.8 and 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.6, 171.3 and 171.1, 156.3, 132.5, 117.9, 71.9 and 71.8, 65.9, 61.7 and 61.6, 60.3 and 60.2, 59.9 and 59.8, 31.1, 26.8, 26.7, 19.3 and 19.1, 17.9 and 17.3, 14.1; IR (film) ν_{max} 3324, 2973, 1729, 1660, 1536, 1214 cm^{-1}; HRMS (ESI) *m/z* 345.1976 (MH^+, $C_{16}H_{28}N_2O_6H^+$ requires 345.1981).



Ethyl 2-((*S*)-2-(((Allyloxy)carbonyl)amino)-3-methylbutanamido)-3-ethyl-3-hydroxypentanoate (5cb). Subjection of β-hydroxyamino ester **4b** (323.2 mg, 1.71 mmol) to the procedure described above for the synthesis of **5aa** with Alloc-Val-OH (**3c**, 514.8 mg, 2.56 mmol, 1.5 equiv) as coupling partner, stirring for 16 h, and purification by flash chromatography (75 mL of SiO_2, 0–4% MeOH in CH_2Cl_2 gradient elution) afforded **5cb** (498.7 mg, 1.34 mmol, 78%) as a colorless liquid that was a 1:1 mixture of diastereomers: ^{1}H NMR

($CDCl_3$, 500 MHz) δ 6.69 and 6.63 (2d, *J* = 8.7 and 8.2 Hz, 1H), 5.97–5.88 (m, 1H), 5.37–5.28 (m, 2H), 5.23 (d, *J* = 10.4 Hz, 1H), 4.62–4.55 (m, 3H), 4.29–4.17 (m, 2H), 4.14–4.09 and 4.08–4.03 (2m, 1H), 2.47 (br s, 1H), 2.25–2.17 and 2.16–2.09 (2m, 1H), 1.59–1.44 (m, 4H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.00 and 0.97 (2d, *J* = 6.8 and 6.8 Hz, 3H), 0.96–0.91 (m, 6H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8 and 171.7, 171.2, 156.2, 132.6, 117.9 and 117.8, 76.2, 65.9, 61.6 and 61.5, 60.2, 56.8 and 56.7, 31.2, 28.4, 26.7, 19.3 and 19.1, 17.7 and 17.2, 14.1, 7.6, 7.5; IR (film) ν_{max} 3336, 2969, 1727, 1659, 1530 cm^{-1}; HRMS (ESI) *m/z* 373.2276 (MH^+, $C_{18}H_{32}N_2O_6H^+$ requires 373.2294).

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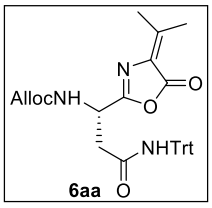
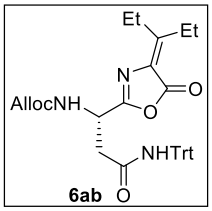


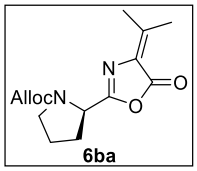
Allyl (*S*)-(3-oxo-1-(5-oxo-4-(propan-2-ylidene)-4,5-dihydrooxazol-2-yl)-3-(tritylamino)propyl)carbamate (6aa). A solution of dipeptide **5aa** (402.9 mg, 0.670 mmol) in 3:1 *t*-BuOH–H_2O (16 mL) at 0 °C was treated with $LiOH·H_2O$ (141.4 mg, 3.37 mmol, 5.0 equiv) and stirred at 0 °C for 4 h. The reaction was quenched with sat aq $KHSO_4$ to adjust the pH to 2, and the resulting mixture was extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude acid (363.6 mg, 0.634 mmol) was then dissolved in anhydrous THF (20 mL), treated with NaOAc (78.1 mg, 0.952 mmol, 1.5 equiv) and Ac_2O (300 μL, 324 mg, 3.17 mmol, 5.0 equiv), then stirred at rt under Ar for 12 h. The reaction was quenched with MeOH (10 mL), stirred at rt for 30 min, diluted with H_2O (10 mL), and extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO_2, 1% Et_3N in 2–10% EtOAc in hexanes gradient elution) afforded **6aa** (252.4 mg, 0.469 mmol,

70%) as a white solid: $[\alpha]^{25}_{D}$ –2.1 (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31–7.23 (m, 9H), 7.17–7.12 (m, 6H), 6.78 (s, 1H), 6.17 (d, *J* = 8.5 Hz, 1H), 5.97–5.82 (m, 1H), 5.32 (d, *J* = 17.1 Hz, 1H), 5.21 (d, *J* = 10.4 Hz, 1H), 5.01–4.91 (m, 1H), 4.64–4.52 (m, 2H), 3.08 (dd, *J* = 15.6, 4.3 Hz, 1H), 2.92 (dd, *J* = 15.5, 4.5 Hz, 1H), 2.35 (s, 3H), 2.19 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.7, 164.8, 161.7, 155.9, 154.5, 144.2 (3C), 132.5, 130.5, 128.6 (6C), 128.0 (6C), 127.2 (3C), 117.7, 70.9, 65.9, 46.9, 37.9, 22.9, 19.7; IR (film) ν_{max} 3286, 2917, 1794, 1673, 1530, 1447, 1158 cm^{-1}; HRMS (ESI) *m/z* 538.2273 (MH^+, $C_{32}H_{31}N_3O_5H^+$ requires 538.2297).

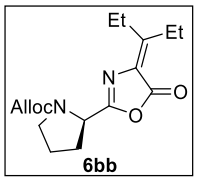


Allyl (*S*)-(3-oxo-1-(5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-yl)-3-(tritylamino)propyl)carbamate (6ab). Subjection of dipeptide **5ab** (342.7 mg, 0.544 mmol) to the saponification procedure described above for the synthesis of **6aa** afforded 316.4 mg (0.526 mmol) of the crude acid. Subjection of this crude mixture to the conditions described previously with NaOAc (65.7 mg, 0.801 mmol, 1.5 equiv), Ac_2O (250 μL, 270 mg, 2.64 mmol, 5.0 equiv), and purification by flash chromatography (60 mL of SiO_2, 1% Et_3N in 1–10% EtOAc in hexanes gradient elution) afforded **6ab** (197.7 mg, 0.349 mmol, 64%) as a white solid: $[\alpha]^{25}_{D}$ –5.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31–7.22 (m, 9H), 7.14–7.11 (m, 6H), 6.78 (s, 1H), 6.15 (d, *J* = 8.6 Hz, 1H), 5.98–5.84 (m, 1H), 5.32 (d, *J* = 17.3 Hz, 1H), 5.21 (d, *J* = 10.5 Hz, 1H), 5.00–4.92 (m, 1H), 4.64–4.52 (m, 2H), 3.10 (dd, *J* = 15.6, 4.4 Hz, 1H), 2.92 (dd, *J* = 15.5, 4.7 Hz, 1H), 2.85–2.71 (m, 2H), 2.69–2.54 (m, 2H), 1.14 (t, *J* = 7.5 Hz, 3H), 1.09 (t, *J*= 7.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.7, 165.4, 164.7, 161.9, 155.9, 144.2 (3C), 132.5, 129.6, 128.6 (6C), 128.0 (6C), 127.2 (3C), 117.6, 71.0, 65.9, 47.0, 37.9, 26.6, 23.4, 12.5, 12.3; IR (film) ν_{max} 3277, 2918,

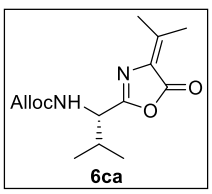
1789, 1648, 1526, 1447, 1268 cm^{-1}; HRMS (ESI) *m/z* 566.2639 (MH^+, $C_{34}H_{35}N_3O_5H^+$ requires 566.2610).



Allyl (*R*)-2-(5-oxo-4-(propan-2-ylidene)-4,5-dihydrooxazol-2-yl)pyrrolidine-1-carboxylate (6ba). Subjection of dipeptide **5ba** (268.6 mg, 0.784 mmol) to the saponification procedure described above for the synthesis of **6aa** afforded 236.1 mg (0.751 mmol) of the crude acid. Subjection of this crude mixture to the conditions described previously with NaOAc (92.8 mg, 1.13 mmol, 1.5 equiv), Ac_2O (360 μL, 389 mg, 3.81 mmol, 5.1 equiv), and purification by flash chromatography (50 mL of SiO_2, 1% Et_3N in 2–10% EtOAc in hexanes gradient elution) afforded **6ba** (157.9 mg, 0.567 mmol, 72%) as a colorless oil: $[\alpha]^{25}_D$ +93 (*c* 0.64, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz, ca. 1.2:1 mixture of rotamers) δ 5.99–5.90 and 5.88–5.78 (2m, 1H), 5.33 and 5.25 (2d, *J* = 17.2 and 18.8 Hz, 1H), 5.22 and 5.13 (2d, *J* = 10.9 and 10.5 Hz, 1H), 4.75–4.68 (m, 1H), 4.65–4.59 and 4.52 (m and dd, *J* = 13.7, 5.1 Hz, 1H), 4.61 (d, *J* = 5.4 Hz, 1H), 3.68–3.59 (m, 1H), 3.59–3.50 (m, 1H), 2.35 and 2.33 (2s, 3H), 2.32–2.26 (m, 1H), 2.24 (s, 3H), 2.18–2.03 (m, 2H), 2.02–1.92 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz, ca. 1.2:1 mixture of rotamers) δ 165.3, 165.1, 163.1 and 162.9, 154.5 and 154.1, 132.8 and 132.6, 130.7 and 130.5, 117.4 and 117.0, 66.0 and 65.9, 55.7 and 55.1, 46.9 and 46.5, 31.2 and 30.4, 24.3 and 23.4, 22.8 and 22.7, 19.7; IR (film) ν_{max} 2917, 1794, 1707, 1675, 1406, 1158 cm^{-1}; HRMS (ESI) *m/z* 279.1325 (MH^+, $C_{14}H_{18}N_2O_4H^+$ requires 279.1300).

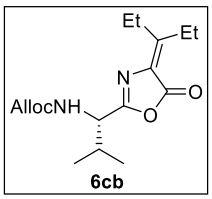


Allyl (*R*)-2-(5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-yl)pyrrolidine-1-carboxylate (6bb). Subjection of dipeptide **5bb** (337.4 mg, 0.911 mmol) to the saponification procedure described above for the synthesis of **6aa** afforded 308.2 mg (0.900 mmol) of the crude acid. Subjection of this crude mixture to the conditions described previously with NaOAc (110.9 mg, 1.35 mmol, 1.5 equiv), Ac_2O (430 μL, 464 mg, 4.55 mmol, 5.1 equiv), and purification by flash chromatography (75 mL of SiO_2, 1% Et_3N in 1–10% EtOAc in hexanes gradient elution) afforded **6bb** (204.8 mg, 0.668 mmol, 73%) as a colorless oil: $[\alpha]^{25}_D$ +83 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, ca. 1.1:1 mixture of rotamers) δ 6.00–5.89 and 5.87–5.76 (2m, 1H), 5.32 and 5.23 (2d, *J* = 17.8 and 18.5 Hz, 1H), 5.22 and 5.12 (2d, *J* = 9.4 and 10.4 Hz, 1H), 4.74 and 4.69 (2dd, *J* = 8.2, 3.0 Hz and 8.1, 3.4 Hz, 1H), 4.65–4.58 and 4.50 (m and dd, *J* = 13.6, 5.2 Hz, 1H), 4.61 (d, *J* = 4.6 Hz, 1H), 3.68–3.60 (m, 1H), 3.59–3.51 (m, 1H), 2.83–2.74 (m, 2H), 2.69–2.59 (m, 2H), 2.34–2.24 (m, 1H), 2.18–2.03 (m, 2H), 2.01–1.91 (m, 1H), 1.17–1.07 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz, ca. 1.1:1 mixture of rotamers) δ 165.2, 165.0 and 164.9, 163.2 and 163.1, 154.5 and 154.2, 132.8 and 132.6, 129.8 and 129.6, 117.4 and 117.1, 66.0 and 65.9, 55.7 and 55.1, 46.9 and 46.5, 31.2 and 30.4, 26.6, 24.3 and 23.6, 23.4, 12.5 and 12.4, 12.3; IR (film) ν_{max} 2975, 1791, 1709, 1668, 1406, 1156 cm^{-1}; HRMS (ESI) *m/z* 307.1625 (MH^+, $C_{16}H_{22}N_2O_4H^+$ requires 307.1613).



Allyl (*S*)-(2-methyl-1-(5-oxo-4-(propan-2-ylidene)-4,5-dihydrooxazol-2-yl)propyl)carbamate (6ca). Subjection of dipeptide **5ca** (394.2 mg, 1.14 mmol) to the

saponification procedure described above for the synthesis of **6aa** afforded 357.9 mg (1.13 mmol) of the crude acid. Subjection of this crude mixture to the conditions described previously with NaOAc (139.4 mg, 1.70 mmol, 1.5 equiv), Ac_2O (540 μL, 583 mg, 5.71 mmol, 5.0 equiv), and purification by flash chromatography (50 mL of SiO_2, 1% Et_3N in 1–10% EtOAc in hexanes gradient elution) afforded **6ca** (257.7 mg, 0.919 mmol, 80%) as a colorless oil: $[\alpha]^{25}_D$ −18 (*c* 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.01–5.89 (m, 1H), 5.34 (d, *J* = 17.1 Hz, 1H), 5.31–5.27 (m, 1H), 5.25 (d, *J* = 10.9 Hz, 1H), 4.64–4.56 (m, 3H), 2.36 (s, 3H), 2.26 (s, 3H), 2.24–2.17 (m, 1H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.9, 162.1, 155.9, 154.7, 132.6, 130.1, 118.0, 66.0, 55.0, 31.3, 22.8, 19.7, 18.9, 17.5; IR (film) ν_{max} 3293, 2917, 1693, 1650, 1536 cm^{-1}; HRMS (ESI) *m/z* 281.1484 (MH^+, $C_{14}H_{20}N_2O_4H^+$ requires 281.1457).



6cb

Allyl (*S*)-(2-methyl-1-(5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-yl)propyl)carbamate (6cb). Subjection of dipeptide **5cb** (338.6 mg, 0.909 mmol) to the saponification procedure described above for the synthesis of **6aa** with 6 h of stirring afforded 307.3 mg (0.892 mmol) of the crude acid. Subjection of this crude mixture to the conditions described previously with NaOAc (109.9 mg, 1.34 mmol, 1.5 equiv), Ac_2O (430 μL, 464 mg, 4.55 mmol, 5.1 equiv), and purification by flash chromatography 65 mL of SiO_2, 1% Et_3N in 1–10% EtOAc in hexanes gradient elution) afforded **6cb** (208.6 mg, 0.676 mmol, 74%) as a colorless oil: $[\alpha]^{25}_D$ −14 (*c* 0.36, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.01–5.90 (m, 1H), 5.33 (dd, *J* = 17.3, 1.6 Hz, 1H), 5.19 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 5.4 Hz, 2H), 4.02 (d, *J* = 6.8 Hz, 1H), 2.58 (q, *J* = 7.6 Hz, 2H), 2.28–2.18 (m, 2H), 2.17–2.09 (m, 1H), 1.11 (t, *J* = 7.4 Hz, 3H), 1.04 (t, *J* = 7.5

Hz, 3H), 1.03 (d, J = 7.3 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.6, 166.5, 157.0, 155.7, 132.9, 121.3, 116.2, 65.2, 60.4, 30.6, 25.4, 24.3, 18.4, 17.0, 12.0, 11.0; IR (film) ν_{max} 3293, 2966, 1693, 1650, 1561 cm^{-1}; HRMS (ESI) *m/z* 309.1746 (MH^+, $C_{16}H_{24}N_2O_4H^+$ requires 309.1770).

General Procedures for Solid-Phase Peptide Synthesis

Attachment of *C*-terminal amino acid to resin. Rink amide MBHA resin (100–200 mesh, 100 μmol) was added to a fritted polypropylene syringe. The resin was swelled in CH_2Cl_2 (10 min), and then in DMF (5 min). The swelling solvents were drained from the resin using a vacuum manifold. After Fmoc deprotection (see below for procedure, repeated twice), the amino acid was coupled to the resin (see below for procedure, repeated twice).

Fmoc Deprotection. The resin (100 μmol) was treated with piperidine (20% solution in DMF, 5.0 mL) and allowed to stand for 5 min. The solution was drained from the resin using a vacuum manifold, and additional piperidine (20% solution in DMF, 5.0 mL) was added. The resulting mixture was stirred twice at 80 °C in a microwave oven for 4 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF (5 × 10 mL).

Peptide coupling. The Fmoc-protected amino acid (500 μmol, 5 equiv) and HBTU (190 mg, 500 μmol, 5 equiv) were dissolved by vortexing in a 0.1 M HOBt solution in NMP (5.0 mL, 500 μmol, 5 equiv). *i*Pr_2NEt (176 μL, 1000 μmol, 10 equiv) was added to this solution, and it was allowed to stand for ca. 1 min. The solution was added to the resin (100 μmol), and the resulting mixture was stirred at 70 °C in a microwave oven for 10 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF (5 × 10 mL). The *N*-terminal amino acid of each peptide (i.e., Arg) was coupled by gently stirring the solution at rt under Ar for ca. 10–12 h after the microwave heating was completed.

Coupling of azlactones 6 with resin-bound peptides. A solution of **6** (500 μmol, 5 equiv, typically employed crude without purification by flash chromatography) and Et_3N (150 μL, 1.08 mmol, 10 equiv) in NMP (10 mL) was added to the resin-bound peptide (100 μmol). The resulting mixture was gently stirred at 80 °C for 24–36 h. The solution was drained from the resin using a fritted polypropylene syringe into a 20 mL glass vial (**note:** the unreacted azlactone present in the solution can be reused), and the resin was rinsed with DMF (5 × 10 mL). Any unreacted amines were then capped by addition of a solution of Ac_2O (1.0 mL, 1.08 g, 10.6 mmol, 106 equiv) and Et_3N (200 μL, 145.2 mg, 1.44 mmol, 14.4 equiv.) in CH_2Cl_2 (5 mL), followed by stirring at rt under Ar for 90 min and washing with CH_2Cl_2 (5 × 10 mL).

Alloc deprotection. The resin-bound peptide (100 μmol) was placed under an Ar atmosphere and treated with a solution of $PhSiH_3$ (700 μL, 613.9 mg, 5.67 mmol, 56.7 equiv) in CH_2Cl_2 (3 mL) with stirring, followed by addition of a solution of $Pd(PPh_3)_4$ (56.8 mg, 49.2 μmol, 0.49 equiv) in CH_2Cl_2 (3 mL). The resulting mixture was stirred at rt under Ar for 20 min. The resin was rinsed with CH_2Cl_2 (5 × 10 mL), and the deprotection protocol was repeated once.

Acetylation of the *N*-terminus of resin-bound peptides. The *N*-terminus of each peptide was capped by addition of a solution of Ac_2O (1.0 mL, 1.08 g, 10.6 mmol, 106 equiv) and Et_3N (200 μL, 145.2 mg, 1.44 mmol, 14.4 equiv.) in CH_2Cl_2 (5 mL), followed by stirring at rt under Ar for 2 h. The resin was then rinsed with CH_2Cl_2 (5 × 10 mL).

Cleavage of peptide from resin and purification. The resin-bound peptide (100 μmol) was treated carefully with a solution of phenol (500 mg, 5.31 mmol), H_2O (500 μL), thioanisole (500 μL, 528.5 mg, 4.26 mmol), ethanedithiol (250 μL, 280.8 mg, 2.98 mmol), and triisopropylsilane (100 μL, 77.3 mg, 488 μmol) in TFA (8.0 mL) in order to avoid the buildup of excess CO_2 pressure in the reaction vessel. The resulting mixture was stirred at rt for ca. 4 h, and

the peptide was precipitated by filtering the mixture and pouring the filtrate into cold Et_2O (40 mL). The resin was rinsed with TFA (3 mL), and the precipitate was collected by centrifugation. The crude peptide was lyophilized and purified by HPLC (see entries for individual peptides for elution conditions).

Synthesis of cyclic and random coil control peptides (S1–S6). Ac-Cys-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Leu-Gln-Cys-NH_2 and Ac-Cys-Trp-Val-Glu-Val-ΔVal-Gly-Orn-Lys-Ile-Leu-Gln-Cys-NH_2 were synthesized according to the previously described general procedures and purified by HPLC. They were then cyclized by stirring in open air for ca. 24 h in a 10 mM phosphate buffer solution (pH 7.5) containing 5% DMSO, affording cyclic controls **S1** and **S4** respectively. Random coil peptides **S2**, **S3**, **S5,** and **S6** were synthesized according to the previously described general procedures. Azlactones **6ca** and **Ac-ΔVal**[4] were used to synthesize **S5** and **S6** respectively.

Peptide Concentration Determination and NMR Sample Preparation. Peptide solutions were prepared in 20 mM sodium phosphate buffer (pH 7), and peptide concentrations were determined spectroscopically based on tryptophan absorbance at 280 nm in 6 M guanidine hydrochloride (Trp $\varepsilon_{280} = 5690\ M^{-1}cm^{-1}$). NMR samples were prepared with 1–3.5 mM peptide in 10% v/v D_2O/H_2O buffered to pD 3.9 with 5000 mM NaOAc-d_3.

[4] El-Baba, S., Nuzillard, J.M., Poulin, J.C., Kagan, H.B. *Tetrahedron* **1986**, *42*, 3851.

Tabulated ^{1}H NMR Data for Peptides

Ac-R-W-V-E-V-**N-G-**O-K-I-L-Q-NH_2 (**1**)

	Amide	α	β	Others (γ, δ, ε)
Arg	8.11	4.38	1.65, 1.71	γ CH_2 1.52; δ CH_2 3.15; ε H 7.14
Trp	8.34	5.09	3.09	H1 10.2; H2 7.25; H4 7.50; H6 7.08; H7 7.34
Val	9.00	4.45	2.04	γ CH_3 0.871, 0.889
Glu	8.55	4.92	1.93, 2.02	γ CH_2 2.28
Val	8.90	4.22	1.96	γ CH_3 0.928
Asn	9.41	4.48	2.77, 3.07	δ NH_2 6.96, 7.64
Gly	8.64	3.75, 4.09		
Orn	7.87	4.62	1.83, 1.87	γ CH_2 1.73; δ CH_2 3.05; ε NH 7.66
Lys	8.51	4.74	1.70	γ CH_2 1.23; δ CH_2 1.36; ε CH_2 2.58; ζ NH_3 7.28
Ile	9.11	4.57	1.89	γ CH_2 1.21, 1.43; δ CH_3 0.836; γ CH_3 0.902
Leu	8.35	4.10	1.09, 1.37	γ CH 0.792; δ CH_3 0.319, 0.529
Gln	8.67	4.33	1.89, 2.05	γ CH_2 2.28; ε NH_2 6.89, 7.36

Ac-R-W-V-E-V-**p-G-**O-K-I-L-Q-NH_2 (**2**)

	H	α	β	Others (γ, δ, ε)
Arg	8.07	4.41	1.64, 1.72	γ CH_2 1.53; δ CH_2 3.16; ε NH 6.88
Trp	8.36	5.13	3.08	H1 10.20; H2 7.23; H4 7.27; H5 7.05; H7 7.49
Val	9.25	4.51	2.05	γ CH_3 0.889, 0.873
Glu	8.59	5.06	2.03, 1.95	γ CH_2 2.33
Val	8.98	4.63	1.99	γ CH_3 0.941
D-Pro		4.38	2.20, 2.40	γ CH_2 2.01, 2.08; δ CH_2 3.88
Gly	8.62	3.80, 4.04		
Orn	7.93	4.66	1.85	γ CH_2 1.71; δ CH_2 3.03, ε NH_3 7.65
Lys	8.48	4.86	1.67	γ CH_2 1.20; δ CH_2 1.28; ε CH_2 2.48
Ile	9.24	4.62	1.88	γ CH_2 1.19, 1.40; δ CH_3 0.816; γ CH_3 0.889
Leu	8.36	4.02	0.966, 1.32	γ CH 0.595; δ CH_3 0.166, 0.445
Gln	8.73	4.33	1.86, 2.03	γ CH_2 2.25; ε NH_2 7.71, 7.12

Ac-R-W-V-E-V-**N**-Δ**Val**-O-K-I-L-Q-NH_2 (**7aa**)

	H	**α**	**β**	**Others (γ, δ, ε)**
Arg	8.12	4.30	1.64	γ CH_2 1.47; δ CH_2 3.11; ε NH 7.11
Trp	8.27	4.97	3.14	H1 10.18; H2 7.23; H4 7.42; H5 7.08; H7 7.49
Val	8.62	4.30	2.01	γ CH_3 0.851
Glu	8.45	4.75	1.90, 1.98	γ CH_2 2.23
Val	8.70	4.18	1.99	γ CH_3 0.915
Asn	9.15	4.61	2.80, 3.01	δ NH_2 6.95, 7.62
ΔVal	9.34			γ CH_3 1.81, 0.917
Orn	7.78	4.49	1.72, 1.76	γ CH_2 1.86; δ CH_2 3.04; ε NH_3 7.64
Lys	8.36	4.57	1.73	γ CH_2 1.26; δ CH_2 1.42; ε CH_2 2.68
Ile	8.76	4.39	1.87	γ CH_2 1.17, 1.42; δ CH_3 0.805; γ CH_3 0.805
Leu	8.33	4.16	1.42, 1.25	γ CH 1.05; δ CH_3 0.484, 0.619
Gln	8.49	4.30	1.91, 2.05	γ CH_2 2.30; ε NH_2 7.12, 7.62

Ac-R-W-V-E-V-**N**-Δ**Env**-O-K-I-L-Q-NH_2 (**7ab**)

	H	**α**	**β**	**Others (γ, δ, ε)**
Arg	8.14	4.27	1.64	γ CH_2 1.47; δ CH_2 3.11; ε NH 7.11
Trp	8.25	4.92	3.18	1H 10.2; H2 7.24; H5 7.10; H7 7.48
Val	8.44	4.25	2.01	γ CH_3 0.856
Glu	8.40	4.66	1.97, 2.09	γ CH_2 2.32
Val	8.58	4.16	2.02	γ CH_3 0.935
Asn	8.98	4.70	2.80, 3.01	δ NH_2 7.00, 7.64
ΔEnv	9.21			γ CH_2 2.139, 2.215; δ CH_3 0.971
Orn	7.85	4.41	1.87	γ CH_2 1.74; δ CH_2 3.04; ε NH_3 7.65
Lys	8.24	4.50	1.77	γ CH_2 1.30; δ CH_2 1.49 ε CH_2 2.75; ζ NH_3 7.41
Ile	8.61	4.30	1.90	γ CH_2 1.18, 1.45; δ CH_3 0.804; γ CH_3 0.879
Leu	8.33	4.19	1.39, 1.48	γ CH 1.23; δ CH_3 0.585, 0.685
Gln	8.62	4.30	2.09	γ CH_2 2.43; ε NH_2 7.15, 7.57

Ac-R-W-V-E-V-**p-ΔVal**-O-K-I-L-Q-NH_2 (**7ba**)

	H	α	β	Others (γ, δ, ε)
Arg	8.08	4.40	1.71, 1.63	γ CH_2 1.513, δ CH_2 3.15, ε CH 7.13
Trp	8.35	5.11	3.06	H1 10.2; H2 7.23; H4 7.50; H6 7.05; H7 7.30
Val	9.15	4.48	2.05	γ CH_2 0.876
Glu	8.55	5.07	1.89, 1.97	γ CH_2 2.16, 2.22
Val	8.95	4.62	2.00	γ CH_3 0.947
D-Pro		4.42	2.19, 2.47	γ CH_2 2.10; δ CH_2 3.88, 3.94
ΔVal	9.25			γ CH_3 1.80, 2.18
Orn	7.77	4.63	1.80, 1.86	γ CH_2 1.73; δ CH_2 3.03; ε NH_3 7.64
Lys	8.48	4.82	1.67	γ CH_2 1.20; δ CH_2 1.30; ε CH_2 2.53
Ile	9.17	4.58	1.87	γ CH_2 1.19, 1.40; δ CH_3 0.814; γ CH_3 0.885
Leu	8.36	4.04	1.01, 1.34	γ CH 0.671; δ CH_3 0.217, 0.470
Gln	8.69	4.32	1.86, 2.03	γ CH_2 2.26; ε NH_2 6.89, 7.33

Ac-R-W-V-E-V-**p-ΔEnv**-O-K-I-L-Q-NH_2 (**7bb**)

	H	α	β	Others (γ, δ, ε)
Arg	8.08	4.39	1.64, 1.71	γ CH_2 1.52; δ CH_2 3.15; ε NH 7.14
Trp	8.32	5.10	3.07	H1 10.2; H2 7.23; H4 7.31; H5 7.05; H7 7.50
Val	9.10	4.48	2.07	γ CH_3 0.884
Glu	8.53	5.09	1.90, 1.97	γ CH_2 2.14, 2.29
Val	8.89	4.61	2.01	γ CH_3 0.955
D-Pro		4.44	2.17, 2.47	γ CH_2 2.09; δ CH_2 3.87, 3.97
ΔEnv	9.33			γ CH_2 2.23, 2.061; δ CH_3 0.969
Orn	7.78	4.62	1.88	γ CH_2 1.74; δ CH_2 3.05; ε NH_3 7.63
Lys	8.44	4.82	1.67	γ CH_2 1.21; δ CH_2 1.34; ε CH_2 2.55
Ile	9.12	4.56	1.88	γ CH_2 1.20, 1.42; δ CH_3 0.823; γ CH_3 0.891
Leu	8.36	4.06	1.36, 1.06	γ CH_2 0.730; δ CH_3 0.258, 0.499
Gln	8.66	4.33	1.89, 2.05	γ CH_2 2.27; ε NH_2 7.12, 7.69

Ac-R-W-V-E-V-Δ**Val-G**-O-K-I-L-Q-NH_2 (**7ca**)

	H	α	β	Others (γ, δ, ε)
Arg	8.08	4.41	1.73, 1.65	γ CH_2 1.54; δ CH_2 3.16; ε NH 7.14
Trp	8.34	5.15	3.04, 3.09	H1 10.2; H2 7.24; H4 7.29; H6 7.06; H7 7.50
Val	9.24	4.54	2.08	γ CH_3 0.880
Glu	8.57	5.14	2.01, 1.93	γ CH_2 2.27, 2.30
Val	9.08	4.38	1.96	γ CH_3 0.994, 0.955
ΔVal	10.3			γ CH_3 1.99, 1.89
Gly	8.63	4.11, 3.73		
Orn	7.98	4.66	1.72	γ CH_2 1.85; δ CH_2 3.03; ε NH_3 7.65
Lys	8.50	4.82	1.67, 1.35	γ CH_2 1.20; δ CH_2 1.28, 1.35; ε CH_2 2.50, 2.45; ζ $NH_3$7.20
Ile	9.26	4.63	1.90	γ CH_2 1.21, 1.41; δ CH_3 0.813; γ CH_3 0.888
Leu	8.35	4.04	0.990, 1.33	γ CH 0.641; δ CH_3 0.194, 0.454
Gln	8.72	4.33	1.87, 2.03	γ CH_2 2.26; ε NH_2 7.12, 7.71

Ac-R-W-V-E-V-Δ**Env-G**-O-K-I-L-Q-NH_2 (**7cb**)

	H	α	β	Others (γ, δ, ε)
Arg	8.08	4.42	1.66, 1.73	γ CH_2 1.54; δ CH_2 3.17; ε NH 7.15
Trp	8.35	5.14	3.04, 3.10	H1 10.2; H2 7.24; H4 7.31; H5 7.07; H7 7.51
Val	9.18	4.52	2.07	γ CH_3 0.885, 0.907
Glu	8.57	5.09	2.22	γ CH_2 1.92, 2.00
Val	9.04	4.41	1.99	γ CH_3 0.965, 0.982
ΔEnv	10.1			γ CH_2 2.36, 2.20; δ CH_3 1.05, 0.992
Gly	8.65	3.76, 4.11		
Orn	7.97	4.67	1.87	γ CH_2 1.74; δ CH_2 3.05; ε NH_3 7.63
Lys	8.53	4.83	1.68	γ CH_2 1.21; δ CH_2 1.30, 1.38; ε CH_2 2.52
Ile	9.23	4.62	1.89	γ CH_2 1.21, 1.42; δ CH_3 0.829; γ CH_3 0.898
Leu	8.34	4.05	1.01, 1.34	γ CH 0.663; δ CH_3 0.224, 0.476
Gln	8.71	4.34	1.88, 2.05	γ CH_2 2.27; ε NH_2 7.71, 7.12

c[Ac-C-R-W-V-E-V-Δ**Val-G**-O-K-I-L-Q-C-NH_2] (**S4**)

	H	**α**	**β**	**Others (γ, δ, ε)**
Cys	8.38	5.23	3.00, 2.41	
Arg	8.73	4.62	1.67, 1.81	γ CH_2 1.51; δ CH_2 3.18; ε NH 7.12
Trp	8.66	5.15	3.10, 2.96	H1 10.2; H2 7.25; H4 7.29; H5 7.01; H7 7.50
Val	9.55	4.61	2.09	γ CH_3 0.888
Glu	8.54	5.16	1.99, 1.90	γ CH_2 2.18, 2.19
Val	9.12	4.40	1.95	γ CH_3 0.968
ΔVal	10.3			δ CH_2 0.985, 1.89
Gly	8.66	3.72, 4.13		
Orn	7.98	4.70	1.86	γ CH_2 1.72; δ CH_2 3.04; ε NH_3 7.64
Lys	8.53	4.99	1.75	γ CH_2 1.38; δ CH_2 1.38; ε CH_2 2.56
Ile	9.40	4.71	1.86	γ CH_2 1.17, 1.41; γ CH_3 0.826; δ CH_3 0.888
Leu	8.35	3.91	0.772, 1.31	γ CH_2 0.379; δ CH_3 -0.338, 0.078
Gln	9.05	4.58	1.83, 2.08	γ CH_2 2.24
Cys	8.94	5.05	2.94, 3.04	

S1

Amino Acid	Hα
Cys	5.22
Arg	4.62
Trp	5.14
Val	4.59
Glu	5.04
Val	4.25
Asn	4.41
Gly	3.69, 4.14
Orn	4.70
Lys	4.97
Ile	4.72
Leu	3.90
Gln	4.57
Cys	5.05

S2

Amino Acid	Hα
Arg	4.18
Trp	4.72
Val	4.00
Glu	4.25
Val	4.09
Asn	4.69
Gly	3.91

S3

Amino Acid	Hα
Asn	4.70
Gly	3.96
Orn	4.36
Lys	4.32
Ile	4.15
Leu	4.40
Gln	4.32

S5

Amino Acid	Hα
Arg	4.18
Trp	4.73
Val	4.01
Glu	4.27
Val	4.15
ΔVal	-
Gly	3.94

S6

Amino Acid	Hα
ΔVal	-
Gly	3.94
Orn	4.35
Lys	4.30
Ile	4.13
Leu	4.38
Gln	4.31

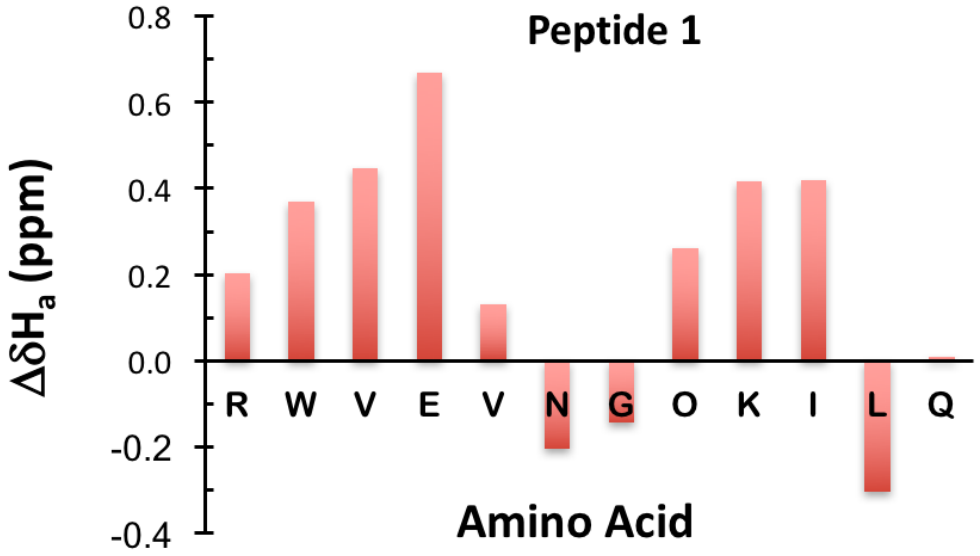


Figure S1. Hα chemical shift differences between the residues in **1** and the corresponding random coil 7-mers **S2** and **S3**.

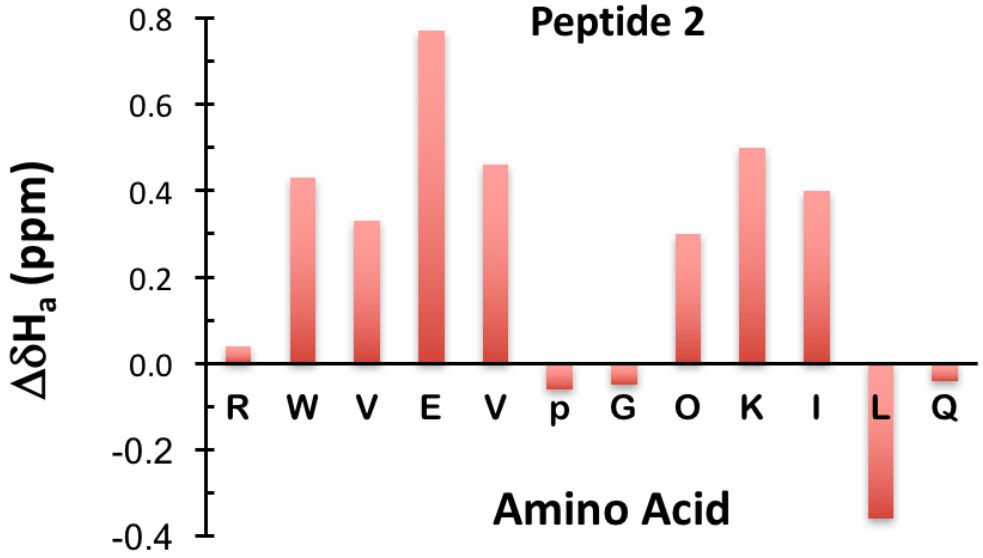


Figure S2. Hα chemical shift differences between the residues in **2** and the random coil values obtained from Wüthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley: New York (1986).

