SUPPORTING INFORMATION

Consequences of Depsipeptide Substitution on the ClpP Activation Activity of Antibacterial Acyldepsipeptides

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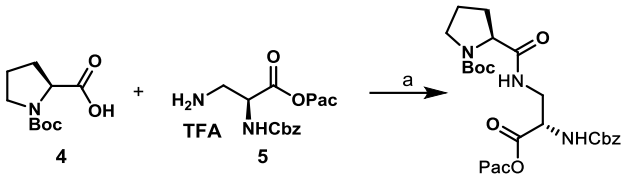
GENERAL EXPERIMENTAL PROCEDURES

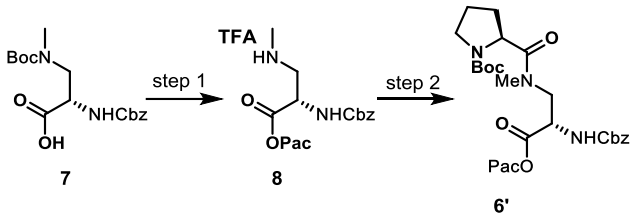
For all synthetic procedures below, H_2O means distilled H_2O unless otherwise indicated. All characterized compounds were purified by preparative thin layer chromatography (SiO_2), eluted with 5% MeOH in DCM, concentrated *in vacuo*, and dried under high vac for ~14 h prior to NMR characterization. NMR samples were prepared in 5 mm tubes with 0.6 mL deuterated solvent. NMR data were all collected on a 300, 400, or 500 MHz (specified below) Varian VNMRS Direct Drive spectrometer equipped with an indirect detection probe. Data was collected at 25 ^oC unless otherwise indicated. Pulse sequences were used as supplied by Varian VNMRJ 4.2 software. All 2D data employed non-uniform sampling (NUS). All NMR data was processed in MestreNova v10. Peak positions are reported after reference centering on deuterated solvent of relevance.

SYNTHESIS



Synthesis of compound 6. HATU (1.2 mmol, 455 mg) was added to a solution of **4** (1.09 mmol, 234 mg) in DMF (2 mL) at 0 °C under an N_2 atmosphere. A pre-mixed solution of **5** (1.09 mmol, 513 mg) and DIPEA (3.27 mmol, 421 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir 12 h at 25 °C under an N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **5** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 30-50% EtOAc in Hexane to yield **6** (507 mg, 84%). ^1H NMR ($CDCl_3$, 500 MHz, mixture of rotamers) δ 7.99 – 7.87 (m, 2H), 7.78 (bs, 0.5H)* 7.68 – 7.60 (m, 1H), 7.56 – 7.47 (m, 2H), 7.39 – 7.23 (m, 5H), 6.27 (bs, 0.5H), 5.93 (bs, 0.5H), 5.71 – 5.54 (m, 1H), 5.35 – 5.25 (m, 1H), 5.18 – 5.02 (m, 2H), 4.69 – 4.52 (m, 1H), 4.33 – 4.15 (m, 2H), 3.71 – 3.53 (m, 2H), 3.52 – 3.31 (m, 1H), 2.25 – 2.02 (m, 2H), 2.02 – 1.81 (m, 2H), 1.44 – 1.24 (m, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz, mixture of rotamers) δ 192.9, 192.6, 175.3, 174.0, 170.2, 169.7, 156.4, 156.1, 155.3, 154.6, 136. 6, 136.3, 134.5, 133.6, 133.5, 129.1, 128.4, 128.1, 127.9, 80.4, 80.2, 67.0, 66.8, 66.7, 61.5, 61.0, 55.3, 54.8, 47.2, 46.9, 40.9, 38.7, 31.5, 30.0, 29.7, 28.4, 28.2, 24.6, 23.7. IR for mixture (film)ν_{max} 3851, 3743, 3647, 3566, 3336, 3062, 3032, 2976, 2953, 2933, 2879, 1759, 1749, 1697, 1681, 1653, 1519, 1508, 1454, 1386, 1367, 1234, 1165, 1192, 1124, 1085, 1070, 966, 920, 775 cm^{-1}; HRESI m/z 576.2316 ($C_{29}H_{35}N_3O_8 + Na^+$ requires 576.2322).

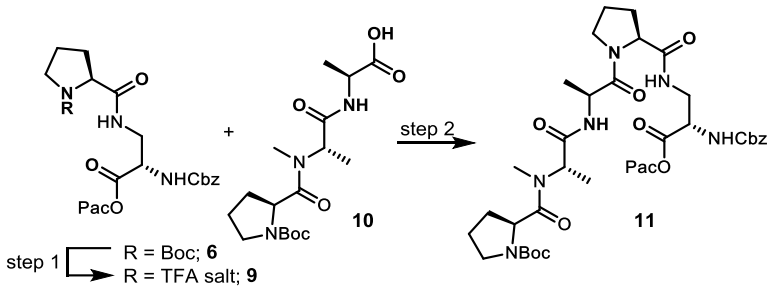
Experimental Note: Peaks labeled with * in the characterization data are exchangeable NH protons from different rotamers.



Synthesis of compound 6'.

Step 1: Neat 2-bromoacetophenone (1.42 mmol, 281 mg) and K_2CO_3 (1.42 mmol, 196 mg) were added to a stirring solution of **7** (1.42 mmol, 500 mg) in anhydrous acetone (10 mL) at 25 °C. The reaction mixture was refluxed for 4 h. Upon completion of the reaction, as indicated via complete conversion of **7** by thin-layer chromotography, the solvent was removed and the residue was partitioned between H_2O (50 mL) and EtOAc (50 mL). The organic layer was collected and the aqueous layer was extracted twice with EtOAc (100 mL). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dissolved in anhydrous DCM (5 mL) and anhydrous TFA (4.25 mL) was added dropwise to this solution at 0 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of starting material by thin-layer chromotography, the solvent was removed via an N_2 stream to yield **8** as the crude TFA salt, which was used without further purification.

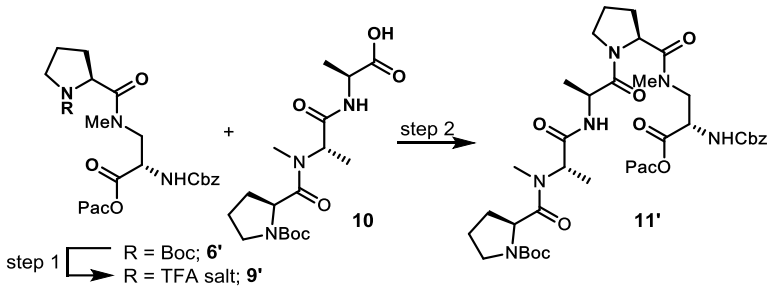
Step 2: HATU (1.56 mmol, 593 mg) was added to a solution of **4** (1.42 mmol, 305 mg) in DMF (2 mL) at 0 °C under an N_2 atmosphere. A pre-mixed solution of **8** (1.42 mmol, 690 mg) and DIPEA (4.26 mmol, 549 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir 12 h at 25 °C under an N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **8** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 30-50% EtOAc in Hexane to yield **6'** (669 mg, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz, mixture of rotamers) δ 7.90 – 7.72 (m, 2H), 7.61 – 7.50 (bs, 1H), 7.47 – 7.36 (m, 2H), 7.34 – 7.15 (m, 5H), 6.41 (bs, 1H), 5.53 – 5.35 (m, 1H), 5.31 – 5.15 (m, 1H), 5.02 (s, 2H), 4.65 – 4.41 (m, 2H), 3.97 – 3.72 (m, 2H), 3.62 – 3.23 (m, 2H), 3.15 – 2.80 (m, 3H), 2.10 – 1.67 (m, 4H), 1.42 – 1.21 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of rotamers) δ 191.6, 175.1, 174.6, 170.2, 170.1, 169.3, 156.4, 156.4, 154.5, 153.7, 136.6, 136.2, 134.6, 134.2, 134.0, 133.9, 129.11, 129.0, 129.0, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 79.7, 79.7, 67.0, 66.8, 66.7, 56.7, 56.6, 54.5, 54.0, 50.5, 50.4, 46.8, 46.6, 38.7, 37.0, 36.7, 30.3, 29.3, 28.5, 28.3, 24.3, 23.5. IR for mixture (film)ν_{max} 3819, 3743, 3722, 3647, 2972, 2954, 2929, 1757, 1749, 1714, 1683, 1697, 1670, 1653, 1635, 1558, 1541, 1519, 1506, 1396, 1338, 1174, 1120, 1083, 756 cm^{-1}.



Synthesis of compound 11.

Step 1: Anhydrous TFA (4.25 mL) was added dropwise to a solution of a **6** (1.09 mmol, 603 mg) in anhydrous DCM (6 mL) at 0 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **6** by thin-layer chromotography, the solvent was removed to yield **9** as the crude TFA salt, which was used without further purification.

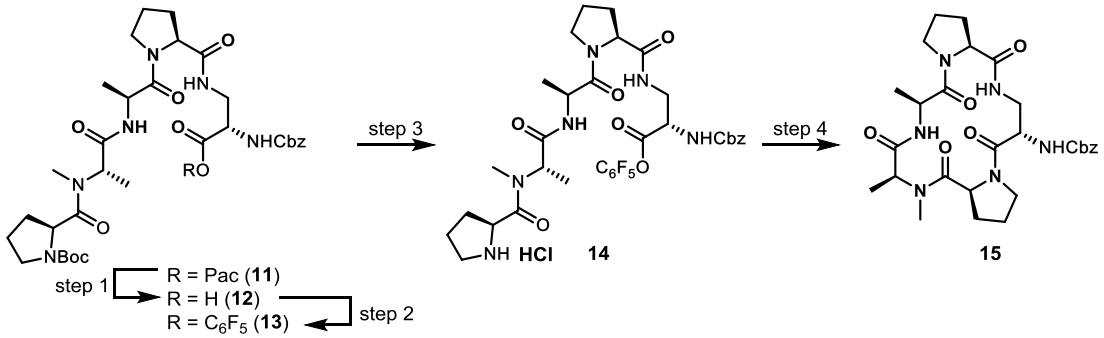
Step 2: HATU (1.2 mmol, 456 mg) was added to a solution of a **10** (1.09 mmol, 404 mg) in DMF (2 mL) at 0 °C under an N_2 atmosphere. A pre-mixed solution of **9** (1.09 mmol, 619 mg) and DIPEA (3.27 mmol, 421 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **9** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-20% acetone in EtOAc to yield **11** (642 mg, 73%). ^{1}H NMR (500 MHz, CD_3CN, 20 °C, major rotamer annotated) δ 7.97 (dd, *J* = 1.4, 7.3 Hz, 2H), 7.69 (tt, *J* = 1.4, 7.6 Hz, 1H), 7.55 (t, *J* = 7.3 Hz, 2H), 7.40 – 7.35 (m, 4H), 7.34 – 7.30 (m, 1H), 7.18 – 7.02 (m, 1H), 6.78 (d, *J* = 7.3 Hz, 1H), 6.34 (d, *J* = 8.3 Hz, 1H), 5.59 (d, *J* = 16.8 Hz, 1H), 5.39 (d, *J* = 16.8 Hz, 1H), 5.15 – 5.03 (m, 3H), 4.60 – 4.47 (m, 3H), 4.29 (dd, *J* = 4.2, 8.5 Hz, 1H), 3.93 – 3.85 (m, 1H), 3.66 – 3.58 (m, 2H), 3.57 – 3.47 (m, 1H), 3.46 – 3.30 (m, 2H), 3.07 – 2.46 (m, 3H), 2.26 – 2.14 (m, 1H), 2.14 – 1.95 (m, 3H), 1.93 – 1.85 (m, 3H), 1.85 – 1.72 (m, 1H), 1.49 – 1.30 (m, 9H), 1.25 (d, *J* = 7.1 Hz, 3H), 1.20 – 1.15 (m, 3H). ^{13}C NMR (CD_3CN, 101 MHz, mixture of rotamers) δ 194.1, 194.0, 193.7, 174.6, 174.5, 173.9, 173.9, 173.7, 173.4, 173.1, 172.6, 172.5, 172.4, 172.4, 172.1, 171.9, 171.9, 171.2, 171.0, 171.0, 171.0, 170.8, 157.0, 155.4, 154.9, 154.5, 137.9, 135.3, 135.3, 134.6, 129.9, 129.9, 129.4, 129.4, 128.9, 128.9, 128.8, 128.7, 128.7, 80.7, 79.7, 79.6, 68.0, 67.9, 67.2, 67.2, 67.1, 61.8, 61.7, 61.6, 57.9, 57.8, 55.9, 55.8, 55.4, 55.4, 55.1, 53.2, 52.6, 52.4, 49.1, 48.1, 47.8, 47.8, 47.7, 47.6, 47.4, 41.6, 41.4, 41.3, 32.3, 31.2, 30.8, 30.8, 30.7, 30.7, 29.9, 29.9, 29.8, 28.7, 28.7, 28.6, 28.6, 28.5, 25.7, 25.7, 25.6, 25.5, 24.8, 24.0, 24.0, 22.8, 18.2, 17.9, 17.8, 17.0, 15.0, 13.8, 13.8, 13.6, 13.6.IR for mixture (film)ν_{max} 3309, 3062, 2976, 2935, 2879, 2245, 1751, 1681, 1647, 1523, 1508, 1454, 1408, 1365, 1234, 1166, 1085, 1070, 966, 920 cm^{-1}; HRESI m/z 829.3751 ($C_{41}H_{54}N_6O_{11}$ + Na^+ requires 829.3748).



Synthesis of compound 11'.

Step 1: Anhydrous TFA (4.6 mL) was added dropwise to a solution of a **6'** (1.18 mmol, 669 mg) in anhydrous DCM (8 mL) at 0 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **6'** by thin-layer chromotography, the solvent was removed to yield **9'** as the crude TFA salt, which was used without further purification.