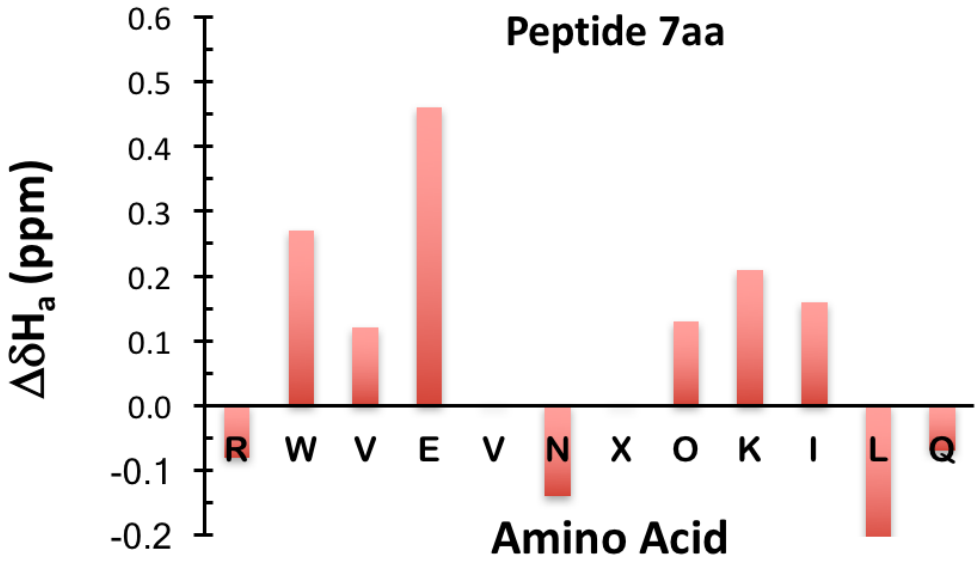


Figure S3. Hα chemical shift differences between the residues in **7aa** and the random coil values obtained from Wüthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley: New York (1986).

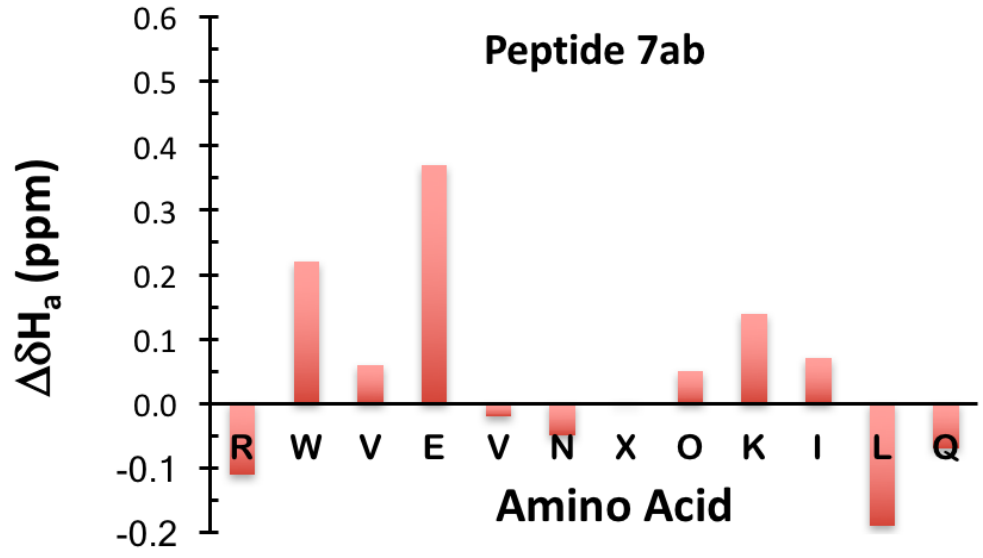


Figure S4. Hα chemical shift differences between the residues in **7ab** and the random coil values obtained from Wüthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley: New York (1986).

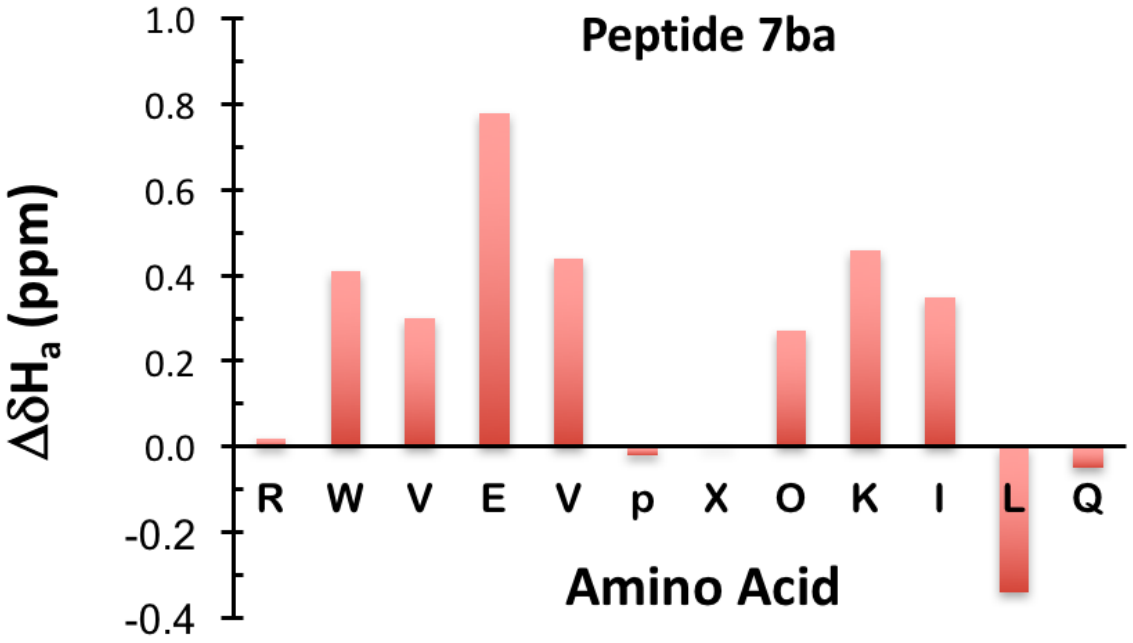


Figure S5. Hα chemical shift differences between the residues in **7ba** and the random coil values obtained from Wüthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley: New York (1986).

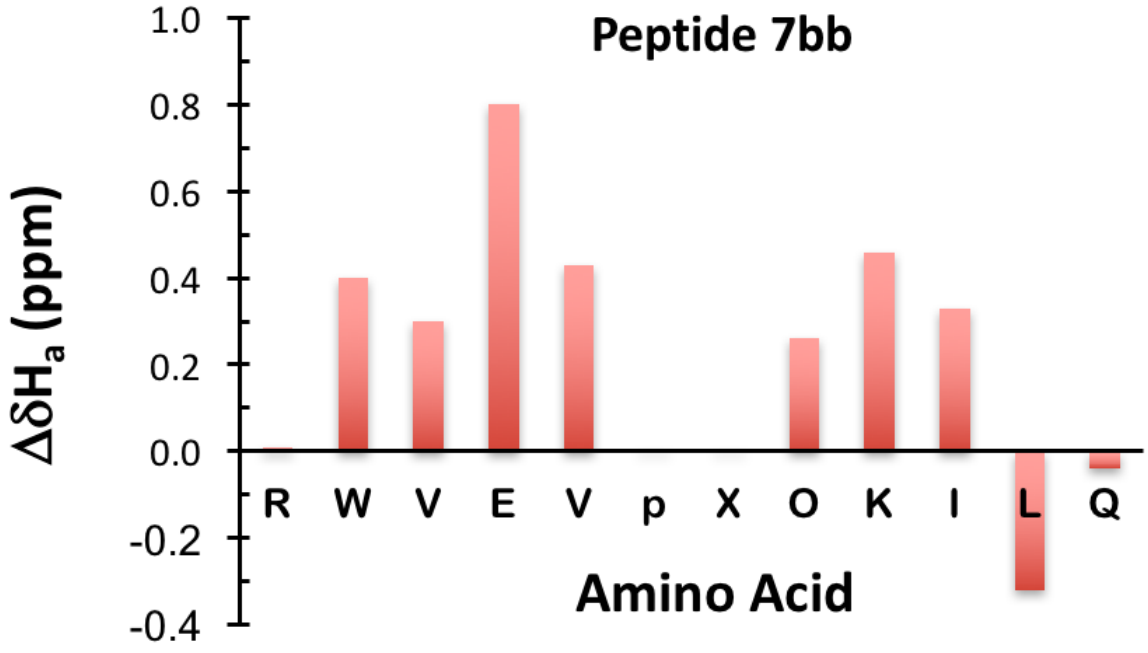


Figure S6. Hα chemical shift differences between the residues in **7bb** and the random coil values obtained from Wüthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley: New York (1986).

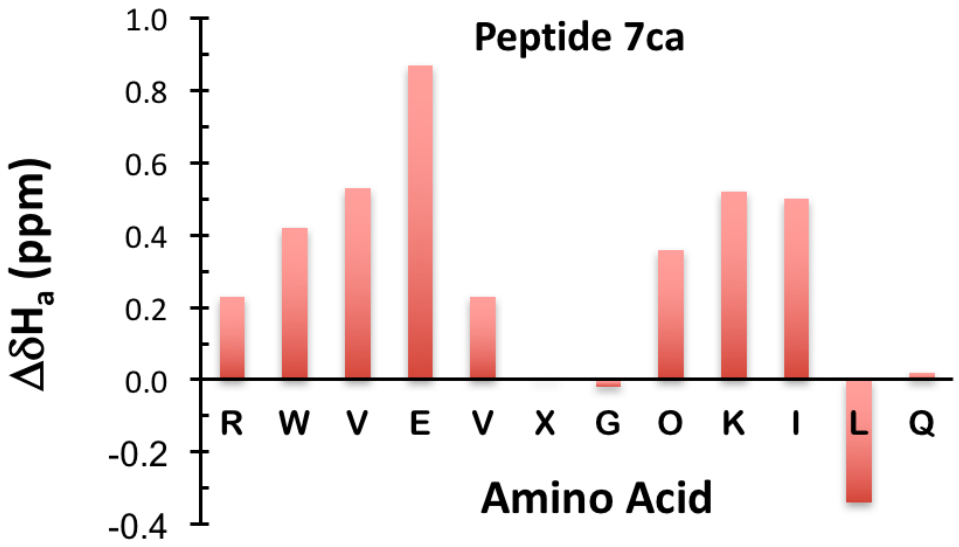


Figure S7. Hα chemical shift differences between the residues in **7ca** and the corresponding random coil 7-mers **S2** and **S3**.

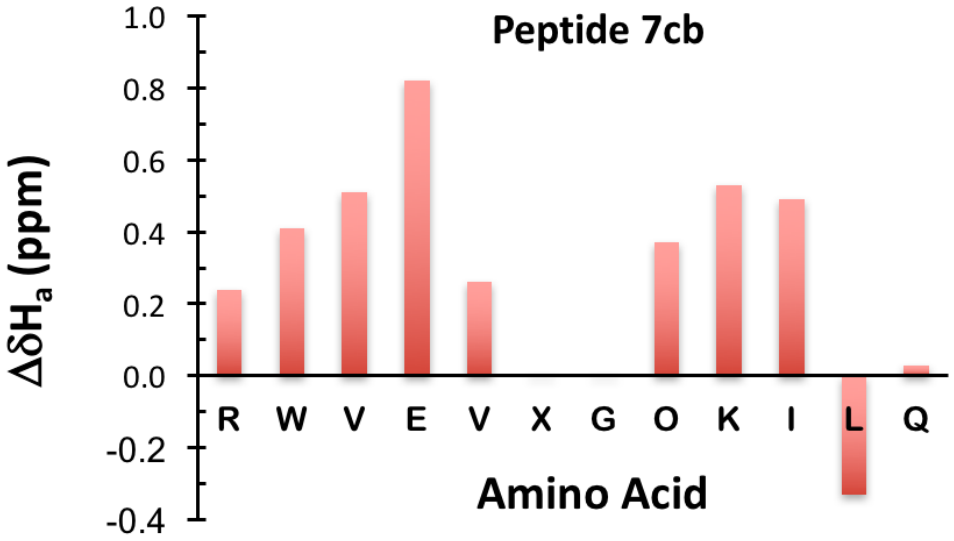


Figure S8. Hα chemical shift differences between the residues in **7cb** and the corresponding random coil 7-mers **S2** and **S3**.

Inter-residue ROESY Cross-peaks

Table S1. Ac-R-W-V-E-V-**N-G**-O-K-I-L-Q-NH_2 (**1**)

Res I	Res II	Peak
2Trp H2,4	**11**Leu δ	S
2Trp H7	**11**Leu δ	M
2Trp α	**11**Leu δ	M
2Trp β	**11**Leu δ	W
2Trp H6	**11**Leu γ	M
2Trp H2,7	**11**Leu α	M
2Trp H2	**11**Leu β	M
2Trp H6	**11**Leu β	W
2Trp β, H7	**11**Leu β	S
2Trp α	**11**Leu α	S
1Arg α	**11**Leu δ	M
1Arg β	**12**Gln H	W
4Glu H	**10**Ile γ	S
2Trp H6,7	**10**Ile α	S
2Trp H2	**10**Ile α, γ	M
5Val γ	**8**Orn H	M
5Val H	**8**Orn H	S
2Trp H2,6,7	**9**Lys γ	S
2Trp H7	**9**Lys α	W
5Val γ	**6**Asn δ	S

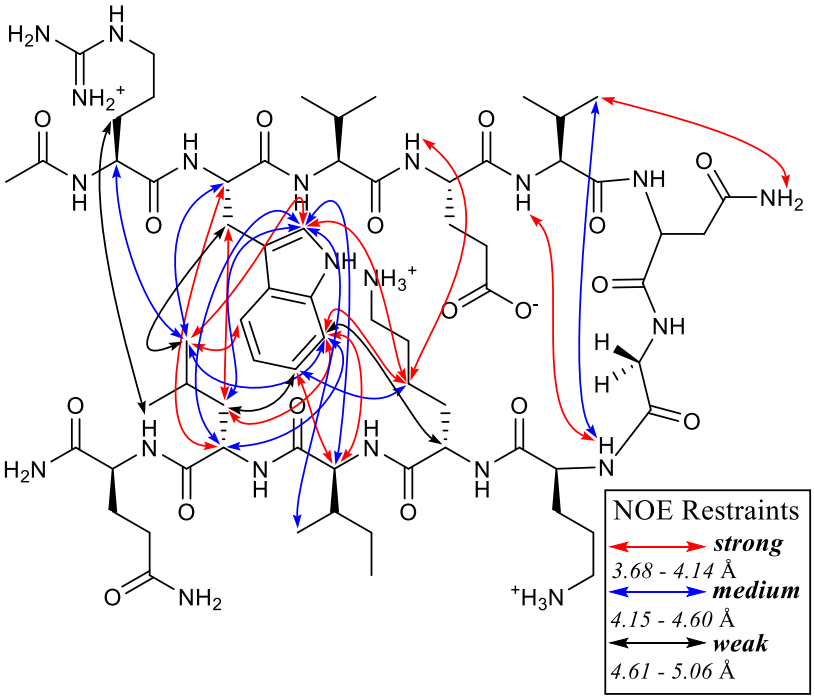


Table S2. Ac-R-W-V-E-V-**p-G**-O-K-I-L-Q-NH_2 (**2**)

Res I	Res II	Peak
2Trp H4	**11**Leu α	M
2Trp α, H2	**11**Leu α	S
2Trp H1,7	**11**Leu δ	S
2Trp α	**11**Leu δ	W
2Trp H1,4	**11**Leu δ	W
2Trp H1,5	**11**Leu γ	M
2Arg α	**11**Leu δ	M
2Trp H2	**9**Lys γ	W
2Trp H4	**9**Lys γ, δ	M
2Trp H1,4	**9**Lys β	S
2Trp α	**10**Ile H	S
2Trp H5	**10**Ile α	S
2Trp H1	**10**Ile α	M
2Trp H4	**10**Ile α	W
4Glu γ	**8**Orn H	M
6D-Pro α	**8**Orn H	M
5Val H	**8**Orn H	M
1Arg δ	**12**Gln α	M
2Trp H2	**12**Gln γ	M
5Val γ	**6**D-Pro δ	S

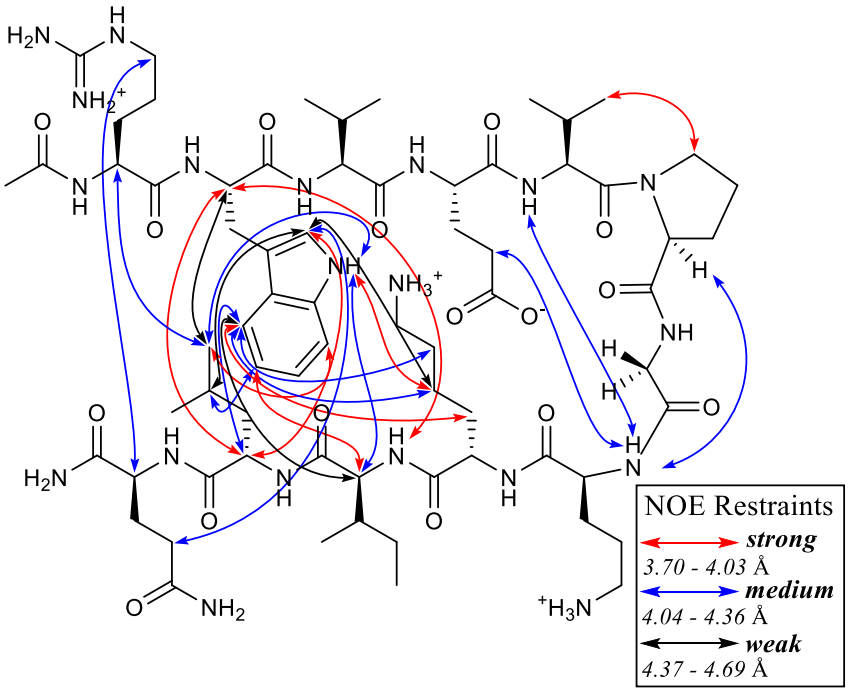


Table S3. Ac-R-W-V-E-V-**N**-ΔVal-O-K-I-L-Q-NH_2 (**7aa**)

Res I	Res II	Peak
1Arg ε	**12**Gln γ	W
1Arg ε	**10**Ile γ	M
2Trp H2	**10**Ile γ	M
2Trp H1,7	**11**Leu δ	W
2Trp H2,4	**11**Leu δ	M
2Trp β	**11**Leu δ	W
2Trp α	**11**Leu δ	M
2Trp α	**11**Leu α	S
2Trp H7	**9**Lys γ	S
2Trp H2	**9**Lys γ	W
2Trp H7	**11**Leu α	S
2Trp H2	**11**Leu α	M
2Trp H7	**10**Ile α	M
2Trp H2	**10**Ile α	W
2Trp H6	**10**Ile α	S
2Trp H2,7	**11**Leu β	S
2Trp H7	**11**Leu δ	S
5Val H	**9**Orn H	M
4Glu γ	**7**ΔVal H	S
4Glu γ	**8**Orn H	S

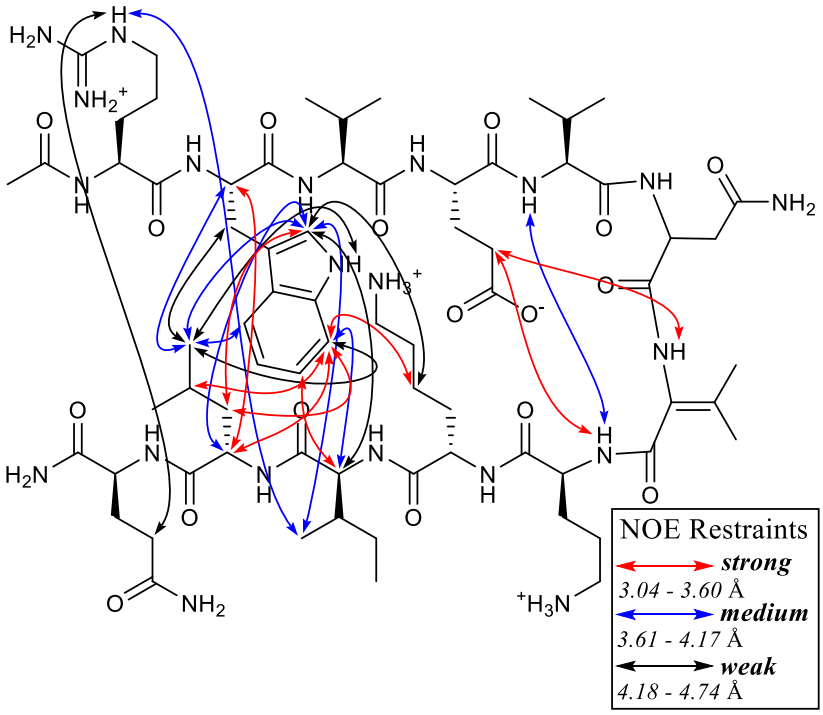


Table S4. Ac-R-W-V-E-V-**N**-Δ**Env**-O-K-I-L-Q-NH_2 (**7ab**)

Res I	Res II	Peak
2Trp H2,7	**11**Leu δ	M
2Trp H1,4	**11**Leu δ	W
2Trp α	**11**Leu δ	S
2Trp H7	**11**Leu β	S
2Trp H5	**11**Leu γ	W
2Trp α	**11**Leu α	S
2Trp H7	**11**Leu α	M
1Arg H	**11**Leu δ	M
2Trp H2	**9**Lys β	M
2Trp H7	**9**Lys β	S
2Trp H2	**9**Lys δ	W
2Trp H7	**9**Lys γ	S
1Arg ε	**9**Lys β	M
2Trp H2	**4**Glu γ	S
1Arg ε	**10**Ile γ	W
5Val H	**8**Orn H	M
11Leu α	**2**Trp H2	W
6Asn δ	**5**Val γ	S

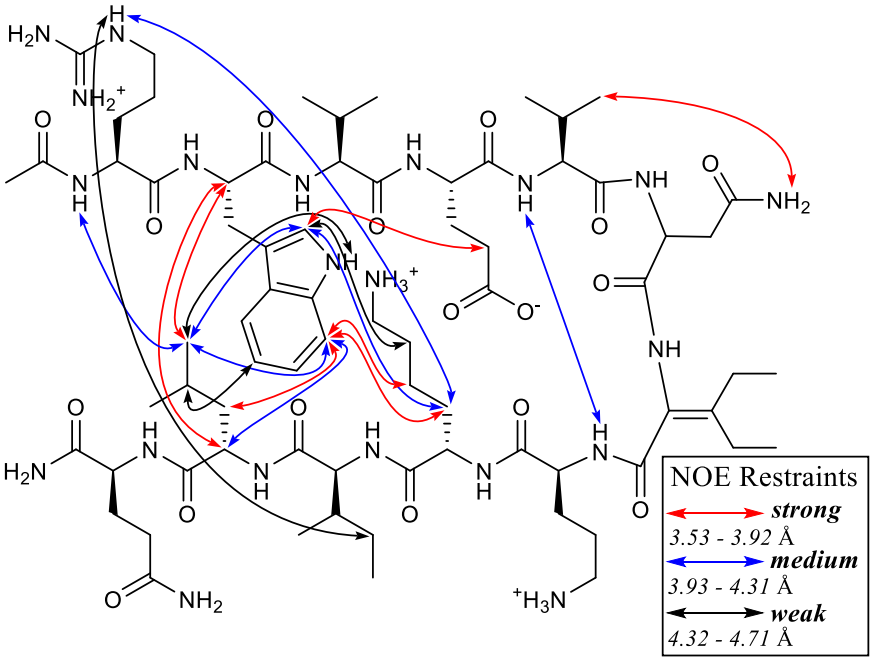


Table S5. Ac-R-W-V-E-V-**p**-ΔVal-O-K-I-L-Q-NH_2 (**7ba**)

Res I	Res II	Peak
2Trp H1,4,7	**11**Leu δ	M
2Trp H6,α	**11**Leu δ	W
2Trp H2	**11**Leu δ	S
2Trp H2,6	**11**Leu γ	W
2Trp H2,7	**11**Leu α	W
2Trp α	**11**Leu α	S
2Trp H7,β	**11**Leu β	S
1Arg H,α	**11**Leu δ	M
2Trp H6	**10**Ile α	S
2Trp H7	**10**Ile H	M
2Trp H2	**10**Ile γ	W
2Trp H7	**9**Lys β	S
2Trp H7,β	**9**Lys γ	M
2Trp H6	**9**Lys δ	W
4Glu H	**9**Lys α	S
4GluH	**10**Ile γ	S
1Arg H	**12**Gln H	S
2Trp α	**12**Gln H	W
5Val H	**8**Orn H	S
5Val γ	**6**D-Pro δ	S

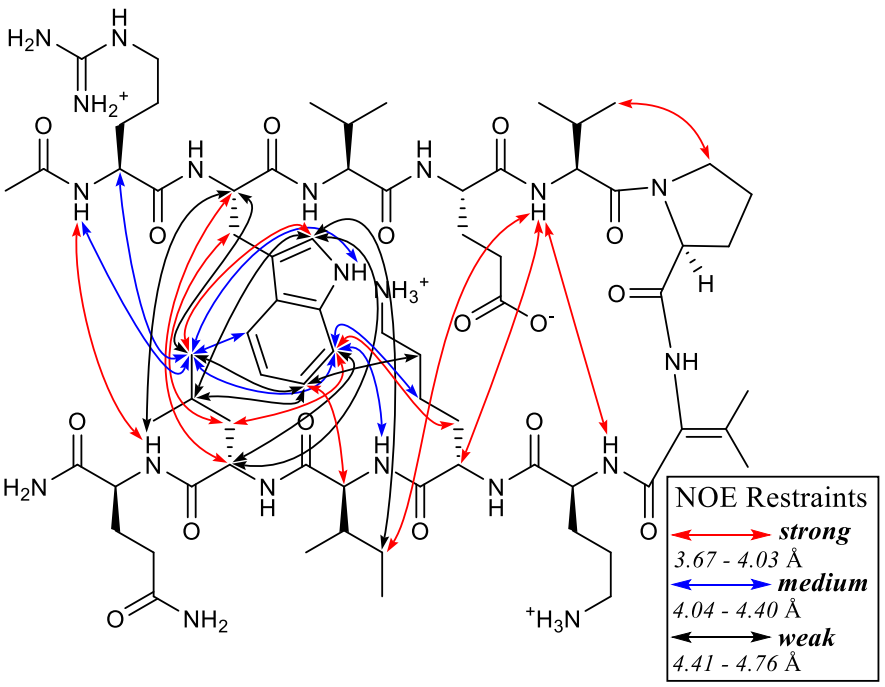


Table S6. Ac-R-W-V-E-V-**p**-ΔEnv-O-K-I-L-Q-NH_2 (**7bb**)

Res I	Res II	Peak
2Trp H1,4	**11**Leu δ	W
2Trp H2,7, α	**11**Leu δ	M
2Trp α	**11**Leu δ	M
2Trp H4, α	**11**Leu α	S
2Trp H2	**11**Leu α	M
2Trp H4	**11**Leu β	M
1Arg α	**11**Leu δ	W
1Arg H	**12**Gln β	W
2Trp H4	**12**Gln α	W
2Trp H4	**9**Lys β	S
2Trp H2	**9**Lys β	M
2Trp H2,5	**9**Lys γ	W
4Trp H2	**9**Lys δ	W
1Trp H4	**9**Lys δ	M
4Glu H	**9**Lys α	S
5Val H	**8**Orn H	S
2Trp H5	**10**Ile α	S
2Trp H2	**10**Ile α	W
2Trp H4	**10**Ile γ	M
4Glu H	**10**Ile γ	S
6D-Pro δ	**7**ΔEnv	S
5Val γ	**6**D-Pro δ	S

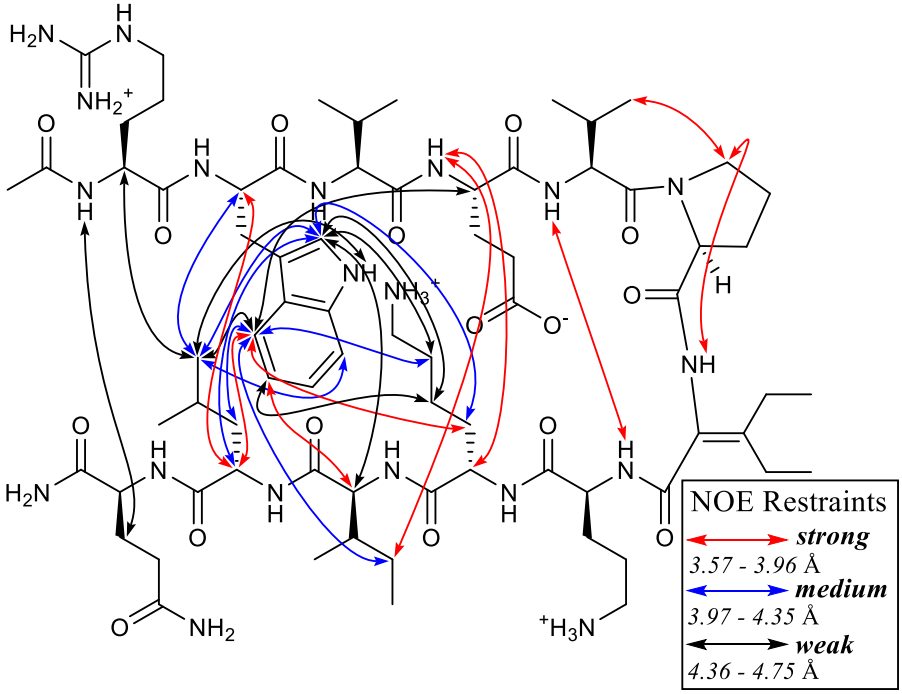


Table S7. Ac-R-W-V-E-V-Δ**Val-G**-O-K-I-L-Q-NH_2 (**7ca**)

Res I	Res II	Peak
2Trp H1,4	**11**Leu δ	W
2Trp H7	**11**Leu δ	M
2Trp H2	**11**Leu δ	S
2Trp α, β	**11**Leu δ	M
2Trp H2,5	**11**Leu γ	M
2Trp H2,4	**11**Leu α	M
2Trp α	**11**Leu α	S
2Trp H2	**11**Leu β	W
1Arg α	**11**Leu δ	M
4Glu β	**11**Leu δ	M
2Trp H2,5	**9**Lys β	M
2Trp H4d	**9**Lys δ	M
2Trp H4	**9**Lys γ	W
2Trp H4	**9**Lys β	S
2Trp H4	**10**Ile α	M
2Trp H2	**10**Ile α	W
2Trp H5	**10**Ile α	S
2Trp H4	**10**Ile γ	S
5Val H	**8**Orn H	S
2Trp H2	**12**Gln γ	W
1Arg H	**12**Gln β	W
6ΔVal γ	**5**Val γ	S

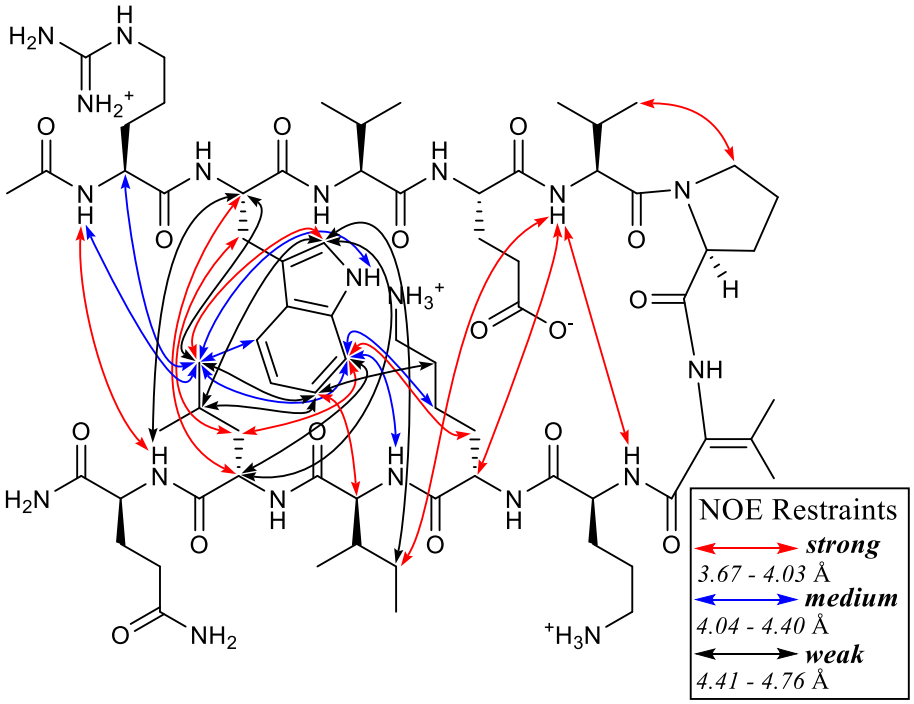


Table S8. Ac-R-W-V-E-V-Δ**Env-G**-O-K-I-L-Q-NH_2 (**7cb**)

Res I	Res II	Peak
2Trp H2	**11**Leu δ	S
2Trp α, H4,5	**11**Leu δ	W
2Trp H7	**11**Leu δ	M
2Trp H2,5	**11**Leu γ	M
2Trp H4,7	**11**Leu γ	W
2Trp α	**11**Leu α	S
2Trp H2	**10**Leu α	W
2Trp H4	**11**Leu α	M
2Trp H5	**10**Ile α	M
2Trp H2	**10**Ile α	W
2Trp H2	**9**Lys β	M
9Lys β, γ, δ	**2**Trp H4	S
9Lys γ, ε	**2**Trp H2	W
2Trp H5	**9**Lys β	M
2Trp H4	**12**Gln γ	S
8Orn β	**5**Val H	S
6ΔEnv δ	**5**Val α	W
1Arg δ	**12**Gln ε	S
1Arg α	**11**Leu δ	S

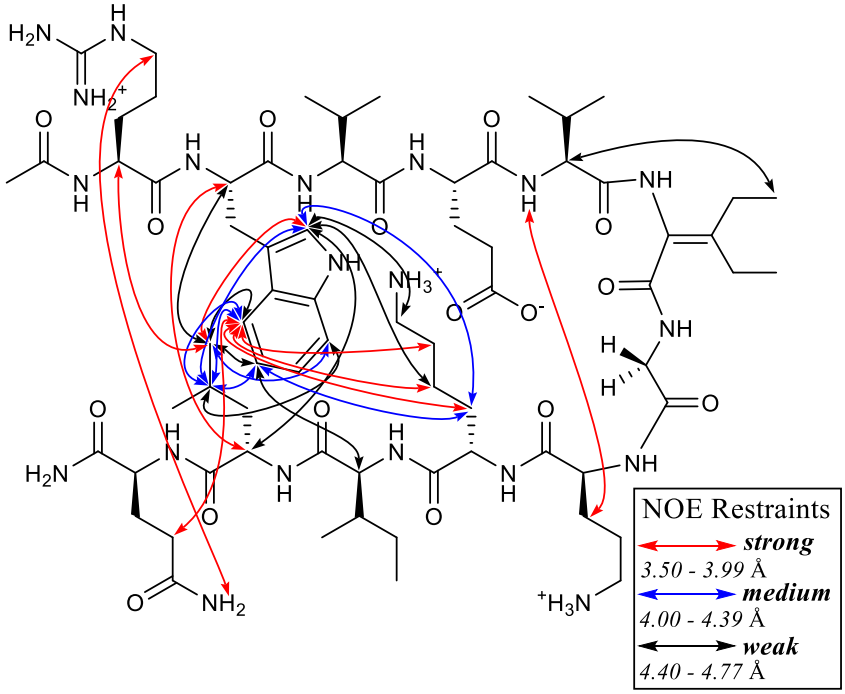
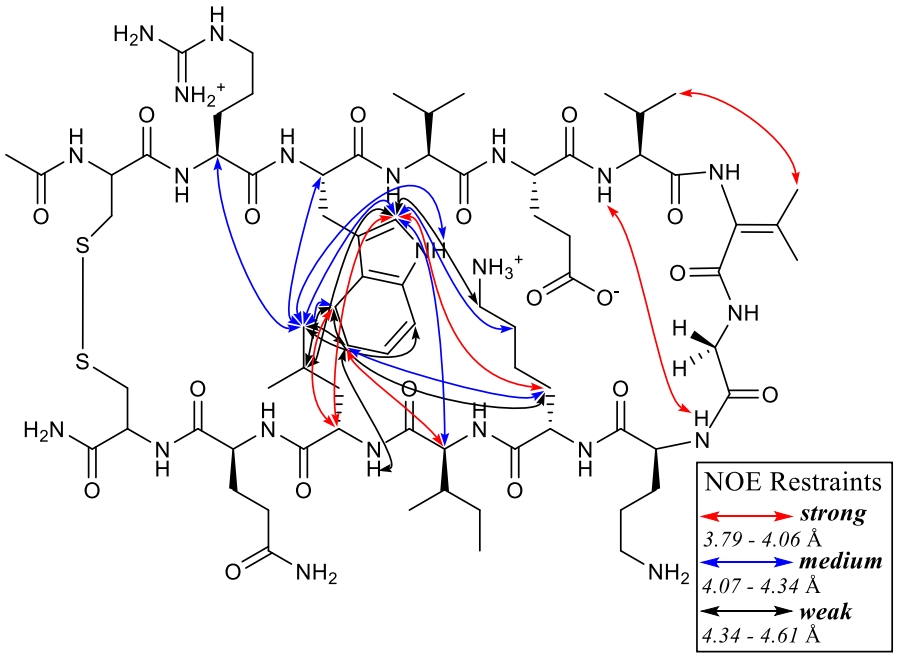


Table S9. c[Ac-Cys-R-W-V-E-V-Δ**Val-G**-O-K-I-L-Q-Cys-NH_2] (**S4**)

Res I	Res II	Peak
2Trp α	**11**Leu δ	M
2Trp H1,2,4	**11**Leu δ	M
2Trp H5,7	**11**Leu δ	W
2Trp H4,2	**11**Leu γ	W
2Trp H4	**11**Leu α	W
2Trp H2	**11**Leu α	S
2Trp α	**11**Leu α	S
2Trp H5	**11**Leu H	W
2Trp H2	**11**Leu H	M
1Arg α	**11**Leu δ	M
2Trp H2	**9**Lys β	S
2Trp H4	**9**Lys β	W
2Trp H5	**9**Lys β	M
2Trp H2	**9**Lys ε	W
2Trp H2	**9**Lys δ	M
2Trp H2	**10**Ile α	M
2Trp H5	**10**Ile α	S
5Val H	**8**Orn H	S
6ΔVal γ	**6Val** γ	S



Proteolysis assays for 1 and its analogues

Pronase E from *Streptomyces griseus* (EC 3.4.24.31) was purchased from EMD Millipore. This mixture of enzymes was dissolved in 1X PBS buffer (10 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl buffer, pH 7.4) at a concentration of 0.128 mg/mL. Then, solutions of each peptide (**1, 7aa, 7ab, 7ca, and 7cb**) in 1X PBS buffer (0.10 mM, 1.5 mL) at 37 °C were treated with an aliquot (5 µL) of the pronase E solution. Aliquots (50 µL) were removed after 0, 30, 60, 90, 150, 210, 270, 360 180, 300, and 360 min. The aliquots were quenched with 25% v/v glacial acetic acid (10 µL), diluted to 75 µL with 1X PBS buffer, and analyzed by HPLC (Phenomenex Jupiter C18, 5 µm particle size, 300 Å pore size, 4.6 × 250 mm, 40 µL injection volume, 10%–60% CH_3CN in H_2O gradient over 50 min, then 95% CH_3CN in H_2O for 10 min, flow rate: 1 mL/min).

Proteolysis assays for 2 and its analogues

Pronase E from *Streptomyces griseus* (EC 3.4.24.31) was purchased from EMD Millipore. This mixture of enzymes was dissolved in 1X PBS buffer (10 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl buffer, pH 7.4) at a concentration of 0.162 mg/mL. Then, solutions of each peptide (**2, 7ba, and 7bb**) in 1X PBS buffer (0.40 mM, 1.5 mL) at 37 °C were treated with an aliquot (10 µL) of the pronase E solution. Aliquots (50 µL) were removed after 0, 30, 60, 90, 150, 210, 270, 360 180, 300, and 360 min. The aliquots were quenched with glacial acetic acid (10 µL), diluted to 75 µL with 1X PBS buffer, and analyzed by HPLC (Phenomenex Jupiter C18, 5 µm particle size, 300 Å pore size, 4.6 × 250 mm, 40 µL injection volume, 10%–60% CH_3CN in H_2O gradient over 50 min, then 95% CH_3CN in H_2O for 10 min, flow rate: 1 mL/min).

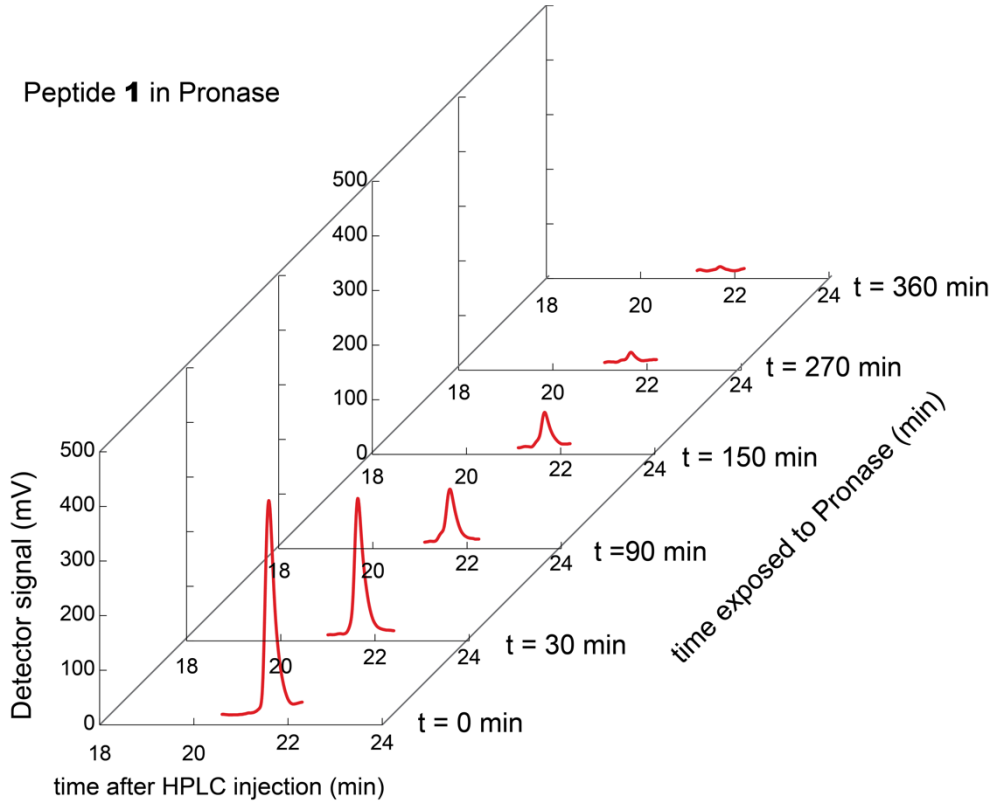


Figure S9. Analytical HPLC traces (monitored at 220 nm) for peptide **1** (0.10 mM) after incubation in Pronase E for up to 360 min.

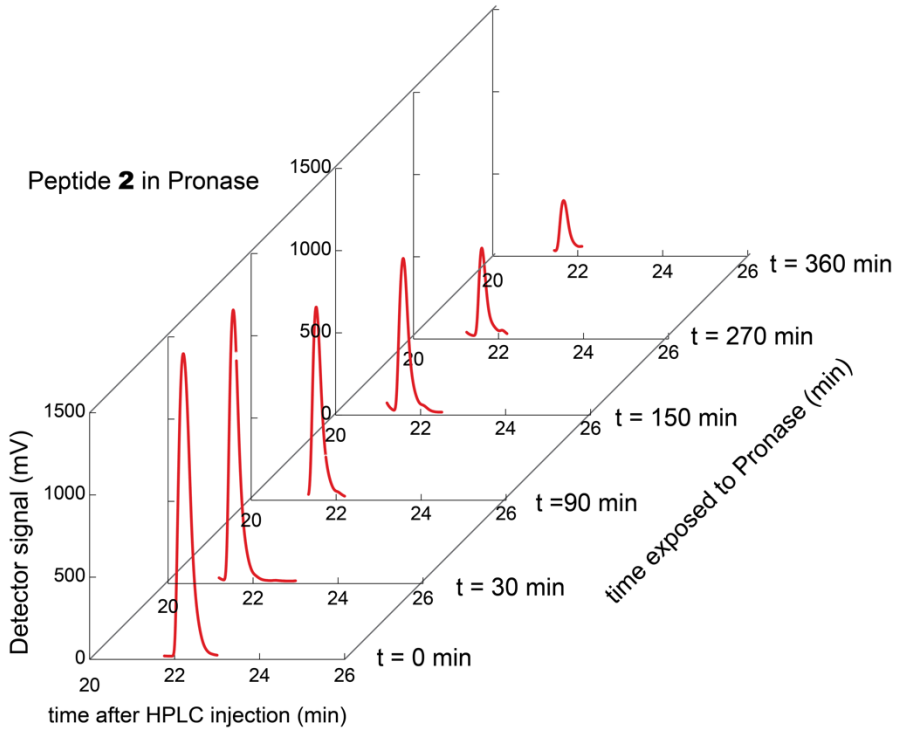


Figure S10. Analytical HPLC traces (monitored at 220 nm) for control peptide **2** (0.40 mM) after incubation in Pronase E for up to 360 min.

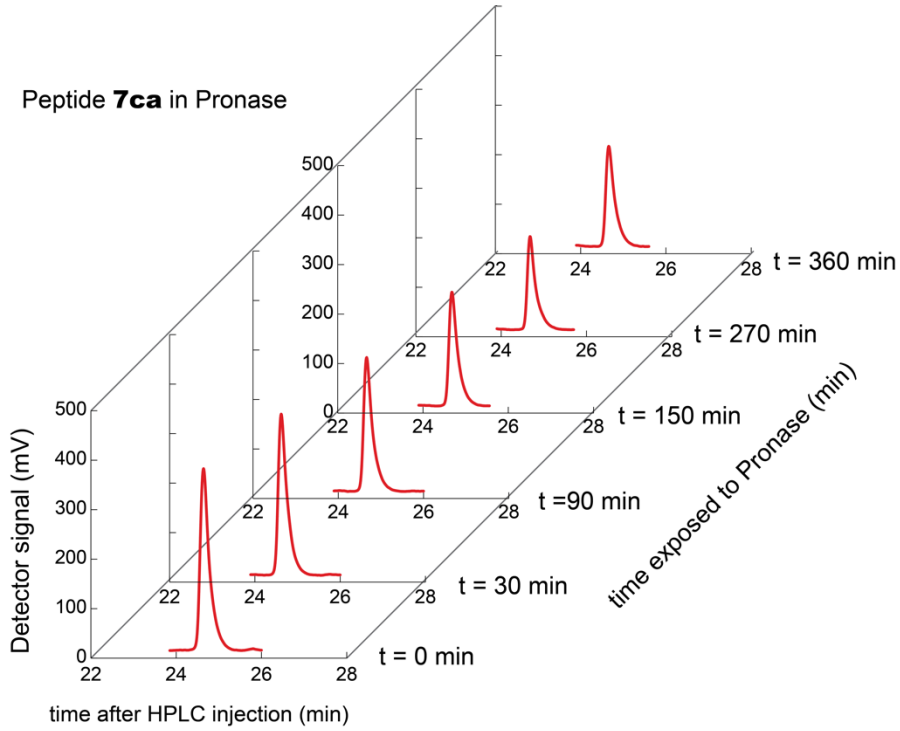


Figure S11. Analytical HPLC traces (monitored at 220 nm) for peptide **7ca** (0.10 mM) after incubation in Pronase E for up to 360 min.

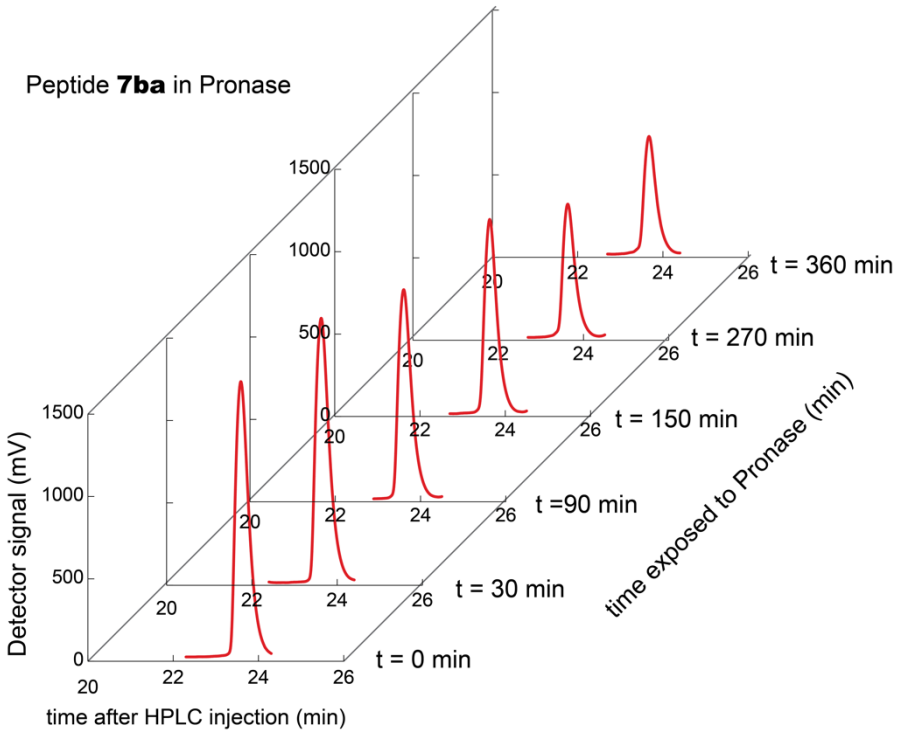


Figure S12. Analytical HPLC traces (monitored at 220 nm) for peptide **7ba** (0.40 mM) after incubation in Pronase E for up to 360 min.

Determination of Percent Folded Values for 1 and 7ca

The percentage of each peptide residing in the folded state was determined using two methods. First, the Hα chemical shifts of residues that form cross-strand hydrogen bonds were compared to the corresponding Hα chemical shifts in random coil 7-mers representing the unfolded structures (δ_0) and to Hα chemical shifts in cyclic controls representing the fully folded structures (δ_{100}). The Hα chemical shifts of residues in peptides **1** and **7ca** were designated as δ_{obs}. The percent folded at each residue was determined by Equation (i):

$$\textbf{Percent Folded} = [(\delta_{obs} - \delta_o)/\delta_{100} - \delta_o)] \text{ X } 100\ \% \qquad \text{(i)}$$

The overall percent folded was calculated by averaging the percent folded values for residues Val3, Val5, Orn8, and Ile10, each of which are involved in cross-strand hydrogen bonds.

Amino Acids	7ca	S4	Random Coil	% Folded	Average % Folded	Standard Error
Val 3	4.534	4.608	4.009	87.64	89.99	2.0
Val 5	4.385	4.397	4.151	95.12		
Orn 8	4.666	4.697	4.354	90.96	**K**	**Std. error**
Ile 10	4.635	4.715	4.134	86.23	9.0	2.0
					ΔG (kcal/mol)	**Std. error (kcal/mol)**
					–1.30	0.13

Amino Acids	1	S1	Random Coil	% Folded	Average % Folded	Standard Error
Val 3	4.447	4.586	4.003	76.16	77.30	2.0
Val 5	4.222	4.249	4.090	83.02		
Orn 8	4.620	4.696	4.364	77.11	**K**	**Std error**
Ile 10	4.564	4.717	4.152	72.92	3.4	0.4
					ΔG (kcal/mol)	**Std. error (kcal/mol)**
					–0.73	0.07

The overall percent folded was also calculated by measuring the differences in chemical shift of the two diastereotopic Hα signals from the glycine residues in **1** and **7ca**. This was done using equation (ii):

$$\textbf{Percent Folded} = (\Delta\delta_{\textbf{Observed}}/\Delta\delta_{100})\ \textbf{X 100 \%} \qquad \text{(ii)}$$

where $\Delta\delta_{\text{Observed}}$ is the difference between the two diastereotopic glycine Hα chemical shifts observed for **1** or **7ca** and $\Delta\delta_{100}$ is the difference between the two diastereotopic glycine Hα chemical shifts of the corresponding cyclic peptide **S1** or **S4**.

Peptide	Gly $H\alpha_a$	Gly $H\alpha_b$	Glycine Splitting	% Folded
Cyclic peptide **(S4)**	4.1322	3.7217	0.4105 ($\Delta\delta_{100}$)	93.7
7ca	4.1141	3.7296	0.3845 ($\Delta\delta_{\text{Observed}}$)	**K**
				14.79
			ΔG (kcal/mol)	**–1.60**

Peptide	Gly $H\alpha_a$	Gly $H\alpha_b$	Glycine Splitting	% Folded
Cyclic peptide **(S1)**	4.1354	3.6865	0.4489 ($\Delta\delta_{100}$)	72.8
1	4.0941	3.7674	0.3267 ($\Delta\delta_{\text{Observed}}$)	**K**
				2.67
			ΔG (kcal/mol)	**–0.58**

Circular Dichroism Experiments

Peptide solutions were prepared in 20 mM sodium phosphate buffer (pH 7.4), and concentrations were determined spectroscopically based on tryptophan absorbance at 280 nm in 6 M guanidine hydrochloride (Trp $\varepsilon_{280} = 5690\ M^{-1}cm^{-1}$). 0.10 mM solutions of **1** and **7ca** in 10 mM sodium phosphate buffer (pH 7.4) and 6 M guanidine hydrochloride were used to perform wavelength scans in duplicate at 25 °C. 0.10 mM solutions of **2** and **7ba** in 10 mM sodium phosphate buffer (pH 7.4) and 2.5 M urea were used to run wavelength scans in duplicate at 25 °C.

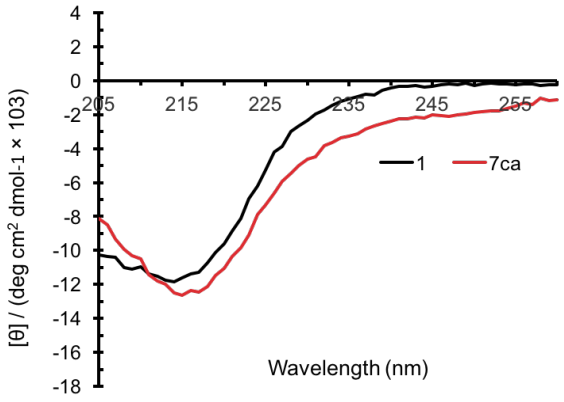


Figure S13. CD wavelength scan for peptides **1** and **7ca** at 25 °C.

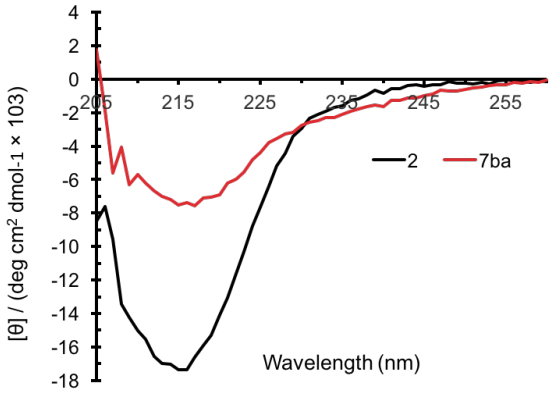


Figure S14. CD wavelength scan for peptides **2** and **7ba** at 25 °C.

NMR Structural Calculations

NOE Distance-restrained Calculations Summary. Manual NMR structural calculations were performed to generate conformational ensembles of all the peptides that were studied. The ensembles were generated using the simulated annealing algorithm CYANA in conjunction with ROESY chemical shift and peak data for the eight peptides (cross-peak data used in the calculations is tabulated and summarized visually in tables **S1-S9**). Dihedral restraints were not used due to the presence of multiple non-standard amino acids (e.g., ornithine, D-proline, ΔVal and ΔEnv) which lack sufficient secondary chemical shift analysis data.

The final ensemble for each peptide consists of the 10 lowest-energy conformations as determined by the CYANA objective-function from 100 preliminary structures. The ensemble shows the expected hydrogen bonding pattern, dihedral angles, and overall secondary structure consistent with the β-hairpin motif.

Table S10. Restraints and Calculation Parameters

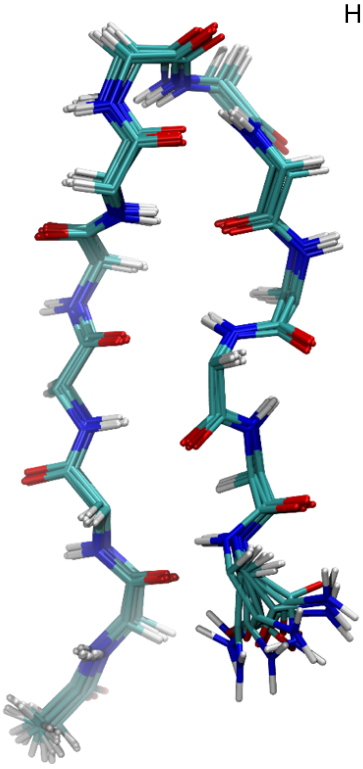
Structure	**1**	**2**	**7aa**	**7ab**	**7ba**	**7bb**	**7ca**	**7cb**
Conformational restraints								
Total Distance	32	28	29	24	29	31	28	31
Restraints								
Cross-strand[b]	31	26	20	22	27	29	28	30
Sequential	1	2	0	2	2	2	0	1
Intra-residue	0	0	0	0	0	0	0	0
Strong Intensity[a]	14	8	9	8	10	12	6	11
Medium Intensity[a]	11	14	9	9	10	9	14	9
Weak Intensity[a]	7	6	11	7	9	10	8	11
Hydrogen Bonds	4	4	4	4	4	4	4	4
Dihedral Restraints[c]	0	0	0	0	0	0	0	0
RMSD from Ideality:								
Backbone (Å)	0.18 ± 0.01	0.40 ± 0.14	0.26 ± 0.12	0.60 ± 0.11	0.26 ± 0.18	0.31 ± 0.11	0.13 ± 0.04	0.34 ± 0.08
Heavy Atom (Å)	0.57 ± 0.08	0.74 ± 0.11	0.72 ± 0.17	1.32 ± 0.34	0.86 ± 0.25	0.77 ± 0.26	0.54 ± 0.08	0.67 ± 0.09

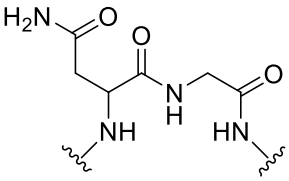
[a] For distance ranges in angstroms for the *strong, medium*, and *weak* restraints see **Tables S1–S9.**
[a] **Tables S1-S9** contain schematic representations of important cross-peaks from the data
[b] The majority of NOEs used in the calculations were cross-strand peaks because of the high quality signals caused by the proximity of the strands
[c] No dihedral restraints were used since our sequences contain non-standard amino acids such as ΔAAs and ornithine which are incompatible the leading software such as DANGLE or TALOS.

NMR structural calculations from NOE peaks

Summary. Out of 100 initial structures, the 10 best conformations per the CYANA objective function, were superimposed based on backbone geometry. Sidechains were omitted for clarity. The structures show the expected beta-hairpin hydrogen bonding and dihedral angles. **Note:** structural ensembles of **7ab** and **7cb** were generated using NOE data from the major conformation of each peptide, as data from the minor conformations were difficult to interpret.

A) Ac-R-W-V-E-V-**N-G**-O-K-I-L-Q-NH_2 (**1**)

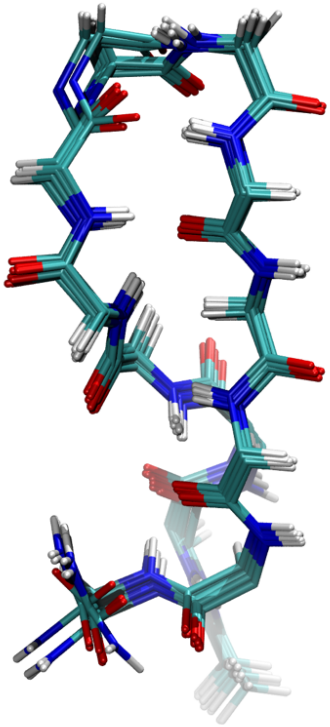


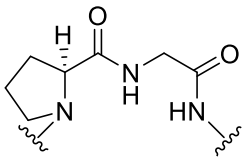


Position	Res.
i	Val
i + 1	Asn
i + 2	Gly
i + 3	Orn

Backbone RMSD (Å):
0.180 ± 0.008

B) Ac-R-W-V-E-V-**p-G**-O-K-I-L-Q-NH_2 (**2**)

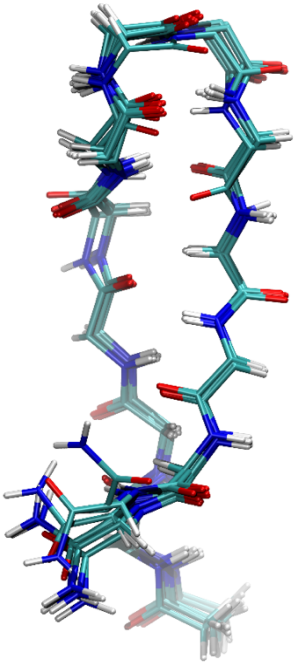


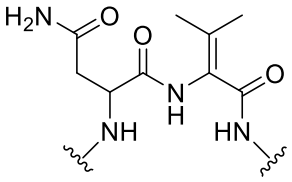


Position	Res.
i	Val
i + 1	D-Pro
i + 2	Gly
i + 3	Orn

Backbone RMSD (Å):
0.396 ± 0.143

C) Ac-R-W-V-E-V-**N-ΔVal**-O-K-I-L-Q-NH_2 (**7aa**)

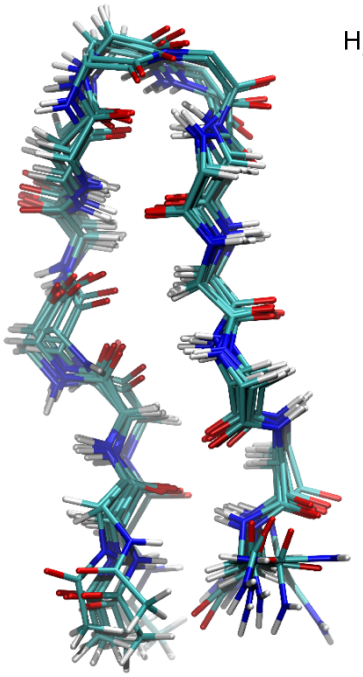


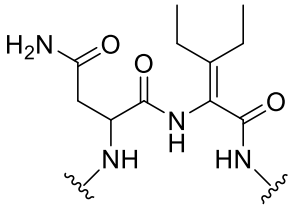


Position	Res.
i	Val
i + *1*	Asn
i + *2*	ΔVal
i + *3*	Orn

Backbone RMSD (Å)
0.256 ± 0.120

D) Ac-R-W-V-E-V-**N-ΔEnv**-O-K-I-L-Q-NH_2 (**7ab**)

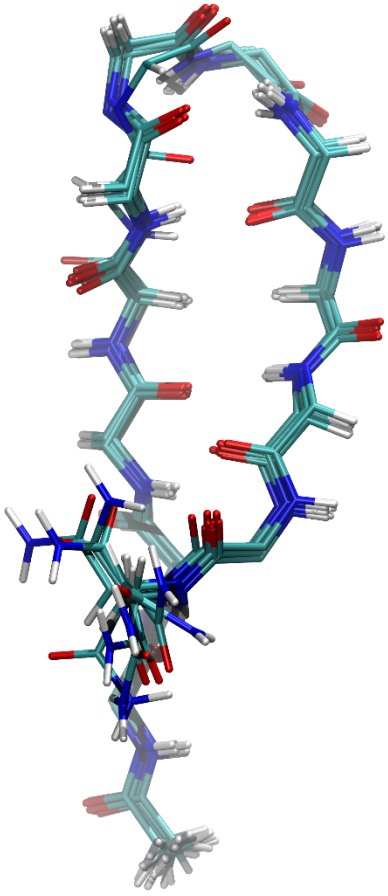


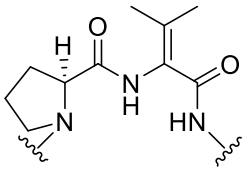


Position	Res.
i	Val
i + *1*	Asn
i + *2*	ΔEnv
i + *3*	Orn

Backbone RMSD (Å)
0.601 ± 0.113

E) Ac-R-W-V-E-V-**p-ΔVal**-O-K-I-L-Q-NH_2 (**7ba**)

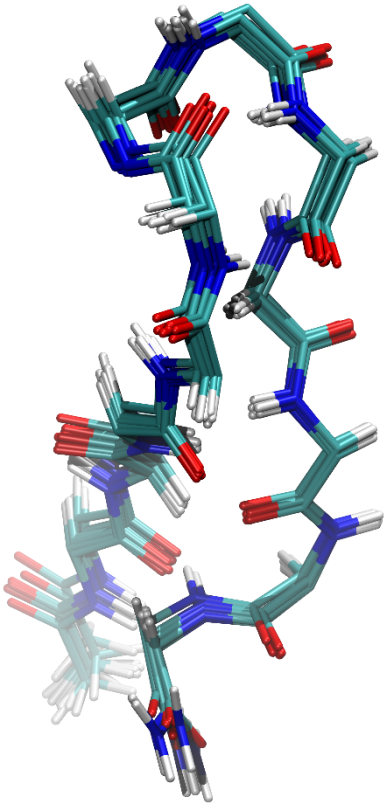


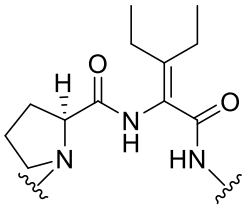


Position	AA
i	Val
i + *1*	D-Pro
i + *2*	ΔVal
i + *3*	Orn

Backbone RMSD (Å):
0.262 ± 0.184

F) Ac-R-W-V-E-**p-ΔEnv**-O-K-I-L-Q-NH_2 (**7bb**)

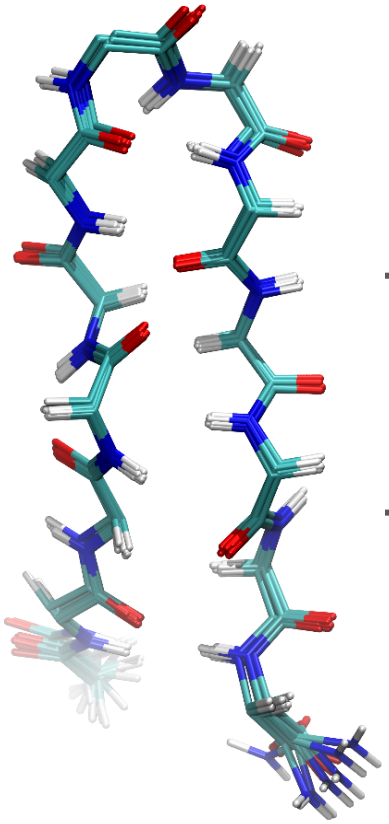


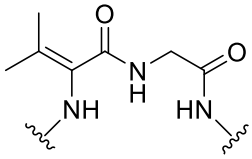


Position	AA
i	Val
i + *1*	D-Pro
i + *2*	ΔEnv
i + *3*	Orn

Backbone RMSD (Å):
0.312 ± 0.108

G) Ac-R-W-V-E- Δ**Val-G**-O-K-I-L-Q-NH_2 (**7ca**)

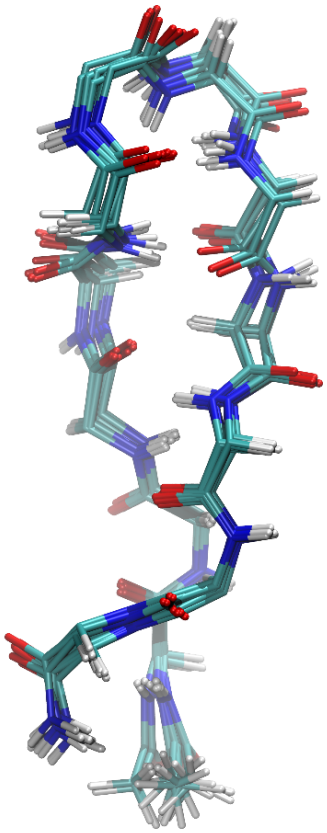


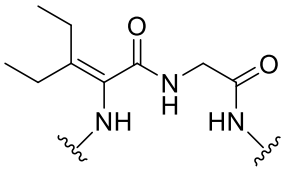


Position	AA
i	Val
i* + *1	ΔVal
i* + *2	Gly
i* + *3	Orn

Backbone RMSD (Å):
0.128 ± 0.037

H) Ac-R-W-V-E-Δ**Env-G**-O-K-I-L-Q-NH_2 (**7cb**)





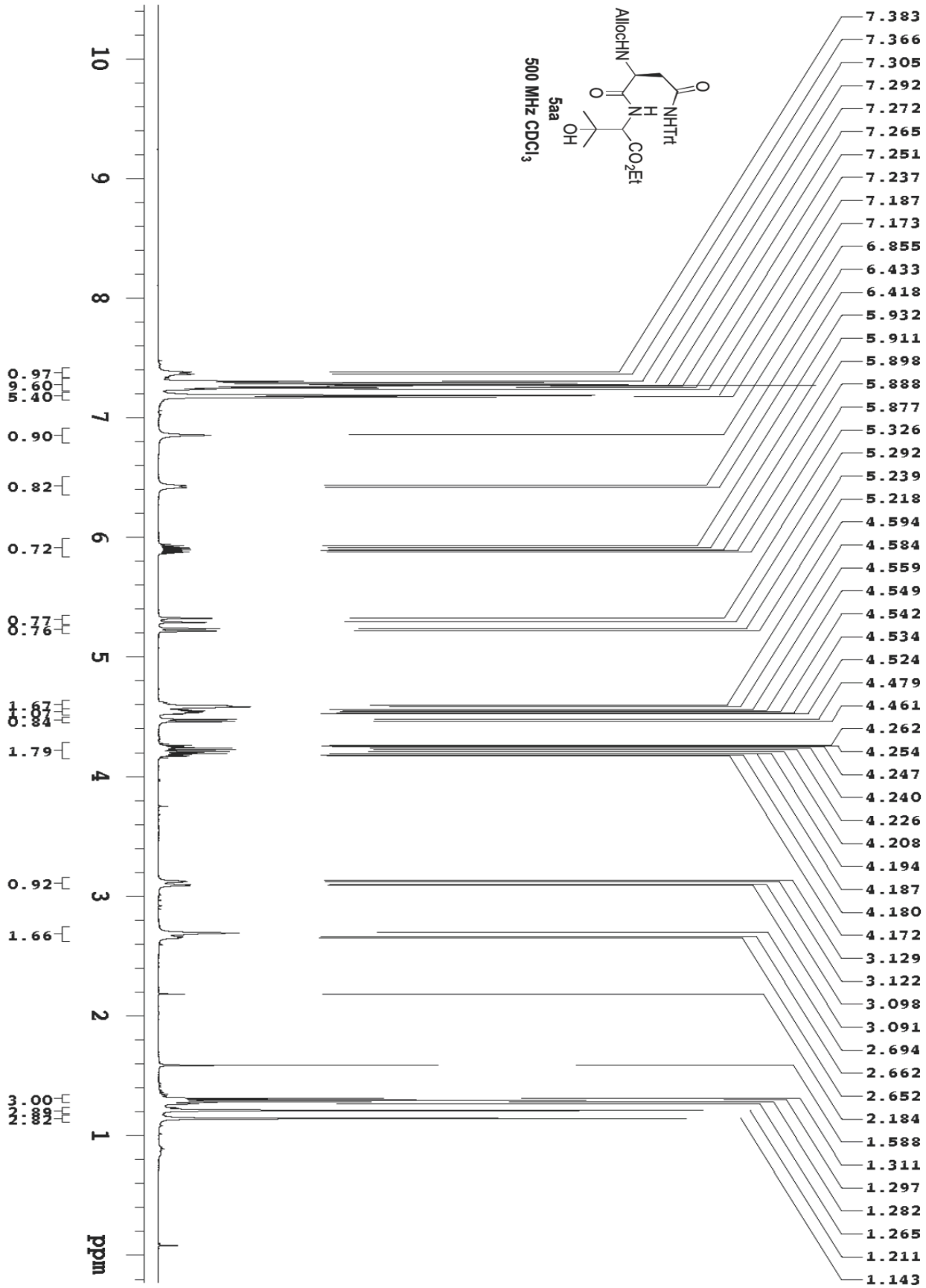
Position	AA
i	Val
i* + *1	ΔEnv
i* + *2	Gly
i* + *3	Orn

Backbone RMSD (Å):
0.335 ± 0.075

Structural Ensembles A–H. Structural ensembles A and B depict known parent β-hairpins **1** and **2** that were used as controls. Ensembles C–G show β-hairpins **7aa–7cb** that contain ΔVal or ΔEnv in either the *i* + *1* or the *i* + *2* position. RMSD values were calculated based on the backbone atoms and only include residues 3–10 to avoid impact of fraying of the ends of the strands where less NOEs were found. Calculations were performed in CYANA,[5] and graphic representations were generated using VMD.[6]

[5] Güntert, P.; Mumenthaler, C.; Wüthrich, K. *J. Mol. Biol.* **1997**, *273*, 283.