Step 2: HATU (1.1 mmol, 418 mg) was added to a solution of **10** (1.0 mmol, 371 mg) in DMF (2 mL) at 0 °C under a N_2 atmosphere. A pre-mixed solution of **9'** (1.0 mmol, 581 mg) and DIPEA (3.0 mmol, 387 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **9'** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-20% acetone in EtOAc to yield **11'** (749 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$, 55 °C, mixture of rotamers) δ 8.40 – 8.12 (m, 1H), 7.99 – 7.78 (m, 2H), 7.70 – 7.54 (m, 1H), 7.54 – 7.40 (m, 2H), 7.41 – 7.16 (m, 5H), 6.77 – 6.56 (m, 1H), 6.28 – 6.06 (m, 1H), 5.67 – 4.96 (m, 6H), 4.95 – 4.26 (m, 5H), 4.21 – 3.28 (m, 7H), 3.26 – 3.08 (m, 2H), 3.05 – 2.85 (m, 2H), 2.83 – 2.66 (m, 1H), 2.41 – 1.61 (m, 9H), 1.53 – 1.15 (m, 11H). ^{13}C NMR ($CDCl_3$, 101 MHz, mixture of rotamers) δ 191.6, 173.7, 170.6, 170.2, 156.3, 154.7, 153.9, 136.8, 134.5, 134.1, 129.1, 128.6, 128.1, 127.9, 116.2, 80.5, 79.6, 67.1, 66.8, 57.0, 56.7, 55.5, 55.0, 54.1, 51.0, 47.9, 47.2, 46.9, 37.4, 30.5, 30.2, 29.8, 29.5, 28.6, 25.1, 24.3, 23.6, 18.0, 16.7, 14.9, 13.5 IR for mixture (film)ν_{max} 3317, 3062, 3034, 2978, 2939, 2877, 2245, 1751, 1649, 1523, 1508, 1452, 1409, 1365, 1249, 1166, 1124, 1083, 1068, 968, 920 cm^{-1}; HRESI m/z 843.3905 ($C_{42}H_{56}N_6O_{11}$ + Na^+ requires 843.3905).



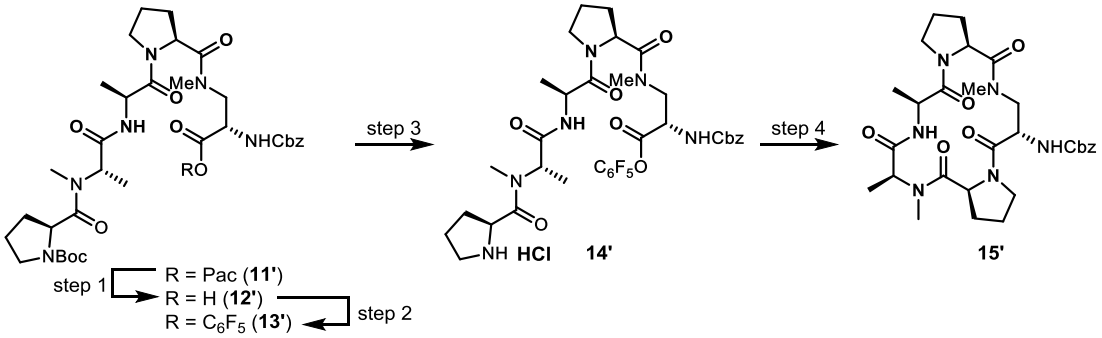
Synthesis of compound 15.

Step 1: Zinc dust (5 mmol, 325 mg) was added to a solution of **11** (0.5 mmol, 402 mg) in 70% aqueous acetic acid (4.2 mL) at 25 °C under a N_2 atmosphere, and the mixture was allowed to stir

12 h at 50 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **11** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and filtered through celite. The filtered zinc was then washed with additional EtOAc (50 mL) and H_2O (50 mL). The filtrate was transferred to a separatory funnel and partitioned between H_2O and EtOAc. The organic layer was subsequently washed three times with 1 M aq. HCl and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield a pale yellow oil. To remove residual acetic acid, the residue was diluted with toluene (20 mL) then concentrated *in vacuo*. This process was repeated a total of three times. The resulting crude pentapeptide free acid **12** was then used without further purification.

Step 2: The pentapeptide **12** was dissolved in DCM (4 mL) and cooled to -20 °C. Neat pentafluorophenol (2.5 mmol, 460 mg) was added followed by EDC•HCl (0.8 mmol, 153 mg). The mixture was stirred 12 h with gradual warming to RT. The solvent was removed *in vacuo* to reveal a pale yellow oily residue **13**, which was used without further purification.

Steps 3 & ***4:*** The crude pentafluorophenol ester **13** was treated with 4 M HCl in 1,4-dioxane (12 mmol, 3 mL) and allowed to react for 6 h at 25 °C. The reaction solution was then diluted with DCM (117 mL) and added dropwise at a rate of ~2 drops / second from an addition funnel into a flask containing a stirring solution of DCM (165 mL) and 1 M aq. $NaHCO_3$ (102 mL). The reaction stirred 12 h at 25 °C, after which, the solution was transferred to a separatory funnel and partitioned between H_2O and DCM. The aqueous layer was subsequently washed with fresh DCM (200 mL). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-30% acetone in EtOAc to yield **15** (173 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 9.8 Hz, 1H), 7.39 – 7.29 (m, 5H), 6.41 (dd, *J* = 2.5, 10.8 Hz, 1H), 5.99 (d, *J* = 8.6 Hz, 1H), 5.25 (dd, *J* = 3.2, 8.6 Hz, 1H), 5.07 (d, *J* = 12.1 Hz, 1H), 5.04 (d, *J* = 12.1 Hz, 1H), 4.99 (dq, *J* = 6.8, 9.8 Hz, 1H), 4.88 (q, *J* = 6.9 Hz, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 4.07 (dd, *J* = 9.1, 14.1 Hz, 1H), 4.02 (t, *J* = 9.1 Hz, 1H), 3.75 (ddd, *J* = 5.5, 7.8, 12.0 Hz, 1H), 3.71 – 3.59 (m, 2H), 3.54 (dt, *J* = 6.5, 7.5, 12.0 Hz, 1H), 2.79 (ddd, *J* = 2.5, 9.1, 14.1 Hz, 1H), 2.72 (s, 3H), 2.43 – 2.30 (m, 1H), 2.24 – 2.01 (m, 3H), 2.00 – 1.75 (m, 4H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.42 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 174.5, 173.1, 171.3, 170.2, 167.0, 157.4, 135.6, 128.7 (2C), 128.6 (2C), 128.5, 68.2, 60.7, 56.5, 56.4, 55.7, 48.8, 48.3, 46.7, 43.4, 31.7, 30.9, 30.8, 23.4, 21.6, 17.9, 15.8. IR (film)ν_{max} 3302, 3059, 2978, 2933, 2881, 1712, 1697, 1643, 1633, 1517, 1454, 1301, 1257, 1112, 1091, 1043, 1028, 1014, 995, 912 cm^{-1}; HRESI m/z 593.2700 ($C_{28}H_{38}N_6O_7$ + Na^+ requires 593.2700).

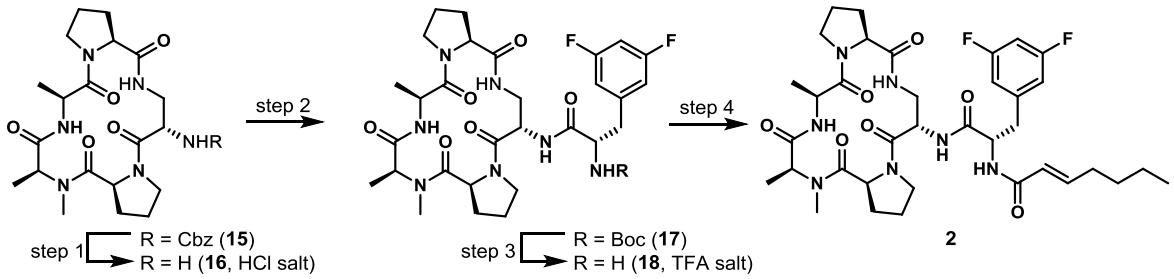


Synthesis of compound 15'.

Step 1: Zinc dust (7.15 mmol, 465 mg) was added to a solution of **11'** (0.614 mmol, 503 mg) in 70% aqueous acetic acid (5 mL) at 25 °C under a N_2 atmosphere, and the mixture was allowed to stir 12 h at 50 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **11'** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and filtered through celite. The filtered zinc was then washed with additional EtOAc (50 mL) and H_2O (50 mL). The filtrate was transferred to a separatory funnel and partitioned between H_2O and EtOAc. The organic layer was subsequently washed three times with 1 M aq. HCl and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield a pale yellow oil. To remove residual acetic acid, the residue was diluted with toluene (20 mL) then concentrated *in vacuo*. This process was repeated a total of three times. The resulting crude pentapeptide free acid **12'** was then used without further purification.

Step 2: The pentapeptide **12'** was dissolved in DCM (6 mL) and cooled to -20 °C. Neat pentafluorophenol (3.07 mmol, 565 mg) was added followed by EDC•HCl (0.982 mmol, 187 mg). The mixture was stirred 12 h with gradual warming to RT. The solvent was removed *in vacuo* to reveal a pale yellow oily residue **13'**, which was used without further purification.

Steps 3 & 4: The crude pentafluorophenol ester **13'** was treated with 4 M HCl in 1,4-dioxane (18 mmol, 4.5 mL) and allowed to react for 6 h at 25 °C. The reaction solution was then diluted with DCM (148 mL) and added dropwise at a rate of ~2 drops / second from an addition funnel into a flask containing a stirring solution of DCM (209 mL) and 1 M aq. $NaHCO_3$ (130 mL). The reaction stirred 12 h at 25 °C, after which, the solution was transferred to a separatory funnel and partitioned between H_2O and DCM. The aqueous layer was subsequently washed with fresh DCM (200 mL). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-30% acetone in EtOAc to yield **15'** (281 mg, 78%). ^{1}H NMR (500 MHz, CD_3CN, -19 °C, major rotamer annotated) δ 8.08 (d, *J* = 3.5 Hz, 1H), 7.43 – 7.24 (m, 5H), 6.63 (d, *J* = 9.9 Hz, 1H), 5.06 (d, *J* = 12.8 Hz, 1H), 5.02 (d, *J* = 12.8 Hz, 1H), 4.96 (d, *J* = 8.4 Hz, 1H), 4.81 (dd, *J* = 3.1, 9.0 Hz, 1H), 4.76 (q, *J* = 6.9 Hz, 1H), 4.43 (ddd, *J* = 4.1, 9.9, 12.0 Hz, 1H), 4.28 (qd, *J* = 3.6, 7.9 Hz, 1H), 3.71 (dd, *J* = 4.1, 15.8 Hz, 1H), 3.58 – 3.46 (m, 2H), 3.45 – 3.32 (m, 2H), 3.15 (dd, *J* = 12.0, 15.8 Hz, 1H), 2.88 (s, 3H), 2.63 (s, 3H), 2.51 – 2.41 (m, 1H), 2.40 – 2.30 (m, 1H), 1.98 – 1.95 (m, 1H), 1.89 – 1.78 (m, 3H), 1.77 – 1.69 (m, 1H), 1.66 – 1.51 (m, 1H), 1.40 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.9 Hz, 3H). ^{13}C NMR (CD_3CN, 101 MHz, -19 °C, mixture of rotamers) δ 174.5, 173.8, 172.1, 172.1, 171.7, 171.3, 171.2, 170.8, 170.6, 168.6, 168.5, 168.1, 156.7, 156.2, 137.5, 137.3, 129.3, 129.1, 129.1, 129.1, 129.0, 129.0, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.2, 67.6, 67.2, 67.1, 59.0, 58.5, 57.3, 55.9, 55.3, 55.0, 54.1, 53.8, 51.1, 50.6, 49.2, 49.1, 48.7, 48.4, 47.5, 47.2. 38.6, 32.8, 32.7, 31.4, 31.0, 30.9, 30.2, 29.8, 29.7, 25.4, 23.2, 22.5, 21.6, 20.5, 17.3, 16.2, 15.6, 14.7. IR for mixture (film) ν_{max} 3294, 3059, 3032, 2976, 2953, 2937, 2881, 1712, 1658, 1643, 1633, 1531, 1512, 1494, 1446, 1435, 1246, 1157, 1112, 1062, 1045, 732, 698 cm^{-1}; HRESI m/z 607.2846 ($C_{29}H_{40}N_6O_7 + Na^+$ requires 607.2856).



Synthesis of compound 2.

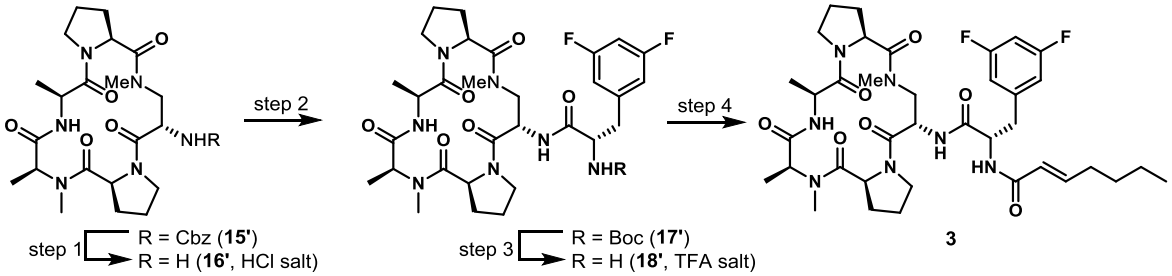
Step 1: 10% Pd/C (22 mg) was added to a solution of **15** (0.172 mmol, 98 mg) in methanol (2 mL), followed by 1M aq. HCl (0.21 mmol, 0.21 mL). With the reaction flask septum sealed, hydrogen gas was delivered to the reaction by a balloon affixed to a syringe. Upon completion of the reaction, as indicated via complete conversion of **15** by thin-layer chromotography, the reaction mixture was filtered through celite in order to remove the Pd/C. Removal of solvent *in vacuo* revealed the peptidolactam **16** as the hydrochloride salt (off-white amorphous solid), which is used without further purification.

Step 2: HATU (0.189 mmol, 72 mg) was added to a solution of Boc-difluorophenylalanine (0.172 mmol, 52 mg) in DMF (2 mL) at 0 °C under a N_2 atmosphere. A pre-mixed solution of **16** and DIPEA (0.516 mmol, 66 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir for 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **16** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to reveal **17** as an amorphous white solid, which was used without further purification.

Step 3: Compound **17** was treated with TFA (0.67 mL) in DCM (2 mL) at 0 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **17** by thin-layer chromotography, the reaction was concentrated with a stream of nitrogen and the resulting residue (**18**) was then stored on the high vac. for 6 h prior to use without further purification.