[6] Humphrey, W., Dalke, A. and Schulten, K., *J. Mol. Graphics* **1996**, *14*, 33. **http://www.ks.uiuc.edu/Research/vmd/**.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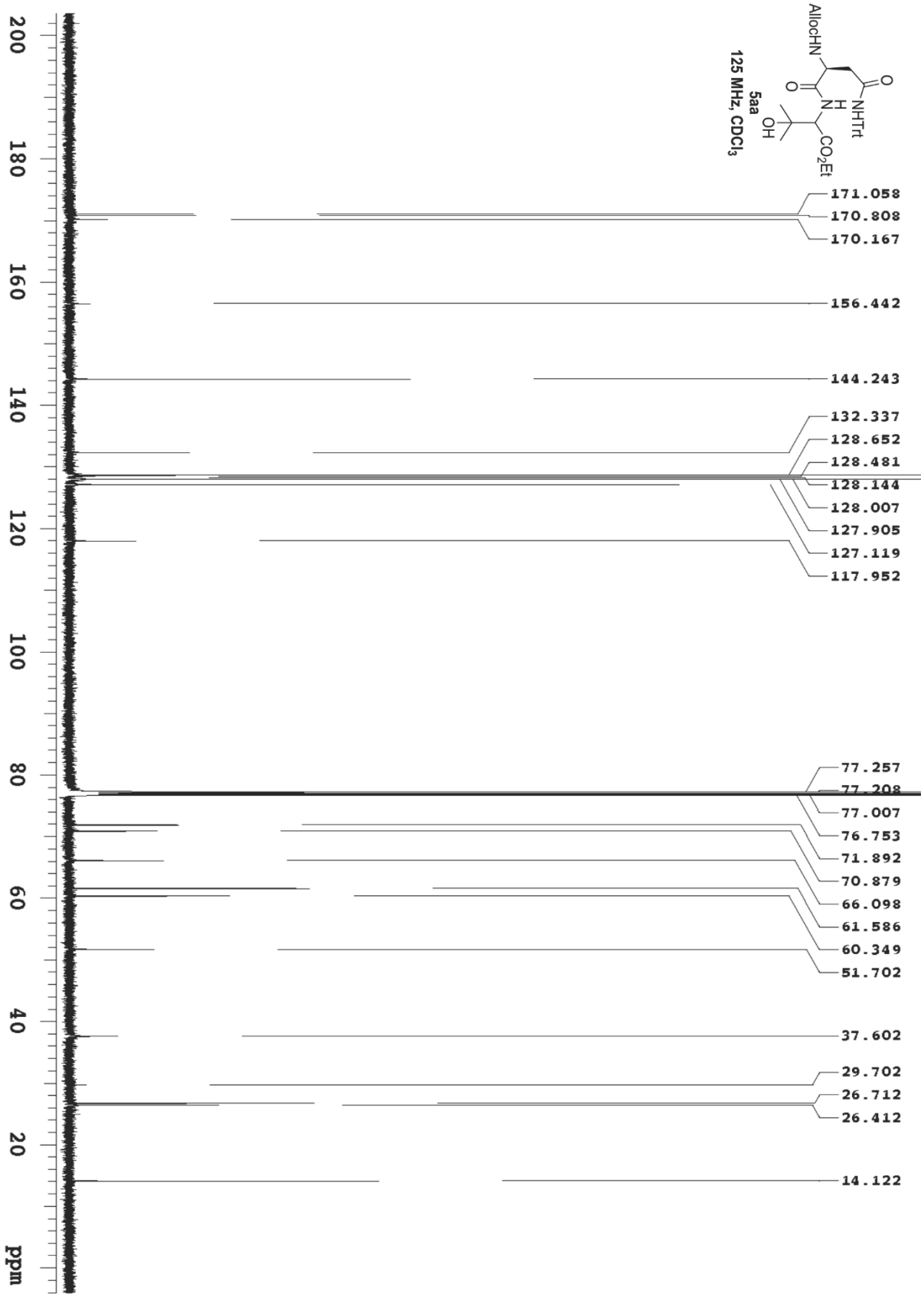
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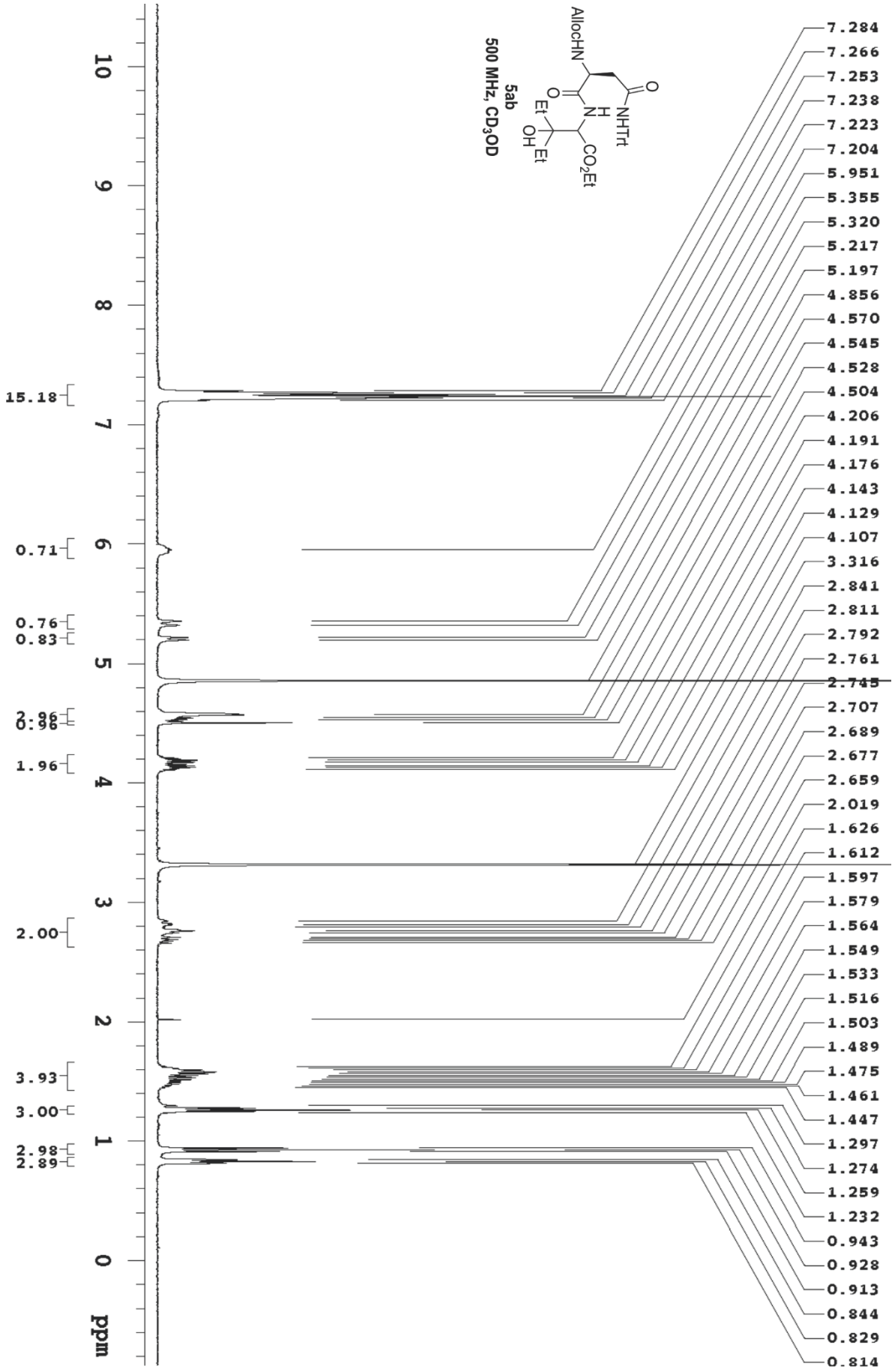
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1.259
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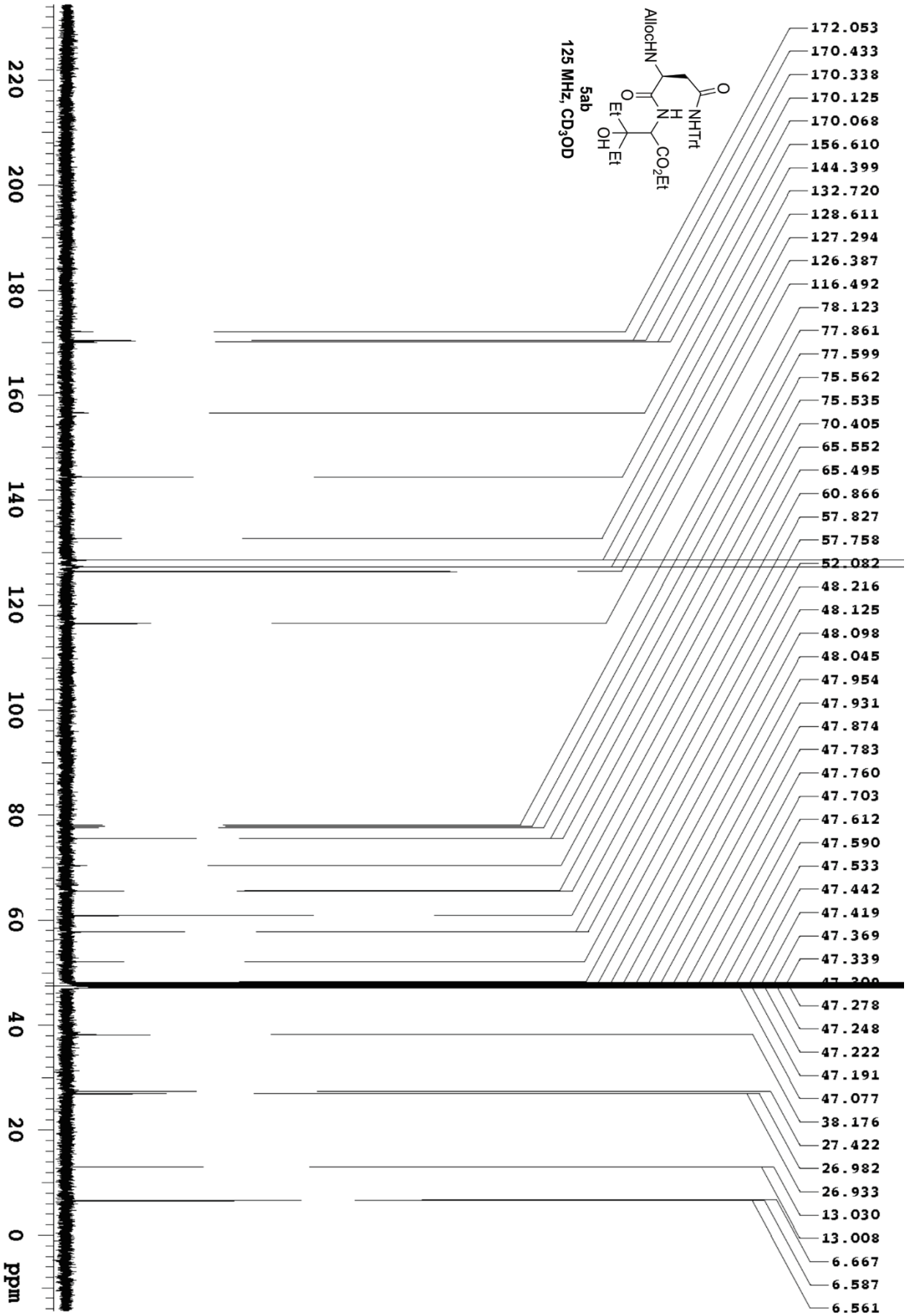
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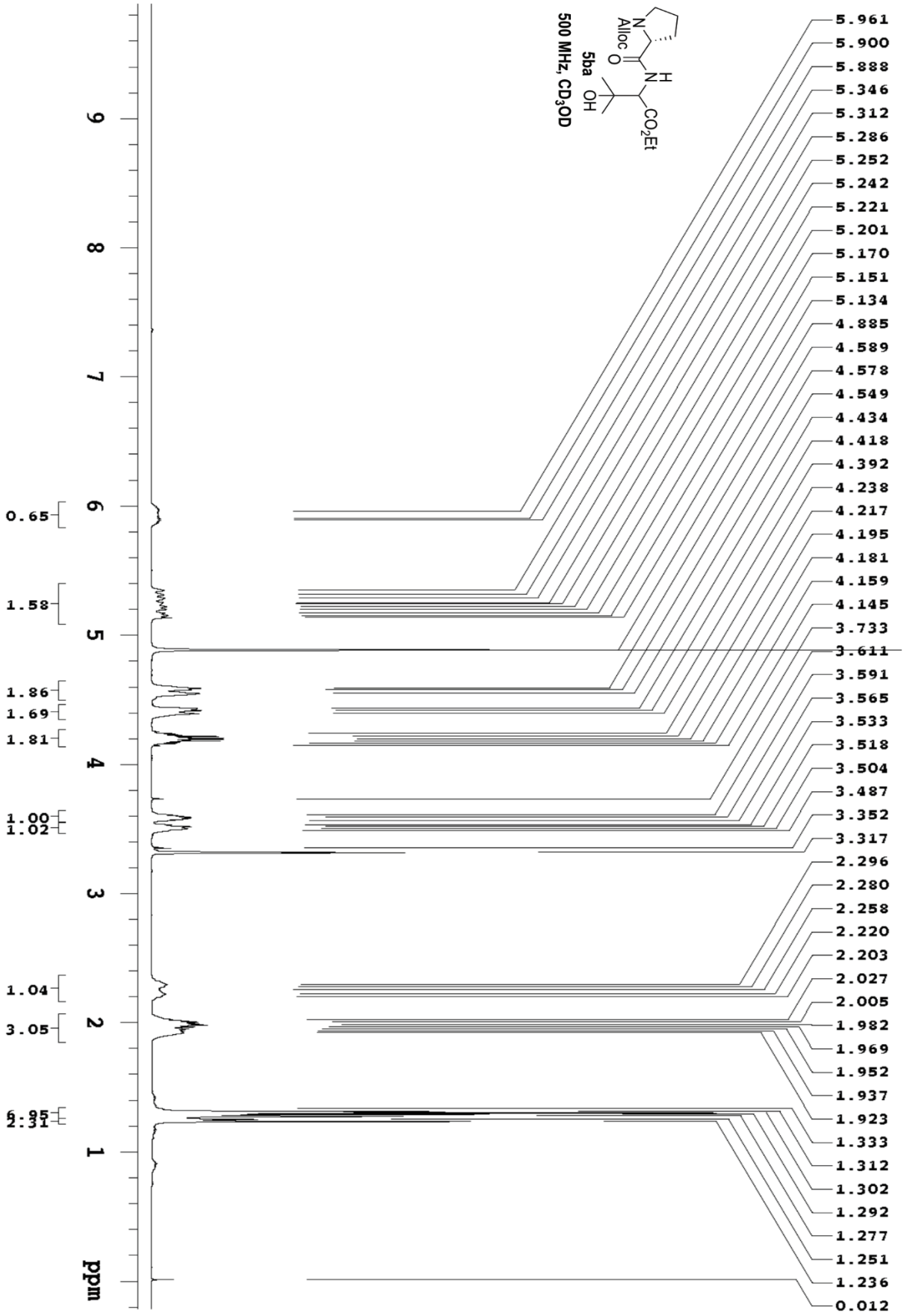
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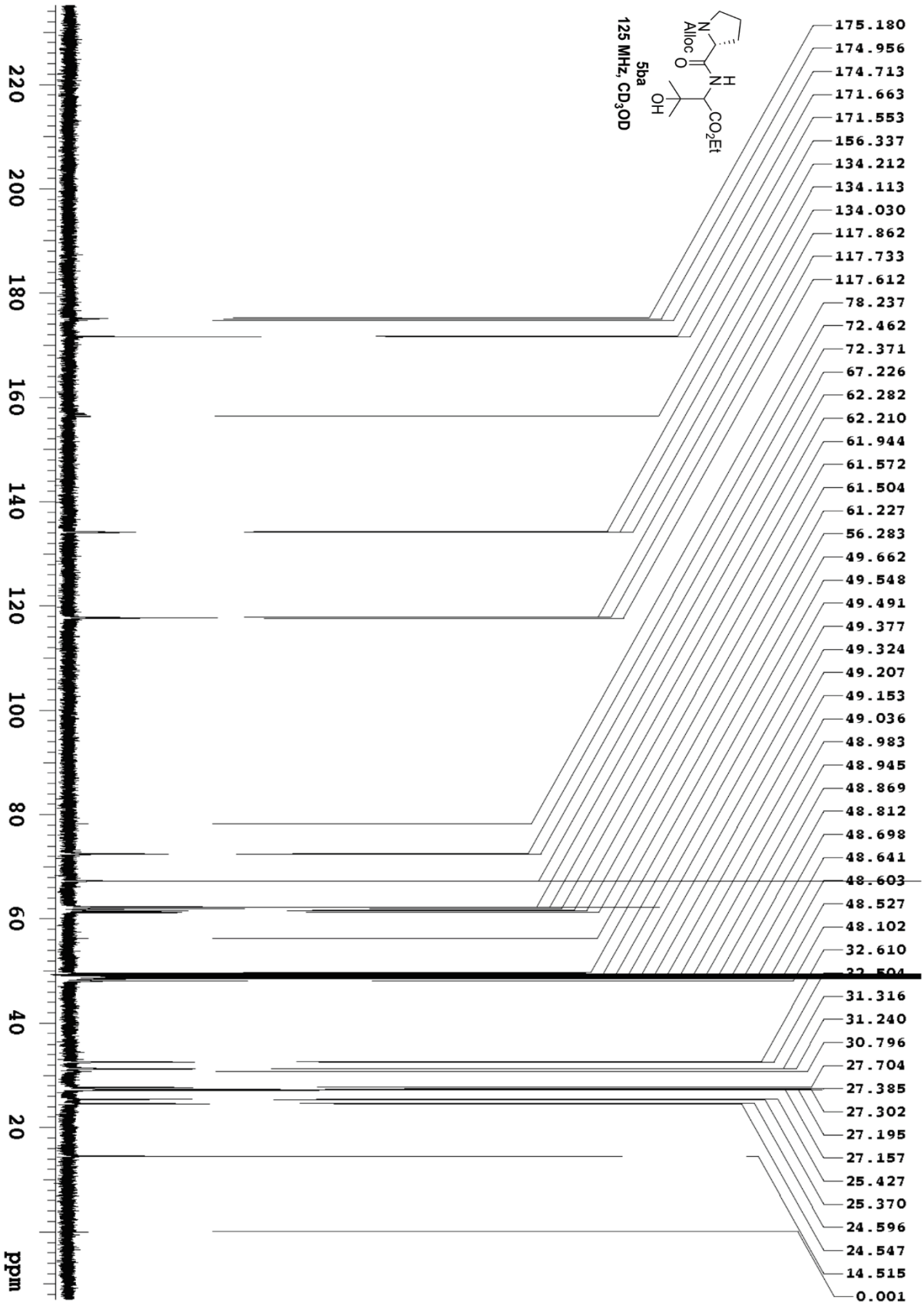
3.565
3.533
3.518
3.504
3.487
3.352
3.317
2.296
2.280
2.258
2.220
2.203
2.027
2.005
1.982
1.969
1.952
1.937
1.923
1.333
1.312
1.302
1.292
1.277
1.251
1.236
0.012
0.65
1.58
1.86
1.69
1.81
1.00
1.02
1.04
3.05
2.31
ppm


5ba
125 MHz, CD3OD
175.180
174.956
174.713
171.663
171.553
156.337
134.212
134.113
134.030
117.862
117.733
117.612
78.237
72.462
72.371
67.226
62.282
62.210
61.944
61.572
61.504
61.227
56.283
49.662
49.548
49.491
49.377
49.324
49.207
49.153
49.036
48.983
48.945
48.869
48.812
48.698
48.641
48.603
48.527
48.102
32.610
31.316
31.240
30.796
27.704
27.385
27.302
27.195
27.157
25.427
25.370
24.596
24.547
14.515
0.001
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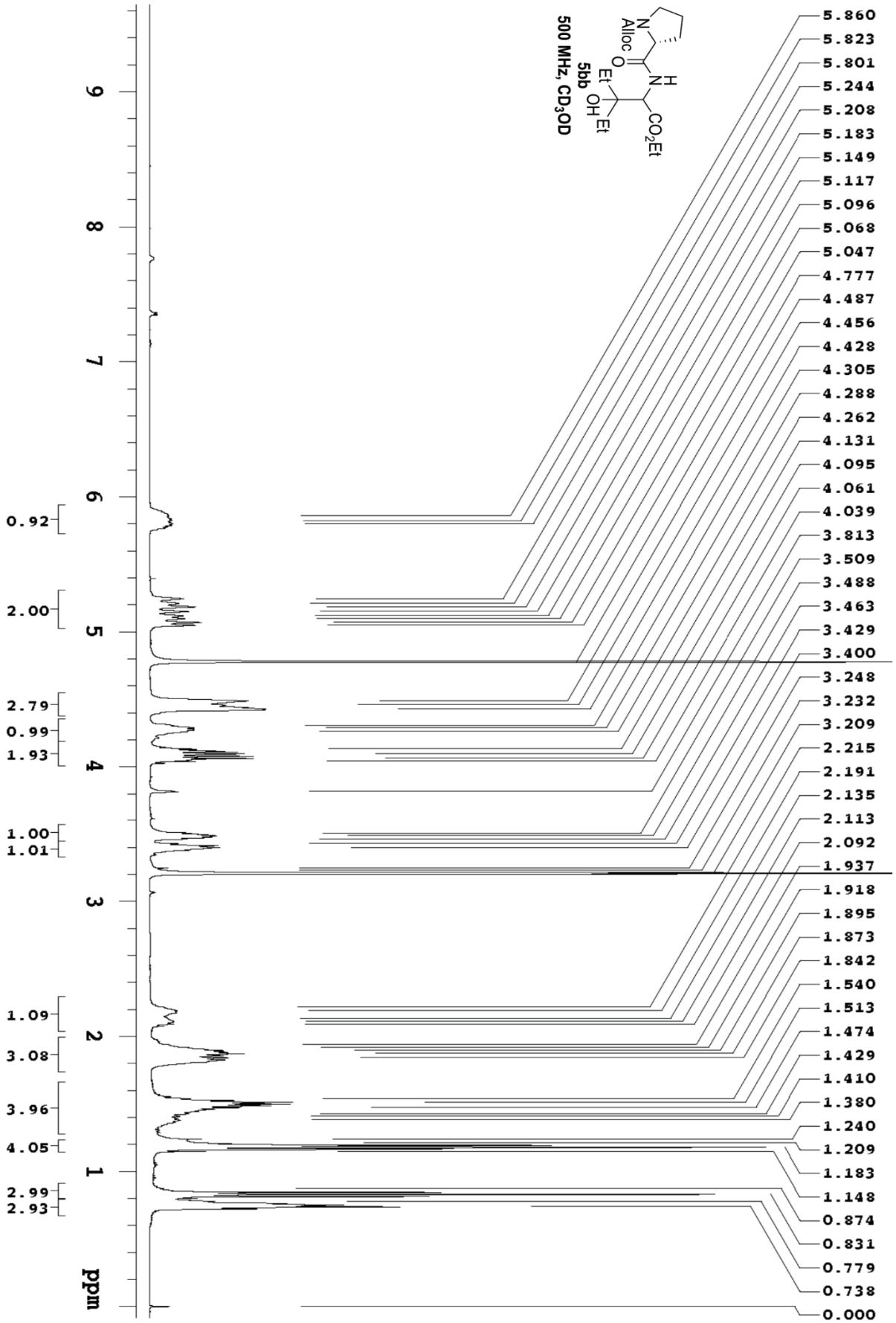
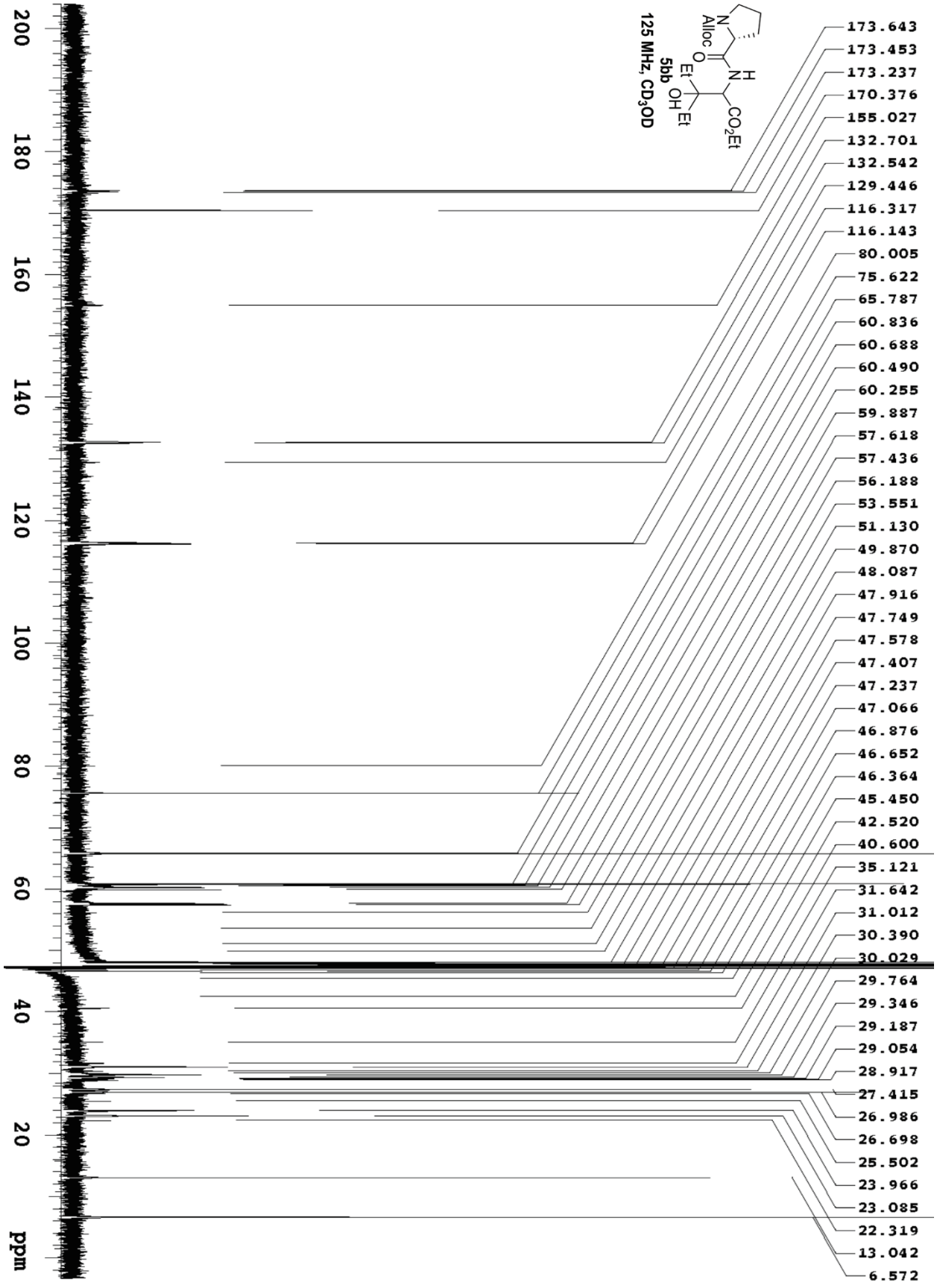

5bb
500 MHz, CD3OD
5.860
5.823
5.801
5.244
5.208
5.183
5.149
5.117
5.096
5.068
5.047
4.777
4.487
4.456
4.428
4.305
4.288
4.262
4.131
4.095
4.061
4.039
3.813
3.509
3.488
3.463
3.429
3.400
3.248
3.232
3.209
2.215
2.191
2.135
2.113
2.092
1.937
1.918
1.895
1.873
1.842
1.540
1.513
1.474
1.429
1.410
1.380
1.240
1.209
1.183
1.148
0.874
0.831
0.779
0.738
0.000
0.92
2.00
2.79
0.99
1.93
1.00
1.01
1.09
3.08
3.96
4.05
2.99
2.93
ppm


5bb
125 MHz, CD3OD
173.643
173.453
173.237
170.376
155.027
132.701
132.542
129.446
116.317
116.143
80.005
75.622
65.787
60.836
60.688
60.490
60.255
59.887
57.618
57.436
56.188
53.551
51.130
49.870
48.087
47.916
47.749
47.578
47.407
47.237
47.066
46.876
46.652
46.364
45.450
42.520
40.600
35.121
31.642
31.012
30.390
30.029
29.764
29.346
29.187
29.054
28.917
27.415
26.986
26.698
25.502
23.966
23.085
22.319
13.042
6.572
200
180
160
140
120
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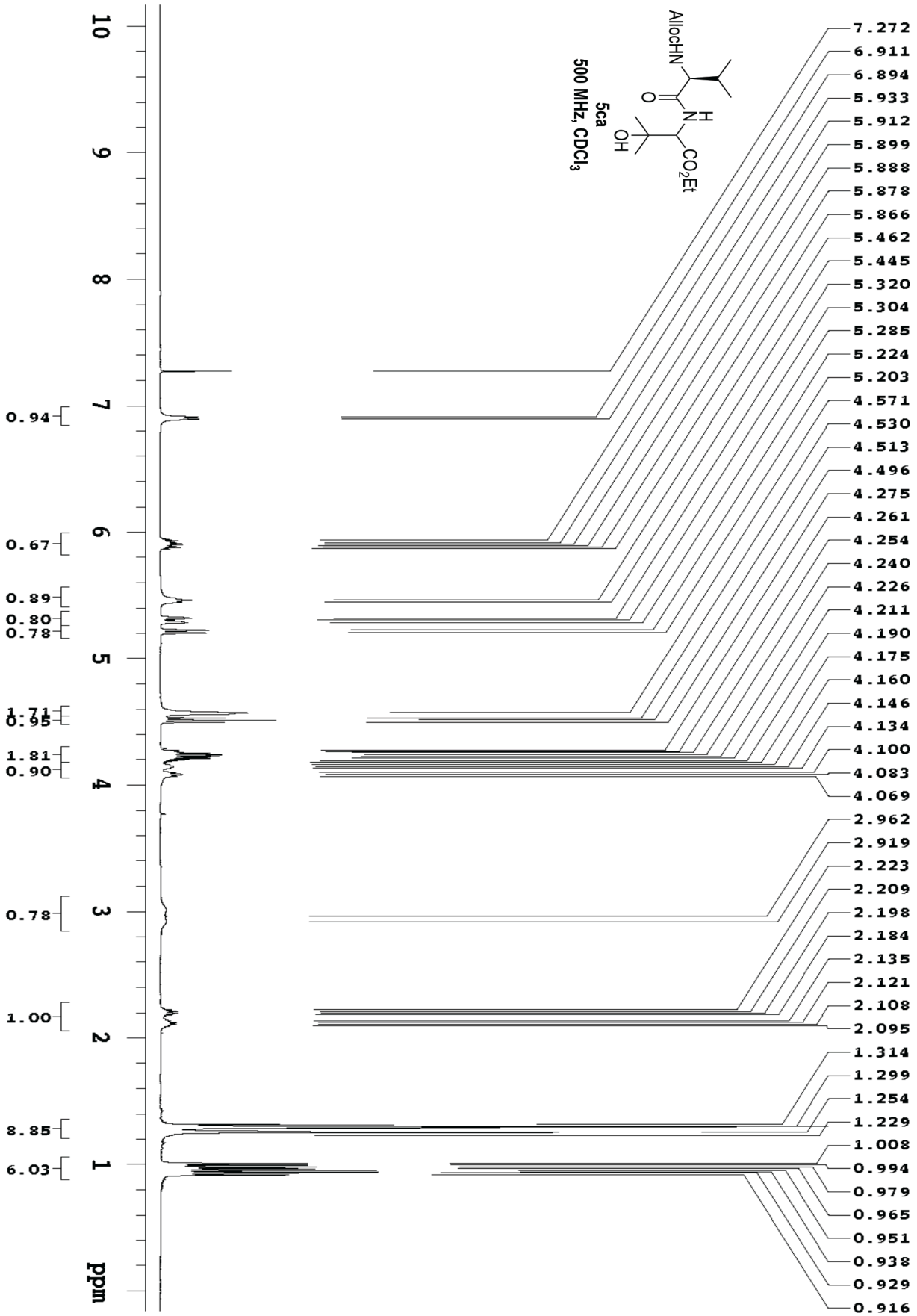
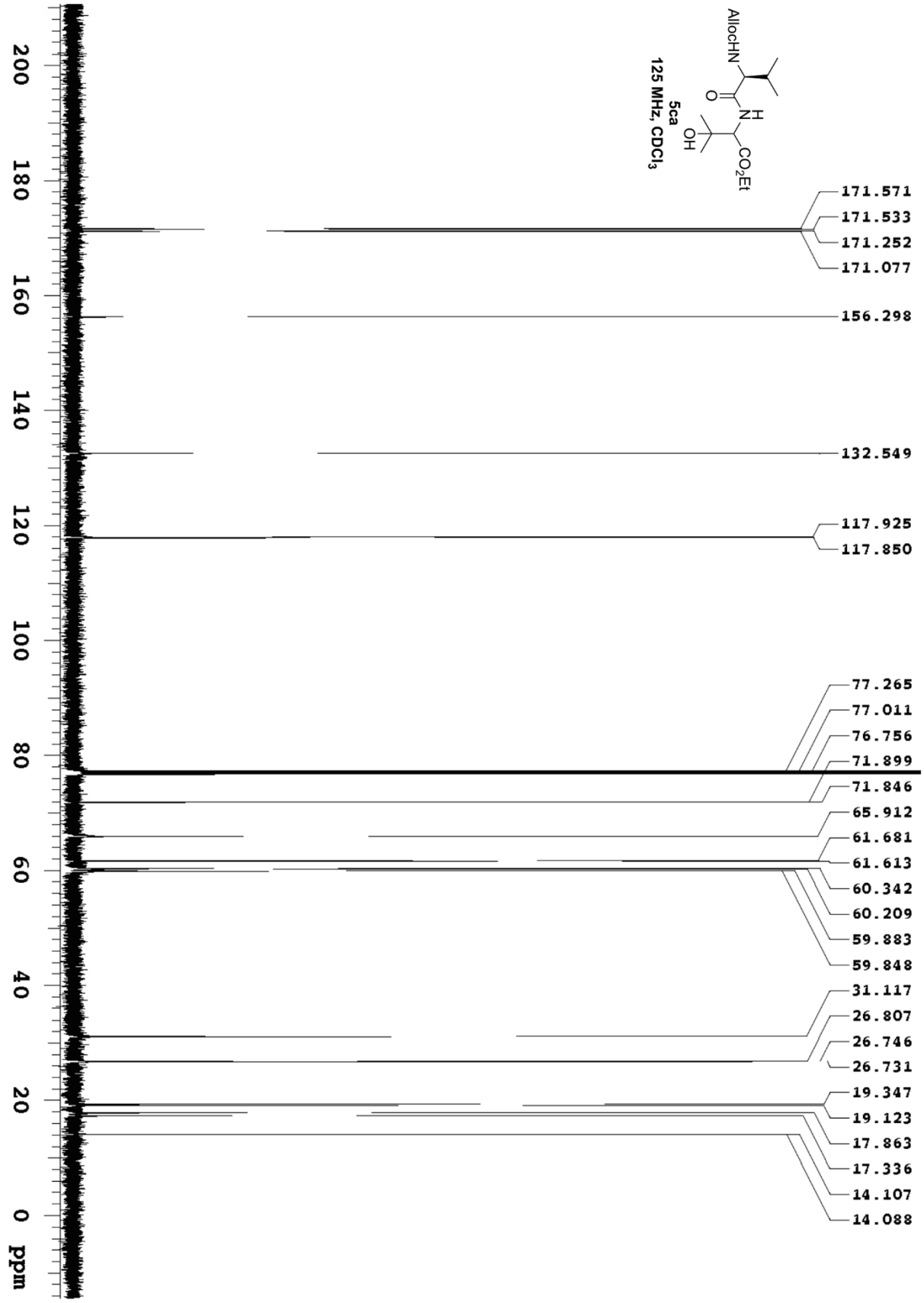
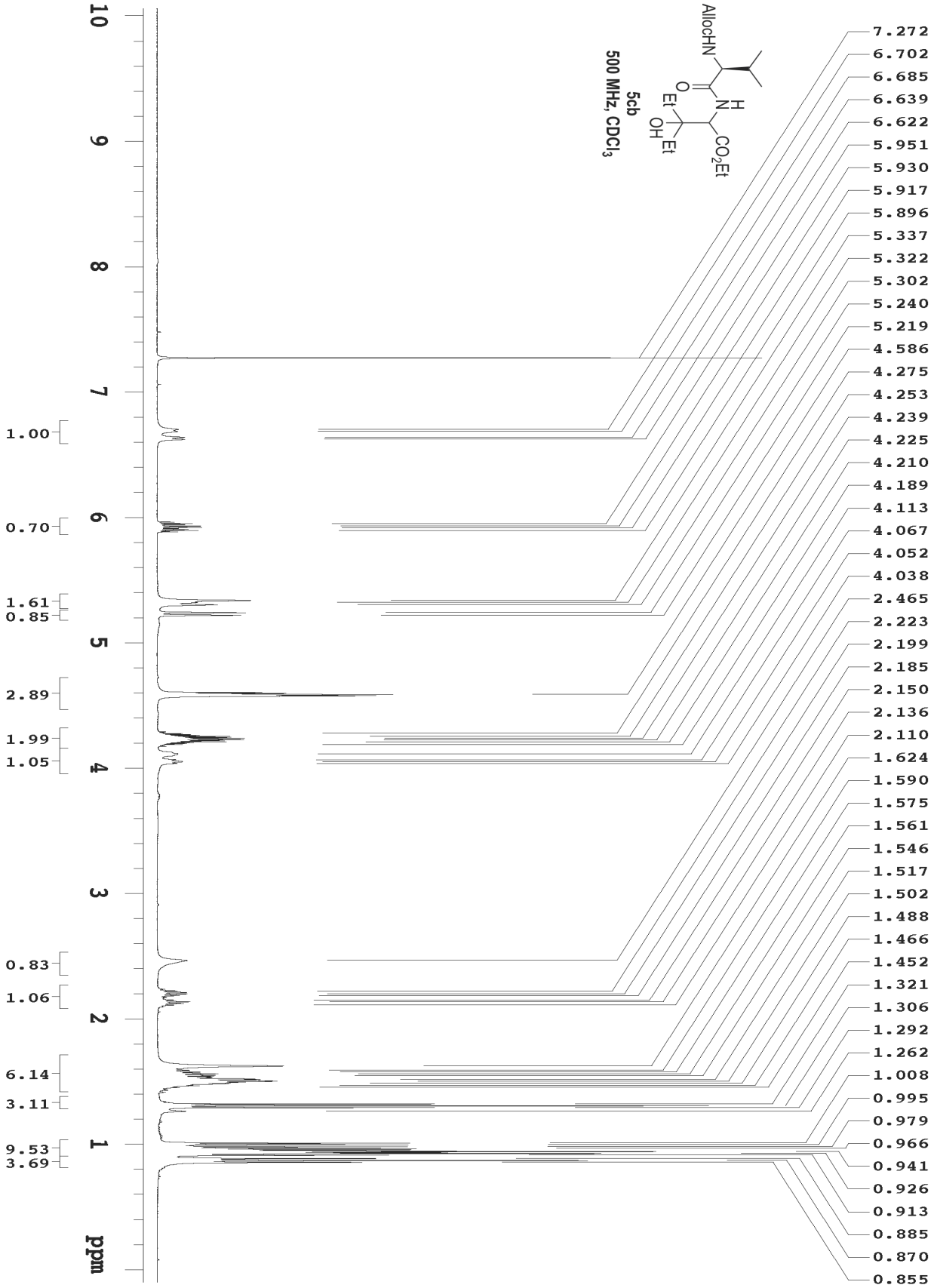
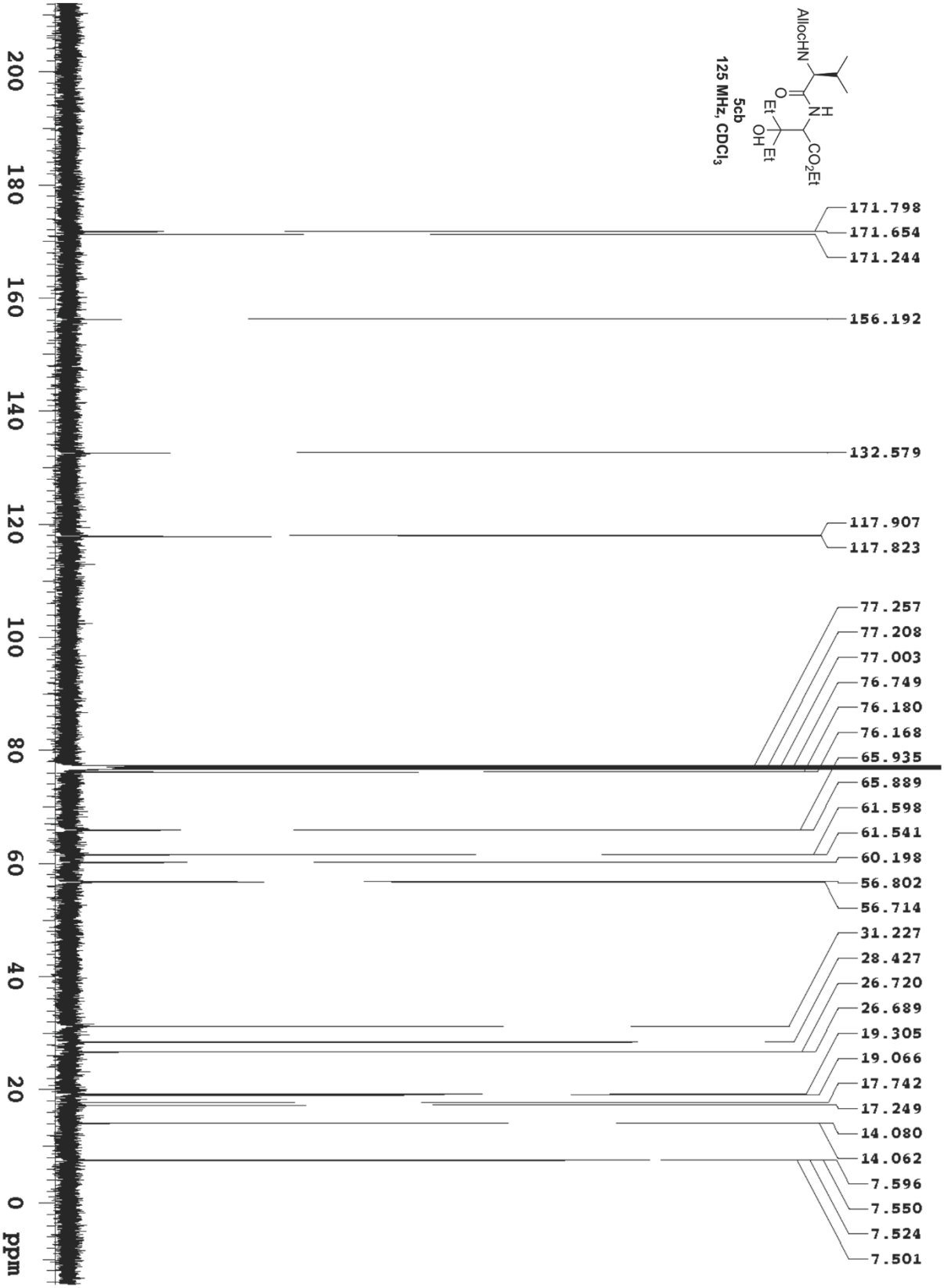
80
60
40
20
ppm