Step 4: HATU (0.189 mmol, 72 mg) was added to a stirring solution of E-2-heptenoic acid (0.172 mmol, 22 mg) in DMF (2 mL) at 0 °C under a N_2 atmosphere. A pre-mixed solution of **18** and DIPEA (0.516 mmol, 66 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir for 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **18** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-20% acetone in EtOAc to yield **2** as a white foam (95 mg, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (d, *J* = 9.8 Hz, 1H), 7.89 (d, *J* = 9.7 Hz, 1H), 7.79 (s, 1H), 7.61 (s, 1H), 6.72 (dt, *J* = 7.1, 15.0 Hz, 1H), 6.67 – 6.54 (m, 3H), 5.84 (d, *J* = 15.0 Hz, 1H), 5.50 (d, *J* = 8.4 Hz, 1H), 5.08 – 4.93 (m, 2H), 4.48 (t, *J* = 9.4 Hz, 1H), 4.31 (d, *J* = 8.3 Hz, 1H), 4.24 (m, 1H), 4.15 (dd, *J* = 10.5, 13.6 Hz, 1H), 3.91 – 3.79 (m, 1H), 3.56 – 3.32 (m, 3H), 3.27 (dd, *J* = 5.1, 14.3 Hz, 1H), 3.03 (dd, *J* = 9.0, 14.3 Hz, 1H), 2.80 – 2.59 (m, 1H), 2.64 (s, 3H), 2.50 – 2.37 (m, 1H), 2.25 – 2.09 (m, 2H), 2.09 – 1.84 (m, 7H), 1.51 (d, *J* = 6.8 Hz, 3H), 1.48 (d, *J* = 6.7 Hz, 3H),

1.45 – 1.26 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.9, 173.9, 172.0, 170.6, 169.8, 168.7, 166.2, 162.5 (dd, J=12.5, 248.6 Hz, 2C), 145.9, 143.4 (t, *J* = 9.2 Hz), 123.1, 112.3 (dd, *J* = 7.6, 24.2 Hz, 2C), 101.8 (t, *J* = 25.2 Hz), 60.8, 57.0, 56.4, 55.9, 54.6, 49.0, 48.6, 46.5, 43.9, 35.8, 32.1, 31.9, 31.0, 30.8, 30.4, 23.3, 22.4, 21.6, 17.9, 15.9, 14.0. IR (film)ν_{max} 3300, 2954, 2929, 2873, 2858, 2358, 2341, 1643, 1595, 1521, 1506, 1446, 1435, 1350, 1317, 1300, 1114, 985, 846 cm^{-1}; HRESI m/z 752.3555 ($C_{36}H_{49}F_2N_7O_7$ + Na^+ requires 752.3559). For specific correlations and peaks as determined by 2D methods please see **Figure S7** and **Table 1**, respectively**.**



Synthesis of compound 3.

Step 1: 10% Pd/C (33 mg) was added to a solution of **15'** (0.15 mmol, 87 mg) in methanol (2 mL), followed by 1M aq. HCl (0.3 mmol, 0.3 mL). With the reaction flask septum sealed, hydrogen gas was delivered to the reaction by a balloon affixed to a syringe. Upon completion of the reaction, as indicated via complete conversion of **15'** by thin-layer chromotography, the reaction mixture was filtered through celite in order to remove the Pd/C. Removal of solvent *in vacuo* revealed the peptidolactam **16'** as the hydrochloride salt (off-white amorphous solid), which is used without further purification.

Step 2: HATU (0.165 mmol, 63 mg) was added to a solution of Boc-difluorophenylalanine (0.15 mmol, 45 mg) in DMF (2 mL) at 0 °C under a N_2 atmosphere. A pre-mixed solution of **16'** and DIPEA (0.45 mmol, 58 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir for 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **16** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to reveal **17'** as an amorphous white solid, which was used without further purification.

Step 3: Compound **17'** was treated with TFA (0.58 mL) in DCM (2 mL) at 0 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **17'** by thin-layer chromotography, the reaction was concentrated with a stream of nitrogen and the resulting residue (**18'**) was then stored on the high vac. for 6 h prior to use without further purification.

Step 4: HATU (0.16 mmol, 62 mg) was added to a stirring solution of E-2-heptenoic acid (0.15 mmol, 19 mg) in DMF (2 mL) at 0 °C under a N_2 atmosphere. A pre-mixed solution of **18'** and DIPEA (0.45 mmol, 58 mg) in DMF (2 mL) was then added to the reaction mixture. The reaction mixture was allowed to stir for 12 h at 25 °C under a N_2 atmosphere. Upon completion of the reaction, as indicated via complete conversion of **18** by thin-layer chromotography, the reaction mixture was diluted with EtOAc (200 mL) and washed three times with 1 M aq. HCl, three times

with saturated aq. $NaHCO_3$, and one time with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) using a gradient of 0-20% acetone in EtOAc to yield **3** as a white foam (57 mg, 51%). Three major rotamers noted by NMR (1.8:1.3:1.0), data is provided for the major rotamer. ^{1}H NMR ($CDCl_3$, 500 MHz) δ δ 8.30 (d, *J* = 9.3 Hz, 1H), 7.04 (d, *J* = 7.9 Hz, 1H), 6.91 – 6.57 (m, 4H), 6.15 (d, *J* = 7.6 Hz, 1H), 5.92 (d, *J* = 15.5 Hz, 1H), 5.51 (d, *J* = 5.2 Hz, 1H), 5.20 – 4.93 (m, 3H), 4.74 – 4.54 (m, 2H), 4.33 (d, *J* = 13.8 Hz, 1H), 3.90 – 3.22 (m, 5H), 3.10 – 2.98 (m, 1H), 2.92 (s, 3H), 2.78 (s, 3H), 2.63 (m, 1H), 2.46 (m, 1H), 2.33 (m, 1H), 2.29 – 2.08 (m, 3H), 2.07 – 1.76 (m, 5H), 1.50 (d, *J* = 6.9 Hz, 3H), 1.43 (m, 5H), 1.38 – 1.23 (m, 2H), 1.03 – 0.72 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 172.9, 172.7, 171.1, 170.9, 165.9, 165.8, 162.0 (2C), 146.5, 143.6, 122.8, 112.5 (2C), 102.5, 61.5, 56.6, 56.5, 54.5, 54.2, 53.9, 50.9, 49.5, 46.4, 38.3, 38.1, 31.8, 31.6, 30.9, 30.7, 30.3, 23.3, 23.1, 22.3, 18.7, 15.7, 13.9. IR for mixture (film) ν_{max} 3358, 2954, 2924, 2852, 2364, 2331, 1716, 1691, 1633, 1558, 1541, 1519, 1506, 1456, 1435, 1417, 1396, 1338, 1114, 956, 821 cm^{-1}; HRESI m/z 766.3740 ($C_{37}H_{51}F_2N_7O_7 + Na^+$ requires 766.3716). For specific correlations and peaks as determined by 2D methods please see **Figure S8** and **Table 2**, respectively**.**

BIOACTIVITY EVALUATION

Protein Purification. *Bacillus subtilis* ClpP (*Bs*ClpP) was overexpressed in BL21 (DE3) *E.coli* cells from New England Biolabs. Overnight cultures were used to inoculate 4 X 1 L LB-broth, which were grown at 37 °C while shaking at 250 rpm to OD_{600} = 0.7-1.0. Prior to induction, the cultures were cooled to ~18 °C. 1000x IPTG was then added to a final concentration of 1 mM IPTG. *Bs*ClpP was expressed for 12-16 h at 18 °C while shaking at 180 rpm. Cells were harvested by centrifugation for 15 min at 10,000 *g* and the pellet was resuspended in ~10 mL Buffer A (50 mM Tris-Cl pH = 8.0, 300 mM NaCl, 10% glycerol) per 1g pellet. The cells were lysed with an Avestin C3 Emulsiflex and the resulting lysate was clarified by centrifugation for 45 min at 28,850 *g*. The filtrate was loaded onto a 5 mL HP HisTrap Column (GE Healthcare), and washed with 5% Buffer B (Buffer A + 500 mM Imidazole) for 20 column volumes before stepwise elution (15%, 30%, 45%, 70%, 100% Buffer B) using a GE Healthcare Lifesciences AKTA FPLC. Fractions were pooled and exchanged into the storage buffer (25 mM HEPES pH = 7.5, 5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 10% glycerol) with a 16/60 S-300 HiPrep Sephacryl SEC (GE Healthcare). Purified protein solutions were concentrated with 50 kDa MWCO Amicon centrifugal concentrator to ~4.5 mg/mL. Final protein concentration was determined with a standard Bradford assay, and >95% purity was confirmed by SDS-PAGE gel analysis. Protein solution aliquots were flash-frozen with liquid N_2 and stored at -80 °C.

DFAP Decapeptide Peptidolysis Assay. 25 nM tetradecameric *Bs*ClpP in activity buffer 1 (25 mM HEPES, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 1mM DTT, 10% glycerol) was incubated with the compound of interest over a range of concentrations at 30 °C for 15 minutes in a flat bottom, non-binding, non-sterile, white polystyrene 96-well plate (Corning 3990). After the pre-incubation period, 1µL of a 1.5 mM Abz-DFAPKMALVPY^{NO2} (Biomatik) solution was added to each assay well to give a final assay concentration of 15 µM fluorogenic decapeptide and final assay volume of 100 µL. Assay plates were incubated at 30 °C and hydrolysis of the fluorogenic peptide was monitored via an i-TECAN Infinite M200 plate reader (excitation: 320 nm; emission: 420 nm). Readings were taken every 30 minutes for 2 hours. All compounds, and 1% DMSO (negative control) were normalized relative to background Abz-DFAPKMALVPY^{NO2} fluorescence (ΔRFU = sample fluorescence – averaged buffer/DFAP fluorescence).

Thermal Shift Assay. All compounds were evaluated at a final assay concentration of 50 μM. 1 μM *Bs*ClpP monomer (71.4 nM tetradecamer) and 2 μL of 5 mM compound of interest prepared in activity buffer 2 (20 mM HEPES pH = 7.0, 100 mM NaCl) in 1.5 mL Eppendorf tubes. SYPRO baseline control and DMSO negative control were prepared similarly. Protein and controls were pre-incubated for 30 min at 25 °C. After pre-incubation, 2 μL of 100X SYPRO Orange Protein Gel Stain, prepared in 100% DMSO and stored in light-free desiccator at 25 °C, was added to the samples and mixed thoroughly for a total volume of 200 μL. The large 200 μL samples were split into 3 x 50 μL wells in 96-well, Hard Shell®, thin-wall PCR Plates (BioRad HSP9601) sealed with optically clear Microseal® 'B' adhesive seals (BioRad MSB1001). The resulting plates were spun in a tabletop PCR plate spinner (VWR 89184-608) in 15 sec intervals until all bubbles were removed. Samples rested in the dark for 10 min and were then evaluated using BioRad CFX96 ™ Real-Time System. Melt curves were prepared in increments of 0.3 °C per minute over a range of 25 °C to 85 °C, with FRET readings taken after a 1 minute hold at each temperature. Melting temperatures were determined by nonlinear fitting to a Boltzmann Sigmoidal Curve using GraphPad Prism.

MIC Determination. *Bacillus subtilus* ATCC 6051 was streaked onto an LB agar plate and grown overnight at 37 °C. Pre-warmed (37 °C) Mueller–Hinton broth (5 mL) was inoculated with 3–5 colonies of *B. subtilis*. The culture was incubated at 37 °C shaking at 250 rpm overnight. The resulting overnight stock solution of *B. subtilis* was diluted 1:100 to provide the assay stock solution. Following standard microdilution protocol[1] compounds **1**–**3** were serially diluted to provide a final well volume of 200 μL. Briefly, 2 μL of compound stock was added to 198 μL of Mueller-Hinton broth. This was then serially diluted 2-fold down the plate by taking 100 μL and transferring to the subsequent well that contained 100 μL of Mueller–Hinton broth. To each well was then added 100 μL of the 1:100 dilution *B. subtilis* assay culture to obtain a final well volume of 200 μL. Plates were incubated overnight at 37 °C and after 16 h, MIC values were determined by visual inspection. Reported MIC values indicate the concentration of the last well containing no visible cell growth. Ampicillin and Kanamycin were used as positive controls. DMSO treatment was used as a negative control. Buffer only wells were included as a control/indicator of bacterial contamination. Each compound was tested in triplicate. Top concentrations for each compound evaluated: Ampicillin (125 μg/mL, 357.7 μM), Kanamycin (125 μg/mL, 258.0 μM), **1** (0.183 μg/mL, 250 nM). **2** (18.25 μg/mL, 25 μM), **3** (18.60 μg/mL, 25 μM). Stock solutions for each compound evaluated: Ampicillin (71.5 mM), Kanamycin (51.6 mM), **1** (50 μM), **2** (5 mM), and **3** (5 mM).

HYDROGEN-DEUTERIUM EXCHANGE EXPERIMENTS

Purified ADEPs **1** and **2** were stored in a desiccator for one week at 25 °C prior to H/D exchange experiments. The NMR probe was pre-equilibrated to either 25° C or 40° C before introduction of the NMR sample. NMR samples were prepared by dissolving compound **1** or **2** in ampule sealed CD_3OD at a concentration of 2 mM. The samples were mixed and promptly transferred to a clean NMR tube. ^{1}H-NMR spectra were acquired at 500 MHz with 8 scans, 1 second delay, sweep width of 8012.8 Hz and 16384 complex points. Timing of each kinetic run was carefully started upon sample mixing. The time of the first collected spectra was designated as t1. Subsequent spectra were collected at 300 second intervals with a 24 second acquisition time. The kinetic runs were terminated when the amide peaks were completely exchanged and no longer visible. The data was

processed in MestReNova software. The data was zero filled to 65536 points with a 0.70 Hz exponential function and baseline correction applied upon processing. The integration of the exchanging amide signal of interest was calibrated to a non-exchanging reference peak and converted to concentration. The concentration versus time data was analyzed using PRISM software. Results for compound **1** are presented in **Figure S3**. No complementary time lapse is shown for compound **2**, as all NH hydrogens had undergone exchange prior to t1 (4:46).

MOLECULAR DYNAMICS SIMULATIONS

Model Peptides. Simulations were performed for both the compounds with ester (**1**) and -NH- (**2**) linkages. Two different initial structures of each compound, S1 and S2 (structure 1 and structure 2, respectively), were prepared using the Chimera molecular modeling package.[2] First, the linear peptides were built, followed by linkage of the *N*- and *C*- terminal residues and subsequent energy minimization to construct the macrocycle. In both the initial structures for (**1**) and (**2**), the amide bonds involving the N atoms of Pro4, NMA5, and Pro7 (**Figure S4**) had the *cis* configuration, while all the other amide bonds were in the *trans* configuration.

Bias-Exchange Metadynamics (BE-META) Simulations. Following preparation in Chimera, each initial structure was solvated using a pre-equilibrated box of water molecules. Enough ions were added to neutralize the charge. Each structure was then energy minimized using the steepest descent algorithm. The minimized system was heated from 5 K to 300 K within 50 ps in an isobaric–isothermal (*NPT*) ensemble, followed by a 100 ps equilibration at a temperature of 300 K and a pressure of 1 bar. In both these equilibration simulations, a harmonic constraint was placed on the peptide heavy atoms, with a force constant of 1,000 kJ/mol·nm^2.

The *NPT* ensemble was used for all BE-META production simulations. The temperature was maintained at 300 K using the V-rescale thermostat,[3] with a time coupling constant of 0.1 ps. The compound and the solvent were coupled to two separate thermostats, to mitigate the "hot solvent–cold solute" problem.[4-5] The pressure was maintained at 1 bar using the Berendsen barostat[6] was used to maintain the pressure at 1 bar, with a time coupling constant of 2.0 ps and an isothermal compressibility of 4.5×10^{-5} bar^{-1}. All bonds involving hydrogen were constrained using the LINCS algorithm.[7] Dynamics of the system were evolved using the leapfrog algorithm, with a time step of 2 fs. Both short-range Lennard-Jones and electrostatic nonbonded interactions were truncated at 1.0 nm. Beyond the cutoff distance, Particle Mesh Ewald (PME) was used for the electrostatic interactions,[8] with a Fourier spacing of 0.12 nm and an interpolation order of 4. A long-range dispersion correction[9] for the energy and pressure was used for the Lennard-Jones interaction beyond the cutoff. All trajectories were saved every 1 ps for subsequent analysis.

All simulations were performed using the OPLS-AA-2005 force field[10] with TIP4P water[11] in Gromacs 4.6.7[12] with the PLUMED 2 plugin.[13] Conformational sampling of the macrocycles was enhanced using BE-META simulations. The collective variables (CVs) in the BE-META simulations consist of two types of 2D biases. The first 2D bias is along ($\phi_i\times\psi_i$), where ϕ/ψ are the backbone dihedral angles of the same residue. The second type of 2D bias is along ψ of one residue and ϕ of the next residue, ($\psi_i\times\phi_{i+1}$). These 2D biases were previously found to enhance the conformational sampling of CPs.[14] Four $\phi_i\times\psi_i$ ($\phi_2\times\psi_2$, $\phi_3\times\psi_3$, $\phi_5\times\psi_5$, and $\phi_6\times\psi_6$) and three $\psi_i\times\phi_{i+1}$ ($\psi_2\times\phi_3$, $\psi_4\times\phi_5$, and $\psi_5\times\phi_6$) were used as CVs for each compound, giving a total of 7 biased replicas (**Figure S5**). Gaussian hills were deposited every 4 ps, with a height of 0.1 kJ/mol and a width of

0.314 rad. Exchanges were attempted every 5 ps between different replicas. For analysis of an unbiased structural ensemble, three neutral replicas were added, giving a total of 10 replicas per system. BE-META production runs were performed for 100 ns for compound **1** and 400 ns for compound **2**.

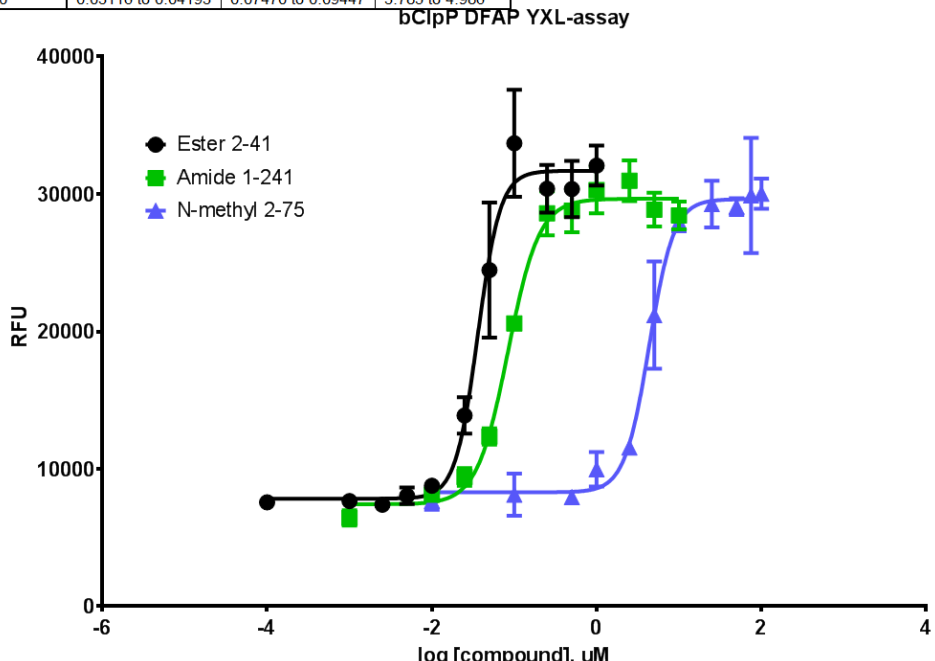
Principal Component and Cluster Analysis. To characterize the structural ensemble of each compound, the last 50 ns of the unbiased replicas were analyzed using dihedral principal component analysis (dPCA) with the ϕ/ψ of all residues.[15-16] It is noted that unintended *cis*/*trans* isomerization of the amide bonds in the *N*-methylated residues and Pro were observed, likely an artifact due to the biasing potentials added on the backbone atoms during the BE-META simulations. Therefore, we only analyzed the conformations in the last 50 ns of the unbiased replicas that had the correct isomer conformations (all three amide bonds involving Pro4, NMA5, and Pro7 were *cis*, as shown in **Figure S4**).

Following dPCA, the population of each cluster was calculated using a density peak-based cluster analysis.[17] For cluster analysis, the principal subspace along the first three principal components (PC1, PC2 and PC3) was divided into 50×50×50 grids. The population of each cluster was determined by summing the population of every grid the cluster contained. Only grids with a population greater than 0.1 were used in cluster analysis. Results for compounds **1** and **2** are shown in **Figure S6.**

As shown in **Figure S6**, structural ensembles of compound **1** from two different initial structures (S1 and S2) converged in 100 ns BE-META simulations. The most populated cluster in water adopted a conformation very similar to the X-ray structure seen in PDB ID 3KTI (backbone RMSD 0.60 Å; **Figure 4A**). However, it is difficult to obtain well-converged results for compound **2**, even after 400 ns BE-META simulations. Compound **2** adopted multiple conformations in water (**Figure 4B**), and the majority of these exposed the alanine -NH- into the solvent and lacked the intramolecular hydrogen bond between the alanine -NH- and the extracyclic 3,5-difluorophenylalanine carbonyl.

FIGURES

	Ester 2-41	Amide 1-241	N-methyl 2-75
LogEC50	-1.506 to -1.377	-1.127 to -1.025	0.5781 to 0.6977
HillSlope	1.982 to 4.399	1.731 to 2.875	1.862 to 3.966
EC50	0.03116 to 0.04193	0.07470 to 0.09447	3.785 to 4.986



	Ester 2-41	Amide 1-241	N-methyl 2-75
Degrees of Freedom	29	29	29
R square	0.9645	0.9840	0.9705

Figure S1. Decapeptide dose-response curves.

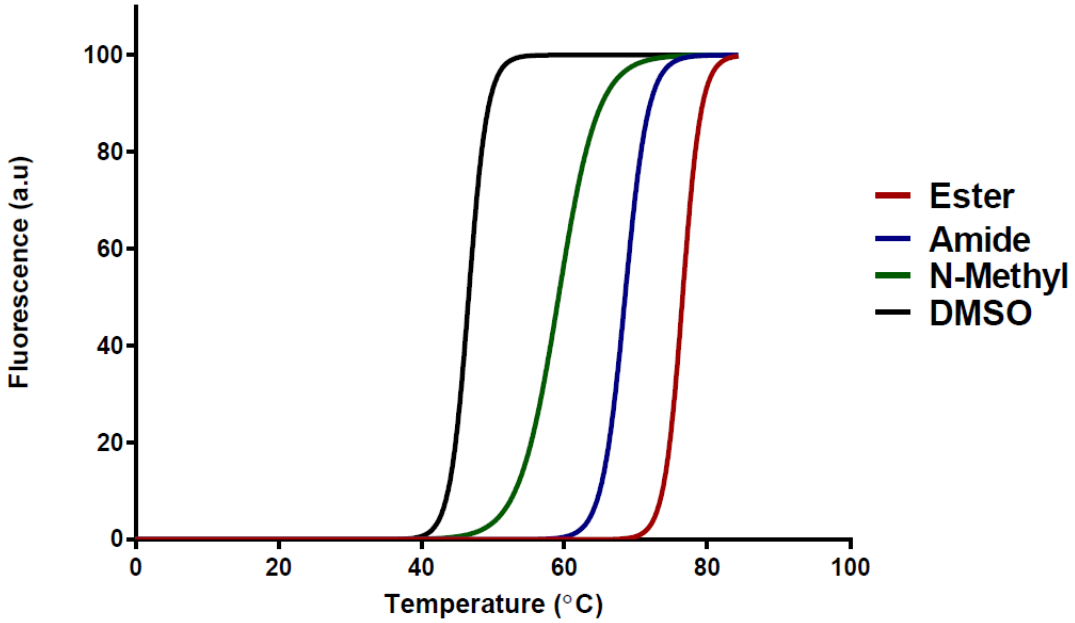


Figure S2. Thermoshift results. DMSO (T_m = 46.6 °C), Ester (**1**, T_m = 76.6 °C), Amide (**2**, T_m = 68.5 °C), N-Methyl (**3**, T_m = 59.2 °C).

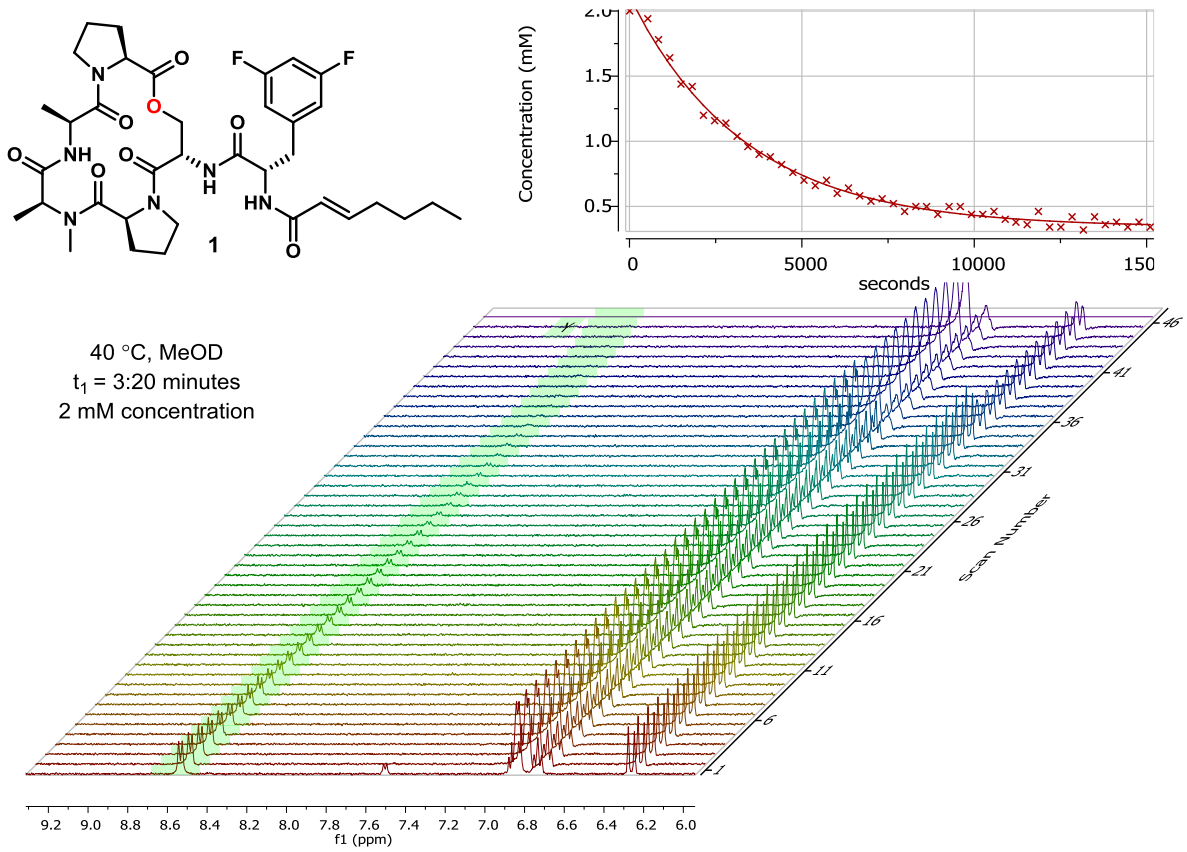


Figure S3. Hydrogen-deuterium exchange results for compound **1**.

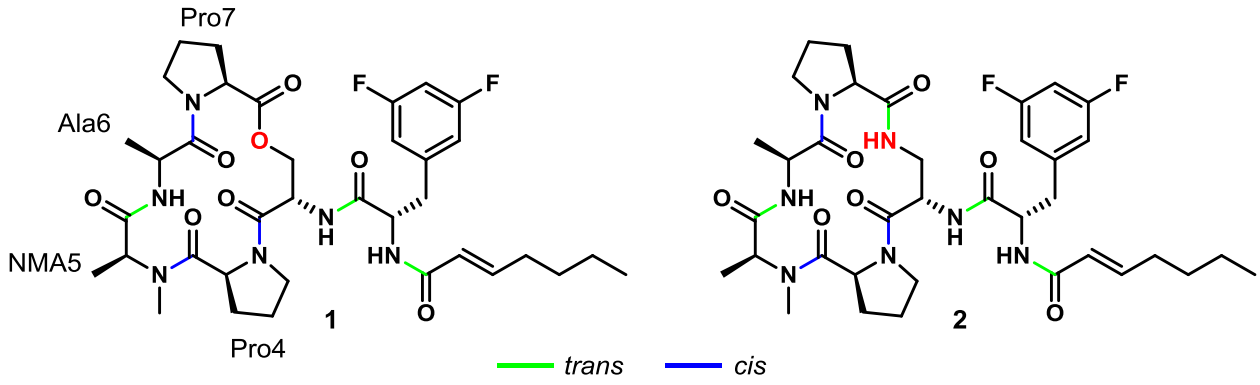


Figure S4. Structure of **1** and **2**, along with the *cis*/*trans* conformations of the amide bonds used as input geometries in MD simulations.