5ca
500 MHz, CDCl3
AllocHN
O
H
N
OH
CO2Et
7.272
6.911
6.894
5.933
5.912
5.899
5.888
5.878
5.866
5.462
5.445
5.320
5.304
5.285
5.224
5.203
4.571
4.530
4.513
4.496
4.275
4.261
4.254
4.240
4.226
4.211
4.190
4.175
4.160
4.146
4.134
4.100
4.083
4.069
2.962
2.919
2.223
2.209
2.198
2.184
2.135
2.121
2.108
2.095
1.314
1.299
1.254
1.229
1.008
0.994
0.979
0.965
0.951
0.938
0.929
0.916
0.94
0.67
0.89
0.80
0.78
1.71
0.95
1.81
0.90
0.78
1.00
8.85
6.03
10
9
8
7
6
5
4
3
2
1
ppm


AllocHN
O
N
H
OH
CO2Et
5ca
125 MHz, CDCl3
171.571
171.533
171.252
171.077
156.298
132.549
117.925
117.850
77.265
77.011
76.756
71.899
71.846
65.912
61.681
61.613
60.342
60.209
59.883
59.848
31.117
26.807
26.746
26.731
19.347
19.123
17.863
17.336
14.107
14.088
200
180
160
140
120
100
80
60
40
20
0
ppm


5cb
500 MHz, CDCl3
AllocHN
H
N
O
Et
OH
Et
CO2Et
7.272
6.702
6.685
6.639
6.622
5.951
5.930
5.917
5.896
5.337
5.322
5.302
5.240
5.219
4.586
4.275
4.253
4.239
4.225
4.210
4.189
4.113
4.067
4.052
4.038
2.465
2.223
2.199
2.185
2.150
2.136
2.110
1.624
1.590
1.575
1.561
1.546
1.517
1.502
1.488
1.466
1.452
1.321
1.306
1.292
1.262
1.008
0.995
0.979
0.966
0.941
0.926
0.913
0.885
0.870
0.855
1.00
0.70
1.61
0.85
2.89
1.99
1.05
0.83
1.06
6.14
3.11
9.53
3.69
10
9
8
7
6
5
4
3
2
1
ppm


5cb
125 MHz, CDCl3
AllocHN
O
H
N
Et
OH
Et
CO2Et
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171.244
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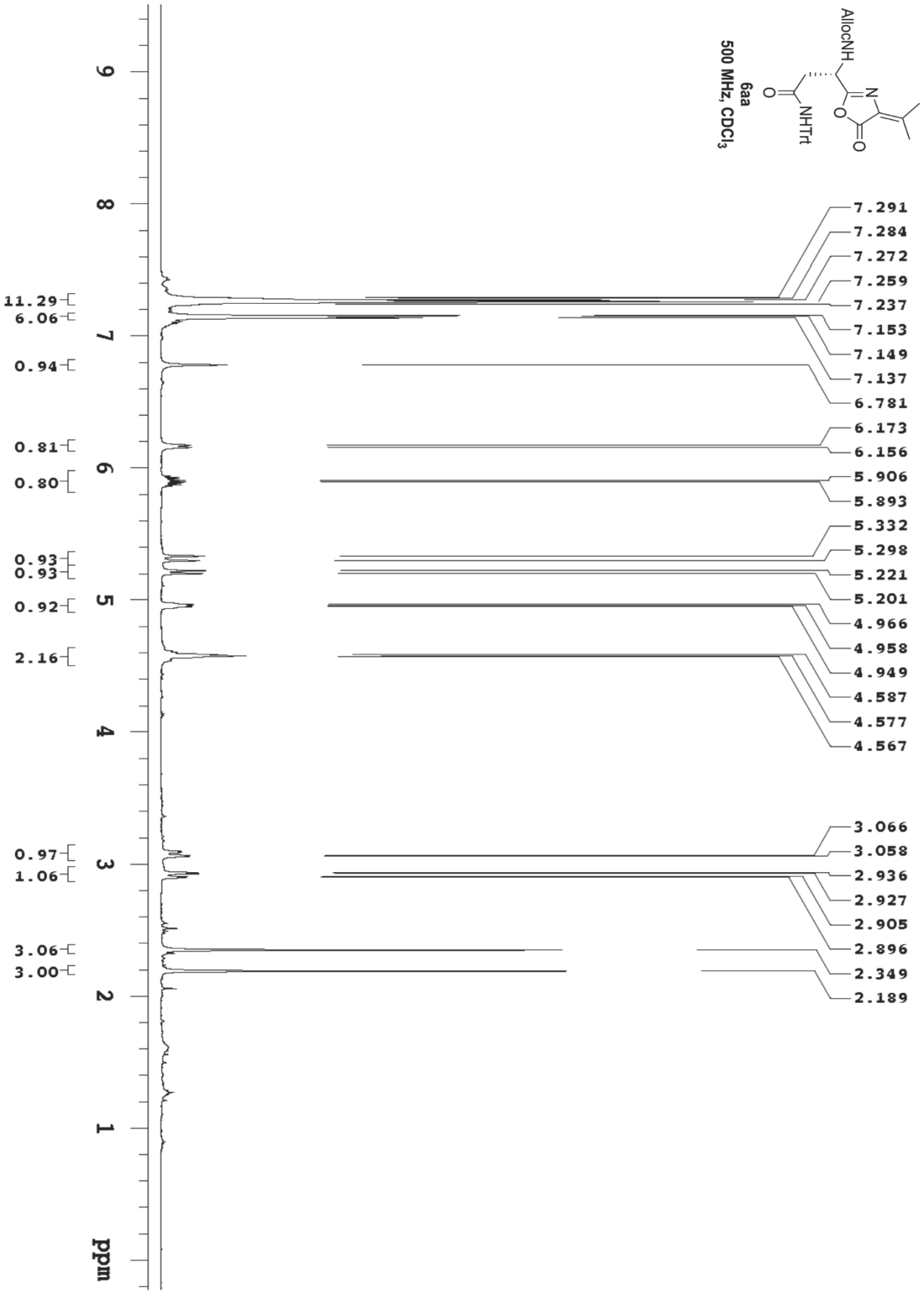
132.579
117.907
117.823
77.257
77.208
77.003
76.749
76.180
76.168
65.935
65.889
61.598
61.541
60.198
56.802
56.714
31.227
28.427
26.720
26.689
19.305
19.066
17.742
17.249
14.080
14.062
7.596
7.550
7.524
7.501
200
180
160
140
120
100
80
60
40
20
0
ppm


6aa
500 MHz, CDCl3
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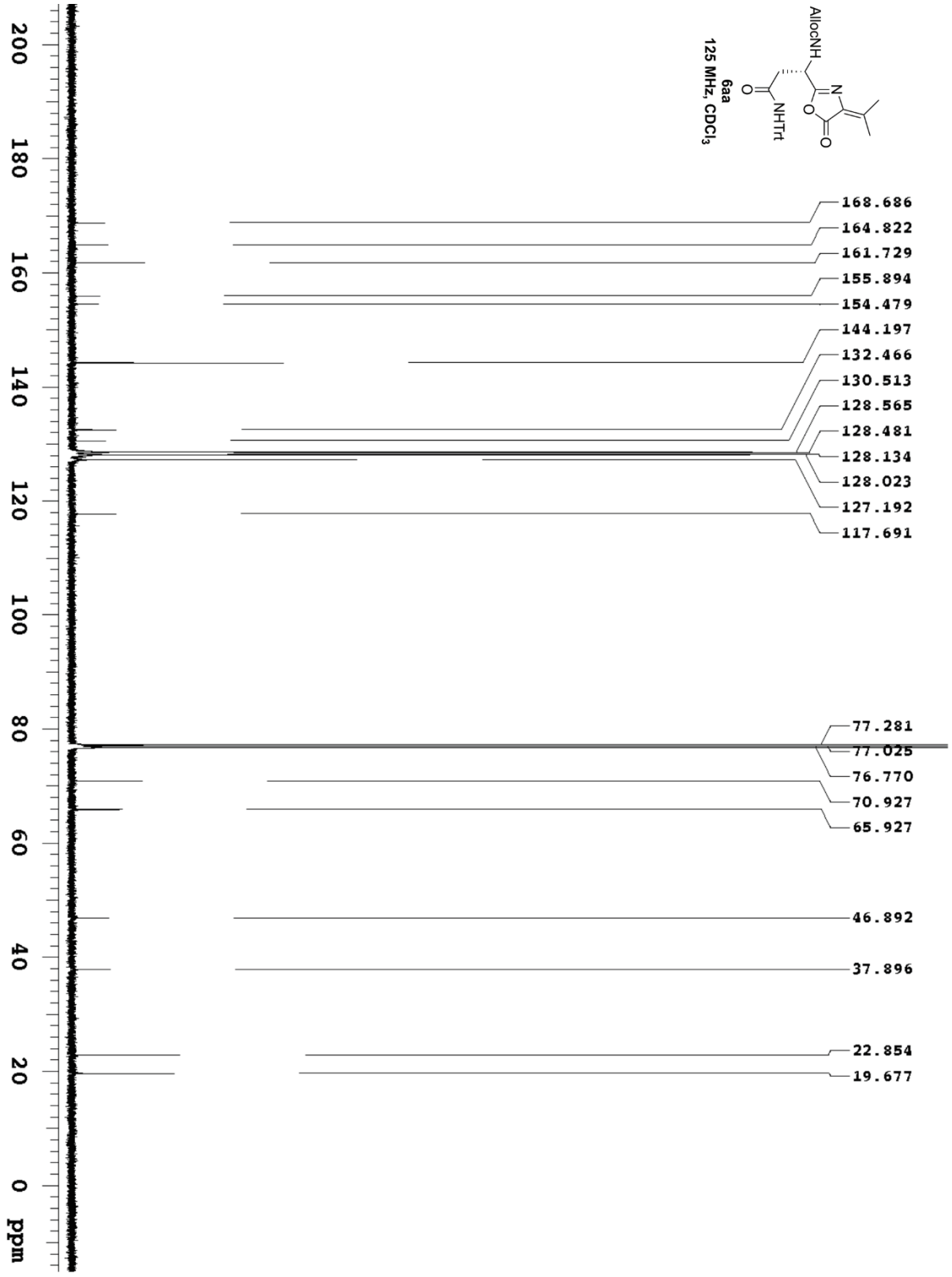
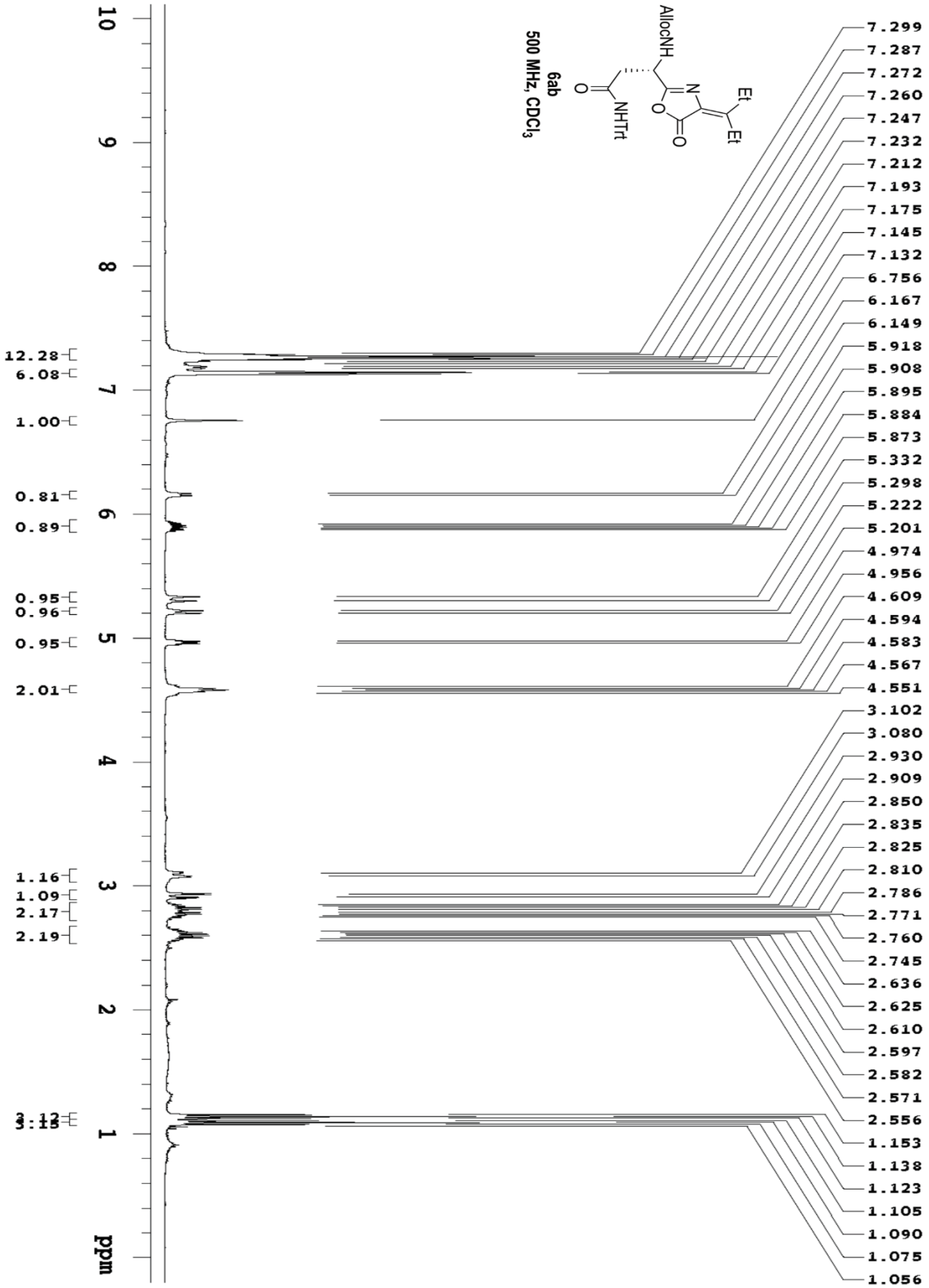
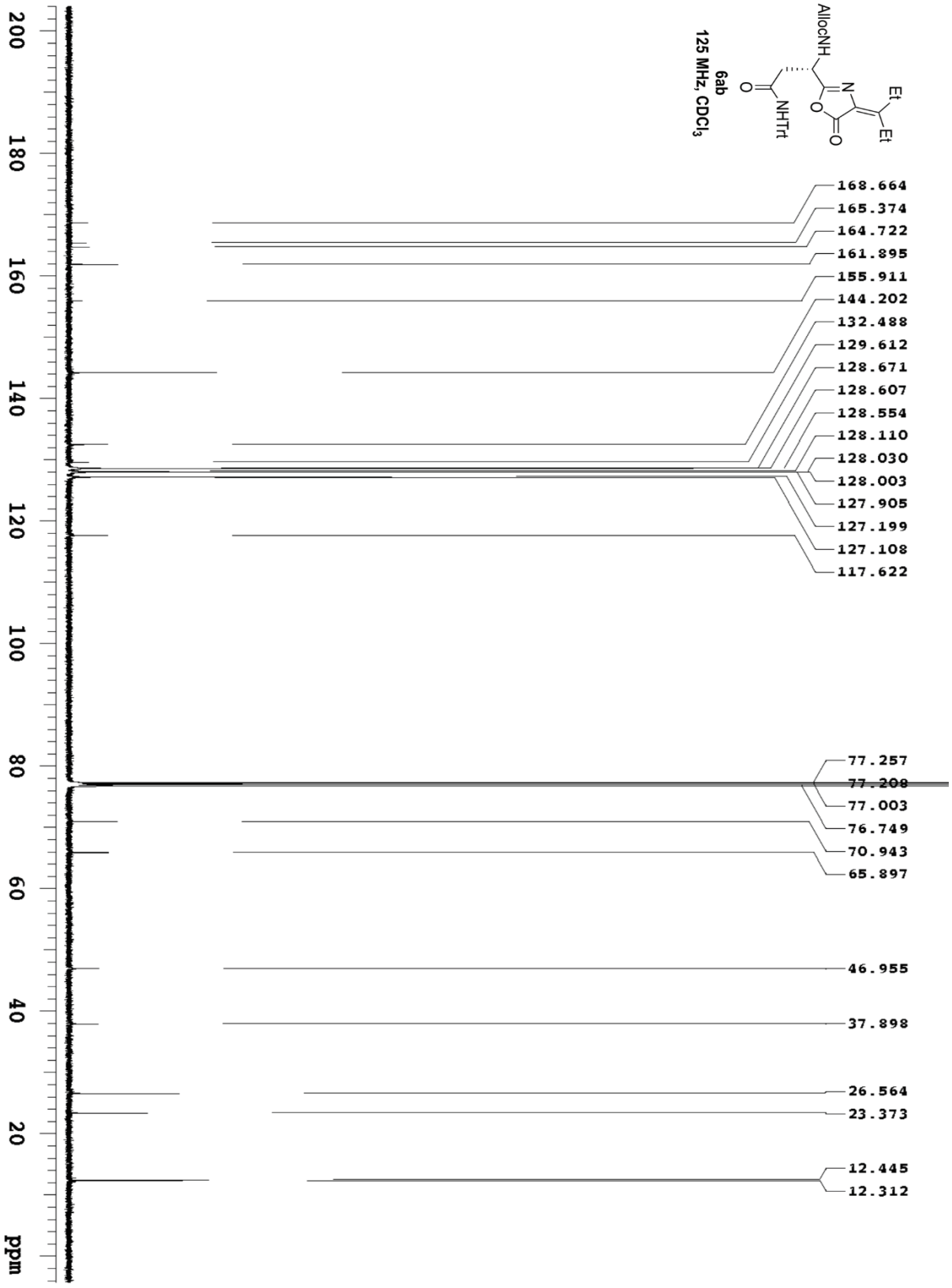
NHTrt
7.291
7.284
7.272
7.259
7.237
7.153
7.149
7.137
6.781
6.173
6.156
5.906
5.893
5.332
5.298
5.221
5.201
4.966
4.958
4.949
4.587
4.577
4.567
3.066
3.058
2.936
2.927
2.905
2.896
2.349
2.189
11.29
6.06
0.94
0.81
0.80
0.93
0.93
0.92
2.16
0.97
1.06
3.06
3.00
ppm


6aa
125 MHz, CDCl3
AllocNH
NHTrt
168.686
164.822
161.729
155.894
154.479
144.197
132.466
130.513
128.565
128.481
128.134
128.023
127.192
117.691
77.281
77.025
76.770
70.927
65.927
46.892
37.896
22.854
19.677
200
180
160
140
120
100
80
60
40
20
0
ppm


6ab
500 MHz, CDCl3
AllocNH
NHTrt
Et
Et
7.299
7.287
7.272
7.260
7.247
7.232
7.212
7.193
7.175
7.145
7.132
6.756
6.167
6.149
5.918
5.908
5.895
5.884
5.873
5.332
5.298
5.222
5.201
4.974
4.956
4.609
4.594
4.583
4.567
4.551
3.102
3.080
2.930
2.909
2.850
2.835
2.825
2.810
2.786
2.771
2.760
2.745
2.636
2.625
2.610
2.597
2.582
2.571
2.556
1.153
1.138
1.123
1.105
1.090
1.075
1.056
12.28
6.08
1.00
0.81
0.89
0.95
0.96
0.95
2.01
1.16
1.09
2.17
2.19
3.12
3.15
10
9
8
7
6
5
4
3
2
1
ppm


6ab
125 MHz, CDCl3
AllocNH
NHTrt
Et
Et
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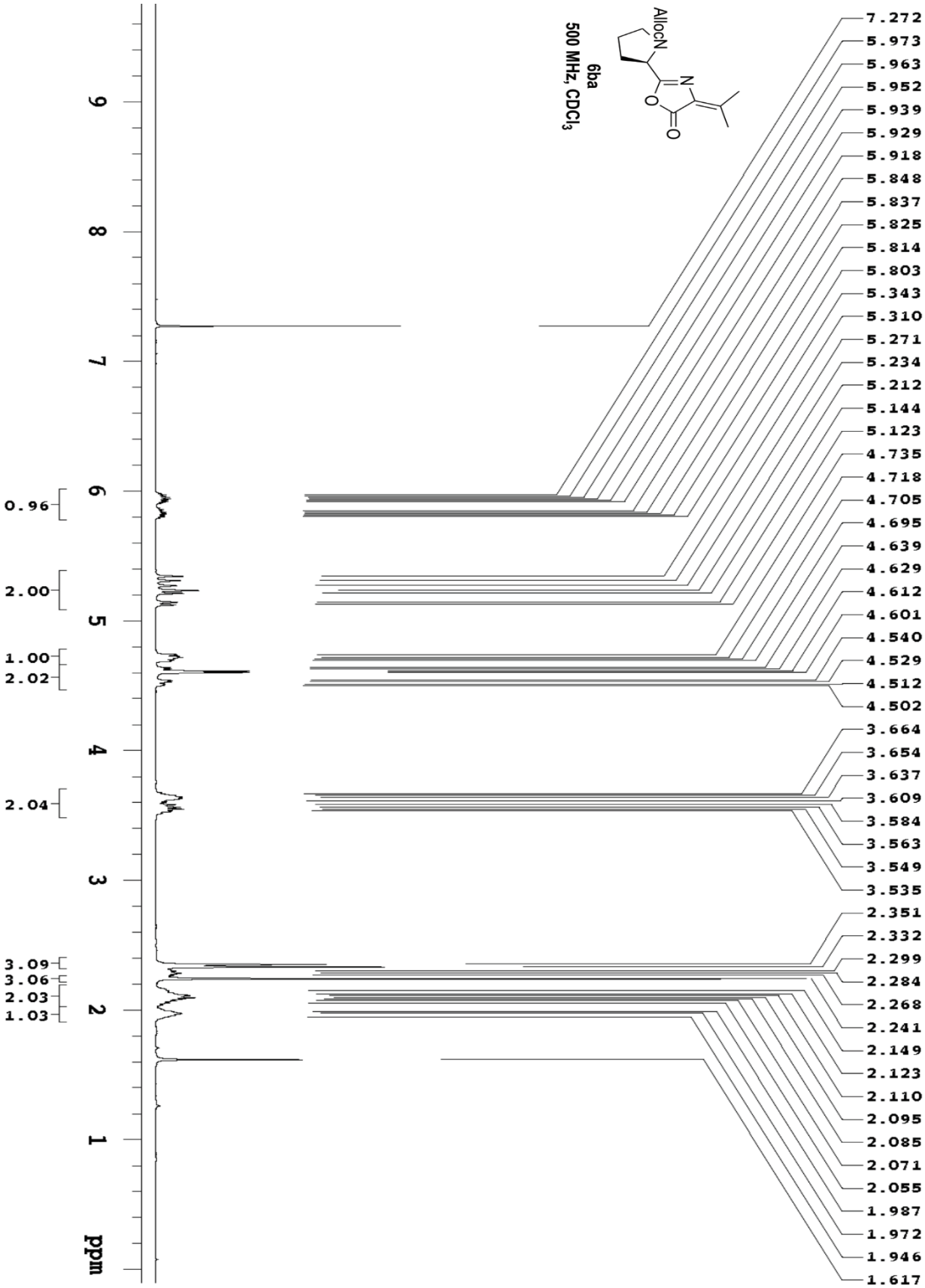
165.374
164.722
161.895
155.911
144.202
132.488
129.612
128.671
128.607
128.554
128.110
128.030
128.003
127.905
127.199
127.108
117.622
77.257
77.208
77.003
76.749
70.943
65.897
46.955
37.898
26.564
23.373
12.445
12.312
200
180
160
140
120
100
80
60
40
20
ppm


6ba
500 MHz, CDCl3
AllocN
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O
O
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5.963
5.952
5.939
5.929
5.918
5.848
5.837
5.825
5.814
5.803
5.343
5.310
5.271
5.234
5.212
5.144
5.123
4.735
4.718
4.705
4.695
4.639
4.629
4.612
4.601
4.540
4.529
4.512
4.502
3.664
3.654
3.637
3.609
3.584
3.563
3.549
3.535
2.351
2.332
2.299
2.284
2.268
2.241
2.149
2.123
2.110
2.095
2.085
2.071
2.055
1.987
1.972
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0.96
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1.00
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2.04
3.09
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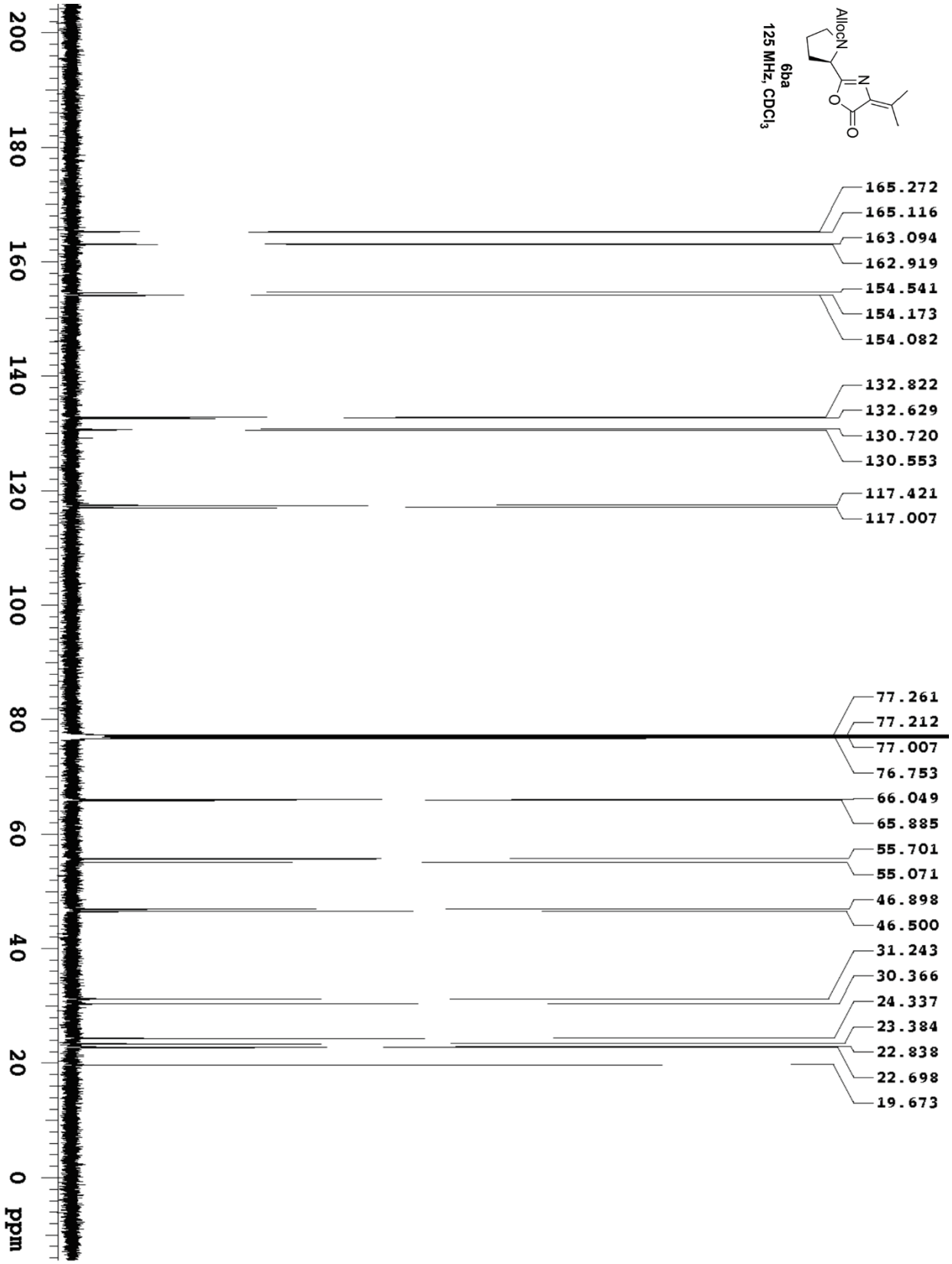
2.03
1.03
ppm


6ba
125 MHz, CDCl3
AllocN
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O
O
O
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165.116
163.094
162.919
154.541
154.173
154.082
132.822
132.629
130.720
130.553
117.421
117.007
77.261
77.212
77.007
76.753
66.049
65.885
55.701
55.071
46.898
46.500
31.243
30.366
24.337
23.384
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22.698
19.673
200
180
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140
120
100
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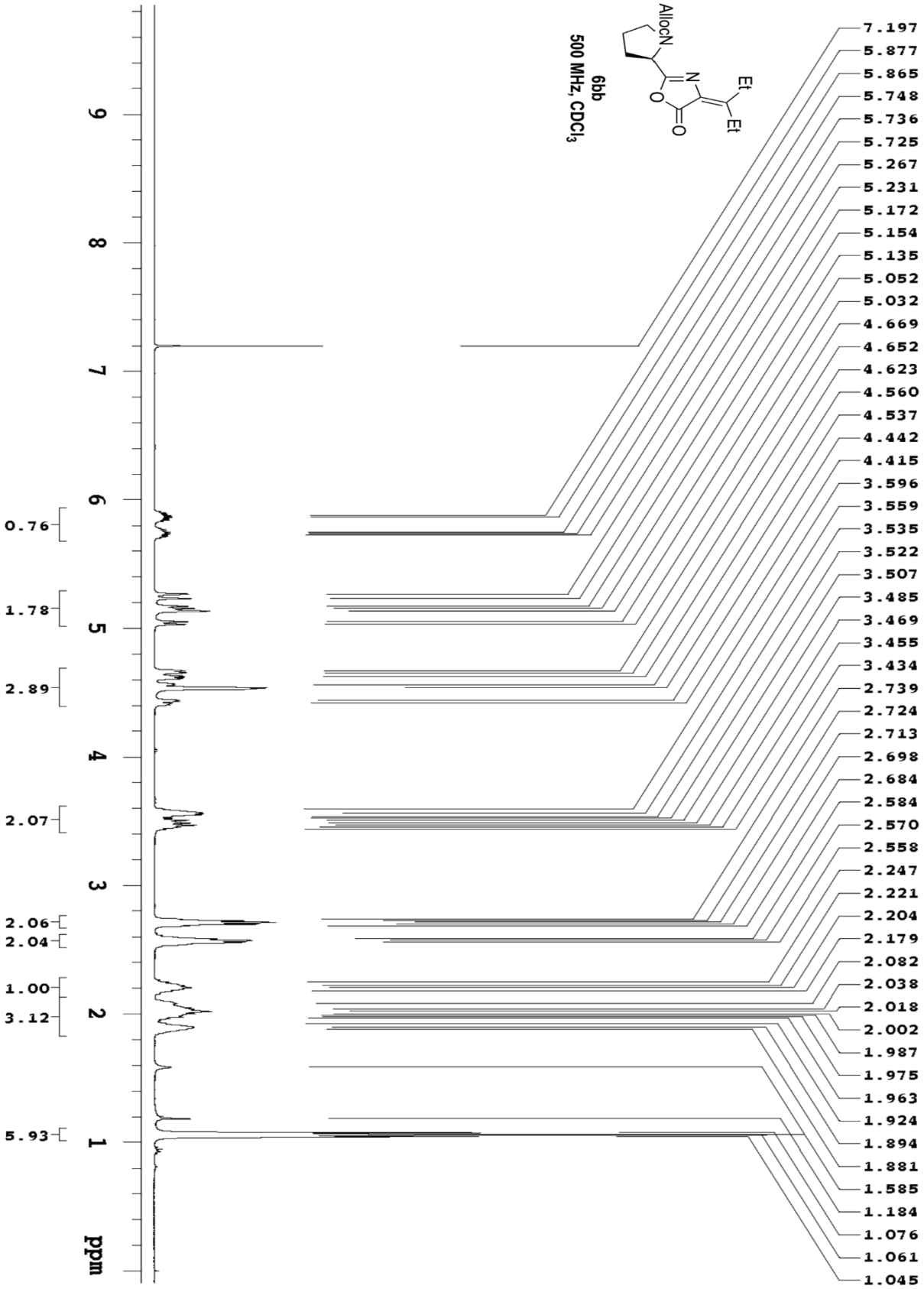
60
40
20
0
ppm


6bb
500 MHz, CDCl3
AllocN
N
O
O
Et
Et
7.197
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5.748
5.736
5.725
5.267
5.231
5.172
5.154
5.135
5.052
5.032
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4.652
4.623
4.560
4.537
4.442
4.415
3.596
3.559
3.535
3.522
3.507
3.485
3.469
3.455
3.434
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2.724
2.713
2.698
2.684
2.584
2.570
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2.247
2.221
2.204
2.179
2.082
2.038
2.018
2.002
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1.924
1.894
1.881
1.585
1.184
1.076
1.061
1.045
0.76
1.78
2.89
2.07
2.06
2.04
1.00
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5.93
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7
6
5
4
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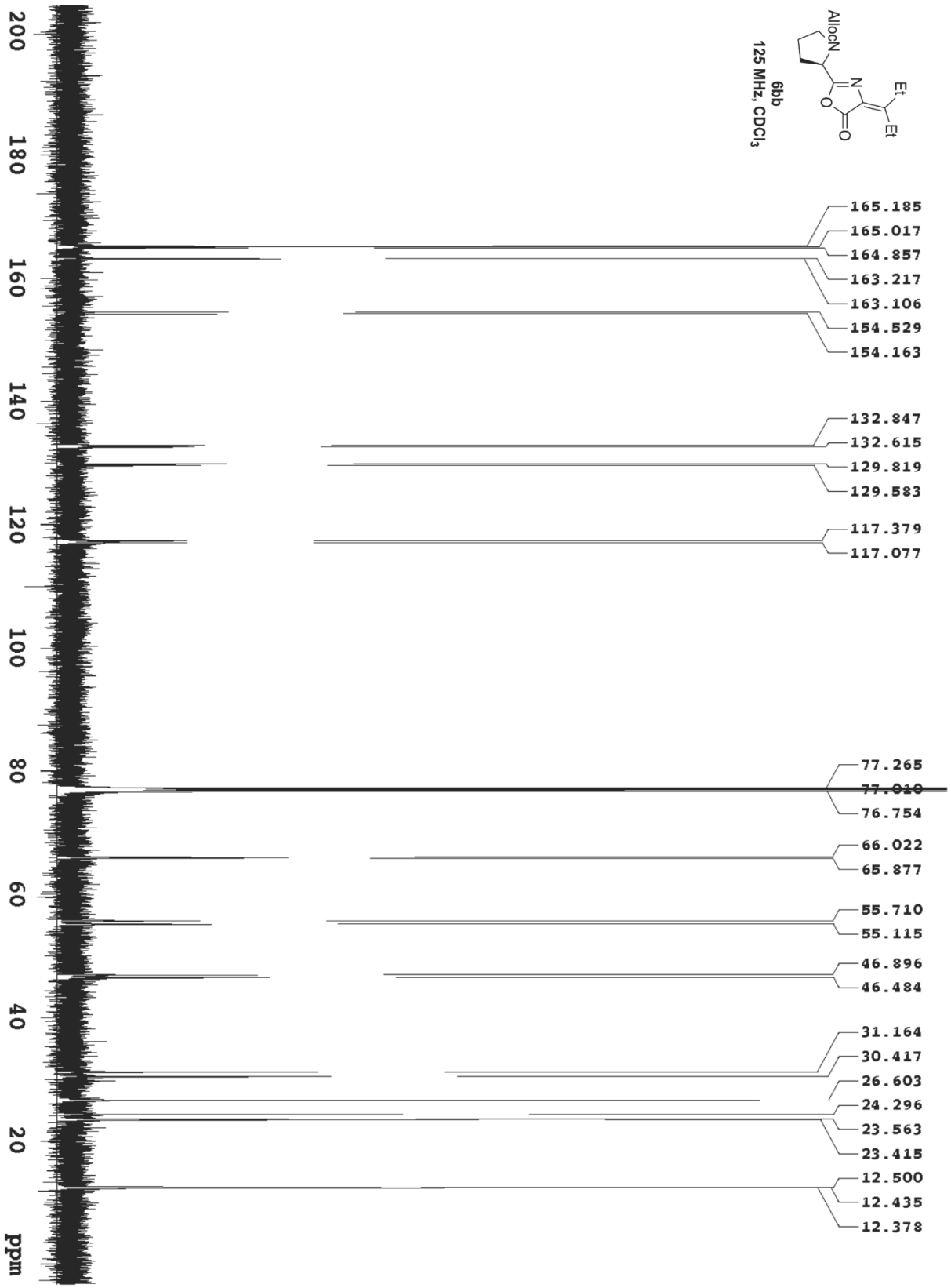
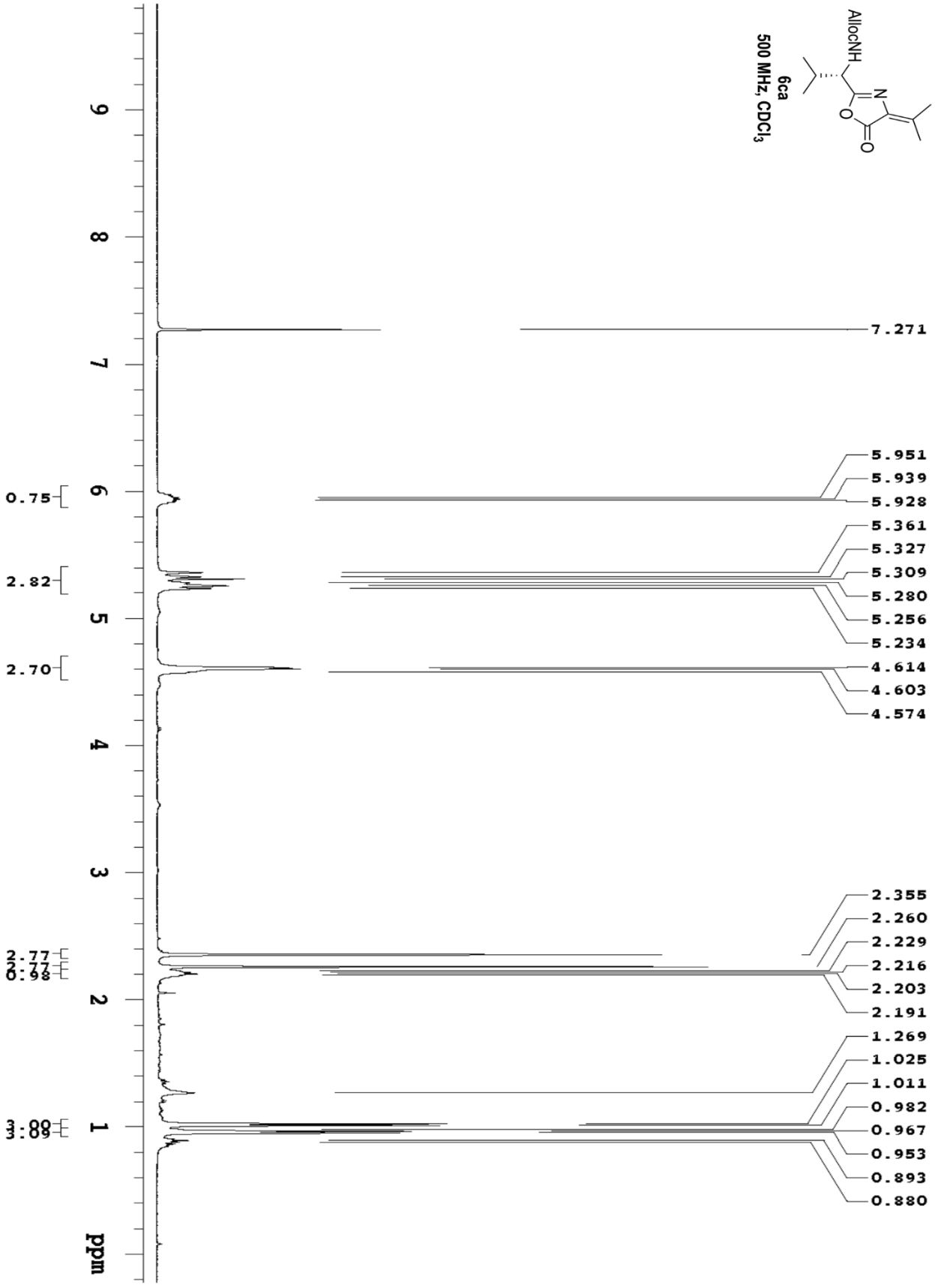
2
1
ppm