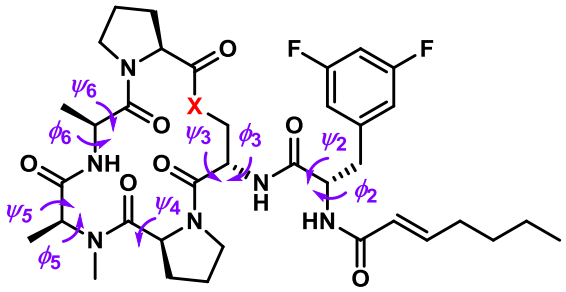


Figure S5. Dihedral angles used in the BE-META simulations used as input in MD simulations.

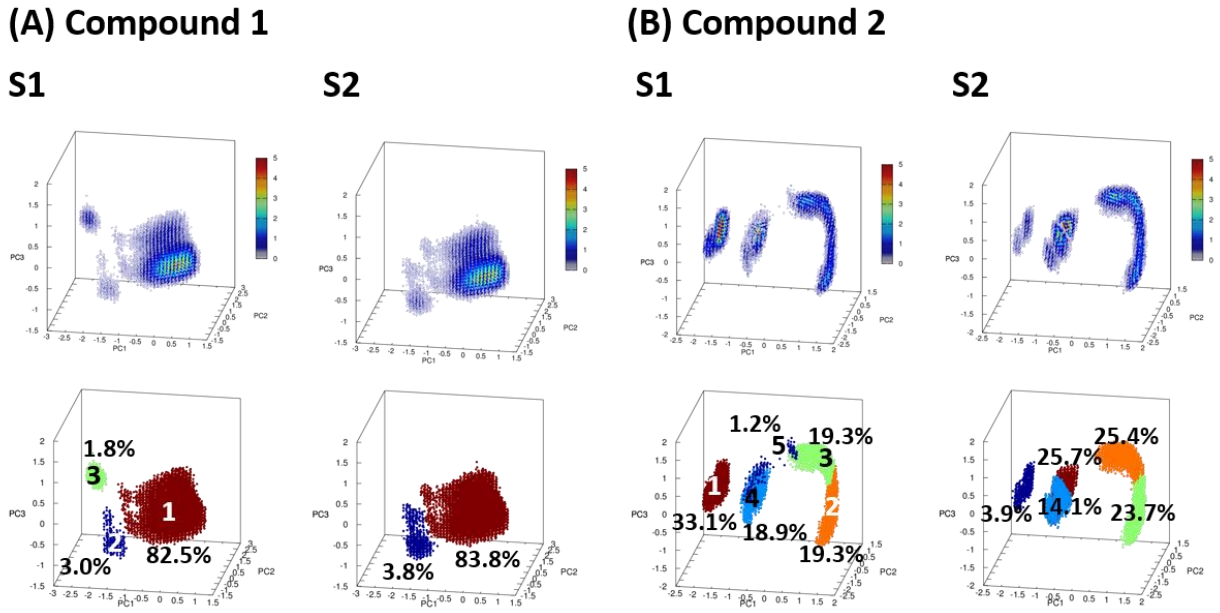
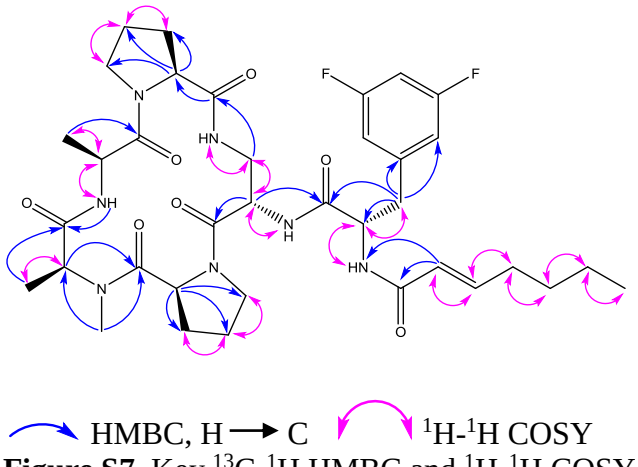
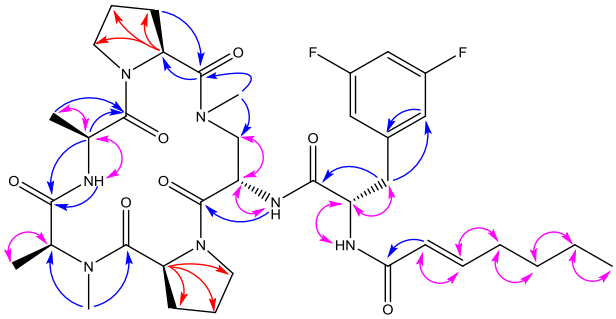


Figure S6. Conformational density profiles as a function of the first three principal components (top) and the corresponding cluster analysis results (bottom) of **(A)** compound **1** and **(B)** compound **2**. Clusters are colored based on their populations with the largest cluster colored in dark red and the smallest cluster colored in dark blue.



HMBC, H → C ^{1}H-^{1}H COSY

Figure S7. Key ^{13}C-^{1}H HMBC and ^{1}H-^{1}H COSY correlations for **2**.



HMBC, H → C ^{1}H-^{1}H COSY HSQC-TOCSY

Figure S8. Key ^{13}C-^{1}H HMBC, ^{1}H-^{1}H COSY and ^{13}C-^{1}H HSQC-TOCSY correlations for **3**.

TABLES

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) Data for **2** in $CDCl_3$ as determined from ^{13}C-^{1}H HMBC, ^{1}H-^{1}H COSY, and ^{13}C-^{1}H HSQC-TOCSY correlations.

Position	δ C, type	δ H, (*J* in Hz)[a]
1		7.89 d (9.7)
2	171.6, C	
3	55.8, CH	3.46 ov.
4		7.61 br.
5	166.1, C	
6	122.9, CH	5.85 d (15.0)
7	145.9, CH	6.73 dt (7.1, 15.0)
8	31.8, CH_2	2.15 ov.
9	30.4, CH_2	1.40 m
10a	22.3, CH_2	1.31 m
10b		1.32 m
11	14.0, CH_3	0.89 t (7.1)
12	54.3, CH	4.49 t (9.4)
13a	43.7, CH_2	2.68 ov.
13b		4.15 m.
14		7.78 br
15	174.7, C	
16	60.5, CH	4.32 d (8.3)
17a	31.9, CH_2	2.04 ov.
17b		2.18 ov.
18a	21.5, CH_2	1.90 ov.
18b		1.94 ov.
19a	46.4, CH_2	3.39 ov.
19b		3.84 m.
20	170.6, C	
21	48.8, CH	5.01 m
22	17.8, CH_3	1.48 d (6.7)
23		8.02 d (9.8)
24	169.7, C	
25	56.4, CH	4.99 ov.
26	15.8, CH_3	1.51 d (6.8)
27	30.9, CH_3	2.65 s
28	173.6, C	
29	56.9, CH	5.50 d (8.4)
30a	30.8, CH_2	1.96 ov.
30b		2.44 m
31a	23.3, CH_2	1.96 ov.
31b		2.02 ov.
32a	48.4, CH_2	3.47 ov.
32b		4.23 m
33	168.1, C	
34a	35.9, CH_2	3.04 dd (9.0, 14.3)
34b		3.29 dd (5.1, 14.3)
35	143.1, C	

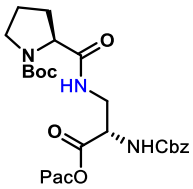
36	112.0, CH	6.62 ov.
37	162.1, C	
38	101.7, CH	6.59 ov.
39	162.1, C	
40	112.0, CH	6.62 ov.
[a]ov: overlapped signal		

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) Data for **3** in $CDCl_3$ as determined from ^{13}C-^{1}H HMBC and ^{1}H-^{1}H COSY correlations.

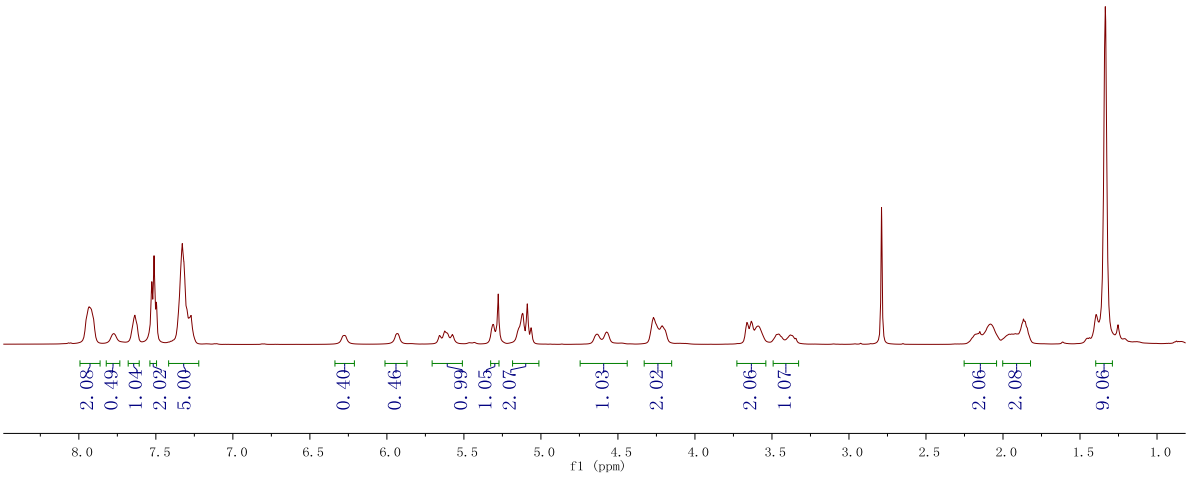
Position	**δ C, type**	**δ H, (*J* in Hz)[a]**
1		6.16 d (7.6)
2	171.1, C	
3	53.9, CH	5.01 ov.[a]
4		7.04 ov.
5	165.9, C	
6	122.8, CH	5.93 d (15.5)
7	146.5, CH	6.86 ov.
8	31.8, CH_2	2.2 ov.
9		-
10a	30.3, CH_2	1.44 ov.
10b	22.3, CH_2	1.35 ov.
11	13.9, CH_3	0.91 ov.
12	54.2, CH	4.67 ov.
13a	54.5, CH_2	4.33 d (13.8)
13b		2.63 m
14	38.3, CH_3	2.93 s
15	173.4, C	
16	61.5, CH	4.65 ov.
17a	31.6, CH_2	2.48 m
17b		2.00 ov.
18a	23.1, CH_2	1.85 ov.
18b	50.9, CH_2	3.82 ov.
19a		3.4 ov.
19b	172.7, C	
20	49.5, CH	5.04 ov.
21	18.7, CH_3	1.46 ov.
22		8.31 d (9.3)
23	170.9, C	
24	56.5, CH	5.14 ov.
25	15.7, CH_3	1.51 d (6.9)
26	30.9, CH_3	2.78 s
27	172.9, C	
28	56.6, CH	5.52 d (5.2)
29	30.7, CH_2	2.35 m
30a		1.96 ov.
30b	23.3, CH_2	1.94 ov.
31a		2.11 ov.
31b	46.4, CH_2	3.77 m.

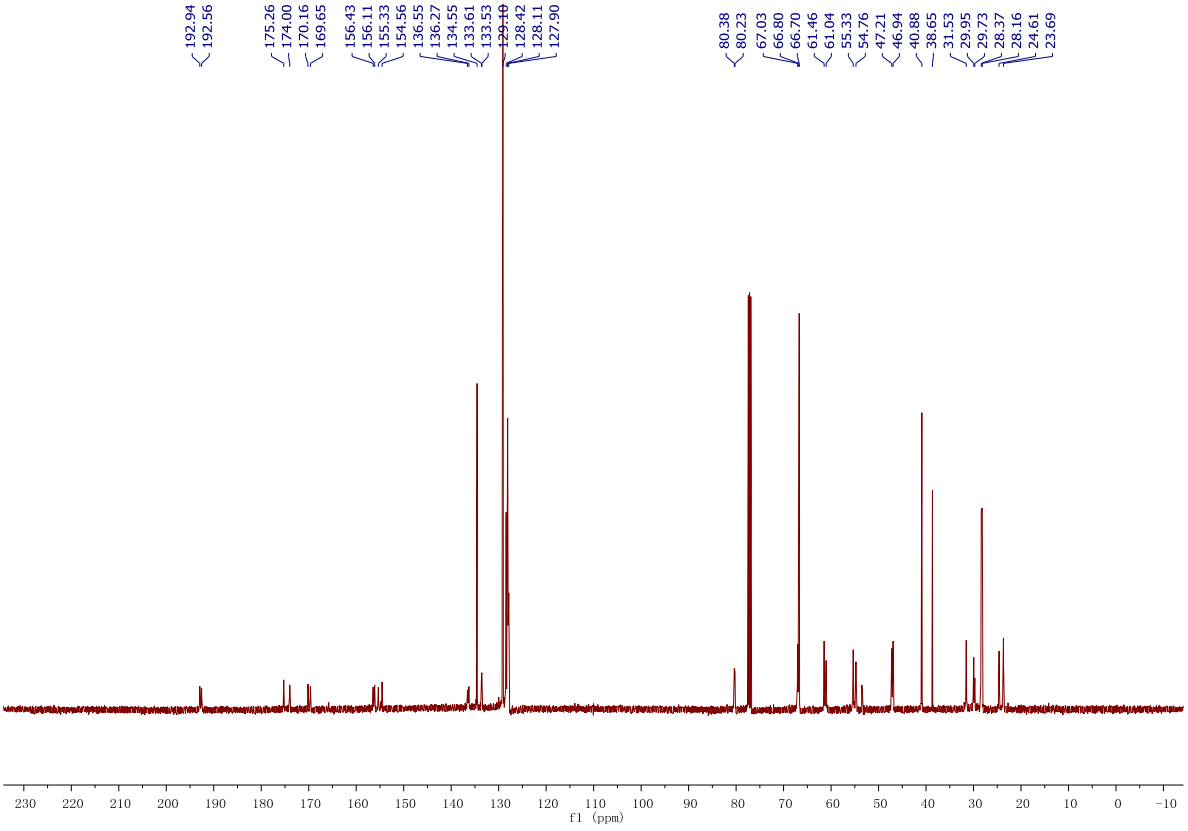
32a		3.56 ov.
32b	165.8, C	
33	38.1, CH_2	3.36 ov.
34a		3.07 ov.
34b	143.6, C	
35	112.5, CH	6.8 ov.
36	162, C	
37	102.5, CH	6.69 ov.
38	162, C	
39	112.5, CH	6.8 ov.
40		6.16 d (7.6)
[a]ov: overlapped signal		

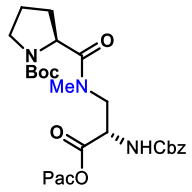
NMR SPECTRA



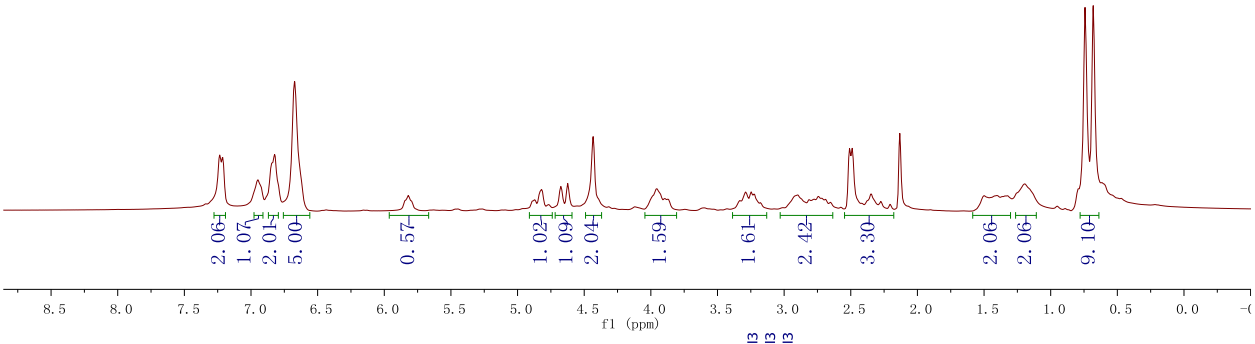
6

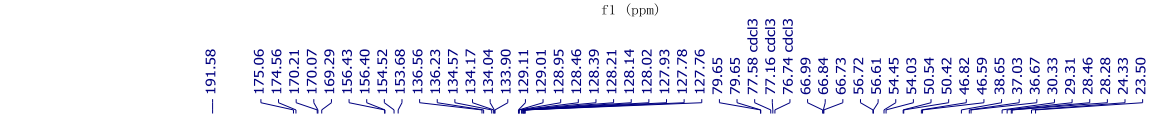





O
N
Boc
MeN
O
NHCbz
PacO

6'


2.06
1.07
2.01
5.00
0.57
1.02
1.09
2.04
1.59
1.61
2.42
3.30
2.06
2.06
9.10
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


191.58
175.06
174.56
170.21
170.07
169.29
156.43
156.40
154.52
153.68
136.56
136.23
134.57
134.17
134.04
133.90
129.11
129.01
128.95
128.46
128.39
128.21
128.14
128.02
127.93
127.78
127.76
79.65
77.58 cdcl3
77.16 cdcl3
76.74 cdcl3
66.99
66.84
66.73
56.72
56.61
54.45
54.03
50.54
50.42
46.82
46.59
38.65
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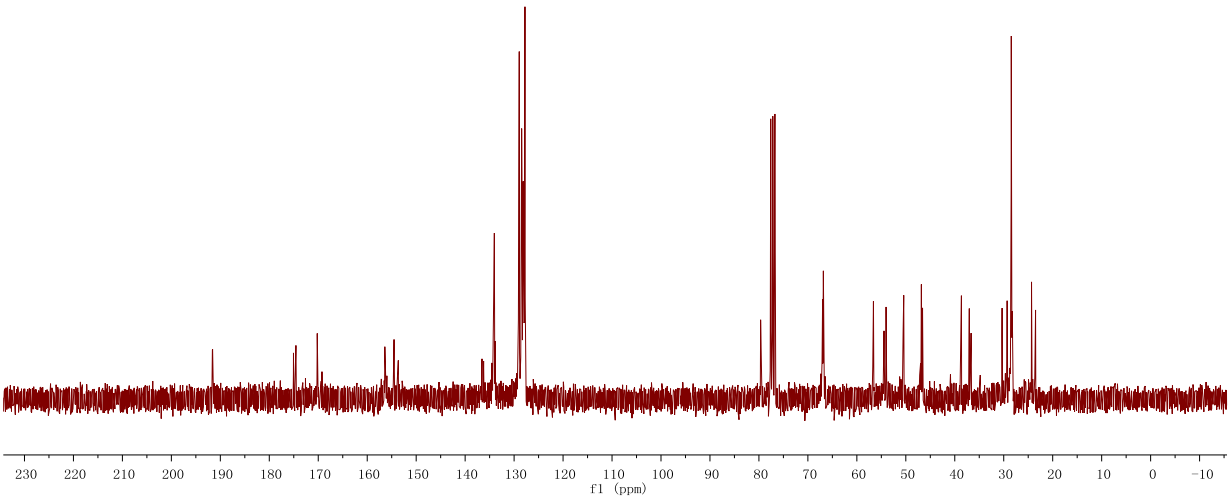
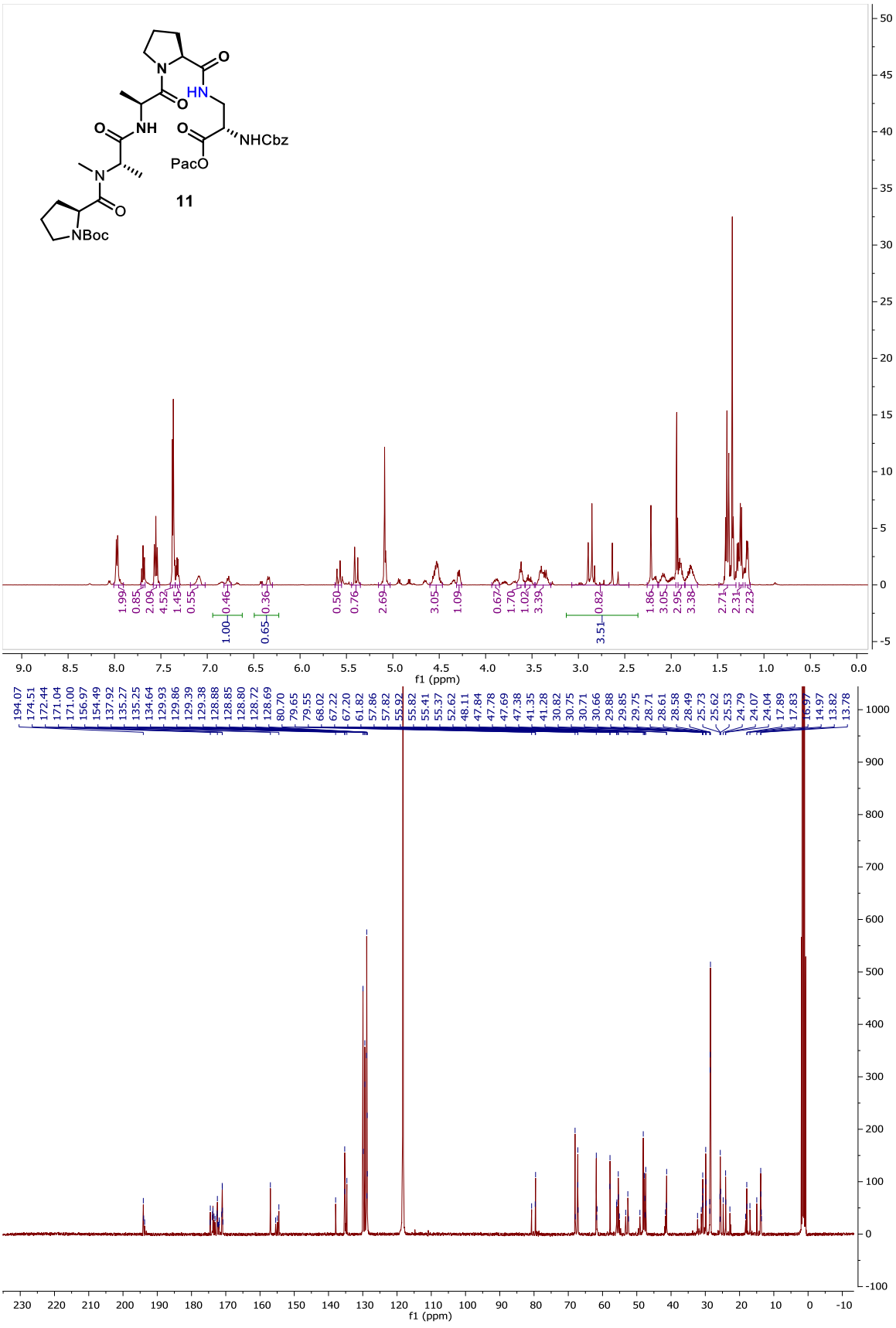
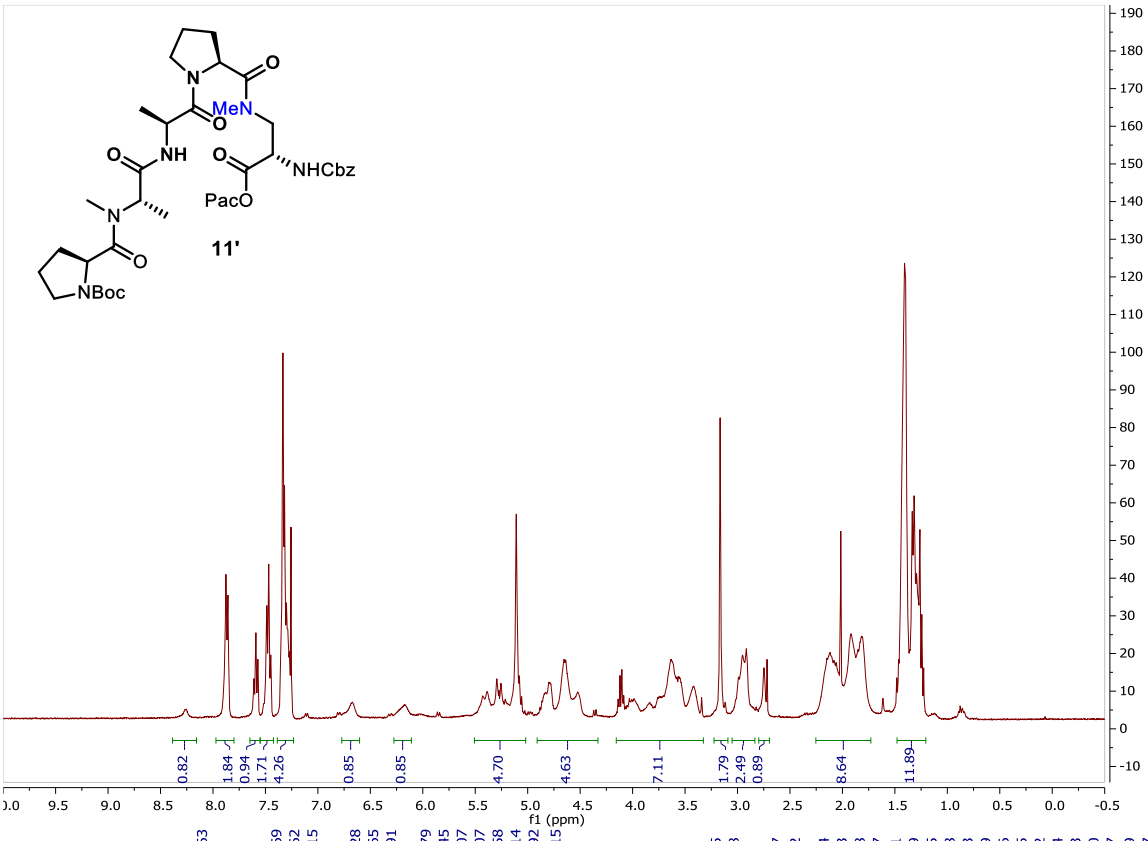
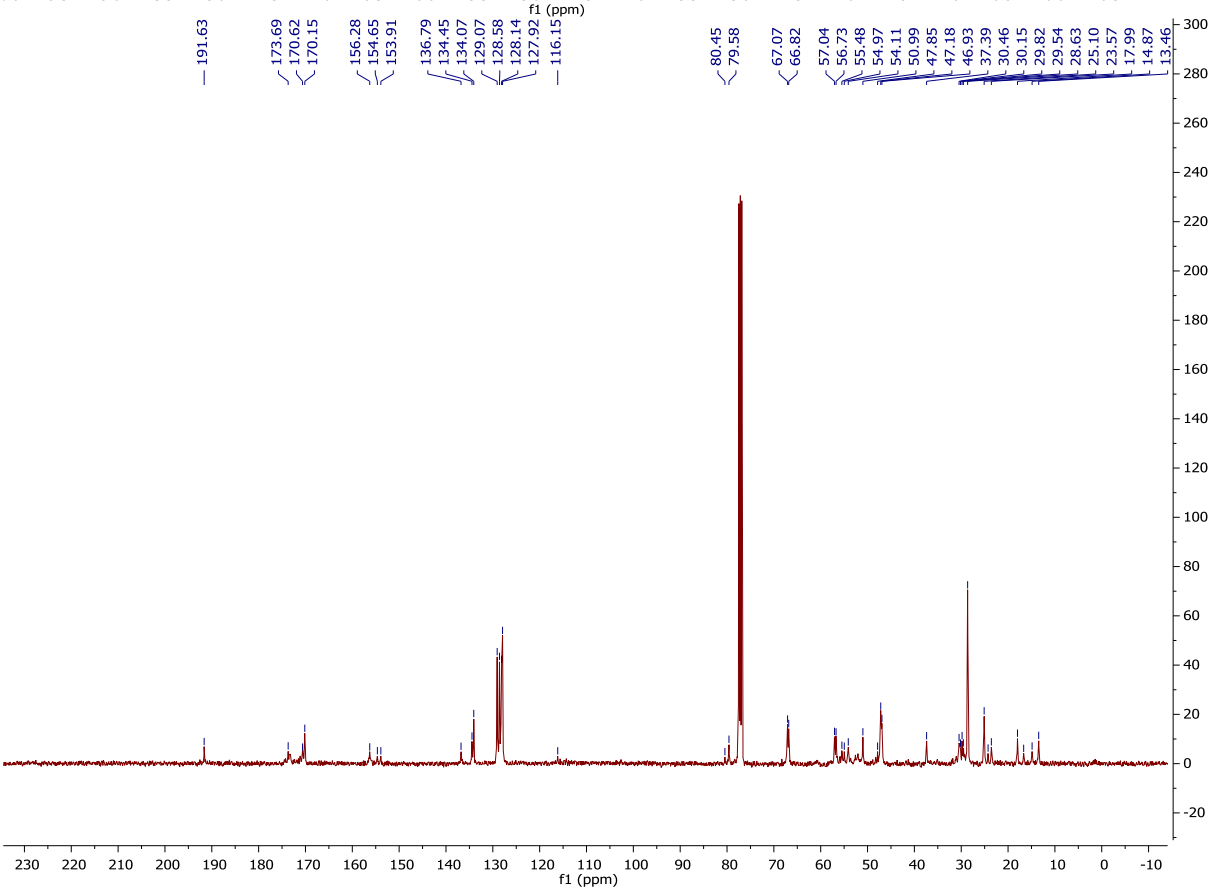
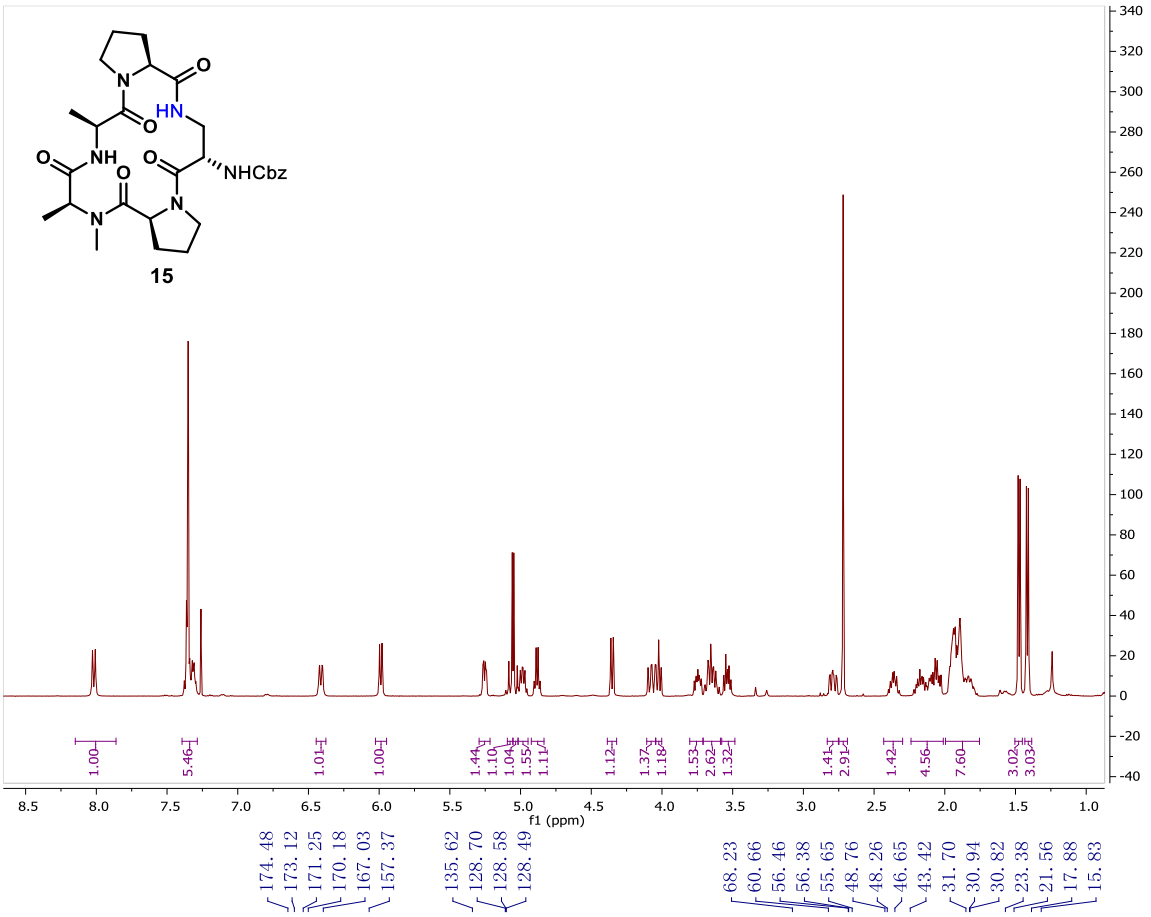
30.33
29.31
28.46
28.28
24.33
23.50