6bb
125 MHz, CDCl3
AllocN
N
O
O
O
Et
Et
165.185
165.017
164.857
163.217
163.106
154.529
154.163
132.847
132.615
129.819
129.583
117.379
117.077
77.265
77.010
76.754
66.022
65.877
55.710
55.115
46.896
46.484
31.164
30.417
26.603
24.296
23.563
23.415
12.500
12.435
12.378
200
180
160
140
120
100
80
60
40
20
ppm


AllocNH
N
O
O
6ca
500 MHz, CDCl3
7.271
5.951
5.939
5.928
5.361
5.327
5.309
5.280
5.256
5.234
4.614
4.603
4.574
2.355
2.260
2.229
2.216
2.203
2.191
1.269
1.025
1.011
0.982
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0.893
0.880
0.75
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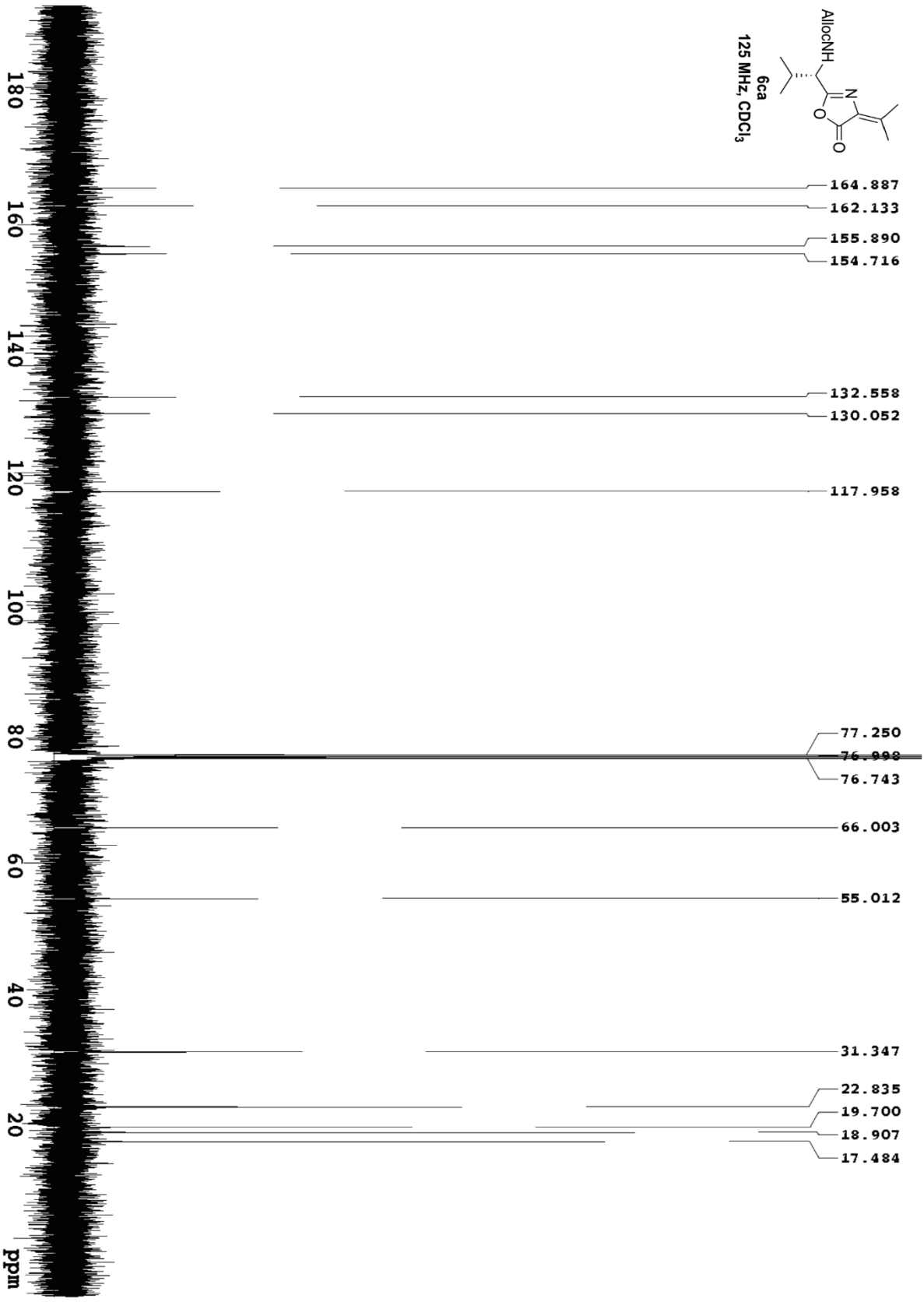
2.70
2.77
0.98
3.00
3.09
ppm


AllocNH
N
O
O
O
6ca
125 MHz, CDCl3
164.887
162.133
155.890
154.716
132.558
130.052
117.958
77.250
76.998
76.743
66.003
55.012
31.347
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19.700
18.907
17.484
180
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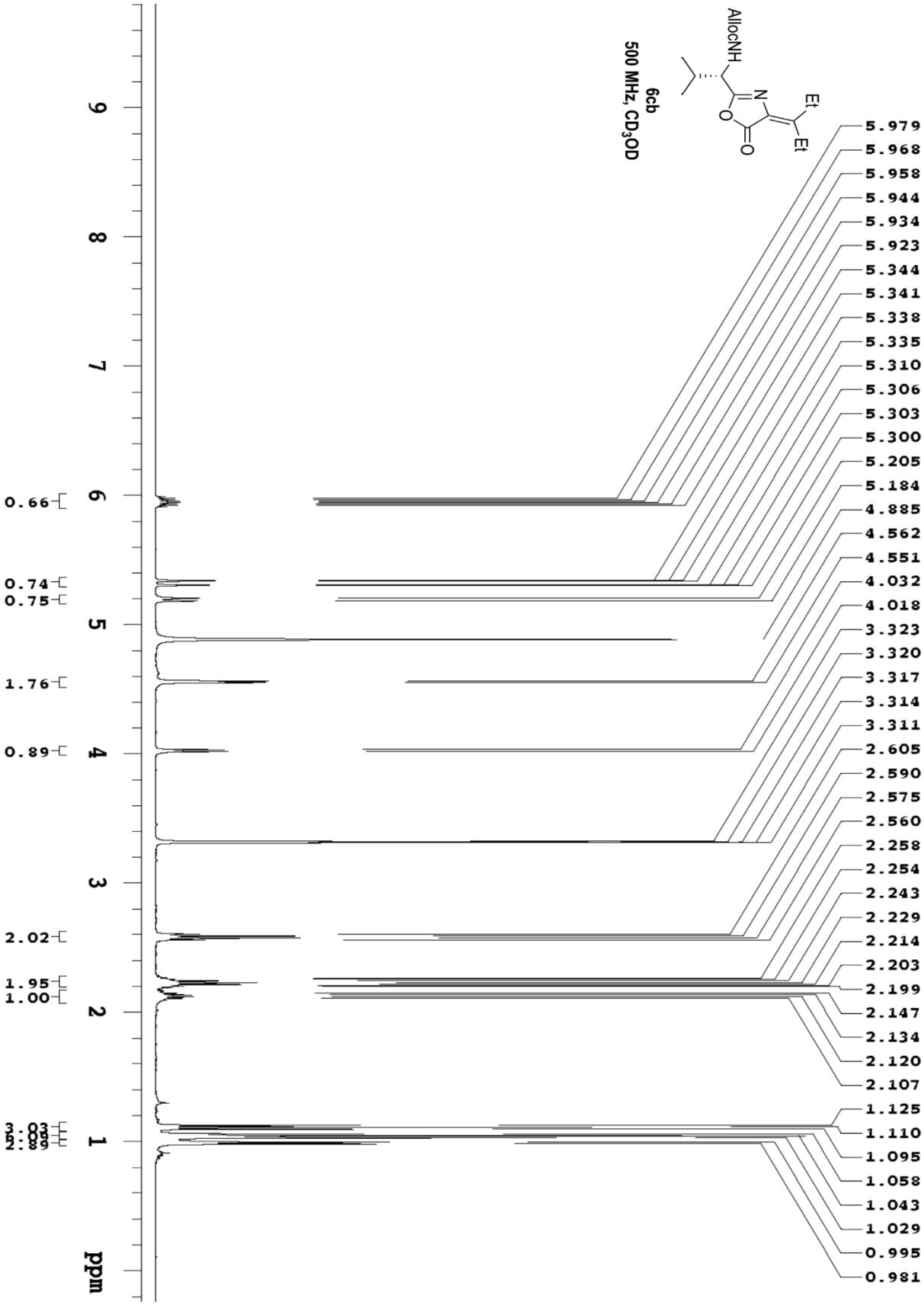
140
120
100
80
60
40
20
ppm


6cb
500 MHz, CD3OD
AllocNH
N
O
O
O
Et
Et
5.979
5.968
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5.934
5.923
5.344
5.341
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5.335
5.310
5.306
5.303
5.300
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5.184
4.885
4.562
4.551
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4.018
3.323
3.320
3.317
3.314
3.311
2.605
2.590
2.575
2.560
2.258
2.254
2.243
2.229
2.214
2.203
2.199
2.147
2.134
2.120
2.107
1.125
1.110
1.095
1.058
1.043
1.029
0.995
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0.74
0.75
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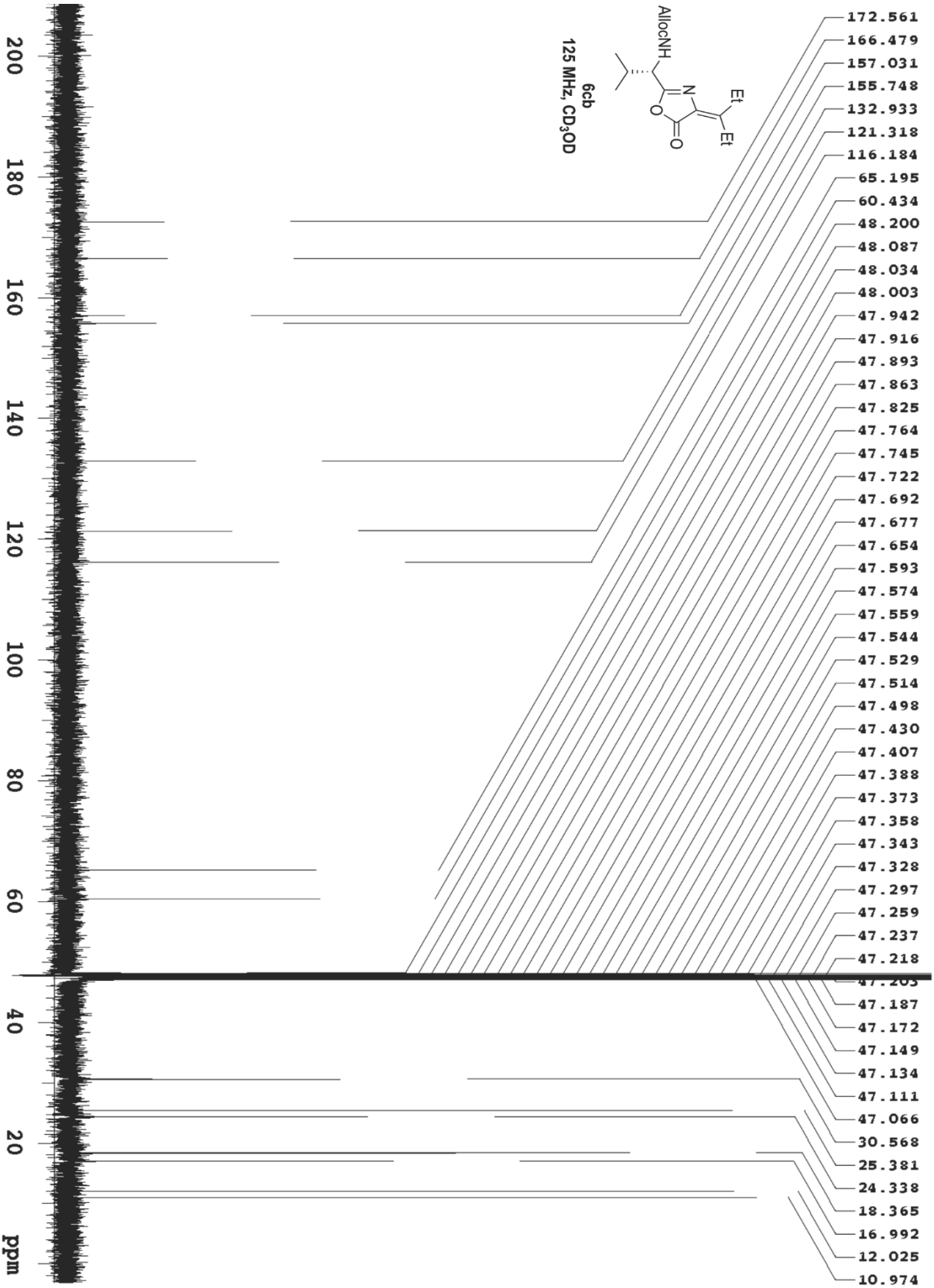
1.95
1.00
3.03
2.89
ppm


6cb
125 MHz, CD3OD
AllocNH
N
O
O
Et
Et
172.561
166.479
157.031
155.748
132.933
121.318
116.184
65.195
60.434
48.200
48.087
48.034
48.003
47.942
47.916
47.893
47.863
47.825
47.764
47.745
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47.373
47.358
47.343
47.328
47.297
47.259
47.237
47.218
47.187
47.172
47.149
47.134
47.111
47.066
30.568
25.381
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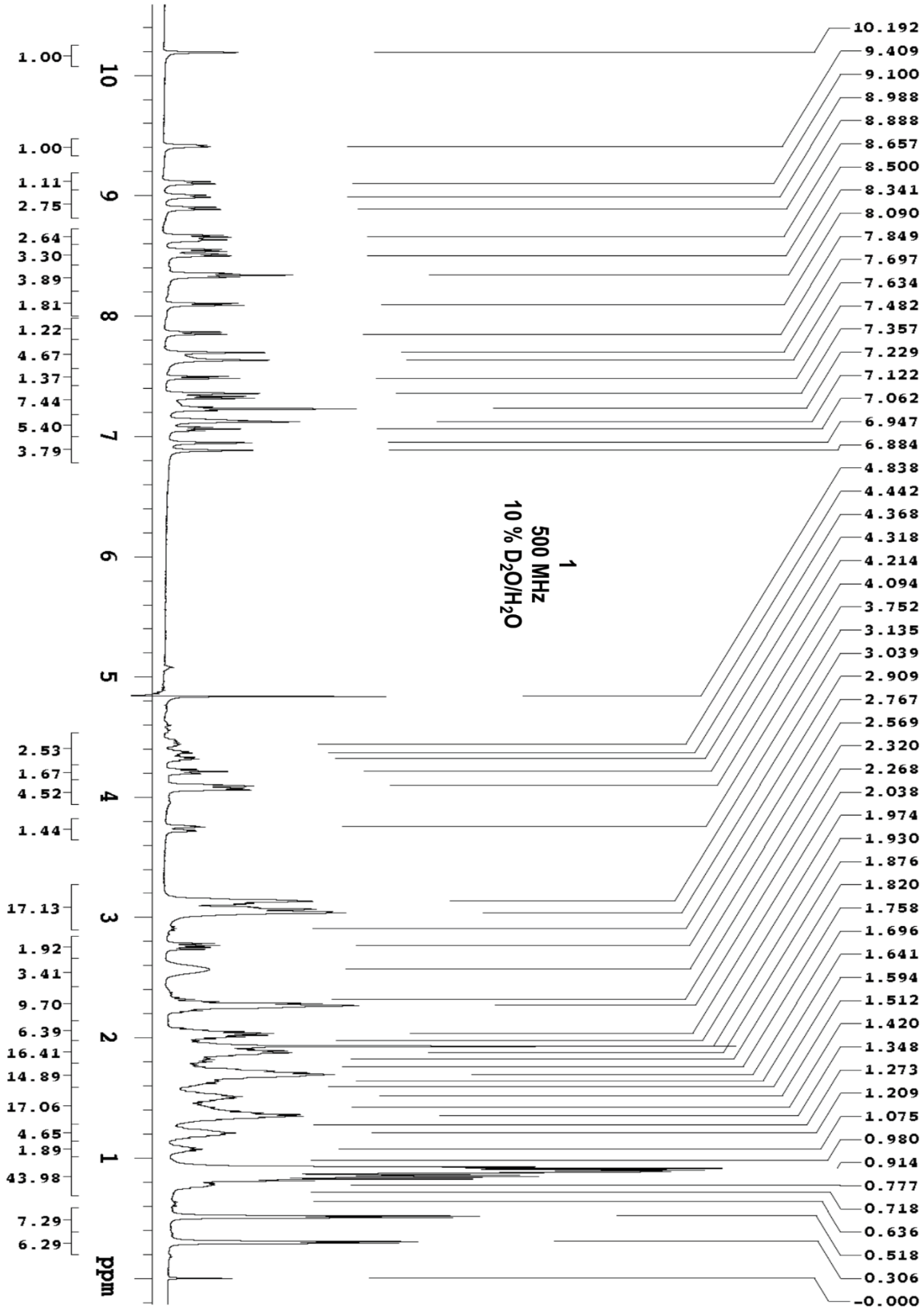
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7.697
7.634
7.482
7.357
7.229
7.122
7.062
6.947
6.884
4.838
4.442
4.368
4.318
4.214
4.094
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3.135
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2.767
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2.320
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1.974
1.930
1.876
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1.420
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0.914
0.777
0.718
0.636
0.518
0.306
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1.00
1.11
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2.64
3.30
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1.81
1.22
4.67
1.37
7.44
5.40
3.79
2.53
1.67
4.52
1.44
17.13
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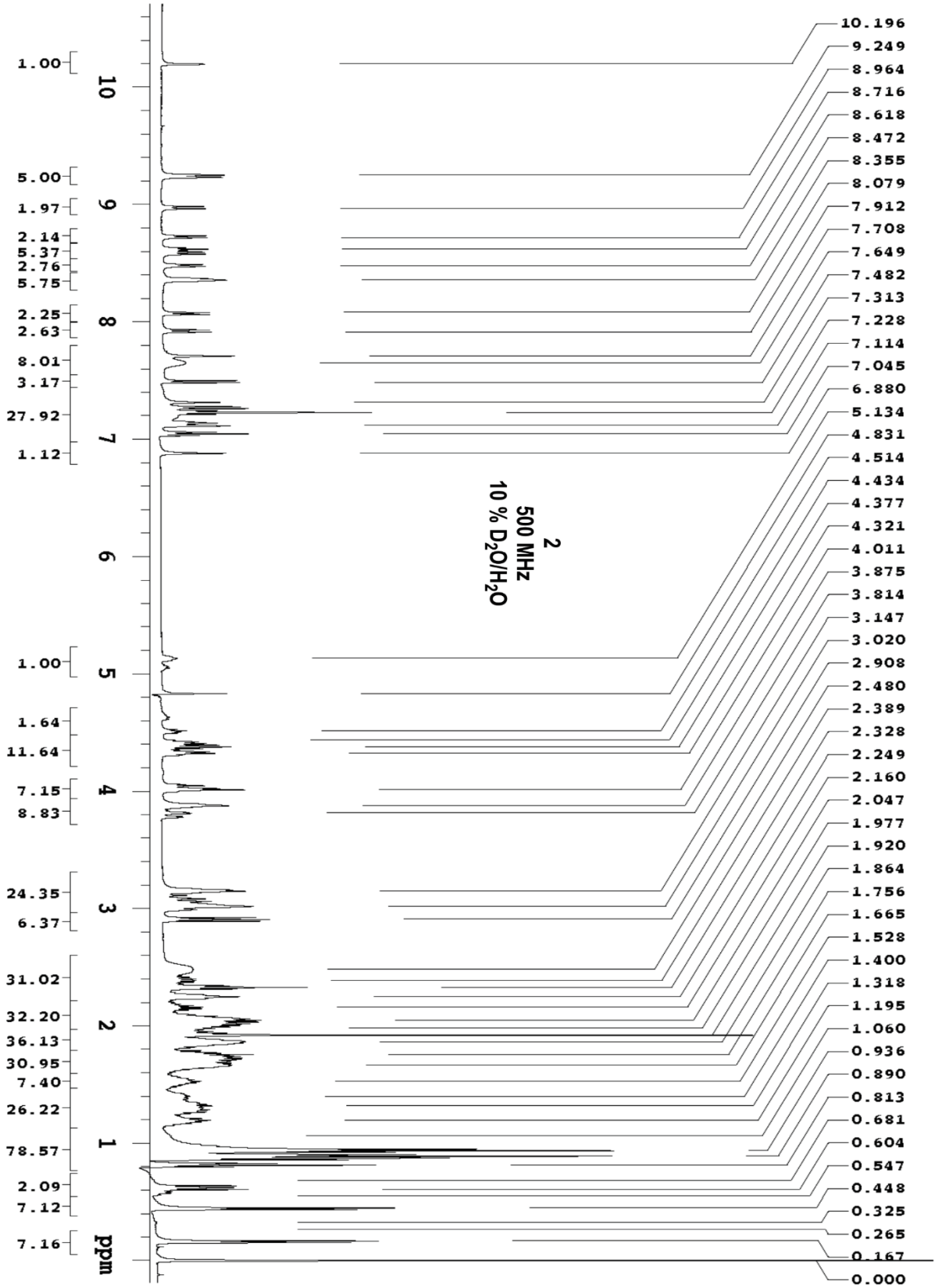
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7.708
7.649
7.482
7.313
7.228
7.114
7.045
6.880
5.134
4.831
4.514
4.434
4.377
4.321
4.011
3.875
3.814
3.147
3.020
2.908
2.480
2.389
2.328
2.249
2.160
2.047
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1.195
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0.890
0.813
0.681
0.604
0.547
0.448
0.325
0.265
0.167
0.000
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5.00
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2.14
5.37
2.76
5.75
2.25
2.63
8.01
3.17
27.92
1.12
1.00
1.64
11.64
7.15
8.83
24.35
6.37
31.02
32.20
36.13
30.95
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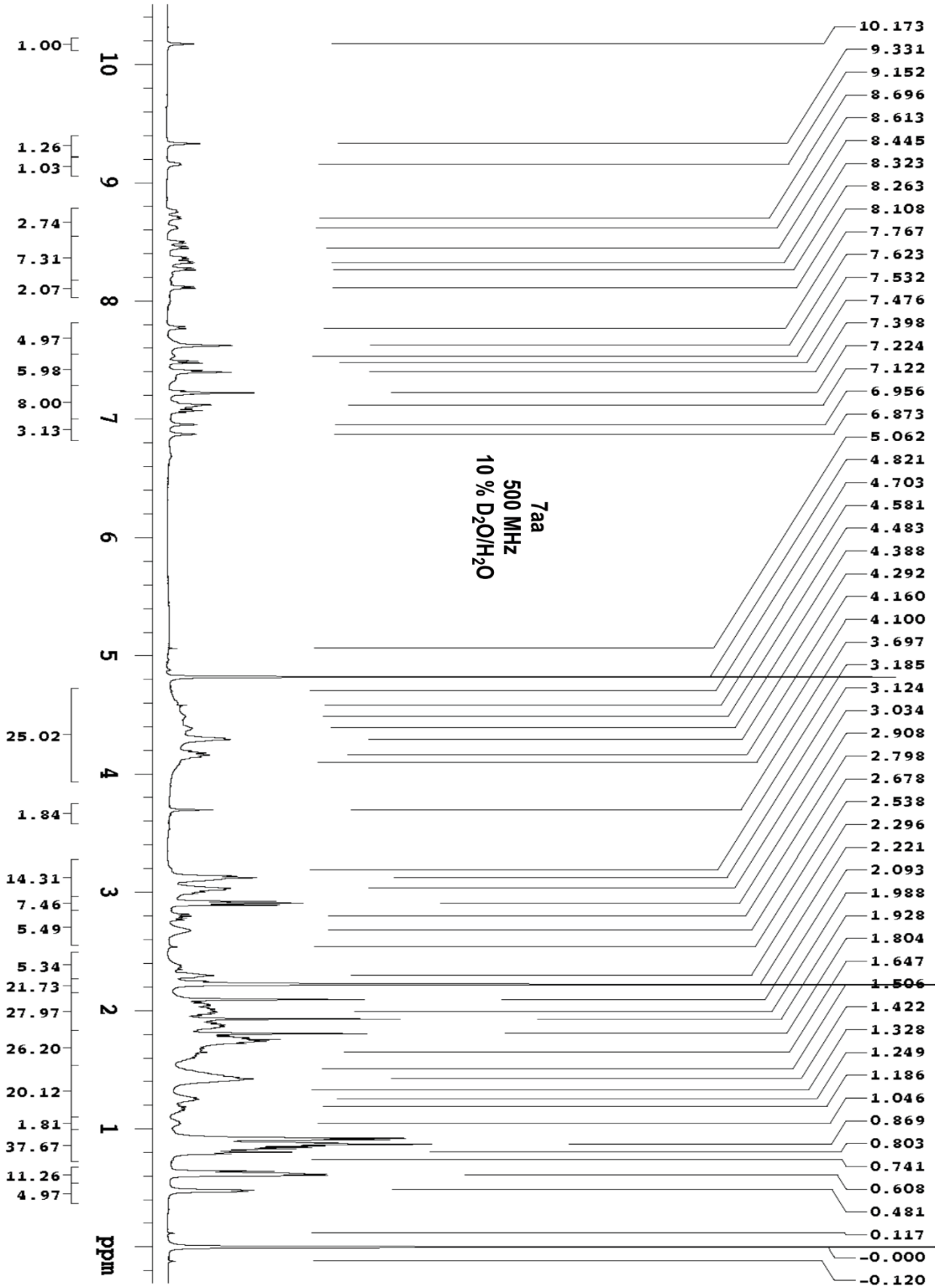
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7.476
7.398
7.224
7.122
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6.873
5.062
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4.703
4.581
4.483
4.388
4.292
4.160
4.100
3.697
3.185
3.124
3.034
2.908
2.798
2.678
2.538
2.296
2.221
2.093
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1.928
1.804
1.647
1.506
1.422
1.328
1.249
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0.741
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3.13
25.02
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14.31
7.46
5.49
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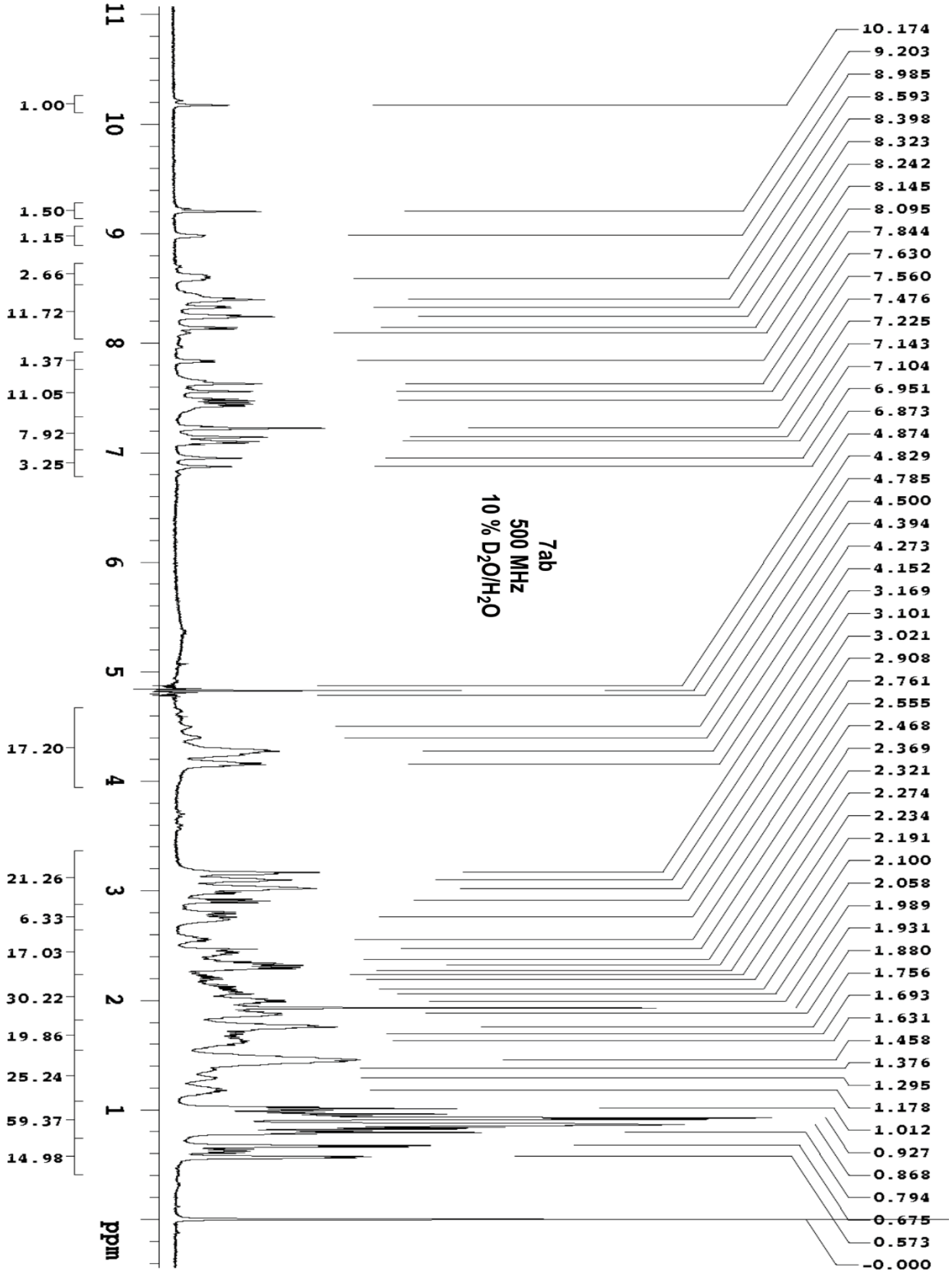
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7.630
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7.476
7.225
7.143
7.104
6.951
6.873
4.874
4.829
4.785
4.500
4.394
4.273
4.152
3.169
3.101
3.021
2.908
2.761
2.555
2.468
2.369
2.321
2.274
2.234
2.191
2.100
2.058
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1.880
1.756
1.693
1.631
1.458
1.376
1.295
1.178
1.012
0.927
0.868
0.794
0.675
0.573
-0.000
1.00
1.50
1.15
2.66
11.72
1.37
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6.33
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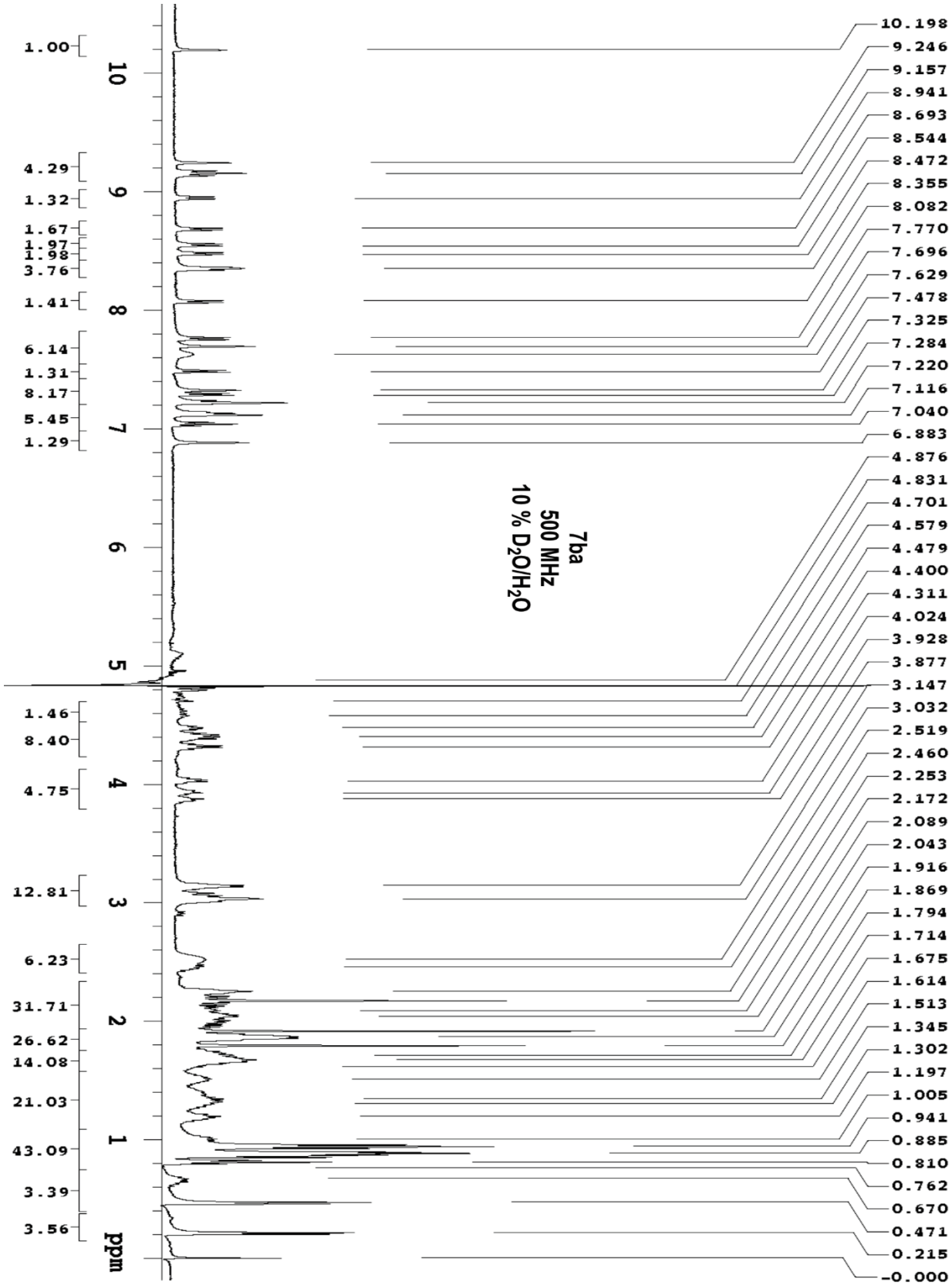
59.37
14.98
ppm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7.770
7.696
7.629
7.478
7.325
7.284
7.220
7.116
7.040
6.883
4.876
4.831
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4.579
4.479
4.400
4.311
4.024
3.928
3.877
3.147
3.032
2.519
2.460
2.253
2.172
2.089
2.043
1.916
1.869
1.794
1.714
1.675
1.614
1.513
1.345
1.302
1.197
1.005
0.941
0.885
0.810
0.762
0.670
0.471
0.215
-0.000
1.00
4.29
1.32
1.67
1.97
1.98
3.76
1.41
6.14
1.31
8.17
5.45
1.29
1.46
8.40
4.75
12.81
6.23
31.71
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9
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7
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4
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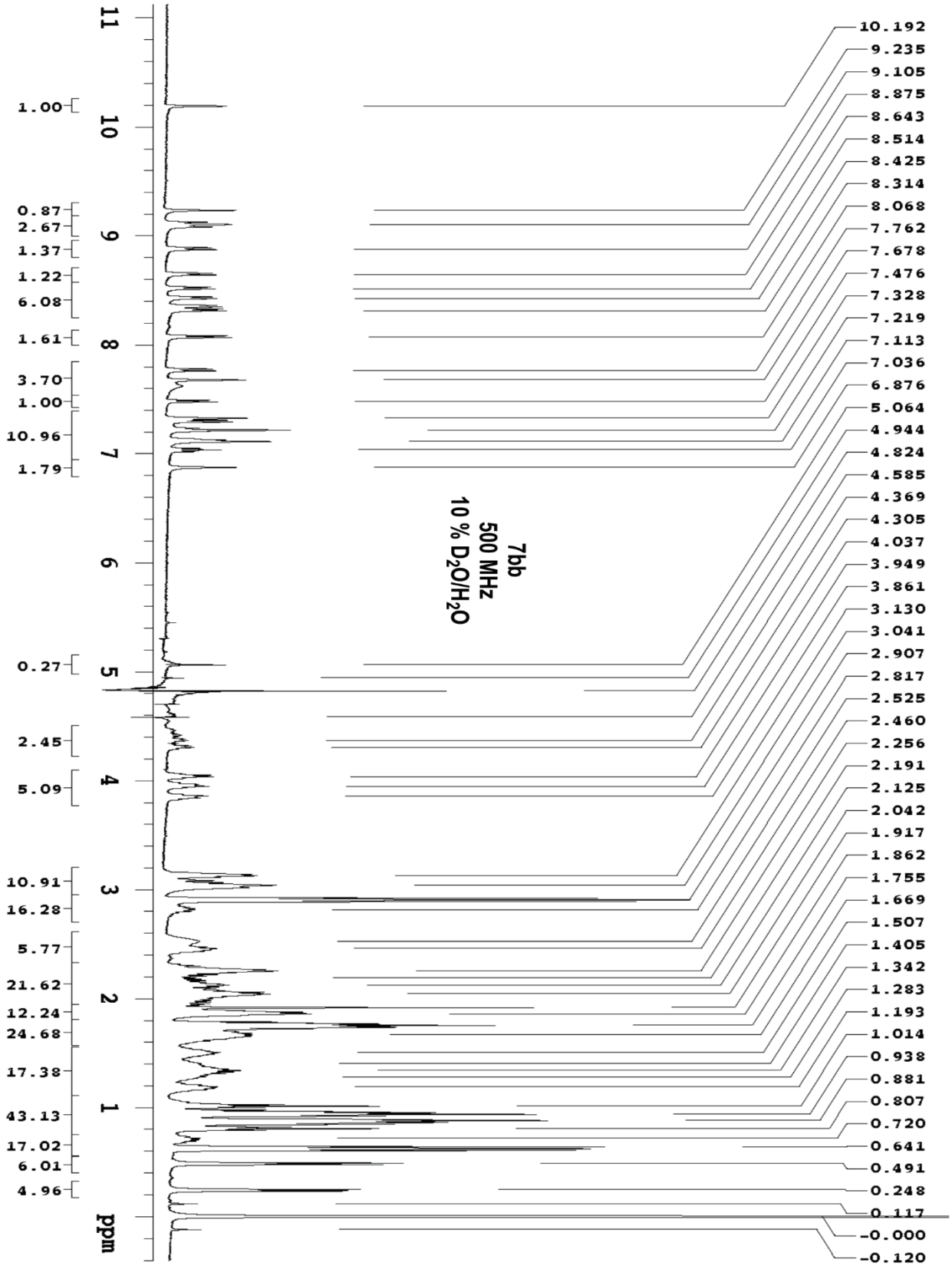
ppm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7.762
7.678
7.476
7.328
7.219
7.113
7.036
6.876
5.064
4.944
4.824
4.585
4.369
4.305
4.037
3.949
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3.130
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2.525
2.460
2.256
2.191
2.125
2.042
1.917
1.862
1.755
1.669
1.507
1.405
1.342
1.283
1.193
1.014
0.938
0.881
0.807
0.720
0.641
0.491
0.248
0.117
-0.000
-0.120
1.00
0.87
2.67
1.37
1.22
6.08
1.61
3.70
1.00
10.96
1.79
0.27
2.45
5.09
10.91
16.28
5.77
21.62
12.24
24.68
17.38
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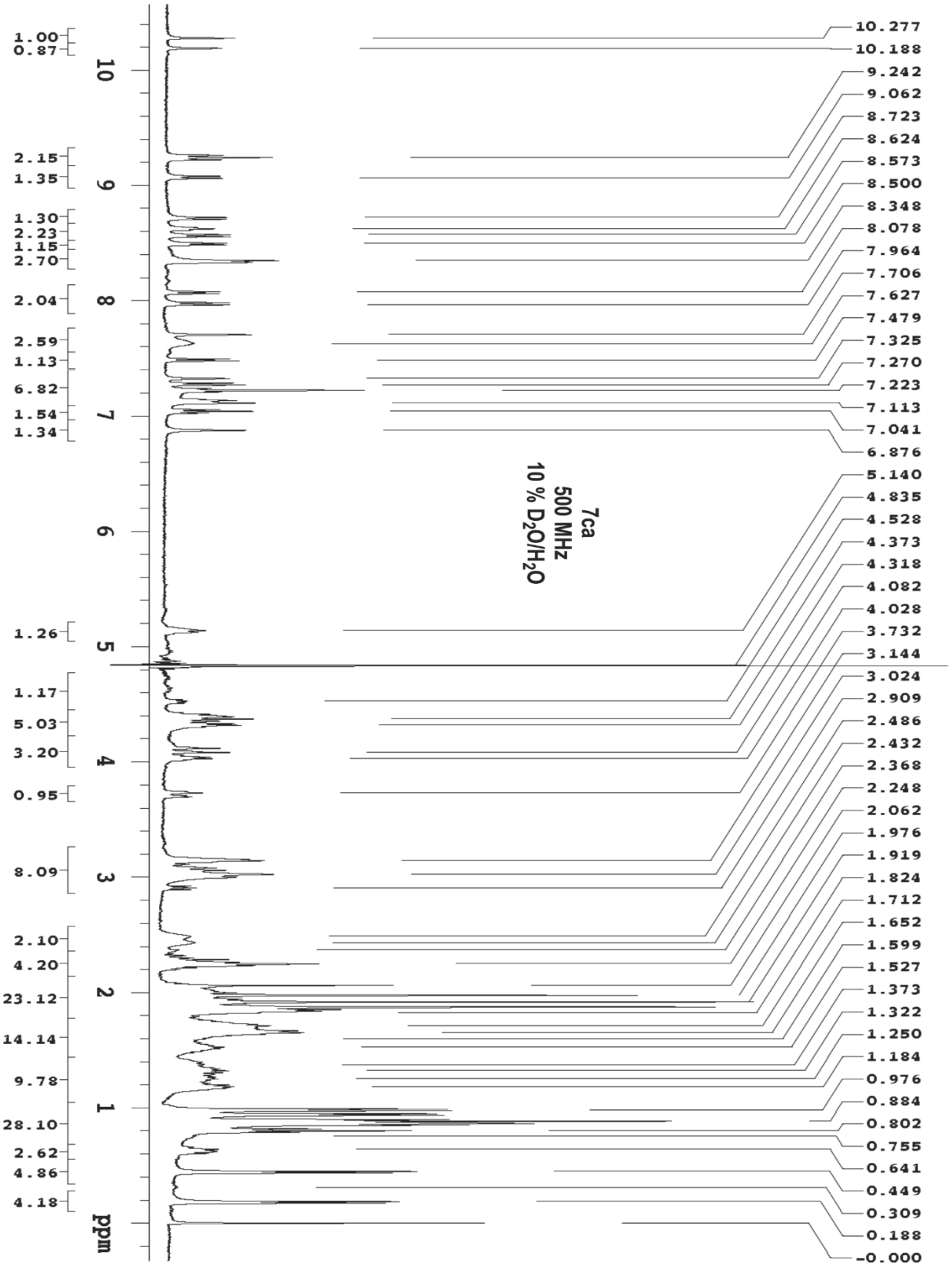
17.02
6.01
4.96
11
10
9
8
7
6
5
4
3
2
1
ppm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8.078
7.964
7.706
7.627
7.479
7.325
7.270
7.223
7.113
7.041
6.876
5.140
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4.528
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4.318
4.082
4.028
3.732
3.144
3.024
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2.432
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2.248
2.062
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1.824
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1.652
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1.527
1.373
1.322
1.250
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0.976
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0.802
0.755
0.641
0.449
0.309
0.188
-0.000
1.00
0.87
2.15
1.35
1.30
2.23
1.15
2.70
2.04
2.59
1.13
6.82
1.54
1.34
1.26
1.17
5.03
3.20
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28.10
2.62
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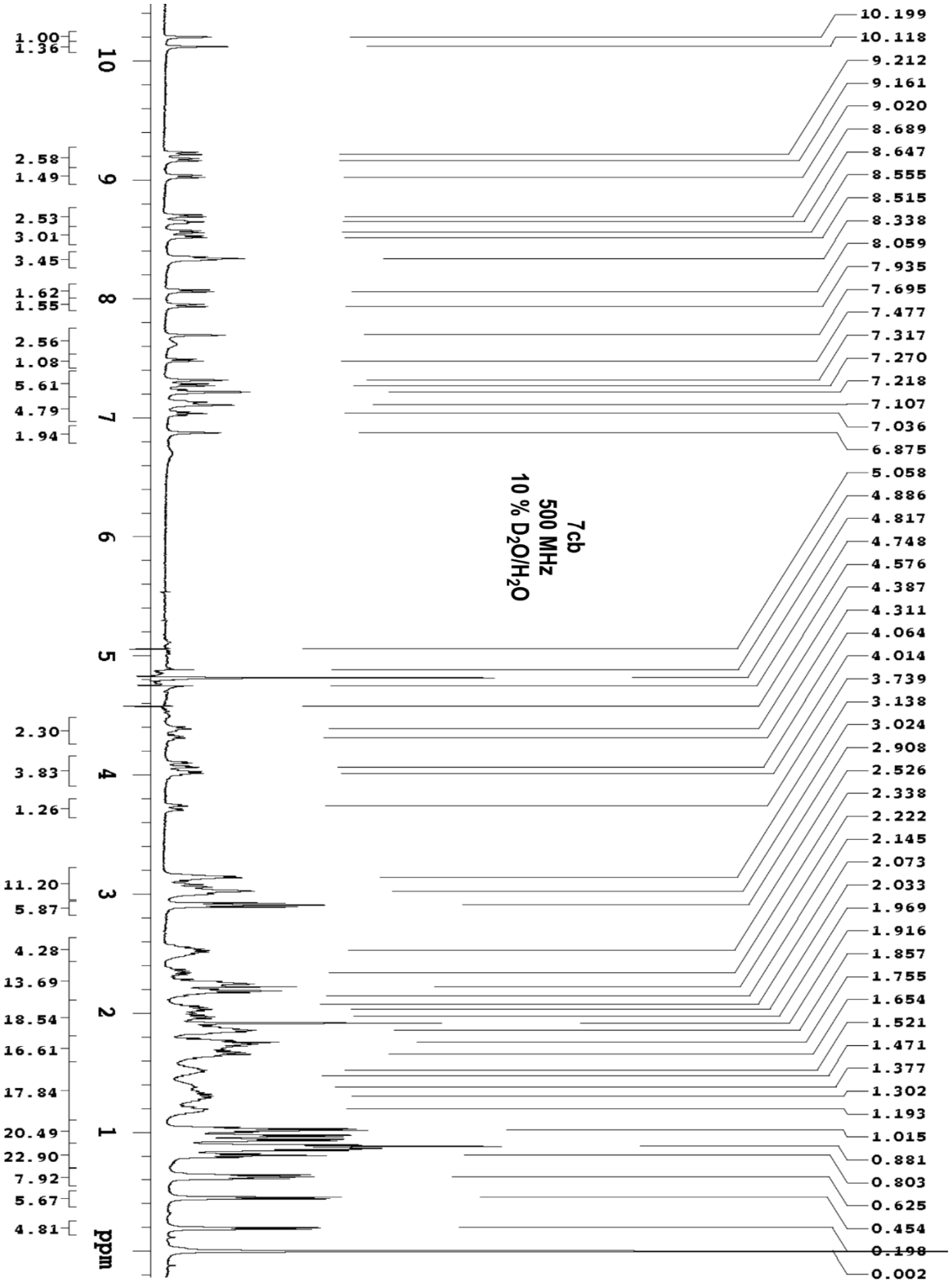
4.18
ppm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10.118
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8.689
8.647
8.555
8.515
8.338
8.059
7.935
7.695
7.477
7.317
7.270
7.218
7.107
7.036
6.875
5.058
4.886
4.817
4.748
4.576
4.387
4.311
4.064
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3.739
3.138
3.024
2.908
2.526
2.338
2.222
2.145
2.073
2.033
1.969
1.916
1.857
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1.654
1.521
1.471
1.377
1.302
1.193
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0.803
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0.454
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1.49
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3.01
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1.62
1.55
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1.08
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4.79
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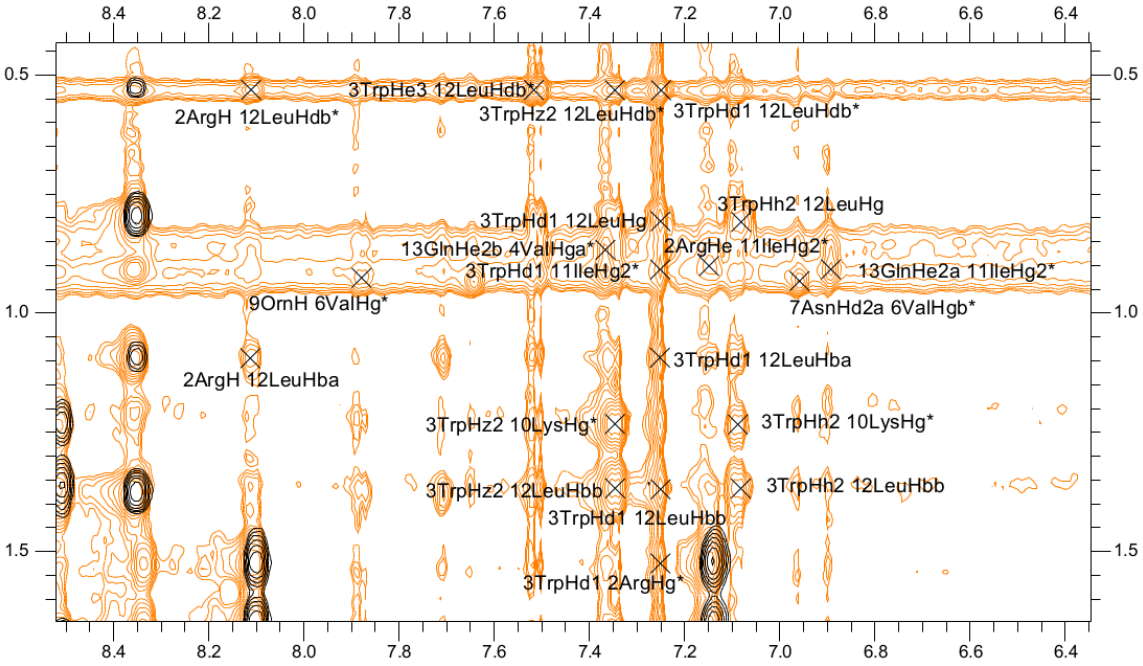
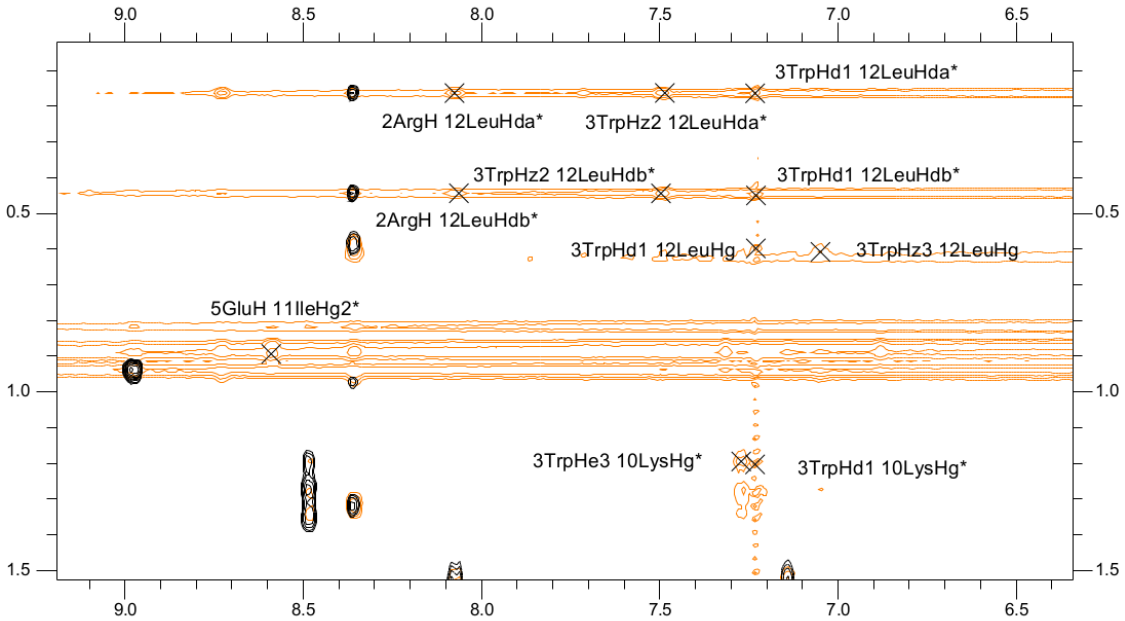
2.30
3.83
1.26
11.20
5.87
4.28
13.69
18.54
16.61
17.84
20.49
22.90
7.92
5.67
4.81
ppm