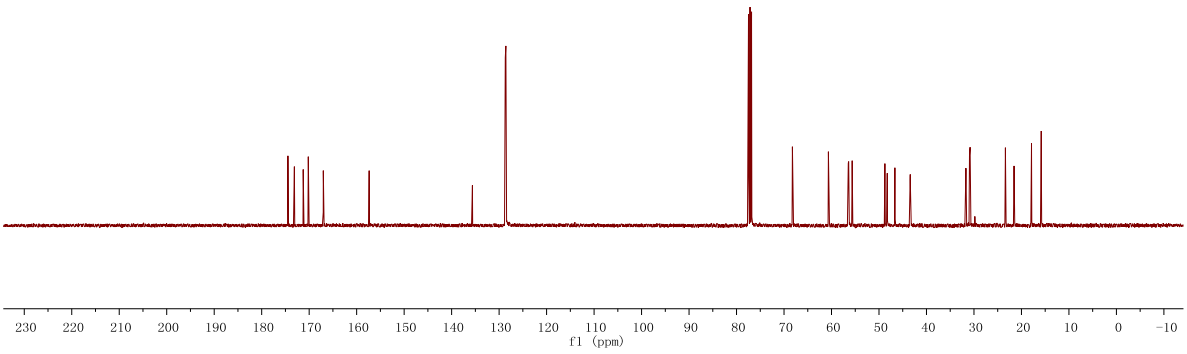

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

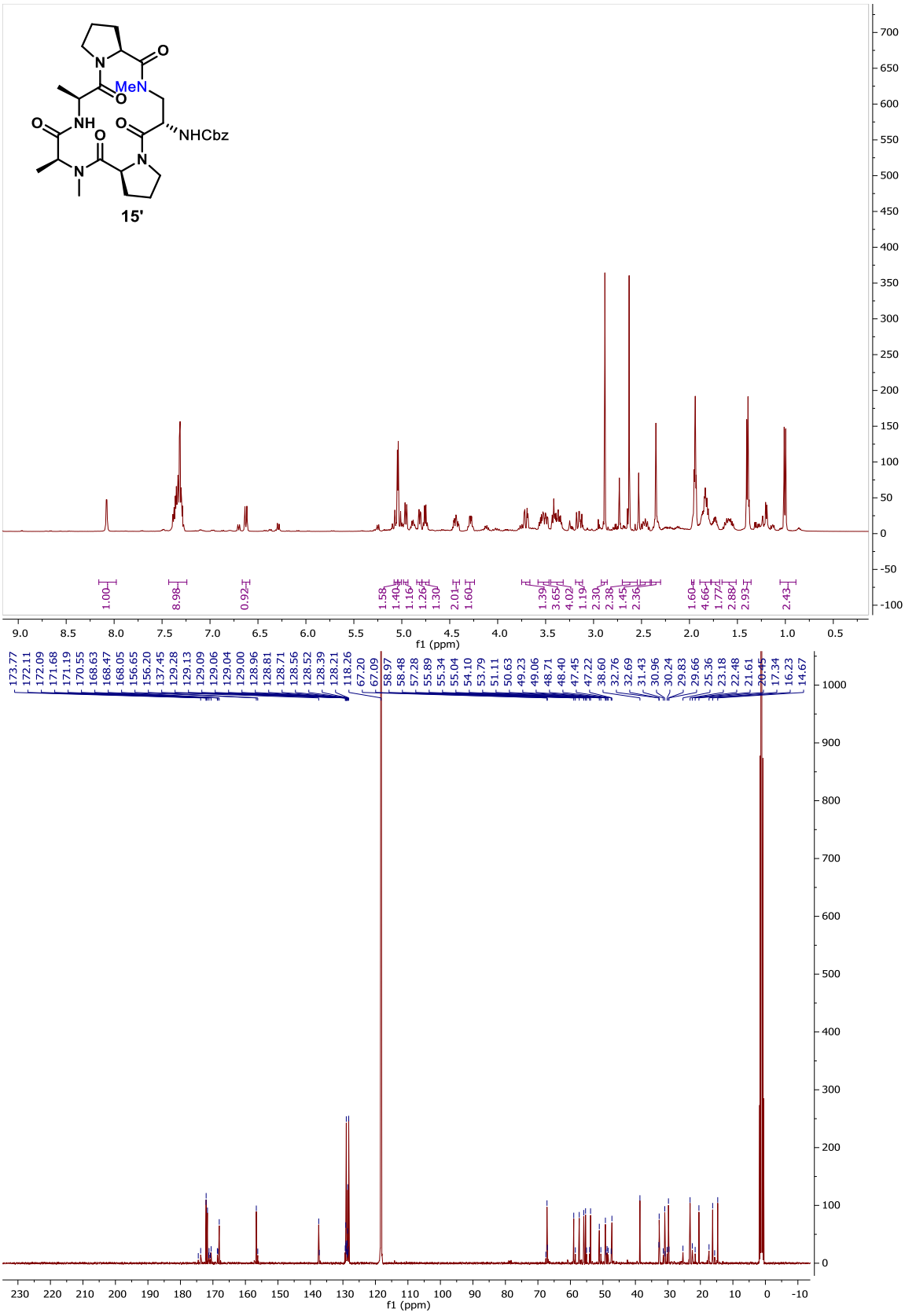

11
f1 (ppm)
f1 (ppm)


MeN
NH
NHCbz
PacO
NBoc
11'
0.82
1.84
0.94
1.71
4.26
0.85
0.85
4.70
4.63
7.11
1.79
2.49
0.89
8.64
11.89
f1 (ppm)


191.63
173.69
170.62
170.15
156.28
154.65
153.91
136.79
134.45
134.07
129.07
128.58
128.14
127.92
116.15
80.45
79.58
67.07
66.82
57.04
56.73
55.48
54.97
54.11
50.99
47.85
47.18
46.93
37.39
30.46
30.15
29.82
29.54
28.63
25.10
23.57
17.99
14.87
13.46
f1 (ppm)


15
f1 (ppm)
1.00
5.46
1.01
1.00
1.44
1.10
1.04
1.55
1.11
1.12
1.37
1.18
1.53
2.62
1.32
1.41
2.91
1.42
4.56
7.60
3.02
3.03
174.48
173.12
171.25
170.18
167.03
157.37
135.62
128.70
128.58
128.49
68.23
60.66
56.46
56.38
55.65
48.76
48.26
46.65
43.42
31.70
30.94
30.82
23.38
21.56
17.88
15.83


f1 (ppm)