Selected Regions of ROESY Spectra. The spectra are presented with ROESY peaks in orange and TOCSY peaks superimposed in black for comparison. Peak labels use the standard CCPNMR nomenclature and their corresponding cross-peaks are marked with a crosshair.

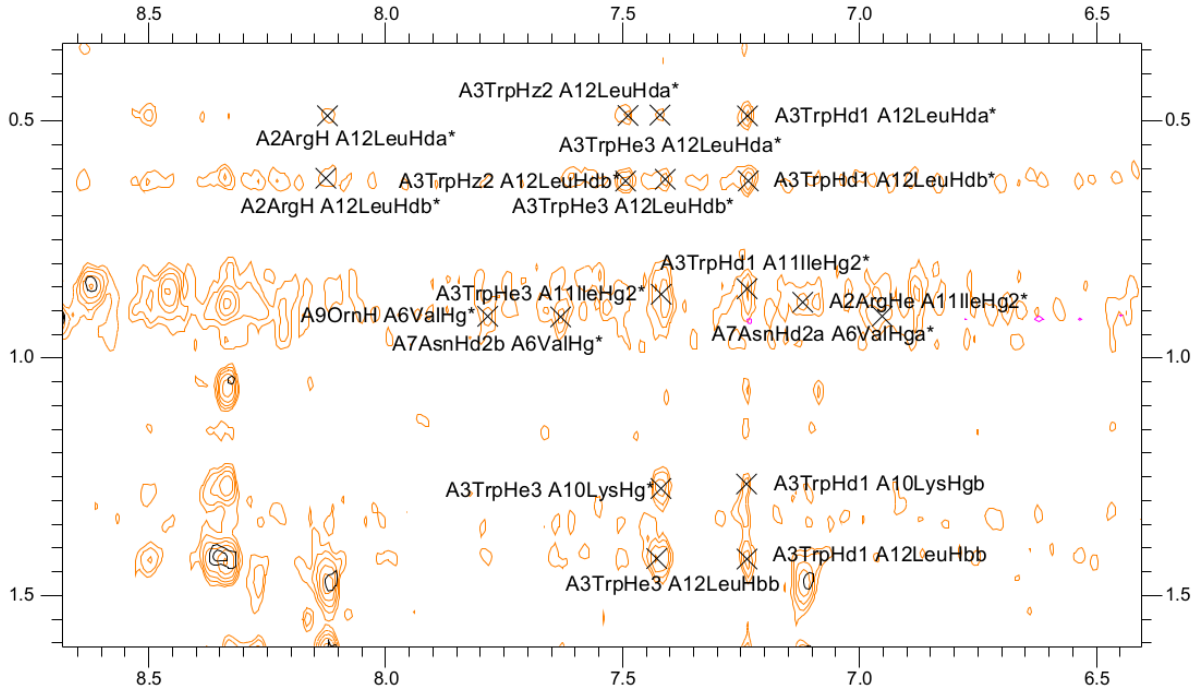
ROESY Spectrum of **1**



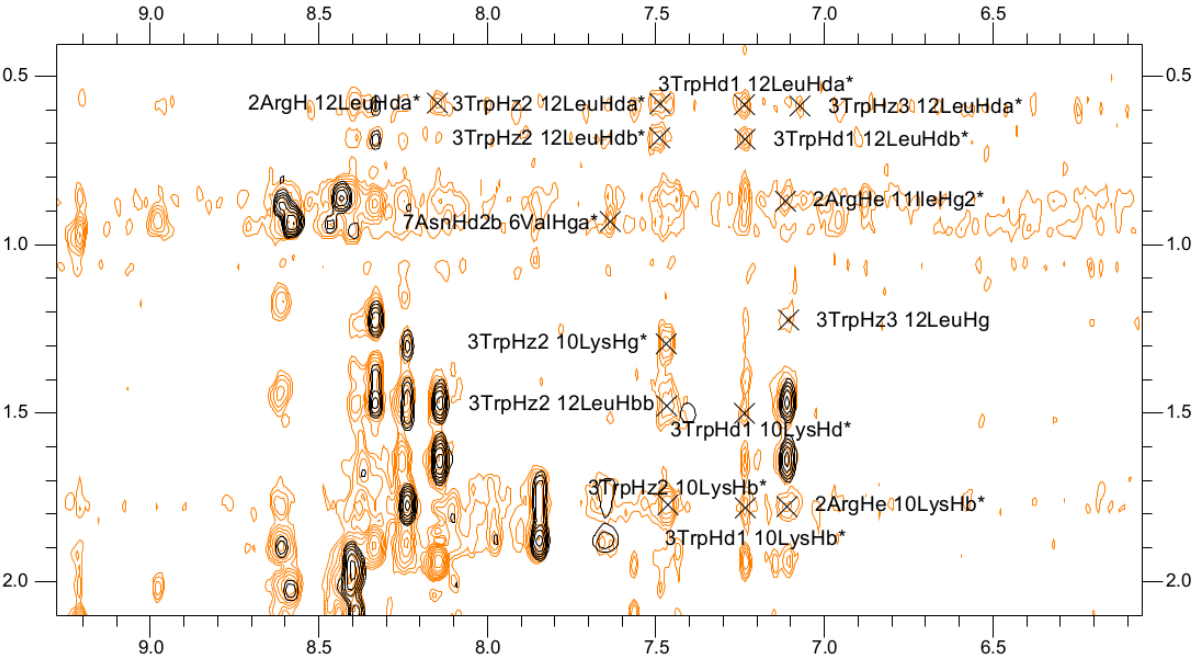
ROESY Spectrum of **2**



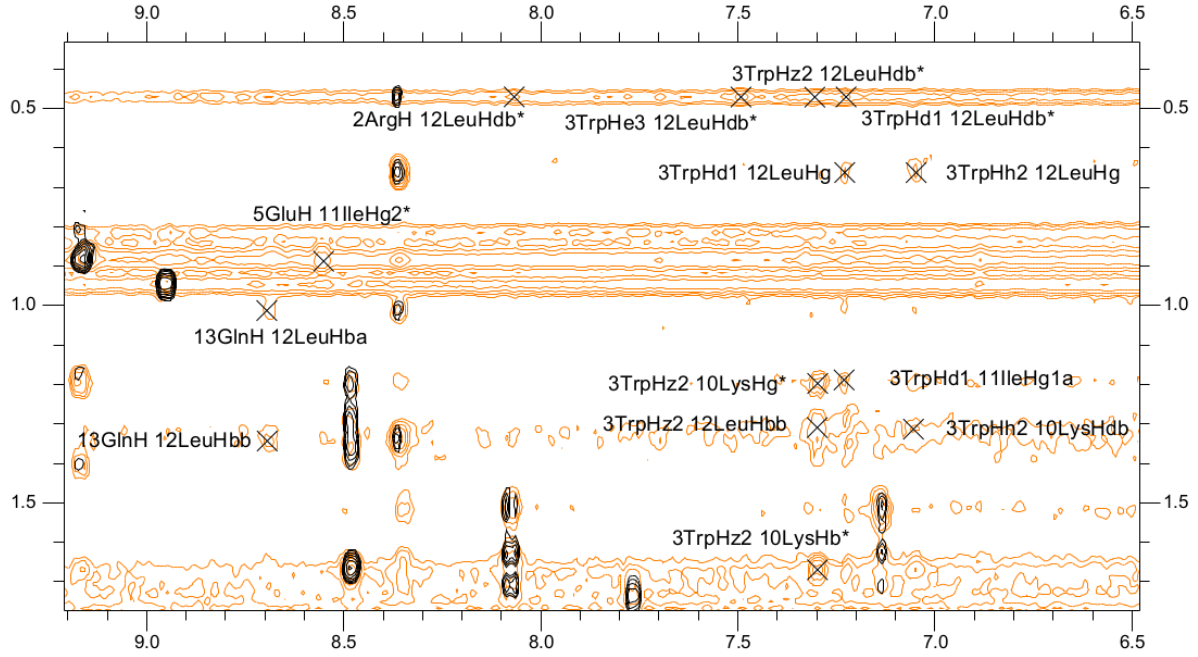
ROESY Spectrum of **7aa**



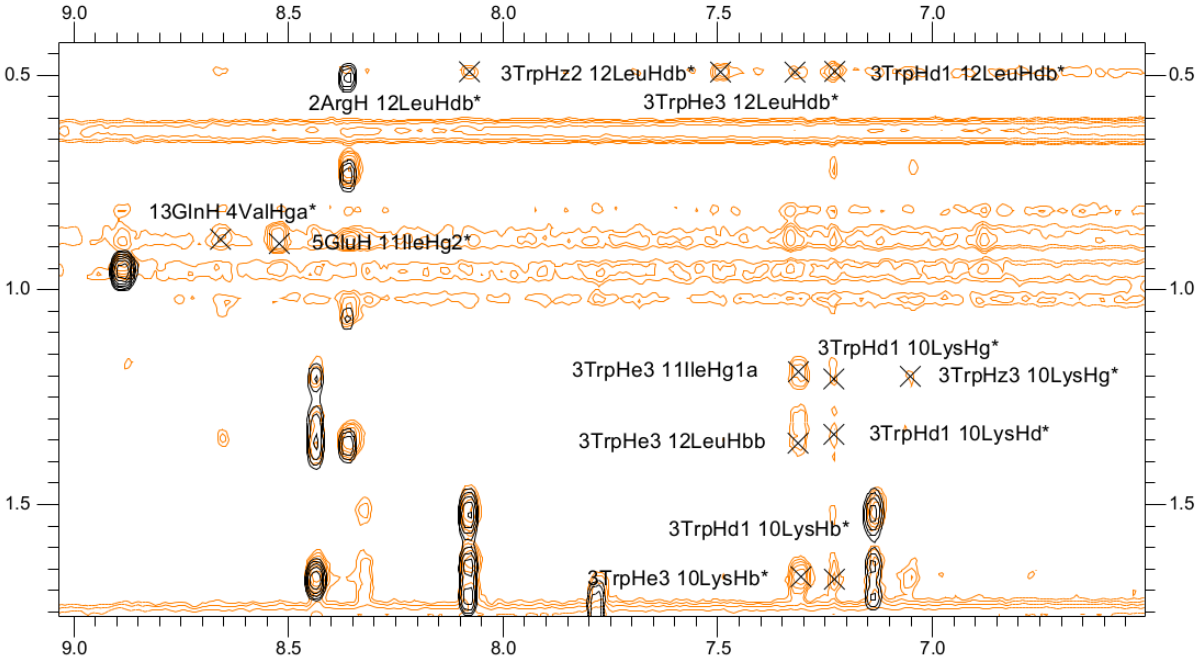
ROESY Spectrum of **7ab**



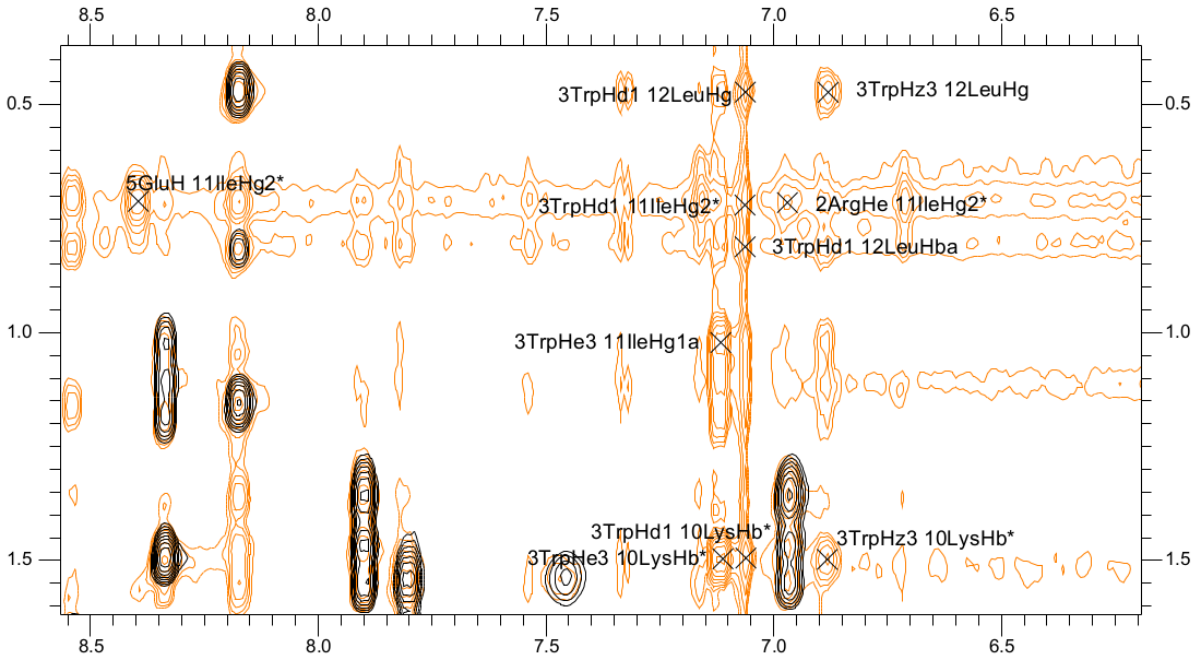
ROESY Spectrum of **7ba**



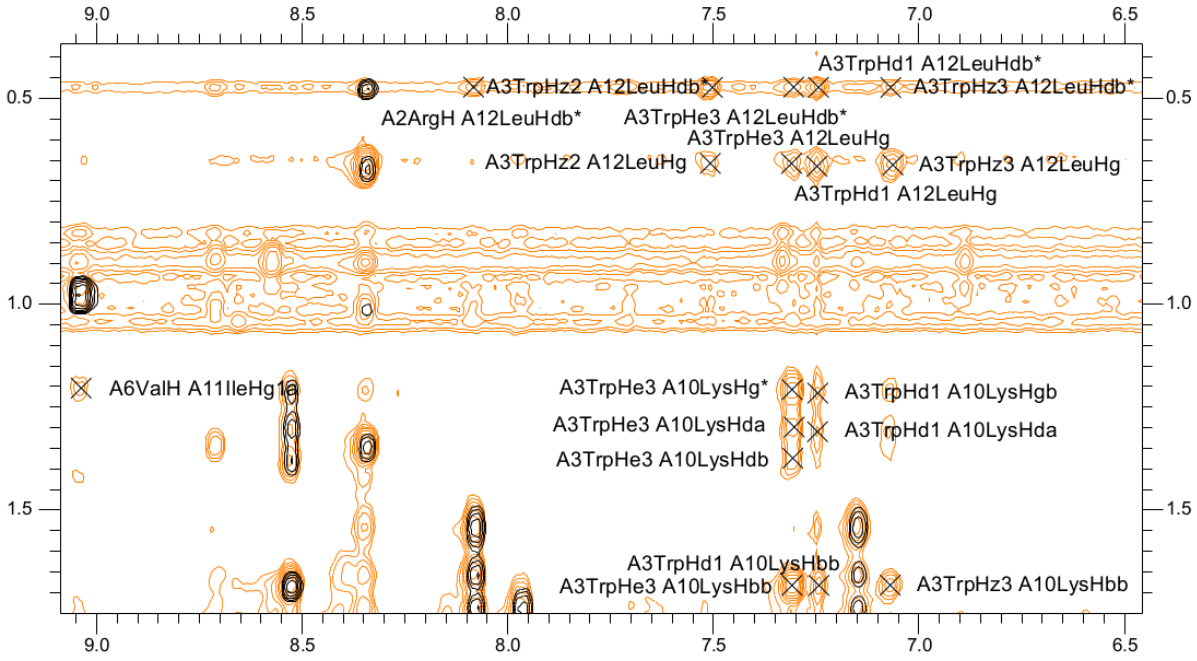
ROESY Spectrum of **7bb**



ROESY Spectrum of **7ca**



ROESY Spectrum of **7cb**



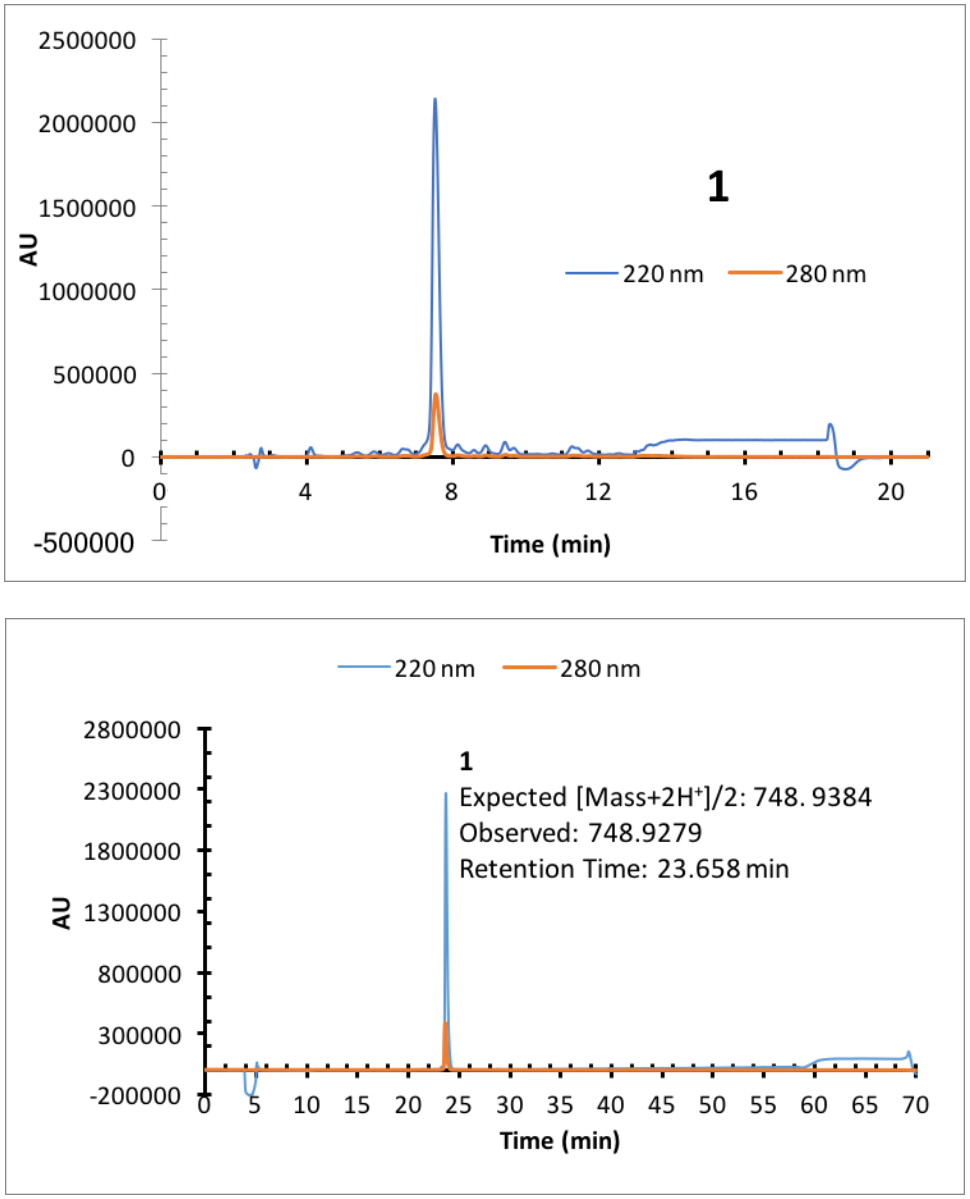


Figure S15. HPLC trace of crude peptide **1** (top). Analytical HPLC trace of peptide **1** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

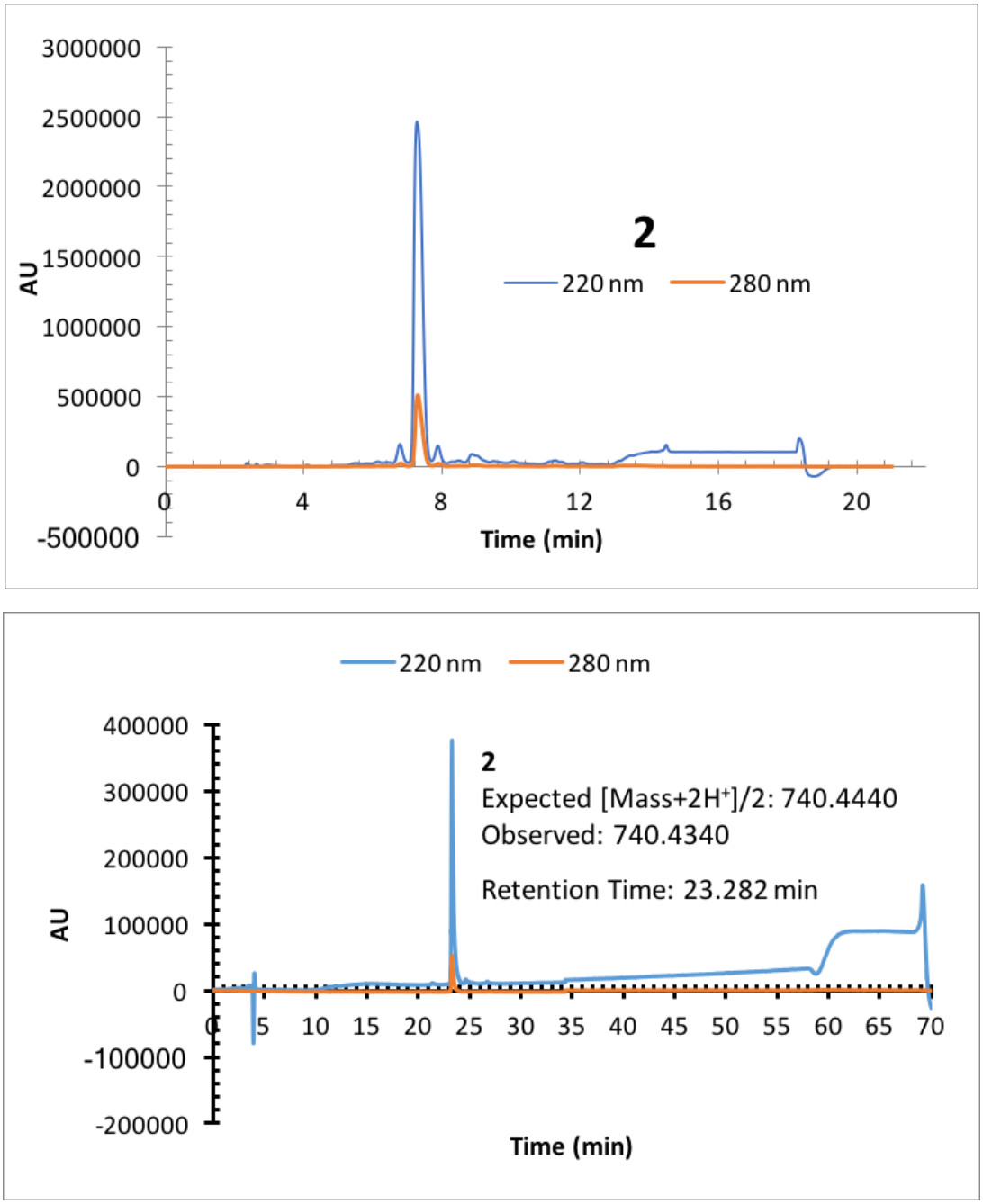


Figure S16. HPLC trace of crude peptide **2** (top). Analytical HPLC trace of peptide **2** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

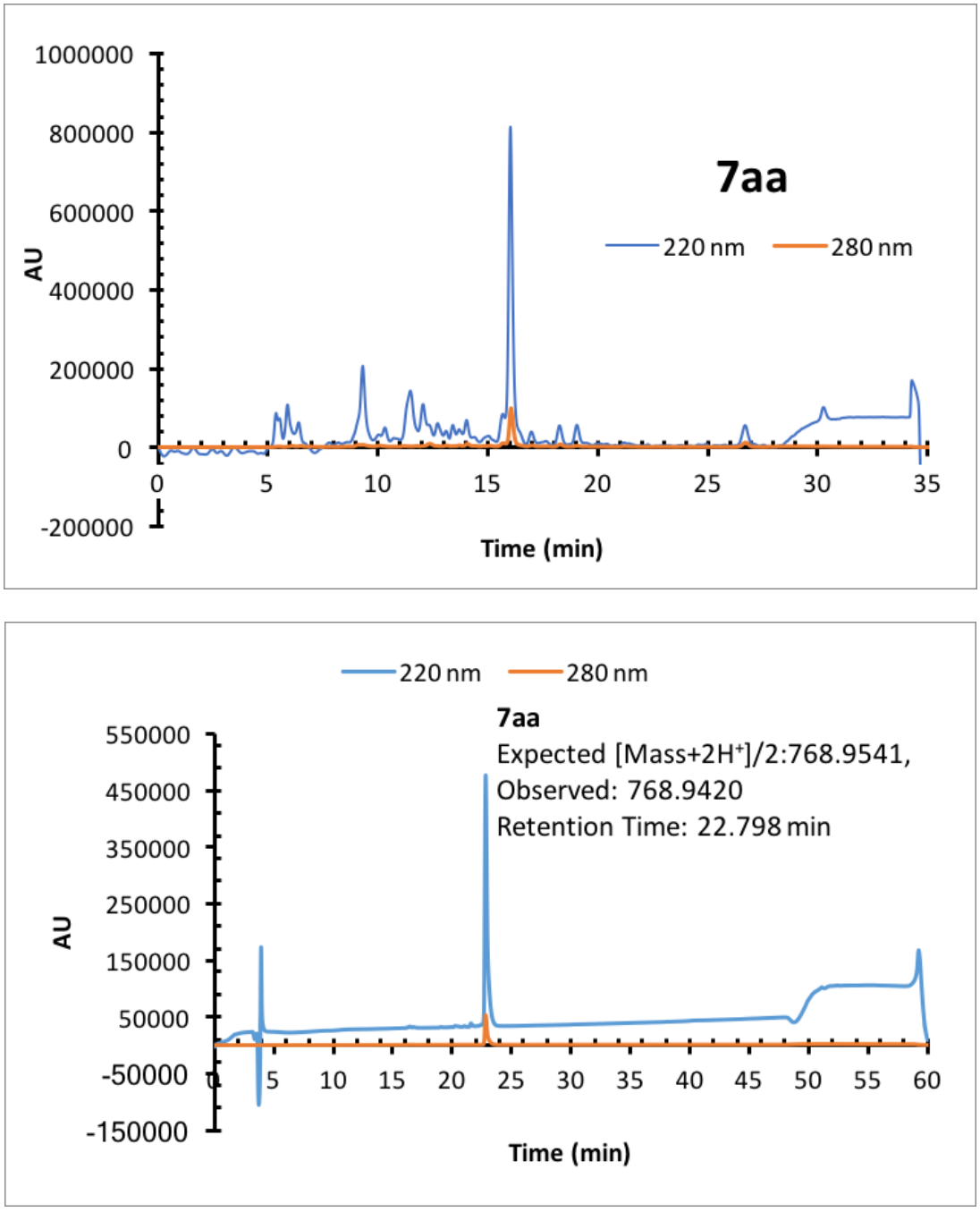


Figure S17. HPLC trace of crude peptide **7aa** (top). Analytical HPLC trace of peptide **7aa** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 20–60% CH_3CN in H_2O (constant 0.1% TFA) over 40 min.