MeN
NH
NHCbz
15'
1.00
8.98
0.92
1.58
1.40
1.16
1.26
1.30
2.01
1.60
1.39
3.65
4.02
1.19
2.30
2.38
1.45
2.36
1.60
4.66
1.77
2.88
2.93
2.43
f1 (ppm)
173.77
172.11
172.09
171.68
171.19
170.55
168.63
168.47
168.05
156.65
156.20
137.45
129.28
129.13
129.09
129.06
129.04
129.00
128.96
128.81
128.71
128.56
128.52
128.39
128.21
118.26
67.20
67.09
58.97
58.48
57.28
55.89
55.34
55.04
54.10
53.79
51.11
50.63
49.23
49.06
48.71
48.40
47.45
47.22
38.60
32.76
32.69
31.43
30.96
30.24
29.83
29.66
25.36
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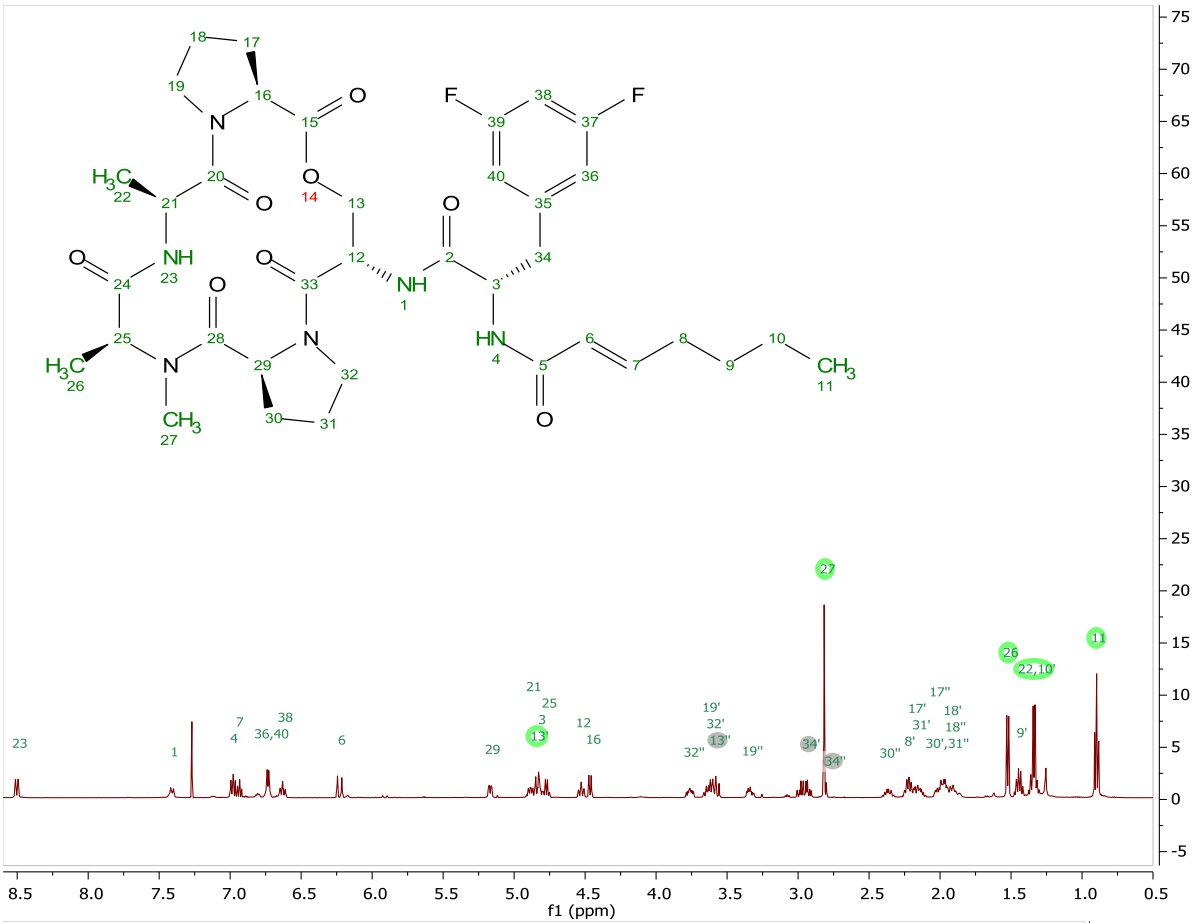
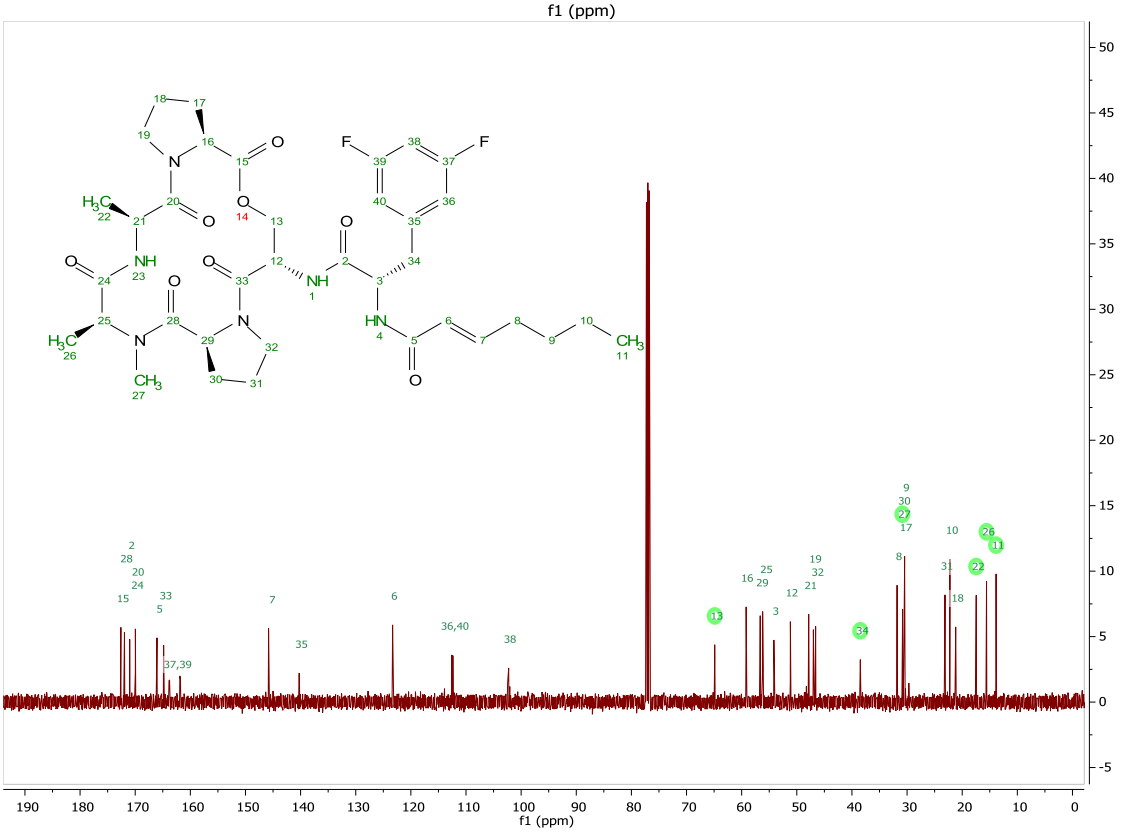
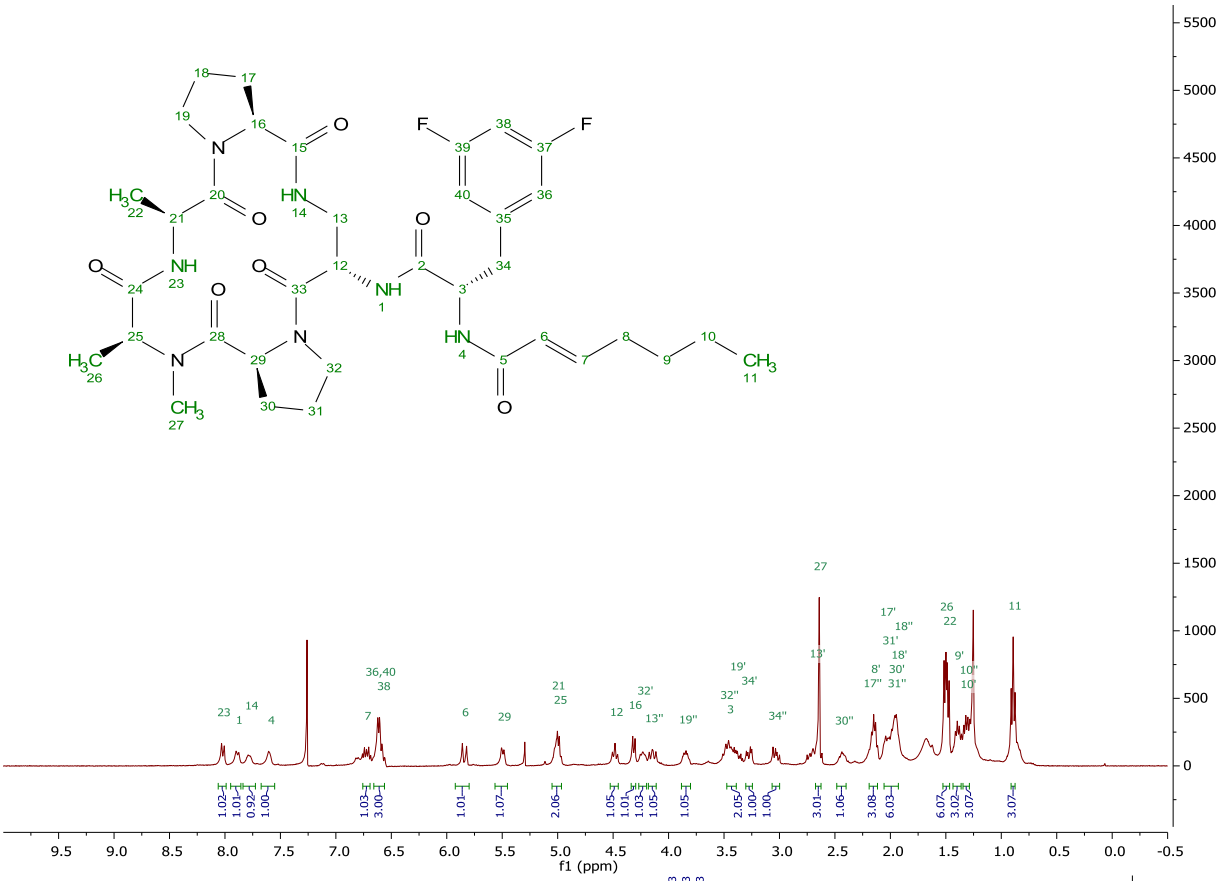
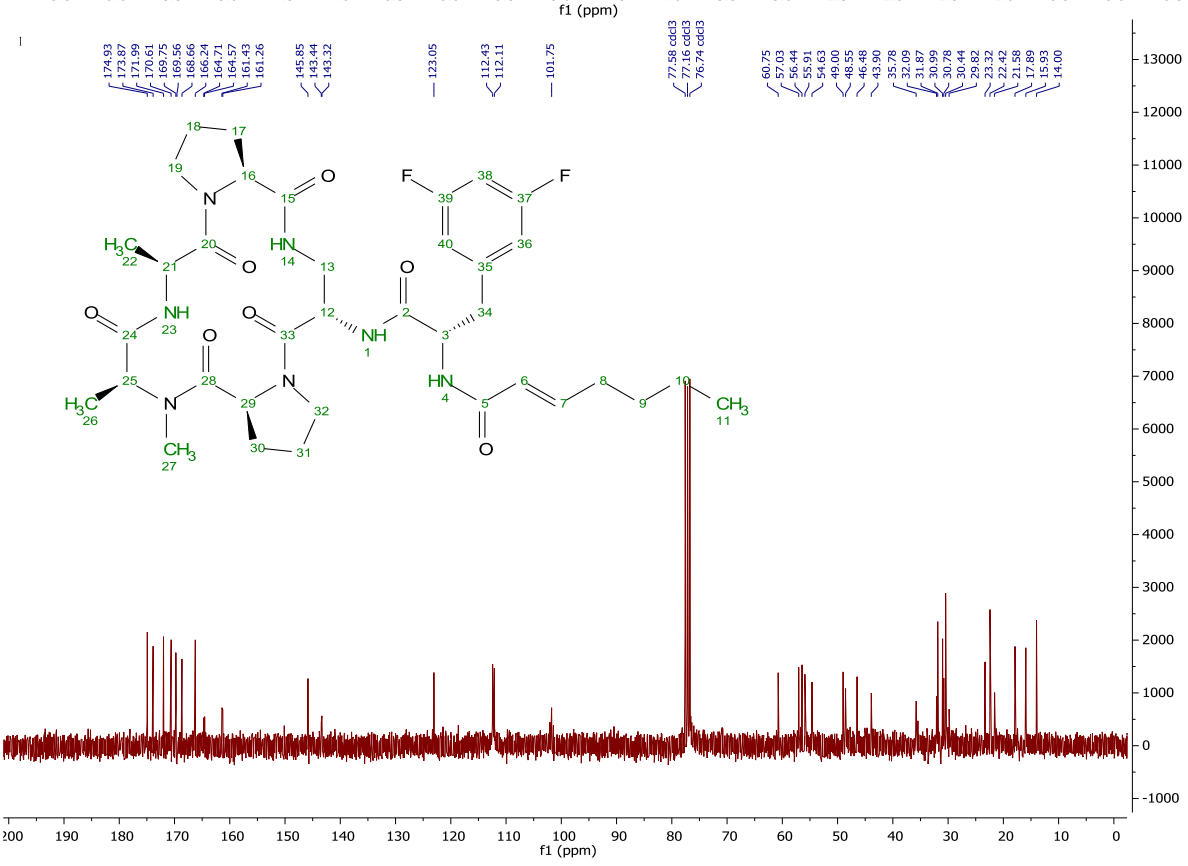
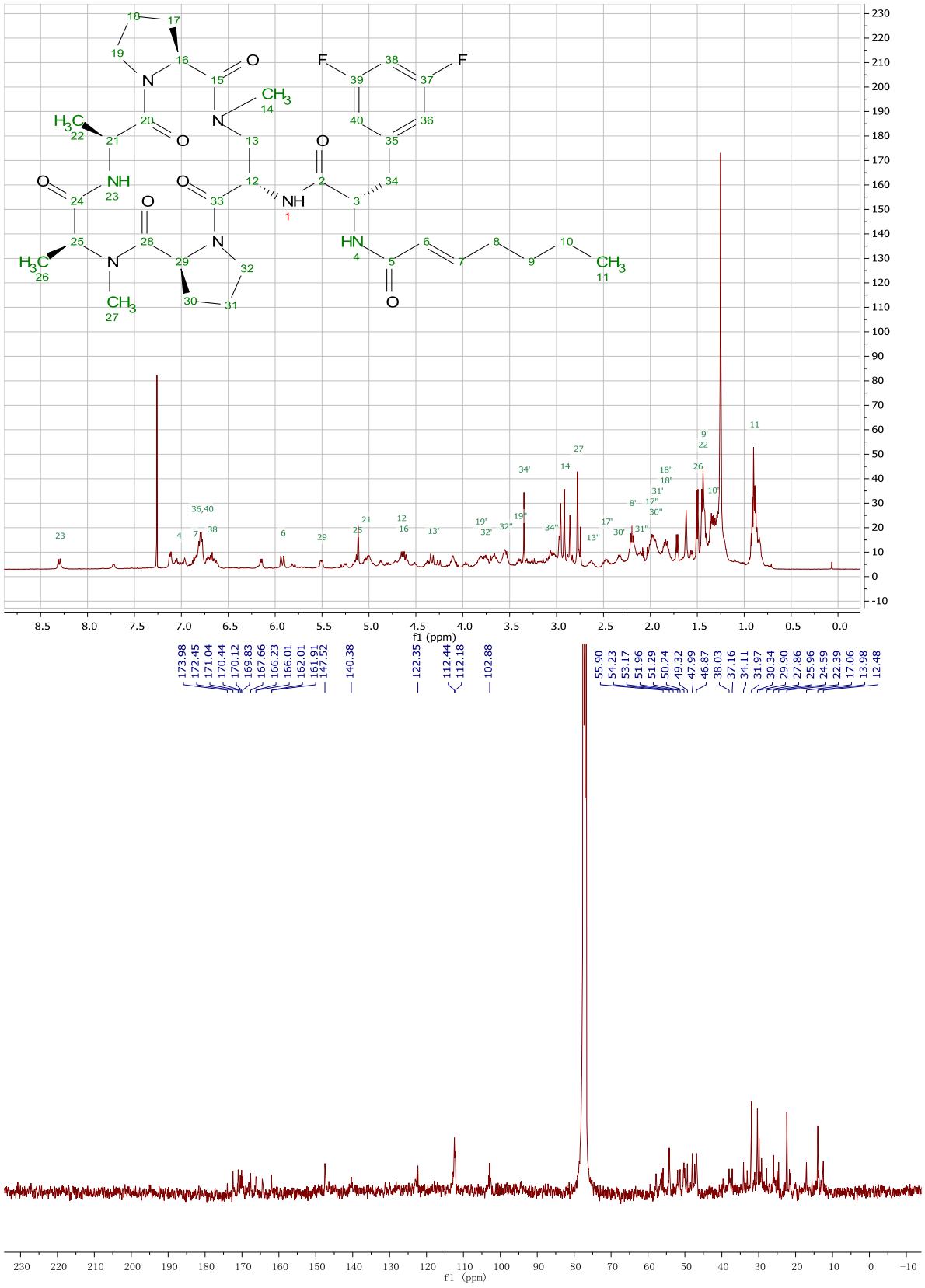
22.48
21.61
20.45
17.34
16.23
14.67
f1 (ppm)


f1 (ppm)


f1 (ppm)


f1 (ppm)


f1 (ppm)


f1 (ppm)
f1 (ppm)

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