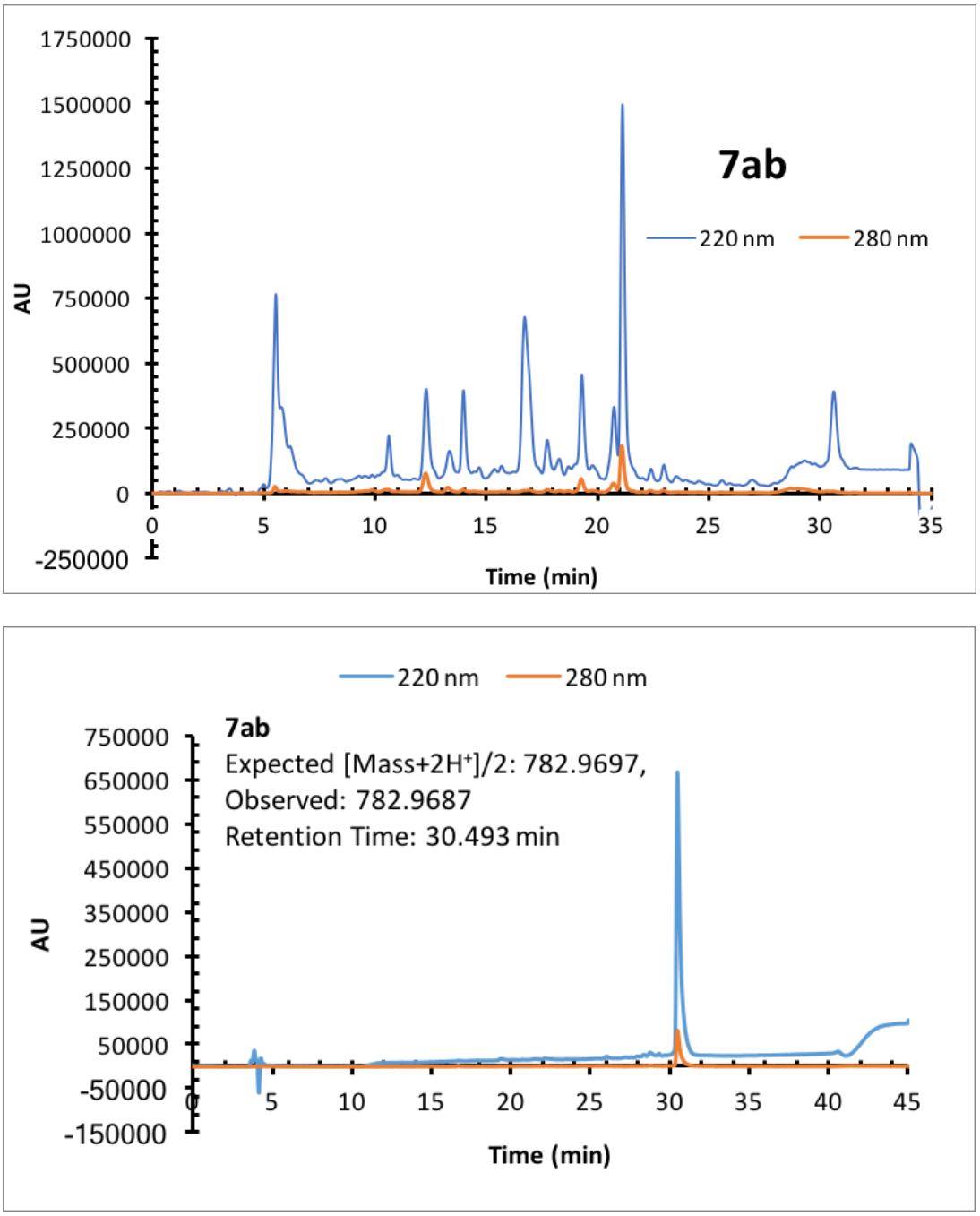
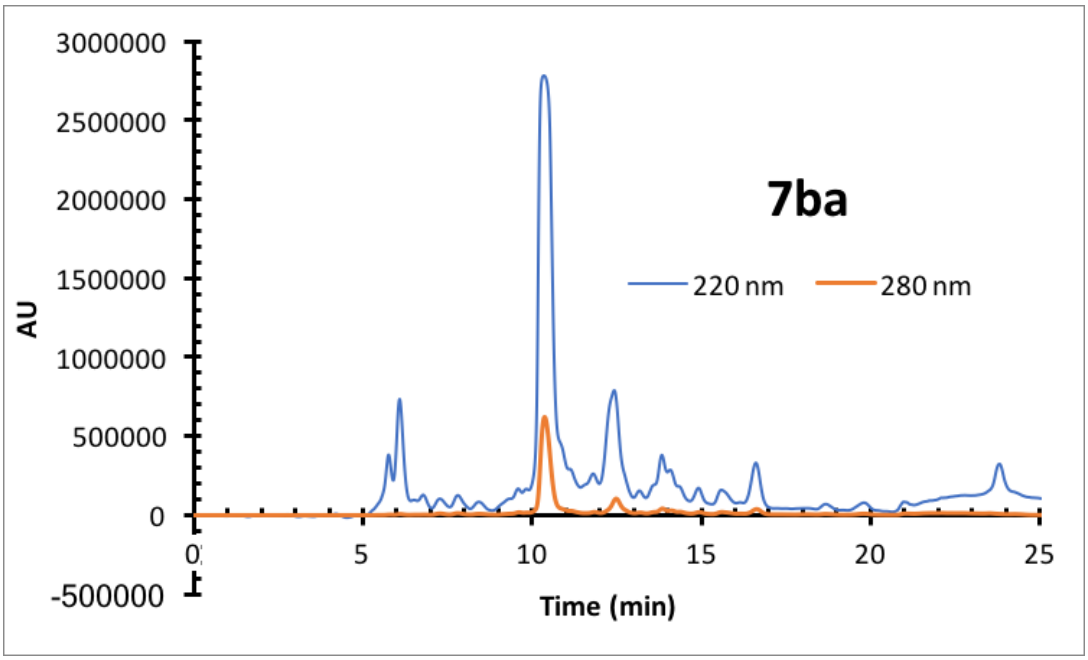


Figure S18. HPLC trace of crude peptide **7ab** (top). Analytical HPLC trace of peptide **7ab** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 38 min (run was terminated ~15 min after the product eluted).



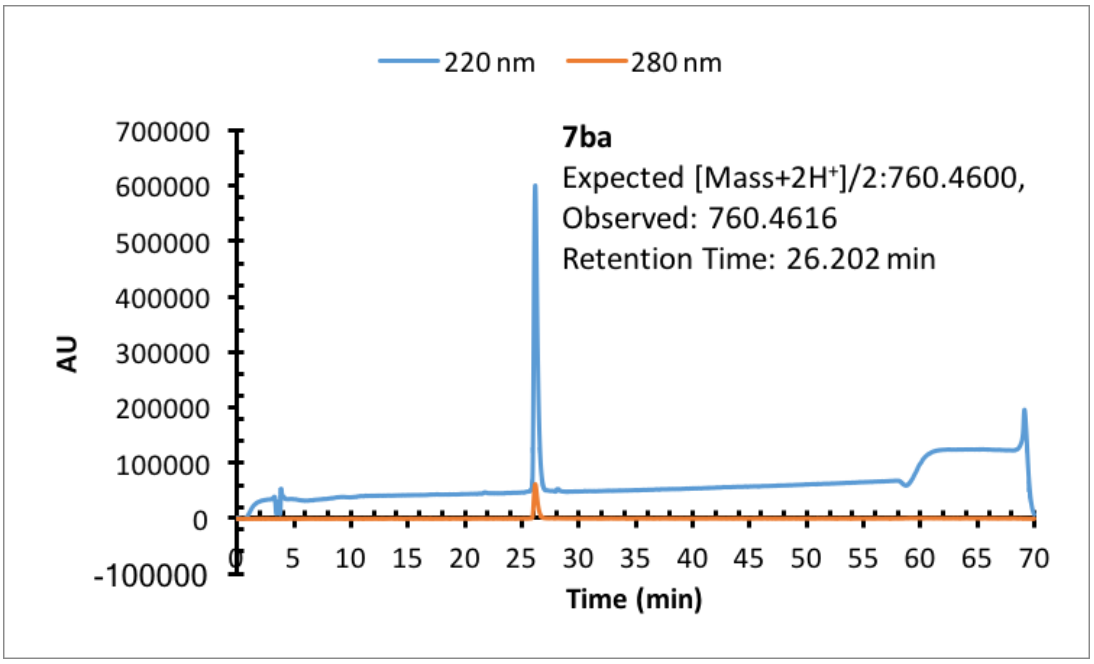


Figure S19. HPLC trace of crude peptide **7ba** (top). Analytical HPLC trace of peptide **7ba** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

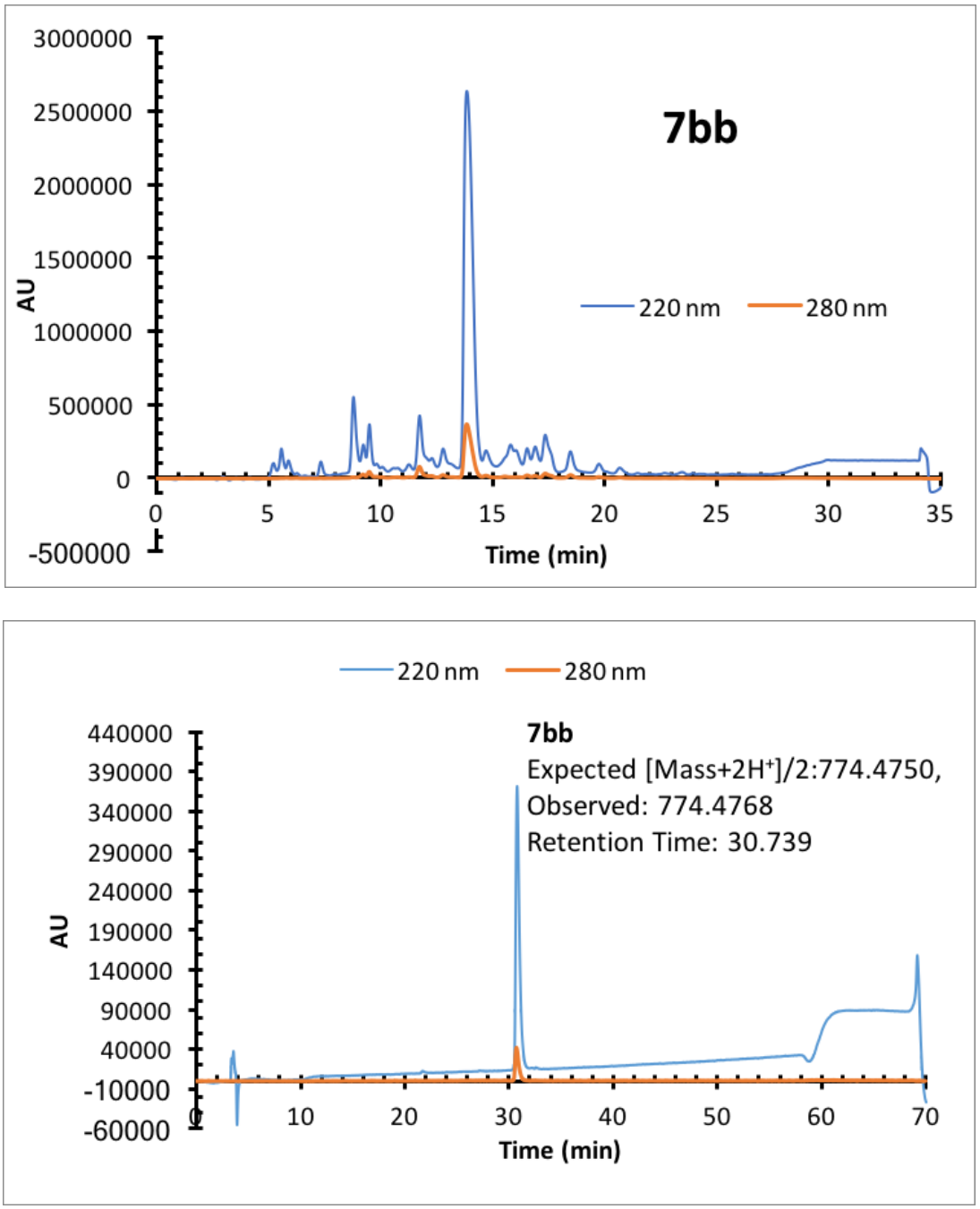


Figure S20. HPLC trace of crude peptide **7bb** (top). Analytical HPLC trace of peptide **7bb** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

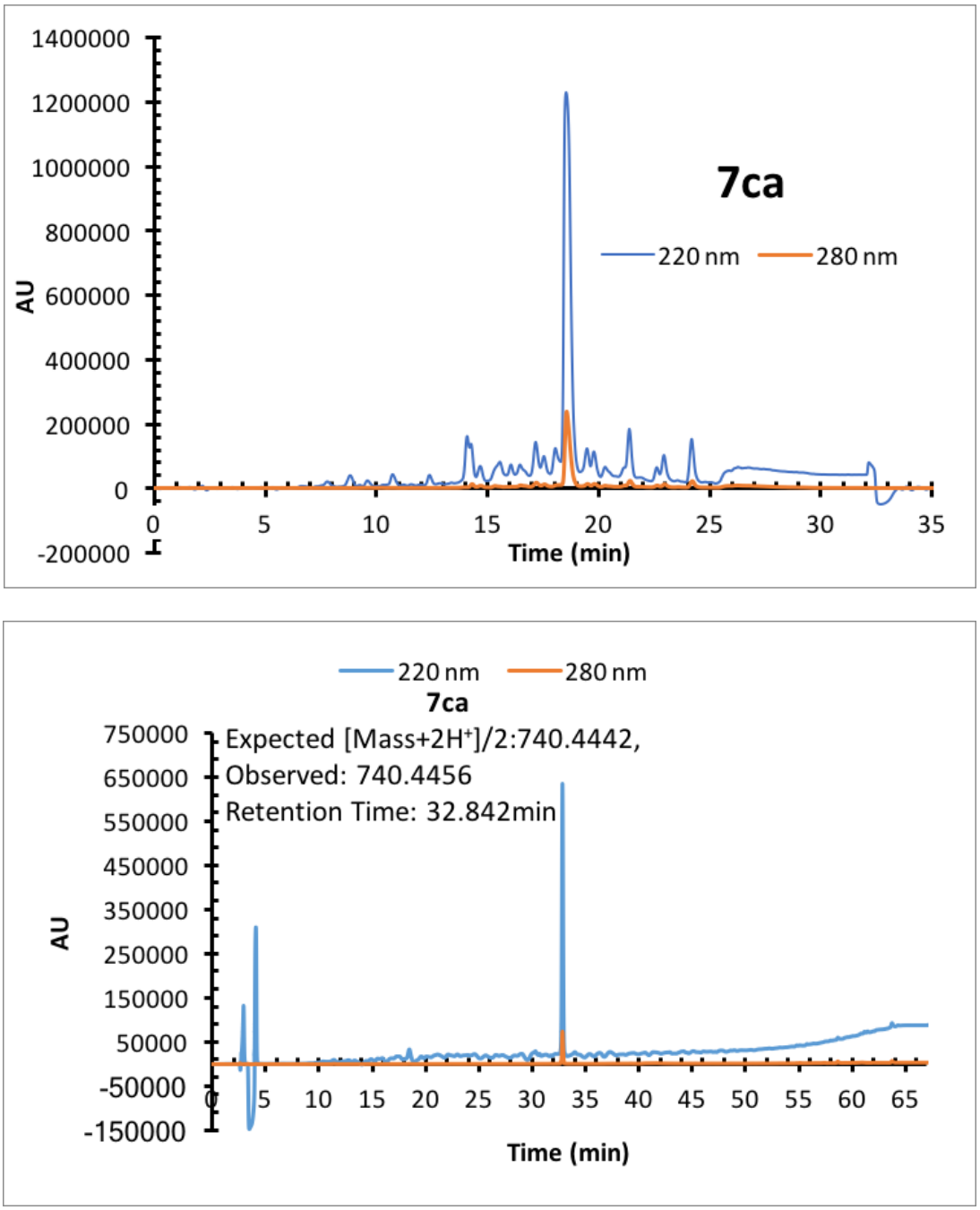


Figure S21. HPLC trace of crude peptide **7ca** (top). Analytical HPLC trace of peptide **7ca** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

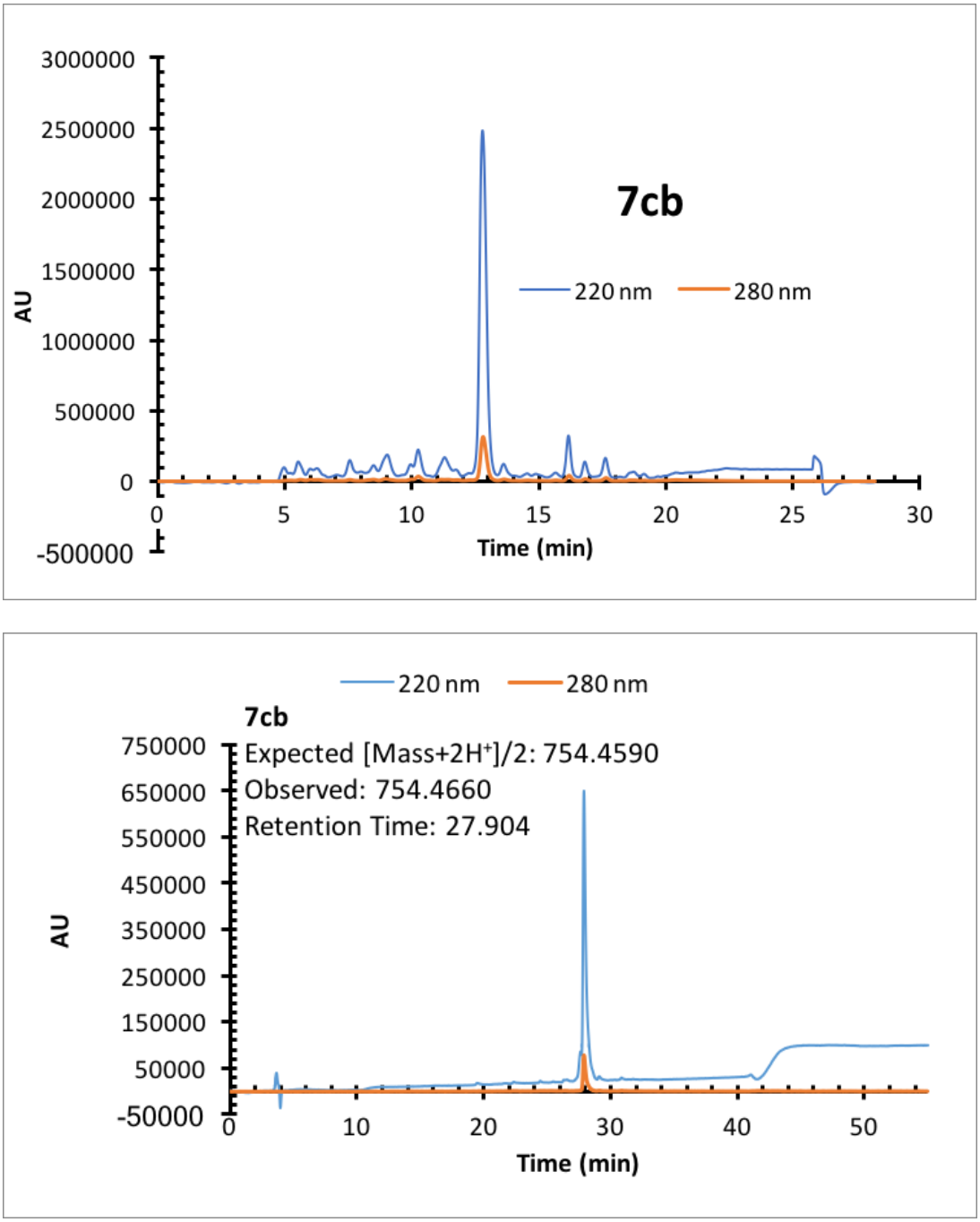


Figure S22. HPLC trace of crude peptide **7cb** (top). Analytical HPLC trace of peptide **7cb** after preparative purification (bottom). Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min (run was terminated ~25 min after the product eluted).

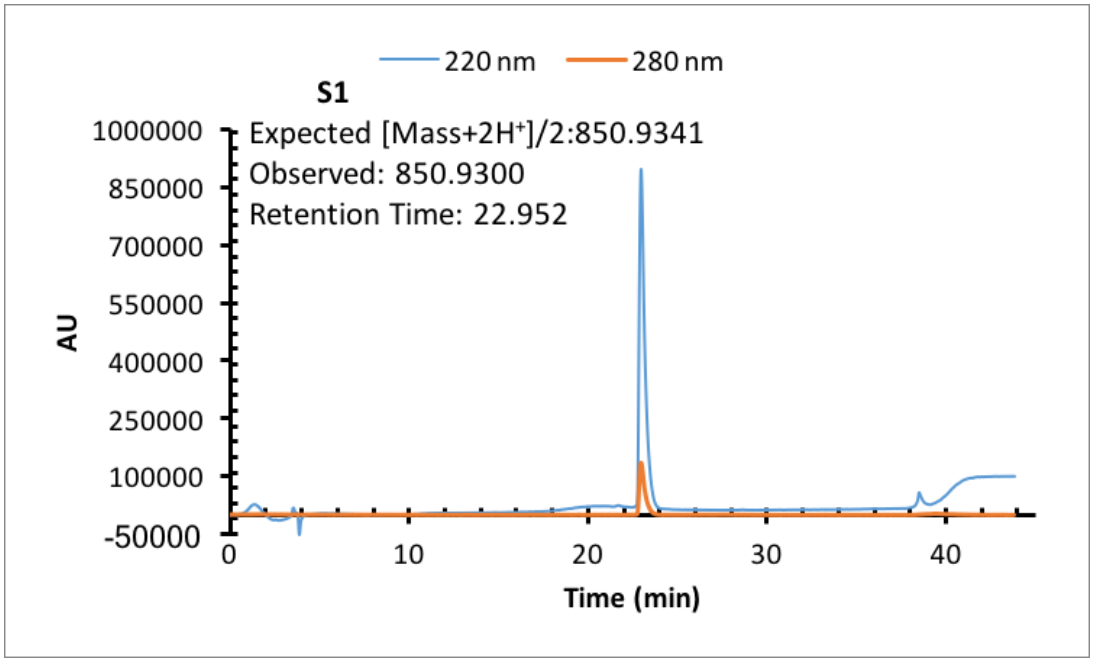


Figure S23. Analytical HPLC trace of peptide **S1** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–40% CH_3CN in H_2O (constant 0.1% TFA) over 25 min.

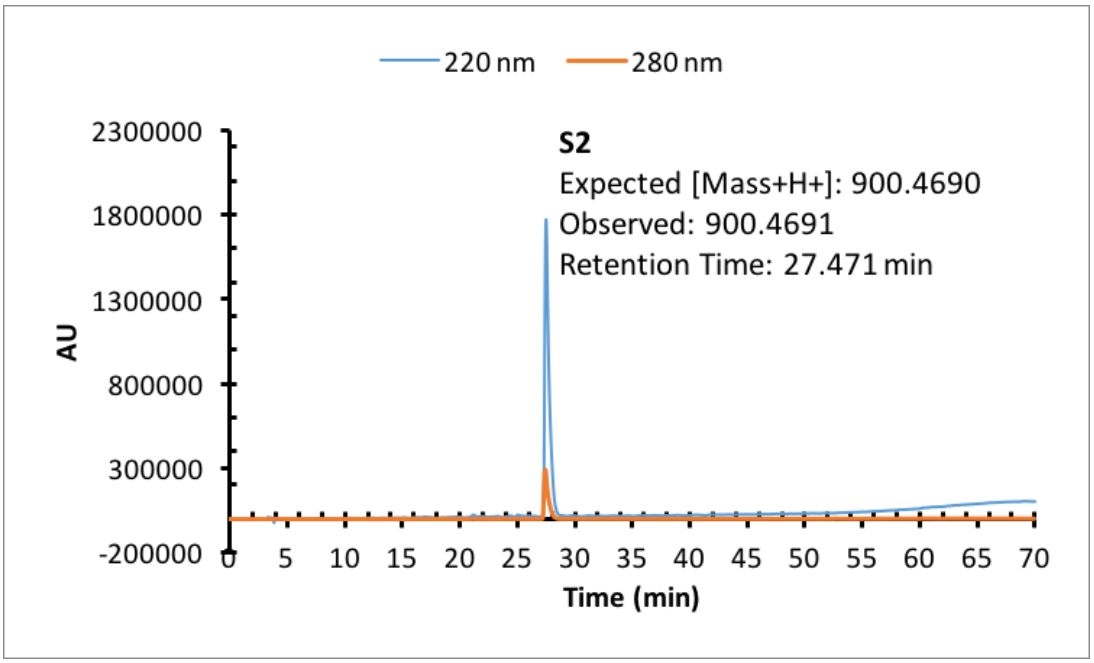


Figure S24. Analytical HPLC trace of peptide **S2** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

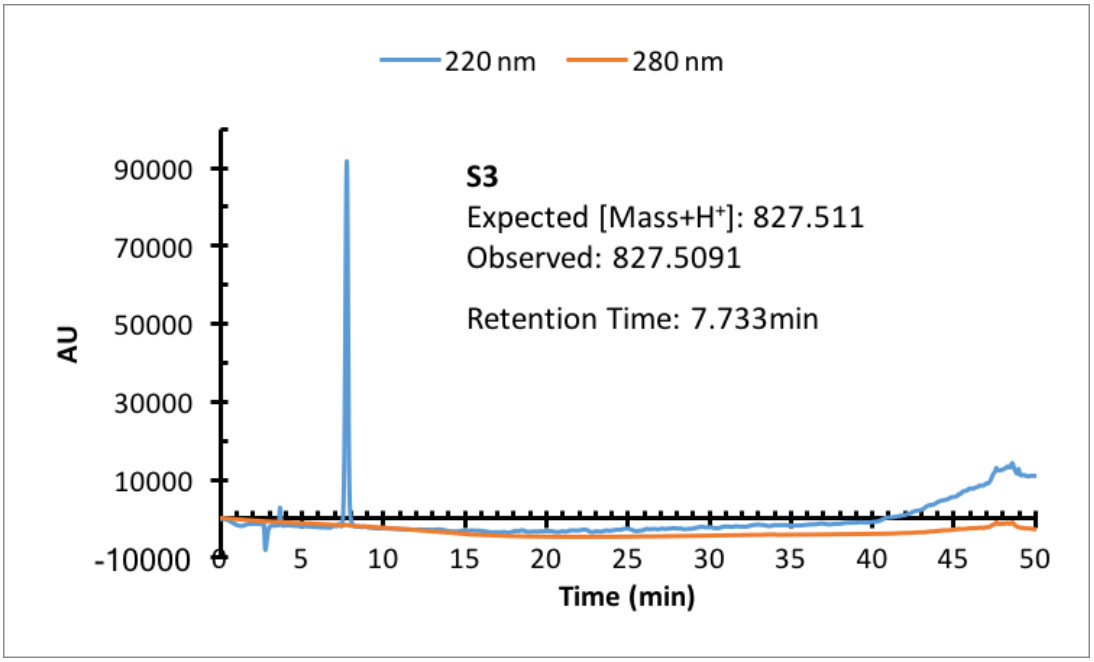


Figure S25. Analytical HPLC trace of peptide **S3** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–28% CH_3CN in H_2O (constant 0.1% TFA) over 35 min.

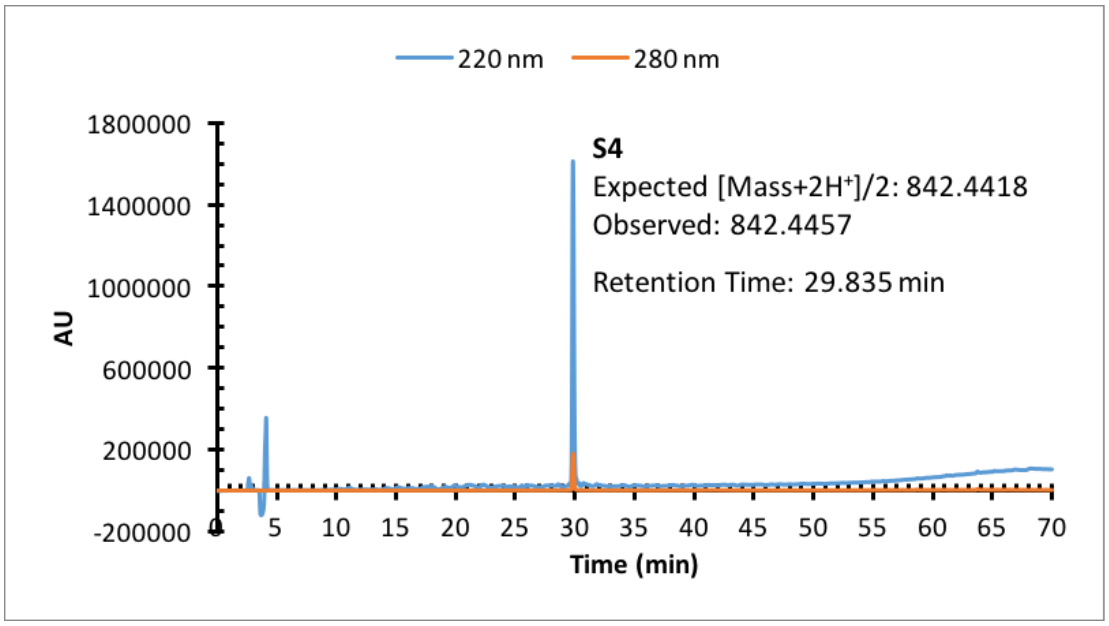


Figure S26. Analytical HPLC trace of peptide **S4** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

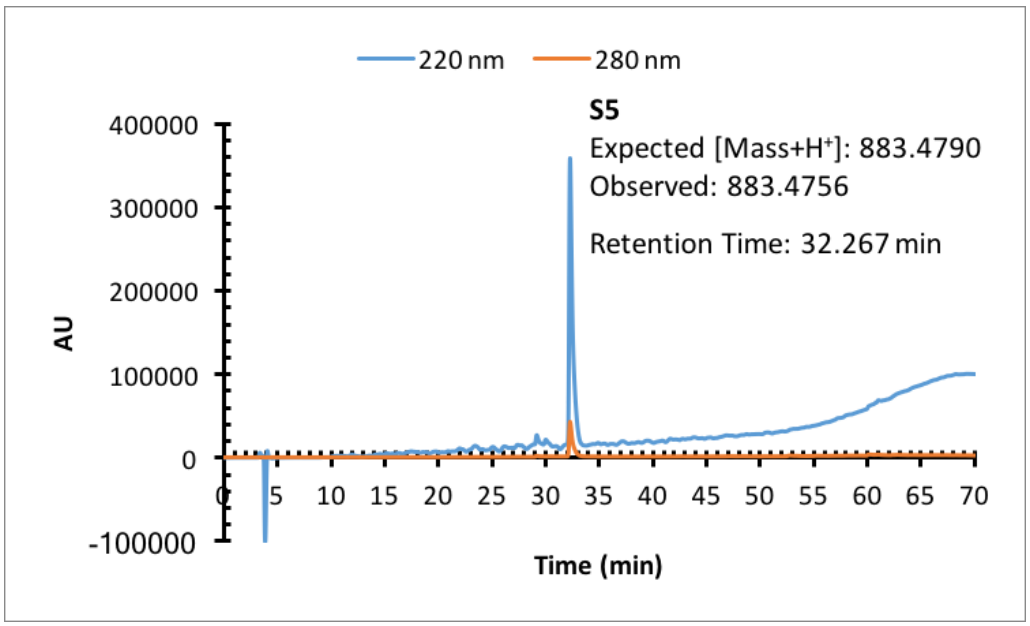


Figure S27. Analytical HPLC trace of peptide **S5** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–60% CH_3CN in H_2O (constant 0.1% TFA) over 50 min.

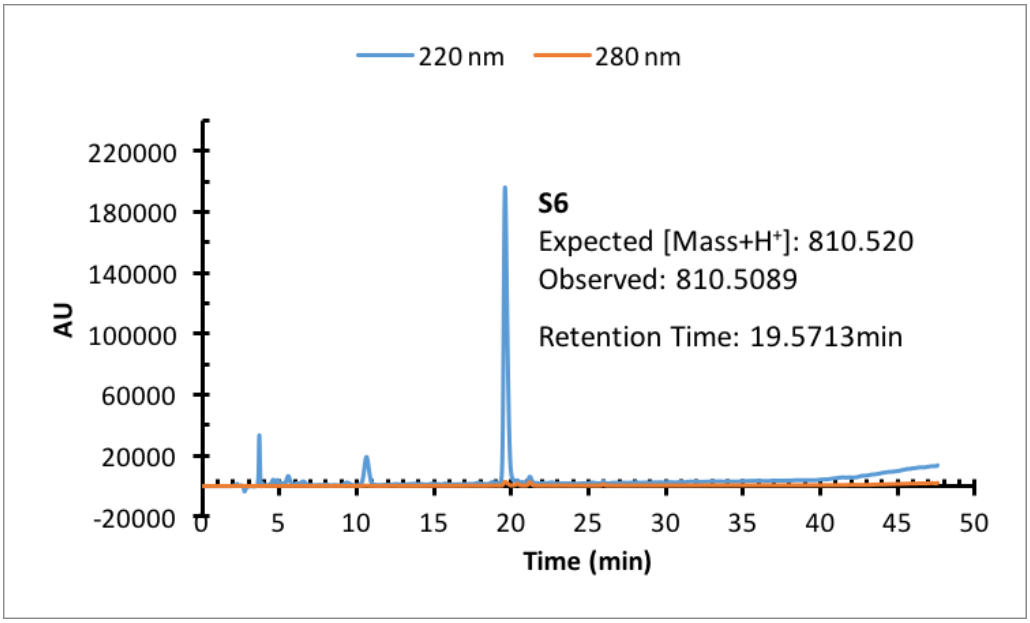


Figure S28. Analytical HPLC trace of peptide **S6** after preparative purification. Sample was injected onto a C18 analytical column (4.6 mm × 25 cm) and eluted using a linear gradient of 10–28% CH_3CN in H_2O (constant 0.1% TFA) over 35 min (run was terminated ~25 min after the product eluted).